A computational systems biology study of the $\lambda$-lac mutants

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We present a comprehensive computational study of some 900 possible “$\lambda$-lac” mutants of the lysogeny maintenance switch in phage $\lambda$, of which up to date 19 have been studied experimentally (Atsumi & Little, PNAS 103: 4558-4563, (2006)). We clarify that these mutants realise regulatory schemes quite different from wild-type $\lambda$, and can therefore be expected to behave differently, within the conventional mechanistic setting in which this problem has often been framed. We verify that indeed, within this framework, across this wide selection of mutants the $\lambda$-lac mutants for the most part either have no stable lytic states, or should only be inducible with difficulty. In particular, the computational results contradicts the experimental finding that four $\lambda$-lac mutants both show stable lysogeny and are inducible. This work hence suggests either that the four out of 900 mutants are special, or that $\lambda$ lysogeny and inducibility are holistic effects involving other molecular players or other mechanisms, or both. The approach illustrates the power and versatility of computational systems biology to systematically and quickly test a wide variety of examples and alternative hypotheses for future closer experimental studies.

The study of bacteriophage $\lambda$ has played a large role in the development of molecular biology [1, 2], and particularly in the understanding of gene regulation [3, 4]. It is a temperate phage which can grow lytically, or remain in a lysogenic state in the host for many generations. While lysogeny as a phenomenon was known since the 1920’ies, and early quantitative studies centered on other systems, coliphage $\lambda$ became the central model system of lysogeny since its discovery in the early 1950’ies [5, 6]. Consequently, $\lambda$ lysogeny has also been the system of choice for mechanistic explanations of gene regulation, ranging from systematic explanations of the data to detailed mathematical models of the kind first presented over twenty years ago [7]. This line of work has been taken up several other groups [8, 9, 10, 11], with the aims to reproduce, in a model, known phenomena, and to shed light on particular aspects of such systems. Together with the lac system this approach has been the prototype for theoretical understanding of gene regulation in prokaryotes, generally taken to be one of the corner-stones of quantitative systems biology [12, 13, 14].

In a recent series of experimental studies by Atsumi & Little [15, 16] the lytic repressor, Cro, was replaced by the Lac repressor, LacR. The stated goal was to continue along the lines of [17] in testing the modularity of the lambda circuit, i.e., in this case, to determine if stable and inducible lysogeny is affected by a change of the lytic repressor protein. Therefore, the authors of [15, 16] constructed mutants in which the cro operon was replaced by the lac repressor operon, lacI, including the Shine-Dalgarno (SD) sequence for lacI. To enable repression of PR and PL, a lac operator site, lacO, was put downstream of the PR and PL transcription initiation sites. Moreover, in these new $\lambda$-lac mutants, some carried an intact OR3 site, only binding CI and hence without any lacI regulation of PRM. Others had the OR3 site replaced with a lacO site, hence disrupting the CI negative control of PRM, but at the same time allowing a negative feed-back from PR. Figure 1 illustrates three different circuits; the wild-type $\lambda$ (WT), circuit A, with intact $\lambda$ OR, and circuit B, where OR3 has been changed into a variant of lacO.

The great advantage of the new $\lambda$-lac mutants from the systems biology point of view is that there are potentially so many of them. While experimental studies have only so far been carried out on a fraction of all defined variants, it should be possible to extend the studies in [16] to many more. Computationally, as we will show, one can survey all variants in one screen, and find clear patterns.

The overall conclusion of our computational study presented here is that the new $\lambda$-lac mutants are not explainable in standard mathematical models of gene regulation. We believe this is of significant interest. First, if $\lambda$ lysogeny cannot be explained, the whole program of computational systems biology may be in trouble. We address this issue in the Discussion. Second, at least for $\lambda$, this program is but the formalization, in terms of defined models and equations, of what is known or accepted in the experimental literature, often for quite some time. Therefore, the implication would be that the functioning of the $\lambda$ lysogeny switch is quite different to what is generally believed.

The problem can be explained by concentrating on one example, the mutant labelled AWCF, displayed in Figure 1 in [16]. This mutant carries a version of lacI instead of cro, a normal $\lambda$ OR region, a strong lacO binding site at PL, an immediately strong lacO binding site downstream of PR, and a (relatively) poor Shine-Dalgarno sequence. It therefore is an example of control circuit A in Fig. 1. This differs from the control scheme of wild-type $\lambda$ in that lacI does not repress CI, because lacR does not bind anywhere in OR. One therefore expects (in the standard framework of $\lambda$) that this mutant can be lysogenized, but that it cannot be induced. As detailed below, the modelling conforms to this pattern in that the kinetic equations has one equilibrium which can be identified with lysogeny, but no equilibrium or other state at high levels of lacR that would lead to lysis. The experimental results (Table 1 and Fig. 3 in [16]) on the contrary show that while indeed it can be lysogenized, it is also inducible at a UV dose only about twice as high.
as in wild-type λ, releasing almost as many phages per cell.

The first message of this paper, taken up again in the Discussion is that all the new λ-lac mutants embody one of two different schemes depending on the OR3 site. Using straight-forward qualitative arguments, as above for AWCF, λ-lac mutants of circuit A type are expected to have stable lysogeny but not be easily inducible. This should also partly be true for circuit B, as CI then does not repress itself. Whether a stable lytic equilibrium can be obtained in these mutants, should depend on the relative strengths of the inserted lacO sites at PRM and PR. These conclusions are supported by the mathematical modelling. We categorize the mutants into three different groups, according to their equilibria, illustrated by Fig. 2. Either the mutants have only the lytic or the lysogenic equilibrium (group 1 or 2), or they have two equilibria and can be placed in group 3, like the wild-type phage.

The results of Atsumi & Little have, we believe, implications far beyond what is actually claimed in [16]. In a well-engineered control system, a module can be replaced by another module of the same characteristics without changing overall system functionality. But one cannot, normally, replace a module with another of different characteristics without either the system being stable against these changes, or the perturbations being taken care of by another additional layer of control. It is the first possibility that we can test in the mathematical model with negative results.

I. RESULTS

A. Equilibria in wild-type λ

The wild-type circuit is known to have two stable equilibria, one lysogenic and one lytic, and the level of CI in lysogeny is fairly well established [9, 18]. In the Atsumi & Little experiments, the PRM promoter was weakened to half its strength, although still enabling the lysogenic state [16]. Therefore, our wild-type lambda system was calibrated to allow a lysogenic equilibrium at ≈ 252 CI with the original promoter strengths, which still allows a lysogenic equilibrium for the weaker promoter. For the latter case, lysogeny is naturally less stable, and has a lower CI level.

B. Equilibria in circuit A

For circuit A, with the intact OR3 site, all variants of lacO at PR and with any Shine-Dalgarno sequence essentially behave in the same way. There is no negative control of PRM other than from CI itself, while PR is inhibited by both CI and lacI. Consequently, the only possible equilibrium is the lysogenic state, where PRM is activated more than PR. The CI level in this state is constant at ≈ 172 molecules, independent of the three parameters (the two lacO sites and the SD sequence). The LacR level however depends on the SD sequence and the lacO at PR. The most

FIG. 1: Illustration of the three different λ circuits. WT) The wild-type λ phage circuit, with auto-repression of cro and CI as well as mutual repression. CI can additionally activate its own production. A) The circuit for mutants with an intact OR3 site, binding only CI. CI then has an intact regulatory circuit for controlling PR and PRM, compared with wild-type, while the original negative feed-back from PR, via cro, has been cut. B) The circuit for mutants with a lacO site instead of the original OR3 site. The negative feed-back from CI on PRM is then cut, while the original negative control from PR is kept.
FIG. 2: Three different groups into which the λ-lac mutants conceptually can be classified. In group 1, there is one equilibrium with higher level of the lytic protein than of the lysogenic protein. In group 2, there is one equilibrium with the opposite characteristics. Group 3 consists of mutants that have two equilibria, one corresponding to the lytic phase, and one with lysogenic properties. The wild-type λ belongs to group 3, with two equilibria in the corresponding mathematical model.

important parameter for the LacR level is the SD sequence. The better translation, the more LacR. Moreover, worse lacO at PR, enables more transcription from PR, also resulting in somewhat higher LacR levels. This is however only relevant at the best translational efficiency. The LacR levels are however always very low, with a maximum of seven molecules for the best SD.

The mutants’ equilibria were computed without IPTG in the system, and with a concentration of $10^{-5}$ M IPTG. The impact of having IPTG present is that the differences in LacR levels, caused by altering binding affinities at PR for the best SD, completely disappears. The promoter activities remain constant, with a PRM activity of about 40 % and PR completely silent. All mutants with circuit A hence fall into group 2, with only one stable lysogenic equilibrium.

C. Equilibria in circuit B

Mutants with circuit B have the additional negative control of lacI on PRM, theoretically allowing a silencing of PRM and a lytic stable equilibrium. Depending on the combination of PRM and PR operators and the Shine-Dalgarno sequence, these mutants can have one or two stable equilibria, see fig 3. The upper plots display the level of LacR and CI at the equilibria, with no IPTG (left) and with $10^{-5}$ M IPTG (right), with the mutants with two equilibria marked with black contours. The lower plots show the corresponding PR and PRM activities. The mutants with this circuit can hence be in either one of the three groups, 1-3.

A stable equilibrium with higher activity of PR than PRM, appears when the mutant have the best PRM lacO sites (circle markers), or in some cases the next best, depending on the PR lacO site. The weaker the PR lacO is (bigger markers), the higher PR/PRM ratio. Since there is always a negative feedback by lacI on PR, the activity of PR is never above 25 % in these ‘lytic’ states. The mutants with two equilibria are the ones with the worst SD sequence but best PRM lacO (XAXE or XAXF), or next best operator and better SD sequences (XBXC or XBXD). For the mutants with best PRM lacO, PR is completely silent at the lysogenic equilibrium, while PRM is fully active. The other equilibrium has an activity of PR that is higher than PRM, but with maximum 25 % activity. For the mutants with next best PRM lacO (squares), their two equilibria are relatively close, with a promoter activity between 0.04-0.15 % for both PR and PRM in both states. Whether this state would in practice correspond to the lytic state or the lysogenic state for the phage is unclear.

Most mutants however, have only one stable state, with a high PRM activity and low PR, corresponding to a lysogenic state in the wild-type λ circuit. The activity of PR is essentially zero for all those cases, since a high CI level shuts down PR. The degree of PRM activity varies, and is in general mostly dependent on the SD sequence, where lower efficiency generates higher PRM activities. Even though the activity of PR is low, and hence the LacR level is low, the negative feed-back on PRM is affected from the occasional transcription from PR. So mutants with efficient SD sequences can still repress PRM in this lysogenic state. For many of these mutants, the PRM promoter is almost fully active, dramatically different from the wild-type lambda where the negative feed-back from CI limits the PRM activity. The CI level is also elevated to around 400 molecules in lysogeny, more than twice the lysogeny level in wild-type λ, with this weakened PRM promoter.
FIG. 3: (AXXX) Plots showing the stable fixed points with corresponding promoter activities for the 180 mutants with the best PL lacO. The two upper plots show the fixed points at 0 IPTG (left) and 1e−5 M (right), while the lower plots show the corresponding PR and PRM activities. The mutants are coded with three parameters; colour, marker type and marker size. The lacO at PRM is indicated with marker type, going from the highest affinity ○ (A) through □ (B), ◊ (C), * (D) and finally ⋆ (E). The PR lacO is indicated by the marker size, with smallest markers for the best operator (A), then increasing marker size with decreasing affinity. The Shine-Dalgarno sequence is indicated by color, with the best efficiency (A) coded as red, then decreasing with orange (B), yellow (C), green (D), blue (E) and finally purple (F). The mutants within circuit B fall into all three possible groups. There are some mutants with one lytic stable fixed point, some with both a lysogenic and a lytic fixed point, while most of them only have the lysogenic fixed point. The mutants with two fixed points are marked with black edges.

**D. Closer look at the 19 experimentally studied mutants**

In Little & Atsumi’s study, only 19 of the 900 mutants were isolated and four of the mutants, all from subgroup A, were examined more closely. The 19 isolated mutants are presented in Table 1, with the experimental lysogenic abilities and our computed stability results.

Two of the mutants they identified, AABF and BAAD, we predict to have two equilibria, i.e. place in group 3. One equilibrium has very low activities for both promoters, and the other with full PRM activity and a silent PR. One other mutant, ABEA, we can place into group 1, with slightly higher PR activity than PRM. The 16 remaining mutants all have only one equilibrium, at higher PRM than PR activity, and hence fall into group 2. For the mutants with circuit A, the promoter activities are not dependent on IPTG levels in the system, while for the others, the promoter activities are in general increased with higher IPTG levels. This is to expect since the negative feedback should be somewhat relieved when IPTG bind LacR.
II. DISCUSSION

A straightforward conclusion of this work is that the new $\lambda$-lac mutants embody different control schemes than wild-type $\lambda$. For this no elaborate modelling is required; case-by-case reasoning as for the AWCF mutant in Introduction suffices. In mutants where the $\lambda$ OR region has been left intact (of the type XWXX where X can be any code) lacI cannot repress cI. In mutants where OR3 has been changed to a version of lacO (of the type XYXX where X can be any code, and Y any code except W), cI does not autorepress itself. In both cases, these mutants should show exceedingly stable lysogeny (if it can be established) because the switches should be much harder to throw in control schemes A and B, than in the wild-type phage.

In the quantitative modelling, every mutant is represented by a set of two equations describing the net production of Cl and LacR molecules per unit time. An equilibrium of these equations means that the net production of both species of molecules vanishes, such that the concentrations are constant. A lysogenic state of the model is an equilibrium where Cl concentration is high and lacR concentration low. The PRM promoter is then activated at a much higher rate than the PR promoter. In models of wild-type $\lambda$ describing the auto-activation of cI, auto-repression of cI and cro, and mutual repression between cI and cro, there is normally both a lysogenic equilibrium and an equilibrium where Cl concentration is very low and Cro concentration is high. In the real system high PR activation goes together with high PL activation and transcription of the $\lambda$ N gene, which turns on the early genes downstream of cro and N [4], and the lysis/lysogeny switch. The equilibrium at high Cro concentration (when it is present in the model) therefore in reality corresponds to sustained high level of Cro for a bacterial generation or more, sufficient to drive the cell to lysis.

We are now ready to state the results of the modelling in more detail. Except for a fraction of the cases in circuit B, the models of the $\lambda$-lac mutants have, unlike wild-type $\lambda$, only one equilibrium, and it is of the lysogenic type. This means that the inductive process, if it is started, is self-limiting, and the switch can never be stably thrown over to lysis. Some of the cases in circuit B have only a 'lytic' equilibrium, albeit with relatively low levels of activation of PR, and these mutants should, one would guess, therefore not be able to form lysogens. One of these examples, mutant ABEA, indeed did not display lysogeny in vivo. A few of the cases in circuit B have two equilibria, one lysogenic and one 'lytic'. Of these AABF and BAAD were studied experimentally in [10]. AABF did not show lysogeny, while BAAD showed what Atsumi & Little refer to as stable and unstable lysogens, where the latter are defective in their growth properties. The four mutants which could be lysogenized and were studied more closely all had only a lysogenic equilibrium in the model, but could nevertheless all be induced experimentally.

We now discuss why we find these results interesting. Biological systems are much harder to model than, say, physical system for reasons which can be classified as (i) unknown parameters and (ii) unknown mechanisms. Models of biological systems should be robust at least to changes in the unknown parameters, which in addition are often likely to vary in vivo. Apart from its colloquial meaning, robustness is also a technical concept in the qualitative theory of dynamical systems, meaning that the equilibria and other characteristics change only smoothly as parameters are varied [20]. Therefore, the behaviour of a biological system, if described as a large system of kinetic equations, should to a large extent be explainable in terms of its equilibria [21, 22]. This has indeed also been the point of view taken in previous modelling of $\lambda$, but which does not seem to hold for the $\lambda$-lac mutants as we have shown here.

So why is there this large discrepancy between the model and the experiments, and what does this imply for $\lambda$? The first and maybe most obvious explanation is that the 19 mutants studied experimentally could be a biased sample, and that if all were screened, then they would conform more closely to the theoretical prediction. This is perhaps unlikely, but supports the utility of the $\lambda$-lac mutants as a test bed, and makes the case that an experimental investigation of many variants would be most interesting.

A second hypothesis is that, in fact, no "lytic" equilibrium is necessary, but that instead a transient rise in Cro is enough to excite genes down-stream of Cro and throw the switch to lysis. This contradicts the original picture of $\lambda$ lysogeny presented in e.g. [4], but is more in line with recent results indicating that Cro is not necessary for lytic induction [18, 19, 23]. However, our comprehensive simulations of the transients ("phase-space plots"), indicate that very few of the mutants have high Lac transients. Especially, the AWCF mutant that experimentally behaves similarly to the wild-type, indeed have no transient rise in LacR (see Fig. 4).

A third hypothesis is that some basic assumption of the modelling may be incorrect. Is this conceivable? Perhaps so. One observation is that the time scales of LacR binding/unbinding have recently been measured in single-cell experiments, and are as long as on the order of a minute [24]. The implied time scales for a looped complex between OR and OL to disassociate may therefore be on the order of a bacterial generation, in which case in vivo occupancy of the operator sites by CI would be far from in equilibrium with the instantaneous CI concentration. Lysogeny would then not be a quasi-equilibrium state, but an intermittently controlled process, where periods of dilution and...
TABLE I: Table displaying the experimentally observed lysogenic properties from Little & Atsumi’s study (from Supplementary material available online [16]), side by side with the computed promoter activities at the equilibria. The two left columns show the experimental results at two different IPTG levels. (U = unstable, S = stable and No indicates that there were no lysogeny).

The computational results are presented as PRM/PR activities, for the stable equilibria identified for each mutant.

| Circuit | Mutant | Lysogeny | PRM/PR |
|---------|--------|----------|--------|
|        | IPTG   | 0        | 10⁻⁵ M |
|        | Experiments | 0        | 10⁻⁵ M |
|        | AABF   | No       | No     | 0.02/0.08,0.96/- | 0.04/0.12,0.99/- |
|        | ABCD   | No       | No     | 0.94/-           | 0.99/-           |
|        | ABEA   | No       | No     | 0.02/0.08        | 0.09/0.01        |
|        | ABEF   | No       | No     | 1/-              | 1/-              |
|        | ACDA   | U        | No     | 0.17/0.002       | 0.78/-           |
|        | ACEF   | No       | No     | 1/-              | 1/-              |
|        | ADCA   | No       | No     | 0.53/-           | 0.88/-           |
|        | ADED   | S & U    | S & U  | 0.99/-           | 0.99/-           |
|        | AECA   | No       | No     | 0.63/-           | 0.91/-           |
|        | AEDF   | No       | No     | 1/-              | 1/-              |
|        | BAAD   | S & U    | S & U  | 0.02/0.02, 1/-   | 0.04/0.03, 1/-   |
|        | BBEF   | No       | No     | 1/-              | 1/-              |
|        | CBCE   | No       | No     | 1/-              | 1/-              |
|        | CCDF   | No       | No     | 1/-              | 1/-              |
|        | DDDA   | No       | NA     | 0.70/-           | 0.98/-           |
|        | AWAЕ   | NA       | S      | 0.41/-           | 0.41/-           |
|        | AWCA   | S & U    | S      | 0.41/-           | 0.41/-           |
|        | AWCD   | S        | S      | 0.41/-           | 0.41/-           |
|        | AWCF   | S        | S      | 0.41/-           | 0.41/-           |

FIG. 4: Phase plots for wild-type λ phage in [16] and the AWCF λ-lac mutant. The left plot shows the phase plot for the wild-type phage, with two quilibria, one lysogenic and one lytic. The right plot shows the corresponding phase plot for the AWCF mutant, belonging to circuit A. This mutant exhibits only one equilibrium, at the lysogenic point.

degradation alternate with sharp bursts of protein synthesis, and such a system would be more easily inducible. Other mechanisms are certainly also possible. The lesson of this study is that the module replacement approach of [16] shows that the λ lysogeny switch may in fact not be a well understood phenomenon.

III. MATERIALS AND METHODS

As already outlined, in the λ-lac mutants in [16], the lacI gene of the E. coli lactose system has been substituted for the cro gene of λ. LacO sites were introduced to maintain transcriptional control, and SD sequences for the lac repressor were inserted. Altogether, Atsumi & Little used five variants of lacO, labelled (A-E), and six variants of
SD, labelled (A-F). In total, the two circuit constructs resulted in 900 possible mutants, identified by a four-letter code as in the example AWCF in the introduction.

Since the experimental genetic constructs fall into two categories, (circuit A and B in Fig. 1), and the wild-type lambda consists of yet another circuit, three separate models were constructed. They differ since the genetic architecture and regulatory mechanisms differ while they are all thermodynamic models, in line with the earlier studies of the lambda switch 7, 8, 25. A short description of the general setup and model assumptions are presented below. For a complete description of all model parameters, see Appendix B and C.

A. General setup

The models all include the left and right operator sites, OL and OR, both binding CI and Cro/LacR. The models allow looping formations between OL and OR, caused by CI forming tetramer and octamer structures 19. The equilibria are obtained from solving the equations describing the net changes in CI and LacR levels, see Eq 1 and 2. CI is a stable protein and is not degraded, only diluted with a continuous rate corresponding to a generation time of \( \tau_{\text{div}} \). LacR is besides diluted by cell division, also degraded with a half-life of \( \tau_{\text{deg}} \). Production of CI from PRM can take place with three different rates, \( R_{\text{uPRM}} \), for the unactivated promoter, \( R_{\text{nPRM}} \) for an activated PRM in a non-looped configuration and finally \( R_{\text{lPRM}} \) for the activated promoter in a looped configuration. The PR promoter has one constant production rate of \( R_{\text{PR}} \).

\[
\frac{dCI}{dt} = -CI \frac{\ln 2}{\tau_{\text{div}}} + 1 \times S_{CI} (R_{\text{uPRM}} P_{\text{uPRM}} + R_{\text{nPRM}} P_{\text{nPRM}} + R_{\text{lPRM}} P_{\text{lPRM}})
\]

\[
\frac{dLacR}{dt} = -LacR ( \frac{\ln 2}{\tau_{\text{div}}} + \frac{\ln 2}{\tau_{\text{lac}}} ) + S_{LacX} R_{PR} P_{PR}
\]

Unactivated transcription from PRM occurs when OR3 and OR2 are unoccupied, while activated transcription requires a CI dimer to be bound at OR2. PR is active with a constant rate as long as OR1 and the lacO at PR remains unoccupied.

B. Looping configurations

In earlier models of the \( \lambda \) phage, the fully occupied OL or/and OR state was treated as one state, with the assumption that OR1 and OR2 forms a cooperative binding 7, 9, 11, 18.

However, theoretically there are cooperative bindings either between CI bound at OR1 and OR2 or at OR3 and OR2, meaning that in principle two separate configurations exist for the fully occupied state. These configurations become important to separate when looping is modeled (for details see Appendix A). Our state description includes both these configurations.
APPENDIX A: LOOPING CONFIGURATIONS

CI molecules form dimers that can bind into each site at the left and right operators, OL and OR. If CI dimers occupy two neighbouring sites, they can interact and bind cooperatively. Dimers bound at OL and OR can also interact with each other, if the DNA forms a loop and allows the molecules to come close enough to each other [18, 19]. If there exist a cooperative interaction between two neighbouring dimers at OR and OL, these can together form an octamer structure, while single dimers at OL and OR can together form a tetrameric structure.

The promoter activities of PRM and PR depend on the CI (and Lac R) binding patterns at OR and OL, and each possible configuration of bound CI and LacR needs to be evaluated. For example, when OR is fully occupied by CI, i.e. CI dimers are bound to all three sites, there are in fact two different configurations, depending on if CI dimers at OR1 and OR2 are bound cooperatively, or dimers at OR2 and OR3. In earlier models of the λ phage switch, before looping between OL and OR had been identified, these two configurations do not need to be separated in the description. The have the same statistical weight, and the same consequence regarding promoter activities [7, 8].

![Diagram of looping configurations](image)

FIG. 5: Illustration of the four possible configurations when OL and OR are both fully occupied by CI. They are all equally probable, i.e. occur with weight 1/4.

However, in a model that acknowledges looping, the two possible fully occupied configurations need to be treated separately, since they allow different looped structures to form. Fig. 5 illustrates the different possible configurations, in the non-looped and the looped state. If CI at OR1 and OR2 are bound cooperatively, and OL2-OL3 likewise, only the octameric structure should be able to form. CI bound at OR3 and OL1 can then never interact and form a tetramer since they are placed on opposite sites of the octamer. The same occurs if the opposite scenario, with OL1-OL2 and OR3-OR2 bound cooperatively. So only two out of the four configurations of fully occupied operators actually allow both an octamer and a tetramer to be formed.

When the cooperative binding energies between neighbouring bound CI are set equal for all interactions, the four configurations are equally probable (each with weight 1/4). In principle, this way of counting configurations, according to the “tilting” of the CI dimer, also means that all configurations could be separated into different tilted versions. However, it is only in the fully occupied state that it becomes necessary to keep them separated. For the configurations where two CI dimers are bound as neighbors but tilted away from each other, i.e. three out of four scenarios, they are so much less probable than the configuration with cooperative binding, that they can be neglected.

APPENDIX B: PARAMETERS

Lac repressor

The wild-type LacR forms relatively stable tetramers [26], while the dimeric form of LacR used in Atsumi & Little’s study, is most likely less stable. The half-life is here taken to be 20 min [27], and the repressor dimerization constant is set to 80 nM [16].

The dimeric mutant is most likely similar in properties to the -32 aa dimeric mutant presented in Chen and Matthews study from 1992 [30]. They measured the apparent operator binding dissociation constant for this mutant to be lower, but concludes that it is not the DNA binding properties that are altered, but the dimerization constant [30]. Therefore, it is assumed to bind as the wild-type repressor to the optimal lacO site, with a Kd of $1.2 \times 10^{-11}$ M.
The affinity for the Lac repressor to the various mutated operator sites was obtained from a study by Betz et al. in 1986 [31]. The dissociation constants for the three first mutations (B,C,D) were experimentally determined in [31]. For the fourth sequence, the dissociation constant were not measure directly, only the β-galactosidase activity, i.e. here the measure of repression of promoter due to binding at the mutated lacO site. By correlating the 15 total measured dissociation constants with their corresponding β-galactosidase activities, also the fourth lacO site affinity was estimated. The binding strengths of the four mutated lacO sites, relative the best operator A, are [ 4.17 10.83 16.67 19.72 ] (B-E).

Regarding IPTG interactions with LacR, one IPTG can bind each LacR monomer, either the free monomer, or each monomer in a multimer. IPTG bound to the repressor lowers its affinity for lacO sites, while nonspecific binding is not affected [32]. The operator affinity for IPTG-bound LacR was here estimated from earlier theoretical models of LacR-IPTG interactions, with ≈ 5 and 30-fold reduced operator affinities (one or two IPTG) [34].

![Diagram of molecular interactions](image-url)

**FIG. 6:** Illustration of all interactions between molecular species in the model. LacR dimerizes and each lacR unit can bind IPTG. LacR dimers with, and without, IPTG can bind to specific operator sites or non-specifically to DNA. CI also dimerizes and can bind to operator sites or to DNA non-specifically.

The IPTG affinity for the LacR monomer is changed when the repressor is bound at an operator site. Moreover, Operator-bound LacR experience a positive cooperative binding, although the IPTG affinity for repressor is being reduced about 20-fold [34, 35]. Our model assumes that the dimerization of Lac repressor is independent of the IPTG binding. Fig. 6 shows all interactions between LacR and IPTG, operators and DNA, that are included in the model.

**CI and Cro**

The CI repressor dimerizes with a dissociation constant, $K_{dCI}$, of $2 \times 10^{-8}$M [8, 18], and binds DNA as a dimer. The CI repressor affinities for the three OR sites have been measured by Koblan & Ackers and for OL by Senear et al. In our model we have used the free energy measurements for the OR, taken at 37 degrees [36]. The affinities at OL sites were estimated from measurements at 20 °C with corrections for higher temperature following the OR measurements [36, 37]. The free energies for forming an octameric or tetrameric looped structure were taken from [18].

In the wild-type λ model the Cro binding affinities were taken from [22, 33]. The unspecific binding of Cro has been taken as in [22]. Cro dimerizes strongly, with a dissociation constant of $1.2 \times 10^{-5}$ [23] and the life-time of Cro is set to be 43 minutes [38]. The number of Cro molecules produced from each cro mRNA has been estimated to 20, followng analysis in [5].
Transcriptional rates

In the wild-type circuit, the unspecific binding of CI was used as free parameter, to calibrate the PR and PRM rates in order to set a lysogenic fix-point of ≈ 252 CI and 3 Cro [9, 18]. The relative rates for PRM, in its three different states, and PR, was taken from experimental measurements [18]. These calibrated rates were then used in the λ-lac models for valid comparisons.

Translational efficiencies

The translational efficiency $S_{CI}$ is assumed to be one CI molecule per transcript [9, 25]. The translational efficiency of the lacI, $S_{LacX}$, is dependent on the Shine-Dalgarno sequence, where the sequences were said to allow gradually lower translational efficiency, from sequence A to F [16, 33]. The best SD sequence is set to generate 23 repressors from each mRNA [28].

To estimate the actual relative translational efficiencies we applied the efficiency-matrix established from experimental data by Barrick et. al [29]. The six sequences were accordingly ranged from higher to lower efficiencies. The obtained number of translations per mRNA, for the optimal SD and the five mutated sequences, is then [23 3.77 3.31 1.24 0.35 0.33] (A-F).

APPENDIX C: TABLES
TABLE II: All parameters included in the model of the $\lambda$-Lac phages, including notation used in main article, plus references. (Ref. 1 and 2 are theoretical studies with further references to be found within.)

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| cell volume                   | $V$      | $2 \times 10^{-15}L$            | [9, 25]   |
| length of bac DNA             | $L_{DNA}$| $5 \times 10^{6}$bp             | [9]       |
| lifetime CI                   | $\tau_{CI}$ | inf                       | [8]       |
| lifetime LacR                 | $\tau_{LacR}$ | 20 min                        | [27]      |
| cell generation time          | $\tau_{div}$ | 34 min                        | [9, 25]   |
| temperature                   | $k_BT$   | 0.617 kcal/mol                  | [8, 9, 25]|

Translational efficiencies [no]

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| translation cl                | $S_{CI}$ | 1                               | [9, 25]   |
| translation lac (A)           | $S_{LacA}$ | 23                             | [28]      |
| translation lac (B)           | $S_{LacB}$ | 3.77                           | [29]      |
| translation lac (C)           | $S_{LacC}$ | 3.31                           | [29]      |
| translation lac (D)           | $S_{LacD}$ | 1.24                           | [29]      |
| translation lac (E)           | $S_{LacE}$ | 0.35                           | [29]      |
| translation lac (F)           | $S_{LacF}$ | 0.33                           | [29]      |

Transcription rates [1/s]

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| $PR$ rate                     | $R_{PR}$ | 1.0171                          | fit to [18]|
| $PRM$ rate, unactive          | $R_{PRM}^{u}$ | 0.0433                        | fit to [18]|
| $PRM$ rate, unlooped         | $R_{PRM}^{nl}$ | 0.3468                      | fit to [18]|
| $PRM$ rate, looped           | $R_{PRM}^{l}$ | 0.2552                        | fit to [18]|

Dimerization and inducer binding [M]

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| Dimerization CI               | $K_{dCI}$ | $1.5 \times 10^{-8}$              | [18, 25] |
| Dimerization LacR             | $K_{dLac}$ | $8 \times 10^{-8}$               | [16]      |
| LacR binding IPTG             | $K_{RI}$ | $1.6 \times 10^{-6}$              | [30]      |
| LacR$_{2\rightarrow O}$ binding IPTG | $K_{dnaR}$ | $3.2 \times 10^{-5}$             | [34, 35] |
| LacR$_{2I\rightarrow O}$ binding IPTG | $K_{dnaRI}$ | $1.6 \times 10^{-5}$            | [34, 35] |

LacR DNA binding [M]

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| LacR$_{2}$ unspec binding     | $dg_{Lacun}$ | $1 \times 10^{-4}$               | [34]      |
| LacR$_{2}$ spec binding (A)   | $K_{dnaRA}$ | $1.2 \times 10^{-11}$            | [30]      |
| LacR$_{2}$ spec binding (B)   | $K_{dnaRB}$ | $5 \times 10^{-11}$              | [31]      |
| LacR$_{2}$ spec binding (C)   | $K_{dnaRC}$ | $1.3 \times 10^{-10}$            | [31]      |
| LacR$_{2}$ spec binding (D)   | $K_{dnaRD}$ | $2 \times 10^{-10}$              | [31]      |
| LacR$_{2}$ spec binding (E)   | $K_{dnaRE}$ | $2.4 \times 10^{-10}$            | [31]      |
| LacR$_{2}$I spec binding      | $K_{dnaRI}$ | $5.6 \times K_{dnaRX}$           | [34]      |
| LacR$_{2}$I$_{2}$ spec binding| $K_{dnaRI2}$ | $30.5 \times K_{dnaRX}$        | [34]      |

CI DNA binding [kcal/mol]

| Parameter                     | Notation | Value                           | Reference |
|-------------------------------|----------|---------------------------------|-----------|
| CI2 unspec binding            | $dg_{CIun}$ | -3.3                            | free parameter |
| $\Delta G_{av1}$              | $dg_{av1}$ | -12.5                           | [36]      |
| $\Delta G_{av2}$              | $dg_{av2}$ | -10.5                           | [36]      |
| $\Delta G_{av3}$              | $dg_{av3}$ | -9.5                            | [36]      |
| $\Delta G_{al1}$              | $dg_{al1}$ | -13.1                           | [36, 37]  |
| $\Delta G_{al2}$              | $dg_{al2}$ | -11.9                           | [36, 37]  |
| $\Delta G_{al3}$              | $dg_{al3}$ | -11.5                           | [36, 37]  |
| $\Delta G_{alcoop}$           | $dg_{coop}$ | -2.8                            | [36]      |
| $\Delta G_{oct}$              | $dg_{oct}$ | -0.5                            | [18]      |
| $\Delta G_{tet}$              | $dg_{tet}$ | -3                              | [18]      |
TABLE III: Additional parameters included in the model of the wild-type phage, including notation used in main article and references. The general parameters and LacR parameters are the same as in Table 1 supplementary materials.

| Parameter                  | Notation | Value               | Reference |
|----------------------------|----------|---------------------|-----------|
| lifetime Cro               | $\tau_{\text{lac}}$ | 43 min              | [38]      |
| translation cro            | $S_{\text{cro}}$ | 20 [no]             | [25]      |
| Dimerization cro           | $K_{d_{\text{cro}}}$ | $1.2 \times 10^{-5} \text{[M]}$ | [25]      |
| cro DNA binding            | $d_{\text{Cround}}$ | -6.5                | [25]      |
| $C_{\text{tro2}}$ unspec binding | $d_{\text{gCround}}$ | -14.4               | [25]      |
| $\Delta G_{\text{or1}}$   | $d_{\text{gor1}}$ | -13.1               | [25]      |
| $\Delta G_{\text{or2}}$   | $d_{\text{gor2}}$ | -15.5               | [25]      |
| $\Delta G_{\text{or3}}$   | $d_{\text{gor3}}$ | -14.5               | [25, 39]  |
| $\Delta G_{\text{ol1}}$   | $d_{\text{gol1}}$ | -13.9               | [25, 39]  |
| $\Delta G_{\text{ol2}}$   | $d_{\text{gol2}}$ | -13.4               | [25, 39]  |
| $\Delta G_{\text{ol3}}$   | $d_{\text{gol3}}$ |                      |           |
