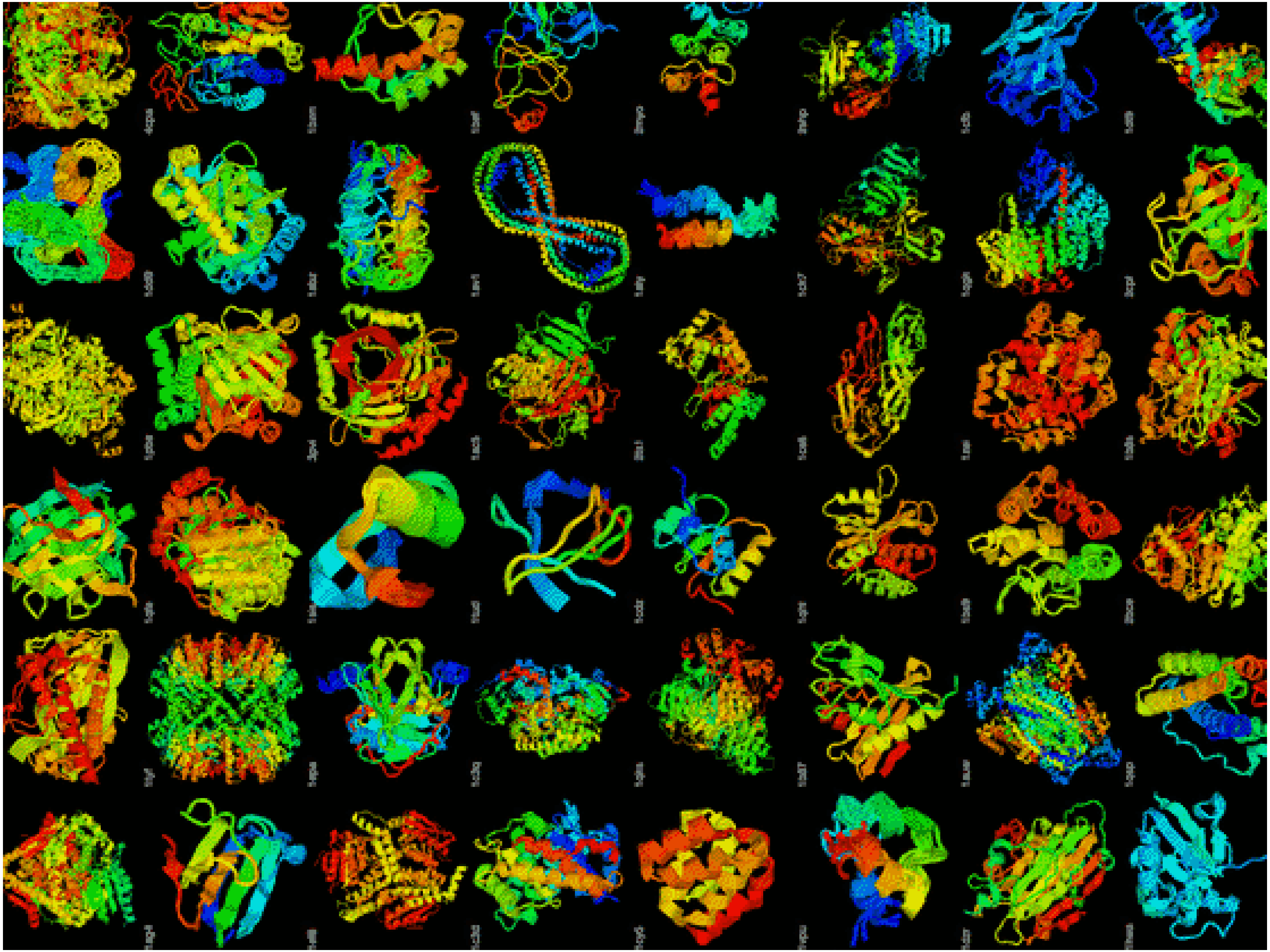
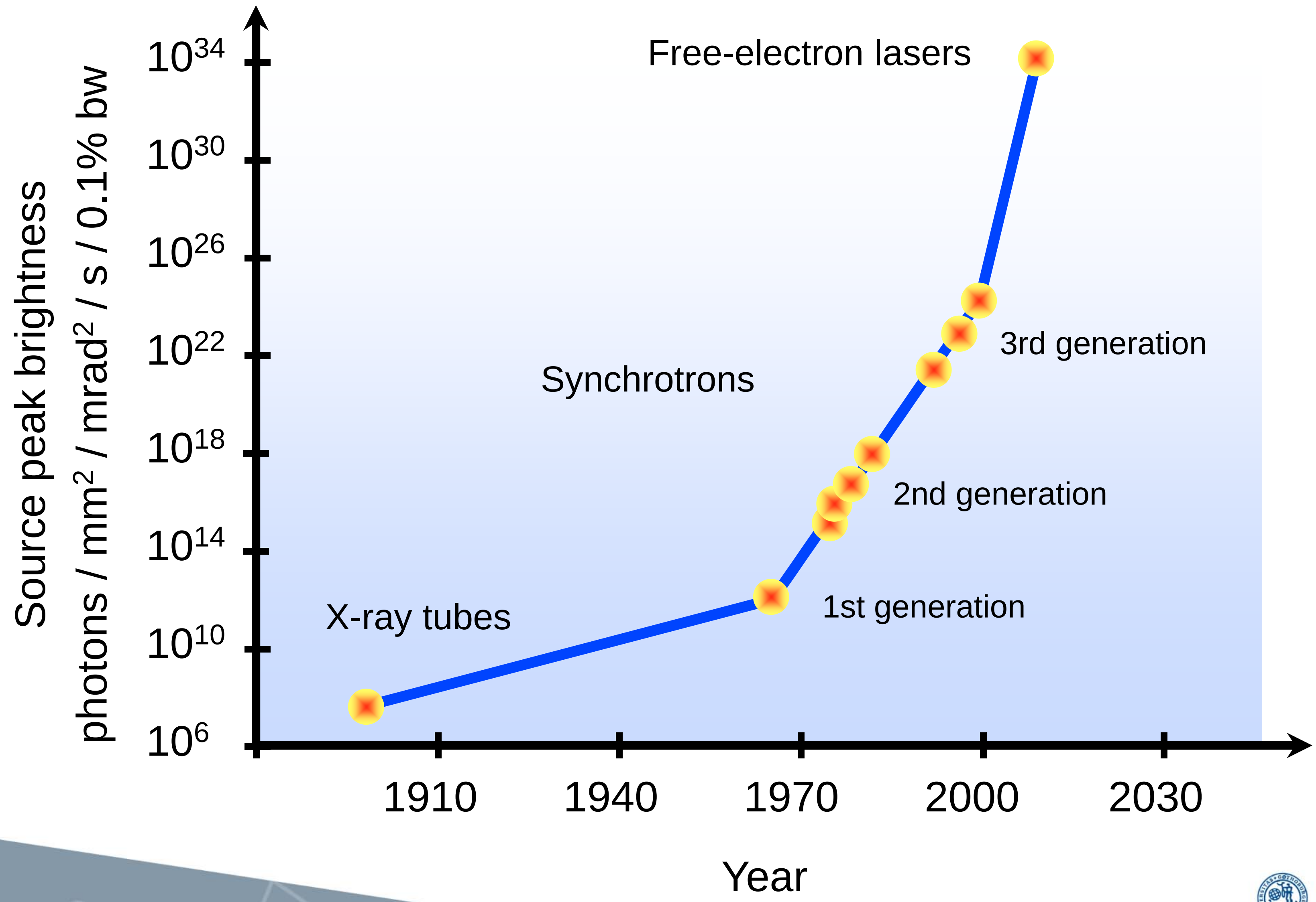
Time resolved diffraction & scattering studies of membrane protein dynamics using XFEL radiation

Richard Neutze
University of Gothenburg

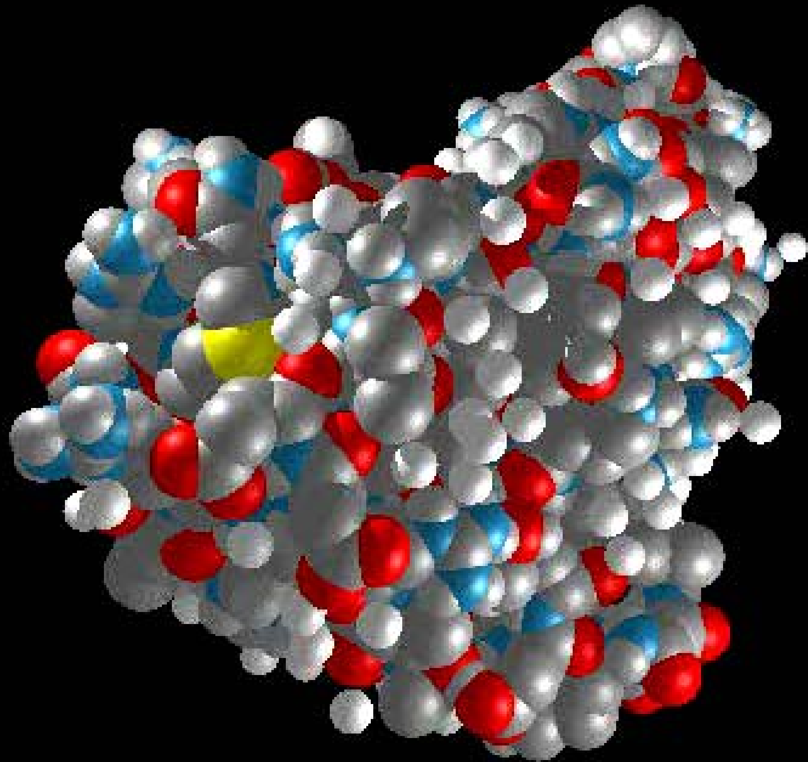


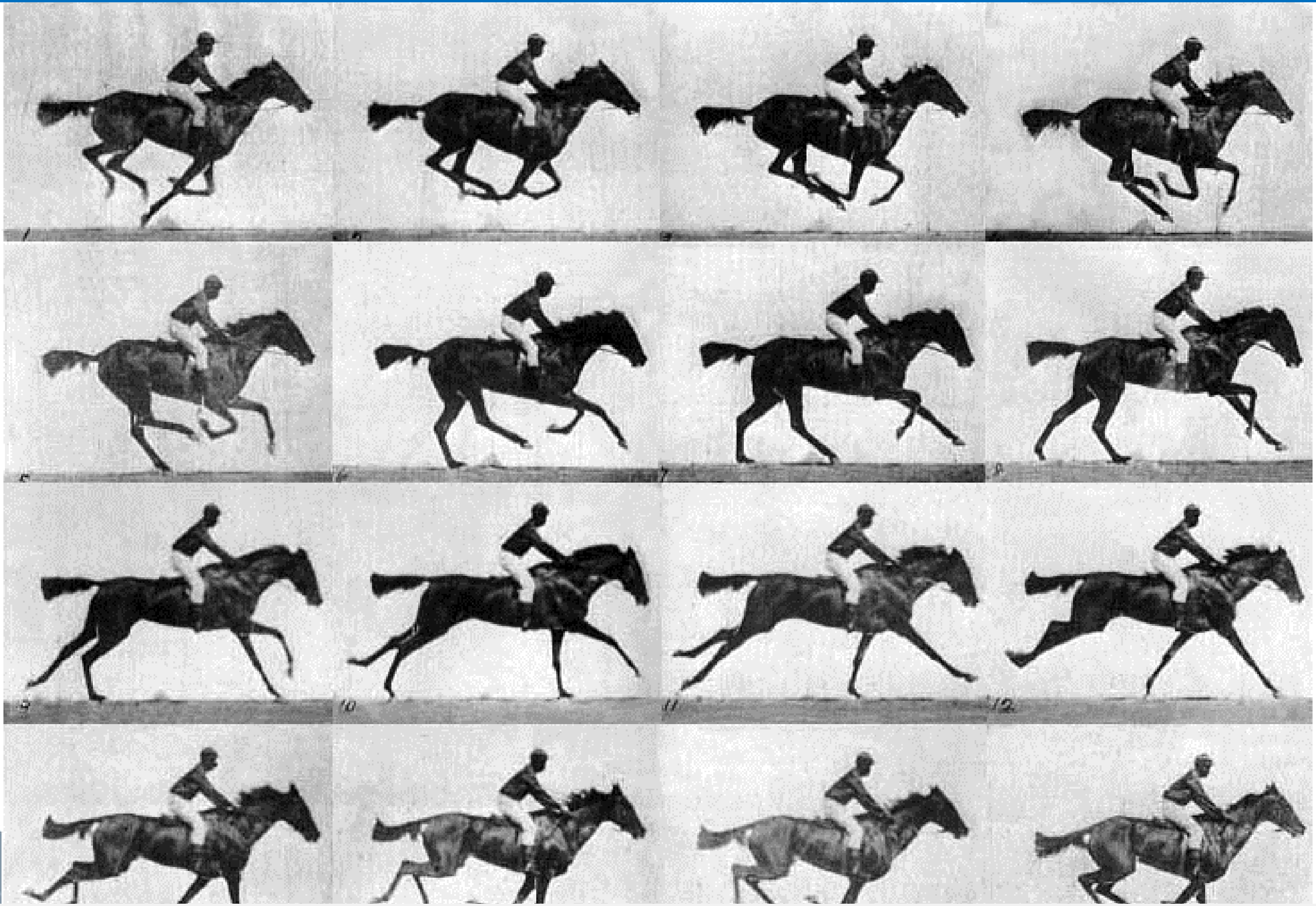
Peak Brilliance



Diffraction before Destruction

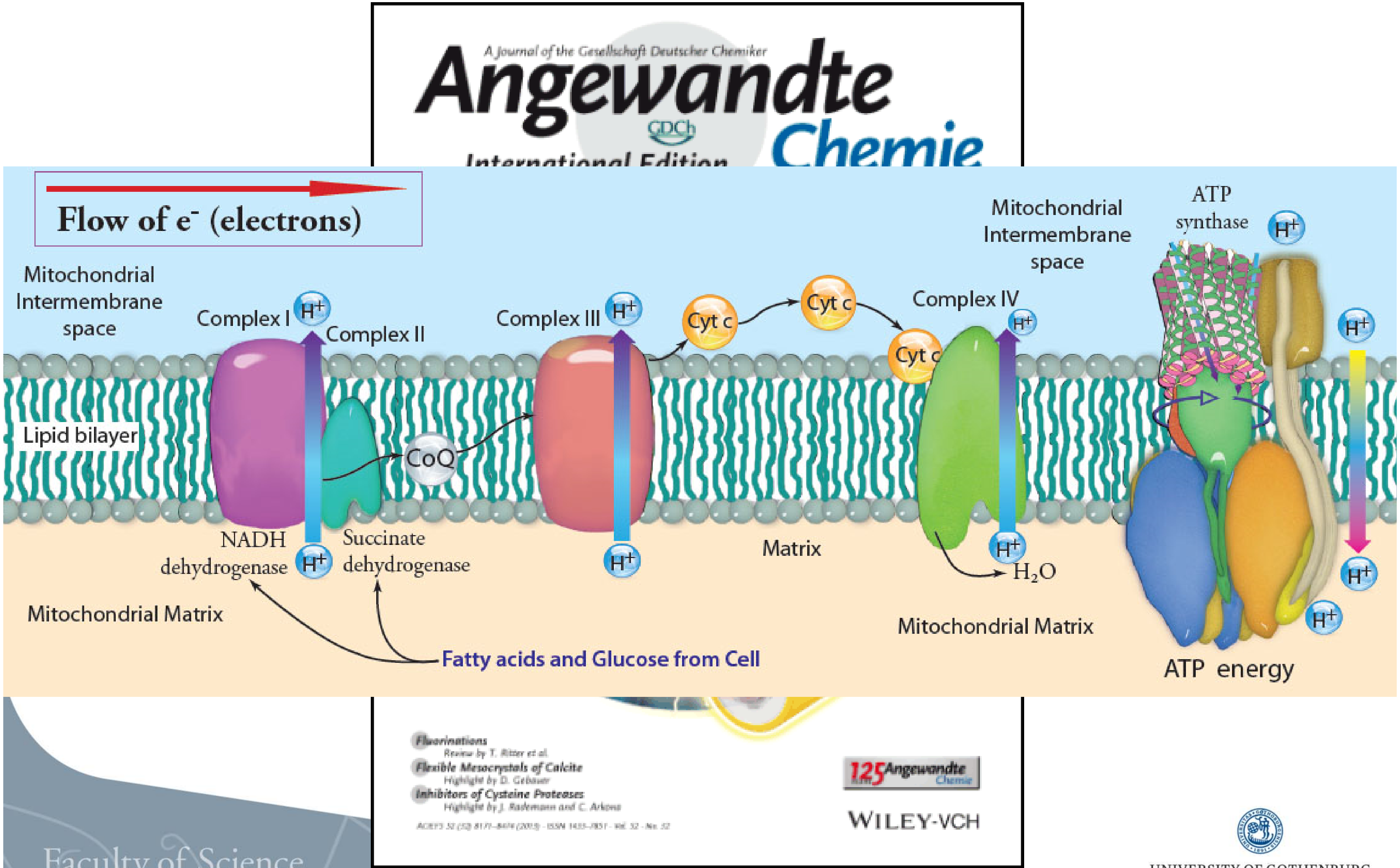


Reaction Dynamics



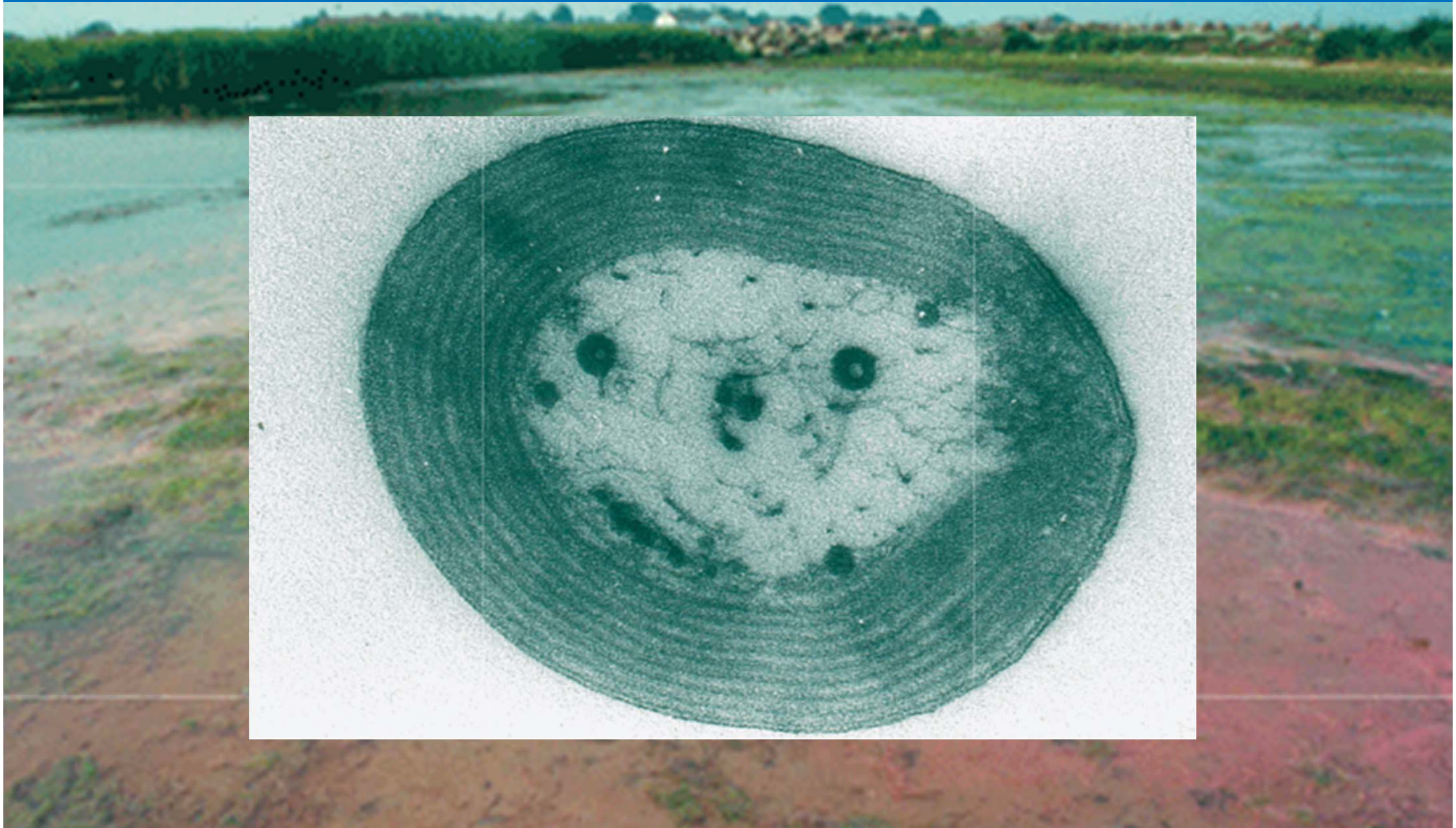
Muybridge's freeze-frame sequence revealed a horse's gait.

Energy Transduction



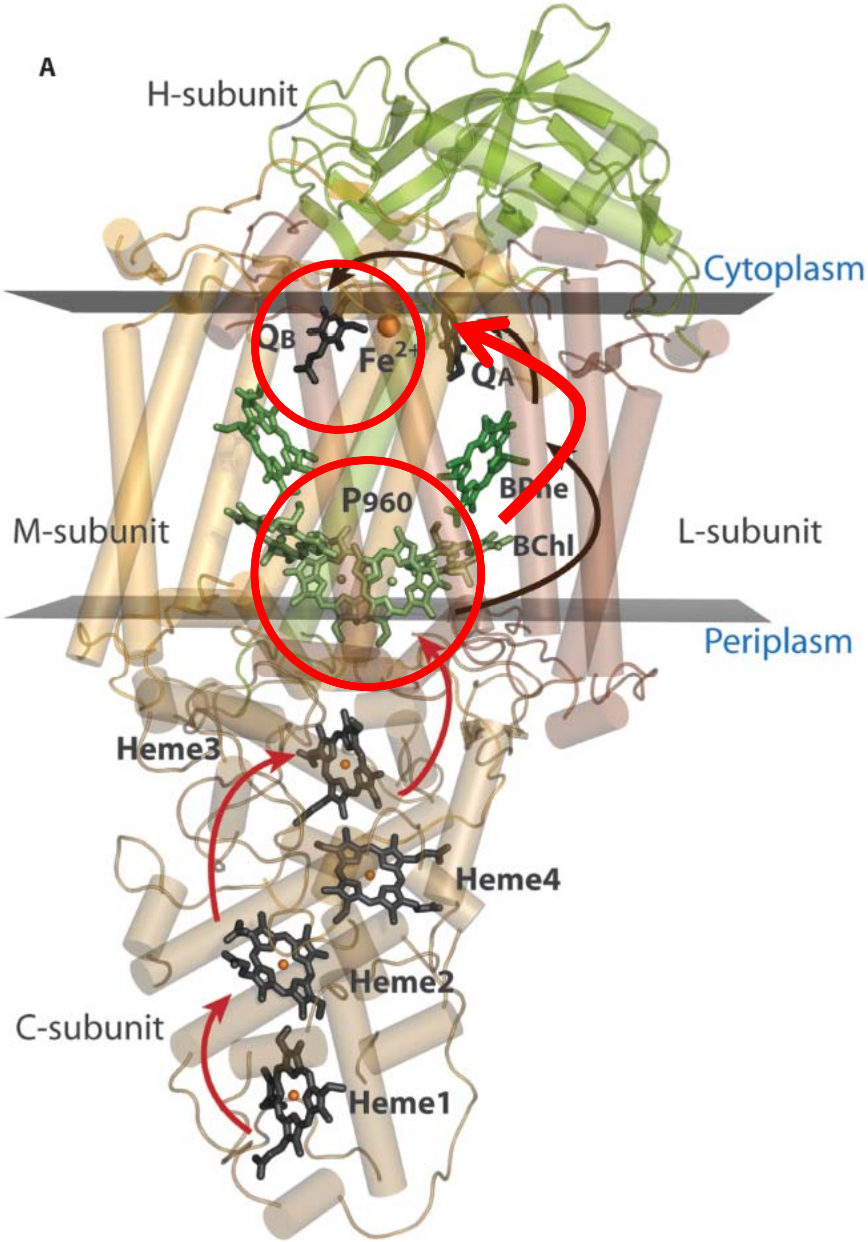
Photosynthetic Proteins

Photosynthetic Purple Bacteria



Blooming purple sulfur bacteria in a coastal lagoon

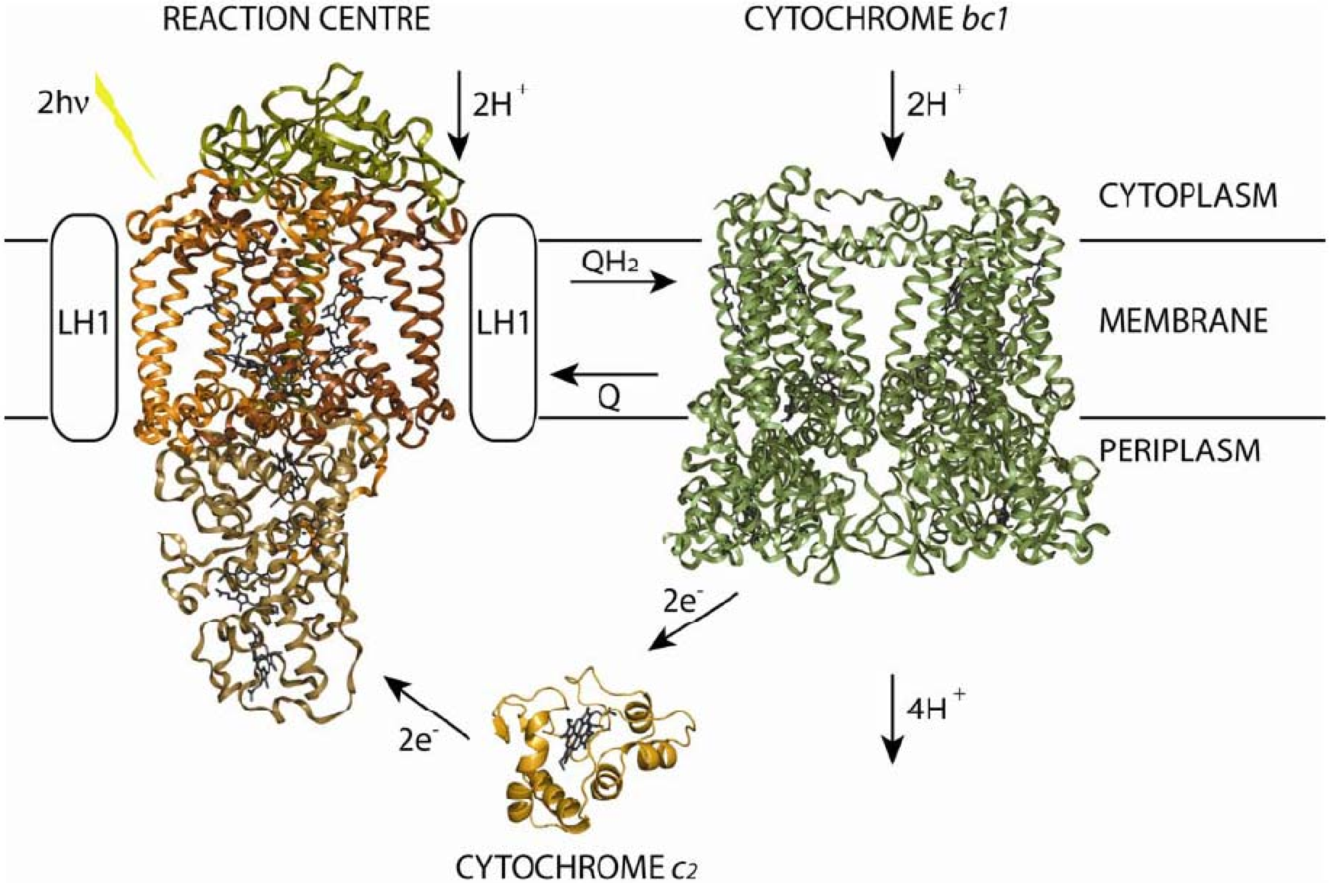
Photosynthetic reaction centres



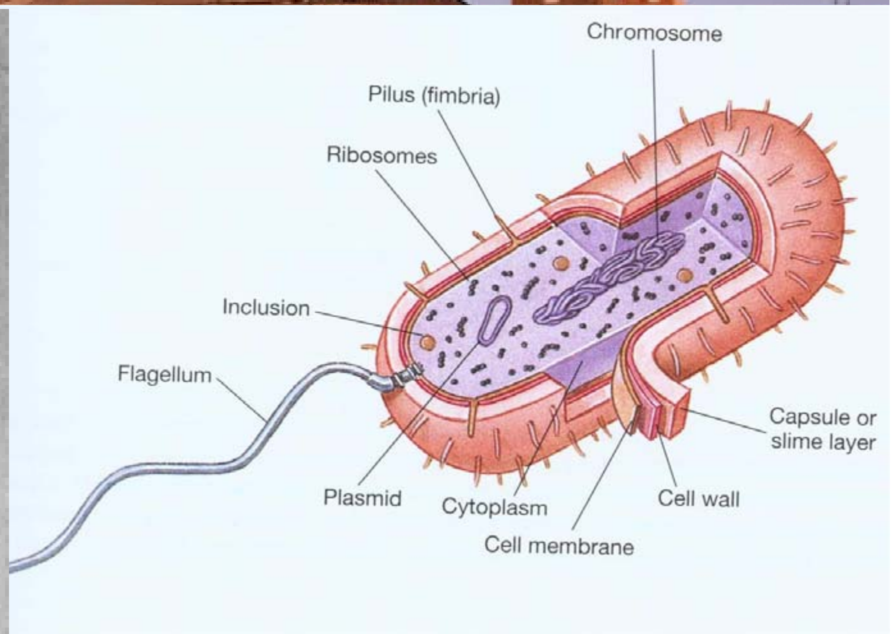
- 135 kDa Membrane protein.
- Complex light driven proton pump.
- Electron movements driven by light.
- Coupled redox reactions pump protons.
- Descendent created O_2 rich atmosphere.

Proton pumping by reaction centres

- Electron movements driven by light.
- Coupled redox reactions pump protons.

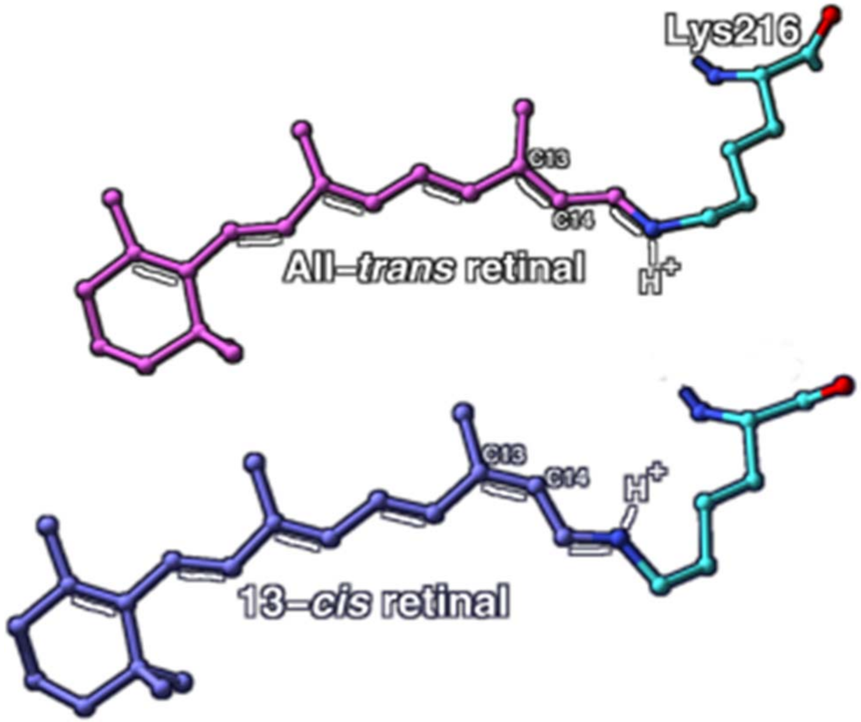


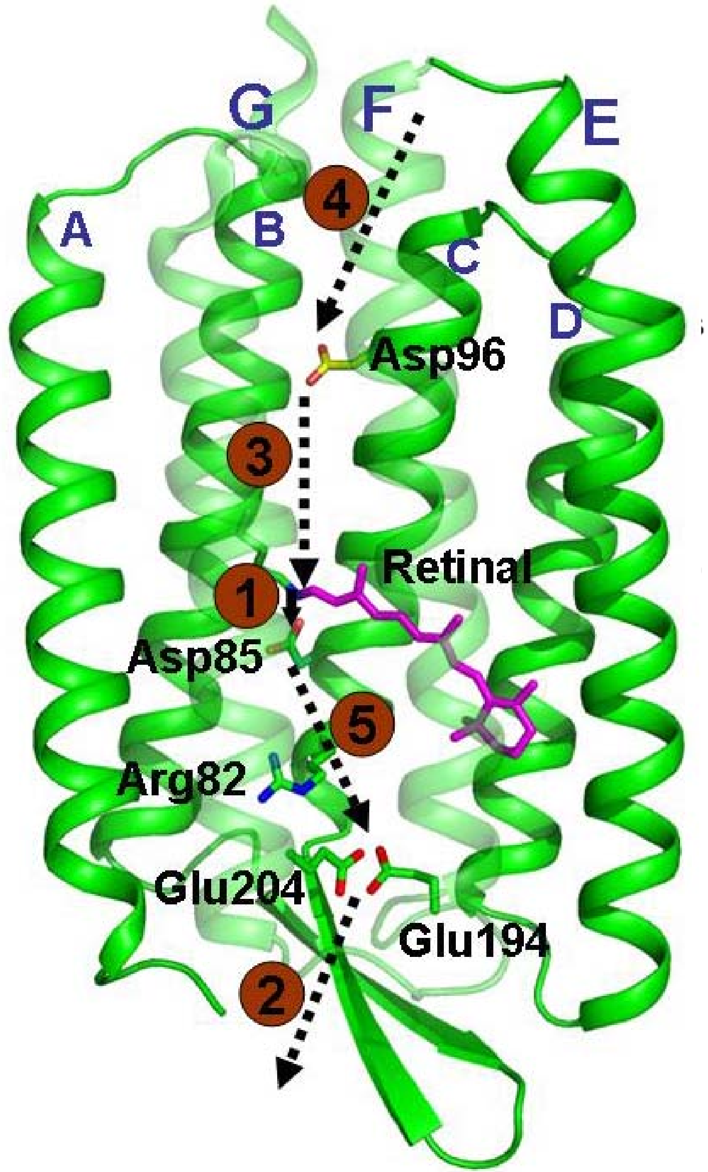
Halo-archaea



Halobacteria Salinarium in high-salt ponds

Bacteriorhodopsin



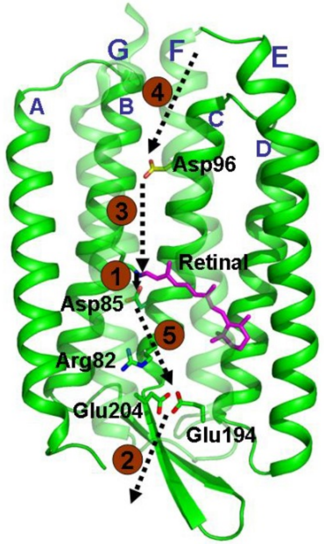


- Retinal isomerisation starts a sequence of structural changes.
- One proton pumped per photon.

Scientific Question

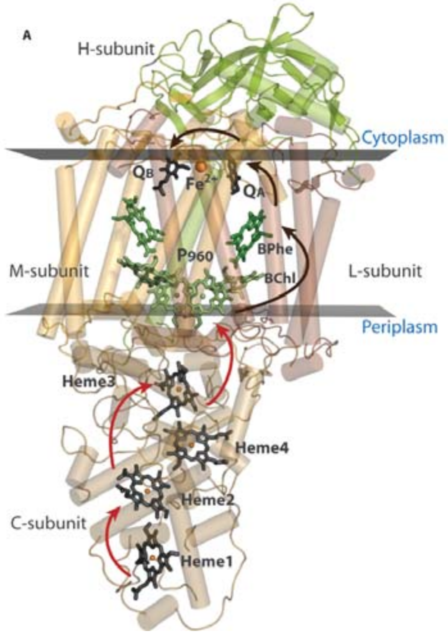
Bacteriorhodopsin:

- What structural changes are needed to achieve proton pumping up-hill against a proton gradient?

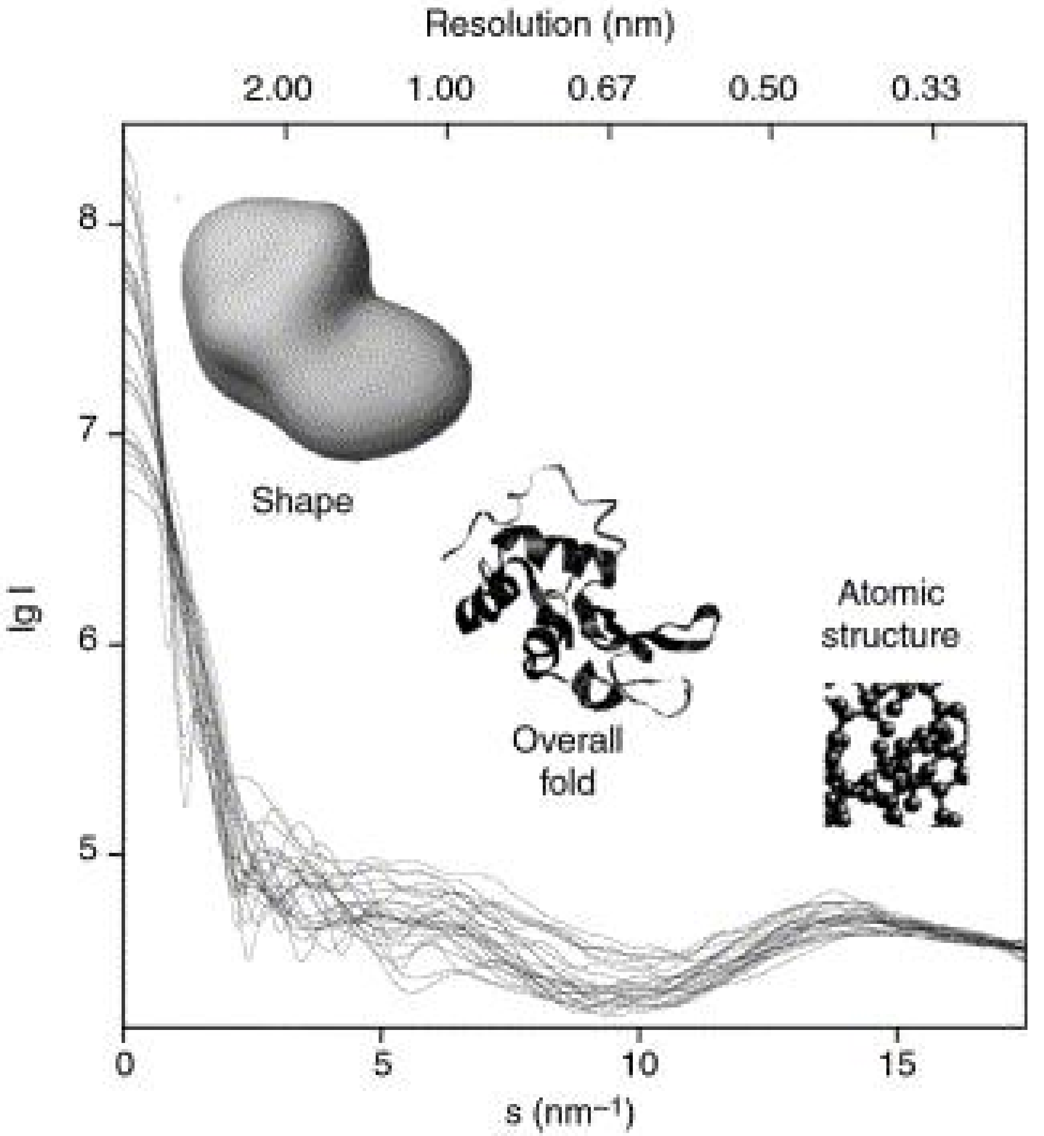


Photosynthetic reaction centres:

- Do ultrafast conformational changes contribute to the primary charge separation reactions of photosynthesis?

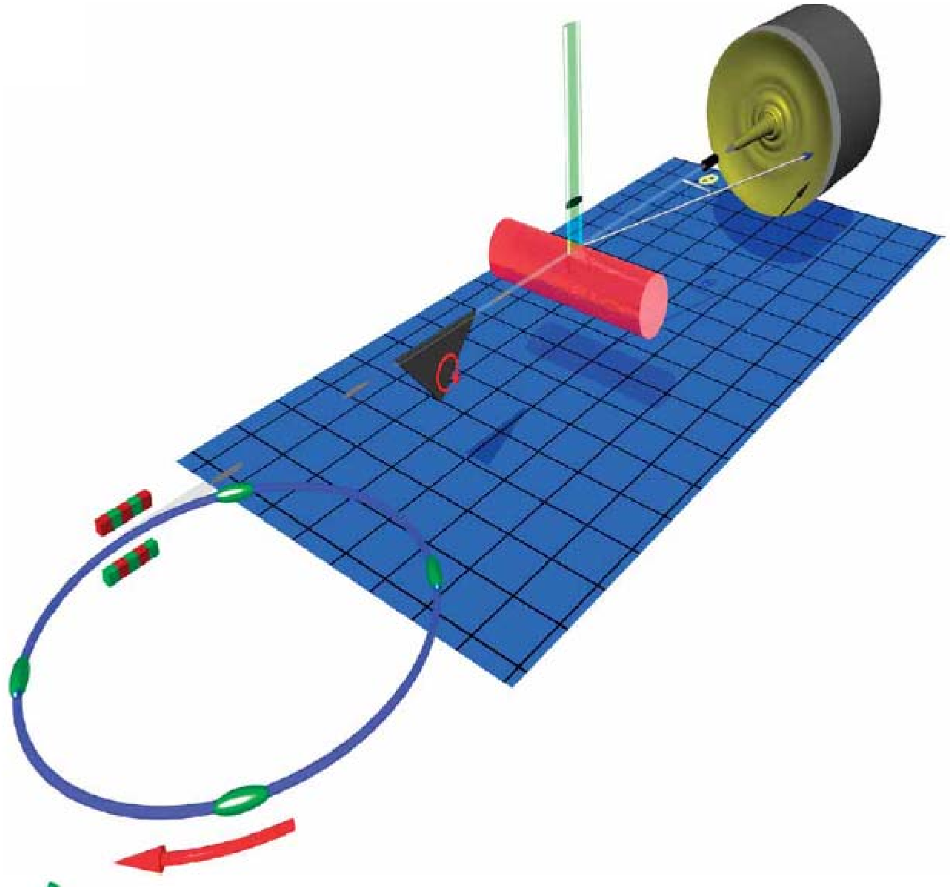


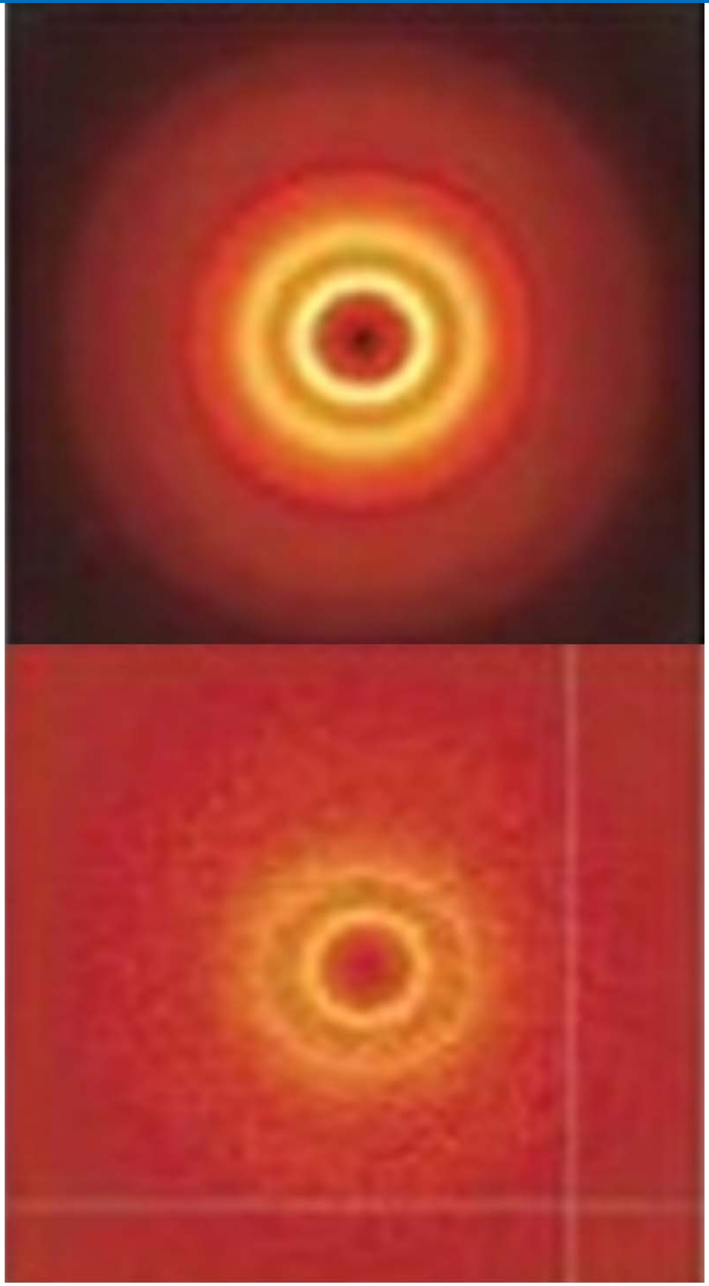
X-ray scattering



Svergun & Koch, *Biophys. Meth*. (2002)

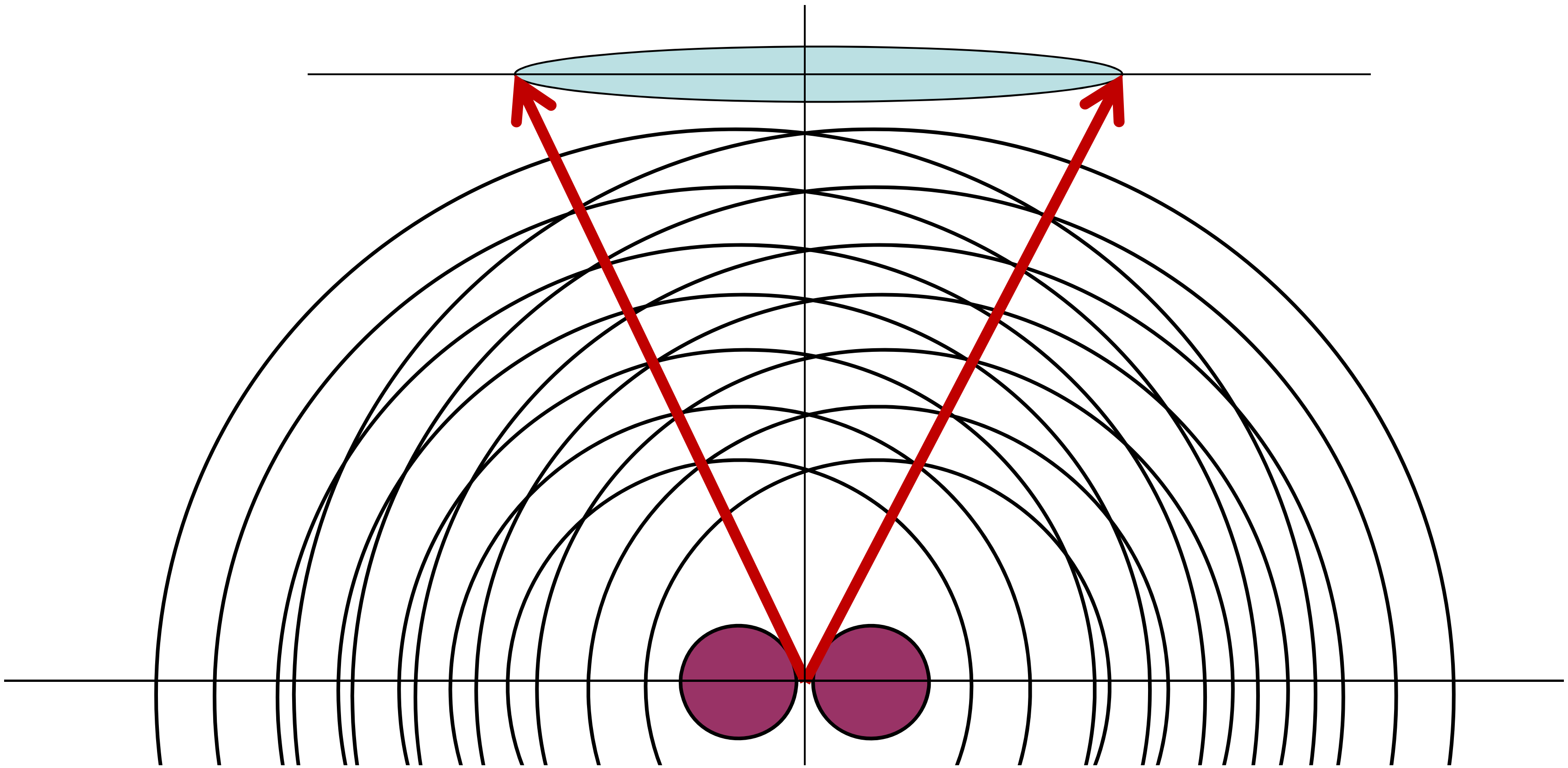
Time-resolved Wide Angel X-ray Scattering



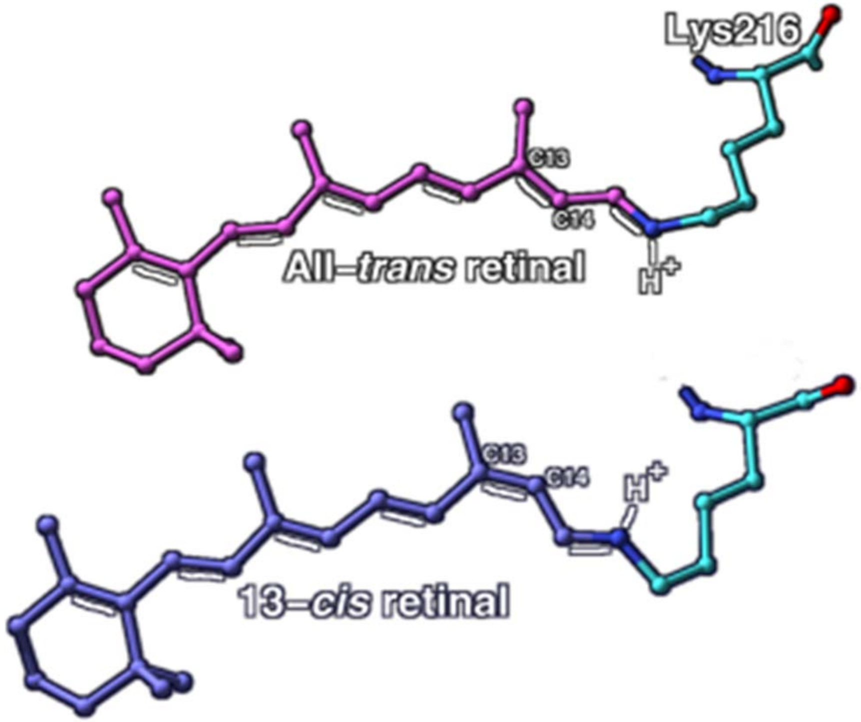


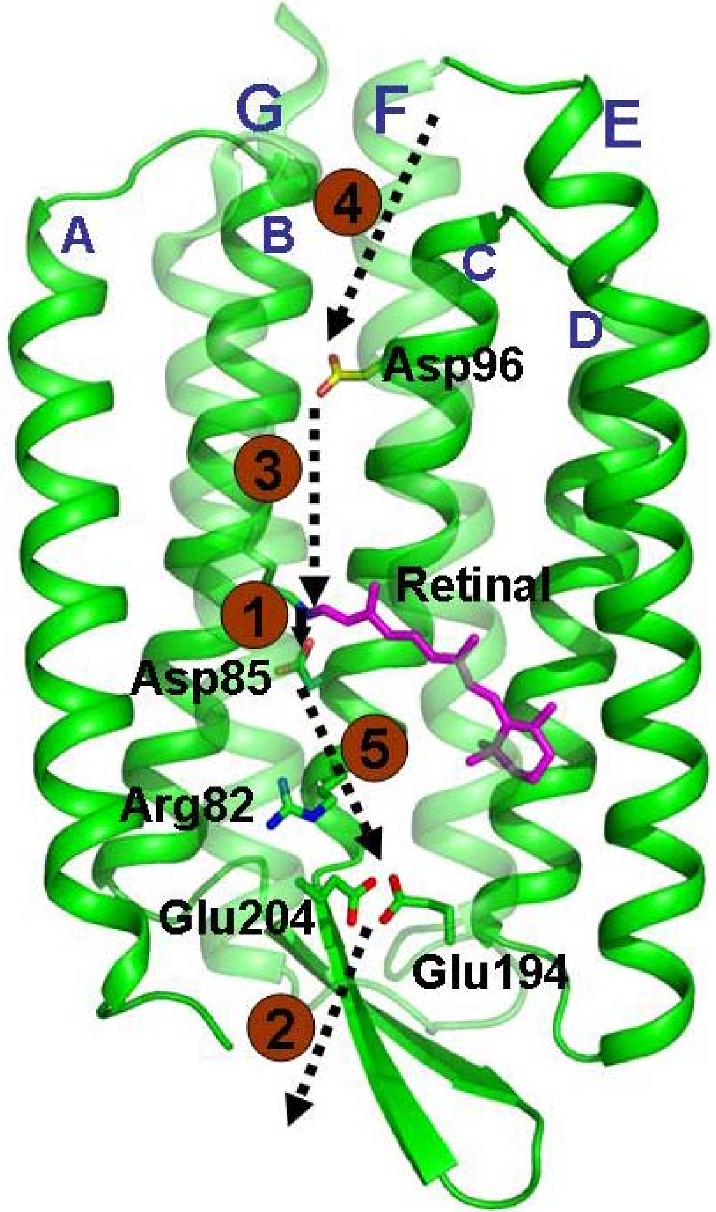
Cammarata et al., Nature Methods (2008)

Time Resolved Wide Angle X-ray Scattering



Bacteriorhodopsin





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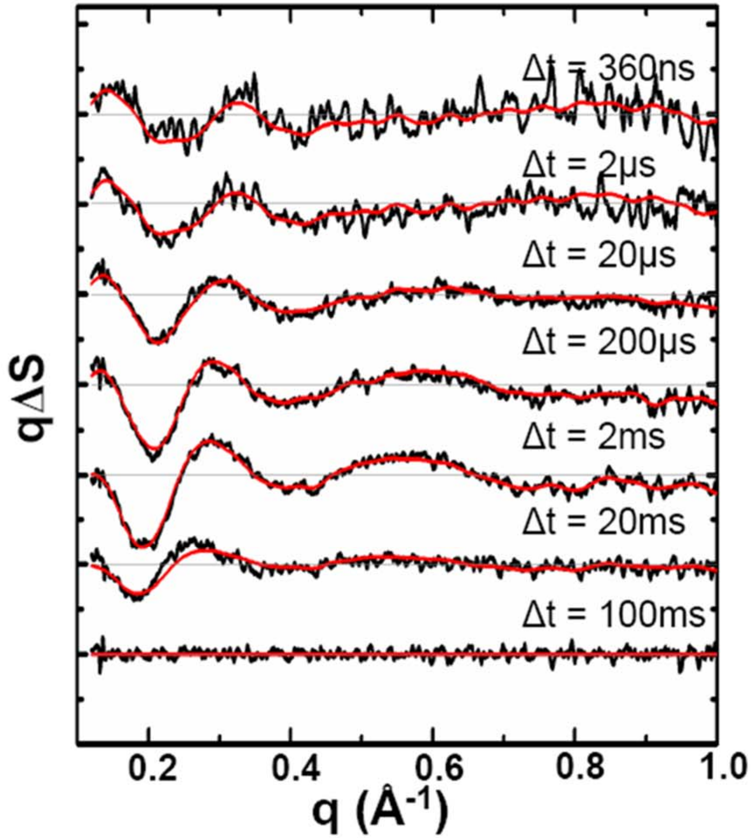
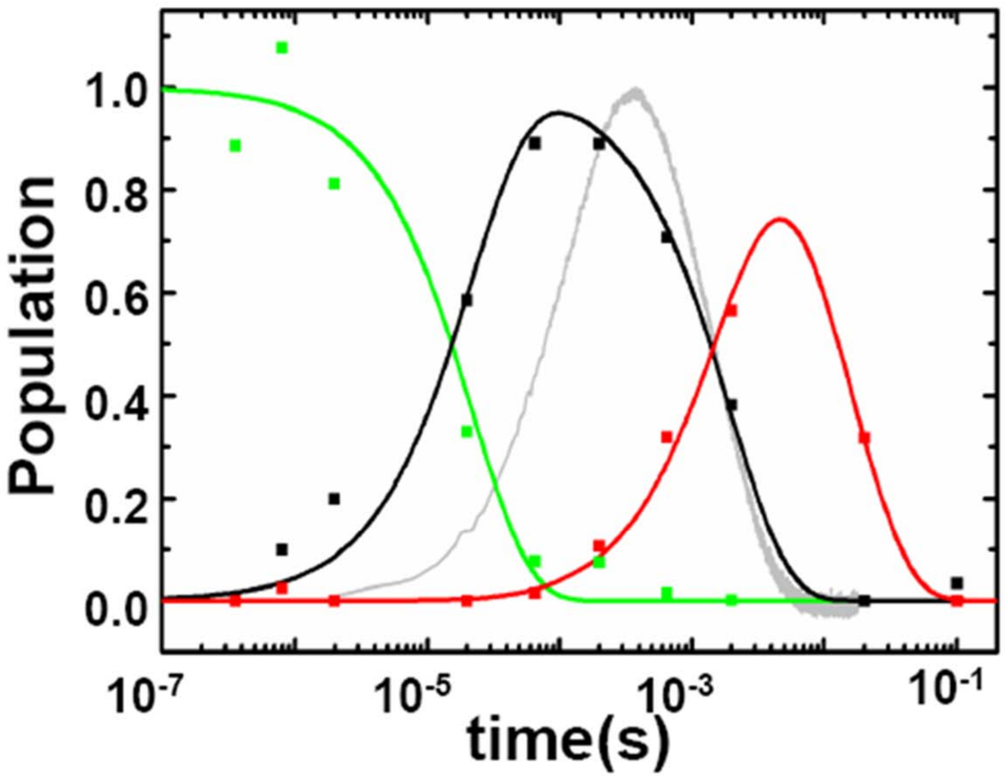
European Synchrotron Radiation Facility

Michael Wulff

Marco Cammarata

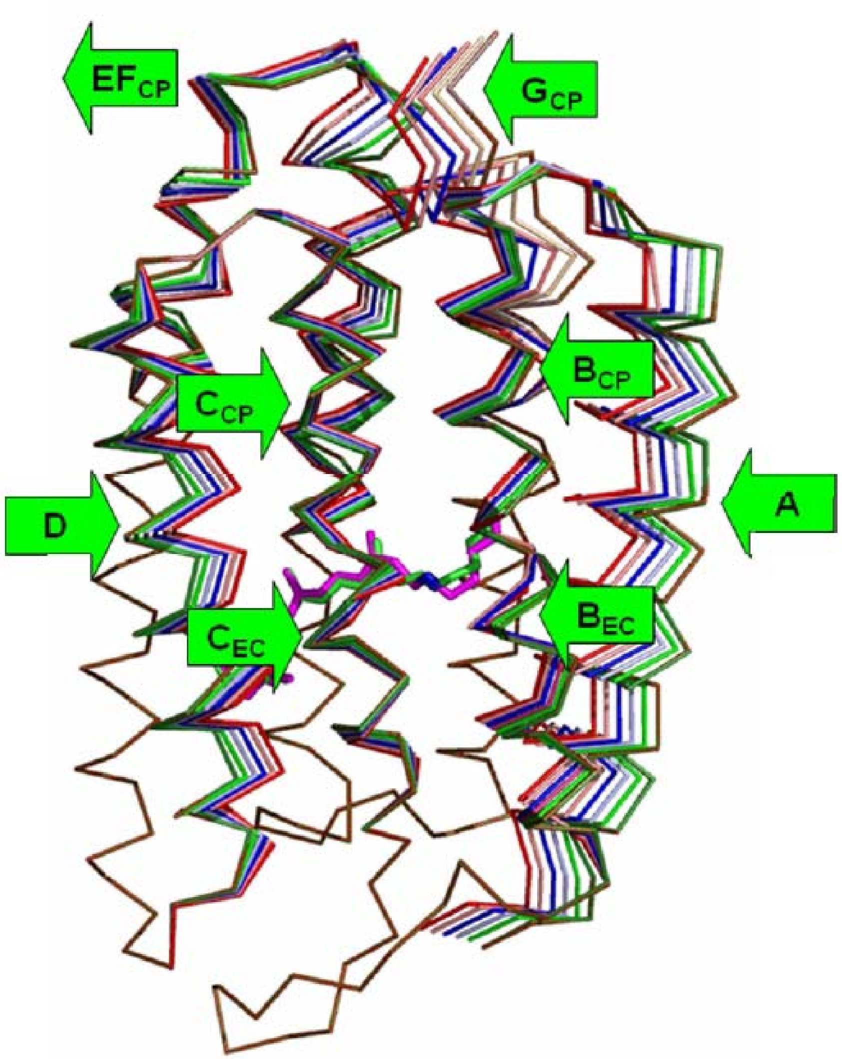
Experimental data & spectral decomposition

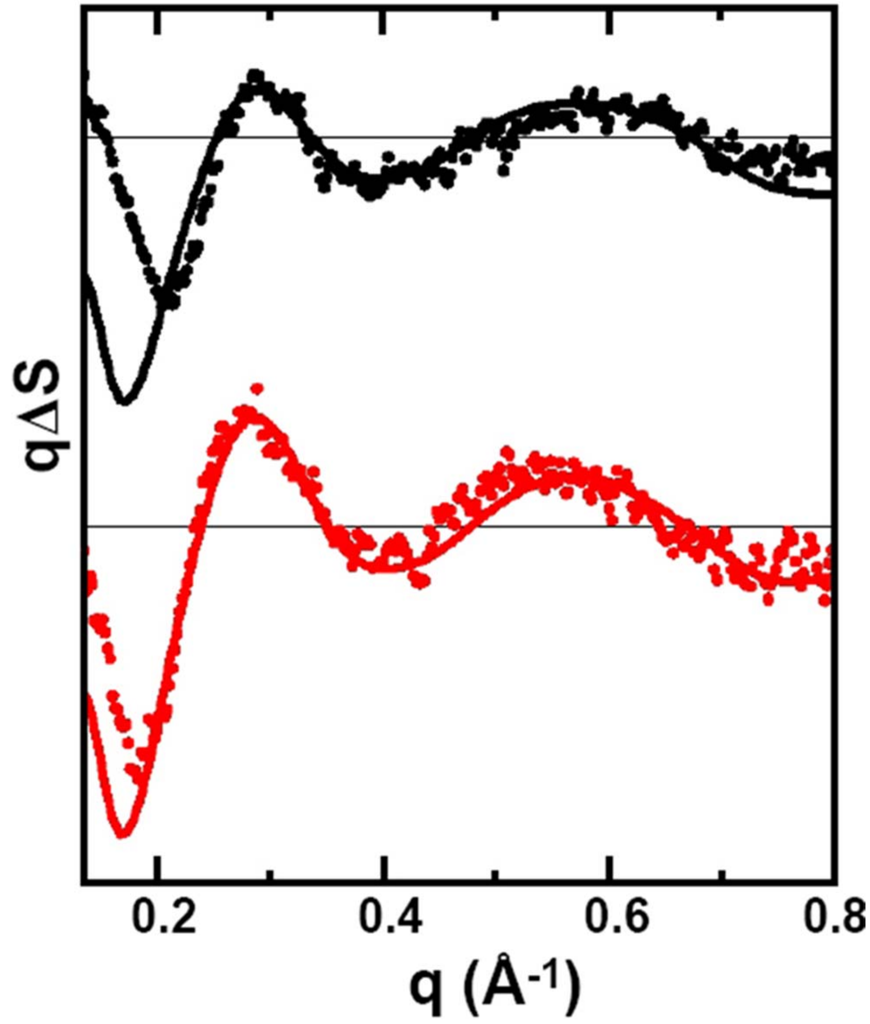




Andersson *et al.*, *Structure* (2008)

Structural Refinement





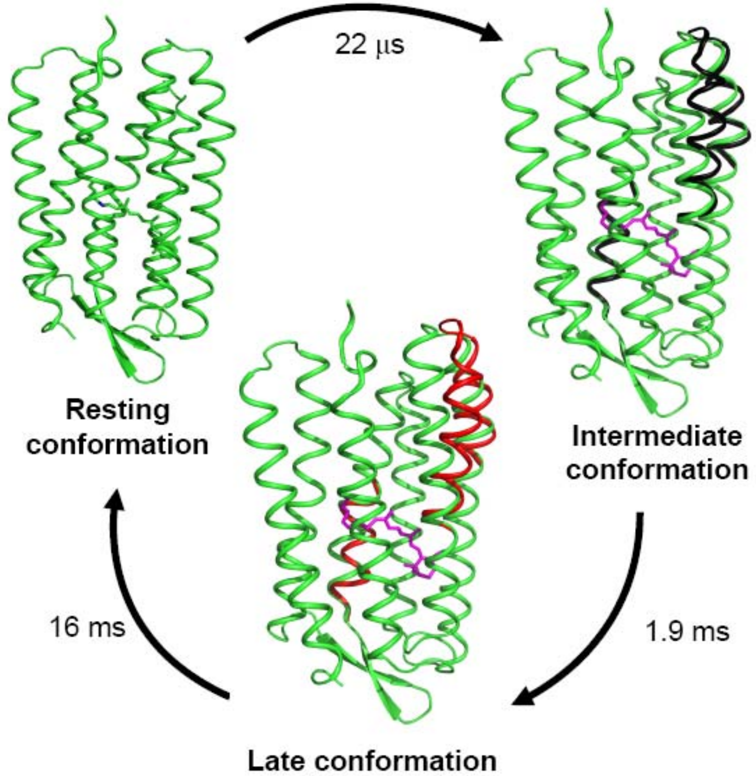
Andersson *et al.*, *Structure* (2008)

Structural Conclusions

• Two intermediate conformations.

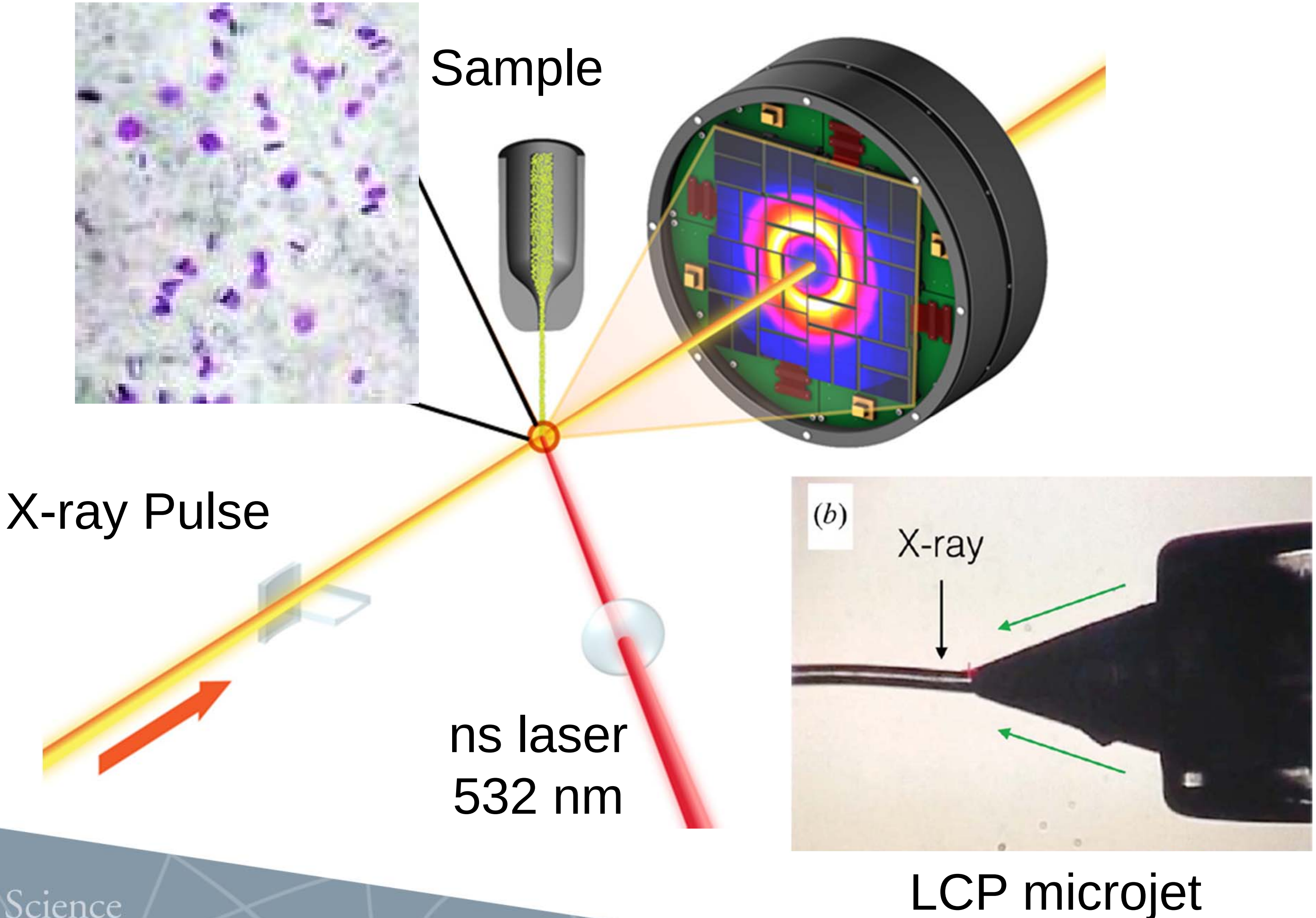
• Movements of helices E, F & C used to model these changes.

• 2/3 of the movement occurs prior to the first proton transfer step.



Andersson *et al.*, *Structure* (2008)

Time Resolved Serial Crystallography

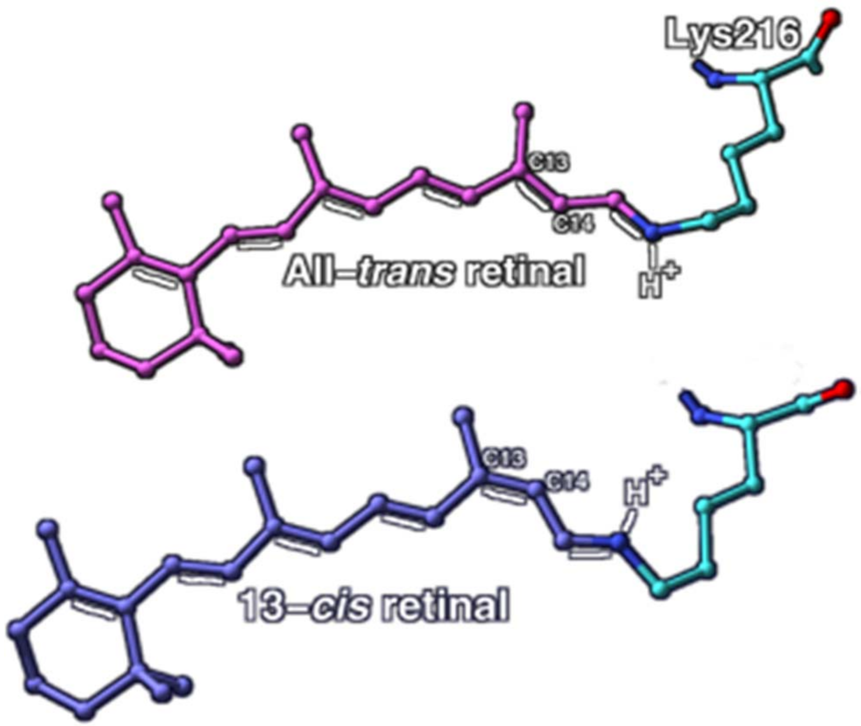


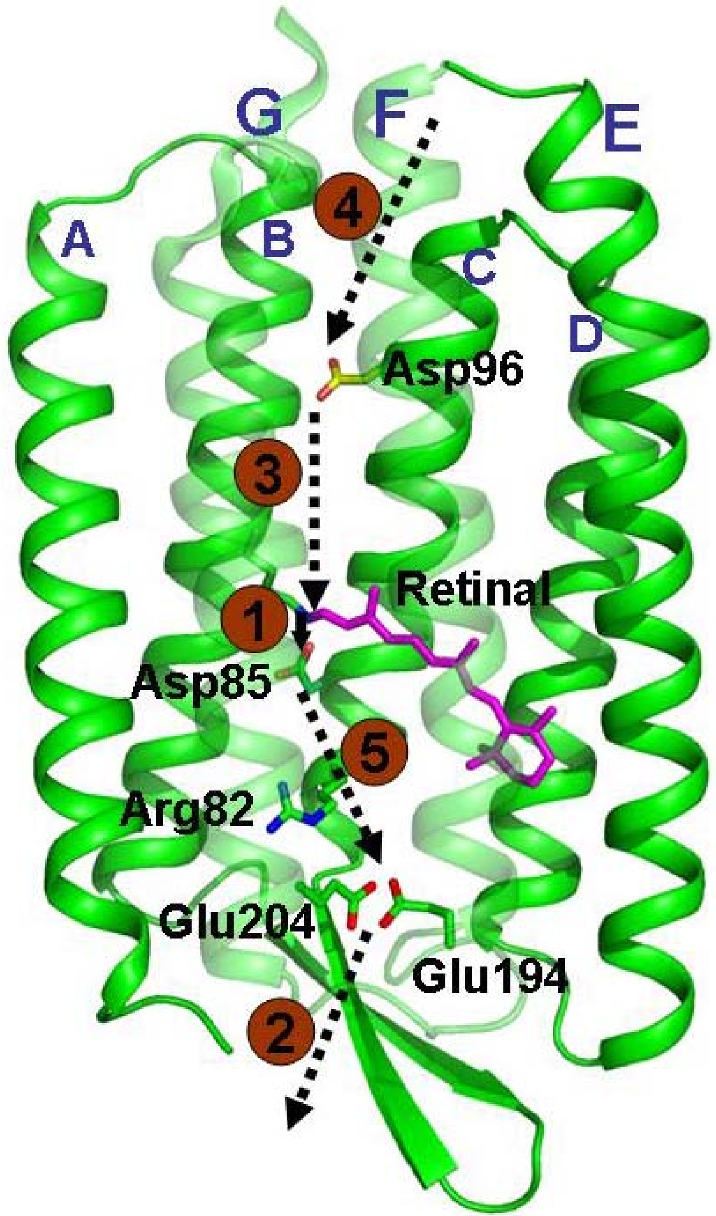
Eriko Nango

So Iwata

SACLA at SPring8
Hyogo, Japan

Bacteriorhodopsin





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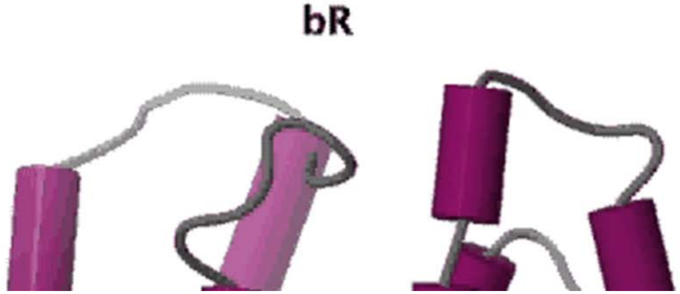
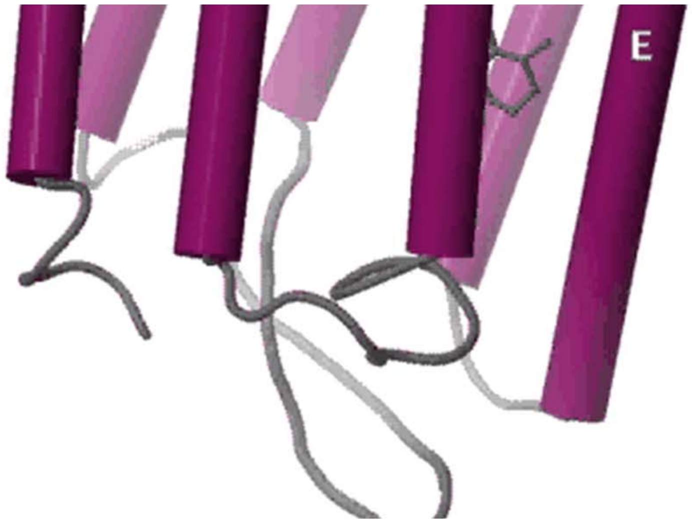
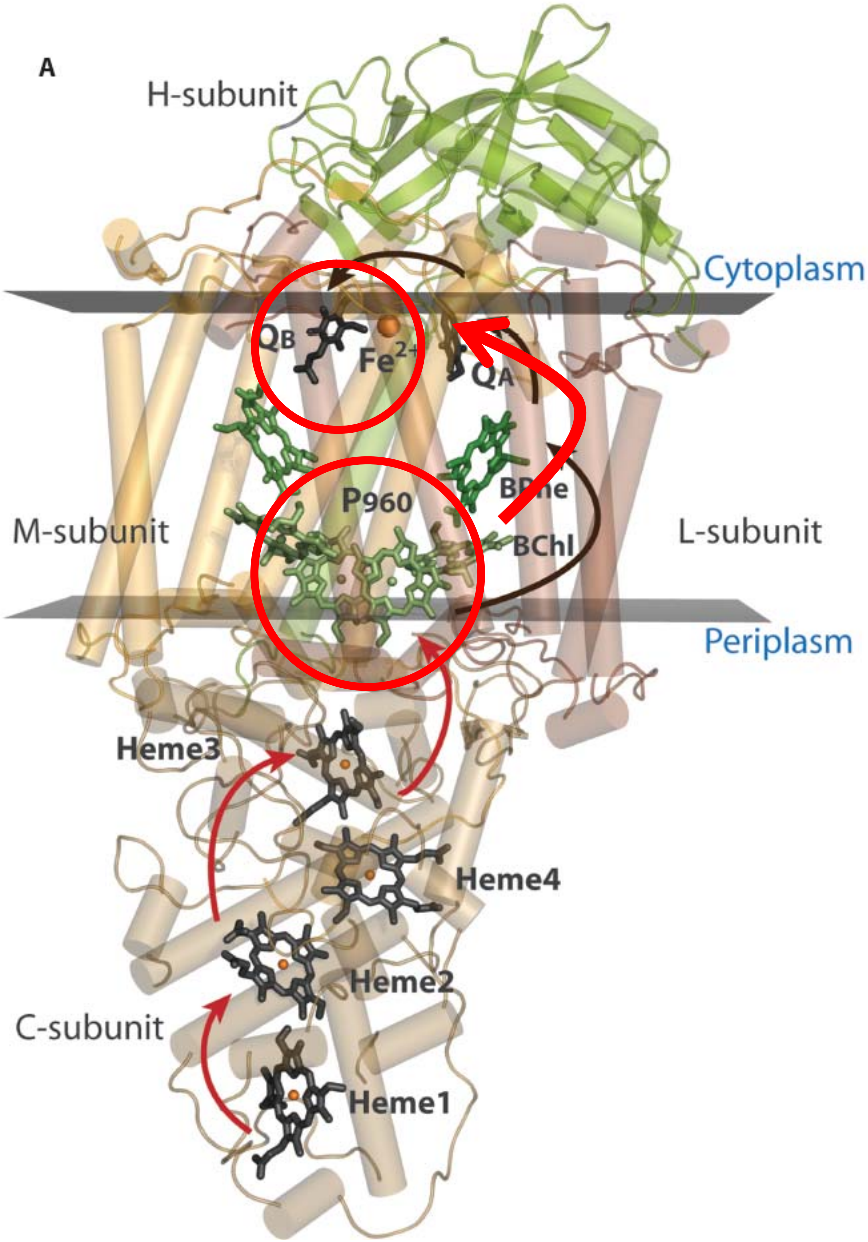
Photocycle of bacteriorhodopsin



- Highly coordinated structural changes control the pKa of key proton-exchange groups.
- Understanding the nature & timing of these events central to untangling the mechanism of proton pumping.



Photosynthetic reaction centres



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- Electron movements driven by light.
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- Descendent created O_2 rich atmosphere.

Time-resolved spectroscopy

Nature **363**, 320 (1993)

ARTICLES

Visualization of coherent nuclear motion in a membrane protein by femtosecond spectroscopy

Marten H. Vos*, Fabrice Rappaport*, Jean-Christophe Lambry*, Jacques Breton† & Jean-Louis Martin*‡

"implicates coherent nuclear motion in the primary electron transfer reaction in functional reaction centres"

Time-resolved spectroscopy

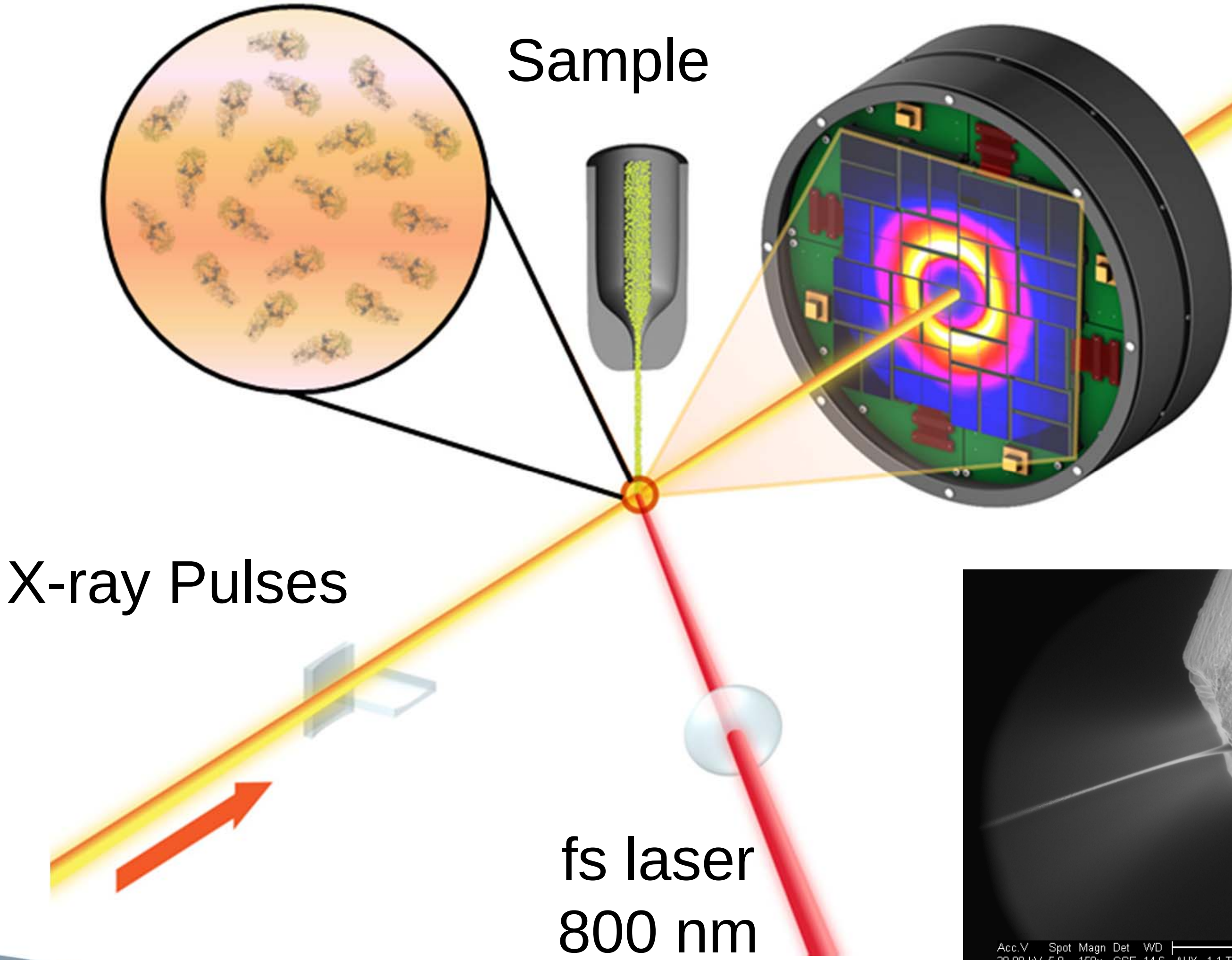
Science **316**, 747 (2007)

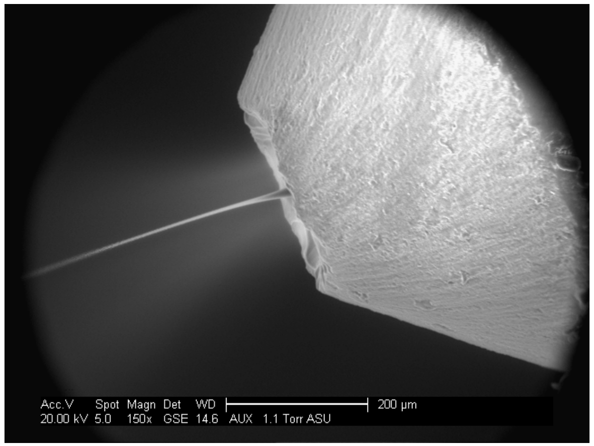
Protein Dynamics Control the Kinetics of Initial Electron Transfer in Photosynthesis

Haiyu Wang,[1,2] Su Lin,[1,2] James P. Allen,[2] JoAnn C. Williams,[2] Sean Blankert,[1,2] Christa Laser,[1,2] Neal W. Woodbury[1,2]*

"These results indicate that initial photosynthetic charge separation is limited by protein dynamics rather than by a static electron transfer barrier"

Time resolved wide angle X-ray scattering



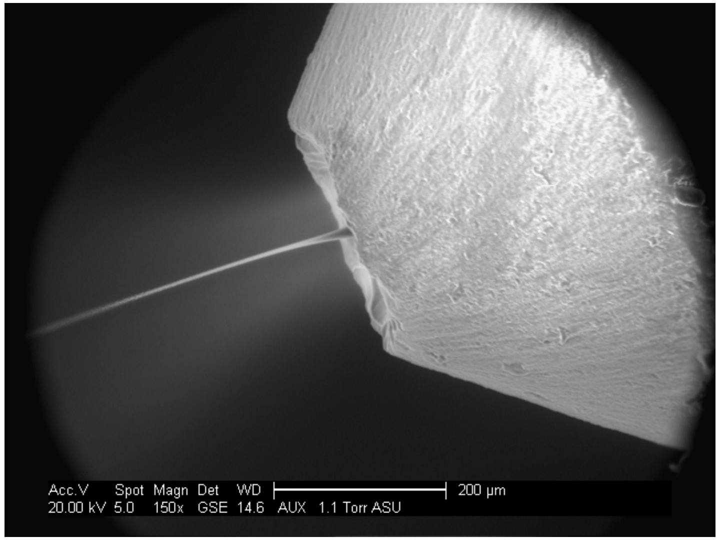


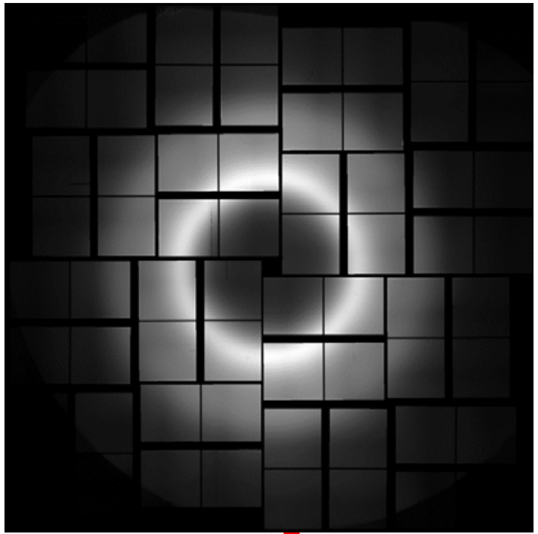
Gas-focused microjet

Linac Coherent Light Source (LCLS)
Stanford, USA

John Spence Henry Chapman Anton Barty Sebastien Boutet

Time resolved wide angle X-ray scattering

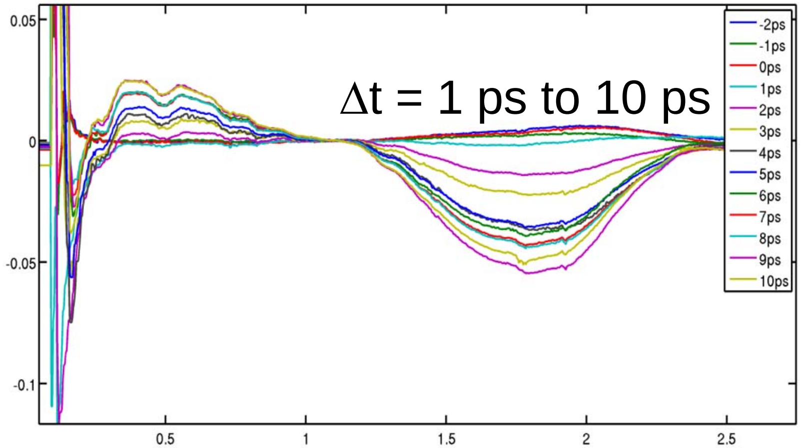




Laser on minus laser off

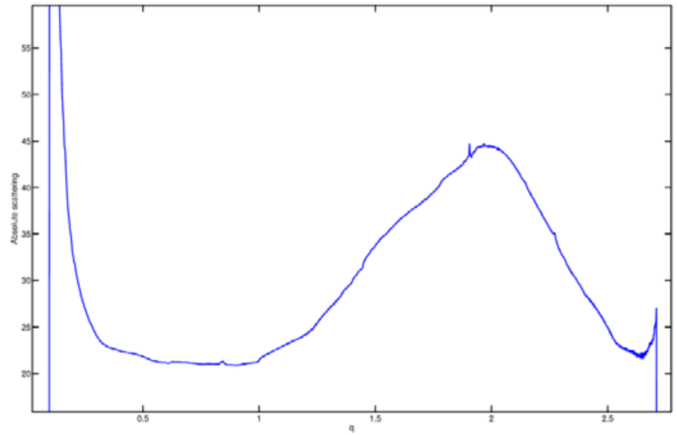
Radial Integration

$\Delta S(q,\Delta t)$



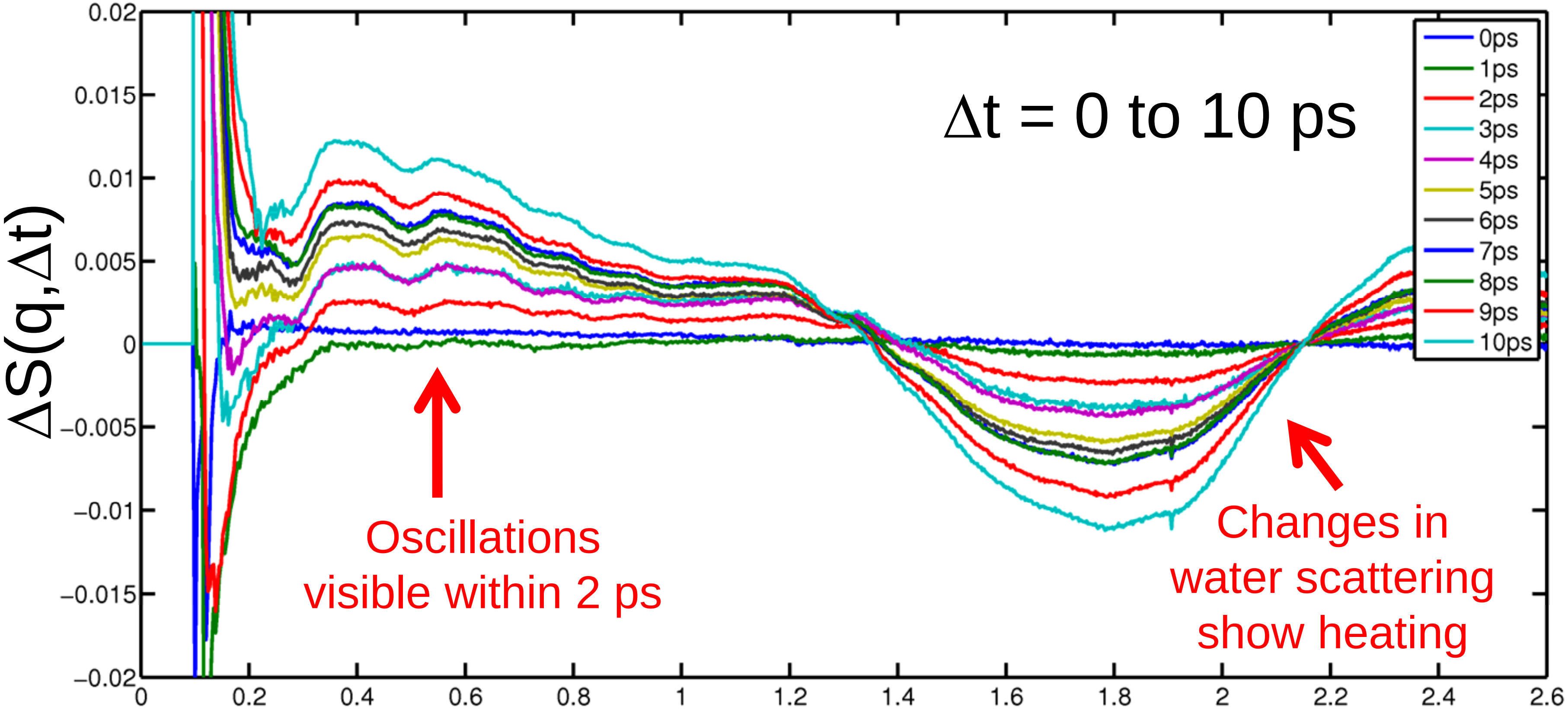
$q = 4\pi \sin\theta/\lambda$

$S(q,\Delta t)$



$q = 4\pi \sin\theta/\lambda$

TR-WAXS: Laser on minus laser off

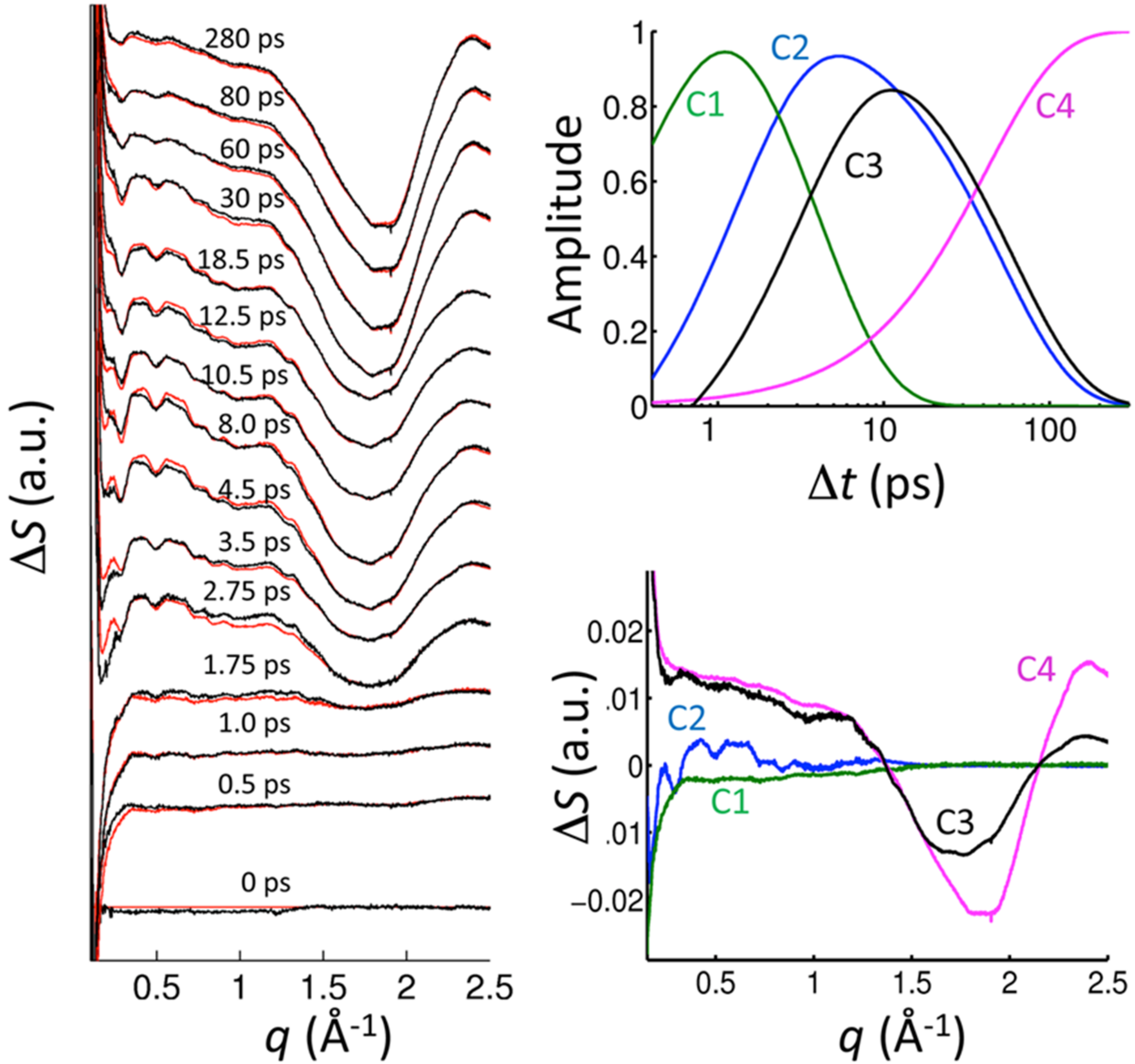


$q = 4\pi \sin\theta/\lambda$ $(Å^{-1})$

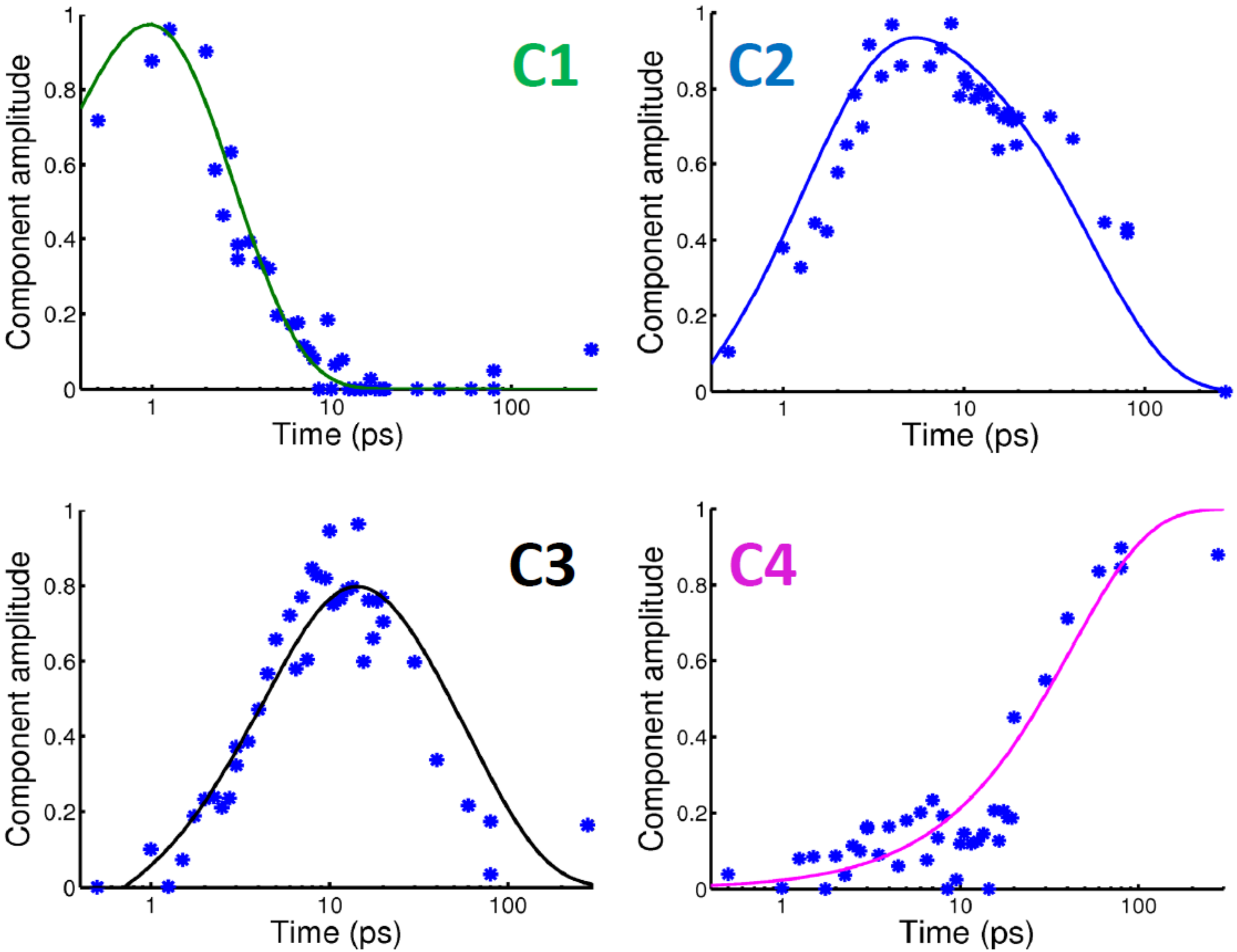
Arnlund *et al.*, *Nature Methods* (2014)

Faculty of Science

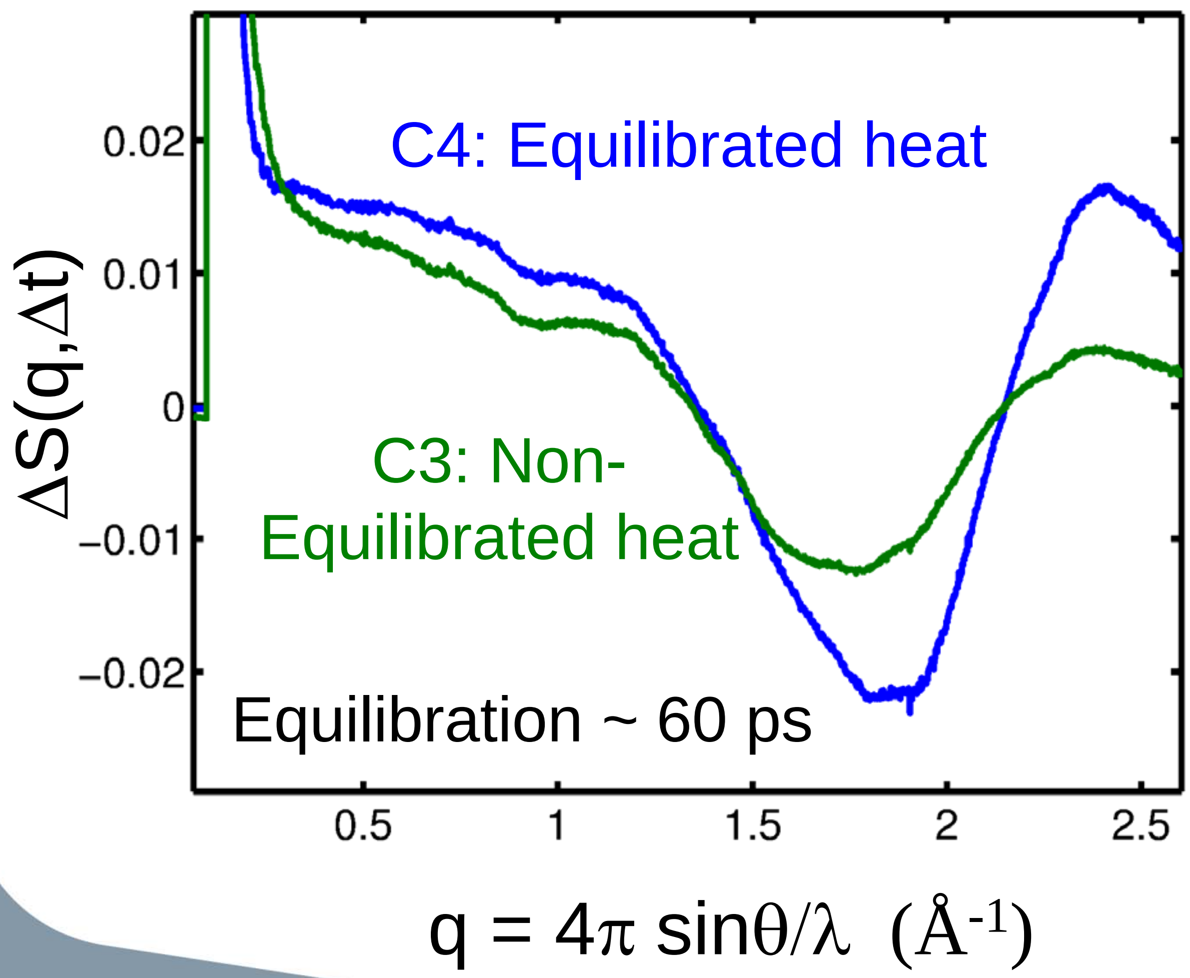
Spectral decomposition

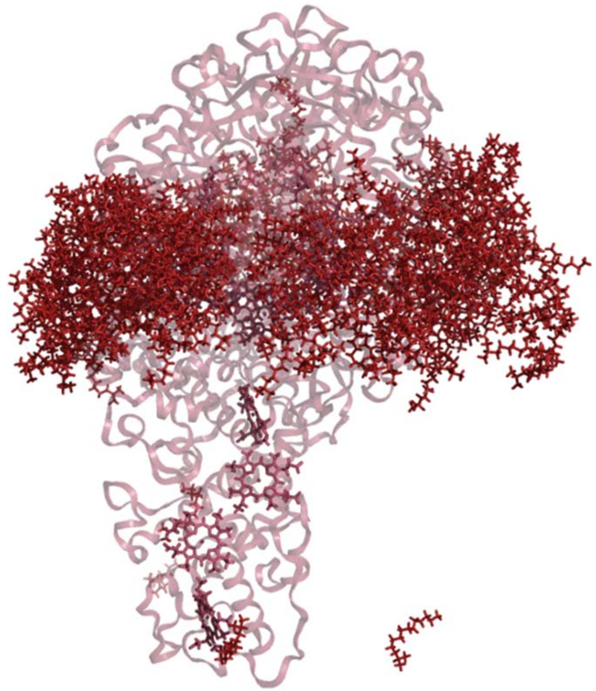


Linear recombination of amplitudes



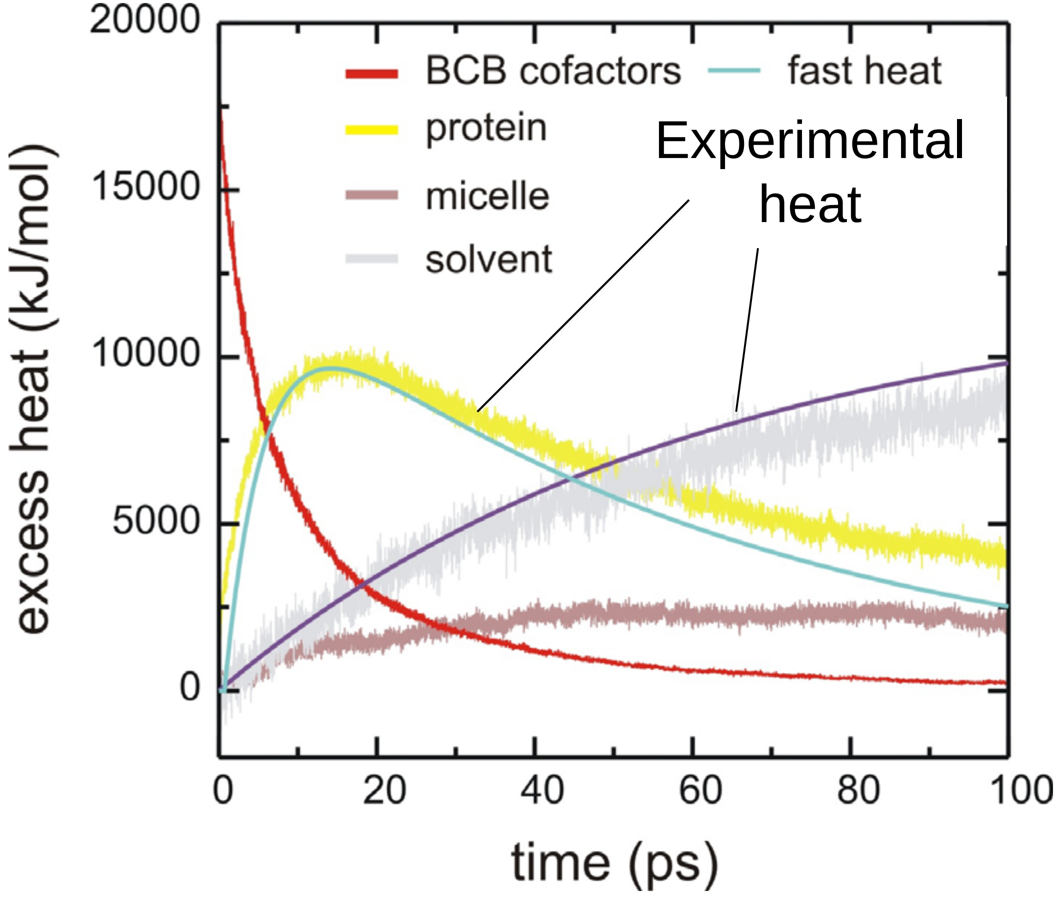
Equilibrated & non-equilibrated heating





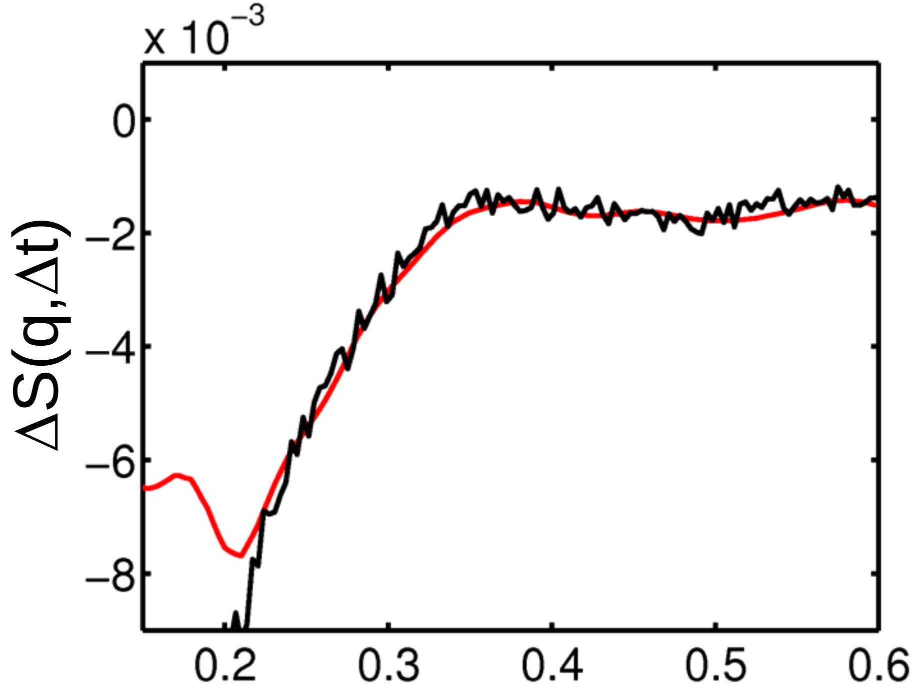
Arnlund *et al.*, *Nature Methods* (2014)

MD simulations: flow of heat

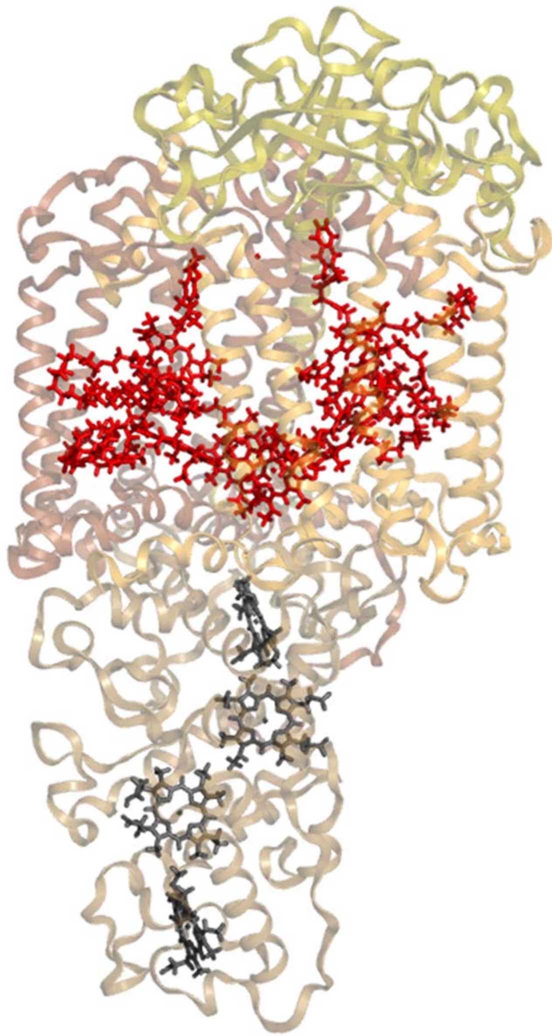


- MD simulations & TR-WAXS measurements both show heat equilibrates within ~ 100 ps.

Ultrafast-component



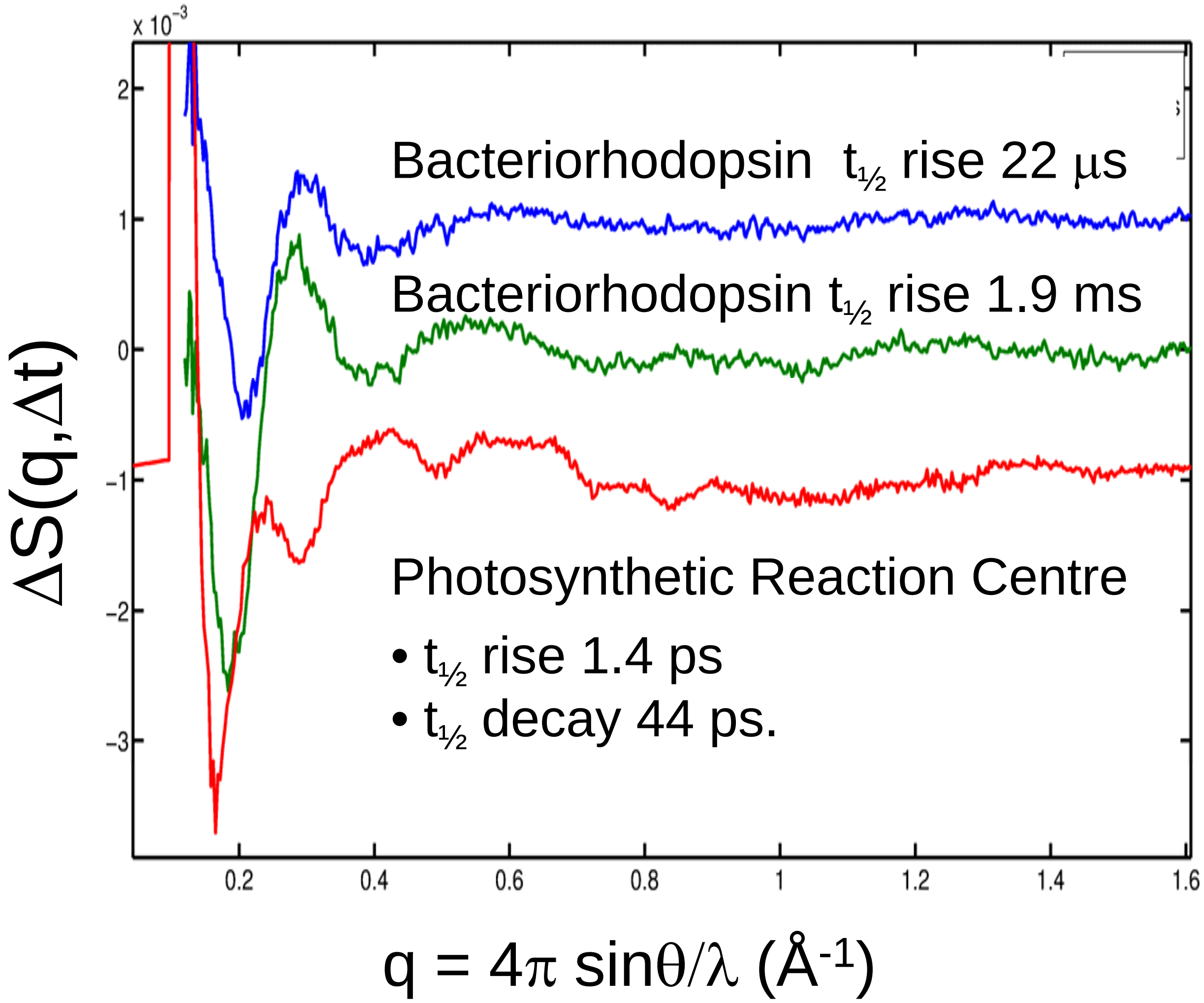
$q = 4\pi \sin\theta/\lambda$ (Å^{-1})



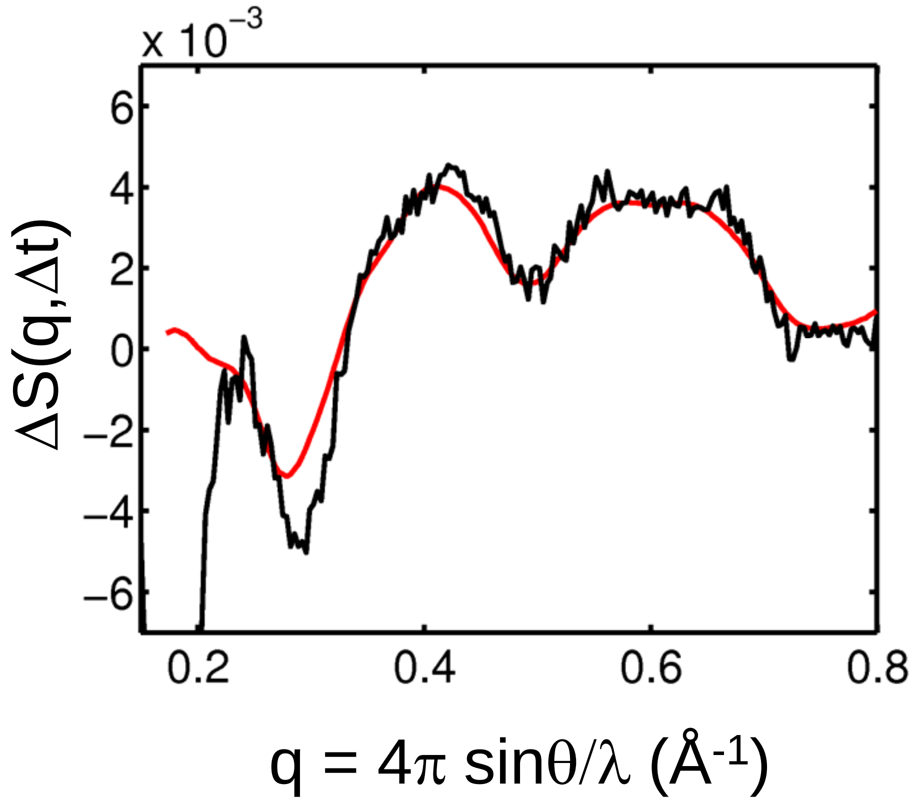
- Fitting with only co-factors moving.
- Rise: Sub-ps.
- $t_{1/2}$ decay ~ 3·2 ps.

Arnlund *et al.*, *Nature Methods* (2014)

c.w. TR-WAXS on bacteriorhodopsin



Fitting protein component

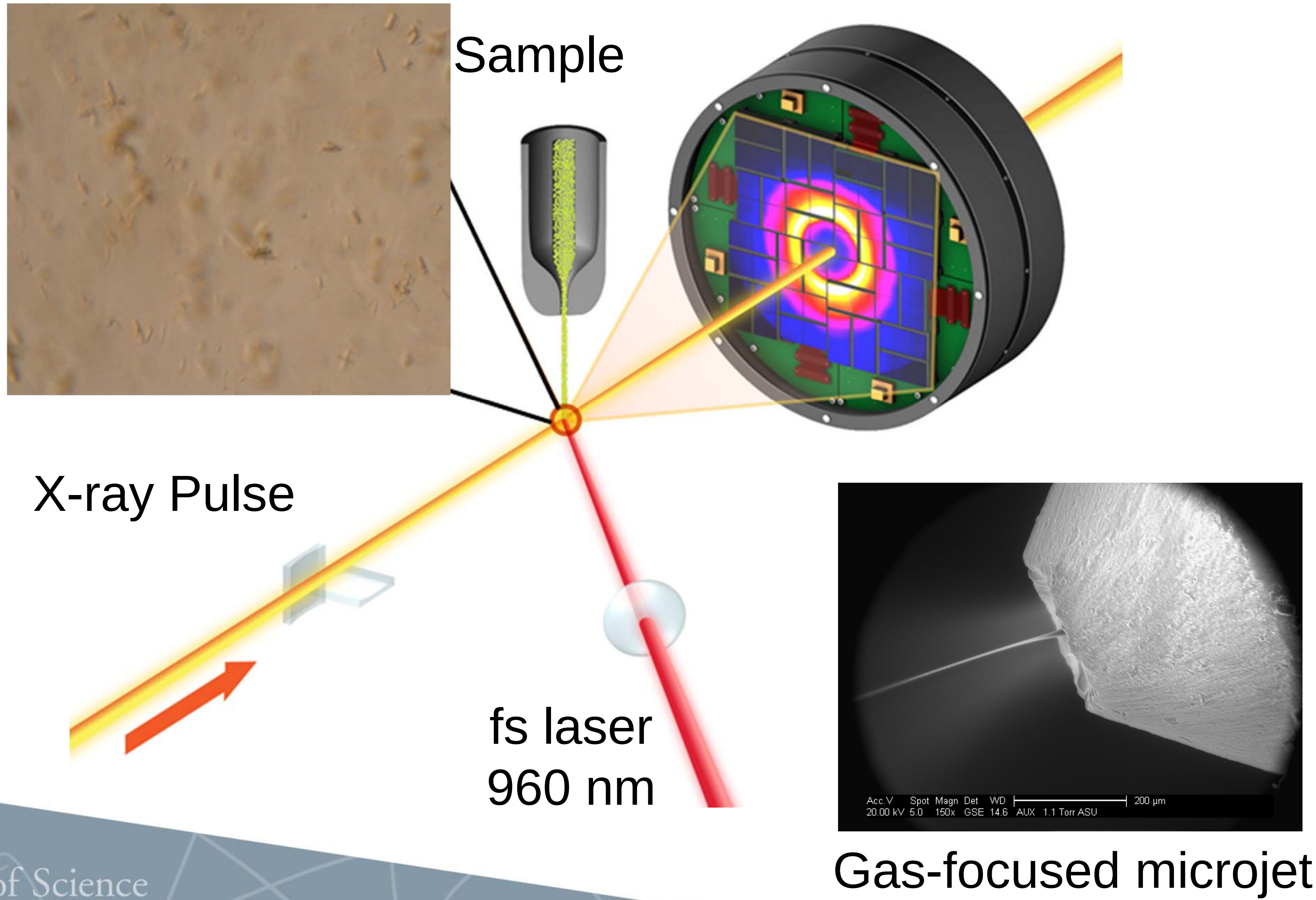


- Fitting with all protein atoms moving.
  - $t_{1/2}$ rise ~ 1·4 ps
  - $t_{1/2}$ decay ~ 44 ps.

Arnlund *et al.*, *Nature Methods* (2014)

Overall conformational change

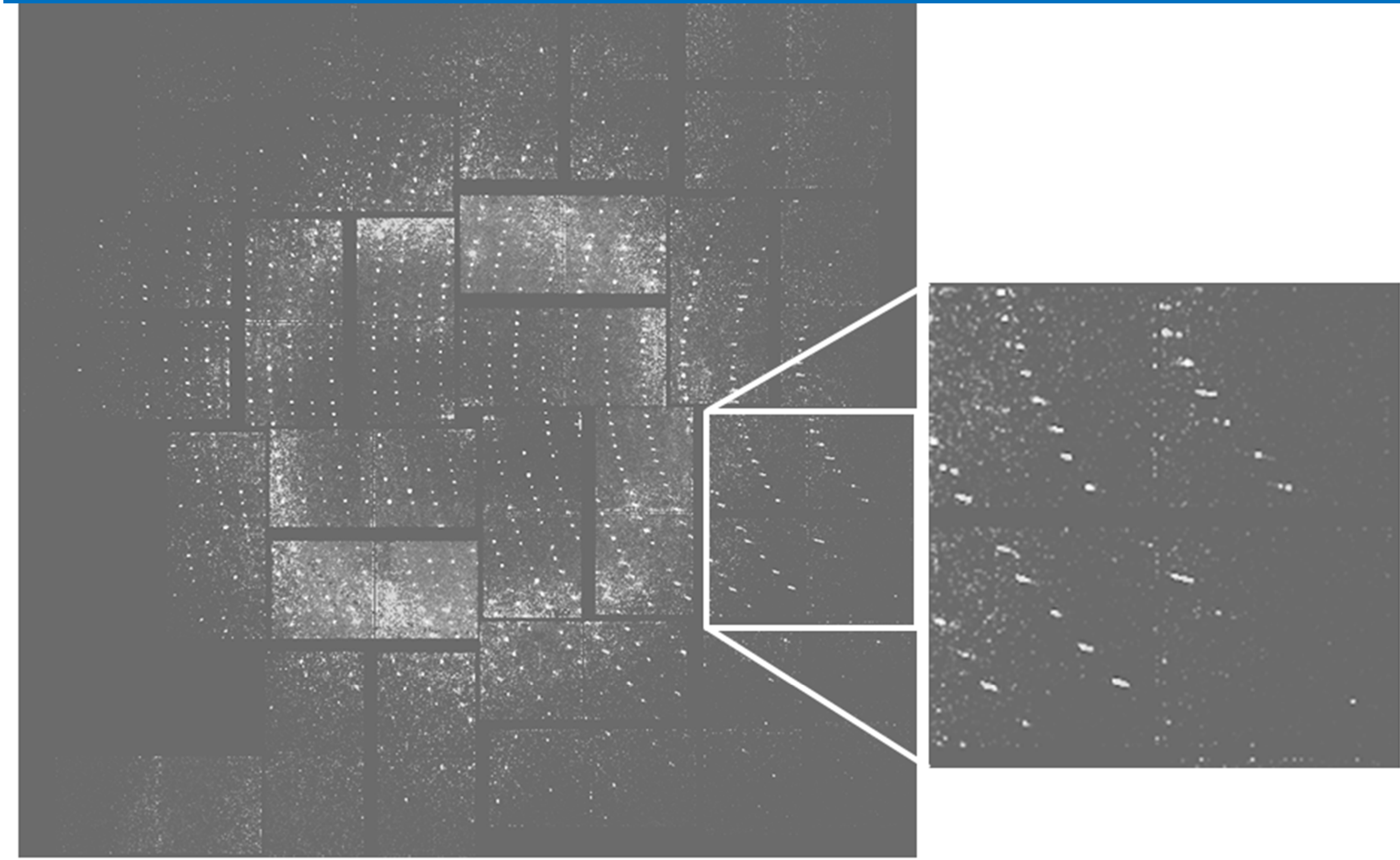
Time Resolved Serial Crystallography



Linac Coherent Light Source (LCLS)
Stanford, USA

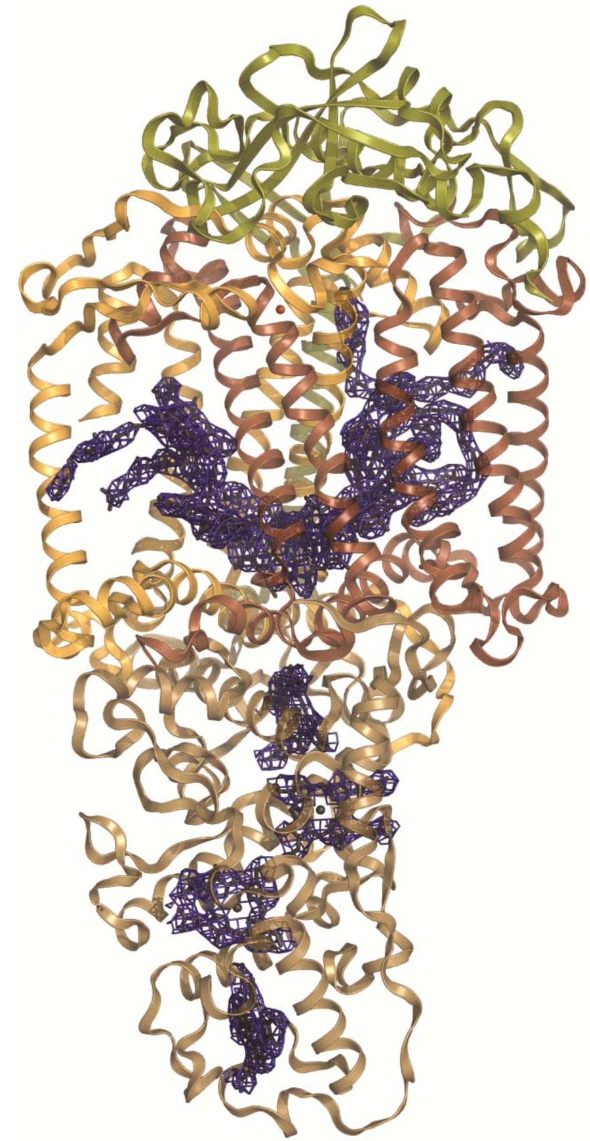
John Spence | Henry Chapman | Anton Barty | Sebastien Boutet

Data extend to 2.8 Å resolution

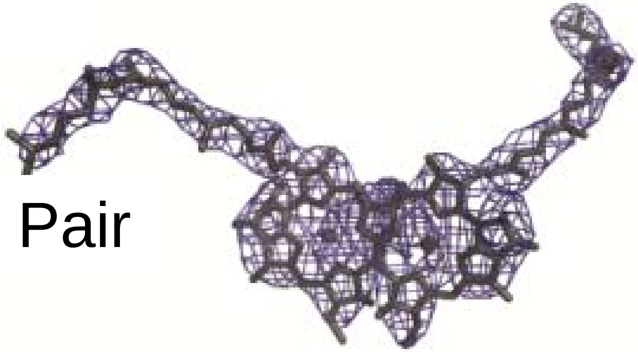


Johansson *et al.*, *Nature Communications* (2013).

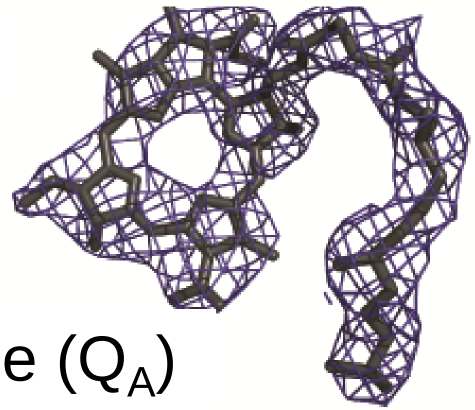
3.5 Å SFX structure



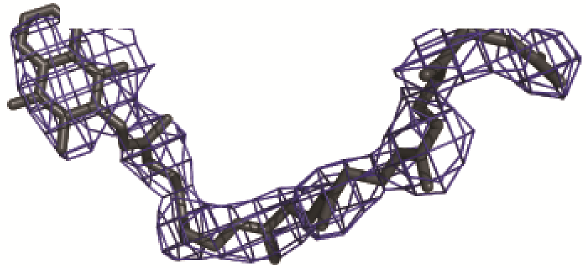
Special Pair



Pheophytin



Menaquinone (Q_A)



Johansson *et al.*, *Nature Communications* (2013)

Ultrafast conformational gating?

- Photosynthetic reaction centres remarkably efficient.
  - Multiple contributing factors.
- Structural changes allow the energy surface for an electron on *the way out* to differ from that *back home*.
- May help extend the lifetime of the charge separated state.

Serial Femtosecond Crystallography @LCLS

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
Gothenburg	***L. Johansson, D. Arnlund***, G. Katona, E. Malmerberg
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
LBNL	S. Marchesini, J. Holton
Cornell	V. Elser, S. Gruner
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*

Time resolved WAXS @ LCLS

Gothenburg	***D. Arnlund, L. Johansson, C. Wikstrand, R. Dods, E. Malmerberg,*** G. Katona, J. Sjöhamn,S. Westenhoff.
CFEL-DESY	***A. Barty, H. Chapman,*** J. Schulz, M. Liang, A. Aquila, T. White, D. Deponte, S. Stern, A. Martin, K. Nass, F. Stellato.
ASU	***J. Spence***, P. Fromme, U. Weierstall, B. Doak, R. Kirian, D.Wang, K. Schmidt, I. Grotjohann, R. Fromme, D. James.
SLAC-LCLS	***S. Boutet, G. Williams,*** M. Seibert, J. Kryzwinski, C. Bostedt, M. Messerschmidt, J. Bozek, W. White, R. Coffee
Uppsala	***J. Davidsson***
MPI Heidelberg	***I. Schlichting***, R. Shoeman, T. Barends, S. Bari
LCLS detectors	C. Kenney, R. Herbst, J. Pines, P. Hart, J. Morse
LCLS accelerator	*Led by P. Emma*
LCLS fs laser	D. Milathianaki, A. Fry.
LLNL	M. Frank
Göttingen	***G. Groenhof***
APS BioCARS	***R. Henning***, I. Kosheleva.
TDU	K. Skov Kjær, T. Brandt van Driel, M. Meedom Nielsen.

Time resolved SFX @ SACLA

Riken	***Eriko Nango, So Iwata, Rie Tanaka,*** Toshiaki Hosaka, Tomoyuki Tanaka, Ayumi Yamashita, Jun Kobayashi, Toshi Arima, Minoru Kubo, Tetsunari Kimura, Song Changyong.
CNRS	***Antoine Royant.***
Tokyo Uni.	***Takanori Nakane***
Gothenburg	Robert Dods.
Uppsala	Jan Davidsson.
JASRI	**Kensuke Tono, Yasumasa Joti,**
Osaka Uni.	Eiichi Mizohata
Postech	Nam Daewoong

Time resolved WAXS @ ESRF

Gothenburg	***Magnus Andersson, Erik Malmerberg***, Sebastian Westenhoff, Gergely Katona, Annemarie Wöhri, Linda Johansson
ESRF	***Michael Wulff,*** Marco Cammarata, Friederike Ewald.
Uppsala	***Jan Davidsson***., Mattias Eklund.

LCLS

Vetenskapsrådet

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SWEGENE
The Postgenomic Research and Technology Programme in South Western Sweden

CHALMERS

SIXTH FRAMEWORK PROGRAMME

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GÖTEBORGS UNIVERSITET

Stiftelsen Olle Engkvist Byggmästare

HFSP
HUMAN FRONTIER SCIENCE PROGRAM
FUNDING FRONTIER RESEARCH INTO COMPLEX BIOLOGICAL SYSTEMS

STINT
Stiftelsen för internationalisering av högre utbildning och forskning

Lundberg Stiftelsen

ESRF

Advanced Photon Source
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PAUL SCHERRER INSTITUT
PSI

MAX-lab