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Review Article

Modeling the nucleoporins that form the hairy pores

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Sitting on the nuclear envelope, nuclear pore complexes (NPCs) control the molecular transport between the nucleus and the cytoplasm. Without definite open or close states, the NPC uses a family of intrinsically disordered nucleoporins called FG-Nups to construct a selective permeability barrier whose functional structure is unclear. Experimental advances have offered high-resolution molecular knowledge of the NPC scaffold and docking of the unfolded FG-Nups, however, the 'hairy' barrier structure still appears as blurred lobes even under the state-of-the-art microscopy. Without accurate experimental visualization, the molecular mechanism for the NPC-mediated transport remains a matter of debate. Modeling provides an alternative way to resolve this long-standing mystery. Here, we briefly review different methods employed in modeling the FG-Nups, arranging from all-atom molecular dynamics to mean-field theories. We discuss the advantage and limit of each modeling technique, and summarize the theoretical insights that, despite certain controversy, deepened our understanding of the hairy pore.

Introduction

The nuclear pore complex (NPC) is the largest molecular channel and the sole intracellular gate in eukaryotic cells that controls nucleocytoplasmic mass exchange [1–5]. To preserve the integrity of the genetic materials, the NPC-mediated transport is highly selective. While small molecules up to 40 kDa can freely diffuse through the NPC, larger macromolecules without the assistance of agents are blocked by a permeability barrier [1,6–8]. This physical barrier is constituted by a family of intrinsically disordered nucleoporins (NPC proteins) called FG-Nups that are rich in phenylalanine-glycine (FG) repeats. It is intriguing that selected macromolecules with nuclear import/export signals (short amino-acid sequences) [9,10] that bind to karyopherins (Kaps) can be transported through the NPC despite the increased size of the Kap–cargo complexes that is entropically unfavorable. The hydrophobic interaction between the Kaps and the FG-Nups has been identified as an important driving force during the trafficking process, although the energetic and spatiotemporal details of the binding/unbinding events remain elusive. As a high-throughput molecular channel, the NPC facilitates more than a thousand macromolecular cargos to translocate per second [11,12], which ensures that the fast interphase cell growth is not limited by the proofreading of biomass exchange across the nuclear envelope. The typical dwell time of cargo in transit is less than 10 ms, a remarkable transport efficiency considering the crowding nanoenvironment of the nuclear pore lumen [12]. How the NPC enables highly selective yet rapid nucleocytoplasmic transport is a fundamental and pressing question in cell biology. The key to resolving this transport paradox of the 'hairy' pore lies in the understanding of the *in vivo* structure of its functional 'hairs'—the FG-Nups. As shown in Figure 1A, the nuclear pore lumen is a rich repository of intrinsically disordered regions dispersed with a variety of FG repeats. Although the amino-acid (AA) sequences of the FG-Nups have been long deciphered, the disordered nature of the protein 'hairs' poses a great challenge for traditional biochemical assays and biophysical techniques to determine the ultrastructure of the nuclear pore lumen [13–18]. Recent structure

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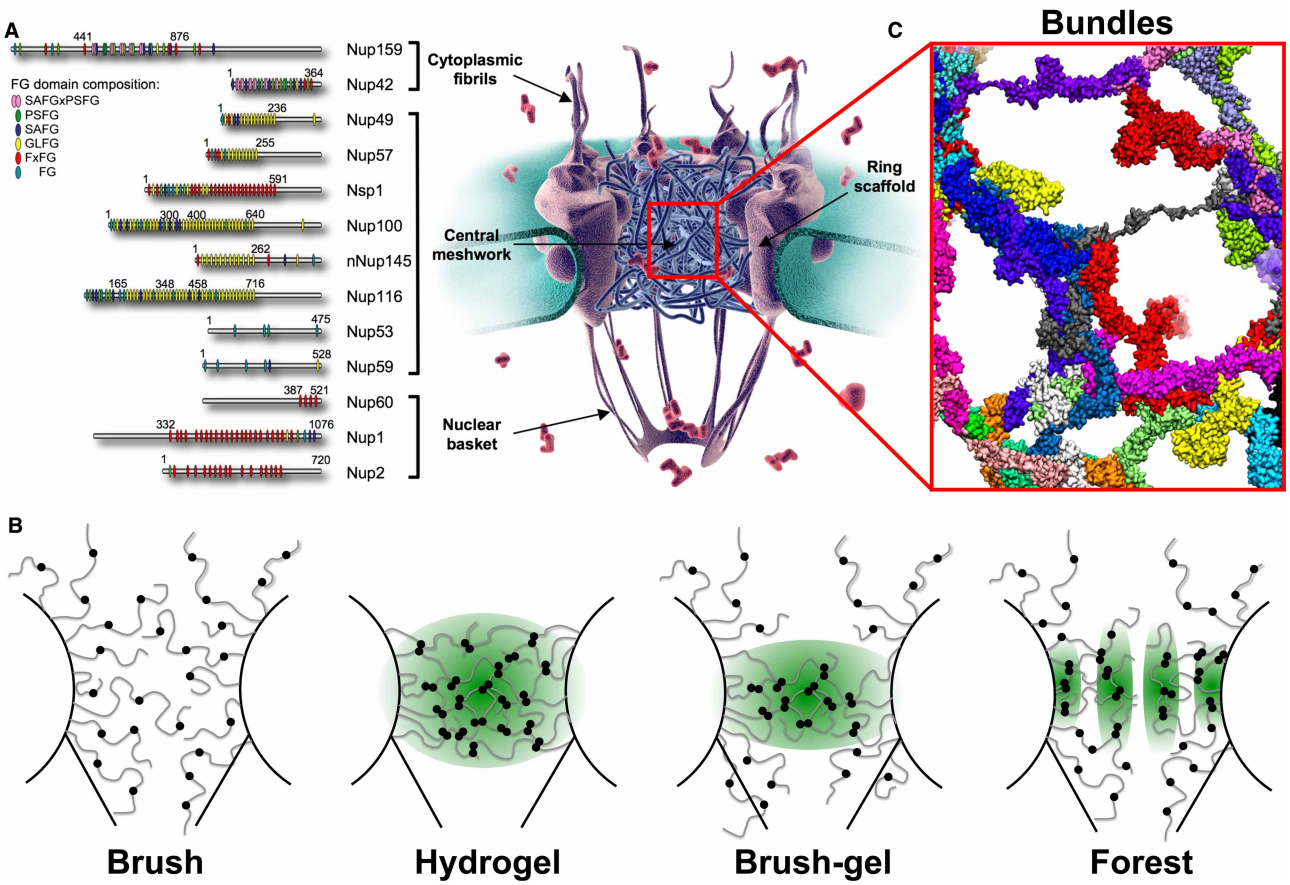

Figure 1. The NPC as a 'hairy' pore.

(**A**) Amino-acid sequences of different FG-Nups and a schematic presentation of the NPC. Reprinted with permission from Ref. [26]. (**B**) Schematic summary of different structural hypotheses of NPC-mediated transport. Dots represent FG motifs of the Nups. Green domains represent hydrogel regions. (**C**) Bundles of FG-Nups (Nsp1) predicted by all-atom simulations. Reprinted with permission from Ref. [27].

resolution of the NPC scaffold based on a divide-and-conquer approach [19,20] has revealed substantial molecular details on this folded part of the protein complex whose typical 8-fold rotational symmetry can be visualized under super-resolution microscope [21,22]. It has been well accepted that the scaffold can be portioned into three rings with the central one well conserved from yeast to human [20,19,23,18]. In contrast, the functional core of the molecular machine, i.e. the unfolded central channel (also termed as central transporter [18,24,25]) of the NPC, is still structurally elusive and mechanistically controversial. Divergent hypotheses have been proposed to explain the structural base of NPC-mediated transport. Despite extensive experimental and computational efforts to test and reconcile conflicting views, consensus has not yet been reached. Nevertheless, the integration of methods and accumulation of data continues to renew our understanding of the NPC. In this review, we focus on the theoretical side of the field and cover many different computational models. The review is organized as the following. We first briefly introduce primary hypotheses on the qualitative structural picture of the NPC. We then review different theoretical approaches that allow more quantitative predictions of the system. These include molecular dynamics (MD) simulations with varying amount of molecular details, mean-field methods, and molecular theory (MT) with explicit considerations of polymer conformations. We discuss the insights from various models and end the review with an outlook of future avenues.

Beyond the dichotomy of brush and gel

The selective nucleocytoplasmic transport mediated by the FG-Nups, with uncompromised efficiency, is remarkable. It is even more so considering the fact that this process does not directly consume energy.

Specifically, energy is only paid to fuel a RanGTP/GDP cycling system [1,28] that directs the movement of Kap–cargo complexes at the entrance and exit of the NPC. Once the complexes enter the nuclear pore lumen, they undergo passive diffusion until their release into either the targeted or home compartment (the success rate is not 100% due to the diffusive nature of the transport [29]). Many hypotheses have been proposed to explain how the FG-Nups enable passive, rapid and selective cargo translocation. Among them, two pioneering ones are the selective phase model [30–34] and the virtual gating model [35,36,3,37]. As first impressions of the NPC, these two paradigms are based on simple intuitions, both plausible yet widely differing from each other. In the selective phase model, the FG-Nups are predicted to fill the central channel by forming a dense hydrogel within which the FG–FG associations are strong and saturated, i.e. of negligible dangling FG motifs [31]. The mesh size of the hydrogel is such that small molecules can freely diffuse through the FG-meshwork while large cargoes get blocked. Kap–cargo complexes, however, can melt the hydrogel by forming non-covalent Kap–FG bonds that are stronger than the FG–FG interactions. The melting process is reversible as the broken FG-meshwork can seal itself after the translocation of the cargo. In this picture, the permeability barrier is purely enthalpic in nature. In contrast, the virtual gating model posits an entropic barrier that is constituted by a highly dynamic, non-cohesive polymer brush of FG-Nups with weak FG–FG associations [36]. To pass through the crowded channel, large Kap–cargo complexes need enthalpic gain from hydrophobic Kap–FG associations to compensate the entropic penalty of excluded volume effect that constrains the conformational freedom of FG-Nups. As schematically shown in the first two panels of Figure 1B, the overall densities, morphologies, and cross-linking levels of the FG-Nups are disparate between the brush and hydrogel models. Although both views could be oversimplified, the 'brush-vs-gel' debate has over the years attracted extensive research attention to the field, and efforts to reconcile these two paradigms have kept moving the field forward.

Recent experimental studies suggested that the arrangement of FG-Nups is inhomogeneous, which could host distinct passageways for different cargoes [17]. This has led to the development of new hypotheses that predict composite gating structures throughout the nuclear pore with both brush-like and gel-like features. One such hybrid model, as illustrated in the third panel of Figure 1B, postulates that there are two non-cohesive FG-brushes at the two exits of the nuclear pore whose center is occluded by a cohesive gel-like structure [26]. In another version of this hybrid model, the central cohesive structure is postulated to have a narrow conduit for the passage of small molecules [38]. A more complicated gating structure has been proposed based on hydrodynamic analyses of individual FG-Nups [39]. It has been observed that different FG-domains have a diversity of cohesiveness dependent on the distribution of FG repeats and charges on their AA sequences [26,39]. Short cohesive FG-Nups such as Nup49 and Nup57 often adopt collapsed conformation, while the longer ones such as Nup100 and Nup116 have non-cohesive subdomains, forming stalk-like extended structures that are docked to the NPC scaffold. Classifying all the FG-Nups into short 'shrubs' and tall 'trees', the forest model [39] contends that the collapsed subdomains of some trees amalgamate to form a central transporter structure suspended by the tree stalks. As shown in the right panel of Figure 1B, the opening of the central transporter provides a passageway for small molecules, whereas large cargoes can translocate through the peripheral zone between the transporter and the shrub-covered NPC scaffold. This model suggests that the central transporter is an intrinsic NPC structure that could explain the blurred 'central plug' observed in electron microscopy (EM) [18,24,25], which has been alternatively interpreted in the literature as cargoes in translocation [40]. Compared with a simple hydrogel, this two-channel model with alternating cohesive and non-cohesive regions is more consistent with single-molecule fluorescence (SMF) observation of peripheral translocation of Kaps [41,42] and EM observation of Kap–cargo complexes near the NPC scaffold [43]. As a 'jigsaw puzzle' approach, the forest model pieces together all the individual FG-Nups assuming no major structural arrangement as they interact.

Besides the above four models summarized in Figure 1B, other models have been proposed to take into consideration of the Kap family as a possible integral component of the transporter. One such model is the reduction in dimension model [44,45], which posits that Kaps collapse the three-dimensional (3D) network of FG-Nups into a two-dimensional (2D) coat of the NPC scaffold. This Kap-rich FG film is responsible to translocate large Kap–cargo complexes, whose diffusion is, therefore, reduced to be 2D and faster than that in 3D space. Another model in this line, the Kap-centric model [46], states that the periphery of the FG-network is loaded with strongly bound Imp β1 (karyopherin β1) that mediate slow cargo transport, whilst the tips of the FG-Nups at the center of the pore allows for faster translocation thanks to the weaker Kap–FG binding there.

While all these qualitative models can explain certain experimental observations, their predictions on the collective structures of FG-Nups are in conflict. This discrepancy is partially due to the uncertainties of

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experimental data. For example, whether the central axis of NPC is void of large cargoes is still under debate because of the controversy in the data analysis of SMF experiments [47]. There exist also apparently conflicting reports on the effects of Kaps as additives to the FG-Nups. Although the early experiment found Kap-induced significant collapse of FG-Nups [48], more recent experiments [46,49–51] seem to agree on the swelling of FG-Nups upon the addition of Kaps. Despite these inconsistencies, it has become more accepted that the FG-network is more than a simple polymer brush or a hydrogel, and rich behaviors can arise due to the interplay between entropic and enthalpic effects [52,53]. Given the complexity of the system, quantitative modeling is needed for deeper insights.

Atoms and fields

Many different quantitative modeling methods have been applied to study the functional structure of FG-Nups. The two ends of the spectrum are all-atom MD simulations and mean-field theories. MD simulation is a standard method to study protein structure and dynamics. It solves the Newtonian equations of motion for particles that represent the atoms/residues of the proteins. A force field, often based on quantum mechanical calculations or calibrated by experimental data, addresses the potential energy between atoms as a function of their distances and connectivity. Classical MD is one of the most accurate approaches to describe the behavior of non-reactive protein/peptide systems at short timescales from nanoseconds to microseconds. However, given the size of the NPC and the time scale of cargo transport, all-atom simulation of the whole system with functionally relevant dynamics is computationally impractical. So far, only small parts of the NPC and a subset of FG-Nups have been scrutinized by all-atom MD simulations [27,54,55]. Gamini *et al.* [27] performed all-atom MD to suggest that FG-Nups tend to form bundles of 2–6 proteins. These bundles, interlinked by single FG-Nups, form a mesh-like structure as shown in Figure 1C. This meshwork of bundles is more structured than the putative hydrogel of disordered single FG-Nups in the selective phase model [31]. It is worth noting that water molecules are not explicitly modeled in these simulations. Recent experimental and explicit-water MD simulations have shown that hydrophobic interaction is not context-free but rather dependent on proximal solvation and charge conditions [56–59]. Being hydrophobic in nature, FG–FG interactions are also expected to be context-dependent, which is a fundamental challenge for implicit-solvent simulations. Other groups have carried out all-atom simulations of smaller systems of FG-Nups with explicit consideration of the solvent effect [60,61]. Raveh *et al.* [61] recognized that the predicted physical extension of FG-Nups heavily depends on the choice of force field for water. Nevertheless, the overall conformation of FG-Nups appears to be highly disordered and unstructured in the explicit-water simulations. Milles *et al.* [60] computationally showed that the conformational flexibility of FG-Nups holds against their binding to Kaps, even when multiple binding sites are involved (Figure 2A). Consistent with NMR [62] and AFM [63,64] observations of rapidly fluctuating FG-Nups, these simulation results suggest that weak and ultrafast multivalent Kap–FG interactions allow the Kap–cargo complexes to translocate in a fast and selective manner [61]. Long timescale MD simulation of short FG repeats and NTF2 (a small Kap responsible for RanGDP import) has further revealed a slide-and-exchange mechanism that provides local molecular details of the transport process [61].

If the FG-Nups are unstructured enough, they can be theoretically treated as flexible polymeric chains. This approximation justifies the application of mean-field approaches [65–69] to study the collective behavior of FG-Nups. Opposed to all-atom simulations of exact many-body interactions, mean-field methods concern the spatial density distribution of coarse-grained FG-polymers and other averaged molecular fields, and how any monomer of the polymers interacts with these fields in approximations of the many-body effects. Here, each monomer represents one or several amino acids. A free energy functional of fields is constructed to account for the entropies of polymers and solvents, as well as the effective enthalpies of intra- and interpolymer interactions that reflect solvent and electrostatic effects. The optimization of the functional regarding to the free energy gives rise to self-consistent solutions for the molecular fields. Vovk *et al.* [68] applied a mean-field theory to study FG-Nups in solution, and explained their phase separation as observed in experiments [70]. The high computational efficiency of the method makes it a powerful tool to calculate the phase diagram of the FG-Nups at various conditions. The theory has been also applied to investigate FG-brushes that are attached to surfaces of simple geometries [69], where the theoretical predictions have found good agreement with numerical simulations [71] and experimental observations [50]. Although the complicated geometry of the NPC scaffold and the chemical heterogeneity of FG-Nups are difficult to treat in an analytical framework, mean-field theories have been developed to study NPC-like nanopore systems with Nup-like polymers and Kap-like particles. Monte Carlo simulation of a polymer–particle system (Figure 2B) has been performed as a reference for the corresponding

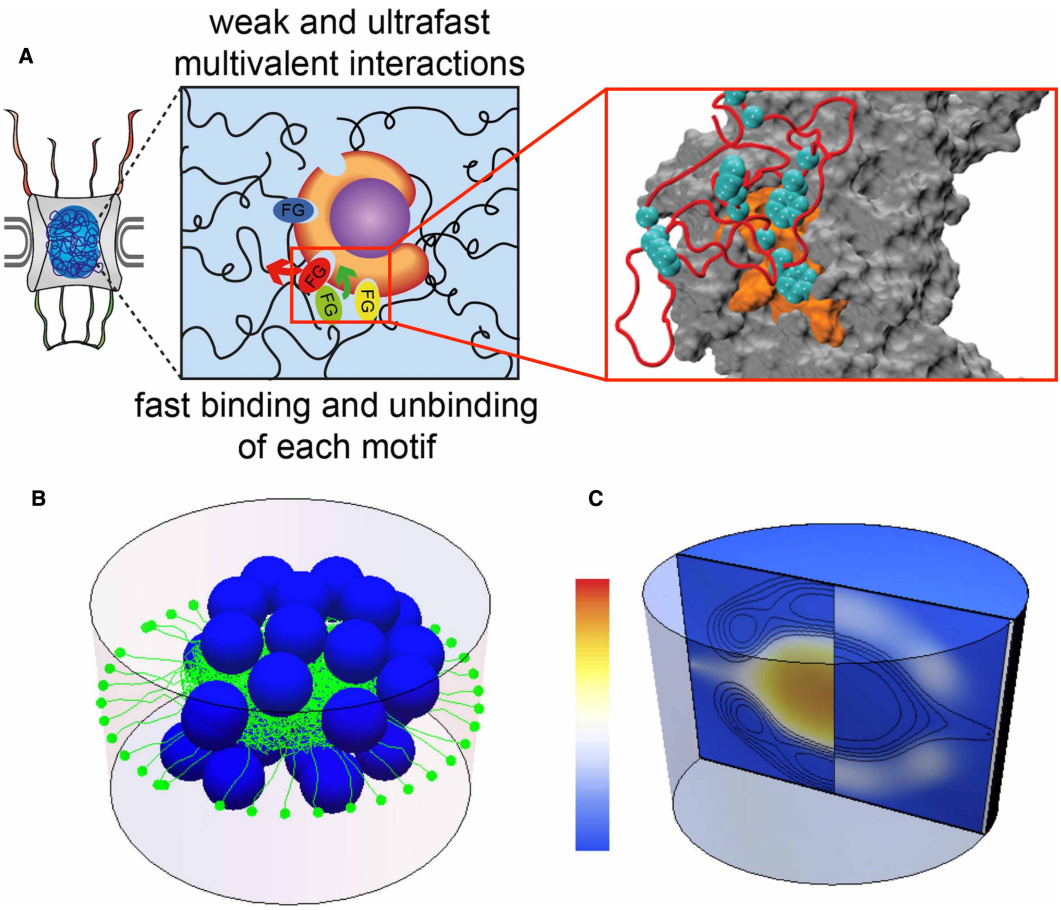

Figure 2. Local and global pictures of the Kap-FG interaction provided by different modeling techniques.
(**A**) All-atom simulation of local Kap–FG interactions reveals a weak and fast multivalent binding scenario. Reprinted with permission from Ref. [60]. (**B**) Monte Carlo simulation of NPC-like nanopore with polymers representing FG-Nups and particles representing Kaps. (**C**) Density profiles (left: polymers, right: particles) of the same nanopore system predicted by a mean-field theory. Reprinted with permission from Ref. [65].

mean-field system [65] (Figure 2C). Systematic mean-field calculations then show that the intermixing between particles and polymers is sensitive to the cohesiveness of the polymer and the particle–polymer attraction [65]. One interesting prediction from mean-field calculations is that, at low FG-Nup concentration, the permeability of Kaps exhibits a non-monotonic dependence on the cohesiveness of the FG-Nups [69]. It is worth noting that in mean-field theories the FG-Nups are often simplified to homopolymers, and the complex molecular interactions are reduced to a single parameter of cohesiveness. The predicted local polymer density is sensitive to the cohesiveness as it governs the free-energy contribution from a quadratic term of the density.

The codes of the 'hairs'

Despite their conformational flexibility, the polymer-like FG-Nups are not uniform in their lengths and AA sequences as shown in Figure 1A. Bioinformatic studies have identified evolutionally conserved patterns in the genetic codes of these functional 'hairs' [72,73]. It is also known that mutations in the sequences of FG-Nups are associated with diseases including cancer and Alzheimer [74,75]. Because all-atom simulations are too expensive and the homopolymer simplification in mean-field approaches is too crucial, coarse-grained (CG) heteropolymer modeling becomes a useful tool to investigate the sequence–structure–function relation of FG-Nups. We will refer to this category of the model simply as CG method, which should not be confounded with the more simplified mean-field approach. In a CG model, one bead or particle maps to one or more amino acids, or just a few atoms

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of one amino acid. Particles interact with each other in a CG potential that includes different energetic components. Brownian dynamics technique is often used in these CG models to reduce the particle–solvent interactions to stochastic forces, so that the explicit solvent molecules can be CG out.

Considerable computational efforts have been devoted to understand the conformational behavior of Nsp1, the most abundant FG-Nup in yeast NPC. Nsp1 is also a typical heteropolymer with two distinct subdomains that contain different FG motifs and charged spacers. Using a CG model, Ando *et al.* [76] showed that the two subdomains have different conformations, with the C-terminal side rich in FxFG motifs and charged spacers being more extended than the N-terminal side rich in FG motifs (Figure 3A). The extended part is anchored to the NPC scaffold like a stalk. The collective morphology of Nsp1s attached to a NPC-like nanopore is biphasic, with a high-density center and low-density periphery (Figure 3B). The condensation degree of the center

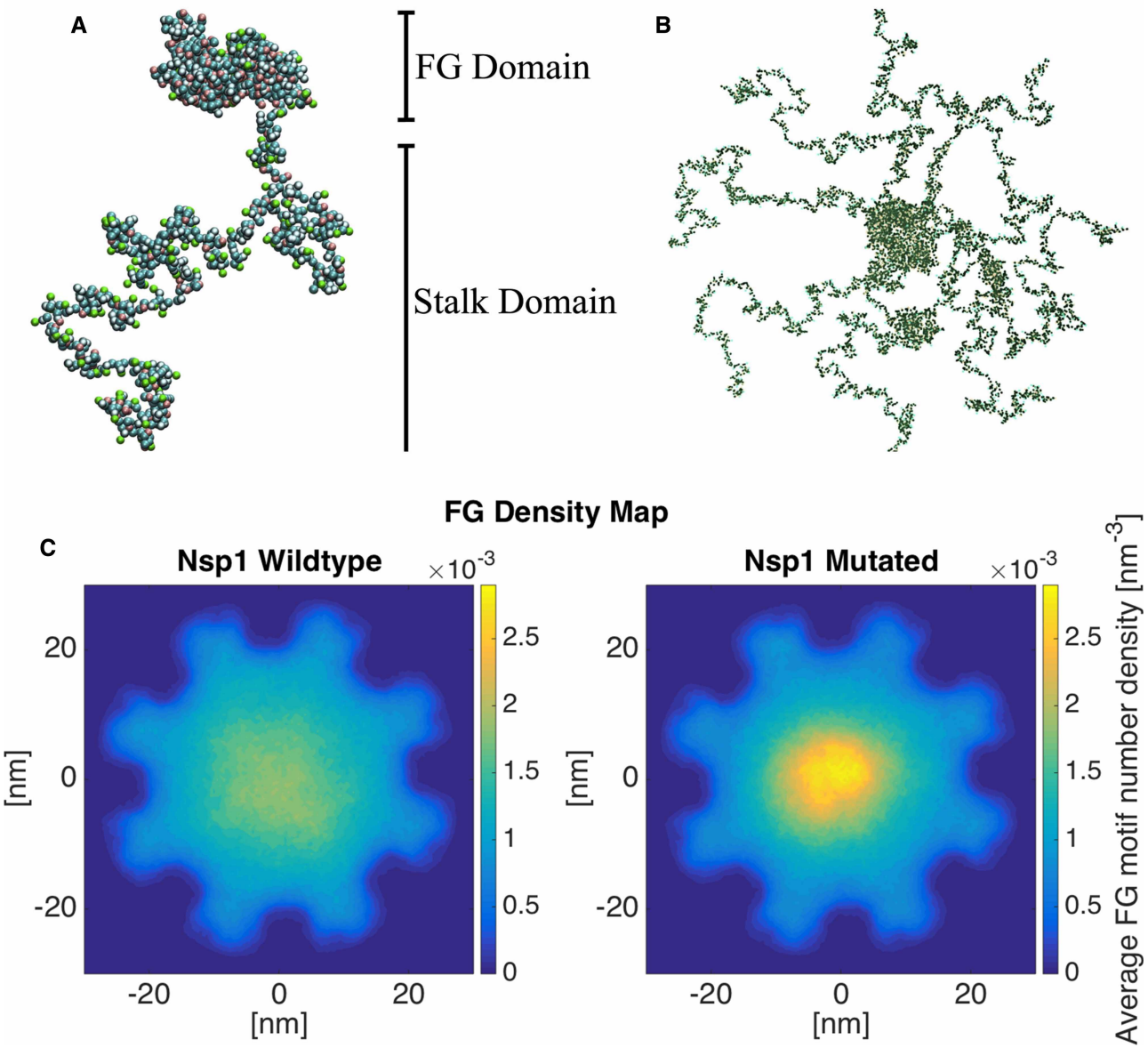

Figure 3. CG models of Nsp1.

(**A**) Typical conformation of a single Nsp1 protein predicted by simulation. (**B**) Simulation snapshot of collective conformation of Nsp1s attached to a nanopore showing biphasic behavior. Reprinted with permission from Ref. [76]. (**C**) FG density maps of wild-type and mutated Nsp1s attached to a nanopore. The mutated Nsp1s have charged residues in the like charge region mutated to neutral. Reprinted with permission from Ref. [77].

depends on the effective interaction strength between the FG-Nups, which could be modulated by Kaps in transit. The charge-poor FG domain of Nsp1 is not purely neutral but contains several charged residues of the same positive type of charge. Similar like charge regions are also found in other FG-Nups such as Nup49 and Nup57. Peyro performed CG simulations to show that these like charge regions prevent FG-Nups to over-collapse (Figure 3C), and argued that a highly condensed barrier might undermine the efficiency of cargo transport [77]. Consistent with this view, the modeling of a NPC-mimic artificial nanopore by Huang and Szleifer reported large free energy barrier for a particle to translocate through a highly condensed phase of polymer, even in the presence of optimized particle–polymer attraction [78].

Whole-NPC simulation and modeling

Understanding of individual FG-Nups each at a time might not be adequate to unveil the gating mechanism of collective FG-Nups as a functional assembly, since the interactions between the disordered proteins could lead to significant conformational rearrangement and even the emergence of high-level structural order. Although atomistic simulation of the whole NPC is not computationally feasible, it is possible to model the entire hairy pore with fair molecular details under proper coarse-graining, and a few such attempts have been made in the last decade [79–88,18]. One of the earliest models of the NPC treats the scaffold as a cylindrical pore and the FG-Nups as flexible filaments [79]. The FG–FG interactions are modeled by collision-induced bonds that subsequently dissociate according to a universal off-rate. The model predicts a Brownian ratchet picture of transport [89], in which a Kap–cargo complexes stay bound to the same FG-Nup until its release [79]. The Brownian motion of the complex in transit is guided by a RanGTP/GDP gradient. This picture is an interesting alternative to the weak multivalent binding scenario as shown in Figure 2A. However, the latter seems to be more consistent with recent experimental and simulation results [60–62]. Theoretical analysis of simple diffusion processes suggests the existence of optimal reversible Kap–FG binding for best transport efficiency [90,91]. Regarding transport selectivity, it has been argued that weak multivalent targeting allows more specific molecular recognition, compared with strong monomeric binding [92]. Nevertheless, the idea of chemical potential gradient from the original Brownian ratchet model could be still very useful in understanding NPC-mediated transport in the context of compartmentalized FG-network revealed by most recent computational works [18,88].

Among all the early whole-NPC models, the two most comprehensive ones are the MD simulation by Ghavami *et al.* [85] and the MT by Tagliazucchi *et al.* [87]. Both models consider the full AA codes of the FG-Nups and all types of molecular interactions involved in the NPC. However, it is worth noting that the two models differ greatly in their methodologies. While the MD simulation employs Lenoard–Jones potential and screened electrostatic potential to describe the two-body interactions, MT uses effective parameters to describe the interaction between monomer and its surrounding molecular fields. Despite the methodological difference, it is instructive to compare the structural predictions from the two comprehensive NPC models. The simulation showed that the native FG-Nups assume a donut-like morphology as shown in Figure 4A, which is disrupted in the case of mutated AA sequences [85]. This predicted barrier structure is consistent with SMF findings of central inert passageway and peripheral channel for Kaps [41,93], but cannot explain the central transporter observed in EM experiment [18,24,25]. A similar center-periphery density difference manifests itself in the gating structure predicted by MT [87], which is more brush-like as shown in Figure 4B. MT is a density functional theory that constructs molecular fields based on the probability distribution of explicit polymer conformations [94]. It is a well-established method for studying polymer-brush systems [95–97,78,98,88]. Both MD and MT have calculated the potential of mean forces (PMFs) of particles transporting through the nuclear pore [86,87], with the results shown in Figure 4C,D, respectively. The former shows how the PMF depends on the size of inert particles, and the latter focuses on the effects of hydrophobicity and charge. For inert particle of the same size (10 nm in diameter), MT predicts a wider and higher energy barrier (notice the units are different in the two panels), which is consistent with the brush-like barrier being more diffusive and less center-void than the donut-like one. Compared with the MD, MT does not appreciate as much the structural difference between native and shuffled AA sequences. However, it demonstrates a cooperative effect between hydrophobic and electrostatic interactions on transporting selected cargoes (Figure 4D). By analyzing the thermodynamics of the transport, MT identifies the entropic penalty as an important component of the free-energy barrier, which is consistent with the recent experimental and theoretical finding of a soft barrier that gradually intensifies with growing inert particle size [84]. Despite their inclusion of the full set of FG-Nups with AA-level resolution, both MD and MT models have only provided trivial gating structures. It is worth noting that the MT model treats all the hydrophobic motifs including FG and non-FG ones as the same. On the other hand, the

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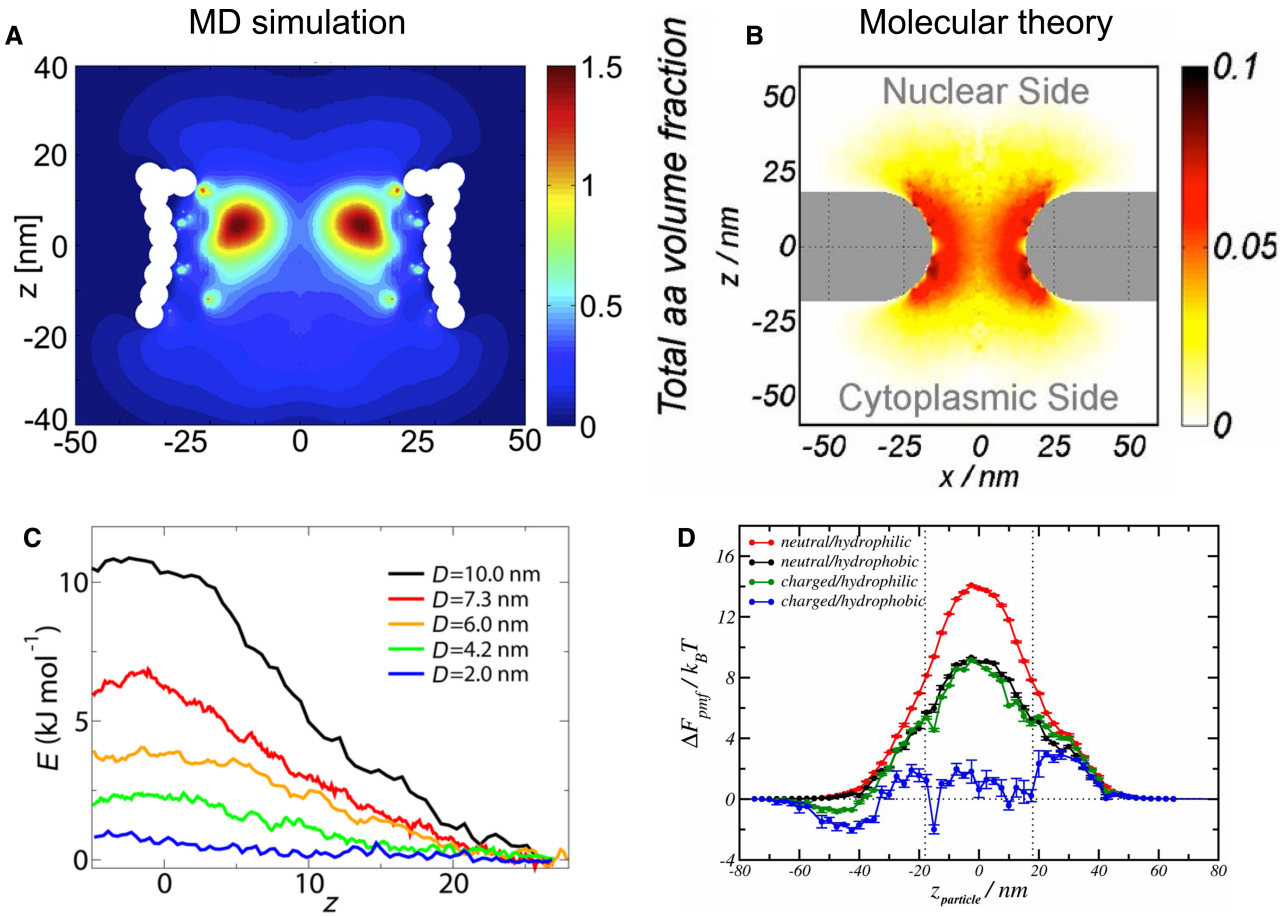

Figure 4. Simulation and molecular theory of the whole NPC.

(**A**) Density map of all FG-Nups predicted by MD simulation. Reprinted with permission from Ref. [85]. (**B**) Volume fraction of all FG-Nups predicted by MT. Reprinted with permission from Ref. [87]. (**C**) PMFs of inert particles of varying sizes along the simulated NPC axis. Reprinted with permission from Ref. [86]. (**D**) PMFs of inert particles (of the same diameter of 10 nm) with different charge and hydrophobicity, predicted by MT. Reprinted with permission from Ref. [87].

MD model has calibrated its force field according to the hydrodynamic properties of individual FG-Nups. However, it does not reproduce the intricate gating structure as speculated by the forest model [39]. Both models predict diffusive spatial distributions of the FG-Nups with more significant overlaps than assumed by the forest model [39], which are consistent with the large thermal fluctuations of the FG-Nups as observed in AFM experiments [63,64].

Towards a comprehensive picture

While each model has its own contribution to the understanding of the hairy pore, it is hard to explain the discrepancy between various theoretical predictions, because different models use different theoretical techniques, parameterizations, and levels of molecular details. Nevertheless, within each theoretical framework, advances have been made based on prior modeling insights and new experimental guidelines. For example, the stoichiometry (copy numbers) of FG-Nups that governs their grafting densities on the scaffold has been recently [18,99] determined to be significantly higher than previous [100,101] reported. Based on this new experimental finding and updated grafting positions of the FG-Nups, Brownian dynamics simulation of the whole NPC has been conducted at a CG level of representing 20 residues in one bead [18]. The new CG simulation suggests that FG motifs are territorially organized *in vivo*.

In this light, a more sophisticated MT has been developed [88] to distinguish different groups of FG motifs as color-marked in Figure 5A. To investigate possible gelation and phase separation of the FG-Nups, the new

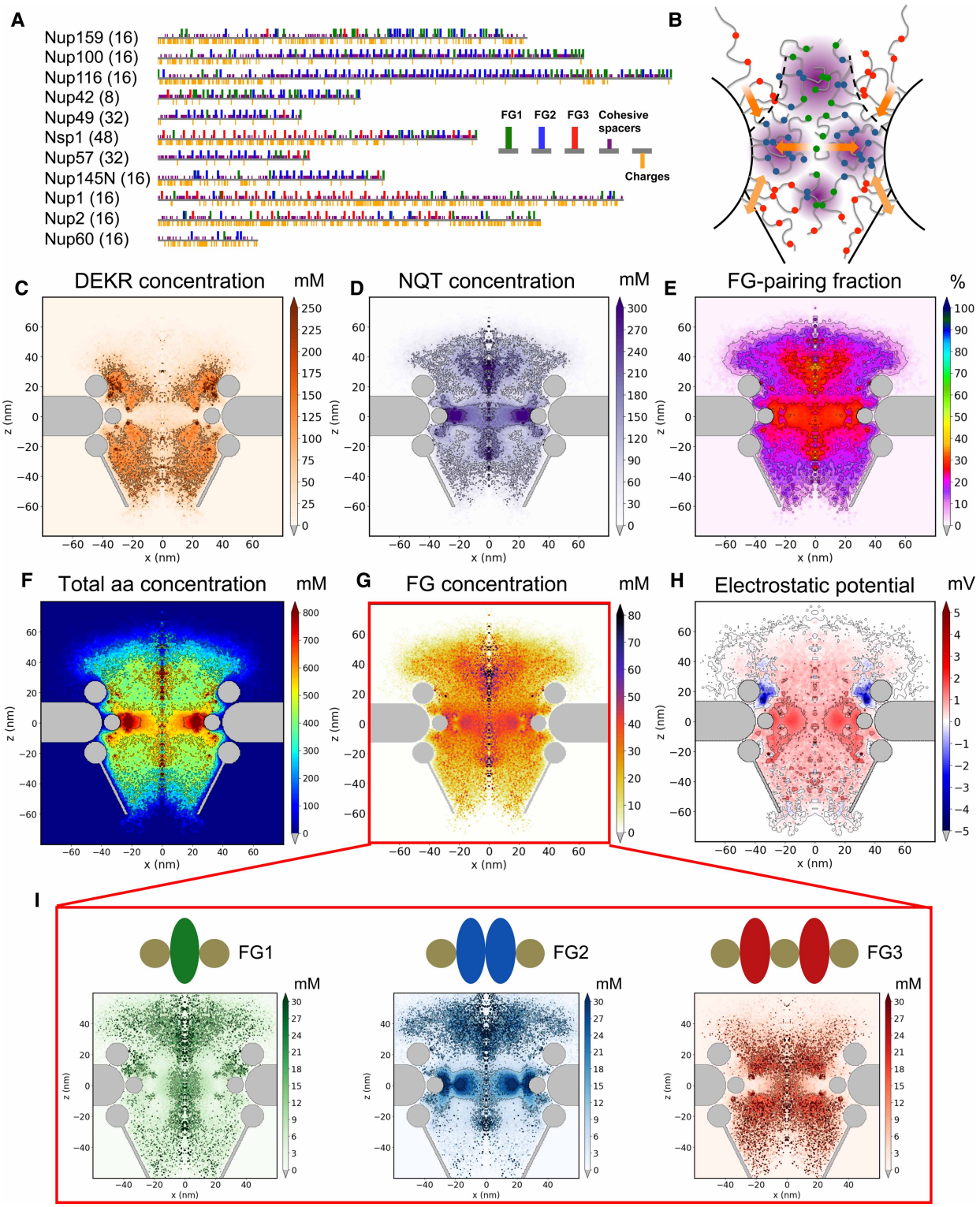

Figure 5. A comprehensive MTP model and its predictions.

(**A**) AA-codes of FG-Nups (**B**) Schematic presentation of the gating structure predicted by MTP. See the main text for more details (**C**) Density field of the charged DEKR (AA code) residues. (**D**) Density field of the cohesive NQT (AA code) residues. (**E**) FG–FG pairing fraction. (**F**) Density map of all the FG-Nups. (**G**) Density map of all FG motifs. (**H**) Self-built electrostatic potential of FG-Nups. (**I**) Territorial organizations of three different FG groups. Reprinted with permission from Ref. [88].

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MT model considers the hydrophobic pairing between FG motifs and the cohesiveness of certain spacers as suggested by recent experiments [32]. The resultant molecular theory with pairing (MTP) model provides an intricate gating structure as schematically shown in Figure 5B, which features both ring and vestibular condensates (purple domains), nano-compartments of different FG motifs (red, blue, and green dots), and electrostatic steering forces for Kaps (orange arrows). A variety of relevant molecular fields have been calculated as shown in Figure 5C–I. Among them, it merits specific attention that a nanoscale 'phase separation' between non-cohesive (Figure 5C) and cohesive spacers (Figure 5D) has profoundly shaped the overall structure of the composite barrier (Figure 5F). MTP predicts an unsaturated FG-network (Figure 5E,G) accompanied by a highly polarized electrostatic potential (Figure 5H). A closer analysis of the FG-network reveals mosaic distributions of three major types of FG motifs (Figure 5I) with single FG in the pore center, GLFG and similar motifs in the high-density ring barrier and the cytoplasmic side, and FxFG and similar motifs in the low-density areas. Together, these modeling results suggest a new gating picture in which the rapid yet selective transport is enabled by multiple routing mechanisms including entropic, electrostatic, and FG steering [88]. Integrating all these essential biophysical factors (some of which are overlooked in oversimplified models), one realizes that a soft barrier without definite shape or complex sub-channels could have everything to mediate fast and path-selective transport. The chemical heterogeneity of the barrier predicted by MTP is in line with the virtual gating hypothesis [3,4,36], but the molecular model also highlights the existence of a physical heterogeneity that is beyond the brush picture. The MTP model stresses the important roles of physical forces in rearranging the FG-Nups when they share a limited space, which are not fully appreciated in the brush picture and the forest model. By studying a reference system with the physical interactions turned off, the MTP model showed that the simple superposition of all the FG-Nups leads to degenerated gating structure of low structural complexity [88], indicating that the whole picture of the nuclear pore lumen is more than the sum of individual FG-Nups [15].

Limitations of current models

Although it is not our goal to review the technical details of different modeling methods in this review, the readers should be reminded that all the current NPC models have their own limitations. For example, mean-field approaches including the MT do not address the dynamics of the FG-Nups. The homopolymer models are able to survey a wide range of polymer-coated nanopores and surfaces to understand the CG hyper phase diagram where the FG-Nups live in, but at the price of losing the ultrastructure and chemical details of the NPC. The MT is able to explicitly consider the chemical heterogeneity of the FG-Nups and their differential physical interactions. However, to gain high computational efficiency, current molecular theories take advantage of the rotational symmetry of the nuclear pore lumen and only solve a 2D problem. A full 3D MT is yet to be built to accurately account for the 8-fold symmetry in the anchorage of the FG-Nups and to capture symmetry breaking events caused by the fluctuation of the FG-Nups and the perturbation from the Kaps. The condensation degree of the barrier would be modulated by Kaps, which is not explicitly modeled in the current molecular theories.

MD simulations are in general more expensive than the mean-field approaches, especially for modeling large assemblies of macromolecules. The accuracy of MD simulation is determined by the quality of the force field. Although standard force fields have been developed and benchmarked for predicting protein structure, these force fields are primarily parameterized for folded proteins and there is no universally accepted force field for disorder proteins. Given the size of the system, implicit water is a reasonable choice for the whole NPC simulation. However, the two-body potentials as adopted in current NPC simulations have compromised accuracy in describing the hydrophobic interaction between nonpolar amino acids. Moreover, the polarization of water and amino acids are expected to depend on the local molecular environment, which poses another challenge for simulating the NPC. Lastly, the orientation-dependent π–π interaction [102] between the FG groups is also difficult to model in a CG MD simulation.

Conclusion and outlook

In summary, theoretical modeling and computer simulations with different molecular scopes have shed lights into the mysterious nuclear pore. These works have not only expanded our knowledge of the disordered nucleoporins, but also enhanced our generic understanding of polymeric behaviors under nano-confinement. The biophysical insights are valuable for the design of bioinspired artificial nanopores and filters. While our understanding of the NPC is still incomplete, the continuous development of theoretical models starts to see a

transition from conflicting qualitative views to convergent quantitative pictures. We also witness a paradigm shift from modeling simple homogeneous systems to investigating the sequence–structure–function relation of the complex molecular machine, thanks to the reducing uncertainty of experimental data and the ever-increasing computational power.

Regarding the future investigation of the NPC, we think there are still many exciting questions to be answered and here is a brief outlook. First, the role of the Kaps as structural elements in the NPC needs to be further studied. Advances in imaging techniques have indicated that different Kaps have distinct preferences in their occupancies of the nuclear pore lumen [93]. Theoretical explanations are needed to understand the differential Kap–Nup interactions and the resultant heterogeneous Kap distributions. Second, the difference between yeast and human NPC and the evolutionary path between them remain to be explored [52]. Most current models have focused on the FG-Nups of the yeast NPC. Expanding our modeling efforts from yeast to human NPC will deepen our understanding of the sequence–structure–function relation of the FG-Nups. Third, it deserves more research to understand how post-translational modifications of the FG-Nups [103,104] affect their gating, their assembly into the NPC, and their interaction with chromatin [105–107]. Such study will allow us to better understand how the nucleocytoplasmic transport is regulated, which places the NPC in a broader and more dynamic cellular context. Understanding the hairy pore under biological regulation can also offer bioinspiration to build smart artificial nanopores [108–111] that respond to external stimuli. Biomimetic nanopores [112] can in return help us understand their biotic analog. We believe the integration between future experimental and modeling efforts holds the key to fully resolving the ultrastructure and the function of the hairy pore.

Perspectives

- *Importance of the field*: Understanding the gating structure of FG-Nups inside the nuclear pore is not only of fundamental importance in cell biology, but can also provide bioinspiration for the rational design of artificial nanodevices. Moreover, such knowledge can help us develop better materials and strategies to deliver drugs into the nucleus.

- *Current thinking*: Comprehensive theory suggests that nanoscale 'phase separation' could play an important role in compartmentalizing the nuclear pore lumen. Multiple steering mechanisms might coexist to enable efficient path-selective transport of Kap–cargo complexes that undergo weak multivalent interactions with the FG-Nups.

- *Future directions*: Although it will remain a great challenge to model the whole NPC with atomistic details, multiscale modeling can be applied to bridge the knowledge gap between the local Kap–FG interaction and the collective morphology of FG-Nups. Beyond the gating structure, the ultimate goal is to understand the function of NPC at a system biology level, such as how it interacts with chromatin and responses to environmental stress.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

AA, amino-acid; CG, coarse-grained; EM, electron microscopy; MD, molecular dynamics; MT, molecular theory; MTP, molecular theory with pairing; NPC, nuclear pore complex; PMFs, potential of mean forces; SMF, single-molecule fluorescence.

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