Biophysical characterization of SMALPs and nanodiscs

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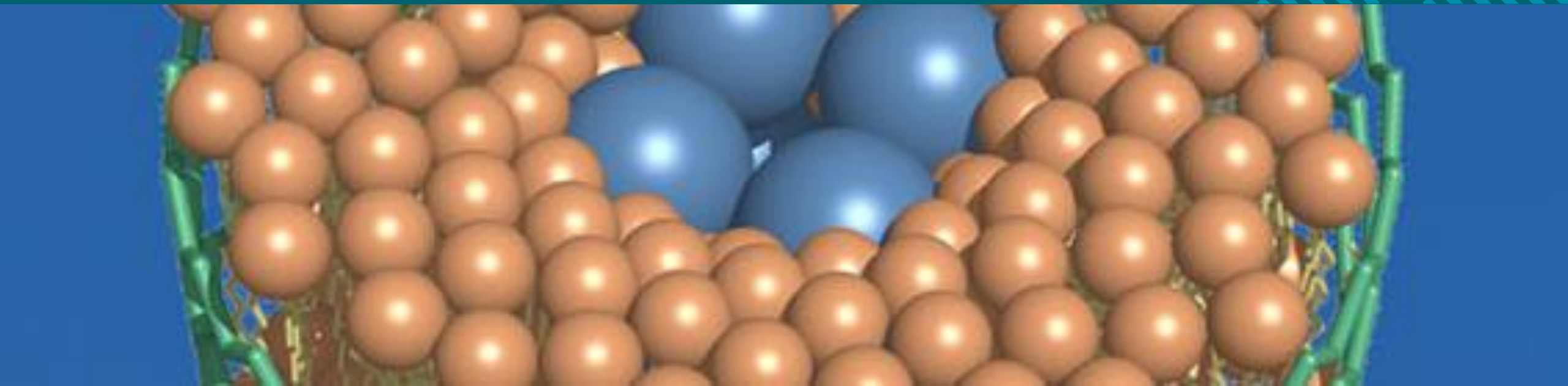


Overview

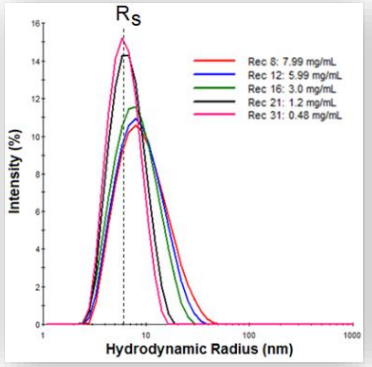
- Light scattering
- SEC-MALS
- DSC
- ITC

Dynamic light scattering (DLS)

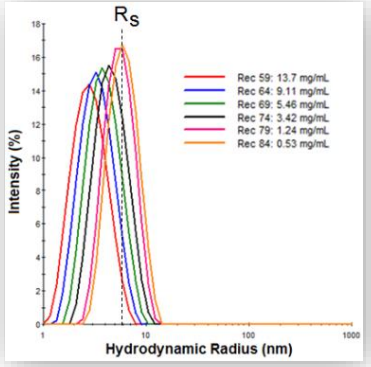
- Size, Charge, and Interaction Parameters
- Proteins, peptides, biopolymers, and nanoparticles
- Screen for aggregation propensity
- Zeta potential
- Rapid aggregate assessment

- Screen For Colloidal Stability
- Multivariate data sets for stability and aggregation metrics from ultra-low volume/concentration assays

Reversible Self-Association



Electrostatic Repulsion



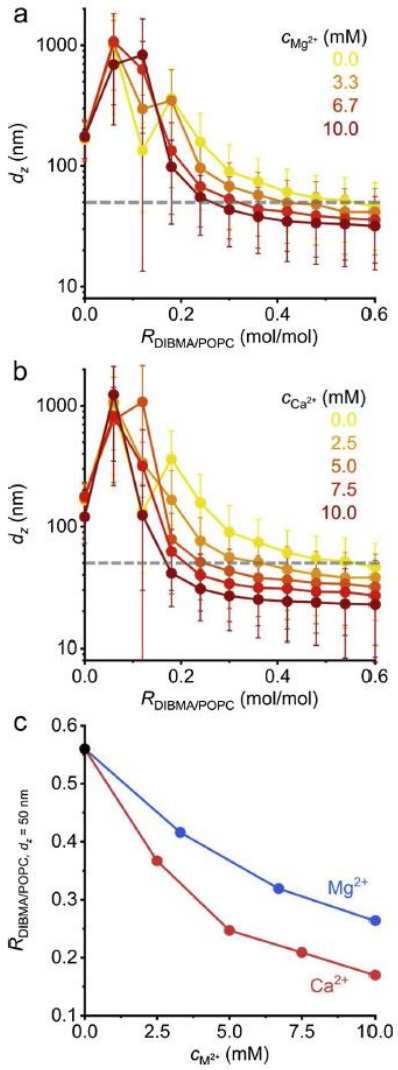
Stability Profile

IgG Stability Profiles For Buffer 1 And Buffer 2

Property	Buffer 1	Buffer 2
k_D (ml/g)	-5.2	31.9
B_{22} (x 10^5 ml mole/g^2)	-1.5	127.5
Z_{Eff}	0.7	4.3
T_m (C)	56	> 56
T_{Agg} (C)	66	> 66
R_S (nm)	5.8	5.7
SubQ Limit (mg/ml)	137	148

Particle size distribution as function of DIMBA/POPC, in divalent cations

Measured by Zetasizer Nano DLS



- Solubilization efficiency of POPC LUVs by DIBMA at 25 °C and increasing cM2+ as monitored by DLS.
- (a,b) z-Average particle diameters, dz, of 5mM POPC as functions of DIBMA/POPC molar ratio, RDIBMA/POPC, in the presence of various concentrations of (a) Mg2+ or (b) Ca2+.
- (c) DIBMA/POPC molar ratios at which particles reached
- dz = 50 nm as derived from data in panels a and b.
- Increasing Mg2+ or Ca2+ concentrations reduced amount of DIMBA required for complete solubilization
- With Mg2+ or Ca2+, for any measured DIMBA/POPC ratio, resulted in particles that were smaller than in the absence of cations

Danielczak et. al, Chem. Phys. Lipids, 221, 30-38 (2019)

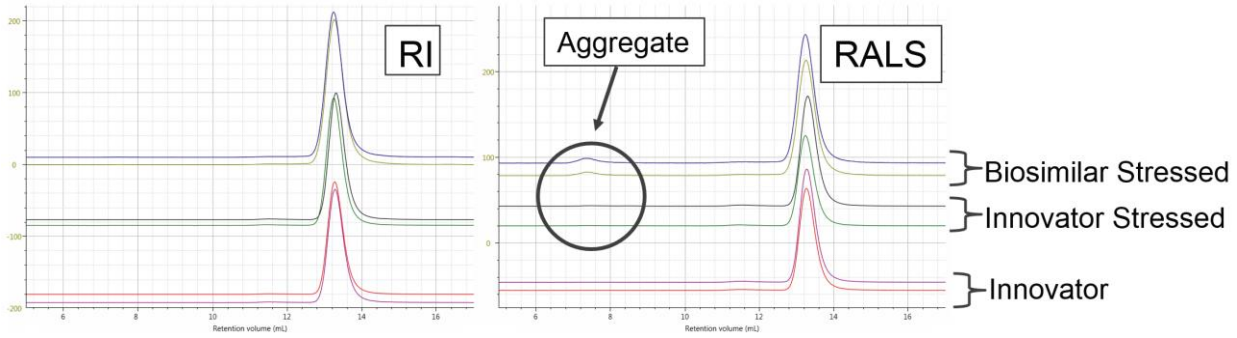
SEC-MALS

- Absolute MW, oligomeric distribution, %Purity, and size from a single injection
- Multi detection SEC includes UV, RI, MALS, and DLS, as well as DSV for intrinsic viscosity
- Increased light scattering sensitivity for detecting trace amounts of HMW species
- Define Oligomeric Baseline
- Measure %Purity & distribution
- Track aggregation & HMW species generation

Denosumab (Prolia® and Xgeva®): Innovator and Biosimilar

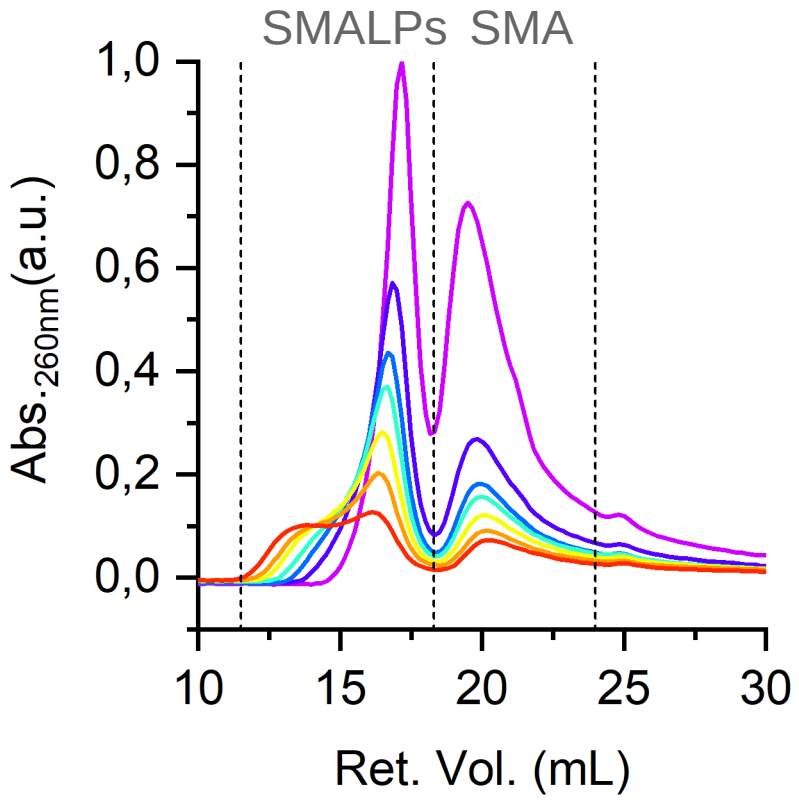
Stressed – Incubation at 30°C

Innovator	Biosimilar
99% monomer	97% monomer
1% dimer	1.5% dimer
	1.5% aggregates



SMA(2:1)-nanodiscs

OMNISEC



Lipid: 4 mM DMPC

Polymer: 0.8 - 4 mM SMA(2:1)

$R_{SMA(2:1)/DMPC}$ (mol/mol) = 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 1

T = 30°C

V_{inj} = 50 µL

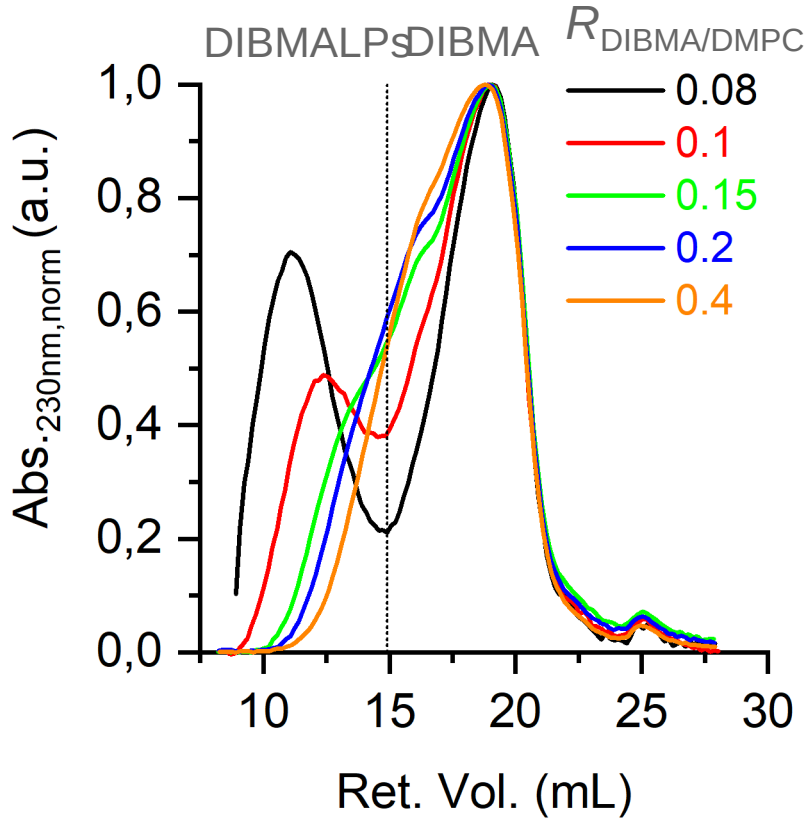
Buffer composition: 50 mM Tris, 200 mM NaCl, pH 7.4

Column: Superose 6 Increase 10/300 GL

Johannes Klingler & Anne Grethen

DIBMA-nanodiscs

OMNISEC



Lipid: 4 mM DMPC

Polymer: 0.3 – 1.6 mM DIBMA

$R_{DIBMA/DMPC}$ (mol/mol) = 0.075, 0.1, 0.15, 0.2, 0.4

T = 30°C

V_{inj} = 50 µL

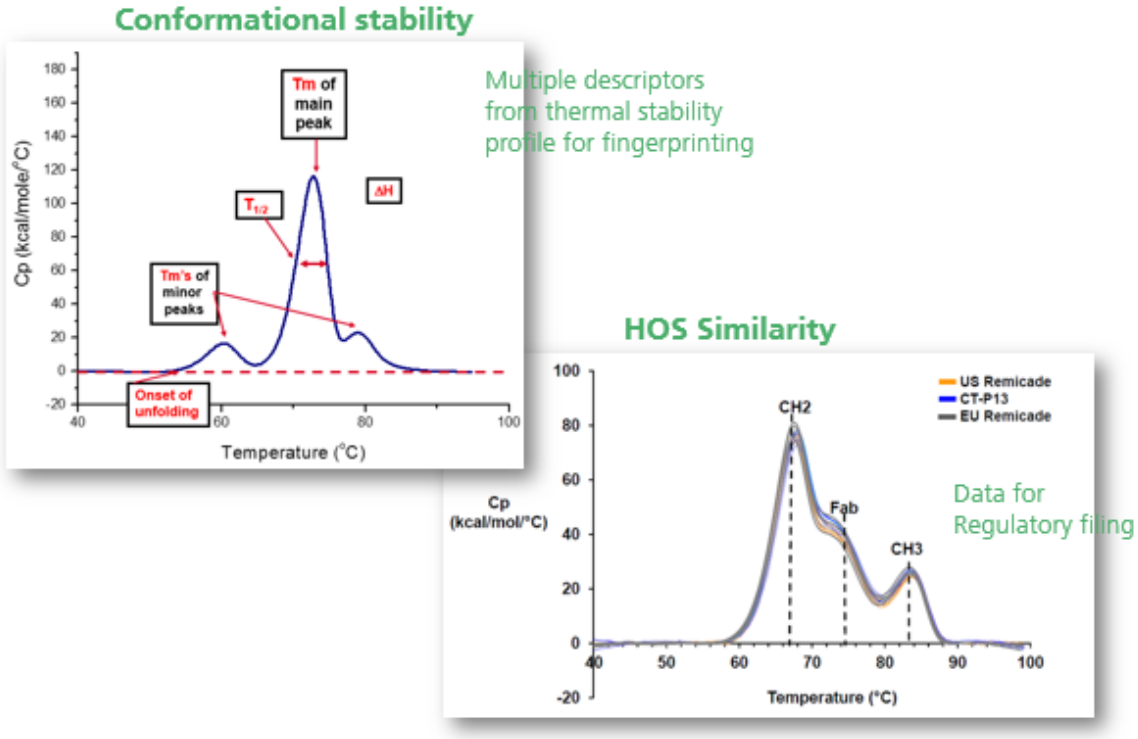
Buffer composition: 50 mM Tris, 200 mM NaCl, pH 7.4

Column: Superose 6 Increase 10/300 GL

Differential Scanning Calorimetry (DSC)

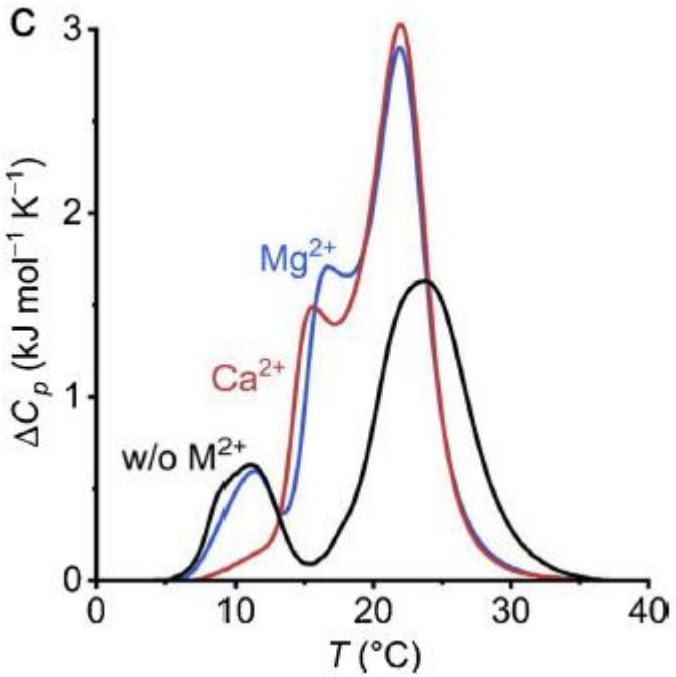
- **Conformational Stability**
- Sensitive to biopolymer domain transitions
- Phase transition reversibility
- T_m correlated with aggregation propensity, formulation stability, and shelf life

- **Screen For Structural Stability**
- High quality thermal phase transition data
- Gold standard, label-free, universal tool for studying thermal stability



Thermal stability by DSC

Gel-to-fluid transition

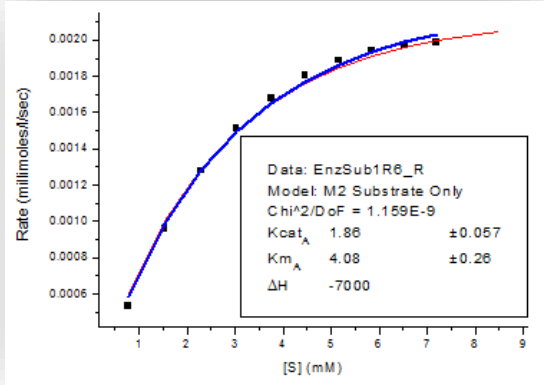


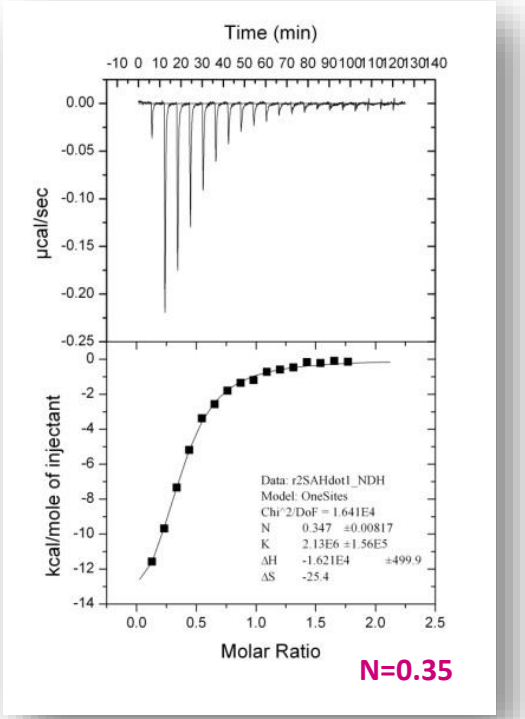
- DSC thermograms showing excess ΔCp, as functions of temperature, T, for 5 mM DMPC and 0.5mM DIBMA without divalent cations or with either 10mM Mg2+ or 7.5mM Ca2+.
- All DIBMALP samples gave rise to relatively broad transitions, which are typical and indicative of nanosized lipid-bilayer patches
- The presence of 10mM Mg2+ or 7.5mM Ca2+ reduced the main transition temperature, Tm, from ~24 °C to ~22 °C.
- Since an increase in the DIBMA/DMPC ratio similarly results in a decrease in Tm, the present observations are in accordance with the above conclusion that divalent cations render DIBMA more efficient in associating with and solubilizing phospholipids.

Danielczak et. al, Chem. Phys. Lipids, 221, 30-38 (2019)

Isothermal Titration Calorimetry (ITC)

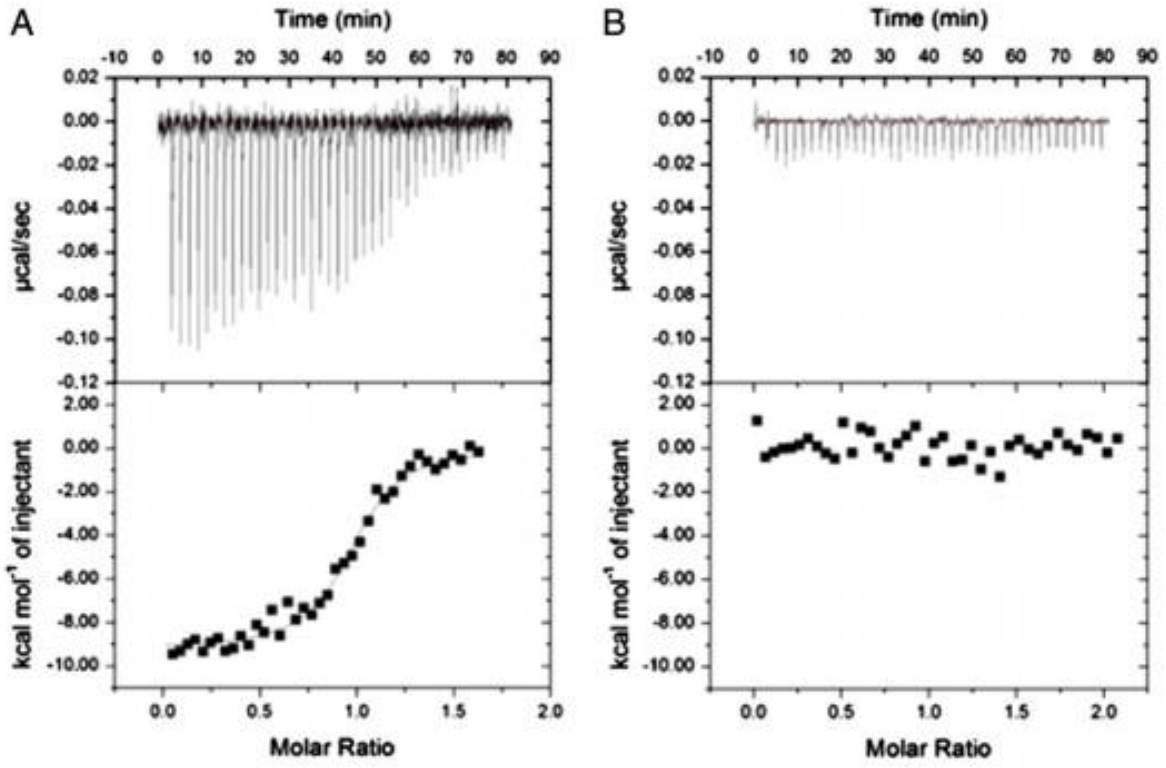
- **Affinity and Function**
- Measures any interactions between two biomolecules in solution or suspension, including nanoparticles and nanodiscs
- Micellization/demicellization
- **Confirm Function**
- High quality affinity data and stoichiometry
- Gold standard, label-free, universal tool for studying biomolecular interactions





FhuA interactions in a detergent-free nanodisc environment

Binding affinity, stoichiometry and thermodynamics using MicroCal VP-ITC



- Binding affinity between Nd-FhuA and TonB32–239. The ITC thermograms show the interaction of Nd-FhuA with TonB in the presence (A) or absence of ferricrocin (B).

Binding affinities and thermodynamics between Nd-FhuA, TonB and ColM.

Titrant	Cell	N	K_D (nM)	ΔH (cal/mol)	ΔS (cal/mol/deg)
TonB	Nd-FhuA apo	–	–	–	–
TonB	Nd-FhuA-Fc	0.977 ± 0.0100	200.4 ± 29.3	−9186 ± 133.4	−0.164
Colicin M	Nd-FhuA apo	0.906 ± 0.0016	3.48 ± 1.09	7969 ± 33.3	65.4
Colicin M	Nd-FhuA-Fc	–	–	–	–
TonB	Nd-FhuA-ColM	–	–	–	–

The estimated errors are based on a $\chi 2$ minimized fit of the experimental data to a single-site binding model using Origin 7.0 software (OriginLab).

Mills et.al, Biochim Biophys Acta, 1838, 364-371(2014)

Summary

- ITC, DSC, DLS, and Multi-detection SEC contribute important information about SMALPs and nanodisc structure, mechanism, thermodynamics, and interactions
- Use in conjunction with other techniques for complete biophysical characterization and development of new methods and products
  - Cyro-EM and other Microscopy
  - SPR
  - FTIR
  - NMR
  - Mass spec
  - CD
  - Analytical ultracentrifugation

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MicroCal PEAQ ITC ★

- Isothermal Calorimetry
- Activity Screening
- K_D, ΔH, ΔS, & n

MicroCal PEAQ DSC ★

- Differential Scanning Calorimetry
- Stability Screening
- T_M & ΔH

Zetasizer (0.6 nm – 10 μm) ★

- Light Scattering
- Stability Screening & Aggregate Detection
- PSD, R_H, T_{Agg}, k_D, B_{22}, & Z_{Eff}

OMNISEC/MALS ★

- Advanced Detection SEC
- Stability Screening
- M_W, R_H, %Purity, & HMW Detection

Mastersizer 3000 (0.01 – 3500 μm)

- Laser Diffraction
- Quality Control
- Particle Size Distribution

Morphology 4 and 4-ID (1 – 1000 μm)

- Digital Imaging & Raman Spectroscopy
- Aggregate Quantification & ID
- Size, Morphology, & Chemical ID

Empyrean

- X-Ray Diffraction
- Polymorph Assessment & Screening
- Excipient & API Form

NanoSight (40 nm – 1 μm)

- Nanoparticle Tracking Analysis
- Aggregate Quantification
- PSD, R_H & SμP Concentration

Zetium

- X-Ray Fluorescence
- Quality Control
- Residual Catalyst & Metal Detection

★ SMALP and nanodisc characterization