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

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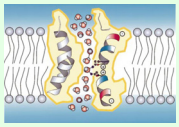
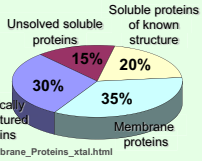
Membrane Proteins

- 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



Murata, K. et al., *Nature*, 2000, *407*.

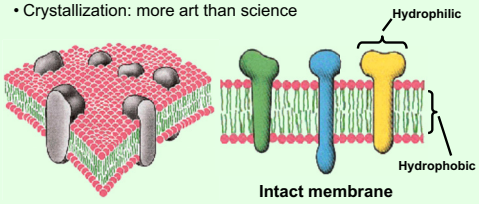


Crystallization of membrane proteins is the challenge

http://www.pdb.org http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html

Challenge: Maintaining Lipidic Domains

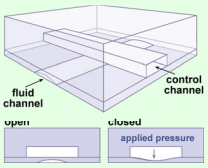
- Amphiphilic nature makes solubilization difficult
- Lateral membrane pressure maintains protein conformation
- Crystallization: more art than science



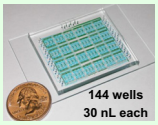
Ostermeier and Michel, *Current Opinion in Structural Biology*, 1997.

Goal: Scaling Down

- Numerous trials needed to determine crystallization conditions
- Membrane proteins available in miniscule quantities
- Microfluidic approach

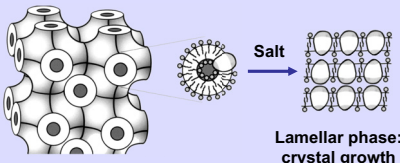


$\sim 10^2 - 10^3$ volume decrease



In-Meso Crystallization

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



Landau and Rosenbusch, *PNAS*, 1996.
Caffrey, *Journal of Structural Biology*, 2003.

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

Present method

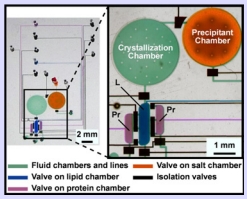
Mixing ~20 µL

Dispensing: µL (manual)

Caffrey, *Journal of Structural Biology*, 2003.

1000-fold volume decrease

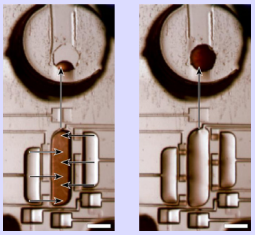
Microfluidics: 20 nL

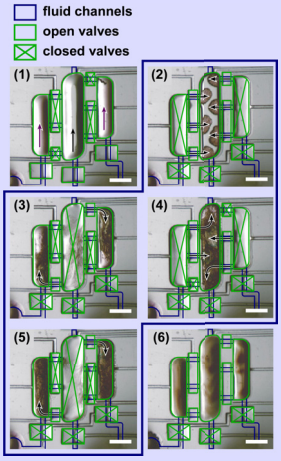


In-situ preparation and screening

Validating Platform with Bacteriorhodopsin

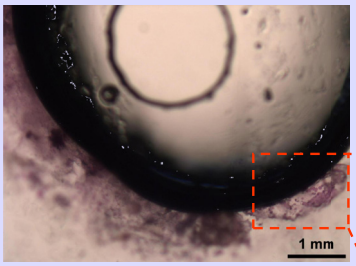
- 13.5 mg/mL bacteriorhodopsin in 25 mM NaH_2PO_4 pH 5.5 and 1.2% β-octyl glucoside mixed 1:1 (v/v) with monoolein
- Precipitant: 2.5 M Sørenson buffer pH 5.5

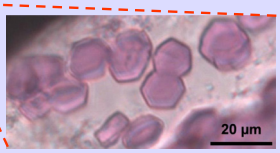




Scale bar: 500 µm

- Total chamber volume = 19.4 nL (9.8 nL protein 9.6 nL lipid)
- 16.0 nL protein per experiment (6.2 nL lost in lines)

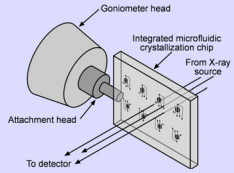




Perry, Roberts, Tice, Gennis, and Kenis, *Crystal Growth & Design*, (submitted).

On Chip X-ray Analysis

- Crystal quality screening
- Structure determination
- Lipid phase diagram studies
- Crystallization science studies

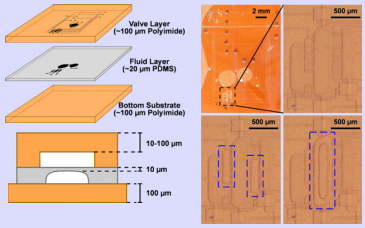


Benefits	Potential Challenges
• No harvesting • No damage to crystals • Compatible with tiny crystals • Crystals can be located optically before cryo-cooling • Ideal for RT screening – Automated screening of arrays – More information per trial	• Scatter from device • Device mounting and rotational limitations

Hybrid PDMS / Polyimide Device

- Challenge: standard µFD materials attenuate X-rays
- Goal: integrate device functionality with X-ray transparency

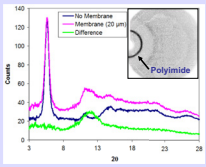
→ **Real-time analysis**

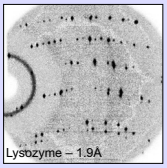


Proof-of-Concept Experiments

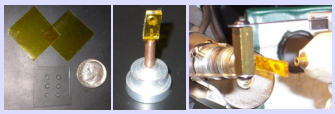
- Minimal attenuation from thin PDMS membrane
- Scatter can be minimized from polyimide
- Adequate S/N for crystallography even on rotating Cu-anode benchtop source
- Compatible with SAXS studies of lipid phase behavior

Benchtop Source



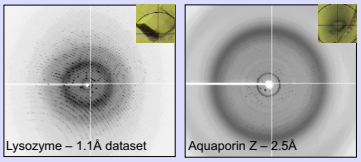


Benchtop X-ray studies done with the help of Dr. Scott Wilson.



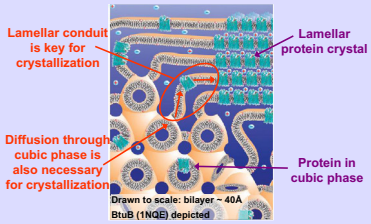
- Compatible with cryo-cooling
- Able to obtain complete datasets
- Comparable to traditional methods

Synchrotron Source



Synchrotron X-ray studies done with the help of Dr. Jean Jakoncic at the National Synchrotron Light Source X6A.

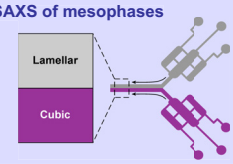
Mechanistic Studies of *In-Meso* Crystallization



Cherezov and Caffrey, *Faraday Discussions* **2007**.
Caffrey, *Crystal Growth & Design* **2008**.
Cherezov et al., *Crystal Growth & Design* **2008**.

- Study diffusion of proteins in mesophases
  - Trends of membrane curvature vs. protein size
  - Preferential partitioning between phases
- Importance of membrane vs. solubility effects

→ **Fluorescence of proteins**
→ **SAXS of mesophases**



Acknowledgements

- Chip design: Griffin Roberts, Josh Tice, Andrew Choi and the Kenis group
- Helpful discussions: Prof. Higdon, Prof. Gennis, Prof. Zukoski
- Proteins: Dr. Berthold, Prof. Rienstra, Dr. Baybert, Dr. Labile and Dr. Kors (ANL), and the Gennis and van der Donk groups
- X-ray studies:Dr. Robinson and Dr. Jakoncic (BNL)

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