Microfluidic Platforms for Membrane Protein 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
- >46,000 structures in Protein Data Bank, but only 280 membrane proteins

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

In-situ Crystallization:
- Most successful method to date
- Protein crystals grown from detergent solubilized solutions

Our Evaporation-Based Crystallization Platform:
- Guarantees a phase change
- Kinetic control achieved using device geometry
- Varying the rate of supersaturation has a profound effect on the outcome

Determining the Solubility Boundary
- Goal is to determine the solubility boundary using a minimal amount of protein

Decoupling Nucleation and Growth
- Problem is that often only showers of crystals are obtained (1.2 only)
- Redissolve small crystals until only a few remain to act as seed crystals (1-4)

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- Amphiphilic nature makes solubilization difficult
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- Crystallization: more art than science

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

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Summary:
- Developed microfluidic membrane protein crystallization devices
- Successfully obtained bacteriorhodopsin crystals on-chip using both in-situ and in-meso methods
- Demonstrated a dilution method to improve crystal quality
- Determined the solubility boundary for 2 forms of a membrane protein

Future Work:
- Characterize and validate microscale in-meso and in-situ crystallization on chip using in-situ X-ray analysis
- Extension to novel membrane proteins
- Scaling out of microfluidic devices to allow for higher throughput parallel processing of crystallization trials

Membrane Proteins

Viscosities: $\eta_{\text{lipid}} \sim 30 \times \eta_{\text{water}}$
Guarantees a phase change

Microfluidic approach
Amphiphilic nature makes solubilization difficult

Malfunction linked to diseases

Extension to novel membrane proteins
Varying the rate of supersaturation has a profound effect on the outcome

Straight flow side to side
Responsible for signal and

Redissolve small crystals until only a few remain to act as seed crystals (1-4)

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