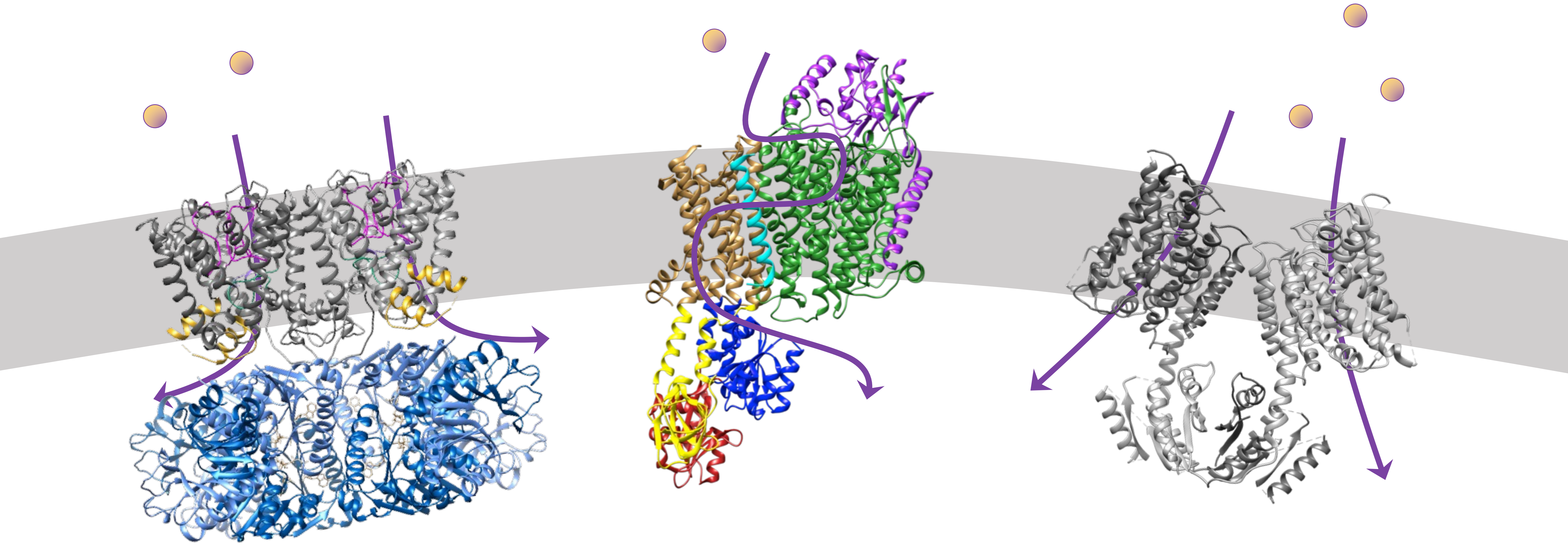


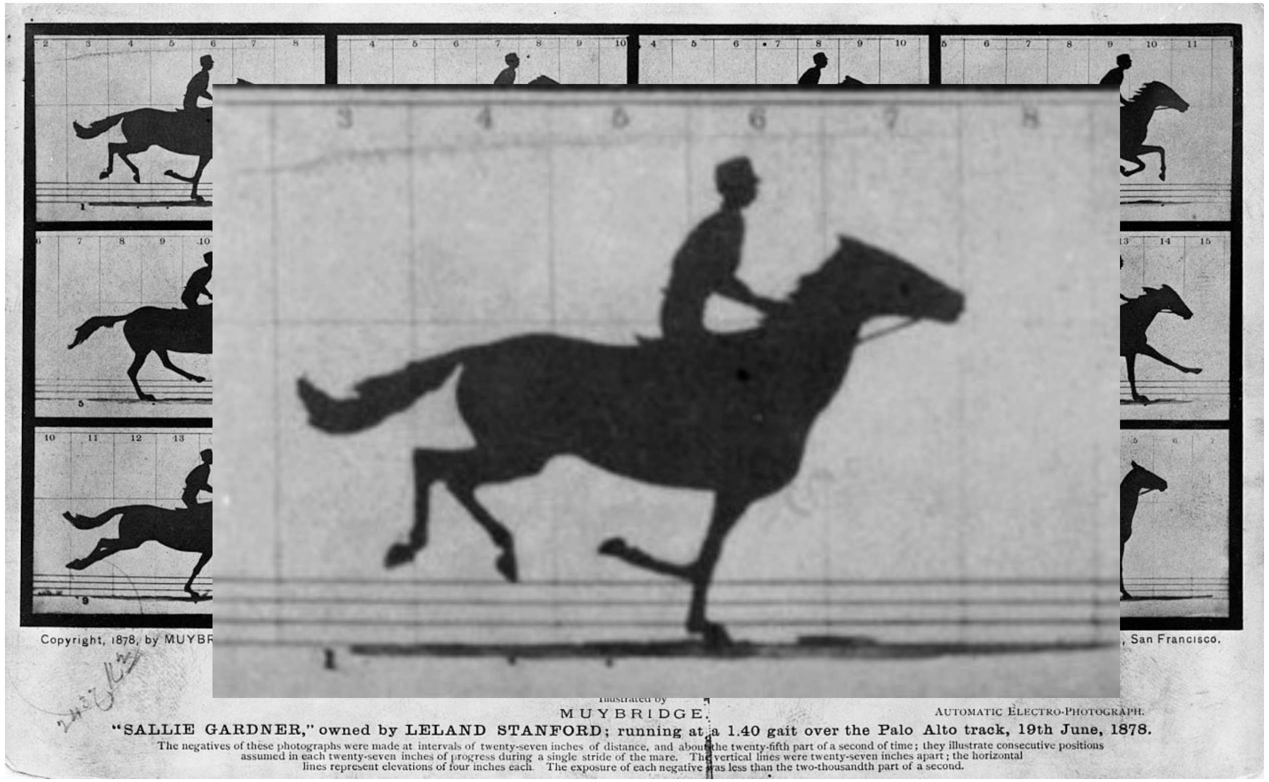
Integrative Structural Biology for Studying Conformational Dynamics of Membrane Transport Proteins

Inga Hänelt

Goethe University Frankfurt

Institute of Biochemistry

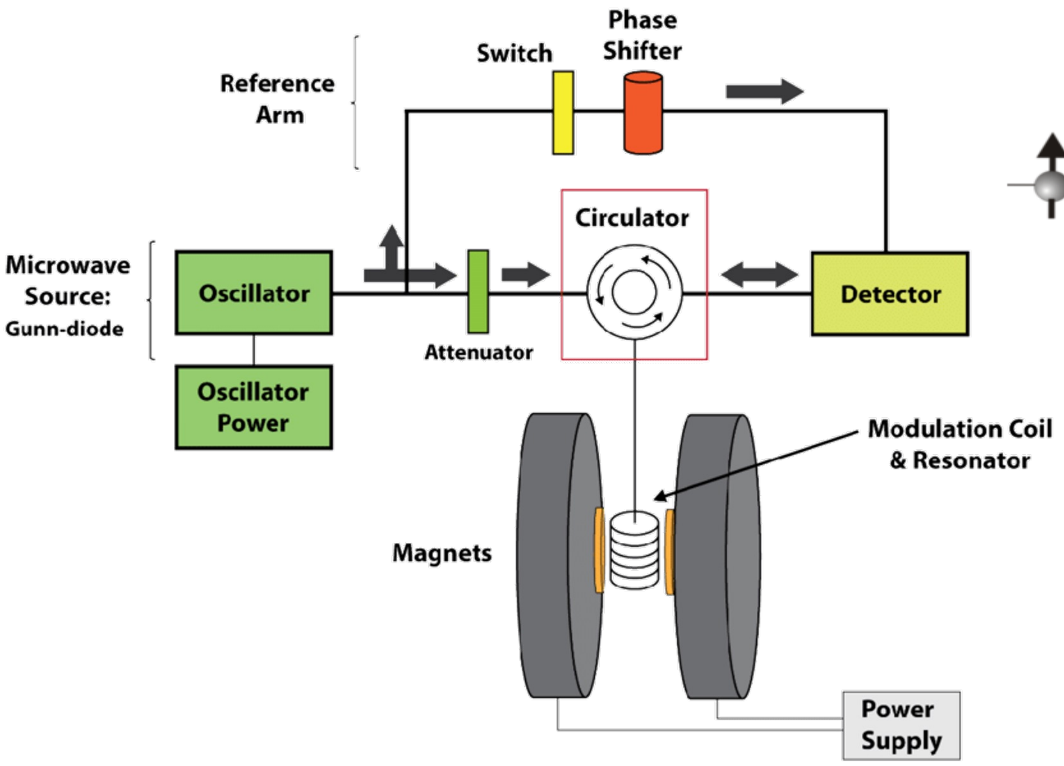
- Single particle cryo-EM
- X-ray crystallography
- Cryo-electron tomography

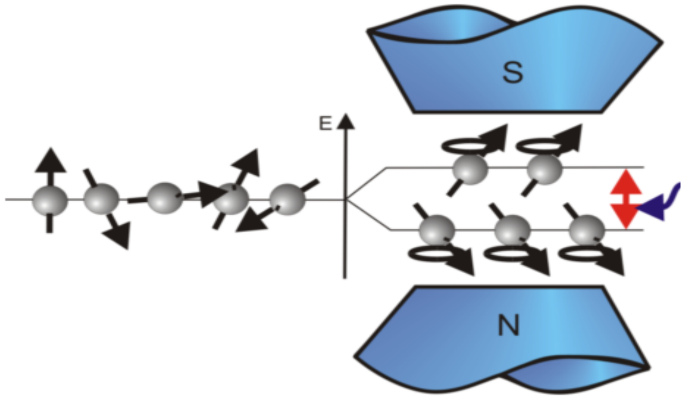


- Single molecule FRET
- hsAFM
- MD simulations
- **Electron paramagnetic resonance spectroscopy (EPR)**

Coupling of the magnetic moment of an unpaired electron with the external magnetic field B_0 => Zeeman effect

$$\Delta E = h\nu = g_e \mu_B B_0$$





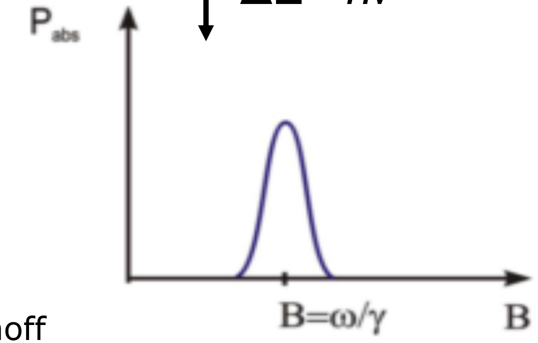
m_s= +1/2

ΔE

m_s= -1/2

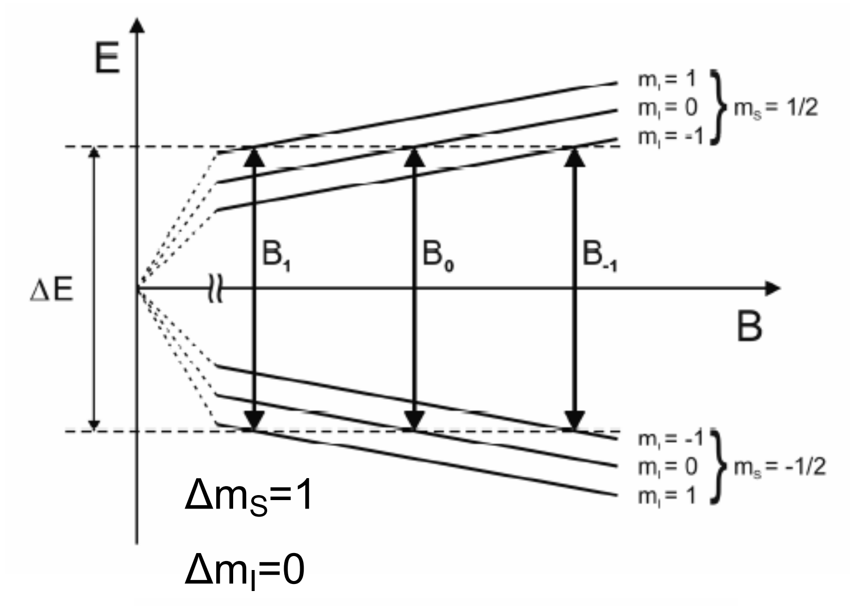
Coupling leads to a splitting of the energy level with ΔE according to the spin orientations

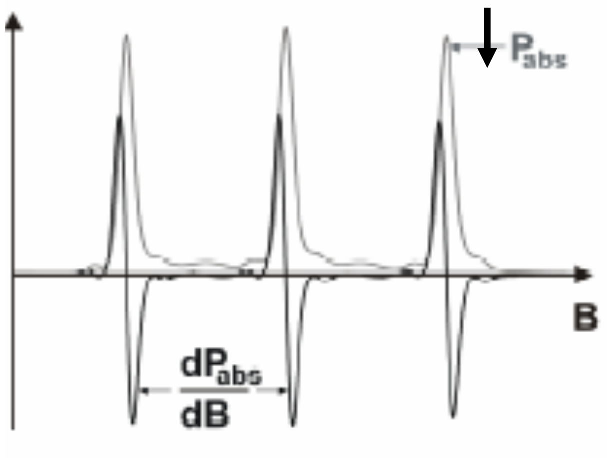
ΔE= *h*ν



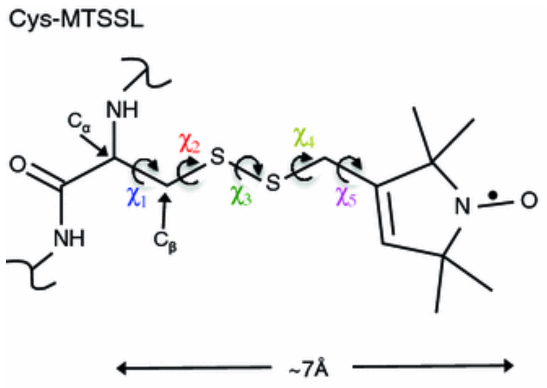
Inducing of transition if the resonance condition ΔE= hν is fulfilled

H.-J. Steinhoff





Diss. C. Beier, 2008



Mostly, MTSSL is attached to site-specific cysteines via disulfide bond

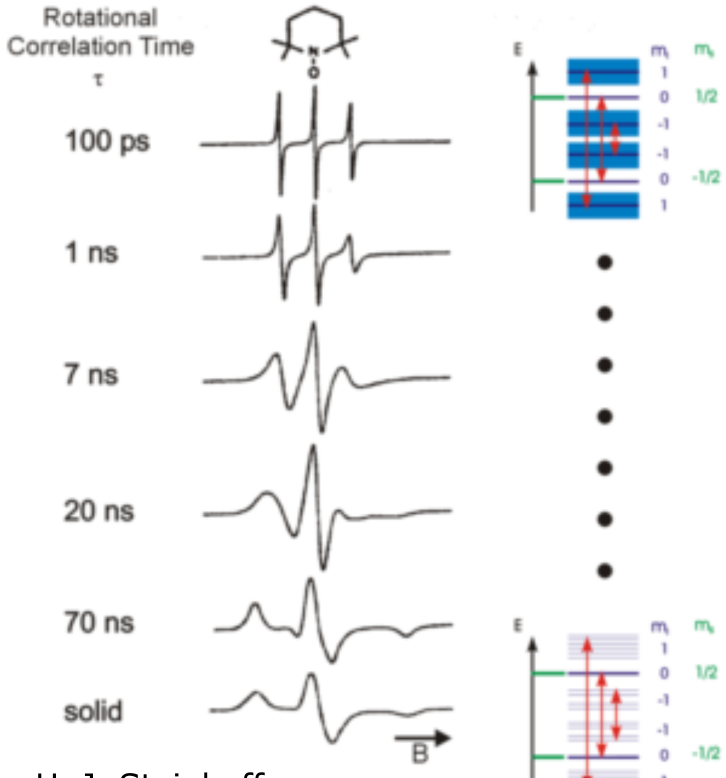
Hyperfine-term: Coupling of the magnetic moment of the electron with the magnetic moment of the nucleus

Nuclear spin I induces a magnetic moment as well

↓

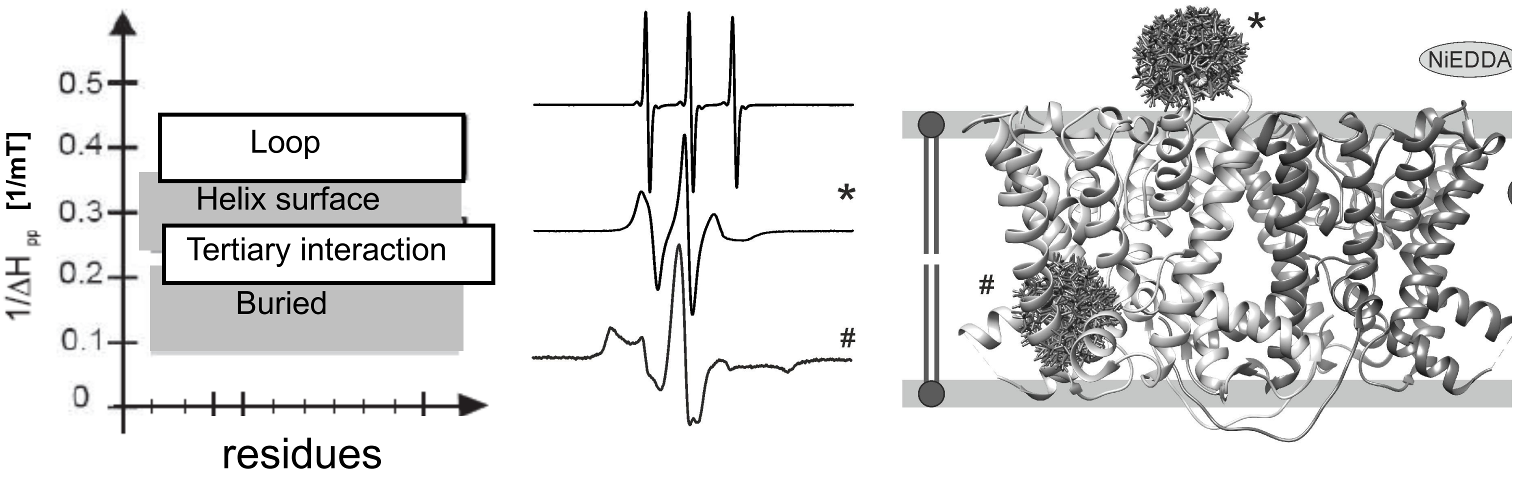
Coupling of the magnetic moments of the electron and nucleus lead to (2I+1) hyperfine splitting (here ^{14}N: I=1)

CWEPR: ROTATION CORRELATION TIME AND RESULTING SPECTRA

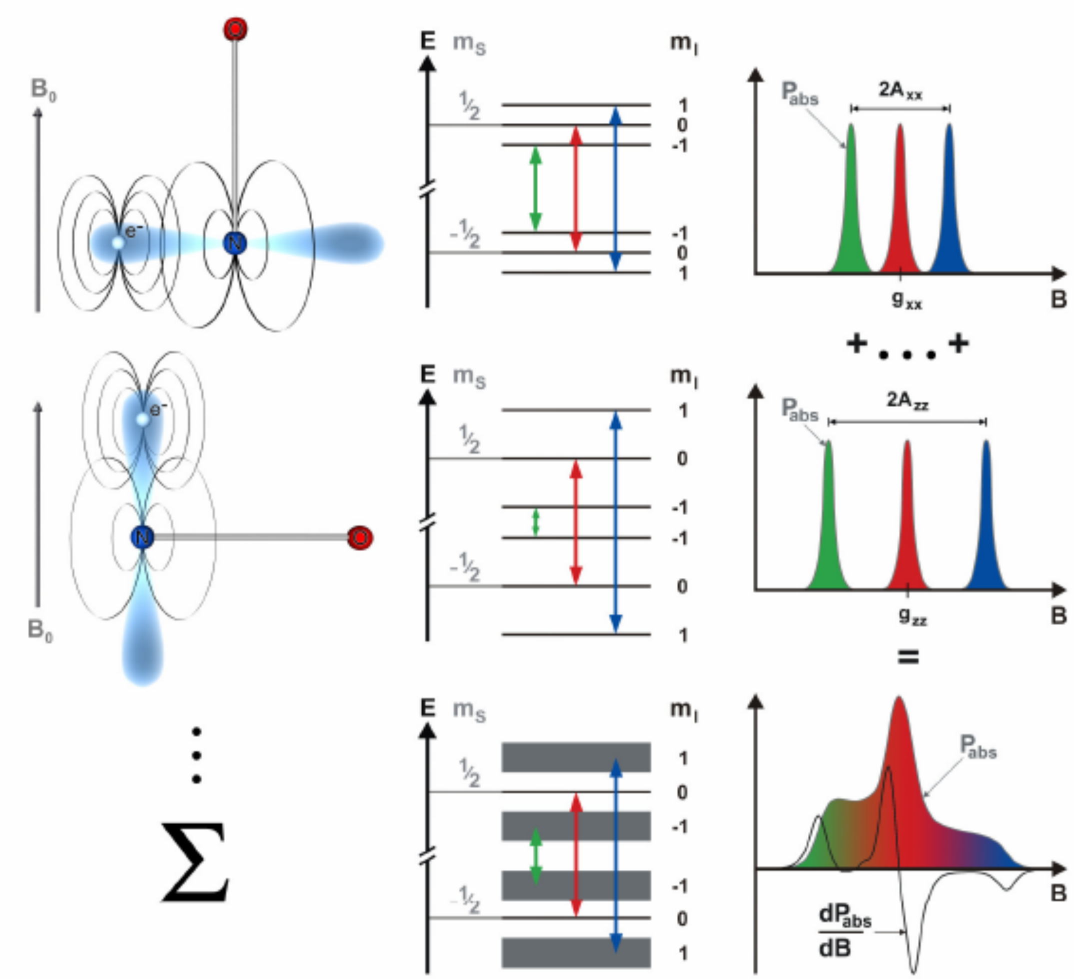


H.-J. Steinhoff

- The higher the viscosity of a liquid or the stronger the interactions with the micro-environment the stronger the spectral broadening
- ΔH_{pp}^{-1}: the inverse of the central resonance line as mobility parameter - the lower ΔH_{pp}^{-1} the lower the mobility

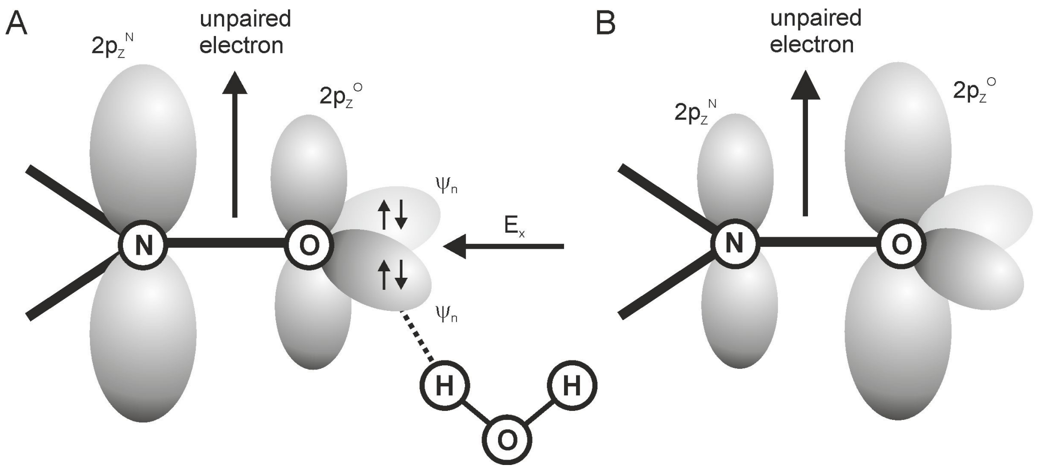


Wunnicke & Hänelt Crystals 2017

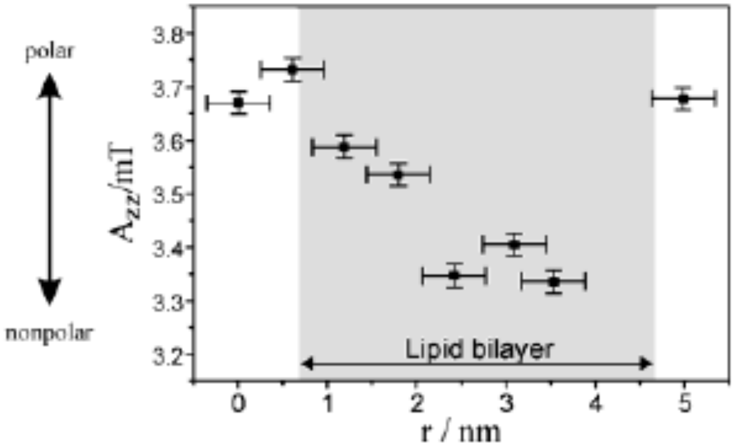


Diss. C. Beier, 2008

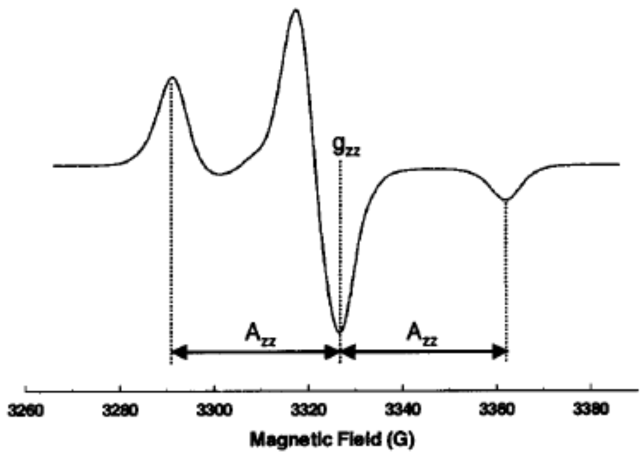
- Depending on the orientation of the p-orbital to the magnetic field the hyperfine splitting differs
- Addition of all possible spectra results in a powder spectrum
- Spectra always recorded as first derivative



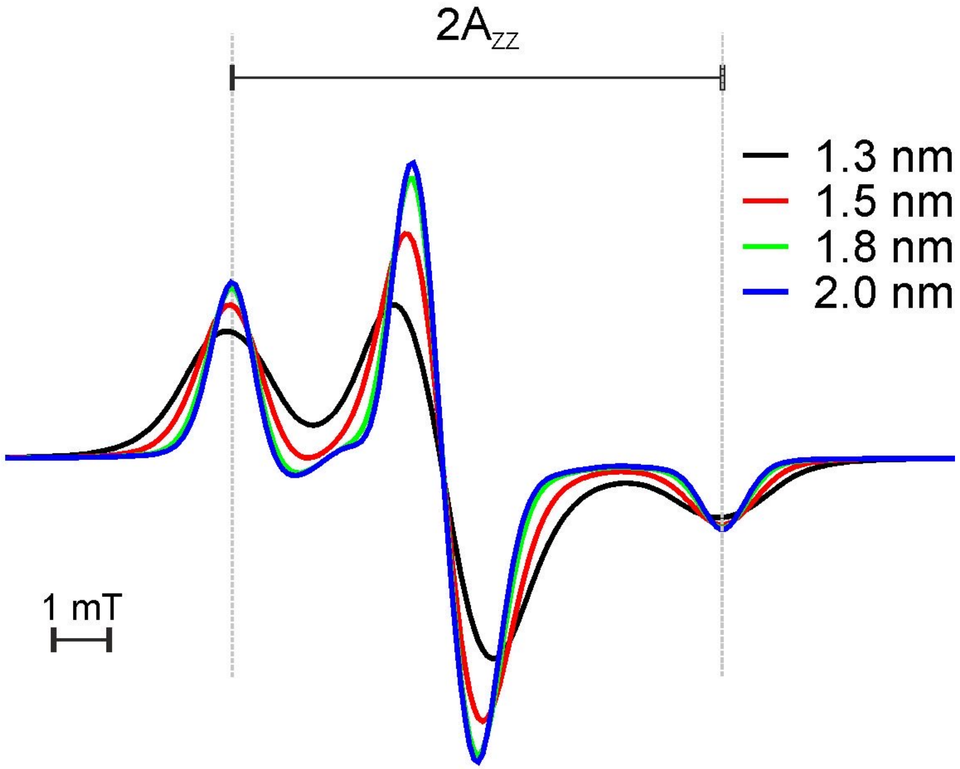
- The hyperfine splitting A_{ZZ} reflects the polarity of the environment: High water accessibility, π-electron density shifted towards the N-atom, strong hyperfine coupling, high A_{ZZ}
- $2A_{zz}$ is the distance between the outer extrema in a powder spectrum



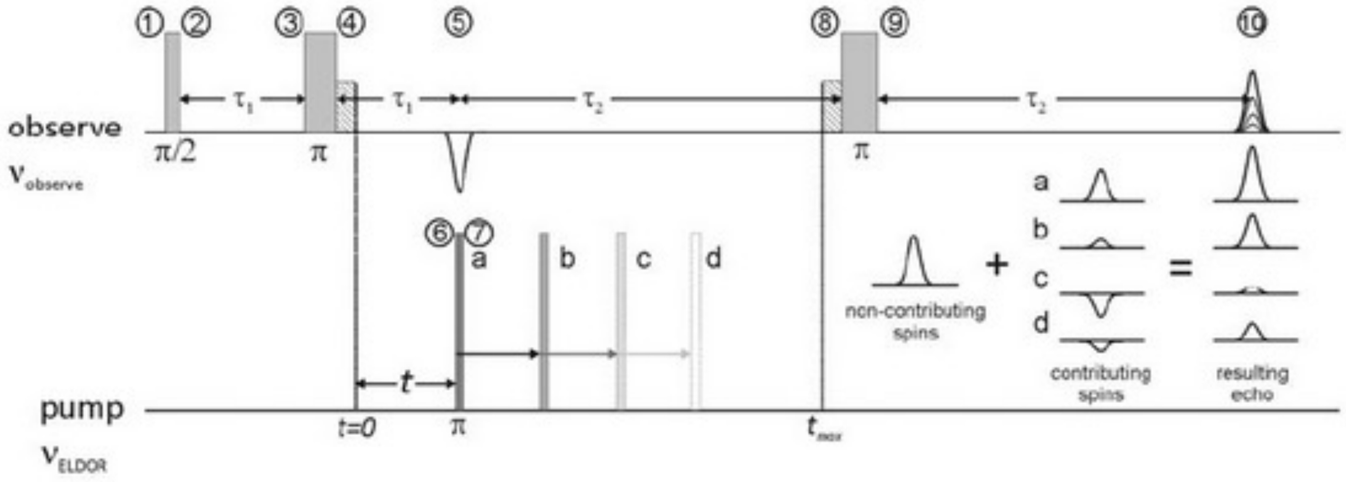
Polarity profile of helix F of bacteriorhodopsin, Savitsky et al. 2004

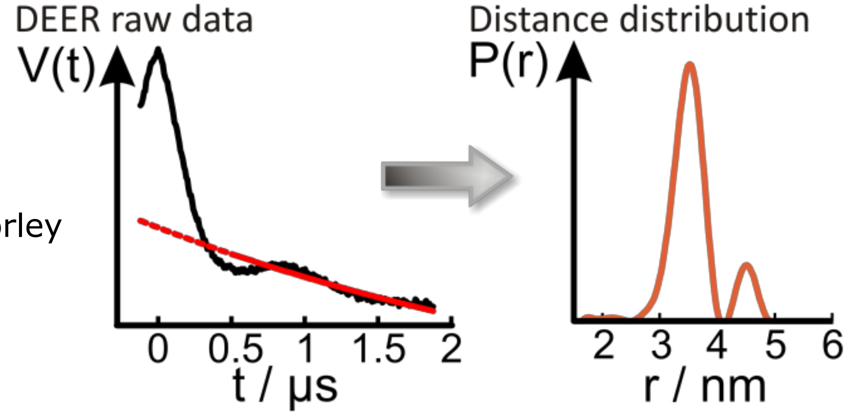


Owenius et al. J Phys Chem 2001

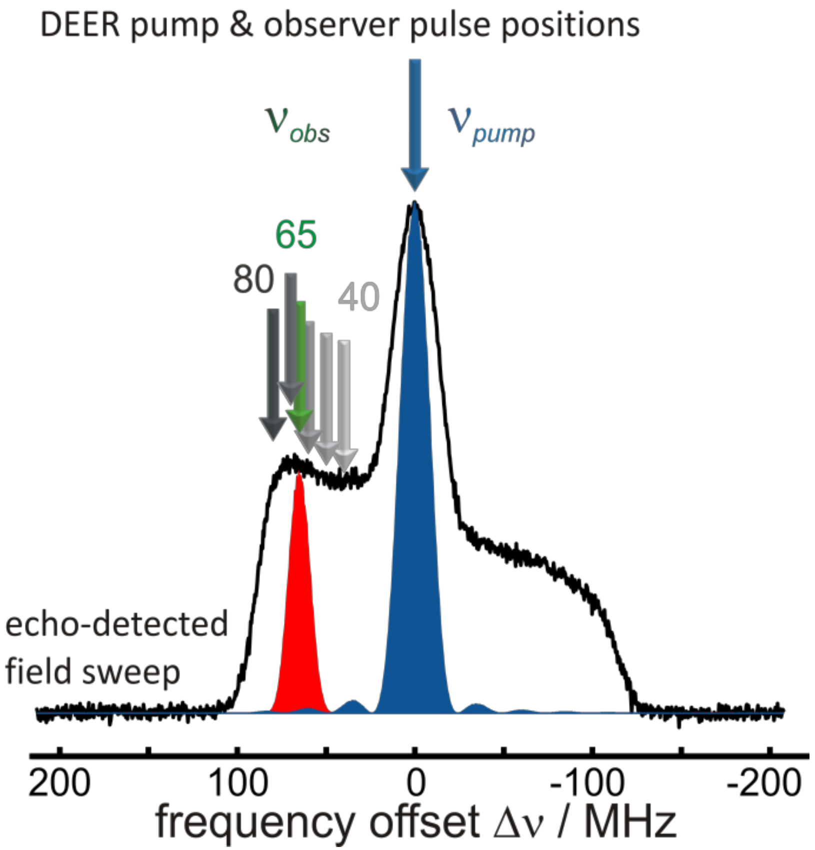


- cw EPR at 160 K: Distances between 0.8 and 1.8 nm can be determined based on dipolar broadened EPR spectra
- Below 0.8 nm: Heisenberg interactions, difficult to fit
- Pulsed EPR: Distances between 1.5 and 8 nm



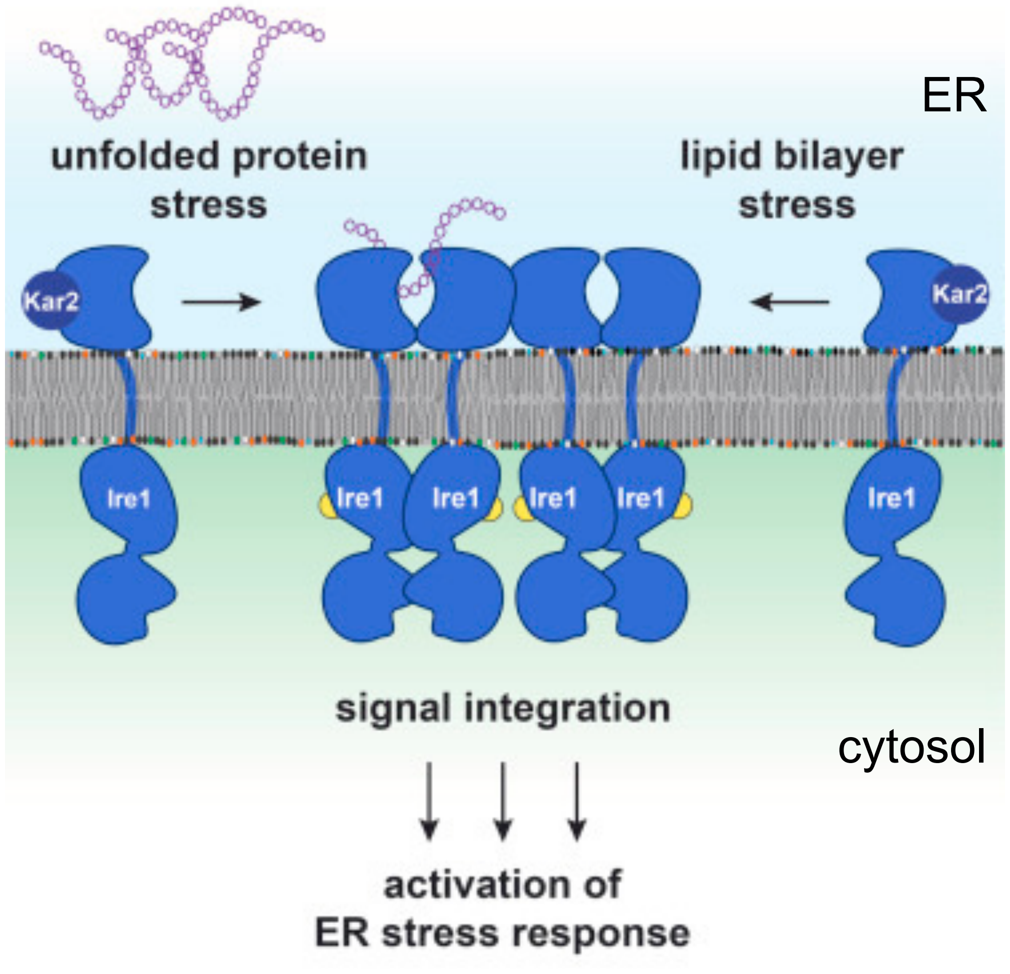


By Gavin W Morley



Klose, 2014

- ‘DEER Analysis’ for Tikhonov regularization and fitting
- ‘MMMx’ for Rotamer Library analysis and distance prediction



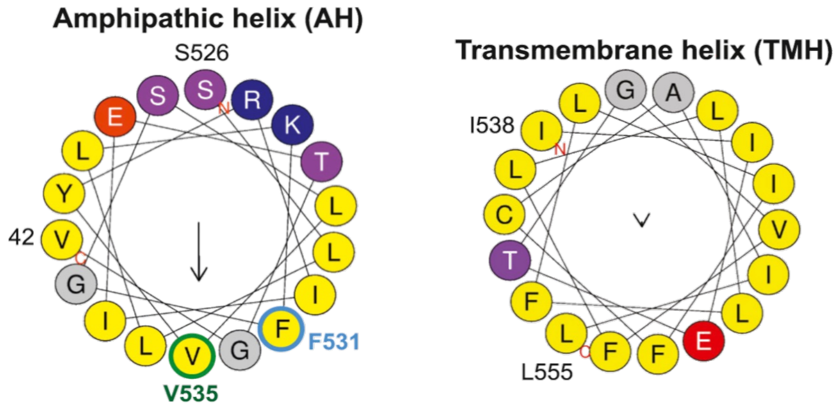
Halbleib et al. Mol Cell 2017

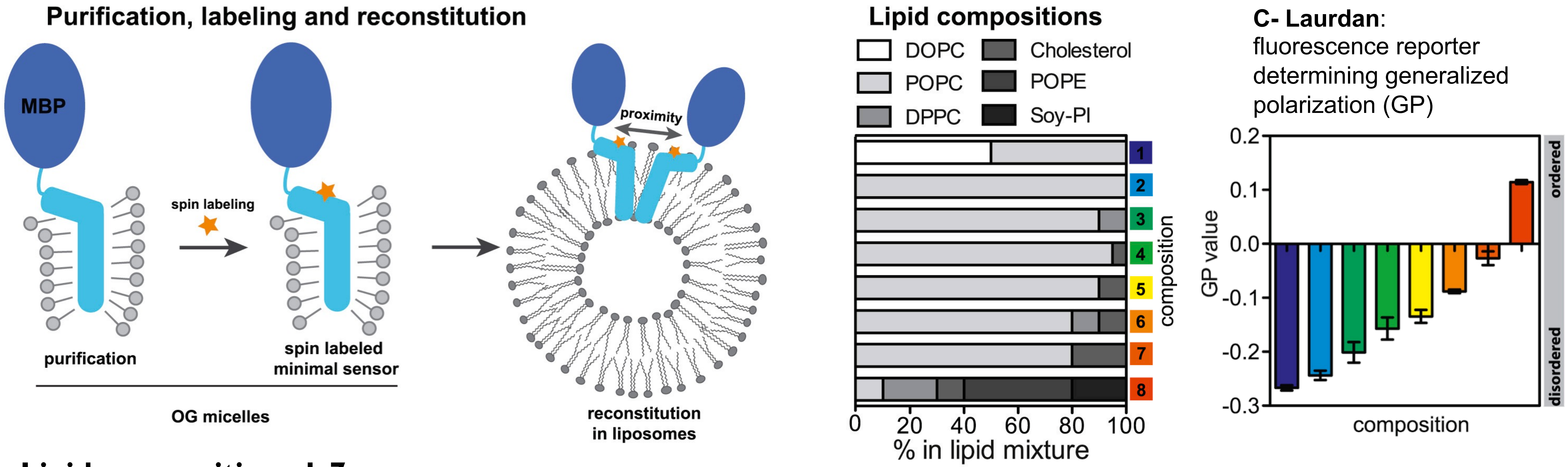
- Unfolded protein response (UPR) is a conserved homeostatic program activated by misfolded proteins in the lumen of the endoplasmic reticulum (ER)
- Aberrant lipid compositions of the ER membrane, referred to as lipid bilayer stress, equally potent in activating the UPR
- Physiological studies revealed impaired UPR in the presence of mutations F531R or V535R disrupting the amphipathic helix

transmembrane helix (TMH)

$_{521}$EKNQNSLLLKFGSLVYRIIETGVFLLLFLIFCAILQRFK$_{559}$

amphipathic helix (AH)





Lipid compositions 1-7:

PC-based for minimal complexity in the lipid headgroup region, differed only in their cholesterol content and the proportion of saturated lipid acyl chains

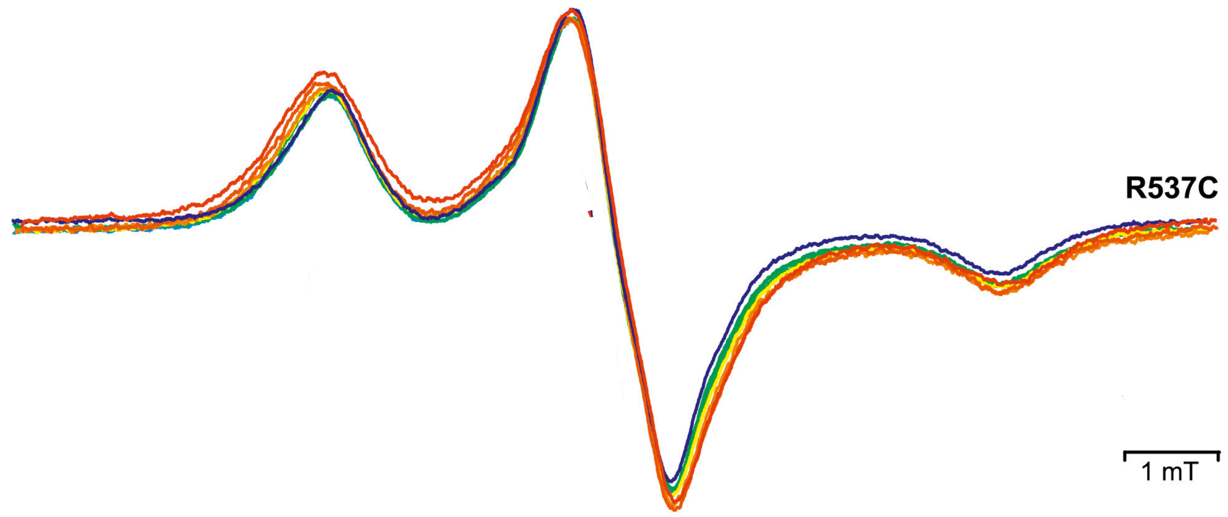
Lipid composition 8:

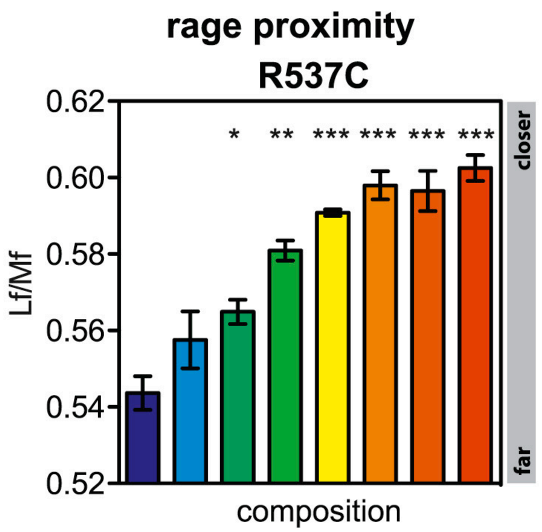
Increased degree of lipid saturation, increased sterol level, and increased PE:PC ratio mimicking lipid bilayer stress

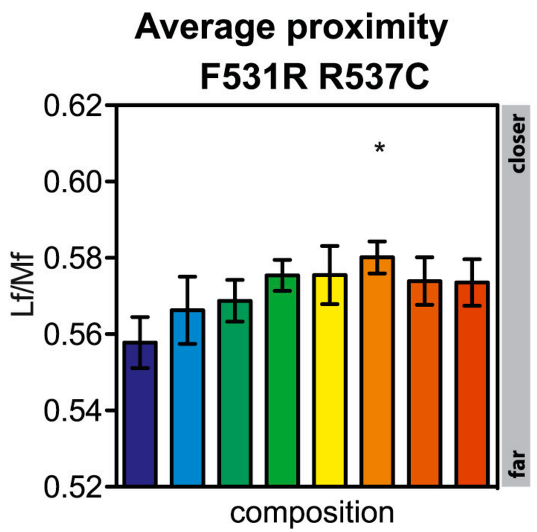
Halbleib et al. Mol Cell 2017

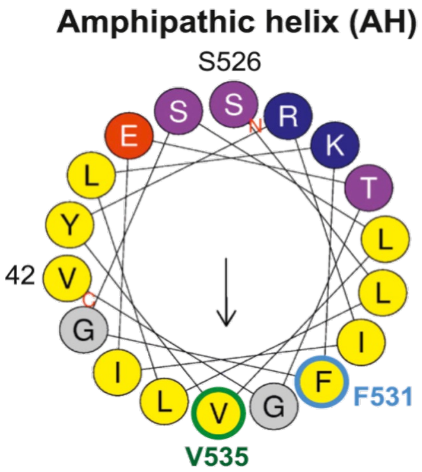
The more ordered the lipid composition, the more the TMHs interact

cw EPR spectra at -115°C



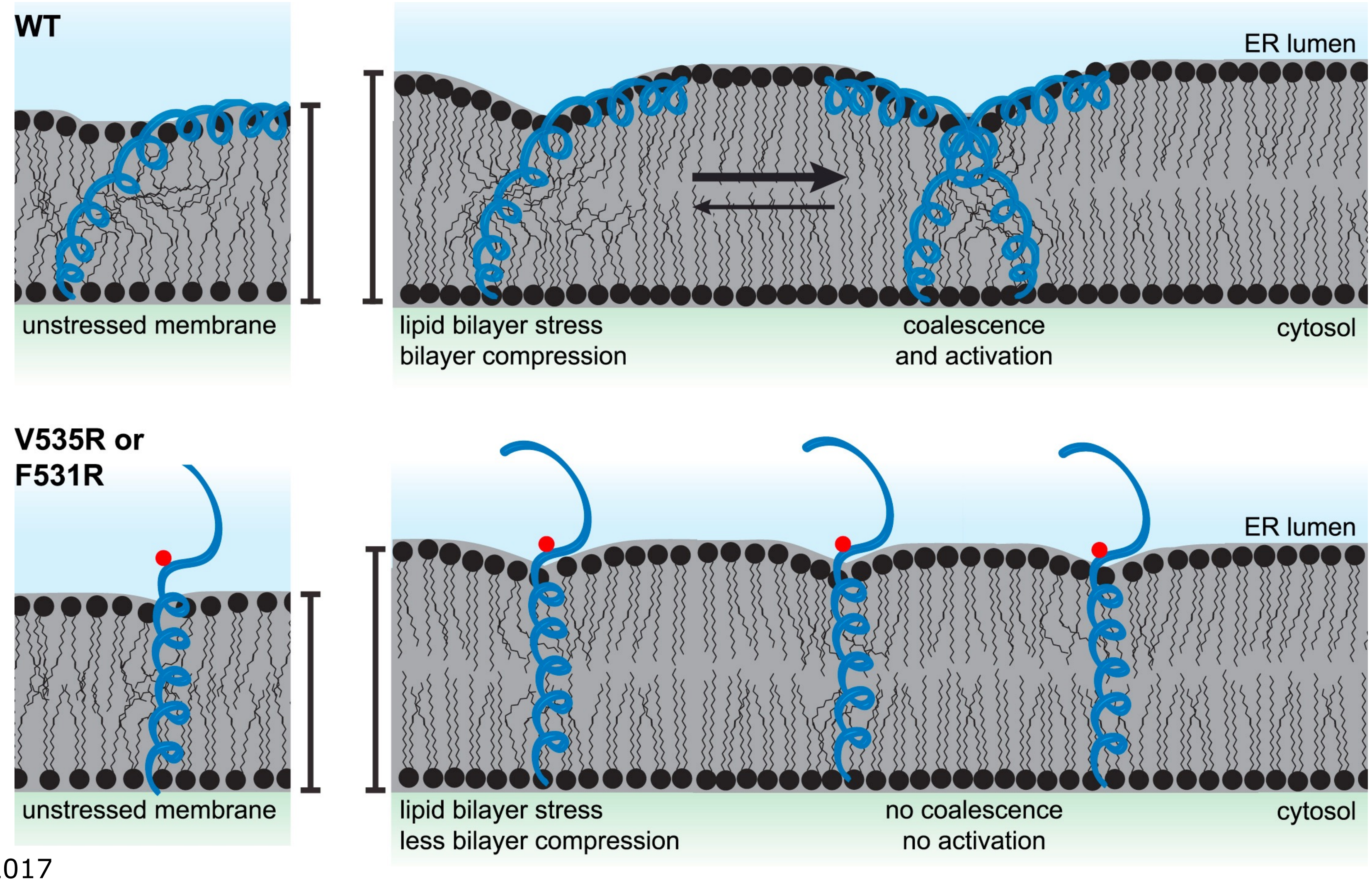




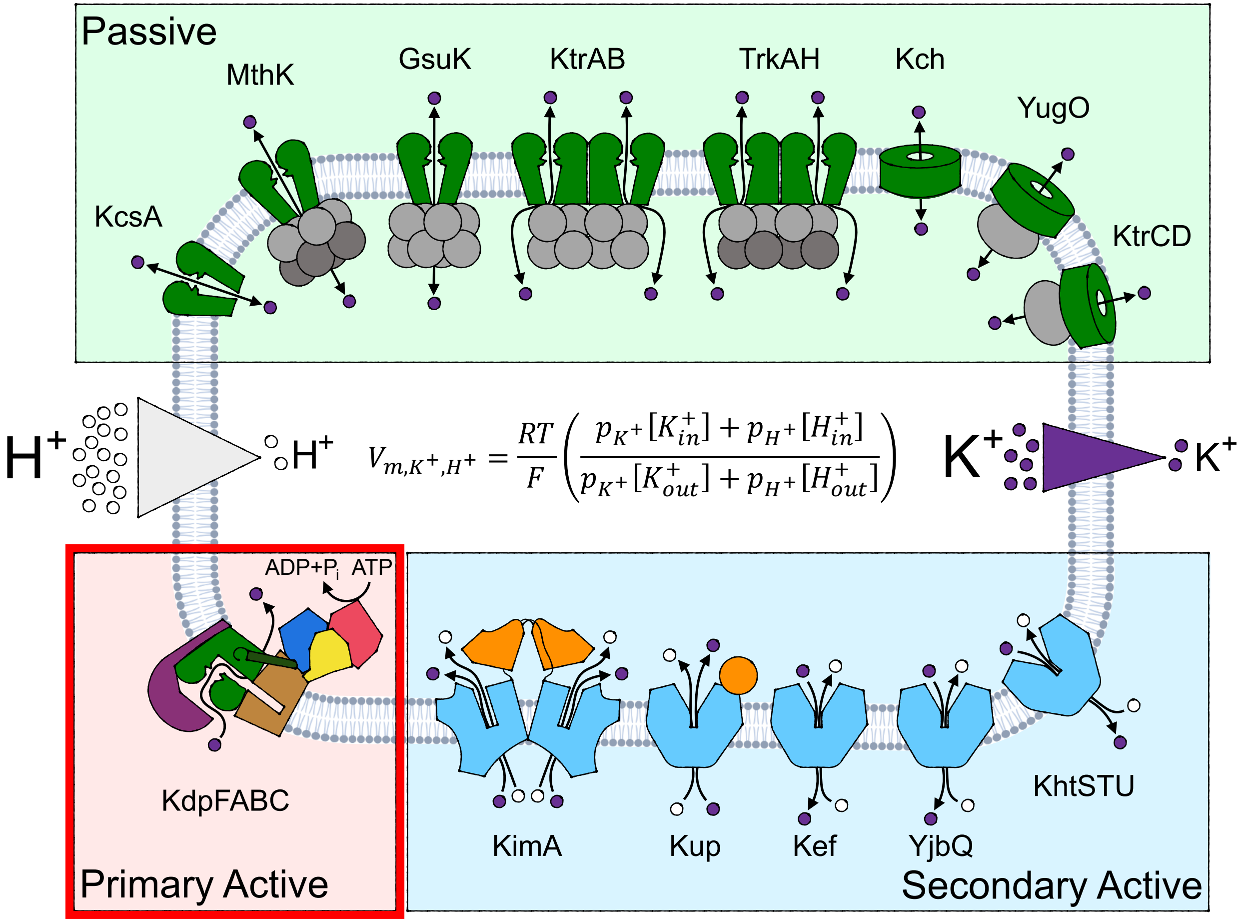


Halbleib et al. Mol Cell 2017

Proposed mechanism of lipid sensing

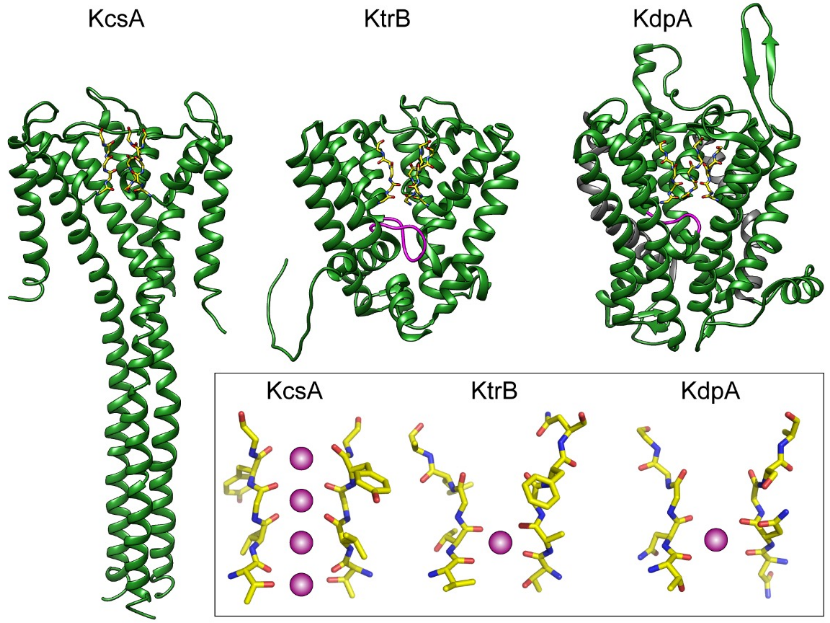


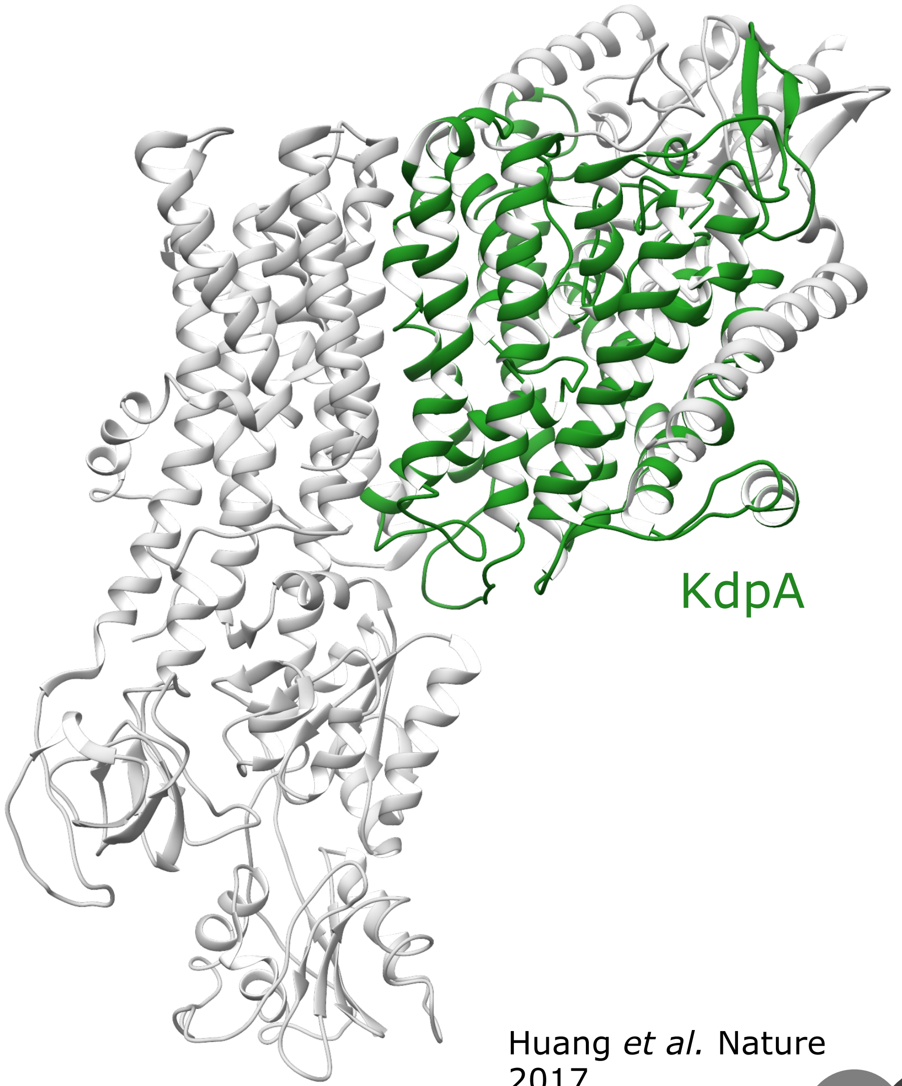
Halbleib et al. Mol Cell 2017



Stautz, Hellmich, Fuss, Silberberg *et al.* JMB 2021

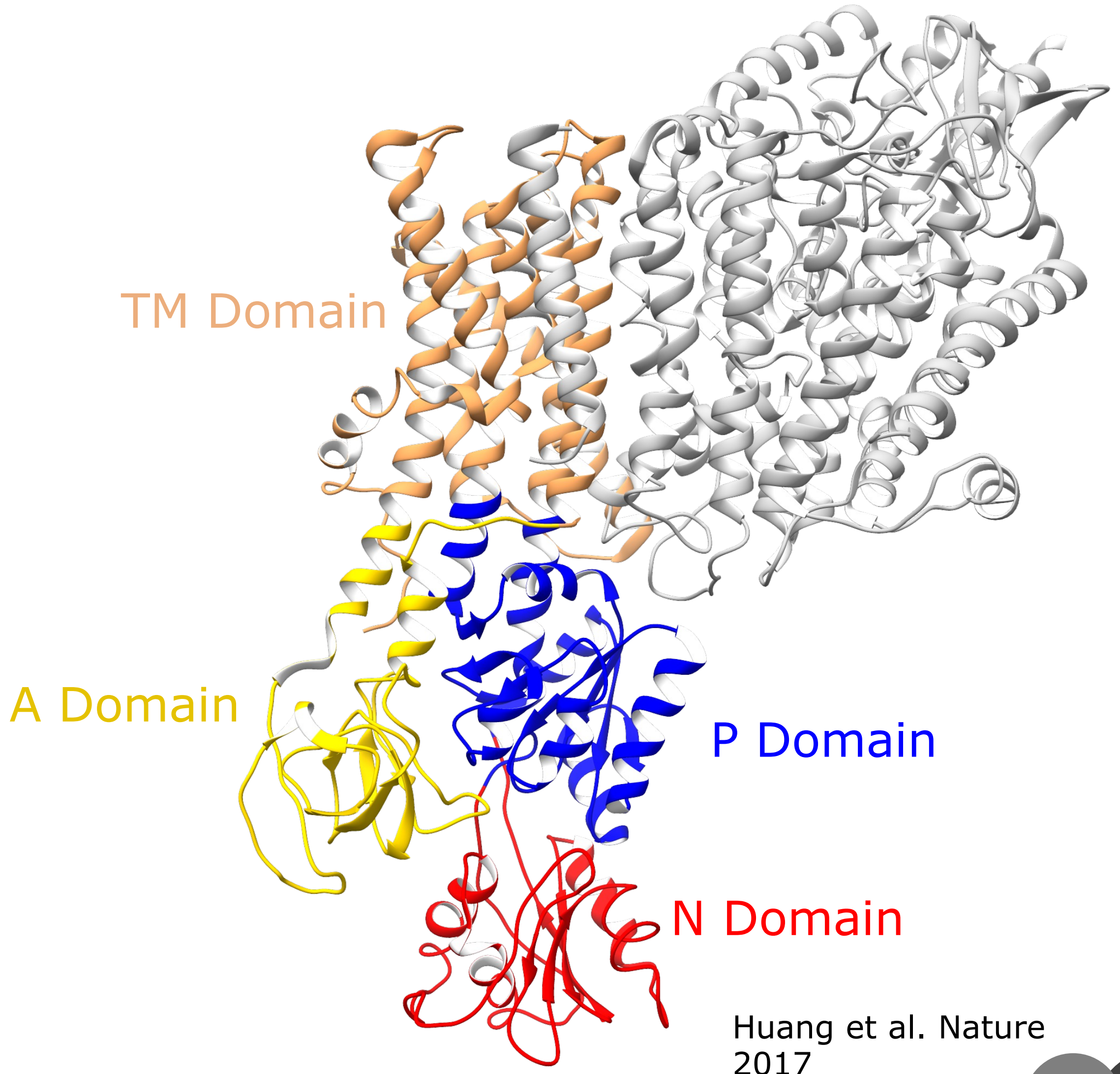
- SKT channel-like selectivity filter
- Intramembrane loop





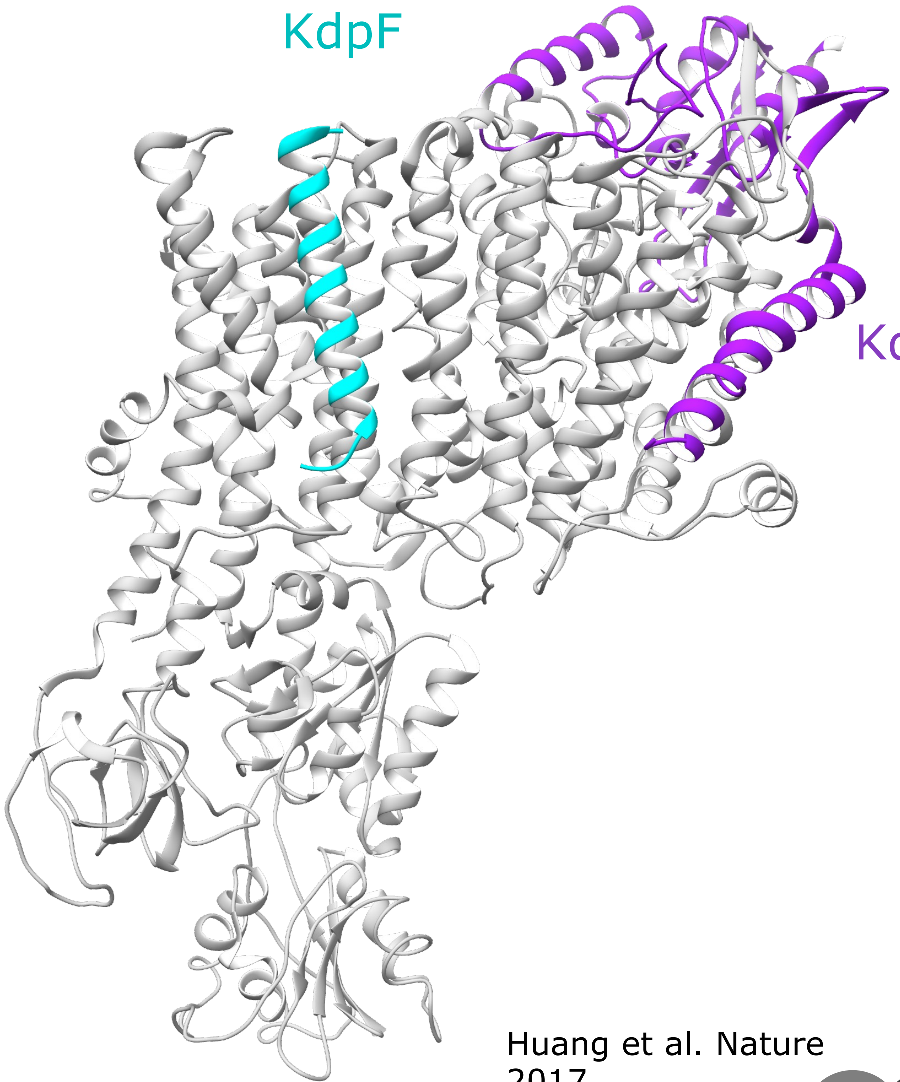
Huang *et al.* Nature 2017

- Smallest P-type ATPase
- Responsible for ATP hydrolysis

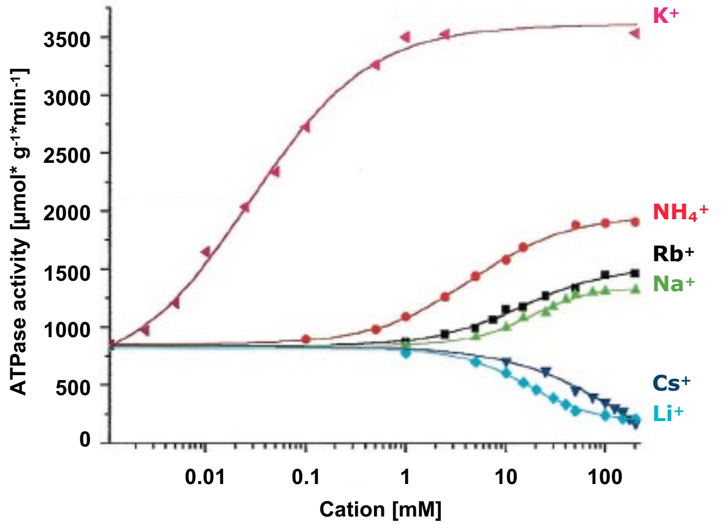


Huang et al. Nature 2017

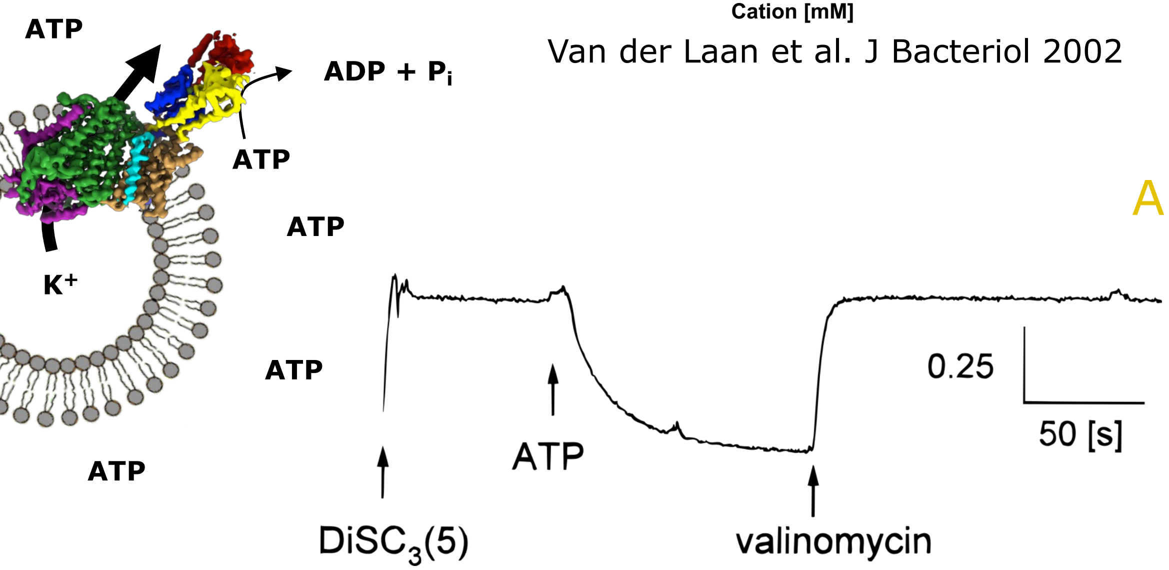
- KdpF: lipid-like stabilizer
- KdpC: unknown function



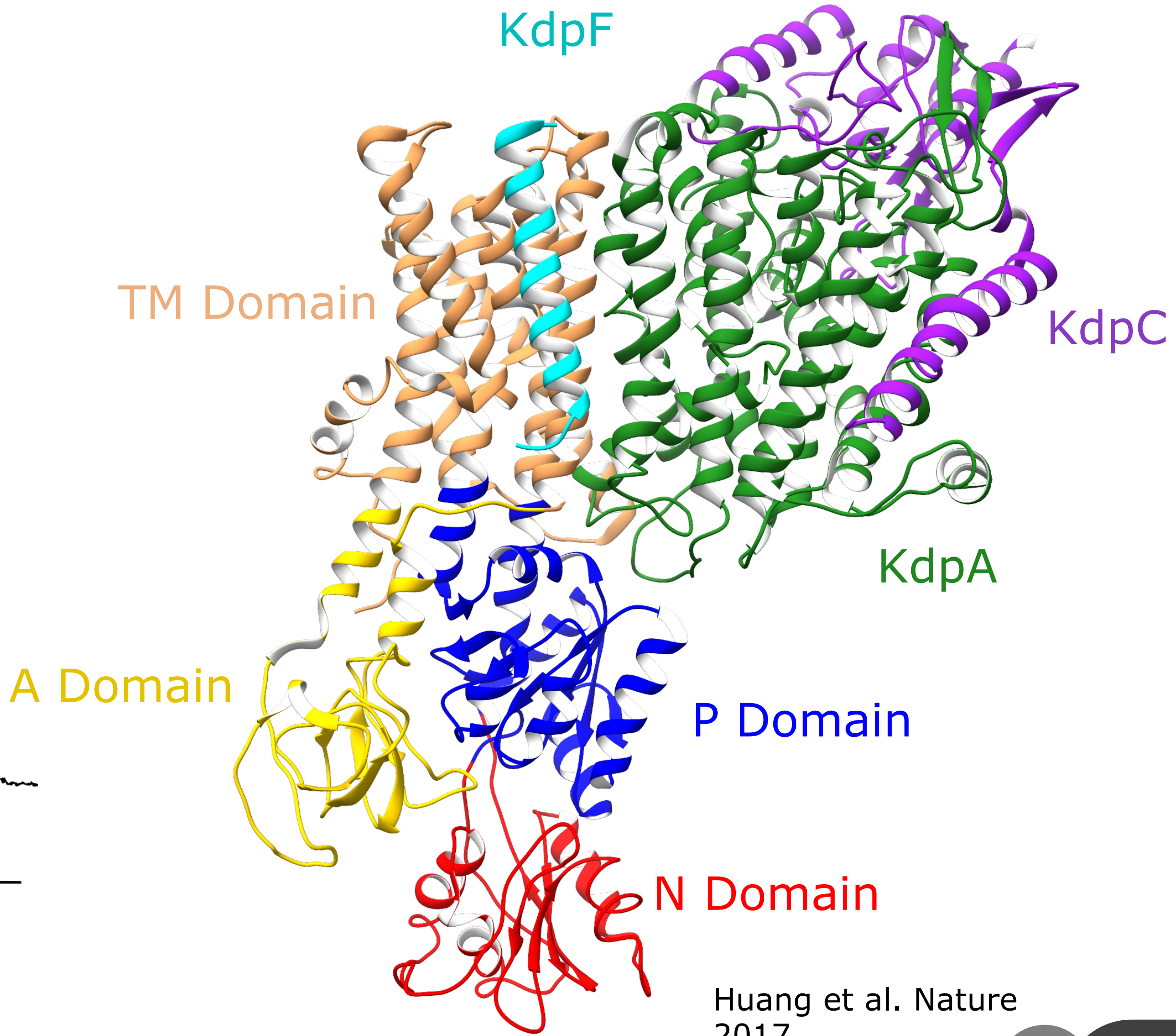
Huang et al. Nature 2017



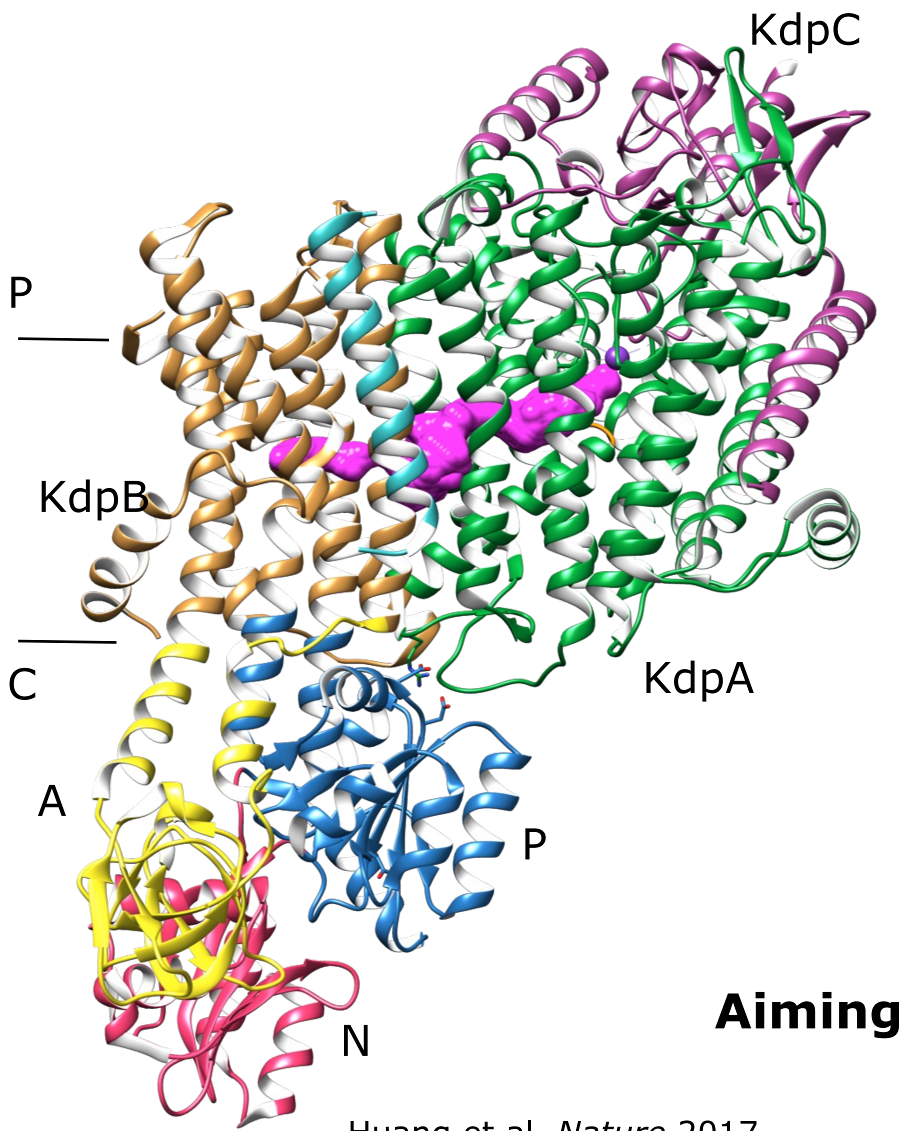
Van der Laan et al. J Bacteriol 2002



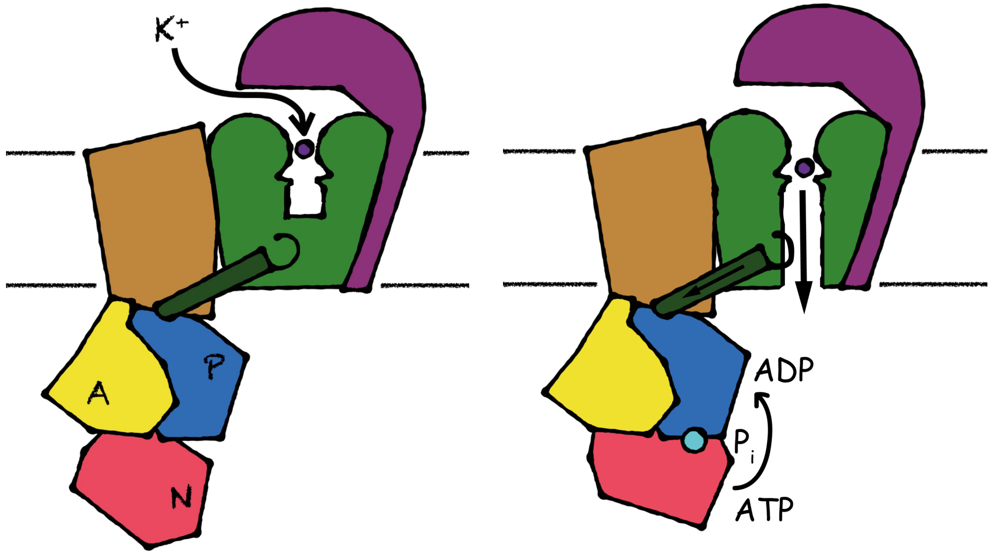
Fendler et al. Biochemistry 1996

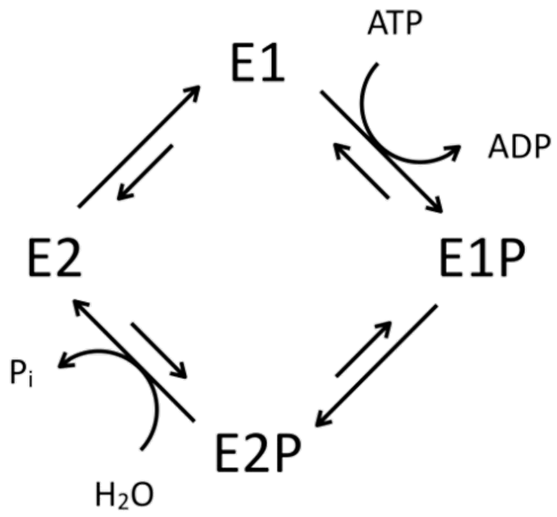


Huang et al. Nature 2017



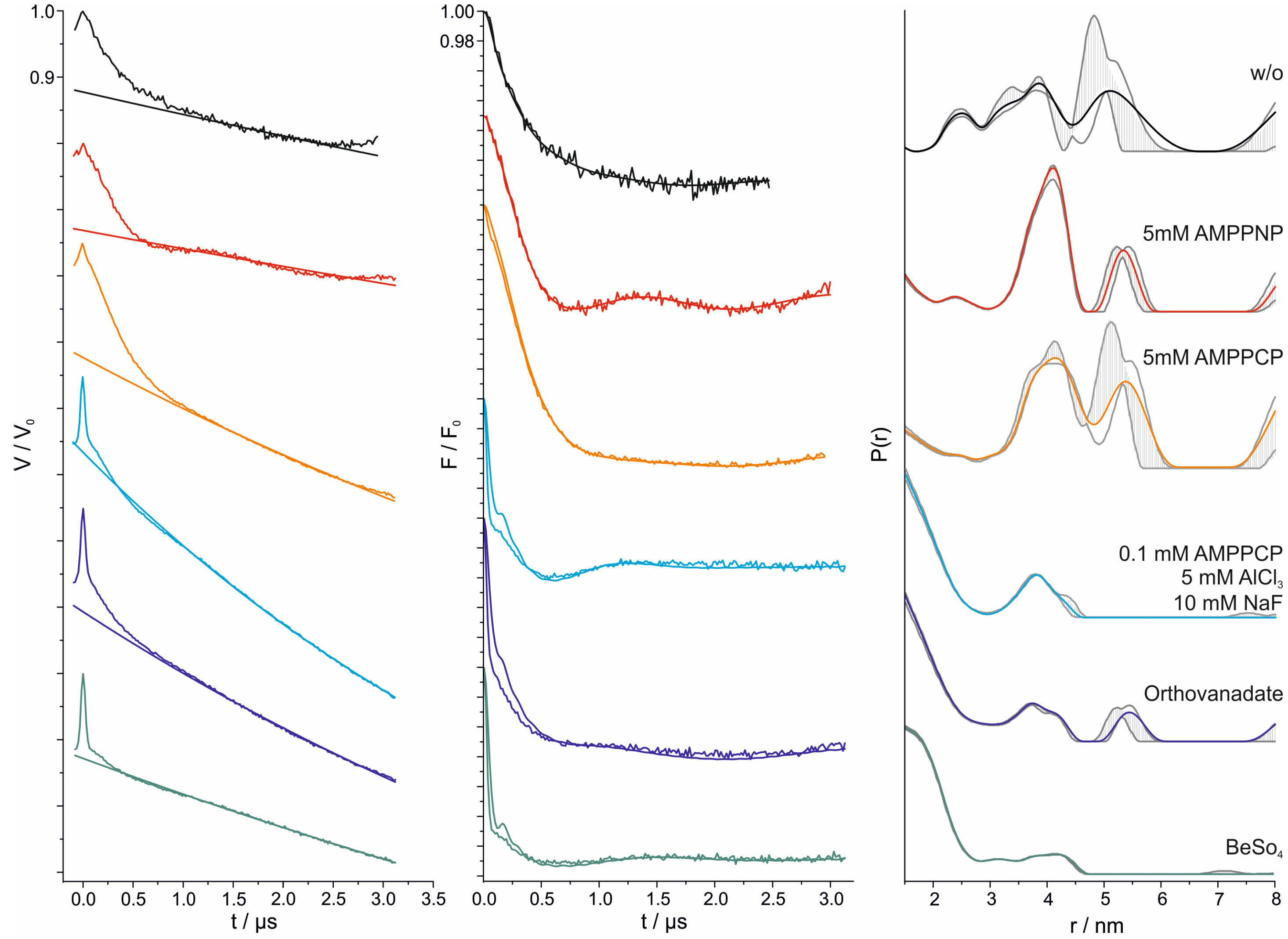
Huang et al. *Nature* 2017

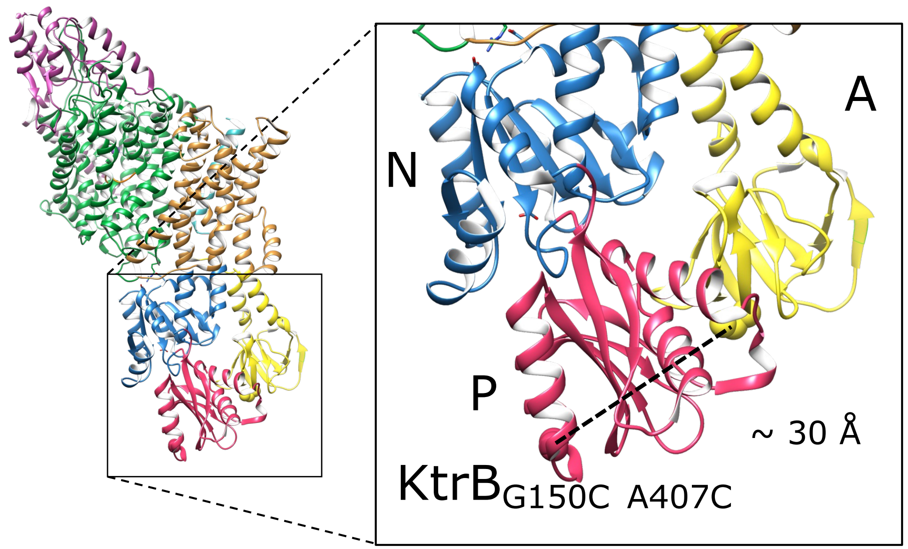


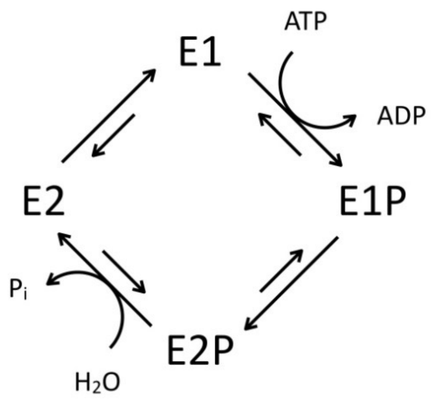


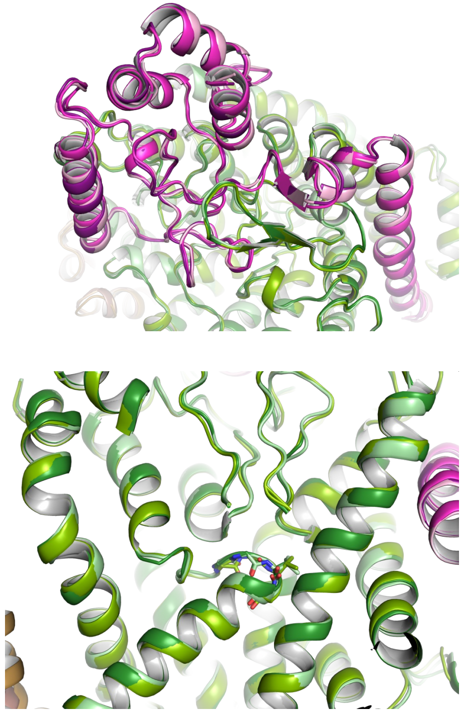
Aiming for E2-P by inhibiting KdpFABC

Aiming for E2-P

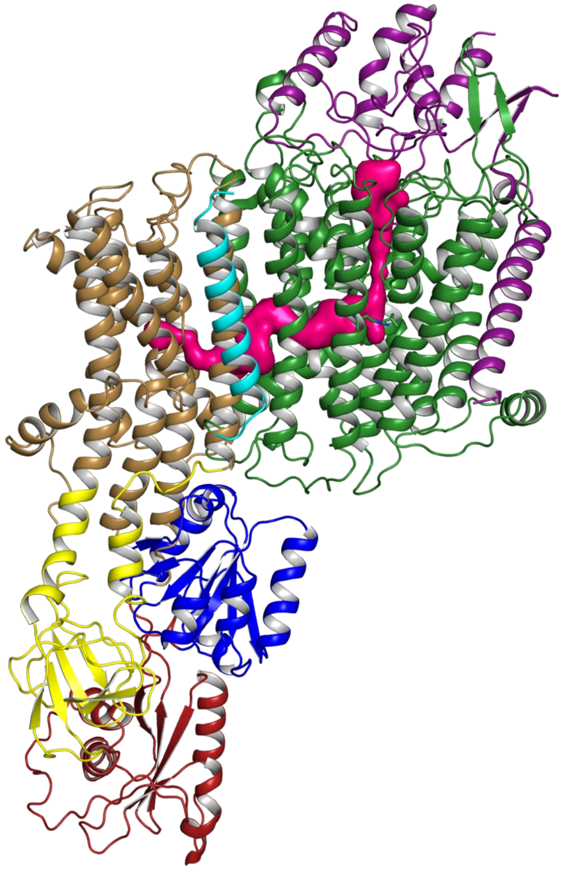


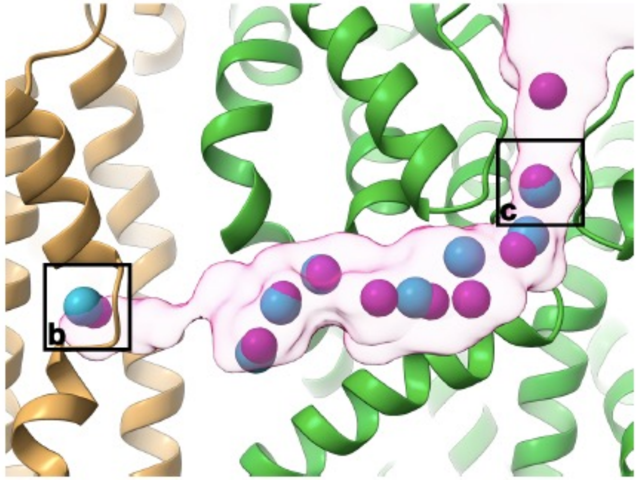


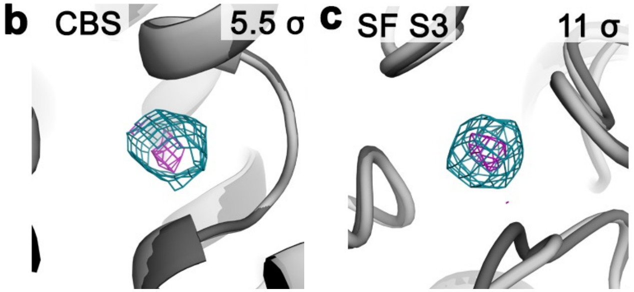




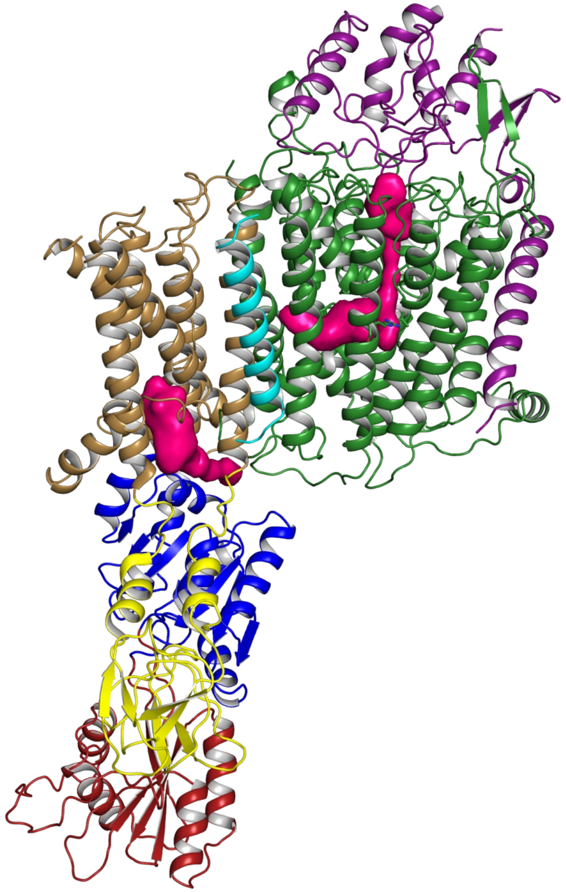
State 1: E1 conformation





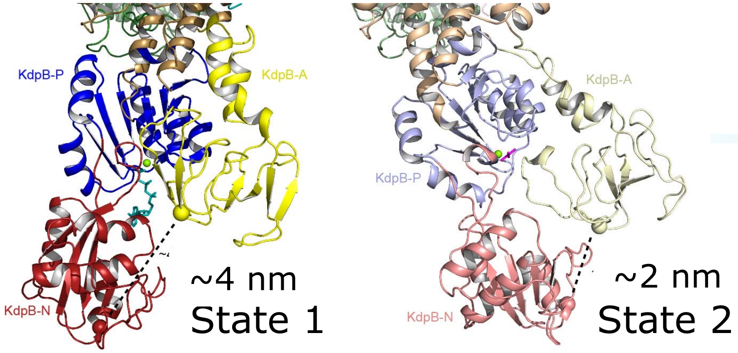


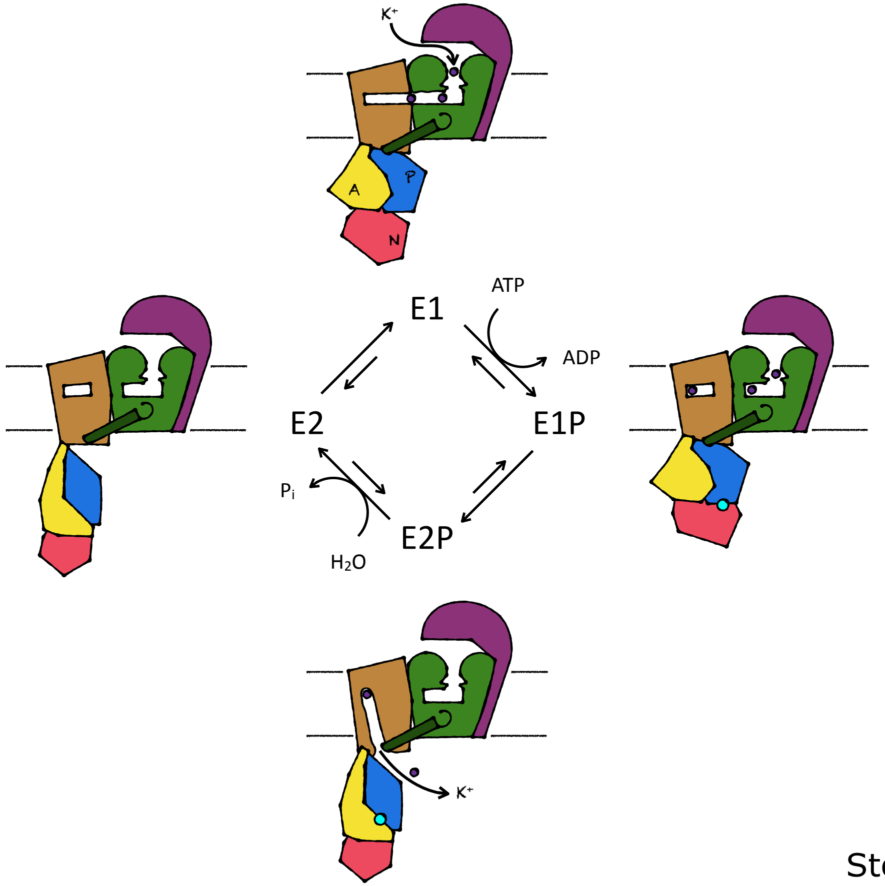
State 2: E2 conformation

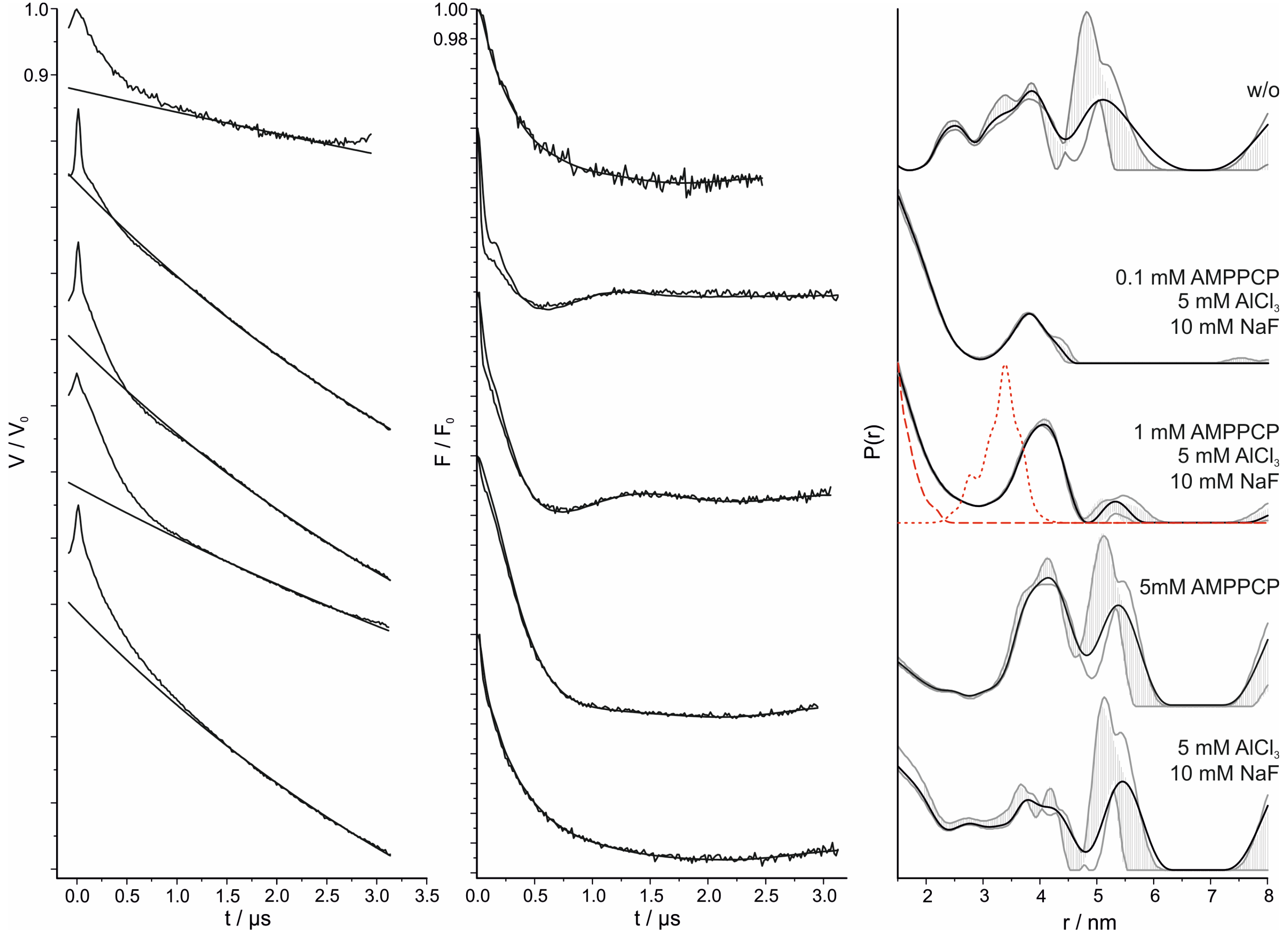


Stock, Hielkema, Tascon et al. Nat Comm 2018

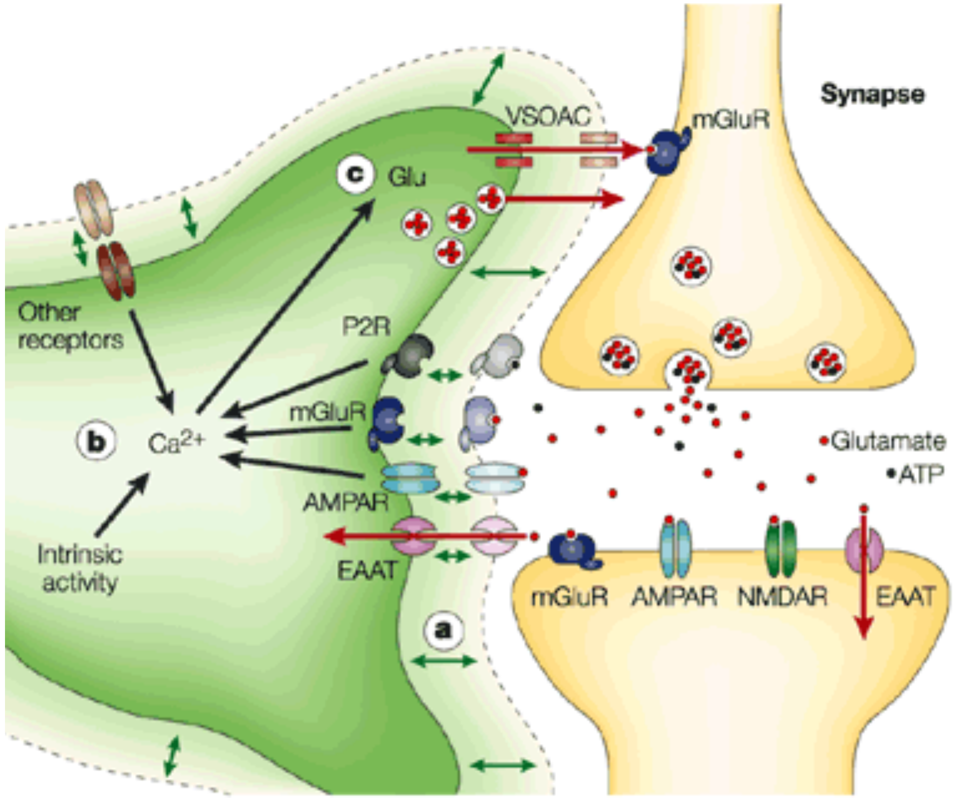
VALIDATION OF CONFORMATIONAL STATES



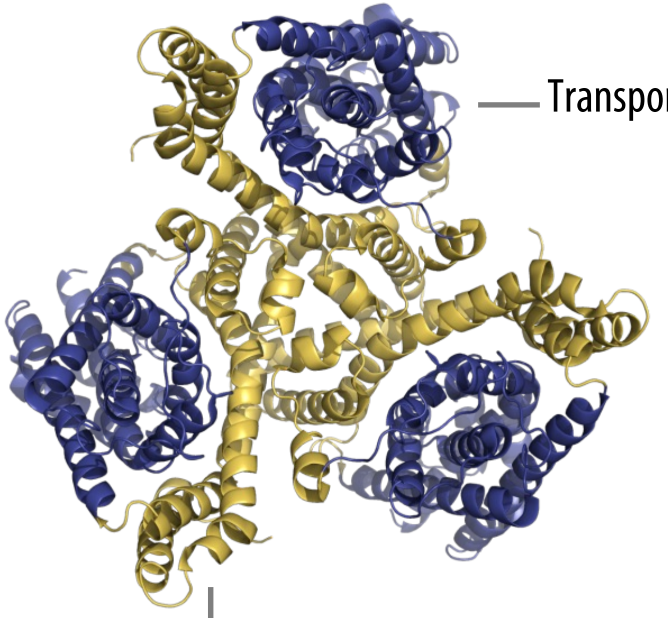


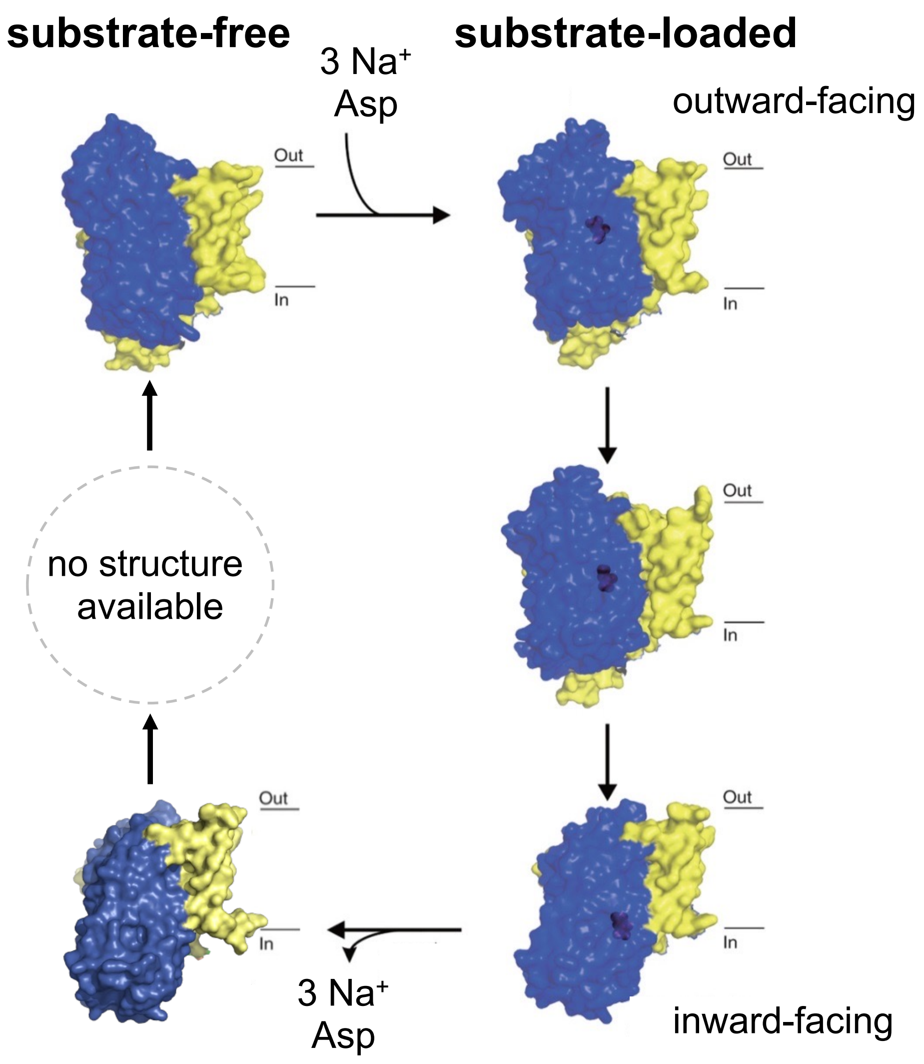


Stock, Hielkema, Tascon et al. Nat Comm 2018

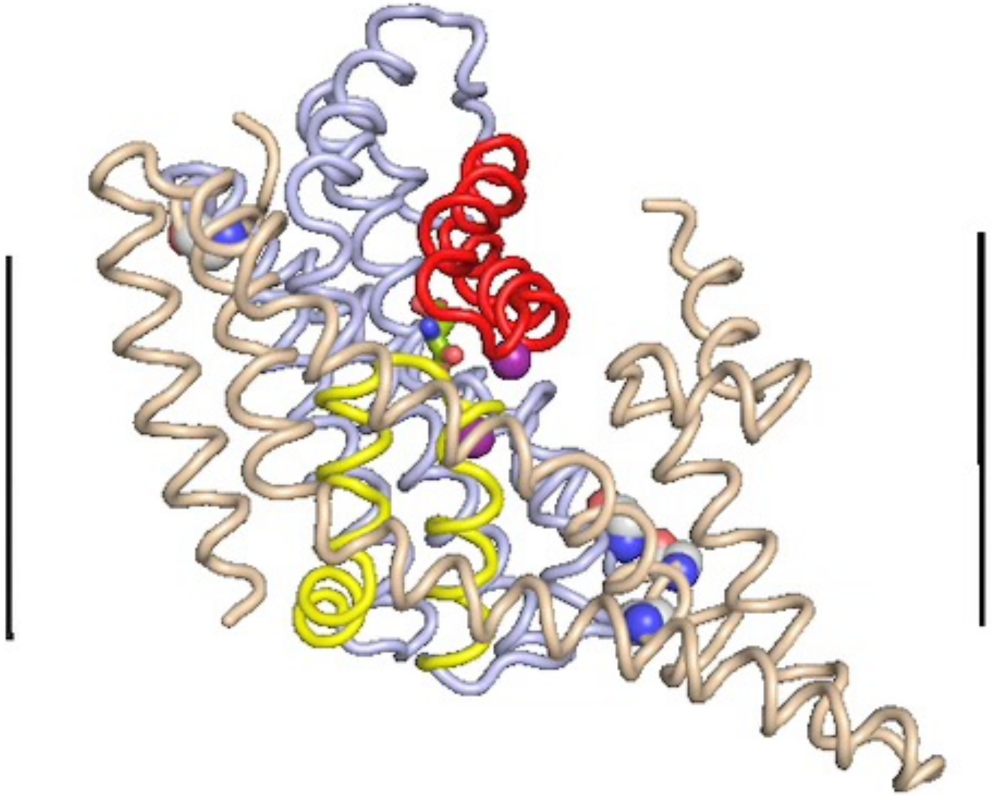


Nedergaard et al. NRN2002



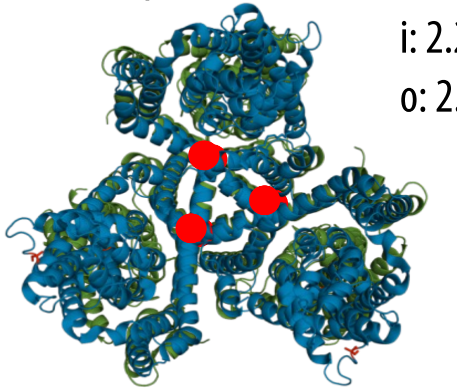


Yernool et al. Nature 2004
Reyes et al. Nature 2009
Verdon et al. NSMB 2012
Jensen et al. NSMB 2013
Verdon et al. Elife 2014

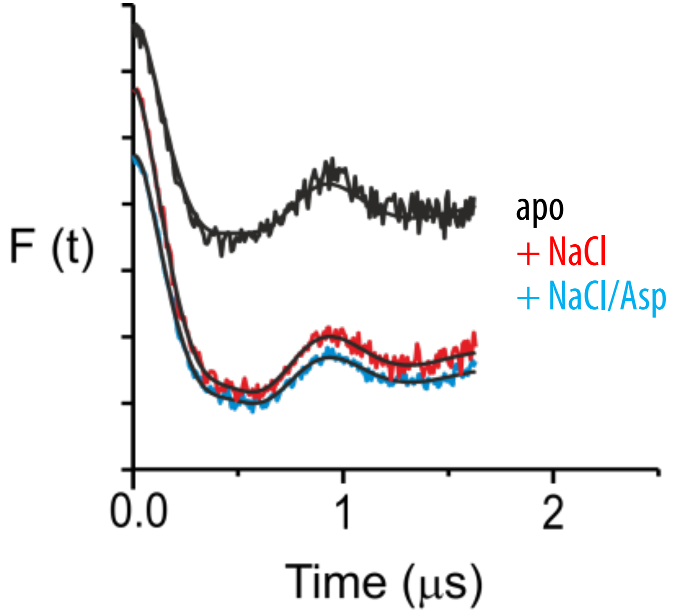


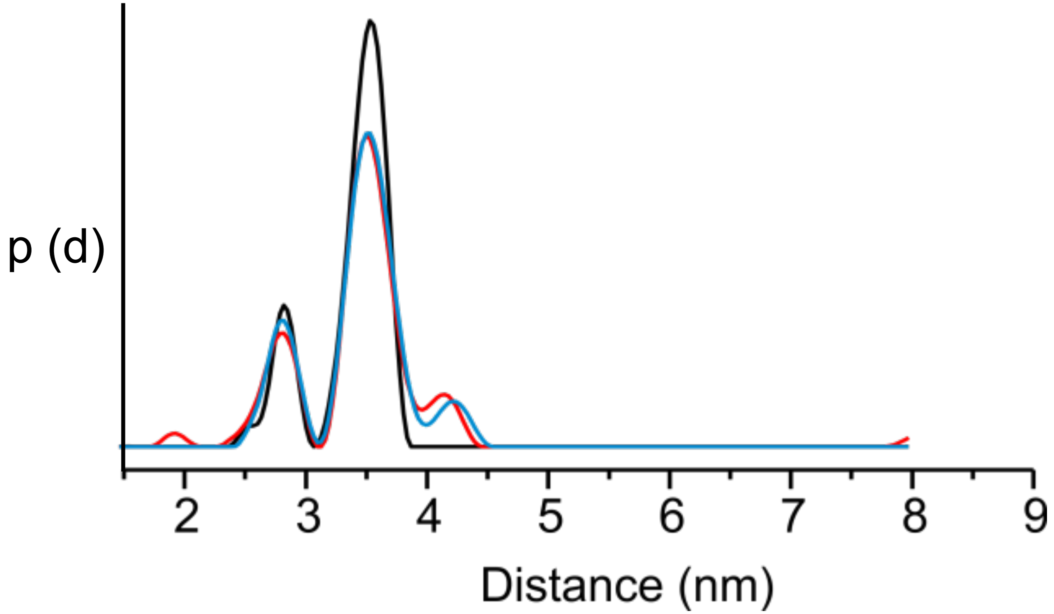
Reyes et al. Nature 2009

V176C-Spin label



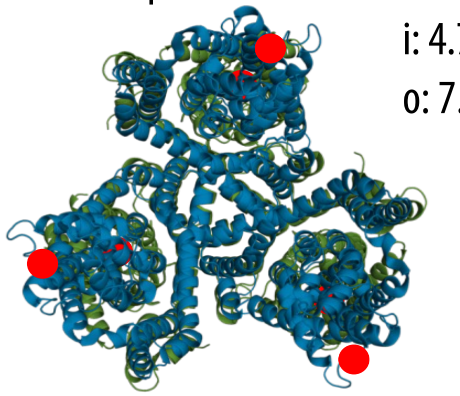
i: 2.2 nm
o: 2.3 nm



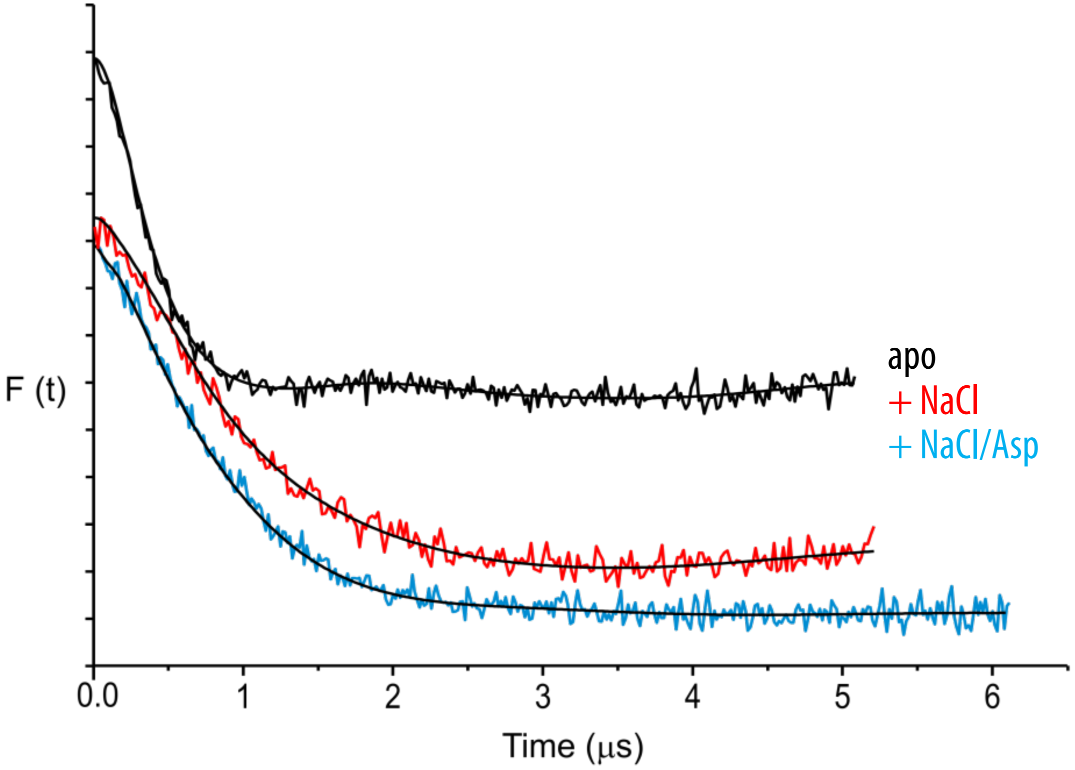


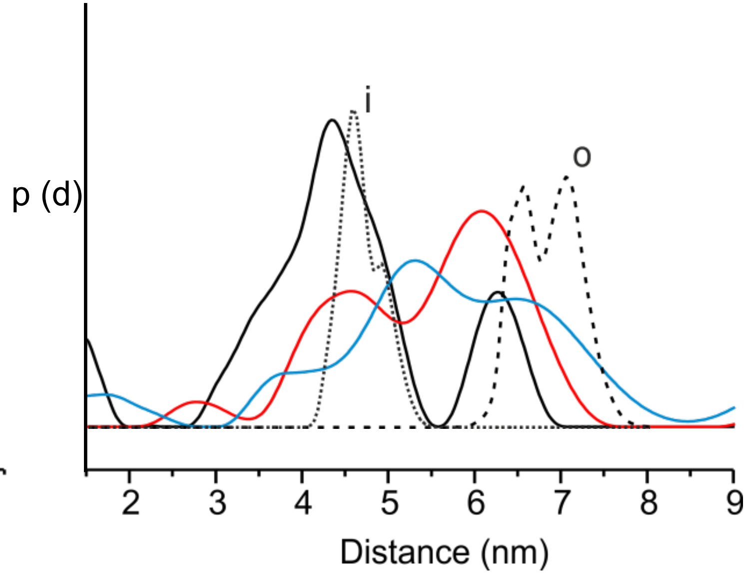
Hänelt et al. NSMB 2013

S331C-Spin label



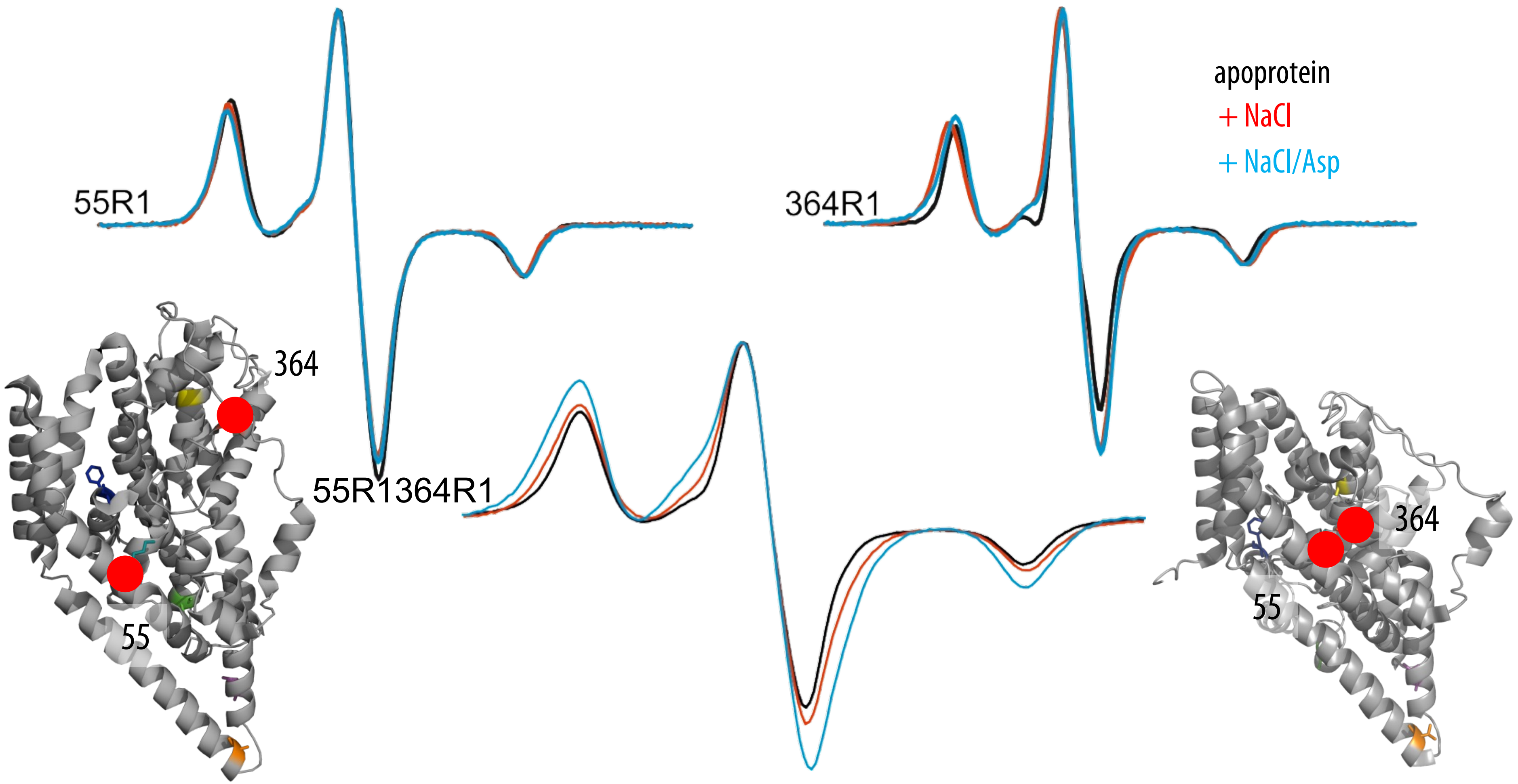
i: 4.7 nm
o: 7.3 nm



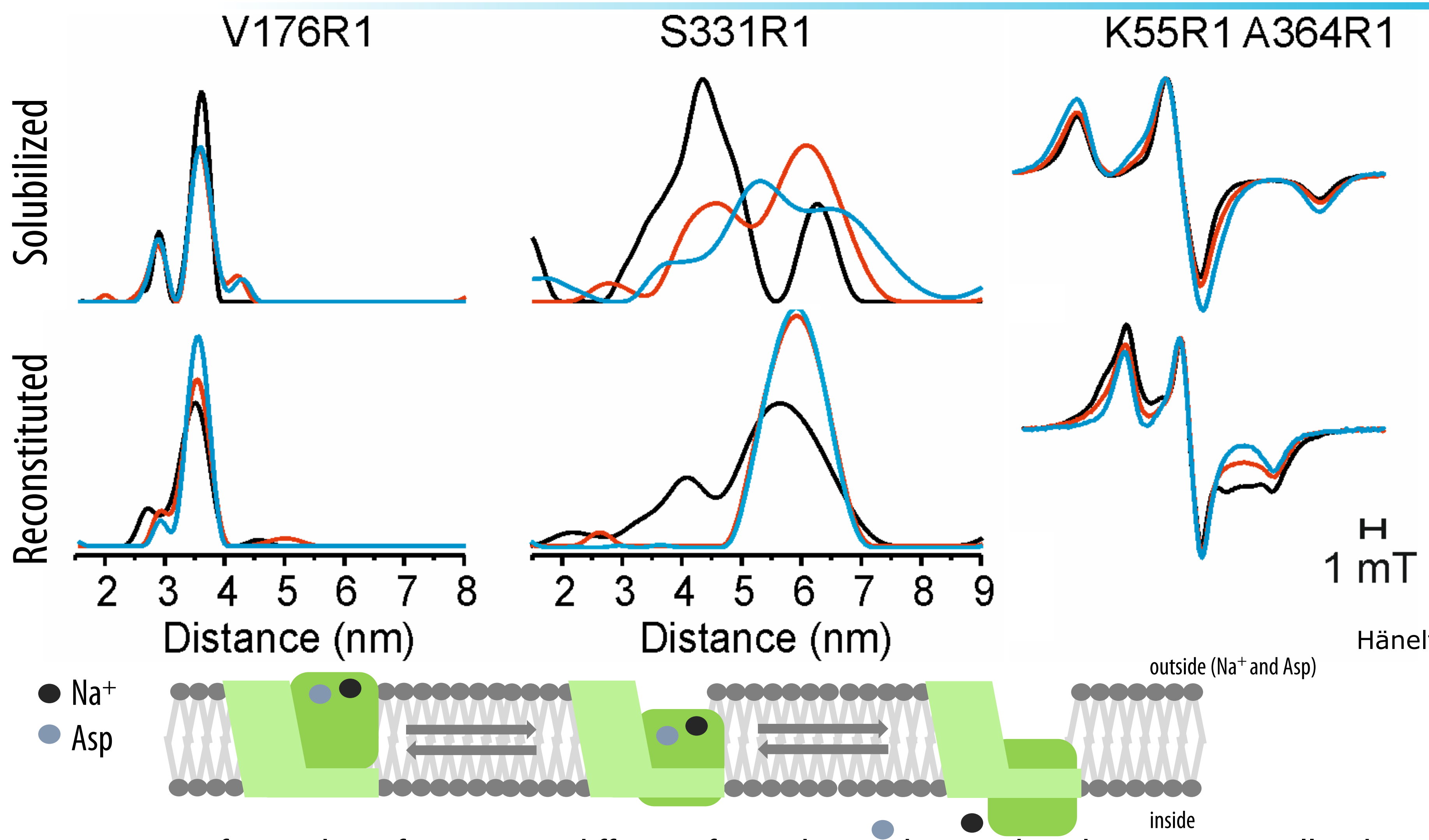


Hänelt et al. NSMB 2013

- Multiple conformational states consistent with equilibrium constants close to unity between the observed transporter conformations
- The transporting domain undergoes an elevator-type movement, which is facilitated by the rigid scaffold of the trimerization domain

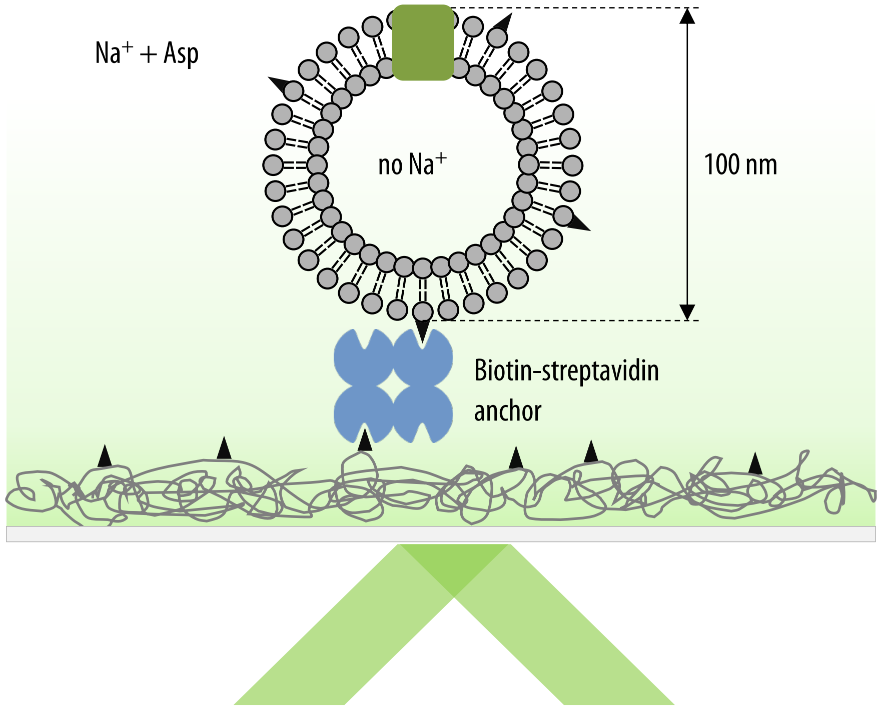


- The transporting domain undergoes an elevator-type movement, which is facilitated by the rigid scaffold of the trimerization domain

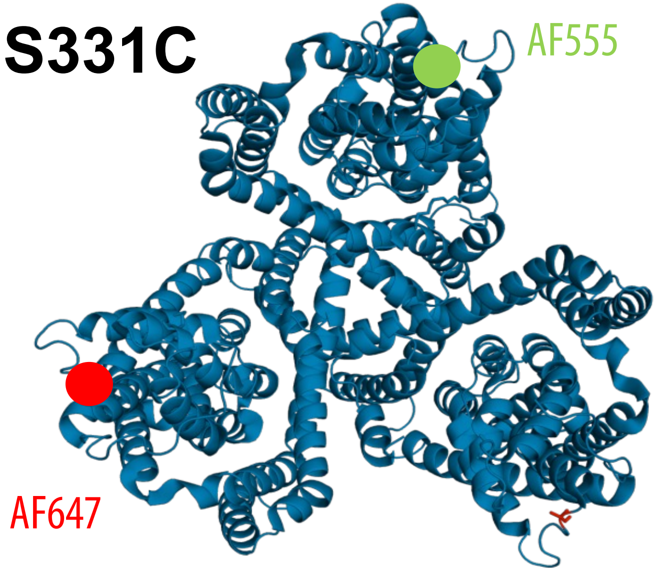


Hänelt et al. NSMB 2013

- Membrane environment favored conformations different from those observed in detergent micelles, but the transport domain remained structurally heterogeneous



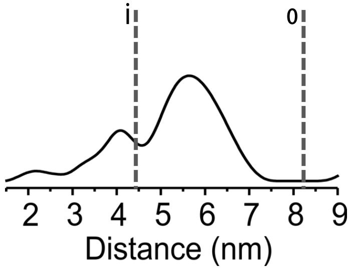
S331C

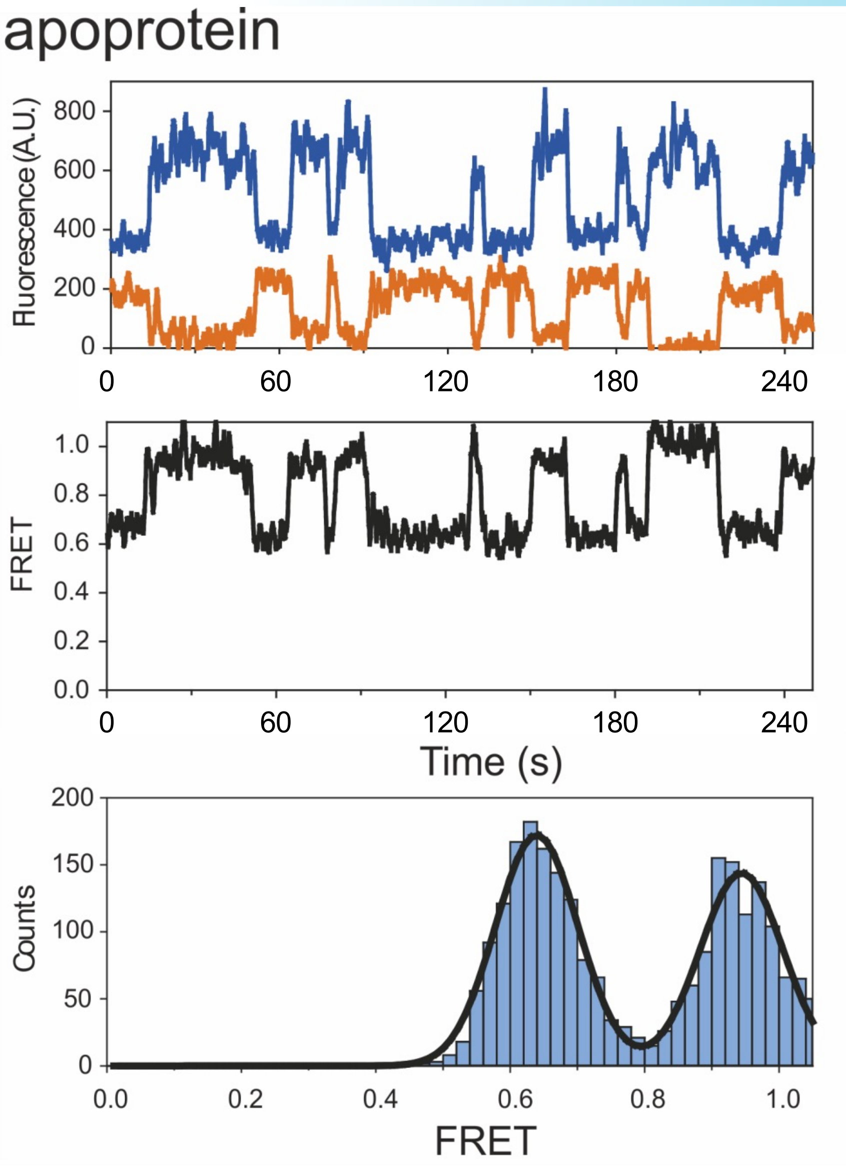


i: 4.70 nm ~ FRET 0.9
o: 7.34 nm ~ FRET 0.4

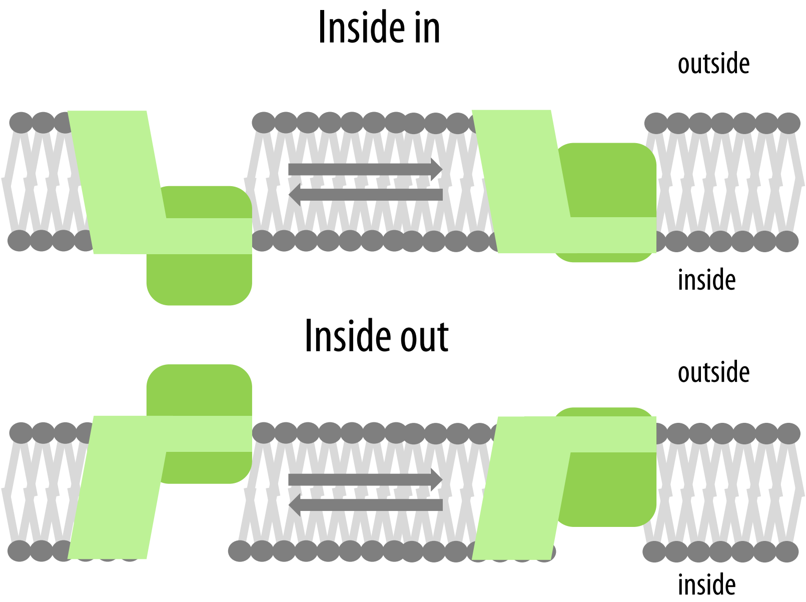
Erkens et al. Nature 2013

TRANSPORT CYCLE ON A SINGLE MOLECULE LEVEL – DYNAMICS OF APO GLT_{PH}



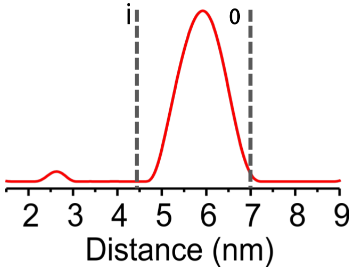


0.6/0.9



Erkens et al. Nature 2013

TRANSPORT CYCLE ON A SINGLE MOLECULE LEVEL – DYNAMICS OF Na^{+}-BOUND GLT_{PH}



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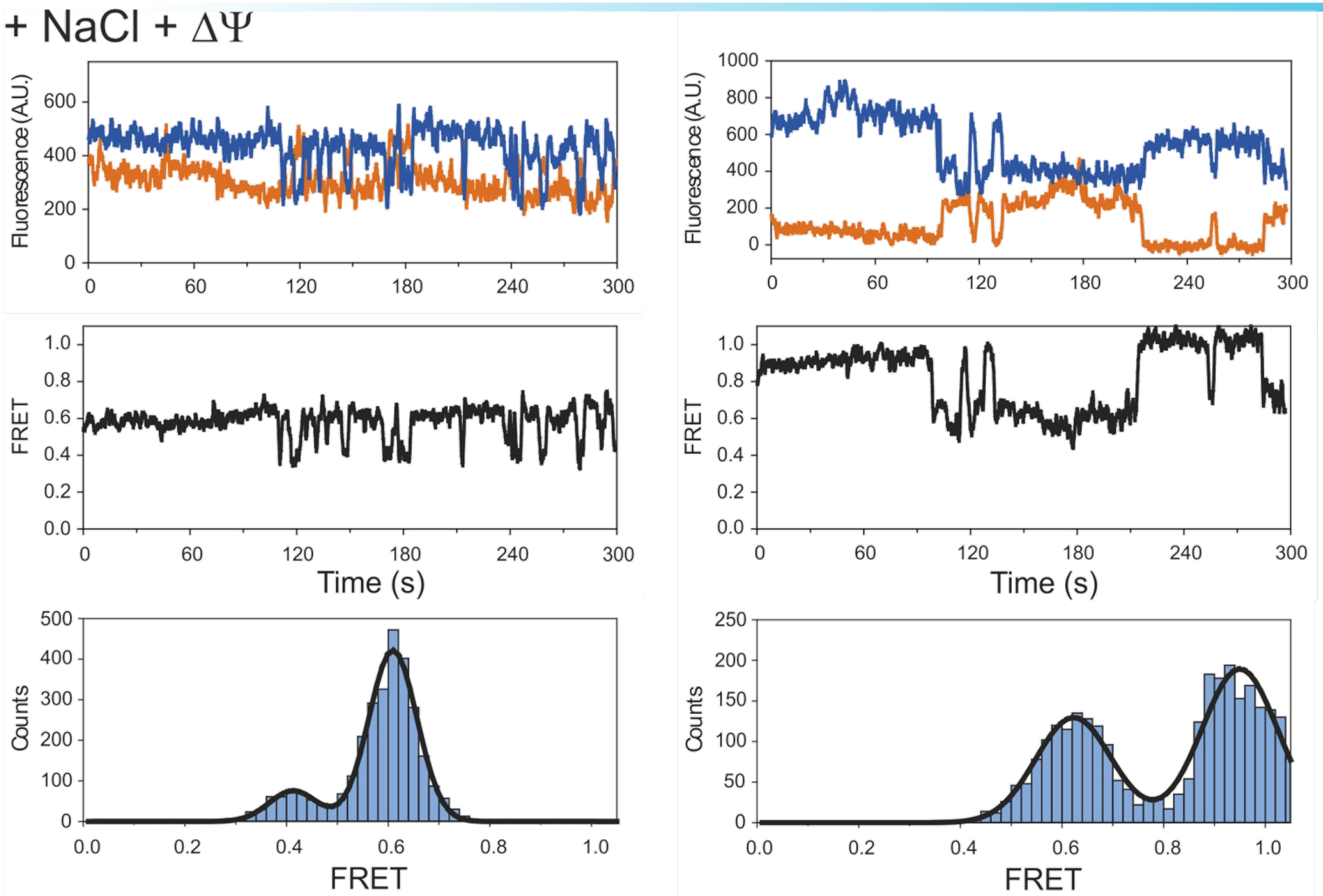
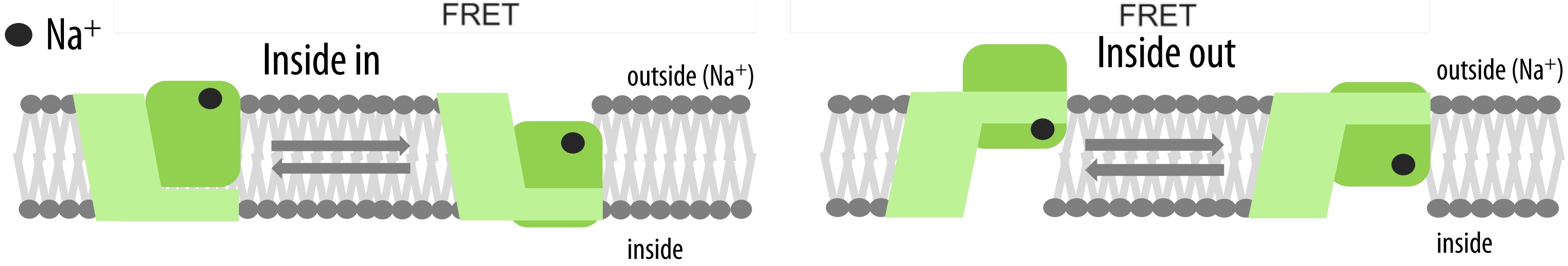
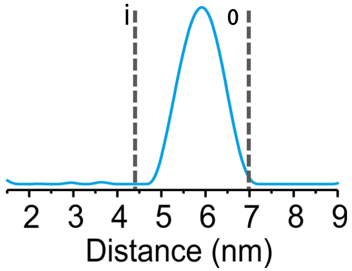


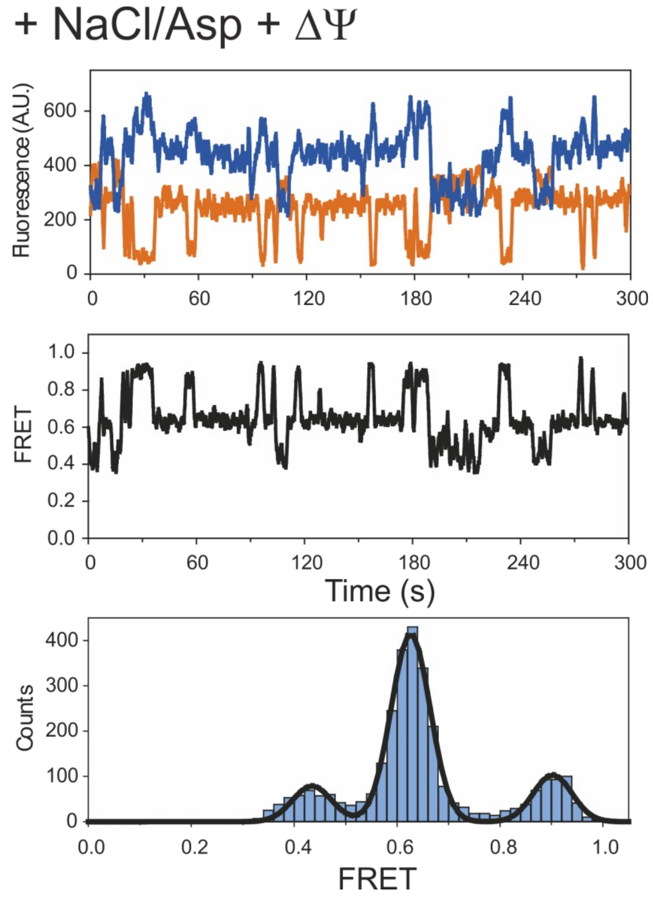
0.6/0.9



Erkens et al. Nature 2013

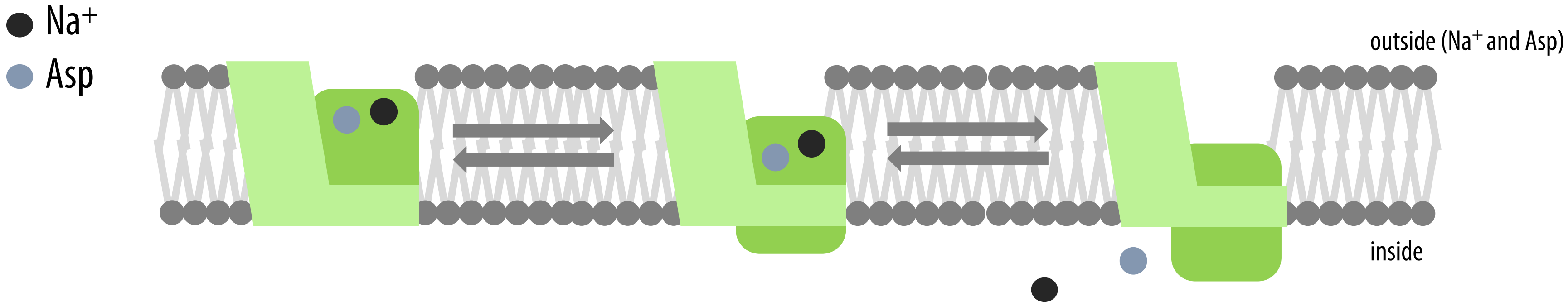
Transport cycle on a single molecule level – dynamics of Na^{+}-bound GLT_{PH}





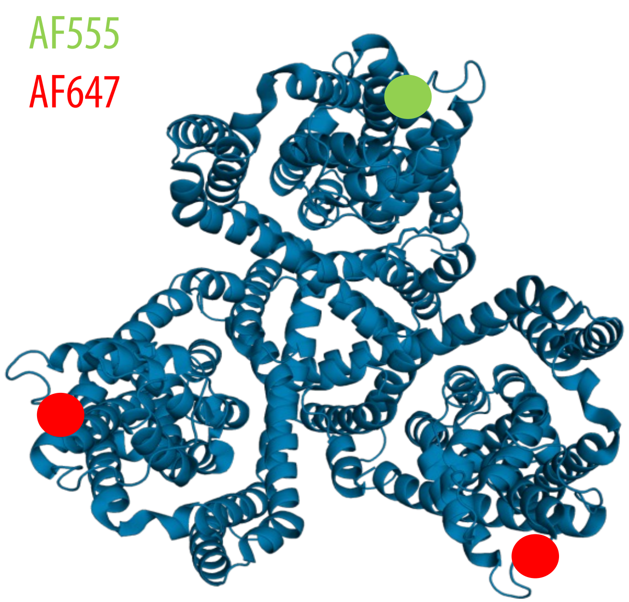
0.4/0.6/0.9

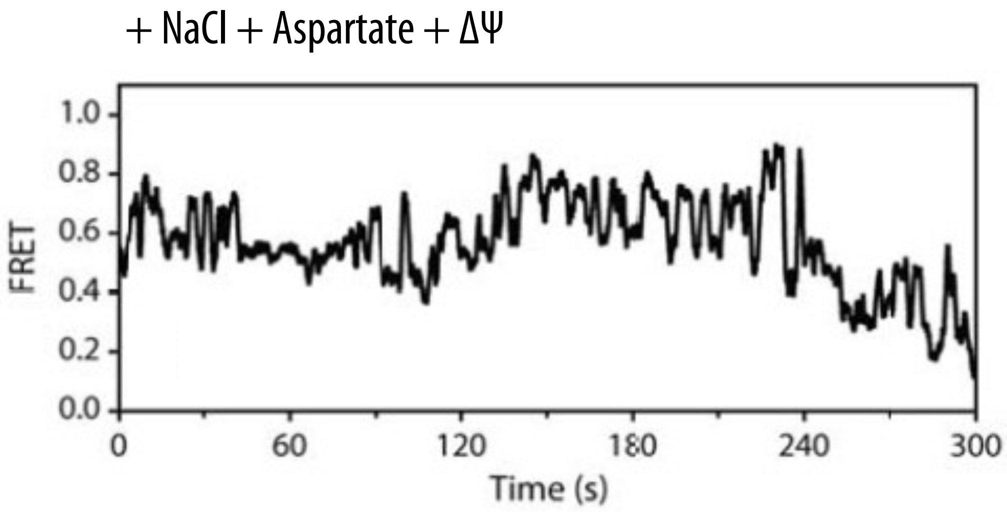
- The intermediate conformation appears to be essential and may hinder the dissipation of the Na^{+} gradient in the absence of Asp



Erkens et al. Nature 2013

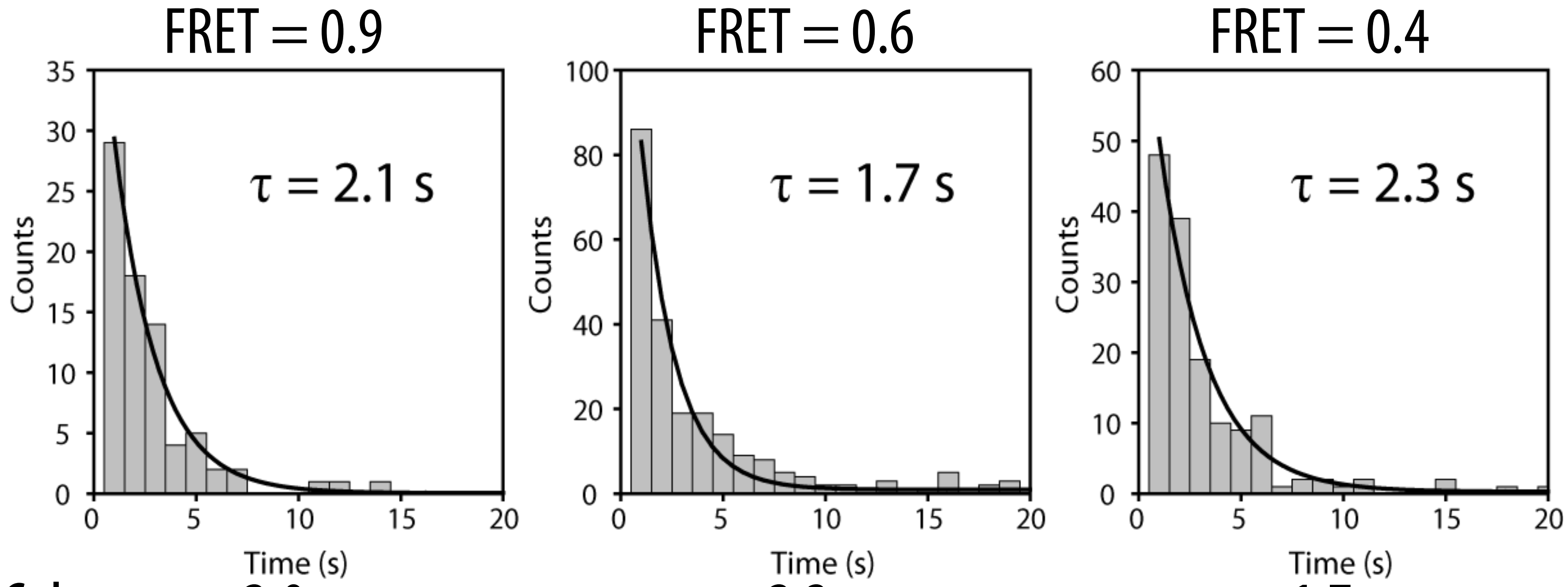
Transport cycle on a single molecule level – no subunit coordination





- Multiple FRET states proposed non-synchronized subunit dynamics within a trimer
- All three subunits move independent of each!

Erkens et al. Nature 2013

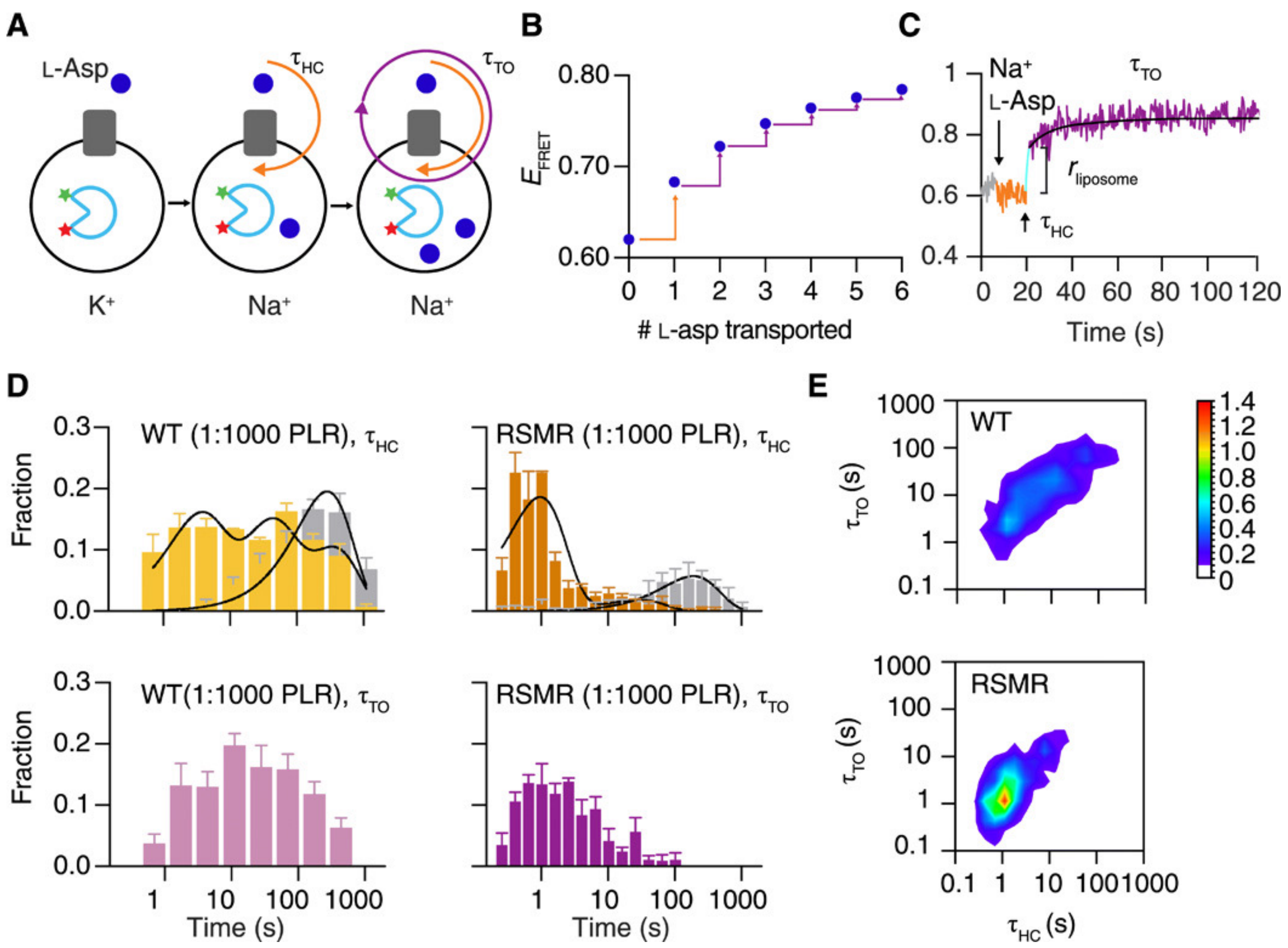


Calc. 2.0 s 2.3 s 1.7 s

- Dwell times of the different FRET levels as determined from single exponential fits agree to those calculated for independent movement
- The dwell time distribution following a single-exponential decay instead of a rise-and-decay function excludes a coordinated e.g. rotary model

Erkens et al. Nature 2013

➔ All three subunits move independent of each other!



Ciftci et al. Science Adv 2020

Movie S6:

High-resolution
HS-AFM movie
Glt_{Ph} in DOPC/DOPE/DOPS (8/1/1) membrane

Conditions: transport (Na^+ + Asp)
Image size: 80nm
Frame rate: $1s^{-1}$

Molecules of interest
exhibiting elevator domain activity
are outlined by white circles

Ruan et al. PNAS 2017

DIRECT VISUALIZATION OF GLT_{PH} ELEVATOR DOMAIN MOVEMENTS BY HS-AFM

Movie S3:

High-resolution single molecule HS-AFM analysis
Glt_{Ph} in a DOPC/DOPE/DOPS (8/1/1) membrane

Conditions: transport (Na^+ + Asp)

Top left: HS-AFM height of the three subunits (red, blue and green traces) as a function of time.
The height scale was arbitrarily set to 0nm corresponding to the farthest indentation the domains visited.
The movement amplitude is 1.85±0.42nm.

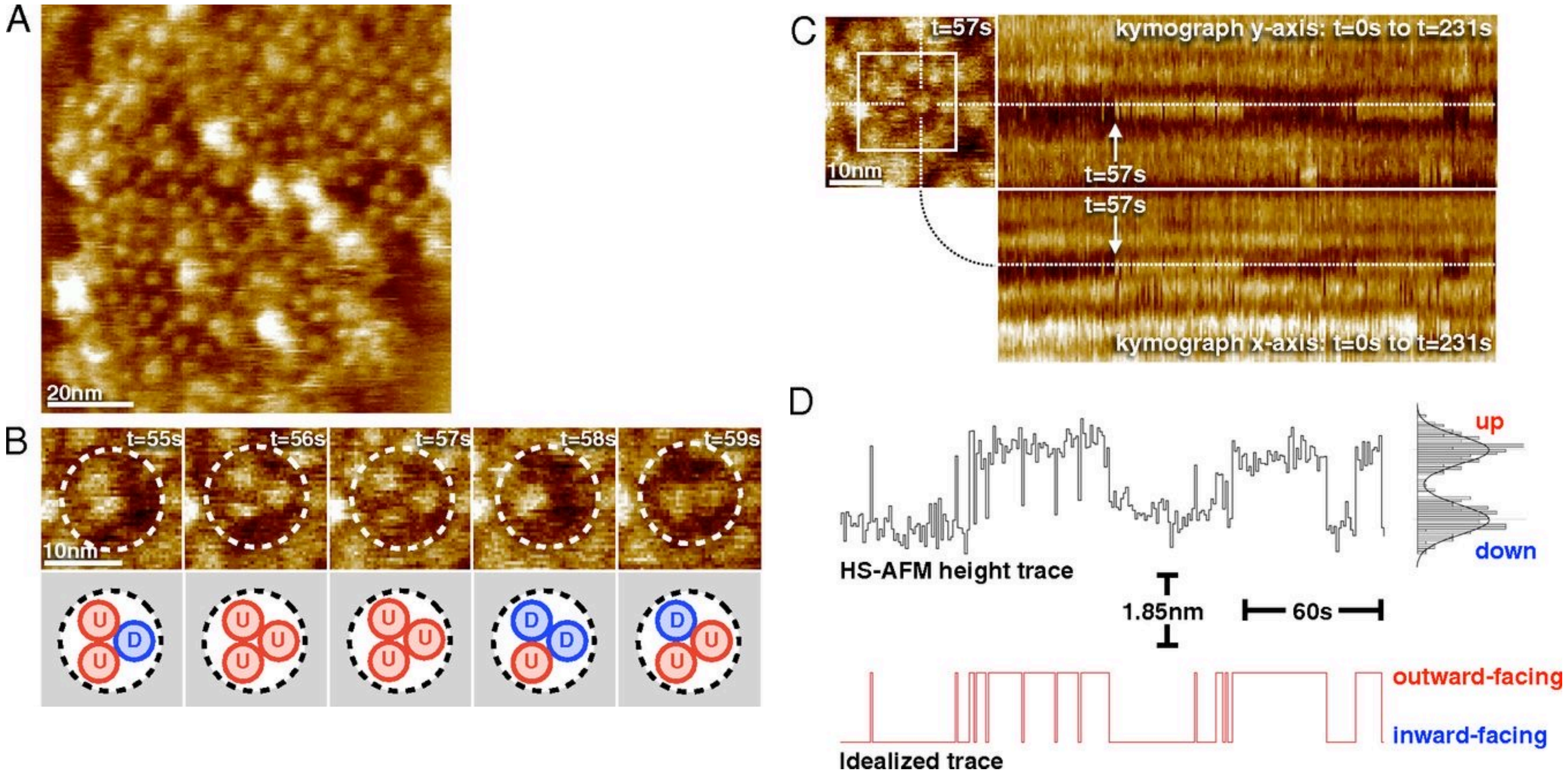
Bottom left: Idealized traces attributing the height variations displayed above to the outward facing (U, up) and inward facing (D, down) states respectively.

Inset top right: Raw data HS-AFM movie of the single Glt_{Ph} trimer analyzed. Image size: 12.8nm.

Inset middle right: Schematic representation of the Glt_{Ph} trimer elevator subunit movements.
Filled circles represent domains in the outward facing (U, up)
and empty circles domains in the inward facing (D, down) states respectively.

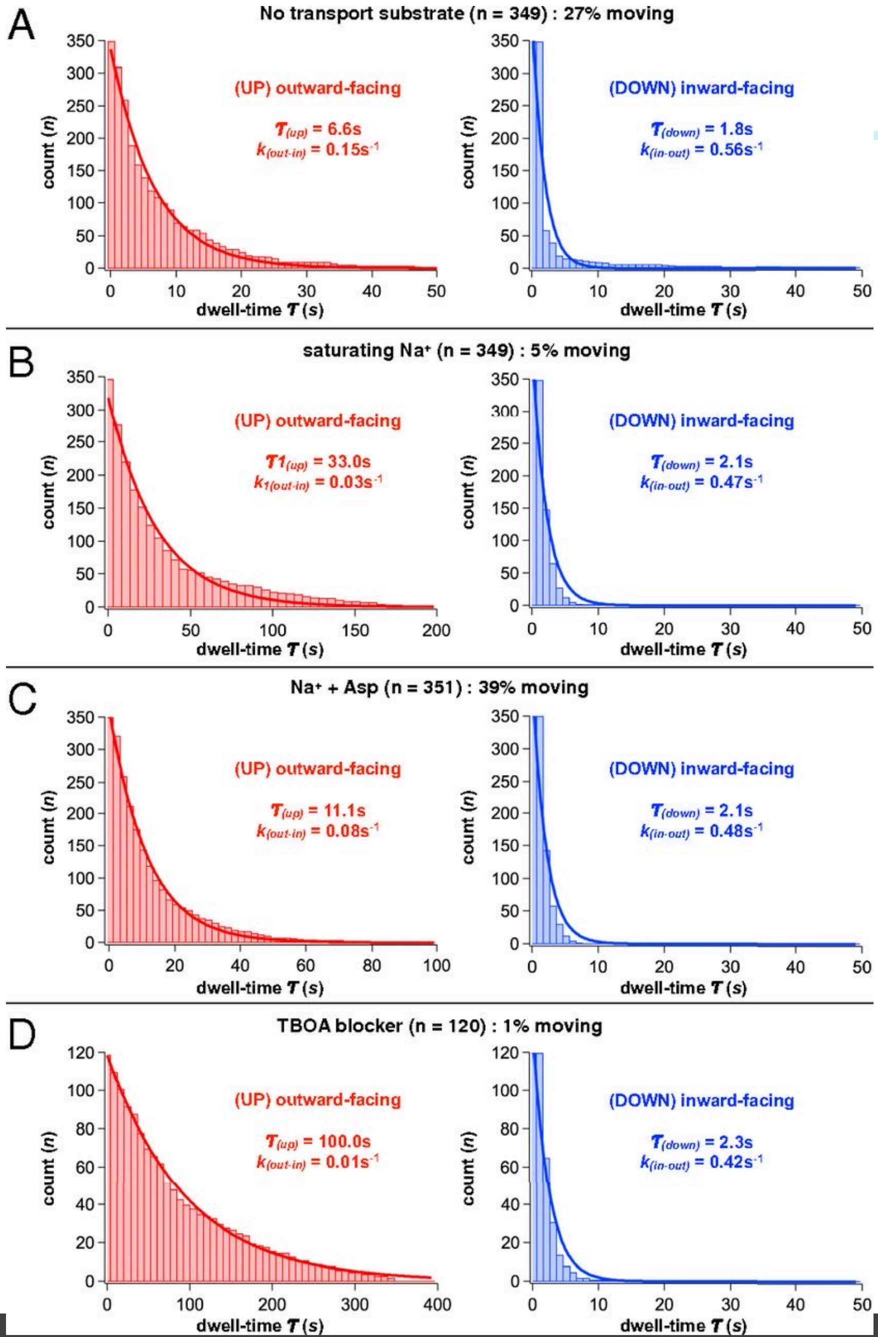
Ruan et al. PNAS 2017

Direct visualization of GLT$_{PH}$ elevator domain movements by HS-AFM



Ruan et al. PNAS 2017

QUANTIFICATION OF THE ELEVATOR DOMAIN DYNAMICS



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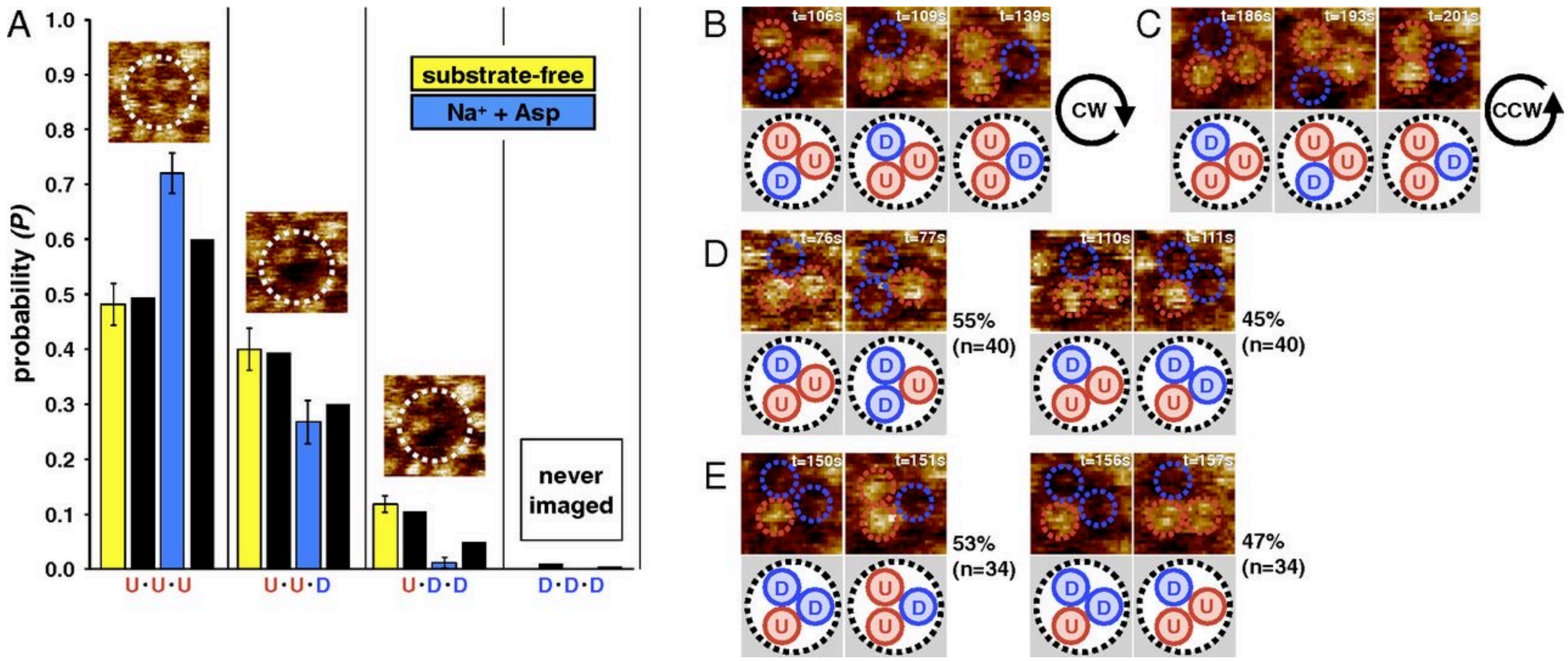
Institute of Biochemistry



Ruan et al. PNAS 2017

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