



# Lecture 7, BCH 8102, 2021 Winter

Paculté de médecine

Membrane Protein Structural Biology: Methods and Applications

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# **Lecture Outline**

#### Part I: Technology

- a) Importance of membrane proteins
- b) A brief history
- c) Where to start?
- d) Old techniques and new discovery
- e) New methodology and old problems

#### **Part II: Structural Determination**

- a) X-ray crystallography: bolts and nuts
- b) Cryo-electron microscopy: bolts and nuts





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# Part I: TECHNOLOGY



### Importance of membrane proteins

- Encoded by some 20-30% genes in typical genome.
- Major components of the mosaic lipid bilayers in cellular membranes
- Mediate cell-to-cell communication and signaling events.
- Disruptions or mutations in humans have been implicated in diseases, such as cardiovascular and metabolic diseases, cancer, rare genetic diseases, ...



#### **Human Membrane Proteome**



#### Half Drug Targets are Membrane Proteins.



(Yildirim et al, Nat Biotech, 2007)





#### **Drug Discovery Work Flow**



(Yin & Flynn, Annu Rev Biomed Eng, 2016)





(Yin & Flynn, Annu Rev Biomed Eng, 2016)



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### **Structural Biology**

- Understanding biology by examining three dimensional (3-D) molecular architectures and their changes.
- Learning life in action with the eyes of atoms: chemical and physical properties of biological matters.
- Structures of biological molecules determine their functions.

Central dogma: Sequence → Structure → Function



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#### **Atomic/Near-Atomic Models of Proteins (2021-3-7)**





#### Atomic/Near-Atomic Models of Membrane Proteins (2021-3-7)





### A brief history: some "atomic" milestones



## Where to start?

- Challenges
- Things to consider
- Right ways to address right questions
- Optical basics





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#### Challenges in Membrane Protein Structural Biology

- Naturally occurred proteins exist in low abundance, with only a few exceptions (*e.g.*, bacteriorhodopsin or aquaporin), and form complexes.
- *E. coli* is often not suitable for producing recombinant membrane proteins of eukaryotic origins.
- No so-called standard protocol of protein extraction, largely due to the complexity of protein-lipid interaction.
- Protocols of purification, crystallization, and *in vitro* reconstitution remain empirical for individual cases.



### Choosing the appropriate expression hosts for recombinant proteins.



E. coli



Sf9 insect cells

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(Zorman et al, Curr Opin Struct Biol, 2015)



**Budding yeast** 





#### Things to consider for membrane protein extraction and purification.

- Cell disruption
- Solubilization agent
  - Detergents
  - Polymers
- Protein engineering
- Column chromatography
- In vitro reconstitution





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#### Ways to study membrane protein structures: optics & spectroscopy.





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#### **Optical diffraction & X-ray diffraction.**



If F(X)=FT[f(x)], then f(x)=IFT[F(X)]

where FT=Fourier transform & IFT=Inverse Fourier transform.

Note: important in X-ray crystallography and 3D reconstruction algorisms.



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### X-ray Crystallography: $\leq$ 3.0



Neuropeptide Y Y<sub>2</sub> Receptor (Tang et al, Nat Comm, 2021)

W116\*\*\*
V110\*\*\*
V110\*\*\*
V110\*\*\*
V110\*\*\*
F307\*\*\*
F307\*\*\*\*
F307\*\*\*
<

Protein-ligand interaction



Drug discovery/design



### Nuclear magnetic resonance (NMR): small/median MP





**Protein dynamics** 



### Cryo-electron microscopy (cryo-EM): ≥ 100 kD



Human D1 and D2 Dopamine Receptors (Zhuang et al, Cell, 2021)

- Ligand/drug-binding sites/kinetics
- Receptor signaling mechanism



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#### Mass spectrometry: native conditions



Native Mass Spestrometry (Calabrese & Radford, Methods, 2018)



Molecular dynamics (MD) simulation: membrane-embedded MP



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(Goossens & Winter, J Chem Inf Model, 2018)



### Lipid cubic phase (LCP) / microdiffraction



(Caffrey, Acta Cryst F, 2015)



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#### Bacteriorhodopsin

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nloaded by Universi

Bacteriorhodopsin

Cholestero

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#### Bacteriorhodopsin (Birefringence)

Lysozyme

#### Bacteriorhodopsin (X-ray damaged)



( Cherezov & Caffrey, Faraday Discuss, 2007)

### Antibodies: antibody fragment (F<sub>ab</sub>)



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(Hino et al, Curr Opin Struct Biol, 2013)



A<sub>2A</sub> adenosine receptor (A<sub>2A</sub>AR) G-protein coupled receptor)

(Hino et al, Nature, 2012)



#### Antibodies: single-chain nanobody





*Camelidae antibodies* (Muyldermans, Annu Rev Biochem, 2013) (Brunner & Schenck, Methods Mol Biol, 2020)

(Brunner et al, eLife, 2020)



### **Protein engineering: fusion proteins**



 $\beta$ 2-adrenergic receptor ( $\beta$ 2-AR) (Rosenbaum et al, Science, 2007)





### **Reconstitution: nanodiscs**



( Denisov & Sligar, Nat Struct Mol Biol, 2016)



TRPM4 (Autzen et al, Science, 2018)



### **Reconstitution: styrene maleic acid lipid particles (SMALPs)**

SMA copolymers SMA-ED SMA-SH SMAd.4 SMA-QA MA-related copolymers 2SM/

(Overduin & Esmaili, SLAS Discovery, 2009)





### **Direct detector: X-ray**

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(Föster et al, Philos Trans A Math Phys Eng Sci, 2019)



#### (https://www.dectris.com/features/featurespilatus3-r/high-dynamic-range/)



### **Direct detector: cryo-EM**



(Koning et al, Annals Anatomy, 2018)



(Yifan Cheng, Cell, 2015)



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### Motion correction / phase plates: cryo-EM



(Yifan Cheng, Cell, 2015)



(Danevet & Baumeister, Curr Opin Struct Biol, 2017)



# Part II: STRUCTURAL DETERMINATION



# X-ray crystallography: bolts and nuts

- Protein preparation
- Crystallization
- Data collection
- Data processing
- Model building




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# Crystallization



(Ghosh et al, Nature Rev Mol Cell Biol, 2015)





(Naomi Chayen, Curr Opin Struct Biol, 2004)

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# Crystallization



(Ghosh et al, Nature Rev Mol Cell Biol, 2015)







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# Crystallization

### In meso lipid cubic phase

![](_page_43_Figure_3.jpeg)

(Ghosh et al, Nature Rev Mol Cell Biol, 2015)

![](_page_43_Picture_5.jpeg)

Bicontinuous cubic phase

![](_page_44_Figure_2.jpeg)

(Caffrey, Acta Cryst F, 2015)

![](_page_44_Picture_4.jpeg)

### **Data Collection**

![](_page_45_Picture_2.jpeg)

(Warren et al, in "The Next Generation in Membrane Protein Structure Determination", 2016)

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## **Data Collection**

![](_page_46_Picture_2.jpeg)

1s exposure

10s exposure

![](_page_46_Picture_5.jpeg)

![](_page_46_Picture_6.jpeg)

(Cherezov & Caffrey, Faraday Discuss, 2007)

# **Data Processing**

XDS

### HKL 2000/3000

HKL2000 v0.98.691g Package Licensed to S. Michi •	
HKL2000	XDSGUI 2018-06-08 running in /nethomes/nho
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Select the detector that describes your experimental site	Folder with XDS configuration and output files Definition in the current directory. The title bar of the XDSGU
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Ok New Site Exit Package Licensed	
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S. Michael Soltis at Stanford Synchrotron Radiation Laboratory	
Colaborative license – Academic Research only	/nethomes/nbome/matthew/Documents/CSHL/data/lipoprote

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Project

### Package Licensed to Iwona Minor at HKL Research, Inc.

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![](_page_48_Picture_5.jpeg)

### Inspection of diffraction data / Preparation for data merging

![](_page_49_Picture_2.jpeg)

![](_page_49_Figure_3.jpeg)

![](_page_49_Picture_4.jpeg)

ling

### Space group / Scaling / Integration

![](_page_50_Picture_1.jpeg)

![](_page_50_Picture_2.jpeg)

![](_page_50_Picture_3.jpeg)

Project	Data	Summary	Index	Strategy	Integrate	Scale	Multi	Macros	Credits	Copyrigh	ts
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<b>Crystal Rota</b>	ation Z:			14.759	0.000	0.003
Detector Ro	tation X:			0.052	-0.004	0.015
Detector Ro	tation Y:			0.054	0.007	0.014
Detector Ro	itation Z:					
Crossfire X:				-0.048	0.000	0.007
Crossfire Y:				0.003	0.002	0.009
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Distance:				177.729	-0.104	0.064
Mosaicity:				0.770	0.003	0.010

![](_page_51_Figure_7.jpeg)

![](_page_51_Picture_8.jpeg)

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reading from a file: B3 1 0020.x reading from a file: B3 2 0001.x reading from a file: B3 2 0002.x reading from a file: B3 2 0003.x reading from a file: B3 2 0004.x reading from a file: B3 2 0005.x reading from a file: B3 2 0006.x reading from a file: B3 2 0007.x reading from a file: B3 2 0008.x reading from a file: B3 2 0009.x reading from a file: B3 2 0010.x reading from a file: B3 2 0011.x reading from a file: B3 2 0012.x reading from a file: B3 2 0013.x reading from a file: B3 2 0014.x reading from a file: B3 2 0015.x reading from a file: B3 2 0016.x reading from a file: B3 2 0017.x reading from a file: B3 2 0018.x reading from a file: B3 2 0019.x reading from a file: B3\_2\_0020.x reading from a file: B3 2 0021.x reading from a file: B3 2 0022.x reading from a file: B3 2 0023.x reading from a file: B3 2 0024.x reading from a file: B3 2 0025.x reading from a file: B3 2 0026.x reading from a file: B3 2 0027.x reading from a file: B3 2 0028.x reading from a file: B3 2 0029.x reading from a file: B3 2 0030.x

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![](_page_52_Picture_4.jpeg)

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### **Output / Statistics**

			Crystal -	Global Refinen	nent		
	Space Group		P21212				
	Resolution		50.00 -	1.89			
	a	30.78	± 0.01	α	90	±	
	b	60.72	± 0.01	β	90	±	
	C	75.44	± 0.01	Y	90	±	
	Cell Volume	140	989.5				
	Mosaicity Ran	ige 0.64	1-0.75				
			Glo	bal Statistics			
Error Scale Factor			U	619 Total I	Number of Reflect	tions	68932
Error Systematic			0.	034 Numb	er of Reflections	Marked for	Rejection 33
Radiation Damage			0,	016 Perce	ntage of Reflection	ons Marked	for Rejection
Anomalous Signal			0.	038 Perce	ntage of Reflection	ons Rejecte	d 0
Internal Non-isomor	phicity		0.0	005 Total I	Rmerge / Rmeas	/ Rpim	0.033 / 0.036 / 0.014

![](_page_53_Figure_3.jpeg)

![](_page_53_Picture_4.jpeg)

![](_page_54_Figure_0.jpeg)

**Output / Statistics** 

![](_page_54_Figure_2.jpeg)

![](_page_54_Picture_3.jpeg)

# **Model Building**

### REFMAC

		العار
		Hel
Job this Do restrained refinement using no prior phase informatio fuput fixed TLS parameters Cycle with arp_waters to analyse solvent model (uses ald version of ARP/wARP Run Cootfindwaters to automatically add remove waters to refined structure Generate weighted difference maps files in CCP4 if format	ni	nput
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### PHENIX

Projects	Favorites					
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Nov2015_PNF         Apr 09 2018 08:37         27         0.3143           Oct2014_5_repr         Apr 09 2018 08:35         240         0.2910           A8C001         Apr 05 2018 09:12         0	Phaser-MR (simple one-component interface) Automated molecular replacement with Phaser-lave only for structures with a single type of component					
	성 영 MRage – automated pipeline 한국 Integrated model identification, preparation, and parallel MR search					
	MR-Rosetta AutoMR combined with Rosetta model improvement and AutoBuild for difficult survetures.					
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	Create ensemble of models for motecular replacement					
	Model building					
	Refinement					
	Cryo-EM					
	Validation					
	Ligands					
	Reflection tools					
	Maps					
	Model tools					
	PDB Deposition					
	Other utilities and Program search					

![](_page_55_Picture_6.jpeg)

1. Fitting the electron density to build a model

![](_page_56_Picture_2.jpeg)

The next step is to fit the amino acid sequence of the protein into the electron density map. You do this "by hand" *in silico*, i.e. using a computer.

Once you have a reasonable fit – i.e. a starting model - you then use computer programs to rotate bond angles, adjust bond lengths, etc. to improve fit between the amino acid sequence and the electron density to arrive at the final protein model.

![](_page_56_Picture_5.jpeg)

## **Model Building**

![](_page_57_Figure_2.jpeg)

![](_page_57_Picture_3.jpeg)

![](_page_57_Picture_4.jpeg)

- 2. Parameters used to assess accuracy of a protein model
  - ii) R-factor (accuracy of "fit")

![](_page_58_Figure_3.jpeg)

![](_page_58_Picture_4.jpeg)

2. Parameters used to assess accuracy of a protein model

ii) R-factor (accuracy of "fit")

![](_page_59_Figure_3.jpeg)

$$R = \frac{\sum ||F_{\rm obs}| - |F_{\rm calc}||}{\sum |F_{\rm obs}|}$$

![](_page_59_Picture_5.jpeg)

2. Parameters used to assess accuracy of a protein model

![](_page_60_Figure_2.jpeg)

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2. Parameters used to assess accuracy of a protein model

iii) Cross Correlation (CC<sub>1/2</sub>)

![](_page_61_Figure_3.jpeg)

CC<sub>1/2</sub> between intensity estimates from half data sets. Primary indicator for use for selecting high resolution cutoff for data processing. Is related to the effective signal to noise of the **data** 

wa

2. Parameters used to assess accuracy of a protein model

iv) B-factor

![](_page_62_Picture_3.jpeg)

Even in a high resolution electron density map, some atoms have tight density around each atom (often in the protein core), while others have "smeared" electron density (often on the protein surface). The smearing of density can arise due to different factors, including movement of the side chain in the crystal. The B-factor or "temperature" fact defines the amount of smearing and thus how accurately one can define the position of a side chain.

2. Parameters used to assess accuracy of a protein model

iv) B-factor

![](_page_63_Figure_3.jpeg)

B-factor values of less than 30 Å<sup>2</sup> signify confidence in an side chains position due to tight electron density, while a temperature value of greater than 60 Å<sup>2</sup> signifies disorder due to smearing of the density. The Protein Data Bank has plots of B-factor versus residue number. Residues on the surface of proteins tend to have higher B-factors!

- 2. Parameters used to assess accuracy of a protein model
  - v) Ramachandran Plots

![](_page_64_Figure_3.jpeg)

ensure that residues are found within the "allowed regions".

### "Table 1" of structural biology works

Extended Data Table 1 | Data processing and refinement statistics

24.5 / 32.9

18151

0.010

1.64

 $R_{\text{work}}/R_{\text{free}}$ 

No. atoms

Protein R.m.s deviations Bond lengths (Å)

Bond angles (°)

	Native*	$[PW_{12}O_{40}^{3}]^{\dagger}$	[(CH <sub>3</sub> ) <sub>3</sub> Pb <sup>-</sup> ]	$(Ta_6Br_{12}^{2+})$	-	
Data collection					-	
Beamline	19-ID-D/23-ID-D <sup>‡</sup>	19-ID-D	19-ID-D	19-ID-D		
Space group Cell dimensions	I 222	I 222	I 222	I 222		
a, b, c (Å)	173.6, 224.8, 253.3	175.5, 227.5, 254.5	$174.6, 225.9, 253.4^{\$}$ $173.6, 225.9, 252.7^{\parallel}$	176.0, 228.0, 253.7	a	b 💉 🖗 🤊
Resolution (Å)	50-3.9 (3.93-3.9)	50-5.0 (5.04-5.0)	50-4.5 (4.54-4.5)	50-5.0 (5.04-5.0)	Contraction of the second	
$R_{\rm sym}$ or $R_{\rm merge}$	16.1 (NA)	13.5 (33.5)	8.7 (NA) <sup>§</sup> 7.1 (NA) <sup>∥</sup>	8.8 (NA)	E SECTION	**
<i>/&lt;0I&gt;</i>	8.8 (0.15)	5.1 (1.4)	$\begin{array}{c} 8.0 \ (0.45)^{\$} \\ 6.1 \ (0.18)^{\parallel} \end{array}$	8.6 (0.50)		
Completeness (%)	99.4 (84.2)	94.4 (47.3)	97.4 (55.9) <sup>§</sup> 94.7 (54.7) <sup>∥</sup>	81.1 (18.3)	A THE AND	A STATE OF
Redundancy	18.9 (2.5)	3.1 (1.7)	$\begin{array}{c} 6.0 \ (2.3)^{\$} \\ 4.3 \ (2.5)^{\parallel} \end{array}$	3.7 (1.3)	1 Product	
Refinement					(Human Al	BCG5/G8, 2016)
Resolution (Å)	25-3.94					
No. reflections	34889					

![](_page_65_Picture_4.jpeg)

# **Cryo-electron microscopy: bolts and nuts**

- Protein preparation.
- Negative-stain screenig
- Data collection
- Data processing
- Model building

![](_page_66_Picture_7.jpeg)

(Same standard as X-ray crystallography)

![](_page_67_Figure_3.jpeg)

![](_page_68_Figure_2.jpeg)

(Abeyrathne & Grigorieff, PLOS ONE, 2017)

![](_page_68_Picture_4.jpeg)

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### (Functionally characterized)

![](_page_69_Figure_3.jpeg)

(Qu/Takahashi/Yang et al, Cell, 2018)

![](_page_69_Picture_5.jpeg)

# **EM Samples: Negative Staining**

- Principle:
  - Embedding objects in a layer of heavy-metal salts that surround the proteins like a shell.
  - Shape of objects are visible in contrast to the optically opaque stains.
- Benefits:
  - Small amount of proteins (0.01 mg/mL)
  - Easy and quick (preparation and imaging)
  - No need of high-end microscope; diagnostic
- Downsides:
  - Low resolution (*e.g.*, high noise from stains)
  - Artifacts (lack of hydration)

![](_page_70_Figure_12.jpeg)

(Brenner & Horne, BBA, 1959)

![](_page_70_Picture_14.jpeg)

# **EM Samples: Protein Concentrations**

- Negative staining: 0.01-0.05 mg/mL
- Cryo: 0.1-5 mg/mL

![](_page_71_Figure_4.jpeg)

![](_page_71_Picture_5.jpeg)
- Supporting grids for cryo-EM
  - · Holey carbon grids
    - Quantifoil
    - C-Flat
    - Lacey
  - Gold grids (Quantifoil UltrAuFoil<sup>®</sup> Holey Gold Films )





Holey carbon

Quantifoil



- Supporting grids for cryo-EM
  - Holey carbon grids
    - Quantifoil
    - C-Flat
    - Lacey
  - Gold grids (Quantifoil UltrAuFoil® Holey Gold Films )







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### **Glow-Discharging**



- Ionization-based plasma.
- Remove the organic contamination.
- Make surface hydrophilic.



**Glow-Discharging** 



(Proteasome, Morris & Fonseca, Acta Cryst D, 2017)

- Different machines.
- Different discharging duration.
- Different air conditions.



#### Sample Freezing with a Plunger







(Commercially available)

# **Storage and Transfer**





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## **Data Collection**

- Very important for data collection
  - Learn to compare different areas (squares and holes) on the grids
  - Good grids, good and fast data collection
- Literature search for similar cases
- Types of EM grids
- Protein concentrations ± additives
- Blotting conditions: time, force, humidity & ethane temperature



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## **Data Collection**

- Most area of a whole grid is likely not ideal.
  - Red: too thick
  - Blue: too thin
  - Green: suitable



## **Data Collection**

- Too thick .
  - Low contrast •
  - Low S/N ratios •
- Too thin •
  - Not thick enough to accommodate particles ٠
  - Protein denaturation •
  - More preferred orientation •
  - Poor support and large motion during ٠ imaging
  - Vulnerable to radiation damage •





### **Data Processing**



The subunit was negatively stained with unrul accetate and air-dried, which accounts for the partial flattening. The tridge of the deep groover tunning bottcontailly, termed *integrino angus*, it created by the balix 69 of 235 rRNA, as later recognized when the X-ray structure of the large subunit was solved. Annotations refer that the structure of the large subunit was solved.

## **Data Processing**

- cryoSPARC (GPU)
- Relion (GPU)
- EMAN/EMAN2
- Frealign/cisTEM
- Xmipps/Scipion
- Spider

. . .

- IMAGIC
- MRC/2dx (2-D crystals/MicroED)





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#### EMAN





## **Data Processing**

### RELION

	RELION-3.0.2: /fdb/app_testdata/cryoEM/RELION	Project Help	
File Jobs Autorun	I/O Searches CTFFIND-4.1 Gctf Running	CIPION Free	tocols ( Data
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Particle extraction Do initial model 30 classification 30 auto-refine 30 multi-body CTF refinement Bayesian polshing Masi: creation Do atto-refine Bayesian polshing Masi: creation Post-processing Local resolution	Number of MPI proc:       1       1       1         Submit to queuer No       2       2         Queue name:       norm       2       2         Queue name:       Ioon       2       2         Queue submit command:       Ioon       2       2         Mamory Per Thread og       1       2       2         Add (exis) SBATCH Directives       3       3       3         Add (exis) SBATCH Directives       3       3       3         Add (exis) SBATCH Directives       3       3       3         Standard submission script:       ELIon/ tempates.common.shi       1       1         Minimum dictated cores per node.       1       3       3         Additional arguments:       7       3       7         Prot.command       Schedule       PLM1	View     Set (2) copy     Denote Except Set (2)     Colorest     Verwitter     4       Protocis     Set (2) copy     Denote Except Set (2)     Colorest     Verwitter     4       Protocis     Set (2) copy     Denote Except Set (2)     Colorest     Verwitter     4       Protocis     Set (2) copy     Denote Colorest     Set (2) copy     Colorest       Protocis     Set (2) copy     Denote Colorest     Set (2) copy     Colorest       Protocis     Protocis     Set (2) copy     Denote Colorest     Set (2) Colorest       Protocis     Set (2) copy     Denote Colorest     Set (2) Colorest       Protocis     Set (2) colorest     Set (2) Colorest     Set (2) Colorest       Protocis     Set (2) colorest     Colorest     Set (2) Colorest	• Provers
		Committed classes core temple ID 11	



# SCIPION

# **Motion Correction**

- Stage drift
- Beam-induced sample motion



(Brilot et al, J Struct Biol, 2012)



(Zivanov et al, IUCrJ, 2019)



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в 10 Last frame Y shift (Å) First frame -> -6 -4 X shift (Å) -10 -8 -2 0 Uncorrected image D С ЗÅ 50 nm **Uncorrected FFT Corrected FFT** Corrected image

(Cheng et al, Cell, 2015)



### **CTF Assessment (Power Spectra)**



**Fig. 8** Assessment of CTF parameters. (**a**) Comparison of theoretically calculated CTF (*left bottom quadrant*) with CTF seen in experimental spectrum. For an accurate CTF determination the Thon rings from both image parts should match accurately. (**b**) Identification of axes of astigmatism which are superimposed over Thon rings of an actual observed power spectrum and compared with the theoretical spectrum. The spectrum of a micrograph shown here indicates that there is a small astigmatism, ~2%, and the axes of ellipse are slightly tilted, shown in *light blue* 

(Costa et al, Meth Mol Biol, 2017)



### **CTF Assessment (Power Spectra)**



Bad: Thon rings only at low resolution

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## **Particle Selection & 2-D Classes**

Manual



Automated (template/deep-learning)





## **Particle Selection & 2-D Classes**





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## **Initial Model & 3-D Classes**

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### **Random Conical Tilt**





(Radermacher et al, J Microsc, 1986)

### **Stochastic Gradient Descent**



(Punjani et al, Nat Methods, 2017)





(Scheres, Meth Enzymol, 2016)



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## **Model Building**

### REFMAC

Job title Do restrained refinement — using no prior phase information		Help
Job title Do restrained refinement  using no prior phase information	2	_
Input fixed TLS parameters     Cycle with arp_waters to analyse solvent model (tuses old version of ARP/wARP)     Run Cootfindwaters to automatically add remove waters to refined structure     Generate weighted difference maps files in CCP1 — format		nput
MTZ in wp links -	Browse	View
FP Sigma	-	
MTZ out wp_linux -	Browse	View
PDB in wp_linux -	Browse	View
PDB out wp linux -	Browse	View
Library wp_linux -	Browse	View
Data Harvesting	-	1
Create harvest file in project harvesting directory		-
Harvest project name wp_linux and dataset name	_	
Refinement Parameters		
Use hydrogen atoms: use if present in file and output to coord Resolution range from minimum Use weighting term 0.3 Vise experimental signas to weight Xray terms Refine isotropic temperature factors Exclude data with freeR label with value of 0	linate file	
Setun Geometric Restraints		E
Setup Non-Crystallographic Symmetry (NCS) Restraints		Ē
Data Output to MTZ file		E
Scaling		Г
Maximum Likelihood Parameters		E
Monitoring		Г
Geometric parameters		Г
		and a

### PHENIX

Projects	Favorites	
Show group: All proves C. Manane	Data analysis	
	Experimental phasing	
New project 🚱 Settings	Molecular replacement	
ID         Last modified         # of jobs         R-free           Nov2015_JODO         Juli 10 2020 02:23 17         0.2836           Jan2015_with R.,.         Aug 12 2019 12:1 23         0.3179	Phaser-MR (full-featured) Maximum-likelihood molecular replacement with Phaser- use for all but simplest cases	
Nov2019_PMP         Apr 09 2018 06-37         27         0.3143           Oct2014_5_repr         Apr 09 2018 08:35         240         0.2910           ABC001         Apr 05 2018 09:12         0	Phaser-MR (simple one-component interface) Automated molecular replacement with Phaser-ace boly for structures with a single type of component	
	성 등 MRage - automated pipeline 한 등 Integrated model identification, preparation, and parallel MR search	
	MR-Rosetta AutoMR combined with Rosetta model improvement and AutoBuild for difficult siruttures	
	Kellptor Modify a molecular replacement asarch model	
	Sculptor - Coot Interface Extension to Coot Gill for running Sculptor Interactively	
	Ensembler Create ensemble of models for molecular replacement	
	Model building	
	Refinement	
	Cryo-EM	
	Validation	
	Ligands	
	Reflection tools	
	Maps	
	Model tools	
	PD8 Deposition	
	Other utilities and Program search	



## **Model Building**







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