Correction

BIOPHYSICS AND COMPUTATIONAL BIOLOGY


The authors note that Figs. 1 and 2 appeared incorrectly. Within these figures, the “Nuclear side” and “Cytoplasmic side” labels were swapped. As a result, the legend for Fig. 1 and some text appeared incorrectly.

On page 3365, right column, first full paragraph, line 4, “cytoplasmic” should instead appear as “nuclear.”

On page 3365, right column, first full paragraph, line 5, “nuclear” should instead appear as “cytoplasmic.”

On page 3365, right column, third full paragraph, line 4, “cytoplasmic” should instead appear as “nuclear.”

On page 3366, left column, first paragraph, line 4, “cytoplasmic” should instead appear as “nuclear.”

On page 3366, left column, first full paragraph, line 5, “nuclear” should instead appear as “cytoplasmic.”

On page 3366, left column, first full paragraph, line 7, “nuclear” should instead appear as “cytoplasmic.”

The corrected figures and the corrected legend for Fig. 1 appear below. This error does not affect the conclusions of the article.

Fig. 1. (A) Geometry of the model NPC. The pore axis is defined as $z$, and the origin is set at the geometrical center of the pore, such that the cytoplasmic and nuclear bulk solutions are located at $z \to \infty$ and $z \to -\infty$, respectively. Schematic representations of the amino acid sequences of the FG-Nups for the native (28) (B) and homogeneous model (C) sequences. The FG-Nups in the homogeneous model sequence contain the same number and type of amino acids as those in the native sequence but in a regular order. The plot shows the different types of amino acids considered in the model: neutral hydrophobic (Hydroph; Ala, Ile, Leu, Phe, Trp, Tyr), Positive (Lys, Arg), Negative (Asp, Glu), Cys, and His (see Tables S1 and S2 and Fig. S1 for model and parameters). For simplicity in the graphical representation, neutral hydrophilic amino acids (Asn, Gln, Gly, Met, Pro, Ser, Thr, Val) are not shown. The figure shows the $z$-positions where the chains are anchored to the rigid protein scaffold.
Fig. 2. Molecular organization of the yeast NPC. Total amino acid (aa) volume fraction (A and D), volume fraction of hydrophobic segments (B and E), and electrostatic potential (C and F) for the native and homogeneous model sequences (Fig. 1). The plots show that homogenizing the amino acid sequence affects the electrostatic potential but not the density of amino acids or the density of hydrophobic amino acids.

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Effect of charge, hydrophobicity, and sequence of nucleoporins on the translocation of model particles through the nuclear pore complex

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The molecular structure of the yeast nuclear pore complex (NPC) and the translocation of model particles have been studied with a molecular theory that accounts for the geometry of the pore and the sequence and anchoring position of the unfolded domains of the nucleoporin proteins (the FG-Nups), which control selective transport through the pore. The theory explicitly models the electrostatic, hydrophobic, sterically conformational, and acid-base properties of the FG-Nups. The electrostatic potential within the pore, which arises from the specific charge distribution of the FG-Nups, is predicted to be negative close to pore walls and positive along the channel. The positive electrostatic potential facilitates the translocation of negatively charged particles, and the free energy barrier for translocation decreases for increasing particle hydrophobicity. These results agree with the experimental observation that transport receptors that form complexes with hydrophilic/neutral or positively charged proteins to transport through the NPC are both hydrophobic and strongly negatively charged. The molecular theory shows that the effects of electrostatic and hydrophobic interactions on the translocating potential are cooperative and nonequivalent due to the interaction-dependent reorganization of the FG-Nups in the presence of the translocating particle. The combination of electrostatic and hydrophobic interactions can give rise to complex translocation potentials displaying a combination of wells and barriers, in contrast to the simple barrier potential observed for a hydrophilic/neutrally translocating particle. This work demonstrates the importance of explicitly considering the amino acid sequence and hydrophobic, electrostatic, and sterically interactions in understanding the translocation through the NPC.

non-additivity | nuclear transport | disordered protein | coarse grain model | mean force potential

Nucleocytoplasmic transport occurs exclusively through protein pores that perforate the nuclear envelope, the nuclear pore complexes (NPCs) (1). Whereas the NPC is permeable to small molecules (e.g., water, ions) that can diffuse freely through it, bigger cargos, such as proteins and mRNA, require the assistance of transport receptors (known as karyopherins or “kaps”) to be effectively transported between the cytoplasm and the nucleus. It is challenging to understand how a cargo that is not able to pass through the pore by itself can successfully traverse the pore on forming a substantially larger kap–cargo complex. Because of its importance to the functioning of eukaryotic cells, this apparent paradox has been the focus of attention of numerous studies throughout the past decade (reviewed in refs. 1–9).

There is no universally agreed picture of the detailed mechanism of selective transport through the NPC, although there is broad agreement that a family of proteins called nucleoporins (Nups) is essential for selective transport through the pore (10–14). The folded domains of the Nups form the outer envelope of the NPC (in contact with the nuclear scaffold), and their intrinsically disordered domains protrude into the inner space of the pore.

These disordered domains, known as FG-Nups due to their high content of phenylalanine-glycine residues (FG-repeats), interact with the translocating particles to set up the permeability barrier that controls selective translocation through the NPC. A definitive transport mechanism remains elusive because directly visualizing FG-Nups and the kap–cargo complex within individual NPCs is at the limits of current single-molecule tracking technology (15–17); therefore, theory (18, 19) and computer simulations (20–22) have been used in an attempt to elucidate the essential features of the translocation process. In a recent coarse-grained molecular dynamics (MD) simulation, the kap–FG interaction was found to be highly dynamic and the FG-Nups formed a layer on the pore walls (20). The kap–cargo complex particle interacts with the FG residues in this layer as it diffuses through the channel. Another simulation study suggested that the translocating particle remains bound to the same Nup for its entire trajectory through the NPC (21). The differences between these works arise due to the choice of the molecular model, which, in neither case, considered the specific sequence and length of the FG-Nups and the particular properties of each amino acid in the sequence (e.g., hydrophobicity, charge).

Until recently, it was believed that hydrophobic interactions were solely responsible for the selectivity of the translocation process (11, 14, 20, 23, 24). According to this view, water-soluble proteins generally present a hydrophilic surface and are repelled by the hydrophobic domains of the FG-Nups, but hydrophobic patches on the surface of kapa interact attractively with the FG-Nups. It was assumed that the main role of charged amino acids in FG-repeats (about 15%) is to stabilize the hydrophobic sequences against self-aggregation and collapse. Although this assumption suggests that the sign and magnitude of the charges do not play important roles, a recent analysis has shown that kapa and kap–cargo complexes are hydrophobic and highly negatively charged, whereas the unfolded Nup domains have a small net positive charge (25), suggesting that electrostatics may be essential for the selective filtering mechanism. The effect of sequence-dependent electrostatic interactions has not been considered in previous simulations and theories; therefore, its contribution to the overall transport process remains unclear.

The goal of the present work is to address the structure of the FG-Nups within the NPC and the molecular factors that...
determine their interactions with the particle. We study the translocation of different model particles to elucidate the role of the different interactions in the system and understand their interplay. Our predictions are based on a molecular theory developed to study the structure, thermodynamics, and transport behavior of responsive polymers end-grafted to surfaces of arbitrary geometry (26, 27) that is extended here to study the translocation of large particles through a nanopore, the NPC. The theory (Methods) is based on a free energy formulation that explicitly treats the size, shape, conformations, and charge state of all the molecular species and accounts for the nontrivial coupling between molecular organization, physical interactions, and chemical equilibrium. Our model for the yeast NPC incorporates the currently available information about the size and shape of the pore, and the sequence and the charge position of each individual FG-Nup (according to the model of Alber et al. (28); for details see Tables S1 and S2). The geometry of the NPC and the native sequence of the FG-Nups are presented in Fig. 1 A and B, respectively. Our calculations show that the FG-Nups present a highly inhomogeneous charge distribution: Negative charges are concentrated on pore walls, and positive charges are located at the center of the pore. This result suggests that FG-Nup sequences are optimized to present a positive electrostatic environment along the pore axis to facilitate transport of negatively charged kap–cargo complexes. The systematic calculations presented in this work show that the interactions between hydrophobic/charged translocating particles and the NPC are qualitatively different from those of hydrophobic/neutral or hydrophilic/charged particles.

Results

Electrostatic Environment Within the NPC Is Highly Inhomogeneous. In Fig. 2 (Left), we show the calculated density profiles and the electrostatic potential within the NPC obtained in the absence of translocating particles in the pore using the information about the amino acid sequences of the FG-Nups (a summary of the properties of each FG-Nup in the system is provided in Table S3). The plots show color maps of the total amino acid volume fraction (Fig. 2A), the volume fraction of hydrophobic amino acids (Fig. 2B), and the electrostatic potential (Fig. 2C) along a vertical cut of the pore (i.e., a plane that contains the pore axis; the scheme of the pore is shown in Fig. L4). The total concentration of amino acids and the concentration of hydrophobic amino acids within the NPC are relatively constant, with the exception of the pore’s center, which shows a slightly lower density and a few spots on the pore’s surface, where there is an enhanced density. There is a very large concentration of FG-Nup segments outside of the NPC, on both the cytoplasmic and nuclear sides, due to the large volume available for the FG-Nups in the outer regions of the pore; this organization significantly reduces the excluded volume repulsions between the FG-Nups. Interestingly, the electrostatic potential within the NPC (Fig. 2C) is highly inhomogeneous and presents pockets of negative electrostatic potential close to the NPC walls, whereas the center of the pore has a positive electrostatic potential.

Positive Electrostatic Environment at the Center of the Pore Is a Direct Consequence of the Native Sequence of the FG-Nups. To examine the effect of the amino acid sequence of the FG-Nups on charge distribution, we have modified the sequences of each of the FG-Nups from the native yeast sequence to a homogeneous one, which has the same total number of amino acids of each type as the native sequences but distributed homogeneously along each FG-Nup chain (the homogeneous model sequences in Fig. 1C and Table S3 illustrate the composition of each FG-Nup). In Fig. 2 D–F, we show the results for the homogeneous case. The volume fraction distributions of all amino acids and their hydrophobic subsets are very similar to those of the native yeast sequences, but the electrostatic potential is much more uniform, and with a much lower absolute value, than in the native case. The highly inhomogeneous electrostatic potential in Fig. 2C is thus a result of the charge distribution along the FG-Nups due to their native amino acid sequence.

Electrostatic and Hydrophobic Interactions Between the Translocating Particle and Pore Are Nonadditive. Our ultimate goal is to understand how the interplay of different interactions allows the translocation of kap–cargo complexes through the pore and blocks the passage of undesired particles. For this purpose, we decided to model translocating particles with well-defined charge and hydrophobicity. It would be possible to generate a particle to model the charge, volume, and hydrophobic segment distribution of a specific protein or kap–cargo complex. However, such calculations would complicate the final goal of this work of elucidating the role of the different interactions in the translocation process. We thus decided to calculate the energetics of translocation of model spherical particles with different surface properties and a radius of 5 nm.

We studied four different particle surfaces: hydrophilic/neutral, hydrophobic/neutral, hydrophilic/charged (with −150 charges per

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**Fig. 1.** (A) Geometry of the model NPC. The pore axis is defined as z, and the origin is set at the geometrical center of the pore, such that the cytoplasmic and nuclear bulk solutions are located at z = −∞ and z = +∞, respectively. Schematic representations of the amino acid sequences of the FG-Nups for the native (28) (B) and homogeneous model (C) sequences. The FG-Nups in the homogeneous model sequence contain the same number and type of amino acids as those in the native sequence but in a regular order. The plot shows the different types of amino acids considered in the model: neutral hydrophilic (Hydrophil; Ala, Ile, Leu, Phe, Trp, Tyr), Positive (Lys, Arg), Negative (Asp, Glu), Cys, and His (see Tables S1 and S2 and Fig. S1 for model and parameters). For simplicity in the graphical representation, neutral hydrophilic amino acids (Asn, Gin, Gly, Met, Pro, Ser, Thr, Val) are not shown. The figure shows the z-positions where the chains are anchored to the rigid protein scaffold.
particle), and hydrophobic/charged (also with $-150$ charges per particle). Our predictions for model cargoes are experimentally testable, for example, by studying the translocation of noble metal nanoparticles and semiconductor quantum dots (QDs) with a well-defined surface chemistry achieved via coating with homogeneous or mixed ligand layers. They are also relevant for the biological problem because they establish the general properties that characterize a translocation-enabled macromolecular complex. The choice of particle charge is based on assuming a charge density of $\approx 0.5$ charges per square nanometer [for a nanoparticle, this corresponds to $\sim 1$ charged ligand every 10 ligands on its surface, a reasonable number as measured and predicted for gold nanoparticles (29)].

In Fig. 3, we display the potential of mean force (pmf) as a function of the distance from the center of the particle to the center of the NPC for the four types of translocating particles. The pmf is the effective potential acting on the particle at a given position, averaged over all the degrees of freedom of all the other molecules in the NPC. In other words, the pmf at a given point is the minimal work required to move the translocating particle from the bulk (i.e., very far from the pore) to that point. The pmf is measured with respect to that in the cytoplasmic and nuclear compartments (the pmf in these compartments is the same because both are 0.15 M, pH 7.2, 1:1 electrolyte solutions). A particle experiencing a potential barrier several times the thermal energy will be unable to pass through the pore, whereas a particle experiencing a flat potential will translocate through the pore at a rate given by the kinetics of chain rearrangement and/or particle diffusion (modeling the kinetics is beyond the scope of this work).

In Fig. 3, we show that the hydrophilic/neutral translocating particle feels a repulsive (positive pmf) interaction that starts at around 15 nm away from the NPC (the positions of the entrances to the NPC are shown by dashed lines) on the cytoplasmic side and decays on the nuclear side at around 20 nm. The interactions away from the NPC reflect the contribution of FG-Nup conformations that extend away from the pore, as observed in Fig. 2A. The repulsive interactions (the only ones relevant for the hydrophilic/neutral particle) arise from two contributions: the osmotic pressure within the pore and the reduction in the number of allowed conformations of the FG-Nups due to the presence of the particle. Henceforth, we will refer to their combination as steric repulsion.

The hydrophobic/neutral nanoparticle (black) curve in Fig. 3 has a shape that is qualitatively very similar to the hydrophilic/neutral curve, with the main difference being the magnitude of the interactions. The weaker repulsion between the NPC and the hydrophobic nanoparticle results from the attractions between the hydrophobic domains of the FG-Nups and the translocating particle. Note, however, that for the strength of hydrophobic interactions used in this calculation, the hydrophobic forces cannot overcome the steric repulsions from the FG-Nups (the effect of the strength of hydrophobic interactions on the pmf is analyzed in Fig. S2).

The green curve in Fig. 3 shows the pmf acting on the hydrophilic/charged model particle. The curve looks very similar to the hydrophilic/neutral pmf, with one important qualitative difference, namely, that on the cytoplasmic side, we observe the presence of a local minimum. This feature arises from the electrostatic interactions, because there is no observed local minimum for the hydrophobic/neutral and hydrophilic/neutral particles. Interestingly, the attraction first appears when the particle is about 45 nm away from the center of the NPC. Inspection of the electrostatic potential distribution in the NPC in the absence of the nanoparticle (Fig. 2C) shows that the electrostatic potential is almost zero at these distances. Therefore, the attractions arise from the conformational reorganization of the FG-Nups induced by the presence of the negatively charged particle, which attracts the positively...
charged FG-Nups at distances from the pore entrance that far exceed the electrostatic screening (about 1 nm for the salt concentration used in this work). In fact, in Fig. S3, we show that inserting a particle on the cytoplasmic side, at z = -45 nm, affects the FG-Nup distribution in a large region between -10 nm < z < -60 nm. Once the hydrophilic/charged translocating particle reaches the region where a relatively high density of the FG-Nups is present (Fig. 2), the pmf becomes repulsive due to the fact that the electrostatic attractions are weaker than the steric repulsions. The quantitative similarity between the black and green curves in Fig. 3 is coincidental, due to the choice of parameters.

A qualitatively different behavior from the other three cases is predicted for the hydrophobic/charged translocating particle (blue curve in Fig. 3). In this case, we see a markedly attractive potential, over 20 nm on the cytoplasmic side, followed by a relatively constant pmf within the NPC, with the exception of the narrow well at around -20 nm and, finally, a repulsive barrier at the exit of the NPC on the nuclear side. An analysis of the different contributions to the pmf (Fig. S4) shows that the narrow well has an electrostatic origin, whereas the repulsive barrier arises from steric and hydrophobic interactions. The effective interaction between the FG-Nups in the NPC and the hydrophobic/charged particle cannot be determined simply from the pmfs of the hydrophilic/charged and hydrophobic/neutral particle (the pmf is nonadditive). For instance, the height of the barrier (maximum of the pmf curve) of the hydrophilic/natural case is lowered by 5.0 k_BT (where k_BT is the thermal energy, 1 k_BT = 2.5 kJ/mol for T = 300 K) by going to either the hydrophilic/natural case or the hydrophilic/charged case. However, making the cargo both hydrophobic and charged lowers the barrier by 12 k_BT, which is higher than the sum of the effects of the individual interactions (10 k_BT). More importantly, the shape of the pmf acting on the hydrophobic/charged particle is markedly different from that for the hydrophilic/natural and hydrophilic/charged cases. There is therefore a synergetic effect that arises from the reorganization of the FG-Nups in the pore due to the presence of the translocating particle that depends on the surface properties of the particle. In SI Text, we show systematic calculations of the pmf as a function of hydrophobicity and charge of the translocating particle (Fig. S2). As expected, hydrophobicity and charge have different effects on the pmf. Therefore, particles presenting different surfaces may experience qualitatively different energy landscapes during the translocation process.

An important conclusion from the pmfs in Fig. 3 for different model particles is that the effective interactions in all these cases cannot be deduced from the knowledge of the spatial organization of the FG-Nups in the NPC in the absence of the translocating objects. In Fig. S3, we show that both the distribution of amino acids and the electrostatic potential within the NPC change on introducing the translocating particle. Another important observation is that the predicted interactions are consistent with experimental observations. Thus, transport receptors (kaps) are negatively charged and hydrophobic (due to hydrophobic pockets on the surface of the kaps) (22), a combination that maximizes the attractive interaction with the pore. Furthermore, a recent study of single-QD tracking in the NPC (17) has found that the kap-capped QDs are attracted to the pore entrance, face a potential barrier on the nuclear side of the channel, and show a Gaussian-like probability distribution (characteristic of a free energy minimum) inside the pore. Inspection of Fig. 3 shows that our results for the pmf acting on the hydrophobic/charged nanoparticle are consistent with these experimental observations. We want to emphasize that a quantitative comparison with the experimental observations has not been attempted in this work, because we did not intend to model the precise charge/hydrophobicity properties of the experimental nanoparticles (e.g., the QDs in the experimental study were modified by the importin-β transport receptor).

Native Sequences of the FG-Nups Are Optimized to Facilitate Transport of Negatively Charged Particles Through the Pore. To show the further importance of the native amino acid sequence, we have calculated the pmfs for FG-Nups with a homogeneous model amino acid sequence (corresponding to Figs. 1C and 2 D–F). In Fig. 4 A and B, we display the pmfs for the hydrophilic/neutral and hydrophobic/neutral model particles for both the native and homogeneous model sequences. The pmfs are essentially identical in both cases. This is not surprising, because these potentials are governed by the steric repulsions and hydrophobic interactions that arise from the distribution of all the amino acids and the hydrophobic ones, respectively; these two distributions are very similar for both types of sequences (Fig. 2 A and B vs. Fig. 2 D and E). The hydrophilic/charged nanoparticles (Fig. 4 C) show important differences in their interactions with the yeast NPC compared with the homogeneous case. For the homogeneous amino acid sequences, the hydrophilic/charged nanoparticles feel a purely repulsive interaction. However, for the native sequence, there is a weak attractive well and the overall potential inside the NPC is lower than that of the homogeneous case. It is clear that these differences arise from the distribution of charged amino acids within the NPC (Fig. 2 C vs. Fig. 2 F). This difference results in a very dramatic change of the effective interaction potential for the case of hydrophobic/charged translocating particles (Fig. 4 D); whereas the homogeneous case presents a purely repulsive pmf, the native NPC shows a complex energy landscape that includes three different regions of the interaction potential as discussed in detail above.

An important conclusion of our work is that transport may be facilitated for negatively charged kap–cargo complexes due not to the overall excess of positive amino acids in the FG-Nups as previously believed (25) (in fact, some FG-Nups have a net negative charge; Table S3) but to the specific charge distribution of the FG-Nups that localizes positive amino acids along the pore axis. It is therefore not surprising that the pmf of the hydrophobic/charged model particles, which bear the closest resemblance to transport receptors and transport receptor–cargo complexes, exhibits the largest contrast between the native and homogeneous model sequences.

**Comparison with Translocation Models.** The most discussed qualitative models for NPC gating are the virtual gate (VG) model (10, 12), the brush model (30), the selective phase (SP) model (11, 23), and the reduction of dimensionality (ROD) model (6, 13). These models differ in the qualitative picture of the distribution of the FG-Nups inside the pore and the mechanism of

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**Fig. 4.** Effect of the amino acid sequence on the pmf for different translocating model particles for the NPC bearing the native amino acid sequences (red curves, sequences in Fig. 1B) and the homogenized amino acid sequences (black curves, sequences in Fig. 1C). The vertical dashed lines indicate the position of the entrances to the NPC in the nuclear and cytoplasmic sides. The horizontal dashed line represents zero interaction.
translocation (5, 31). Note that these models provide only a qualitative description of the translocation process, whereas our theory provides quantitative predictions. In the VG model, the FG-Nups block the pore (possibly leaving a narrow channel along the center that allows unhindered translocation of small proteins) and impose an entropic penalty to the passage of large molecules. Interactions between the FG residues and the surface of transport receptors decrease the height of the barrier and allow translocation. The brush model, a variation of the VG model, proposes that the entropic filter is formed by stretched brush-like FG-Nup chains. The FG-Nups in the SP model block the passage through the pore by forming a gel-like structure cross-linked by FG–FG interactions. The main characteristic of the SP model is a gel-like structure, where the mobility of the chains is highly reduced due to transient FG–FG interactions. The transport receptor–cargo complex penetrates the gel by dissociating the cross-links. The ROD model proposes that the FG-Nups form a collapsed layer on pore walls and a channel is formed in the center of the pore. The kap–cargo complexes can slide through the pore by interacting with the FG domains in the collapsed layer (the transport rate is increased by the 1D character of the process).

Our calculations predict that the FG-Nups are close to homogeneously distributed within the pore, in qualitative agreement with both the VG and SP models but not with the ROD model, which postulates a protein-free channel along the pore axis. Note, however, that the ROD model assumes the presence of Kaps within the pore [consistent with experimental findings for the NPC in neuroblastoma cells (32)] that collapse the FG-Nups, as observed in synthetic planar brushes of cys-nup62 (33). In our model, the NPC is free of Kaps and a direct comparison with the ROD model is not possible (to the best of our knowledge, the average end-to-end distance of the amino acids is not affected (i.e., no collapse to the walls is observed), even when the strength of the hydrophobic interactions in our system is dramatically increased (Fig. S5).

As predicted by our theory, the inner structure of the pore also differs quantitatively from a fully extended brush (proposed in the brush model) because the average end-to-end distance of the grafted chains in our calculation is 6.7-fold smaller than their maximum contour length (Table S4). A further differentiation between the SP and VG models is outside the scope of the theory because the theory makes no predictions about the mobility of the chains. Therefore, we cannot distinguish between the jammed gel-like or fluid brush-like structures proposed by the PS and VG models, respectively. The close to homogeneous amino acid distribution found in our work also agrees with the extended bundles of FG-Nups observed in MD simulations by Miao and Schulten (22, 34). We attribute this discrepancy to the following:

i) Difference in the modeled systems: The MD simulations are for a planar surface grafted with 100-aa-long FG-Nups, whereas we model the sequences of the FG-Nups determined for yeast grafted inside a pore that mimics the experimental size and shape of the yeast NPC. We have shown in previous work (27) that increasing chain length and decreasing the radius of curvature of the pore may disfavor the formation of chain aggregates in comparison to the homogeneous system.

ii) MD simulations in the study by Miao and Schulten (22) are initialized in a fully stretched configuration of the 100-aa model FG-Nups and run for a few microseconds. As those authors point out, the observed morphology may correspond to a kinetically frozen structure instead of an equilibrium structure.

iii) Our theory neglects inhomogeneities in the angular coordinate, and therefore cannot predict the formation of bundles of chains along this coordinate.

The pmf of a model cargo translocating through the pore predicted by our theory is a free energy criterion that determines what types of objects can be transported. The results of our model agree with previous experimental evidence that suggests (as considered by the VP and SP models) that hydrophobic interactions decrease the translocation energy barrier for kap–cargo complexes. We propose here that the charge distribution along the FG-Nups helps to lower this barrier for negatively charged cargos. Rout et al. (12) have pointed out that virtual gating can be improved by a nonsymmetrical distribution of FG-domains within the pore. We predict here that the nonsymmetrical distribution of hydrophobic and charged domains and the nonadditivity of interactions give rise to complex translocation potentials, which may help to explain the complex translocation behaviors observed in single-molecule experiments (17).

Conclusions

In conclusion, we have presented a theoretical study of the structure and translocation of model particles in the yeast NPC system. In this set of calculations, the pore is treated with all the details of the number, sequence, anchoring position, and length of the intrinsic disordered FG-Nups available from experimental observations on yeast. Even so, our model of the FG-Nups is coarsen in the sense that it makes no distinction between the hydrophobicities of the different amino acids, does not explicitly incorporate hydrogen bonding, and does not include specific interchain binding [e.g., as observed in the formation of gels with Asn-rich FG sequences (35)]. We have also omitted specific binding interactions between the model particles and the FG domains in the FG-Nups; these interactions have been proposed to play a role in the kap-mediated translocation mechanism (11, 21, 23, 36, 37). The association between the kaps and the FG domains is weak (36, 38) and dynamical (22); therefore, the nature of this interaction is probably between high-affinity ligand–receptor binding and the hydrophobic interaction modeled here. Our work suggests that generic hydrophobicity and negative charge are necessary for translocation of both homogeneous and patched nanoparticles (Fig. S6, in which we show the pmfs for several charge distributions on the surface of the particle). Therefore, it raises the question as to whether highly specific ligand–receptor interactions are needed for successful crossing of the NPC.

Our most important findings are as follows. First, the charge distribution along the FG-Nups (originating from their native sequence) and the tethering position of the FG-Nups create a positively charged environment along the pore axis. This environment could facilitate the passage of the negatively charged kap–cargo complexes. Second, the different interactions in this system are highly nonadditive, and their combination can give rise to complex translocation potentials depending on the properties of the translocating object. This nonadditive behavior results from the reorganization of the flexible FG-Nups in the pore due to the presence of the particle. Moreover, nonadditivity implies that understanding how hydrophobic/neutral and hydrophilic/charged particles interact with the NPC is not enough to conclude how a hydrophobic/charged particle interacts with it. The optimization of the structure is the result of the minimization of the free energy for the overall system and is not reproduced by considering individual contributions only. This is a common theme in soft-matter systems that can change their molecular organization on interaction with the environment, which is also found in many other subcellular systems (e.g., cell membranes).

The mechanism of transport of large molecules through the NPC is complex because it depends on the change in molecular organization of the FG-Nups in the presence of the translocating particle. Our methodology provides a way to study the details of the interactions and the energy landscape within the pore. Future work will focus on the transport of specific protein complexes through the yeast NPC and on transport of kaps through synthetic pores coated with specific molecular modifiers.
Methods

We model the NPC as a rigid hourglass pore with the dimensions experimentally determined for yeast (28, 39). The disordered proteins, FG-Nups, are tethered to the inner surface of the pore based on the structural model in the study by Alber et al. (28) (additional information is provided in Fig. 1 and SI Text). The proteins are modeled with a coarse-grained model that represents each FG-Nup as a freely jointed chain in which each bead models 1 amino acid. Our model accounts for the sequence of each FG-Nup, the anchoring positions of the FG-Nups, and the conformational statistics and appropriate excluded volume of the disordered proteins. The 20 amino acids are divided into six groups: hydrophobic, hydrophilic, positive, negative, cations, anions, and histidine. The last two are considered separately due to their pKa, and the possibility of charge regulation.

To model the NPC, we use a molecular theory that explicitly considers the shape, size, conformation, charge and charge distribution, and intermolecular and intramolecular interactions of all the molecular species in the system. The theory is formulated by writing down system free energy. In general terms, we write (26, 40, 41):

$$ F = -\Delta G_{\text{mix}} = -\sum_{i}^{n} G_{i} - \sum_{i<j}^{n} E_{ij}$$

$$+ \sum_{i}^{n} \left( \left( -\frac{1}{2} \sum_{j \neq i}^{n} \frac{1}{r_{ij}} - \frac{1}{2} \sum_{j \neq i}^{n} \frac{1}{r_{ij}} \right) + \sum_{j \neq i}^{n} \frac{1}{r_{ij}} \right)$$

$$\text{where the term } S^{\text{mix}} \text{ represents the mixing entropy of water molecules, ions,}
\text{anions, cations, and hydroxyl ions (i.e., } \alpha_{w}, \alpha_{c}, \alpha_{H}, \text{ and } \alpha_{OH}^{m}, \text{ respectively). } S^{\text{conf}} \text{ is the conformational entropy of the FG-Nup; } E^{\text{mix}} \text{ is the energy of the van der Waals (vdW) attractions between amino acids (the repulsive interactions are modeled in our theory as a packing constraint, as discussed in SI Text); } E_{\text{elec}} \text{ is the electrostatic energy; } E_{\text{vdW}} \text{ is the free energy associated with the acid-base chemical reactions of the amino acids; and } E_{\text{hydrophobic}} \text{ is the energy of the vdW interactions between the amino acids and the translocating particle. Each of these terms is a function of the distributions of the different molecular species, the probabilities of the different FG-Nup conformations, and the fraction of charged and uncharged amino acids. We find the extremum of } F \text{ with respect to these functions to determine the equilibrium structure of the system. In SI Text, we present a detailed description of the free energy expression, the molecular model, the minimization procedure, and the numerical calculation. For the pmf calculation, we determine the free energy of the system in the presence of the translocating object and subtract from this value the free energy of the system, where the particle is in the bulk solution (far away from the pore).}

Note Added in Proof. While this paper was under review, Serdiuk et al. (42) showed that negatively charged sub-3-nm nanoparticles (which lack specific transport receptors on their surface) can translocate through the NPC and localize in the cell nucleus, whereas positively charged nanoparticles of the same size stay in the cytoplasm; these observations are consistent with our predictions.

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