Extreme genetic code optimality from a molecular dynamics calculation of amino acid polar requirement

Thomas Butler and Nigel Goldenfeld
Department of Physics and Institute for Genomic Biology, University of Illinois at Urbana Champaign, 1110 West Green Street, Urbana, IL 61801 USA

Damien Mathew and Zaida Luthey-Schulten
Center for Biophysics and Computational Biology, Department of Chemistry and Institute for Genomic Biology, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave, Urbana, IL 61801 USA

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A molecular dynamics calculation of the amino acid polar requirement is presented and used to score the canonical genetic code. Monte Carlo simulation shows that this computational polar requirement has been optimized by the canonical genetic code more than any previously-known measure. These results strongly support the idea that the genetic code evolved from a communal state of life prior to the root of the modern ribosomal tree of life.

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The genetic code is one of life’s most ancient and universal features\(^1\,^2\). It summarizes how RNA transcripts are translated into amino acids to form proteins, and is shared by all known cells, across the three domains of life, with only a few very minor variations\(^3\,^4\). Representing a complex series of biochemical steps that comprise all known cell’s translation apparatus, the canonical genetic code is a mapping from the space of triplets of nucleotide codons to the space of amino acids. Sequences of codons then correspond to protein sequences, and ultimately give rise to each organism’s phenotype.

Almost immediately after its elucidation, attempts were made to explain the assignment of codons to amino acids. It was noticed that amino acids with related properties were grouped together, which would have the effect of minimizing translation errors\(^5\,^6\,^7\). In order to determine whether or not this was a genuine correlation or simply a fluctuation reflecting the limited size of the codon table, the canonical genetic code was compared to samples of randomly-generated synthetic codes, starting with early but inconclusive Monte Carlo work of Alff-Steinberger\(^8\), and compellingly revisited with larger sample sizes by Haig and Hurst\(^9\). Depending on the measure used to characterize or score the sampled codes, high degrees of optimality have been reported. For example, using an empirical measure of amino acid differences referred to below as the “experimental polar requirement” (EPR) \(^10\,^11\), Freeland and Hurst calculated that the genetic code is “One in a million”\(^12\). More recently, it has been shown that when coupled to known patterns of codon usage, the canonical code (and the codon usage) is simultaneously optimized with respect to point mutations and to the rapid termination of peptides that are generated with frame shift errors\(^13\).

These results are generally interpreted to imply that the canonical genetic code had to have undergone a period of evolution, and was not simply a frozen accident\(^14\,^15\). While it was long assumed that code evolution would be lethal, it has been recently shown how a genetic code can evolve along with a dynamic refinement of the precision of translation\(^16\,^17\,^18\). Under vertically-dominated evolutionary dynamics, the optimization of the code is relatively weak: the system gets trapped in “local minima” and is neither strongly optimized nor converged to a unique code. On the other hand, if the evolutionary dynamics is horizontally-dominated, with genes shared between organisms (as is the case with contemporary microbes\(^19\)), modularity of structures such as the translation apparatus and the genome emerges naturally\(^20\), and optimization is strong, rapid and convergent to a universal genetic code, suggesting that early life was communal in nature and collective in its dynamics\(^18\). Thus, the extent of optimality of the canonical genetic code is of great interest, because the greater the level of optimization, the more likely it is that the genetic code evolved when life was communal in character.

The purpose of this Letter is to set a lower bound on the level of optimality of the canonical genetic code. We do this by using molecular dynamics to construct a measure of code optimality without any input from experiment, specifically by simulating the equilibrium behavior of free amino acids in water-pyridine solutions, resembling those of the original polar requirement experiments, and constructing a “computational polar requirement” (CPR) by analysis of certain two-point correlation functions. We then use Monte Carlo simulation to determine the level of code optimality, and find that the level is so high that a new and detailed error analysis is required to ensure statistically significant assessment of very small probabilities. We also explore the dependence of our re-
sults on the sensitivity of code optimality to the scale of code variations. Our results lend strong support to evolutionary scenarios for the structure of the genetic code, with a level of optimization that would only be attainable from some form of collective dynamics. We also report indications that the dynamics involved the refinement over evolutionary time of a primitive translation machinery that was ambiguous, generating a statistical ensemble of related proteins (“statistical proteins”) rather than a unique protein, as is now the case.

Molecular Dynamics of the Polar Requirement: The experimental polar requirement is a chromatographic measure of amino acid affinity to a water-pyridine solution that was originally motivated by a simple stereochemical theory of the origin of the genetic code. This measure is related to, and strongly correlated with, several other amino acid measures, such as hydrophobicity and Grantham polarity, but is not simply related to these other measures. The original polar requirement experiments used partition chromatography. In the EPR experiments, water/dimethylpyridine (DMP) ratios ranging from 40-80% mole fraction water were used for each amino acid measured. When the chromatographic factor, $R_m$, was plotted as a function of mole fraction water in log-log scale, a linear trend was observed for each amino acid. The slope of the corresponding best fit line was taken to be the amino acid’s EPR.

The methods used for obtaining the computational polar requirement numbers (CPR) have been reported elsewhere and are summarized here. The distribution of solute molecules across the water/DMP interface is related to the equilibrium solvent environment surrounding the molecules in a binary solution similar to that used in the experiments. Trends in the local water density of a solvated amino acid in water/DMP solutions were found to be linear functions of mole fraction water. The slopes of these linear trends were used to obtain a set of computed CPR values. To quantitatively measure the differences in local solvent environment, molecular dynamics (MD) calculations were performed using NAMD2 with an NPT ensemble and the Charmm 27 force field. A pressure of 1.01325 bar and temperature of 300K were maintained for each simulation. The systems consisted of a single amino acid molecule in a box of water and randomly placed DMP molecules of a determined water/DMP ratio. For each amino acid at least four systems, each with a different water/DMP ratio, were simulated. Radial distribution functions (RDFs) of water relative to the amino acid side chains were calculated from the equilibrated MD trajectories using VMD (Visu-}

![FIG. 1: Scatter plot showing the relationship between RDF peak slope and experimental polar requirement for all amino acids. The straight line is a guide to the eye.](image-url)
calculations, we used the values from \cite{12} as listed in table \ref{table1}. Finally, \(d^q(x, y)\) is a metric on the space of amino acids. For the polar requirement, the metric is taken to be \(d^q(x, y) = |x - y|^q\) over the polar requirement values corresponding to the given amino acid.

The appropriate quantity to compute is the probability \(P_b = Pr(O > O_1)\) that a random realization is more optimal than the canonical code. To compute \(P_b\), we count the number of randomly generated codes that are more optimal than the canonical code and divide by the total number of random codes generated. \(P_b\) is invariant to uniform linear rescaling of the amino acid polar requirement data, and is smaller for more optimal codes while including the effects of the large number of codes that can be explored, rather than the simple linear scale provided by the bare optimality score.

The error in the computed \(P_b\) can be estimated using an analytical realization of bootstrap resampling. Simulated data sets for bootstrap are created by randomly sampling optimality scores from the original data set. When the samples are drawn from the original set, there are only two alternatives: a more, or less optimal code can be sampled, with probability \(P_b = N_{O>O}/N_{total}\) of drawing a random code better than the canonical code. Since the number of better codes in a sample is the number whose error we wish to estimate, we can regard drawing a better code as a step to the right with probability \(P_b\) in a one dimensional random walk. The known formulas for the asymmetric one dimensional random walk allow us to immediately write down the bootstrap error estimate in the limit of infinitely many resampled sets, i.e. the exact bootstrap estimate. For metrics under which the code is fairly optimal (i.e. \(P_b \ll 1\)), we obtain the variance in \(P_b\) to be

\[
\text{var}(P_b) = \text{var}\left[\frac{N_{O>O}}{N_{total}}\right] = \frac{P_b(1 - P_b)}{N_{total}^2} \approx \frac{N_{O>O}}{N_{total}^2} \quad (2)
\]

To obtain a reasonable estimate of error, or to compare the results of different metrics on the space of amino acids, the number of more optimal codes, \(N_{O>O}\), from the random sample must be sufficiently large (\(\sqrt{N_{O>O}} \ll N_{O>O}\), or about \(N_{O>O} = 10\) as a reasonable minimum).

When the computational polar requirement difference squared is used in the amino acid metric \(P_b = (19 \pm 4.36) \times 10^{-8}\). In contrast, with the experimental polar requirement, \(P_b = (26.5 \pm 1.63) \times 10^{-7}\), and order of magnitude improvement. To assess the impact of tyrosine (which had the largest variation between the CPR and EPR values) on these results we redid the calculation of \(P_b\) for the CPR, but with tyrosine replaced with the value from the EPR. The result is \((P_b = (9.3 \pm 1.0) \times 10^{-7})\).

To test the sensitivity of the results for the CPR, we varied each element of \(W_{e,c'}\) independently by \(\pm 0.1 \times W_{e,c'}\) and repeated the calculation of \(P_b\). This led to results that were statistically indistinguishable from the results reported above. Shorter computations (justified by the faster convergence due to decreased optimality) for the EPR indicate a similar level of robustness. With a \(W_{e,c'}\) uniform among nearest neighbors we saw substantial increases in \(P_b\) in agreement with \cite{3}. However, the CPR continued to be superior to the EPR, with the CPR yielding \(P_b = (3.7 \pm 0.61) \times 10^{-5}\) and the EPR yielding \(P_b = (11.8 \pm 1.1) \times 10^{-5}\).

Varying the value of \(q\) in the metric \cite{30} provides a further probe to explore the optimization of the genetic code. Increasing the value of \(q\) is equivalent to emphasizing the role of larger and larger differences between the amino acid intended, and the one generated by point mutation. Thus, if \(P_b\) reduces for increased values of \(q\), the code (along with \(W_{e,c'}\) evolved to suppress the effects of rarer, possibly catastrophic errors that may be generated by point mutations. This may happen primarily by evolving small elements of \(W_{e,c'}\) where \(c \rightarrow c'\) is catastrophic, or vice versa. Conversely, if \(P_b\) reduces for smaller values of \(q\), the code evolved to both mitigate the possibility of these catastrophic errors, and to minimize the effects of frequent, small errors. Varying \(q\) we find that the canonical genetic code is most optimal for \(q\) between one and two with significant increases outside this regime in either direction (Fig. \ref{fig2}). This indicates that the genetic code is optimized for minimizing errors according to their size with no undue emphasis to larger or smaller errors. Given the relative weakness of the code when emphasizing large errors, evolution must have favored organisms that discarded or edited fatally flawed proteins over evolving the code to make them less likely at the cost of reducing its ability to minimize the more frequent moderate and minor errors. The weakness of the canonical code when minor errors are emphasized (\(q < 1\)) suggests that while the code was still evolving, minor errors were on the whole less important biologically, as would be expected in evolutionary dynamics \cite{17, 18} that utilized ambiguity tolerance in early proteins \cite{6, 21}.

**Optimality analysis of alternative codes and measures:**
A selection of variant codes were also analyzed using the CPR. Our findings, displayed in table \ref{table1} were consistent with the previous findings of Knight in that the alternative codes did not show marked improvements in optimality over the canonical code \cite{13}. This is consistent with our expectation that evolutionary pressure to optimize the code with respect to the polar requirement was eased after the last universal ancestral state.
We also tested Grantham polarity, which has been argued in a survey of genetic code optimality under different amino acid measures to be the amino acid measure most optimized by the genetic code. The results yield $P_b = (285 \pm 16.88) \times 10^{-8}$, or an order of magnitude higher than with the CPR metric, leading to the conclusion that the CPR is the most effective known metric for optimization of the genetic code. Previous computations evaluated $P_b$ by generating 100,000 random codes. Scaling our results to the size of these original simulations, we see that the EPR and the Grantham polarity have virtually identical scores. Scaling the errors for the CPR and the Grantham polarity to errors assessed from only 100,000 codes, we get for the CPR, $P_b = (0.19 \pm 0.44) \times 10^{-3}$ and for the Grantham polarity, $P_b = (2.85 \pm 1.69) \times 10^{-5}$. These results are within a standard deviation and a half of each other, and are therefore not different in a statistically meaningful way.

In conclusion, earlier estimates of code optimality were understated by a statistically significant amount. The extent of optimality revealed here further supports the notion that the genetic code must have evolved during an early communal state of life.

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