

MATHEMATICAL MODELLING OF BACTERIAL MERCURY RESISTANCE

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Abstract

A mathematical model of mercury resistance was designed which describes the following reactions: the cellular uptake and volatilisation of Hg^{2+} , binding of the DNA by the regulator, *mer* protein synthesis, and dilution of quantities by cell growth. A total of 66 biological experiments were then selected from the scientific literature from studies of Tn21 and Tn501 in *E. coli* at 37 °C. These experiments were repeated in the computer simulation and the information from their 489 data points was incorporated into the 16 parameters of the model using the Metropolis-Hastings algorithm.

This model is very useful biology for four reasons. Firstly, it shows whether the data from existing biological experiments are consistent with each other or not. Secondly, it predicts the previously unknown concentrations of *mer* proteins in cells of each mercury phenotype. In addition, it challenges the hypotheses that the rates of uptake and volatilisation are always equal in resistant cells and that the plasmid copy number effects replicated by the model are caused by the saturation of MerT in the membrane. Thirdly, the model can guide the design of future experiments. This guidance can minimise the use of laboratory resources and will ensure that sufficient data are created for every parameter in the model under standardised conditions. Finally, the modelling has identified many areas for future biological research: the absolute concentrations of *mer* proteins, the significance of MerC and MerD, plasmid copy number effects and substrate inhibition, the three uptake processes (non-*mer* import, MerA transport, and non-MerA transport), the order of DNA + MerR + Hg^{2+} binding, the nature of toxicity, and the concentrations of mercury in each of the five cellular binding sites.

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Abbreviations

$D(x, \mu)$	The closeness of fit between x (the simulated output) and μ (the experimental data).
Genome equivalent	As in "plasmid copies per genome equivalent". One set of the <i>mer</i> genes.
Hg	Mercury, but its chemical form (e.g. Hg^{2+} , Hg^0) is unknown.
Hg^0	Elemental mercury.
Hg^{2+}	Ionic mercury.
<i>in vitro</i>	The required chemical components are mixed together in known concentrations in the absence of cells.
<i>in vivo</i>	Experiments are performed in cells.
K_D	The equilibrium dissociation constant, i.e. $k_{\text{off}}/k_{\text{on}}$.
k_{max}	The population ceiling.
$\log(P(D \theta))$	The log of the posterior probability.
<i>mer</i> (ΔR)-o/p- <i>TP</i> Δ (<i>CA</i>) <i>D'</i> - <i>lacZ</i>	The <i>merR</i> gene is deleted, <i>merT</i> and <i>merP</i> are present, <i>merC</i> and <i>merA</i> are deleted, and the <i>merD</i> gene is truncated and fused with that of <i>lacZ</i> .
<i>merT</i> , MerT	The <i>merT</i> gene (in italics) and MerT proteins, respectively
<i>merR'</i>	A truncated <i>merR</i> gene that does not produce a functional protein.
$\Delta merR$ mutant	All <i>mer</i> genes intact except for <i>merR</i> .
M.I.C.	Minimal inhibitory concentration, e.g. of HgCl_2 on cell growth.
<i>mer</i>	Mercury resistance.
membranar	Of or relating to the cell membrane.
non- <i>mer</i>	Not involving the <i>mer</i> proteins.
ODE	Ordinary differential equation.
OD_{600}	Optical density of cells measured at wavelength 600 nm.
o/p	The <i>mer</i> operator/promoter region.

O_t	Total <i>mer</i> operator DNA.
OR	Operator bound by MerR.
O	Free operator.
OR*	Operator bound by MerR-Hg ²⁺ .
Parameter	The rate constant for a chemical reaction. The abbreviations for all parameters are defined in Table 3.1.
r	The exponential growth rate.
R, T, P, C, A, D	MerR, MerT, MerP, MerC, MerA, MerD.
P_R, P_T	The <i>merR</i> and <i>merT</i> promoters, respectively.
S	Free Hg inside the cell.
S'	Free Hg in the external medium.
S_t	The total amount of Hg in the cell.
S'_t	The total amount of Hg in the medium and bound in T*.
Simulated output	The output from the ODE solver.
Tn	Transposon, e.g. Tn501.
V_s	The volume of an average cell in the population.
V_{sp}	The volume of external medium around an average cell in the population.
[X]	The concentration of X.
$[X]_{max}$	The maximum concentration of X.
X^*	X bound by Hg, i.e. X-Hg ²⁺
X_t	The total amount of X.
²⁰³ X	Radio-labelled isotope of X.
X_{all}	The simulated output which is closest to all data sets.
X_{sub}	The simulated output which is closest to a subset of data sets.

Chapter 1: Introduction

This chapter begins with an explanation of why a mathematical model of mercury resistance can be so useful to biology. In summary, the model can be used to:

- Complement or challenge the existing biological knowledge on which the mathematical model is based.
- Show which experimental data sets are consistent with each other or not.
- Identify gaps in our existing knowledge and to guide the design of future biological experiments.
- Quantify the trade-off between the metabolic cost of increasing the rates of *mer* protein synthesis and the metabolic benefit of reducing the amount of Hg^{2+} binding to the cell.
- Infer the fitness of the *mer* operon at each stage during its evolution.
- Optimise the bioremediation of mercury contamination in the environment.

Finally, this chapter ends with an overview of the other chapters of the thesis.

1.1 Philosophical considerations

If the meaning of “thesis” is to be construed as a statement or theory, then I can present this work as complementing or challenging our existing knowledge of mercury resistance. This knowledge was gained from biological experiments which created data by measuring the underlying cellular processes. These data are used to argue for theories and new hypotheses about how these cellular processes work. Therefore, our existing scientific knowledge is the combination of all data and the scientific ideas based upon them.

Our scientific knowledge is in the dynamic state of being maintained or disproved but continues on a trajectory of becoming ever more refined and complete. Our current theory is often the most consistent with the data. However, it may be incompatible with some data which may misrepresent the underlying cellular process. Alternatively, the theory may indeed be correct for a limited set of circumstances but should be extended to incorporate new data. These new data may be experimental data derived from future biological experiments. In addition, the concept of data should be extended to include simulated data predicted by a mathematical model which is itself based upon our existing knowledge.

As the biologist replicates the realistic biological conditions of a cellular process in the laboratory, likewise, the mathematical modeller captures the essential details of this same process in a set of equations. Biological experiments create data by

adding the essential chemicals at their relevant biological concentrations to the assay mixture and then by taking experimental measurements. Likewise, a mathematical model predicts data after replicating the conditions of the same cellular process with the essential chemical entities (and their reactions) at their relevant biological concentrations with a computer to produce a simulated output. It is precisely because of this similarity that a mathematical model can complement or challenge the biology (and vice versa). In fact, the model could not test the biology or make any relevant predictions if it were not based upon our existing knowledge. Admittedly, the model could include purely theoretical components which test competing hypotheses or it could include mathematical expressions such as $y = x^2 + 1$ which conveniently fit the overall shape of the experimental data. Preferably, however, a biological experiment should create the data required to distinguish between these hypotheses and provide conclusive evidence that these biological components really exist. This avoids having a model which is non-intuitive and unrelated to the biology or which contains too many parameters than the existing data can inform.

Therefore, the first advantage of a mathematical model of mercury resistance is it tests our existing biological knowledge. The biological experiments are of prime importance since the accuracy and precision of the model must depend upon the quality of the experimental data. However, some experimental conditions may not be biologically realistic or the experimental methods may have been performed in error. Therefore, secondly, the model can be used to identify the data from existing biological experiments which are consistent with each other or not. This is a more informative, less expensive, and quicker alternative to repeating each biological experiment in the laboratory. Finally, the model can guide the design of future biological experiments. This approach should utilise laboratory resources effectively and ensure that biologists are creating informative data which distinguish between every parameter in the model.

1.2 Structure of the thesis

The thesis is an ordinary differential equation/quasi-steady-state model of mercury resistance in *E. coli* at 37 °C which is based on experimental data for the Tn21 and Tn501 operons.

The modelling process begins with Chapter 2. This reviews the relevant biology of mercury resistance and identifies suitable components for the model. Chapter 3 then justifies the design of a model with 16 parameters which describe: the

cellular uptake and volatilisation of Hg^{2+} , binding of the DNA by the regulator, *mer* protein synthesis, and dilution of quantities by cell growth.

Chapter 4 summarises the 66 biological experiments which are replicated in the final computer simulation. Then Chapter 5 describes how the information from their 489 data points is incorporated into the posterior distributions for the 16 parameters of the model using the Metropolis-Hastings algorithm.

Chapter 6 reports and discusses the simulation results. Firstly, values are found for the parameters in the model which give the closest fit of the simulated output through the experimental data points. Then, once our existing biological knowledge is incorporated into a mathematical model of mercury resistance, the model is used to create new knowledge. Nine experiments are then designed to create data about every parameter in the model under standardised conditions.

Finally, eight conclusions are stated in Chapter 7, each of which is a significant contribution to scientific research. In addition, the biological applications of the model are stated.

Chapter 2: The Relevant Biology

This review identifies the components for a mathematical model of mercury resistance in *E. coli*. It integrates data for Tn21 (R100) from *Shigella flexneri*; and Tn501 from *Pseudomonas aeruginosa*. Both of these operons encode a set of proteins, pairs of which are 82 to 89 % homologous in their amino acid sequences (Misra *et al.*, 1994; Misra *et al.*, 1985). Therefore, to make this review comprehensive, the knowledge from these two highly related and overlapping sources is combined and structured into the following sections:

- An overview of each mercury resistance (*mer*) protein in the chemical reduction of Hg^{2+} to Hg^0 .
- How previous studies have shown that the mercury (Hg) phenotype is caused by the combination of *mer* proteins in the cell.
- The relative effects of synthesis, degradation, and growth on net *mer* protein concentrations.
- Three sections on the reductase, transporter, and regulatory proteins.
- The toxicity of Hg on protein synthesis and cell growth.
- An original classification of Hg cellular binding sites.
- Suitable components for a model of Hg resistance.

2.1 Overview

Inorganic mercury (Hg^{2+}) has no known biological function, but will bind reversibly to components of the cell cytoplasm and membrane. The nature of these cellular binding sites is generally unknown, but Hg^{2+} will bind strongly to the sulphhydryl groups (R-SH) of isolated proteins (Casas and Jones, 1979). Therefore, it is assumed that mercury toxicity is the effect that Hg has on the function of the molecule to which it binds (Beppu and Arima, 1969).

These toxic effects can be reversed by reducing Hg^{2+} to non-toxic Hg^0 . Figure 2.1 shows that Hg detoxification requires the proteins encoded by the *mer* operon:

- MerR, which regulates expression of the *mer* genes from the DNA.
- MerT, MerP (and MerC in Tn21), which transport Hg^{2+} from the periplasm across the inner membrane.
- MerA, which catalyses the reduction of cytoplasmic Hg^{2+} to Hg^0 .
- MerD, which is assumed to down-regulate expression from the operon.

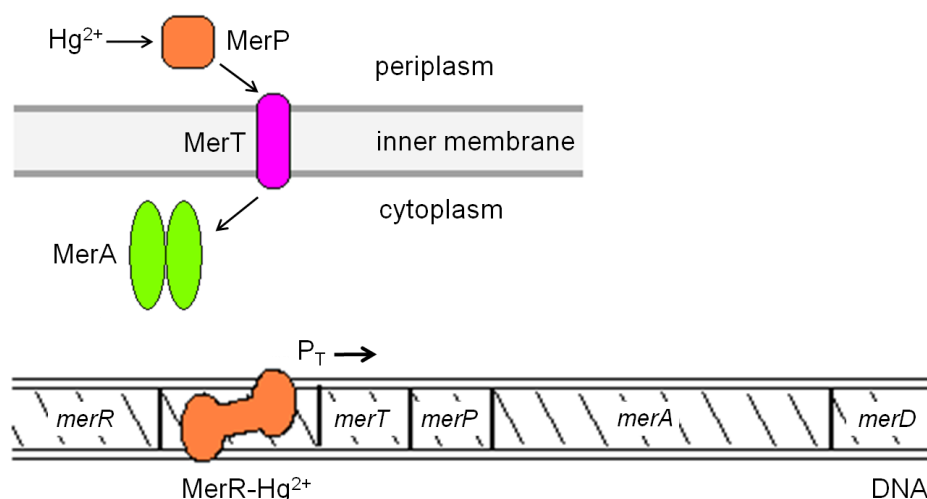


Figure 2.1 The roles of the *mer* proteins. MerR-Hg²⁺ binds to the *mer* promoter (P_T) to increase synthesis of the transporter (MerP and MerT) and reductase (MerA) proteins. This allows more Hg^{2+} to be transported from MerP in the periplasm, across the inner membrane by MerT, and into the cytoplasm where it is detoxified by MerA to Hg^0 . This volatile product then diffuses from the cell and can be identified by peaks at 128 seconds and a mass to charge ratio of 200 on the GCMS spectrum (Summers and Silver, 1972).

2.2 Phenotypes and gene products

Induction of Hg resistance

The rate of volatilisation of Hg^{2+} to Hg^0 is greater in induced cells than in uninduced cells. Induced cells are those which have recently been exposed to Hg. The transition from the uninduced to induced states can take several hours while cells gradually resume their growth (Chang, Hong, Oguseitan, and Olson, 1993). During induction, metabolic resources are consumed to protect from and to reverse Hg-binding, and to repair any damage caused to cellular components.

Hg phenotypes

Foster, Nakahara, Weiss, and Silver (1979) cloned 23 x pDU202*mer*::Tn801 insertion mutant plasmids into *E. coli*. These cells were compared for their Hg sensitivities, volatilisation rates, and for the total amounts of Hg bound to each cell. These authors then classified cells into four phenotypes based upon which set of *mer* R100 genes had been inactivated by the Tn801 insertion. These Hg phenotypes are: resistant, sensitive, hypersensitive, and micro-constitutive.

For example, the growth of resistant cells is inhibited when the concentration of HgCl_2 in the media exceeds 74 μM . However, the corresponding concentrations for sensitive (including the plasmidless control) and hypersensitive cells are much lower at $11.1 \pm 0.7 \mu\text{M}$ and $1.7 \pm 0.2 \mu\text{M}$, respectively. Further, the volatilisation

rate of resistant cells increases from 0.05 % (uninduced) to 100 % (i.e. a half-life of 0.5 min) following 60 minutes induction with 5 μM HgCl_2 . However, micro-constitutive sensitive cells always volatilise Hg^{2+} with intermediate half-lives (25–45 minutes) irrespective of any previous induction. Finally, hypersensitive cells induced with 0.2 μM HgCl_2 do not volatilise Hg^{2+} , but within 30 minutes bind three to five times more Hg than all non-induced cells.

These phenotypes can be explained by cleaving *mer* DNA (pDU202, R100) into two fragments called EcoR1-H and EcoR1-I. Whereas both fragments cloned together (contiguously and in the correct order) confers Hg resistance, each fragment alone confers a phenotype deficient in reductase activity, i.e. EcoR1-H confers hypersensitivity and EcoR1-I confers sensitivity. Therefore, the intact gene for mercuric reductase must bridge the EcoR1 cleavage site. Further, since hypersensitive cells bind more Hg, and, since this binding is inducible, the transport and regulatory genes of *mer* must reside on EcoR1-H (Foster, Nakahara, Weiss, and Silver, 1979). In conclusion, it is the combination of regulation, transport, and reduction, which confer each of the Hg phenotypes.

Induced *mer* peptides

Both *in vitro* and *in vivo* studies show that HgCl_2 induces transcription of the *mer* genes with a Hill coefficient of 2.3–2.6. This ultrasensitive response allows steady-state *mer* mRNA expression to increase by 70- to 140-fold within 30–60 minutes of adding the inducer, Hg^{2+} (Rouch, Parkhill, and Brown, 1995; Ralston and O'Halloran, 1990). However, to date, no equivalent data have been reported for the translation rates of *mer* mRNAs into their respective proteins. Therefore, the hypothesis that *mer* mRNA and protein synthesis rates are generally proportional to each other remains to be disproved.

The set of Tn501 and Tn21 proteins which are expressed more strongly in the presence of HgCl_2 was first identified by Jackson and Summers (1982a). All peptides were labelled in *E. coli* minicells with [^{35}S]methionine or ^{14}C -amino acids and separated by denaturing gel electrophoresis alongside a molecular weight standard. An autoradiograph of the gels shows that both Tn501 and Tn21 (pRR130) produce new bands corresponding to peptides of sizes 69 and 12 kDa in the presence of HgCl_2 . In addition, Tn21 has new bands appearing at 14 and 15.1 kDa, and Tn501 has two faint bands appearing in the 14–17 kDa range.

Specifically, the 15.1, 14, and 12 kDa peptides are encoded from the EcoR1-H fragment of Tn21 (pRR134), along with a 40 kDa peptide thought to be the amino-terminus of the MerA monomer. Since these peptides are inducible and confer a hypersensitive phenotype, this fragment must encode the transport (MerTPC) and regulatory (MerR) functions of *mer* (Jackson and Summers, 1982a).

The remaining 69 kDa peptide is modified *in vivo* to a 66 kDa form (Jackson and Summers, 1982b). Only these two peptides and the 40 kDa peptide from pRR134 precipitate with anti-reductase antibodies to purified Tn501 reductase antigen. Since the mass of the Tn501 antigen and the peptide are identical, this 66 kDa peptide must be the MerA monomer (Jackson and Summers, 1982a).

Interestingly, the *mer* proteins are located in different compartments of the cell. Electrophoresis of cell fractions reveals that the 15.1 (MerT) and 14 kDa (MerC) peptides are located in the inner membrane and the 12 kDa peptide (MerP) is located in the soluble fraction, i.e. in the cytosol or periplasm (Jackson and Summers, 1982b). More precisely, N-terminal sequencing of the purified 7.5 kDa MerP monomer shows it loses a signal peptide as the protein relocates to the periplasm (Sahlman and Jonsson, 1992).

Finally, the location of MerA is found from the distribution of reductase activity in cells chemically treated to remove their cell walls (i.e., in spheroplasts). The vast majority of activity is found in intact (1,980 units) and lysed (1,780 units) spheroplasts (i.e. in the soluble cell fraction). In contrast, very little activity is observed in the lysed spheroplast pellet (180 units) or is segregated into the supernatant of intact spheroplasts (60 units) along with the known periplasmic enzyme alkaline phosphatase. Therefore, MerA must reside in the cytoplasm (Summers and Sugarman, 1974).

Table 2.1 reviews the locations and sizes of the *mer* peptides found either by experimentation or predicted from their respective DNA sequences. Small inconsistencies in these values may be caused by proteolytic modification (i.e. for MerA and MerP), or by the aberrant migration of some hydrophobic peptides through the gels (Hobman, personal communication). The remaining peptides, MerR and MerD, may exist in too small a quantity to be detected or, alternatively, their presence on gels may be obscured by co-migration with peptides of a similar size (Ni'Bhriain and Foster, 1986).

Table 2.1 The locations and sizes of the *mer* peptides of Tn21. Data are from Jackson and Summers (1982a)^a, Jackson and Summers (1982b)^b, Mukhopadhyay, Nucifora, and Misra (1991)^c, Ni'Bhriain and Foster (1986)^d, and Yoon (1994)^e.

Peptide	Location	Size found by experimentation		Size predicted from DNA sequence ^d
		kDa ^a	kDa ^d	kDa
Reductase: MerA	Cytoplasm	69, 66	65, 62	58.9
Transporters: MerT	Inner membrane	15.1, 16.5	17, 16	12.5
MerP	Periplasm	12, 13 ^b	9.8, 9.5	9.5, 7.5
MerC	Inner membrane	14	14	15
Regulators: MerR	(Cytoplasm)	ND	16 ^e	15.9
MerD	(Cytoplasm)	ND	13.5 ^c	12.7

2.3 Protein concentrations

Degradation

Jackson and Summers (1982b) state that the *mer* peptides remain relatively intact in labelled minicells for at least 95 minutes at 37 °C. Unfortunately, the quantitative data supporting this statement were not published. However, a low-quality autoradiograph of minicell samples does show that the 12, 14.1, 15.1, 66, and 69 kDa bands are still visible at this final time point. In addition, no new bands are observed representing any non-specific degradative products of the *mer* peptides. Finally, the authors report that the sum of the 66 and 69 kDa peptides is always a constant fraction of total labelled minicell protein. This would imply that, up to 95 minutes following its synthesis, the 69 kDa MerA monomer is not significantly degraded beyond the 66 kDa form.

Dilution by cell growth

The above experiments were conducted in minicells, which are achromosomal cells incapable of cell division. In contrast, a growing *E. coli* cell may divide up to four times during any similar 95 minute period. This process will dilute the quantities of all existing *mer* proteins into an increasing cell volume. Thus, with every division cycle, the quantities of each *mer* protein will be partitioned equally into two separate daughter cells. For example, after four cell divisions, only 1×0.5^4 of their original quantity will remain in each cell. Therefore, compared to the global effects of protein synthesis and dilution by cell growth, any loss of the *mer* proteins by degradation is expected to be relatively insignificant.

Protein synthesis

Whether the previous assumption is correct or not, densitometric analysis shows that *merT* mRNA is 1.6 times more abundant than *merA* in induced maxicells. Interestingly, a similar, 1.73-fold, relationship exists in the relative amounts of the MerT and MerA proteins (Philippidis, Malmberg, Hu, and Shottel, 1991). However, it would be incorrect to assume that a set of proteins will always exist in the same relative quantities as their underlying mRNAs. This is because the observed protein quantity is the net effect of two processes which create protein (DNA transcription and mRNA translation) and three processes which destroy it (mRNA degradation, protein degradation, and dilution by cell growth). Since each DNA, mRNA or protein chain varies in its chemical sequence and length, so will the rates of each of these five processes. Therefore, a proportional relationship existing initially between the quantities of different mRNAs would not usually be maintained in their respective proteins.

Indeed, experimental data do show that proportional relationships between the *mer* mRNAs are created at DNA transcription (Gambill and Summers, 1992). This is because genes closer to the promoter are transcribed into mRNA more frequently than genes at the distal end of the operon. For example, β -galactosidase is synthesised 10-to 15-fold more frequently when *lacZ* is inserted in the proximal gene *merA* than in the distal gene *merD* (Lee, Gambill, and Summers, 1989). In general, the further a gene is located from its promoter, the greater the likelihood that RNA polymerase will have already dissociated from or paused at an upstream site on the DNA template. For example, in Tn21, there exists an unidentified site between *merC* and *merA* where the majority of transcripts appear to terminate prematurely (Gambill and Summers, 1992).

Relative and absolute concentrations

In criticism of the literature, the absolute concentrations of the *mer* proteins in *E. coli* are seldom measured. What is often reported are the relative mRNA or protein synthesis rates found by densitometry or inferred from the synthesis of a *mer*-reporter fusion protein. For example, β -galactosidase is a reporter protein encoded by the *lacZ* gene. This enzyme cleaves its substrate into a blue product which can easily be quantified with a spectrophotometer. To create the fusion, the *lacZ* gene is inserted in place of (or overlapping) the *mer* gene it is reporting on. For example, a *merA'*-*lacZ* fusion exchanges the majority of the *merA* gene with that of the *lacZ* gene. Therefore, the *merA* gene will no longer encode a viable

MerA protein while the *lacZ* gene is transcribed under the control of the *merT* promoter from the natural position of *merA* on the DNA.

However, the physical insertion of *lacZ* into the *mer* region itself may affect the validity of its reporting. This is because the *lacZ* gene alone is 3075 bp in length which is over ten times longer than the 273 bp gene of *merP*. It is almost two times longer than the 1695 bp *merA* gene in Tn21 (Stefan, Radeghieri, Gonzalez Vara y Rodriguez, and Hochkoeppler, 2001; Misra *et al.*, 1984; Misra *et al.*, 1984). Therefore, not only are more ribonucleotides and amino acids required to synthesise (and to be degraded in) the reporter protein (or mRNA), but the sequences of the *lac* reporter and the reported *mer* species will have different chemical properties. Secondly, *lacZ* displaces all *mer* genes lying distally to the insertion by 3075 bp downstream of their natural positions on the DNA. For example, a *merC::Tn5* insertion in pDU1003 reduces *merA* expression to 0.3–2.0 % of wild type (Ni'Bhriain, Silver, and Foster, 1983).

The data provided by a gene fusion could be of the form: "The DNA transcription of gene *X* is so many times that of gene *Y*". For example, "The DNA transcription (from the natural position) of *merA* is 10- to 15-times that of *merD*". This is a valid inference because the rates of mRNA translation, mRNA degradation and protein degradation are identical for a reporter synthesised from different positions on the DNA. Therefore the proportional relationships created at DNA transcription are preserved in the synthesised reporter proteins. Alternatively, the relative data could be of the form: "The synthesis of *X* under condition *a* is so many times the synthesis of the same *X* under condition *b*". For example, "The derepressed synthesis of MerA from the naked DNA is 100-times less than the activated synthesis of MerA from DNA-MerR-Hg²⁺". In general, these statements are useful, but fail to position these proportional relationships on the scale of magnitude of small and large absolute values.

To their credit, Rinderle, Booth, and Williams (1983) have reported the absolute induced MerA dimer concentration as $\sim 10^{-6}$ M (pRR130, Tn21, after 8 hours growth in 10^{-4} M HgCl₂). For the remaining *mer* proteins, it is still possible to predict an upper bound on their absolute concentrations. For example, the 32 kDa MerR dimer has been over-expressed to 15–20 % of total cellular protein using the *tac* promoter (Yoon, 1994). Therefore, the absolute MerR concentration expressed from the weaker *mer* promoter must be less than $\sim 5.1 \times 10^{-4}$ M. MerT has also been over-expressed, but not successfully quantified (Hobman and

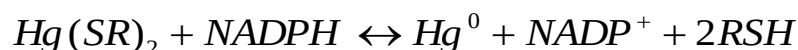
Brown, 1996). However, it is possible for a cytoplasmic enzyme such as MerA to be over-expressed to 25–60 % of total cellular protein, whereas the concentration of MerT is limited by the capacity of the membrane to 1–3 % of total cellular protein (Philippidis, Malmberg, Hu, and Shottel, 1991). Thus, due to the unequal capacity of the two cellular compartments, as the membrane becomes saturated with MerT, its proportional relationship to MerA in the cytoplasm can no longer be maintained (Nakahara *et al.*, 1979).

2.4 MerA enzyme

Reduction

Mercuric reductase is a dimeric NADPH-dependent flavoenzyme which catalyses the conversion of Hg^{2+} into the non-toxic, volatile form, Hg^0 (Equation 2.1). Free Hg^{2+} is not a substrate for the reaction, but must be bound to the –SH group of a thiol compound such as 2-mercaptoethanol, glutathione or cysteine (Rinderle, Booth, and Williams, 1983; Miller, Ballou, Massey, Williams, and Walsh 1986).

Equation 2.1 The reduction of Hg^{2+} to Hg^0 by MerA.



In vitro properties

There are two properties of the MerA reaction which occur *in vitro* when the composition of the assay mixture is biologically unrealistic. The first is biphasic kinetics. Reduction of Hg^{2+} proceeds with a rapid velocity which shifts to a slower steady state within one minute. This initial phase can be eliminated completely by preincubating the enzyme with the substrates NADPH and 2-mercaptoethanol. Secondly, MerA forms an irreversible inhibitory complex with the synthetic compound EDTA. This is a metal chelator which is also a substrate for MerA when complexed with Hg^{2+} (Rinderle, Booth, and Williams, 1983).

Substrate inhibition

In addition, MerA is inhibited by free Hg^{2+} which binds reversibly to an already formed MerA- Hg^{2+} complex. This inhibition is more noticeable at high Hg^{2+} concentrations once the reduction rate begins to decline. However, below 40–50 μM Hg^{2+} , the MerA enzyme-catalysed reaction fits a simple two-parameter Michaelis-Menten model (Nakahara *et al.*, 1979; Philippidis, Schottel, and Hu 1990). The first parameter is v_{max} , the maximum reduction rate. The second is K_A , the substrate concentration at which the reduction rate is 50 % of v_{max} .

2.5 MerT, MerP, and MerC transporters

Hg uptake

The net uptake of Hg^{2+} by intact cells also fits a Michaelis-Menten model (Philippidis, Shottel, and Hu, 1990). Uptake occurs simultaneously by two processes: *mer* transport and non-*mer* import. *mer* transport is the shuttling of Hg^{2+} between paired cysteine residues in the proteins MerP, MerT, and MerA (Rossy *et al.*, 2004). Non-*mer* import is the passive or facilitated diffusion, or active transport of Hg independently of, or, in the absence of any *mer* transporters. In both cases, Hg must enter the cell through the biological membrane before it can be volatilised by MerA in the cytoplasm.

MerA volatilisation can be studied separately from Hg uptake by permeabilising cell membranes with ether. This allows small molecules such as Hg^{2+} and NADPH to equilibrate instantaneously between the cell interior and the external medium. In permeabilised cells, the initial rate of volatilisation is $16.8 \mu\text{M Hg}^{2+} \text{ min}^{-1}$ (with 1.0 mM NADPH and $40 \mu\text{M Hg}(\text{NO}_3)_2$). In intact cells, this rate is four-fold lower at $4.13 \mu\text{M Hg}^{2+} \text{ min}^{-1}$ (Philippidis, Shottel, and Hu, 1990).

The rate of volatilisation in intact cells is assumed to measure the rates of *mer* transport and non-*mer* import combined. Firstly, the quantity of Hg bound to induced resistant cells is barely detectable (Summers and Kight-Olliff, 1980). This implies that an insignificant proportion of Hg entering the cell is free to leave by non-*mer* export, i.e. most cytoplasmic Hg^{2+} is volatilised by MerA. Hence, if these assumptions are correct, uptake of Hg across the biological membrane must be rate-limiting on volatilisation.

The magnitudes of each uptake process

Experimenters are yet to isolate *mer* transport from both non-*mer* uptake and *mer* transport combined. However, the relative significance of each process can be inferred. For example, Wilson, Leang, Morby, Hobman, and Brown (2000) reported that induced resistant cells volatilise 66.4 % of total HgCl_2 in ten minutes (pBR322, *mer*, 23.4 plasmid copies per genome, Tn501). However, control cells (pBR322, *mer*(ΔTP)A), capable of non-*mer* uptake alone, volatilise only 9.2 % as much. Further, with ATP synthesis inhibited, the volatilisation rates of intact cells (but not of permeabilised cells) are reduced to a similar 6.4–12.8 % (pDU1003, 38.1 plasmid copies per genome, R100) (Philippidis, Shottel, and Hu, 1990; Kiyono, Uno, Omura, and Pan-Hou, 2000). Together, these data indicate

that *mer* transport is an energy-dependent process and that, in induced resistant cells, transport is substantially quicker than non-*mer* uptake.

Single transporter mutants

Intermediate between the phenotypes of resistant and control cells are the single *mer* transporter mutants (Hamlett, Landale, Davis, and Summers, 1992). In the absence of MerT or MerP (or both), *merA* mutants bind two- to three-times less Hg than when all transporter proteins are present (pNH10, Tn21, *merTPC*(ΔA), *merR* *in trans* on pNH9). However, single *merC* mutants show no appreciable differences in cell growth or Hg-binding indicating a less essential role for MerC in Hg resistance. A similar pattern is observed when MerA is intact. Again, single mutants are intermediate in phenotype between the wild type (pBR*merBS*2, Tn501) and double transporter mutants (*merR*(ΔTP)*AD*). Specifically, a *merT* deletion confers a lower rate of volatilisation than does a deletion of *merP*, with 16.0 and 19.9 % more HgCl₂ remaining after 10 minutes than in induced resistant cells, respectively (Wilson, Leang, Morby, Hobman, and Brown, 2000).

2.6 The MerR regulator

Regulation

MerR is synthesised from the third open reading frame on the bottom DNA strand at positions 548–144 on the *mer* DNA. Therefore, Lund, Ford, and Brown (1986) digested Tn501 with restriction endonuclease HaeIII and isolated the fragment containing the *merR* promoter (P_R, positions 500-700). This promoter fragment was ligated into control plasmid pRZ5225 so that P_R would direct expression of the *lacZ* gene. They found that β -galactosidase synthesis from the resulting plasmid (pRZ-H-12) decreases with MerR provided *in trans* (on pAC-E₅E_L). Hence, MerR must inhibit its own synthesis independently of the presence (from 24.0 to 11.0 Miller units) or absence of HgCl₂ (from 22.3 to 10.9 Miller units).

The *merTP(C)AD* genes are transcribed from the *merT* promoter (P_T), which is oriented in the opposite direction to *merR*. Therefore, Lund, Ford, and Brown (1986) studied plasmids in which expression of the *lacZ* gene is directed from P_T. They found that β -galactosidase synthesis (pDU1161, *mer*(ΔR)-P_R-o/p-P_T-*merA'*-*lacZ*) decreases without HgCl₂ (56.1 Miller units) and increases with HgCl₂ (684.9 Miller units) when MerR is present *in trans* (on pAC-EZ-1, *mer*⁺). However, in the absence of MerR, synthesis from P_T is intermediate at 100.9 and 101.8 Miller units, respectively. Thus MerR is a repressor of P_T in the absence of HgCl₂, and an activator in the presence of HgCl₂ (Figure 2.2).

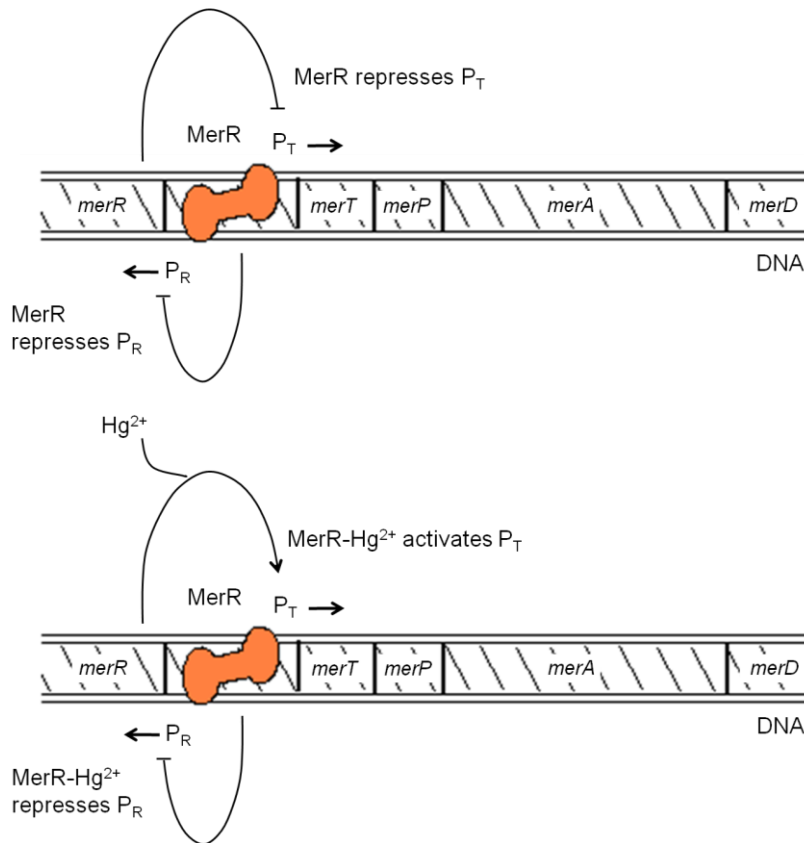


Figure 2.2 Repression and activation by MerR. MerR controls the expression from the *merR* (P_R) and *merT* (P_T) promoters. (Top) In the absence of Hg^{2+} , MerR represses both P_T and P_R . (Bottom) Once Hg^{2+} binds to MerR, the MerR- Hg^{2+} complex (orange) activates expression of *merTP(C)AD* from P_T , but continues to repress synthesis of MerR from P_R .

MerR- Hg^{2+} binding

At concentrations of 10^{-8} – 10^{-5} M, Tn21 MerR exists exclusively as a dimer (Shewchuk *et al.*, 1989) and will equilibrate rapidly with a single atom of Hg^{2+} . Exchange of Hg^{2+} in and out of the MerR- Hg^{2+} complex occurs within seconds, even in the presence of a 100-fold excess of competing thiols, a 1000-fold excess of Zn^{2+} , Cd^{2+} , Ag^+ or Tl^{2+} , or a stoichiometric amount of operator DNA (Shewchuk, Verdine, and Walsh, 1989). During this equilibrium, the total of all MerR forms ($MerR_t$) is partitioned between free MerR dimer, free Hg^{2+} and the MerR- Hg^{2+} complex. For example, at an Hg^{2+} concentration of $K_D = 10^{-7}$ M, $MerR_t$ is partitioned equally with 50 % in free MerR and 50 % in MerR- Hg^{2+} (Shewchuk, Verdine, and Walsh, 1989).

MerR-DNA binding

MerR and MerR- Hg^{2+} bind a single region of dyad symmetry between the -35 and -10 hexamers of P_T . This region is protected by the regulator in assays with DNase I, dimethyl sulphate or hydroxyl radicals (Shewchuk, Verdine, and Walsh,

1989). Generally-speaking, the MerR_t concentration required for 50 % of operator DNA to be bound by MerR (or by MerR-Hg^{2+}) is four- to ten-fold greater in the presence of HgCl_2 ($K_D \approx 10^{-10}$ M of MerR, O'Halloran, Frantz, Shin, Ralston, and Wright, 1989; Parkhill, Ansari, Wright, Brown, and O'Halloran, 1993).

The contrasting data of Shewchuk, Verdine, and Walsh (1989), conducted at 4 °C, show the opposite relationship. Since their dissociation rates (k_{off}) of pre-formed DNA-MerR and DNA-MerR- Hg^{2+} complexes were similar (half-lives: 51 min and 49 min, respectively), these authors attributed the observed decrease in K_D in the presence of HgCl_2 to faster binding (k_{on}) of MerR- Hg^{2+} to the DNA over MerR alone. However, this conclusion contrasts the results published by Parkhill and Brown (1990) who found that the association of MerR to the DNA is reduced to 40 % in the presence of HgCl_2 .

Open complex formation

The *mer* DNA can be transcribed *in vitro* by adding the required chemical components to the reaction mixture. These include MerR, HgCl_2 , *E. coli* RNA polymerase (RNAPol), ribnucleotide triphosphates, and a DNA template containing the truncated *merR'* and *merT'* genes: *merR'-P_R-o/p-P_T-merT'*. In the absence of purified MerR, only a 74 nucleotide mRNA transcript (*merR'*) is synthesised from P_R . Then, as the concentrations of MerR and Hg^{2+} increase, a 104 nucleotide transcript (*merT'*) is observed while the transcription of *merR'* from P_R decreases reciprocally. However, *merT'* is not detectable in the absence of HgCl_2 and presence of MerR (O'Halloran, Frantz, Shin, Ralston, and Wright, 1989).

The open complex for P_T is RNAPol-DNA-MerR- Hg^{2+} while that of P_R is RNAPol-DNA. Since the DNA-MerR- Hg^{2+} complex is formed from DNA, MerR and Hg^{2+} , there are two hypotheses for the order in which these components bind. The first is $\text{DNA-MerR} + \text{Hg}^{2+} \rightarrow \text{DNA-MerR-Hg}^{2+}$, i.e. free MerR binds the DNA, after which Hg^{2+} binds DNA-MerR. The second is $\text{MerR-Hg}^{2+} + \text{DNA} \rightarrow \text{DNA-MerR-Hg}^{2+}$, i.e. Hg^{2+} cannot bind DNA-MerR, but MerR must first dissociate from the DNA complex before MerR- Hg^{2+} can bind. To date, the experiment has not been conducted which would test which (or both) of these hypotheses is true. Each predicts that, in the presence of Hg^{2+} , a greater concentration of MerR_t is required for 50 % of the DNA to be in the DNA-MerR or DNA-MerR- Hg^{2+} forms.

Nonetheless, this experiment can be performed mentally as follows. Modifying the Hg^{2+} concentration in the cell would disturb the rapid equilibrium of the fixed

quantity MerR_t into MerR-Hg^{2+} and free MerR. For example, an increase in Hg^{2+} would increase the proportion of MerR_t partitioned into MerR-Hg^{2+} at the expense of free MerR. The first hypothesis, $(\text{DNA} + \text{MerR}) + \text{Hg}^{2+} \rightarrow \text{DNA-MerR-Hg}^{2+}$, assumes that free MerR is the only molecule which can bind the DNA. Therefore a decrease in free MerR means less DNA-MerR complex is being formed to which Hg^{2+} can bind to form DNA-MerR-Hg^{2+} . This decrease in DNA-MerR-Hg^{2+} with increasing Hg^{2+} can be tested experimentally. However, it creates two problems for the cell. Firstly, that the rate of *mer* protein synthesis from DNA-MerR-Hg^{2+} should decrease as Hg^{2+} increases in the cell. Secondly, in order to increase DNA-MerR-Hg^{2+} , the cell would have to synthesise more MerR_t to provide the necessary partitioned free merR to bind to the DNA.

Alternatively, the second hypothesis, $(\text{MerR} + \text{Hg}^{2+}) + \text{DNA} \rightarrow \text{DNA-MerR-Hg}^{2+}$, implies, simply, that MerR-Hg^{2+} does not bind DNA as strongly as does free MerR. Again, increasing Hg^{2+} would cause MerR_t to equilibrate into more MerR-Hg^{2+} and less free MerR. However, assuming both MerR and MerR-Hg^{2+} can bind the DNA independently, an increase in MerR-Hg^{2+} would mean more DNA is partitioned into the activating state (DNA-MerR-Hg^{2+}). This result has been tested indirectly in experiments which show a positive correlation between *mer* protein synthesis and increasing Hg^{2+} . In fact, it is not difficult to envisage that the MerR-Hg^{2+} complex can bind the DNA when there exists a *merR* mutant (A89V; S131L) which can bind to, distort, and activate transcription from P_T even in the absence of HgCl_2 (Parkhill, Ansari, Wright, Brown, and O'Halloran, 1993).

Operator distortion

The 19 bp spacing between the -35 and -10 hexamers of the *mer* promoters is two bp longer and 68° out of phase than those normally observed in *E. coli* (Condee and Summers, 1992). However, this spacing is essential for the behaviour of the *mer* operon, and has been reproduced artificially by altering spacer size (Parkhill and Brown, 1990), isolating MerR activating mutants (Parkhill, Ansari, Wright, Brown, and O'Halloran, 1993), and by modifying the global level of DNA supercoiling using Coumermycin A₁ (Condee and Summers, 1992). In summary, MerR bends P_T towards itself, winds the DNA by 19° , and forms an inactive complex with RNA polymerase. In contrast, Mer-Hg^{2+} unwinds the DNA by 33° which realigns and shortens the spacing between the -10 and -35 elements. This allows optimal access by the σ^{70} subunit of RNA polymerase in forming the open transcriptional complex (Ansari, Bradner, and O'Halloran, 1992; Ansari, Chael, and O'Halloran, 1995; Caslake, Ashraf and Summers, 1997).

MerD

It is not clear how MerD regulates the expression of the *mer* genes. For example, Mukhopadhyay, Yu, Nucifora, and Misra (1991) propose that MerD competes directly with MerR for occupancy of the *mer* promoter region. To the contrary, Champier, Duarte, Michaud-Soret, and Covès (2004) found that neither MerD from pDU1358 nor from Tn4378 can bind to *mer* DNA. Instead, binding of MerD to a site on MerR may reduce the affinity of MerR to the *mer* promoter in the presence of Hg^{2+} .

The consequences of mutating the *merD* gene are also unpredictable. For example, a Tn5 insertion in *merD* of Tn21 has no effect on inducible MerA activity in *E. coli* (Ni'Bhriain, Silver, and Foster, 1983; Brown *et al.*, 1986) but will increase β -galactosidase synthesis from *merA'*-*lacZ* by 2- to 3-fold. Similarly, *merD* mutants may grow more or less quickly, or at the same rate as wild-type cells (Mukhopadhyay, Yu, Nucifora, and Misra, 1991; Nucifora, Silver, and Misra, 1989; Ni'Bhriain, Silver, and Foster, 1983). Thus, overall, the mechanism of MerD co-regulation in Tn501 and Tn21 is unknown and the evidence for a significant regulatory effect of MerD on Hg resistance is not convincing.

2.7 Hg toxicity

Toxicity as unbalanced rates

The relative rates of Hg uptake and volatilisation determine how much Hg is bound within each cellular compartment. Specifically, the concentration of Hg^{2+} in the cytoplasm determines the induction of *mer* protein synthesis through the partitioning of MerR_t into activating (MerR-Hg^{2+}) and repressing forms (MerR). Simultaneously, however, Hg inhibits protein synthesis itself – including that from the *mer* operon. Thus, the Hg phenotypes—resistant, sensitive and hypersensitive— indicate the level at which toxic Hg is allowed to accumulate inside the cell.

If Hg uptake is relatively fast and volatilisation is slow, Hg will accumulate to a high concentration in the cell. This means, potentially, that a greater proportion of MerR bound by Hg^{2+} is available to activate the *mer* operon. However, simultaneously, elevated levels of Hg will reduce protein synthesis and confer the hypersensitive phenotype. For example, in the absence of volatilisation, β -galactosidase synthesis decreases with *mer* transport (pRZ-ES-1, *merRTPA'*-*LacZ*, 89.7 Miller units) compared to sensitive cells containing P_T alone (pRZ-ES-M1, *merR*(Δ TPA)-*LacZ*, 275.0) (Lund and Brown, 1987).

Alternatively, if Hg uptake is slow and volatilisation is fast, volatilisation may remove Hg^{2+} in the cell cytoplasm, but the relatively slow transfer to MerA of toxic Hg^{2+} from non-specific sites in the membrane will confer the sensitive phenotype. Thus, inhibition of protein synthesis by Hg and the lower proportion of MerR in the activating form will reduce expression from P_T . For example, in the presence of (a two-fold over-expressed) MerA, β -galactosidase synthesis decreases (pAC-AH-52, *mer*(ΔRTP)A–*LacZ* + pRZ-ES-M1, *merR*(ΔTPA)–*LacZ*, 125.0 Miller units) compared to when MerA is absent (pRZ-ES-M1, *merR*(ΔTPA)–*LacZ*, 275.0) (Lund and Brown, 1987).

Finally, Hg resistance is conferred when uptake and volatilisation are balanced. It occurs, for example, by complementing pAC-AH-52 (over-expressed MerA, minimal inhibitory concentration (MIC) = 3.7 μM HgCl_2) with pRZ-EX-1 (*merRTP*(ΔA), MIC <1.8 μM). Interestingly, these cells are more resistant (MIC = 66.3 μM) than those in which MerA is expressed from the wild-type *Tn501* promoter (pAC-E_SE_L, *merRTPA*, MIC = 47.9 μM). Further, this full complement of *mer* genes confers the highest induced β -galactosidase synthesis (1658.5 Miller units, pRZ-ES-1 (*merRTP*(ΔA)) + pAC-AC-52) (Lund and Brown, 1987).

In conclusion, binding of Hg^{2+} to the *mer* proteins should avoid the toxic effects of free Hg^{2+} binding to other molecules in the cell. MerP should scavenge Hg^{2+} from the periplasmic space and the outer face of the inner membrane. MerT, in addition to accepting Hg^{2+} directly from MerP, should protect the cytoplasm by binding any Hg^{2+} which has not been sequestered within MerR or reduced by MerA (Hamlett, Landale, Davis, and Summers, 1992).

Subtoxic Hg

Very often, Hg is added to cells at a concentration which is below the threshold known to inhibit protein synthesis and growth. For example, Nakahara, Silver, Miki, and Rownd (1979) reported the growth of *E. coli* KP245 cells in Difco nutrient broth. Sensitive cells (pRR132, *Tn21*, *mer*($\Delta RTPA$)) grew normally in subtoxic HgCl_2 concentrations from 0.0 to 2.0 μM , but did not grow above 5.0 μM . Hypersensitive cells (pRR134, *merRTP*(ΔA)) grew into late log-phase in 0.1 μM , but at a lower rate than without HgCl_2 . For doses 0.5–5.0 μM HgCl_2 , hypersensitive cell growth stopped within one hour and then declined.

Moore and Walsh (1989) performed an identical experiment, except with *E. coli* HB101 cells in 2 x YT liquid medium. Sensitive cells (pKK223, *mer*[−]) grew more

slowly in 25 μM than without HgCl_2 and stopped growing after one hour in 50 μM . Hypersensitive cell (pMMA558a559 and pMM Δ 85, Tn501) growth slowed in 0.5 μM and stopped in more than 5 μM HgCl_2 . Finally, resistant cell (pJOE114) growth was reduced in 250 μM HgCl_2 (increased from 50 μM after one hour).

These reductions in cell growth rates were correlated by Nakahara, Silver, Miki, and Rownd (1979) to the loss of cellular RNA. Cells were induced (or not) with 0.25 μM HgCl_2 then resuspended in tryptone broth containing 1.0–10 μM HgCl_2 and ^{14}C -uracil. After 10 minutes, the amount of ^{14}C -uracil incorporated into trichloroacetic acid-precipitable material was measured and normalised to that incorporated by the control (no HgCl_2 , 100 %). In subtoxic HgCl_2 concentrations of less than 5 μM , incorporation by sensitive and uninduced hypersensitive cells was indistinguishable from resistant cells (pRR130, *merRTPA*). However in 7–10 μM HgCl_2 , uracil incorporation for all cells except resistant cells fell quickly to less than 10 %. Finally, induced hypersensitive cells incorporated only 80 % as much uracil as the control when resuspended in 1.25 μM and about 20 % in 5 μM HgCl_2 .

Since Hg does not affect the *in vitro* activity of RNAPol (Ralston and O'Halloran, 1990), it may cause extensive degradation of mRNA by binding to an inhibitor of ribonuclease I (Beppu and Arima, 1969). Thus, with fewer mRNA molecules to translate into proteins, the rate of down-stream protein synthesis also decreases in the presence of Hg. For example, [^{35}S]methionine incorporation into all *E. coli* proteins is normal after 15 minutes with a subtoxic 0.8 μM HgCl_2 , but is three- to four-fold lower with a toxic 2 μM (Dempsey *et al.*, 1978). These effects include the expression from P_7 after 20 minutes, which decreases by 24 % after increasing from 1 to 10 μM HgCl_2 (Tn21, *merR-o/p-T'-lacZ*). Similarly, expression in the $\Delta merR$ mutant decreases by 7.3 % in 1 μM , and by 21.0 % in 10 μM HgCl_2 (Shewchuk, Verdine, Nash, and Walsh, 1989).

It is worth noting that Hg sensitivities can vary by five- to 10-fold depending on the choice of *E. coli* strain, plasmid copy number, and growth medium (Nakahara, Silver, Miki, and Rownd, 1979; Moore and Walsh, 1989). Similarly, β -galactosidase synthesis can vary by two-fold with different batches of the same media and stocks of HgCl_2 (Ross, Park and Summers, 1989). A significant cause of this variation is through the interaction of Hg with molecules in the external medium. For example, binding may reduce the bioavailability of the metal to the cell (Chang, Hong, Oguseitan, and Olson, 1993) or increase the (facilitated)

diffusion of the Hg-complex across the membrane (Golding *et al.*, 2002). Thus, cysteine (but not leucine or methionine – which lack thiol groups) increases Hg uptake, whereas glutathione decreases it (Ndu, Mason, Zhang, Lin, and Visscher, 2012). Organic supplements also affect the concentration of chloride ions in the medium. This is significant since 96 % of toxicity (in *Pseudomonas fluorescens*) is correlated to the mole fraction of metal in Hg(II)-chloride complex forms (but not to the fraction as free Hg^{2+}). Of particular note is HgCl_2 due to its high permeability through the lipid membrane. Finally, the inorganic cations, Ca^{2+} and Mg^{2+} , decrease toxicity, possibly by reducing the permeability of the cell to Hg or by competing directly for Hg-binding sites (Farrel, Germida, and Huang, 1993).

2.8 Hg binding sites

Concentration of binding sites

Hg diffuses into the cell interior and will activate transcription of P_T even in the absence of the MerP and MerT transporters (Hansen and Sorensen, 2000). Net uptake of Hg then continues until the rates of Hg import and export are in equilibrium. For example, when an *E. coli* cell (*mer*⁻) is incubated for 10 minutes in 5 μM HgCl_2 , the internal Hg concentration equilibrates at about $\sim 200 \mu\text{M}$ while that of the external media remains relatively unchanged (Kiyono and Pan-Hou, 1999). Theoretically, each cell contains a maximum number of (non-*mer*) binding sites which become saturated (i.e. 100 % bound) when the internal Hg concentration reaches between 3.8 mM (Chen and Wilson, 1997) and 32 mM (Huang *et al.*, 2003). This is similar to the concentration of total cellular thiols, i.e. 8.0 mM (Ledwidge, *et al.*, 2005). In both *E. coli* (Bae, Wu, Kostal, Mulchandani, and Chen, 2003) and in *Pseudomonas oleovorans* (Horitsu, Takagi and Tomoyeda, 1978), 80 % of internal Hg is bound to the cytoplasm while 20 % is bound to the membrane.

The equilibrium concentration of internal Hg may be increased in four ways. The first is to increase the total Hg concentration in the assay. For example, both sensitive (pRR132, *mer*⁻) and hypersensitive (pRR134, *merRTP*(ΔA)) cells will bind the same amount of Hg when the total Hg concentration exceeds 50 μM (Nakahara, Silver, Miki, and Rownd, 1979). The second method is to increase the rate at which Hg enters the cell, for example, by expressing the transporters MerT and MerP (Bae, Mehra, Mulchandani, and Chen, 2001). Similarly, cells treated with membrane-perturbing agents such as toluene, ether or sodium deoxycholate, bind as much Hg as induced hypersensitive cells (Nakahara, Silver, Miki, and Rownd, 1979). Thirdly, the number of non-*mer* binding sites may be

increased. Cells overexpressing MerT and MerP alone will saturate at 3.8 mM Hg bound. However, if GST-PMT metallothionein, which is rich in cysteine residues, is co-expressed to 25 % of total protein, the internal Hg concentration saturates at 24.1 mM Hg (Chen and Wilson, 1997). Finally, the number of *mer*-Hg²⁺ binding sites may be increased, for example, by over-expressing MerR (Song *et al.*, 2004) or MerP (Huang, *et al.*, 2003).

Classification of binding sites

The cellular sites to which Hg can bind are either accessible to MerA volatilisation or inaccessible to MerA volatilisation. Generally, MerA-accessible binding sites are located in the cytoplasm and MerA-inaccessible sites are located in the membranes and periplasm. The role of *mer* transport is to relocate Hg²⁺ from MerA-inaccessible sites to MerA-accessible sites so that MerA may reduce Hg²⁺ to Hg⁰. To date, experimental data have not distinguished between Hg-binding sites, so Table 2.2 and Figure 2.3 subclassifies them into five hypothetical groups.

Hg may bind to sites which are inaccessible to both MerA and the *mer* transporters. This will include the negligible amount of Hg bound to the outside of the cell. This quantity can be estimated from the amount of Hg bound to the whole cell in the presence of MerA volatilisation and *mer* transport, but excluding the amount of Hg²⁺ in the Hg²⁺-*mer* complexes. For example, Summers and Kight-Olliff (1980) found that the total amount of Hg bound to induced resistant cells (R702) was barely detectable.

External Hg is hypothesised to diffuse rapidly into the periplasm through the pores of the outer membrane (Philippidis, Schottel, and Hu, 1990). Although the periplasm may contain an unequal concentration of binding sites than the external medium, Hg may equilibrate relatively quickly between the two compartments. Therefore, in the absence of *mer* transport, it is the rate of non-*mer* import from membranar sites which will limit the transfer of Hg²⁺ to MerA-accessible sites. However, despite the permeability barrier of the inner membrane, Hg is still found to bind predominantly in the cytoplasm (Bae, Wu, Kostal, Mulchandani, and Chen, 2003). Therefore, the majority of total cellular binding sites (greater than 80 %) are MerA-accessible and these can be increased by expressing MerA and MerR to form the *mer*-Hg²⁺ complexes (cytoplasm).

Table 2.2 Classification of Hg binding sites.

Hg binding site	Definition
Inaccessible	Sites inaccessible to both MerA and the transporters.
<i>mer</i> -Hg ²⁺ (membrane)	Hg ²⁺ bound in the MerT-Hg ²⁺ and MerP-Hg ²⁺ complexes.
<i>mer</i> -Hg ²⁺ (cytoplasm)	Hg ²⁺ bound in the DNA-MerR-Hg ²⁺ , MerR-Hg ²⁺ and MerA-Hg ²⁺ complexes.
Membranar	Sites accessible to but not bound to MerT and MerP and not accessible to MerA. Hg ²⁺ is shuttled from MerT-Hg ²⁺ to MerA through MerA transport or through the non-MerA transport from MerT-Hg ²⁺ to intracellular thiols in the cytoplasm.
Cytoplasmic	Sites accessible to but not bound by MerA in the absence of <i>mer</i> transport.

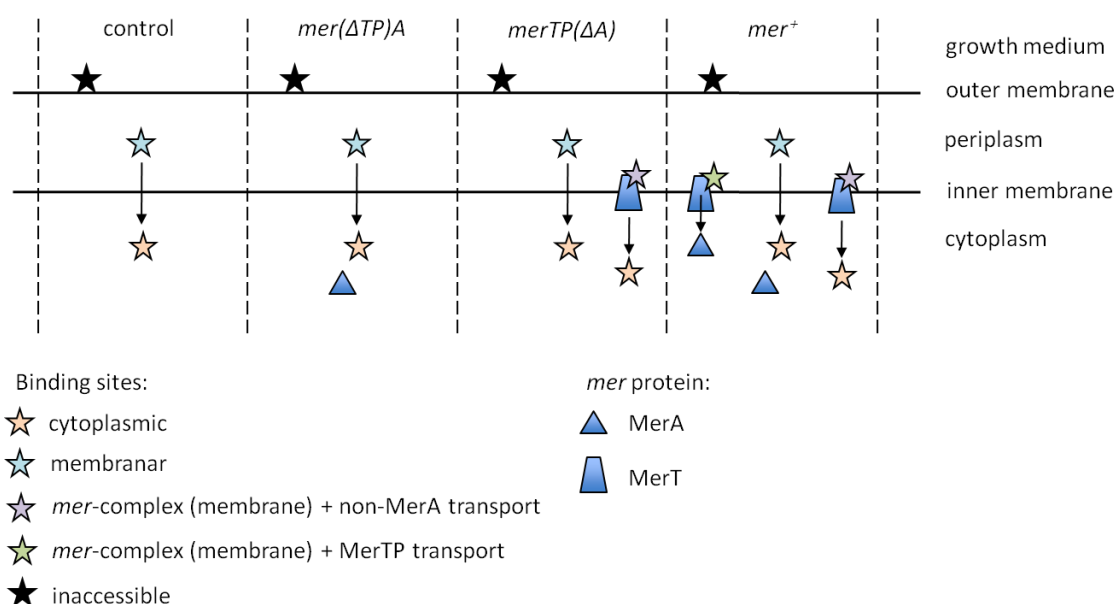


Figure 2.3 Binding sites for control, sensitive, hypersensitive, and resistant cells.

Hg is taken up from the growth medium by non-*mer* import, MerA transport or non-MerA transport. Hg always binds to cytoplasmic, membranar, and inaccessible sites in the cell. In addition, Hg²⁺ forms a complex with the *mer* proteins (Table 2.2).

Membranar sites are accessible to MerT and MerP but not to MerA. Initially, MerT and MerP bind Hg²⁺ into the *mer*-Hg²⁺ complexes (membrane). For example, MerP binds Hg²⁺ with a K_D of 3.7 μ M in a four-fold excess of cysteine (Tn21, Sahlman and Jonsson, 1991). Unfortunately, the kinetics of MerT-Hg²⁺ binding is still unknown. Nevertheless, the primary mechanism by which membranar Hg becomes accessible to MerA is via the shuttling of Hg²⁺ from MerT-Hg²⁺ to MerA, and/or via the non-MerA transport from MerT-Hg²⁺ directly into the cytoplasm. To a lesser extent, Hg is transferred to MerA by non-*mer* import.

In hypersensitive cells (*merRTP*(ΔA)), Hg will be distributed in the membrane, cytoplasm, and *mer*-complexes (membrane). However, if the membrane capacity is only 1–3 % of total cellular protein (i.e. 7.7×10^{-5} M), the quantity of Hg^{2+} in MerT- Hg^{2+} or MerP- Hg^{2+} should be negligible. Therefore, it is possible to speculate that: 1) MerT (Wilson, Leang, Morby, Hobman and Brown, 2000) and MerP (Huang, *et al.*, 2003) can individually bind and transport Hg^{2+} in the absence of the other transporter protein; and, 2) these transporters not only concentrate/retain Hg in membranar sites (by association-dissociation of Hg^{2+}), thus increasing the rate of non-*mer* import across the inner membrane, but, that MerT itself can physically transport Hg^{2+} across the inner membrane by non-MerA transport even if its shuttling partner, MerA, is absent.

In support of MerA transport, the cytoplasmic loop of Tn501 MerT does interact transiently with the N-terminal region of MerA in both the presence and absence of HgCl_2 (Schue, Glendinning, Hobman and Brown, 2008). In addition, Rossy *et al.* (2004) synthesised a MerT peptide (ppMerT) and found that MerA transport to the N-terminal of *Ralstonia metallidurans* CH34 MerA (MerAa) is faster than non-MerA transport to other thiols. For example, 100 μM of preformed ppMerT- Hg^{2+} solution (mixed with 80 μM HgCl_2) transfers most of its Hg^{2+} to 100 μM MerAa in 10 minutes at room temperature. In contrast, incubation with 100 μM MerP, 8 mM glutathione, or 200 μM L-cysteine results in only half of the ppMerT losing its metal. These results are important because the concentrations of these thiols are biologically realistic. However, although the catalytic core of MerA does not require its N-terminal domain for *in vitro* activity (Ledwidge *et al.*, 1989), no study has demonstrated the significance of the N-terminal on volatilisation in whole cells nor have they demonstrated the individual rates of MerA transport and non-MerA transport across a plasma membrane.

2.9 Conclusions: suitable components for a model

The roles and cellular locations of the *mer* proteins are well understood. Each protein appears to remain stable over the course of biological experiments although no study has quantified their set of absolute concentrations. The significance of MerC and MerD has not been satisfactorily demonstrated. Therefore, three essential components for a model of Hg resistance are: regulation by MerR, transport by MerT and MerP, and reduction by MerA.

Saturation of the membrane capacity may cause a change in the relative concentrations of MerT and MerA. Nonetheless, uptake of Hg across the biological

membrane is still assumed to be rate limiting on volatilisation. Uptake occurs by three processes – non-*mer* import, MerA transport, and non-MerA transport. The only uptake process which has been studied in isolation is non-*mer* import/export. Therefore, this can be a component of a model separate from transport by MerT.

The cytoplasmic concentration of Hg determines the rate of *mer* protein synthesis through the partitioning of the regulator, MerR_t, into activating (MerR-Hg²⁺) and repressing forms (MerR). Although the order of DNA + MerR + Hg²⁺ binding is unknown, both MerR and MerR-Hg²⁺ may individually bind the DNA. In contrast to the activating effect of Hg²⁺ on *mer*, Hg can simultaneously inhibit general protein synthesis and cell growth. However, since a subtoxic concentration of Hg is used in most experiments, and, since the nature of Hg toxicity is still unknown, a toxicity component in a model may be omitted.

Finally, the interaction of Hg with chemicals in the external growth medium affects its uptake. Total Hg uptake saturates at 3.8–32 mM, with 80 % bound in the cytoplasm and 20 % in the membrane. These binding sites are now classified into five hypothetical groups. Therefore, new experimental data on cellular Hg concentrations are required so that a two-compartment model can be expanded to include periplasmic, membranar, cytoplasmic, and external compartments.

Chapter 3: The Model

This chapter outlines a model of our existing knowledge of Hg resistance. The model is composed of a set of differential and algebraic equations which determine how the concentrations of each entity changes with time. These equations are simplified with quasi-steady-state approximations and extended to conserve the significant quantity of Hg^{2+} bound in the *mer*- Hg^{2+} complexes. This process leaves 16 parameters which describe the following reactions: uptake and volatilisation, operator states, protein synthesis, and, dilution by cell growth. This model excludes entities which are not significant to Hg resistance, mechanisms which are currently unknown, and reactions which cannot be distinguished from one another because of insufficient experimental data.

3.1 The full model

This two-compartment model represents an average cell in the population surrounded by its dedicated volume of growth medium (or buffer). Thus, every cell has an internal volume, v_s , and an external volume, v_{sp} . When a quantity of external Hg, S' , in the volume v_{sp} , enters the cell, it becomes the quantity, S , in the new volume v_s , with concentration $[S] = S/v_s$. For example, 1 L of assay mixture may contain 0.78×10^{11} cells, each of volume $v_s = 4.4 \times 10^{-15}$ L, and growing at rate 0.0268 min^{-1} . Therefore, $v_{sp} = \{1 \text{ L} - (4.4 \times 10^{-15} \text{ L} \times 0.78 \times 10^{11} \text{ cells})\} / (0.78 \times 10^{11} \text{ cells}) = 1.28 \times 10^{-11} \text{ L}$. Then, if Hg is added to v_{sp} at $[S'] = 1 \times 10^{-6} \text{ M}$, $S' = 1 \times 10^{-6} \text{ mols/L} \times 1.28 \times 10^{-11} \text{ L} = 1.28 \times 10^{-17} \text{ mols}$. If all of S' were to enter v_s , $[S] = 1.28 \times 10^{-17} \text{ mols} / 4.4 \times 10^{-15} \text{ L} = 2.91 \times 10^{-3} \text{ M}$ and $[S'] = 0 \text{ M}$.

Within these two compartments are the six *mer* entities: S' (unbound external Hg), S (unbound internal Hg), T (combined MerT and MerP transporters, as justified below), A (MerA), R (MerR), and O_T (total *mer* operator DNA). Each entity has an initial state which is its concentration (in Molar) at time = 0 (in minutes) in the simulation. These entities change their state by interacting in chemical reactions. Each reaction has a rate constant which is the value of one of the 16 parameters in the model. Figure 3.1 organises these parameters by the following reactions: uptake and volatilisation; operator states; protein synthesis; and, dilution of entities by cell growth.

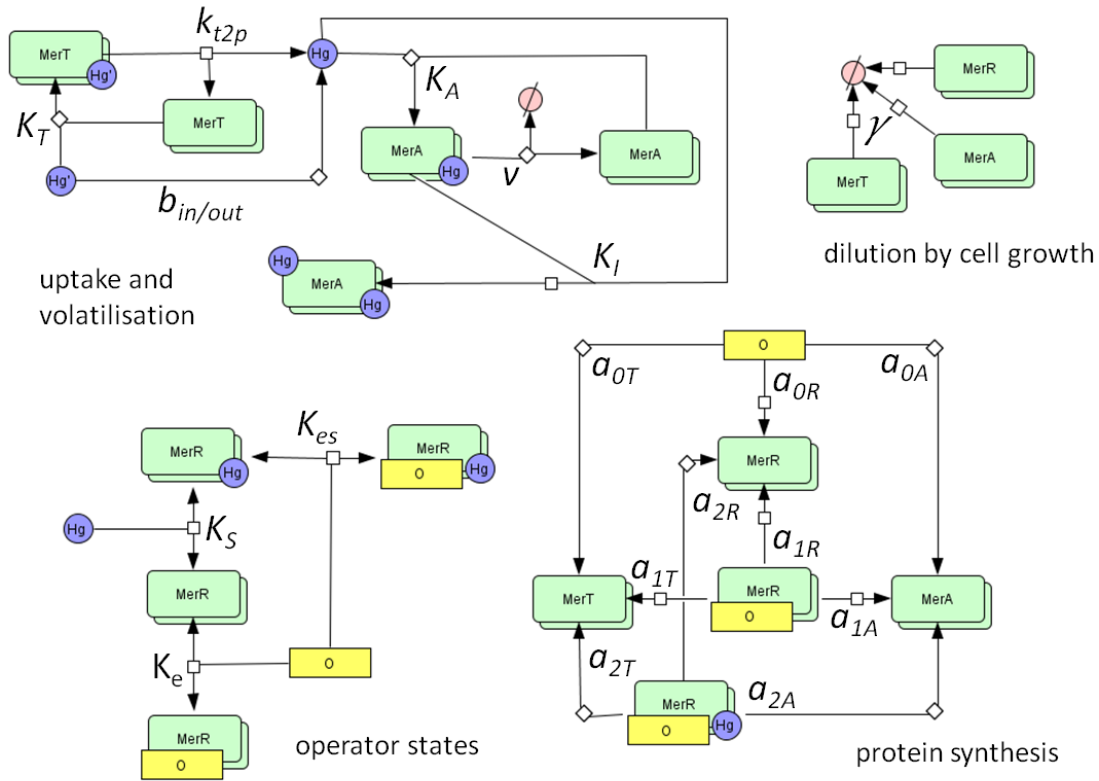


Figure 3.1 Model summary. The entities of the system are the *mer* proteins (green), operator DNA (yellow) and Hg and Hg' (blue). These entities interact via chemical reactions, shown by arrows. Each reaction occurs with a rate constant which is a parameter in the model. These parameters fall into four groups: uptake and volatilisation; operator states; synthesis; and, dilution by cell growth. In the final model, the number of parameters is reduced further by expressing a_{0T} , a_{0R} , a_{1T} , a_{1A} , a_{1R} , a_{2T} , a_{2A} and a_{2R} all relative to a_{0A} as $a_{0T/0A}$, $a_{0R/0A}$, a_1 , a_2 , and a_R . For example, $a_{2A} = a_{0A} \cdot a_2$.

Table 3.1 defines the reactants, products, and rate constants of each reaction. The reaction rate depends on the number of ways a molecule of one entity can combine with another (i.e. on their concentrations) and on the frequency of one unit of each of these entities colliding with the correct orientations and energies to undergo a chemical reaction (i.e. the rate constant).

An entity is involved in a number of reactions which are grouped together into one differential equation (Equation 3.1). A reaction in which the concentration of the entity decreases with time represents a negative term in the equation. Conversely, a reaction in which the concentration of the entity increases with time represents a positive term in the equation. Overall, each differential equation describes the total change in the concentration of the same entity with time.

Table 3.1 Definition of model parameters.

Parameter	Reaction	Reaction rate	Definition	Units
b_{in}	$[S'] \rightarrow [S]$	$b_{in} [S'] (v_{sp}/v_s)$	Non- <i>mer</i> import of Hg^{2+} into cell	min^{-1}
b_{out}	$[S] \rightarrow [S']$	$b_{out} [S]$	Non- <i>mer</i> export of Hg^{2+} from cell	min^{-1}
k_{t2p}	$[T^*] \rightarrow [T] + [S]$	$k_{t2p} [T^*] (v_{sp}/v_s)$	Non-MerA transport of Hg^{2+} into cell	min^{-1}
v	$[A^*] \rightarrow [A] + NULL$	$v [A^*]$	Reduction of Hg^{2+} to Hg^0	min^{-1}
a_{0A}	$[O] \rightarrow [A]$	$a_{0A} [O]$	MerA synthesis from (free) DNA (no regulator bound)	min^{-1}
$a_{0T/0A}$	$[O] \rightarrow [T]$	$(a_{0T/0A} \times a_{0A}) \times [O]$	MerT synthesis from DNA / MerA synthesis from DNA	dimensionless
$a_{0R/0A}$	$[O] \rightarrow [R]$	$(a_{0R/0A} \times a_{0A}) \times [O]$	MerR synthesis from DNA / MerA synthesis from DNA	dimensionless
a_1	$[OR] \rightarrow [A]$	$a_{0A} \times a_1 [OR]$	MerA synthesis from DNA-MerR / MerA synthesis from DNA	dimensionless
a_1	$[OR] \rightarrow [T]$	$(a_{0T/0A} \times a_{0A}) \times a_1 [OR]$	MerT synthesis from DNA-MerR / MerT synthesis from DNA	dimensionless
a_2	$[OR^*] \rightarrow [A]$	$a_{0A} \times a_2 [OR^*]$	MerA synthesis from DNA-MerR- Hg^{2+} / MerA synthesis from DNA	dimensionless
a_2	$[OR^*] \rightarrow [T]$	$(a_{0T/0A} \times a_{0A}) \times a_2 [OR^*]$	MerT synthesis from DNA-MerR- Hg^{2+} / MerT synthesis from DNA	dimensionless
a_R	$[OR] \rightarrow [R]$	$(a_{0R/0A} \times a_{0A}) \times a_R [OR]$	MerR synthesis from DNA-MerR / MerR synthesis from DNA	dimensionless
a_R	$[OR^*] \rightarrow [R]$	$(a_{0R/0A} \times a_{0A}) \times a_R [OR^*]$	MerR synthesis from DNA-MerR- Hg^{2+} / MerR synthesis from DNA	dimensionless
K_e	$[R] + [O] \leftrightarrow [OR]$	$k_{e+} [R][O], k_{e-} [OR]$	[MerR] for which 50 % of O_t is DNA-MerR (at equilibrium)	M
K_{es}	$[R^*] + [O] \leftrightarrow [OR^*]$	$k_{es+} [R^*][O], k_{es-} [OR^*]$	[MerR] for which 50 % of O_t is DNA-MerR- Hg^{2+}	M
K_S	$[R] + [S] \leftrightarrow [R^*]$	$k_{s+} [R][S], k_{s-} [R^*]$	[S] for which 50 % of $MerR_t$ is MerR- Hg^{2+}	M
K_A	$[A] + [S] \leftrightarrow [A^*]$	$k_{a+} [A][S], k_{a-} [A^*]$	Equilibrium constant for MerA _t and MerA- Hg^{2+}	M
K_T	$[T] + [S'] \leftrightarrow [T^*]$	$k_{t+} [T][S'], k_{t-} [T^*]$	[S'] for which 50 % of $MerT_t$ is MerT- Hg^{2+}	M
K_I	$[A^*] + [S] \leftrightarrow [^*A^*]$	$k_{i+} [A^*][S], k_{i-} [^*A^*]$	Equilibrium constant for MerA- Hg^{2+} , Hg^{2+} -MerA- Hg^{2+} and Hg^{2+}	M
γ	$[X_t] \rightarrow NULL$	$\gamma [X_t]$	Cell growth (<i>mer</i> protein dilution)	min^{-1}

Equation 3.1 Full model including radioactive (^{203}X) entities.

$$\begin{aligned}
\frac{d[S]}{dt} &= k_{t2p}[T^*] + b_{in}[S'] - b_{out}[S] - k_{a+}[S][A] + k_{a-}[A^*] - k_{i+}[S][A^{203}] + k_{i-}[^*A^{203}] \\
&\quad - k_{i+}[S][A^*] + k_{i-}[^*A^*] - k_{s+}[S][R] + k_{s-}[R^*] - \gamma[S] \\
\frac{d[^{203}S]}{dt} &= k_{t2p}[T^{203}] + b_{in}[^{203}S'] - b_{out}[^{203}S] - k_{a+}[^{203}S][A] + k_{a-}[A^{203}] - k_{i+}[^{203}S][A^{203}] \\
&\quad + k_{i-}[^{203}A^{203}] - k_{i+}[^{203}S][A^*] + k_{i-}[^{203}A^*] - k_{s+}[^{203}S][R] + k_{s-}[R^{203}] - \gamma[^{203}S] \\
\frac{d[S']}{dt} &= (-b_{in}[S'] + b_{out}[S] - k_{t1+}[S'][T] + k_{t1-}[T^*] + \gamma[S']) \frac{v_s}{v_{sp}} \\
\frac{d[^{203}S']}{dt} &= (-b_{in}[^{203}S'] + b_{out}[^{203}S] - k_{t1+}[^{203}S'][T] + k_{t1-}[T^{203}] + \gamma[^{203}S']) \frac{v_s}{v_{sp}} \\
\frac{d[OR]}{dt} &= k_{e+}[R][O] - k_{e-}[OR] - \gamma[OR] \\
\frac{d[OR^*]}{dt} &= k_{es+}[R^*][O] - k_{es-}[OR^*] - \gamma[OR^*] \\
\frac{d[OR^{203}]}{dt} &= k_{es+}[R^{203}][O] - k_{es-}[OR^{203}] - \gamma[OR^{203}] \\
\frac{d[O]}{dt} &= -k_{e+}[R][O] + k_{e-}[OR] - k_{es+}[R^*][O] + k_{es-}[OR^*] - k_{e+}[R^{203}][O] + k_{e-}[OR^{203}] - \gamma[O] \\
\frac{d[A^*]}{dt} &= k_{a+}[S][A] - k_{a-}[A^*] - k_{i+}[S][A^*] + k_{i-}[^*A^*] - k_{i+}[^{203}S][A^*] + k_{i-}[^{203}A^*] - v[A^*] - \gamma[A^*] \\
\frac{d[A^{203}]}{dt} &= k_{a+}[^{203}S][A] - k_{a-}[A^{203}] - k_{i+}[S][A^{203}] + k_{i-}[^*A^{203}] - k_{i+}[^{203}S][^{203}A] + k_{i-}[^{203}A^{203}] \\
&\quad - v[A^{203}] - \gamma[A^{203}] \\
\frac{d[A]}{dt} &= \alpha_{0a}[O] + \alpha_{1a}[OR] + \alpha_{2a}([OR^*] + [OR^{203}]) - k_{a+}[S][A] + k_{a-}[A^*] + v[A^*] \\
&\quad - k_{a+}[^{203}S][A] + k_{a-}[A^{203}] + v[A^{203}] - \gamma[A] \\
\frac{d[^*A^*]}{dt} &= k_{i+}[S][A^*] - k_{i-}[^*A^*] \\
\frac{d[^{203}A^*]}{dt} &= k_{i+}[^{203}S][A^*] - k_{i-}[^{203}A^*] \\
\frac{d[^*A^{203}]}{dt} &= k_{i+}[S][A^{203}] - k_{i-}[^*A^{203}] \\
\frac{d[^{203}A^{203}]}{dt} &= k_{i+}[^{203}S][A^{203}] - k_{i-}[^{203}A^{203}] \\
\frac{d[T^*]}{dt} &= k_{t1+}[S'][T] - k_{t1-}[T^*] - k_{t2p}[T^*] - \gamma[T^*] \\
\frac{d[T^{203}]}{dt} &= k_{t1+}[^{203}S'][T] - k_{t1-}[T^{203}] - k_{t2p}[T^{203}] - \gamma[T^{203}] \\
\frac{d[T]}{dt} &= \alpha_{0t}[O] + \alpha_{1t}[OR] + \alpha_{2t}([OR^*] + [OR^{203}]) - k_{t1+}[S'][T] + k_{t1-}[T^*] + k_{t2p}[T^*] - \gamma[T] \\
&\quad - k_{t1+}[^{203}S'][T] + k_{t1-}[T^{203}] + k_{t2p}[T^{203}] \\
\frac{d[R]}{dt} &= \alpha_{0r}[O] + \alpha_{1r}[OR] + \alpha_{2r}([OR^*] + [OR^{203}]) - k_{e+}[R][O] + k_{e-}[OR] - k_{s+}[S][R] + k_{s-}[R^*] - \gamma[R] \\
&\quad - k_{s+}[^{203}S][R] + k_{s-}[R^{203}] \\
\frac{d[R^*]}{dt} &= k_{s+}[S][R] - k_{s-}[R^*] - k_{es+}[R^*][O] + k_{es-}[OR^*] - \gamma[R^*] \\
\frac{d[R^{203}]}{dt} &= k_{s+}[^{203}S][R] - k_{s-}[R^{203}] - k_{es+}[R^{203}][O] + k_{es-}[OR^{203}] - \gamma[R^{203}]
\end{aligned}$$

3.2 Quasi equilibria

Some chemical reactions equilibrate more rapidly than slower reactions in the system. For example, a mixture of MerR_t and Hg^{2+} will equilibrate within seconds into free MerR, MerR-Hg^{2+} , and free Hg^{2+} . There are in fact two parameters in this reversible reaction: the association (k_{s+}) of MerR and Hg^{2+} and the dissociation (k_{s-}) of the MerR-Hg^{2+} complex back into MerR and Hg^{2+} (Equation 3.2)

Equation 3.2 Differential equations for the binding of Hg^{2+} to MerR.

$$\begin{aligned}\frac{d[R]}{dt} &= -k_{e+}[R][O] + k_{e-}[OR] - k_{s+}[R][S] + k_{s-}[R^*] \\ \frac{d[R^*]}{dt} &= k_{s+}[R][S] - k_{s-}[R^*] - k_{es+}[R^*][O] + k_{es-}[OR^*] \\ \frac{d[S]}{dt} &= -k_{s+}[R][S] + k_{s-}[R^*]\end{aligned}$$

At equilibrium, $k_{s-}/k_{s+} = K_S$ is equivalent to $[\text{MerR}][\text{Hg}^{2+}]/[\text{MerR-Hg}^{2+}]$. In addition, only one molecule of MerR_t (per plasmid copy per genome equivalent) exists as DNA-MerR or DNA-MerR- Hg^{2+} . Therefore, the quantity bound to the DNA is negligible compared to that of free MerR and MerR- Hg^{2+} . These two observations allow the number of parameters in the model to decrease by replacing three differential equations in rapid equilibrium with algebraic relations for $[\text{MerR}]$ and $[\text{MerR-Hg}^{2+}]$, given $[\text{Hg}^{2+}]$ and $[\text{MerR}_t]$ (Equation 3.3).

Equation 3.3 The quasi equilibrium of MerR_t into MerR and MerR- Hg^{2+} .

$$\begin{aligned}\frac{d[R]}{dt} &= -k_{s+}[R][S] + k_{s-}[R^*] = 0 \\ \frac{[R][S]}{[R^*]} &= \frac{k_{s-}}{k_{s+}} = K_S \rightarrow [R^*] = \frac{[R][S]}{K_S} \\ [R_t] &= [R] + [R^*] + ([OR] + [OR^*]) \rightarrow [R^*] = [R_t] - [R] \\ [R_t] - [R] &= \frac{[R][S]}{K_S} \rightarrow [R] = [R_t] \frac{K_S}{[S] + K_S} \\ [R] + [R^*] &= [R_t] \frac{K_S}{[S] + K_S} + [R_t] \frac{[S]}{[S] + K_S} = [R_t]\end{aligned}$$

In conclusion, MerR_t is conceptualised as equilibrating instantaneously into $[\text{MerR}]$ and $[\text{MerR-Hg}^{2+}]$ at each time point in the computer simulation from known values of $[\text{Hg}^{2+}]$ and $[\text{MerR}_t]$ which are themselves changing according to their respective differential equations (Equation 3.1).

3.3 Operator states

Equation 3.4 shows how the differential equations for operator states were reduced into quasi-steady-state forms.

Equation 3.4 Derivation of operator quasi equilibria.

$$\begin{aligned}
\frac{d[OR]}{dt} &= 0 = k_{e+}[R][O] - k_{e-}[OR] \rightarrow \frac{[R][O]}{K_e} = [OR] \\
\frac{d[OR^*]}{dt} &= 0 = k_{es+}[R^*][O] - k_{es-}[OR^*] \rightarrow [OR^*] = \frac{[R^*][O]}{K_{es}} \\
\frac{d[OR^{203}]}{dt} &= 0 = k_{es+}[R^{203}][O] - k_{es-}[^{203}OR^*] \rightarrow [OR^{203}] = \frac{[R^{203}][O]}{K_{es}} \\
\frac{d[A]}{dt} &= \alpha_{0a}[O] + \alpha_{1a}[OR] + \alpha_{2a}[OR^*] + \alpha_{2a}[OR^{203}] - \dots \\
&= \alpha_{0a}[O] + \alpha_{1a} \frac{[R][O]}{K_e} + \alpha_{2a} \frac{[R^*][O]}{K_{es}} + \alpha_{2a} \frac{[R^{203}][O]}{K_{es}} - \dots \\
&= [O_T] \left(\frac{\alpha_{0a} + \alpha_{1a} \frac{[R]}{K_e} + \alpha_{2a} \frac{[R^*]}{K_{es}} + \alpha_{2a} \frac{[R^{203}]}{K_{es}}}{1 + \frac{[R]}{K_e} + \frac{[R^*]}{K_{es}} + \frac{[R^{203}]}{K_{es}}} \right) - \dots
\end{aligned}$$

3.4 MerT quasi-steady-states

Equation 3.5 shows how the differential equations for non-MerA transport were reduced into quasi-steady-state forms.

Equation 3.5 Derivation of non-MerA transport quasi equilibria.

$$\begin{aligned}
\frac{d[S]}{dt} &= k_{t2p}[T^*] \rightarrow \frac{d[S]}{dt} = k_{t2p} \frac{[T_t][S']}{K_T + [S']} \\
\frac{d[S']}{dt} &= (-k_{t1+}[S'][T] + k_{t1-}[T^*]) \frac{v_s}{v_{sp}} \rightarrow \frac{d[S']}{dt} = \left(-k_{t2p} \frac{[S'] [T_t]}{K_T + [S']} \right) \frac{v_s}{v_{sp}} \\
\frac{d[T^*]}{dt} &= k_{t1+}[S'][T] - k_{t1-}[T^*] - k_{t2p}[T^*] \rightarrow \frac{d[T^*]}{dt} = 0 \\
\frac{d[T]}{dt} &= -k_{t1+}[S'][T] + k_{t1-}[T^*] + k_{t2p}[T^*] \rightarrow \frac{d[T]}{dt} = 0 \\
0 &= k_{t1+}[S'][T] + [T^*](-k_{t1-} - k_{t2p}) \rightarrow [T^*] \frac{(k_{t1-} + k_{t2p})}{k_{t1+}} = [S'][T] \rightarrow [T^*] = \frac{[S'] [T]}{K_T} \\
[T_t] &= [T] + [T^*] = [T] + \frac{[S'] [T]}{K_T} \rightarrow [T] = [T_t] \frac{K_T}{K_T + [S']}
\end{aligned}$$

3.5 Substrate inhibition

Equation 3.6 shows how the differential equations for MerA volatilisation were reduced into quasi-steady-state forms, including substrate inhibition.

Equation 3.6 Derivation of MerA volatilisation quasi equilibria.

$$\begin{aligned}
0 &= (-k_{a+}[S][A] + k_{a-}[A^*]) + (-k_{a+}[^{203}S][A] + k_{a-}[A^{203}]) + v[A^*] + v[A^{203}] \\
0 &= [A^*](k_{a-} + v) + [A^{203}](k_{a-} + v) - k_{a+}[S][A] - k_{a+}[^{203}S][A] \\
[S][A] + [^{203}S][A] &= [A^*]\frac{(k_{a-} + v)}{k_{a+}} + [A^{203}]\frac{(k_{a-} + v)}{k_{a+}} \\
\frac{[A]([S] + [^{203}S])}{K_A} &= [A^*] + [A^{203}] \\
0 &= (k_{a+}[S][A] - k_{a-}[A^*]) - v[A^*]; \quad 0 = (k_{a+}[^{203}S][A] - k_{a-}[A^{203}]) - v[A^{203}] \\
[A^*] &= \frac{[S][A]}{K_A}; \quad [A^{203}] = \frac{[^{203}S][A]}{K_A} \\
0 &= [S][A^*] - K_I[*A^*] \rightarrow [*A^*] = \frac{[S][A^*]}{K_I} \\
0 &= [^{203}S][A^*] - K_I[^{203}A^*] \rightarrow [^{203}A^*] = \frac{[^{203}S][A^*]}{K_I} \\
0 &= [S][A^{203}] - K_I[*A^{203}] \rightarrow [*A^{203}] = \frac{[S][A^{203}]}{K_I} \\
0 &= [^{203}S][A^{203}] - K_I[^{203}A^{203}] \rightarrow [^{203}A^{203}] = \frac{[^{203}S][A^{203}]}{K_I} \\
[A_t] &= [A] + [A^*] + [A^{203}] + [*A^*] + [^{203}A^*] + [*A^{203}] + [^{203}A^{203}] \\
[A_t] &= [A] + \frac{[S][A]}{K_A} + \frac{[^{203}S][A]}{K_A} + \frac{[S][A^*]}{K_I} + \frac{[^{203}S][A^*]}{K_I} + \frac{[S][A^{203}]}{K_I} + \frac{[^{203}S][A^{203}]}{K_I} \\
[A_t] &= [A] + \frac{[S][A]}{K_A} + \frac{[^{203}S][A]}{K_A} + \frac{[S]\frac{[S][A]}{K_A}}{K_I} + \frac{[^{203}S]\frac{[S][A]}{K_A}}{K_I} + \frac{[S]\frac{[^{203}S][A]}{K_A}}{K_I} + \frac{[^{203}S]\frac{[^{203}S][A]}{K_A}}{K_I} \\
[A_t] &= [A] + \frac{[S][A]}{K_A} + \frac{[^{203}S][A]}{K_A} + \frac{[S]^2[A]}{K_A K_I} + 2\frac{[^{203}S][S][A]}{K_A K_I} + \frac{[^{203}S]^2[A]}{K_A K_I} \\
[A] &= \frac{[A_t]K_A}{K_A + [S] + [^{203}S] + \frac{[S]^2}{K_I} + 2\frac{[^{203}S][S]}{K_I} + \frac{[^{203}S]^2}{K_I}}
\end{aligned}$$

3.6 Total [Hg²⁺] bound to a cell

MerT-Hg²⁺ is considered part of S_t, i.e. the total amount of internal Hg²⁺. When Hg²⁺ enters the cell, it passes between two compartments of different volumes. Concentrations in [S'_t] are converted into concentrations in [S_t] by multiplying by v_{sp}/v_s (Puc, Kotnik, Mir, and Miklavčič, 2003). The concentration of Hg²⁺ bound to

the cell is the quantity of free Hg^{2+} ($S \cdot v_s$) plus the quantity of Hg^{2+} in T^* ($[T^*] \cdot v_s$), R^* , A^* , and $*A^*A$, all divided by the volume of the cell (v_s) (Equation 3.7).

Equation 3.7 The total concentration of Hg^{2+} bound to a cell.

$$[S_t] = [S] + [R^*] + [A^*] + 2[*A^*]$$

3.7 Conservation of mass

It is important not to ignore the quantity of Hg^{2+} bound in the MerX-Hg^{2+} complexes because $[\text{MerR-Hg}^{2+}]$ and $[\text{MerA-Hg}^{2+}]$ can be on the same order of magnitude as $[S_t]$. Thus, in finding the quasi equilibrium, all forms of Hg^{2+} must be partitioned so that the total mass of S_t (or S'_t) be maintained (Equation 3.8). Please note that, compared to $[S']$, the quantity in $[\text{MerT-Hg}^{2+}]$ is negligible.

Equation 3.8 Conservation of mass in the quasi equilibria.

$$[S_t] = [S] + [R^*] + [A^*] + 2[*A^*] + [^{203}A^*] + [*A^{203}]$$

$$[^{203}S_t] = [^{203}S] + [^{203}R] + [^{203}A] + 2[^{203}A^{203}] + [^{203}A^*] + [*A^{203}]$$

$$[S_t] = [S] + \frac{[R_t][S]}{K_s + [^{203}S] + [S]} + [A_t] \frac{[S] + 2 \frac{[S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I}}{K_A + [S] + [^{203}S] + \frac{[S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I} + \frac{[^{203}S]^2}{K_I}}$$

$$[^{203}S_t] = [^{203}S] + \frac{[R_t][^{203}S]}{K_s + [^{203}S] + [S]} + [A_t] \frac{[^{203}S] + 2 \frac{[^{203}S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I}}{K_A + [S] + [^{203}S] + \frac{[S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I} + \frac{[^{203}S]^2}{K_I}}$$

Solving these equations requires complicated algebra. But the trick is to notice that $[S_t]/[S] = [^{203}S_t]/[^{203}S]$ (Equation 3.9).

Equation 3.9 Reformulating the quasi equilibrium partition for $[S]$ and $[^{203}S]$.

$$A = B \left(1 + \frac{D}{E + N + B} + F \frac{1 + 2 \frac{B}{K_I} + 2 \frac{N}{K_I}}{G + B + N + \frac{B^2}{H} + 2 \frac{NB}{H} + \frac{N^2}{H}} \right)$$

$$C = N \left(1 + \frac{D}{E + N + B} + F \frac{1 + 2 \frac{N}{K_I} + 2 \frac{B}{K_I}}{G + B + N + \frac{B^2}{H} + 2 \frac{NB}{H} + \frac{N^2}{H}} \right)$$

$$\frac{A}{B} = \frac{C}{N} \rightarrow \frac{AN}{C} = B \rightarrow N = \frac{BC}{A}$$

$$A = [S_t], B = [S], C = [^{203}S_t], D = [R_t], E = K_s$$

$$F = [A_t], G = K_A, H = K_I, N = [^{203}S]$$

These new expressions for [S] and [²⁰³S] are then substituted back into the original equations to eliminate these variables (Equation 3.10).

Equation 3.10 Substitution of new terms for [S] and [²⁰³S] into Equation 3.8.

$$A = B \left(1 + \frac{D}{E + \left(\frac{BC}{A}\right) + B} + F \frac{1 + 2\frac{B}{K_I} + 2\left(\frac{BC}{A}\right)}{G + B + \left(\frac{BC}{A}\right) + \frac{B^2}{H} + 2\frac{\left(\frac{BC}{A}\right)B}{H} + \frac{\left(\frac{BC}{A}\right)^2}{H}} \right)$$

$$C = N \left(1 + \frac{D}{E + N + \left(\frac{AN}{C}\right)} + F \frac{1 + 2\frac{N}{K_I} + 2\left(\frac{AN}{C}\right)}{G + \left(\frac{AN}{C}\right) + N + \frac{\left(\frac{AN}{C}\right)^2}{H} + 2\frac{N\left(\frac{AN}{C}\right)}{H} + \frac{N^2}{H}} \right)$$

Then, with only one variable remaining, further rearrangments and gatherings of like terms gives a polynomial with coefficients *a*, *b*, *c*, *d* and *e* (Equation 3.11).

Equation 3.11 The rearrangement of Equation 3.10 as two polynomial equations.

$$0 = aB^4 + bB^3 + cB^2 + dB + e$$

$$a = 2C^2 + 2AC + \frac{C^3}{A} + C^2 + CA + A^2$$

$$b = 2FAC + 2FC^2 + 2FA^2 + 2FCA + EC^2 + DC^2 + C^2H + ACH + A^2H + EA^2 + DA^2 + CAH + 2DAC + 2ECA - CA^2 - A^3 - C^3 - AC^2 - 2C^2A - 2A^2C$$

$$c = DA^2H + EA^2H + EACH + DACH + FAHC + 2FA^2E + 2FCAE + CGAH + GA^2H + FA^2H - AC^2H - A^2CH - EA^3 - 2ECA^2 - AEC^2 - CA^2H - A^3H$$

$$d = FA^2HE + EGA^2H + DGA^2H - CGA^2H - GA^3H - EA^3H - A^2ECH$$

$$e = -EGA^3H$$

$$0 = aN^4 + bN^3 + cN^2 + dN + e$$

$$a = 2A^2 + 2CA + \frac{A^3}{C} + A^2 + AC + C^2$$

$$b = 2FCA + 2FA^2 + 2FC^2 + 2FAC + EA^2 + DA^2 + A^2H + CAH + C^2H + EC^2 + DC^2 + ACH + 2DCA + 2EAC - AC^2 - C^3 - A^3 - CA^2 - 2A^2C - 2C^2A$$

$$c = DC^2H + EC^2H + ECAH + DCAH + FCHA + 2FC^2E + 2FACE + AGCH + GC^2H + FC^2H - CA^2H - C^2AH - EC^3 - E2AC^2 - CEA^2 - AC^2H - C^3H$$

$$d = FC^2HE + EGC^2H + DGC^2H - AGC^2H - GC^3H - EC^3H - C^2EAH$$

$$e = -EGC^3H$$

Equations 3.12 and 3.13 show analogous equations for the absence of MerR and MerA, respectively.

Equation 3.12 Polynomial equations in the absence of MerR.

$$\begin{aligned} 0 &= aB^2 + bB + c & 0 &= aN^2 + bN + c \\ a &= C + A & a &= C + A \\ b &= DA - AC - A^2 + EA & b &= DC - AC - C^2 + EC \\ c &= -A^2E & c &= -C^2E \end{aligned}$$

Equation 3.13 Polynomial equations in the absence of MerA.

$$\begin{aligned} 0 &= aB^3 + bB^2 + cB + d \\ a &= 2C + \frac{C^2}{A} + A \\ b &= 2FA + 2CF + AH + CH - A^2 - 2CA - C^2 \\ c &= GAH + FAH - CAH - A^2H \\ d &= -GA^2H \end{aligned}$$

$$\begin{aligned} 0 &= aN^3 + bN^2 + cN + d \\ a &= 2A + C + \frac{A^2}{C} \\ b &= 2FC + 2FA + CH + AH - C^2 - 2AC - A^2 \\ c &= GCH + FCH - ACH - C^2H \\ d &= -GC^2H \end{aligned}$$

Finally, these roots are approximated numerically using the Newton-Raphson algorithm. Starting with an initial guess of [S] as $x_0 = [S_t]$, a closer estimate for the positive root of $f(x) = 0$ is the x-intersection of the tangent line to the curve at $f(x_0)$. This x-intersect is x_{n+1} . This step of the algorithm is then iterated with $x_n = x_{n+1}$ until $x_n - x_{n+1}$ is acceptably small (until the accuracy of the C++ compiler causes $x_{n+1} > x_n$). x_{n+1} is then accepted as the root, i.e. $f(x_{n+1}) \cong 0$ (Equation 3.14).

Equation 3.14 Newton-Raphson method for finding roots of cubic equations.

$$y - f(x_n) = f'(x_n)(x_{n+1} - x_n) \rightarrow x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)} = \frac{3ax_n^4 + 2bx_n^3 + cx_n^2 - e}{4ax_n^3 + 3bx_n^2 + 2cx_n + d}$$

3.8 Reduced model

Equation 3.15 shows a reduction of the full model in Equation 3.1 and Equation 3.16 provides an example of some initial conditions in the simulation.

Equation 3.15 Reduced model including radioactive (^{203}X) entities.

$$\begin{aligned}\frac{d[S_t]}{dt} &= b_{in}[S'] - b_{out}[S] + k_{t2p}[T^*] - v[A^*] - \gamma[S_t] \\ \frac{d[^{203}S_t]}{dt} &= b_{in}[^{203}S'] - b_{out}[^{203}S] + k_{t2p}[^{203}T^*] - v[A^{203}] - \gamma[^{203}S_t] \\ \frac{d[S'_t]}{dt} &= \left(-b_{in}[S'] + b_{out}[S] - k_{t2p}[T^*] + \gamma[S'_t] \right) \frac{v_s}{v_{sp}} \\ \frac{d[^{203}S'_t]}{dt} &= \left(-b_{in}[^{203}S'] + b_{out}[^{203}S] - k_{t2p}[^{203}T^*] + \gamma[^{203}S'_t] \right) \frac{v_s}{v_{sp}} \\ \frac{d[A_t]}{dt} &= \zeta \frac{\alpha_{0A} + \alpha_{0A}\alpha_1 X + \alpha_{0A}\alpha_2(Y+Z)}{1+X+Y+Z} - \gamma[A_t] \\ \frac{d[T_t]}{dt} &= \zeta \frac{\alpha_{0T/0A}\alpha_{0A} + \alpha_{0T/0A}\alpha_{0A}\alpha_1 X + \alpha_{0T/0A}\alpha_{0A}\alpha_2(Y+Z)}{1+X+Y+Z} - \gamma[T_t] \\ \frac{d[R_t]}{dt} &= \zeta \frac{\alpha_{0R/0A}\alpha_{0A} + \alpha_{0R/0A}\alpha_{0A}\alpha_R(X+Y+Z)}{1+X+Y+Z} - \gamma[R_t]\end{aligned}$$

Protein synthesis rates are proportional to $[O_t]$ which exists in four operator states:

$$1 = \frac{[O]}{[O]}; \quad X = \frac{[OR]}{[O]} = \frac{[R]}{K_e}; \quad Y = \frac{[OR^*]}{[O]} = \frac{[R^*]}{K_{es}}; \quad Z = \frac{[OR^{203}]}{[O]} = \frac{[R^{203}]}{K_{es}}$$

Total MerX concentrations (X_t) are conserved and equilibrate instantaneously into free MerX and MerX-Hg²⁺ forms:

$$\begin{aligned}[R] &= [R_t] \frac{K_s}{[S] + [^{203}S] + K_s}; \quad [R^*] = [R_t] \frac{[S]}{[S] + [^{203}S] + K_s}; \quad [R^{203}] = [R_t] \frac{[^{203}S]}{[S] + [^{203}S] + K_s} \\ [T^*] &= [T_t] \frac{[S']}{[S'] + [^{203}S'] + K_T}; \quad [T^{203}] = [T_t] \frac{[^{203}S']}{[S'] + [^{203}S'] + K_T} \\ [A^*] &= [A_t] \frac{[S]}{[S] + [^{203}S] + K_A + \frac{[S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I} + \frac{[^{203}S]^2}{K_I}} \\ [A^{203}] &= [A_t] \frac{[^{203}S]}{[S] + [^{203}S] + K_A + \frac{[S]^2}{K_I} + 2 \frac{[^{203}S][S]}{K_I} + \frac{[^{203}S]^2}{K_I}}\end{aligned}$$

Protein synthesis rate is scaled to the logistic growth rate variable, γ . The constant $r_{\max}$ is the theoretical maximum growth rate for *E. coli*, i.e. $\log(2)/20$, r is the exponential growth rate, k is the current cell density, and $k_{\max}$ is the maximum possible cell density in this growth medium (i.e. the population ceiling).

$$\zeta = [O_t] \frac{\gamma}{r_{\max}}; \quad \gamma = r \left(1 - \frac{k}{k_{\max}} \right)$$

As $[S_t]$ (the total concentration of cellular Hg) becomes diluted by cell growth, the external volume surrounding an average cell in the population, v_{sp} , will decrease. However, since $v_{sp} \gg v_s$, $[S'_t]$ (the total concentration of external Hg) will simultaneously increase at the rate γ scaled by the relative volumes of the two compartments, v_s/v_{sp} .

Equation 3.16 Example list of initial conditions for the reduced model.

$$[S_t] = 0M; [S'_t] = 10^{-6}M; [A_t] = 1.65 \times 10^{-8}M; [T_t] = 8.02 \times 10^{-8}M; [R_t] = 9.98 \times 10^{-7}M$$

$$v_{sp} = 2.425 \times 10^{-12}L; v_s = 414552 \times 10^{-15}L$$

$$\frac{[O]}{[O_t]} = 3.355 \times 10^{-7}; \frac{[OR]}{[O_t]} = 9.999$$

$$\gamma = r \left(1 - \frac{k}{k_{\max}} \right) = 0.0268 \text{ min}^{-1} \times \left(1 - \frac{0.15}{5.595} \right) = 0.0261 \text{ min}^{-1}$$

$$\zeta = [O_t] \frac{\gamma}{r_{\max}} = \frac{1/6.022 \times 10^{23} \text{ mols}}{414552 \times 10^{-15}L} \times 1.22 \times 3.39 \times \frac{0.0261}{\log(2)/20} = 1.23 \times 10^{-8}M$$

Finally, Equation 3.17 shows the subset of non-radioactive equations in a *mer*(ΔRT)A mutant.

Equation 3.17 Subset of non-radioactive equations in a *mer*(ΔRT)A mutant.

$$\frac{d[S_t]}{dt} = b_{in}[S'] - b_{out}[S] - v \frac{[A_t][S]}{K_A + [S] + \frac{[S]^2}{K_I}} - \gamma[S_t]$$

$$\frac{d[S'_t]}{dt} = (-b_{in}[S'] + b_{out}[S] + \gamma[S'_t]) \frac{v_s}{v_{sp}}$$

$$\frac{d[A_t]}{dt} = \zeta \alpha_{0A} - \gamma[A_t]$$

3.9 Limitations of the experimental data

Most of the experimental data is in a form which is difficult to combine with a mathematical model. Firstly, their experimental designs do not distinguish between multiple, correlated parameters. For example, some key measurements in Hg resistance are not measured directly: $[Hg^{2+}]$ in the cell cytoplasm, membrane and periplasm; the *in vivo* partitioning of MerR into activating and repressing forms; and, the absolute protein concentrations and cell growth rates. Secondly, the reporting of experimental methods often lacks sufficient detail to be repeated faithfully. Finally, experiments do not conform to a set of standardised conditions. One useful set could be: *E. coli* cells + growth in LB medium at 37 °C + *mer* genes from Tn501 or Tn21 on low-copy number plasmids. These factors must necessarily diminish not only the quality of the experimental data, but also of the predictions by any model based on those data.

In general, our existing biological knowledge restricts the number of parameters in the model. Firstly, the model may exclude insignificant details. For example, MerC is not essential for Hg resistance and is not present in Tn501 (Hamlett,

Landale, Davis, and Summers, 1992). Also, the rates of protein degradation are assumed to be negligible, as are the toxic effects from a subtoxic Hg concentration. Information about intermediate reactions steps may also be omitted. For example, the MerR monomer dimerises rapidly and exclusively into a dimer at concentrations $10^{-8} - 10^{-5}$ M (Shewchuk *et al.*, 1989). Thus, *mer* proteins can be modelled as being synthesised directly from the DNA without the intermediate steps of mRNA synthesis and degradation or dimerisation.

Secondly, there are unknown components in the system, which, until proved otherwise, are assumed to be insignificant. For example, the significance and mechanism of MerD co-regulation are unclear. Similarly, it is unknown whether the bioavailability/toxicity of Hg changes as it passes from the growth medium into the chemical environment of the cell. Even the nature of these unknown Hg binding sites must be conceptualised in a general sense. Therefore, each cell has a total concentration of non-*mer* binding sites which is represented in the model as the non-saturating equilibrium: $b_{in}[S'] = b_{out}[S]$.

Thirdly, the literature may lack sufficient data to distinguish between correlated parameters. Since the goal is to create a comprehensive model with the fewest number of parameters possible, one solution is to combine multiple parameters into one process. For example, the data do not distinguish adequately between MerP and MerT or between the uptake of Hg^{2+} into periplasmic, membranar and cytoplasmic compartments. Therefore MerP is combined into MerT, which transports Hg between just two compartments – the external medium and the whole cell. Alternatively, the model may exclude the least significant of two or more processes. For example, there are two hypotheses for the order in which $Hg^{2+} + MerR + DNA$ can bind. However, $MerR-Hg^{2+} + DNA \rightarrow DNA-MerR-Hg^{2+}$ is considered to be the most significant. Finally, MerA transport and non-MerA transport have insufficient experimental data to distinguish them. So, non-MerA transport was chosen in order that transport can still occur in $\Delta merA$ mutants. This decision also separates the roles of MerA and MerT exclusively into volatilisation and transport, respectively. This allows these processes to be modelled independently by simple algebraic forms.

In conclusion, this chapter has described the mathematical model of Hg resistance. The next two chapters will summarise the experimental data and the Metropolis-Hastings algorithm used to estimate distributions of values for the 16 parameters in the model.

Chapter 4: The Experimental Data

This chapter defines the set of experimental conditions that all included experiments must have in common. Experiments which do not meet these criteria or which are not reported in sufficient detail to be repeated accurately are omitted from the simulation. Where possible, further information was obtained from the original research scientists and assumptions were made so that each experimental method should be complete. Appendix A provides full details of these experiments.

4.1 Standardised conditions

Experiments which are included in the simulation meet the following criteria:

- Any strain of *E. coli* cells.
- Rich or minimal medium, or buffer, at 37 °C.
- *mer* genes from Tn501 or Tn21.
- Plasmid copy number can be estimated.
- Experimental methods are described in sufficient detail to be repeated.
- Data are relevant to the parameters in the model.

4.2 Standard format of included data

Full details of the included experiments are provided in Appendix A. These experiments are grouped by research paper and structured in the following format:

- Experimental conditions and methods.
- Measurements of data from original figures.
- Tables of extracted data values.
- Calculations, unit conversions, and standard deviations of data points.
- Comments.

For ease of reading, these 70 experiments are summarised in Table 4.1.

4.3 Estimation of experimental conditions

Where information is missing from the reported experimental methods, these details are generally assumed. Firstly, some assumptions are reasoned from general knowledge. For example, it should take 20 seconds to mix the reactants and take a reading of the initial volatilisation rate.

Table 4.1 Summary of the included literature. Data sets from 70 experiments are grouped by experimental technique (in brackets) and, where applicable, by the combinations of *mer* genes. For example, *merR-T(ΔA)* uptake = non-*mer* import/export + (the induction of) non-MerA transport. Also shown are the bibliographic references for each data set, the numbers of data points (and their standard deviations), the choices of growth medium (MM, minimal medium; RM, rich medium) and plasmid type, whether MerD is present, and the plasmid copy number per genome equivalent (or the DNA concentration, for some *in vitro* experiments). An experiments is classified as *in vitro* when the required chemical components are mixed together in known concentrations in the absence of cells. Therefore, volatilisation assays on cell extracts (data sets 51, 53, 55, 58–63) are not classified as *in vitro* because the unknown MerA concentration depends on the induction of protein synthesis in growing cells.

A. Protein synthesis from P_T – *in vitro* (abortive transcription)

Data set	Reference	Data points	Standard deviations	Plasmid	Growth medium	MerD	Plasmid copies per genome equivalent
1	Ralston and O'Halloran (1990)	26	0.3	Tn501	<i>in vitro</i>	No	0 (0.4e-9 M DNA)
2	Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	2	0.4	Tn501	<i>in vitro</i>	No	0 (0.4e-9 M DNA)

B. Protein synthesis from P_T – control cells (β-galactisidase synthesis, luciferase synthesis)

3	Rouch, Parkhill, and Brown (1995)	15	0.3	Tn501	MM	No	5
4	Figure 5 of Condee and Summers (1992)	15	0.2	Tn21	RM	No	16.8
5	Caguiat, Watson, and Summers (1999)	6	0.3	Tn21	MM	No	23.4
6	Shewchuk, Verdine, Nash, and Walsh (1989)	3	0.3	Tn21	RM	No	23.4
7	Figure 4 of Condee and Summers (1992)	9	0.2	Tn21	RM	No	16.8
8	Hansen and Sorensen (2000)	8	0.2	Tn21	MM	No	1
9	Park, Wireman and Summers (1992)	2	0.1	Tn21	RM	No	23.4
10	Figure 8 of Condee and Summers (1992)	2	0.2	Tn21	RM	No	16.8
11	Ross, Park, and Summers (1989)	4	0.19	Tn21	RM	No	23.4
12	Caslake, Ashraf and Summers (1997)	18	0.35	Tn21	RM	No	23.4

C. Protein synthesis from P_T – *merR-T(ΔA)* (β-galactisidase synthesis)

13	Ni'Bhriain, Silver, and Foster (1983)	1	0.2	Tn21	RM	No	1.3
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D. Protein synthesis from P_T – merR-TA (β-galactisidase synthesis)

Data set	Reference	Data points	Standard deviations	Plasmid	Growth medium	MerD	Plasmid copies per genome equivalent
14	Nucifora, Chu, Silver, and Misra (1989)	4	0.3	Tn21	RM	Yes	23.4+1.3

E. Protein synthesis from P_T – merR-TA (densitometry, enzyme purification)

15	Lee, Gambill and Summers (1989) – [MerT]/[MerA]	1	0.6	Tn21	MM	Yes	16.8
16	Philippidis, Malmberg, Hu, and Schottel (1991) – [MerT/MerA]	1	0.6	Tn21	RM	Yes	1.2
17	Rinderle, Booth, and Williams (1983) – [MerA]	1	0.7	Tn21	RM	Yes	13.2

F. Protein synthesis from P_R – control cells (β-galactisidase synthesis, alkaline phosphatase synthesis)

18	Park, Wireman and Summers (1992)	2	0.1	Tn21	RM	No	16.8
19	Caslake, Ashraf and Summers (1997)	8	0.35	Tn21	RM	No	16.8

G. Protein synthesis from P_R – merR-T(ΔA) (β-galactisidase synthesis)

20	Foster and Brown (1985)	2	0.3	Tn21	RM	No	23.4+1
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H. Protein synthesis from P_R – merR-TA (β-galactisidase synthesis)

21	Foster and Brown (1985)	2	0.3	Tn21	RM	Yes	16.8+23.4
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I. Binding of MerR (and MerR-Hg²⁺) to the DNA – *in vitro* (gel electrophoresis-densitometry)

22	Parkhill, Blair, Hobman, and Brown (1998)	2	0.4	Tn501	<i>in vitro</i>	No	0 (5e-11 M DNA)
23	O'Halloran, Frantz, Shin, Ralston, and Wright (1989)	27	0.22	Tn501	<i>in vitro</i>	No	0 (4e-12 M DNA)
24	Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	20	0.45	Tn501	<i>in vitro</i>	No	0 (5e-12 M DNA)
25	Shewchuk, Verdine, and Walsh (1989)	16	0.3	Tn501	<i>in vitro</i>	No	0 (1e-12 M DNA)

J. Binding of Hg²⁺ to MerR – *in vitro* (gel filtration, equilibrium ultrafiltration, free dialysis)

26	Zeng, Stålhandske, Anderson, Scott, and Summers (1998)	6	0.3	Tn21	<i>in vitro</i>	No	0
27	Song <i>et al.</i> (2004)	4	0.1, 0.067	Tn21	<i>in vitro</i>	No	0
28	Shewchuk <i>et al.</i> (1989)	6	0.3	Tn21	<i>in vitro</i>	No	0

K. Uptake of Hg²⁺ – control cells (atomic absorption spectroscopy, scintillation spectroscopy)

Data set	Reference	Data points	Standard deviations	Plasmid	Growth medium	MerD	Plasmid copies per genome equivalent
29	Sone, Pan-Hou, Nakamura, Sakabe, and Kiyono (2010)	3	0.1	NA	RM	No	0
30	Hamlett, Landale, Davis, and Summers (1992)	12	0.35	NA	RM	No	0
31	Bae, Mehra, Mulchandani, and Chen (2001)	3	0.2	NA	RM	No	0
32	Kiyono, Sone, Nakamura, Pan-Hou, and Sakabe (2009)	3	0.1	NA	RM	No	0
33	Nakahara, Silver, Miki, and Rownd (1979)	7	0.6	NA	RM	No	0
34	Foster, Nakahara, Weiss, and Silver (1979)	6	0.35	NA	RM	No	0
35	Bae, Wu, Kostal, Mulchandani, and Chen (2003)	1	0.4	NA	RM	No	0
36	Kiyono, Omura, Fujimori, and Pan-Hou (1995)	4	0.1	NA	RM	No	0
37	Kiyono and Pan-Hou (1999)	4	0.1	NA	RM	No	0
38	Pan-Hou, Kiyono, Kawase, Omura, and Endo (2001)	1	0.1	NA	RM	No	0
39	Ni'Bhriain, Silver, and Foster (1983)	24	0.35	NA	RM	No	0
40	Hsieh, Chen, Chang, Endo, and Huang (2007)	2	0.1	NA	Buffer	No	0
41	Sone, Nakamura, Pan-Hou, Itoh, and Kiyono (2013)	1	0.3	NA	RM	No	0
42	Inoue, Kusano, and Silver (1996)	6	0.4	NA	RM	No	0
43	Nagata, Kiyono and Pan-Hou (2006)	1	0.1	NA	RM	No	0
44	Song <i>et al.</i> (2004)	6	0.29	NA	RM	No	0

L. Uptake of Hg²⁺ – *merR-T(ΔA)* (scintillation spectroscopy)

45	Hamlett, Landale, Davis, and Summers (1992)	12	0.35	Tn21	RM	No	16.8
46	Nakahara, Silver, Miki, and Rownd (1979) – induction	5	0.4	Tn21	RM	No	13.2
47	Foster, Nakahara, Weiss, and Silver (1979)	20	0.35	Tn21	RM	No	1.3
48	Nakahara, Silver, Miki, and Rownd (1979)	14	0.6	Tn21	RM	No	13.2
49	Ni'Bhriain, Silver, and Foster (1983)	24	0.35	Tn21	RM	No	8

M. Uptake of Hg²⁺ – *mer(ΔR)-TA* (scintillation spectroscopy)

50	Foster, Nakahara, Weiss, and Silver (1979)	20	0.35	Tn21	RM	Yes	1.3
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N. Volatilisation of Hg²⁺ – *merR-TA* (scintillation spectroscopy)

Data set	Reference	Data points	Standard deviations	Plasmid	Growth medium	MerD	Plasmid copies per genome equivalent
51	Philippidis, Malmberg, Hu, and Schottel (1991)	21	0.1	Tn21	MM	Yes	1.22
52	Wilson, Leang, Morby, Hobman and Brown (2000); Schue, Glendinning, Hobman, and Brown (2008)	22	0.3	Tn501	MM	Yes	23.4
53	Nakahara <i>et al.</i> (1979) – K _A = 9e-6 M, cell extracts	1	0.3	Tn21	RM	Yes	13.2
54	Foster, Nakahara, Weiss, and Silver (1979) – resistant and micro-constitutive cells	13	0.3	Tn21	RM	Yes	1.3
55	Nakahara, Silver, Miki, and Rownd (1979) – induction	6	0.3	Tn21	RM	Yes	13.2
56	Laddaga, Chu, Misra, and Silver (1987)	8	0.2	Tn21	RM	Yes	23.4
57	Figure 4 of Ni'Bhriain, Silver, and Foster (1983) – induction	15	0.3	Tn21	RM	Yes	23.4
58	Philippidis, Malmberg, Hu, and Shottel (1991) – K _T = 3.6e-6 M	1	0.3	Tn21	MM	Yes	31.8
59	Philippidis, Malmberg, Hu, and Shottel (1991) – K _T = 3.8e-6 M	1	0.3	Tn21	MM	Yes	1.22
60	Philippidis, Malmberg, Hu, and Shottel (1991) – K _T = 4.7e-6 M	1	0.3	Tn21	MM	Yes	27.3
61	Philippidis, Malmberg, Hu, and Shottel (1991) – K _T = 5.1e-6 M	1	0.3	Tn21	MM	Yes	50.6
62	Tables 3 and 7 and Figure 4 of Ni'Bhriain, Silver, and Foster (1983) – resistant whole cells and extracts, and one $\Delta merR$ cell extract	11	0.3	Tn21	RM	Yes	1.3, 13.2, 23.4, 8
63	Philippidis, Malmberg, Hu, and Shottel (1991) – K _T = 6.0e-6 M	1	0.3	Tn21	MM	Yes	57.1

O. Volatilisation of Hg²⁺ – *merR-(ΔT)A* (scintillation spectroscopy)

64	Wilson, Leang, Morby, Hobman and Brown (2000)	4	0.3	Tn501	MM	Yes	23.4
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P. Volatilisation of Hg²⁺ – *in vitro* (scintillation spectroscopy)

65	Fox and Walsh (1982) – v = 800 min ⁻¹	1	0.3	Tn501	<i>in vitro</i>	No	0
66	Moore, Miller, and Walsh (1992) – v = 810 min ⁻¹	1	0.3	Tn501	<i>in vitro</i>	No	0
67	Moore, Distefano, Zydowsky, Cummings, and Walsh (1990) – v = 340 min ⁻¹	1	0.3	Tn501	<i>in vitro</i>	No	0
68	Fox and Walsh (1982) – K _A = 12e-6 M	1	0.3	Tn501	<i>in vitro</i>	No	0
69	Moore, Distefano, Zydowsky, Cummings, and Walsh (1990) – K _A = 5e-6 M	1	0.3	Tn501	<i>in vitro</i>	No	0
70	Moore, Miller, and Walsh (1992) – K _A = 5e-6 M	1	0.3	Tn501	<i>in vitro</i>	No	0

Secondly, methods are sometimes referenced from a second paper or inferred from similar experiments by the same scientists. For example, Hamlett, Landale, Davis, and Summers (1992) refer to Nakahara, Silver, Miki, and Rownd (1979) for methods of Hg binding. Therefore, all the experimental conditions, including the growth curves, are applied.

Thirdly, cell mass, cell volume, genome equivalents per cell, and optical density are estimated continuously in the simulation from the growth rate (Figure 4.1).

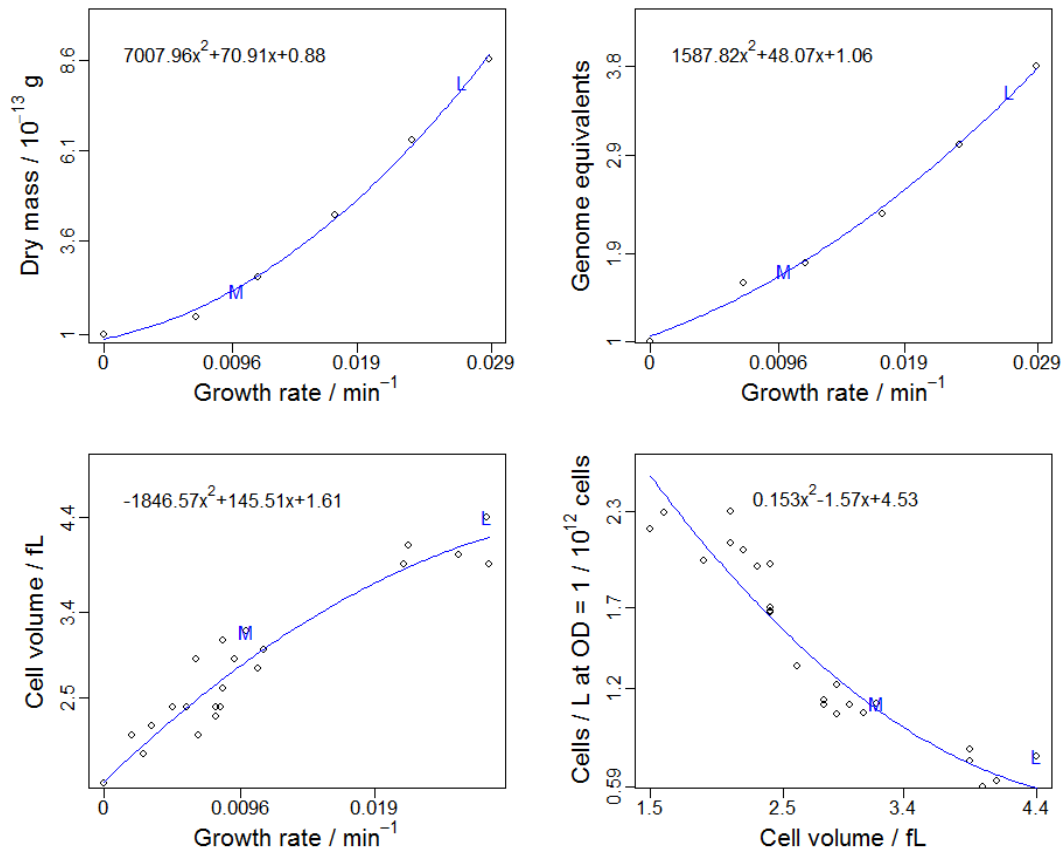


Figure 4.1 Estimation of *E. coli* constants. Given the cell growth rate, values for dry cell mass (Top left), cell volume (Bottom left), genome equivalents per cell (Top right) and cell concentration (Bottom right) are inferred (Volkmer and Heinemann, 2011; Bremer and Dennis, 1996). Values for exponential growth in Luria-Bertani (L) and minimal media (M) and lines of best fit are shown in blue. Since the average mass of an *E. coli* protein is 40 kDa (Savageau, 1986), the protein concentration in exponentially growing cells in LB is $(4.5e-13 \text{ g}/40000 \text{ Da})/4.4e-15 \text{ L} = 2.56e-3 \text{ M}$. Similarly, the DNA concentration is $3.54 \text{ genome equivalents} \times (1/6.022e23 \text{ mols})/4.4e-15 \text{ L} = 1.34e-9 \text{ M}$.

Each type of growth medium is classified as either rich (RM) or minimal (MM) and the logistic growth for each is defined by Figure 4.2. For simplicity, cells which are transferred from one type of medium into another (or into buffer) while experimental measurements are being taken are assumed to grow at their previous rates. However, if chloramphenicol, tetracycline or rifampin is added to the assay mixture, growth and synthesis rates are set to zero.

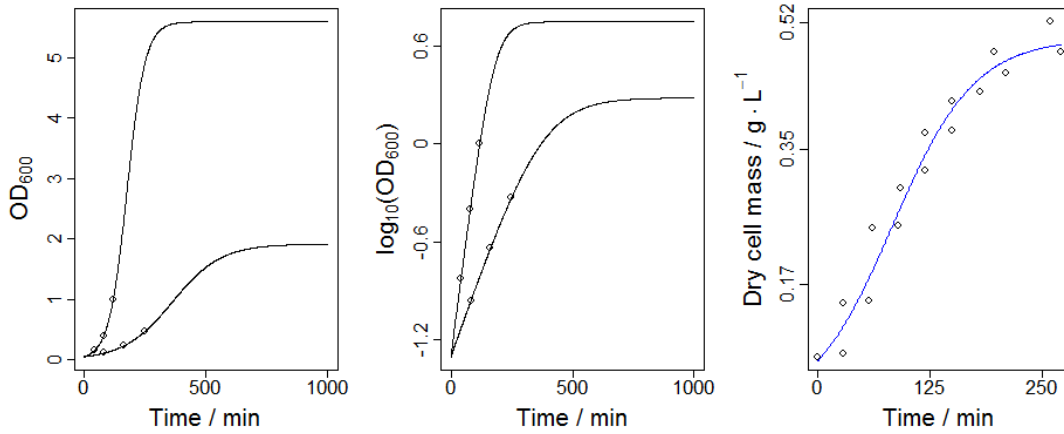


Figure 4.2 Logistic growth curves. The growth of *E. coli* in rich (RM) or minimal medium (MM) is defined. In RM, early-, mid- and late log-phases are $OD_{600} = 0.15, 0.4, 1.0$, respectively. In MM, these values are $OD_{600} = 0.11, 0.23, 0.47$. Exponential growth rates are $r = 0.0268 \text{ min}^{-1}$ (RM) and 0.01 min^{-1} (MM) (Volkmer and Heinemann, 2011). Population ceilings, k_{max} , for overnight cultures grown to stationary phase are $OD_{600} = 5.595$ (RM) and $OD_{600} = 1.915$ (MM) (Tao, Bausch, Richmond, Blattner, and Conway, 1999; Sezonov, Joseleau-Petit, and D'Ari, 2007; Thakur, Brown, Sama, Jackson, and Dayie, 2010). (Left and Middle) Cells grown from $OD_{600} = 0.05$ through early-, mid-, and late log-phases (circles). (Right) The fit to Figure 1 of Nakahara, Silver, Miki and Rownd (1979) after converting from Klett units to dry cell mass as stated in their paper: $0.17 \text{ g (dry weight) cells/L} = 75 \text{ Klett units}$. Similarly, 75 Klett is $OD_{550} = 0.5$ (Hamlett, Landale, Davis, and Summers, 1992).

Finally, the number of copies per genome equivalent of each *mer* plasmid is assumed from Table 4.2 using average values from multiple references. The experiments which are omitted from the simulation are described in Table 4.3.

Table 4.2 Plasmid copy numbers. The total concentration of *mer* promoters in the cell is $(1/6.022 \times 10^{23}) / (\text{cell volume}) \times \text{genome equivalents per cell} \times \text{plasmid copies per genome equivalent}$. However, when the number of genome equivalents is not reported, it is estimated from the rates of exponential growth in minimal medium (Figure 4.2). Therefore, values of plasmid copies per cell are converted to plasmid copies per genome equivalent by dividing by 1.72 (underlined values).

Plasmids (and derivatives)	Copies per genome equivalent	References
pBR322 (pWR2/10, pSJ51, pRB28, pDU1003, pJOE114)	$(\underline{25/1.72} + 78/2.45 + \underline{57/1.72} + 67/2.45 + \underline{17.5/1.72})/5 = 23.4$	Ni'Bhriain, Silver, and Foster (1983); Philippidis, Malmberg, Hu, and Shottel (1991); Chambers, Prior, Barstow, and Minton (1988); Sambrook, Fritsch, and Maniatis (1989); Morby, Hobman, and Brown (1985).
pACYC184 (pSA1, pSJ43), pACYC177 (pDG106, pCC206/207, pNH9)	$(18 + 15/3.54 + 16/3.54 + 124/2.45 + \underline{11/1.72})/5 = 16.8$	Chang and Cohen (1978); Hiszczynska-Sawicka and Kur (1997); Philippidis, Malmberg, Hu, and Shottel (1991); Sambrook, Fritsch, and Maniatis (1989).
ColE1 (pRR130/132/134, pNH6)	$(1.3 \times 6.5 + 18)/2 = 13.2$	Nakahara <i>et al.</i> (1979); Cabello, Timmis, and Cohen (1976).
NR1 (R100, pDU202, pDU1161)	$(1.4 + 3/2.45)/2 = 1.3$	Taylor, Greenberg, and Rownd (1977); Philippidis, Malmberg, Hu, and Shottel (1991).
pUB5572 (pRZH-18)	8	Ni'Bhriain, Silver, and Foster (1983).
pRZ5255	7	Lambert and Reznikoff (1985).
pUC9	$\underline{600/1.72} = 348.8$	Chambers, Prior, Barstow, and Minton (1988); Sambrook, Fritsch, and Maniatis (1989).
pUC19	$140/2.45 = 57.1$	Philippidis, Malmberg, Hu, and Shottel (1991).

Table 4.3 Excluded literature. Experiments from these papers are omitted from the simulation due to insufficient methodology, non-standardised experimental conditions, or for providing information about parameters which are not relevant to the mathematical model.

Paper	Figure/table	Reason for exclusion
Bae, Mehra, Mulchandani, and Chen (2001)	Figure 3: Bioaccumulation of Hg^{2+} after 1 hour.	Time-course data from Figure 4 are included instead which saturate at an order of magnitude greater.
Foster and Ginnity (1985)	Table 2: β -galactosidase activities.	Mixtures of multicopy number plasmids.
Hamlett, Landale, Davis, and Summers (1992)	Table 2: Volatilisation from whole cells. Table 4: Volatilisation from cell extracts. Figure 7: Induction of β -galactosidase.	Not known whether assays were performed on whole cells or cell extracts. In Table 2, the value from cell suspensions for pNH6 is 5.69 units whereas, in Table 4, the value from cell extracts for pNH6 is almost identical at 5.8 units. Post-doc did not calibrate equipment correctly resulting in negative values for β -galactosidase activities (Hamlett, personal communication).
Hansen and Sorensen (2000)	Figure 2: GFP and luciferase activities.	Conducted at 30 °C and room temperature, respectively.
Huang <i>et al.</i> (2003)	Figure 1a: Adsorption isotherm for Hg^{2+} in <i>E. coli</i> BL21 (DE3).	No parameter in model for saturation of binding sites. Also, extreme Hg^{2+} concentrations equilibrating up to 3 mM in the external media are highly toxic to the cell.
Kiyono, Uno, Omura, and Pan-Hou (2000)	Figures 1 and 5: Uptake.	Interpretation of y-axis scale of " $\times 10^{-3}$ cpm / 2×10^9 cells" gives unrealistic values of bound Hg^{2+} .
Kusano, Inoue, and Silver (1990)	Figure 4: Volatilisation from whole cells with <i>Thiobacillus</i> MerA only.	Since the kinetics of the <i>Thiobacillus merA</i> gene can be estimated from their data on cell-free extracts, in the absence of a transport system, these data would measure non- <i>mer</i> uptake. However, the data presented in Figure 4 are misleading since cells with the control vector (pUC18) appear to volatilise more $^{203}\text{Hg}^{2+}$ than with pUC18 + <i>merA</i> .
Kusano, Ji, Inoue, and Silver (1990)	Figures 5 and 7a: Whole cell binding by control cells.	Cell densities are unknown. Data are highly variable and much lower in magnitude than in similar papers.
Lee, Livrelli, Park, Totis, and Summers (1993)	Figure 1: β -galactosidase and Alkaline phosphatase activities.	No experimental details.
Livrelli, Lee, and Summers (1993)	Figure 3: β -galactosidase activities.	No experimental details.
Lund and Brown (1987)	Table 2: Volatilisation from cell extracts.	Insufficient experimental details.

Paper	Figure/table	Reason for exclusion
Lund and Brown (1987) Lund and Brown (1989)	Tables 4 and 5: β -galactosidase synthesis. Figure 2: β -galactosidase synthesis.	Cell concentrations are unknown. For plasmid pAC-EH-3, the <i>merR</i> gene is over-expressed from the chloramphenicol acetyltransferase promoter of pACYC184 which means the relationship between [MerR] and [MerA] is not the same as in wild-type <i>mer</i> .
Lund, Ford, and Brown (1986)	Figure 3: β -galactosidase synthesis. Table 2 and 3: β -galactosidase synthesis.	Induction time unknown. Mixtures of multicopy number plasmids.
Nakahara <i>et al.</i> (1979)	Figure 1: Volatilisation from whole and broken cells.	Cell concentrations are unknown.
Nakahara, Silver, Miki, and Rownd (1979)	Figure 3b: Saturation of Hg^{2+} binding sites.	No parameter in model for saturation of Hg binding sites.
Ni'Bhriain, Silver, and Foster (1983)	Table 5: β -galactosidase synthesis (except for plasmid pDU1124). Table 7: β -galactosidase synthesis and volatilisation from cell extracts (except for volatilisation with pDU1003 <i>merR25::Tn5</i>).	Mixtures of multicopy number plasmids. Significant polarity effects for <i>mer</i> genes distal of the Tn5 insertion.
Nucifora, Silver, and Misra (1989)	Figure 3b: β -galactosidase synthesis.	Induction time is unknown.
Park, Wireman, and Summers (1992)	Table 3: Alkaline phosphatase synthesis from P_R . Table 4: Gel retardation assay for MerR-operator binding.	No parameter in model. DNA concentration is unknown.
Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	Figures 2a and 2b: β -galactosidase synthesis. Table 2: Affinity of MerR for Hg^{2+} .	For plasmid pAC-EH-3, MerR is over-expressed at an unknown rate from the chloramphenicol acetyltransferase promoter of pACYC184. No parameter in model.
Parkhill, Blair, Hobman, and Brown (1998)	Figure 2: β -galactosidase synthesis.	MerR is over-expressed from the chloramphenicol acetyltransferase promoter of pACYC184.
Parkhill and Brown (1990)	Figure 3: Association of MerR for promoter. Figure 2: β -galactosidase synthesis.	No parameter in model; association and dissociation constants are combined into the equilibrium dissociation constant, K_e . For plasmid pAC-EH-3, MerR is over-expressed from chloramphenicol acetyltransferase promoter of pACYC184.
Philippidis, Schottel, and Hu (1990); Philippidis, Malmberg, Hu, and Shottel (1991)	Figures 1, 2 and 3: Reduction of Hg^{2+} by intact and permeabilised cells with high copy number plasmids (67–140 copies per cell). Table 2: Relative synthesis of MerT and MerA with increasing plasmid copy number.	Only the data for R100 (3 copies per cell) may be absent from copy number effects. The underlying cause(s) of these effects are not satisfactorily understood and are difficult to model without known MerA and MerT/P (membrane) concentrations.

Paper	Figure/table	Reason for exclusion
Ross, Park and Summers (1989)	Tables 2, 6 and 7: β -galactosidase synthesis.	Their normalisation process is too complicated. So only actual β -galactosidase activities from Table 5 are used.
Rouch, Parkhill, and Brown (1995)	Figure 1c: Luciferase synthesis from <i>merR</i> -o/p- <i>TPAD-lux</i> .	Copy number of pUCD615 is unknown.
Sahlman and Skarfstad (1993)	Figure 1: Binding of Hg^{2+} by MerP.	No parameter in model.
Shewchuk <i>et al.</i> (1989)	Figure 2: Dimerisation of MerR.	No parameter in model.
Shewchuk, Verdine, Nash, and Walsh (1989)	Table 2: β -galactosidase synthesis with $10\text{e-}6\text{ M Hg}^{2+}$. Figure 2b: Dimerisation of MerR.	In wild-type and in ΔmerR , β -galactosidase values with $10\text{e-}6\text{ M Hg}^{2+}$ are lower than with $1\text{e-}6\text{ M Hg}^{2+}$ by 15-25 %. This indicates inhibition of protein synthesis by toxic Hg. No parameter in model.
Shewchuk, Verdine, and Walsh (1989)	Figure 4: Dissociation of MerR from operator.	No parameter in model.
Zeng, Stålhandske, Anderson, Scott, and Summers (1989)	Table 2: K_{off} for MerR- Hg^{2+} complex.	No parameter in model.

Chapter 5: Parameter estimation

Each experiment from Table 4.1 provides information about a subset of parameters in the model. For example, a volatilisation assay provides information about the binding of MerA to Hg^{2+} (K_A) and about the maximum rate of reduction of Hg^{2+} into Hg^0 (v). The computer simulation repeats these biological experiments with the same initial concentrations and methods as the original research paper. However, each time these experiments are simulated, the parameters in the model take different values. Therefore, after multiple simulations, the set of parameter values will be found which gives the closest overall fit of the simulated output through the experimental data points.

5.1 The computer simulation

The purpose of the computer simulation is to replicate the biological experiments which created the experimental data. This is achieved by choosing the relevant subset of parameters in the model, the initial concentrations of each entity, and the simulation time. These equations are then solved numerically by an ODE solver to produce a simulated output for each set of parameter values. The CVODE (Sundials) solver in C++ was chosen with the BDF method and Newton Iteration (Hindmarsh *et al.*, 2005) and each experiment was run as a separate thread (using pthread.h).

The simulated output is compared to the experimental data for its closeness of fit. This process is similar to least squares, i.e. fitting a curve (the simulated output) through a fixed set of coordinates (the experimental data). However, instead of calculating the sum of the squares of the differences between the two sets of values, this closeness of fit is found by a probability density function. This approach has the following advantages:

- To integrate experimental data and prior knowledge. Experimental data can be the combined result of multiple parameter interactions, e.g. *in vitro* MerA synthesis requires: K_e , K_{es} , K_S , a_{0A} , a_1 , and a_2 . In contrast, each prior distribution integrates previous knowledge about one parameter.
- To give a weighting to each data point (i.e. its standard deviation) which accounts for the actual (or estimated) variation associated with the experimental technique used to create it.
- To provide a posterior probability distribution of parameter values rather than a single set which gives the closest fit.

5.2 The posterior probability

The posterior, $P(\theta|D)$, is the probability that the parameter values, θ , are true given the data, D (Equation 5.1). It is the product of the likelihood, $P(D|\theta)$, which is the closeness of fit between the simulated output and the experimental data (Figure 5.1), and the prior, $P(\theta)$, which is the closeness of fit between each parameter value and its prior distribution. When taking the ratio of the proposed (ϕ) and current (θ) posterior probabilities, the normalising constant, $P(D)$, cancels. This leaves $P(D|.)$ and $P(.)$ which are calculated in the Metropolis-Hastings algorithm.

Equation 5.1 The posterior probability.

$$P(\theta|D) = \frac{P(D|\theta)P(\theta)}{P(D)}$$

In practice, all equations are converted into logarithmic forms. This ensures that parameter values remain positive and that the results of calculations are not too small or large for the computer to store in memory. Therefore, log-likelihoods are added to log-priors to give the log-posteriors for each set of parameter values.

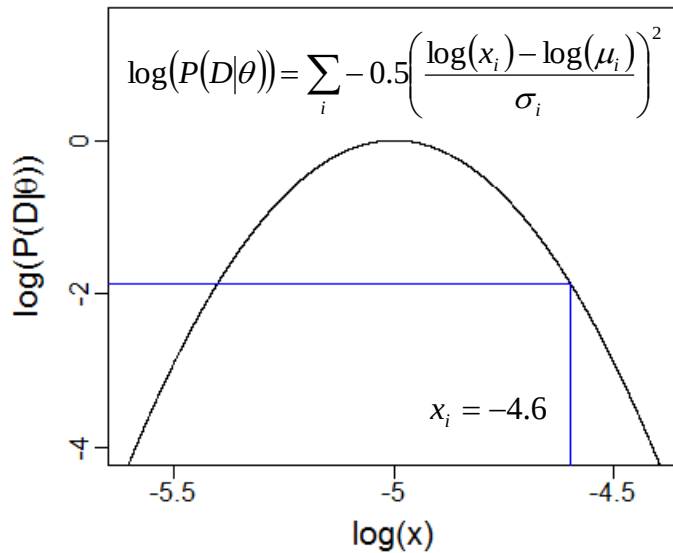


Figure 5.1 Calculating the log-likelihood. An experimental data point is modelled as a log-normal distribution with $\log(\mu_i) = -5.0$, and $\sigma_i = \sqrt{(\log(0.3)^2 + 1)} = 0.29$. For example, μ_i could represent a protein concentration at 10 minutes. The (weighted) closeness of fit between x_i (the simulated output) and μ_i is calculated by a log-likelihood function, i.e. $\log(P(D|\theta))$. For example, the log-density of $\log(x_i) = -4.6$ is $\log(P(D|\theta)) = -1.9$ on the above distribution. Therefore, the closer the fit between x_i and μ_i , the higher is $\log(P(D|\theta))$.

5.3 The Metropolis-Hastings algorithm

This Metropolis-Hastings algorithm uses random (Gaussian) numbers to sample the state space of an unknown function. For example, a state space may consist of all possible pairs of values of the parameters a and b (Figure 5.2).

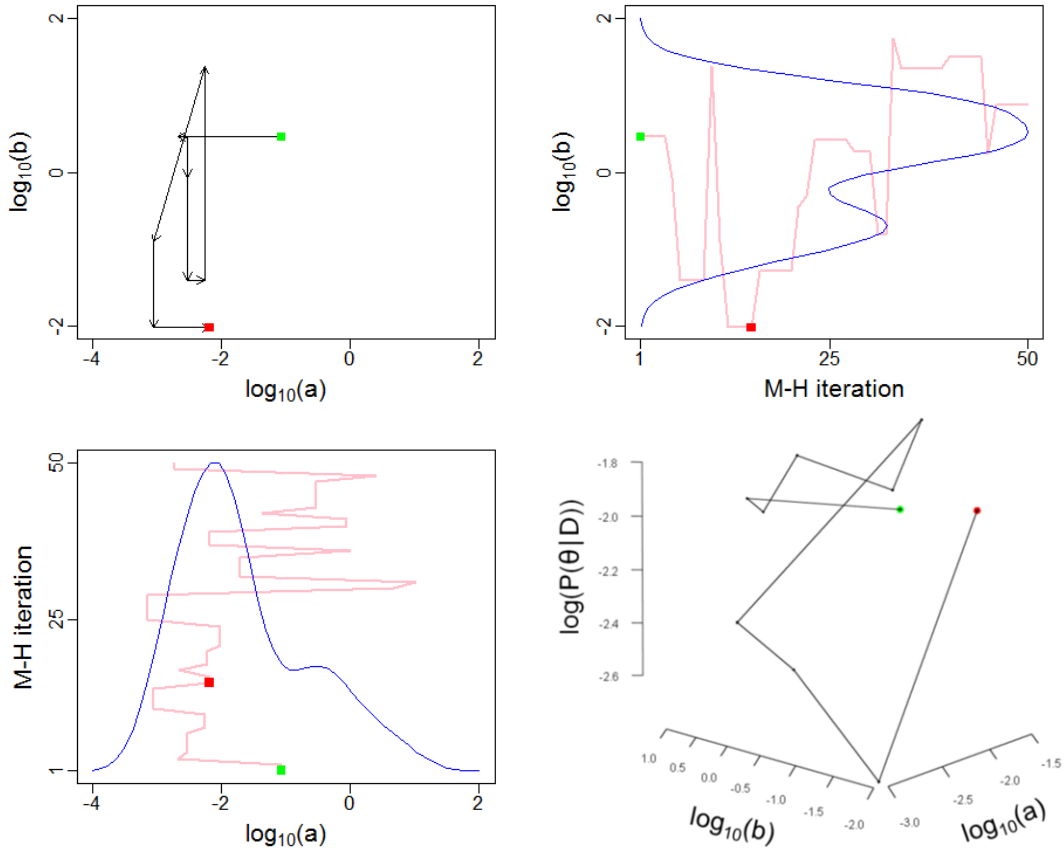


Figure 5.2 Sampling the state space of the parameters a and b with the Metropolis-Hastings algorithm. (Top left) Arrows show the path of the random walk of 15 outer iterations, which begins at the green square and ends on the red square. Notice, there are not 15 distinct points; a move is only accepted in approximately 1 out of every 4 proposed moves. (Bottom right) A 3D view showing that the random walk spends more time in regions of higher posterior probability. (Top right and bottom left) The same random walk (pink line) is extended to 50 outer iterations. The posterior distributions for each parameter are shown in blue.

Starting with the current parameter values, $\theta = (a_n, b_n)$, a new set of values, $\varphi = (a_{n+1}, b_n)$, is generated from the proposal distributions for each parameter. Each proposal distribution has a standard deviation which is tuned so that moves to (a_{n+1}, b_n) , are accepted with a probability of 0.19 – 0.35.

The decision to move from (a_n, b_n) to (a_{n+1}, b_n) occurs by comparing the current posterior probability, $P(D|\theta)$, to that of the proposed parameter pair, $P(D|\phi)$. If $P(D|\phi) > P(D|\theta)$, the move is always accepted. However, to ensure the random walk explores regions of lower probability, if $P(D|\phi) < P(D|\theta)$, a move is accepted if a uniform random number between 0 and 1 is less than $\alpha = P(D|\phi)/P(D|\theta)$. Thus, an $\alpha < 1$ but nearer to 1 will be accepted more frequently than an $\alpha < 1$, but nearer to 0.

An acceptance or rejection of a move is one (inner) iteration of the algorithm. Therefore, to minimise the effects of random chance, a simulation usually consists of $>10^5$ iterations which together record the course of a random walk. This walk will sample multiple regions of the state space but will spend more time in regions of higher posterior probability. Therefore by plotting each parameter (value) against density, one is able to visualise the posterior distribution which represents the previously unknown function of the state space.

Chapter 6: Computer simulation

There are four objectives for this chapter. The first is to find values for the parameters in the model which give the closest fit of the simulated output through the experimental data points. Secondly, these data sets are analysed for any correlations between closeness of fit and experimental technique, *mer* gene combination, plasmid type, growth medium, or the presence of MerD. Thirdly, once all our existing biological knowledge has been successfully integrated into a model of Hg resistance, this model will predict:

- The absolute concentrations of *mer* proteins in cells of each Hg phenotype in the presence and absence of Hg.
- The dynamics of every reaction in the model including the pairs of $[S]$ and $[S']$ for which uptake and volatilisation rates are balanced.
- That plasmid copy number effects occur by an unknown mechanism which is not captured in the model.

Finally, nine experiments are designed which create data about every parameter in the model under standardised conditions.

6.1 The first simulation – including all experiments

Table 6.1 lists the set of parameter values which give the closest fit of the first simulated output (x_{all}) through the 504 data points of 70 experiments. This fit corresponds to a maximum log-posterior of $\log(P(D|\theta))_{max} = -7336.92$.

These provisional results suggest that the rate constant for non-*mer* import (b_{in}) is on the same order of magnitude as the for non-MerA transport (k_{t2p}). In addition, the rate constant for non-*mer* export (b_{out}) is two orders of magnitude lower than that for volatilisation (v). The binding of Hg^{2+} by MerR (K_S) is four orders of magnitude stronger than by MerA (K_A), and MerR (K_e) binds the DNA 454 times more strongly than does MerR- Hg^{2+} (K_{es}). MerT synthesis is always 4.57 times greater than that of MerA ($a_{0T/0A}$) and synthesis from DNA-MerR (a_1 , a_r) is always lower than the respective synthesis from the naked DNA (a_{0T} , a_{0A} , a_{0R}). Finally, fully activated synthesis of MerT from DNA-MerR- Hg^{2+} , with rate $(a_{0T} \cdot a_2) \cdot [O_T]$, is 210 times higher than its synthesis from the naked DNA (or 469 times higher than its fully repressed synthesis from DNA-MerR with rate $(a_{0T} \cdot a_1) \cdot [O_t]$).

Table 6.1 Values from a simulation including all experiments. These parameter values give the closest fit of the simulated output through the 504 data points of 70 experiments. Also shown are the means and standard deviations (log-scale) of the posterior distributions and the standard deviations (log-scale) of the proposal distributions.

Parameter	Units	Closest fit value	Mean	Standard deviation	Standard deviations of proposal distribution
b_{in}	min^{-1}	2.54e1	2.54e1	6.56e-2	6.82e-2
b_{out}	min^{-1}	2.08	2.07	4.94e-2	7.90e-2
K_S	M	1.15e-9	1.07e-9	2.49e-1	1.66e-1
k_{t2p}	min^{-1}	1.92e1	2.05e1	4.18e-1	9.21e-2
K_A	M	2.97e-5	2.87e-5	9.59e-2	1.38e-1
v	min^{-1}	5.95e2	6.88e2	1.65e-1	1.29e-1
K_e	M	4.53e-13	4.07e-13	2.76e-1	2.86e-1
K_{es}	M	2.06e-10	2.02e-10	1.04e-1	2.03e-1
a_{0A}	min^{-1}	1.13	9.80e-1	1.83e-1	8.29e-2
$a_{0T/OA}$	-	4.57	5.87	3.92e-1	1.20e-1
$a_{0R/OA}$	-	3.48e2	4.09e2	2.16e-1	9.21e-2
a_1	-	4.48e-1	4.52e-1	3.99e-2	1.13e-1
a_2	-	2.10e2	2.14e2	4.78e-2	1.20e-1
a_R	-	3.09e-2	3.12e-2	5.71e-2	1.11e-1
K_T	M	1.25e-6	1.24e-6	8.96e-2	1.20e-1
K_I	M	1.95e-4	2.12e-4	2.14e-1	5.62e-1

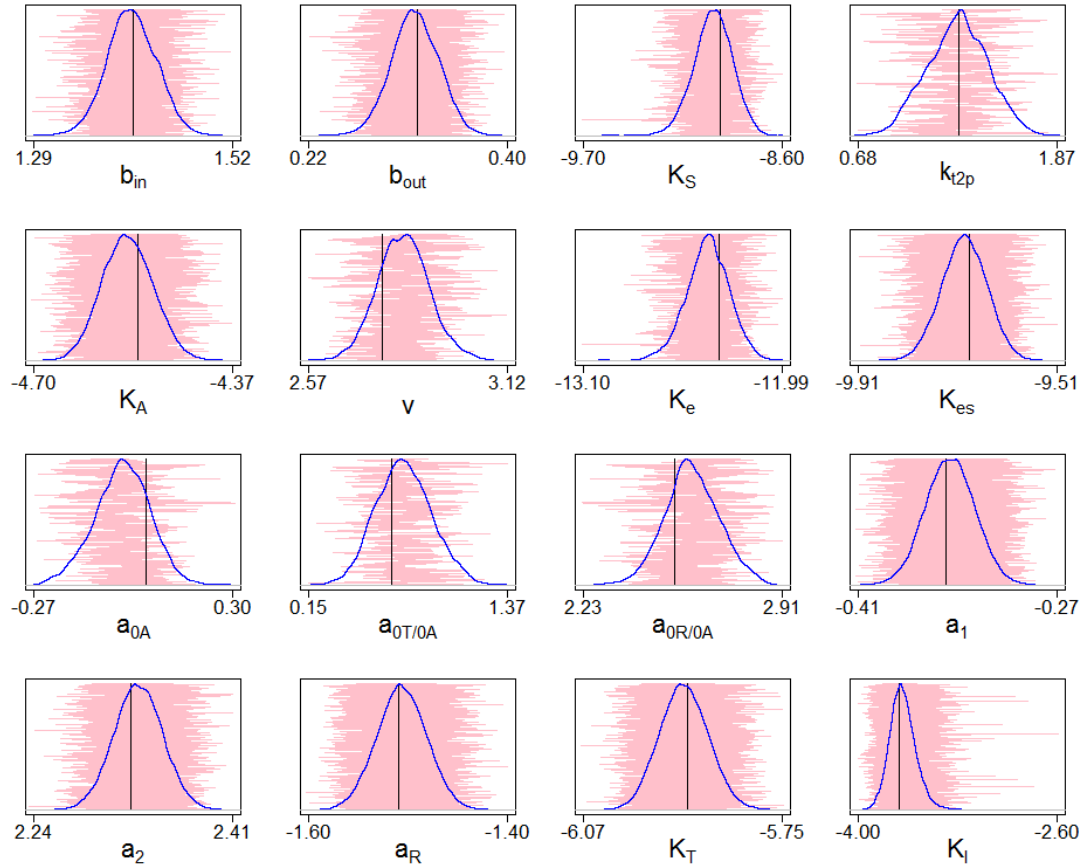


Figure 6.1 Posterior distributions from a simulation including all experiments. For each parameter, the posterior distributions (blue), random walks of 3105349×16 iterations (pink), and closest-fit values from Table 6.1 (black lines) are plotted on a $\log_{10}$ scale.

The first simulation requires 3105349×16 iterations for the random walks of all 16 parameters to converge on log-posterior distributions (Figure 6.1). Several random walks are correlated which means the experimental data only give information about the combined effects of their respective parameters. For example, a_{0A} and v are inversely correlated. In experiments measuring v , the concentration of MerA is never fixed. Therefore the value of a_{0A} in the simulation, which determines the MerA concentration, can decrease as v increases (and vice versa) to produce the same rate of volatilisation $= v \cdot [A_t] \cdot [S] / ([S] + K_A + [S]^2 / K_I)$. Similarly, non-MerA transport, non-mer import and export are correlated, as are k_{t2p} and $a_{0T/0A}$. These parameter correlations always indicate there are too many parameters in the model for the existing data to inform. However, this situation can result as much from a need for improved experimental design as it does from a model which tries to capture more than the essential components of a system.

6.2 Comparing the simulated output and experimental data

The average fold-distance, $D(x, \mu)$, is defined as the closeness of fit between x (the simulated output) and μ (the experimental data) (Equation 6.1). This alternative to the log-likelihood is more intuitive and allows the fits of different data sets to be compared fairly without being weighted by a standard deviation.

Equation 6.1 The proximity of the simulated output and experimental data. The function $D(x, \mu)$ is defined as the mean of the non-weighted fold-distances between pairs of values of the simulated output (x) and the experimental data (μ) (Figure 6.2). Using $\log(x_n/\mu_n)^2 = \log(\mu_n/x_n)^2$, the log-likelihood, $\log(P(D|\theta))$, is rearranged to give the fold-ratio of one simulated output point (x_n) to its corresponding experimental data point (μ_n). Then, $D(x, \mu) = 10$ is chosen so that data sets which are, on average, 10-fold smaller (or greater) than x will be excluded from subsequent simulations.

$$\log(P(D|\theta)) = -0.5 \times \left(\frac{\log(x_n) - \log(\mu_n)}{\sigma} \right)^2 \rightarrow e^{\sigma \sqrt{\frac{\log(P(D|\theta))}{-0.5}}} = \frac{x_n}{\mu_n}$$

$$\mu_1 = \mu_2 = 1; \quad \frac{x_1}{\mu_1} = \frac{1}{10}; \quad \frac{x_2}{\mu_2} = 10; \quad \sigma = 0.3$$

$$-0.5 \times \left(\frac{\log(\mu_1/10) - \log(\mu_1)}{\sigma} \right)^2 = -0.5 \times \left(\frac{\log(10\mu_2) - \log(\mu_2)}{\sigma} \right)^2 = -29.45$$

$$D(x, \mu) = \frac{\frac{\mu_1}{x_1} + \frac{x_2}{\mu_2}}{2} = \frac{e^{\sigma \sqrt{\frac{\log(P(D_1|\theta))}{-0.5}}} + e^{\sigma \sqrt{\frac{\log(P(D_2|\theta))}{-0.5}}}}{2} = 10$$

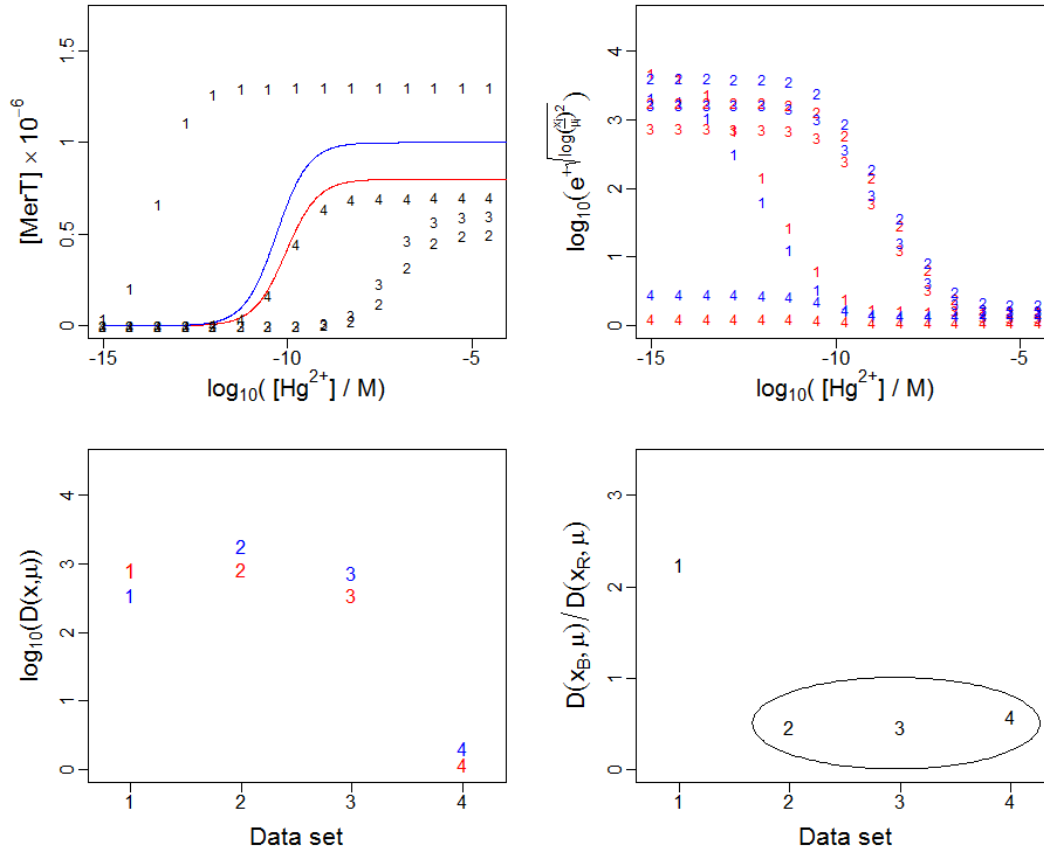


Figure 6.2 The proximity of the the simulated output and experimental data.

(Top left) The simulated outputs, x (red, R; blue, B), and the data sets, μ (1, 2, 3, and 4). (Top right) The fold-distances between each individual x_n and μ_n . (Bottom left) The average fold-distances, $D(x, \mu)$, between the red and blue simulated outputs and each data set. (Bottom right) The ratio of the average fold-distances, $D(x_B, \mu) / D(x_R, \mu)$, shows that data sets 2, 3, and 4 form a group separate from data set 1.

Of all the data sets included in the first simulation, four differ from x_{all} by $D(x_{all}, \mu) > 10$ -fold (i.e. $\mu < x_{all}/10$ or $\mu > 10x_{all}$) (Table 6.2). These relatively large $D(x_{all}, \mu)$ values suggest that the respective data sets are influencing the position of x_{all} for one of the following reasons:

- The experimental methods are not replicated faithfully in the simulation.
- The biological experiments were originally performed in error.
- Their data are genuine statistical outliers.
- The respective components of the model are not realistic.

Therefore, since alternative data about the same parameters are available with a closer fit to x_{all} , these four experiments are excluded from all subsequent simulations. This causes $\log(P(D|\theta))$ to decrease immediately from -7336.92 to -4732.52 with the parameter values from Table 6.1.

Table 6.2 Four excluded experiments. Four data sets were identified from the first simulation with $D(x_{all}, \mu) > 10$. Even when excluded from the second simulation, each data set is still $D(x_{sub}, \mu) > 14.15$ -fold away from the second simulated output (x_{sub}).

Experiment	$D(x_{all}, \mu)$	$D(x_{sub}, \mu)$
Protein synthesis: Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	13.04	14.15
Uptake: Inoue, Kusano, and Silver (1996)	33.11	26.69
Nagata, Kiyono, and Pan-Hou (2006)	20.80	26.75
Song <i>et al.</i> (2004)	1402.52	1542.90

6.3 The second simulation – excluding a subset of experiments

Table 6.3 lists the set of parameter values which give the closest fit of the second simulated output (x_{sub}) through the 489 data points of 66 experiments. This fit corresponds to a $\log(P(D|\theta)) = -4679.91$.

These final results show that the rate constant for non-*mer* import (b_{in}) is 2.31 times greater than that for non-MerA transport (k_{t2p}). Similarly, the rate constant for non-*mer* export (b_{out}) is 230.91 times smaller than that for volatilisation (v). The equilibrium concentration of Hg^{2+} for 50 % of $MerA_t$ to be bound by Hg^{2+} is four orders of magnitude higher than that for $MerR_t$. The equilibrium concentration of MerR for 50 % of the DNA to be bound by MerR (K_e) is 310.63 times less than that for $MerR-Hg^{2+}$ (K_{es}). MerT synthesis is always 4.85 times greater than that of MerA ($a_{0T/0A}$) and synthesis from DNA-MerR (a_1 , a_R) is always lower than the respective synthesis from the naked DNA (a_{0T} , a_{0A} , a_{0R}). Specifically, repression by MerR of its own promoter (P_R) is 14.72 times that of its repression of P_T . Finally, fully activated synthesis of MerT from DNA-MerR- Hg^{2+} , with rate $(a_{0T} \cdot a_2) \cdot [O_t]$, is 252 times higher than its synthesis from the naked DNA (or 542 times higher than its fully repressed synthesis from DNA-MerR with rate $(a_{0T} \cdot a_1) \cdot [O_t]$).

Figure 6.3 shows that the second simulation requires 3200001×16 iterations for the random walks of all 16 parameters to converge on log-posterior distributions. In particular, the parameter k_{t2p} requires more iterations than for the simulation with all experiments included. This suggests that some information about k_{t2p} is lost in the excluded experiments. However, since their exclusion does not result in any new parameter correlations, the included experiments must still provide sufficient information about all parameters in the model.

Table 6.3 Values from a simulation excluding a subset of experiments. These parameter values give the closest fit of the simulated output through the 489 data points of 66 experiments. Also shown are the means and standard deviations (log-scale) of the posterior distributions and the standard deviations (log-scale) of the proposal distributions.

Parameter	Units	Closest fit value	Mean	Standard deviation	Standard deviation of proposal distribution
b_{in}	min^{-1}	4.34e1	4.34e1	5.69e-2	6.82e-2
b_{out}	min^{-1}	2.75	2.74	4.06e-2	7.90e-2
K_S	M	2.26e-9	2.29e-9	2.06e-1	2.32e-1
k_{t2p}	min^{-1}	1.88e1	2.15e1	4.07e-1	1.35e-1
K_A	M	2.28e-5	2.36e-5	1.01e-1	1.38e-1
v	min^{-1}	6.35e2	6.83e2	1.61e-1	8.85e-2
K_e	M	7.34e-13	7.36e-13	2.38e-1	1.96e-1
K_{es}	M	2.28e-10	2.30e-10	1.04e-1	2.03e-1
a_{0A}	min^{-1}	7.44e-1	7.19e-1	1.77e-1	8.29e-2
$a_{0T/0A}$	-	4.85	5.28	3.79e-1	1.20e-1
$a_{0R/0A}$	-	8.88e2	9.64e2	2.07e-1	9.21e-2
a_1	-	4.65e-1	4.69e-1	3.77e-2	1.13e-1
a_2	-	2.52e2	2.54e2	4.59e-2	1.20e-1
a_R	-	3.16e-2	3.11e-2	5.71e-2	1.11e-1
K_T	M	8.06e-7	8.41e-7	8.83e-2	1.20e-1
K_I	M	2.46e-4	2.58e-4	2.02e-1	5.62e-1

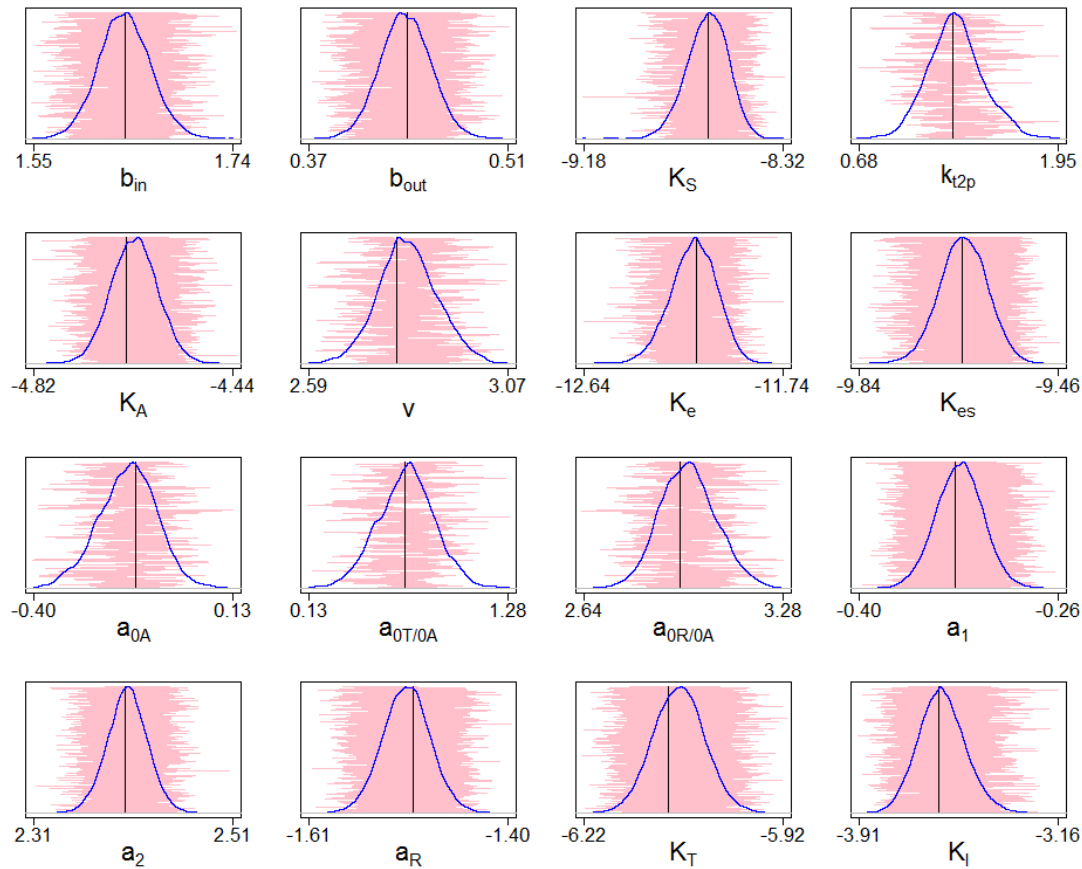


Figure 6.3 Posterior distributions from a simulation excluding a subset of experiments. For each parameter, the posterior distributions (blue), random walks of 3200001×16 iterations (pink), and closest-fit values from Table 6.3 (black lines) are plotted on a $\log_{10}$ scale.

6.4 Correlations to closeness of fit

So far, the $D(x, \mu)$ values have been used to exclude four experiments which may not have been replicated faithfully in the simulation. This suggests that the assumptions made about their respective experimental methodologies are potentially incorrect. In addition, a set of standardised experimental conditions were assumed so that a wide range of data sets could be included. Therefore, experiments are grouped into the following categories so that any correlations to their $D(x, \mu)$ values can reveal whether these assumptions are incorrect:

- The combination of *mer* genes. This indicates which processes are being measured simultaneously, e.g. *merR-T(ΔA)*, non-*mer* uptake + (the induction of) non-MerA transport.
- Experimental technique (*in vitro* or *in vivo*, indirect/relative or absolute measurements, accuracy of technique, or research laboratory).
- Plasmid type (control cell, Tn501, or Tn21).
- Growth medium (rich or minimal media, or buffer).
- Whether MerD is present or not.
- Plasmid copy number.
- (Subtoxic Hg^{2+} concentration in the cell).

Table 6.4 shows all data sets grouped by experiment type (technique and *mer* gene combination), then ordered internally by their $D(x_{sub}, \mu)$ values. Figure 6.4 then plots each category against the $D(x_{sub}, \mu)$ values of each data set.

With all experiments included, $1.02 < D(x_{all}, \mu) < 9.24$ for the included data, and $13.04 < D(x_{all}, \mu) < 1402.52$ for the (to be) excluded data. Then, with four experiments excluded, $1.04 < D(x_{sub}, \mu) < 9.06$ for the included data, and $14.15 < D(x_{sub}, \mu) < 1542.9$ for the excluded data. Thus, the position of x_{all} is different to x_{sub} because the knowledge integrated into the values of the parameters in the model changes between simulations. Some data sets are closer to x_{sub} than to x_{all} while others are farther away. But, overall, the included data are closer to x_{sub} ($\log(P(D|\theta)) = -4679.91$) than to x_{all} ($\log(P(D|\theta)) = -7336.92$).

Although $D(x, \mu)$ measures the average closeness of x to μ , it does not position x as above or below μ in magnitude. Therefore, $D(x_{all}, \mu) / D(x_{sub}, \mu)$ positions μ values relative to the position half way between both simulated outputs, i.e. $(x_{all} + x_{sub})/2$. Thus, data with $D(x_{all}, \mu) / D(x_{sub}, \mu) > 1$ are, on average, closer to x_{all} than to x_{sub} . Data with $D(x_{all}, \mu) / D(x_{sub}, \mu) < 1$ are, on average, closer to x_{sub} .

Whether all data sets are clustered on one side of $(x_{all} + x_{sub})/2$ does not matter. What matters is which categories of data sets are clustered closely together.

Figure 6.4 shows that data sets from the following experiment types are particularly close to x_{sub} (i.e. $D(x_{sub}, \mu) < 2$ -fold):

- E. Protein synthesis from P_T – *merR-TA* (densitometry, enzyme purification), in particular [MerT/MerA].
- F. Protein synthesis from P_R – control cells (β -galactisidase synthesis, alkaline phosphatase synthesis).
- J. Binding of Hg^{2+} to MerR – in vitro
- O. Volatilisation of Hg^{2+} – *merΔ(TP)* mutant cells

For the remaining experiment types, at least one of their data sets is particularly close to x_{sub} (except for types A, C, D, G, H, M, which only contain one data set each). The reason that four experimental types have such a close fit is perhaps that the information provided by their data is consistent with or is not contradicting that of another experiment type. For example, the MerT concentration can always be 4.85 times that of MerA, while the (correlated) value of k_{t2p} can increase or decrease accordingly.

Since the experiments with minimal and rich media are dispersed equally and widely between the $D(x_{all}, \mu) / D(x_{sub}, \mu) > 1$ and $D(x_{all}, \mu) / D(x_{sub}, \mu) < 1$ groups (Figure 6.5), the assumption to model protein synthesis rate as proportional to growth rate has not been invalidated. In addition, there is no evidence of the difference in using $Hg(NO_3)_2$ or $HgCl_2$ to supply Hg^{2+} ions to the cells. This leads to two hypotheses for $HgCl_2$ and $Hg(NO_3)_2$: 1) their toxicities are similar (or are both insignificant); and 2) their rates of uptake are similar. Firstly, there is no toxicity component in the model because experiments were assumed to use a subtoxic concentration of Hg. Secondly, only a single cellular uptake experiment was performed in buffer (Hsieh, Chen, Chang, Endo, and Huang, 2007) for a $D(x_{sub}, \mu) = 5.19$. This is not enough data to disprove the hypothesis that media composition affects the bioavailability of Hg. In addition, the data sets from experiments with or without MerD are not clustered separately. Similarly, the assumption that the data for Tn501 and Tn21 are interchangeable is also not violated.

Table 6.4 A summary of simulations one and two. Simulation one includes all experiments numbered 1 to 70. Simulation two includes all experiments except for the excluded experiments: 2, 42–44. Data sets are grouped by experiment type and ordered by the fold-differences, $D(x_{sub}, \mu)$, between values of the simulated output of the second simulation (x_{sub}) and values of the mean (unique) data points (μ). Several experiments measured multiple values (i.e. repeat readings) for the dependent variable (e.g. [S]) for a single value of the independent variable (e.g. time). Therefore the numbers shown in brackets on which $D(x, \mu)$ is calculated apply to the number of unique data points; one value for each value of the independent variable. This ensures that the closeness of fit between x and μ is calculated fairly for each data set as the fold distance to the mean of the repeat readings and not the mean of the average fold distances of the repeat readings. The standard deviations of each data point are also shown.

A. Protein synthesis from P_T – *in vitro*

Data set	Reference	Data points	Standard deviations	Simulation one		Simulation two	
				Mean likelihood of one data point	$D(x_{all}, \mu)$	Mean likelihood of one data point	$D(x_{sub}, \mu)$
1	Ralston and O'Halloran (1990)	26	0.3	-7.31	3.15	-8.31	3.40
2	Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	2	0.4	-20.61	13.04	-21.94	14.15

B. Protein synthesis from P_T – control cells

3	Rouch, Parkhill, and Brown (1995)	15	0.3	-1.35	1.64	-0.780	1.45
4	Figure 5 of Condee and Summers (1992)	15	0.2	-6.33	2.04	-5.23	1.91
5	Caguiat, Watson, and Summers (1999)	6 (2)	0.3	-16.50 (-3.36)	2.18	-17.36 (-4.10)	2.36
6	Shewchuk, Verdine, Nash, and Walsh (1989)	3 (2)	0.3	-8.99 (-6.84)	3.03	-9.59 (-7.41)	3.17
7	Figure 4 of Condee and Summers (1992)	9	0.2	-19.12	3.44	-17.20	3.23
8	Hansen and Sorensen (2000)	8	0.2	-20.50	3.60	-22.09	3.78
9	Park, Wireman and Summers (1992)	2	0.1	-87.78	3.76	-102.23	4.18
10	Figure 8 of Condee and Summers (1992)	2	0.2	-28.74	4.56	-28.73	4.55
11	Ross, Park, and Summers (1989)	4 (3)	0.19	-24.36 (-29.56)	4.31	-28.16 (-34.34)	4.83
12	Caslake, Ashraf and Summers (1997)	18 (4)	0.35	-11.48 (-14.89)	6.75	-12.51 (-16.34)	7.40

C. Protein synthesis from P_T – *merR-T(ΔA)*

13	Ni'Bhriain, Silver, and Foster (1983)	1	0.2	-18.61	3.39	-23.11	3.90
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D. Protein synthesis from P_T – *merR-TA*

Data set	Reference	Data points	Standard deviations	Simulation one		Simulation two	
				Mean likelihood of one data point	$D(x_{all}, \mu)$	Mean likelihood of one data point	$D(x_{sub}, \mu)$
14	Nucifora, Chu, Silver, and Misra (1989)	4	0.3	-6.58	2.97	-6.75	3.01

E. Protein synthesis from P_T – *merR-TA*

15	Lee, Gambill and Summers (1989) – [MerT]/[MerA]	1	0.6	-3.67e-4	1.02	-7.72e-3	1.08
16	Philippidis, Malmberg, Hu, and Schottel (1991) – [MerT/MerA]	1	0.6	-0.141	1.38	-9.41e-2	1.30
17	Rinderle, Booth, and Williams (1983) – [MerA]	1	0.7	-1.86	3.86	-1.30	3.09

F. Protein synthesis from P_R – control cells

18	Park, Wireman and Summers (1992)	2	0.1	-9.52	1.55	-10.47	1.58
19	Caslake, Ashraf and Summers (1997)	8 (2)	0.35	-6.20 (-1.42)	1.80	-6.12 (-1.31)	1.76

G. Protein synthesis from P_R – *merR-T(ΔA)*

20	Foster and Brown (1985)	2	0.3	-27.48	9.24	-26.97	9.06
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H. Protein synthesis from P_R – *merR-TA*

21	Foster and Brown (1985)	2	0.3	-21.12	7.03	-20.66	6.88
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I. Binding of MerR (and MerR-Hg²⁺) to the DNA – *in vitro*

22	Parkhill, Blair, Hobman, and Brown (1998)	2	0.4	-0.802	1.66	-0.804	1.66
23	O'Halloran, Frantz, Shin, Ralston, and Wright (1989)	27	0.22	-8.67	2.50	-8.36	2.46
24	Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)	20	0.45	-3.02	3.02	-2.81	2.90
25	Shewchuk, Verdine, and Walsh (1989)	16	0.3	-6.91	3.05	-6.81	3.02

J. Binding of Hg²⁺ to MerR – *in vitro*

Data set	Reference	Data points	Standard deviations	All experiments combined		Subset of experiments	
				Mean likelihood of one data point	D(x _{all} , μ)	Mean likelihood of one data point	D(x _{sub} , μ)
26	Zeng, Stålhandske, Anderson, Scott, and Summers (1998)	6	0.3	-0.312	1.27	-0.308	1.27
27	Song <i>et al.</i> (2004)	4	0.1, 0.067	-8.75	1.52	-8.75	1.52
28	Shewchuk <i>et al.</i> (1989)	6	0.3	-2.16	1.86	-2.15	1.86

K. Uptake of Hg²⁺ – control cells

29	Sone, Pan-Hou, Nakamura, Sakabe, and Kiyono (2010)	3	0.1	-2.39	1.24	-0.528	1.11
30	Hamlett, Landale, Davis, and Summers (1992)	12 (4)	0.35	-0.903 (-0.614)	1.47	-0.319 (-0.158)	1.22
31	Bae, Mehra, Mulchandani, and Chen (2001)	3	0.2	-6.18	2.02	-2.68	1.59
32	Kiyono, Sone, Nakamura, Pan-Hou, and Sakabe (2009)	3	0.1	-12.27	1.64	-12.15	1.64
33	Nakahara, Silver, Miki, and Rownd (1979)	7 (4)	0.6	-0.458 (-0.319)	1.61	-0.610 (-0.401)	1.71
34	Foster, Nakahara, Weiss, and Silver (1979)	6 (3)	0.35	-3.38 (-3.25)	2.44	-2.84 (-2.69)	2.25
35	Bae, Wu, Kostal, Mulchandani, and Chen (2003)	1	0.4	-3.54	2.90	-2.11	2.27
36	Kiyono, Omura, Fujimori, and Pan-Hou (1995)	4	0.1	-53.33	2.81	-36.55	2.35
37	Kiyono and Pan-Hou (1999)	4	0.1	-68.01	3.21	-49.80	2.71
38	Pan-Hou, Kiyono, Kawase, Omura, and Endo (2001)	1	0.1	-57.69	2.93	-87.09	3.74
39	Ni'Bhriain, Silver, and Foster (1983)	24 (4)	0.35	-8.54 (-6.93)	3.68	-10.73 (-9.01)	4.42
40	Hsieh, Chen, Chang, Endo, and Huang (2007)	2	0.1	-144.92	5.49	-135.57	5.19
41	Sone, Nakamura, Pan-Hou, Itoh, and Kiyono (2013)	1	0.3	-12.03	4.35	-16.38	5.57
42	Inoue, Kusano, and Silver (1996)	6	0.4	-38.28	33.11	-33.71	26.69
43	Nagata, Kiyono and Pan-Hou (2006)	1	0.1	-460.58	20.80	-540.09	26.75
44	Song <i>et al.</i> (2004)	6	0.29	-312.16	1.40e3	-320.43	1.54e3

L. Uptake of Hg²⁺ – *merR-T(ΔA)*

45	Hamlett, Landale, Davis, and Summers (1992)	12	0.35	-9.90e-2	1.17	-0.151	1.21
46	Nakahara, Silver, Miki, and Rownd (1979) – induction	5	0.4	-2.26	2.34	-1.41	1.96
47	Foster, Nakahara, Weiss, and Silver (1979)	20 (8)	0.35	-1.59 (-1.49)	1.83	-1.99 (-1.96)	2.00
48	Nakahara, Silver, Miki, and Rownd (1979)	14 (8)	0.6	-1.11 (-0.831)	2.17	-1.04 (-0.692)	2.03
49	Ni'Bhriain, Silver, and Foster (1983)	24 (8)	0.35	-14.97 (-14.75)	6.69	-14.03 (-13.81)	6.29

M. Uptake of Hg^{2+} – *mer*(ΔR)-TA

Data set	Reference	Data points	Standard deviations	All experiments combined		Subset of experiments	
				Mean likelihood of one data point	$D(x_{\text{all}}, \mu)$	Mean likelihood of one data point	$D(x_{\text{sub}}, \mu)$
50	Foster, Nakahara, Weiss, and Silver (1979)	20 (10)	0.35	-8.56 (-8.61)	4.27	-7.93 (-7.97)	4.04

N. Volatilisation of Hg^{2+} – *merR*-TA

51	Philippidis, Malmberg, Hu, and Schottel (1991)	21	0.1	-10.65	1.59	-11.50	1.62
52	Wilson, Leang, Morby, Hobman and Brown (2000); Schue, Glendinning, Hobman, and Brown (2008)	22 (6)	0.3	-5.99 (-2.34)	1.91	-5.27 (-1.75)	1.75
53	Nakahara <i>et al.</i> (1979) – $K_A = 9\text{e-}6$ M, cell extracts	1	0.3	-7.91	3.30	-4.78	2.53
54	Foster, Nakahara, Weiss, and Silver (1979) – resistant and micro-constitutive cells	13 (7)	0.3	-3.70 (-6.64)	2.98	-3.78 (-6.71)	3.00
55	Nakahara, Silver, Miki, and Rownd (1979) – induction	6	0.3	-6.60	2.98	-7.17	3.11
56	Laddaga, Chu, Misra, and Silver (1987)	8	0.2	-18.52	3.38	-17.24	3.24
57	Figure 4 of Ni'Bhriain, Silver, and Foster (1983) – induction	15 (5)	0.3	-9.01 (-9.23)	3.63	-9.33 (-9.56)	3.71
58	Philippidis, Malmberg, Hu, and Shottel (1991) – $K_T = 3.6\text{e-}6$ M	1	0.3	-6.83	3.03	-12.44	4.46
59	Philippidis, Malmberg, Hu, and Shottel (1991) – $K_T = 3.8\text{e-}6$ M	1	0.3	-6.18	2.87	-13.35	4.71
60	Philippidis, Malmberg, Hu, and Shottel (1991) – $K_T = 4.7\text{e-}6$ M	1	0.3	-6.83	3.03	-17.27	5.83
61	Philippidis, Malmberg, Hu, and Shottel (1991) – $K_T = 5.1\text{e-}6$ M	1	0.3	-9.70	3.75	-18.90	6.33
62	Tables 3 and 7 and Figure 4 of Ni'Bhriain, Silver, and Foster (1983) – resistant whole cells and extracts, and one $\Delta merR$ cell extract	11 (9)	0.3	-17.67 (-19.32)	6.45	-17.59 (-19.08)	6.38
63	Philippidis, Malmberg, Hu, and Shottel (1991) – $K_T = 6.0\text{e-}6$ M	1	0.3	-10.93	4.07	-22.38	7.44

O. Volatilisation of Hg^{2+} – *mer*(ΔTP) mutant cells

64	Wilson, Leang, Morby, Hobman and Brown (2000)	4	0.3	-4.24e-2	1.09	-7.31e-3	1.04
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P. Volatilisation of Hg^{2+} – *in vitro*

Data set	Reference	Data points	Standard deviations	All experiments		Subset of experiments	
				Mean likelihood of one data point	$D(x_{\text{all}}, \mu)$	Mean likelihood of one data point	$D(x_{\text{sub}}, \mu)$
65	Fox and Walsh (1982) – $v = 800 \text{ min}^{-1}$	1	0.3	-0.487	1.34	-0.296	1.26
66	Moore, Miller, and Walsh (1992) – $v = 810 \text{ min}^{-1}$	1	0.3	-0.529	1.36	-0.329	1.28
67	Moore, Distefano, Zydowsky, Cummings, and Walsh (1990) – $v = 340 \text{ min}^{-1}$	1	0.3	-1.74	1.75	-2.17	1.87
68	Fox and Walsh (1982) – $K_A = 12\text{e-}6 \text{ M}$	1	0.3	-4.55	2.47	-2.28	1.90
69	Moore, Distefano, Zydowsky, Cummings, and Walsh (1990) – $K_A = 5\text{e-}6 \text{ M}$	1	0.3	-17.62	5.94	-12.76	4.55
70	Moore, Miller, and Walsh (1992) – $K_A = 5\text{e-}6 \text{ M}$	1	0.3	-17.62	5.94	-12.76	4.55

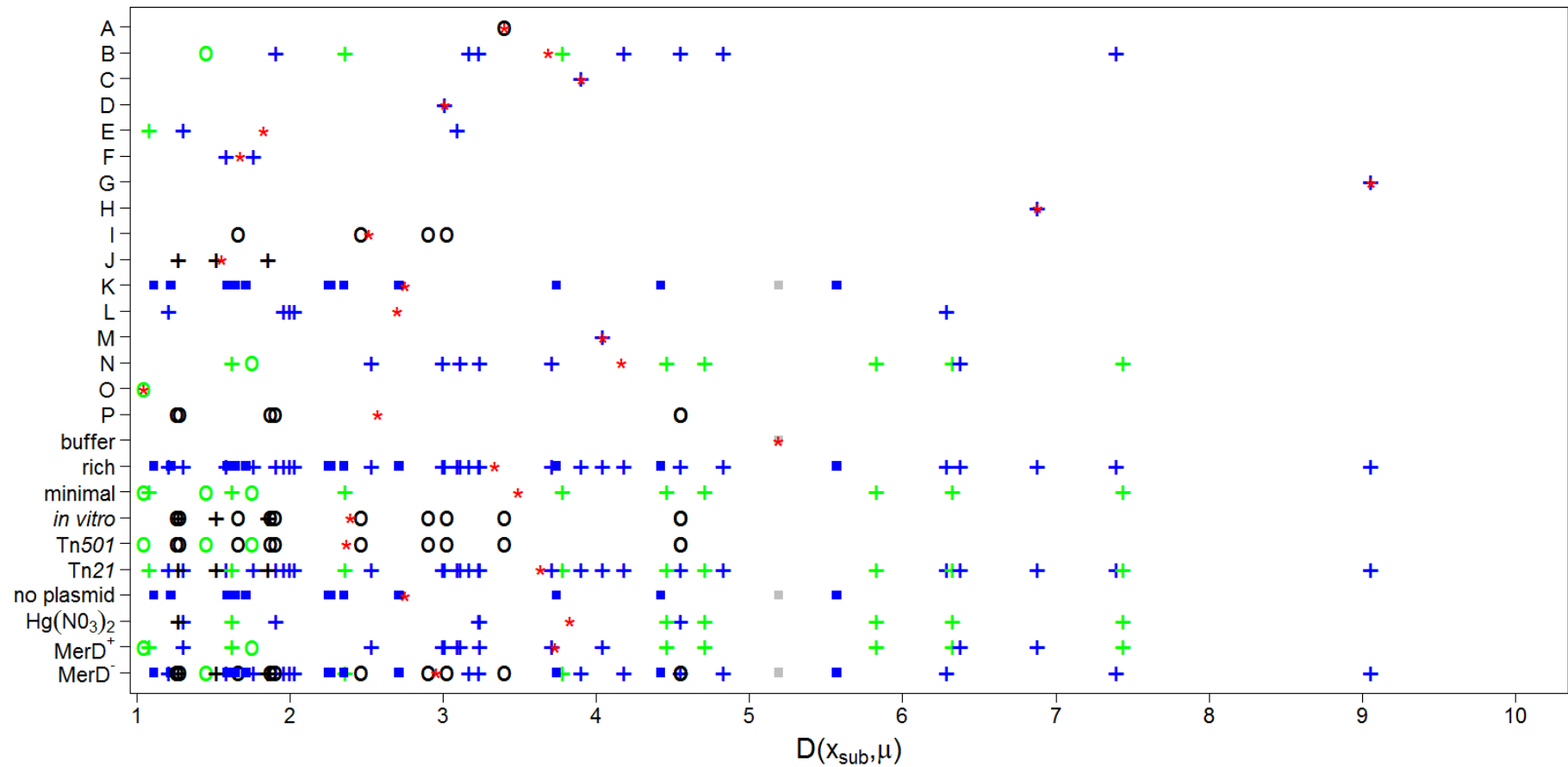


Figure 6.4 Correlations to closeness of fit. Data sets 1:70 are grouped by experiment type as stated in Table 6.4 and are ordered by their fold-distances to the simulated output of the second simulation, $D(x_{\text{sub}}, \mu)$. The symbols for the medium and plasmid used in the experiments are: + (Tn21), O (Tn501), ■ (no plasmid), minimal medium (green), rich medium (blue), *in vitro* (black), cells in buffer (grey). Experiments are further categorized as adding Hg^{2+} as $\text{Hg}(\text{NO}_3)_2$ instead of HgCl_2 , or whether MerD is present. Finally, the means of each group of included data sets are shown by a red star: *.

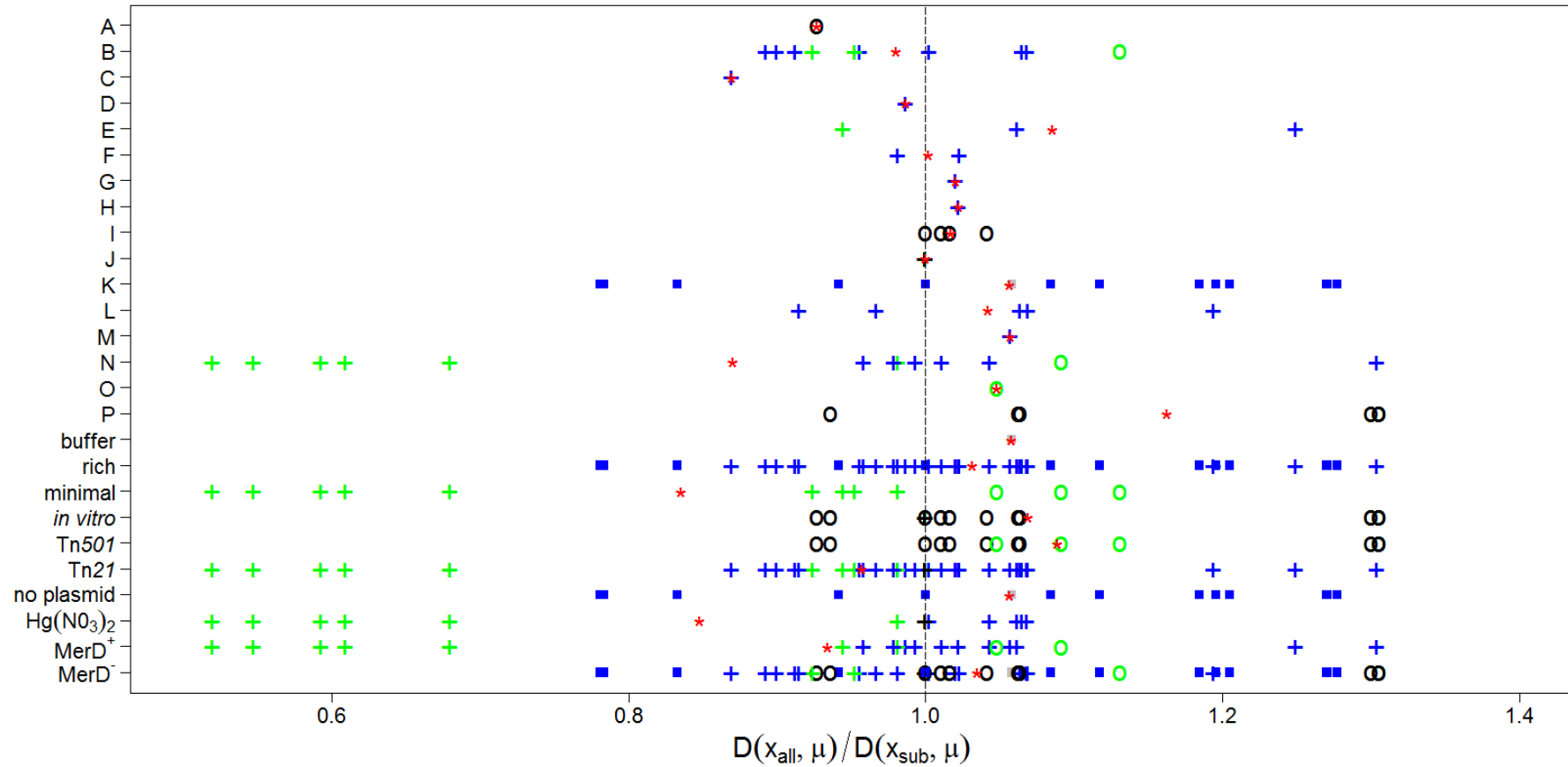


Figure 6.5 Clustering of experiments. Data sets 1:70 are grouped by experiment type as stated in Table 6.4 and are ordered by their $D(x_{all}, \mu) / D(x_{sub}, \mu)$ values, i.e. $D(x_{all}, \mu) / D(x_{sub}, \mu) < 1$ means that x_{all} is closer to μ . The symbols for the medium and plasmid used in the experiments are: $+$ (Tn21), $\circ$ (Tn501), $\blacksquare$ (no plasmid), minimal medium (green), rich medium (blue), *in vitro* (black), cells in buffer (grey). Experiments are further categorized as adding Hg^{2+} as $Hg(NO_3)_2$ instead of $HgCl_2$, or whether MerD is present. Finally, the means of each group of included data sets are shown by a red star: *.

Finally, overall, although the group of *in vitro* experiments is not clustered separately from the *in vivo* experiments, the data for the parameter K_T in the group 'N. Volatilisation of Hg^{2+} – *merR-TA*' from Philippidis, Malmberg, Hu, and Schottel (1991) are clustered together. Thus their positions away from $(x_{\text{all}} + x_{\text{sub}})/2$ may indicate that this experiment type is inconsistent with the others. Therefore, Figure 6.6 and Table 6.5 show the effects on the estimates of parameter values of including or excluding these K_T experiments.

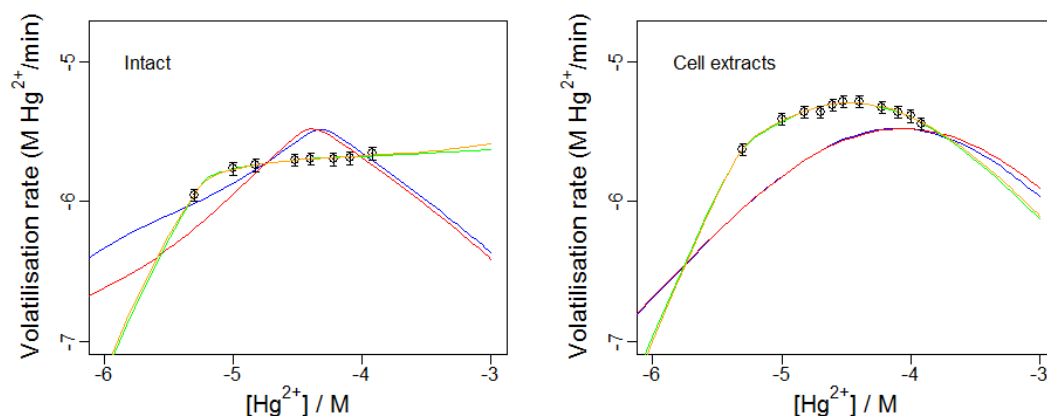


Figure 6.6 Volatilisation in intact (Left) and cell extracts (Right). Raw data are from Figures 2 and 3 of Philippidis, Malmberg, Hu, and Schottel (1991). The simulated outputs for the closest fit values are shown for the subset of experiments with the K_T experiments included (blue), or excluded (red), and for the Philippidis, Malmberg, Hu, and Schottel (1991) experiment simulated by itself with the K_T experiments included (green) or excluded (orange).

The main observation is that the estimated value for the parameter K_T changes very little if the K_T experiments are excluded from a simulation of the Philippidis, Malmberg, Hu, and Schottel (1991) experiment alone (included: $4.59\text{e-}6$ M vs. excluded: $2.53\text{e-}6$ M). However, there is an order of magnitude difference in the estimate of this parameter when the K_T experiments are excluded from the subset simulation of all 66 experiments (included: $8.06\text{e-}7$ M vs. excluded: $9.33\text{e-}8$ M). Therefore, we can conclude that the K_T and the Philippidis, Malmberg, Hu, and Schottel (1991) experiments are consistent with each other (as expected), but that the Philippidis, Malmberg, Hu, and Schottel (1991) experiment is not consistent with other data sets in the subset simulation that also provide information on K_T .

Table 6.5 Parameter values for Figure 6.6. These parameter values give the closest fit of the simulated outputs through the data points of experiments in four different simulations: (subset) 484+5 data points of 61+5 experiments, (subset excluding K_T) 484 data points of 61 experiments, (Philippidis with K_T) 21+5 data points of 1+5 experiments, (Philippidis without K_T) 21 data points of 1 experiment. Here, 'Philippidis' refers to the volatilisation data from Philippidis, Malmberg, Hu, and Schottel (1991). Maximum likelihoods are shown below each column, where the bracketed figures indicate the contribution to the likelihood of the Philippidis experiment.

Parameter	Units	Subset	Subset excluding K_T	Philippidis with K_T	Philippidis without K_T
b_{in}	min^{-1}	4.34e1	6.27e1	1.72e1	11.1
b_{out}	min^{-1}	2.75	3.34	5.90e2	9.32e2
K_S	M	2.26e-9	1.90e-9	8.95e-15	9.86e-14
k_{t2p}	min^{-1}	1.88e1	3.91	5.11e2	4.37e2
K_A	M	2.28e-5	1.90e-5	6.89e-10	3.13e-9
v	min^{-1}	6.35e2	7.10e2	3.45	2.82
K_e	M	7.34e-13	5.00e-13	3.84e-6	6.18e-6
K_{es}	M	2.28e-10	2.38e-10	2.30e-11	7.75e-11
a_{0A}	min^{-1}	7.44e-1	5.34e-1	1.03e3	8.56e2
$a_{0T/OA}$	-	4.85	6.14	3.26e-2	2.54e-2
$a_{0R/OA}$	-	8.88e2	1.65e3	5.86e7	3.27e7
a_1	-	4.65e-1	4.78e-1	4.08e2	5.52e2
a_2	-	2.52e2	2.66e2	1.38e-18	1.19e-15
a_R	-	3.16e-2	3.09e-2	2.84e-9	3.30e-16
K_T	M	8.06e-7	9.33e-8	4.59e-6	2.53e-6
K_T	M	2.46e-4	3.27e-4	8.50e-5	9.81e-5
Maximum likelihoods:		-4679.91 (-241.57)	-4540.25 (-271.91)	-1.75 (-0.761)	-0.740 (-0.740)

6.5 Examples from each model component

Cellular uptake of Hg

For the data of Nakahara, Silver, Miki, and Rownd (1979), the average fold-distance between x_{sub} and μ is $D(x_{sub}, \mu) = 1.71$ for control cells and 1.96 for *merR-T(ΔA)* (Figure 6.7). These data show the uptake of $2 \mu\text{M } ^{203}\text{HgCl}_2$ into control cells and into hypersensitive cells induced (or not) with $0.2 \mu\text{M HgCl}_2$. In control cells, $[^{203}\text{S}_t]_{max}$ saturates at $2.16\text{e-}5 \text{ M}$ through the equilibrium of non-*mer* import and export. In hypersensitive cells, total uptake increases because non-*mer* uptake and non-MerA transport are combined. With a steady-state $[T_t]$, uptake is $[^{203}\text{S}_t]_{max} = 3.09\text{e-}5 \text{ M}$. With an induced $[T_t]$, uptake is higher, at $[^{203}\text{S}_t]_{max} = 2.54\text{e-}4 \text{ M}$.

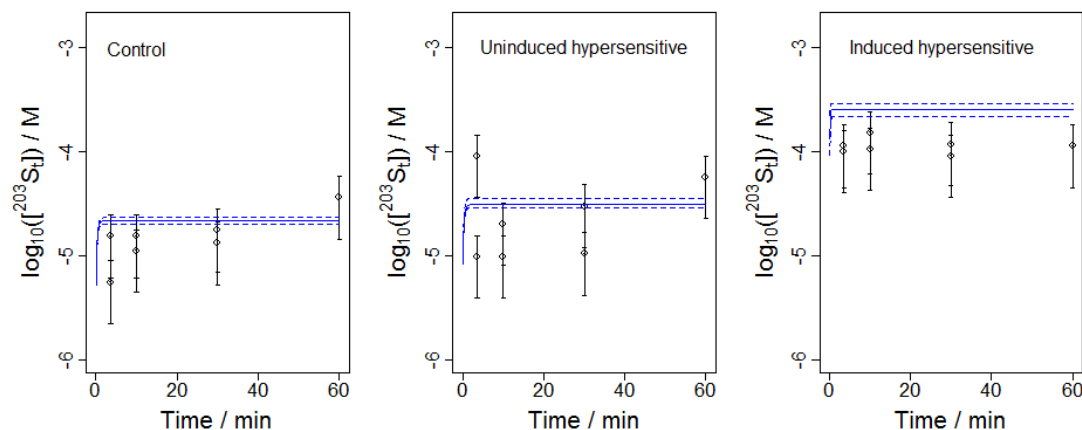


Figure 6.7 Whole-cell binding of $2\ \mu\text{M}$ $^{203}\text{HgCl}_2$ by control (Left) and hypersensitive cells. Hypersensitive cells are induced (Right) or not (Middle) with $0.2\ \mu\text{M}$ HgCl_2 for 60 min. Raw data are from Figure 3 of Nakahara, Silver, Miki, and Rownd (1979). The second simulated outputs are shown in blue for the closest fit values (solid line) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed lines).

Figure 6.8 compares the results of two similar methods. One experiment induces with $0.2\ \mu\text{M}$ HgCl_2 followed by $2\ \mu\text{M}$ $^{203}\text{HgCl}_2$ (Foster, Nakahara, Weiss, and Silver, 1979) and the other induces with $0.25\ \mu\text{M}$ HgCl_2 followed by $5\ \mu\text{M}$ $^{203}\text{HgCl}_2$ (Ni'Bhriain, Silver, and Foster, 1983). It is therefore surprising that cells should bind more $^{203}\text{HgCl}_2$ when induced with a lower HgCl_2 concentration and with less $^{203}\text{HgCl}_2$ added (and vice versa). Instead, the model predicts that the difference between control uptake and induced uptake is directly correlated to the induced $[T_t]$ and the total amount of $^{203}\text{HgCl}_2$ available to bind. Therefore, the simulated output fits between these two data sets: $D(x_{\text{sub}}, \mu) = 2.25$ (control), 2.00 (*merR*- $T(\Delta A)$) and $4.42, 6.29$, respectively.

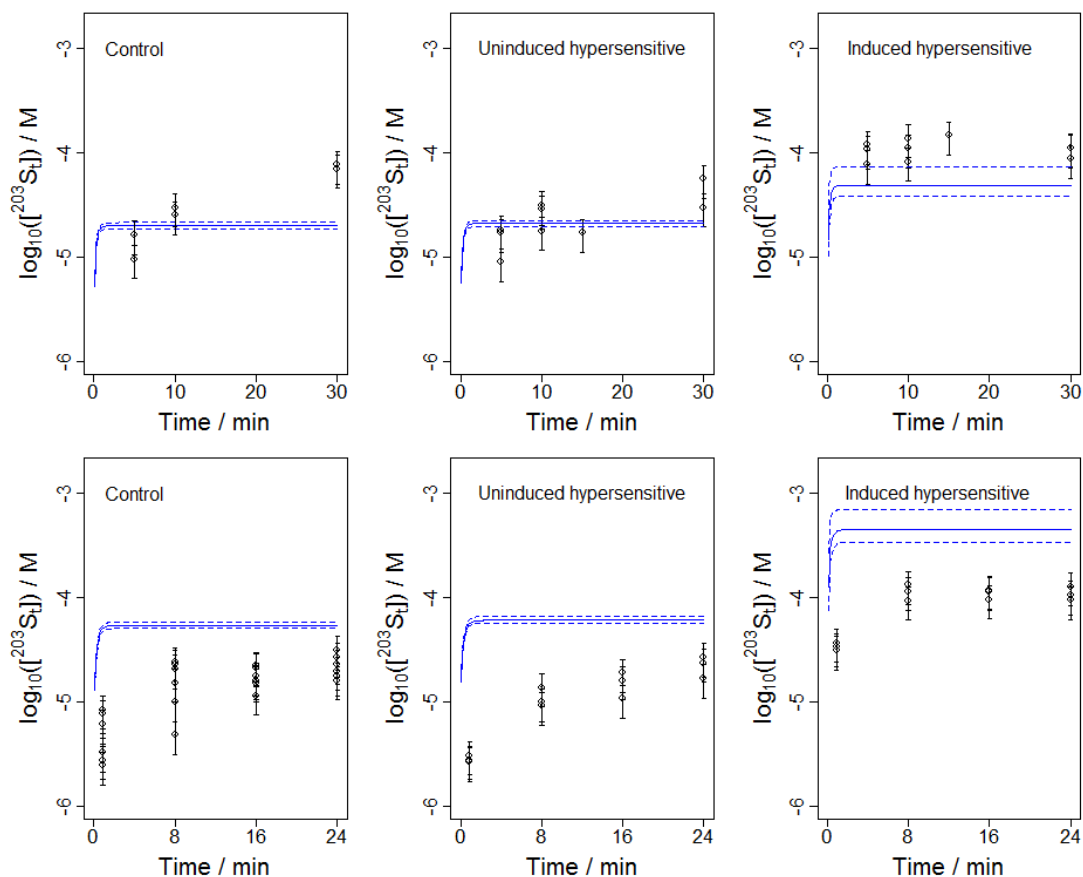


Figure 6.8 Whole-cell binding of $^{203}\text{HgCl}_2$ by control and hypersensitive cells. Cells are induced or not with $0.2 \mu\text{M}$ HgCl_2 for 45.47 minutes before $2 \mu\text{M}$ $^{203}\text{HgCl}_2$ is added (Top) or with or without $0.25 \mu\text{M}$ HgCl_2 for 60 minutes before $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ is added (Bottom). Raw data are from (Top) Figure 2 of Foster, Nakahara, Weiss, and Silver (1979) and (Bottom) Figure 5 of Ni'Bhriain, Silver, and Foster (1983). The second simulated outputs are shown in blue for the closest fit values (solid line) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed lines).

MerR-(Hg^{2+})-DNA binding

Figure 6.9 shows the binding of DNA by MerR and MerR- Hg^{2+} with $D(x_{\text{sub}}, \mu) = 2.90$ (Parkhill, Ansari, Wright, Brown, and O'Halloran, 1993). The R_t concentrations required for 50 % binding of the DNA can be estimated from this figure as $1.18\text{e-}10 \text{ M}$ (with Hg^{2+}) and $7.34\text{e-}13 \text{ M}$ (no Hg^{2+}). The value for without Hg^{2+} is identical to the parameter $K_e = 7.34\text{e-}13 \text{ M}$, whereas the value with Hg^{2+} is less than $K_{\text{es}} = 2.28\text{e-}10 \text{ M}$ because MerR_t is not 100 % saturated with Hg^{2+} .

Figure 6.9 also shows that the simulated output always lies above the data in the absence of Hg^{2+} . This means that the model is consistently over-estimating the

fraction of operator bound by MerR in the absence of Hg^{2+} . This shift in the position of the simulated output is caused by included data from other reactions in the model which are also estimating the parameter K_e . For example, the synthesis of MerR is inversely correlated to K_e . A decrease in K_e causes an increase in $[\text{OR}]/[\text{O}_t]$ and therefore a decrease in the value of repressed synthesis/derepressed synthesis of alkaline phosphatase (since $a_{1R} \ll a_{0R}$) and a closer fit to the data of Figure 6.13 (Top left). Thus, overall, a decrease in K_e makes the fit to the whole DNA-binding component is more distant, while simultaneously causing a closer fit to these particular data for MerR synthesis.

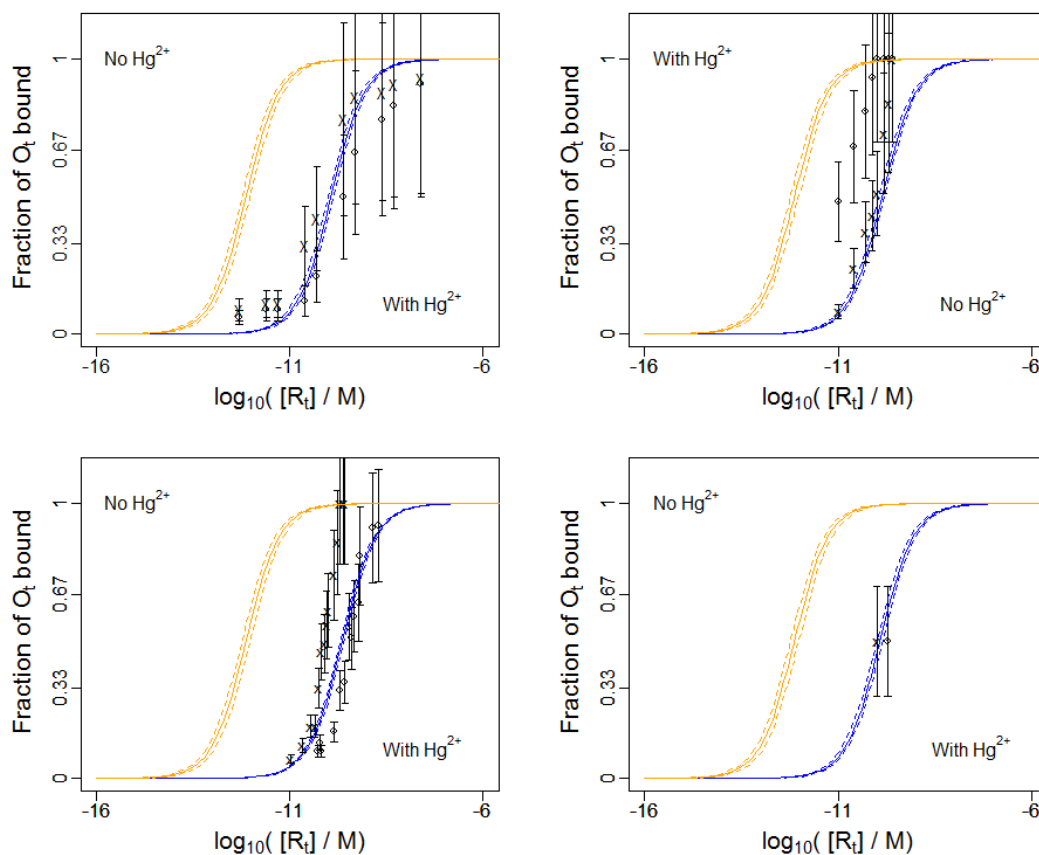


Figure 6.9 The fraction of *mer* operator bound by MerR in the presence (O) and absence (x) of HgCl_2 . Raw data are from (Top left) Figure 3 of Parkhill, Ansari, Wright, Brown, and O'Halloran (1993); (Top right) Figure 3 of Shewchuk, Verdine, and Walsh (1989); (Bottom left) Figure 5 of O'Halloran, Frantz, Shin, Ralston, and Wright (1989); (Bottom right) Table 2 of Parkhill, Blair, Hobman, and Brown (1998). The second simulated outputs are shown for the closest fit values (solid lines: blue (Hg^{2+}); orange (no Hg^{2+})) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed lines).

The methods of O'Halloran, Frantz, Shin, Ralston, and Wright (1989) took many hours to interpret. However, the final decision was to include the data exactly as

stated. These authors reported that if their experiment is repeated with a 1:1 ratio of (5.4e-11 to 2e-9 M) MerR_t and HgCl₂, or, with a fixed 0.5e-6 M HgCl₂ and MerR_t (5.4e-11 and 2e-9 M), it “gave the same result (data not shown)”. Note also, that this same behaviour was stated for the transcriptional runoff assay in their paper where “further enhancement of *merT* transcription was not observed when excess mercuric ion (up to 1 µM) was added to the incubation mixture”. Therefore, this is interpreted as meaning that R_t is saturated with HgCl₂ such that increasing the HgCl₂ concentration has no additional effect. Hence the [R]/K_e = 0 term was disregarded from the model equations for this experiment. In conclusion, without making a reference to the knowledge that other experiments show that MerR is not 100 % saturated with Hg²⁺ at 1e-6 M HgCl₂, this experiment in itself cannot be deemed incorrect. Since D(x_{sub}, µ) = 2.46 is a reasonably close fit, there remains no reason to exclude this experiment or to believe the assumptions made in interpreting it are incorrect. Finally, it is the data of Shewchuk, Verdine, and Walsh (1989) with the least close fit, D(x_{sub}, µ) = 3.02. This may occur because of the opposite relationship in the positions of the data in the presence and absence of Hg²⁺ than the other three data sets and to the simulated outputs themselves.

MerR + Hg²⁺ binding

Figure 6.10 can be used to calculate the fraction of MerR_t from Figure 6.9 that is bound by Hg²⁺. Equation 6.2 then shows that, for the [Hg²⁺] used by Parkhill, Ansari, Wright, Brown, and O’Halloran (1993) of 0.75 µM HgCl₂, 99.70 % of R_t would be bound by Hg²⁺ when 50% of the DNA is bound by MerR or MerR-Hg²⁺. Further, if 99.9999 % of R_t were to be bound by Hg²⁺, then the Hg²⁺ concentration would have to be 5e-3 M HgCl₂.

Equation 6.2 The fraction of MerR_t bound by Hg²⁺.

$$[S_t] = [S] + [R_t] \frac{[S]}{[S] + K_s} = 0.750 \times 10^{-6} M = [S] + 118 \times 10^{-10} M \frac{[S]}{[S] + 2.26 \times 10^{-9} M}$$

$$\rightarrow [S] = 0.7499 \times 10^{-6} M; [R^*] = 1.1765 \times 10^{-10} M; \frac{[R^*]}{R_t} = 0.9970$$

The results of Song *et al.* (2004) shown in Figure 6.11 are a good fit to the data with D(x_{sub}, µ) = 1.52. However, notice that the concentration of free Hg²⁺ required for 50 % of R_t to be bound is greater than K_s = 2.26e-9 M. This is because, unlike in Figure 6.9, the quantity of R_t is much greater and, hence, the loss of Hg from S_t in the form of R* becomes significant. This significance is demonstrated in Figure 6.10. Increasing [R_t] means that the [S] required for 50 % of [R_t] to be in the [R*] form is still the same, i.e. = K_s. However, in order

to achieve this free $[S]$, more $[S_t]$ must be added to the system. In other words, a greater quantity of Hg becomes bound in R^* so less Hg is available as free S . So, for example, if $[S_t] = K_s$ is added, if the $[R_t] = 1.18\text{e-}10\text{ M}$, then $[R^*] = 5.823\text{e-}11\text{ M}$ is not very significant compared to $[S] = 2.2017\text{e-}9\text{ M}$ and $[S_t] = 2.2599\text{e-}9\text{ M}$. This results in $[R^*]/[R_t] = 0.4934$. However, if $[R_t]$ is $60\text{e-}6\text{ M}$, then very little Hg ($8.51\text{e-}14\text{ M}$) is as free $[S]$; most is as $[R^*] = 2.2599\text{e-}9\text{ M}$. This results in $[R^*]/[R_t] = 3.77\text{e-}5$. Therefore, overall, as $[R_t]$ increases, so does the concentration of S_t required for 50 % of R_t to be as R^* .

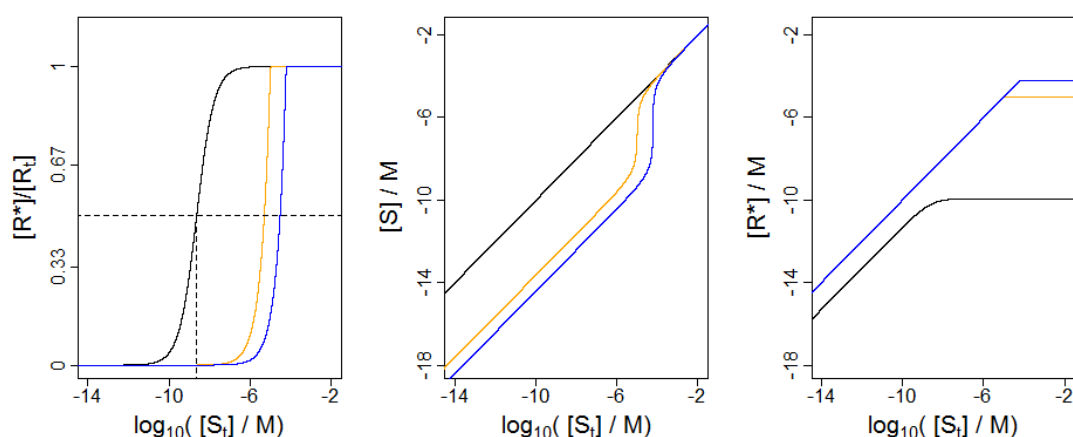


Figure 6.10 The fraction of MerR_t as MerR-Hg^{2+} . (Left) The fraction of MerR_t (R_t) as MerR-Hg^{2+} (R^*) for $[\text{MerR}_t] = 1.18\text{e-}10\text{ M}$ (black), $10\text{e-}6\text{ M}$ (orange), and $60\text{e-}6\text{ M}$ (blue). (Middle) The concentrations of free Hg^{2+} ($[S]$). (Right) The concentrations of R^* .

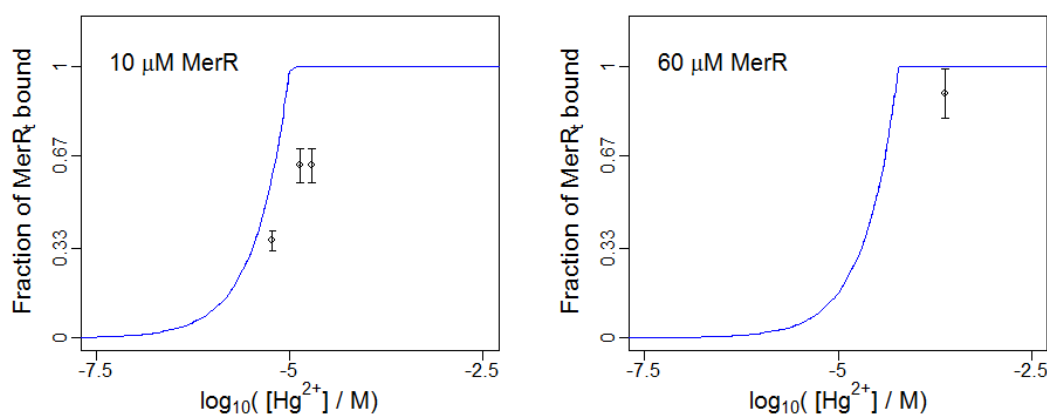


Figure 6.11 The fraction of MerR dimer bound by $^{203}\text{Hg}^{2+}$. (Left) $10\text{ }\mu\text{M}$ MerR with 5 to $20\text{ }\mu\text{M}$ $^{203}\text{HgCl}_2$ in Tris-HCl buffer for 15 minutes. (Right) $60\text{ }\mu\text{M}$ MerR with a four-fold excess of $^{203}\text{HgCl}_2$ in Tris-HCl buffer for 60 minutes. Raw data are from Figures 2 and 3 of Song *et al.* (2004). The second simulated outputs are shown in blue for the closest fit values (solid line or x) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed lines and *).

Protein synthesis

The simulated outputs on Figure 6.12 show that the maximum MerT synthesis rate is achieved when 100 % of R_t is in the R^* form. This was calculated previously to occur when $[\text{HgCl}_2] > 5\text{e-}3 \text{ M}$. Note that one data set is from an *in vivo* experiment (Rouch, Parkhill and Brown, 1995) and the other is *in vitro* (Ralston and O'Halloran, 1990). The fits for these two experiments are, respectively, $D(x_{\text{sub}}, \mu) = 1.45$ and 3.40 . Clearly, a lower $[\text{Hg}^{2+}]$ is needed for a 50 % MerT synthesis rate when HgCl_2 is added to the outside of the cell since uptake concentrates Hg in the cytoplasm. Also, the difference in induction magnitude (138-fold vs. 479-fold) is explained by the fact that neither of these increases are relative to the same base point (ideally, 0 M Hg^{2+}), but relative to the synthesis from $3.72\text{e-}9 \text{ M}$ and $1\text{e-}10 \text{ M Hg}^{2+}$, respectively.

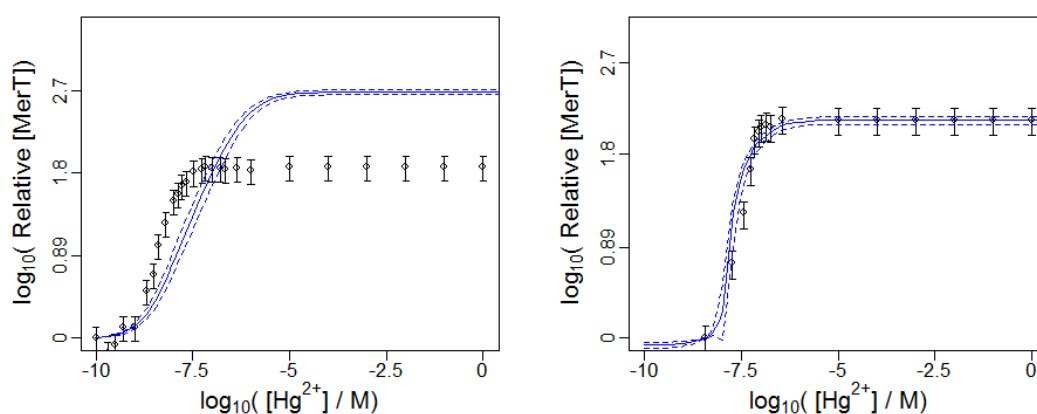


Figure 6.12 Protein synthesis from P_T . (Left) *in vitro* transcriptional activity from P_T . (Right) β -galactosidase activities from $merT'$ - $lacZ$ fusions after 60 minutes induction with HgCl_2 . Raw data are from (Left) Figure 1 of Ralston and O'Halloran (1990) and (Right) Figure 1 of Rouch, Parkhill, and Brown (1995). The second simulated outputs are shown in blue for the closest fit values (solid line) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed lines).

Additional fits for protein synthesis are shown in Figure 6.13: MerR synthesis, $D(x_{\text{sub}}, \mu) = 1.58$, Park, Wireman, and Summers (1992); relative MerT concentration, $D(x_{\text{sub}}, \mu) = 4.83$, Ross, Park, and Summers (1989); absolute MerA concentration, $D(x_{\text{sub}}, \mu) = 3.09$, Rinderle, Booth, and Williams (1983); and MerT concentration relative to MerA concentration, $D(x_{\text{sub}}, \mu) = 1.08$, Lee, Gambill and Summers (1989). Each of these experimental conditions is very specific, using an Hg^{2+} concentration at different positions on the induction curve of Figure 6.12 (*in vivo*).

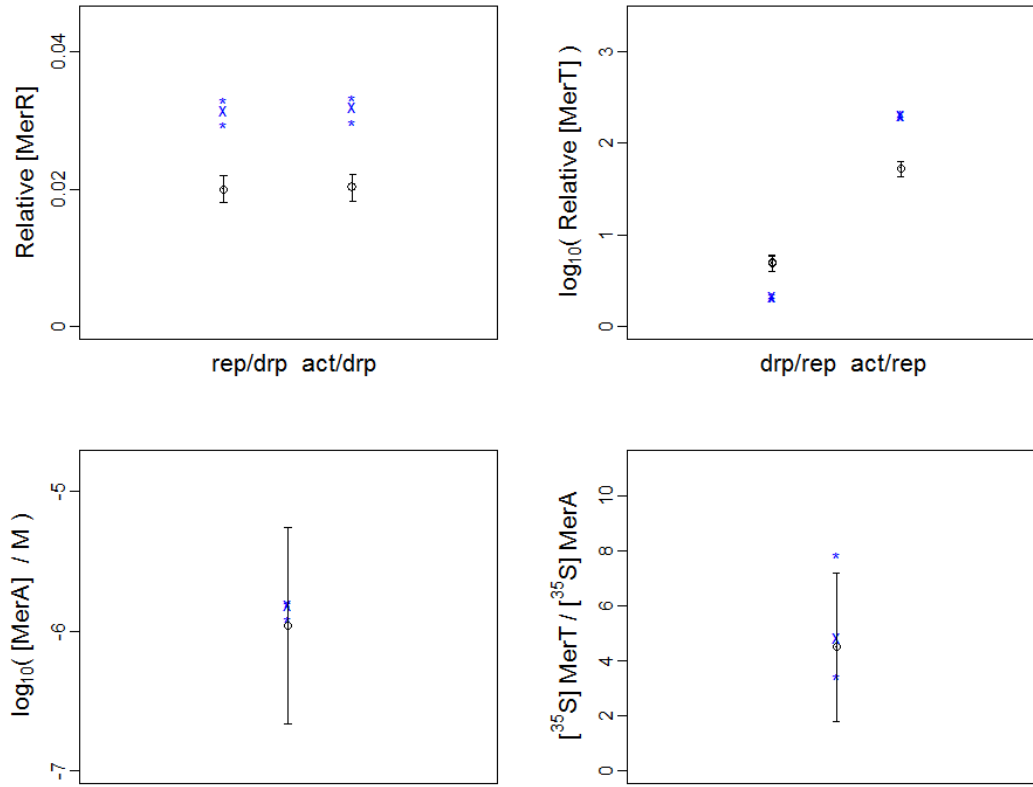


Figure 6.13 Protein synthesis. (Top left) Alkaline phosphatase synthesis from *phoA-merR'* after 20 minutes induction with 2 μM HgCl_2 . Abbreviations: rep = repressed synthesis; drp = derepressed synthesis; act = activated synthesis. (Top right) β -galactosidase synthesis from *merT'-lacZ* after 20 minutes induction with 1 μM HgCl_2 . (Bottom left) MerA concentration after growth in HgCl_2 . (Bottom right) Relative concentrations of induced MerT and MerA in Tn21. Raw data are from (Top left) Table 4 of Park, Wireman, and Summers (1992), (Top right) Table 5 of Ross, Park, and Summers (1989), (Bottom left) the text of Rinderle, Booth, and Williams (1983), and (Bottom right) Lee, Gambill and Summers (1989). The second simulated outputs are shown in blue for the closest fit values (x) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (*).

The model predicts that the maximum possible induction of P_T is equivalent to $a_{2T}/a_{1T} = (a_2 \cdot a_{0A} \cdot a_{0T/0A}) / (a_1 \cdot a_{0A} \cdot a_{0T/0A}) = 542.0$ times. Similarly, $a_{1T}/a_{0T} = 0.465$ times. The parameter $a_{0T/0A}$ has a value of 4.848 which is course the value shown in Figure 6.13 for the data of Lee, Gambill and Summers (1989). The relative $[R]$ is generally independent of the presence of Hg^{2+} ; 0.0315823 in the absence and 0.031951 in the presence of Hg^{2+} (Park, Wireman, and Summers, 1992). R_t will increase marginally in the presence of Hg^{2+} due to the lower affinity of R^* for the DNA leaving a greater fraction of P_R in the derepressed state to transcribe at the higher rate a_{0R} . If all operator is bound by MerR (or MerR- Hg^{2+}), the model predicts that $a_{1R}/a_{0R} = a_R \cdot (a_{0A} \cdot a_{0R/0A}) / (a_{0A} \cdot a_{0R/0A}) = 0.0315822$.

Volatilisation and non-MerA transport

For the data of Philippidis, Malmberg, Hu, and Schottel (1991), $D(x_{\text{sub}}, \mu) = 1.62$ (Figure 6.14). With $v_s = 4.0344\text{e-}15$ L, $v_{\text{sp}} = 2.4250\text{e-}12$ L, $[^{203}\text{S}'_t] = 5.0083\text{e-}6$ M, $[\text{S}] = 5.1951\text{e-}6$ M, $[\text{T}_t] = 2.5389\text{e-}5$ M, and $[\text{A}_t] = 5.2367\text{e-}6$ M, the rate of non-*mer* import is $b_{\text{in}} \cdot [^{203}\text{S}'_t] = 2.17\text{e-}4$ M min⁻¹, while that of non-MerA transport is greater at $k_{t2p} \cdot [^{203}\text{T}^*] = k_{t2p} \cdot [^{203}\text{T}^*] = 4.12\text{e-}4$ M min⁻¹. However, the maximum rate of volatilisation ($d(v \cdot [\text{A}^*])/d[\text{S}] = 0 \rightarrow [\text{S}] = \sqrt{(K_I \cdot K_A)}$) is $v \cdot [\text{A}_t] \cdot [\text{S}] / (K_A + [\text{S}] + [\text{S}]^2 / K_I) = 2.0670\text{e-}3$ M min⁻¹ (when $[\text{S}] = \sqrt{(K_I \cdot K_A)} = 7.477\text{e-}5$ M). Thus, firstly, uptake rate can exceed that of volatilisation. Secondly, the uptake rate of induced non-MerA transport is usually greater than that of non-*mer* import. However, non-MerA transport will saturate (99.99 %) at: $k_{t2p} \cdot [\text{T}_t] \cdot 0.999 = 4.78\text{e-}4$ M min⁻¹ when $b_{\text{in}} \cdot [\text{S}'] = 0.3497$ M min⁻¹ (Equation 6.3). However, non-*mer* import is modelled as non-saturating since in no experiment is $[\text{S}'_t]$ near to $8.06\text{e-}3$ M (i.e. $[\text{S}'_t]$ is usually in the non-saturating range of $1\text{--}10\text{e-}6$ M).

Equation 6.3 99.99 % saturation of non-MerA transport.

$$\frac{[\text{S}'_t][\text{T}_t]}{K_T + [\text{S}'_t]} = [\text{T}_t] \cdot 0.9999; \frac{[\text{S}'_t]}{K_T + [\text{S}'_t]} = 0.9999; [\text{S}'_t] = \frac{0.9999 K_T}{0.0001} = 0.00806199 \text{ M}$$

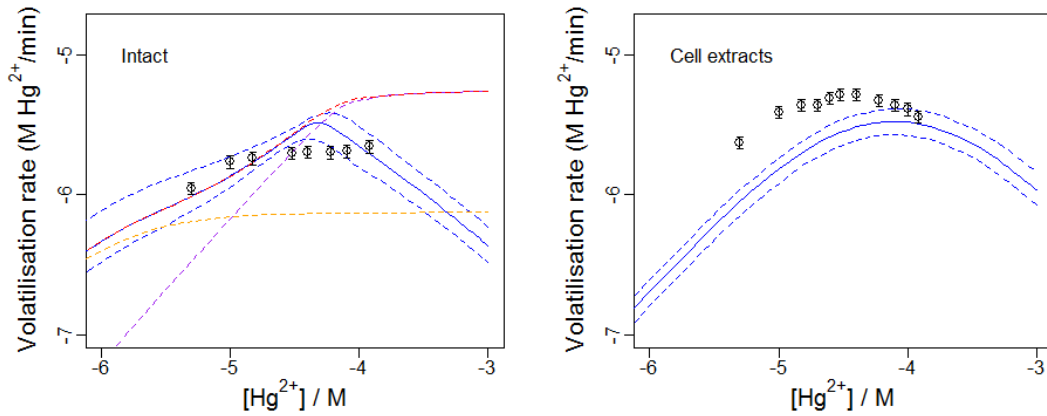


Figure 6.14 Volatilisation in intact (Left) and cell extracts (Right). Raw data are from Figures 2 and 3 of Philippidis, Malmberg, Hu, and Schottel (1991). The second simulated outputs are shown in blue for the closest fit values (solid line) and for one standard deviation either side of the mean simulated outputs when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 (dashed blue lines). To aid the interpretation of this graph, substrate inhibition has been removed from the equations for MerA (dashed red line) and the individual contributions to this curve of non-*mer* uptake (orange dashed line) and non-MerA transport (purple dashed line) are shown.

6.6 Absolute concentrations

The following experiment was designed and simulated. Five phenotypes of non-induced cells were grown in Hg for 120 minutes from $OD_{600} = 0.15$ with growth rate parameters: $r = 0.0268$ and $k_{max} = 5.595$. Each cell contains 1.22 copies of P_T and P_R per genome equivalent. The five phenotypes, shown in Figure 6.15, are: control (Δmer), resistant, hypersensitive ($\Delta merA$), sensitive ($\Delta merT$), and micro-constitutive ($\Delta merR$). The values in Table 6.6 summarises the following text.

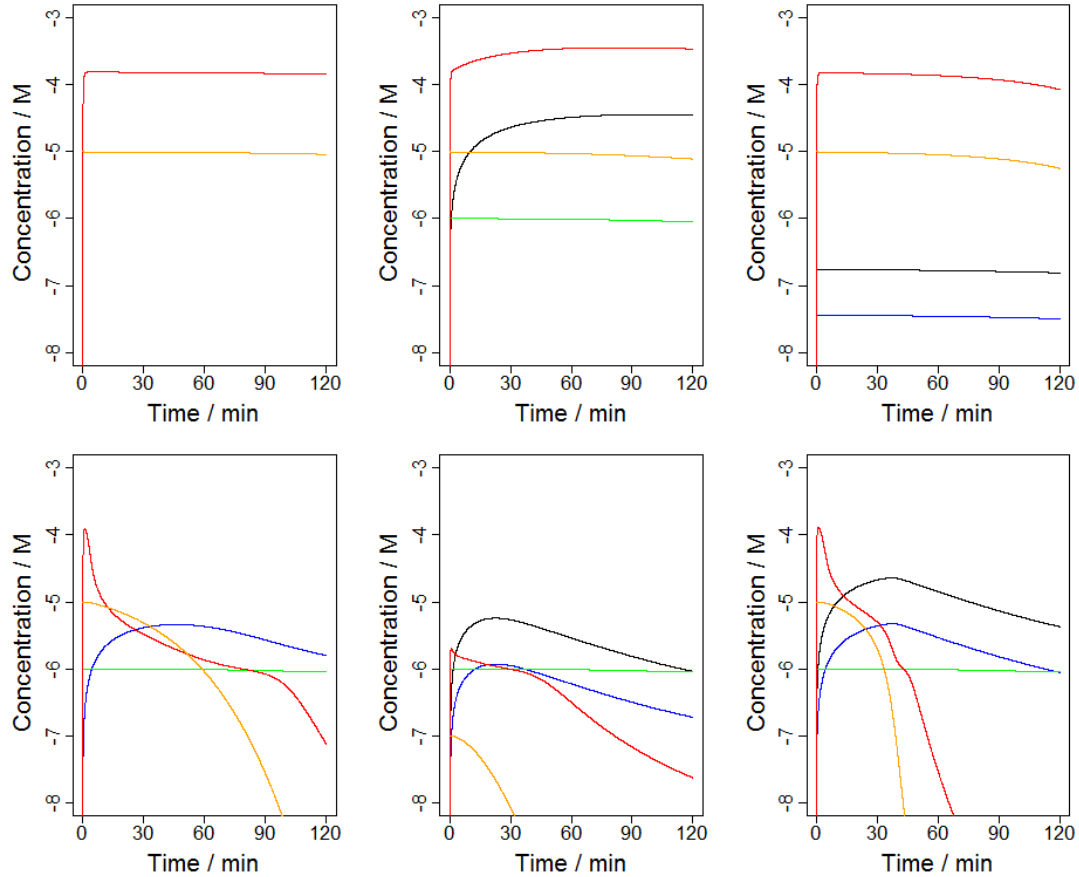


Figure 6.15 Predicted absolute concentrations in cells of each phenotype. (Top left) Control cells with $[S'_t] = 1e-5$ M. (Top middle) Hypersensitive cells with $[S'_t] = 1e-5$ M. (Top right) Micro-constitutive cells with $[S'_t] = 1e-5$ M. (Bottom left) $\Delta merT$ mutants with $[S'_t] = 1e-5$ M. (Bottom middle) Resistant cells with $[S'_t] = 1e-7$ M. (Bottom right) Resistant cells with $[S'_t] = 1e-5$ M. Each *mer* species is S'_t (orange), S_t (red), R_t (green), T_t (black), and A_t (blue).

At time = 0, the repressed steady-state concentrations in the absence of Hg are: $8.02e-8$ M (MerT), $1.65e-8$ M (MerA), and $9.98e-7$ M (MerR). In the derepressed state, the equivalent steady-state concentrations are higher: $1.73e-7$ M (MerT), $3.56e-8$ M (MerA) and 0 M (MerR). Following the addition of Hg, $[R_t]$ remains relatively constant in all phenotypes, as does $[T]$ and $[A]$ in micro-constitutive

cells. In all cases, MerT and MerA are synthesised proportionally such that $[T]/[A] = 4.85$. Once the greater quantity of S_t has been volatilised by MerA, MerT and MerA reach a maximum concentration after which they decline as protein synthesis rate becomes lower than the rate of dilution by cell growth.

In control cells, the quantities of Hg equilibrate quickly between the internal and external compartments of the cell by 3.50 minutes. Then $[S]$ and $[S']$ gradually decline to compensate for the declining growth rate. Control cells are almost identical to micro-constitutive cells except that the latter transport and volatilise more $[S]$. Thus, whereas $[S_t]_{\max}$ in control cells is $1.552e-4$ M at 3.50 minutes, it is $1.515e-4$ M and is achieved more quickly in 2.97 minutes in micro-constitutive cells (both with $[S'_t] = 10 \mu\text{M}$ added).

For example, at $[S] = 1.515e-4$ M and $[S'] = 9.922e-6$ M, the dilution rate is $g \cdot [S] = 3.942e-6$ M min^{-1} , non-*mer* export is $[S] \cdot b_{\text{out}} = 4.168e-4$ M min^{-1} , and non-*mer* import is $b_{\text{in}} \cdot [S'] = 4.304e-4$ M min^{-1} . However, in these micro-constitutive cells, non-MerA transport is adding to $[S_t]$ a further $k_{t2p} \cdot [T^*] = 1.3972e-6$ M min^{-1} , i.e. adding an extra 0.32 % of uptake. Volatilisation also occurs at a low rate in micro-constitutive cells taking away $v \cdot [A_t] \cdot [S] / ([S] + K_A + [S]^2/K_I) = 5.947e-6$ M min^{-1} , i.e. an additional 1.4 % of loss. Hence, in summary, micro-constitutive cells bind less $[S_t]$ because the increase in $[S_t]$ by non-MerA transport rate is less than its loss by volatilisation.

$[S_t]_{\max}$ in sensitive cells is the lowest so far at $1.243e-4$ M (0.97 minutes). This occurs more quickly than in control or micro-constitutive cells. Sensitive cells contain *merA* expressed from an activated P_T , but non-MerA transport is absent. MerA becomes induced to $4.574e-6$ M at 46.08 minutes but to a lower level than in resistant cells. This is because MerT is absent to increase $[S_t]$ while MerA is present to reduce it. Hence, in sensitive cells, a lower $[S_t]$ results in a lower induction of P_T . In contrast, $[S_t]_{\max}$ in resistant cells is $1.295e-4$ M at 1.06 minutes. MerA is induced to a maximum of $4.723e-6$ M, and MerT is induced to $2.290e-5$ M both at 37.38 minutes when $[S_t] = 1.989e-6$ M. With only $[S'_t] = 0.1 \mu\text{M}$ added, $[S_t]_{\max} = 2.011e-6$ M is achieved at 0.84 minutes, and $[A]_{\max} = 1.179e-6$ M and $[T]_{\max} = 5.714e-6$ M at 22.60 minutes when $[S_t] = 1.087e-6$ M. The maximum theoretical induction of MerA in resistant cells is $[A_t] = 1.24711e-9 \cdot (a_{0A} \cdot a_2) / (r \cdot (1 - 0.15/k)) = 8.969e-6$ M, for this experiment.

In hypersensitive cells, $[S]_{\max} = 3.608\text{e-}4$ M at 82.72 minutes. This is 2.79 times greater than the $[S]_{\max}$ of resistant cells. This concentration is so high, that $[S'_t]$ reduces significantly to $8.898\text{e-}6$ M (from $1\text{e-}5$ M) as Hg binds to the cells. Full induction of MerT is reached at 103.87 minutes, when $[T] = 3.606\text{e-}5$ M and $[S] = 3.546\text{e-}4$ M. This is 1.57 times higher than the induced $[T]$ of resistant cells. Hypersensitive induction is higher because $[S_t]$ is higher due to the absence of volatilisation. Hence, a higher $[S_t]$ results in a higher induction of P_T .

Table 6.6 Steady-state, induced and maximum concentrations. For control (non-*mer*), hypersensitive ($\Delta merA$), micro-constitutive ($\Delta merR$), sensitive ($\Delta merT$) and resistant cells, the steady-state concentrations of MerT, MerA, and MerR, and the concentrations and times of maximum induction (with $[S'_t] = 1\text{e-}5$ M) and maximum $[S_t]$ (maximum Hg inside the cell) are shown.

	non- <i>mer</i>	$\Delta merA$	$\Delta merR$	$\Delta merT$	resistant
Steady-state (M) [MerT] [MerA] [MerR]			1.73e-7 3.56e-8 0		8.02e-8 1.65e-8 9.98e-7
$[S_t]_{\max}$ (M)	1.552e-4	3.608e-4	1.515e-4	1.243e-4	1.295e-4
Time (minutes)	3.50	82.72	2.97	0.97	1.06
Induced (M) [MerT] [MerA]		3.606e-5		4.574e-6	2.290e-5 4.723e-6
Time (minutes)		103.87		46.08	37.38

6.7 Analysis of model components

The reactions of Hg resistance, uptake, volatilisation, operator-binding, and protein synthesis are shown in Figures 6.16 and 6.17. These figures plot the dynamics of the system as cells in steady state are induced with $10\text{ }\mu\text{M}$ Hg for 45 minutes. Chloramphenicol is added to inhibit both protein synthesis and growth when the concentrations of MerA and MerT are near their highest induced concentrations for this particular Hg concentration. This is followed by two doses of Hg; $10\text{ }\mu\text{M}$ is added at 60 minutes, and $1\text{ }\mu\text{M}$ is added at 100 minutes.

The most interesting observations are that MerA and MerT are induced proportionally, such that $[T]/[A] = 4.85$. Secondly, if the same dose of Hg is added to fully induced cells, the presence of MerT and MerA prevents Hg from reaching a high level in the cell. However, $[S_t]_{\max} = 1.295\text{e-}4$ M at 1.064 minutes vs. $1.146\text{e-}5$ M at 60.117 minutes, i.e. the model predicts that excess Hg is not sequestered away within the *mer* proteins. What happens is that $d[S_t]/dt$ increases sharply following each dose and it is $[S_t]$ which determines the fraction of A_t in the A^* and $*A^*$ forms. With the addition of $[S'_t] = 10\text{ }\mu\text{M}$, non-MerA transport is only saturates at 92.46 % at 60.177 minutes and occurs at rate

$3.538 \times 10^{-4} \text{ M min}^{-1}$. This rate is much less than that of volatilisation, $7.576 \times 10^{-4} \text{ M min}^{-1}$, for which MerA is 70.49 % saturated with one Hg molecule. However, non-*mer* import is $4.289 \times 10^{-4} \text{ M min}^{-1}$. This makes total uptake ($3.538 \times 10^{-4} \text{ M min}^{-1} + 4.289 \times 10^{-4} \text{ M min}^{-1} = 7.827 \times 10^{-4} \text{ M min}^{-1}$) greater than volatilisation. Hence, the excess [S] that accumulates in the cell, leaves the cell by non-*mer* export, at $2.528 \times 10^{-5} \text{ M min}^{-1}$.

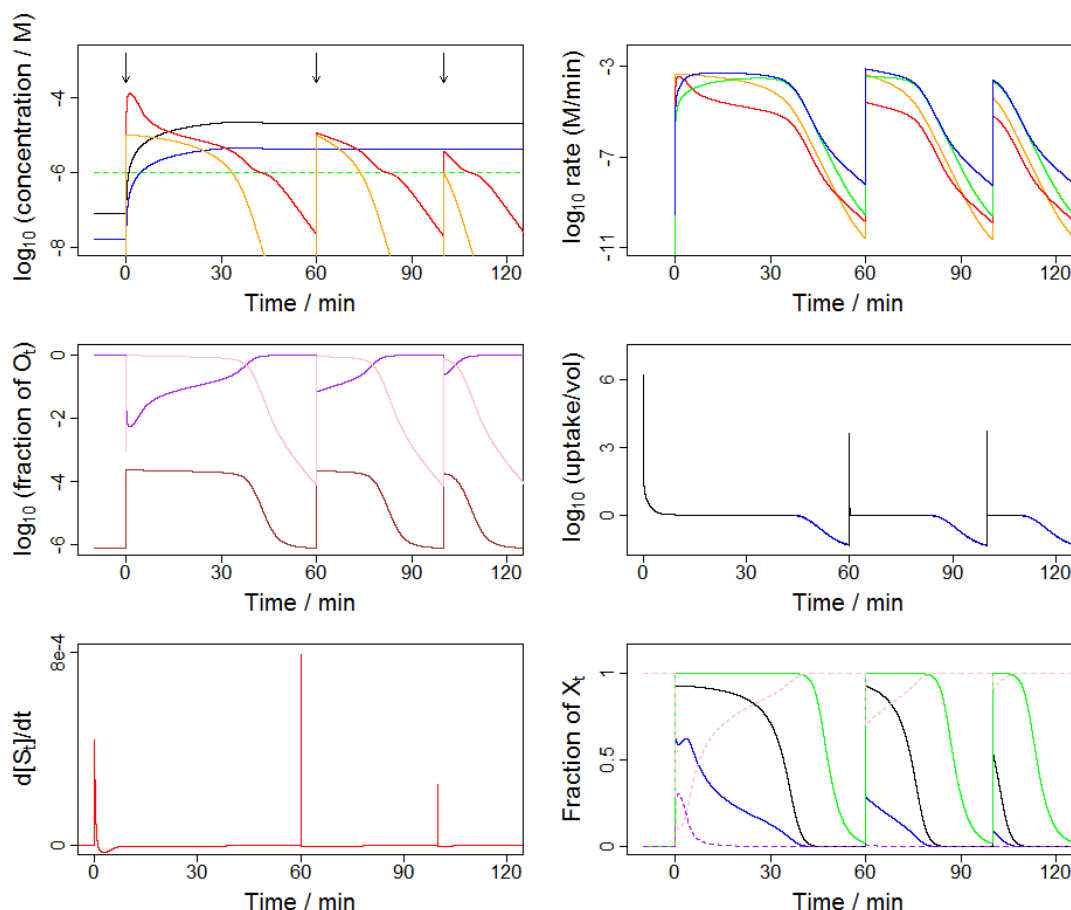


Figure 6.16 Analysis of model components. (Top left) The absolute concentrations of the *mer* entities after two doses of $10 \mu\text{M}$ Hg at 0 and 60 minutes and one dose of $1 \mu\text{M}$ Hg at 100 minutes. S_t (red), S'_t (orange), R_t (green), T_t (black), A_t (blue). (Top right) The change in each term of the $d[S_t]/dt$ differential equation: non-*mer* export (red), non-*mer* import (orange), non-MerA transport (green), volatilisation (blue). (Middle left) The fraction of O_t in the forms: O (brown); OR (purple); OR* (pink). (Middle right) The ratio of uptake to volatilisation: uptake is higher (black); volatilisation is higher (blue). (Bottom left) $d[S_t]/dt$. (Bottom right) The fraction of X_t bound by Hg^{2+} : A^* (blue); A (pink); $*A^*$ (purple); R^* (green); T^* (black).

The binding of Hg by MerA is four orders of magnitude weaker than the binding of Hg by MerR. Therefore, these two *mer* proteins have different K_m parameters for the concentration of free S at which 50 % of the total protein is bound ($K_S =$

2.26e-9 M, $K_A = 2.28e-5$ M). Before the addition of Hg ($= 0$ M), $[R]/[R_t] = 1.0$. This coincides with $[O]/[O_t] = 7.355e-7$ and $[OR]/[O_t] = 9.999$ at steady state ($dR/dt = 0$) when $[R_t] = 9.981e-7$ M. Thus, although the parameter for derepressed synthesis of MerR is relatively high, $a_{0R} = a_{0A} \cdot a_{0R}/a_{0A} = 660.89$, and the parameter, a_{1R} , for repressed synthesis of MerR is relatively low, $a_{0R} \cdot a_R = 20.87$, since $[R]$ is high, MerR is bound to the DNA for the majority of the time. This maintains the operator in the repressed state. Hence, derepressed MerR synthesis is $6.062e-13 \text{ min}^{-1}$ and repressed synthesis is greater at $2.603e-8 \text{ min}^{-1}$. MerT shows a similar relationship for its derepressed ($3.311e-15 \text{ min}^{-1}$) and repressed ($2.093e-9 \text{ min}^{-1}$) synthesis rates even though $a_{0T} = a_{0A} \cdot a_{0T/0A} = 3.609 \text{ min}^{-1}$, and $a_{1T} = a_{0T} \cdot a_1 = 1.678 \text{ min}^{-1}$.

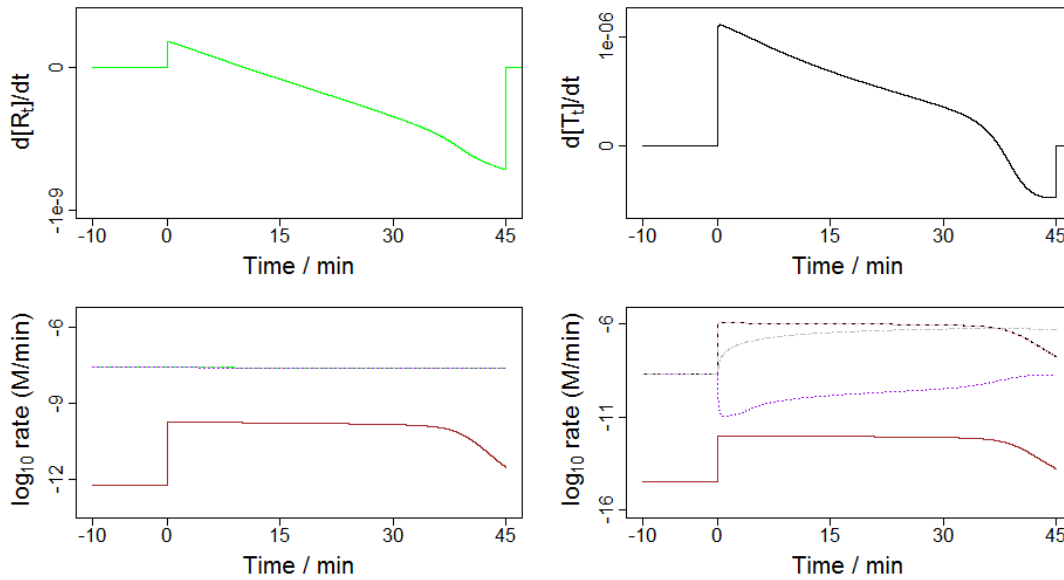


Figure 6.17 Analysis of model components. (Top left) The dynamics of the *mer* entities after a doses of 10 μ M Hg at 0 minutes. (Top left) $d[R_t]/dt$. (Top right) $d[T_t]/dt$. (Bottom left) Protein synthesis of R_t from P_R : repressed or activated synthesis (purple); derepressed synthesis (brown); dilution (grey); total synthesis (green). (Bottom right) Protein synthesis of T_t from P_T : activated synthesis (pink); repressed synthesis (purple); derepressed synthesis (brown); dilution (grey); total synthesis (black).

In general, whether Hg is present or not, the majority fraction ($1 - 7.355e-7$) at 0 minutes to 0.9943 at 1.060 minutes of O_t is in the OR or OR* states, respectively. Due to the increased K_D of R^* for the operator than of R to the operator, derepressed MerR synthesis increases in the presence of Hg^{2+} : $6.062e-13 \text{ min}^{-1}$ (0 minutes) to $1.868e-10 \text{ min}^{-1}$ (0.7575 minutes). This causes an marginal increase in $[R_t]_{max}$ which returns back towards a new steady-state level

as $[S_t]$ declines: $9.981\text{e-}7$ M (0 minutes) to $9.999\text{e-}7$ M (10.0615 minutes) to $9.876\text{e-}7$ M (130 minutes).

Finally, MerT (and MerA) synthesis increases in the presence of Hg and continues to increase until 45 minutes when protein synthesis is inhibited. Derepressed MerT synthesis increases and decreases analogously to derepressed MerR synthesis except that derepressed MerT synthesis is in the order of magnitude $3.311\text{e-}15$ min^{-1} to $1.020\text{e-}12$ min^{-1} (maximum synthesis at 0.7575 minutes). In general, MerR synthesis is equivalent to its repressed synthesis and MerT synthesis equivalent to its activated or repressed synthesis (the other synthesis rates are insignificant in comparison). Therefore, the parameters a_{0A} and a_{0T} can be excluded from future versions of the model. For example, even the repressed MerT synthesis rate is higher than this: $2.093\text{e-}9$ min^{-1} (at steady state) to the minimum rate = $1.137\text{e-}11$ min^{-1} (1.0625 minutes). In comparison, activated MerT synthesis reaches a maximum rate of $1.126\text{e-}6$ min^{-1} at 0.781 minutes.

Notice that, for each induction, non-MerA transport and volatilisation are equal only at the points where their lines cross on each sub-graph (for example, between 60.008 and 60.0085 minutes. This is the point at which non-MerA transport rate becomes lower than volatilisation rate. Equation 6.4 shows that the non-*mer* import and export rates are in equilibrium in non-growing control cells when $[S'] = 6.342\text{e-}2 \times [S]$. For example, if $[S'] = 10\text{e-}6$ M, $[S] = 1.577\text{e-}4$ M.

Equation 6.4 The $[S']/[S]$ for which non-*mer* import and export are equal.

$$b_{in}[S'] = b_{out}[S]$$

$$b_{in}(x[S]) = b_{out}[S]$$

$$x = \frac{[S']}{[S]} = \frac{b_{out}}{b_{in}} = 6.342 \times 10^{-2}$$

A similar equation is derived for how many times more is $[S]$ than $[S']$ when the uptake and loss rates are equal in non-growing resistant cells (Equation 6.5). The ratio $[S]:[S'] = x$ changes with $[S]$, and the root of the quadratic equation can be found numerically.

The pairs of concentrations of $[S]$ and $[S']$ for which uptake and loss are equal are plotted as black lines in Figure 6.18 for $[A_t] = 4.723\text{e-}6$ M (induced resistant cells) and for $1.654\text{e-}8$ M (at steady state). The colour-coded area above each black line represents the pairs $([S'], [S])$ for which loss > uptake and below each black line represents the pairs $([S'], [S])$ for which uptake > loss in the

differential equation $d[S_t]/dt$. Thus, starting with any $([S'], [S])$, at time = 0 minutes, $[S] = 0$ M (or is extremely small). This means that uptake is always greater than loss until $[S]$ increases in the cell. At high concentrations of $[S']$ in induced cells, transport becomes saturated and non-*mer* import > non-MerA transport. However, at low concentrations of $[S']$, non-MerA transport is the dominant uptake rate.

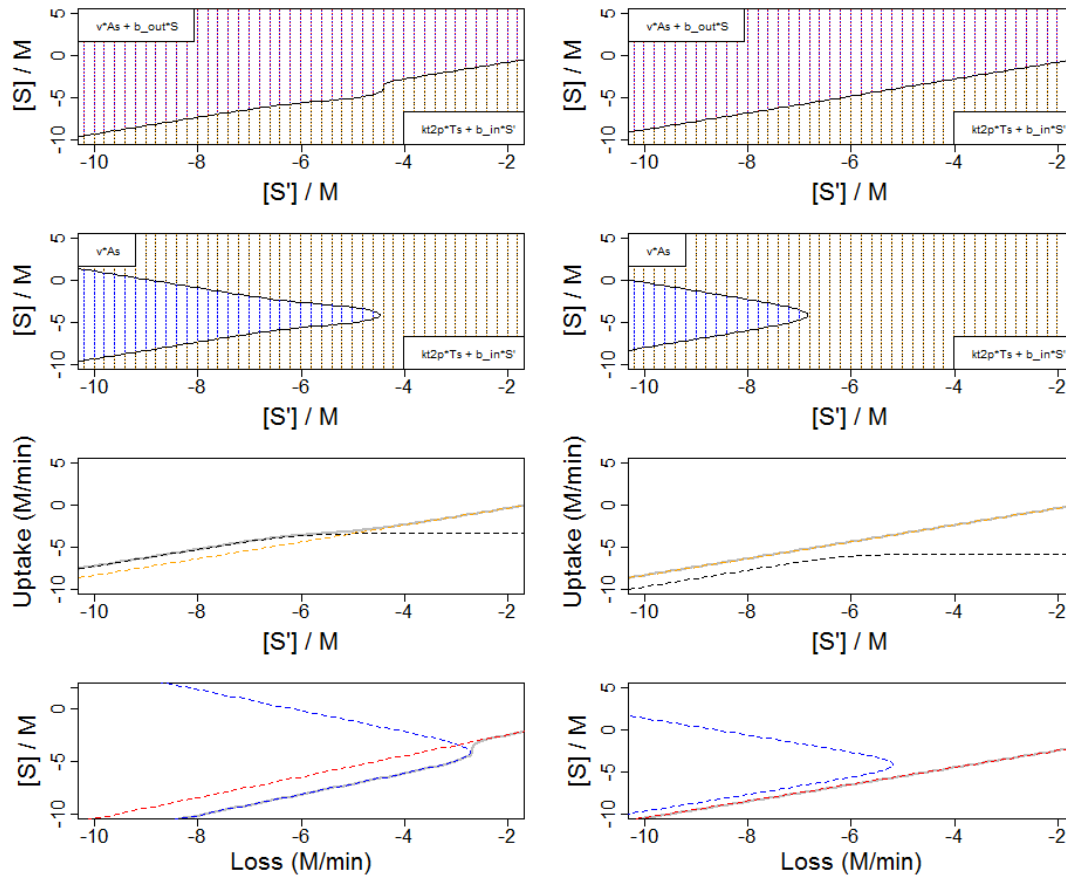


Figure 6.18 Pairs of $[S]$ and $[S']$ for which the rates of Hg^{2+} uptake and loss are equal. (Left) Induced $[A_t] = 2.99e-6$ M and $[T_t] = 1.21e-5$ M. (Right) Steady-state $[A_t] = 1.655e-8$ M and $[T_t] = 8.023e-8$ M. (Top) Pairs of $[S]$ and $[S']$ for which volatilisation + non-*mer* export = non-MerA transport + non-*mer* import; (Centre top) volatilisation = non-MerA transport + non-*mer* import. (Centre bottom) Uptake rate: total uptake (full grey line); non-MerA transport (black dotted); non-*mer* import (orange). (Bottom) Loss rate: total loss (full grey line); volatilisation (blue dotted); non-*mer* export (red). In all graphs, $v_{sp} = 2.425e-12$ L and $v_s = 4.14552e-15$ L.

Equation 6.5 [S] and [S'] for which uptake and loss of Hg²⁺ are equal.

$$b_{out}[S] + v[A^*] = k_{t2p}[T^*] + b_{in}[S']$$

$$b_{out}[S] + \frac{v[A_t][S]}{[S] + K_A + \frac{[S]^2}{K_I}} = \frac{k_{t2p}[T_t](x[S])}{(x[S]) + K_T} + b_{in}(x[S])$$

$$0 = ax^2 + bx + c$$

$$a = (b_{in}[S]^4 + b_{in}[S]^3 K_I + b_{in}[S]^2 K_A K_I)$$

$$b = \left(\begin{aligned} &b_{in}[S]K_T K_A K_I + b_{in}[S]^3 K_T + k_{t2p}[T_t][S]^3 + k_{t2p}[T_t][S]K_A K_I \\ &+ b_{in}[S]^2 K_I K_T + k_{t2p}[T_t][S]^2 K_I \\ &- b_{out}[S]^3 K_I - b_{out}[S]^2 K_A K_I - b_{out}[S]^4 - v[A_t][S]^2 K_I \end{aligned} \right)$$

$$c = (-b_{out}[S]^2 K_I K_T - b_{out}[S]K_A K_I K_T - b_{out}[S]^3 K_T - v[A_t][S]K_I K_T)$$

Something very interesting happens by comparing uptake to loss by volatilisation only. The effect of uptake is to concentrate Hg in the cell above the concentration of Hg in the external medium. Due to substrate inhibition, volatilisation saturates after $[S] = \sqrt{(K_I \cdot K_A)} = 7.477e-5$ M. Therefore, whenever $[S'_t] \geq 3.327e-05$ M, uptake is always greater than volatilisation and volatilisation is rate limiting on itself. Under these circumstances, the volatilisation rate cannot be increased further even though uptake is increasing $[Hg_t]$. This explains the results of Philippidis, Malmberg, Hu, and Shottel (1991) in Figure 6.14. It is only when volatilisation reduces $[S_t]$ and, hence, when $[S'_t]$ goes below $3.327e-05$ M, that uptake may or may not become limiting on the rate of volatilisation (depending on $[S]$). Finally, the position of $[S']$ on Figure 6.18 at which uptake is always greater than volatilisation is $1.453e-7$ M with micro-constitutive non-MerA transport and volatilisation.

6.8 Copy number effects

The model predicts that the steady-state and induced concentrations of all *mer* proteins saturate with increasing plasmid copy number (Figure 6.19).

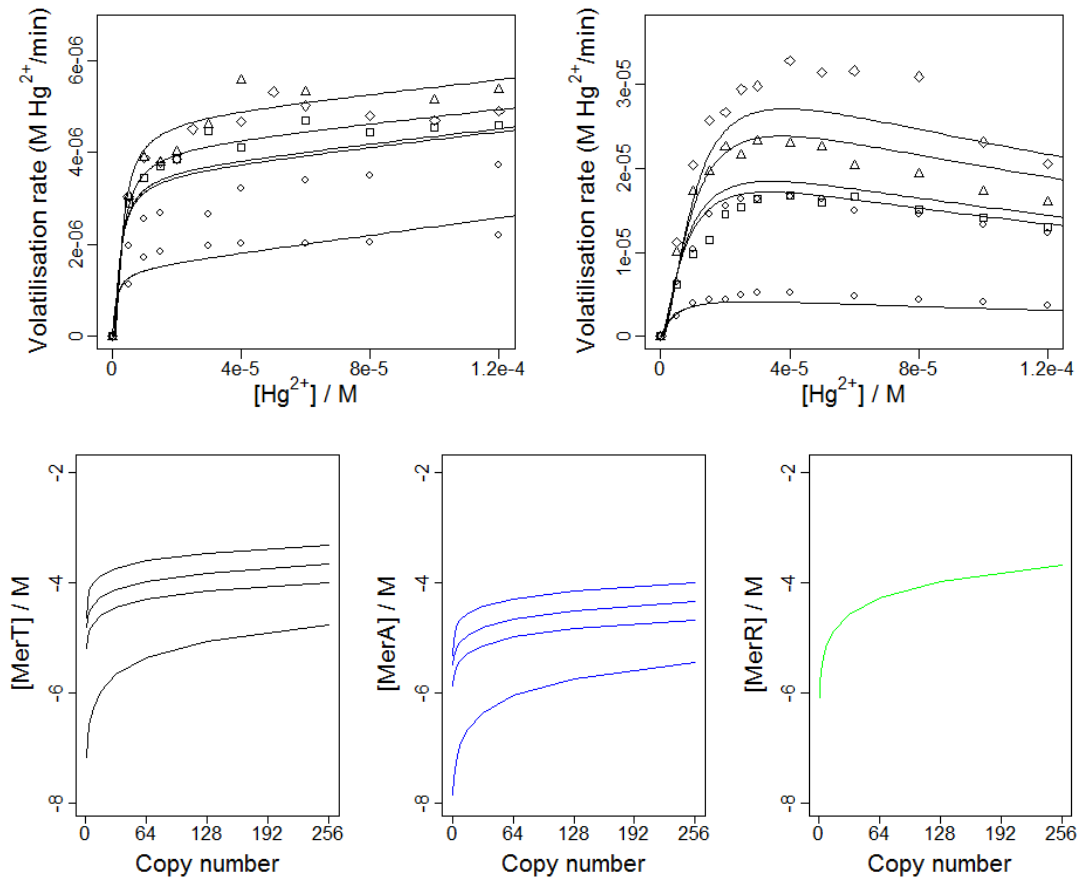


Figure 6.19 The effect of plasmid copy number on the volatilisation rate in intact cells. (Top) The experiment of Philippidis, Malmberg, Hu, and Schottel (1991) was replicated with the following parameters {6.52, 1.09e-13, 1.80e-11, 2.48, 4.12e-6, 2.98e1, 1.78e-14, 3.89e-7, 2.92e2, 4.24, 7.44e2, 5.74e-19, 3.19e3, 4.27e-3, 8.22e-07, 1.66e-4} for a maximum likelihood of -177.59. Plasmid copies per genome equivalent: R100 * (3/2.45), pBRmer ○ (67/2.45), pDU1003 □ (78/2.45), pACYCmer ◇ (124/2.45), pUCmer △ (140/2.45). (Bottom) A similar experiment was performed with plasmid numbers per genome equivalent doubled from 1 until 512 copies. Cells were induced with Hg^{2+} concentrations of 0, 0.1e-6, 1e-6 and 10e-6 M for 45 minutes. Induced concentrations are shown in black (MerT), blue (MerA), and green (MerR).

6.9 Experimental design

Finally, the model can be used to inform which biological experiments should be performed in the future. These are the minimal set of experiments which, ideally, distinguish between each parameter in the model and which contain just enough data points to allow the computer simulation based on those data points to converge. This was achieved by designing nine *in silico* experiments under standardised conditions and then using the model to predict a set of experimental data points for each experiment. The simulated output was then fitted to these data points to show that the nine experiments would indeed be sufficient if performed in a real laboratory.

Details of each experiment and their data points are shown in Figure 6.20. These were designed to allow some variation between the model predictions and the unperformed biological results. This will ensure that the full behaviour of the response is captured. In addition, all the experiments are *in vitro* in order to minimise the cost and difficulty of each assay.

The experiment for MerA does not distinguish between the parameters of binding (K_A) and volatilisation (v). This is due to practical considerations; as soon as MerA binds its substrate, that substrate is converted immediately into product. Similarly, a distinction is not made between the transport parameters, K_T and k_{t2p} . What is anticipated is that biologists will design a clever future experiment which can measure non-MerA transport in the absence of non-*mer* uptake.

As expected, the numerical simulation found the same set of closest-fit parameter values from Table 6.3. However, this time the experiments have been designed with a mathematical model so that any unnecessary correlations between parameters are removed. The correlations between K_A and v and between K_T and k_{t2p} still persist, but their random walks mix very well since these parameters are studied in isolation from other parameters (Figure 6.21).

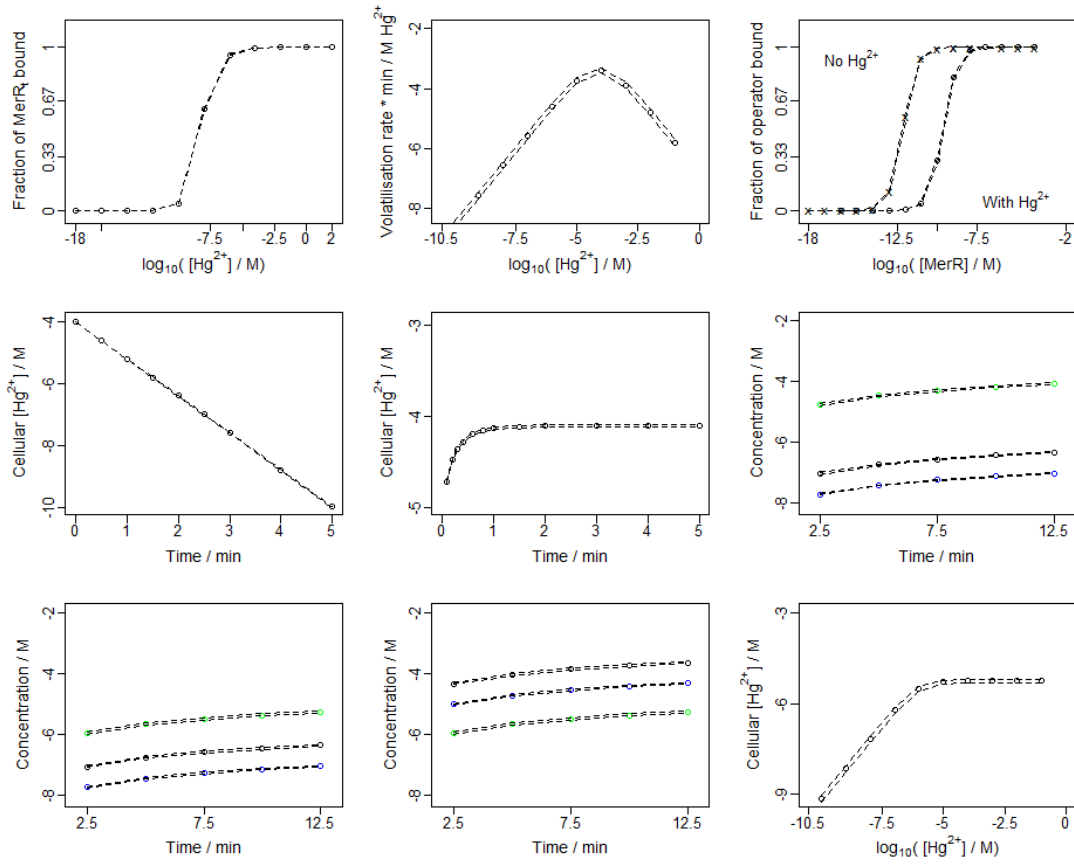


Figure 6.20 Nine experiments. Experiments are numbered from top to bottom and from left to right. The parameters being measured in each experiment are shown in brackets. The data points are the closest-fit values (points). One standard deviation either side of the means when 1000 sets of parameter values are sampled from the posterior distributions of Figure 6.3 are shown as dashed lines. **1.** MerR-Hg²⁺ (K_S): Mix Hg²⁺ and 10e-6 M MerR_t. Measure MerR-Hg²⁺/MerR_t at equilibrium. **2.** v-MerA-Hg²⁺ (K_A , v): Mix Hg²⁺ with 1e-6 M MerA_t. Measure initial volatilisation rate. **3.** MerR(-Hg²⁺) + DNA (K_e , K_{es}): Mix MerR with or without a saturating amount of Hg²⁺ (i.e. 1e-2 M) and 4e-12 M *mer* operator DNA. Measure fraction of operator DNA bound at equilibrium. **4.** Non-*mer* export (b_{out}): Inhibit cell growth and protein synthesis ($OD_{cells} = 0.15$, $r = 0.0268$, $k_{max} = 5.595$). Mix cells with Hg²⁺ such that $[S] = 1e-4$ M at equilibrium. Wash cells with fresh medium containing no Hg²⁺ so that $[S'] = 0$ M and measure $[S]$ at each time point. **5.** Non-*mer* uptake (b_{in} , b_{out}): Mix inhibited cells with 5e-6 M Hg²⁺ and measure $[S]$ at each time point. **6.** DNA → MerX (a_{0A} , $a_{0T/0A}$, $a_{0R/0A}$). Mix 1e-8 M *mer* operator DNA with transcriptional components. Measure protein concentrations of MerA, MerT, and MerR' (truncated, not a regulator). **7.** DNA-MerR → MerX (a_1 , a_R). Mix 1e-8 M *mer* operator DNA with 1e-3 M MerR (saturating). Measure protein concentrations of MerA, MerT, and MerR'. **8.** DNA-MerR-Hg²⁺ → MerX (a_2). Mix 1e-8 M *mer* operator DNA with 1e-3 M MerR (saturating) and 1e-2 M Hg²⁺ (saturating). Measure protein concentrations of MerA, MerT, and MerR'. **9.** Non-MerA transport (K_T , k_{t2p}): Add Hg²⁺ to the outside of cells ($OD_{cells} = 0.15$, $r = 0.0268$, $k_{max} = 5.595$) with membranes containing 1e-6 M MerT. Measure initial rates of transport as the amount of Hg²⁺ on the internal side of the membrane and as MerT-Hg²⁺ after 0.3 minutes.

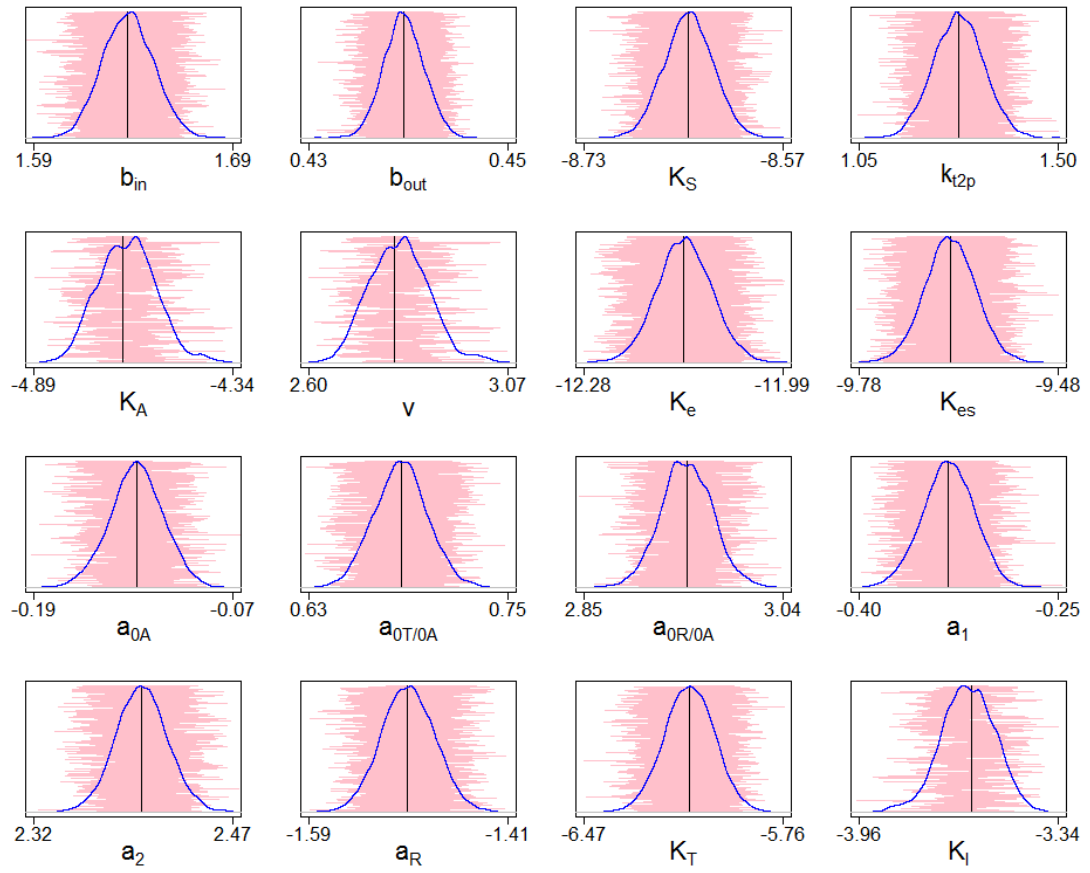


Figure 6.21 Posterior distributions from a simulation of nine experiments. For each parameter, the posterior distributions (blue), random walks of 766172×16 iterations (pink), and the closest-fit values (black lines) are plotted on a $\log_{10}$ scale.

CHAPTER 7: Conclusions

This chapter summarises the significant contributions of this thesis to scientific research. A section is dedicated to each of the eight conclusions which have been found by a mathematical model of mercury resistance.

7.1 Our knowledge of Hg resistance is consistent with the model

The model is based on the following schema. Hg^{2+} enters the cell and binds to the regulator, MerR. MerR controls the expression from the *merR* (P_R) and *merT* (P_T) promoters. In the absence of Hg^{2+} , MerR represses both P_T and P_R . Once Hg^{2+} enters the cell, it binds to MerR. The MerR- Hg^{2+} complex activates expression of *merTP(C)AD* from P_T , but continues to repress the synthesis of MerR from P_R . Thus, MerR- Hg^{2+} increases the synthesis of the transporter (MerP and MerT) and reductase (MerA) proteins. This allows more Hg^{2+} to be transported from MerP in the periplasm, across the inner membrane by MerT, and into the cytoplasm where it is detoxified by MerA to Hg^0 . This volatile product then diffuses from the cell.

The simulation results show that, in induced resistant cells, the rate of non-*mer* import is significantly lower than that of non-MerA transport. Similarly, the rate of non-*mer* export is less than that of volatilisation. The equilibrium concentration of free Hg^{2+} for 50 % of MerR_t to be bound by Hg^{2+} is four orders of magnitude less than that for MerA_t . The equilibrium concentration of MerR_t for 50 % of the DNA to be bound by MerR is 310.63 less than in the presence of Hg^{2+} . MerT synthesis is always 4.85 times greater than that of MerA and synthesis from DNA-MerR is always lower than the respective synthesis from the naked DNA. Specifically, repression by MerR of its own promoter is 14.72 times that of its repression of P_T . Finally, fully activated synthesis of MerT from DNA-MerR- Hg^{2+} is 252 times higher than its synthesis from the naked DNA (or 542 times higher than its fully repressed synthesis from DNA-MerR).

7.2 Closeness of fit is not correlated to experimental variables

The closeness of fit of each data set to the simulated output is not correlated to experiment type, choice of media or plasmid, the chemical form of Hg^{2+} , or to the presence of MerD. Therefore, the assumption to include data from a wide range of experiments is still valid.

7.3 When Hg uptake is and is not rate-limiting on volatilisation

A higher rate of Hg uptake by non-*mer* import and non-MerA transport relative to volatilisation causes MerA to be maximally saturated with Hg^{2+} . Under these

circumstances, volatilisation occurs at its maximum rate, so the concentration of excess Hg in the cell accumulates. However, with a low external concentration of Hg, the rates of volatilisation and uptake are in balance. In this case, uptake can correctly be described as being rate-limiting on volatilisation.

7.4 Hg is not sequestered away within the Mer-Hg²⁺ complexes

When uptake and volatilisation rates are not equal, excess Hg accumulates inside the cell and binds to non-*mer* binding sites. Therefore the hypothesis that Hg is carefully sequestered away within the *mer* proteins of resistant cells in the sequence MerP → MerT → MerA, is not predicted by the model.

7.5 The model predicts absolute *mer* protein concentrations

The model predicts the protein and Hg²⁺ concentrations in cells of each Hg phenotype: control, sensitive, hypersensitive, micro-constitutive, and resistant. These predictions are consistent with our current biological knowledge.

7.6 Plasmid copy number effects are observed in the model

Plasmid copy number effects are captured in the current model.

7.7 Experimental design

Nine experiments are designed to create data about every parameter in the model under standardised conditions. This demonstrates how the model can be used in collaboration with biologists in order to design informative experiments.

7.8 The model identifies areas for future research

The roles and cellular locations of the *mer* proteins are well understood. Each protein appears to remain stable over the course of biological experiments. However, no study has quantified the set of absolute *mer* protein concentrations or demonstrated conclusively the significance of MerC and MerD.

Saturation of the membrane capacity with increasing plasmid copy number may change the relative concentrations of (membrane-bound) MerT and (cytoplasmic) MerA. However, this has not been proven experimentally and the model does not support this hypothesis.

Hg uptake is hypothesised to occur by three processes: non-*mer* import, MerA transport, and non-MerA transport. Each of these processes needs to be studied in isolation.

The cytoplasmic concentration of Hg determines the rate of *mer* protein synthesis through the partitioning of the regulator, MerR_t, into activating (MerR-Hg²⁺) and repressing forms (MerR). However, the order of DNA + MerR + Hg²⁺ binding is unknown. In contrast to the activating effect of Hg²⁺ on the *merT* promoter, Hg can simultaneously inhibit general protein synthesis and cell growth. However, the nature of Hg toxicity is still unknown.

Finally, the interaction of Hg with chemicals in the external growth medium affects its uptake. Total Hg uptake may saturate at 3.8–32 mM, with 80 % bound in the cytoplasm and 20 % in the membrane. These binding sites are now classified into five hypothetical groups. Therefore, new experimental data on cellular Hg concentrations are required so that the model can be expanded to include periplasmic, membranar, cytoplasmic, and external compartments.

7.9 Applications of the model

The metabolic trade-off between synthesis and toxicity

The model can be used to quantify the trade-off between the metabolic cost of increasing the rates of *mer* protein synthesis and the metabolic benefit of reducing the amount of Hg²⁺ binding to the cell. This can be accomplished by assigning the number of ATP molecules required to synthesise each *mer* protein (and for volatilisation and transport) and by assigning the number of ATP molecules used to repair the damage caused by Hg²⁺ binding to the cell. The system can then be simulated to find the solution that minimises the total number of ATP expended by the cell when different concentrations of Hg²⁺ are added to the external medium.

The evolutionary fitness of the *mer* operon

The above procedure can be used for the *mer* operon at each stage during its evolution. For example, what would be the fitness of the cell (measured in ATP spent) if only the MerA enzyme were present? Would the cell conserve more ATP if the MerR regulator were added to the system, or if the MerT/P transporter were added to the membrane? Can the parameter values of the full *mer* operon be modified to achieve a higher conservation of ATP and perhaps predict how the *mer* operon will evolve in the future?

Optimisation of Hg²⁺ bioremediation

What set of parameter values and what combinations of *mer* proteins will remove Hg²⁺ contamination from the environment most efficiently and at the lowest cost?

How long will it take to decontaminate the area? Can Hg^{2+} be removed from other sources, such as that accumulated in human tissue?

In general, these questions are best answered using the computer model because thousands of biological experiments can be tested very quickly *in silico*.

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Appendix A – Standard formatted data

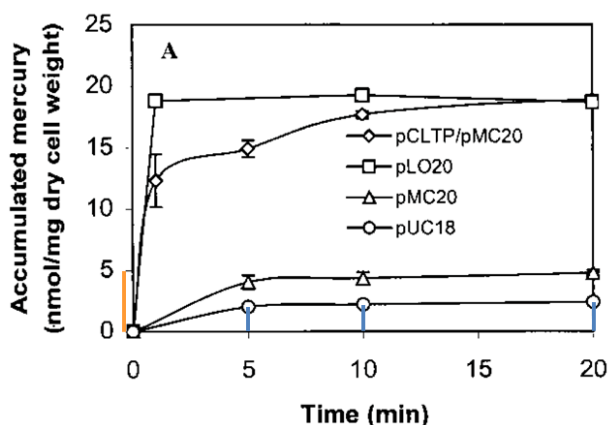
Full details of the experiments which are included in the computer simulation are grouped by research paper and structured in the following format:

- Experimental conditions and methods.
- Measurements of data from original figures.
- Tables of extracted data values.
- Calculations: unit conversions, and standard deviations of data points.
- Comments.

A.1 Bae, Mehra, Mulchandani, and Chen (2001)

Experimental conditions

Organism	<i>Escherichia coli</i> JM109
Plasmids	pUC18 (cloning vector)
Temperature	37 °C
Media	LB
Experiments	Grow cells overnight and resuspend at OD ₆₀₀ = 1 in media with 5 µM ²⁰³ Hg ²⁺ . Measure ²⁰³ Hg ²⁺ in the supernatant by cold-vapor atomic absorption spectroscopy.



Whole-cell binding of 5 µM ²⁰³Hg²⁺ by control cells (O). Raw data are from Figure 4 of Bae, Mehra, Mulchandani, and Chen (2001).

Extraction and unit conversion of data

Time / min	Cellular ²⁰³ Hg ²⁺	
	nmol / mg (dry weight) cells	M
0	0	0
5	1.98	1.08e-4
10	2.12	1.16e-4
20	2.36	1.29e-4

Calculations

Unit conversions	To convert from nmol Hg ²⁺ /mg cells to M Hg ²⁺ , multiply by 1e-9/5.76e-6. At growth rate = 0.0268*(1 - 5.595/5.595) = 0.00 min ⁻¹ , cell mass = 8.80e-14 g, cell volume = 1.61e-15 L. So the volume of 1 mg cells is (1e-3 g/8.80e-14 g) * 1.61e-15 L = 1.83e-5 L.
Standard deviations	20 %

A.2 Bae, Wu, Kostal, Mulchandani, and Chen (2003)

Experimental conditions

Organism	<i>Escherichia coli</i> JM109
Plasmids	pUC18Not
Temperature	37 °C
Media	LB
Experiments	Grow cells overnight and resuspend at OD ₆₀₀ = 1.0 in buffer with 5 µM HgCl ₂ . Wash cells twice with saline. Measure ²⁰³ HgCl ₂ bound to cells by cold-vapor atomic absorptions spectrophotometry.

Whole-cell binding of 5 µM ²⁰³HgCl₂ by control cells. Raw data are from the text of Bae, Wu, Kostal, Mulchandani, and Chen (2003).

Time / min	Binding of HgCl ₂	
	nmol / mg (dry weight) cells	M
60	3.1	1.69e-4

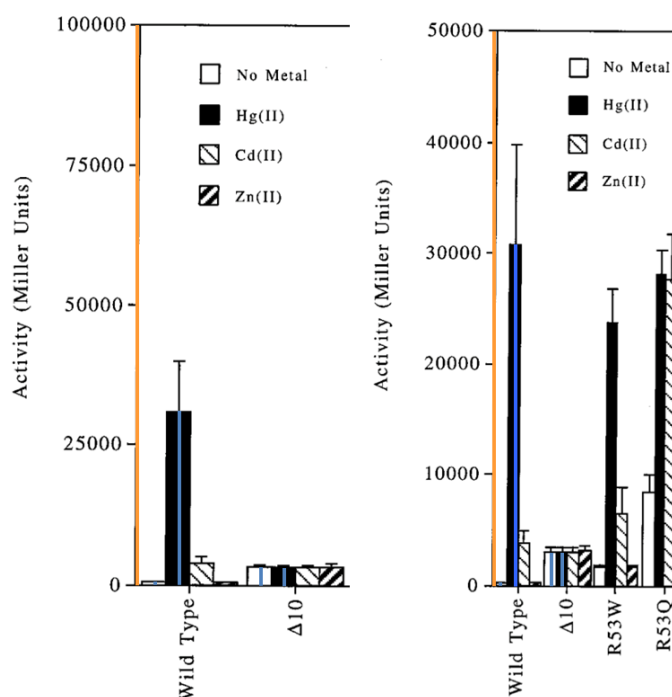
Calculations

Unit conversions	To convert from nmol HgCl ₂ /mg cells to M HgCl ₂ , multiply by 1e-9/5.76e-6. At growth rate = 0.0268*(1 - 5.595/5.595) = 0.00 min ⁻¹ , cell mass = 8.80e-14, cell volume = 1.61e-15 L. So the volume of 1 mg cells is (1e-3 g/8.80e-14 g) * 1.61e-15 L = 1.83e-5 L.
Standard deviations	40 %

A.3 Caguiat, Watson, and Summers (1999)

Experimental conditions

Organism	<i>Escherichia coli</i> CAG1574
Plasmids	Tn21 pWR2 (pBR322): <i>merR</i> -o/p- <i>T'</i> - <i>lacZ</i> pWR10 (pBR322): <i>mer(ΔR)</i> -o/p- <i>T'</i> - <i>lacZ</i>
Temperature	37 °C
Media	M9
Experiments	Grow cells to mid-log phase. Add 2 µM HgCl ₂ for 30 min. Measure β-galactosidase activity.



β-galactosidase activities from *merT'*-*lacZ* fusions after 30 minutes induction with 2 μM HgCl₂. Raw data are from Figures 2 and 3 of Caguiat, Watson, and Summers (1999).

Extraction and unit conversion of data

State	β-galactosidase activity	Relative β-galactosidase concentration
Repressed	287.08	1
	5999.45	1
Derepressed	3014.35	10.50
	3014.35	10.50
	3317.54	0.55
	3222.75	0.54
Activated	30813.4	107.33
	31090.05	5.18

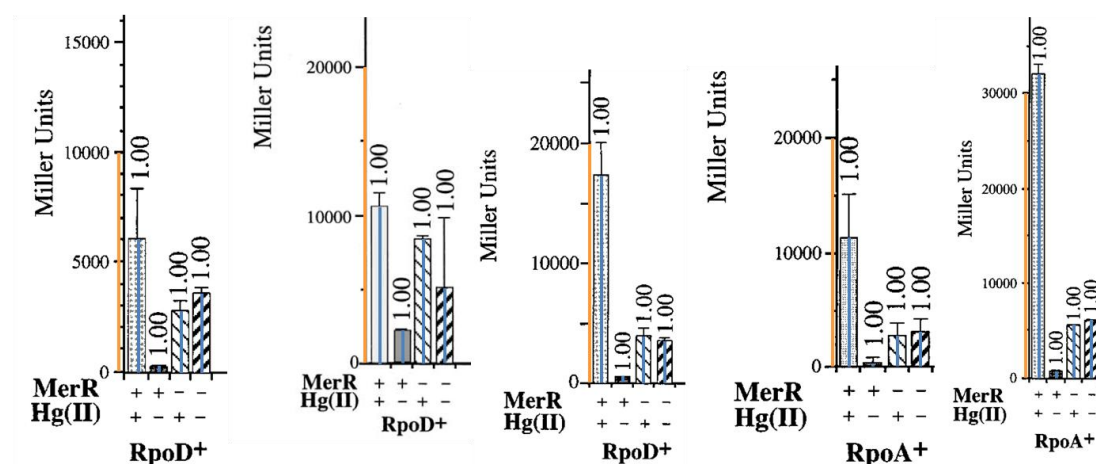
Calculations

Unit conversions	<p>Values are normalised to the β-galactosidase activity observed for the repressed state in each data set.</p> <p>$r = 0.01$; $k = 1.915$; $y_0 = 0.23$; $y_{30} = 0.298$</p> <p>$A = (k - y_0)/y_0$</p> <p>$k/(1+(\exp(1)^{-r*t}) * A) = y_t$</p>
Standard deviations	30 %

A.4 Caslake, Ashraf and Summers (1997)

Experimental conditions

Organism	<i>Escherichia coli</i> CAG1574
Plasmids	pWR2 (pBR322, Tn21): <i>merR</i> -o/p- <i>T'</i> - <i>lacZ</i> pWR10 (pBR322): <i>mer(ΔR)</i> -o/p- <i>T'</i> - <i>lacZ</i> pSA1, pNH9 (pACYC184): <i>merR</i> -o/p pSJ43 (pACYC184): <i>phoA-mer(ΔR)</i> -o/p- <i>T'</i> - <i>lacZ</i>
Temperature	37 °C
Media	LB
Experiments	Dilute overnight cultures 1:20 in LB and grow for an additional 50 Klett units. Add 1 μM HgCl ₂ for 20 min. Measure β - galactosidase and alkaline phosphatase activities.

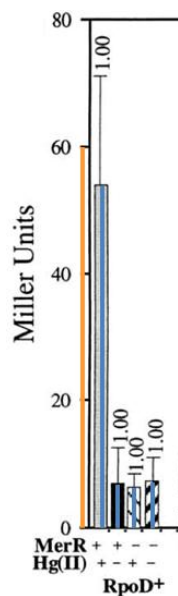


β-galactosidase activities from *merT'*-*lacZ* fusions after 20 minutes induction

with 1 μM HgCl₂. Raw data are from Figures 2a, 3, 5a, 6a and 7a of Caslake, Ashraf and Summers (1997).

Extraction and unit conversion of data

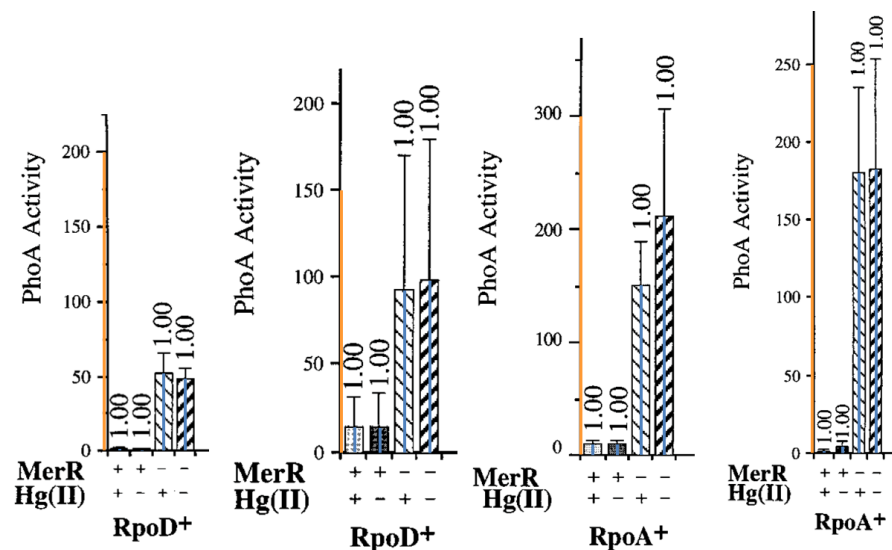
State	β-galactosidase activity	Relative β-galactosidase concentration
Repressed	278.37	1
	2215.19	1
	509.80	1
	246.41	1
	690.79	1
	690.79	1
Derepressed	2826.55	10.15
	8417.72	3.8
	3921.57	7.69
	2669.41	10.83
	5625	8.14
	3618.84	13
	5063.29	2.29
	3490.20	6.85
	3039.01	12.33
	6118.42	8.86
Activated	6102.78	21.92
	10601.27	4.79
	17333.33	34.00
	11334.70	46.00
	32023.03	46.36



β-galactosidase activities from *merT'*-*lacZ* fusions after 20 minutes induction with 1 μM HgCl₂. Raw data are from Figure 4a of Caslake, Ashraf, and Summers (1997).

Extraction and unit conversion of data

State	β-galactosidase activity	Relative β-galactosidase concentration
Repressed	6.79	1
Derepressed	6.28	0.925
	7.20	1.06
Activated	53.82	7.93



Alkaline phosphatase activities from *phoA*-*merR'* fusions after 20 minutes induction with 1 μM HgCl₂. Raw data are from Figures 2b, 4b, 5b, 6b and 7b of Caslake, Ashraf and Summers (1997).

Extraction and unit conversion of data

State	Alkaline phosphatase activity	Relative alkaline phosphatase concentration
Repressed (- Hg)	0.296 14.14 9.40 3.98	0.00617 0.144 0.0444 0.0218
Derepressed: - Hg	48.00 98.48 211.49 182.58	1 1 1 1
+ Hg	51.56 92.68 150.39 180.32	1 1 1 1
Activated (+ Hg)	0.889 14.14 9.40 0.853	0.0172 0.153 0.0625 0.00473

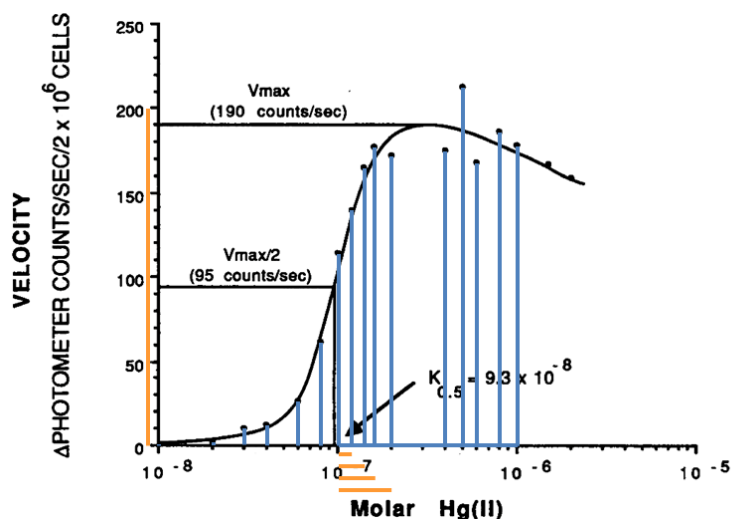
Calculations

Unit conversions	β -galactosidase activities are normalised to the value observed for the repressed state in each data set. Alkaline phosphatase activities are normalised to the value observed for the derepressed state in each dataset.
Standard deviations	35 %

A.5 Condee and Summers (1992)

Experimental conditions

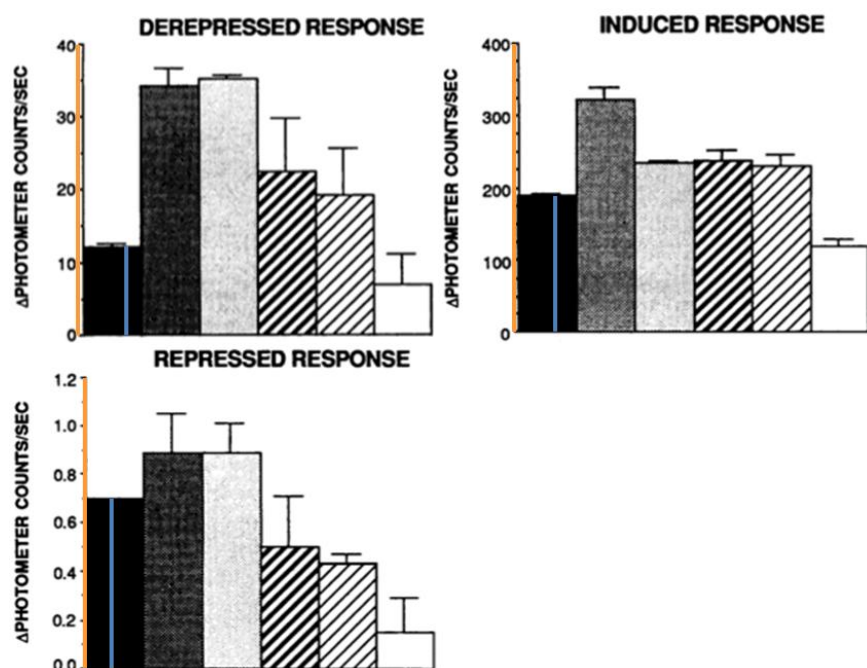
Organism	<i>Escherichia coli</i> K-12 (CB806)
Plasmids	Tn21 pCC306 (pACYC177): <i>merR</i> -o/p-T'- <i>luxAB</i> pCC307 (pACYC177): <i>merΔ(R)</i> -o/p-T'- <i>luxAB</i>
Temperature	Growth: 37 °C Measurements: 35 °C
Media	LB
Experiments	<p>Luciferase activities: Grow cells to mid-log phase and maintain in exponential growth at rate = $\log(6e11/4e11)/15 = 0.027 \text{ min}^{-1}$. Add 5 μL of cell suspension to 995 μL luciferase reaction medium (LRM) with $\text{Hg}(\text{NO}_3)_2$. For the repressed condition only, add 50e-6 L cell suspension to 950 μL LRM. Measure bioluminescence at 35 °C using an ATP-Photometer linked to a strip-chart recorder.</p> <p>Figure 4 of their paper: Data are reported as total (cumulative) photometer counts for 2e6 cells.</p> <p>Figure 5 of their paper: Rates are reported as the maximum change in photometer counts per second for 2e6 cells.</p> <p>Figure 8 of their paper: Add 1 μM $\text{Hg}(\text{NO}_3)_2$ without coumermycin A₁ for 15 minutes. Rates are reported as the change in photometer counts per second for 3e6 cells.</p>



Maximum change in photometer counts from *merT'*-*luxAB* fusions after 15 minutes induction with $\text{Hg}(\text{NO}_3)_2$. Each data point is reported as the maximum change in total photometer counts per second per 2×10^6 cells. This is interpreted as the maximum concentration of luciferase achieved once $\text{Hg}(\text{NO}_3)_2$ and luciferase reaction medium have equilibrated in the cell. Raw data are from Figure 5 of Condee and Summers (1992).

Extraction and unit conversion of data

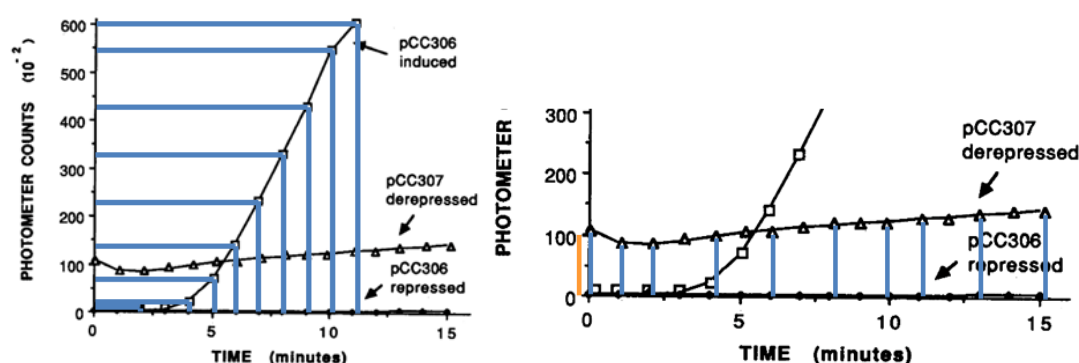
$[\text{HgNO}_3] / \text{M}$	Maximum velocity (Δ in photometer counts / sec / 2×10^6 cells)	Relative luciferase concentration
1×10^{-8}	0.70	1
2×10^{-8}	1.75	2.5
3×10^{-8}	9.77	13.96
4×10^{-8}	11.87	16.96
6×10^{-8}	26.18	37.40
7×10^{-8}	61.08	87.26
1×10^{-7}	113.79	162.56
1.2×10^{-7}	139.62	199.46
1.4×10^{-7}	164.40	234.86
1.6×10^{-7}	176.61	252.30
2×10^{-7}	171.73	245.33
4×10^{-7}	174.52	249.31
5×10^{-7}	212.57	303.67
6×10^{-7}	167.54	239.34
8×10^{-7}	186.04	265.77
1×10^{-6}	177.66	253.80



Maximum change in photometer counts from *merT'*-*luxAB* fusions after 15 minutes induction with $1\mu\text{M}$ $\text{Hg}(\text{NO}_3)_2$. Each data point is reported as the change in total photometer counts per second per 3×10^6 cells. This is interpreted as the maximum concentration of luciferase achieved once $\text{Hg}(\text{NO}_3)_2$ and luciferase reaction medium have equilibrated in the cell. Raw data are from Figure 8 of Condee and Summers (1992).

Extraction and unit conversion of data

State	Velocity (Δ in photometer counts / min / 3×10^6 cells)	Relative luciferase concentration
Repressed	0.70	1
Derepressed	12.22	17.46
Activated	188.19	268.84



Total photometer counts from *merT'*-*luxAB* fusions after induction with $1\mu\text{M}$ $\text{Hg}(\text{NO}_3)_2$. Each data point is reported as the total (and therefore cumulative) photometer count, which is proportional to the concentration of luciferase in the cell. Raw data are from Figure 4 of Condee and Summers (1992).

Extraction and unit conversion of data

Time / min	Velocity (Δ in photometer counts / min / 2×10^6 cells)	Relative luciferase concentration
Repressed	$4.8/15 = 0.32$	1
Derepressed	$(132.95 - 80.68)/(13 - 2) = 4.75$	14.84
Activated		
0	$0.32 - 0 = 0.32$ (repressed)	1.0
2	$(6.49 - 0.32)/2 = 3.09$	9.66
4	$(19.46 - 6.49)/2 = 6.49$	20.28
5	$(68.11 - 19.46)/1 = 48.65$	152.03
6	$(137.84 - 68.11)/1 = 69.73$	217.91
7	$(228.65 - 137.84)/1 = 90.81$	283.78
8	$(327.57 - 228.65)/1 = 98.92$	309.13
9	$(424.86 - 327.57)/1 = 97.29$	304.03
10	$(543.24 - 424.86)/1 = 118.38$	369.94

Calculations

Unit conversions	Figures 4 and 8 of their paper: Values are normalised to the luciferase activity observed for the repressed state in each data set. Figure 5 of their paper: Values are normalised to the maximum luciferase activity when cells are induced with 10^{-8} M $\text{Hg}(\text{NO}_3)_2$.
Cell constants	Cell concentration: $5\text{e-}6 \text{ L} * 4\text{e}11 \text{ cells/L} = 2\text{e}6 \text{ cells}$. $2\text{e}6 \text{ cells} / (995\text{e-}6 + 5\text{e-}6 \text{ L}) = 2\text{e}9 \text{ cells/L}$.
Standard deviations	20 %

Comments

The change in total photometer counts is assumed to be proportional to the concentration of luciferase enzyme. Since the *lux* genes are fused within *merT*, the concentration of the reporter, luciferase, is assumed to be proportional to that of an intact MerT (with its transport function disabled). The degradation/dilution rates for luciferase and MerT are also assumed to be equal.

During the assay, both $\text{Hg}(\text{NO}_3)_2$ and luciferase reaction media (LCM) must diffuse into the cell. LCM is readily absorbed through cell membranes (Condee and Summers, 1992) and is expected not to limit the rate of light production. Further, since $\text{Hg}(\text{NO}_3)_2$ is not volatilised from the system, I expect luciferase to reach a maximum concentration when the concentration of $\text{Hg}(\text{NO}_3)_2$ is also at a maximum, i.e. when $\text{Hg}(\text{NO}_3)_2$ has equilibrated between the internal and external compartments of the cell.

At inducing concentrations of $10^{-7} - 10^{-6}$ M $\text{Hg}(\text{NO}_3)_2$, the maximum luciferase concentration is observed to saturate and then decrease in value after 10^{-6} M (Figure 5 of their paper). This may be due to the inhibition of luciferase synthesis by toxic $\text{Hg}(\text{NO}_3)_2$. To discount these toxic effects, firstly, data after 10^{-6} M $\text{Hg}(\text{NO}_3)_2$ are ignored and it is assumed that the luciferase concentration would saturate and be maintained at this value if toxic effects were not present. The final data point in Figure 4 of their paper is also excluded. The decrease in this value below the previous time point is also expected to be

due to the toxic effects of $\text{Hg}(\text{NO}_3)_2$ and/or LRM becoming limiting on the rate of the luciferase reaction following 10 minutes of induction.

Cells are assumed to grow at 0.027 min^{-1} when diluted 1:1000 in LRM. The growth rate in LRM is unknown. However, the steady-state concentrations in the repressed and derepressed states are not observed to decrease with time (Figure 4 of paper). This suggests that the levels of synthesis and degradation/dilution by growth are maintained at the levels before the cells were transferred from LB into LRM.

A.6 Foster and Brown (1985)

Experimental conditions

Organism	<i>Escherichia coli</i> DU5003
Plasmids	pDU3321 (R100-1): <i>mer</i> (ΔR)-o/p- <i>TPC</i> (ΔAD) pDU3316 (R100-1): <i>merR</i> -o/p- <i>TPC</i> (ΔAD) pDU1355 (pBR322): <i>LacZ</i> - <i>merR'</i> -o/p- <i>TP</i> (ΔCAD) pDU1125 (pACYC184): <i>merR</i> -o/p- <i>TPCAD</i> pDU1126 (pACYC184): <i>mer</i> (ΔR)-o/p- <i>TPCAD</i>
Temperature	37 °C
Media	LB
Experiments	Grow cells to early-log phase in LB. Add $0.5 \mu\text{M}$ HgCl_2 for 30 min. Measure β -galactosidase activity.

β -galactosidase activities from the *merR*-*lacZ* fusion plasmid pDU1355 with pDU3316/21 (*merR*, R100) after 30 minutes induction with $0.5 \mu\text{M}$ HgCl_2 .

Raw data are from Table 2 of Foster and Brown (1985).

State	β -galactosidase activity	Relative β -galactosidase concentration
Repressed (- Hg)	541	0.328
Derepressed:		
- Hg	1650	1
+ Hg	1855	1
Activated (+ Hg)	468	0.252

β -galactosidase activities from the *merR*-*lacZ* fusion plasmid pDU1355 with pDU1125/6 (*merR*, pACYC184) after 30 minutes induction with $0.5 \mu\text{M}$ HgCl_2 .

Raw data are from Table 2 of Foster and Brown (1985).

State	β -galactosidase activity	Relative β -galactosidase concentration
Repressed (- Hg)	570	0.222
Derepressed:		
- Hg	2573	1
+ Hg	2473	1
Activated (+ Hg)	534	0.216

Calculations

Unit conversions	Values are normalised to the β -galactosidase activity observed in the derepressed state for each data set.
Standard deviations	30 %

A.7 Foster, Nakahara, Weiss, and Silver (1979)

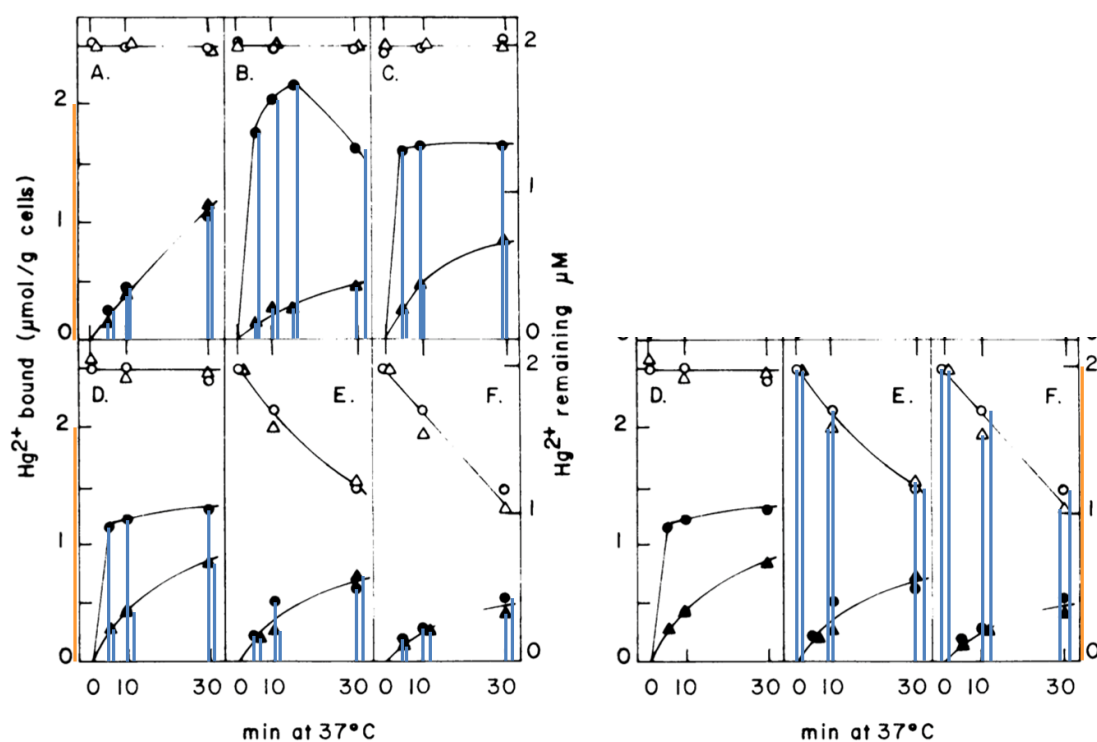
Experimental conditions

Organism	<i>Escherichia coli</i> DU1040
Plasmids	All plasmids are pDU202mer::Tn801 mutants (R100) Whole-cell binding: pDU3307, pDU3321: <i>mer</i> (ΔR)-o/p- <i>TPCAD</i> (micro-constitutive) pDU3303, pDU3316, pDU3302: <i>merR</i> -o/p- <i>TPC</i> (ΔAD) (hypersensitive) Volatilisation: pDU3303-1, pDU3305, pDU3307, pDU3321, pDU3324: <i>mer</i> (ΔR)-o/p- <i>TPCAD</i> (micro-constitutive)
Temperature	37 °C
Media	Tryptone
Experiments	Whole-cell binding: Grow cells to 0.22 g (dry weight) cells/L. Add 0.2e-6 M HgCl ₂ . Harvest cells at 0.34 g (dry weight) cells/L and resuspend in fresh media at 0.68 g (dry weight) cells/L. Add 2 μ M ²⁰³ HgCl ₂ and rifampin. Measure ²⁰³ HgCl ₂ directly from the assay mixture using scintillation spectroscopy. For cell-bound ²⁰³ HgCl ₂ , measure radioactivity remaining on the nitrocellulose filter after washing and filtering cells. Further methods are assumed to be identical to those in Ni'Bhriain, Silver, and Foster (1983) and Nakahara, Silver, and Rownd (1979). Volatilisation: Methods are assumed to be identical to those in Ni'Bhriain, Silver, and Foster (1983), except with chloramphenicol in the assay mixture for measurements of initial volatilisation rates and half-lives of loss.

Volatilisation of 5 μ M ²⁰³HgCl₂ by resistant and micro-constitutive cells. Raw data are from Table 3 of Foster, Nakahara, Weiss, and Silver (1979).

Cell state	Initial rate of ²⁰³ HgCl ₂ loss from assay mixture	
	μ mol/min/g cells	M / min
Micro-constitutive:		
Induced	0.12, 0.098, 0.084, 0.077	3.36e-8, 2.74e-8, 2.35e-8, 2.15e-8
Uninduced	0.14, 0.12, 0.12, 0.12	3.92e-8, 3.36e08, 3.36e08, 3.36e08
Resistant:		
Induced	7	1.96e-6
Uninduced	0.0035	9.80e-10

Cell state	Time / min	% ²⁰³ HgCl ₂ remaining
Micro-constitutive	35	50
Resistant:		
Induced	0.5	50
Uninduced	90	94



Whole-cell binding of $2 \mu\text{M}$ $^{203}\text{HgCl}_2$ by micro-constitutive (E, F), hypersensitive (B, C, D) and control cells (A). Cells are induced (●) or not (○) with $0.2 \mu\text{M}$ HgCl_2 for 45.47 minutes. Raw data are from Figure 2 of Foster, Nakahara, Weiss, and Silver (1979).

Extraction and unit conversion of data

Time / min	$^{203}\text{HgCl}_2$ remaining in assay mixture / M	
	Micro-constitutive	
	Induced	Uninduced
0	1.98e-6 1.99e-6	1.97e-6 1.98e-6
10	1.70e-6 1.70e-6	1.58e-6 1.54e-6
30	1.17e-6 1.16e-6	1.22e-6 1.03e-6

Time / min	Cellular $^{203}\text{HgCl}_2$ in $\mu\text{mol} / \text{g cells}$					
	Micro-constitutive		Hypersensitive		Control cells	
	Induced	Uninduced	Induced	Uninduced	Induced	Uninduced
5	0.22 0.19	0.19 0.13	1.75 1.59 1.14	0.13 0.25 0.27	0.24	0.14
10	0.51 0.28	0.26 0.25	2.03 1.63 1.21	0.26 0.46 0.42	0.44	0.37
15	/	/	2.14	0.25	/	/
30	0.62 0.53	0.72 0.40	1.61 1.64 1.29	0.44 0.83 0.83	1.03	1.13

Time	Cellular $^{203}\text{HgCl}_2$ / M					
	Micro-constitutive		Hypersensitive		Control cells	
	Induced	Uninduced	Induced	Uninduced	Induced	Uninduced
5	1.49e-5 1.28e-5	1.28e-5 8.78e-6	1.18e-4 1.07e-4 7.70e-5	8.78e-6 1.69e-5 1.82e-5	1.62e-5	9.46e-6
10	3.45e-5 1.89e-5	1.76e-5 1.69e-5	1.37e-4 1.10e-4 8.18e-5	1.76e-5 3.11e-5 2.84e-5	2.97e-5	2.50e-5
15	/	/	1.45e-4	1.69e-5	/	/
30	4.19e-5 3.58e-5	4.86e-5 2.70e-5	1.09e-4 1.11e-4 8.72e-5	2.97e-5 5.61e-5 5.61e-5	6.96e-5	7.64e-5

Calculations

Unit conversions	<p>Whole-cell binding: To convert from $\mu\text{mol HgCl}_2/\text{g cells}$ to M HgCl_2, multiply by $1\text{e-}6/0.0148$. At growth rate = $0.0220 \cdot (1 - 0.34/0.498) = 0.00698 \text{ min}^{-1}$, cell mass = $1.716\text{e-}13 \text{ g}$, cell volume = $2.536\text{e-}15 \text{ L}$. So 1 g cells is $1 \text{ g}/1.716\text{e-}13 \text{ g/cell} = 5.83\text{e}12 \text{ cells} = 2.536\text{e-}15 \text{ L} \cdot 5.83\text{e}12 = 0.0148 \text{ L}$.</p> <p>Induction time: $r = 0.0220$; $k = 0.498$; $y_0 = 0.22$; $y_t = 0.34$ $A = (k - y_0)/y_0$ $t = \log(-1 + k/y_t)/A)/-r = 45.47 \text{ min}$</p> <p>Volatilisation: To convert from $\mu\text{mol HgCl}_2/\text{min/g cell}$ to $\text{M HgCl}_2/\text{min}$, multiply by $(1\text{e-}6)/3.571$. These units are the rates of loss of Hg^{2+} from the assay mixture. The cell concentration in the assay mixture is $0.28 \text{ g (dry weight) cells/L}$. So values normalised to 1 g cells/L should be divided by $1/0.28 = 3.571$.</p>
Standard deviations	<p>Whole-cell binding: 35 %</p> <p>Volatilisation: 30 %</p>

A.8 Fox and Walsh (1982)

Experimental conditions

Organism	<i>In vitro</i>
Plasmids	Tn501 pVS1
Temperature	37 °C
Media	Sodium phosphate buffer, 1 mM 2-mercaptoethanol, 200 μM NADPH, 100 μM $^{203}\text{HgCl}_2$.
Experiments	<i>In vitro</i> volatilisation assay

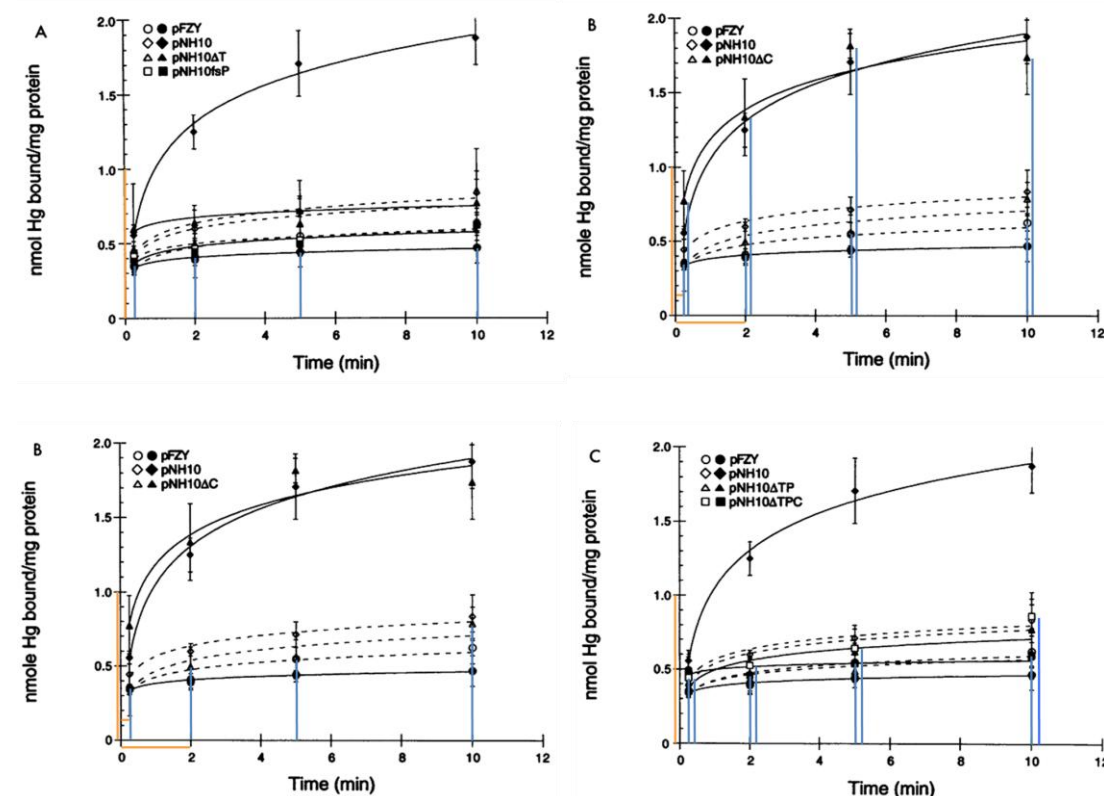
K_m and v of MerA

Parameter	Value
K_m	12 $\mu\text{M HgCl}_2$
v	800 min^{-1}

A.9 Hamlett, Landale, Davis, and Summers (1992)

Experimental conditions

Organism	<i>Escherichia coli</i> MC1061-5, JM103, SK1592
Plasmids	Tn21 pNH10 (monocopy): <i>mer</i> (ΔR)-o/p-TPCA'-LacZ pNH9 (pACYC177): <i>merR</i> -o/p-T'
Temperature	37 °C
Media	Tryptone broth
Experiments	Whole-cell binding: Grow cells to 0.17 g (dry weight) cells/L. Add 0.2 μ M HgCl ₂ for 60 min. Harvest cells and resuspend in fresh media. Add 2 μ M ²⁰³ HgCl ₂ and chloramphenicol. Add 0.1 ml cell suspension to a filter and wash twice with 5 ml broth (Clark, Weiss, and Silver, 1977). The fresh media is assumed to be in contact with the cells for 3.5 seconds. Measure ²⁰³ HgCl ₂ bound to filter by scintillation spectroscopy.



Whole-cell binding of 2 μ M ²⁰³HgCl₂ by *merA* mutants. Cells are induced (●) or not (○) with 0.2 μ M HgCl₂ for 60 minutes. Raw data are from Figure 8 of Hamlett, Landale, Davis, and Summers (1992).

Extraction and unit conversion of data

mer(ΔR)-o/p-TP(ΔC)A'-LacZ + merR

Time / min	Cellular ²⁰³ HgCl ₂			
	μmol / g protein		/ M	
	Induced	Uninduced	Induced	Uninduced
0.27	0.765	0.341	3.10e-5	1.38e-5
2	1.331	0.488	5.39e-5	1.98e-5
5	1.812	0.546	7.34e-5	2.21e-5
10	1.741	0.780	7.05e-5	3.16e-5

mer(ΔR)-o/p-(ΔTPC)A'-LacZ + merR

Time / min	Cellular ²⁰³ HgCl ₂			
	μmol / g protein		/ M	
	Induced	Uninduced	Induced	Uninduced
0.27	0.440	0.435	1.78e-5	1.76e-5
2	0.457	0.522	1.85e-5	2.11e-5
5	0.541	0.642	2.19e-5	2.60e-5
10	0.599	0.858	2.43e-5	3.47e-5

Control

Time / min	Cellular ²⁰³ HgCl ₂			
	μmol / g protein		/ M	
	Induced	Uninduced	Induced	Uninduced
0.27	0.348	0.348	1.41e-5	1.41e-5
2	0.397	0.404	1.61e-5	1.64e-5
5	0.436	0.546	1.77e-5	2.21e-5
10	0.466	0.622	1.89e-5	2.52e-5

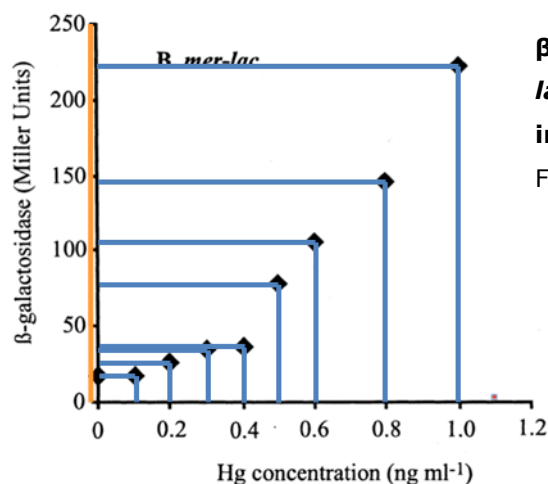
Calculations

Unit conversions	<p>Whole-cell binding: $r = 0.0220$; $k = 0.498$; $y_0 = 0.17$; $t = 60$ $A = (k - y_0)/y_0$ $y_t = k/(1 + A \cdot \exp(1)^{-r \cdot t}) = 0.329$ g (dry weight) cells/L.</p> <p>To convert from μmol HgCl₂/g protein to M HgCl₂, multiply by $1e-6/0.0247$. At growth rate $= 0.0220 \cdot (1 - 0.329/0.498) = 0.00747$ min⁻¹, protein mass = $1.05e-13$ g/cell, cell volume = $2.595e-15$ L. So 1 g protein is $1 \text{ g}/1.05e-13 \text{ g/cell} = 9.524e12$ cells = $2.595e-15 \text{ L} \cdot 9.524e12 = 0.0247$ L.</p>
Standard deviations	Whole-cell binding: 35 %

A.10 Hansen and Sorensen (2000)

Experimental conditions

Organism	<i>Escherichia coli</i> MT102-PIR
Plasmids	Tn21 pRB28 (mini-Tn5 delivery vector pUT- <i>mer-lac</i>): <i>merR-o/p-T'-lacZYA</i>
Temperature	37 °C
Media	ABB1 with 0.2 % glucose (very similar to M9)
Experiments	β-galactosidase activities: Add HgCl ₂ to cells at OD ₆₀₀ = 0.02 and grow for 4 hours to OD ₆₀₀ = 0.16 (Hansen, personal communication). Measure β-galactosidase activity.



β-galactosidase activities from *merT'*-*lacZ* fusions after 215.62 minutes induction with HgCl₂. Raw data are from Figure 2 of Hansen and Sorensen (2000).

Extraction and unit conversion of data

HgCl ₂		<i>merR</i> -o/p- <i>T'</i> - <i>LacZ</i> YA	
ng ml ⁻¹	M	β-galactosidase activity	Relative β-galactosidase concentration
0	0	17.07	1.00
0.1	3.68e-10	17.07	1.00
0.2	7.37e-10	25.60	1.50
0.3	1.10e-9	34.14	2.00
0.4	1.47e-9	36.14	2.12
0.5	1.84e-9	77.81	4.56
0.6	2.21e-9	105.92	6.21
0.8	2.95e-9	145.58	8.53
1.0	3.68e-9	221.89	13.00

Calculations

Unit conversions	Values are normalised to the maximum β-galactosidase activity when cells are not induced with HgCl ₂ . Molecular mass of HgCl ₂ = 271.496 g/mol. To convert from ng/ml to mol/L, multiply by (1e-6/271.496).
Cell constants	Induction time: $r = 0.01$; $k = 1.915$; $y_0 = 0.02$; $y_t = 0.16$ $A = (k - y_0)/y_0$ $t = \log(-1 + k/y_t)/A)/-r = 215.62 \text{ min}$
Standard deviations	20 %

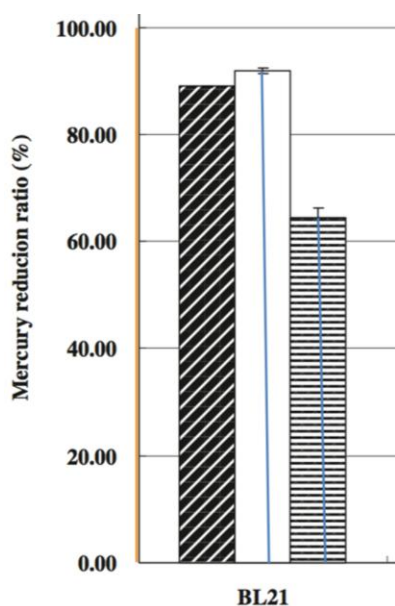
Comments

The mini-Tn5 plasmid (pUT/Km-*mer-lac*) contains the *tnp* transposase gene, the product of which inserts the *mer-lac* cassette randomly and uniquely into the bacterial chromosome. The Tn5 insertion is stable following conjugation and cells will lose the marker (Pip^r, piperacillin resistance), associated with the original pUT delivery plasmid (Herrero, de Lorenzo, and Timmis, 1990). Therefore, a single copy of the *mer* genes is assumed to be present in this experiment.

A.11 Hsieh, Chen, Chang, Endo, and Huang (2007)

Experimental conditions

Organism	<i>Escherichia coli</i> BL21 (DE3)
Plasmids	N/a
Temperature	37 °C
Media	Tris buffer
Experiments	Grow cells from OD ₆₀₀ = 0.6 for 6 hours. Resuspend cells in buffer at 2 g (dry weight) cells/L with 10 µM or 25 µM HgCl ₂ for 2.5 hours. Measure ²⁰³ HgCl ₂ in supernatant by atomic absorption spectrometry.



Whole-cell binding of ²⁰³HgCl₂ by control cells. Data are the percentage of mercury absorbed after 2.5 hours with initial concentration 10 µM HgCl₂ (middle) and 25 µM HgCl₂ (right). Raw data are from Figure 4 of Hsieh, Chen, Chang, Endo, and Huang (2007).

Extraction and unit conversion of data

Initial ²⁰³ HgCl ₂ concentration / M	% bound
10e-6	92.0
25e-6	64.5

Calculations

Standard deviations	10 %
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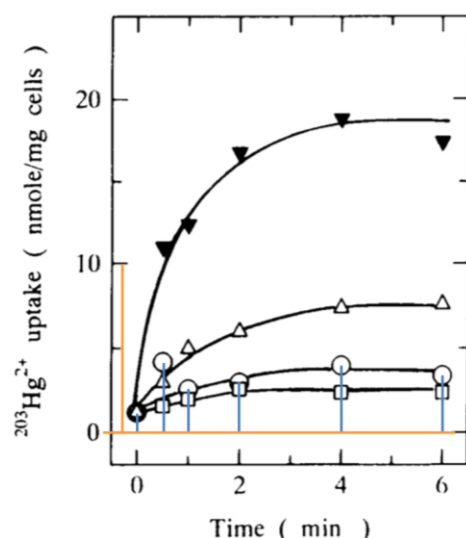
Comments

Stationary-phase cells are resuspended in buffer, so I assume no growth occurs over the 2.5 hours.

A.12 Inoue, Kusano, and Silver (1996)

Experimental conditions

Organism	<i>Escherichia coli</i> JM109
Plasmids	pKK223-3 expression vector
Temperature	37 °C
Media	LB
Experiments	Grow cells to 50 Klett units. Add 2.5 μM $^{203}\text{HgCl}_2$ with chloramphenicol. Add 0.5 ml of cell suspension to a filter and wash three times with 5 ml broth. Measure $^{203}\text{HgCl}_2$ bound to cells by scintillation spectroscopy.



Whole-cell binding of 5 μM $^{203}\text{HgCl}_2$ by control cells (O). Raw data are from Figure 4 of Inoue, Kusano and Silver (1996).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	nmol / mg cells	M
0.125	1.14	1.98e-4
0.5	4.13	7.17e-4
1	2.63	4.57e-4
2	2.97	5.16e-4
4	4.00	6.94e-4
6	3.42	5.94e-4

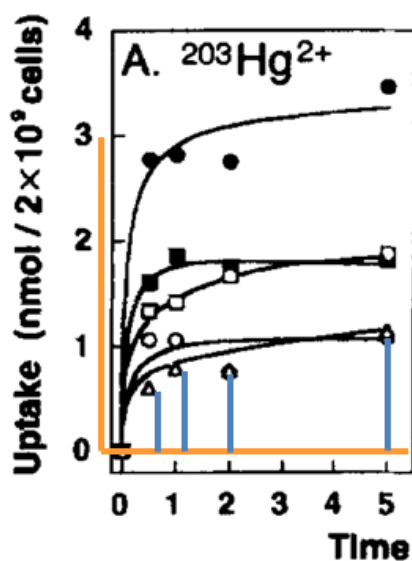
Calculations

Unit conversions	50 Klett units is $\text{OD}_{600} = 0.333$. To convert from nmol HgCl_2/mg cells to M HgCl_2 , multiply by $1\text{e-}9/5.76\text{e-}6$. At growth rate = $0.0268 \times (1 - 0.333/5.595) = 0.0252 \text{ min}^{-1}$, cell mass = $7.12\text{e-}13$, cell volume = $4.10\text{e-}15 \text{ L}$. So the volume of 1 mg cells is $(1\text{e-}3 \text{ g} / 7.12\text{e-}13 \text{ g}) \times 4.10\text{e-}15 \text{ L} = 5.76\text{e-}6 \text{ L}$.
Standard deviations	40 %

A.13 Kiyono, Omura, Fujimori, and Pan-Hou (1995)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pBluescript II SK(+)
Temperature	37 °C
Media	LB
Experiments	Grow cells to $OD_{600} = 1.0$ (late log-phase). Resuspend cells in media with $5 \mu\text{M } ^{203}\text{HgCl}_2$ and chloramphenicol. Add 0.5 ml of cell suspension to a filter and wash three times with 5 ml broth. Measure $^{203}\text{HgCl}_2$ bound to cells by scintillation spectroscopy.



Whole-cell binding of $5 \mu\text{M } ^{203}\text{HgCl}_2$ by control cells (Δ). Raw data are from Figure 4 of Kiyono, Omura, Fujimori, and Pan-Hou (1995).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	nmol / 2e9 cells	M
0	0	0
0.5	0.58	$7.40\text{e-}5$
1	0.76	$9.69\text{e-}5$
2	0.75	$9.57\text{e-}5$
5	1.11	$1.42\text{e-}4$

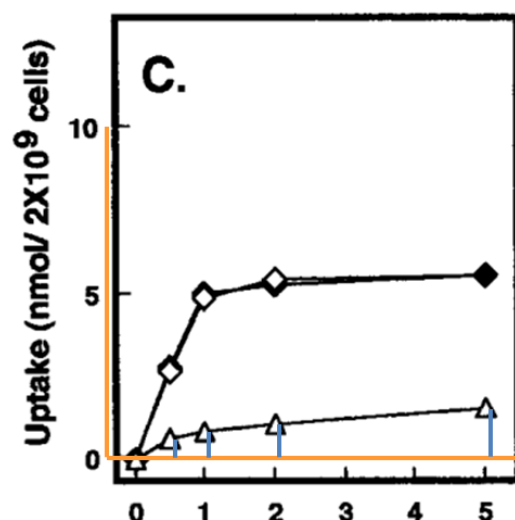
Calculations

Unit conversions	To convert from nmol $\text{HgCl}_2/2\text{e}9$ cells to M HgCl_2 , multiply by $1\text{e-}9/7.84\text{e-}6$. At growth rate = $0.0268 \cdot (1 - 1/5.595) = 0.0220 \text{ min}^{-1}$, cell volume = $3.918\text{e-}15 \text{ L}$. So $2\text{e}9$ cells is $2\text{e}9 \cdot 3.918\text{e-}15 \text{ L} = 7.84\text{e-}6 \text{ L}$
Standard deviations	10 %

A.14 Kiyono and Pan-Hou (1999)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pBluescript II SK(+)
Temperature	37 °C
Media	LB
Experiments	Grow cells to $OD_{600} = 1.0$ (late log-phase). Resuspend cells in media with $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ and chloramphenicol. Add 0.5 ml of cell suspension to a filter and wash three times with 5 ml broth. Measure $^{203}\text{HgCl}_2$ bound to cells by scintillation spectroscopy.



Whole-cell binding of $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ by control cells (Δ). Raw data are from Figure 4 of Kiyono and Pan-Hou (1999).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	nmol / 2e9 cells	M
0	0	0
0.5	0.54	6.89e-5
1	0.78	9.95e-5
2	0.99	1.26e-4
5	1.44	1.84e-4

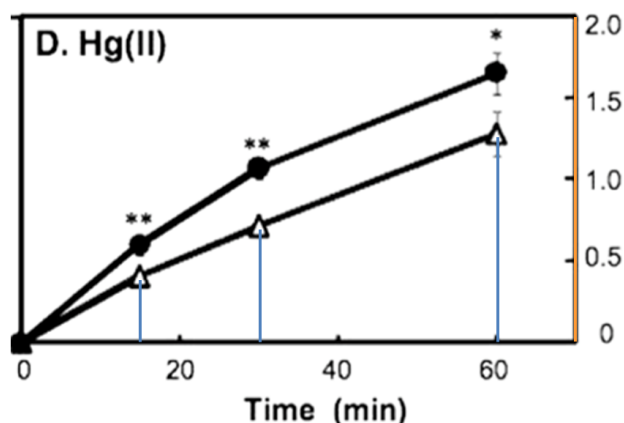
Calculations

Unit conversions	To convert from nmol $\text{HgCl}_2/2\text{e}9$ cells to M HgCl_2 , multiply by $1\text{e}-9/7.84\text{e}-6$. At growth rate = $0.0268 \cdot (1 - 1/5.595) = 0.0220 \text{ min}^{-1}$, cell volume = $3.918\text{e}-15 \text{ L}$. So $2\text{e}9$ cells is $2\text{e}9 \cdot 3.918\text{e}-15 \text{ L} = 7.84\text{e}-6 \text{ L}$
Standard deviations	10 %

A.15 Kiyono, Sone, Nakamura, Pan-Hou, and Sakabe (2009)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pKF19k cloning vector
Temperature	37 °C
Media	LB
Experiments	Grow cells to OD ₆₀₀ = 1.0. Resuspend cells in media with 10 µM ²⁰³ HgCl ₂ and chloramphenicol. Add 0.5 ml of cell suspension to a filter and wash three times with 5 ml broth. Measure ²⁰³ HgCl ₂ bound to cells by atomic absorption spectrometry.



Whole-cell binding of 10 µM ²⁰³HgCl₂ by control cells (Δ). Raw data are from Figure 3 of Kiyono, Sone, Nakamura, Pan-Hou, and Sakabe (2009).

Extraction and unit conversion of data

Time / min	Cellular ²⁰³ HgCl ₂	
	nmol / 2e9 cells	M
0	0	0
15	0.38	4.85e-5
30	0.69	8.80e-5
60	1.26	1.61e-4

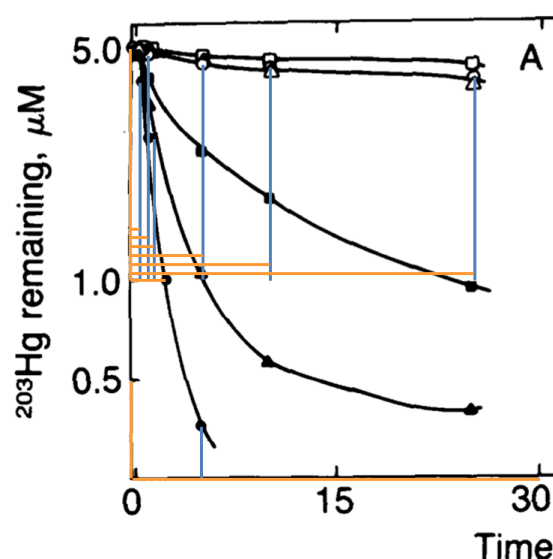
Calculations

Unit conversions	To convert from nmol HgCl ₂ /2e9 cells to M HgCl ₂ , multiply by 1e-9/7.84e-6. At growth rate = 0.0268*(1 - 1/5.595) = 0.0220 min ⁻¹ , cell volume = 3.918e-15 L. So 2e9 cells is 2e9*3.918e-15 L = 7.84e-6 L
Standard deviations	10 %

A.16 Ladagga, Chu, Misra, and Silver (1987)

Experimental conditions

Organism	<i>Escherichia coli</i> strain HB101
Plasmids	pDU1003 (pBR322): <i>merR</i> -o/p-TPCAD
Temperature	37 °C
Media	Tryptone
Experiments	Grow cells to 50 Klett units (mid log-phase). Add 2.5 µM Hg(NO ₃) ₂ for 60 min. Resuspend cells and measure loss of 5 µM ²⁰³ Hg(NO ₃) ₂ from the assay mixture. Further methods are assumed to be identical to those in Ni'Bhriain, Silver, and Foster (1983).



Volatilisation of 5 μM $^{203}\text{Hg}(\text{NO}_3)_2$ by induced (●) and uninduced (○) resistant cells. Raw are data from Figure 1 of Laddaga, Chu, Misra and Silver (1987).

Extraction and unit conversion of data

Time / min	$^{203}\text{Hg}(\text{NO}_3)_2$ remaining in assay mixture / M	
	Induced	Uninduced
0	5e-6	5e-6
0.68	4.42e-6	/
1.27	/	4.87e-6
1.73	3.43e-6	/
2.61	1.0e-6	/
5.34	2.65e-7	4.72e-6
10.26	/	4.69e-6
25.31	/	4.51e-6

Calculations

Standard deviations	20 %
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Comments

I assume no cell growth or protein synthesis occurs of uninduced cells in 5 μM $^{203}\text{Hg}(\text{NO}_3)_2$.

A.17 Lee, Gambill and Summers (1989)

Experimental conditions

Organism	<i>Escherichia coli</i> K-12 strain DS410
Plasmids	Tn21 (pDG106): <i>merR</i> -o/p-TPCAD
Temperature	37 °C
Media	M9 with 0.5 % casamino acids
Experiments	Data are not shown, but authors refer to Jackson and Summers (1982a) for experimental details. Grow cells to 90–120 Klett units. Add 1 μM HgCl_2 to 8×10^{11} minicells/L ($\text{OD}_{620} = 0.2$) and label induced <i>mer</i> polypeptides for 15 mins. Increase concentration to 5 μM HgCl_2 for a further 45 minutes. Quantify the relative concentrations of the <i>mer</i> proteins by densitometry.

Relative concentrations of induced MerT and MerA polypeptides in R100. Raw data are from Lee, Gambill, and Summers (1989).

Relative concentration	
MerT	MerA
4.5	1

Calculations

Standard deviations	60 %
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Comments

MerA is assumed to be in a form which can volatilise HgCl_2 and that all MerT is located in the membrane and can transport HgCl_2 .

Irradiated cells do not grow since their chromosomal DNA is not intact. However, protein synthesis from the *mer* plasmids will occur during labelling. The rate of *mer* synthesis is scaled to reflect the amount of cellular resources during cell growth before irradiation.

A.18 Moore, Distefano, Zydowsky, Cummings, and Walsh (1990)

Experimental conditions

Organism	<i>In vitro</i>
Plasmids	Tn501
Temperature	37 °C
Media	Buffer, 1 mM 2-mercaptoethanol
Experiments	<i>In vitro</i> volatilisation assay

K_m and v of MerA

Parameter	Value
K_m	5 μM HgCl_2
v	340 min^{-1}

A.19 Moore, Miller, and Walsh (1992)

Experimental conditions

Organism	<i>In vitro</i>
Plasmids	Tn501
Temperature	37 °C
Media	Sodium phosphate buffer, 1 mM 2-mercaptoethanol, 200 μM NADPH
Experiments	<i>In vitro</i> volatilisation assay

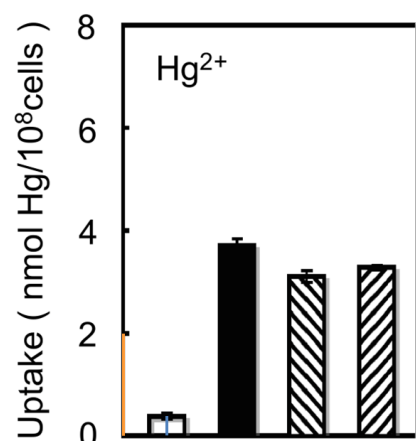
K_m and v of MerA

Parameter	Value
K_m	5.0 μM HgCl_2
v	810 min^{-1}

A.20 Nagata, Kiyono, and Pan-Hou (2006)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pKF18k
Temperature	37 °C
Media	LB
Experiments	Grow cells to mid-exponential phase. Resuspend cells in media with 16 μM Hg^{2+} for 10 minutes. Measure $^{203}\text{Hg}^{2+}$ bound to cells by atomic absorption spectrometry.



Whole-cell binding of 16 μM $^{203}\text{Hg}^{2+}$ by control cells after 10 minutes (white bar).

Raw data are from Figure 2 of Nagata, Kiyono, and Pan-Hou (2006).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{Hg}^{2+}$	
	nmol / 1e10 cells	M
10	0.379	9.27e-6

Calculations

Unit conversions	To convert from nmol $\text{Hg}^{2+}/1\text{e}10$ cells to M Hg^{2+} , multiply by $1\text{e}-9/4.09\text{e}-5$. At growth rate = $0.0268 \cdot (1 - 0.4/5.595) = 0.0249 \text{ min}^{-1}$, cell volume = $4.09\text{e}-15 \text{ L}$. So $1\text{e}10$ cells is $1\text{e}10 \cdot 4.09\text{e}-15 \text{ L} = 4.09\text{e}-5 \text{ L}$
Standard deviations	10 %

A.21 Nakahara *et al.* (1979)

Experimental conditions

Organism	<i>Escherichia coli</i>
Plasmids	Tn21 NR1 (R100), pRR130 (ColE1)
Temperature	37 °C
Media	Sodium phosphate buffer
Experiments	Cell-free volatilisation assay

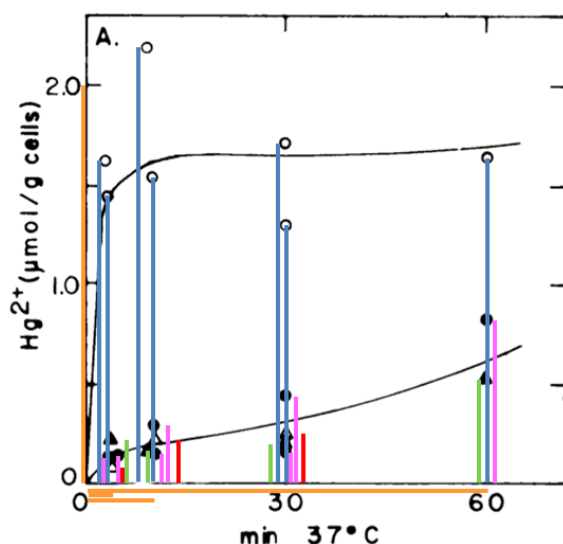
K_m of MerA

Parameter	Value
K _m	8 µM – 10 µM HgCl ₂

A.22 Nakahara, Silver, Miki, and Rownd (1979)

Experimental conditions

Organism	<i>Escherichia coli</i> KP245, NR1
Plasmids	Tn21 pRR130 (ColE1): <i>merR</i> -o/p-TPCAD, <i>EcoRI</i> -H + <i>EcoRI</i> -I fragments pRR132 (ColE1): <i>mer</i> (Δ <i>R</i>)-o/p-(ΔTPCA)D, sensitive, <i>EcoRI</i> -I pRR134 (ColE1): <i>merR</i> -o/p-TPC(ΔAD), hypersensitive, <i>EcoRI</i> -H
Temperature	37 °C
Media	Tryptone broth
Experiments	Whole-cell binding: Grow cells to 0.17 g (dry weight) cells/L. Add 0.2 µM HgCl ₂ for 60 min. Harvest cells and resuspend in media at 0.28 (dry weight) cells/L. Add 2 µM ²⁰³ HgCl ₂ and chloramphenicol. Add 0.1 ml of cell suspension to a filter and wash twice with 5 ml broth (Clark, Weiss, and Silver, 1977). The fresh media is assumed to be in contact with the cells for 3.5 seconds. This is consistent with Shewchuk, Verdine, and Walsh (1989) who completed the processes of washing with 2 ml buffer and filtering mixtures of MerR and Hg ²⁺ within 15 seconds time intervals. Measure ²⁰³ HgCl ₂ bound to filter by scintillation spectroscopy. For induction of hyperbinding, measurements are the average of the 5- and 10 minute results. Induction of volatilisation: Grow and induce cells as for binding experiments, then measure initial rates of volatilisation of 5 µM ²⁰³ HgCl ₂ , in chloramphenicol, after 20 seconds (Hobman, personal communication).



Whole-cell binding 2 µM ²⁰³HgCl₂ by sensitive (triangles) and hypersensitive cells (circles). Cells are induced (white) or not (black). Raw data are from Figure 3 of Nakahara, Silver, Miki, and Rownd (1979).

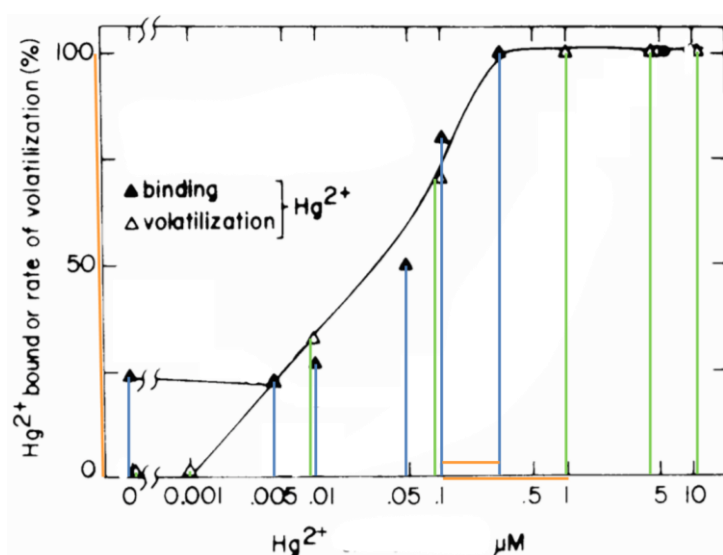
Extraction and unit conversion of data

Hypersensitive

Time / min	Cellular $^{203}\text{HgCl}_2$			
	$\mu\text{mol} / \text{g cells}$		/ M	
	Induced	Uninduced	Induced	Uninduced
3.65	1.62	0.14	1.13e-4	9.72e-6
	1.44	0.13	1.00e-4	9.03e-5
10.02	1.53	0.29	1.06e-4	2.01e-5
	2.19	0.14	1.52e-4	9.72e-6
30	1.30	0.43	9.03e-5	2.99e-5
	1.70	0.15	1.18e-4	1.04e-5
60	1.63	0.82	1.13e-4	5.69e-5

Sensitive

3.65	0.08	0.22	5.56e-6	1.53e-5
10.02	0.22	0.16	1.53e-5	1.11e-5
30	0.25	0.19	1.74e-5	1.32e-5
60	/	0.52	/	3.61e-5



Induction of volatilisation and hyperbinding of $^{203}\text{HgCl}_2$ by resistant (Δ) and hypersensitive cells ($\blacktriangle$). Raw data are from Figure 4 of Nakahara, Silver, Miki, and Rownd (1979).

Extraction and unit conversion of data

HgCl_2 / M	% volatilisation	% binding	normalised volatilisation	normalised binding
0	1.03	23.59	0.0103	0.237
1e-9	1.22	/	0.0122	/
5e-9	/	22.37	/	0.225
1e-8	32.41	26.41	0.325	0.265
5e-8	/	49.62	/	0.498
1e-7	70.11	79.89	0.702	0.802
2.9e-7	/	99.62	/	1
1e-6	99.53	/	0.997	/
5e-6	99.72	/	0.999	/
1e-5	99.81	/	1	/

Calculations

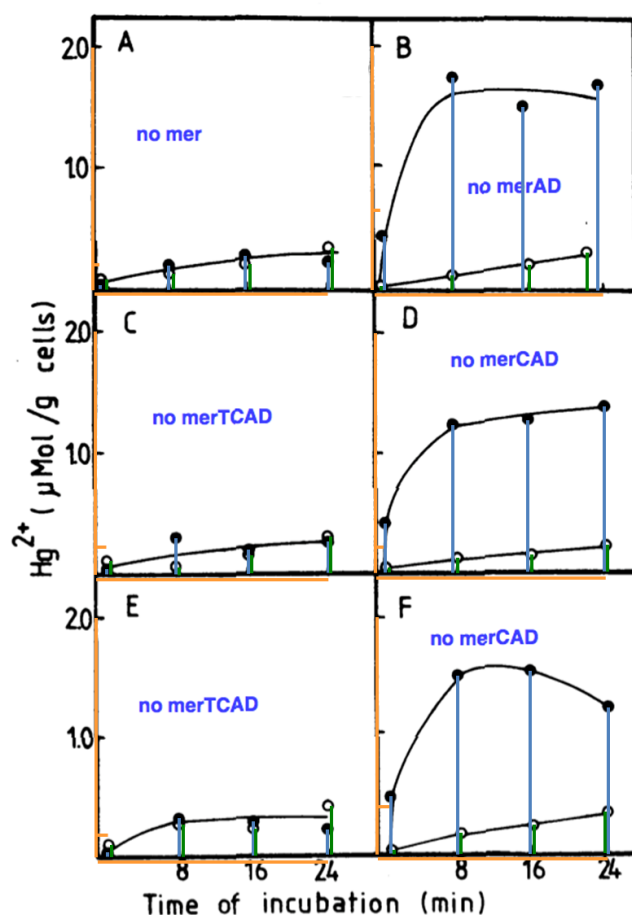
Unit conversions	<p>Whole-cell binding: $r = 0.0220$; $k = 0.498$; $y_0 = 0.17$; $t = 60$ $A = (k - y_0)/y_0$ $y_t = k/(1 + A \cdot \exp(1)^{-r \cdot t}) = 0.329$</p> <p>To convert from $\mu\text{mol HgCl}_2/\text{g cells}$ to M HgCl_2, multiply by $1\text{e-}6/0.0144$ At growth rate $= 0.0220 \cdot (1 - 0.329/0.498) = 0.00747 \text{ min}^{-1}$, cell mass $= 1.803\text{e-}13 \text{ g/cell}$, cell volume $= 2.595\text{e-}15 \text{ L}$. So 1 g cells is $1 \text{ g}/1.803\text{e-}13 \text{ g/cell} = 5.546\text{e}12 \text{ cells} = 2.595\text{e-}15 \text{ L} \cdot 5.546\text{e}12 = 0.0144 \text{ L}$</p>
Standard deviations	<p>Whole-cell binding: 60 % Induction of binding: 40 %. Induction of volatilisation: 30 %.</p>

A.23 Ni'Bhriain, Silver, and Foster (1983)

Experimental conditions

Organism	<i>Escherichia coli</i> ED2030 <i>recA</i> and C600
Plasmids	<p>Whole-cell binding: pDU1179-80: <i>merR</i>-o/p-(ΔTPCAD) pDU1181-2: <i>merR</i>-o/p-TP(ΔCAD) all derived from cloning pDU1003 (R100) proximal to the <i>mer</i>::Tn5 insertions into pUB5572 pDU991 (pRR134 + pUB5572): <i>merR</i>-o/p-TPC(ΔAD)</p> <p>β -galactosidase activities: pDU1124 (<i>merA</i>::Mu d <i>amp lac</i>; derivative of R100-1)</p> <p>Volatilisation: pDU1003: <i>merR</i>-o/p-TPCAD pDU1047 and pDU1073 are <i>merD</i>::Tn5 derivatives of pDU1003</p> <p>Volatilisation from whole and broken cells: pDU202 (R100-1) pRR130 (ColE1 + R100 <i>EcoRI</i> fragment H and I) pDU962 and pDU1003 (pRR130 + pBR322) pDU1125 (pDU1003 + pUB5572)</p>
Temperature	37 °C
Media	Tryptone, LB
Experiments	<p>Whole-cell binding: Grow cells to 50-70 Klett units. Add $0.25 \mu\text{M Hg}^{2+}$ for 60 min. Harvest cells and resuspend in media. Add $5 \mu\text{M }^{203}\text{HgCl}_2$ and tetracycline. Add 0.1 ml of cell suspension to a filter and wash twice with 5 ml broth (Clark, Weiss, and Silver, 1977). The fresh media is assumed to be in contact with the cells for 3.5 seconds.</p> <p>Volatilisation: Induce cells for 60 mins with HgCl_2 then add $5 \mu\text{M }^{203}\text{HgCl}_2$. This paper and Nakahara, Silver, Miki, and Rownd (1979) both refer to Shottel (1978) for further methods. Nakahara, Silver, Miki and Rownd (1979) also refer to Weiss, Murphy, and Silver (1977). However, Schottel (1978) perform the assay <i>in vitro</i>, whereas the other two papers conduct their experiments <i>in vivo</i>. Therefore the methods of Weiss, Murphy, and Silver (1977) will supplement the information provided in this and the Nakahara, Silver, Miki and Rownd (1979) papers. These assumptions are similar to the methods in an earlier paper, Schottel, Mandal, Clark, and Silver (1974). Assumptions: grow cells to mid-log phase (75 Klett units, 0.17 g (dry weight) cells/L) then add HgCl_2. Harvest cells and resuspend in assay buffer at 2.75 g (dry weight)/L.</p>

Experiments	<p>Dilute 10-fold into assay mixture containing $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ and chloramphenicol, resulting in a cell concentration of $0.28 \text{ g (dry weight) cells/L}$. Count radioactivity remaining in assay mixture with a scintillation spectrometer. The initial rates of volatilisation are assumed to be taken at 20 seconds (Hobman, personal communication).</p> <p>Volatilisation from whole cells and broken cells: Induce cells for 60 mins with $5 \mu\text{M}$ HgCl_2. For the broken cell treatment, following induction, full cells are assumed to be suspended in buffer at $2.75 \text{ g (dry weight)/L}$ and lysed. Remove the particulate material (membranes and ribosomes) by centrifugation. (No equilibrium of Hg^{2+} is assumed to occur between MerT-Hg^{2+} and the buffer containing no thiols.) Dilute the broken cell contents 10-fold into the assay mixture containing $5 \mu\text{M}$ $^{203}\text{HgCl}_2$.</p> <p>β -galactosidase activities: Grow cells to early-log phase in LB. Add $0.5 \mu\text{M}$ HgCl_2 for 30 min. Measure β -galactosidase activity.</p>
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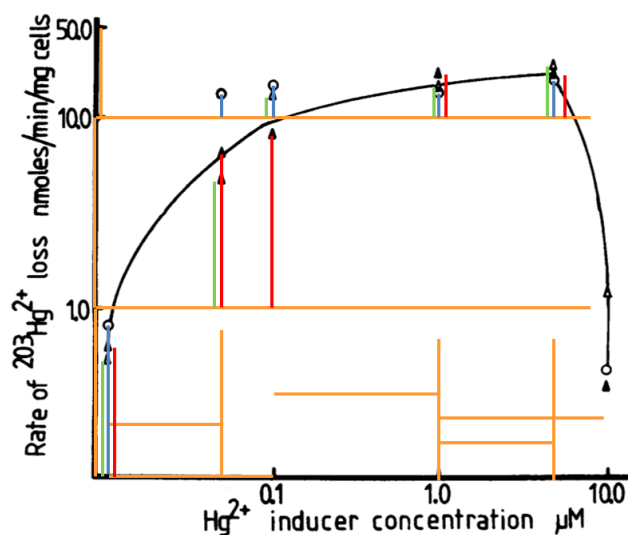


Whole-cell binding of $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ in *merA* mutants with (B, D, F) and without functional *mer* transport (A, C, E). Induced (●), not induced (○). Raw data are from Figure 5 of Ni'Bhriain, Silver, and Foster (1983).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$ in $\mu\text{mol} / \text{g cells}$			
	<i>merR</i> -o/p- <i>TPC</i> (ΔAD): B <i>merR</i> -o/p- <i>TP</i> (ΔCAD): D, F		<i>merR</i> -o/p-($\Delta TPCAD$): A, C, E	
	Induced	Uninduced	Induced	Uninduced
0.9	0.44 0.41 0.48	0.035 0.040 0.036	0.032 0.043 0.036	0.08 0.11 0.10
8	1.74 1.23 1.51	0.12 0.13 0.18	0.20 0.30 0.32	0.13 0.063 0.27
16	1.50 1.26 1.54	0.21 0.14 0.25	0.28 0.20 0.29	0.21 0.15 0.23
24	1.68 1.38 1.24	0.31 0.22 0.35	0.23 0.26 0.21	0.35 0.30 0.41

Time / min	Cellular $^{203}\text{HgCl}_2 / \text{M}$			
	<i>merR</i> -o/p- <i>TPC</i> (ΔAD): B <i>merR</i> -o/p- <i>TP</i> (ΔCAD): D, F		<i>merR</i> -o/p-($\Delta TPCAD$): A, C, E	
	Induced	Uninduced	Induced	Uninduced
0.9	3.33e-5 3.11e-5 3.64e-5	2.65e-6 3.03e-6 2.73e-6	2.42e-6 3.26e-6 2.73e-6	6.06e-6 8.33e-6 7.58e-6
8	1.32e-4 9.32e-5 1.14e-4	9.09e-6 9.85e-6 1.36e-5	1.52e-5 2.27e-5 2.42e-5	9.85e-6 4.77e-6 2.05e-5
16	1.14e-4 9.55e-5 1.17e-4	1.59e-5 1.06e-5 1.89e-5	2.12e-5 1.52e-5 2.20e-5	1.59e-5 1.14e-5 1.74e-5
24	1.27e-4 1.05e-4 9.39e-5	2.35e-5 1.67e-5 2.65e-5	1.74e-5 1.97e-5 1.59e-5	2.65e-5 2.27e-5 3.11e-5



Initial rates of volatilisation of $5 \mu\text{M}$ $^{203}\text{HgCl}_2$ in cells with wild-type *mer* (○) and *merD* mutant plasmids (△, ▲). Raw data are from Figure 4 of Ni'Bhriain, Silver, and Foster (1983).

Extraction and unit conversion of data

HgCl ₂ inducer concentration	Initial rate of ²⁰³ HgCl ₂ loss from assay mixture	
M	nmol min ⁻¹ mg ⁻¹ cells	M / min
1e-8	0.89 0.77 0.69	2.49e-7 2.16e-7 1.93e-7
4.8e-8	20.16 8.24 6.99	5.65e-6 2.31e-6 1.96e-6
1e-7	24.18 9.24 18.89	6.77e-6 2.59e-6 5.29e-6
1e-6	20.58 29.05 23.12	5.76e-6 8.14e-6 6.48e-6
4.96e-6	26.30 28.84 32.86	7.37e-6 8.08e-6 9.20e-6

β-galactosidase activities from *merA::Mu d amp lac* fusion plasmids after 30 minutes induction with 0.5 μM HgCl₂. Raw data are from Table 5 of Ni'Bhriain, Silver, and Foster (1983).

State	β-galactosidase activities	Relative β-galactosidase concentration
Uninduced	5	1
Induced	374	74.8

Volatilisation of 5 μM ²⁰³HgCl₂ from whole and broken cells with wild-type *mer* plasmids. Raw data are from Table 3 of Ni'Bhriain, Silver, and Foster (1983). Values are adjusted since only 90 % of the original reductase activity remains after breaking open cells (Weiss, Murphy, and Silver, 1977).

Plasmid	Initial rate of ²⁰³ HgCl ₂ loss from assay mixture			
	μmol Hg ²⁺ min ⁻¹ g cells ⁻¹		M Hg ²⁺ / min	
	Whole cells	Broken cells	Whole cells	Broken cells
pDU202	22	9	6.16e-6	1.11*2.52e-6
pDU1125	22	53	6.16e-6	1.11*1.48e-5
pRR130	19	38	5.32e-6	1.11*1.06e-5
pDU962	23	40	6.44e-6	1.11*1.12e-5
pDU1003	22	53	6.16e-6	1.11*1.48e-5

Volatilisation of 5 μM ²⁰³HgCl₂ from broken cells with a *merR* mutant plasmid.

Raw data are from Table 7 of Ni'Bhriain, Silver, and Foster (1983).

Plasmid	Initial rate of ²⁰³ HgCl ₂ loss from assay mixture, as a percentage of wild type
pDU1003 <i>merR25::Tn5</i>	0.5 %

Calculations

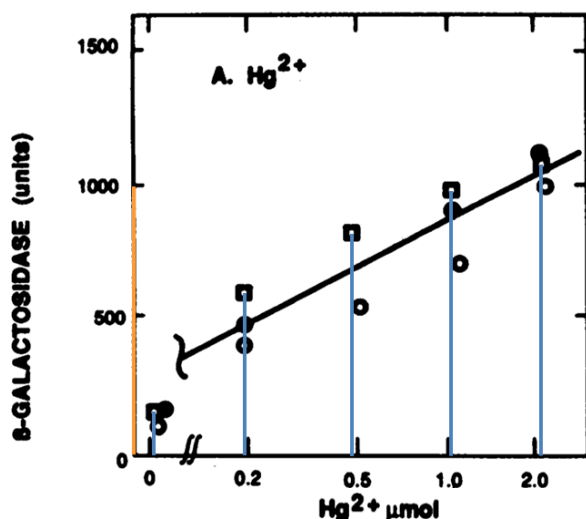
Unit conversions	Whole-cell binding: $r = 0.0220$; $k = 0.498$; $y_0 = 0.136$; $t = 60$ $A = (k - y_0)/y_0$ $y_t = k/(1 + A \cdot \exp(1)^{-r \cdot t}) = 0.291$
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Unit conversions	<p>To convert from $\mu\text{mol HgCl}_2/\text{g cells}$ to M HgCl_2, multiply by $1\text{e-}6/0.0132$. At growth rate = $0.0220 \cdot (1 - 0.291/0.498) = 0.00914 \text{ min}^{-1}$, cell mass = $2.11\text{e-}13 \text{ g/cell} = 4.74\text{e}12 \text{ cells} = 2.79\text{e-}15 \text{ L} \cdot 4.74\text{e}12 = 0.0132 \text{ L}$</p> <p>Volatilisation: To convert from $\mu\text{mol HgCl}_2/\text{min/g cell}$ to $\text{M HgCl}_2/\text{min}$, multiply by $(1\text{e-}6)/3.571$. These units are the rates of loss of HgCl_2 from the assay mixture. The cell concentration in the assay mixture is $0.28 \text{ g (dry weight) cells/L}$. So values normalised to 1 g cells/L should be divided by $1/0.28 = 3.571$.</p> <p>β -galactosidase activities: Values are normalised to the β-galactosidase activity observed when cells are not induced with HgCl_2.</p>
Standard deviations	<p>Whole-cell binding: 35 %</p> <p>Volatilisation: 30 %</p> <p>β-galactosidase activities: 20 %</p>

A.24 Nucifora, Chu, Silver, and Misra (1989)

Experimental conditions

Organism	<i>Escherichia coli</i> DU5111
Plasmids	Tn21 pDU1161 (<i>merA::Mu d amp lac</i> ; derivative of R100, with a Tn5 insertion in <i>merR</i>): <i>mer</i> (ΔR)-o/p-TPCA'- <i>lacZ</i> -(ΔD) pDU1003 (<i>merR</i> in trans): <i>merR</i> -o/p-TPCAD
Temperature	37 °C
Media	LB
Experiments	Grow cells to exponential phase. Add HgCl_2 for 60 min. Measure β -galactosidase activity.



β -galactosidase activities from *merA'*-*lacZ* fusions after 60 minutes induction with HgCl_2 ($\square$). Raw data are from figure 5 of Nucifora, Chu, Silver and Misra (1989).

Extraction and unit conversion of data

HgCl_2 / M	β -galactosidase activity	Relative β -galactosidase concentration
0	162.651	1
0.2e-6	604.418	3.716
0.5e-6	829.317	5.099
1e-6	987.952	6.074
2e-6	1086.345	6.679

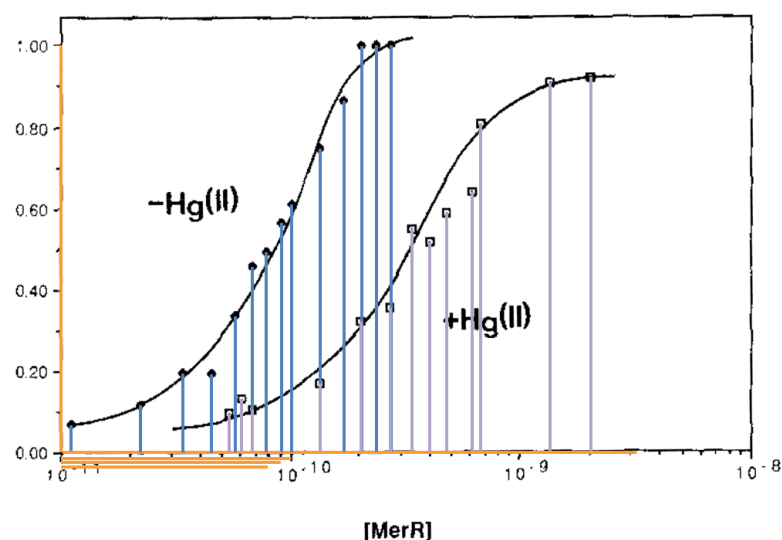
Calculations

Unit conversions	<p>Values are normalised to the β-galactosidase activity observed when cells are not induced with HgCl_2.</p> <p>Induction time: $r = 0.0268$; $k = 5.595$; $y_0 = 0.15$; $y_{60} = 0.6765$ $A = (k - y_0)/y_0$ $k/(1+(\exp(1)^{-r*t}) * A) = y_t$</p>
Standard deviations	30 %

A.25 O'Halloran, Frantz, Shin, Ralston, and Wright (1989)

Experimental conditions

Organism	N/a: experiments conducted <i>in vitro</i>
Plasmids	Tn501 pTO90-16 (a <i>merR</i> -o/p-T' fragment from pT040)
Temperature	24 °C
Media	N/a
Experiments	Incubate 4×10^{-12} M operator DNA with $0.5 \mu\text{M}$ HgCl_2 . Separate equilibrated complexes by gel electrophoresis and quantify bound and unbound fractions by densitometry.



The fraction of *mer* operator bound by MerR in the presence and absence of HgCl_2 . Raw data are from Figure 5 of O'Halloran, Frantz, Shin, Ralston, and Wright (1989).

Extraction and unit conversion of data

[MerR] / M	Fraction of MerR bound to operator	
	no HgCl ₂	with HgCl ₂
1.1e-11	0.07	/
2.2e-11	0.12	/
3.4e-11	0.19	/
4.5e-11	0.19	/
5.4e-11	/	0.10
5.6e-11	0.33	/
6.1e-11	/	0.13
6.7e-11	0.46	0.10
7.93e-11	0.49	/
9e-11	0.56	/
1.0e-10	0.61	/
1.4e-10	0.74	0.17
1.7e-10	0.86	/
2.0e-10	0.997	0.32
2.4e-10	0.999	/
2.7e-10	0.999	0.35
3.4e-10	/	0.55
4.0e-10	/	0.51
4.7e-10	/	0.59
6.1e-10	/	0.64
6.7e-10	/	0.81
1.4e-9	/	0.91
2.0e-9	/	0.92

Calculations

Standard deviations	Values of K _D are given as $4.2 \pm 0.7 \times 10^{-10}$ M (with HgCl ₂) and $1.4 \pm 0.4 \times 10^{-10}$ M (no HgCl ₂). Therefore 22 % is chosen as the s.d. calculated as the average of $(0.7/4.2 = 0.17)$ and $(0.4/1.4 = 0.29)$.
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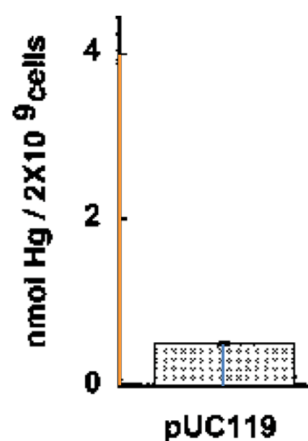
Comments

These results, conducted at 24 °C, may differ from those performed at 37 °C.

A.26 Pan-Hou, Kiyono, Kawase, Omura, and Endo (2001)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pUC119
Temperature	37 °C
Media	LB
Experiments	Grow cells to OD ₆₀₀ = 1.0 (late log-phase). Resuspend cells in media with 16 µM HgCl ₂ for 24 hours. Measure ²⁰³ HgCl ₂ bound to cells by cold-vapor atomic absorption spectroscopy.



Whole-cell binding of $16 \mu\text{M } ^{203}\text{HgCl}_2$ by control cells after 24 hours. Raw data are from Figure 4 of Pan-Hou, Kiyono, Kawase, Omura, and Endo (2001).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	nmol / 2×10^9 cells	M
1440	0.506	6.45×10^{-5}

Calculations

Unit conversions	To convert from nmol $\text{HgCl}_2 / 2 \times 10^9$ cells to M HgCl_2 , multiply by $1 \times 10^{-9} / 7.84 \times 10^{-6}$. At growth rate = $0.0268 \times (1 - 1/5.595) = 0.0220 \text{ min}^{-1}$, cell volume = $3.918 \times 10^{-15} \text{ L}$. So 2×10^9 cells is $2 \times 10^9 \times 3.918 \times 10^{-15} \text{ L} = 7.84 \times 10^{-6} \text{ L}$
Standard deviations	10 %

Comments

Cells are assumed to be grown to $\text{OD}_{600} = 1.0$ then chloramphenicol is added to the assay mixture.

A.27 Park, Wireman, and Summers (1992)

Experimental conditions

Organism	<i>Escherichia coli</i> K12, CAG1574, CB806
Plasmids	Tn21 pSJ51 (pBR322): <i>merR</i> -o/p-T'- <i>lacZ</i> and a ΔmerR derivative pSJ43 (pBR322): <i>phoA</i> - <i>merR</i> '-o/p-T'- <i>lacZ</i> , with MerR <i>in trans</i> on pNH9 (p15A)
Temperature	37 °C
Media	LB
Experiments	Grow cells to 25 Klett units. Add $2 \mu\text{M } \text{HgCl}_2$ for 20 min. Measure β -galactosidase or Alkaline phosphatase activities.

β -galactosidase activities from *merT'*-*lacZ* fusion plasmids after 20 minutes induction with 2 μ M HgCl₂. Raw data are from Table 2 of Park, Wireman and Summers (1992).

State	β -galactosidase activity	Relative β -galactosidase concentration
Repressed	657	1
Derepressed	(2435+2296)/2	3.60
Activated	19837	30.19

Alkaline phosphatase activities from *phoA*-*merR'* fusion plasmids after 20 minutes induction with 2 μ M HgCl₂. Raw data are from Table 3 of Park, Wireman and Summers (1992).

State	Alkaline phosphatase activity	Relative alkaline phosphatase concentration
Repressed (- Hg)	31	0.0200
Derepressed: - Hg + Hg	1547 (-Hg), 1436 (+Hg)	1 1
Activated (+ Hg)	29	0.0202

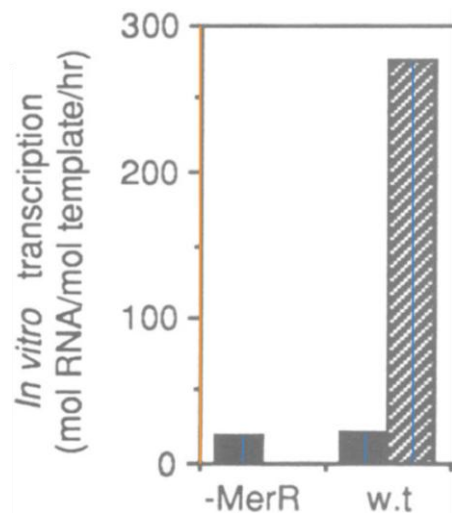
Calculations

Unit conversions	β -galactosidase activities are normalised to the value observed for the repressed state in each data set. Alkaline phosphatase activities are normalised to the value observed for the derepressed state in each dataset.
Standard deviations	10 %

A.28 Parkhill, Ansari, Wright, Brown, and O'Halloran (1993)

Experimental conditions

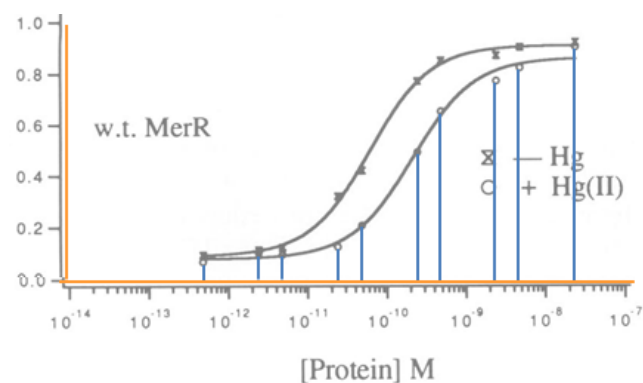
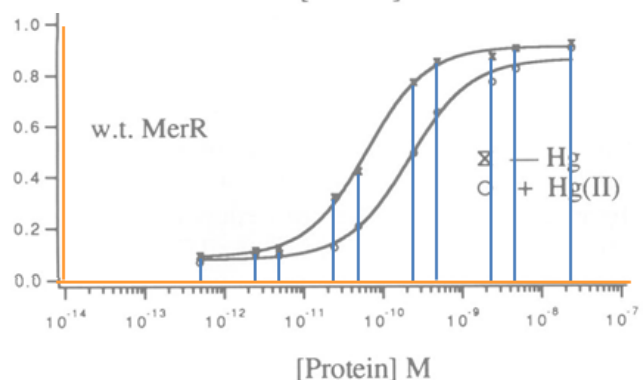
Organism	N/a: experiments conducted <i>in vitro</i>
Plasmids	Tn501 Abortive transcription: A <i>mer</i> (ΔR)-o/p- <i>T'</i> operator fragment from pTO40. Gel retardation: Operator DNA is a HindIII-EcoRV fragment of pAA15. MerR is purified from pTO90-16.
Temperature	Abortive transcription: 37 °C Gel retardation: 10-12 °C
Media	Buffer
Experiments	Abortive transcription: Incubate 0.4e-9 M operator DNA, 50e-9 M MerR, and 10 μ M HgCl ₂ for 45 min. Start reaction and continue for 15 mins. Gel retardation: Incubate 5e-12 M operator DNA with MerR in the presence or absence of 0.75 μ M HgCl ₂ . Separate equilibrated complexes by gel electrophoresis and quantify bound and unbound fractions with a phosphoimager.



***in vitro* transcription rates from *merT'* with or without 15 minutes induction with 10 μM HgCl_2 .** Raw data are from Figure 2 of Parkhill, Ansari, Wright, Brown, and O'Halloran (1993).

Extraction and unit conversion of data

State	<i>in vitro</i> transcription rate	Relative transcription rate
Repressed	20.91	1
Derepressed	18.82	0.900
Activated	275.54	13.177



The fraction of *mer* operator bound by MerR with and without HgCl_2 . Raw data are from Figure 3 of Parkhill, Ansari, Wright, Brown, and O'Halloran (1993).

Extraction and unit conversion of data

[MerR] / M	Fraction of MerR bound to operator	
	with HgCl ₂	no HgCl ₂
5e-13	0.06	0.09
2.5e-12	0.09	0.11
5e-12	0.09	0.11
2.5e-11	0.12	0.32
5e-11	0.21	0.42
2.5e-10	0.50	0.78
5e-10	0.66	0.86
2.5e-9	0.78	0.88
5e-9	0.83	0.91
2.5e-8	0.91	0.93

Calculations

Unit conversions	Values are normalised to the transcription rate observed for the repressed state.
Standard deviations	Abortive transcription: 40 % Gel retardation: Values of K_D are given as $2.0 \pm 1.4 \times 10^{-10}$ M (with HgCl ₂) and $0.5 \pm 0.1 \times 10^{-10}$ M (no HgCl ₂). Therefore, 45 % is chosen as the s.d. calculated as the average of $(1.4/2.0 = 0.7)$ and $(0.1/0.5 = 0.2)$.

Comments

Gel retardation results, conducted at 10–12 °C, may differ from those performed at 37 °C.

A.29 Parkhill, Blair, Hobman, and Brown (1998)

Experimental conditions

Organism	<i>E. coli</i> CSH26ΔrecA
Plasmids	Tn501 pRZH-2 (operator DNA) pTO90-16 (purified MerR)
Temperature	10 °C
Media	N/a
Experiments	Incubate 5×10^{-11} M operator DNA with MerR in the presence or absence of $0.75 \mu\text{M}$ HgCl ₂ . Separated equilibrated complexes by gel electrophoresis and quantify bound and unbound fractions with a phosphorimager.

The fraction of *mer* operator bound by MerR in the presence and absence of HgCl₂. Raw data are from Table 2 Parkhill, Blair, Hobman, and Brown (1998)

Fraction of MerR bound to operator	[MerR] / M	
	with HgCl ₂	no HgCl ₂
0.5	1.9×10^{-10}	1.0×10^{-10}

Calculations

Standard deviations	40 %
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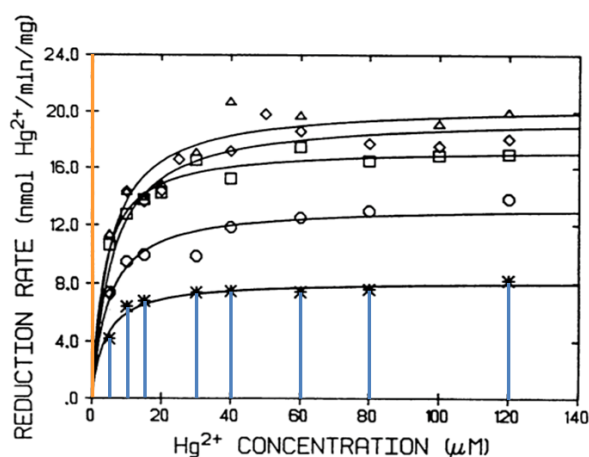
Comments

These results, conducted at 10 °C, may differ from those performed at 37 °C.

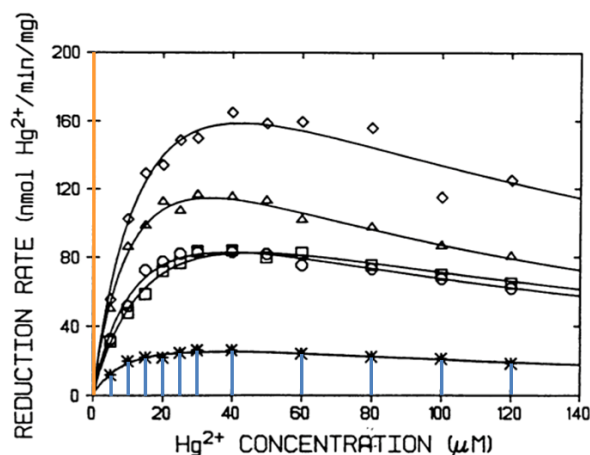
A.30 Philippidis, Malmberg, Hu, and Schottel (1991)

Experimental conditions

Organism	<i>Escherichia coli</i> strain C600 r^-m^+ and MM294A
Plasmids	R100: <i>merR-o/p-TPCAD</i> (3/2.45 copies per genome)
Temperature	37 °C
Media	Volatilisation: Luria-Bertani Densitometry: M9 with 1 % casamino acids
Experiments	<p>Volatilisation: Grow cells in 10 μM $\text{Hg}(\text{NO}_3)_2$ from $\text{OD}_{650} = 0.05$ to 0.4. Add 20 μM $\text{Hg}(\text{NO}_3)_2$ to cells and grow from $\text{OD}_{650} = 0.4$ to 0.6. Harvest cells, concentrate 10-fold in buffer, and keep on ice. (Permeabilise cells with a brief treatment of ethyl ether on ice or prepare cell extracts by French press or sonication at 4 °C). Dilute cells 10-fold in assay mixture with a 40 μM mixture of $^{203}\text{Hg}(\text{NO}_3)_2$ and $\text{Hg}(\text{NO}_3)_2$. Measure initial rates of $^{203}\text{Hg}(\text{NO}_3)_2$ loss from the assay by scintillation spectroscopy during the first minute at intervals of 20 seconds. Normalise values to the total cellular protein concentration in the assay mixture: 0.25–0.29 g/L for intact cells and cell-free extracts; 0.18–0.22 g/L for permeabilised cells. (Philippidis, personal communication).</p> <p>Densitometry: Grow cells to 2×10^{11} cells/L. Irradiate with UV for 6 min to produce maxicells with degraded chromosomal DNA. Resuspend cells at 4×10^{11} cells/L. Add 10 μM $\text{Hg}(\text{NO}_3)_2$ and label induced <i>mer</i> polypeptides for 90 mins (Sancar, Hack, and Rupp, 1979). Separate <i>mer</i> proteins by fluography and quantify their relative concentrations by densitometry.</p>



Initial rates of volatilisation of $\text{Hg}(\text{NO}_3)_2$ in intact (left) and permeabilised cells (right) with plasmid R100. Raw data are from Figures 2 and 3 of Philippidis, Malmberg, Hu, and Schottel (1991).



Extraction and unit conversion of data

Hg(NO ₃) ₂ /M	Initial rate of ²⁰³ Hg(NO ₃) ₂ loss from assay mixture			
	nmol min ⁻¹ mg ⁻¹ protein		M / min	
	Intact	Permeabilised	Intact	Permeabilised
5e-6	4.16	11.85	1.12e-6	2.37e-6
10 e-6	6.36	19.56	1.72e-6	3.91e-6
15 e-6	6.76	21.93	1.83e-6	4.39e-6
20 e-6	/	21.93	/	4.39e-6
25 e-6	/	24.59	/	4.92e-6
30 e-6	7.32	26.07	1.98e-6	5.21e-6
40 e-6	7.47	26.07	2.02e-6	5.21e-6
50 e-6	/	/	/	/
60 e-6	7.43	23.70	2.01e-6	4.74e-6
80 e-6	7.57	21.93	2.04e-6	4.39e-6
100 e-6	/	20.44	/	4.09e-6
120 e-6	8.18	18.07	2.21e-6	3.61e-6

Relative concentrations of induced MerT and MerA polypeptides in R100. Raw data are from Table 2 of Philippidis, Malmberg, Hu, and Schottel (1991).

Normalised band intensity		Relative concentration	
MerT	MerA	MerT	MerA
0.69	0.40	6.29	1

K_t of MerT transport

Parameter	Values
K _t /V _{sp}	3.8 μM, 4.7 μM, 3.6 μM, 5.1 μM and 6.0 μM Hg(NO ₃) ₂

Calculations

Unit conversions	<p>Induction time: $r = 0.0268$; $k = 5.595$; $y_0 = 0.05$; $y_t = 0.4$ $A = (k - y_0)/y_0$ $t = \log((-1 + k/y_t)/A) / -r = 80.02$ mins</p> <p>$r = 0.0268$; $k = 5.595$; $y_0 = 0.4$; $y_t = 0.6$ $A = (k - y_0)/y_0$ $t = \log((-1 + k/y_t)/A) / -r = 16.59$ mins</p> <p>Volatilisation: To convert from mol min⁻¹ mg⁻¹ protein to M/min, divide by (1 ml/0.27 g)/1000 = 1/270 L for intact cells, and divide (1 ml / 0.20 g)/1000 = 1/200 L by for permeabilised cells. These units are the rates of loss of Hg(NO₃)₂ from the assay mixture.</p> <p>Densitometry: The number of monomers in MerT and MerA is assumed to be the same (Hobman, personal communication). The molecular masses of monomers quoted in this paper are MerA is 62 kDa, and MerT is 17 kDa. MerA relative concentration = $(0.40/62)/(0.40/62) = 1$ MerT relative concentration = $(0.69/17)/(0.40/62) = 6.29$</p>
Standard deviations	<p>Volatilisation: 10 % Densitometry: 60 %</p>

Comments

Since treatment with ether made the cells completely permeable to Hg(NO₃)₂, data for cell extracts and permeabilised cells are assumed to be equivalent (Philippidis, Shottel, and

Wu, 1990). Thus, MerA and MerR remain in the cell extract, but MerT/P and Hg^{2+} bound to the transporters are removed in the cell pellet following centrifugation of the broken cells.

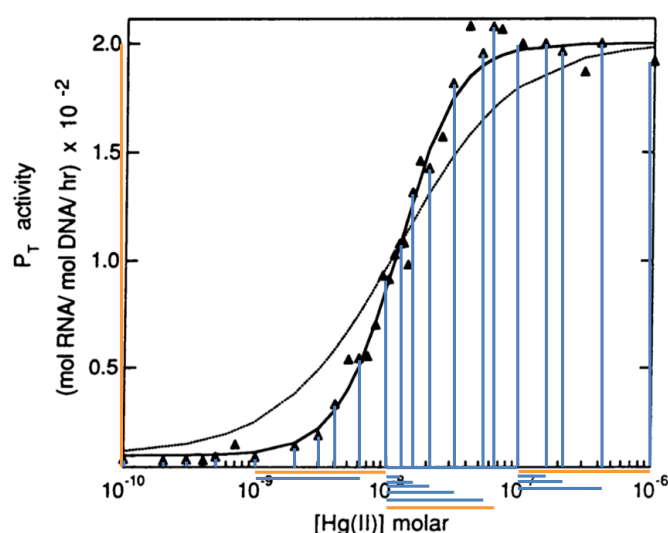
For densitometry measurements, the same assumptions apply as for Lee, Gambill and Summers (1989).

Initial volatilisation rate plateaux with increasing $\text{Hg}(\text{NO}_3)_2$ for each copy number of plasmid. Thus both *non-mer* import and *mer* transport are either saturated or the rate of one of these processes is increasing but negligible. However, increasing the plasmid copy number, and hence, the MerT, MerP concentration, increases the magnitude of the plateaux. So this increase cannot be due to *non-mer* import which is either saturated or negligible. Thus, *mer* transport is the most significant process and maintains relatively the same K_T with increasing plasmid copy number. This represents the K_T for *mer* transport since export of Hg^{2+} is expected to be negligible as most Hg^{2+} is volatilised by MerA.

A.31 Ralston and O'Halloran (1990)

Experimental conditions

Organism	<i>in vitro</i>
Plasmids	Tn501 pGMer: <i>me</i> (ΔR)-o/p-(ΔT)
Temperature	37 °C
Media	Buffer
Experiments	Abortive transcription (<i>in vitro</i>): Incubate 0.4×10^{-9} M DNA, 2.5×10^{-9} M MerR dimer, and HgCl_2 for 45 mins. Initiate transcription, and after 30 mins, quantify ^{32}P in the RNA product by scintillation spectroscopy.



***In vitro* transcriptional activity of P_T .** Raw data are from Figure 1 of Ralston and O'Halloran (1990).

Extraction and unit conversion of data

[HgCl ₂] / M	P _T activity	Relative P _T activity
1e-10	0.0281	1.00
2e-10	0.0194	0.690
3e-10	0.0238	0.847
5e-10	0.0367	1.31
1e-9	0.0367	1.31
2e-9	0.0907	3.23
3e-9	0.140	4.98
4e-9	0.289	10.28
6.3e-9	0.505	17.97
1e-8	0.883	31.42
1.3e-8	1.052	37.44
1.6e-8	1.292	45.98
2.1e-8	1.406	50.04
3.3e-8	1.810	64.41
5.5e-8	1.952	69.47
6.7e-8	2.078	73.95
1e-7	1.996	71.03
1.6e-7	2.000	71.17
2.2e-7	1.963	69.86
4.4e-7	2.002	71.25
1e-6	1.911	68.01

Calculations

Standard deviations	30 %
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A.32 Rinderle, Booth, and Williams (1983)

Experimental conditions

Organism	<i>Escherichia coli</i> KP245
Plasmids	R100 pRR130 (ColE1): <i>merR</i> -o/p-TPCA, <i>EcoRI</i> -H + <i>EcoRI</i> -I fragments
Temperature	37 °C
Media	Difco lactose-broth
Experiments	Grow cells from a 2 % inoculum for 8 hours in Difco lactose-broth with 27 mg/L HgCl ₂ . Purify MerA.

MerA concentration after growth in HgCl₂. Raw data are from the text of Rinderle, Booth, and Williams (1983).

MerA	
Percentage of total soluble protein	Concentration / M
0.1	1.09e-6

Calculations

Unit conversions	At stationary phase, soluble protein is approximately 0.7e-13 g per cell, cell volume is 1.61e-15 L. The average molecular mass of an <i>E. coli</i> protein is 40 KDa (Savageu, 1986). Therefore, the soluble protein concentration is: (0.7e-13 / 40000) / 1.61e-15 = 0.00109 M. Concentration of MerA dimer: (0.1/100) * 0.00109 = 1.09e-6 M Molecular mass of HgCl ₂ = 271.496 g/mol. 27e-3 / 271.396 = 9.95e-5 M HgCl ₂ .
Standard deviations	70 %

Comments

After 8 hours of growth, cells are assumed to be in stationary phase by the following calculation:

$$r = 0.02683333; k = 5.595; t = 8 \times 60$$

$$y_0 = (2/100) \times k$$

$$A = (k - y_0)/y_0$$

$$y_t = k/(1 + A \times \exp(1)^{-r \times t}) = 5.594 \text{ (i.e., } k)$$

A.33 Ross, Park, and Summers (1989)

Experimental conditions

Organism	<i>Escherichia coli</i> K12, CAG1574
Plasmids	Tn21 pWR2 (pBR322): <i>merR</i> -o/p- <i>T'</i> - <i>lacZ</i> pWR10 (pBR322): <i>mer</i> (ΔR)-o/p- <i>T'</i> - <i>lacZ</i> pNH9 (pACYC177): <i>merR</i> -o/p- <i>T'</i>
Temperature	37 °C
Media	LB
Experiments	Dilute overnight cultures 1:100 in LB and grow for an additional four generations. Add 1 μM HgCl_2 for 20 min. Measure β -galactosidase activity.

β -galactosidase activities from *merT'*-*lacZ* fusion plasmids after 20 minutes

induction with 1 μM HgCl_2 . Where applicable, MerR is provided *in trans* on pNH9. Raw data are from Table 5 of Ross, Park, and Summers (1989).

State	β -galactosidase activity	Relative β -galactosidase concentration
Repressed		
pWR2 + pNH9	483	1
pWR10 + pNH9	208	1
Derepressed		
pWR10	1012	4.89
	1041	5.00
Activated		
pWR2 + pNH9	10620	21.99
pWR10 + pNH9	11085	53.29

Calculations

Unit conversions	Values are normalised to the β -galactosidase activity observed for the repressed state in each data set.
Standard deviations	Values are given as 483 ± 188 , 10620 ± 502 , 1012 ± 122 , and 1041 ± 205 . Therefore, 19 % is chosen as the s.d., calculated as the average of $(188/483 = 0.39)$, $(502/10620 = 0.04)$, $(122/1012 = 0.12)$ and $(205/1041 = 0.20)$.

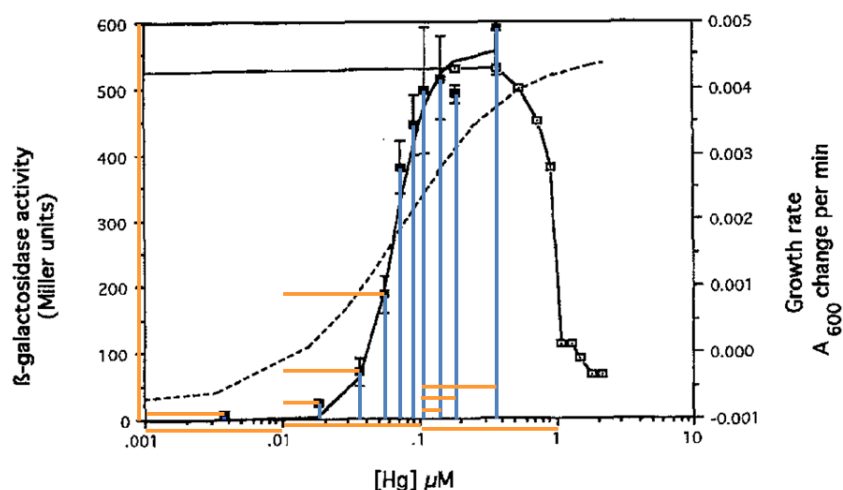
Comments

The authors note that β -galactosidase activities varied up to two-fold from different batches of media and different stocks of HgCl_2 .

A.34 Rouch, Parkhill, and Brown (1995)

Experimental conditions

Organism	<i>Escherichia coli</i> TG2
Plasmids	Tn501 pRZH18 (pRZ5255): <i>merR-o/p-T'-LacZ</i>
Temperature	37 °C
Media	M9 supplemented with casamino acids and glucose
Experiments	Grow cells to OD ₆₀₀ = 0.6–0.7. Add HgCl ₂ for 60 min. Measure β -galactosidase activity.



β -galactosidase activities from *merT'-lacZ* fusions after 60 minutes induction with HgCl₂. Raw data are from Figure 1 of Rouch, Parkhill, and Brown (1995).

Extraction and unit conversion of data

[HgCl ₂] / M	β -galactosidase activity	Relative β -galactosidase concentration
3.72e-9	4.18	1
1.83e-8	22.59	5.40
3.62e-8	70.29	16.82
5.51e-8	187.45	44.84
7e-8	378.24	90.49
9e-8	443.52	106.11
1e-7	496.23	118.72
1.39e-7	512.13	122.52
1.8e-7	491.21	117.51
3.52e-7	590.80	141.34

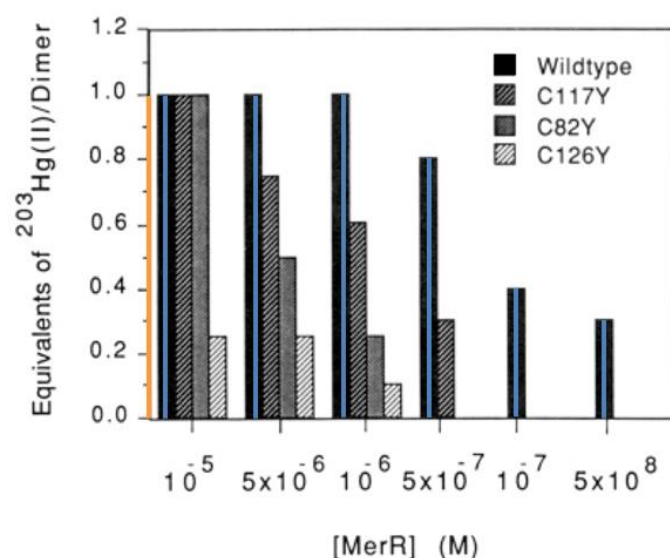
Calculations

Unit conversions	Values are normalised to the β -galactosidase activity when cells are induced with 3.72e-9 M HgCl ₂ .
Standard deviations	30 %

A.35 Shewchuk *et al.* (1989)

Experimental conditions

Organism	<i>Escherichia coli</i> JM109
Plasmids	Tn21 MerR purified from plasmid pWR2
Temperature	Samples kept on ice before HPLC gel filtration
Media	Buffer
Experiments	Gel filtration: Incubate MerR with a 10-fold molar excess of $^{203}\text{Hg}^{2+}$ in the presence of 1 mM 2-mercaptoethanol for 30 minutes. Load samples onto an HPLC gel filtration column and quantify the fractions of bound and unbound $^{203}\text{HgCl}_2$ by scintillation counting.



Binding of HgCl_2 by MerR. Raw data are from Figure 4 of Shewchuk *et al.* (1989).

Extraction and unit conversion of data

[MerR] / M	Equivalents of $^{203}\text{HgCl}_2$ per MerR dimer
1e-5	1
5e-5	1
1e-6	1
5e-7	0.80
1e-7	0.40
5e-8	0.30

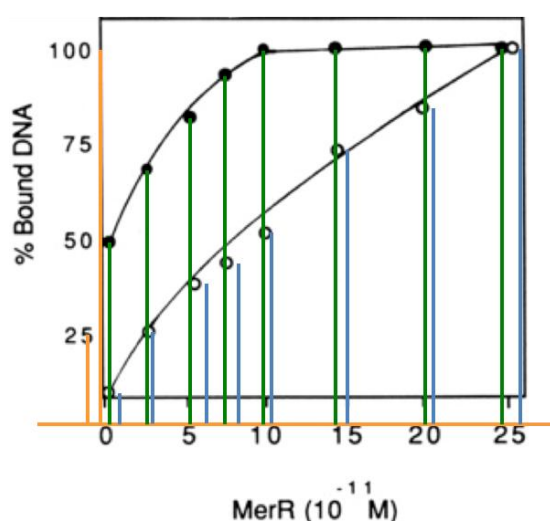
Calculations

Standard deviations	Gel filtration: 30 %
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A.36 Shewchuk, Verdine, and Walsh (1989)

Experimental conditions

Organism	N/a
Plasmids	Tn501 Operator fragment: pJOE114 MerR purified from pTO90-16; a <i>merR</i> -o/p- <i>T'</i> fragment from pT040, (O'Halloran and Walsh, 1987).
Temperature	4 °C
Media	N/a
Experiments	Gel retardation: Incubate operator 1e-12 M DNA with MerR in the presence or absence of 1 μ M HgCl ₂ . Separated equilibrated complexes by gel electrophoresis and quantify bound and unbound fractions with densitometry.



The fraction of *mer* operator bound by MerR in the presence (●) and absence (○) of HgCl₂. Raw data are from Figure 3 of Shewchuk, Verdine, and Walsh (1989).

Extraction and unit conversion of data

[MerR] / M	Fraction of MerR bound to operator	
	no HgCl ₂	with HgCl ₂
1e-11	0.083	0.48
2.5e-11	0.24	0.68
5e-11	0.37	0.81
7.5e-11	0.43	0.93
1e-10	0.51	1.00
1.5e-10	0.73	1.00
2e-10	0.84	1.00
2.5e-10	1.00	1.00

Calculations

Standard deviations	Gel retardation: 30 %
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Comments

These results, conducted at 4 °C, may differ from those performed at 37 °C.

A.37 Shewchuk, Verdine, Nash, and Walsh (1989)

Experimental conditions

Organism	<i>Escherichia coli</i> JM109
Plasmids	Tn21 pWR2 (pBR322): <i>merR</i> -o/p- <i>T'</i> - <i>lacZ</i>
Temperature	37 °C
Media	T-broth
Experiments	β -galactosidase assay: Dilute overnight cultures 1:100 into T-broth and grow for an additional 60 minutes. Add 1 μM HgCl_2 for 20 min. Measure β - galactosidase activity.

β -galactosidase activities from *merT*-*lacZ* fusion plasmids after 20 minutes

induction with 1 μM HgCl_2 . Raw data are from Table 2 of Shewchuk, Verdine, Nash, and Walsh (1989).

State	β -galactosidase activity	Relative β -galactosidase concentration
Repressed	77	1
Derepressed	838 777	10.88 10.09
Activated	10500	136.36

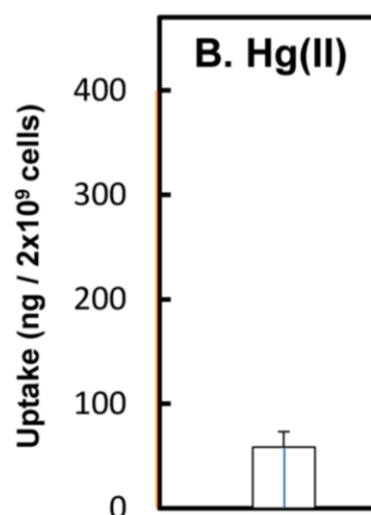
Calculations

Unit conversions	Values are normalised to the β -galactosidase activity observed for the repressed state.
Standard deviations	β -galactosidase activities: 30 %

A.38 Sone, Nakamura, Pan-Hou, Itoh, and Kiyono (2013)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pKF19k
Temperature	37 °C
Media	LB
Experiments	Grow cells to $\text{OD}_{600} = 1.0$ (late log-phase). Resuspend cells in media with 10 μM $^{203}\text{HgCl}_2$ and chloramphenicol for 30 minutes. Measure $^{203}\text{HgCl}_2$ bound to cells by atomic absorption spectrometry.



Whole-cell binding of 5 μM $^{203}\text{HgCl}_2$ by control cells after 30 minutes. Raw data are from Figure 3 of Sone, Nakamura, Pan-Hou, Itoh, and Kiyono (2013).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	ng / 2e9 cells	M
30	57.59	2.71e-5

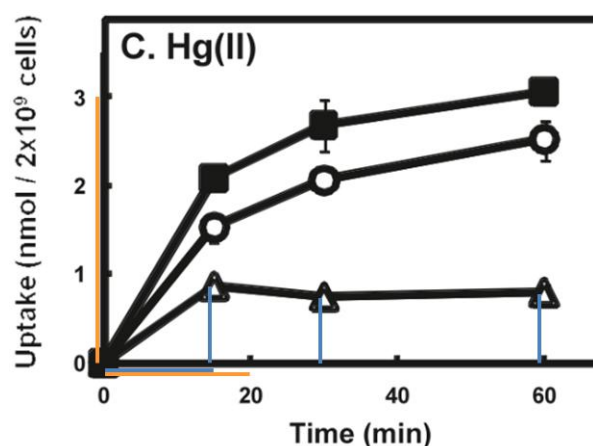
Calculations

Unit conversions	To convert from ng HgCl_2 /2e9 cells to g/L HgCl_2 , multiply by $1\text{e-}9/7.84\text{e-}6$. At growth rate = $0.0268 \cdot (1 - 1/5.595) = 0.0220 \text{ min}^{-1}$, cell volume = $3.918\text{e-}15 \text{ L}$. So 2e9 cells is $2\text{e}9 \cdot 3.918\text{e-}15 \text{ L} = 7.84\text{e-}6 \text{ L}$. Then, to convert from g/L to M Hg^{2+} , divide by molecular mass of $\text{HgCl}_2 = 271.496 \text{ g/mol}$
Standard deviations	30 %

A.39 Sone, Pan-Hou, Nakamura, Sakabe, and Kiyono (2010)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	pKF19k (control vector)
Temperature	37 °C
Media	LB
Experiments	Grow cells to $\text{OD}_{600} = 1.0$ (late log-phase). Resuspend cells in media with $10 \mu\text{M } ^{203}\text{HgCl}_2$ and chloramphenicol. Add 0.5 ml of cell suspension to a filter and wash three times with 5 ml broth. Measure $^{203}\text{HgCl}_2$ bound to cells by atomic absorption spectrometry.



Whole-cell binding of $10 \mu\text{M } ^{203}\text{HgCl}_2$ by control cells (Δ). Raw data are from Figure 2 of Sone, Nakamura, Sakabe, and Kiyono (2010)

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$	
	nmol / 2e9 cells	M
0	0	0
15	0.86	1.10e-4
30	0.74	9.44e-5
60	0.78	9.95e-5

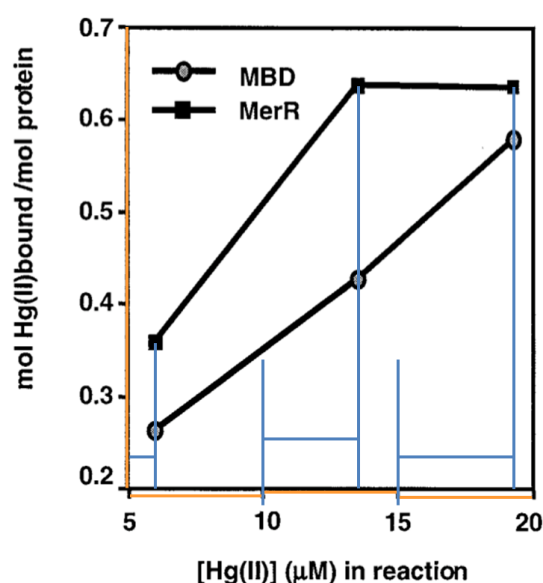
Calculations

Unit conversions	To convert from nmol HgCl ₂ /2e9 cells to M HgCl ₂ , multiply by 1e-9/7.84e-6. At growth rate = 0.0268*(1 - 1/5.595) = 0.0220 min ⁻¹ , cell volume = 3.918e-15 L. So 2e9 cells is 2e9*3.918e-15 L = 7.84e-6 L
Standard deviations	10 %

A.40 Song *et al.* (2004)

Experimental conditions

Organism	<i>Escherichia coli</i> XL1-Blue
Plasmids	Tn21 pASK-IBA3 (over-expressing MerR from pNH9)
Temperature	Equilibrium ultrafiltration and free dialysis: room temperature Whole-cell binding: 37 °C
Media	LB
Experiments	<p>Whole-cell binding: Grow cells to OD₆₀₀ = 0.5. Add anhydrotetracycline for 3 hours ([MerR] = 6e5–9e5 molecules/cell). Add 3 µM HgCl₂. Centrifuge cells and measure radioactivity in supernatant and pellet by scintillation spectrometry.</p> <p>Equilibrium ultrafiltration: Incubate 10 µM MerR with a 5 to 20 µM ²⁰³HgCl₂ in Tris-HCl buffer for 15 minutes. Quantify the fractions of bound and unbound ²⁰³HgCl₂ by ultrafiltration and scintillation counting.</p> <p>Free dialysis: Incubate 60 µM MerR with a 4-fold molar excess of ²⁰³HgCl₂ in Tris-HCl buffer for 60 minutes. Quantify the fractions of bound and unbound ²⁰³HgCl₂ by ultrafiltration and scintillation counting.</p>



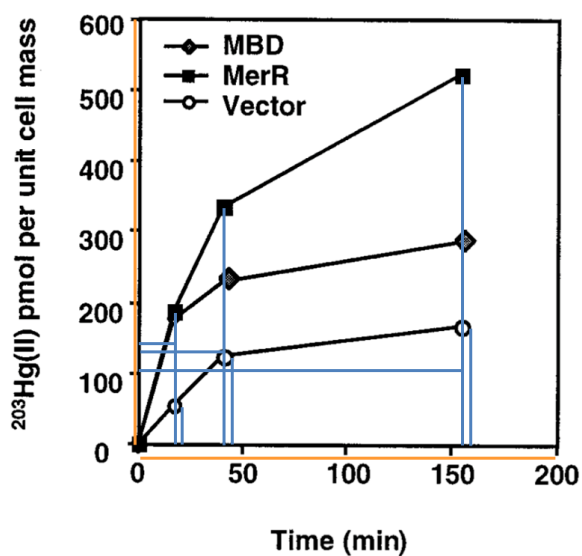
Quantification of ²⁰³Hg²⁺ bound by MerR by equilibrium ultrafiltration. Raw data are from Figure 2 of Song *et al.* (2004).

Extraction and unit conversion of data

$[^{203}\text{HgCl}_2] / \text{M}$	Fraction of MerR dimer bound by $^{203}\text{Hg}^{2+}$
6e-6	0.358
13.58e-6	0.636
19.35e-6	0.636

Quantification of $^{203}\text{Hg}^{2+}$ bound by MerR by equilibrium by free dialysis. Raw data are from the text of Song *et al.* (2004).

$[^{203}\text{HgCl}_2] / \text{M}$	$[\text{MerR}] / \text{M}$	Fraction of MerR dimer bound by $^{203}\text{Hg}^{2+}$
240e-6	60e-6	0.90



Whole-cell binding of $^{203}\text{HgCl}_2$ by cells with a control vector (○) and over-expressed MerR (■). Raw data are from Figure 3 of Song *et al.* (2004).

Extraction and unit conversion of data

Time / min	Cellular $^{203}\text{HgCl}_2$			
	pmol / OD_{600}		/ M	
	Vector	MerR	Vector	MerR
17.75	52.39	0185.24	1.35e-8	4.78e-8
41.13	122.25	334.30	3.15e-8	8.62e-8
158.44	165.28	519.54	4.26e-8	1.34e-7

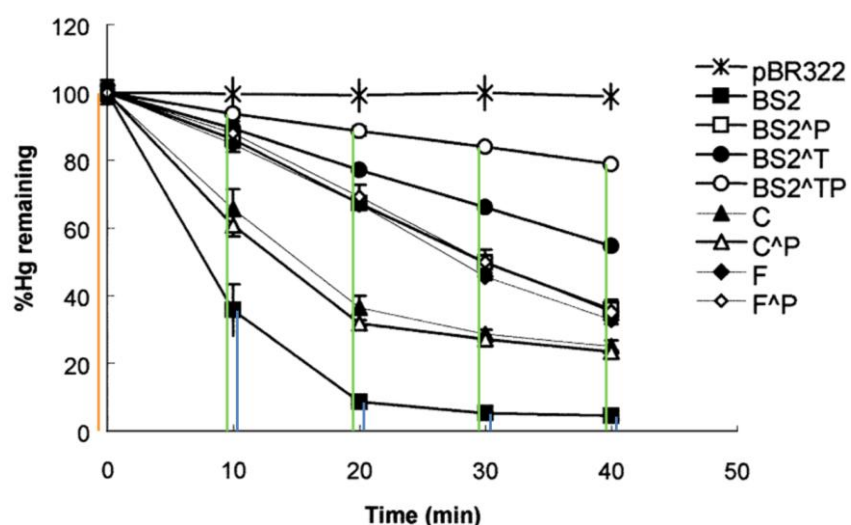
Calculations

Unit conversions	<p>After 3 hours growth from $\text{OD}_{600} = 0.5$, cells are assumed to be in stationary phase. At growth rate = 0.00, cell mass = $8.80\text{e-}14$, cell volume = $1.61\text{e-}15$ L. So the volume of 1 mg cells is $(1\text{e-}3 \text{ g}/8.80\text{e-}14 \text{ g}) \cdot 1.61\text{e-}15 \text{ L} = 1.83\text{e-}5 \text{ L}$.</p> <p>To convert from pmol $^{203}\text{HgCl}_2$/ mg cells to M $^{203}\text{HgCl}_2$, multiply by $1\text{e-}12/1.83\text{e-}5 \text{ L} = 5.46\text{e-}8$.</p> <p>6e5 to 9e5 MerR molecules/cell 1 mol = $6.022\text{e}23$ (Avogadro's number) $(7.5\text{e}5/6.022\text{e}23) \text{ mol}/1.61\text{e-}15 \text{ L} = 7.74\text{e-}4 \text{ M}$</p>
Standard deviations	<p>Equilibrium ultrafiltration: 10 % Free dialysis: 6.7 % Whole-cell binding: 29 %</p>

A.41 Wilson, Leang, Morby, Hobman, and Brown (2000); Schue, Glendinning, Hobman, and Brown (2008)

Experimental conditions

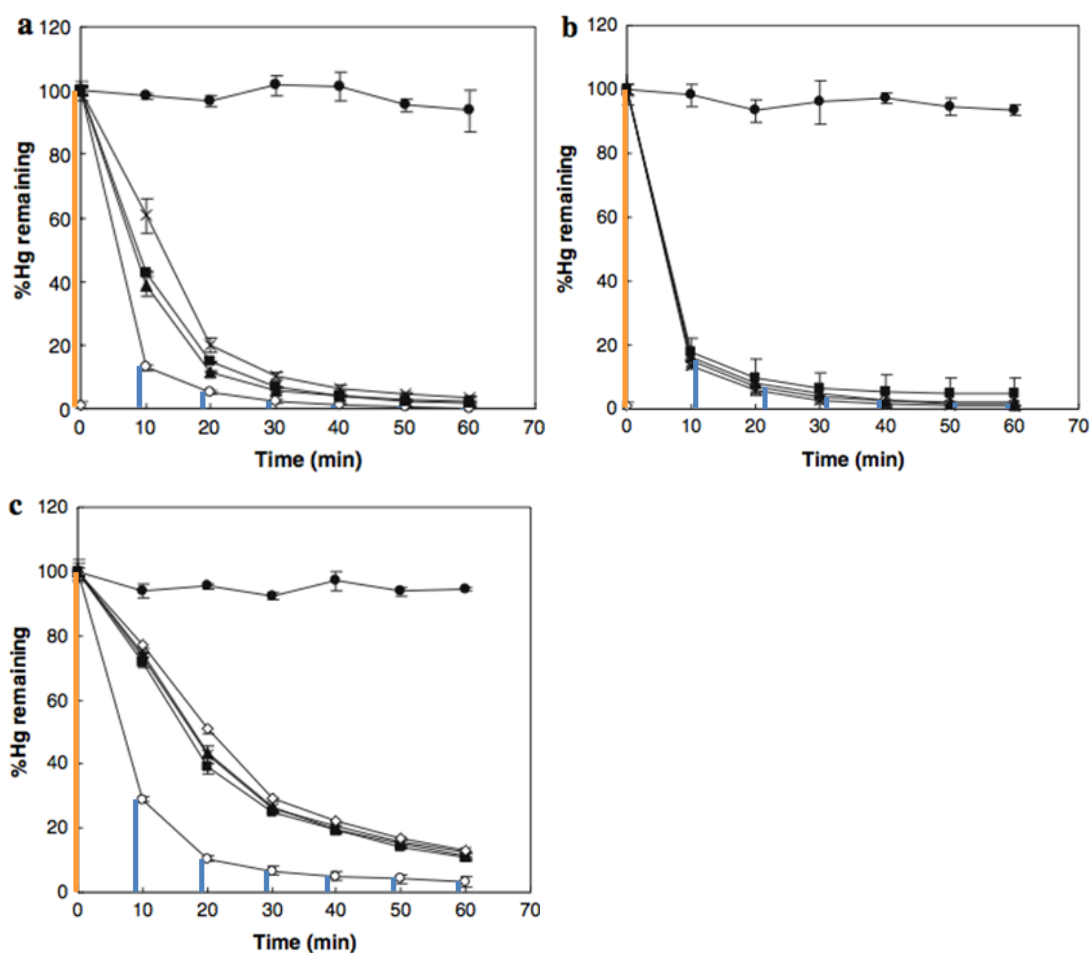
Organism	<i>Escherichia coli</i> TG2
Plasmids	Tn501 pBRmer BS2 (pBR322): <i>merRTPAD</i> pUC18/19, pBR322
Temperature	37 °C
Media	M9
Experiments	Grow cells to mid-log phase ($OD_{600} = 0.35-0.4$). Add 1 $\mu\text{g/ml}$ HgCl_2 for 60 min. Suspend 2×10^8 cells in 100 μL M9 media with 5 μM HgCl_2 . Add cell suspension to 5 ml buffer with 1 $\mu\text{Ci/ml}$ $^{203}\text{HgCl}_2$. Count radioactivity remaining in assay mixture by scintillation spectrometry



Volatilisation of $^{203}\text{HgCl}_2$ by cells with wild-type *mer* (■), and *merTP* (○) mutant plasmids. Raw data are from Figure 2 of Wilson, Leang, Morby, Hobman, and Brown (2000).

Extraction and unit conversion of data

Time / min	Proportion of $^{203}\text{HgCl}_2$ remaining	
	<i>merR</i> -o/p- <i>TPA</i>	<i>merR</i> -o/p-(Δ <i>TP</i>) <i>A</i>
10	0.336	0.939
20	0.0838	0.888
30	0.0496	0.839
40	0.0413	0.789



Volatilisation of $^{203}\text{HgCl}_2$ in cells with wild-type *mer* plasmids (O). Raw data are from Figure 4 of Schue, Glendinning, Hobman, and Brown (2008).

Extraction and unit conversion of data

Time / min	Proportion of $^{203}\text{HgCl}_2$ remaining merR-o/p-TPA
10	0.131 0.151 0.290
20	0.053 0.0693 0.103
30	0.023 0.0335 0.064
40	0.0008 0.0263 0.050
50	0.003 0.0167 0.043
60	0.0001 0.0144 0.034

Calculations

Unit conversions	Molecular mass of $\text{HgCl}_2 = 271.496 \text{ g/mol}$. $1\text{e-}3/271.396 = 3.68\text{e-}6 \text{ M HgCl}_2$.
Cell concentrations	$5\text{e-}6 \text{ mol/L} * 100\text{e-}6 \text{ L} = 5\text{e-}10 \text{ mol}$. $5\text{e-}10 \text{ mol}$ in $100\text{e-}6 + 0.005 \text{ L} = 9.8\text{e-}8 \text{ M}$ $2\text{e}8 \text{ cells}$ in $100\text{e-}6 + 0.005 \text{ L} = 3.92\text{e}10 \text{ cells/L}$.
Standard deviations	30 %

Comments

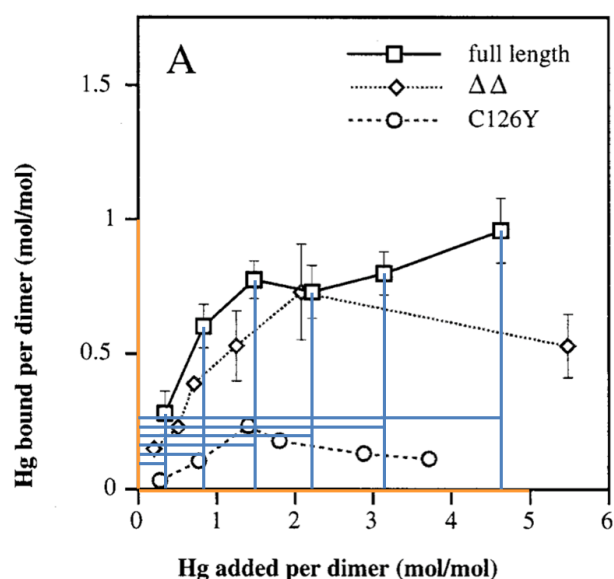
Following induction, $9.8\text{e-}8 \text{ M HgCl}_2$ and $1\text{e-}6 \text{ Ci/ml } ^{203}\text{HgCl}_2$ are added to the cells as a mixture. Therefore, a percentage decrease in one is assumed to reflect the same percentage decrease in the other. However, the concentration of the $^{203}\text{HgCl}_2$ tracer is insignificant in quantity so can be ignored (Hobman, personal communication).

In deleting the *merT* and *merP* genes, the *merA* gene is moved into the promoter-proximal position which is occupied by *merT* in the wild-type. Therefore, MerA is synthesised at the MerT rate in the *merTP* deletion mutant.

A.42 Zeng, Stålhandske, Anderson, Scott, and Summers (1998)

Experimental conditions

Organism	N/a: experiments conducted <i>in vitro</i>
Plasmids	Tn21 MerR purified from plasmid pWR2
Temperature	4 °C
Media	N/a
Experiments	Incubate $1 \mu\text{M} - 2 \mu\text{M}$ MerR dimer with $\text{Hg}(\text{NO}_3)_2$ giving Hg/MerR_2 ratios from 0.2 to 10. After 1 hour, quantify the fractions of bound and unbound Hg^{2+} by ultrafiltration.



Binding of Hg^{2+} by MerR. Raw data are from Figure 5 of Zeng, Stålhandske, Anderson, Scott, and Summers (1998).

Extraction and unit conversion of data

$^{203}\text{Hg}(\text{NO}_3)_2$ added per dimer (mol/mol)	Fraction of MerR dimer bound by $^{203}\text{Hg}(\text{NO}_3)_2$
0.336	0.276
0.808	0.602
1.48	0.776
2.20	0.729
3.14	0.800
4.65	0.962

Calculations

Standard deviations	30 %
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Comments

These results, conducted at 4 °C, may differ from those performed at 37 °C.