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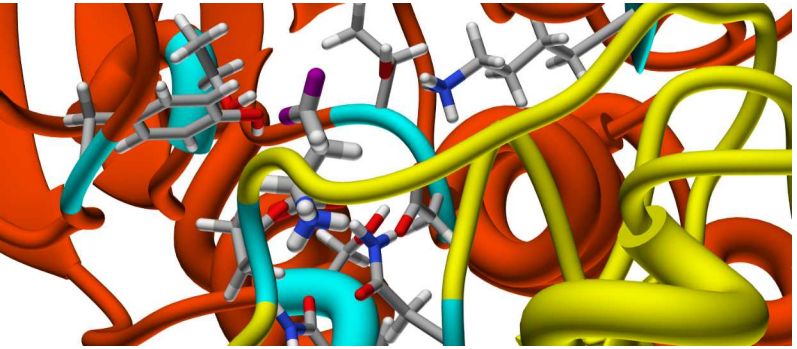

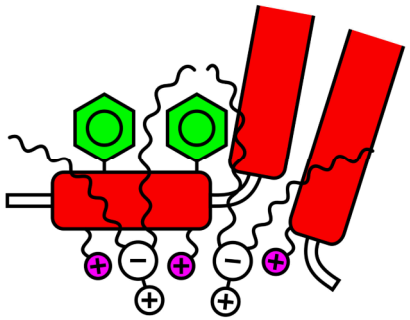

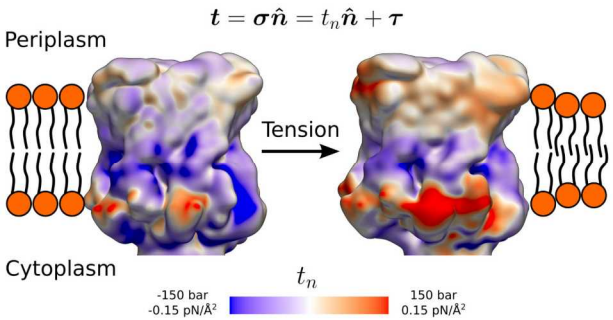

Computational "microscopy": Probing sub-cellular function with molecular modeling

Juan Vanegas

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Center for Biological and Material Sciences

Sandia National Laboratories

U.S. DEPARTMENT OF ENERGY

National Nuclear Security Administration

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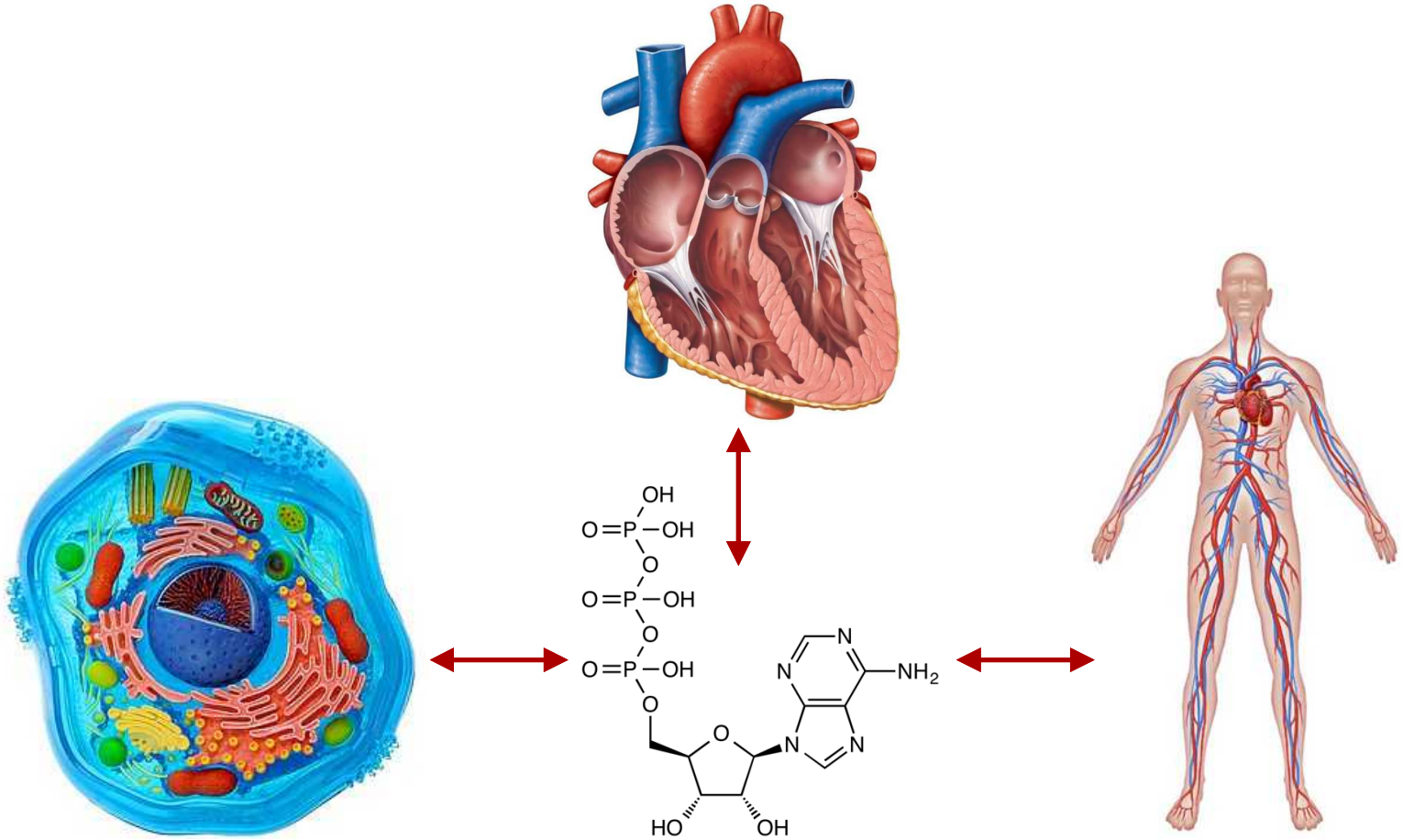
From molecules to organisms

- Biological function across the scales is intimately connected to the interaction of atoms and molecules at the nm level



Molecular machines

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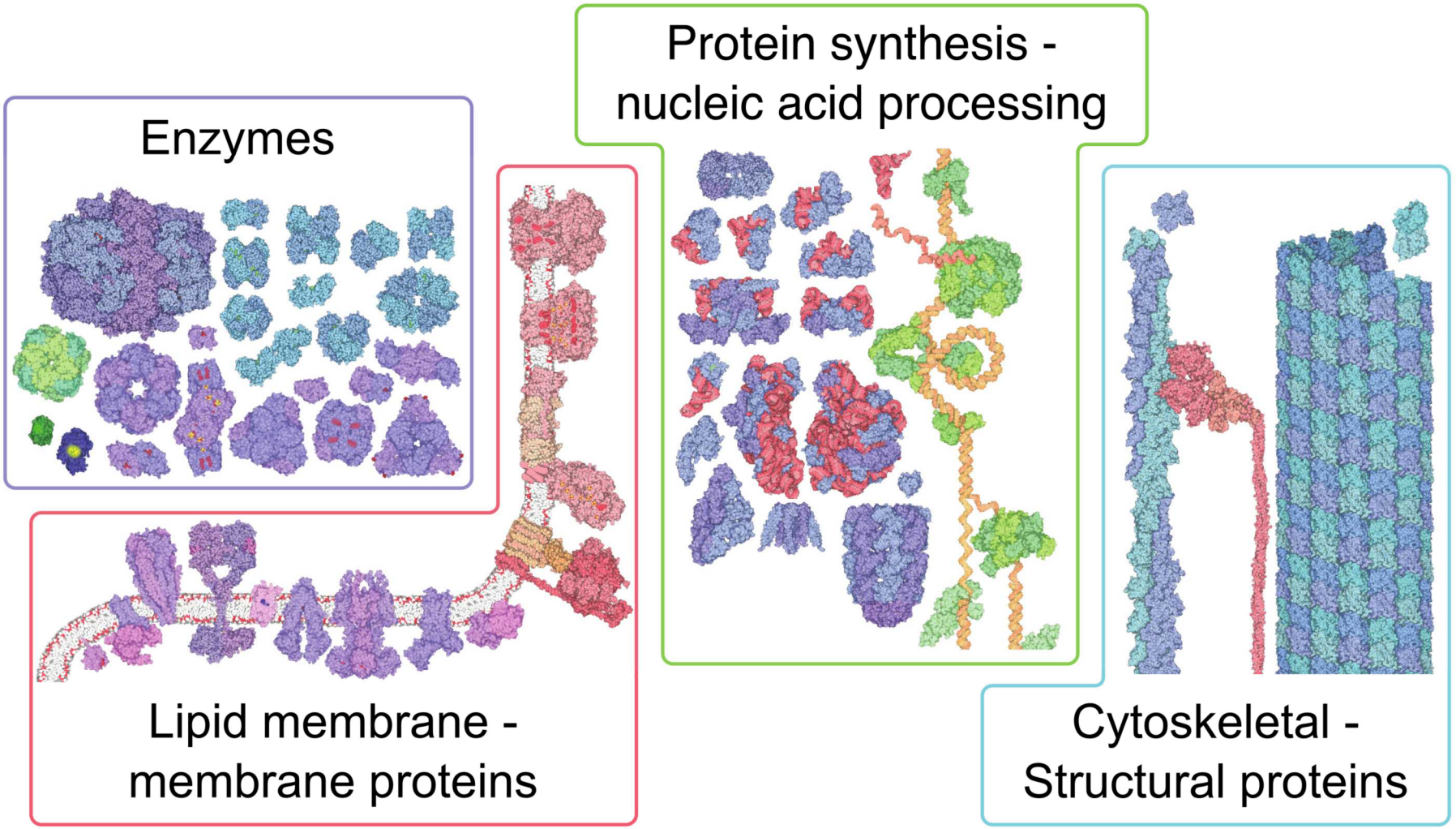


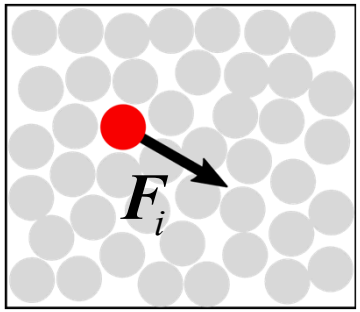
Images from rcsb.org

Computational "microscopy"

- How can we probe structure-function relationships in molecular systems with computational models?
- Molecular dynamics (MD) simulations
  - Classical trajectories of interacting point particles

$$\boldsymbol{F}_i = m_i \ddot{\boldsymbol{r}}_i = -\nabla_{\boldsymbol{r}_i} V(\boldsymbol{r}_1, \ldots, \boldsymbol{r}_N)$$

$$\boldsymbol{r}_i(t + \Delta t) = 2\boldsymbol{r}(t) - \boldsymbol{r}(t - \Delta t) + \ddot{\boldsymbol{r}}(t)\Delta t^2 + O(\Delta t^4)$$



  - Interaction potentials

$$V(\boldsymbol{r}_1, \ldots, \boldsymbol{r}_N) = V_{\text{VdW}} + V_{\text{Coul}} + V_{\text{bonds}} + V_{\text{angles}} + V_{\text{torsions}} + \ldots$$

$$\sum_{i=1}^{N} \sum_{j=i+1}^{N} \left(4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right)$$

$$\sum_{\text{angles}} \frac{u_i}{2} \left(\theta_i - \theta_{i,0} \right)^2$$

$$\sum_{\text{bonds}} \frac{k_i}{2} \left(l_i - l_{i,0} \right)^2$$

MD in action: Surfactant self-assembly

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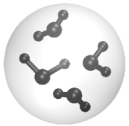
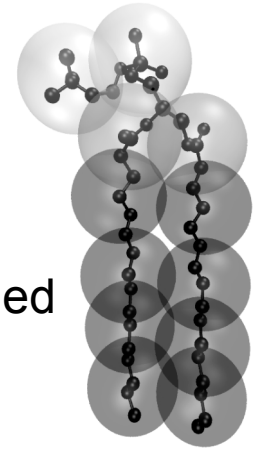
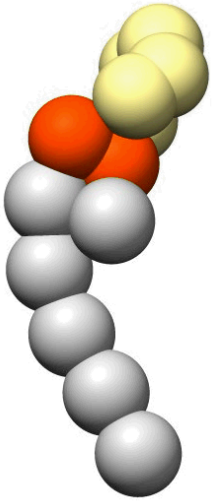


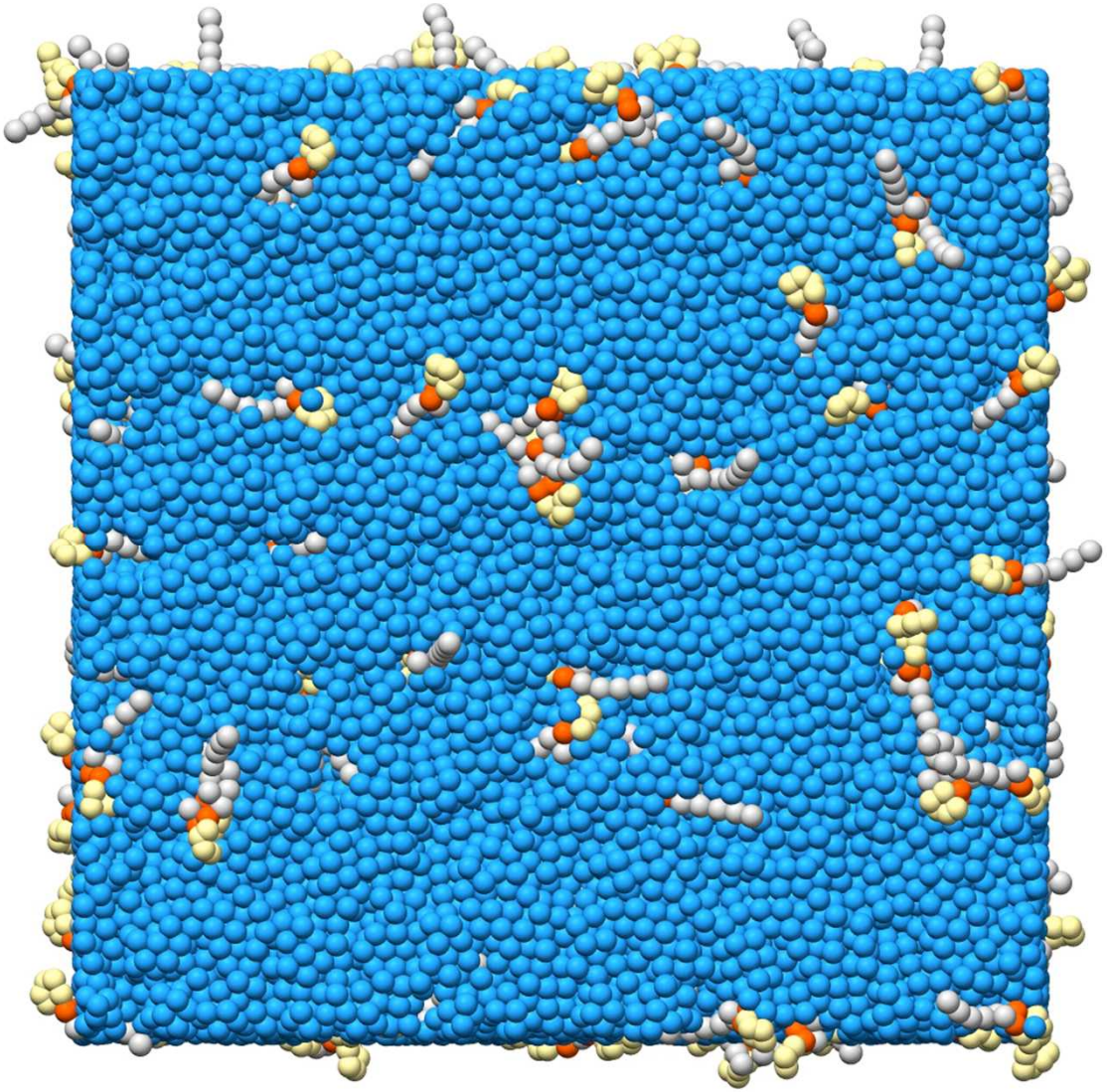


MARTINI Coarse-grained model

CG Water



CG Surfactant



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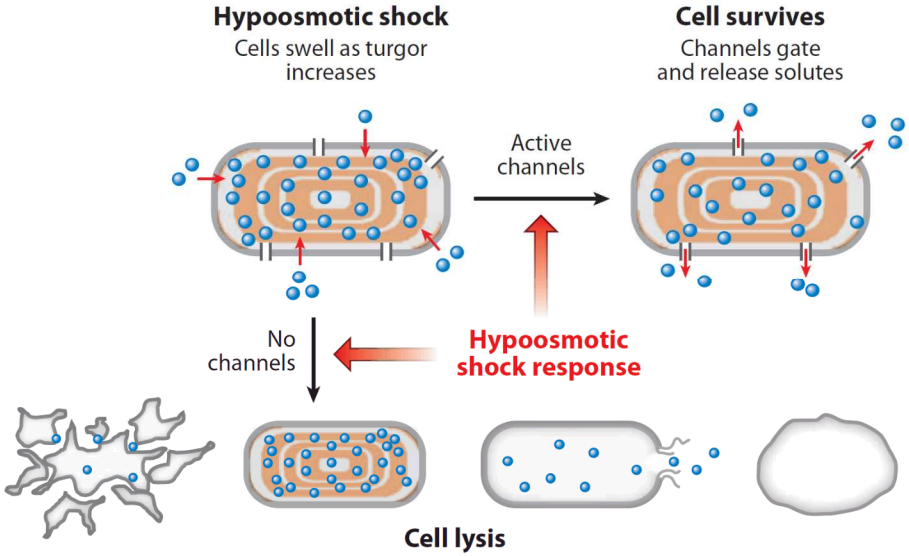
Mechanical signal transduction in the bacterial MscL channel

Mechanosensation in biology

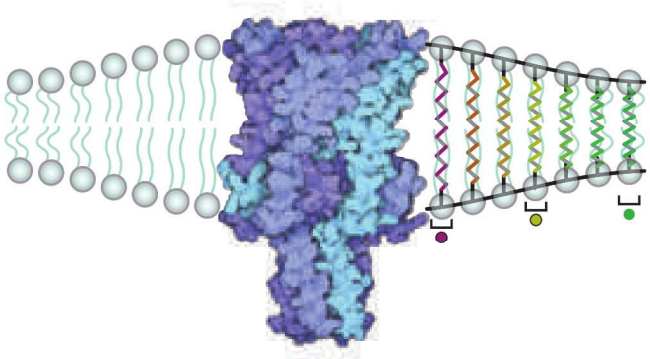
- Conversion of external/mechanical stimuli into electrochemical signals
  - Sensing touch, hearing (vibrations), movement (balance)
  - Sensing gravity (gravitropism)
  - Sensing changes in cell volume/shape (growth and development)
  - Vascular pressure control
  - Osmotic regulation



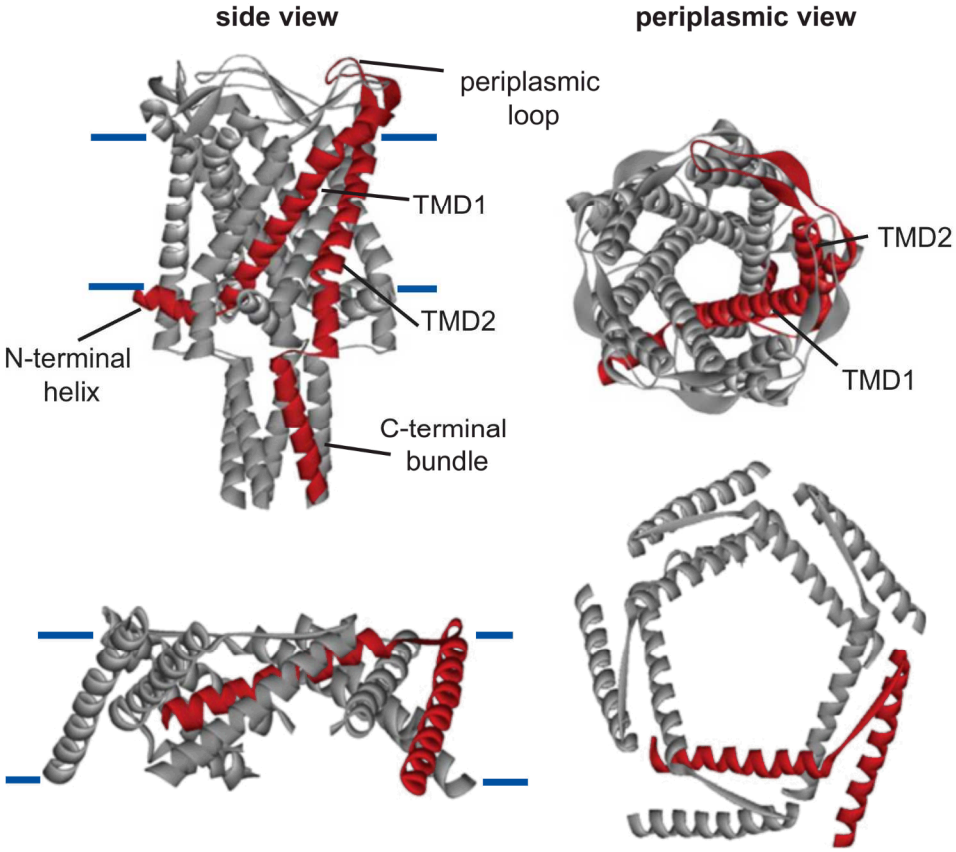
Naismith & Booth. *Annu. Rev. Biophys.* 41, 157–77 (2012)

Bacterial MscL channel

- Mechanosensitive channel of large conductance
  - Unselective channel with large opening – cell's last resort before lysis



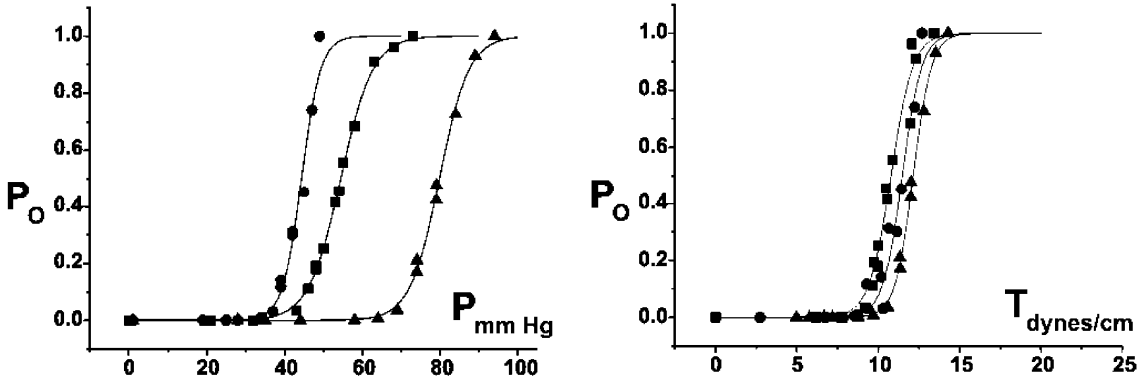
Phillips et al. *Nature.* 459, 379-385 (2009)



Iscla & Blount. *Biophys. J.* 103, 169-174 (2012)

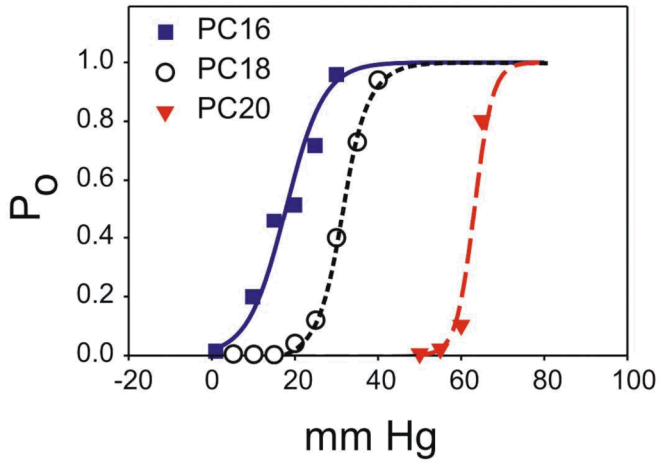
What actuates the channel?

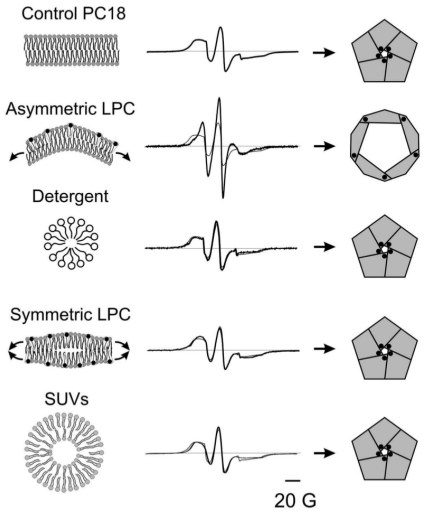
- Channel senses tension in the membrane, not pressure differences

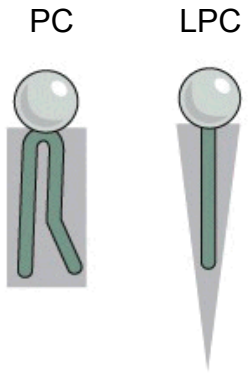


Moe & Blount. *Biochemistry.* 44, 12239-12244 (2005)

- Gating is sensitive to bilayer thickness and conical (lyso) lipids



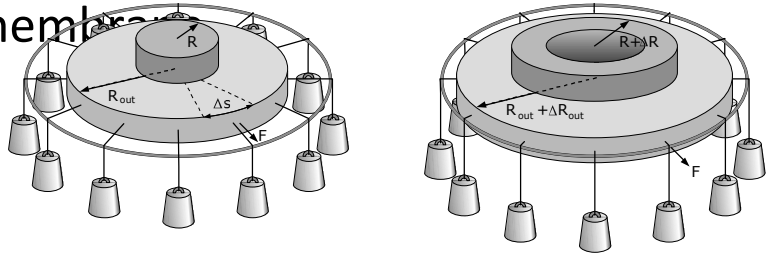




Perozo et al. *Nat. Struct. Biol.* 9, 696-703 (2002)

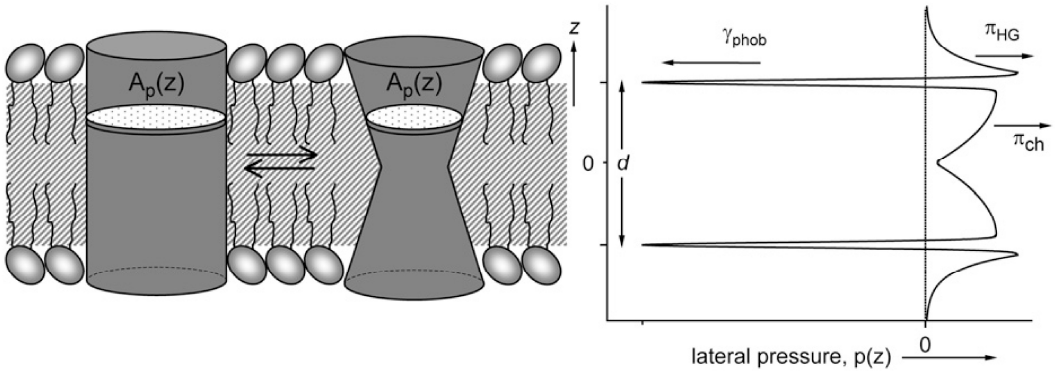
Local stress, elasticity and gating

- Gating energetics can be estimated by various models
  - Continuum models that characterize elastic deformation of membrane



Wiggins & Phillips. *Biophys. J.* 88, 880-902 (2005)
Phillips et al. *Nature.* 459, 379-385 (2009)
Reeves et al. *Phys. Rev. E* 78, 041901 (2008)

  - Internal stress or "lateral pressure" in the membrane may modulate



Marsh. *Biophys. J.* 93, 3884-3899 (2007)

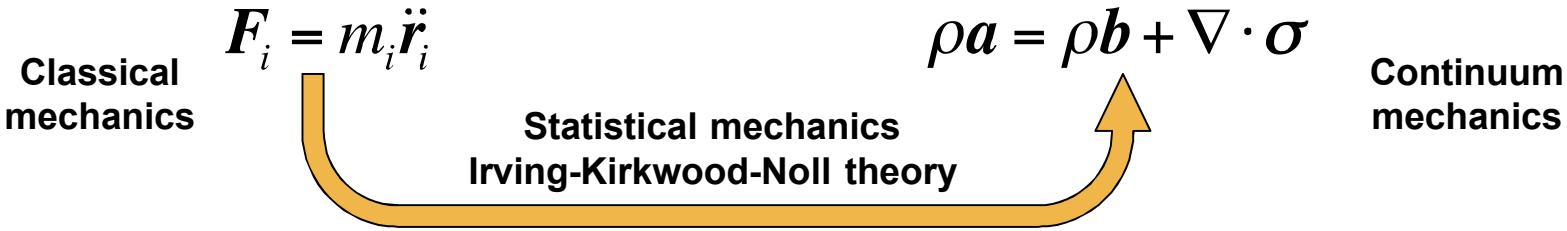
  - Changes in area due to pressure/surface tension

$$W = \int P(z)\Delta A(z)\,dz$$

Ollila et al. *Biophys. J.* 100, 1651-1659 (2011)
Gullingsrud & Schulten. *Biophys. J.* 86, 3496-3509 (2004)

Bridging continuum & molecular models

- Continuum fields can be obtained from particle-based MD simulations through ensemble averaging



$$\nabla \cdot \boldsymbol{\sigma} = \nabla \cdot \left(\sum_{i=1}^{N} \left\langle m_i \boldsymbol{v}_i \otimes \boldsymbol{v}_i \delta(\boldsymbol{r}_i - \boldsymbol{x}) \right\rangle \right) - \sum_{i=1}^{N} \left\langle \boldsymbol{F}_i \delta(\boldsymbol{r}_i - \boldsymbol{x}) \right\rangle$$

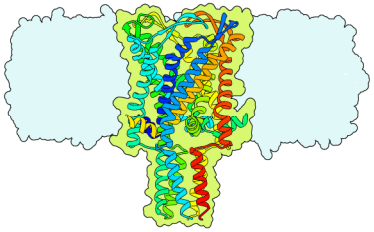
- Microscopic stress field (3D) from MD simulations (time avg)

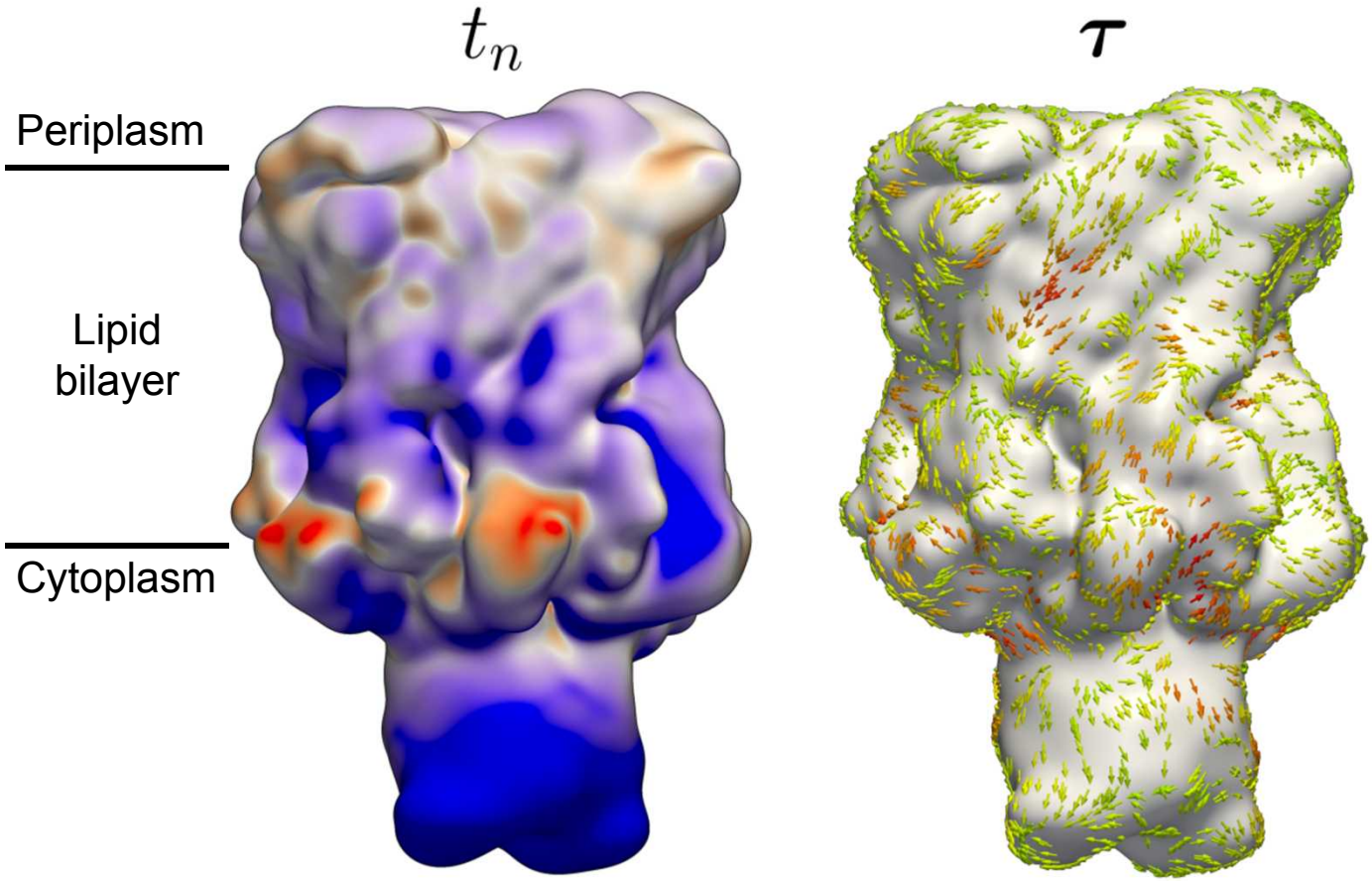
$$\boldsymbol{\sigma} = \frac{1}{N_\tau} \sum_{t}^{N_\tau} \left[-\sum_{i}^{N} m_i \boldsymbol{v}_{i(t)} \otimes \boldsymbol{v}_{i(t)} w(\boldsymbol{r}_{i(t)} - \boldsymbol{x}) + \frac{1}{2} \sum_{\substack{i,j \\ i \neq j}}^{N} \boldsymbol{f}_{ij(t)} \otimes \boldsymbol{r}_{ij(t)} B(\boldsymbol{x}; \boldsymbol{r}_{i(t)}, r_{j(t)}) \right]$$

Vanegas, Torres-Sanchez, & Arroyo. *J. Chem. Theory Comput.* 10, 691-702 (2014)
Torres-Sanchez, Vanegas, & Arroyo. *Phys. Rev. Lett.* 114, 258102 (2015)

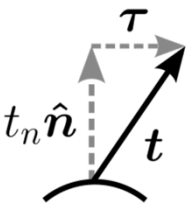
Local stress on the surface of MscL

- MscL simulated in a membrane patch (~500 lipids)
- 3D stress visualized as traction vector on surface





$$\boldsymbol{t} = \boldsymbol{\sigma}\hat{\boldsymbol{n}} = t_n\hat{\boldsymbol{n}} + \boldsymbol{\tau}$$



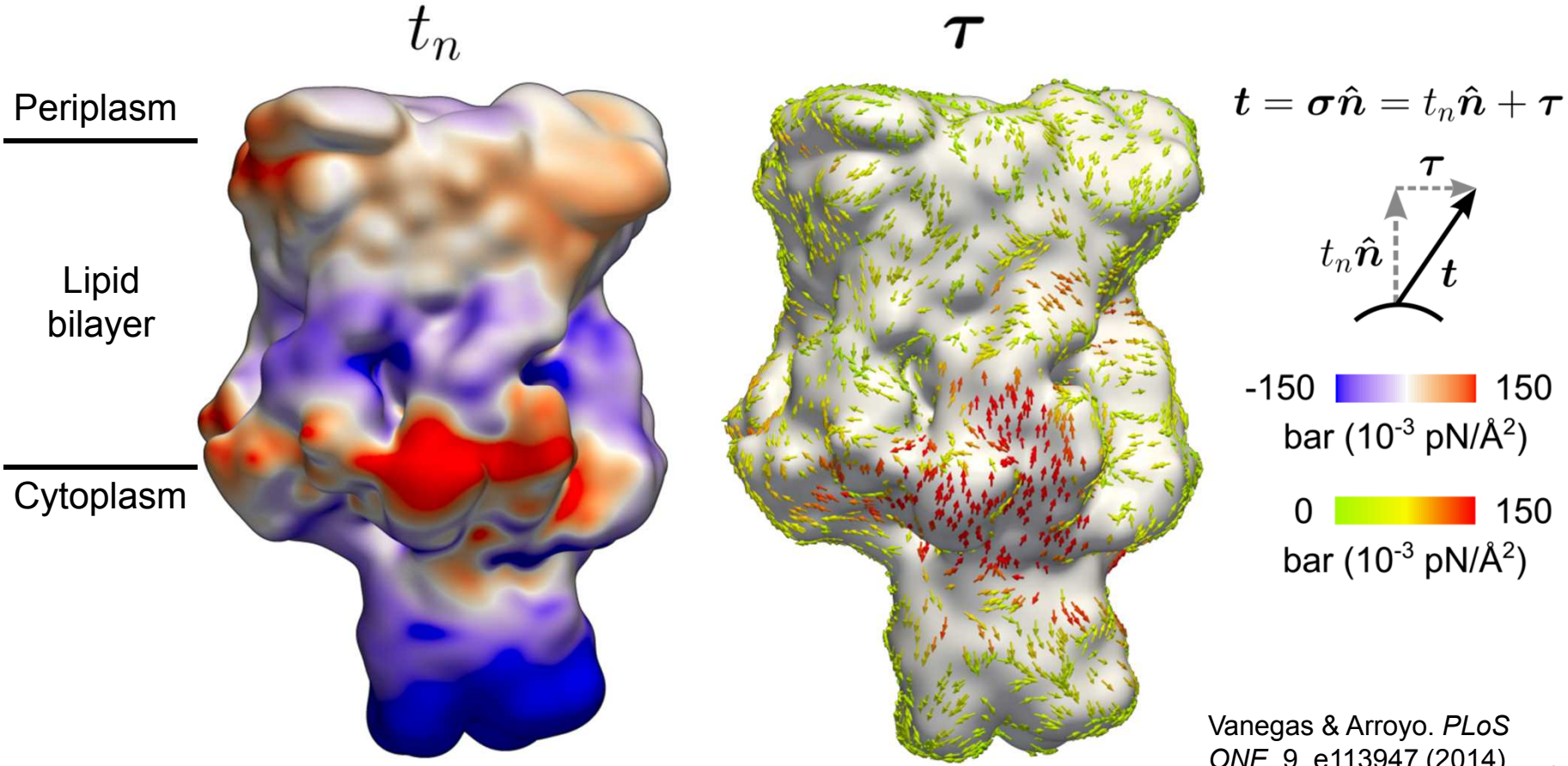
-150 150
bar (10^{-3} pN/Å^2)

0 150
bar (10^{-3} pN/Å^2)

Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

Stress “hot-spots” under tension

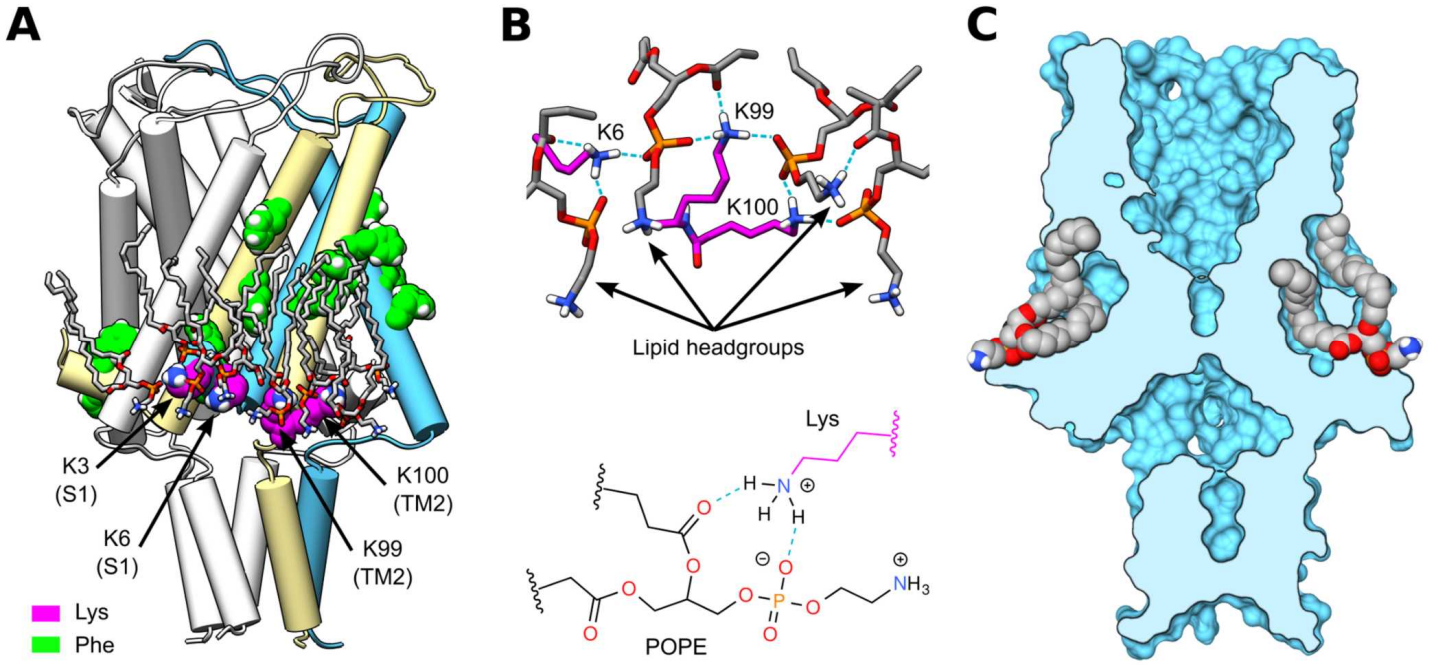
- Membrane under tension (P_L=-20 bar, ~6 % lateral stretch)
- Channel remains in closed quasi-equilibrium state



Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

Lipid binding on the cytoplasmic side

- Two lipids associate with each MscL monomer at specific sites
  - **Locations match stress hot-spots**
  - + Charged lysines on C-terminus and TM2 (K3, K6, K99, K100) interact with electronegative oxygens on the POPE lipid headgroup
  - Lipid tails accommodate in hydrophobic cavities – preference for 16 vs 18 carbon tail (8 out of 10) of POPE



Vanegas & Arroyo. *PLoS ONE*. 9, e113947 (2014)

Energetics of lipid unbinding

- What is the interaction energy between bound lipids and MscL?
  - Lipid unbinding kinetics under a pulling force

$$d\phi/dt = (1-\phi)k_d \Rightarrow \phi(t) = 1 - e^{-k_d t}$$

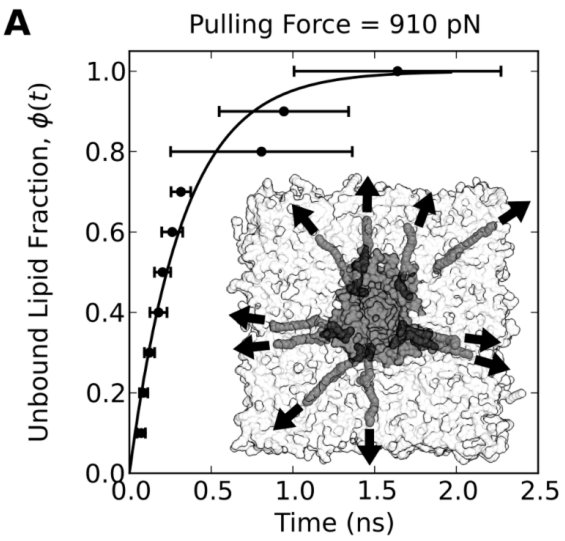
  - Simple Arrhenius-Bell model (assuming electrostatic interaction dominates)

$$E_b(f) = E_0 - fx_b$$

$$k_d(f) = 1/t_d e^{-(E_0 - fx_b)/k_B T}$$

$$k_d(f) = 1/t_{off} e^{fx_b/k_B T}$$

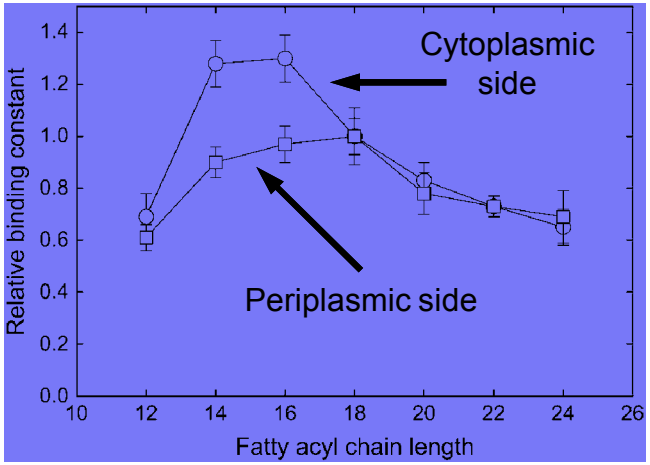
  - Energy barrier in range of 10-13 $k_B T$



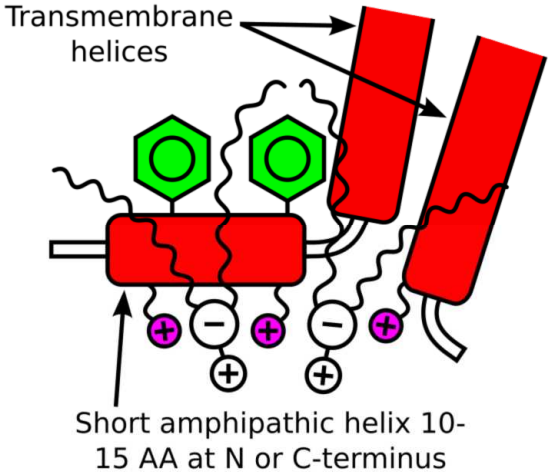
Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

A resilient association

- Association of lipids with MscL observed experimentally
  - Higher affinity for cytoplasmic side and 16 carbon tails
- Lipid binding to MscL is simple and robust
  - Charged interactions + geometric confinement (lipid tail)



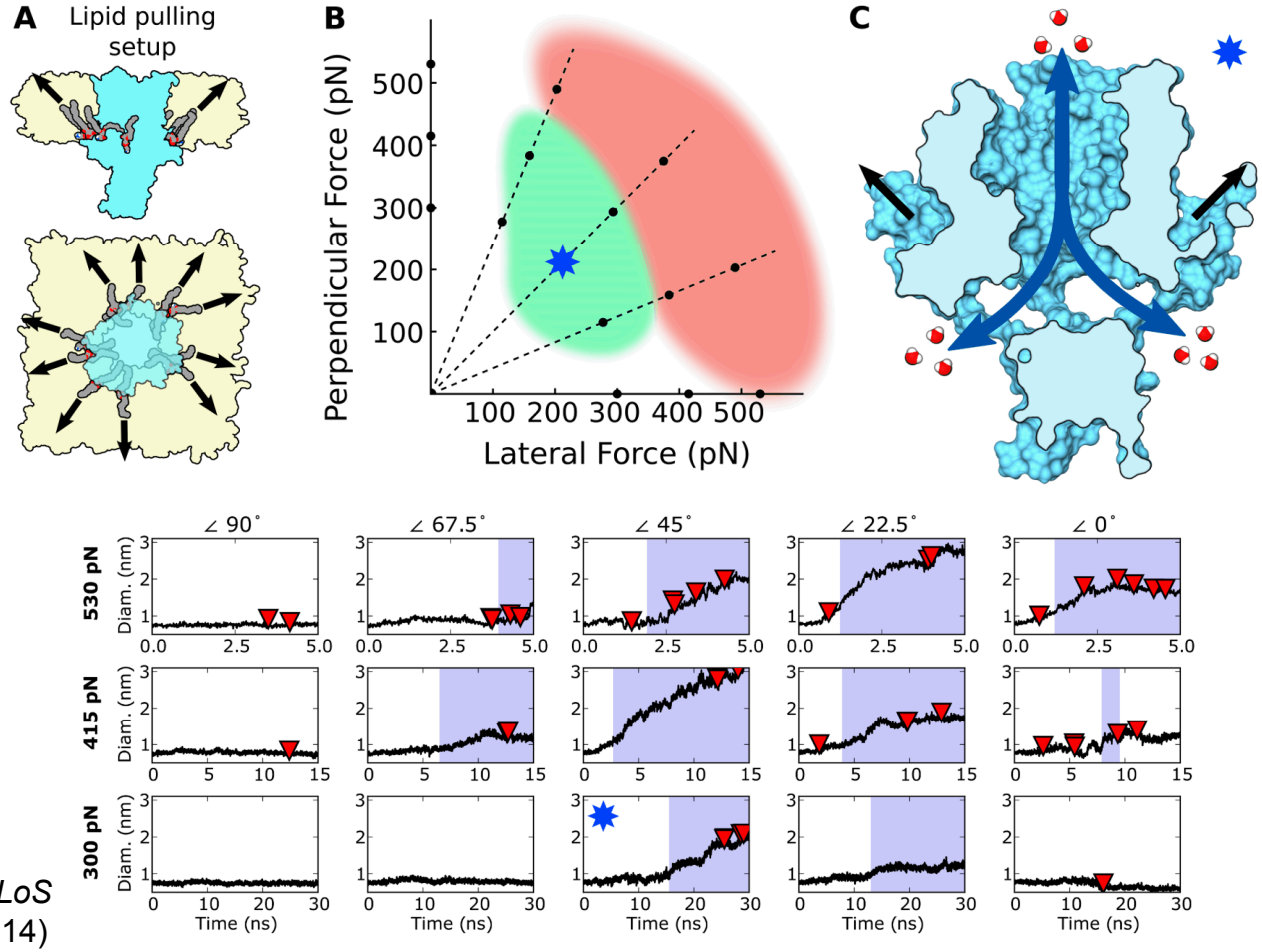
Powl et al. *Biophys. J.* 93, 113-122 (2007)



Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

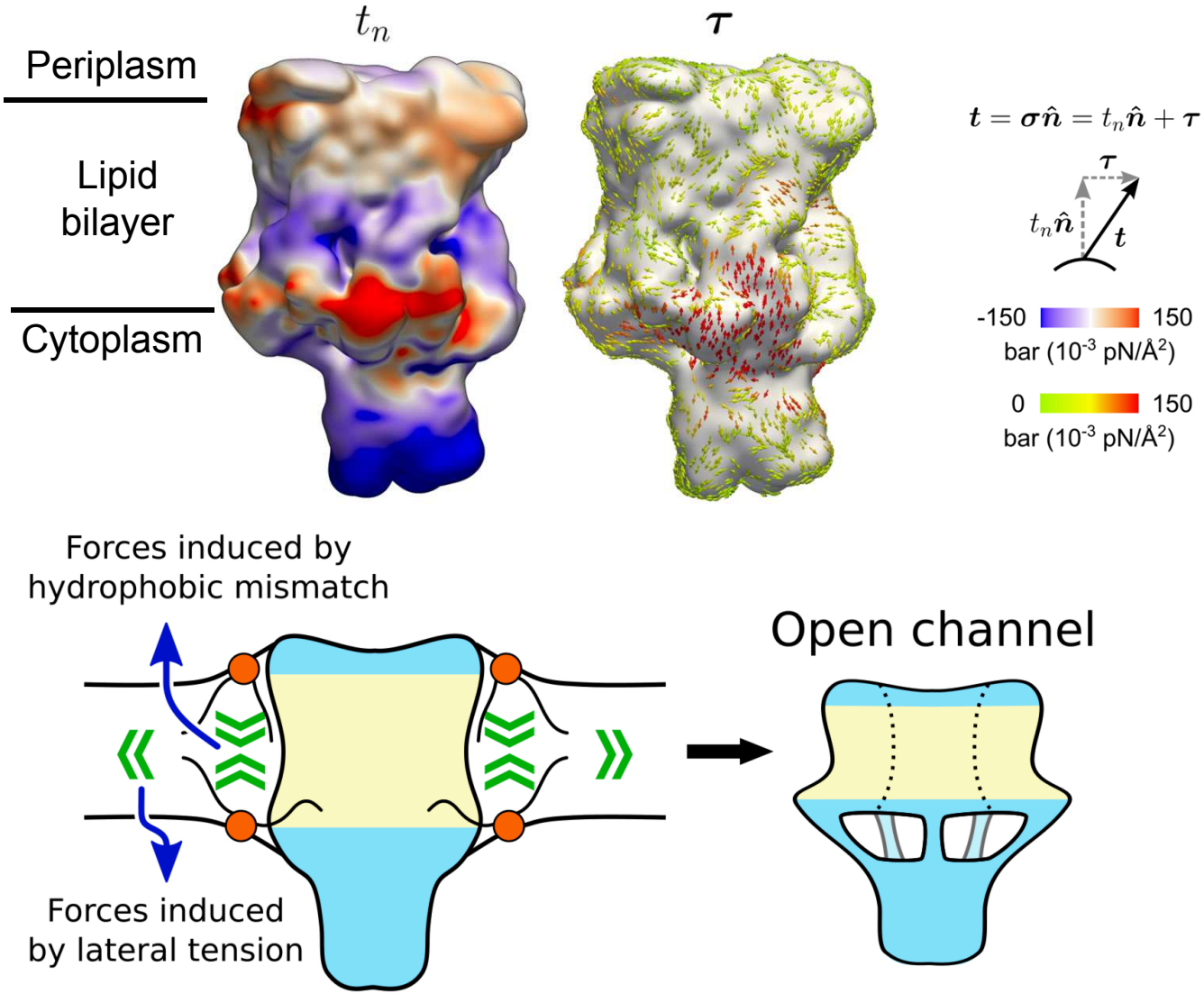
Actuating the channel in steered MD

- MscL gating by membrane tension is slow (microseconds)
  - Can the channel be actuated by pulling on the tightly-bound lipids?



Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

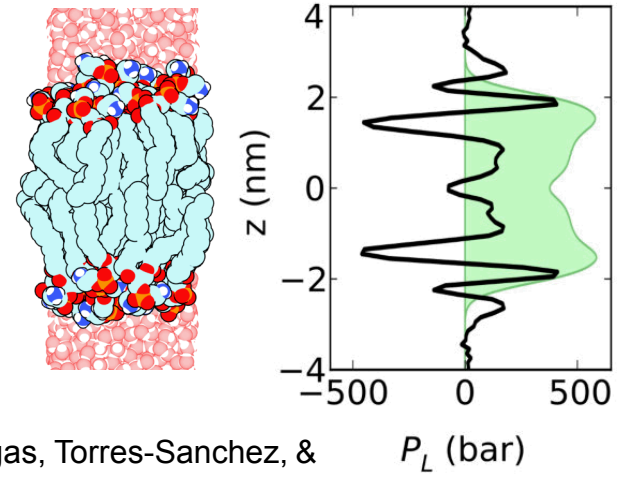
A framework for force transduction



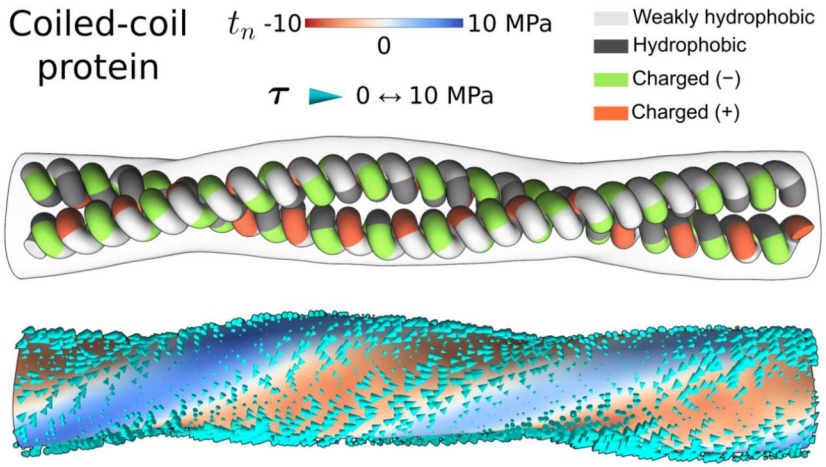
Vanegas & Arroyo. *PLoS ONE.* 9, e113947 (2014)

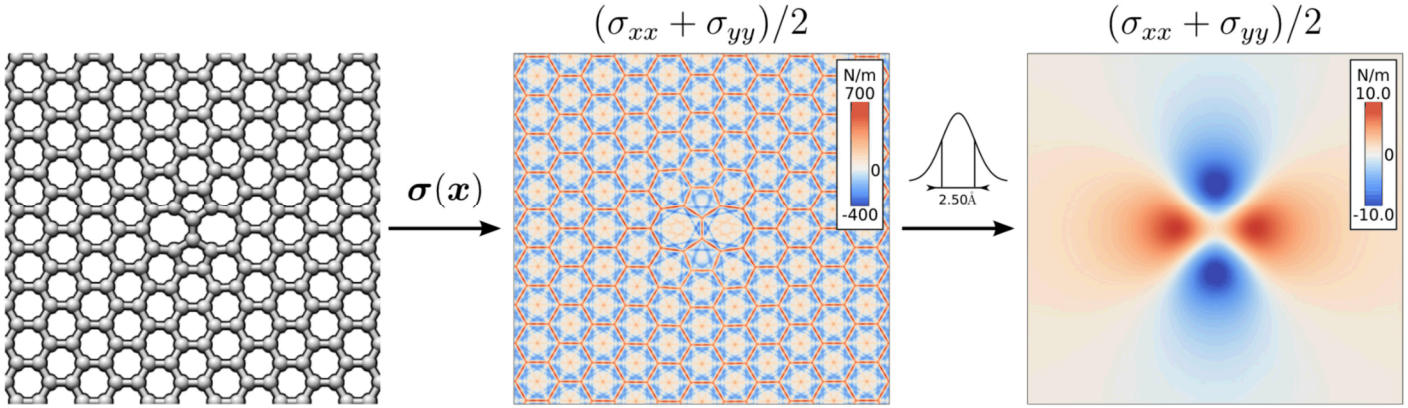
Other applications of local stress

- More info/code available at mdstress.org



Vanegas, Torres-Sanchez, & Arroyo. *J. Chem. Theory Comput.* 10, 691-702 (2014)



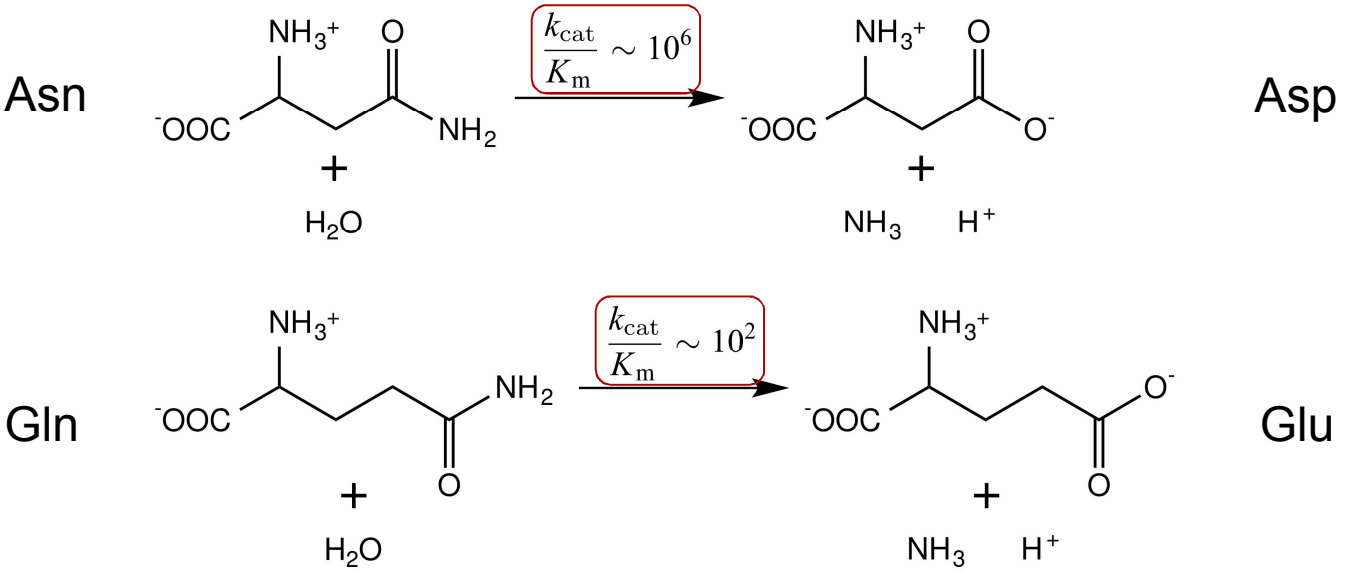


Torres-Sanchez, Vanegas, & Arroyo. *Phys. Rev. Lett.* 114, 258102 (2015)

Catalysis and specificity in L-Asparaginase, an anticancer enzyme

L-Asparaginase: Starving cancer cells

- Drug treatment against acute lymphoblastic leukemia
- L-ASP degrades amino acids Asn and Gln – starves certain cancer cells that can't synthesize these



- L-ASP type II from *E. coli* (Elspar ®) is most widely used clinically

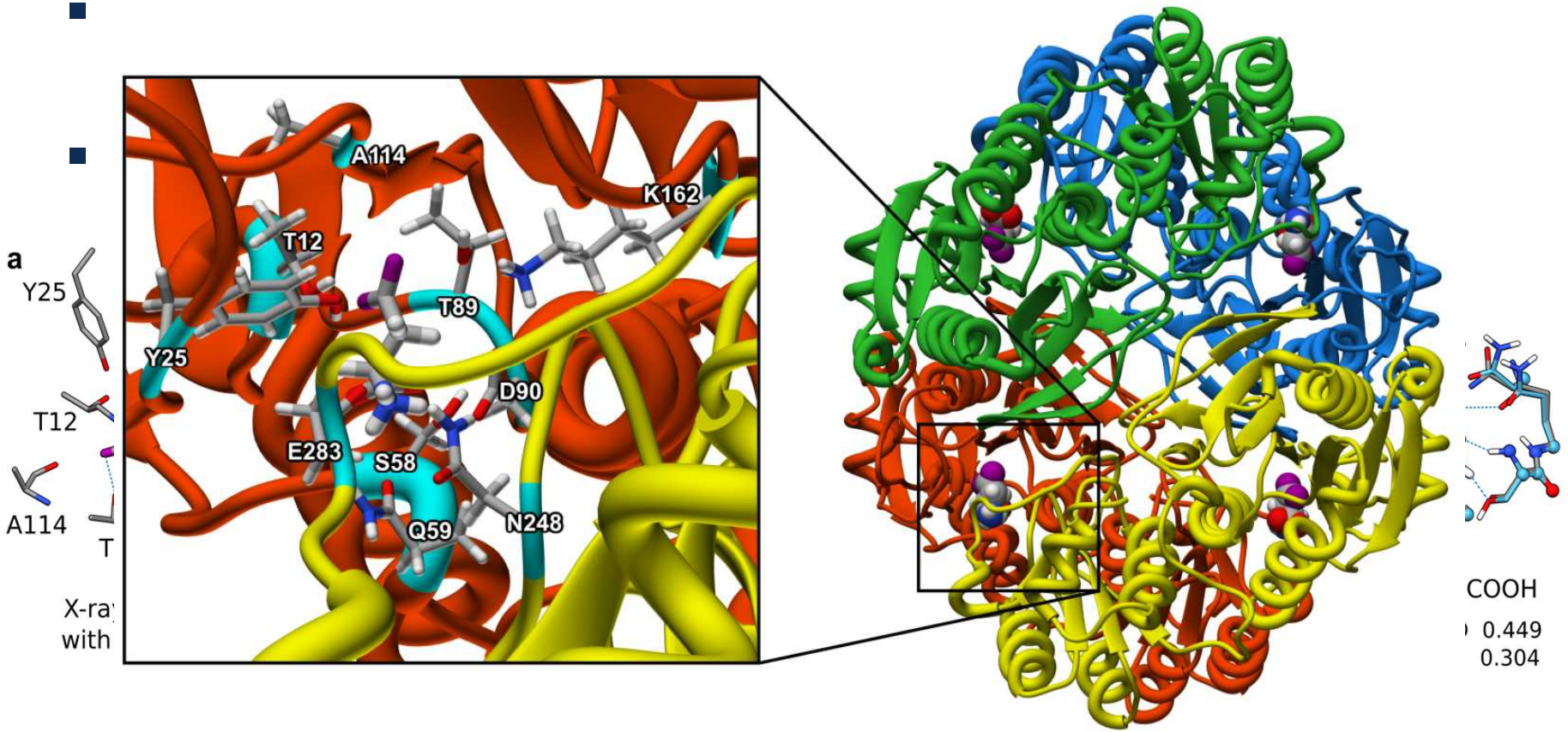
Improving L-ASP's anticancer properties

Sandia National Laboratories

- *E. coli* L-ASP has low catalytic rate – needs high doses for effective treatment (toxic side effects)
- Secondary glutaminase activity is also problematic
  - Linked to toxic side effects – precludes completion of treatment
  - Some cancers require degradation of both Asn and Gln
- How to optimize L-ASP for cancer treatment?
  - Tune substrate specificity – modulate glutaminase activity
  - Improve catalytic rate
- Despite 40+ years of clinical use, complete reaction mechanism remains unknown – limits rational engineering

Hidden proton in L-ASP's product

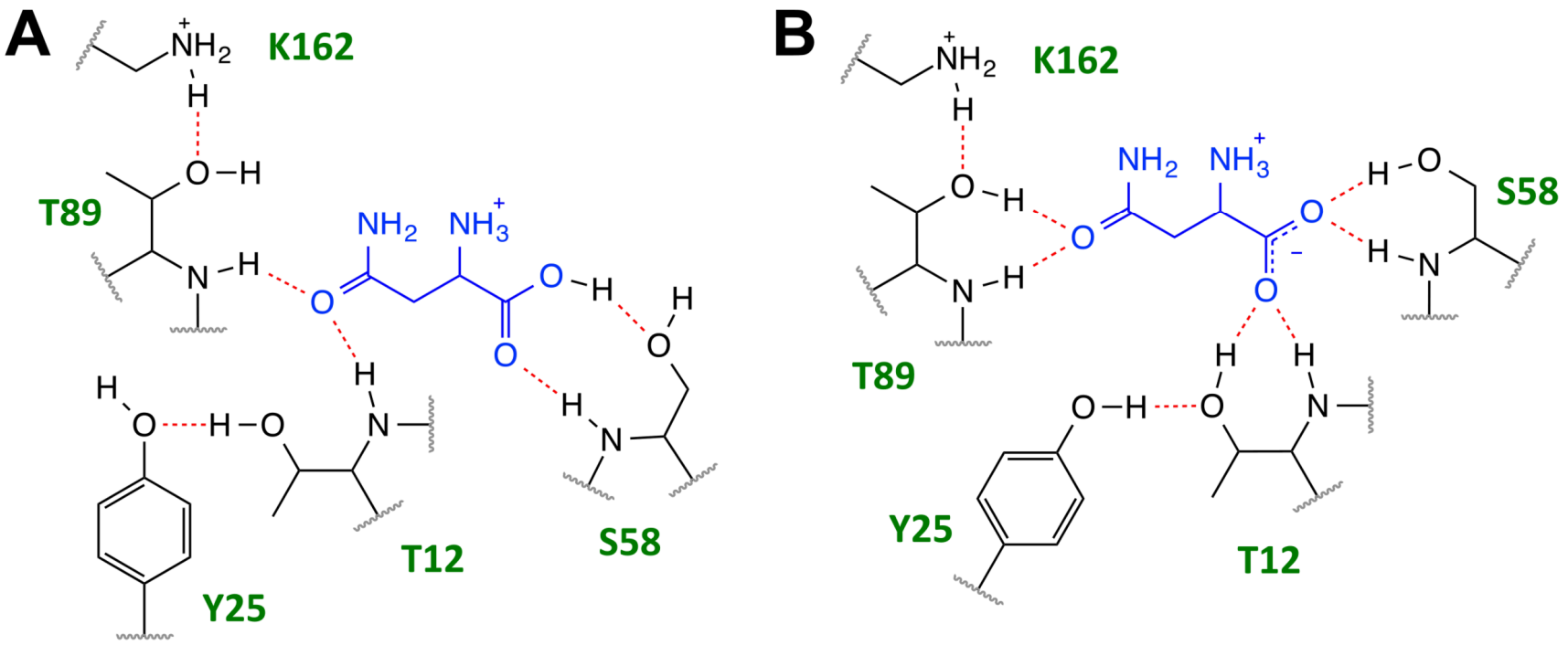
- Deamidation mechanisms rely heavily on crystal structure (PDB 1NNS) of L-ASP with the reaction product Asp



Anishkin, Vanegas et al. *J. Mol. Biol.* 427, 2867-2885 (2015)

Substrate vs product conformations

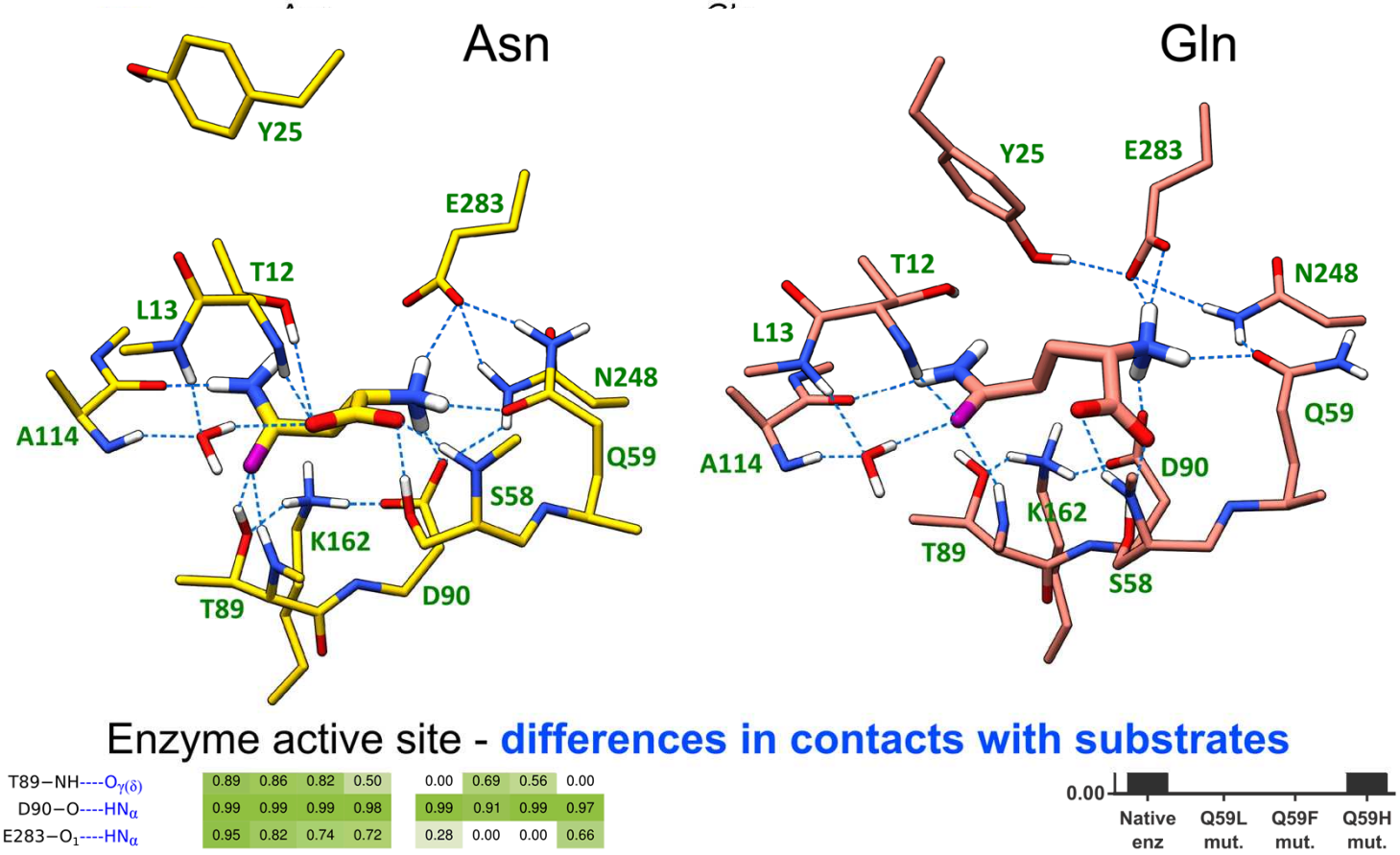
- Asn substrate quickly rearranges in L-ASP active site
  - Unprotonated α-COO^- in Asn substrate induces large reorientation
  - T12, S58 and T89 “clamp” onto oxygens of Asn



Anishkin, Vanegas et al. *J. Mol. Biol.* 427, 2867-2885 (2015)

Asn vs Gln: Tuning L-ASP's activity

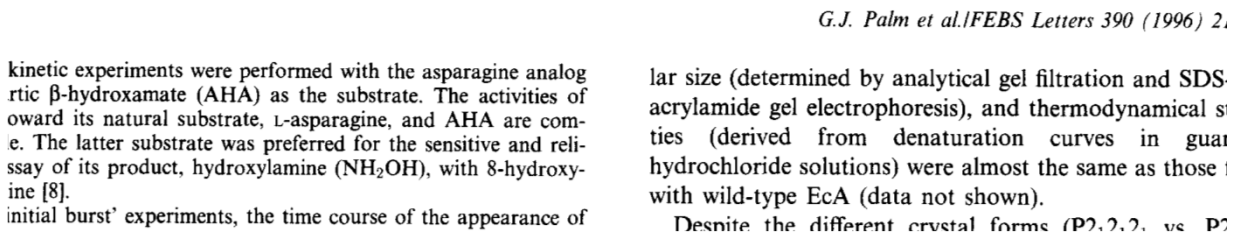
- Can we alter L-ASP's active site to eliminate Gln degradation?
- Additional methylene in Gln affects substrate orientation



Chan et al. *Blood.* 123, 3596-5606 (2014); Anishkin, Vanegas et al. *J. Mol. Biol.* 427, 2867-2885 (2015)

L-ASP's mechanism puzzle

- Experiments strongly support double displacement mechanism with an acyl-enzyme covalent intermediate



G.J. Palm et al./FEBS Letters 390 (1996) 2

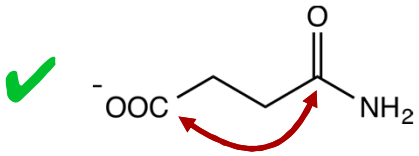
kinetic experiments were performed with the asparagine analog rtic β-hydroxamate (AHA) as the substrate. The activities of oward its natural substrate, L-asparagine, and AHA are com- e. The latter substrate was preferred for the sensitive and reli- ssay of its product, hydroxylamine (NH_2OH), with 8-hydroxy- ine [8].
initial burst' experiments, the time course of the appearance of

lar size (determined by analytical gel filtration and SDS- acrylamide gel electrophoresis), and thermodynamical s ties (derived from denaturation curves in gua hydrochloride solutions) were almost the same as those with wild-type EcA (data not shown).
Despite the different crystal forms (P2₁2₁2₁ vs. P

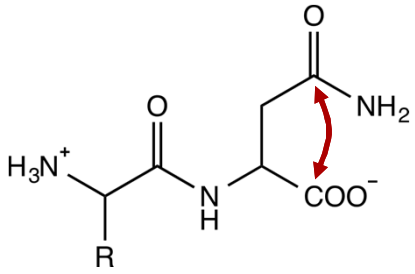
Palm et al. *FEBS Lett.* 390, 211-216 (1996)

- L-ASP's threonine T12 and T89 are possible nucleophiles
  - T89V and T12A single mutants both reduce k_{cat} ~100,000 fold
- Absolute requirement of carboxyl group 2-3 carbons from amide

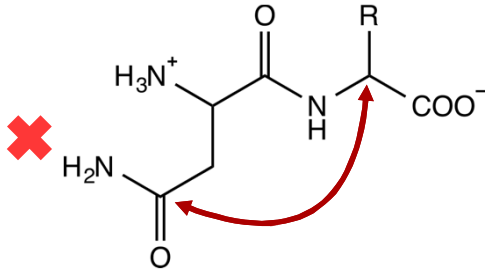
succinamate



NH_3^+-Xxx-Asn-COO^-

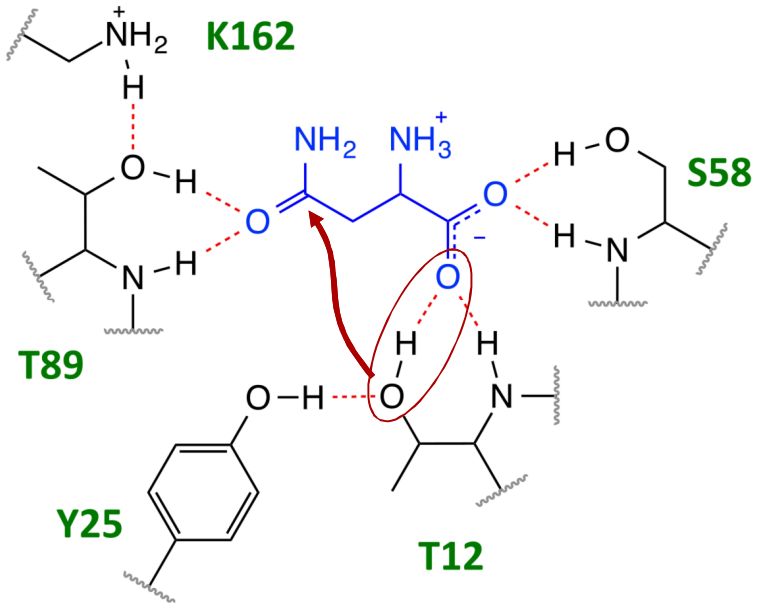


NH_3^+-Asn-Xxx-COO^-



Initial stages of the reaction

- Focus on nucleophilic attack by T12 onto γ-carbon of Asn



- Where does T12-OH proton go? Can substrate α-COO^- accept it?
- Is the acyl-enzyme intermediate stable?

Anishkin, Vanegas et al. *J. Mol. Biol.* 427, 2867-2885 (2015)

Probing the reaction with QM

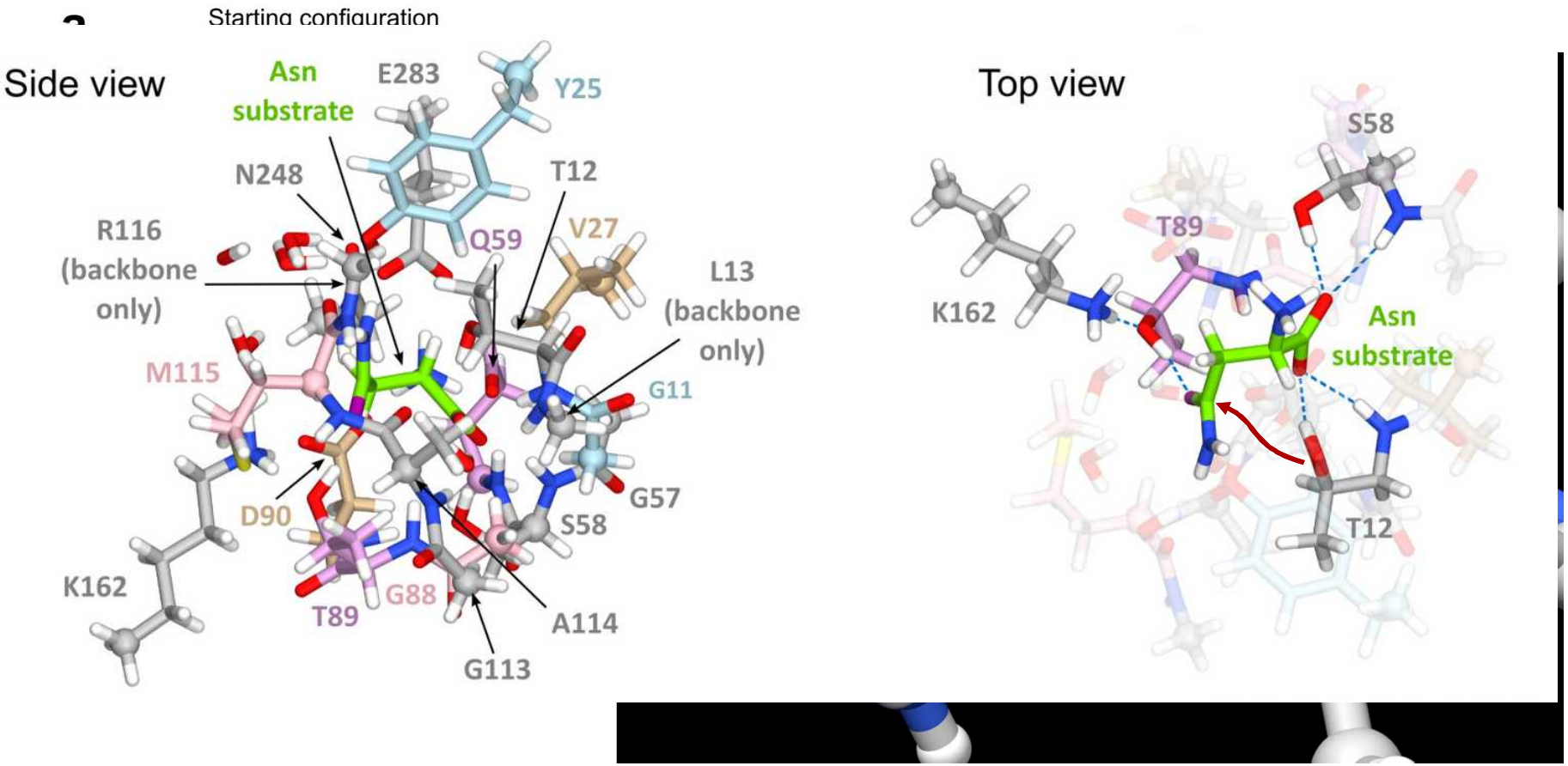
- Classical MD simulations not suitable to follow chemical rxns
- Use QM self-consistent field methods (Hartree-Fock, density functional theory)
  - Born-Oppenheimer approx. – separate wavefunction into nuclear and electronic components
  - Treat heavy nuclei as fixed point particles – focus on electrons
  - Iteratively solve time-independent Schrödinger eqtn. $H_{elec}\Psi_0 = E_{elec}\Psi_0$
  - *Ab initio* MD – classical trajectories of nuclei in quantum potential

$$\boldsymbol{F}_I = M_I\ddot{\boldsymbol{R}}_I = -\nabla_I V^{BO}(\boldsymbol{R}_I) = -\nabla_I \min_{\Psi_0}\left\{\left\langle \Psi_0 \middle| H_{elec} \middle| \Psi_0 \right\rangle\right\}$$

- Explore kinetics with *ab initio* MD based on regular MD results
- Calculate relative energies from stationary structures at various steps of reaction

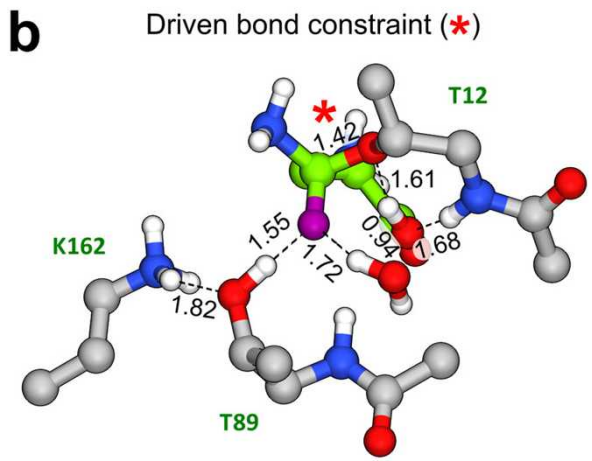
Direct nucleophilic attack by T12

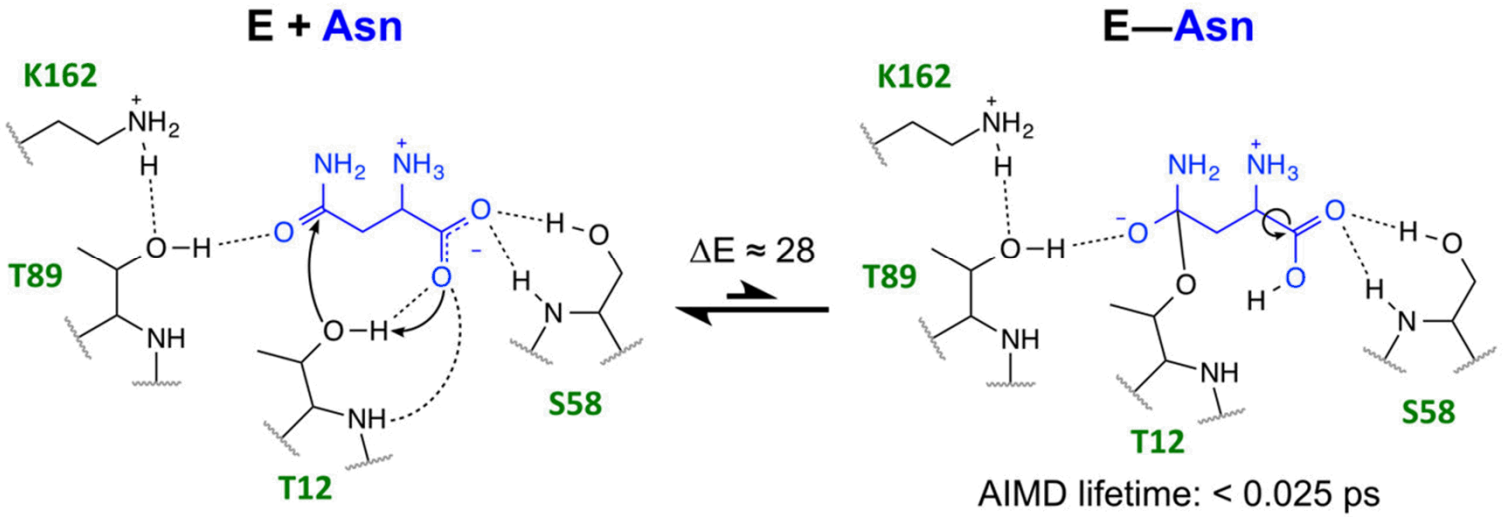
- QM system = active site amino acids + 4 waters – 239 atoms (α-carbons fixed)



Direct nucleophilic attack by T12

- T12-OH proton spontaneously transfers to substrate α-COO^-
- Enzyme-substrate covalent intermediate unstable (<0.025 ps)
- Relative energy of optimized end states ~28 kcal/mol





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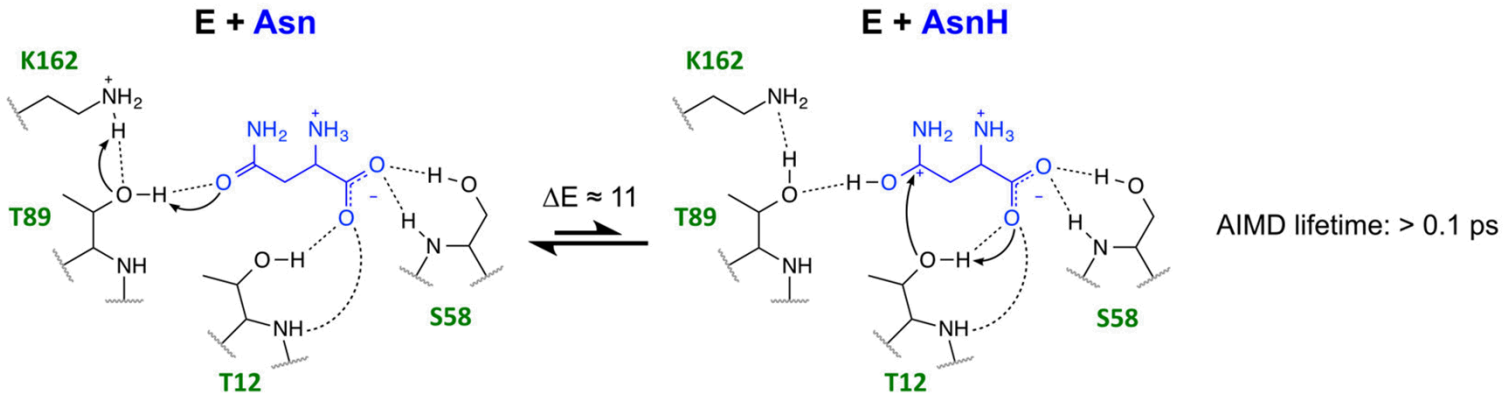
Pre-protonation stabilizes reaction

- Two-step attack by T12 is more kinetically favorable
  - Amide oxygen is first protonated by K162-T89 proton bridge



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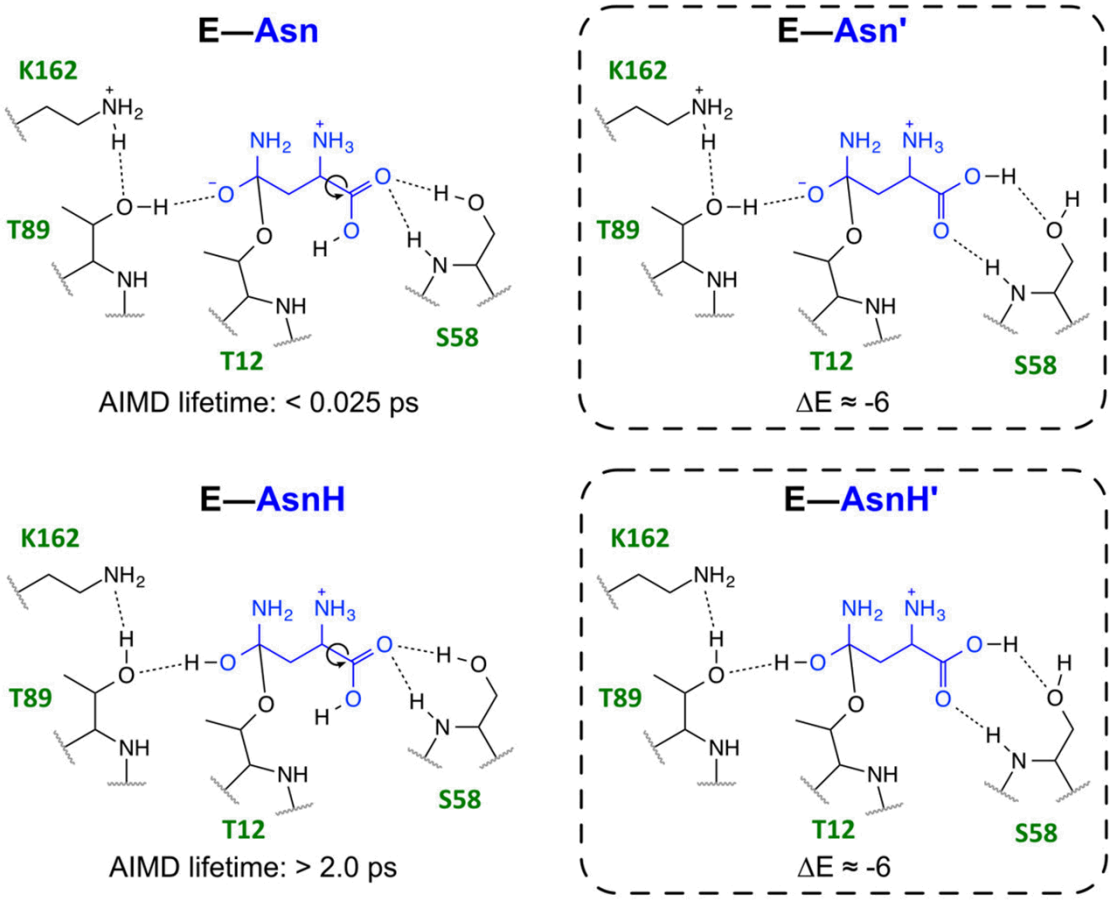
α-COOH prefers paired H-bonding

- Protonated α-COOH prefers a “paired” H-bonding pattern with S58 instead of being “clamped”



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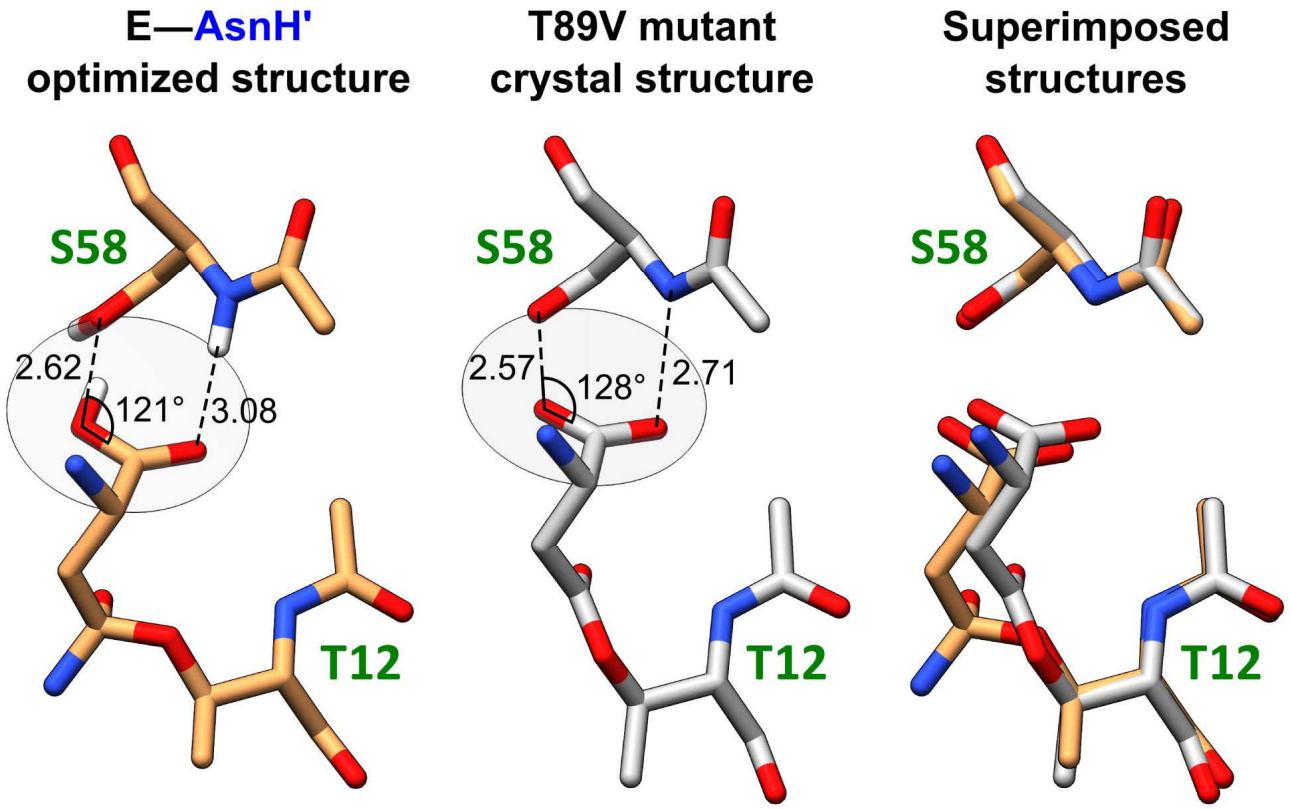
QM structure similar to T89V mut.

- E-AsnH' configuration similar to acyl-enzyme intermediate in T89V crystal structure (PDB 4ECA)
  - WT + mutant structures resemble intermediates, not initial state



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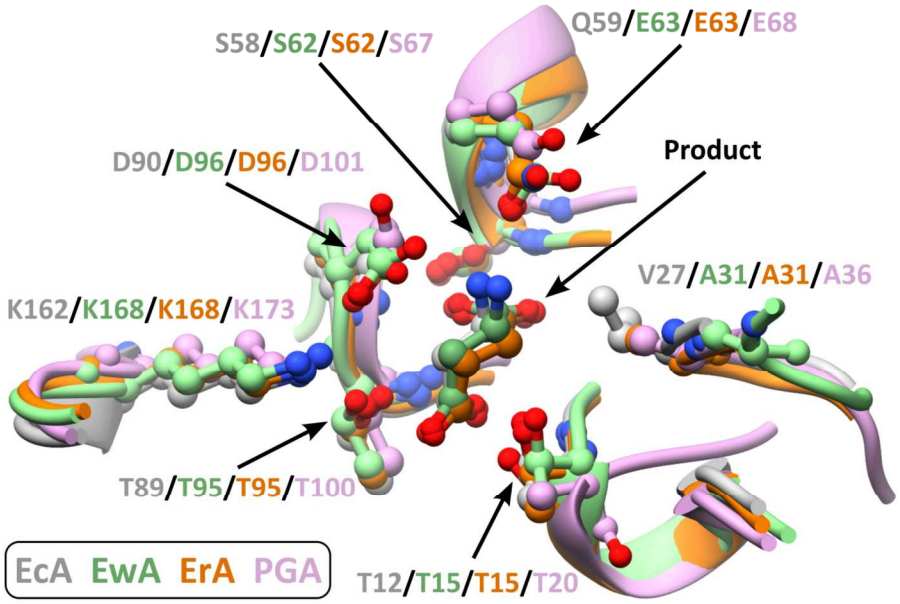
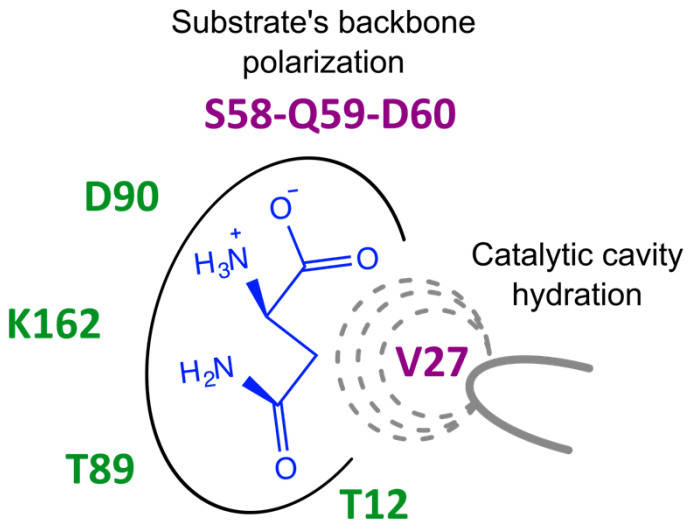
Ongoing work/future directions

- Increasing catalytic rate of L-ASP requires knowledge of complete reaction mechanism
  - Mapping all reactions including energy barriers with QM/MM
- Comparison of E. coli L-ASP's structural features with enzymes from homologs (*Erwinia, Pseudomonas, Helicobacter*)





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