RICE UNIVERSITY

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Center for Theoretical Biological Physics

How to Understand Molecular Transport through Channels: The Role of Interactions

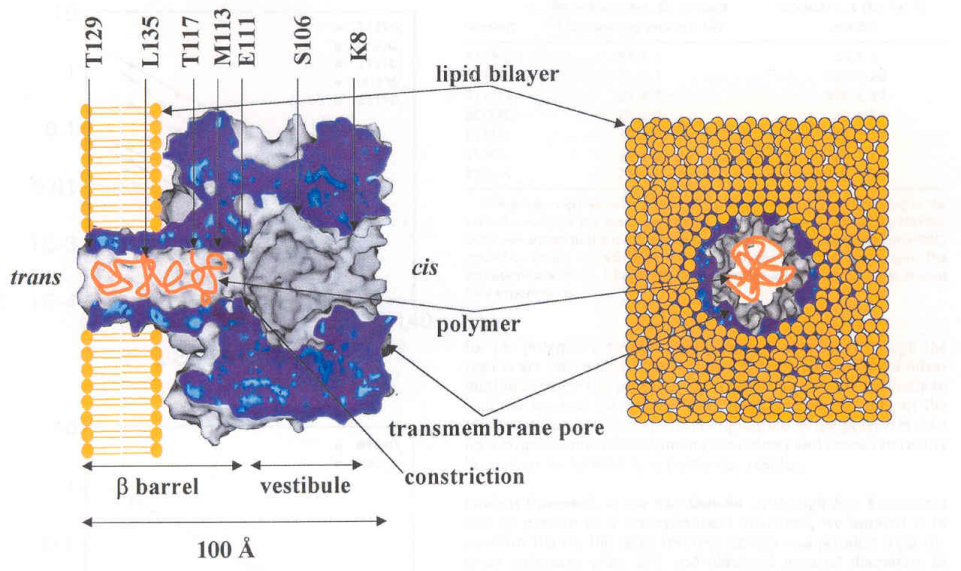
Transport Through Channels

Chromatography

Chemistry, Physics

Oil pumping

Industry



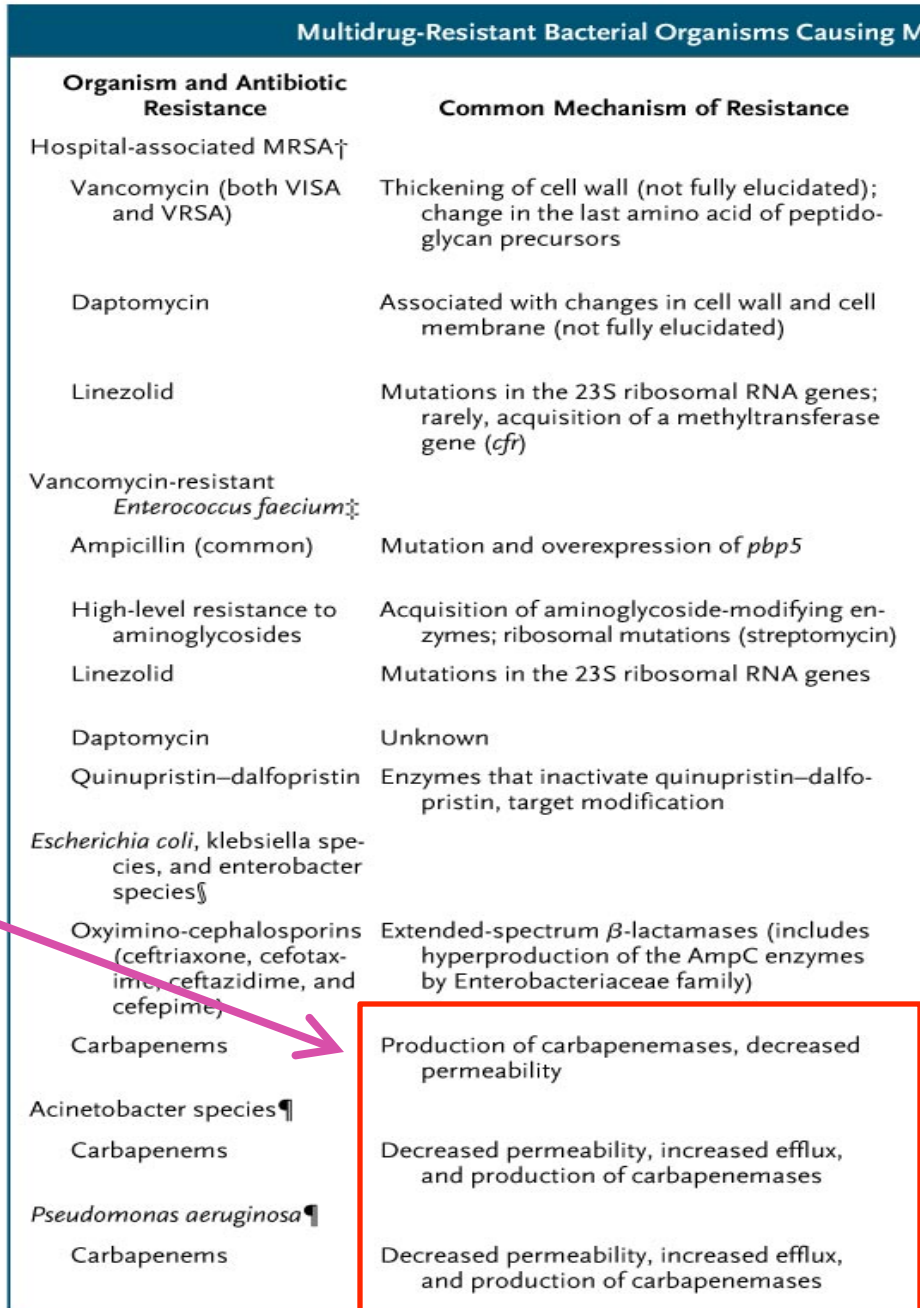
Translocation through membranes

Biology

Example: Resistance to Antibiotics

Major medical problem:

1) bacteria are developing resistance to drugs
2) Very few new anti-bacterial compounds
3) Mechanisms of resistance are unclear in many cases
4) One of the most important mechanisms – "permeability barrier"



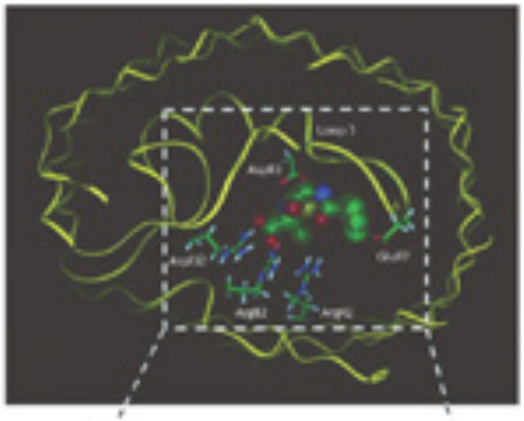
Multidrug-Resistant Bacterial Organisms Causing M

Organism and Antibiotic Resistance	Common Mechanism of Resistance
Hospital-associated MRSA†	
Vancomycin (both VISA and VRSA)	Thickening of cell wall (not fully elucidated); change in the last amino acid of peptidoglycan precursors
Daptomycin	Associated with changes in cell wall and cell membrane (not fully elucidated)
Linezolid	Mutations in the 23S ribosomal RNA genes; rarely, acquisition of a methyltransferase gene (*cfr*)
Vancomycin-resistant *Enterococcus faecium*‡	
Ampicillin (common)	Mutation and overexpression of *pbp5*
High-level resistance to aminoglycosides	Acquisition of aminoglycoside-modifying enzymes; ribosomal mutations (streptomycin)
Linezolid	Mutations in the 23S ribosomal RNA genes
Daptomycin	Unknown
Quinupristin–dalfopristin	Enzymes that inactivate quinupristin–dalfopristin, target modification
Escherichia coli, klebsiella species, and enterobacter species§	
Oxyimino-cephalosporins (ceftriaxone, cefotaxime, ceftazidime, and cefepime)	Extended-spectrum β-lactamases (includes hyperproduction of the AmpC enzymes by Enterobacteriaceae family)
Carbapenems	Production of carbapenemases, decreased permeability
Acinetobacter species¶	
Carbapenems	Decreased permeability, increased efflux, and production of carbapenemases
Pseudomonas aeruginosa¶	
Carbapenems	Decreased permeability, increased efflux, and production of carbapenemases

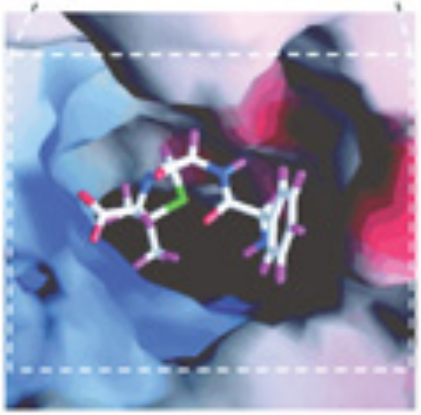
N. Eng. J. Med. **360**, 439 (2009)

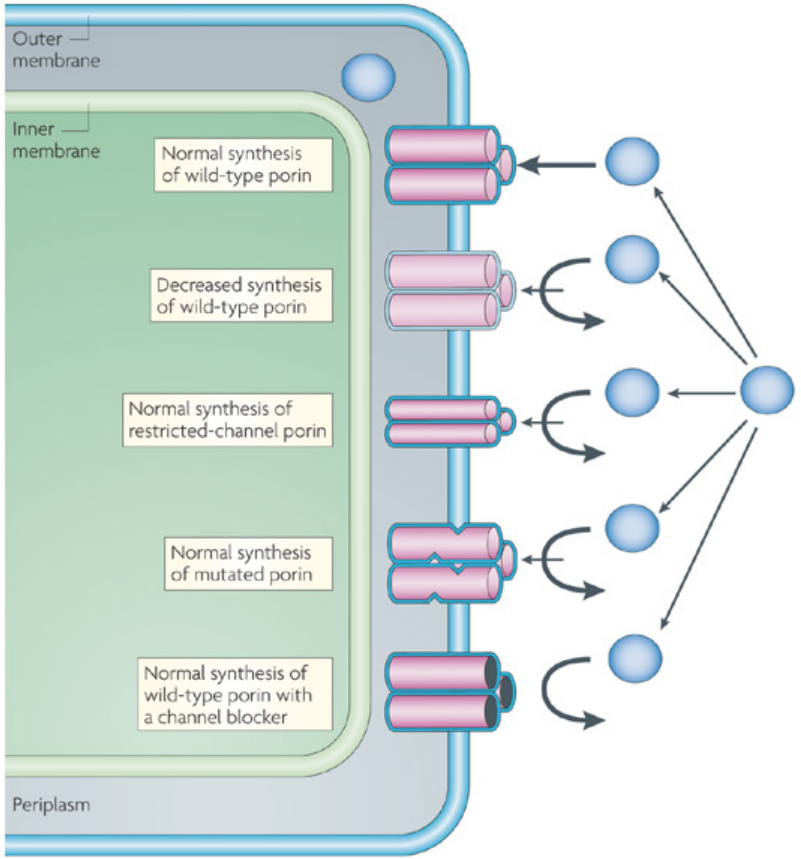
Example: Resistance to Antibiotics

A key resistance mechanism in Gram-negative bacteria is the prevention of the antibiotic uptake via channel proteins porins.



Antibiotic docking to porin channels



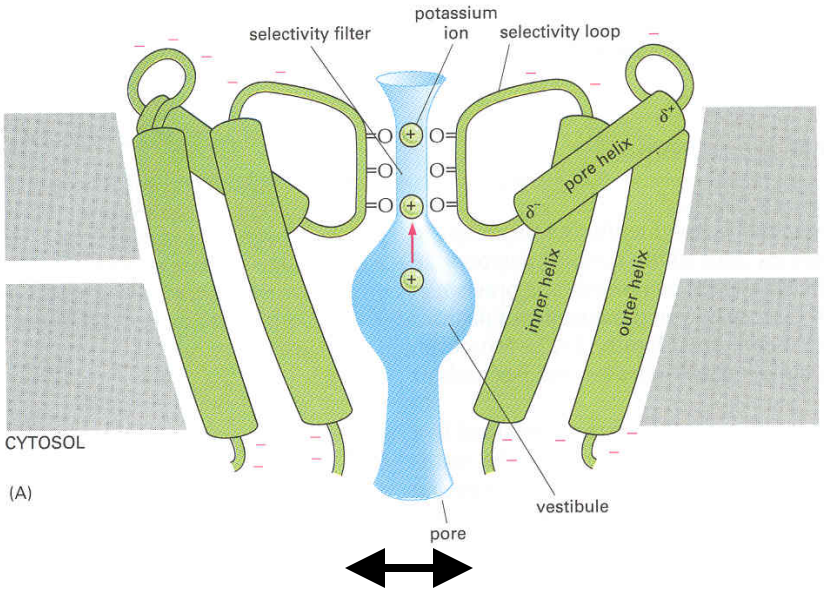


Nature Reviews | Microbiology

Multidrug resistance mechanisms associated with porin modification

Nat. Rev. Microbiol., **6**, 893, 2008

Membrane Protein Channels: 2 types

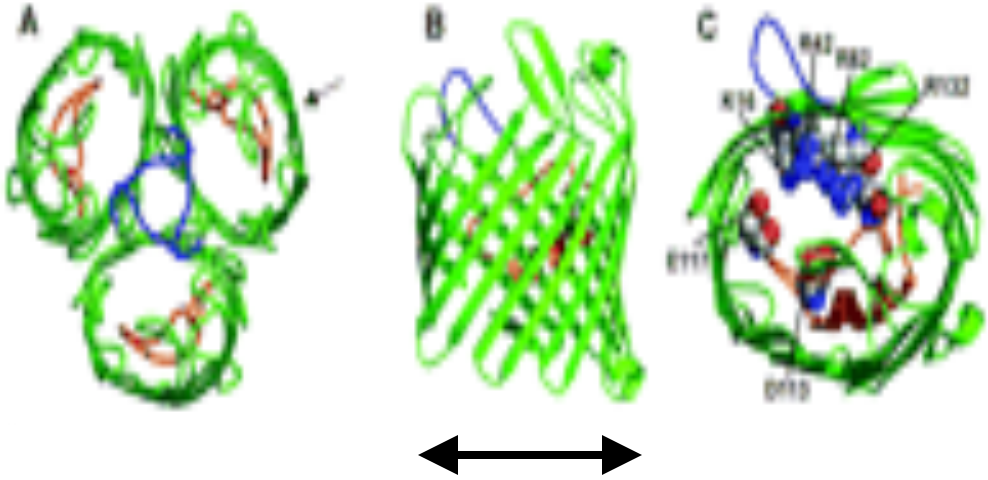


0.1 nm

Ion Channels

Active Transporters

Highly efficient and very selective



2 nm

Large water-filled proteins

Assumed: Passive Transporters

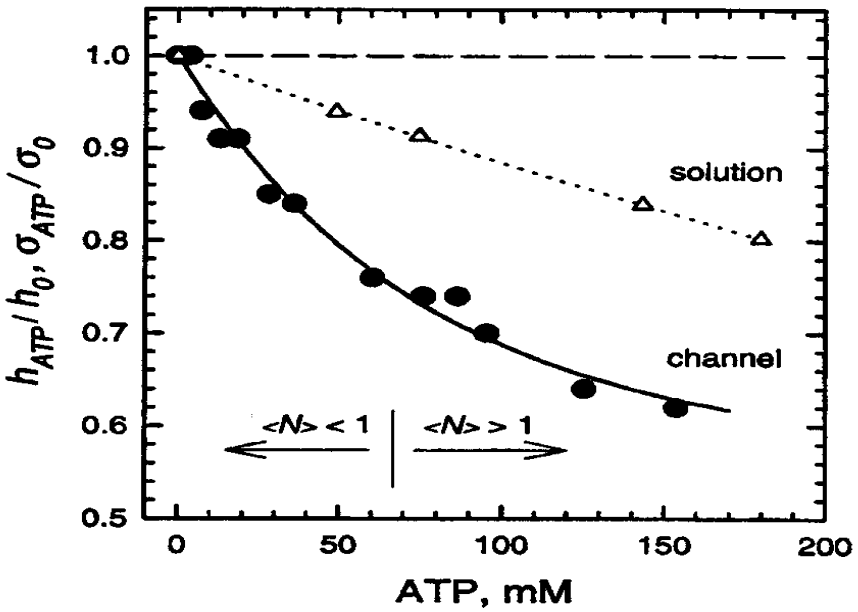
Low efficiency and selectivity

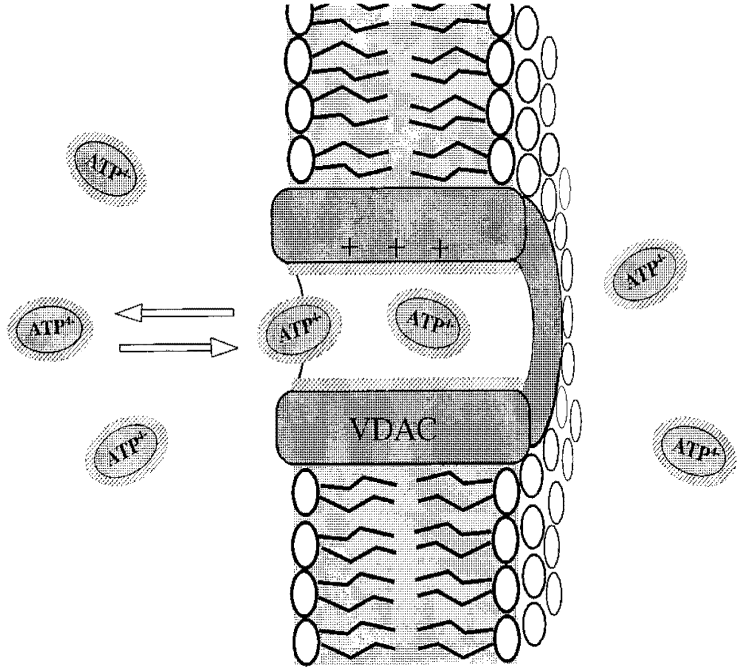
BUT …

Large Membrane Pores: Selectivity

Transport of ATP molecules through mitochondrial channel VDAC studied by current fluctuation analysis

Biophys. J. **74**, 2365 (1998)





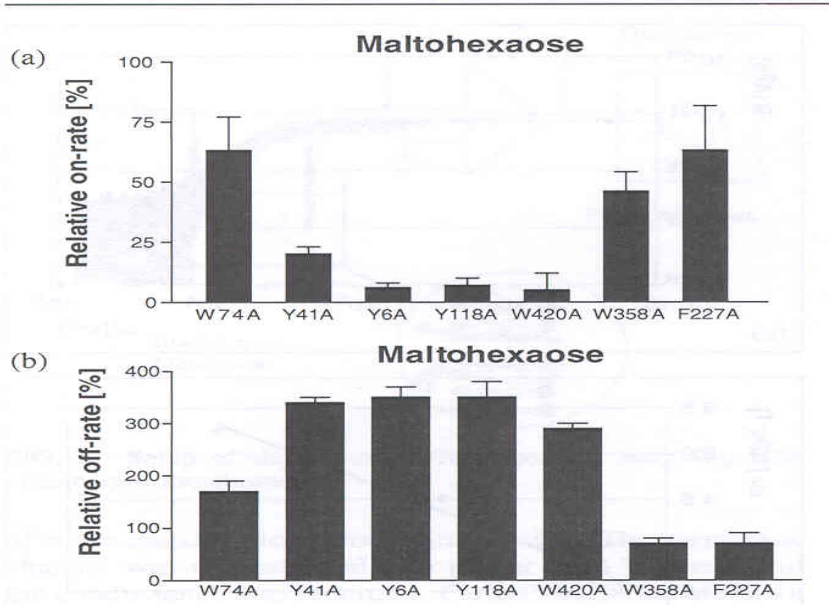
The effect of ATP addition on VDAC channel and bulk solution conductance

Lower conductance in the channel means that ATP interacts with the pore stays longer in the channel

Large Membrane Pores: Selectivity

Transport of sugar molecules through maltoporin LamB channel studied with current fluctuations

Phys. Rev. Lett. **86**, 5624 (2001)



Maltose molecules interact specifically with channel residues – this is the reason for selectivity and for the efficiency

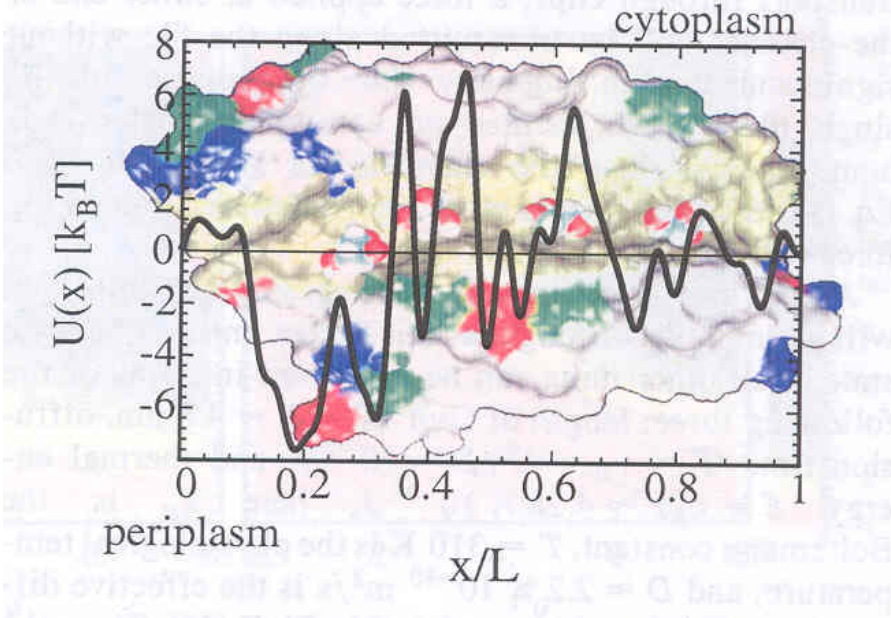
effect of mutations with respect to the wild type on kinetic rates

Theoretical Efforts:

Molecular Dynamics Computer Simulations:

Stimulated by increasing amount of structural information

K. Schulten, M. Ceccarelli, I. Kosztin, R.D. Coalson, A. Aksimentiev…



Problems with full-atomic MD simulations: can describe systems with <100,000 atoms for few ns, not enough for real biological transport systems

Coarse-grained MD and/or more phenomenological physical-chemical analytical models. But there is a lot of confusion!

Theoretical Efforts:

Molecular transport through channels and pores: Effects of in-channel interactions and blocking

Wolfgang R. Bauer*† and Walter Nadler‡

PNAS, **103**, 11446 (2006)

*Medizinische Universitätsklinik 1, Josef Schneider Strasse 2, D-97080 Würzburg, Germany; and ‡Department of Physics, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931-1295

Edited by Nicholas J. Turro, Columbia University, New York, NY, and approved June 5, 2006 (received for review March 3, 2006)

Facilitated translocation of molecules through channels and pores is of fundamental importance for transmembrane transport in biological systems. Several such systems have specific binding sites inside the channel, but a clear understanding of how the interaction between channel and molecules affects the flow is still missing. We present a generic analytical treatment of the problem that relates molecular flow to the first passage time across and the number of particles inside the channel. Both quantities depend in different ways on the channel properties. For the idealized case of noninteracting molecules, we find an increased flow whenever there is a binding site in the channel, despite an increased first passage time. In the more realistic case that molecules may block the channel, we find an increase of flow only up to a certain threshold value of the binding strength and a dependence on the sign of the concentration gradient, i.e., asymmetric transport. The optimal binding strength in that case is analyzed. In all cases the reason for transport facilitation is an increased occupation probability of a particle inside the channel that overcomes any increase in the first passage time because of binding.

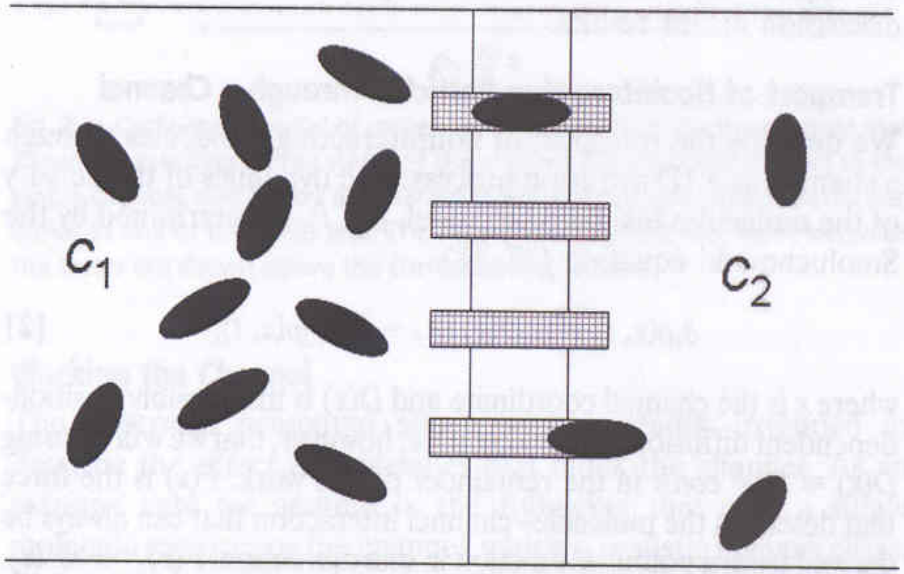


Fig. 1. Basic biological situation. A membrane separates two baths with molecular concentrations c_1 and c_2. The baths are connected by channels (hatched rectangles), allowing only access to a single molecule.

WRONG! Infinite interactions – no current!

Theoretical Approaches:

1) Continuum models – transport through the channels is viewed as a motion of the particle in the effective 1D potential created by interactions with the pore

Berezhkovskii, Bezrukov, *J. Chem. Phys.*, **119**, 3943 (2003); *Chem. Phys.*, **319**, 342 (2005); *Biophys. J.*, **88**, L11 (2005); *J. Chem. Phys.*, **127**, 115101.

2) Discrete models- translocation dynamics is viewed as hopping between discrete binding sites.

T. Chou, *Phys. Rev. Lett.*, **80**, 85 (1998), *J. Chem. Phys.*, **110**, 606 (1999)

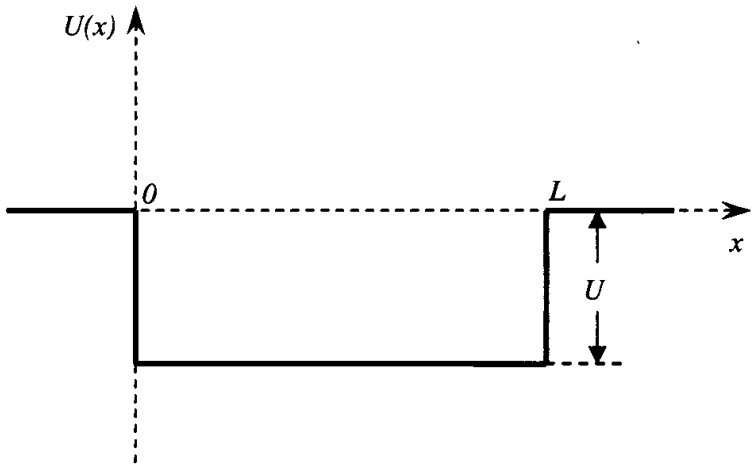
A.Kolomeisky, *Phys. Rev. Lett.*, **98**, 048105 (2007), *J. Chem. Phys.*, **128**, 085101 (2008)

A. Zilman, Biophys. J. 96, 1235 (2009), Phys. Rev. Lett., 103, 128103 (2009)

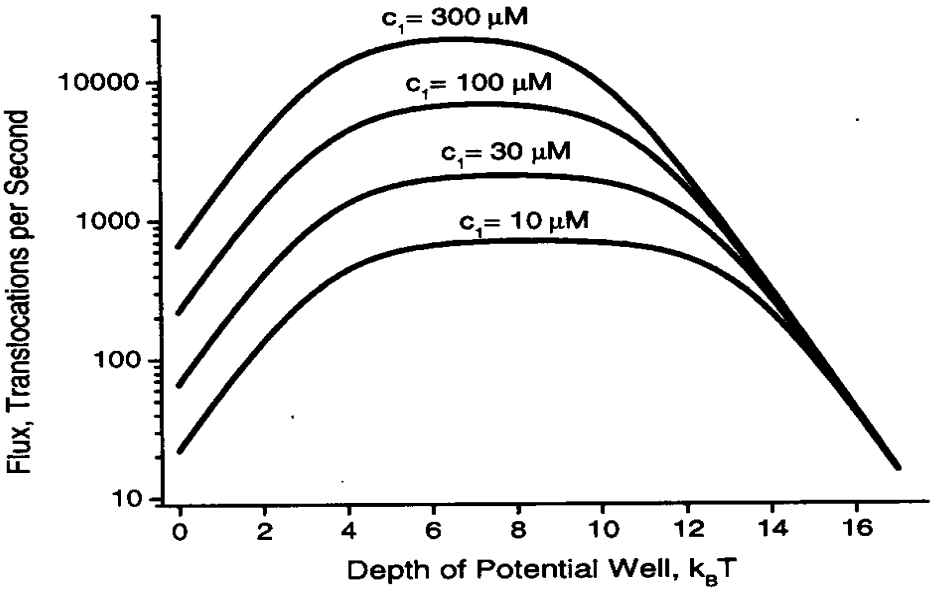
Theoretical Efforts:

Channel-Facilitated Membrane Transport Models – Berezhkovskii and Bezrukov (NIH)

Idea: 1D diffusion in the effective potential created by interactions with the pore



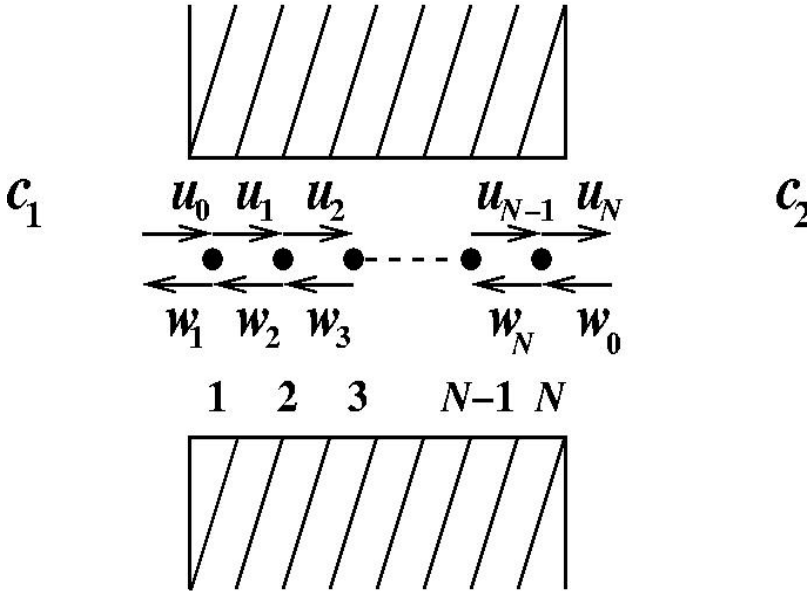
Optimal attraction between the channel and the molecule



Theoretical Efforts:

Discrete-state stochastic models:

Idea: transport of the channel can be viewed as a sequence of transitions between several binding sites in the pore.



Important theoretical result:

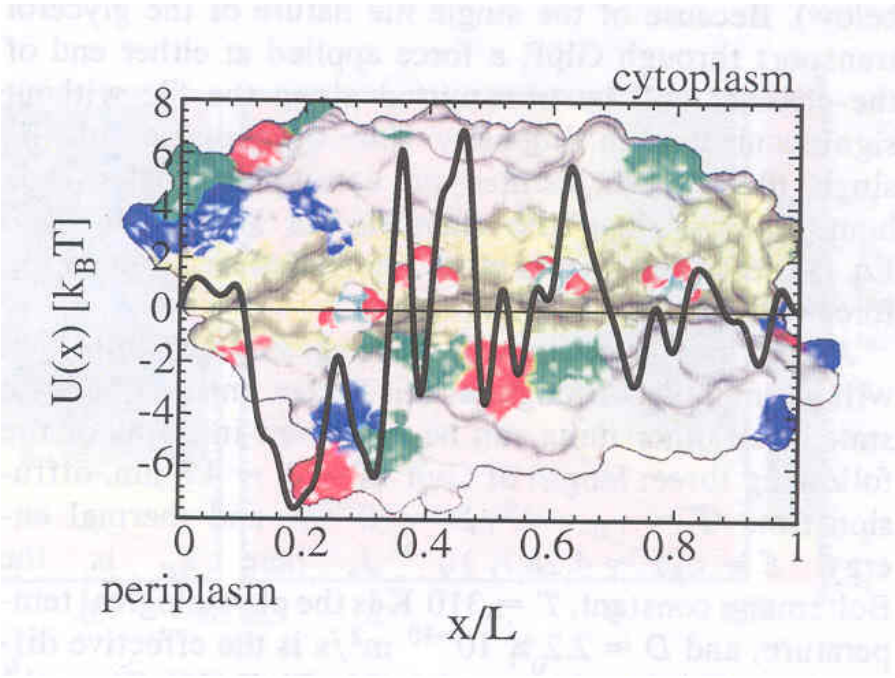
Continuum and discrete models can be mapped into each other

But discrete models probably describe real biological translocation better:

1) Binding sites are real
2) It is hard to measure potentials, but can be "measured" by MD (potential of mean forces)

Theoretical Problems:

1) What is the fundamental role of interactions (molecule/pore and intermolecular)? By what mechanisms they control the channel flux?



2) There are **attractive and repulsive** binding sites. Why?

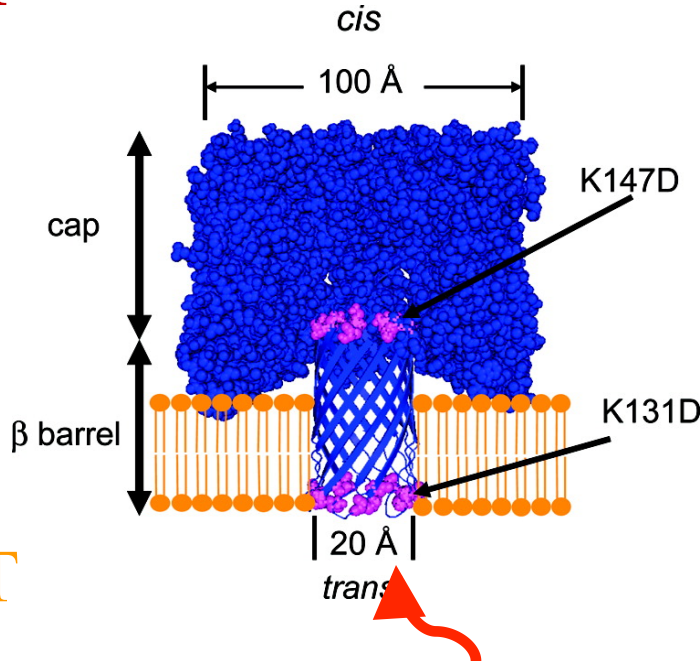
3) Spatial distribution of interaction potentials?

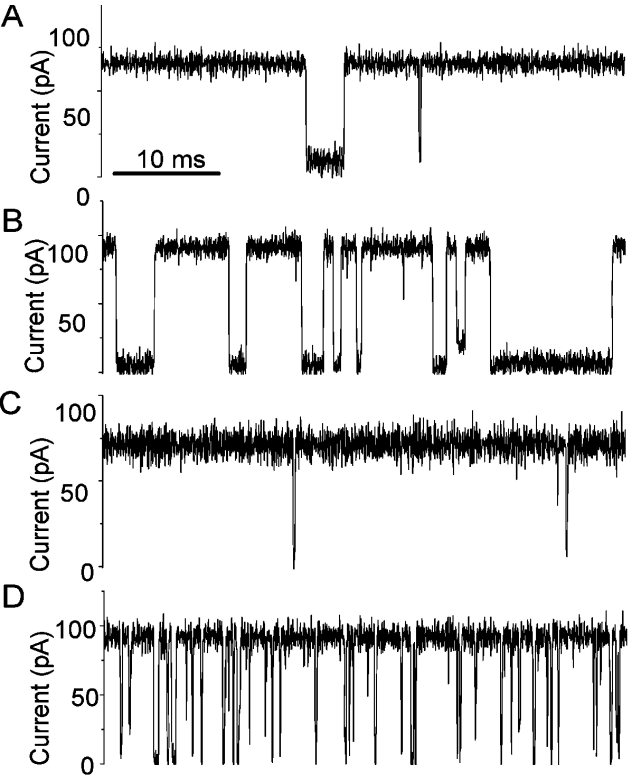
Potential of Mean Forces for glycerol conduction – through aquaglyceroporin

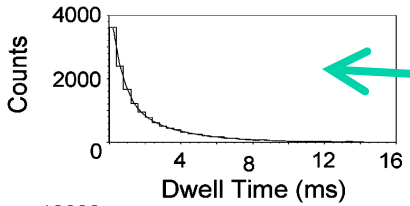
Phys. Rev. Lett., **93**, 238102 (2004)

Single-Molecule Experiments

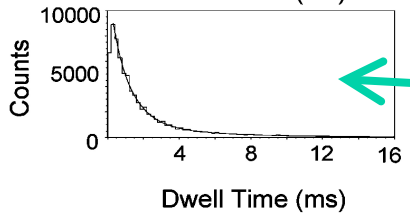
L. Movileanu and coworkers investigated transport of polypeptides through modified α-hemolysin channel: *JACS*, **129**, 14034 (2007); JACS, 130, 4081 (2008)



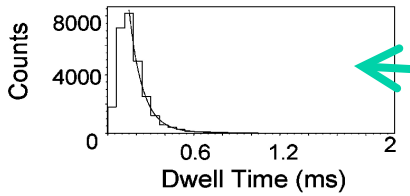




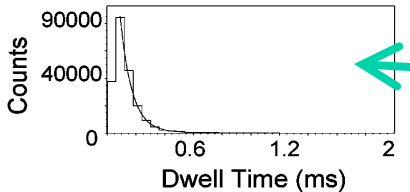
WT



$K131D_7$ - entrance



$K147D_7$ - exit



$K131D_7$ / $K147D_7$

Single-Molecule Experiments

Observations: spatial distribution of the binding sites strongly affect the particle current; *JACS*, **129**, 14034 (2007).

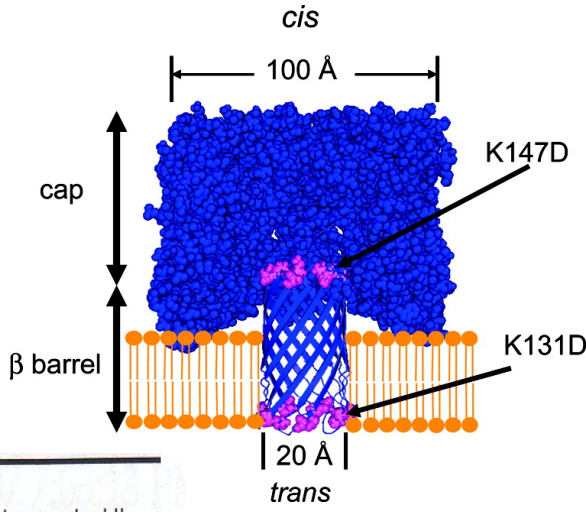


Table 3. The Rate Constants of Dissociation k_{off-1}, k_{off-2}, k_{off-2}^{trans}, and k_{off-2}^{cis} of the Interaction between Cationic Polypeptides and αHL Pores at a Transmembrane Potential of +80 mV[a]

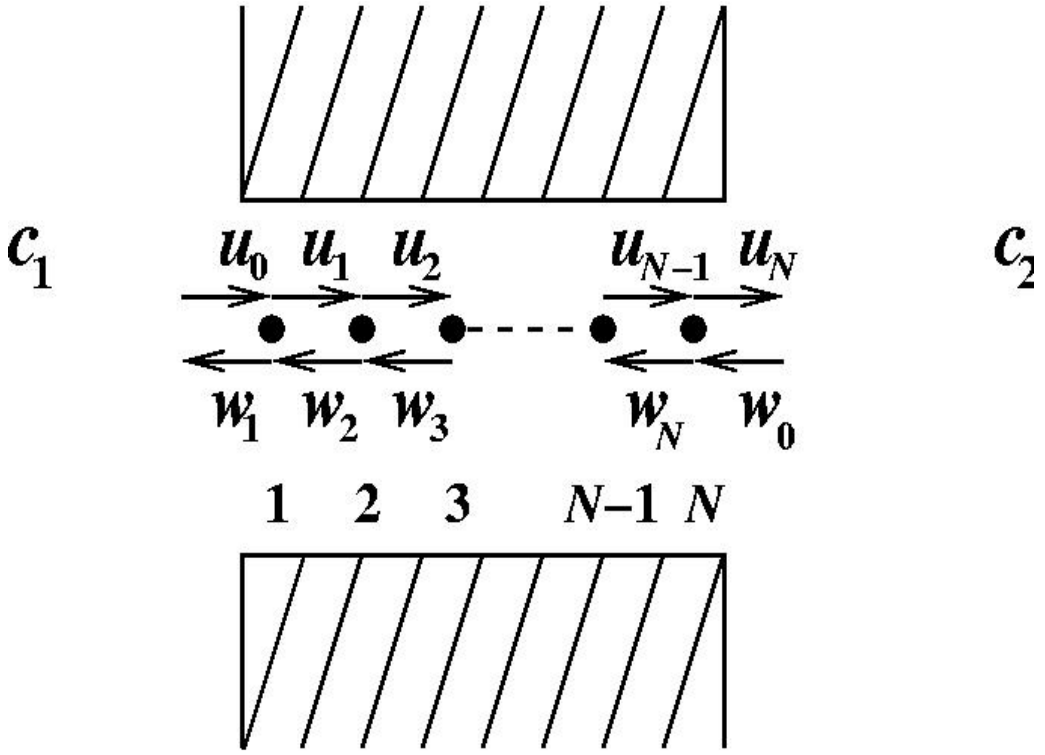
peptide	protein pore	k_{off-1} (s^{-1}) × 10^{-3}	k_{off-2} (s^{-1}) × 10^{-3}	k_{off-2}^{trans} (s^{-1}) × 10^{-3}	k_{off-2}^{cis} (s^{-1}) × 10^{-3}
Syn B2	WT-αHL	1.1 ± 0.4	0.37 ± 0.02	0.29 ± 0.01	0.14 ± 0.01
	$K131D_7$	3.2 ± 2.0	0.33 ± 0.04	0.20 ± 0.03	0.12 ± 0.02
	$K147D_7$	N/A[b]	7.2 ± 1.2	N/A[c]	7 ± 2
	$K131D_7/K147D_7$	N/A[b]	11 ± 1	N/A[c]	10 ± 1
Cox IV	WT-αHL	0.76 ± 0.01	0.11 ± 0.01	0.050 ± 0.002	0.052 ± 0.002
	$K131D_7$	2.1 ± 1.3	0.16 ± 0.04	0.15 ± 0.04	0.009 ± 0.003
	$K147D_7$	N/A[b]	4.8 ± 0.6	N/A[c]	5.1 ± 0.6
	$K131D_7/K147D_7$	N/A[b]	2.2 ± 0.2	N/A[c]	2.0 ± 0.2
AK	WT-αHL	9.3 ± 0.9	1.3 ± 0.1	0.04 ± 0.01	1.2 ± 0.5
	$K131D_7$	2.5 ± 0.1	0.57 ± 0.02	0.21 ± 0.01	0.34 ± 0.03
	$K147D_7$	7.9 ± 3.9	1.3 ± 0.5	N/A[c]	1.3 ± 0.3
	$K131D_7/K147D_7$	N/A[b]	7.6 ± 2.0	N/A[c]	6.2 ± 2.0

Currents through channels for different positions of binding sites

Our Theory

N-binding sites model

Particles do not interact with each other



Entrance rates:

$$u_0 = k_{on} c_1, \; w_0 = k_{on} c_2$$

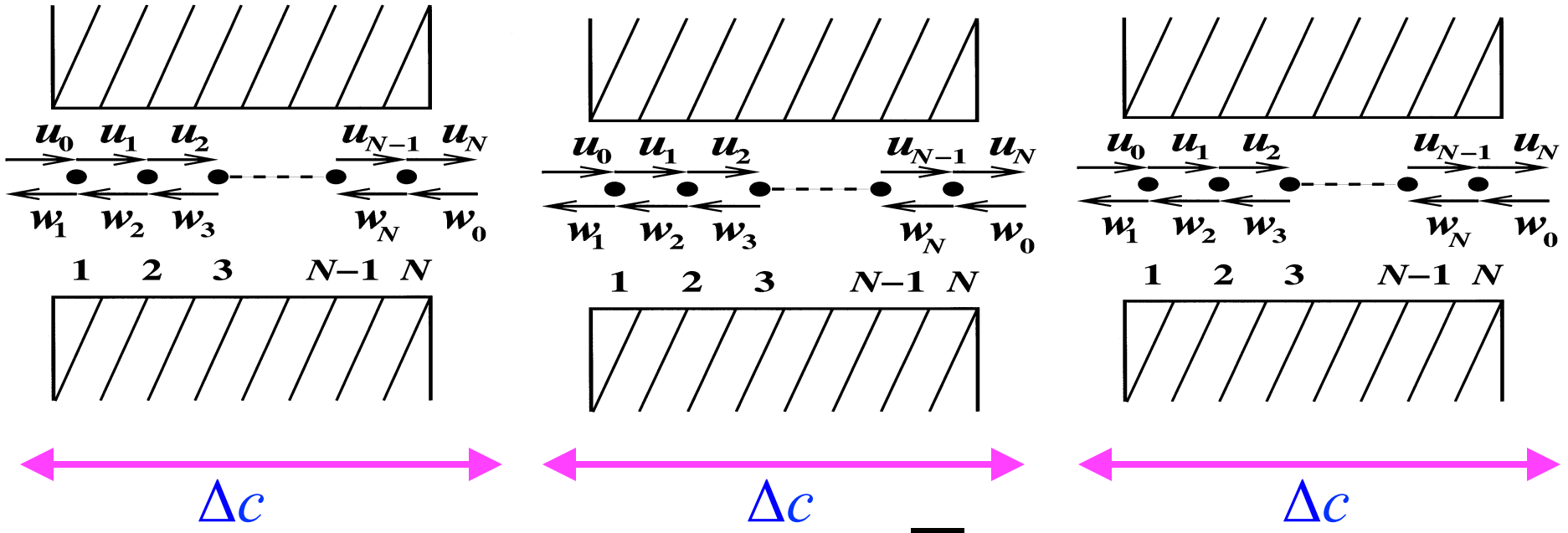
Exit rates:

$$w_1 = u_N = k_{off}$$

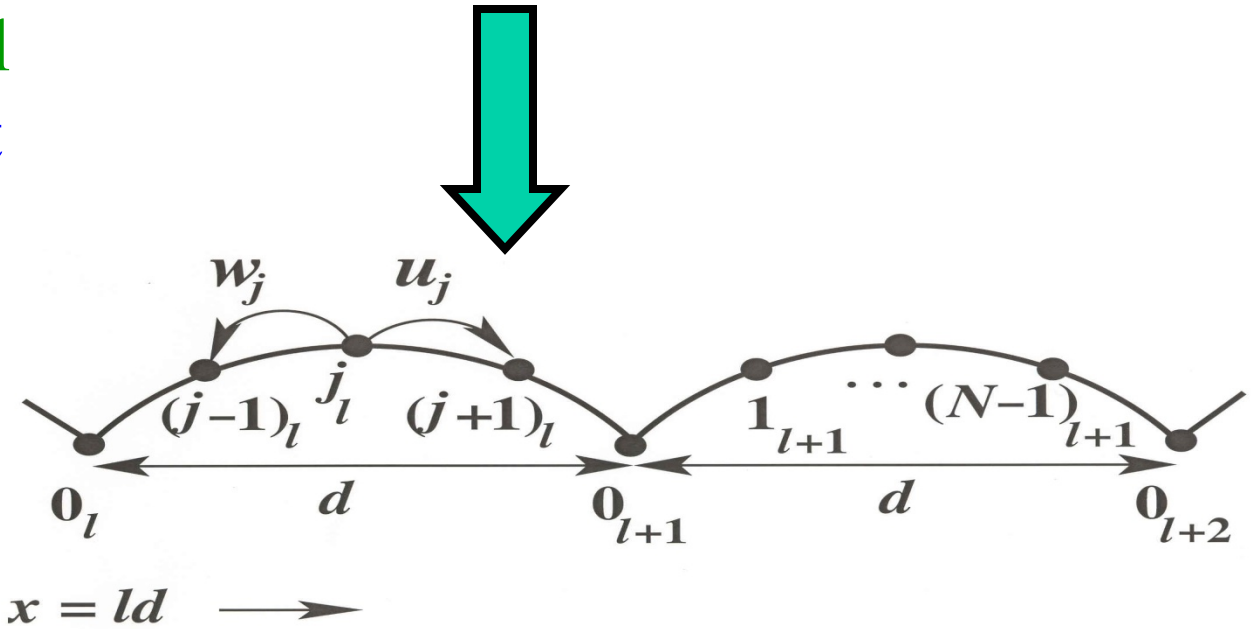
Current depends on the concentration gradient $\Delta c = c_1 - c_2$

Single particle motion through the channels

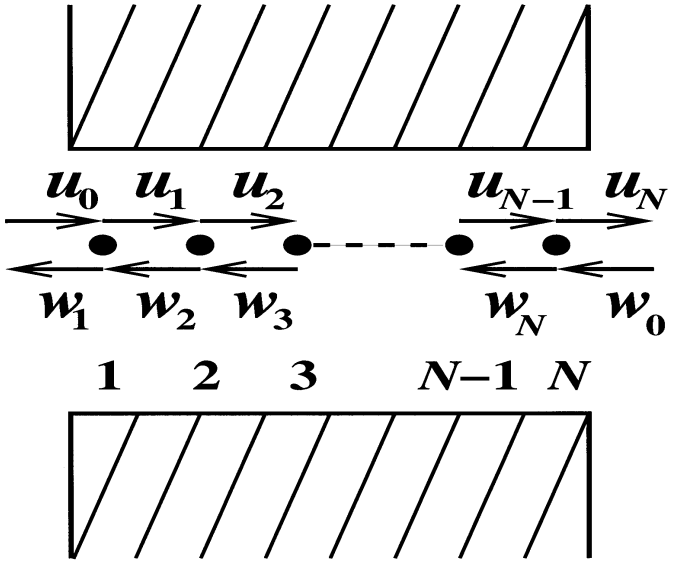
Our Theory

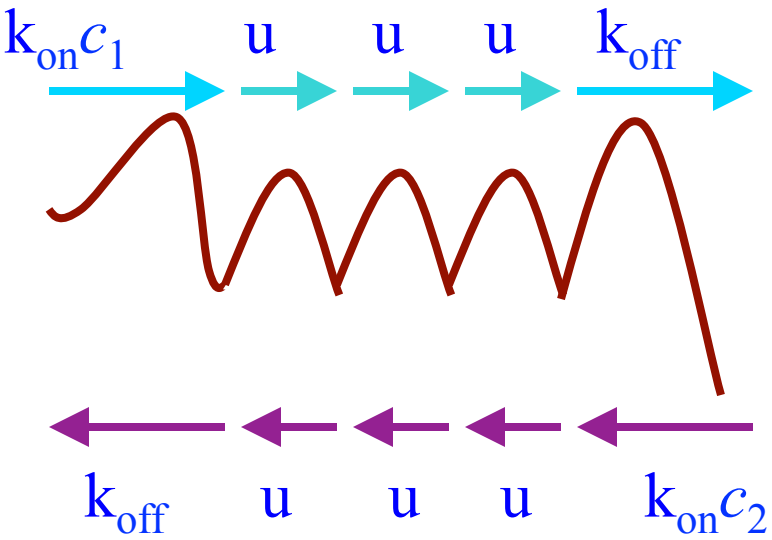


N-site binding model for channel transport can be mapped into the single-particle hopping model on the (*N*+1)-periodic lattice



Our Theory





Dynamic properties can be calculated explicitly

B. Derrida, *J. Stat. Phys*. **31**, 433 (1983)

A.B. Kolomeisky and M.E. Fisher, *Ann. Rev. Phys. Chem*. **58**, 675 (2007)

Particle current through the channel:

$$J = \frac{k_{on}(c_1 - c_2)}{2[1 + \frac{k_{on}(c_1 + c_2)N}{2k_{off}}][1 + \frac{k_{off}(N-1)}{2u}]}$$

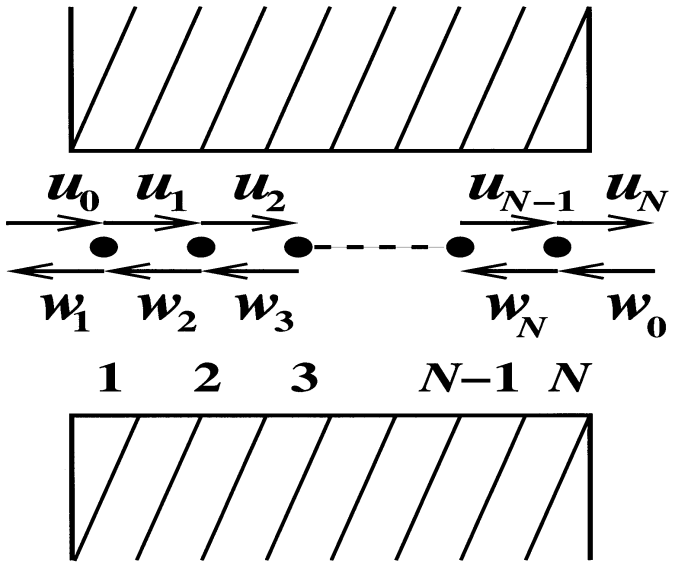
Our Approach:

Our goal:

To investigate effect of interactions on molecular transport through cellular membranes using discrete-state stochastic models.

2 types of interactions considered:

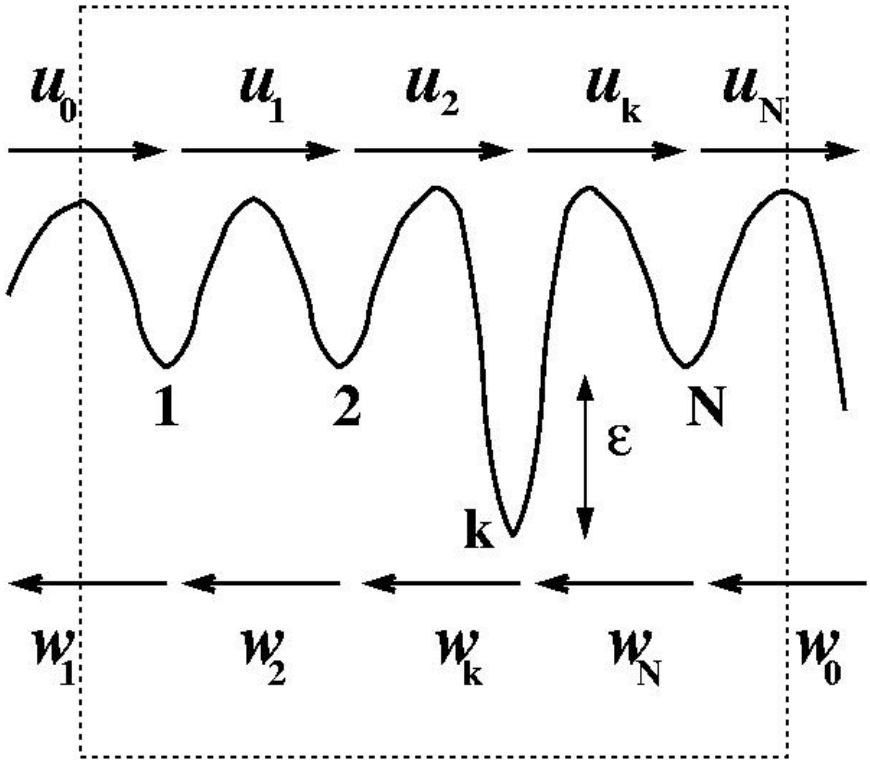
1) Molecule-Nanopore
2) Intermolecular



Molecule/Nanopore Interactions

To test the role of interactions consider a specific model:

1) Channel with N binding sites;
2) Only one particle can be found in the channel;
3) Mostly uniform channel
4) Assume that the binding site k is special with a potential ε
5) Zero particle concentration on one side of the channel (to the right) – to simplify calculations
6) Concentration gradient is supported by other processes

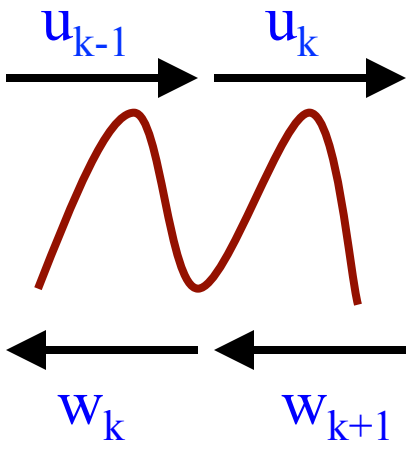


Questions:

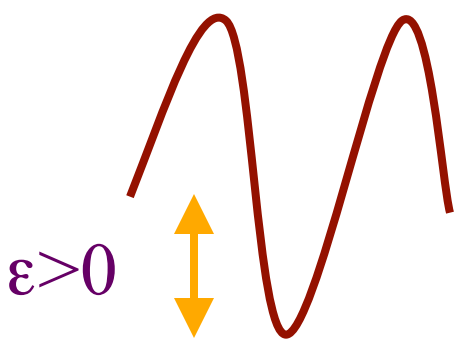
How current depends on k and on ε

Molecule/Nanopore Interactions

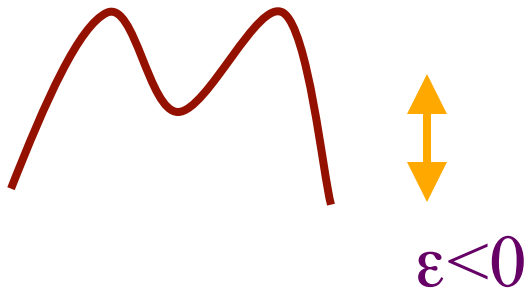
Consider dynamics near the *k*-th binding site:



$\varepsilon=0$ – no interactions



attractive site



repulsive site

Detailed balance-like arguments (but note that no equilibrium – no detailed balance!)

$$\frac{u_{k-1}(\varepsilon)}{w_k(\varepsilon)} = \frac{u_{k-1}(\varepsilon=0)}{w_k(\varepsilon=0)}x, \quad \frac{u_k(\varepsilon)}{w_{k+1}(\varepsilon)} = \frac{u_k(\varepsilon=0)}{w_{k+1}(\varepsilon=0)}(1/x)$$

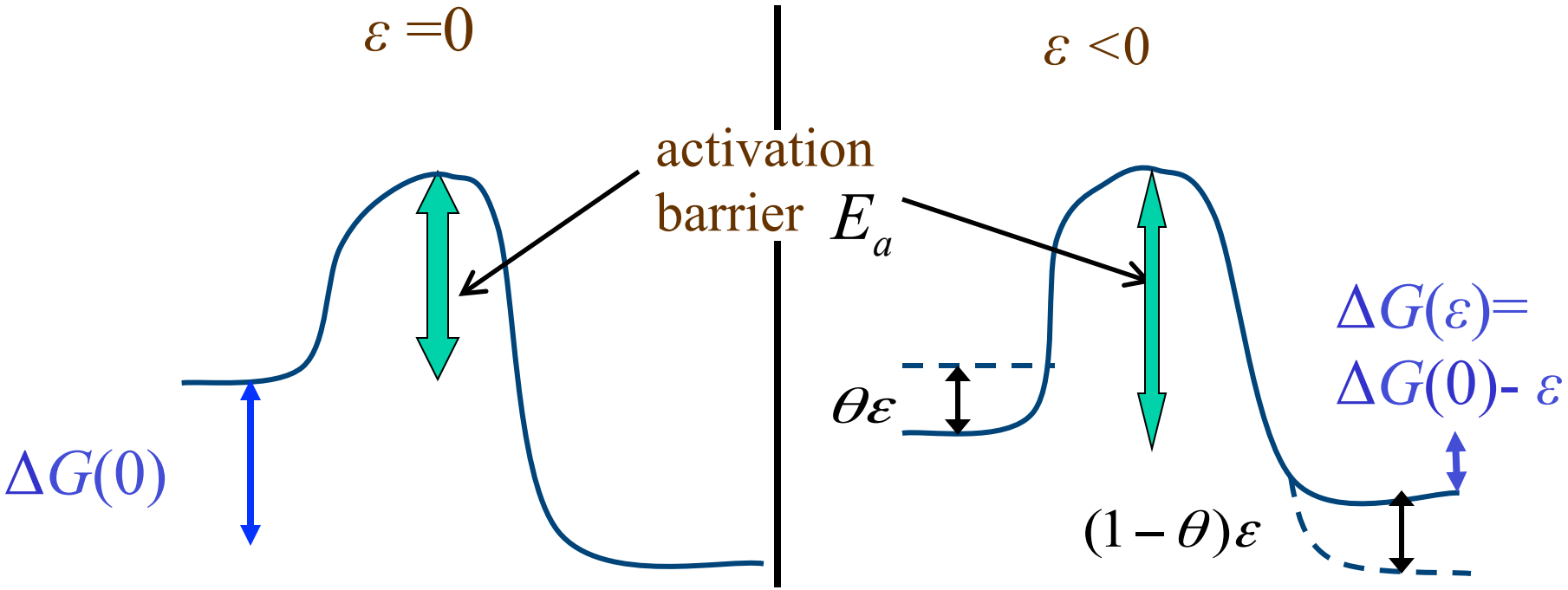
$$x = \exp\left(\frac{\varepsilon}{k_B T}\right)$$

$$u_{k-1}(\varepsilon) = u_{k-1}x^{\theta},\ w_{k+1}(\varepsilon) = w_0 x^{\theta},\ u_k(\varepsilon) = u_k x^{\theta-1},\ w_{k+1}(\varepsilon) = w_{k+1}x^{\theta-1}$$

Molecule/Nanopore Interactions

Interaction-distribution factors $0<\theta<1$

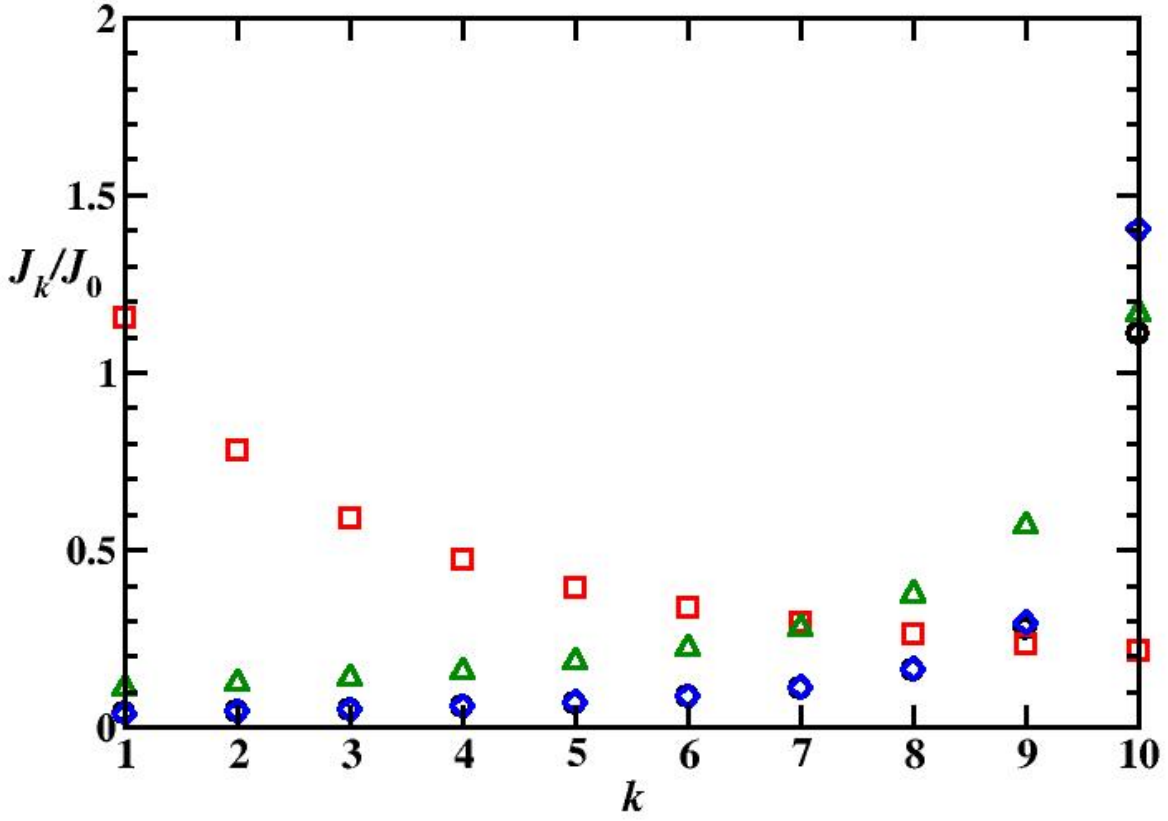
$$u_{k-1}(\varepsilon) = u_{k-1} e^{\frac{\theta\varepsilon}{k_B T}} \qquad w_k(\varepsilon) = w_k e^{\frac{(1-\theta)\varepsilon}{k_B T}}$$



$$u_{k-1} \cong e^{-E_a / k_B T}$$

Molecule/Nanopore Interactions

The ratio of particle currents for different positions of the binding site k for the channel with N=10 binding sites **from our exact theory**



J_0 – flux in the uniform channel without interactions

ε/k_BT=5, u/u_0=0.1, θ=0.5- attraction

ε/k_BT=-5, u/u_0=0.1, θ=0.5-repulsion

ε/k_BT=5, u/u_0=10, θ=0.5- attraction

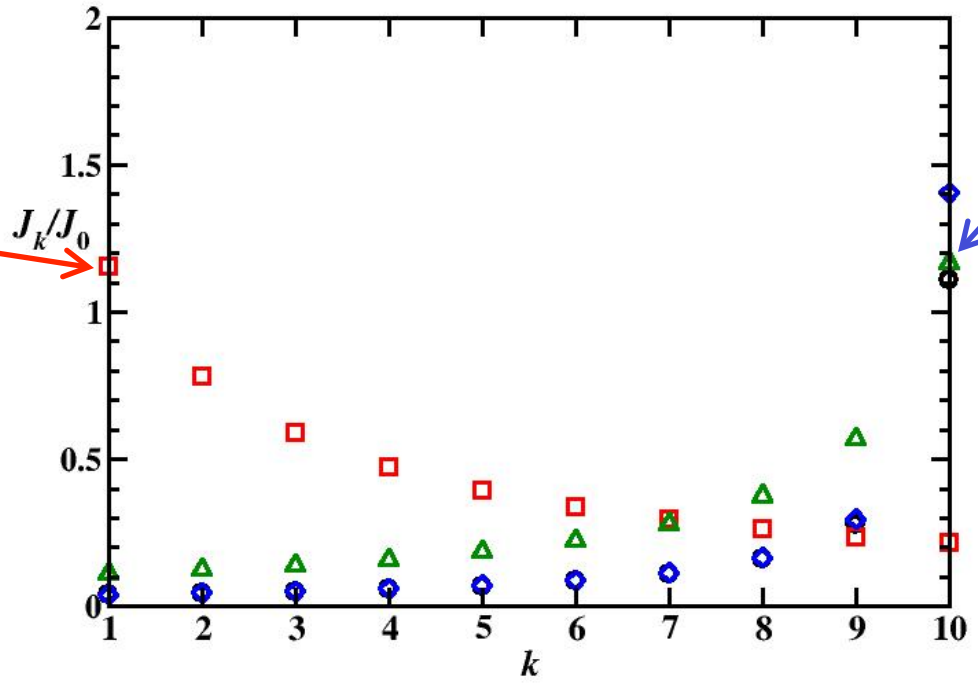
ε/k_BT=5, u/u_0=0.1, θ=0- attraction

Molecule/Nanopore Interactions

Exact results - surprising:

1) for attractive interactions the largest flux is obtained when the binding site at the exit
2) for repulsive interactions the largest flux is obtained when the binding site at the entrance

repulsive binding sites

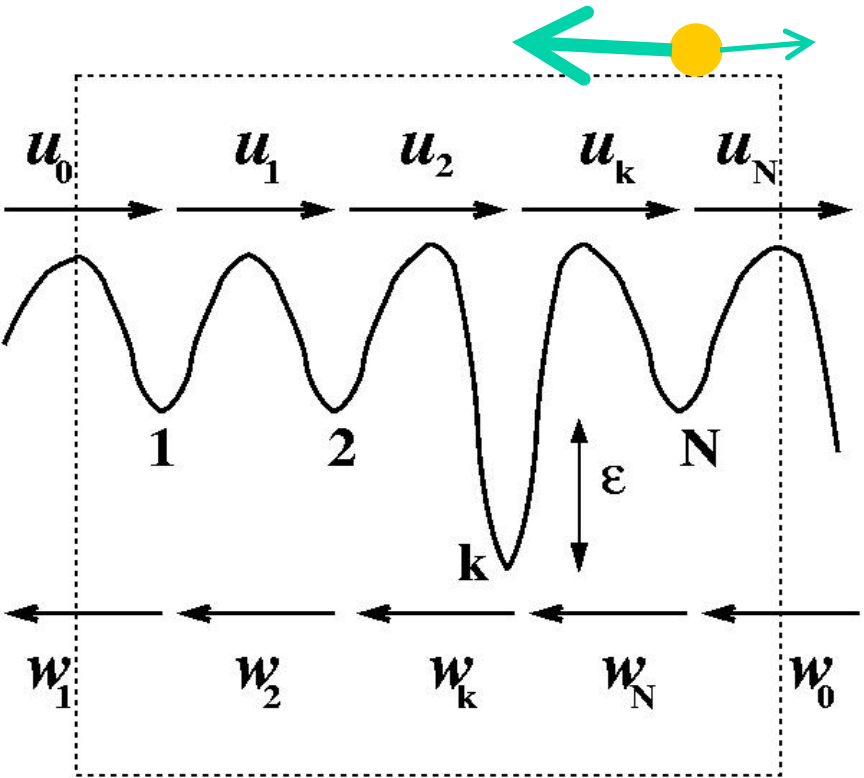


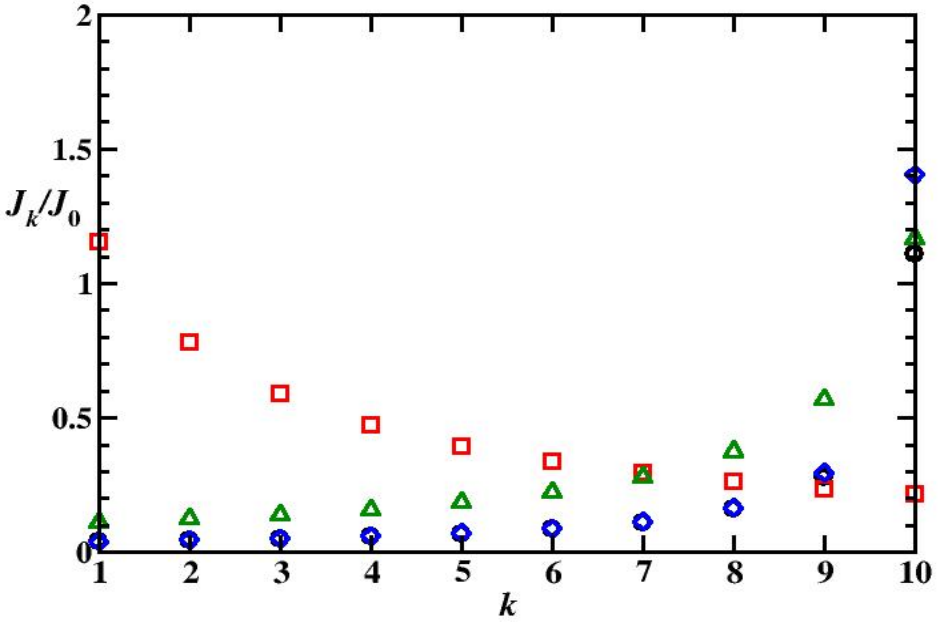
attractive binding sites

Molecule/Nanopore Interactions

Mechanism: control of local concentration of particles

For attractive interactions the binding site can be viewed as a trap, the particle that already passed tends to return back, lowering the overall flux

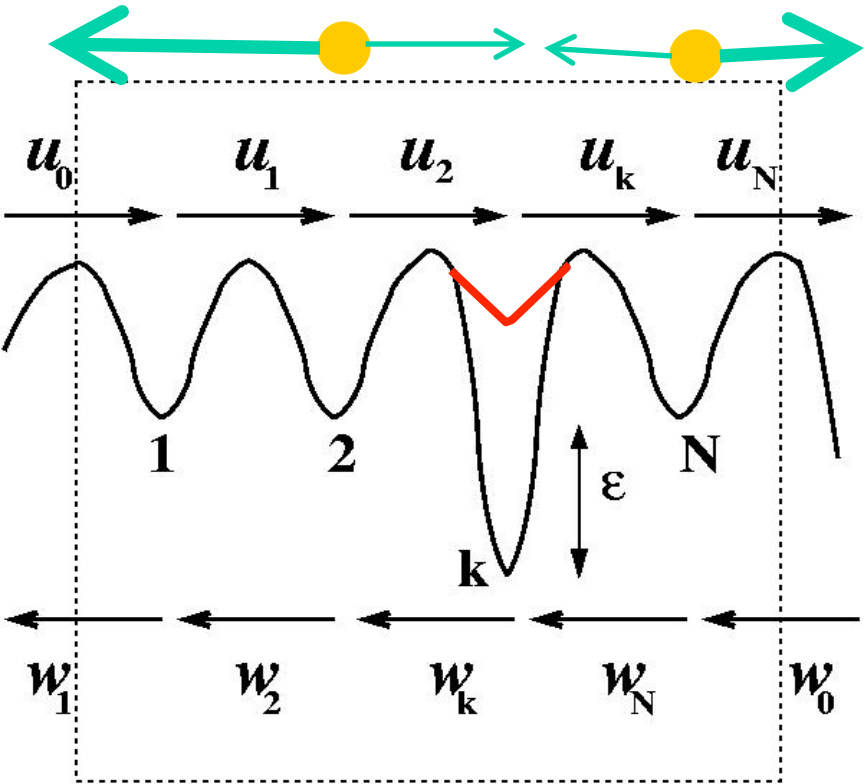


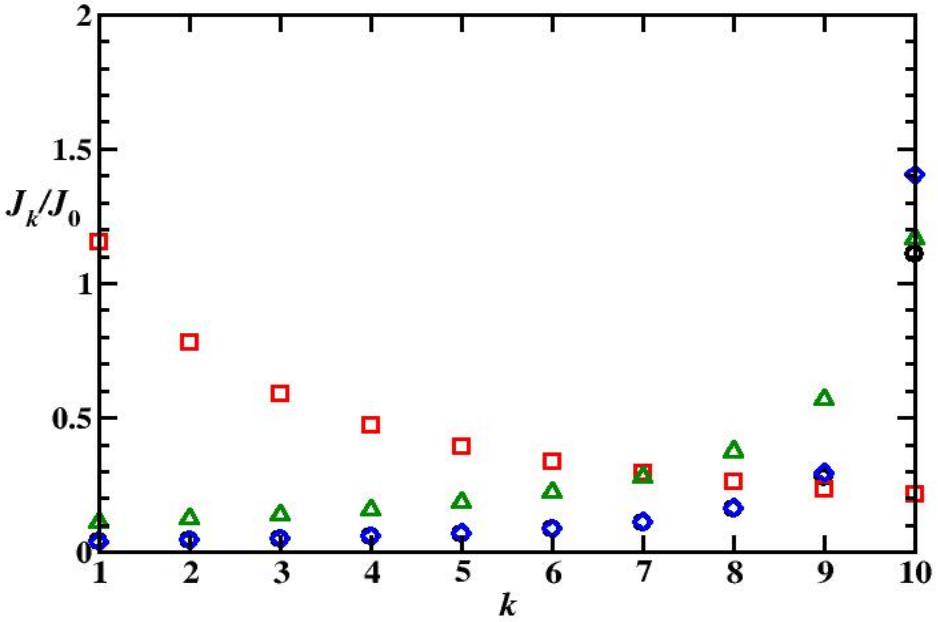


Molecule/Nanopore Interactions

Mechanism: control of local concentration of particles

For repulsive interactions the binding site can be viewed as a barrier, the particle that already passed cannot return back, and this leads to increasing the overall flux





Molecule/Nanopore Interactions

Our theoretical results in agreement with single-molecule observations: translocation is faster if the attractive binding site at the exit

JACS, **129**, 14034 (2007).

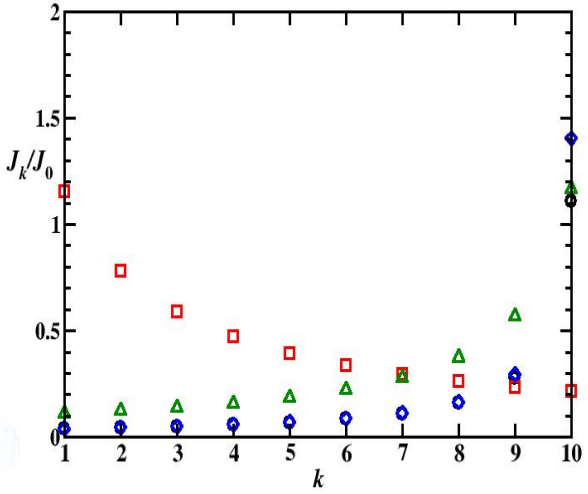
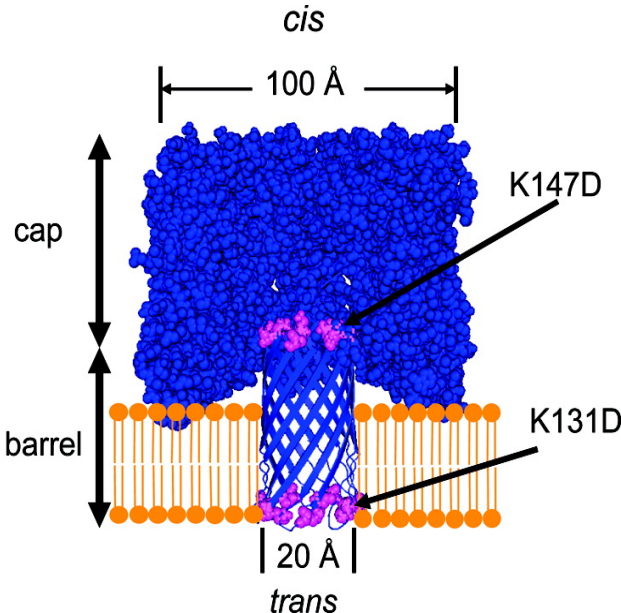


Table 3. The Rate Constants of Dissociation k_{off-1}, k_{off-2}, k_{off-2}^{trans}, and k_{off-2}^{cis} of the Interaction between Cationic Polypeptides and αHL Pores at a Transmembrane Potential of +80 mV[a]

peptide	protein pore	k_{off-1} $(s^{-1}) \times 10^{-3}$	k_{off-2} $(s^{-1}) \times 10^{-3}$	k_{off-2}^{trans} $(s^{-1}) \times 10^{-3}$	k_{off-2}^{cis} $(s^{-1}) \times 10^{-3}$
Syn B2	WT-αHL	1.1 ± 0.4	0.37 ± 0.02	0.29 ± 0.01	0.14 ± 0.01
	$K131D_7$	3.2 ± 2.0	0.33 ± 0.04	0.20 ± 0.03	0.12 ± 0.02
	$K147D_7$	N/A[b]	7.2 ± 1.2	N/A[c]	7 ± 2
	$K131D_7/K147D_7$	N/A[b]	11 ± 1	N/A[c]	10 ± 1
Cox IV	WT-αHL	0.76 ± 0.01	0.11 ± 0.01	0.050 ± 0.002	0.052 ± 0.002
	$K131D_7$	2.1 ± 1.3	0.16 ± 0.04	0.15 ± 0.04	0.009 ± 0.003
	$K147D_7$	N/A[b]	4.8 ± 0.6	N/A[c]	5.1 ± 0.6
	$K131D_7/K147D_7$	N/A[b]	2.2 ± 0.2	N/A[c]	2.0 ± 0.2
AK	WT-αHL	9.3 ± 0.9	1.3 ± 0.1	0.04 ± 0.01	1.2 ± 0.5
	$K131D_7$	2.5 ± 0.1	0.57 ± 0.02	0.21 ± 0.01	0.34 ± 0.03
	$K147D_7$	7.9 ± 3.9	1.3 ± 0.5	N/A[c]	1.3 ± 0.3
	$K131D_7/K147D_7$	N/A[b]	7.6 ± 2.0	N/A[c]	6.2 ± 2.0



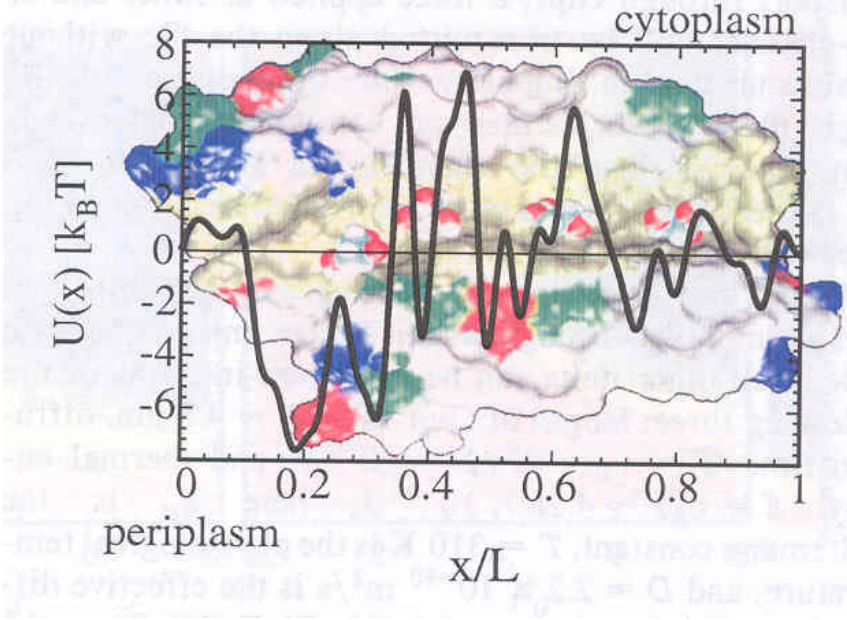
Molecule/Nanopore Interactions

Our theory can be extended to more complex interactions.

Our predictions: the most optimal flux is achieved when attractive sites cluster near the exit and repulsive sites are near the entrance.

But are biological channels are optimized for this function?

Not clear!

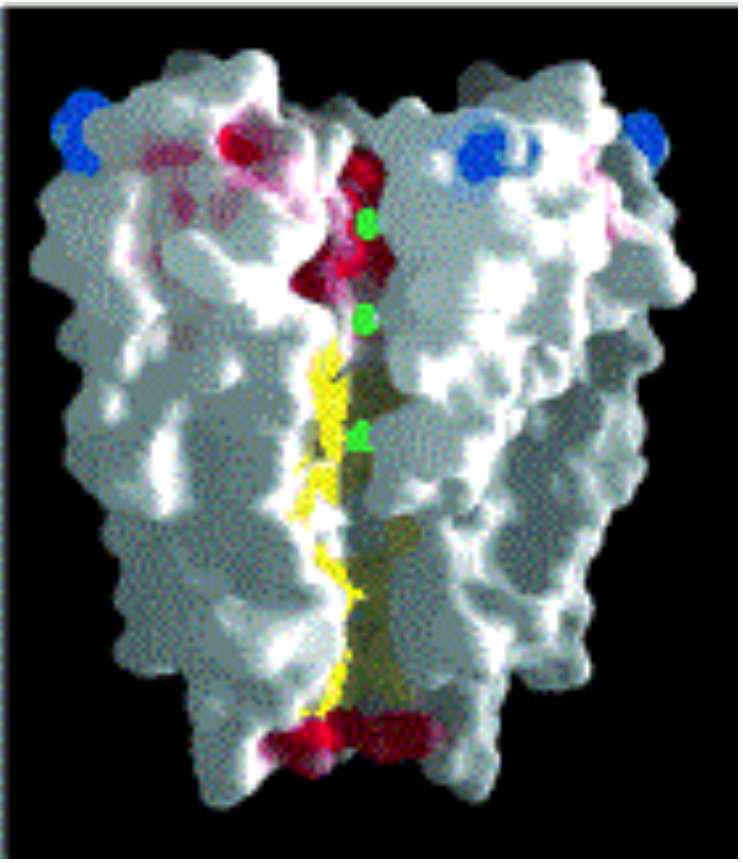


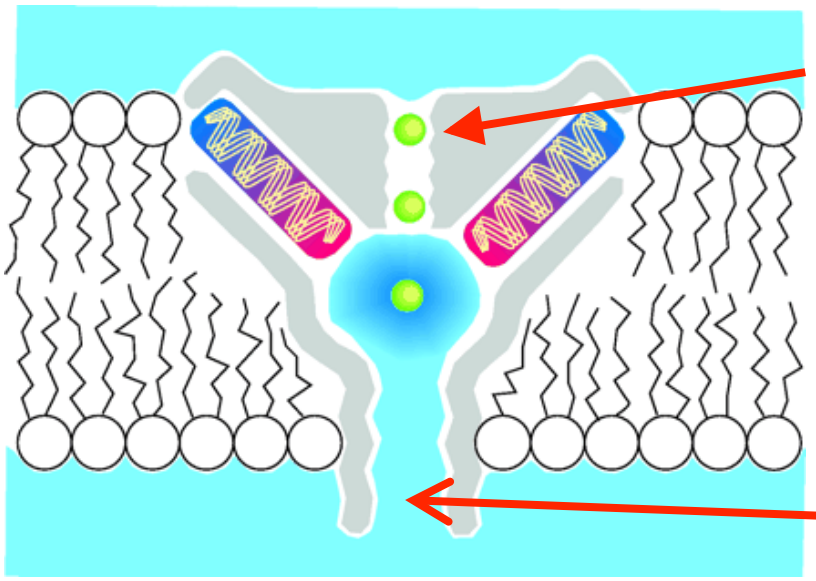
Potential of Mean Forces for glycerol conduction – through aquaglyceroporin

Phys. Rev. Lett., **93**, 238102 (2004)

Transport through K^+ Channels

Mechanism of Transport of K^+ through Potassium Channels:



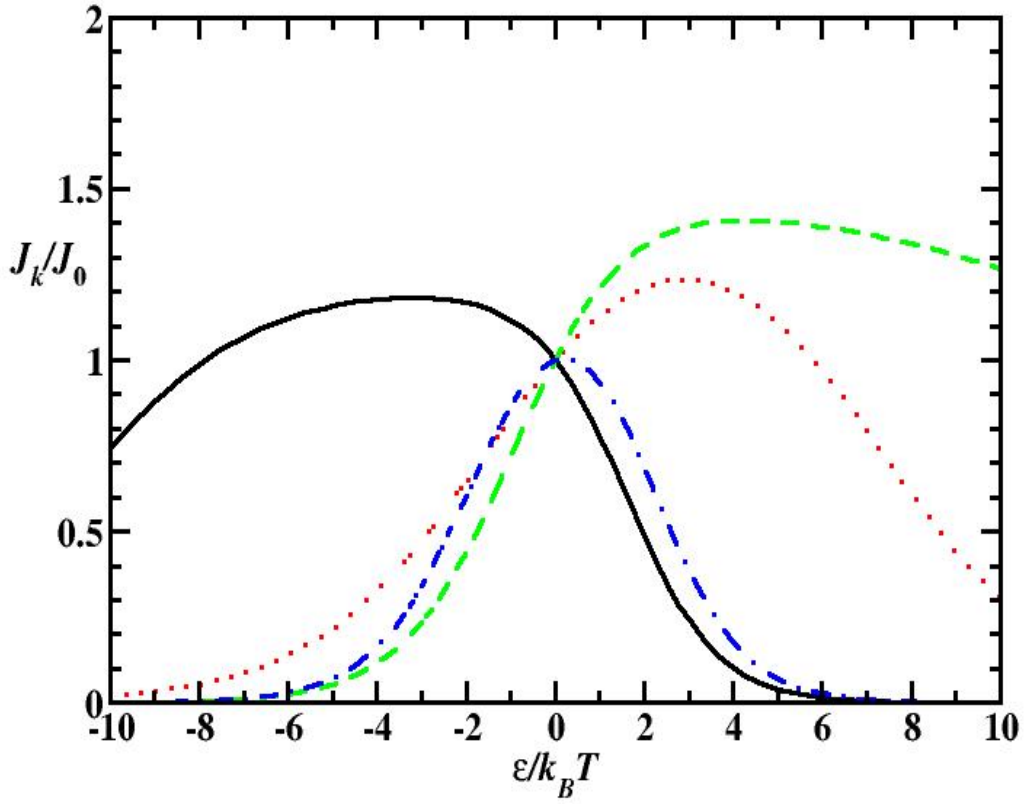


Red – negative groups
Blue- positive groups
Yellow- hydrophobic groups

Science, **280**, 69 (1998)

Molecule/Nanopore Interactions

The ratio of particle currents as a function of interaction strength for the channel with N=10 binding sites



Strength of interactions is an important parameter for channel transport

k=1, u/u_0=0.1, θ=0.5

k=10, u/u_0=0.1, θ=0.5

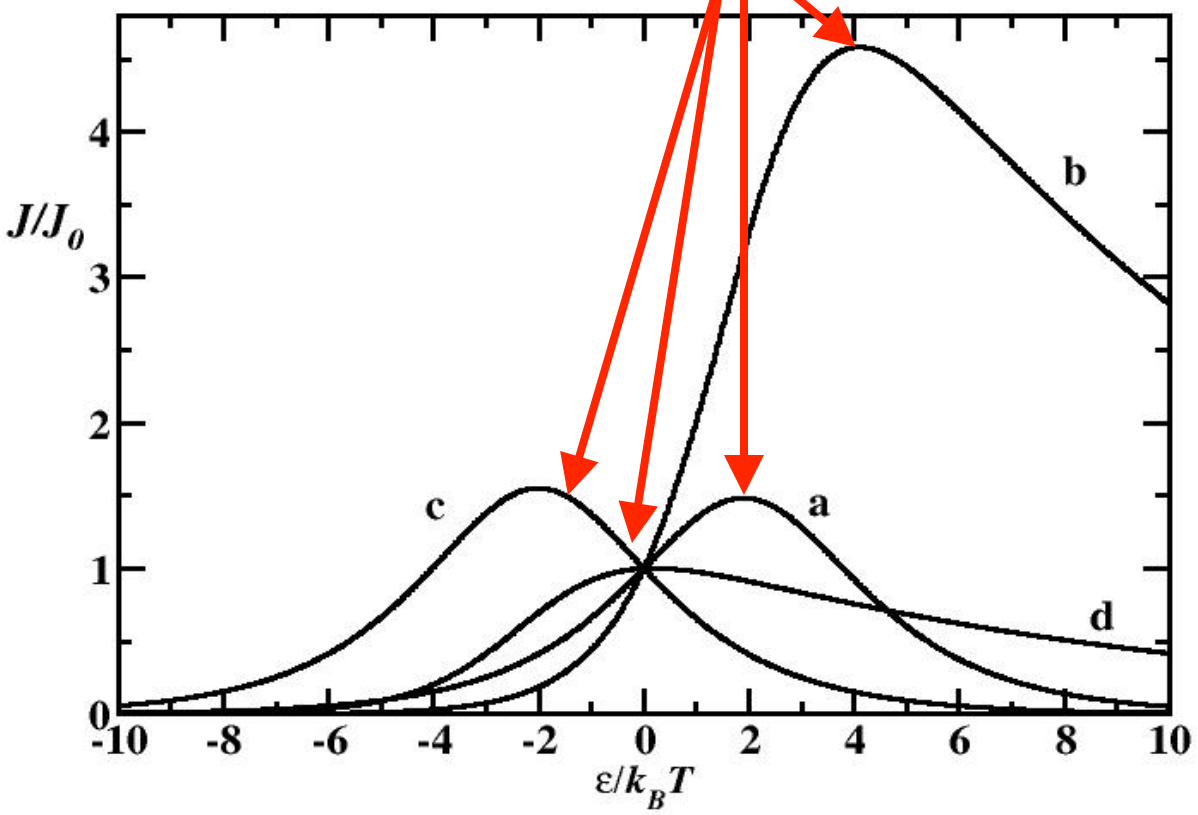
k=10, u/u_0=0.1, θ=0.8

k=5, u/u_0=0.1, θ=0.9

Molecule/Nanopore Interactions

Relative currents as a function of interaction strength for N=1 model

Most optimal interaction $\varepsilon^* \approx 0$



Parameters for maltodextrin translocation, k_{on}=15 $\mu M^{-1}s^{-1}$, k_{off}=500 s^{-1}

assume c_2=0,

a) c_1=10 μM,θ=0.5

b) c_1=10 μM, θ=0.9

c) c_1=500 μM,θ=0.5

d) c_1=500 μM,θ=0.9

Molecule/Nanopore Interactions

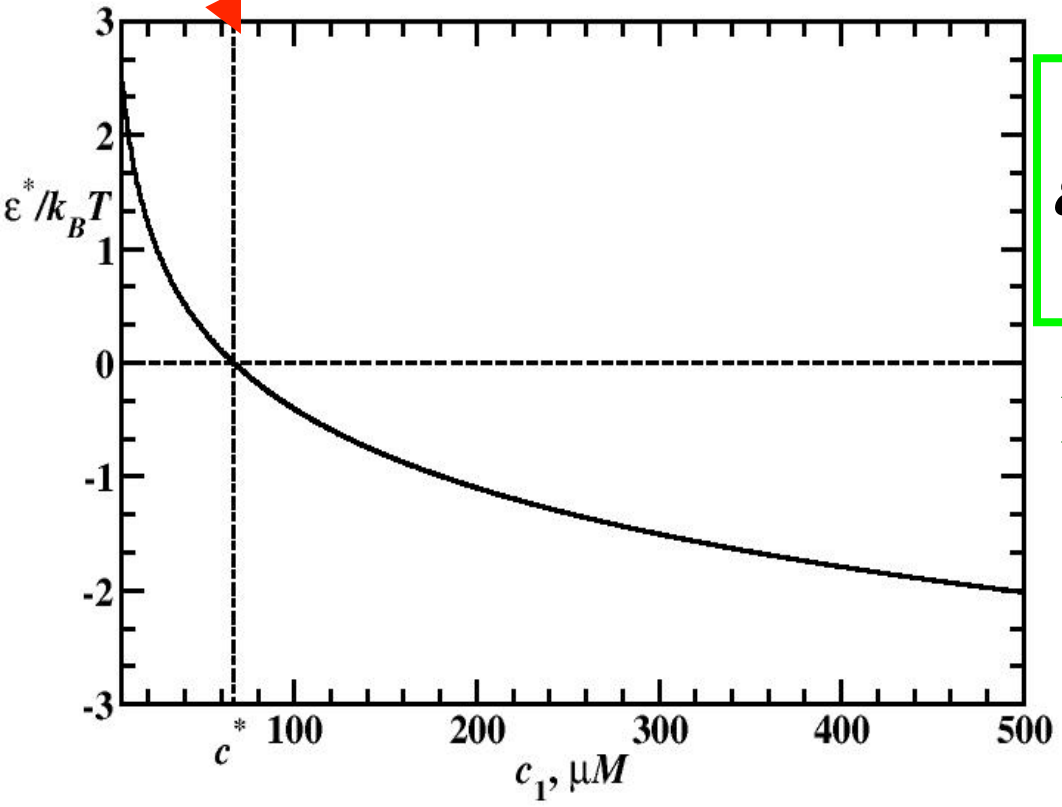
Most optimal interaction as a function of c_1 (assuming c_2=0)

Molecular flux increases

$c_1 < c^*$ - for attractive site

$c_1 > c^*$ - for repulsive site

c^*-critical concentration



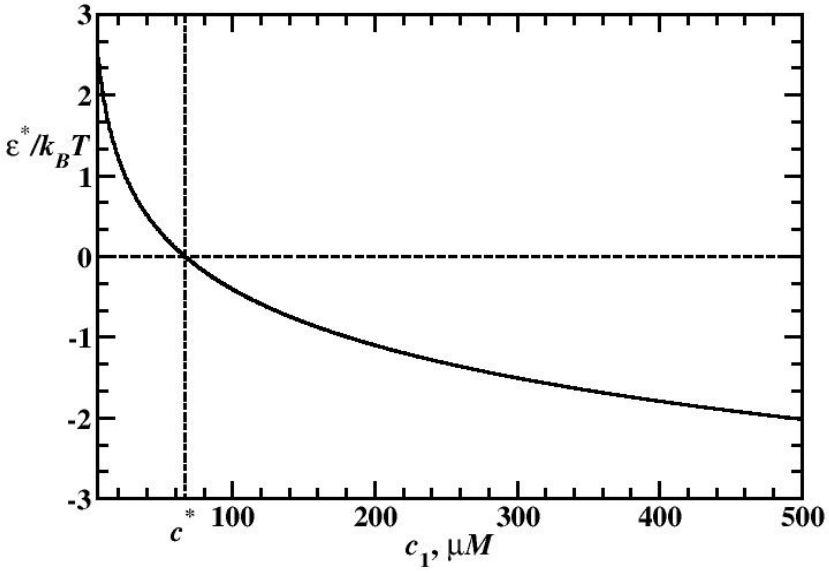
For N=1:

$$\varepsilon^* = k_B T \ln\left[\frac{\theta}{1-\theta}\frac{2k_{off}}{k_{on}(c_1+c_2)}\right]$$

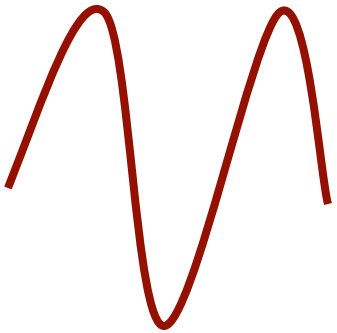
For large concentration gradients – the most optimal interaction is negative, for small gradients – the most optimal is positive

Molecule/Nanopore Interactions

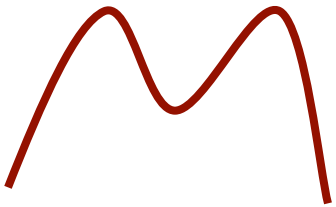
Surprising results:
at some conditions the repulsive site provides the most optimal flux!



attractive site



repulsive site



Stationary conditions: the flux into the channel is equal to the flux out. Then for large concentrations outside the particle must stay short time inside, i.e., the binding site is repulsive

Molecule/Nanopore Interactions

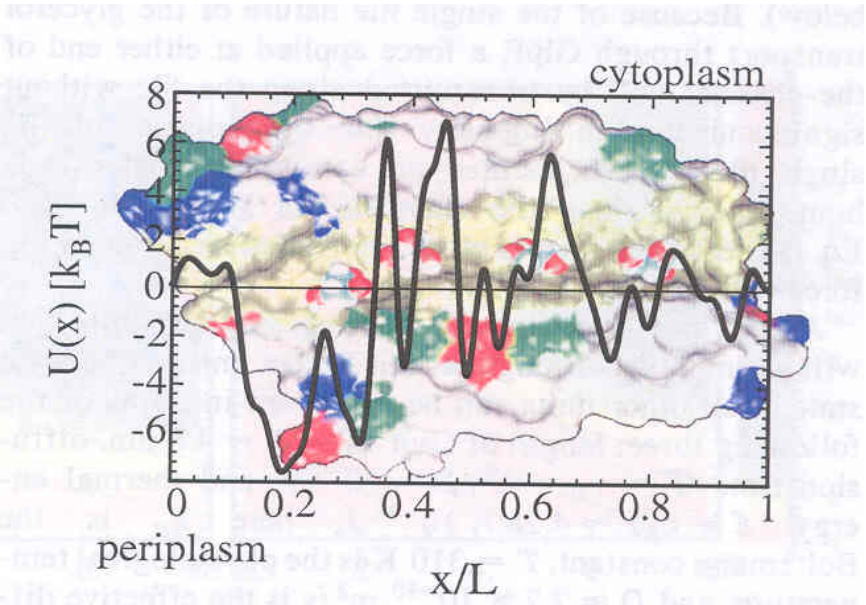
Analogy with entering the bus

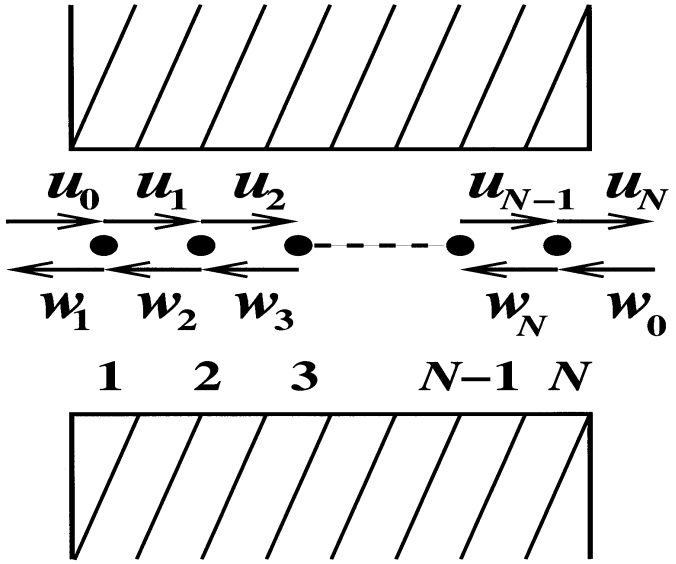
Intermolecular Interactions

More than 1 molecule might fit inside the channel during translocation.

Current theoretical view: molecules do not interact except hard-core exclusion, no correlations in their motion is assumed (mean-field).

Biophys. J., **96**, 1235 (2009), *Phys. Rev. Lett.*, **103**, 128103 (2009)

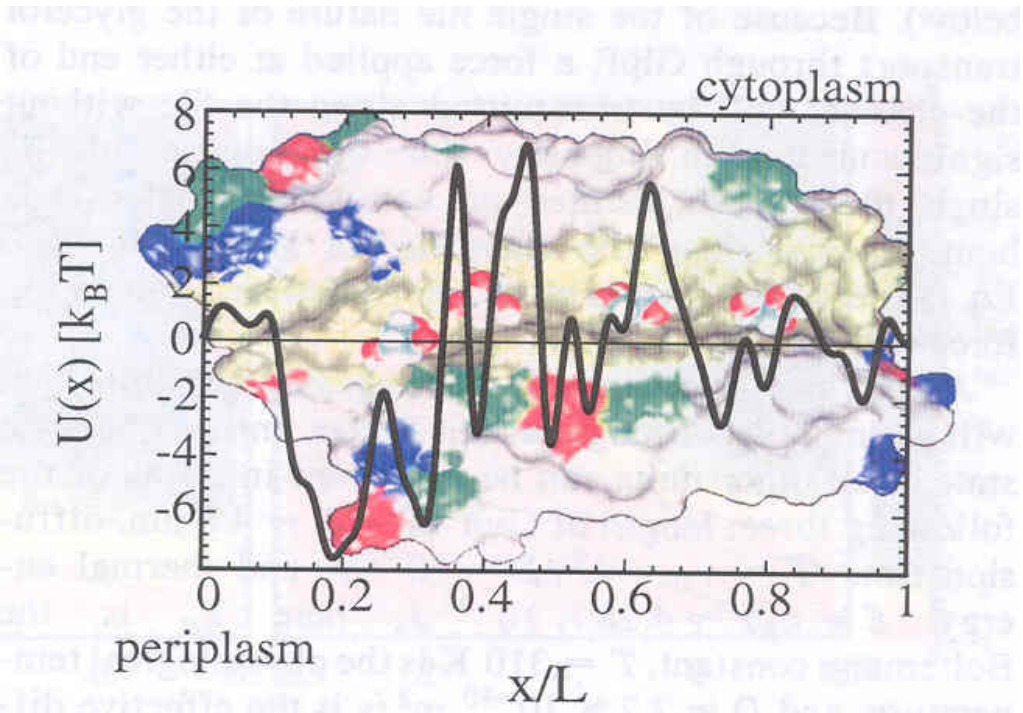


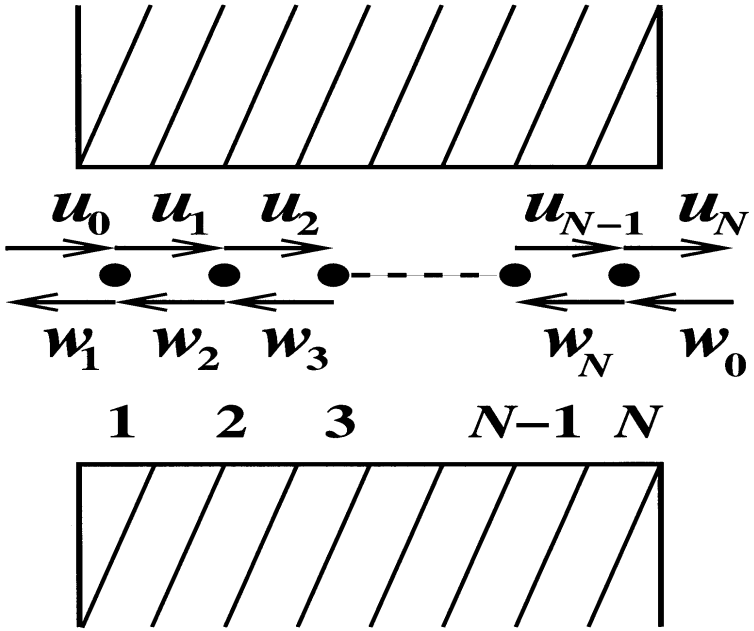


Intermolecular Interactions

Our hypothesis:

molecules can interact with each other in the biological channels, and this could modify the particle flux – it turned out to be important for some ion channels transport

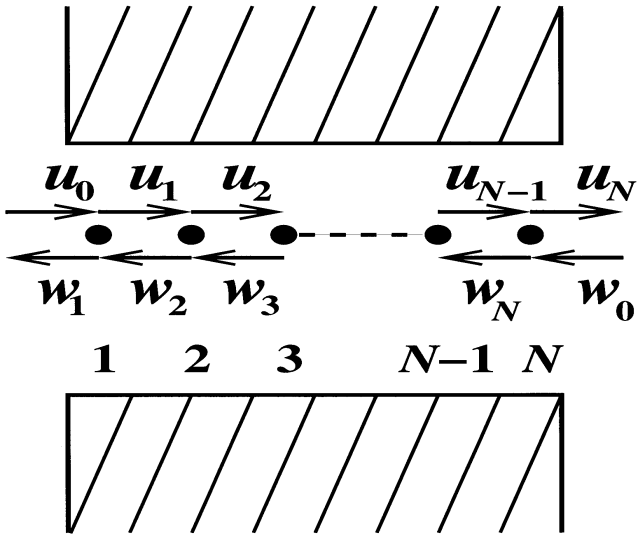


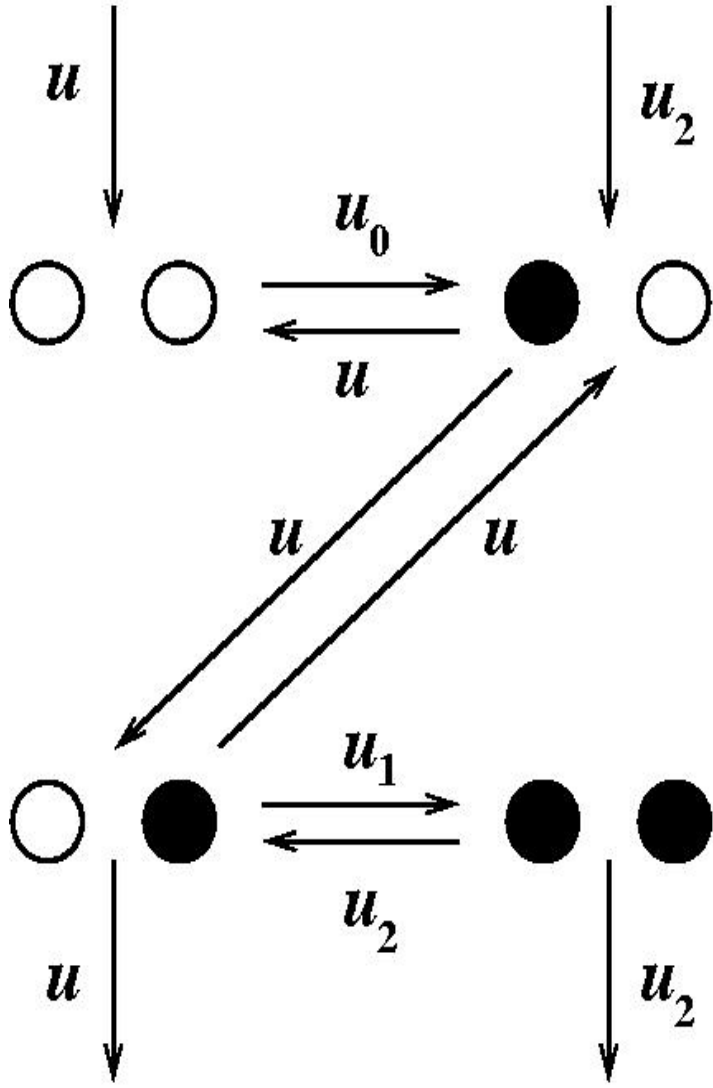


Intermolecular Interactions

To investigate explicitly intermolecular interactions consider N=2 model:

1) No molecule/nanopore interactions;
2) More than 1 particle can be found in the channel
3) Particle interact with each other with energy ε





Intermolecular Interactions

4 possible configurations: (0,0); (1,0); (0,1); (1,1)

Limiting case $\varepsilon \longrightarrow -\infty$:

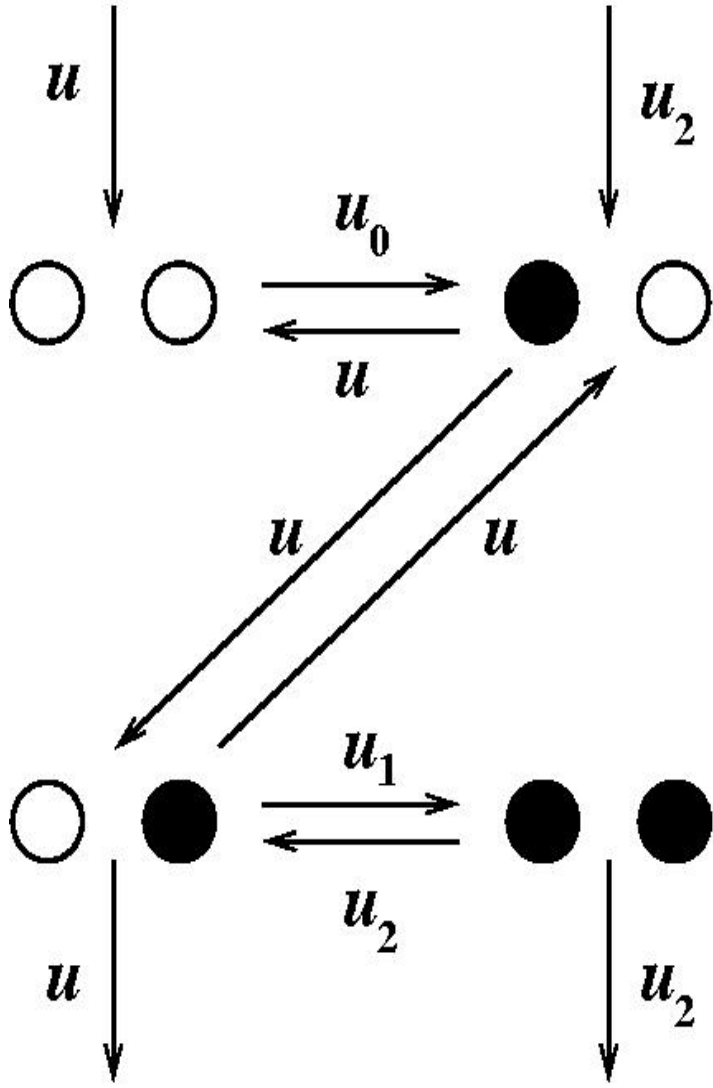
Single particle in the channel

Detailed balance arguments:

$$\frac{u_1}{u_2} = \frac{u_0}{u} x,$$

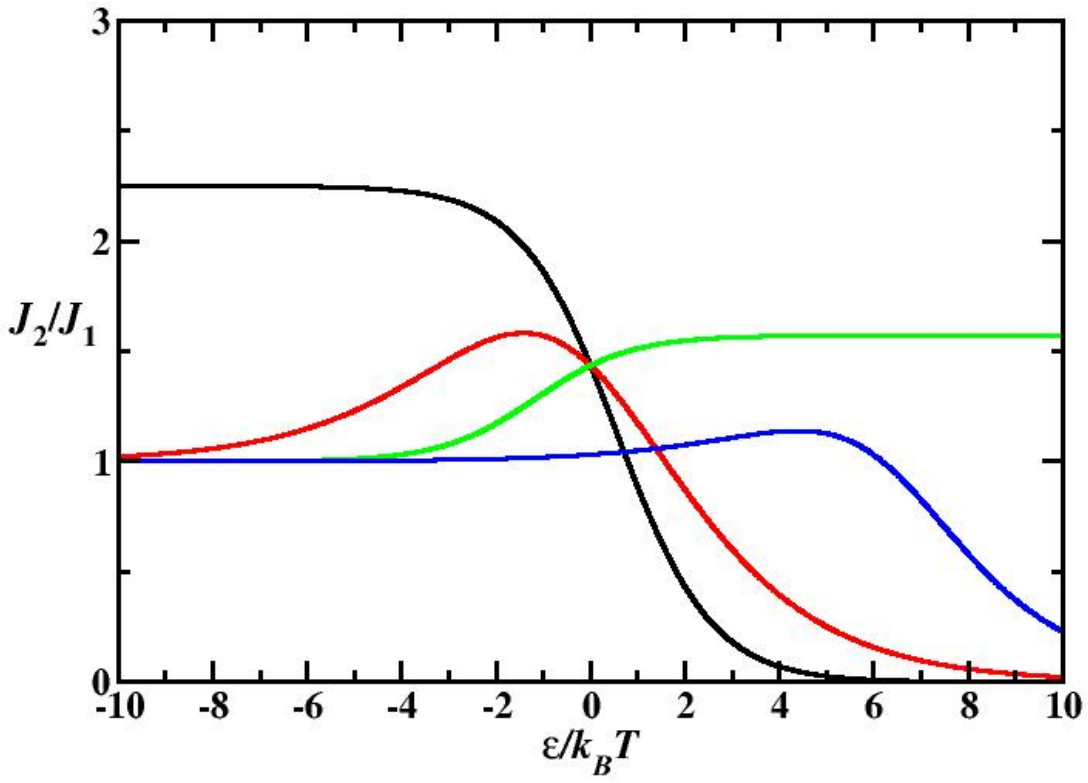
$$x = \exp\left(\frac{\varepsilon}{k_B T}\right)$$

$$u_1 = u_0 x^{\theta}, u_2 = u x^{\theta-1}$$



Intermolecular Interactions

Ratio of particle currents as a function of intermolecular interaction for the channel with N=2 binding sites. J_1 is the current for $\varepsilon \rightarrow -\infty$



u/u_0=0.1, θ=0

u/u_0=0.1, θ=0.5

u/u_0=0.1, θ=1

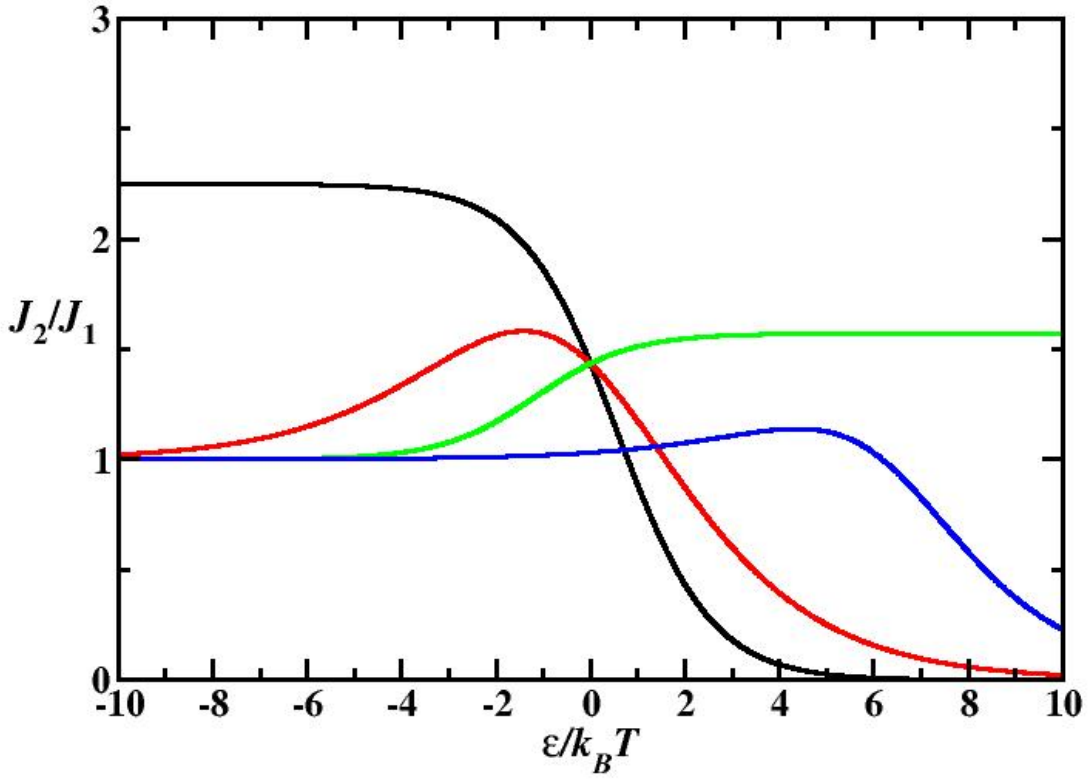
u/u_0=10, θ=0.5

Intermolecular Interactions

Complex behavior that depends on the parameter θ:

For $0<\theta<1$ – non-monotonous behavior with optimal interaction where the flux is maximal.

Optimal interaction could be attractive or repulsive!



$u/u_0=0.1$, $\theta=0$

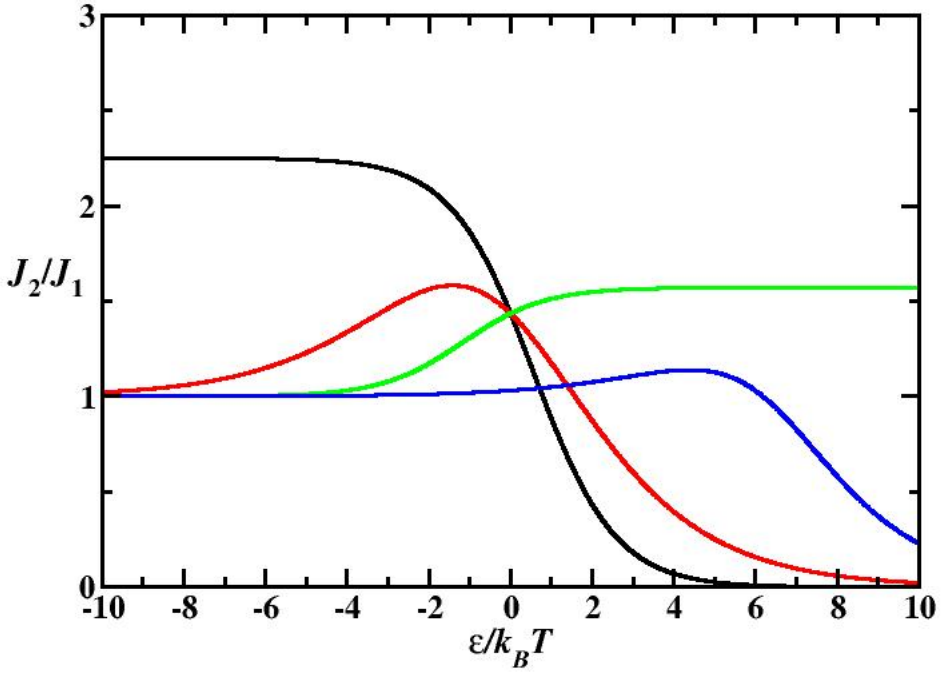
$u/u_0=0.1$, $\theta=0.5$

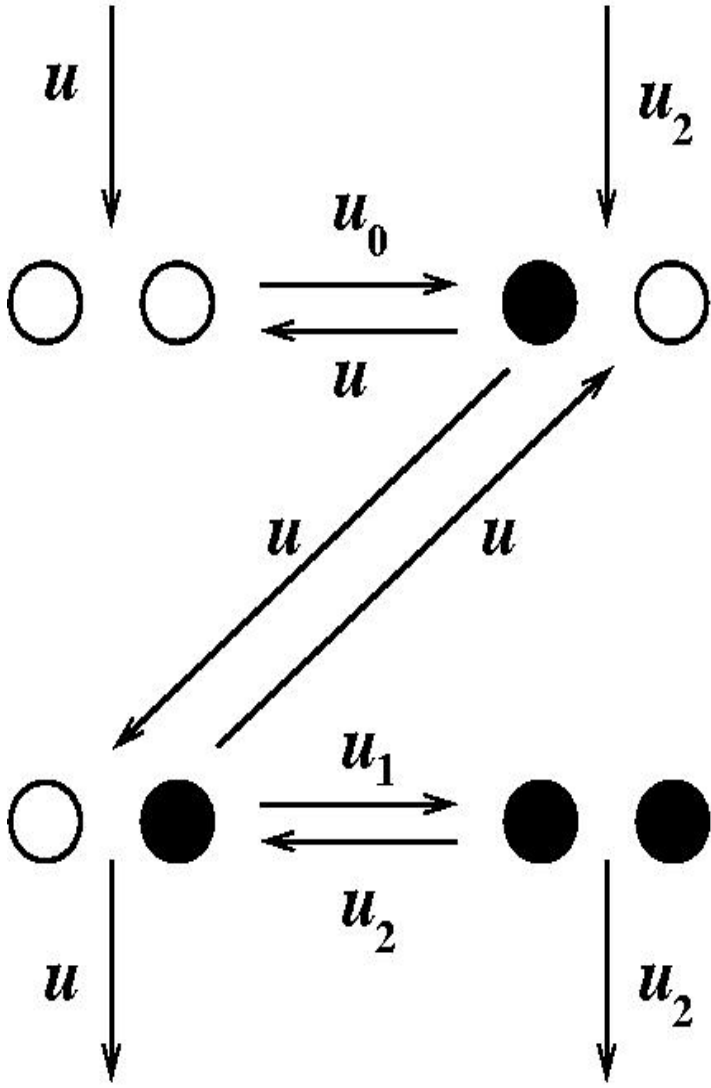
$u/u_0=0.1$, $\theta=1$

$u/u_0=10$, $\theta=0.5$

Intermolecular Interactions

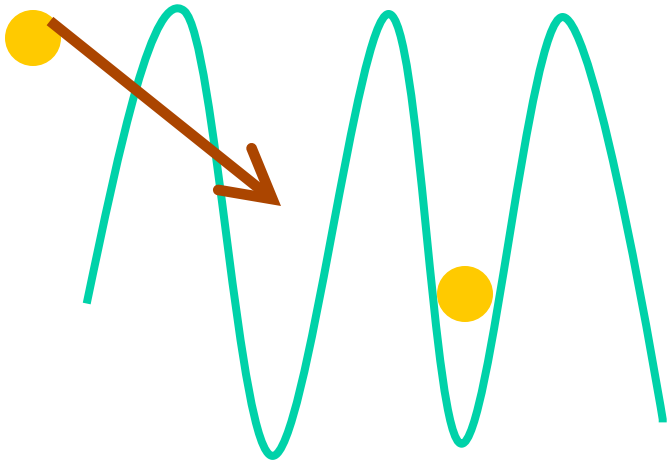
Mechanism: particle in the channel might catalyze or inhibit the entrance or exit of another one, changing the dynamics and modifying the current





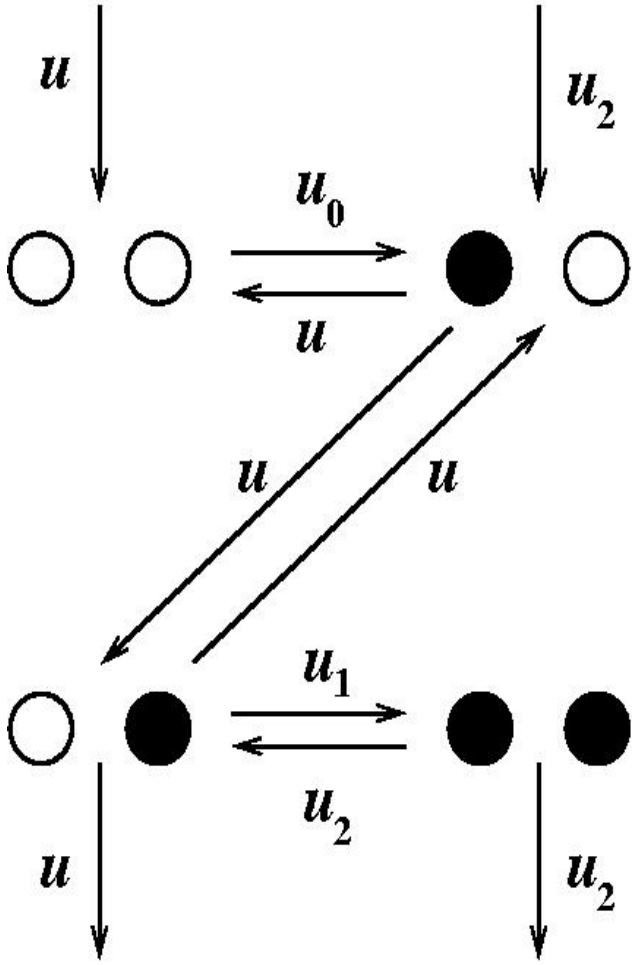
Intermolecular Interactions

For attractive interactions:



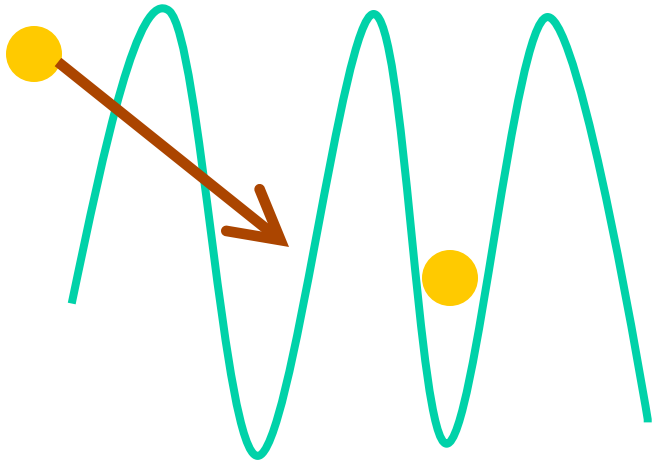
1) Increases the flux of other particles into the channel;

2) Reduces the flux out of the channel



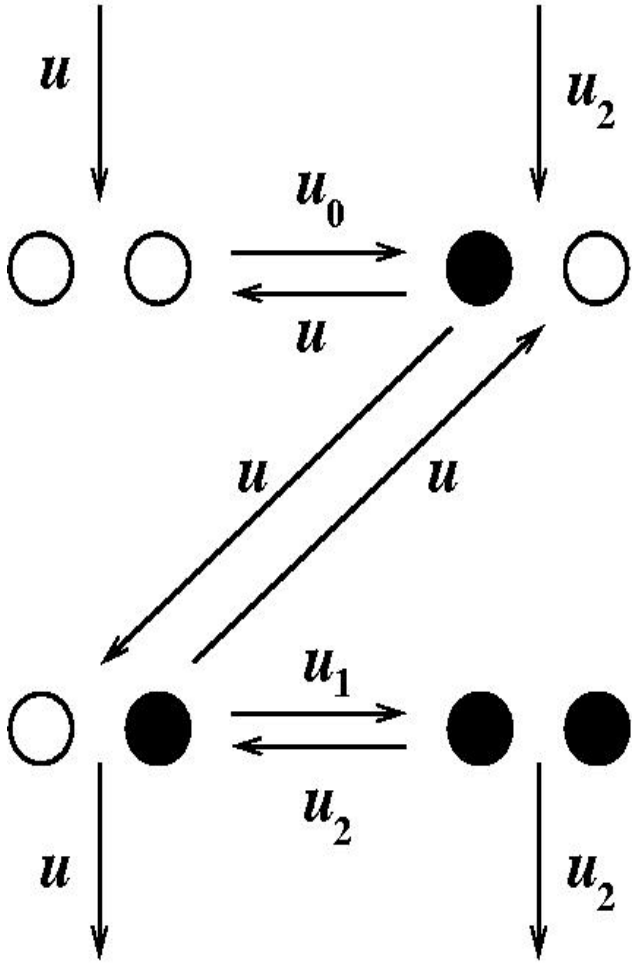
Intermolecular Interactions

For repulsive interactions:



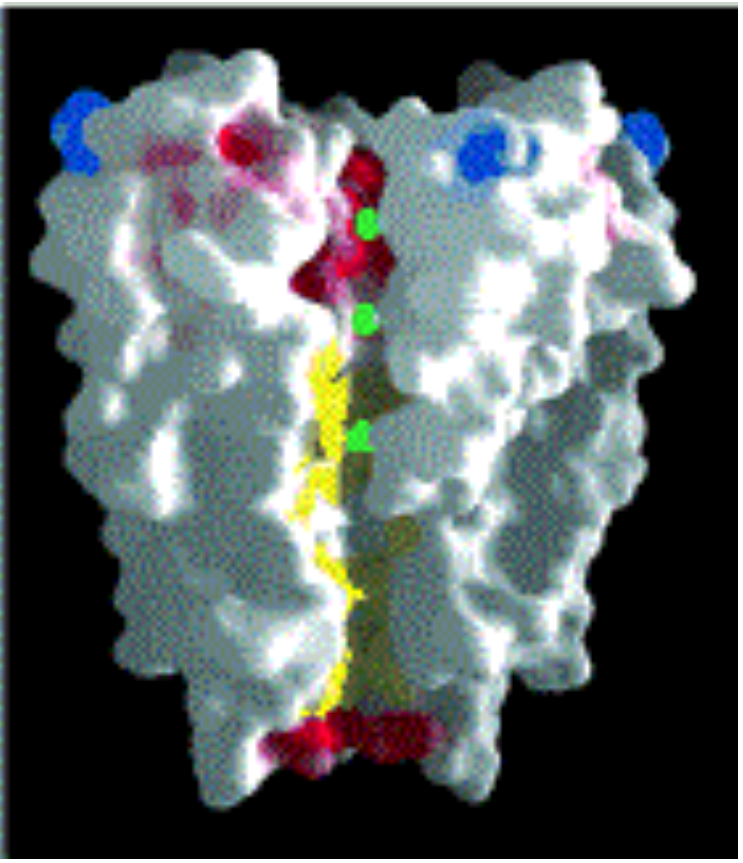
1) Decreases the flux of other particles into the channel;

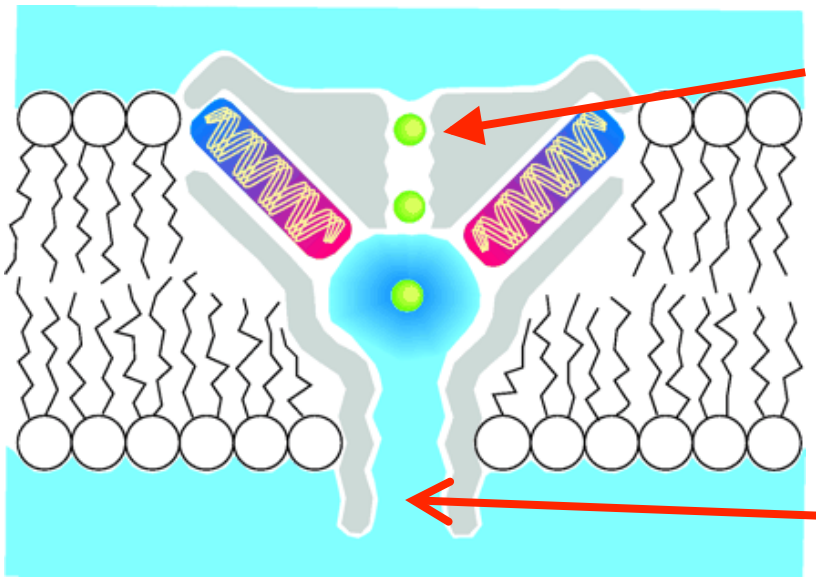
2) Increases the flux out of the channel



Transport through K^+ Channels

Mechanism of Transport of K^+ through Potassium Channels:





Red – negative groups
Blue- positive groups
Yellow- hydrophobic groups

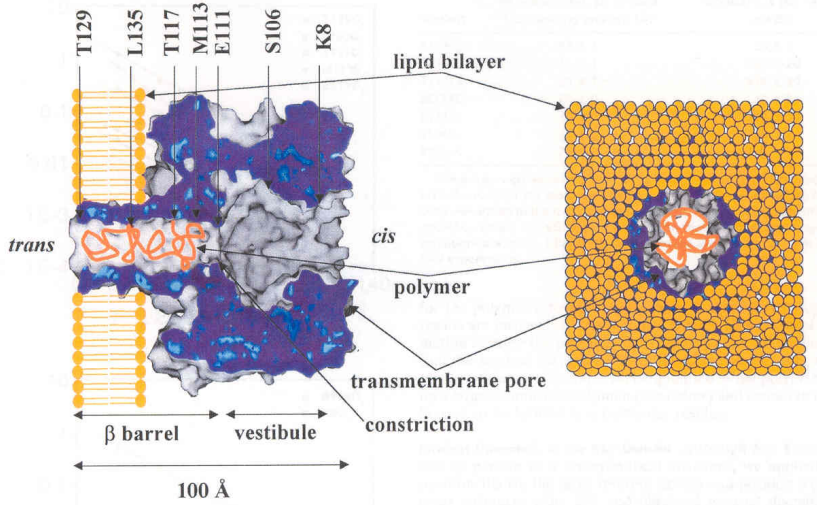
Science, **280**, 69 (1998)

What Did We Learn?

- **Molecules can be moved through channels by modifying the spatial distribution of binding sites (potential of interactions)**
- **Another important factor in controlling the channel transport – strength of interactions**
- **Both negative and positive interactions might accelerate the particle currents**
- **We argue that interactions between the molecules can also influence the flux across the nanopores**

Comments and Future Directions

1) Real biological channels are complex structures, far away from uniform cylindrical channels assumed in theory
2) In many cases the transport is complicated by external field and complex short-range and long-range interactions
3) Separation of mixtures



It is necessary to combine experimental, analytical and computational methods in order to elucidate mechanisms of biological transport

CONCLUSIONS

- A theoretical approach based on **discrete-state stochastic models** for molecular transport through biological channels is developed
- The mechanisms of interactions are investigated using simple discrete-state models
- Molecule/Nanopore interactions might control the transport across channels via strength and/or spatial distributions
- Both attractive and repulsive binding sites might produce the optimal flux
- Intermolecular interactions can also influence transport across the channels