Function changing mutations in glucocorticoid receptor evolution correlate with their relevance to mode coupling

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Nonlinear effects in protein dynamics are expected to play role in function, particularly of allosteric nature, by facilitating energy transfer between vibrational modes. A recently proposed method focusing on the non-Gaussian shape of the population near equilibrium projects this information onto real space in order to identify the amino acids relevant to function. We here apply this method to three ancestral proteins in glucocorticoid receptor (GR) family and show that the mutations that restrict functional activity during GR evolution correlate significantly with locations that are highlighted by the nonlinear contribution to the near-native configurational distribution. Our findings demonstrate that nonlinear effects are not only indispensable for understanding functionality in proteins, but they can also be harnessed into a predictive tool for functional site determination.

A. Introduction

Mechanisms of information transfer and function in proteins continue to be challenging problems where different points of view compete. The ensemble view, i.e., that a ligand binding event triggers allostery by modifying the free energy landscape is now a commonly recognized paradigm [1–3]. The so called “population shift” picture is helpful in understanding allostery without shape change and finds support from recent NMR studies [4]. Models which focus on mechanistic aspects, such as the suppression of a certain vibrational mode or energy transfer between two modes [5–9] are also recognized paradigm [1–3]. The so called “population shift” picture is helpful in understanding allostery within allostery with [11, 12]. The central idea of the method is to quantify the nonlinear contribution to the near-native configurational distribution. Our findings demonstrate that nonlinear effects are not only indispensable for understanding functionality in proteins, but they can also be harnessed into a predictive tool for functional site determination.

B. Extracting information on mode coupling

Consider a protein composed of $N$ amino acids. Let the Cartesian coordinates of carbon-alpha ($C_\alpha$) atoms be stored in the vector $\mathbf{R}$ of length $3N$, encoding a coarse representation of the protein’s spatial arrangement, or configuration. The configurational probability distribution, $p(\mathbf{R})$ can be derived numerically from an $M \times 3N$ real-valued matrix, where $M$ is the number of snapshots acquired by sampling sufficiently long MD trajectories. This configurational distribution is then used to determine fluctuations around the mean structure, $\delta \mathbf{R} = \mathbf{R} - \langle \mathbf{R} \rangle$, where $\langle \cdot \rangle$ indicates averaging over time and multiple MD trajectories generated using different random seeds.

Within the framework of elastic network models, the configurational distribution is most conveniently expressed in terms of “modal fluctuations”

$$\delta \mathbf{r} = \Gamma^{-1/2} \delta \mathbf{R}$$

where $\Gamma = \langle \delta \mathbf{R} \delta \mathbf{R}^T \rangle$ is the covariance matrix associated with real-space fluctuations. A general analytical expression for $p(\delta \mathbf{r})$ in terms of Hermite tensor polynomials was originally proposed by Flory [13]:

$$p(\delta \mathbf{r}) = \frac{e^{-\delta^2/2}}{(2\pi)^{3N/2}} \sum_{\nu=0}^{\infty} C_{\nu} H_{\nu}(\delta \mathbf{r}) ,$$

where $H_{\nu}$ denotes the Hermite tensor polynomial of rank $\nu$, and the coefficients $C_{\nu}$ follow from the orthogonality relation $\int H_{\nu}(\delta \mathbf{r}) H_{\mu}(\delta \mathbf{r}) d\mathbf{r} = (\nu!)^{3N} \delta_{\nu,\mu}$.

In $\{\mathbf{r}\}$ basis, all expansion coefficients $C_{\nu \neq 0}$ in Eq. 2 vanish for a perfectly elastic network. As a result, the configurational distribution of such a linear system is separable into $3N$ identical Gaussian functions with unit standard deviation and zero mean:

$$p^{(g)}(\delta \mathbf{r}) = \prod_{m=1}^{3N} \exp[-(\delta r_m)^2/2] \sqrt{2\pi} .$$

The superscript $(g)$ implies the Gaussian product form of $p$. All vibrational modes of the linear system are represented in an identical fashion in this normalized form of the distribution. Yet, their physical difference is evident from and encoded in the corresponding eigenvalues and eigenvectors of $\Gamma$. 

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Nonlinearity can be introduced into this picture without coupling the vibrational modes, by simply adding higher-order terms to the corresponding harmonic oscillator Hamiltonians:

$$H_m = \frac{p_m^2}{2\mu_m} + \frac{1}{2} k_m (\delta r_m)^2 + \sum_{n=2}^{\alpha_{n,m}} (\delta r_m)^n$$

(4)

where \(\mu_m\) and \(k_m\) are the effective mass and the spring constant for the \(m^{th}\) mode, and \(\alpha_{n,m}\) indicate the strength of higher-order terms in appropriate units, all of which can in principle be derived from the underlying dynamics. The resulting configurational distribution function can then be expressed as

$$p^{(s)}(\delta r) = \prod_m p_m(\delta r_m) .$$

(5)

where

$$p_m(\delta r_m) = \frac{\exp[-(\delta r_m)^2/2]}{\sqrt{2\pi}} \left[ 1 + \sum_{\nu=3}^{\infty} \frac{c_{\nu}^m H_\nu(\delta r_m)}{\sqrt{2\pi}} \right] .$$

(6)

Note that, \(H_\nu\) above is now the ordinary Hermite polynomial of rank \(\nu\). Eqs. (4) describe the most general separable distribution for the variables \{\(\delta r_m\}\), hence the superscript \(s\).

An arbitrary configurational distribution can be expressed in a similar fashion, starting from Eq. (2) [11]:

$$p^{(s)}(\delta R) = p^{(s)}(\delta r)/\sqrt{\det \Gamma} .$$

(8)

Next, the difference between \(p(\delta R_i)\) and \(p^{(s)}(\delta R_i)\) is measured along each Cartesian component of the position vector \(R_i\) of residue \(i\) by means of the Jensen-Shannon (JS) divergence \(d_{js}[p, p^{(s)}]\) defined as [19]:

$$d_{js}[p, p^{(s)}] = \frac{1}{2} \left[ d_{kl}(p, M) + d_{kl}(p^{(s)}, M) \right]$$

(9)

where \(M = \frac{1}{2}(p+p^{(s)})\) and \(d_{kl}(p, q)\) is the Kullback-Leibler divergence that is given by

$$d_{kl}(p, q) = \int_{-\infty}^{\infty} p(x) \ln \left( \frac{p(x)}{q(x)} \right) dx .$$

(10)
An advantage of using JS divergence is that it is symmetric, therefore immune to the possibility that one of the two distributions may vanish at certain points (Kullback-Leibler divergence yields infinity for such instances).

Following the recipe above, we calculate the mode-coupling score for each amino acid in a protein, as the sum of JS divergences calculated along the components of $R_i$. Note that, comparing $p$ and $p'(\delta)$ aminoacid rather than in their full domain not only yields a testable output (locations relevant to mode coupling in the protein), but is also more robust to stochastic fluctuations inherent to the method, simply because of reduced dimensionality. That is, histograms for spatial fluctuations of single aminoacids can be represented with much fewer bins compared to joint distributions $p$ or $p'(\delta) for the whole protein, therefore they are sufficiently well sampled along the MD trajectories. Variations in the output as a function of the simulation length as well as between independent MD runs are investigated in Section 12.

An earlier version of this prescription was applied to motor protein myosin II with encouraging results 12. We here consider the ancestral chain of GR proteins and show that the outlined analysis reveals a significant bias in mode-coupling scores for experimentally validated function-altering mutations in the family.

C. Glucocorticoid receptors and evolutionary data

Glucocorticoid receptors are a class of endogenous steroid hormones that regulate inflammatory and stress responses, growth, development, and apoptosis 20–26. GR positively regulates transcription through a process known as transactivation in which the ligand-bound GR dislocates from cytoplasm to enter cell nucleus where it activates transcription 27. Its paralogous counterpart, mineralocorticoid receptor (MR) is mainly responsible for regulating electrolyte homeostasis 28. While GR binds glucocorticoid hormone cortisol 29, MR acts as a host for aldosterone, 11-deoxycorticosterone (DOC) and with a lesser affinity for cortisol 30. Through phylogenetic analysis, the sequence and the crystal structure of their common ancestor AncCR, as well as the ancestral GR proteins in cartilaginous fish (AncGR1) and in bony vertebrates (AncGR2) have been determined 31, 32. It has been shown that AncGR, similar to AncGR1, indiscriminately binds to DOC, aldosterone and cortisol. On the other hand, AncGR2 exclusively binds cortisol and is not activated by aldosterone and DOC.

Considerable effort has been devoted to understanding the basis of ligand specificity in the evolution of GR 31, 32. Structural variations are minute, with $< 1\AA$ RMSD difference between AncGR1 and AncGR2 33. Among the 36 mutations that transform AncGR1 to AncGR2, it has been shown that two strictly conserved mutations, S106P and L111Q (group X), are sufficient to increase cortisol specificity. S106P changes the architecture of ligand binding pocket and allows L111 to be located at a closer position to the ligand. The effect of L111Q is biochemical rather than mechanical, since it creates an additional hydrogen bond between 111Q and the cortisol, which lacks in DOC and aldosterone binding. Three additional mutations, L29M, F98I and S212F (group Y), wipe out the affinity towards DOC and aldosterone. However, AncGR1+X+Y structure cannot activate transcription due to the damaged hydrogen bond network which destabilizes the activation-function helix. Two further mutations, N26T and Q105L (group Z), are necessary in order to reestablish the hydrogen bond network and thereby stabilize the structure. All together, AncGR1+X+Y+Z, captures AncGR2 phenotype. A recent study found that XYZ mutations correlate with a measure based on the difference in fluctuation amplitudes of a residue in principal vibrational modes of ancestral GR proteins 31.

Besides historically occuring mutations, another study on alternative evolutionary pathways that restore AncGR2 phenotype 32 demonstrated that, among a set of suggested alternatives, only the mutation pair Q114L/M197I recovers cortisol sensitivity similar to the historical set of permissive mutations, albeit with a loss of associated transcriptional function.

D. Specifics on molecular dynamics simulations

Crystal structures of AncCR, AncGR1, and AncGR2 are publicly available at PDB with accession codes 2Q3Y, 3RY9, and 3GN8, respectively. MD simulations of each were carried out on Tesla K20 GPUs by means of Amber 14 Molecular Dynamics Package 34 using ff14sb force-field 35. Ligand molecules were also included in the simulations and their parametrization were done with Antechamber using generalized Amber force field 36. All simulations were performed in $(N,P,T)$ ensemble with explicit water solvent and with Langevin dynamics which maintained the temperature at 310 K and the pressure at 1 bar. No rigid bonds were assumed. 1 fs timesteps were used between successive frames while trajectories were captured every 1000 frames, i.e. 1 ps apart, throughout 128 ns long simulations performed for each sample. Each protein was simulated four times with the same initial condition but different random number generator seeds. Before further analysis, trajectories were aligned by means of the backbone $C_{\alpha}$ atoms. Discarding the first 7 ns of each simulation for equilibration, this protocol resulted in $5 \times 10^5$ snapshots, derived from $\approx 500$ ns long simulations of the near-native dynamics for each protein.

E. Mode coupling in GR proteins and comparison with evolutionary data

A substantial contribution from marginal anharmonicity is observable in the amplitude distributions of the
conformational free energy impact of a mode pair by considering the (dimensionless) protein’s biological function is our focus in this study.

Information content of such deviation from marginal analysis exemplified by the difference between the two rows of Fig. 2. (1 \( p \) \( \neq \) \( \bar{v} \)) = 32 are also shown in red.

We start by asking whether certain mode pairs stand out in the above analysis. One can assess the overall impact of a mode pair by considering the (dimensionless) conformational free energy

\[
F[p] = -\int p(\vec{\delta}r) \ln p(\vec{\delta}r) d\vec{\delta}r = \frac{1}{M} \sum_{i=1}^{M} \ln p(\vec{\delta}r_i) \quad (11)
\]
calculated with and without the mode coupling contribution from the mode pair \((m, n)\) in Eq. (9). For this purpose, we approximated \( p(\vec{\delta}r_i) \) by the one- and two-body terms spelled out in Eq. (7). We then defined \( p_{m-n}(\vec{\delta}r_i) = p(\vec{\delta}r_i) |_{c_{\mu,v}^{m-n} = c_{\mu,v}^{m-n} = 0} \), in which the mode-coupling contribution of the pair \((m, n)\) is discarded. The difference \( \Delta_{mn} = F[p_{m-n}] - F[p] \) is a measure of the impact of the interaction between modes \( m \) and \( n \) on protein’s behavior near equilibrium. \( \Delta_{mn} \) for all pairs composed out of slowest 25 modes of each protein are given in Fig. 4. It is interesting that \( \Delta_{mn} \) displays a power-law dependence with a scaling exponent \( \sim -0.8 \) over more than two decades on the rank order of the pair \((m, n)\) (Fig. 3b). An exhaustive analysis over all mode pairs was not performed due to its heavy computational cost.

We found that the highest-impact mode pairs are 1-3 for AncCR and AncGR1; and 1-7 and 2-6 for AncGR2. Spatial fluctuations associated with these mode pairs coincide with helices 7 and 10, along with the loop region preceding helix-7. Indeed, these helices form part of the ligand binding pocket, while the loop before helix-7 is where the two X mutations are located. We additionally observed a region on helix-9 with high sequence conservation score also involved in mode coupling, which, to our knowledge, has not been highlighted in earlier studies.

We next performed the analysis outlined in Section B in order to derive a mode-coupling score for each aminoacid. The resulting score vectors obtained over the full data set (four trajectories) separately for each member of the GR family are shown as a heat map superimposed onto the proteins’ three-dimensional structure in Fig. 4. We observed that the loop (100-110) preceding helix-7 yields considerably high scores in all proteins, despite the fact that this loop and the nearby helix-7 exhibit the largest structural variability between AncGR1 and AncGR2 \[32\]. Furthermore, the same region also accommodates 4 of the 6 (XYZ-)mutations mentioned above. These observations hint at the relevance of mode coupling to the evolutionary history of function in the GR protein’s lineage, which we investigate below in further detail.

Note that, the location of the X-mutation S106P consistently has one of the highest scores in all proteins. Considering that S106P \textit{alone} decreases activation in AncGR indepedent of the ligand type \[32\] suggests that the mechanism underlying the activity loss is mechanical in origin, rather than biochemical (to which the present method is insensitive). The opposite is true for the second X mutation L111Q which recovers cortisol speci-

\[ \text{FIG. 1. Marginal distributions for the amplitudes of six slowest modes of AncGR2. Anharmonicity is most discernible in the first three and gradually disappears for faster modes. Analytical approximations derived from Eq. (6) with a cut-off at } v = 32 \text{ are also shown in red.} \]

\[ \text{FIG. 2. Pairwise joint probability distributions given as heat maps for the amplitudes of three slowest modes in AncGR2. High probability regions are shown in yellow. First row corresponds to the MD data for pairs 1-2, 1-3, and 5-6, respectively. Second row gives the product of corresponding marginal distributions. It is evident that joint distributions of slow modes can not be captured by Eq. (9), meaning mode-coupling corrections must be included.} \]
FIG. 3. (a) Pairwise mode coupling scores for slowest 25 modes of the three members of GR protein lineage. (b) Mode-coupling scores $\Delta_{mn}$ of rank-ordered mode pairs for the three proteins. The straight line segment (black) corresponds to a power-law decay with an exponent $-0.8$.

Specificity [32] by allowing formation of a hydrogen bond with cortisol. Mode-coupling score of location 111 shows no significant deviation from the mean. On the other hand, the synthetic mutations Q114L/M197I in AncGR1 - that also recover cortisol specificity and disrupt communication between cortisol binding and transcriptional activity - coincide with the two mode-coupling peaks in AncGR1 located on helix-7 and helix-10.

Complementing these observations, an objective evaluation of the correlation between mode-coupling scores and the AncGR1 $\rightarrow$ AncGR2 mutation set is desirable. For this purpose, we use the recall analysis where mutation sites under consideration are labelled as the target set and their rankings are inspected in the full residue list sorted according to mode-coupling scores. The result is presented as a recall curve which is a plot of the fraction of the target set elements ($y$-coordinate) observed in a given fraction ($x$-coordinate) of the list picked from the top. In absence of correlation between the target set and the scoring function, one expects to see the recall to remain on the diagonal upto statistical fluctuations. A recall curve remaining significantly above the diagonal indicates a positive correlation, since it reflects the fact that the aminoacids in the target set come with higher-than-average scores.

Fig. 5 shows the outcome of the recall analysis for the three proteins in the GR family. In all cases, we observe no visible correlation between mode-coupling scores and the complete set of mutations accompanying each evolutionary step. Focusing on the function changing XYZ-mutations only, we first note an overall positive correlation with B-values (variance of fluctuations around equilibrium for each $C_\alpha$ atom), due to the fact that these mutations are located mostly on the loop regions. It is striking that the mode-coupling based ordering yields better recall values in all three proteins. We furthermore observe that the recall performances improve slightly when mode-coupling scores are divided by the B-values in or-

FIG. 4. (a) Cartoon representation of the three studies proteins. Helices 3 (29-54), 7 (108-125), and 10 (180-210) are shown in red, yellow, and purple, respectively. Loop region preceding helix 7 is colored in blue with activation-function helix (AF-h) (220-232) in magenta. Helices 3, 7, and 10 alongside with helix 1 (not shown) form a part of ligand binding pocket. AF-h is essential for transcriptional activity. (b) Mode-coupling scores mapped onto the corresponding protein structures where hotter colors represent higher scores. Significant activity is observed on helices 7 and 10, and around loop regions.

FIG. 5. Recall analysis for AncCR, AncGR1, AncGR2. For each protein, mode-coupling scores and B-values (rms amplitude of $C_\alpha$ fluctuations) were used for ranking residues. Target residues were set to be XYZ-mutation locations or the locations of all mutations that occured between two evolutionary steps. All figures show a significant positive bias towards XYZ-mutations when mode-coupling scores are used. This pattern is lost when B-values are used for scoring.
FIG. 6. Self consistency of the score vectors increases with the length of the MD trajectory. \( \langle \cos \theta \rangle_T \) is the average value of the dot product of two normalized score vectors obtained from different time windows of size \( T \).

der to factor out the bias mentioned above. This is the central result of the present work which, together with a similar observation on myosin II [12], lends support to the thesis that coupling between vibrational modes is a key physical mechanism in protein function.

F. Robustness wrt data acquisition period

Since most proteins carry out their function in time scales beyond the reach of computer simulations, it is natural to ask how sensitive above results are to the simulation time window. We investigated the robustness of our findings by re-analyzing the data in varying time intervals. To this end, we divided each trajectory into \( N_T \) fragments of \( T \) ns each (\( T = 1, 2, 4, 8, \ldots, 128 \)) and calculated mode-coupling scores by using each fragment separately. We then compared score vectors for each interval pair with identical lengths by measuring the angle between them. This was done by evaluating the dot product of the two vectors after setting their mean to zero (by a constant shift) and rescaling them to unit length. The mean \( \langle \cos \theta \rangle_T \) and the standard deviation \( \sigma_T \) of the obtained dot products were recorded separately for each interval length \( T \). Results shown in Fig. 6 confirm that the analysis detailed in section D yields progressively more consistent results with increasing \( T \).

G. Conclusion

While it is natural from a physical point of view to postulate that nonlinear effects mitigate energy transfer within a protein [9], precisely how the nonlinearity observed in protein dynamics can be fruitfully exploited to yield biologically relevant predictions is unclear. Even the relevance of nonlinear effects to protein function is far from being universally acknowledged. While part of the literature (such as on discrete breathers [41][44]) attests to its importance, there is substantial amount of past and recent work which investigate mechanisms of protein function within a linear (harmonic) framework or at the level of principal component analysis [8][10][16][45][46]. By demonstrating that functionally critical mutations along the evolutionary descent which relates three ancestral proteins of the GR family are highlighted in an analysis of the nonlinear contribution to dynamics, the present work emphasizes the significance of nonlinearity, in particular that beyond marginal anharmonicity, to protein function.

The selective power of the mode-coupling analysis for functionally relevant sites (in GR protein family reported here and in myosin II earlier [12]) is suggestive. However, it is also evident from the data that not all known functional locations come with high mode-coupling scores. Given the complexity of the system and the multitude of factors beyond protein dynamics that play role in functionality, this is only expected. Applying the analysis on carefully constructed toy nonlinear models may help clarify the mechanistic role played by the aminoacids that score high in the present analysis. Such information could be useful for characterizing the proteins on which the current approach may be expected to be successful in future.

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