On-Chip X-ray Analysis and Mechanistic Studies of *In-Meso* Crystallization

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**Membrane Proteins**
- Responsible for signal and material/energy transduction
- Common drug targets
- Malfunction linked to diseases
- ~10,000 membrane proteins in humans
- ~56,000 structures in Protein Data Bank, but only 433 membrane proteins

**Challenge: Maintaining Lipidic Domains**
- Amphiphilic nature makes solubilization difficult
- Lateral membrane pressure maintains protein conformation
- Crystallization: more art than science

**Goal: Scaling Down**
- Numerous trials needed to determine crystallization conditions
- Membrane proteins available in minuscule quantities
- Microfluidic approach

**In-Meso Crystallization**
- Self-assembling lipid/water phases
- Maintains protein in a more "native" environment
- Averages concerns for solubilization and denaturation
- Concentration induced phase change drives crystallization

**Mixing of Lipids and Aqueous Media**
- Viscosities: η(lipid) ~ 30 x η(water)
- Mixing via tendril-whorl type flow
- Use pneumatic valves over chambers to drive fluid motion
- Uniform mixture achieved in ~1 min

**Validating Platform with Bacteriorhodopsin**
- 13.5 mg/mL bacteriorhodopsin in 25 mM NaH2PO4 pH 5.5 and 1.2% β-ocyt glucoside mixed 1:1 (v/v) with mononolein
- Precipitant: 2 M Sørenson buffer pH 5.5

**On Chip X-ray Analysis**
- Crystal quality screening
- Structure determination
- Lipid phase diagram studies
- Crystallization science studies

**Benefits**
- No harvesting
- No damage to crystals
- Compatible with tiny crystals
- Crystals can be located optically before cryo-cooling
- Ideal for RT screening
- Automated screening of arrays
- More information per trial

**Potential Challenges**
- Scatter from device
- Device mounting and rotational limitations

**Proof-of-Concept Experiments**
- Minimal attenuation from thin PDMS membrane
- Scatter can be minimized from polyimide
- Adequate S/N for crystallography even on rotating Cu-anode benchtop source
- Compatible with SAXS studies of lipid phase behavior

**Mechanistic Studies of *In-Meso* Crystallization**
- Study diffusion of proteins in mesophases
  - Trends of membrane curvature vs. protein size
  - Preferential partition between phases
- Importance of membrane vs. solubility effects
- Fluorescence of proteins
- SAXS of mesophases

**Summary**
- Developed microfluidic chips for *in-meso* membrane protein crystallization
- Validated on chip *in-meso* crystallization using bacteriorhodopsin
- Developed X-ray transparent chip designs

**Ongoing Work**
- Extension to novel membrane proteins
- Scaling out of microfluidic devices to allow for higher throughput parallel processing of crystallization trials
- Studies of lipid phase behavior
- Develop the science of *in-meso* crystallization

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