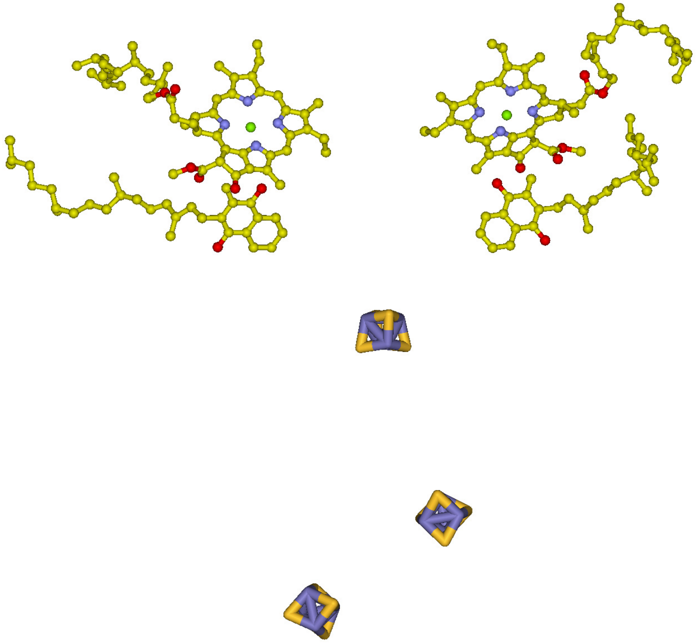


Meccanismi del trasporto di elettroni nei centri di reazione fotosintetici

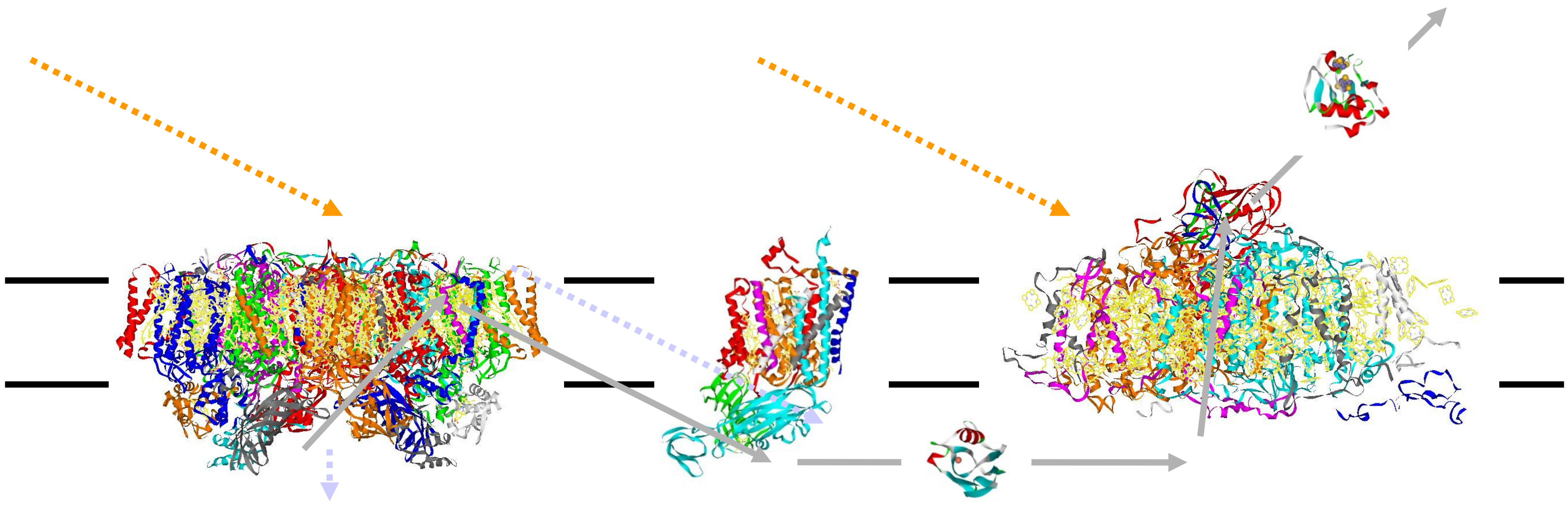
Stefano Santabarbara

Istituto di Biofisica, Consiglio Nazionale delle Ricerche

Milano, Italy

Accademia dei Georgofili, Firenze, 24 Maggio 2012

Photosynthesis: Overview



- Photosystem II

$2H_20+2PQ+ 4h\nu \rightarrow$

$2PQ^{--} + 0_2 + 4H^+$

Cytochrome b_6f

$PQH_2 + PC^{II} \rightarrow$

$PQ+PC^{I} + 2H^+$

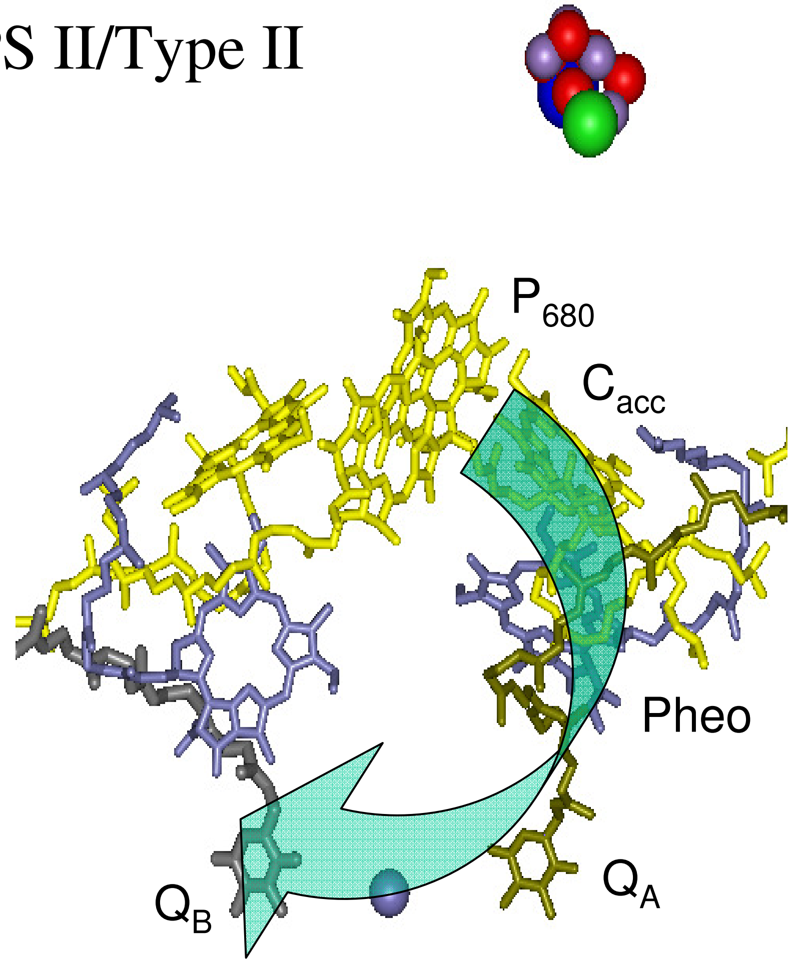
- Photosystem I

$PC^{I} + Fd^{ox} + h\nu \rightarrow$

$PC^{II} + Fd^{red}$

Comparing *Photosystem II* and *Photosystem I*

PS II/Type II

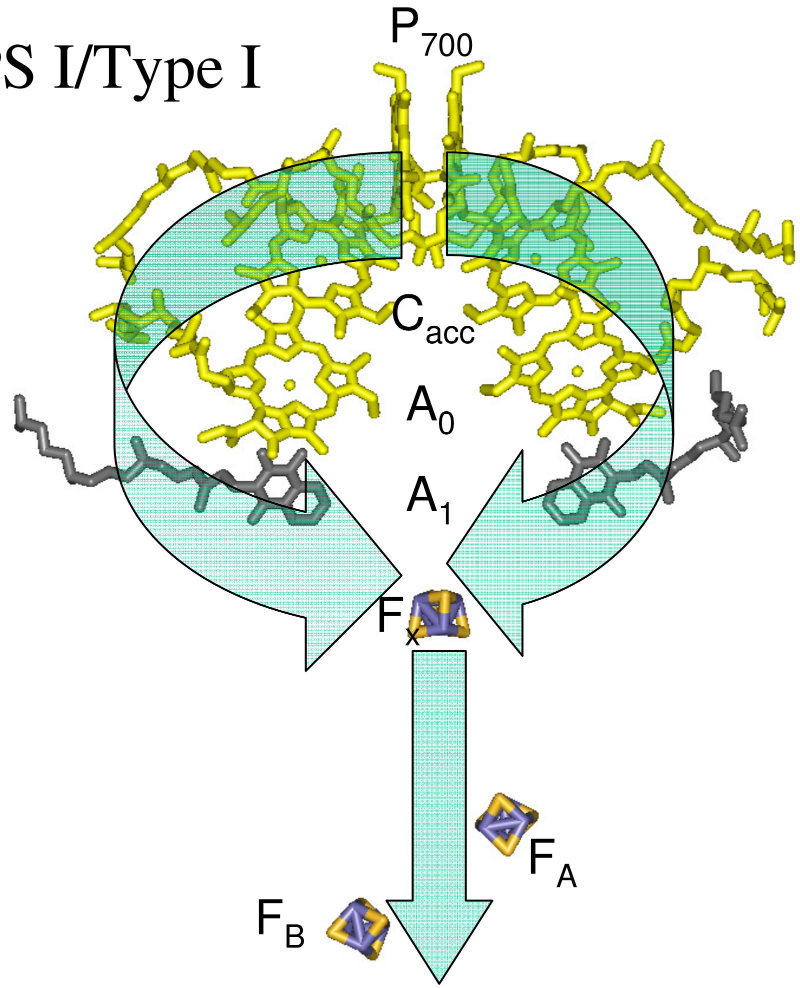


• $Q_A^- \rightarrow Q_B$
• $Q_A^- \rightarrow Q_B^-$

•Asymmetric ET

• Two-electron gate

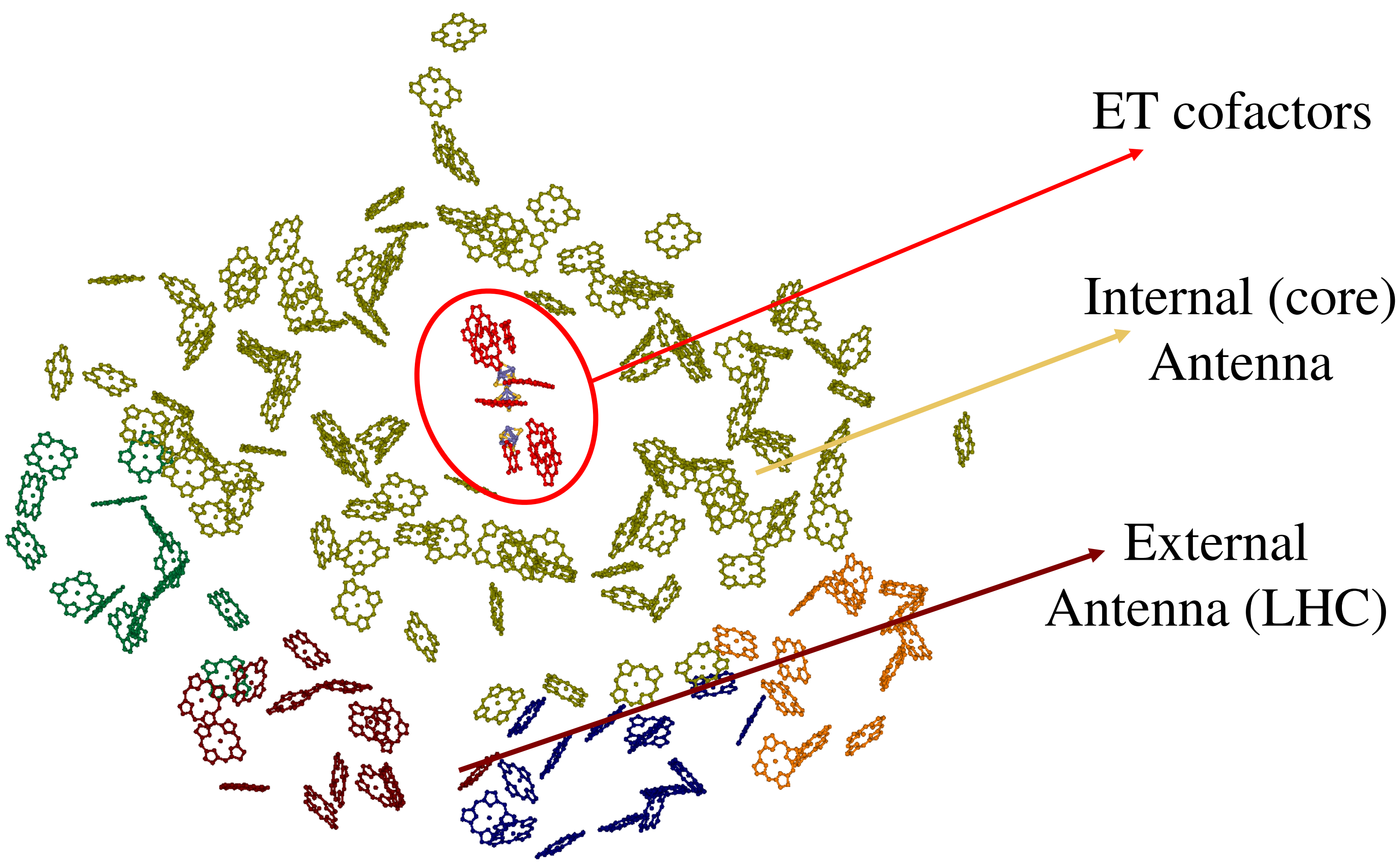
PS I/Type I



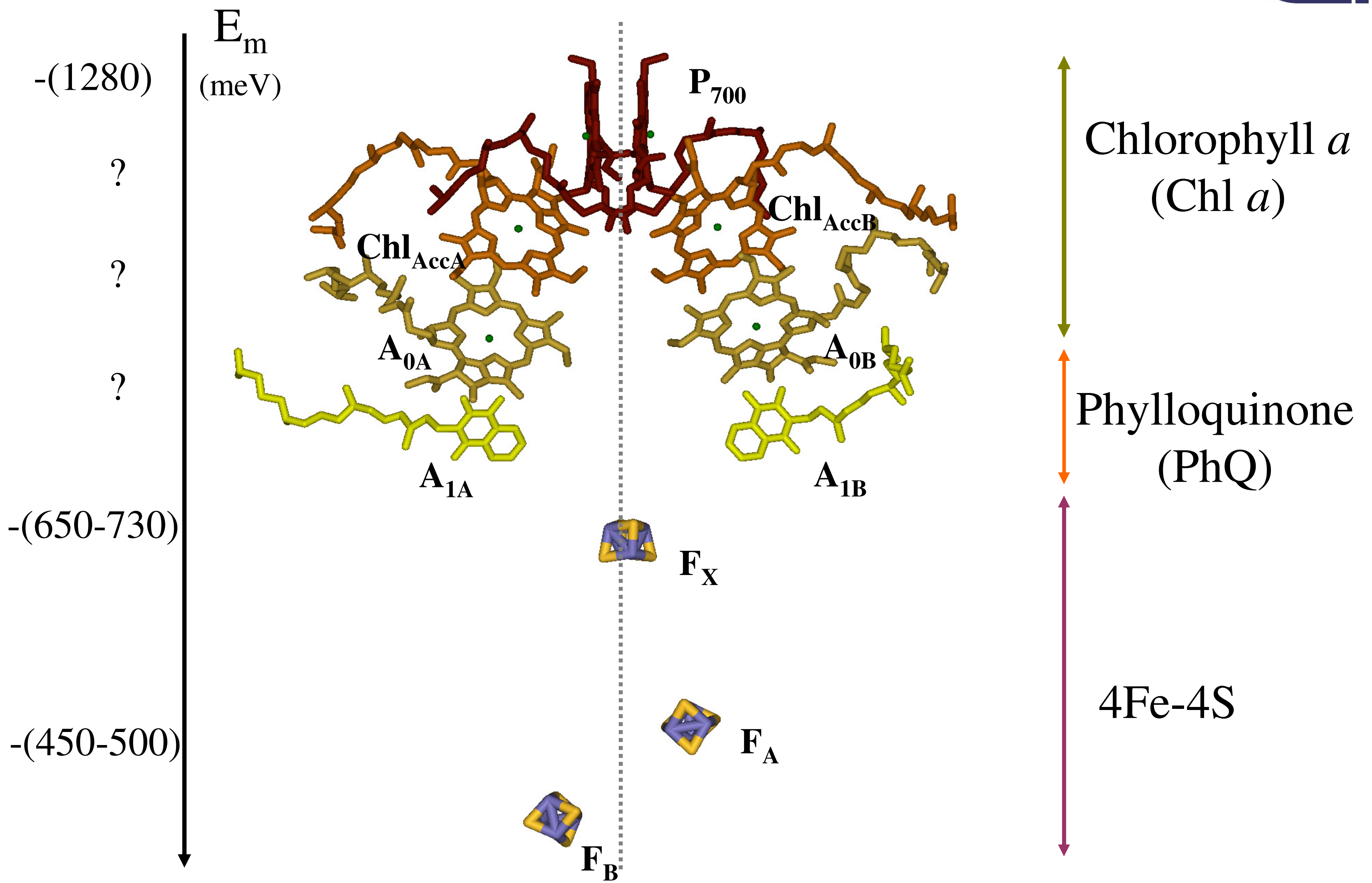
• Symmetric ET

• One-electron chemistry

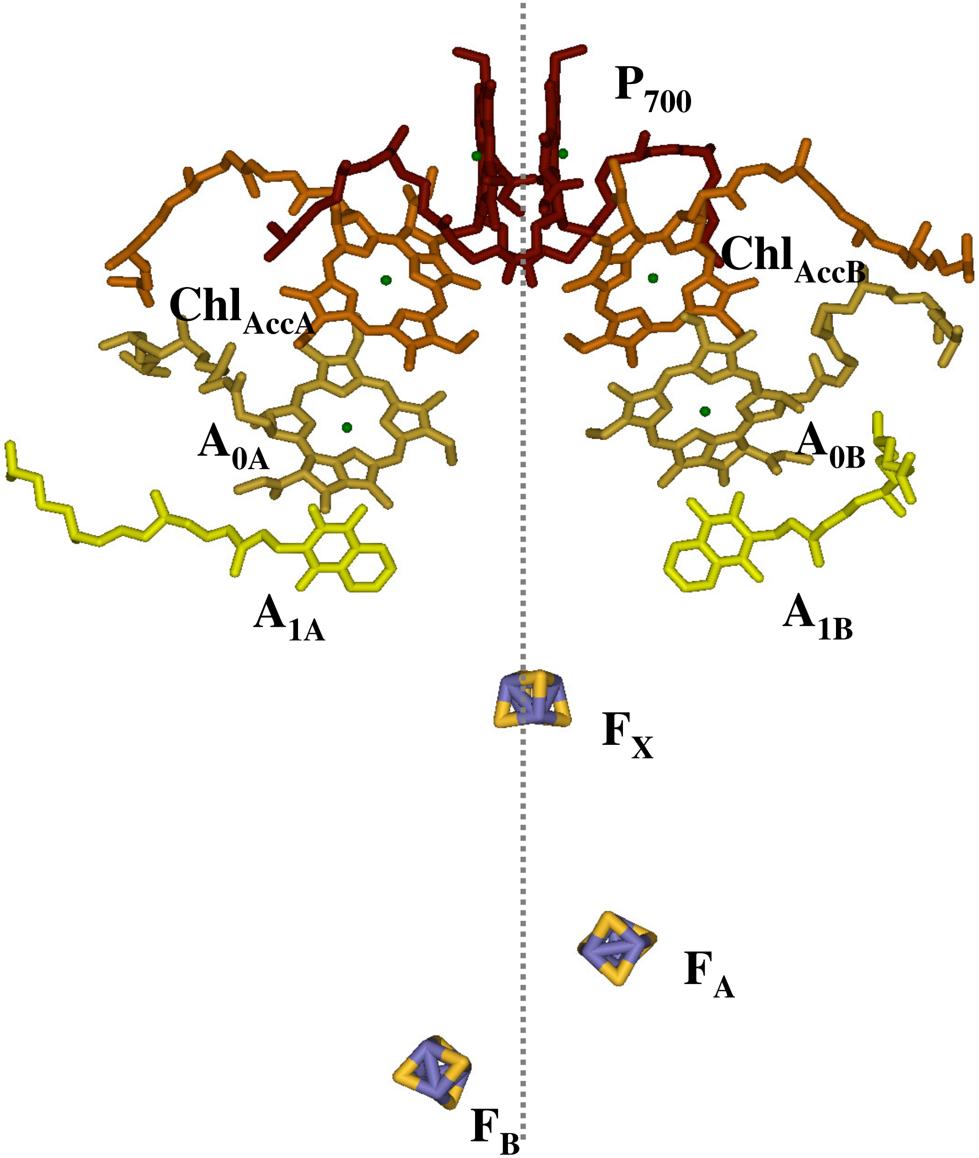
Photosystem I: electron transfer cofactors

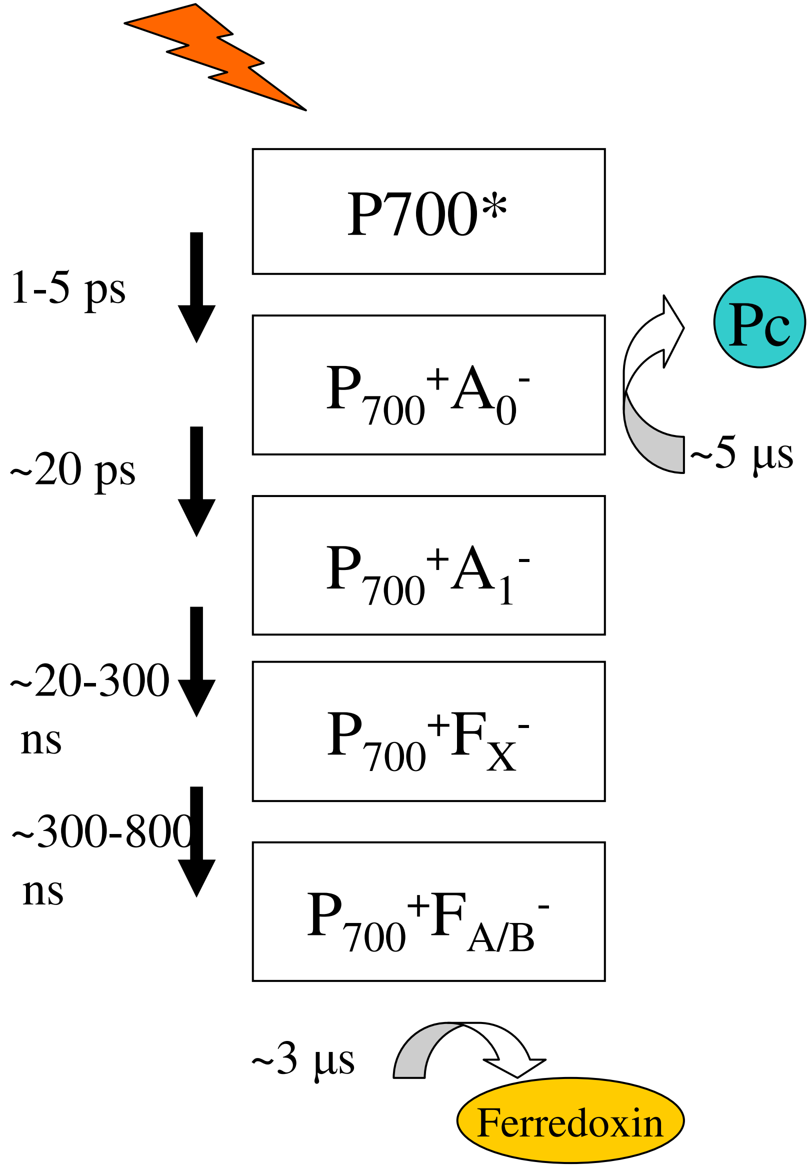


Photosystem I: electron transfer cofactors

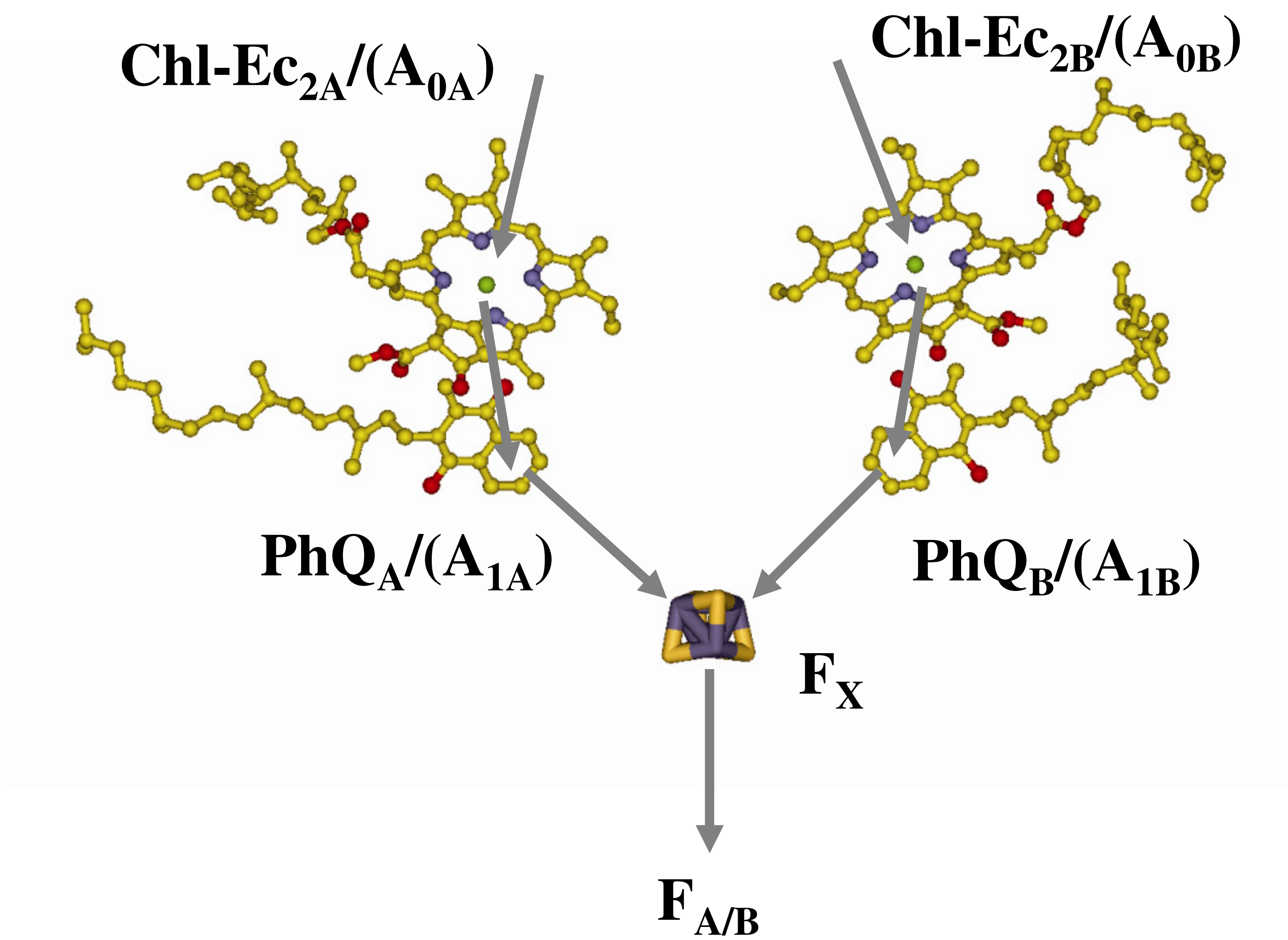


Photosystem I: timing of ET reactions.

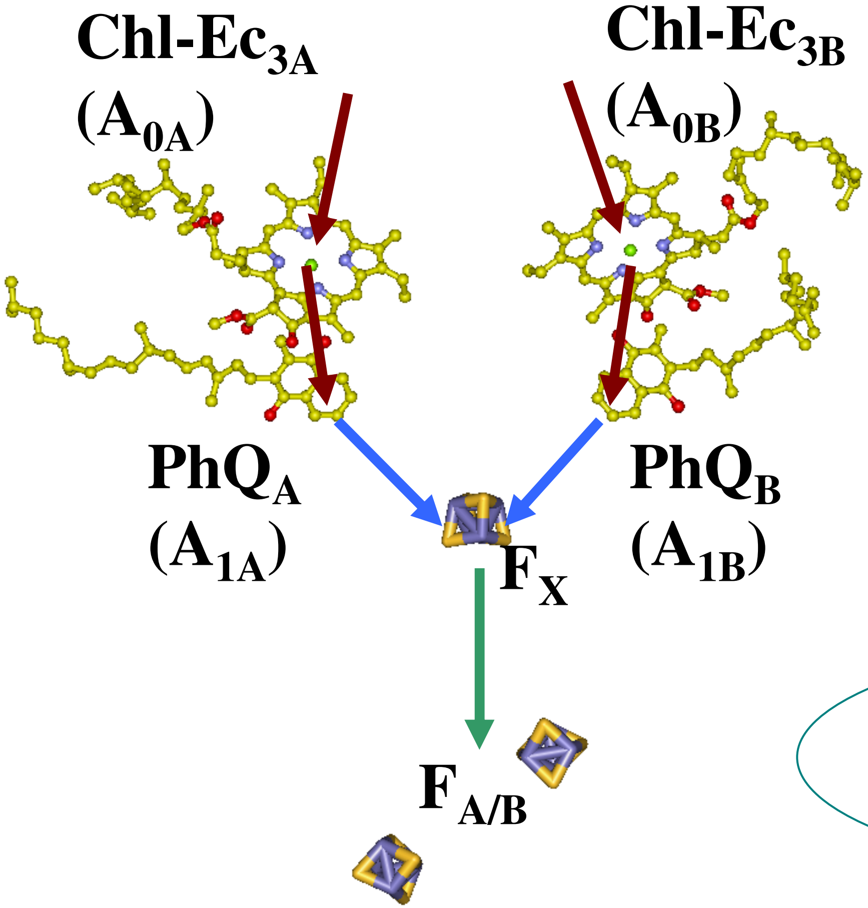




Secondary Electron Transfer *in* *Photosystem I*



How to go from symmetric to asymmetric?

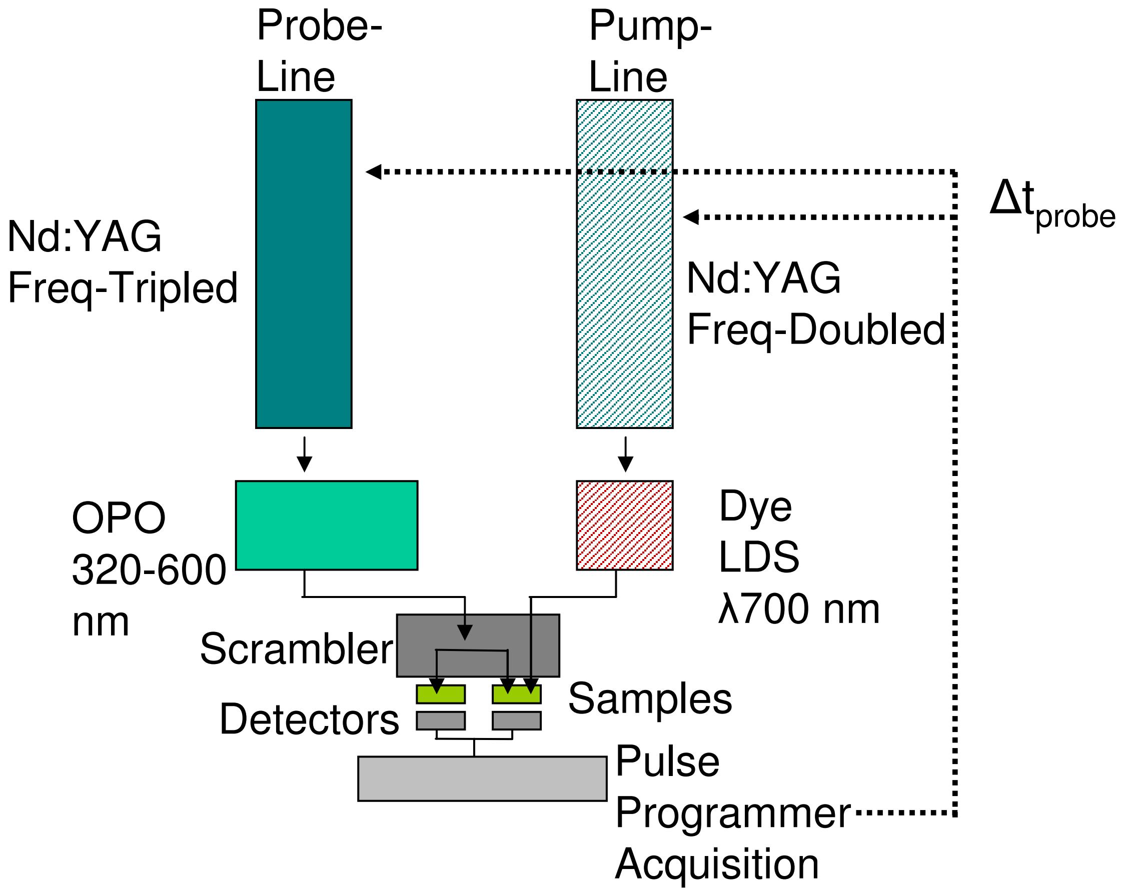


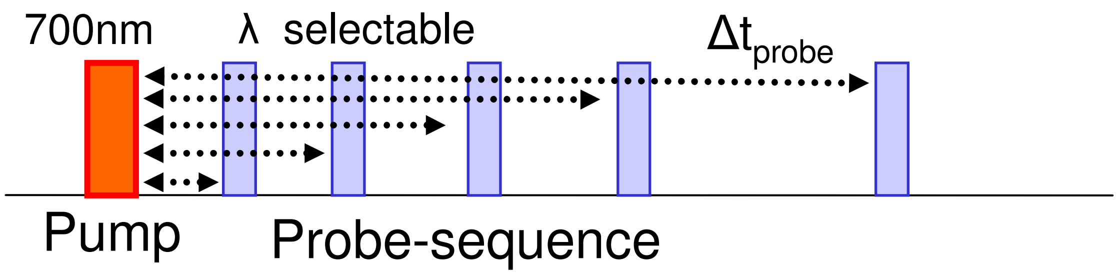
- Control of directionality

- Control of ET rate, stability of semi-quinone

- Control of successive ET reactions

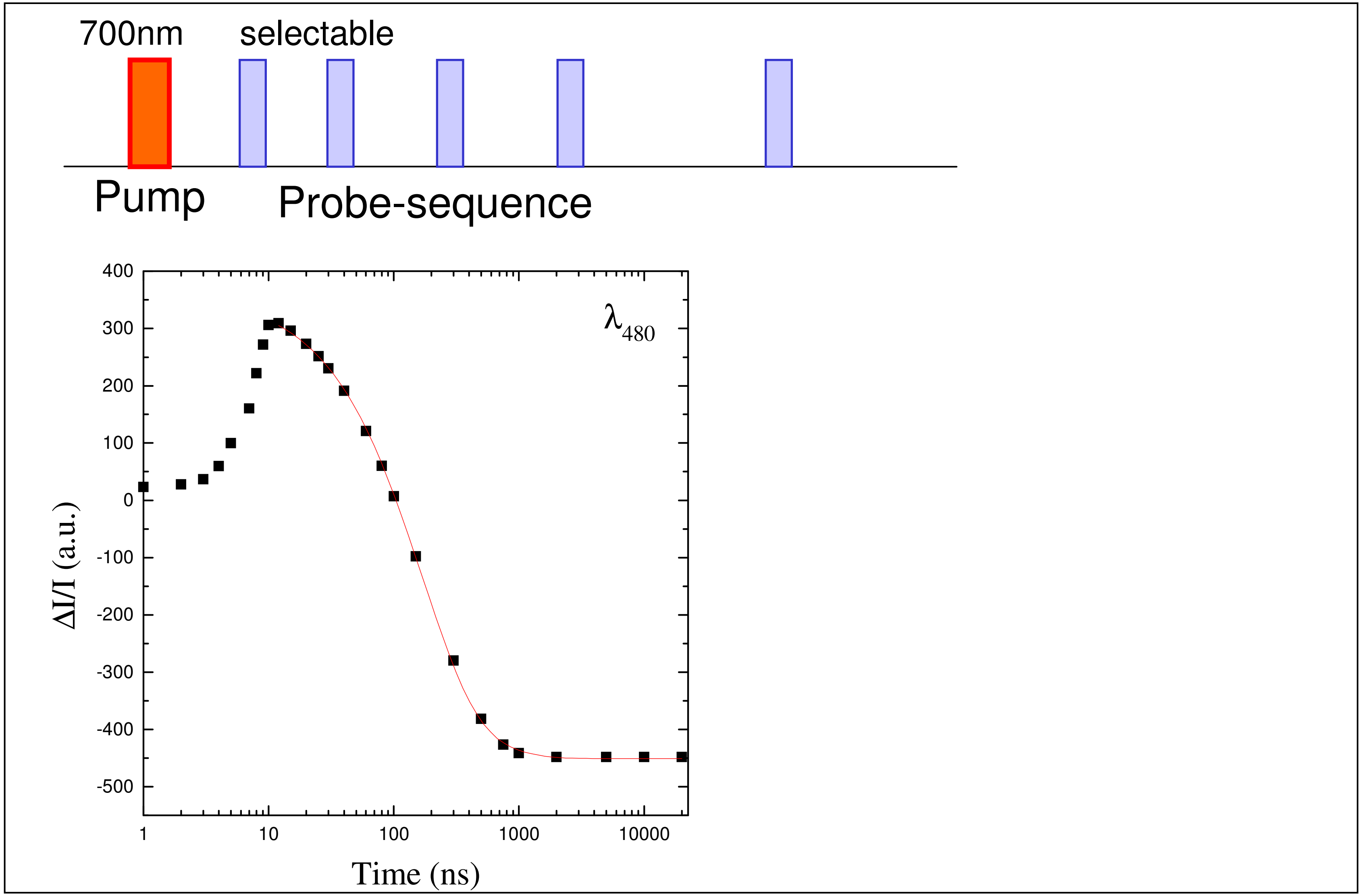
Pump-Probe set up





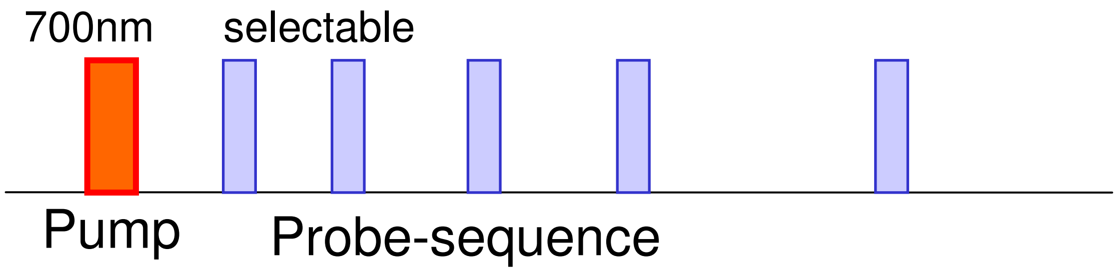
Strategies of Investigation: *spectroscopy*

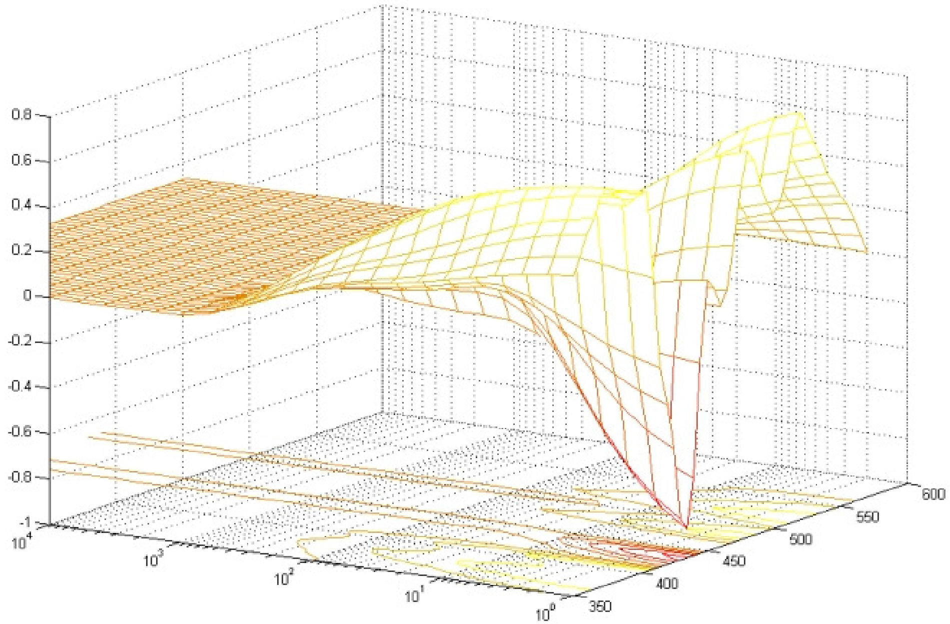
- Pump-Probe in the nanoseconds time-range



Strategies of Investigation: *spectroscopy*

- Pump-Probe in the nanoseconds time-range

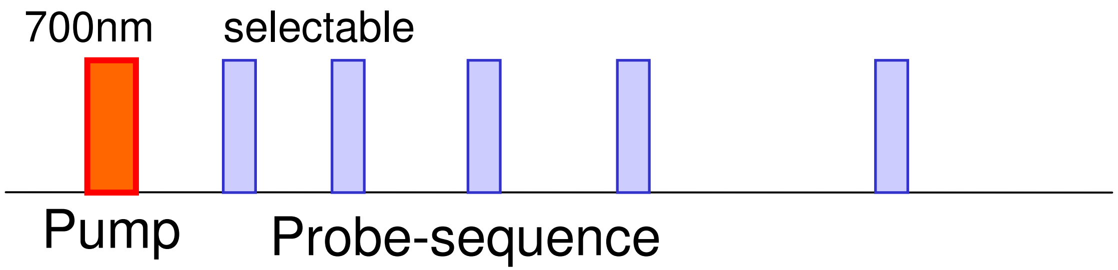


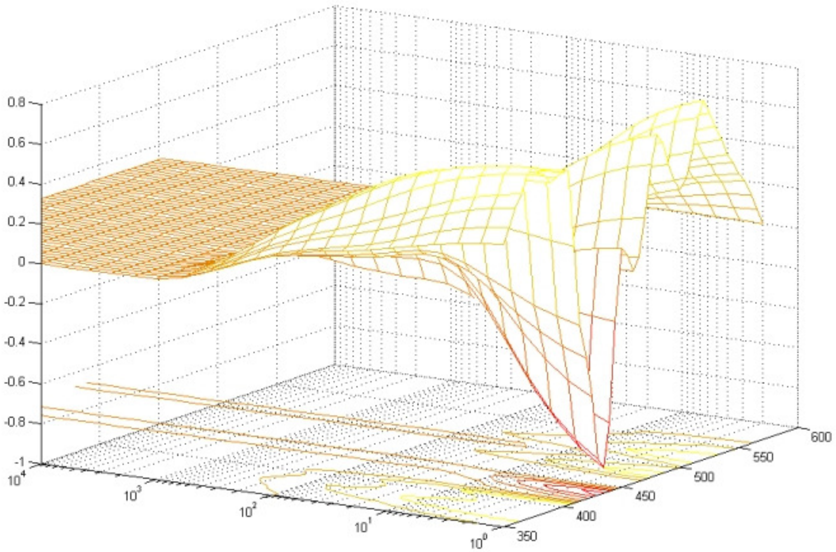


- And obtains a surface like this…

Strategies of Investigation: *spectroscopy*

- Analysis: fit with a sum of exponents:





$$\frac{\Delta I}{I}(t,\lambda) = \sum_{i=1}^{n} A_i(\lambda)\cdot e^{-\frac{t}{\tau_i}} + A_\infty$$

Signal

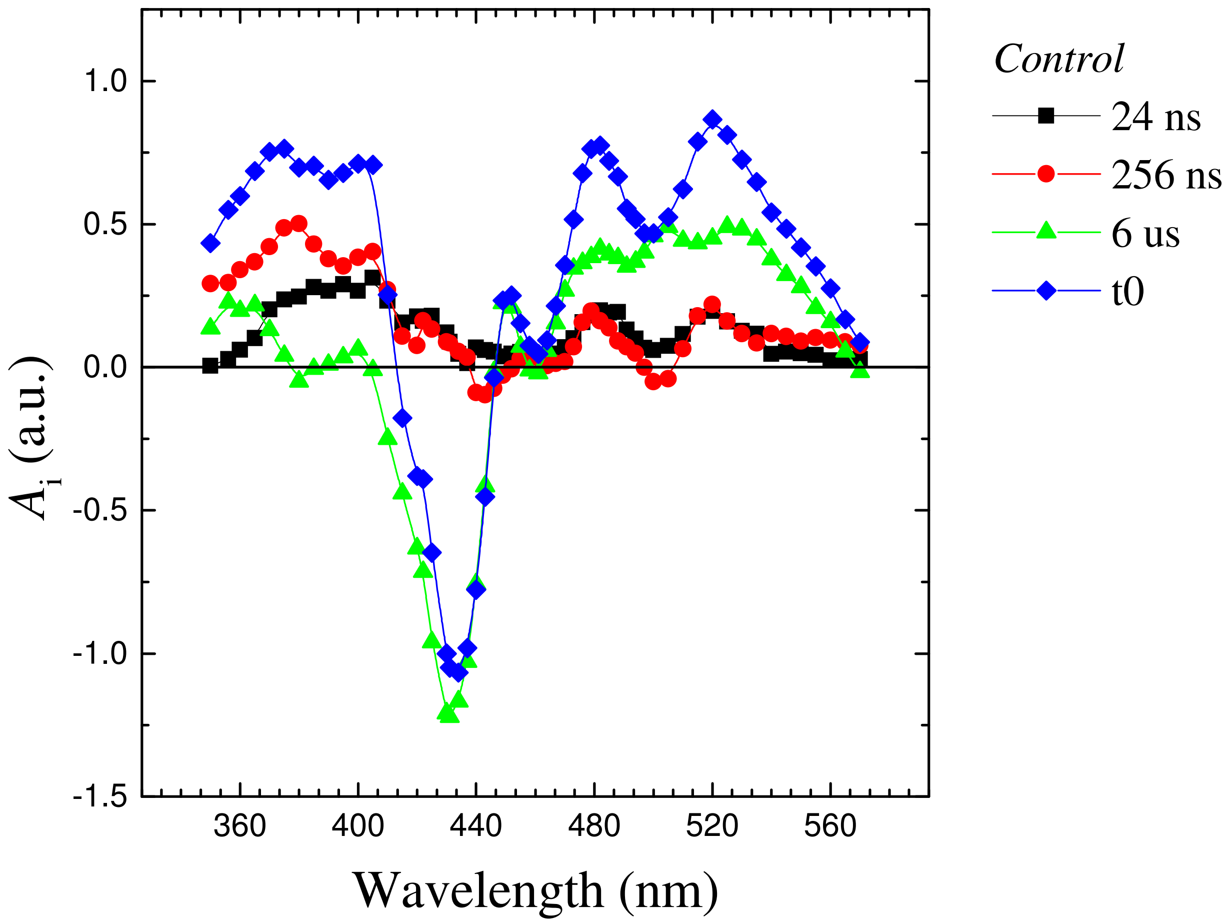
Amplitude

Lifetime

Rate~τ^{-1}

Strategies of Investigation: *spectroscopy*

- Decay Associated Spectra (DAS)



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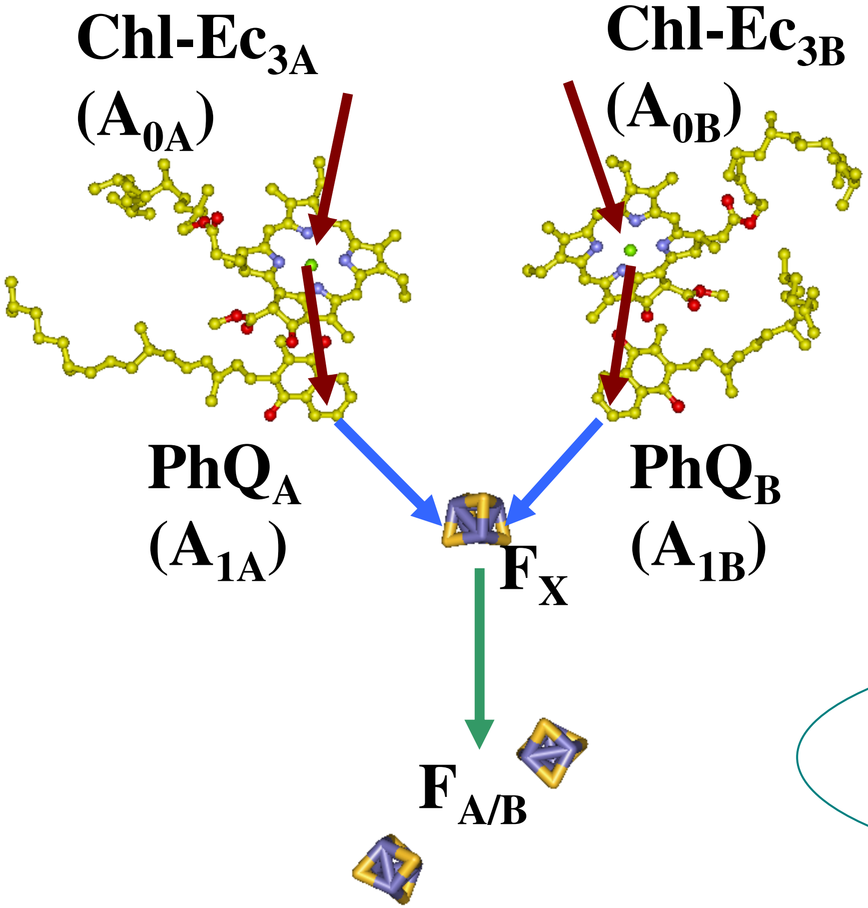
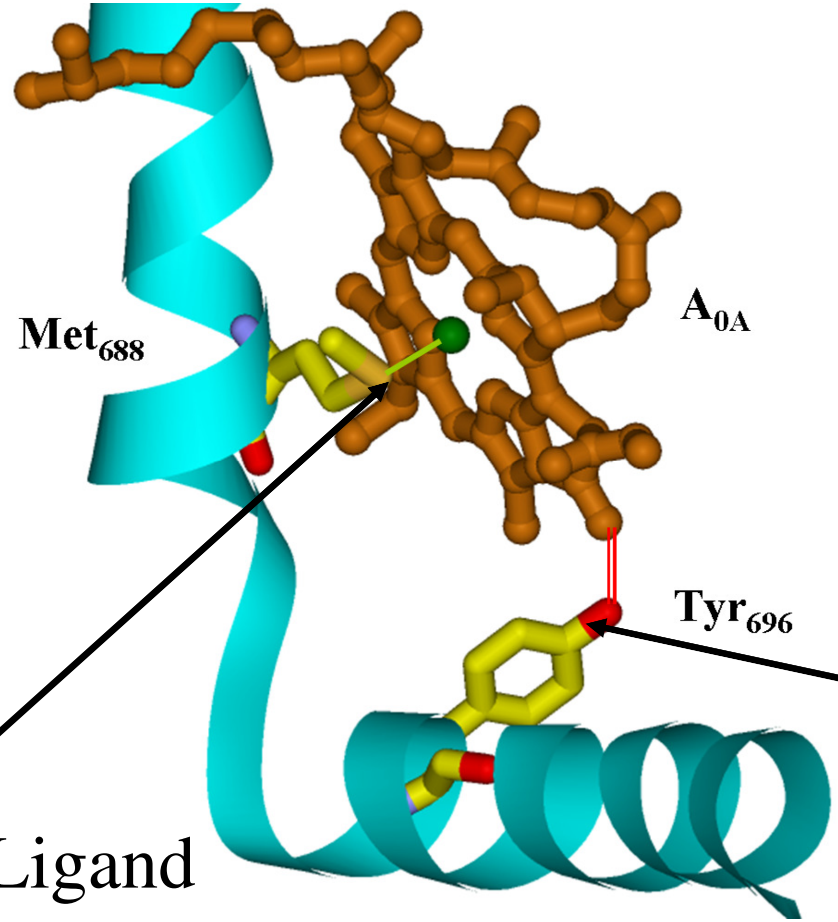


- Control of directionality

- Control of ET rate, stability of semi-quinone

- Control of successive ET reactions

- Chlorophyll A_0 binding site

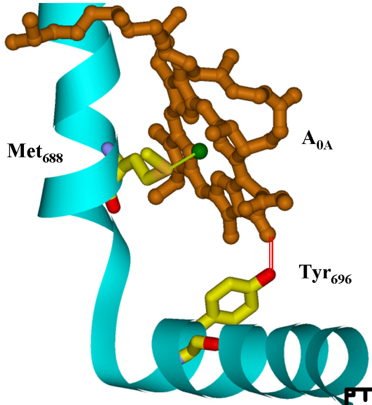


- H-bond (Chl Macrocycle)

- Axial Ligand

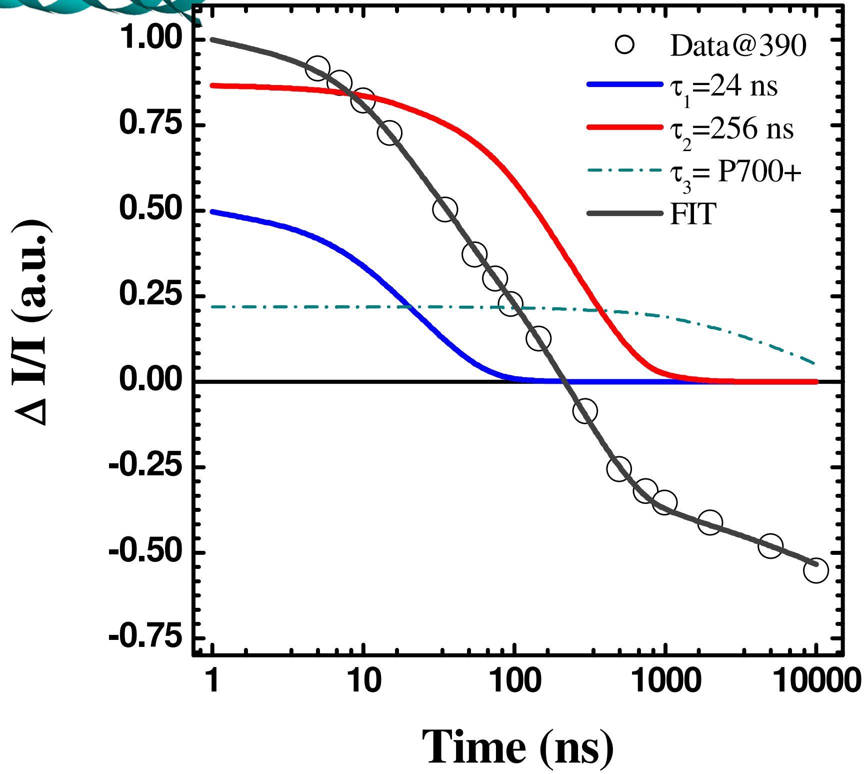
PsaA subunit

1: *Control of directionality*

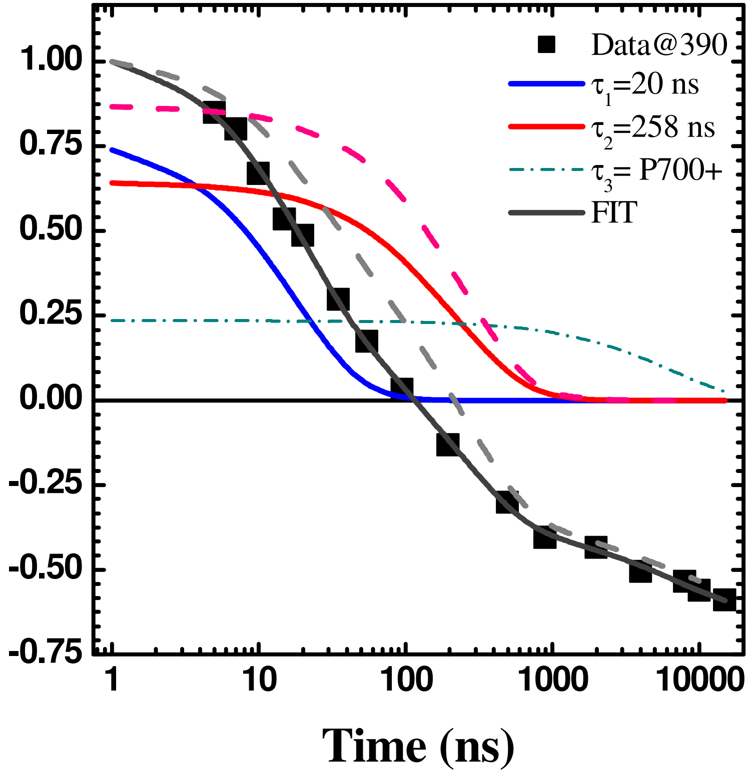


Mutants of A_{0A} binding site

Wild-type

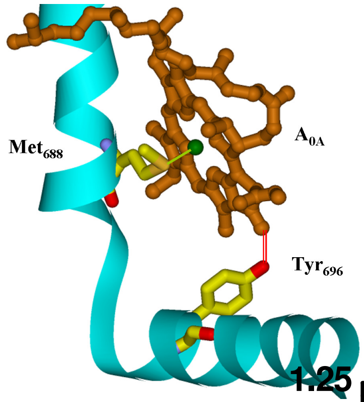


PsaA-F696Y

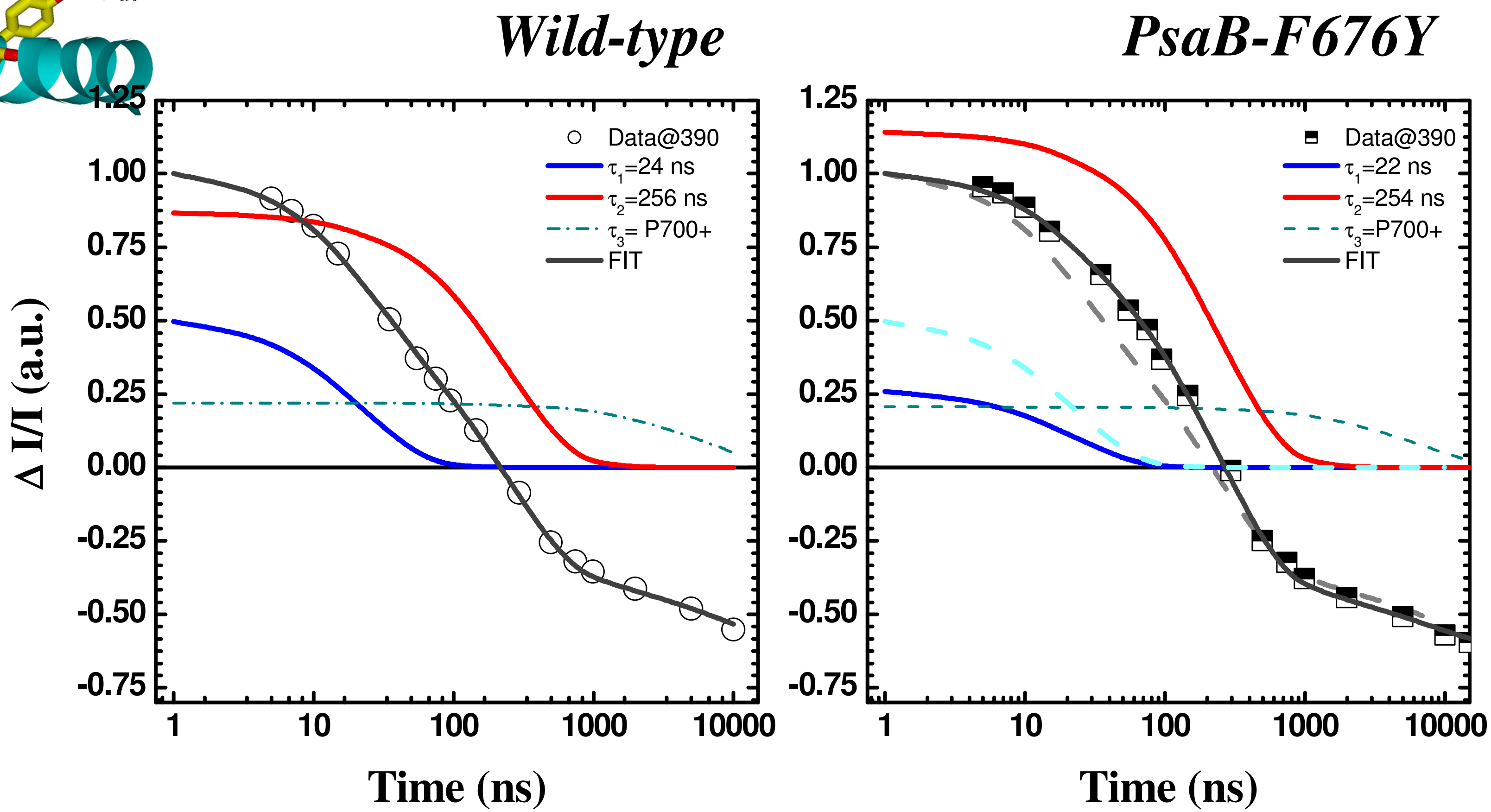


Li X. *et al.* PNAS (2006) *103,* 2144/Byrdin M. et *al.* (2006) BBA *1757*, 1521

1: *Control of directionality*



Mutants of A_{0B} binding site



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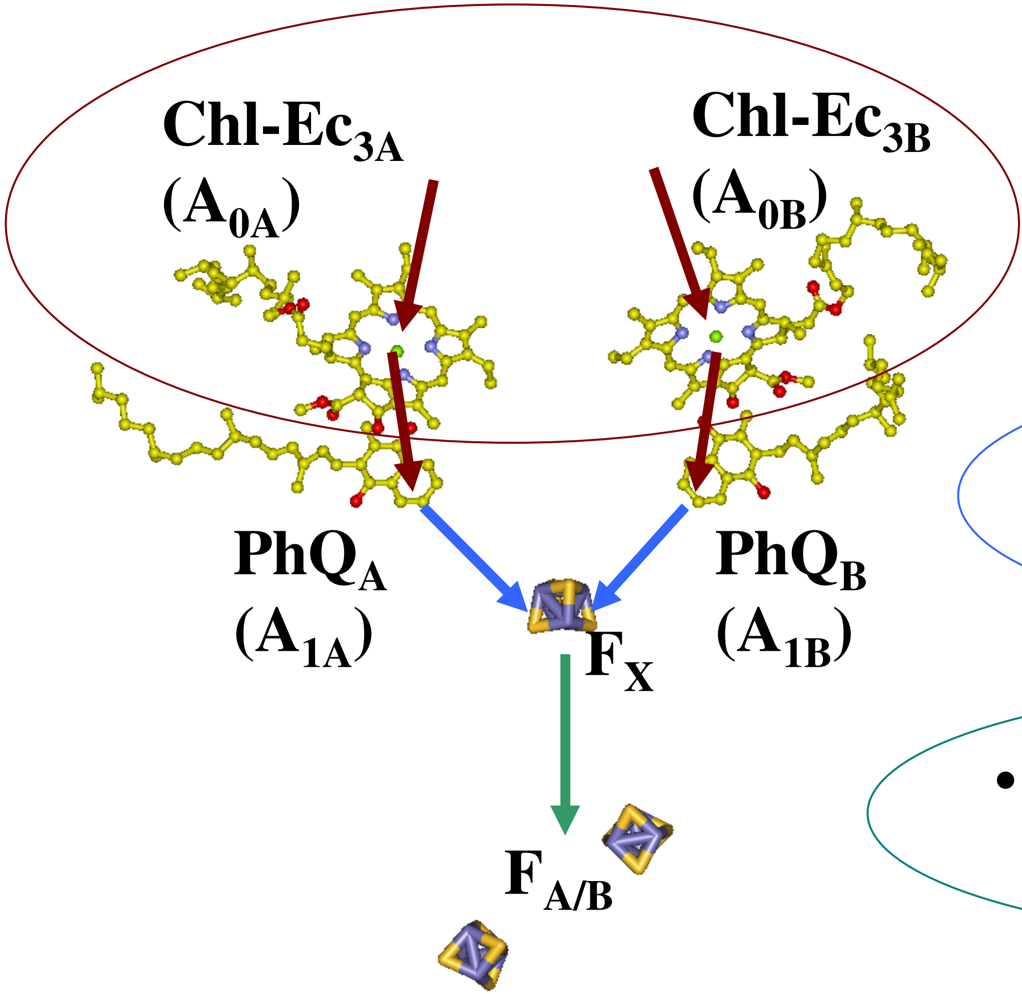
How to go from symmetric to asymmetric?

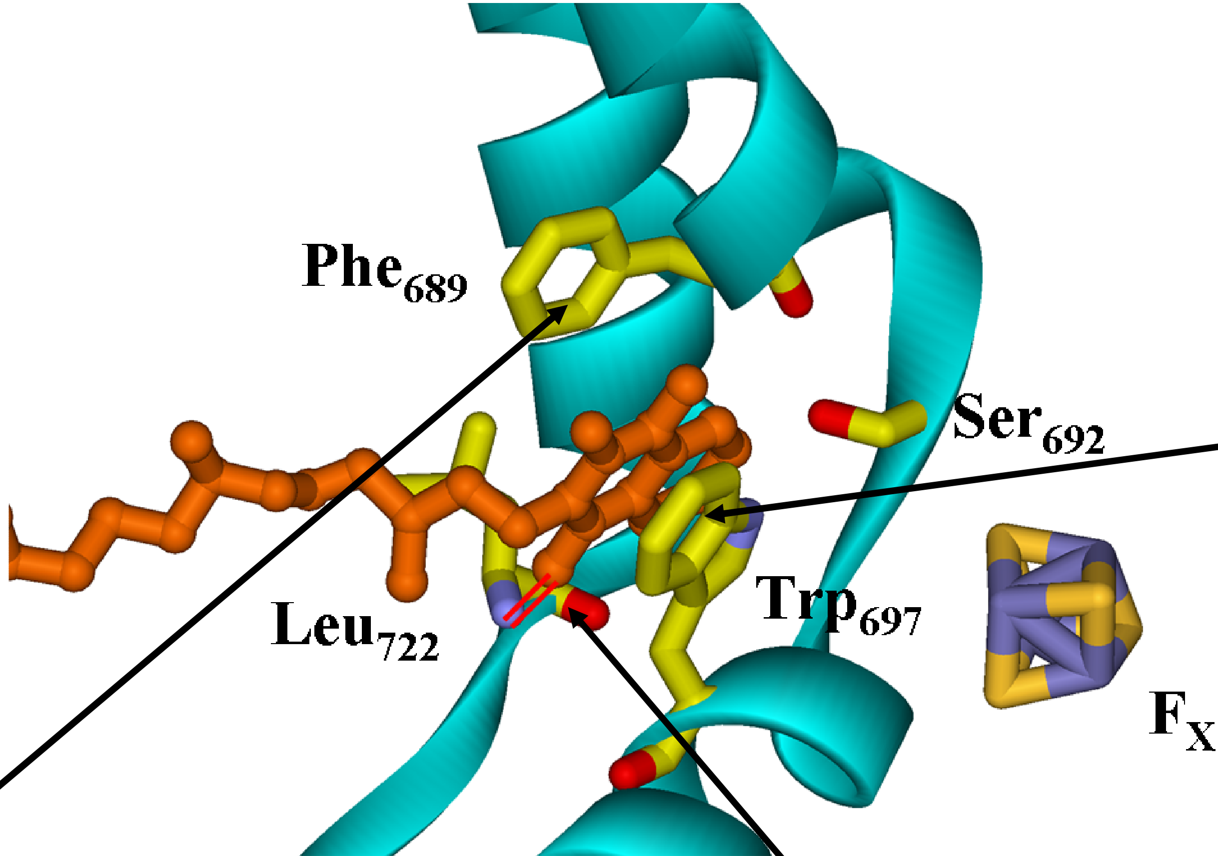


- Control of directionality: OK

- Control of ET rate, stability of semi-quinone

- Control of successive ET reactions

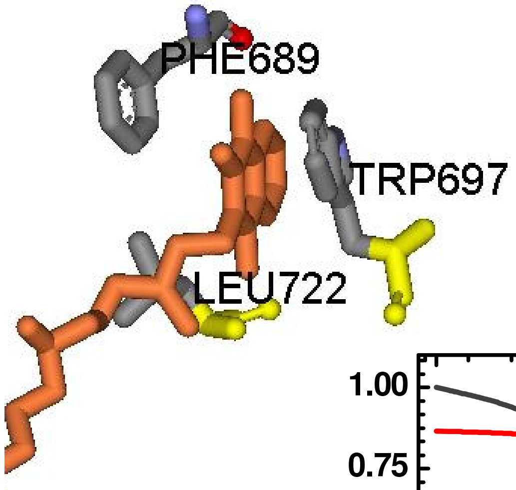
- The phylloquinone (A_1) binding site



- π-π orbital stacking
- Distal π-π stacking ?
- H-bond

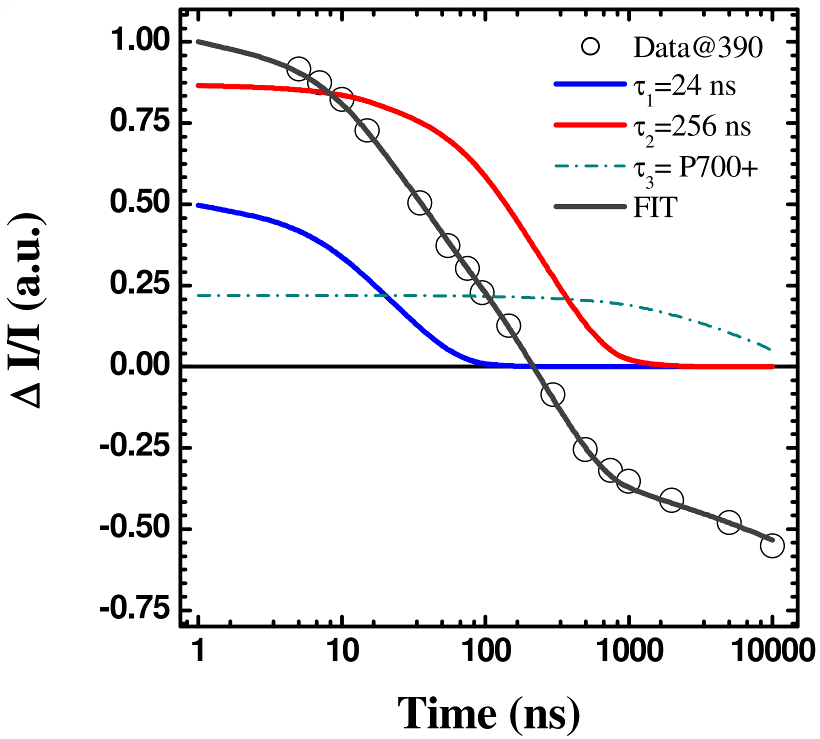
PsaA subunit

2: Control of electron transfer kinetics

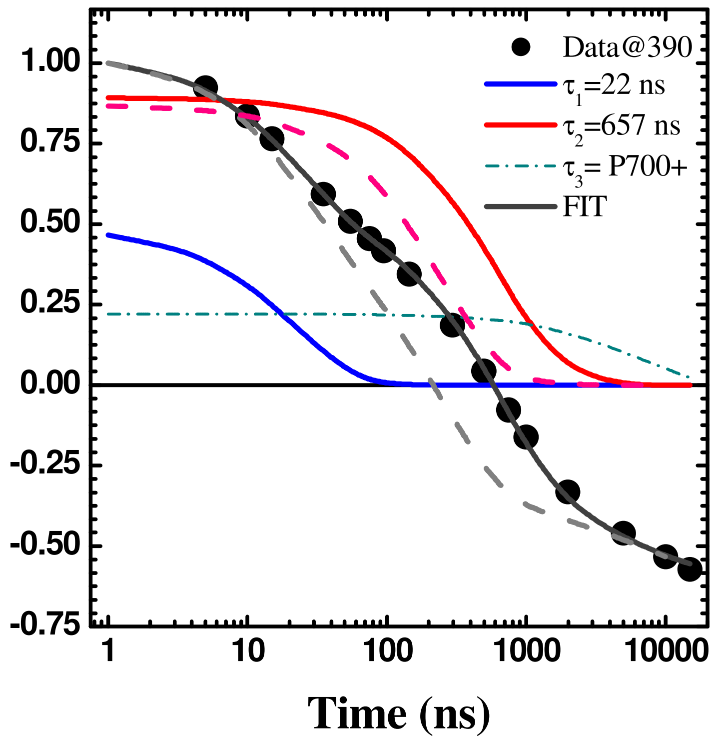


Mutants of A_{1A} binding site: *slower* kinetics

Wild-type

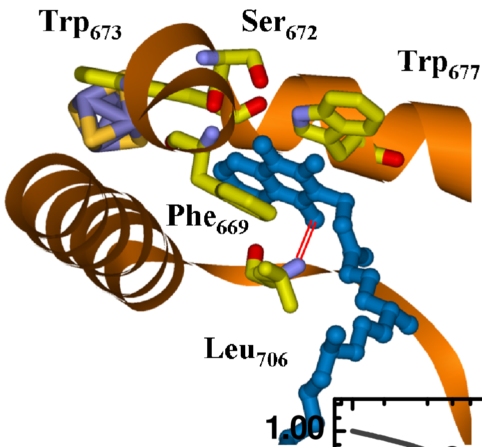


PsaA-W697F

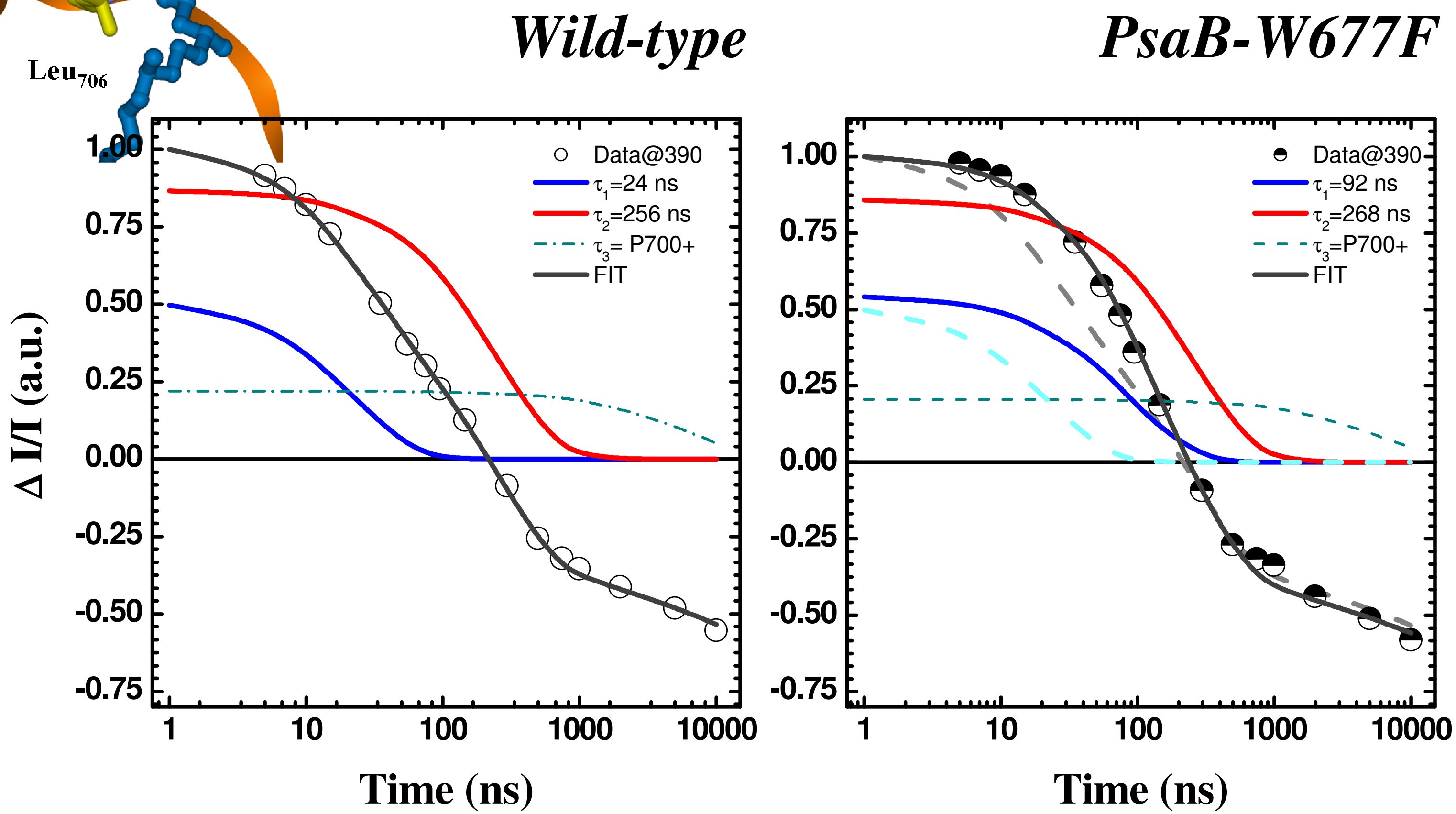


Guergova-Kuras M. *et al.* PNAS (2001) *98,* 4437/Santabarara *et al.* (2008) Photochem. Photobiol. *84*, 1381

2: Control of electron transfer kinetics

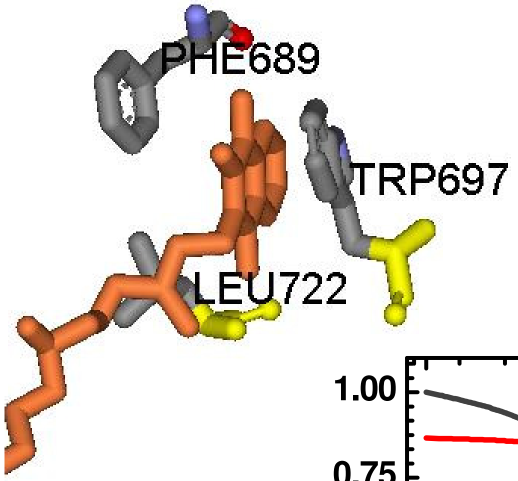


Mutants of A_{1B} binding site: *slower* kinetics



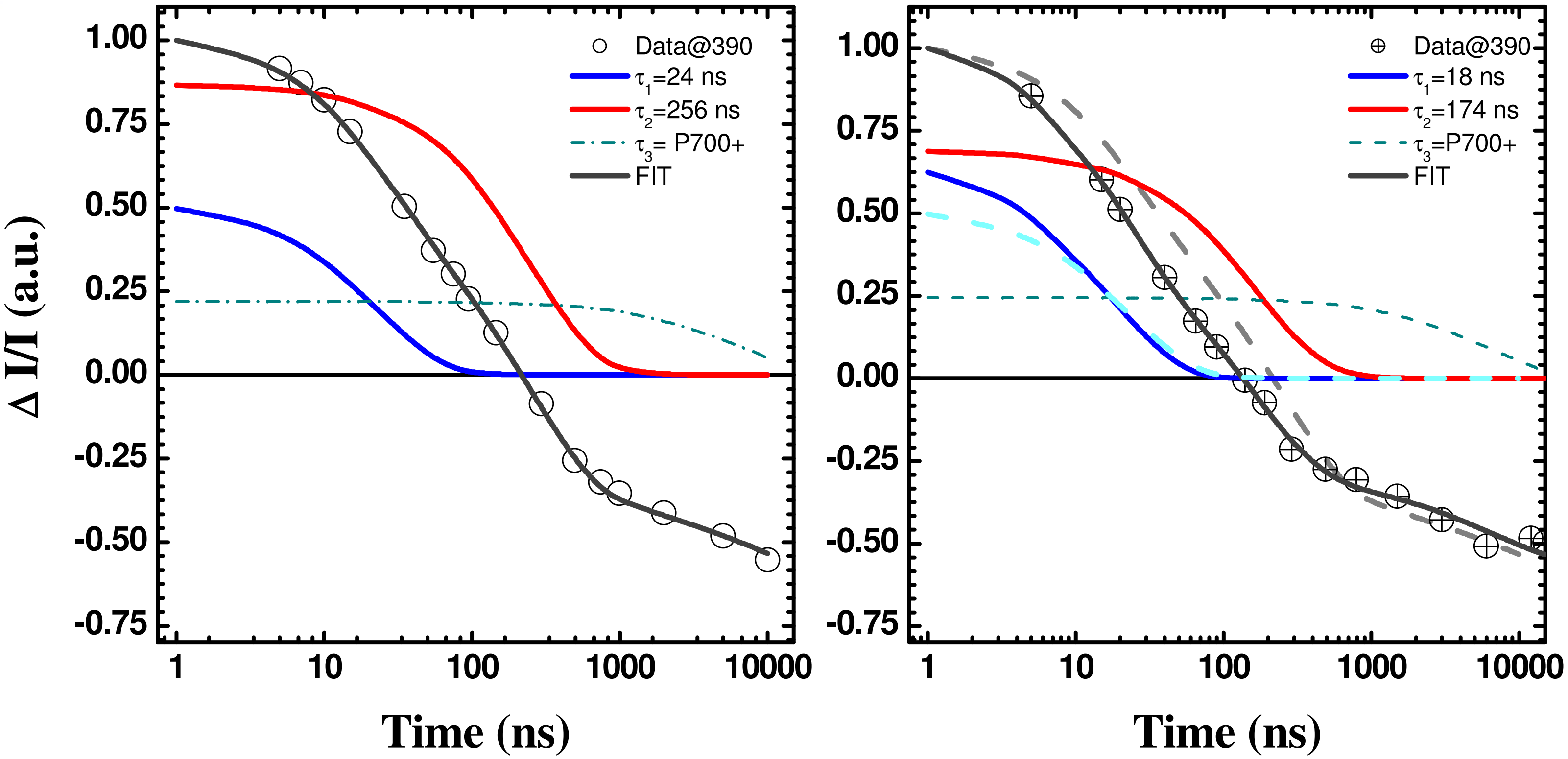
Guergova-Kuras M. *et al.* PNAS (2001) *98,* 4437

2: Control *of electron transfer kinetics*



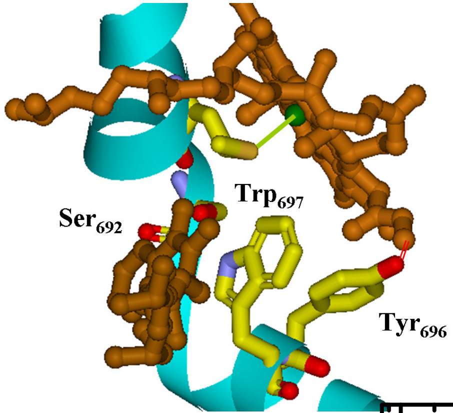
Mutants of A_{1A} binding site: *faster* kinetics

Wild-type ***PsaA-L722T***



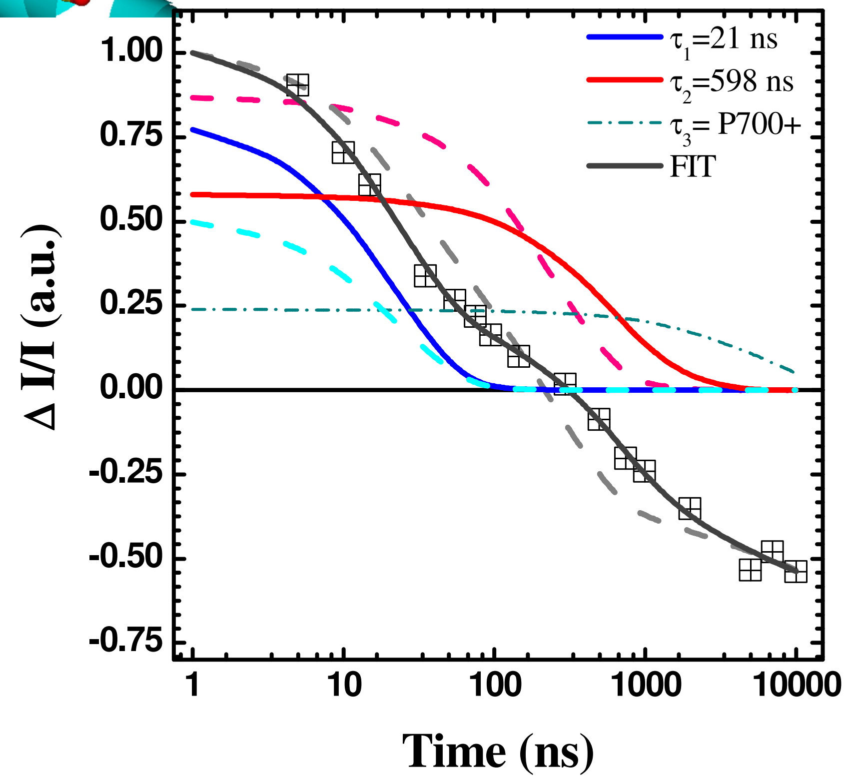
Santabarbara *et al.* (2010) J. Phys. Chem. B. *114,* 9300

3: *Control of directionality and ET kinetics*

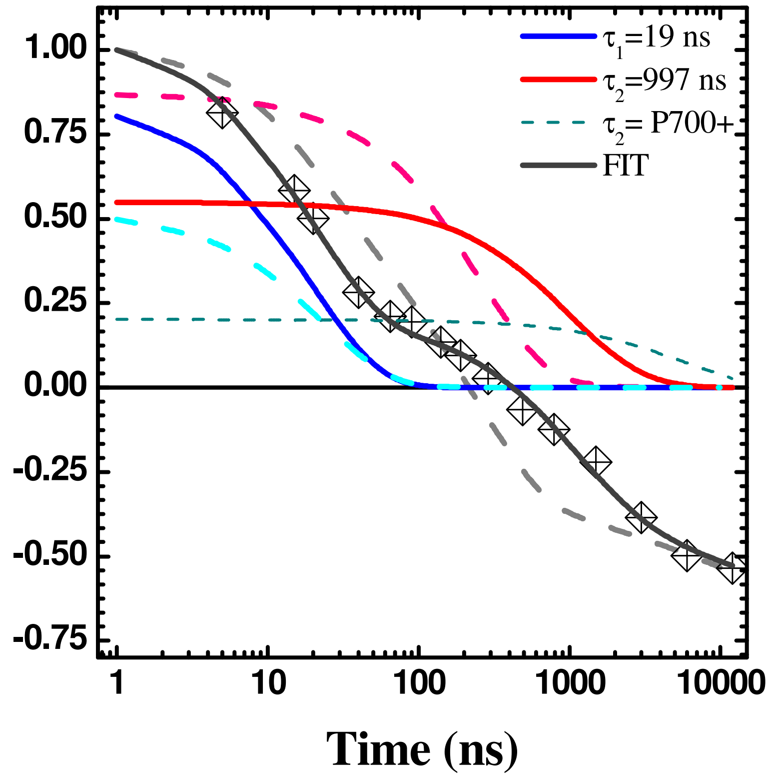


Combination of A_{0A} and A_{1A} binding site mutants

PsaA-F696Y/W697F

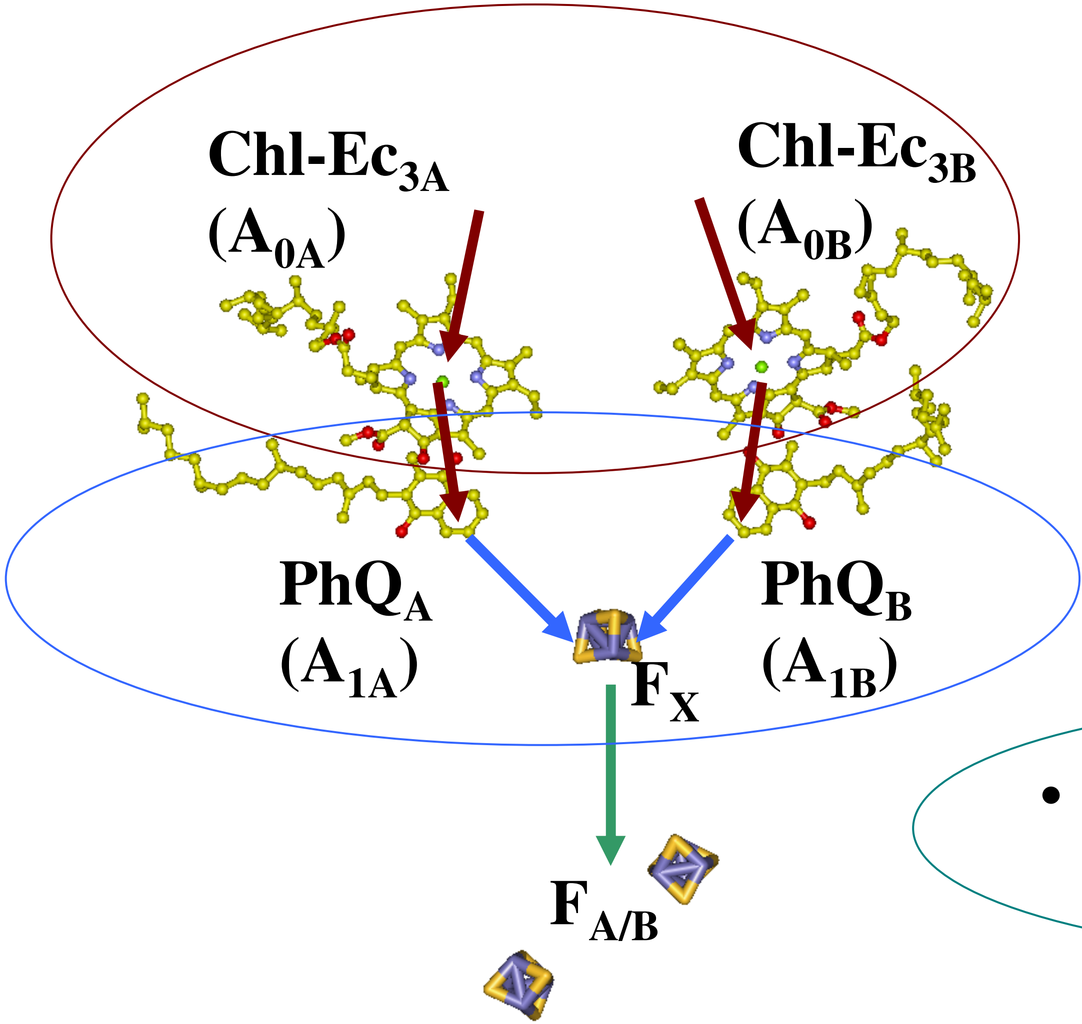


PsaA-F696Y/S692A



Santabarbara S. *et al.* Photochem. Photobiol. (2008) *84,* 1381

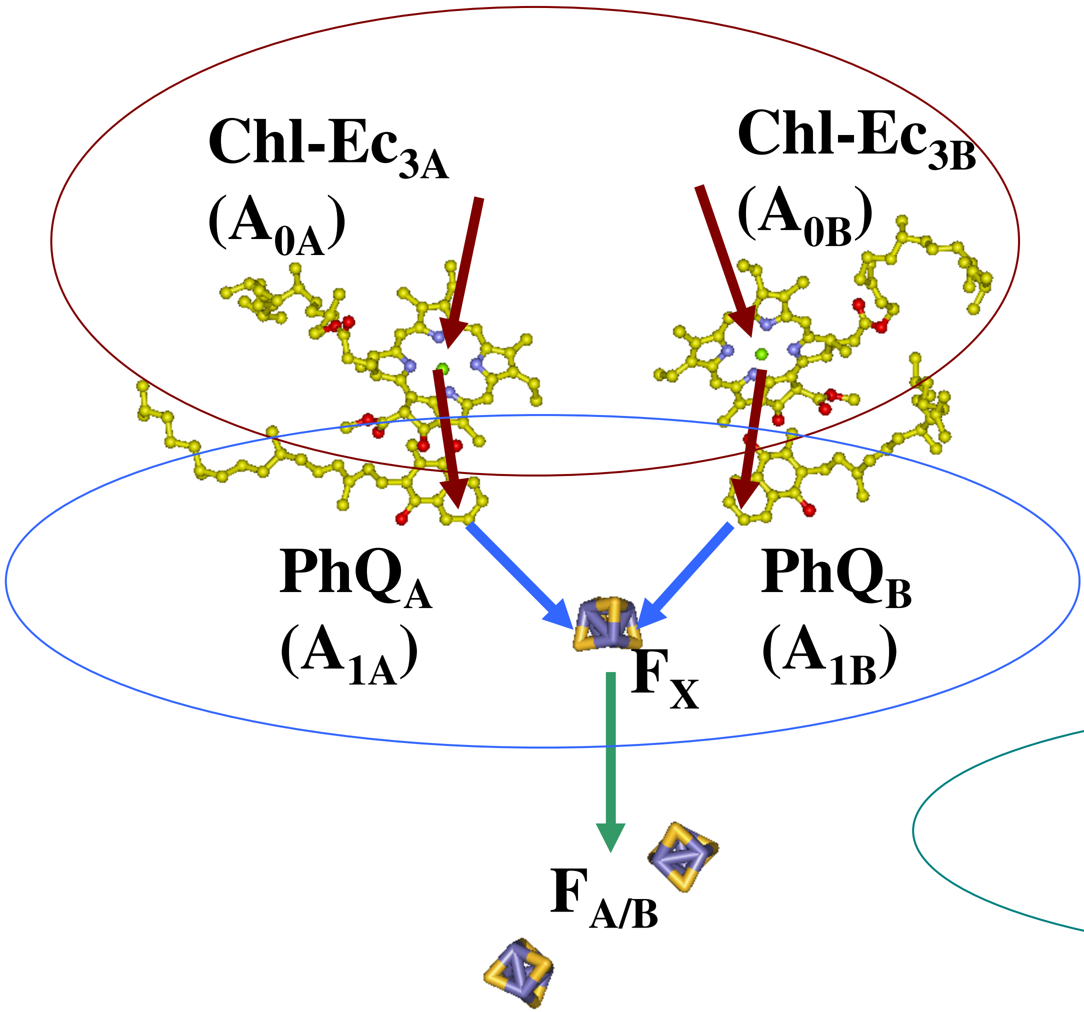
How to go from symmetric to asymmetric?



- Control of directionality: OK
- Control of ET rate, stability of semi-quinone: OK (sort of..)
- Control of successive ET reactions

•Two single point-mutations which combines…

How to go from symmetric to asymmetric?



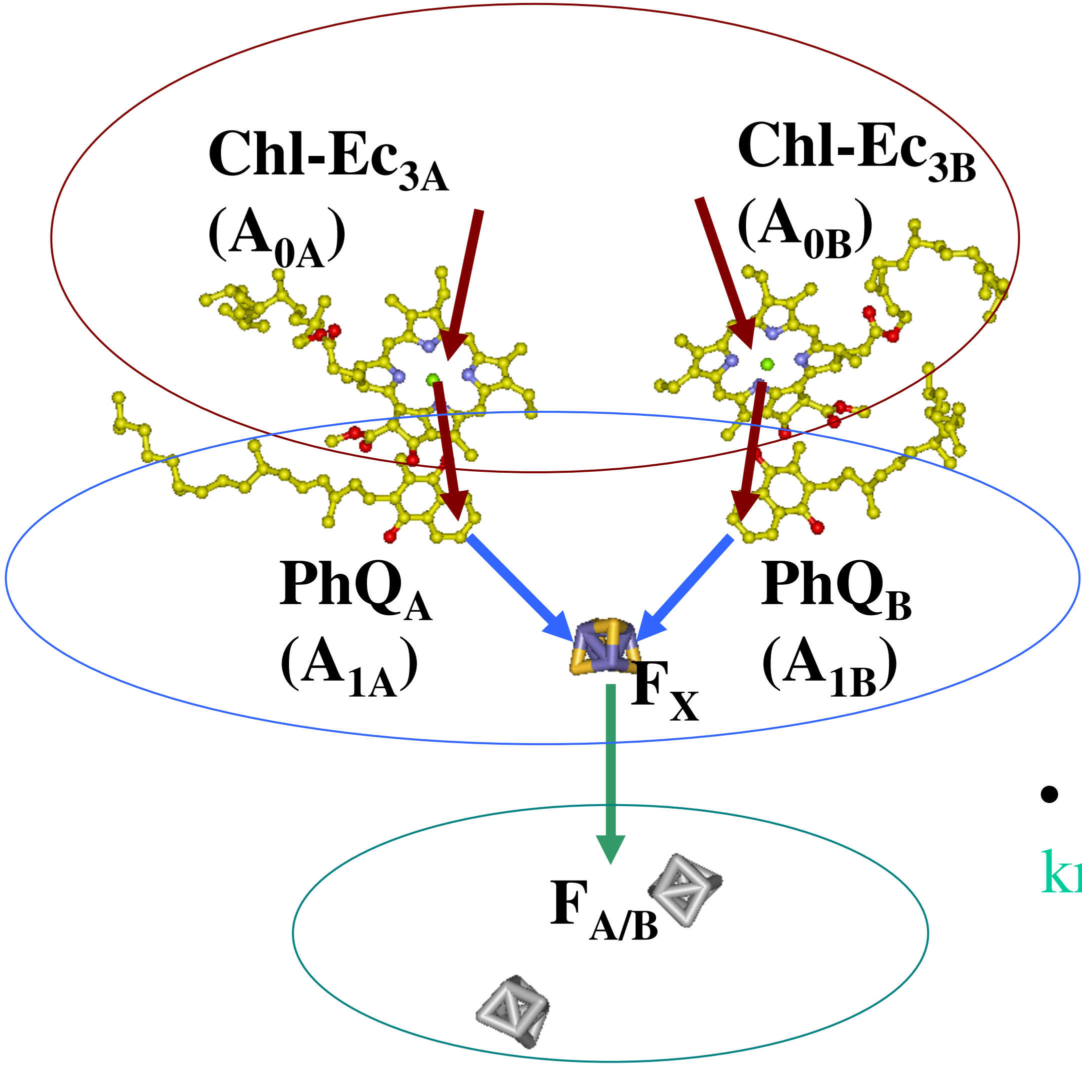
- Control of directionality: OK

- Control of ET rate, stability of semi-quinone: OK (sort of..)

Control of successive ET reactions

•Two single point-mutations which combines…
this is (very) asymmetric but very different from PS II

How to go from symmetric to asymmetric?



- Control of directionality: OK
- Control of ET rate, stability of semi-quinone: OK (sort of…)
- $F_{A/B}$ bound to PsaC, can be knocked out by deletion: OK

- Still.. very different from PS II, all ends up in F_X…

4: *Understanding* *control of ET kinetics*

Marcus Equation

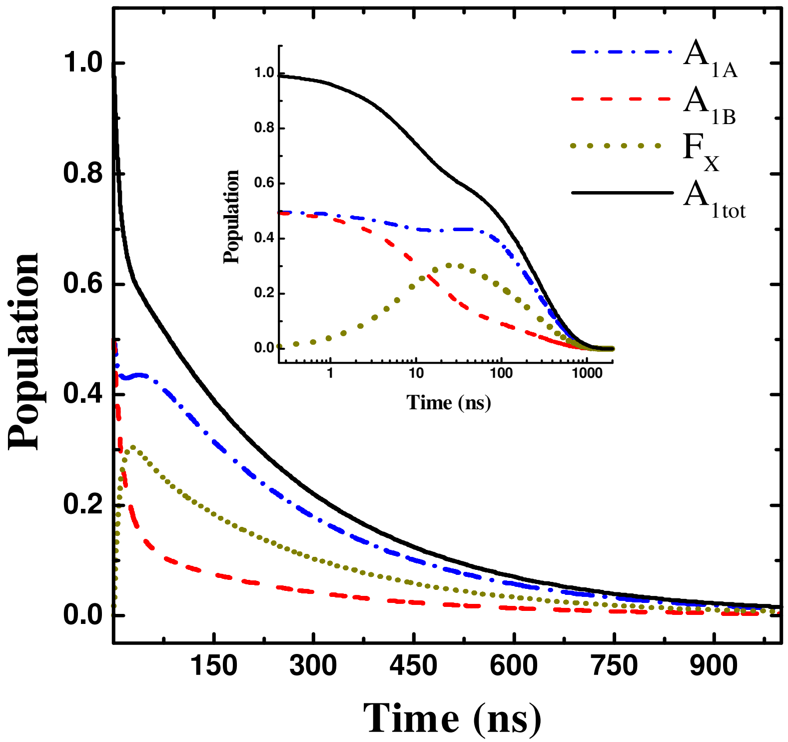
$$k_{et} = \frac{2\cdot\pi}{\hbar}\cdot\frac{|H_{DA}^{0}|^{2}\cdot e^{-\beta\cdot X_{DA}}}{\sqrt{2\pi\lambda_t k_b T}}\cdot e^{-\frac{(\Delta G+\lambda_t)^2}{4\lambda_t\cdot k_b T}}$$

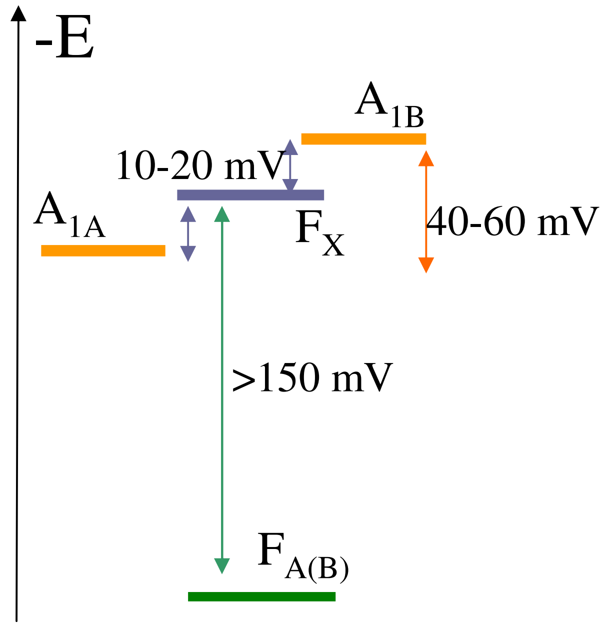
Edge-to-edge donor-acceptor distance

Driving force

Reorganisation energy

Simulated Kinetics



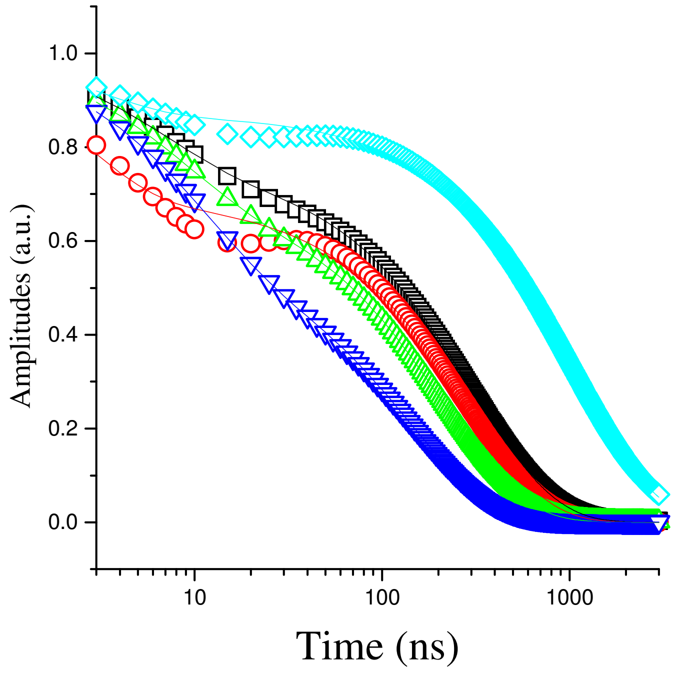


$\overline{\lambda} = 0.65 - 0.75$ eV

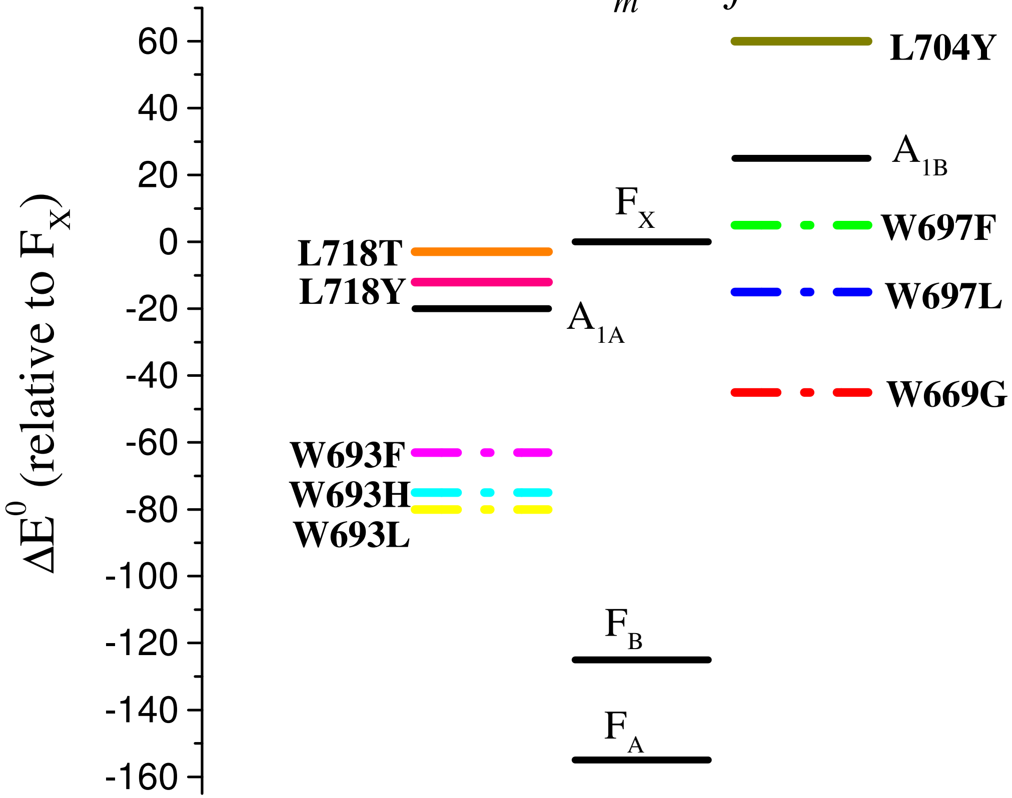
τ: 9.5ns 25ns 255ns

4: *Understanding control of ET kinetics*

Simulated Kinetics



Simulated E_m^0 shifts

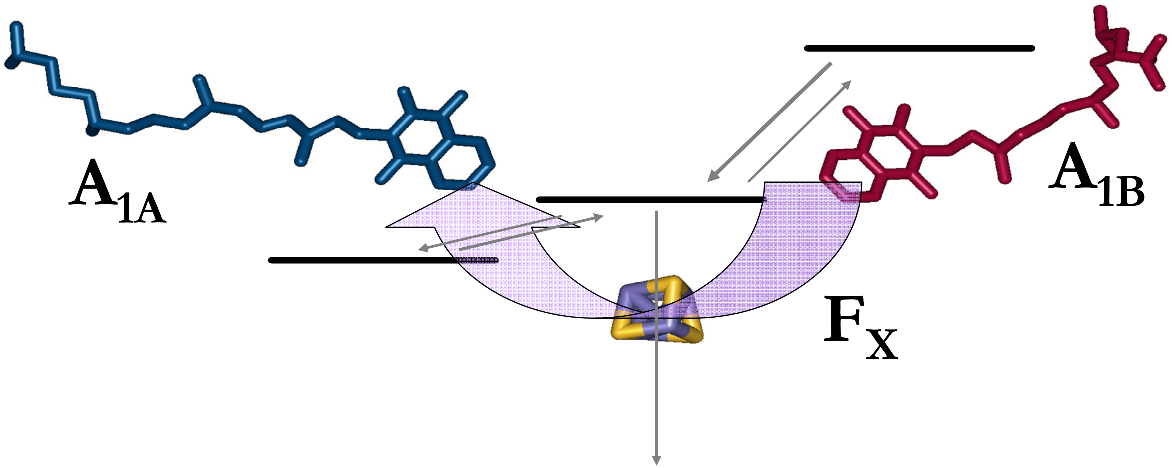


- Experimental decays are described satisfactorily (by change in ΔG^0).
- Some (small) discrepancies relating the amplitudes of the phases

Control of electron transfer kinetics

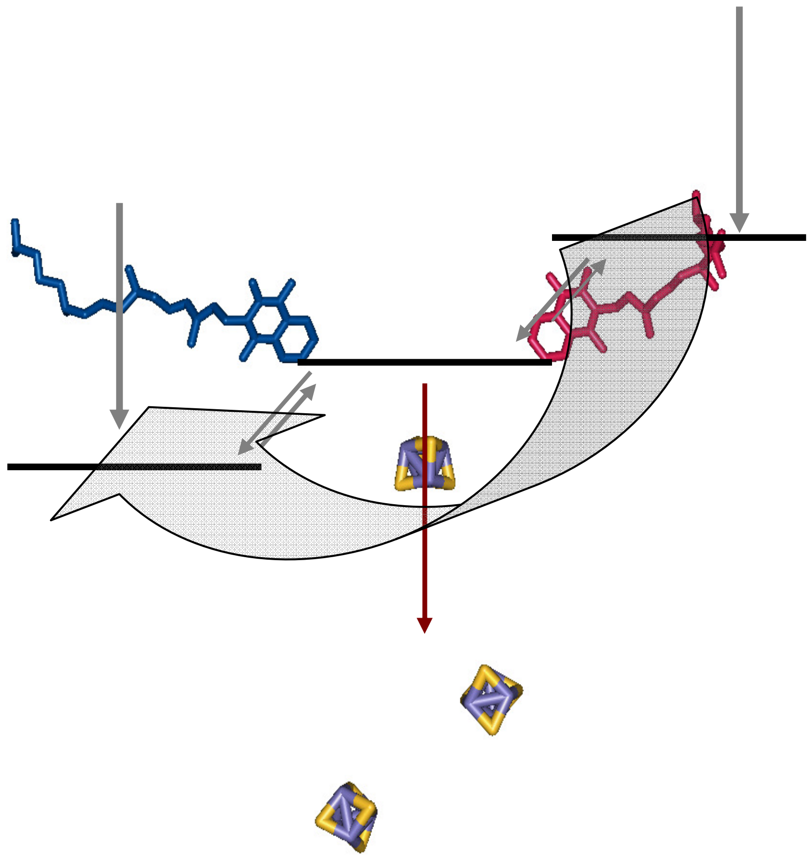
• The two quinones are **non-identical** under physical-chemical point of view.

• the energetic asymmetry is determined by protein cofactor interaction **(and can be modulated)**



• This asymmetry makes possible the occurrence of electron **inter-quinone electron transfer.**

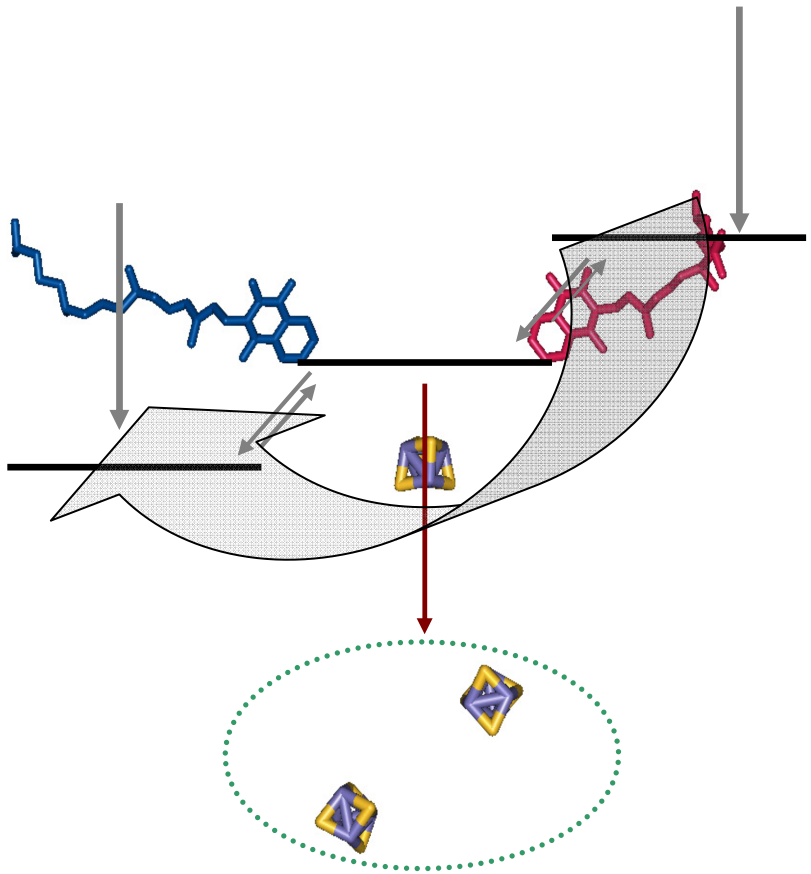
How to make PS I PS II-like??.....



• if the interpretation of the data is correct (i.e. there is inter-quinone ET….)

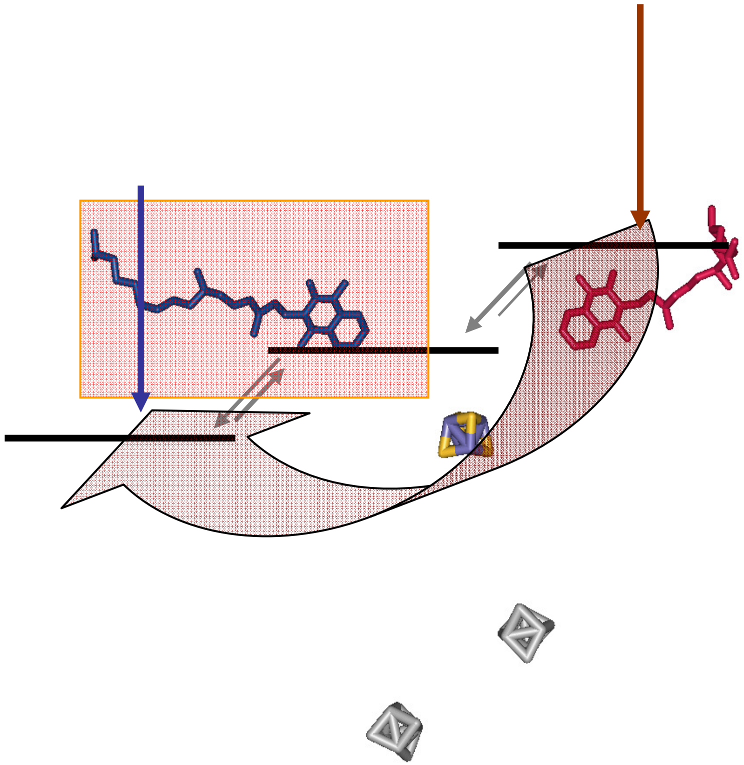
• **this is *similar* to what occurs in PS II….**

How to make PS I PS II-like??.....



- if the interpretation of the data is correct, there is inter-quinone, which is *similar* to PS II.
- **by removing PsaC (which binds $F_{A/B}$)**

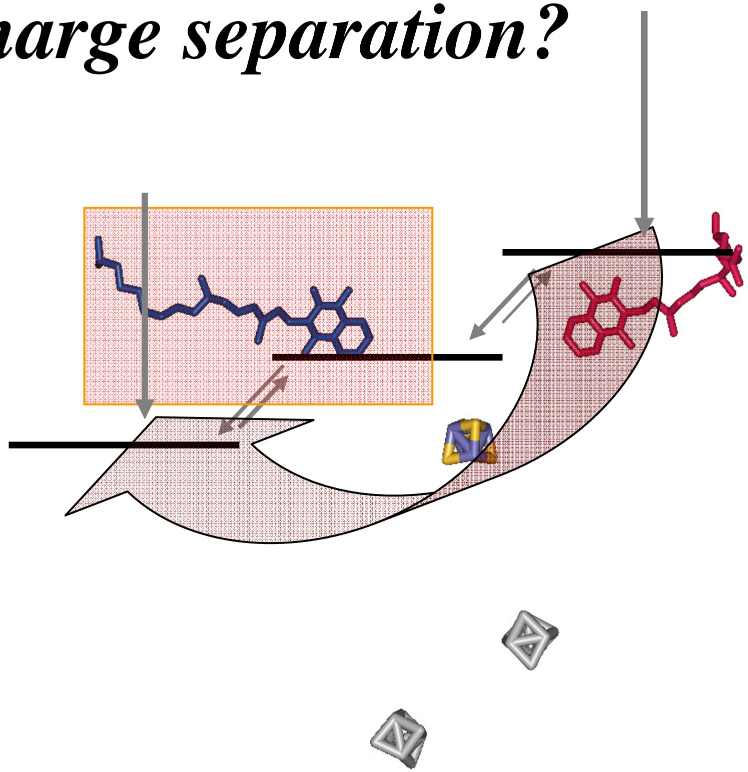
How to make PS I PS II-like??.....



• if the interpretation of the data is correct, there is inter-quinone ET, which is *similar* to PS II.

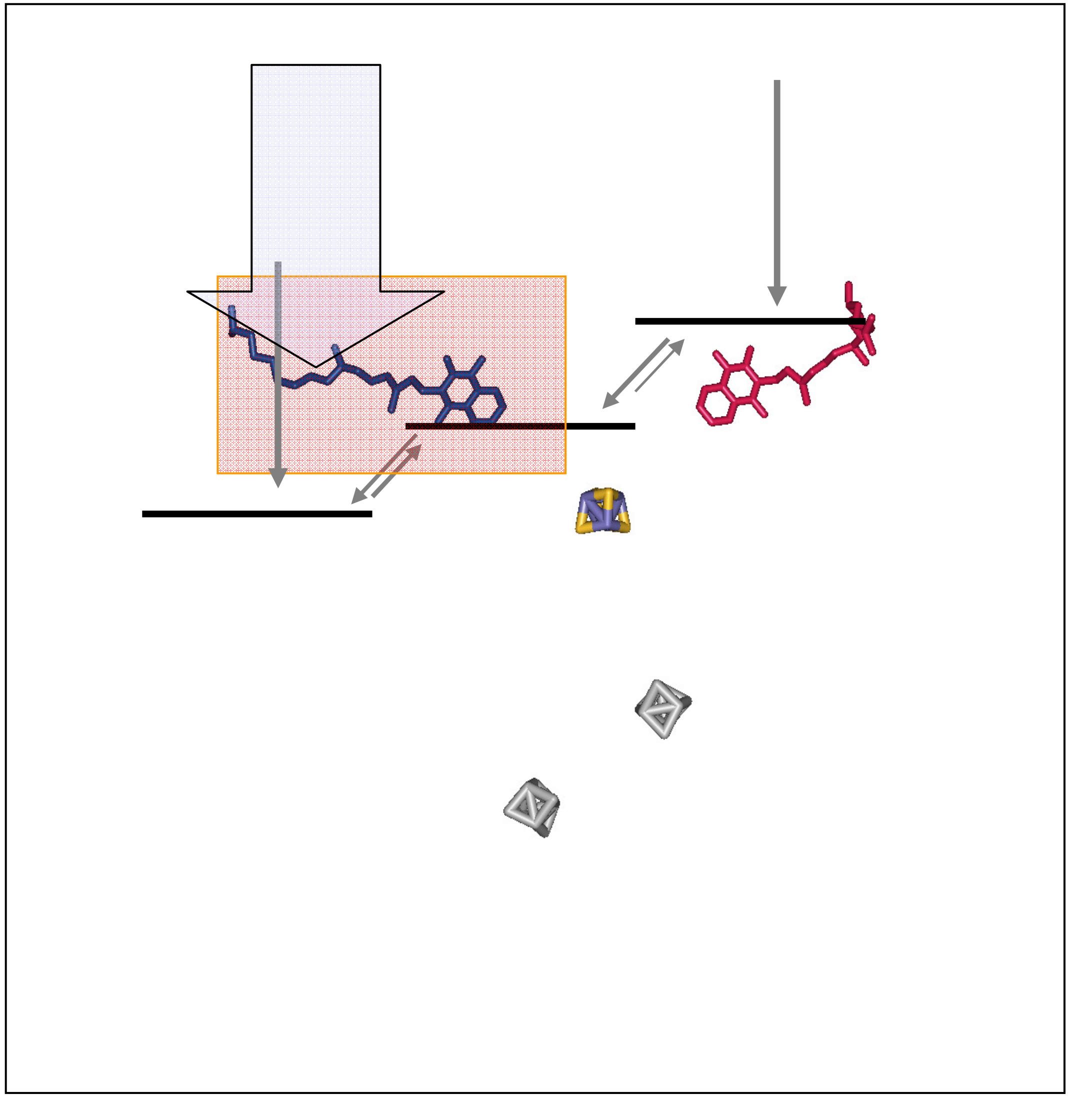
• by removing PsaC (which binds $F_{A/B}$) **the electrons will all end up in A_{1A}** (directly or via the $A_{1B} \rightarrow F_X$ pathway)

• ***What will happen after a second charge separation?***



• *Can the semi-quinone be sufficiently stable (long-lived) to allow for 1- second charge separation 2-reduction to quinole?*
• *Will it be reduced directly from A_{0A} or via A_{1B}/F_X?*
• *Can charge recombination between A_{1A}^- and P_{700}^+ be avoided?*

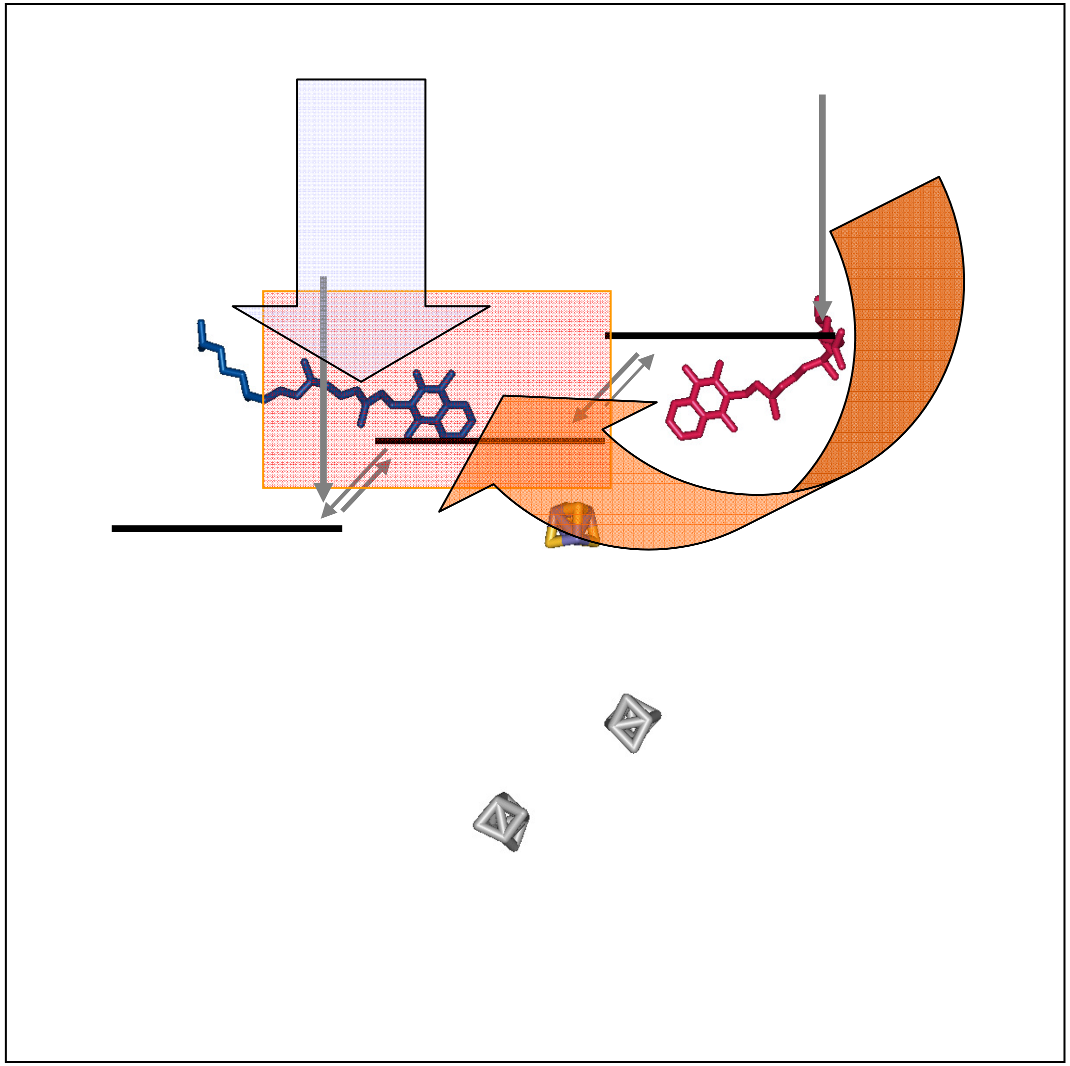
How to make PS I PS II-like??.....



- ***What will happen after a second charge separation?***

- *That either THIS way.*

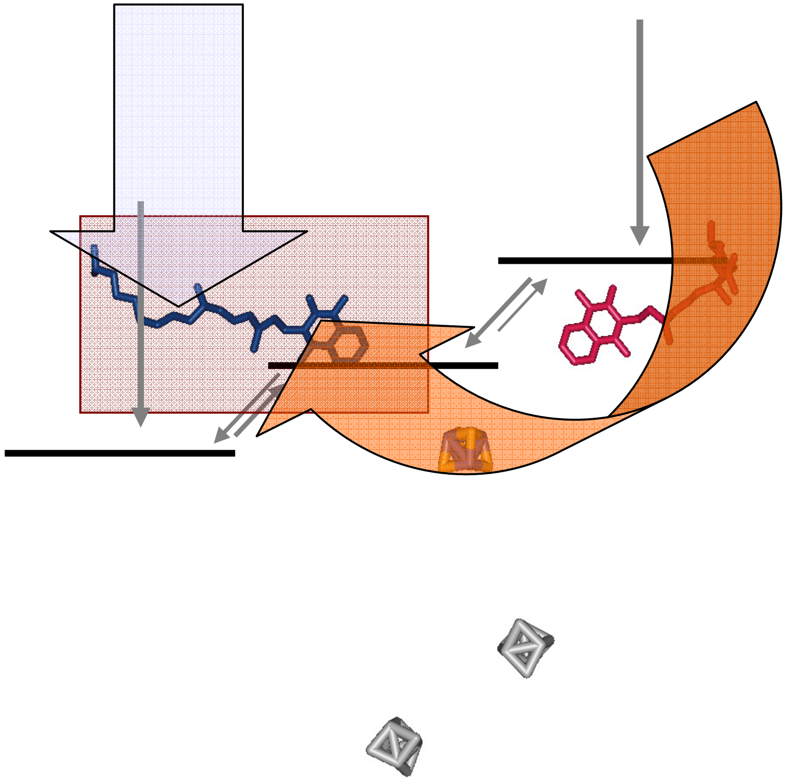
How to make PS I PS II-like??.....



- ***What will happen after a second charge separation?***

- *That either THIS way.*
- *Or THIS way*

How to make PS I PS II-like??.....



- ***What will happen after a second charge separation?***

- *That either THIS way.*
- *Or THIS way*
- *A_1^- would act a terminal acceptor (almost like Q_B^- in PS II) and eventually be fully reduced (A_1^{--}).*

Conclusions

Control of directionality

• the probability of utilisation of two ET branches is controlled by properties of cofactors involved in primary charge separation (A_0)

Control of semi-quinone stability

• the lifetime of semiquinone is controlled by interaction of A_1 with the binding site, and it can be stabilised to live ~1.5 μs (or longer).

Asymmetry in the redox properties determines an inter-quinone ET

This has significant implication to understand the evolution of RC functionality

Acknowledgments

Fabrice Rappaport , **IBPC, Paris**
Kevin Redding, **Arizona State University**

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Istituto di Biofisica, CNR
Sez. Fisiologia Vegetale Fotosintesi, University of Milan

Acknowledgments

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Saul Purton & Mike Evans, **University College, London**

Analysis and Theory of Pulsed EPR experiments:

Ilya Kuprov & Peter Hore, **University of Oxford**

TR-EPR:

Donatella Carbonera, **University of Padoa**

Gerd Kothe, **University of Freiburg**

Oleg Poulektov, **Argonne Nat. Lab.**