# High-throughput Screening of 2D Crystallization

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Table 1. 3D structures of membrane proteins determined by electron         crystallography         Atomic resolution structures are highlighted in hold									
Integral Membrane Protoin	Resol	Vear							
integral membrane Protein	(Å)	rear	Reference						
Bacteriorhodopsin	7.0	1975	(Henderson and Unwin)						
Bacteriorhodopsin	6.5	1983	(Leifer and Henderson)						
Bacteriorhodopsin	3.5	1990	(Henderson, Baldwin et al.)						
Plant LHC-II	3.4	1991	(Kühlbrandt and Wang)						
Porin PhoE	6.0	1991	(Jap, Walian et al.)						
Acetylcholine receptor	9.0	1993	(Unwin)						
Frog Rhodopsin frog	6.5	1997	(Unger, Hargrave et al. )						
Bacteriorhodopsin	3.0	1997	(Kimura, Vassylyev et al.)						
Plant photosystem II RC	8.0	1998	(Rhee, Morris et al.)						
Neurospora H <sup>+</sup> -ATPase	8.0	1998	(Auer, Scarborough et al.)						
Gap junction channel	7.5	1999	(Unger, Kumar et al.)						
NhaA Na/ $H^+$ antiporter	7.0	2000	(Williams)						
Glycerol channel GlpF	6.9	2000	(Stahlberg, Braun et al.						
Halorhodopsin	5.0	2000	(Kunji, von Gronau et al.)						
Aquaporin-1	3.8	2000	(Murata, Mitsuoka et al.)						
Glutathione transferase	6.0	2002	(Holm, Morgenstern et al.)						
Oxalate transporter OxIT	6.5	2002	(Hirai, Heymann et al.)						
Ca <sup>2+</sup> -ATPase	6.5	2002	(Xu, Rice et al.)						
Bovine Rhodopsin	5.5	2003	(Krebs, Edwards et al.)						
EmrE multidrug transporter	7.0	2003	(Ubarretxena-Belandia et al.)						
Eye lens Aquaporin 0	1.9	2005	Gonen, Cheng et al.)						
Acetylcholine receptor	4.0	2005	(Unwin)						
Human aquaporin 2	4.5	2005	(Schenk, Werten et al.)						
Plant Aquaporin SoPIP2	5.0	2005	(Kukulski, Schenk et al.)						
Sec YEG complex	8.0	2005	(Bostina, Mohsin et al.)						
Glutathione transferase	3.2	2006	(Holm, Bhakat et al.)						
Gap junction channel	7.0	2007	(Oshima, Tani et al.)						
Aquaporin-4	2.8	2009	(Tani, Mitsuma et al.)						
hCTR1 Cu transporter	7.0	2009	(De Feo, Aller et al.)						

# 2DX has been applied to a number of membrane proteins

#### PROS

Membrane proteins studied in their natural lipid bilayer environment.

Molecular packing is less constrained and can accommodate conformational changes.

Membrane proteins in a 2DX are fully accessible to the aqueous medium.

#### CONS

Screening of 2D crystallization trials is slow and optimal 2D crystals for structure

determination at high-resolution are relatively rare.

Data collection is tedious as many crystals and different tilts have to be merged in dataset.

2D crystals are flimsy.

Obtained 3D map suffers from anisotropic resolution.

# General strategy to 2D crystallize membrane proteins

Expression



Solubilization

Purification

2D crystallization

Imaging







#### Our current 2DX pipeline



Data Analysis

Robotic grid handling and automated image acquisition by EM

# The membrane protein targets

Protein	Organism	(Putative) Function	MW (kDa)	pI	TMD	N-term	% TM	Screens
E2P1	M.voltae	Protease	25.9	8.7	4	Inside	45	4
E2P2	M.maripaludis	Protease	25.9	5.0	4	Inside	39	3
E2P3	M.marisnigri	Protease	31.8	5.3	7	Inside	59	2
E2P4	M.hungatei	Protease	32.9	7.8	9	Inside	61	2
P2A3	S.oneidensis	Cation efflux family protein	32.5	5.4	5	Inside	36	5
Rhomboid PA3086	P.aeruginosa	Intramembrane protease	31.8	9.9	6	Inside	45	4
YkgB-D332	E.coli	Unknown	21.9	6.0	3	Inside	38	1
YkgB-D36	E.coli	Unknown	21.8	5.7	3	Inside	37	4
β1-adrenergic receptor	M.gallopavo	G-protein coupled receptor	54.1	9.3	7	Outside	33	3
Rhomboid GlpG	E.coli	Intramembrane protease	31.3	9.2	6	Inside	46	1
Cytochrome b561	P.aeruginosa	Electron carrier activity	20.6	9.6	4	Inside	47	1
E1 protein	Semliki Forest Virus	Inducer of membrane fusion	47.4	7.6	0(1)	Outside	5	4
P40B7	B.subtilis	Sporulation kinase C	48.8	6.3	2	Inside	10	1
Kdp-ATPase (4 subunits)	E.coli	High-affinity K-pump	159.2	5.2-9.4	17	3 in, 1out	31	1
P39H10	K. pneumoniae	Diguanylate cyclase	45.7	6.5	2	Inside	11	1

PI: isoelectric point; TMD: transmembrane domain; N-Term: location of N-teminus; %TM: % of transmembrane sequence ; Screens: Numl

# Purity of the membrane protein targets



#### Behavior of membrane protein targets in detergent

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



#### Making sure we know the detergent concentration of the membrane protein

#### Colorimetric detection

Condensation reaction of furfural derivatives (generated by dehydration of sugars in concentrated H2SO4) with aromatic molecules such as phenol > spectrophotometer

- Nasty reactants
- Only possible to use on glucosidic/maltosidic or bile-salt derived detergents.

#### TLC – Thin layer chromatography

Based on mobile phase and detection method, detergents and lipids can be both identified and quantified.

#### Drop shape

Detergents modify surface tension of aqueous solutions. Both shapes and contact angles of drops are affected.





#### 2D crystallization in parallel



#### Standard conditions for initial screen

Lipids: DMPC, DOPC, POPC, DOPG, E.coli polar lipid extract **pH:** 6.0 (20mM MES), 7.0 (20mM TES), 8.0 (20mM TES) LPRs: 0.25, 0.75, 1.5 **Cations (mM):**  $Na^+(100), Mg^{2+}(10)$ 

	1	2	3	4	5	6	7	8	9	10	11	12
	v	DOPC	POPC	DOPG	v	DOPC	POPC	DOPG	v	DOPC	POPC	DOPG
A	Λ	0.25	0.75	1.5	Λ	0.25	0.75	1.5	Λ	0.25	0.75	1.5
D	DMPC	DOPC	POPC	E.coli	DMPC	DOPC	POPC	E.coli	DMPC	DOPC	POPC	E.coli
D	0.25	0.75	1.5	0.25	0.25	0.75	1.5	0.25	0.25	0.75	1.5	0.25
C	DMPC	DOPC	DOPG	E.coli	DMPC	DOPC	DOPG	E.coli	DMPC	DOPC	DOPG	E.coli
C	0.75	1.5	0.25	0.75	0.75	1.5	0.25	0.75	0.75	1.5	0.25	0.75
n	DMPC	POPC	DOPG	E.coli	DMPC	POPC	DOPG	E.coli	DMPC	POPC	DOPG	E.coli
	1.5	0.25	0.75	1.5	1.5	0.25	0.75	1.5	1.5	0.25	0.75	1.5
Г	v	DOPC	POPC	DOPG	v	DOPC	POPC	DOPG	v	DOPC	POPC	DOPG
Ľ	Λ	0.25	0.75	1.5	Λ	0.25	0.75	1.5	Λ	0.25	0.75	1.5
F	DMPC	DOPC	POPC	E.coli	DMPC	DOPC	POPC	E.coli	DMPC	DOPC	POPC	E.coli
Ľ	0.25	0.75	1.5	0.25	0.25	0.75	1.5	0.25	0.25	0.75	1.5	0.25
	DMPC	DOPC	DOPG	E.coli	DMPC	DOPC	DOPG	E.coli	DMPC	DOPC	DOPG	E.coli
G	0.75	1.5	0.25	0.75	0.75	1.5	0.25	0.75	0.75	1.5	0.25	0.75
п	DMPC	POPC	DOPG	E.coli	DMPC	POPC	DOPG	E.coli	DMPC	POPC	DOPG	E.coli
	1.5	0.25	0.75	1.5	1.5	0.25	0.75	1.5	1.5	0.25	0.75	1.5

pH6.0, pH7.0, pH8.0 Rows A-D contain 100mM Na<sup>+</sup>; Rows E-H contain 10mM Mg<sup>2+</sup>

#### Making mixed micelles in the optimal detergent



# Negative staining on a 96-well format









#### Automated loading and imaging of specimens

Need to load and image 96 grids per crystallization block



# Automated loading and imaging of specimens



# Automated loading and imaging of specimens



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#### Leginon controls robot and 2DX imaging



LEGINON is a system designed for automated collection of images by TEM (NRAMM at Scripps)



# Define montage image area (e.g. 3x3)





Take a images at low mag

▼



Pick target squares using square finder (histogram criteria)



Take images of selected squares



Pick targets from square images (histogram criteria)



Take images at intermediate mag for evaluation



#### 2DX screening evaluation and outcome



Currently evaluation is carried out by an expert. We need to develop algorithms for automated evaluation of the screens.

# Laboratory Information Management System (LIMS)

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SW Cut	12	0	50	11.92		0	485: 1.0 M zinc chloride	pH 2	i mM	1.25	1	467: 1.0 M Na-citrate pH
OC CUR	10	0	50	18.58		0	478: 1.0 M magnesium	sulp 2	5 mM	1.25	1	467: 1.0 M Na-citrate pH
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# SESAME by Zsolt Zolnai at the University of Wisconsin-Madison

# After a long story, how did we perform?

Protein	Organism	(Putative) Function	MW (kDa)	pI	TMD	N-term	% TM	Screens	Best Outcome
E2P1	M.voltae	Protease	25.9	8.7	4	Inside	45	4	Sheets, Tubes
E2P2	M.maripaludis	Protease	25.9	5.0	4	Inside	39	3	Sheets, Tubes,
E2P3	M.marisnigri	Protease	31.8	5.3	7	Inside	59	2	Sheets, Tubes
E2P4	M.hungatei	Protease	32.9	7.8	9	Inside	61	2	Sheets, Tubes
P2A3	S.oneidensis	Cation efflux family protein	32.5	5.4	5	Inside	36	5	Helical crystals
Rhomboid PA3086	P.aeruginosa	Intramembrane protease	31.8	9.9	6	Inside	45	4	Lipidic structures
YkgB-D332	E.coli	Unknown	21.9	6.0	3	Inside	38	1	Tubes
YkgB-D36	E.coli	Unknown	21.8	5.7	3	Inside	37	4	Helical crystals
β1-adrenergic receptor	M.gallopavo	G-protein coupled receptor	54.1	9.3	7	Outside	33	3	Sheets, Tubes
Rhomboid GlpG	E.coli	Intramembrane protease	31.3	9.2	6	Inside	46	1	Aggregates
Cytochrome b561	P.aeruginosa	Electron carrier activity	20.6	9.6	4	Inside	47	1	Lipidic structures
E1 protein	Semliki Forest Virus	Inducer of membrane fusion	47.4	7.6	0(1)	Outside	5	4	Sheet crystals
P40B7	B.subtilis	Sporulation kinase C	48.8	6.3	2	Inside	10	1	Vesicles, Sheets
Kdp-ATPase (4 subunits)	E.coli	High-affinity K-pump	159.2	5.2-9.4	17	3 in, 1out	31	1	Sheets, Tubes
P39H10	K. pneumoniae	Diguanylate cyclase	45.7	6.5	2	Inside	11	1	Sheets, Tubes

PI: isoelectric point; TMD: transmembrane domain; N-Term: location of N-teminus; %TM: % of transmembrane sequence ; Screens: Number of screens

#### **Current developments**

# 1) Following on Remigy's steps



2) Do dialysis with much less protein sample: 0.3 mgs for 96 conditions





#### TRANSCONTINENTAL EM INITIATIVE FOR MEMBRANE PROTEIN STRUCTURE (TEMIMPS)

NIH funded:

PSI-Biology: Centers for Membrane Protein Structure Determination (U54)

Team of 6 investigators with extensive experience in 2DX of membrane proteins:

Stokes, David L. - NYU SoM/NYSBC - Program Director

Engel, Andreas - Case Western Reserve - Co-PI

Gonen, Tamir - Univ. of Washington - Co-PI

Love, James D. - NYCOMPS - Co-PI

Penczek, Pawel A. - Univ of Texas - Houston - Co-PI

Ubarretxena, Iban - Mount Sinai SoM - Co-PI

#### Goals:

- Solve structures of eukaryotic membrane proteins that are relevant for human health and disease.
- Study their molecular mechanisms in their natural lipid environment.
- Accelerate membrane protein structure determination from 2DX.

# Multiple positions available (http://temimps.nysbc.org/jobs/):

#### Acknowledgements

Changki Kim Minghui Hui Martin Vink David Stokes

NYSBC KD Derr Ruben Diaz Bill Rice

NRAMM Bridget Carragher Clint Potter Jim Pulokas Anchi Cheng

NIH R01 GM081817

NYCOMPS James Love Wayne Hendrickson

JKD instruments John Koss Kevin Damico

SESAME University of Wisconsin Zsolt Zolnia

GN Biosystems University of Wisconsin Jiang Huang