KITP, UCSB, Jan 14 2010

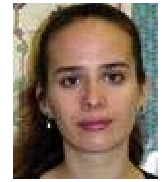
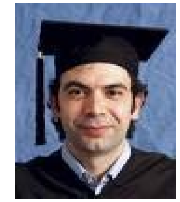
# Selectivity of transport through the nuclear pore complex: from nuclear import to biosensors

Anton Zilman

Theoretical Biology and Biophysics Group and Center for Nonlinear Studies

Theoretical Division, Los Alamos National Laboratory, Los Alamos, USA

# Coauthors




Tijana Jovanovic-Talisman

Stefano Di Talia

Jackie Tetenbaum-Novatt

Marcelo Magnasco

Reiner Peters

Mike Rout

Brian Chait

*Rockefeller University*

***Reported in***

Zilman, Di Talia, Chait, Rout, Magnasco, ***PLoS Computational Biology (2007)***

Zilman, ***Biophysical Journal (2009)***

Jovanovic-Talisman, Tetenbaum-Novatt, McKenney, Zilman, Peters, Rout, Chait, ***Nature (2009)***

Zilman, Jovanovic-Talisman, Di Talia, Chait, Rout, Magnasco, ***(2009) in review***

# Plan

Overview of function

Overview of function-relevant structure

Overview of current views of selectivity

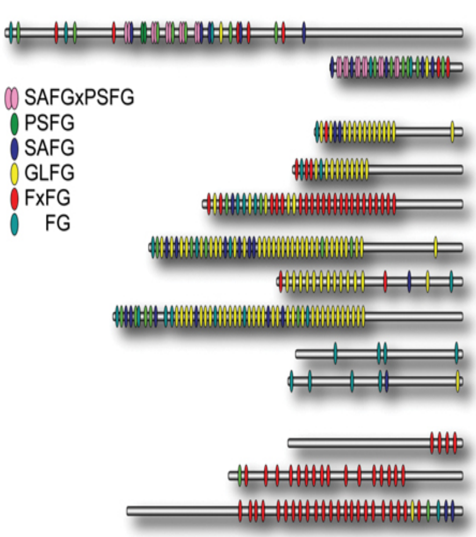
Basic physics: our model

Comparison to experiments

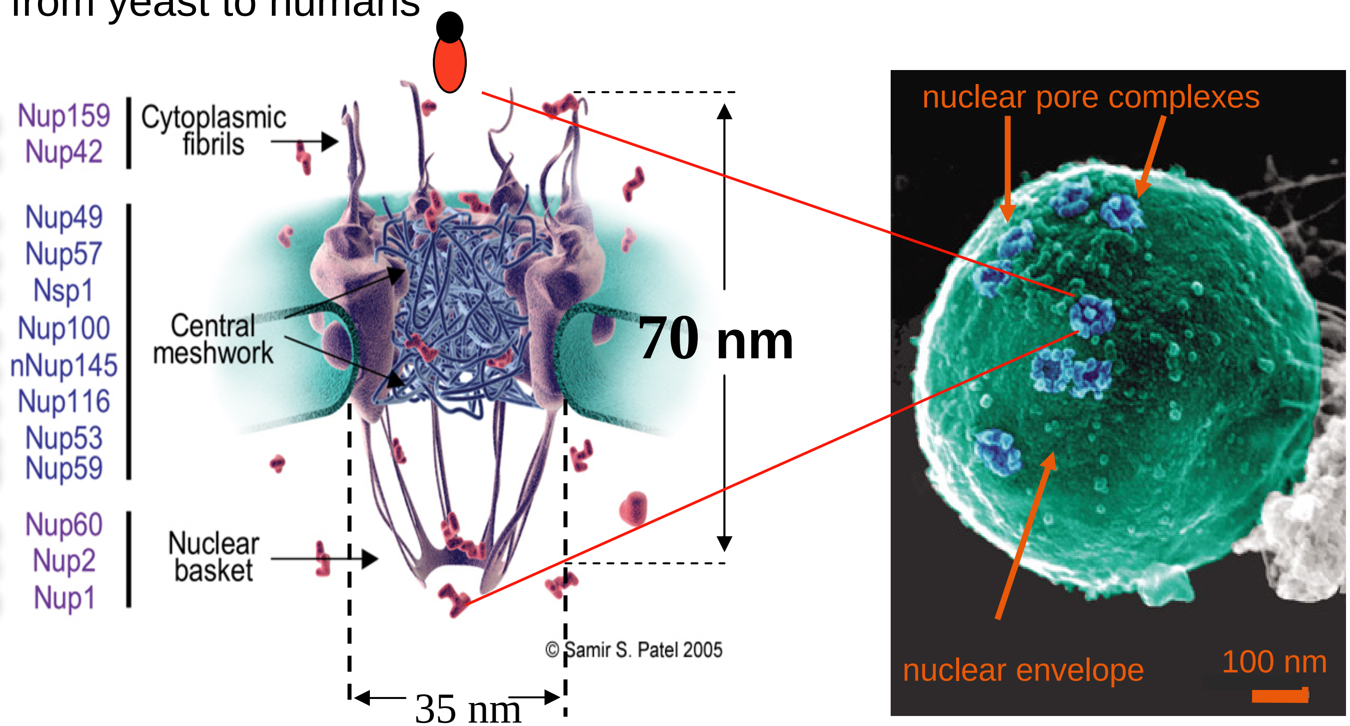
# Nuclear pore complex

Gates all transport between nucleus and cytoplasm in all eukaryotic cells

Relatively conserved from yeast to humans




Patel et al., Cell 2007




Kiseleva, 2005

Hundreds of cargoes per second in both directions

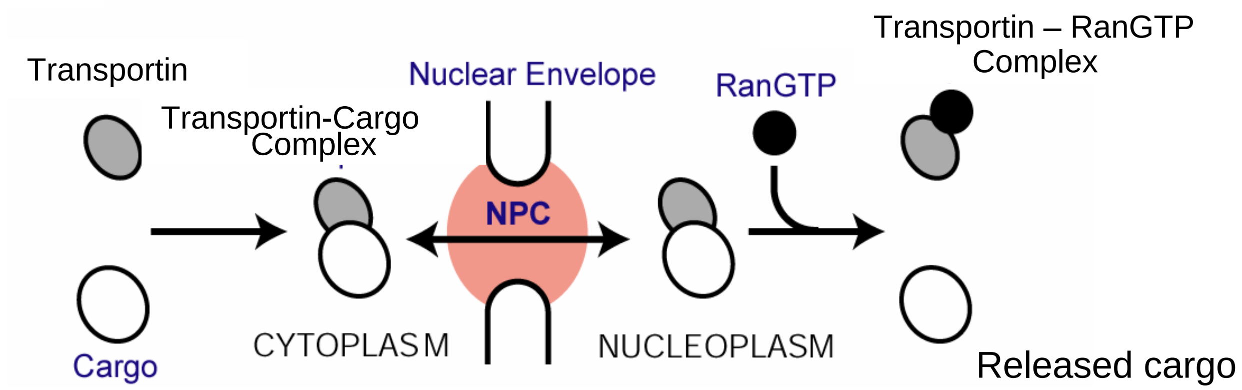
Directionality: the cargo is sequestered in the nucleus

Selectivity and efficiency are energy-independent

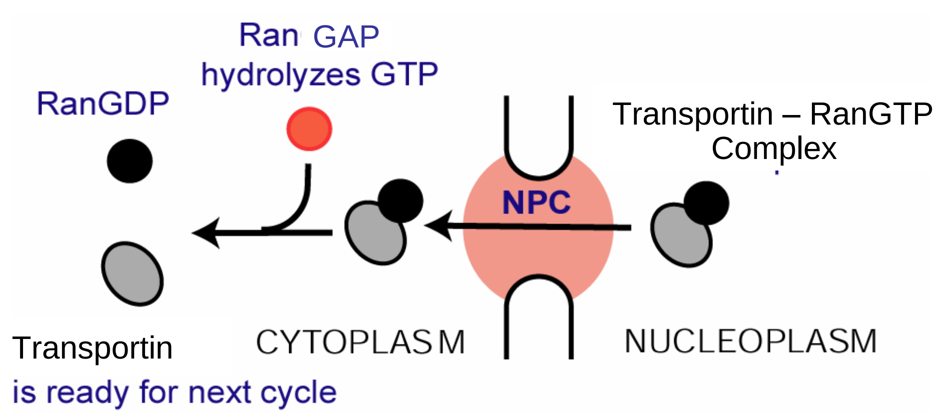
Important paradigm: applications in gene therapy, viral infections, artificial nano-sorting devices

# Functional scheme: import

*Forward process*




*Inverse process*




Nuclear *RanGTP* creates gradient of the karyopherin-cargo complexes

One *GTP* is used per one transported cargo

# NPC is an example of an ‘always open’ channel

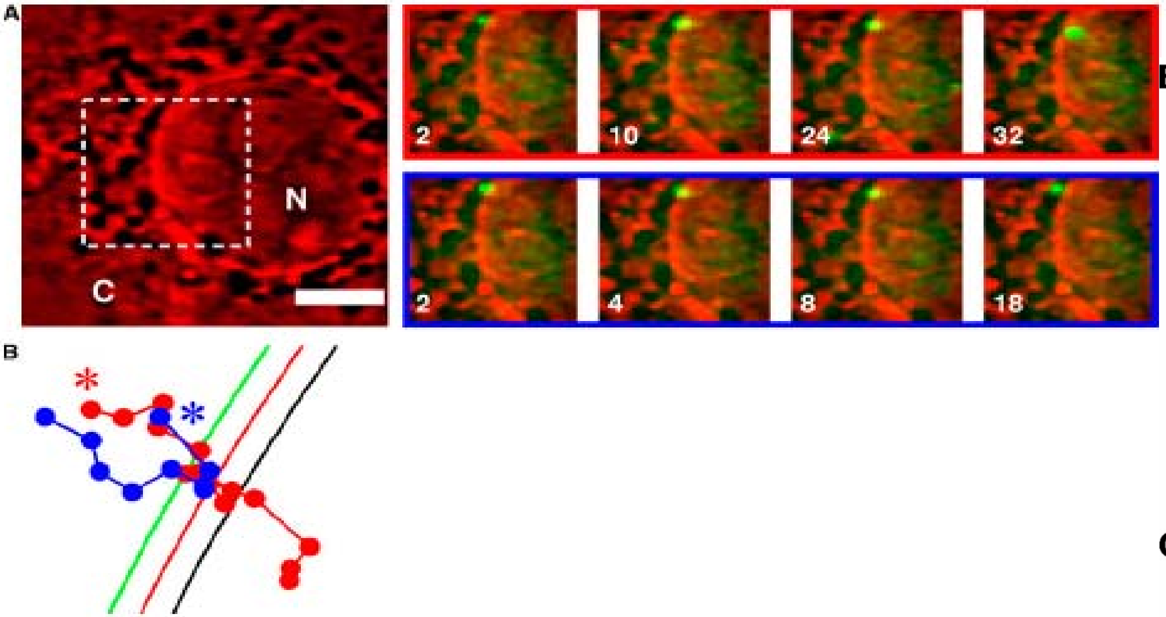
‘Does not have ‘moving parts’ (at least any obvious ones)

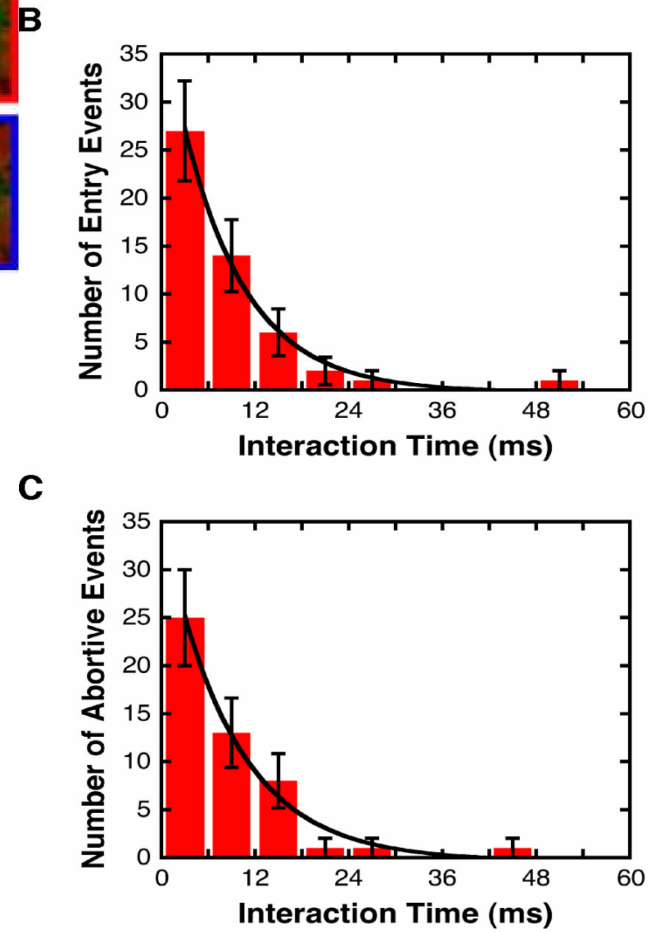
Don’t use metabolic energy *directly* during the transport event

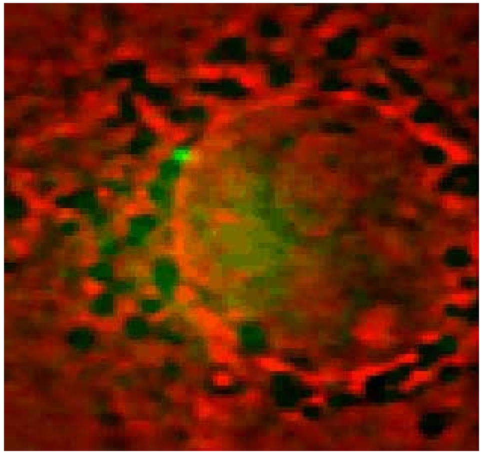
Transport occurs by some sort of ‘facilitated diffusion’

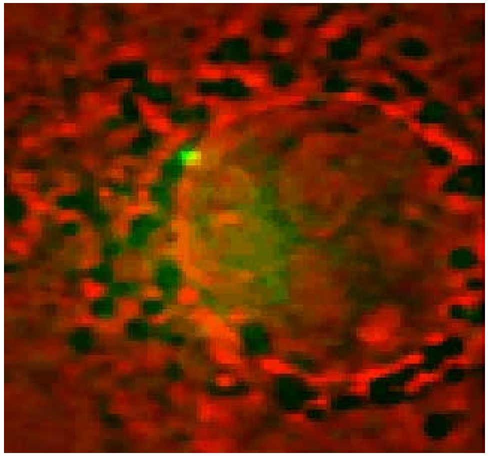
Still directional and *selective:*
transports only specific molecules but not the others

# Single molecule tracking:  stochastic dynamics





Yang and Musser, JCB (2006)

# FG nups: major determinant of the selecitivity

~13 different types (in yeast). Some have vertebrate homologues.
Known for the FG residues repeats.

**Natively unfolded:**
**are unfolded proteins unfolded when no one is looking?**

Biophysical evidence: radii of gyration

Biochemical evidence: protease action

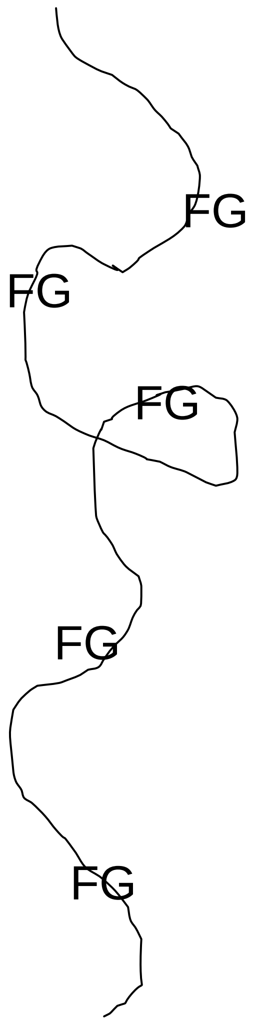
Structural and bioinformatic evidence: aminoacid composition

Cell biology evidence: intracellular gold immuno-labeling

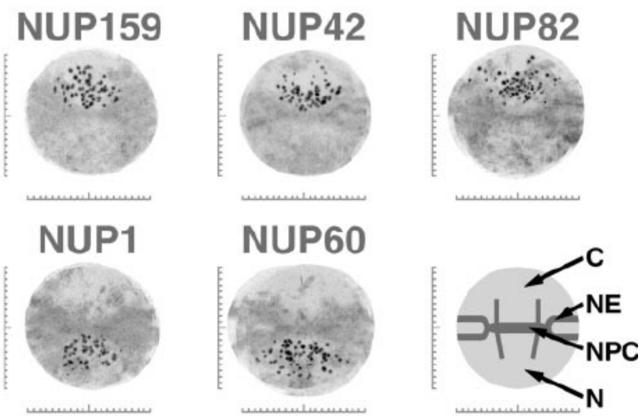
Imaging evidence: electron microscopy

Crysallographic evidence: lack of thereof

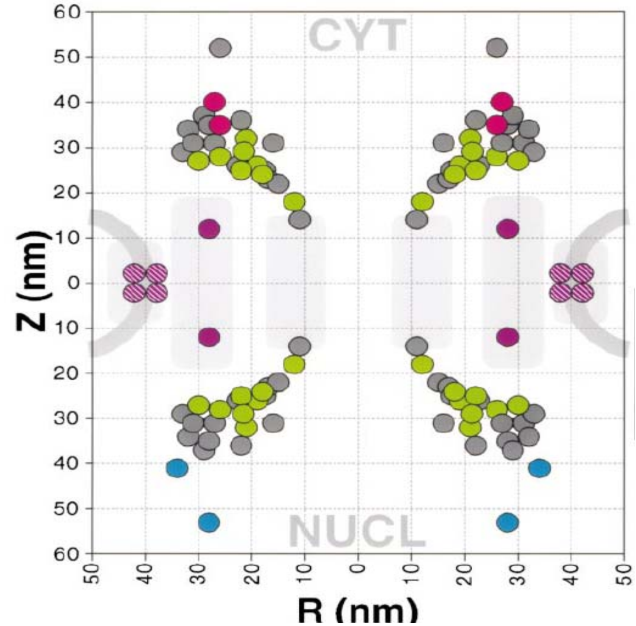
Physical evidence: in vitro AFM measurements

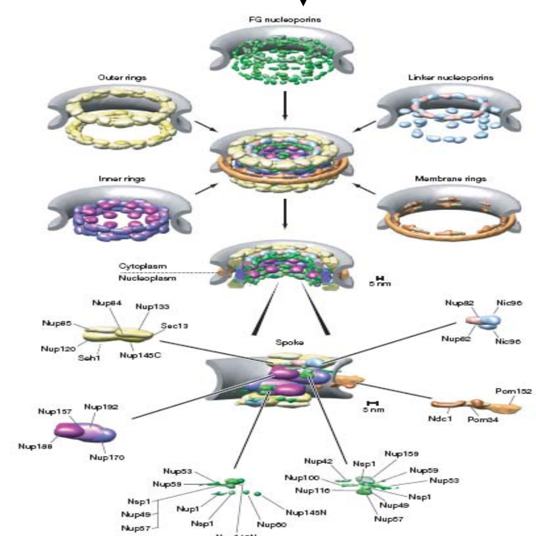


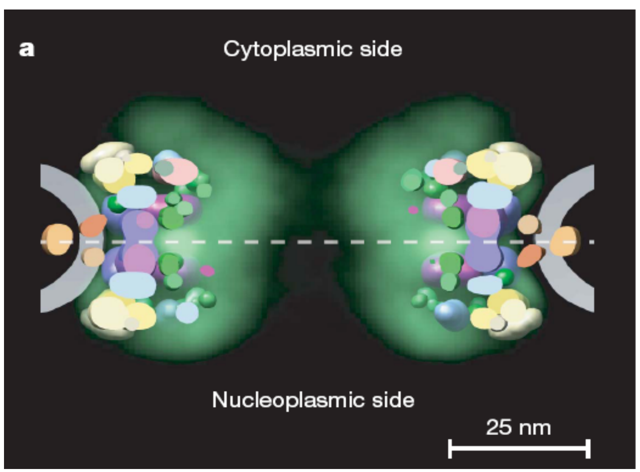
# Distribution of the FG nups: how do we know




Rout et al.,JCB 2000



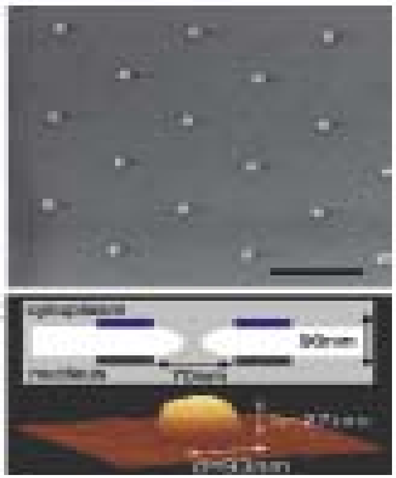



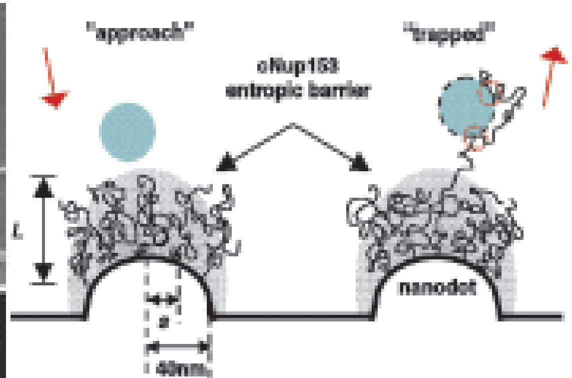




Alber et al.,Nature 2007

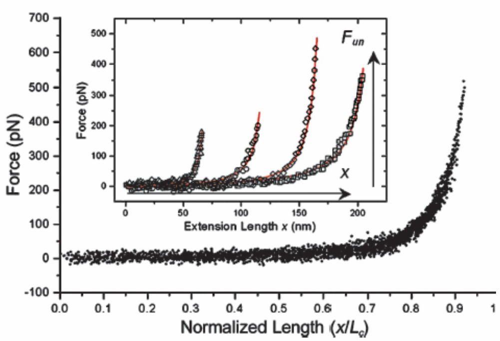
# Conformation and physical chemistry of the filaments

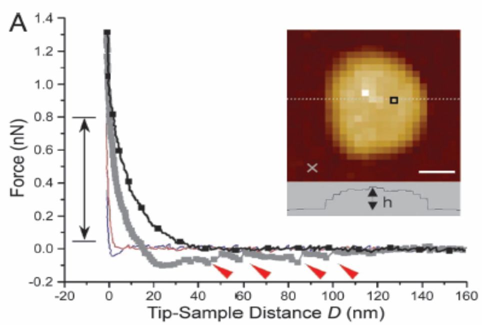
*Some form a brush on a surface*

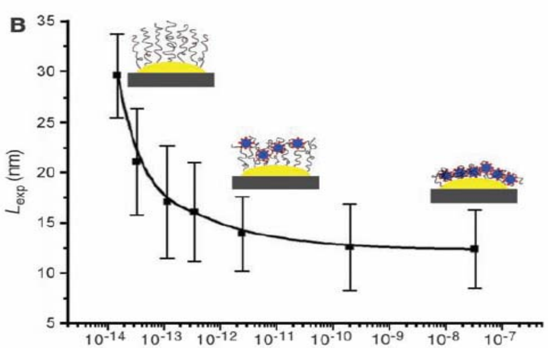





Lim et al, PNAS 2006, Science 2007



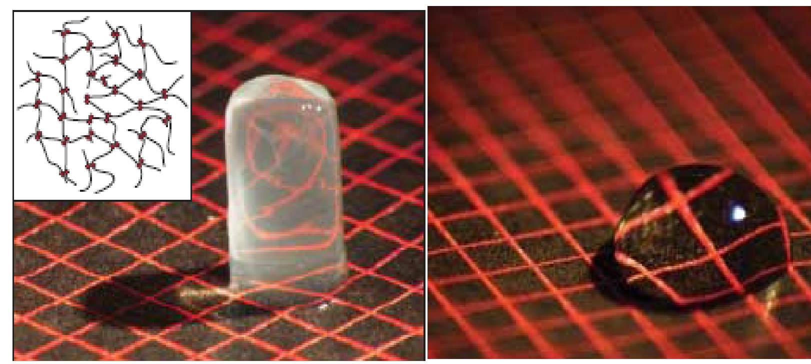



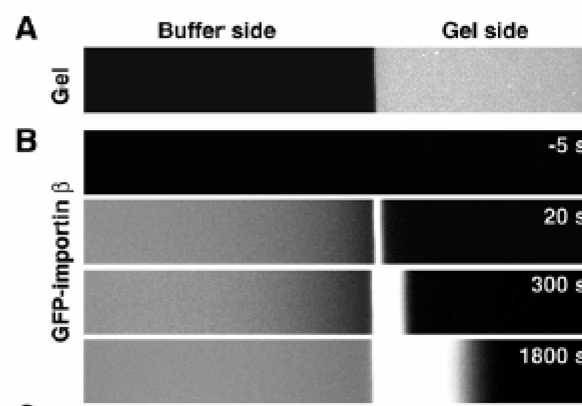




*Some form a gel in bulk*

WT Nsp1

F-Y mutant

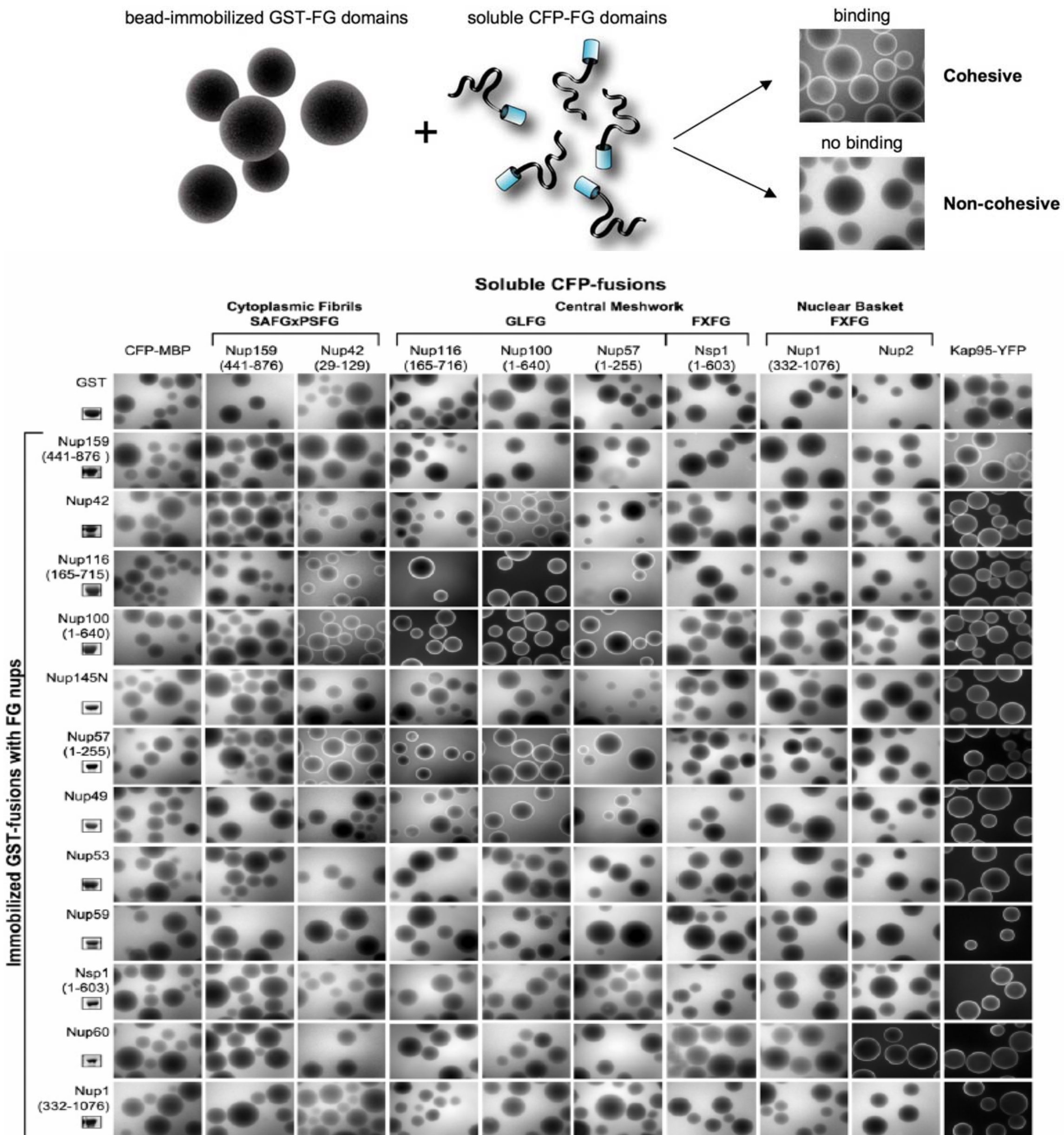





Frey et al, Science 2006, Cell 2007

# Systematic study (in yeast)

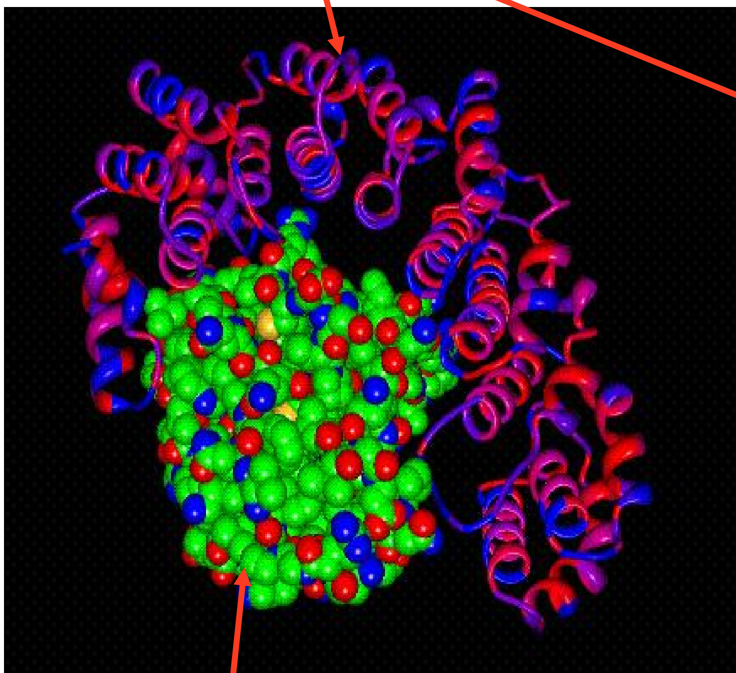
*Some are  mutually ‘cohesive’.*

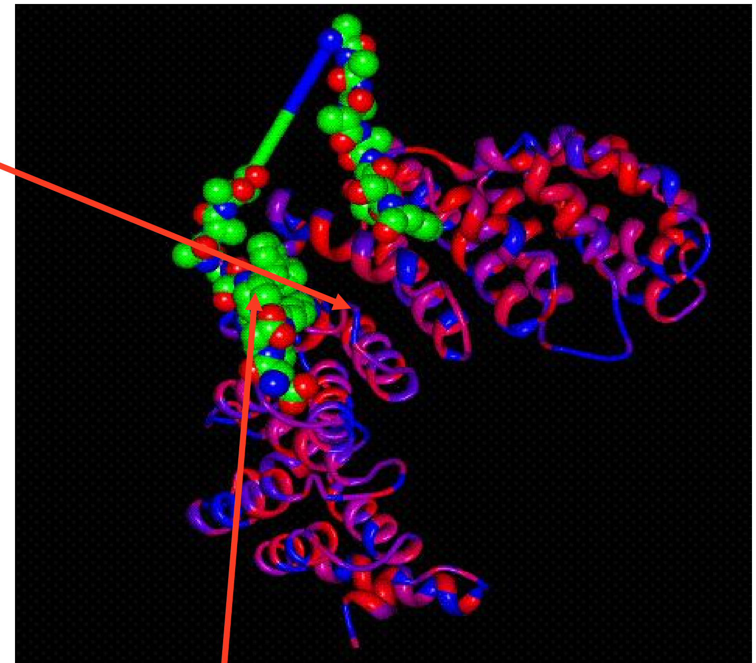



Patel et al, Cell, 2007

# Transport factors bind flexible filaments

Conserved alpha-helical repeats





Bound cargo

Bound piece of a filament

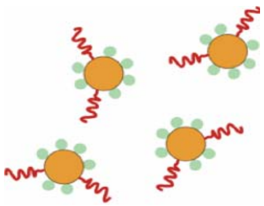
Binding affinity ~ 10 nM
Binding times - minutes

Binding affinity ~ 100-1000 nM
**Binding time < 1 msec????**

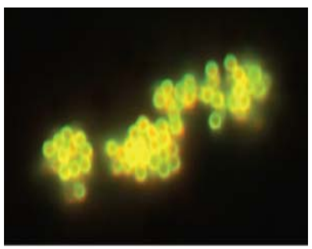
# Kap-FG nup binding affinities: discrepancies

## Typical assay

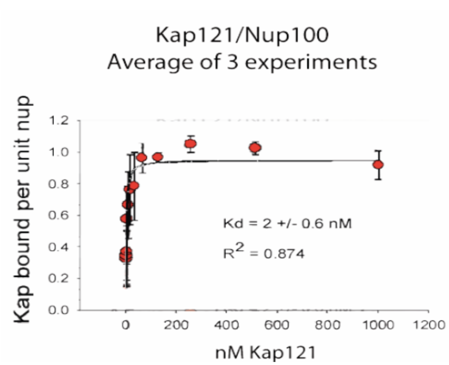
Beads with FG nups



Add Kaps



Elute and measure

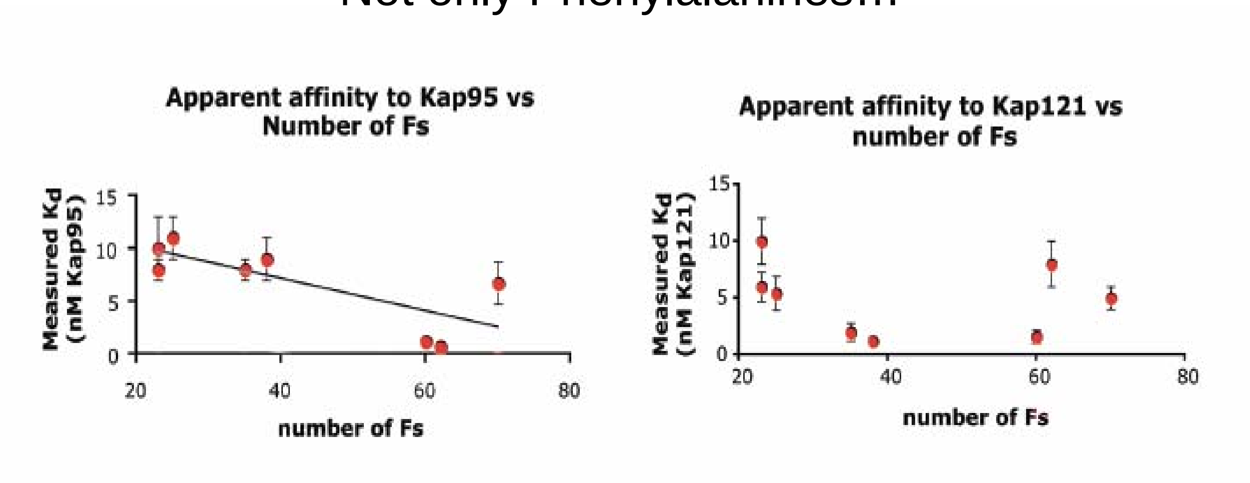



Jaclyn Novatt, Ph.D Thesis, Rockefeller 2009

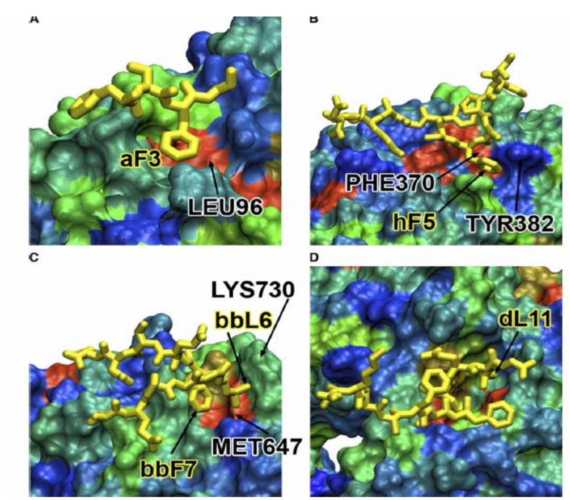
| Kap | FG-nup (fragment) | Additional molecules present | Non-specific competitor | Apparent $K_d$ (nM) | Method | Reference | Apparent $K_d$ from this study for full-length nup (nM) |
|---|---|---|---|---|---|---|---|
| Kap95 | Nup1 (aa 423-816) | - | 30mg/mL BSA | 350 | Microtiter plate | (Bayliss, Littlewood et al. 2002) | 7 +/- 2 |
| Kap95 | Nup1 (aa 332-1076) | - | 1mg/mL BSA | 7.9 +/- 1.7 | Sepharose resin | (Pyhtila and Rexach 2003) | 7 +/- 2 |
| Kap95 | Nup1Δ36 (aa 332-1040) | - | 1mg/mL BSA | 2500 +/- 700 | Sepharose resin | (Pyhtila and Rexach 2003) | 7 +/- 2 |
| Kap95 | Nup1ΔN (aa 332-1076) | Kap60 + Cbp80 NLS-MBP | 1mg/mL BSA | 0.40 +/- 0.02 | Sepharose resin | (Gilchrist, Mykytka et al. 2002) | 7 +/- 2 |
| Kap95 | Nup1ΔN (aa 332-1076) | Kap60 | 1mg/mL BSA | ≤ 0.05 +/- 0.01 | Sepharose resin | (Gilchrist, Mykytka et al. 2002) | 7 +/- 2 |
| Kap95 | Nup1 (aa 332-1076) | Kap60 | 1mg/mL BSA | ≤ 0.05 | Sepharose resin | (Pyhtila and Rexach 2003) | 7 +/- 2 |
| Kap95 | Nup1Δ36 (aa 332-1040) | Kap60 | 1mg/mL BSA | 11.2 +/- 1.4 | Sepharose resin | (Pyhtila and Rexach 2003) | 7 +/- 2 |
| Importin β (vertebrate Kap95) | Nup153-C (Nup1), aa 609-1475) | - | 30 mg/mL BSA | 9 +/- 2.5 | Microtiter Plate | (Ben-Efraim and Gerace 2001) | 7 +/- 2 |
| Kap95 | Nsp1 (aa 262-603) | - | 30 mg/mL BSA | 160 +/- 40 | Microtiter plate | (Bayliss, Littlewood et al. 2002) | 0.7 +/- 0.1 |

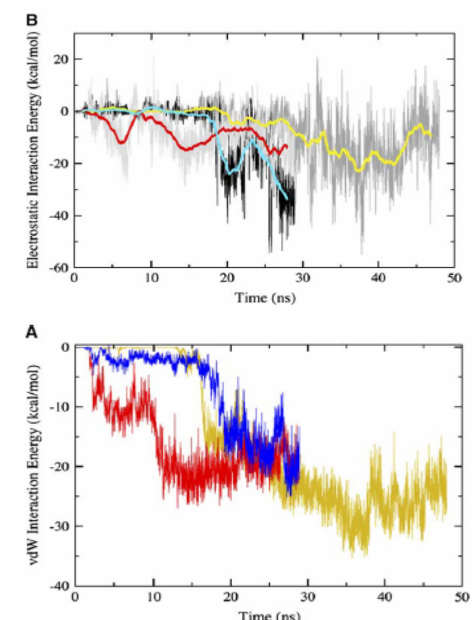
# The nature of Kap – FG nup interaction

Not only Phenylalanines…



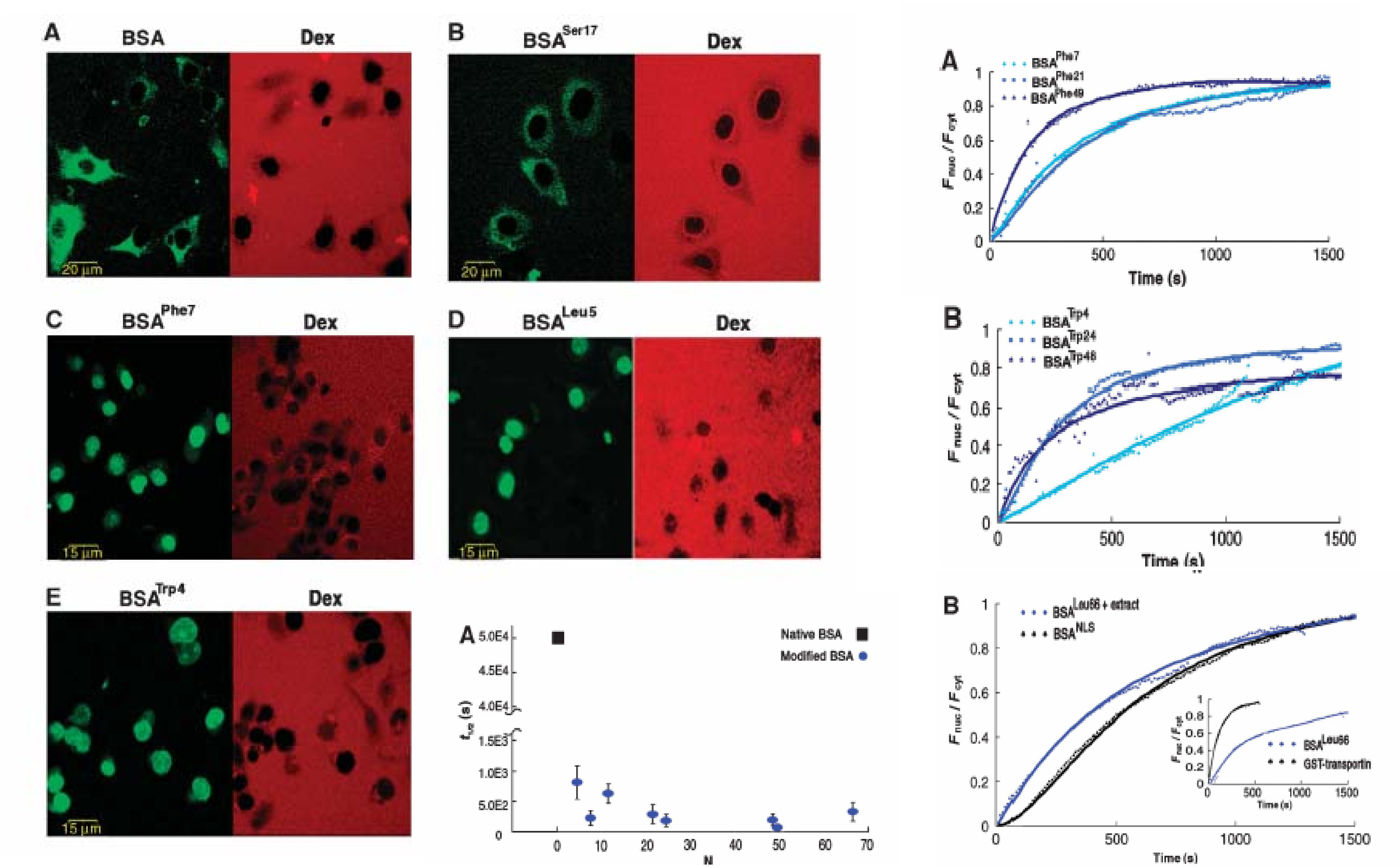

MD simulations




Isgro and Schulten, 2006

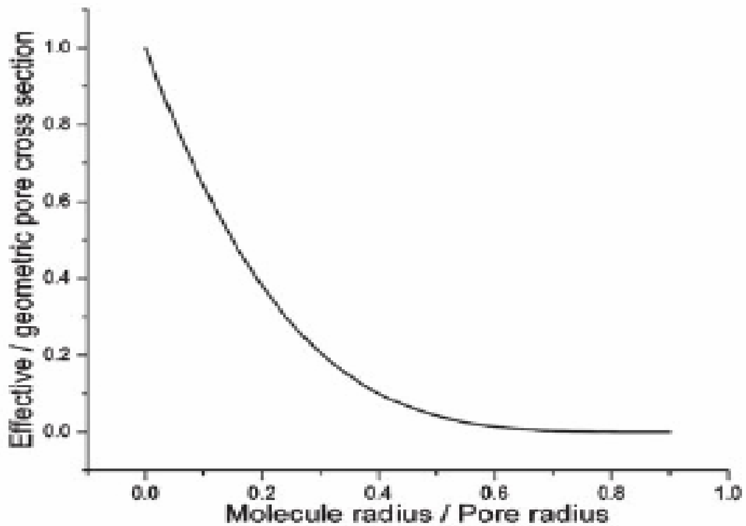
# Hydrophobicity is nevertheless important




Naim et al, EMBO J, 2009

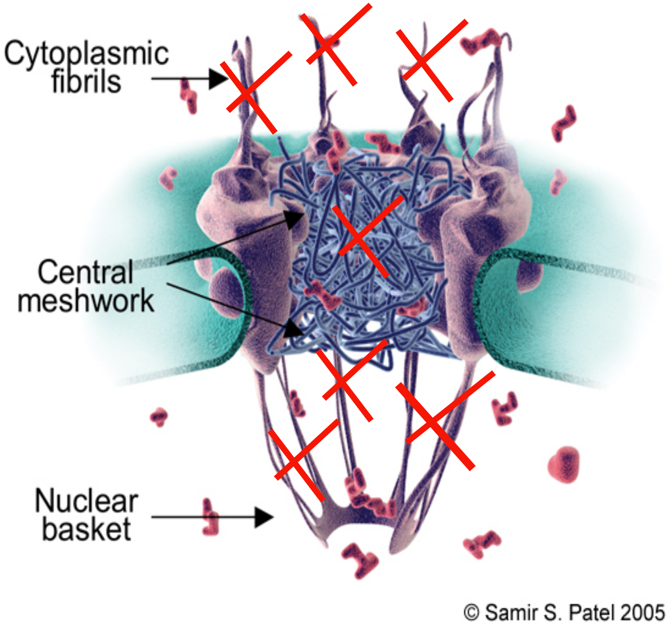
# Size selectivity for neutral particles

| | Non-binding particles (molecules) | Binding particles (transport factors) |
|---|---|---|
| Selectivity | Equivalent to that of a molecular sieve with cylindrical pores of 4–5 nm radius and 45 nm length[43] | High selectivity achieved by signal sequences on cargos that are bound by nuclear transport receptors |
| Exclusion limit | 8–10 nm diameter[43] | ~40 nm diameter[81] |
| Transport rates (molecules/NPC/second/μM) | GFP (2.8 nm radius): 3[45]<br>BSA (3.35 nm radius): <0.1[29] | NTF2 (2.36 nm radius): ~100[45]<br>Transportin (4.13 nm radius): 65[29] |
| Directionality | Bidirectional | Primarily bidirectional. However, directionality imposed outside the NPC by Ran cycle |




Peters, BioEssays, 2009

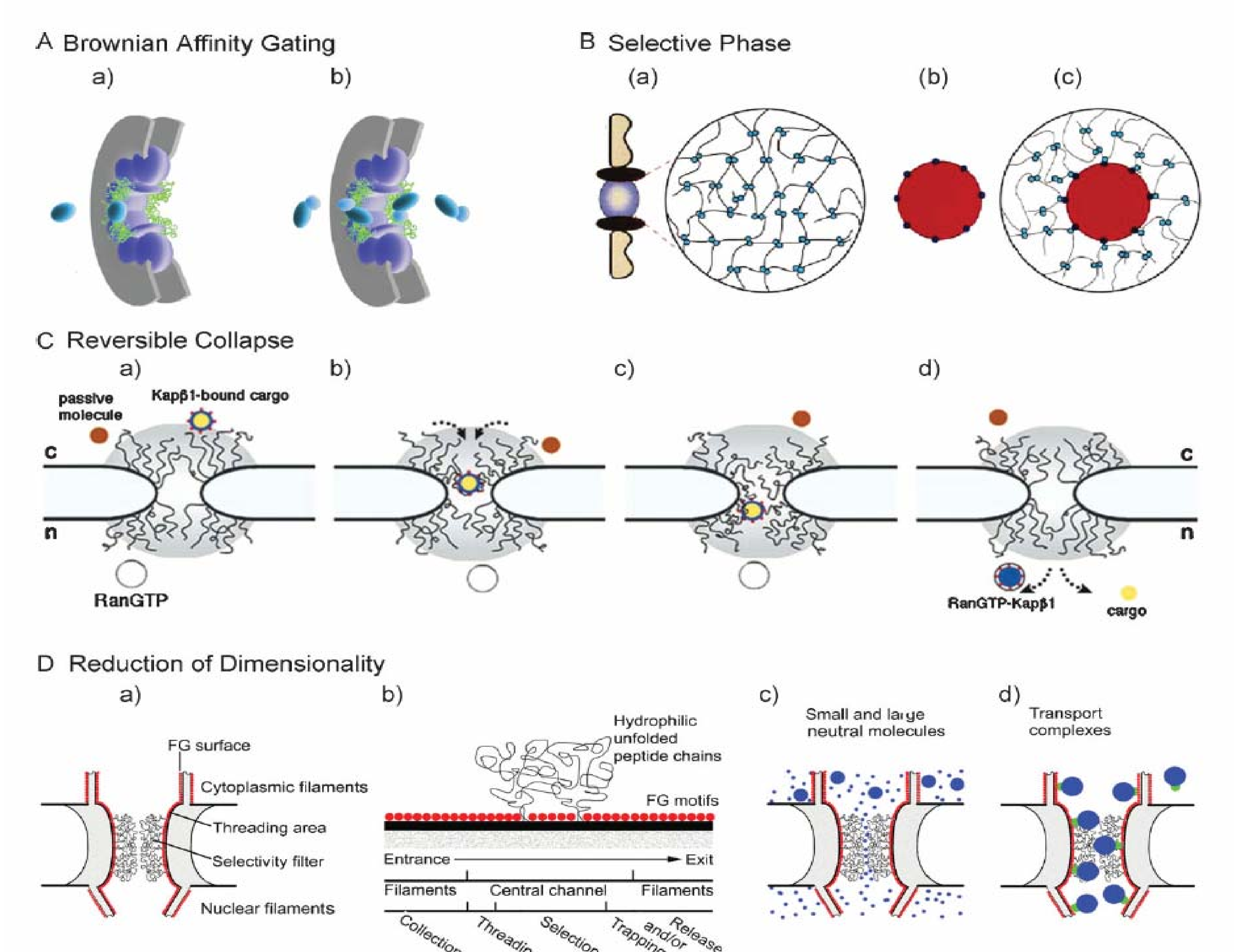
# Robustness of the NPC transport




Knockout of up to 50% of filaments in the central core does not impair viability

Strawn et al, Nature Cell Biol 2004

# The Nuclear Pore Complex: *oily spagetty* or a *gummy bear?*




Weiss, Cell 2005, Peters, BioEssays 2009

# Existing scenarios and models

‘Brush’ model

‘Gel’ model

‘Hydrophobic partitioning’ model

‘Brownian affinity’ aka ‘Virtual gating’ model

‘Affinity gradient’ model

‘The oily spagetti’ model

‘Dimensionality reduction’ model

‘Stepping stone’ model

More scenarios ........

Give a lot of importance to (yet not fully known) details of the FG nup conformation inside the pore

# Summary about structure and energetics

Some things are reasonably certain:

- Transport is a probabilistic process

- Translocation occurs by some sort of ‘facilitated diffusion’, without direct input of metabolic energy during transport

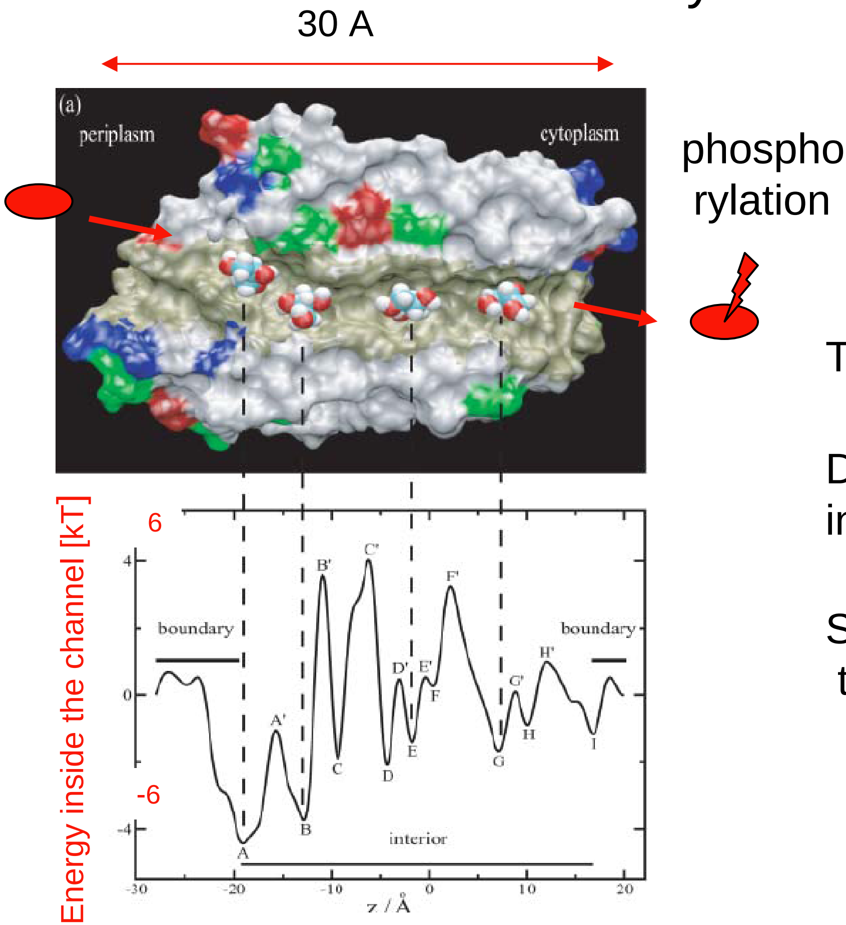
- Cognate molecules bind and are trapped inside the channel

- Selectivity is not based on size, but on the interaction of the cargoes with the channel

- Disruption of binding disrupts transport

# Other examples: porins

## Glycerol facilitator GlfP




Liu, Grayson and Schulten
Biophysical Journal 2005

Conducts linear polyalcohols

Glycerol is the preferred molecule

Transported more efficiently than smaller molecules

Directionality: glycerol is phosphorylated
inside the cell preventing backward transport

Selectivity and efficiency are related
to the potential inside the channel

## Maltoporin

Conducts maltose

Maltose transiently binds inside

Kullman et al 2006

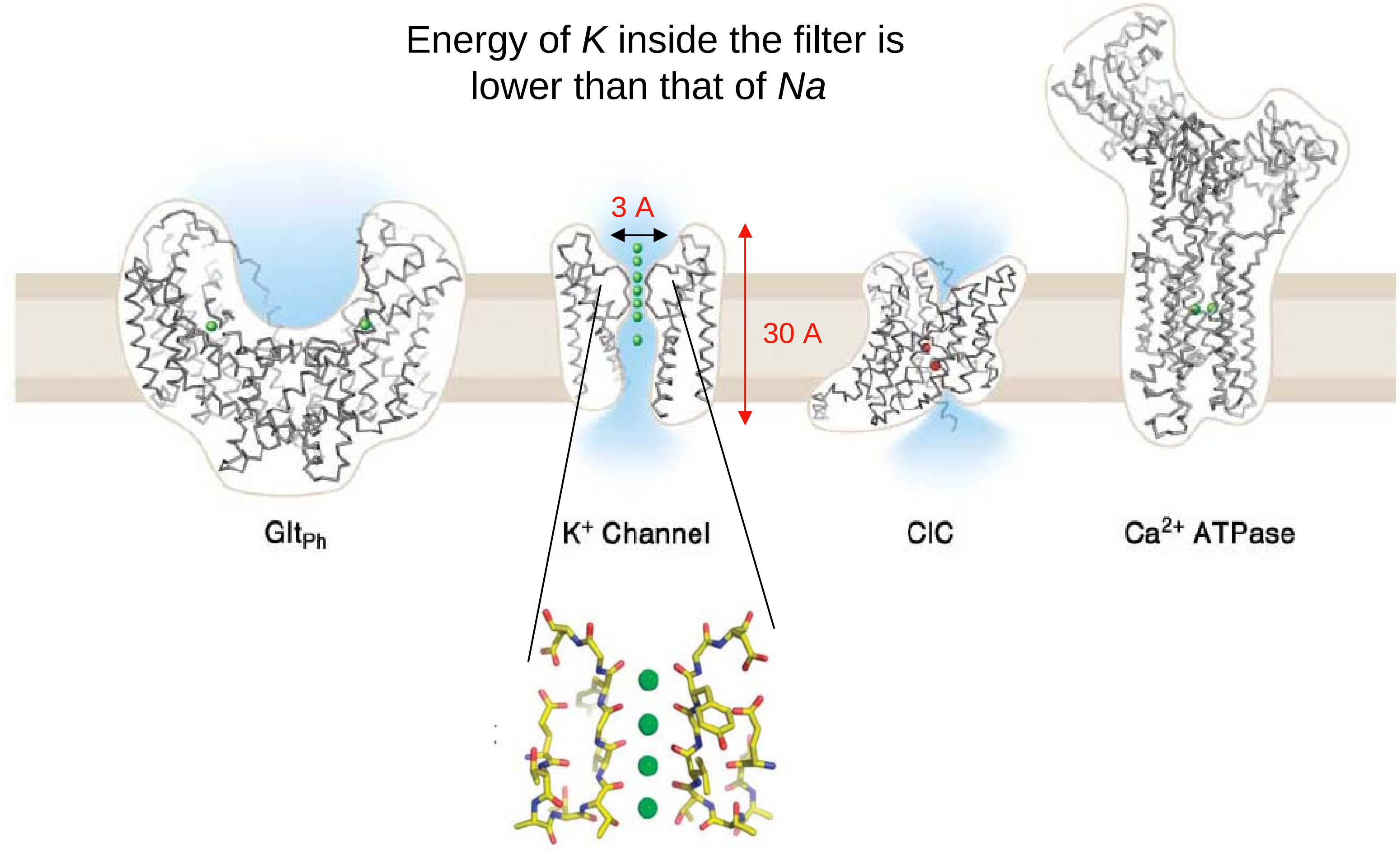
# Classical (but distant) example: ion channels

Conduct ions down the concentration or electric field gradient: directionality

Transports *K* with 10000 – fold preference over Na

*Na* is smaller than *K* !

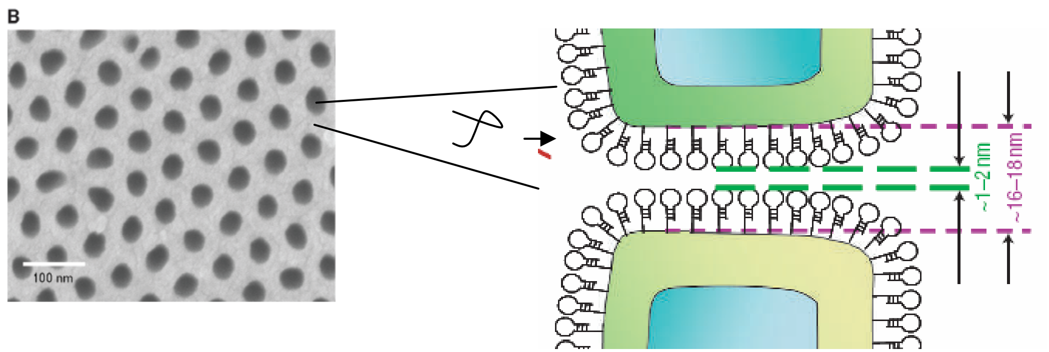
Energy of *K* inside the filter is lower than that of *Na*

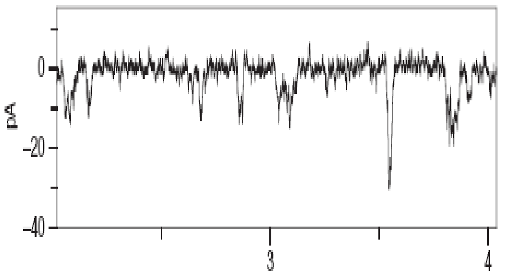


# In vitro models: artificial channels and biosensors

## Nanopores functionalized single stranded DNA

Kohli et al, **Science (2004),** Iqbal et al, **Nature Nano (2008**)

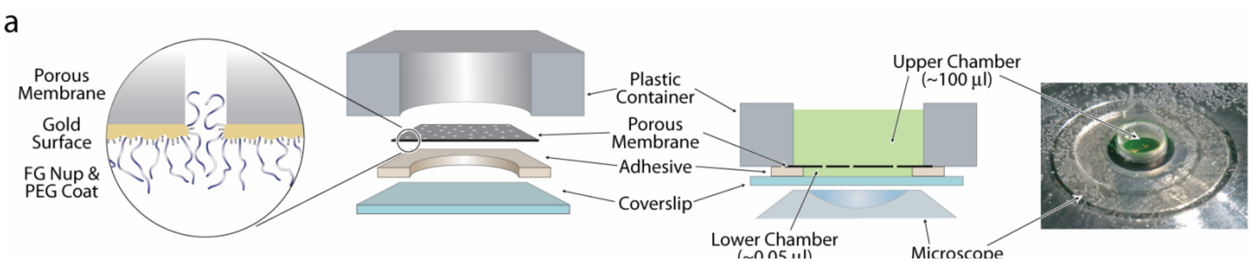



Discriminates between different DNA sequences

## Nanopores functionalized with nuclear pore filaments

Jovanovic-Talisman et al, **Nature (2009)**




Discriminates between different proteins

# In vitro models: artificial channels and biosensors

**Nanopores functionalized with synthetic polymers**

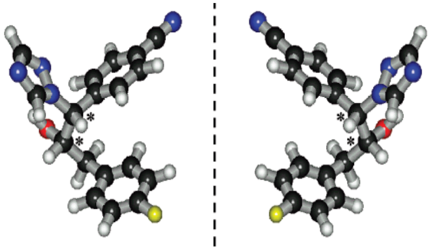
Caspi et al, **Nano Lett** (2008)

Selectively transport transporter-bound cargoes

**Nanopores functionalized with antibodies**

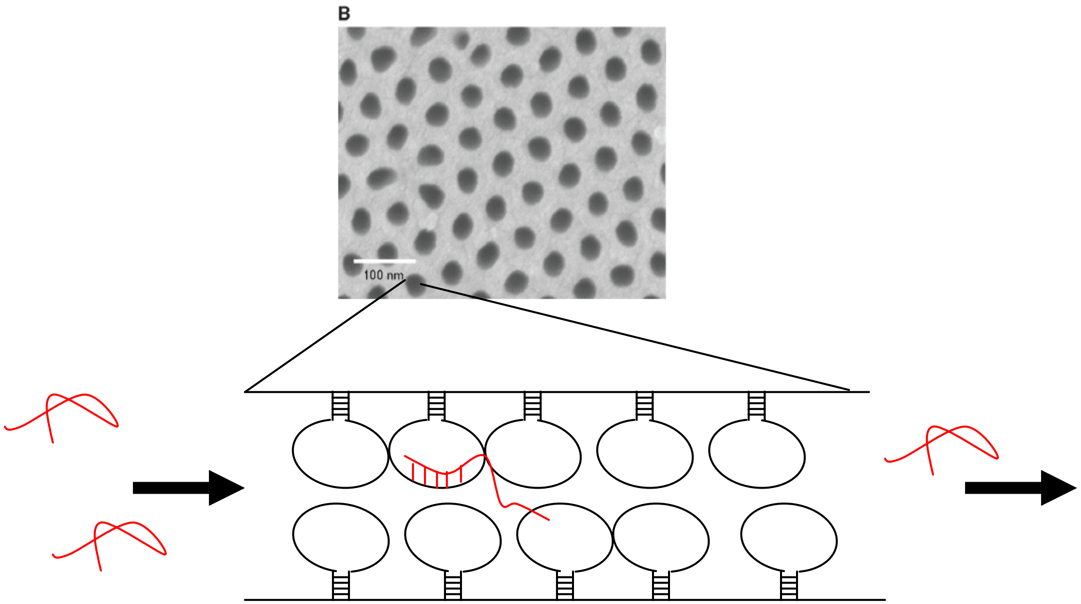
Lee et al, **Science** 1998

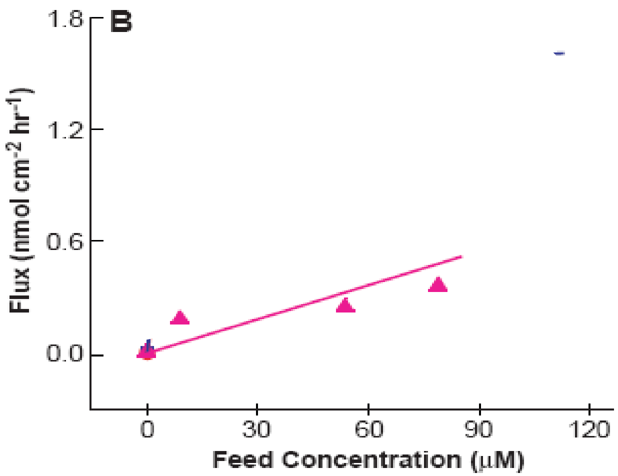
Discriminate between molecular enantiomers



Etc…

# DNA-functionalized nano-channels




Kohli et al, **Science (2004)**

# How does one make sense of all this?

**Fundamental biological and physical question:**
**how does one make a selective channel that is ‘always open’?**

**Are there general principles in a wider context?**

**What is the right framework to think about it?**

**What are the core phenomena that need to be explained?**

# Minimal model

**Needs to answer:**

How the transport is selective?

How binding enhances diffusion without clogging?

What is the role of  confinement and jamming?

How selectivity is maintained in the presence of non-specific competition?

**Minimal model with only two ingredients**

**1. binding (trapping) and 2. confined space**

Stochastic description of transport  based on physical principles

Can be tested in controlled experiments

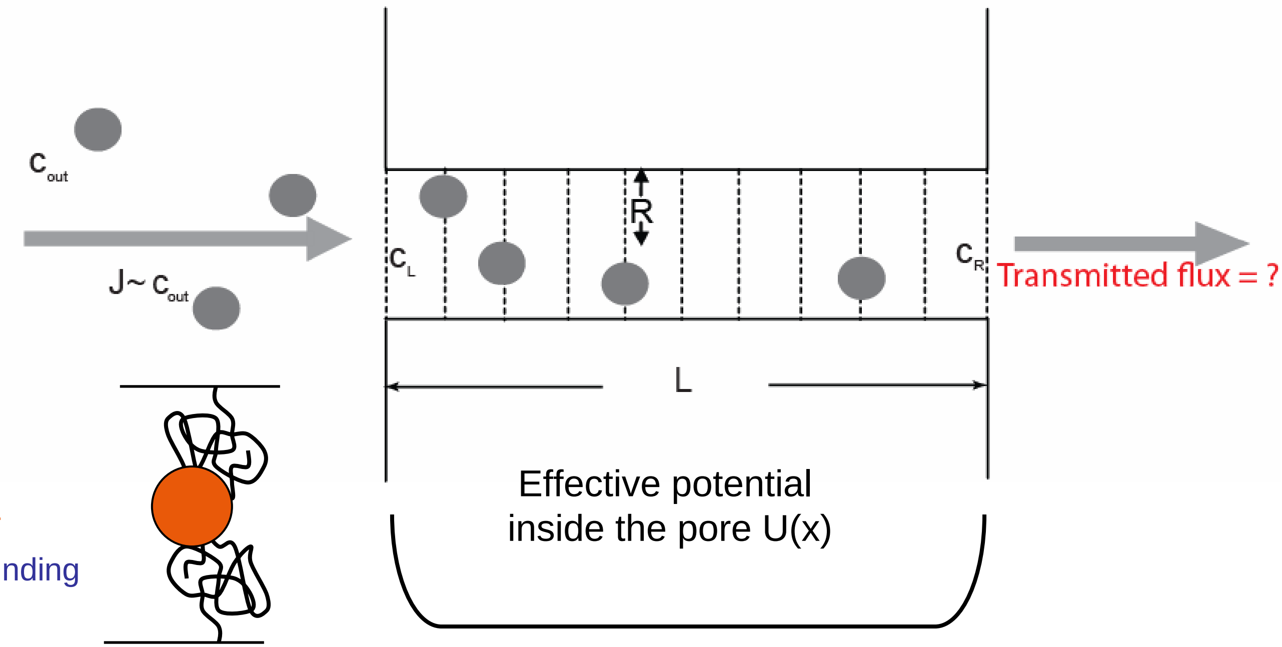
# Important concepts

- **Directionality**
- **Selectivity**
- **Efficiency (rate)**
- **Speed (time)**

# The role of trapping

U(x)= repulsion due to filaments –

attraction due to binding




## Diffusion at steady state

Entering flux

$$J = \alpha(c_L^\infty - c_L) R D_{\text{out}} = F$$

Exiting flux

$$J_{\text{out}} = \alpha c_R R D_{\text{out}} = F$$

Flux inside

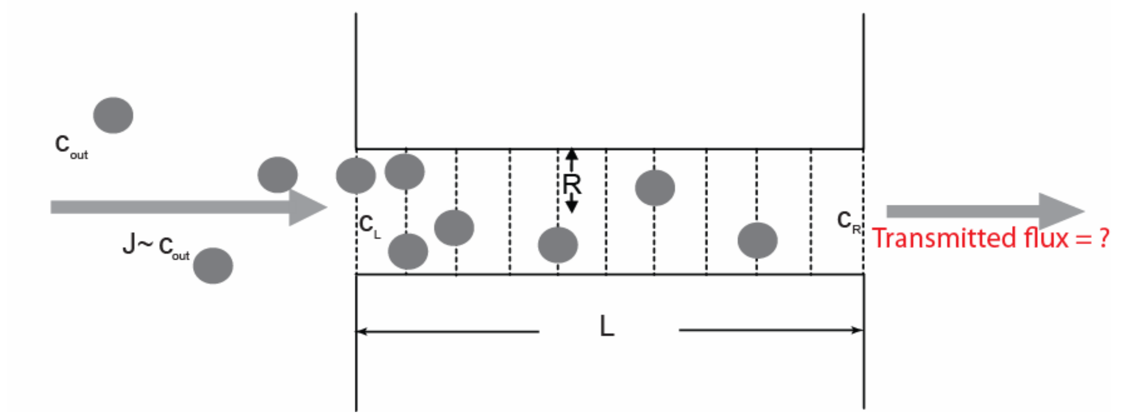
$$F = \frac{c_L^{1} - c_R^{1}}{\int_0^L e^{U(x)/T}\, dx}$$

Facilitated flux through the channel

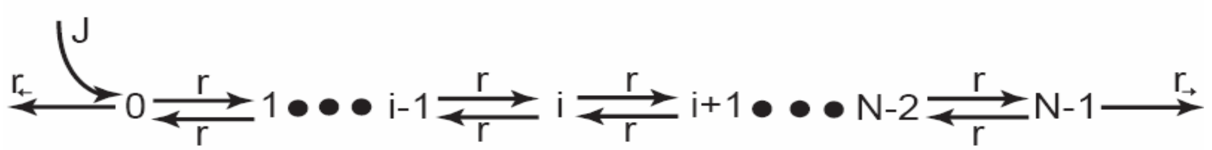
$$J_{\text{out}} / J = \frac{1}{2 + \frac{\alpha}{\beta} \frac{D_{\text{out}}}{D_{\text{in}}} \frac{L}{R} e^{-U/kT}}$$

Increases with the binding strength U

# Inter-particle interactions inside the channel




## Further model reduction: one-dimensional hopping




Diffusion inside

$$r = D_{in} / a^2$$

Exit rate is reduced due to binding

$$r_{\rightarrow} = r_{\leftarrow} \sim e^{-E/kT}$$

## Equation for occupancy probabilities

$$\frac{dp_i}{dt} = -2rp_i + rp_{i+1}(1 - p_i) + rp_{i-1}(1 - p_i)$$

# Jamming: saturation and selectivity

Particles inside the channel prevent entrance of the new ones and interfere with each other

Probability to enter

$$P_{in} = 1 - p_1$$

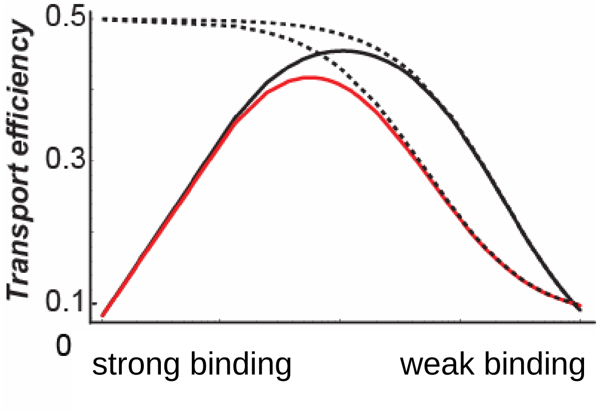
Probability to translocate

$$P_{\rightarrow} = \frac{1}{2 + \frac{\alpha}{\beta}\frac{D_{\text{out}}}{D_{\text{in}}}\frac{L}{R}e^{-E}}$$

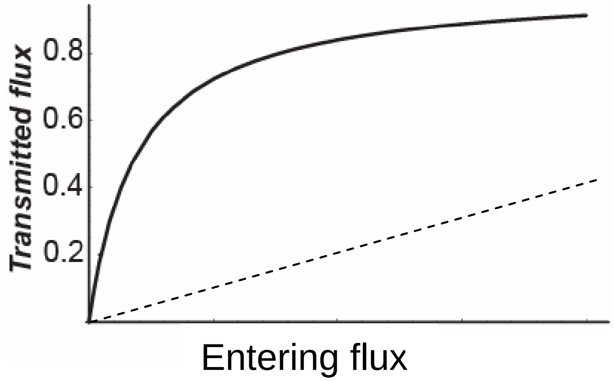
Total transport efficiency

$$J_{\text{out}}/J = \frac{1}{2+\frac{\alpha}{\beta}\frac{D_{\text{out}}}{D_{\text{in}}}\frac{L}{R}e^{-E} + \frac{J}{D_{out}/SR}e^{E}\left(1+\frac{\alpha}{\beta}\frac{D_{\text{out}}}{D_{\text{in}}}\frac{L}{R}e^{-E}\right)}$$

Optimality

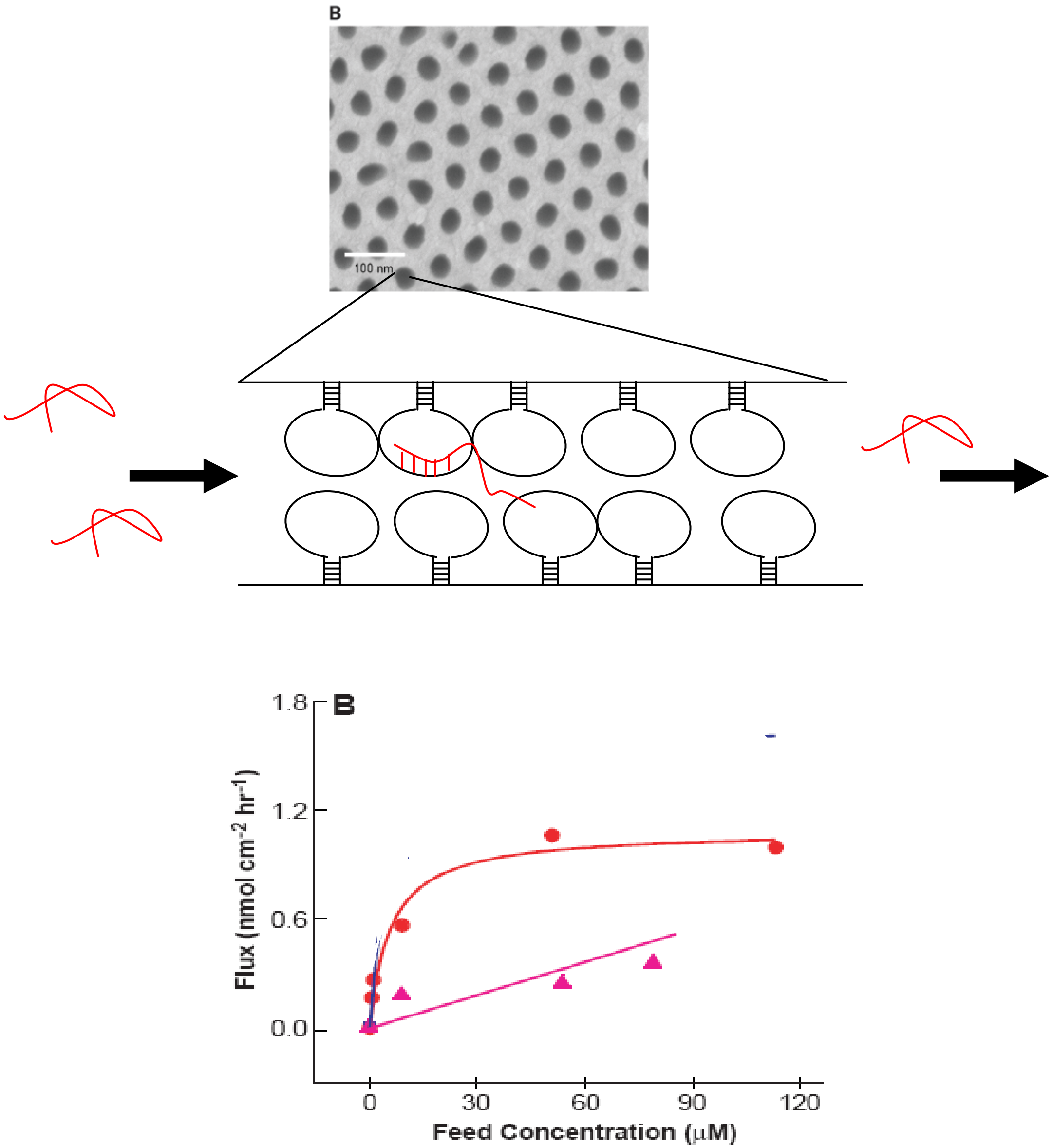



Saturation



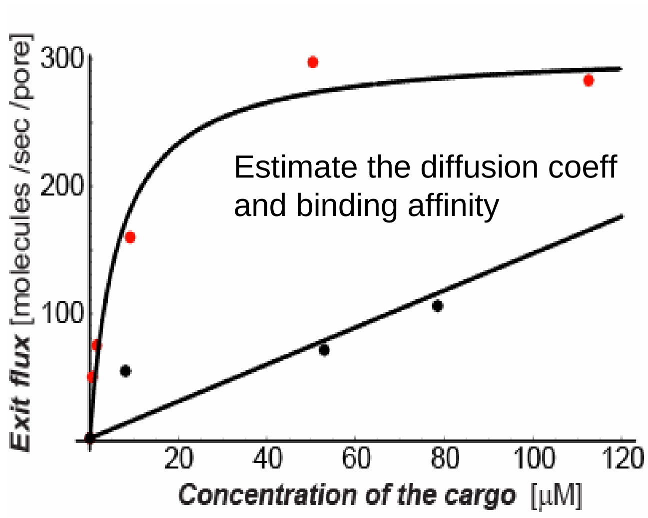
Does it work?

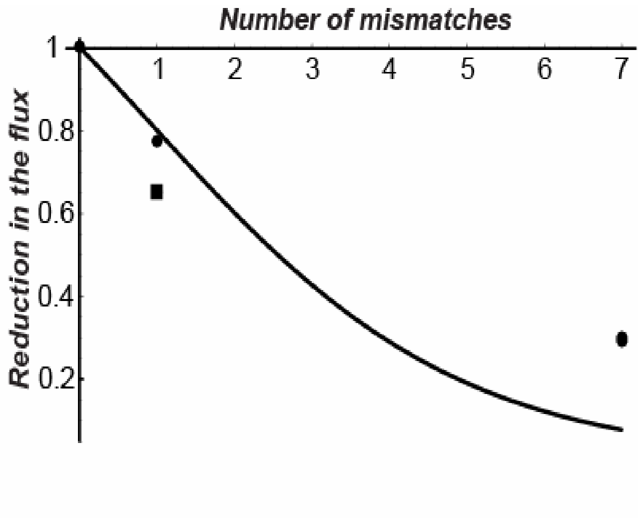
# DNA-functionalized nano-channels



# Comparison with experiment

Flux of ssDNA through DNA-functionalized channels




Saturation of the flux - confirmed

Facilitation of flux by binding - confirmed

Existence of the optimal binding – not yet

# Non-specific competition

The binding energies are not high – sometimes several *kT* only

Anything can bind to anything with several *kT* energy

In the cell, specific molecules have to be transported in the presence of several orders of magnitude higher amounts of others

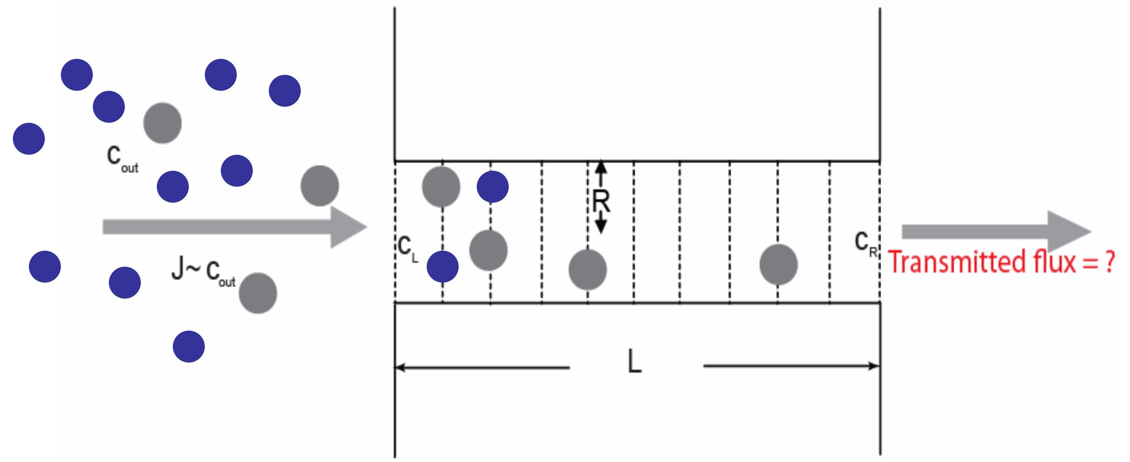
Artificial molecular sorters: detecting minute amounts of specific molecules?

Similar problems: separation membranes (e.g. reverse osmosis)

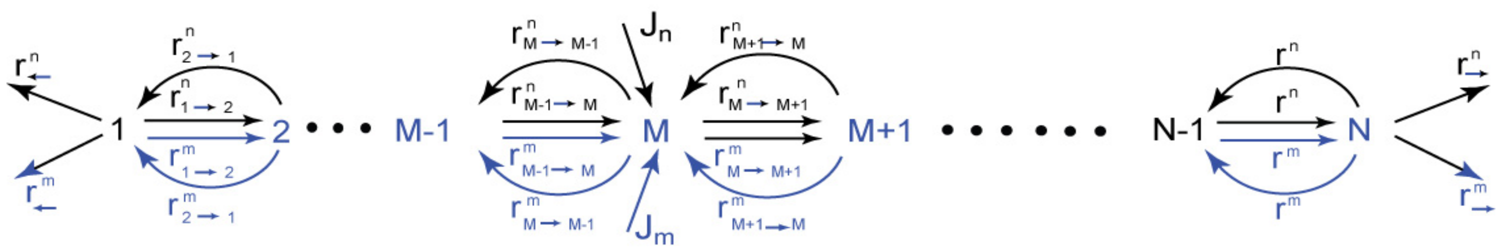
One solution: ‘non-fouling’ surfaces – difficult to achieve desired functionalization

Other mechanisms?

# Non-specific competition: model




*Channel of N positions (sites)*

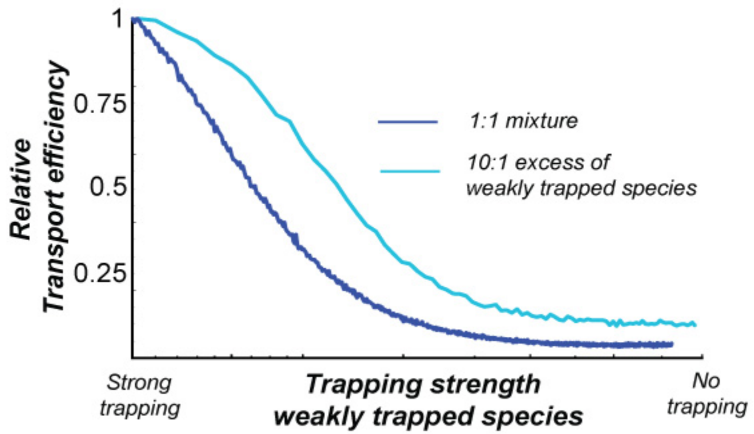



Simulations

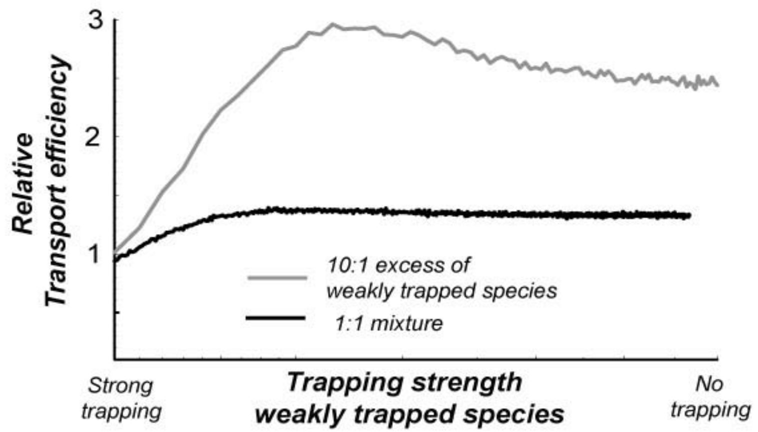
$$P^n_{i\to i\pm1} = \frac{R_{i\to i\pm1}}{(J_n + J_m)(1 - o(M)) + \sum_i [R_{i\to i\pm1} + Q_{i\to i\pm1}]}$$

# Strongly trapped specific  particles filter out the non-specific

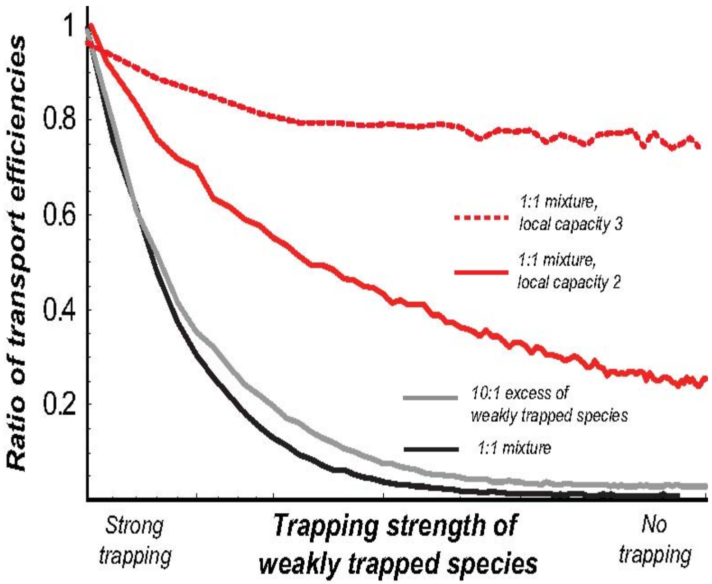
Weakly trapped species

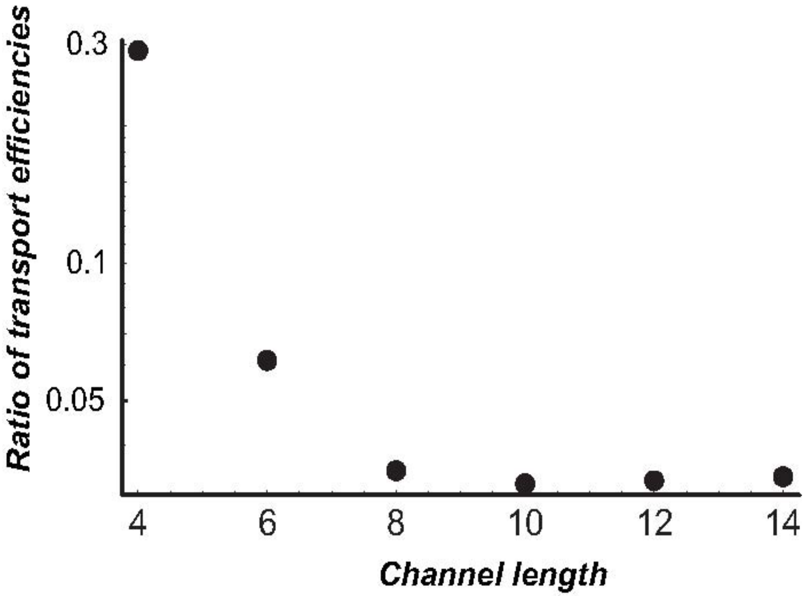



Strongly trapped species



# Selectivity increases with channel length and decreases with width




Purely kinetic, non-equilibrium mechanism

Holds for a very general choice of kinetic profiles

Confinement and sequential transport are critical

# Modeling the experiments

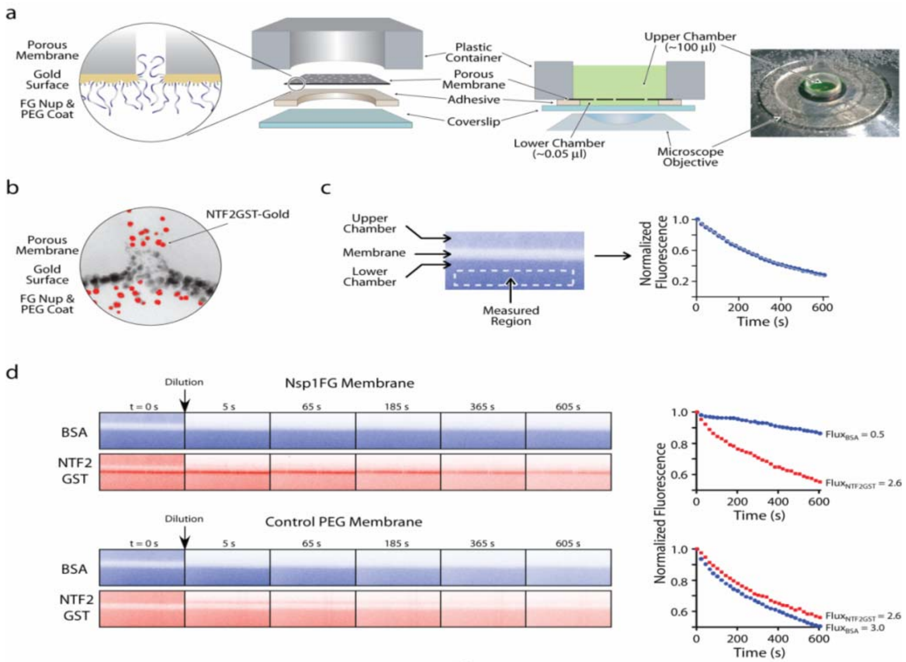
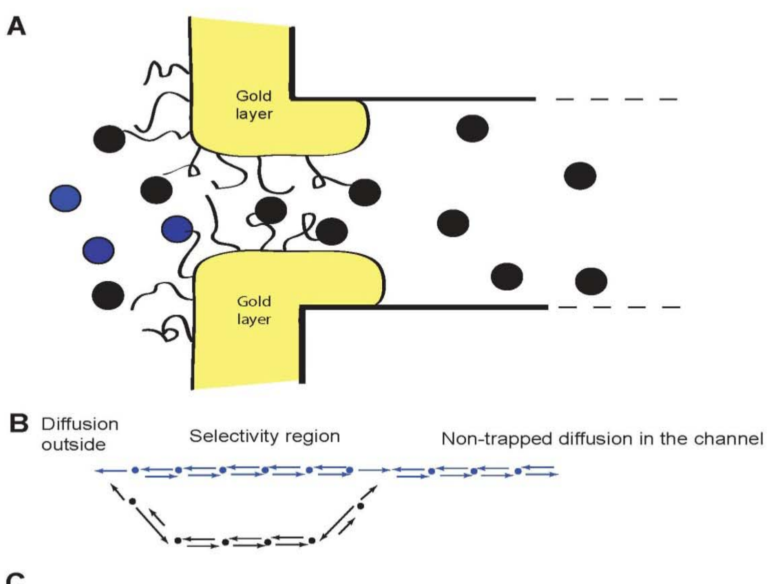
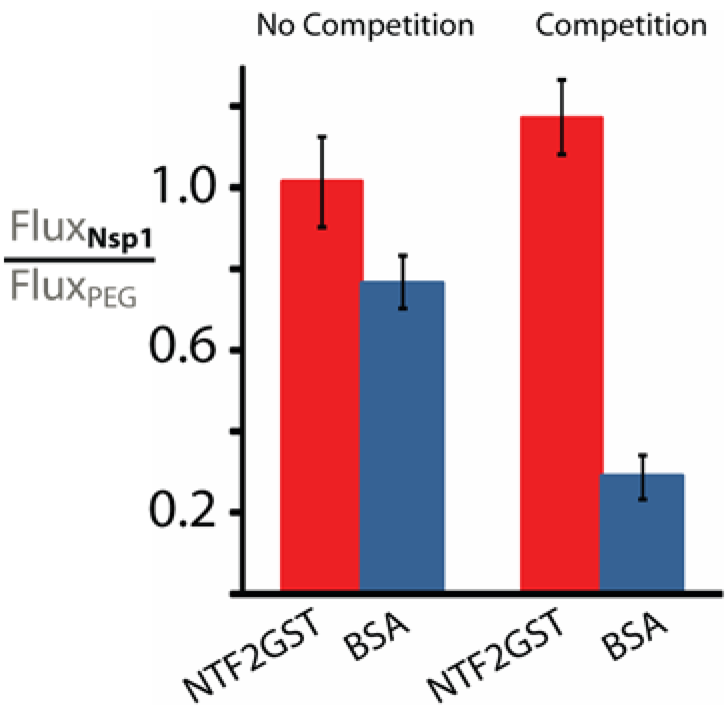



**Figure 1.**

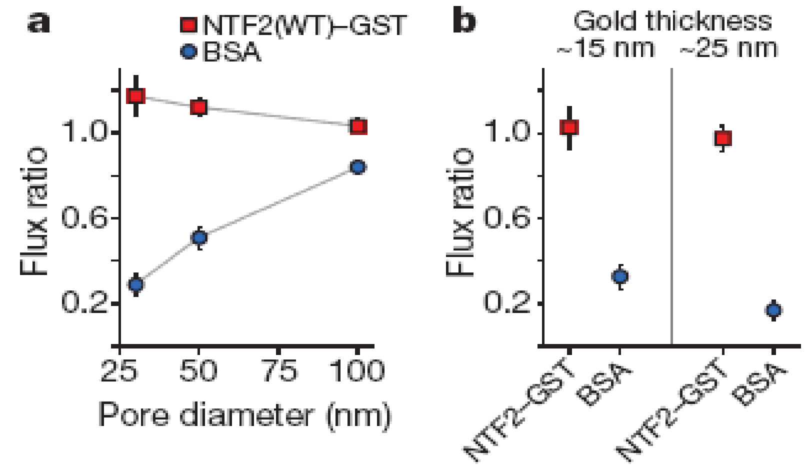


# Comparison with experiment

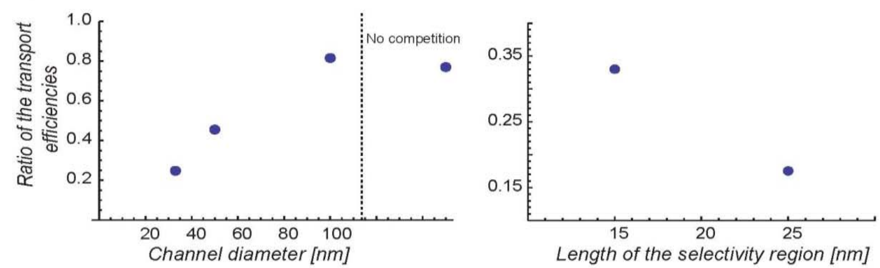
## Experimental results




A




C



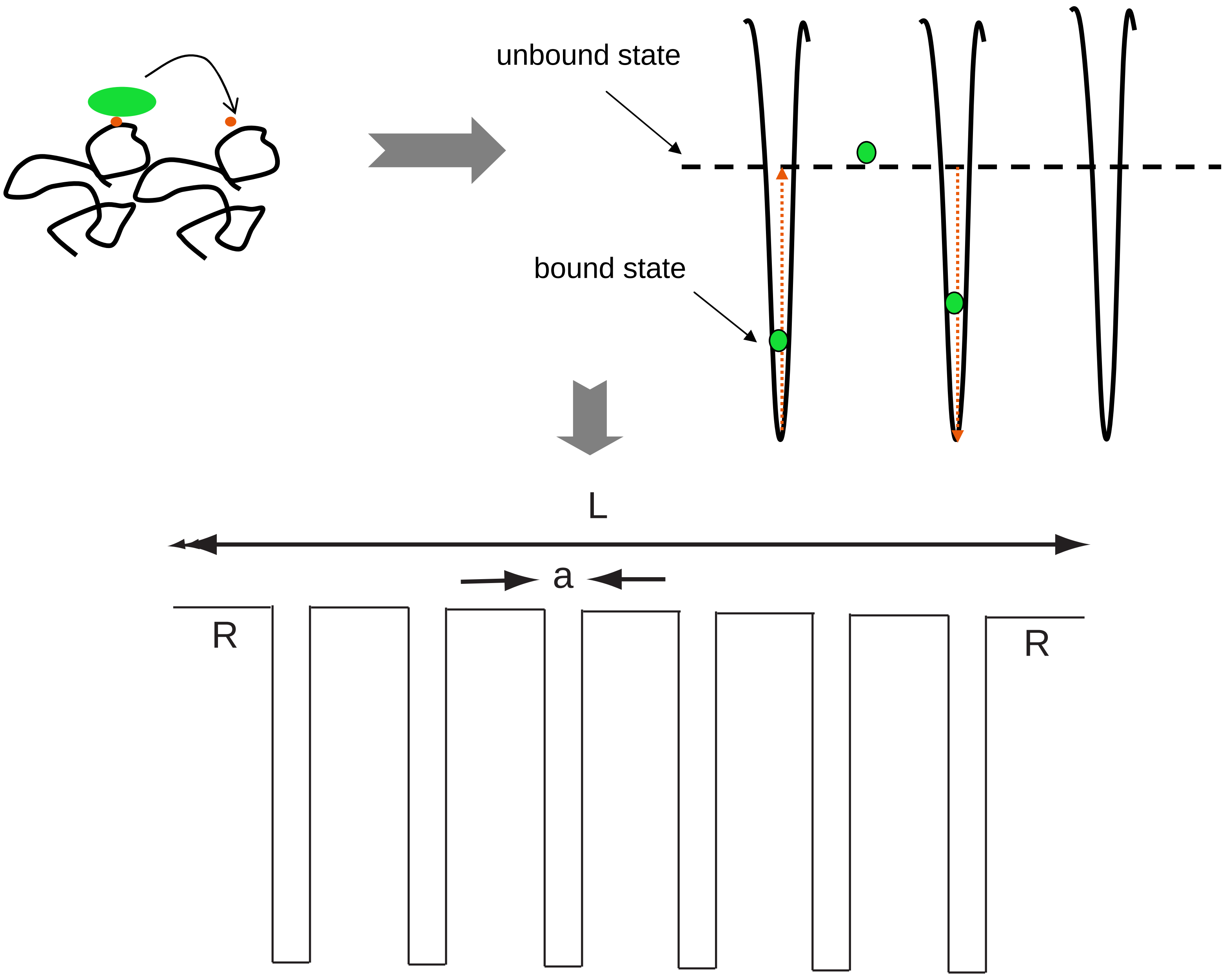

Transport of weakly binding non-specific cargoes is inhibited

Transport of specific cargoes is enhanced

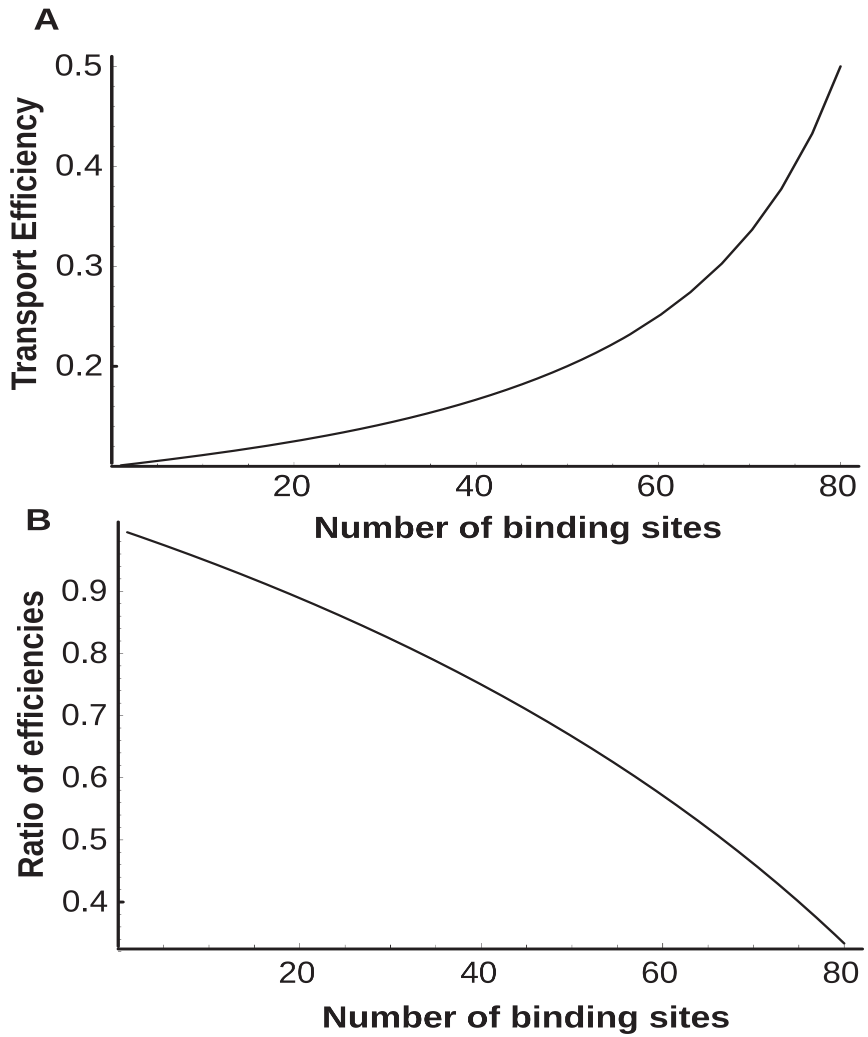
Effect increases with length, decreases with width

Tijana Jovanovic-Talisman et al, Nature 2009

# More reality: discrete binding sites

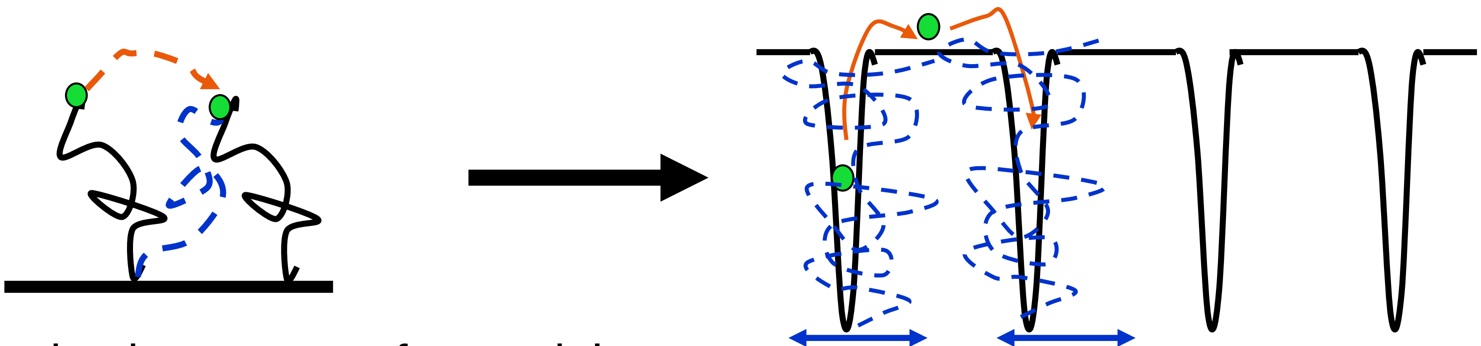


# Efficiency as a function of the number of binding sites




In the region of high efficiency, the transport is sensitive to the numbers of sites

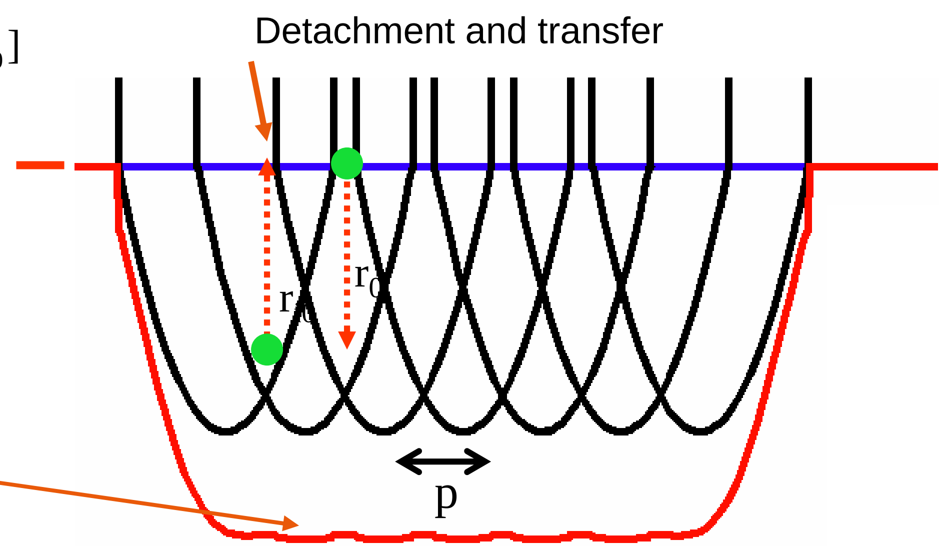
# Fluctuating filaments: effective potential



Diffusion in an array of potentials

$$\frac{\partial P_i}{\partial t} = D\frac{\partial^2 P_i}{\partial x^2} - \frac{\partial}{\partial x} P_i \frac{\partial}{\partial x} U_i - w_{i0}P_i + w_{0i}P_0$$

$$\frac{\partial P_0}{\partial t} = D\frac{\partial^2 P_0}{\partial x^2} - \frac{\partial}{\partial x} P_0 \frac{\partial}{\partial x} U_0 + \sum_i \left[w_{i0}P_i - w_{0i}P_0\right]$$




## Effective potential

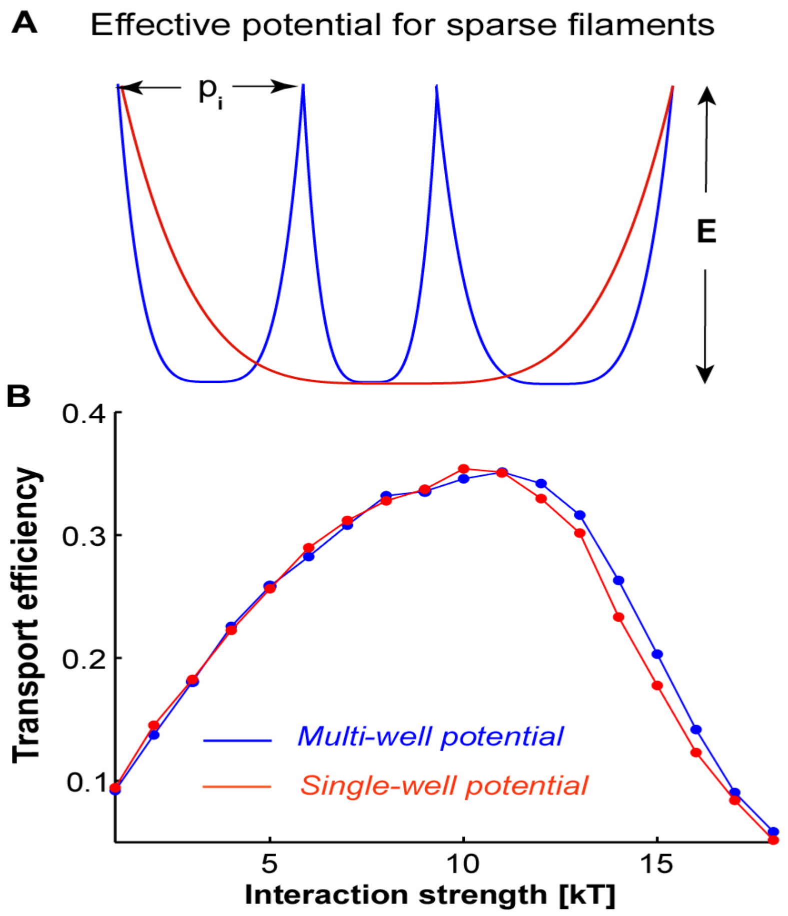
$$\frac{\partial P_{tot}}{\partial t} = D\frac{\partial^2 P_{tot}}{\partial x^2} - \frac{\partial}{\partial x} P_{tot} \frac{\partial}{\partial x} U_{eff}$$

$$U_{eff}(x) = -T\ln\left(1 + \sum_i e^{-U_i(x)/T}\right)$$

Transport properties are not very sensitive to the shape of the effective potential

# Sparse wells

Robust even in the case of sparse wells




Transport properties are identical in single- and multi well potential:
explains why deletion of up to third of the filaments does not abolish transport

# Summary and conclusions

Minimal model: trapping and confinement

Molecular details are integrates into effective affinities and diffusion coefficients

Binding increases flux

Due to confinement, strongly binding specific cargoes
filter out non-specific  competitors

Selectivity must be considered in the presence of multiple species:
transport factors are part of the 'machine'

Theory explains behavior of artificial channels that mimic the NPC

Theory provides guidelines for design of nano-bio-sensors

Hints that theoretical physics can be useful for biology

**One of the principal objects of research in my department of knowledge is to find the point of view from which the subject appears in the greatest simplicity.**

***Josaiah Willard Gibbs***