



2DX

2DX WORKSHOP 2016

August 23 - 26, 2016

Henning Stahlberg

C-CINA, Biozentrum, University of Basel

2DX WORKSHOP 2016



2DX

Workshop Organization

Nikhil Biyani

Ricardo Righetto

Robert McLeod

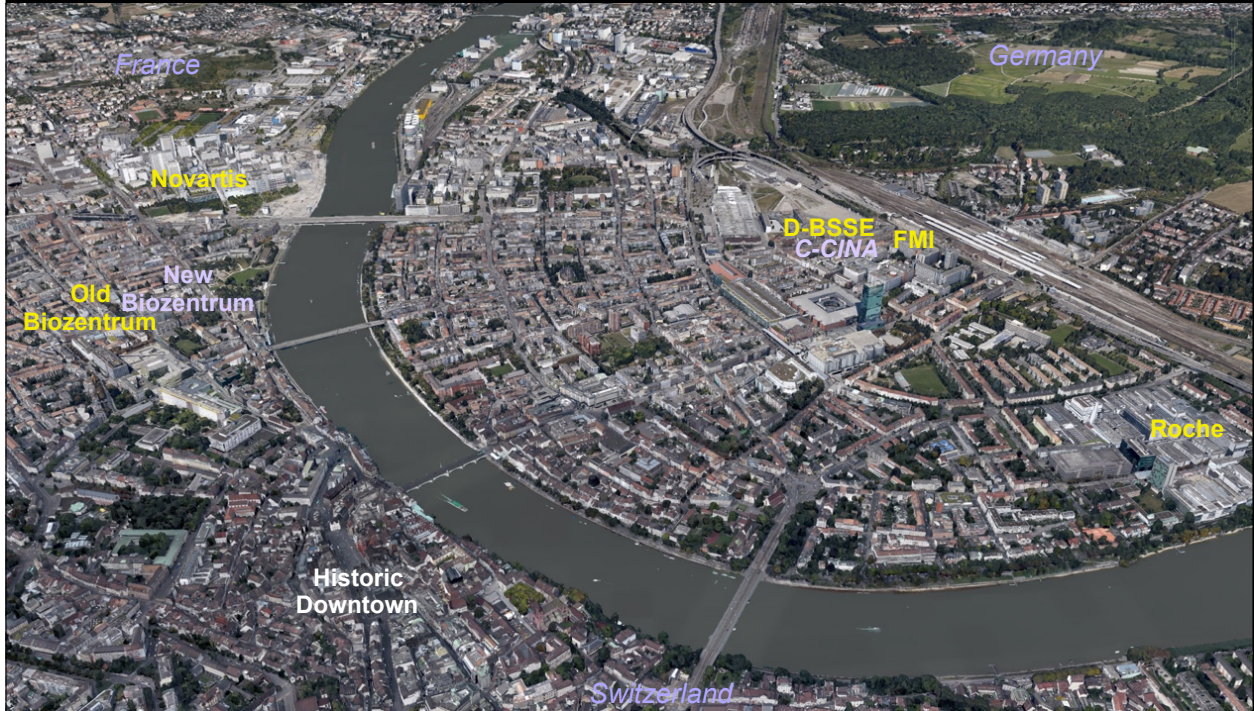
Henning Stahlberg

Organization

Karen Bergmann

Heidi Brönnimann

Basel



A Swiss City of Science at the border to Germany and France

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BIOZENTRUM
Universität Basel
The Center for Molecular Life Sciences

The University of Basel

- The University of Basel was founded in 1460, it is Switzerland's oldest University
- Rich in history:
 - Erasmus of Rotterdam
 - Paracelsus
 - Daniel Bernoulli
 - Jacob Burckhardt
 - Leonhard Euler
 - Friedrich Nietzsche
 - Carl Jung

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The Biozentrum, University of Basel



- The University of Basel was founded in 1460, it is Switzerland's oldest University
- The Biozentrum was founded in 1971, it is the largest department in the Faculty of Science at the University of Basel
- Budget of about 60 Mio CHF/yr
- Regularly more than 200 scientific publications/year, many in top-tier journals
- New home as of 2017

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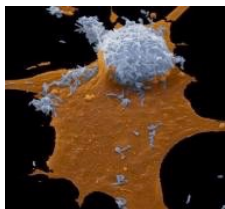
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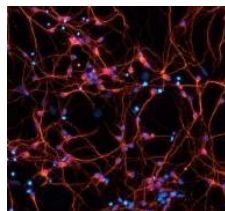
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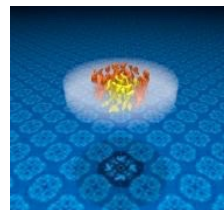
Growth
& Development



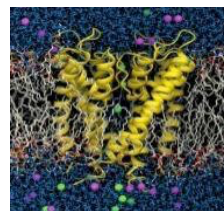
Infection Biology



Neurobiology



Structural Biology
& Biophysics



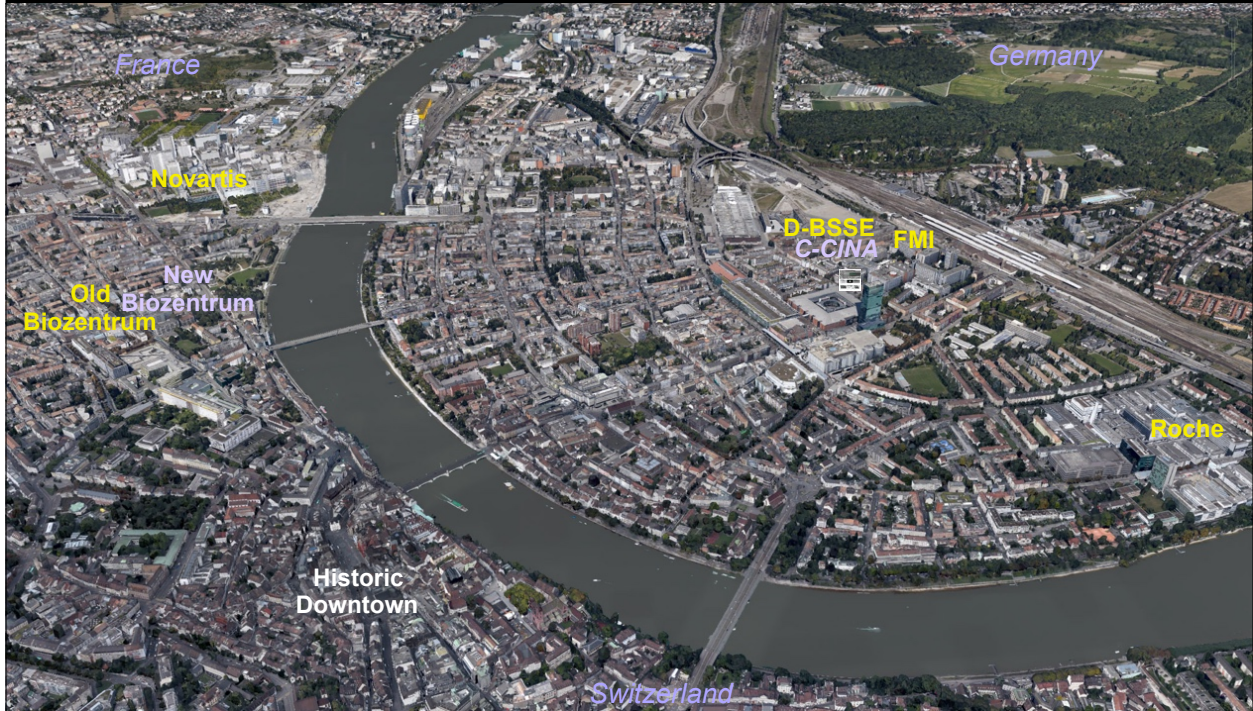
Computational
& Systems Biology

Searching to understand the fundamental mechanisms of life

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Basel



C-CINA is an outpost of the Biozentrum

C-CINA
The Center for
Cellular Imaging and NanoAnalytics

c-cina.org

Upcoming Conference:
ICON-2016
Nanoscopy Conference
June 2016

1 | 2 | 3 | 4 | 5 | 6

NEURODEGENERATION
Spreading of prionoid fibrils of proteins alpha-synuclein and tau are likely behind several neurodegenerative diseases...
[read more...](#)

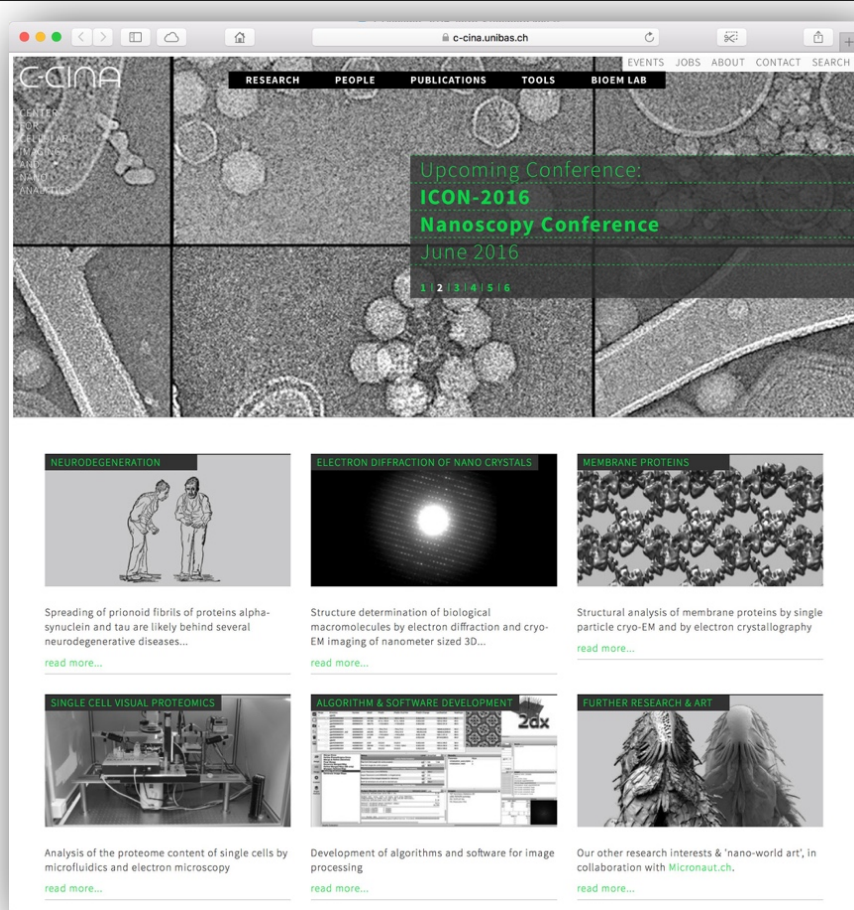
ELECTRON DIFFRACTION OF NANO CRYSTALS
Structure determination of biological macromolecules by electron diffraction and cryo-EM imaging of nanometer sized 3D...
[read more...](#)

MEMBRANE PROTEINS
Structural analysis of membrane proteins by single particle cryo-EM and by electron crystallography
[read more...](#)

SINGLE CELL VISUAL PROTEOMICS
Analysis of the proteome content of single cells by microfluidics and electron microscopy
[read more...](#)

ALGORITHM & SOFTWARE DEVELOPMENT
Development of algorithms and software for image processing
[read more...](#)


FURTHER RESEARCH & ART
Our other research interests & 'nano-world art', in collaboration with [Micronaut.ch](#).
[read more...](#)




c-cina.org

Electron Microscopes in C-CINA


FEI Quanta200/3View
Serial Block Face SEM



Zeiss Merlin/3View
Serial Block Face SEM




FEI Versa3D
Dual-Beam FIB-SEM




SEM

Philips CM100
100 kV, CCD
negative stain EM, sections




Philips CM10
100 kV, CCD
negative stain EM, sections




TEM


FEI T12
120kV, F416 CMOS
Cryo-EM screening




Philips CM200F
200kV, F416 CMOS
Cryo-EM




FEI Talos
200kV, Ceta 16M CMOS
Cryo-EM and STEM

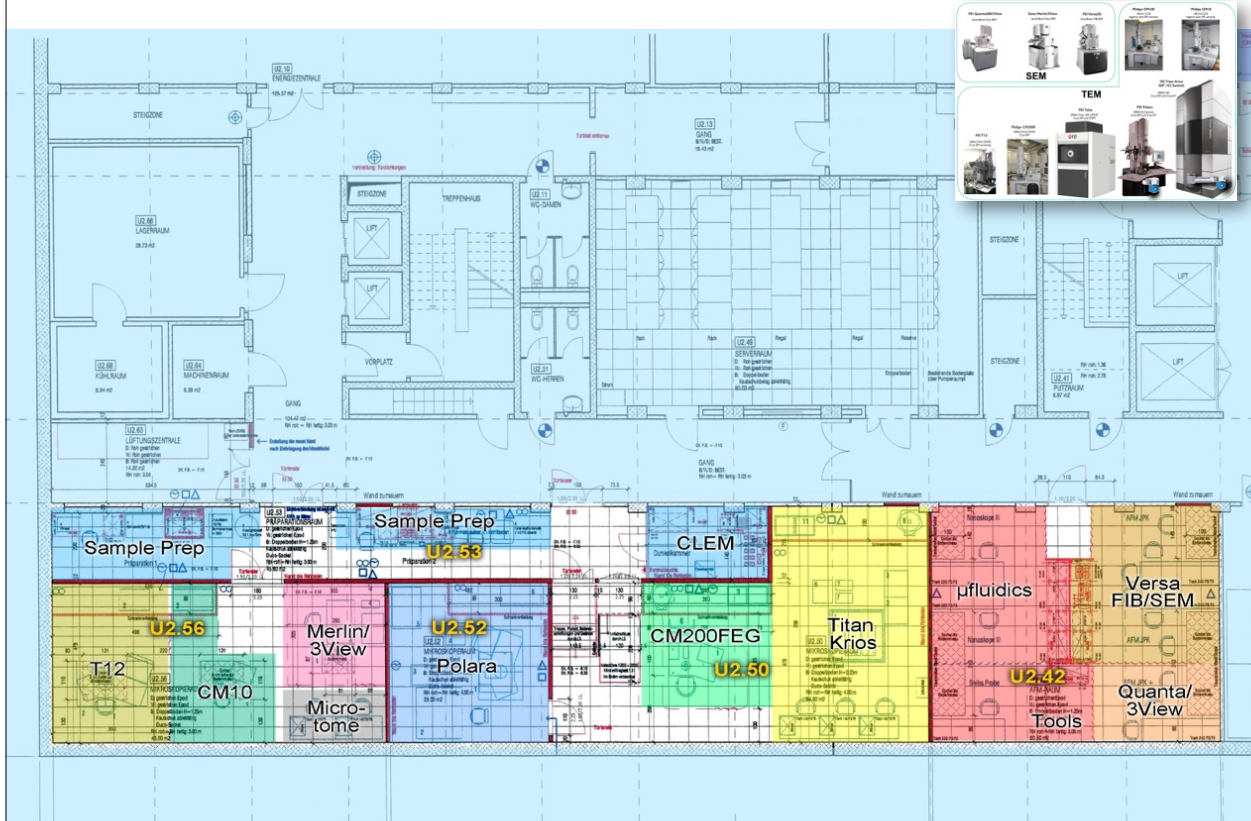


FEI Polara
300kV, K2 Summit
Cryo-EM and Cryo-ET



FEI Titan Krios
GIF / K2 Summit
300kV, GIF
Cryo-EM and Cryo-ET





C-CINA Suite
in New Biozentrum:
(2018)

In U-3 basement:

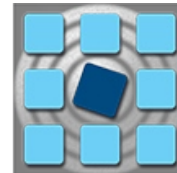


On 5th floor:

CM100
(100kV)

Versa3D
(FIB-SEM)

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Nikhil Biyani



Robb McLeod



Ricardo Righetto



Daniel Castaño-Diez



Karen Bergmann

BIOZENTRUM

Universität Basel
The Center for Molecular Life Sciences



Uni Basel



Expression



Purification



2D Crystallization



Sample Prep



Imaging










Image Processing















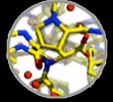
Model Building

Electron Crystallography

	1985	1995	2005	2015	2020
 Expression	Prokaryotic		Eukaryotic	Cell-Free	
 Purification			FPLC	Detergent Drop-Box	Liposomes
 2D Crystallization	Dialysis Machine		Automation		No Crystals
 Sample Prep	Sugar embedding		Carbon Sandwich	Holey Carbon cryo-EM	Automation
 Imaging	FEG	Stable Stages	High-quality Vacuum	Auto-mation Detectors	New Phase Plates Cs-Correct.
 Image Processing	MRC		2dx, IPLT	ML, PCO	Hybrid Processing
 Model Building		Modeling	MD refinement	Docking	Multi-Resolution Automation

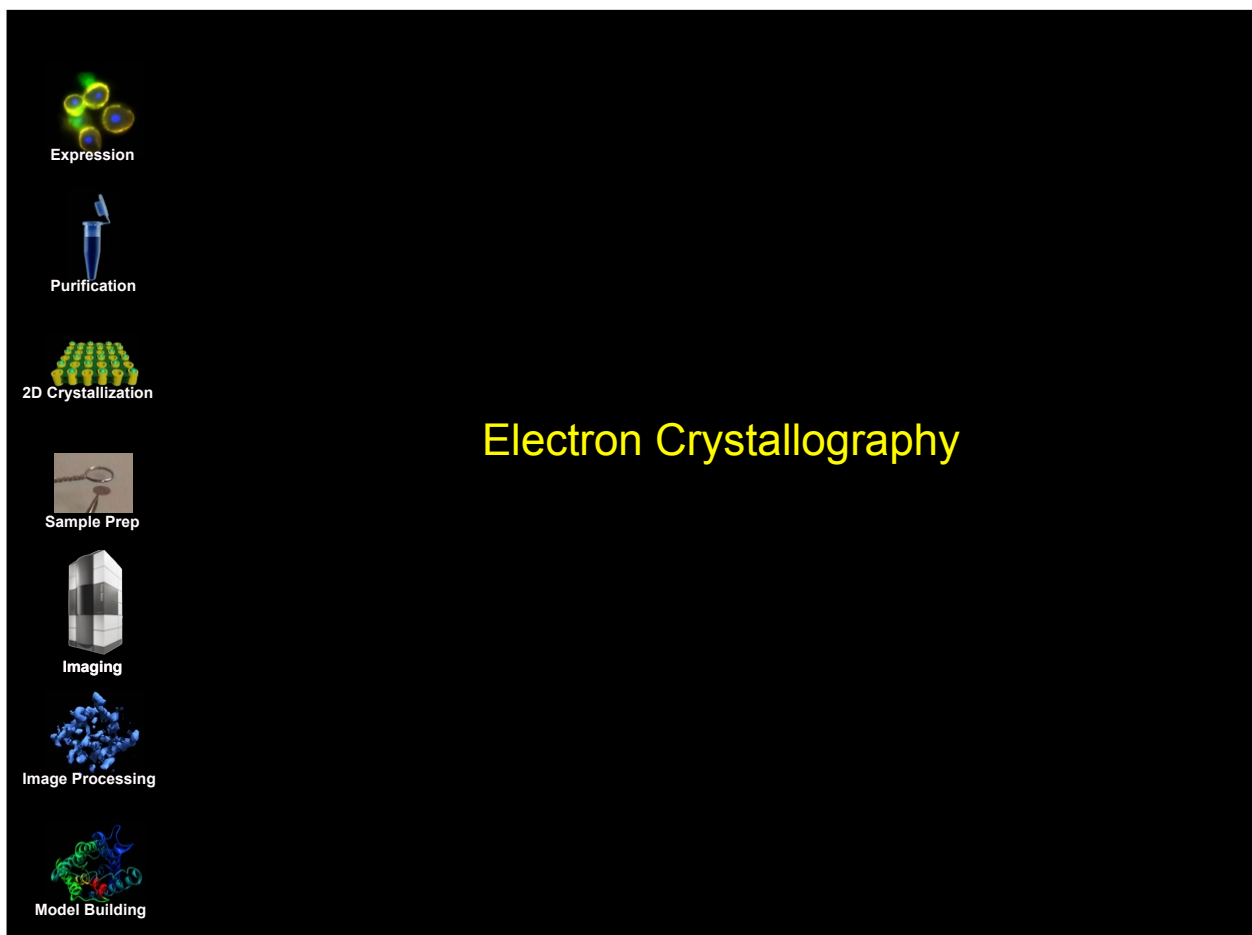
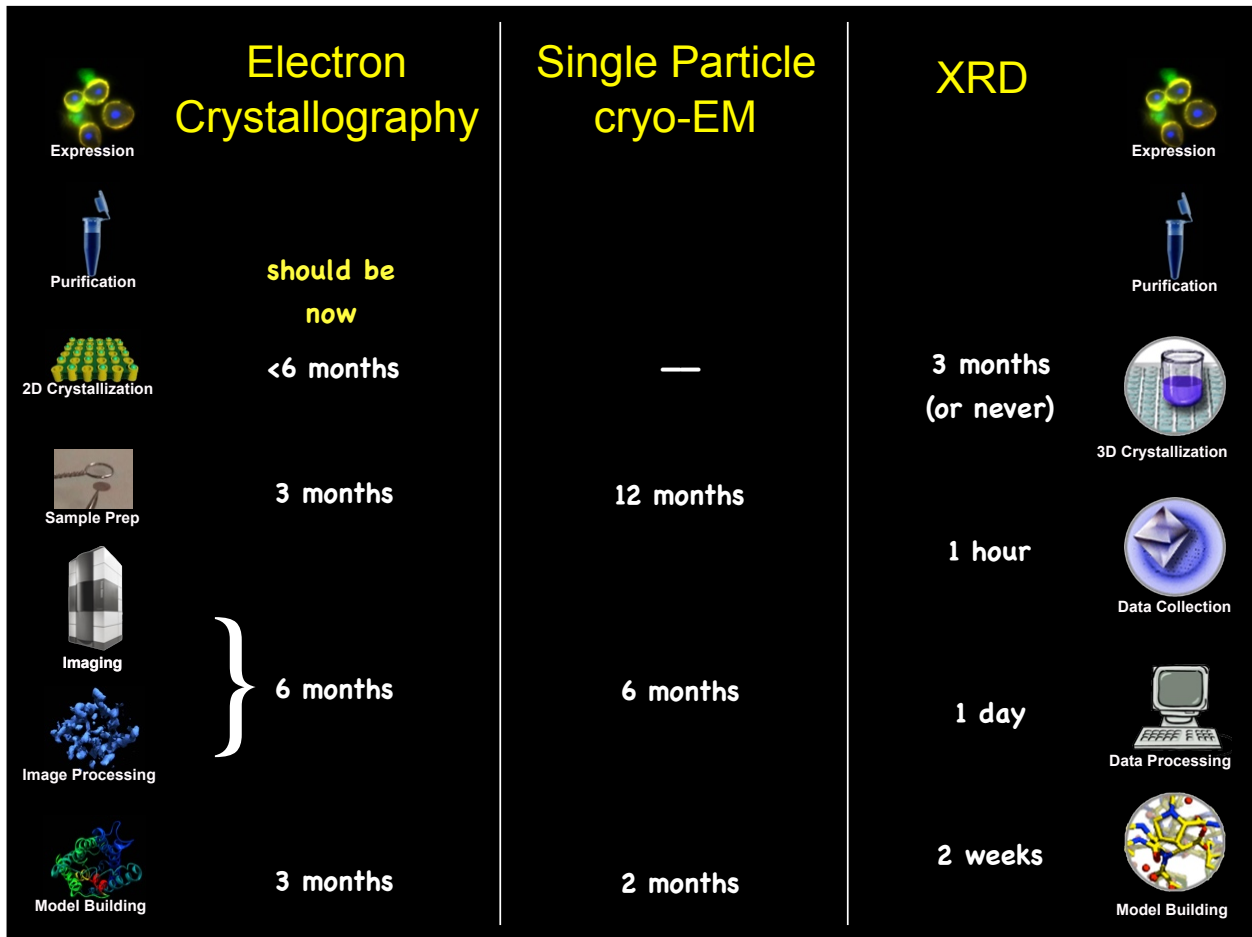
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	Electron Crystallography	XRD
 Expression		 Expression
 Purification		 Purification
 2D Crystallization	2 years	3 months (or never)
 Sample Prep	1 years	 3D Crystallization
 Imaging	} 4 years	1 hour
 Image Processing		1 day
 Model Building	6 months	 Data Collection
		 Data Processing
		 Model Building
	6 months	2 weeks
	3 months	
	3 months	
	3 months	

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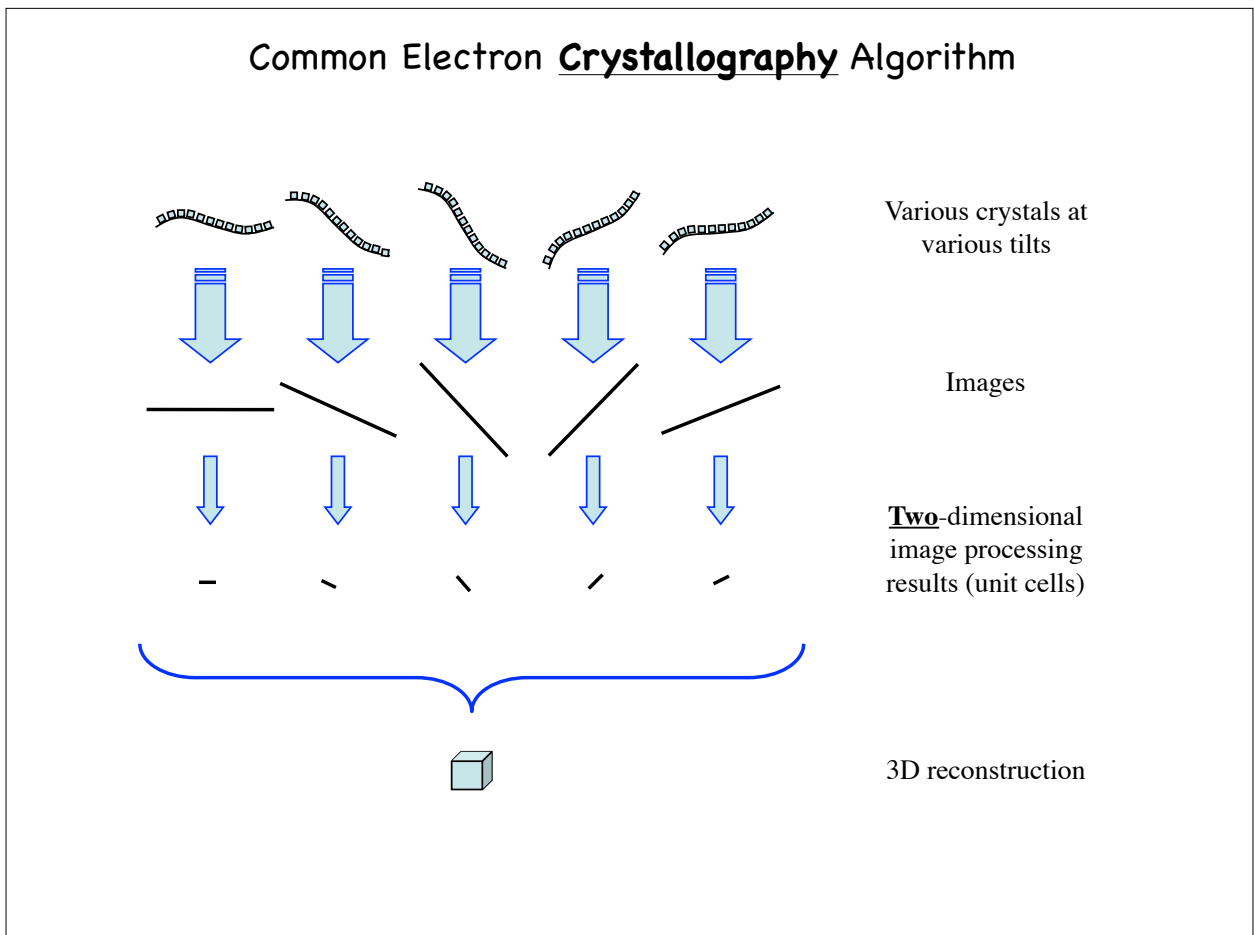
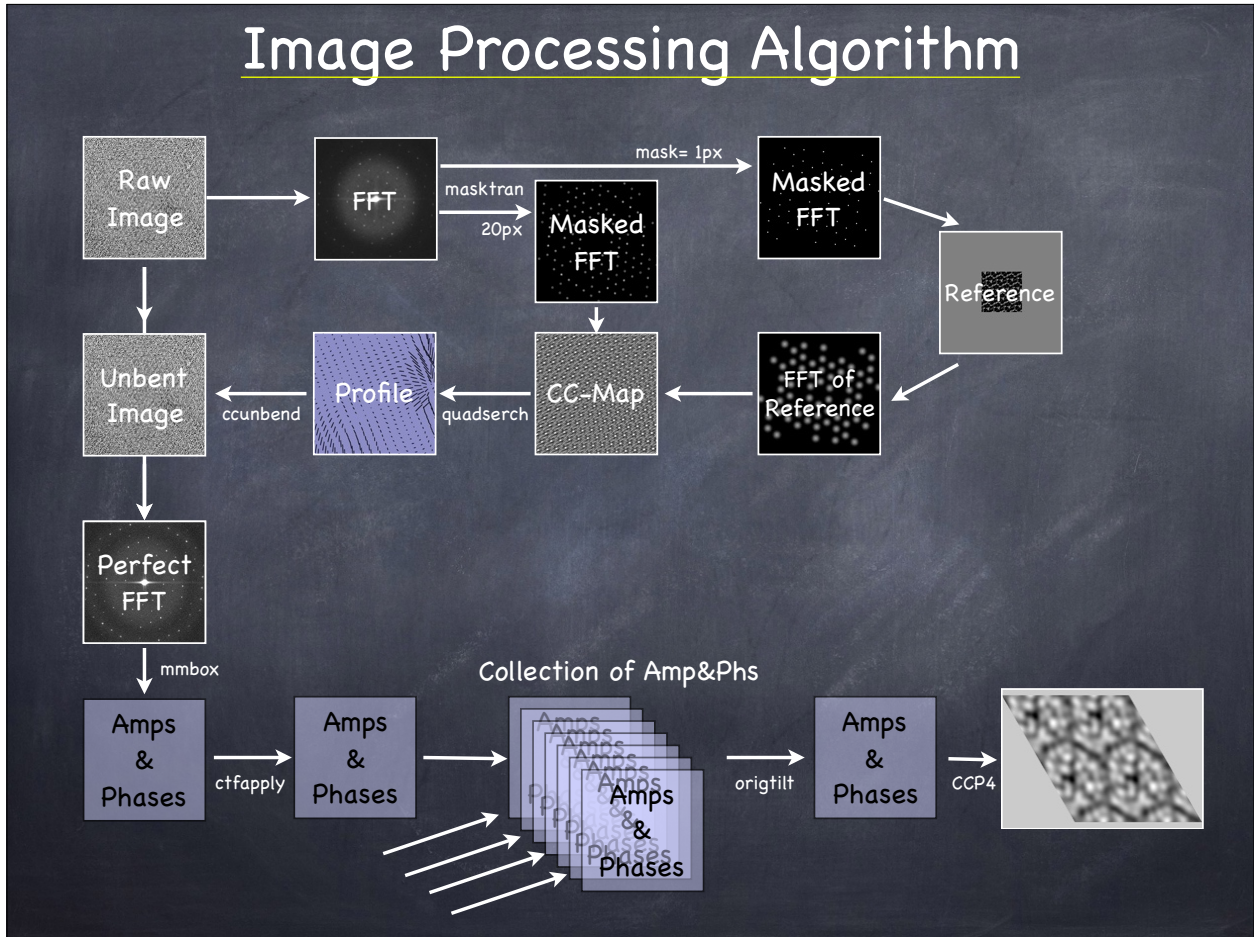


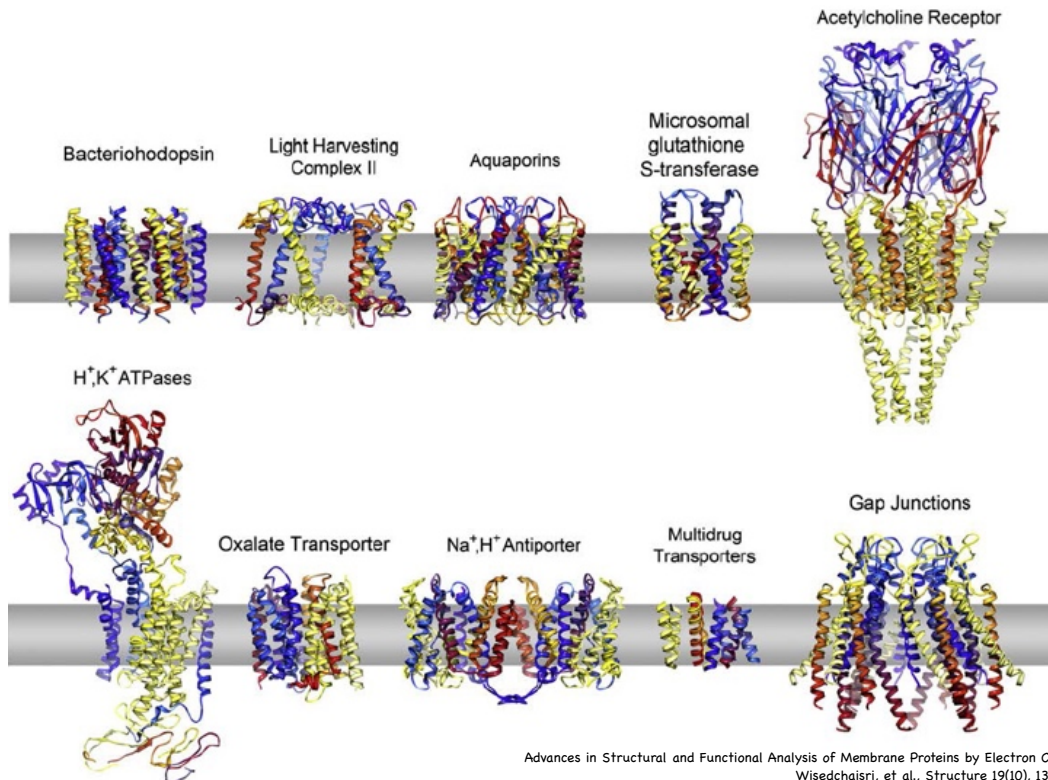
Image Processing Algorithm



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Membrane Protein Structures determined by Electron Crystallography



Advances in Structural and Functional Analysis of Membrane Proteins by Electron Crystallography
Wisedchaisri, et al., Structure 19(10), 1381-1393 (2011)

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Electron Crystallography Literature

MRC Software:

- Henderson & Unwin, Nature 257, 28-32 (1975)
- Amos & Henderson & Unwin, Prog. Biophys. molec. Biol. 29, 183-231 (1982)
- Henderson et al., J. Mol. Biol. 213, 899-929 (1990)

2dx Software: (2dx.org)

- Gipson et al., J. Struct. Biol. 157, 64-72 (2007)
- Gipson et al., J. Struct. Biol. 160(3), 375-384 (2007)
- Arbeit et al., In Book: *Electron Crystallography of Soluble and Membrane Proteins*, Methods in Molecular Biology, Vol. 955, Chapters 10, 11, and 18 (2013)

Reviews:

- Kühlbrandt: Quarterly Review of Biophysics 25(1), 1-49 (1992)
- J. Struct. Biol. Special Issue on Electron Crystallography: JSB 160(3) (2007).
- Book: Glaeser et al., *Electron Crystallography of Biological Macromolecules*, Oxford Univ. Press, USA, 476 pages (2007).
- **Abeyrathne et al., Comprehensive Biophysics Vol 1.19, 277-310 (2012)**
- **Book: Schmidt-Krey & Cheng (eds.): *Electron Crystallography of Soluble and Membrane Proteins: Methods and Protocols*, Methods in Molecular Biology Vol. 955 (2013)**

Electron Microscopy Analysis of 2D Crystals of Membrane Proteins

Priyanka D. Abeyrathne¹, Marcel Arbeit¹, Fabian Kebbel¹, Daniel Castano-Diez¹,
Kenneth N. Goldie¹, Mohamed Cham¹, Ludovic Renault², Werner Kühlbrandt^{3,*}, and
Henning Stahlberg^{1,*}

Comprehensive Biophysics 1.19 (2012)

1 Tables

Table 1. Structures of membrane proteins analyzed by electron crystallography, and the protein production, purification, and crystallization conditions. Future, updated versions of this table will be maintained at <http://2dx.org>. Crystallization Method: DI = Dialysis; BB = Biobeads; LM = Lipid Monolayer; SP = Salt Precipitation; FU = Fusion.

Family	Protein	Resolu- tion 2D [Å]	Resolu- tion 3D [Å]	PDB/ EMD	Origin	Expressed in	Conc [mg/ml]	Lipid added	LPR [w/w]	Detergent	T [°C]	pH	Salts [mM]	Non- ionic agents [%]	Time	Crystalliza- tion Method	Crystal type	Reference
Ion Channels	AmbB - Ammonium gas ion channel	12			<i>E. coli</i>	<i>E. coli</i>	0.4	DMPC	1	DM	20	8	250 NaCl, 0.6 Na ₂ S ₂ O ₈	-	10d	DI	sheets	(Conroy et al., 2004)
	Annexin A5 - Ca ²⁺ -specific ion channel	6.5			Rat	<i>E. coli</i>	0.1	DOPC / DOPS	-	none	20	7.4	150 NaCl, 2 CaCl ₂ , 3 Na ₂ S ₂ O ₈	-	3-4 d	LM	sheets	(Oling et al., 2000)
	MscL - mechanosensitive ion channel	15			<i>E. coli</i>	<i>E. coli</i>	1	<i>E. coli</i> lipid	0.45	Triton X-100	4	8	100 KCl	-	5 h	BB	vesicle	(Saint et al., 1998)
	VDAC - voltage dependent anion channel	18			Potato											-	tubules	(Hoogenboom et al., 2007)
Potassium Channels	KcsA potassium channel	6			<i>S. lividans</i>	<i>E. coli</i>	1-2	DMPC / Sodium cholate	-	DDM	RT	7.5	100 KCl, 1 EDTA	-	3 d	DI	-	(Li et al., 1998)
	KirBac3.1 potassium channel	9			<i>M. magnetotacticum</i>	<i>E. coli</i>	1	DOPC	0.6-1	DM	20 / 37	8	100 KCl, 3 Na ₂ S ₂ O ₈ , 75 MgCl ₂	-	7 d	BB	sheets	(Kuo et al., 2005)
	MioK1 - Cyclic Nucleotide-Modulated K-Channel	16			<i>M. loti</i>	<i>E. coli</i>	0.5	<i>E. coli</i> lipid		DM	25 / 37	6.7	20 KCl, 1 BaCl ₂	-	5 d	DI	sheets	(Chiu et al., 2007)
Ion Antiporters	CIC-ec1 - chloride proton antiporter from <i>E. coli</i>	6.5			<i>E. coli</i>	<i>E. coli</i>		POPC	0.4	DM	4	7	25 NaCl, 20 MgCl ₂ , 0.8 Na ₂ S ₂ O ₈	-	several days	DI	sheets	(Mindell et al., 2001)
		4			<i>E. coli</i>	<i>E. coli</i>	0.8	<i>E. coli</i> lipid	0.2-0.5	DDM	37	4	25 KAc, 150 KCl, 0.1 GdCl ₃ , 3 Na ₂ S ₂ O ₈	5-10 glycerol	4-6 d	DI	tubes	(Williams et al., 1999)
	NhaA - Na ⁺ - H ⁺ antiporter from <i>E. coli</i>	7			<i>E. coli</i>	<i>E. coli</i>	0.5	<i>E. coli</i> lipid	0.2-0.5	DDM	37	4	25 KAc, 150 KCl, 0.1 GdCl ₃ , 3 Na ₂ S ₂ O ₈	5-10 glycerol	4-6 d	DI	tubes	(Williams, 2000)
		7		3F11	<i>E. coli</i>	<i>E. coli</i>	0.5	<i>E. coli</i> lipid	0.2-0.5	DDM	37	4	25 KAc, 150 KCl, 0.1 GdCl ₃ , 3 Na ₂ S ₂ O ₈	5-10 glycerol	4-6 d	DI	tubes	(Appel et al., 2009)
	NhaP1 - Na ⁺ - H ⁺ antiporter from <i>M. jannaschii</i>	6			<i>M. jannaschii</i>	<i>E. coli</i>	1	<i>E. coli</i> lipid	0.4-0.55	DDM	37	4	200 NaCl, 25 Acetate	10 glycerol	5-7 d	DI	tubes	(Vinothkumar et al., 2005)
		7			<i>M. jannaschii</i>													tubes
	TetA - secondary tetra-cycline transporter	17			<i>E. coli</i>		1	DMPC/POPC	0.5-1.5	DDM (lipids in ratio)		7.4	10 Tris, 150 NaCl, 40 MgCl ₂			DI		(Yin et al., 2000)

	22 August 2016 MONDAY	23 August 2016 TUESDAY	24 August 2016 WEDNESDAY	25 August 2016 THURSDAY	26 August 2016 FRIDAY
09:00		Introduction to Workshop, Henning Stahlberg	2D Crystallization, Philippe Ringler	Electron Diffraction of NanoCrystals, Jan Pieter Abrahams	Single Particle Processing from 2D Crystals, Ricardo Righetto
09:15					
09:30		Electron Microscope: The Hardware, Eric van Genderen	Sample Preparation for Cryo-EM of membrane proteins, Mohammed Chamli	Merging in 2D, Henning Stahlberg	Applications of 2D Crystallography, Cristina Paulino
09:45					
10:00		Fourier Transforms, Henning Stahlberg	Lattice Determination and tilt Geometry, Henning Stahlberg		
10:15					
10:30		Coffee Break - 10:25-10:45 (C-CINA near Football Table)			
10:45		Fourier Transforms Applications, Henning Stahlberg	Crystallographic Symmetries, Henning Stahlberg	Merging in 3D, Henning Stahlberg	Tools from Dynamo, Daniel Castano-Diez
11:00					
11:15		CTF Correction, Henning Stahlberg	Crystal Unbending, Henning Stahlberg	Missing Cone, Nikhil Biyani	MloK1, Julia Kowal
11:30					
11:45		Introduction to Motion Correction and ZORRO, Robert McLeod	Movie Mode Unbending, Henning Stahlberg	Quality Evaluation, Henning Stahlberg	Tips for highest resolution data, Henning Stahlberg
12:00					
12:15				Group Photo (C-CINA)	Discussion
12:30		Lunch (Canteen SV)			
14:00		Introduction to 2DX (NB)	Get Lattice (HS)	2D Merging (HS)	Porting project to RELION/FREALIGN (Ricardo Righetto)
14:30		Image Processing Pipeline (NB)	Get Defocus (HS)	Refine Phase Origins (HS)	
15:00		Project Initialization (NB)	Refine Lattice (HS)	3D Merging (HS)	
15:30		External softwares (NB)	Unbending I & II (HS)	Generate Image Maps (HS)	
16:00		Coffee Break - 16:00-16:30 (C-CINA near Football Table)			
16:30	Arrival/ Optional Software Installation (C-CINA)	FFT/Periodogram (HS)	Movie Mode Unbending (HS)	Selecting good images (NB)	Participant Dataset/ C-CINA Guided Tour
17:00		CTF/Thon Rings (HS)	CTF Correction (HS)	Missing cone (NB)	
17:30		Automated Pipeline (HS)	Generate Map (HS)	Quality Evaluation (NB)	
18:00		Dinner (Science Lounge)		Workshop Dinner	Departure
19:30		Poster Presentation #1	Poster Presentation #2		