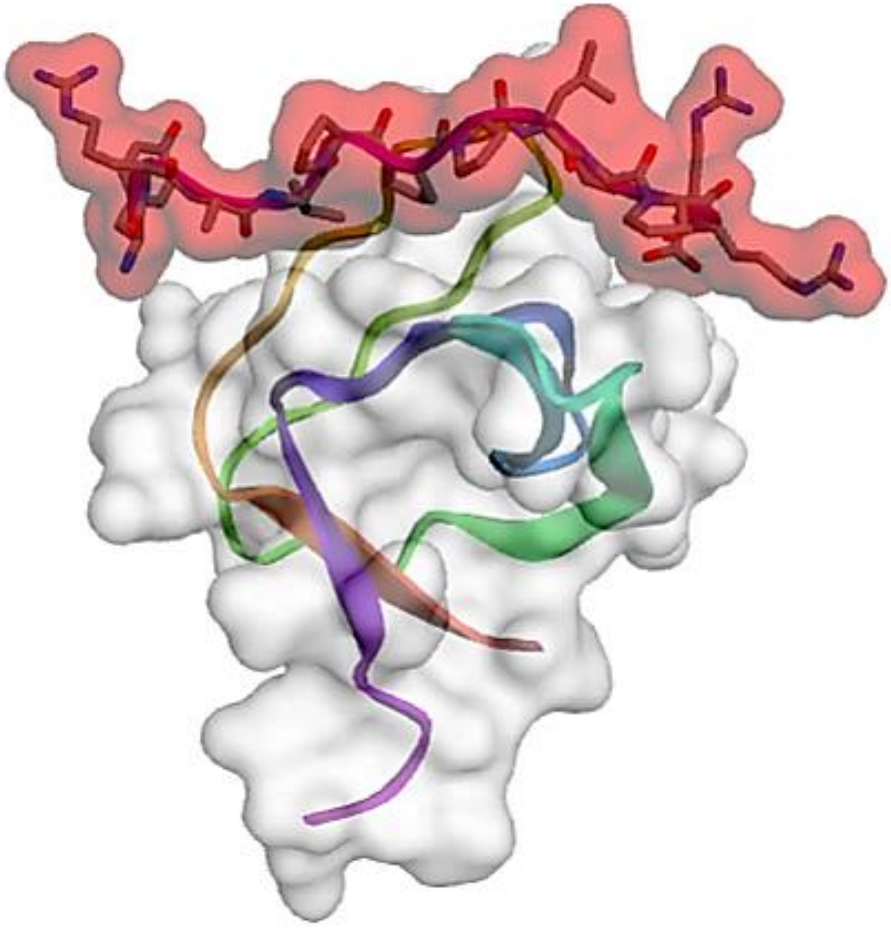
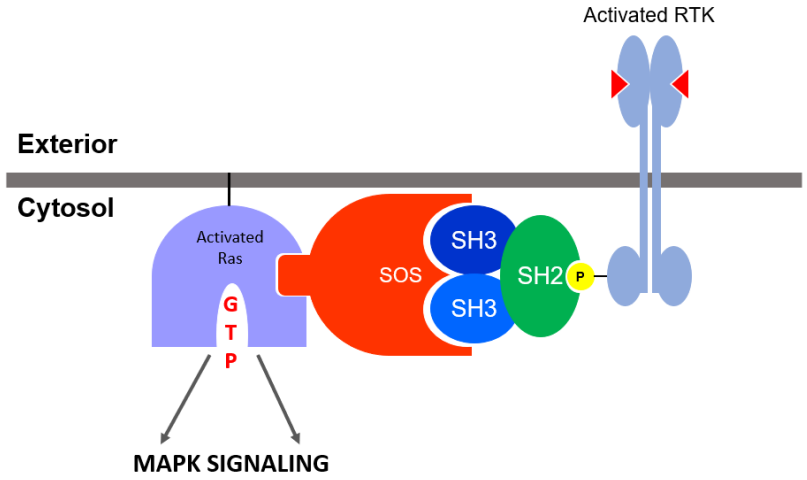
Physiologically-relevant crowding effects on a protein-peptide interaction



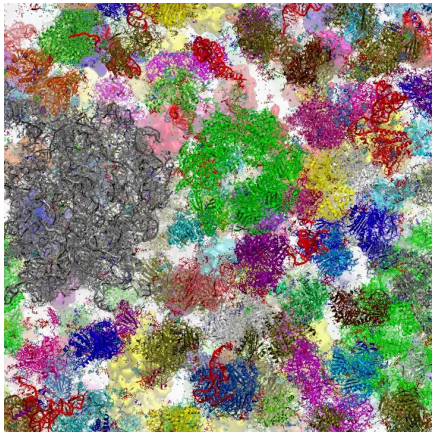
Sam Stadmiller
Pielak Lab
UNC Chapel Hill
April 10, 2018

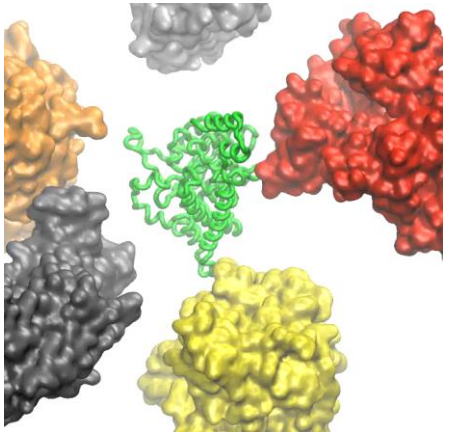
From fundamentals to applications

Biophysics of cell signaling



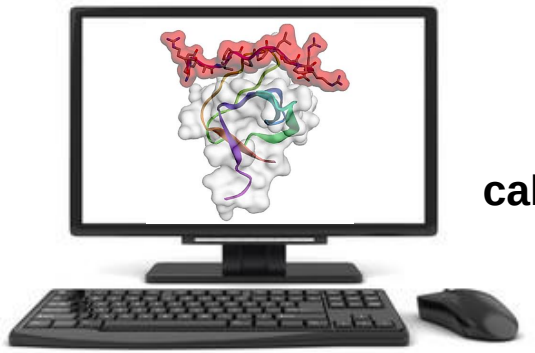
Computation

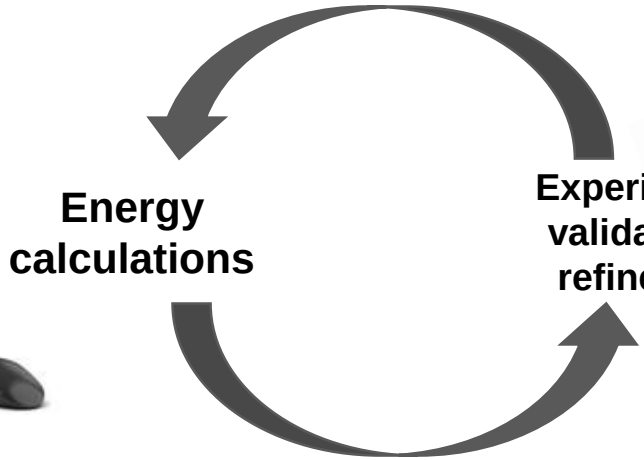




Yu *et al.* eLife. (2016)

Biotherapeutic design and development



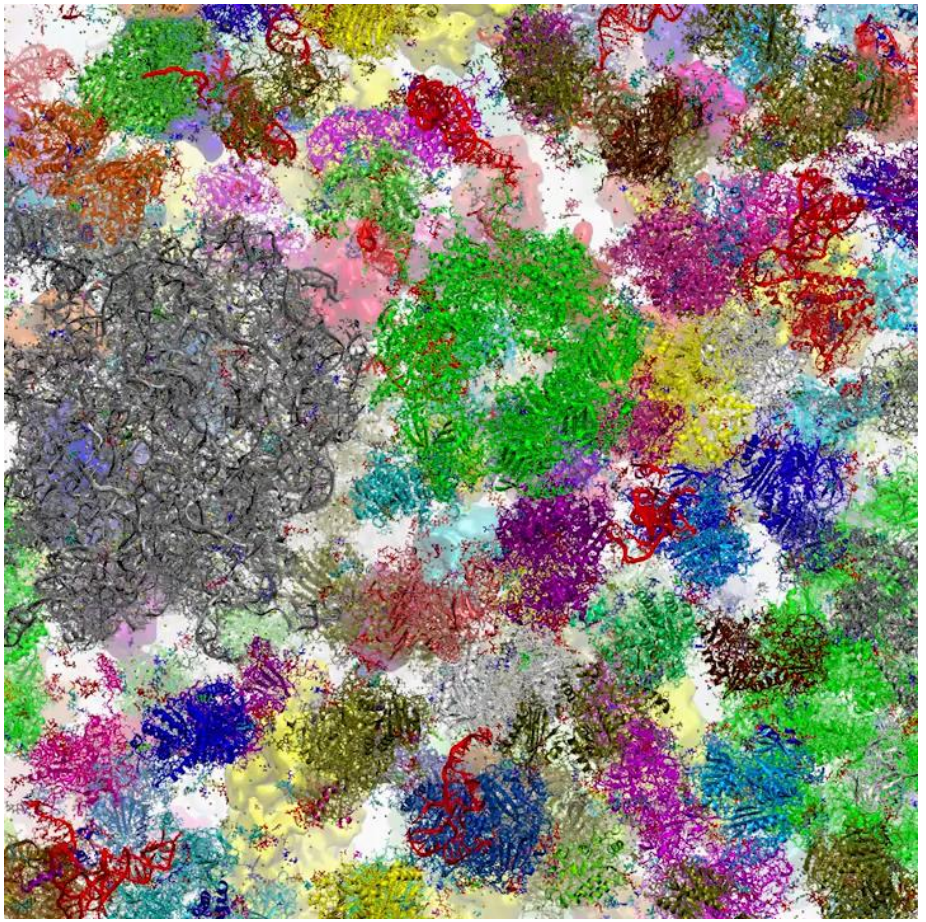


Cells are crowded and dynamic

Most experiments

Buffer
< 10 g/L

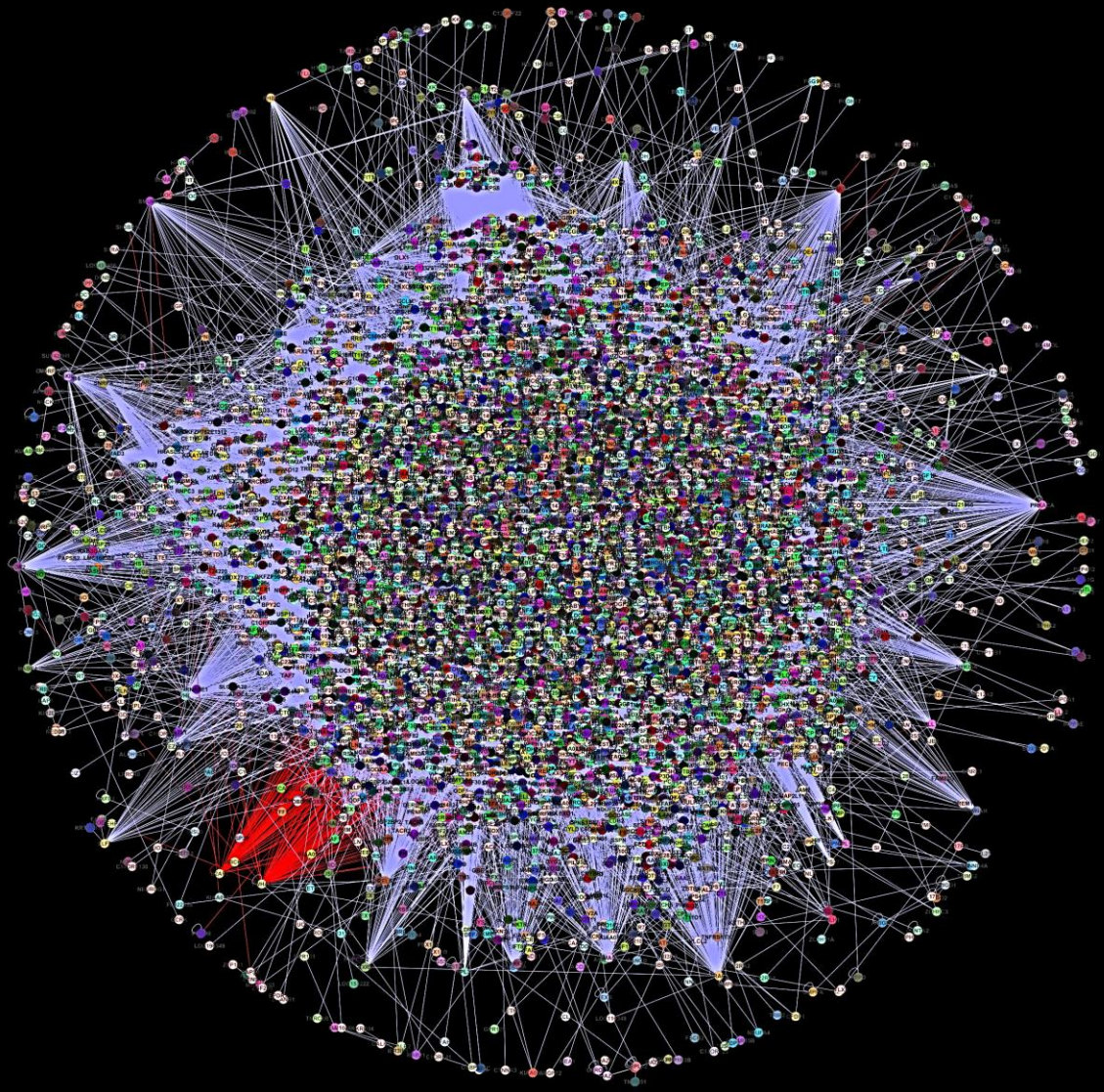
Proteins' native environment



~ 300 g/L macromolecules

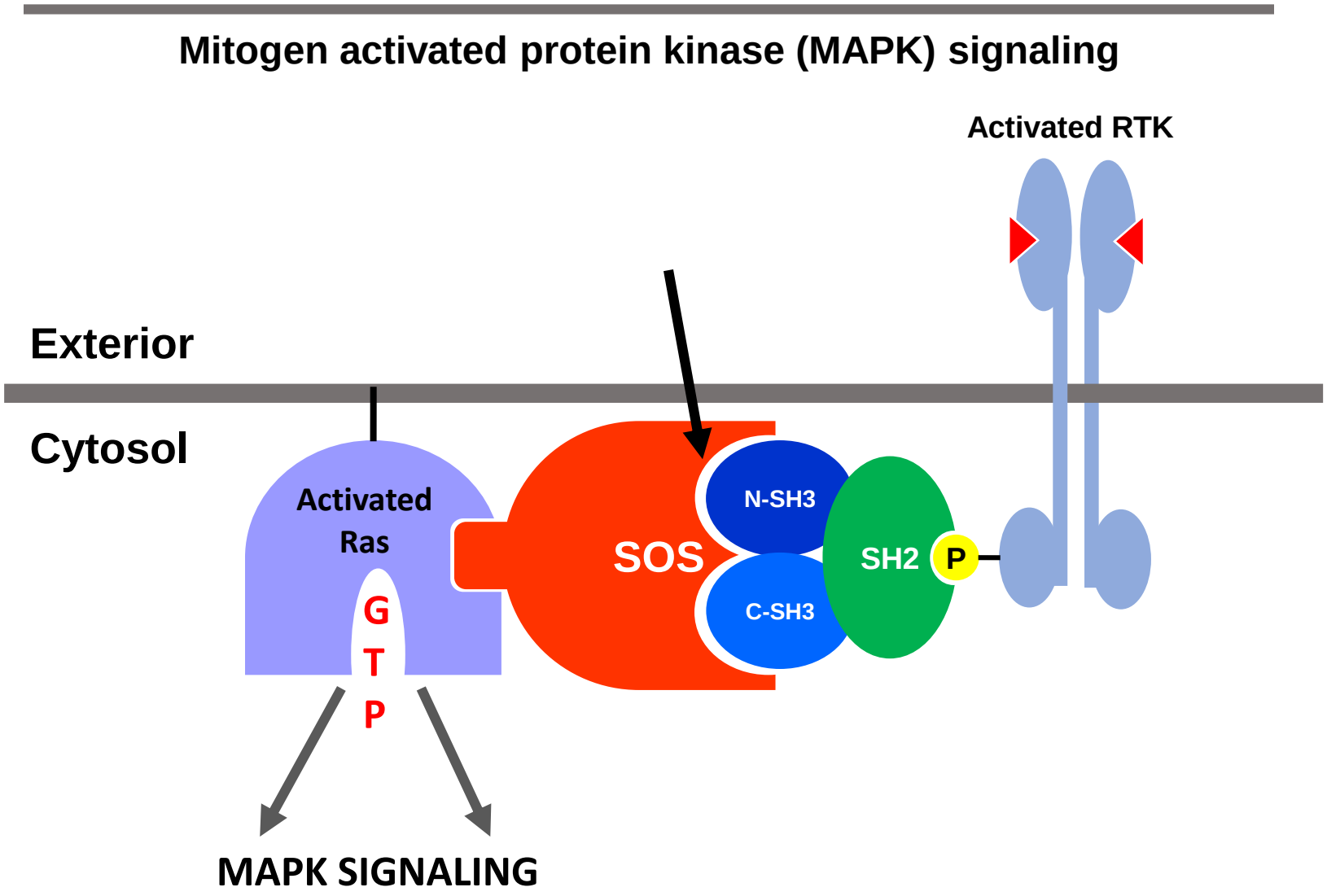
Yu *et al.* eLife. (2016)

Protein-protein interactions are essential to life



A. Garrow, Human Interactome, 2006, https://www.flickr.com/photos/andytrop/5234326542/in/album-72157625527595330/

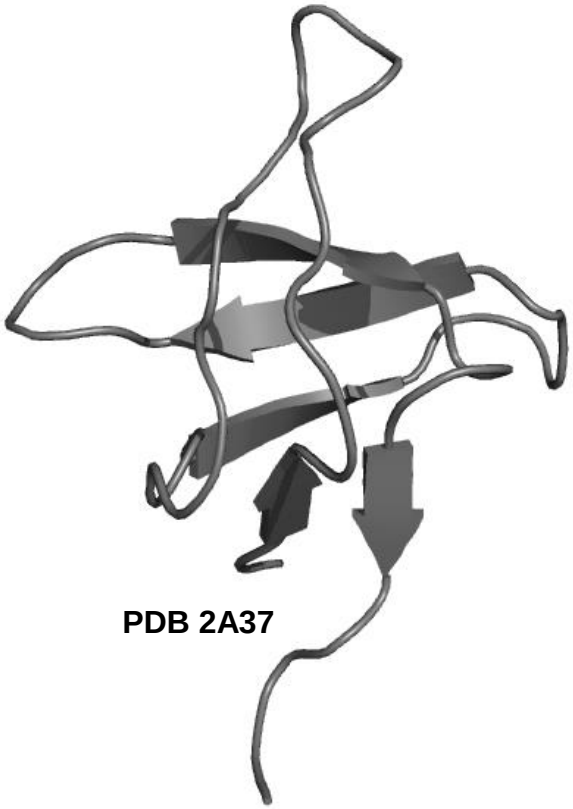
SH3 interactions are important for cell signaling



Adapted from: Lodish, Molecular Cell Biology

Protein and peptide of interest

N-terminal SH3 domain from adaptor protein

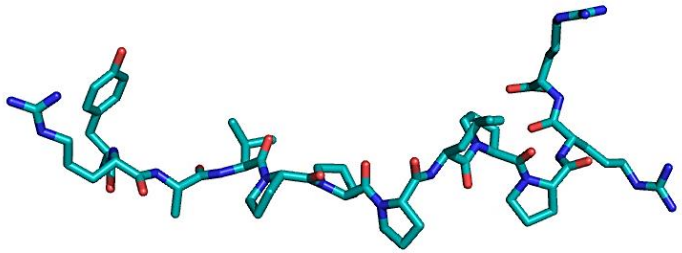


PDB 2A37

6.8 kDa
pI 4.8

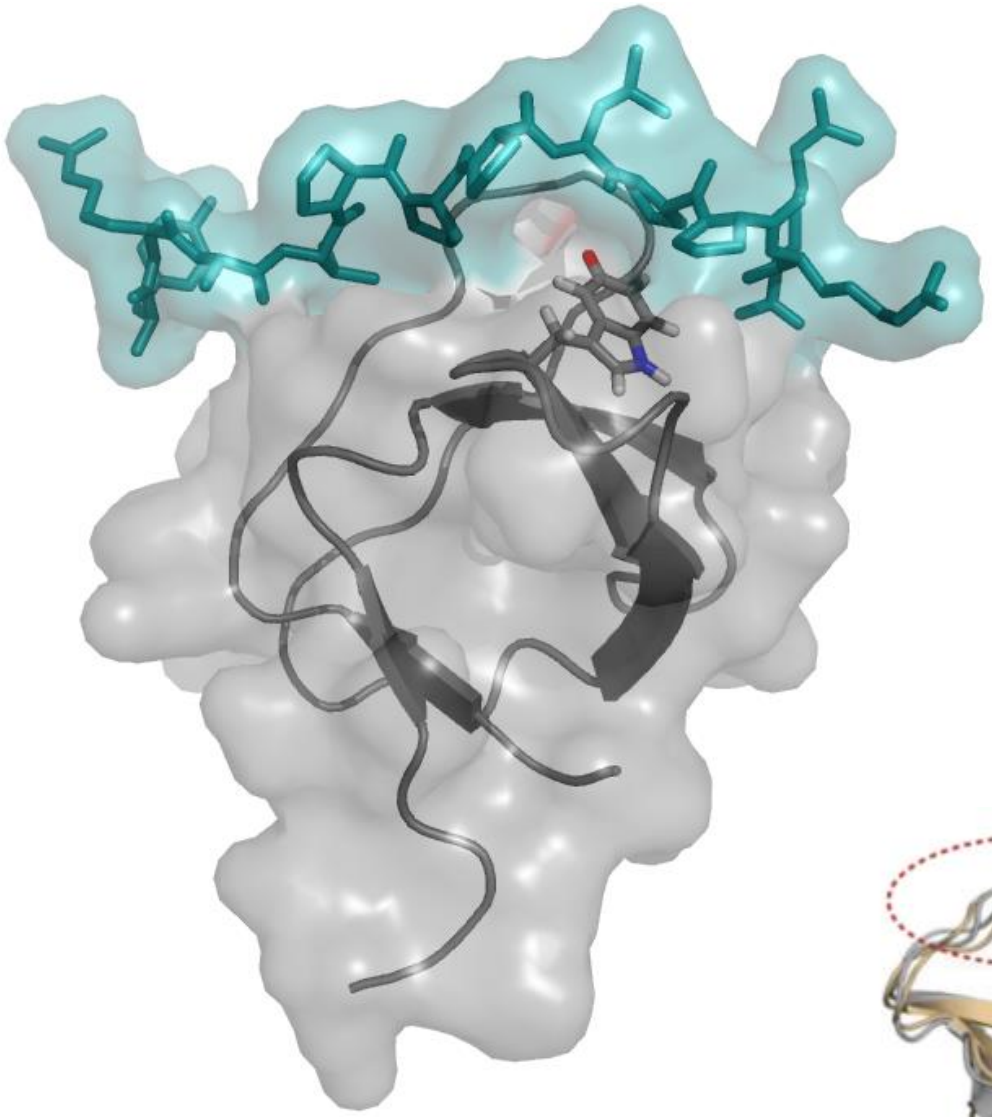
SOS peptide

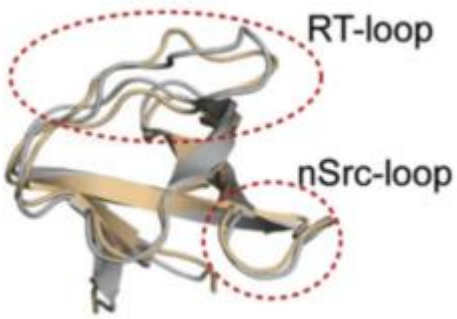
Sequence



1.4 kDa
pI 11.7

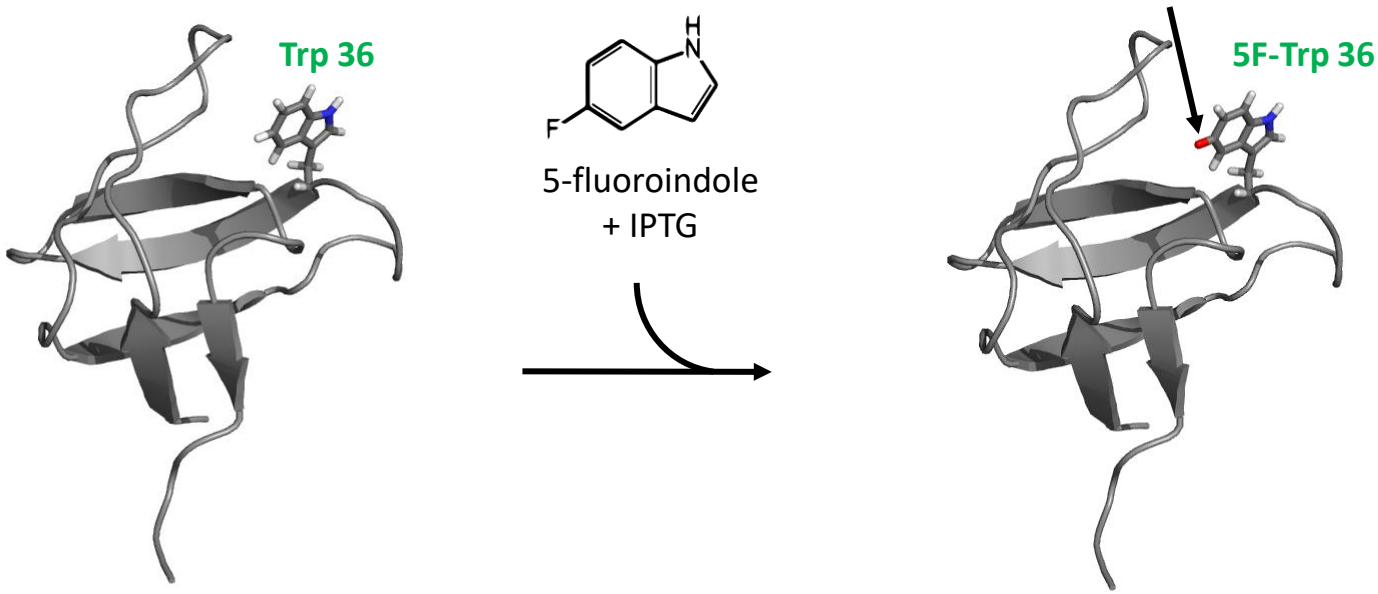
Predicted bound structure

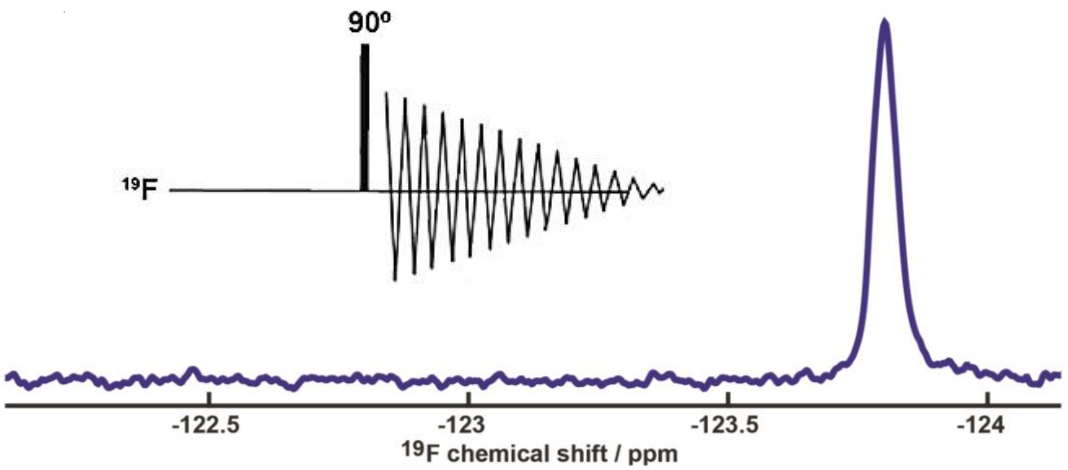




Kurcinski, M. *et al.* Nuc. Acids Res. (2015)
http://biocomp.chem.uw.edu.pl/CABSdock/

^{19}F labeling of SH3





^{19}F NMR advantages

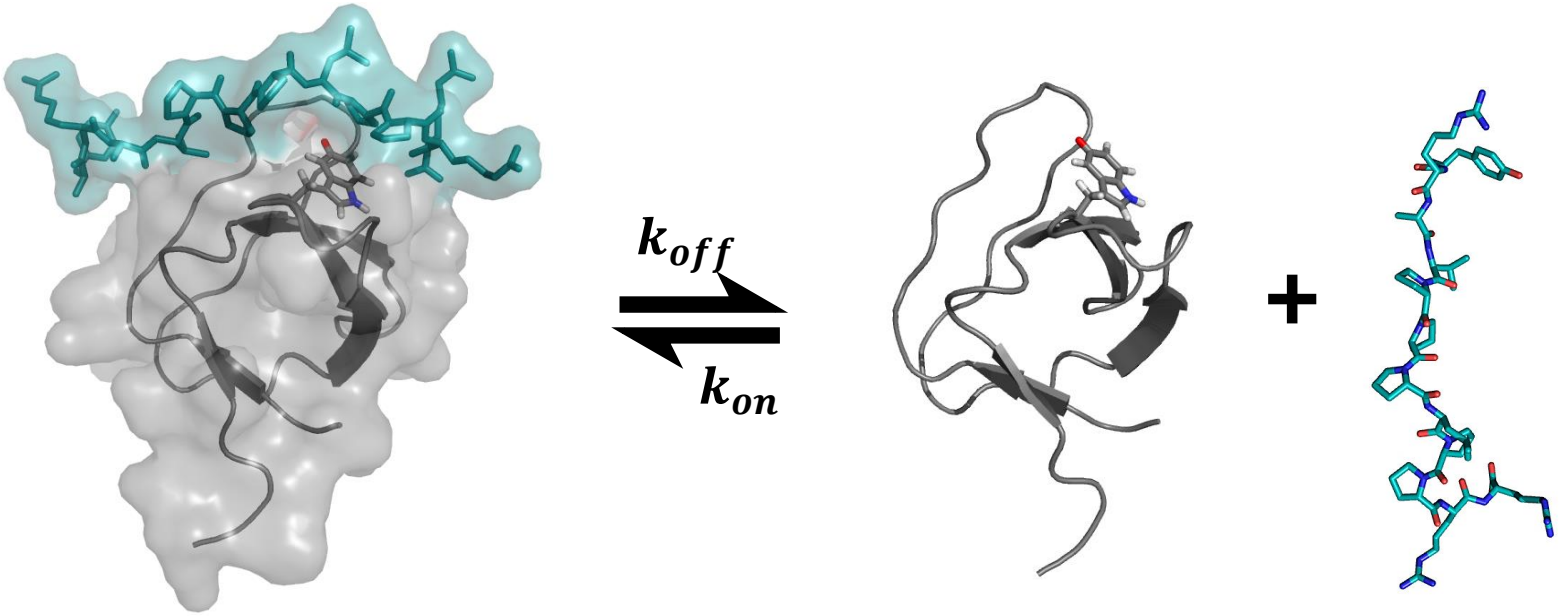
100% abundant

Sensitivity (83% proton)

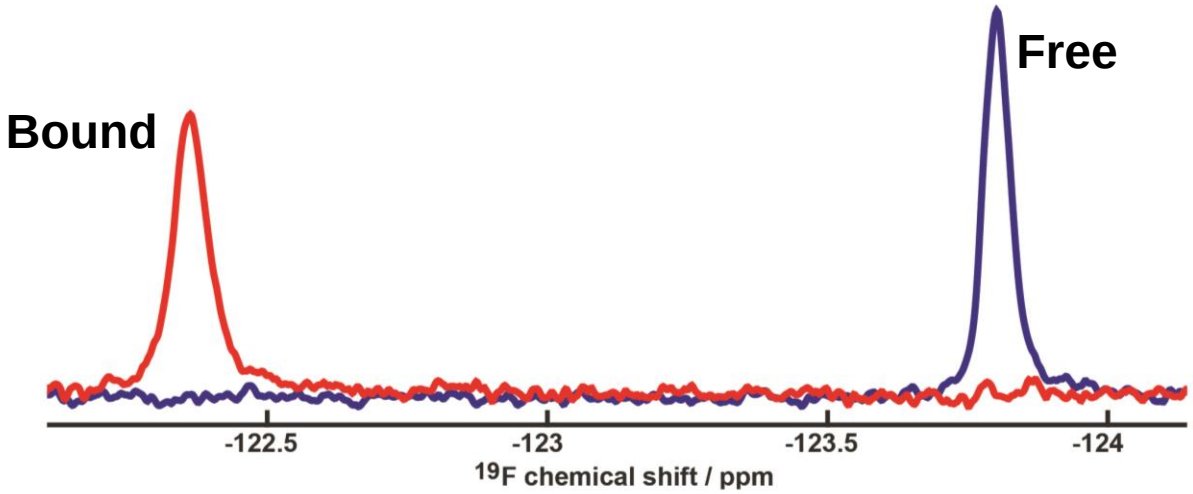
Rare in biology

Easily incorporated into proteins

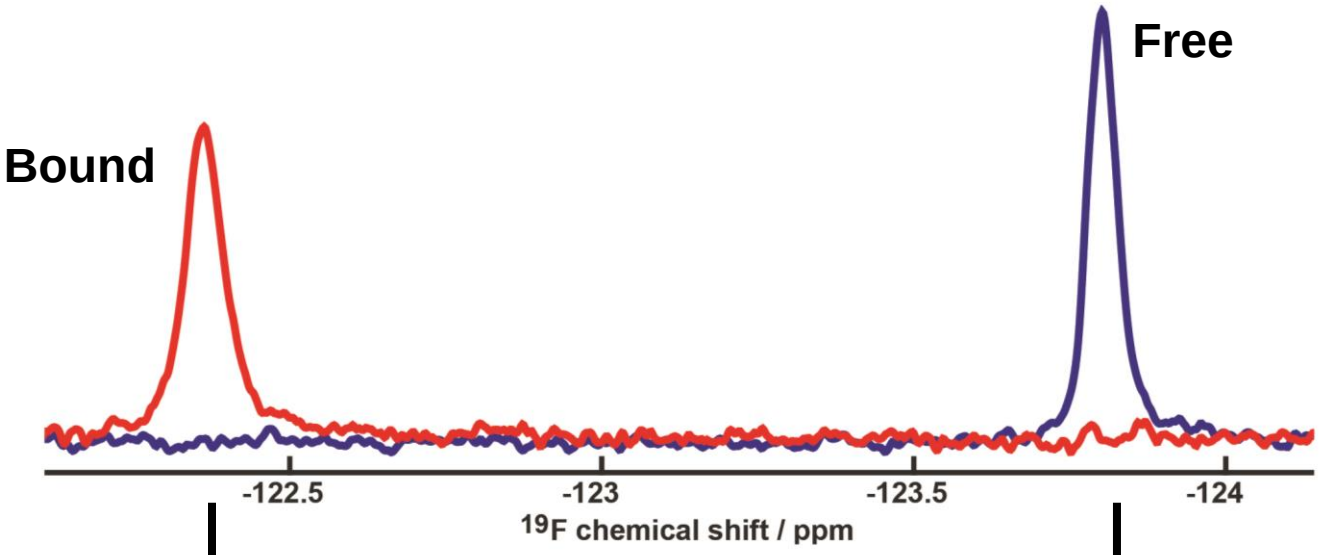
Peptide binding affects ^{19}F chemical environment



$$k_{ex} = k_{on}[peptide] + k_{off}$$



NMR lineshapes are information-rich



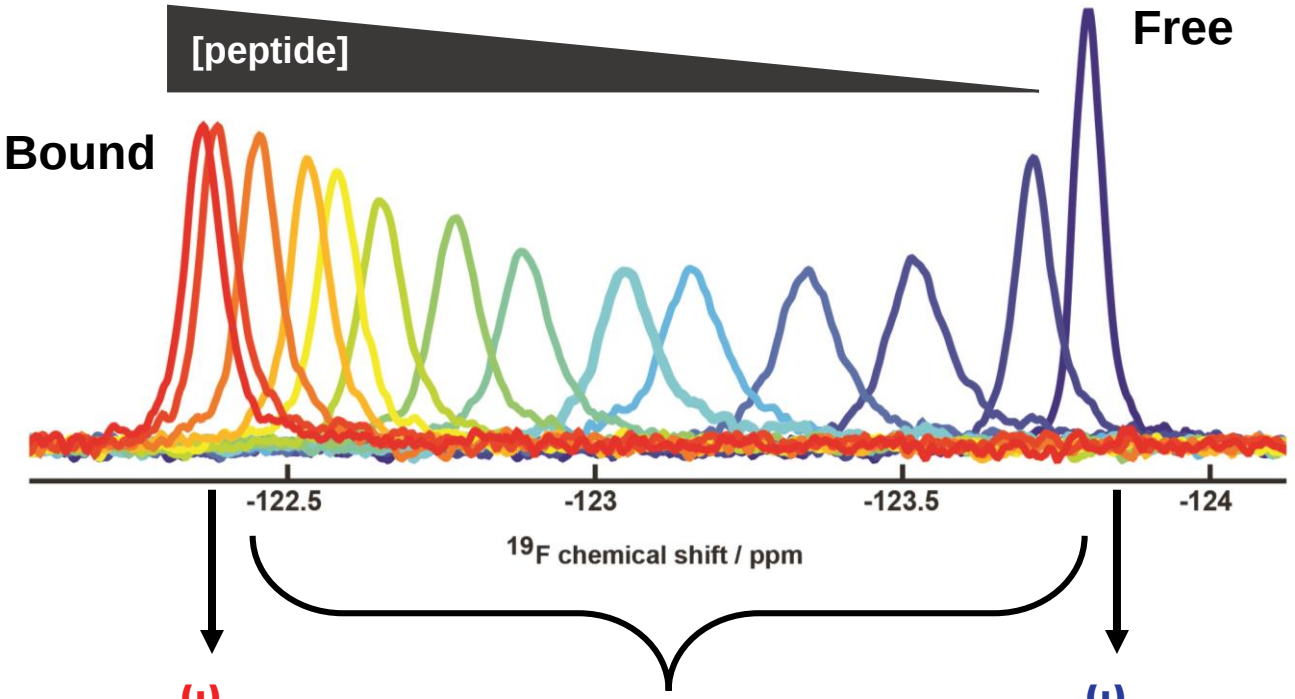
ω_B p_B R_{2B}

ω_A p_A R_{2A}

Lineshape = solution to Bloch equations

with Chris Waudby

NMR lineshapes are information-rich

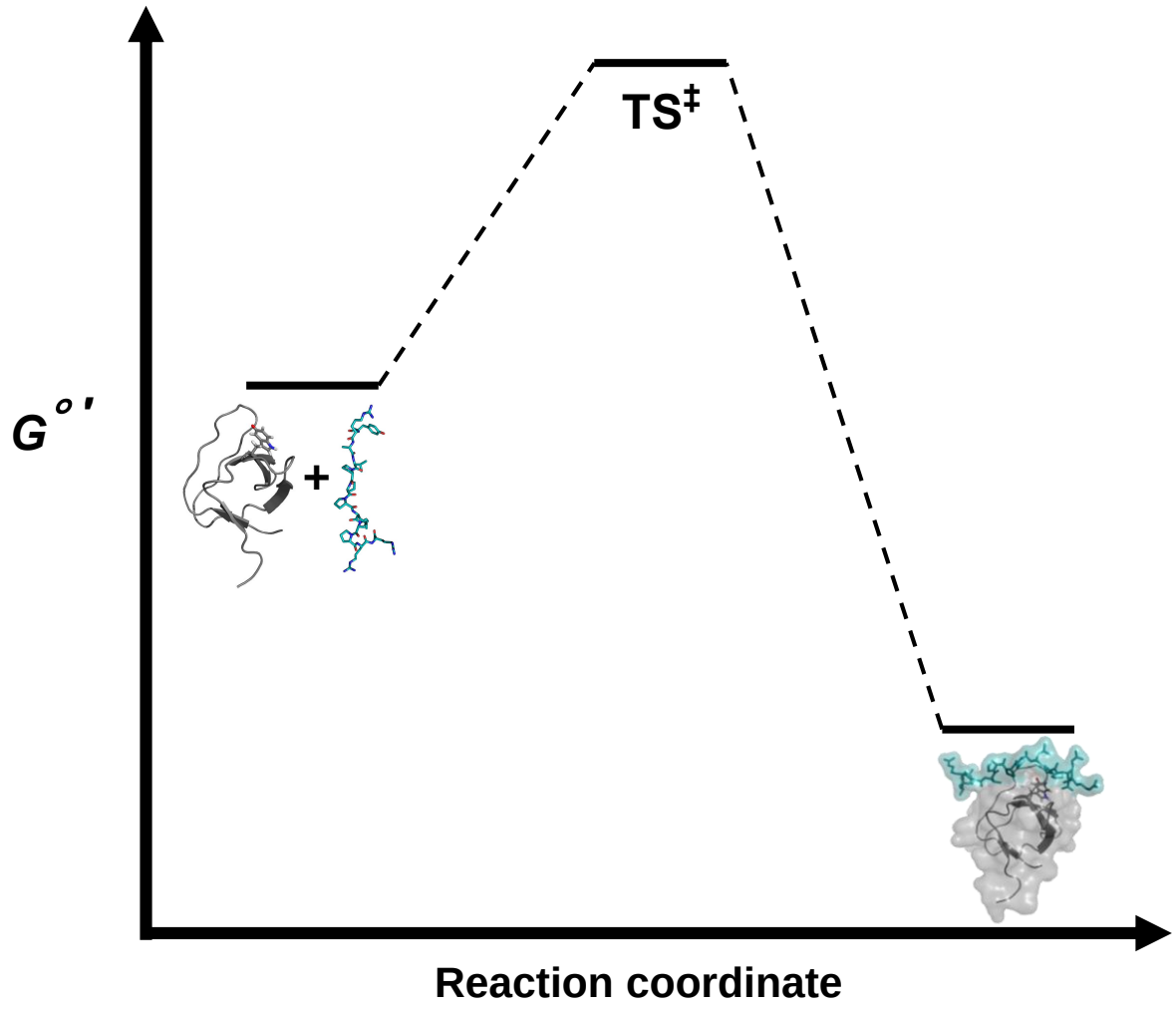


Lineshape = solution to Bloch-McConnell equations

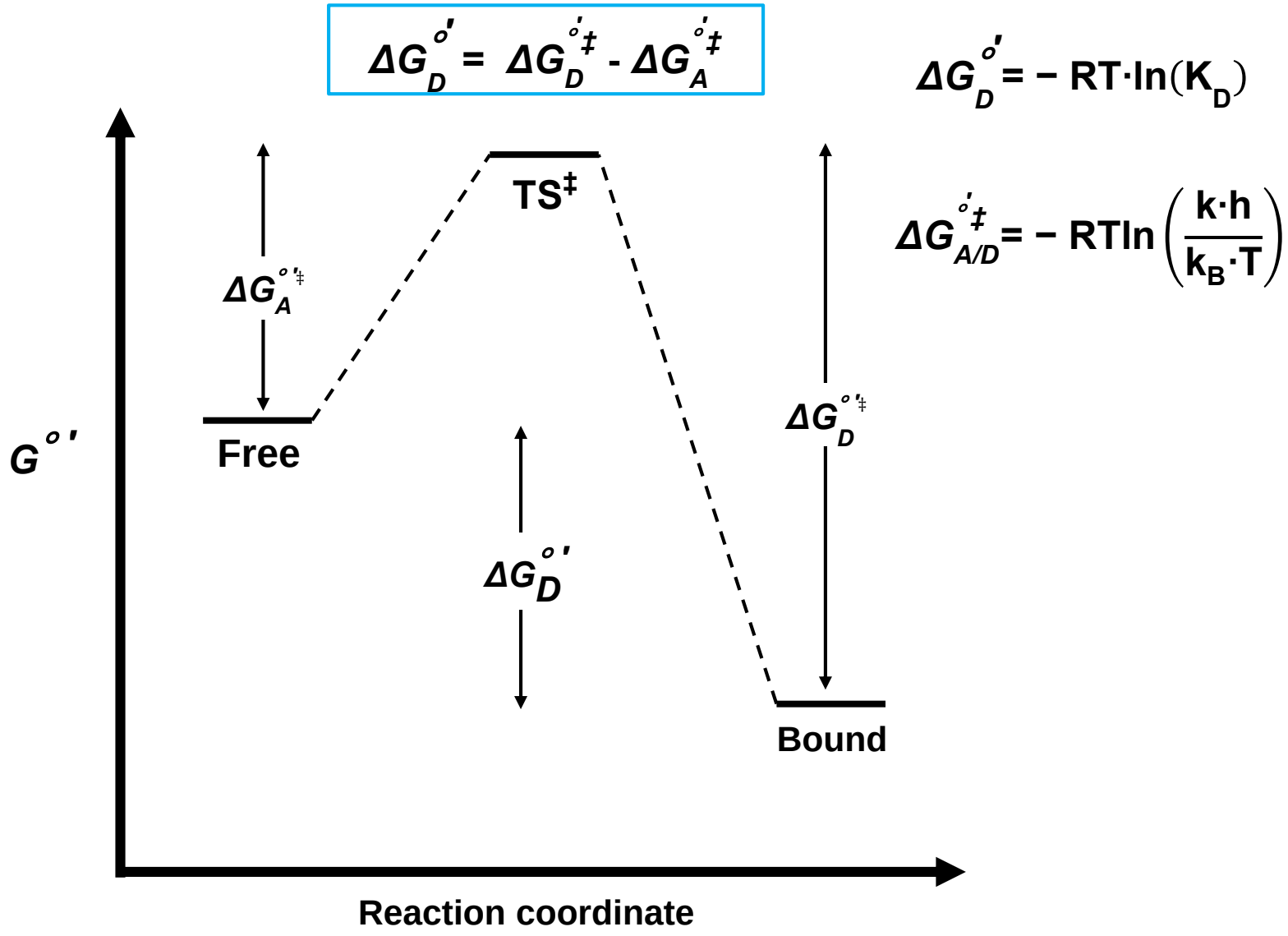
K_D, k_{ex}, [SH3]=constant, [peptide]

with Chris Waudby

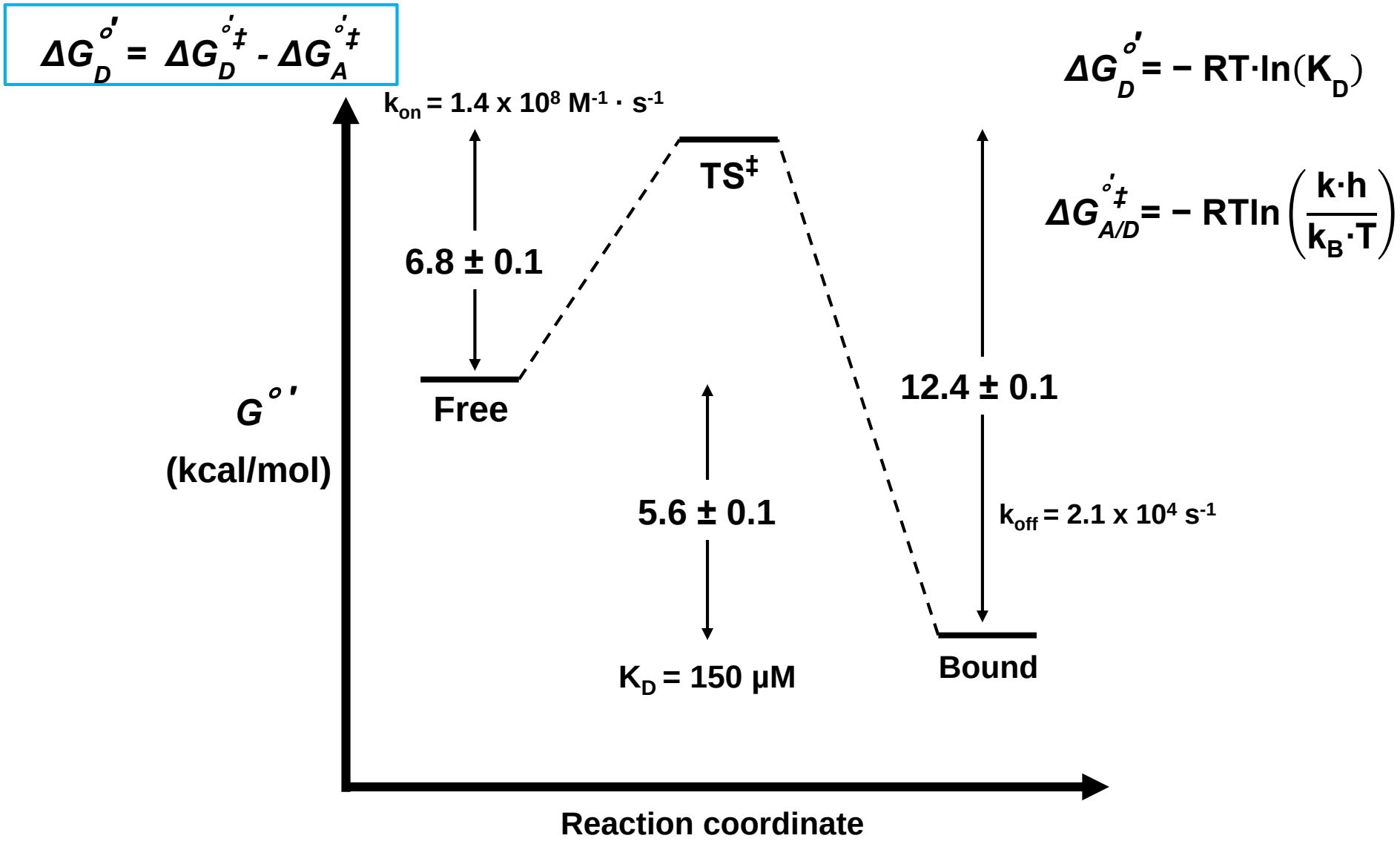
Protein association and free energy diagrams



Protein association and free energy diagrams

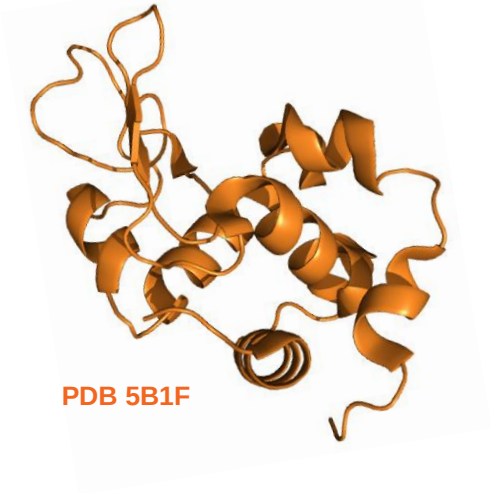


SH3-peptide interaction in buffer



Investigating macromolecular crowding effects

Lysozyme



Ovalbumin

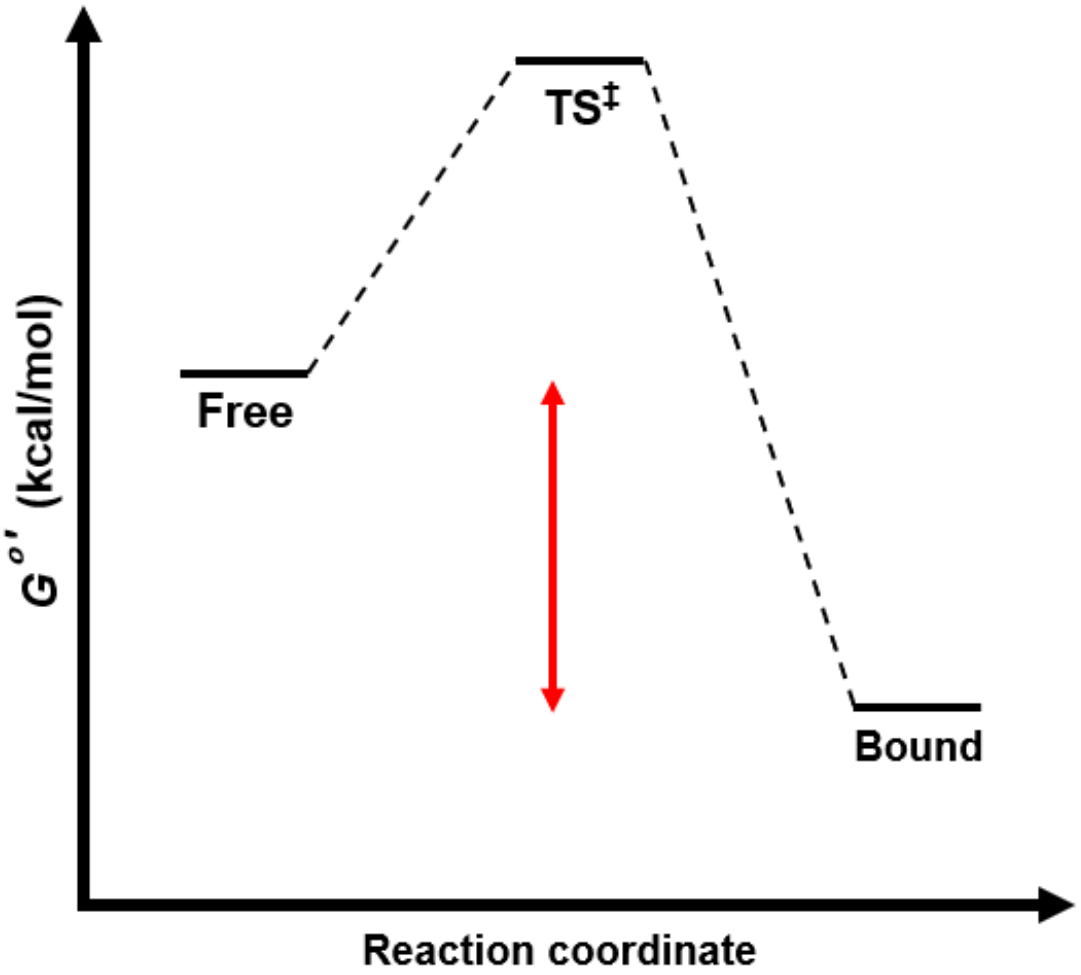
BSA

	Lysozyme	Ovalbumin	BSA
MW (kDa)	14.4	43	66
pI	9	5	6
Charge at pH 7.4	+ 7	- 12	- 18

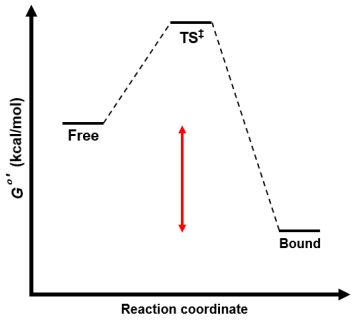
http://protcalc.sourceforge.net

Electrostatic crowder-SH3 interactions slightly perturb binding affinity

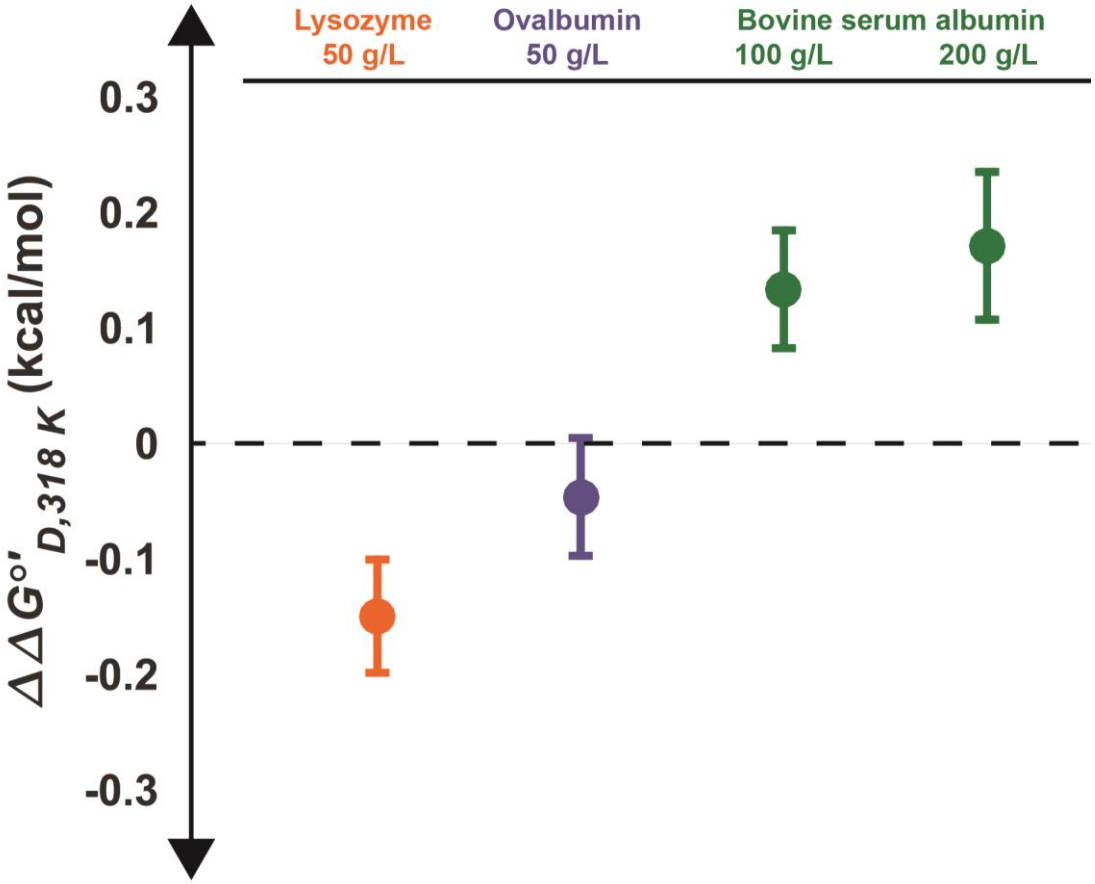
$$\Delta\Delta G_D^{\circ\prime} = \Delta G_{D,crowder}^{\circ\prime} - \Delta G_{D,buffer}^{\circ\prime}$$



Electrostatic crowder-SH3 interactions slightly perturb binding affinity



$$\Delta\Delta G_D^{\circ\prime} = \Delta G_{D,crowder}^{\circ\prime} - \Delta G_{D,buffer}^{\circ\prime}$$

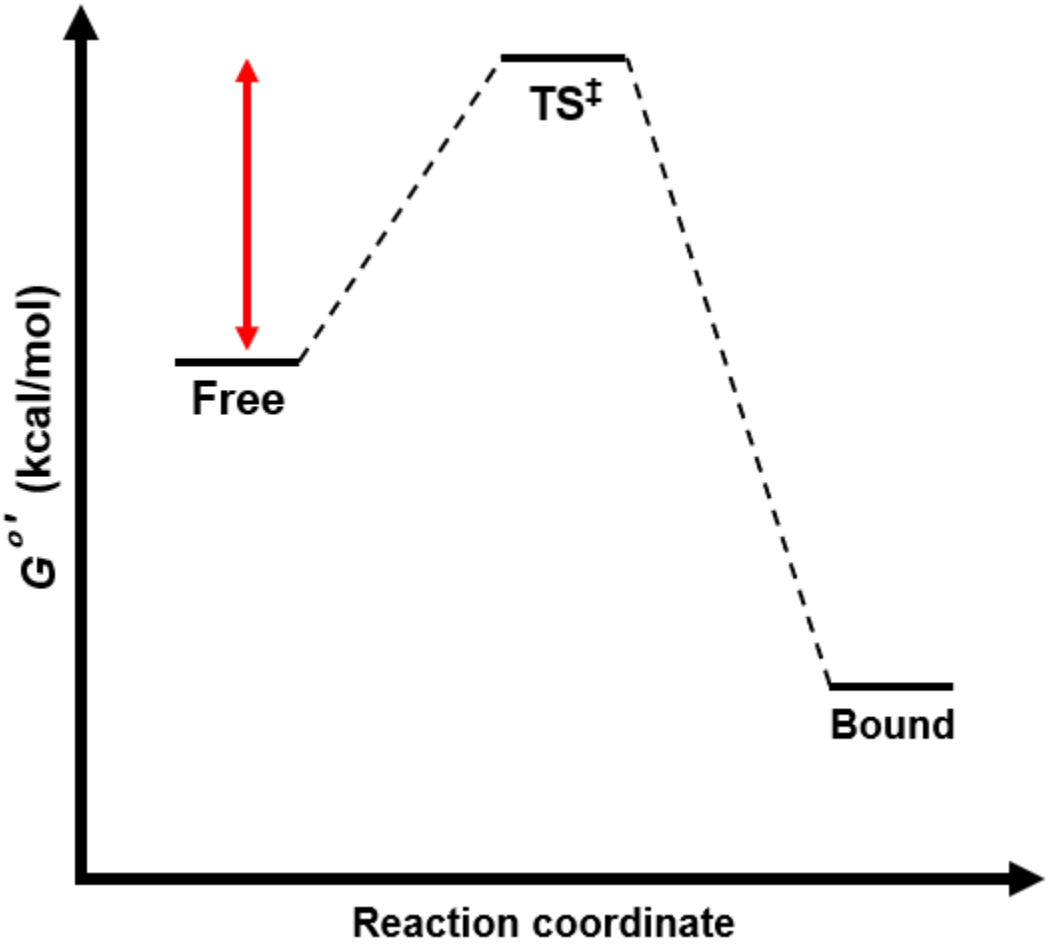


Stabilizes complex

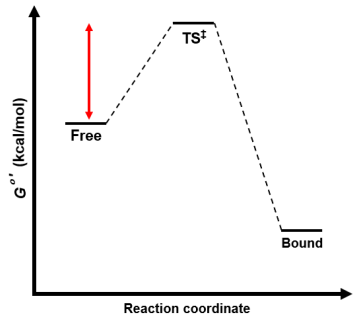
Destabilizes complex

Crowder charge dictates kinetic effects

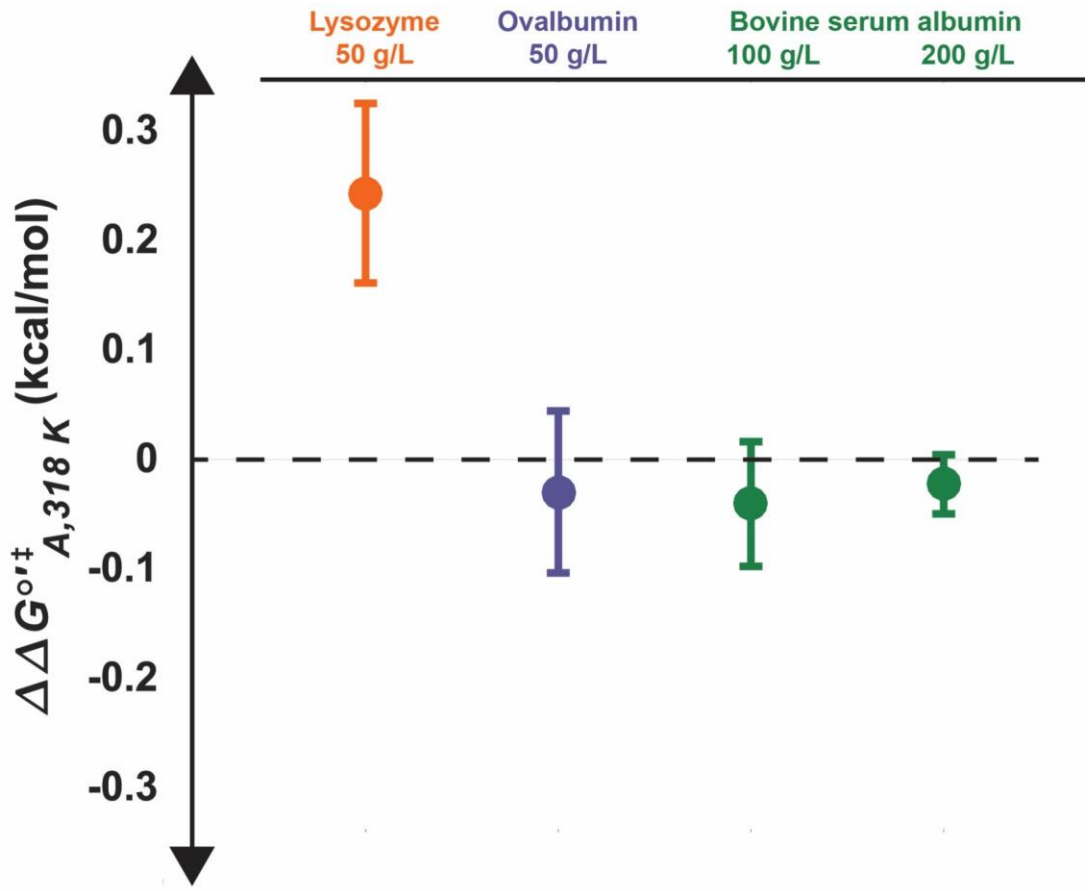
$$\Delta\Delta G_A^{\circ\prime\ddagger} = \Delta G_{A,\,crowder}^{\circ\prime\ddagger} - \Delta G_{A,buffer}^{\circ\prime\ddagger}$$



Crowder charge dictates kinetic effects



$$\Delta\Delta G_A^{\circ\prime\ddagger} = \Delta G_{A,\,crowder}^{\circ\prime\ddagger} - \Delta G_{A,buffer}^{\circ\prime\ddagger}$$



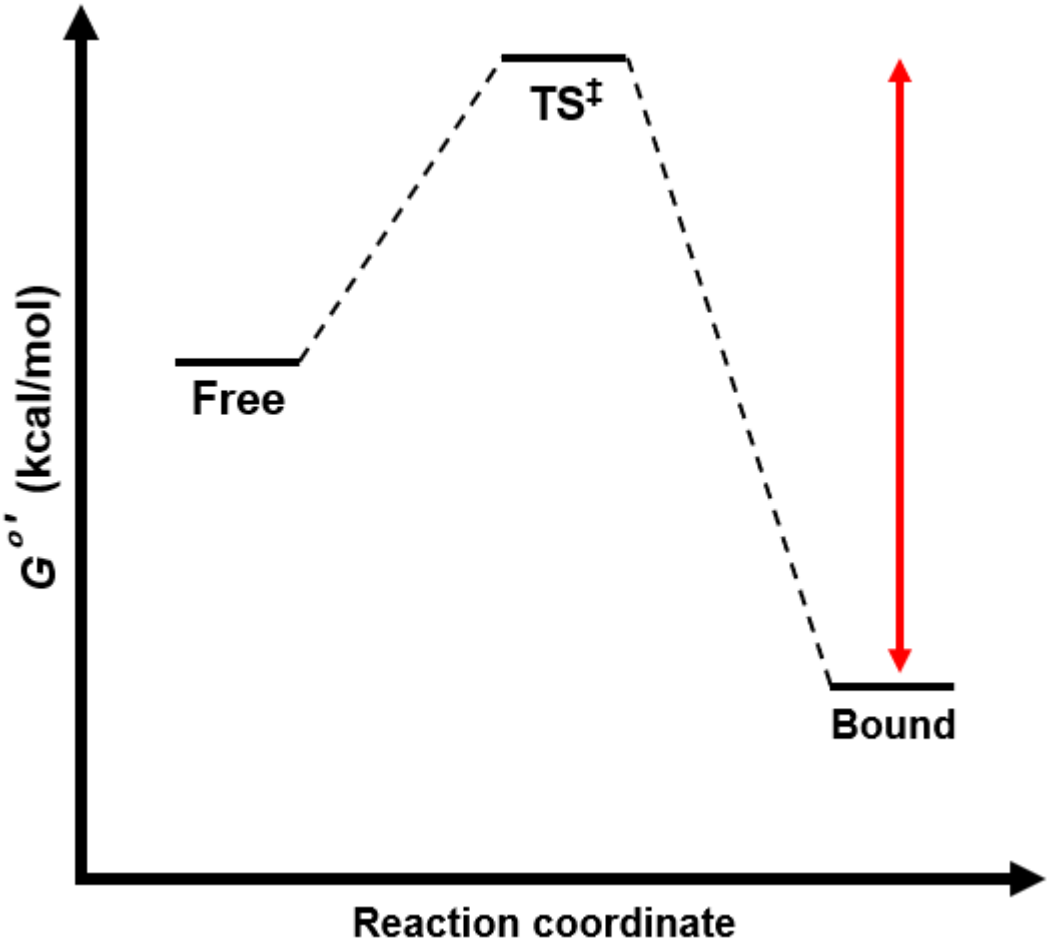
Increases barrier to association

Decreases barrier to association

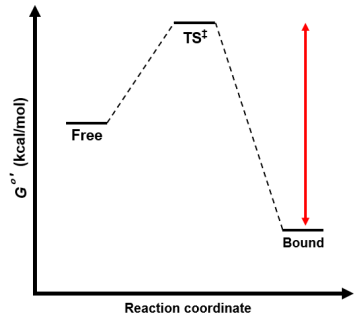
Only positively charged lysozyme affects the association rate constant

Crowder charge dictates kinetic effects

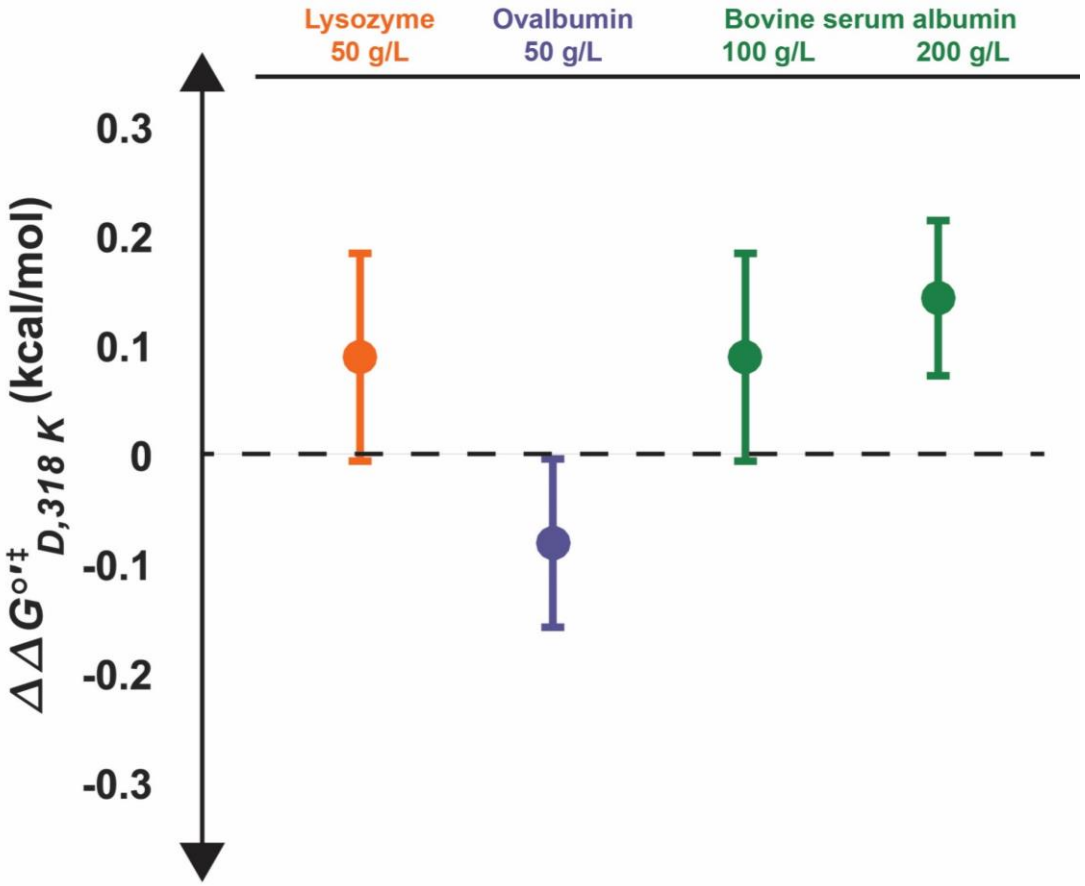
$$\Delta\Delta G_D^{\circ\prime\ddagger} = \Delta G_{D,\,crowder}^{\circ\prime\ddagger} - \Delta G_{D,buffer}^{\circ\prime\ddagger}$$



Crowder charge dictates kinetic effects



$$\Delta\Delta G_D^{\circ\prime\ddagger} = \Delta G_{D,\,crowder}^{\circ\prime\ddagger} - \Delta G_{D,buffer}^{\circ\prime\ddagger}$$

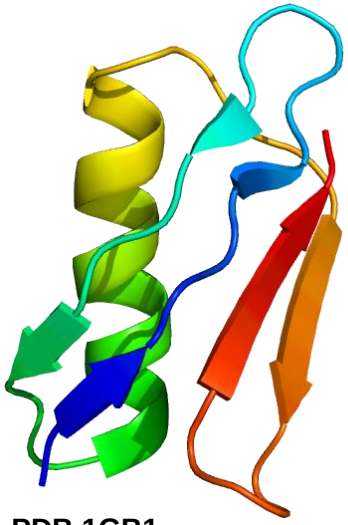


Increases barrier to dissociation

Decreases barrier to dissociation

Only high concentrations of negatively charged BSA affects the dissociation rate constant

Future Crowded Conditions

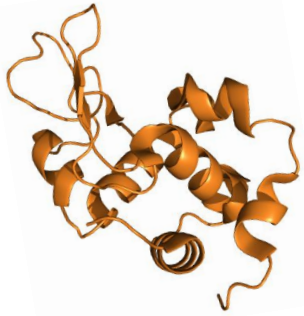


PDB 1GB1

Cell lysates

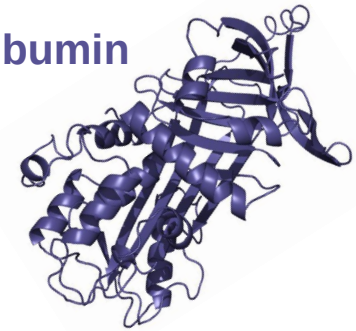
+

Lysozyme



BSA

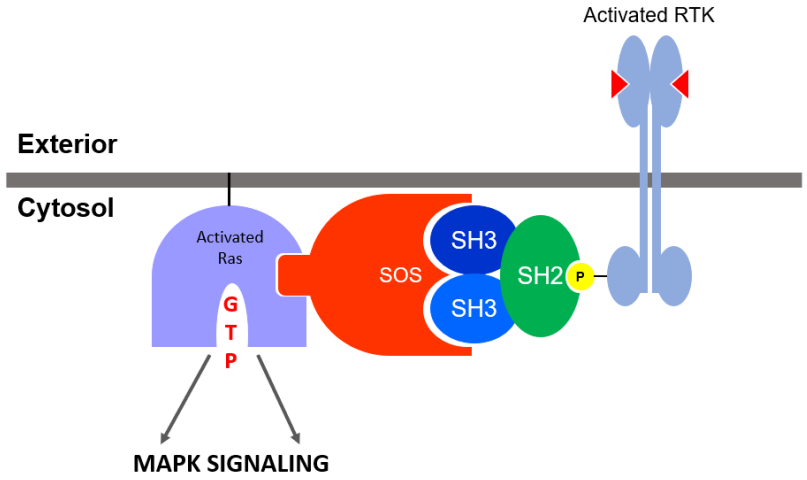
Ovalbumin



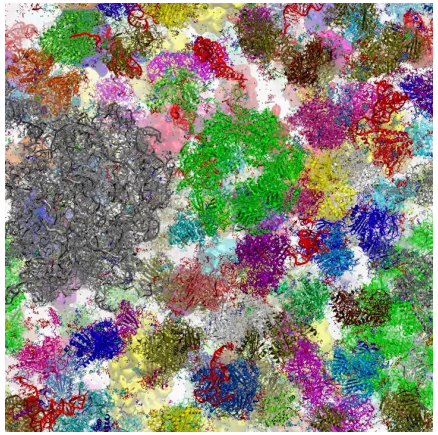
Investigate concentration and size dependence (if any)

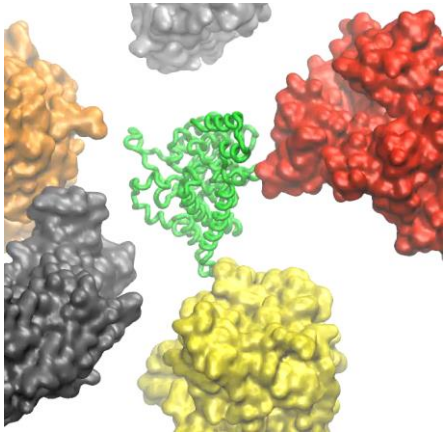
From fundamentals to applications

Biophysics of cell signaling



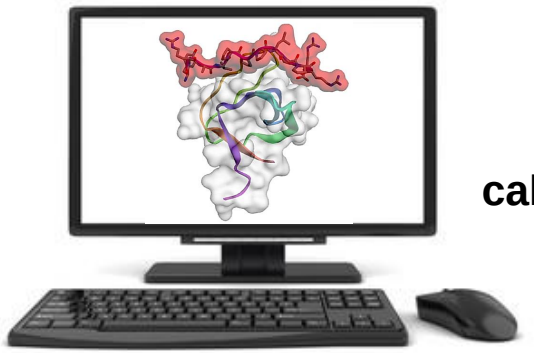
Computation

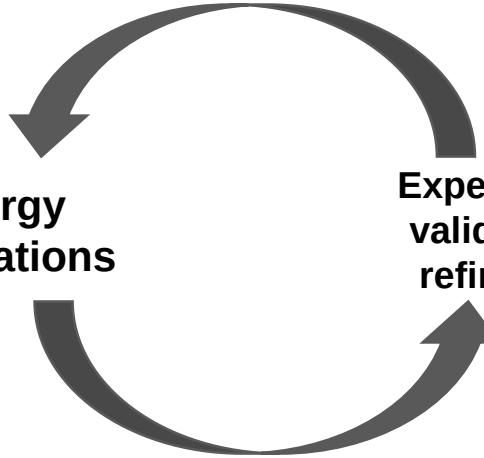




Yu *et al.* eLife. (2016)

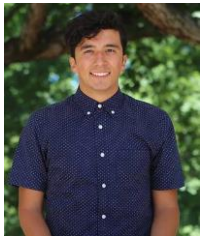
Biotherapeutic design and development





Acknowledgments

Pielak Lab



NMR
Greg Young

Lineshape Analysis
Chris Waudby, UCL

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