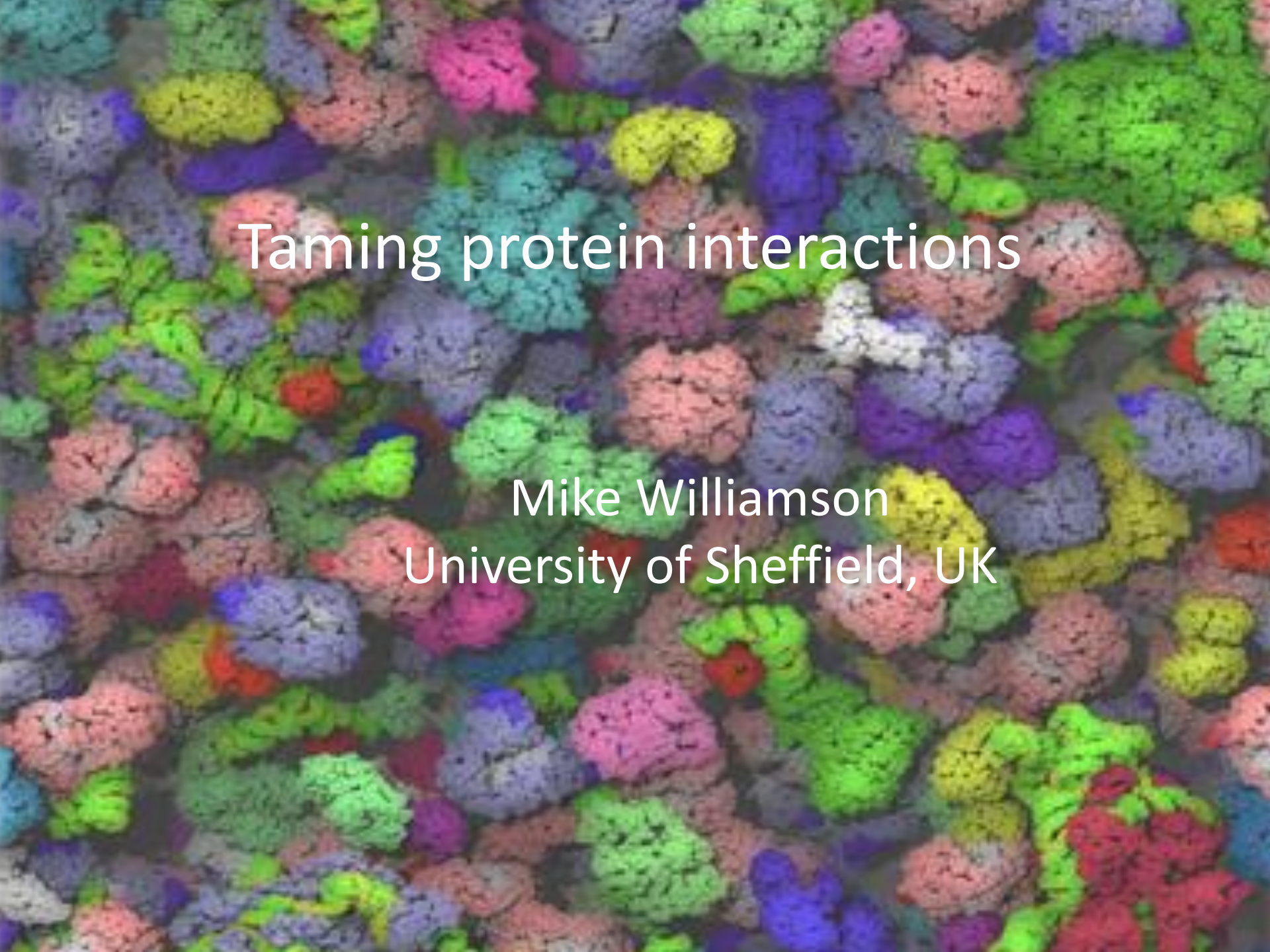

# Taming protein interactions

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# Outline

Part I

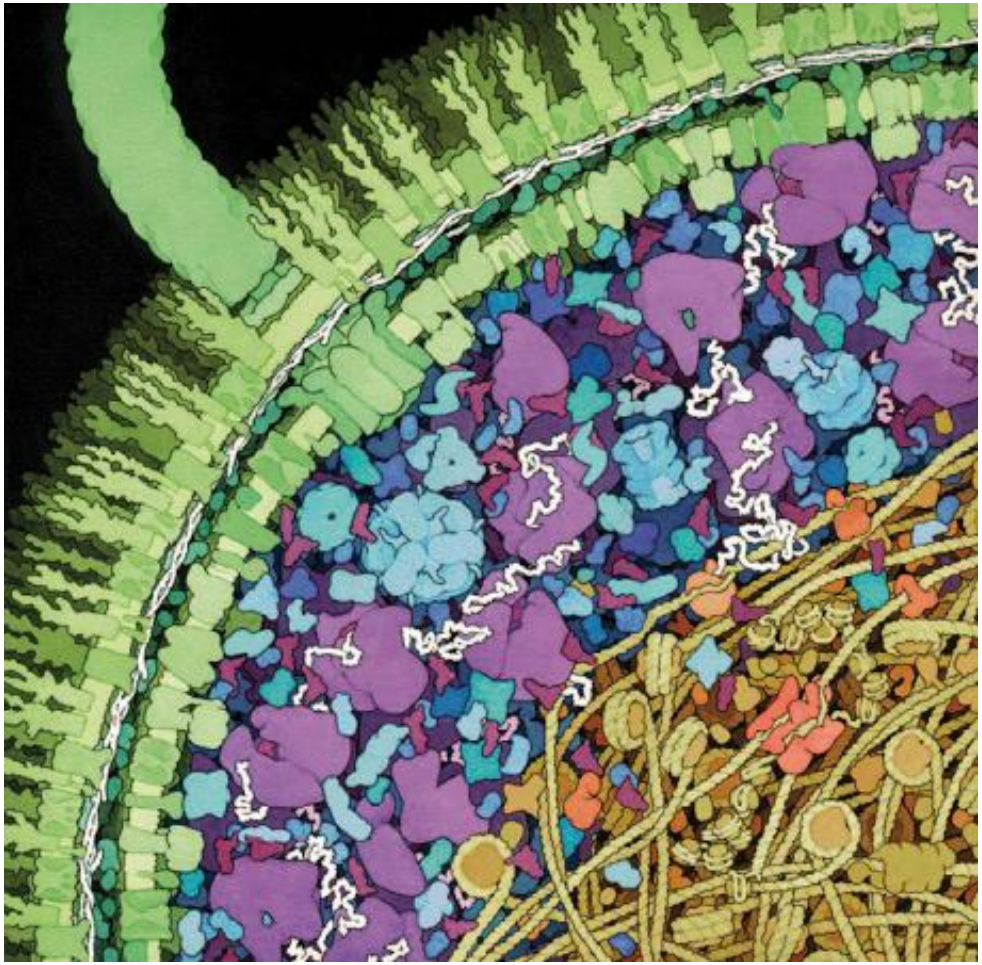
- Protein interactions in the cell
- The importance of water

Part II

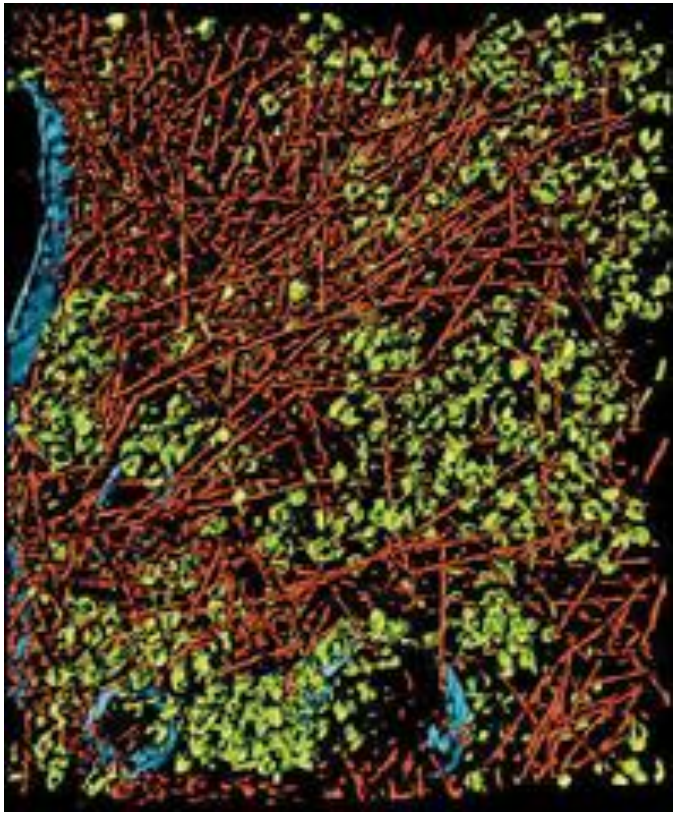
- The Hofmeister series, denaturants and osmolytes
- What to do about interactions: remove or tame?

# Protein interactions in the cell

Cell is very crowded: almost the same density as in a protein crystal



*E. coli*
David Goodsell, Scripps



*Dictyostelium* by cryoelectron tomography (*Science* 2002 **289** 1209)

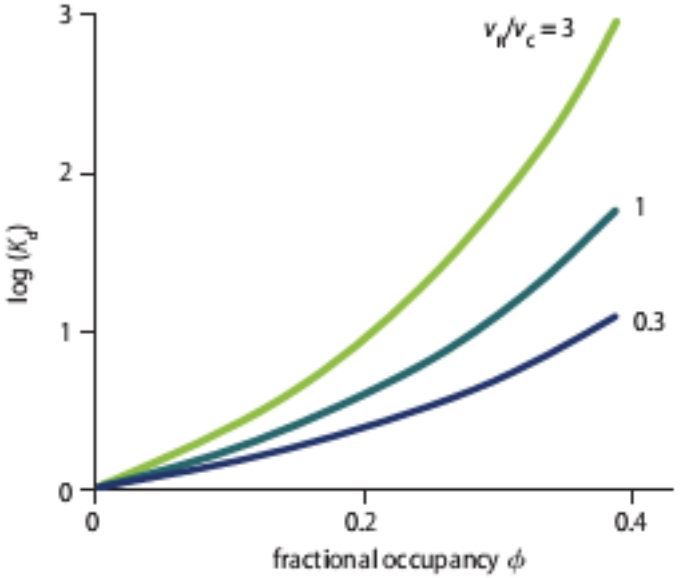
Mean distance between proteins is usually < 100 Å, often < 50 Å

Average protein diameter is about 35 Å – so only a few Å in between

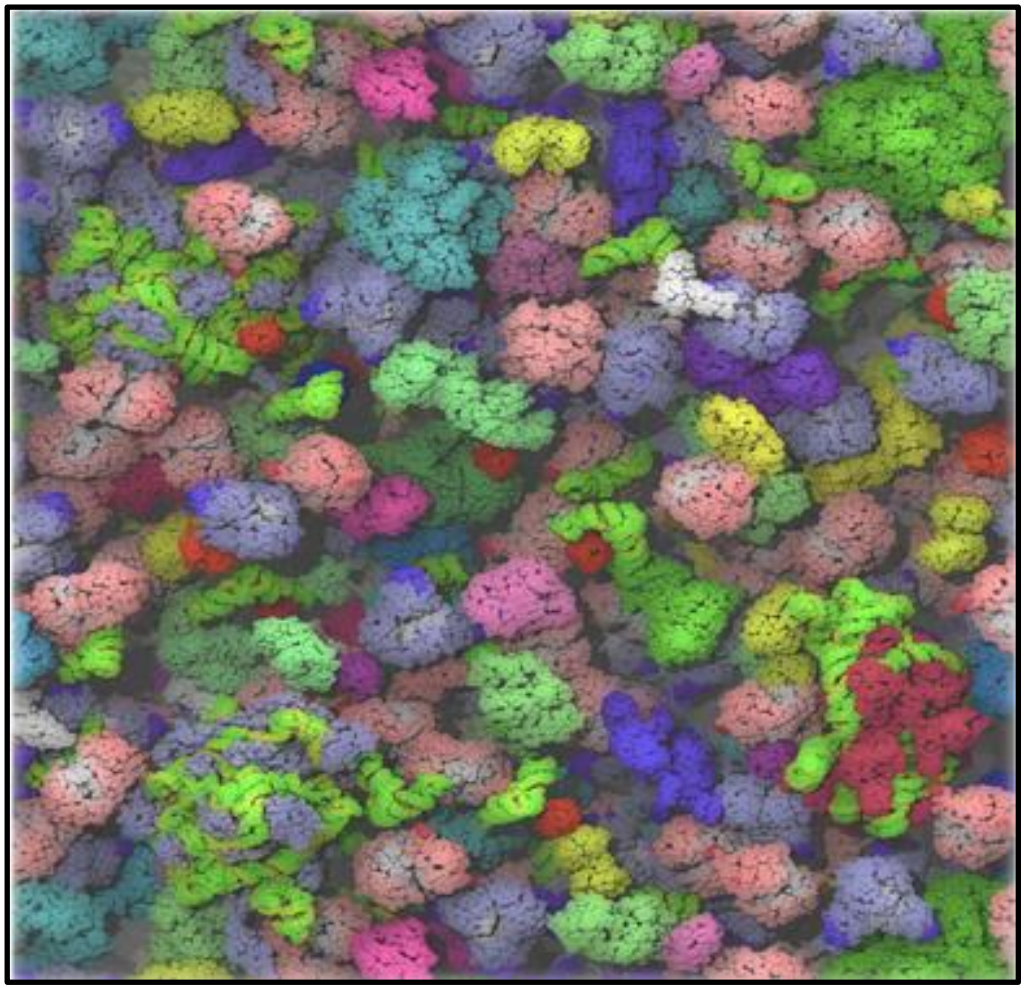
Proteins occupy up to 40% of total volume in cells
(Compare this with the packing density of spheres which is about 65%!)

***Cellular/macromolecular crowding***

One consequence is that protein/protein interactions are much stronger than they would be in dilute solution – by roughly a factor of 10



Is it really like this in cells? *Yes!*



Simulation based on excluded volume

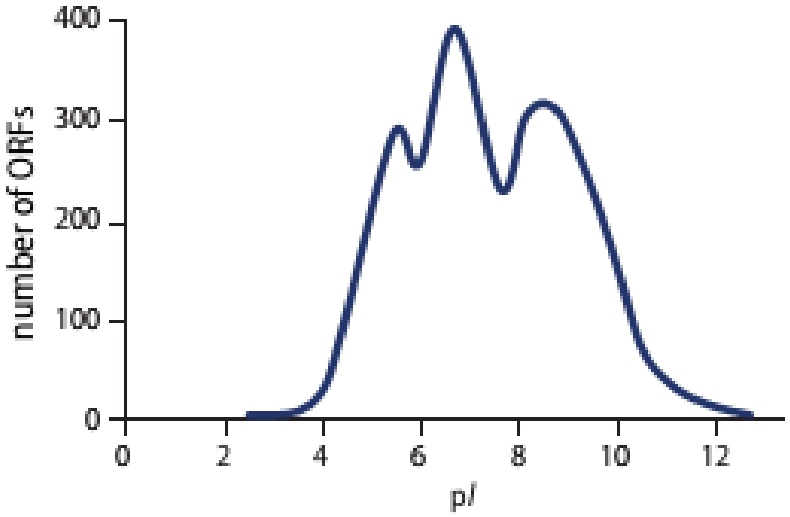
Simulation based on excluded volume plus protein/protein interactions (hydrophobic, charge/charge, etc)

If it's as bad as this, how does anything move in cells at all?

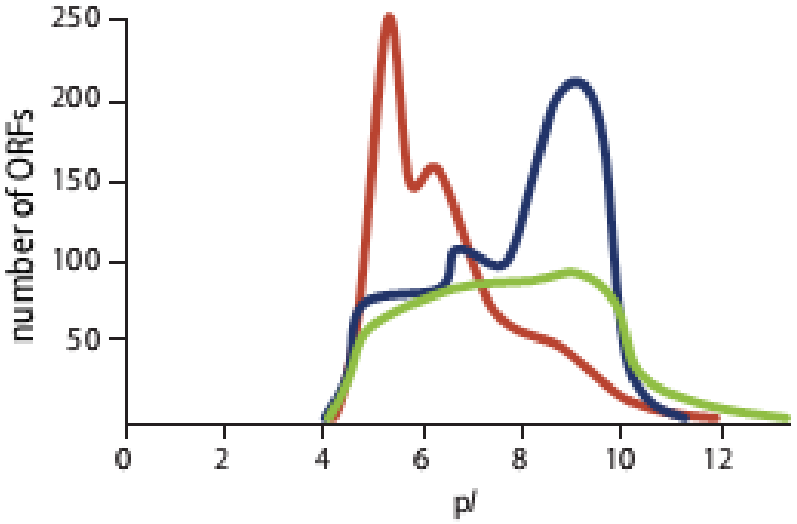
a) Proteins clustering round the membranes
- mobility in the mitochondrion is greater than you would expect

NB The implication of this is that proteins are not randomly distributed / diffusing in the cell, they are clustered in particular locations, particularly at membranes

b) Proteins have evolved to be able to cope with interactions




Predicted pI for proteins in *Drosophila*
*Genome Res* 2001 **11** 703




Cytoplasmic – mainly -ve
Integral membrane – mainly +ve
Nuclear
Membrane – mainly -ve

Proteins have evolved to bind weakly to each other (except for strong functional binding)

What do we see when we look at the protein interactome (ie, the set of interactions made by proteins)?

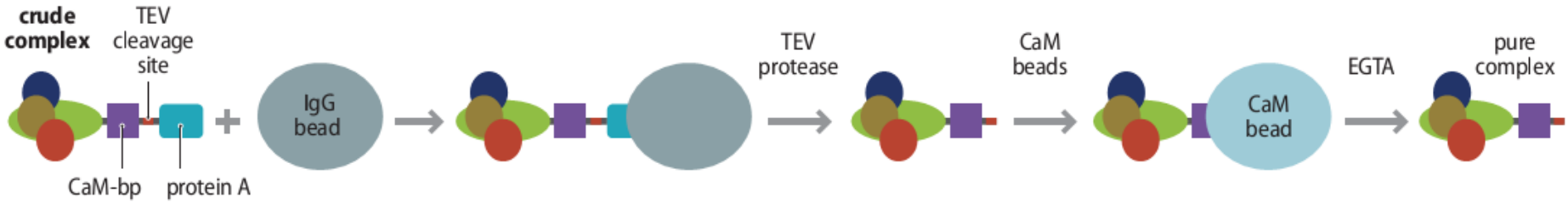
Several studies, usually by two-hybrid methods (pairwise) or TAP-tagging (sets of proteins)

Not a lot of agreement yet between studies – ie still a lot of 'noise'

- About 2/3 of all proteins had at least one partner
- Many proteins involved in more than one complex

Is this a reliable picture?

Probably not.
TAP-tagging requires several washing steps. These will remove anything bound weakly




Complexes with affinities weaker than about 0.1 µM will get washed off and will not be observed.
TAP-tag genome-wide studies have so far found almost **no** observations of metabolic complexes (glycolysis, TCA cycle, biosynthetic pathways) despite reasonably good evidence that they must exist.

So probably almost all proteins in the cell function bound to other proteins (some weak, some strong)

Some evidence from **in-cell NMR**.
Label proteins with $^{15}N$ and import them into cells.
NB In order to see these proteins, they are overexpressed at fairly high levels

If the proteins interact with other cellular components enough to slow down their rotational correlation time significantly, then the signals will be broadened so much that we will not be able to see signals from them.

Some proteins are completely invisible – ie, they are bound enough to other cellular components that it slows down the rotation

Others remain sharp, and look similar to the spectra of the protein in dilute solution. In particular ubiquitin; and intrinsically disordered proteins such as Tau and α-synuclein.

So proteins **can** avoid interactions with other proteins if they choose to – most do not choose to.

**SUMMARY**: Almost all proteins make functionally important interactions with other proteins *in vivo,* though in many cases weakly (> 0.1 μM) (therefore transient)

# What does the surface of a protein look like?

In particular, what is the role of water?

Proteins have a hydration layer on the surface.
The water molecules in this layer are slightly more tightly bound, slower to move away, less compressible, and more ordered than bulk water.

But the layer is no more than 2 molecules thick.

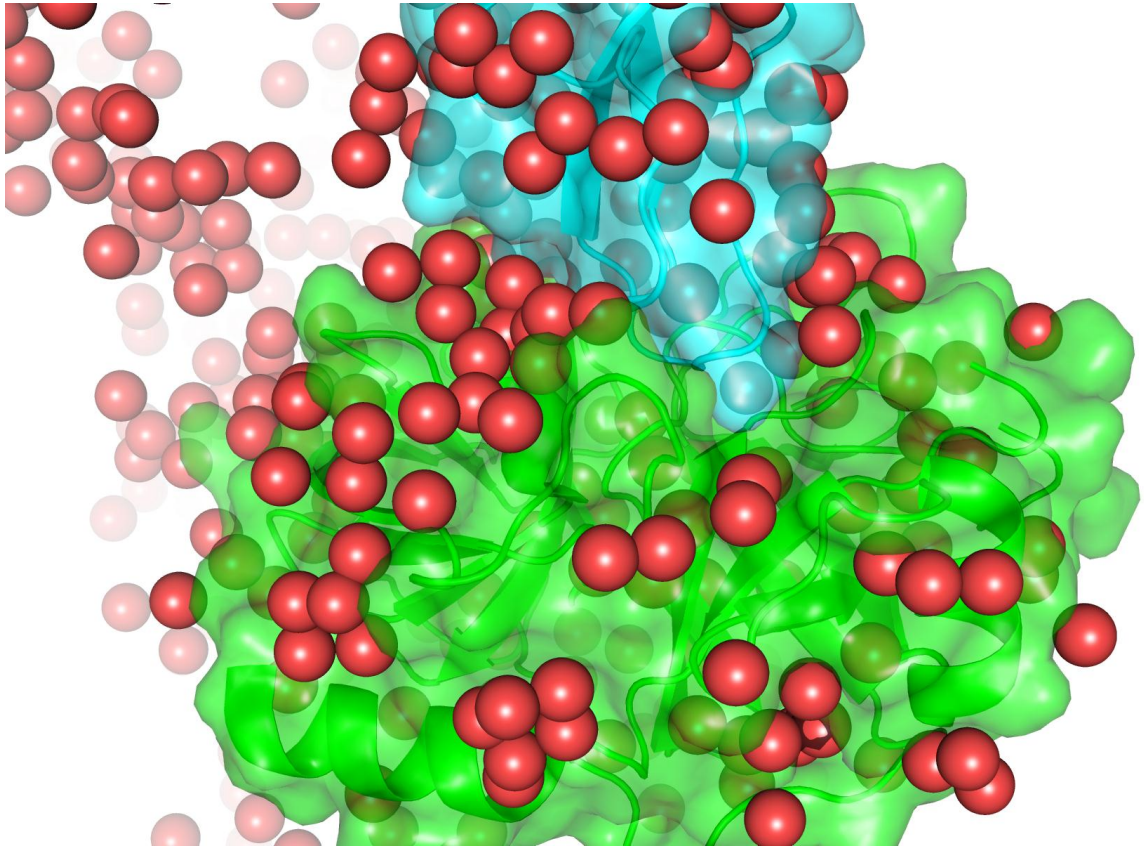
When you remove water completely from a protein, it becomes much less active.
But you only have to add back about 50 waters to regain activity; full activity requires a few hundred – less than a single layer thick.

NMR studies and MD showed that most hydration layer water has only slightly longer correlation time than bulk (maybe 10 ps compared to 2.5 ps for bulk): the only hydration water significantly retarded is in pits and cavities on the surface.

Are protein interfaces similar, ie also hydrated?
Water is commonly found ‘filling up the gaps’ in protein/protein interfaces

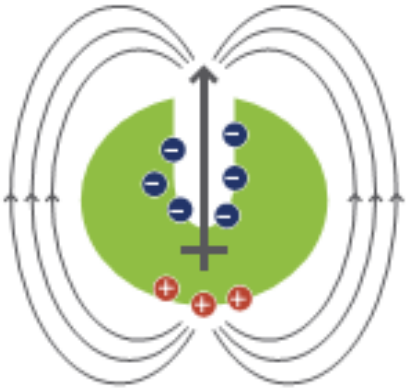
Trypsin (green) and BPTI (blue). Waters in magenta.

Lots of waters in the interface, even buried deep.



Water screens surface charges very effectively

But the distribution of charges on proteins generates a field around the protein which steers other proteins towards them



Eg barnase/barstar
Come together at a rate $10^{10}$ $M^{-1}$ $s^{-1}$
This is about 10 times **faster** than the diffusion-controlled limit

Eg2 trypsin/BPTI
On-rate is $10^{6}$ $M^{-1}$ $s^{-1}$
Both proteins are positively charged
Mutate K15 in BPTI to alanine
– reduces positive charge on BPTI so reduces electrostatic repulsion
- but **de**creases the on-rate by 250x

Thus, at a distance, a protein is mainly an electric field: surface features do not matter until you get very close.
Two proteins colliding will typically be in contact for 0.4 ns, but then remain closely associated for ≈ 6 ns, allowing them time to explore surface features quite extensively (correlation time typically a few ns)

Water molecules get out of the way in ps, ie much more rapidly, and thus present no barrier to binding

# To come to the point….

- Proteins have evolved to make multiple weak and transient interactions
- The normal environment of proteins in the cell is to be surrounded by high concentrations of other proteins
- Proteins are very shortsighted – they can only see surface features at very close range

So what is the problem when proteins are at high concentration
(eg drug formulation; overexpression into inclusion bodies; amyloid)?

- High concentration of protein makes intermolecular interactions more likely (same arguments as for 3D domain swapping)

Implies the solution is to stop proteins unravelling and keep themselves to themselves

# Part II: Weak interactions with counterions and osmolytes

Why do denaturants (urea, guanidium) denature proteins?

They interact with, and stabilise, the unfolded protein
- specifically the peptide backbone

## Hofmeister series

$F^- \sim SO_4^{2-} > HPO_4^{2-} > acetate^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$
(there is another series for cations but less important)

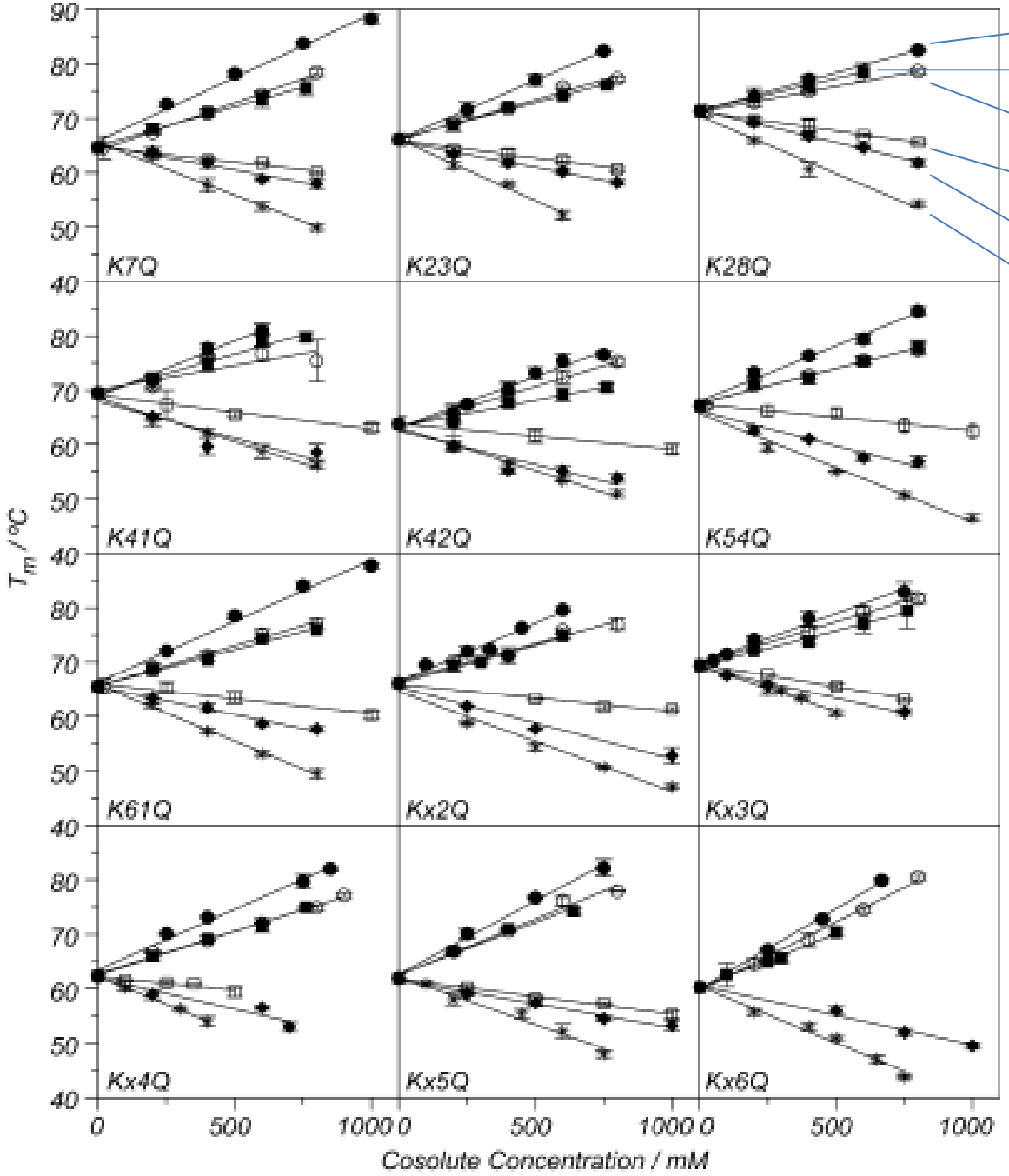
Decrease solubility of nonpolar molecules (salting out)
Strengthen hydrophobic interactions

Stabilise protein

Increase solubility of nonpolar molecules (salting in)
Weaken hydrophobic interactions

Interact with hydrophilic parts (eg peptide backbone)

Destabilise protein

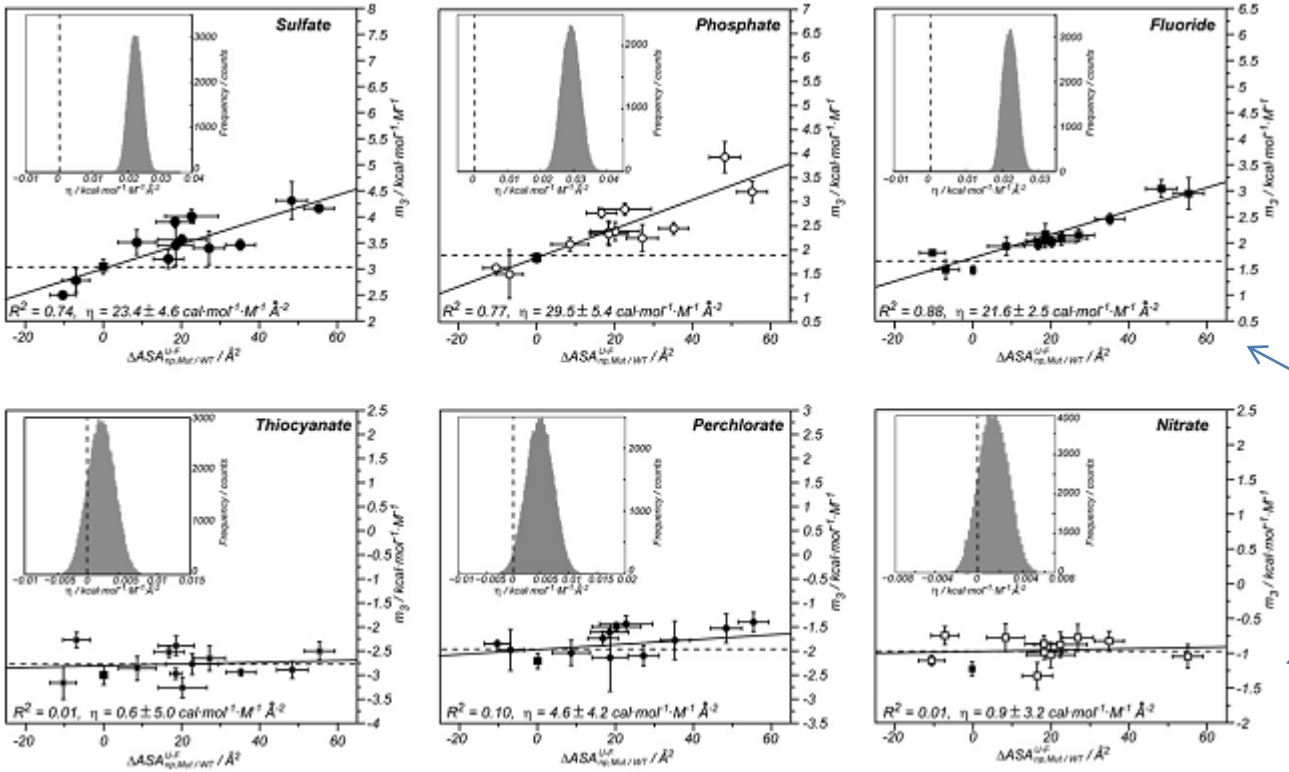



ie, fairly nonspecific effects

Mutants of protein L
Tadeo, Milet *et al* (2009) Biophys J **97,** 2595

All these ions have a **lower** concentration at air/water interface than bulk water
- presumably also true for protein/water interface, ie these ions do not interact much with folded protein

Consequence is that they increase the surface tension of water: this stabilises the folded conformation, because the denatured state has a larger surface area exposed to solvent




Dependence of stability on counterion concentration (slope of graphs on previous slide)

Stabilising effect of ions correlates with surface hydrophobic area

Destabilising ions have no correlation

Change in nonpolar surface area

Implies two different effects:

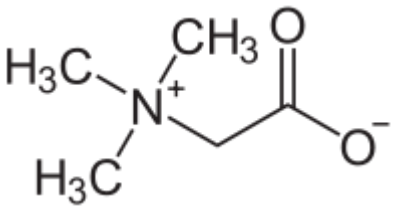
(a) Destabilising ions act the same way as denaturants: interact preferentially with peptide backbone of unfolded protein
(b) Stabilising ions work mainly by increasing the surface tension of the water and therefore stabilise the folded form (least surface area)

This is what we are looking for!

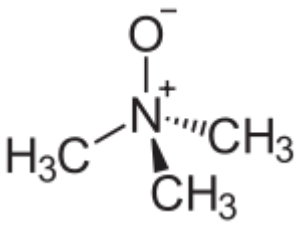
Osmolytes ('compatible solutes') have same effect
Stabilise proteins against denaturation due to unfavourable solution conditions, eg

- High salt (halophiles)
- High temperature (thermophiles)
- Low temperature (psychrophiles)
- High pressure (barophiles)

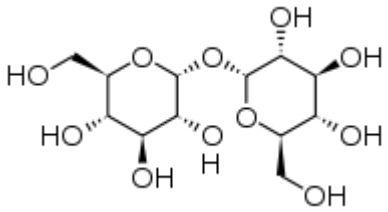
Wide range of chemical structures, eg glycine betaine, various non-reducing glucose derivatives such as trehalose, proline, glutamate



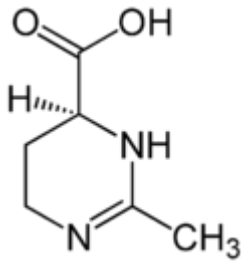

Glycine betaine




TMAO




trehalose




ectoine

In general, they do not interact with the protein directly
Organism produces enough that they counteract the destabilising effect but without overcompensating.

# Conclusions

Implication is that stabilisation against unwanted aggregation does not require keeping proteins apart, but does require keeping proteins tucked in, ie minimising local unfolding

Thus we should be looking at the ions towards the left of the Hofmeister series, which work by raising surface tension
- but not necessarily the most extreme

Eg sulphate, phosphate, acetate

(trifluoroacetate sometimes acts as a magic cosolute)

Thanks to:

Dr Jim Gilmour (Sheffield)

BBSRC