## 2DX Workshop, September 10th 2008


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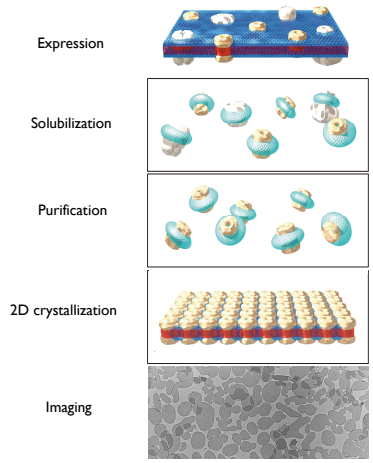
## Obtaining optimal 2DXs is the bottle-neck

**Table 1. 3D structures of unique membrane proteins determined by electron crystallography.**

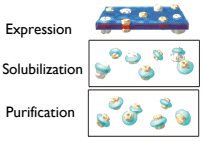
| Integral Membrane Protein | Resolution, Å | Year | Reference |
|---|---|---|---|
| **Eye lens Aquaporin-0** | 1.9 | 2005 | (Gonen, Cheng et al. 2005) |
| **Rat Aquaporin-4** | 3.2 | 2006 | (Hiroaki et al. 2006) |
| **Glutathione transferase** | 3.2 | 2006 | (Holm, Bhakat et al. 2006) |
| **Plant LHC-II** | 3.4 | 1994 | (Kühlbrandt et al. 1994) |
| **Bacteriorhodopsin** | 3.5 | 1990 | (Henderson, Baldwin et al. 1990) |
| **Red cell Aquaporin-1** | 3.8 | 2000 | (Murata, Mitsuoka et al. 2000) |
| **Acetylcholine receptor** | 4.0 | 2005 | (Unwin 2005) |
| **Human aquaporin 2** | 4.5 | 2005 | (Schenk, Werten et al. 2005) |
| Halorhodopsin | 5.0 | 2000 | (Kunji, von Gronau et al. 2000) |
| Plant Aquaporin SoPIP2 | 5.0 | 2005 | (Kukulski, Schenk et al. 2005) |
| Porin PhoE | 6.0 | 1991 | (Jap, Walian et al. 1991) |
| Rhodopsin frog p2 | 6.5 | 1997 | (Unger, Hargrave et al. 1997) |
| $Ca^{2+}$-ATPase | 6.5 | 2002 | (Xu, Rice et al. 2002) |
| Oxalate transporter OxlT | 6.5 | 2002 | (Hirai, Heymann et al. 2002) |
| Glycerol channel GlpF | 6.9 | 2000 | (Stahlberg, Braun et al. 2000) |
| NhaA Na/ $H^+$ antiporter | 7.0 | 2000 | (Williams 2000) |
| EmrE multidrug transporter | 7.0 | 2003 | (Ubarretxena-Belandia, Baldwin et al. 2003) |
| Gap junction channel | 7.5 | 1999 | (Unger, Kumar et al. 1999) |
| Sec YEG complex | 8.0 | 2005 | (Bostina, Mohsin et al. 2005) |
| Plant photosystem II RC | 8.0 | 1998 | (Rhee, Morris et al. 1998) |
| Neurospora $H^+$-ATPase | 8.0 | 1998 | (Auer, Scarborough et al. 1998) |

One bottle-neck is getting 2D crystals that are large enough and diffract to high resolution!

## General strategy to 2D crystallize membrane proteins




## A high-throughput screening for 2DX




Need mg quantities of purified and stable MP

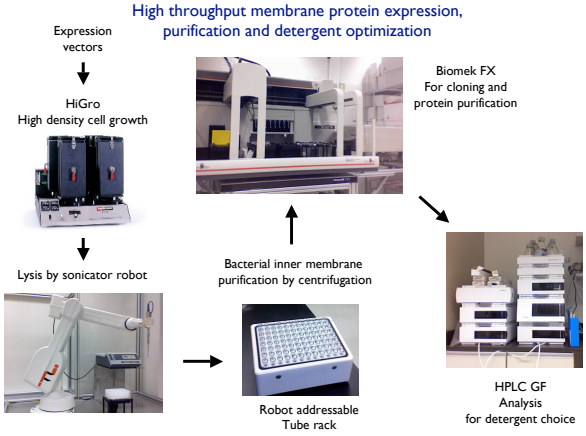
Need sequence variation: access to homologs

New York Consortium on Membrane Protein Structure (www.nycomps.org)

>10000 multispan membrane proteins selected from 92 prokaryotic genomes

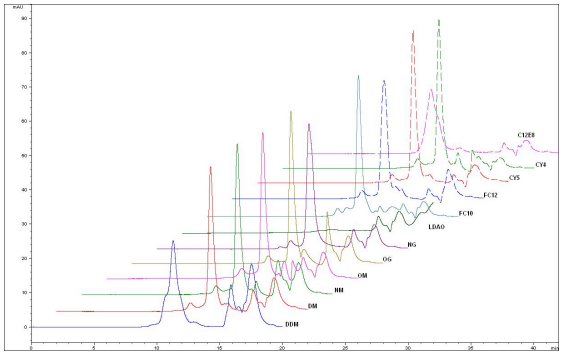
## A HT screening for 2DX: protein production

High throughput membrane protein expression, purification and detergent optimization

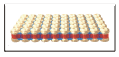



## A HT screening for 2DX: protein selection

A given protein and its homologs are selected based on their behavior on SEC



## A HT SCREENING FOR 2DX: CRYSTALLIZATION

2DX 

Variables in 2DX by dialysis: the crystallization space is "small"

Protein concentration: 0.4-1 mg/ml

Lipid type: DMPC, DOPC, POPC, DOPG, E. coli lipids

Lipid-to-protein-ratio (LPR, mg-mg): 0.2-1.5

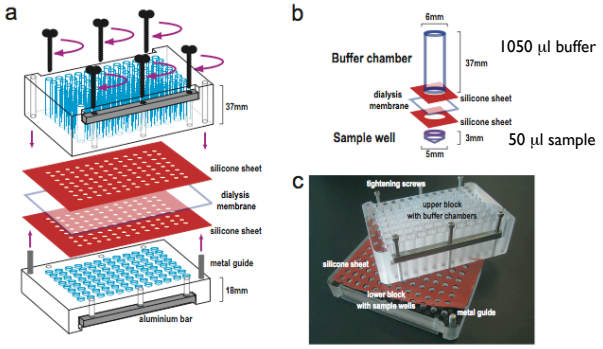
Detergent type and concentration: DDM or OG

No precipitants used

Buffer: pH 6-8, 100 mM NaCl and divalent cations, reducing agents

Set up 2D crystallizations on a 96-well format

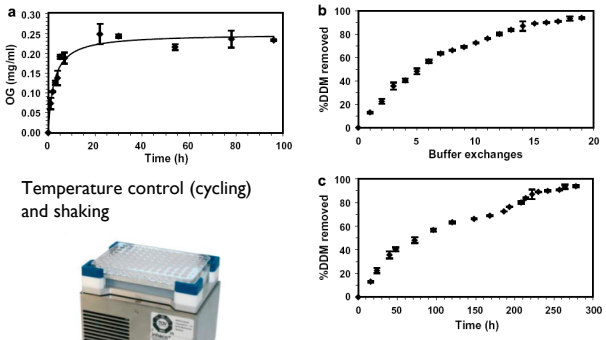
## A HT SCREENING FOR 2DX: 96-WELL 2DX BLOCK




Vink, M., Derr, KD, Love, J., Stokes, D.L., & Ubarretxena-Belandia, I., 2007
A high-throughput strategy to screen 2D crystallization trials of membrane proteins. *JSB*, **160**, 294-304

## A HT SCREENING FOR 2DX: 96-WELL 2DX BLOCK

Efficient Low and High CMC Detergent Dialysis



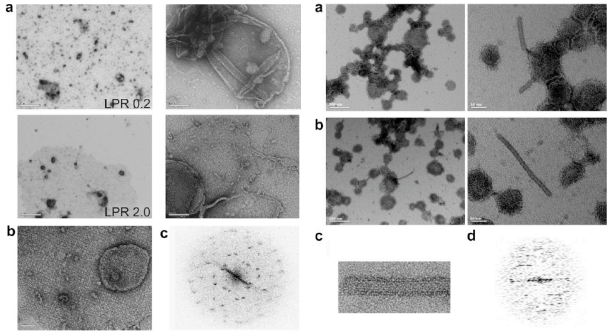

Temperature control (cycling) and shaking



## 2DX WERE OBTAINED WITH LH2 AND COPA

Purified LH2 (0.5 mg/ml) in LDAO
Mixed with DOPC in OG
Dialyzed against 10 mM Hepes, pH 7.5, 100 mM NaCl for 24-h at 30 °C

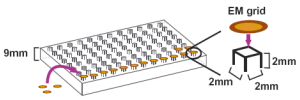
Purified CopA (0.5 mg/ml) in 0.01% DDM
Mixed with DOPC in C12E8
Dialyzed against buffer for 7-d at 20 °C



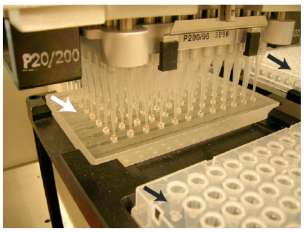

## A HT SCREENING FOR 2DX: NS ON A 96-WELL FORMAT

Imaging 

Need to prepare 96 negatively stained specimens to evaluate each dialysis block by EM



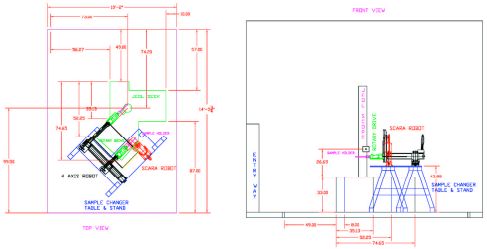

96-format robot operated negative staining procedure using cooper grids, "nitrocellulose" carbon and 0.25% UA




## A HT SCREENING FOR 2DX: GRID LOADING AND IMAGING SYSTEM

Imaging 

Need to load and image 96 grids per crystallization block



Picks grid from a 96-well anodized platform by suction, transfers grid to the stage, inserts the stage. Removes stage and places grid back in platform

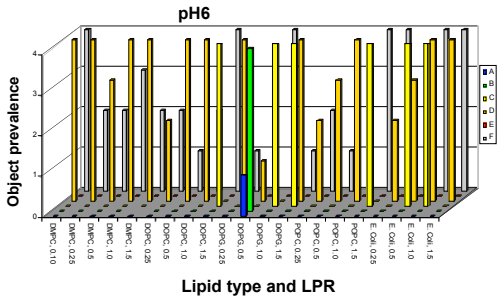
Transfer device and grid imaging is managed by LEGINON

Provides user with a montage of low magnification pictures per grid. Selected grids can be automatically imaged at high magnification

System implemented in JEOL 1230. ~100 grids imaged per day

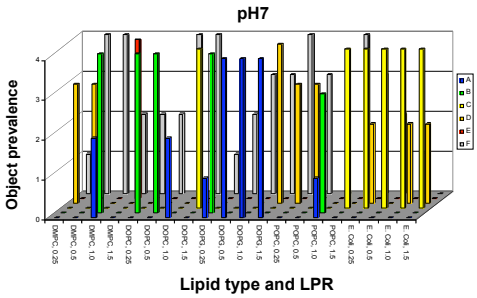
## A HT SCREENING FOR 2DX: AN EXAMPLE

Sample: P2A3
Detergent: DDM
LPR 1.0



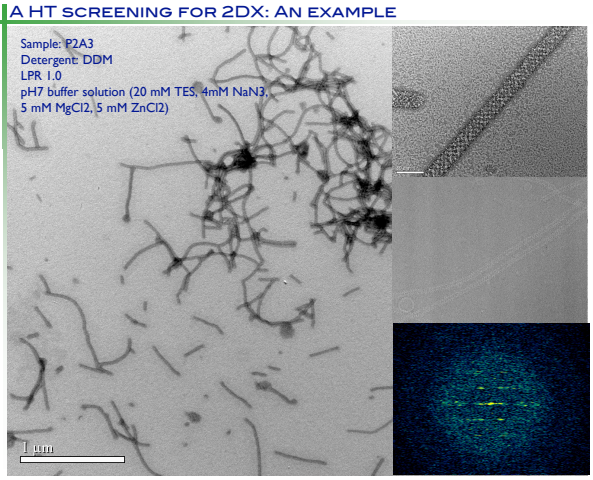

A: Crystal lattice
B: Tubular vesicle, sheets and physical growth
C: Vesicle and vesicle cluster
D: Protein aggregate and lipid aggregate
E: Lipid string
Blank: Failure, large precipitate (checked by light microcopy), bad staining, broken carbon, etc

## A HT SCREENING FOR 2DX: AN EXAMPLE



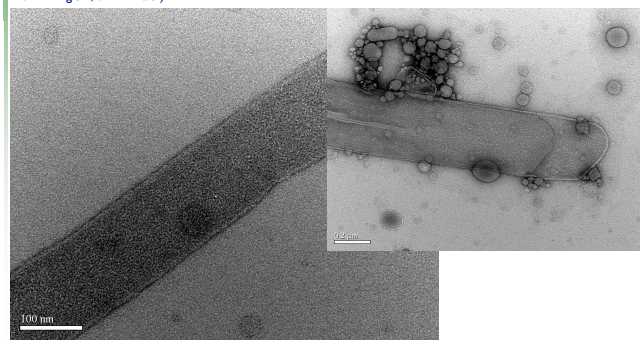

## A HT SCREENING FOR 2DX: AN EXAMPLE

Sample: P2A3
Detergent: DDM
LPR 1.0
pH7 buffer solution (20 mM TES, 4mM NaN3,
5 mM MgCl2, 5 mM ZnCl2)




## A HT SCREENING FOR 2DX: AN EXAMPLE

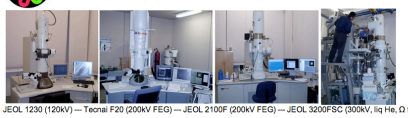
Sample: MCV
Lipid: DMPC
Detergent: Foscholine-12
LPR 1.0
pH6 buffer solution (20 mM MES, 4mM NaN3,
5 mM MgCl2, 0 mM NaCl)




## A HT SCREENING FOR 2DX: IN PRACTICE

1- Screen 100 membrane proteins, derived from the NYCOMPS pipeline, for 2D crystallization in next 2 years
2- Initial screen of 2x96 conditions per protein
3- Identify proteins that crystallize and redo a targeted screening for diffraction at high-resolution
4- Start 3D structure determination using NYSBC microscope facilities

**New York Structural Biology Center**



JEOL 1230 (120kV) — Tecnai F20 (200kV FEG) — JEOL 2100F (200kV FEG) — JEOL 3200FSC (300kV, liq He, Ω filter)

Welcome to the Cryoelectron Microscopy Facility

www.nysbc.org

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