Proposed title: “Coarse-Grained Normal Mode Analysis in Structural Biology”
Short title: “Normal Mode Applications”

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Summary
The realization that experimentally observed functional motions of proteins can be predicted by coarse-grained normal mode analysis (NMA) has renewed interest in applications to structural biology. Notable applications include the prediction of biologically relevant motions for proteins and supramolecular structures driven by their structure-encoded collective dynamics; the refinement of low-resolution structures including those determined by cryo-electron microscopy; and the identification of conserved dynamic patterns and mechanically key regions within protein families. Additionally, hybrid methods that couple atomic simulations with deformations derived from coarse-grained NMA are able to sample collective motions beyond the range of conventional molecular dynamics simulations. Such applications have provided great insight into the underlying principles linking protein structures to their dynamics, and their dynamics to their functions.

Introduction

Recent advances in sequencing and structural genomics indicate that the canonical sequence-to-structure-to-function paradigm is insufficient for understanding and controlling the mechanisms of biomolecular interactions and functions. Because molecular structures are dynamic rather than static, information regarding their dynamics is required to establish the link between structure and function. Normal mode analysis (NMA) has re-emerged in recent years as a powerful method for elucidating the structure-encoded dynamics of biomolecules. NMA has been applied to proteins since the early 1980s[1;2]. However, its usefulness in structural biology has been exploited only recently, after the observation that the collective motions predicted by NMA for folded structures are highly robust and bear *functional* significance[3-5]. Although the actual motions of macromolecules in solution are very complex, involving transitions between innumerable conformations, the success of NMA hinges upon the fact that the motions near native state conditions are much simpler and more robust. Structural changes are dominated by the inter-residue contact topology of the folded state implying that the most probable deformations are those requiring the smallest energy ascent in the multidimensional energy landscape.

It is plausible that the motions NMA predicts are functional if one considers that each protein functions only if it is folded into its equilibrium/native structure, and each equilibrium

structure encodes a unique *equilibrium dynamics*. Furthermore NMA yields a unique analytical solution for the modes of motion accessible at equilibrium (near a global energy minimum). Thus the equilibrium dynamics predicted by NMA, and the structure-encoded collective motions in general, ought to be functional, based on the premise that each protein has evolved to optimally achieve its biological function.

Elastic network models and coarse-grained NMA

Building upon the ability of NMA to predict the most probable cooperative motions of biomolecular structures, much of the increased utilization of NMA in recent years has resulted from the introduction of computationally simpler, elastic network (EN) models. These EN models replace detailed atomic potentials by uniform harmonic potentials between interacting atom or residue pairs[6-8]. These and subsequent studies have demonstrated that the large scale, collective motions predicted by NMA are insensitive to both the model and the details of the force fields used, provided that the topology of inter-residue contacts in the native structure is accurately modeled[6-11]. Given the computational efficiency of coarse-grained NMA, a convenient methodology has been (i) to map the protein structure onto its EN model, (ii) perform a coarse-grained NMA using an EN model of suitable resolution to generate 'alternative' structures sampled during equilibrium fluctuations, (iii) use the NMA-generated 'alternative' forms to characterize the natural dynamics or reconstruct structures at their atomic level representation. This three-step procedure and associated applications are summarized in Figure 1. Below, we briefly describe the tasks indicated by this figure and discuss the various applications to structural biology.

I. Mapping the structure into reduced models that maintain contact topology

The most common model adopted in coarse-grained NMA involves a single-site-per-residue representation where the sites are identified by the C^{α}-atoms and connected by uniform springs. The dynamics of such an interconnected bead-and-spring model can be described by the Gaussian Network Model (GNM) or an EN model using a potential of the form

$$V = \frac{\gamma}{2}\left[\sum_{i,j}^{N}\left(\boldsymbol{R}_{ij} - \boldsymbol{R}_{ij}^{0}\right)\bullet\left(\boldsymbol{R}_{ij} - \boldsymbol{R}_{ij}^{0}\right)f\left(R_{ij}^{0}\right)\right] \qquad (1)$$

for the GNM and

$$V = \frac{\gamma}{2}\left[\sum_{i,j}^{N}\left(\left|R_{ij}\right| - \left|R_{ij}^{0}\right|\right)^{2} f\left(R_{ij}^{0}\right)\right] \qquad (2)$$

for the EN model. Here γ is the uniform spring constant; $\boldsymbol{R}_{ij}^{0}$ and $\boldsymbol{R}_{ij}$ are the original and instantaneous distance vectors between residues *i* and *j*, R_{ij}^{0} and R_{ij} are the corresponding magnitudes; the summation is performed over the pairs of residues/nodes filtered through the function $f(R_{ij}^{0})$ that selects the interacting pairs. $f(R_{ij}^{0})$ is either the Heaviside function based on an interaction cutoff distance of R_c ($f(R_{ij}^{0}) = -1$ if $R_{ij}^{0} \le R_c$, and zero otherwise)[10;11], or an exponentially decaying function in distance[9].

Lower resolution models have been adopted to examine larger biomolecular assemblies where groups of residues are clustered into unified sites[12;13], or rigid blocks (RTB and BNM)[14;15]. Related methods (QEDM) effectively quantize the shape of the structure without directly identifying specific residues or group of residues [16;17]. A reduction in the number of nodes by one order of magnitude increases the computation speed by three orders of magnitude since NMA computing time scales with N^3. Notably, the global motions computed by such coarse-grained NMA maintain their fundamental characteristics that can be related to functional mechanisms[13].

II. Performing NMA with EN models: Functional deformations and critical sites

NMA depends upon the eigenvalue decomposition of the Hessian matrix – a 3Nx3N matrix composed of the second derivatives of the potential, V, with respect to residue fluctuations. Thus for an EN model potential (Eq. 2), one obtains 3N-6 normal mode vectors describing anisotropic deformations. In the case of the GNM, the Hessian is replaced by the NxN Kirchhoff matrix, $\mathbf{\Gamma}$, describing the inter-residue contact topology, such that N-1 isotropic modes are obtained. The B-factors computed by the GNM yield good agreement with X-ray crystallographic data[18] and NMR order parameters[19]. However, the mechanisms of deformations cannot be characterized unless a 3N-dimensional Hessian is used in NMA.

An exciting contribution of NMA to structural biology is its ability to provide insight about large-scale and long-time conformational motions of proteins which tend to be inaccessible to standard MD techniques. Recent applications to very large supramolecular assemblies include the ribosome[20;21] and viral capsids[22;23]. In general, a few of the low-frequency modes, $\boldsymbol{u_j}$, predicted by NMA exhibit a large degree of overlap,

$$I_j = \frac{\left|\boldsymbol{u}_j \cdot \boldsymbol{\Delta r}\right|}{\left|\sum_i^{3N} u_{ij}^2\right|^{1/2} \left|\sum_i^{3N} \Delta r_i^2\right|^{1/2}} \qquad (3)$$

with the vector describing the motion between two known conformations, $\boldsymbol{\Delta r}$ [11]. Overlap values exceeding 80% suggest that the structures (open and closed) have an intrinsic tendency to reconfigure along a small set of low-frequency modes, even if the fully evolved conformational change may involve a passage over a conformational energy barrier. Recently, it has been shown that only minimal information about the target structure is required to drive one structure to the other by a linear combination of low-frequency normal modes[24].

The utility of NMA becomes particularly significant when combined with experimental data. Notable applications that provide insights on functional mechanisms include the study of muscle myosin ATPase regulation[25] or flexibility[26;27], the modulation of protein flexibility during the RNA polymerase cycle[28], and the elucidation of ribosomal machinery[20;21].

Although these coarse-grained, C^{α}-based NMA methods lack any sequence specificity, there is increasing evidence for their ability to identify functional and structural roles of individual residues. Many studies have identified residues that impart inherent stability and are critical

for folding[29-31], as well as forming binding "hot spots"[32], catalytic residues[33] and deformable residues[34].

III. Applications in structural biology: Utility in predicting structure and dynamics

Flexible docking. A major application of normal modes is the identification of potential conformational changes, e.g. of enzymes upon ligand binding[11;35]. In particular, it has been shown that over half of 3800 known protein motions (inferred from different conformations of the same protein) can be approximated by perturbing the original structures along the direction of their two low-frequency normal modes[36]. Such results suggest that that the protein structures may have evolved to accommodate or facilitate biologically functional conformational changes. The functional mechanisms are indeed more readily accessible provided that they coincide with the smoothest ascent directions in the neighborhood of the global energy minimum, i.e. those along the lowest frequency modes. The fact that the observed changes coincide with those predicted by the slowest NMA modes should not be a coincidence but a design principle favored by nature. Building on the notion that NMA can be used to identify potential motions induced by binding, a computationally tractable way to generate a set of docking targets has been proposed[35].

Cryo-EM structure modeling. Recently there have been several applications of NMA to low-resolution cryo-electron microscopy (cryo-EM) structure modeling. This experimental data is naturally low-resolution, being reconstructed by averaging over multiple images of many molecules from several different angles. Additionally, the imaged molecules often undergo structural changes along with vibrations making it very difficult to extract high-resolution structural information. Several groups[16;17;37] have constructed EN models of pseudo-atomic representations for a given cryo-EM map and calculated the resulting distortions due to normal modes as an aid in the refinement of the raw cryo-EM data to produce higher-resolution structural information. Alternatively, a procedure for the flexible docking of atomic or residue level structures into cryo-EM has been suggested by using the NMA mode shapes calculated for either these pseudo-atomic EN or homology-based structures[37-40].

Domain Identification. Because these elastic networks quickly identify coupled motions it is possible to partition the protein into various domains[9]. Recently, this idea of decomposing proteins into domains based upon their structural topology has been automated[41] and applied to identifying domains that have been recombined or swapped during evolution[42].

Steering MD simulations and exploring non-equilibrium dynamics. As discussed above, the low-frequency modes from NMA are able to capture the *collective* dynamics of proteins. A recent application of this fact is to steer MD simulations along these dominant modes of motion using hybrid methods that combine MD and harmonic modes[43-45]. Specifically a hybrid MD-NMA simulation protocol has been implemented where motions along the direction of the slowest few modes are coupled to a temperature bath and thus amplified to study the unfolding and large-scale domain motions of peptides and proteins[43;44]. The inverse of this approach, namely, that the normal modes of a protein can be extracted from an applied driving force in a MD simulation[46] has also recently been shown.

Drawing on similar insight, it has been suggested that one can minimize steric clashes and interpolate between two conformations of a protein using the modes from an EN model[47] to characterize this transition. Because the harmonic approximation of NMA remains valid only near the equilibrium structure, an alternative method to escape the local minima surrounding the native state involves the iterative calculation of successive EN models deformed along one or several low-frequency modes[48]. This method allows "cracking" or partial unfolding of the underlying EN structure suggesting that such unfolding or "proteinquakes" may be coupled to collective motions[49;50].

High throughput examination of families of proteins. Fold families such as globins[51], and protein superfamiles[52] in general have been compared using NMA-based methods to identify common and distinctive structural-dynamic features. For the test case of proteases, these salient dynamic features from GNM calculations combined with data mining techniques in an unsupervised learning technique have been shown to identify the highly conserved catalytic triad[53]. More recently the minima in the slowest modes (global hinge centers) have been shown to be co-localized near catalytic residues in a representative set of enzymes[33]. These results indicate that there is a great deal of information about functional residues to be extracted from the comparative coarse-grained NMA of protein family members.

Databases/servers of molecular motion. The logical extension of family analysis is the compilation and update of web accessible databases housing NMA-based calculations for all available protein structures. Several such databases have been constructed including iGNM[54], ProMode[55], ElNémo[56], WEBnm[57], and MolMovDB[58] that allow the user to browse pre-calculated data and/or submit structures for NMA.

Conclusions and perspectives

The past five years have seen a renewed interest in NMA-inspired methods since they provide a biologically relevant and unique analytical solution for the equilibrium dynamics of biomolecules. The successes of NMA indicate that the three-dimensional structures contain the requisite information to determine functional motions. Because the most collective, or global, modes of motion predicted by NMA are insensitive to the details of models and energy parameters, but rather depend upon the topology of inter-residue contacts at equilibrium; justifies the widespread use of more efficient coarse-grained EN models described here. Such approaches are now being used, in conjunction with experimental studies, to unravel the supramolecular dynamics and long time-scale motions or large structures otherwise inaccessible via conventional simulations.

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Figure 1. Overview of various applications and methodologies of elastic network models to the GroEL-GroES complex. The elastic network model (ENM) (b) requires an initial input structure (a), typically an atomic-resolution structure such as the one at the far left colored according to secondary structural elements. As is noted in the text, a lower-resolution structure such as one representing the cryo-EM map (c) can also be used as an input for constructing an ENM. Often to process supramolecular assemblies, a further coarse-graining (d) is adopted. The figure shows the low resolution ENM in which only every 20^{th} residue is used to define the nodes. Once the ENM is constructed, various motions are calculable by NMA, ranging from the level of the entire molecule to domains to individual residues. Panel (e) illustrates the global motions computed for the GroEL-GroES complex (PDB code 1gru)[59], which reveal a counter-rotation of the GroES-bound (*trans*) ring with respect to the lower (*cis*) ring as shown by the magenta arrows. The structure has been colored by increasing mobility from blue to red, showing the mobility increases with increasing distance from the interface between the *cis*-*trans* rings and from the cylindrical axis of symmetry. Panel (f) illustrates the motions of the individual subunits composed each of three domains (apical, red; intermediate, green; equatorial, blue) that can be obtained from analysis of the ENM. The top diagram shows the ATP-bound form of a subunit in the *trans*-ring and the lowest diagram is its unliganded counterpart in the *cis*-ring. Applying the deformations from the first (slowest) mode calculated by NMA analysis to the *trans*-ring monomer produces the middle structure, demonstrating the intrinsic (structure-encoded) ability of the subunit to reconfigure into the closed form assumed in the *cis* ring, consistent with the successive interchanges of the subunit conformations between the two forms upon binding and dissociation of the cap to either ring during the chaperonin cycle. From these calculations, databases of global motions (g) have been constructed and (h) several important additional applications of these motions and deformations have been indicated.

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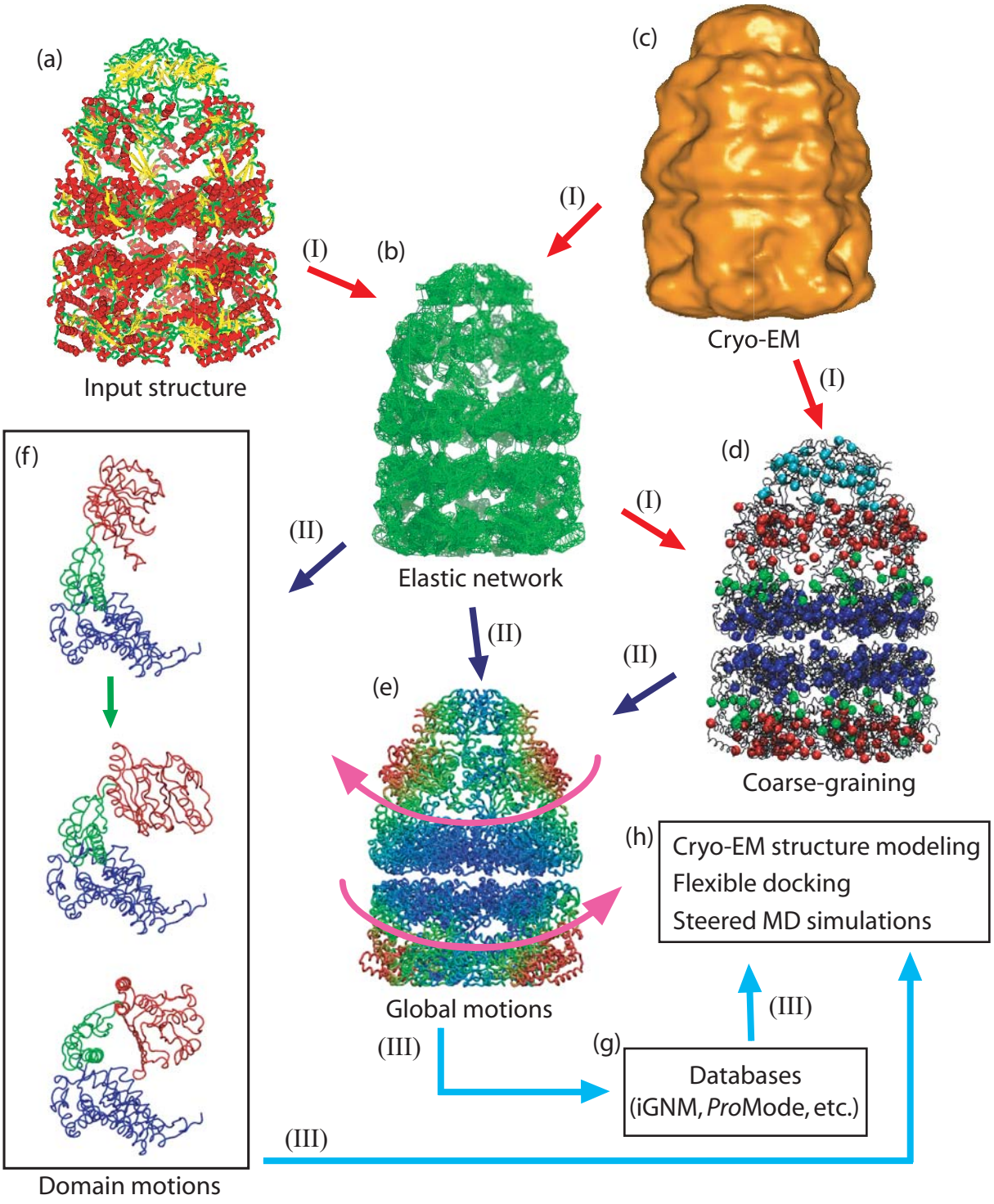
(a)
Input structure
(I)
(b)
Elastic network
(I)
(c)
Cryo-EM
(I)
(d)
Coarse-graining
(II)
(II)
(e)
Global motions
(II)
(f)
Domain motions
(III)
(III)
(g)
Databases
(iGNM, ProMode, etc.)
(III)
(h)
Cryo-EM structure modeling
Flexible docking
Steered MD simulations