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Biophysical characterization of SMALPs and nanodiscs

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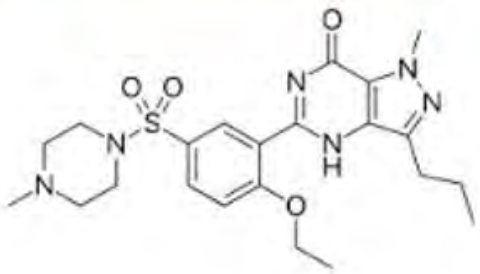
Provider of industry leading analytical instrumentation for the characterization of

Molecules



Particles

Materials

Along with the expertise and understanding of how

Molecular Properties → **Control** → Particle Size, Shape, & Interactions → **Drives** → Product Performance

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Malvern Panalytical Biosciences Group Solutions and Instrumentation for SMALP and nanodisc characterization

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

MicroCal PEAQ DSC ★

- Differential Scanning Calorimetry
- Stability Screening
- T_M & ΔH

MicroCal PEAQ ITC ★

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

- **Driving advances in biophysical characterization** by engaging and collaborating with researchers to improve understanding of biomolecular interactions and to accelerate development of more effective drugs
- From molecular interactions to successful drug products

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Zetasizer Nano & APS

Submicron Light Scattering

- **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

SLS | DLS | ELS

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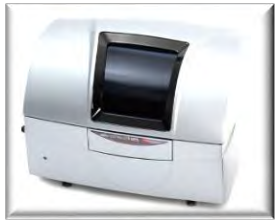
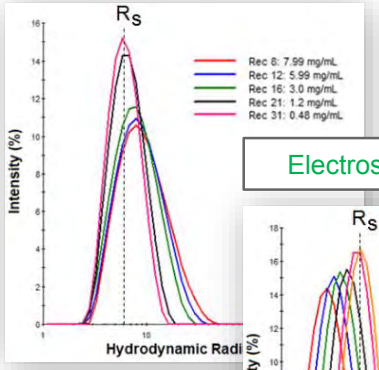
R_H | R_S | k_D

Z_{Eff} | B_{22} | T_{Agg}

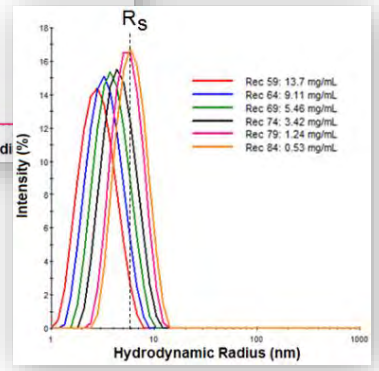
Distribution



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

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Probing molecular interactions of poly(styrene-co-maleic acid) with lipid matrix models to interpret the therapeutic potential of the co-polymer

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Particle size and zeta potential measurements with Zetasizer NanoZS DLS

System[a]	Hydrodynamic diameter[b] (nm)	Zeta potential[b] (mV)
A	1453 ± 21.5	−3.62 ± 0.42
B	1746 ± 31.8	−8.54 ± 0.72
C	1501 ± 24.2	−4.18 ± 0.47
D	1739 ± 28.7	−8.26 ± 0.57
E	1420 ± 38.1	+12.9 ± 1.12
F	1579 ± 25.4	−8.72 ± 0.50
G	1507 ± 42.9	−17.1 ± 1.54
H	1852 ± 36.3	−26.5 ± 1.06
	194.8 ± 1.4	

[a] Compositions of the systems are as follows: A, DSPC; B, DSPC + SMA; C, DSPC + CHOL; D, DSPC + CHOL + SMA; E, DSPC + DODAB + CHOL; F, DSPC + DODAB + CHOL + SMA; G, DSPC + DCP + CHOL; H, DSPC + DCP + CHOL + SMA.

[b] Mean ± SD (n = 3).

- Samples: Neat and SMA-incorporating MLVs at pH 7.0
- The observed slight increase in the mean hydrodynamic diameter of the MLVs in presence of SMA was due to incorporation of the co-polymer within the bilayers
- Zeta potential measurements revealed significant change in the vesicle surface charge in presence of the anionic co-polymer.

Banerjee et. al, Biochim Biophys Acta, 1818, 537-550 (2012)

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OMNISEC/MALS

SEC/LS | MALS | DLS | DSV

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Advanced Detection & MALS SEC

- **Absolute M_W, 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

R_H | M_W | R_g

C | [η] | Shape

%Purity | Distribution

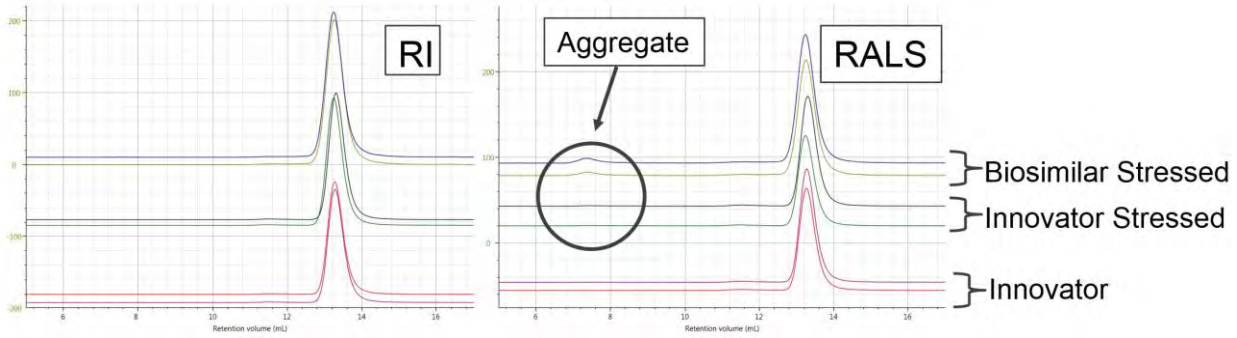
HMW Species Detection

- **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



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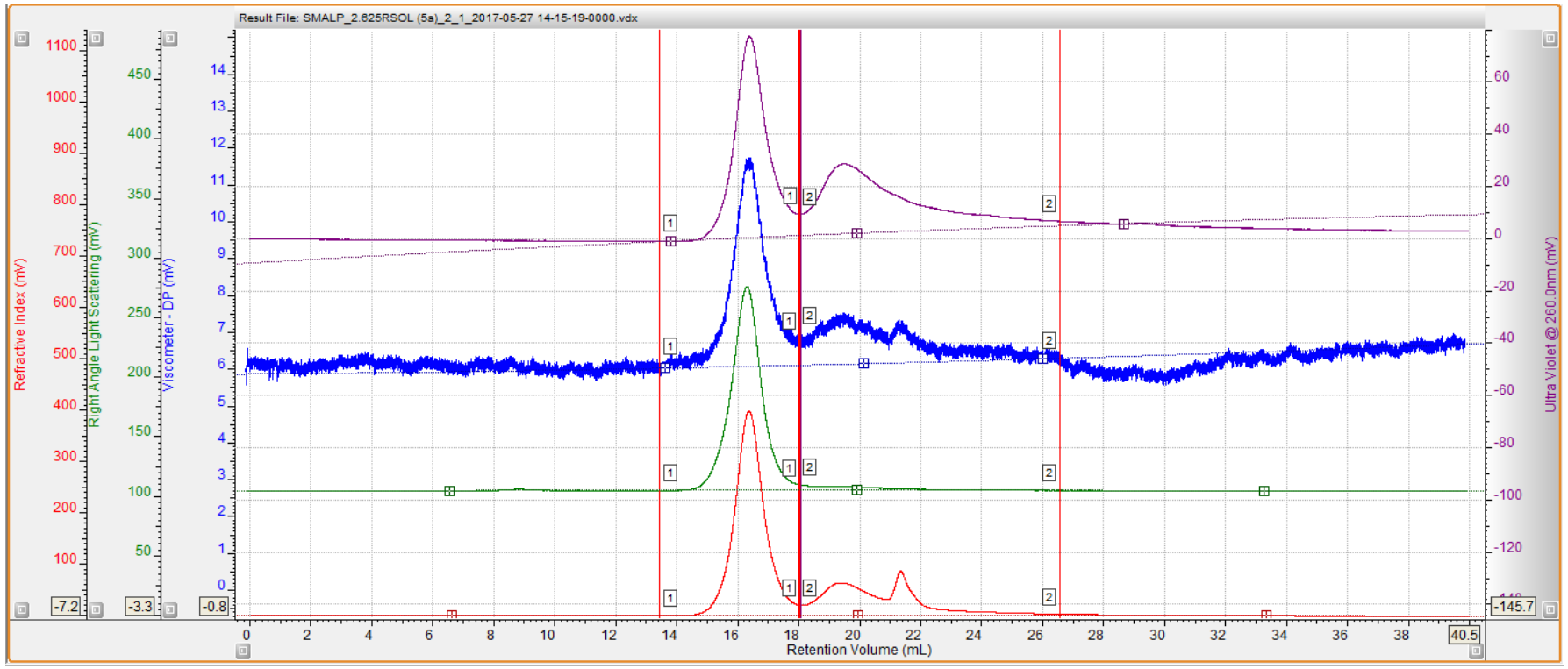
SMALP analysis (SMA distribution) – OMNISEC data



UV area: 192 mVmL

UV area [1]: 99 mVmL

UV area [2]: 93 mVmL

***RI*, *RALS*, *DP*, A_{260}**

→ Nearly 50% of SMA is free in solution

Molecular Biophysics
Keller Lab

Johannes Klingler, Malvern Panalytical webinar, 2017

TECHNISCHE UNIVERSITÄT
KAISERSLAUTERN

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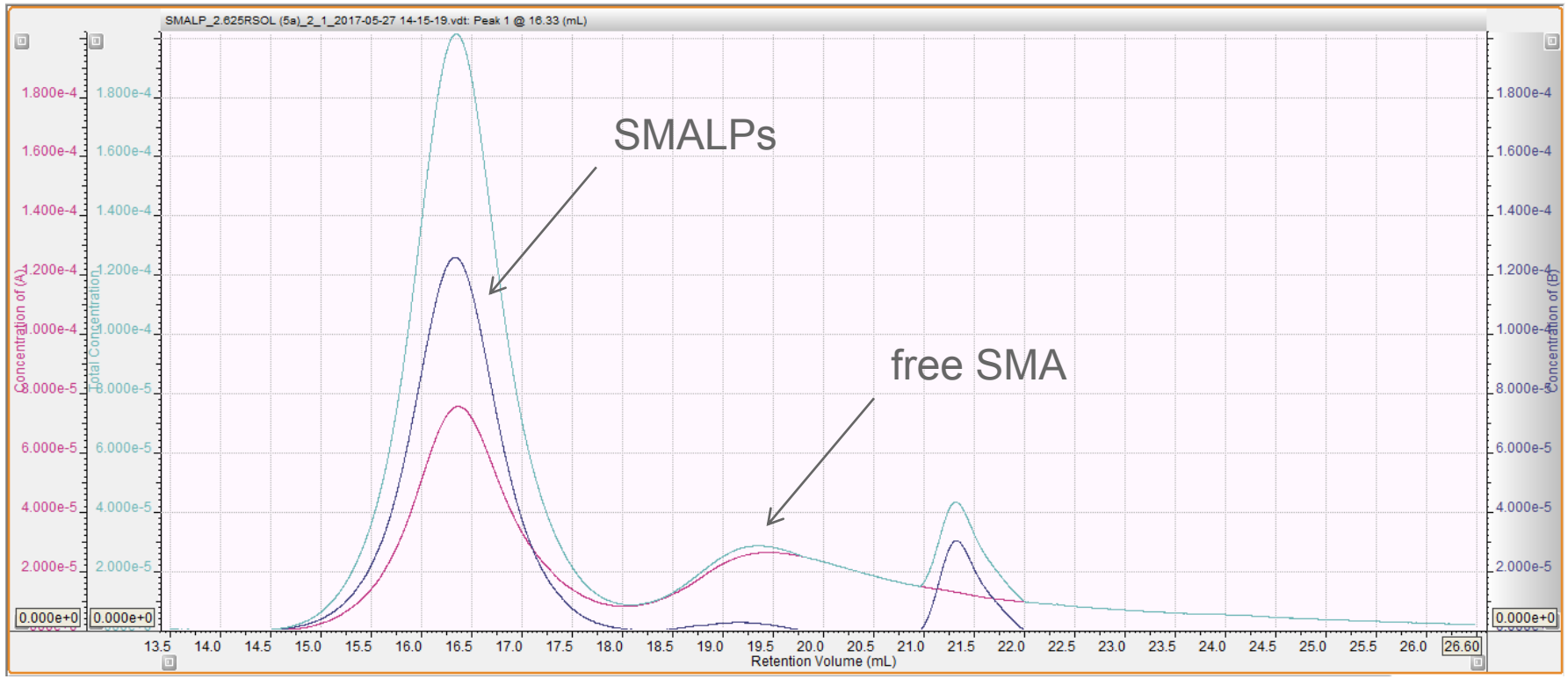
SMALP analysis (SMA distribution) – OMNISEC data



c_{SMA}, c_{DMPC}, c_{total}

→ The second peak is virtually lipid-free

Johannes Klingler, Malvern Panalytical webinar, 2017

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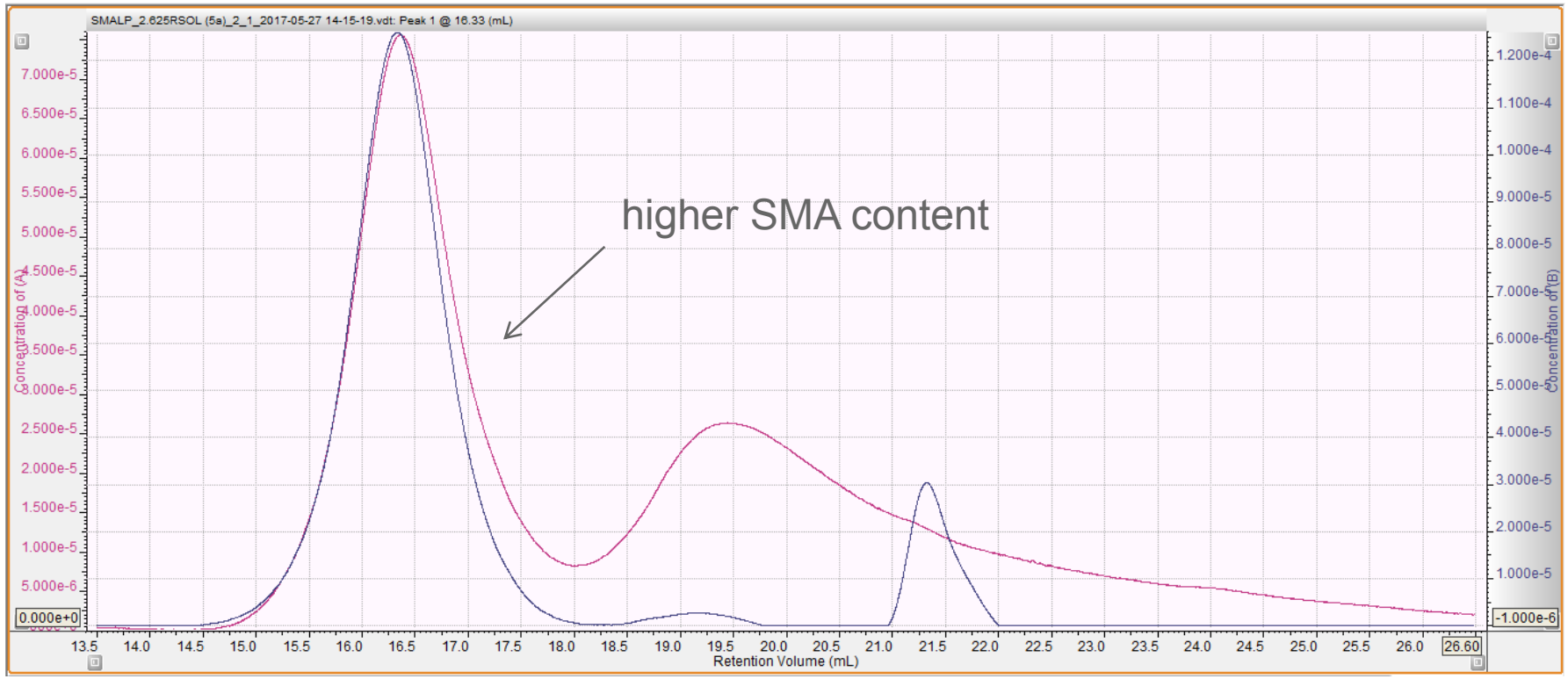
SMALP analysis (SMA distribution)



c_{SMA}, c_{DMPC}

→ SMA is more abundant in the right flank of the SMALP peak

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SMALP analysis (SMALP size and composition) – OMNISEC data

M_w: 161 kg mol^{-1}

M_w/M_n: 1.05

$[\eta]$: 0.049

R_h: 5.0 nm

$X_{SMA/DMPC}$: 0.40

c_{SMA}, c_{DMPC}, c_{total}, R_h, $[\eta]$, M_w

→ SMALP masses and sizes are narrowly distributed
→ SMA contributes 40% to the total mass of SMALPs

© Ma

Summary and conclusions: OMNISEC

- Nearly 50% of SMA is free in solution
- SMALPs show relatively narrow size and mass distributions
- SMA contributes a significant amount to the SMALP mass
- Combining data on hydrodynamic size and mass confirms disc shape of SMALPs

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MicroCal PEAQ DSC

DSC

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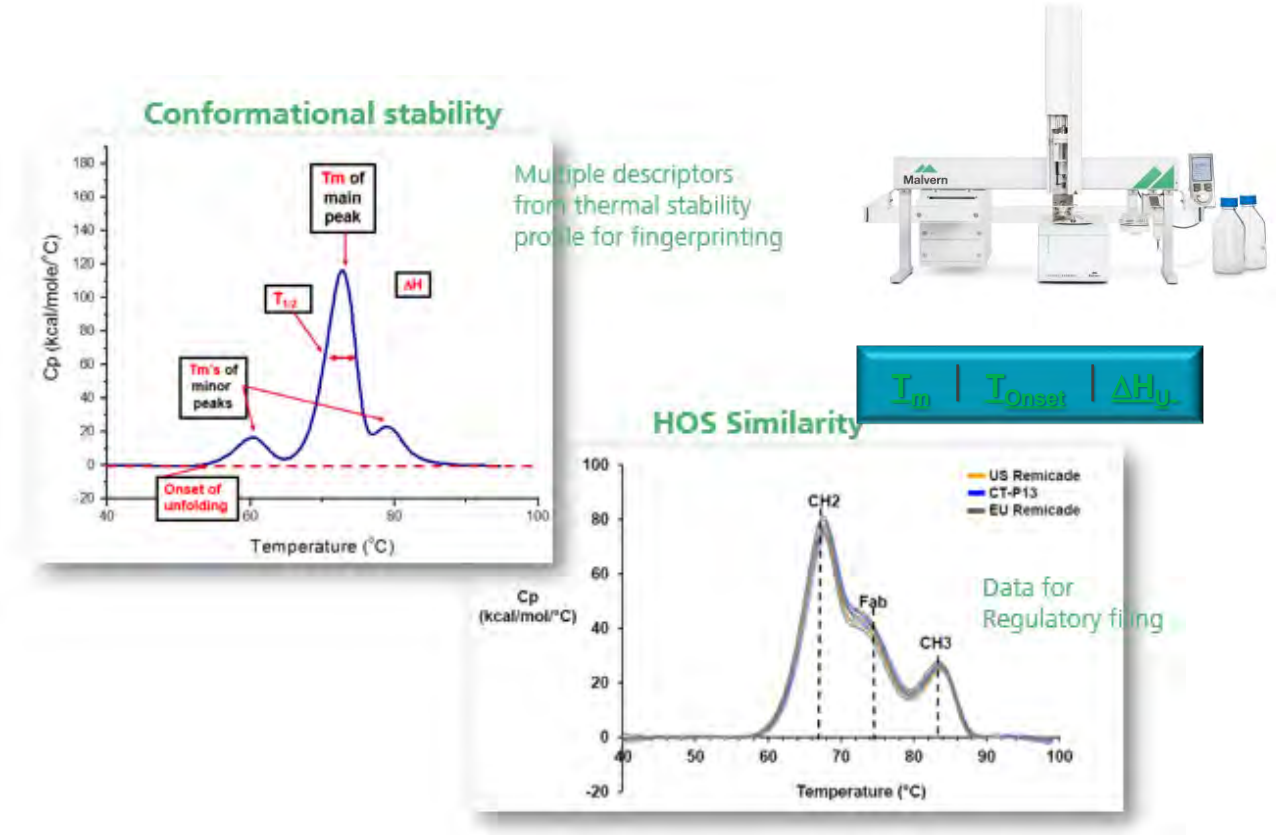
Differential Scanning Calorimetry

- **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



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Probing molecular interactions of poly(styrene-co-maleic acid) with lipid matrix models to interpret the therapeutic potential of the co-polymer

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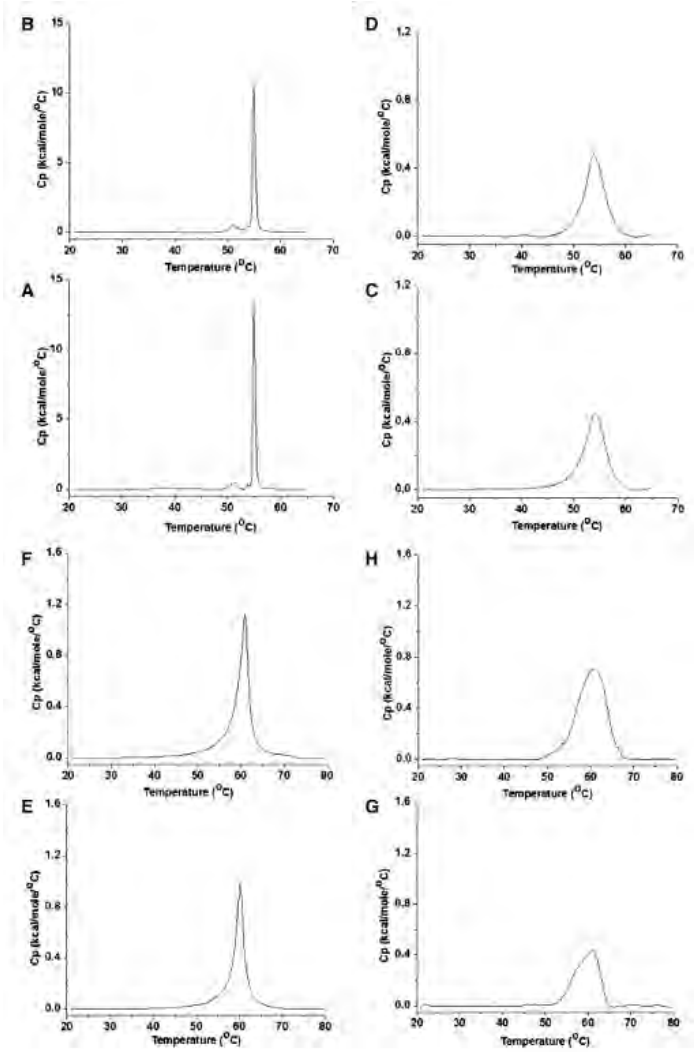
Thermal stability using MicroCal VP-DSC



- Study the thermotropic phase behavior of liposomes from which the molecular interactions between the co-polymer and phospholipids were quantitatively probed
- Representative DSC heating scans of
- A, DSPC; B, DSPC+SMA;
- C, DSPC+CHOL; D, DSPC+CHOL+SMA;
- E, DSPC+DODAB+CHOL; F, DSPC+DODAB+CHOL+SMA;
- G, DSPC+DCP+CHOL; H, DSPC+DCP+CHOL+SMA
- Multilamellar vesicles prepared in hepes buffered saline (10mM Hepes+150 mM NaCl, pH 7.0).
- Changes in T_M, enthalpy and peak broadening provide insights into the mechanism and interaction between SMA and the MLVs

Banerjee et. al, Biochim Biophys Acta, 1818, 537-550 (2012)

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MicroCal PEAQ ITC

Isothermal Titration Calorimetry

- **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

ITC

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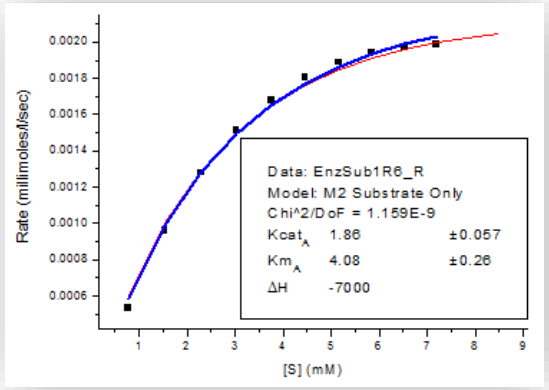
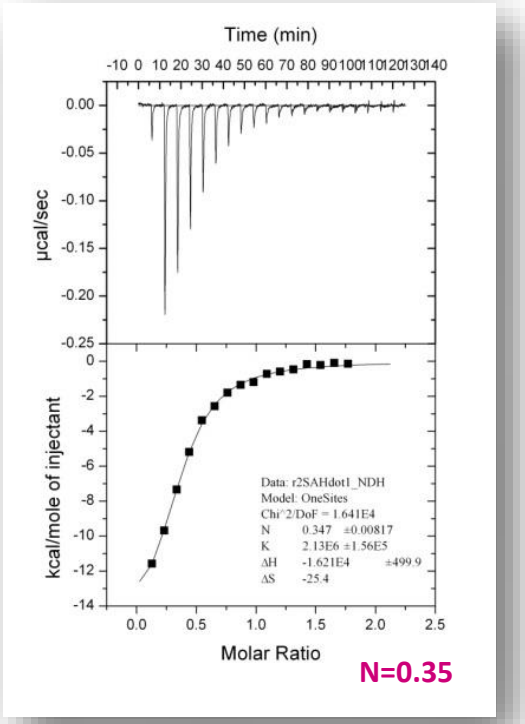
K_D

n

ΔH

ΔS





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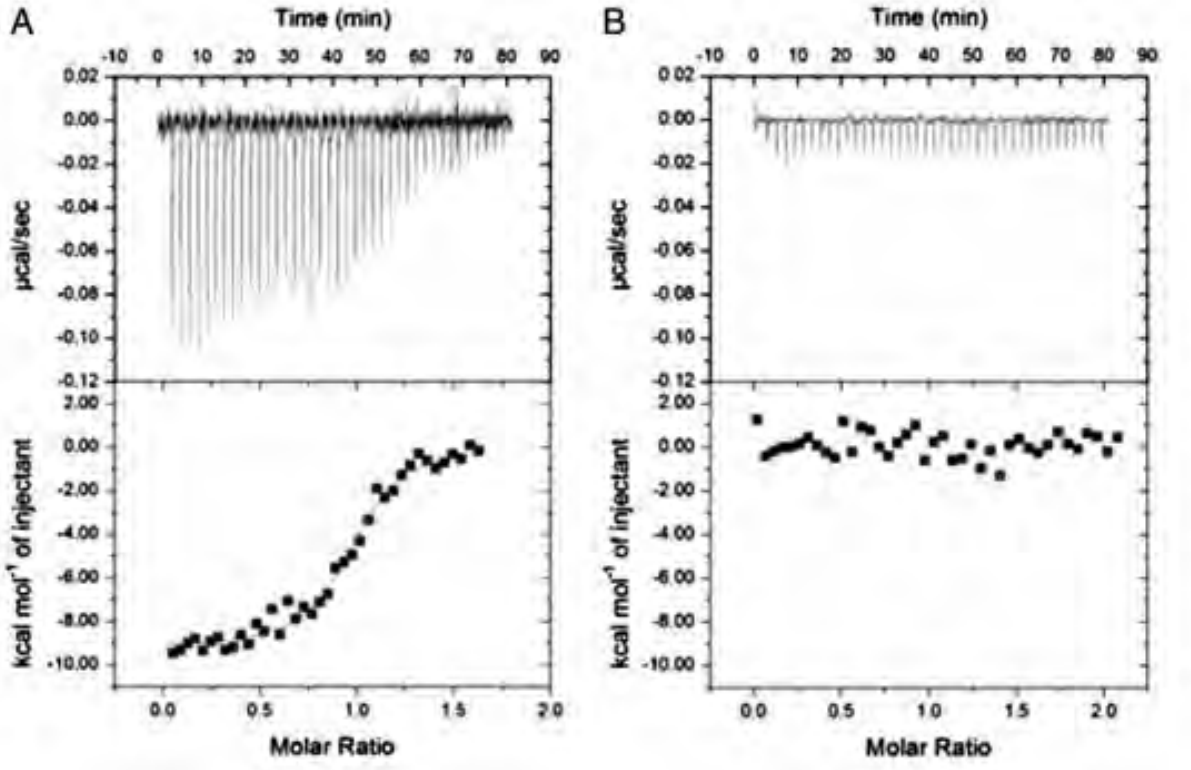
FhuA interactions in a detergent-free nanodisc environment

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)

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Summary

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

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Malvern Panalytical – Solutions and Instrumentation for Bioscience

MicroCal PEAQ ITC ★

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

Viscosizer TD (0.9 – 120 cP)

- Taylor Dispersion Analysis & Viscosity
- Stability Screening
- R_S, k_D, & η

Morphology G3-ID (1 – 1000 µm)

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

OMNISEC/MALS ★

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

Empyrean

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

Mastersizer 3000 (0.01 – 3500 µm)

- Laser Diffraction
- Quality Control
- Particle Size Distribution

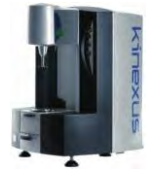
MicroCal PEAQ DSC ★

- Differential Scanning Calorimetry
- Stability Screening
- T_M & ΔH

NanoSight (40 nm – 1 µm)

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

Kinexus



- Rotational Rheometry
- Quality Control
- η, G', and G"

Zetasizer (0.6 nm – 10 µm) ★

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

Archimedes (250 nm – 5 µm)

- Resonant Mass Measurement
- Aggregate Quantification & ID
- PSD, R_m, m_B, & SµP Concentration

Zetium

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

★ SMALP and nanodisc characterization

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