Mechanisms of diffusion in nuclear pore complex mimics

by

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Mechanisms of diffusion in nuclear pore complex mimics

Thesis directed by Assistant Professor Loren Hough

Placeholder text for abstract.

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Chapter 1

Introduction

1.1 The nuclear pore complex is a unique filter

How many pores are there in a typical yeast cell? Human cell?

1.1.1 Structure

The nuclear pore is a very large complex, about 120 MDa total. It has eightfold symmetry. It consists of a ring of ordered proteins and disordered proteins filling the center of the ring. The disordered proteins are called FG Nups. Some of the ring proteins are also called Nups, but not FG nups. There's an inner and an outer ring that I need to learn more about. There's a structure called the nuclear basket on the nuclear side and the cytoplasmic filaments on the cytoplasm side. Dimensions vary but are about 50 nm in diameter and maybe 50 nm high for the rings but I'm not sure about that. There is disagreement about how far the Nups extend out of the pore, so the total height of the complex might be anything between 50 and 200 nm. There are some recent cryo EM studies of the pore that are probably going to be very useful here (Rout, I think, and at least one other group.)

1.1.2 Transport factors

Transport factors (TFs) are ordered proteins that carry cargo through the NPC. While there are various types, they share several features in common, most notably the fact that all known transport factors have more than one hydrophobic binding pocket which binds to FG repeats. In fact, many TFs have several binding pockets. Likewise, the binding affinity between TFs and FG Nups remains unknown for most TFs. Estimates of dissociation constant K_D vary from nanomolar to millimolar, depending on the environment (cellular, buffer, etc.) in which the measurement is made [**?**]. There are many types of TF, of which some of the most important are the importins and exportins (karyopherins), NTF2, and mRNA exporters.

The karyopherins (Kaps) are the most-studied family of TFs. They are also known (in human cells?) as importins and exportins. The twenty or so different Kaps are responsible for most nucleocytoplasmic transport [1]. Kaps typically consist of multiple HEAT repeats, a helical motif which conveys structural flexibility [2]. Most Kaps bind their cargo directly via a nuclear localization signal (NLS, for nuclear import) or nuclear export signal (NES, for nuclear export). NLS and NES are 5-7 amino acid tags found on cargo []. However, Kap95? (importin β) uses the adaptor protein Kap60? (importin α) to bind its cargo. In general, Kaps are on the order of 100 kDa in size, well above the passive permeability limit []. Kaps may contribute to the selectivity barrier.

Unlike the karyopherins, nuclear transport factor 2 (NTF2) does not transport a wide variety of cargo across the NPC. Instead, NFT2 is focused on maintaining the Ran gradient needed for transport. It transports

I need to decide whether to call them transport factors (TFs) or nuclear transport receptors (NTRs) throughout my paper. We exclusively call them TFs in my lab and a few other labs but I think NTR is more common in the field as a whole. For now I will call them TFs. TFs carry cargo in and out of the nucleus, through the NPC. All known TFs have at least two sites that bind to the FG repeats on the Nups, and many have more than that (up to 10 or so, it's unclear). The

importins (called karyopherins in yeast, and I'm not sure whether exportins are also karyopherins) are a major set of transport factors. They are 95 kDa or larger and do most of the carrying of proteins (I think). They are formed from many HEAT repeats, which are flexible helical structures, and have many binding sites. I didn't study these. I studied NTF2, which is a much smaller protein. It's a homodimer whose components are each 14 kDa, so it's a 28 kDa dimer. Each dimer has one FG binding site. NTF2 carries Ran through the nuclear pore. I need a section about the Ran cycle, which means I actually need to understand the Ran cycle. NTF2 is near the cutoff for passively crossing through the pore, but it still travels through at least 30 times faster than similarly-sized inert proteins. I've also worked with domains of Mex67, which I think is an mRNA exporter. I need to learn more about mRNA export, as well as transport of large cargo with multiple TFs bound at once. TFs bind to cargo since cargo has a nuclear localization signal (for entering the nucleus, NLS) or a nuclear export signal (for leaving the nucleus, NES), which is a short peptide tag that binds to transport factors.

1.1.3 FG nucleoporins

The central channel of the pore is filled with disordered FG nucleoporins (FG Nups). Disordered proteins have no secondary structure. FG Nups typically consist of an ordered domain that anchors them to the wall of the channel, and a totally disordered domain that sticks out into the channel. The disordered domain has several to tens of phenylalanine-glycine binding motifs which bind to TFs. There are several binding motifs, which all incorporate FGs; for instance, FSFG, GLFG, etc (from that figure I always use in my slides). There are about a hundred FG nups total (number of individual proteins) in the pore, and maybe ten or so different types of Nups. Since each FG Nup has many binding sites, there are about 1000 FG binding motifs in each pore. The Nups are hard to visualize since they are disordered. Averaging techniques tend to smear them out into nothing. Early research suggested that they formed a central plug or "transporter", but more recent work suggests that there is no central structure, just disordered proteins (the AFM study from Lim or Lemke group).

1.1.4 Ran cycle

I don't know nearly as much about the Ran cycle as I should. I always forget which way it works. Basically, the chemical energy needed for selective transport comes from a gradient in RanGTP/GDP. Ran can carry either GTP or GDP. One form helps the cargo unbind from the TF once it has transited the pore, then the state needs to change (GTP/GDP) so that Ran unbinds from the TF. NTF2 doesn't work this way because it's job is to carry Ran through the pore to maintain the gradient. The proteins that cause Ran to change phosphorylation state are called RanGAP and RanGEF. All of this means that the selective transport itself doesn't use external energy sources; it is driven by a concentration gradient set up by the Ran cycle.

1.2 IDPs are important

I'm not sure whether this should be a section or subsection or where exactly it should go. I want to give some broader context for intrinsically disordered proteins (IDPs) and their cellular functions. IDPs play a role in phase separation. Intrinsically disordered regions of proteins are also very important. About 30% of eukaryotic proteins are disordered or contain significant disordered regions. Selectivity through weak, multivalent binding is a hallmark of disordered protein function. IDPs can form "fuzzy" complexes or act as hubs, since they can often bind to multiple binding partners. Some become ordered upon binding and some do not. I don't know exactly what other cellular functions IDPs are involved in - signaling pathways, I think.

1.3 Experimental observations

Should I have a separate section for computational studies, or include them in experimenal work?

1.3.1 Passive permeability barrier

There is a cutoff around 30 kDa where molecules stop being able to passively transit the pore at any appreciable rate. Work from the Timney lab suggests that the passive permeability barrier is not a sharp cutoff, but broad.

1.3.2 Single-molecule studies (Kinetics of transport?)

Lots of people have done single-molecule studies of the NPC. They need to use superresolution microscopy and can't really see more than the length of time that it takes to passage the pore. They measure things like dwell time in the pore and the proportion of successful transit attempts. Some of these studies lead to estimations of the transport kinetics, i.e. on and off rates for Nup and transport factor interactions. Many of these come from SPR studies or stopped-flow anisotropy measurements. The SPR measurements also lead to estimations of layer height compaction or extension when various TFs are flowed across a grafted monolayer of FG Nups. These experiments can often result in contradictory data. There are some good review articles for single-molecule studies, and also some of the techniques I've described here aren't single molecule.

1.3.3 Effect of transport factors

The presence of transport factors may make the selectivity barrier more robust. SPR measurements as discussed above give estimates of layer compaction or extension, and populations of tight- or weak-binding TFs. Other studies (?) suggest that crowding with TFs might help reduce non-specific interactions and increase the selectivity of transport.

1.3.4 Permeability barrier/flux studies

People have measured the flux through the pore in vitro and in vivo in various ways. One group developed OSTR, where they seal an NPC to the pore of a membrane and measure fluorescence flux of TFs through that pore. The Gorlich group (Ribbeck) have permeabilized cells and injected fluorescent transport factors and watched how long it took them to localize to the

nucleus. These measurements show selectivities of 20-150 (ish) fold flux of TFs as compared to inert proteins.

1.3.5 Nup deletion, etc.

Some people have deleted subsets of Nups to see which are necessary for transport. I don't know much about these studies. None of the asymmetric Nups are necessary. I think that about half of the mass of Nups can be deleted without significant consquences to transport, showing how robust the NPC is.

1.4 Theoretical models of the NPC

Many theoretical models exist, both qualitative and quantitative.

1.4.1 Entropic barrier model

The entropic barrier model postulates that Nups remain disordered within the pore (should talk about experimental evidence for and against). In this model, inert proteins are kept out of the pore through an entropic barrier, because their entry into the pore would restrict the possible conformations of the disordered Nups. TFs can get into the pore, in contrast, because the binding energy offsets the entropic penalty. This is one of the two main qualitative models of the pore, the other being the hydrogel or selective phase model. The Rout lab supports this model. I think most labs other than the Gorlich lab are beginning to support this model, but I'm not sure.

1.4.2 Hydrogel model

The hydrogel model is the other main qualitative NPC model. The Gorlich group supports this model. This model postulates that the Nups interact via their FG motifs (discuss evidence in previous section, maybe?) and form a hydrogel. A dynamic hydrogel. Inert proteins are kept from passing through because they can't get through the gel mesh, but transport factors disrupt the crosslinks by binding to FGs and "melt" through.

1.4.3 Forest/intermediate models

This model proposes that a mix of the entropic barrier model and selective phase model are at play.

1.4.4 Effect of crowding

Some people (Zilman paper, etc) have modeled the effect of crowding on the selectivity barrier. A lot of overlap with effect of transport factors.

1.4.5 Effect of transport factors

Same as previous section sort of. Kap-centric models of the NPC (short for karyopherin-centric, for the class of TFs called karyopherins) propose that a permanent population of Kaps lives within the NPC. This strengthens the selectivity barrier.

1.4.6 Energy landscape models

Most quantitative models of the nuclear pore are energy landscape models. These are typically computational studies that require a fair amount of detail and assumptions about the pore. They incorporate effects of charge, hydrophobicity, specific binding interactions, etc. Generally the result is a picture of the free energy landscape encountered by TFs and by inert proteins as the travel along the axis of the pore.

1.5 Synthetic NPCs

Many different groups have attempted to make synthetic nuclear pore complexes, but they are generally not very successful.

1.5.1 Gold nanopores (Rout group)

One of Loren's colleagues in New York grafted FG Nups onto a gold-coated nanopore and monitored flux through the pore. She saw low (less than 10-fold) selectivity. I'm not sure whether

other nanopore-based approaches have been tried.

1.5.2 Hydrogels (Gorlich)

The Gorlich group keeps making hydrogels out of Nups and testing the entry of various proteins. They take Nsp1 or fragments of it or other Nups that spontaneously form hydrogels in buffer, and let them form a gel. Then they introduce fluorescently-tagged TFs and inert proteins and monitor the progression of the fluorescent front into the gel. They see very high (100 or more) partition coefficients, indicating that the TFs really bind very strongly to the gel. They do not see rapid exit from the gel as would be required for rapid transport.

1.5.3 Other models (peptide hydrogels, DNA origami, etc)

There are a grab-bag of other models that I need to learn more about. Some groups have done similar things to what we want to do, making hydrogels out of non-aggregating Nup peptides and a pentameric crosslinking domain. There was a group that made an NPC ring out of DNA origami and attached Nups at particular points, though I'm not sure how that can be used to test selectivity because I can't see how to anchor the rings into a membrane.

1.6 Conclusions of introduction?

NPCs are interesting and important.

Chapter 2

Modeling

Selective filters made of biopolymers are used in living and synthetic systems to control the localization and movement of molecules, nanoparticles, viruses and other organisms [3]. These filters regulate access to genetic material (the nuclear pore complex, or NPC), cells (the pericellular matrix), tissues (the extracellular matrix), and organs (mucus). In addition to their protective role, polymeric biomaterials are physical barriers that can inhibit drug delivery. Microbial biofilms can sequester antibiotics in their pericellular matrix, hindering treatment of infections [**?**]. The extracellular matrix and mucosal layers limit drug delivery in cancer and other diseases [3]. How particle binding affects motion and filtering is unclear. Binding to the pericellular matrix facilitates uptake of nanoparticles by single cells [**?**], and transport factors that bind to proteins in the NPC move rapidly through it [**?**]. In contrast, binding inhibits the uptake of nanoparticles that bind to airway mucus [**?**, **?**, **?**]. Many viruses minimize binding interactions, allowing human papillomavirus and human immunodifficiency virus to move nearly unrestricted in cervical mucus under certain conditions [**?**, **?**], although antibody binding can slow these interactions [**?**]. Improved design of drug delivery vehicles and synthetic selective filters requires understanding what distinguishes these behaviors. While particle size, charge, and binding interactions are known to affect filtering [3], the physical principles that underlie mobility and transport in polymeric biomaterials are not fully understood.

Among these filters, the NPC is tuned for selective passage enabled by binding. The NPC selectively filters molecular traffic between the nucleus and cytoplasm of eukaryotic cells, making

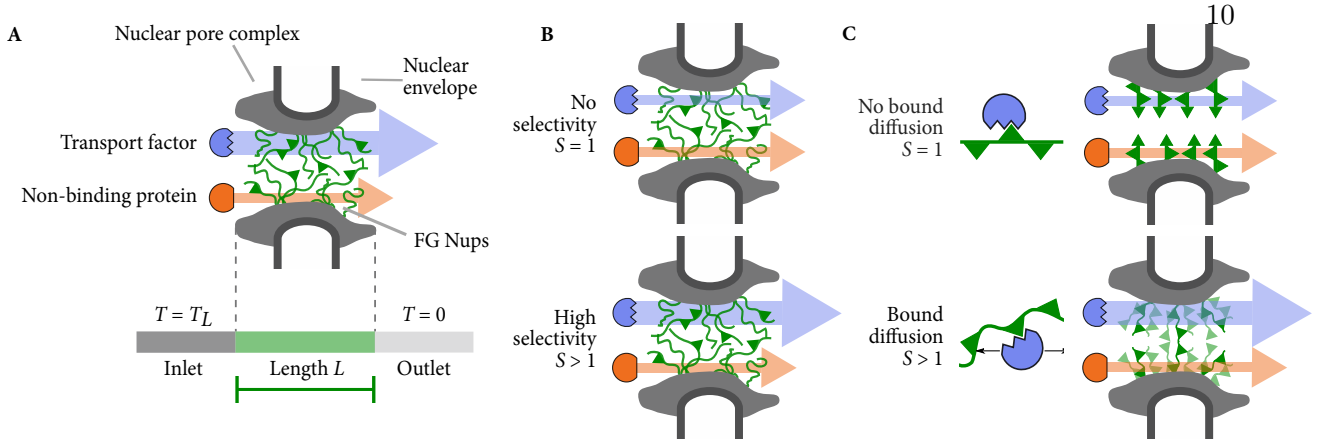

Figure 2.1: Schematics of the nuclear-pore complex and model. (A) The nuclear pore complex (gray) is filled with FG Nups (green polymers) that selectively passage transport factors that bind to FG Nups (blue) while blocking non-binding proteins (red). The central channel of the pore has length L. Protein concentration is high on the left (inlet) and low on the right (outlet). (B) Selectivity quantifies the degree of selective transport through the pore. A non-selective pore with $S = 1$ has the same flux for a transport factor as for a non-binding protein (top). A selective pore with $S > 1$ has a larger flux for a transport factor than a non-binding protein (lower). (C) The bound diffusion coefficient quantifies the mobility of a bound transport factor. A transport factor may be immobile (top) or mobile (lower) when bound.

it important for diverse processes including gene regulation and translation [**?**]. Transport occurs through the central channel, ∼50 nm in diameter and ∼100 nm long. The selective barrier filling the central channel is made from disordered proteins, the FG nucleoporins (FG Nups), which contain repeated phenylalanine-glycine (FG) motifs Figure 2.1. Transport factors (TFs) that directly bind to the FG repeats can cross the NPC and carry cargo with them [**?**]. Transport through the NPC is remarkably fast, with pore residence times ∼10 ms [**?**]. Binding between FG Nups and TFs shows diffusion-limited on-rates and transient binding of individual FG repeats to TFs [4, 5]. How the FG Nups both block passage (of non-binding molecules) and facilitate passage (of binding molecules) is not fully understood, making the NPC an ideal system to dissect the principles of binding-controlled selective transport.

Models of the NPC selective barrier have proposed that the FG Nups may form an entropic brush [6], a dynamic hydrogel [7, 8], an intermediate state between a brush and gel [9], or liquid droplets [10]. These mechanisms may be modulated by spatial organization [**?**, 11] and binding of

TFs to multiple FG repeats [12,13]. Attempts to distinguish these models have been hindered by the pore's small size, the redundancy and multiple copies of FG Nups, and contradictory experimental results on FG Nups and TF binding [9]. Some FG Nup fragments form less-dynamic hydrogels *in vitro* [8], but remain highly dynamic within cells [5]. Molecular dynamics simulations find highly dynamic FG Nups, though the degree and extent of motion depends on the affinity of FG repeats for each other and for TFs [9,14]. Crowding and competition modulate affinity [**?**] and may contribute to selective transport [15]. However, the connection between the amino-acid level behavior of the FG-TF interaction and macroscopic transport selectivity remains unclear. Here we address the central contradiction of selective transport through the NPC: how does binding of TFs to FG Nups within the pore increase the flux rather than decreasing it [3, 16]?

Using a biophysical model, we demonstrate that TF diffusion and binding are sufficient for selective transport, as long as binding only partially immobilizes TFs. Binding increases the local concentration, and these molecules contribute to the flux if mobile. Thermally-driven diffusion of TFs bound to flexible tethers gives sufficient particle mobility to produce selectivity similar to experimental measurements. Tether flexibility also allows bound TFs to hop between tethers, further enhancing selectivity.

Biophysical model of transport through the NPC

We consider a minimal model of the central channel of the NPC containing FG Nups homogeneously anchored Figure 2.1. This model is sufficiently general to describe the common features of a range of biopolymer filters. The NPC, unlike most other biopolymer filters, has a wide capture area that may increase transport rates [17]. In order to focus on basic principles of transport, we neglect this effect. A varying free energy landscape along the axis of the NPC may play a role in selective transport [15, 18–20]. However, the NPC is robust to deletion of all asymmetric Nups and many Nup combinations, indicating that spatial variation in pore properties is not necessary [**?**, **?**]. Experiments *in vitro* with simplified, homogeneous Nup composition produced selective transport [**?**, **?**].

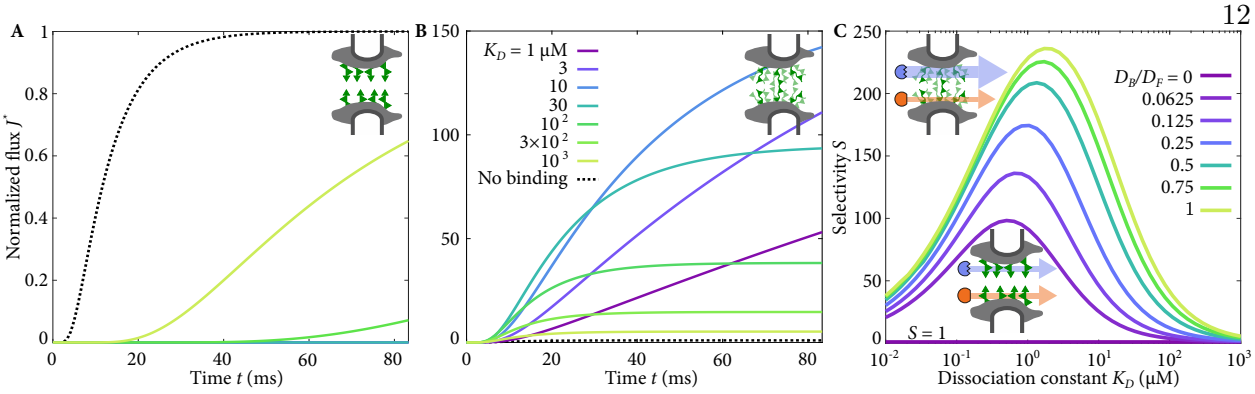

Figure 2.2: Flux through the pore and selectivity for TFs with varying bound mobility. (A) Flux as a function of time when TFs are immobile while bound, with varying binding affinity as in (B). (B) Flux as a function of time when TFs are mobile while bound with $D_B = D_F$, with varying binding affinity. (C) Selectivity as a function of dissociation constant with varying bound diffusion coefficient.

Rapid transport requires TF-FG Nup binding, while a protein similar to a TF but unable to bind FGs is excluded. Therefore, in our model we compare two proteins that are identical, except that one binds FG Nups and the other does not. As a model TF, we consider nuclear transport factor 2 (NTF2) [**?**]. NTF2 is small (∼5 nm) relative to the diameter (∼50 nm) and length of the pore (∼100 nm), suggesting that passage of NTF2 does not require large-scale molecular rearrangements that have been proposed for larger molecules [**?**, 12]. Because of the small size of NTF2 we neglect effects of steric crowding, which can enhance selectivity in a transport model [15]. NTF2 appears not to be actively released from the pore, suggesting that selective transport is an intrinsic property of the NPC [15, 21], and in contrast to actively released karyopherins [**?**, 12, 21, 22].

Transport through the NPC requires entry into the pore, passage, and exit. In single-molecule measurements, most of the transport time is spent in a random walk within the central channel [**?**, 19]. We therefore assume that entry and exit rates are determined by binding kinetics (see Supporting Information, section **??** for the model when entry and exit are rate-limiting.) The directional bias in TF transport is controlled outside the NPC through a concentration difference between the nucleus and cytoplasm generated by the Ran-GTP system [**?**]. In our model, we impose a fixed concentration difference across the pore.

We consider a channel of length L filled homogeneously with Nups that separates two reservoirs Figure 2.1A. Within the channel are free transport factor (concentration T), free FG Nups (N), and bound TF-FG complex (C), with total Nup concentration $N_t = N + C$. TF diffusion within the channel ($0 < x < L$) is described by the reaction-diffusion equations

$$\frac{\partial T}{\partial t} = -k_{\text{on}} T N + k_{\text{off}} C + D_F \frac{\partial^2 T}{\partial x^2}, \tag{2.1}$$

$$\frac{\partial C}{\partial t} = k_{\text{on}} T N - k_{\text{off}} C + D_B \frac{\partial^2 C}{\partial x^2}. \tag{2.2}$$

TF-FG interaction has on-rate constant k_{on}, off-rate k_{off}, and dissociation constant $K_D = k_{\text{off}}/k_{\text{on}}$. We include competition between TFs for FG binding sites [20]. The diffusion constants of free (D_F) and bound (D_F) TFs are spatially constant. The fixed reservoir TF concentrations are T_L (inlet, left) and 0 (outlet, right).

The flux of transport factor out of the pore $J = -D_F \, \partial T/\partial x|_{x=L}$. We numerically integrated the full equations. Because flux measured in experiments is typically linearly proportional to TF concentration [10, 23], TF concentration likely remains below binding saturation in the NPC. Therefore, we also solved eqns. (2.1, 2.2) analytically in the low binding limit. We define the transport selectivity S as the ratio of steady-state flux of a binding versus a non-binding species Figure 2.1B

$$S = \frac{J_{\text{binding}}(t \to \infty)}{J_{\text{non-binding}}(t \to \infty)}. \tag{2.3}$$

2.0.1 *

No selective transport occurs if bound TFs are immobile If TF-FG Nup binding immobilizes the TF, the bound-state diffusion coefficient $D_B = 0$. For immobile bound TFs, transport is not selective: the steady-state flux $J = D_F T_L/L$ for both binding and non-binding proteins, so $S = 1$ (figs. 2.2, **??**). The binding TF accumulates within the pore, but its immobility means it does not enhance transport compared to the non-binding case. Notably, this effect is independent of binding kinetics. Prior to steady state, binding slows transport Figure 2.2A. In systems such as airway mucus, immobilization may increase the time available for degradation or active clearance,

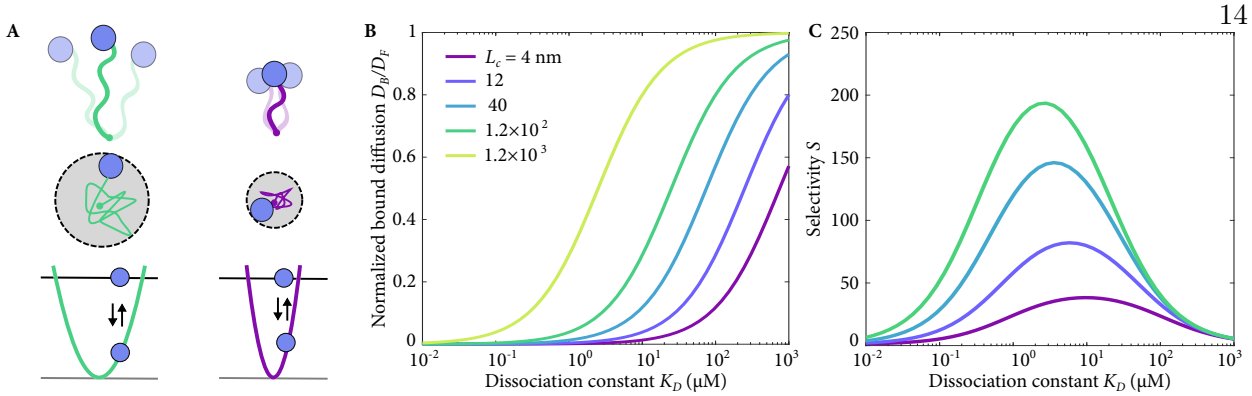

Figure 2.3: (A) Schematic of the flexible tether model of bound-state diffusion. FG Nups are treated as entropic springs that constrain the motion of TFs more (top and center left, longer FG Nup) or less (top and center right, shorter Nup), which corresponds to changing width of the harmonic potential well (lower). (B) Ratio of bound to free diffusion coefficient as a function of dissociation constant, with varying polymer length in the tethered-diffusion model. (C) Selectivity as a function of K_D, with varying polymer length in the tethered-diffusion model.

consistent with the observation that binding tends to inhibit selective transport in those systems [**?**, **?**, **?**]. This effect is related to the binding-site barrier seen in antibody delivery to tumors [**?**], and observations that non-binding nanoparticles are often more effective in drug delivery to tumors than binding particles [3].

Our model is related to the classic problem of molecular transport through an oil membrane separating two aqueous reservoirs [**?**]. The relative concentration of a species just inside the oil barrier to the concentration in water is called the partition coefficient. The steady-state flux through the membrane is directly proportional to the partition coefficient (Supporting Information, section **??**, fig. **??**). By analogy, one might expect the TF-FG binding affinity to determine the flux across the pore. However, binding is different from partitioning. In systems where the increase in intra-pore concentration arises from binding, the effective diffusion coefficient is typically inversely proportional to the partition coefficient, making the flux independent of binding affinity [16]. This result led us to consider whether TFs may be mobile while bound to FG Nups.

2.0.2 *

Bound-state diffusion allows selective transport When bound TFs are mobile, selective trans-

port occurs with a selectivity up to 240 for a conservative set of parameters (figs. 2.2B,C, **??**, Supporting Information, section **??**). Remarkably, this selectivity is comparable to experimental measurements of NTF2 versus GFP flux (Table 2.1). The interplay between binding kinetics and diffusion leads to an optimal dissociation constant $\sim$1 μM for maximum selectivity Figure 2.2C. Selectivity decreases for high K_D because binding is too weak to significantly increase TF concentration in the pore. For low K_D, tight binding causes the concentration of bound complexes to become approximately constant across the pore. Because diffusive flux is driven by a concentration gradient, this washing out of the gradient by tight binding decreases flux and selectivity.

Our model predicts that selectivity is increased by increasing binding on-rate constant k_{on} Figure **??**. Consistent with this, the on-rate constants of TF-FG Nup interactions have been measured to be diffusion limited [4, 5]. Large k_{on} makes transport more selective because fast binding kinetics relative to diffusive motion are necessary to maintain steep concentration gradients within the pore. High FG Nup concentration (as measured experimentally) leads to large N_t and low D_F, both of which increase selectivity. Decreasing D_F or increasing the length of the pore both reduce the magnitude of the flux and increase selectivity (figs. **??**, **??**). Therefore, varying TF free diffusion coefficient and pore length involves a trade-off between transit time and selectivity.

Table 2.1: Comparison between experimental results for NTF2 and GFP (a similarly-sized non-binding protein) and model predictions. Flux measured in units of molecules per pore per second.

Method	Cell type	Species	Flux	Selectivity	Notes
OSTR	*Xenopus*	NTF2 GFP	91–123 3.3–3.8	24–37	[24]
OSTR	*Xenopus*	NTF2 GFP	47.3 1.1	43	[25]
Permeabilized cells	HeLa	NTF2 GFP	250 2	125	[7]
Model	–	Binding Non-binding	2–480 2	1–240	This work

Mechanisms of bound transport factor mobility

Our result that bound-state diffusion is required for selective transport raises a mechanistic question: how can TFs move while bound to FG Nups? Here we consider two experimentally based mechanisms: movement of the bound TF due to the intrinsic flexibility of the FG Nups [**?**] and multivalent binding that allows hopping of TFs between neighboring Nups [26].

2.0.3 *

FG Nup flexibility allows tethered diffusion Previous measurements have found that FG Nups are flexible and dynamic [**?**, 5, 27]. Although FG Nups are attached at one end to the inner surface of the NPC scaffold, chain flexibility allows a TF bound far from the tethered end to move. Flexible polymers behave as entropic springs [**?**] if they are not highly stretched. Therefore, a bound TF diffuses while attached to a spring-like tether, which can be represented as diffusion in a harmonic potential well Figure 2.3A. The width of the harmonic well is related to the effective length of the flexible domain: if FG Nups are not crosslinked, the effective length is the full FG Nup length, while if they are crosslinked or entangled, the length is reduced [7]. The probability density of a TF that binds to the center of a well at $x = 0$ is $P(x,t) = e^{-\frac{x^2}{2\alpha(t)}}/\sqrt{2\pi\alpha(t)}$, where $\alpha(t) = (1 - e^{-2kD_F\beta t})/(k\beta)$, k is the spring constant of FG Nup tethering and $1/\beta = k_BT$ is the thermal energy [28]. The TF mean-squared displacement (MSD) is then $\langle x^2(t)\rangle = \int_{-\infty}^{\infty} P(x,t)x^2dx = \alpha(t)$. The typical TF MSD during a binding event can be determined from the probability density of binding time $\rho(t) = \exp(-t/\tau)/\tau$, where $\tau = 1/k_{\text{off}}$ is the mean bound lifetime:

$$\overline{\langle x^2\rangle} = \int_0^{\infty} \rho(t')\langle x^2(t')\rangle dt' = \frac{2D_FL_c\ell_p}{L_c\ell_pk_{\text{off}} + 3D_F}. \tag{2.4}$$

Here we assume that the spring constant is that of a worm-like chain polymer $k = 3/(2\beta L_c\ell_p)$, where L_c is the contour length and ℓ_p the persistence length [**?**].

Because FG-TF interactions have fast binding kinetics [4, 5], we estimate the bound diffusion

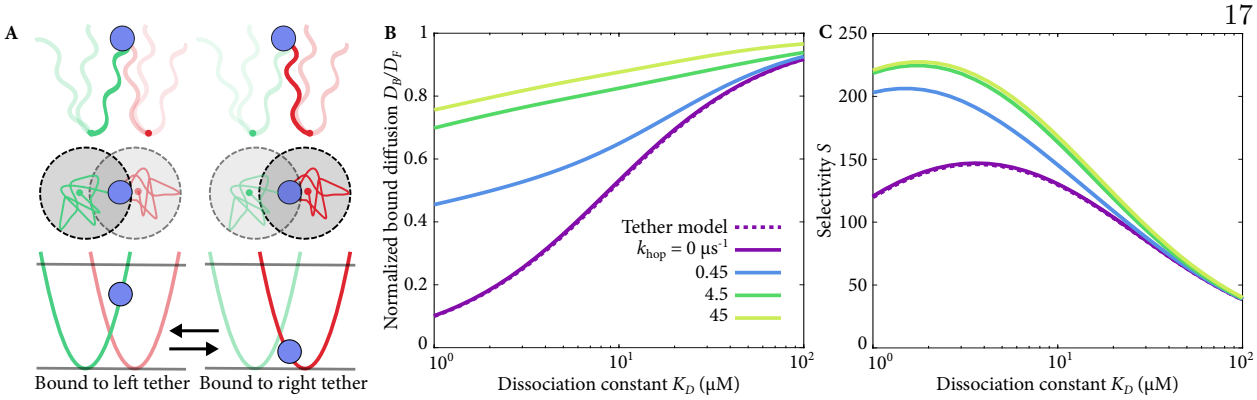

Figure 2.4: (A) Schematic of the inter-chain hopping model of bound-state diffusion. FG Nups are treated as entropic springs that constrain the motion of TFs, and inter-chain hopping allows a TF to move from one FG Nup (top and center left, green Nup) to another (top and center right, red Nup) without unbinding, which corresponds to switching from one harmonic potential well to another (lower). (B) Ratio of bound to free diffusion coefficient as a function of dissociation constant, with varying hopping rate in the inter-chain hopping model. (C) Selectivity as a function of K_D with varying hopping rate. FG Nup contour length $L_c = 40$ nm in (B, C).

coefficient by averaging over many binding events, while considering only bound motion, giving

$$D_B \approx \frac{\overline{\langle x^2 \rangle}}{2\tau} = \frac{D_F L_c \ell_p k_{\text{off}}}{L_c \ell_p k_{\text{off}} + 3D_F} = \frac{D_F}{1 + 3\frac{D_F}{D_P}}. \tag{2.5}$$

Here $D_P = L_c \ell_p k_{\text{off}}$ controls the bound-state diffusion coefficient: higher D_P corresponds to a lower constraint of the TF by the tether and greater bound mobility. Bound mobility increases with increasing chain length, flexibility of the polymer, or decreasing binding lifetime. When D_P is large ($D_F/D_P \ll 1$), D_B approaches D_F, since the long, flexible chains barely affect TF motion during the short binding event. For small D_P ($D_F/D_P \gg 1$), TF motion is inhibited by a short tether, giving $D_B \approx D_P/3 \ll D_F$. This result highlights that the kinetics of TF-FG Nup interaction are a primary determinant of the bound mobility: the faster the binding kinetics, the higher the bound diffusion constant.

Flexible disordered proteins typically have low persistence lengths [29], so we estimate $\ell_p \approx 1$ nm. If the on-rate constant is diffusion limited, $k_{\text{on}} = 10^{-3} \mu\text{M}^{-1}\,\mu\text{s}^{-1}$ [4, 5], the binding affinity determines the off rate. Disordered FG Nups have $L_c \approx 100$–280 nm (250–700 amino acids long [**?**] with a contour length per amino acid ≈ 0.4 nm). For our conservative parameters, tethered diffusion alone predicts significant selectivity $\sim$200.

2.0.4 *

Inter-chain hopping increases selectivity The tethered diffusion mechanism is constrained by a trade-off: tighter binding increases the TF concentration in the pore, but hinders motion. Multivalent TF-FG interactions can relax this constraint, because a TF can bind simultaneously to more than one FG Nup, moving hand-over-hand while remaining bound [30]. Consistent with this, TFs may slide between nearby FG sites rather than fully unbinding and re-binding [26]. If the newly-bound FG repeat is on a neighboring chain, the FG tether site that constrains TF motion moves while the TF remains bound. We model inter-chain hopping with a TF that undergoes tethered diffusion when bound to an FG Nup and hops between neighboring, randomly distributed tethers Figure 2.4. Numerical simulations of this model determined the bound diffusion coefficient (fig. **??**, Supporting Information, section **??**). We note that intra-chain hopping does not change the flux, since the anchor point of the tethering chain is not changed; therefore we neglect it.

Inter-chain hopping increases selectivity most for tight binding and short chains, the parameter regime where tethered diffusion gives limited selectivity (figs. 2.4, **??**, **??**). Hopping may therefore be important for FGs that form transient crosslinks: if FG Nups are highly crosslinked, our model suggests that inter-chain hopping is the key mechanism of TF movement. For weaker binding and longer chains, inter-chain hopping leads to a modest increase in selectivity.

Discussion

A key puzzle of the NPC is how transport-factor binding allows rapid transport through the pore. Binding typically immobilizes the bound particle, and so the increase in concentration resulting from binding does not, in general, result in increased flux. The biophysical theory we developed includes diffusion of TFs due to thermal fluctuations, binding to polymeric tethers, and the hopping of bound species between these tethers. Thus we identified principles of selective transport resulting from binding Figure 2.1, emphasizing that bound-state mobility is essential for selective transport Figure 2.2. Binding increases the local concentration, and any bound mobility

increases the flux. We characterized two mechanisms to obtain bound-state mobility and found that thermally-driven diffusion of TFs bound to flexible tethers and rapid binding kinetics [4, 5] allow TF mobility, leading to selectivity similar to that observed experimentally Figure 2.3. In addition, tether flexibility enables multivalent bound particles to hop between binding regions Figure 2.4 [12, 13], further enhancing selectivity. Mobility of bound or partitioned molecules occurs in many biological contexts, suggesting that the mechanisms we study here may be broadly applicable [**?**,31].

Our model for selective transport by tethered diffusion generalizes to a range of FG-FG interactions [9], if we decrease the effective chain length L_c for cohesive FG Nups. For short chains the selectivity simply due to chain flexibility is modest, suggesting that other mechanisms, like hopping, may be important. Our model suggests that transient cross-linking of FG repeats proposed to occur within the pore may serve to increase the viscosity and therefore the selectivity. Crosslinks need not be actively melted by TFs to enhance selectivity Figure **??**.

Our model provides a quantitative tool to evaluate selective transport. Materials formed *in vitro* by spontaneous self-assembly of FG Nups [8] or transient crosslinking by alpha-helical peptides [32] show strong selective *entry*. Using published data, we predicted whether these gels also showed selective *transport* (table **??**). Most synthetic gels are predicted to have $S < 10$, less than the selectivity of NTF2 in cells (table 2.1). The predicted selectivity of one hydrogel is $S \approx 200$, apparently the most selective synthetic gel to date [8].

2.0.5 *

Overcoming the limitations of binding Binding, even in the presence of bound-state motion, limits selectivity. Biological systems appear to have developed strategies to avoid this, for example, by using true partitioning. Lipid domains in complex membranes partition proteins [**?**]. Membraneless organelles spontaneously assembled from low-complexity proteins and nucleic acids can localize a molecule without immobilizing it [**?**]. Because membraneless organelles are fluid, the constraints imposed in our NPC model by binding are released. Our work thereby suggests a benefit of phase-separated droplets to cells: they provide significantly higher selectivity than can occur with

immobilizing binding. This may be especially important for spatially complex assemblies [**?**].

Though we show it is not necessary, the active dissolution of polymeric biomaterials has been proposed to occur in the NPC [7]. This strategy is used by *Helicobacter pylori* to penetrate the gastric mucus [**?**]. Because the particularly dense extracellular matrix of solid tumors blocks the motion of particles, especially larger nanoparticles, ECM dissolution has been used to enhance drug delivery [**?**]. Unfortunately, this approach may not be universally applicable: breaking down the ECM surrounding tumors may promote cancer metastasis [**?**].

2.0.6 *

Design principles of selective transport by binding

Filtering by polymeric biomaterials occurs in many systems for particles of different sizes: for example, nutrients reach our intestinal walls while larger molecules are excluded. However, controlling the selective transport of similarly-sized molecules by tuning specific interactions has proven elusive. In drug delivery applications, inert nanoparticles are typically more effective at penetrating extracellular spaces and reaching their cellular targets [3]. Because biopolymer filters are the first point of contact of nanoparticles used for drug delivery, specific targeting of transport through mucus may enhance the effectiveness of drug delivery. If NPC-like bound mobility as described in our model could be achieved in these systems, it would increase the rates of transport and drug delivery.

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