Biophysical characterization of SMALPs and nanodiscs

Verna Frasca, Ph.D.
Malvern Panalytical
Verna.Frasca@Malvern.com
Malvern Panalytical

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

- **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
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

### Stability Profile

<table>
<thead>
<tr>
<th>Property</th>
<th>Buffer 1</th>
<th>Buffer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_D (M)^{-1}$</td>
<td>3.2</td>
<td>3.7</td>
</tr>
<tr>
<td>$B_{22} (x10^{-5} M/Mg/mol)$</td>
<td>1.5</td>
<td>127.5</td>
</tr>
<tr>
<td>$Z_{Eff}$</td>
<td>0.7</td>
<td>4.3</td>
</tr>
<tr>
<td>$T_{Agg} (°C)$</td>
<td>&gt;56</td>
<td>&gt;56</td>
</tr>
<tr>
<td>$R_S (nm)$</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>SubQ Limit (mg/ml)</td>
<td>137</td>
<td>148</td>
</tr>
</tbody>
</table>

**Reversible Self-Association**

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

Particle size and zeta potential measurements with Zetasizer NanoZS DLS

<table>
<thead>
<tr>
<th>System</th>
<th>Hydrodynamic diameter (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1453 ± 21.5</td>
<td>−3.62 ± 0.42</td>
</tr>
<tr>
<td>B</td>
<td>1746 ± 31.8</td>
<td>−8.54 ± 0.72</td>
</tr>
<tr>
<td>C</td>
<td>1501 ± 24.2</td>
<td>−4.18 ± 0.47</td>
</tr>
<tr>
<td>D</td>
<td>1739 ± 28.7</td>
<td>−8.26 ± 0.57</td>
</tr>
<tr>
<td>E</td>
<td>1420 ± 38.1</td>
<td>+12.9 ± 1.12</td>
</tr>
<tr>
<td>F</td>
<td>1579 ± 25.4</td>
<td>−8.72 ± 0.50</td>
</tr>
<tr>
<td>G</td>
<td>1507 ± 42.9</td>
<td>−17.1 ± 1.54</td>
</tr>
<tr>
<td>H</td>
<td>1852 ± 36.3</td>
<td>−26.5 ± 1.06</td>
</tr>
</tbody>
</table>

- 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.

OMNISEC/MALS

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

- Define Oligomeric Baseline
- Measure %Purity & distribution
- Track aggregation & HMW species generation

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

Stressed – Incubation at 30°C

<table>
<thead>
<tr>
<th></th>
<th>Innovator</th>
<th>Biosimilar</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Purity</td>
<td>99% monomer</td>
<td>97% monomer</td>
</tr>
<tr>
<td>Distribution</td>
<td>1% dimer</td>
<td>1.5% dimer</td>
</tr>
<tr>
<td>HMW Species</td>
<td>1.5% aggregates</td>
<td>1.5% aggregates</td>
</tr>
</tbody>
</table>
SMALP analysis (SMA distribution) – OMNISEC data

UV area: 192 mVmL
UV area [1]: 99 mVmL
UV area [2]: 93 mVmL

→ Nearly 50% of SMA is free in solution

Johannes Klingler, Malvern Panalytical webinar, 2017
The second peak is virtually lipid-free

Johannes Klingler, Malvern Panalytical webinar, 2017
SMALP analysis (SMA distribution)

SMALP peak with annotation:
- SMA is more abundant in the right flank of the SMALP peak

Graph showing retention volume vs. concentration with an indication of higher SMA content.

Johannes Klingler, Malvern Panalytical webinar, 2017
SMALP analysis (SMALP size and composition) – OMNISEC data

\[ M_w : 161 \text{ kg mol}^{-1} \]
\[ M_w / M_n : 1.05 \]
\[ [\eta] : 0.049 \]
\[ R_h : 5.0 \text{ nm} \]
\[ X_{\text{SMA/DMPC}} : 0.40 \]

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

Johannes Klingler, Malvern Panalytical webinar, 2017
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
MicroCal PEAQ DSC

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

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

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

© Malvern Panalytical 2018
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).

<table>
<thead>
<tr>
<th>Titrant</th>
<th>Cell</th>
<th>N</th>
<th>$K_d$ (nM)</th>
<th>$\Delta H$ (cal/mol)</th>
<th>$\Delta S$ (cal/mol/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TonB</td>
<td>Nd-FhuA</td>
<td>1</td>
<td>20.4 ± 5.6</td>
<td>-918.6 ± 133.4</td>
<td>-0.164</td>
</tr>
<tr>
<td>TonB</td>
<td>apo</td>
<td>1</td>
<td>0.010 ± 0.003</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>Colicin</td>
<td>Nd-FhuA-M</td>
<td>0.906 ± 0.001</td>
<td>3.48 ± 1.09</td>
<td>79.8 ± 32.3</td>
<td>65.4</td>
</tr>
<tr>
<td>Colicin</td>
<td>apo</td>
<td>0.0016</td>
<td>1.09 ± 0.09</td>
<td>32.3 ± 65.4</td>
<td></td>
</tr>
</tbody>
</table>

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).
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
## Malvern Panalytical – Solutions and Instrumentation for Bioscience

### MicroCal PEAQ ITC
- **Isothermal Calorimetry**
- Activity Screening
- K_D, ΔH, ΔS, & η

### 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

### Viscosizer TD (0.9 – 120 cP)
- Taylor Dispersion Analysis & Viscosity
- Stability Screening
- R_g, k_D, & η

### Empyrean
- **X-Ray Diffraction**
- Polymorph Assessment & Screening
- Excipient & API Form

### NanoSight (40 nm – 1 μm)
- Nanoparticle Tracking Analysis
- Aggregate Quantification
- PSD, R_H, & S_app Concentration

### Archimedes (250 nm – 5 μm)
- Resonant Mass Measurement
- Aggregate Quantification & ID
- PSD, R_W, m_B, & S_app Concentration

### Morphology G3-ID (1 – 1000 μm)
- Digital Imaging & Raman Spectroscopy
- Aggregate Quantification & ID
- Size, Morphology, & Chemical ID

### Mastersizer 3000 (0.01 – 3500 μm)
- Laser Diffraction
- Quality Control
- Particle Size Distribution

### Kinexus
- Rotational Rheometry
- Quality Control
- η, G’, and G”

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

★ SMALP and nanodisc characterization