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New generation of elastic network models

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The intrinsic flexibility of proteins and nucleic acids can be grasped from remarkably simple mechanical models of particles connected by springs. In recent decades, Elastic Network Models (ENMs) combined with Normal Model Analysis widely confirmed their ability to predict biologically relevant motions of biomolecules and soon became a popular methodology to reveal large-scale dynamics in multiple structural biology scenarios. The simplicity, robustness, low computational cost, and relatively high accuracy are the reasons behind the success of ENMs. This review focuses on recent advances in the development and application of ENMs, paying particular attention to combinations with experimental data. Successful application scenarios include large macromolecular machines, structural refinement, docking, and evolutionary conservation.

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Introduction

Almost two decades ago, Tirion was the first to realize that functional protein motions can be captured using a greatly simplified harmonic potential by Normal Mode Analysis (NMA) [1••]. Shortly afterward, several coarse-grained versions of Tirion's elastic network model (ENM) were proposed, in which a given conformation of the protein was approximated by a set of particles (e.g., residues represented by Cα atoms) interconnected by elastic springs. Two main types of ENM were established: the anisotropic network model (ANM) [2,3], which corresponds directly to the coarse-grained version of Tirion's approach, and the Gaussian network model (GNM) [4], which is a one-dimensional simplification limited to the evaluation of the mean squared displacements and cross-correlations between atomic fluctuations magnitude. From these seminal works, ENMs have proven over the years to be an effective approach to understanding the intrinsic dynamics of biomolecules [5,6•,7].

Although there are many variations to reduce the complex biomolecular structures into a network of nodes and springs, the basic assumption (and limitation) of ENMs is that the potential energy is described by a quadratic function around a minimum energy conformation:

$$V = \sum_{i<j} K_{ij}(r_{ij} - r_{ij}^0)^2 \quad (1)$$

where the superindex 0 indicates the initial conformation, r_{ij} is the distance between atoms i and j, and K_{ij} is a spring stiffness function. The intrinsic dynamics of an ENM is mostly assessed by NMA. In this classical mechanics technique, all the complex motions around an initial conformation are decoupled into a linear combination of orthogonal basis vectors, the so-called normal modes. The modes are computed solving by diagonalization the following generalized eigenvalue problem:

$$\mathbf{HU} = \boldsymbol{\lambda}\mathbf{TU} \quad \text{where} \quad \mathbf{U} = (\mathbf{u}_1, \mathbf{u}_2, ..., \mathbf{u}_N), \quad (2)$$

where $\mathbf{H}$ is the Hessian matrix (partial second derivatives of the potential energy), $\mathbf{T}$ the kinetic energy matrix, and $\boldsymbol{\lambda}$ is a diagonal matrix with the λ_k eigenvalues associated to the kth normal mode $\mathbf{u}_k$. As the frequencies ($\omega_k = \lambda_k^2/2\pi$) are directly proportional to the energy required for the movement, high frequency modes describe local motions whereas low frequency modes represent collective (large-scale) conformational changes. Most importantly, it has been widely confirmed by many studies that ENM's lowest-frequency normal modes often give a reasonable description of experimentally observed functional motions (see review articles for further methodological details including experimental validation [5,6•,8,9]). Following the structure–dynamics–function paradigm, these collective modes have been conserved during evolution as they represent the mechanical deformations of lowest energetic cost. Even though the shown usefulness, the validity of ENM–NMA fluctuations is limited to small excursions around the equilibrium conformation. However, larger deformations can be obtained by iteratively applying small displacements along the lowest modes [10].

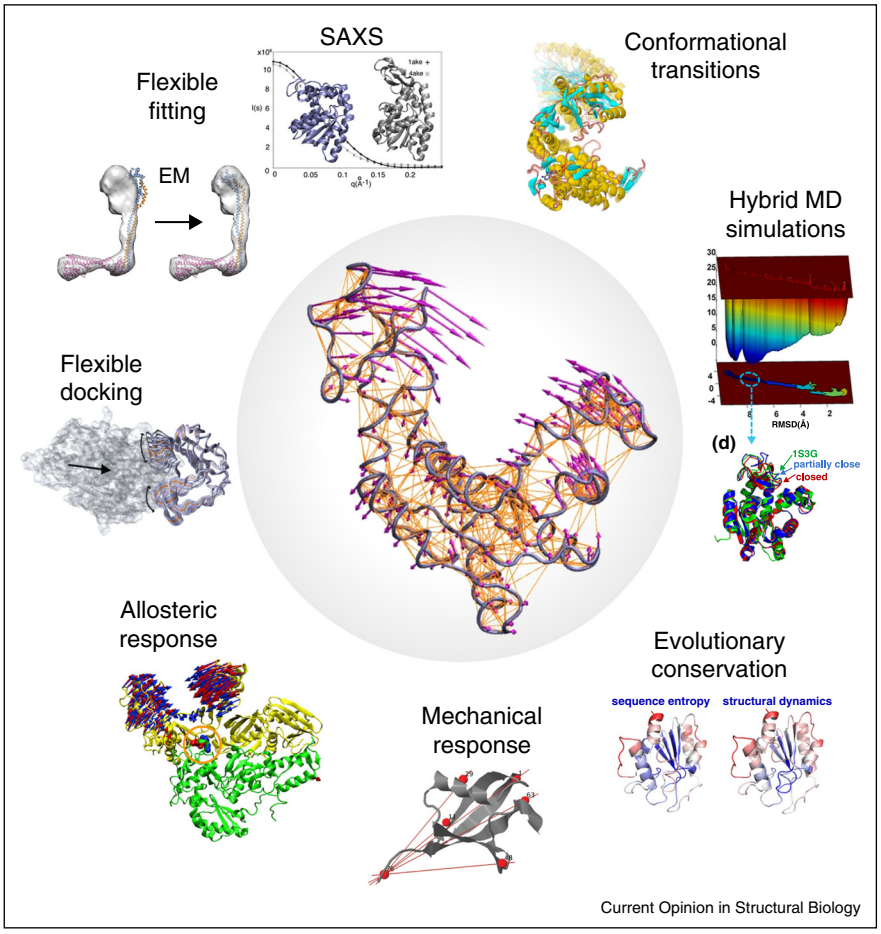
There is an overwhelming literature validating the use of ENM in multiple scenarios. This review focuses on the recent progress of ENM for characterizing macromolecular flexibility, predicting functional conformational changes, and assisting in the interpretation of structural experimental data, paying close attention to hybrid

methods that combine ENM with simulations and/or experimental data.

Trends in ENM development

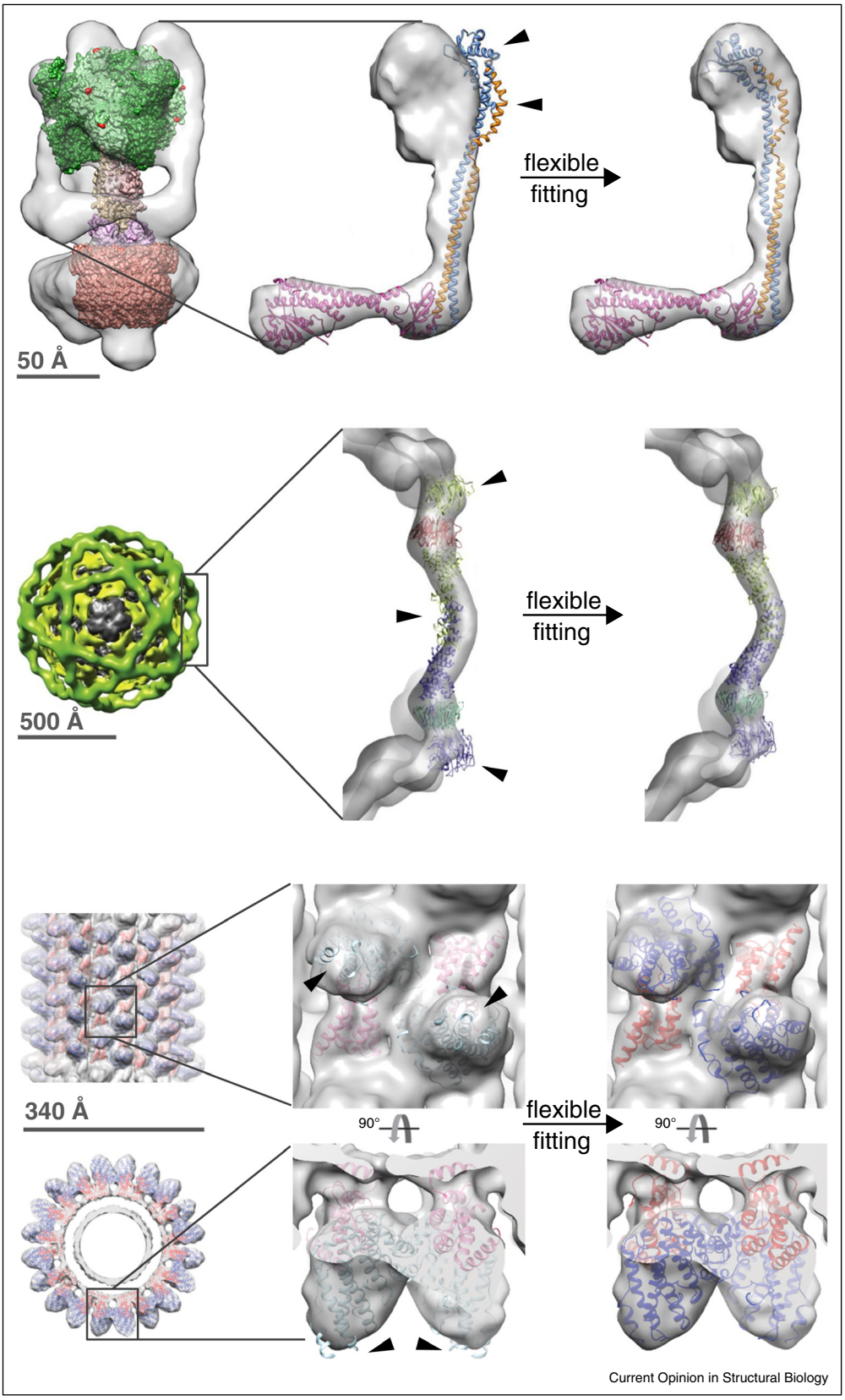
The agreement between the observed functional motions and the lowest frequency modes extracted with NMA is relatively well preserved independently of model or potential details, evidencing the approach's robustness as long as the 3D contact topology is reasonably maintained. In the past, many research efforts were dedicated to further simplifying ENMs and extend the application range to larger systems. Notable reductions can be obtained by grouping atoms into clusters [11,12] or into rotational and translational blocks [13,14] without substantial loss of accuracy. Even from low-resolution 3D reconstructions, it is still possible to obtain insight into the macromolecular global flexibility [15,16], revealing the importance of the shape in determining the lowest-frequency normal modes. Other authors have used more sophisticated schemes to reduce the main computational bottleneck of the NMA, the diagonalization of the Hessian matrix, for example, by calculating only the relevant normal modes of interest [17] or considering the system symmetry [18–20]. Another elegant way to reduce the computational cost is working in internal coordinates (IC). Using dihedral angles as ICs instead of Cartesian coordinates reduces the number of degrees of freedom, leading to substantial savings in computational resources. Moreover, the implicit maintenance of the covalent structure preserves the model geometry and minimizes the potential distortions frequently observed in Cartesian approaches. Based on early works by Go and Levitt [21,22] that established the complete mathematical framework, several authors successfully developed different coarse-grained ENM approximations using torsion angles as variables [23–25]. Despite the convenience of ICs [26], the vast majority of current approaches are Cartesian-based because of their simplicity (i.e., the

Figure 1



Representative ENM application scenarios. The ENM application range is currently expanding from the prediction and analysis of biologically relevant motions to serve as a conformational sampling engine in multiple structural biology scenarios. Enclosed by different application examples, a Tirion's elastic network (orange segments) of the adenylate kinase protein (gray tube) is illustrated together with the corresponding lowest energy normal mode (pink arrows). The central image was generated using iMod [23] and VMD, and the remaining images were reproduced with permission from Refs. [23,61,69,75,77,79,86,89].

Figure 2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kinetic energy matrix is reduced to the identity) and straightforward implementation. In the opposite direction from increasing coarseness, the parameterization of the potential energy (e.g., the definition of spring stiffness constants) for better reproduction of the observed intrinsic flexibility also led to interesting advances. There is an extensive literature dealing with different variations of the ENM that add complexity to the network by considering, for instance, chemical bond information [27], backbone secondary structure [28], side chain identity [29], atom type [30,31], or anharmonicity [32].

In most studies thus far, the spring constants and model parameters have been fitted to obtain atomic fluctuations according to the crystallographic B-factors, even achieving an almost perfect fit [33,34]. Although crystalline environment symmetry can be accounted for [20], crystal packing effects, internal static disorder, and refinement errors prevent B factors from being a fully reliable measure of intrinsic flexibility [35,36•]. Validating ENMs against Nuclear Magnetic Resonance (NMR) ensemble data is a more suitable option, but missing data would artificially increase the structural diversity. High-resolution structures solved in multiple conformations provide a reliable but partial picture of flexibility because they are usually limited to a few states, and information about intermediates is sparse or non-existent. Despite these drawbacks, principal component analysis of crystallographic and NMR ensembles has proven that the normal modes capture the collective conformational variability of proteins fairly well [37]. However, the development of a gold standard for benchmarking intrinsic flexibility in solution remains an open question.

An interesting alternative to refining ENMs is found in direct comparison with Molecular Dynamics (MD) simulations. In fact, there is a good correspondence between protein Essential Dynamics (ED) extracted from atomistic MD simulations and ENM-based NMA [38,39]. To the best of our knowledge, Hinsen was the first to validate ENM with a short MD simulation to derive an improved spring stiffness function [40]. ENM force constants can be directly calculated from MD variance–covariance matrices by inversion [41], iterative adjustment [42], or entropy maximization [43]. In this line, we extracted simple connectivity rules to obtain more realistic ENM spring forces and atomic fluctuations by comparison with a database of representative MD trajectories [44]. Our improved ENM was more robust against protein size or fold variations and captured the flexibility of NMR structural ensembles better than other standard ENMs. Advances in computer hardware and software have now made it possible to run MD simulations at longer timescales, even reaching milliseconds of simulation. Bahar and coworkers, showed how ANM lowest-frequency modes naturally facilitate interconversion between the most probable distinct conformations sampled by micro-to-millisecond MD trajectories of two protein conformational transitions [45•]. We think that future accessibility to very long MD simulations [46] of different systems will be crucial in developing a more faithful description of the intrinsic flexibility based on ENMs.

Broadening the ENM application range

Providing an exhaustive coverage of the many applications of ENM–NMA (Figure 1) is out of the scope of this review. Here we focus in ENM-based hybrid methods that enable the interpretation of the structural dynamics information extracted from complementary biophysical techniques and simulations, especially for large macromolecules and supramolecular complexes.

Huge macromolecular machines

Large macromolecular complexes are the main actors of biological processes. Understanding how they work and how they move is critical to understanding cellular function and is among the most challenging tasks for current structural biology. Unfortunately, their large size and the long time scale of their functional motions are often prohibitive for traditional MD. In this context, ENM has arisen as a powerful alternative to yield molecular insights into the fluctuations of macromolecular complexes and the mechanisms of their large-scale functional rearrangements from a single atomic structure. For example, the swollen motion of complete virus capsids or the functional motions of the ribosome have been successfully characterized by ENM [47]. An interesting source of large supramolecular complexes can be found in membrane proteins. The functional mechanisms of ion channels or membrane receptors and transporters appear to be dominated by a few collective motions, independently of the membrane environment (for a complete review, see [8]). There is plenty of room to improve and extend the applicability of ENM in the study of the relevant biological processes occurring in huge size systems at longer time scales (e.g., considering solvent damping effects). In this context, we significantly extended the applicability of our internal coordinates ENM towards much larger systems (>100K residues), such as long actin filaments or microtubules, by effectively reducing the computational burden of the diagonalization step using parallel strategies with multicore technology [48].

(Figure 2 Legend) Flexible fitting of atomic structures into electron density maps using several ENM-based strategies. Fitting of the peripheral stalk of the eukaryotic V-ATPase (top panel), the coat protein complex II coat assembly (middle panel), and two domains of clathrin adaptors (bottom panel) using iMODFIT [56•], NMFF [59], and DEN-DireX [67], respectively. The atomic structures are represented with ribbons and the EM maps in gray. Reproduced with permission from Refs. [61,63,90].

The majority of the work in this area has been confined to proteins, and only limited attention has been paid to investigating nucleic acids. Nevertheless, there are impressive examples of the power of ENM and NMA to reveal different functional motions of the ribosome [47,49,50] or well-packed RNAs [51]. Additionally, the ability of normal modes of ANMs to capture the spatial variance observed in a collection of 16 RNA ensembles, many of which are riboswitches, has been confirmed [52•]. Recently, different ENMs have been optimized to reproduce the flexibility of RNA and DNA structures using a large data set of experimentally determined structures and MD simulations [53]. Additional validation of ENMs against MD simulations of RNAs and SHAPE experiments has also been recently reported [54].

Hybrid methods for structural refinement and simulation

In the current integrative structural biology context, the merging of information from electron microscopy (EM) and atomic resolution techniques [55] constitutes a fruitful scenario for ENM to decipher the dynamics of essential macromolecular complexes. Computational flexible fitting techniques based on ENM–NMA enable the dynamic interpretation of low/medium-resolution EM data captured in different functional states in terms of available atomic structures [56•,57,58] (Figure 2). In these techniques, an initial atomic structure is iteratively deformed using the conformational space spanned by their lowest frequency modes for improving the density overlap with a target EM map. Impressive results have been obtained in the study of the infective swelling motion of the Cowpea chlorotic mottle virus by flexibly fitting the closed crystallized structure into the mature 3D EM reconstruction [59]. Other representative examples of ENM–NMA flexible fitting applications include the structural characterization of the ribosomal machinery [47,60], ATPases [61,62], the coat protein complex II cage [63], and several virus capsids [64,65]. The deformable elastic network (DEN) is also based on an ENM, but instead of using NMA to guide the low-resolution refinement, it employs torsion-angle MD to fit either X-ray or cryo-EM data [66,67]. NMA-based elastic iterative 3D-to-2D alignment has been integrated into the 3D EM reconstruction process [68]. Other hybrid strategies exploit the synergism of ENMs with Small Angle X-ray Scattering using NMA [69] or Newton–Raphson [70] methods. Notice that in these hybrid strategies the experimental data naturally constraints the amplitude and the relative importance of the modes alleviating one of the ENM–NMA drawbacks.

Combining ENM with atomistic simulations is another remarkable hybrid approach. Collective modes have been successfully incorporated into MD simulations to speed up large-scale domain motions [71–73]. For example, Bahar and colleagues took advantage of the ANM collective motions selected by a Monte Carlo-Metropolis algorithm to generate transition pathways in close agreement with detailed full-atom MD simulations [74]. The combination of internal coordinates NMA and MD umbrella sampling has also proven useful to describe ligand-driven conformational transitions [75]. By numerically solving the equations of motion of an ENM [76] or by NMA–ANM [77], the relative mechanical responses of different residue pairs under unfolding tension forces can be quantitatively described.

Protein docking

In the context of *ab initio* prediction of protein complexes from their isolated structures, a comprehensive analysis of two thousand unbound-to-bound transitions revealed that the changes observed in one-third of the cases can often be described using the deformation modes computed from the unbound structures [78]. Zacharias pioneered the use of a few lowest modes to improve the quality of near-native docking solutions, at least for several test cases [79]. Other approaches such FiberDock [80], SwarmDock [81], or EigenHex [82] effectively exploited ENM vibrational modes to account for flexibility and improve the docking predictability. SwarmDock, one of highest performing methods in the Critical Assessment of Prediction of Interactions (CAPRI) contest, uses only a few low frequency modes as a component of the optimization vector to model transitions between unbound and bound conformations. The employ of ENM–NMA in small-molecule docking is rather limited likely because it fails to describe local changes [83].

Intrinsic dynamics conservation

The intrinsic dynamics predictable by ENM–NMA appears as a major determinant in protein–protein and protein–ligand interactions, allosteric response modulation, and assembly mechanisms. Moreover, in many cases, the conservation of low frequency modes throughout the evolution has been well characterized [84–86]. The validation of several ENMs for the comparative analysis of protein dynamics across structures with different conformations and within a protein family has also been recently addressed [87]. In terms of the evolutionary selection of structures and their intrinsic dynamics, the predictions of dynamical structural couplings (allosteric), critical residues (hot spots), or mutagenic effects are quite interesting research topics. For more information about the subject, the reader is referred to excellent review articles [88••,89].

Conclusions and perspectives

Although we remain intrigued by the simplicity of the approach and are aware of its limitations, ENM has proven successful over a wide range of applications. Pushing up against the limits of methodology, research will continue to improve and design new ENMs for recovering large scale conformational changes in large macromolecules with higher accuracy. In this endeavor,

structural data from a great variety of experimental sources such as X-ray crystallography, NMR, EM, SAXS, among others, as well as extended MD atomistic simulations, will provide crucial information for the successful parameterization of new ENMs. Undoubtedly, ENM-based hybrid approaches will continue to play important roles by enabling the dynamic interpretation of the structural information of supramolecular structures and assemblies from complementary structural biophysical techniques and simulations. Bridging structural dynamics and evolution with ENM will also open new interesting perspectives. Moreover, the efficiency, scalability, and robustness of the approach will definitely contribute to maintaining its popularity and extend the application range in which an efficient engine to explore large scale collective motions is needed, particularly in a structural integrative framework.

Conflict of interest

Nothing declared.

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- •• of outstanding interest

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