Time-Resolved Serial Femtosecond Crystallography at Free Electron Lasers

Marius Schmidt

Jan. 25, 2017

National Science Foundation STC

BioXFEL

Time-Resolved Crystallography

goals

- extract kinetics and dynamics
- kinetic mechanism
- rate coefficient
- barriers of activation

and

- molecular structures of reaction intermediates

for

- *reversible* reactions

and

- *irreversible* (catalyzed) reactions

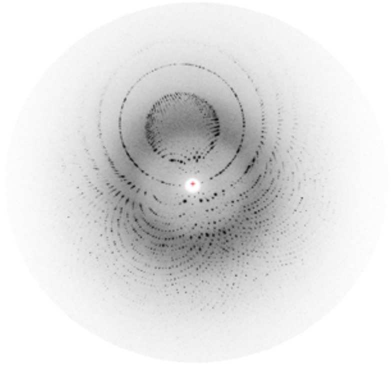
from

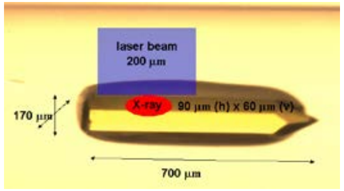
crystallographic data *alone*

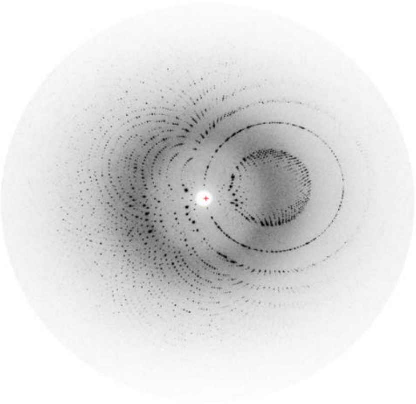
Time-Resolved Crystallography Works with Difference Maps

time resolved scattering patterns

corresponding dark exposures



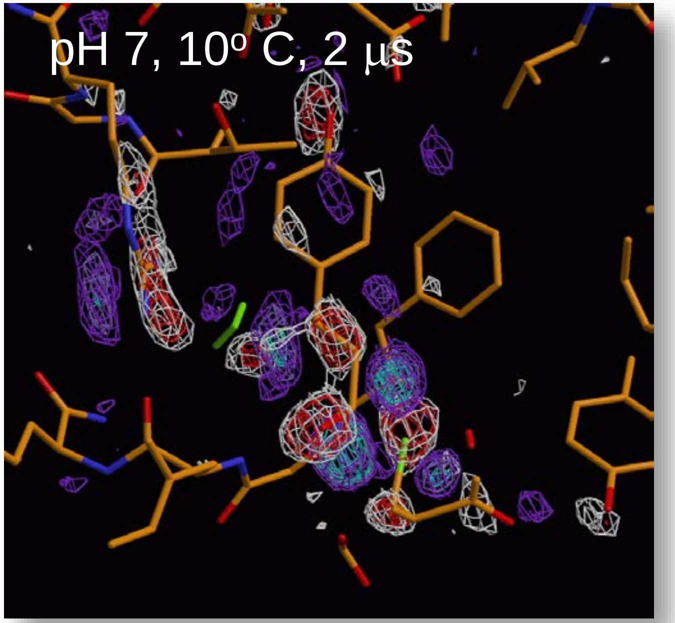




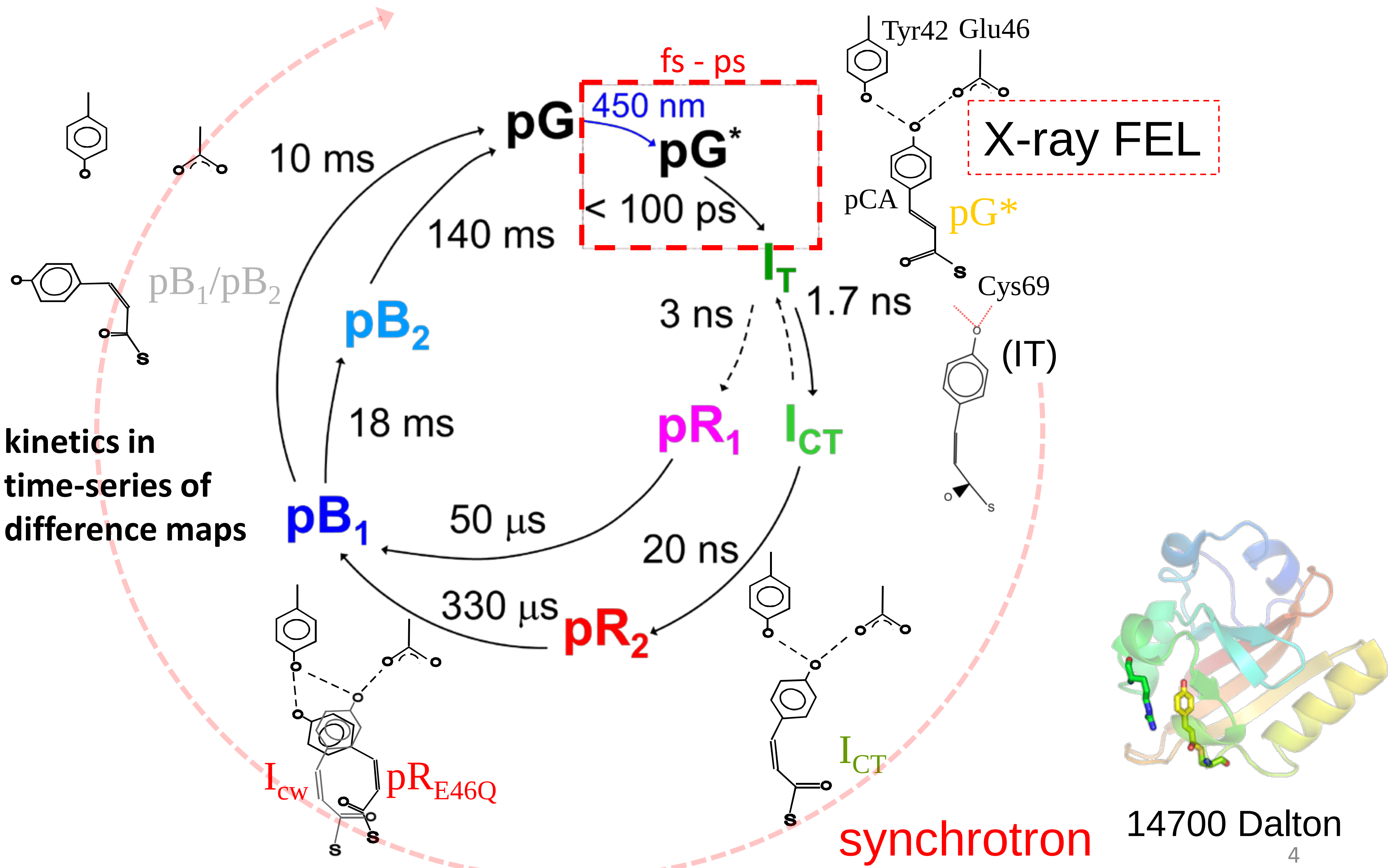
spacegroup	$P6_3$, hexagonal
unit cell [Å]	a=66.8 c=41
resolution [Å]	0.9 [cryo] 1.5 [Laue] 1.5 [LCLS]

Pump-probe
Mix-and-inject
T-jump
…

difference electron density maps $\Delta\rho(x,y,z,t)$



Photocycle of Photoactive Yellow Protein
a bio-molecular reaction



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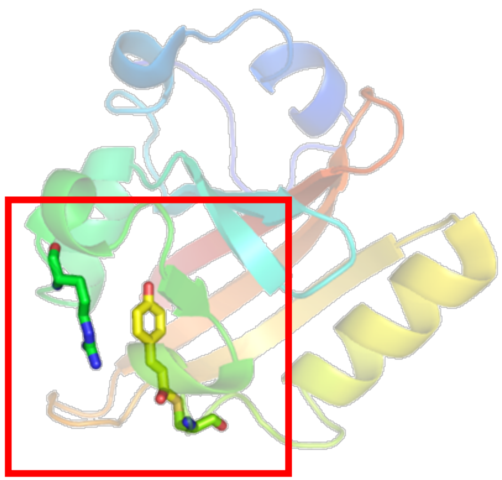
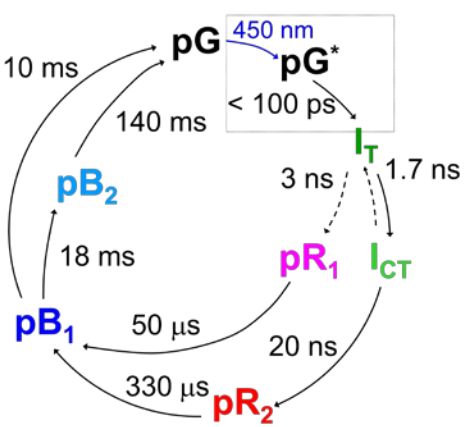
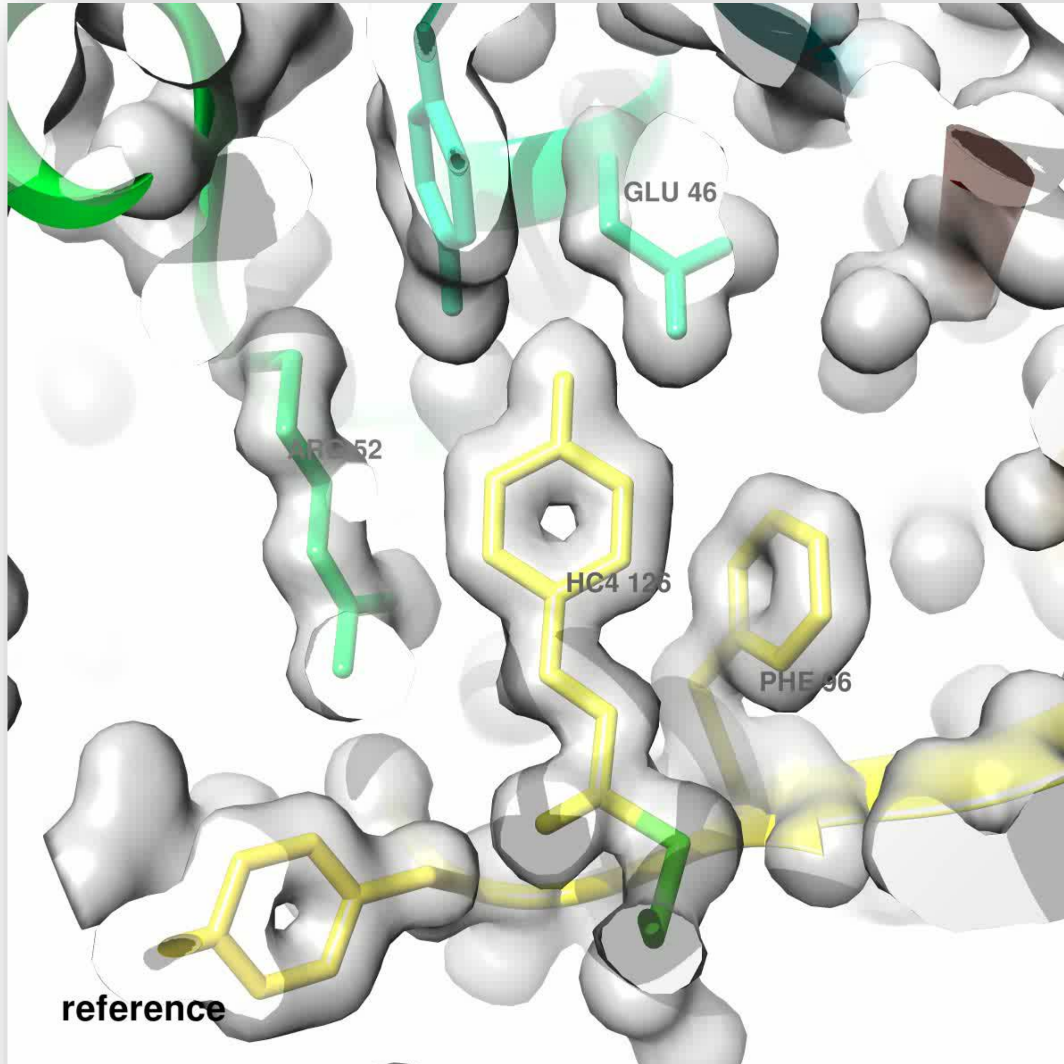
Movie
the Interpretation of the Kinetics

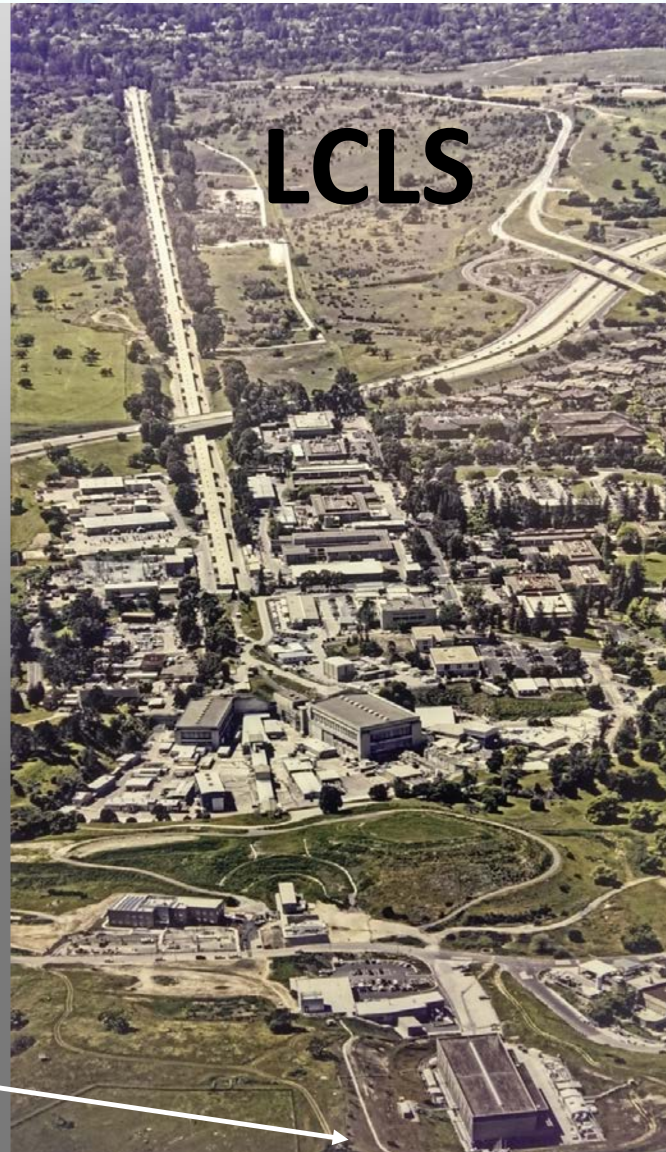
Schmidt et al., Acta Cryst D, 2013
assisted by singular value decomposition







The SFX Revolution: X-ray Free Electron Lasers



40 fs X-ray pulses
10^{12} photons/pulse
1 µm focal spot
9.5 keV (~1.3 Å)
120 Hz repetition

CXI instrument

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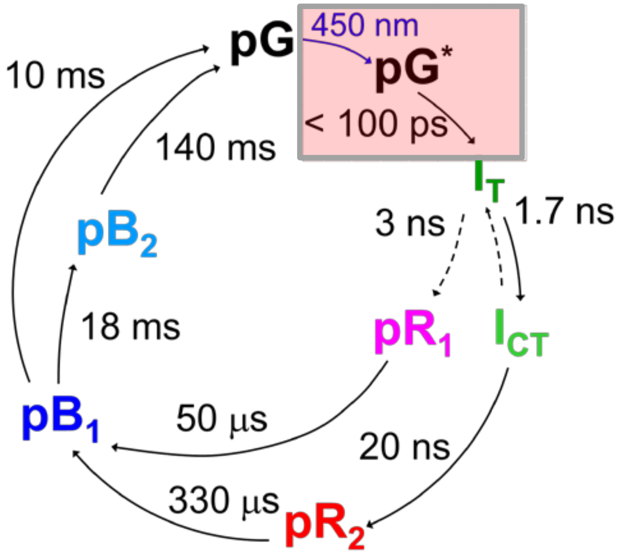
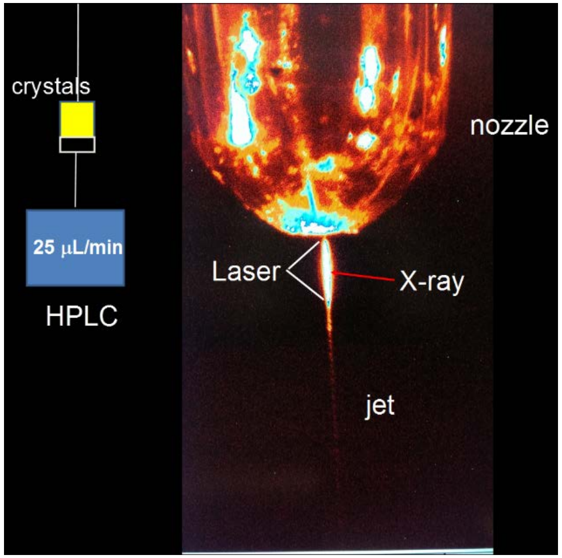
The SFX Revolution: X-ray Free Electron Lasers

Goals

- time-resolved crystallography at the X-ray FEL
- microcrystals rather than macrocrystals
- near atomic resolution
- femtosecond time resolution
- bio-medically relevant systems





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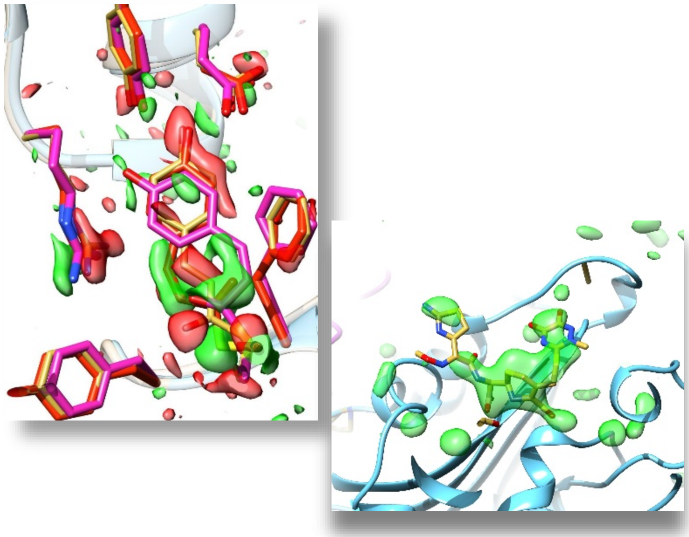
Our Recent Work at Free Electron Lasers

LCLS

I. TR-SFX with near atomic resolution
LCLS beamtime LD62
Tenboer et al., 2014, Science



II. TR-SFX with fs time resolution
LCLS beamtime LG09
Pande et al., 2016, Science

III. Mix-and-inject experiment,
LCLS beamtimes LK17 and LN50
foundation of structure based enzymology, Kupitz et al., 2016, Struct. Dyn.

IV. Experiments at LCLS + SACLA

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I. Time-Resolved Serial Femtosecond Crystallography

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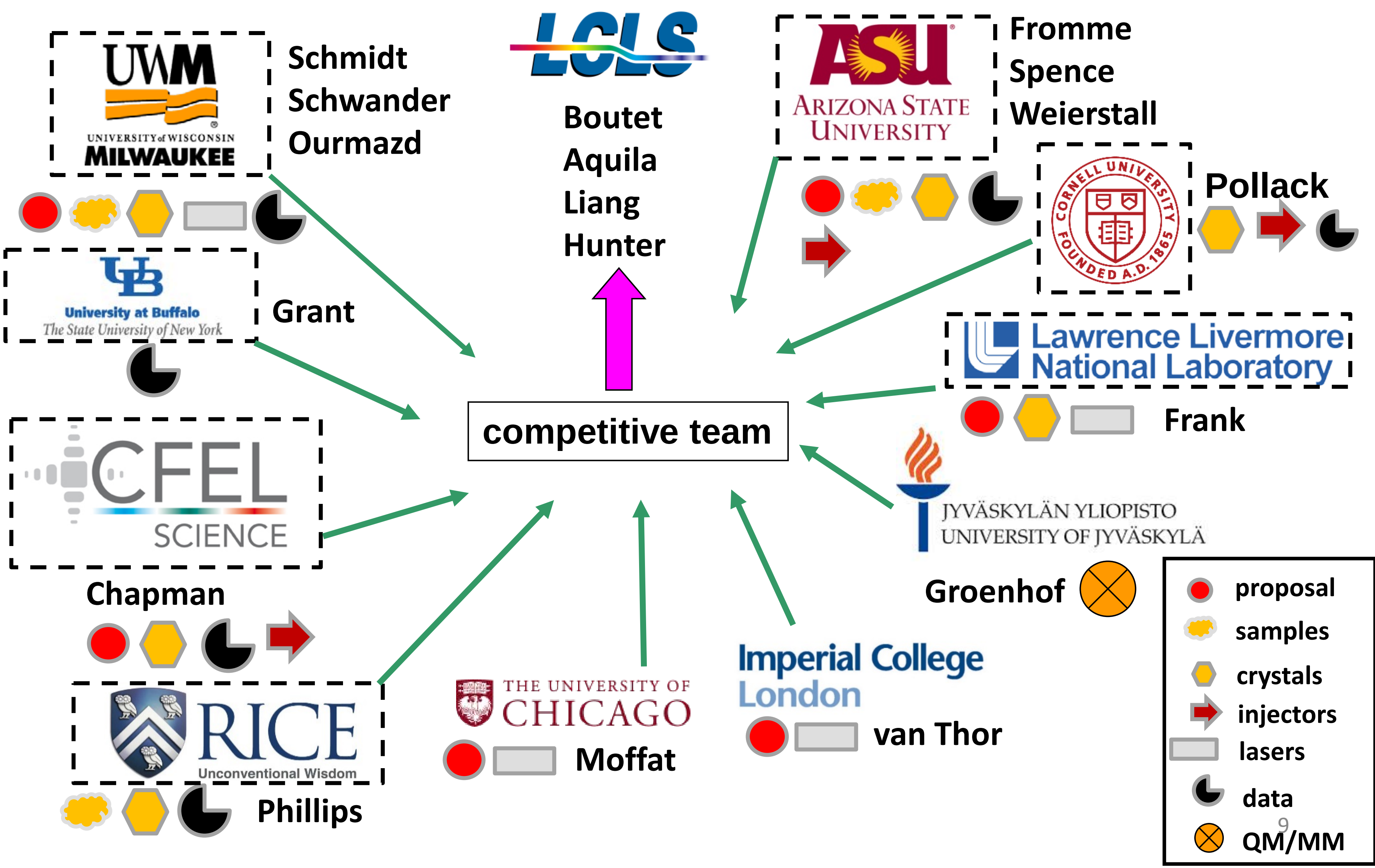


BioXFEL strategic plan

“the development and application of free-electron X-ray lasers to structural biology, with growing emphasis on time-resolved imaging”

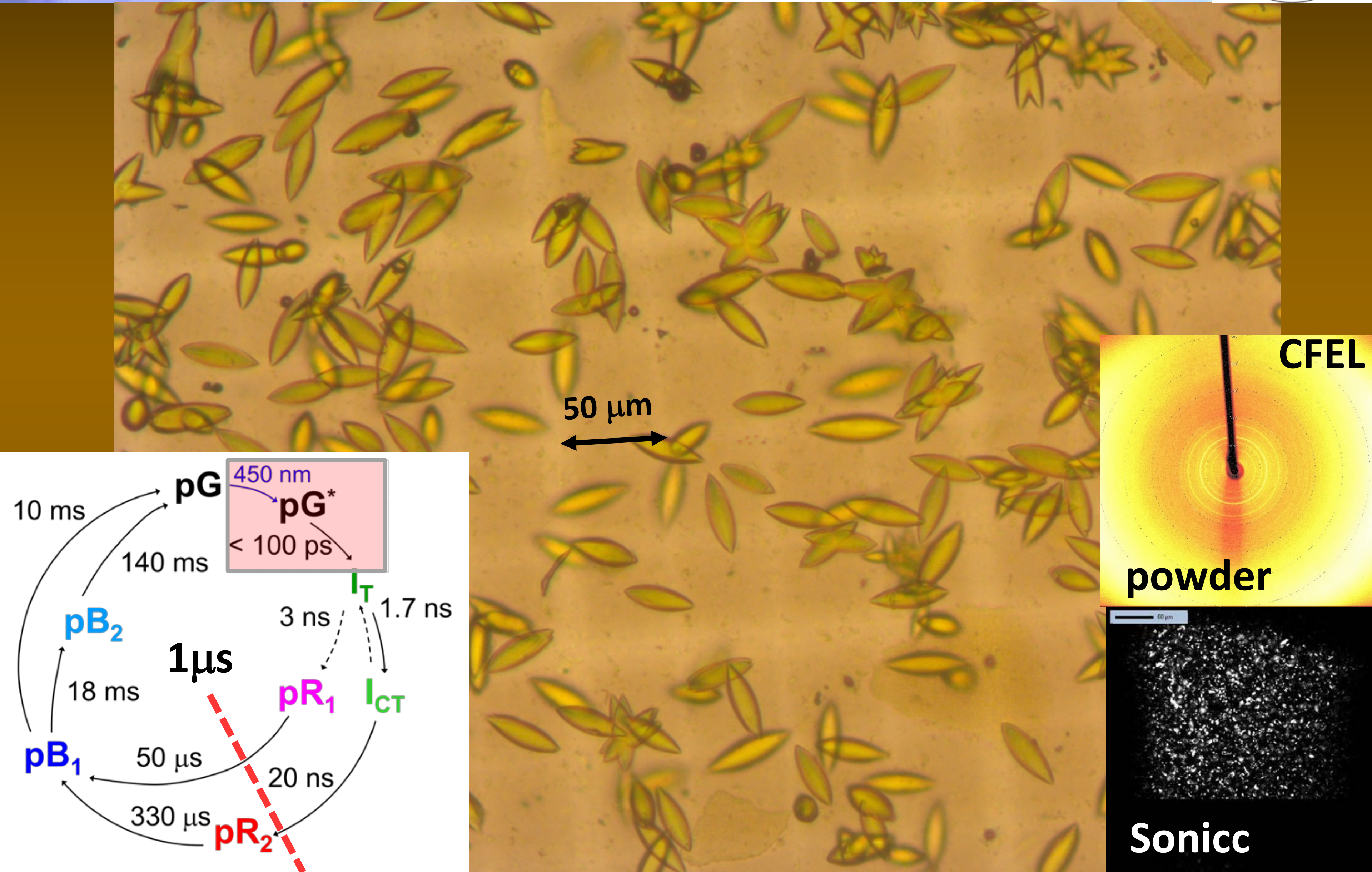
1. develop nanocrystallography, including use of viscous media.

2. achieve ***time-resolved nanocrystallography*** and imaging of dynamics, on timescales from femtoseconds to seconds.

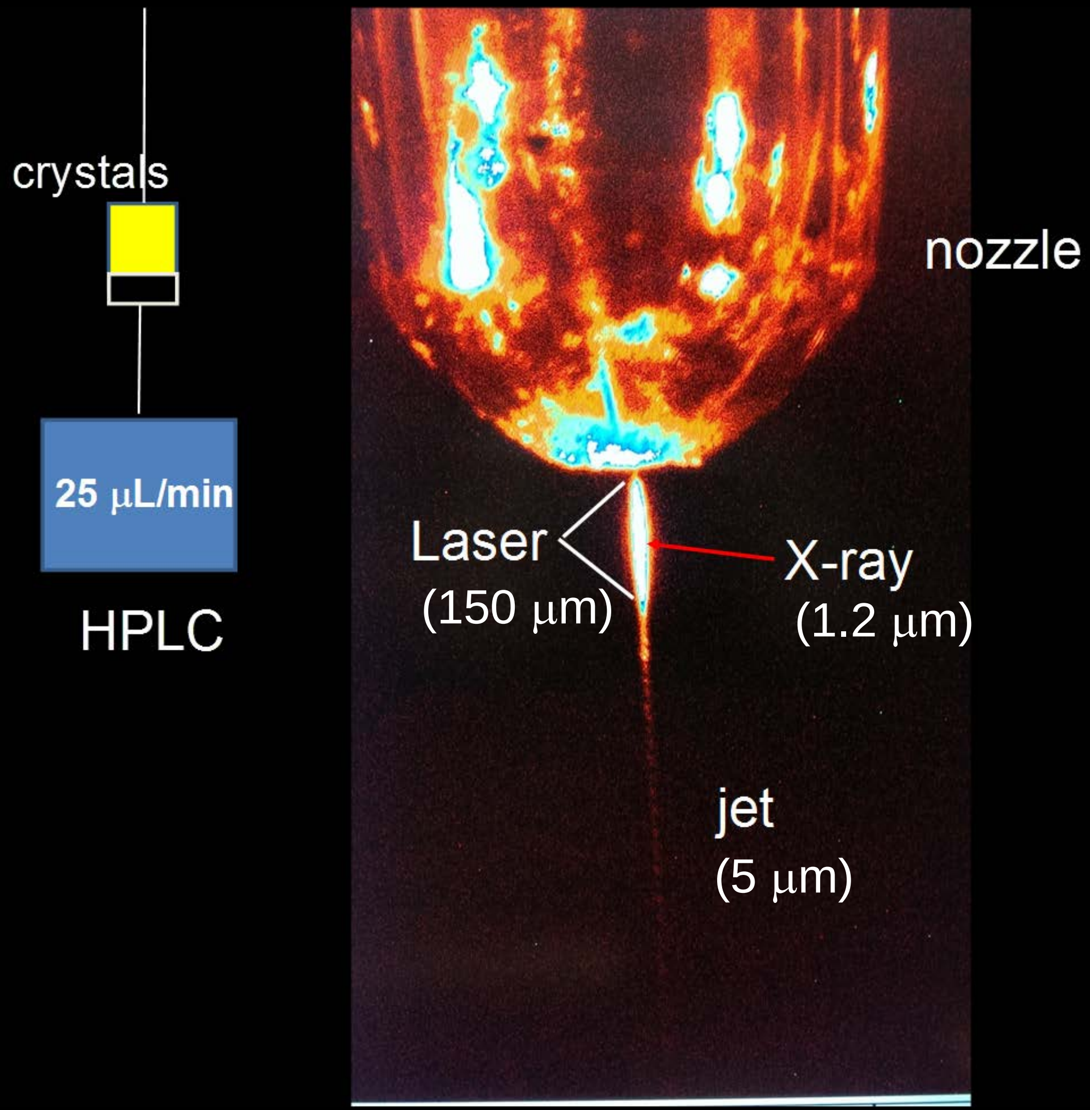
3. apply these methods widely to ***important biological targets***.

4. enable the determination of structure and function without crystals using an X-ray laser.

These Crystals are a Factor 10 too Large!!! -

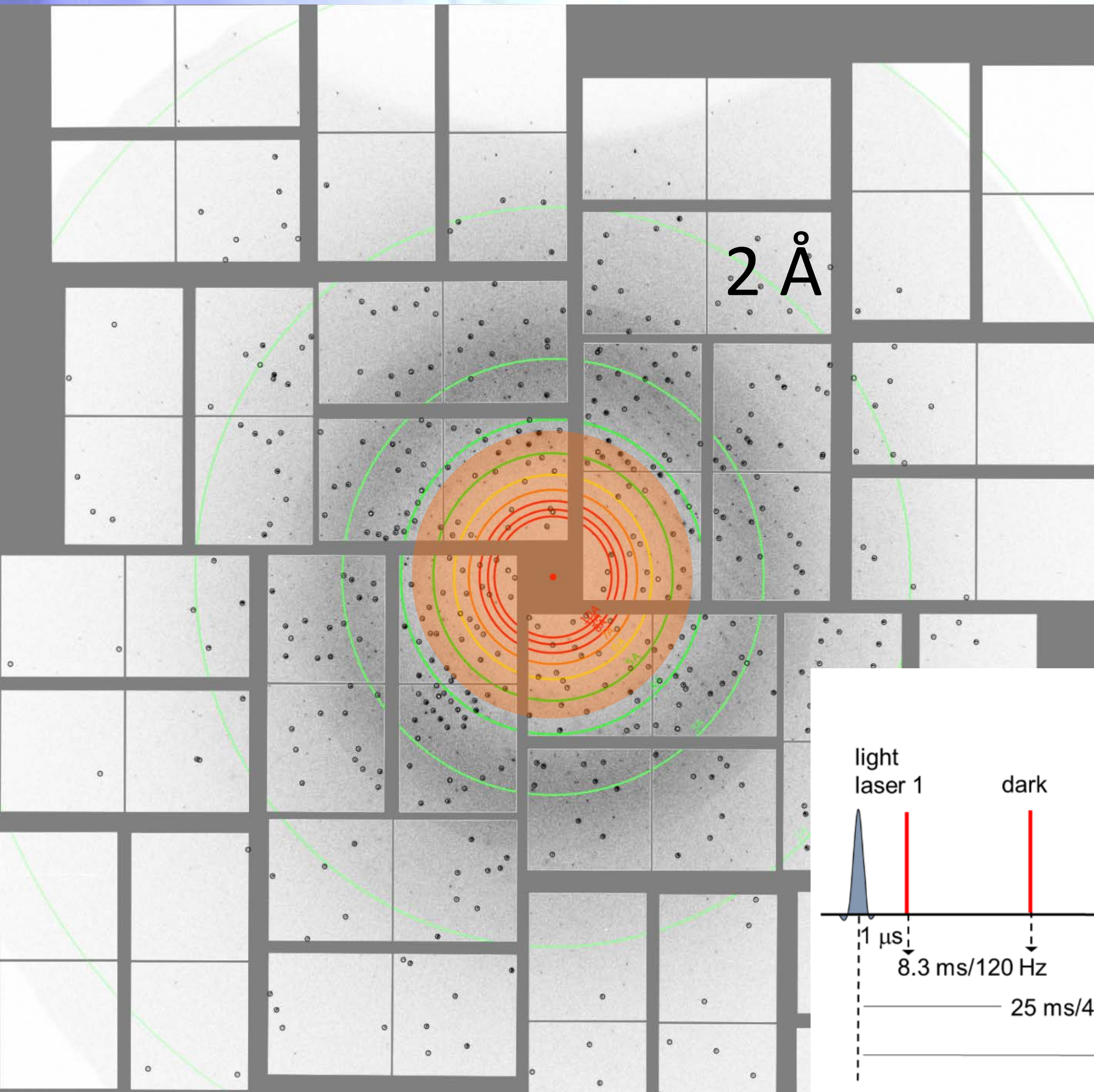


Nano/microcrystal Injection
Gas Dynamic Virtual Nozzle (GDVN)



B. Doak/J. Spence

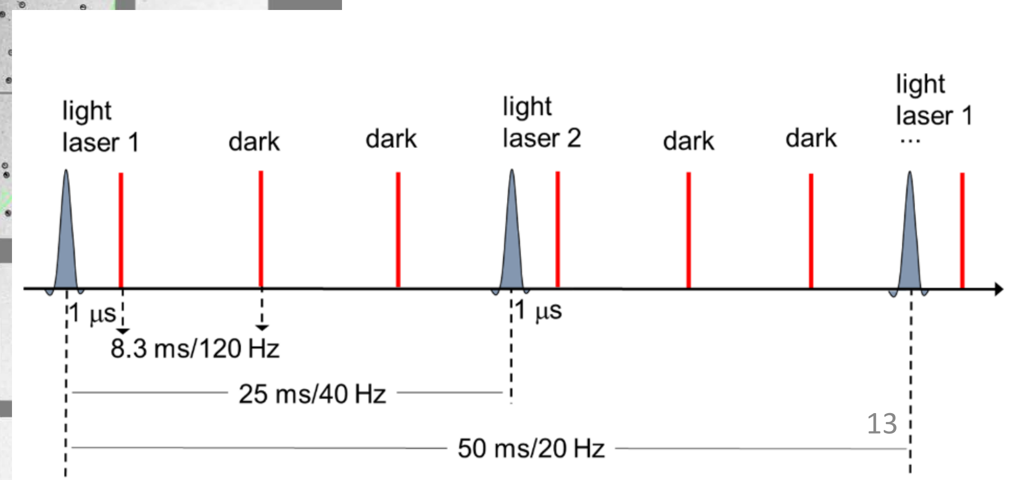
Diffraction Patterns
Special Detector, CSPAD



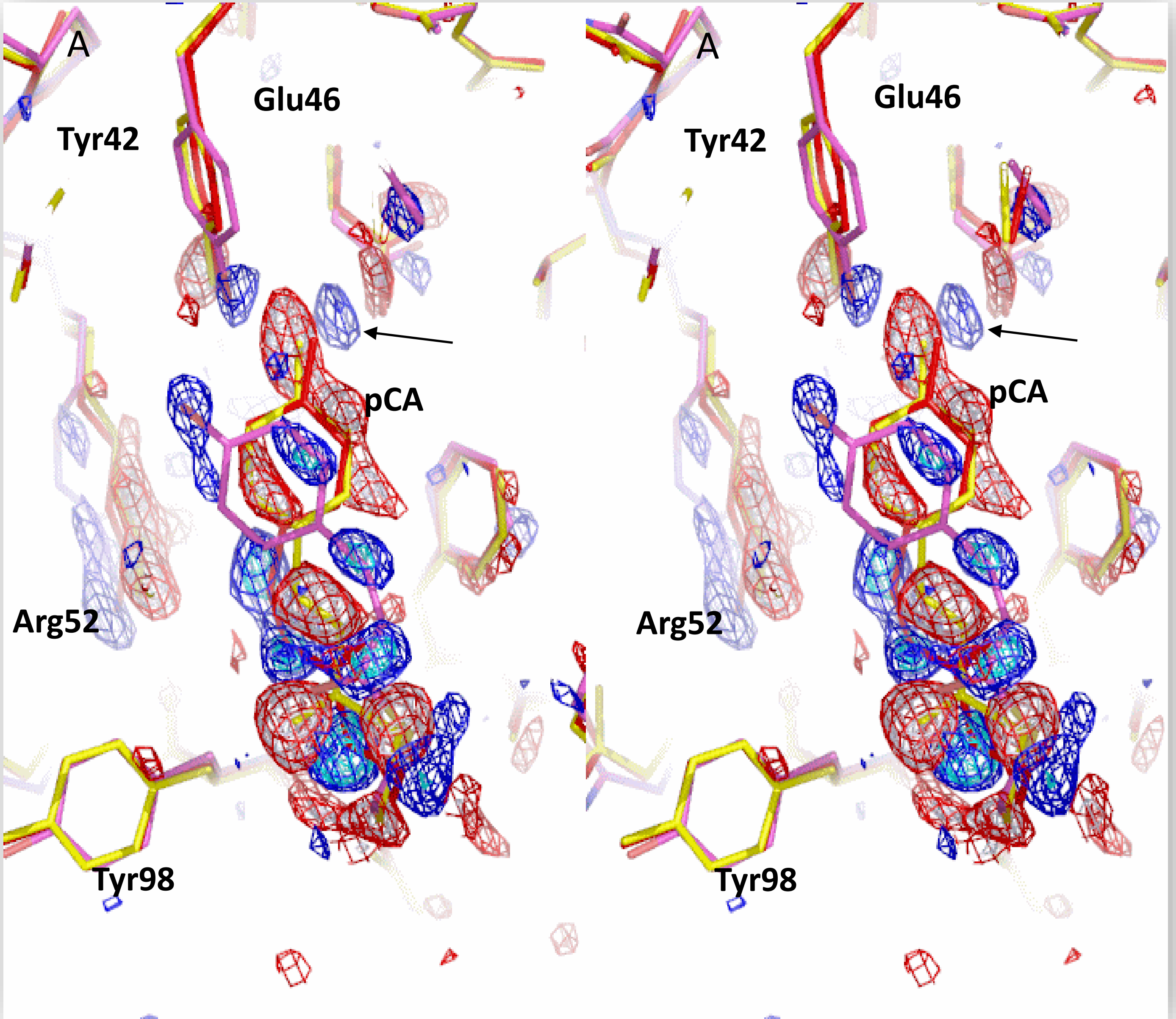
high quality diffraction

data analysis software
- CrystFel (T. White, DESY)

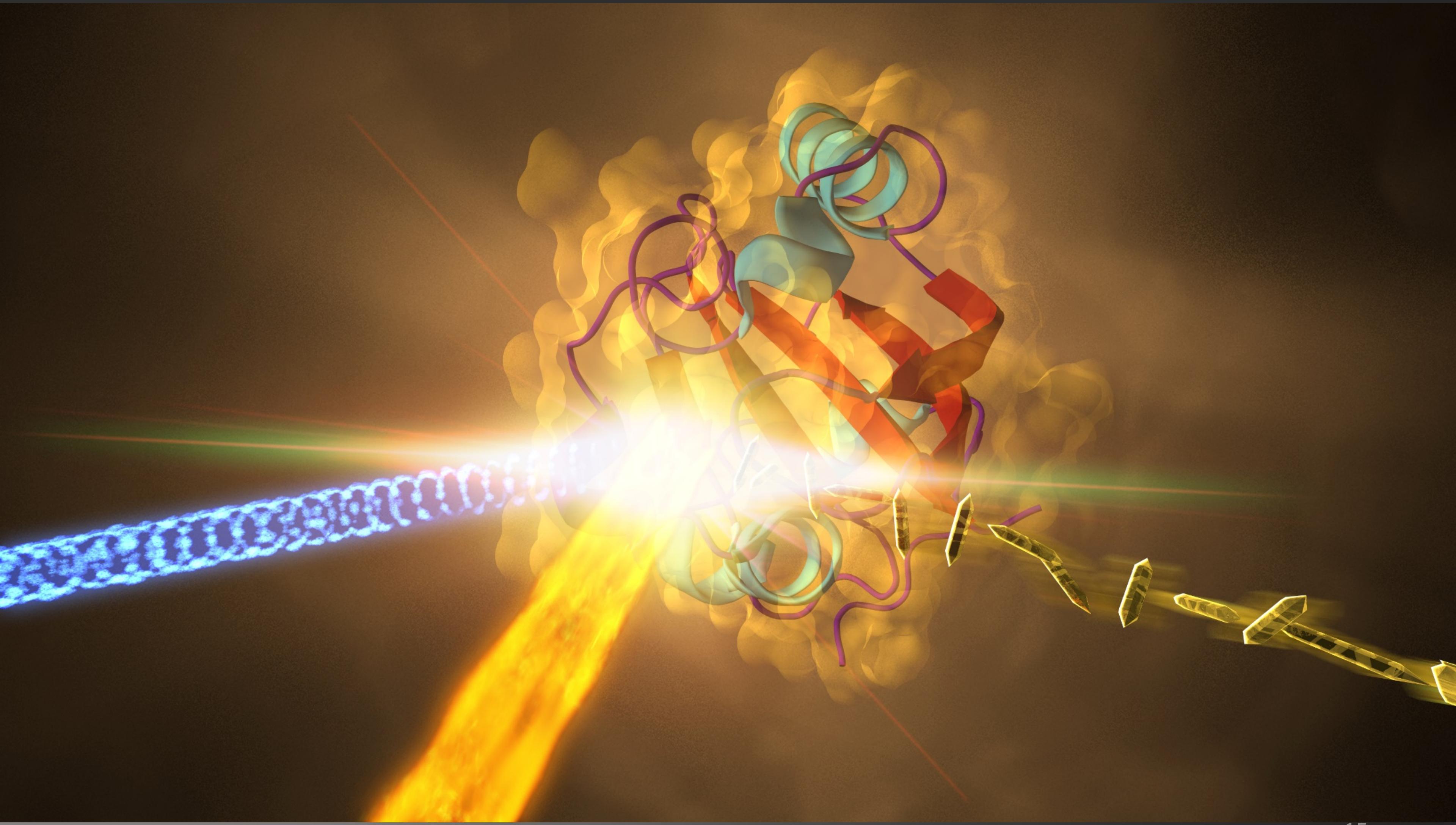
data collection
40 Hz Laser pulses, 120 Hz X-rays
- laser-on (pump-probe)
- dark
- dark



Difference Electron Density Maps
Resolution: 1.6 Å, Delay: 1 μs, Stereo



I. The Fastest Camera in the World "in 2014 "
Movie with 25 Trillion ! Pictures per Second



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II. Enables Movies on Ultrafast Time-Scales

- Structural changes are small
- Low photoactivation yield with femtosecond laser pulses

  (no chance with macroscopic PYP crystals)
- Exponential approach to chemical kinetics may be invalid
- ***Excited state*** dynamics rather than ground state dynamics
- New restraints for refining structure (no thermal equilibrium)
- QM/MM approaches for structural interpretation necessary

  (computational support necessary)

complexity

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Laser Excitation with 900 fs Laser Pulses

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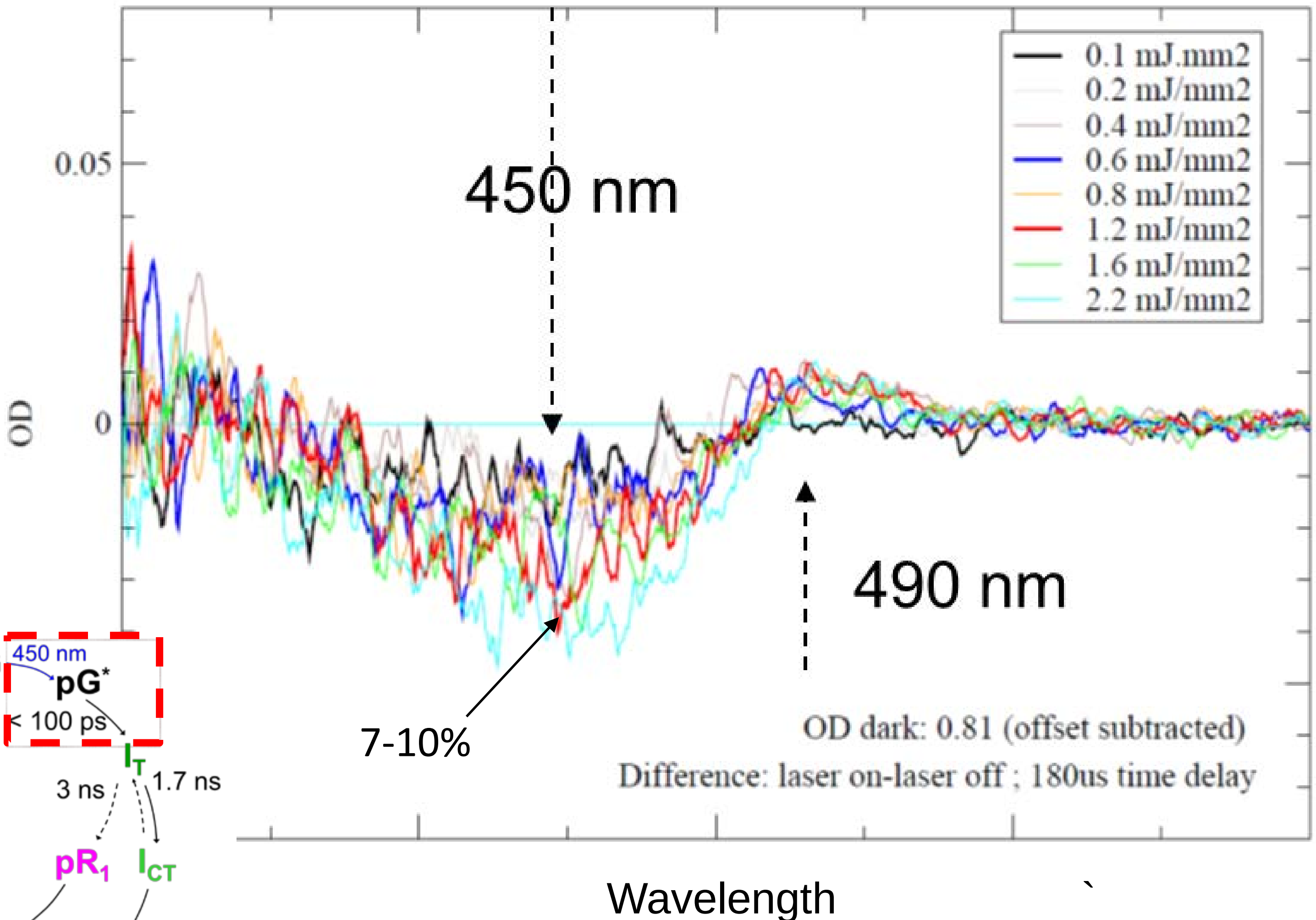
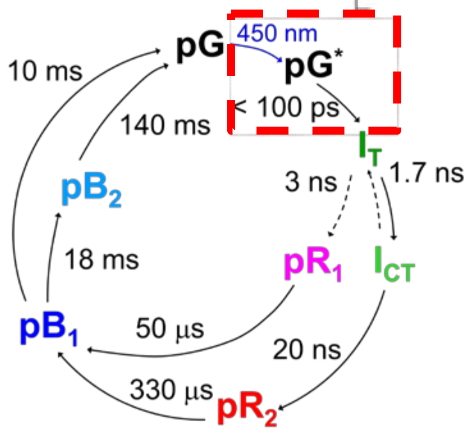
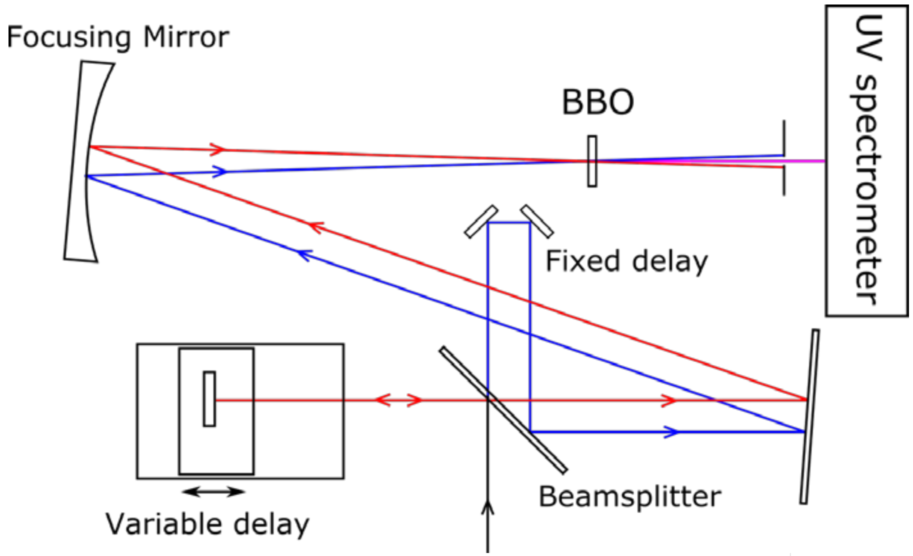
BioCARS (Moffat) 900 fs laser + UWM fast spectrometer (ANDOR)



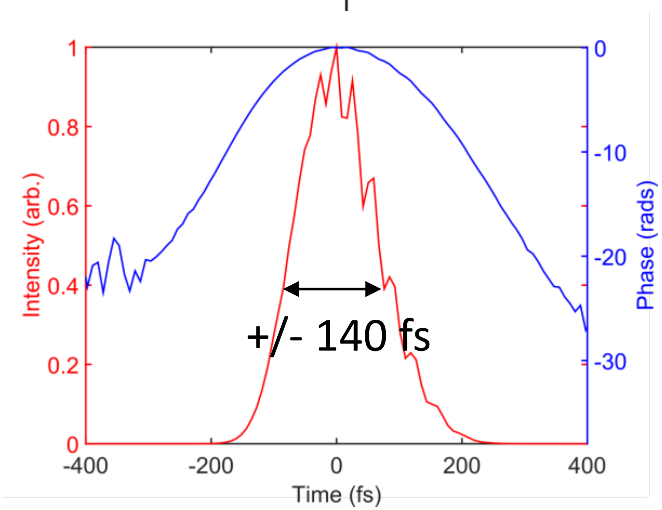


fs Laser Pulse Characterization at CXI/LCLS

Second Harmonic Generation Frequency Resolved Optical Gating (SHG-FROG, J. van Thor)



- temporal pulse profile
- spatial profile not shown



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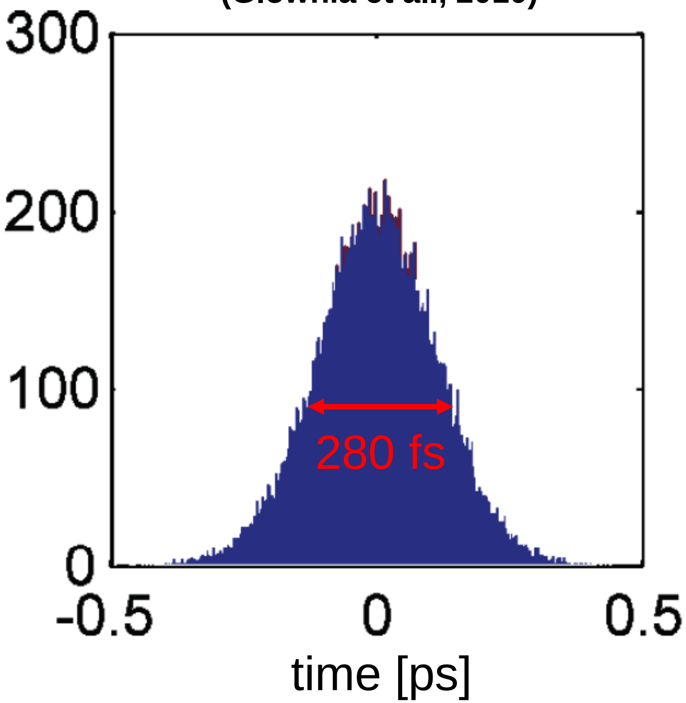
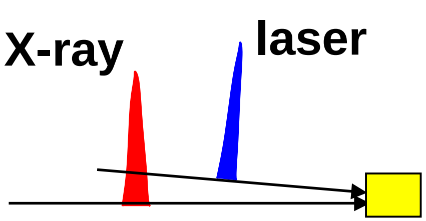
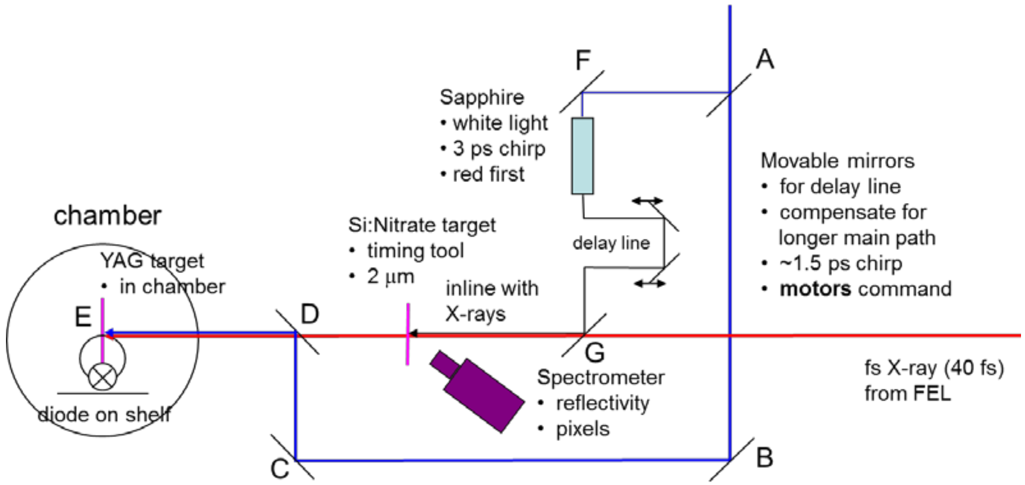
Jitter: Time-Tool Needed

LCLS jitter

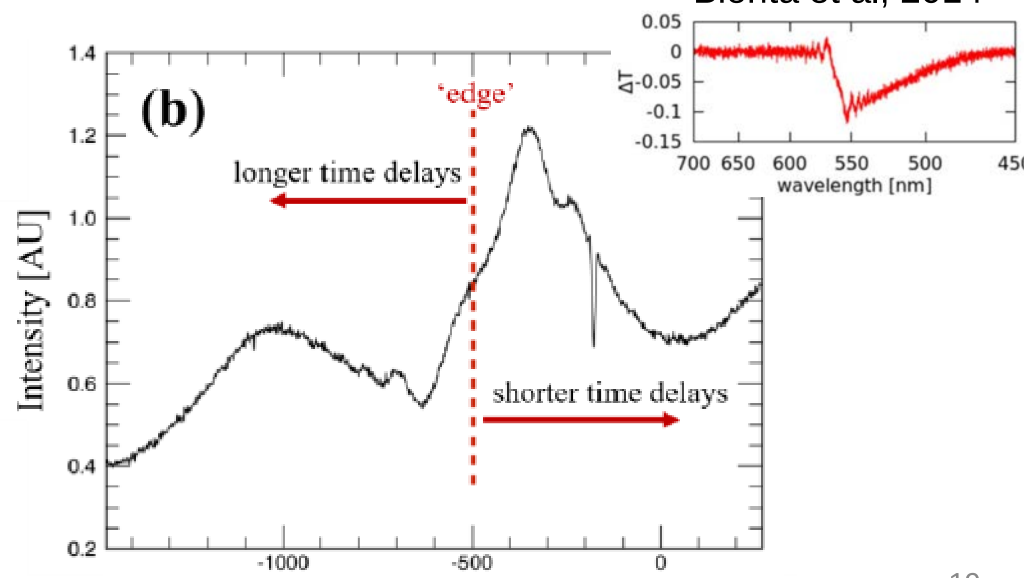
(Glownia et al., 2010)







Bionta et al, 2014



delay [fs] = pixel = wavelength

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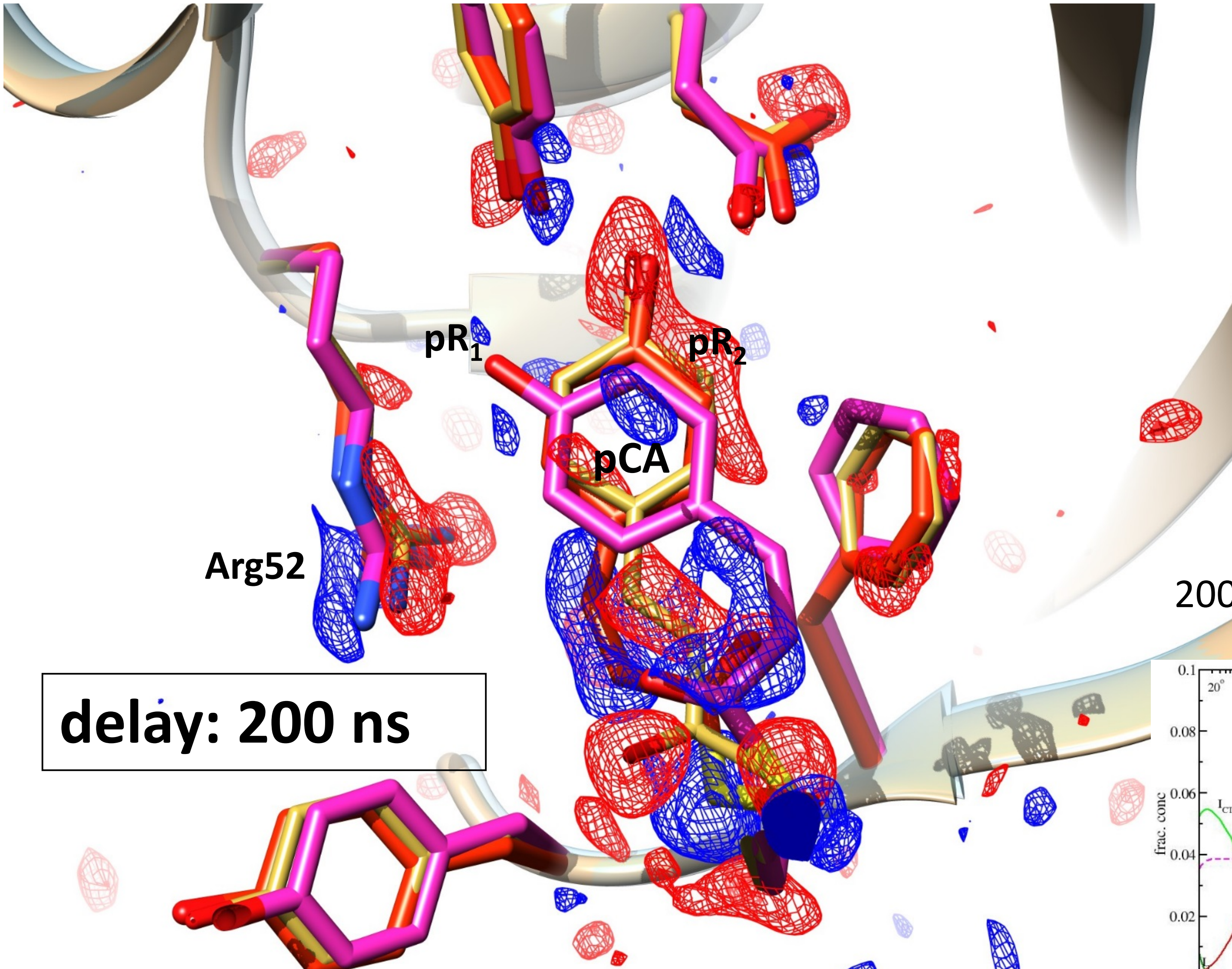
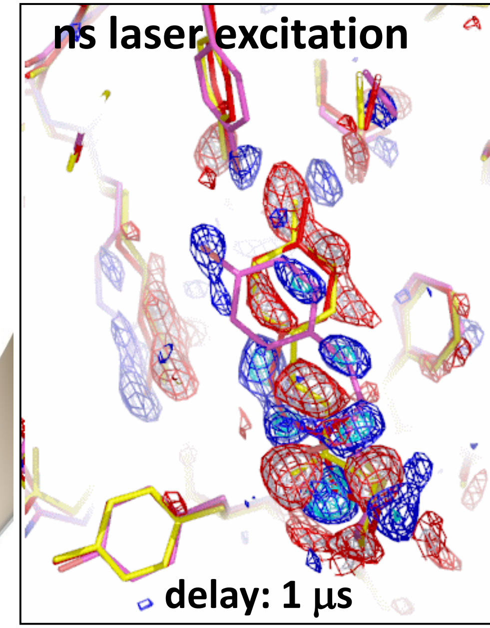
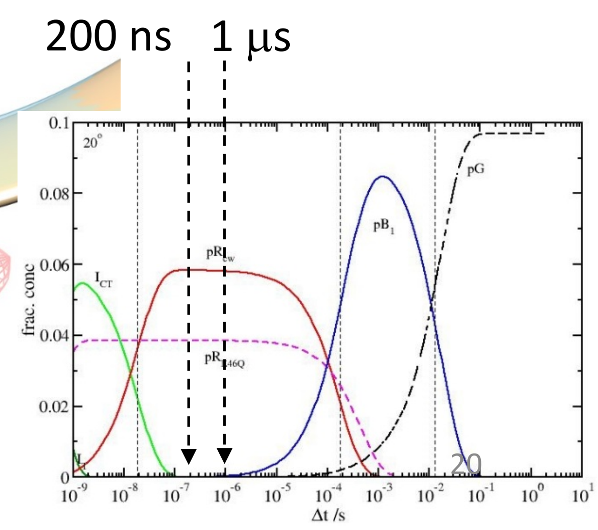
Reaction Initiation: Femtosecond-Laser Pump: 140 fs (450 nm blue), Probe: 40 fs X-ray







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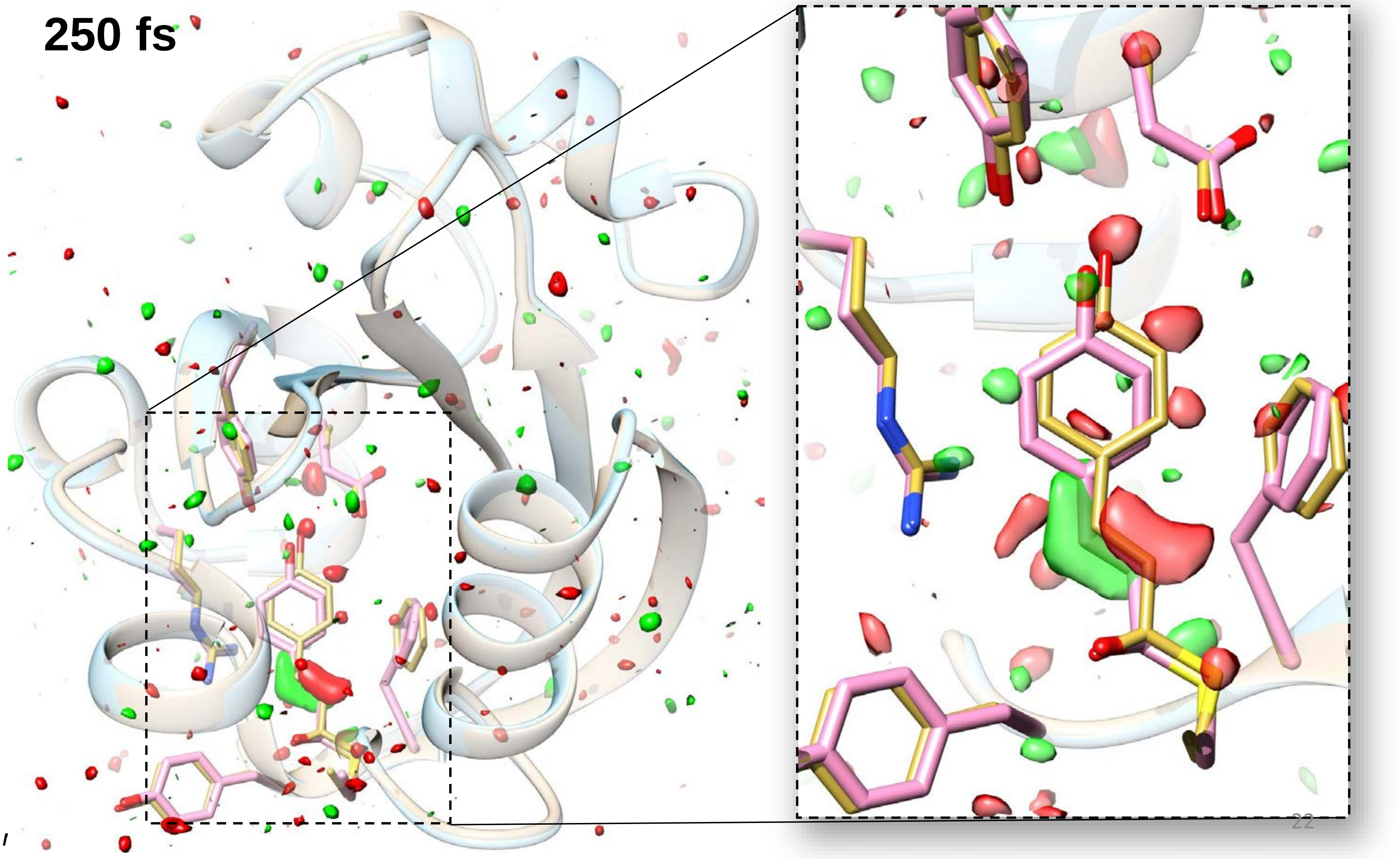
The Fastest Camera in the World fs-Laser Excitation, fs Time Delays, Resolution 1.6 Å

Time	# indexed	R-split [%]	CC*	Completeness	Multiplicity
142 fs	38606	9.01 (26.05)	0.997 (0.962)	99.92 (100)	714.75 (48.5)
269 fs	38786	9.16 (26.08)	0.997 (0.959)	100 (100)	695.25 (49.6)
455 fs	37563	9.86 (26.58)	0.996 (0.957)	99.84 (100)	637.31 (57.7)
699 fs	43617	7.53 (16.94)	0.998 (0.984)	99.92 (100)	931.65 (124.9)
799 fs	44892	7.57 (19.84)	0.998 (0.974)	99.84 (100)	961.51 (121.1)
856 fs	44470	7.61 (17.60)	0.997 (0.983)	99.92 (100)	955.96 (112.9)
915 fs	44180	7.58 (18.75)	0.998 (0.981)	99.84 (100)	927.25 (106.3)
1023 fs	45880	7.53 (17.19)	0.998 (0.978)	99.76 (100)	971.3 (123.7)
3 ps	76411	5.43 (14.36)	0.999 (0.989)	100 (100)	1685.8 (145.9)

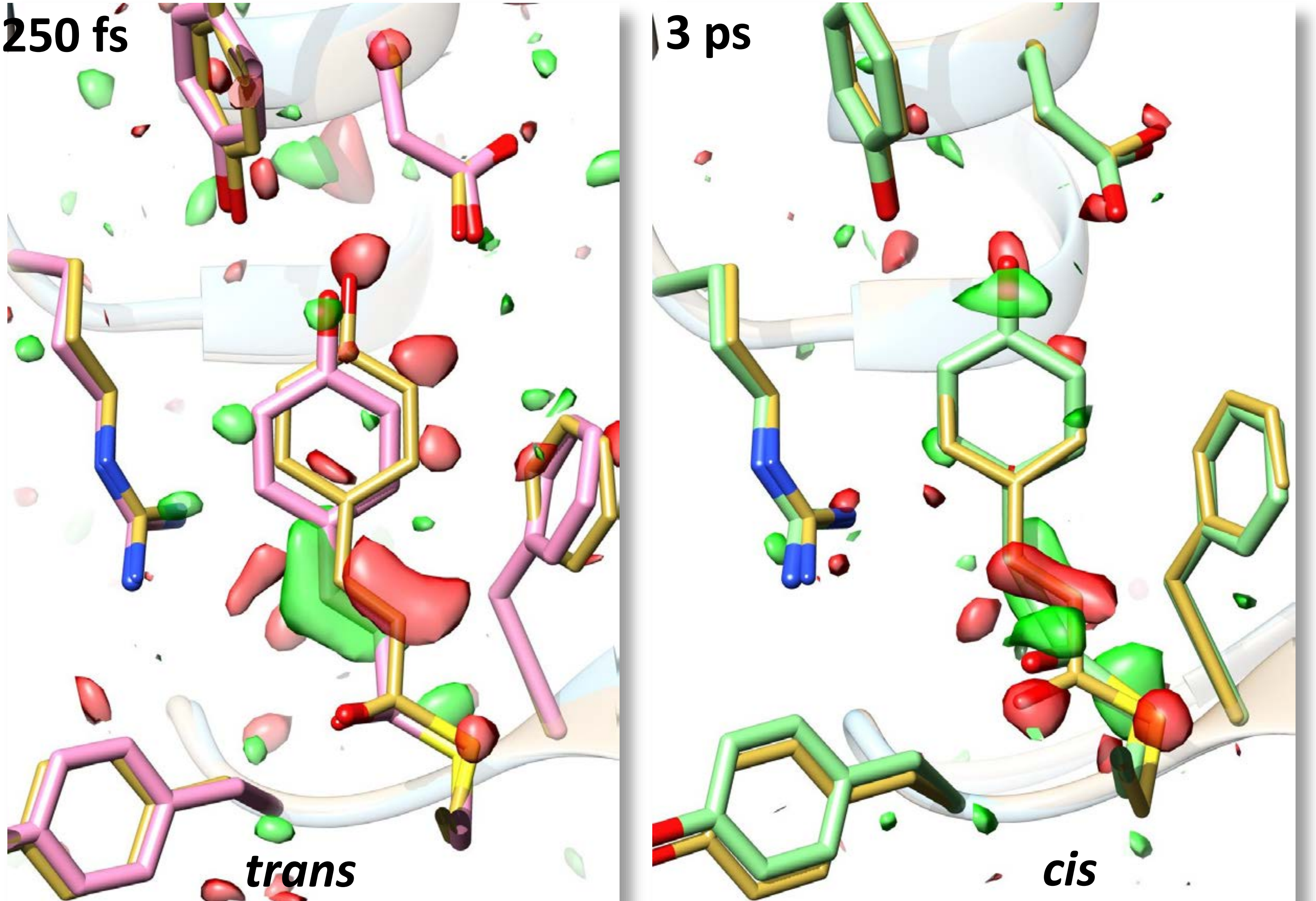
Trans to *Cis* Isomerization in PYP

Pande et al. Science 2016



Trans to *Cis* Isomerization in PYP

Pande et al. Science 2016



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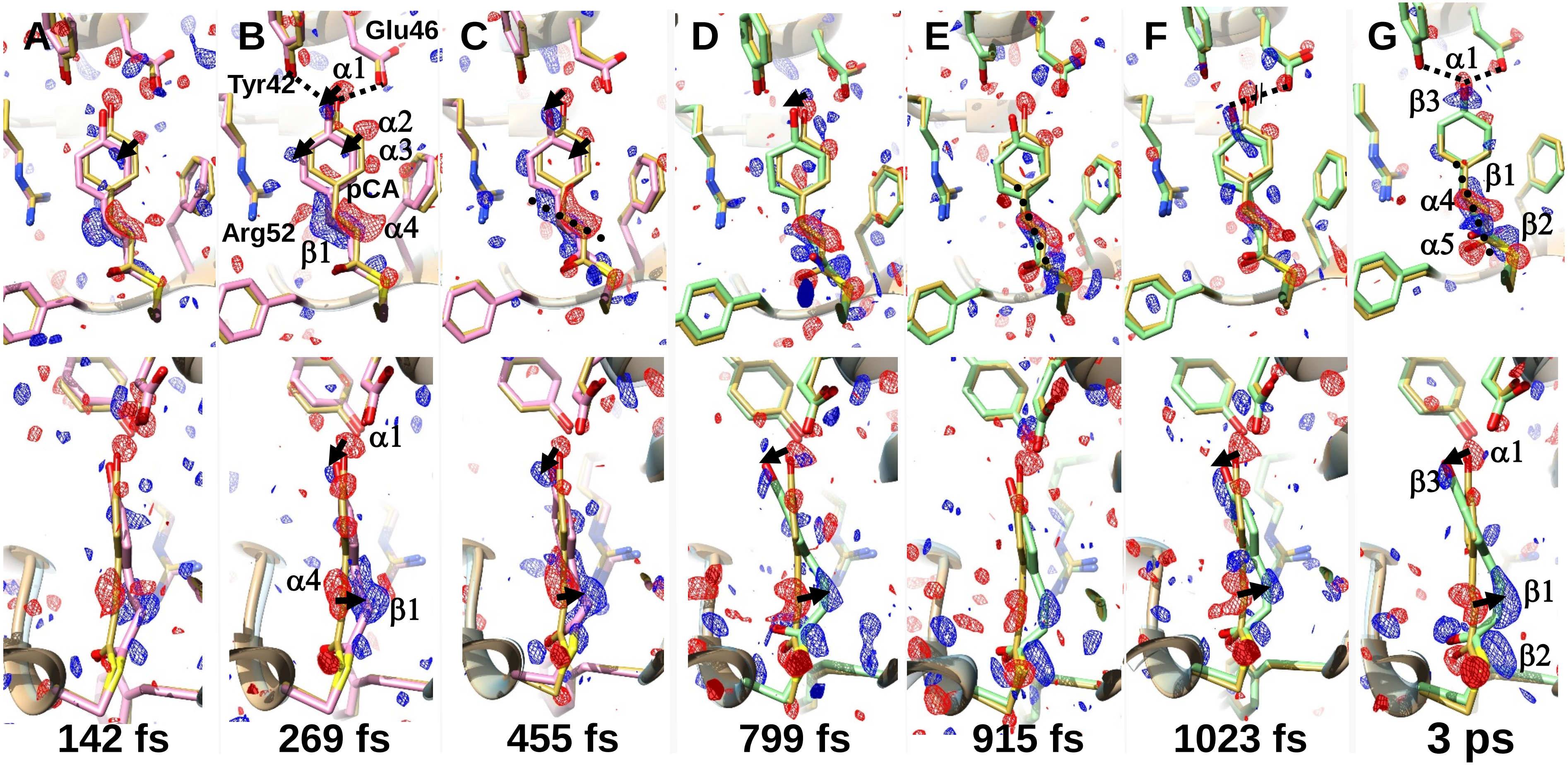
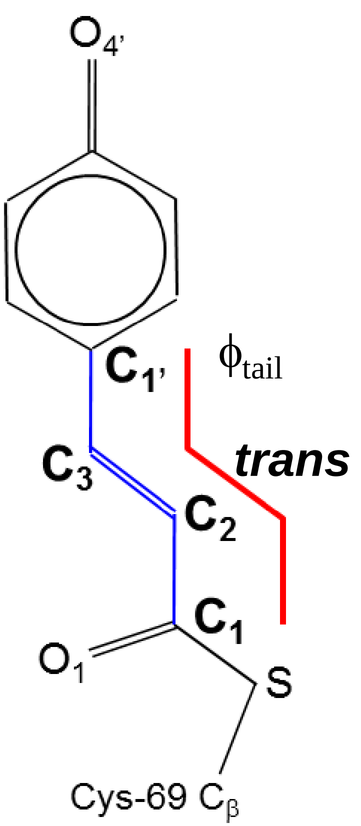
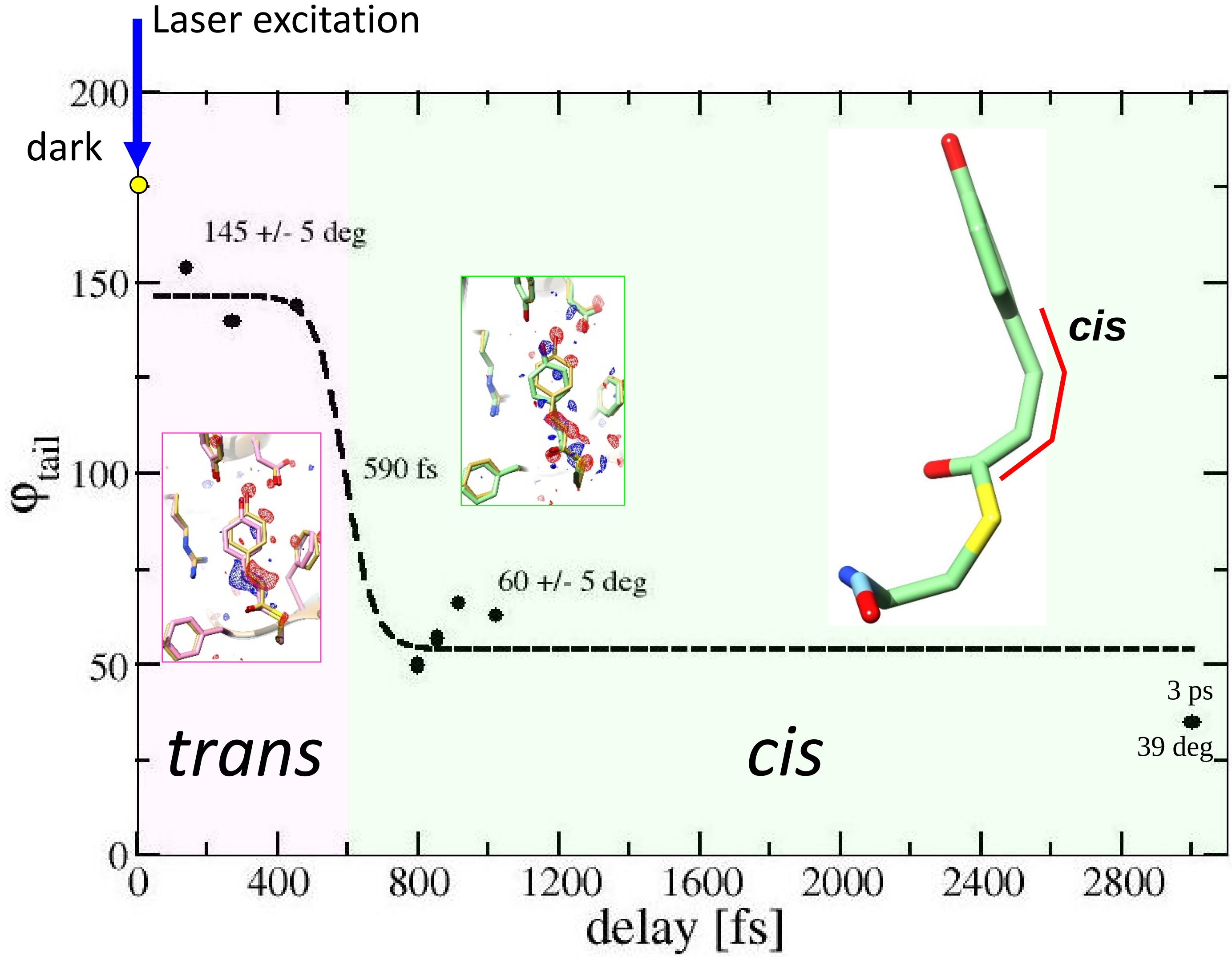
Movie: *Trans* to *Cis* Isomerization in PYP

Pande et al. Science 2016



Trans to *Cis* Isomerization in PYP
- Torsional Dynamics





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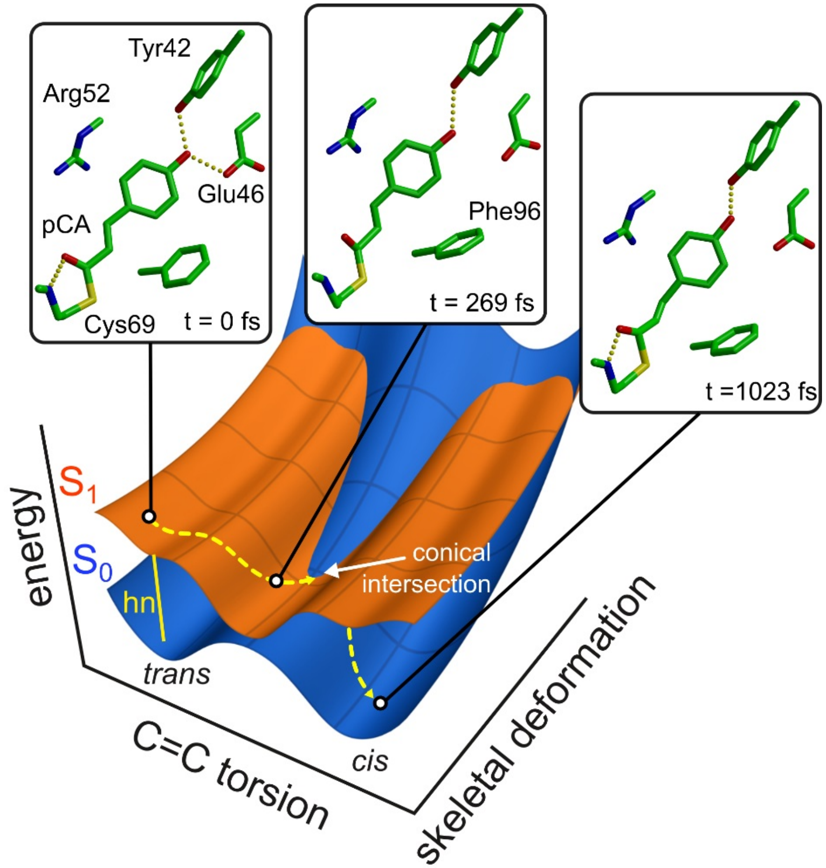
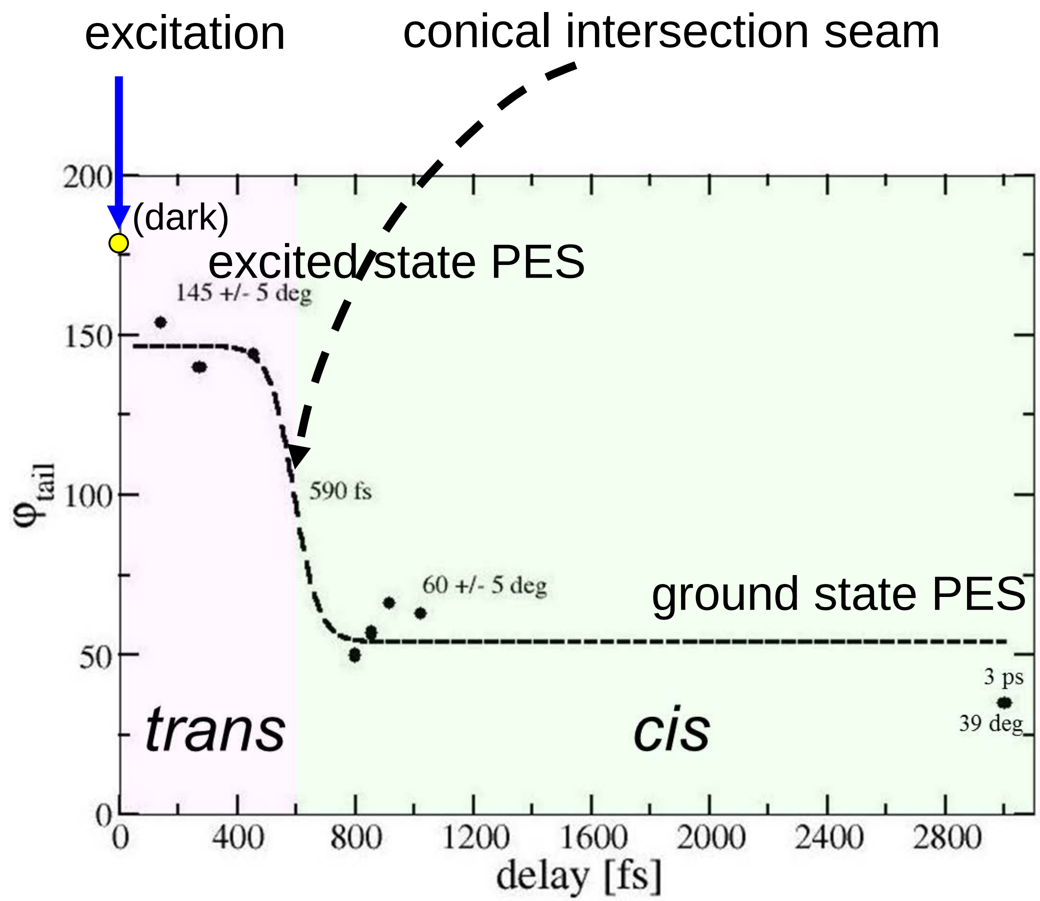
Transition Through the Conical Intersection (long sought after)

Theory: QM/MM calculations (Gerrit Groenhof)
- highly complex
- computer intensive



Experiment: TR-SFX (Pande et al.)



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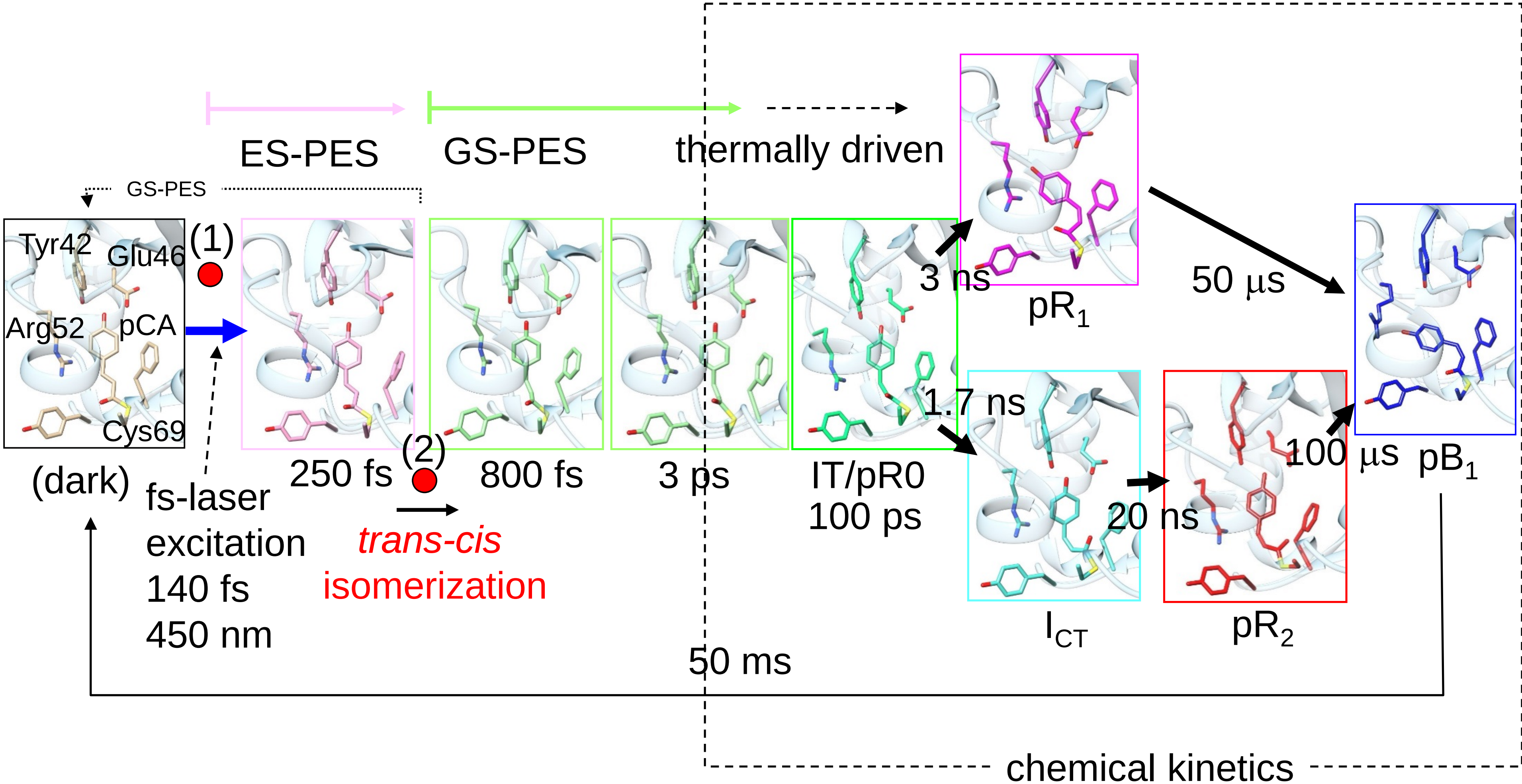
Ultimate Goal: Structural Characterization of a Reaction, from Fundamental Motions to the End … Reached!



● Born-Oppenheimer approximation _not_ valid

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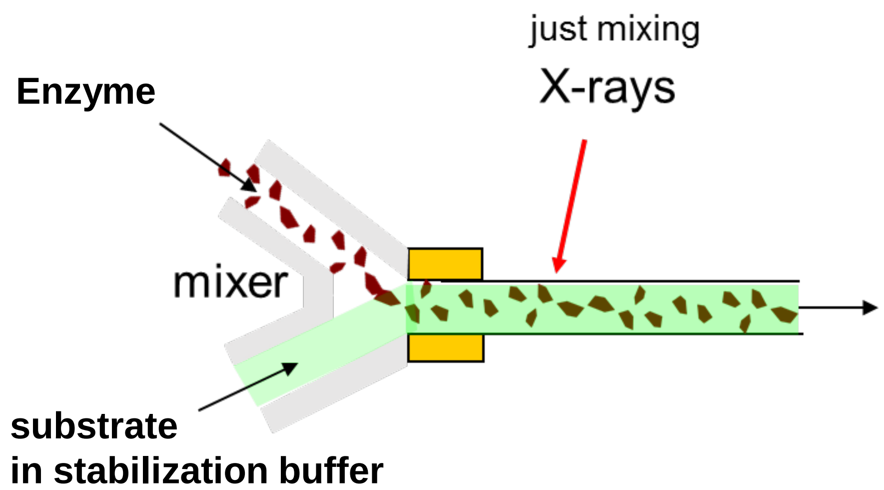
fs TR-SFX at Near Atomic Resolution Successful

Key Messages

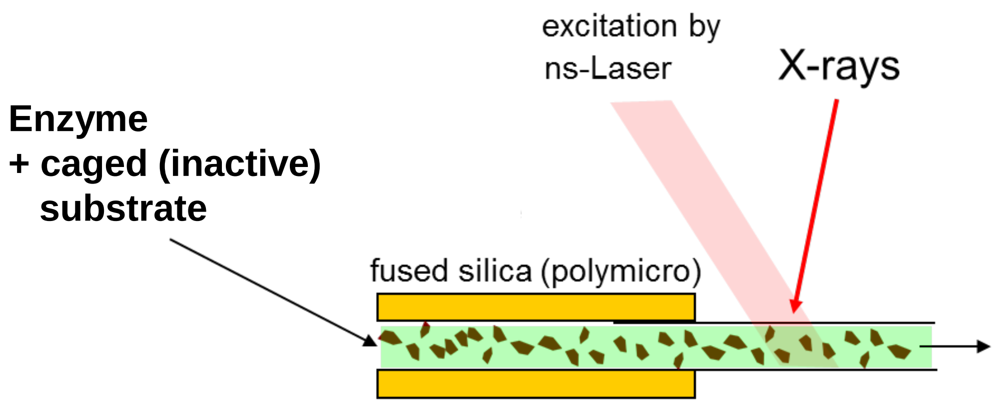
- Structures from SFX are the same as those from more conventional methods
- TR-SFX works at X-ray FEL at high, near atomic resolution
- TR-SFX works with ***fs*** time-resolution
- structural characterization of *trans/cis* isomerizations
- electronic excited state dynamics!!!
- transition through conical intersection
- opportunity to observe laser induced damage
- optical control of reaction dynamics

III. The Holy Grail of Time-Resolved Crystallography: Single Turnover Kinetics, Mixing

mix-and-inject (Schmidt, Adv. Cond. Mat. Phys, 2013)



pump-probe



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III. The Holy Grail of Time-Resolved Crystallography: Single Turnover Kinetics, Mixing

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L.A. Sluyterman, M. J. M. De Graaf, 1969
The activity of papain in the crystalline state
Biochem Biophys Acta

The rate of conversion of dissolved substrate by a suspension of enzyme crystals is governed by the rate of diffusion and the reaction rate of the substrate inside the crystal. *If the crystal is thin enough* the diffusion is not rate limiting.

Diffusion is governed by Fick's laws in 3D
Especially Fick's 2nd law is pretty illuminating and can be solved in 3D assuming certain simplifications

$$\boxed{D\nabla^2 C = \frac{\partial C}{\partial t}}$$

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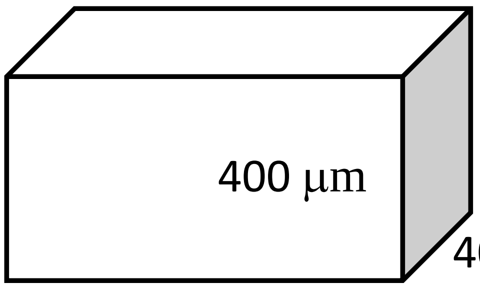
Calculation, Simulation, Experiment

D of Glucose: $5 \times 10^{-6}\ cm^2/s$

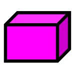
Diffusion into *glycogen-phosphorylase* crystals



400 µm

1600 µm

Crystal size	From solving Fick's 2nd law	num. simul.	***measured***
1600 µm × 400 µm × 400 µm	τ_L = **16 s**	25 s **Geremia** et al., 2006	< 1min **Johnson, Hajdu**, 1990
40 µm × 40 µm × 40 µm	τ_L = 100 ms	-/-	
4 µm × 4 µm × 4 µm	τ_L = 1 ms	-/-	ms-time resolution

40 µm  40 µm

40 µm

4 µm  4 µm

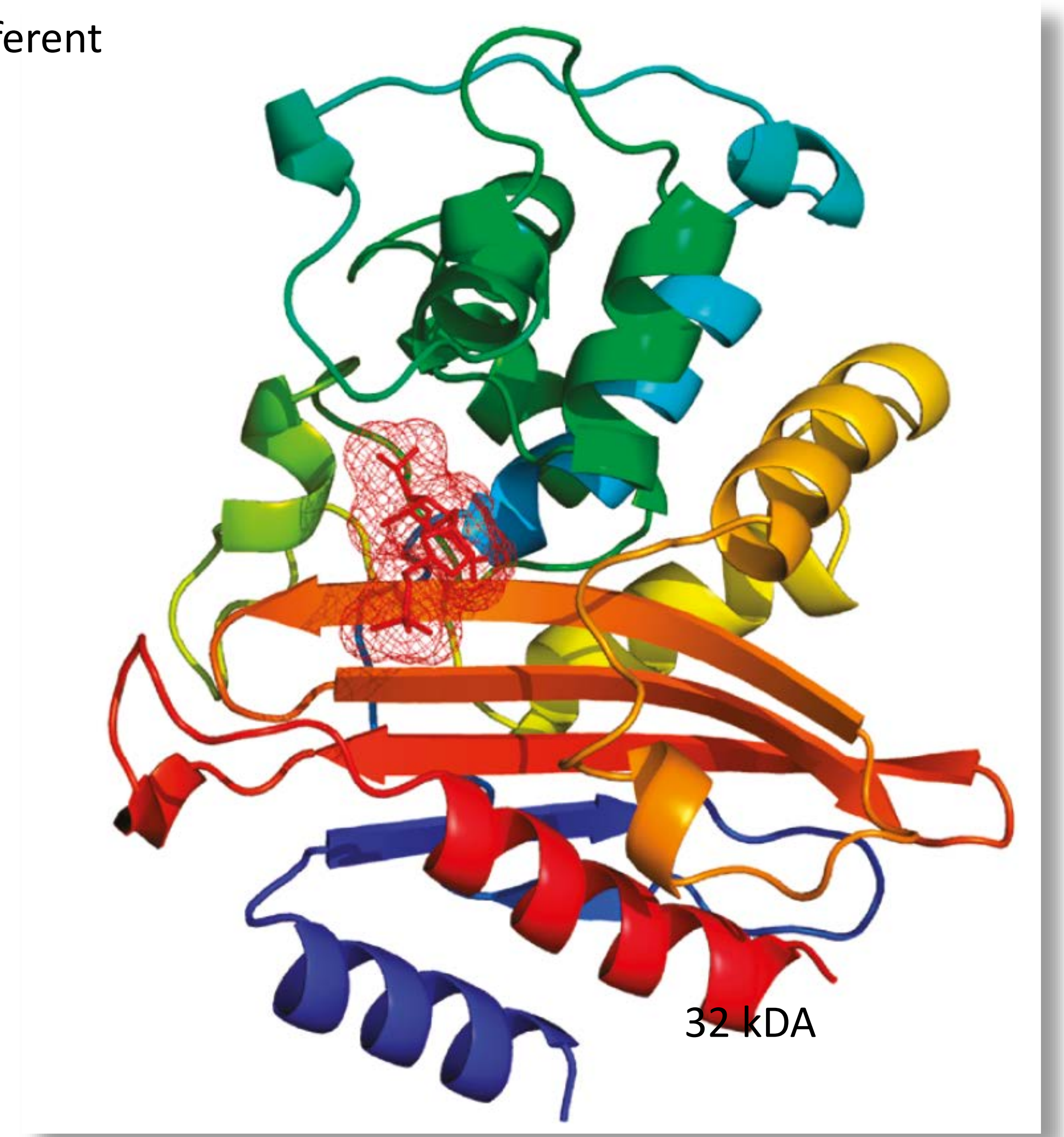
4 µm

diffraction before destruction

Highly Relevant Enzyme, β-Lactamase

catalytic turnover for different substrates:

- from 10 ms to 10 min



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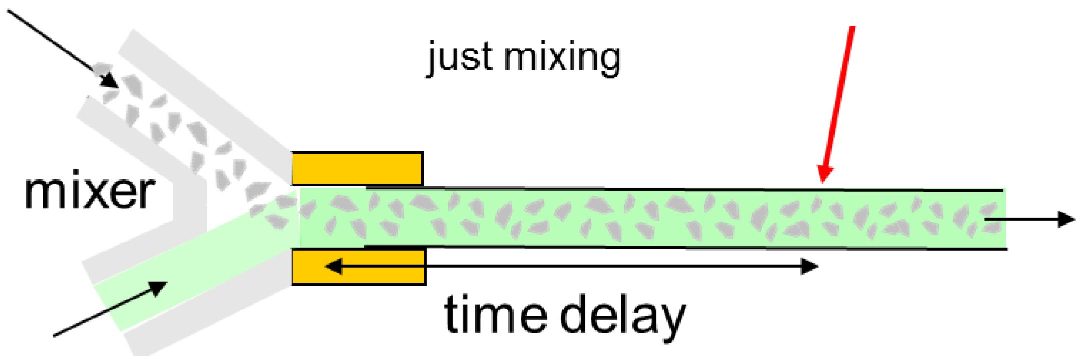
Mix-and-Inject Experiment at LCLS/CXI

LCLS beamtime LK17

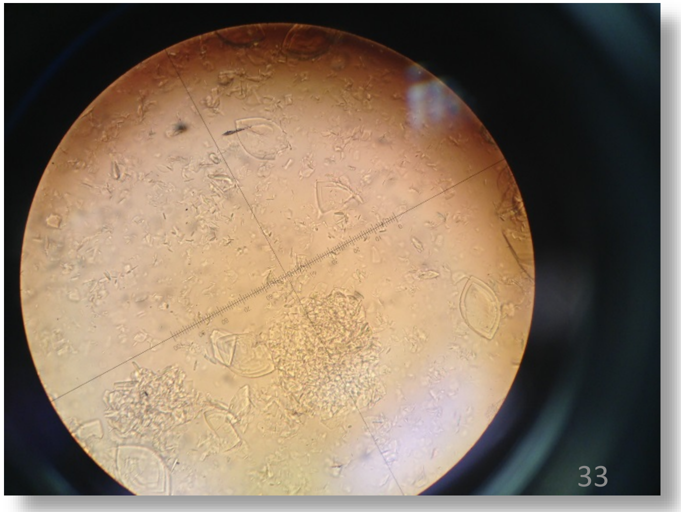
enzyme microcrystal slurry

X-rays



substrate + stabilization buffer

new construct



spacegroup	$P2_12_12_1$, orthorhombic
unit cell [Å]	a=78 b=96 c=111
resolution [Å]	synchrotron: 2.1 [cryo]

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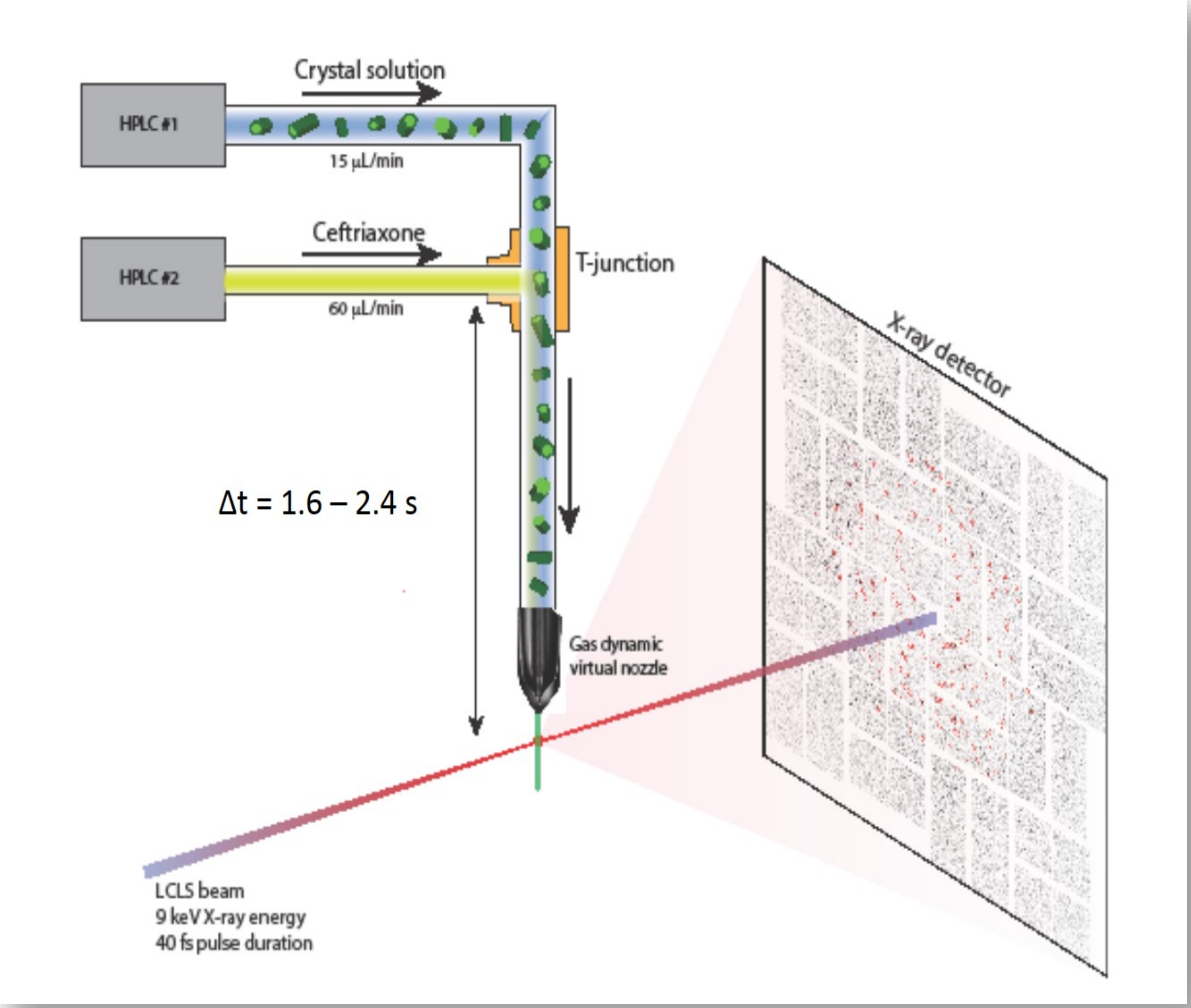
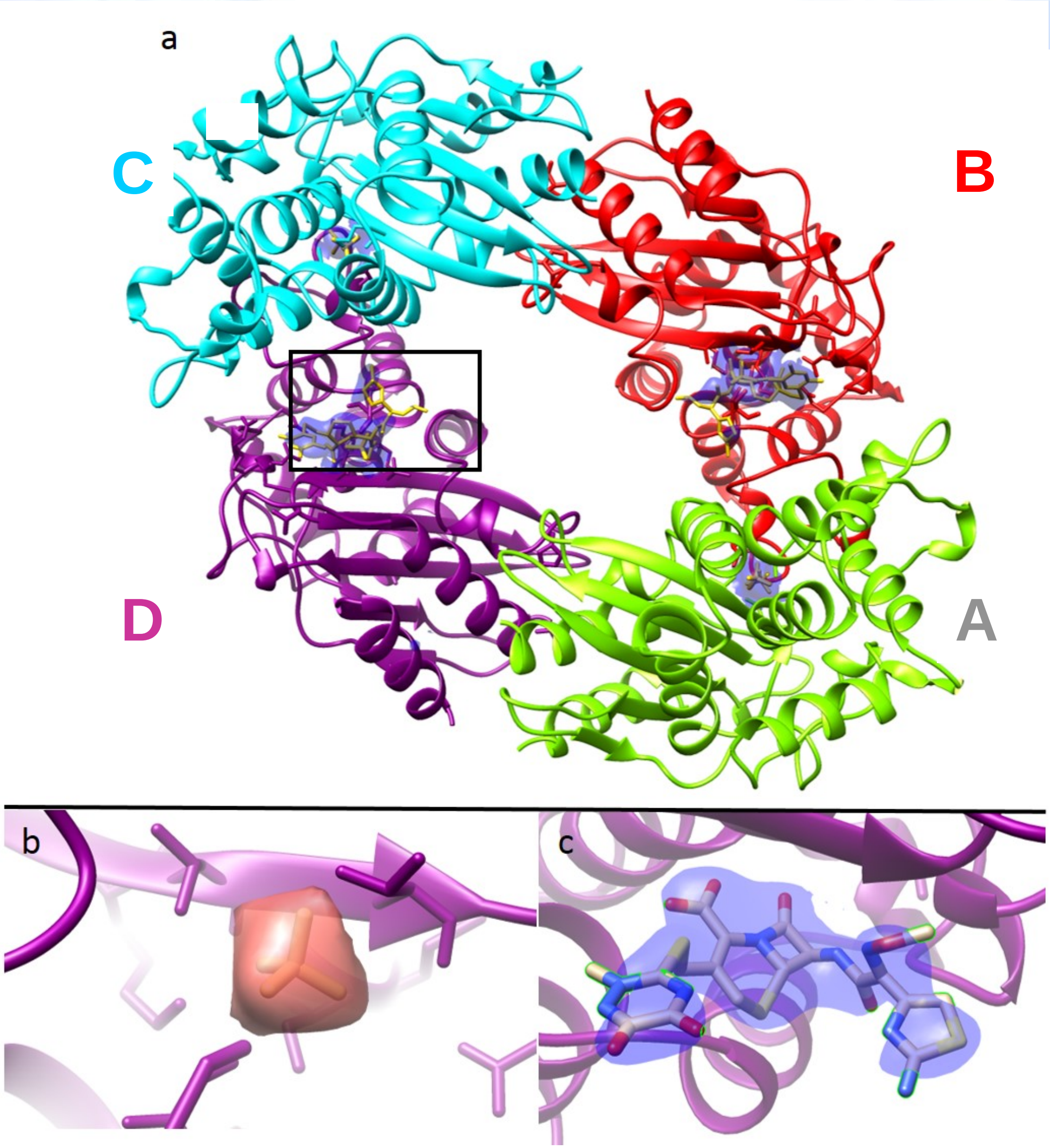
Mix-and-Inject Experiment at LCLS/CXI

LCLS beamtime LK17, Dec 2015



‘On-the-Fly’



Summary

Time-resolved serial crystallography at the LCLS

- light to ligand triggered reactions

  PYP and β-lactamase
- small to large protein complexes

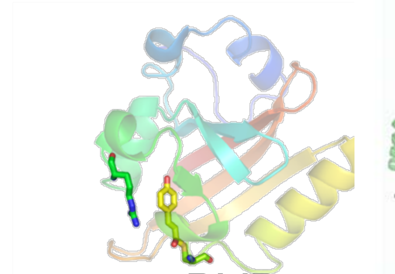
  PYP to photosystem II
- model systems to biomedically relevant
- ultrafast to slower timescales

  femtosecond to millisecond
- single time point to movie
- *Future*

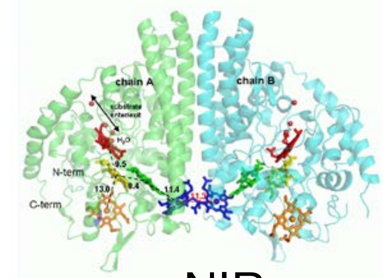
  crystals to single particles

  ***movies* in ultra slow motion**

Evolution

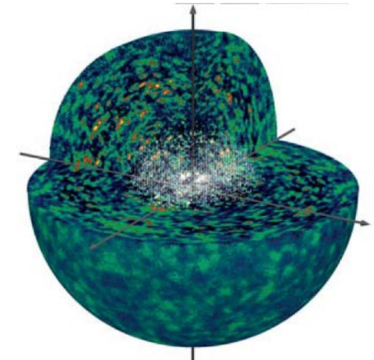


PYP

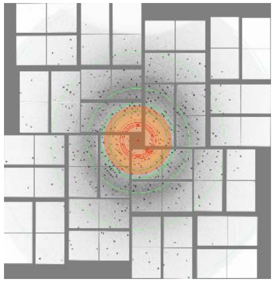


ccNIR

β-lactamase



photosystem II



discrete

continuous

BioXFEL
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