Serial femtosecond crystallography of a photosynthetic reaction centre

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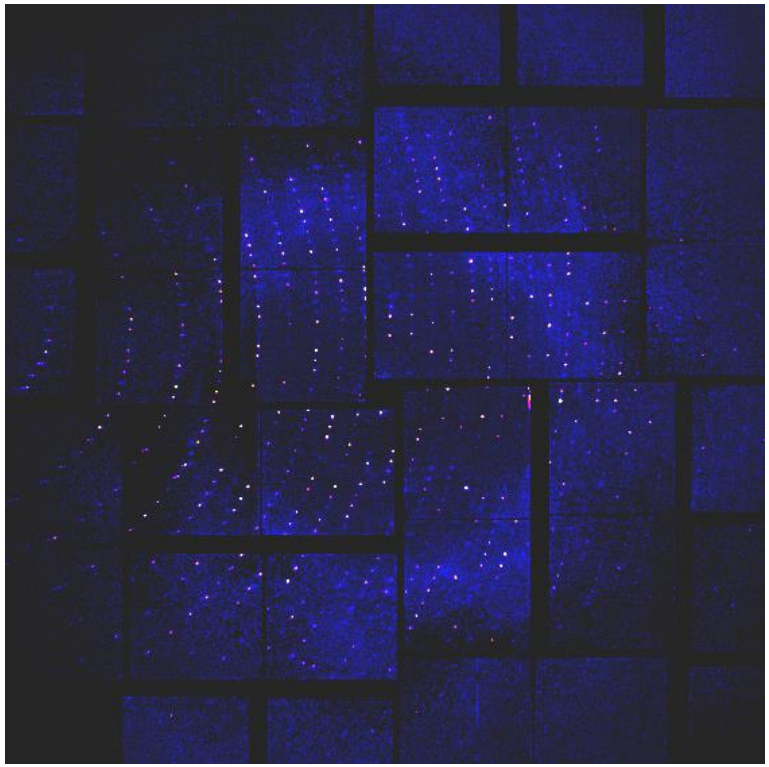


SFX collaboration

Gothenburg	***R. Neutze, L. Johansson, D. Arnlund***, ***A. Sharma***, E. Malmerberg C. Wickstrand, J. Sjöhamn, Weixiao Y. Wahlgren
CFEL-DESY	***H. Chapman,*** J. Schulz, A. Barty, M. Liang, A. Aquila, T. White, D. Deponte, S. Stern, A. Martin, C. Caleman, K. Nass, F. Stellato, F. Wang, H. Fleckenstein, L. Gumprecht, L. Holmegaard, N. Coppola, S. Bajt, M. Barthelmess,
ASU	***J. Spence***, ***P. Fromme***, U. Weierstall, B. Doak, M. Hunter, R. Kirian, X.Wang, K. Schmidt, I. Grotjohann, R. Fromme
SLAC-PULSE	M. Bogan, D. Starodub, R. Sierra, C. Hampton, D. Loh
SLAC-LCLS	***S. Boutet***, ***G. Williams,*** M. Seibert, J. Kryzwinski, C. Bostedt, M. Messerschmidt, J. Bozek, W. White, R. Coffee
Uppsala	J. Hajdu, Nic Timneanu, J. Andreasson, M. Seibert, F. Maia, M. Svenda, J. Davidsson
MPG CFEL ASG	***I. Schlichting,*** R. Shoeman, L. Lomb, S. Kessemeyer, T. Barends, J. Steinbrener, M. Bott, D. Rolles, S. Epp, A. Rudenko, L. Strüder, R. Hartmann, L. Foucar, N. Kimmel, P. Holl, T. Barends, J. Ullrich
LLNL	S. Hau-Riege, M. Frank
CAMP Team	*Led by* ***J. Ullrich*** *and* ***I. Schlichting***
LCLS detector	C. Kenney, R. Herbst, J. Pines, P. Hart, J. Morse
Accelerator	*Led by P. Emma*

Vetenskapsrådet

GÖTEBORGS UNIVERSITET

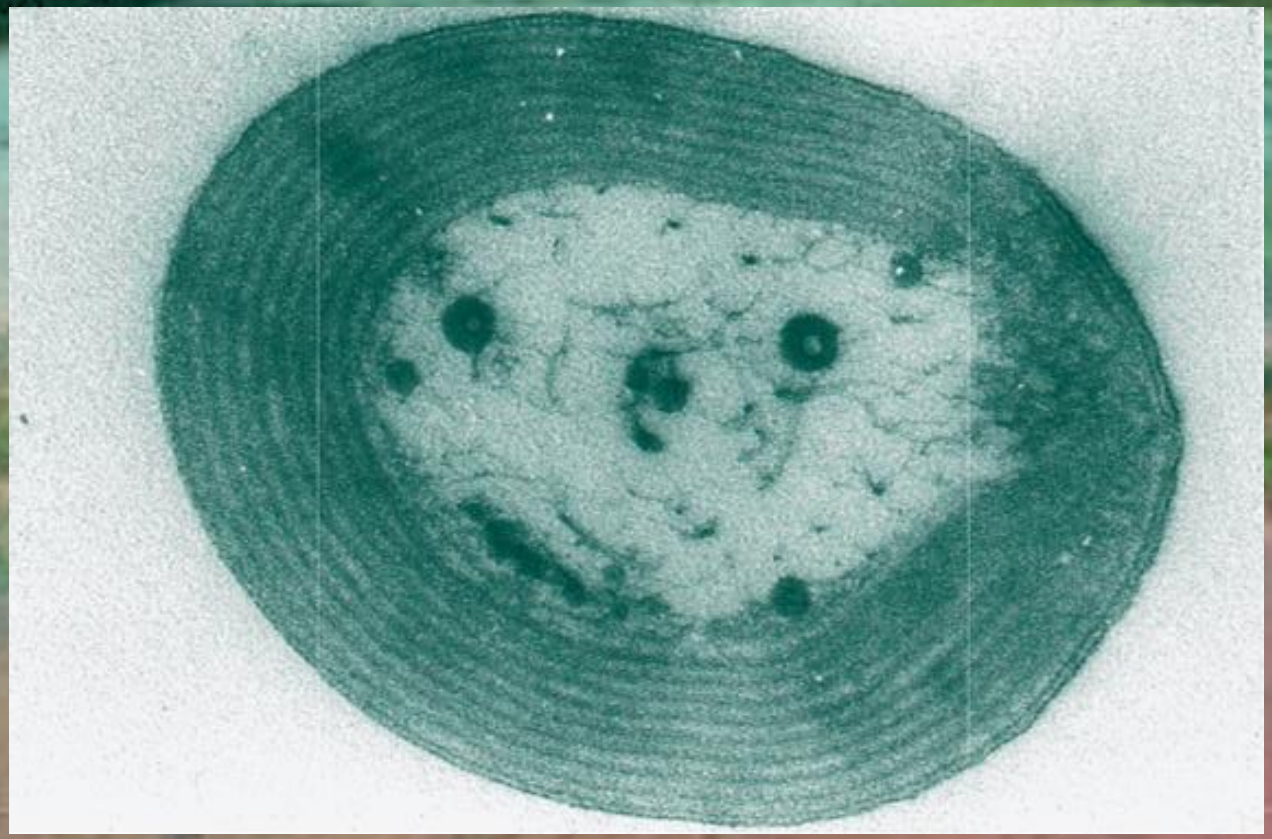
CHALMERS

Lundberg Stiftelsen

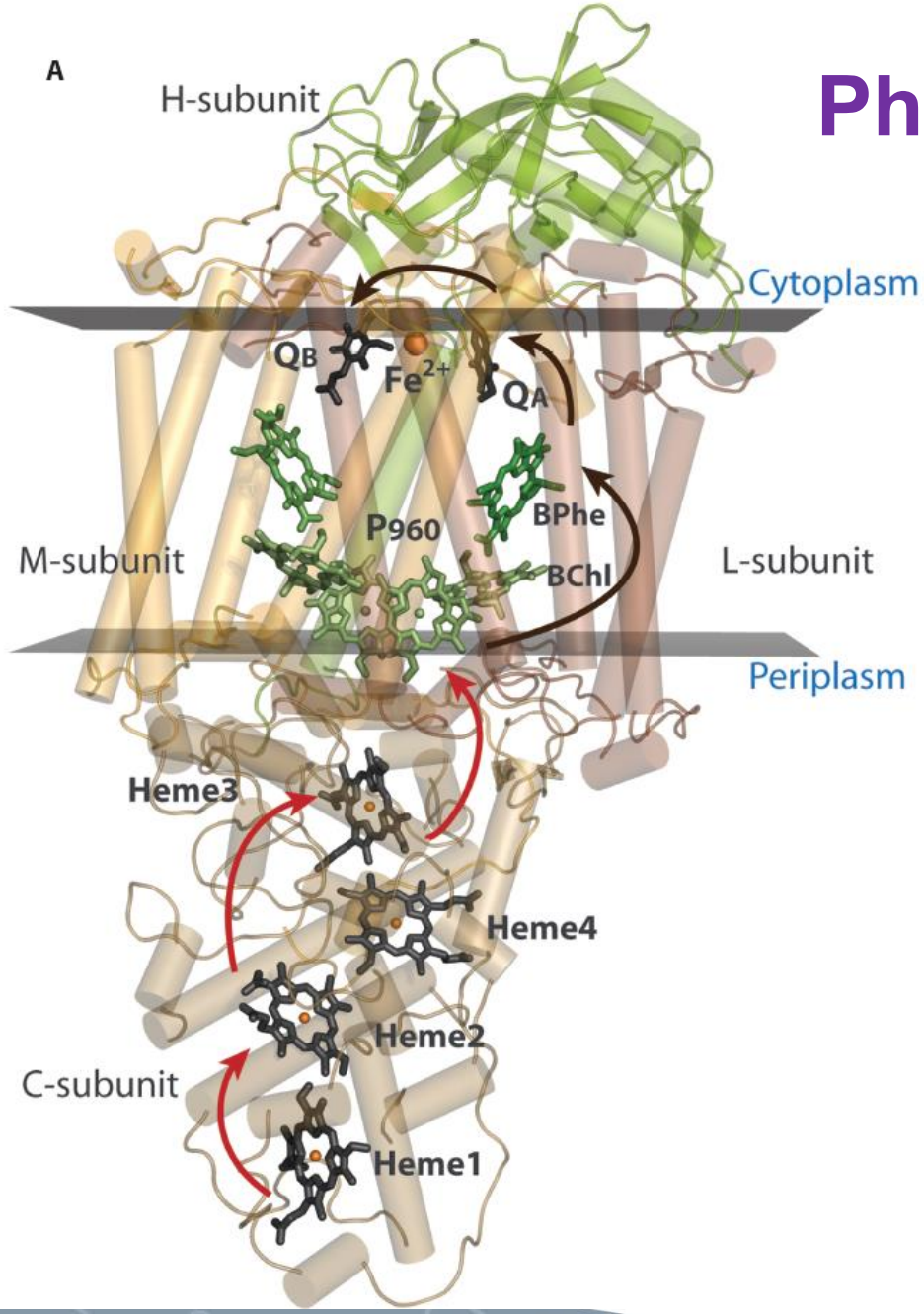
Stiftelsen Olle Engkvist Byggmästare

HUMAN FRONTIER SCIENCE PROGRAM
FUNDING FRONTIER RESEARCH INTO COMPLEX BIOLOGICAL SYSTEMS

Purple photosynthetic bacteria

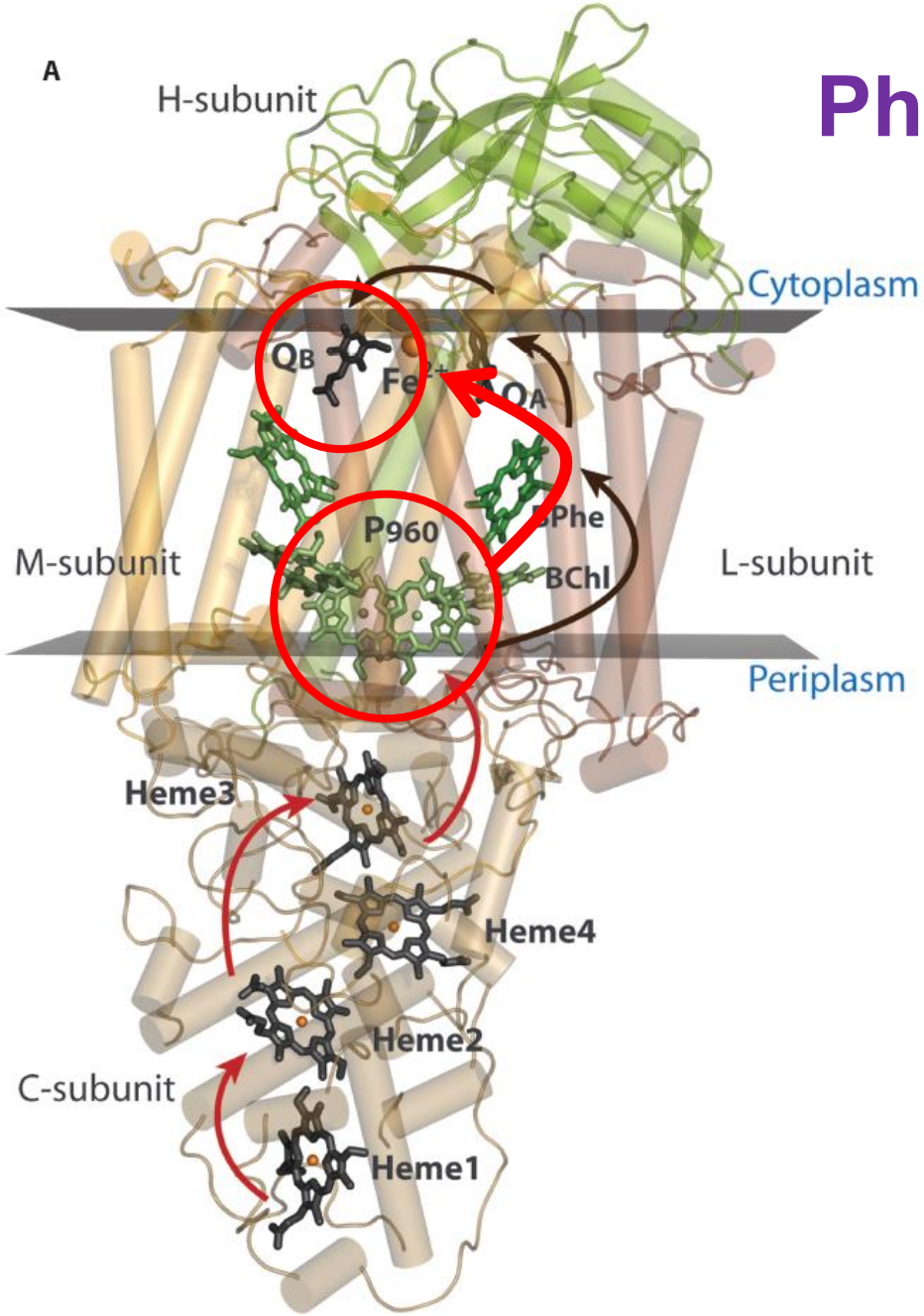


Blooming purple nonsulfur bacteria in a coastal lagoon



Photosynthetic Reaction Centre

- MEMBRANE PROTEIN
- 135 kDa
  - 4 subunits
  - H, L, M, cytochrome c
- 13 cofactors
  - 4 heme irons
  - 4 bacteriochlorophyll
  - 2 bacteriopheophytin
  - menaquinone
  - non-heme iron
  - ubiquinone

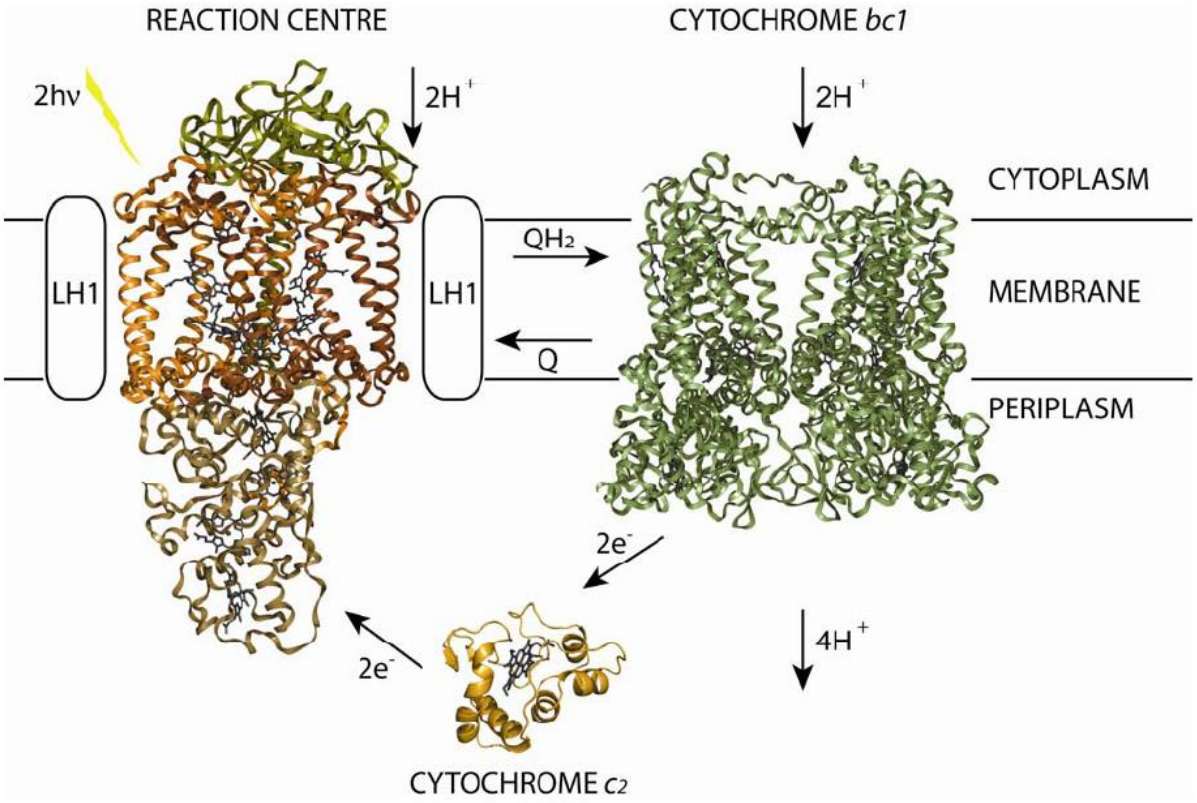


Photosynthetic Reaction Centre

- Light photo-excites special pair of chlorophylls (P_{960}).
- Electron transferred to mobile quinone (Q_B).
- fs to ms time-scales.

Electron & proton movements

- Complex light driven proton pump.
  - Electron movements driven by light.
  - Coupled redox reactions pump protons.
- Descendent created O_2 rich atmosphere.



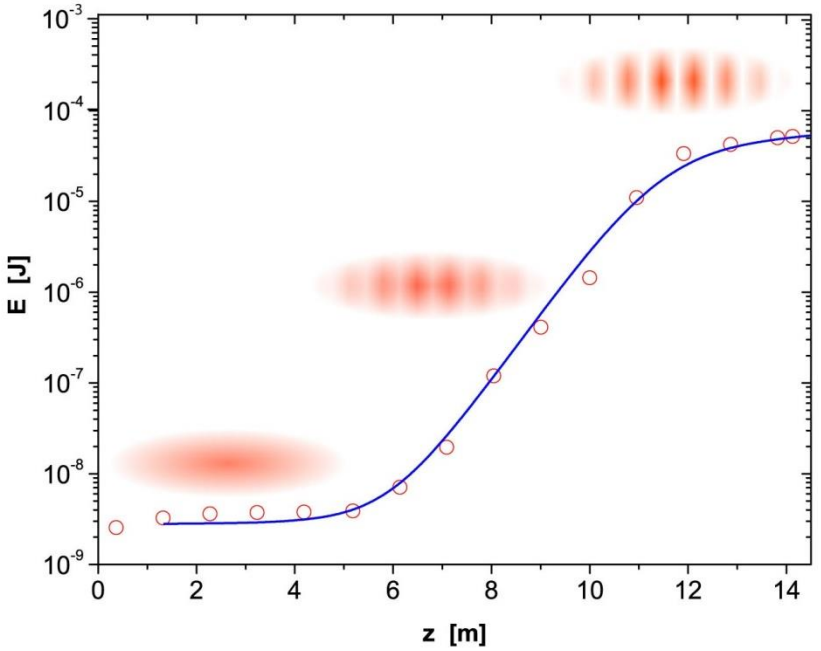
Linac Coherent Light Source (LCLS)
Stanford, USA

SLAC NATIONAL ACCELERATOR LABORATORY


LCLS
SLAC
NATIONAL ACCELERATOR LABORATORY
132 m long undulator

Microbunching of electrons

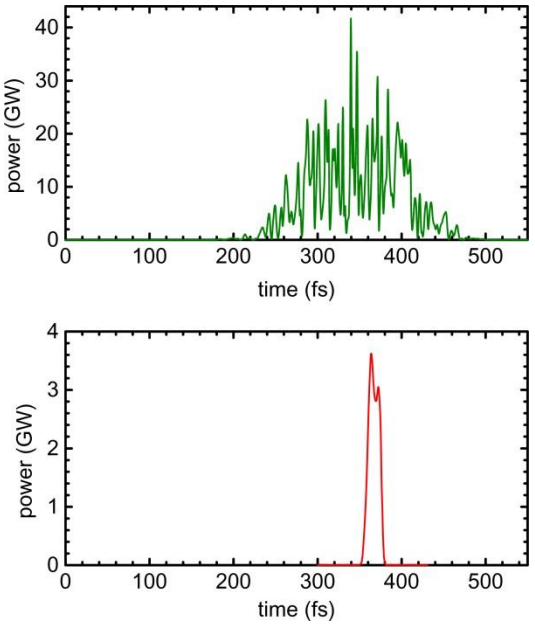
- Electrons form periods that matches the emission wavelength
  - Constructive interference
  - Exponential amplification of beam intensity



Self-amplified sponaneous emission and seeding

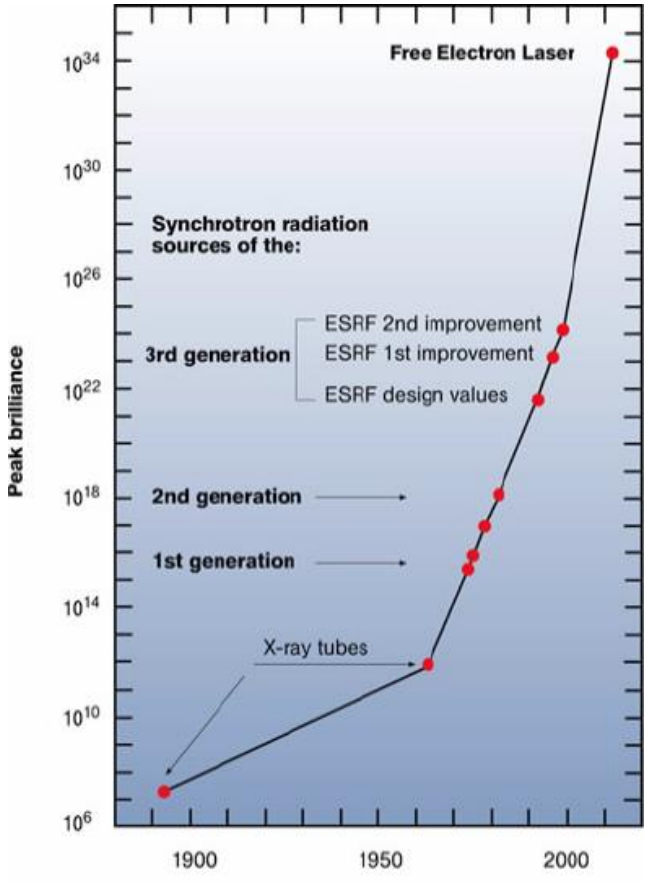
- Shot noise gets amplified leading to instability in:
  - Beam position
  - Temporal profile
  - Wavelength (spectrum)
  - Intensity

- Seeding can stabilize, but there are no X-ray seed lasers
  - Solution is recycling cleaned XFEL radiation



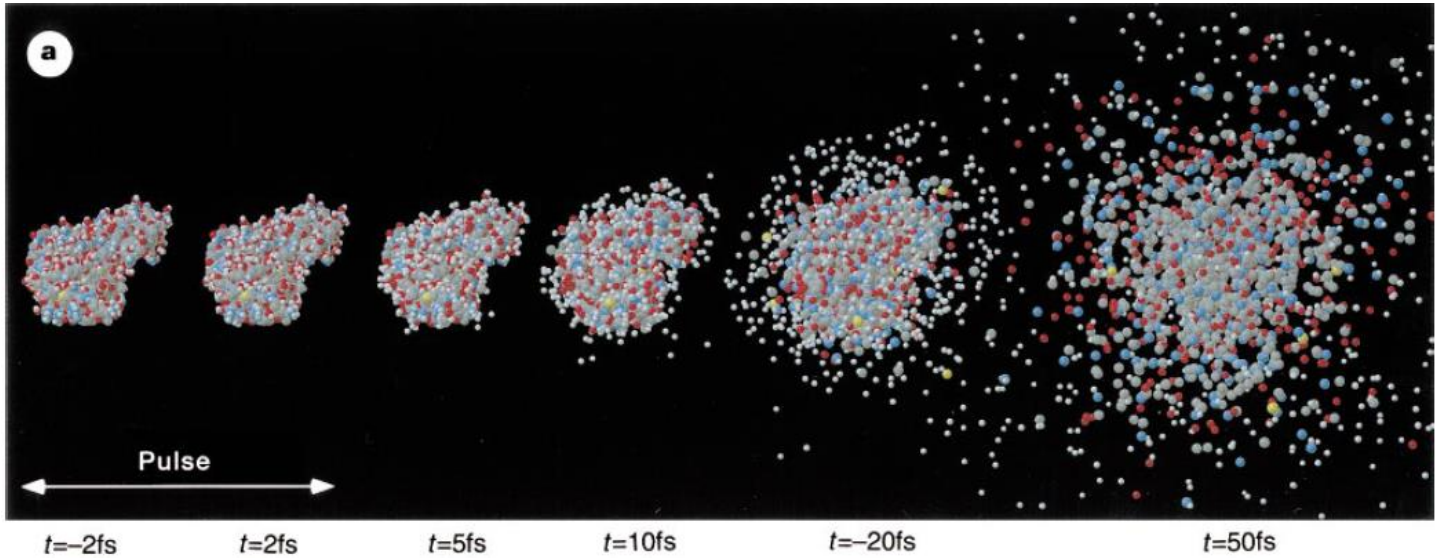
Follath (2007)

Free Electron Lasers and Structural Biology



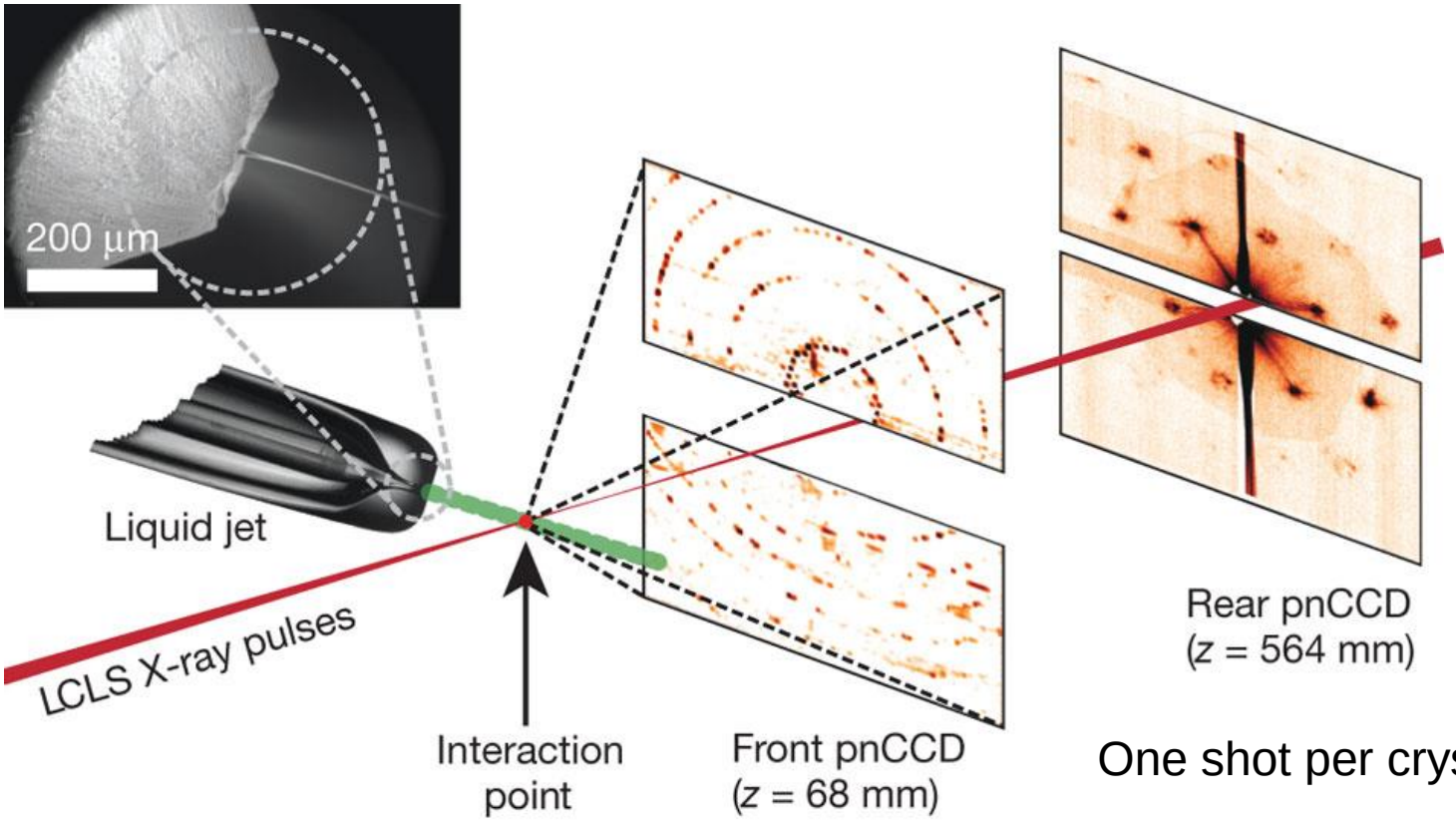
- First hard XFEL source was LCLS in Stanford, US (2009)
- Exploit microcrystals
- Time-resolved studies

Henderson's limit: 20 MGy, Garman's 30 MGy



Neutze et al. *Nature* 2000

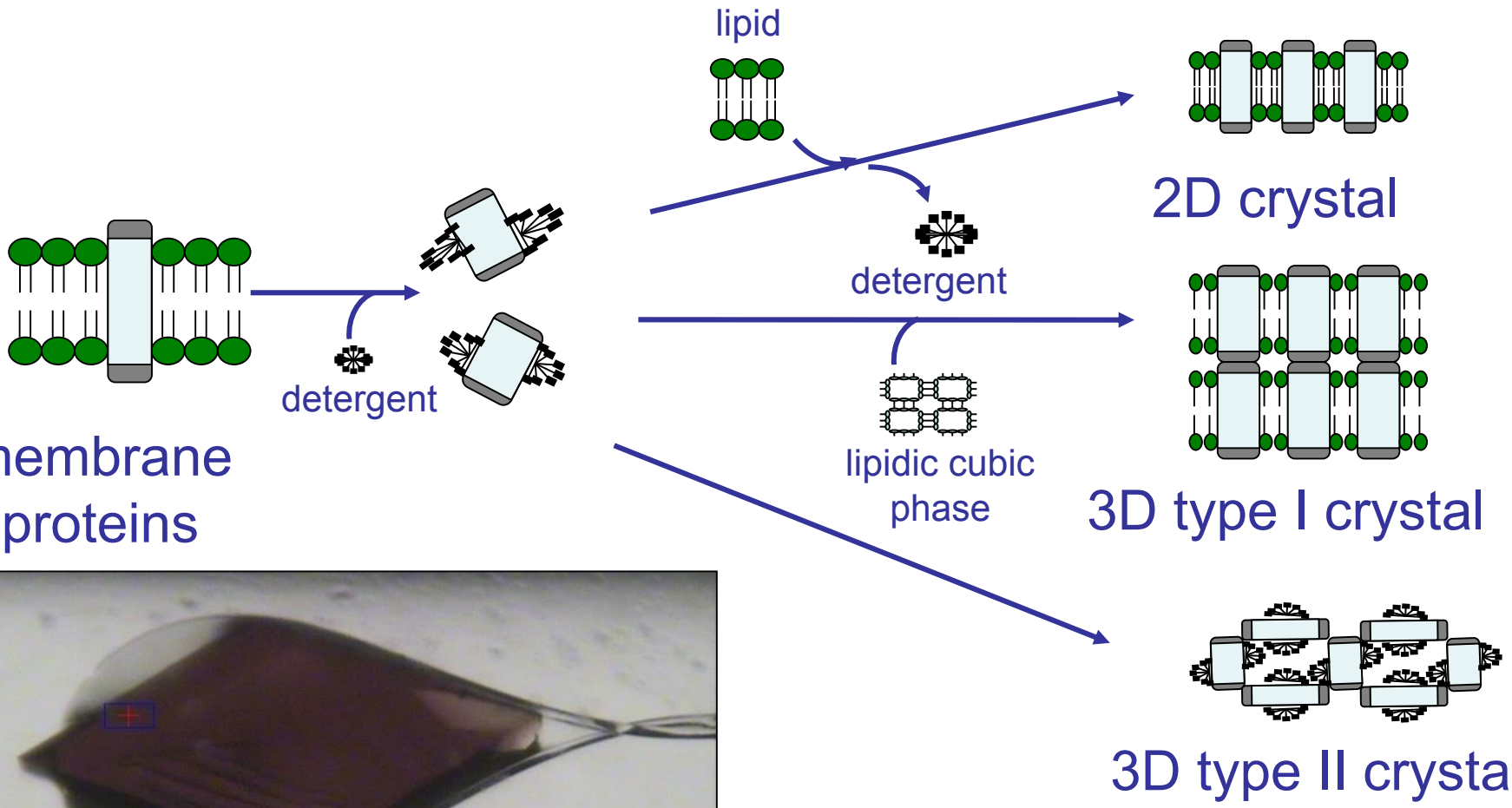
Paradigm shift in data collection

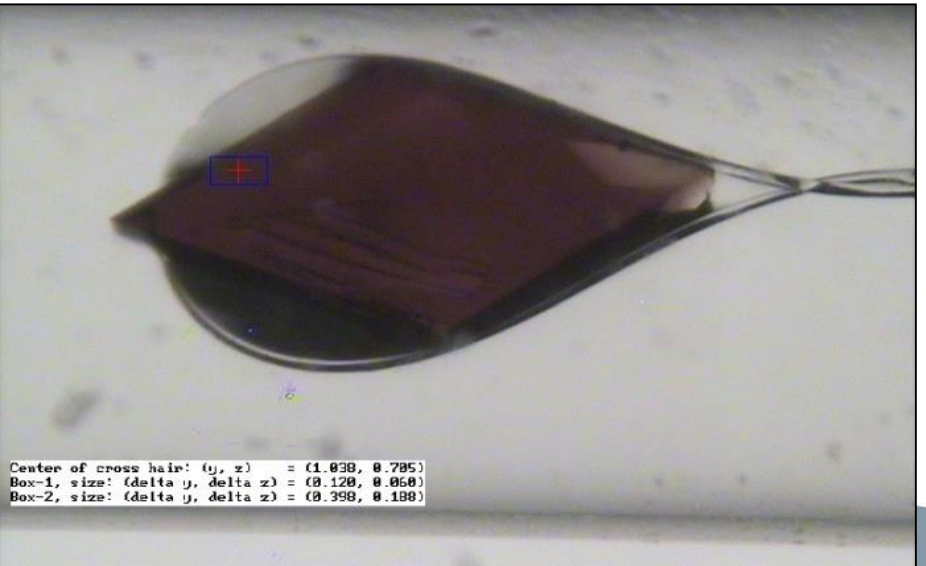


One shot per crystal

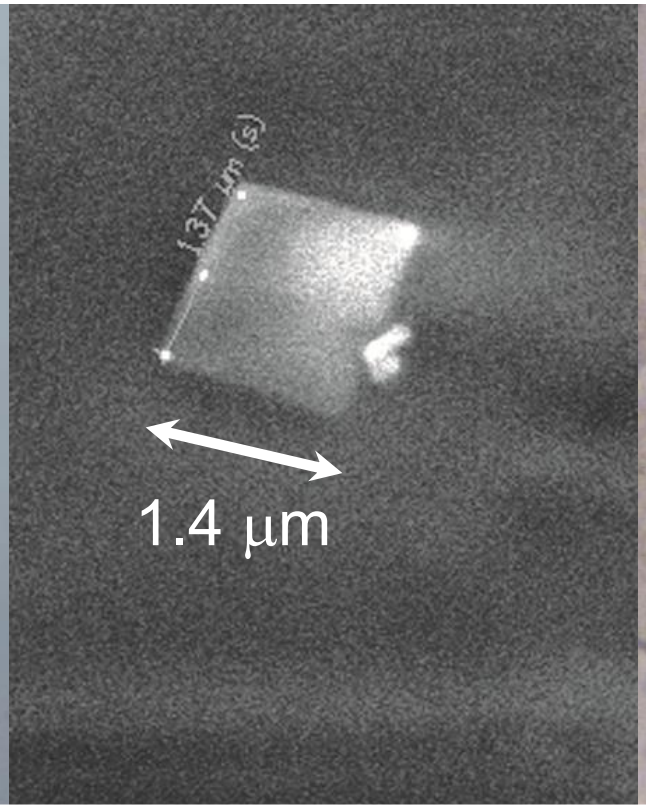
Rotation not possible

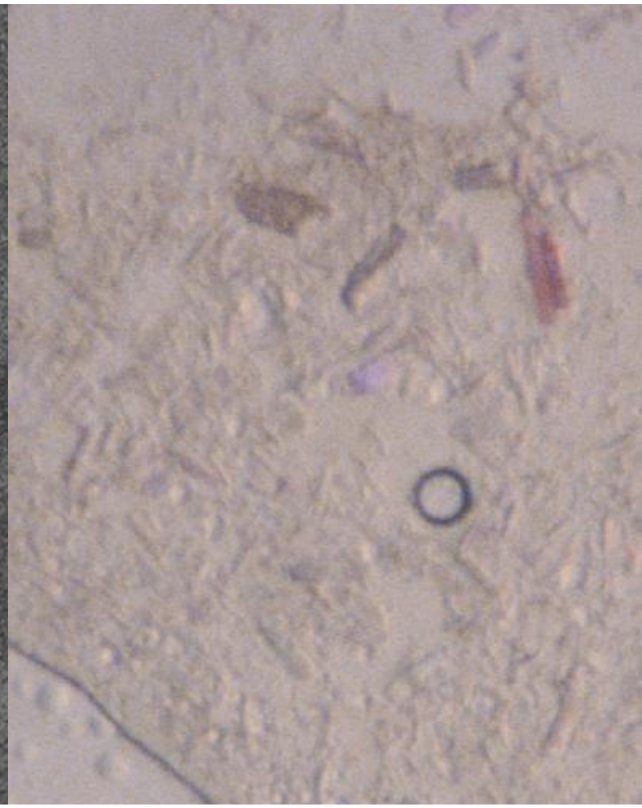
Membrane Protein Crystallisation





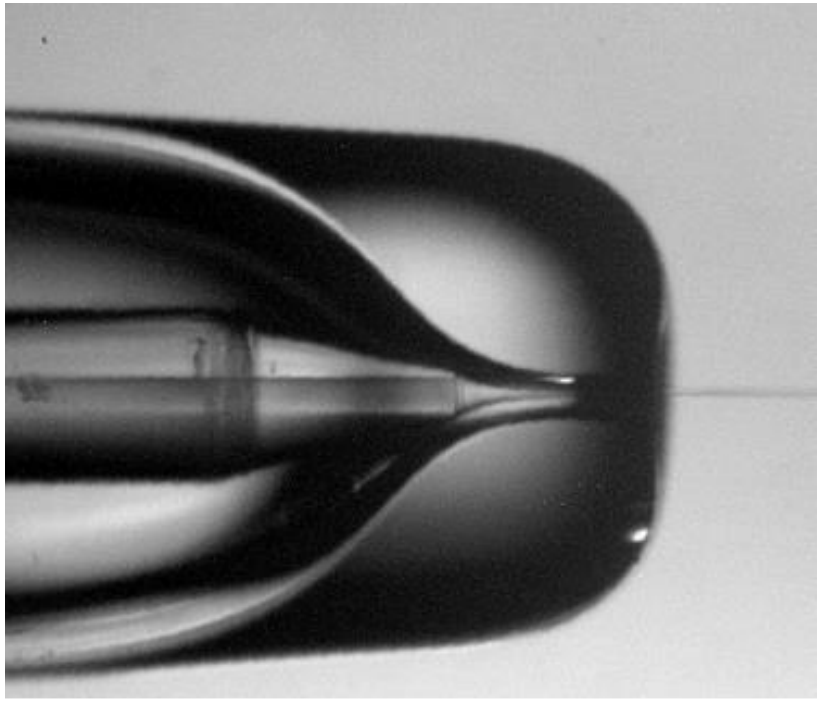
Micro-crystallization of *Bl. viridis* photosynthetic reaction centre

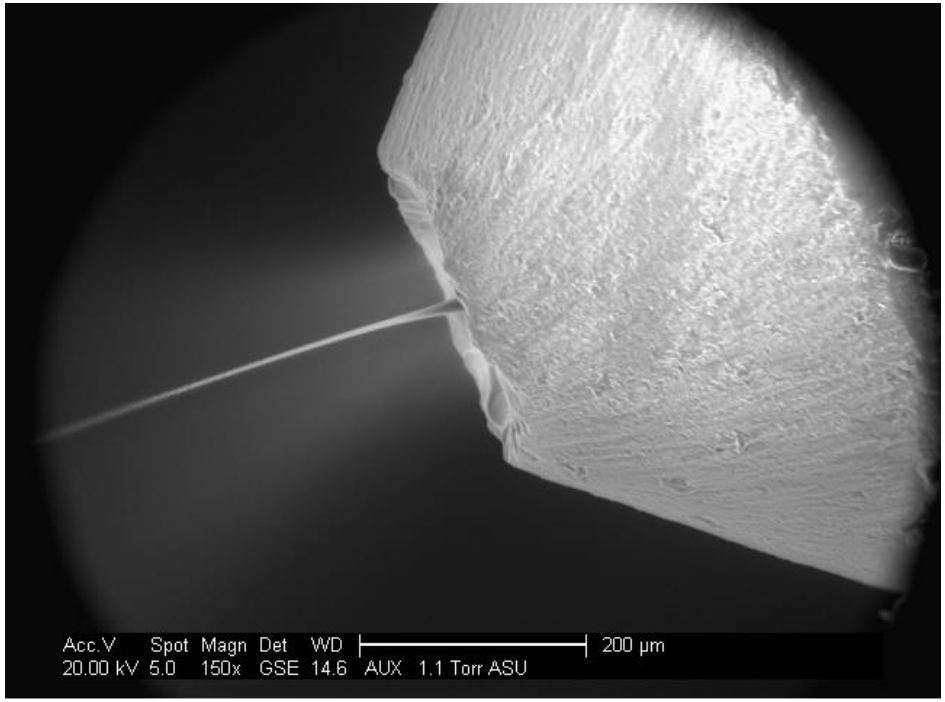




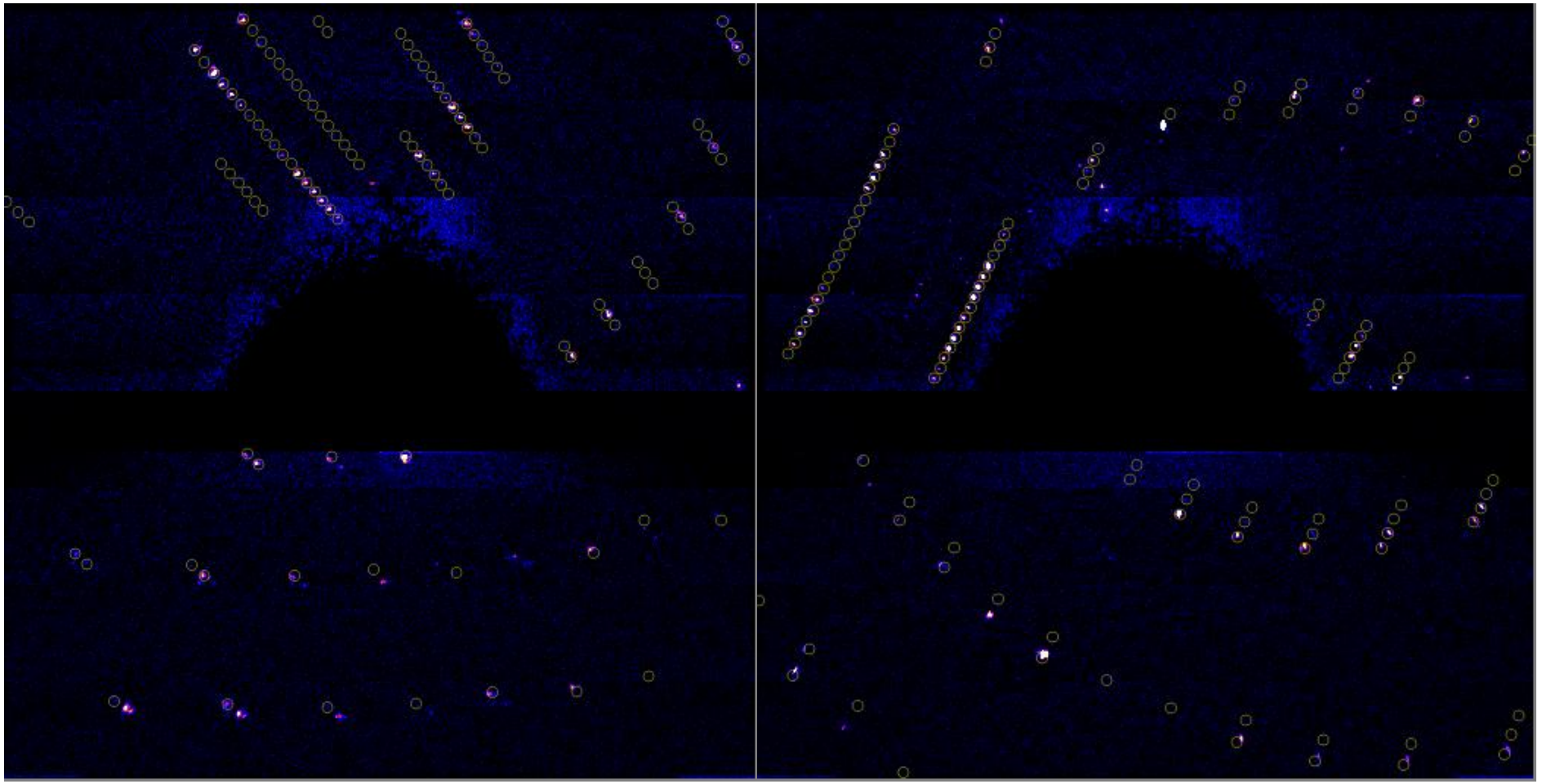
- Crystals grow in a lipidic sponge phase medium.
  - Linda Johansson, David Arnlund.

Micro-crystal injection





- Liquid jet ≤ 4 μm in diameter.
- Developed at Arizona state University.
  - John Spence, Uwe Weierstall, Bruce Doak, Petra Fromme, Dan DePonte, David Shapiro.



- Data processing algorithms developed at CFEL, Hamburg.
  - Henry Chapman, Thomas White, Anton Barty, Richard Kirian *et al.*
- ~1500 hits; 265 images processed; 90 minutes beamtime.

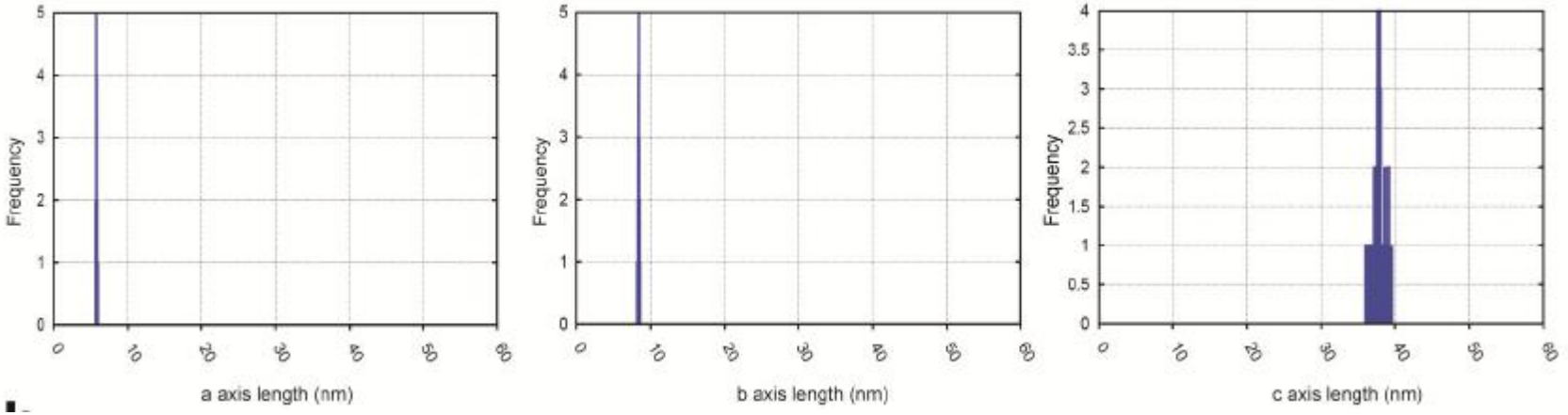
$P\ 4_3\ 2_1\ 2$, a=b=223 Å, c=114 Å

Deisenhofer, Michel et al
Nobel prize (1988)

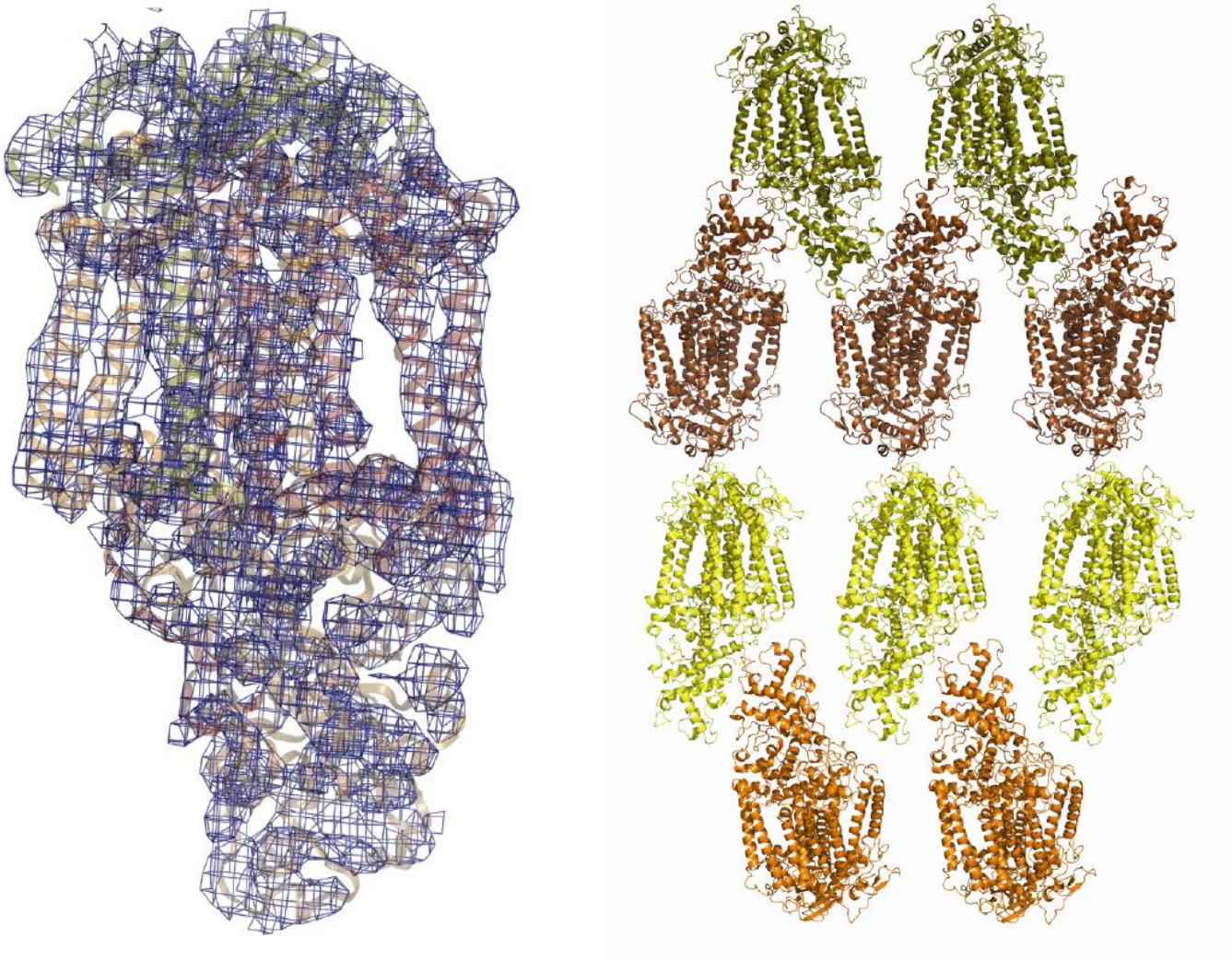
$P2_12_12$, a=85 Å, b=139 Å, c=178 Å, Large sponge phase crystals

Wöhri et al Biochemistry (2009), Wöhri et al Science (2010)

+ various unpublished RC crystal forms while trying to get the LHI-RC complex (at least 3 diffracting better than 5 Å)

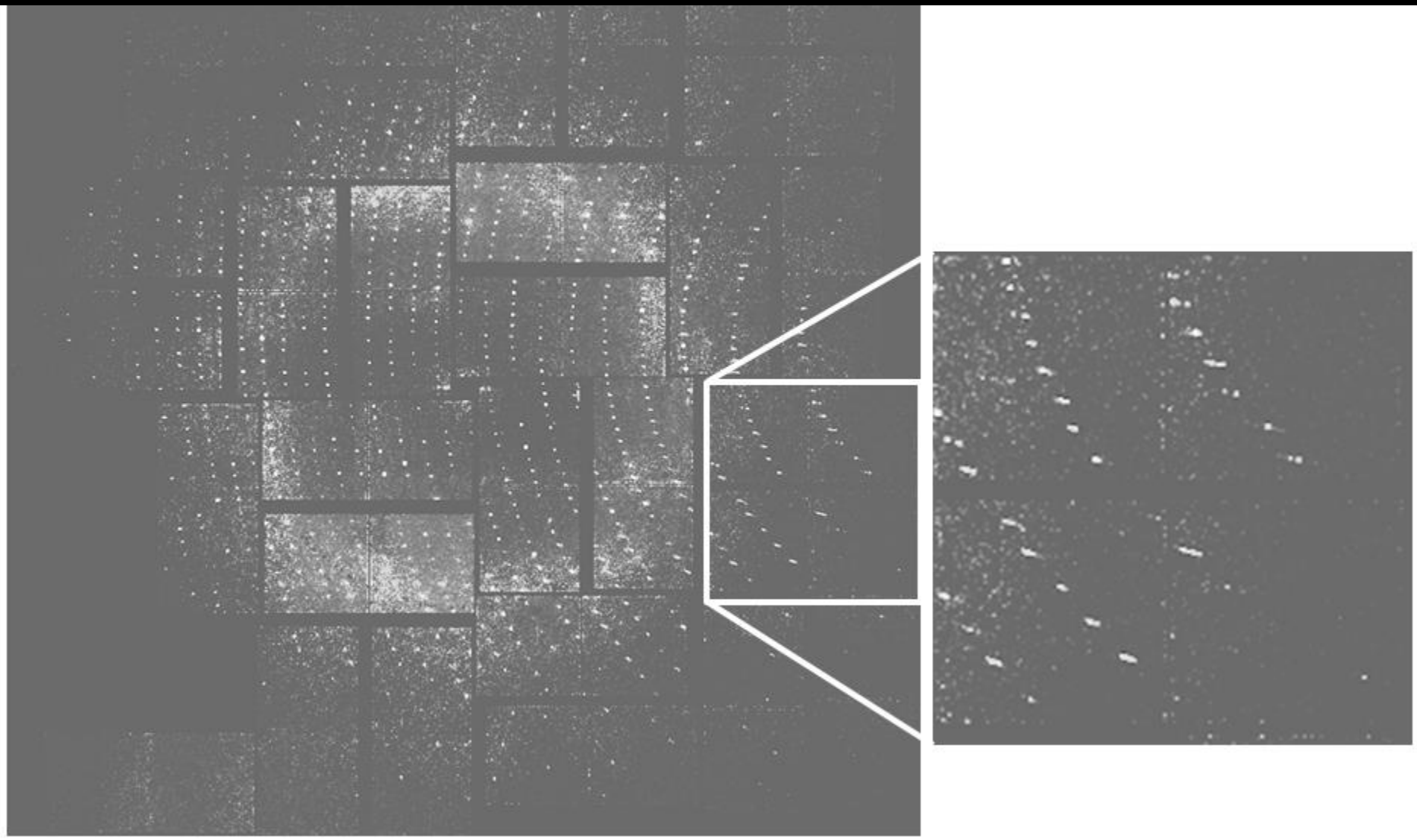


8.2 Å serial crystallography structure of a photosynthetic reaction center



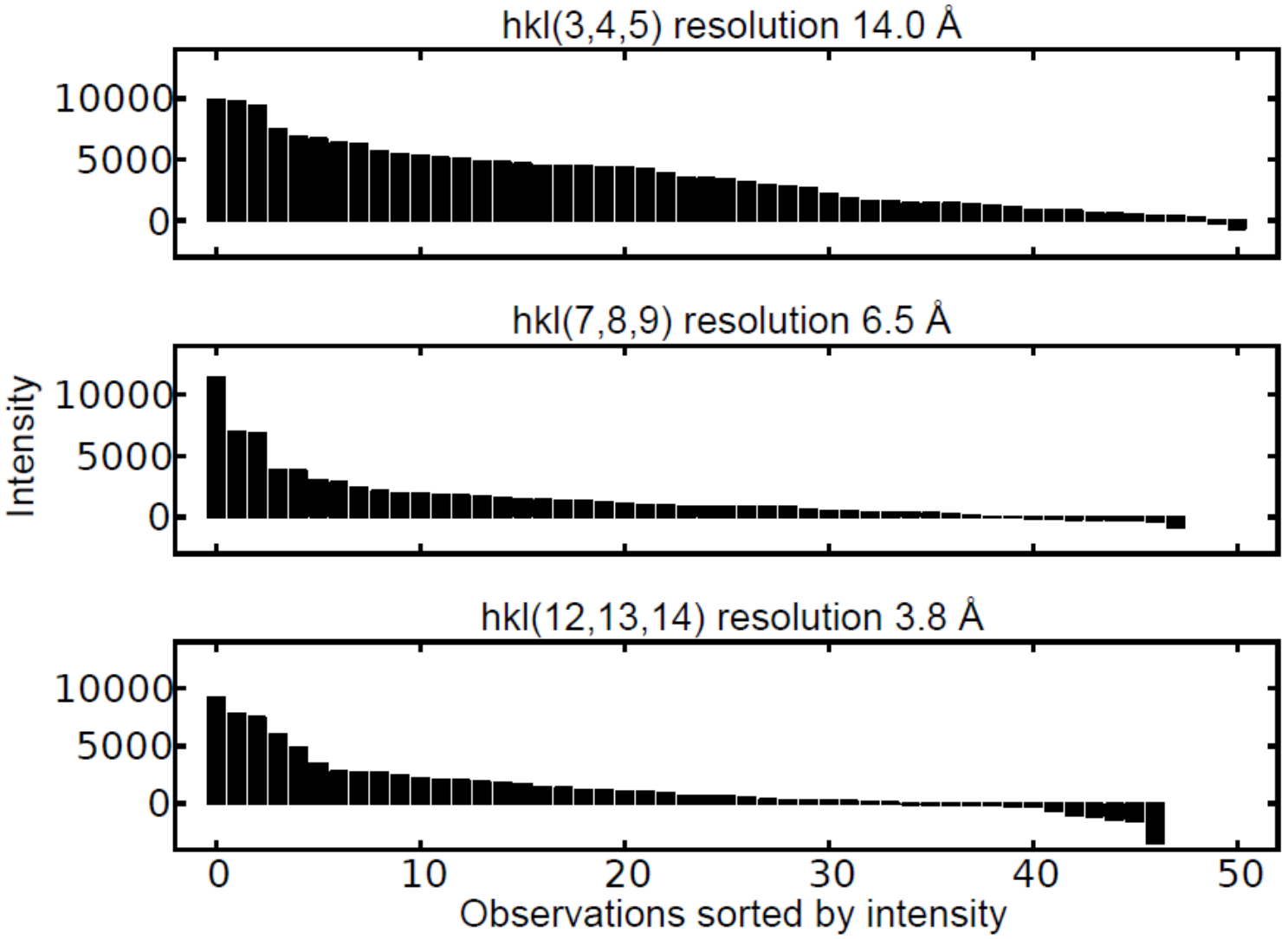
$P2_12_12_1$ a = 58 Å, b = 85 Å, c = 376 Å

Data extends to 2.8 Å resolution using 1.4 Å X-rays



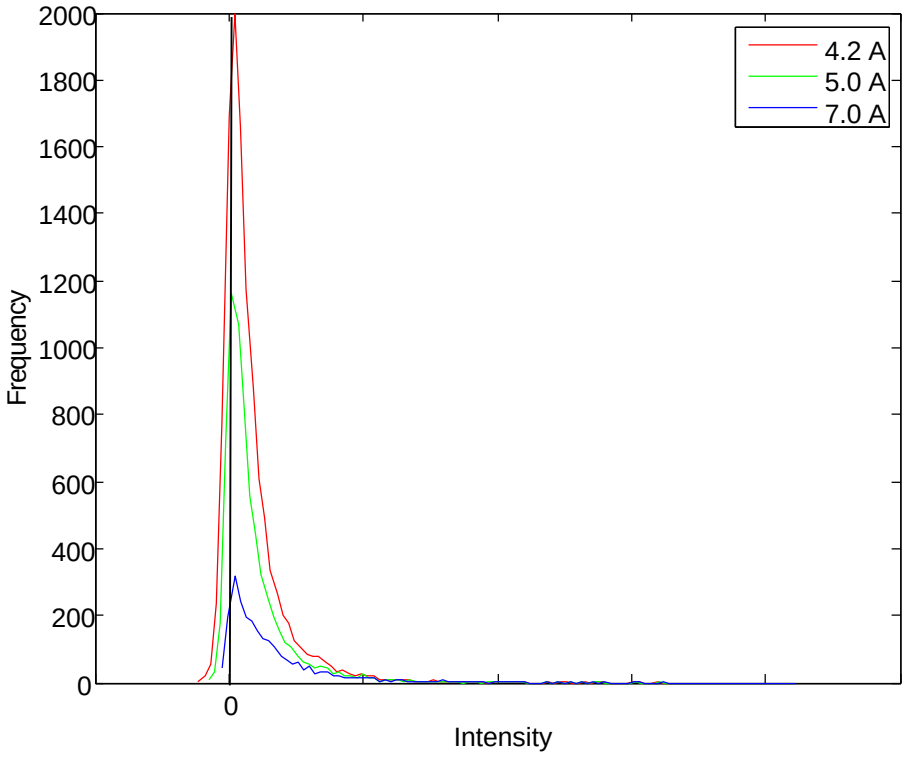
Data collection & refinement	
Total number of recorded images	2,744,614
Number of confirmed diffraction patterns	5,767
Number of indexed images	**1,175**
Space group	$P2_12_12_1$
a, b, c (Å)	57.9, 84.8, **384.3**
Completeness (%)	99.1 (93.4)
Multiplicity	**27.0** (27.6)
Overall R_{split} on I (%)	36.5 (52.7)
Mean I/σ(I)	3.50 (2.0)
$CC_{1/2}$ §	0.54 (0.32)
Refinement resolution limits (Å)	49.6 - 3.50
R_{work} /R_{free}	29.4 / 32.7

Representative reflections

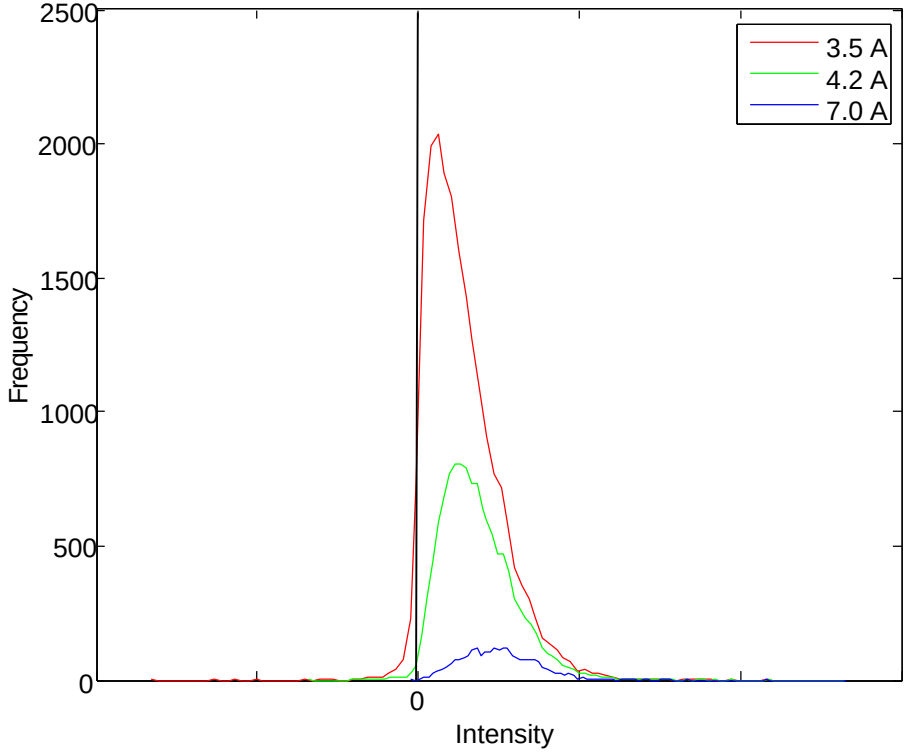


Intensity distribution of merged reflections (acentric)

Synchrotron data



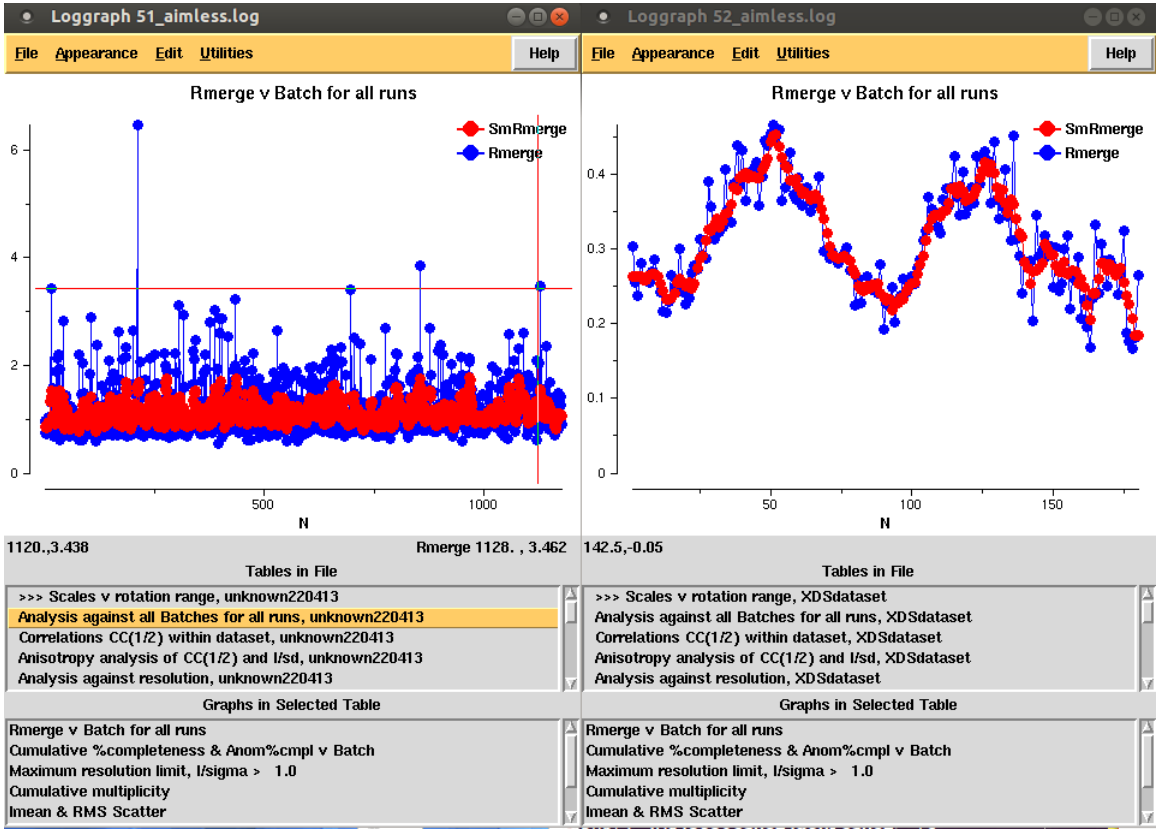
XFEL data



Rmerge vs batch

XFEL data

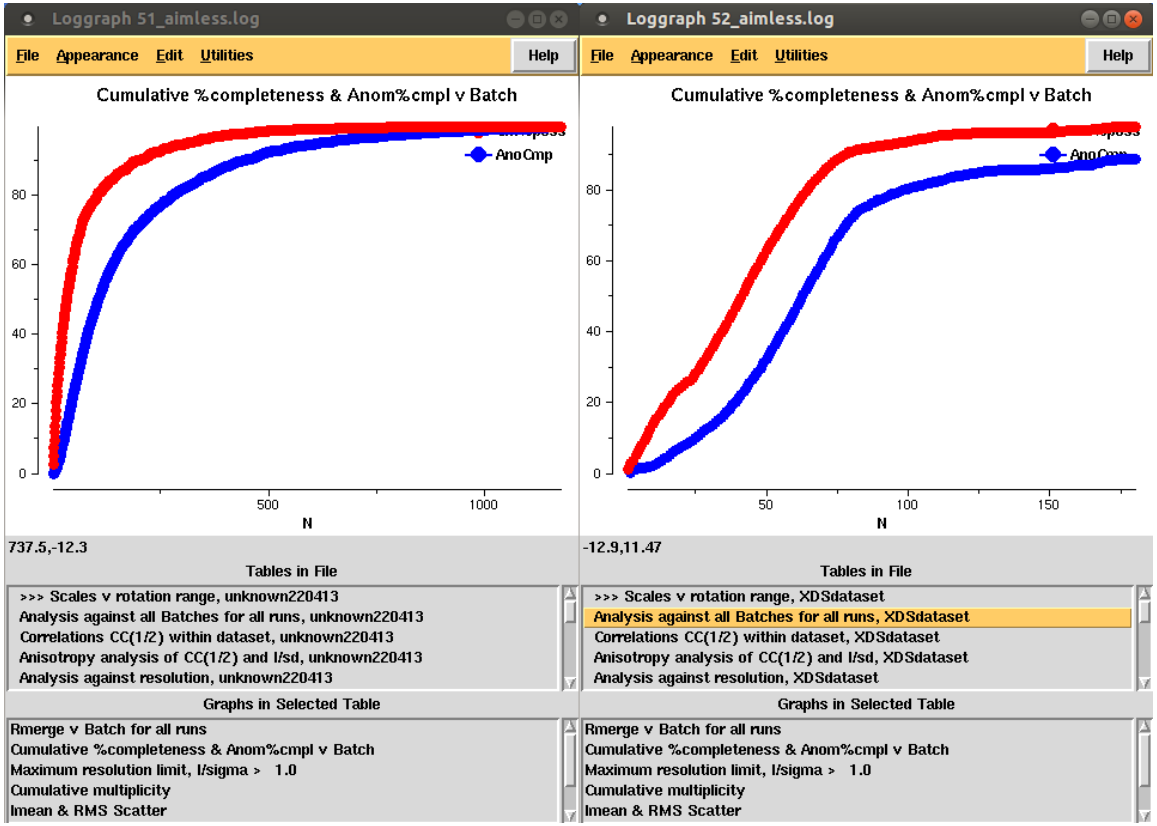
Synchrotron data



Completeness vs batch

XFEL data

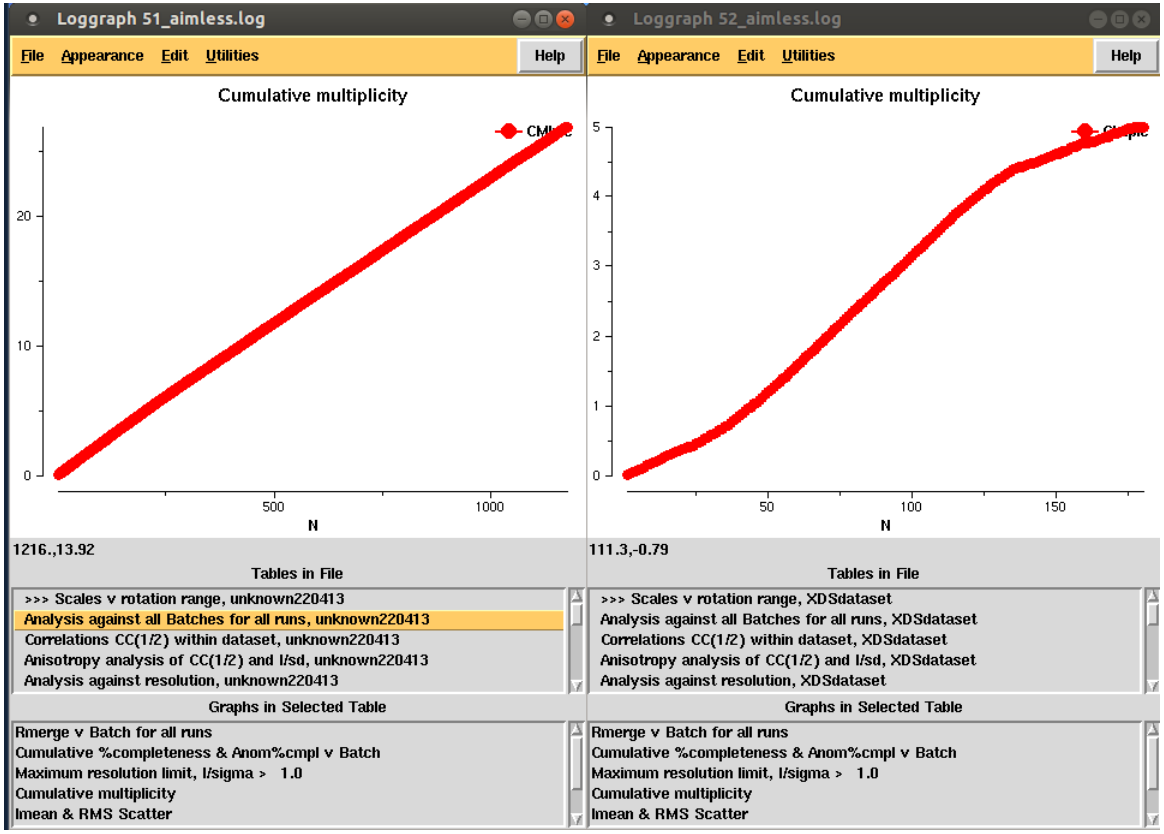
Synchrotron data



Cumulative multiplicity

XFEL data

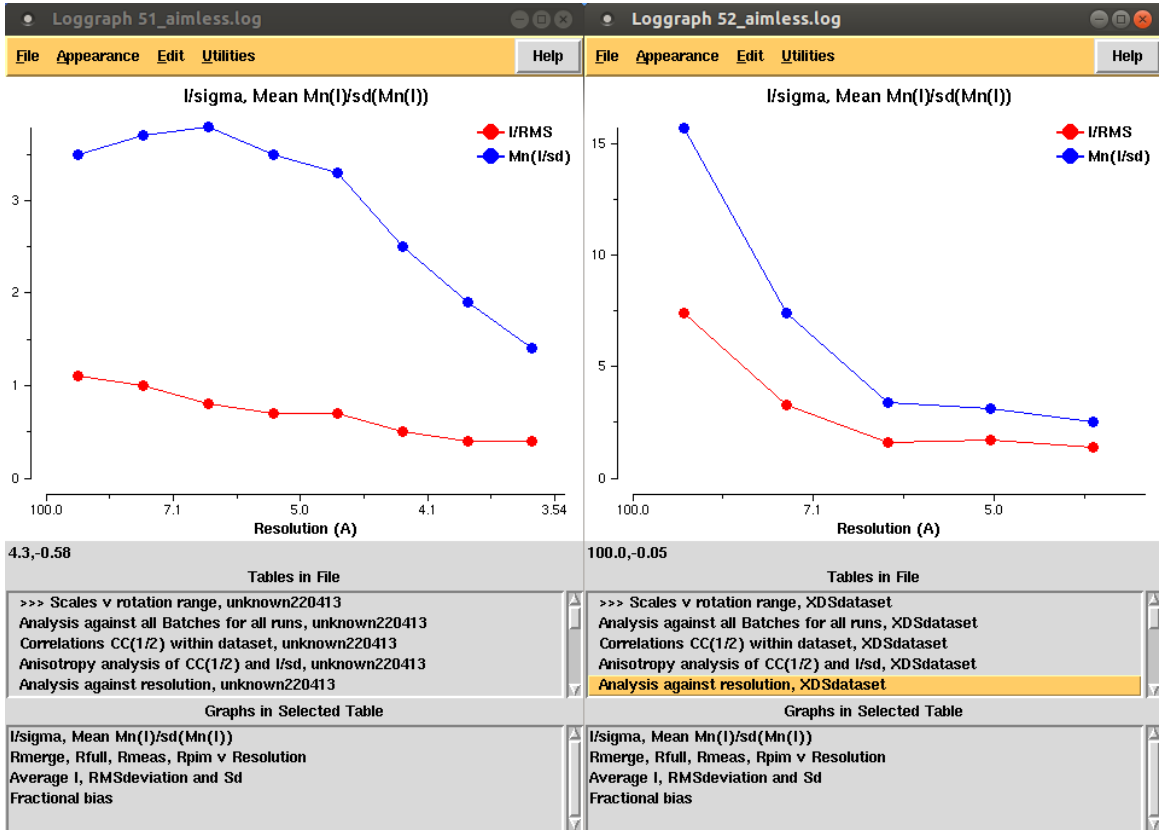
Synchrotron data



I/sigma vs resolution

XFEL data

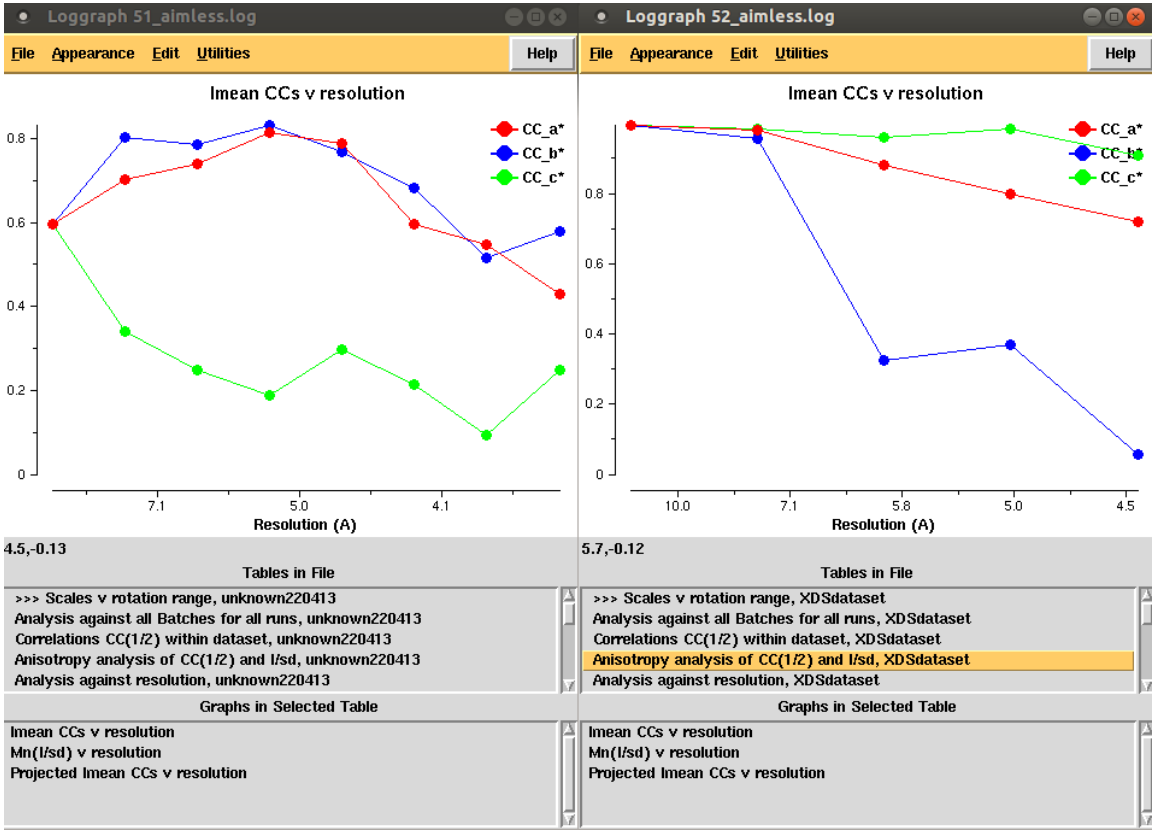
Synchrotron data



Anisotropy of CC1/2

XFEL data

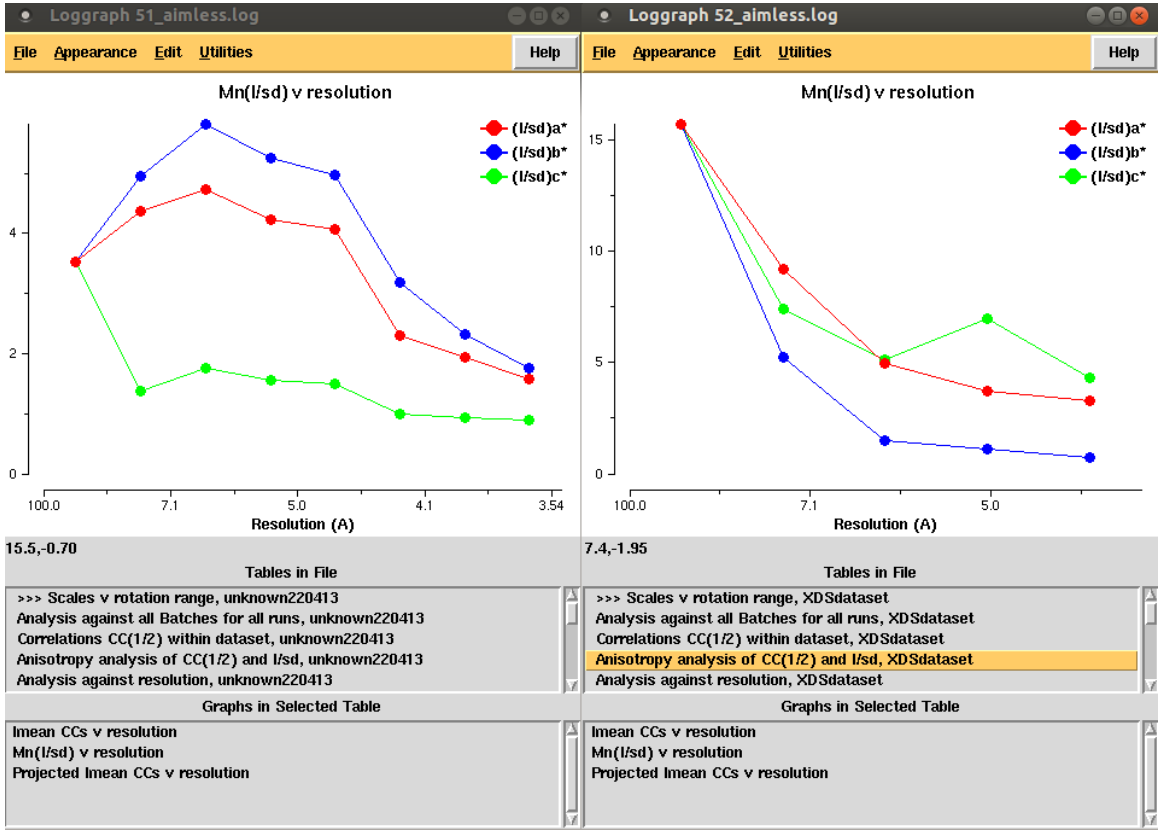
Synchrotron data



Anisotropy of I/sigma

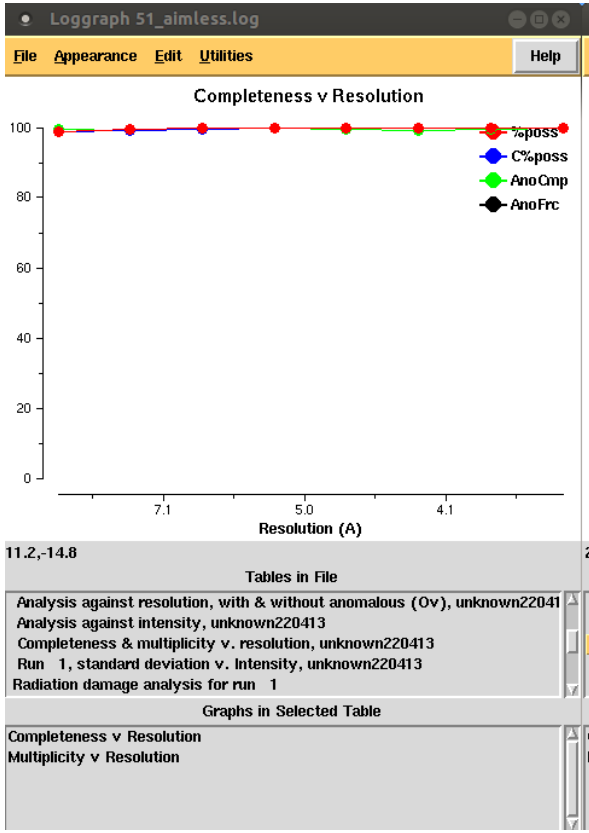
XFEL data

Synchrotron data

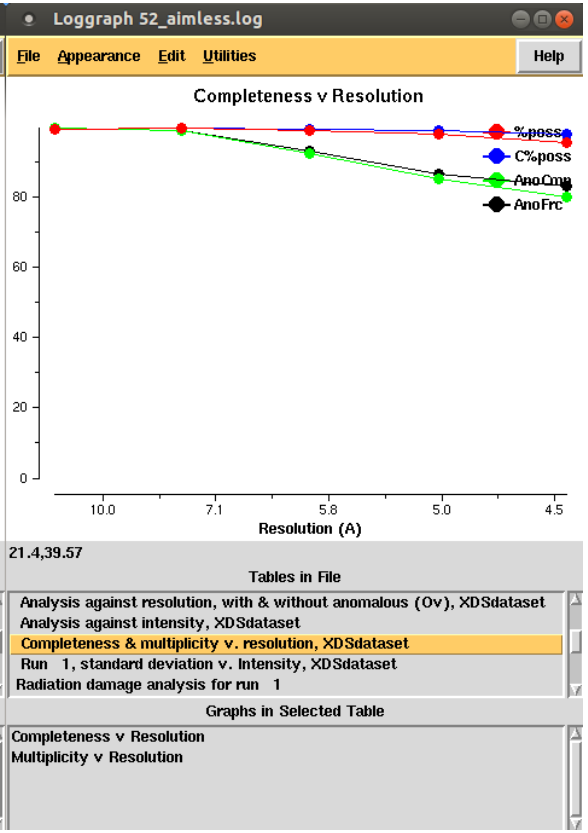


Completeness vs resolution

XFEL data



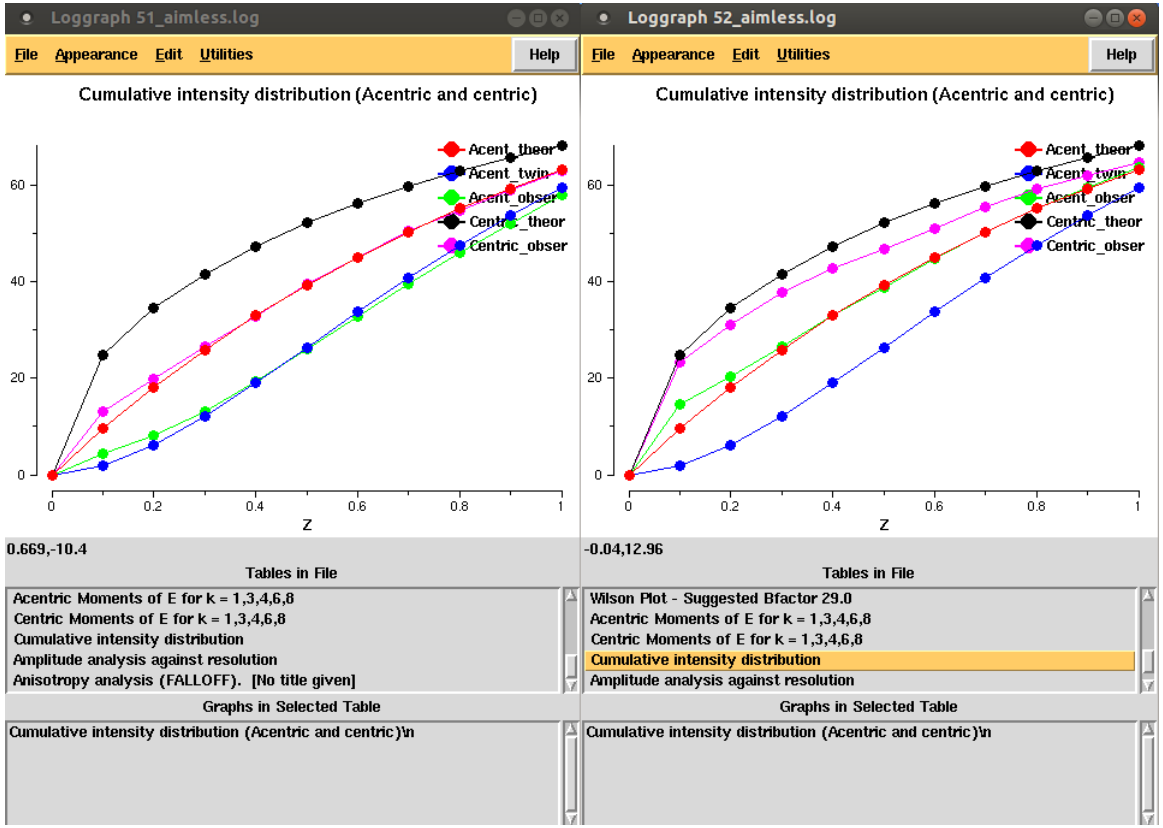
Synchrotron data



Cumulative intensity distribution

XFEL data

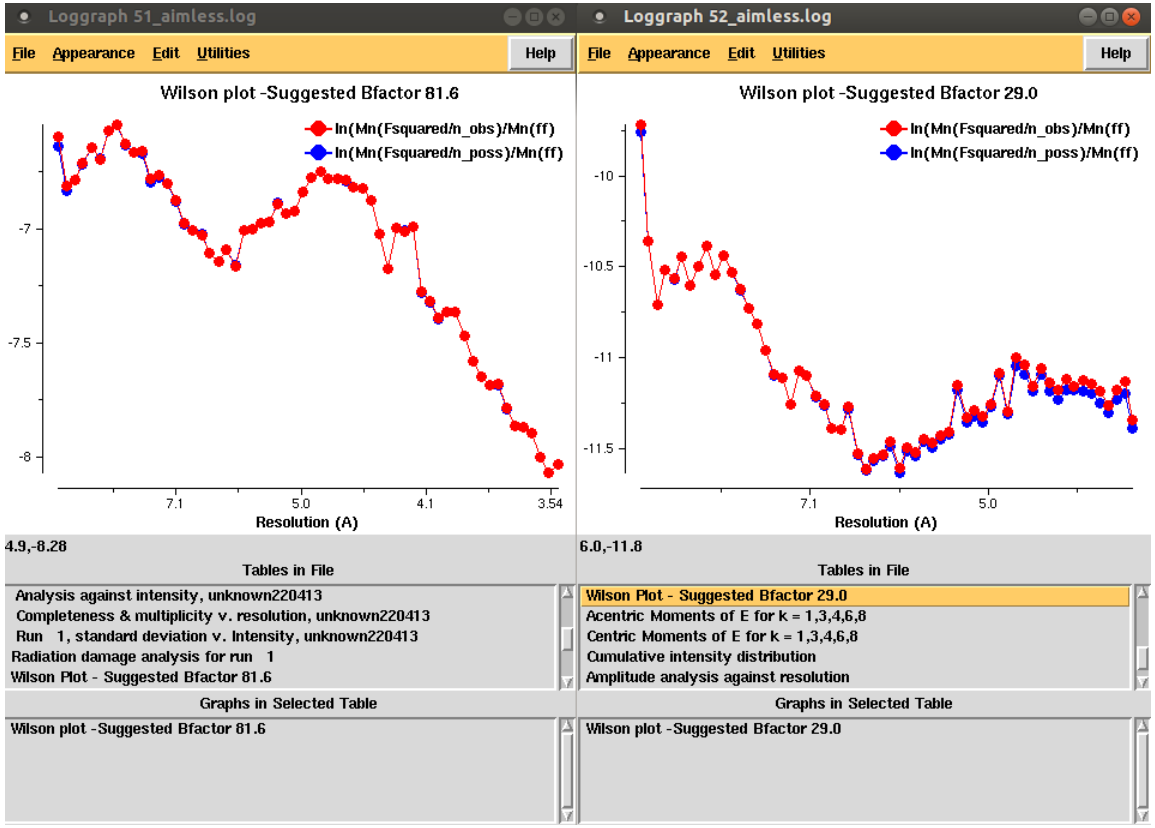
Synchrotron data



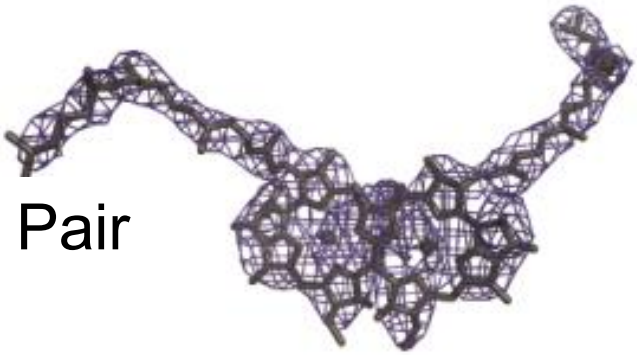
Wilson plot

XFEL data

Synchrotron data

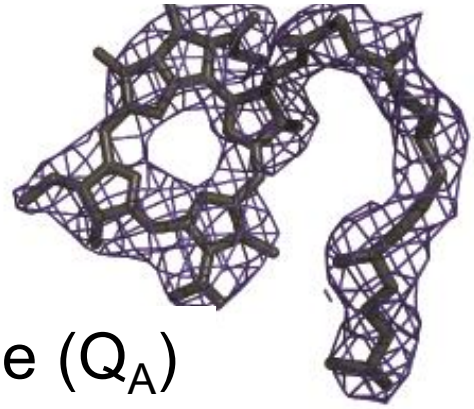


3.5 Å SFX structure



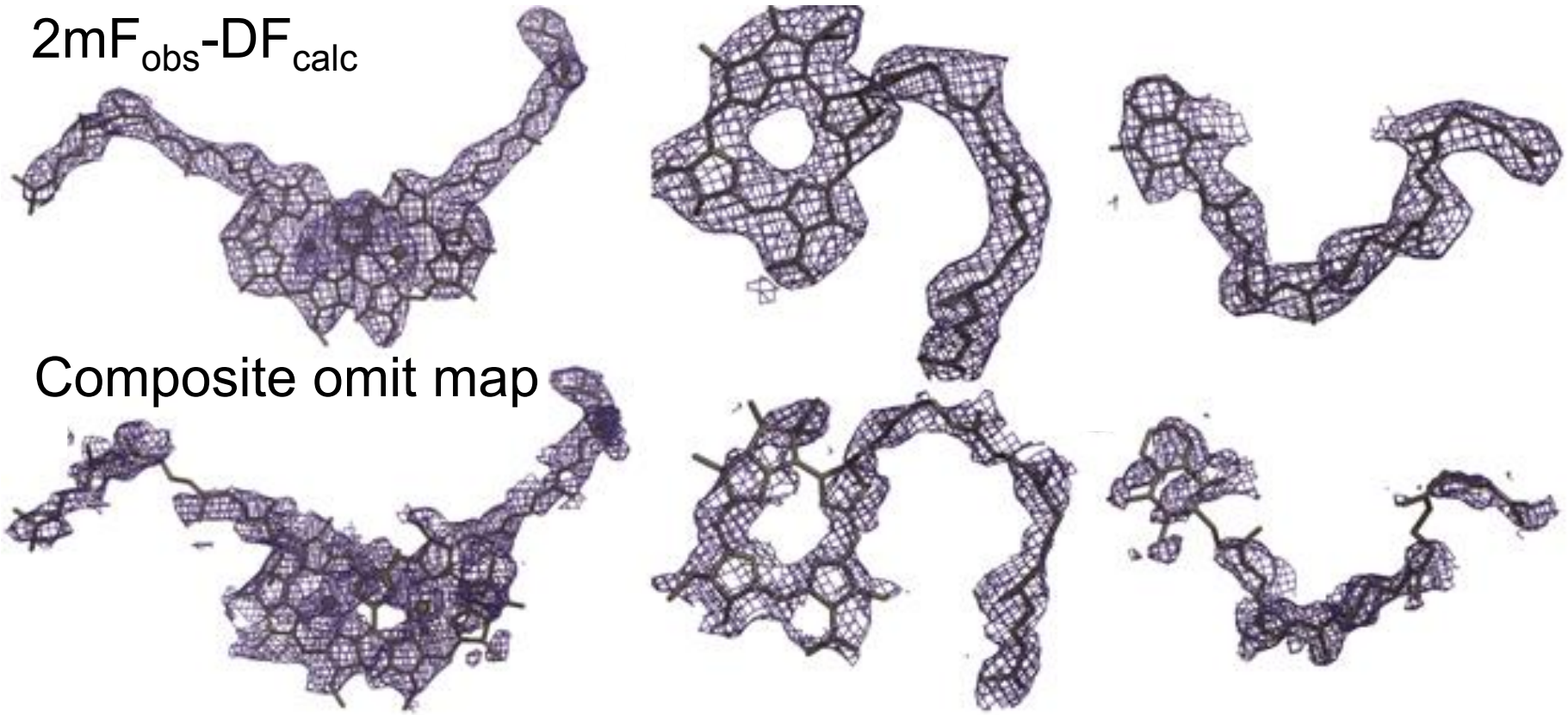
Special Pair

Pheophytin

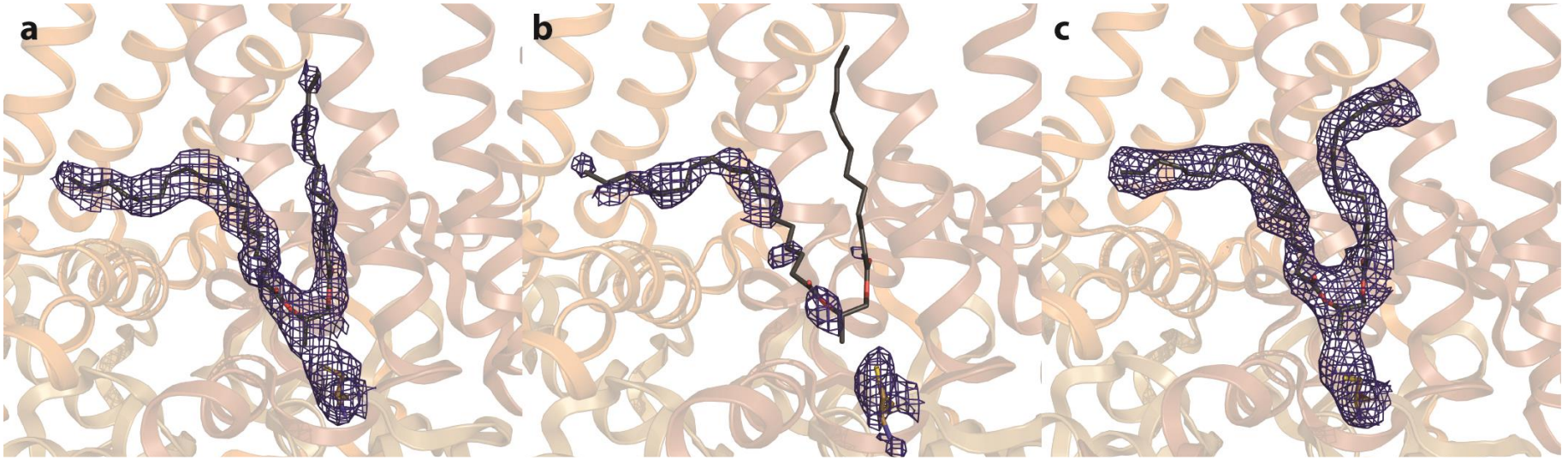


Menaquinone (Q_A)

Composite omit map



Thioether bond



100 K,
4.4 MGy

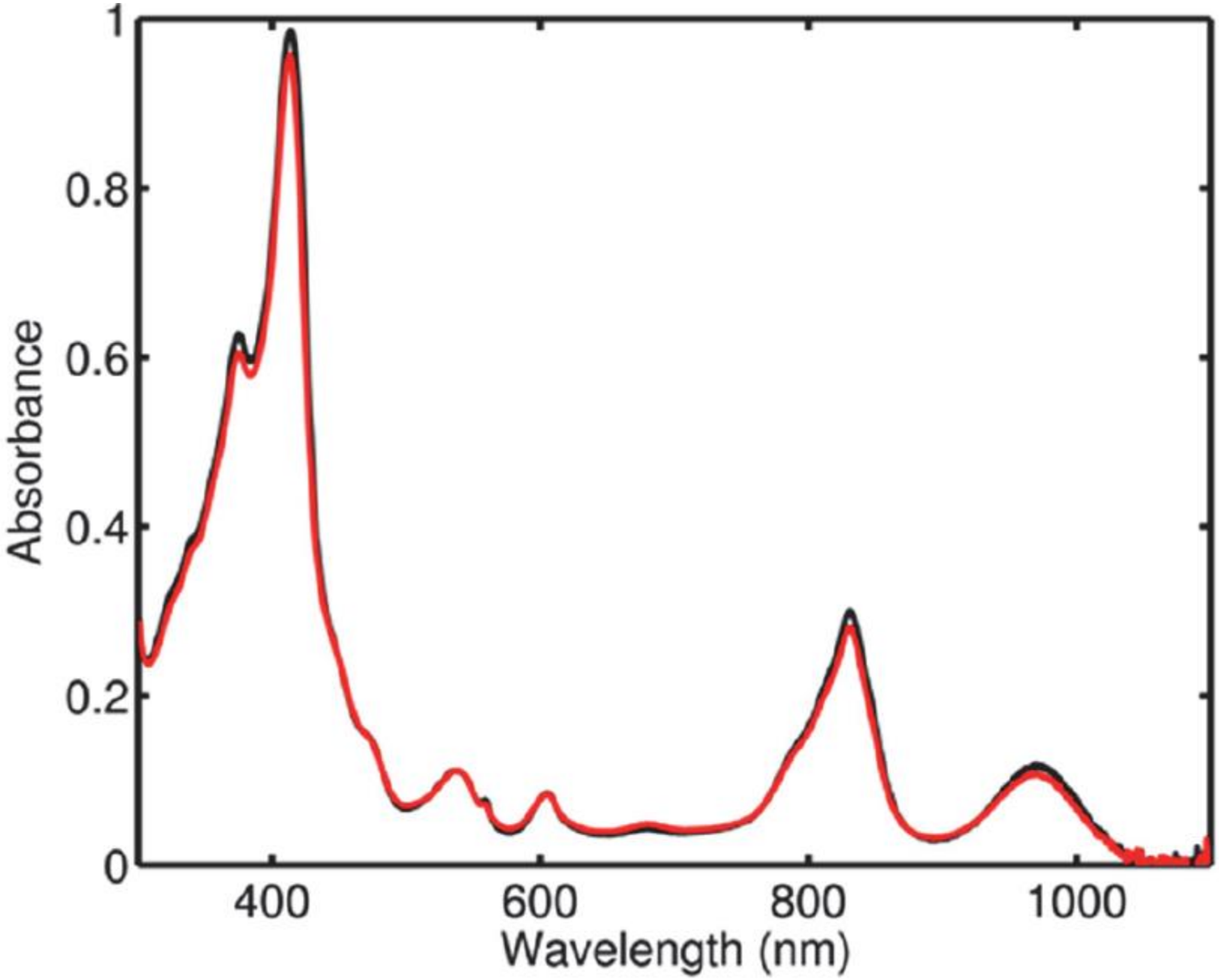
100 K,
77 MGy

RT SFX
33 MGy

- A thioether bond links the tetraheme subunit N-terminal cysteine to a diacylglycerol molecule.
  - Susceptible to radiation damage.

Opinion (Conclusion?)

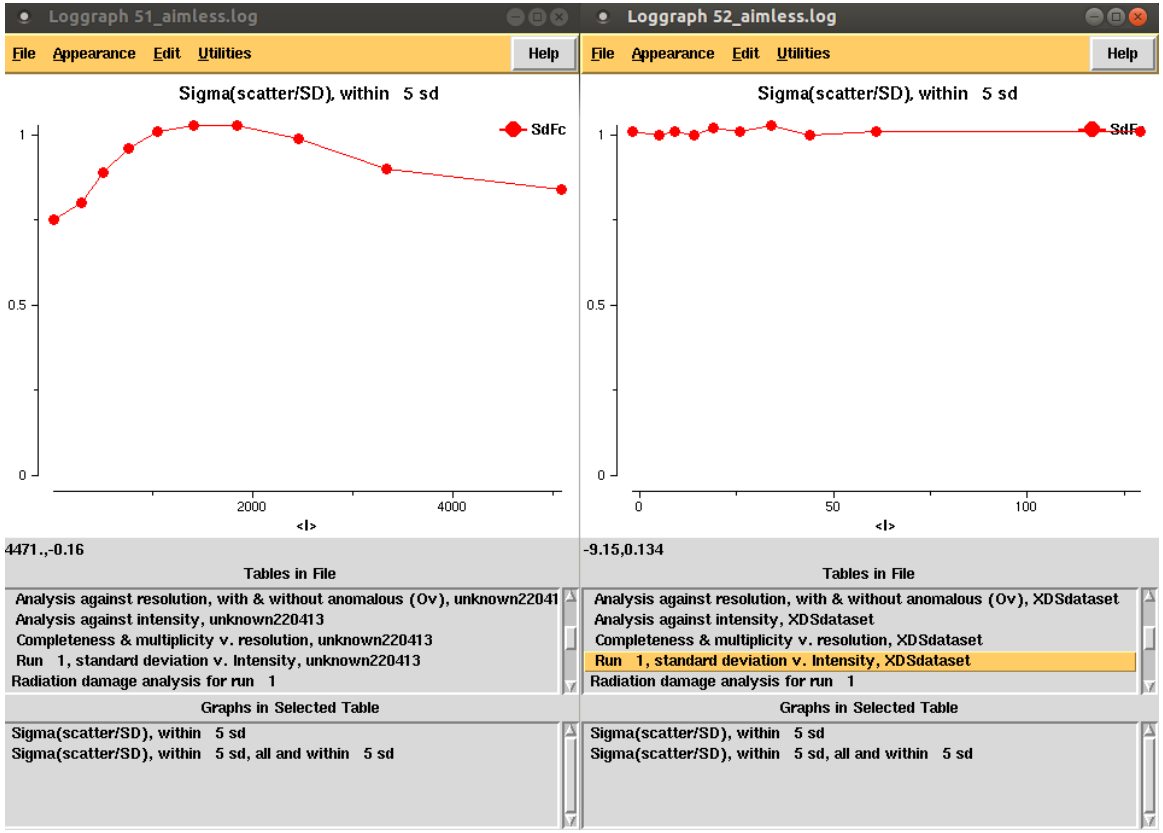
- Oscillation data collection is just one of the many ways reflection intensities can be estimated
  - What are the most useful alternatives for synchrotrons?
- Cross crystal averaging often advantageous
- Radiation damage much less apparent
  - SFX better
    - Microseconds to Henderson, more intense problematic
- Serial crystallography for pump-probe studies of irreversible reactions
  - Intense microfocus beamlines will be very useful
  - Detector readout rate need to keep up with intensity


Absorbance
1
0.8
0.6
0.4
0.2
0
400
600
800
1000
Wavelength (nm)

Sigma correction

XFEL data

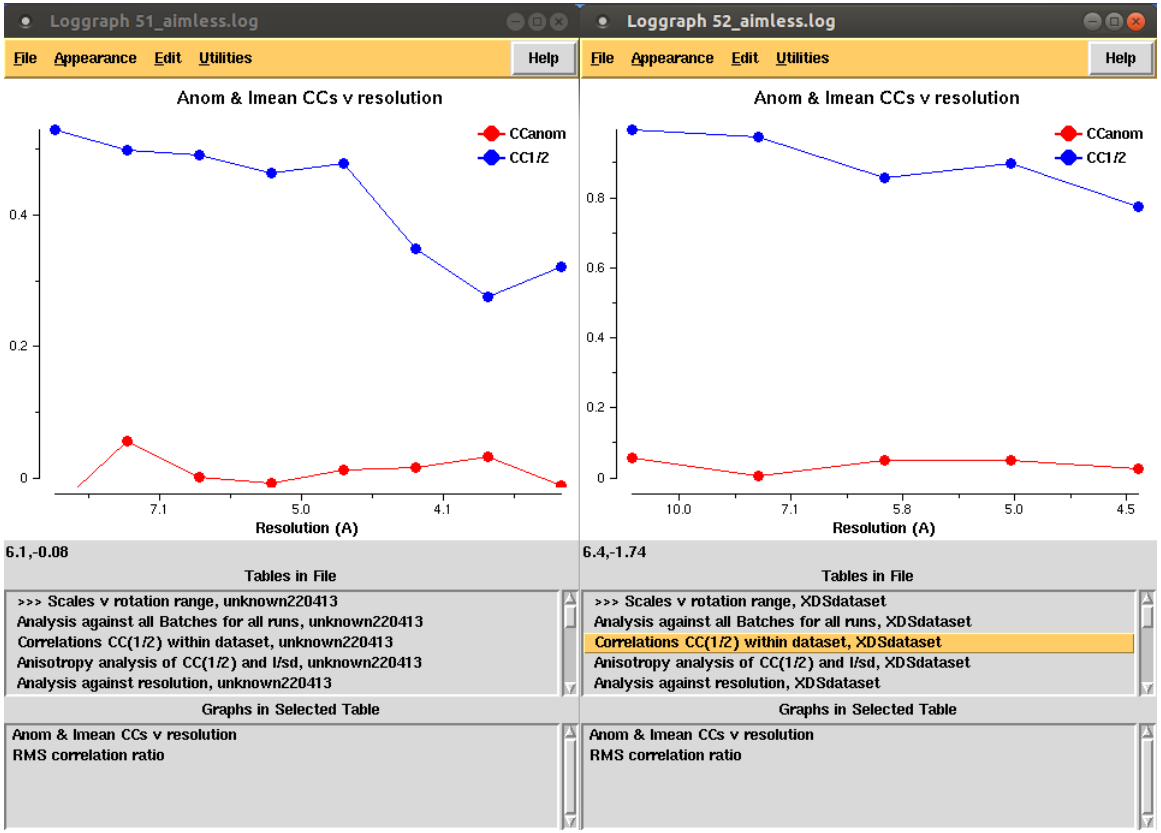
Synchrotron data



CC1/2 vs resolution

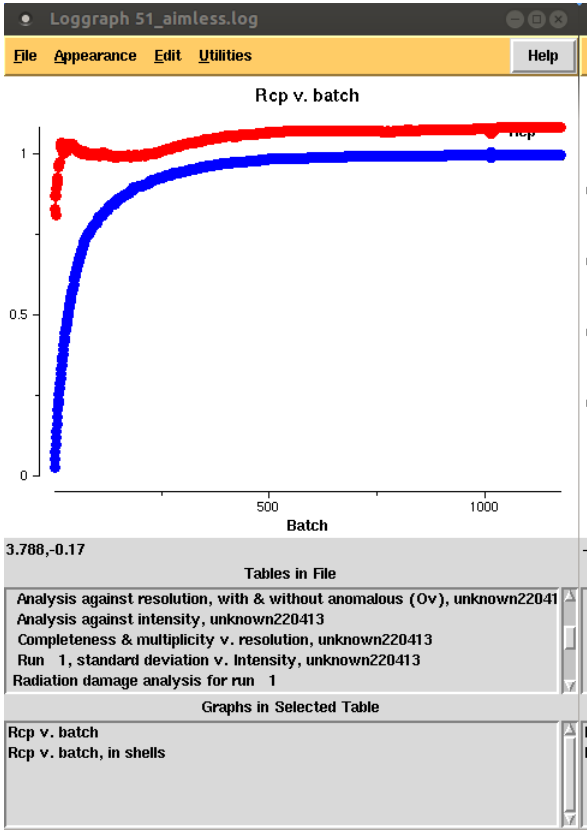
XFEL data

Synchrotron data

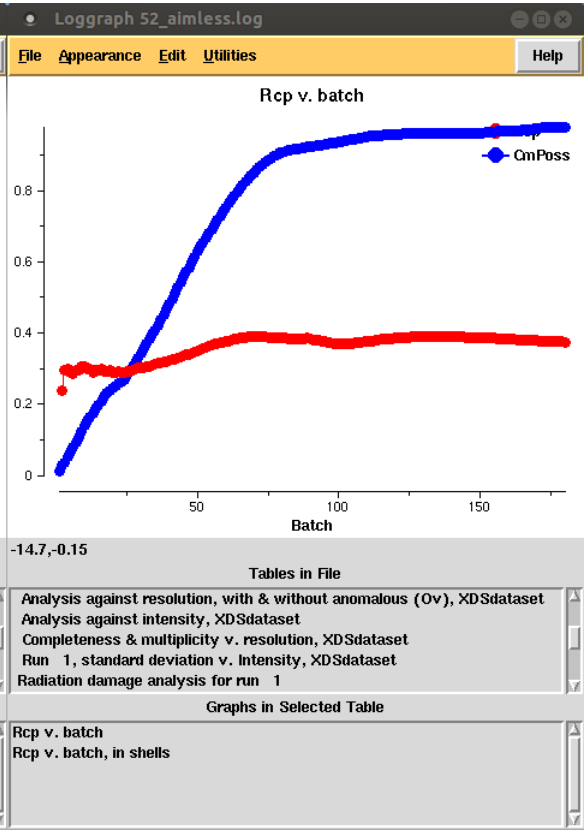


Rcp vs batch

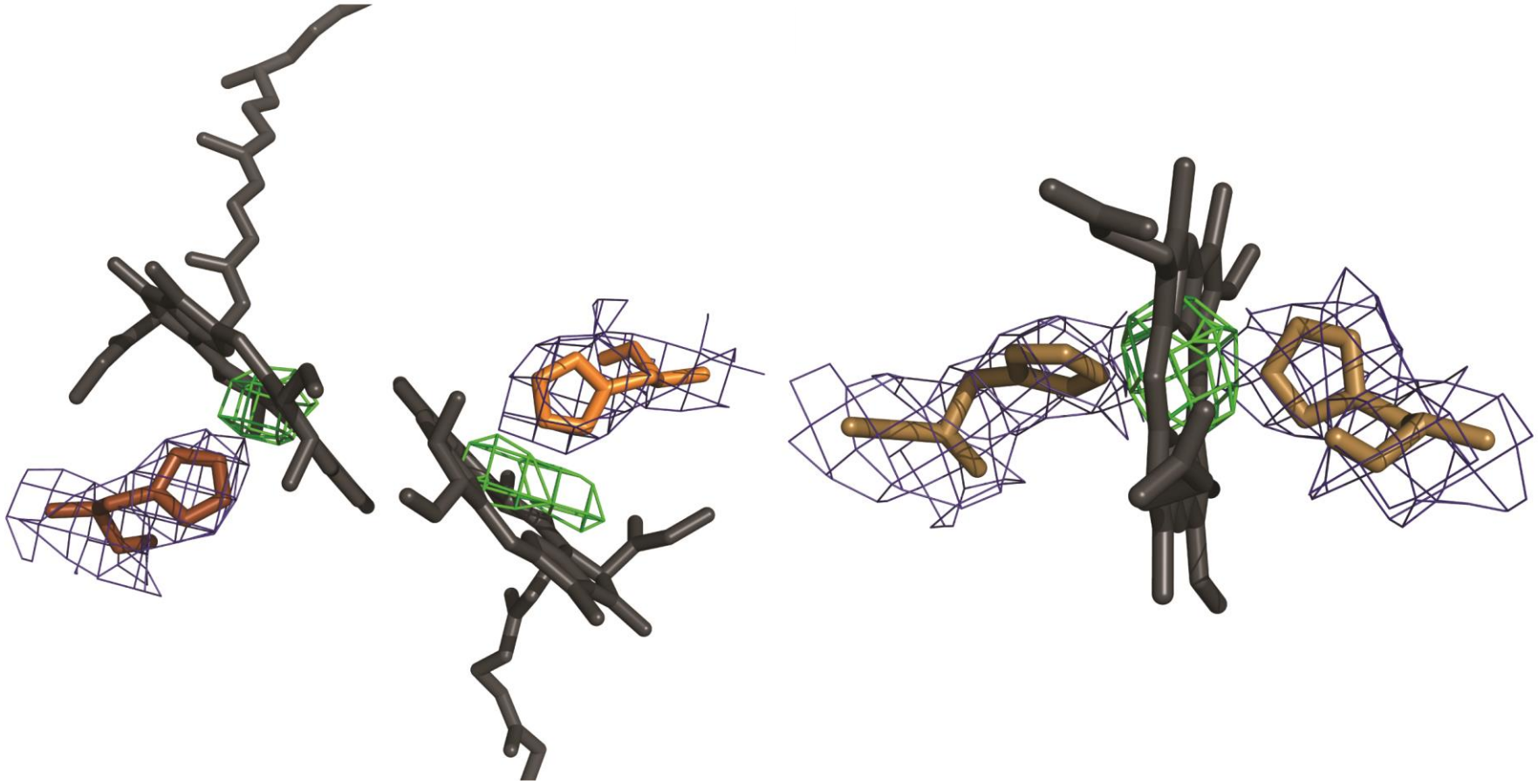
XFEL data



Synchrotron data



Metal Centres



Green: mF_{obs}-DF_{calc} calculated with Mg^{2+} & Fe^{2+} ions removed.