What determines the binding affinity?

M1 ZHAI 20210430

Contents

- Binding affinity
- Assays for binding affinity
- Overall determinants for binding affinity
- Different binding affinity between in vivo and in vitro
- Structure prediction of macromolecular complexes

Binding affinity

- The binding affinity is the strength of the interaction between two (or more than two) molecules that bind reversibly (interact).
- It is translated into physicochemical terms in the dissociation constant (Kd), the latter being the concentration of the free protein that occupies half of the overall sites of the second protein at equilibrium.

$$[\mathrm{A}] + [\mathrm{B}] \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftarrows}} [\mathrm{AB}], \qquad K_{\mathrm{d}} = \frac{[\mathrm{A}][\mathrm{B}]}{\mathrm{AB}} = \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}}.$$

- The binding affinity can also be translated in physical terms into the Gibbs free energy of dissociation.

$$\Delta G_{\mathrm{d}} = -RT \ln \frac{K_{\mathrm{d}}}{c_0} = \Delta H_{\mathrm{d}} - T\Delta S_{\mathrm{d}},$$

- The binding affinity is related to the Gibbs free energy of association (▵ Ga) as

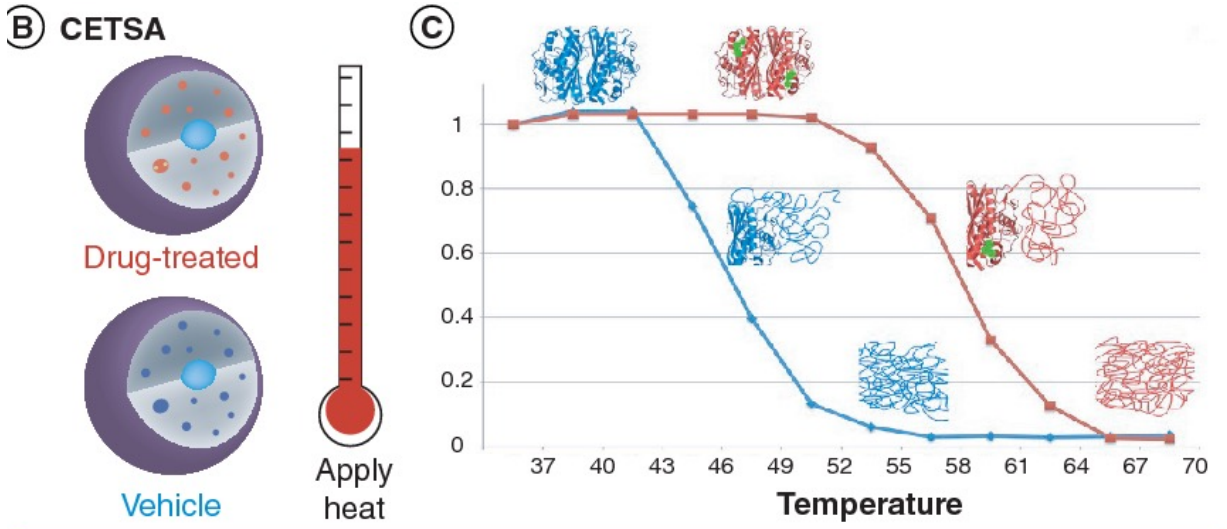
$$\Delta G_{\mathrm{a}} = -\Delta G_{\mathrm{d}}. \qquad \Delta G_{\mathrm{a}} = \Delta G_{\mathrm{bond}} + \Delta G_{\mathrm{entropy}},$$

Binding affinity

- Correct and precise estimation of the binding affinity is crucial throughout these essential drug design stages.
- This high demand has facilitated the development of a number of different techniques to assess or predict ligand binding.

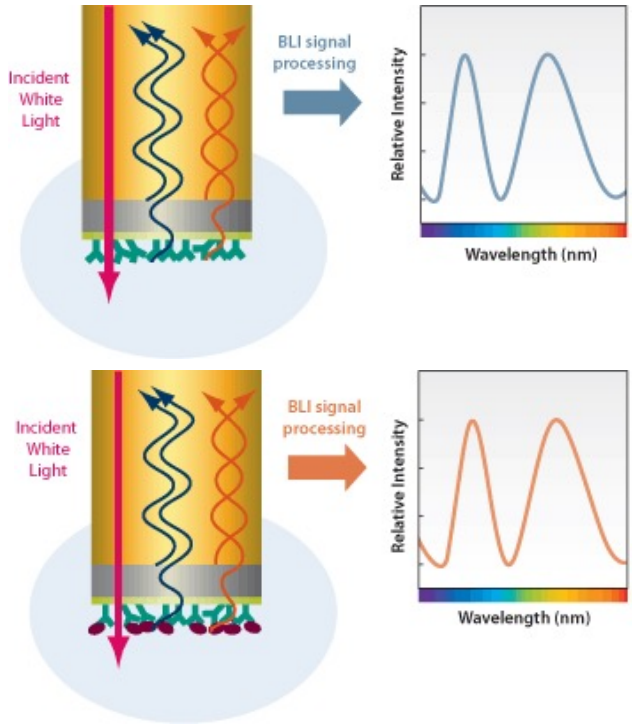
CETSA

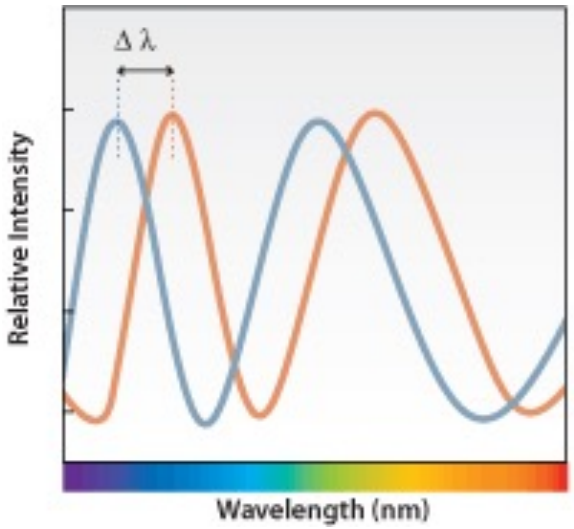
- Cellular thermal shift assay
- The method allows studies of target engagement of drug candidates in a cellular context.
- Melting temperature (Tm) shift assays

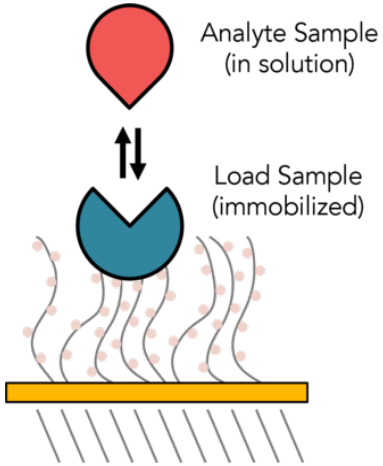


BLI

- Biolayer interferometry
- Label-free technology for measuring biomolecular interactions







Overall determinants for binding affinity

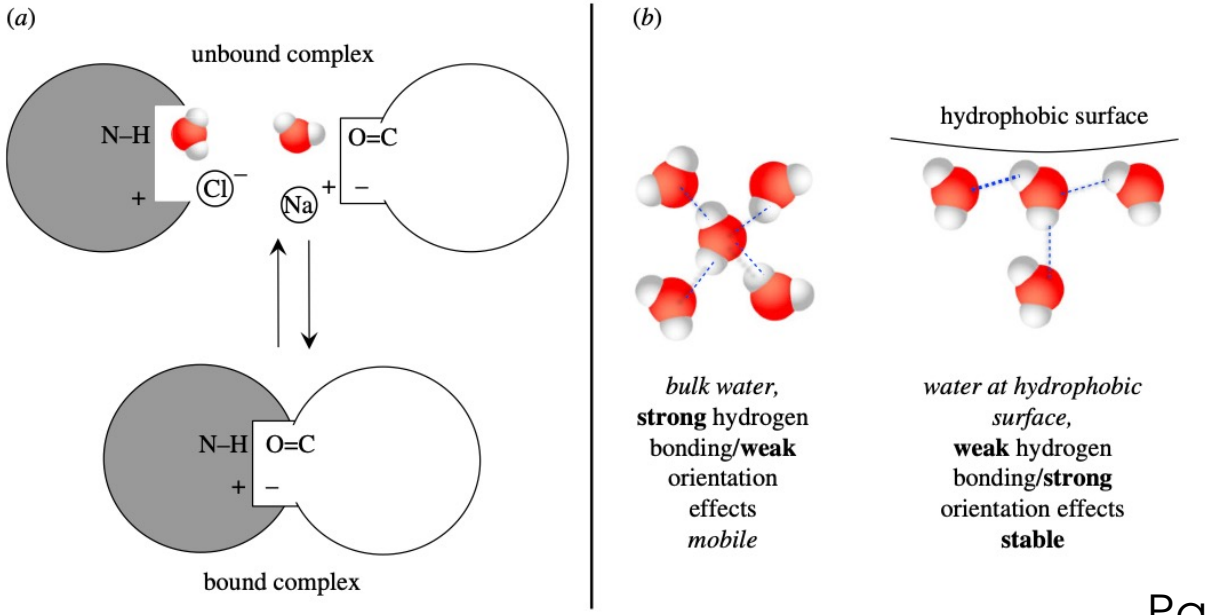
- Buried surface area
- Hot spots and anchor residues
- Allosteric regulators and non-interface affinity modifiers

Overall determinants for binding affinity

- The buried surface area (BSA) is defined as the surface buried away from the solvent when two or more proteins or subunits associate to form a complex.
- The BSA has been the primary descriptor to be related to binding affinity, and more specifically, to the intrinsic bond energy.

$$\Delta G_{\text{bond}} = 0.025 \cdot \text{BSA}.$$

- BSA is a macroscopic descriptor for the hydrophobic interactions.



Panagiotis L., *J. R. Soc. Interface*, **2012**

Overall determinants for binding affinity- Hot spots and anchor residues

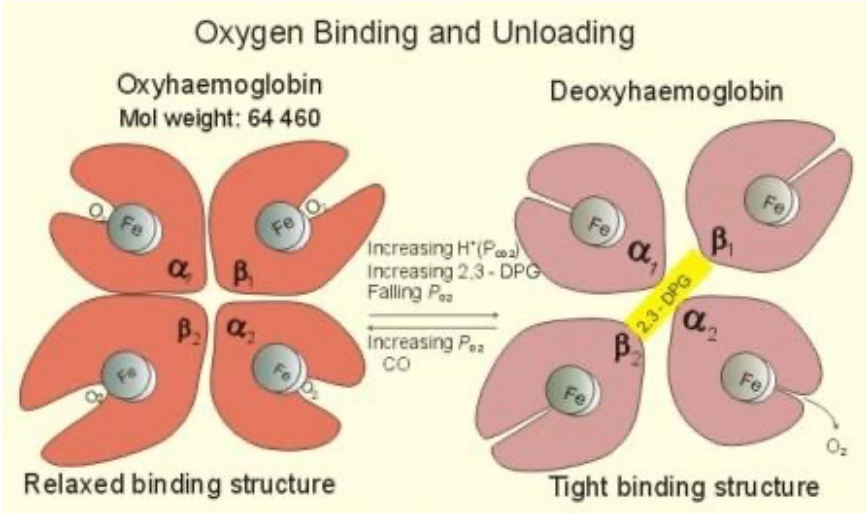
- In the context of protein-protein interactions, the term "hot spot" refers to a residue or cluster of residues that makes a major contribution to the binding free energy.
- They are most often found in central regions of the interface .
- Their amino acid composition differs from that of nonhot-spot residues.

Overall determinants for binding affinity- Hot spots and anchor residues

- Anchor chains act as ready-made recognition motifs by acquiring native-like conformations before any physical interaction with the receptor.
- Anchors are proposed to reduce the number of possible binding pathways and therefore avoid structural rearrangements.
- Anchor residues must provide most of the specificity necessary for protein–protein recognition whereas other important residues on the interface contribute to the stabilization.

Overall determinants for binding affinity- Allosteric regulators and non-interface affinity modifiers

- Definiton: account for regulation of a protein by a change in its tertiary structure induced by a small molecule.
- Changes in the dynamics or structure of a protein by a modulator.
- Such changes shift the population of the inactive protein to its active form, thereby significantly altering its binding affinity.
- The binding of oxygen to haemoglobin.



Allison AC. *Br Med J.*, **1954,** *1,* 290-4

Different binding affinity between in vivo and in vitro

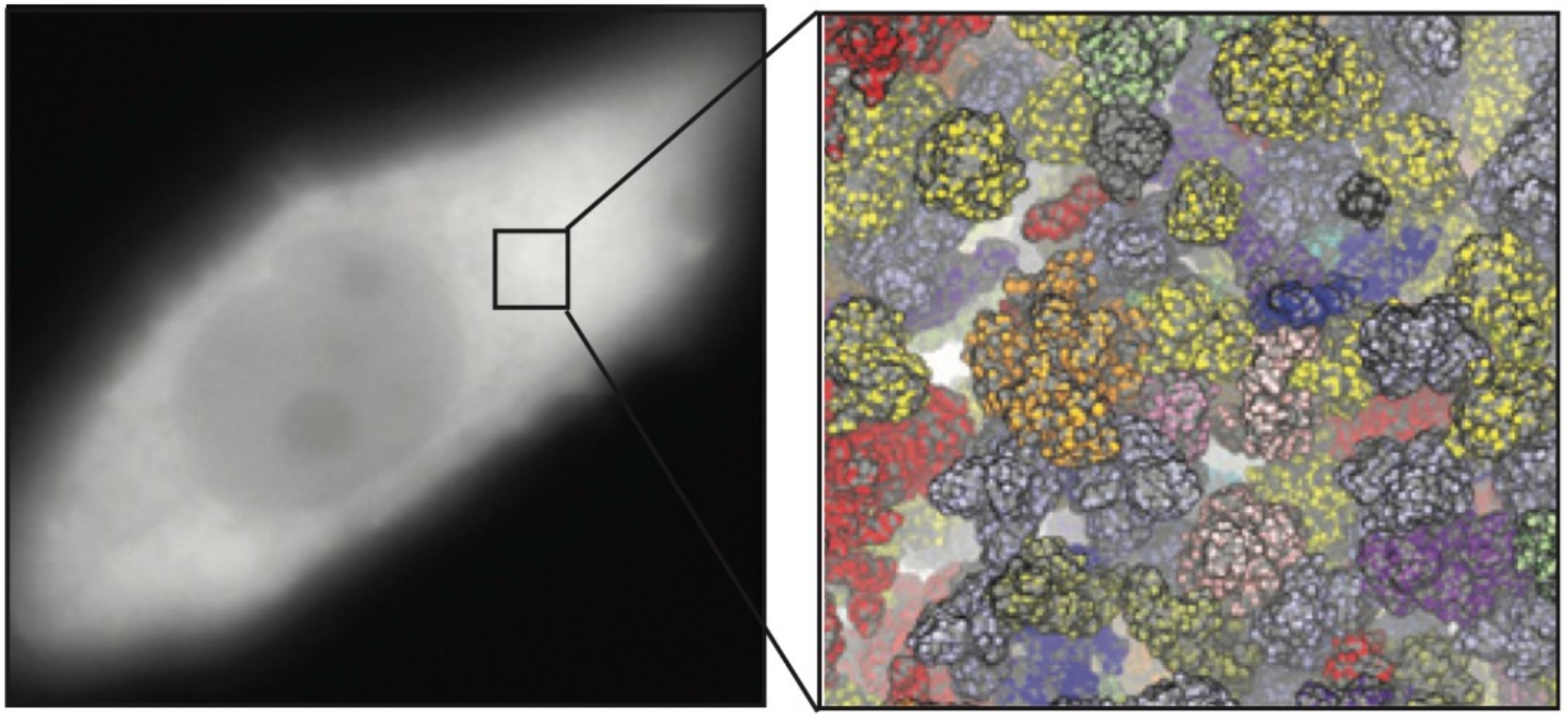
- Experimental aspect
- Cell environment

Different binding affinity between in vivo and in vitro- Experimental aspect

- Lack of contextual data (target physiology, pathology and micro-environment) in samples and an assay platform capable of probing the interactome.
- Binding differences between soluble and membrane-bound forms of target.
- Performed in non-native environments, are restricted to relatively simple matrices such as buffer.

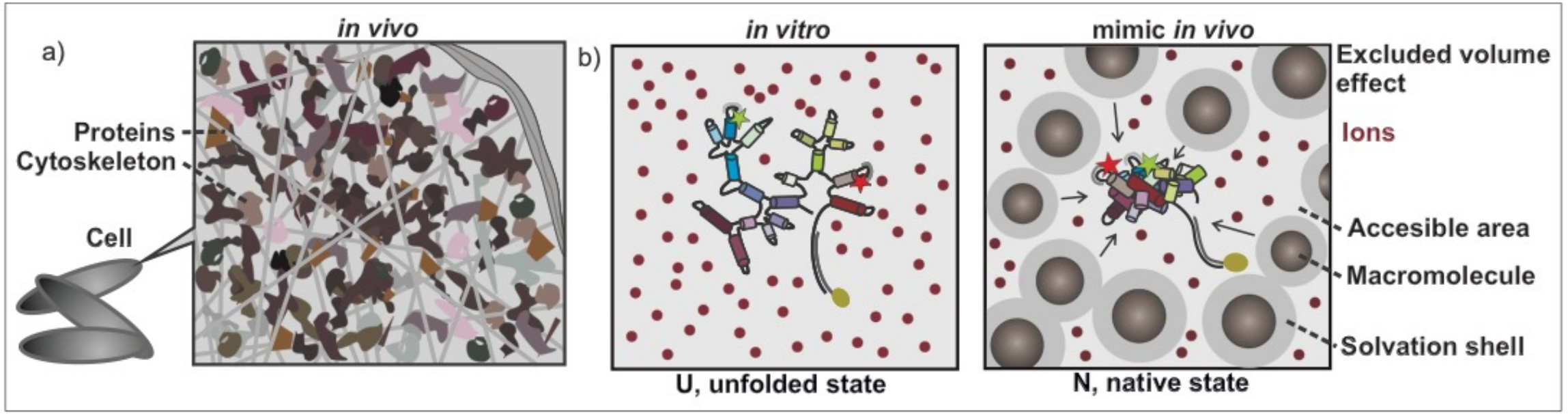
Different binding affinity between in vivo and in vitro -Macromolecular crowding

- Refers to a phenomenon that alters the properties of molecules in a solution when high concentrations of macromolecules such as proteins are present.



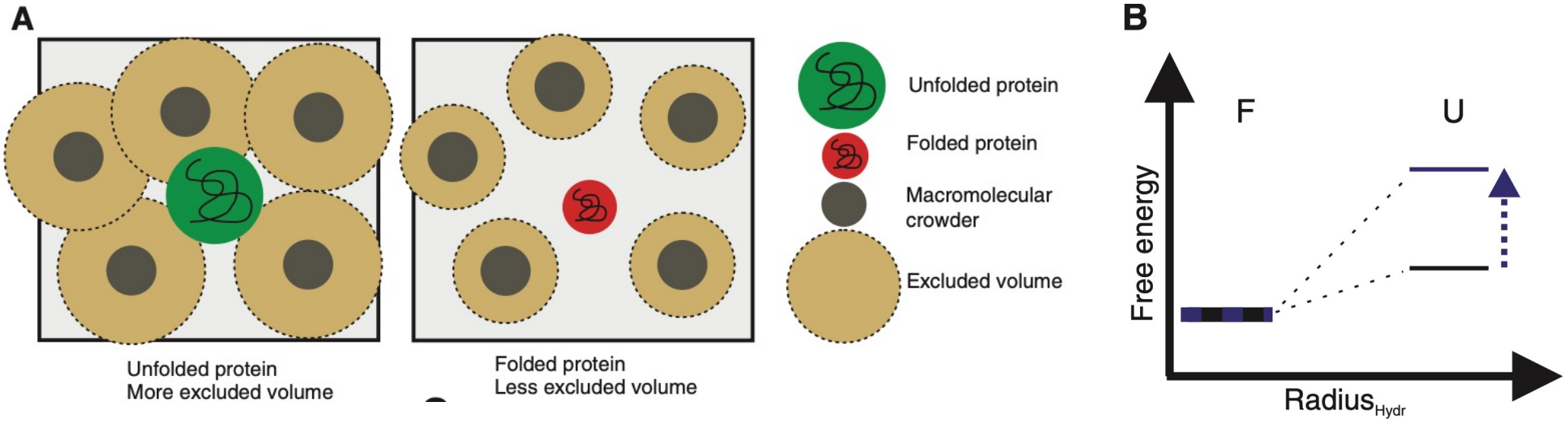
David G., *Biol.Chem.*, **2016**, *397*, 37

Macromolecular crowding-excluded volume effect



Erica Fiorini, *CHIMIA International Journal for Chemistry*, **2015**, *207–212*

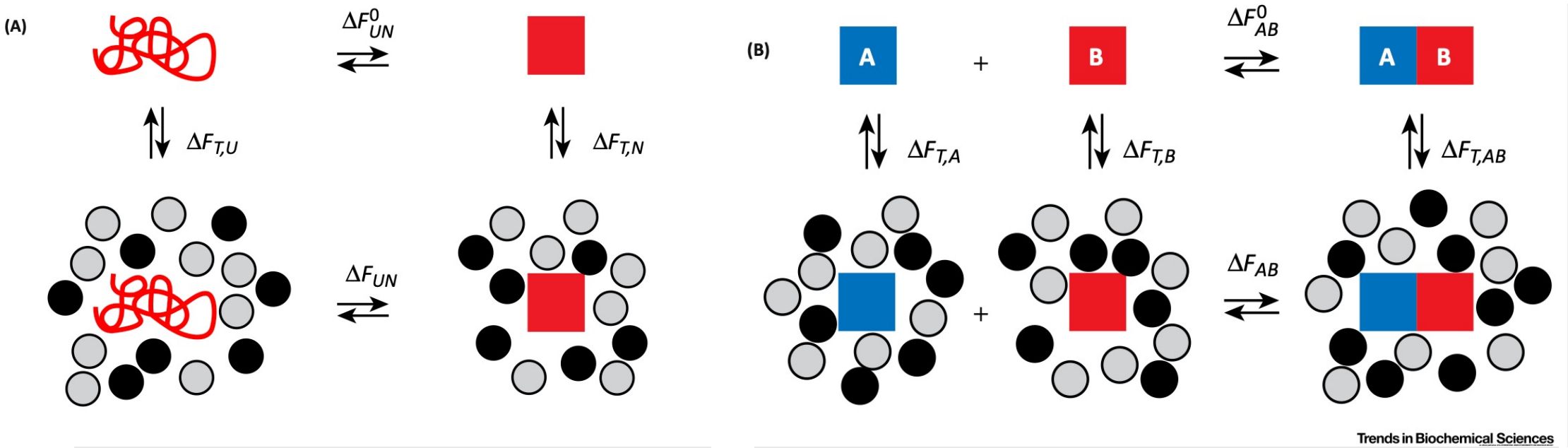
Macromolecular crowding-excluded volume effect



proteins are more stable in a crowded solution of macromolecules compared to dilute aqueous solution

David G., *Biol.Chem.*, **2016**, 397, 37

Macromolecular crowding-excluded volume effect

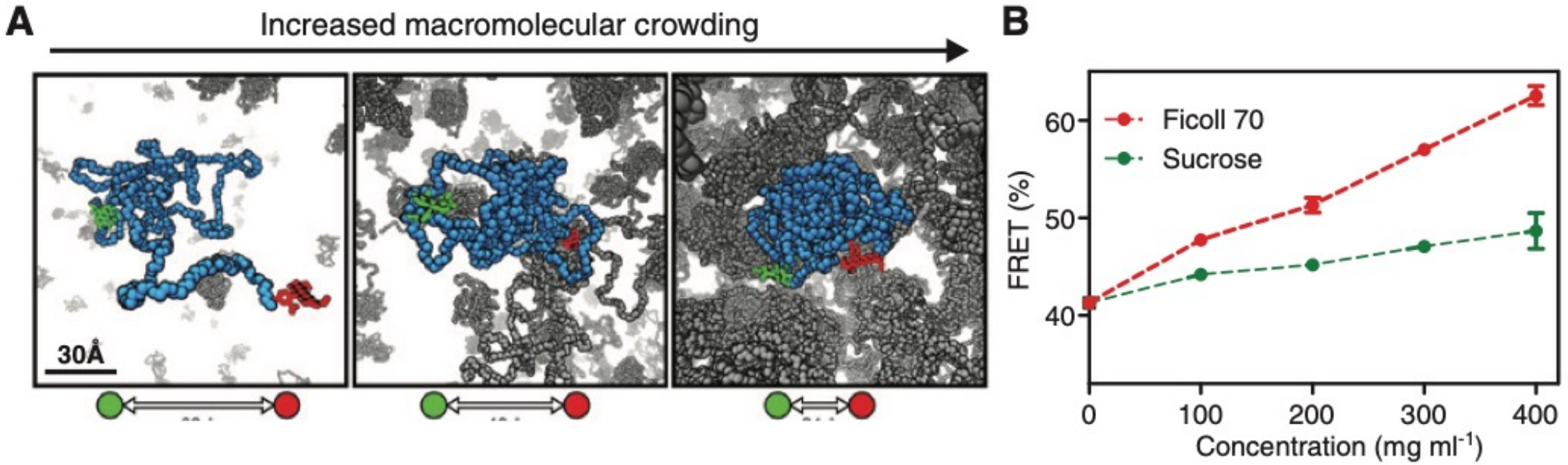


$$\Delta\Delta F_{UN} = \Delta F_{UN} - \Delta F^0_{UN} = \Delta F_{T,N} - \Delta F_{T,U}$$

$$\Delta\Delta F_{AB} = \Delta F_{AB} - \Delta F^0_{AB} = \Delta F_{T,AB} - (\Delta F_{T,A} + \Delta F_{T,B})$$

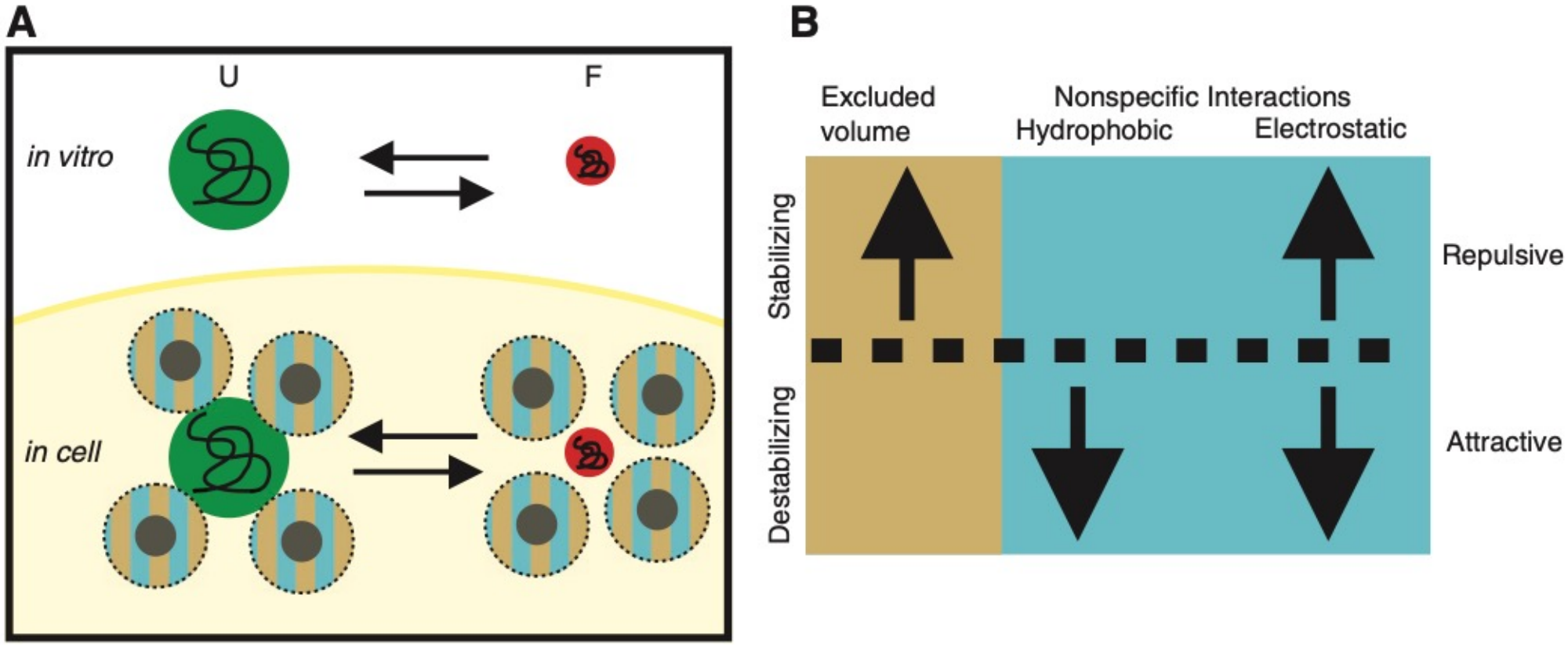
Germán Rivas., *Trends in Biochemical Sciences*, **2016**, *41*

Analysing macromolecular crowding effects in the living cell



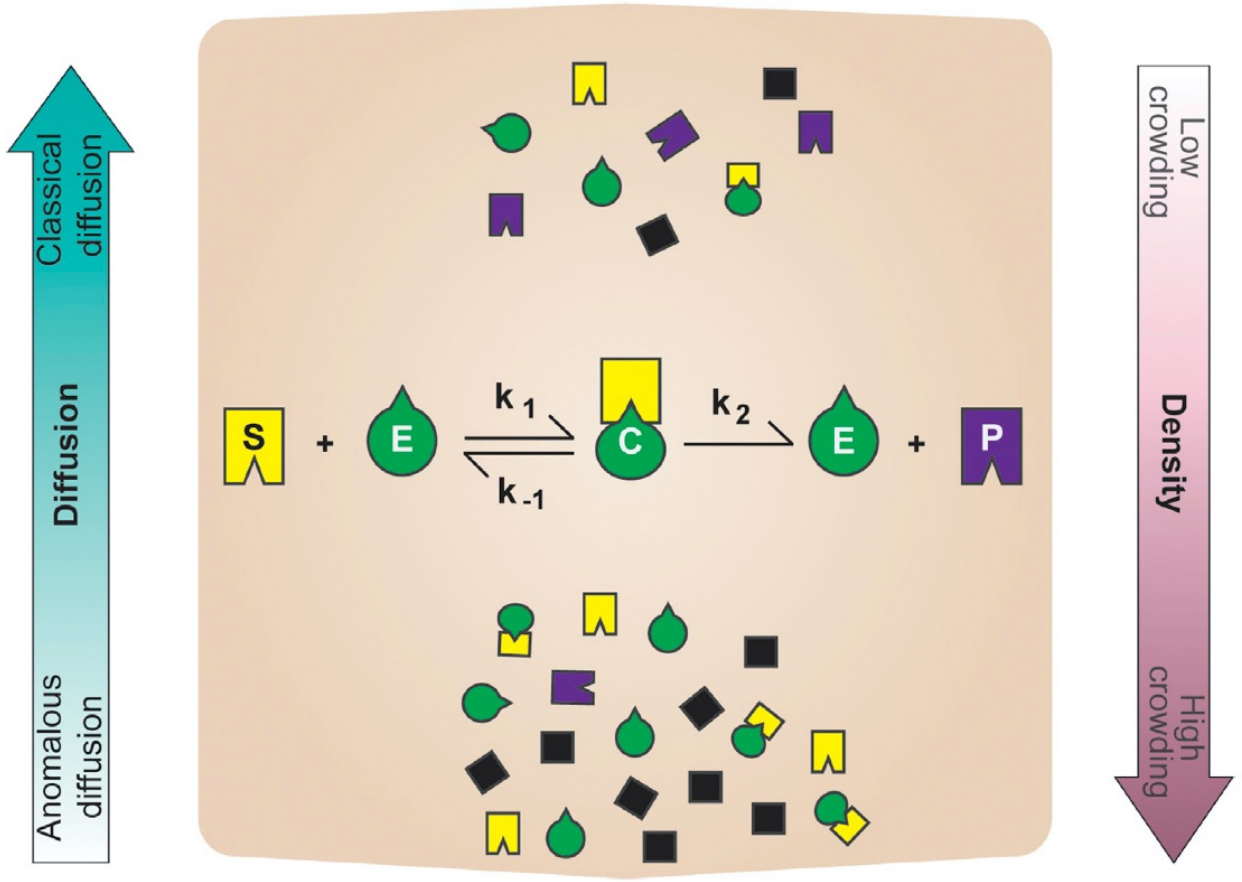
David G., *Biol.Chem.*, 2016, *397*, 37

Macromolecular crowding-excluded volume effect and nonspecific interactions



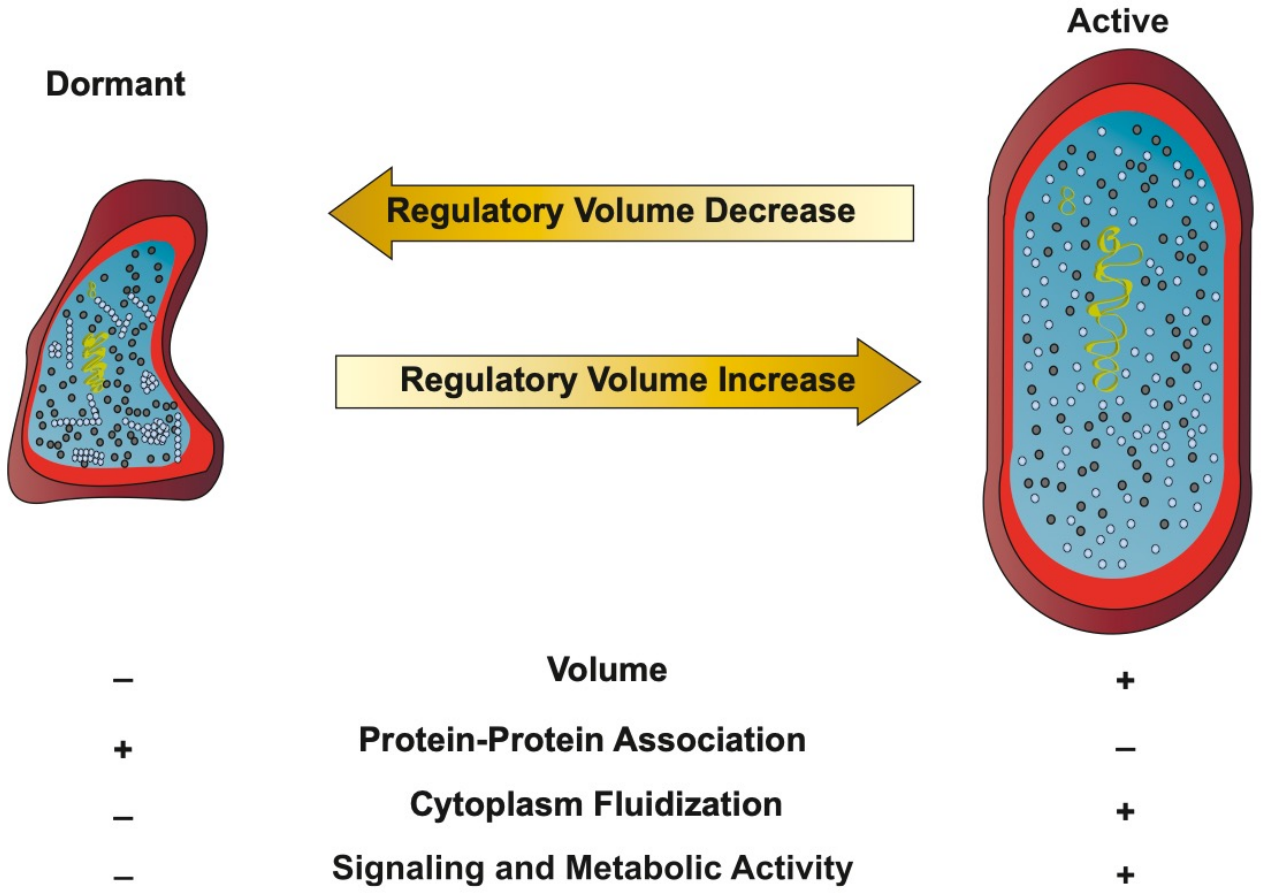
David G., *Biol.Chem.*, **2016,** *397,* 37

Macromolecular crowding affects diffusion and the rates of enzyme-catalyzed reactions.



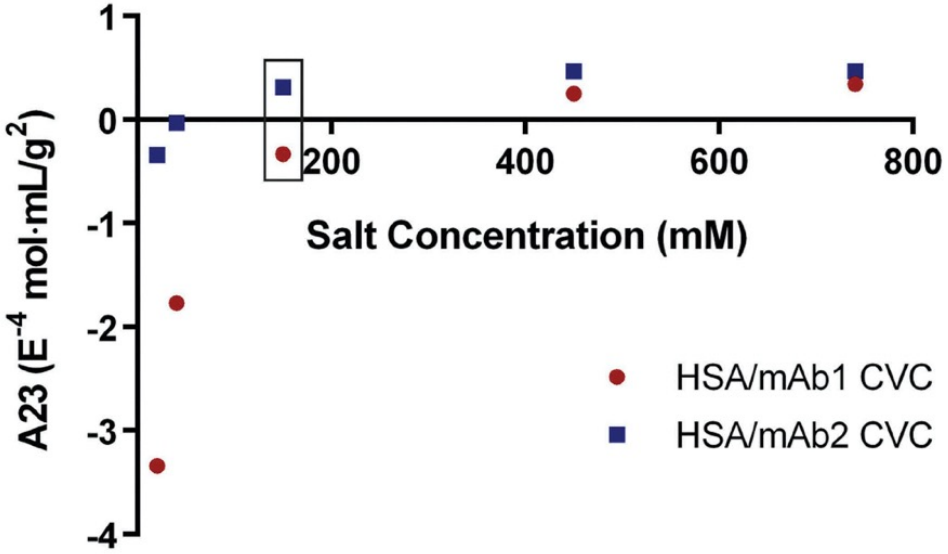
Ma´rcio A. Mourã o, *Biophysical Journal*, **2014**, *107*, 2761

Modulation of cellular volume to control physiological processes via macromolecular crowding



Ma´rcio A. Mourã o, *Biophysical Journal,* **2014,** *107,* 2761

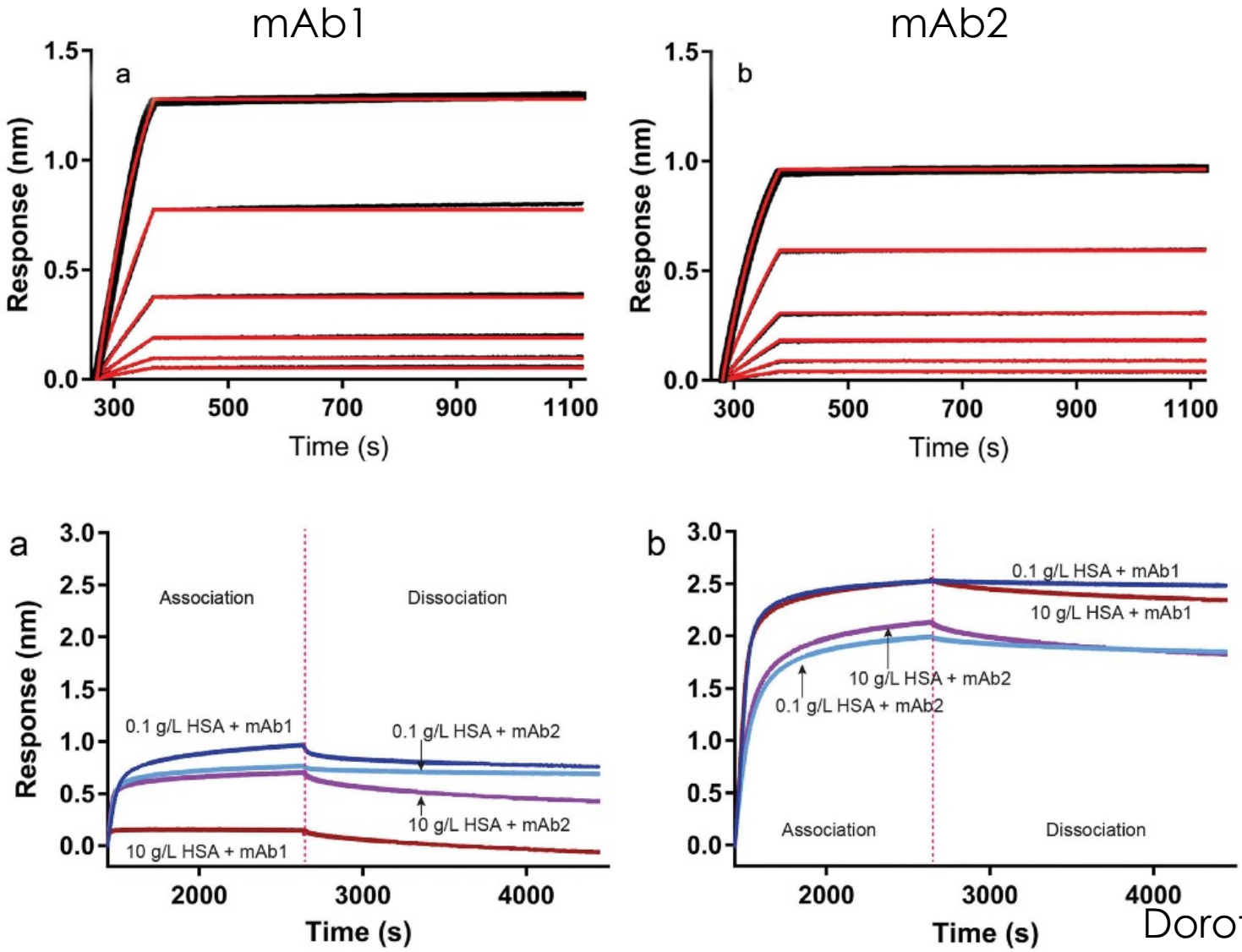
A specific example- the effects of macromolecular crowding on antibody function ：HSA&mAbs



Ionic strength dependence of mAb1/HSA and mAb2/HSA cross interactions measured by CG-MALS

Dorothy M Kim, *mAbs*, ***2019***, 1319

A specific example- the effects of macromolecular crowding on antibody function

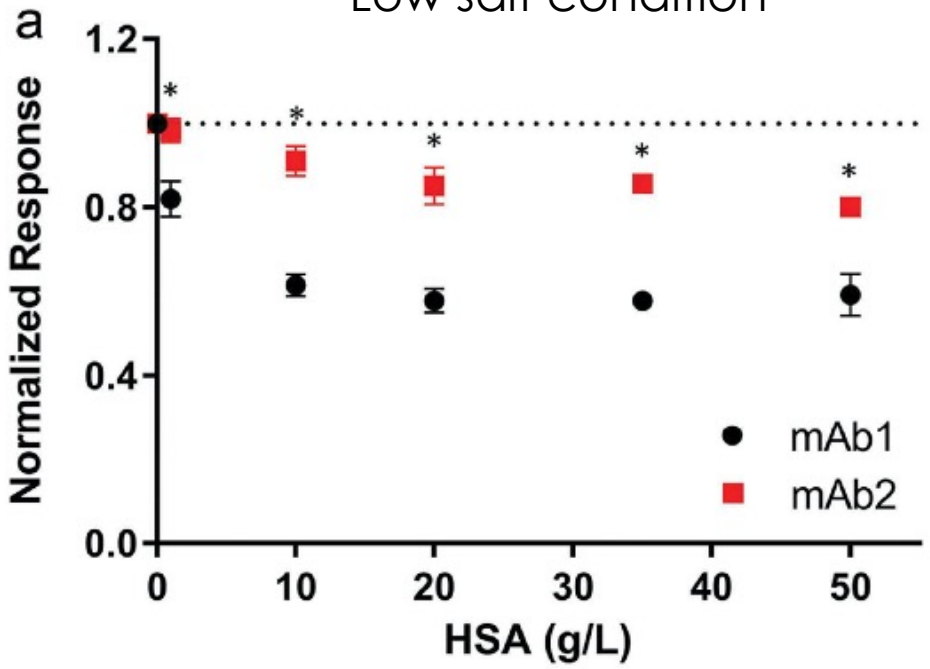


Binding of 40 nM mAb1 and mAb2 to biotinylated antigen in the absence and presence of HSA was observed by biolayer interferometry at 10 (panel a) and 137 mM NaCl (panel b) in phosphate buffer

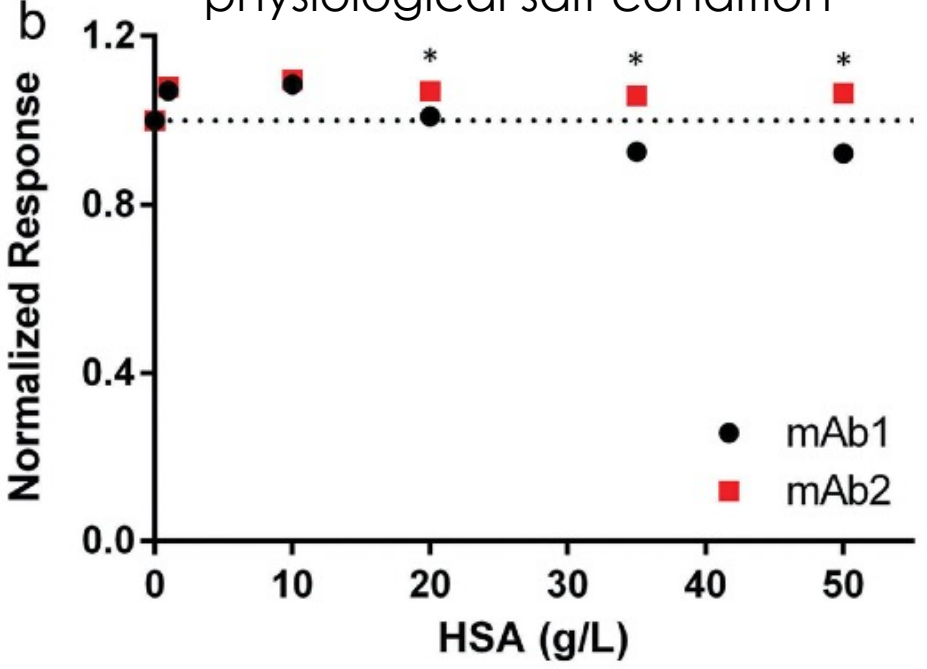
Dorothy M Kim, *mAbs*, ***2019***, 1319 [23]

A specific example- the effects of macromolecular crowding on antibody function

Low salt condition



physiological salt condition



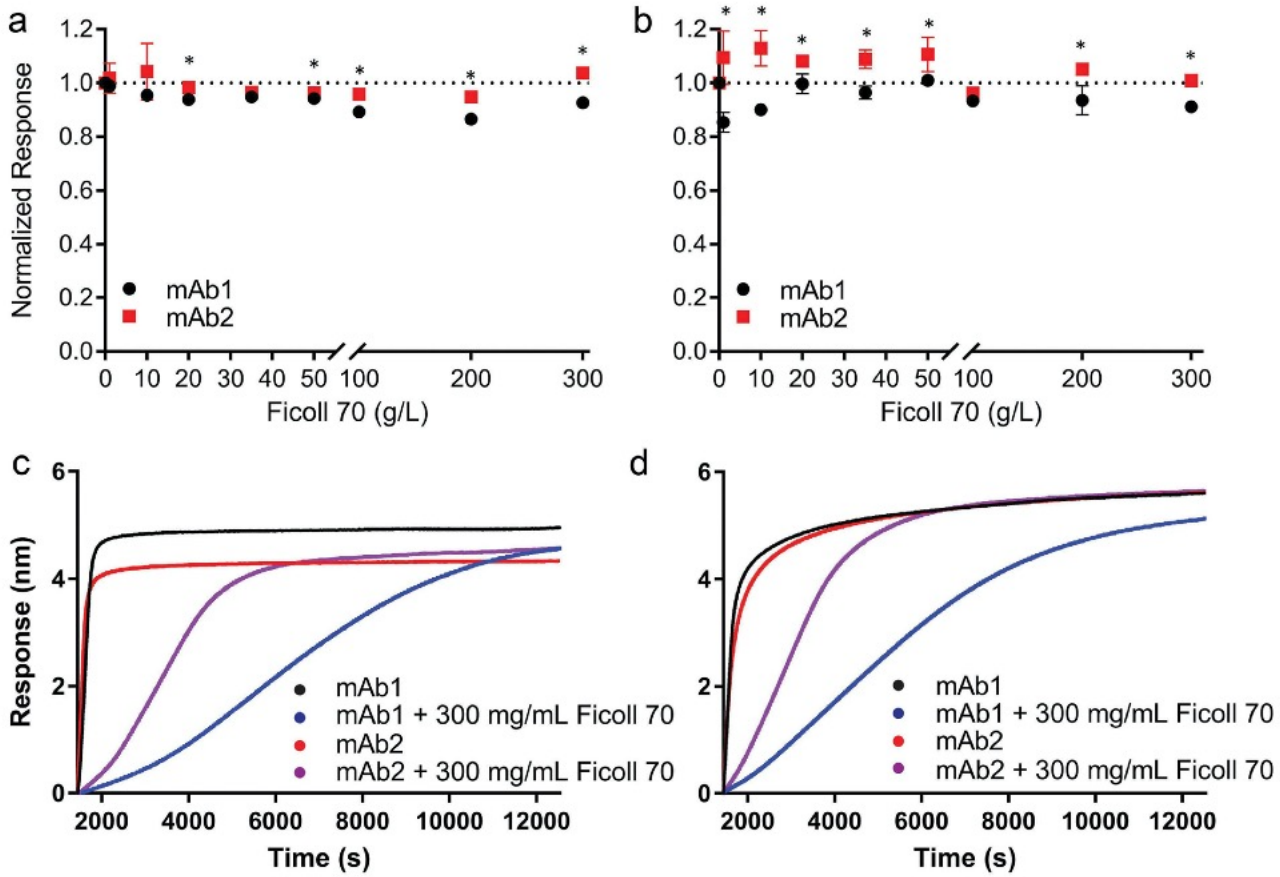
Dorothy M Kim, *mAbs*, ***2019***, 1319

A specific example- the effects of macromolecular crowding on antibody function

- The crowding agent ficoll 70 does not produce the same effect on mAb binding to antigen

Low salt condition

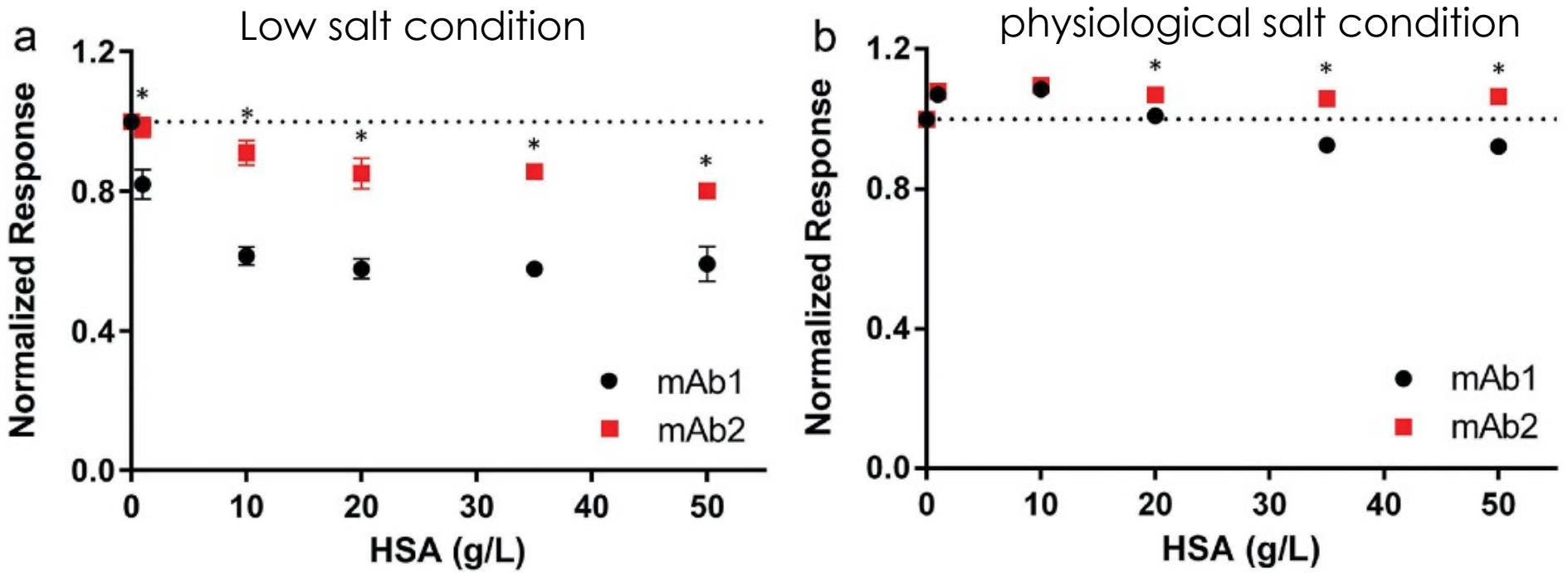
physiological salt condition



Dorothy M Kim, *mAbs*, ***2019***, 1319

Binding affinity

- The physicochemical complexity of the solvent-accessible surface areas presented by different proteins plays a fundamental role in the diversity of non-specific macromolecular interactions.



Dorothy M Kim, *mAbs*, ***2019***, 1319

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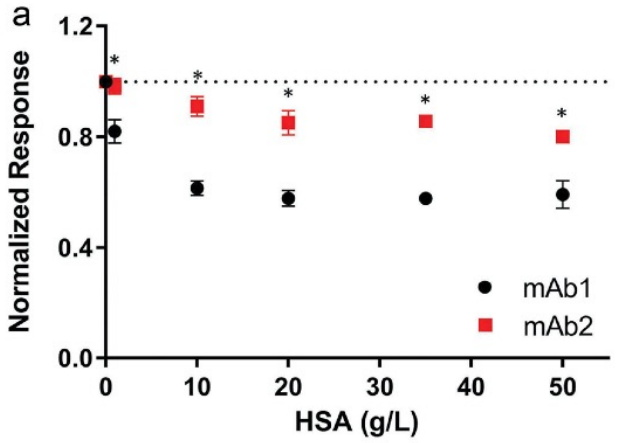
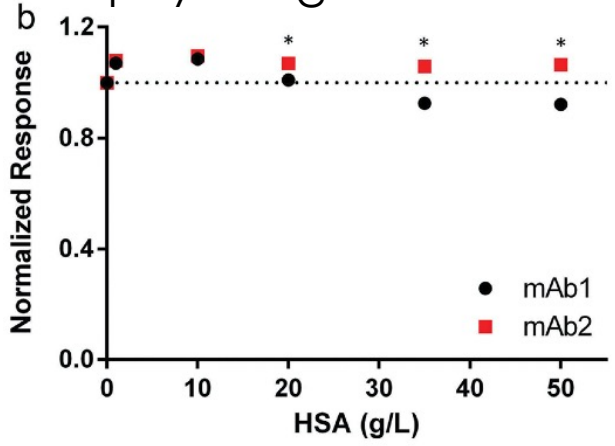
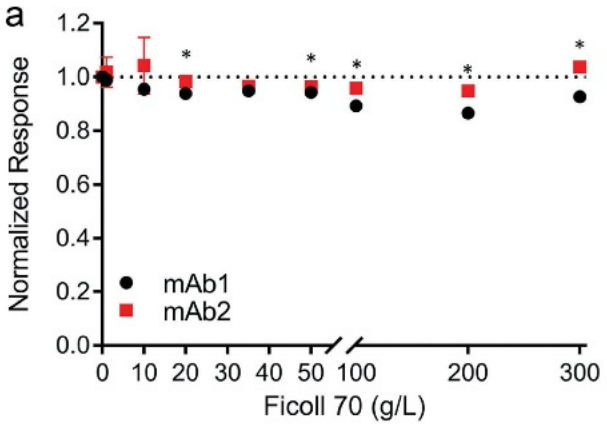
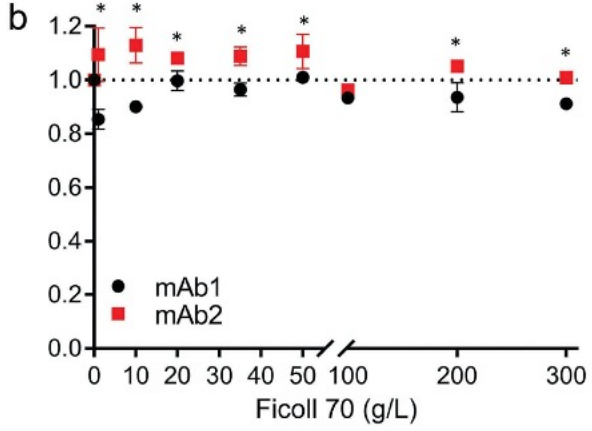
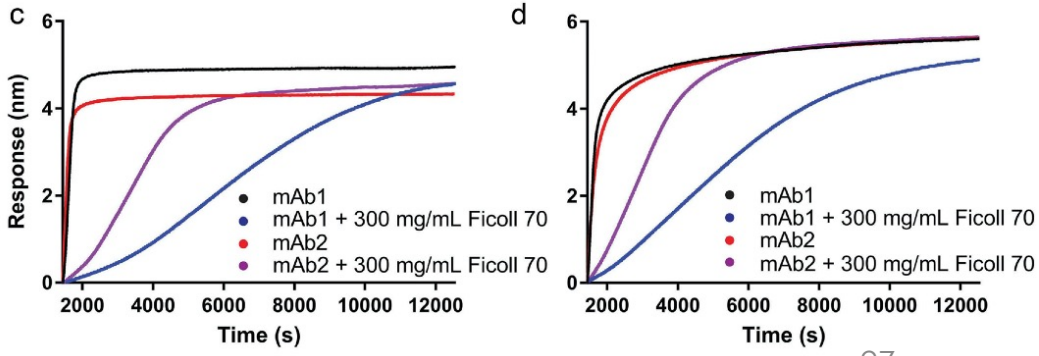


physiological salt condition









Dorothy M Kim, *mAbs*, ***2019***, 1319

Different macromolecular crowding agent cause different results

Differential effect of HSA and RNase A as crowder proteins

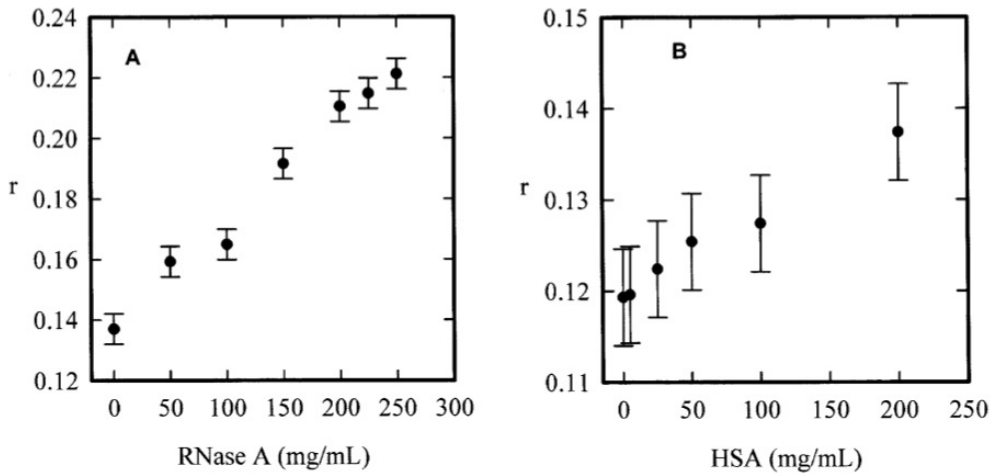


Figure 2. Steady-state fluorescence anisotropy (r) of labeled apoMb as a function of crowder concentration. (A) ApoMb-ANS in RNase A solutions; [apoMb-ANS] = 80 μM; λ_{exc} = 393 nm and λ_{em} = 465 nm. (B) ApoMb-Fl in HSA solutions; [apoMb-Fl] = 2 μM; λ_{exc} = 460 nm and λ_{em} = 520 nm. $[apoMb]_T$ = 100 μM. T = 20°C.

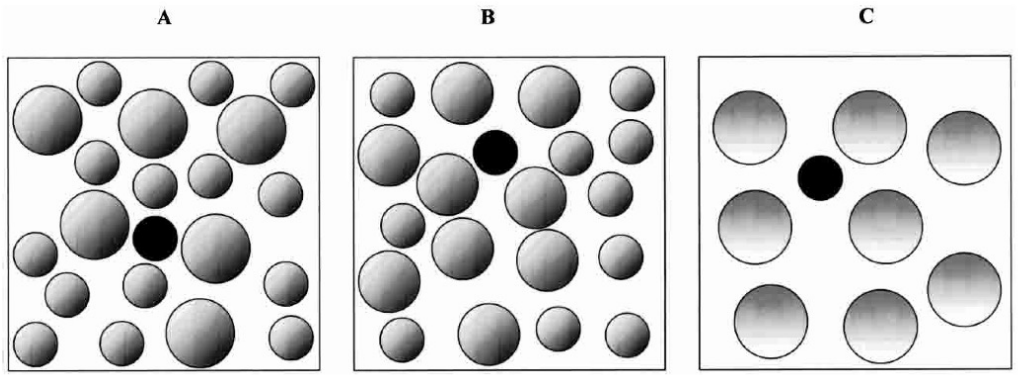


Figure 5. Idealized scaled representation of excluded and free volume in crowded solutions. (A) 200 mg/mL RNase A solution containing monomers and tetramers of the crowder. (B) 200 mg/mL RNase A solution containing monomers and trimers, and (C) 200 mg/mL HSA solution. All species are represented as spherical particles of equivalent volume, assuming an hydration of 0.3 g of water/g protein. The black circle represents an apoMb monomer molecule.

Silvia Z., *Protein Sci.*, **2004**, *13*, 2960

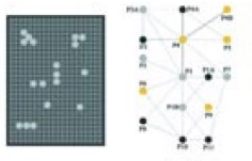
Macromolecular crowding

- Contrary to the typical in vitro media, the intracellular environment is densely packed with macromolecules.
- Excluded volume effect + nonspecific interaction
- Polymer crowders do not consistently produce an effect on ligand binding, and may even have totally different effect on different proteins

Structure prediction of macromolecular complexes

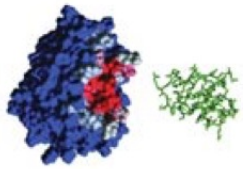
- Scheme of a typical drug discovery process.

Target identification

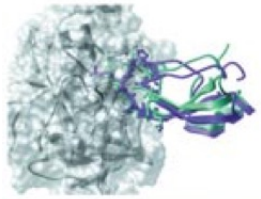


1. Establish protein–protein interaction — Correlated mRNA expression profiles; correlated evolution; domain fusion patterns; automated literature mining

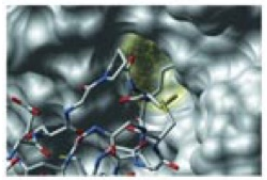
Target characterization



2. Locate interface — Surface analysis; hydrophobicity profiles; 3D cluster analysis; residue conservation

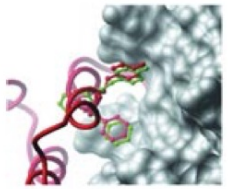


3. Modeling protein–protein interaction — Rigid-body docking; energy minimization; side-chain refinement; flexible docking

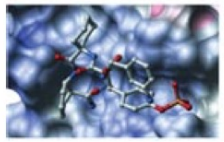


4. Finding putative small-molecule pockets — Analysis of 'hot spots'; surface concavities

Lead discovery and optimization



5. Mimicking interface — Energy minimization; graphic modeling



6. Ligand docking — Flexible ligand docking; grid or explicit receptor representations; MC minimization

Structure prediction of macromolecular complexes

- Docking: developed but still influenced by several factors

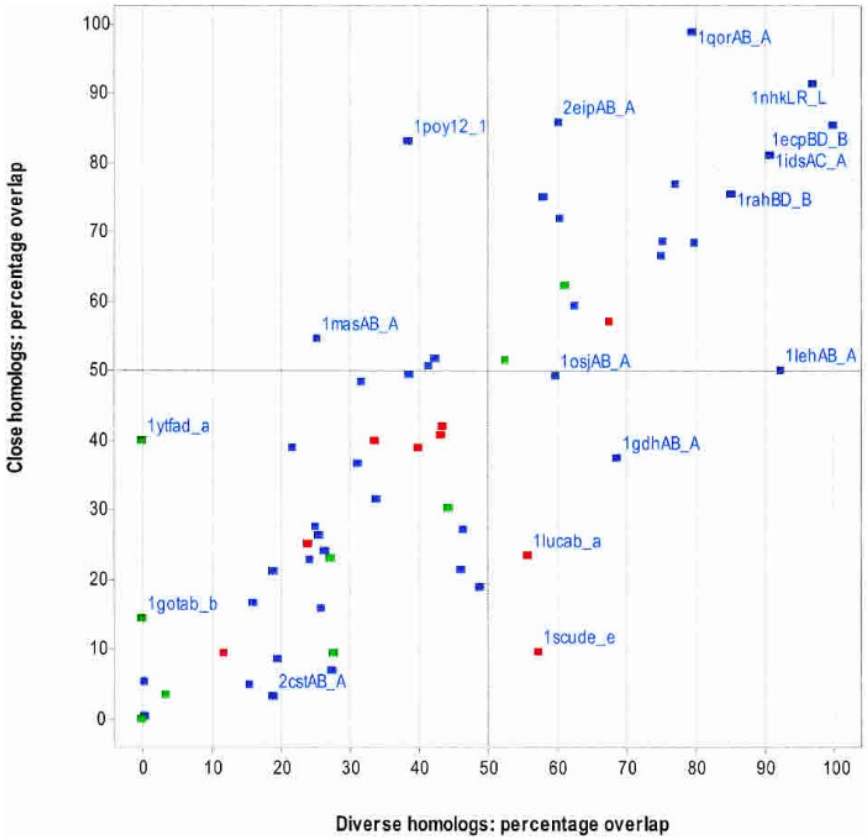
- Proteins are not static structures.

- The binding site is not always conserved or cannot always be identified.

- Current docking methods cannot distinguish whether two proteins will bind or not, (predict the binding affinity).

Structure prediction of macromolecular complexes

The binding site is not always conserved or cannot always be identified.



The most conserved surface patch on a protein was rarely found to share >50% residue overlap with the real interface.

The data set consists of 42 chains that form homodimers, 12 chains that form heterodimers, and 10 chains that form transient complexes as described

Overall, the results suggest that one will have a small chance (17/64) of correctly predicting 50% of the interface residues

Caffrey DR., *Protein Sci.*, **2004**, 190

Structure prediction of macromolecular complexes

Current docking methods cannot distinguish whether two proteins will bind or not, (predict the binding affinity).

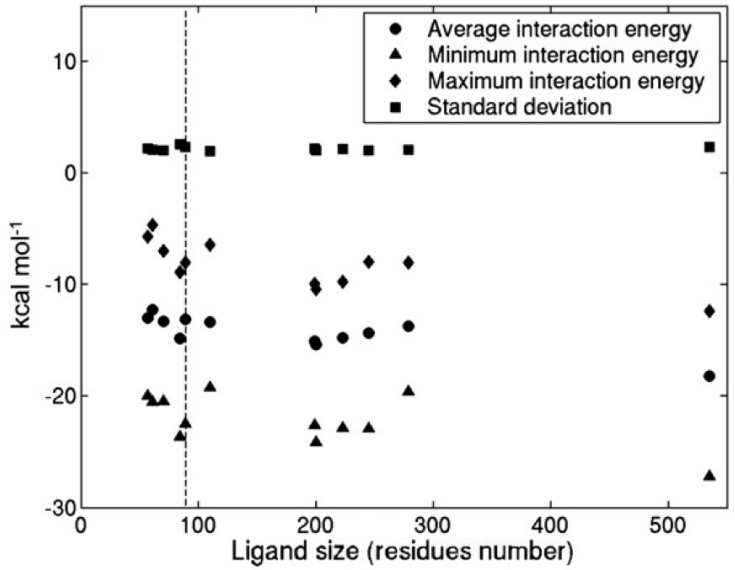
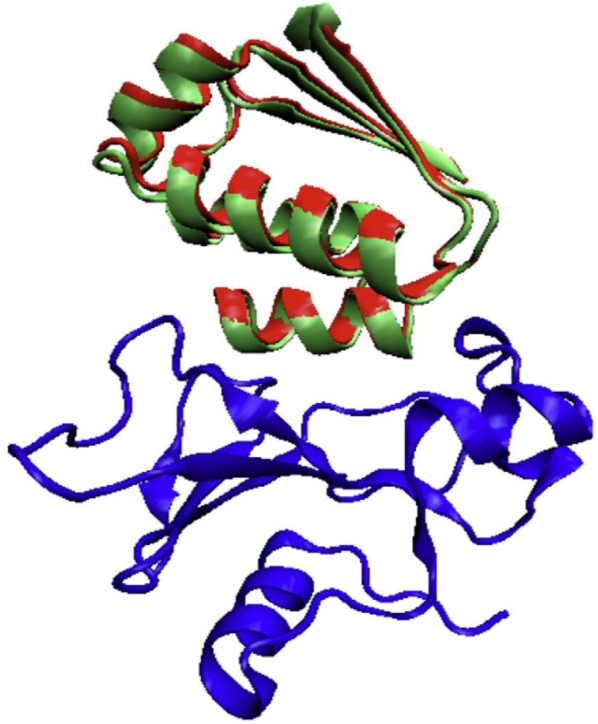


Fig. 3. Statistical data concerning the energy maps obtained for barnase. The vertical broken line crosses points corresponding to the experimental partner (barstar).

Barstar-barnase complex



.Sacquin-Mora S. et al., *J. Mol. Biol.*, **2008**, *382*, 1276

Summary

- Correct and precise estimation of the binding affinity is crucial throughout these essential drug design stages.
- CETSA and BLI would be effective method to estimate binding affinity.
- Buried surface area, hot spots and anchor residues and allosteric regulators and non-interface affinity modifiers would be the determinant of binding affinity.
- Macromolecular crowding may account for the difference of binding affinity between in vivo and in vitro.
- Some problems still need to be resolved for the prediction of binding affinity.