Weill Cornell Medicine

High-Speed Atomic Force Microscopy (HS-AFM): The Dawn of Dynamic Structural Biochemistry

Simon Scheuring

Bio-AFM-Lab.com

ScheuringLab

Bio-AFM-Lab

Weill Cornell Medicine

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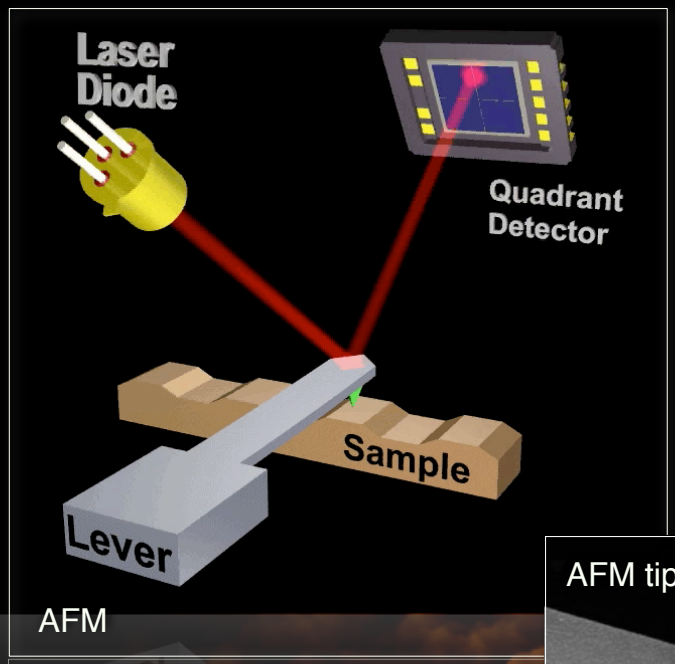
High-Speed Atomic Force Microscopy (HS-AFM)

Atomic Force Microscopy (AFM)

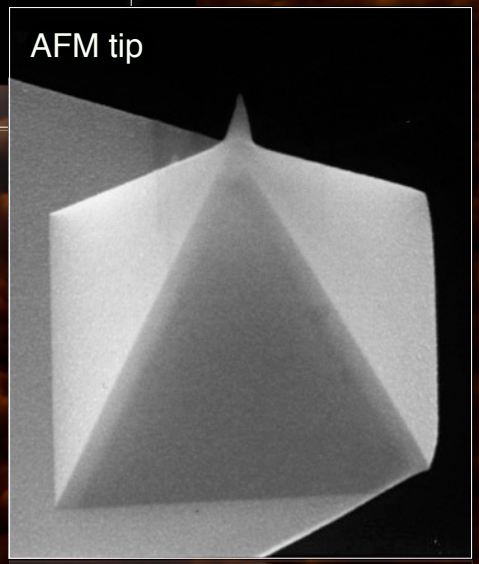
Simon Scheuring



Lever length: ~ μm
Tip radius: ~ nm

Analogy
AFM - Phonograph

Mounting: ~ mm
Tip radius: ~ μm



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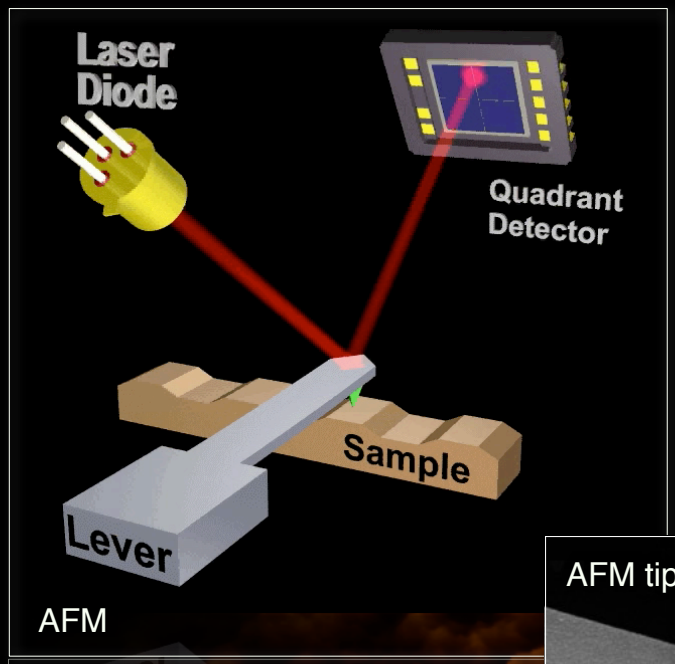
Atomic Force Microscopy (AFM)

Simon Scheuring



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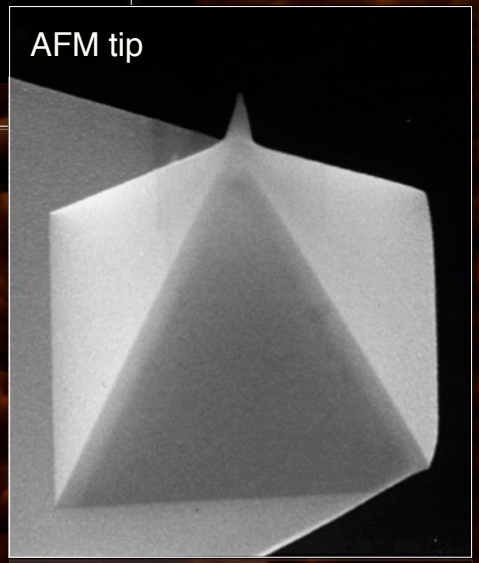

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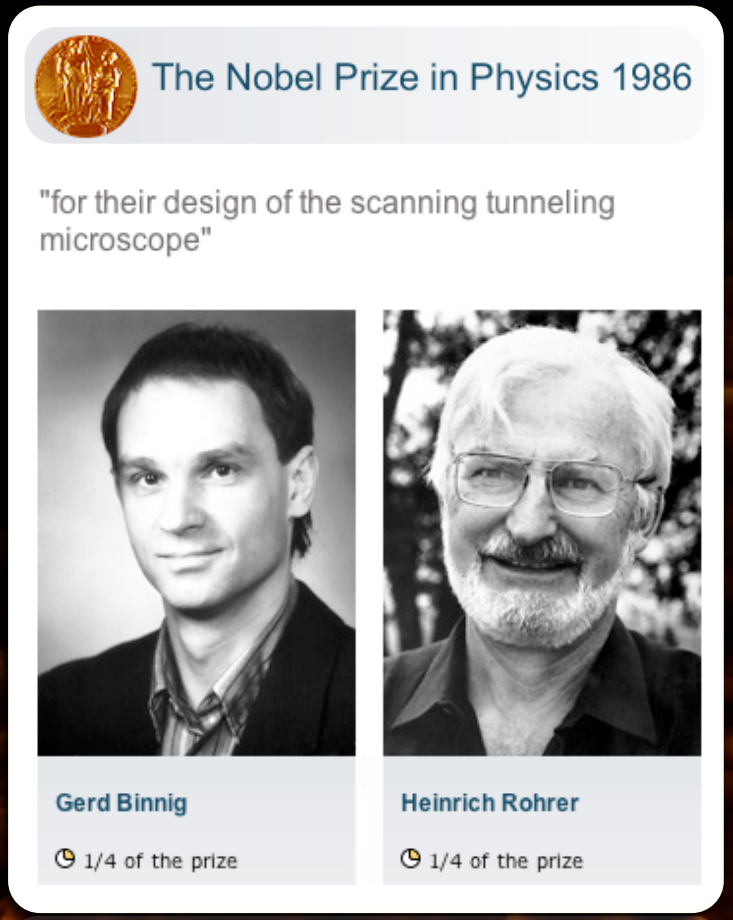
The Promise... We Will Take Movies!

Gerd Binnig about dynamic AFM imaging in 1992



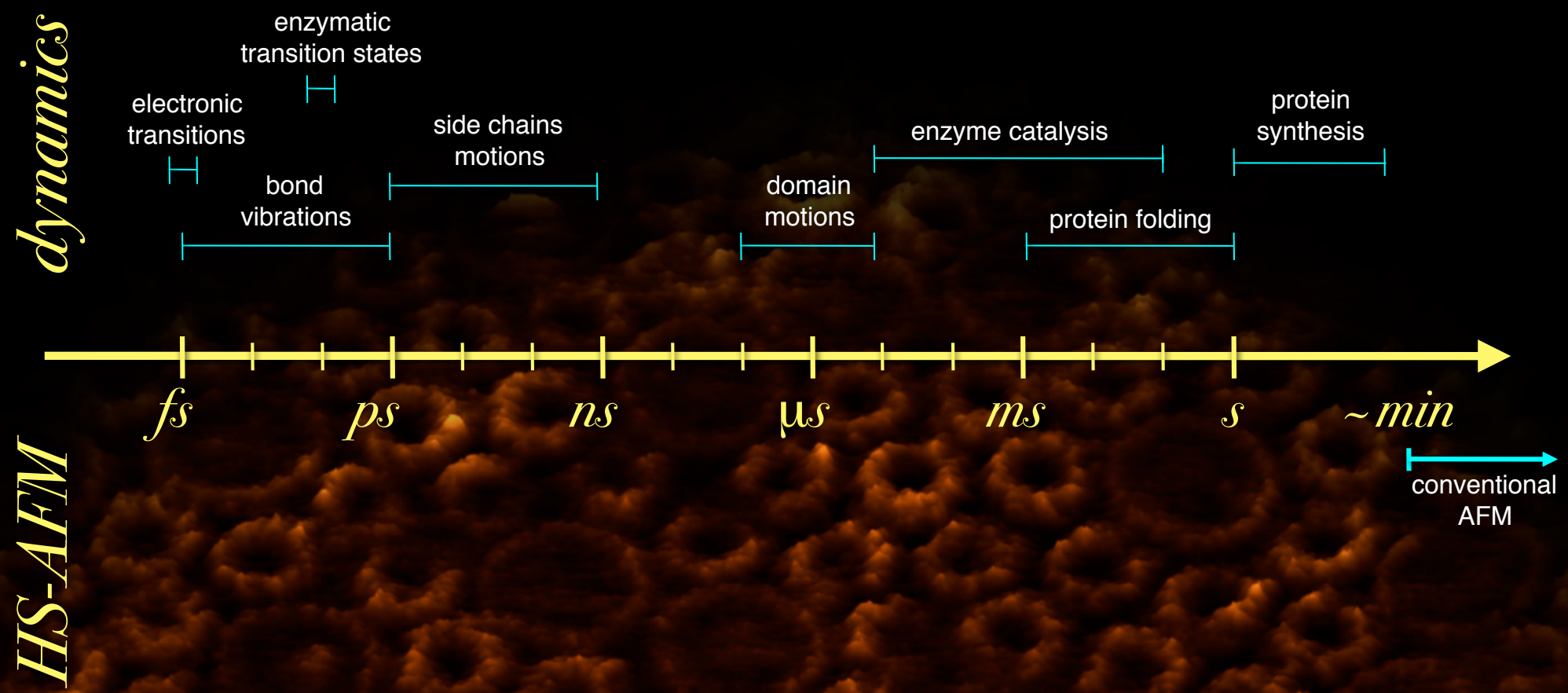
In biology, use of the force microscope will probably become quite common because of its ability to deliver films of processes.

Binnig, G. Ultramicroscopy 1992, 42, 7.

Atomic Force Microscope (HS-AFM)

Simon Scheuring

Dynamics in Biology



PNAS, 2001, 98: 12468-12472
Chemical Reviews, 2014, 114 (6): 3120–3188

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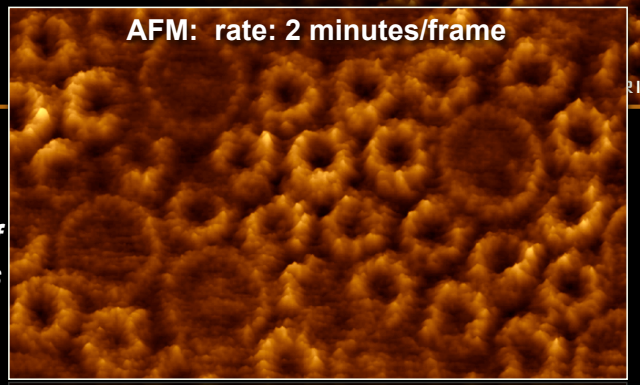

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enzymatic transition states
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side chains motions
bond vibrations
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fs *ps* *ns* *μs* *ms* *s* *~min*

conventional AFM

HS-AFM

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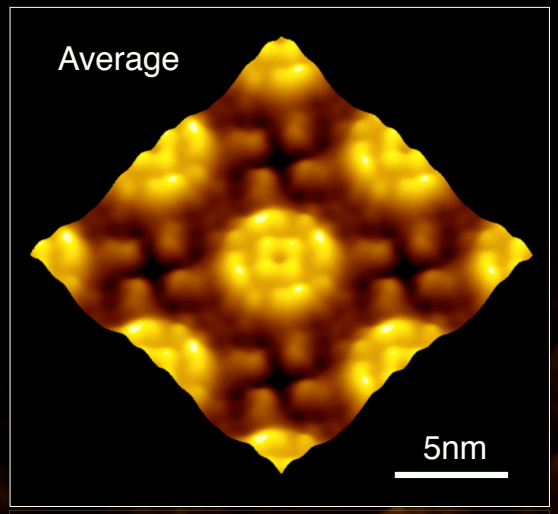
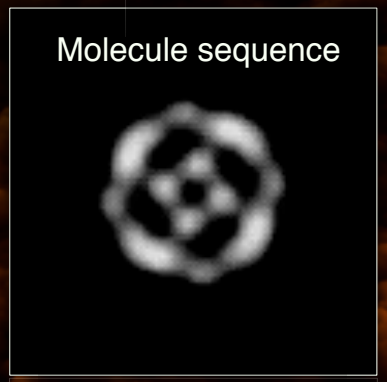
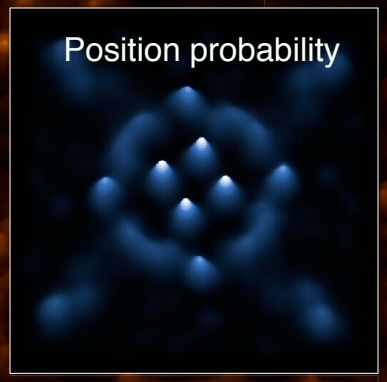
The Detour... Morphing!

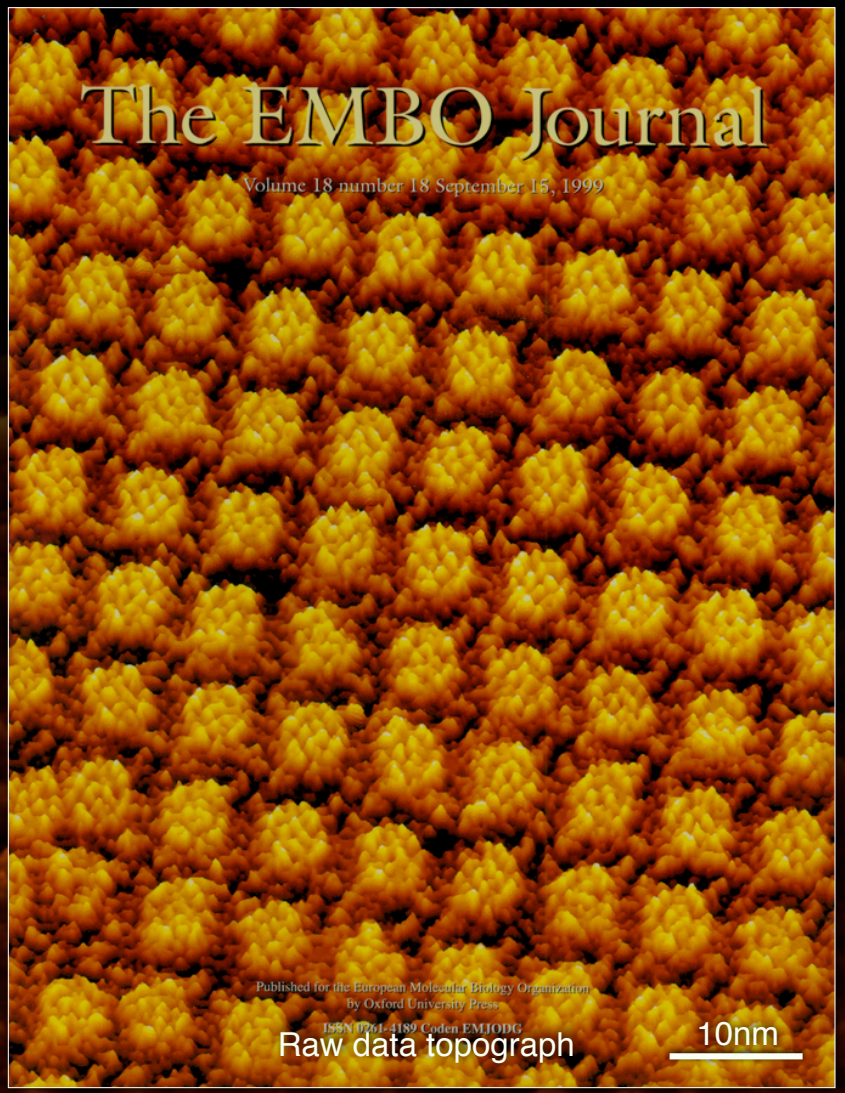
Simon Scheuring

High-resolution imaging and image processing









EMBO J, 1999, 18 (18): 4981-4987
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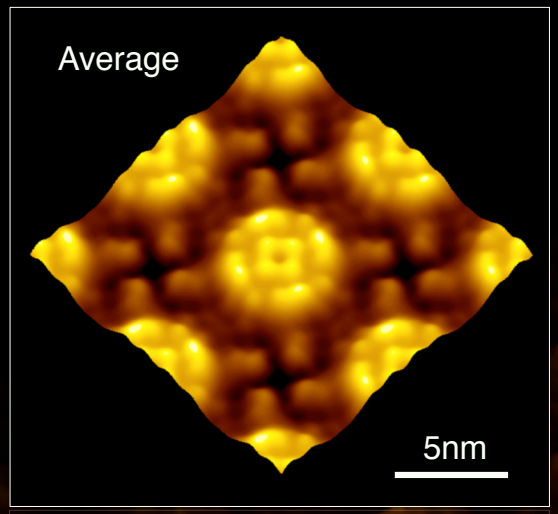

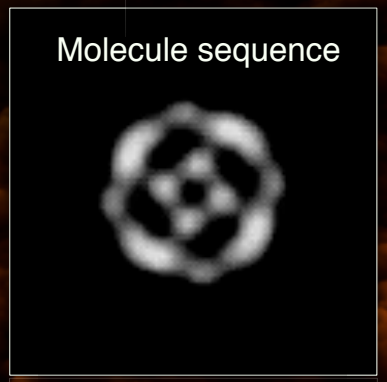

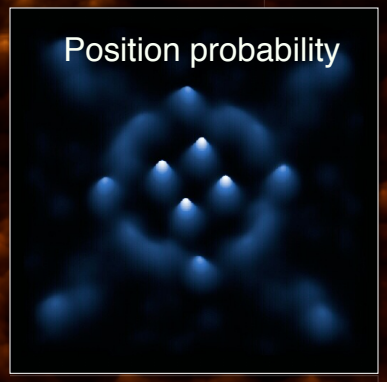

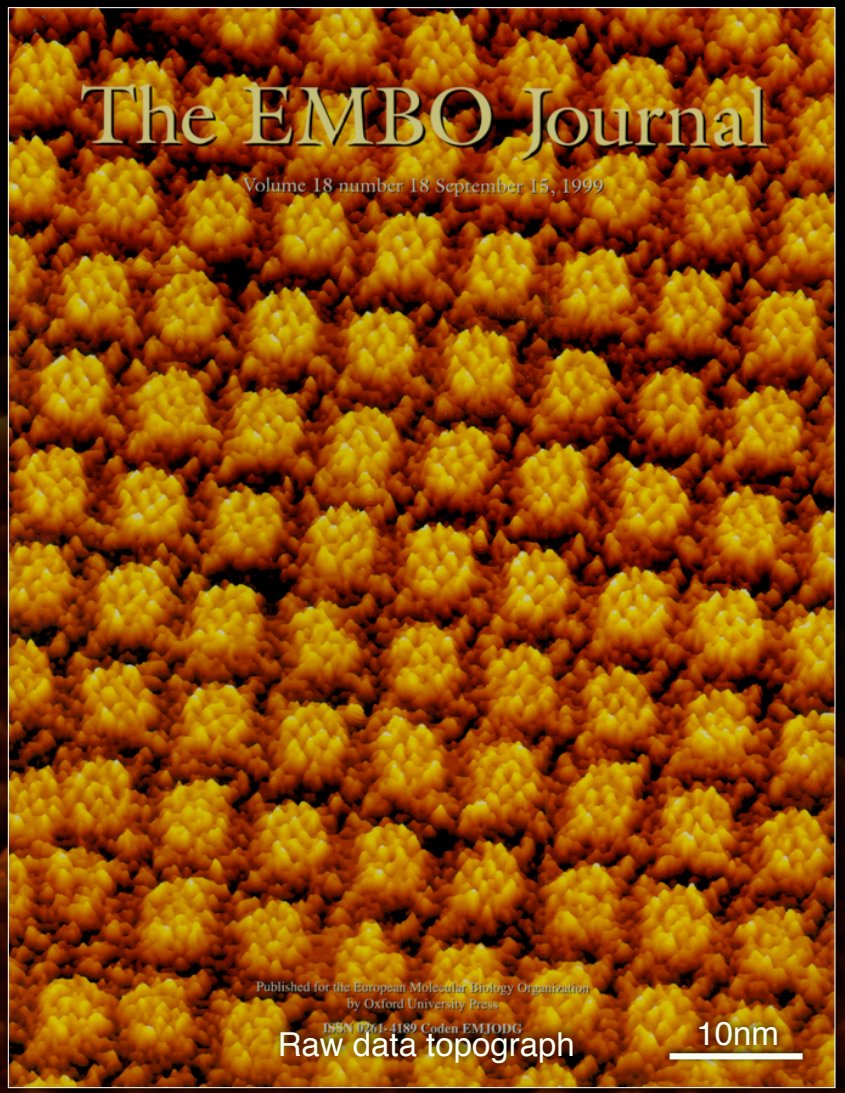

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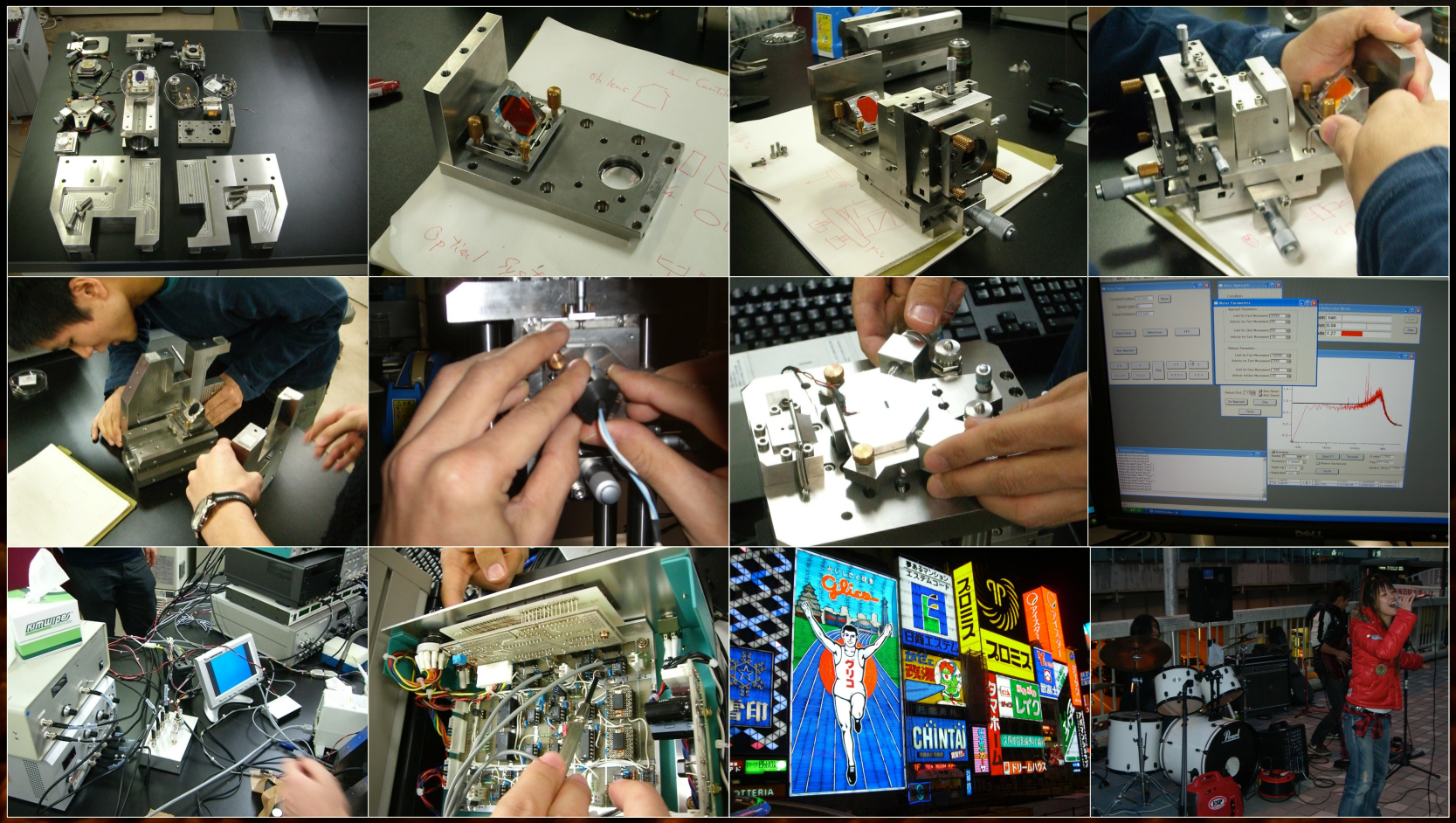
Bio-AFM-Lab

HS-AFM: 1st Prototype

HS-AFM: 1st Prototype: January/February 2008



Acknowledgement: Prof ANDO, Kanazawa University, Japan
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High-Speed Atomic Force Microscope (HS-AFM)

Simon Scheuring

HS-AFM: 1st prototype built in Paris, 2008

Part of the setup

Dirt on mica - 03 / 09 / 2008

~20 images / second
~50 milliseconds per image
~100 microseconds per line

Reminder:
Conventional AFM:
Contact Mode: ~1 image / minute
Oscillating Mode: ~1 image / 10 minutes

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High-Speed Atomic Force Microscope (HS-AFM)

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The high-speed atomic force microscope (HS-AFM)

HS-AFM

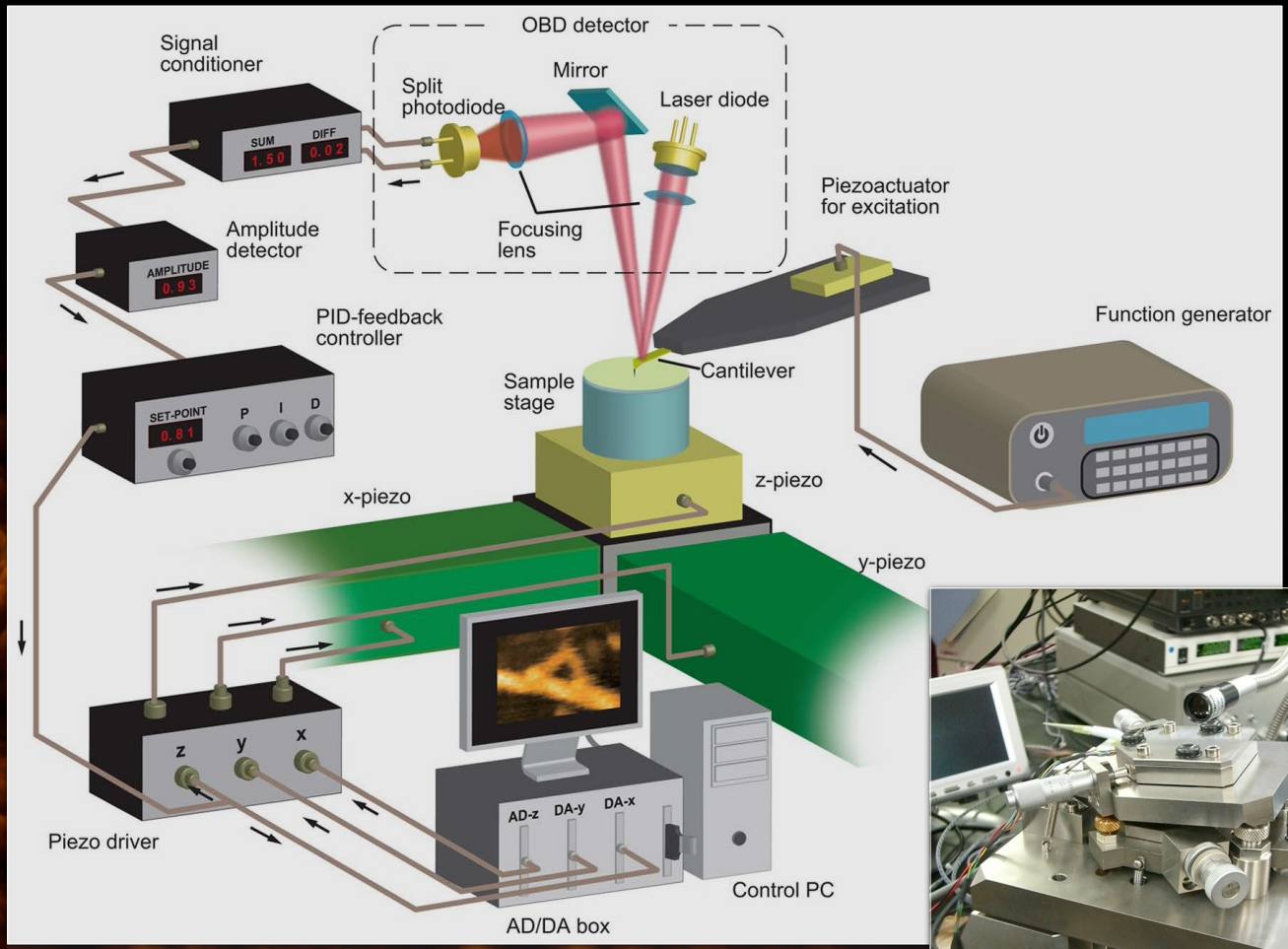
Bio-compatibility:

- in buffer solution
- at ambient pressure
- at ambient temperature

Speed:

about 1000 times faster than conventional AFM

- short cantilevers
- small piezo elements
- dummy piezos
- fast electronics (wave det.)
- dynamic feedback
- etc



Ando, Uchihashi & Scheuring
Chemical Reviews, 2014, 114 (6): 3120–3188

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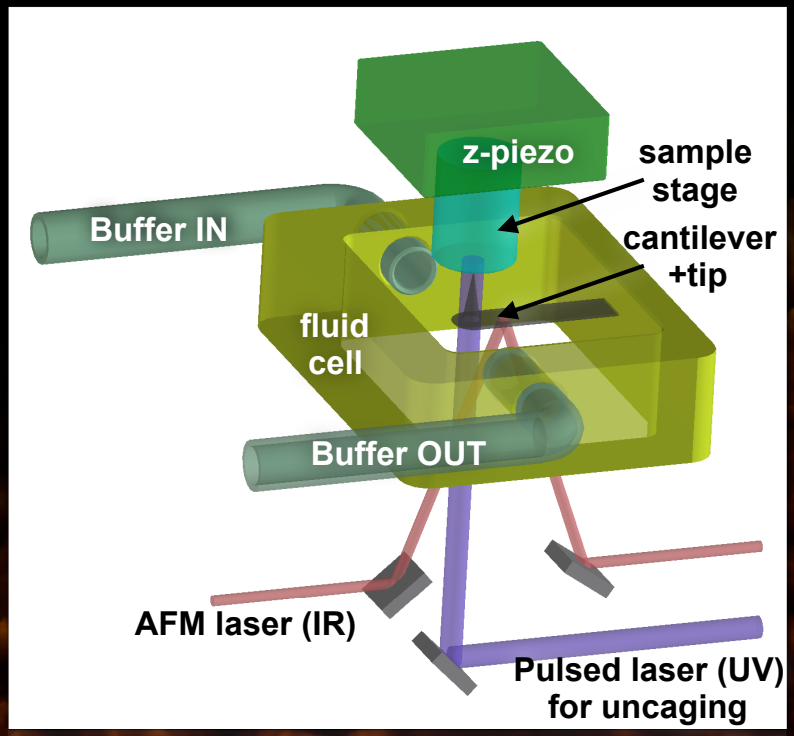
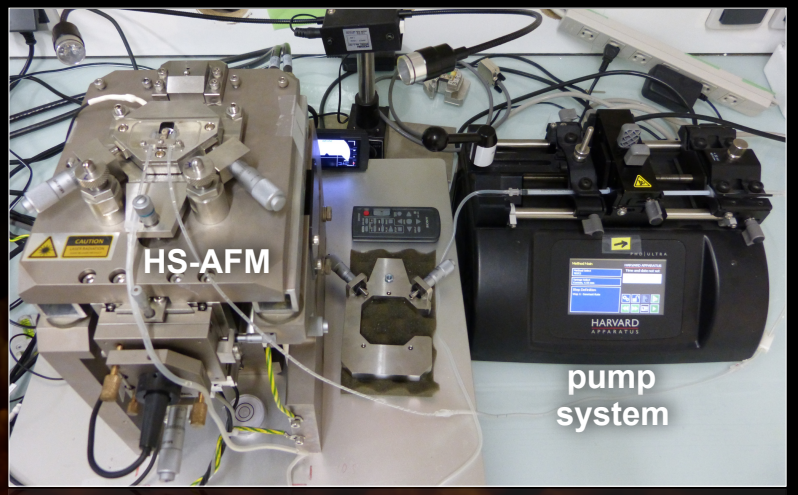
Bio-Enhanced HS-AFM

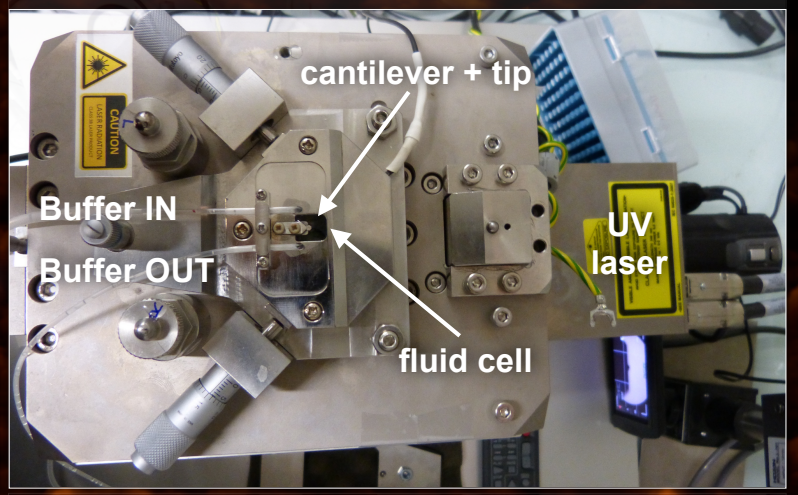
Simon Scheuring

Ultra-violet (UV) pulsed laser &
Pump buffer exchange systems



Uncaging caged molecules
Adding/Removing Agonists/Antagonists





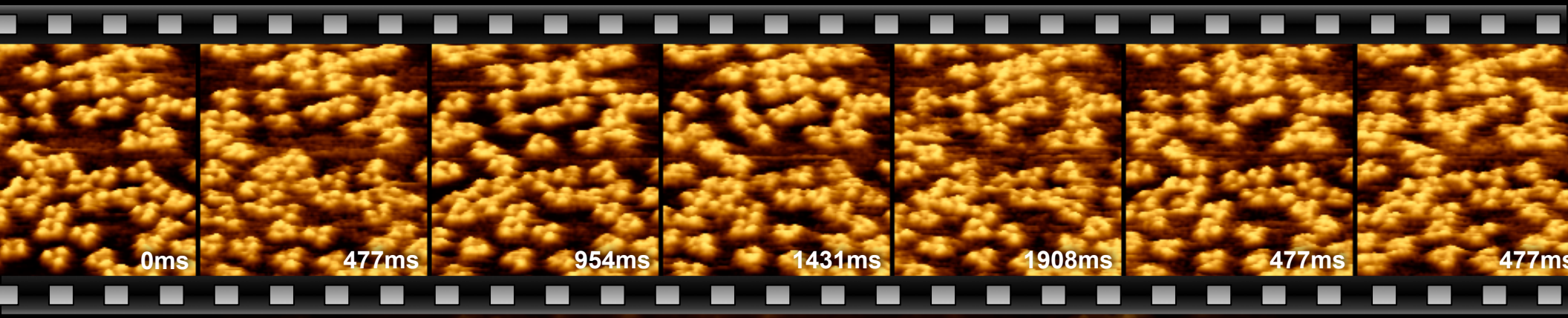
Nature Nanotechnology, 2016, 11: 783-790

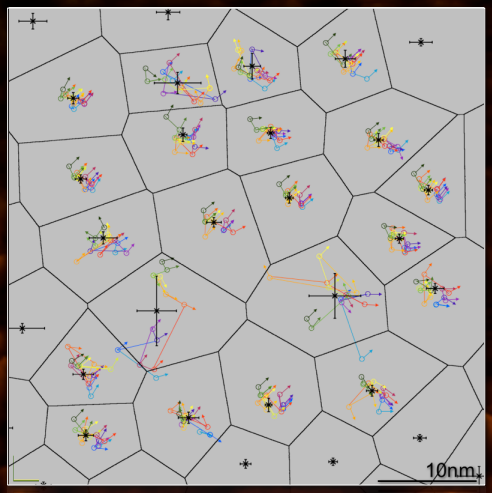
Bio-AFM-Lab

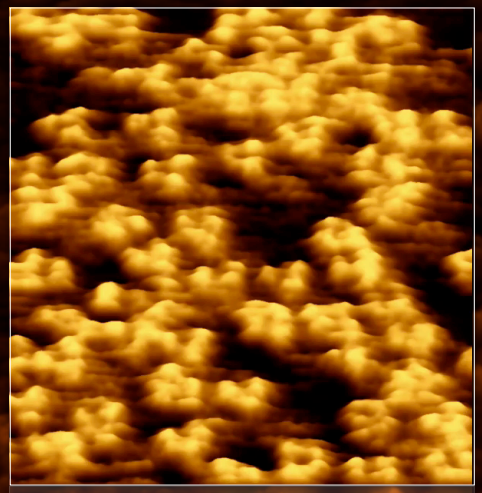
OmpF Diffusion and Interaction In Membrane

Simon Scheuring

E. coli outer membrane porin F (OmpF) in *E. coli* lipids : Mobile molecules







frame rate: 477ms (2.1 frames/second)
scan size: 75nm (750Å)
image size: 300 · 300 pixels
cantilever resonance frequency: 600kHz
z-piezo resonance frequency: 350kHz
feedback-loop bandwidth: 100kHz
cantilever spring constant *k*: 0.15N/m
$A_{(free)}$ ~ 1nm / $A_{(tap)}$ ~ 0.9 x $A_{(free)}$
applied force (approximation!): $F = k \cdot \Delta d / Q$ = ~10pN
Impulse per tap: ~$1 \cdot 10^{-18}$N·s = ~1aN·s
taps per pixel: 600000/(2.1·300^2·2) = 1.6 taps/p

Nature Nanotechnology, 2012, 7 (8): 525-529

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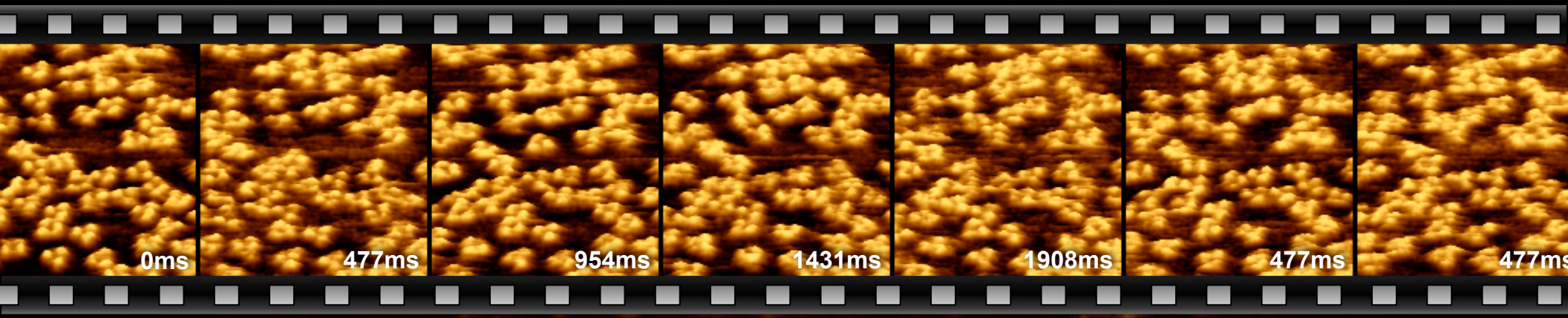

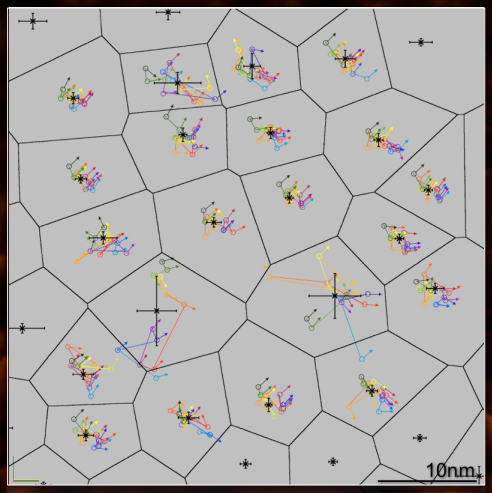

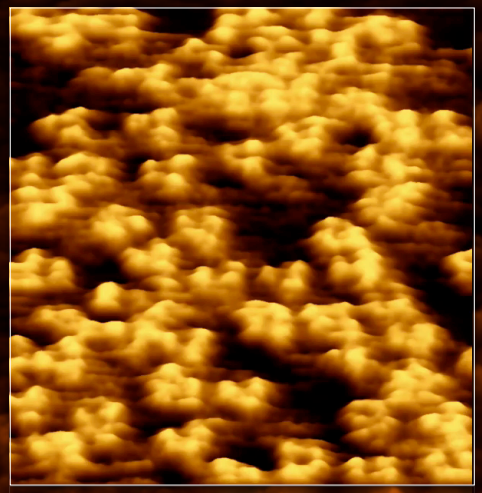

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Rotational Diffusion Of Unlabeled OmpF

Simon Scheuring

HS-AFM at **100** frames/second

HS-AFM movie (100 f/s)
5 times slowed speed
full movie length: 20000ms (20s)

10ms^{-1} frame rate !!!

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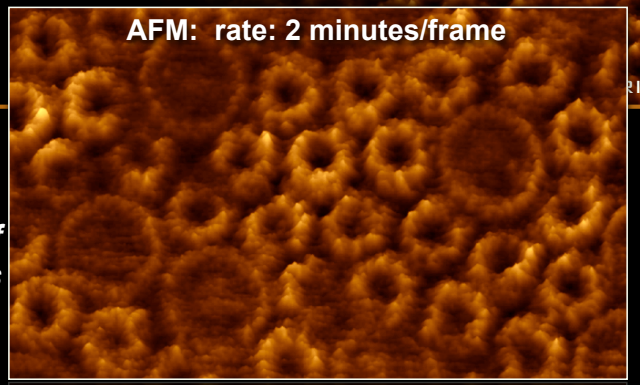
The Solution... High-Speed AFM



Dynamics in Biology

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Simon Scheuring*, & James Sturgis

dynamics

enzymatic transition states
electronic transitions
side chains motions
bond vibrations
domain motions
enzyme catalysis
protein synthesis
protein folding

fs *ps* *ns* *μs* *ms* *s* *~min*

HS-AFM

image scan HS-AFM
conventional AFM

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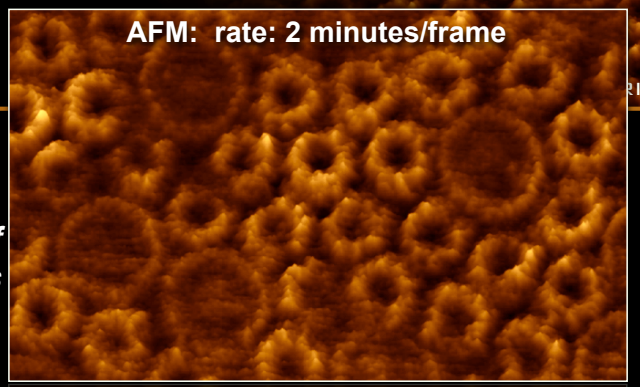

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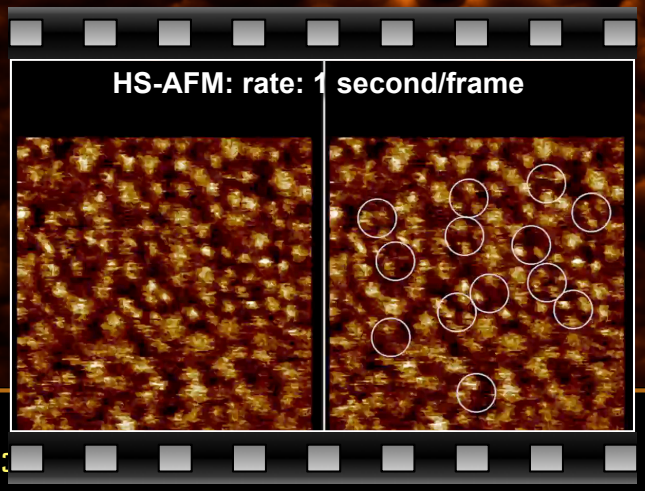


Direct visualization of glutamate transporter elevator mechanism in substrate transport by high-speed AFM.
PNAS, 2017, 114 (7):1584-1588.
Yi Ruan, Atsushi Miyagi, Xiaoyu Wang, Mohamed Chami, Olga Boudker* & Simon Scheuring*

PNAS, 2001, 98: 12468-12472
Chemical Reviews, 2014, 114 (6): 3

Bio-AFM-Lab

Weill Cornell Medicine

Simon Scheuring

High-Speed Atomic Force Microscopy (HS-AFM) Reveals the Inner Workings of the MinDE Protein Oscillator

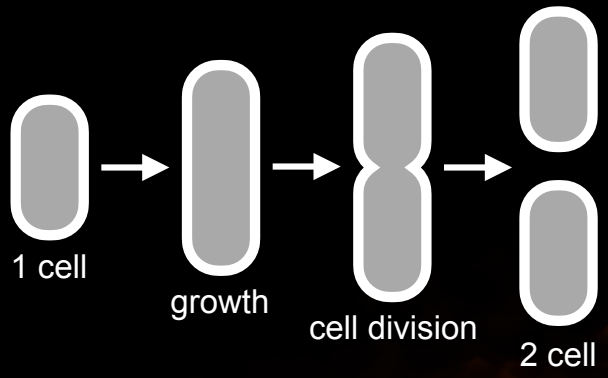
Atsushi Miyagi, Beatrice Ramm, Petra Schwille & Simon Scheuring

Collaboration with
Petra Schwille laboratory
Max Planck Institut, Martinsried, Germany

HS-AFM Deciphers MinDE Pattern Formation

Simon Scheuring

Bacterial cell division



need for a molecular ruler to define the midcell

The Min system

MinD is a membrane associating ATPase

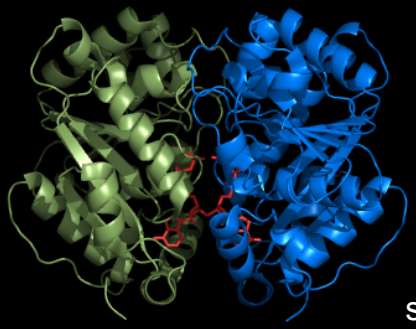
MinE is a membrane and MinCD associating and ATPase activation protein (AAP)

MinC is an FtsZ inhibitor, antagonizing its structure assembly when in a high enough concentration

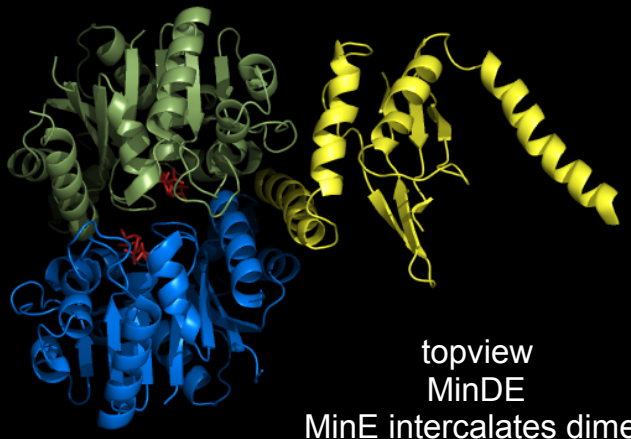
FtsZ is a bacterial cytoskeletal protein and forms a ring around the longitudinal midpoint, or septum, of the cell

Structure (PDB 3Q9L / PDB 3R9J)

topview
MinD
~6nm x ~4nm



sideview
MinD
height: ~5nm



topview
MinDE
MinE intercalates dimer

Bio-AFM-Lab

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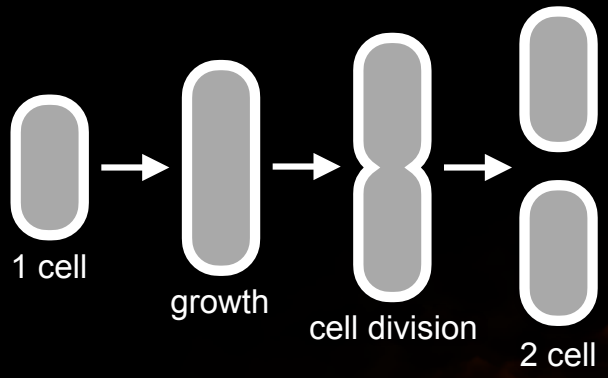
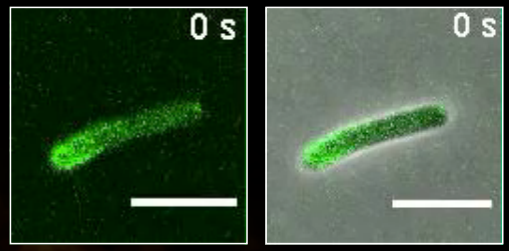


need for a molecular ruler to define the midcell

The Min system is the molecular ruler



MinDE oscillations from pole to pole (Schwille lab)

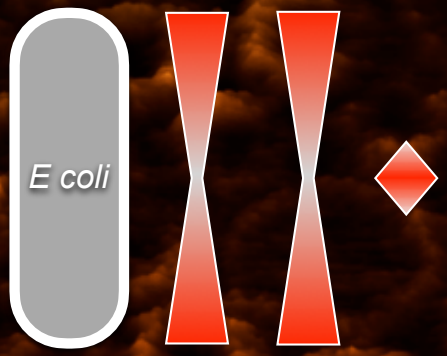
Plac: sfGFP-MinDMinE in *E.coli* HL1 (ΔMinDE)

4 proteins are involved

FtsZ is the belt
MinC inhibits FtsZ

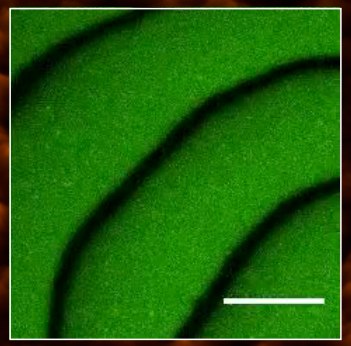
MinD is oscillator
MinE is oscillator

MinC follows MinDE



time averaged [conc]

MinD and MinE: necessary and sufficient



eGFP-MinD

in vitro reconstitution of oscillatory pattern formation with MinD and MinE only (Schwille lab)

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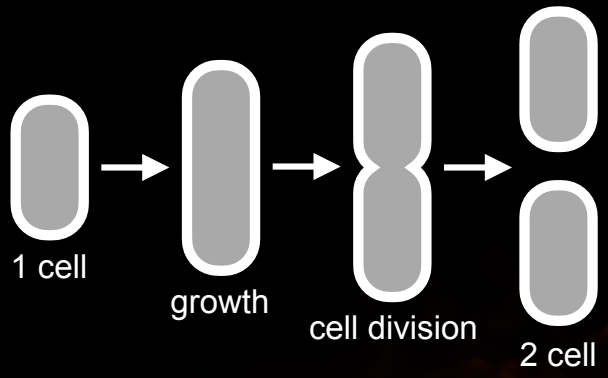

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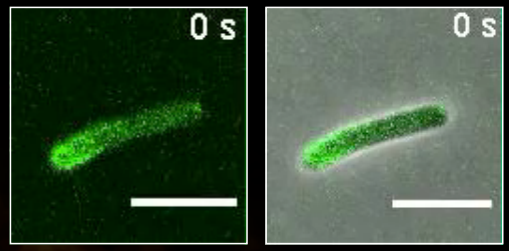

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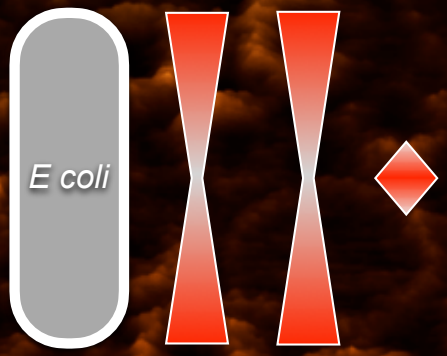

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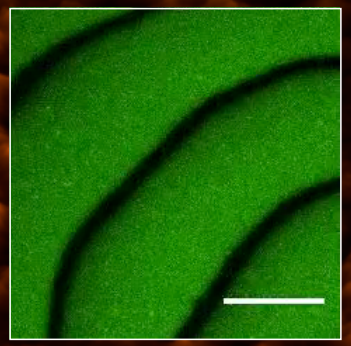

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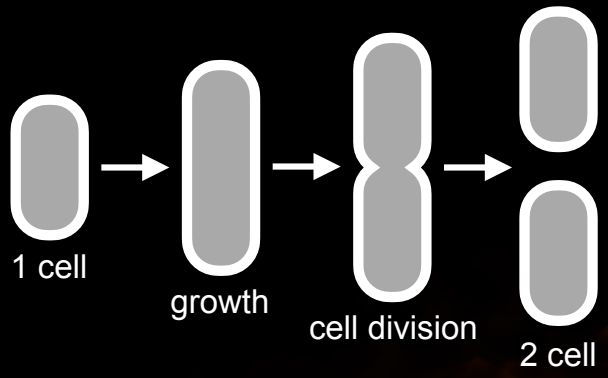
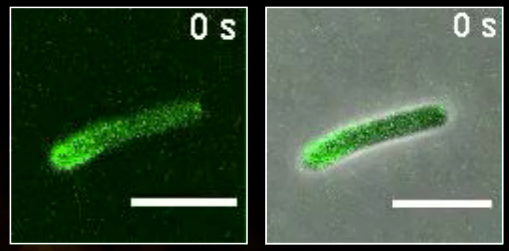
Bacterial cell division



need for a molecular ruler to define the midcell

The Min system is the molecular ruler



MinDE oscillations from pole to pole (Schwille lab)

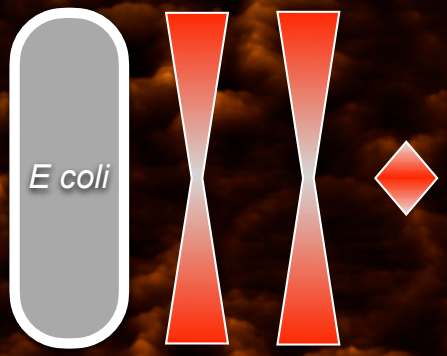
Plac: sfGFP-MinDMinE in *E.coli* HL1 (ΔMinDE)

4 proteins are involved

FtsZ is the belt
MinC inhibits FtsZ

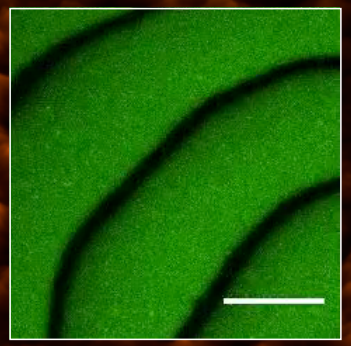
MinD is oscillator
MinE is oscillator

MinC follows MinDE



time averaged [conc]

MinD and MinE: necessary and sufficient



eGFP-MinD

in vitro reconstitution of oscillatory pattern formation with MinD and MinE only (Schwille lab)

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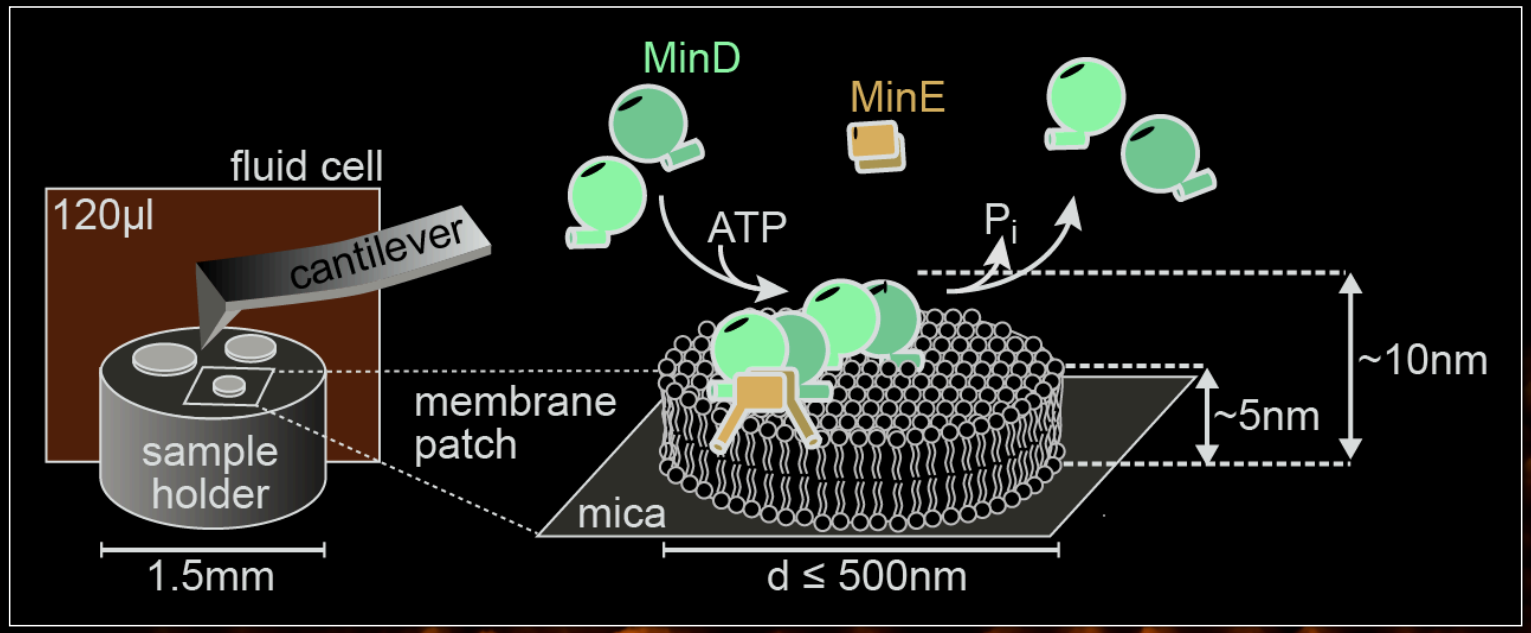
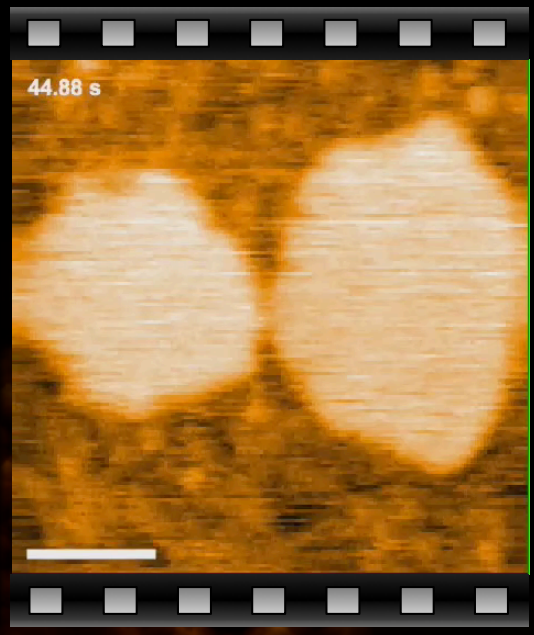
HS-AFM Deciphers MinDE Pattern Formation

Simon Scheuring

The experimental setup: HS-AFM / nanometer scale *E.coli* membrane patches on the surface





Experimental setup schematic

HS-AFM:
cantilever: 8µm long / ~600kHz resonance frequency / 150pN/nm force constant
operation mode: amplitude modulation / ~1.0nm free amplitude / ~0.9nm setpoint amplitude
optimized feedback parameters with adynamic feedback loop / free amplitude control

On the support: E. coli membrane patches of ~50nm < diameter ~500nm

In solution: typically 1µM MinD / 1µM MinE / 2.5mM ATP

HS-AFM movie
1.02s/frame
scale bar: 100nm

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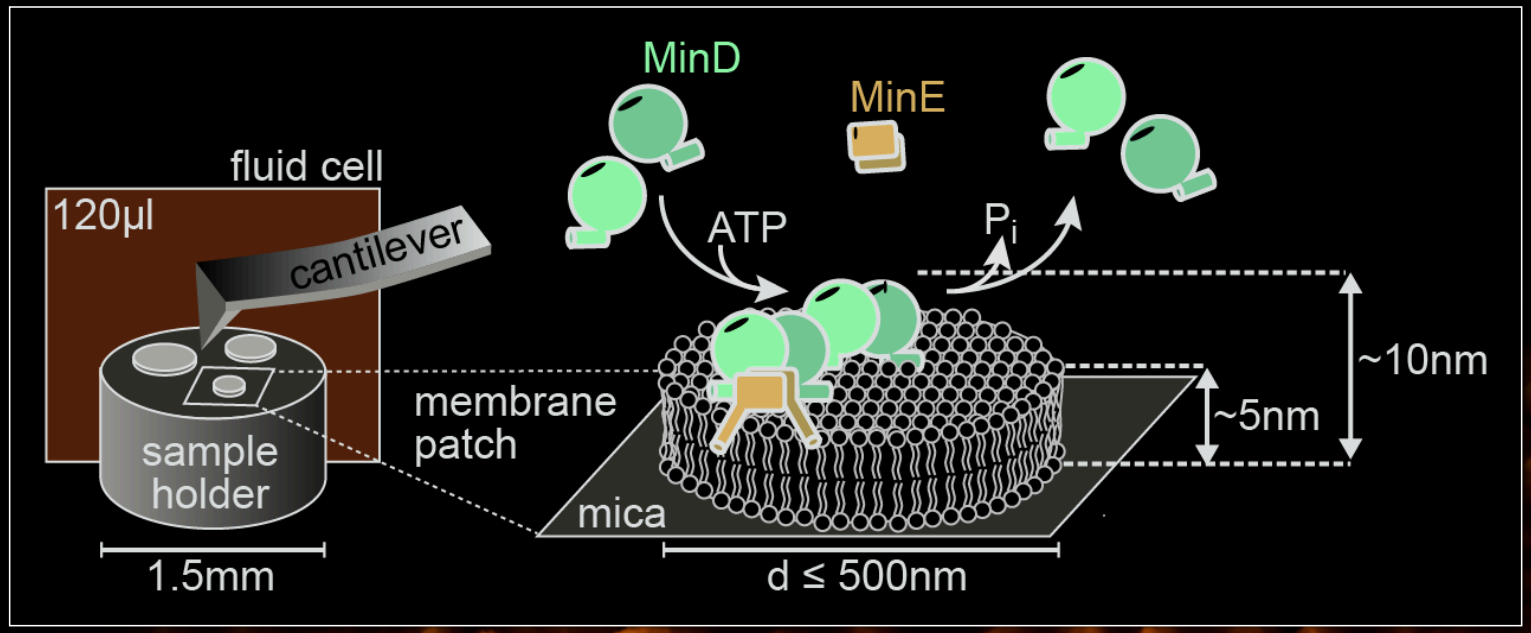

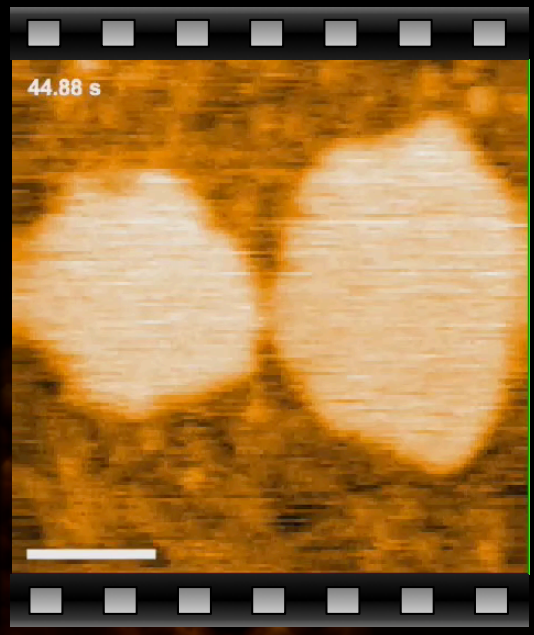

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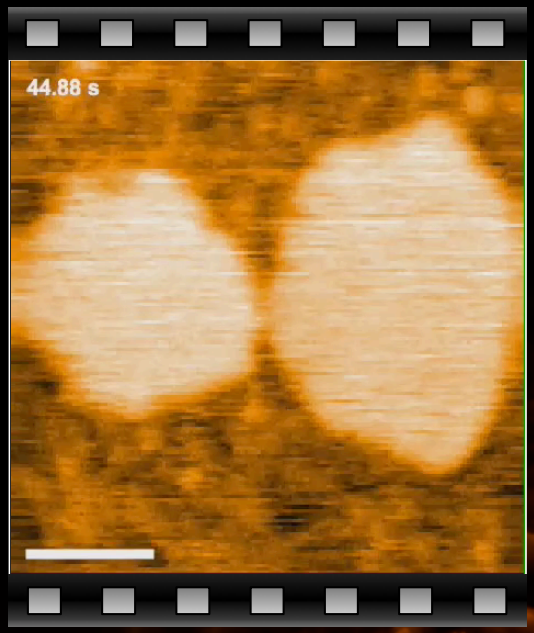
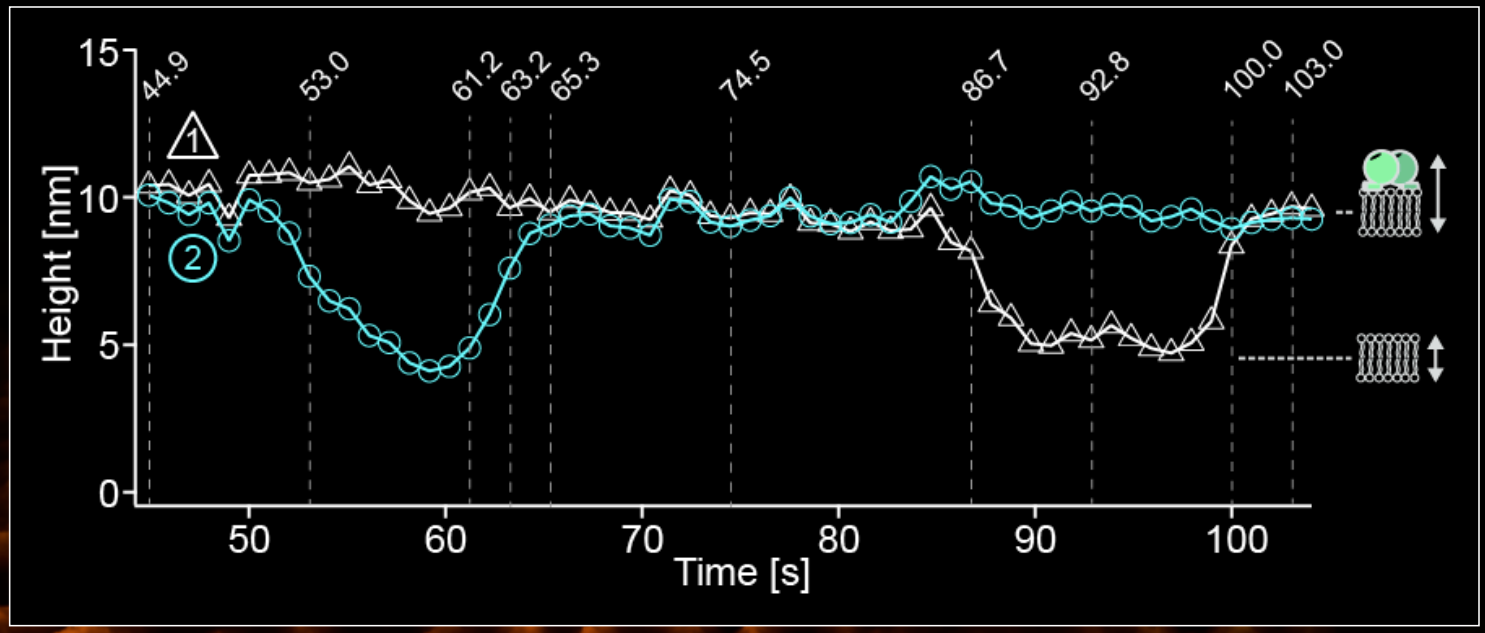
MinDE creates 'point oscillations' on nanometric membrane patches





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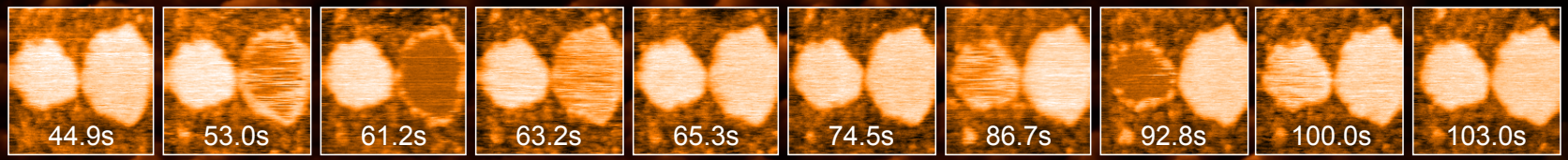

HS-AFM allows direct visualization of nanometer scale 'point oscillations' of MinDE on membrane patches
MinD covered membrane patches have a height of ~10nm
bare lipid bilayers have a height of ~5nm

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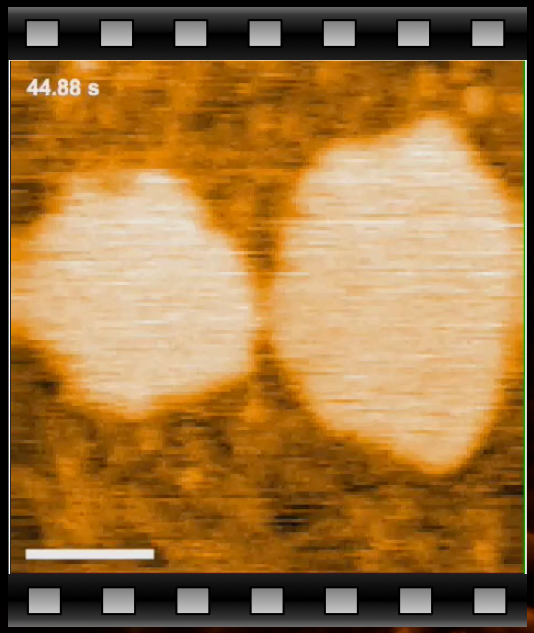

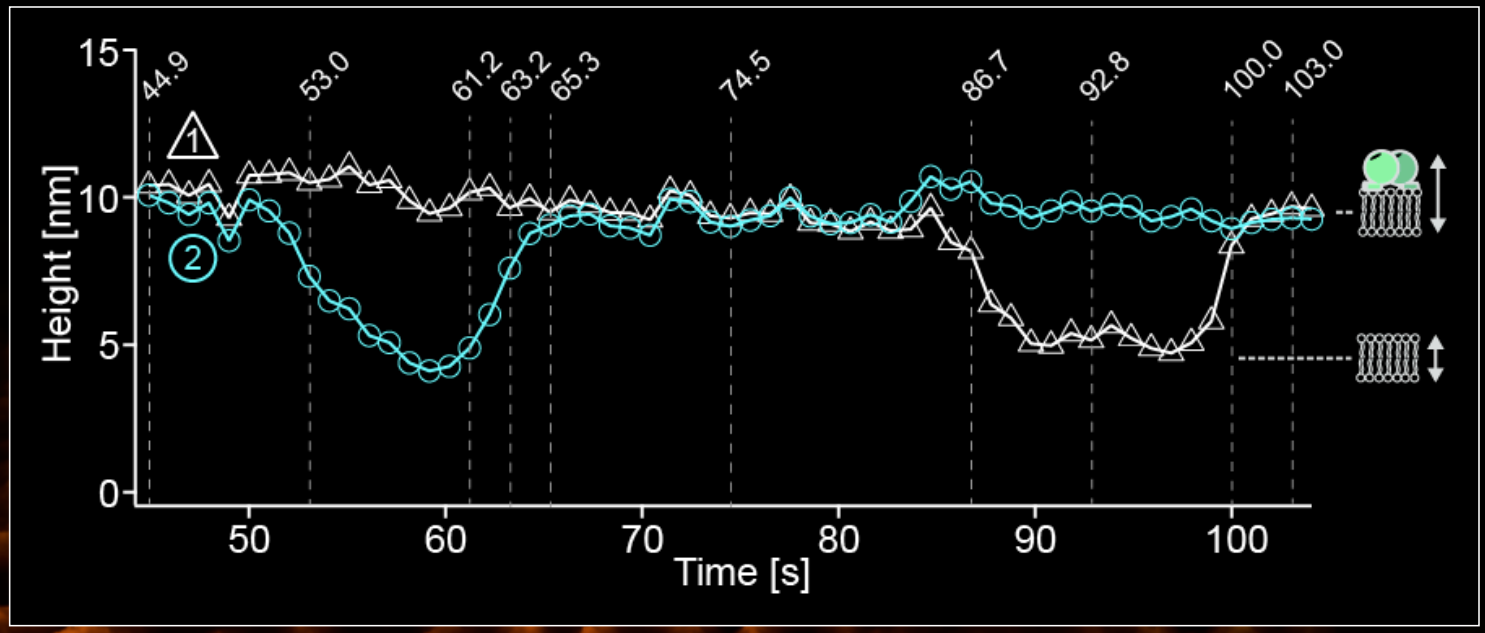

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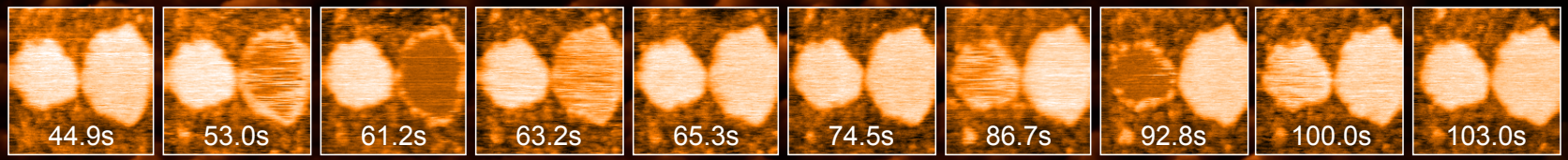

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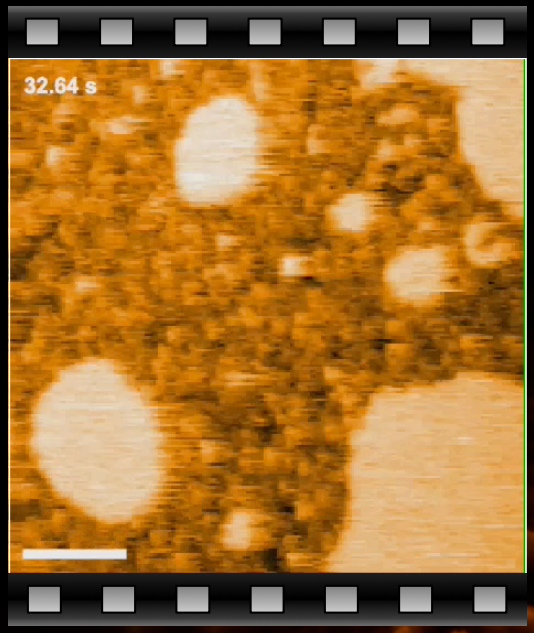
'point oscillations' display different periodicity, notably t_{ON} varies



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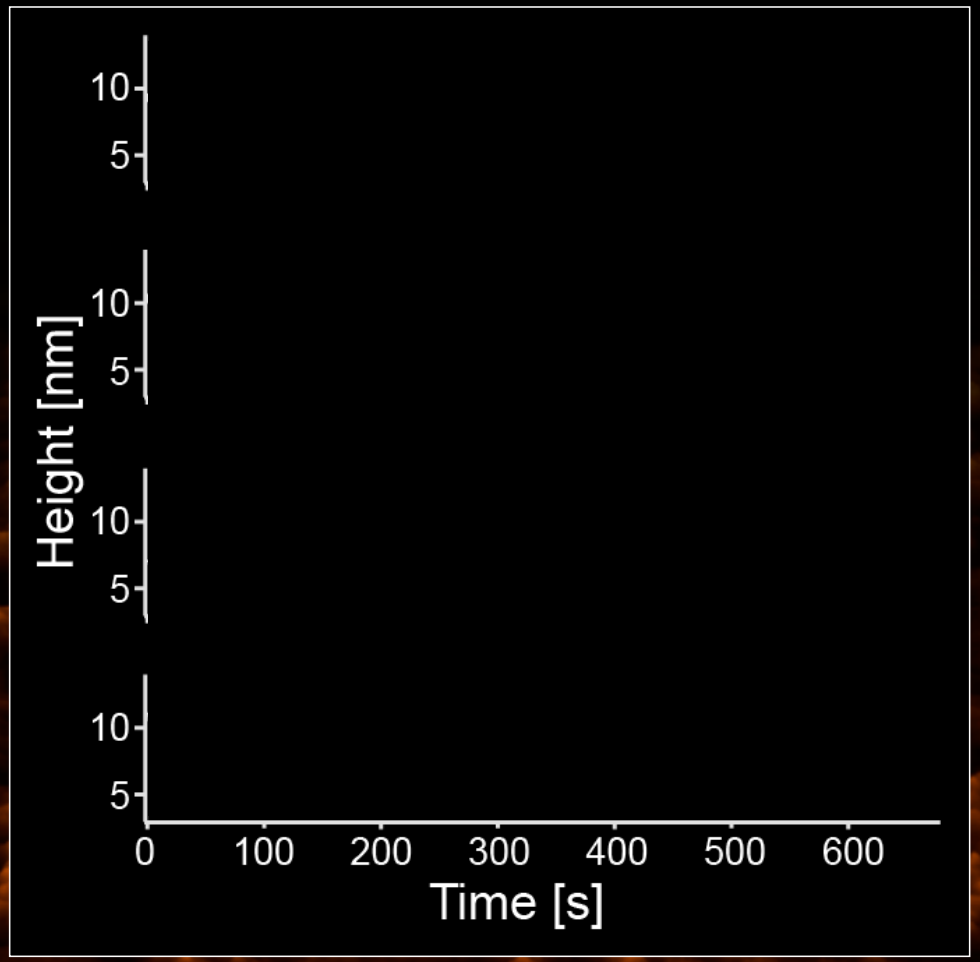

HS-AFM of unlabeled MinDE monitors 'point oscillations' on membrane patches over more than 10 minutes

Visual inspection I:
Larger patches display longer oscillation periodicity

Visual inspection II:
It is notably the membrane associated state that is elongated on larger patches

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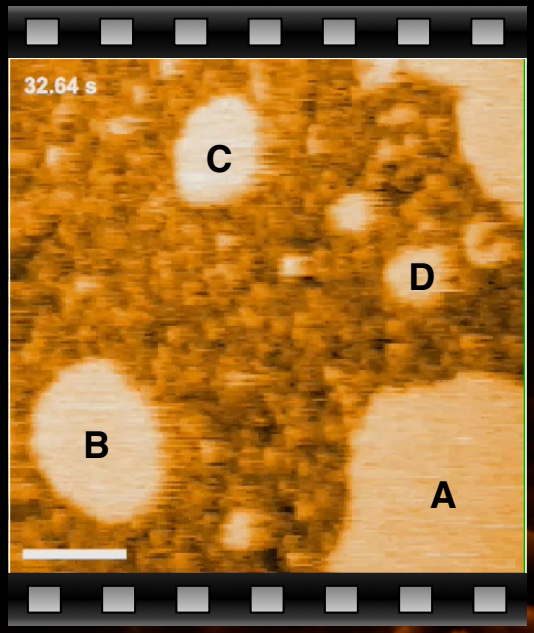

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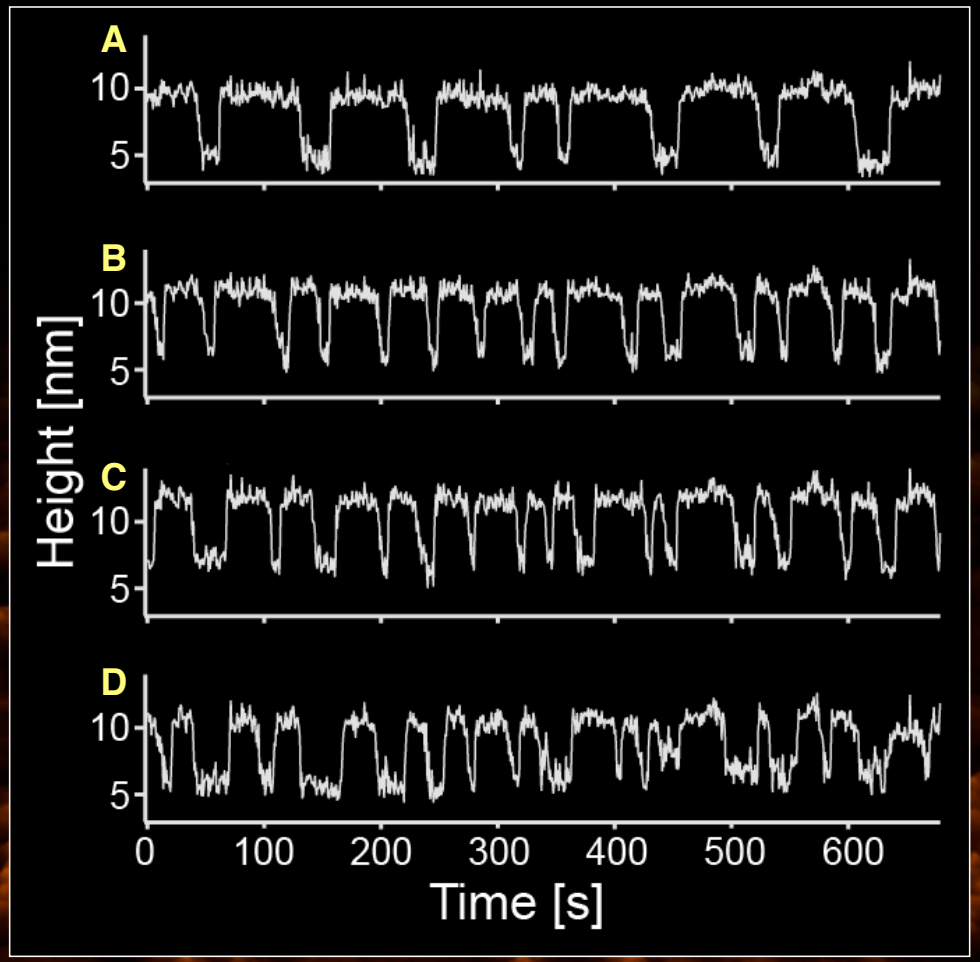

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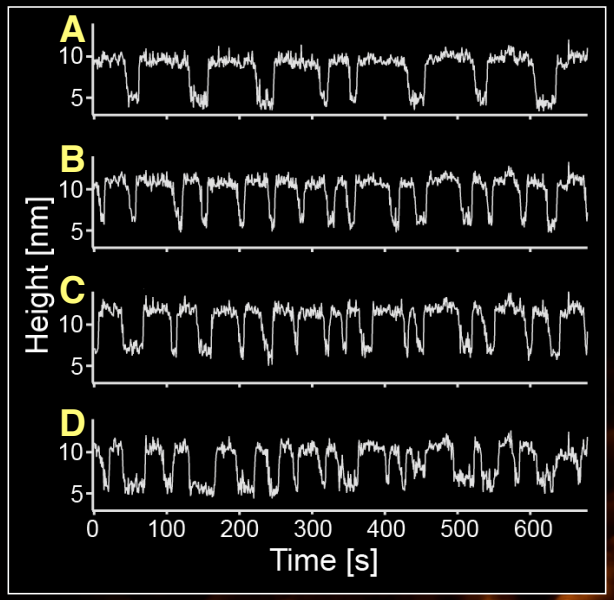

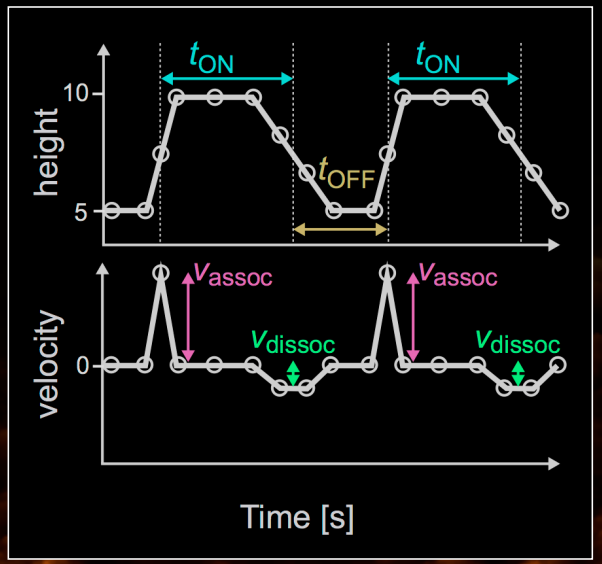

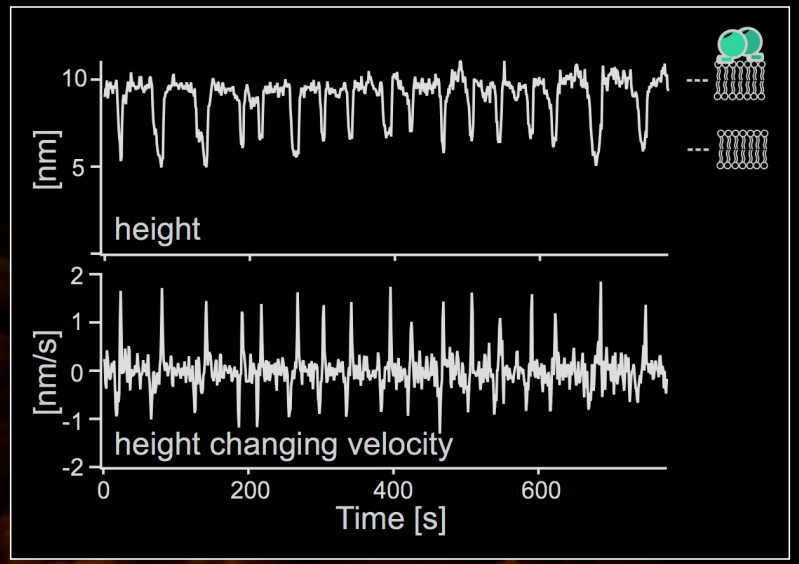

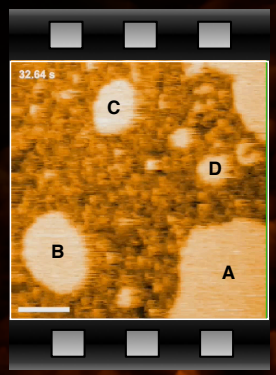

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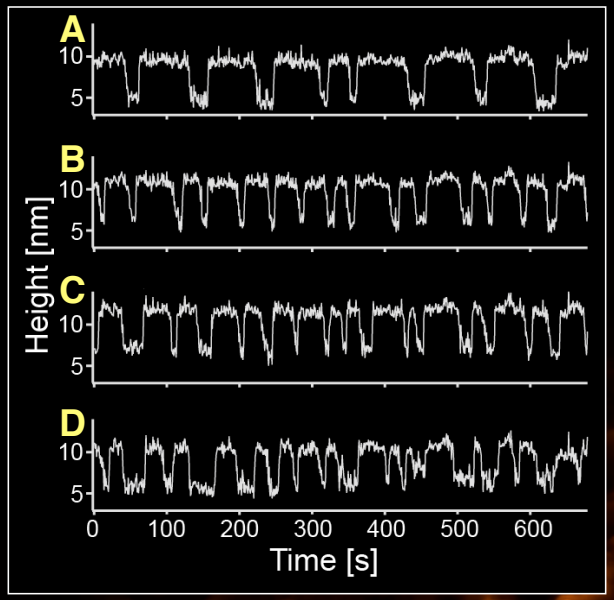

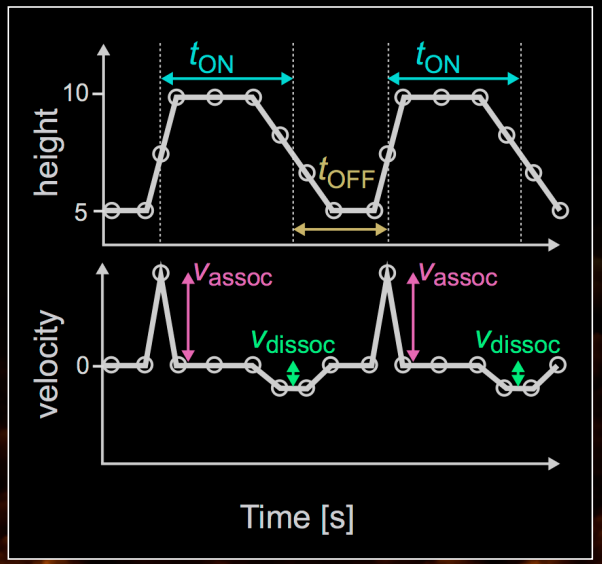

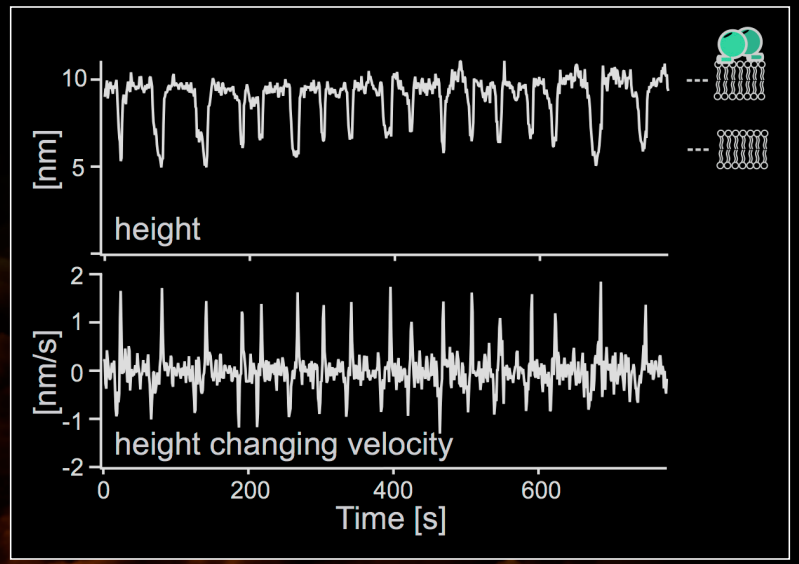

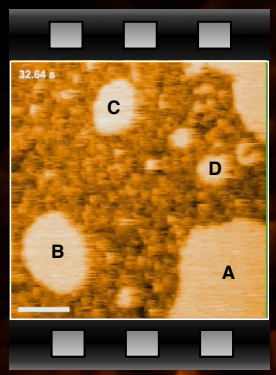

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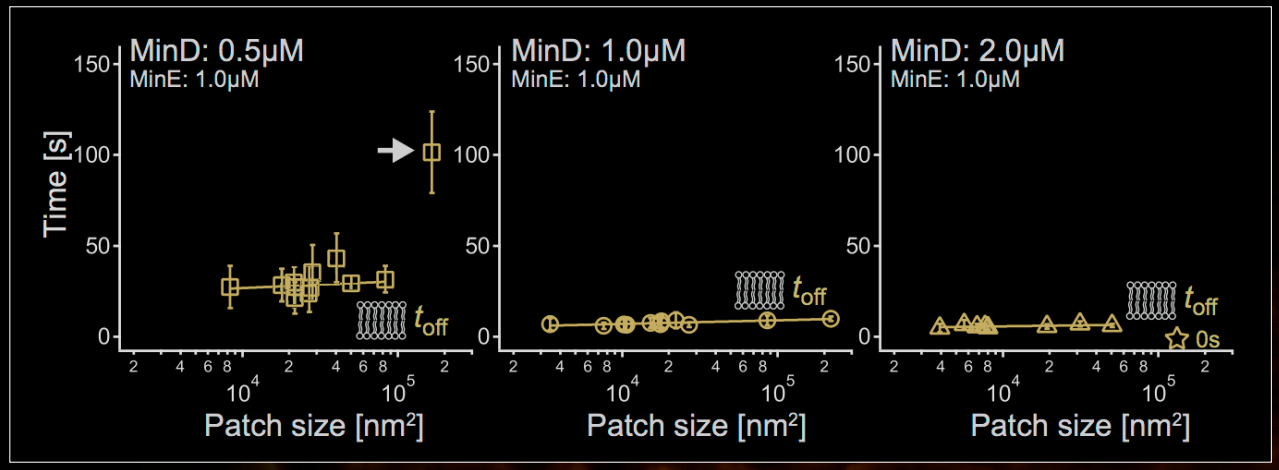
toff, va, ton, vd as a fuction of MinD concentration and patch size



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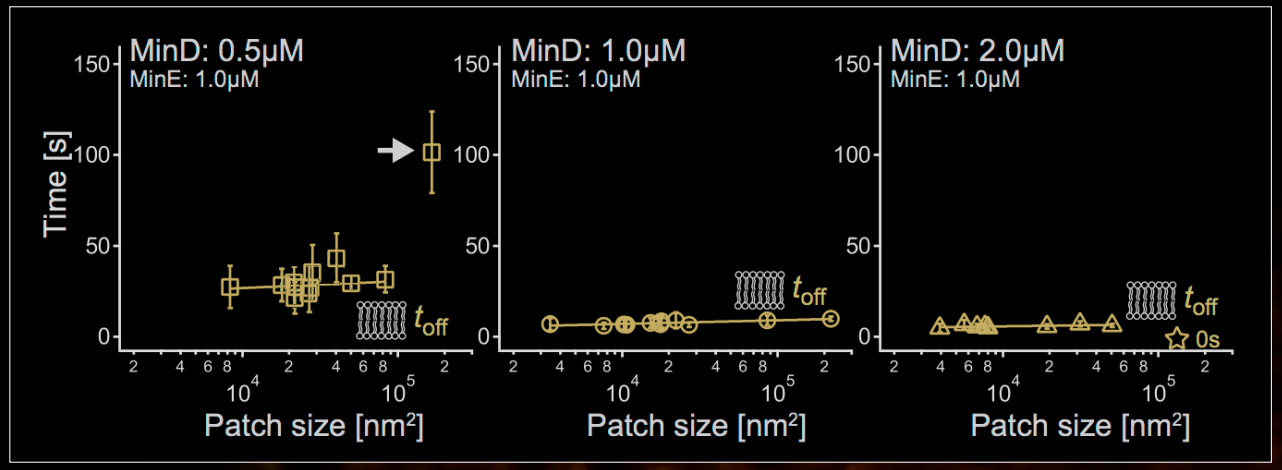

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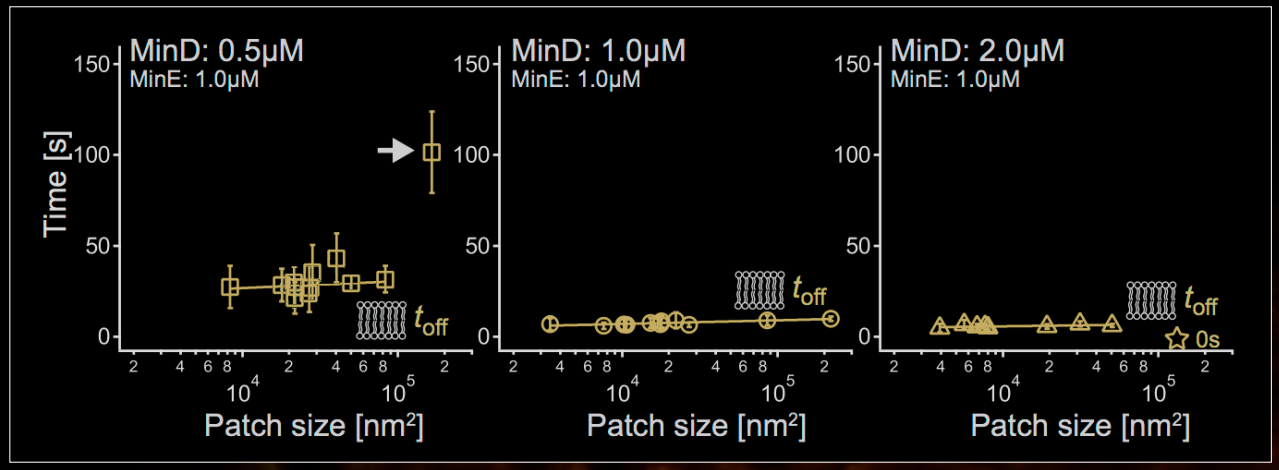

t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
MinD membrane seeding

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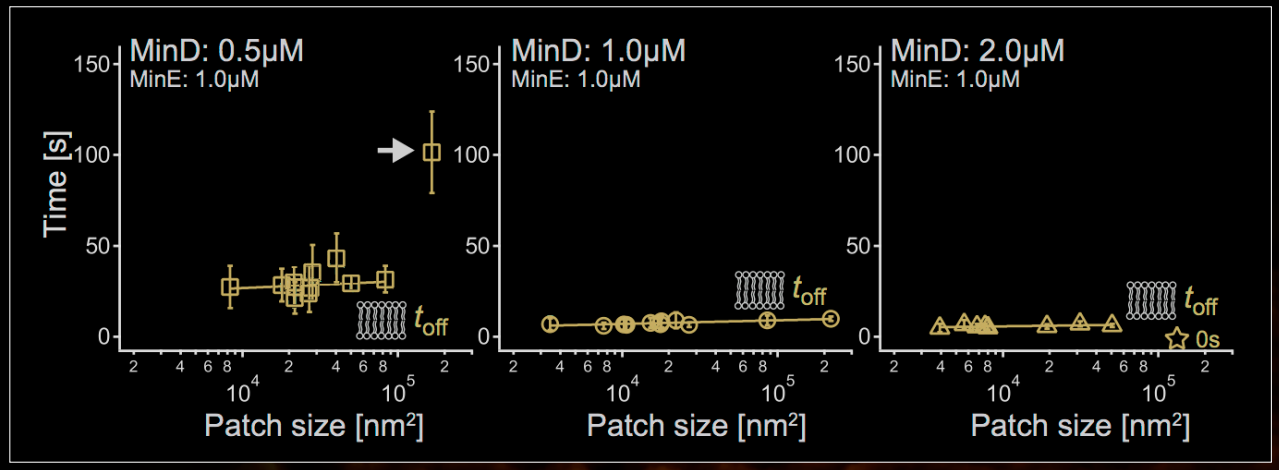

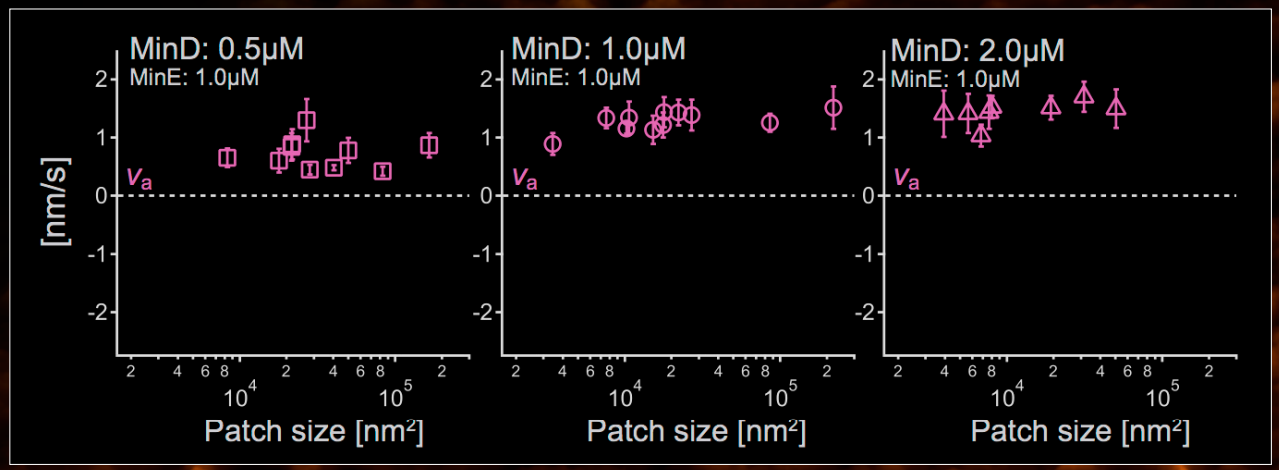

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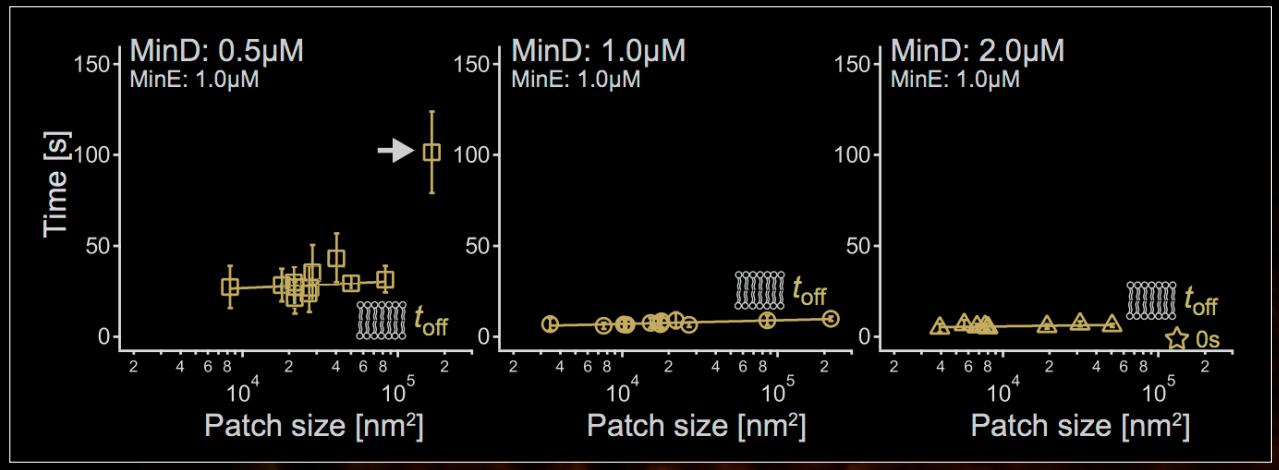

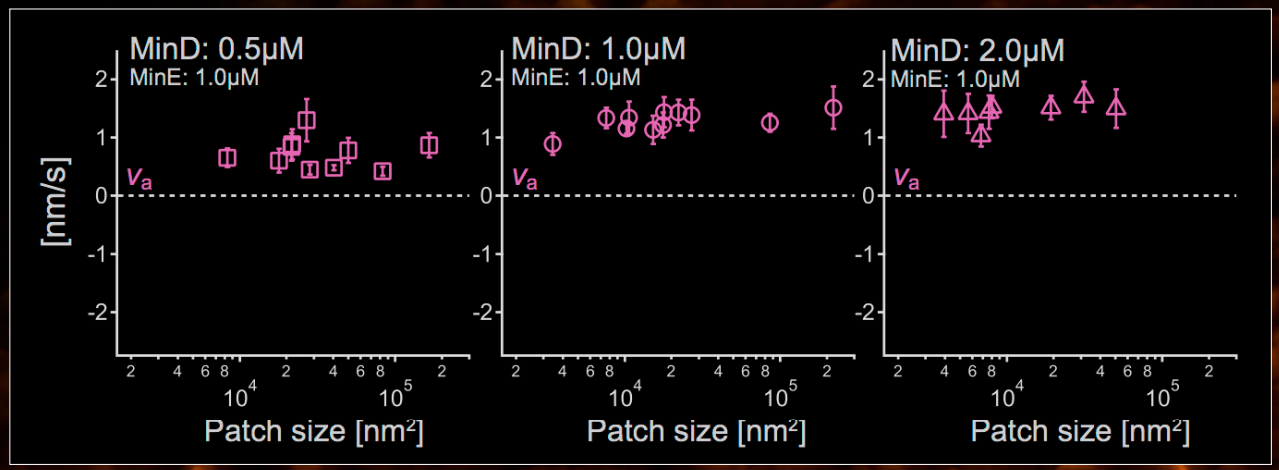

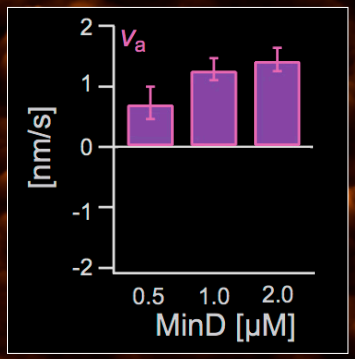

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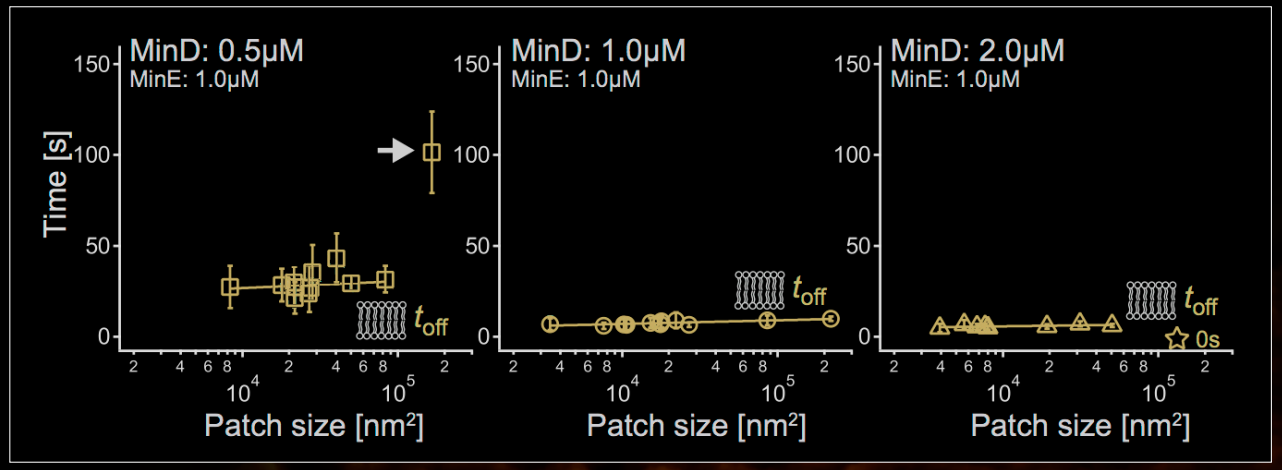

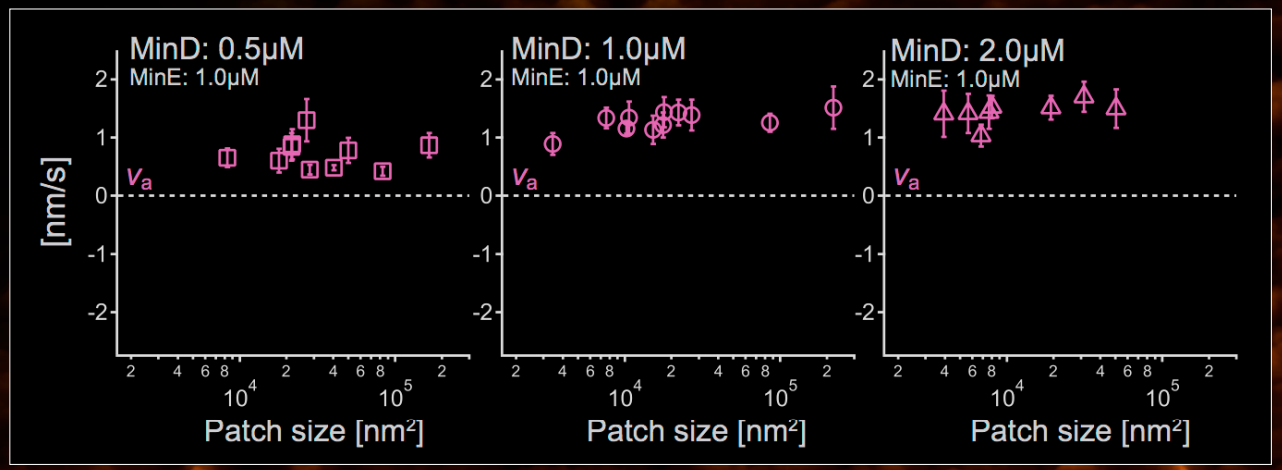

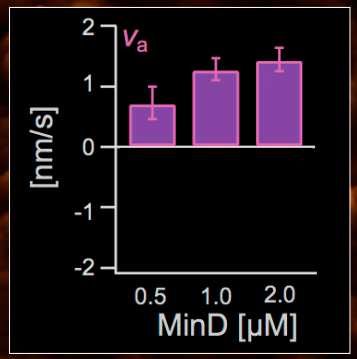

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v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinD dependent
↑ [MinD] = v_a ↑
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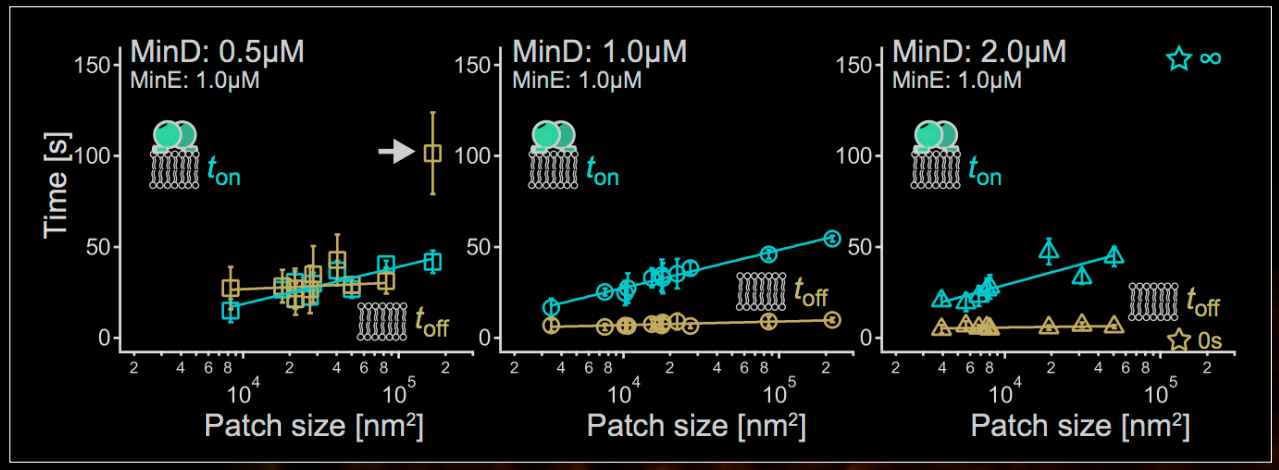

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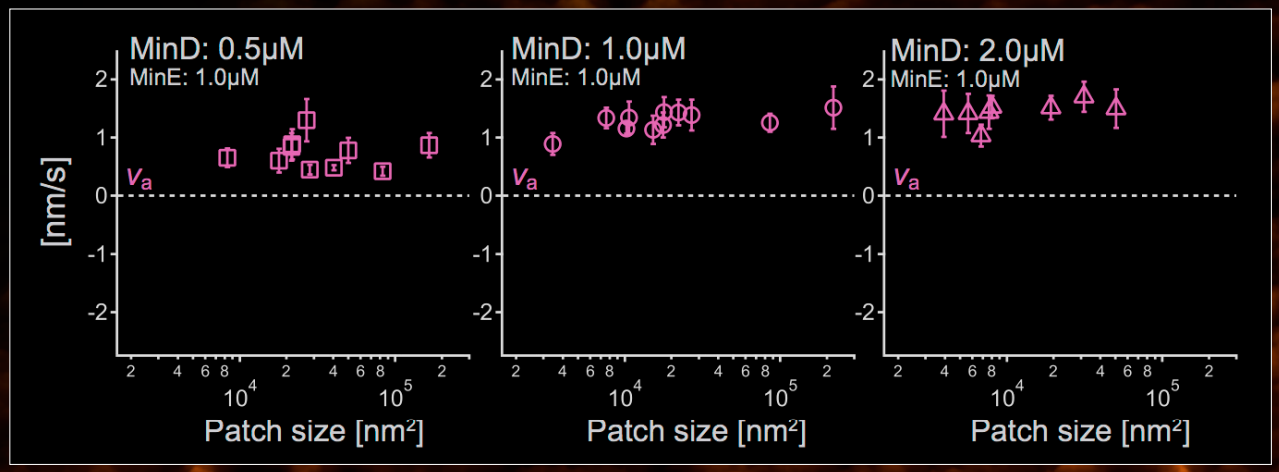

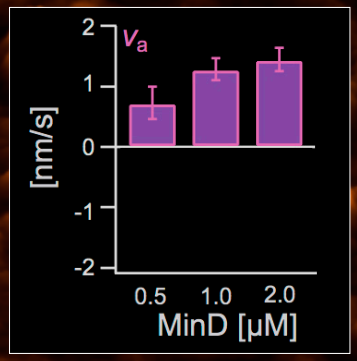

v_a is patchsize independent
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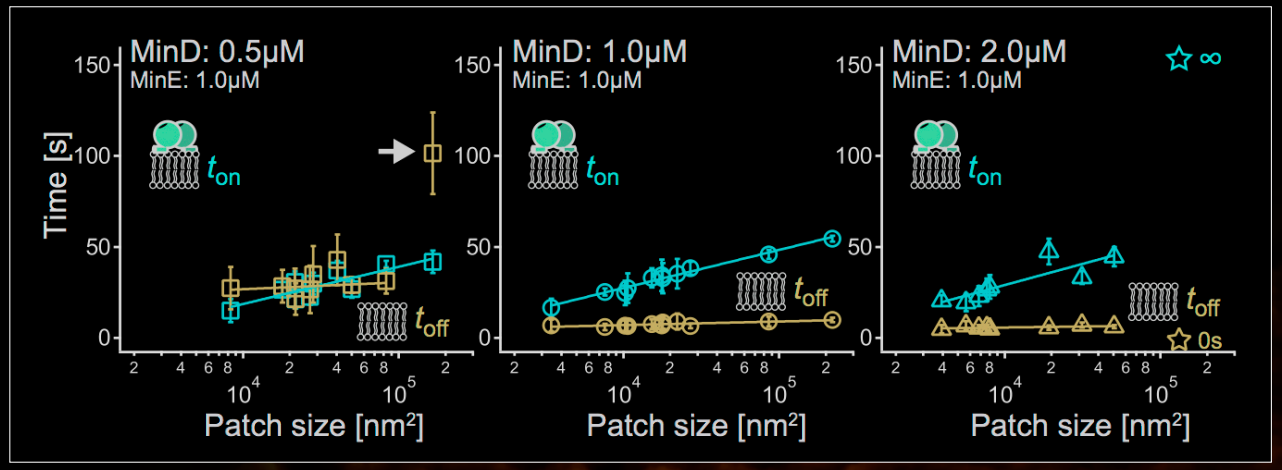

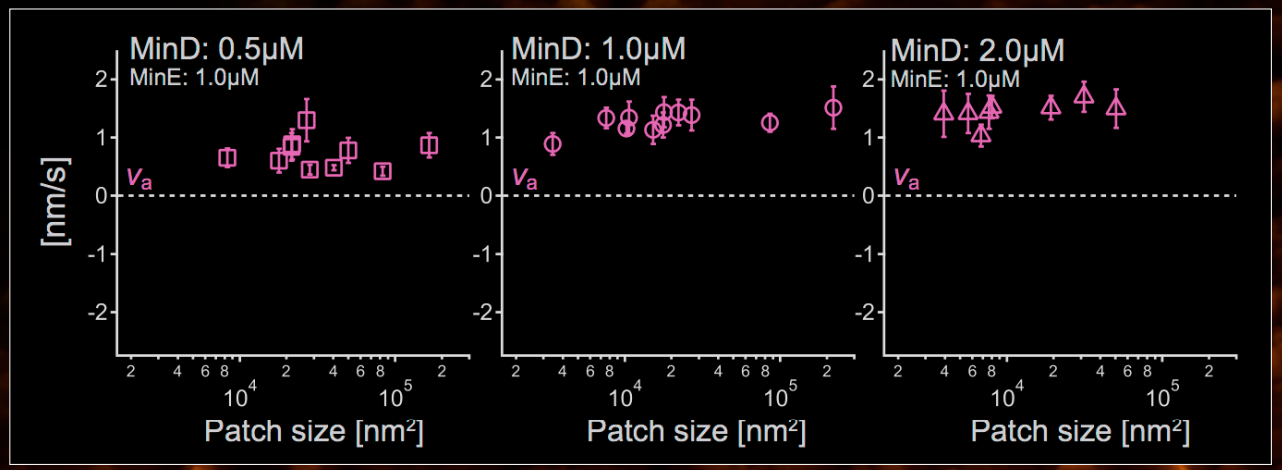

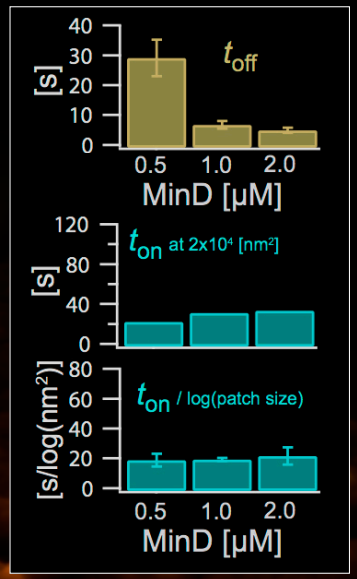

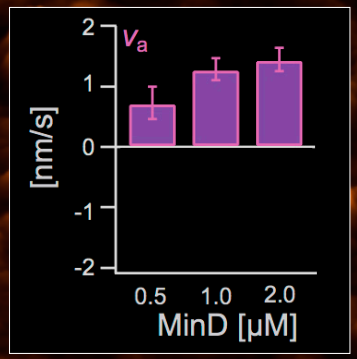

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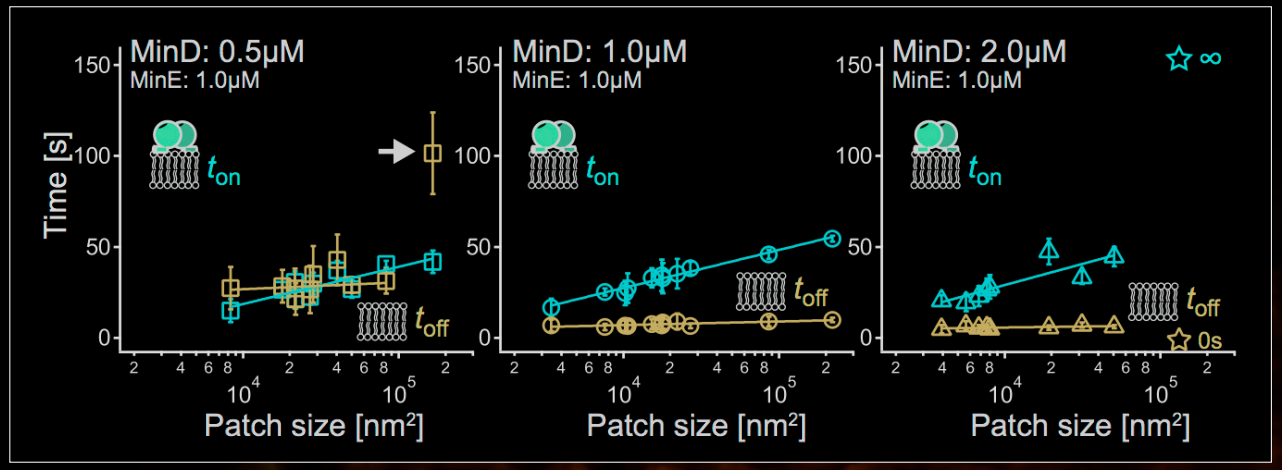
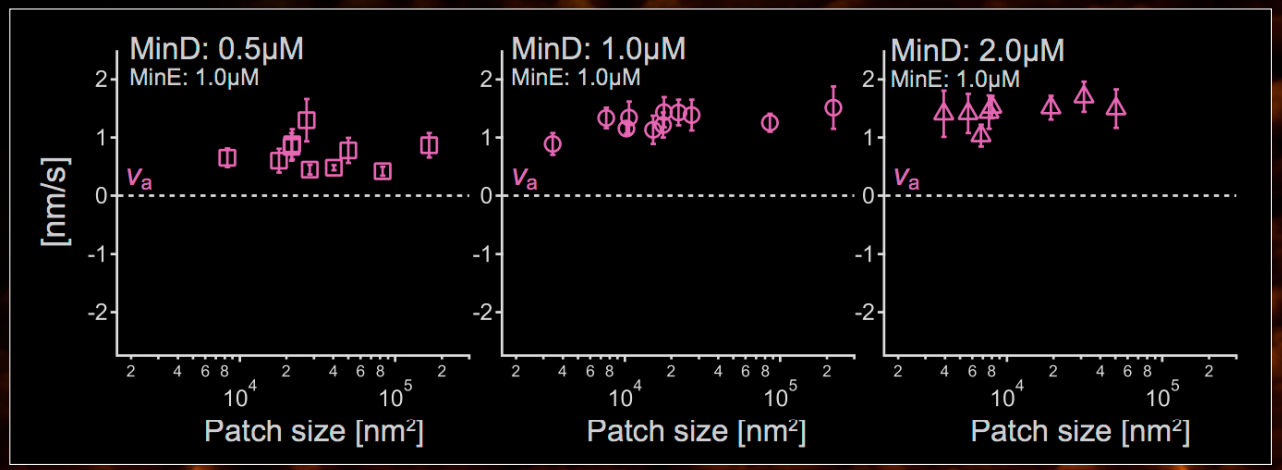
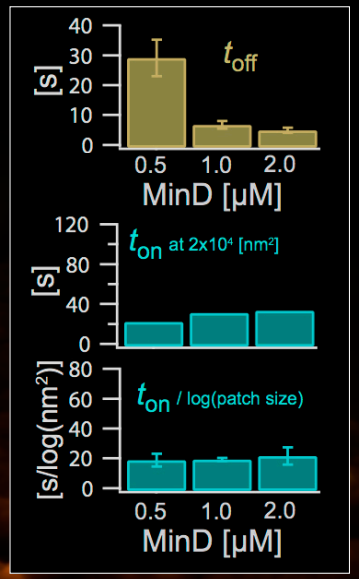
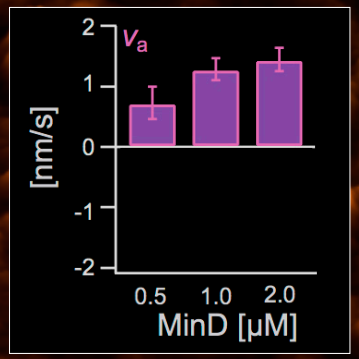








t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
MinD membrane seeding

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinD dependent
↑ [MinD] = v_a ↑
MinD membrane seeding

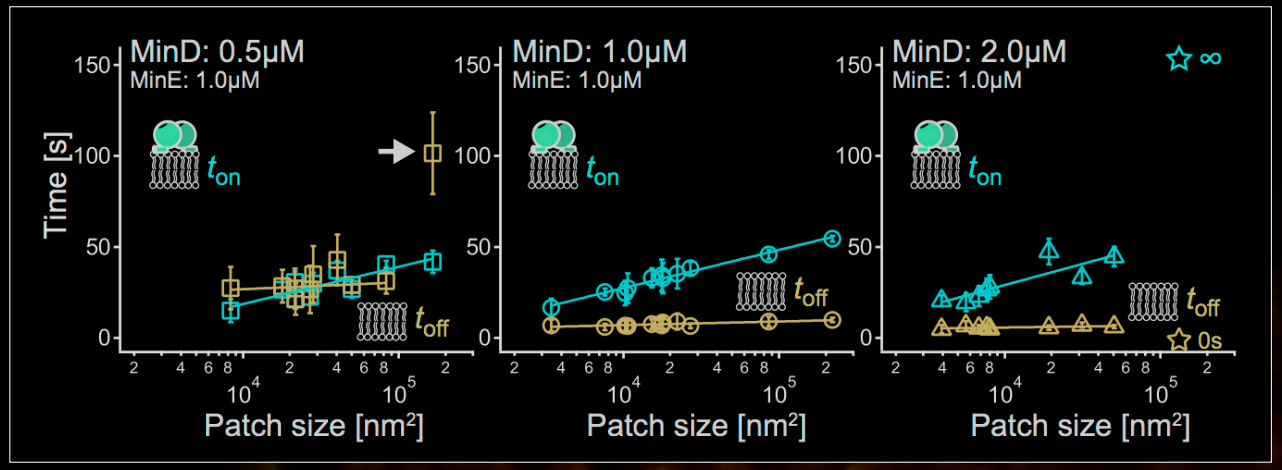
t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is slighly MinD dependent
↑ [MinD] = t_{on} ↗
? + slight MinD replenish

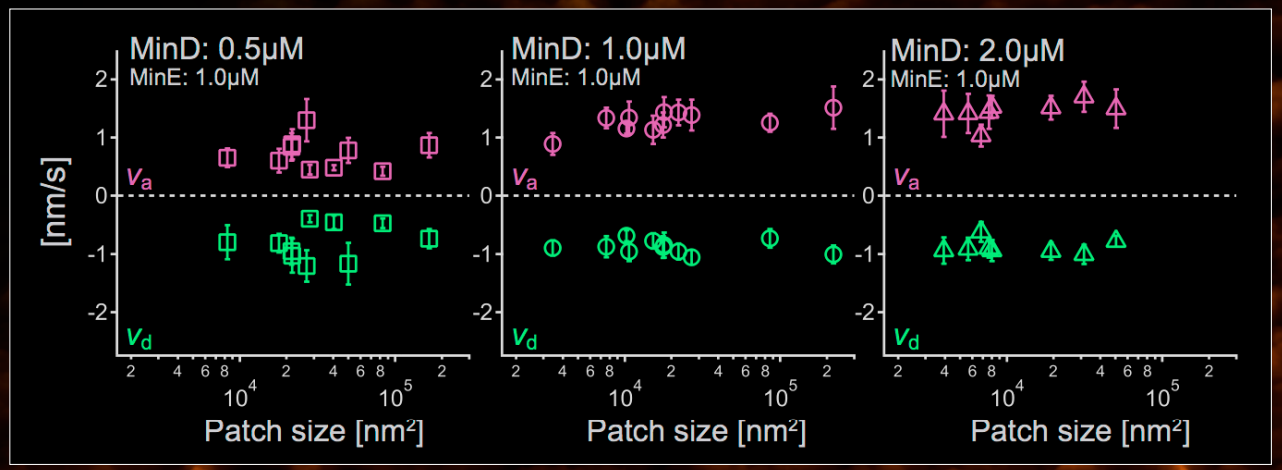
Bio-AFM-Lab

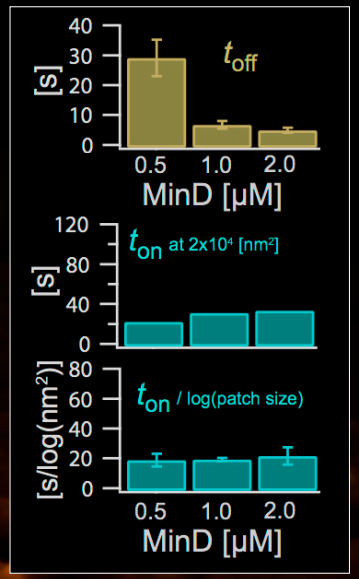
HS-AFM Deciphers MinDE Pattern Formation

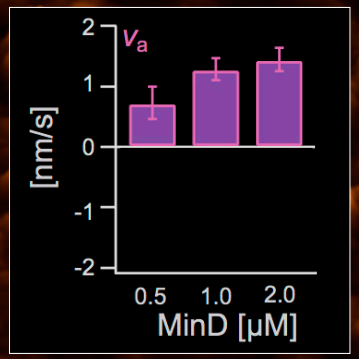
Simon Scheuring

toff, va, ton, vd as a fuction of MinD concentration and patch size









t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
MinD membrane seeding

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinD dependent
↑ [MinD] = v_a ↑
MinD membrane seeding

t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is slighly MinD dependent
↑ [MinD] = t_{on} ↗
? + slight MinD replenish

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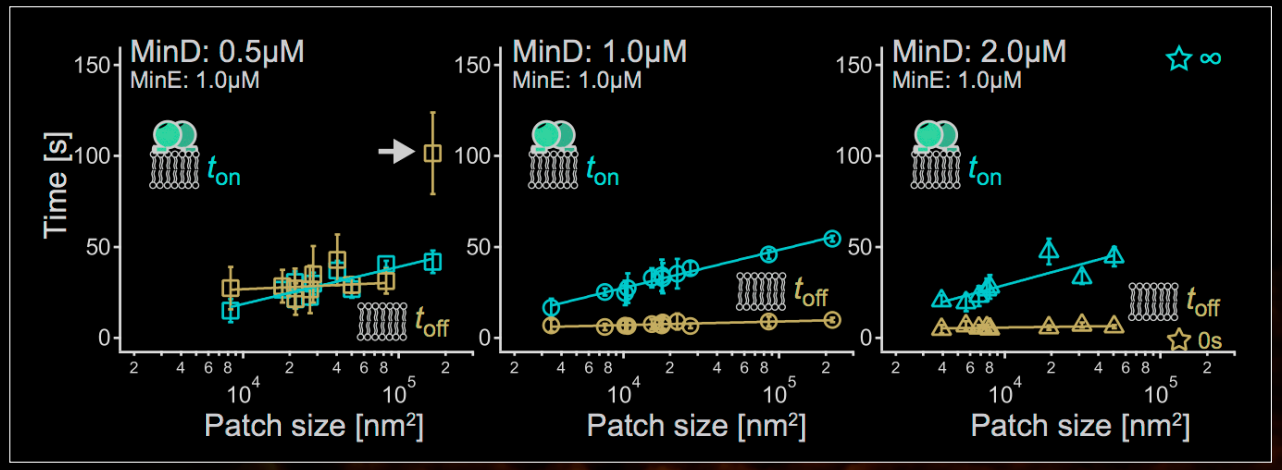

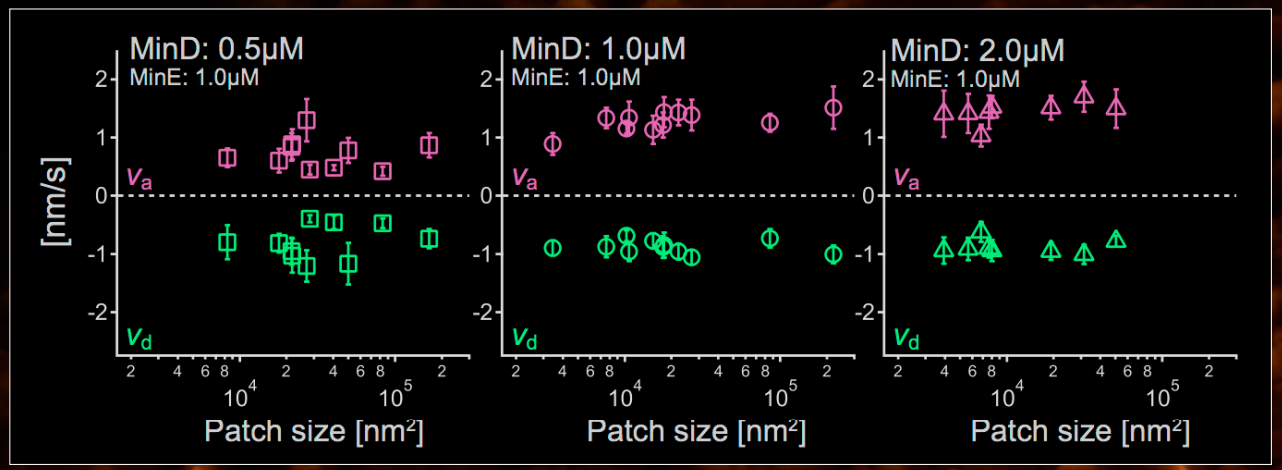

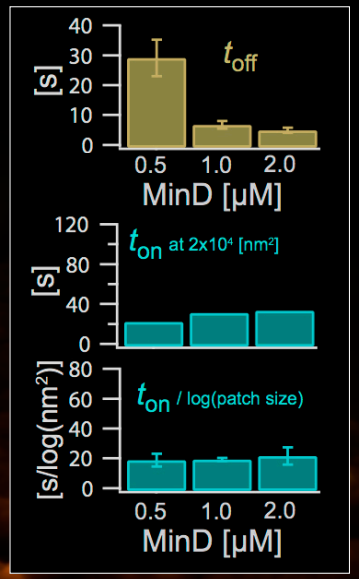

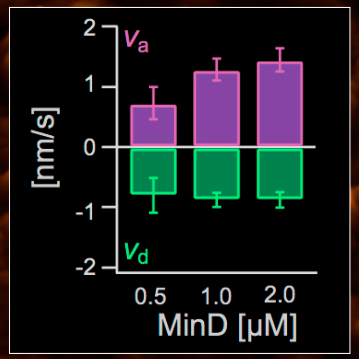

t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
MinD membrane seeding

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinD dependent
↑ [MinD] = v_a ↑
MinD membrane seeding

t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is slighly MinD dependent
↑ [MinD] = t_{on} ↗
? + slight MinD replenish

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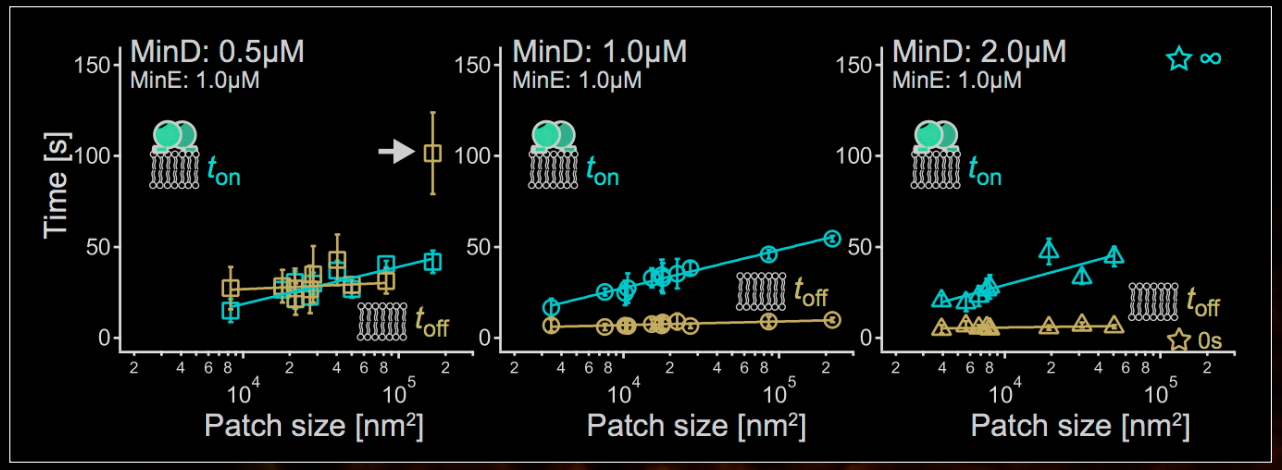
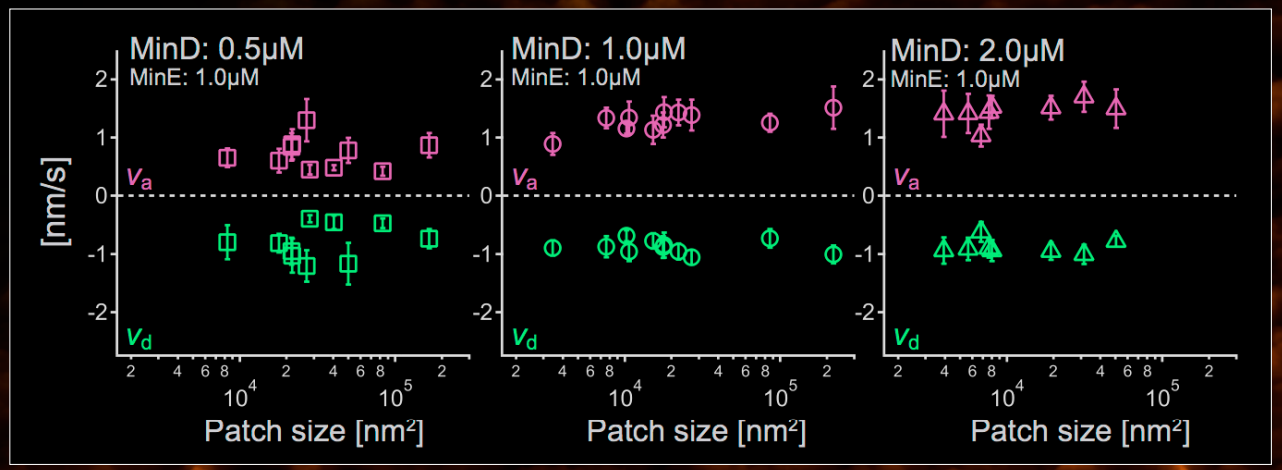
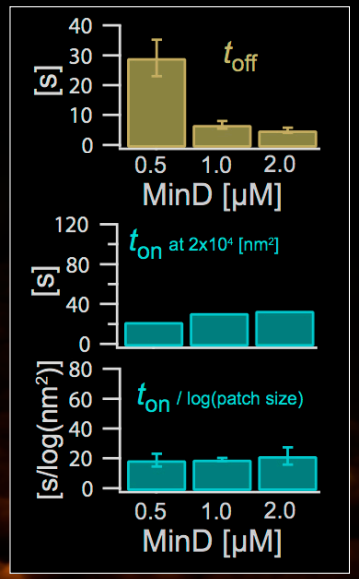
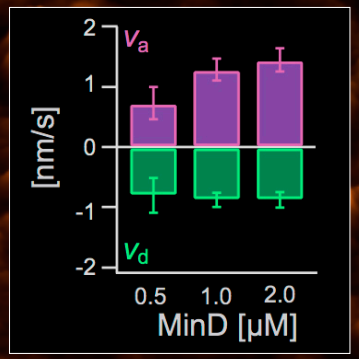
Simon Scheuring

toff, va, ton, vd as a fuction of MinD concentration and patch size









t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
MinD membrane seeding

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinD dependent
↑ [MinD] = v_a ↑
MinD membrane seeding

t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is slighly MinD dependent
↑ [MinD] = t_{on} ↗
? + slight MinD replenish

v_d is patchsize independent
→ [membrane] = v_d ←
v_d is MinD independent
→ [MinD] = v_d ←
no MinD binding during v_d

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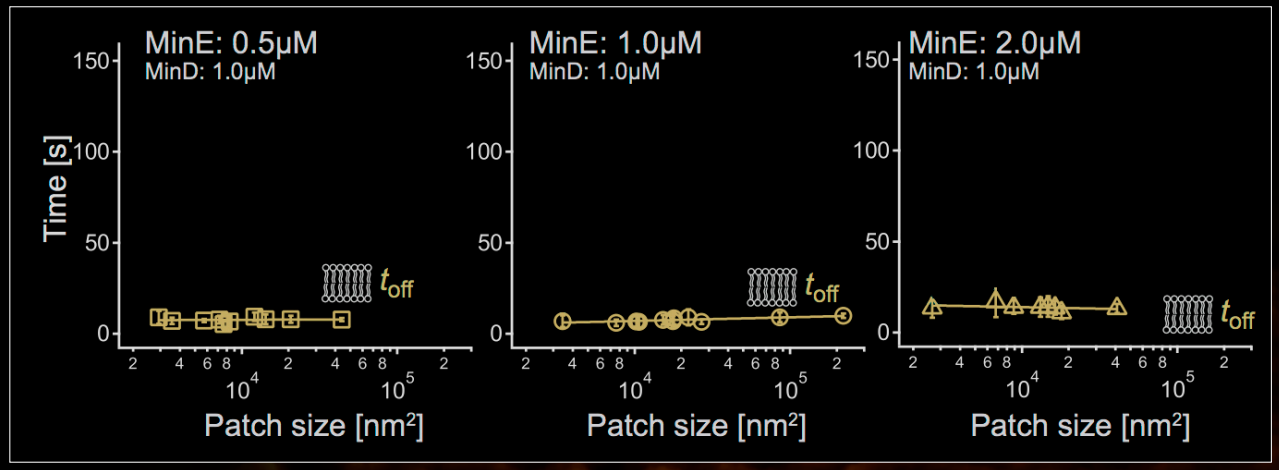
Simon Scheuring

toff, va, ton, vd as a fuction of MinE concentration and patch size



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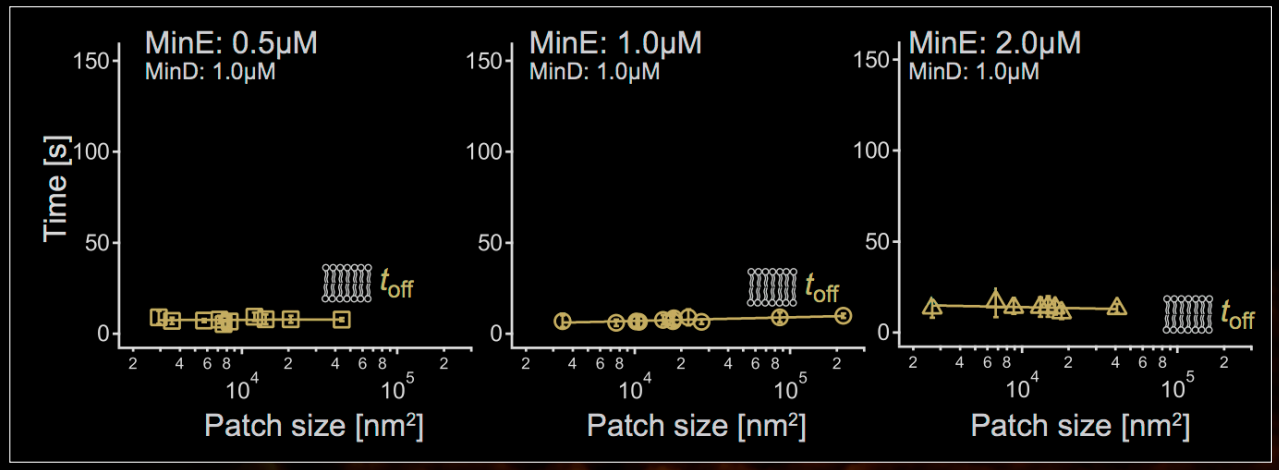

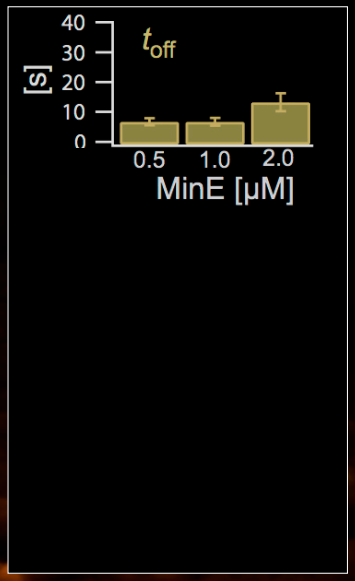

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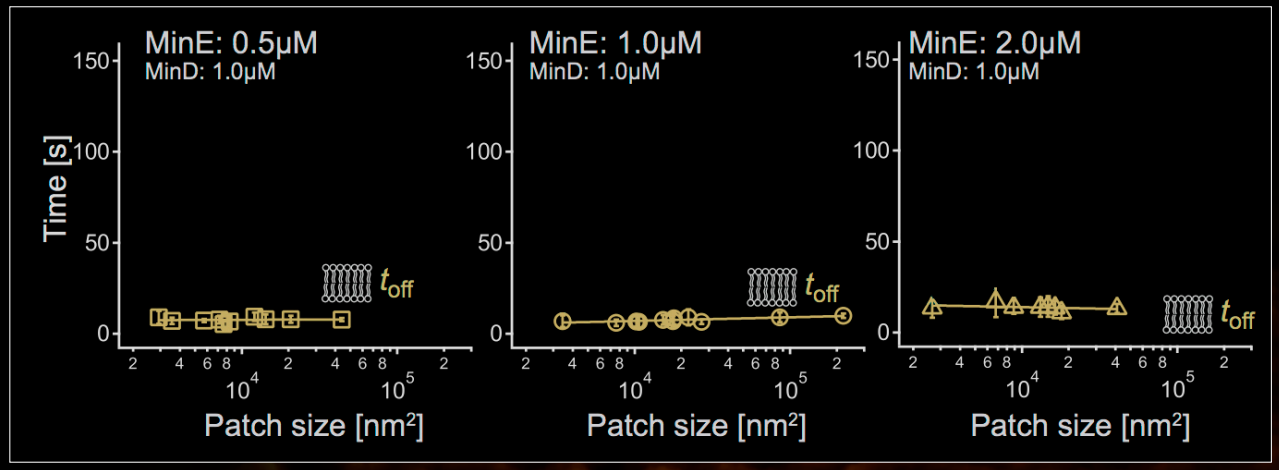

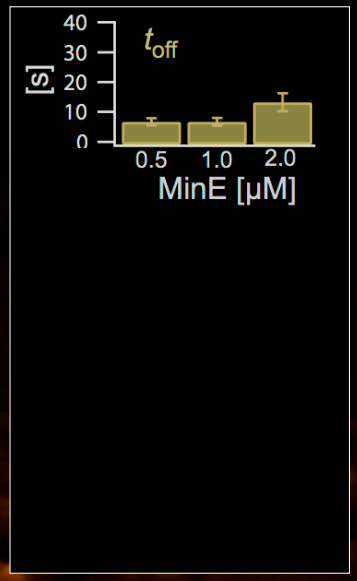

t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

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HS-AFM Deciphers MinDE Pattern Formation

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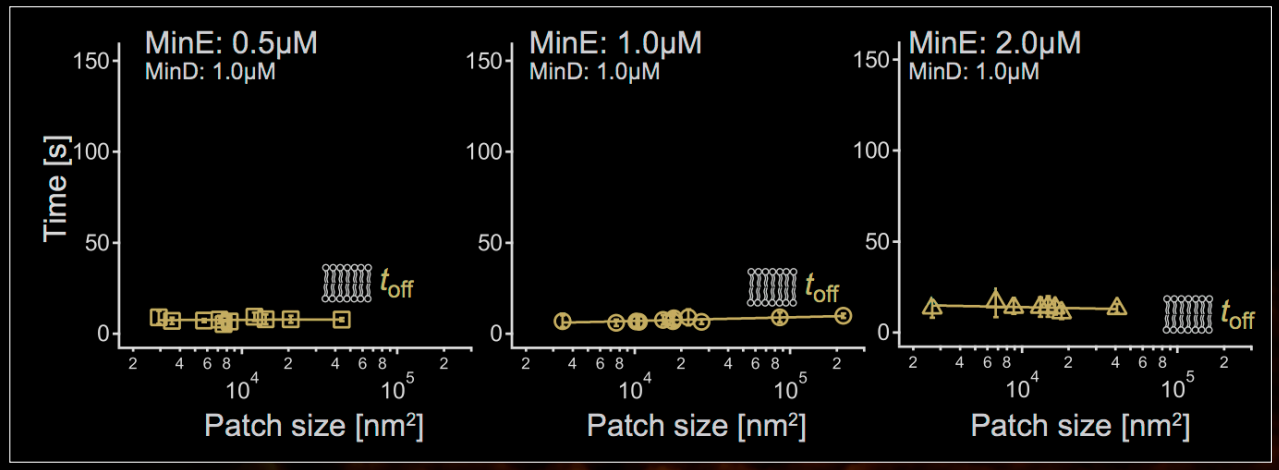
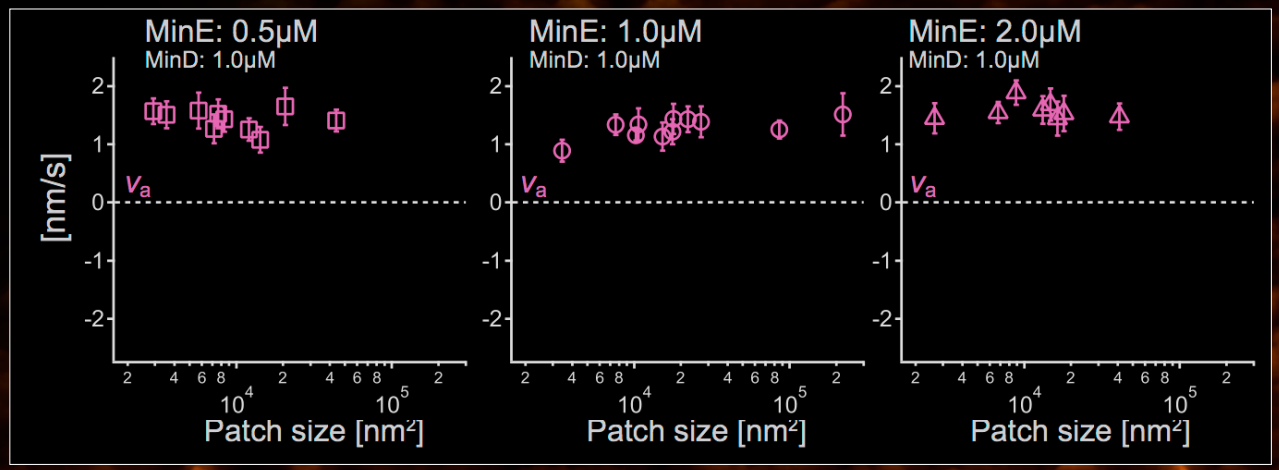
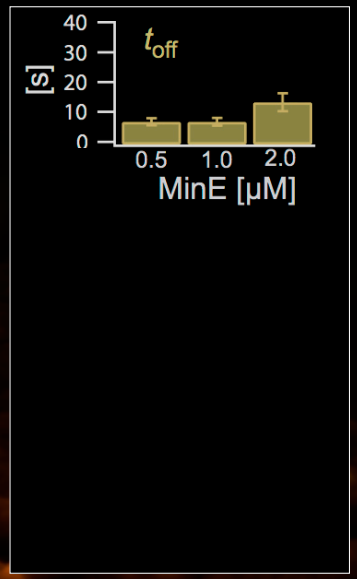
toff, va, ton, vd as a fuction of MinE concentration and patch size







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MinE activation of seed MinD

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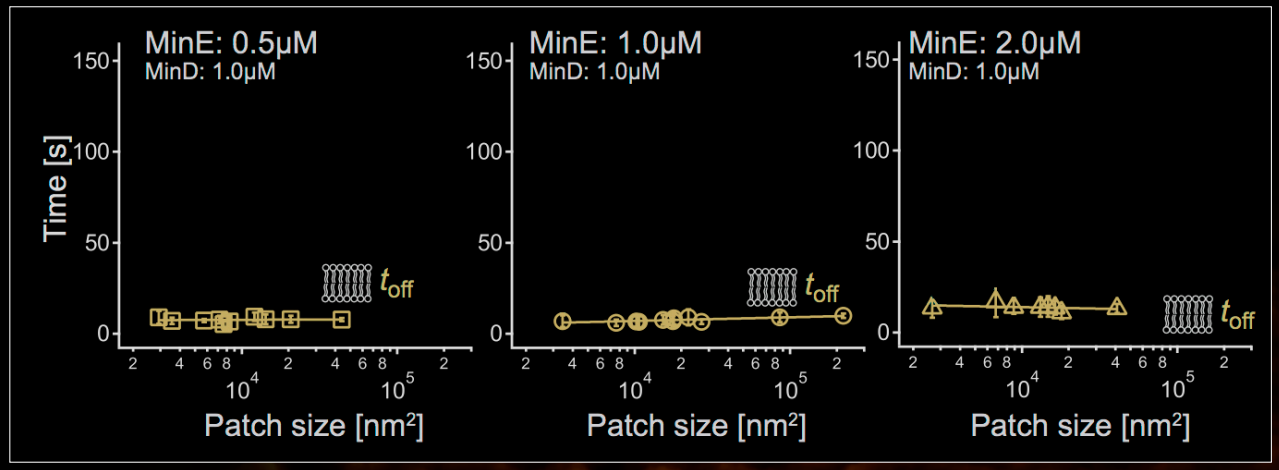

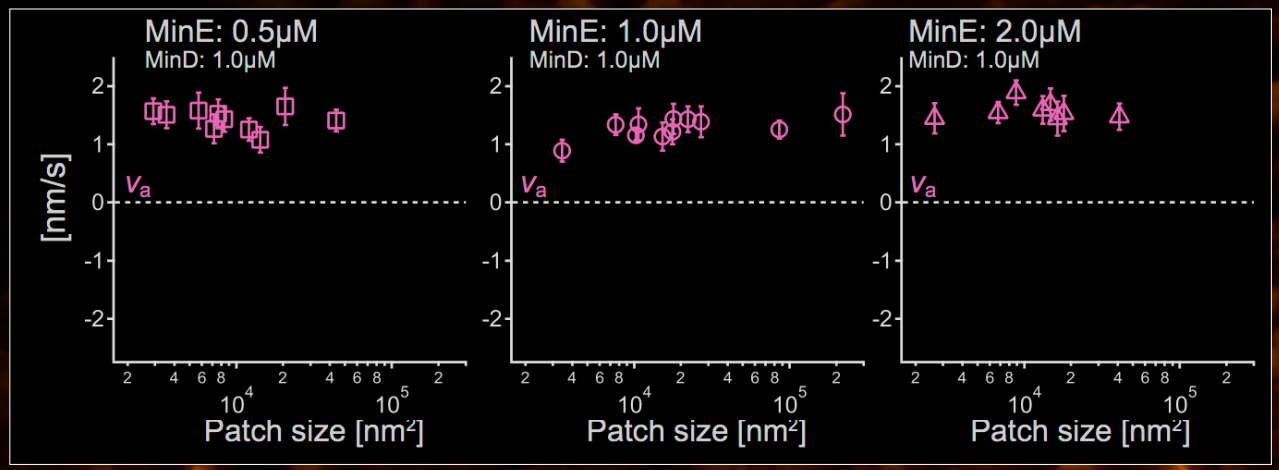

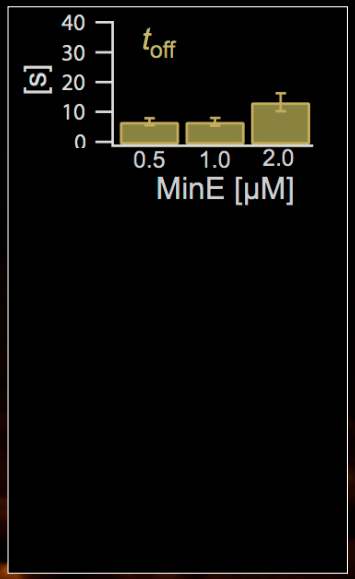

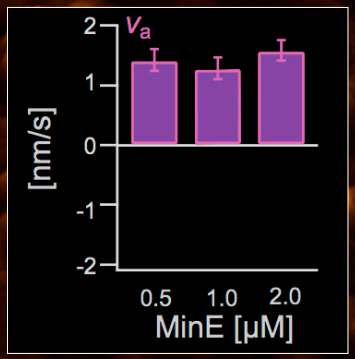

t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

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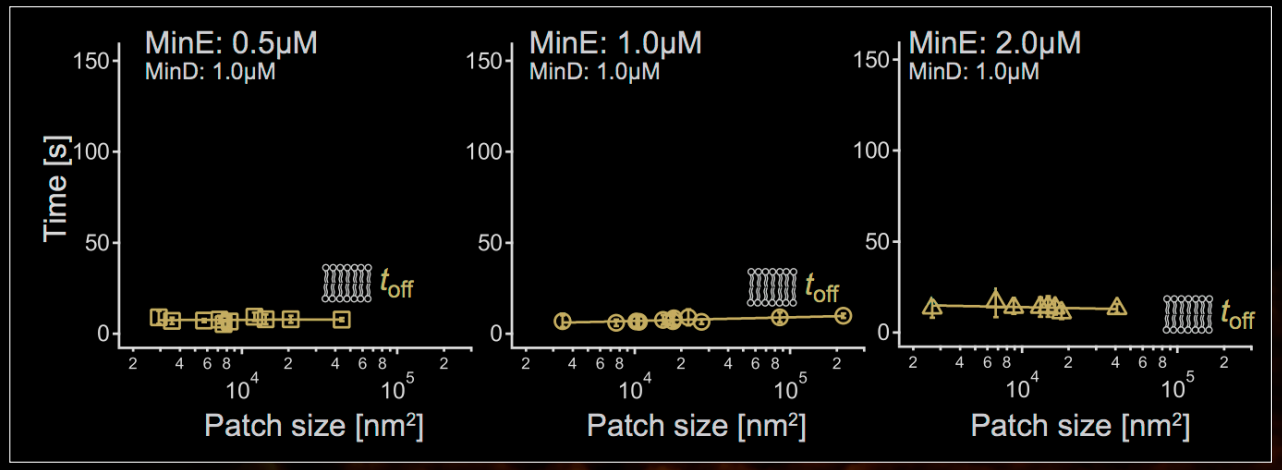
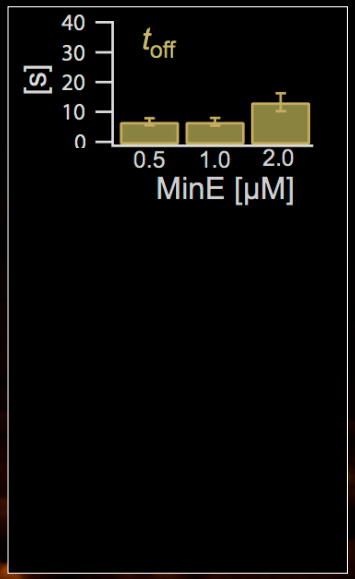
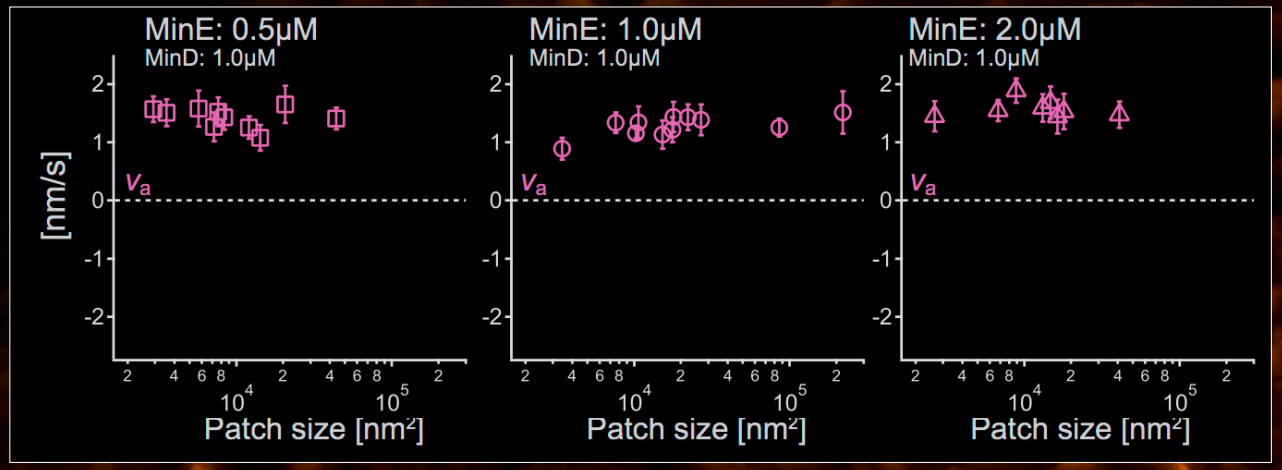
toff, va, ton, vd as a fuction of MinE concentration and patch size

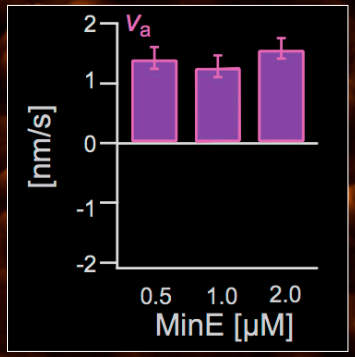




t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinE independent
→ [MinE] = v_a ←
no MinE binding during v_a





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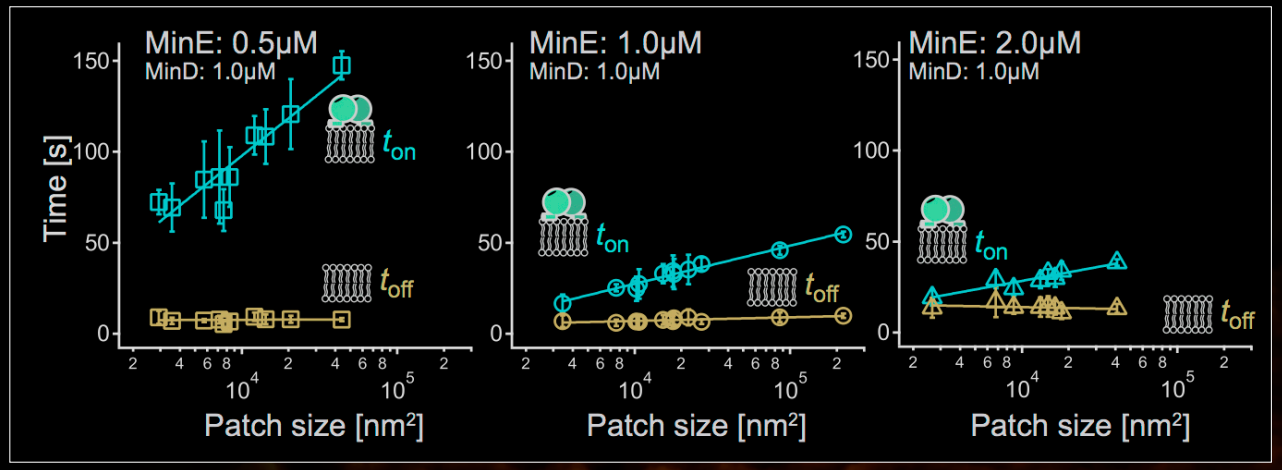
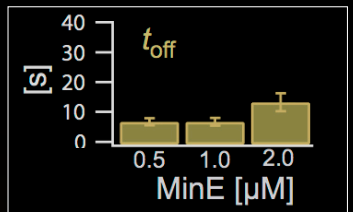
Simon Scheuring

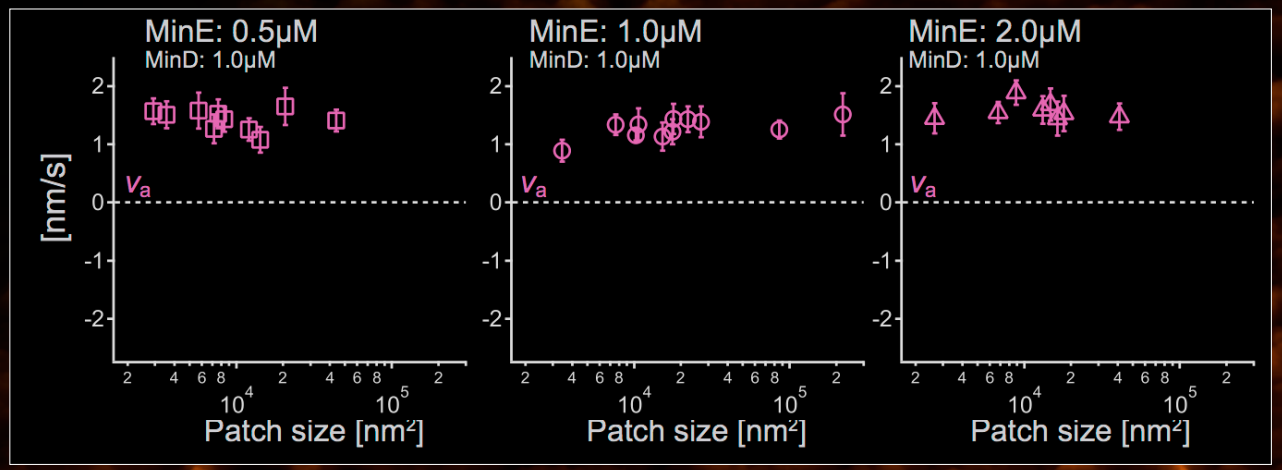
toff, va, ton, vd as a fuction of MinE concentration and patch size

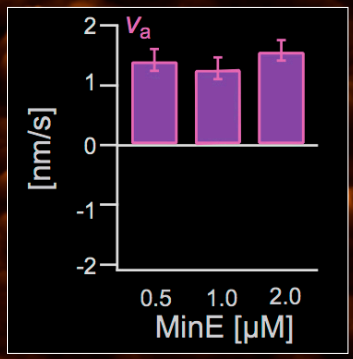




t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinE independent
→ [MinE] = v_a ←
no MinE binding during v_a





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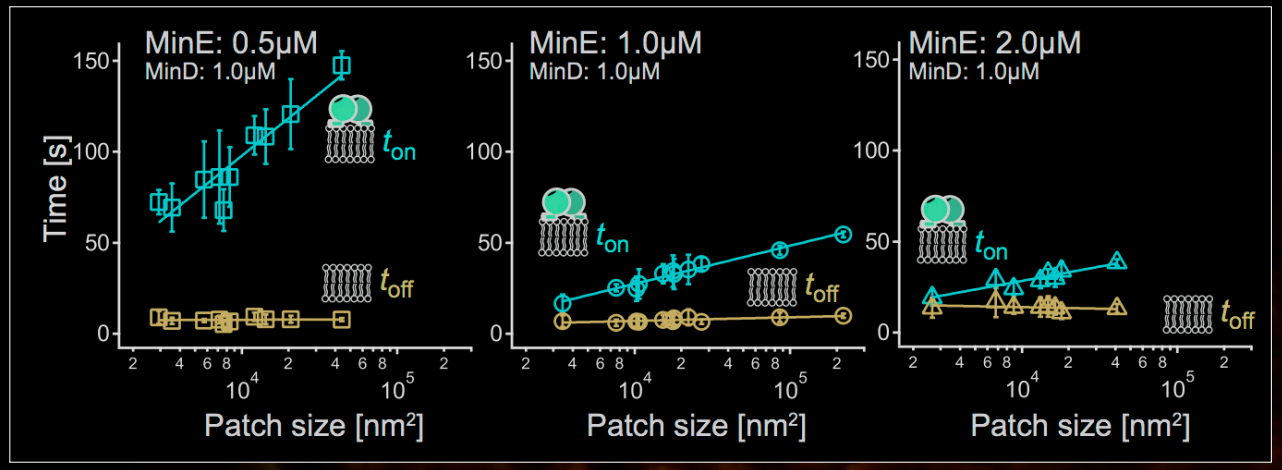
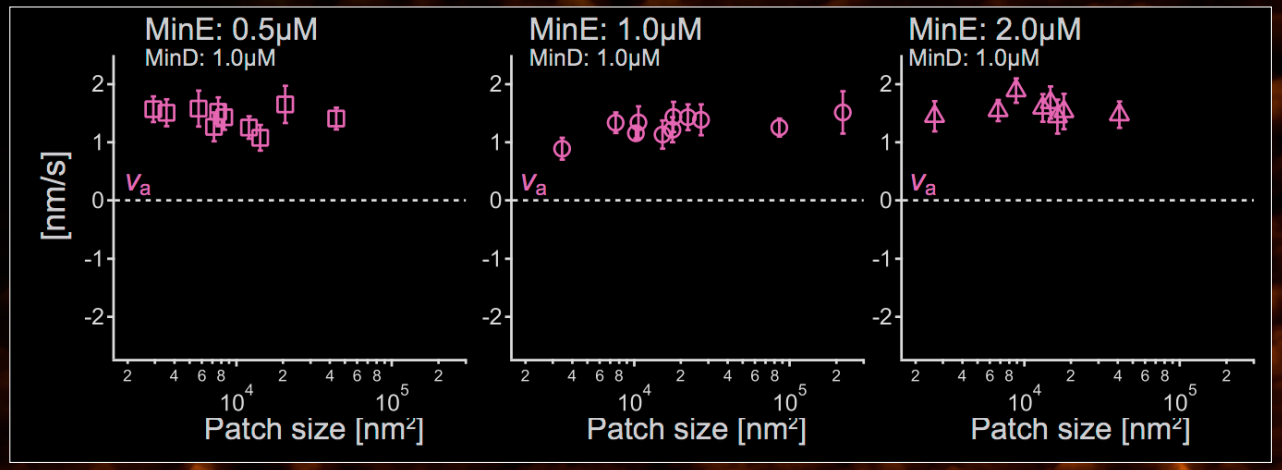
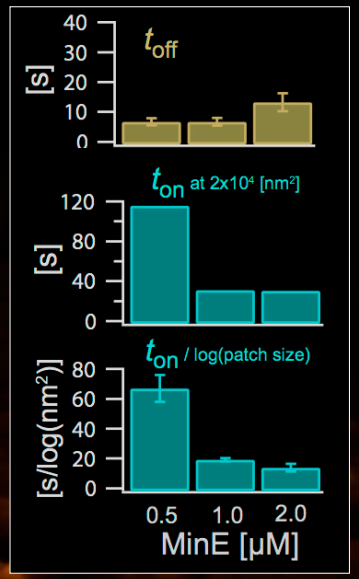
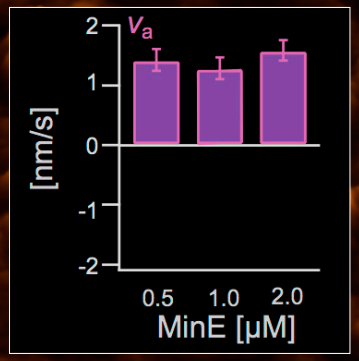
toff, va, ton, vd as a fuction of MinE concentration and patch size









t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinE independent
→ [MinE] = v_a ←
no MinE binding during v_a

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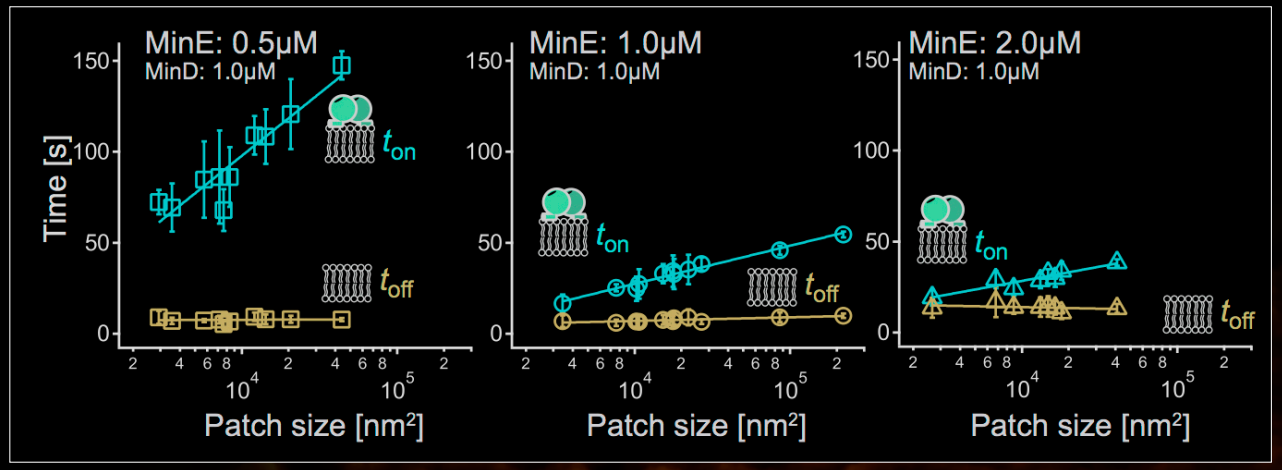
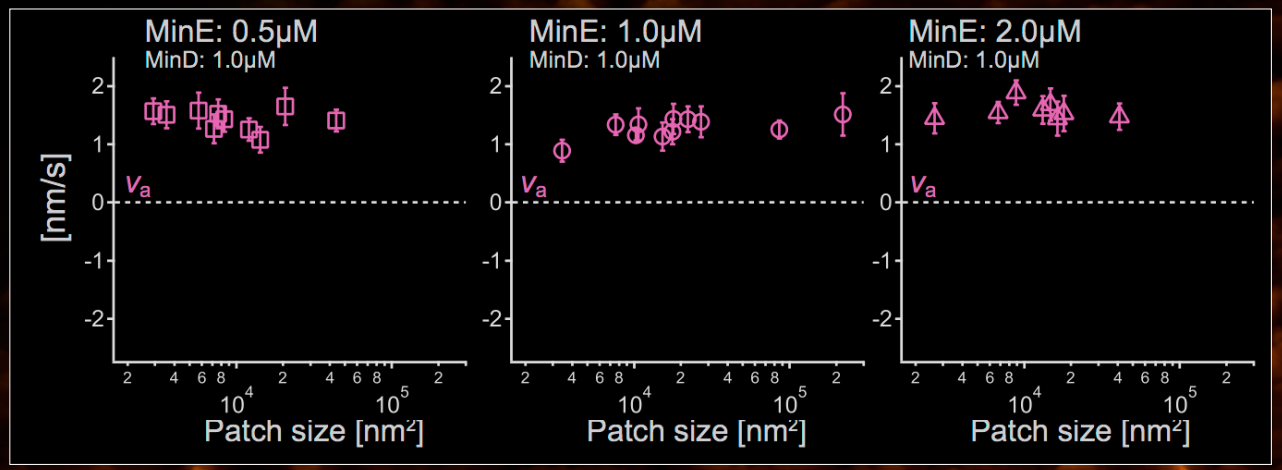
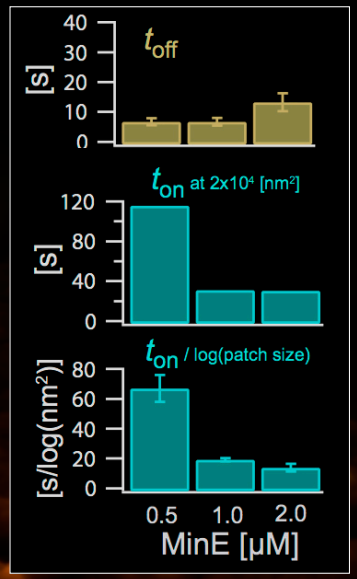
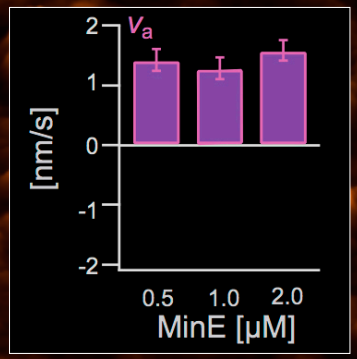
toff, va, ton, vd as a fuction of MinE concentration and patch size









t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinE independent
→ [MinE] = v_a ←
no MinE binding during v_a

t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is MinE dependent
↑ [MinE] = t_{on} ↓
patchsize dependence is MinE dependent
↑ [MinE] = [membrane] ↓
large patch - incr. act. barrier
MinE activation of MinD
MinE lowers act. barrier

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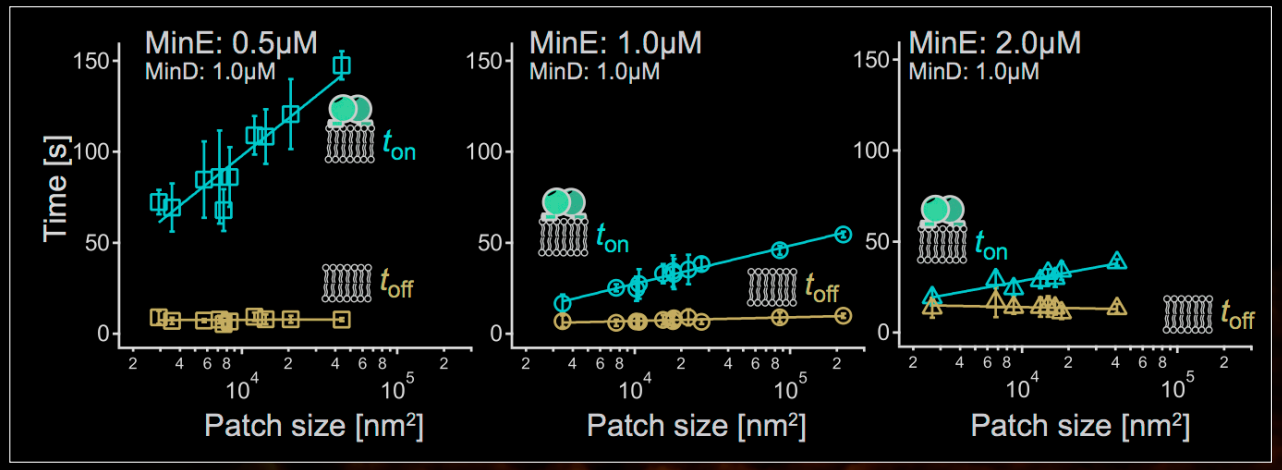
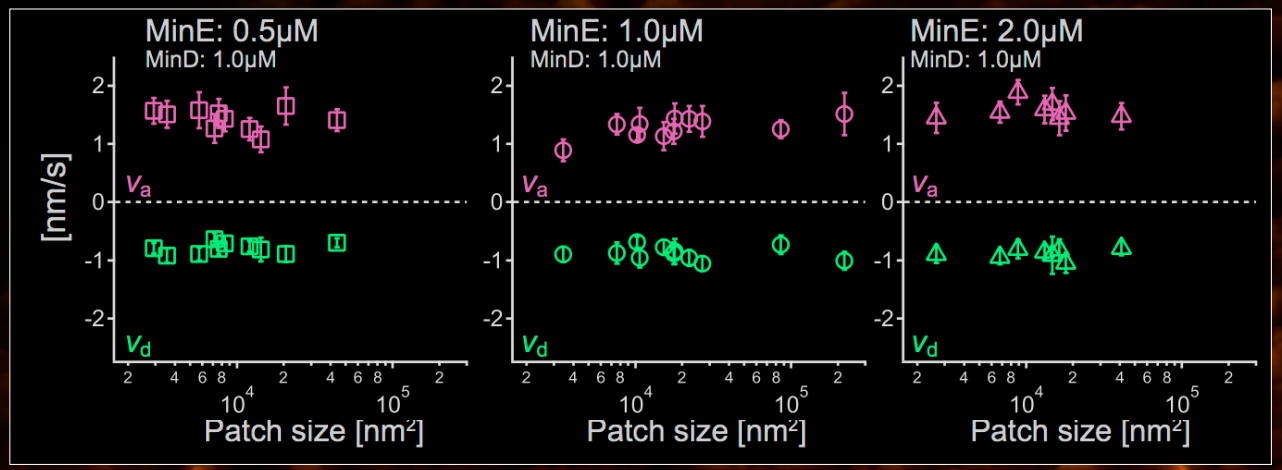
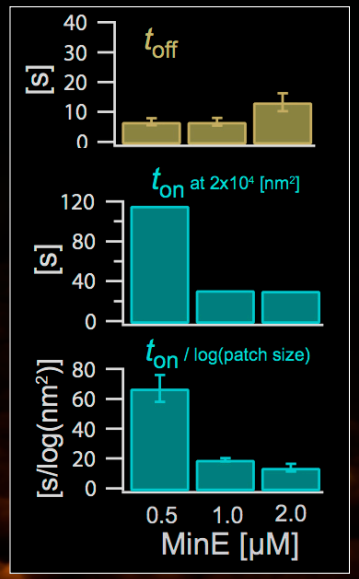
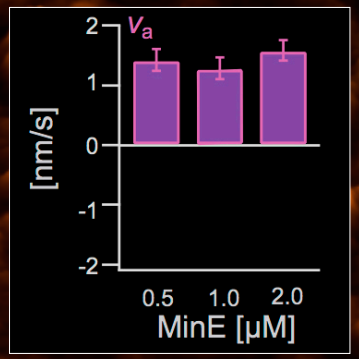








t_{off} is patchsize independent
→ [membrane] = t_{off} ←
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinE activation of seed MinD

v_a is patchsize independent
→ [membrane] = v_a ←
v_a is MinE independent
→ [MinE] = v_a ←
no MinE binding during v_a

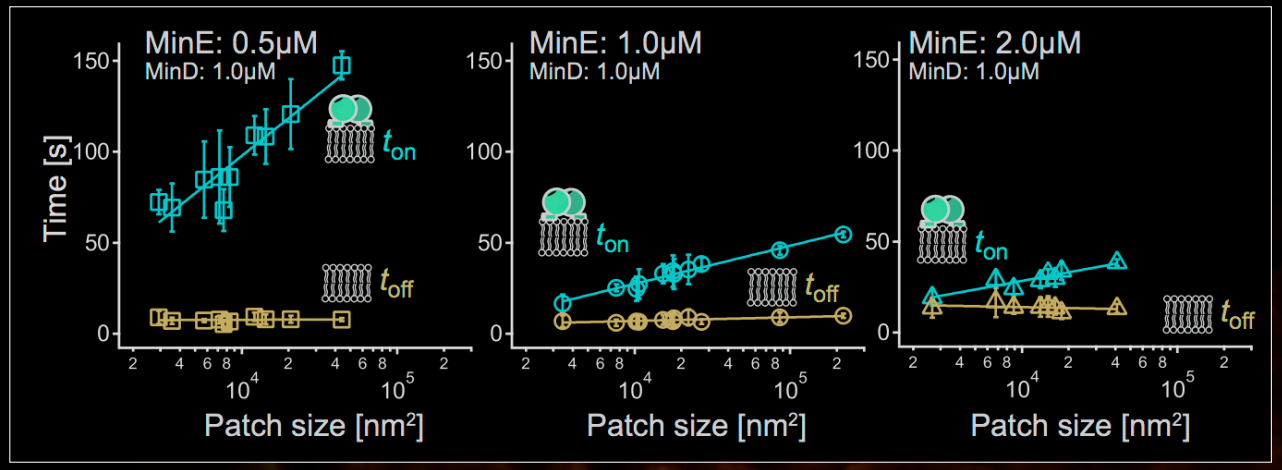
t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is MinE dependent
↑ [MinE] = t_{on} ↓
patchsize dependence is MinE dependent
↑ [MinE] = [membrane] ↓
large patch - incr. act. barrier
MinE activation of MinD
MinE lowers act. barrier

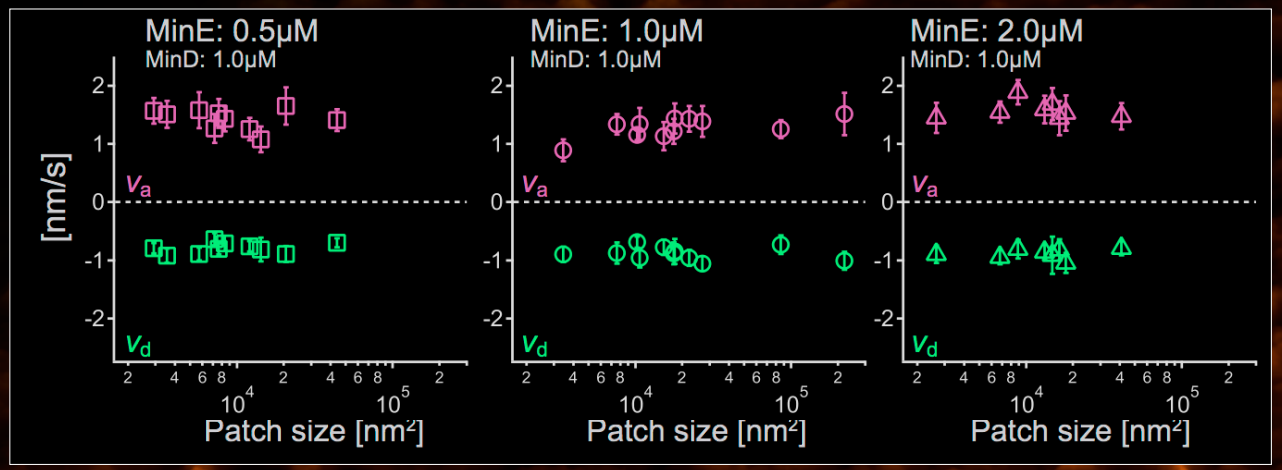
Bio-AFM-Lab

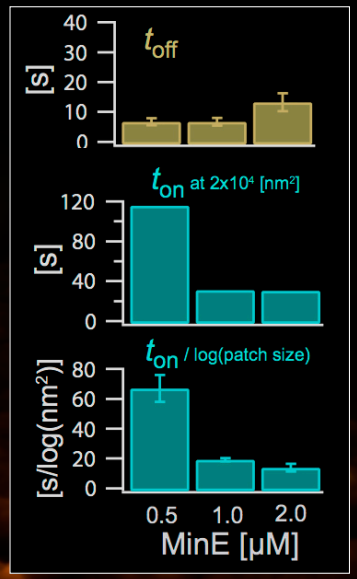
HS-AFM Deciphers MinDE Pattern Formation

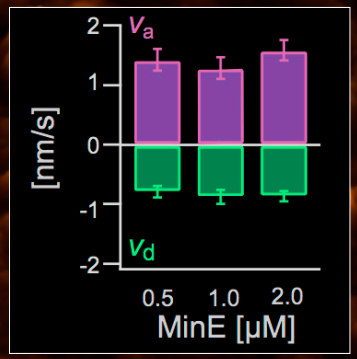
Simon Scheuring

toff, va, ton, vd as a fuction of MinE concentration and patch size









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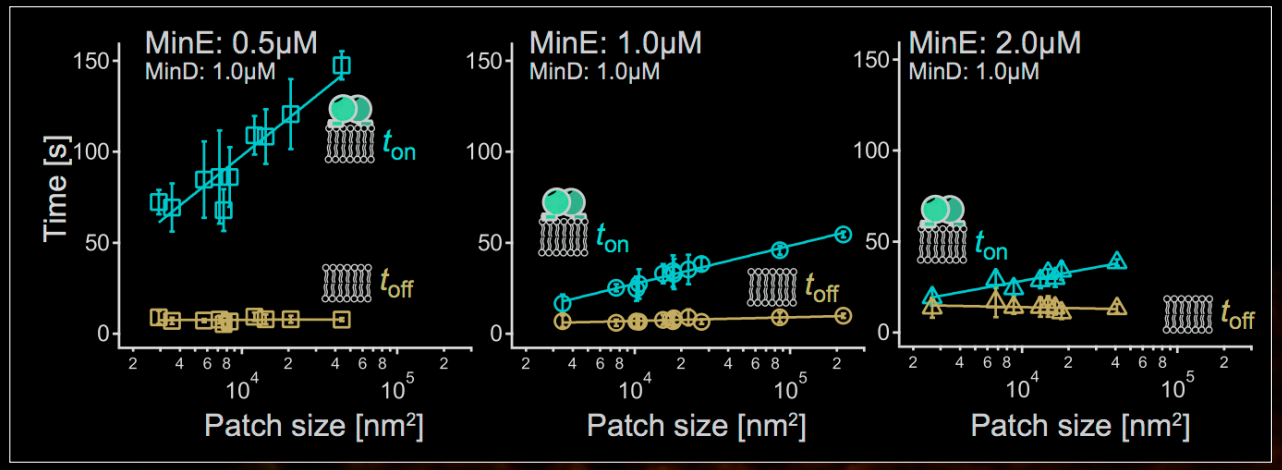

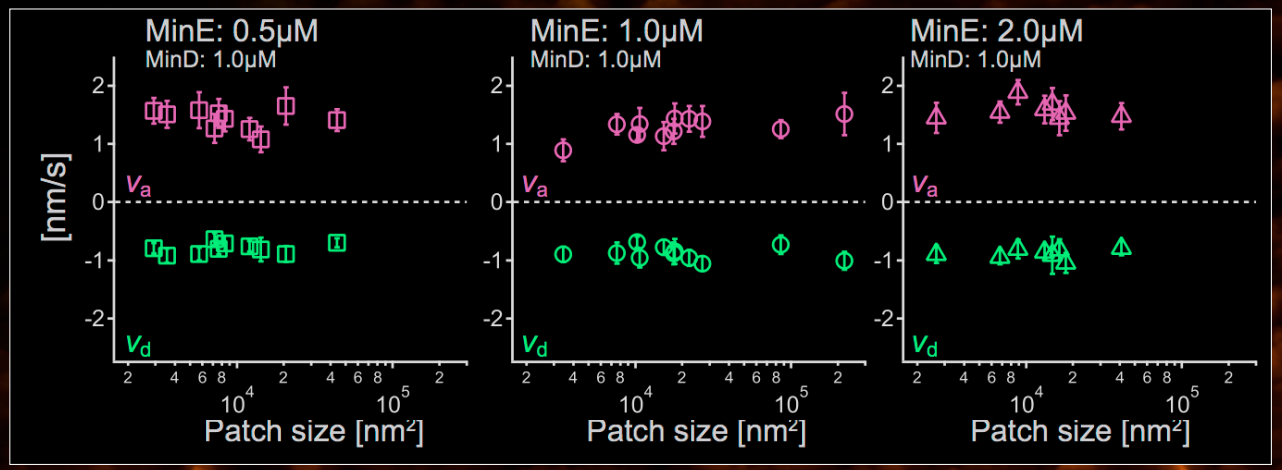

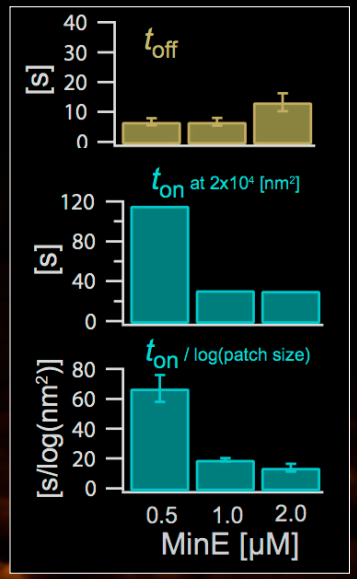

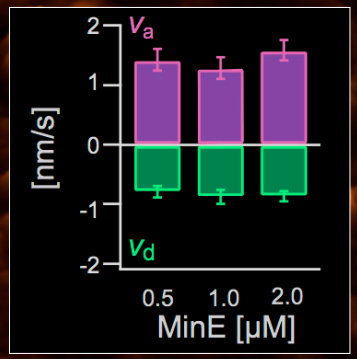

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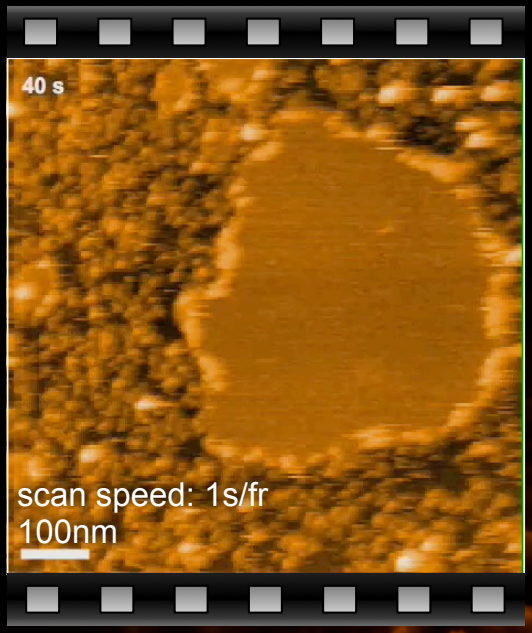
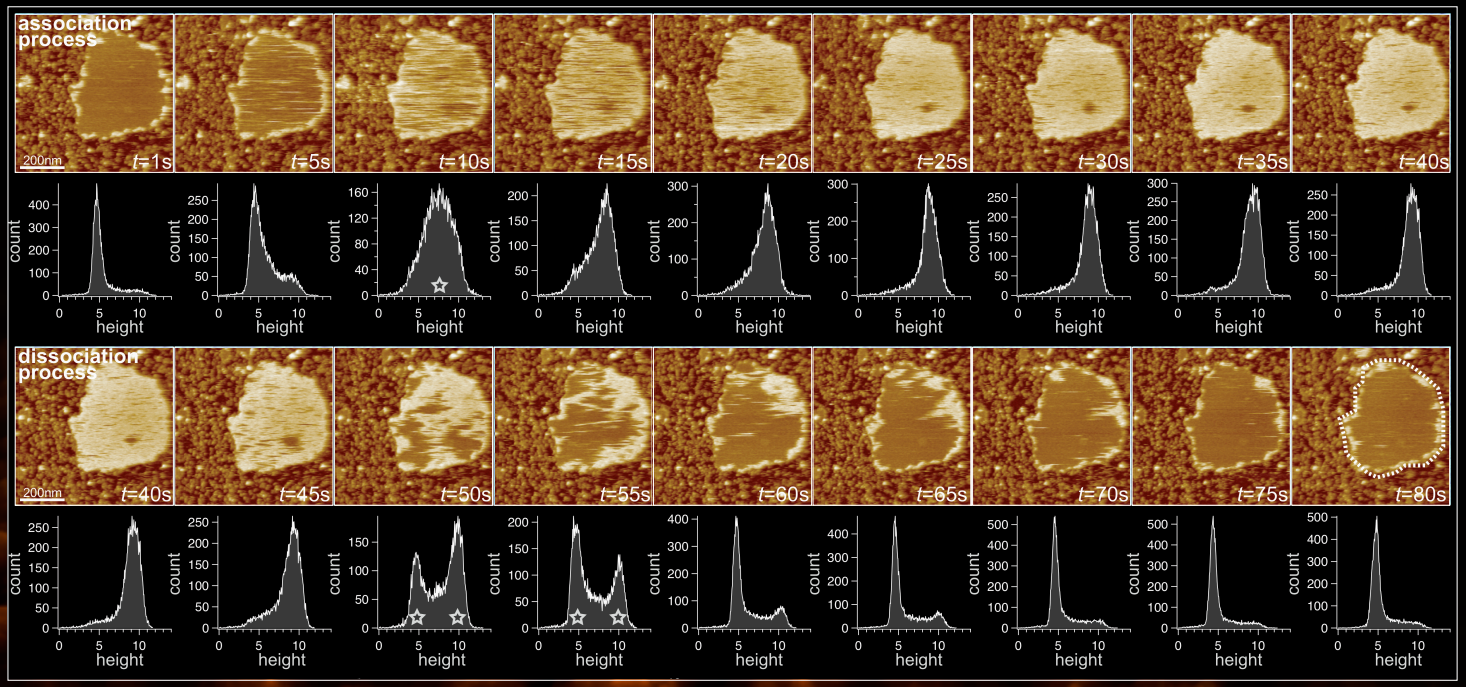
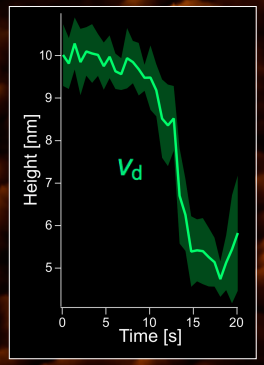
Morphologial differences of the cooperative association and dissociation processes







HS-AFM unravels morphological differences between the association (v_a) and the dissociation (v_d) processes.

Association: gradual increase from 5nm- to the 10nm state - fast diffusing molecules
Dissociation: 5nm- and 10nm states coexist - membrane subdomains dissociate

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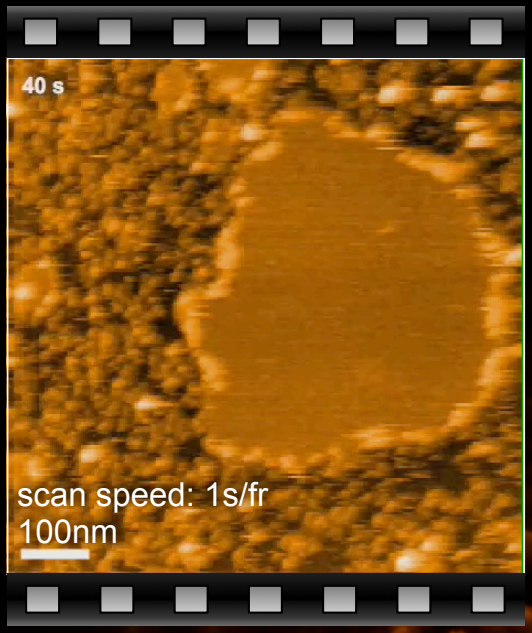

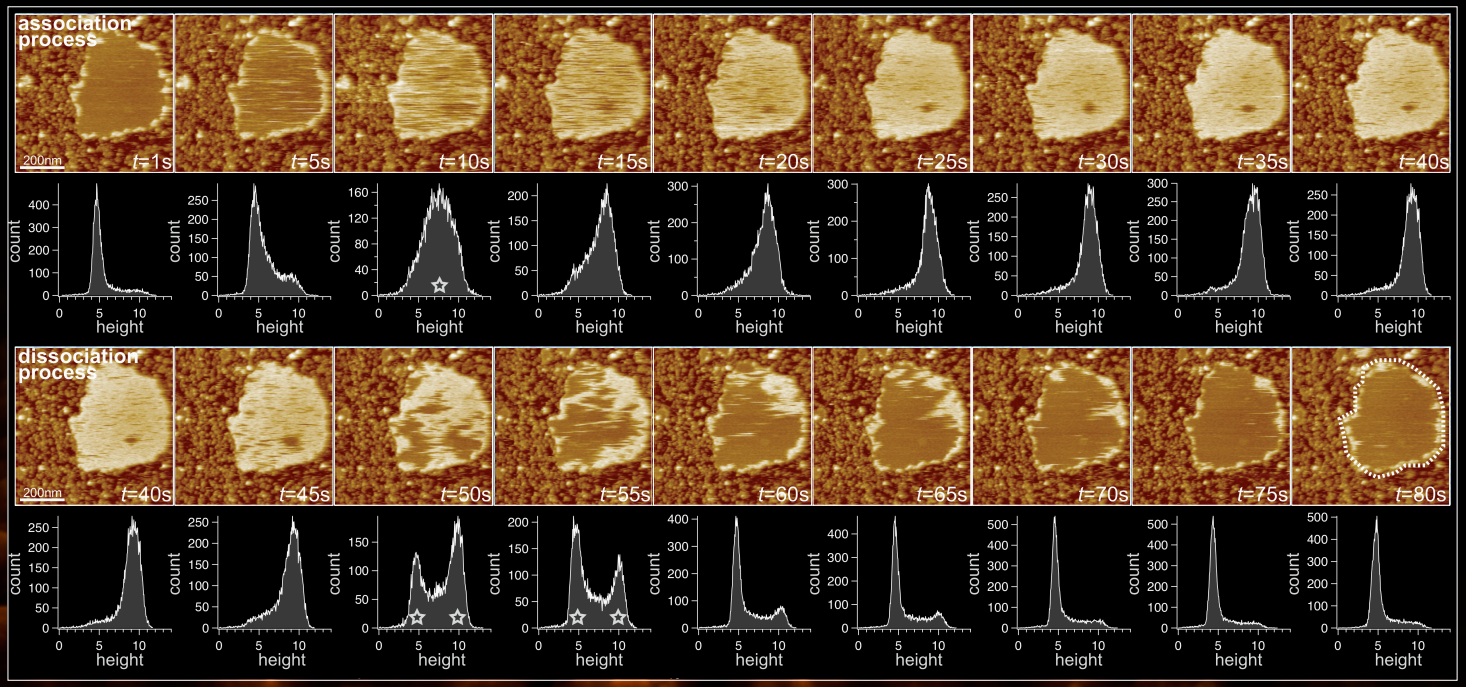

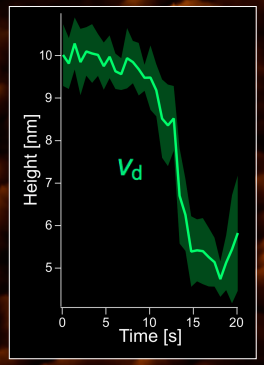

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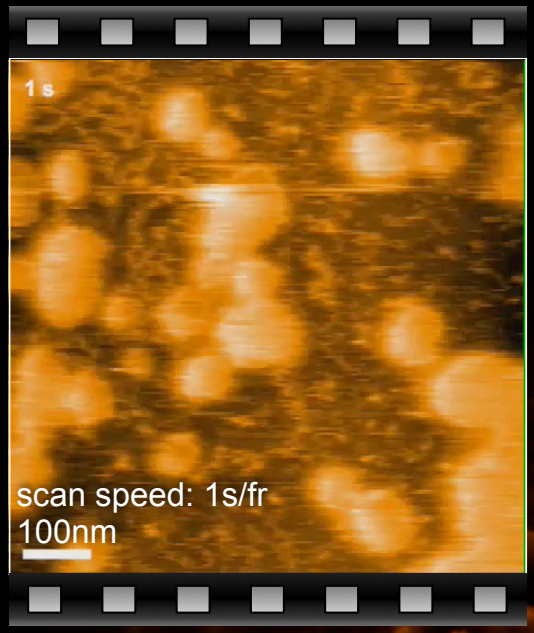
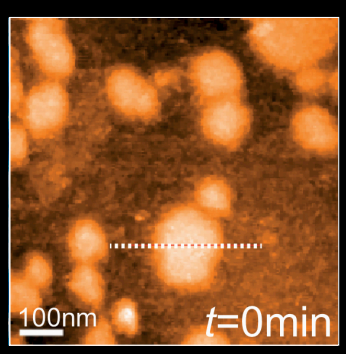
MinD forms filaments on the membrane patches

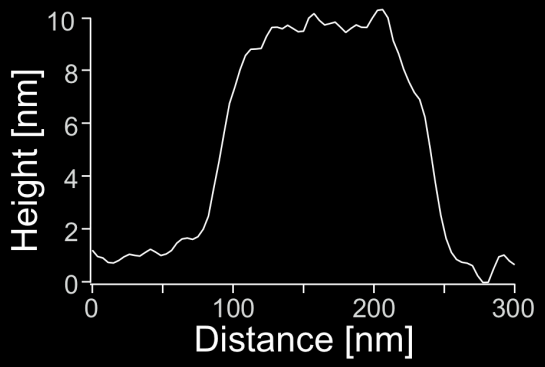


MinD forms stable filaments on membranes

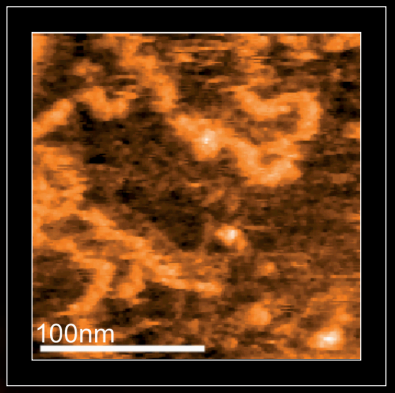
The persistence length *L* of MinD filaments
***L* = 27.1 +/- 9.6nm**

corresponding to about 7 MinD

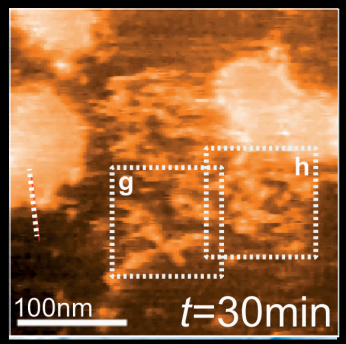


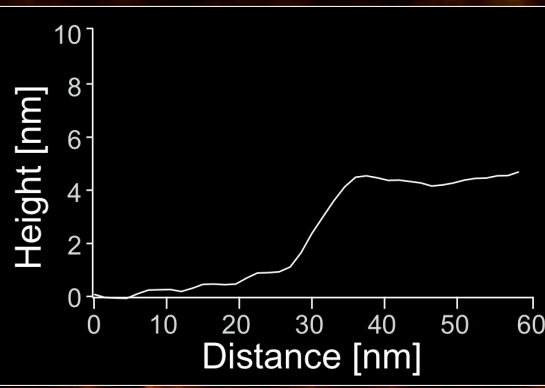


1µM MinD + 2.5mM ATP (0µM MinE): 10nm state over >10min

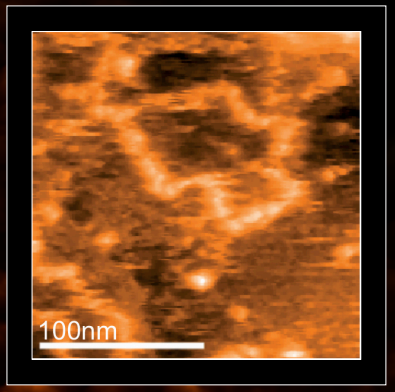


MinD-filaments





MinD pushed off the membranes



MinD-filaments

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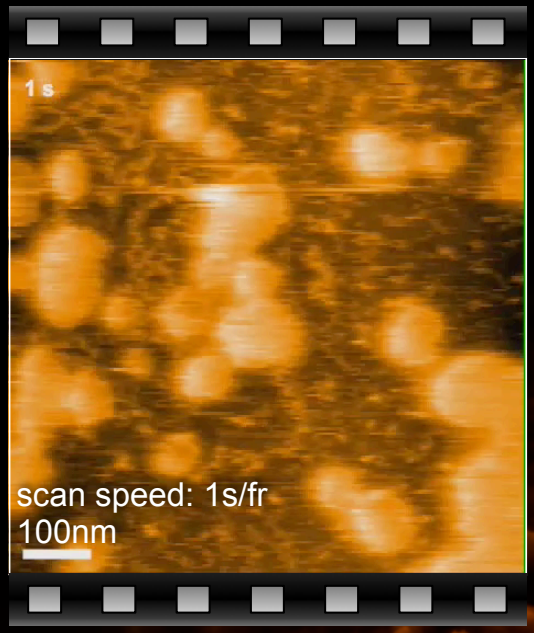
MinD forms filaments on the membrane patches



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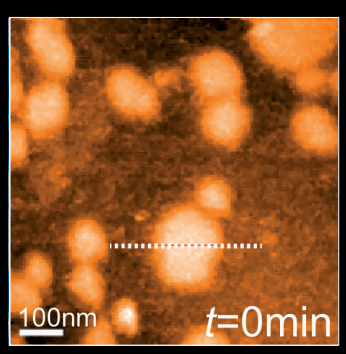

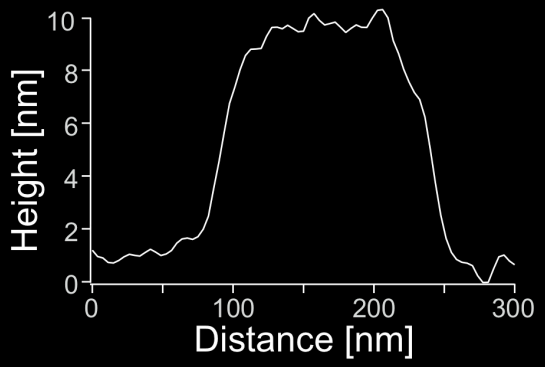

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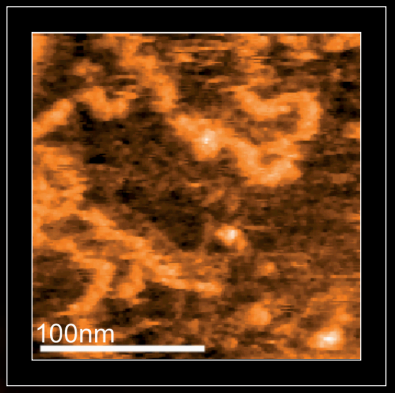

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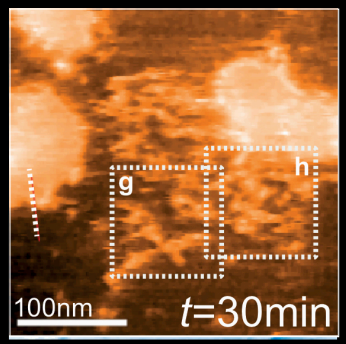

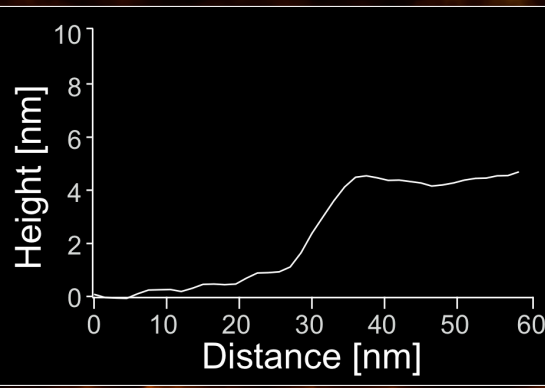

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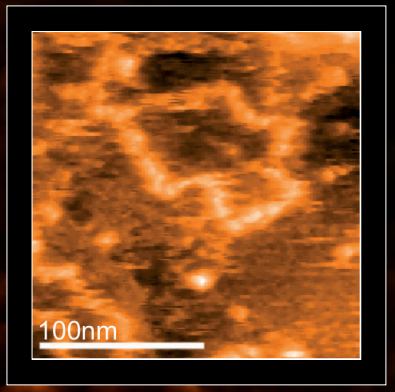

MinD-filaments

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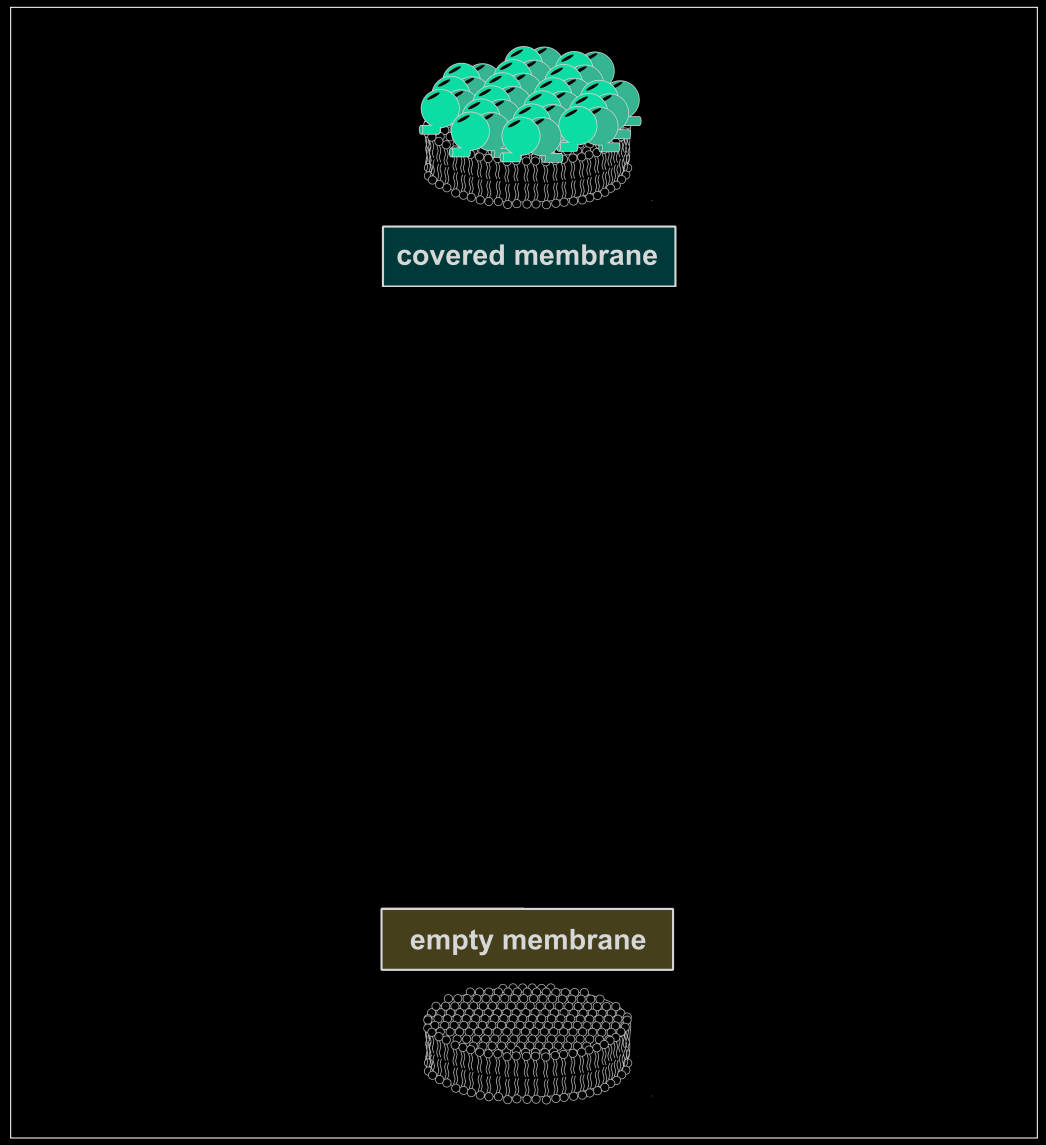

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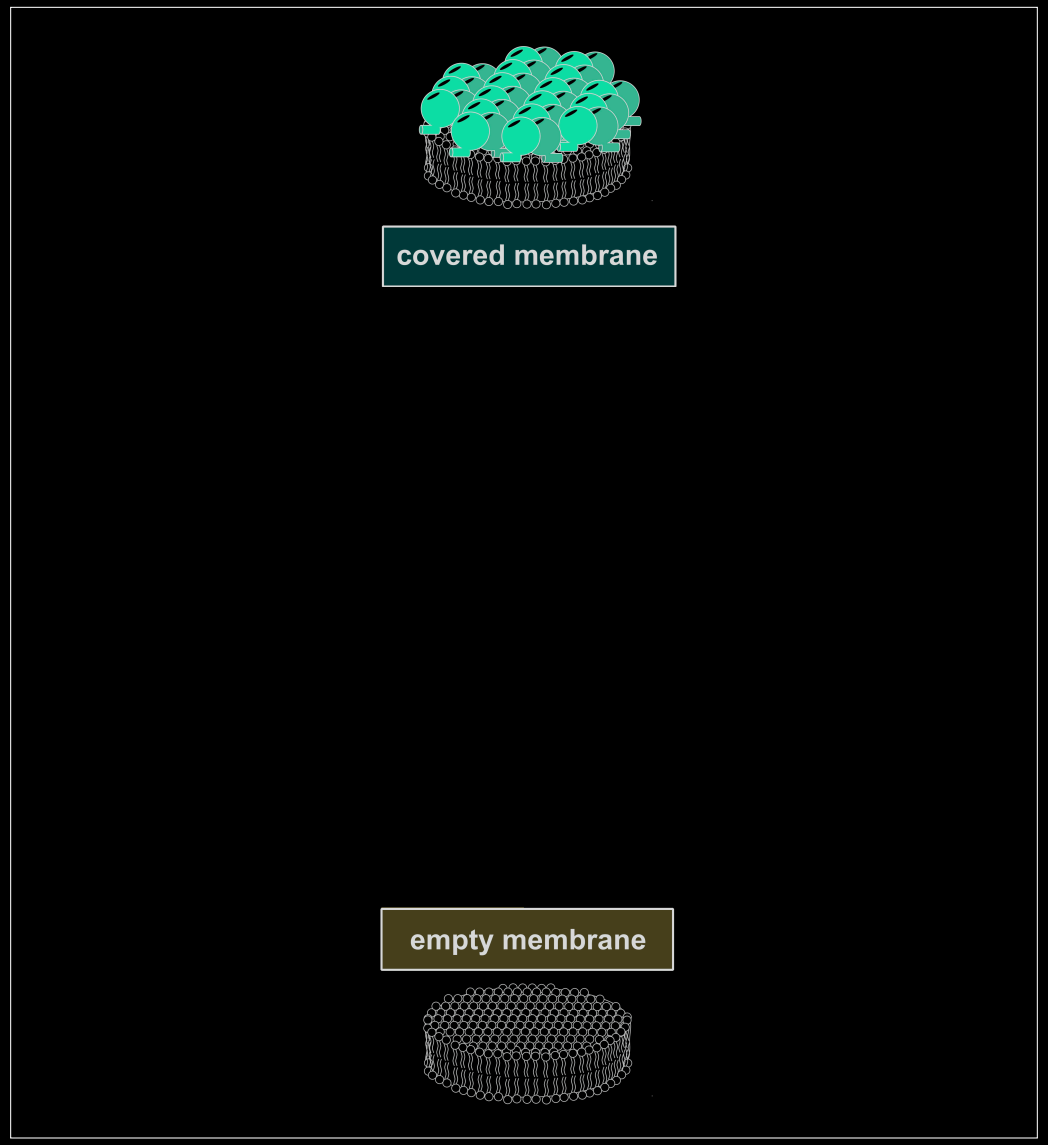

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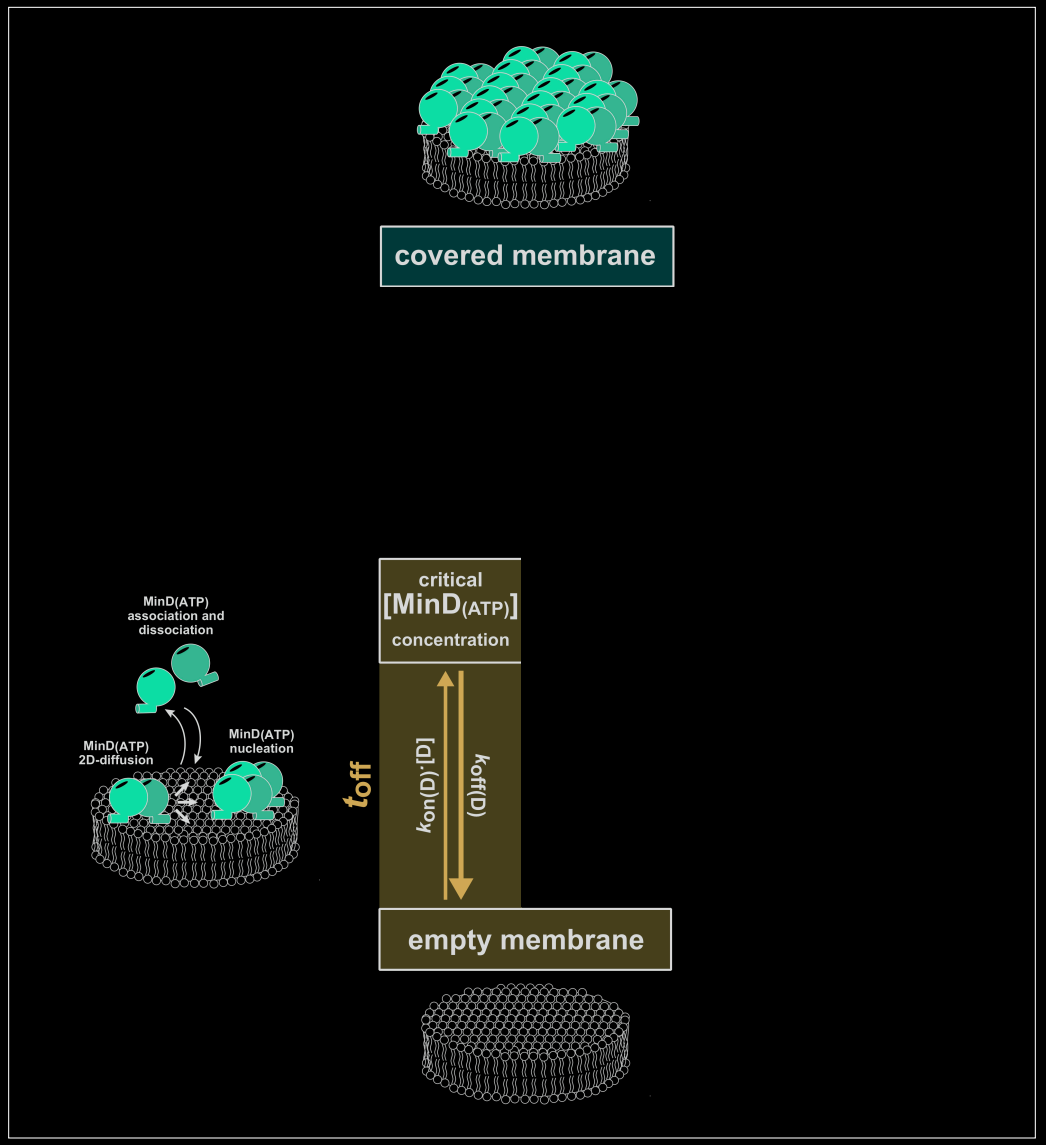

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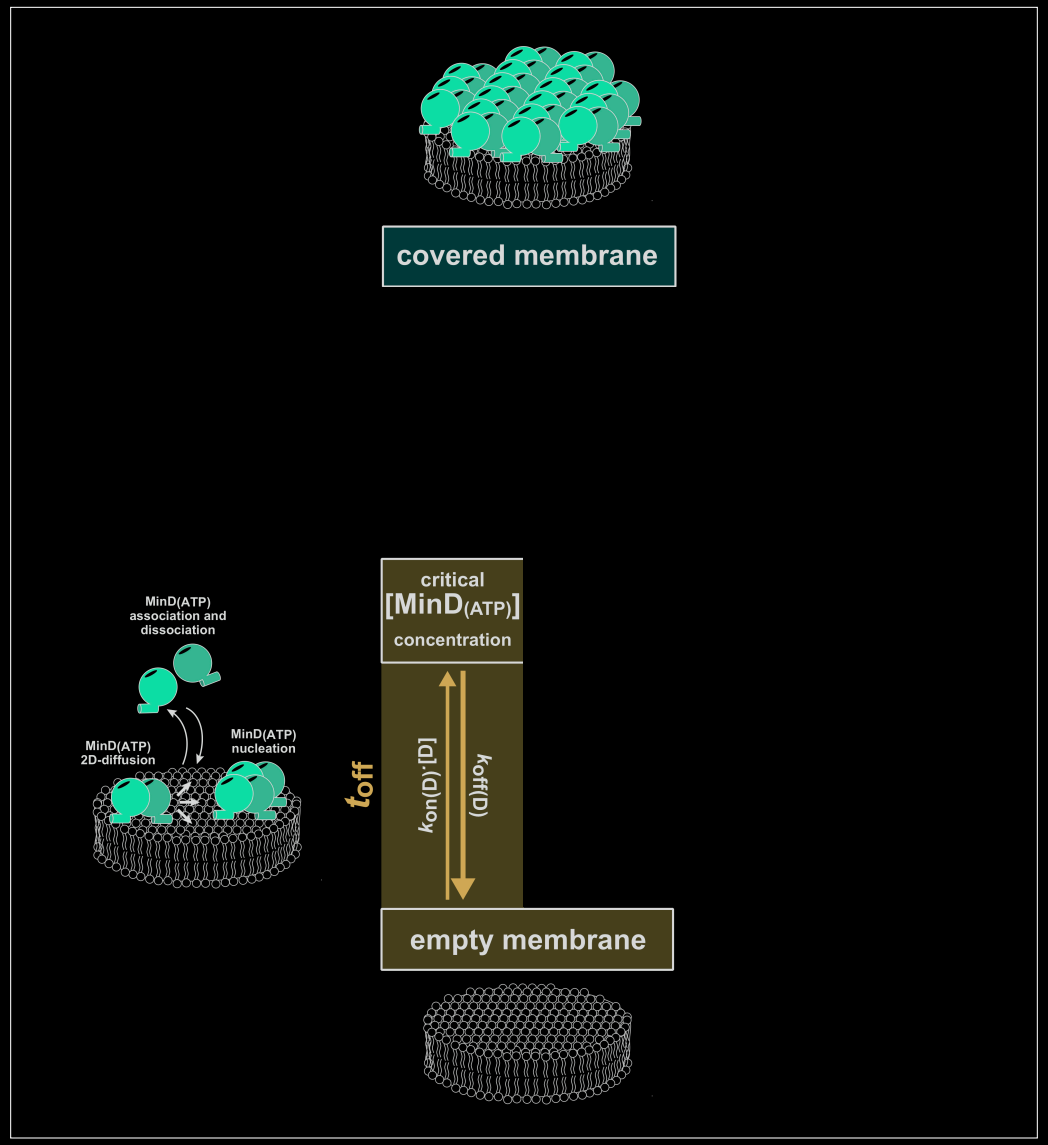

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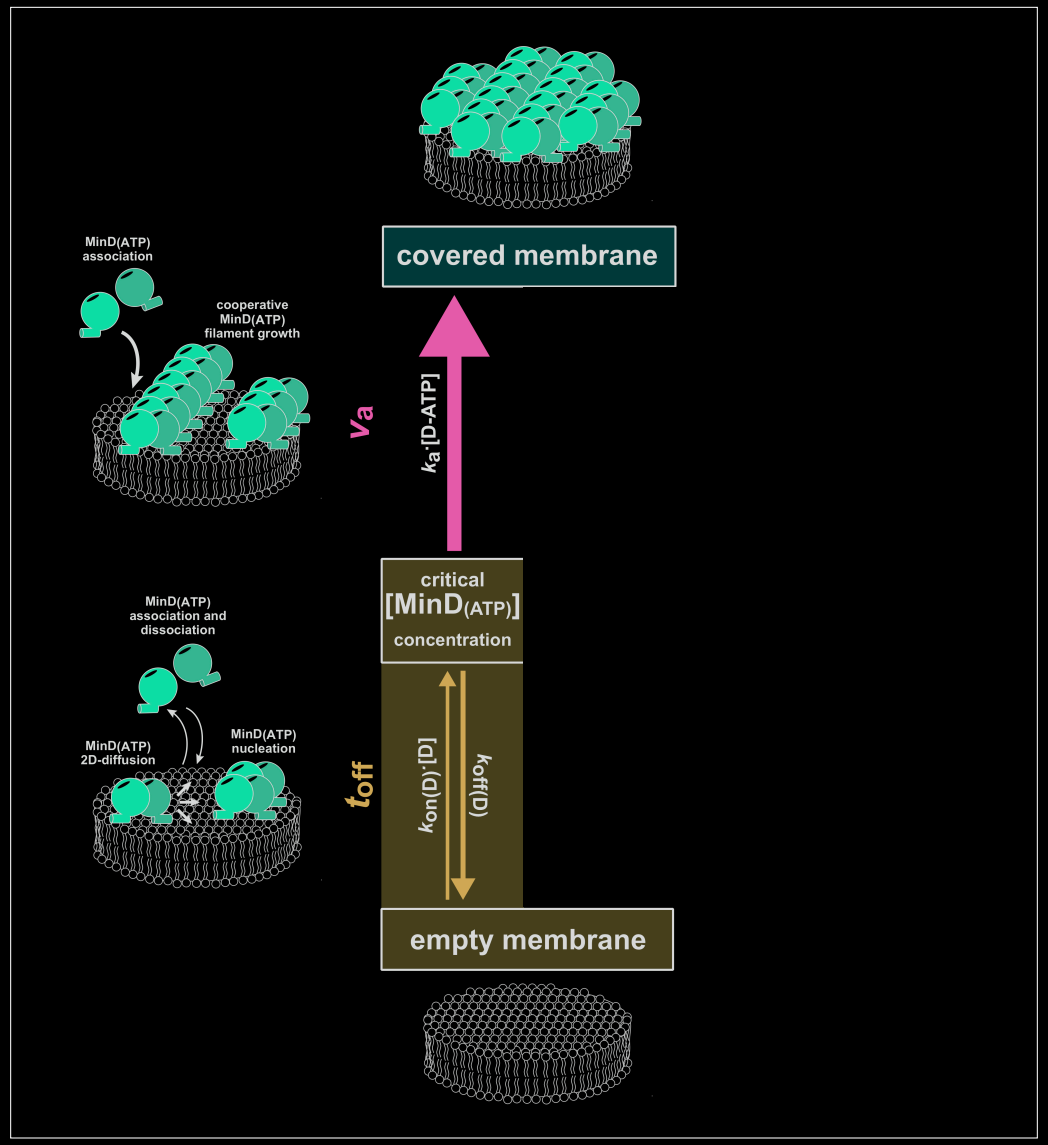

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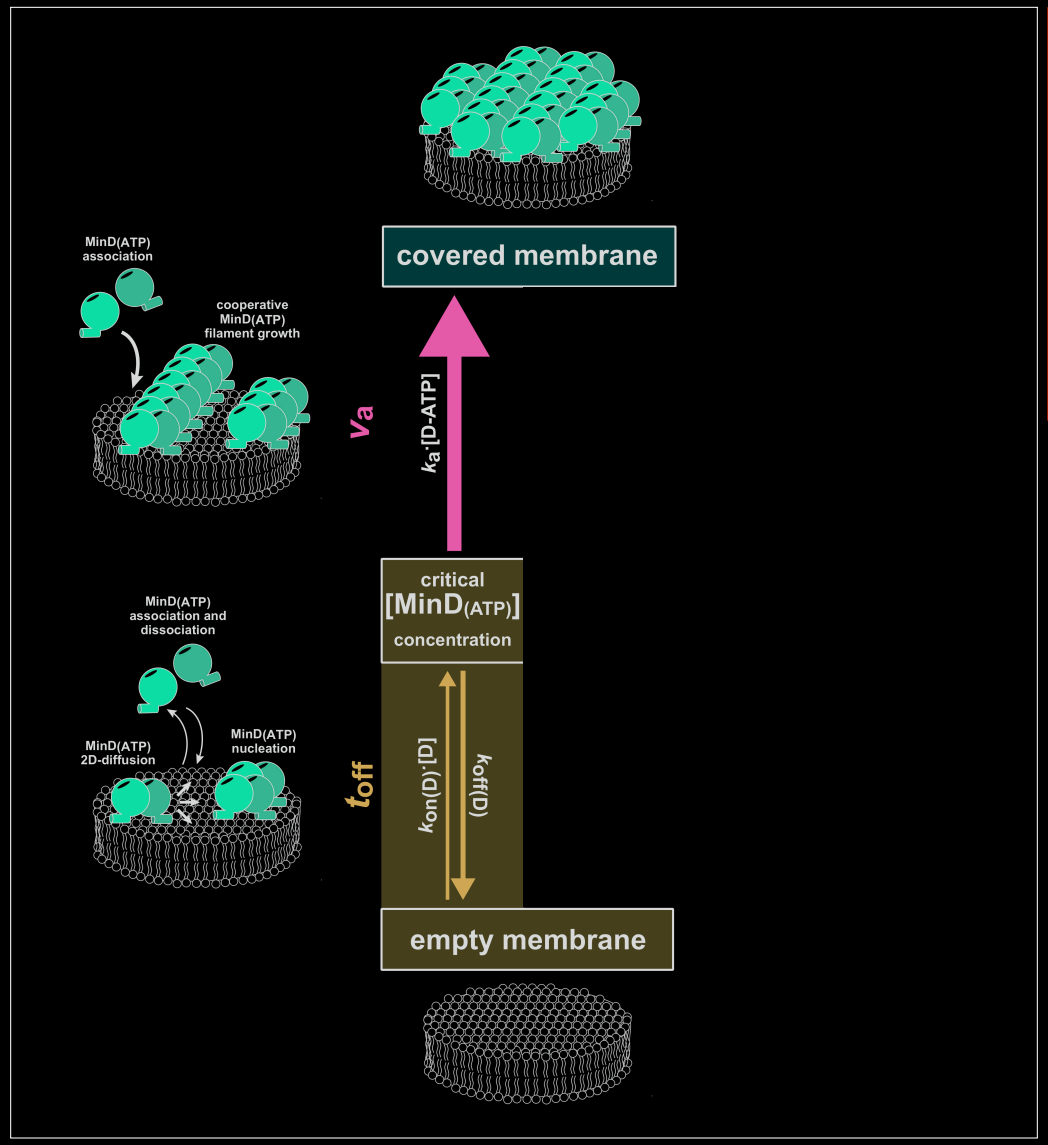

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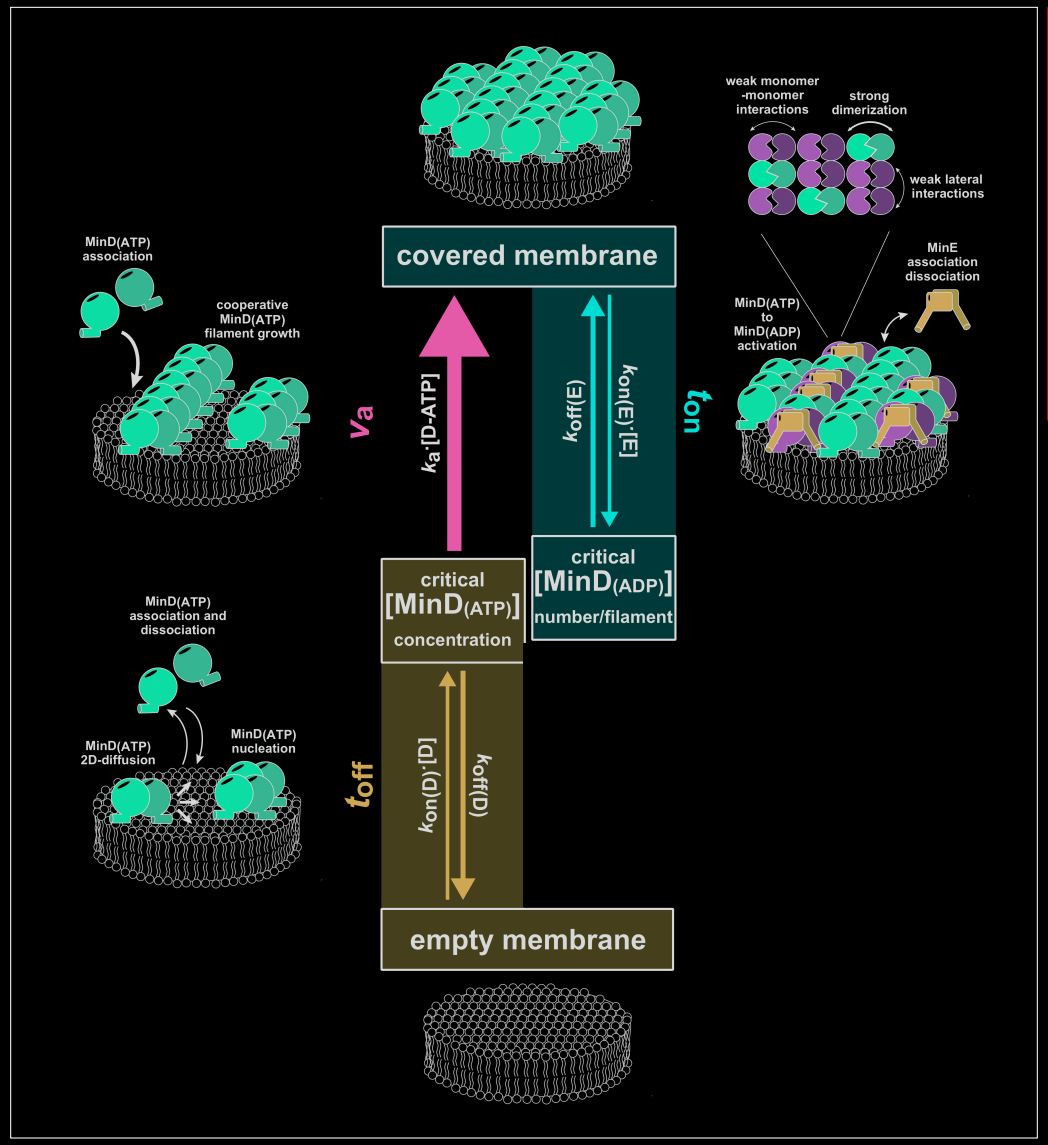

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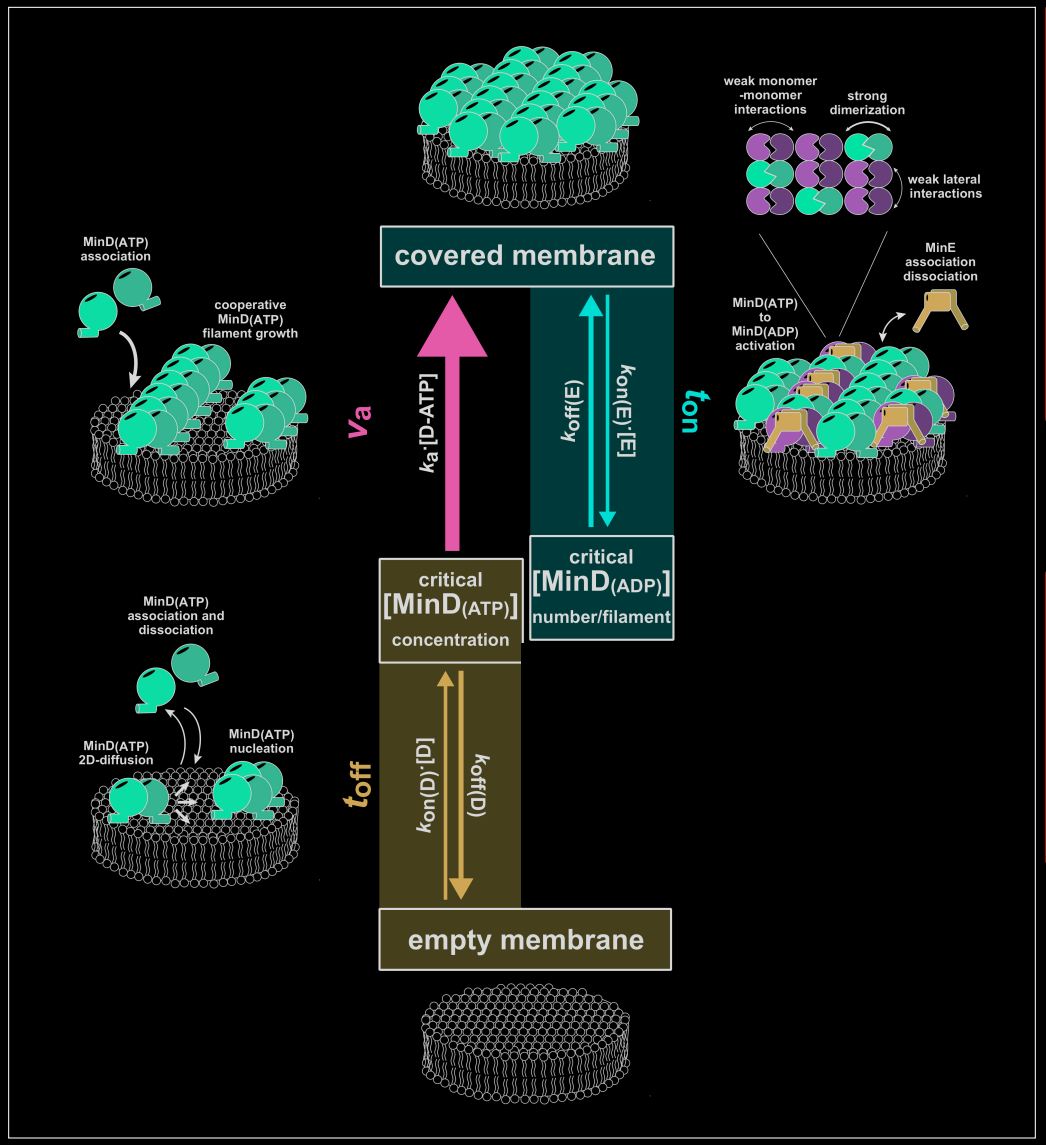

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v_d is MinD independent
→ [MinD] = v_d ←
v_d is MinE independent
→ [MinE] = v_d ←
MinD NO binding during v_d
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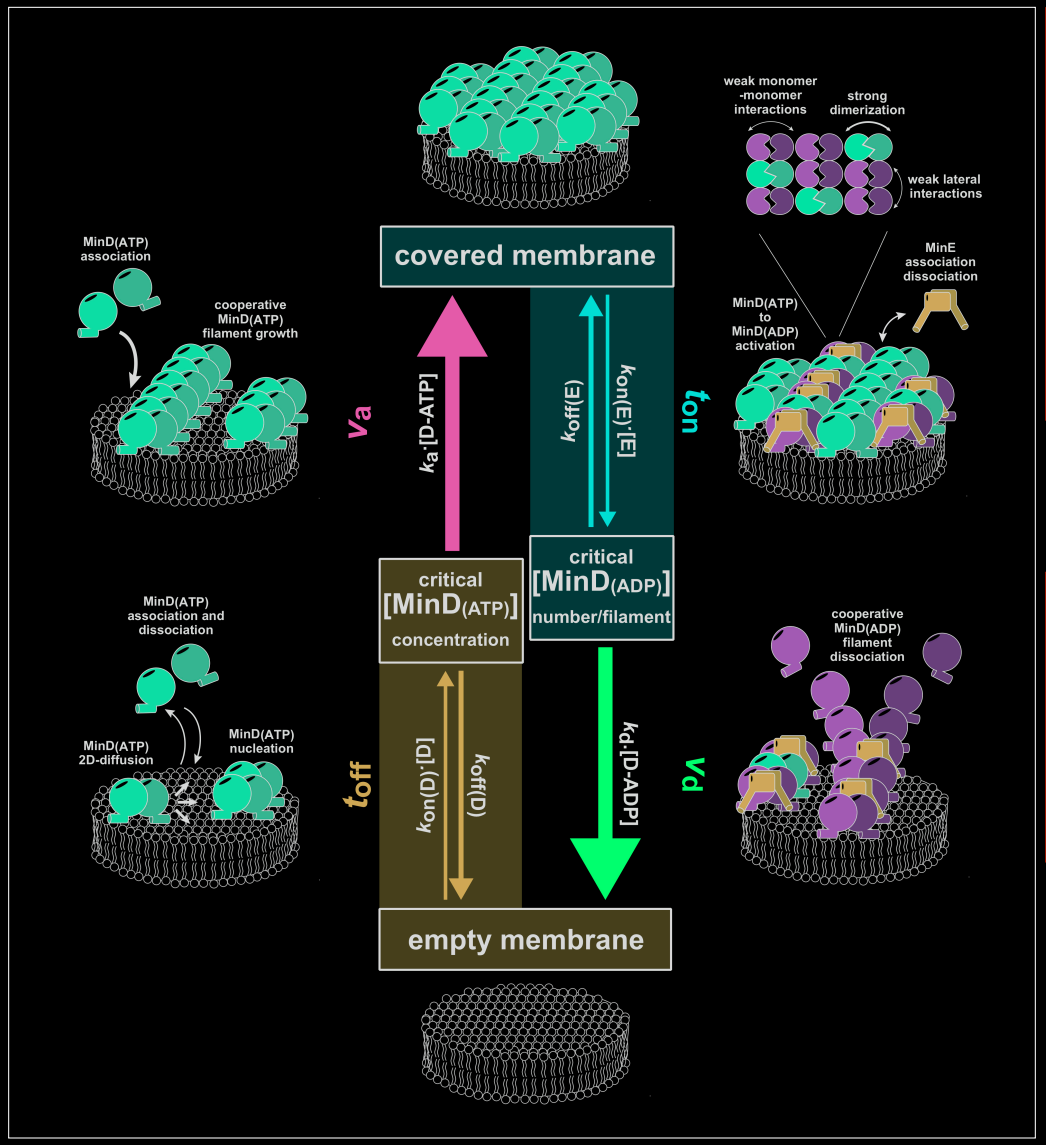
→ [membrane] = t_{off} ←
t_{off} is MinD dependent
↑ [MinD] = t_{off} ↓
t_{off} is slightly MinE dependent
↑ [MinE] = t_{off} ↗
MinD membrane seeding during t_{off}
MinE slight activation of seed MinD



t_{on} is patchsize dependent
↑ [membrane] = t_{on} ↑
t_{on} is slighly MinD dependent
↑ [MinD] = t_{on} ↗
t_{on} is MinE dependent
↑ [MinE] = t_{on} ↓
patchsize dependence is MinE dependent
↑ [MinE] = [membrane] ↓
MinD slight replenish
MinE activation of MinD during t_{on}
larger patch - increased activation barrier
MinE lowers activation barrier

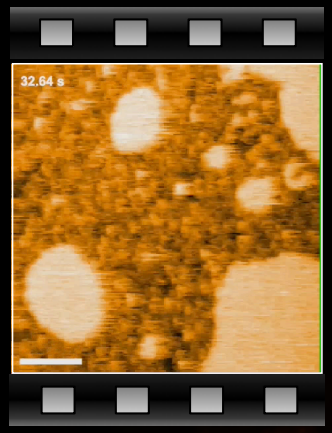
v_d is patchsize independent
→ [membrane] = v_d ←
v_d is MinD independent
→ [MinD] = v_d ←
v_d is MinE independent
→ [MinE] = v_d ←
MinD NO binding during v_d
MinE NO binding during v_d

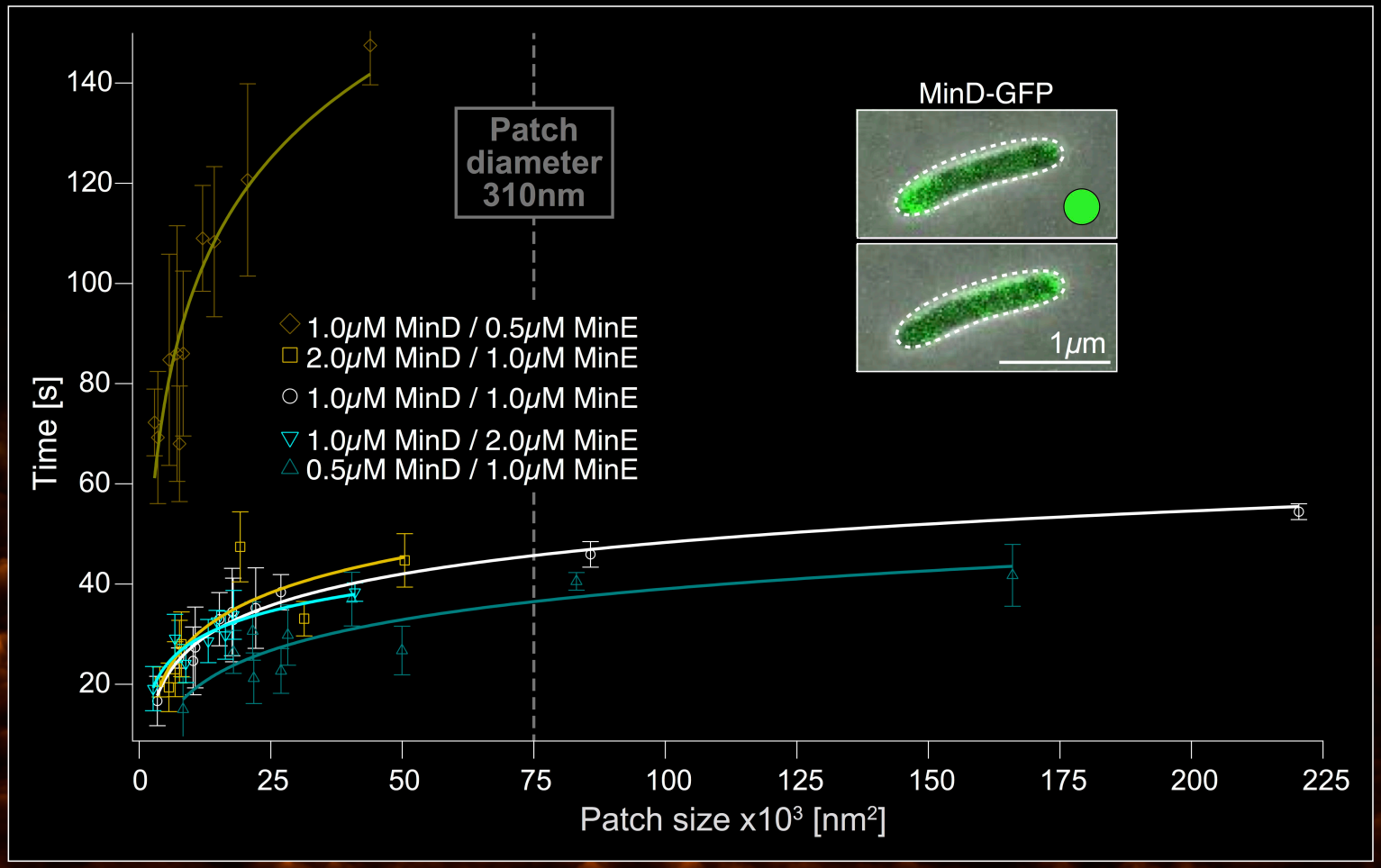
Bio-AFM-Lab

HS-AFM Deciphers MinDE Pattern Formation

Simon Scheuring

Potential explanation why small patches are not 'viable' in vivo





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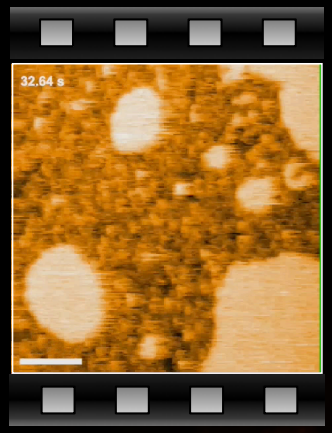
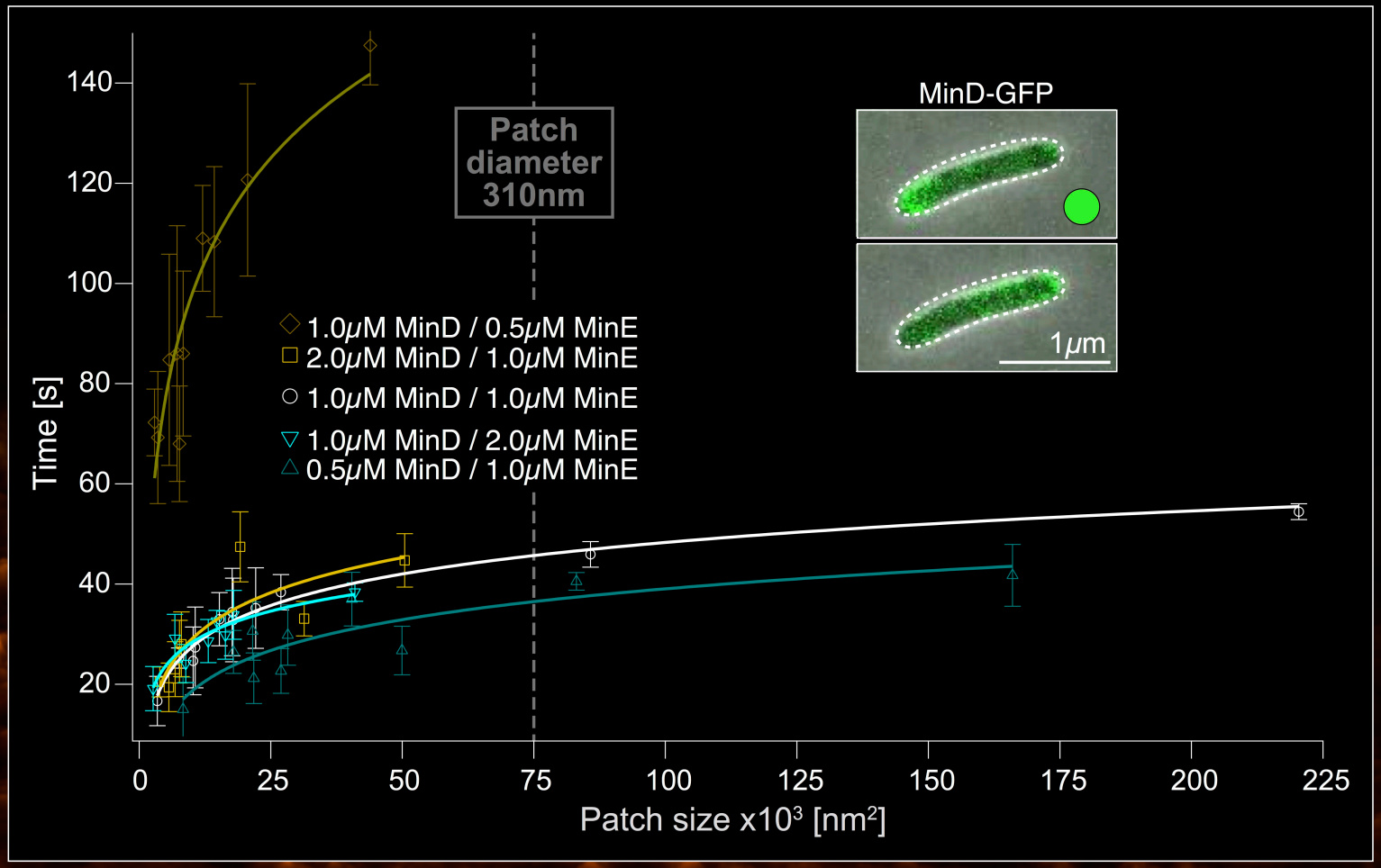




Bio-AFM-Lab

Weill Cornell Medicine

Direct visualization of glutamate transporter elevator mechanism by high-speed AFM

Yi Ruan, Atsushi Miyagi, Xiaoyu Wang, Mohamed Chami,
Olga Boudker & Simon Scheuring

Collaboration with
Olga Boudker laboratory
Weill Cornell Medicine, New York, USA

PNAS, 2017, 114(7):1584-1588; DOI: 10.1073/PNAS.1616413114

Bio-AFM-Lab

HS-AFM of GLT_{PH} «Elevator» Movements

Simon Scheuring

Glutamate transporters 'recycle' glutamate from the synaptic cleft

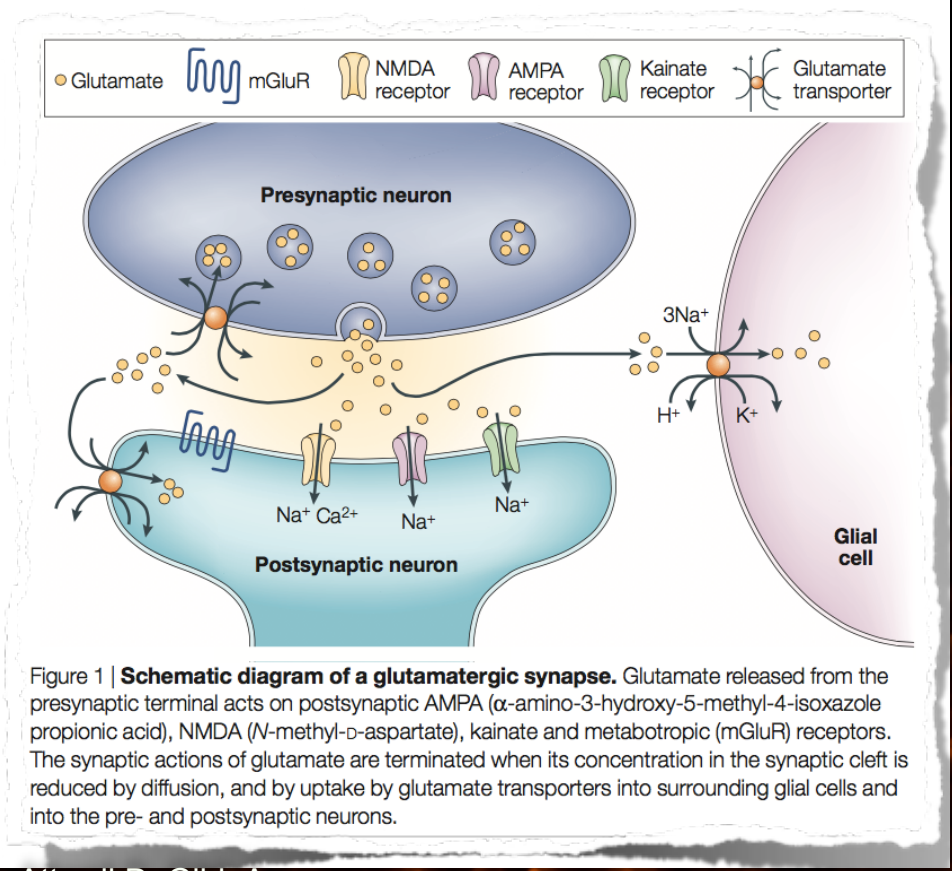
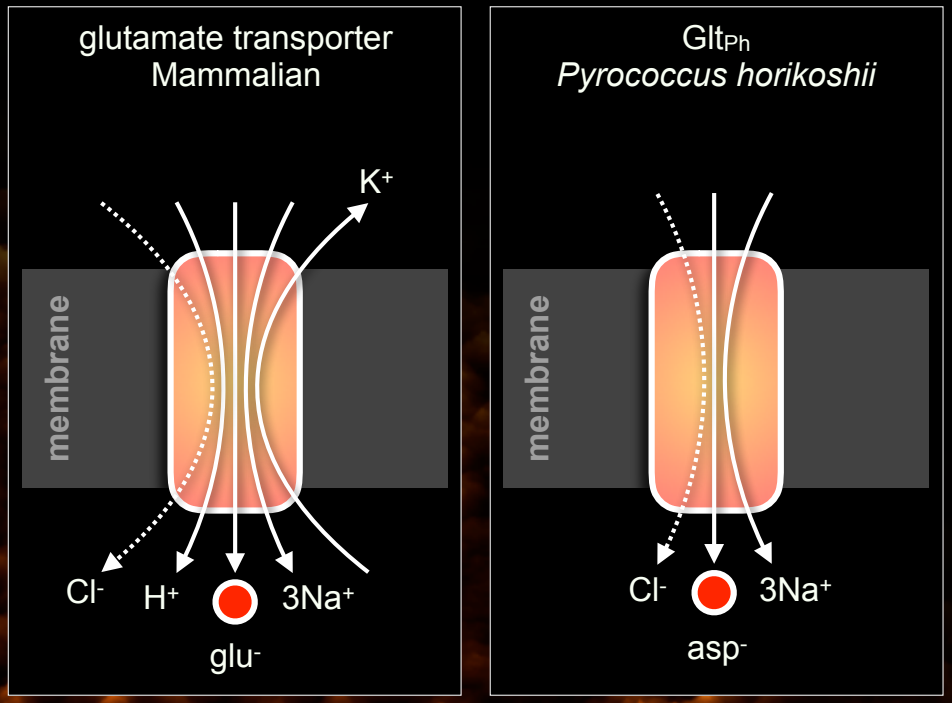


Figure 1 | **Schematic diagram of a glutamatergic synapse.** Glutamate released from the presynaptic terminal acts on postsynaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), NMDA (*N*-methyl-D-aspartate), kainate and metabotropic (mGluR) receptors. The synaptic actions of glutamate are terminated when its concentration in the synaptic cleft is reduced by diffusion, and by uptake by glutamate transporters into surrounding glial cells and into the pre- and postsynaptic neurons.

Attwell D, Gibb A.
Neuroenergetics and the kinetic design of excitatory synapses
Nat Rev Neurosci. 2005;6(11):841-9



GLT_{Ph} shares **37% amino acid identity** with human excitatory amino acid transporter 2 (hEAAT2)

PNAS, 2017, 114(7):1584-1588; DOI: 10.1073/PNAS.1616413114

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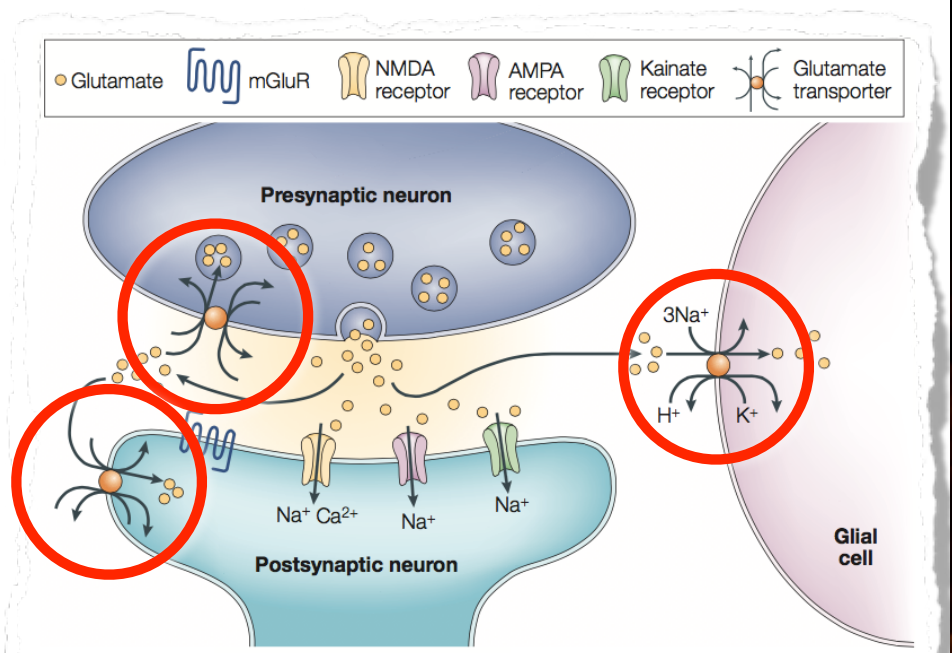

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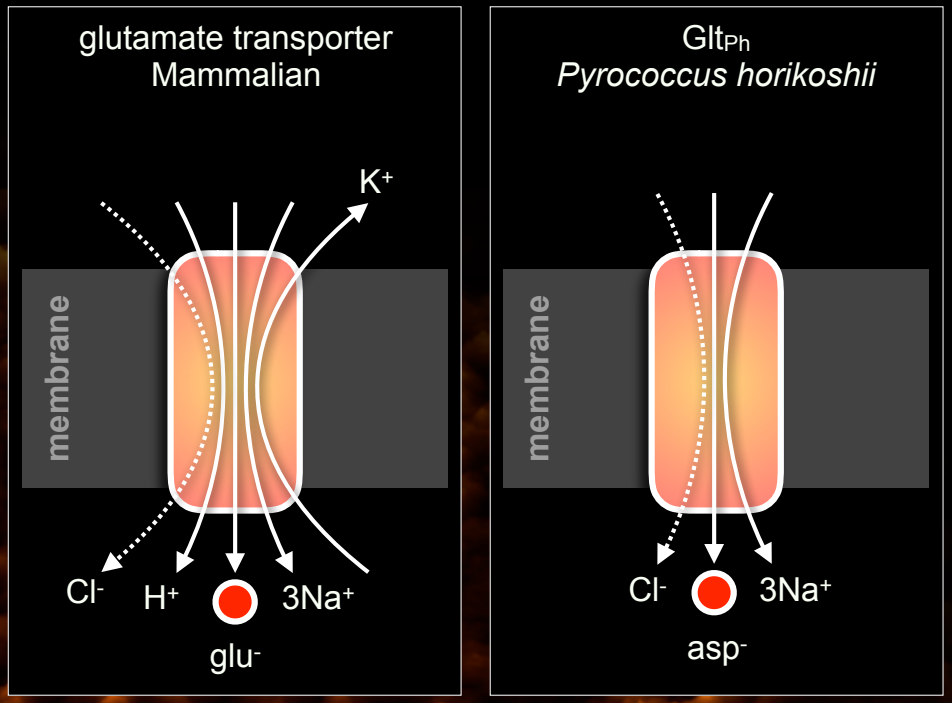

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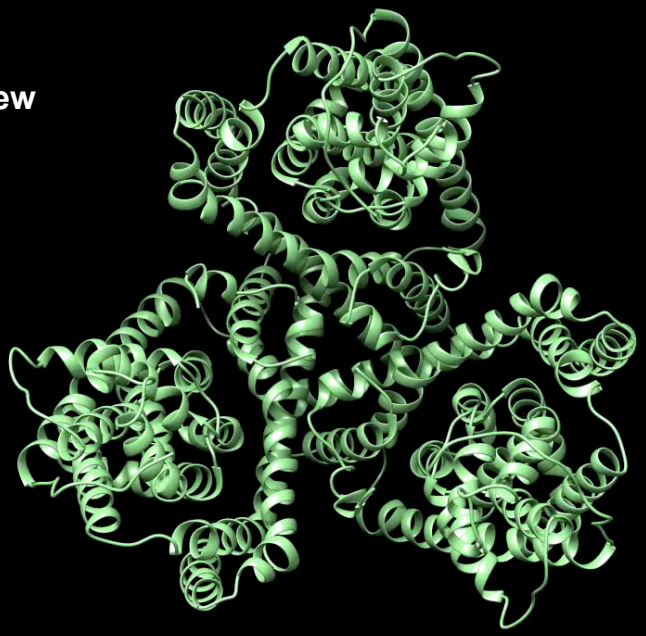
HS-AFM of GLT_{PH} «Elevator» Movements

Simon Scheuring

Structures of the Glt_{Ph} elevator domain conformational changes: ~1.5nm to ~2.0nm

TOP view



SIDE view

Structures: Boudker-lab

outward-facing Glt_{Ph} **PDB 2NWX** ⟷ inward-facing Glt_{Ph} **PDB 3KBC** ⟷ outward-facing Glt_{Ph} **PDB 2NWX**

Morphing: UCSF Chimera

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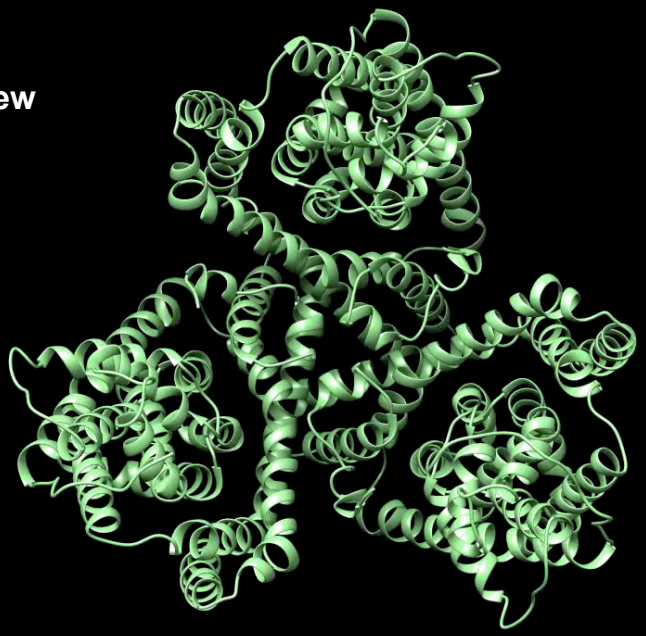

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Bio-AFM-Lab

HS-AFM of GLT_{PH} «Elevator» Movements

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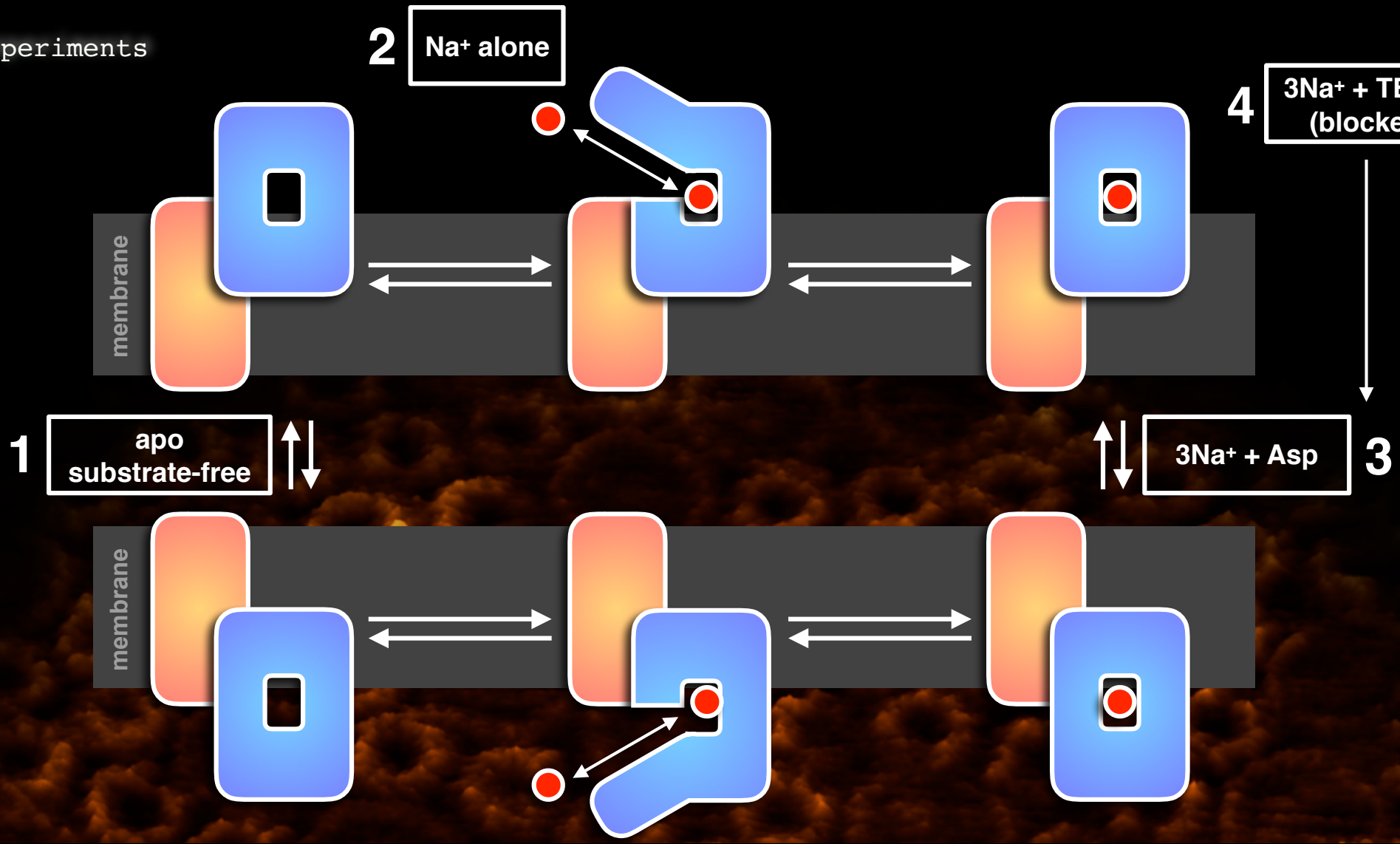
Experiments



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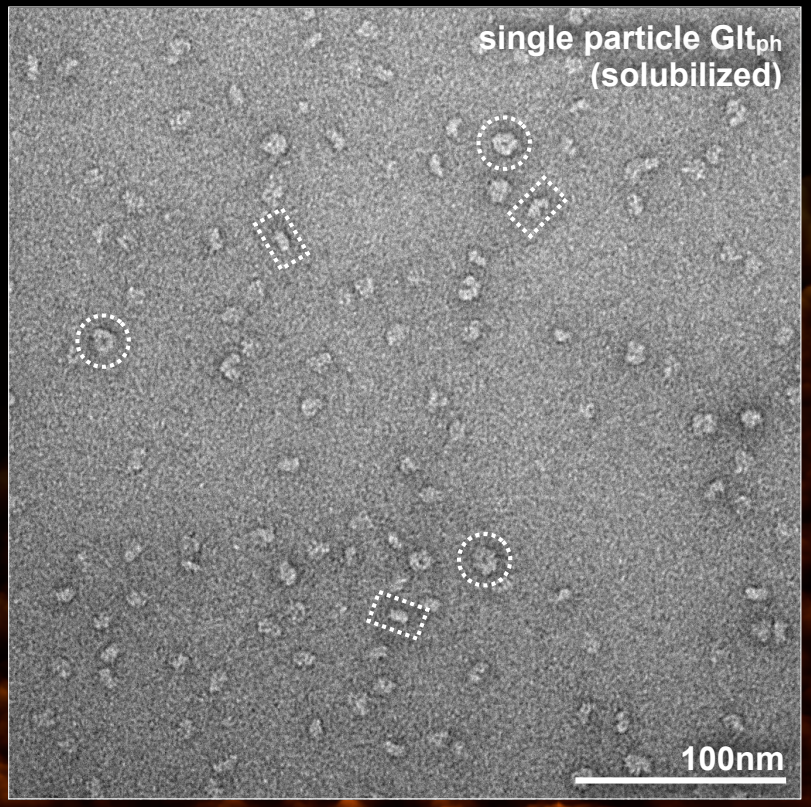
HS-AFM of Glt_{Ph} «Elevator» Movements

Simon Scheuring

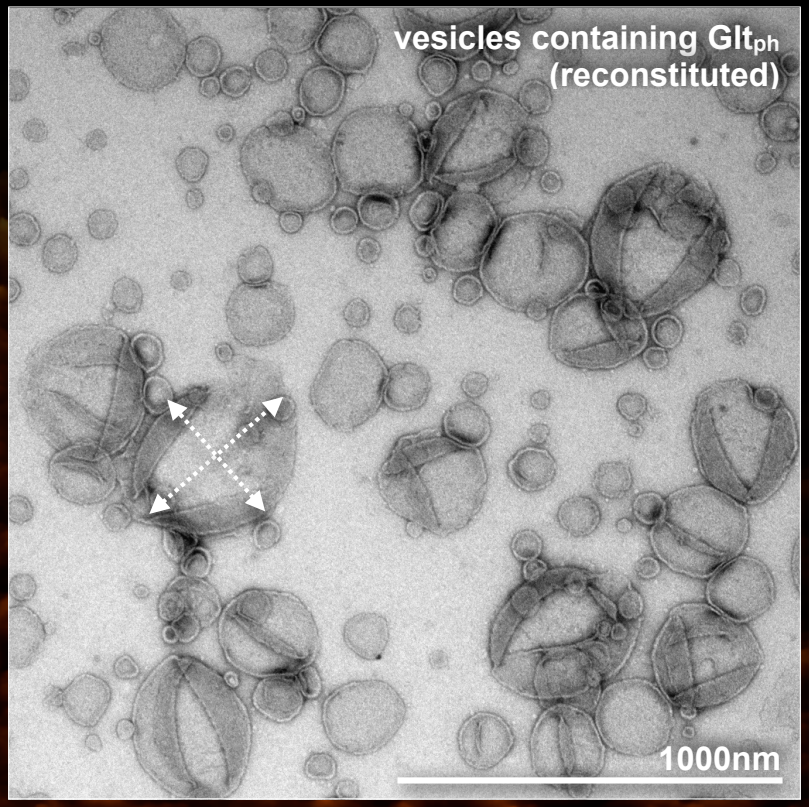
Glt_{Ph} reconstitutions checked by EM

before reconstitution



Top views (circles) / Side views (rectangles)

after reconstitution (LPR 1)



Vesicles with up to 500nm diameter

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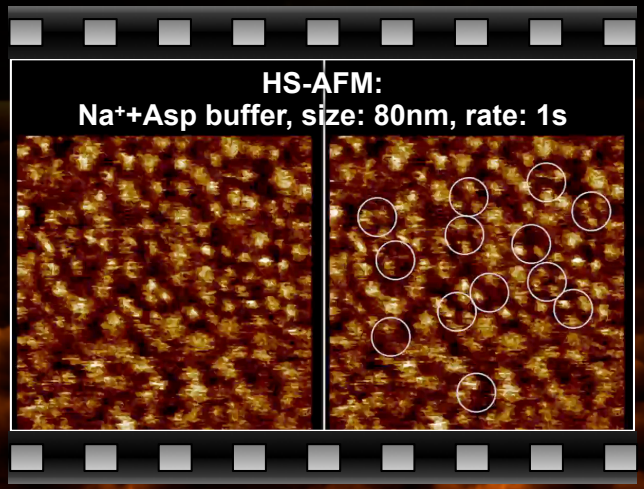
Bio-AFM-Lab

HS-AFM of Glt_{Ph} «Elevator» Movements

Simon Scheuring

Direct visualization of the movements of the Glt_{Ph} elevator domains



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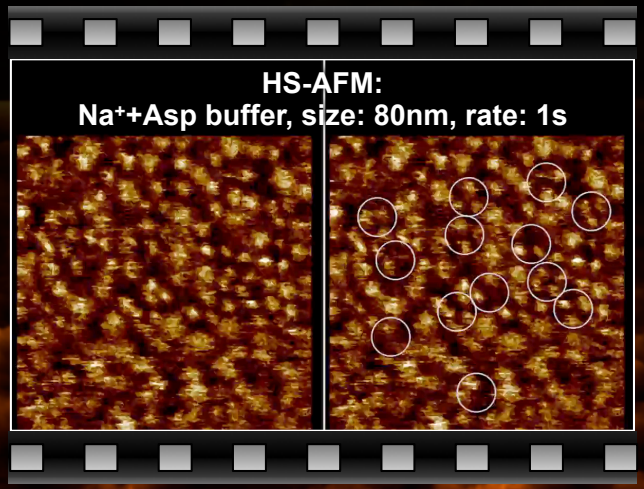

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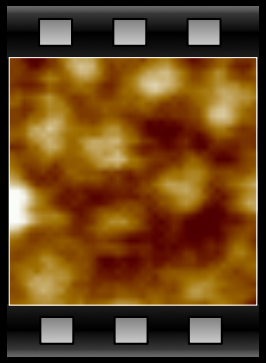
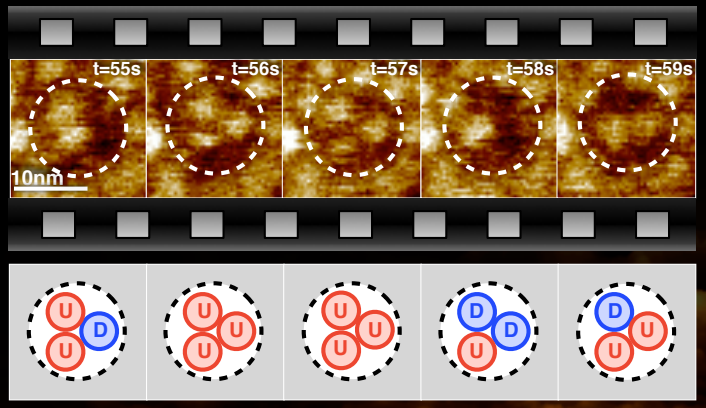
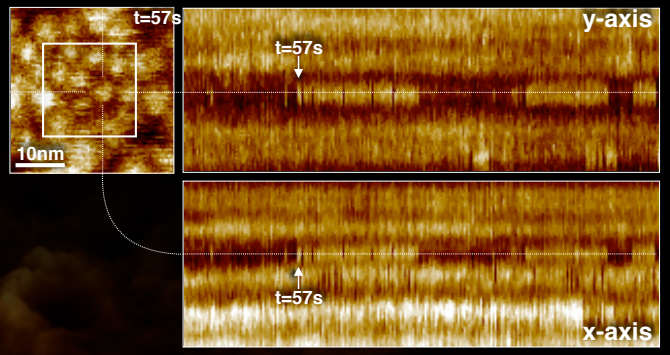


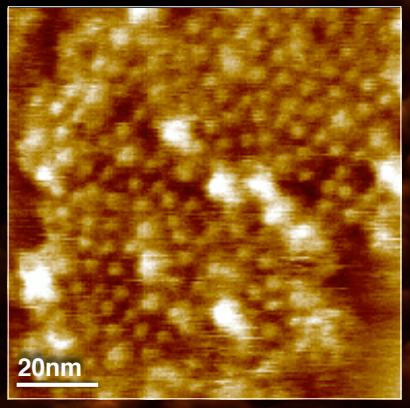
HS-AFM movie



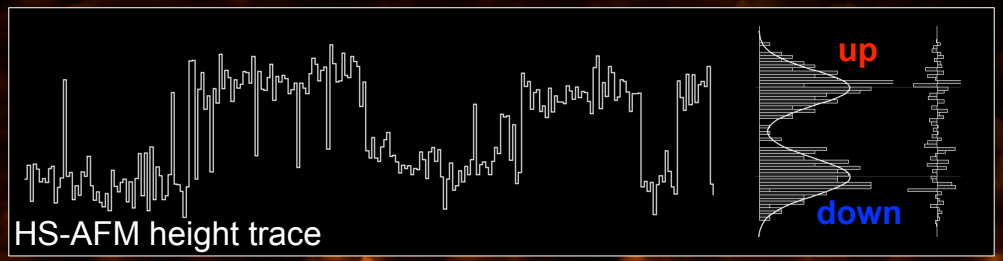
Single molecule states

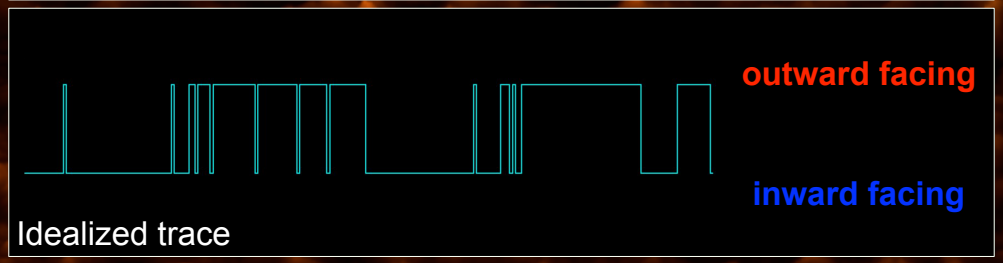


kymographs: t=0s to t=231s



HS-AFM frame





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Bio-AFM-Lab

HS-AFM of $GltPh$ «Elevator» Movements

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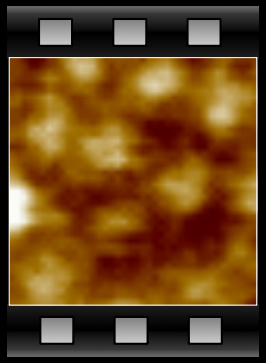
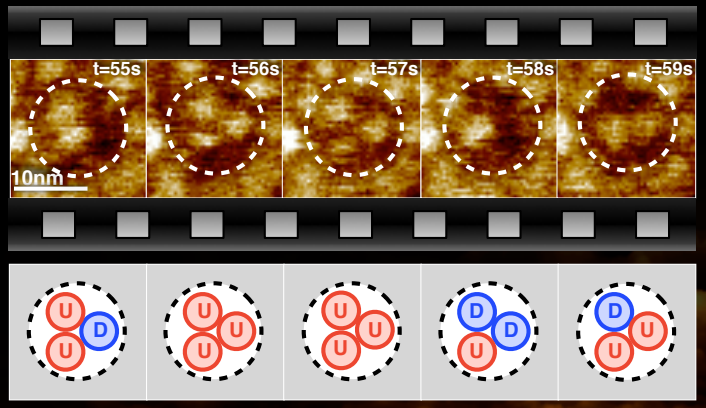
Direct visualization of the movements of the Glt_{Ph} elevator domains



HS-AFM movie



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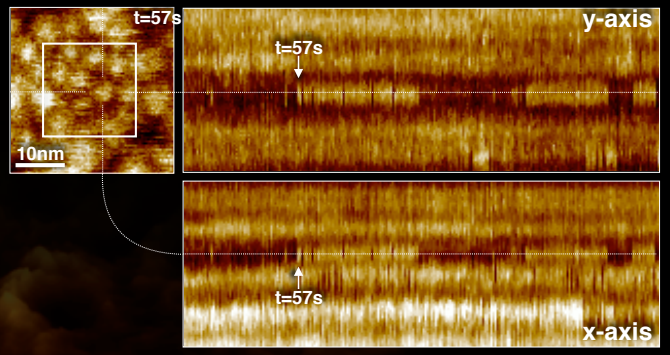

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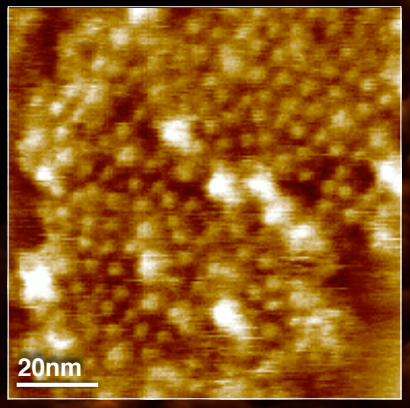
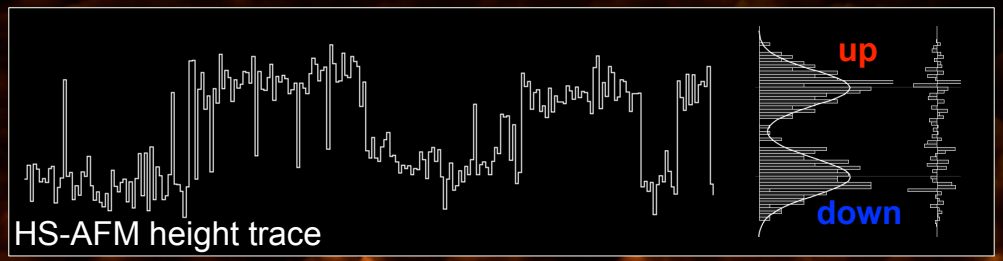
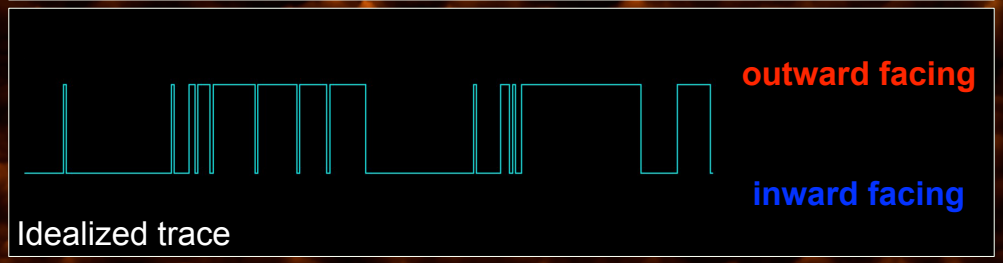


HS-AFM frame





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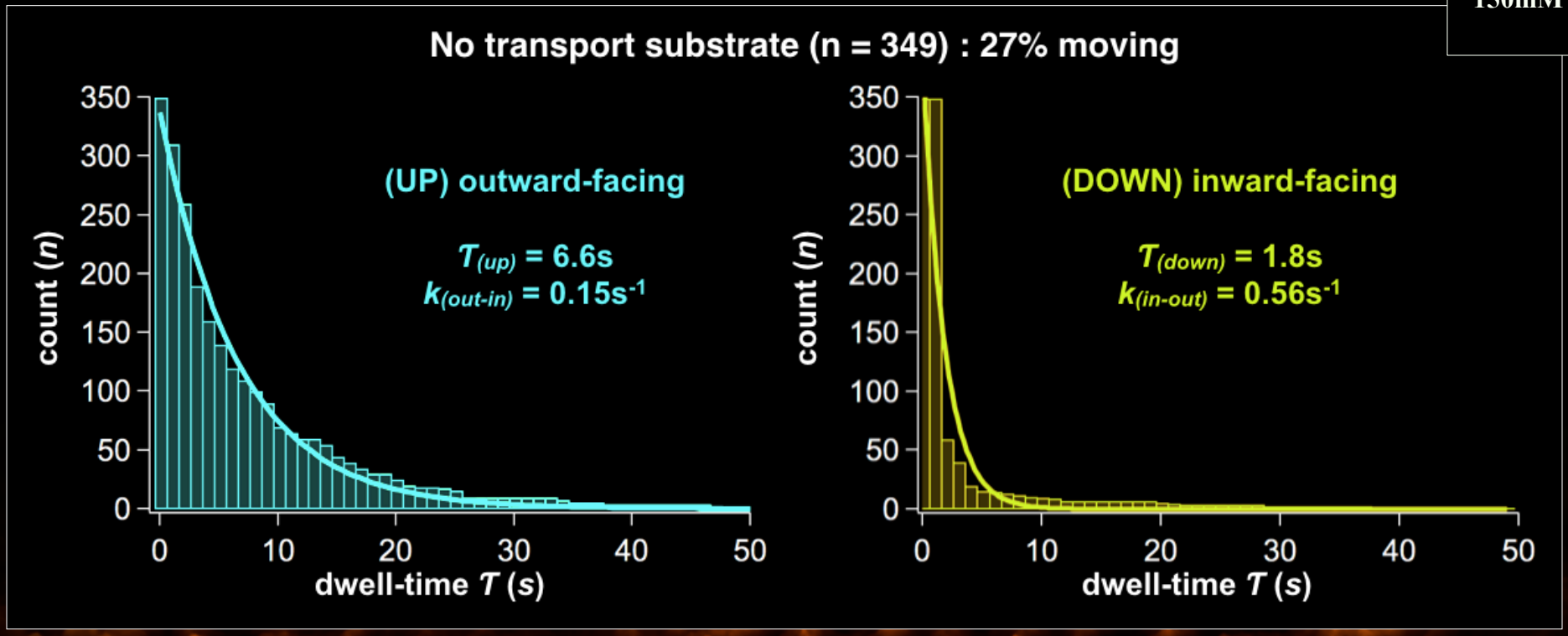
HS-AFM of GLT_{PH} «Elevator» Movements

Simon Scheuring

Apo-conditions: NO substrates / Elevator domain motion

20mM Tris
pH 7.5
150mM KCl



In the absence of subrates, the elevator domains move efficiently (empty)
The UP state is favored over the DOWN state

PNAS, 2017, 114(7):1584-1588; DOI: 10.1073/PNAS.1616413114

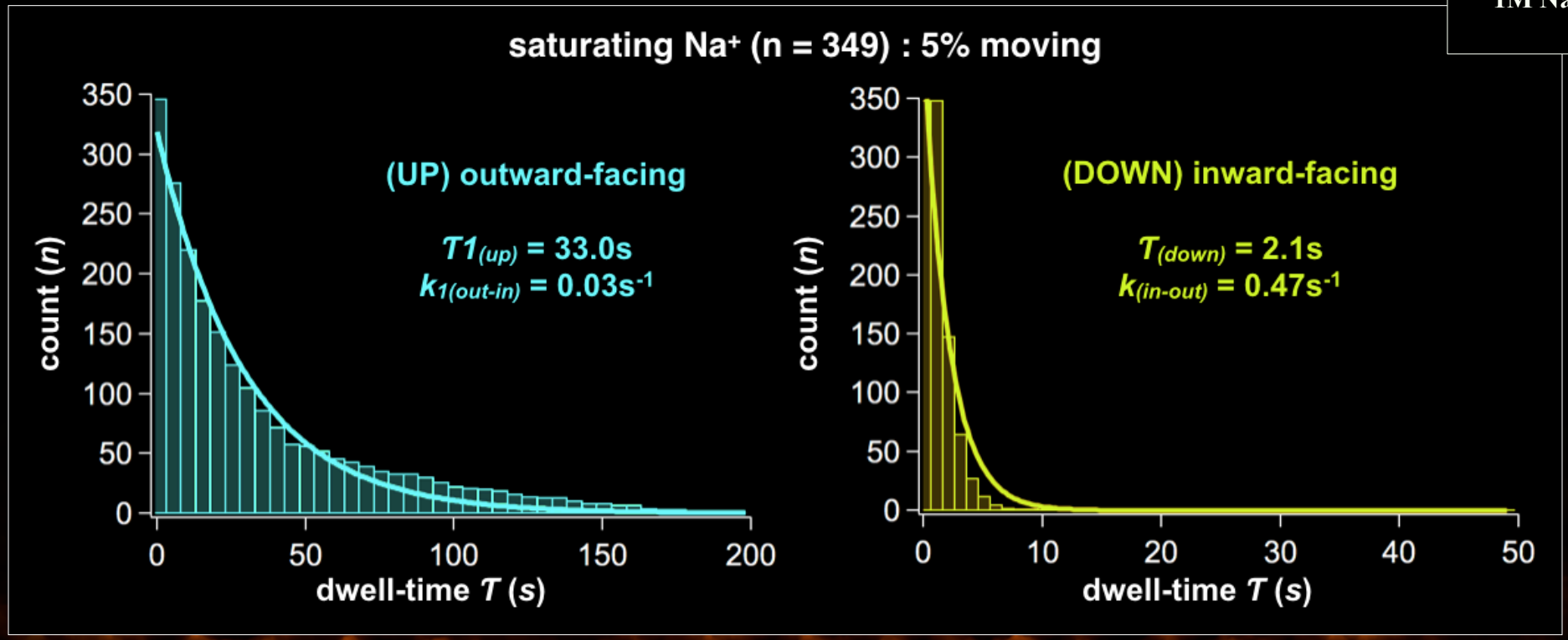
Bio-AFM-Lab

HS-AFM of GLTPH «Elevator» Movements

Simon Scheuring

Saturating (>10times kD) Na+ conditions: Elevator domains stall

20mM Tris
pH 7.5
1M NaCl



In abundance of Na+, but absence of aspartate, the elevator domains stall
The DOWN state is only short lived

PNAS, 2017, 114(7):1584-1588; DOI: 10.1073/PNAS.1616413114

Bio-AFM-Lab

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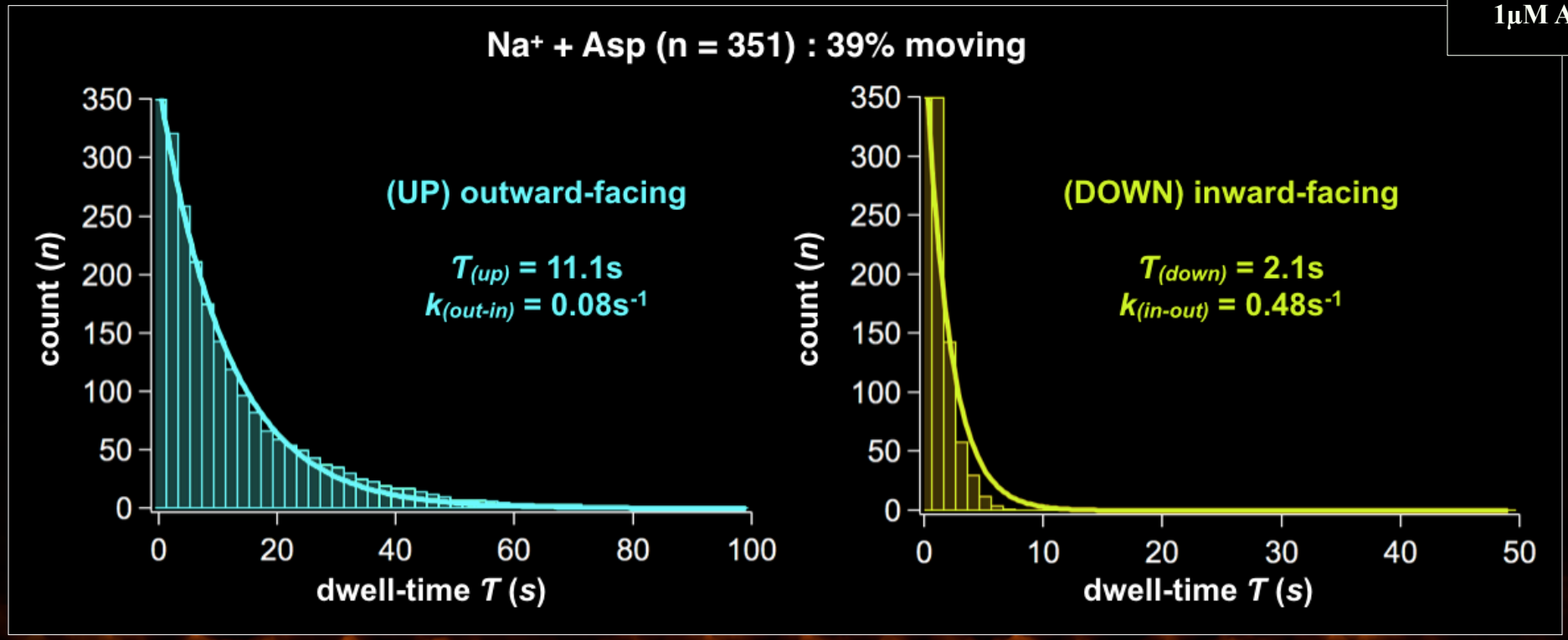
Simon Scheuring

Transport conditions: Na+ and Asp / Elevator domain motion

20mM Tris
pH 7.5
150mM NaCl
1µM Asp



In the presence of both subrates, the elevator domains move efficiently / slighly slower than in apo conditions
The UP state is favored over the DOWN state

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Bio-AFM-Lab

HS-AFM of GLTPH «Elevator» Movements

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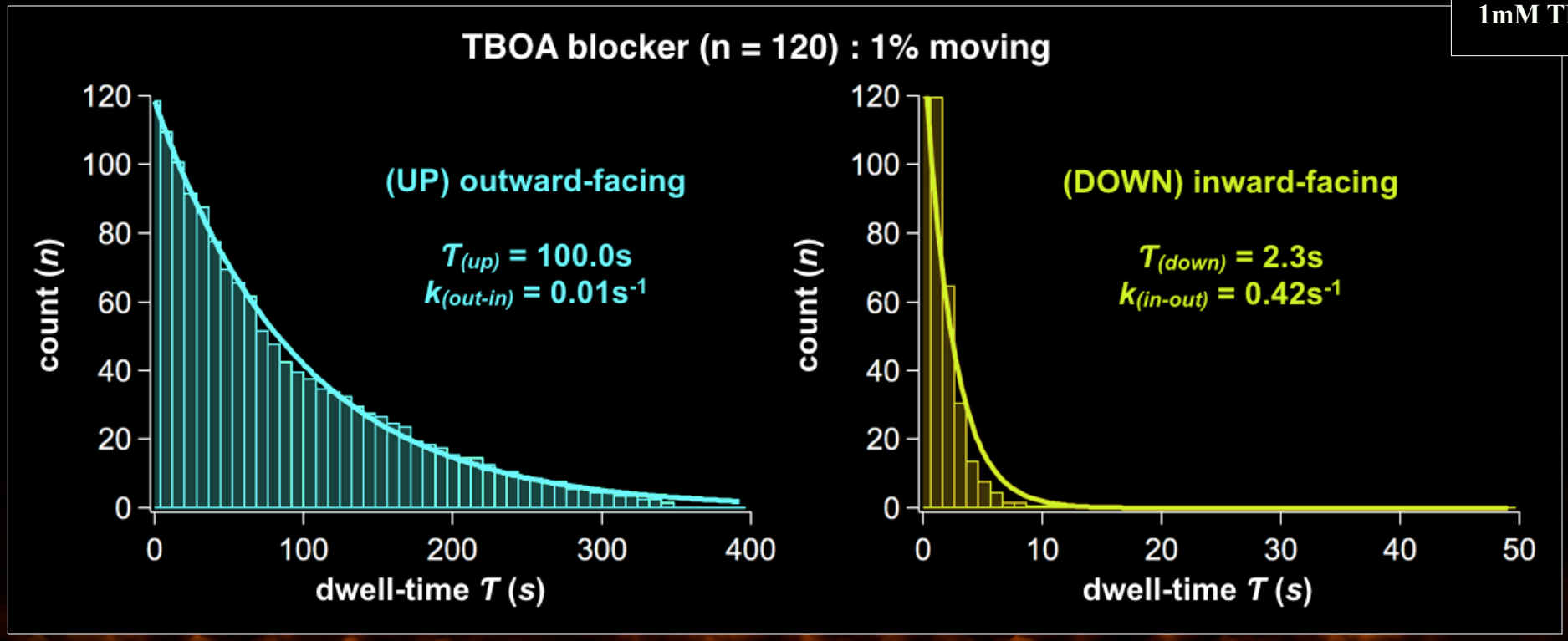
TBOA conditions: Block / Elevator domains stall

20mM Tris
pH 7.5
150mM NaCl
1mM TBOA



In abundance the presence of TBOA (DL-threo-beta-benzyloxyaspartate), the elevator domains stall
The DOWN state is only short lived

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Bio-AFM-Lab

HS-AFM of GLT_{PH} «Elevator» Movements

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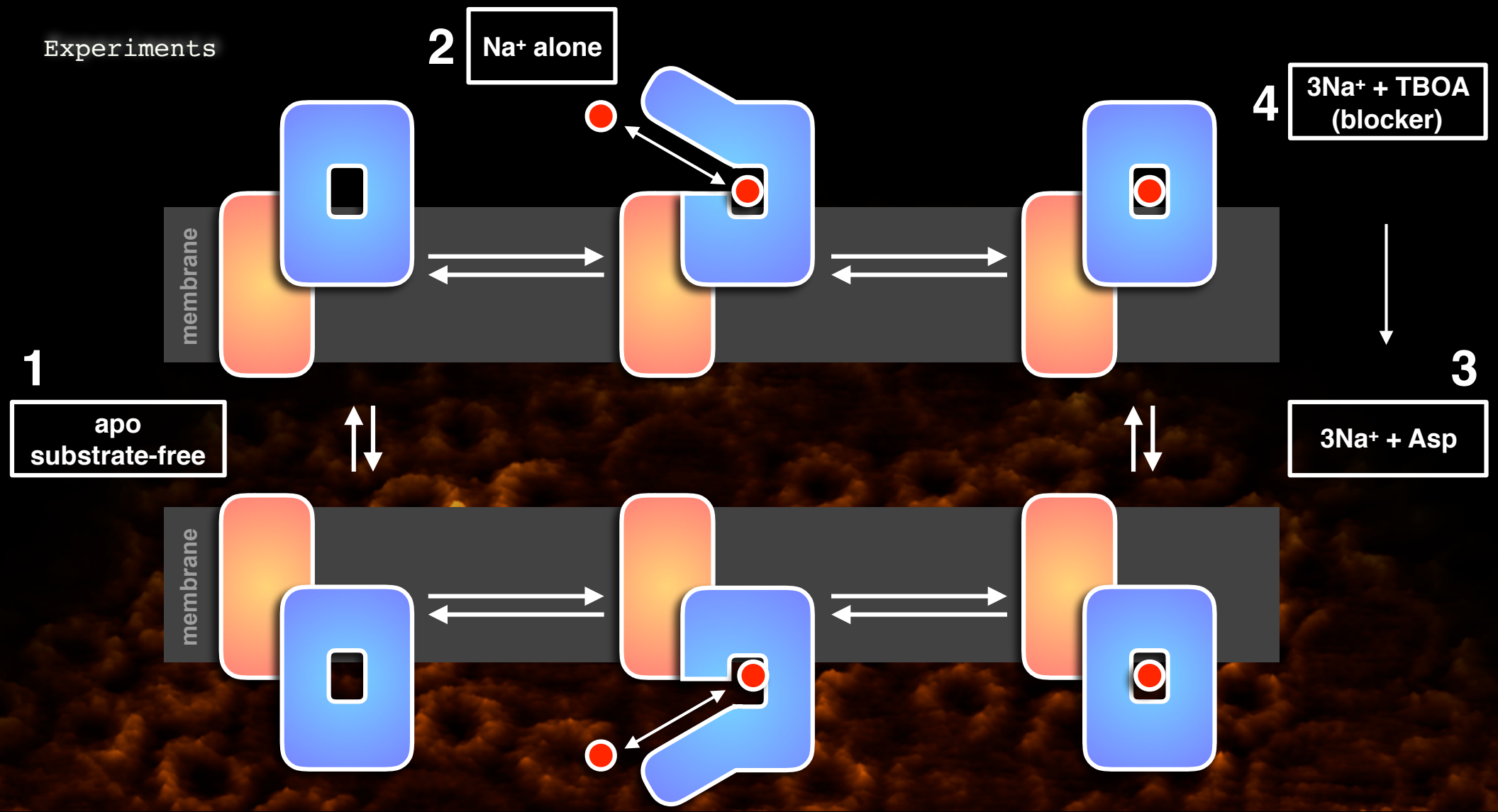

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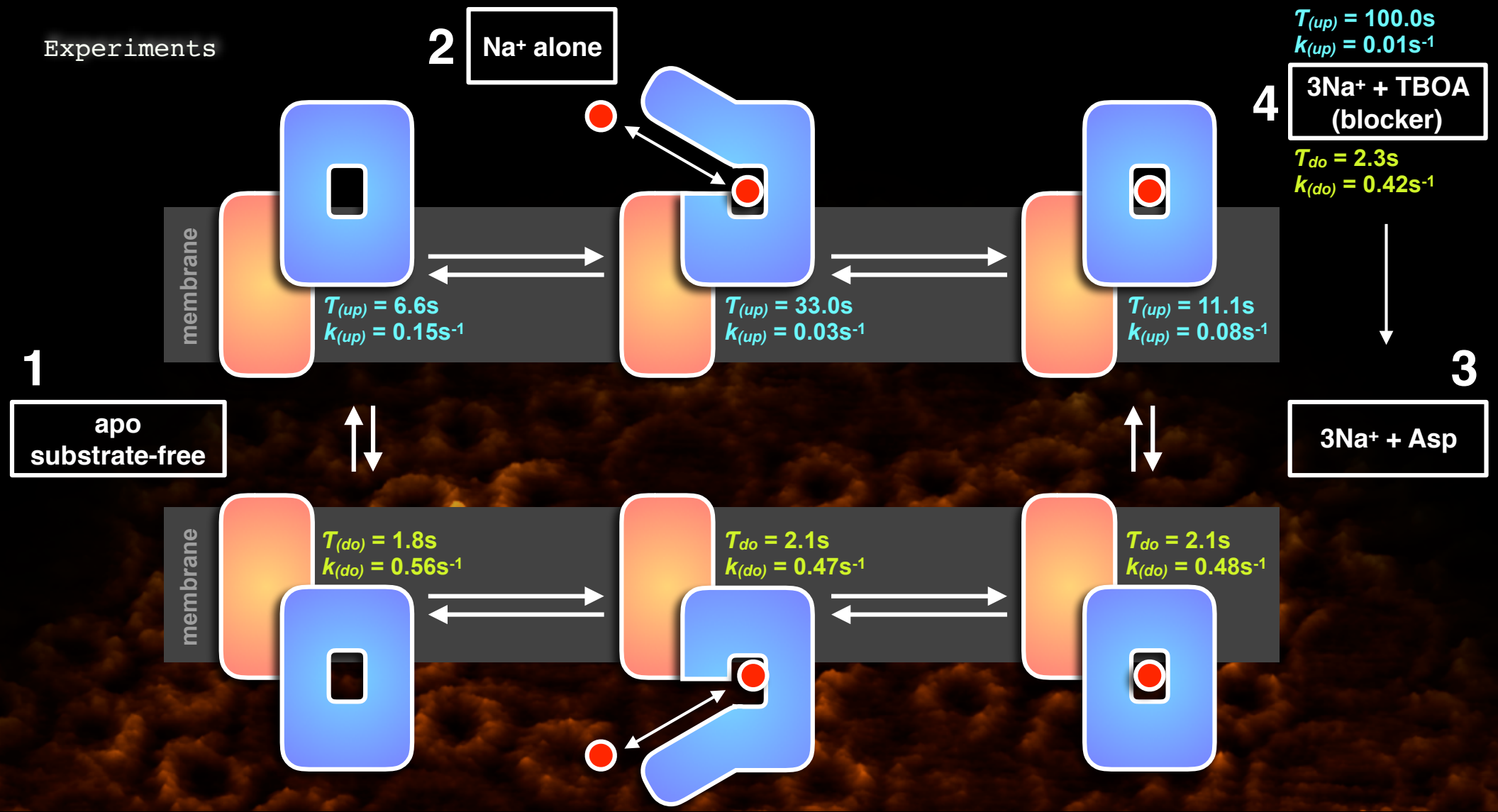

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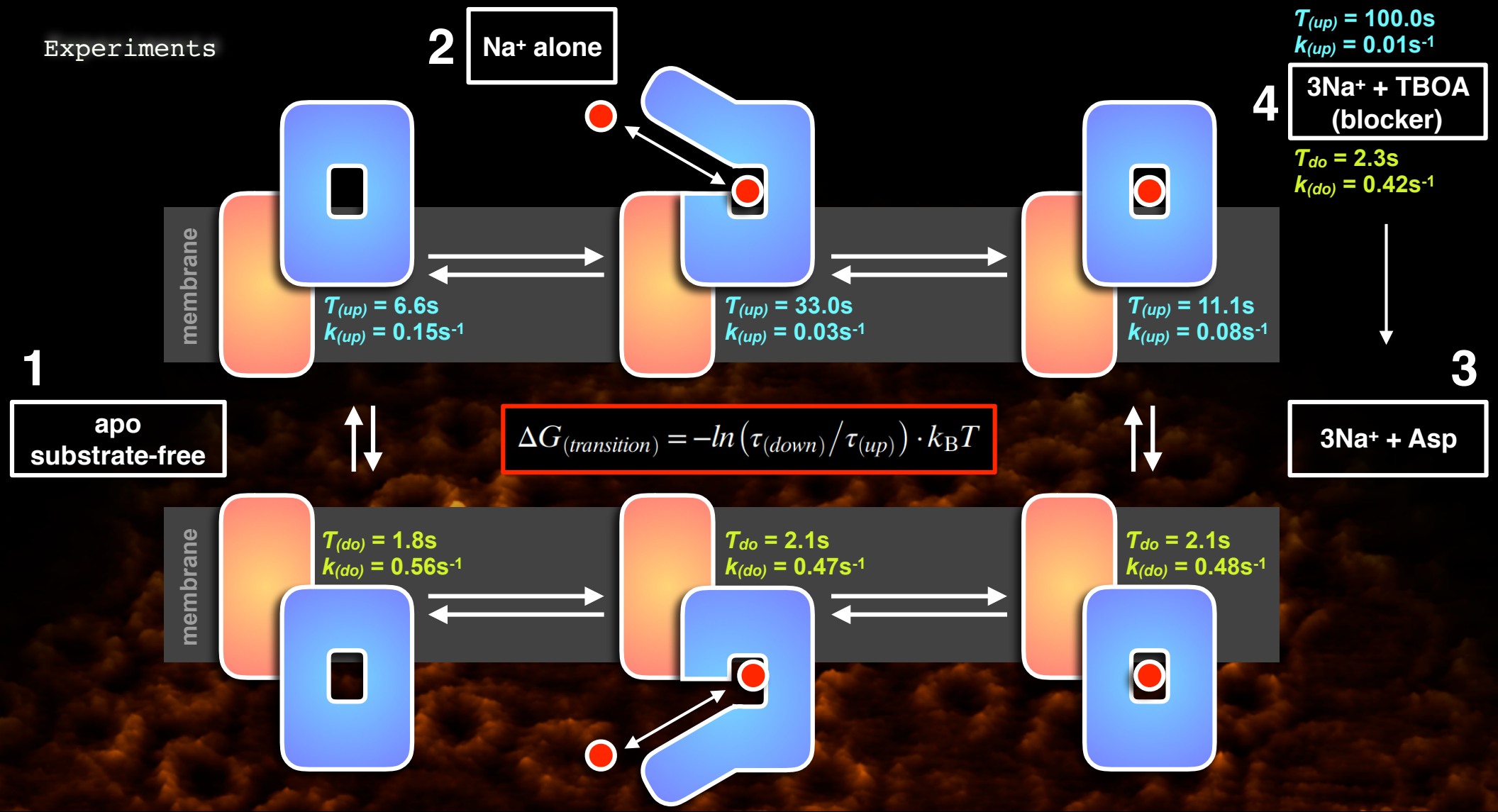

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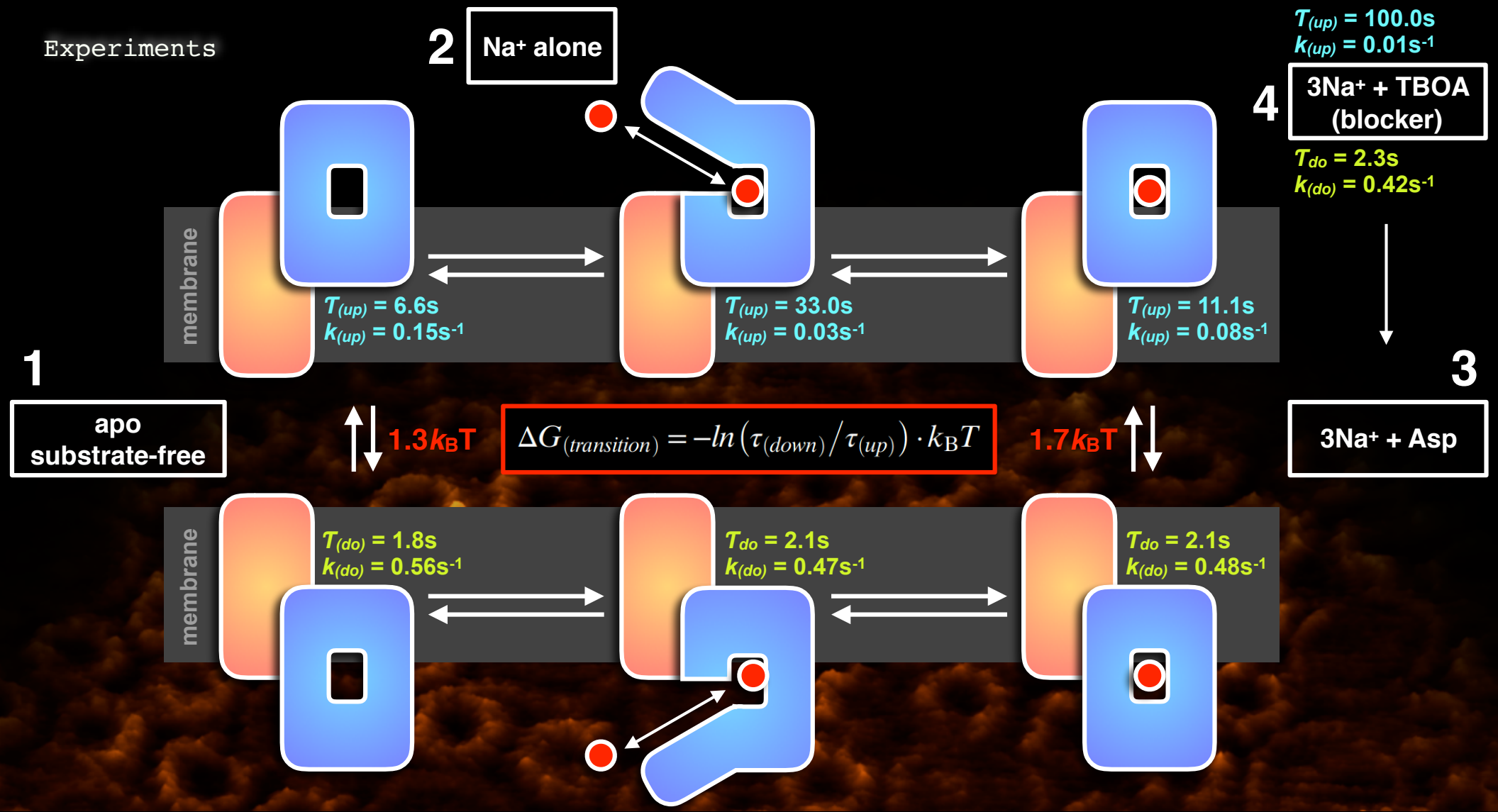

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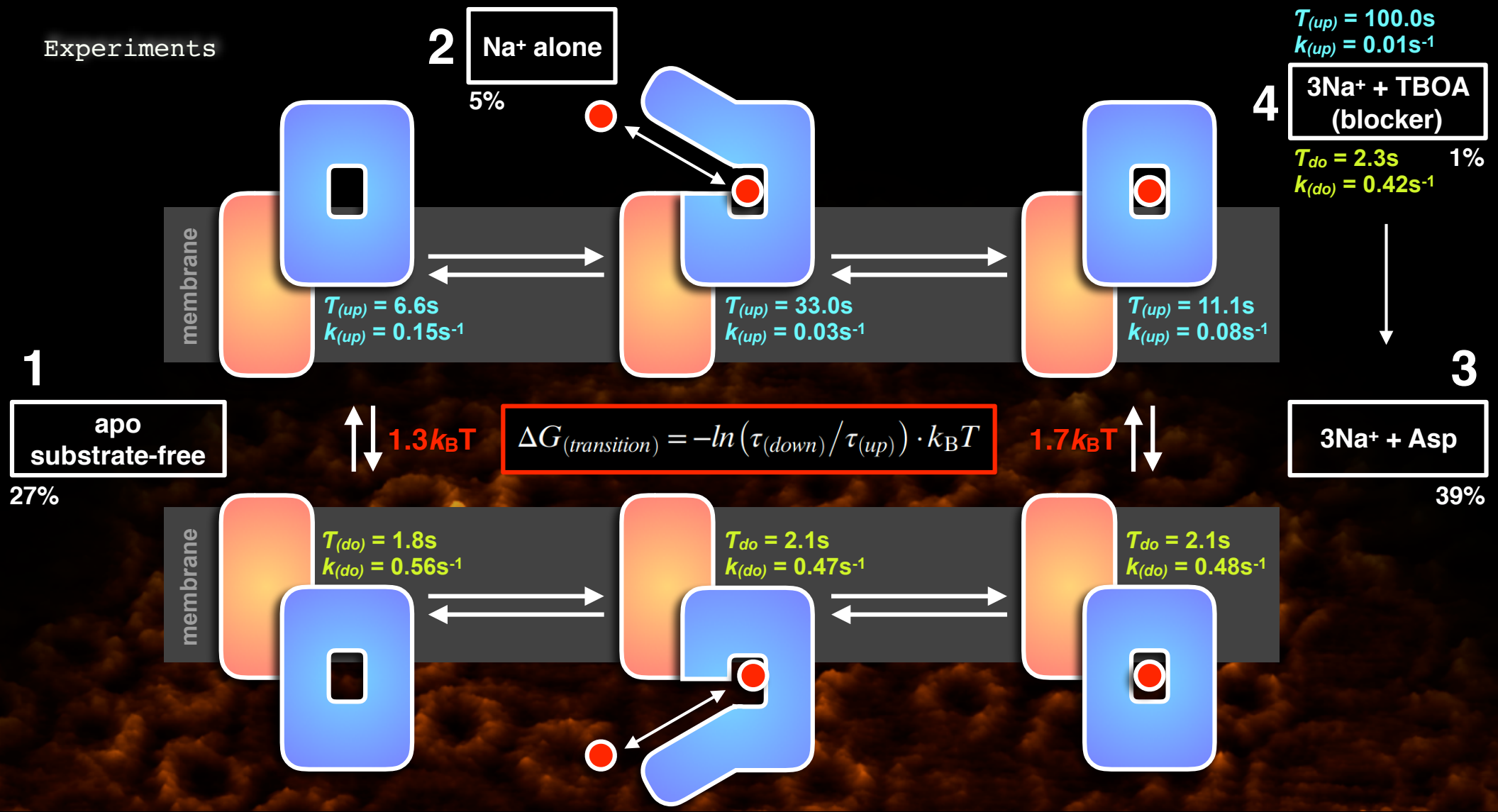

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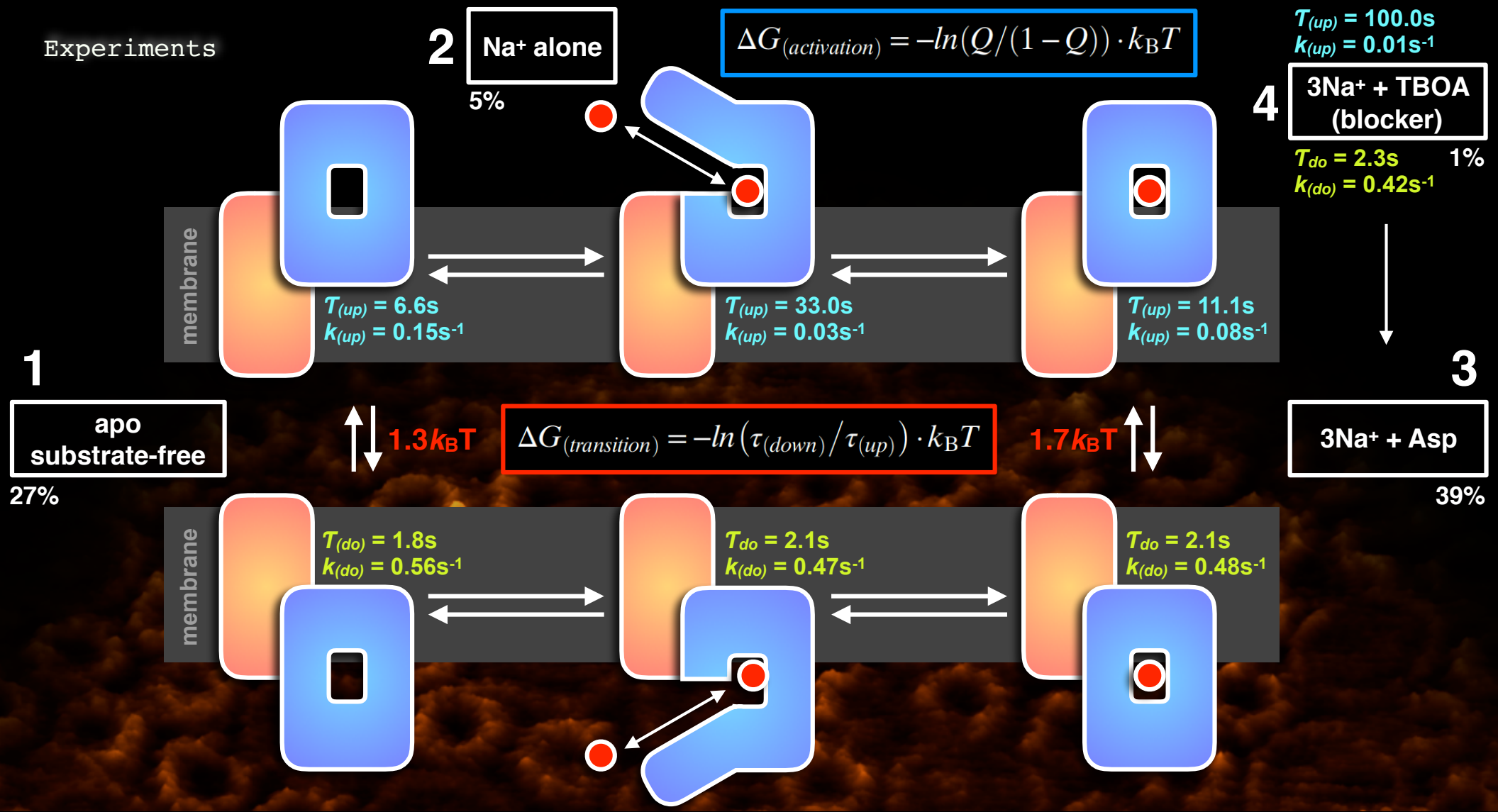

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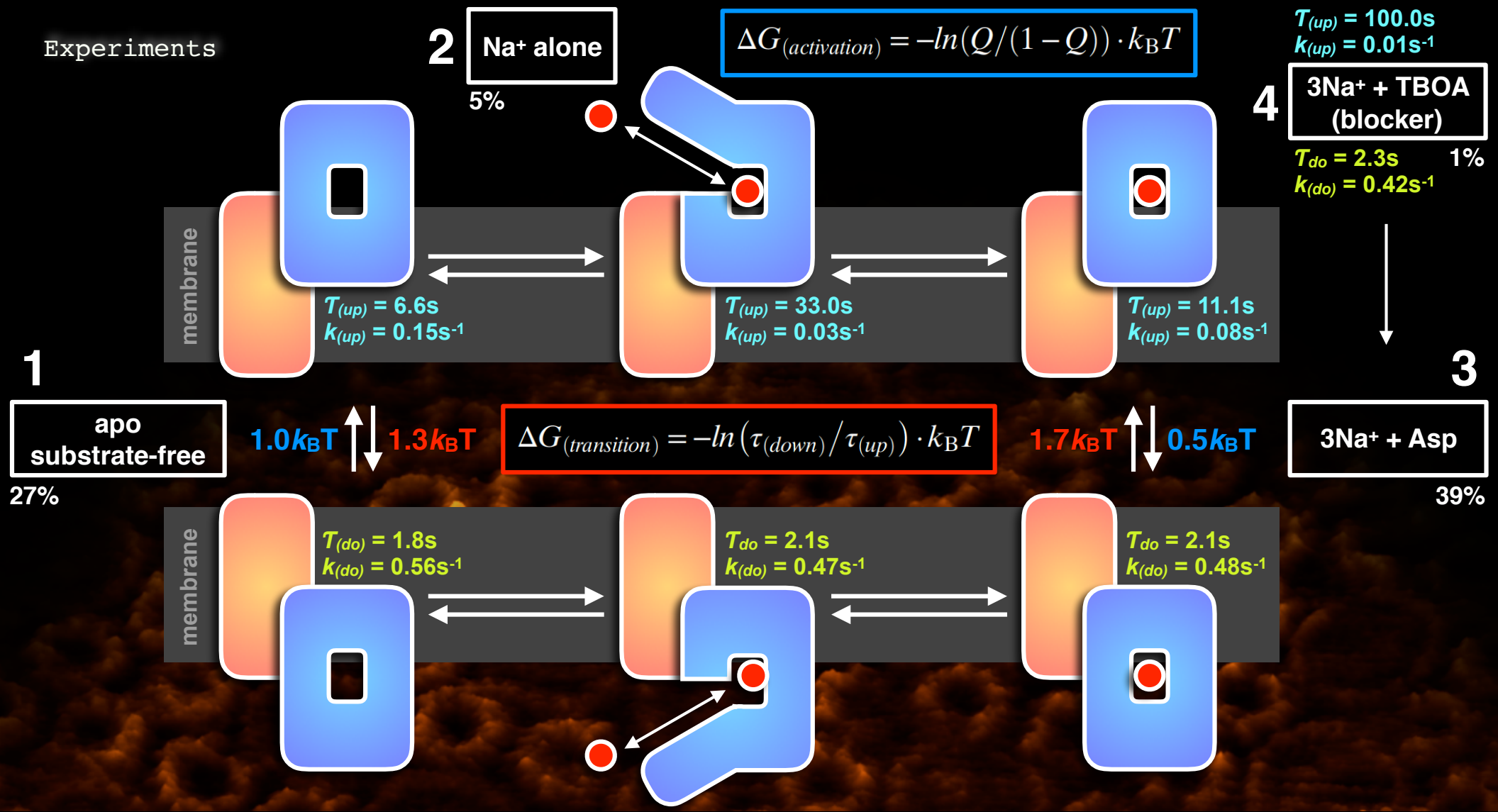

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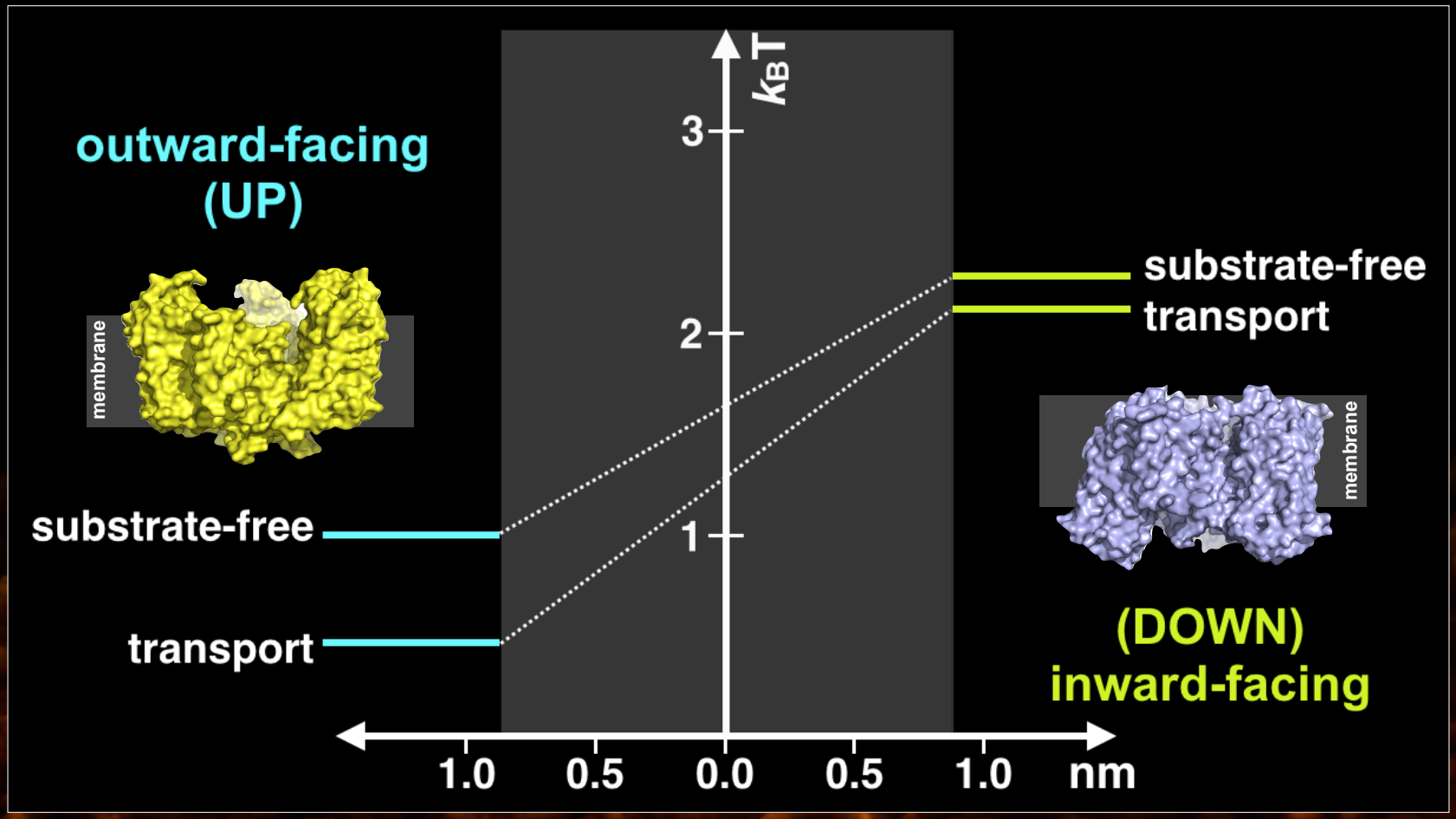
Glutamate transporters 'recycle' glutamate from the synaptic cleft



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HS-AFM of GLTPH «Elevator» Movements

Simon Scheuring

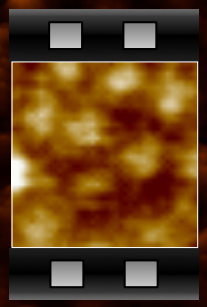
Complete non-cooperativity of transporter domain action

Single transport domain lifetimes and state probabilities

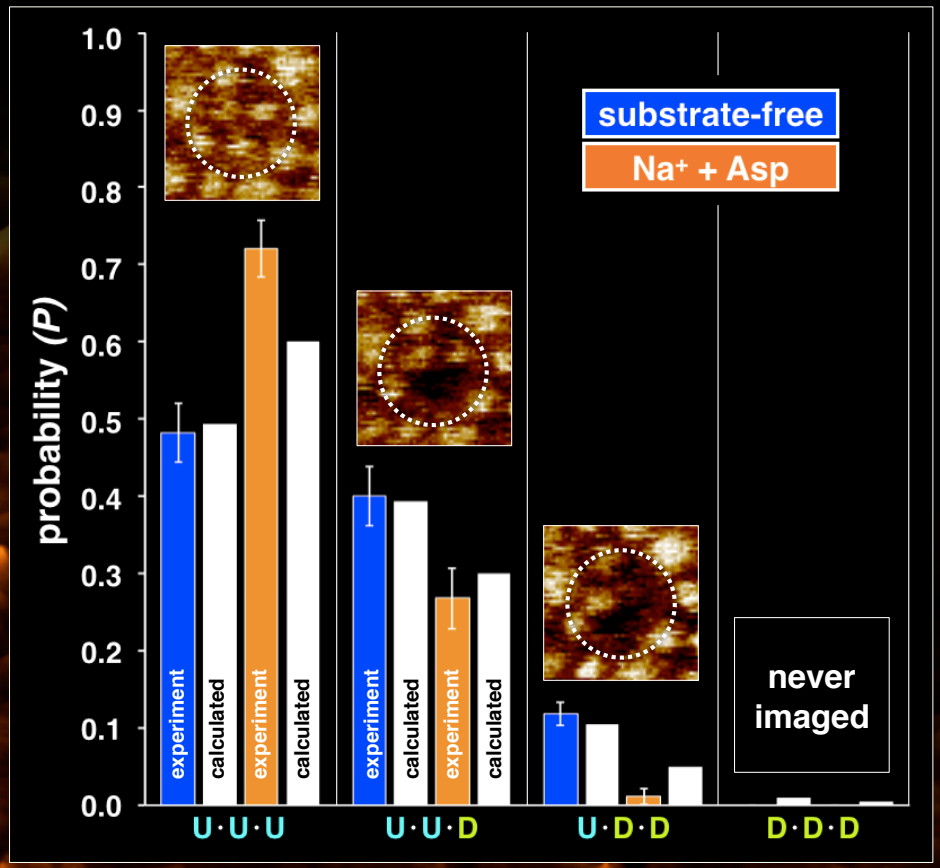
	lifetime outward-facing	lifetime inward-facing	probability outward-facing	probability inward-facing
substrate-free	$T_{(up)}$ = 6.6s	$T_{(do)}$ = 1.8s	P_U = 0.79	P_D = 0.21
Na^+ + Asp	$T_{(up)}$ = 11.1s	$T_{(do)}$ = 2.1s	P_U = 0.84	P_D = 0.16

Calculated probabilities for non-cooperative action

	UUU	UUD	UDD	DDD
substrate-free	0.49	0.39	0.12	0.01
Na^+ + Asp	0.60	0.34	0.05	0.004



Single molecule HS-AFM movie



Experimental data vs non-cooperative model

PNAS, 2017, 114(7):1584-1588; DOI: 10.1073/PNAS.1616413114

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HS-AFM of GLTPH «Elevator» Movements

Simon Scheuring

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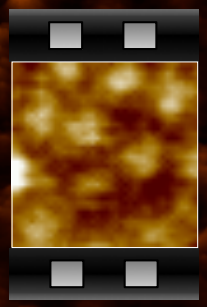

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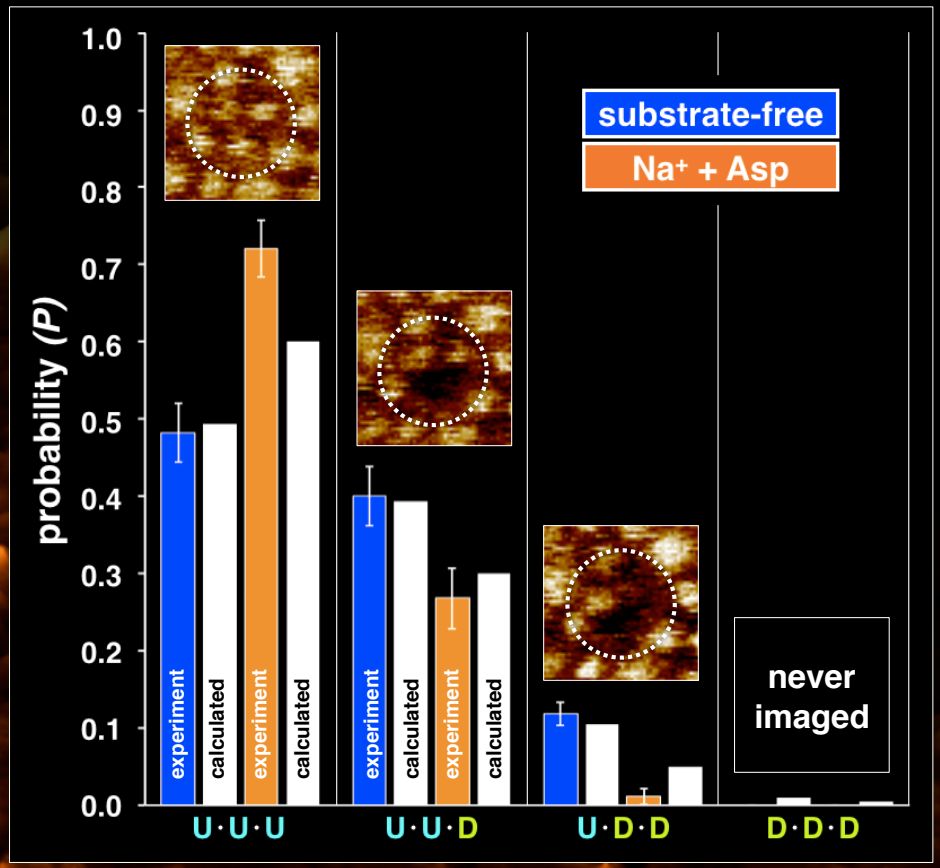

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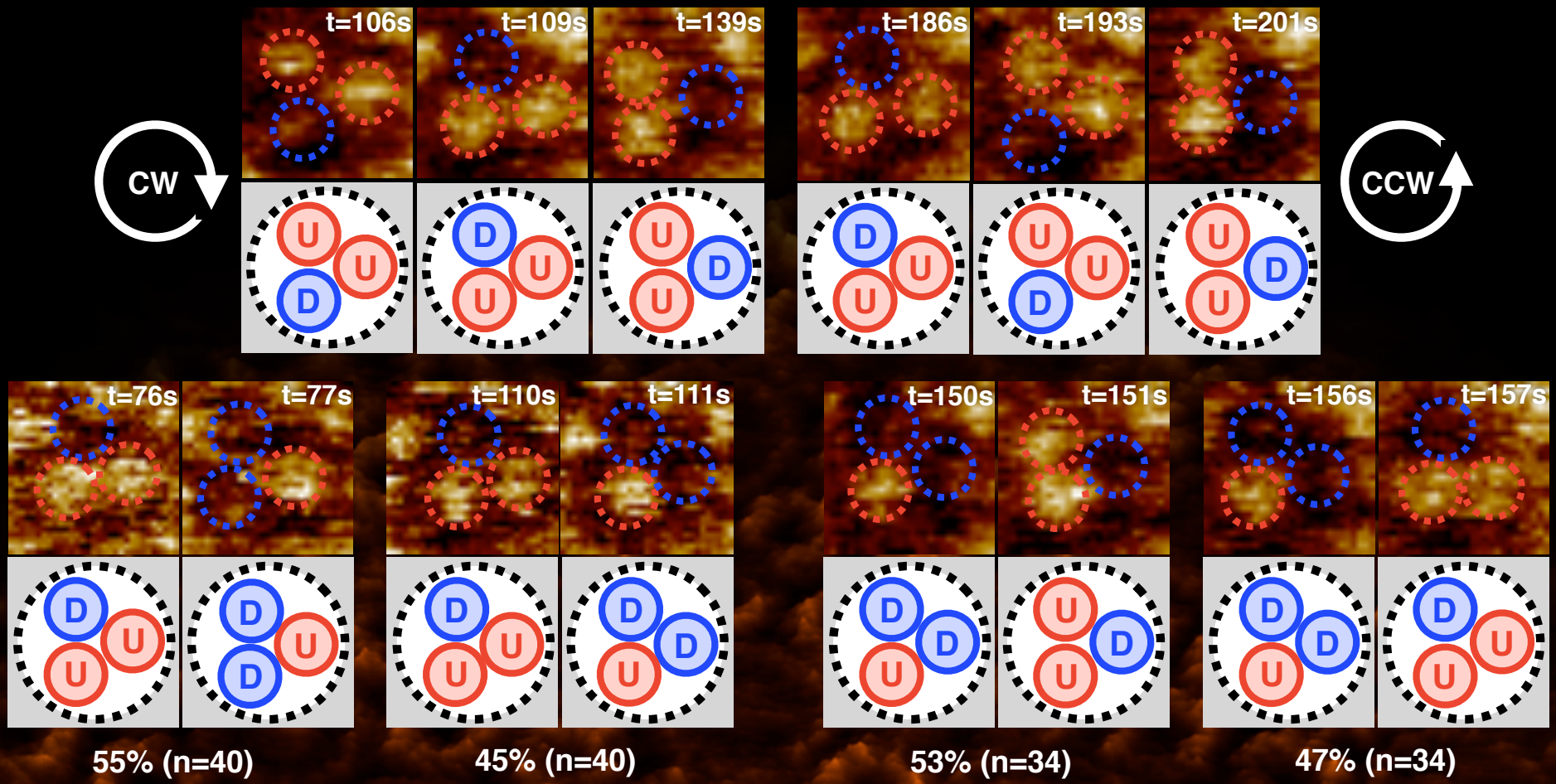

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Weill Cornell Medicine

Thank You !

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