Simulation and analysis of \textit{in vitro} DNA evolution

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We study theoretically the \textit{in vitro} evolution of a DNA sequence by binding to a transcription factor. Using a simple model of protein-DNA binding and available binding constants for the Mnt protein, we perform large-scale, realistic simulations of evolution starting from a single DNA sequence. We identify different parameter regimes characterized by distinct evolutionary behaviors. For each regime we find analytical estimates which agree well with simulation results. For small population sizes, the DNA evolutional path is a random walk on a smooth landscape. While for large population sizes, the evolution dynamics can be well described by a mean-field theory. We also study how the details of the DNA-protein interaction affect the evolution.

The concept of evolution has not only fundamentally shaped our view of biology, but also found rich and profound applications in bioengineering and biotechnology. In particular, \textit{in vitro} evolution has been widely used to evolve DNA\textsuperscript{[1]}, RNA\textsuperscript{[2]} and proteins\textsuperscript{[3]}. In evolution, mutations at the molecular level are selected at the functional level. A quantitative theory of an evolutionary process would require a quantitative understanding of the selection process (e.g. fitness function, landscape, selection pressure, etc.). While this is in general difficult to achieve for natural or laboratory evolution, there are simple cases where such a quantitative description is readily available. Based on experiments of RNA virus evolution\textsuperscript{[4]}, Levine and colleagues\textsuperscript{[5, 6, 7]} studied a simple model of evolutionary process in a smooth landscape in which the fitness of an individual is given as the sum of many individual contributions that can be mutated independently. Their studies found good agreement between theory and simulations for small population sizes and for equilibrium mean field theory, but the evolution dynamics turned out to be pathological in large population sizes where extremely rare mutations are exponentially amplified, yielding infinite speed of evolution. Peng \textit{et al.}\textsuperscript{[8]} proposed DNA binding to proteins as a model system for evolution in a smooth landscape and studied a model where a large population of long DNA molecules were subjected to high mutation rates and selected by how strongly they bind to a protein (the histone-octamer was mentioned as a possible example). Their model can be well described by a continuum equation and they have shown that the average distance to the highest affinity sequence exponentially approaches its equilibrium value\textsuperscript{[8]}.

In a recent experiment, Dubertret \textit{et al.}\textsuperscript{[9]} studied the \textit{in vitro} evolution of DNA sequences via binding to the Mnt repressor protein. The system of DNA-\textit{mnt} is perhaps the best experimentally characterized system of sequence-specific DNA-protein binding,\textsuperscript{[10, 11, 12]} and is particularly suited for a thorough quantitative study of molecular evolution. Specifically, the binding constants of the WT DNA sequence and many other sequences, including all the sequences one point-mutation away from the WT, are measured experimentally.\textsuperscript{[10]} Furthermore, it has been demonstrated that the binding energy of a sequence can be approximately decomposed as the sum of the contributions from the individual bases.\textsuperscript{[10, 12]} This \textit{additive} form of binding energy greatly simplifies the analysis—it enables us to perform realistic large scale simulations as well as to obtain analytic solutions and estimates in various cases. In our study, we explore various regimes of experimentally accessible parameters and we find very distinct evolution dynamics in different regimes.

I. MODELS AND METHODS

We assume that the binding energy between a DNA molecule and the \textit{mnt} protein is given by the sum of the contributions from individual base pairs,

\[ E(S) = \sum_i \epsilon_i(S_i), \]  

(1)
where $S_i \in A, C, G, T$ is the base at the $i$-th position of the DNA sequence and $e_i(S_i)$ is the contribution to the binding energy from the $i$-th position for which we use the experimentally determined value in Ref. 1. The relative binding constants are then $K(S) = \prod_i K_i(S_i) = \prod_i e^{-\beta e_i(S_i)}$. We start with a population size $N$ of DNA molecules of the same sequence that is significantly different from the WT, avoiding the potential problem of enrichment dominating the evolution (i.e. sequences very close to the WT in the initial pool being amplified exponentially). An iteration of the evolutionary process consists of an amplification with mutation followed by a selection. During amplification the population is doubled $I$ times (corresponding to, e.g. $I$ cycles of PCR), so that the population size is increased to $2^I N$. We assume that at each duplication there is an error rate of $r$ per base (see the appendix for more on this). The population is then subject to a selection process via binding to the WT an evolution path. Each path contains the six required mutations from WT, a sequence that differs from WT at one position (19), we denote the highest affinity sequence by SS. We start with a population size $N$ of WT. We denote the number of iterations required $I(\text{dashed lines})$, and $I = 1$ are held fixed. The various regimes (random walk, middle ground, and crossover to mean field) are indicated.

Simulation. The binding site for the $mnt$ repressor consists of 17 important base pairs, at positions 3 through 19. For our starting sequence, we chose, by random mutations from WT, a sequence that differs from WT at one position (19). We denote the number of iterations required $t_M$. We iterate the evolutionary process until at least 90% of the DNA molecules are WT, and we denote the number of iterations required $t_M$ and record the number of molecules coming from each different path.

### II. RESULTS

Some of the key quantities from a single simulation run are the fraction of WT that was produced through minimum paths, $f_{\text{min}}^{\text{WT}}$, the number of minimum paths used, $n_{\text{min}}$, and the fraction of the WT produced through the best single path in the simulation, $f_{\text{best}}^{\text{WT}}$. Fig. 1 shows how these quantities depend on the population size (averaged over many simulations). We see that $f_{\text{min}}^{\text{WT}}$ is small for very small $N$ and is very close to 1 for large $N$, with a fairly sharp transition, whereas $f_{\text{best}}$ slowly decreases from 1 with increasing $N$. This indicates that we may expect to find qualitatively different behavior for small and large population sizes.

#### Small $N$: Random Walk. Let us first consider the case of $N = 1$ and $I = 1$, i.e. a single DNA molecule is duplicated once and one of the two molecules is selected at each iteration. If there are no mutations during the duplication, the two molecules are identical and nothing
interesting can happen. If there is a mutation of type \text{5

i (the chance of multiple mutations in the same duplication is negligible), the binding constant of the copy will be \(\Delta K_i\) times that of the original, and the chance of selecting the mutant is \(\frac{\Delta K_i}{1+\Delta K_i}\), which is high for favorable mutations \((\Delta K_i > 1)\) and low for unfavorable ones. The DNA molecule will thus perform a biased random walk: 

The molecule will make a step whenever a mutant is selected, and the steps that improve the binding to the protein are favored over the steps that reduce the binding. Considering the probability of making each step, we find that we have exactly a random walk in the energy landscape given by the binding energy—in equilibrium, \(\text{Prob}(S) \propto e^{-\beta E(S)}\).

Now consider a population size \(N > 1\), keeping \(I = 1\). As long as \(N\) is sufficiently small \((N \ll 1/r)\), the chance of a mutation happening in any single iteration is very low. When a mutation happens, it will almost certainly either “die out” (disappear from the population) or spread through the whole population before the next mutation occurs, so that most of the time the population consists of \(N\) identical DNA molecules. During selection, the chance of choosing a particular combination of DNA molecules is proportional to the product of their binding constants. Thus, if there are \(m\) mutants of type \(i\) in the population at iteration \(t\), the chance that there will be \(m + j\) mutants in the population the next iteration is \((\Delta K_i)^{2j}\) times the chance that there will be \(m - j\) mutants (as we select exactly half the DNA, the combinatorics are identical). The probabilities \(p^N_+(m)\) that \(m\) identical mutants in a population of size \(N\) will spread through the whole population \((p_+)\) or will die out \((p_-)\) then satisfy

\[
p^N_+(N - m) = (\Delta K_i)^{2m} p^N_-(m). \tag{2}
\]

Including the probability that a mutant will be created and selected in the first place, we find the rate \(R_i\) at which a single mutation of type \(i\) will cause the whole population to be replaced:

\[
R_i = \frac{N r}{3} \cdot \frac{\Delta K_i}{\Delta K_i + 1} p^N_+(1) = \frac{N r}{3} \cdot \frac{(\Delta K_i)^{2N} - (\Delta K_i)^{2N-1}}{(\Delta K_i)^{2N} - 1}
\]

\[
\approx \left\{ \begin{array}{ll}
\frac{N r}{3} \left(1 - \frac{1}{\Delta K_i}\right), & \Delta K_i > 1 \\
0, & \Delta K_i < 1
\end{array} \right. \tag{3}
\]

where Eq. \(2\) and the condition \(p^N_+(m) + p^N_-(m) = 1\) are used, and the approximation is valid for \(|2N \log \Delta K_i| \gg 1\). The population again describes a random walk, but this time the energy landscape is \(2N - 1\) times the binding energy of a single DNA molecule—in equilibrium, \(\text{Prob}(N \text{ copies of } S) \propto e^{-\beta(2N-1)E(S)}\).

\[5\] Mutation “type” specifies both the position and the bases involved.

FIG. 2: Average number of different bases between DNA pool and WT as function of time, and the exponential fit. \(r = 10^{-4}, N = 256, I = 1, \text{ averaged over } 8192\text{ runs.}\)

The average time needed to improve the DNA pool by one base relative to WT can now be estimated as

\[
\langle T \rangle \approx \left[ \sum_i \frac{N r}{3} \left(1 - \frac{1}{\Delta K_i}\right) \right]^{-1} + \frac{\log(N)}{\log(2\Delta K/1+\Delta K)} \tag{4}
\]

where \(\Delta K\) is a typical value for \(\Delta K_i\). The first term is the time required to create a “seed” mutation: The sum is over all possible correct mutations, \(\frac{1}{\Delta K_i}\) is the chance of each mutation occurring in a given iteration, and \(1 - \frac{1}{2\Delta K}\) is the chance that the mutation survives. The second term is the time required for the mutation to spread through the population (for each mutant, \(\frac{2\Delta K}{1+\Delta K}\) is the average number of surviving children after one iteration), which is negligible for small \(N\). Since the first term, which dominates, is inversely proportional to the distance from WT (i.e. the number of terms in the sum), the average speed of the DNA pool will be proportional to that distance. Fig. 2 shows the distance from WT as a function of time, and except in the beginning, it can be almost perfectly fitted to an exponential—this is similar to the result in [8], which is for a very different regime. The corrections for the beginning are precisely what we would expect from the second term: It reduces the speed when the speed is large and causes a short delay. Our result for the evolution speed in the random walk (RW) regime is very similar to the one found in [4] for a birth-rate model: As long as the second term of equation [4] is negligible, the evolution speed of the DNA population is proportional to the mutation rate and to the population size \(N\).

Given a sequence \(S\), the chance that mutation \(i\) will be the next surviving favorable mutation (in the limit of small \(N r\) and large \(N\)) is simply

\[
P_{RW_mut}(S, i) = \frac{1 - \frac{1}{\Delta K_i}}{\sum_j 1 - \frac{1}{\Delta K_j}}. \tag{5}
\]
where the sum is over all possible favorable mutations to $S$. The chance of a given path $\pi$ will thus be $P_{\text{RW path}}(\pi) = \prod_n P_{\text{RW mut}}(S_n, \pi_n)$. Fig. 3 compares these predicted values with simulation results (which are Poisson distributed), and as we can see, they agree very well—the total observed normalized variance is 745.2, vs. expected 720. The approach to the small limit for the population before the next favorable mutation occurs.

When $I > 1$, the random walk approach clearly doesn’t work when the second term of equation 4 exceeds the first term: $A = 1$, averaged over 131,072 runs.

In Fig. 1 we found that for large $N$, almost all the WT is produced through minimum paths. Looking at the full parameter range we have explored, for $r = 10^{-4}$ and $I = 20$ the contribution to WT through minimum paths is about 98% for the largest $N$ we can simulate, and for all other parameters it’s well above 99% for large $N$—thus we expect any set that includes all the minimum paths to give a reasonable result. To get even more accurate results, we allow one erroneous mutation (this already increases the number of paths from 720 to 156,960) and verify that this is only a minor correction.

Let $n_{SS}^0, \pi$ be the amount of WT produced from one molecule through $\pi$, i.e. number of WT sequences at time $t_M$ originating from a single molecule of sequence SS existing at the beginning of the experiment, through any specific path $\pi$. Once we know all the chemical potentials $\mu(t)$ used for the selections, we can use a set of recursion relations to find the average $\langle n_{SS}^0, \pi \rangle$ and the variance $\text{Var}(n_{SS}^0, \pi)$, as well as the probability $P_{\pi}(n_{SS}^0, \pi) = P(n_{SS}^0 > 0)$ that a single SS molecule at time $t = 0$ will yield some nonzero amount of WT at $t_M$ through $\pi$ (details in the appendix).

In the mean field regime, the amount of WT produced through each minimum path should be relatively constant from one experiment to the next, which gives us a lower bound for the population size:

$$N > \max_{\pi} \frac{\text{Var}(n_{SS}^0, \pi)}{(n_{SS}^0, \pi)^2}. \quad (6)$$

Table II shows these bounds for a selection of parameters $I$ and $r$. The dependence on $r$ is roughly $N \sim r^{-m}$, where $m = 6$ is the number of different bases between SS and WT.

The speed of evolution in the mean field regime can be easily estimated in the case of $I = 1$. With $I = 1$, half the population is removed during each selection, thus the chemical potential for the selection will be close to the median binding energy in the population, and any DNA with significantly higher binding energy than the majority will almost certainly survive. In the very first iteration of the evolution process, a fraction $r/3$ of the DNA will get each of the $m$ “correct” mutations. In the following iterations, almost all of these improved DNA will survive the selections, and the amount of improved DNA
will thus roughly be doubled in each iteration. After

$$T_0 \approx 1 + \frac{\log(\frac{3}{m' T r})}{\log(2)}$$

(iterations, the improved DNA will have replaced the original population, i.e. most of the DNA will only have $m$ errors relative to WT.

Once the whole population has been improved by one base, the process is repeated. However, there have already been $T_0$ iteration in which the improved DNA could improve further through mutations, and this even more improved DNA has been amplified at the same rate as the regular improved DNA, i.e. the “seed fraction” of improved DNA will now be $\frac{1}{2} T_0 (m-1) r$ (the factor $\frac{1}{2}$ is because only the copy gets mutated in each amplification). The time required to improve the DNA pool by one base is thus roughly

$$T(m') \approx 1 + \frac{\log(\frac{3}{m' T r})}{\log(2)},$$

where $m'$ is the number of errors left. Corrections include that some of the improved DNA will be lost during selection, reducing the effective amplification from 2 to $\frac{2 A K}{A K + r}$, and higher order corrections to the factor $T$ in the seed fraction. These and other corrections can be addressed by considering an infinite length model.

Fig. 4 shows how the average number of errors changes with time, and we see that the evolution speed is almost constant—using $T(2)$ from equation 8 gives a very good fit. The first improvement takes somewhat longer, as expected, and so does the last improvement: For most of the DNA, the error at position 4 will be the last to be corrected, and the binding energy difference for that mutation is much smaller than for the others, thus the effective amplification is significantly smaller.

The effects of large $I$ and various analytical estimates for the mean field regime are discussed in the appendix.

The Middle Ground. The argument used for the evolution speed in the mean field regime is qualitatively valid for all $N > \frac{1}{r}$ (the factor $T$ in the seed fraction does not fully apply until $N > \frac{1}{r}$), thus we expect a smooth transition from the RW evolution behavior, random with on average exponential approach, to the mean field behavior, constant speed. However, while all the key quantities shown in fig. 4 vary smoothly with the population size, there is a significant region where $f^W_T \approx 1$ and $f^{WT} > 0.5$, i.e. typically a single minimum path dominates. In this region the average contribution of the different paths vary far more than in the RW and MF regimes (Fig. 5), and the region can be considered a third parameter regime. Fig. 6 shows the result of a simulation in the middle ground regime, and while there in this simulation are 11 minimum paths that contribute WT, 70% of it comes from a single path. Note that all the minimum paths used here are “probable” ones (fairly red).

As a single minimum path dominates in each simulation, the average contribution of the different paths should depend strongly on how often that path dominates. Given that the overall evolution behavior is similar to that of the mean field regime, we can use as a first guess the probabilities $P_{\pi f}^W(n_0 SS, \pi)$ from the large $N$ discussion (we here add the superscript MF to emphasize that these are mean field calculations). Fig. 6 shows the results from simulations plotted against this estimate, and there is a very accurate relationship between them (it is not linear), at least for the most probable paths—for the less probable paths, statistical errors are large. The middle ground region corresponds fairly well to $\frac{1}{r} < N < (\sum P_{\pi f}^{MF}(n_0 SS, \pi))^{-1}$, i.e. the regime ends approximately when the population is large enough that, using the mean field chemical potentials, we would expect to find at least some WT in most simulations. There is a very large crossover region between the MF
FIG. 6: Tree of evolution to WT (the root is SS) for a sample simulation with $N = 2^{20}$, $r = 10^{-4}$, and $I = 1$. Node size shows the sum over all the iterations of the amount of the sequence (from that path) present during the simulation. Leaf size (square) shows the amount of WT produced (all nodes annotated with a square are WT). The color of the squares show the probability of that path using the mean field calculation.

regime and the middle ground, and a smaller region between the middle ground and the RW regime (Fig. 1).

Fig. 6 also shows the predictions (or “best guess” for the middle ground) for the various regimes plotted against each other, and it is clear that they are very different, though they are somewhat correlated.

**Experimental Signatures.** It is difficult to completely and directly test the above theoretical analysis experimentally—one would need to sequence a large number of DNA. A more practical choice is to consider only the distance from a sequence to WT, i.e. the number of positions at which they differ, and study its variance in different regimes. In the random walk regime, at most times the DNA pool consists of only a single sequence, thus the variance of this distance is, at any given time in any given run, almost zero, but the variance from one run to the next can be very large. As we increase the population size, the variance within a run increases somewhat, but the variance between runs decreases drastically, and above the middle ground we have almost perfect coherence (Fig. 7).

Fig. 8 shows how the distribution of the distances changes through (a part of) a simulation, and it confirms our earlier assumptions: Only once the whole population has been “upgraded” does further improvement start to become significant, i.e. there are at most two distances with significant population at any one time.

FIG. 7: The variance of the number of errors relative to WT as a function of the time (iteration). $r = 10^{-4}$ and $I = 1$ for all graphs, while $N = 2^{20}$ for a), $N = 2^{18}$ for b) and $N = 2^{32}$

FIG. 8: Distribution of distances from WT as function of time. $r = 10^{-4}$, $I = 1$, $N = 2^{39} \approx 5.5 \cdot 10^{11}$

### III. CONCLUSION

Our simulation and analysis show that in the simple case of additive binding energy the evolution behavior of DNA-protein binding can be understood quantitatively and rather completely. Depending on the population size and the mutation rate, the evolutionary process exhibits distinct behaviors in three parameter regimes. Our results are fairly general as long as the potential is mainly additive and can be used to make sense of experimental data.

It is noteworthy that evolutionary processes via molecular breeding such as the one discussed here are fundamentally different from those where the fitness of an individual depends solely on itself (e.g. on its genotype) and does not depend on others in the population. In the latter case the fitness landscape is fixed and the more fit the better, while in the former case the fit-
ness landscape is dynamic and you just have to be better than the average—to be even better does not increase your fitness. The two cases can lead to, for example, very different evolution dynamics for large population sizes.

The additivity of the binding energy gives rise to a smooth landscape, which greatly simplifies the analysis. The inclusion of a small perturbative non-additive part to the potential would not change the picture, but would nonetheless provide insights to the cases of more general potentials and fitness functions.

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IV. APPENDIX

A. Approximations

Mutation rates. In the main paper we assumed that all mutation rates were the same regardless of which base (A,C,G or T) was mutated and which base it mutated into, while in reality these rates can be very different. While this assumption was done to simplify the analysis, the variations rates will depend on the specific experimental conditions under which the PCRs are performed, and it might thus not have sufficed to choose just one set of base dependent mutation rates.

The simulations, the mean field recursion relations and the analysis of the random walk regime can all easily be altered to include different mutation rates. Note that the exact results for the equilibrium distributions in the random walk regime only hold (in the correct limit) when mutation rates are the same for opposite mutations. The evolution speed for the mean field regime is also still essentially correct, although the least likely mutations will typically occur last and will take longer, as the seed fraction is smaller (this is also addressed in the infinite length model).

Non-specific binding. In the main paper we always started out with a DNA sequence that binds strongly to the protein, such that favorable mutations can be selected quickly. If we start out with a very poor sequence, or, in the simulations, increase the non-specific binding strength of the protein, then even highly favorable mutations will be only weakly selected, as non-specific binding will dominate.

Figure 9 shows how the average number of errors changes through the iterations when we start with a sequence with 10 errors (the usual ones and 4 more) and various values for the non-specific binding strength. For high non-specific binding strengths, none of the mutations are selected strongly, and “nothing happens” until, by chance, several very good mutations combine, yielding a sequence that binds significantly more strongly than by non-specific binding alone - the DNA pool then abruptly improves by all those mutations at once, and the process then proceeds as usual. Also, the original sequence now contains several (3) very weak errors, and once only these remain, the “one mutation at a time” assumption fails.

Note that a very large population size is required in order to be certain that a sufficiently good sequence will be produced (exponentially increasing the further into the non-specific binding regime we are), and the random walk regime no longer exists - for small N, most experiments would never generate the WT sequence.

B. More on Mean Field

Mean Field Recursion Relations. Here we derive a set of recursion equations in the MF regime. We assume that the number of iterations $t_M$ and the chemical potentials $\mu(t)$ are known from a mean field simulation, as discussed in the main paper.

Let $n_t^{S,\pi}$ be the number of WT sequences at time $t_M$ originating from a single molecule of sequence $S$ existing a time $t$ in the experiment, through a specific path $\pi$ (which takes $S$ to WT). Let $P_+(n_t^{S,\pi})$ be the chance that the molecule produces some nonzero amount of WT through $\pi$, $\langle n_t^{S,\pi} \rangle$ be the expected amount, and $\text{Var}(n_t^{S,\pi}) = \langle (n_t^{S,\pi})^2 \rangle - \langle n_t^{S,\pi} \rangle^2$ be the variance.

Given these quantities for time $t'$ right after a selection, we can compute the values for time $t$ right before the selection:

$$P_+(n_t^{S,\pi}) = p_t \cdot S \cdot P_+(n_{t'}^{S,\pi})$$
$$\langle n_t^{S,\pi} \rangle = p_t \cdot S \cdot \langle n_{t'}^{S,\pi} \rangle$$
$$\langle (n_t^{S,\pi})^2 \rangle = p_t \cdot S \cdot \langle (n_{t'}^{S,\pi})^2 \rangle$$

$$p_t = \frac{1}{1 + e^{E(S) - \mu(t)}}$$

Similarly, given the quantities for $t'$ right after a cycle of PCR, we can compute the values for time $t$ right before the selection:
the PCR:

\[ P_0(n_t^{S,\pi}) = P_0(n_t^{S,\pi'}) \left[ 1 - (1 - r)^L P_+(n_t^{S,\pi'}) \right] - \frac{r}{3} (1 - r)^{L-1} P_+(n_t^{S,\pi'}) \]

\[ \langle n_t^{S,\pi} \rangle = \langle n_t^{S,\pi'} \rangle + (1 - r)^L \langle n_t^{S,\pi'} \rangle + \frac{r}{3} (1 - r)^{L-1} \langle n_t^{S,\pi'} \rangle, \]

where \( S' \) is the sequence reached by performing the first mutation in path \( \pi \) on \( S \), \( \pi' \) is the remaining path, and \( P_0(x) = 1 - P_+(x) \).

The first term of equation \[\text{13}\] \( P_0(n_t^{S,\pi'}) \), is the chance that the original, unchanged DNA molecule does not produce any WT at time \( t_M \) (through path \( \pi \)). The second term is the corresponding chance for the possibly mutated copy: If it was not mutated (chance \( (1 - r)^L \)), the chance of producing WT through path \( \pi \) is \( P_+(n_t^{S,\pi'}) \). If it was mutated exactly the right way, i.e., according to the first step of path \( \pi \) (chance \( \frac{r}{3} (1 - r)^{L-1} \)), the chance of producing WT through the remaining part \( \pi' \) of path \( \pi \) is \( P_+(n_t^{S,\pi'}) \), while any other mutation would mean it is off the right path.

Equation \[\text{14}\] is straightforward: \( \langle n_t^{S,\pi'} \rangle \) is the contribution from the unchanged molecule, and the other terms are for the possibly mutated copy. The equation for \( \langle (n_t^{S,\pi'})^2 \rangle \) follows immediately. These equations cover the case where we only allow single mutations; there are additional terms when we allow multiple mutations (i.e., multiple steps along the path).

We trivially know that \( P_+(n_t^{\text{WT},-}) = \langle n_t^{\text{WT},-} \rangle = \langle (n_t^{\text{WT},-})^2 \rangle = 1 \), and the values are zero for all other sequences at that time. By applying the above equations for all selections and PCRs in the experiment, in reverse order, we find the desired quantities for time \( t = 0 \). The results of this calculation for \( r = 10^{-4} \) and \( I = 1 \) are shown in Fig. \[\text{11}\].

**Large \( I \).** As discussed for \( I = 1 \), evolution works by first producing a seed fraction of improved DNA which is then amplified through subsequent iterations. Unlike the \( I = 1 \) case, we can no longer consider all improved DNA (with a given number of errors) to be equivalent:

The maximum amplification (in one iteration) for DNA that is better than the majority of the population is \( 2^I \). However, for this amplification to actually occur, the binding constant of the DNA that is to be amplified must be at least \( 2^I \) times that of the typical DNA in the population. For \( 2^I \gg \Delta K \), the amplification will simply be \( \Delta K \) per iteration.

The highest possible amplification in our model is thus \( \Delta K(\text{WT}) \approx 2^{15} \). For very small \( I \) we expect the number of iterations required to improve by one base (equations 7 and 8), and thus the total number of iterations \( t_M \), to be approximately inversely proportional to \( I \) (this holds for all \( I \) for the infinite length model \[\text{13}\]). As we increase \( I \), the various amplifications are gradually saturated (approach \( \Delta K \)), and for \( I > 15 \) they are all saturated. Increasing \( I \) beyond this only serves to increase the effective rate of mutation. Table \[\text{III} \] shows how \( t_M \) depends on \( I \), and this matches our expectations well.

| \( I \) | 1   | 2   | 4   | 6   | 10  | 15  | 20  | 30  | 50  | 100 |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| \( t_M \) | 151 | 80  | 46  | 36  | 28  | 25  | 22  | 21  | 20  |     |

**TABLE III:** Number of iterations for mean field simulations. \( r = 10^{-4} \) for all values.

For large \( I \), the amplification of a given DNA sequence in an iteration is approximately the ratio of its binding constant to that of the typical DNA in the population, thus DNA that is much better than the average will be amplified far more strongly. In particular, DNA that mutates to WT during the very first iteration will receive the maximum possible amplification, and this process is the one that contributes the most WT in a simulation.

We can estimate this contribution and the corrections due to DNA that does not quite reach WT the first iteration, as well as the variation from this process. These calculations are carried out below and give us an estimate for the lower bound of the mean field regime for large \( I \):

\[ N > \max_{\pi} \frac{\text{Var}(n_{\text{WT}}(t_M))}{\langle n_{\text{WT}}(t_M) \rangle^2} \propto \left( \frac{3}{Tr} \right)^m. \]

**FIG. 10:** Lower bound for mean field regime calculated from recursion relations vs. analytical estimate, as function of \( I \). \( r = 10^{-4} \).

**Mean field estimates: Large \( I \).** When \( I \) is sufficiently large, the chemical potential for the selections is much lower than even the binding energy of WT, thus the chance that a DNA molecule will survive a selection is proportional to the binding constant of the sequence. The chance that each initial error will be corrected during any given iteration (\( I \) cycles of PCR) is \( \frac{r}{3} \), but for each iteration that mutation \( i \) has not happened, the DNA will be amplified by a factor \( \Delta K_i \) less than those that
have reached WT. The number of WT molecules created (through all paths), as a function of time, is approximately

$$\langle n_{WT}(t) \rangle \approx f(t)2^{t-m} \left( \frac{I_r}{3} \right)^m \prod_i \left( \frac{1}{1-(\Delta K^i)^{-1}} \right),$$

(16)

where $i$ runs over the mutated positions, and $f(t)$ is a function describing the ratio of WT molecules that would have survived all the selections so far. The powers of 2 is the total number of PCRs minus the number of cycles in which the DNA mutated (ignoring simultaneous mutations), and the last factor corrects for mutations that didn’t happen the first iteration.

The main source of variance is whether or not a starting molecule produces a WT molecule that survives the first selection:

$$\text{Var} \left( \langle n_{WT}(t) \rangle \right) \approx \left( \langle n_{WT}(t) \rangle \right)^2$$

$$\approx \left( 2^{t+m-1} \frac{f(t)}{f(1)} \right)^2 \frac{2^{t-m}}{m!} \left( \frac{I_r}{3} \right)^m f(1)$$

(17)

$$\approx \frac{2^m}{m!} \left( \frac{3}{I_r} \right)^m \prod_i \left( \frac{1-(\Delta K^i)^{-1}}{\Delta K^i} \right)^2$$

(18)

where we have used $f(1) = \prod_i \Delta K^i$ — this assumes that, before the selection for the first iteration, most of the DNA sequences have not been mutated, which fixes the chemical potential $\mu_1$. The first factor of eq. (17) is the average number of WT molecules that a WT molecule that survives the first selection would produce by time $t_M$, squared, and the remaining factors are the chance that such a molecule is produced; the expected number of WT molecules produced through a single path during the $I$ cycles of PCR in the first iteration times the chance $f(1)$ that each WT molecule survives the first selection.

We eliminated $f(t)_{t_M}$ from equation (17) by using equation (18) setting $\langle n_{WT}(t) \rangle \approx 1$. From this we can get a rough estimate for the lower bound of the mean field regime by using $\langle n_{WT}(t) \rangle \approx \frac{1}{m!}$, as for $I = 1$. However, for large $I$ we can do better by calculating the relative contributions of individual paths directly:

$$\langle n_{WT}(t) \rangle \approx f(t)2^{t-m} \left( \frac{I_r}{3} \right)^m \prod_i \left( \frac{1}{1-(\Delta K^i)^{-1}} \right)^2$$

$$g_i(\pi) = \frac{1}{i!} \sum_{j=1}^{i-1} \frac{g_j(\pi_j)}{(i-j)!} \frac{1}{\Delta K(\pi) - 1},$$

(19)

(20)

where $\pi_j$ means the last $j$ steps of path $\pi$, and $\Delta K(\pi)$ is the total change in binding constant, i.e. the product of the $\Delta K$ for the individual mutations of path $\pi$. Knowledge of initial sequence is implicit.

Using equation (19) to eliminate $f(t)$ in equation (17) we find the bound for the MF regime directly:

$$N > \max_\pi \frac{2^m}{m!} \left( \frac{3}{I_r} \right)^m \frac{\Delta K(\pi)}{(g_m(\pi))^2}$$

(21)

**Mean field estimates: $I = 1$.** A DNA molecule that has higher binding energy than the average will typically survive a selection, and vice versa. The simplest estimate we can use for the behavior of the average binding energy is a linear change from the binding energy of SS to that of WT.

Given that a DNA molecule mutates from SS to WT during the $t_M$ iterations of the simulation, we estimate that it will survive all the selections iff the number of good mutations is always at least $t_M^m$. The number of ways this can happen is approximately $\frac{t_M^m}{m!}$.

There must be a mutation at the first iteration, giving the factor $m$ and leaving approximately $(t_M)^{m-1}$ combinations for the remaining mutations. Given $m$ points randomly distributed on a circle of length $m$, there is with probability 1 exactly one point for which, when traveling clockwise from that point, you will always have visited more points than the length you have traveled (assuming $t_M \gg m$, this continuum case should be good enough) — thus, the chance that we started from the right point/mutation is $\frac{1}{m}$.

From this, the number of WT molecules produced from a single SS molecule in $t_M$ iterations is about

$$\langle n_{WT}(t_M) \rangle \approx 2^{t_M-m} \left( \frac{3}{3} \right)^m \left( \frac{t_M}{m} \right)^{m-1},$$

(22)

where the powers of two are the number of iterations where the DNA didn’t change (and thus was duplicated).

The simulation ends when there are about as many WT molecules as there were SS molecules at the beginning:

$$1 \approx \langle n_{WT}(t_M) \rangle$$

$$\downarrow$$

$$t_M \approx m + m \log_2 \left( \frac{3}{M} R \right) - (m-1) \log_2(t_M).$$

(23)

This is very similar to $T_0 + \sum_{m'=1}^{m-1} T(m')$, using equations 7 and 8 and $\log_2(t_M) \approx m \log_2(T)$ — most of the discrepancy corresponds to the higher order corrections $\frac{1}{\log^2(T)}$ found for the infinite length model [18].

Regarding the variance $\text{Var}(\langle n_{WT}(t_M) \rangle)$ of the number of WT molecules produced from a single SS molecule through a single path $\pi$, any duplication that occurs before the WT is reached will only yield a factor of 2 — the two molecules must independently develop to the WT — while duplication once the WT is reached gives a factor of 4. Because of this, the variance is dominated by the extremely rare events where all the good mutations happen very early (the first $m + \Delta$ iterations), and the WT molecules are then simply duplicated until the simulation ends:

$$\text{Var}(\langle n_{WT}(t_M) \rangle) \approx \langle n_{WT}(t_M) \rangle^2$$

$$\approx \sum_{\Delta \geq 0} 2^{t_M-2m-\Delta} \left( \frac{3}{3} \right)^m \frac{(m-2+\Delta)!}{\Delta!(m-2)!}$$

(24)

$$= 2^{2t_M-m-1} \left( \frac{3}{3} \right)^m.$$

(25)
If the DNA is mutated to WT in the first \( m + \Delta \) iterations (\( \Delta \) is the number of “idle” iterations), it will then be duplicated \( t_M - m - \Delta \) times, giving a contribution \( 2^{2t_M - 2m - 2\Delta} \). Also, the DNA will be duplicated during each idle iteration, but this merely increases the chance that a molecule will reach WT, and thus gives a factor \( 2^\Delta \).

The number of ways the mutations can happen is \( \frac{(m+2+N)!}{\Delta!(m-2)!} \), assuming that there must be a mutation the first iteration — we have ignored the possibility of multiple mutations in one cycle of PCR, which is a significant correction. Equation 25 follows by using

\[
\sum_{\Delta \geq 0} x^{-\Delta} \frac{(n + \Delta)!}{\Delta!n!} = \left( \sum_{n \geq 0} x^{-n} \right)^{n+1}. \tag{26}
\]

We can then use the average \( \langle n_{\pi WT}(t_M) \rangle_\pi = \frac{1}{t_M} \) to estimate (very roughly) the lower bound for the mean field regime.

For \( r = 10^{-4} \) we estimate \( t_M \approx 65 \) and thus \( \text{Var}(n_{\pi WT}(t_M)) \approx 5.8 \times 10^{18} \). The actual values are \( t_M = 83 \) and \( \text{Var}(n_{\pi WT}(t_M)) \approx 1.5 \times 10^{12} \). The somewhat odd fact that \( t_M \) is pretty far off, while the variance, which depends very strongly on \( t_M \), is reasonably close, is not entirely unexpected: Towards the end of the simulation, many WT molecules will be selected away (as the chemical potential for the selection approaches the binding energy of WT), which will increase \( t_M \). However, this will also affect the variance correspondingly, such that using the solution from equation 23 in equation 25 will still give the right value. Considering that we have used an extremely simple approximation for selection (and completely ignored the \( \Delta K \) for the various mutations), the estimate seems very reasonable. As a check, when using very large energies for the mutations (while keeping the ratios fixed), the simulations yield \( t_M = 65 \) for the mean field regime.

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FIG. 11: Tree of evolution to WT for mean-field regime. The root is SS, the leaves are WT produced through individual paths, the edges are mutations. Only minimum paths are included. Size of internal nodes is given by the sum of the size of the leaves.