No signature of natural selection in patterns of protein structural divergence

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Abstract

Proteins diverge during biological evolution. At sequence level, different sites evolve at different rates, mainly due natural selection. In contrast, it has been suggested that observed patterns of structural divergence are not a signature of natural selection but, rather, of the response of protein structure to random mutations. Here, we have systematically studied whether there is any signal of natural selection in patterns of protein structural evolution. We model evolution as follows: (1) proteins are Elastic Networks of amino acids, (2) a mutation at a site perturbs the springs that connect it to its neighbors, (3) selection is either not considered (by fixing all mutations) or included by fixing mutants according to a stability-based fitness function. We analyzed variation of structural divergence among sites and among normal modes. We compared predicted and observed patterns for several protein families. We found very good agreement between predicted and empirical structural divergence patterns whether natural selection is considered or not. For all cases studied, including selection does not improve model fit. Therefore, observed patterns can be explained in terms of mutational robustness of the structure. In a word, we found no evidence of natural selection in patterns of structural divergence.

1 Introduction

Proteins diverge during biological evolution, which is evident in the variation of the aminoacid sequences and the resulting structural, dynamical and functional changes. It is known that the structure diverges much more slowly than the sequence, that the structural divergence occurs mainly along the lower energy vibrational modes and that there is a structurally conserved core. These facts are difficult to interpret because most of the studies made so far are purely empirical. To go forward in this sense, the mechanistic model "Linearly Forced – Elastic Network Model" (LF - ENM) was developed , which predicts the change in the equilibrium position of protein sites as the result of random mutations, not subjected to natural selection [1]. Applying this model, it was shown that the experimental patterns of structural change can be reproduced without resorting to natural selection [3,4]. This result call into question interpretations based on the assumption that everything that is conserved or that varies is related to the biological function.

While natural selection apparently little affects structural divergence patterns, at the level of aminoacid sequences different sites evolve at different speeds mainly due to natural selection. Purely mutational evolutional models, such as the LF-ENM, cannot account for this fact. To explain such patterns of sequence variation, natural selection must be modeled. We have recently proposed a mechanistic stress model of evolution [5], which is based on the idea that a mutant is viable to the extent that it spends time in the active conformation. This model has been successfully used to account for the average evolutionary variation from site to site [6].

Considering this scenario, we set out to study the role of natural selection at the structural divergence level with a different approach: (1) using the LF - ENM to simulate mutations and (2) either not selecting them or fixing them according to the stress model fitness function. We will show that the agreement between experimental and simulated structural divergence profiles is high either considering or not natural selection and that, including natural selection, does not improve the model fit.

2 Results and discussion

We aim to study the role of natural selection on the structural divergence of proteins by comparing experimental proteins with simulated mutants obtained either considering or not natural selection. To do this, we first selected diverse families of proteins from the Database of Multiple Structural Alignments of Homologous HOMSTRAD according to the criteria explained in Methods. We extracted from HOMSTRAD the multiple structural alignments and the superimposed coordinates of all of the proteins. Then, for each family, we selected the most structurally representative protein as the reference "ancestor" protein and we considered the other proteins different lineages in a "star tree" that begins with the ancestor. For each lineage, we extracted aligned aminoacids and calculated the "branch length", which we considered as the number of mutated sites in the aligned region. After that, we simulated multiple mutants of the ancestor using the LF - ENM and selecting each single mutation according to the probability of fixation given by the stress model. To account for different selection regimens, we gave the average probability of acceptance of mutations different values: ≈ 1 (no selection, all mutants are accepted), ≈ 0.9 (weak selection), ≈ 0.5 (medium selection) and ≈ 0.1 (strong selection). Finally, we calculated measures of structural variability of aligned sites on Cartesian coordinates and projected on the normal modes of the reference protein. We compared these measures for experimental and theoretical profiles in order to find out whether there is any signal of natural selection.

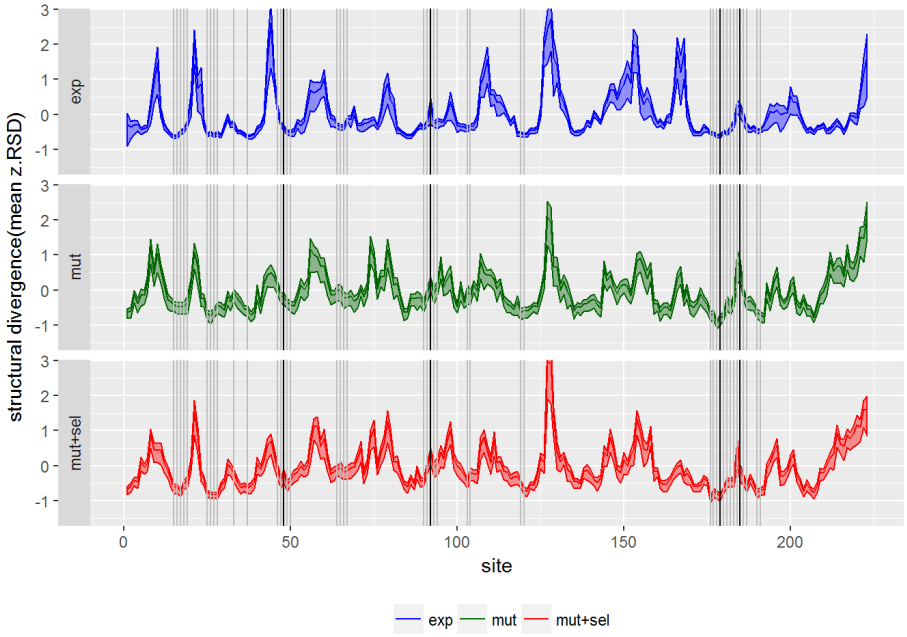
Cartesian coordinates

For each family we calculated experimental and theoretical $< zRSD_i >$ profiles as explained in Methods and we calculated the correlation coefficient (CC) between these profiles. The results are shown in Table 1.

Table 1 shows that, for all families studied, the CC between the experimental profile and the different theoretical profiles are very good (≈ 0.72) and that, accounting for natural selection, does not seem to improve the agreement.

Figure 1 shows the $< zRSD_i >$ profiles obtained for the reference protein of the Serin Proteases family and figure 2 shows the same protein colored according to the different $< zRSD_i >$ profiles. The active site of the protein and its neighborhood are shown in both figures. It can be noticed in figure 1 and figure 2 that the qualitative similarity between experimental and theoretical profiles, with any selection regimen, is high, even in the active site of the protein and in the active site´s neighborhood. A high qualitative similarity is observed for the other families too (data not shown).

Figure 1: $< zRSD_i >$ experimental and theoretical profiles obtained for the Serine Proteases family. Only no selection and strong selection theoretical profiles are shown. The active site of the reference protein corresponds to the vertical black lines and its neighborhood corresponds to the vertical gray lines.

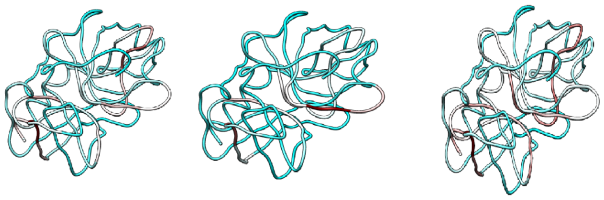


2.0.1 Active site distance

To focus on the active site and on its neighborhood, we calculated the CC between $< zRSD_i^{experimental} > - < zRSD_i^{theoretical} >$ and the distance of each site to its closest active site. If there was any signal of natural selection we would expect that the difference between these profiles would be negative for sites near the active site and close to 0, positive or negative, for distant sites. Thus, we would expect a positive correlation coefficient. Table 3 shows the results obtained for all enzymatic families.

It can be noticed in Table 3 that the CC obtained for some cases have the predicted sign but are very low. Moreover, as we suspected that this slight CC might be due to the fact that more divergent sites have less information and that their variability tend to be underestimated, we repeated the analysis only on the conserved core of proteins (sites of the ancestor with no gaps on the

Figure 2: Reference protein of the Serine Proteases family colored according to $< zRSD_i >$ experimental and theoretical profiles. Only no selection and strong selection theoretical profiles are shown. The active site of the reference protein corresponds to the gold sites and its neighborhood corresponds to orange sites.



whole structural alignment). We noticed that, for all cases, taking out divergent and noisy sites diminished the CC to a very low value (data not shown). These results are more proof that there is no evidence of natural selection on structural divergence of proteins even in the active site neighborhood.

Normal modes coordinates

For each family, we calculated theoretical and experimental $< P_n >$ profiles as explained in Methods and we calculated the CC between these profiles. The results are shown in Table 2.

Table 2 shows that the CC between the experimental profiles and theoretical profiles is high (≈ 0.73) and that natural selection does not improve the fit, another proof of the lack of natural selection on the structural evolution of proteins.

2.1 Stress model verification

As we used here a fitness function based on the stress model, we must verify that sequence evolutionary rates obtained for these families by this model correlate better with experimental sequence profiles than evolutionary rates of a purely mutational model. To do this, for each family we calculated the number of times we had mutated each site on the subset of simulated mutants under each different selection regimens. Then, we correlated obtained profiles with site´s evolutionary rate obtained from ConsurfDB. Results are shown in Table 4.

It can be observed in Table 4 that the stress model indeed predicted the site´s evolutionary rate at a great extent. Thus, we proved that our selection function is suitable and that not finding differences between different regimens

profiles means that there is no signature of natural selection on protein structure evolution.

3 Methods

3.1 ENM

We consider the backbone fluctuations of a protein around its equilibrium conformation to be described by a coarse - grained "Elastic Network Model" (ENM), which represents a protein as a network of sites connected by springs. In general, the ENM potential is of the form:

$$V_{wt} = \frac{1}{2} \sum_{i} \sum_{i<j} k_{ij} (d_{ij} - d_{ij}^0)^2 \tag{1}$$

where k_{ij} is the force constant of the spring connecting sites i and j, d_{ij} is the distance between sites i and j and d_{ij}^0 is the equilibrium distance between these sites. These distances are calculates as the modules of $\mathbf{d}_{ij} = \mathbf{r}_i - \mathbf{r}_j$ and $\mathbf{d}_{ij}^0 = \mathbf{r}_i^0 - \mathbf{r}_j^0$ respectively, being $\mathbf{r}$ the position of a given site and $\mathbf{r}_0$ the equilibrium position of the site.

LF - ENM

To simulate mutants of a protein we used the "Linearly Forced - Elastic Network Model" (LF - ENM). This model simulates the effect of a single mutation by perturbing the equilibrium lengths of the ENM springs: $d_{ij}^0 \rightarrow d_{ij}^0 + \Delta_{ij}$, where Δ_{ij} are picked independently from the same uniform distribution, which satisfies $< \Delta_{ij} >= 0$ and $Var(\Delta_{ij}) = \sigma^2$. Following this, the mutant's potential is of the form:

$$V_{mut} = \frac{1}{2} \sum_{i} \sum_{i<j} k_{ij} [d_{ij} - (d_{ij}^0 + \Delta_{ij})]^2 \tag{2}$$

Then, the LF - ENM is obtained from expanding Eq. 2 up to second order. The potential is expressed in terms of "forces" directed along the contacts of the mutated site with lengths of the form $f_{ij} = k_{ij} \Delta_{ij}$. Finally, the equilibrium structure of the mutant $\mathbf{r}_{mut}^0$ is the value of $\mathbf{r}$ that minimizes V_{mut}. Using Eqs. 1 and 2 and after some algebra we find the structural variation due to the mutation of a protein of N sites:

$$d\mathbf{r}^0 \equiv \mathbf{r}_{mut}^0 - \mathbf{r}_{wt}^0 = \mathbf{K}_{wt}^{-1} \mathbf{f} \tag{3}$$

being $\mathbf{r}$ a $3N$ vector of coordinates, $\mathbf{f}$ a $3N$ vector of forces and $\mathbf{K}$ a $3N$ x $3N$ stiffness matrix, which represents the network s topology and the spring force constants.

Stress Model of protein evolution

The stress model of protein evolution predicts the acceptance probability of single mutations. The model is based on the idea that a mutant is viable to the extent that it spends time in the active conformation, which will depend

on mutational changes of the stability of the active conformation. The fixation probability of a mutant is modeled as:

$$P_{fix} \propto C^F_{mut}\rho_{mut}(\mathbf{r}_{active})/C^F_{wt}\rho_{wt}(\mathbf{r}_{active}) \tag{4}$$

where wt stands for wild-type, mut for mutant, C^F is the concentration of folded protein and $\rho(\mathbf{r}_{active})$ its probability of adopting the active conformation. Assuming that C_{mut}/C_{wt} is equal to the ratio of partition functions, from basic statistical physics it follows that:

$$P_{fix} \propto e^{-\beta\Delta V^*} \tag{5}$$

where $\Delta V^* = V_{mut}(\mathbf{r}_{active}) - V_{wt}(\mathbf{r}_{active})$ is the energy difference between the mutant and the wild-type in the active conformation and β represents the selective pressure. Lower values of β imply weaker selective pressure and higher values of β imply stronger selective pressure. From Eqs. 1 and Eqs. 5 we get:

$$P^i_{fix} = e^{-\beta\frac{1}{2}\sum_{j\neq i} k_{ij}\Delta^2_{ij}} \tag{6}$$

3.2 Two-nodes per site evolution model

As we previously found that sequence evolutionary rates are better reproduced using a model that considers both alpha carbons (C_α) and geometric centers (ρ) of aminoacids [] than using a one-node per site model, in this work we represented proteins by means of the two-nodes per site model. The ENM potential of Eq. 1 can be rewritten in terms of C_α and ρ distances:

$$V_{wt} = \frac{1}{2}\sum_i\sum_{i<j} k_{C_{\alpha_i}C_{\alpha_j}}(d_{C_{\alpha_i}C_{\alpha_j}} - d^0_{C_{\alpha_i}C_{\alpha_j}})^2 + \frac{1}{2}\sum_i\sum_{i<j} k_{C_{\alpha_i}\rho_j}(d_{C_{\alpha_i}\rho_j} - d^0_{C_{\alpha_i}\rho_j})^2 + \frac{1}{2}\sum_i\sum_{i<j} k_{\rho_i C_{\alpha_j}}(d_{\rho_i C_{\alpha_j}} - d^0_{\rho_i} \tag{7}$$

where $d_{n_in_j}$ is the distance between nodes n_i and n_j (n is either C_α or ρ), $k_{n_in_j}$is the force constant of the spring connecting these nodes, and $d^0_{n_in_j}$ is the equilibrium spring length.

A mutation at site i will replace ρ_i, affecting only the parameters of the energy function related to this node. Following [], we model a mutation at site i by adding random perturbations to the lengths of the springs connected to ρ_i: $d^0_{\rho_i\rho_j} \rightarrow d^0_{\rho_i\rho_j} + \Delta_{\rho_i\rho_j}$ and $d^0_{\rho_i C_{\alpha_j}} \rightarrow d^0_{\rho_i C_{\alpha_j}} + \Delta_{\rho_i C_{\alpha_j}}$. We can again express the potential of the mutant in terms of "forces" directed along the C_α and ρ contacts of the mutated site with lengths of the form $f_{\rho_i\rho_j} = k_{\rho_i\rho_j}\Delta_{\rho_i\rho_j}$ and $f_{\rho_i C_{\alpha_j}} = k_{\rho_i C_{\alpha_j}}\Delta_{\rho_i C_{\alpha_j}}$. Then, we use the same criteria explained for the one-node per site model to get the structure of the mutant using Eq. 3, being now $\mathbf{K}$ a $6N$ x $6N$matrix and $\mathbf{f}$ a column vector of length $6N$.

To calculate ΔV^*, we use Eq. 7 and Eqs. 5 to get:

$$\Delta V^* = \frac{1}{2}\sum_{i\neq j}(k_{\rho_i C_{\alpha_j}}\Delta^2_{\rho_i C_{\alpha_j}} + k_{\rho_i\rho_j}\Delta^2_{\rho_i\rho_j}) \tag{8}$$

and to rewrite the fixation probability as follows:

$$P^i_{fix} = e^{-\beta\frac{1}{2}\sum_{i\neq j}(k_{\rho_i C_{\alpha_j}}\Delta^2_{\rho_i C_{\alpha_j}} + k_{\rho_i\rho_j}\Delta^2_{\rho_i\rho_j})} \tag{9}$$

For the special case of the "Anisotropic Network Model" (ANM), which gives a spring force constant of 1 to nodes at a distance $< R_0$ (the contacts) and of 0 to nodes at a distance $> R_0$, we get:

$$< P^i_{fix} >= e^{-\beta \frac{1}{2} < \Delta^2_{ij} > (CN_{i_{C_\alpha \rho}} + CN_{i_{\rho\rho}})} \tag{10}$$

being $CN_{i_{c_\alpha \rho}}$ and $CN_{i_{\rho\rho}}$the number of C_α and ρ contacts of ρ_i respectively.

3.3 Experimental dataset

We selected 8 families of proteins from the Database of Multiple Structural Alignments of Homologous HOMSTRAD. In the dataset, there are representatives of the major structural classes: all alpha, all beta, alpha and beta and small proteins. We looked for families that possess multiple structural alignments with more than 12 proteins and with an alignment length greater than 50 sites. For each family, we obtained the multiple structural alignment and the superimposed coordinates of the proteins. Then, we selected a reference "ancestor" protein. To do this, we calculated the average structure of the family and selected the protein with the lower "Mean Square Deviation" (MSD) between its structure and the average structure. We approximated each family tree topology by a "star tree" that begins with the ancestor protein. Each lineage corresponds to a pair alignment of each of the other proteins with the ancestor protein and the corresponding"branch length" is the number of mutated sites.

Theoretical dataset

For each family and for each lineage we simulated 100 mutants of the reference protein following a path of substitutions composed of various evolutionary steps, each of them comprising a single substitution. The steps were simulated by first picking one random site l of the reference protein, obtaining a set of forces f_{lj} for each of the j (C_α and ρ)contacts of ρ_l and the reaction force over ρ_l, and calculating the mutant´s structure from Eq. 3. The **K** matrix used is $6N$ x $6N$ and is based on a two-nodes per site model that considers both C_α and ρ interactions. After we obtained the structure of the mutant, we calculated the probability of fixation of the mutation from Eq. 10 and, with this value, we calculated the logical variable Accept = $P_{fix} \geq$ runif(0,1). If Accept was TRUE, we accepted the mutation and the evolutionary step was finished. Else, we rejected the trial mutation and tried again until we had mutated the number of mutated sites that corresponds to the lineage.

We simulated mutants with different selection regimens; No selection $< P_{fix} > \approx 1$, weak selection $< P_{fix} > \approx 0.9$, medium selection $< P_{fix} > \approx 0.5$ and strong selection $< P_{fix} > \approx 0.1$. To obtain these $< P_{fix} >$ we gave β different values according to the following equation:

$$\beta^{regimen} = -ln(< P^{regimen}_{fix} >)/(-\beta \frac{1}{2} < \Delta^2_{ij} > (< CN_{C_\alpha \rho} > + < CN_{\rho\rho} >) \tag{11}$$

being the average over all ρ_i of the corresponding reference protein. It is remarkable that each family has a different value of β.

Structural variability measures

For each family and for each subset, experimental or theoretical with different regimens of selection, we obtained the coordinates of aligned and nonaligned sites of each protein relative to the reference protein. For experimental subsets, aligned and nonaligned sites of each lineage were obtained from the multiple structure alignment provided from HOMSTRAD. For theoretical subsets, we considered that there were not nonaligned sites (no gaps or insertions). We obtained experimental protein structures from the pdb file of superimposed coordinates also provided by HOMSTRAD and theoretical protein structures from the simulation output. With this data we calculated the following measures of structural variability.

3.3.1 Cartesian coordinates

For each family and for each subset we calculated the average structural variation in Cartesian coordinates. To do this, for each comparison between a protein and the reference protein of the corresponding family we calculated the root square deviation of each aligned C_{α_i} (RSD_i) as follows:

$$RSD_i = ((\Delta x_i)^2 + (\Delta y_i)^2 + (\Delta z_i)^2)^{1/2} \tag{12}$$

being Δx_i, Δy_i and Δz_i the difference in the x_i, y_i and z_i Cartesian coordinates of the C_{α_i} of both proteins respectively. Then, we calculate z-normalized profiles. Finally, for each family and for each subset, we averaged these profiles to obtain $< z\mathbf{RSD}_i >$ profiles.

3.3.2 Normal modes coordinates

For each family and for each subset we calculated average structural variation on normal modes coordinates. To do this, the $\mathbf{K}$ matrix of the reference protein of each family was replaced by the $\mathbf{K}_{eff}$ matrix, which was calculated like in [] and which accounts for the effective movements of the aligned C_α. Both not aligned C_α and all ρ where taken away to calculate $\mathbf{K}_{eff}$. We calculated normal modes as follows:

$$\mathbf{K}_{eff}\mathbf{q}_n = \Lambda_n \mathbf{q}_n \tag{13}$$

where Λ_n and $\mathbf{q}_n$ are the n eigenvalue and eigenvector respectively. There are $3N-6$ non-zero eigenvalues, which correspond to the vibrational modes, numbered $n = 1, 2, ..., 3N$–7. Then, for each mutant, we calculated the contribution of each normal mode to the total structural variation (P_n) by projecting structural differences of the aligned C_α on each normal mode n of the reference protein. We normalized profiles so that they added up to 1. Finally, for each family and for each subset, we averaged these profiles to obtain $< \mathbf{P}_n >$ profiles.

Model parameters

To completely specify the model, we must specify parameters for $\mathbf{K}$ and $\mathbf{f}$. To calculate $\mathbf{K}$, as we mentioned before, we used the ANM. We chose $R_0 = 7.5$ as the cut off value after the optimization using different values (data not shown). To calculate $\mathbf{f}_{lj}$, given a mutation at ρ_l, each node j (C_α and ρ) in contact with ρ_l was assigned a force directed along the $\rho_l - j$ contact and ρ_l was assigned a

Family	no selection	week selection	medium selection	strong selection
Serin Proteases	0.67	NC	NC	0.70
Azurin - Plastocyanins	0.61	NC	NC	0.65
Phospholipases	0.66	NC	NC	0.67
Fatty acid binding proteins	0.74	NC	NC	0.79
Globins	0.69	NC	NC	0.67
RNA recognition motif	0.75	NC	NC	0.75
Snake toxins	0.82	NC	NC	0.78
SH3 homology domain	0.78	NC	NC	0.74
Mean	0.72	NC	NC	0.72

Table 1: Correlation coefficient (CC) between experimental ¡$zRSD_i$¿ profiles and theoretical ¡$zRSD_i$¿ profiles selected under different selection regimens: no selection, weak selection, medium selection and strong selection

Family	no selection	week selection	medium selection	strong selection
Serin Proteases	0.83	NC	NC	0.82
Azurin - Plastocyanins	0.69	NC	NC	0.71
Phospholipases	0.59	NC	NC	0.56
Fatty acid binding proteins	0.85	NC	NC	0.88
Globins	0.77	NC	NC	0.73
RNA recognition motif	0.56	NC	NC	0.46
Snake toxins	0.66	NC	NC	0.69
SH3 homology domain	0.94	NC	NC	0.95
Mean	0.74	NC	NC	0.73

Table 2: Correlation coefficient (CC) between experimental ¡P_n¿ profiles and simulated mutants ¡P_n¿ profiles selected under different selection regimens: no selection, weak selection, medium selection and strong selection

reaction force. The magnitudes of each $\mathbf{f}_{lj}$, which depends on the value of Δ_{lj}, were randomly picked from a uniform distribution in the interval $[-f_{max}, f_{max}]$. The forces for different contacts were picked independently. Since f_{max} does not affect the results, we set $f_{max} = 2$. We can think of the range $[-f_{max}, f_{max}]$ as a continuous approximation of the perturbations introduced by the mutations, covering from mutations between physicochemically similar amino acids ($f \approx 0$) up to mutations between very different amino acids ($f \leq f_{max}$).

3.4 Tables

Family	no selection	week selection	medium selection	strong selection
Serin Proteases	0.15	NC	NC	0.16
Azurin - Plastocyanins	-0.04	NC	NC	-0.06
Phospholipases	-0.05	NC	NC	-0.05
Fatty acid binding proteins	0.13	NC	NC	0.1
Globins	0.01	NC	NC	0.01

Table 3: Correlation coefficient (CC) between $< zRSD_i^{experimental} > - < zRSD_i^{theoretical} >$ and the distance of each site to its closest active site. The theoretical profiles correspond to mutants selected under different selection regimens: no selection, weak selection, medium selection and strong selection