ID23-2 Fixed Energy Microfocus

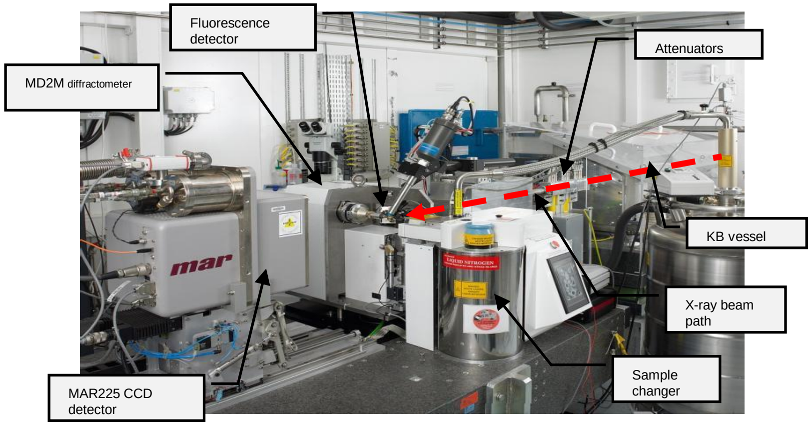
Max Nanao, Shibom Basu

ESRF | The European Synchrotron

ID23-2 HISTORY

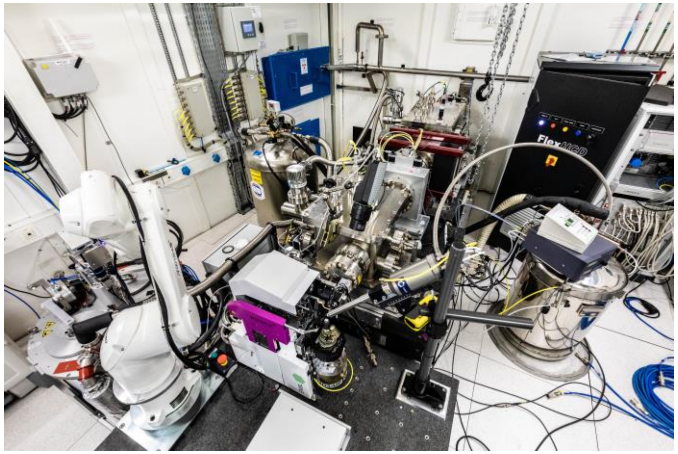
• Microfocus beamline dedicated to MX

- Stability
- Ease of use: No microfocus expertise required
- Consistent user experience w/ other ESRF MX

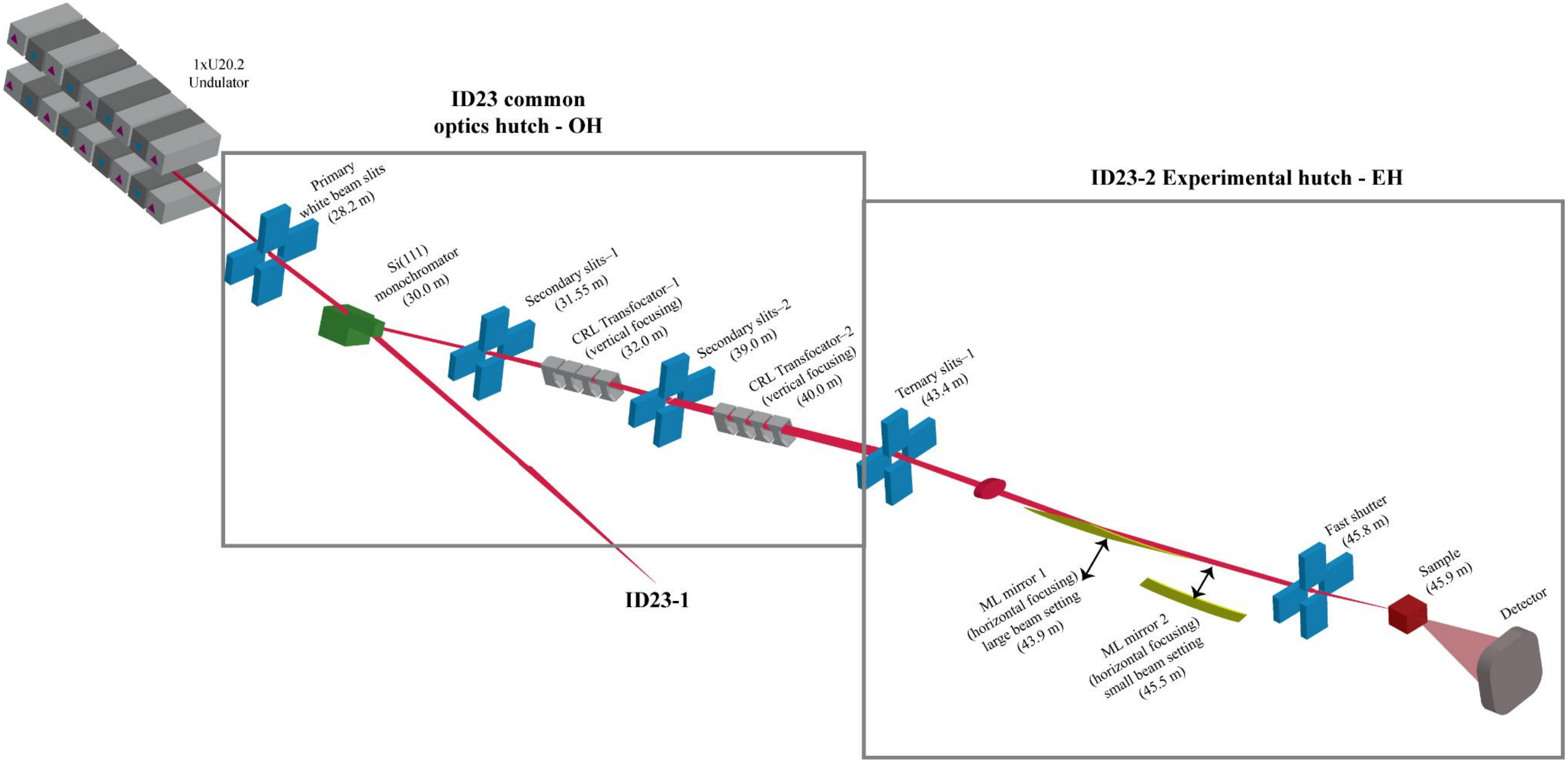


•History

- Operation since 2006 (KB, MAR 225)
- Incremental upgrades:
  - *Detector 2014 (Pilatus 3 2M)*
  - *Multilayer optics 2015*
- Major upgrade, first users July 12 2017
- EBS, Spring 2020 (source u23->u20)



ID23-EH2 LAYOUT



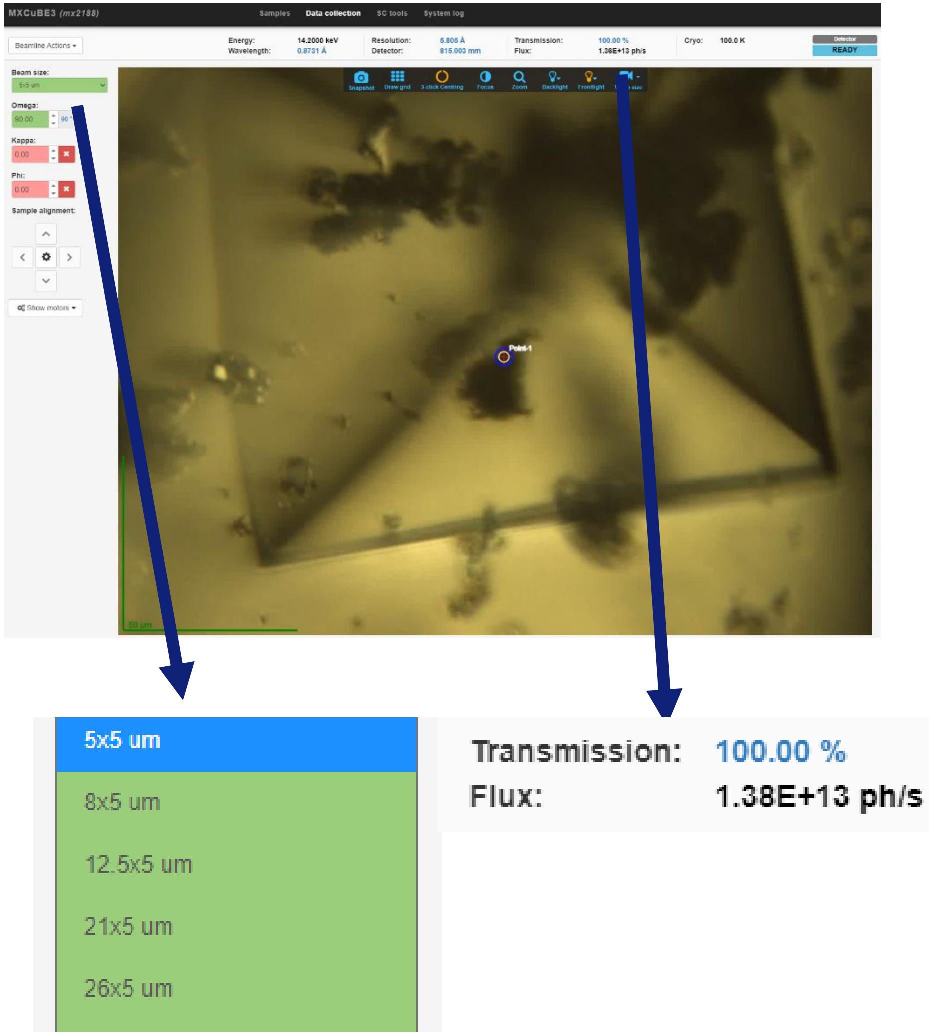
ID23-2 SAMPLE POSITION

+Variable vertical focus+high flux

+MD3Up diffractometer

- +Very fast mesh scans
- +Excellent OAV
- +Split beamstop
- +Apertures
- +Excellent beam visualisation
- +Mini Kappa goniometry
- +Plate Gripper

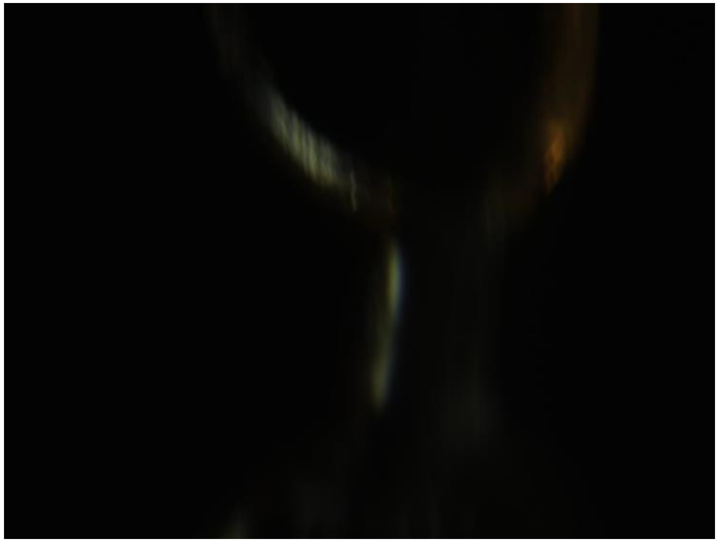
+New FLEX HCD. UNIPUCKS ONLY,



Energy: 14.2000 keV
Wavelength: 0.8731 Å
Resolution: 5.806 Å
Detector: 815.003 mm
Transmission: 100.00 %
Flux: 1.38E+13 ph/s
Cryo: 100.0 K
Detector READY
Beam size: 5x5 um
Omega: 90.00
Kappa: 0.00
Phi: 0.00

Transmission: 100.00 %
Flux: 1.38E+13 ph/s

ID23-2 SAMPLE POSITION

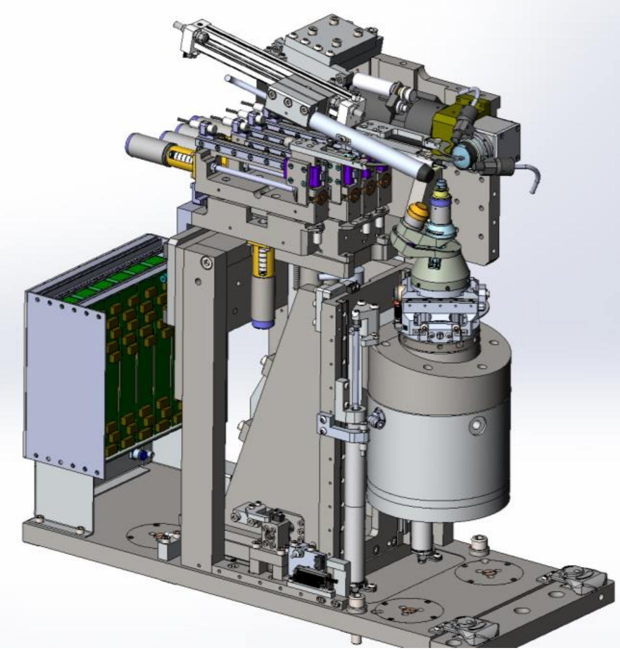


+Variable vertical focus

MD3Up diffractometer

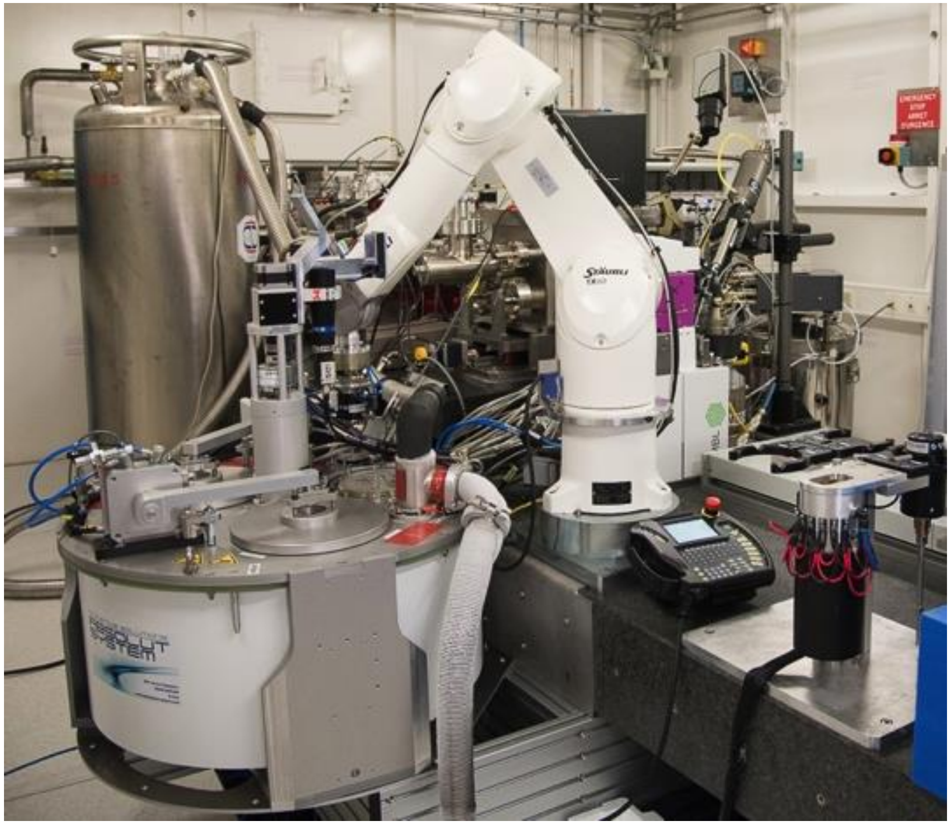
- +Very fast mesh scans
- +Excellent OAV
- +Split beamstop
- +Apertures
- +Excellent beam visualisation
- +Mini Kappa goniometry
- +Plate Gripper
- +Test bed for serial devices

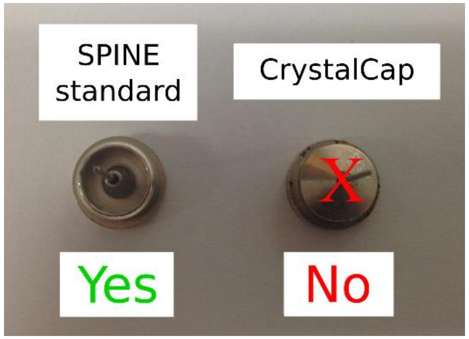
+New FLEX HCD. UNIPUCKS ONLY,



ID23-2 SAMPLE CHANGER

- +Variable vertical focus
- +MD3Up diffractometer
  - +Very fast mesh scans
  - +Excellent OAV
  - +Split beamstop
  - +Apertures
  - +Excellent beam visualisation
  - +Mini Kappa goniometry
  - +Plate Gripper
  - +Test bed for serial devices
- **+FLEX HCD. UNIPUCKS ONLY**



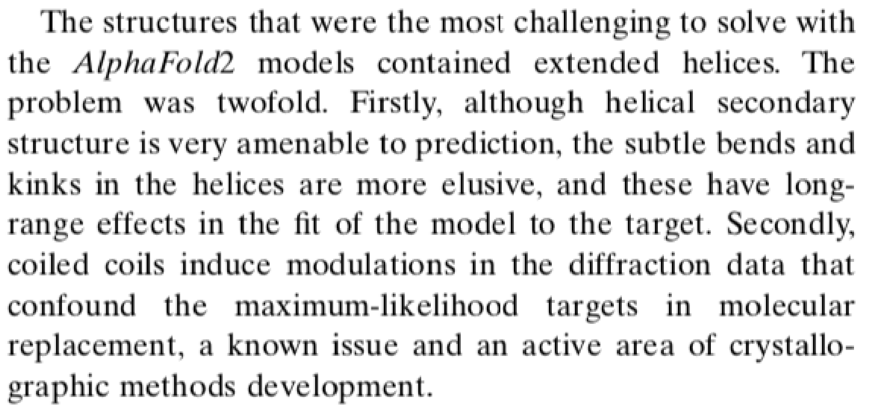


RECENT HIGHLIGHT

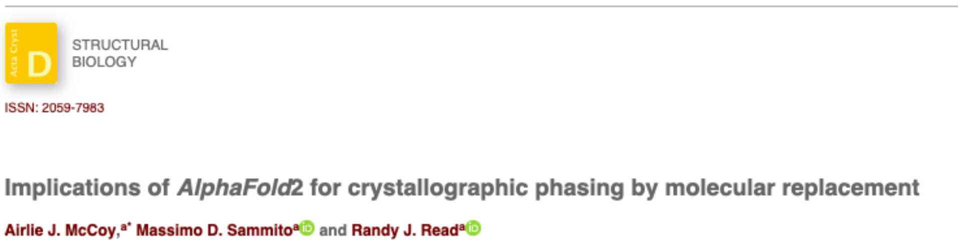
- 200 Amino acids
- 8/AU
- C2221
- 2.9 Å diffraction

RECENT HIGHLIGHT

- 200 Amino acids
- 8/AU
- C2221
- 2.9 Å diffraction
- ARCIMBOLDO_ LITE also failed
- Alphafold on target and similar molecules failed



The structures that were the most challenging to solve with the *AlphaFold*2 models contained extended helices. The problem was twofold. Firstly, although helical secondary structure is very amenable to prediction, the subtle bends and kinks in the helices are more elusive, and these have long-range effects in the fit of the model to the target. Secondly, coiled coils induce modulations in the diffraction data that confound the maximum-likelihood targets in molecular replacement, a known issue and an active area of crystallographic methods development.



STRUCTURAL
BIOLOGY

ISSN: 2059-7983

Implications of *AlphaFold*2 for crystallographic phasing by molecular replacement

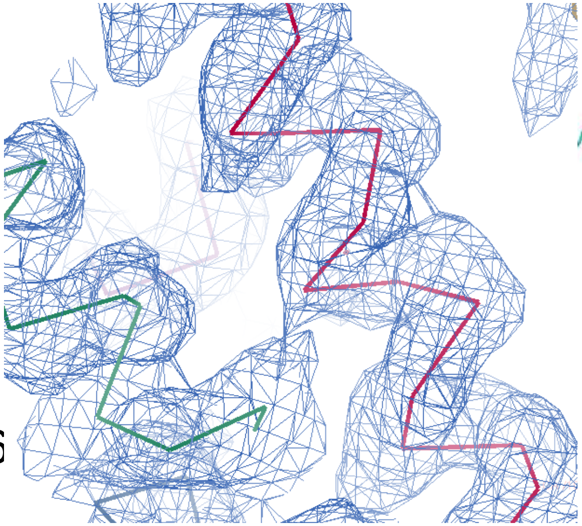
Airlie J. McCoy,[a*] **Massimo D. Sammito**[a] and **Randy J. Read**[a]

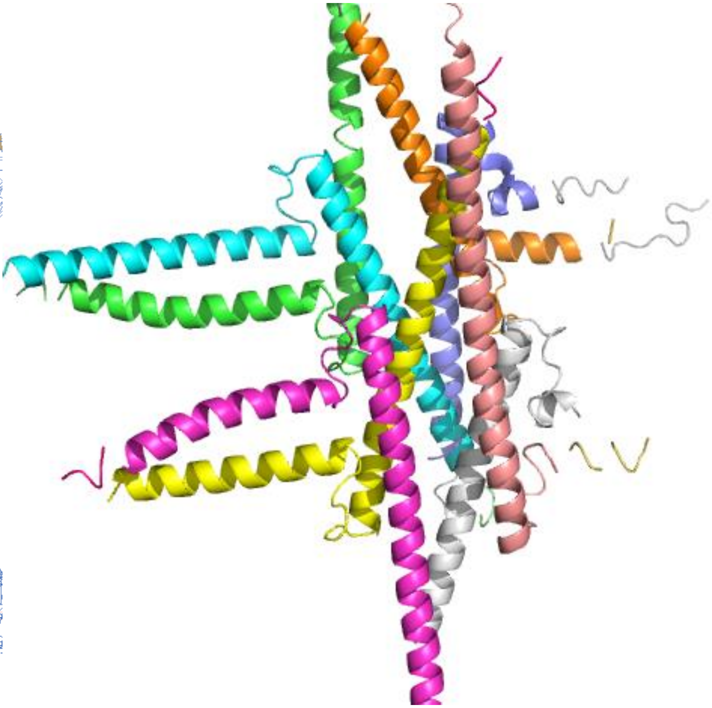
Volume 78| Part 1| January 2022| Pages 1-13

RECENT HIGHLIGHT

- 200 Amino acids
- 8/AU
- C2221
- 2.9 Å diffraction
- ARCIMBOLDO_ LITE also failed
- Alphafold on target and similar molecules failed

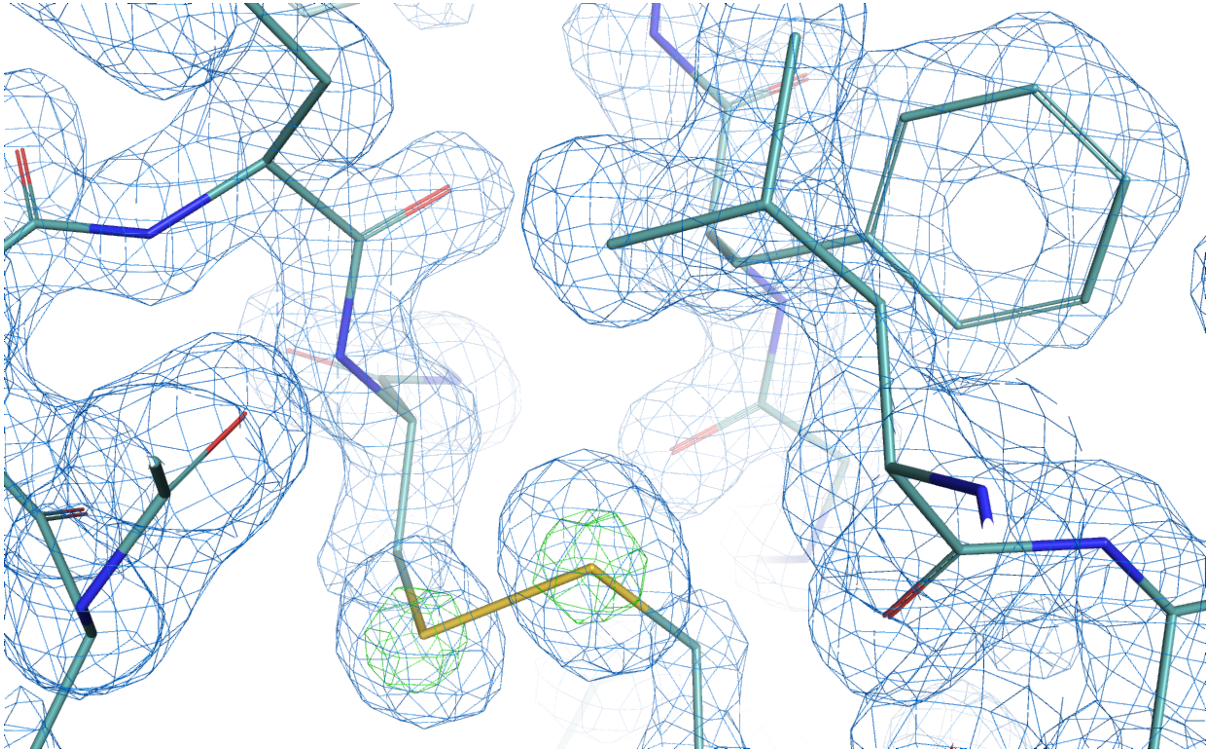
- ID23-2 (~3 e- f”)
- Helical dataset
  - 8X multiplicity
  - <I/σ(I)> inner, overall = 44, 11
- SeMet (48)
- 12 sites found
  - ano peak heights 6 11 σ





RECENT HIGHLIGHT

- ID23-2 S-SAD
  - 14.2 kEV (0.18 e- f”)
  - Small beam setting
    - 3x1.5 μm^2 FWHM
  - 300x50x50 μm^3 xtal
- Helical dataset
  - 1.2 Å
  - 90x multiplicity
  - <I/σ(I)> inner, overall = 75, 30
- 6 sites found
  - ano peak heights 12.6-14 σ



Experimental electron density (1.5 σ) with final model. Model phased diff ano at 14 σ.

Described in 23-2 rebuild paper. Images+meta data uplaoded to Zenodo, unmerged data uploaded to PDB (in progress)

ID23-2 FUTURE

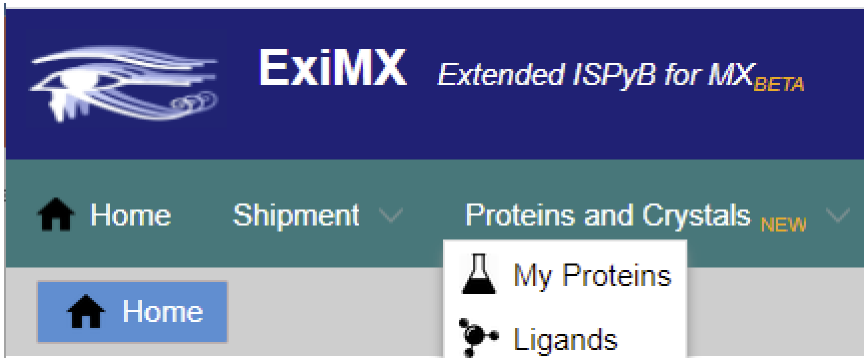
- **Improve mesh+helical performance**
  - Enable ROI
  -
- **Software**
  - Deploy S.Basu’s automated crystfel of meshes
  - Improve automatic HCA+GA of MeshandCollect

AUTOPROCESSING

+New compute nodes added, more cores/process

+Automated processing of mesh and collect data with HCA (ccCluster) and GA (CODGAS)

+SDF files with multiple ligands for automatic ligand fitting

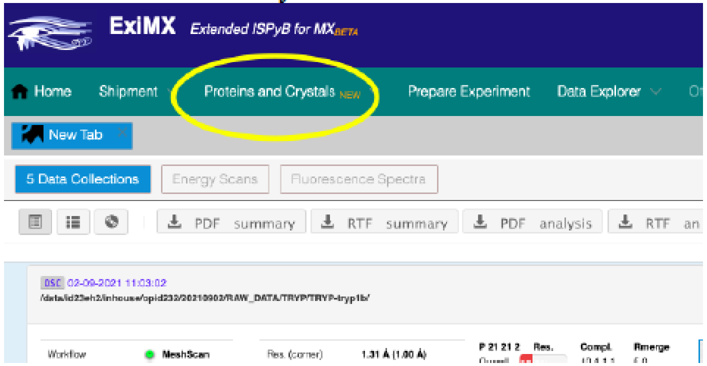


tinyurl.com/iiqgypj9

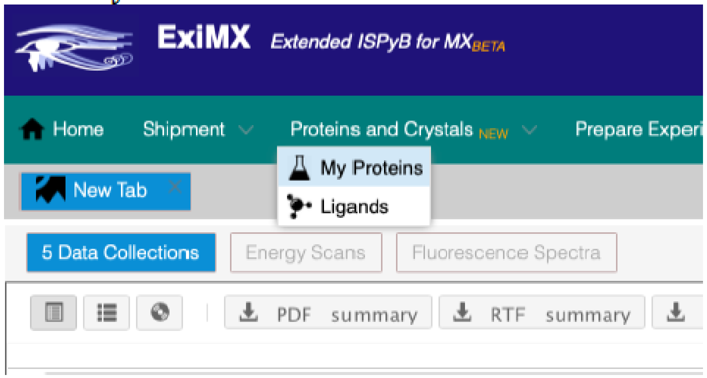
AUTOPROCESSING –EBI ALPHAFOLD

- **Provide a UniprotID**
  - PDB checked for Uniprot. If exists, that PDB is used for MR
  - EBI Alphafold Database checked
    - *Trimmed full length model → MR*
    - *Trimmed, domain-ified (phenix.process_predicted_model) → MR each domain*

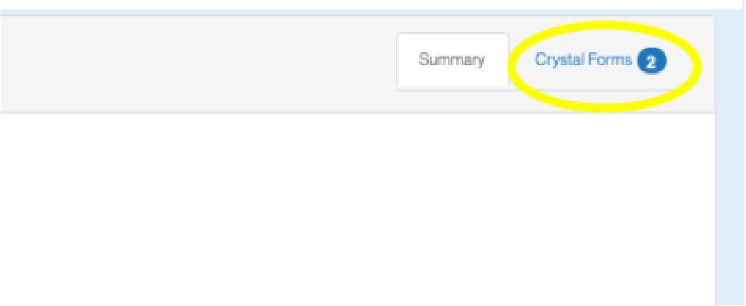
1) Go to Proteins and Crystals



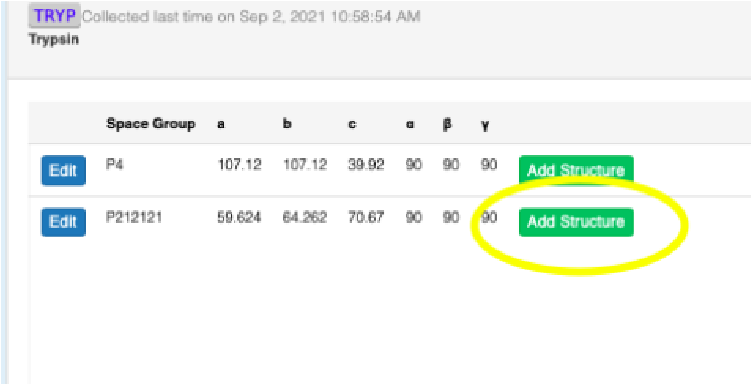
2) Select My Proteins



3) Go to Crystal Forms



4) For the correct crystal form (if there are multiple), "Add Structure"

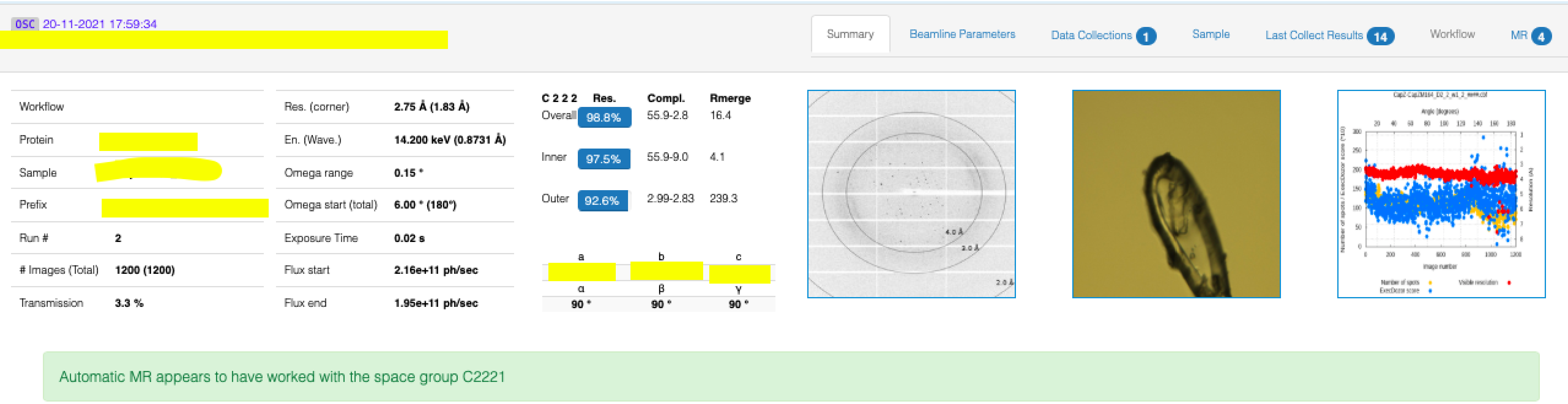


TRYP Collected last time on Sep 2, 2021 10:58:54 AM
Trypsin

	Space Group	a	b	c	α	β	γ	
Edit	P4	107.12	107.12	39.92	90	90	90	Add Structure
Edit	P212121	59.624	64.262	70.67	90	90	90	Add Structure

Can be specified at the last minute – even during beam time!

ALPHAFOLD RESULTS



OSC 20-11-2021 17:59:34

[redacted]

Workflow	
Protein	[redacted]
Sample	[redacted]
Prefix	[redacted]
Run #	**2**
# Images (Total)	**1200 (1200)**
Transmission	**3.3 %**

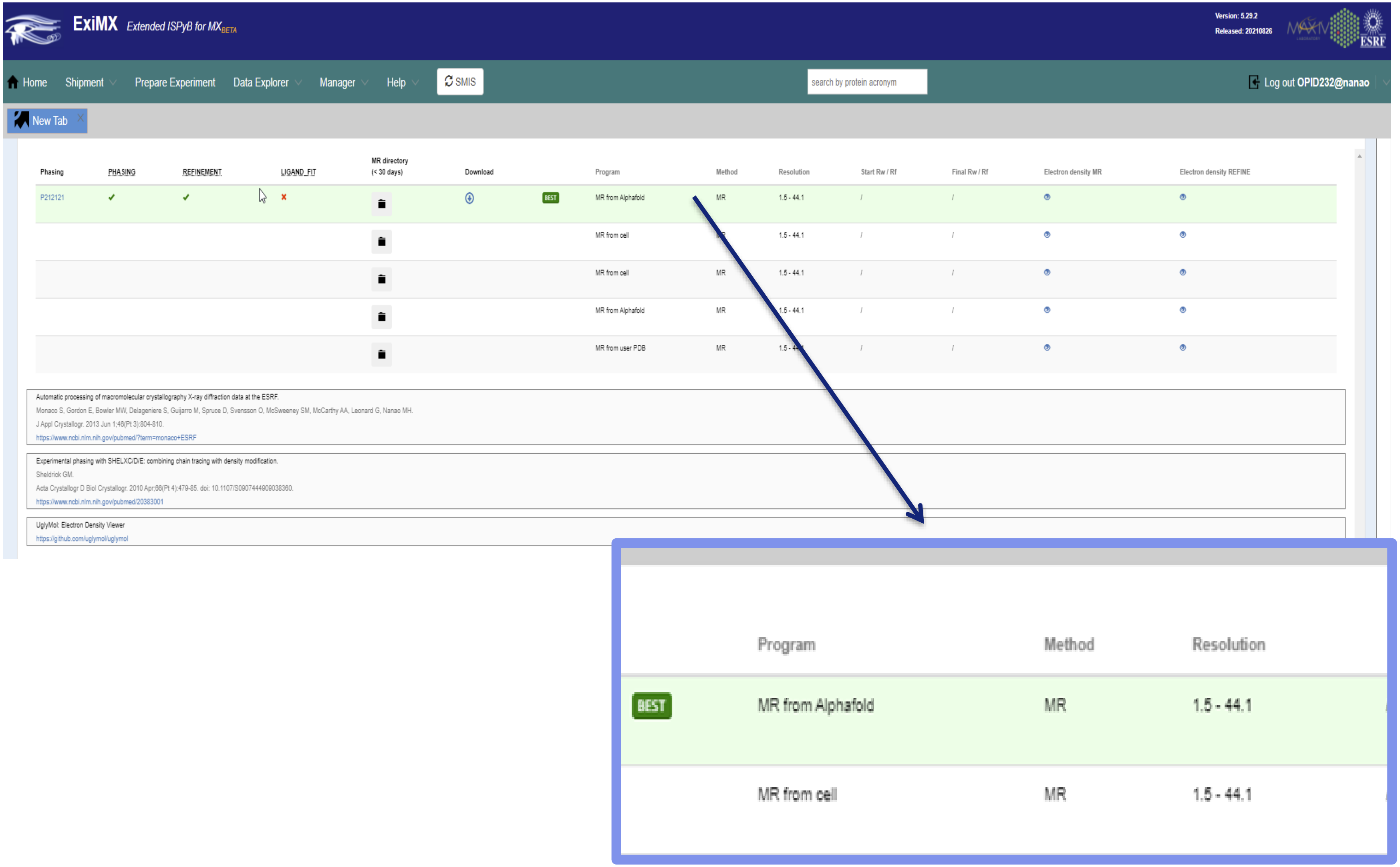
Res. (corner)	**2.75 Å (1.83 Å)**
En. (Wave.)	**14.200 keV (0.8731 Å)**
Omega range	**0.15 °**
Omega start (total)	**6.00 ° (180°)**
Exposure Time	**0.02 s**
Flux start	**2.16e+11 ph/sec**
Flux end	**1.95e+11 ph/sec**

C 2 2 2	**Res.**	**Compl.**	**Rmerge**
Overall	98.8%	55.9-2.8	16.4
Inner	97.5%	55.9-9.0	4.1
Outer	92.6%	2.99-2.83	239.3

a	b	c
[redacted]	[redacted]	[redacted]
α	β	γ
90 °	**90 °**	**90 °**

Automatic MR appears to have worked with the space group C2221

ALPHAFOLD RESULTS



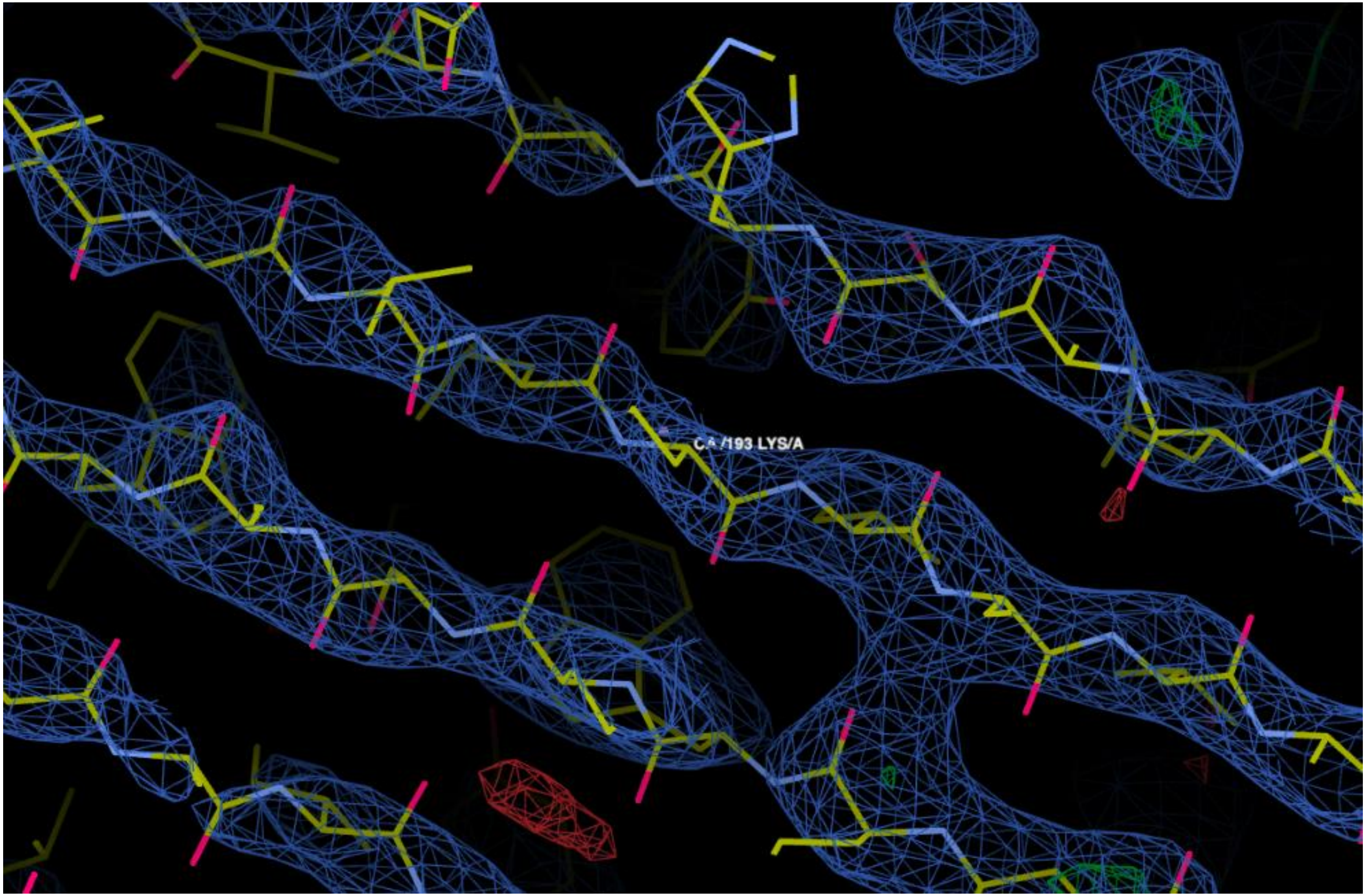
Phasing	PHASING	REFINEMENT	LIGAND_FIT	MR directory (< 30 days)	Download		Program	Method	Resolution	Start Rw / Rf	Final Rw / Rf	Electron density MR	Electron density REFINE
P212121	✓	✓	✗	■	⊕	BEST	MR from Alphafold	MR	1.5 - 44.1	/	/	●	●
				■			MR from cell	MR	1.5 - 44.1	/	/	●	●
				■			MR from cell	MR	1.5 - 44.1	/	/	●	●
				■			MR from Alphafold	MR	1.5 - 44.1	/	/	●	●
				■			MR from user PDB	MR	1.5 - 44.1	/	/	●	●

Automatic processing of macromolecular crystallography X-ray diffraction data at the ESRF.
Monaco S, Gordon E, Bowler MW, Delageniere S, Guijarro M, Spruce D, Svensson O, McSweeney SM, McCarthy AA, Leonard G, Nanao MH.
J Appl Crystallogr. 2013 Jun 1;46(Pt 3):804-810.
https://www.ncbi.nlm.nih.gov/pubmed/?term=monaco+ESRF

Experimental phasing with SHELXC/D/E: combining chain tracing with density modification.
Sheldrick GM.
Acta Crystallogr D Biol Crystallogr. 2010 Apr;66(Pt 4):479-85. doi: 10.1107/S0907444909038360.
https://www.ncbi.nlm.nih.gov/pubmed/20383001

UglyMol: Electron Density Viewer
https://github.com/uglymol/uglymol

	Program	Method	Resolution
BEST	MR from Alphafold	MR	1.5 - 44.1
	MR from cell	MR	1.5 - 44.1


CA /193 LYS/A