

Mapping the Intramolecular Vibrational Energy Flow in Proteins Reveals Functionally Important Residues

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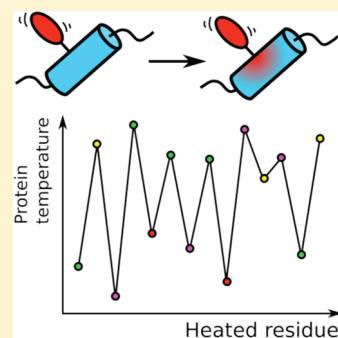
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S Supporting Information

ABSTRACT: Unveiling the mechanisms of energy relaxation in biomolecules is key to our understanding of protein stability, allostery, intramolecular signaling, and long-lasting quantum coherence phenomena at ambient temperatures. Yet, the relationship between the pathways of energy transfer and the functional role of the residues involved remains largely unknown. Here, we develop a simulation method of mapping out residues that are highly efficient in relaxing an initially localized excess vibrational energy and perform site-directed mutagenesis functional assays to assess the relevance of these residues to protein function. We use the ligand binding domains of thyroid hormone receptor (TR) subtypes as a test case and find that conserved arginines, which are critical to TR transactivation function, are the most effective heat diffusers across the protein structure. These results suggest a hitherto unsuspected connection between a residue's ability to mediate intramolecular vibrational energy redistribution and its functional relevance.

SECTION: Biophysical Chemistry



Vibrational energy transfer in proteins is particularly complex because proteins can be simultaneously highly anisotropic and strongly correlated environments at a molecular level. High-resolution protein structures carry functional information that is hidden from immediate inspection, particularly for mechanisms involving large amplitude motions, allostery, and relaxation.^{1–3} Some mechanisms involve conformational dynamics that are hard to access by current experimental and computational techniques. For example, mutational correlations between residues far apart in the protein structure have been associated with pathways of heat diffusion,⁴ although the structural nature of the correlations is still debatable. The pathways of heat diffusion in a protein molecule subsequent to an external excitation such as ligand binding, radiation, heat shocks, or chemical reactions, may play important roles in maintaining the narrow temperature ranges in which proteins function.^{5,6} In most proteins, relaxation of excess energy must be efficient, and, therefore, important dissipation mechanisms may be widespread in protein families. Experiments such as the reversible denaturation of nucleic acids by excitation of an attached quantum dot⁷ and the structural changes in flash-cooling cryocrystallography⁸ have already conceptually proven the possibility of modulating structures by perturbing biomolecular energy flow. In photosynthetic complexes, by contrast, full dissipation seems to be avoided for a fairly long time as the excitation energy from absorbed light is quantum-coherently funneled to a reaction center several nanometers away.^{9–12}

The mechanisms of energy relaxation within proteins have been characterized by theoretical models: Globally, proteins display anomalous subdiffusion, resembling vibrational energy propagation in fractals or percolated clusters.¹³ Locally, rates of vibrational energy relaxation from selected modes are estimated to be on the subpicosecond time scale at room temperature, in agreement with experimental measurements.⁶ However, the role of specific structural elements has been elusive and the subject of debate.^{14,15} Backbone atoms were shown to be the main transporters of energy in a peptide helix.¹⁶ HEME propionate side chains, in turn, funnel vibrational energy perturbations arising from ligand detachment directly into the solvent, avoiding the protein atoms.^{17,18} These findings provide compelling evidence that structurally directed mechanisms of vibrational energy relaxation exist. Therefore, mapping the structural linchpins of this energy flow may provide useful insights for the design of new amino acid sequences and ligands to modulate protein activity.

Ligand binding domains (LBDs) of nuclear hormone receptors (NRs) are examples of globular, compact, and mostly α -helical proteins for which allosteric mechanisms have been correlated with intramolecular signaling pathways.¹⁹ They are target proteins for estrogen-derived contraceptives, corticoids, progesterone,

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vitamins A and D, and thyroid hormones, to name a few, comprising important binders of currently available pharmaceuticals.²⁰ Various crystallographic structures of LBDs of NRs were obtained in the past decade, revealing a mostly preserved α -helical sandwich fold, which totally buries ligands and suggests a common overall mechanism for ligand-mediated transcription regulation (Supporting Information, Figure S1). At the same time, novel ligands and mutations cause unsuspected functional responses by perturbing structural elements far from the mutation sites.^{21,22} Unveiling key functional residues are, thus, of utmost importance.

In this work, we take on the LBD of the β -subtype thyroid hormone receptor (TR β) as our model system to study intramolecular energy transfer mechanisms and show that residues that are outstanding heat diffusers through the protein are also functionally vital. Using molecular dynamics simulations, we are able to identify key residues for the transactivation regulation function of TR β by quantifying the ability of the residue side chains to transfer excess vibrational energy to the protein. Our computational approach is based on a variation of the anisotropic thermal diffusion (ATD) strategy suggested by Ota and Agard,⁴ which consists of modeling single residue heating in an artificially cooled protein. We extend their method by quantifying the thermal energy dissipated to the rest of the protein environment resulting from heating every residue individually in separate simulations. By computationally mutating each side chain to alanine (or glycine), we discriminate side-chain from backbone contributions, providing chemical insights into protein intramolecular energy flow.

Figure 1 schematically depicts the LBD of TRs and illustrates the technique we propose to map thermal diffusion and side-chain contributions. The protein structure is cooled to 10 K, and the atoms of a single residue are coupled to an independent heat bath held at 300 K. Release of the chilled protein heat bath allows for the vibrational energy flow from the heated residue to the remainder of the structure. Although the vibrational energy is heterogeneously distributed during the course of this nonequilibrium process, after a fixed elapsed time, the protein reaches an average temperature. In order to evaluate side-chain contributions to the overall thermal energy transfer, the computational experiment is repeated with each residue individually mutated to glycine (Supporting Information, Figures S2–S4) or alanine. We focus primarily on Ala mutations because its standard Ramachandran dihedral is less prone to promote structural distortions in experimental constructs. Thus, one ATD set of simulations comprises 260 independent runs, one for each residue, to evaluate final average protein temperatures after 30 ps of nonequilibrium simulation time. Each nonequilibrium run was repeated 20 times using multiple snapshots obtained from an equilibrium 300 K run, from which water was removed. These energy dissipation runs are short, such that the structures extracted from the equilibrium run are mostly preserved. Thus, these simulations sample kinetic energy dissipation mechanisms out of an ensemble of 300 K structures, although the coupling of energy flow with large amplitude motions of the protein is neglected.^{23,24} The removal of surrounding water molecules reduces the noise-to-signal ratio and favors intramolecular relatively to solvent-mediated mechanisms of energy flow. In the presence of water, important alternative dissipation paths are likely to exist, and some internal transfers may become damped. The difference between native and alanine-mutated protein temperatures is attributed to the ability of native side chains to

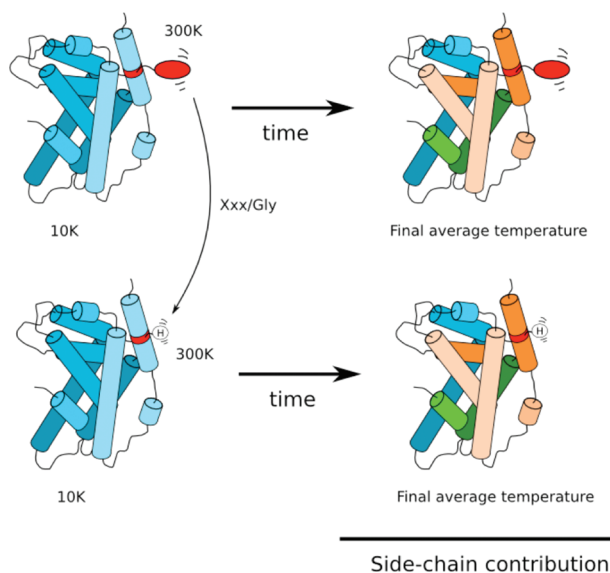


Figure 1. Schematic representation of an NR LBD and the heat diffusion modeling experiment. The LBD is a compact α -helical sandwich (Figure S1). A single residue is thermalized to 300 K in a 10 K protein. After 30 ps, the average temperature of the protein is evaluated. Then, one residue is independently mutated to glycine or alanine, and the computational experiment is repeated. The difference between the final average temperatures reached by the protein with native or mutated side chains is the side chain contribution of the mutated residue to the vibrational energy transfer.

transfer their excess vibrational energy to the rest of the protein. Moreover, the residues identified as the most relevant heat diffusers were experimentally mutated to alanine, and the effect of these mutations in the transactivation function of TRs was evaluated. Our findings show that residues that are protagonists in the mechanisms' intramolecular signaling are also functional linchpins. Additional details of the computational and experimental procedures are available as Supporting Information.

A typical vibrational energy relaxation profile is provided in Figure 2A. Arginine 429 is coupled with a heat bath at 300 K. The high temperature of the residues in the vicinity of position 429 shows that energy flows effectively through the protein backbone. Furthermore, significant heat transfer occurs from Arg429 to residues with which it forms close contacts in the folded structure (Glu311 and Asp383). This energy is then dissipated through the backbone in vicinities of these residues (Figure 2B). To provide a global picture of these mechanisms, we define a thermal diffusion map (TDM) in which the thermal response of all residues (y -axis) is plotted as a function of every heated residue (x -axis). The TDM mimics the contact map, but with a more spread and diffuse connectivity (Figure 2C,D). Therefore, the backbone, close-contact, and secondary backbone mechanisms are roughly representative of all heat diffusion experiments.

The final protein average temperatures resulting from heating every residue are summarized in Figure 3A. Using this heating protocol, protein temperatures ranged from 22 to 36 K. Therefore, different residues have different capabilities of transferring vibrational energy to the rest of the protein body. There are no obvious systematic correlations between energy transfer efficiency and residue charge, mass, or side-chain structure and chemistry. However, arginines seem to be particularly capable of transferring heat, as many of them stand out from the average.

The thermal response profile is smoothed when residues are individually mutated to alanine, as shown in Figure 3B. The difference between native and alanine-mutated thermal responses reflects the contribution from native side chains to the overall vibrational energy transfer. Figure 3C displays the side chain contributions. Closer visual inspection of Figure 3A,B (and

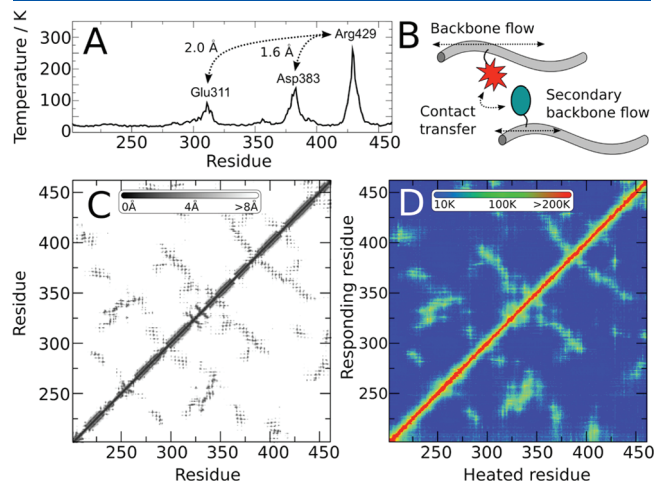


Figure 2. Paths for thermal diffusion. (A) Example of the heating of Arg429 and the corresponding heat transfer along the protein main chain and to nearby residues. (B) Schematic representation of the most relevant mechanisms of heat transfer. (C) Contact map and (D) TDM of TR β LBD; the temperature goes from cold (blue) to hot (red).

further detailed in Table S1), reveals that 9 out of 10 Arg residues of the TR β LBD are featured among the most relevant residues for heat transfer (Figure 3C), being accompanied by Lys342. The single arginine of TR β , which is not conserved in TR α , and the three TR α arginines, which are not conserved in TR β , transfer heat to the LBDs, much like the average residue (Figures S1–S4 and Table S2). Thus, Arginines conserved between subtypes are the most important thermal diffusers.

We conjecture that side-chain contribution to energy transfer could be correlated with functional roles played by residues and detectable in site-directed mutagenesis experiments (contributions from the backbone cannot be similarly discriminated, since single-point mutations preserve backbone structure). Alanine mutants (mimicking the computational construct) for each of the nine most outstanding arginines and Lys342 of TR β were obtained. Without exception, reduction of the transactivation function of TR β (Figure 3F) was observed. Mutation of the one arginine (R391) that does not belong to this group of relevant thermal diffusers does not impair the transactivation function. In most cases, arginine residues do not play obvious structural roles in transactivation, transrepression, or dimerization.²⁵

The charged and rigid guanidinium group of arginines may be the reason behind their distinct role in thermal energy transfer, but only if structurally connected to the protein core. This is the case of the Arg residues conserved within TR subtypes. Four of these residues (Arg338, Lys342, Arg383, and Arg429) participate on charge clusters that are required for LBD stability,²⁶ and three of them belong to the binding cavity (Arg282, Arg316, and Arg320). Conversely, Arg residues and other charged residues

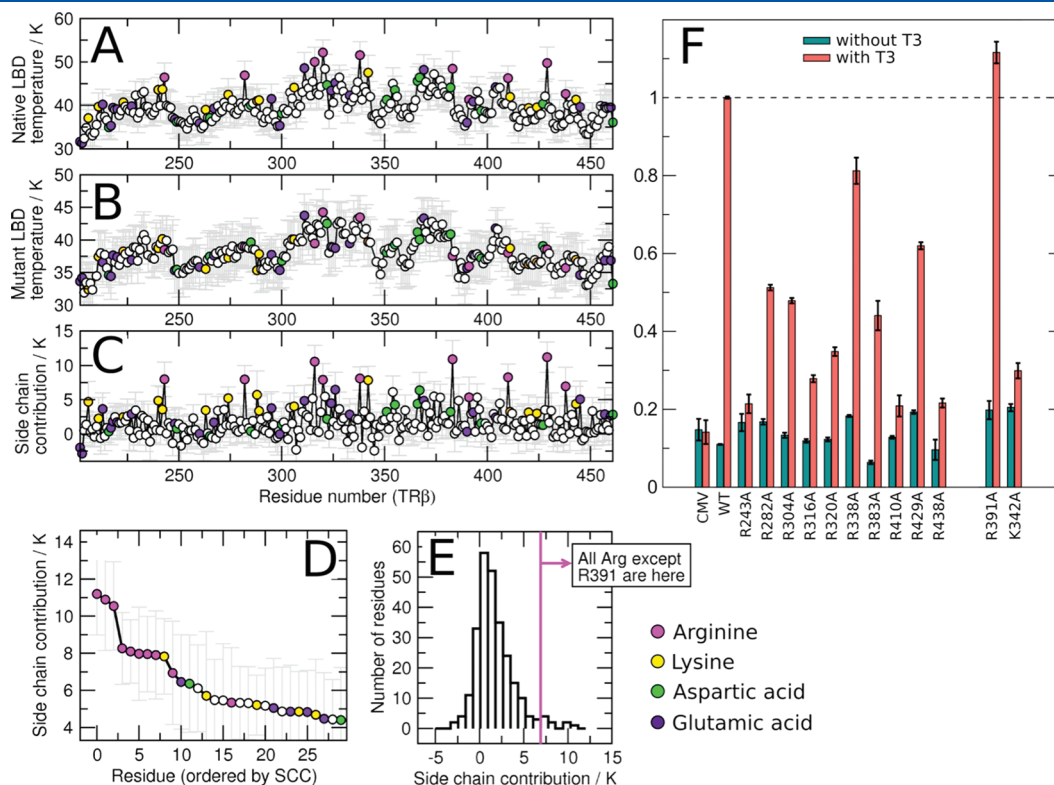


Figure 3. Computing side chain contributions for heat transfer. (A) Thermal response of native TR β to heating of each residue. (B) Thermal response of TR β mutants in which the heated residue was mutated to alanine. (C) Difference between A and B, displaying side chain contributions. (D,E) Most important side chain contributions come from arginines. Similar profiles for glycine mutants and for TR α are available as Figures S2–S4, Supporting Information. (F) Mutation of all TR β arginines but Arg391 impairs the receptor's function in transactivation assays. Lys342, the only non-arginine residue that belongs to the top 10 thermal diffusers is also functionally relevant.

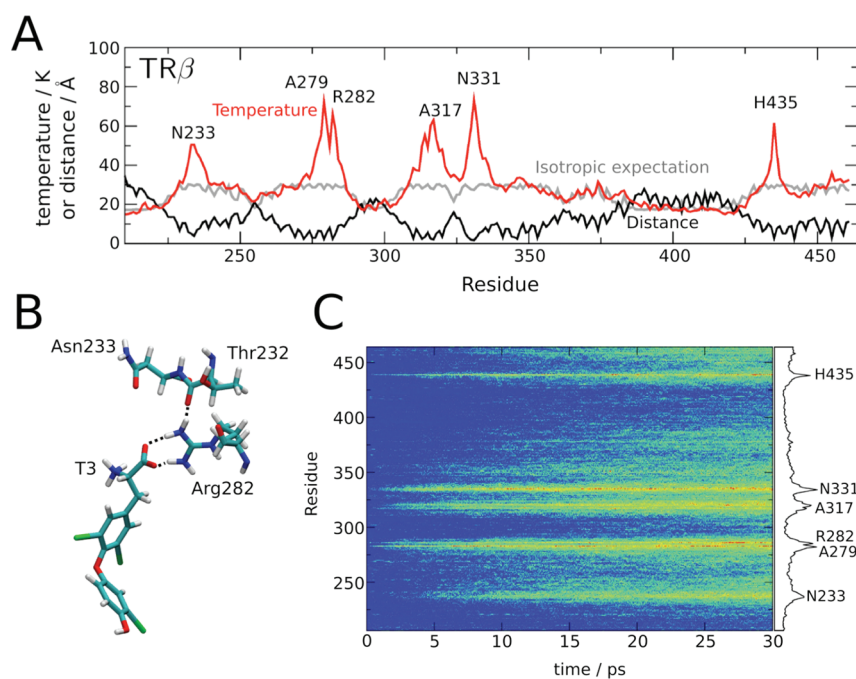


Figure 4. (A) LBD response to ligand heating is highly anisotropic. (B) Thermal response of Asn233 involves an indirect path of energy flow and, thus, is time-delayed (C). Additional ligand-heating simulations are shown in the Supporting Information, Figure S6.

not noticeable as thermal diffusers are at the protein surface and might be relevant for solubility only (Supporting Information, Figure S5). That is, within charged residues, conserved and important arginines seem to interact more effectively with the protein core (Figures S1 and S5) of TRβ LBD. Remarkably, all identified arginines, in addition to Lys342, have been found to be targets for mutations in patients with resistance to thyroid hormone syndrome (RTH) (Table S2).²⁵ It has been traditionally difficult to link RTH mutations to specific impairments in TR functions, and previous hypotheses to explain their effects centered on the disruption of LBD dynamics.²⁷ Thus, mutation of these structural linchpins that mediate energy transfer leads to disruption of protein function in a manner that causes human disease.

Thermal response to ligand heating was also investigated, and is particularly interesting since ligands form only noncovalent contacts with LBDs. A typical profile LBD response to heating of the natural ligand T3 is shown in Figure 4A. Strongest responses are highly anisotropic and arise from the vicinity of polar active site residues Arg282, Arg316, Arg320, Asn331, and His435. Although residue Asn233 does not belong to the binding pocket, heating of Asn233 was systematically observed for various ligands (Figure S5). Structural analysis shows that the Asn233 response results from an indirect energy flow path involving a salt bridge between T3 and Arg282, which in turn forms a hydrogen bond with Thr232 (Figure 4B). Because of this indirect, multistep path of energy flow, Asn233 heating is time-delayed relative to other elements, as shown in Figure 4C. Therefore, ligand excess kinetic energy can relax to the protein through structurally directed mechanisms, and multistep connectivities can modulate the time-dependence of the vibrational energy propagation. Specifically for TRs, strong responses on position 331 are appealing as it contains the single amino acid substitution (TRβ-Asn331/TRα-Ser277) that provides subtype selectivity to many ligands.^{28–30} Also, TRβ selective ligand Triac promotes a smaller thermal response in TRβ than it does in TRα (Supporting Information,

Figure S6), which is consistent with our recent findings showing that Triac is less attached to the TRβ binding cavity in spite of its β-selectivity.³⁰

The existence of nonrandom structural linchpins for protein energy flow has evolutionary implications, as mechanisms to dissipate kinetic energy perturbations may favor stable protein folding. At the same time, correlations between functionality and energy flow might arise only indirectly from the residues' connectivity, or may be simply related to the residue's ability to couple the motions of separate protein regions, as originally suggested by the ATD method.⁴ Yet, the present study of the mechanisms of intramolecular energy flow singles out individual residues that are highly relevant to TR function and disease, and can be easily adapted to other biomolecules. Indeed, we have performed auxiliary simulations of thermal diffusion on the LBD of the peroxisome proliferator activated receptor-γ and on a hyperthermostable variant of a GH11 Xylanase.³¹ Arginines emerge as important thermal diffusers in both cases (Supporting Information Figure S7). However, their role is less prominent for the xylanase because the residue thermal energy transfer is overall greater for this engineered thermo-stable protein. We suggest that mechanisms of energy flow are relevant in proteins whose functions are not related to the absorption of radiation and light-harvesting, and can be explored to shed additional light into the complexity of protein structure–function relationships.

Other methods to study intramolecular energy flow in proteins were recently proposed which are capable of exploring the global networks of energy flow in proteins in a much more systematic way than the present approach.^{32,33} The time scales associated with distinct energy transfer pathways have been evaluated from the time-correlation function of the interatomic energy flux,³² whereas the local energy diffusivities have been computed in the frequency domain via normal-mode analyses.³³ These methods, however, have not yet been explored as tools to reveal functionally relevant residues. A remarkable exception is

the Markovian stochastic model of information diffusion proposed by Bahar and collaborators,²⁴ who show a connection between signal transduction events and the fluctuation dynamics of the protein and find that functionally active residues are efficient mediators of communication using different proteins.

Summarizing, we map the mechanisms by which vibrational energy relaxes in proteins by computationally heating each residue individually in an artificially cooled structure. By computationally mutating each side chain to glycine or alanine, we associate the ability of each residue in diffusing heat to its chemical nature, and we identify arginines as the most important heat diffusers in the case of the LBD of the TR. These arginines are experimentally identified as crucial for the TR's transactivation function, thus revealing that mapping heat diffusion can be used profitably as a means of identifying functionally relevant residues. This study also suggests a strategy to understand correlations between protein stability and their ability to dissipate local perturbations. The discovery that arginines are particularly important in this context may provide a novel interpretation for the evolutionary selection of this amino acid. Finally, the methodology can be easily applied to understand vibrational energy relaxation in a variety of proteins.

■ ASSOCIATED CONTENT

S **Supporting Information.** Detailed description of Materials and Methods; sequence comparison between TR α and TR β and their α -helical secondary structure (Figure S1); thermal responses for the TR α subtype and other proteins (Figures S2–S8); most pronounced side-chain contributions to thermal response for TRs (Table S1) and functional data (Table S2). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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