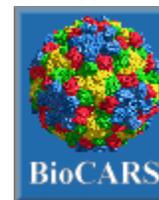
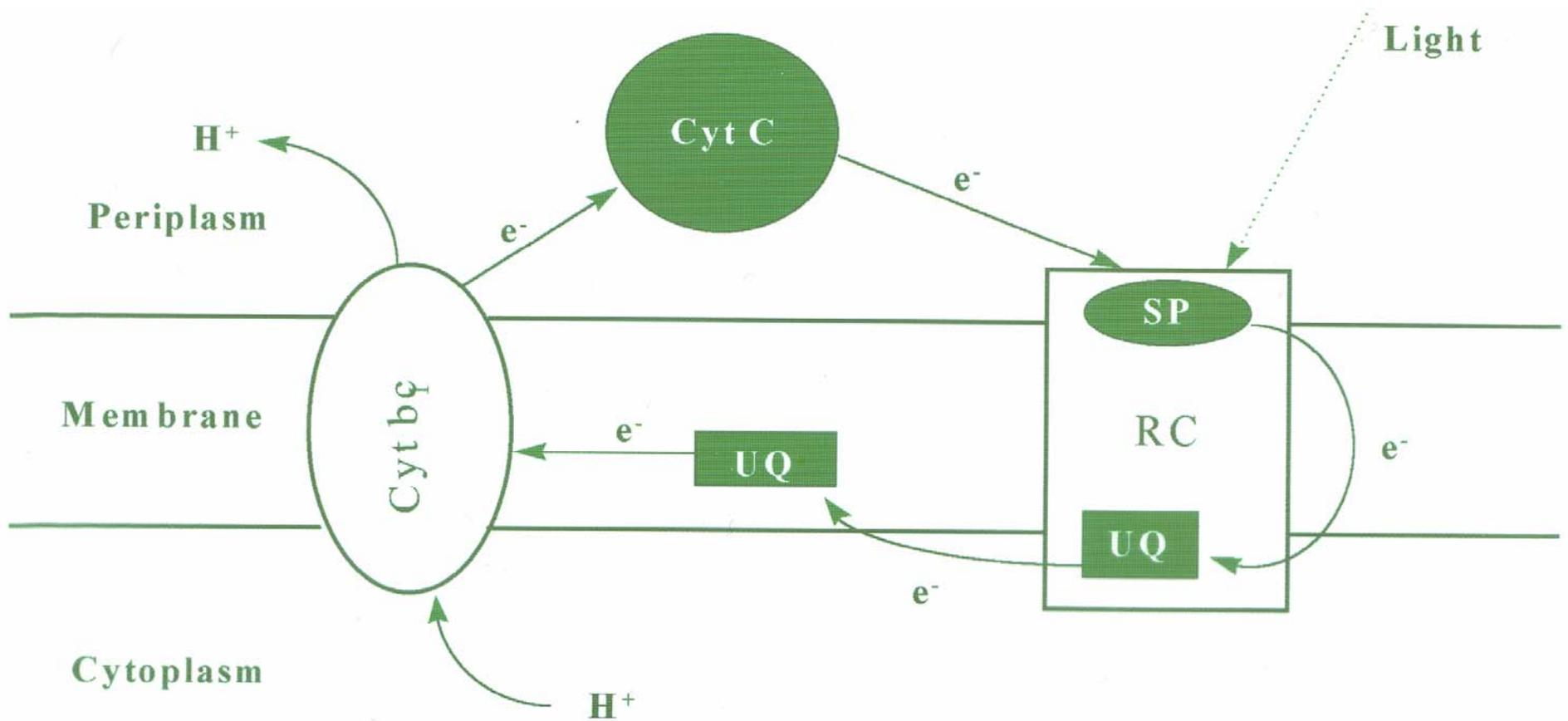


Investigating Photosynthesis Using Synchrotron Pulses

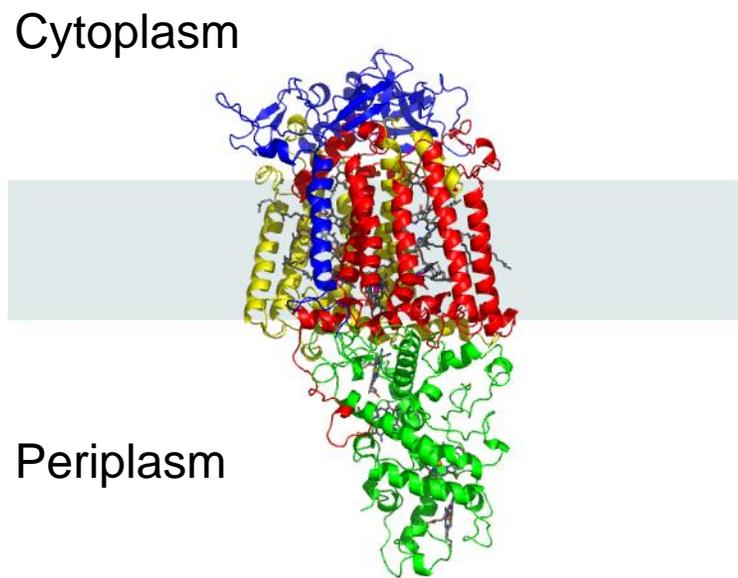
**Department of Chemistry
Institute for Biophysical Dynamics
Consortium for Advanced Radiation Sources
University of Chicago
U.S.DOE, Office of Basic Energy Sciences,
Division of Chemical Sciences**



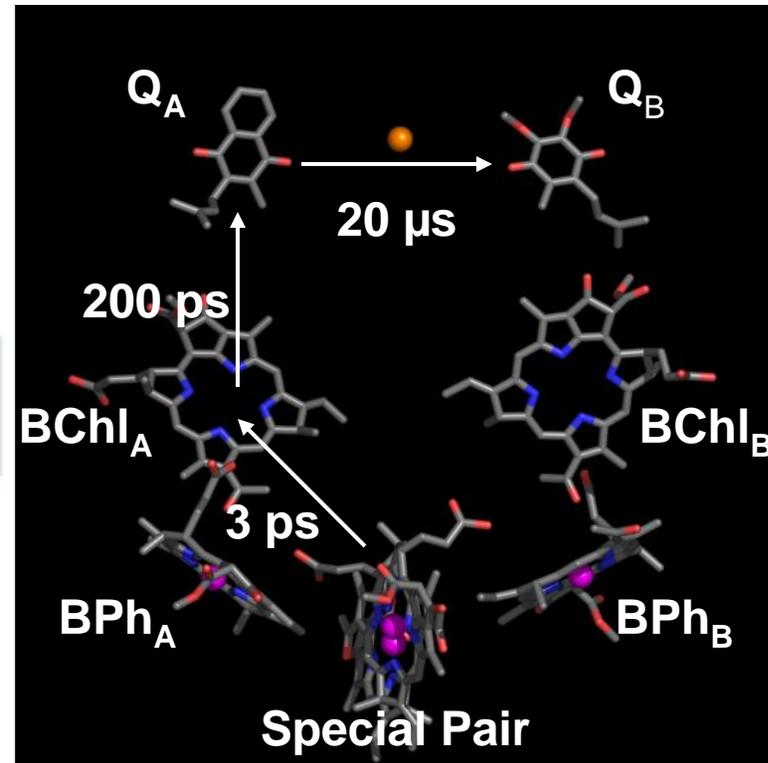
The Photosynthetic Process in Bacteria



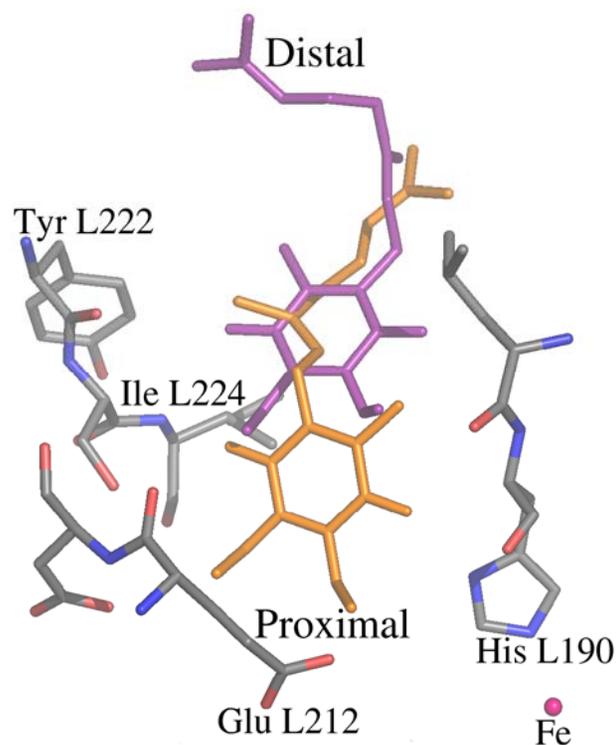
The photosynthetic reaction center of *Blastochloris viridis*



- C Subunit
- L Subunit
- M Subunit
- H Subunit

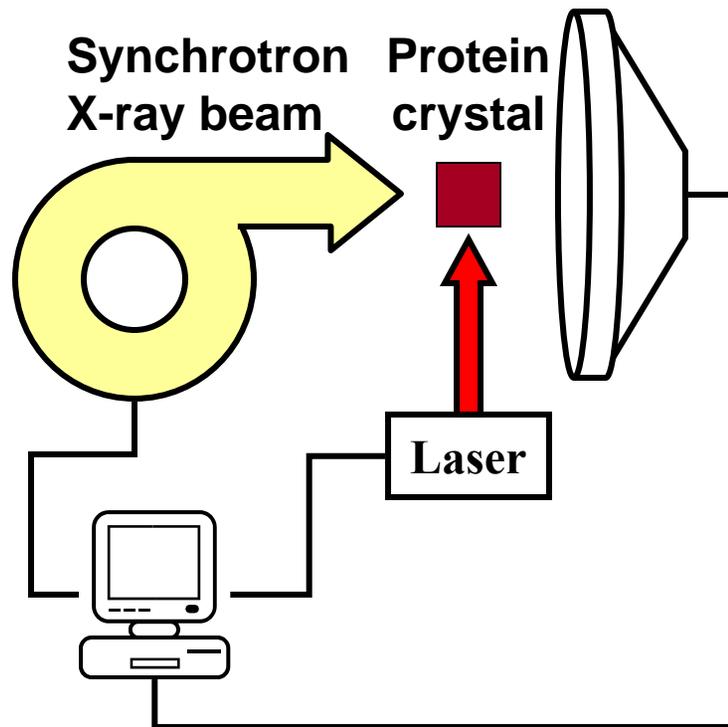


Two positions for Q_B

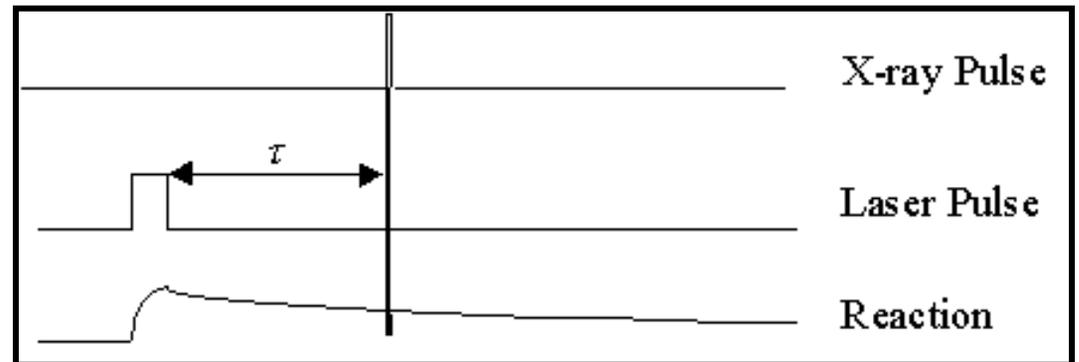


- 1985 – 1PRC *B. viridis*
 - Q_B poor occupancy
 - proximal position
- 1991 – xRCR *R. sphaeroides*
 - MR from 1PRC
 - proximal position
- 1994 – 1PCR *R. sphaeroides*
 - new xtal form ($P3_121$)
 - distal position
- 1995 – 1PRC (revised)
 - proximal position
- 1997 – 1AIG/1AIJ *R. sphaeroides*
 - Freeze-trapping
 - Distal position
- 1997 – 2PRC *B. viridis*
 - UQ2-reconstituted
 - Proximal position
- 1997 – 1PRC_{new} *B. viridis*
 - Reanalysis of original dataset
 - Distal position

Experimental design

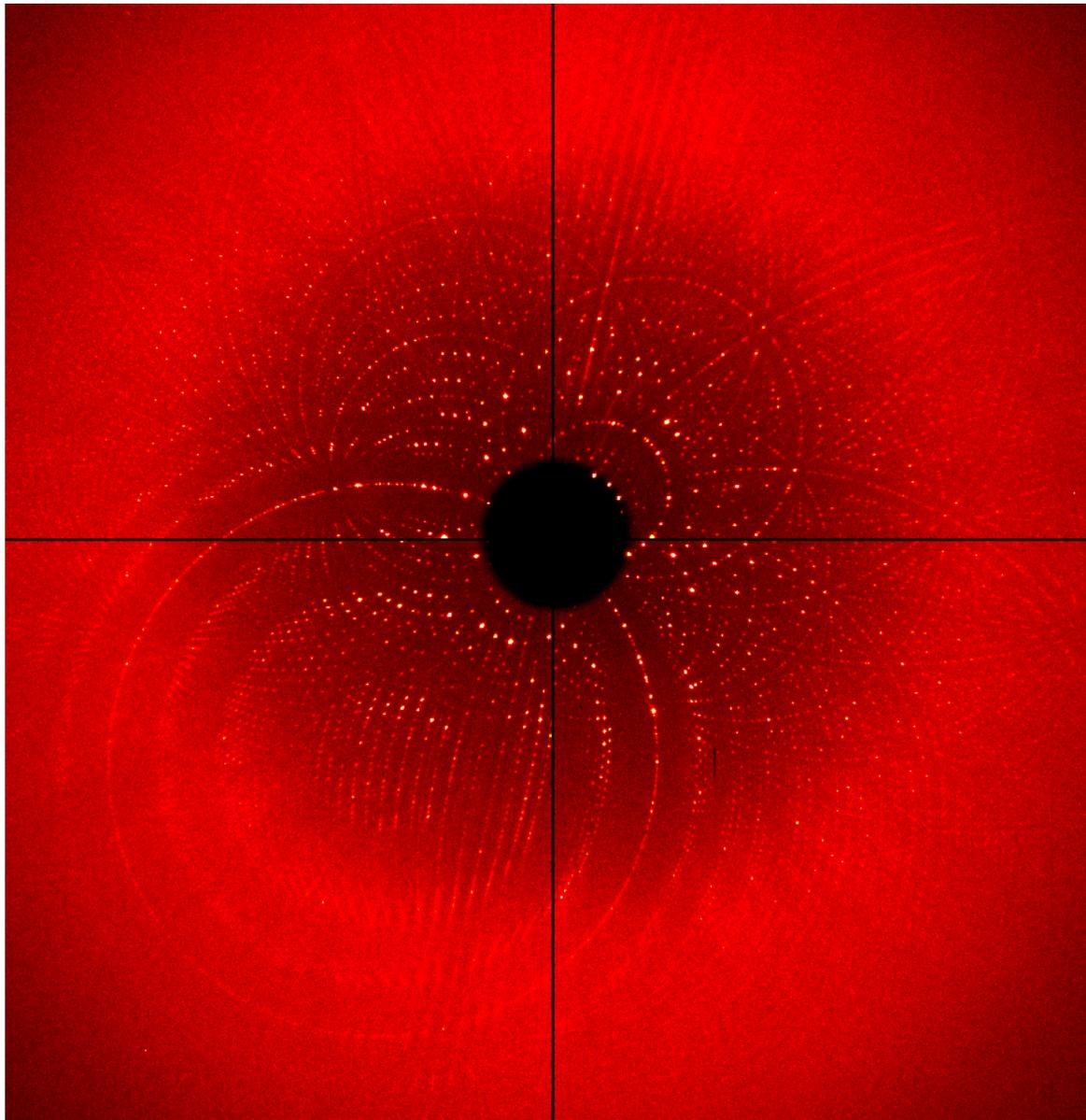


Schematic Diagram



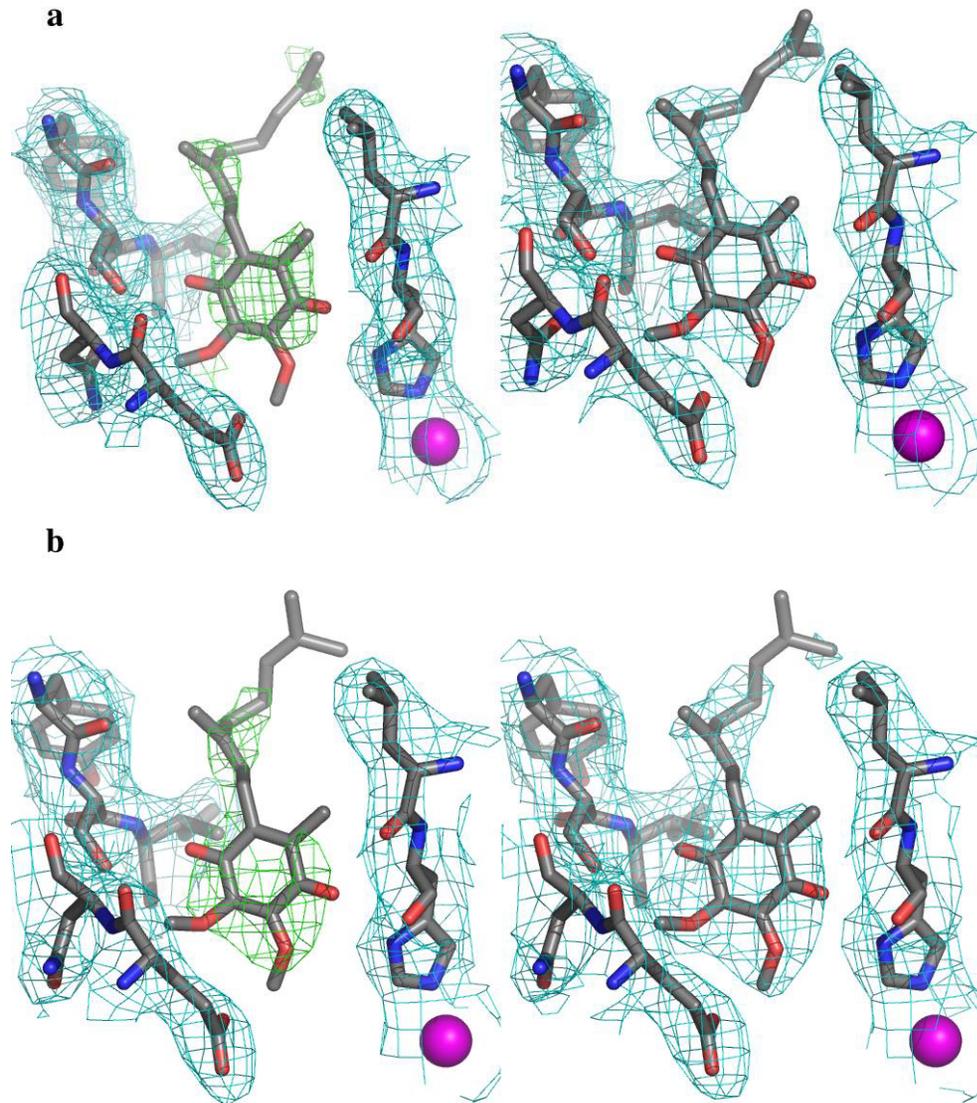
Experimental Time Sequence

Laue Diffraction Pattern of Photosynthetic Reaction Center

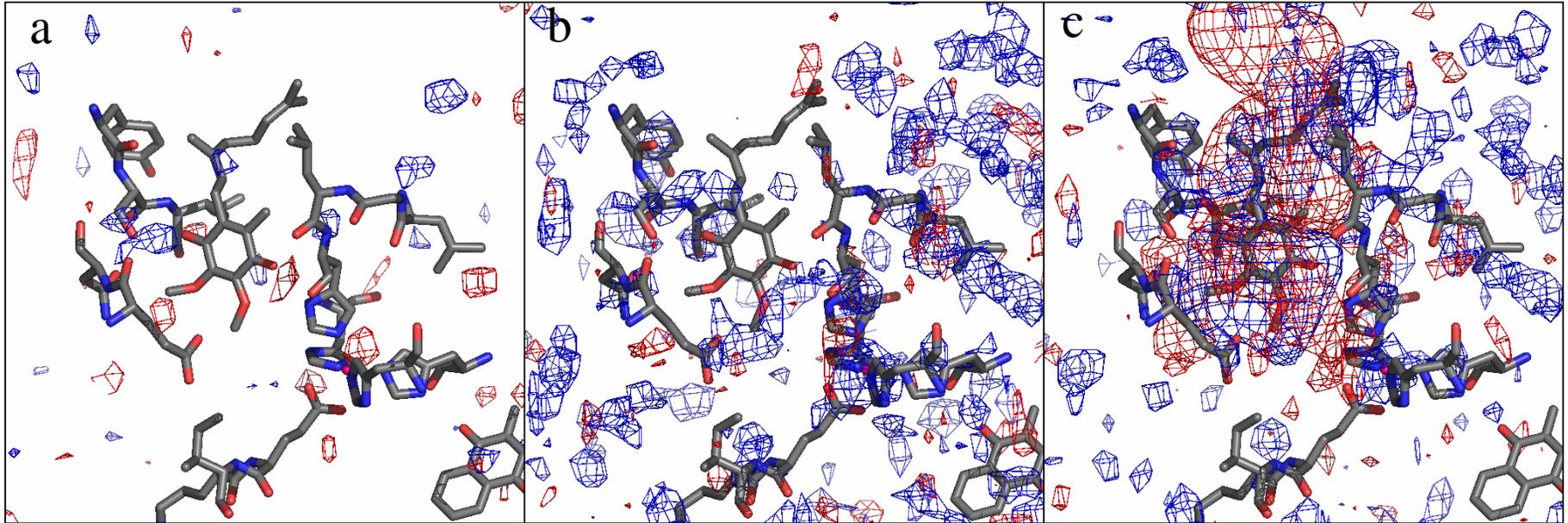


- 73.2 μsec
Dark
Exposure
- CARS
APS-ANL
Undulator
- Resolution
 $\sim 3.3 \text{ \AA}$

Dark structure for the Q_B site



Laue data analysis



$F_{\text{light}} - F_{\text{dark}}$ maps, negative electron density **red**, positive density **blue**.

a) experimental structure factors,

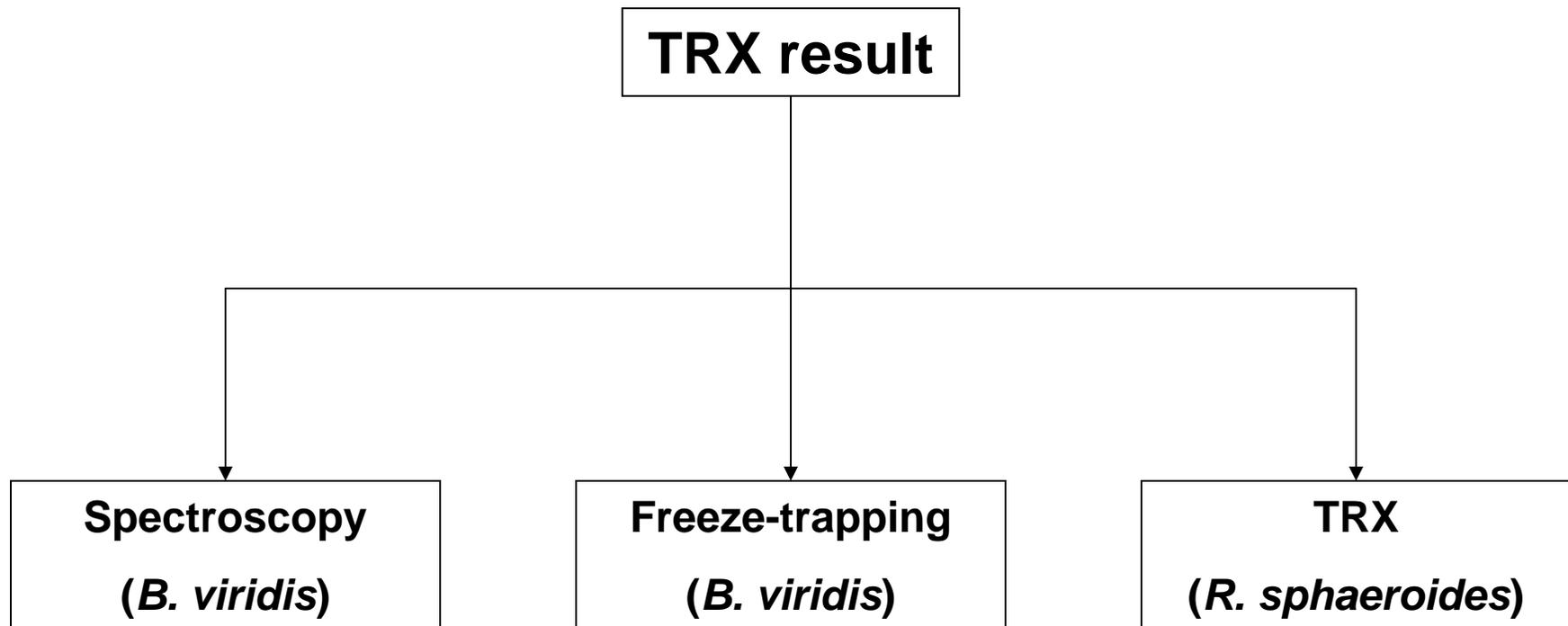
b) $F_{\text{dark}} = \sigma(F_{\text{dark}})$, $F_{\text{light}} = \sigma(F_{\text{light}})$,

c) simulation, dark: 10% distal/proximal, light: 20% proximal.

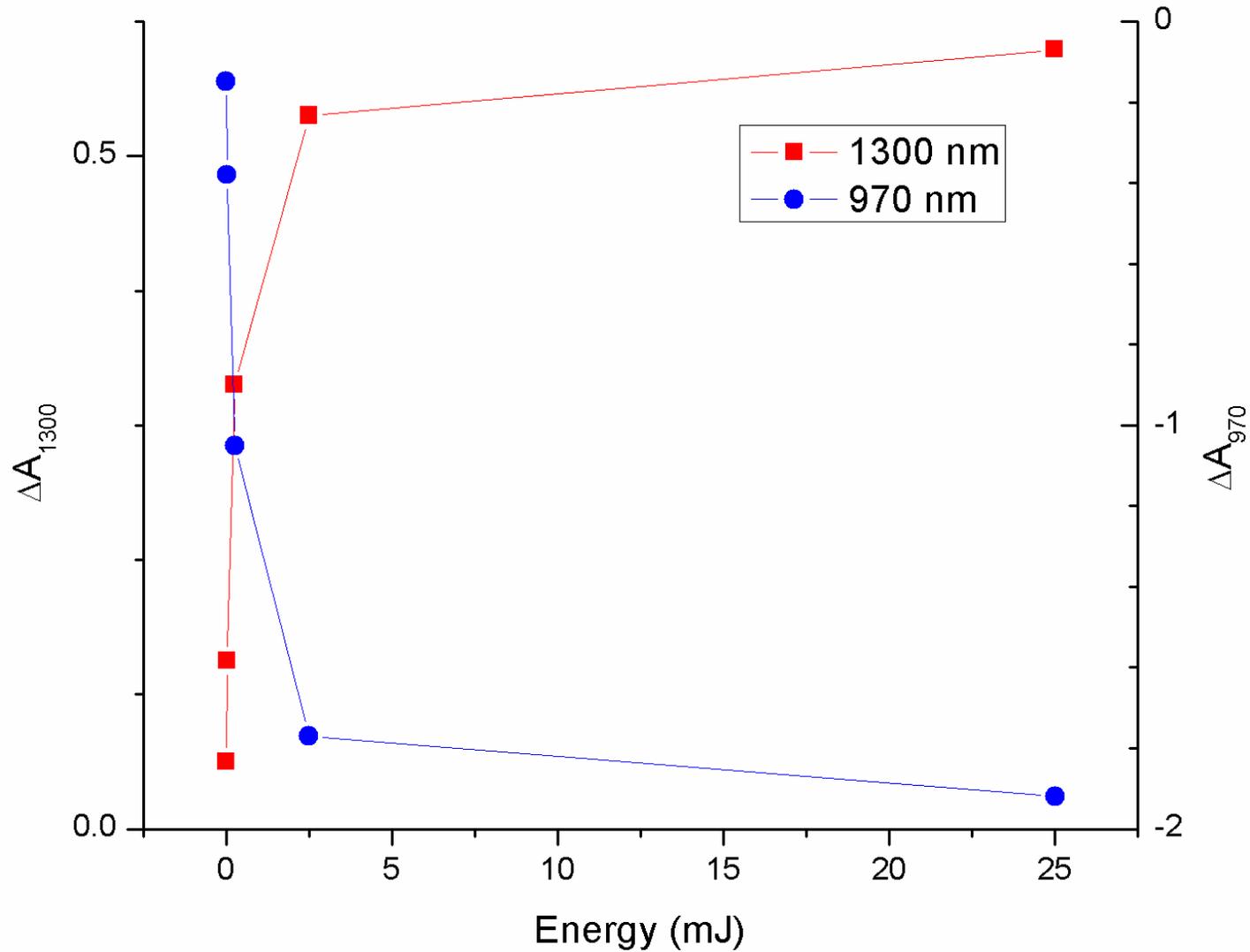
Whither the 'conformational gate'?

Q_B appears only in the proximal binding site at room temperature in *B. viridis*.

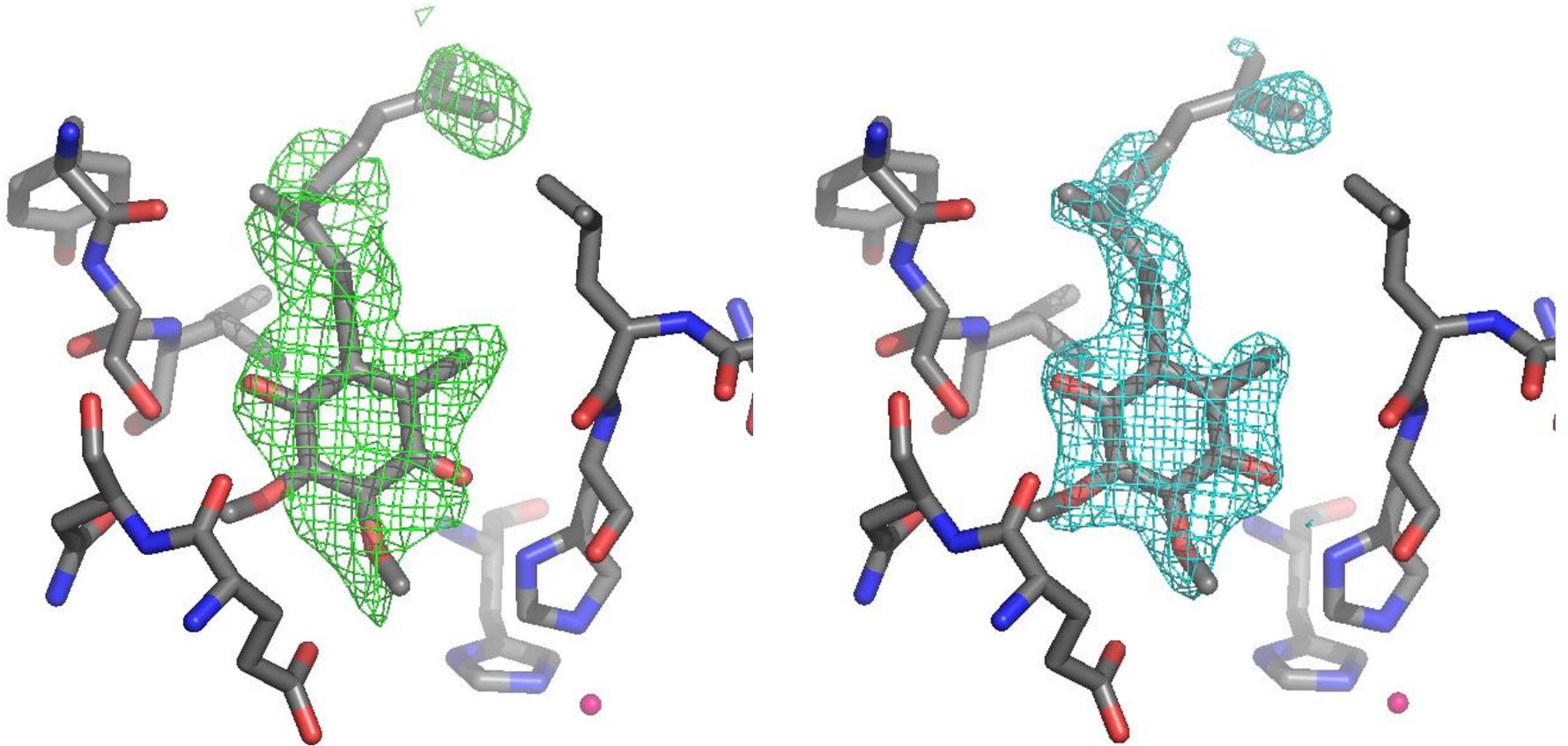
There is no large-scale movement of Q_B accompanying electron transfer.



Quantification of Photoactivation



Q_B active site at 2.1 Å

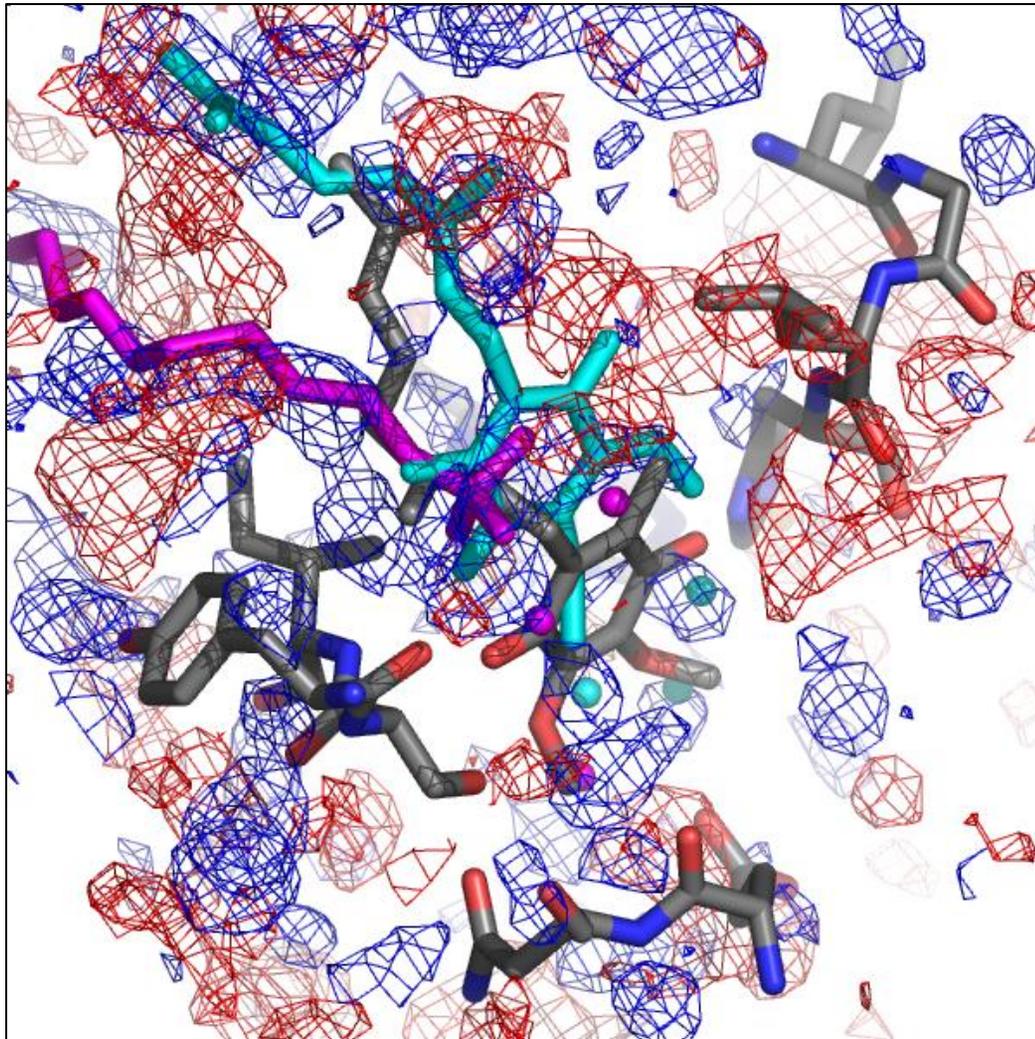


$2F_o - F_c$ (a) SA-omit map, (b) refined model. Contours 1.5σ .

RQ_A : 0.89

RQ_B : 0.19

Tripartite model and $F_o - F_c$ map



Occupancy Refinement

Proximal - 0.57

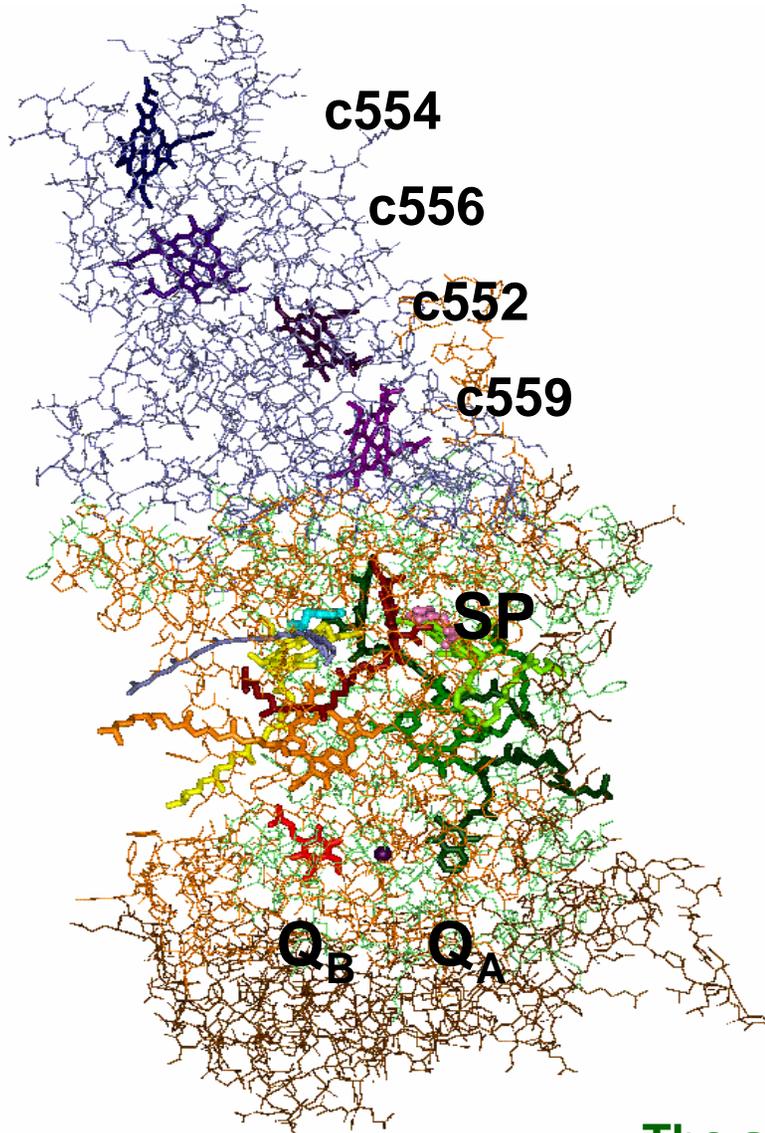
Distal - 0.17

Empty - 0.21

TOTAL - 0.95

B-factor 24.7 Å²

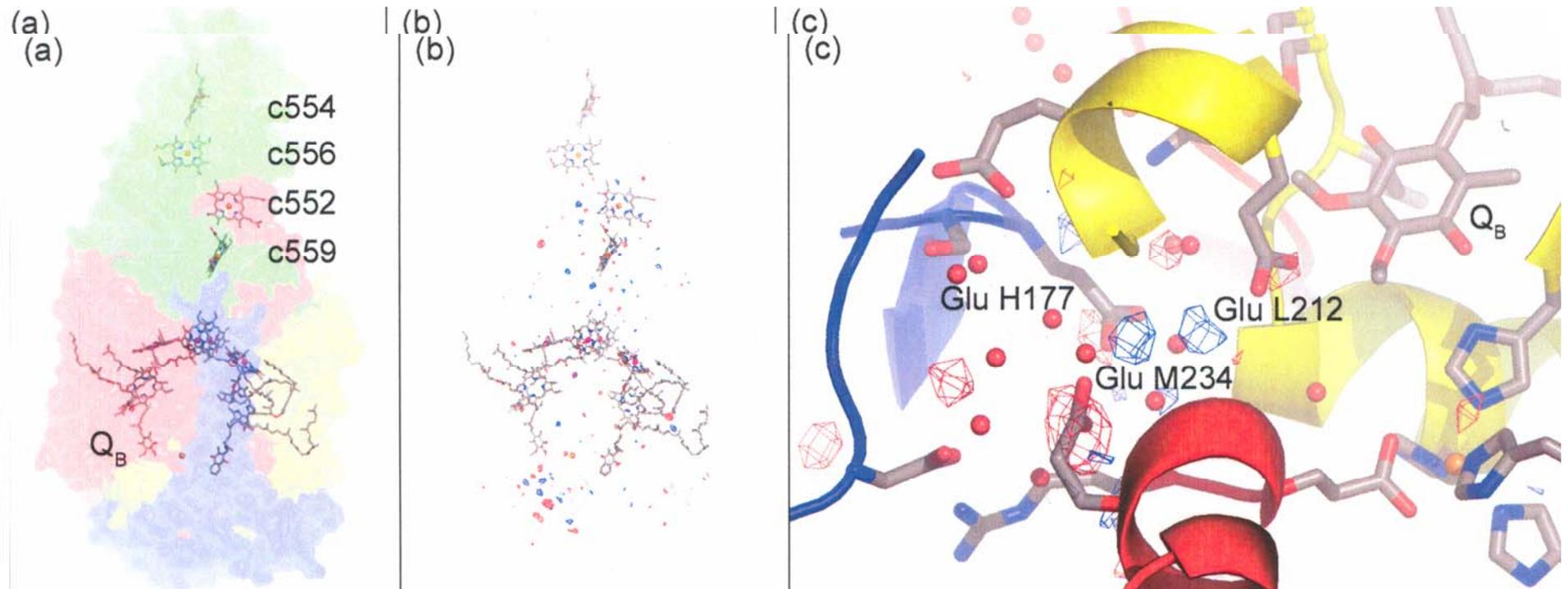
RQ_BQ: 0.42



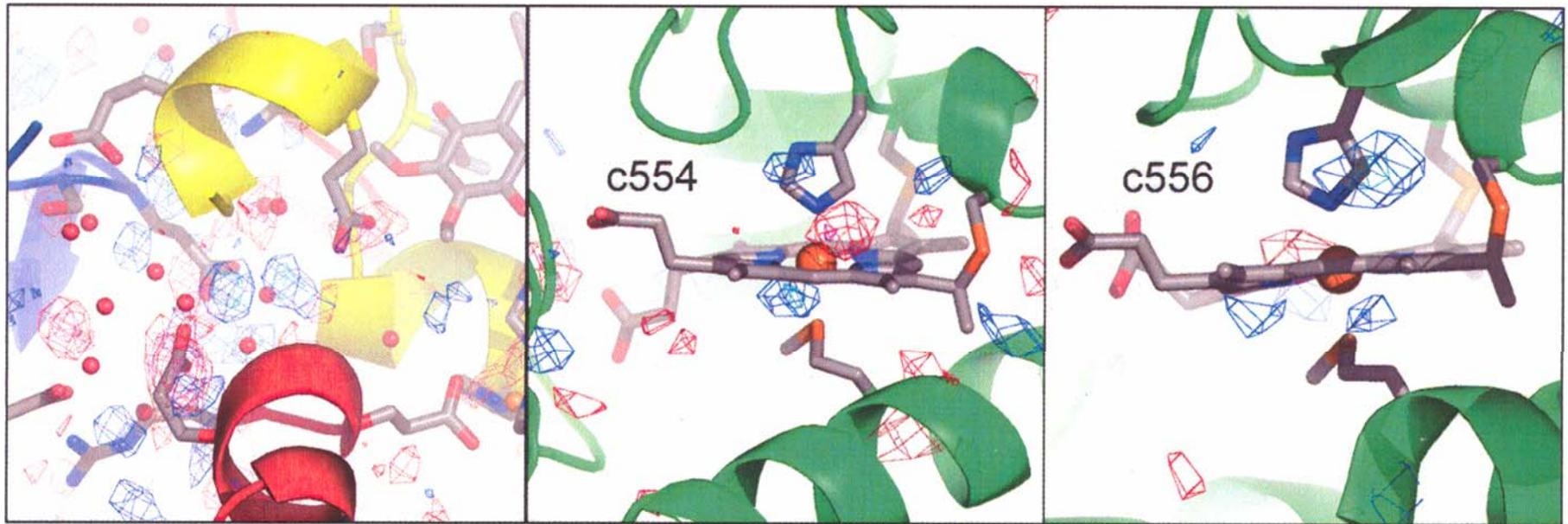
Cofactors in Photosynthetic Reaction Center of *B. Viridis*.

The active branch is on the right of the figure

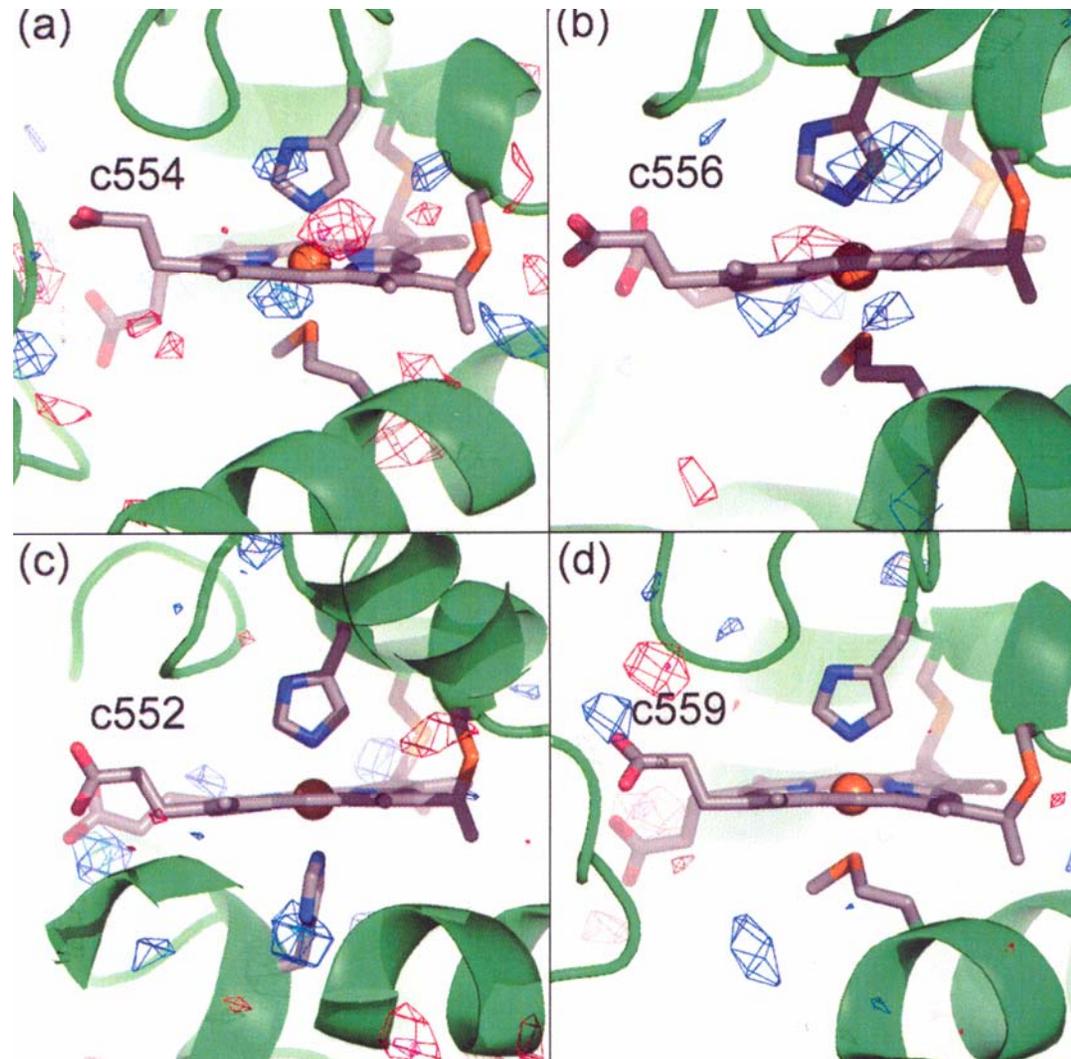
RC Complex of *B. Viridis*



Major Regions of Radiation Damage



Difference Fourier map of the four hemes of the cytochrome

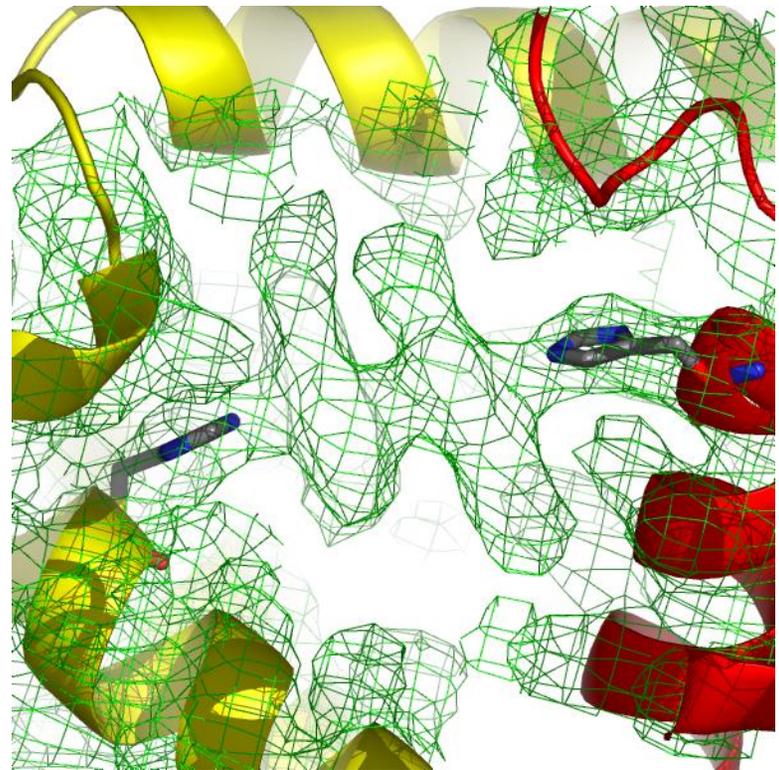


Summary of Results

- **Quinone occupancy 0.6 ± 0.1 , assume distal occupancy 0.2**
- **Extent of photoactivation 50%**
- **Due to slower rate of charge recombination, difference map contains 25% 'light' structure**
- **$0.2 \times 0.5 \times (1 - 0.25) = 7.5\%$ occupancy shift, which is observable, but not observed**
- **Freezing is not an artifact**

Future experiments

- $Q_A^-Q_B^- \rightarrow Q_AQ_B^-$ in *Rhodobacter sphaeroides*
- Improve efficiency of photoactivation, optimize radiation dose, correct timing
- $Q_A^-Q_B^- + 2H^+ \rightarrow Q_A + Q_BH_2$ in *Blastochloris viridis* (requires flowing of reagents)



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