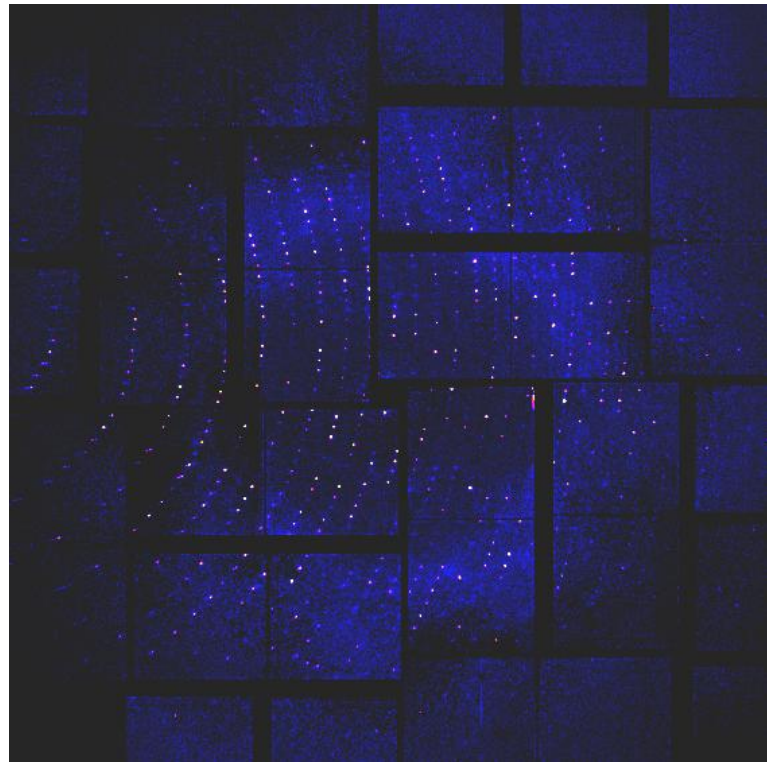


# *Structure determination of a photosynthetic reaction centre by serial femtosecond crystallography*

Gergely Katona

Department of Chemistry  
University of Gothenburg



# SFX collaboration

|                      |                                                                                                                                                                                                                                        |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Göteborg</b>      | <b>R. Neutze, L. Johansson, D. Arnlund</b> , E. Malmerberg C. Wickstrand, J. Sjöhamn, A. Sharma.                                                                                                                                       |
| <b>CFEL-DESY</b>     | <b>H. Chapman</b> , 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, |
| <b>ASU</b>           | <b>J. Spence, P. Fromme</b> , U. Weierstall, B. Doak, M. Hunter, R. Kirian, X. Wang, K. Schmidt, I. Grotjohann, R. Fromme                                                                                                              |
| <b>SLAC-PULSE</b>    | M. Bogan, D. Starodub, R. Sierra, C. Hampton, D. Loh                                                                                                                                                                                   |
| <b>SLAC-LCLS</b>     | <b>S. Boutet, G. Williams</b> , M. Seibert, J. Kryzwiniski, C. Bostedt, M. Messerschmidt, J. Bozek, W. White, R. Coffee                                                                                                                |
| <b>Uppsala</b>       | J. Hajdu, Nic Timneanu, J. Andreasson, M. Seibert, F. Maia, M. Svenda, <b>J. Davidsson</b>                                                                                                                                             |
| <b>MPG CFEL ASG</b>  | <b>I. Schlichting</b> , R. Shoeman, L. Lomb, S. Kessemeier, 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                         |
| <b>LLNL</b>          | S. Hau-Riege, M. Frank                                                                                                                                                                                                                 |
| <b>CAMP Team</b>     | Led by <b>J. Ullrich</b> and <b>I. Schlichting</b>                                                                                                                                                                                     |
| <b>LCLS detector</b> | C. Kenney, R. Herbst, J. Pines, P. Hart, J. Morse                                                                                                                                                                                      |
| <b>Accelerator</b>   | Led by <b>P. Emma</b>                                                                                                                                                                                                                  |

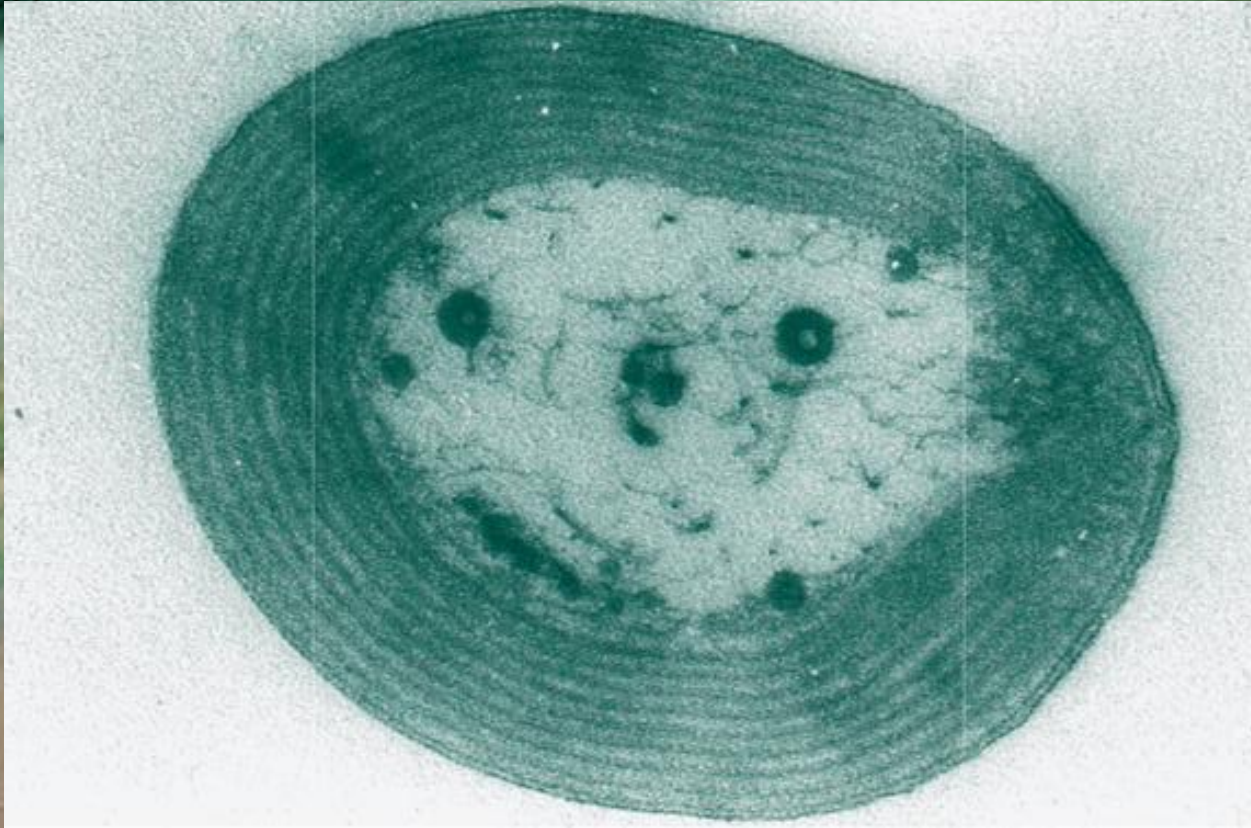


# Photosynthetic Proteins

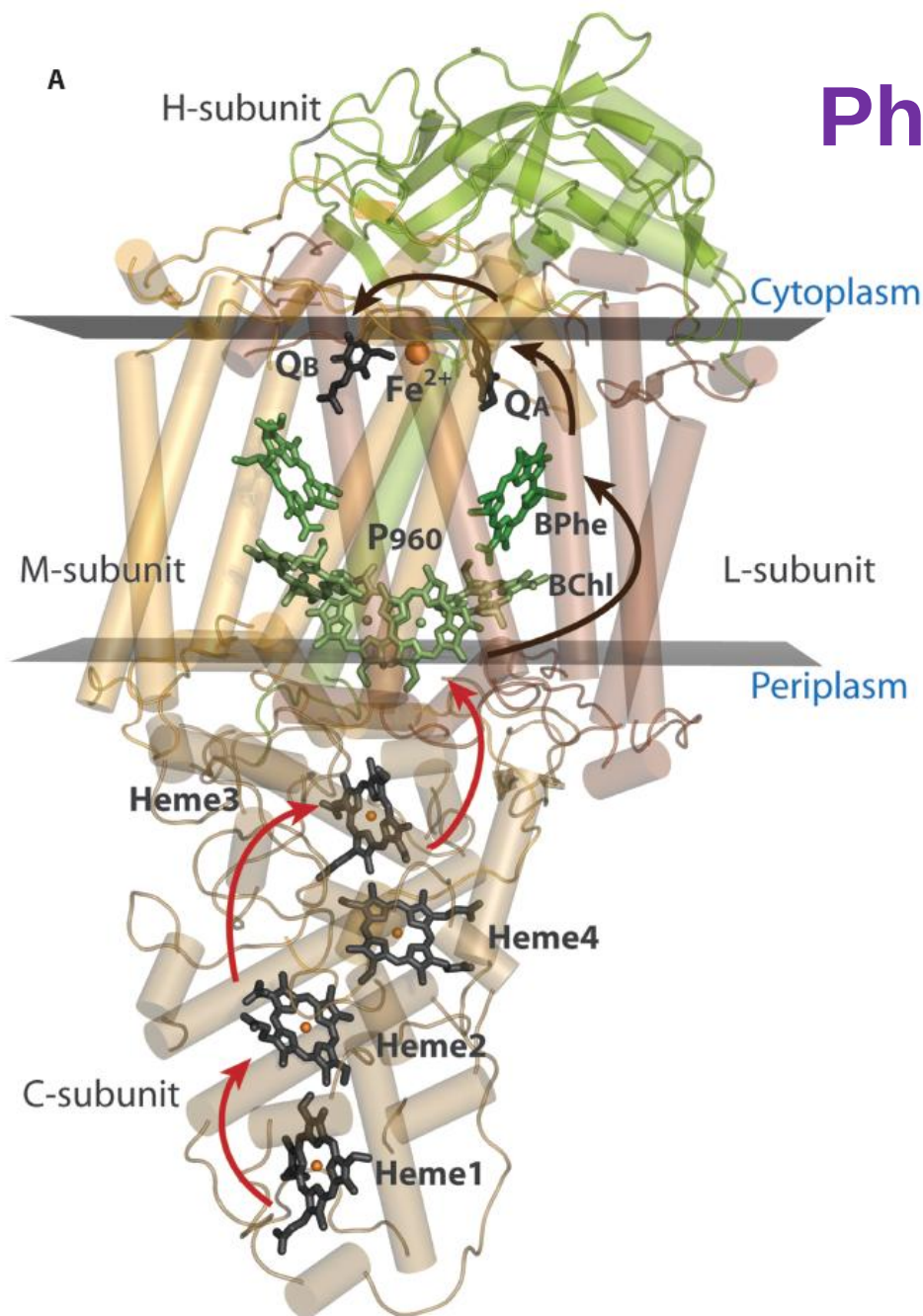




# Photosynthetic Bacteria



Blooming purple sulfur bacteria in a coastal lagoon

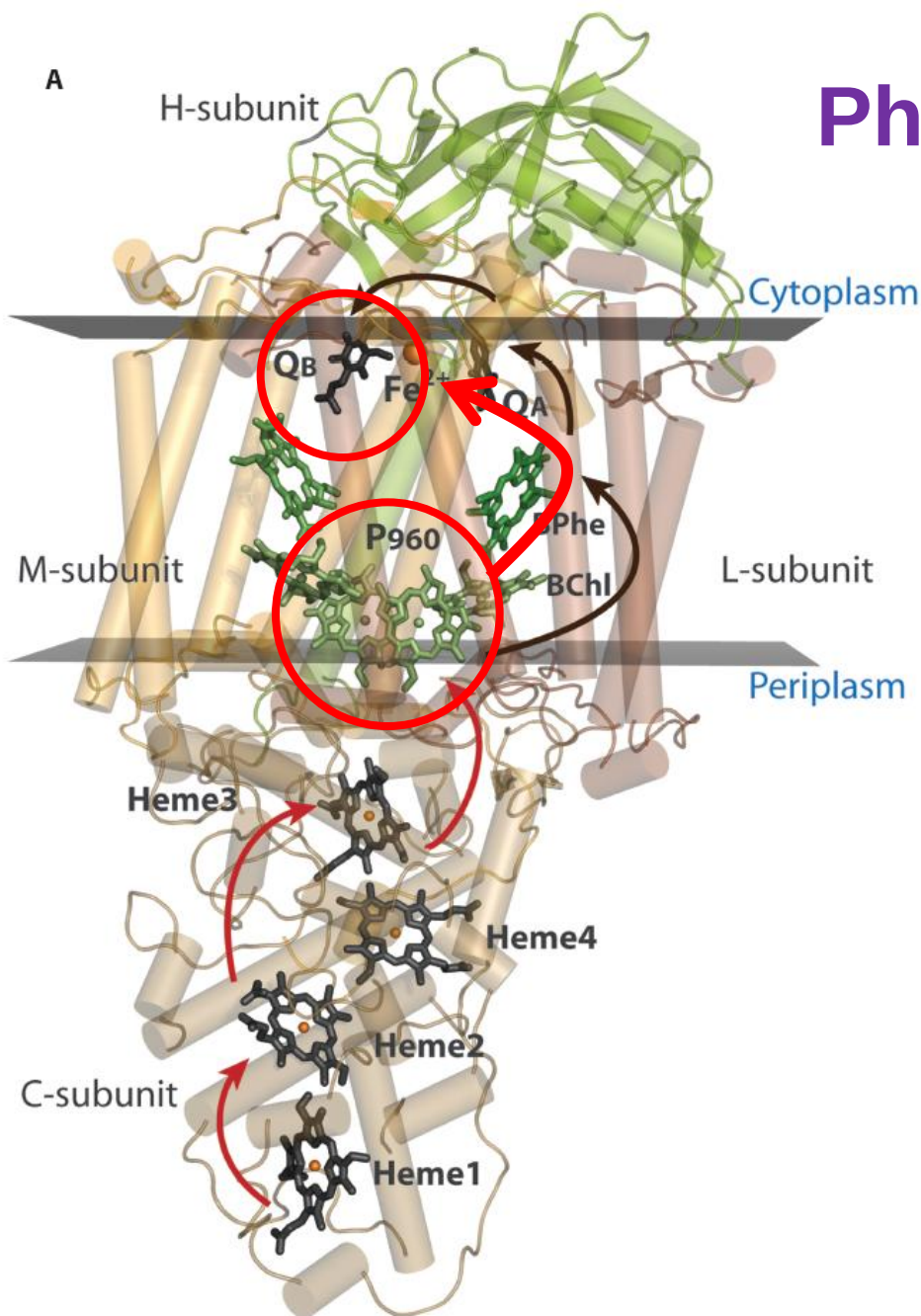


# Photosynthetic Reaction Centre

- 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



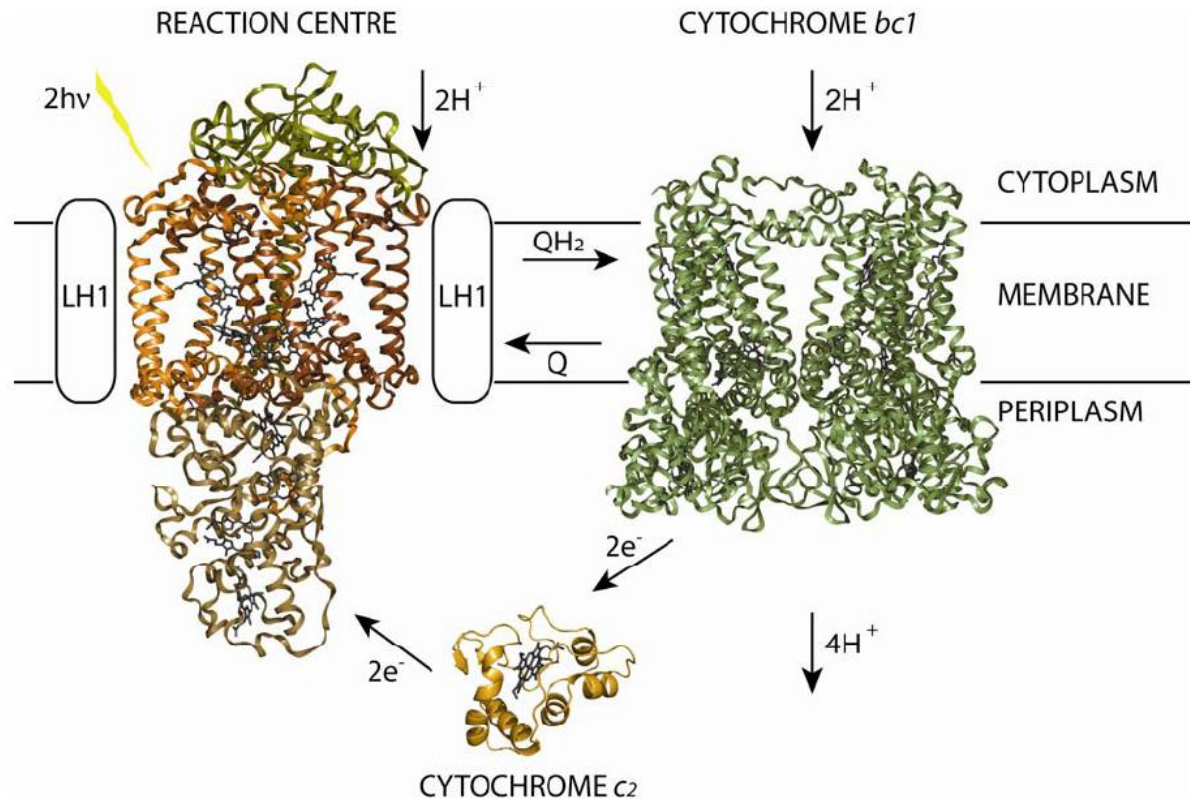
# Photosynthetic Reaction Centre



- Light photo-excites special pair of chlorophylls (P<sub>960</sub>).
- Electron transferred to mobile quinone (Q<sub>B</sub>).
- 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<sub>2</sub> rich atmosphere.





# Linac Coherent Light Source (LCLS) Stanford, USA

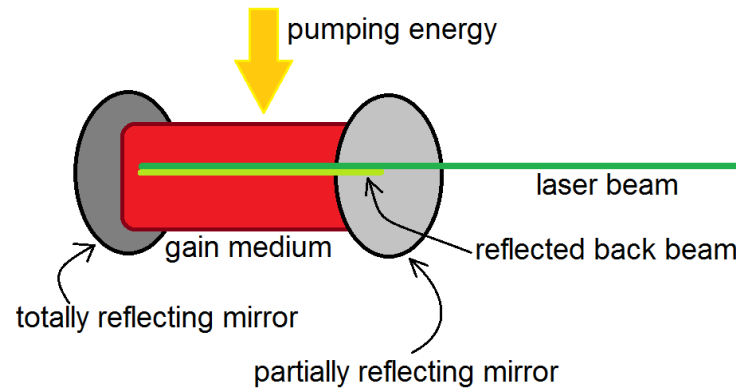




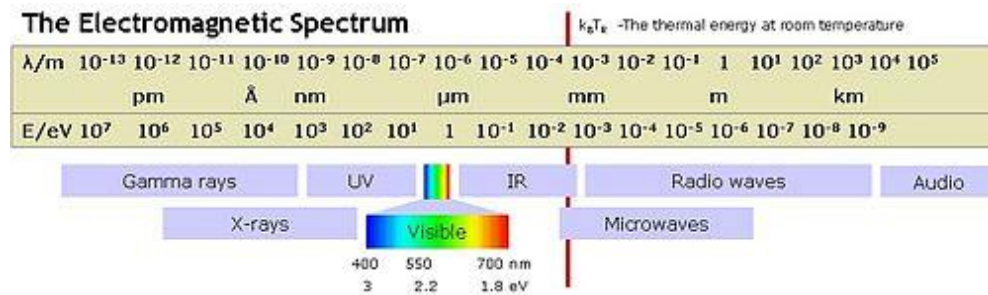


132 m long undulator

# Conventional and free electron laser



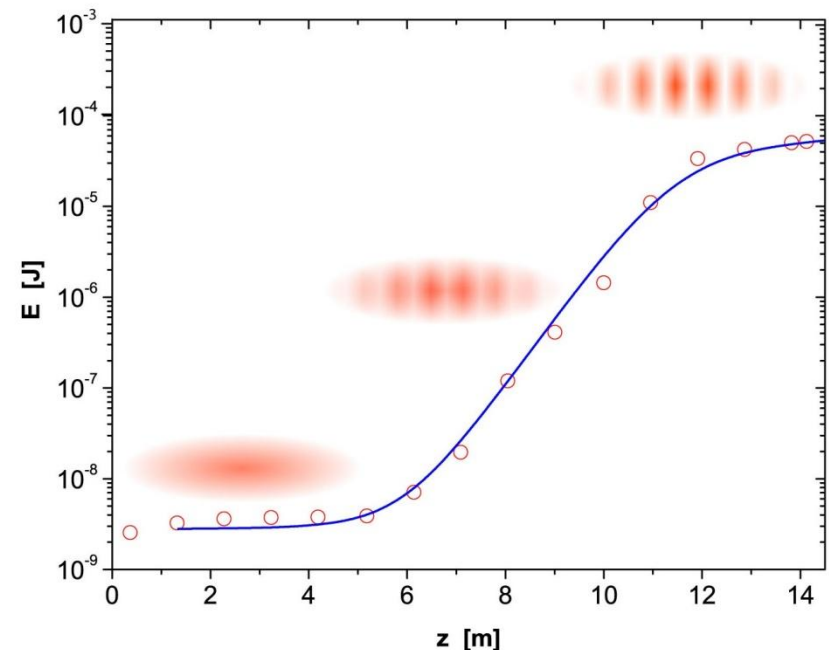
**The Electromagnetic Spectrum**





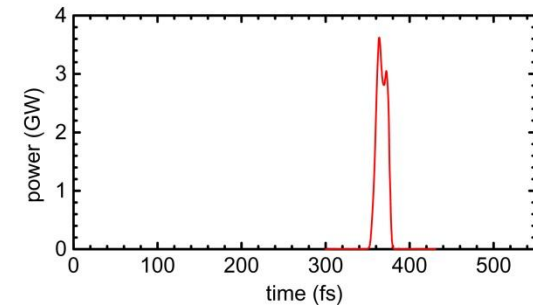
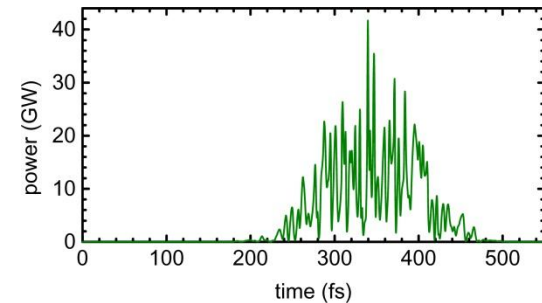
# Microbunching of electrons

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



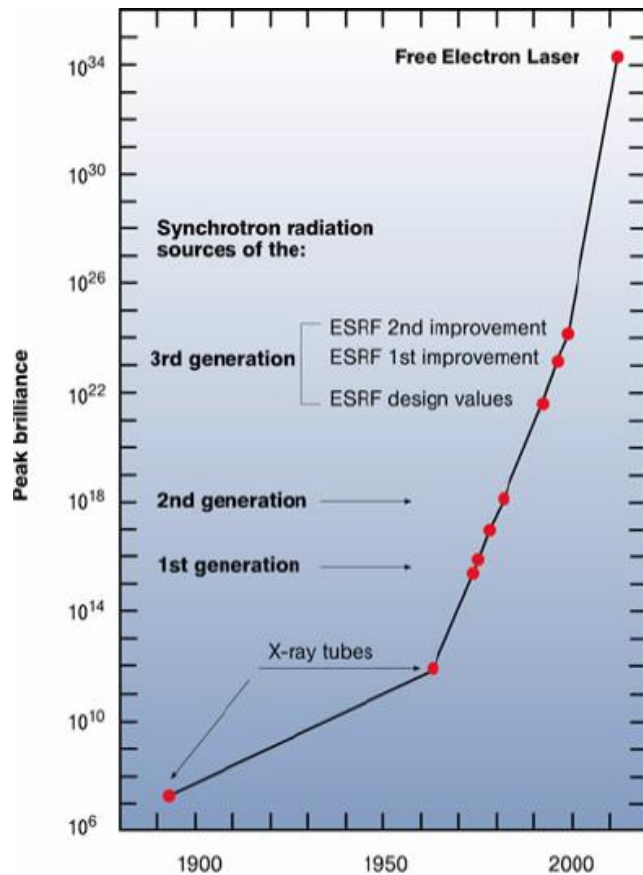
# Self-amplified sponaneous emission and seeding

- 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
  - Cleaning XFEL radiation



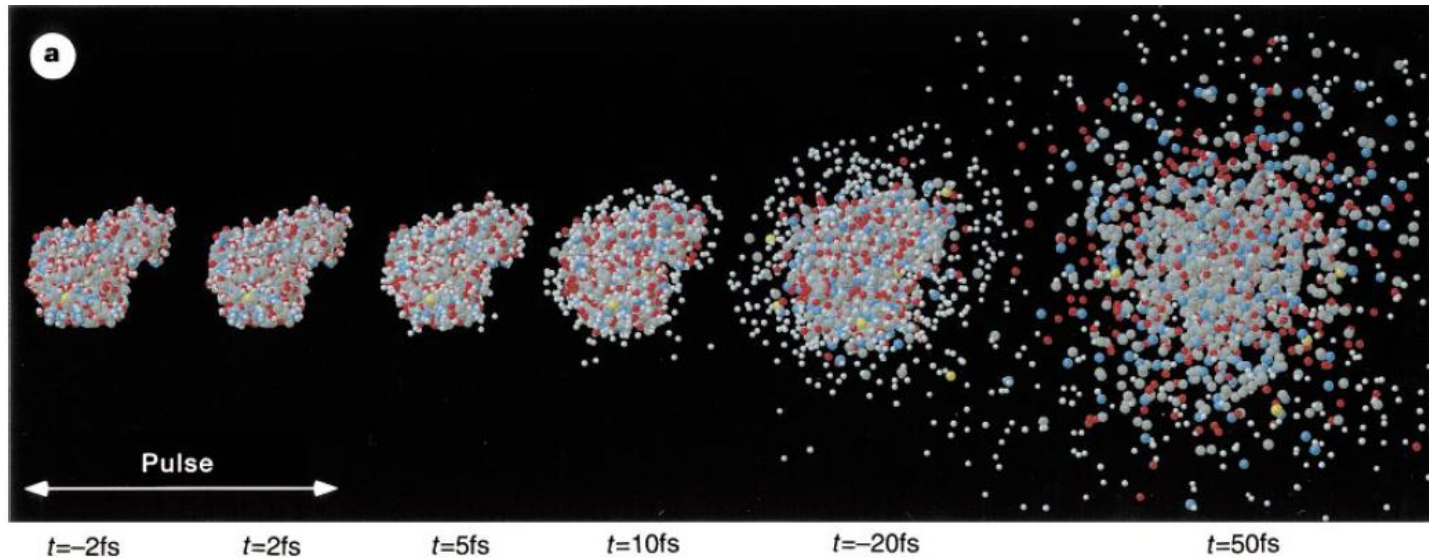


# Free Electron Lasers and Structural Biology



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

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

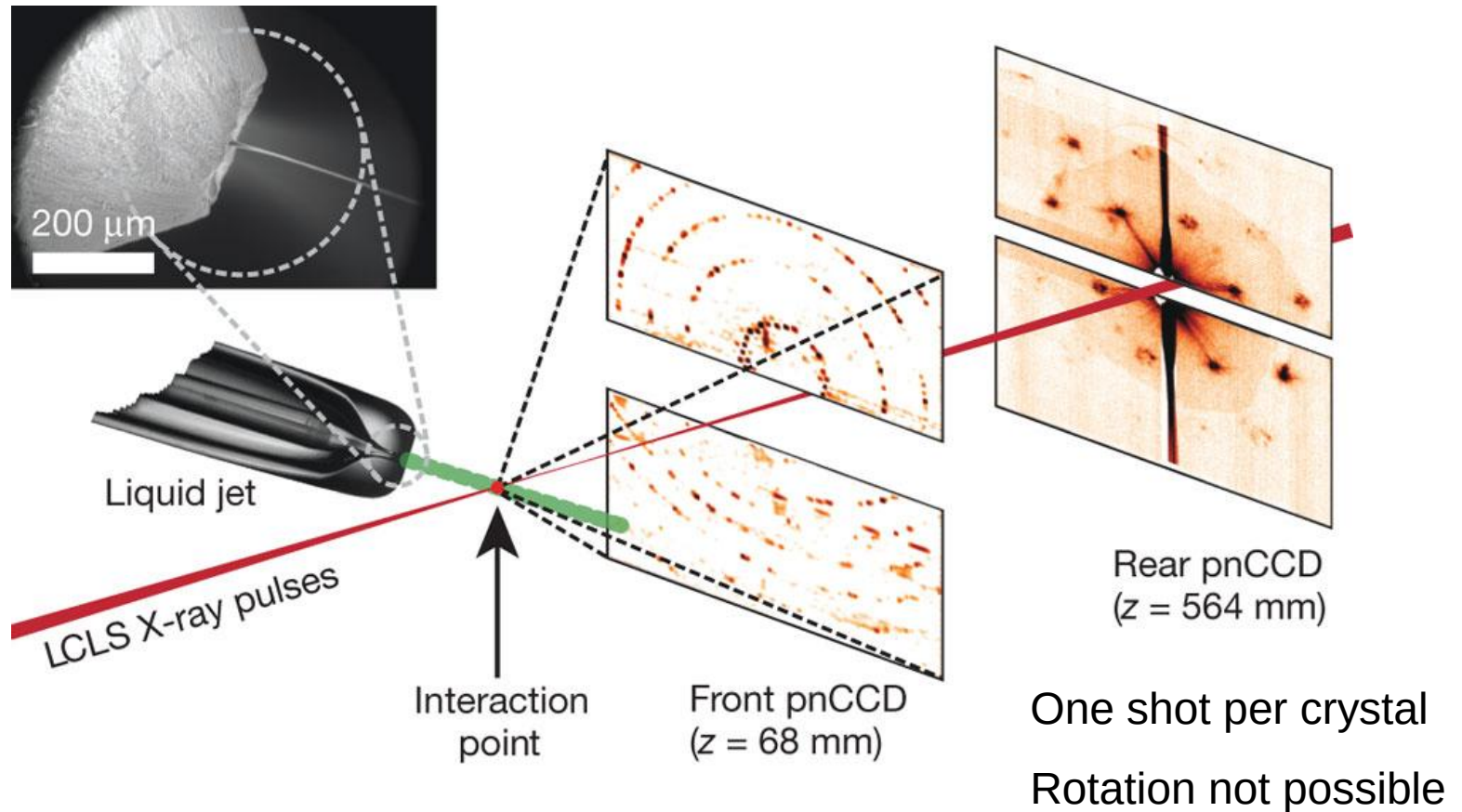


Defeating radiation damage

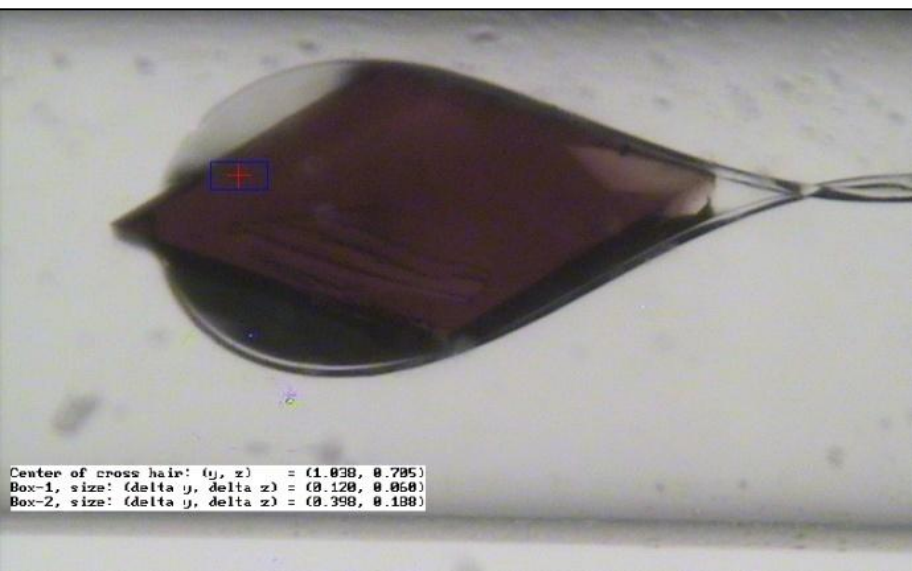
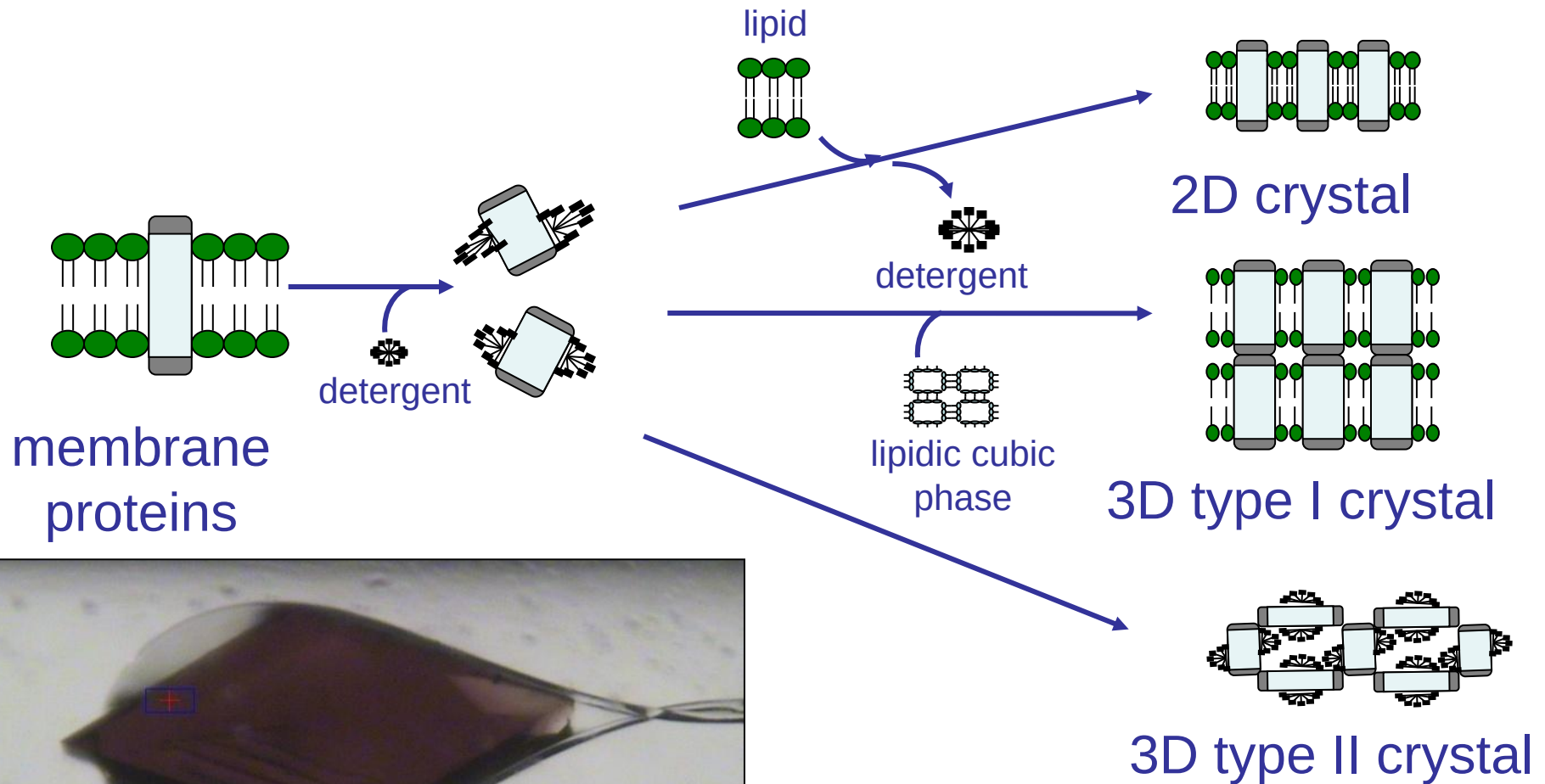
Neutze et al. *Nature* 2000



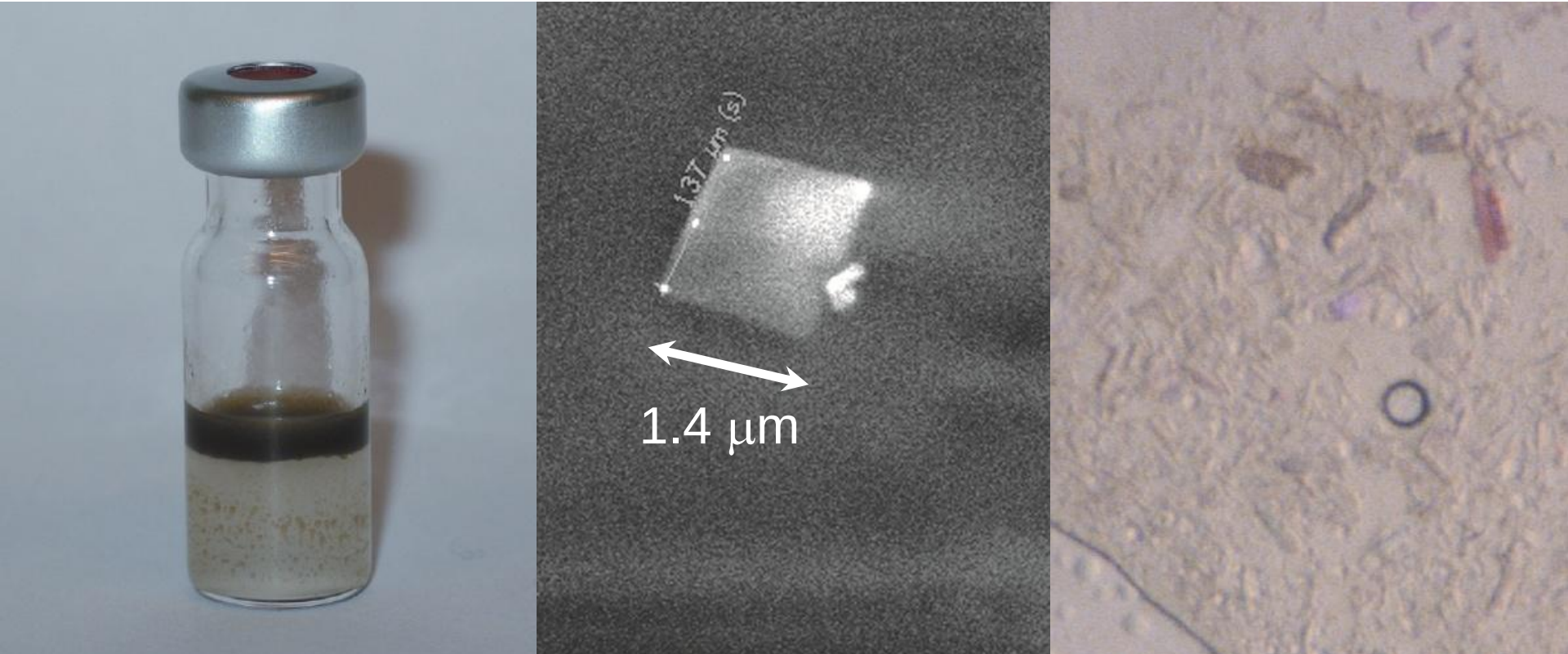
# Paradigm shift in data collection



# Membrane Protein Crystallisation



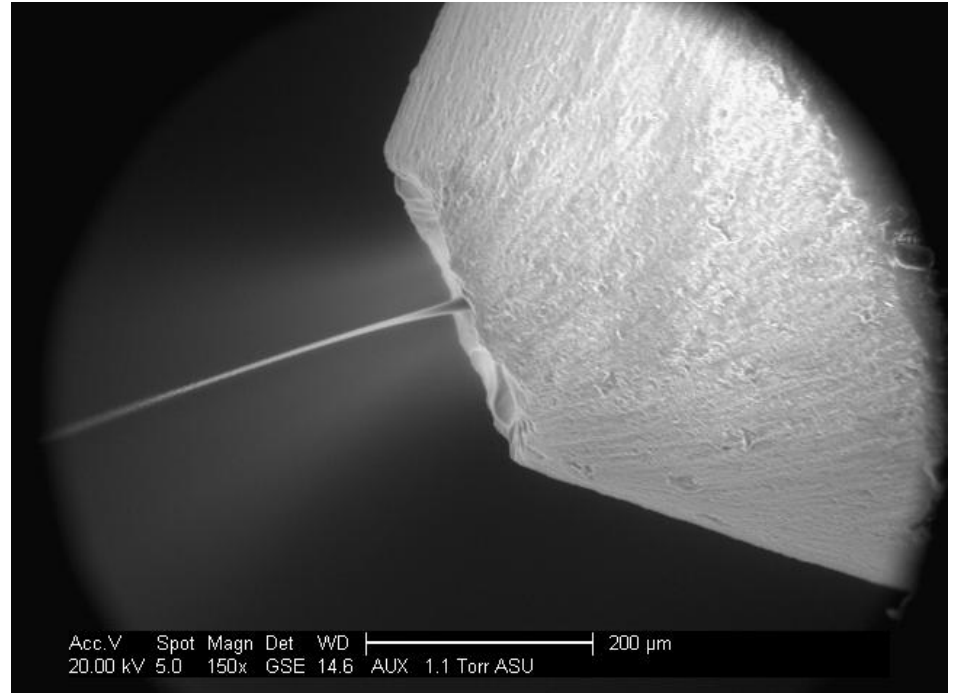
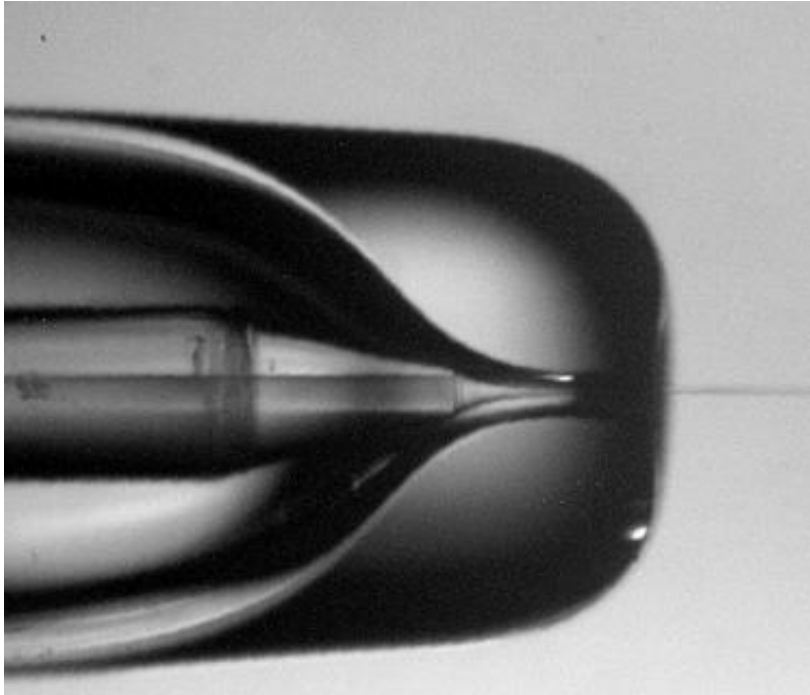
# Micro-crystallization of *Bl. viridis* photosynthetic reaction centre



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



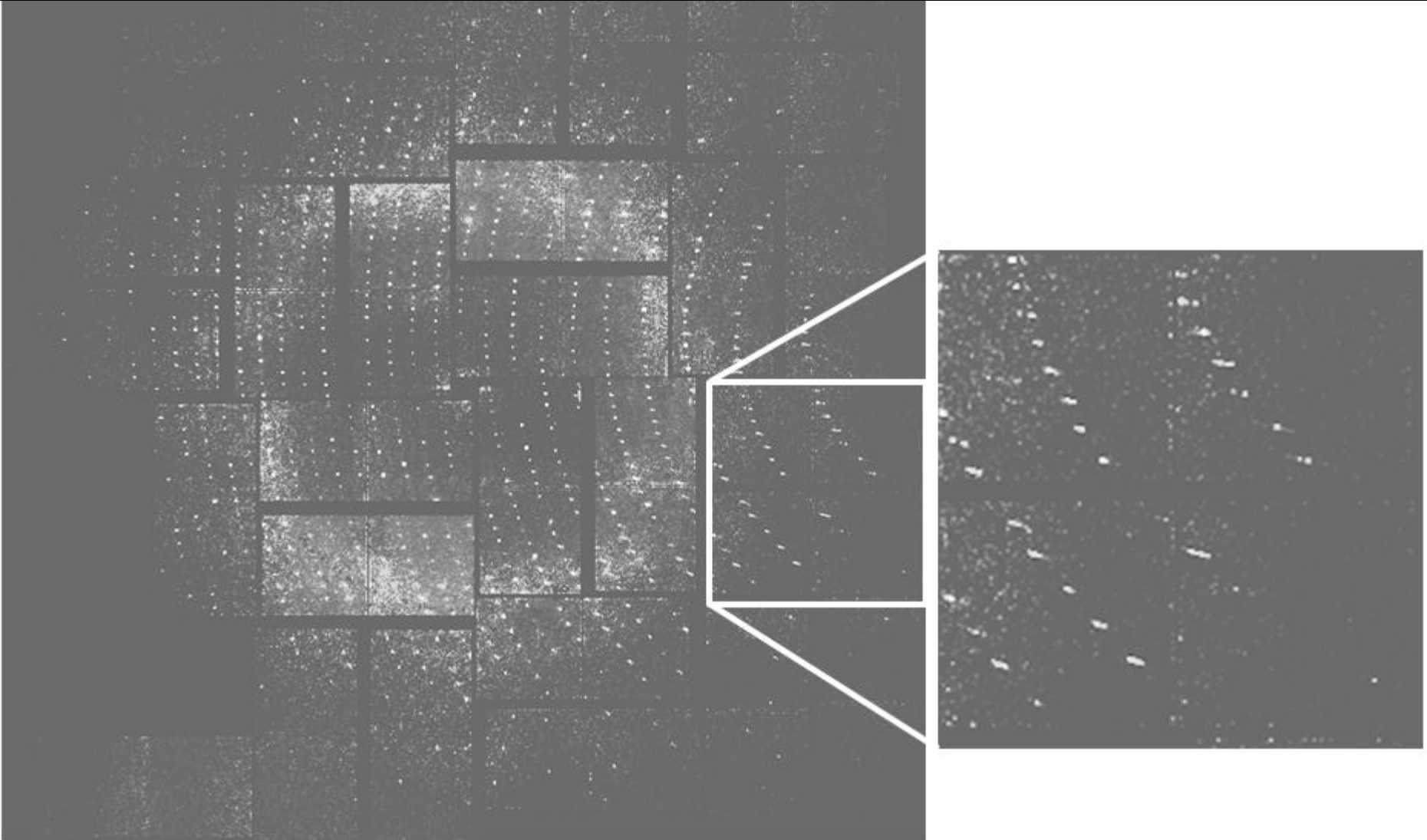
# Micro-crystal injection



- Liquid jet  $\leq 4 \mu\text{m}$  in diameter.
- Developed at Arizona state University.
  - John Spence, Uwe Weierstall, Bruce Doak, Petra Fromme, Dan DePonte, David Shapiro.



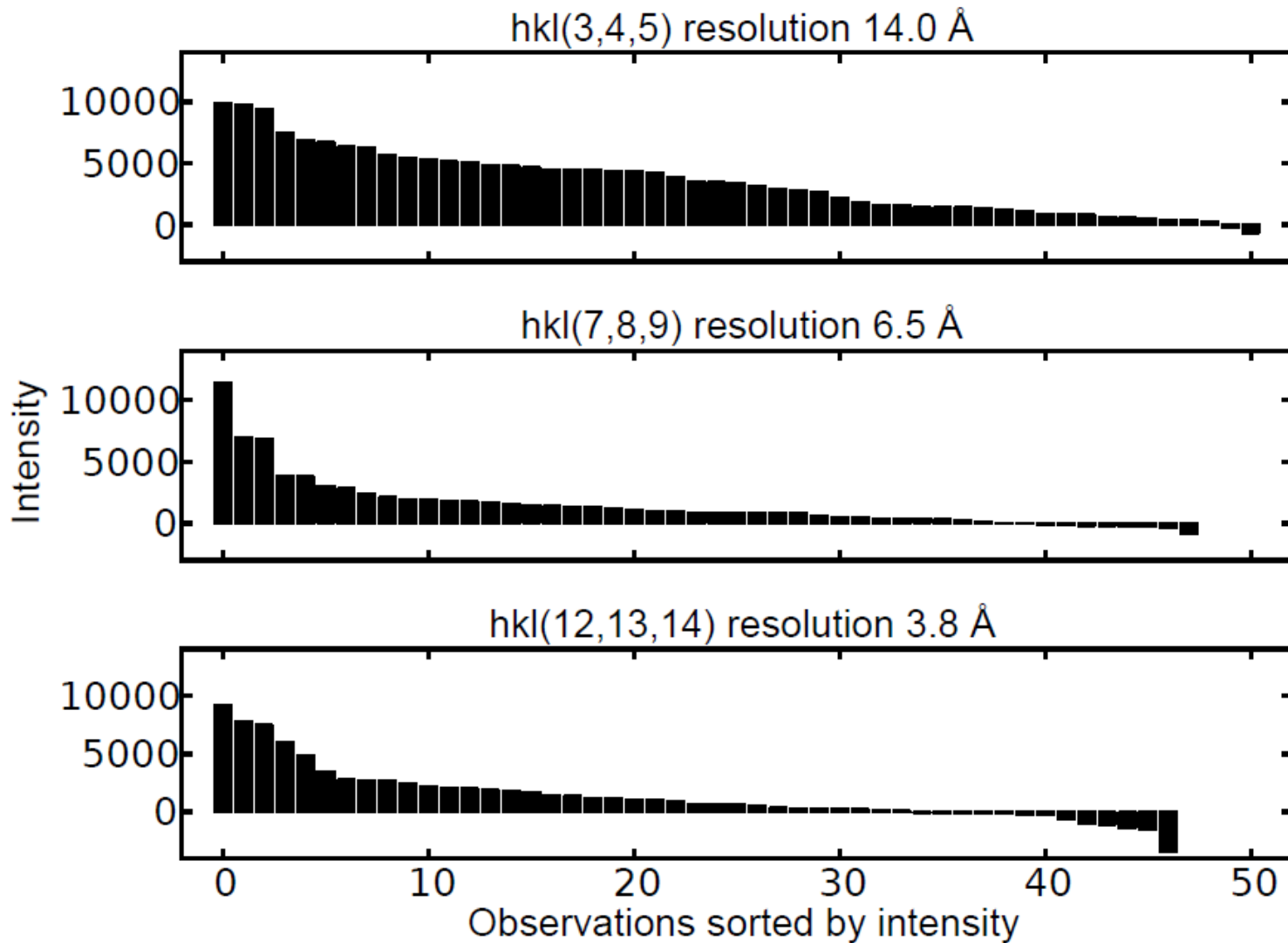
**Data extends to 2.8 Å resolution using 1.4 Å X-rays**



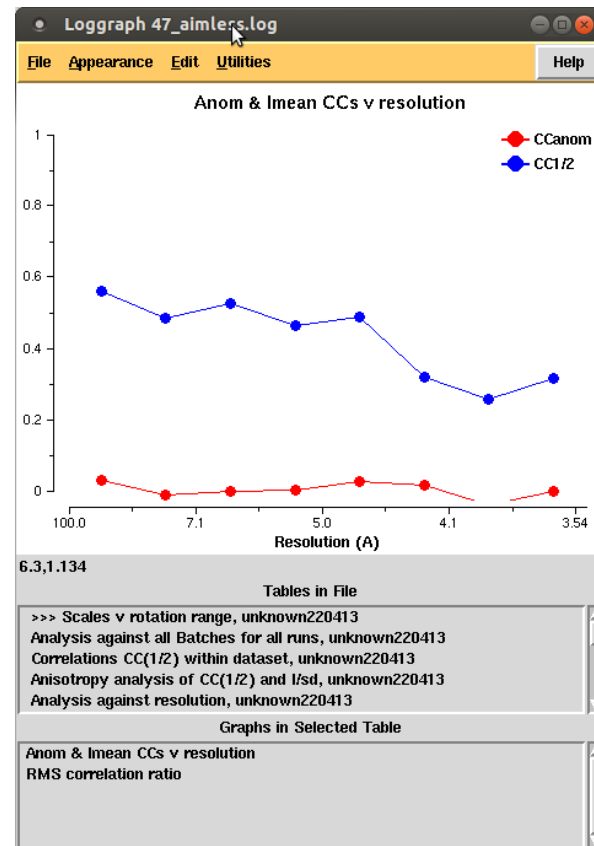
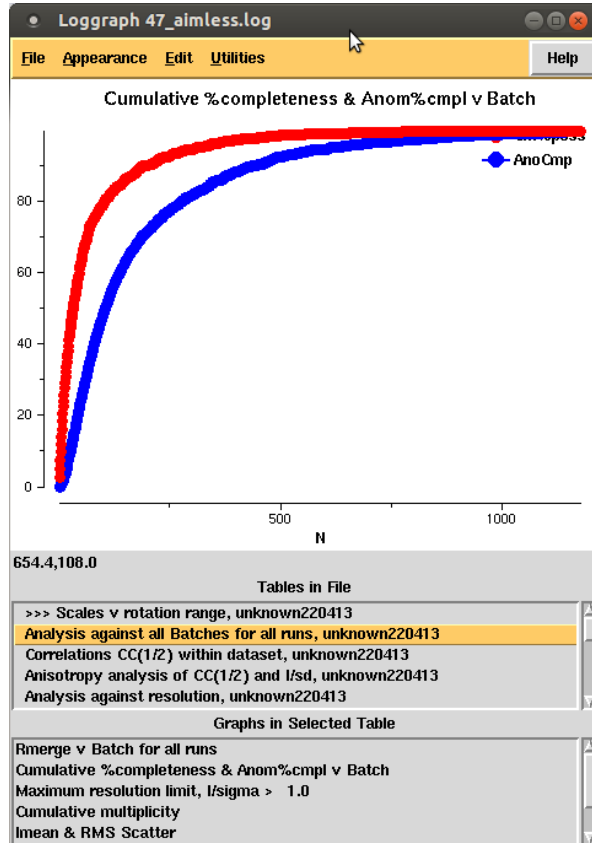


| Data collection & refinement             |                                               |
|------------------------------------------|-----------------------------------------------|
| Total number of recorded images          | 2,744,614                                     |
| Number of confirmed diffraction patterns | 5,767                                         |
| Number of indexed images                 | <b>1,175</b>                                  |
| Space group                              | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> |
| a, b, c (Å)                              | 57.9, 84.8, <b>384.3</b>                      |
| Completeness (%)                         | 99.1 (93.4)                                   |
| Multiplicity                             | <b>27.0</b> (27.6)                            |
| Overall R <sub>split</sub> on I (%)      | 36.5 (52.7)                                   |
| Mean I/σ(I)                              | 3.50 (2.0)                                    |
| CC <sub>1/2</sub> <sup>s</sup>           | 0.54 (0.32)                                   |
| Refinement resolution limits (Å)         | 49.6 - 3.50                                   |
| R <sub>work</sub> /R <sub>free</sub>     | 29.4 / 32.7                                   |

# Representative reflections

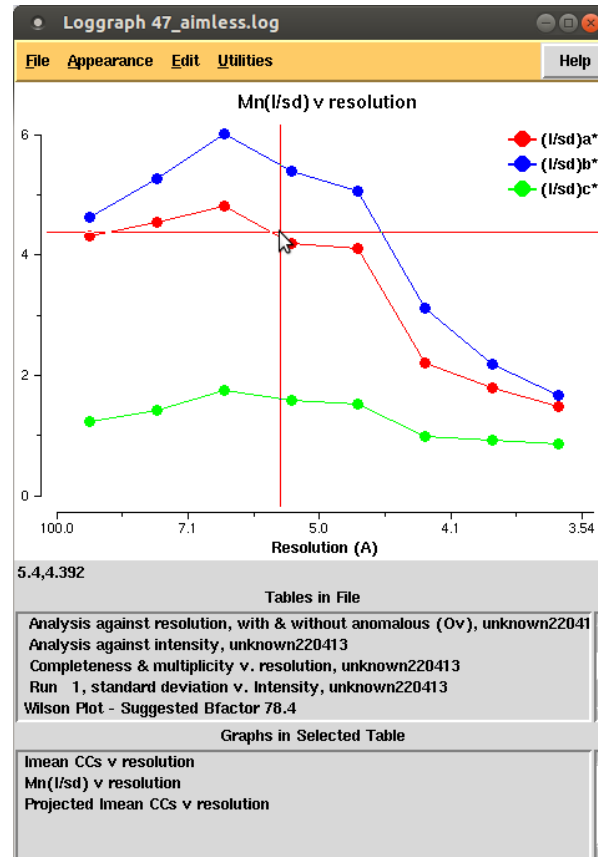
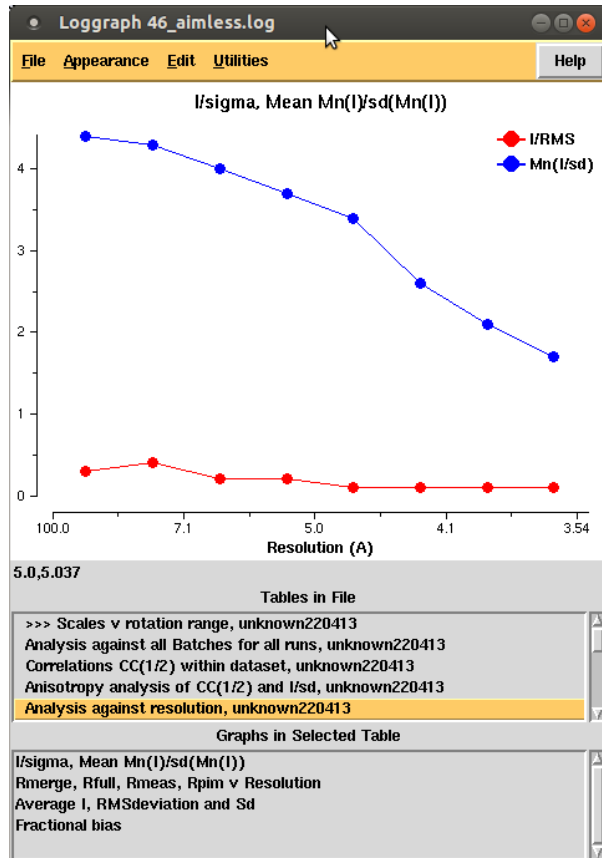


# Completeness, CC

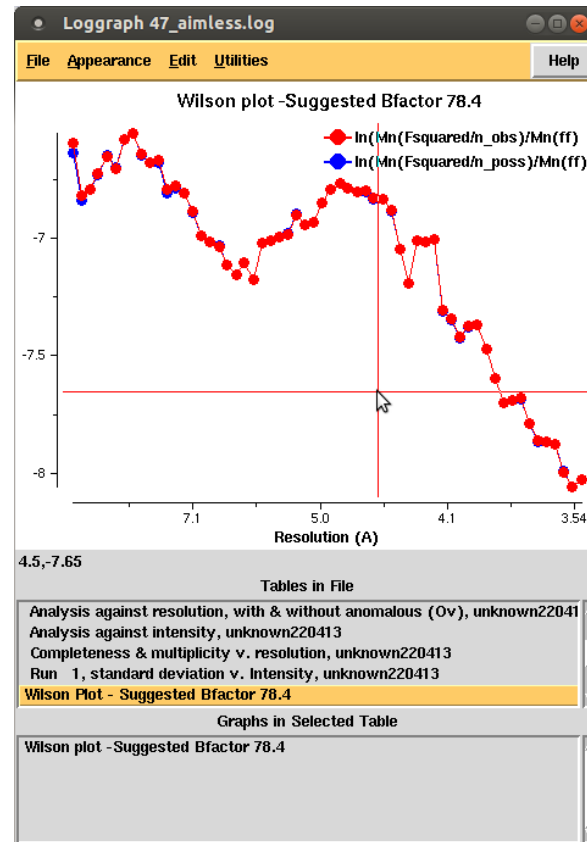
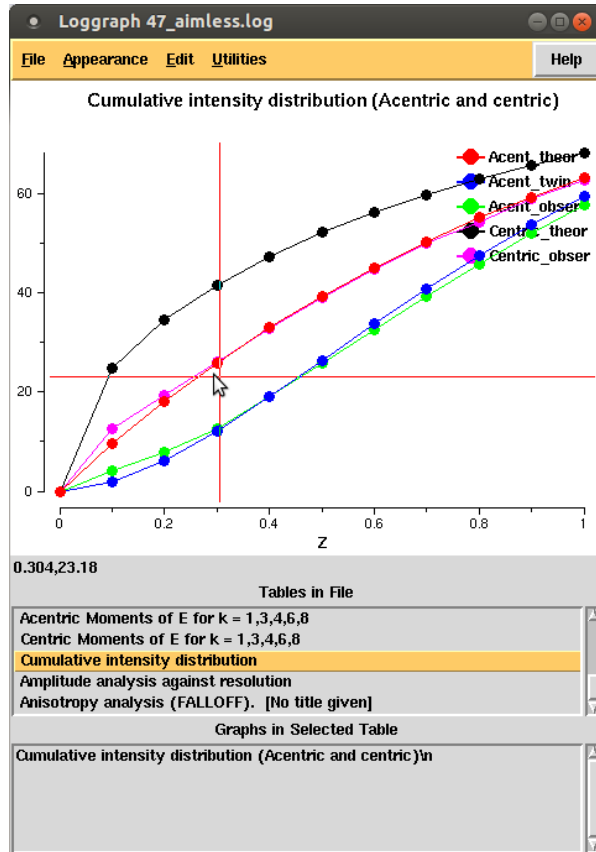




# I/sigma and anisotropy

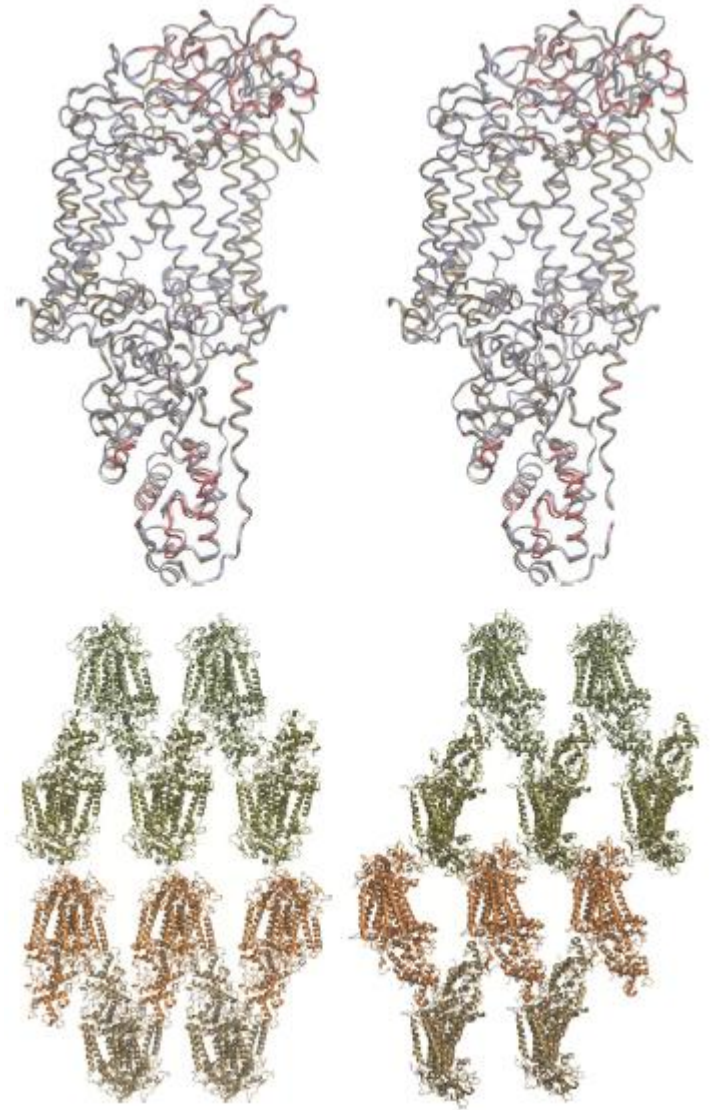


# Intensity distribution



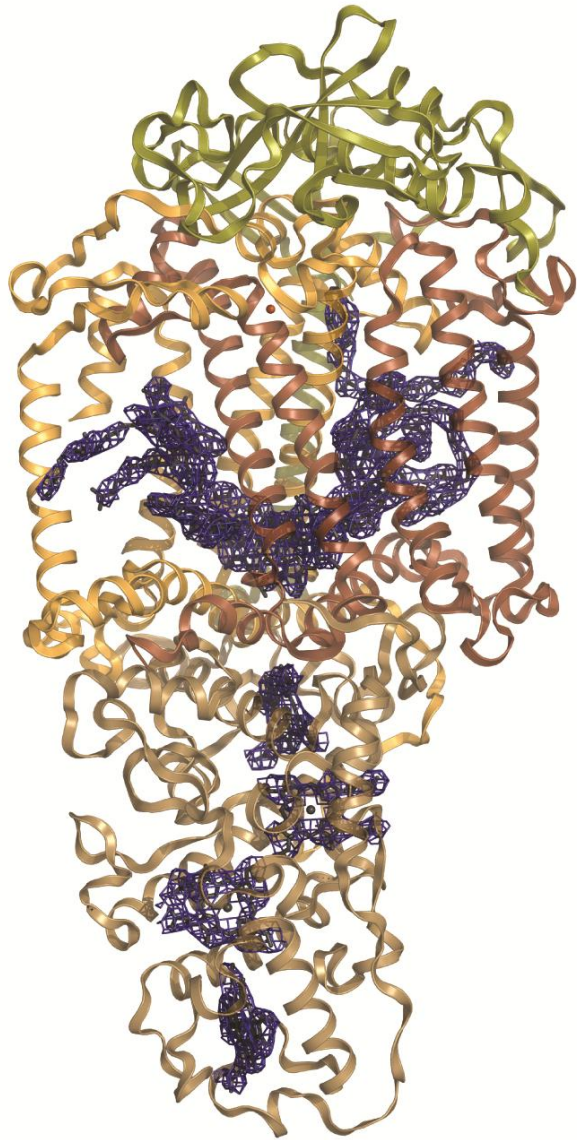
# The new crystal form

- Small changes in the backbone
- Crystal packing differences
- Longer c-axis

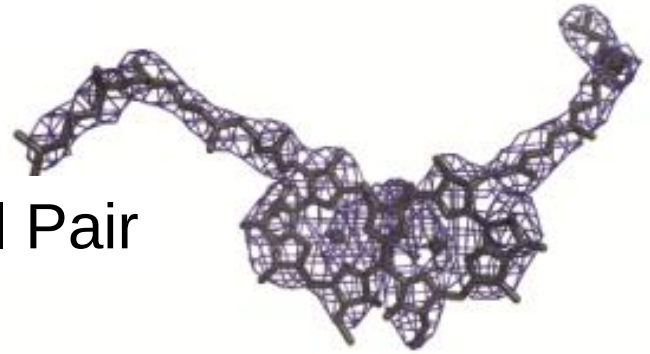




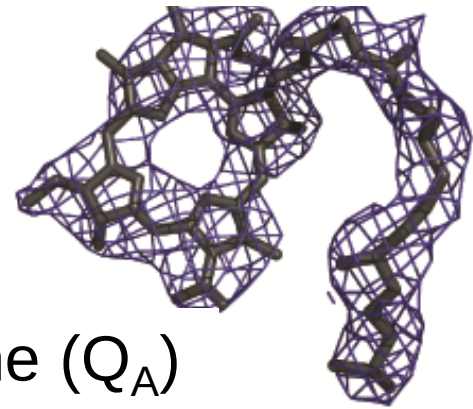
# 3.5 Å SFX structure



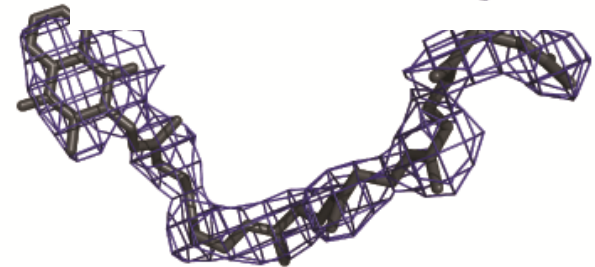
Special Pair



Pheophytin

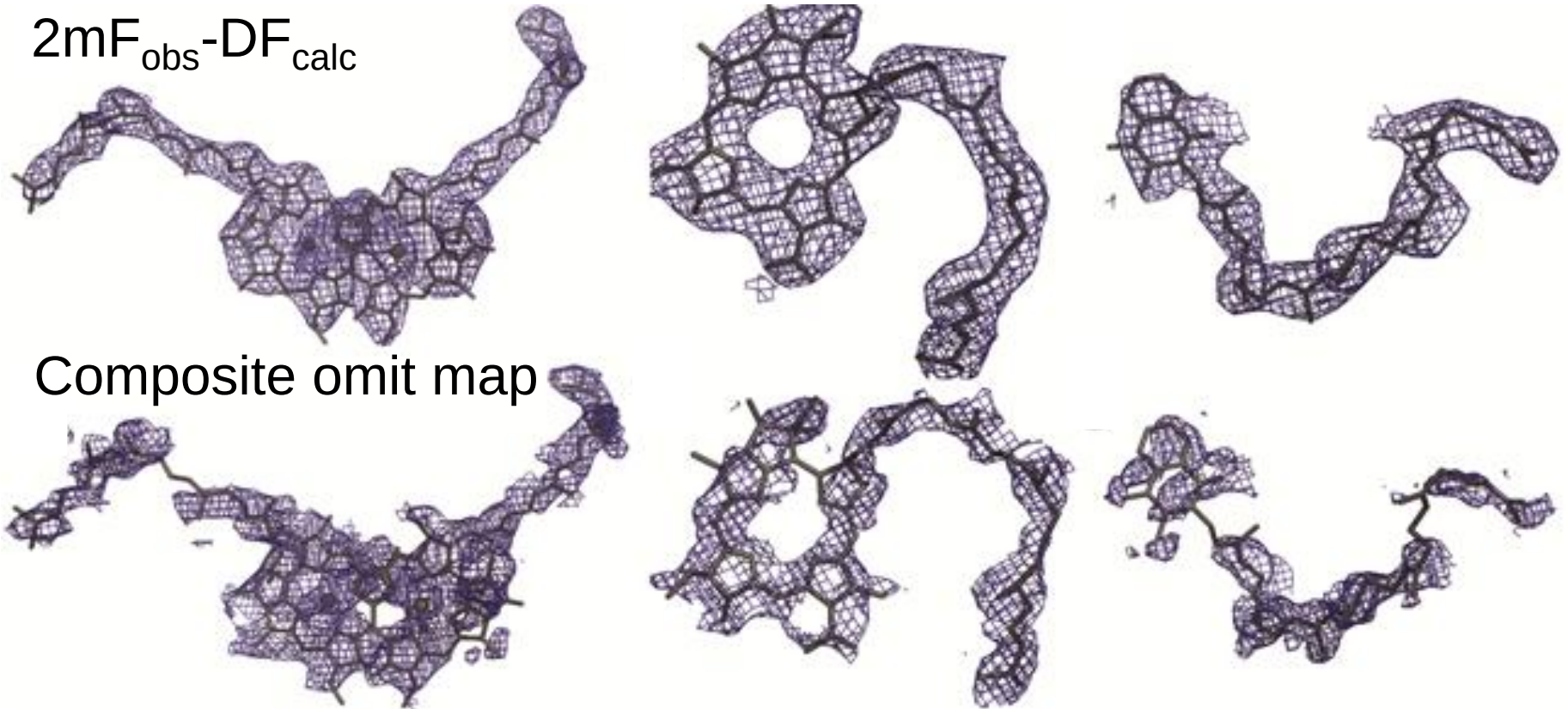


Menaquinone ( $Q_A$ )



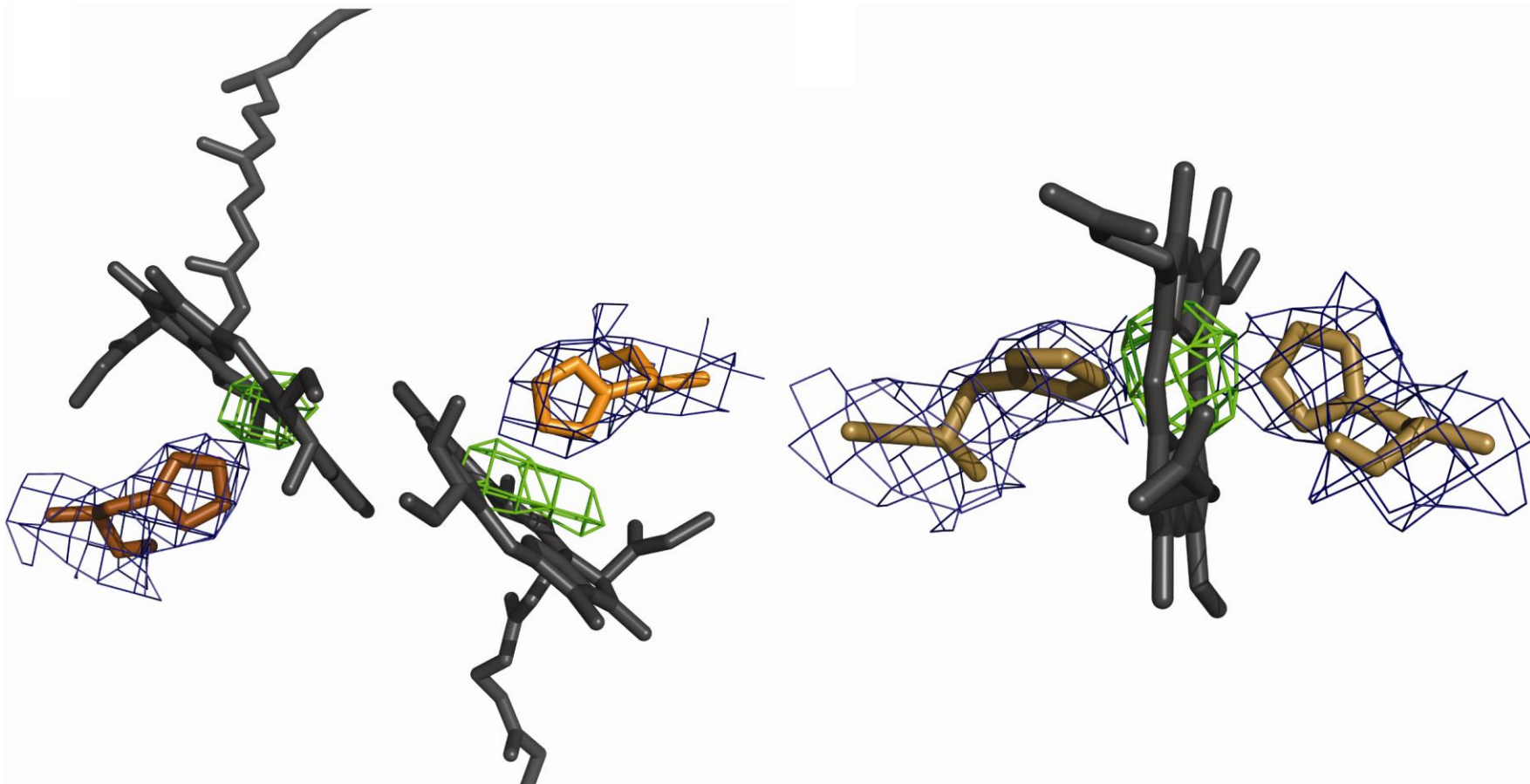
# Composite omit map

$2mF_{\text{obs}} - DF_{\text{calc}}$



Composite omit map

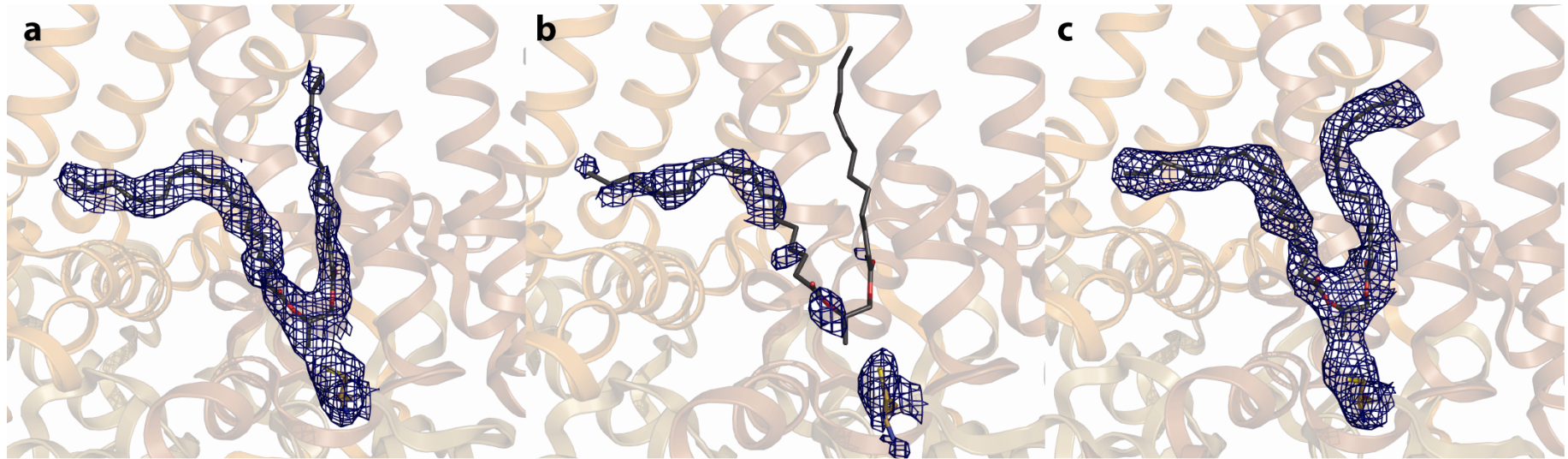
# Metal Centres



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



# 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.



# Summary

- “Diffraction before destruction” works in practice
  - May offset radiation damage
- Particular advantage:
  - Small crystals
  - Time-resolved studies

# SFX collaboration

|                      |                                                                                                                                                                                                                                        |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Göteborg</b>      | <b>R. Neutze, L. Johansson, D. Arnlund</b> , E. Malmerberg C. Wickstrand, J. Sjöhamn, A. Sharma.                                                                                                                                       |
| <b>CFEL-DESY</b>     | <b>H. Chapman</b> , 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, |
| <b>ASU</b>           | <b>J. Spence, P. Fromme</b> , U. Weierstall, B. Doak, M. Hunter, R. Kirian, X. Wang, K. Schmidt, I. Grotjohann, R. Fromme                                                                                                              |
| <b>SLAC-PULSE</b>    | M. Bogan, D. Starodub, R. Sierra, C. Hampton, D. Loh                                                                                                                                                                                   |
| <b>SLAC-LCLS</b>     | <b>S. Boutet, G. Williams</b> , M. Seibert, J. Kryzwiniski, C. Bostedt, M. Messerschmidt, J. Bozek, W. White, R. Coffee                                                                                                                |
| <b>Uppsala</b>       | J. Hajdu, Nic Timneanu, J. Andreasson, M. Seibert, F. Maia, M. Svenda, <b>J. Davidsson</b>                                                                                                                                             |
| <b>MPG CFEL ASG</b>  | <b>I. Schlichting</b> , R. Shoeman, L. Lomb, S. Kessemeier, 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                         |
| <b>LLNL</b>          | S. Hau-Riege, M. Frank                                                                                                                                                                                                                 |
| <b>CAMP Team</b>     | Led by <b>J. Ullrich</b> and <b>I. Schlichting</b>                                                                                                                                                                                     |
| <b>LCLS detector</b> | C. Kenney, R. Herbst, J. Pines, P. Hart, J. Morse                                                                                                                                                                                      |
| <b>Accelerator</b>   | Led by <b>P. Emma</b>                                                                                                                                                                                                                  |



Vetenskapsrådet



*Knut och Alice  
Wallenbergs  
Stiftelse*



GÖTEBORGS UNIVERSITET



CHALMERS

**SWE**GENE  
The Postgenomic Research and Technology Programme in South Western Sweden



Lundberg Stiftelsen



Stiftelsen Olle Engkvist Byggmästare



HUMAN FRONTIER SCIENCE PROGRAM  
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