Using high-throughput DLS to detect and quantify the effect of osmolytes on protein associations

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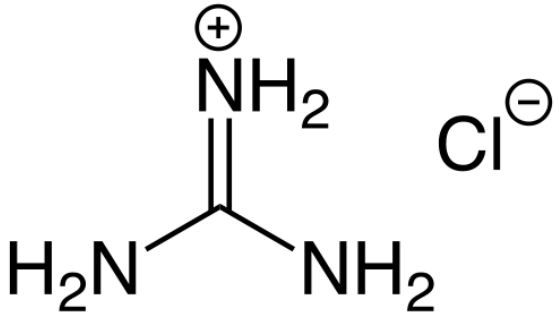
Osmolytes are small organic molecules synthesized in osmotically stressed organisms and tissues to maintain cell volume (P.H. Yancey)

More generally: small organic molecules affecting protein stability

Examples:

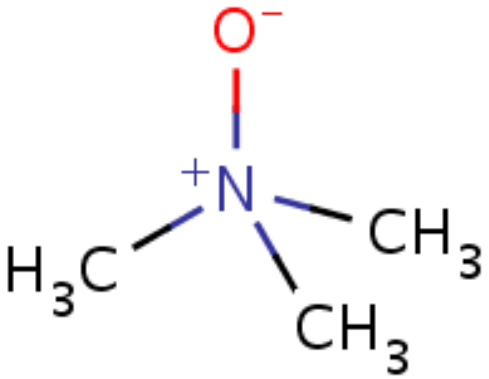
- small polyols (e.g. glycerol, sorbitol)
- amino acids (e.g. β-alanine, glycine betaine, taurine)
- Trimethylamine-N-oxide (TMAO)
- Urea
- Guanidine hydrochloride (GuHCl)

Guanidine Hydrochloride



MW 95.5
Neutral at pH 6-8
Very strong protein denaturant

TMAO

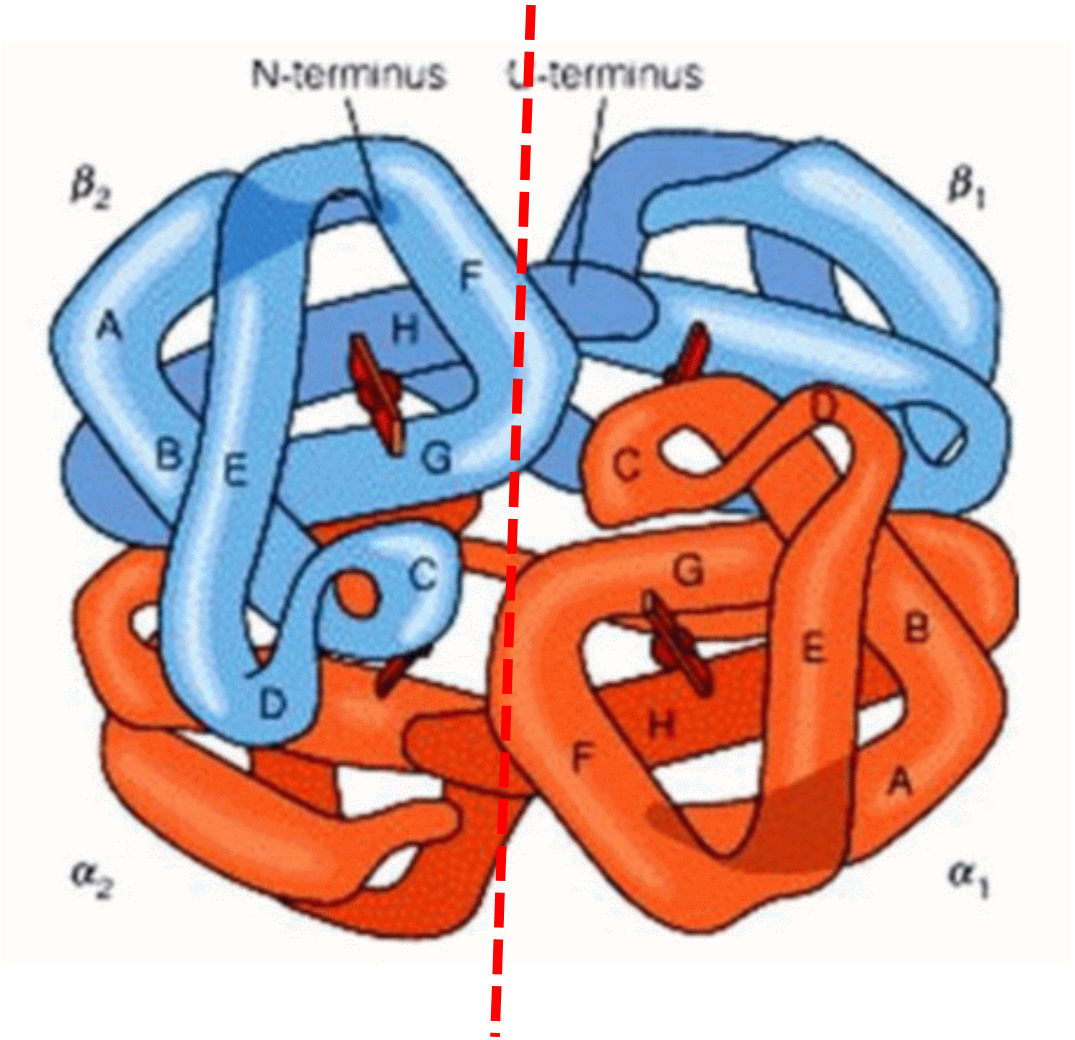


MW 75
Neutral at pH 6-8
Strong stabilizer of proteins against denaturation

High concentrations of TMAO can counteract the denaturing effect of GuHCl on proteins.

What effects do these two osmolytes have on noncovalent protein associations?

Hemoglobin: a hetero-tetramer
$\alpha_2\beta_2$ can reversibly dissociate into 2 $\alpha\beta$ dimers



Biochemistry 4: 1203-1213 (1965)

Dissociation of Human CO-Hemoglobin by Urea, Guanidine Hydrochloride, and Other Reagents*

Kazuo Kawahara, Annette G. Kirshner, and Charles Tanford

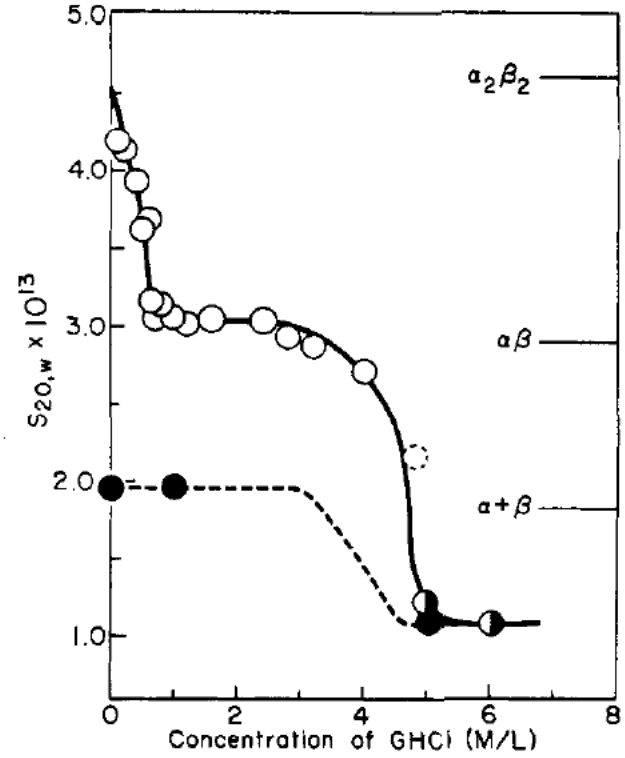
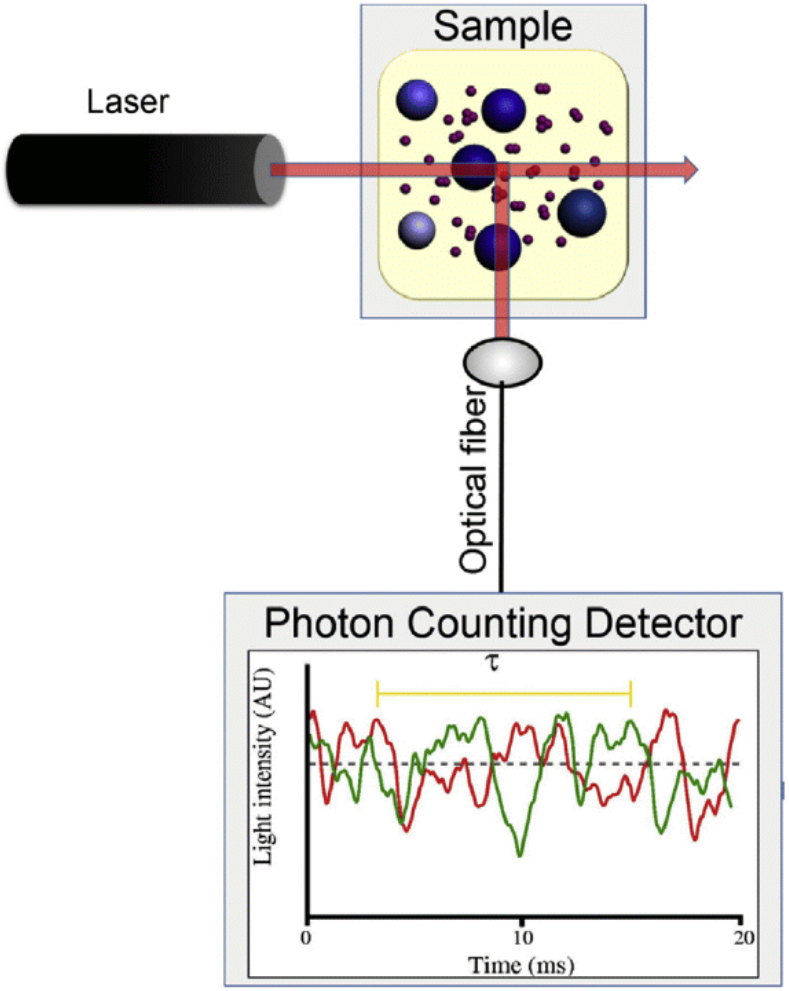
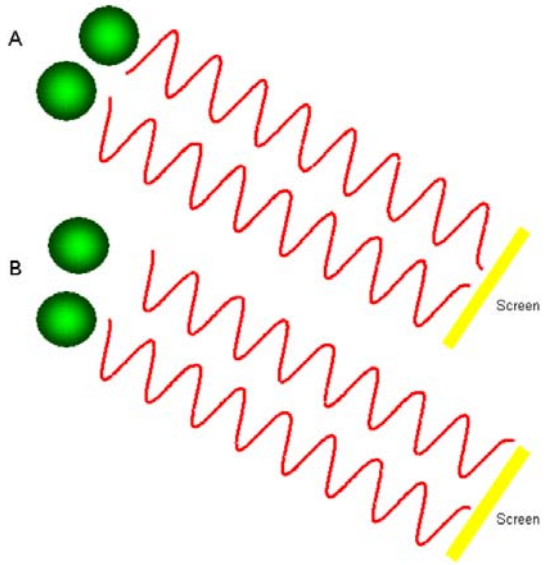
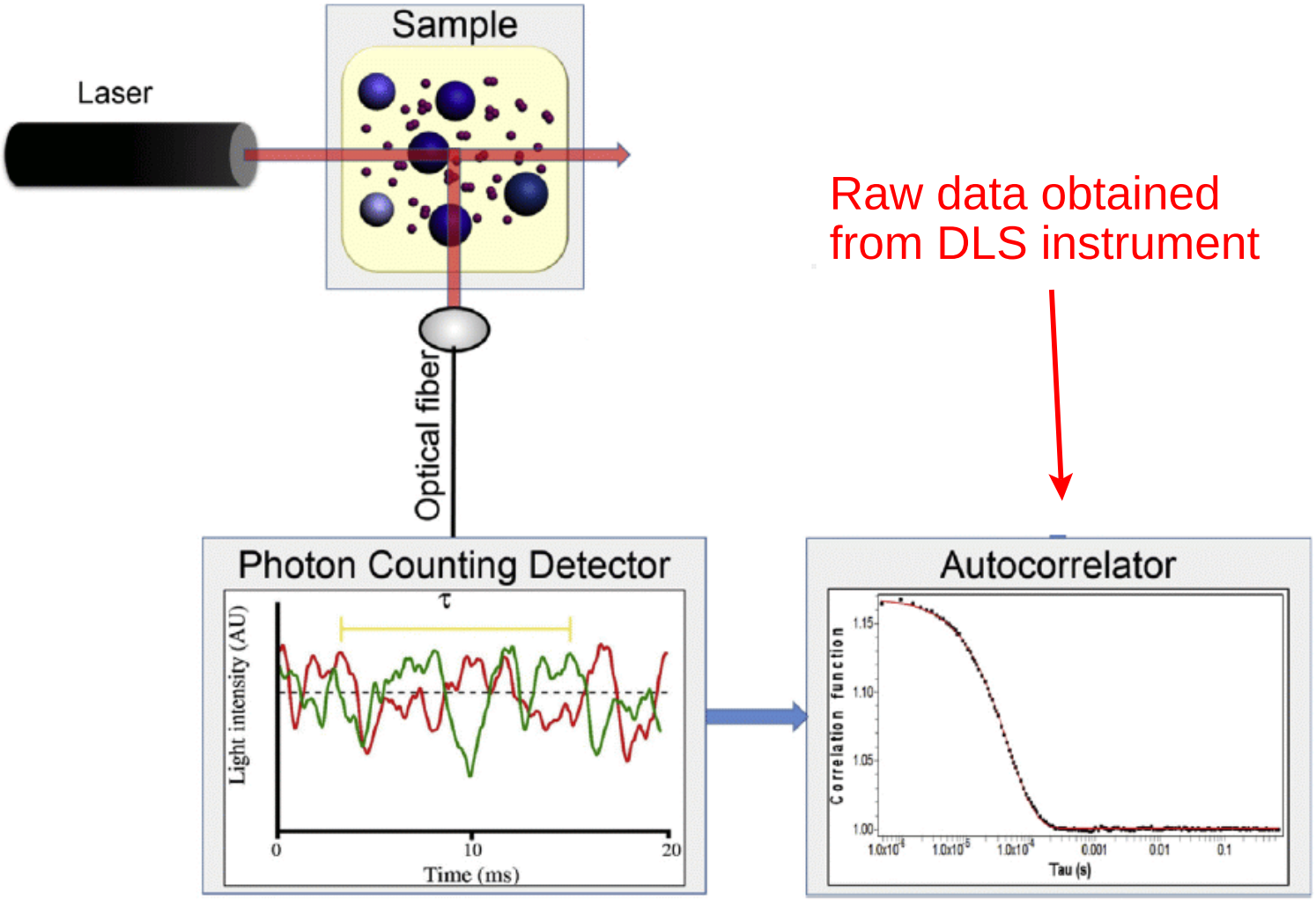


FIGURE 3: Effect of guanidine hydrochloride on the sedimentation coefficients of hemoglobin and myoglobin.

Dynamic Light Scattering

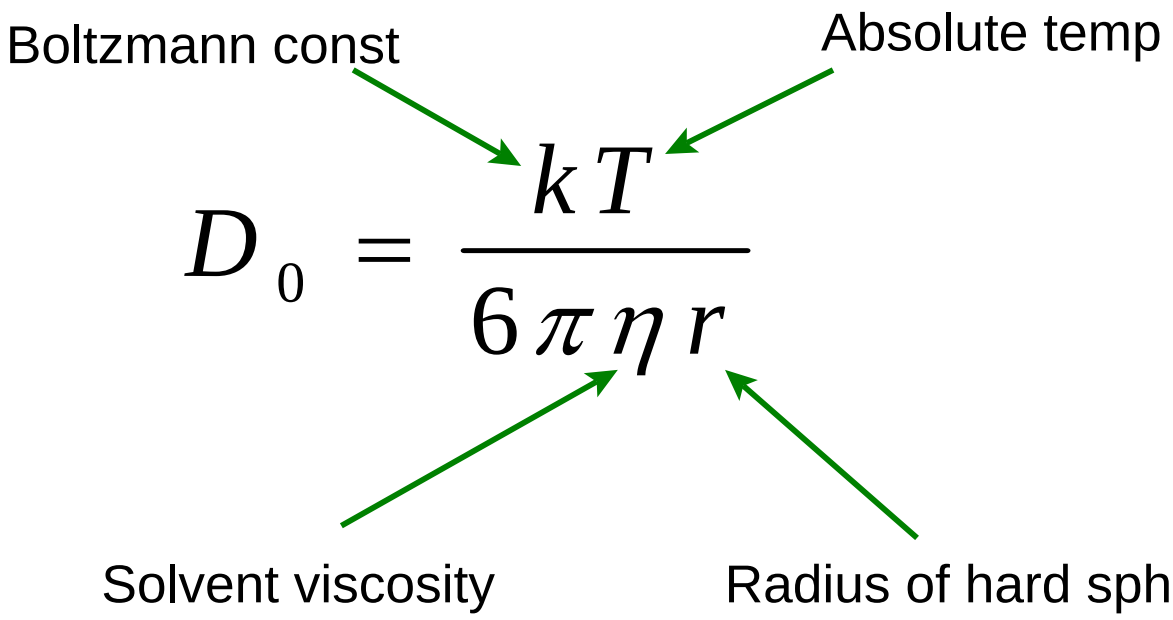






Raw data obtained
from DLS instrument

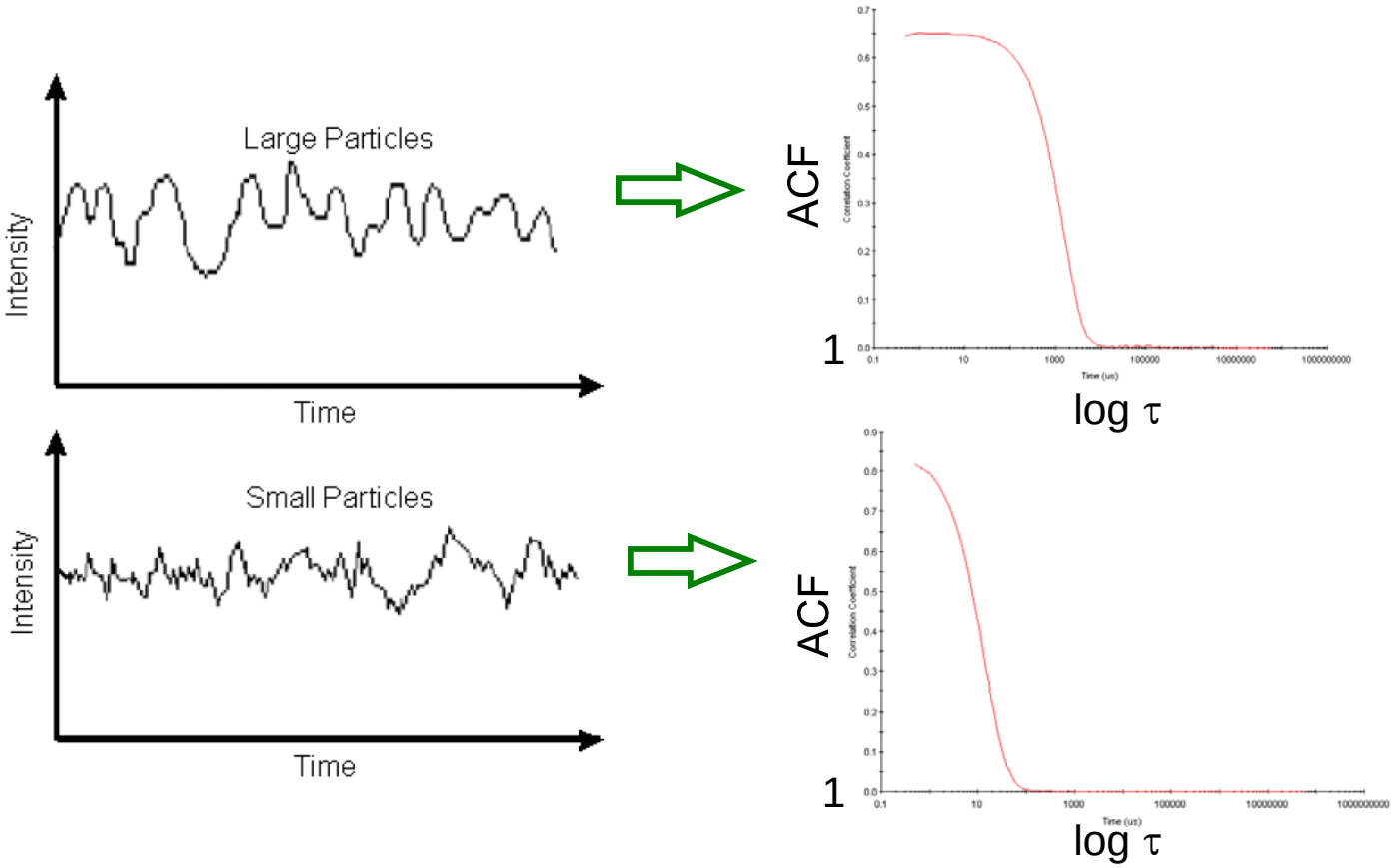
Stokes-Einstein relation for the diffusion coefficient of an isolated hard sphere in a continuum fluid



The larger the hard sphere, the smaller the diffusion coefficient

$$ACF(\tau) = 1 + \beta \exp\left(-2Dq^2\tau\right)$$

Large mass → small D → slower fluctuations
Small mass → large D → more rapid fluctuations



Concentration-dependent ACF of Zn-insulin
(Attri et al, 2010)

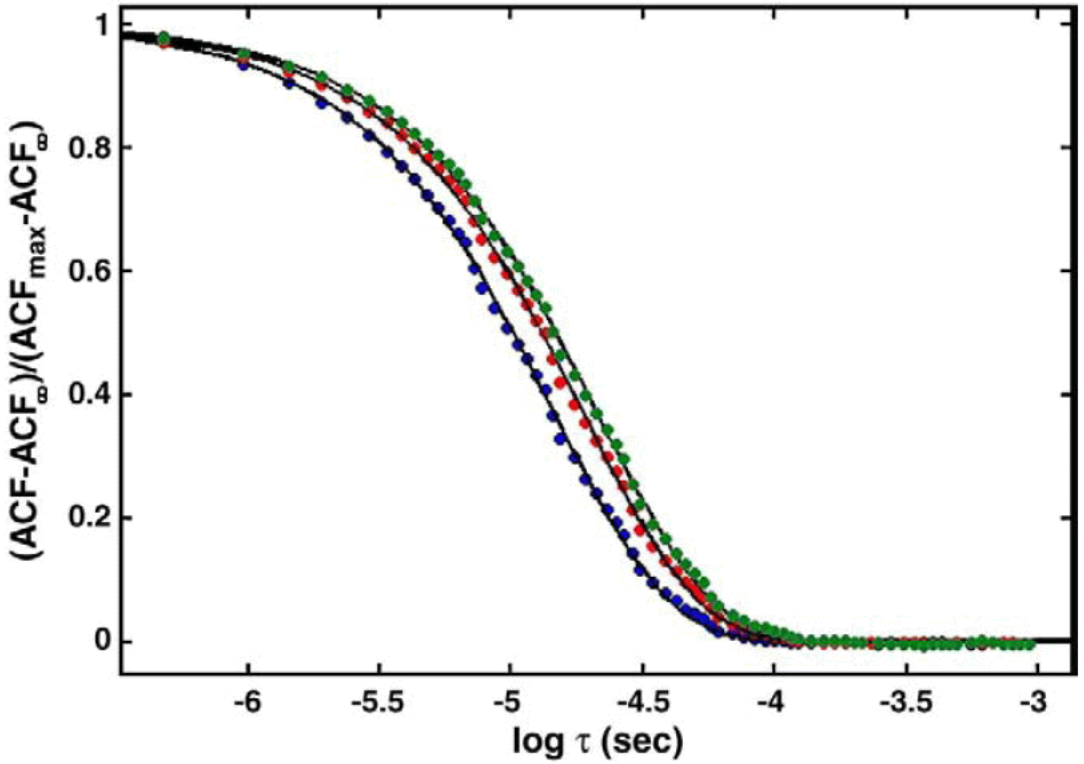
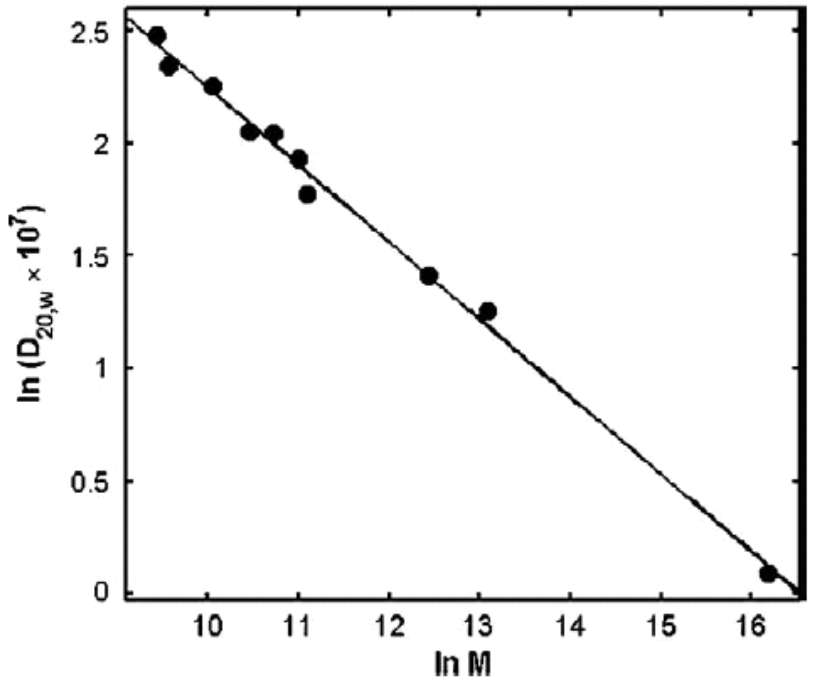


Fig. 2. Normalized autocorrelation functions measured for the following insulin concentrations: 4.99 g/l (green), 2.52 g/l (red), and 0.48 g/l (blue), plotted together with curves calculated according to the best fit of Eq. (1) to the respective data set.

For globular (quasi-spherical) proteins

Attri et al (2010)



$$D \propto 1/M^{1/3} \propto 1/r_{eff}$$

agrees well with predictions of Stokes-Einstein relation

Diffusion coefficient of *i*-mer

Assuming validity of Stokes-Einstein relationship between D and M

$$\frac{D_i}{D_1} = \left(\frac{M_1}{M_i} \right)^{1/3} = i^{-1/3}$$

Diffusion coefficient of dimer

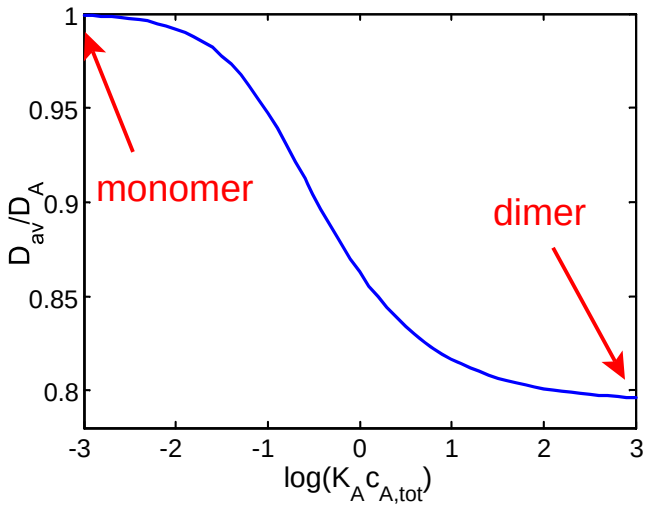
$$D_2 = D_1 \times 2^{-1/3} = 0.78 D_1$$

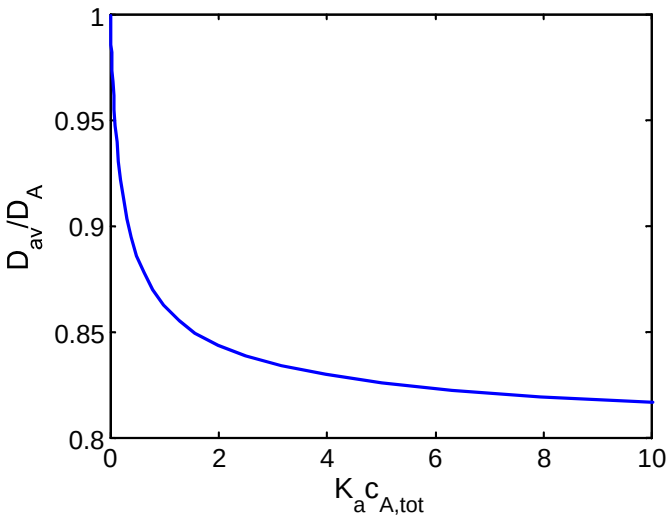
The “average” diffusion coefficient measured in a mixture of diffusing solutes is intensity weighted

$$D_{exp} = D_z = \frac{\sum_i I_i D_i}{\sum_i I_i} = \frac{\sum_i c_i M_i^2 D_i}{\sum_i c_i M_i^2}$$

For reversible monomer-dimer self-association

$$D_{A_2} \cong .79 D_A$$





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Self-association of Zn-insulin at neutral pH: Investigation by concentration gradient-static and dynamic light scattering

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ABSTRACT

Equilibrium self-association of Zn-insulin at pH 7.0 was characterized over the range 0.3–5 mg/mL by simultaneous measurement of static and dynamic light scattering. Analysis of static light scattering yielded a concentration-dependent weight-average molecular weight, and analysis of dynamic light scattering yielded a concentration-dependent intensity-average diffusion coefficient. The concentration dependence of both quantities may be accounted for to within experimental precision by a simple model, according to which the basic structural unit of Zn-insulin at concentrations exceeding 0.3 mg/mL is a hexamer H. With increasing total protein concentration, hexameric protomers may self-associate in accordance with an isodesmic scheme in which a protomer may add to any prexisting oligomer H_n to form H_{n+1} with an invariant stepwise equilibrium association constant.

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Free-Solution, Label-Free Protein-Protein Interactions Characterized by Dynamic Light Scattering

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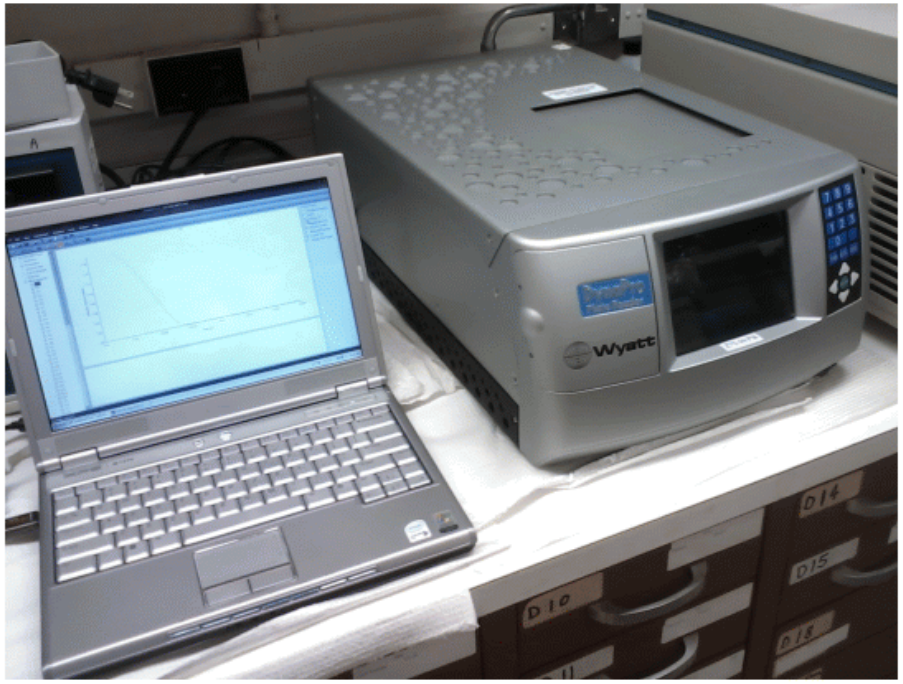
ABSTRACT We report a free-solution, label-free method for quantitative characterization of macromolecular interactions using dynamic light scattering, a temperature controlled plate reader, and a multiwell concentration gradient. This nondestructive technique enabled determination of stoichiometry of binding, equilibrium dissociation constant, and thermodynamic parameters, as well as the impact of temperature, buffer salinity, and a small-molecule inhibitor. The low volume capability of dynamic light scattering reduced the required sample to 426 pmol/experiment, with detection limits for 150-kDa proteins anticipated to be in the low femtomole range.

High throughput CG-DLS

Hamilton NIMBUS microplate pipetting robot

Prepares up to 96 samples of varying composition in individual microplate wells under computer control.

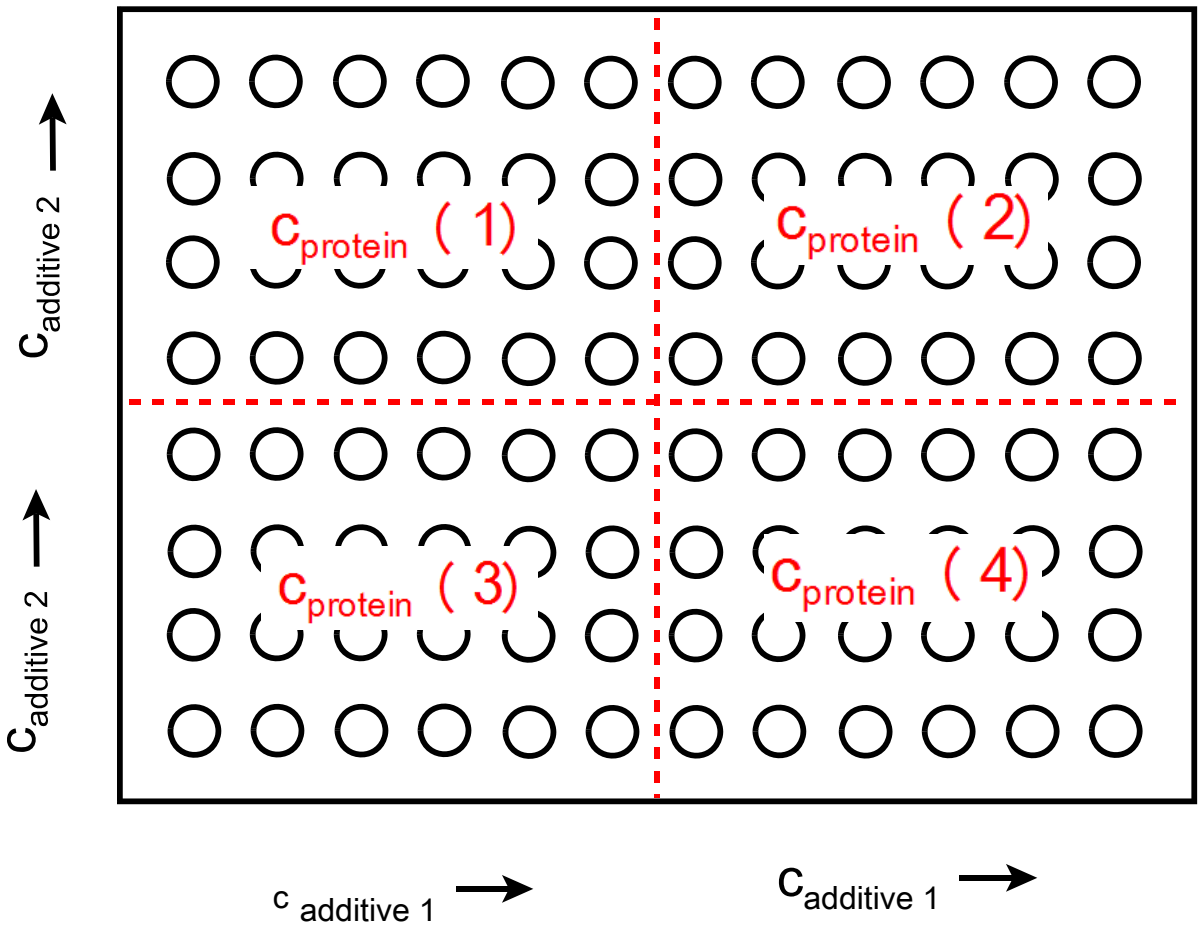


Wyatt Dynapro DLS microplate reader

Measures the scattering autocorrelation function in each of up to 96 microplate wells

Example: measuring protein self-association as a function of the concentrations of protein, additive 1 and additive 2

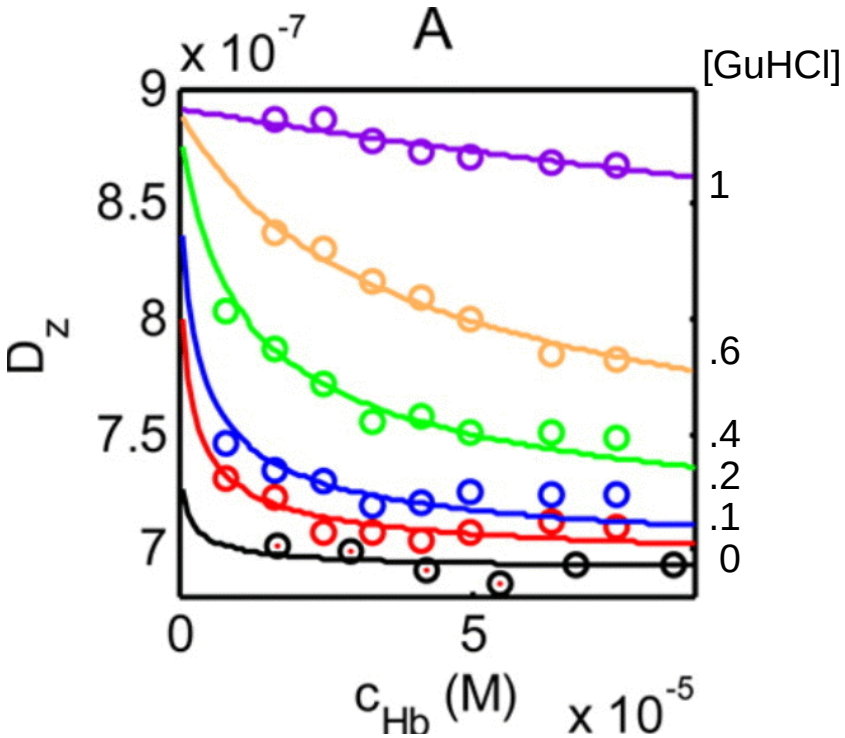
Design of a composition gradient on a 96 well microplate

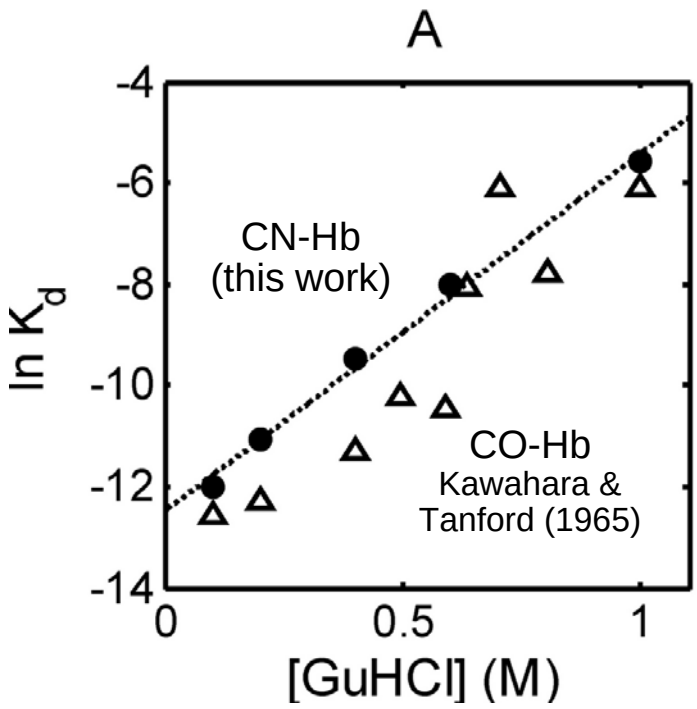


CN-Hb tetramer -dimer dissociation

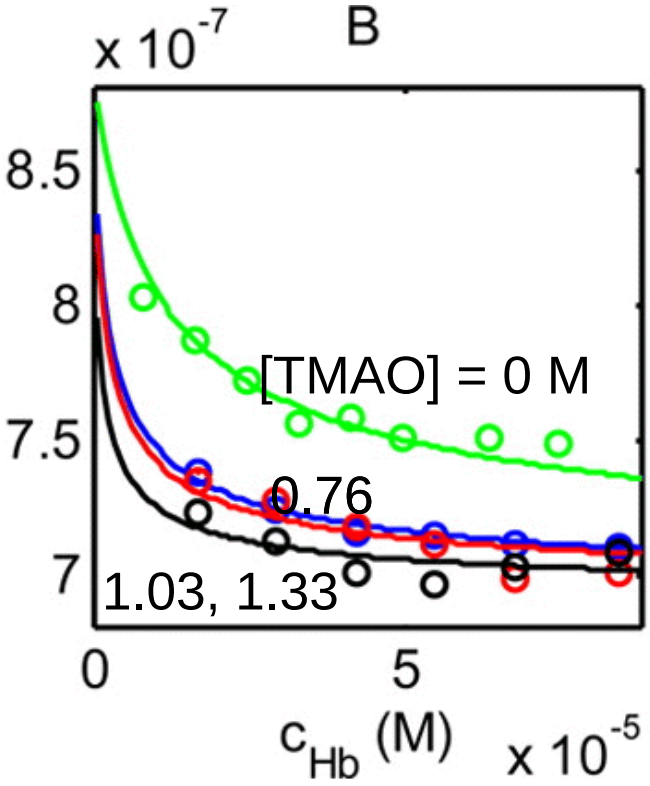
Wu and Minton (2013)

$$\alpha_2\beta_2 \underset{}{\overset{K_d}{\rightleftharpoons}} 2\alpha\beta \qquad \left(K_d = 1/K_a\right)$$

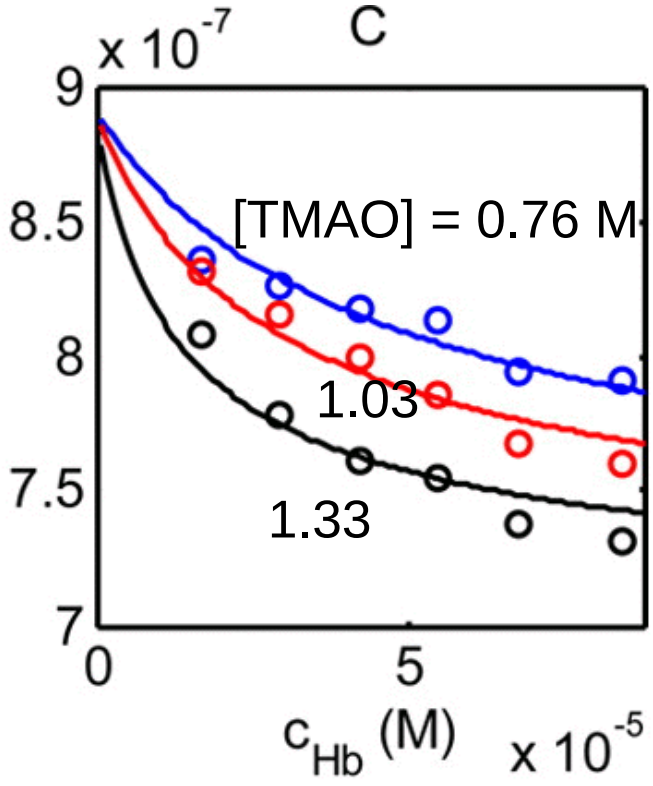




[GuHCL] = 0.4 M



[GuHCL] = 0.8 M



Model: GuHCl and TMAO act independently on the free energy of CNHb dissociation

$$RT \ln K_d = -\Delta G_d = -\left(\Delta G_d^0 + \Delta g_G \left[GuHCl\right] + \Delta g_T [TMAO]\right)$$

$$K_d = c_{dim}^2 \big/ c_{tet}$$

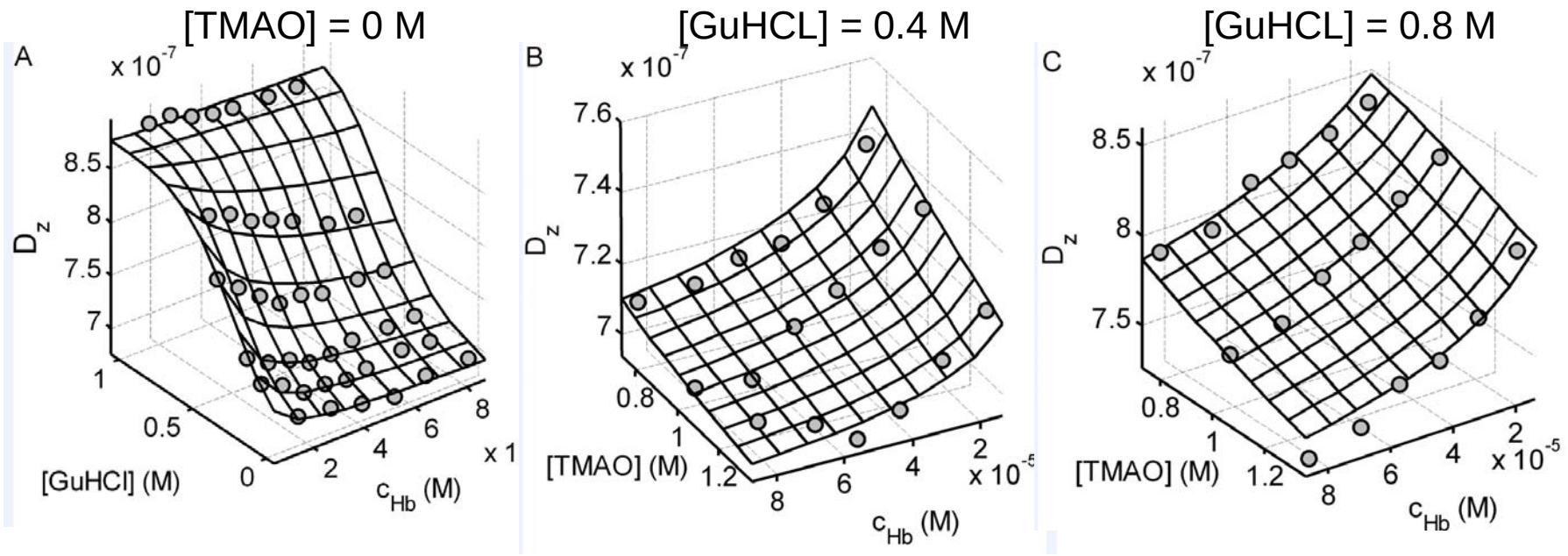
$$c_{Hb,tot} = 0.5 c_{dim} + c_{tet}$$

$$D_z = \frac{c_{dim} D_{dim} + 4 c_{tet} D_{tet}}{c_{dim} + 4 c_{tet}}$$

Given values of ΔG_d^o, Δg_G, Δg_T, D_{dim}, and D_{tet},
one can calculate D_z as a function of $c_{Hb,tot}$, [GuHCl] and [TMAO].

Model: GuHCl and TMAO act independently on the free energy of CNHb dissociation

$$RT \ln K_d = -\Delta G_d = -\left(\Delta G_d^0 + \Delta g_G \left[GuHCl\right] + \Delta g_T \left[TMAO\right]\right)$$



Global fit of model to all data with bf parameters:

D_{tet} = 6.85 Fick, D_{dim} = 8.77 Fick, ΔG_d^o = 7.2 kcal/mol,
Δg_G = -4.5 kcal/mol-M, Δg_T = 1.1 kcal/mol-M

Conclusions

1. Free energy of HbCN dissociation to half-molecules decreases linearly with increasing [GuHCl].

2. Free energy of dissociation increases linearly with increasing [TMAO].

3. Effects of GuHCl and TMAO are additive: modes of interaction are independent.

4. Experimental finding that $D_{tet} = 0.78\ D_{dim}$ is in excellent agreement with prediction of Stokes-Einstein.

Conclusions

5. The combination of Hamilton NIMBUS pipetting robot and Wyatt Dynapro DLS plate reader provides an excellent high-throughput and high-resolution method for screening the effect of small cosolutes upon protein stability, specific protein associations, and aggregation.

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