

Time-resolved serial crystallography studies of conformational changes in cytochrome c oxidase

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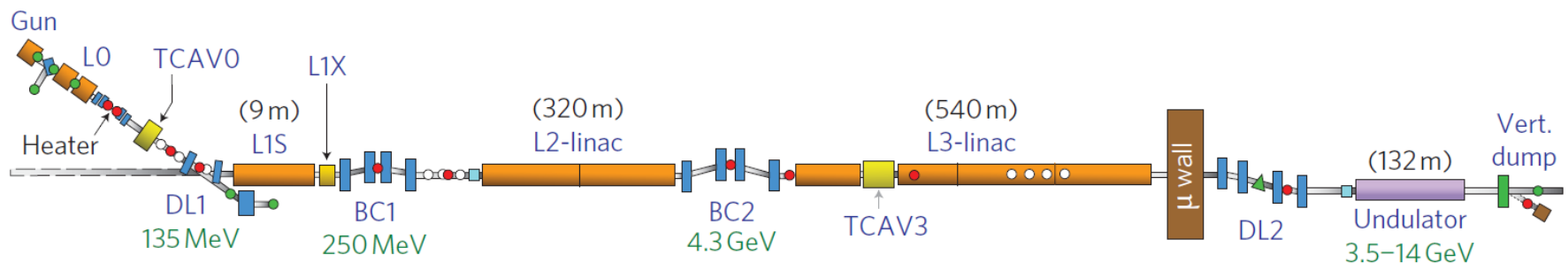


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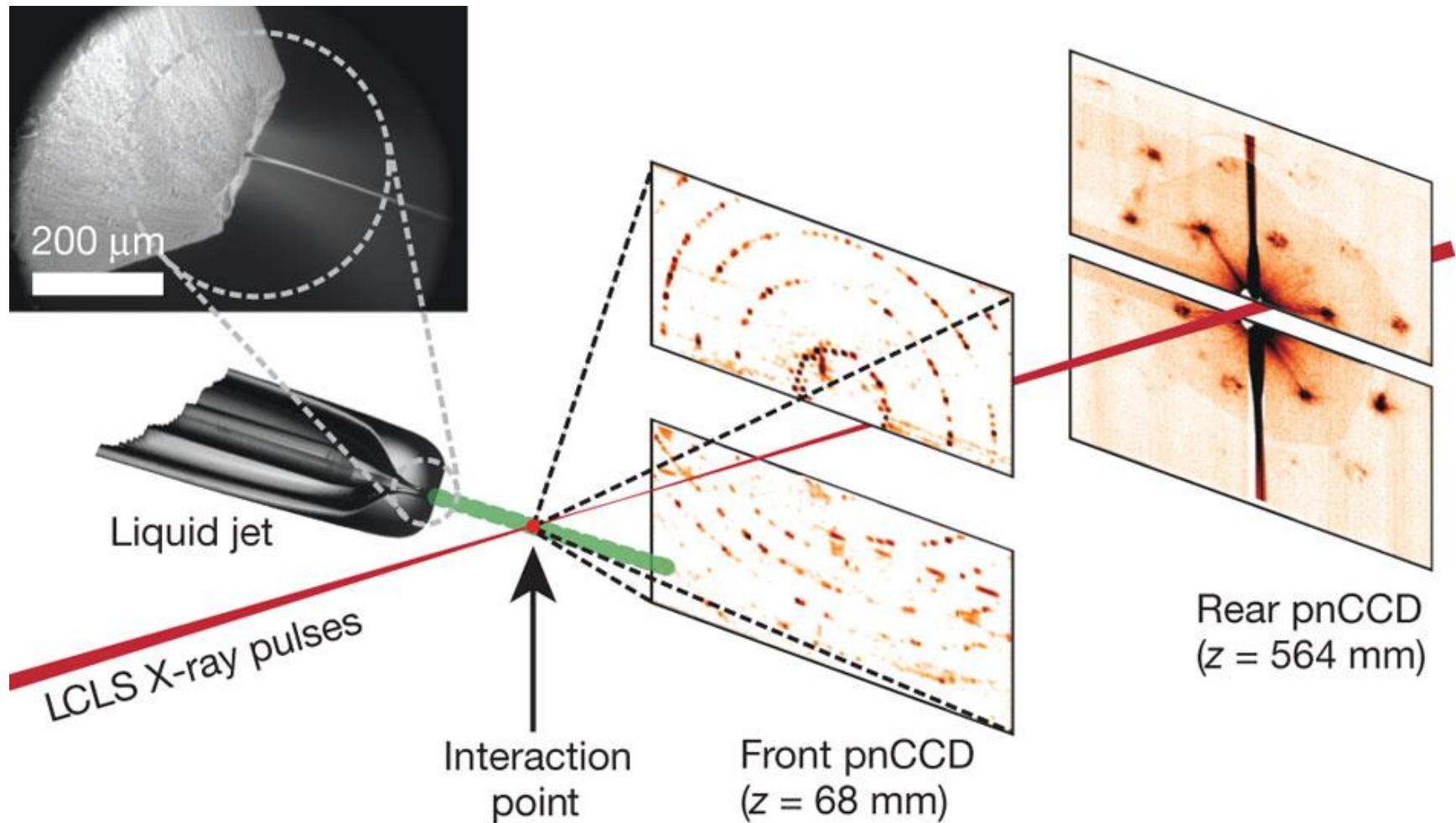
First lasing and operation of an ångstrom-wavelength free-electron laser

P. Emma¹*, R. Akre¹, J. Arthur¹, R. Bionta², C. Bostedt¹, J. Bozek¹, A. Brachmann¹, P. Bucksbaum¹, R. Coffee¹, F.-J. Decker¹, Y. Ding¹, D. Dowell¹, S. Edstrom¹, A. Fisher¹, J. Frisch¹, S. Gilvich¹, J. Hastings¹, G. Hays¹, Ph. Hering¹, Z. Huang¹, R. Iverson¹, H. Loos¹, M. Messerschmidt¹, A. Miahnahri¹, S. Moeller¹, H.-D. Nuhn¹, G. Pile³, D. Ratner¹, J. Rzepiela¹, D. Schultz¹, T. Smith¹, P. Stefan¹, H. Tompkins¹, J. Turner¹, J. Welch¹, W. White¹, J. Wu¹, G. Yocky¹ and J. Galayda¹

NATURE PHOTONICS | VOL 4 | SEPTEMBER 2010 |



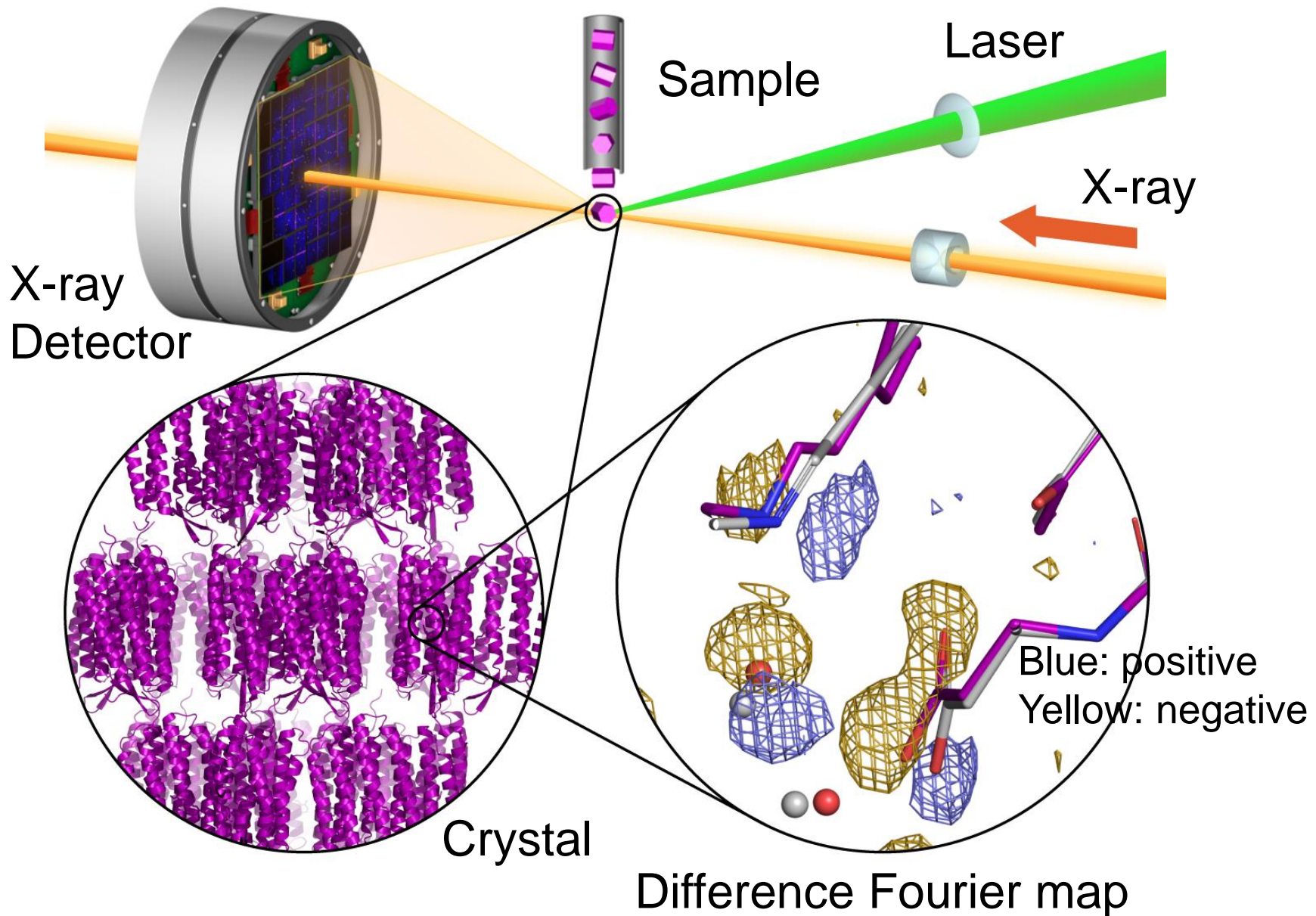
Serial femtosecond crystallography



Chapman *et al*, *Nature* **470** 73 (2011)

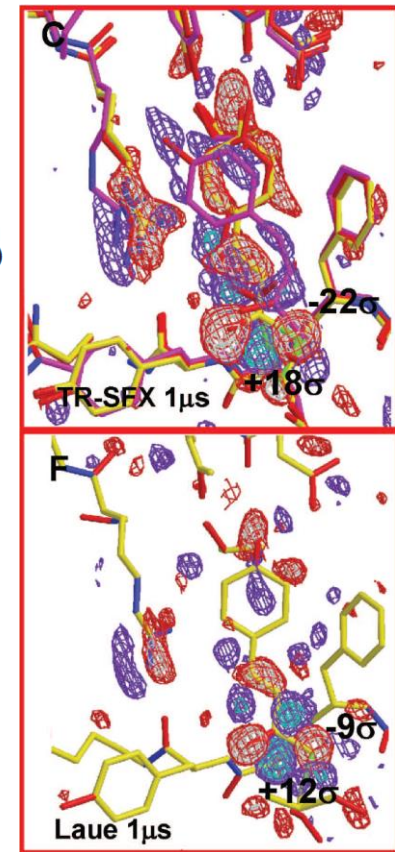


Time-resolved serial crystallography

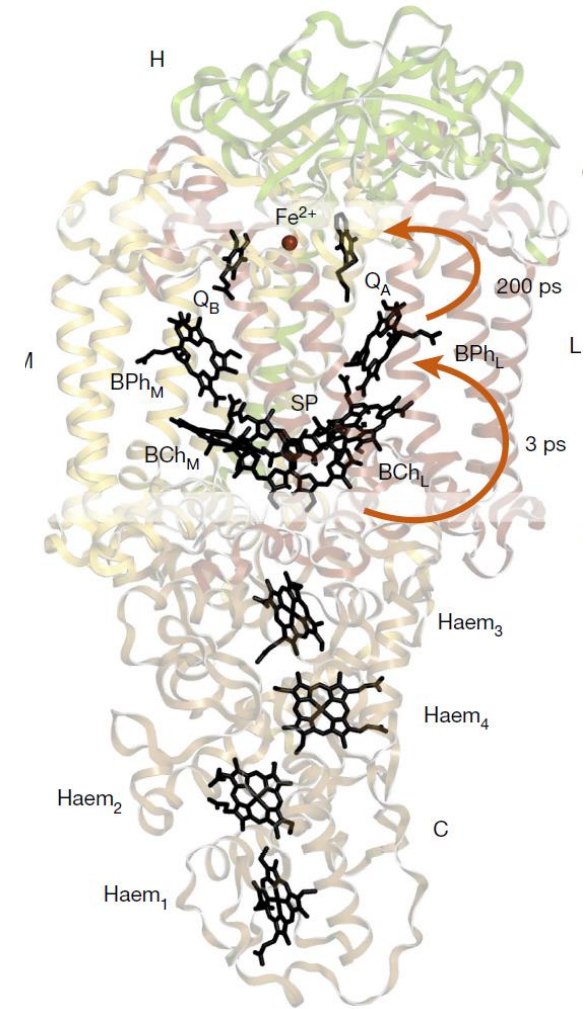
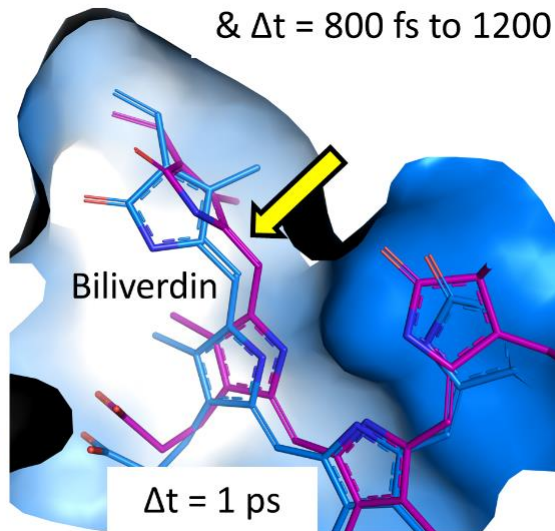
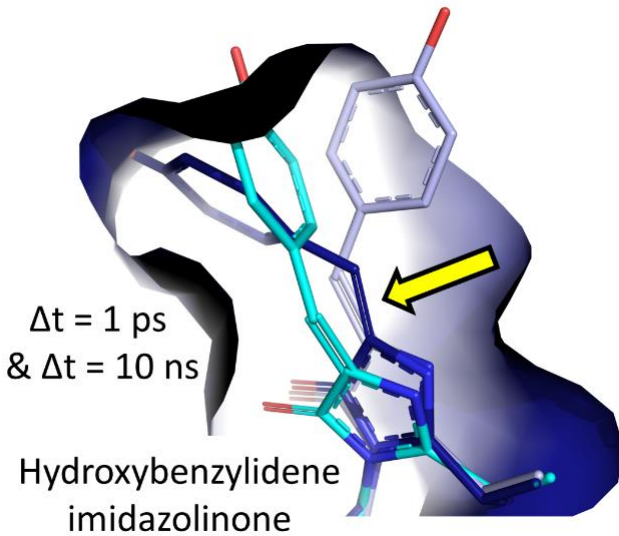
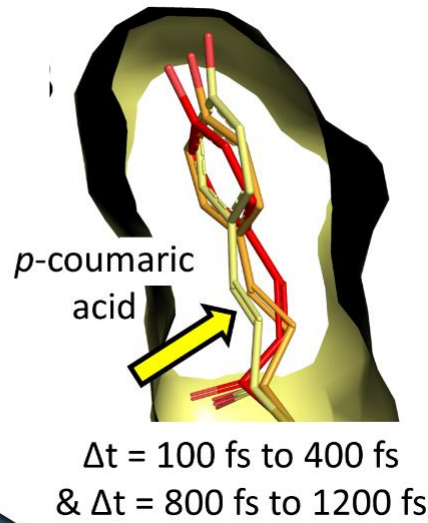
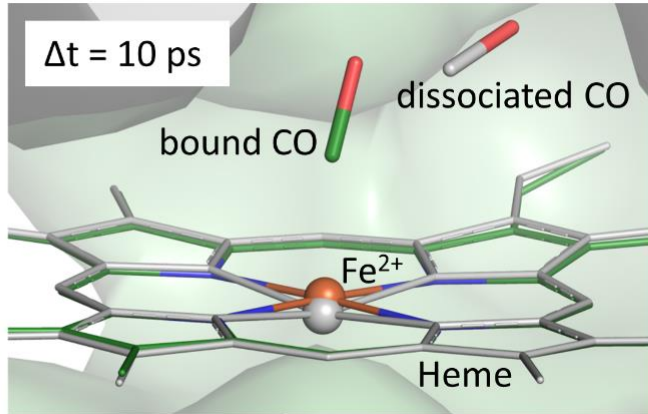


Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein

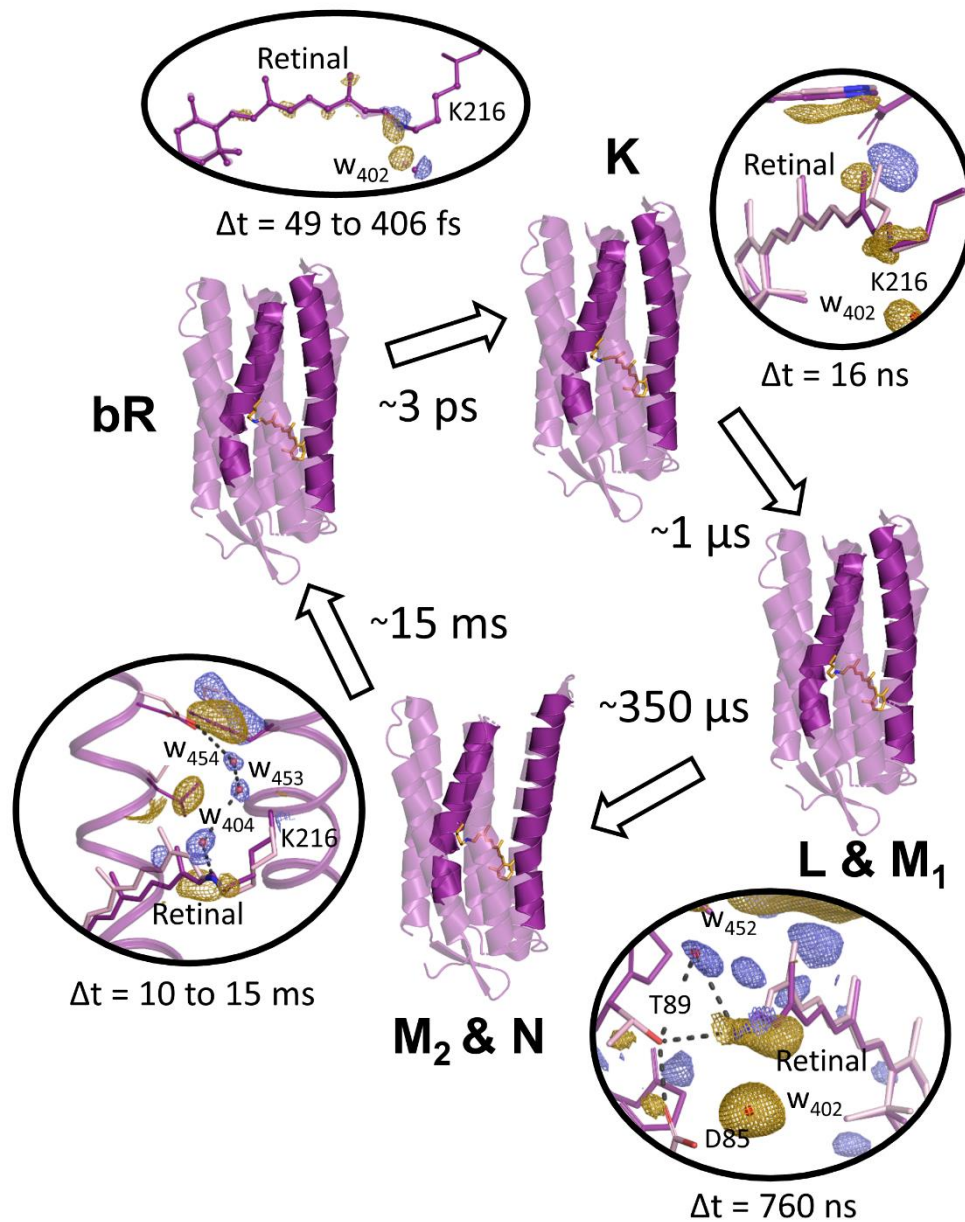
Jason Tenboer,¹ Shibom Basu,² Nadia Zatsepin,³ Kanupriya Pande,¹
 Despina Milathianaki,⁴ Matthias Frank,⁵ Mark Hunter,^{5*} Sébastien Boutet,⁴
 Garth J. Williams,⁴ Jason E. Koglin,⁴ Dominik Oberthuer,⁶ Michael Heymann,⁷
 Christopher Kupitz,^{2†} Chelsie Conrad,² Jesse Coe,² Shatabdi Roy-Chowdhury,²
 Uwe Weierstall,³ Daniel James,³ Dingjie Wang,³ Thomas Grant,⁸ Anton Barty,⁷
 Oleksandr Yefanov,⁷ Jennifer Scales,¹ Cornelius Gati,^{6,7} Carolin Seuring,⁶
 Vukica Srajer,⁹ Robert Henning,⁹ Peter Schwander,¹ Raimund Fromme,²
 Abbas Ourmazd,¹ Keith Moffat,^{9,10} Jasper J. Van Thor,¹¹ John C. H. Spence,³
 Petra Fromme,² Henry N. Chapman,^{6,7} Marius Schmidt^{1‡}



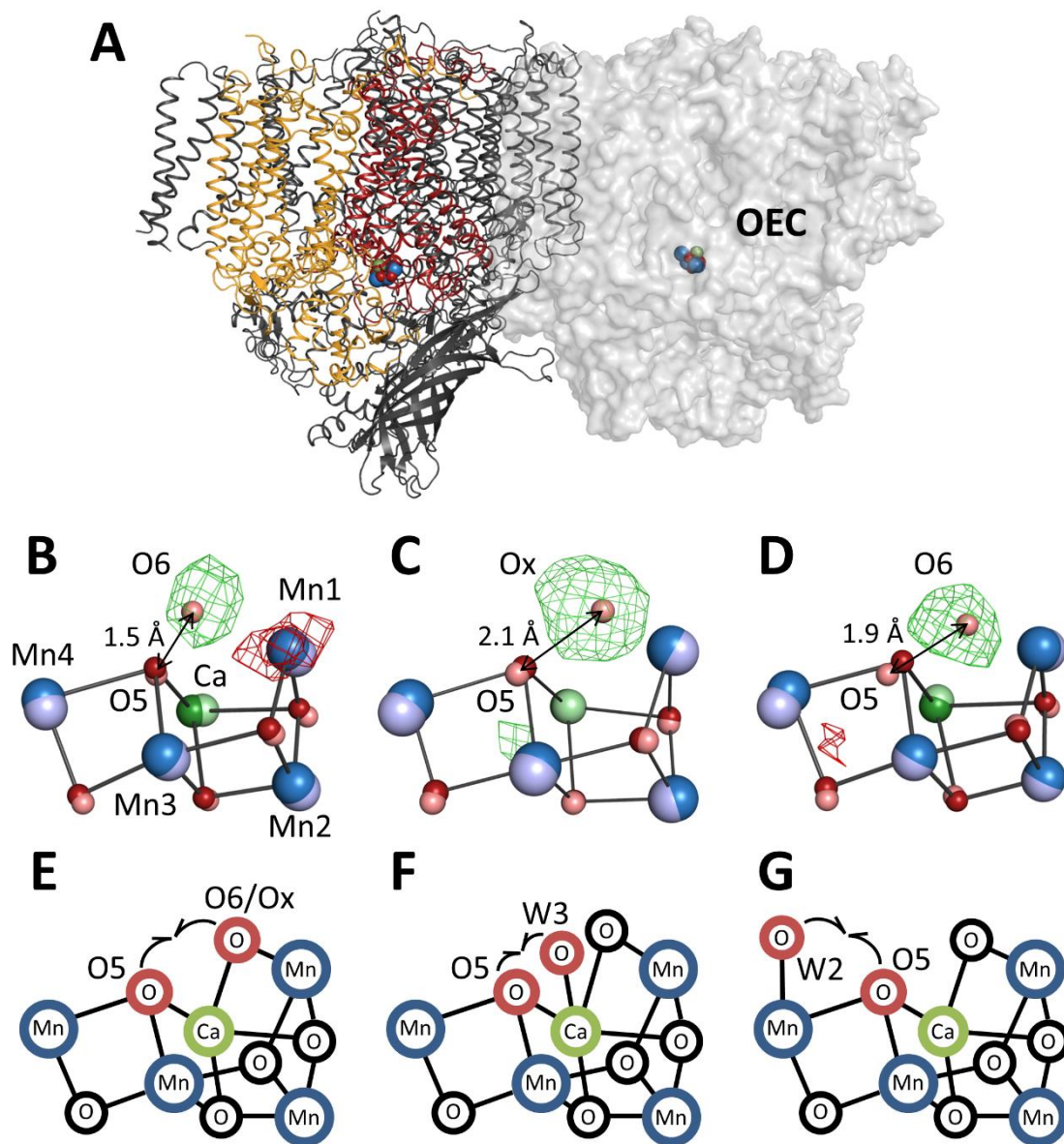
Ultrafast motions in light-sensitive proteins



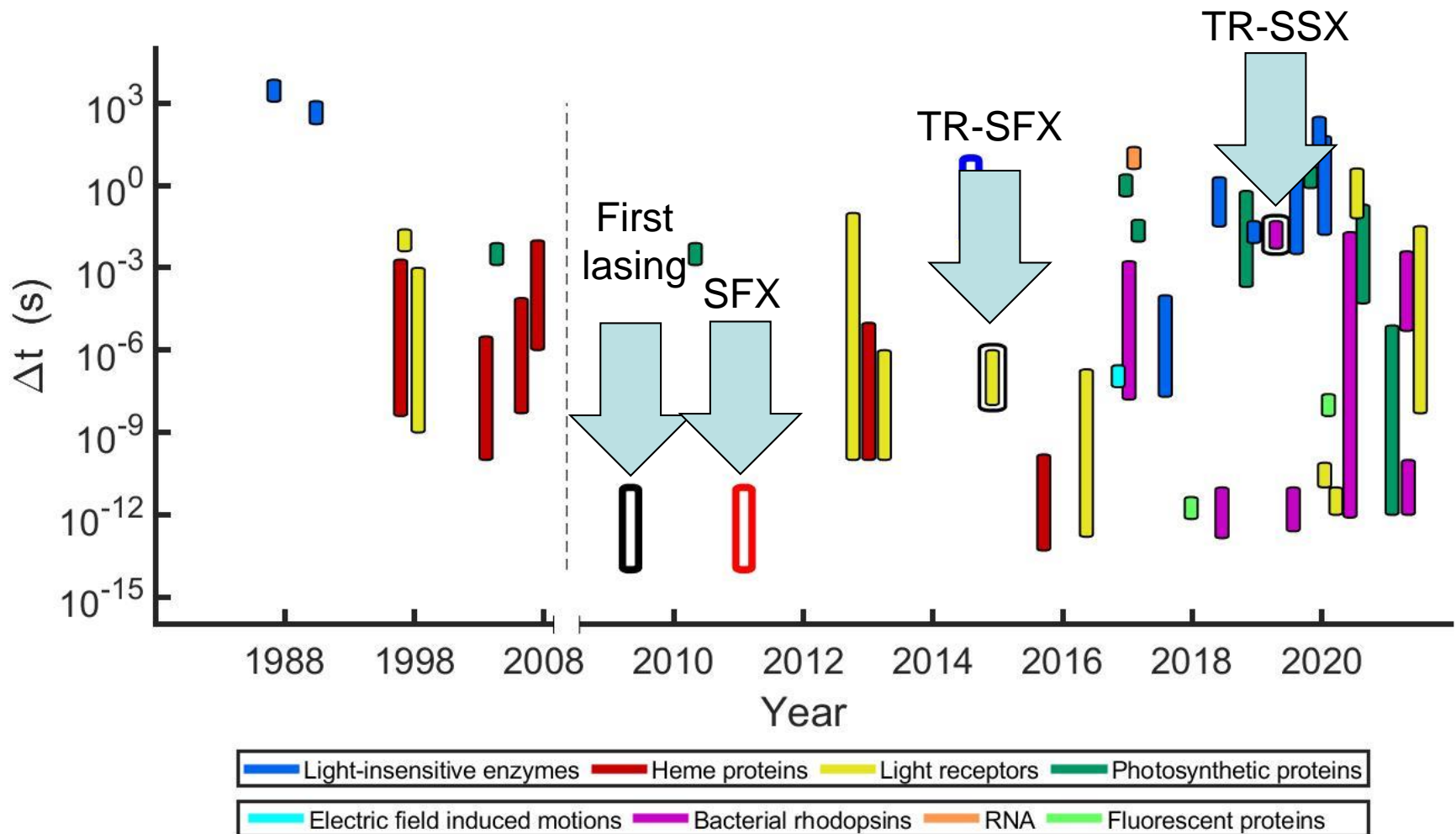
Evolving motions in retinal proteins



Structural changes during photosynthesis



Time-resolved diffraction



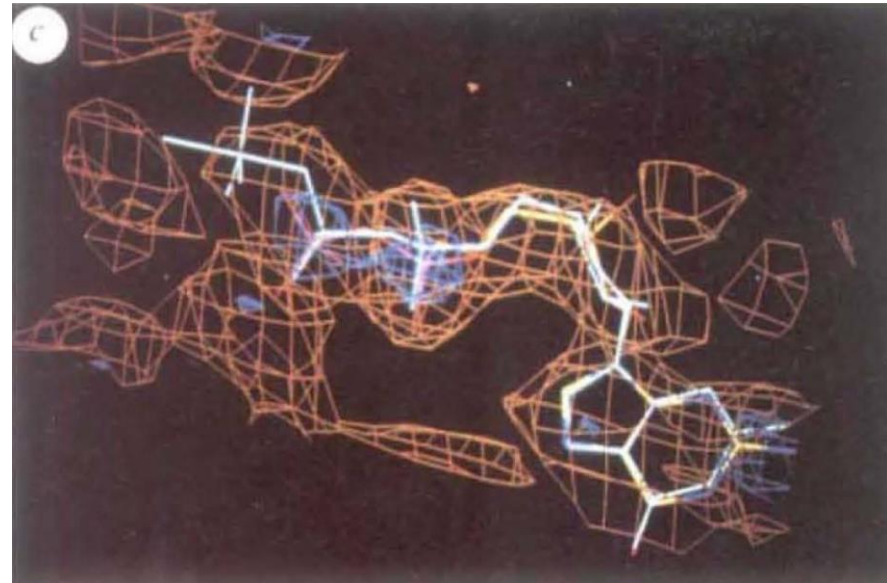
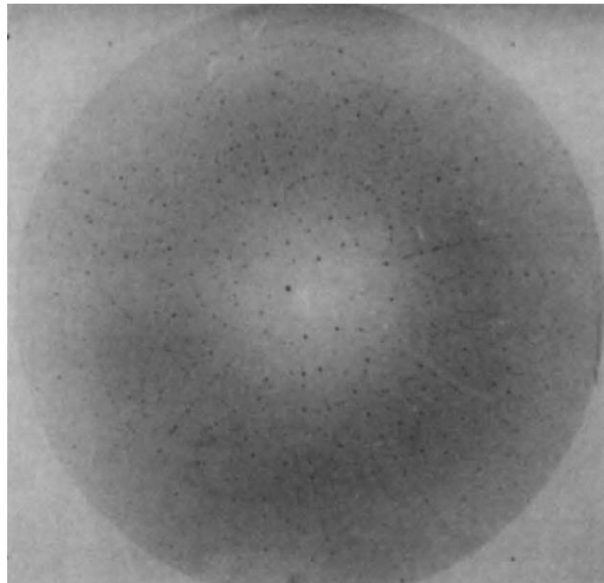
Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis

Ilme Schlichting*, **Steven C. Almo^{†‡}**, **Gert Rapp[§]**, **Keith Wilson[§]**,
Kyriakos Petratos[§], **Arno Lentfer[§]**, **Alfred Wittinghofer***,
Wolfgang Kabsch*, **Emil F. Pai***, **Gregory A. Petsko^{†‡}** & **Roger S. Goody^{*||}**

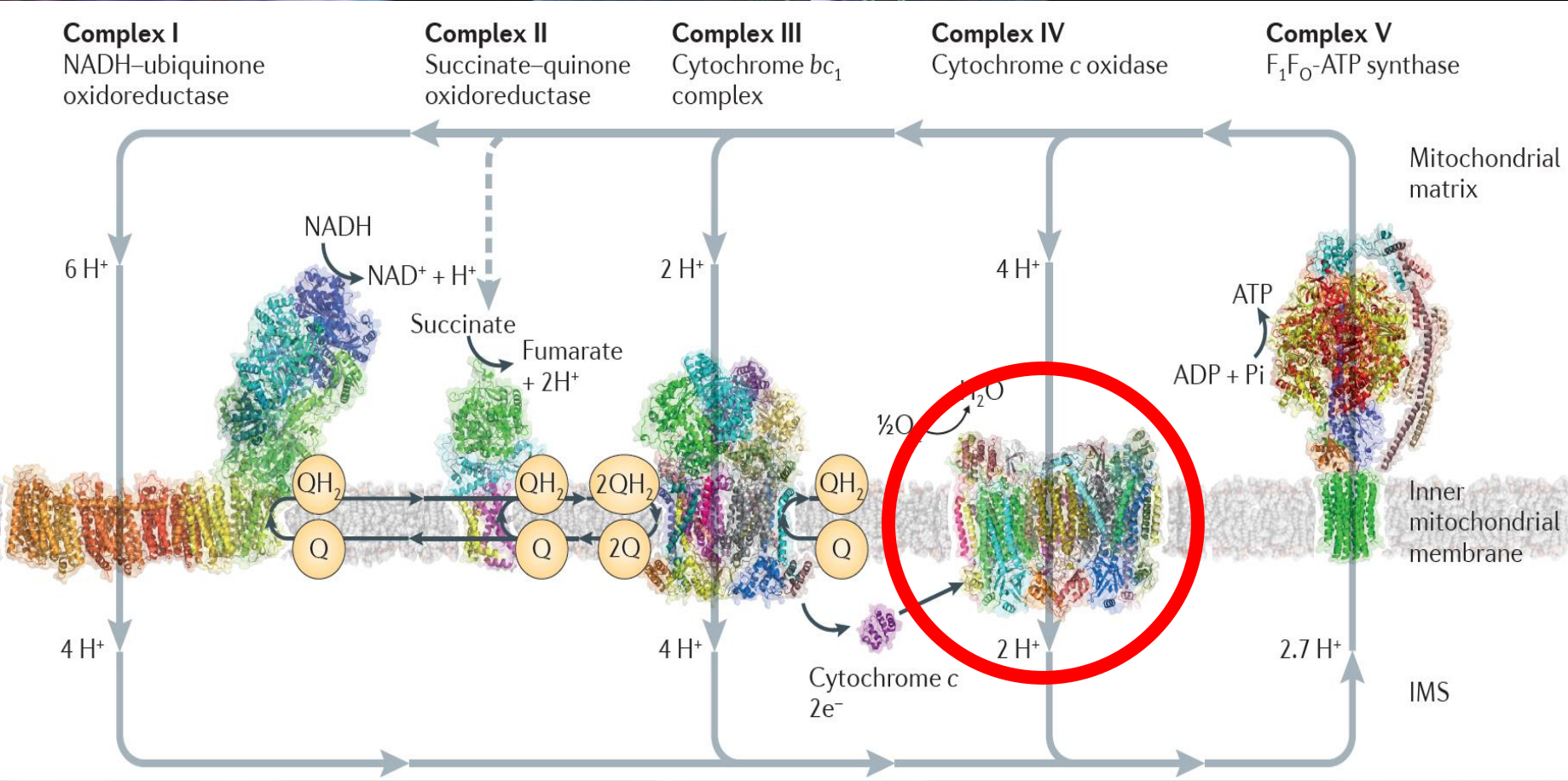
* Abteilung Biophysik, Max-Planck-Institut für Medizinische Forschung, 6900 Heidelberg, FRG

† Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

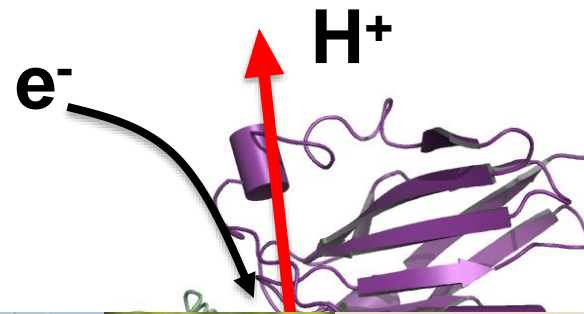
§ EMBL Outstation, DESY, 2000 Hamburg, FRG



Respiratory chain



Cytochrome c oxidase



Gisela
Brändén

Doris
Zoric

Jonatan
Johannesson

Emil
Sandelin

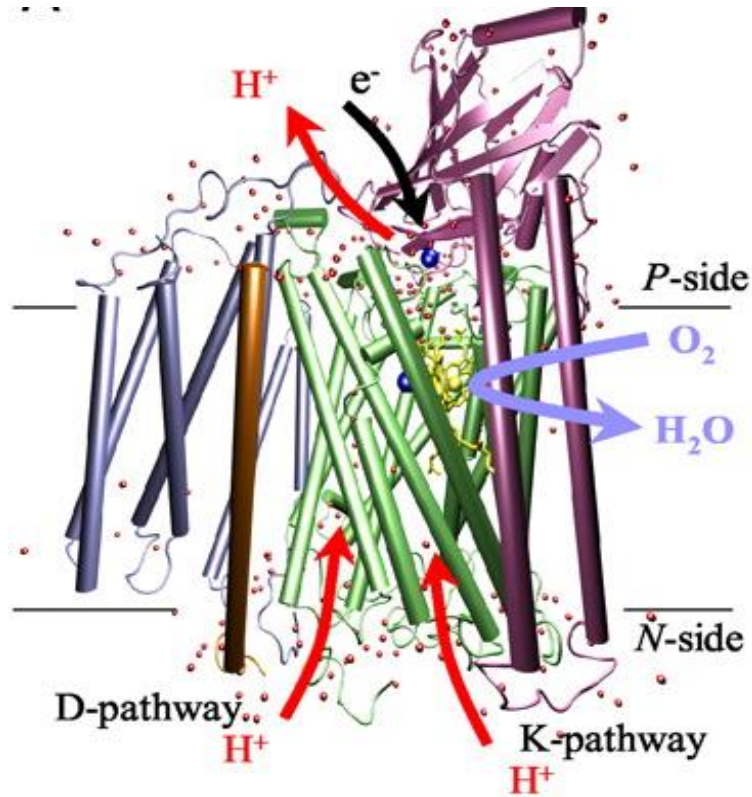
Arpitha
Kabinale

Adams
Vallejos

Swagatha
Ghosh

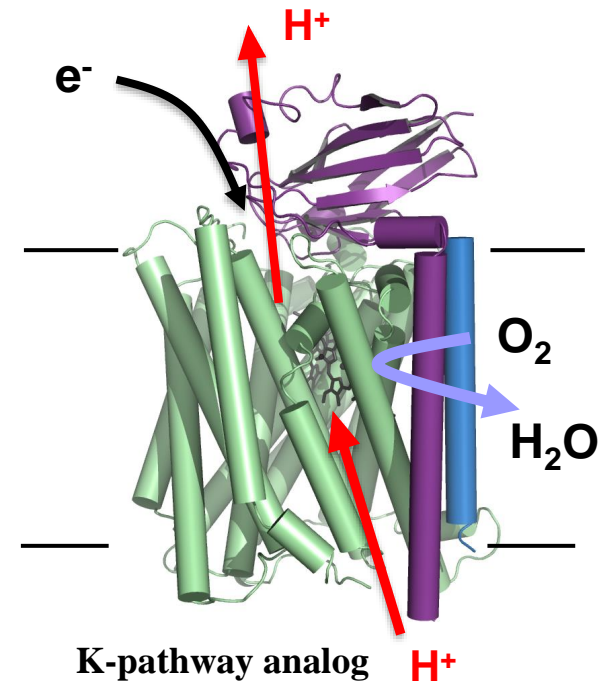
*ba*₃-type CcO

Cytochrome c oxidase



*aa*₃-type CcO

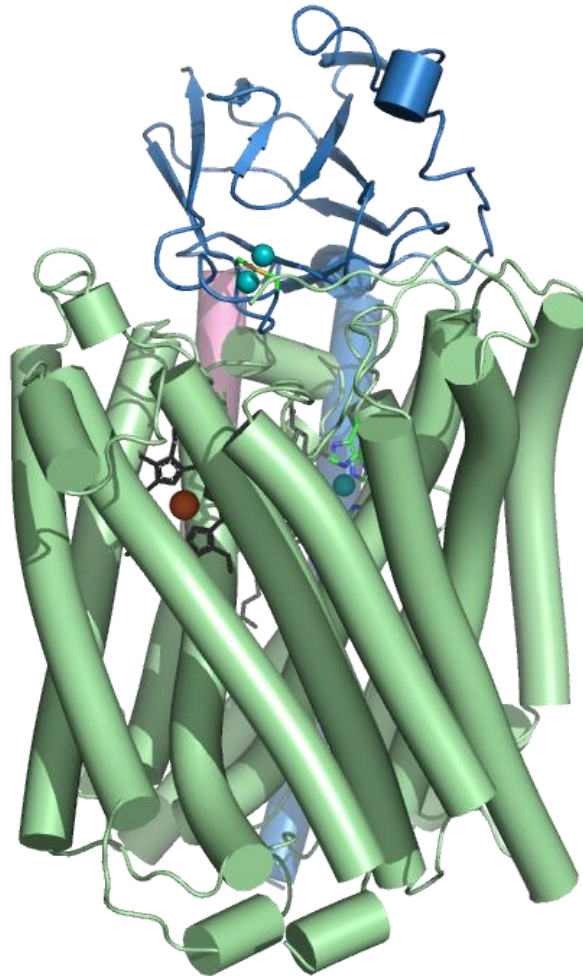
Mitochondria



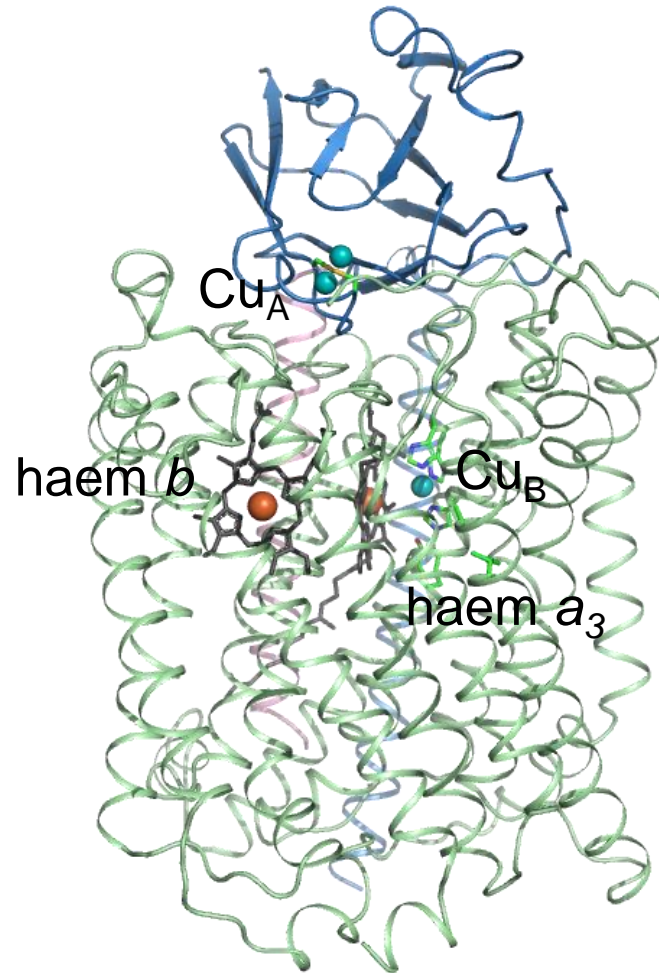
*ba*₃-type CcO

Thermus thermophilus

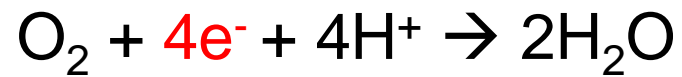
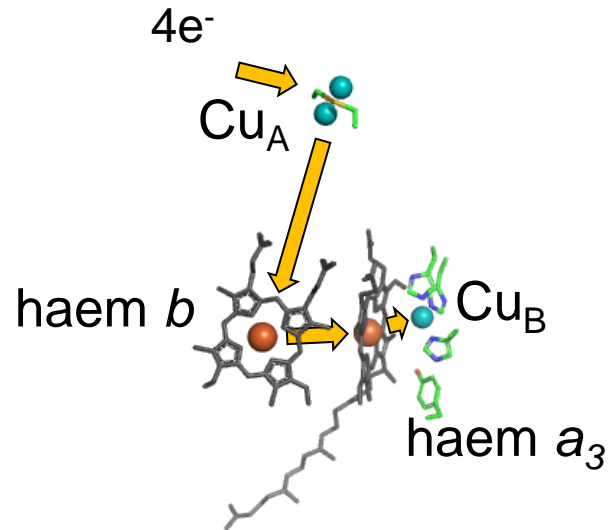
*ba*₃-type cytochrome c oxidase



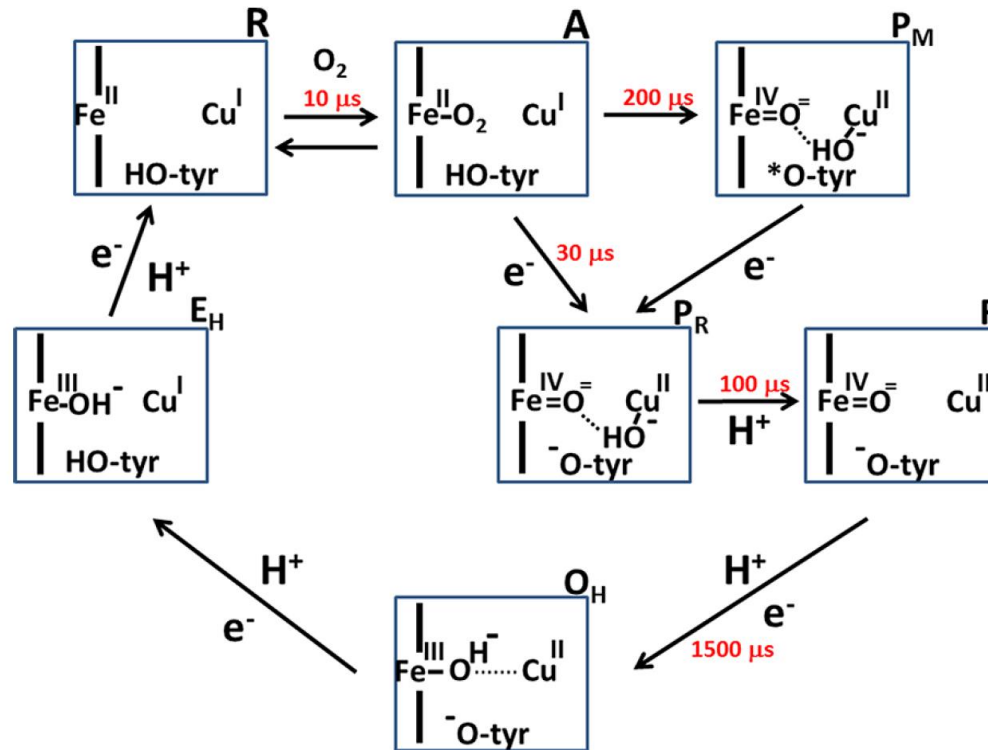
*ba*₃-type cytochrome c oxidase



Electron transfer steps

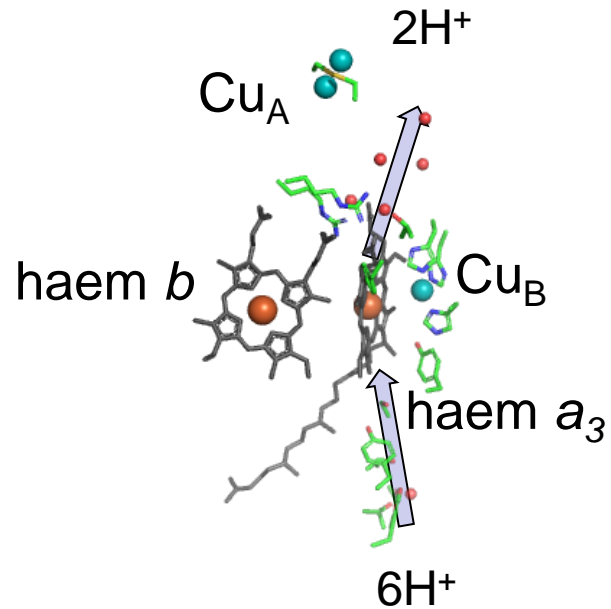


Electron transfer steps



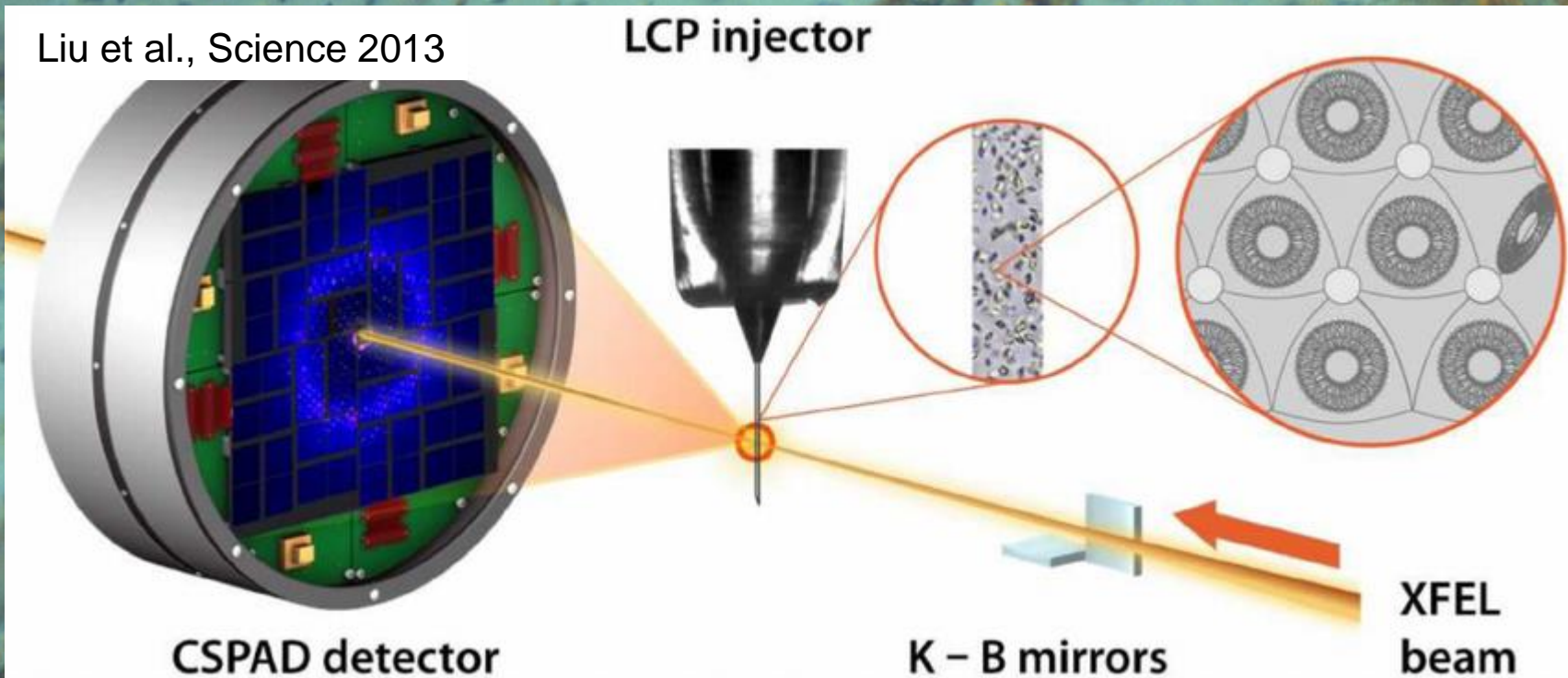
Belevich, I., et al. (2007). PNAS 104: 2685

Proton transfer steps



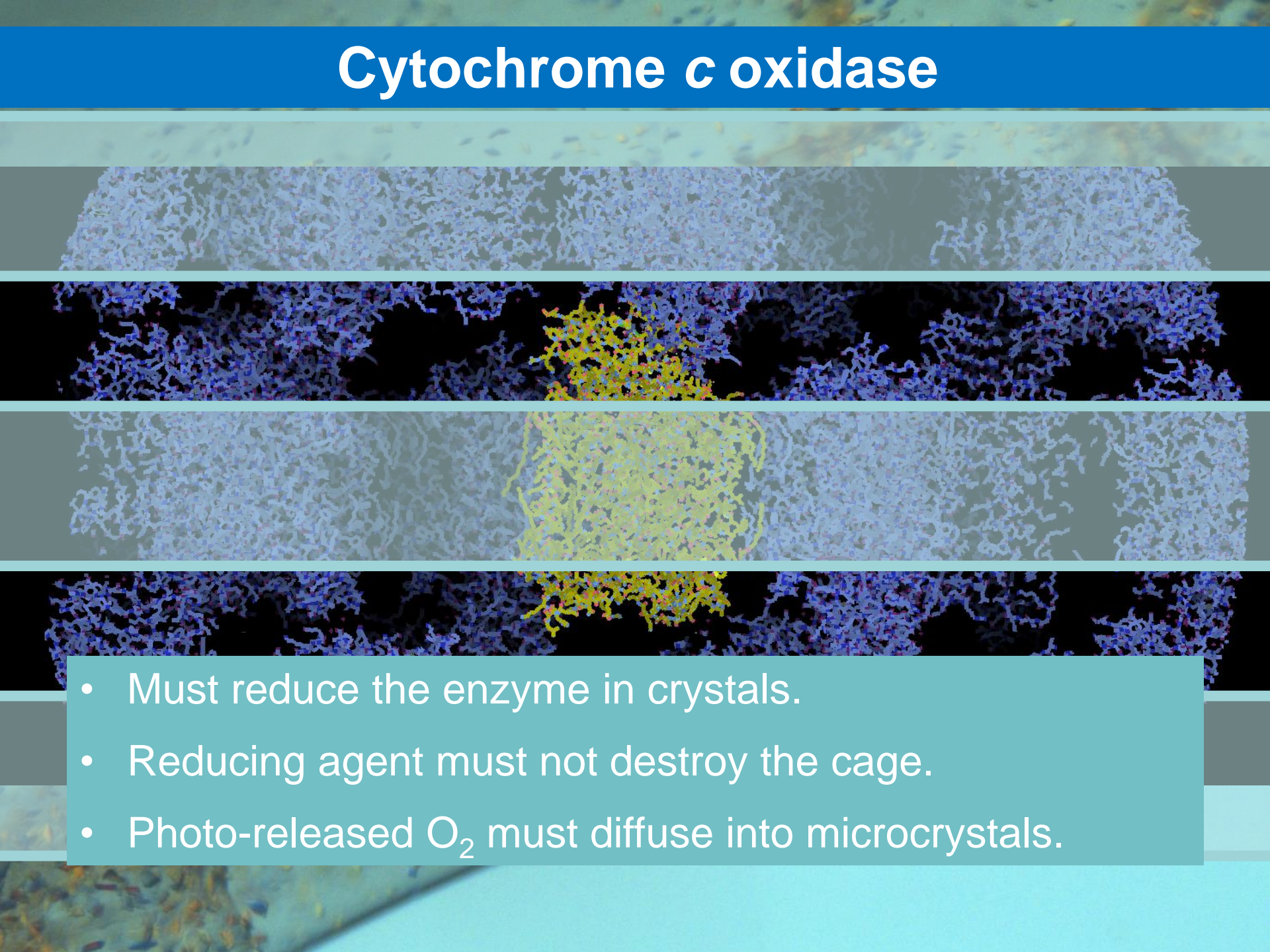
Cytochrome c oxidase

Liu et al., Science 2013



- Microcrystals grown in a lipidic cubic phase.
- Injected using an LCP injector.

Cytochrome c oxidase

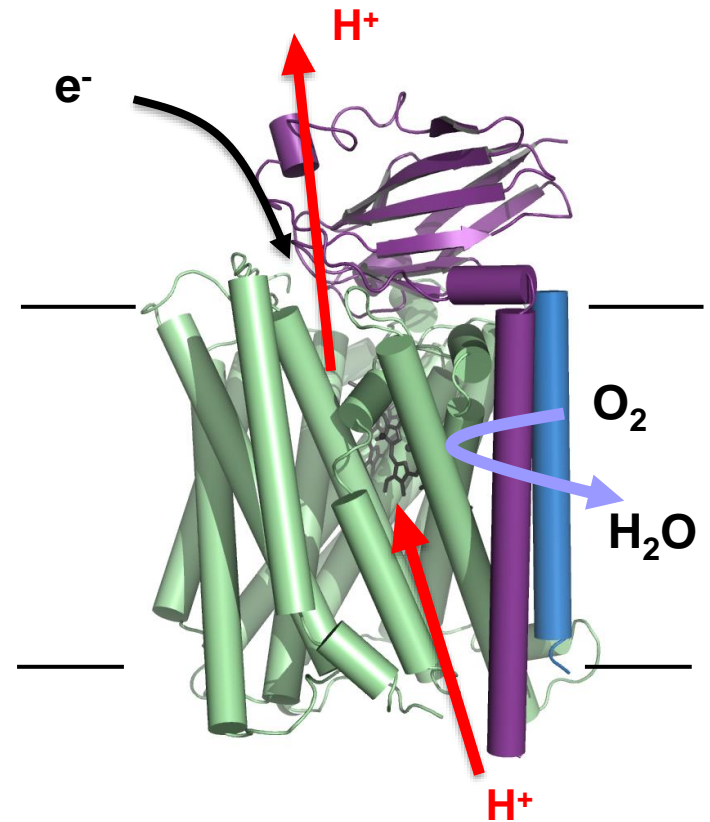
- 
- Must reduce the enzyme in crystals.
 - Reducing agent must not destroy the cage.
 - Photo-released O₂ must diffuse into microcrystals.

TR-SFX @ SwissFEL, EU-XFEL & LCLS



Conclusions

- TR-SFX studies of CcO using photocaged O_2 are possible.





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Cecilia Safari

Rebecka Andersson

Peter Dahl

Carl Johan Wallentin

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Emma V. Beale

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Abdullah Kahraman

Dmitry Ozerov



Sebastien Boutet

Christopher Kupitz

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Raymond Sierra



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Mohammad Vakilli

