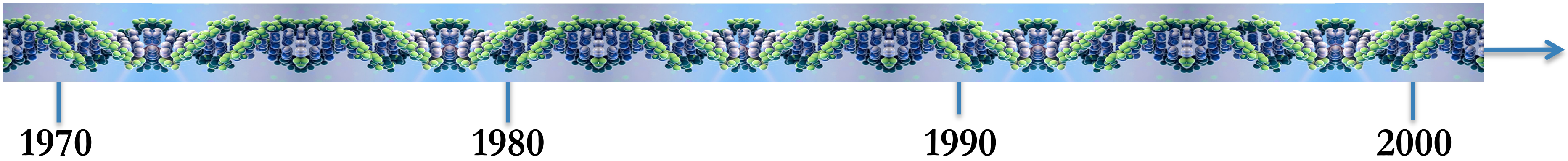
What X-ray footprinting can tell you about in protein interactions and conformation

Corie Ralston, Sayan Gupta
Lawrence Berkeley National Laboratory

Berkeley Lab

A BRIEF HISTORY OF OH· FOOTPRINTING



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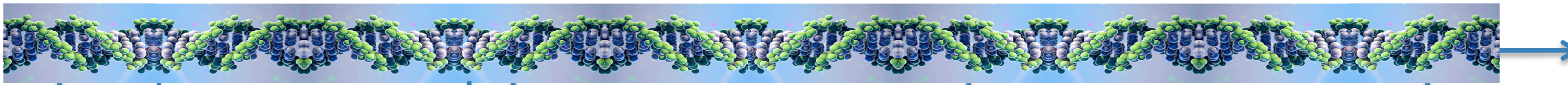

1970 | 1980 | 1990 | 2000

Chemical modification methods developed

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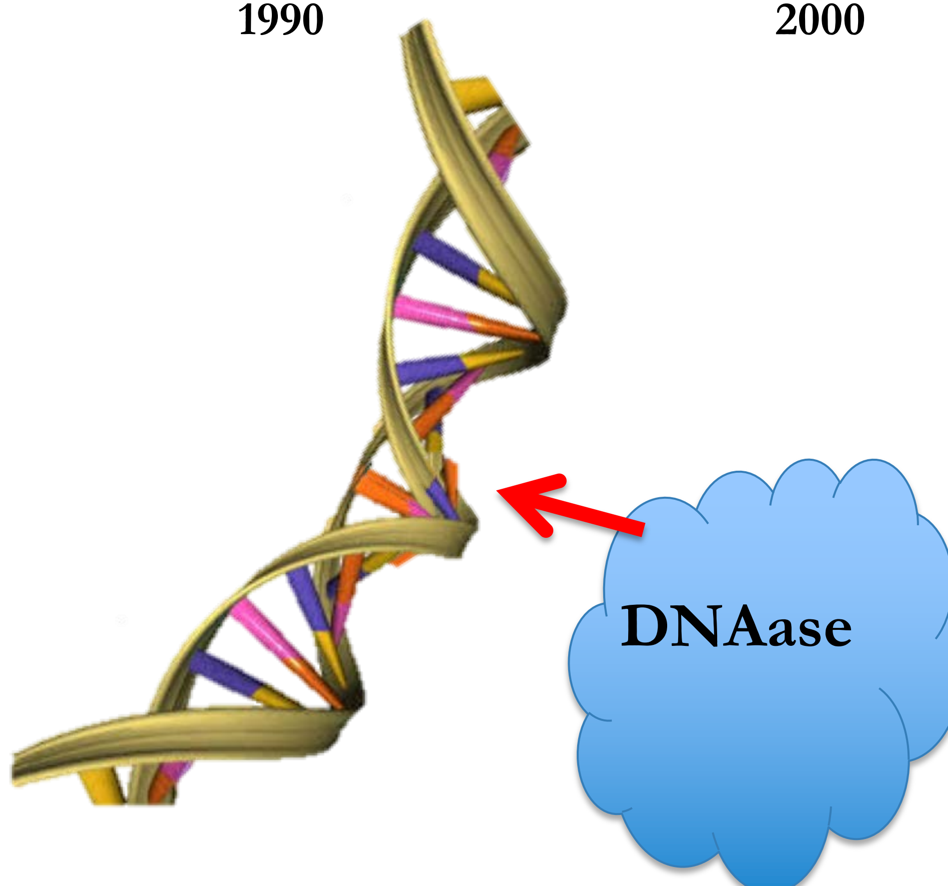

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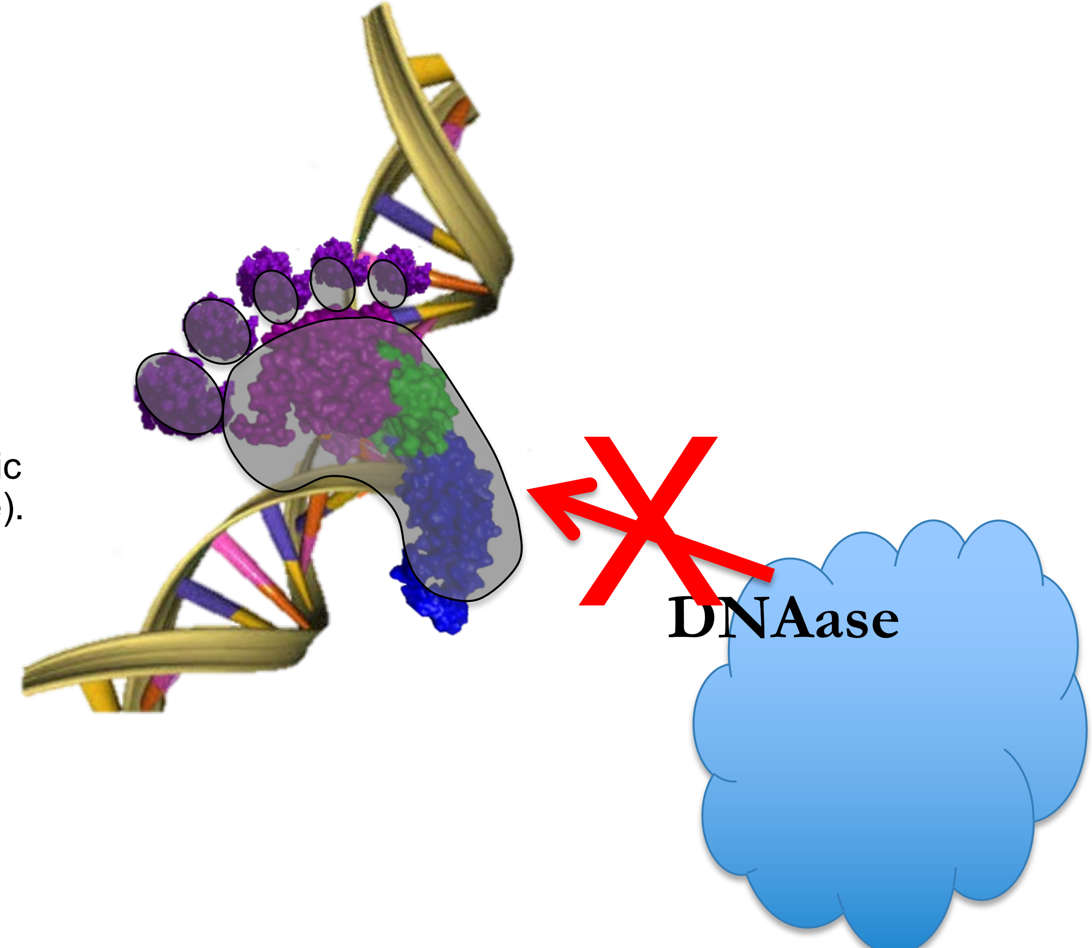

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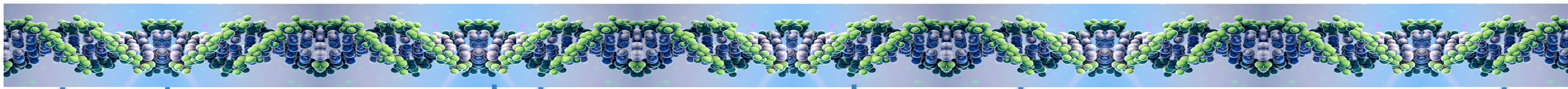

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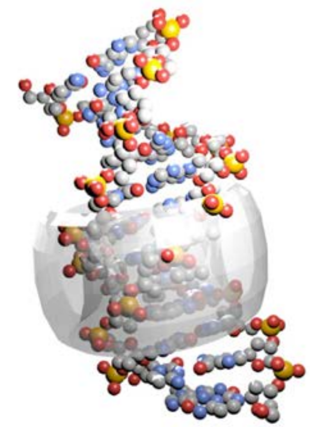

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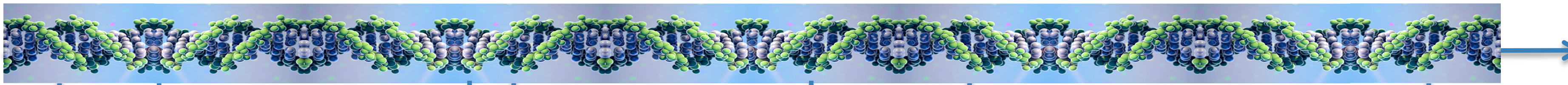

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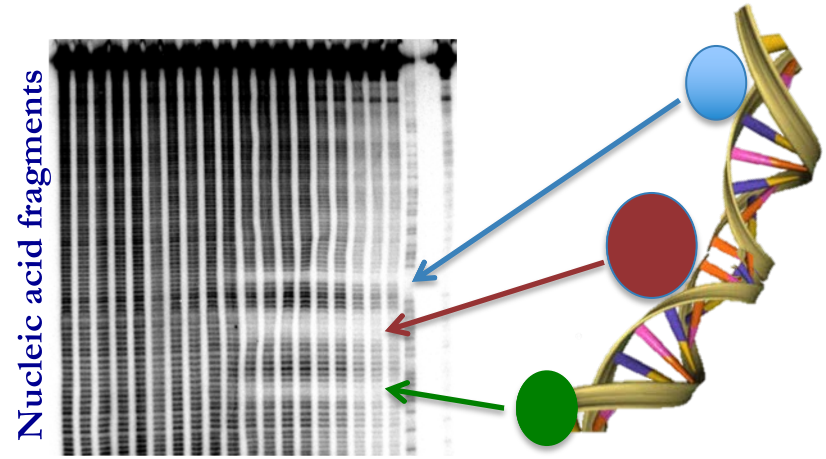

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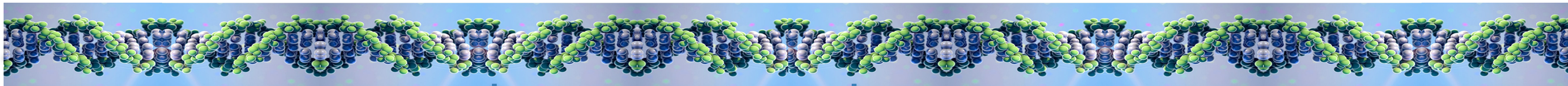

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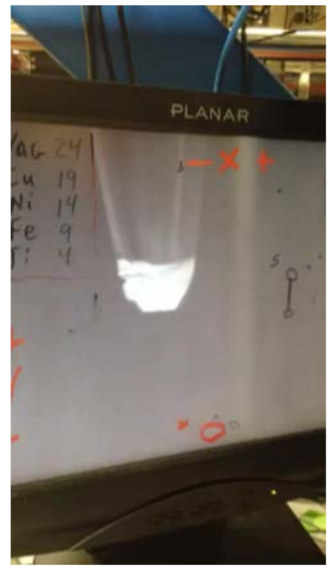

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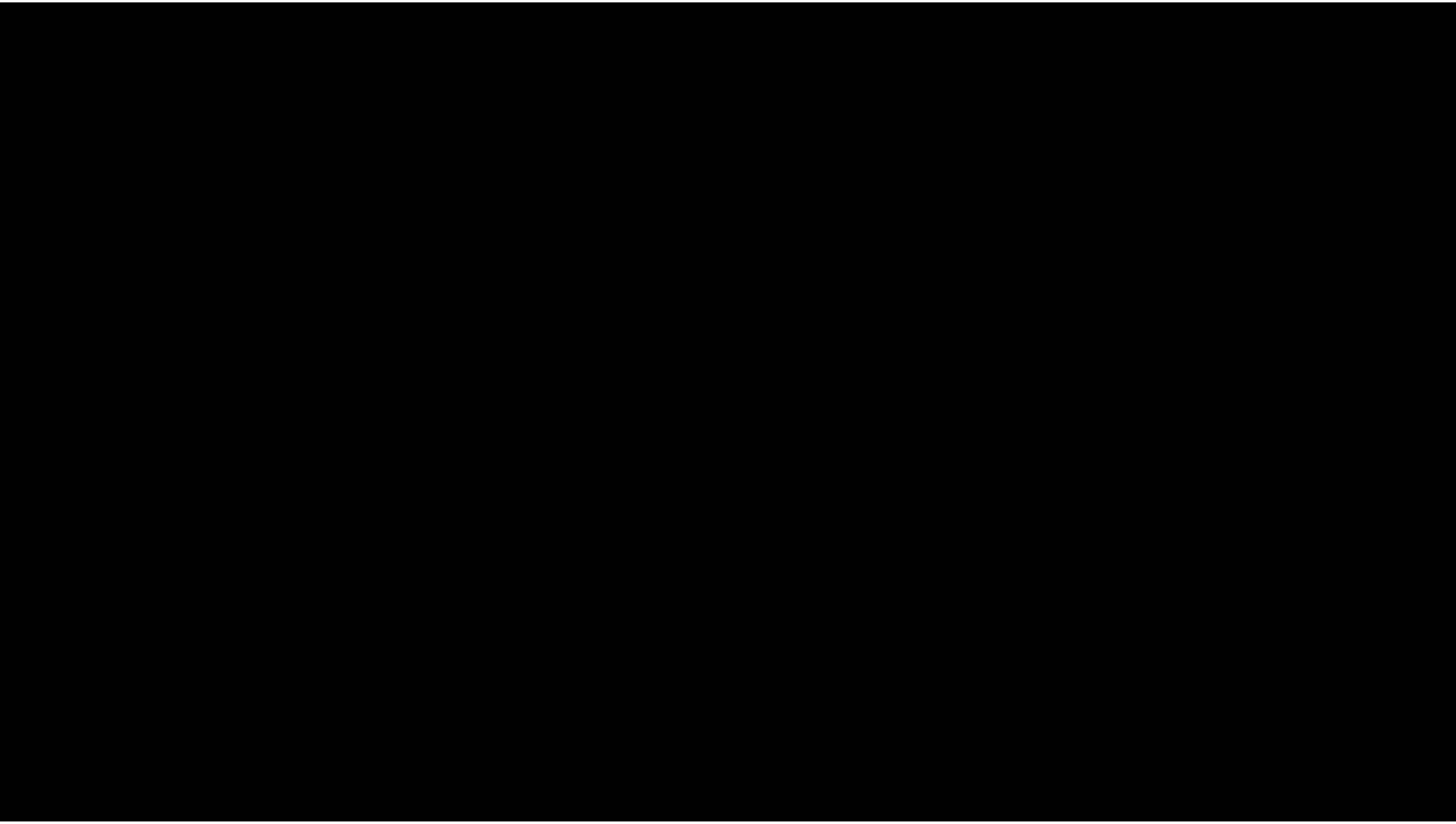
PROTEIN $OH^{\cdot}$ FOOTPRINTING



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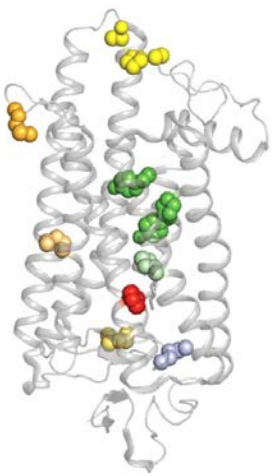

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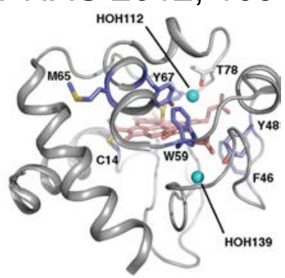

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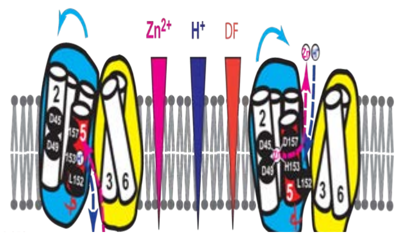

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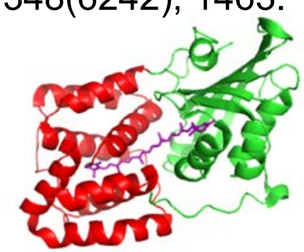

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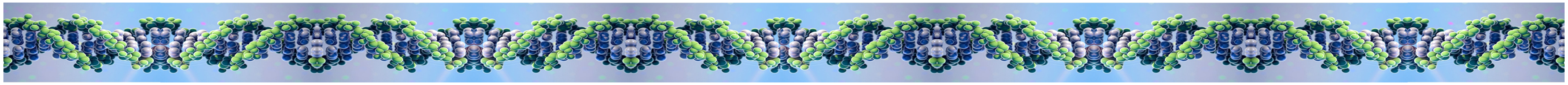

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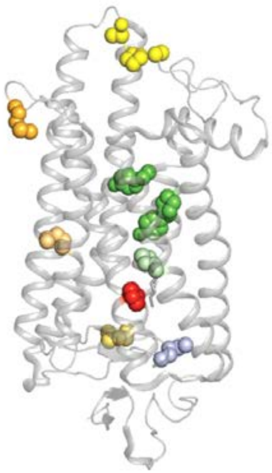

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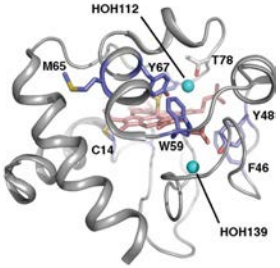

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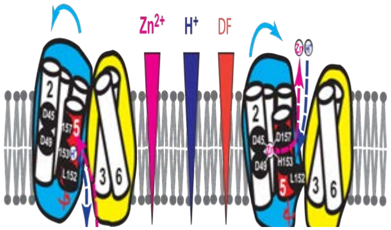

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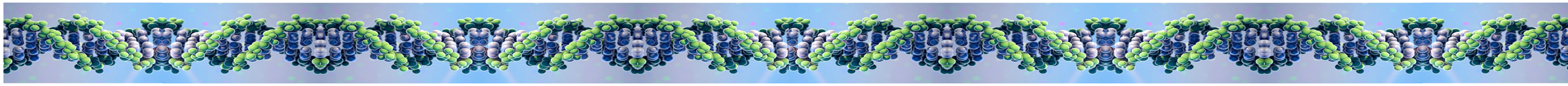

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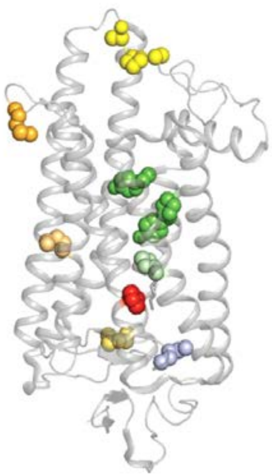

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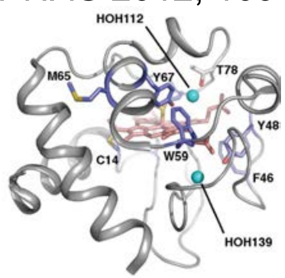

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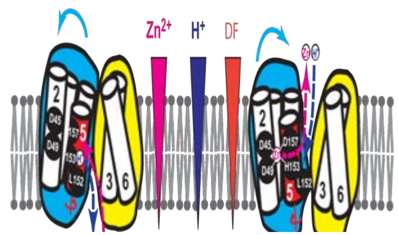

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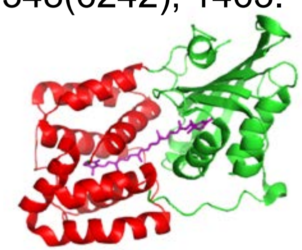

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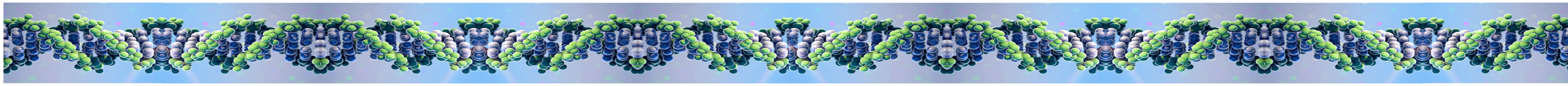

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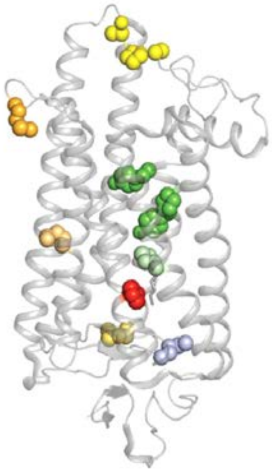

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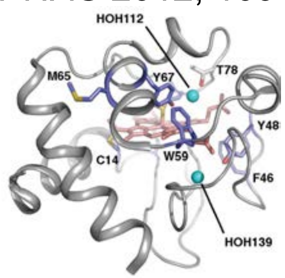

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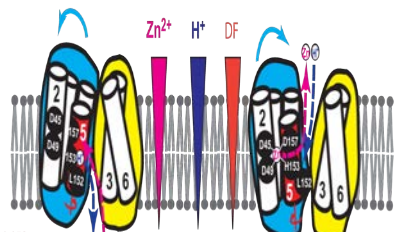

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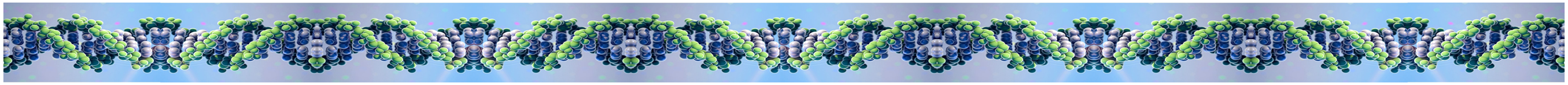

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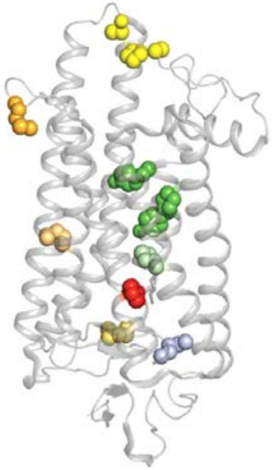

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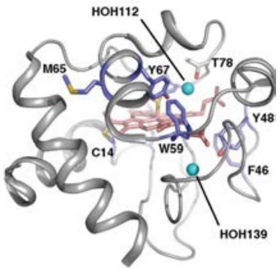

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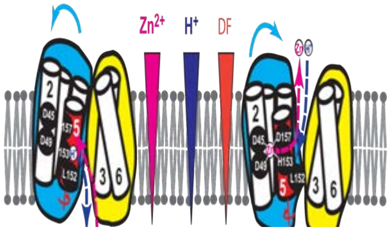

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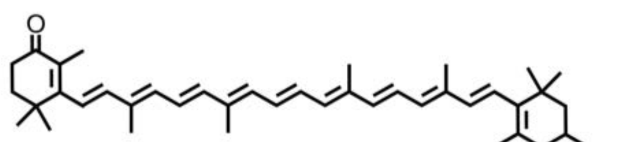
UNTANGLING PHOTOPROTECTION IN CYANOBACTERIA

Photosynthetic organisms turn light into chemical energy through a complex interplay of proteins

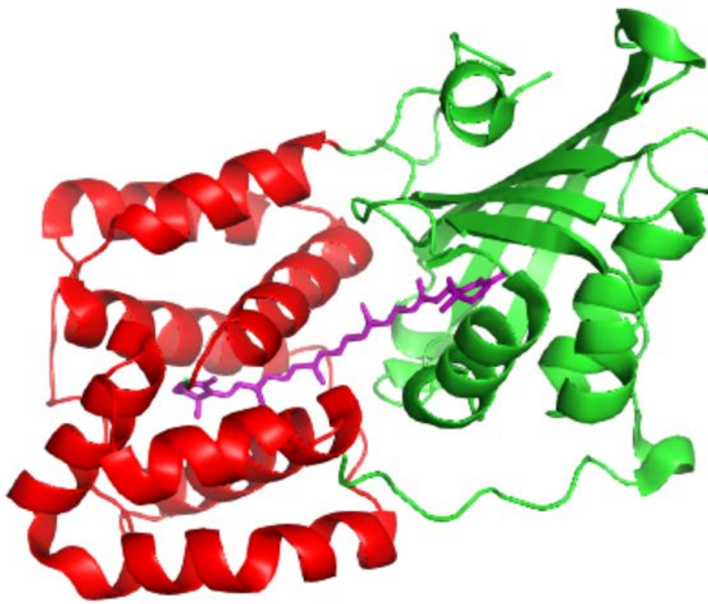
Kerfeld Lab - MSU

A KEY PLAYER: THE ORANGE CAROTENOID PROTEIN

- Small protein consisting of two domains
- Contains a light-sensitive pigment carotenoid
- Inactive “orange” form has been crystallized
- Active “red” form has not been crystallized



3’-hydroxyechinenone (hECN)



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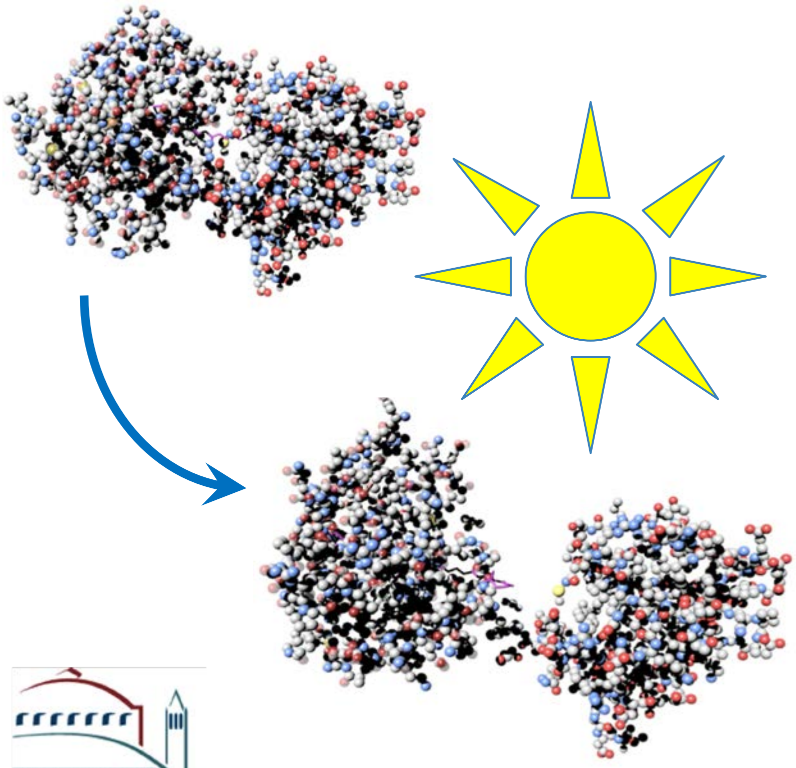
- Light converts OCP from the inactive to active state



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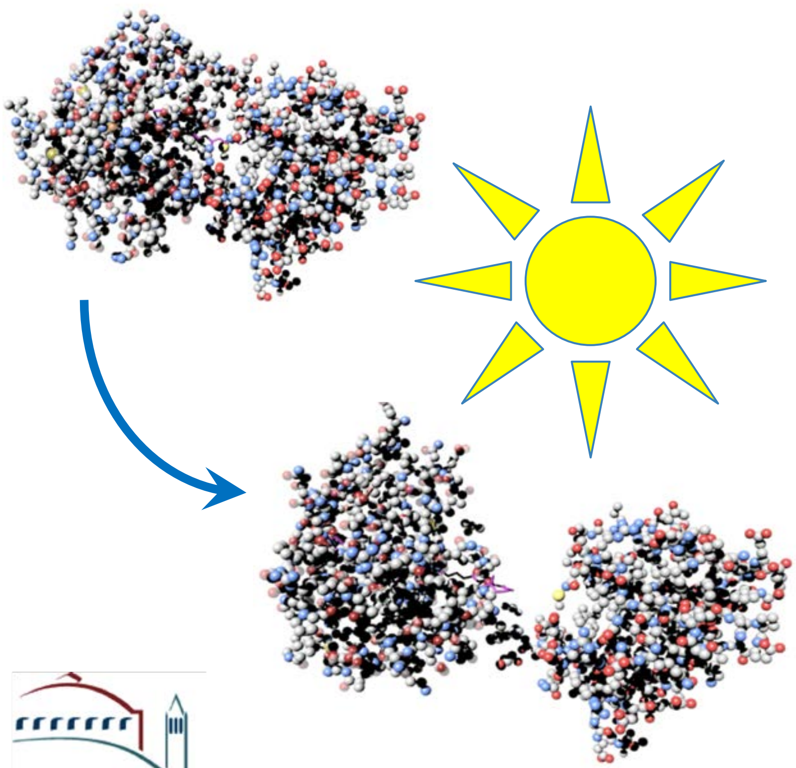
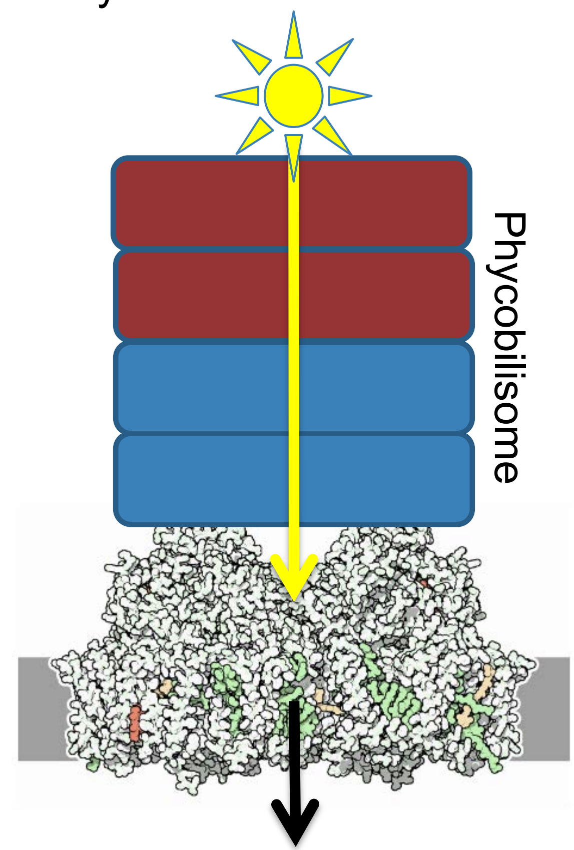


The Phycobilisomes are the light-harvesting “antennae” for cyanobacteria



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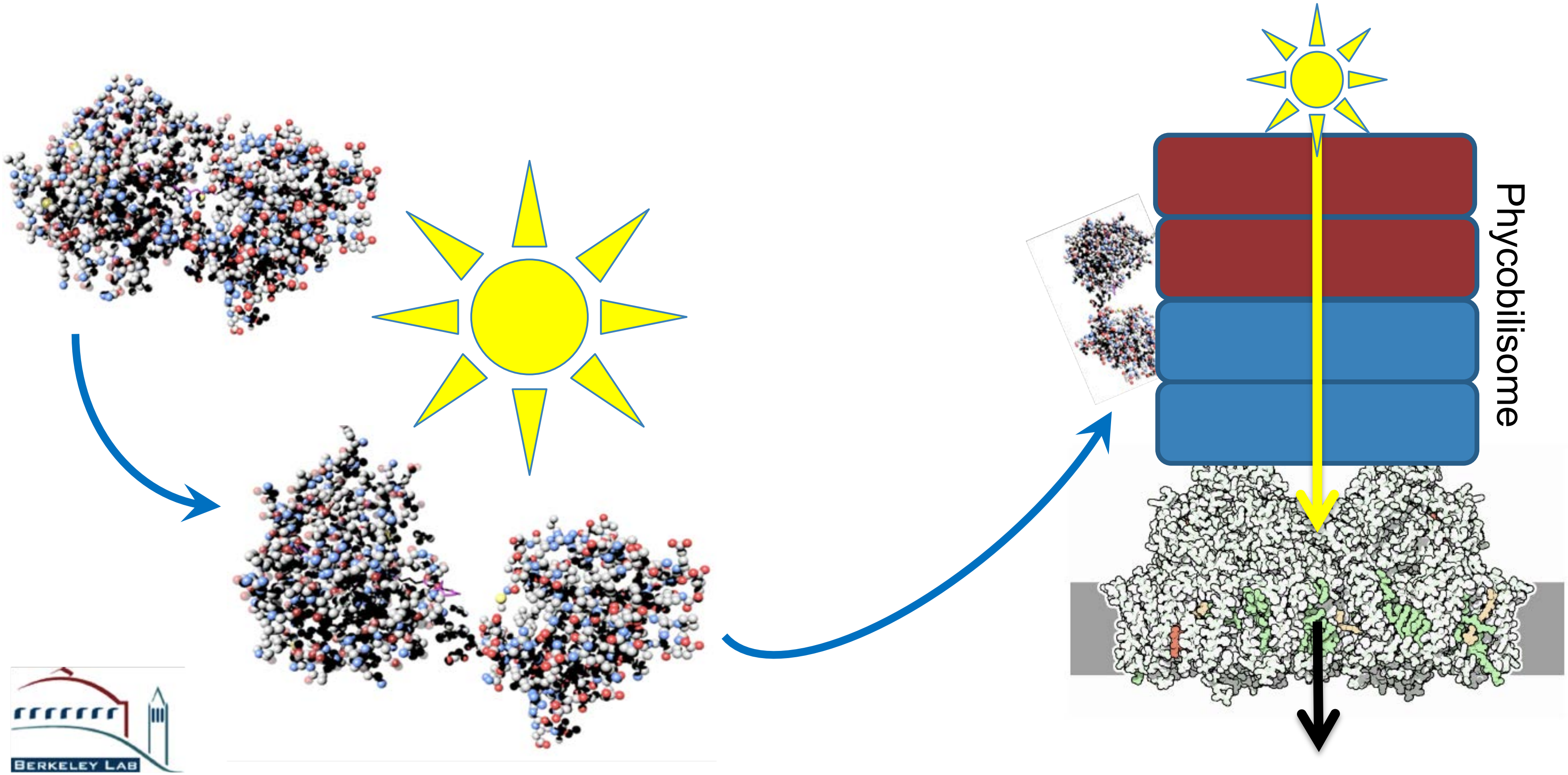
- OCP then binds to the phycobilisome



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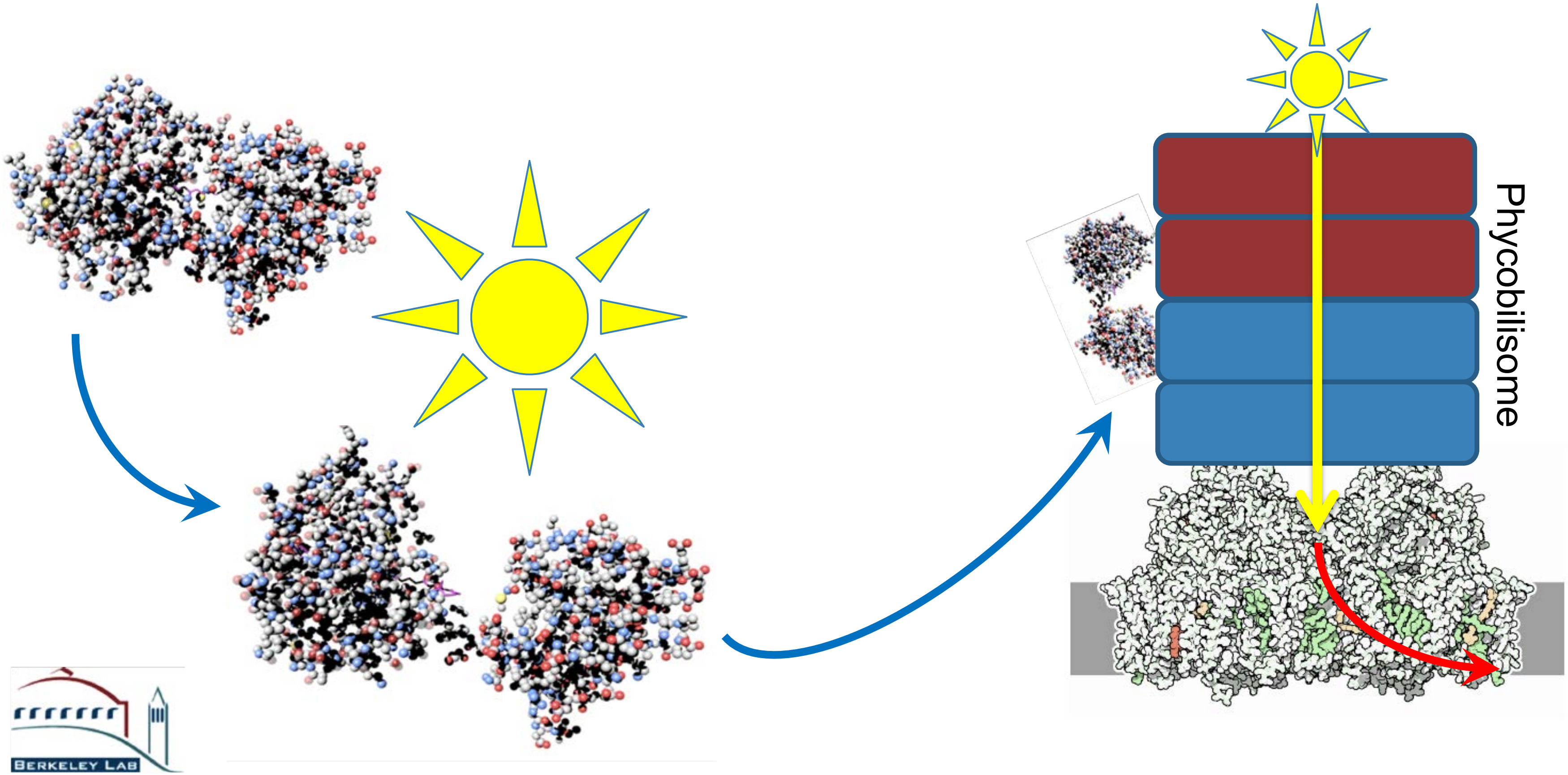

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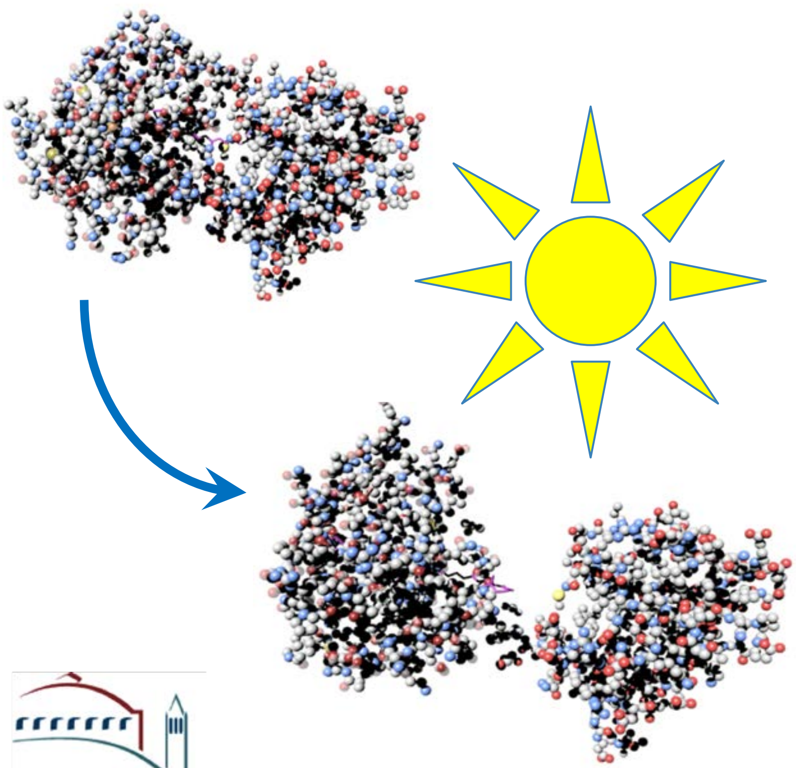

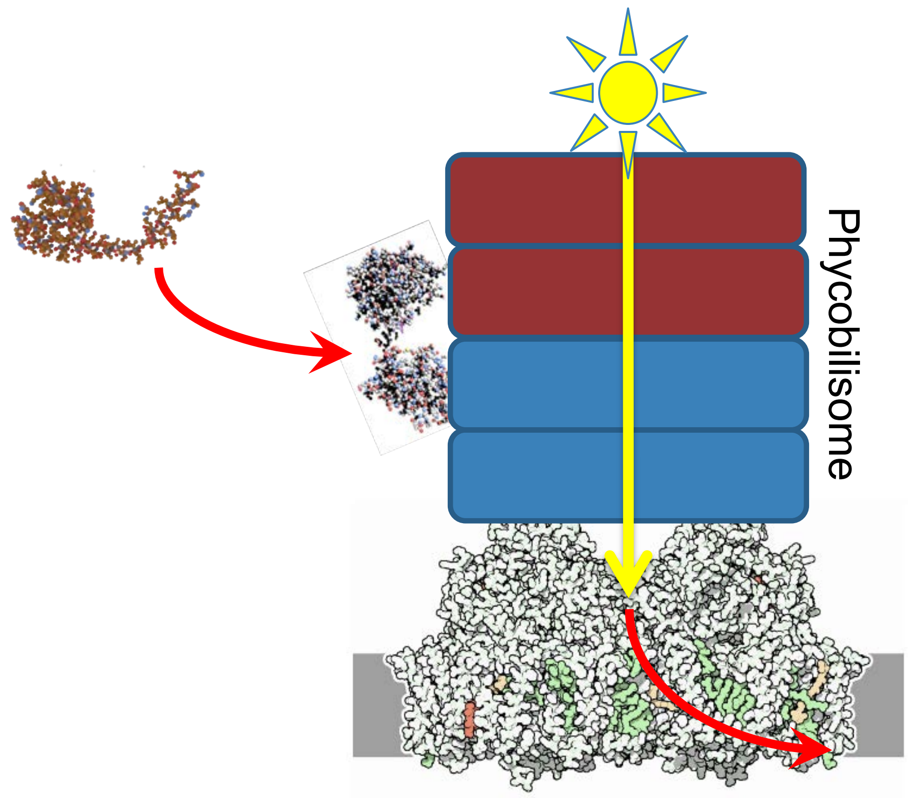

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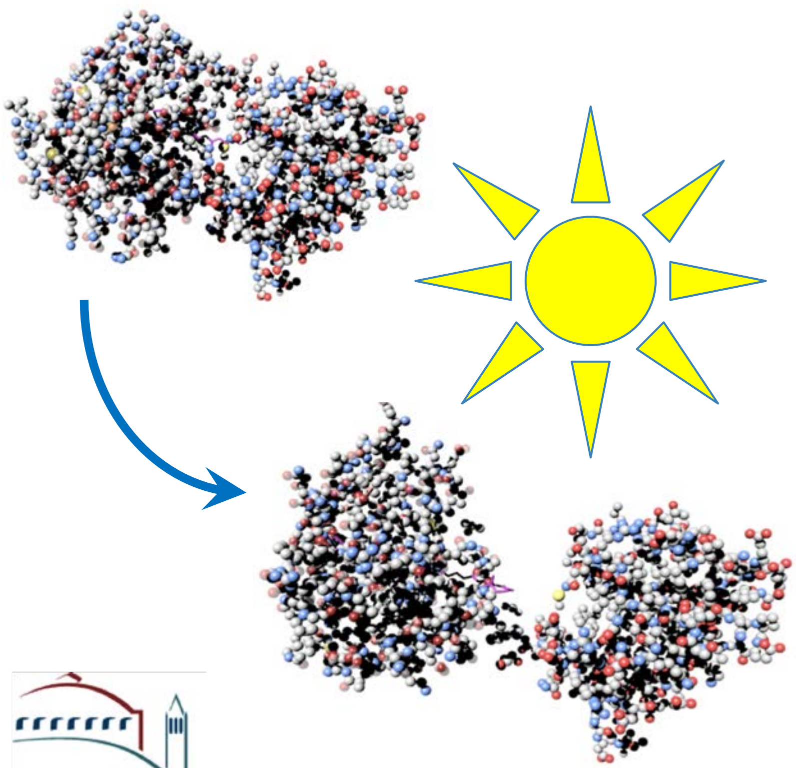

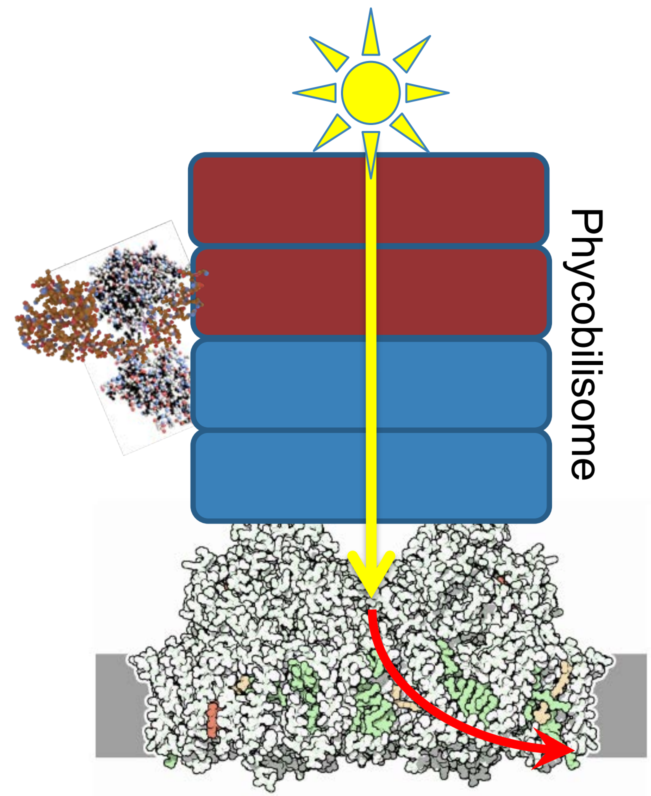

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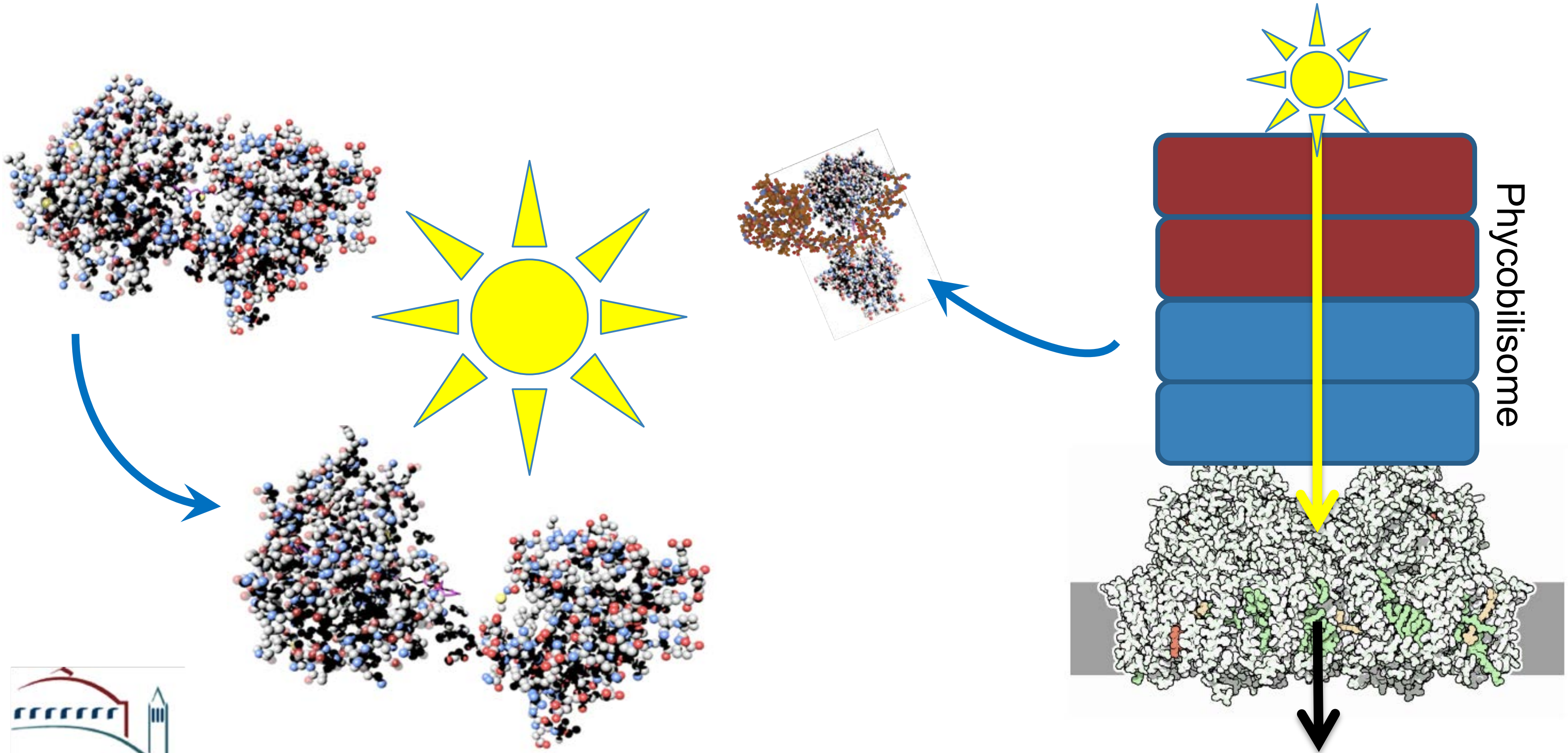
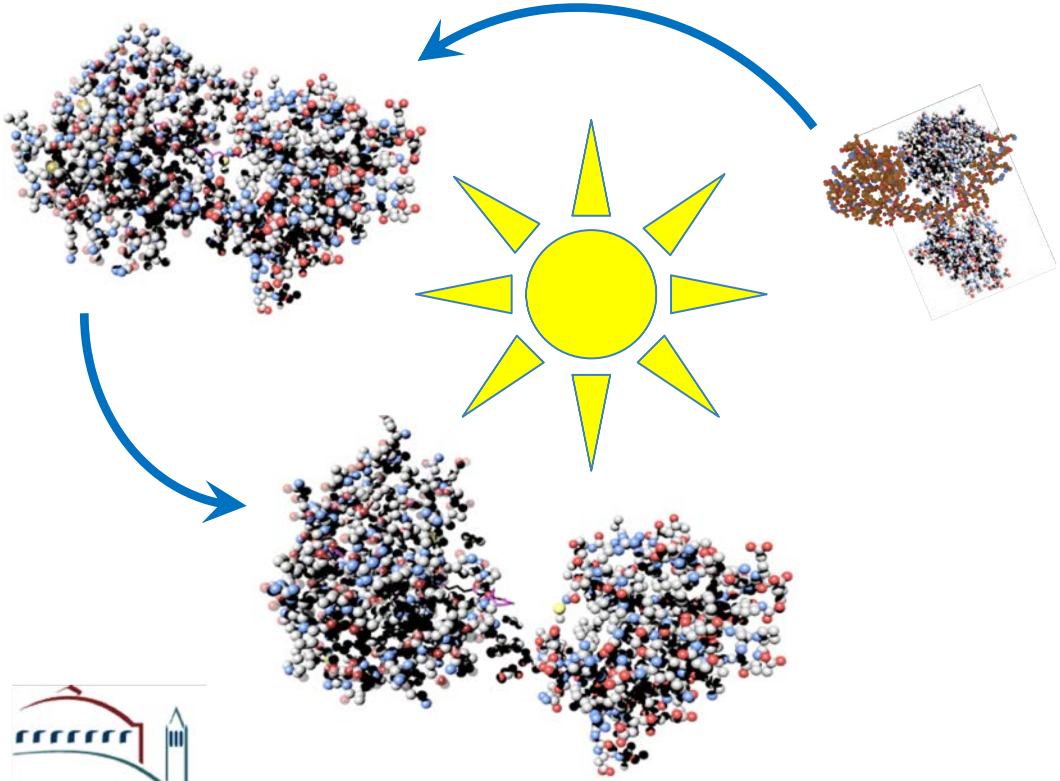
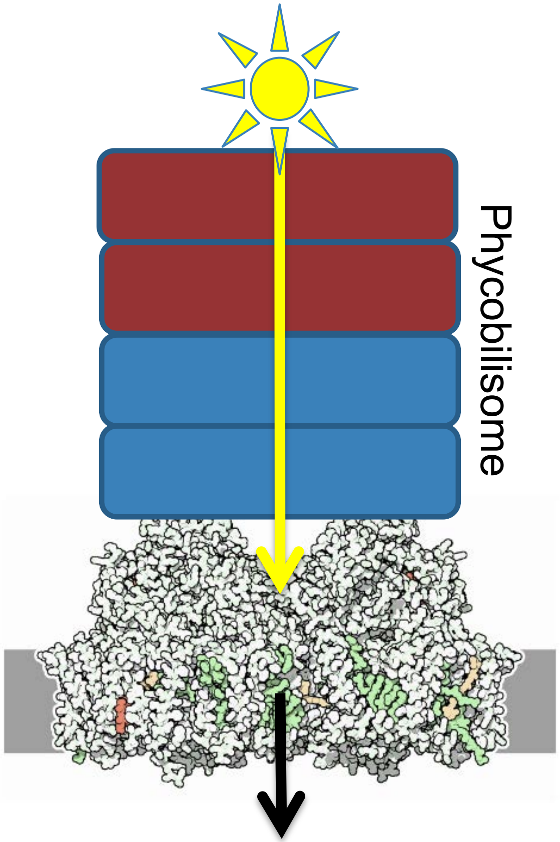


HOW DOES OCP PREVENT LIGHT-RELATED DAMAGE?

- Light converts OCP from the inactive to active state
- OCP then binds to the phycobilisome
- Light energy is dissipated as heat
- FRP protein binds to OCP and helps it dissociate from the phycobilisome
- FRP also facilitates OCP conversion back to inactive state





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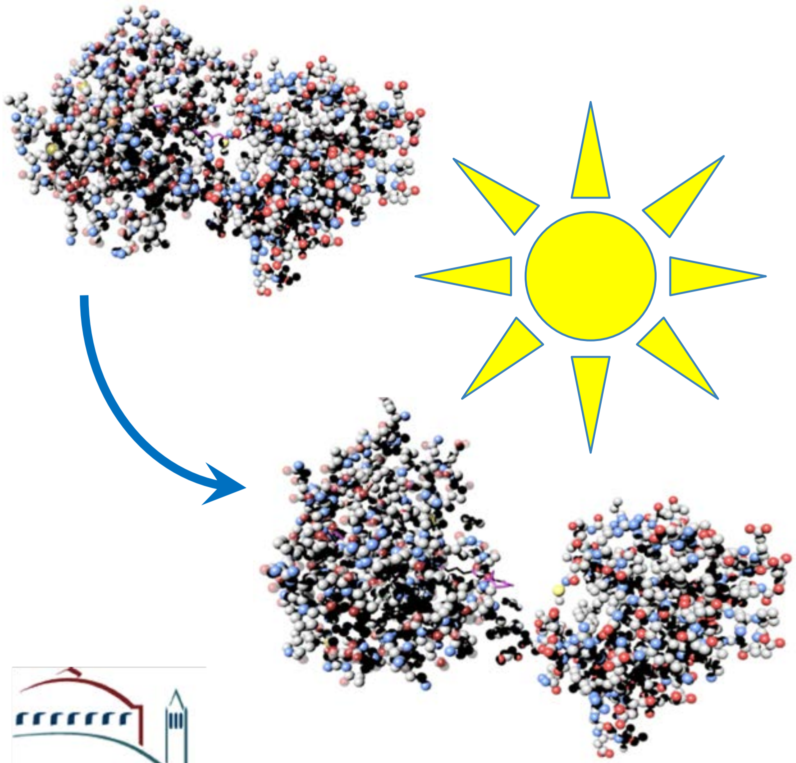
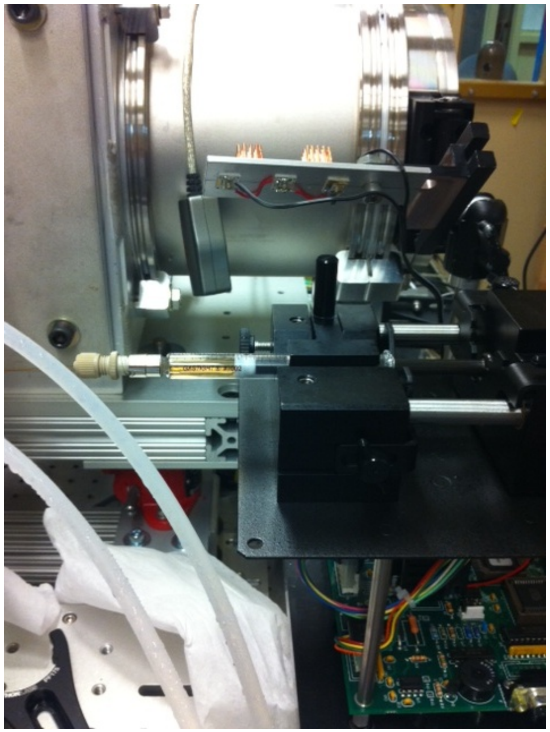
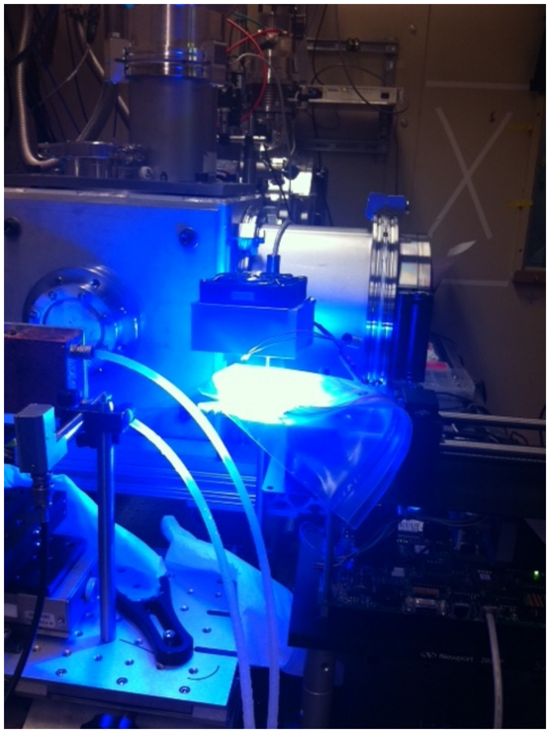
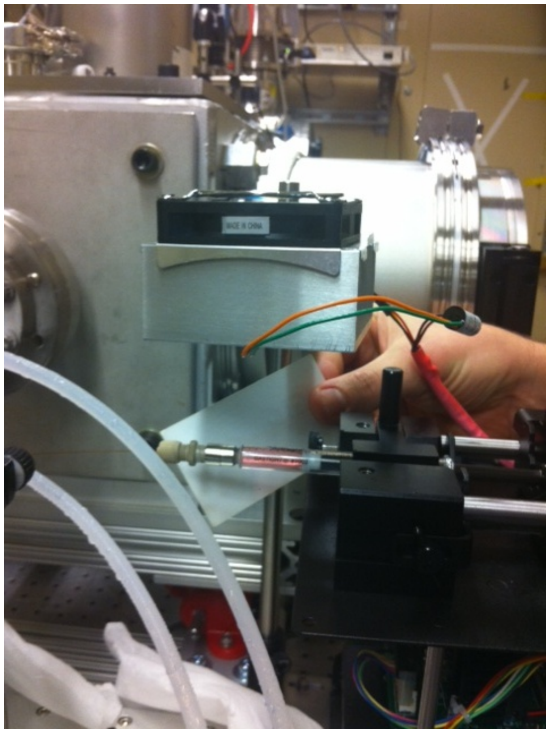


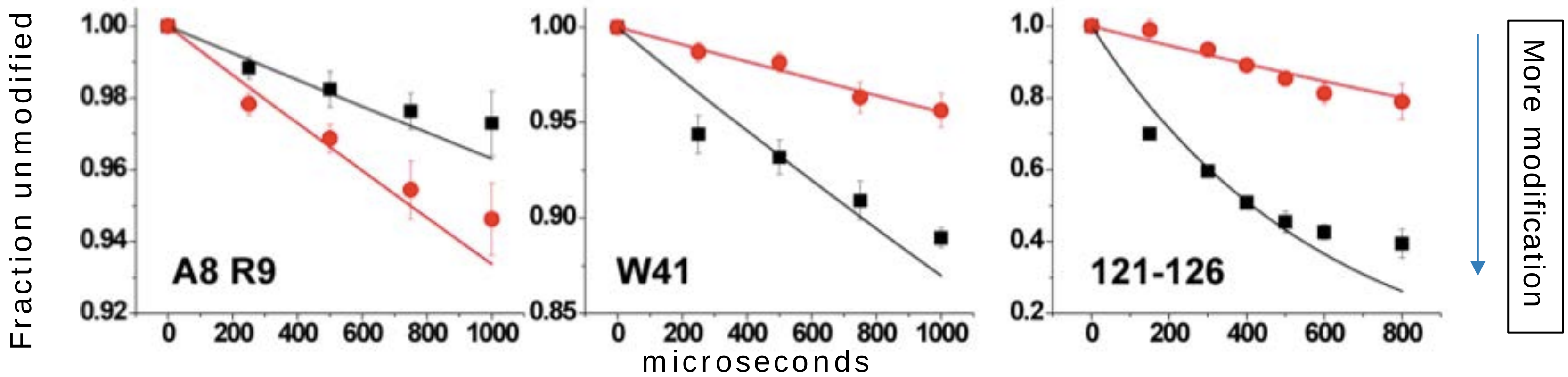
OCP ORANGE TO RED CONVERSION VIA LIGHT

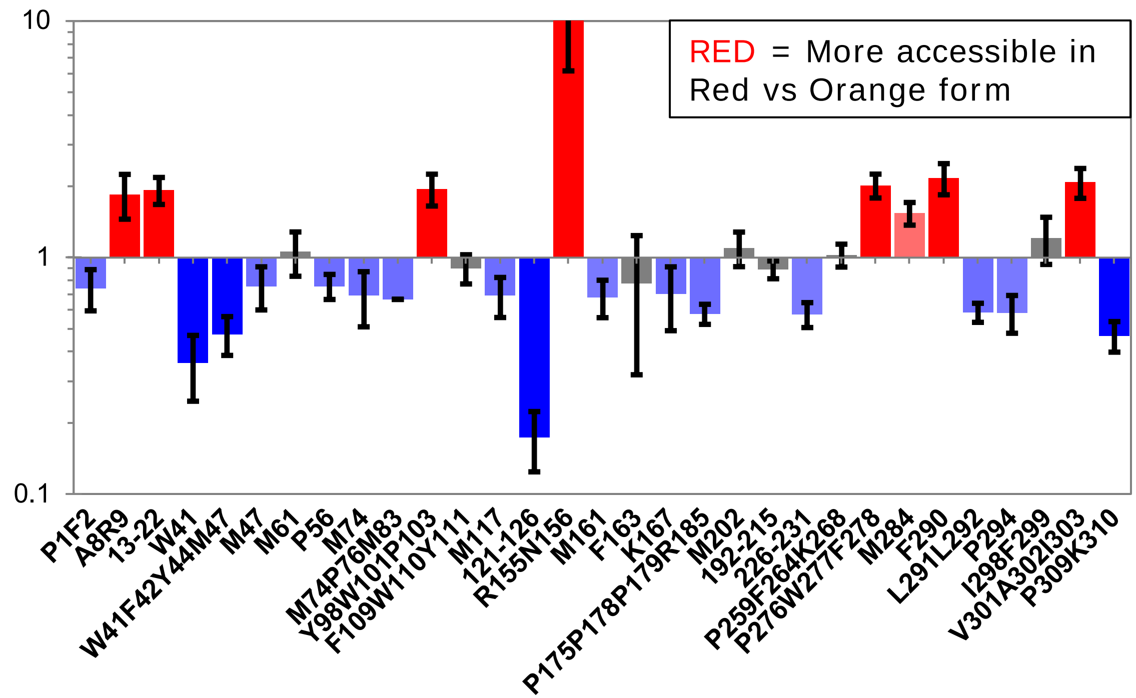




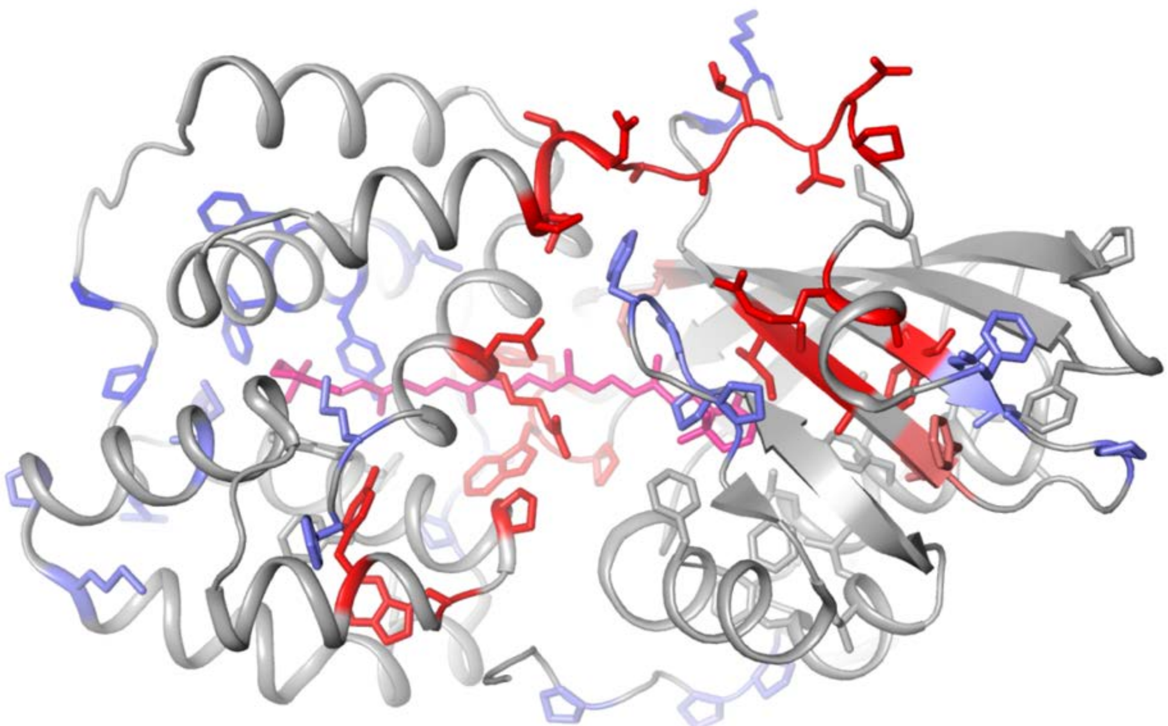


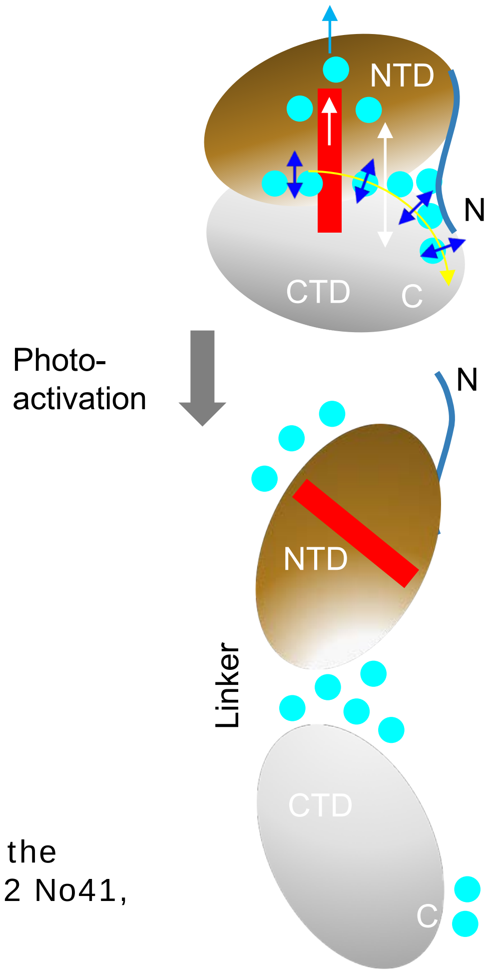
PROTECTIONS OBSERVED USING X-RAY FOOTPRINTING





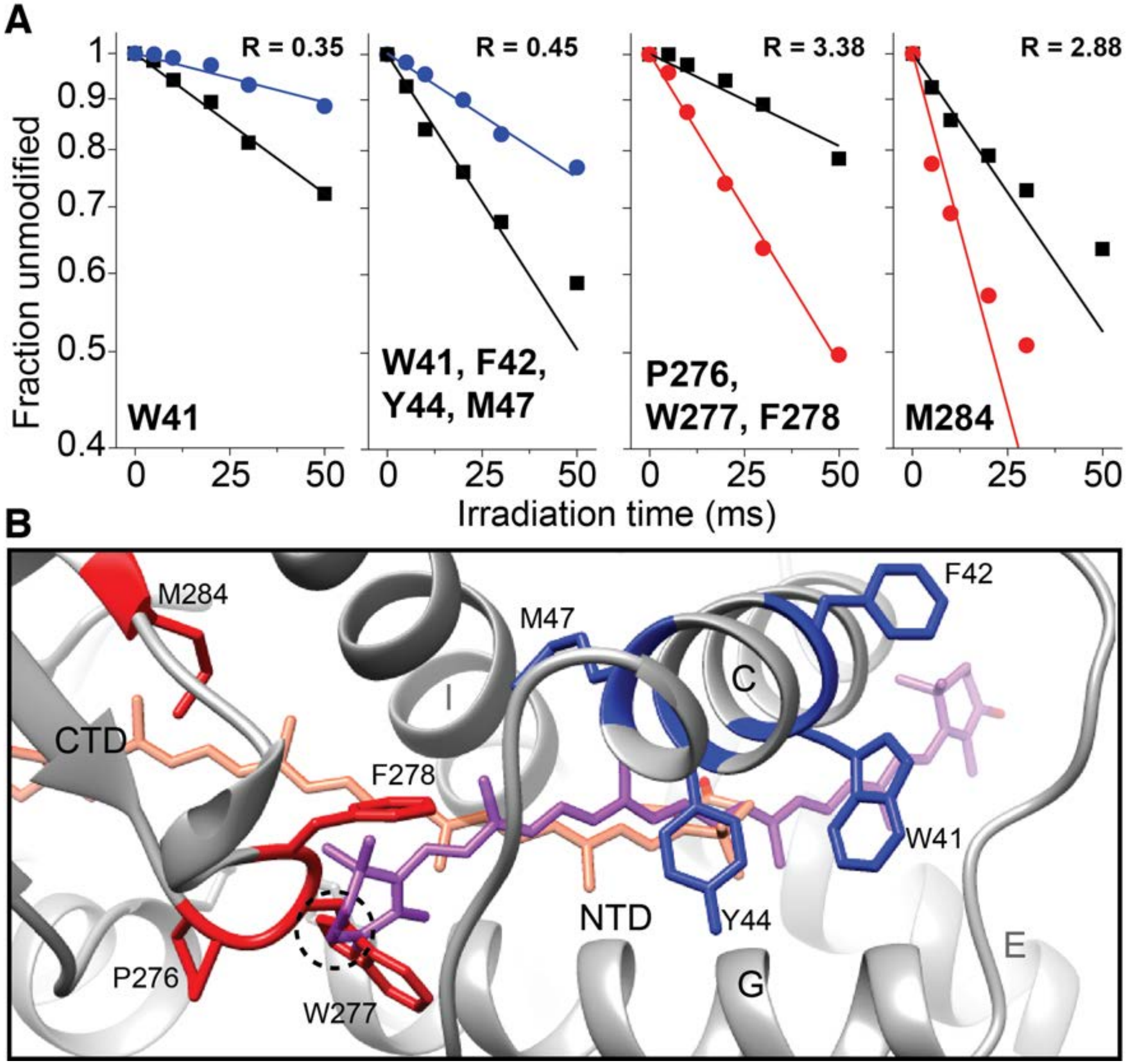
FOOTPRINTING DATA SUPPORTS DOMAIN SEPARATION





Gupta, Guttman et al, "Local and global structural drivers for the photoactivation of the orange carotenoid protein," *PNAS*, V112 No41, E5567, 2015.

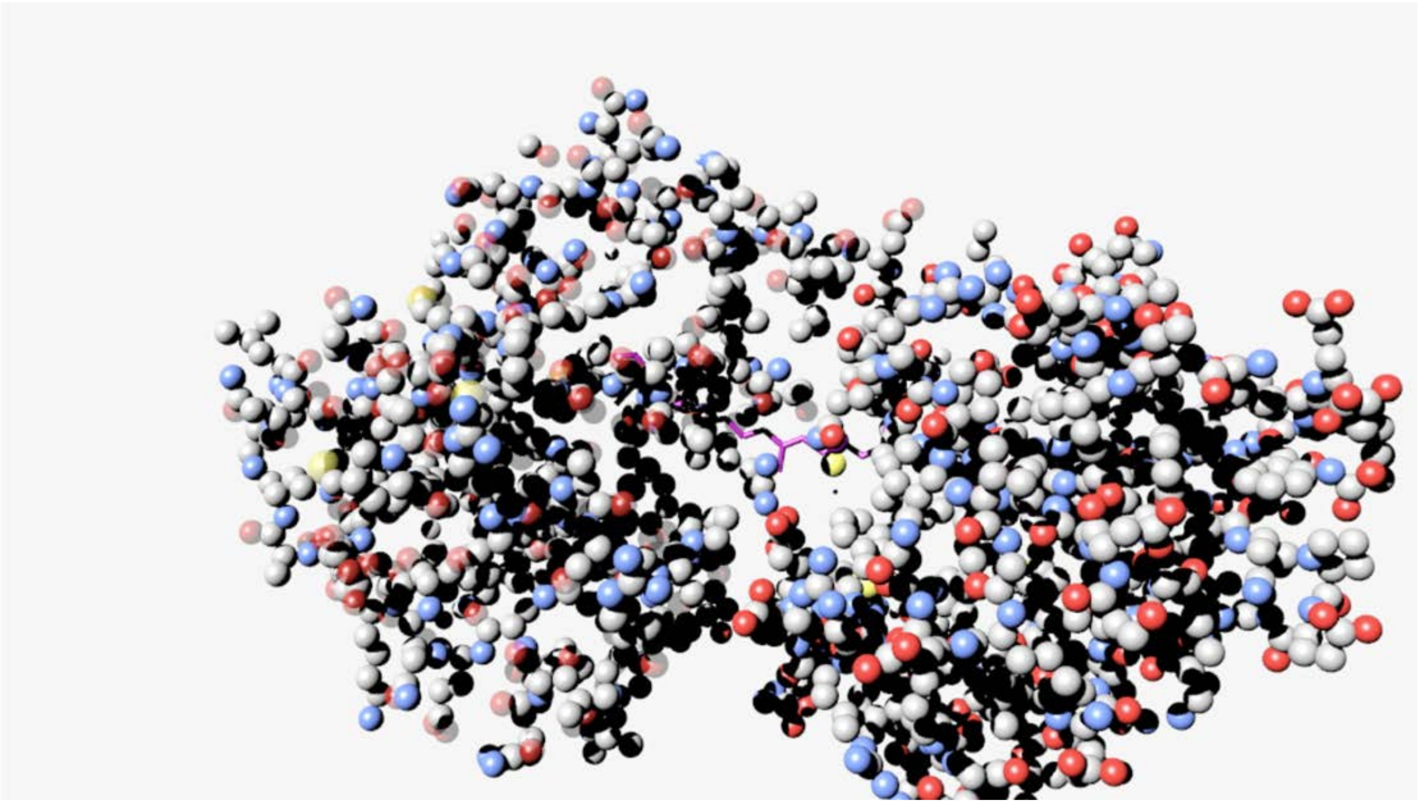
CAROTENOID MOVEMENT WITHIN THE PROTEIN



More modification

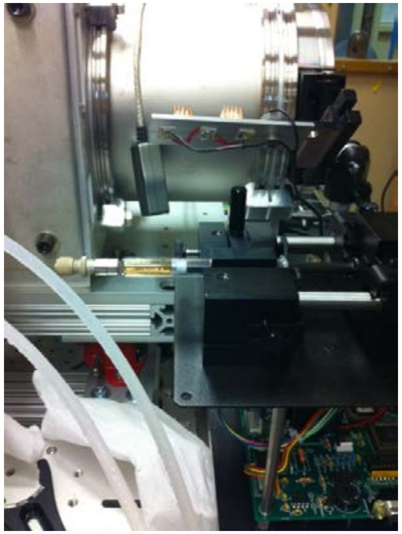
Leverenz, Gupta et al, "Carotenoid translocation in the Orange Carotenoid Protein activates a photoprotective mechanism in cyanobacteria," *Science* V348, 6242, p1463, 2015.

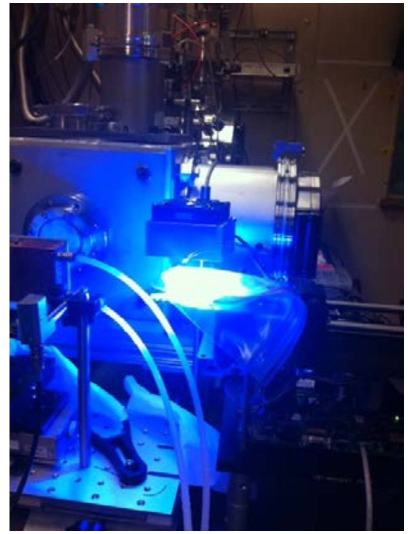
CAROTENOID MOVEMENT WITHIN THE PROTEIN

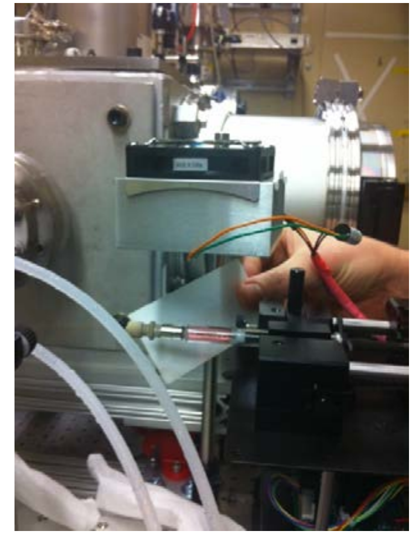


BERKELEY LAB

TIME RESOLVED ORANGE TO RED CONVERSION







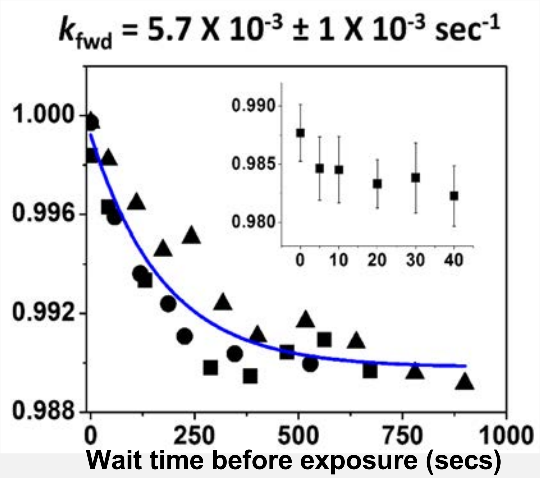
TIME RESOLVED ORANGE TO RED CONVERSION

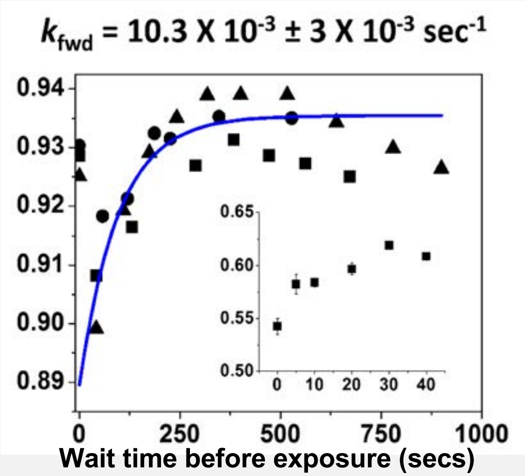
R155,156

W41, Y44, M47

ORANGE TO RED

Exposure time constant at: 0.3ms

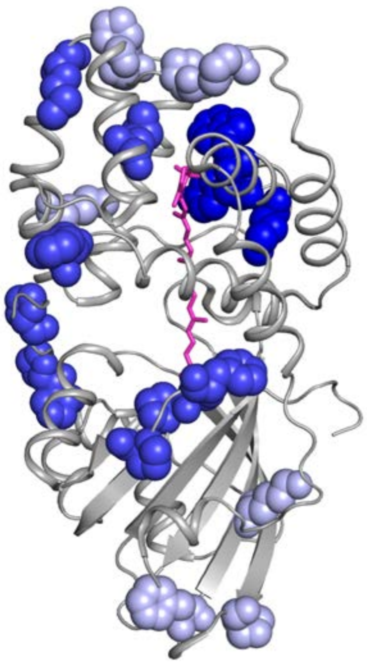




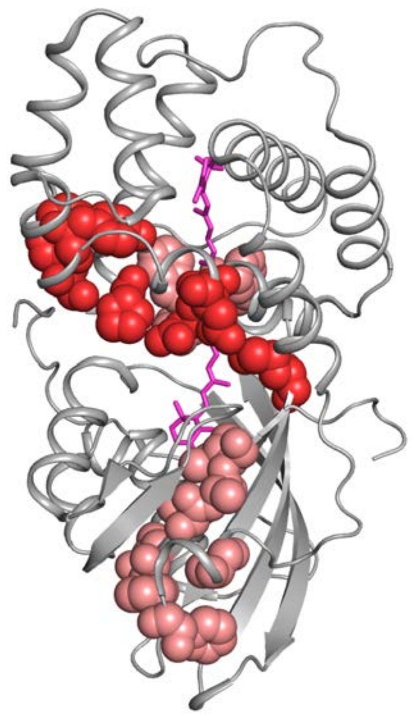
More accessible

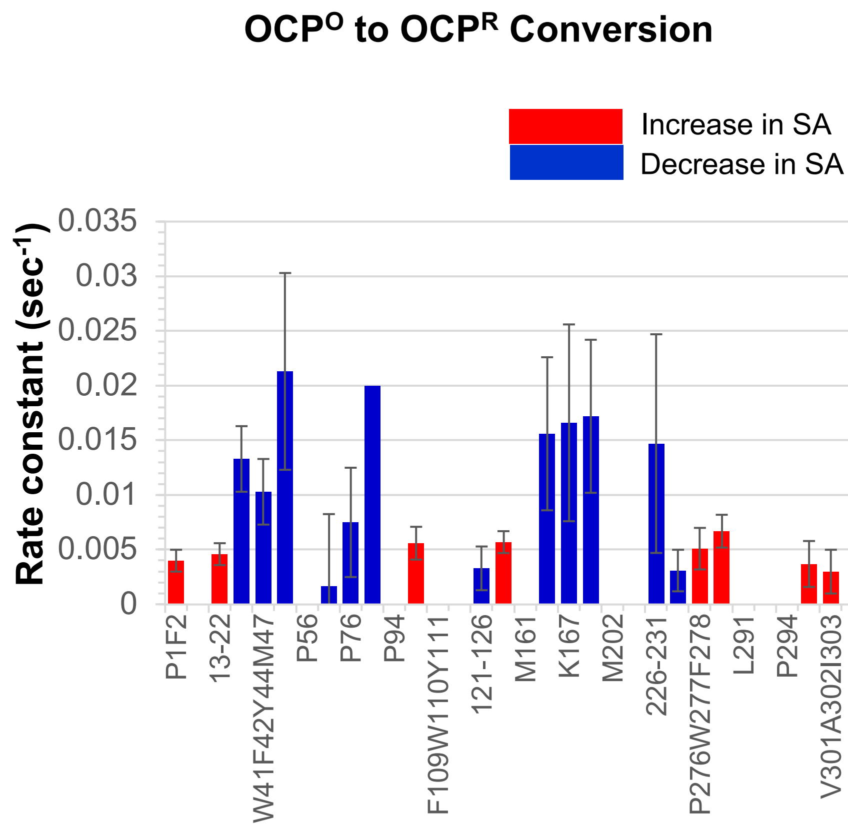
PICTORAL VIEW OF ORANGE TO RED CONVERSION

Decrease in SA



Increase in SA

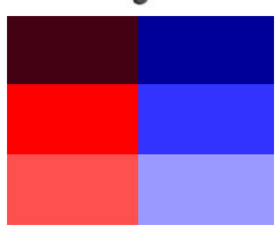




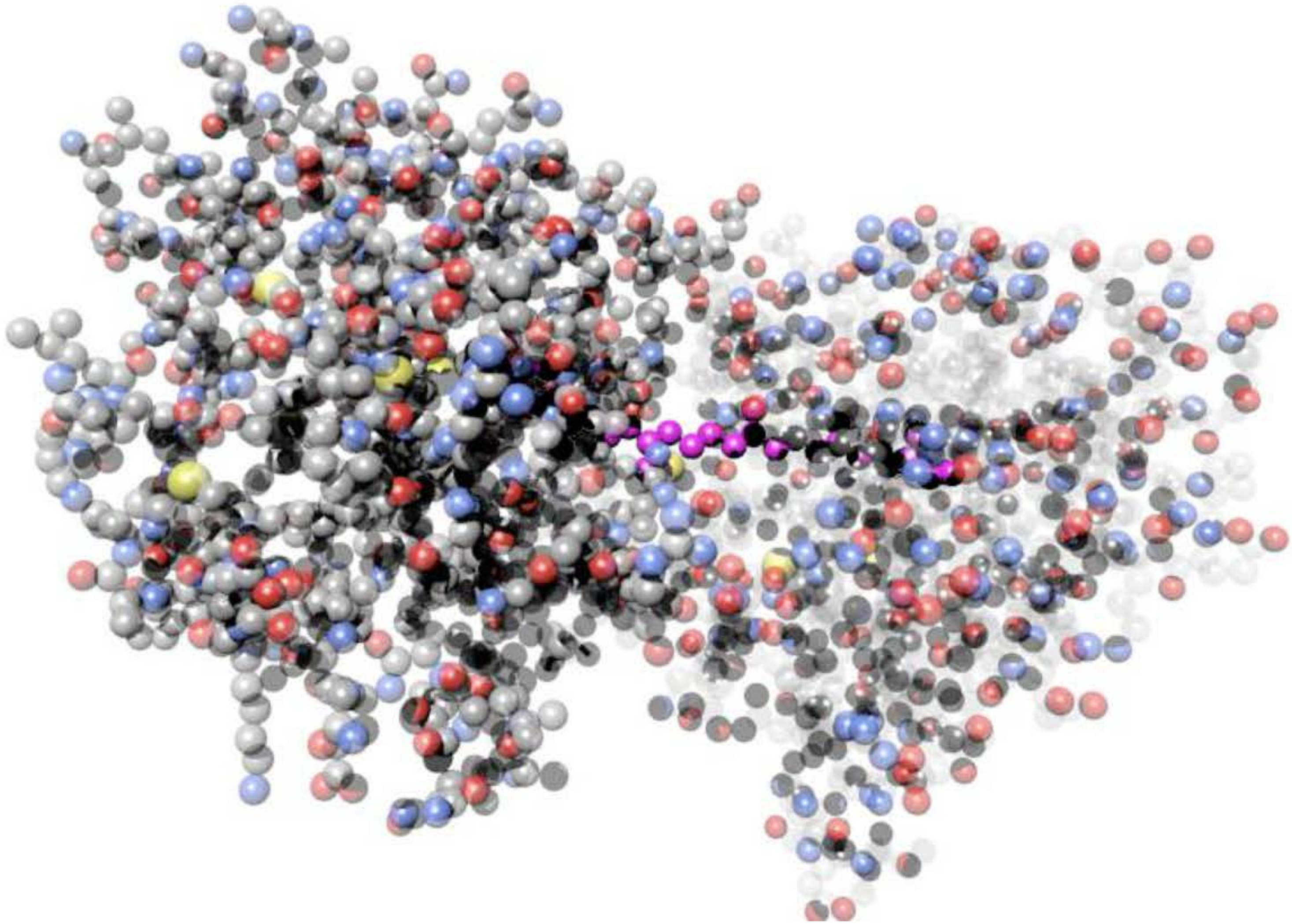
> 0.010 s^{-1} (Fast)

< 0.010 to > 0.005 s^{-1}

> 0.005 s^{-1} (Slow)



PICTORAL VIEW OF ORANGE TO RED CONVERSION

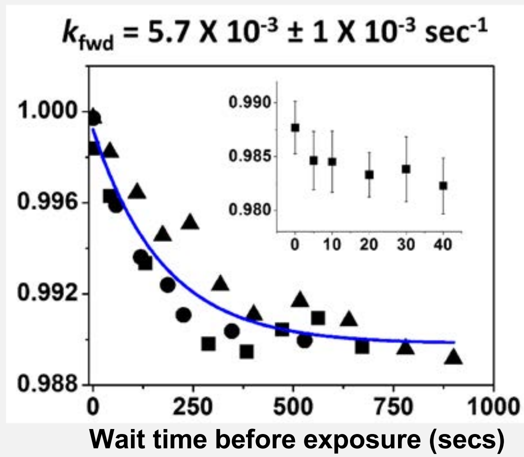


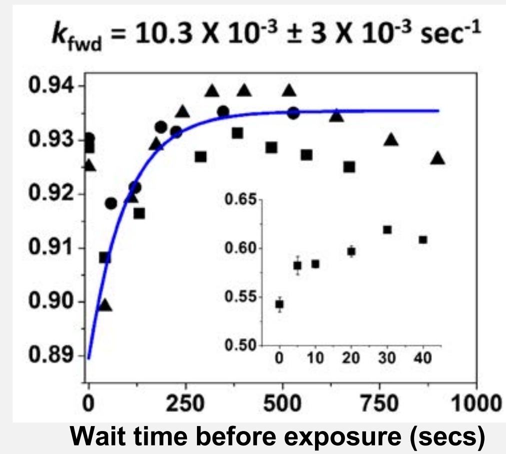
TIME RESOLVED RED TO ORANGE CONVERSION

Exposure time constant at: 0.3ms

R155,156 | **W41, Y44, M47**

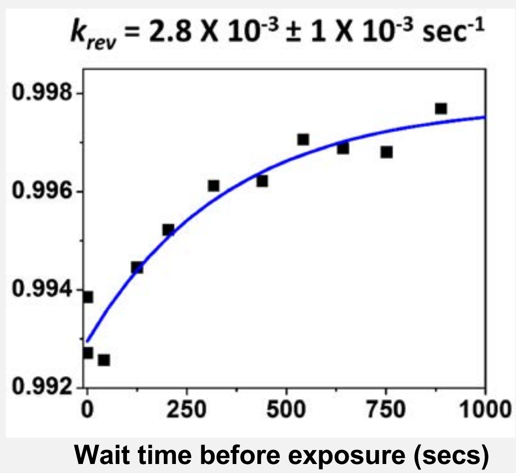
ORANGE TO RED

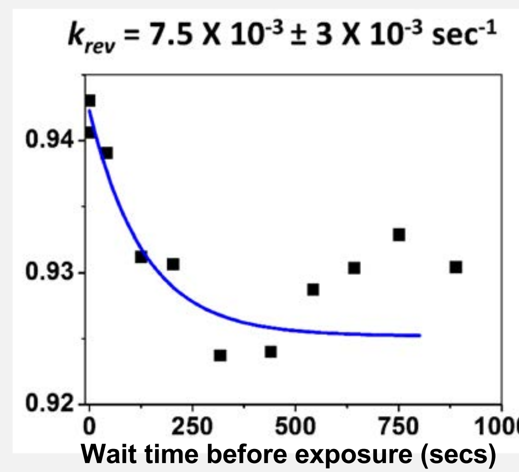




More accessible

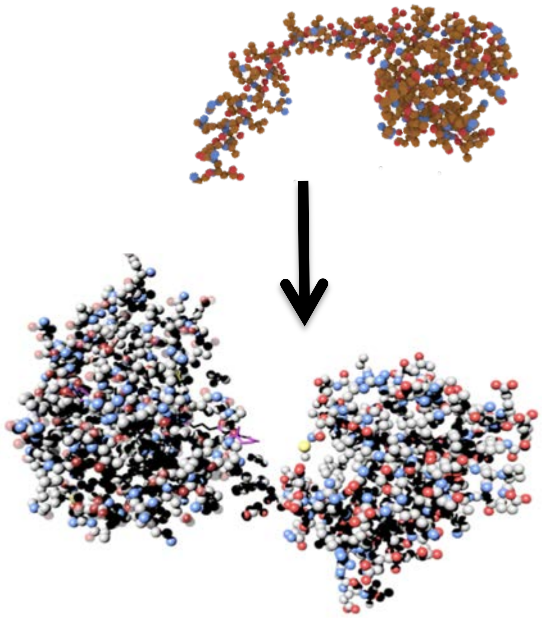
RED TO ORANGE

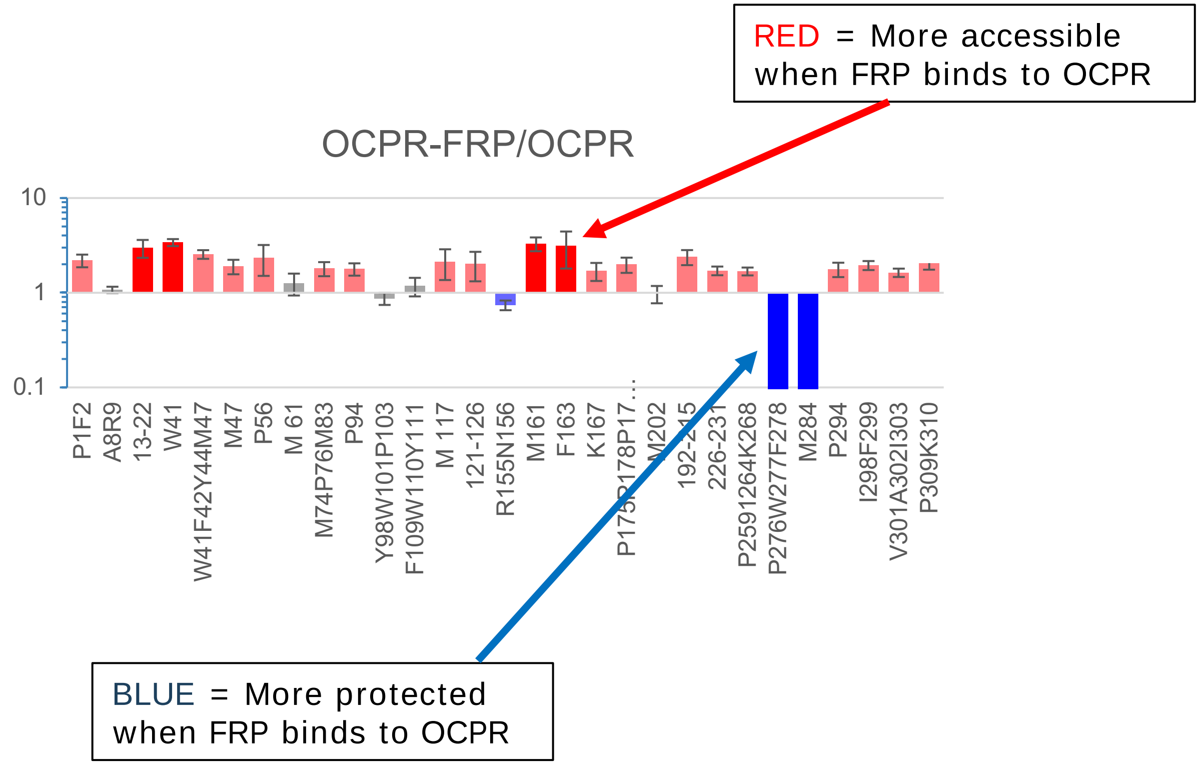




More accessible

PROTECTION OF OCP WHEN FRP BINDS





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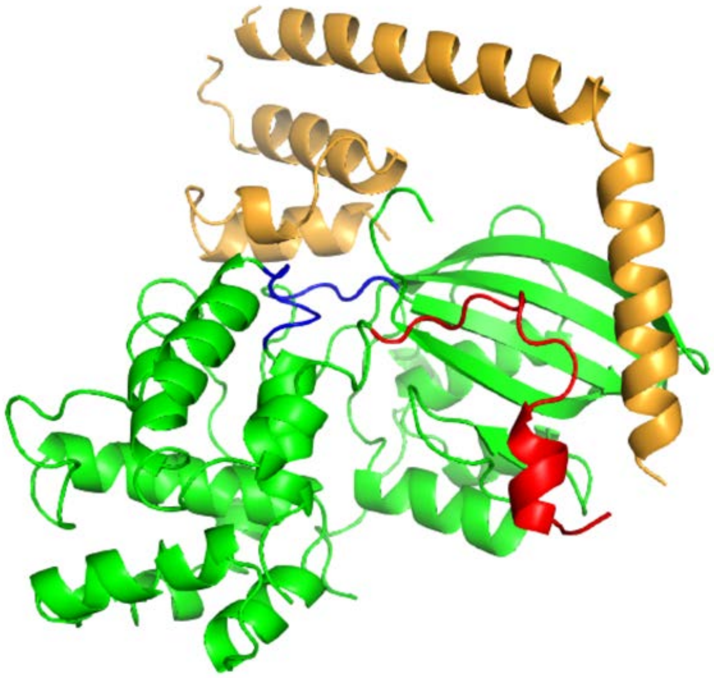
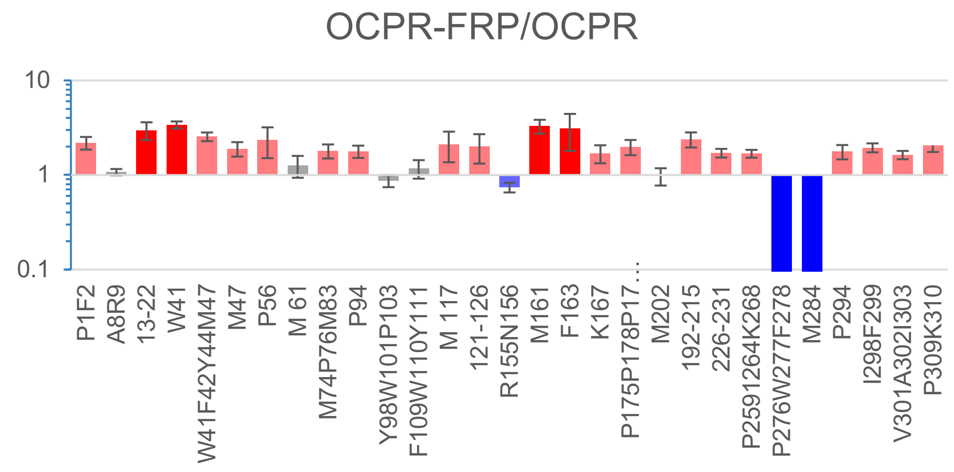
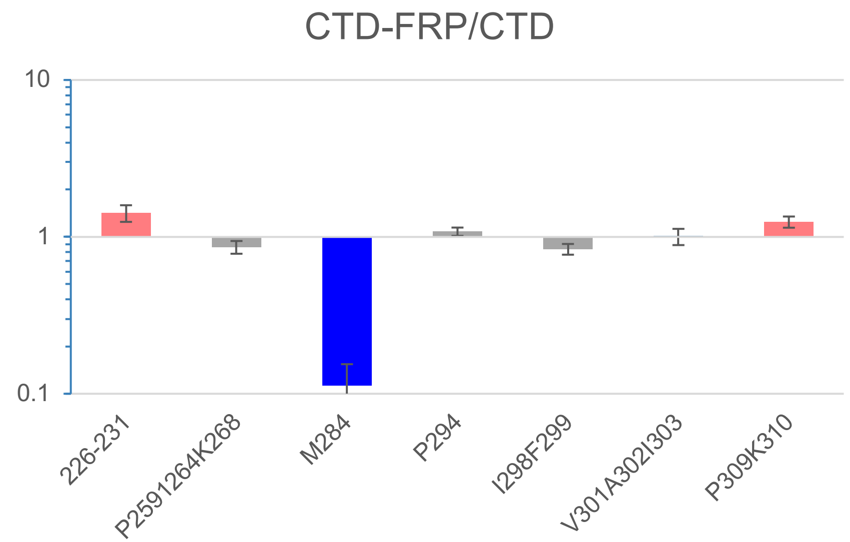
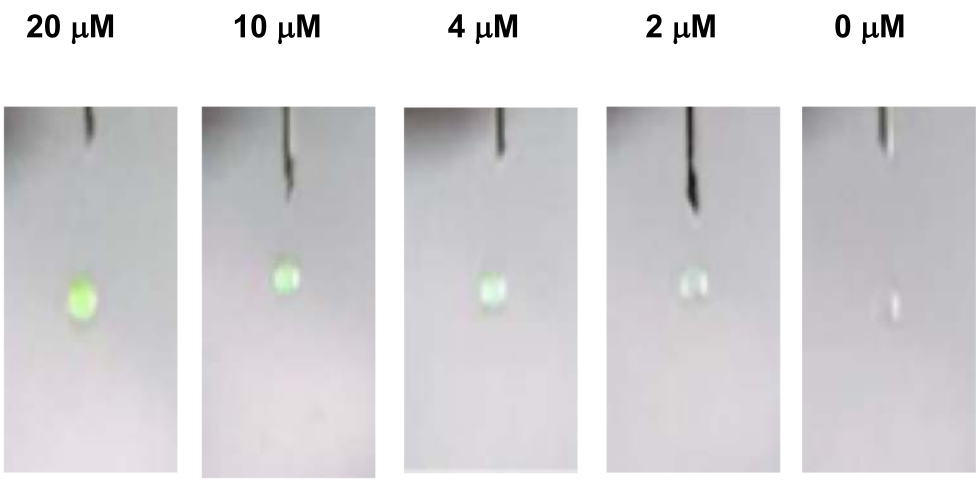




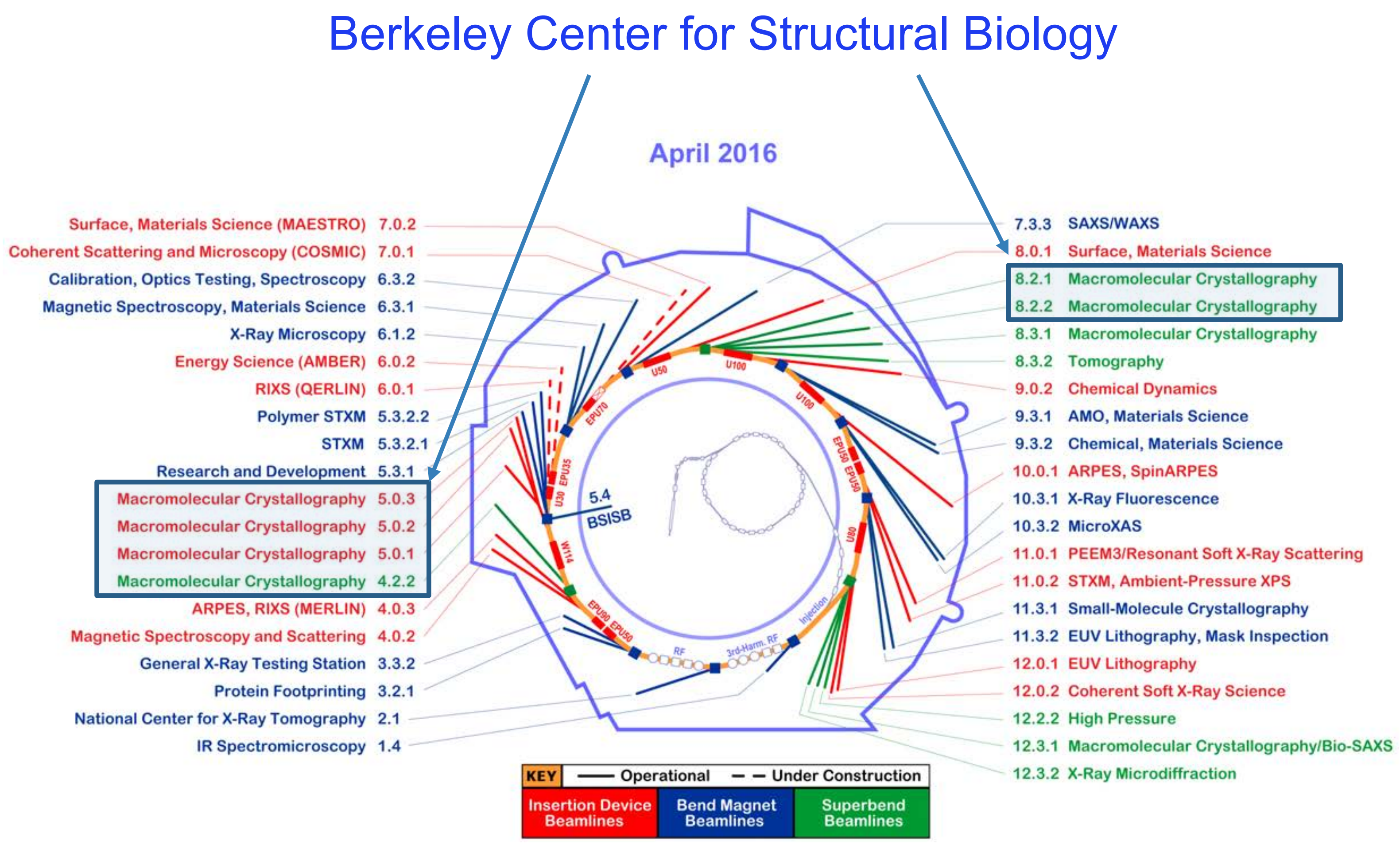


WHAT’S NEXT?

- Continue investigating OCP/PB/FRP as well as other systems
- Set up mixing experiment to enable faster time-resolved studies
- Investigate footprinting in live cells
- Develop drop-on-demand for achieving even higher doses

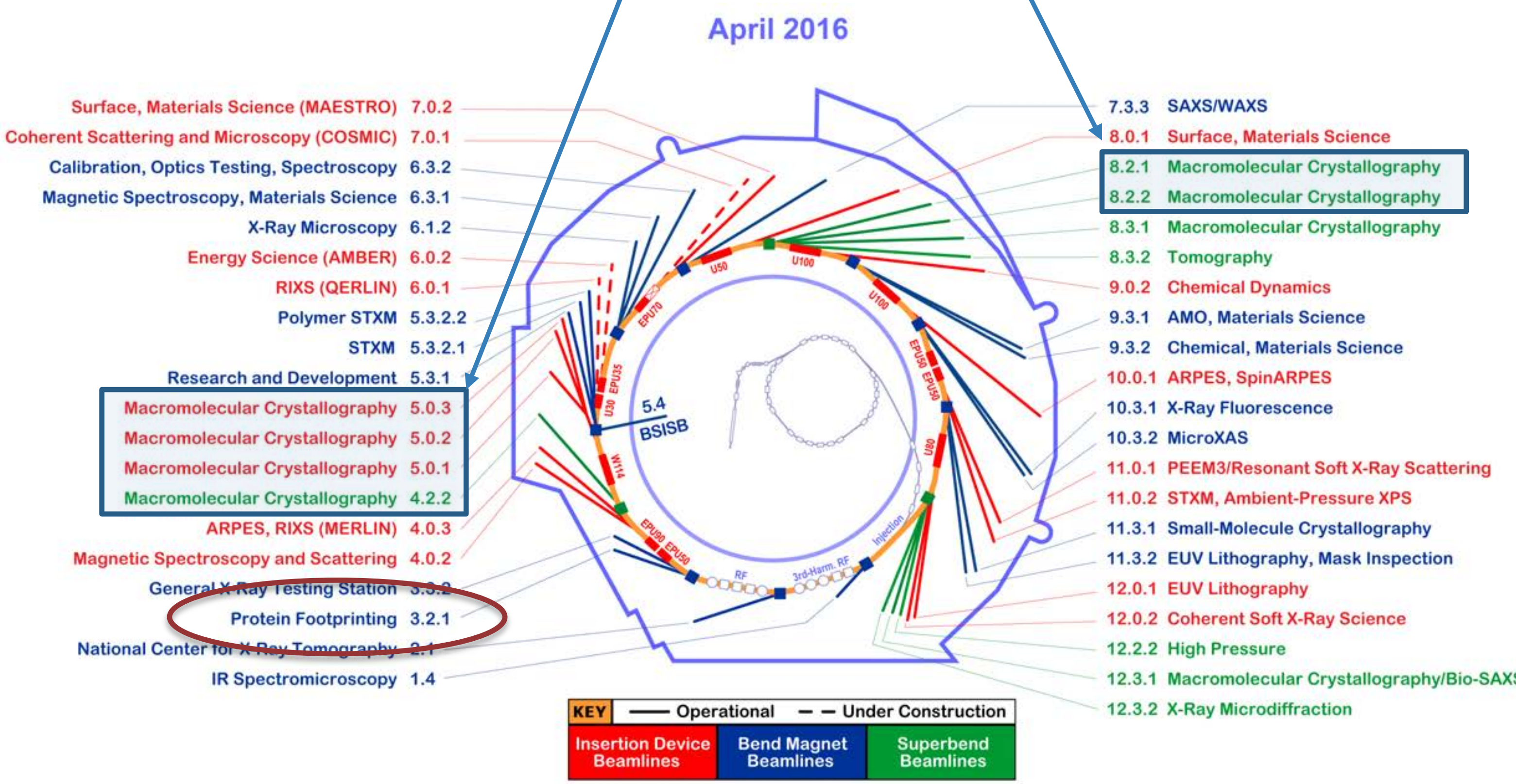


ALS SYNCHROTRON – CRYSTALLOGRAPHY AND MORE



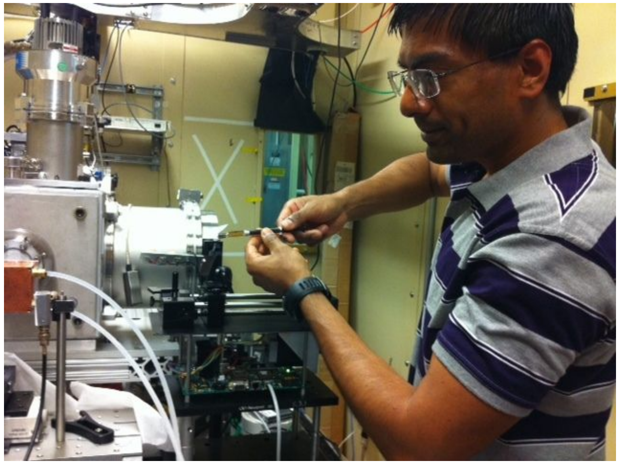
ALS SYNCHROTRON – CRYSTALLOGRAPHY AND MORE

Berkeley Center for Structural Biology



THANKS TO…

Bringing Footprinting to the ALS
Mark Chance (Case Western)
Jen Bohon (Case Western, NSLSII)
Sayan Gupta (NSLS, now ALS)



Mass Spec
Chris Petzold (JBEI)
Leanne Chan (JBEI)

Collaborations
Kerfeld Lab - MSU
Craik Lab – UCSF
Marqusee Lab – UCB
Merritt Lab – Stanford
Ajo-Franklin Lab – LBNL
Fu Lab - JHU

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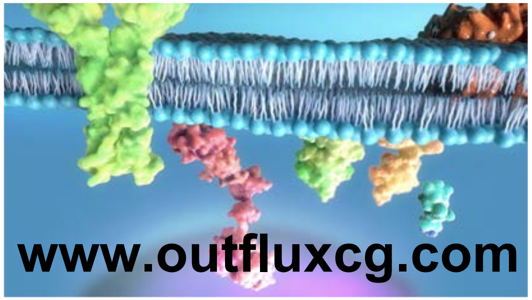
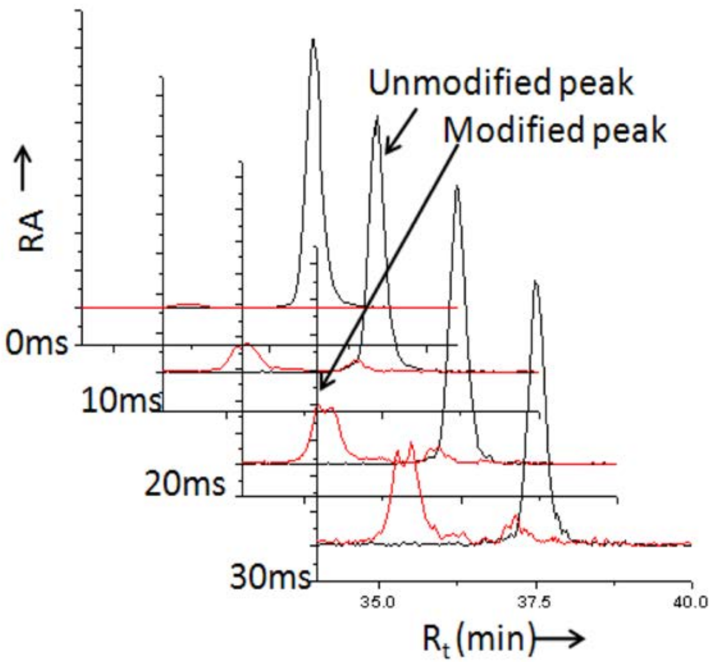
LDRD through Physical Biosciences Division from 2012-2014
HUGE THANKS to Mark Chance, Cheryl Kerfeld, Dax Fu

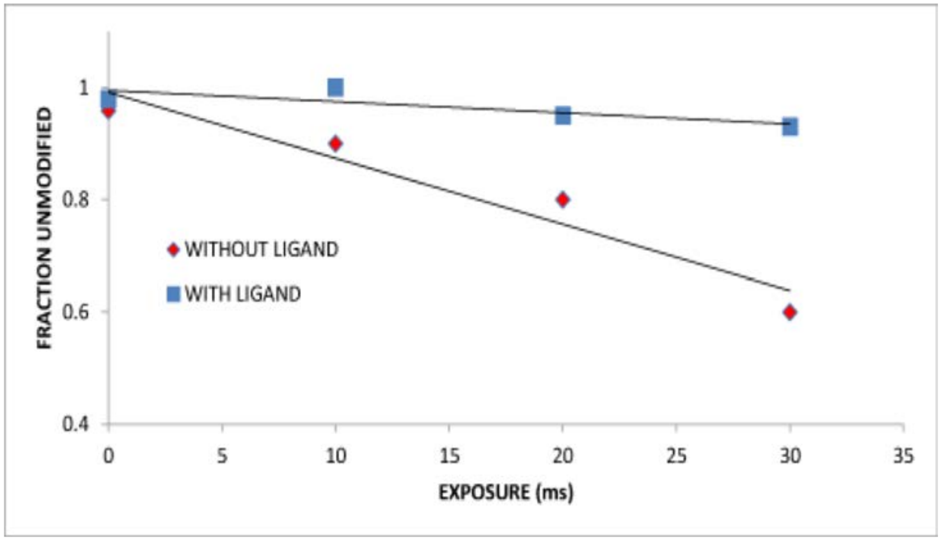


OMG I got an R01!!

Extra Slides Follow

Dose and Exposure – Some Practical Aspects





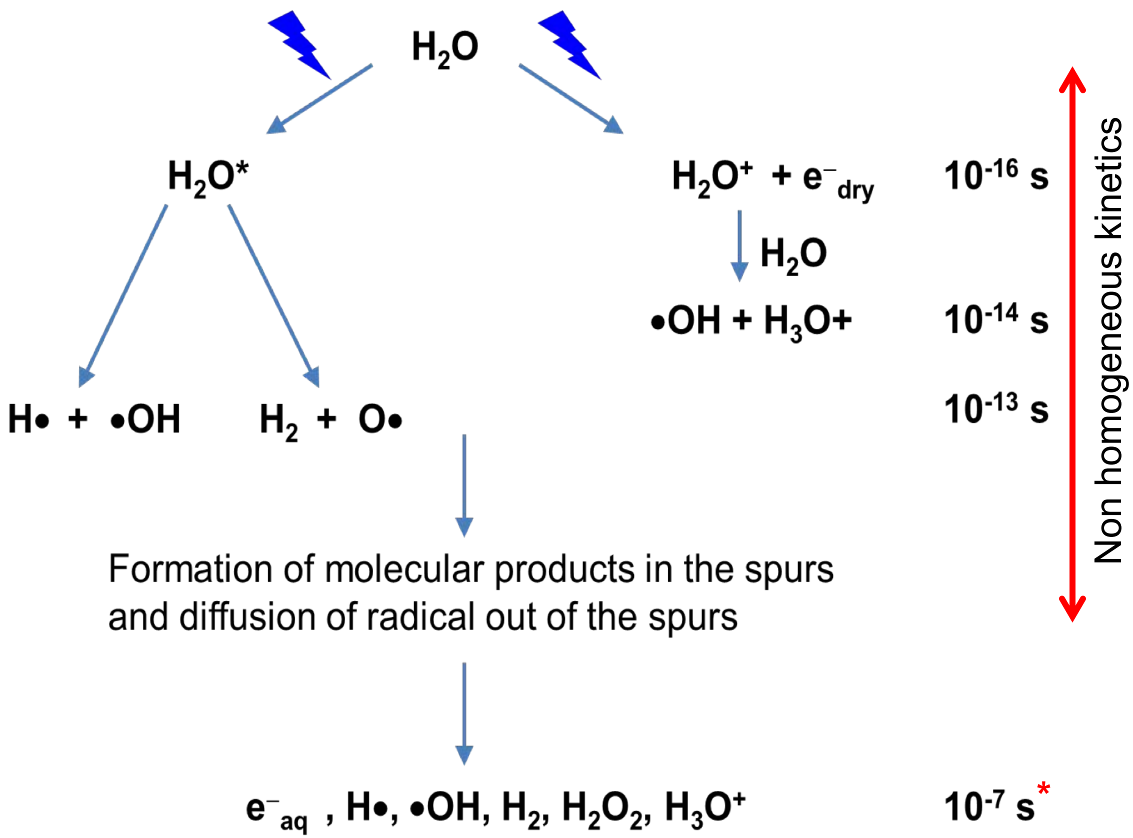
- **Fraction Unmodified = 1-[modified/(total of mod+unmod)]**
- **Limit exposure to stay in linear region**

Table 1. Rate Constants for Reaction of Amino Acids with Hydroxyl Radical and Hydrated Electrons[a]

	HO^-		e_{aq}^{-1}	
substrate	rate (M^{-1} s^{-1})	pH	rate (M^{-1} s^{-1})[b]	pH
Cys	3.5×10^{10}	7.0	1.0×10^{10}	−7
Trp	1.3×10^{10}	6.5−8.5	3.0×10^{8}	7.8
Tyr	1.3×10^{10}	7.0	2.8×10^{8}	6.6

X-Ray Radiolysis of Water

- **Water radiolysis & primary radical products**



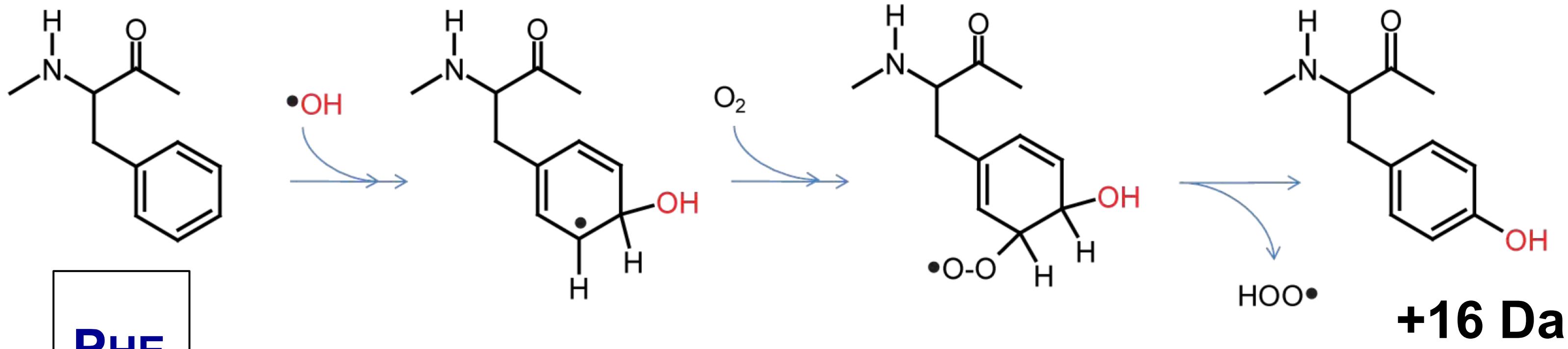
- **Secondary radical product**

$$e_{aq}^- + e_{aq}^- = H_2 + 2OH^-$$
$$e_{aq}^- + \bullet OH = OH^-$$
$$e_{aq}^- + H_3O^+ = H\bullet + H_2O$$
$$e_{aq}^- + O_2 = \bullet O_2^-$$
$$H\bullet + H\bullet = H_2$$
$$H\bullet + O_2 = \bullet HO_2$$
$$\bullet OH + \bullet OH = H_2O_2$$
$$\bullet OH + H\bullet = H_2O$$
$$H_3O^+ + OH^- = H_2O$$

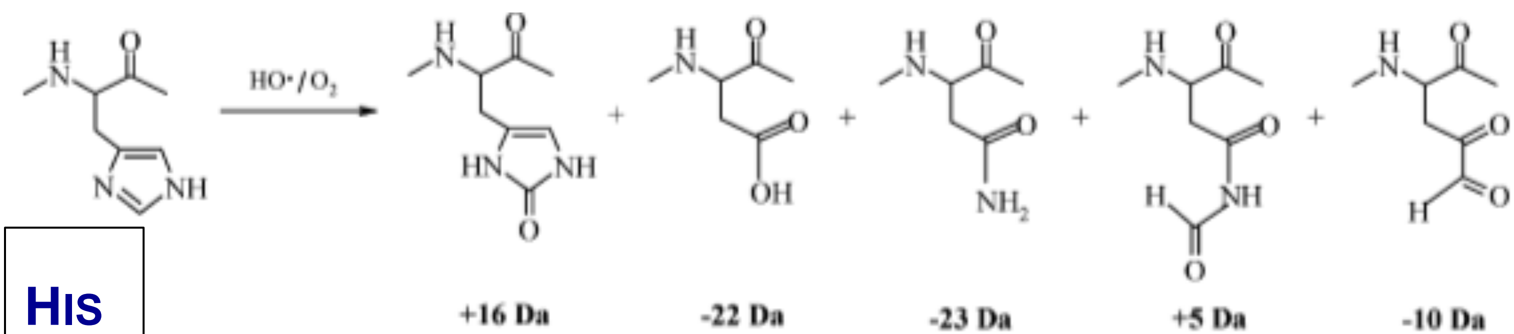
- **OH reacts within 1 to 5 molecular diameters of the site of formation***

Gupta et. al. JSR. 2014. 21(Pt 4):690-9 / Pryor WA. A. R. Physiol. 1988. 48, 657-667
Buxton et al. JPC Ref. D. 1988. 17-34

Variation in Reactions by Residue



Phe



His

Dealing with Residue-Specific Reactivity

Chemical Reviews, 2007, Vol. 107, No. 8 **3519**

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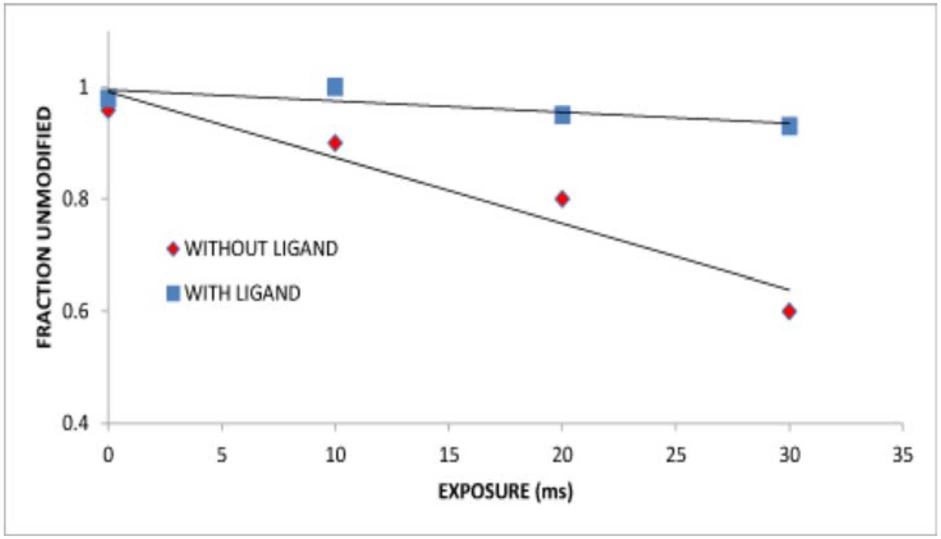
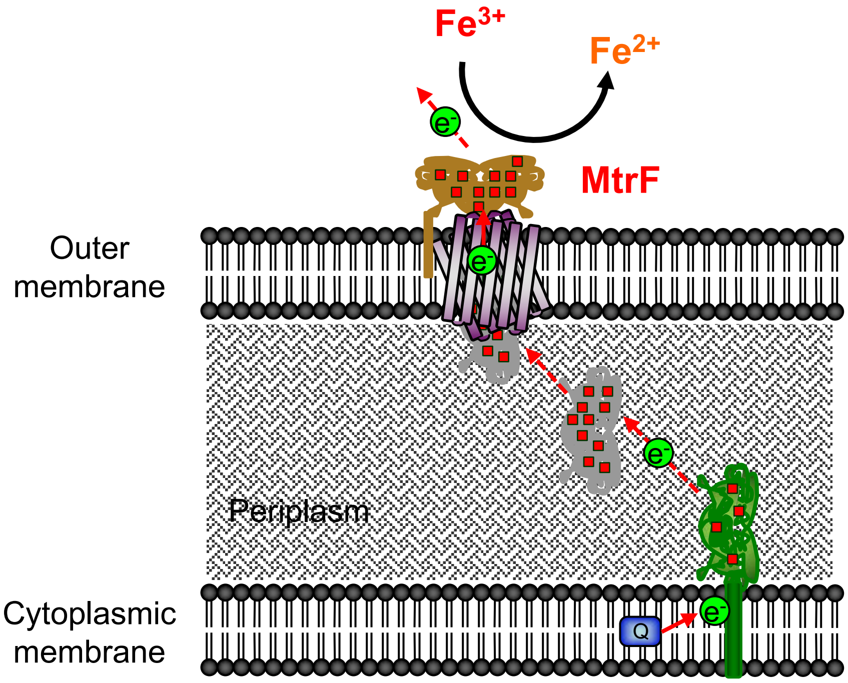
Tyr	1.3×10^{10}	7.0	2.8×10^{8}	6.6
Met	8.5×10^{9}	6−7	4.5×10^{7}	7.3
Phe	6.9×10^{9}	7−8	1.6×10^{7}	6.9
His	4.8×10^{9}	7.5	6.0×10^{7}	−7
Arg	3.5×10^{9}	6.5−7.5	1.5×10^{8}	6.1
cystine	2.1×10^{9}	6.5	1.5×10^{10}	6.2
Ile	1.8×10^{9}	6.6	N/A	N/A
Leu	1.7×10^{9}	~6	$<1 \times 10^{7}$	6.5
Val	8.5×10^{8}	6.9	$<5 \times 10^{6}$	6.4
Pro	6.5×10^{8}	6.8	2.0×10^{7}	6.7
Gln	5.4×10^{8}	6.0	N/A	N/A
Thr	5.1×10^{8}	6.6	2.0×10^{7}	7.0
Lys	3.5×10^{8}	6.6	2.0×10^{7}	7.4
Ser	3.2×10^{8}	~6	$<3 \times 10^{7}$	6.1
Glu	2.3×10^{8}	6.5	$1\text{--}2 \times 10^{7}$	5.7−7
Ala	7.7×10^{7}	5.8	1.2×10^{7}	7.4
Asp	7.5×10^{7}	6.9	1.8×10^{7}	7.0
Asn	4.9×10^{7}	6.6	1.5×10^{8}	7.3
Gly	1.7×10^{7}	5.9	8.0×10^{8}	6.4

[a] http://allen.rad.nd.edu/browse compil.html. [b] Davies, M. J.; Dean, R. T. *Radical-mediated protein oxidation: from chemistry to medicine*; Oxford University Press: 1997; pp 44−45.

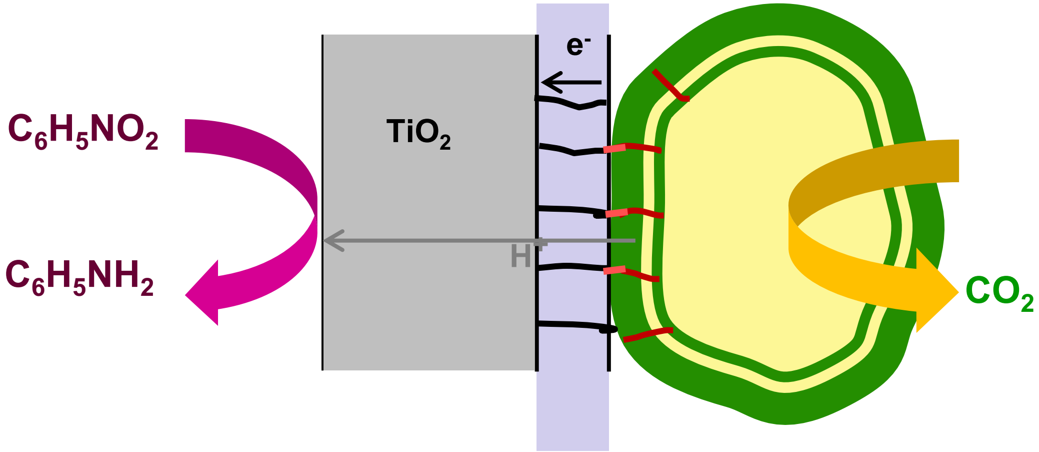


EXAMPLE 1: TOWARDS BIOHYBRIDS

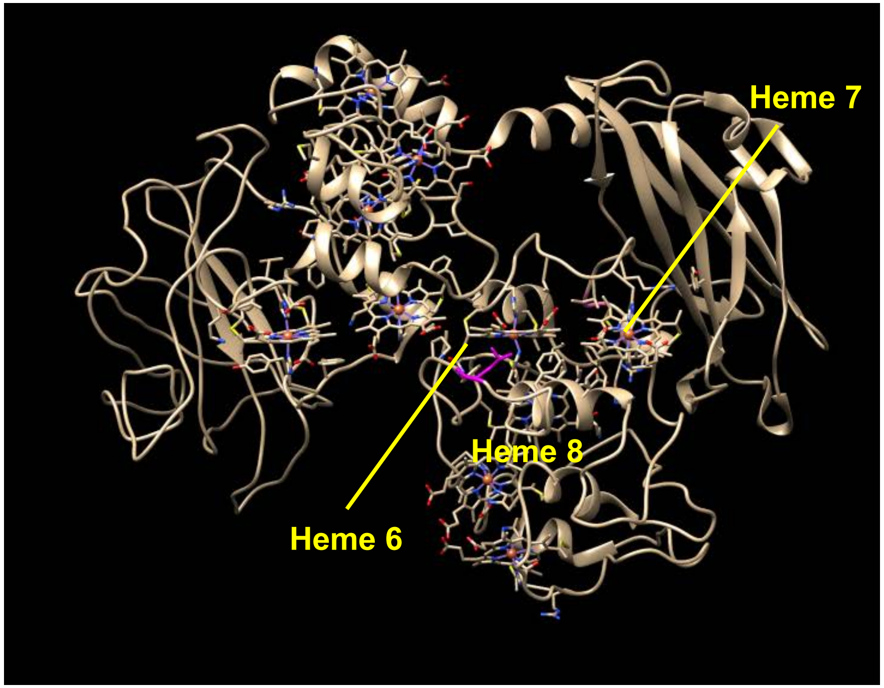


Electron machinery in *Shewanella oneidensis*

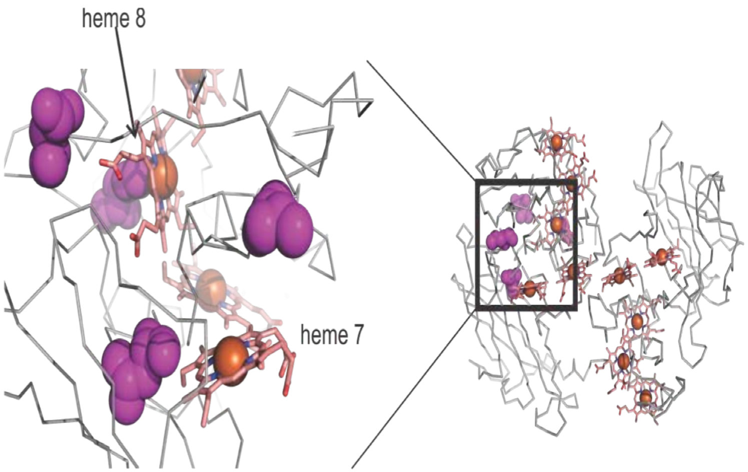
Goal: Engineering the nanoscale interface between living microbes and inorganic materials



X-RAY FOOTPRINTING SHOWS MTRF PROTECTIONS



Areas around hemes 7 and 8 of MtrF are protected by Fe_2O_3



Fukushima et al, “The electron transfer protein MtrF binds to its inorganic substrate through electrostatic interactions created by tertiary structure,” **paper accepted to JACS**.