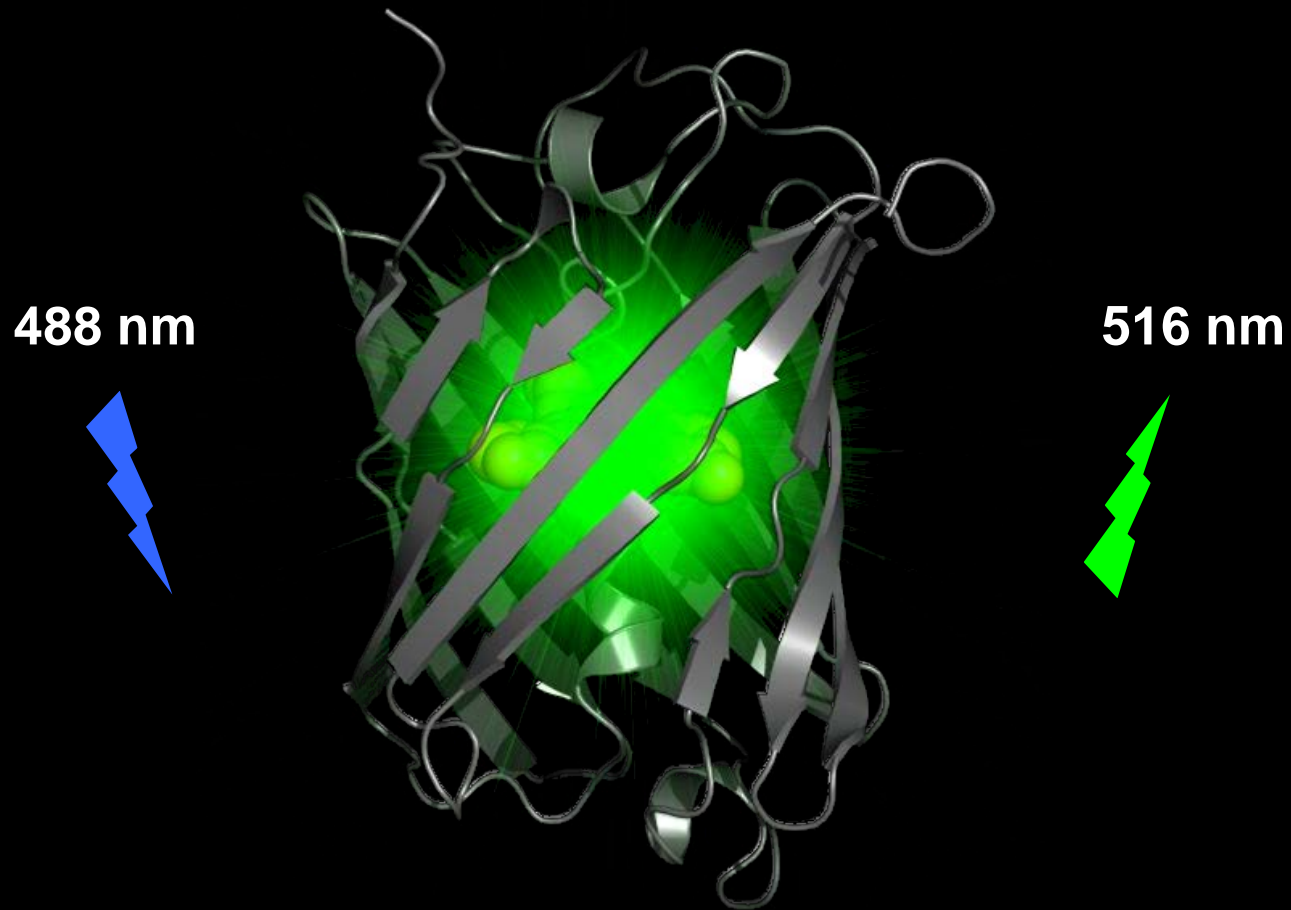
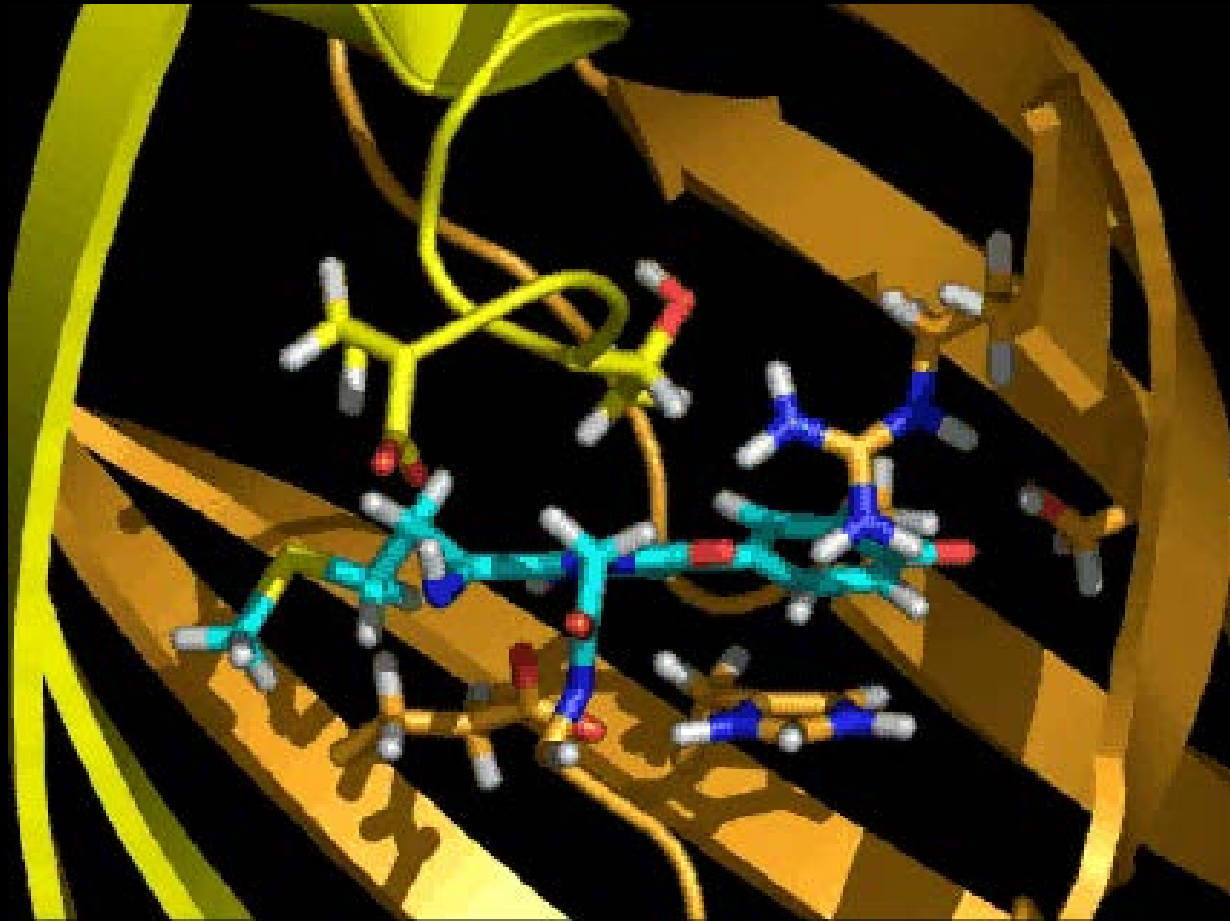


*Understanding and Engineering
Improved Phototransformable
Fluorescent Proteins for Advanced
Microscopy*

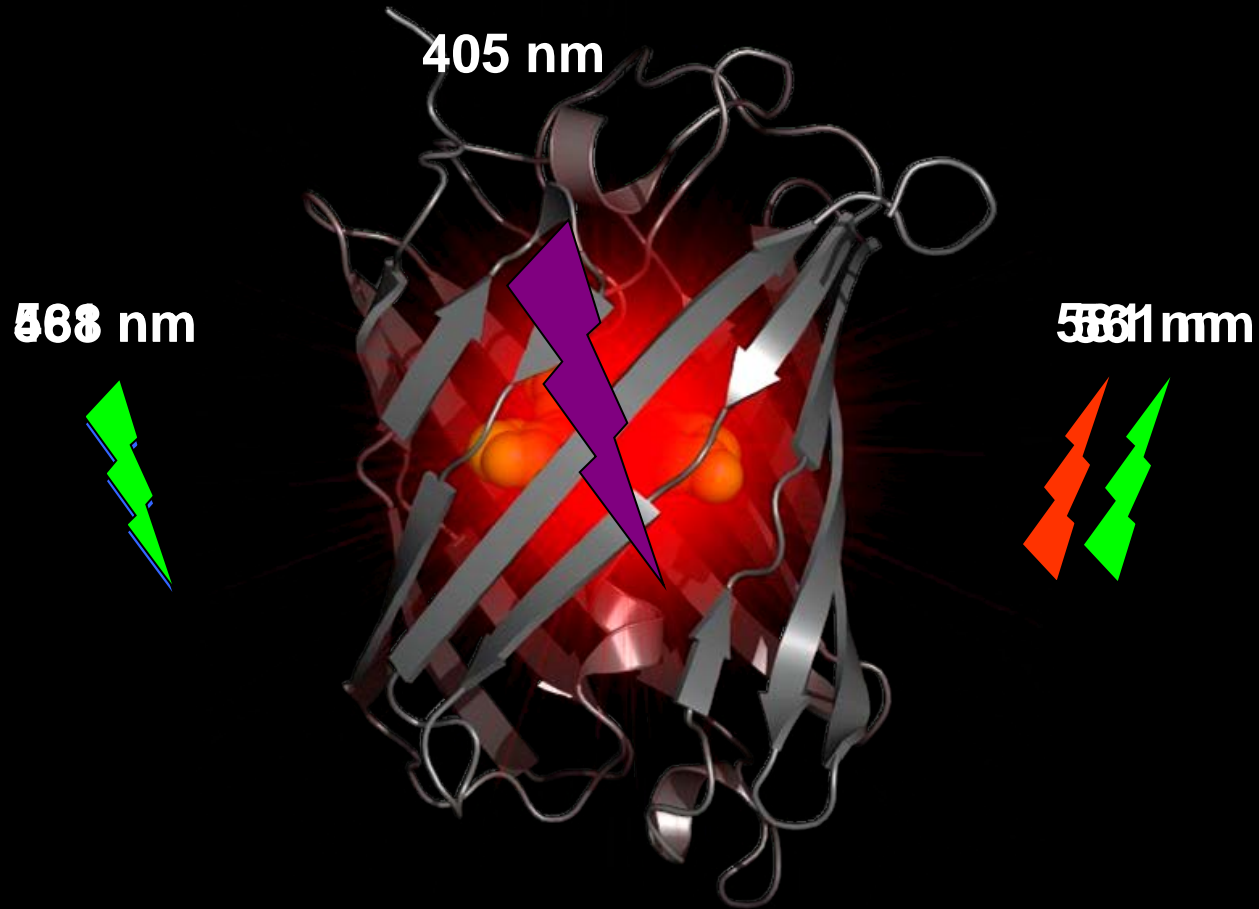
GFP Fluorescence



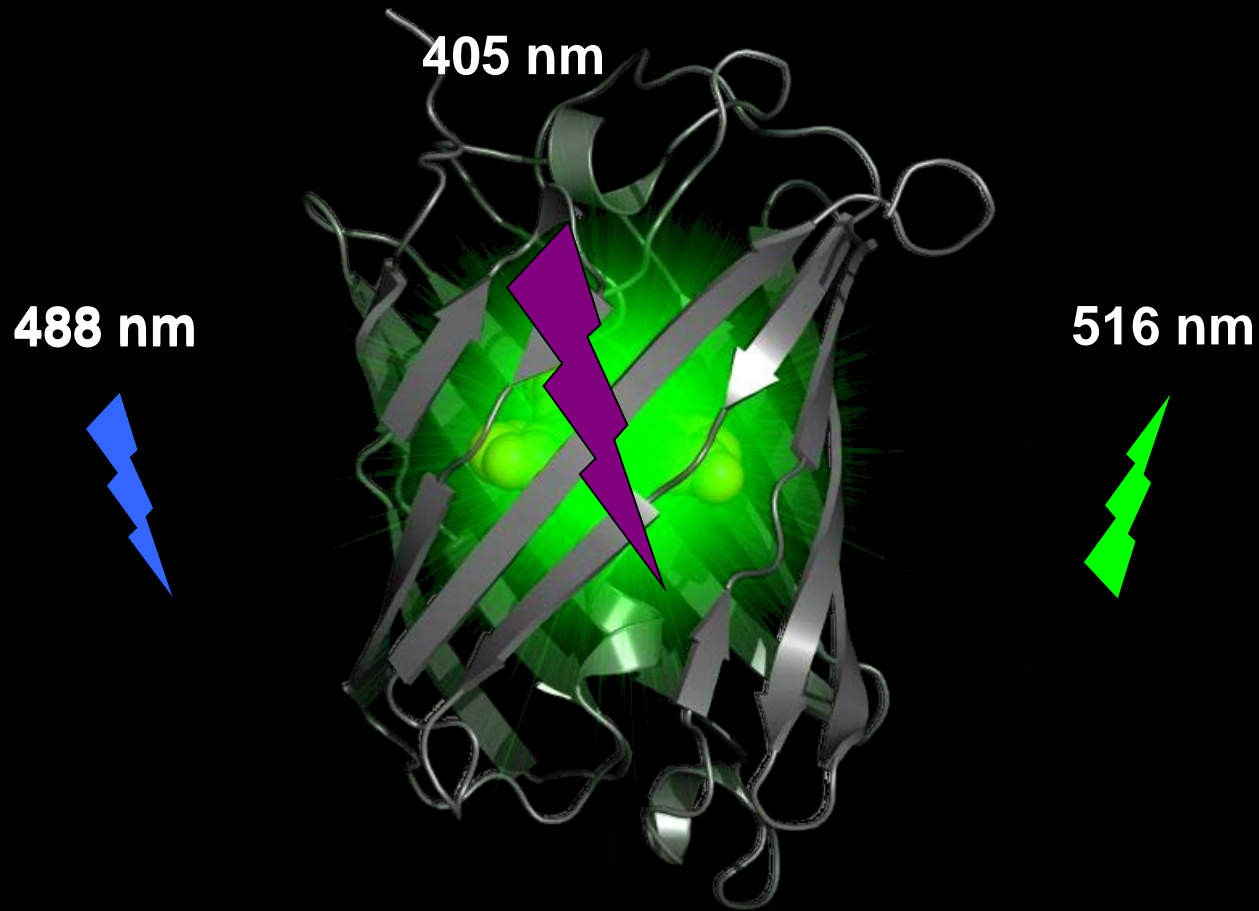
Fluorescence states are linked to structural dynamics



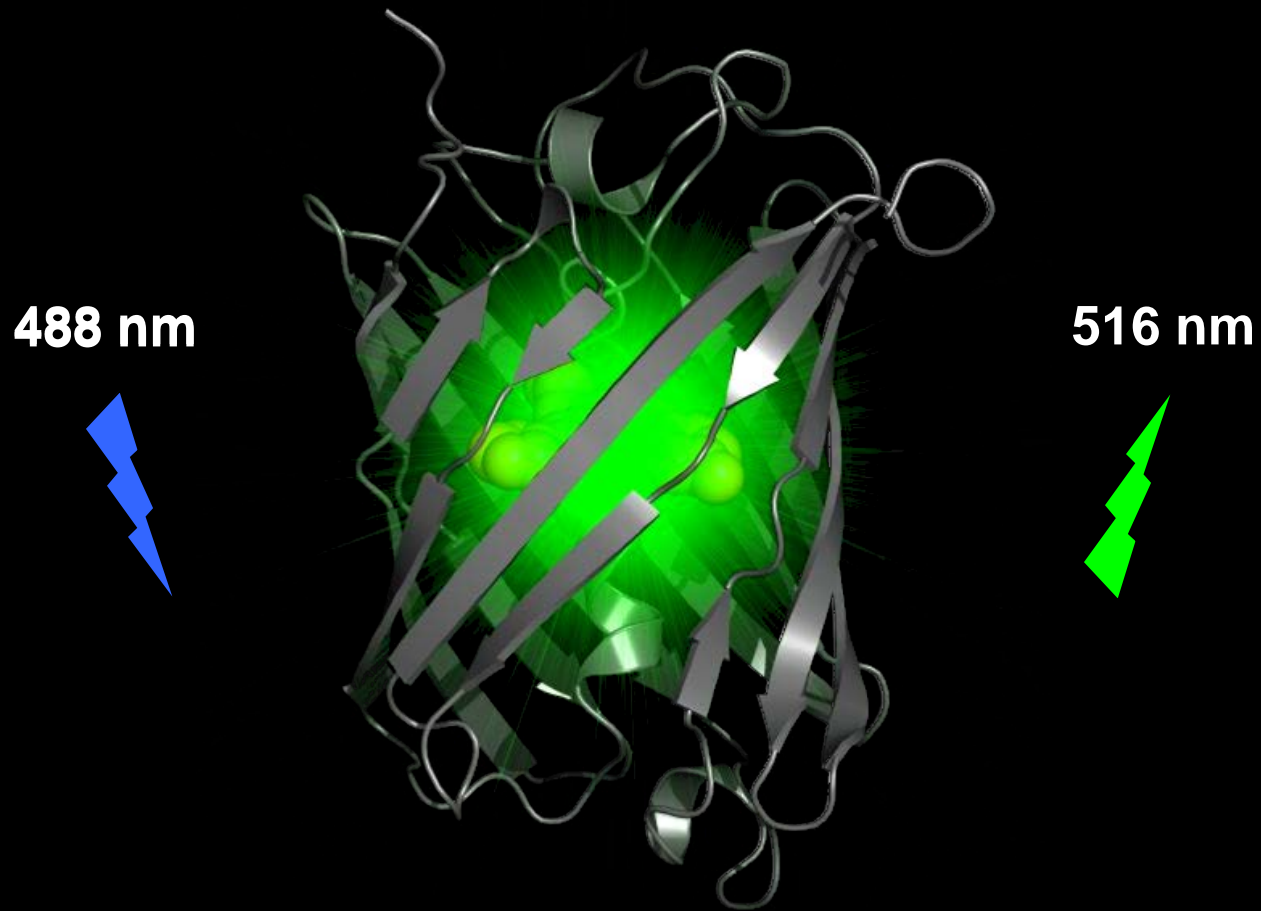
Photoconvertible fluorescent proteins



Photoswitchable fluorescent proteins

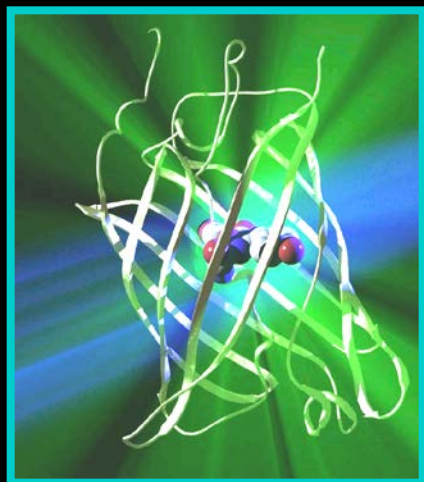


Photoblinking



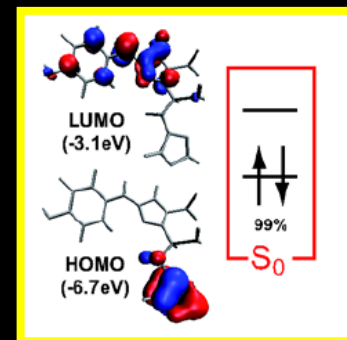
Photobleaching





*Kinetic X-ray
crystallography*

Structure



Simulations

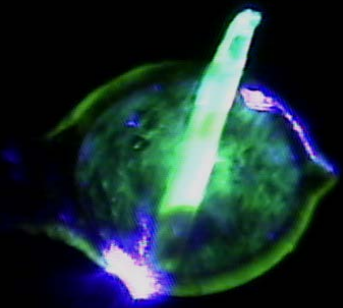
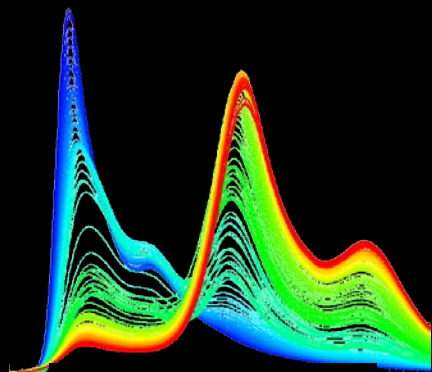
*Dynamics
QC/MM*

Mechanism



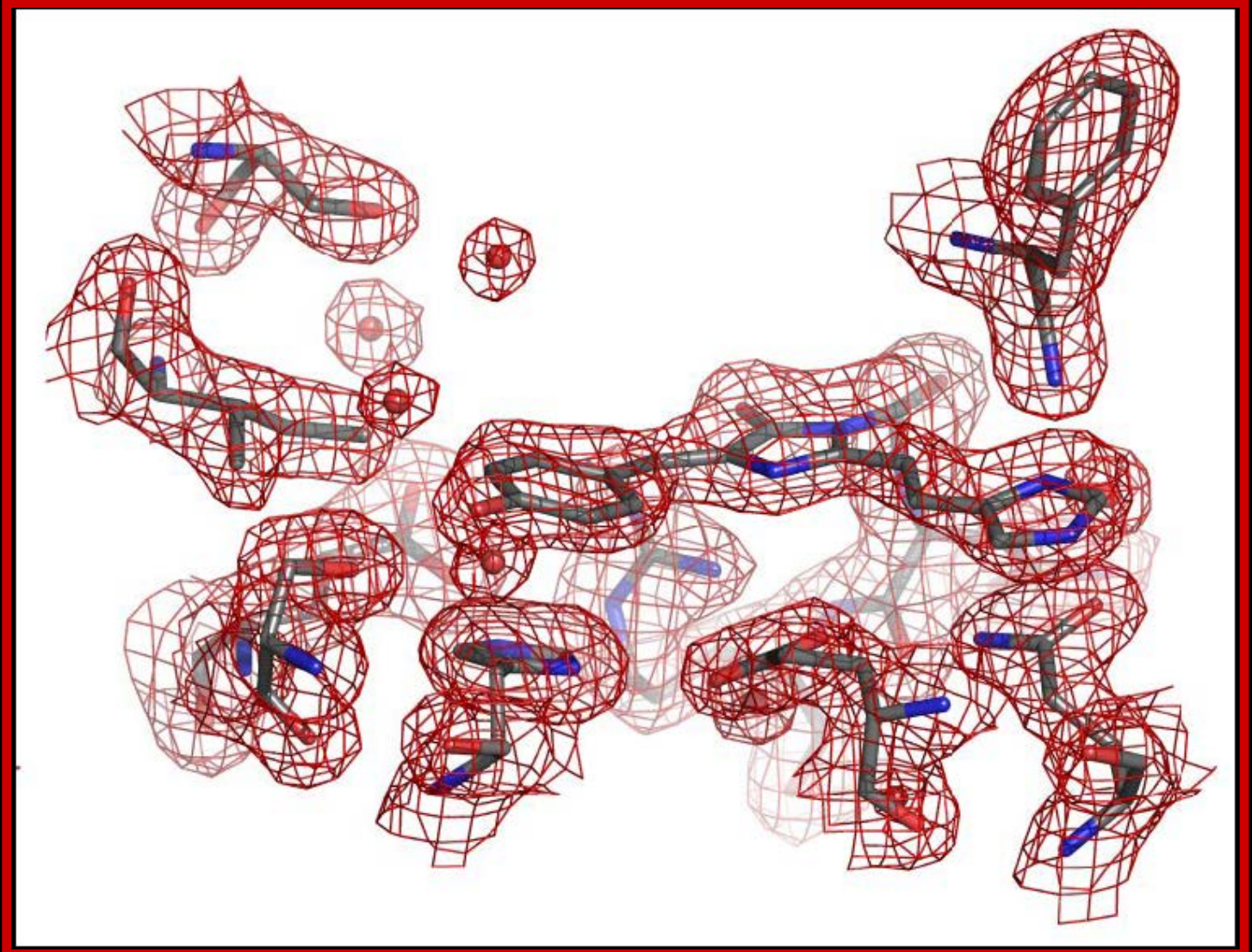
*(in crystallo) Optical
Spectroscopy*

*Fluorescence
behaviour*

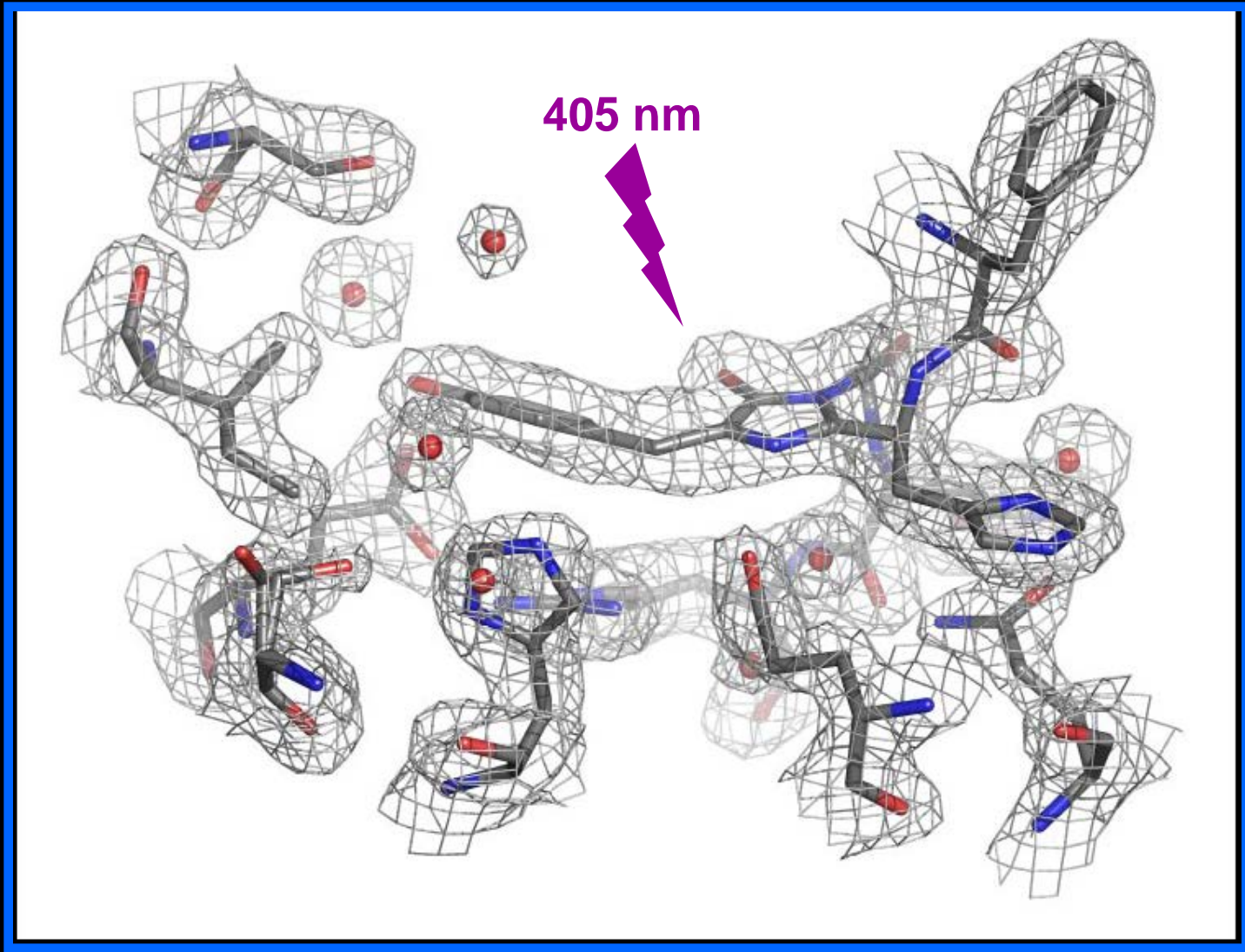


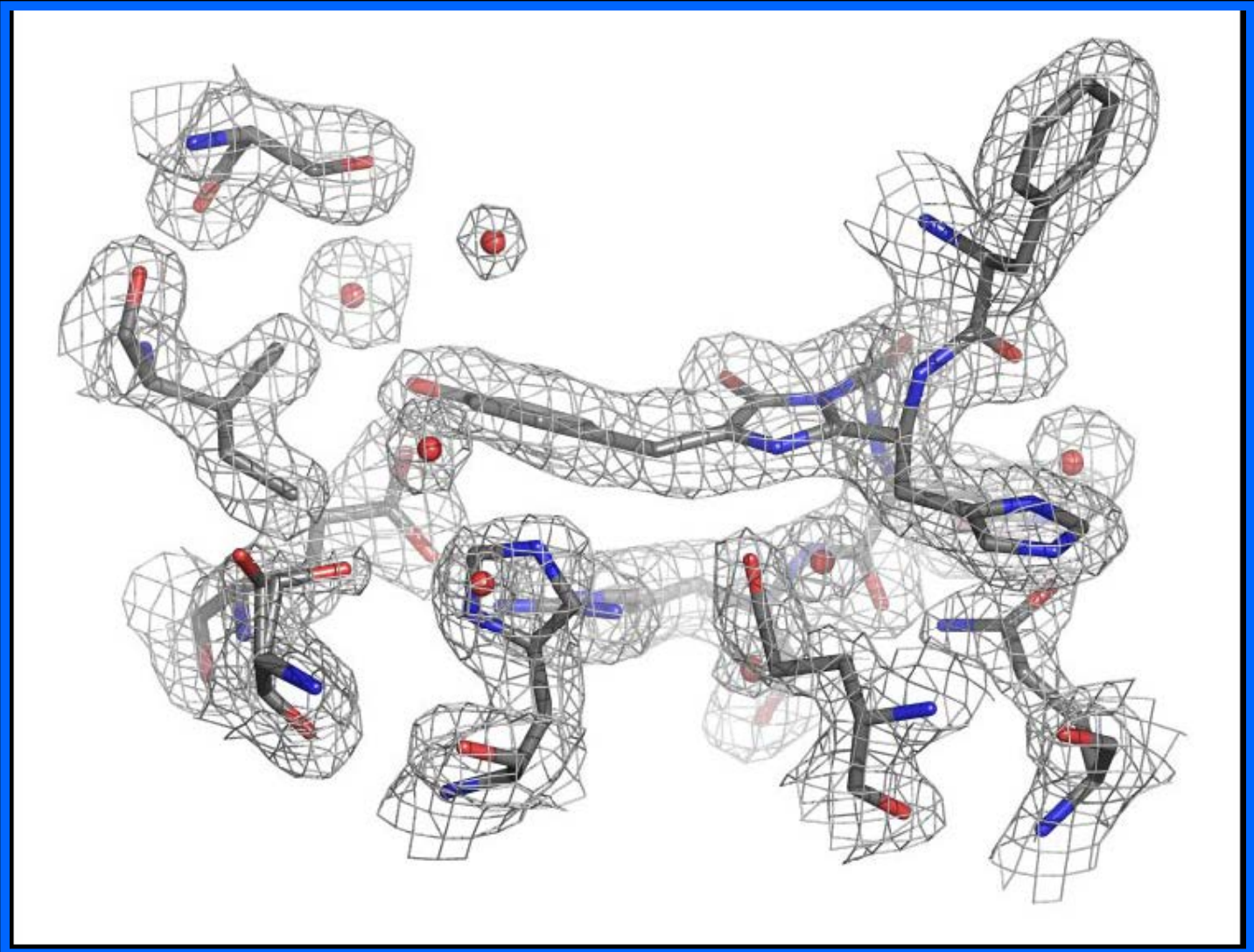


Photoconversion



Photoswitching

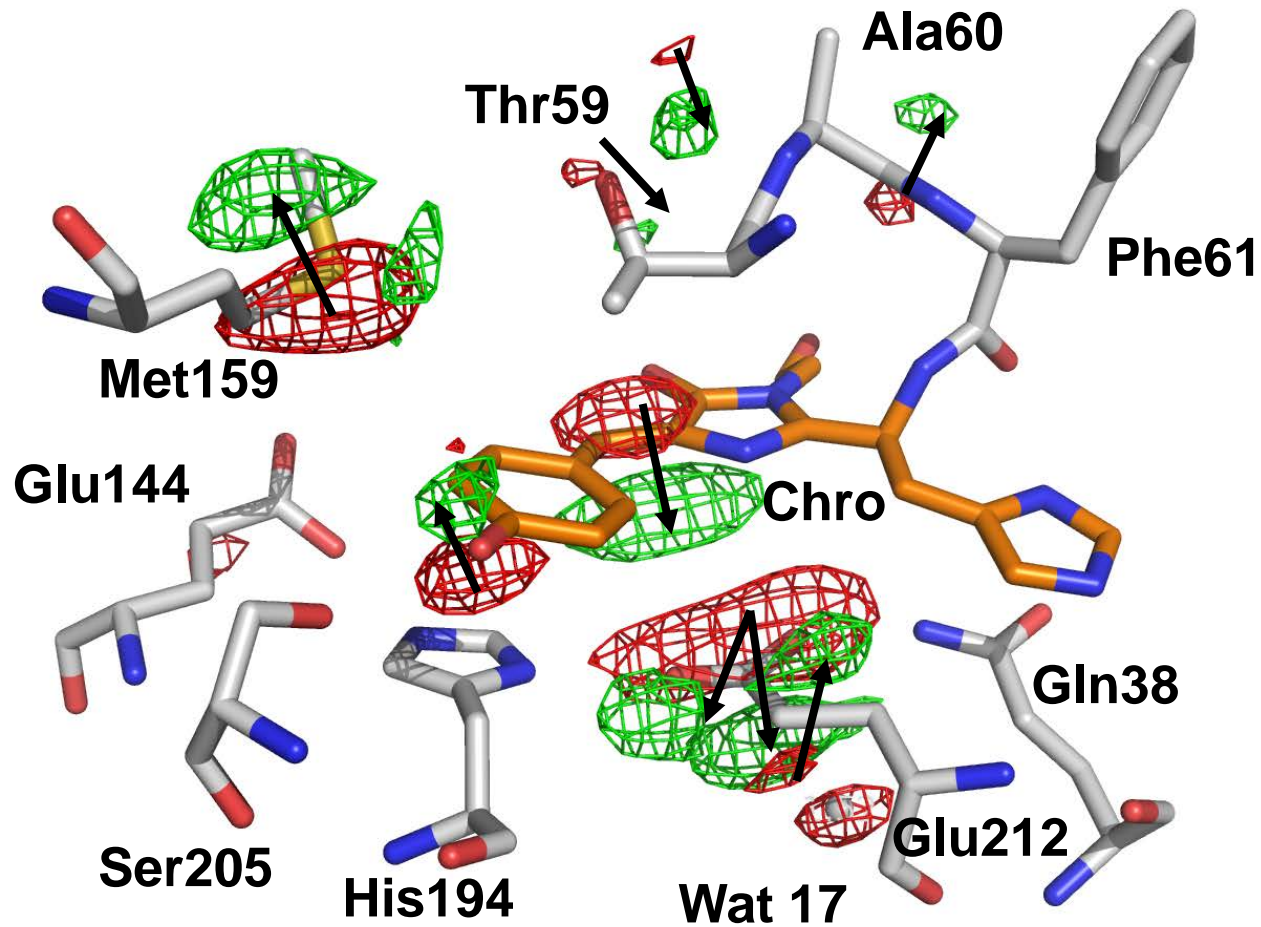




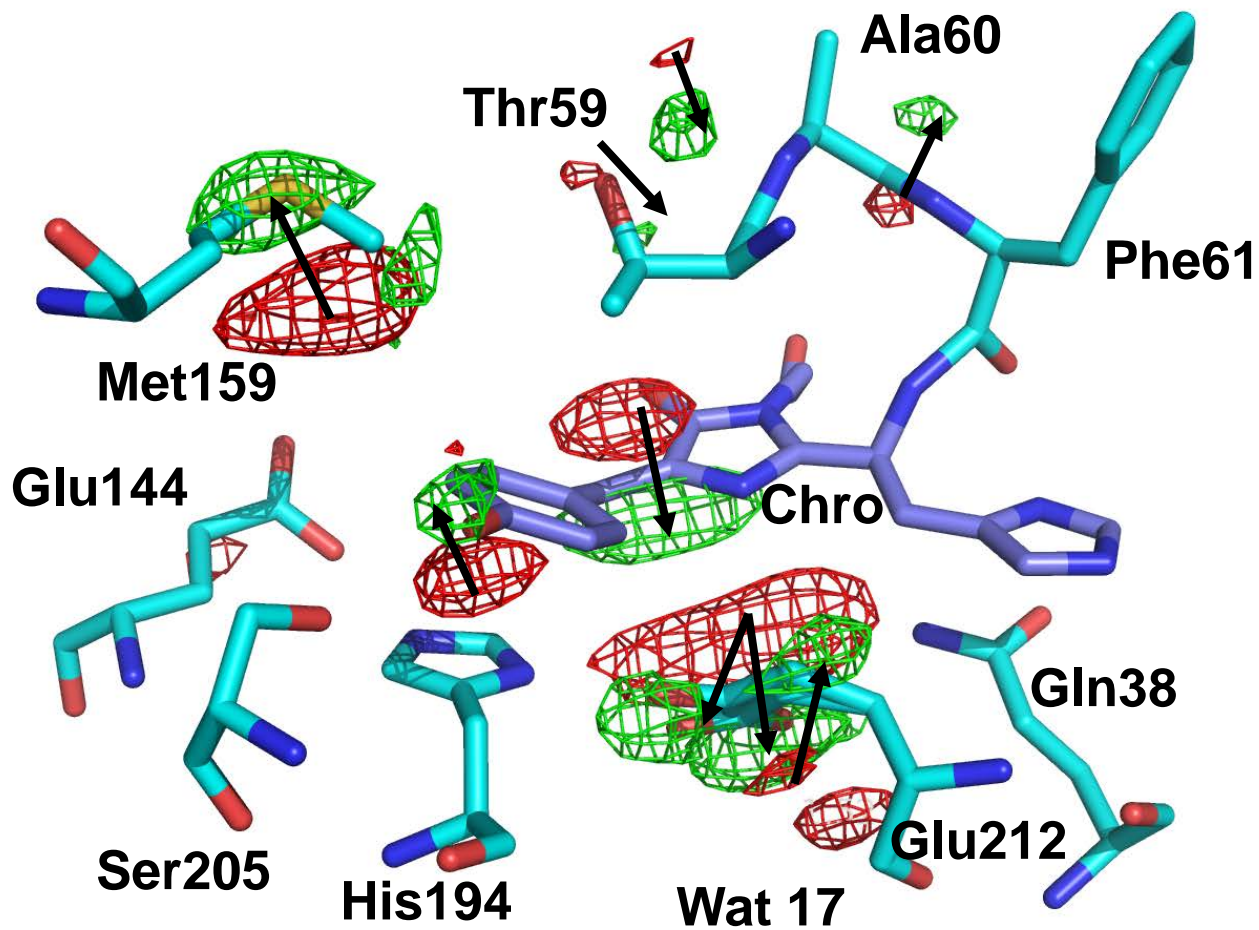
Blinking

Adam *et al*, *JACS*, 2009, 131, 18063
Roy *et al*, *JACS*, 2011, 133, 18586

Blinked state: Structural changes



Blinked state: Structural changes



ON

$+e^-$

Chro

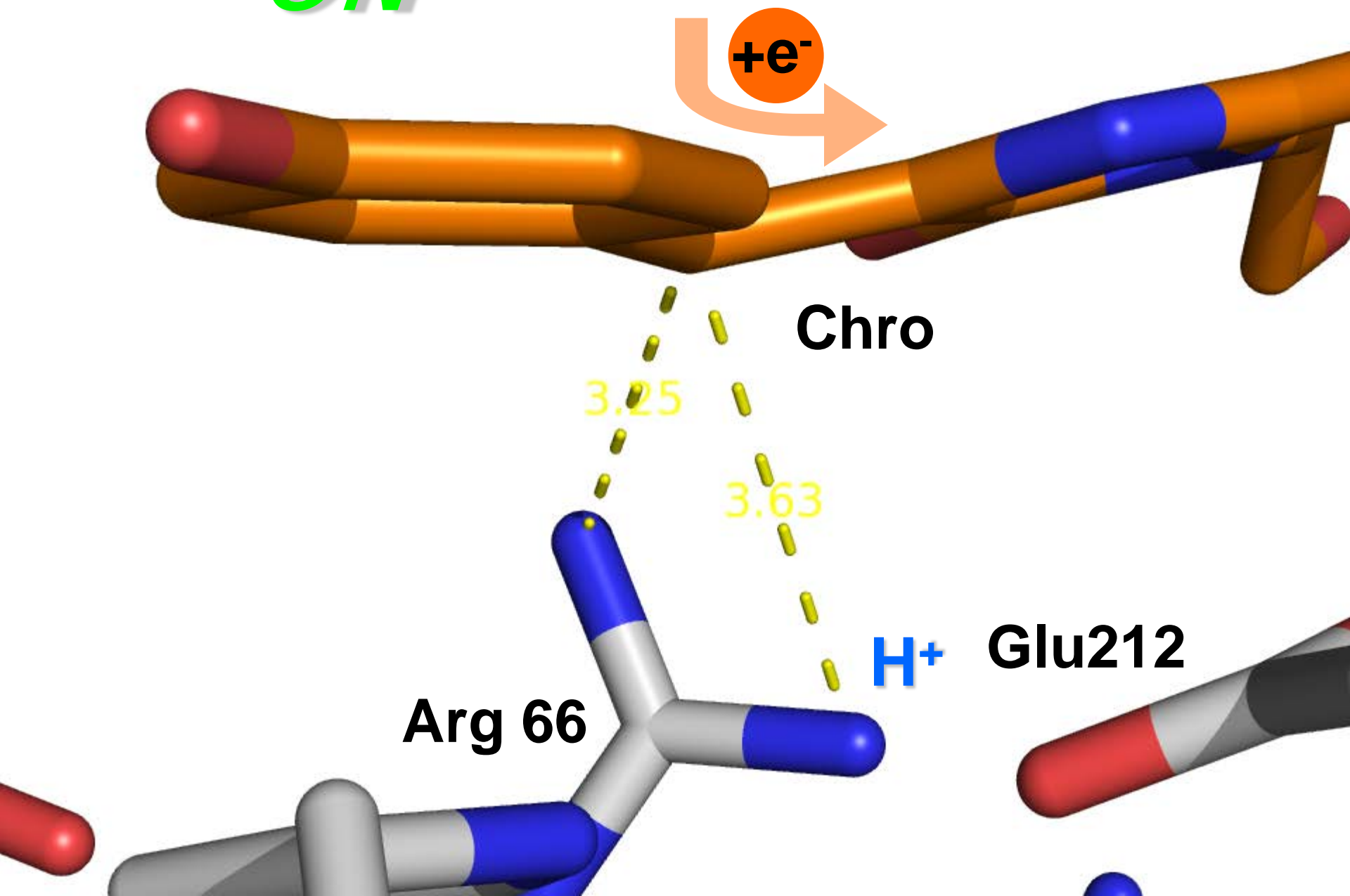
3.25

3.63

H^+

Glu212

Arg 66



OFF

*Chromophore transient
protonation on C_{α} atom.*

Chro

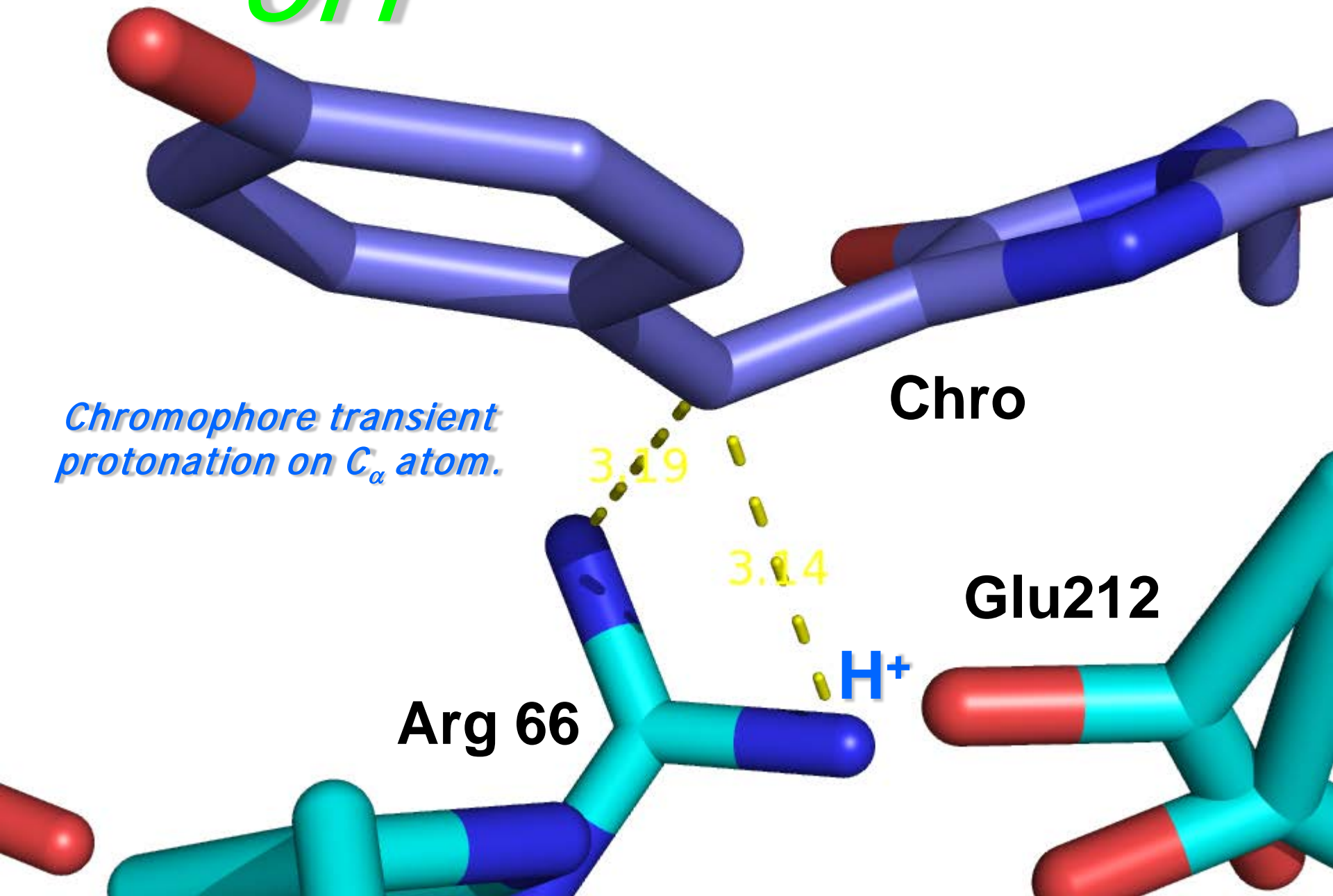
3.19

3.14

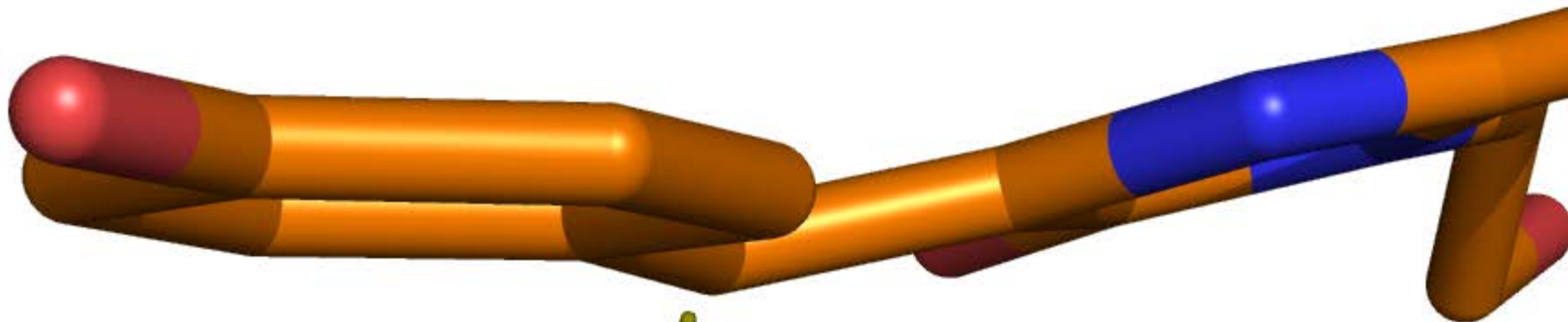
H⁺

Arg 66

Glu212



ON



H⁺Chro

Back to fluorescent state

3.25

3.63

Arg 66

Glu212



OFF

*Chromophore transient
protonation on C_{α} atom.*

Chro

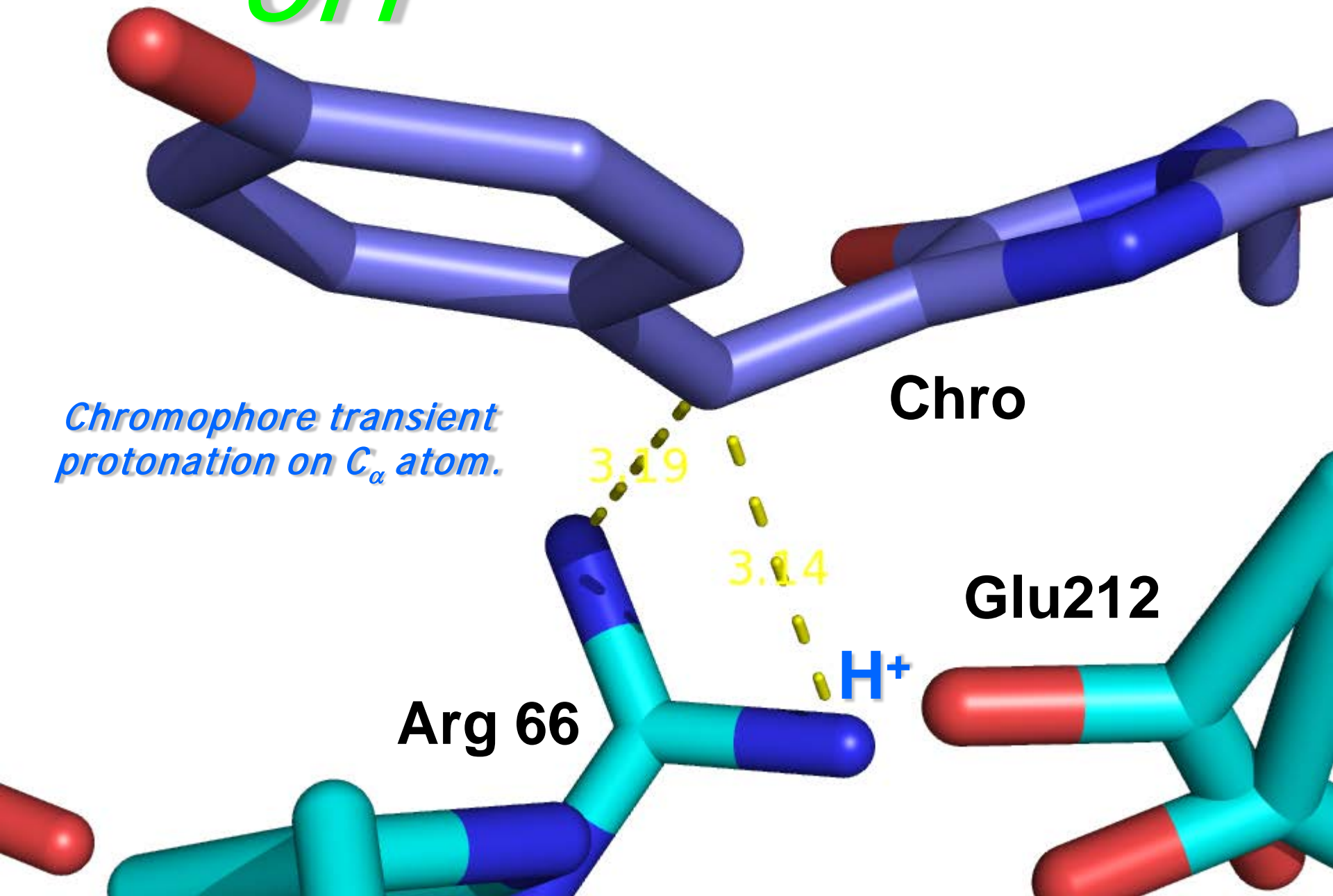
3.19

3.14

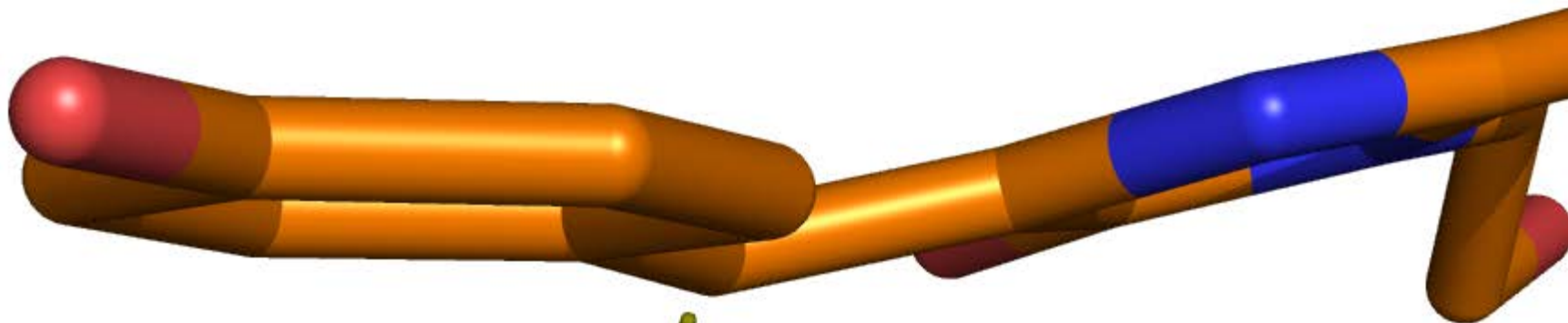
H⁺

Arg 66

Glu212



ON



H⁺Chro

Back to fluorescent state

3.25

3.63

Arg 66

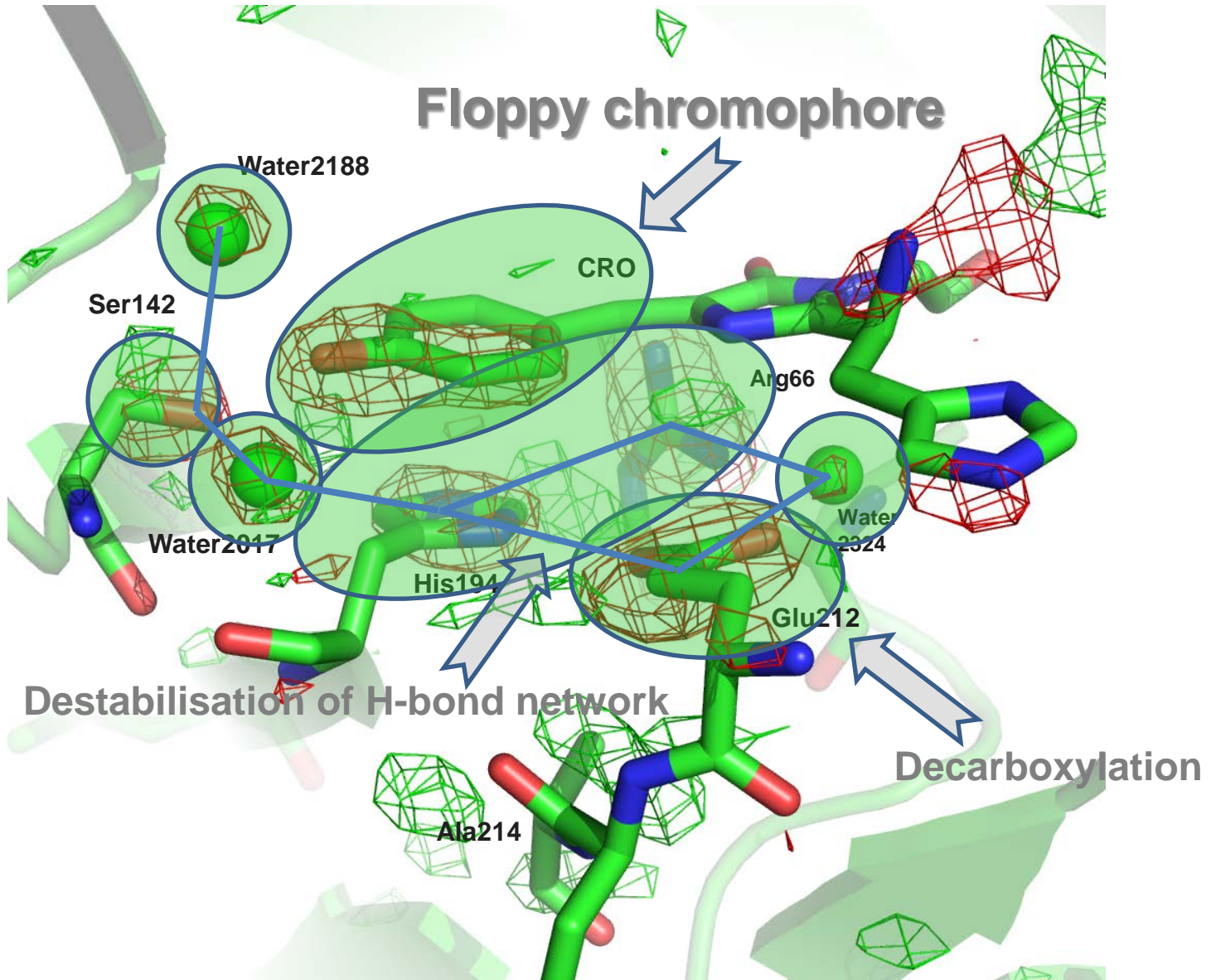
Glu212



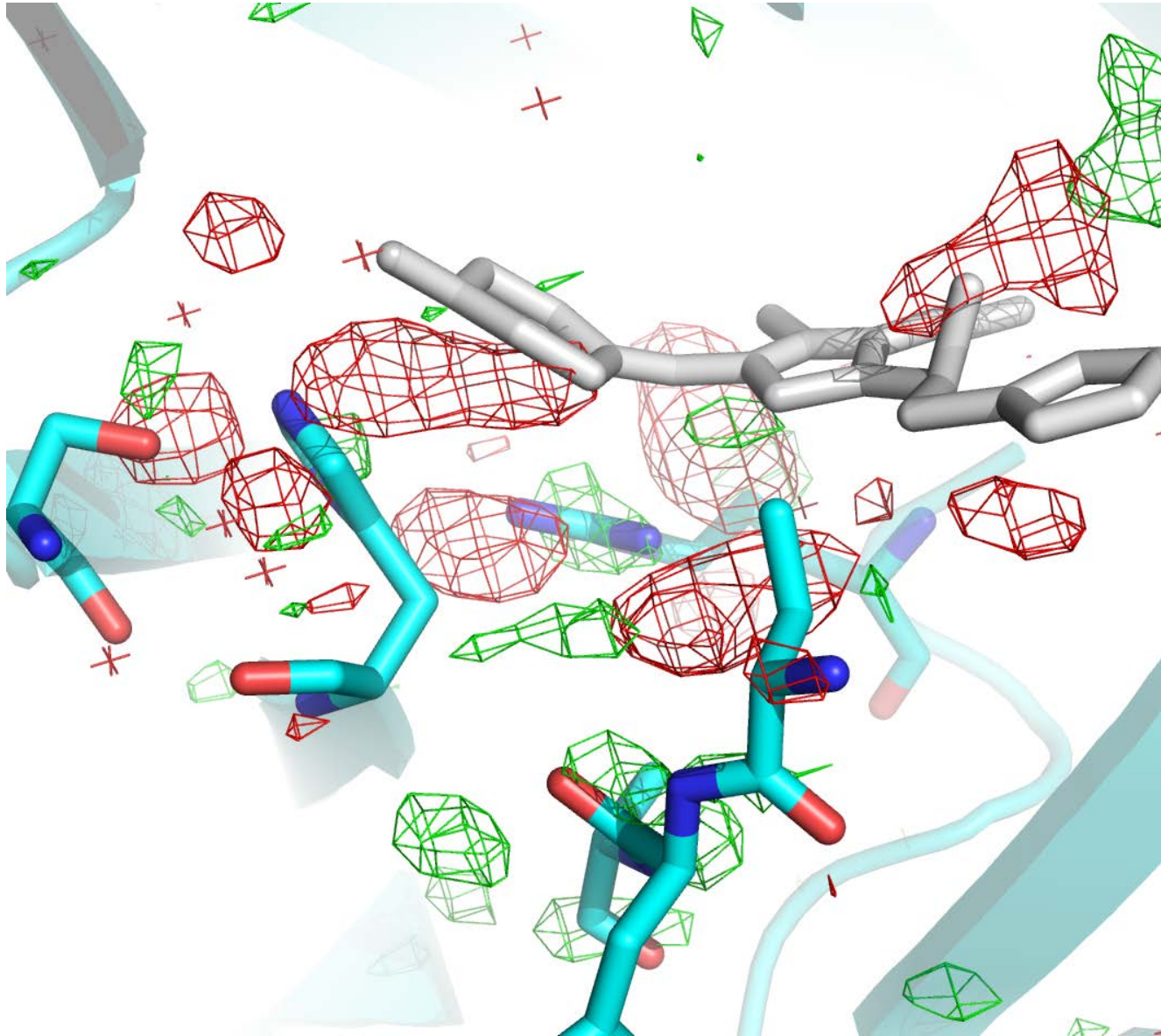


Photobleaching

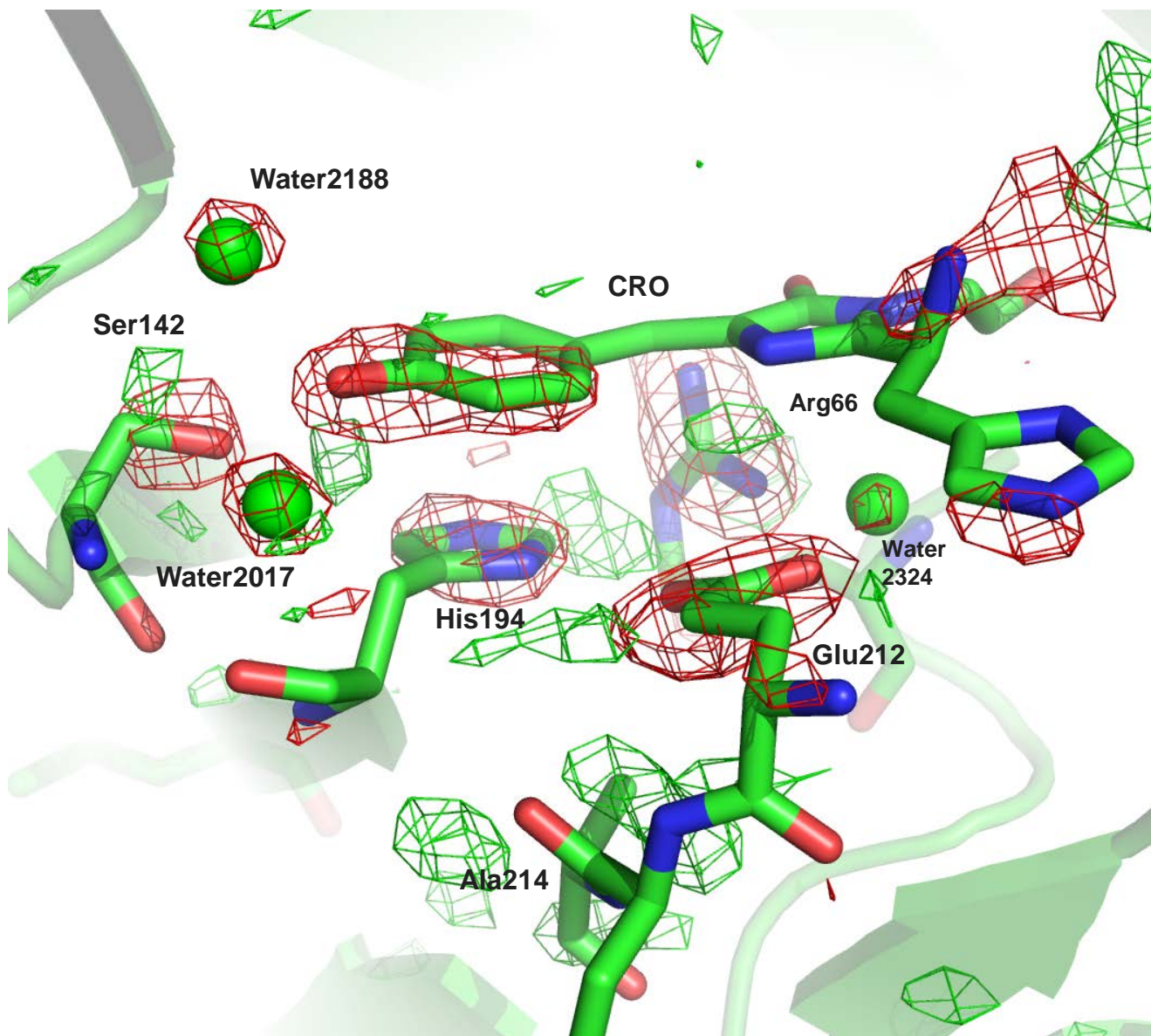
IrisFP Photobleaching

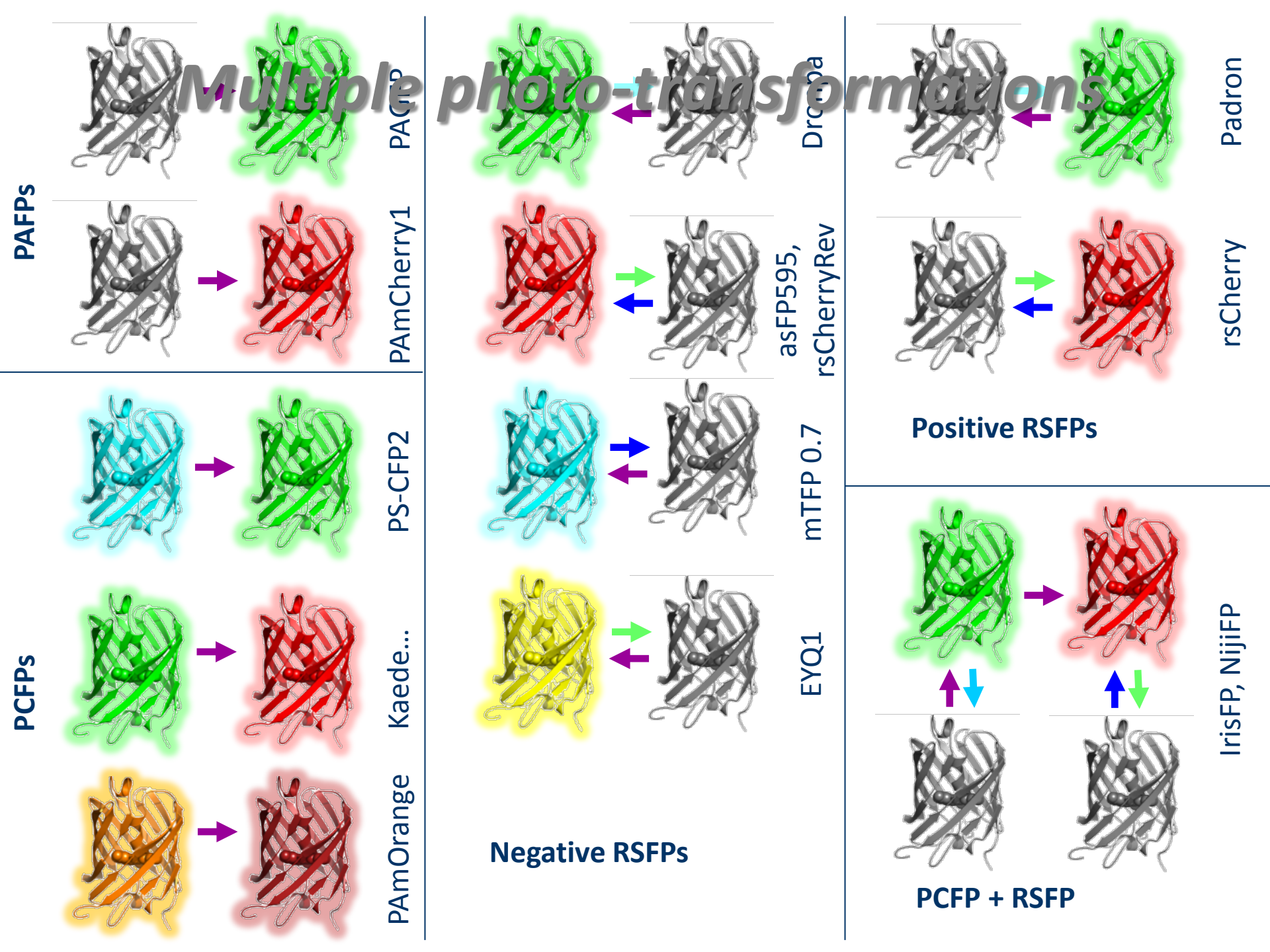


IrisFP Photobleaching



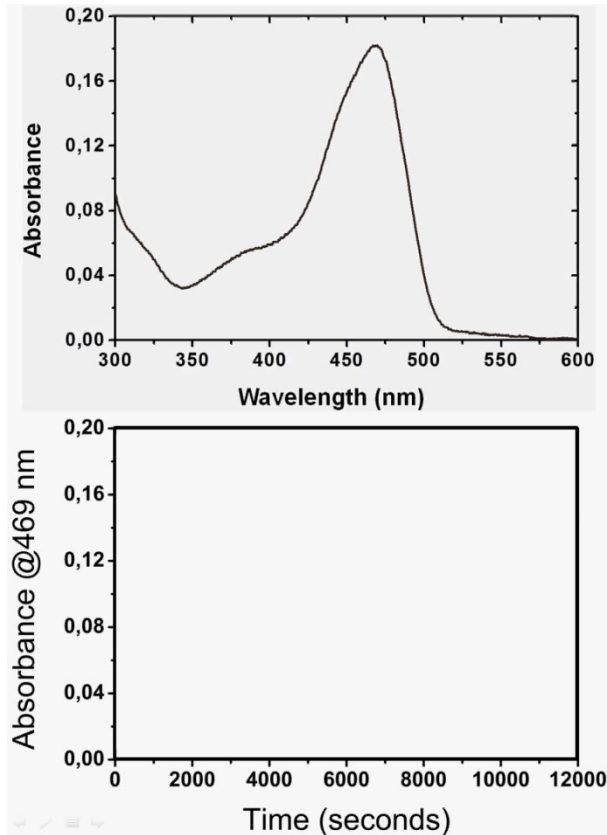
IrisFP Photobleaching



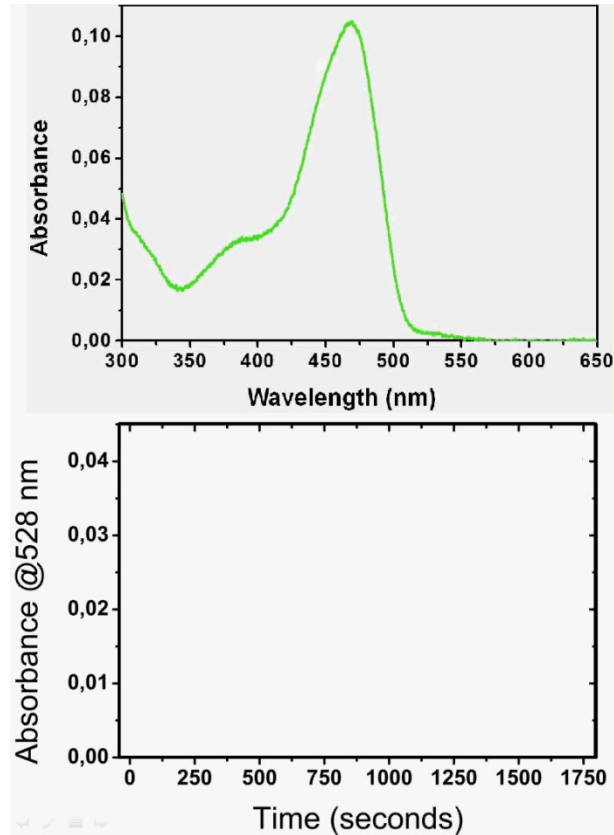


NijiFP (Dendra2 F173S): Multiple phototransformations *in vitro*

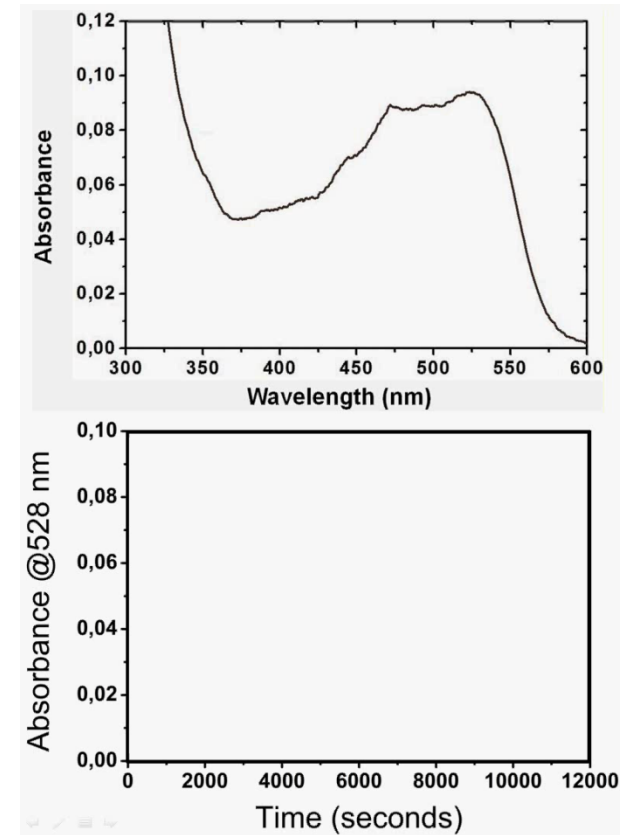
Green on/off switchings



Green-red photoconversion



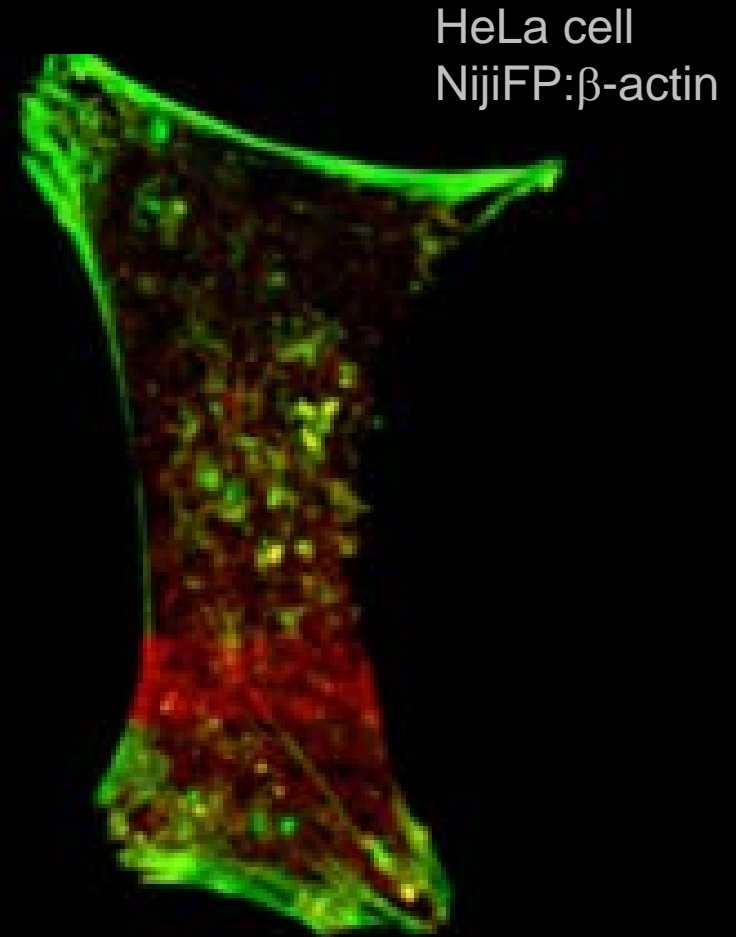
Red on/off switchings



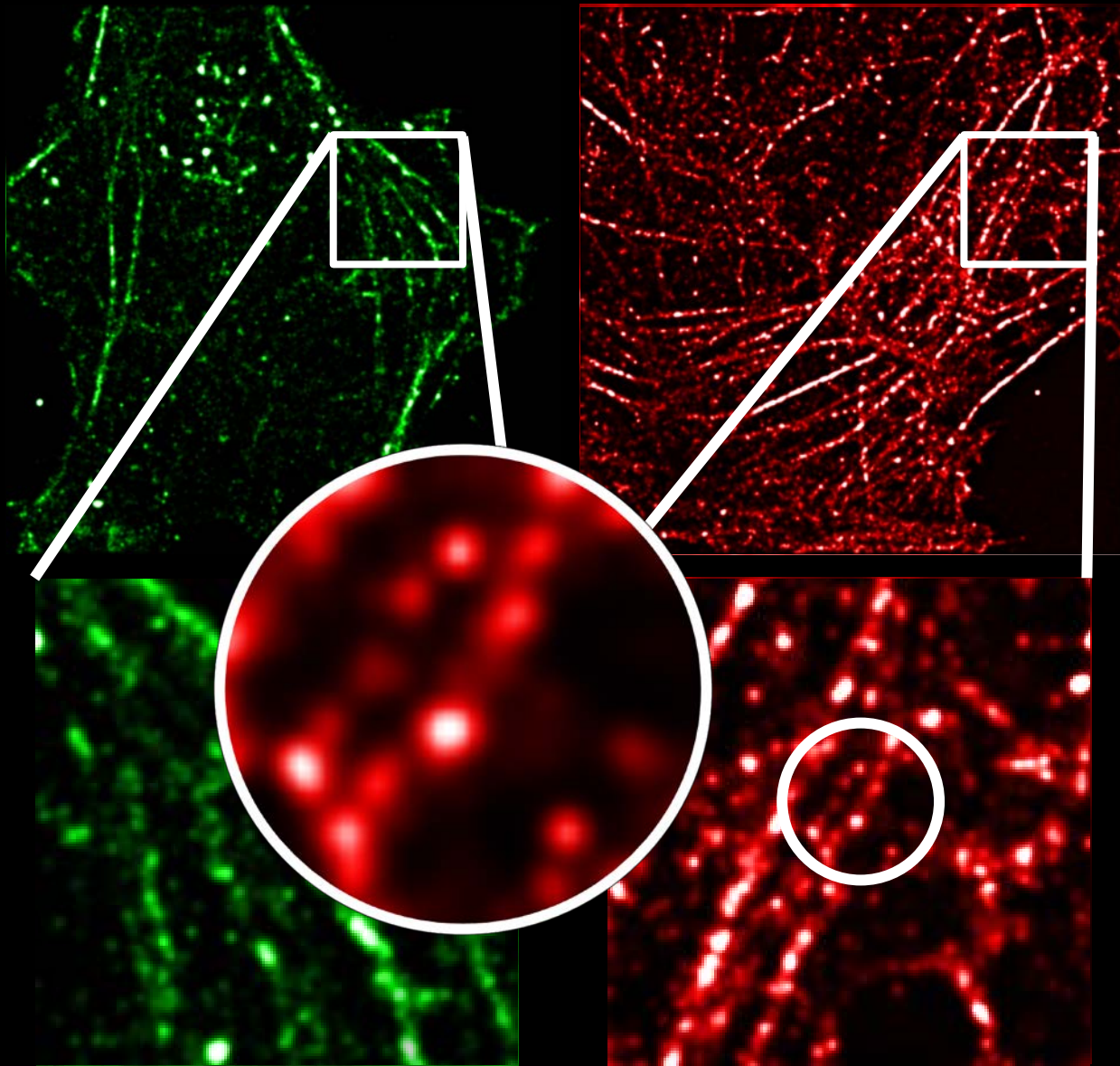
- ✓ Excellent brightness
- ✓ High contrast between on and off states
- ✓ Good resistance to photofatigue

Pulse-chase experiments in live-cells

- ✓ Green and red diffusing molecules can be observed differentially
- ✓ Dynamic tracking experiment
- ✓ Combination with PALM
(*Fuchs et al, (2010) Nat. Methods 7, 627-630*)



PALM on eukaryotic cells



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