Mapping the Crystallization Slot for Membrane Proteins

Patrick J. Loll, W. William Wilson, and Charles Henry

Second Virial Coefficient ($B_{22}$)

Series expansion of thermodynamic activity

$$\Pi = RTc \left( \frac{1}{MW} + B_{22}c + \cdots \right)$$

$B_{22} \neq 0$ for non-ideal solution

$B_{22}$ and solubility are highly correlated

Negative $B_{22}$
Solubility decreases

Positive $B_{22}$
Solubility increases
These are all soluble proteins.

Is $B_{22}$ predictive for membrane proteins as well?
Yes.

**Hypothesis:**
Cloud point is *surrogate marker* for crystallization slot

\[ B_{22} \times 10^4 \text{(mol mL}^{-1}\text{g}^{-2}) \]

PEG concentration (% w/v)

-10
-8
-6
-4
-2
0

cloud growth zone

crystallization slot

OmpF Porin
Problems with using light scattering to measure $B_{22}$

- Technical problems
  - Fiddly (dust)
  - Slow, requires lots of material
- Fundamental problems

Multiple species confound analysis

Self-Interaction Chromatography

- Form of affinity chromatography
  - Initially demonstrated by Przybycien
  - Developed in modern form by Lenhoff’s group
- Advantages over light scattering
  - Widely used instrumentation (LC)
  - Signal not so dependent on buffer composition
    - Compatible with polymers and micelles
  - Easily automated
- Practice
  - Immobilize protein of interest on stationary phase
  - Inject protein of interest in mobile phase
  - Monitor position of elution peak
Attractive self-interactions $\Rightarrow$ longer retention times

Obtaining virial coefficients from SIC

$$B_{22} = B_{HS} + \frac{k}{\rho_s \phi}$$

Tessier PM, Lenhoff AM, Sandler S I Biophys J 2002, 82, 1620-1631.
Obtaining virial coefficients from SIC

\[ B_{22} = B_{HS} + \frac{k}{\rho_s \phi} \]

- Hard sphere component
- \( k = \frac{V_r - V_o}{V_o} \) (retention factor)
- immobilization density
- phase ratio (surface area/V_o)

Tessier PM, Lenhoff AM, Sandler S I Biophys J 2002, 82, 1620-1631.

Yes, this works...

Lysozyme, pH 4.2
Yes, this works… …and we can do it on a small scale.

Model Membrane Proteins

- Proteorhodopsin (pR)
  - Isolated from ocean bacterioplankton
  - Bacteriorhodopsin homolog (α-helical)
  - Can be produced in large quantities

- OmpX
  - *E. coli* outer membrane protein
  - 8-stranded β barrel
Can We Immobilize Membrane Proteins?

Exposed to green light (520 nm)  After exposure to blue light (472 nm)

*Proteorhodopsin will photobleach at pH 12, but not 7*

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*Examples of “routine” $B_{22}$ measurements: Dependence of $B_{22}$ on pH*

*Wild Type*  *Mutant*

flow rate: 0.1 mL/min, injection volume 1 µL, wavelength 520nm.
Effect of Salt Concentration

Instrumental conditions: mobile phase pH 9, 20mM MES, flow rate 0.1 mL/min, injection volume 1 µL, wavelength 520nm.

Mixed Surfactant Studies

SIC conditions: Mobile phase: 20mM MES, 50mM NaCl, pH 7, flow rate: 20 µL/min, injection volume 0.5 µL, wavelength 280nm.
Can we do this for other membrane proteins?

![Graph showing OmpX](image)

Buffer: 0.5% C8E4, 20 mM TRIS-HCl, 20 mM NaCl, pH 8.5, PEG (MW 4,000)

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Glycerol as a small amphiphile

![Graph showing glycerol and C8E4](image)

SiC conditions: Mobile phase: 0.1 M sodium acetate, 0.2M CaCl₂, pH 4.5; flow rate: 25 µL/min, injection volume 0.5 µL
Summary

• SIC is a useful tool to measure $B_{22}$
  – Rapid
  – Uses small amounts of protein
• SIC can measure interactions in complicated solutions
• Works with membrane proteins

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