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Time resolved crystallography: Optical-Control of Photoisomerisation populations in a Reversibly Switchable Fluorescent Protein

Establishing time-resolved macromolecular serial crystallography as a technique for measuring light-induced structural differences

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Imperial College London
Jasper van Thor Group

Workshop on Chemical Dynamics and XFELs
11/12/2019

RS-Kiiro

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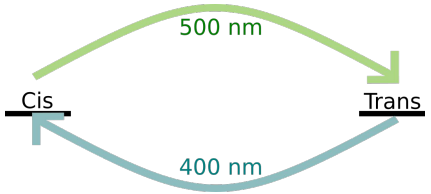
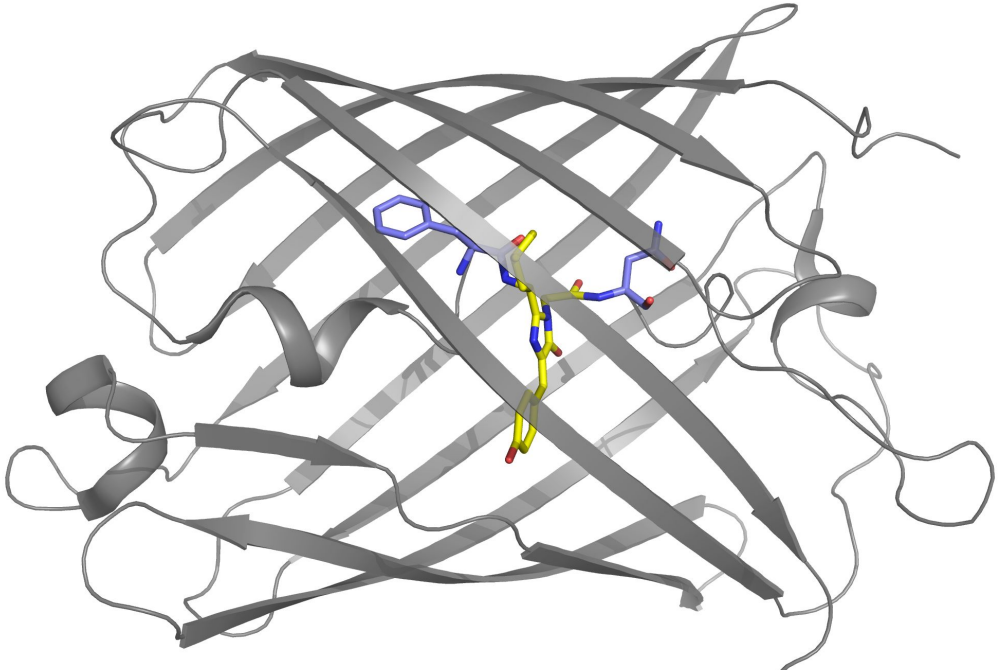
- Mutant from Skylan-NS (similar to GFP)
- 20% photolysis yields from fs excitation





RS-Kiiro

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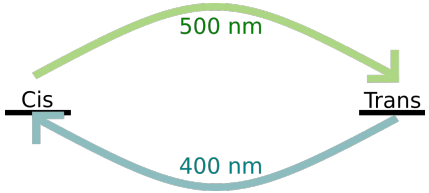
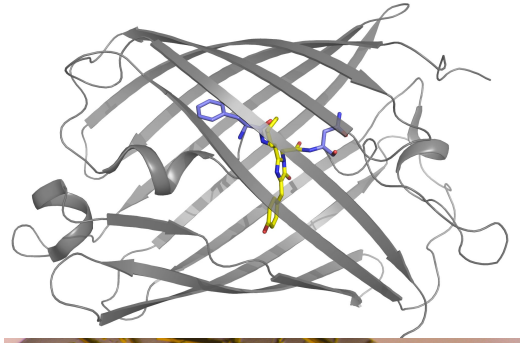
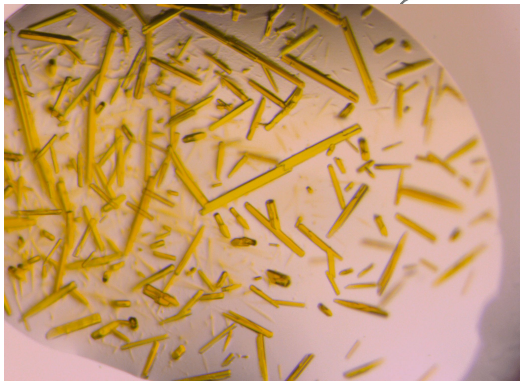
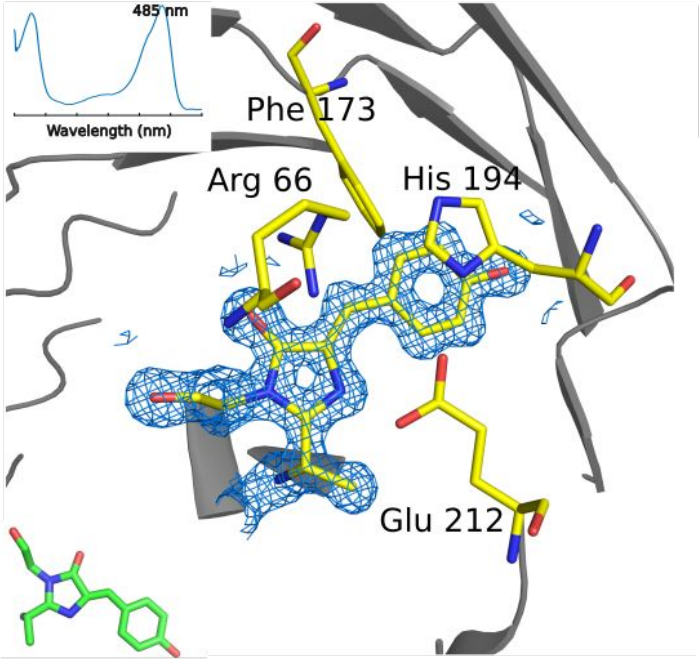
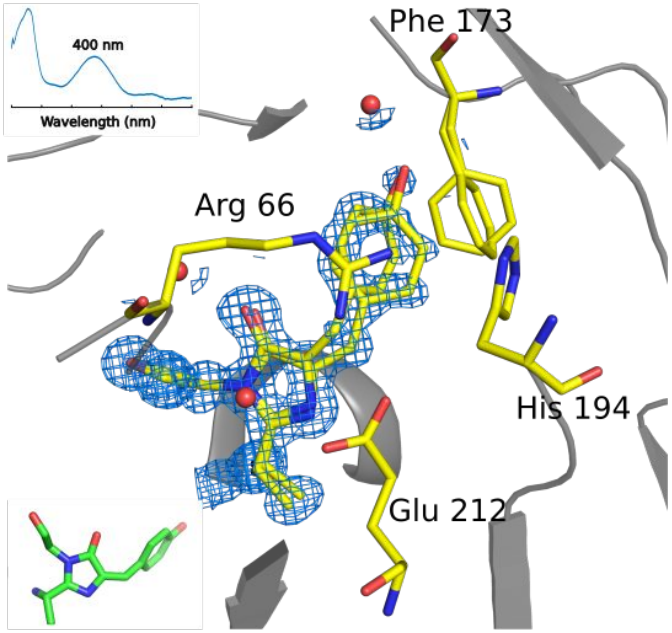
- 20% photolysis yields from fs excitation
- Crystals in batch up to 0.94 Å resolution overnight











Flash Photolysis in Crystals

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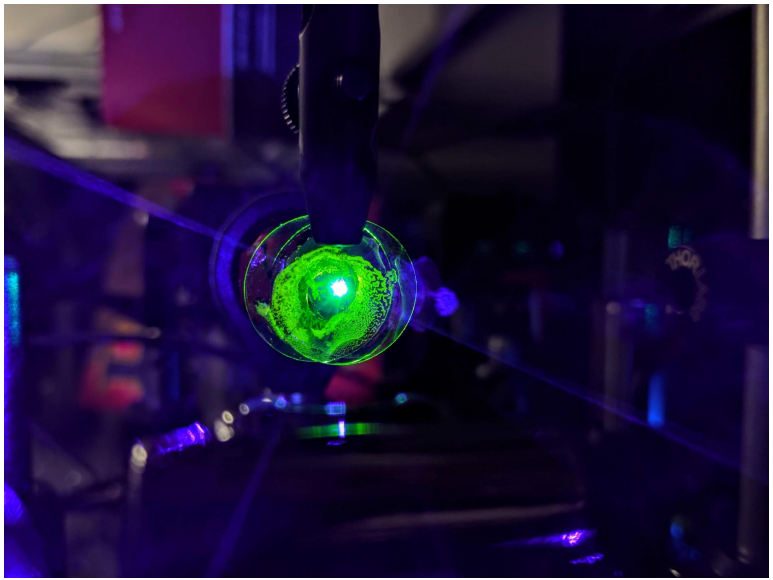
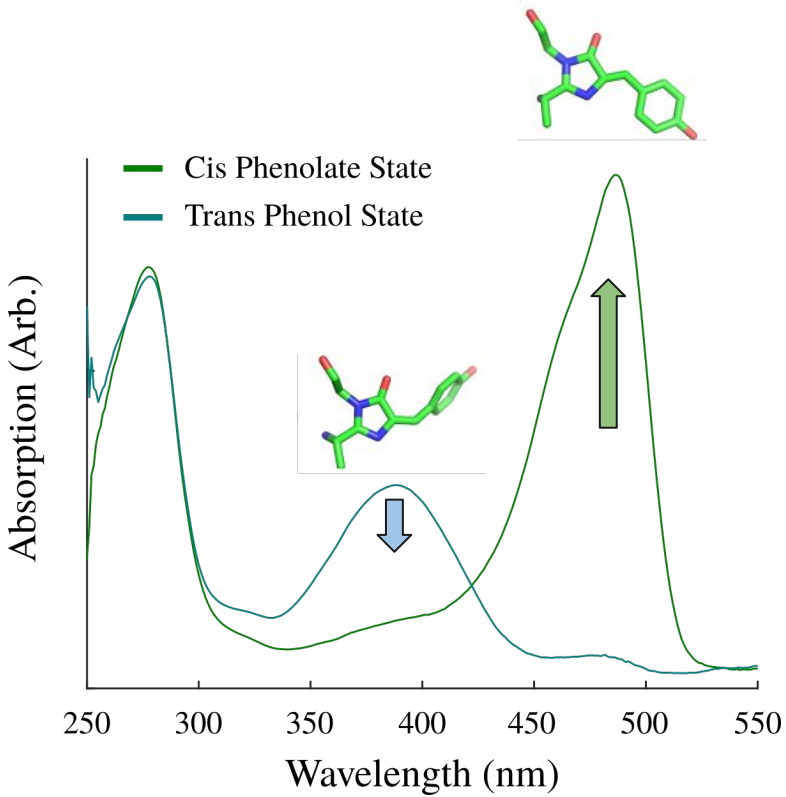
- Power titrations on crystal pancake samples
- Back reaction has the highest yield so preconvert then pump ‘backwards’.
- Power titration: 1 ms pulses of 405 nm, probe with 500 nm





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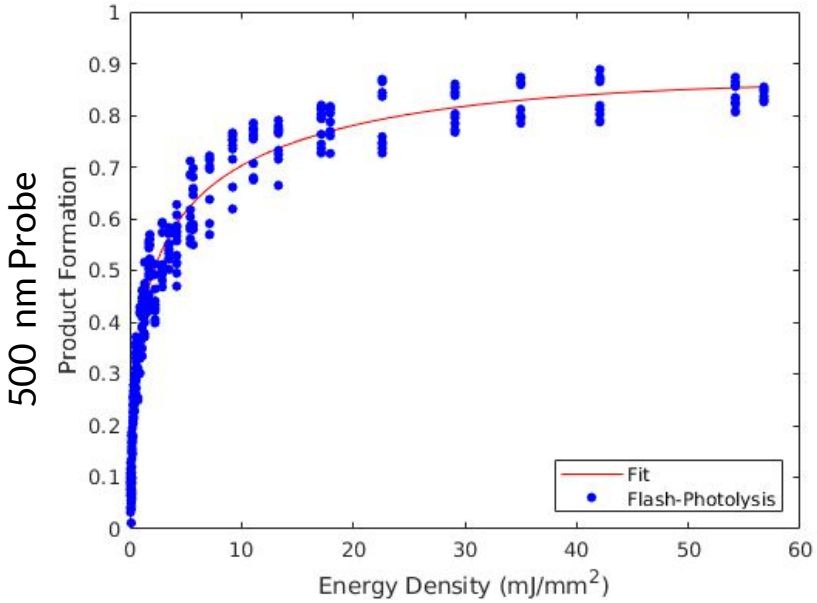

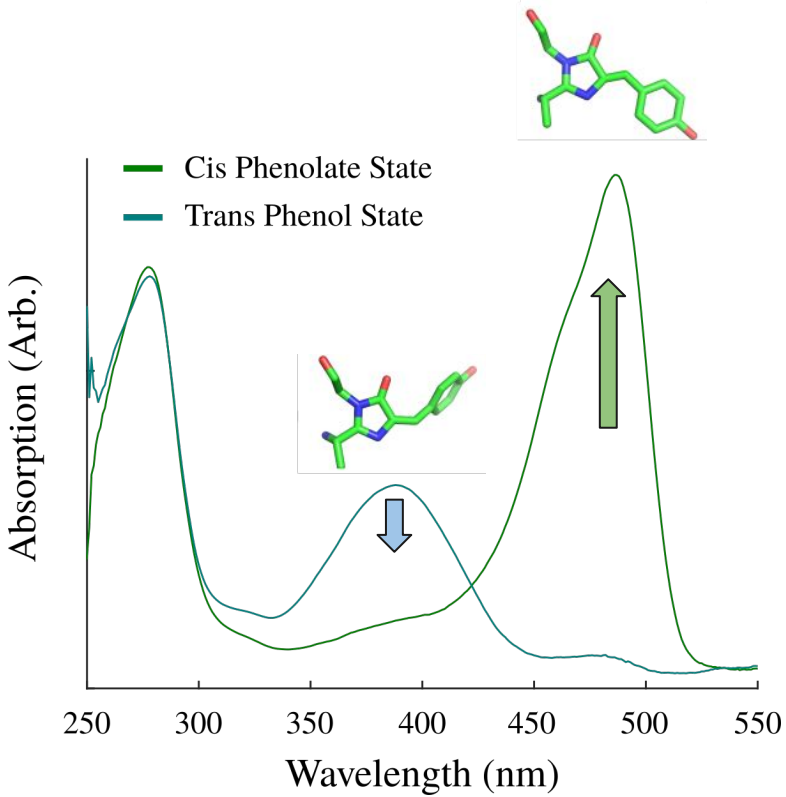

Can time-resolved serial crystallography resolve these differences ?

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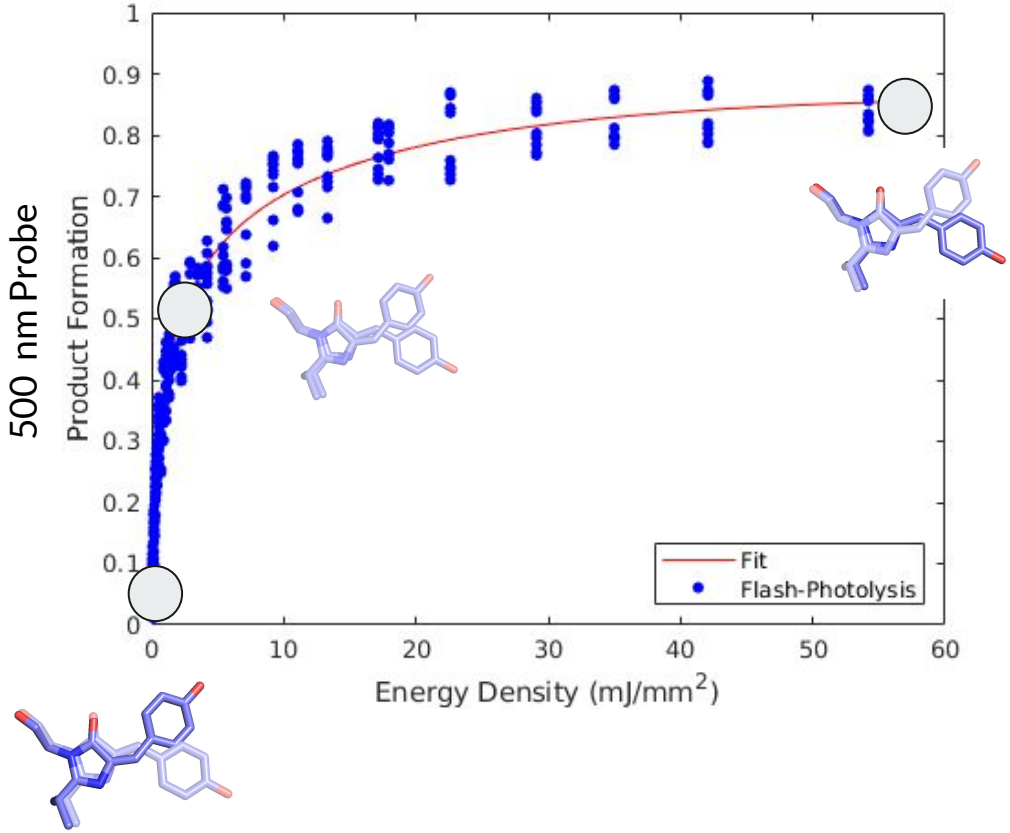

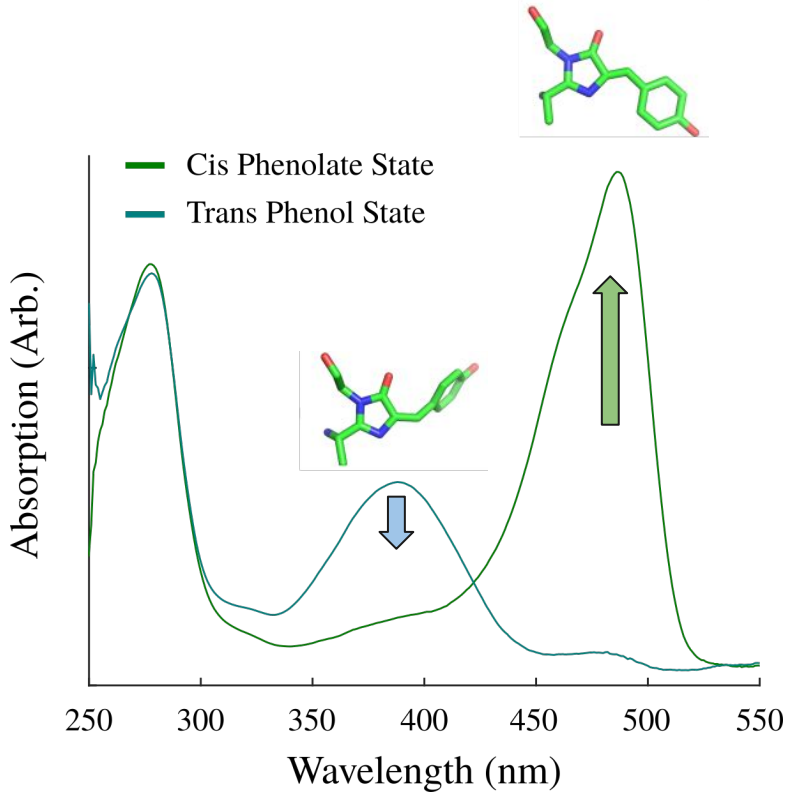

Time-Resolved Serial Crystallography

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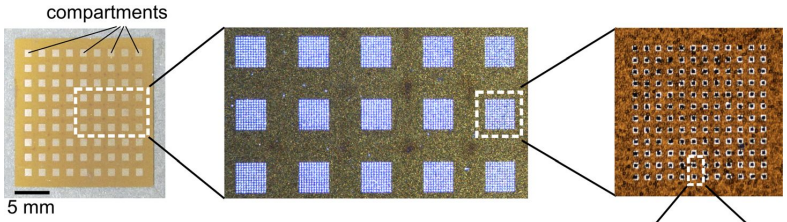
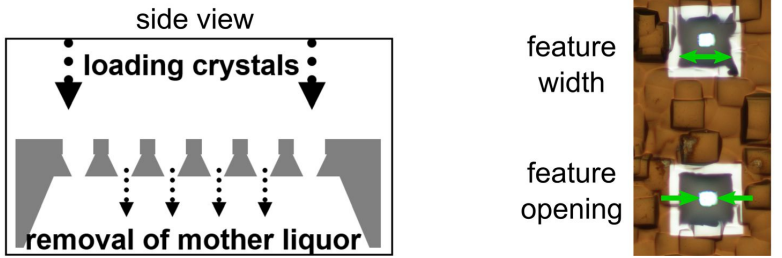
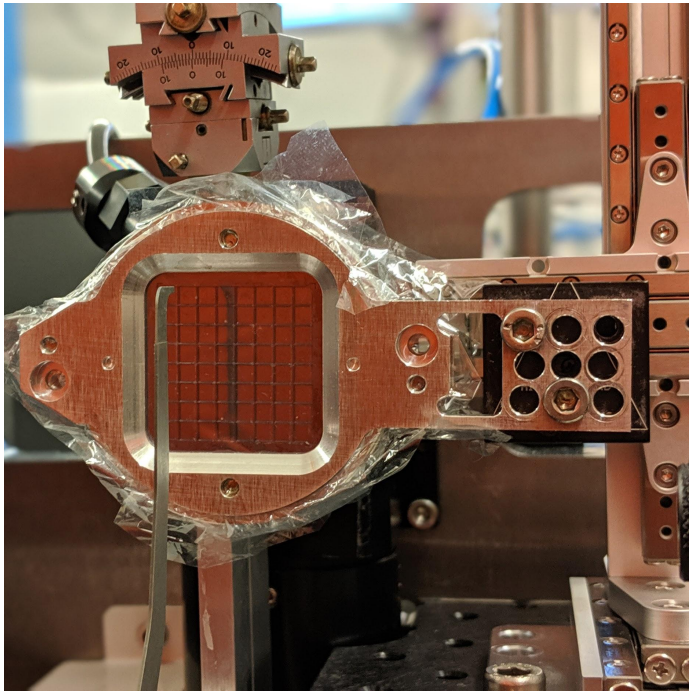
- Collect in random orientations over a large number of crystals (~10,000 per a dataset) to access the full Ewald sphere





Mueller, Miller et al. 2015



Fixed Target Serial Crystallography

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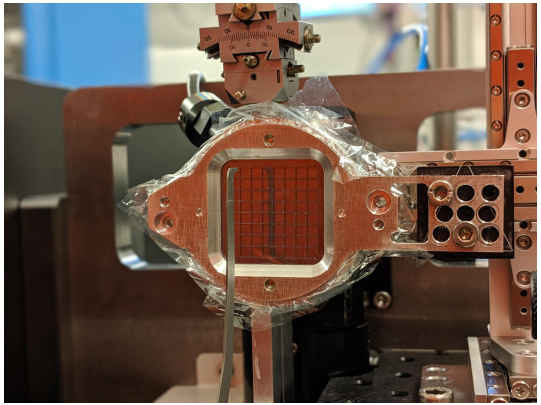
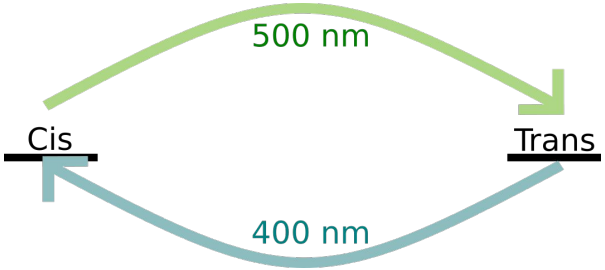
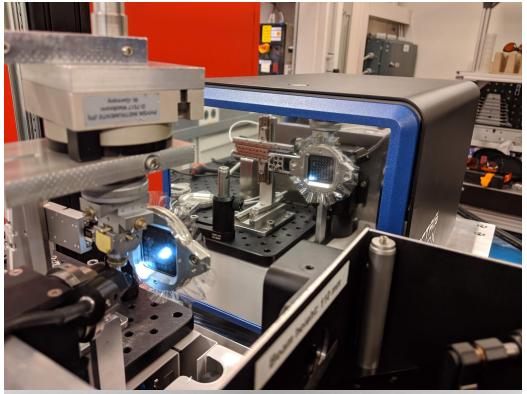
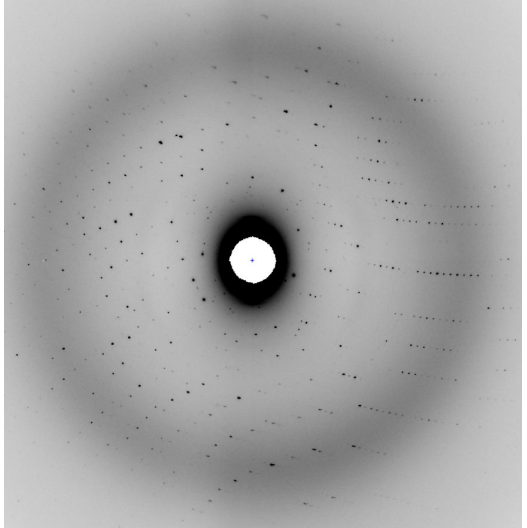
- Pre-convert with 500 nm, pump with 405 nm, probe with x-ray diffraction









Pre-convert
500 nm

Flash
405 nm
1 ms
Varied Pulse Energy

X-Ray Probe
~ 12 KeV
20 ms

Fixed Target Serial Crystallography

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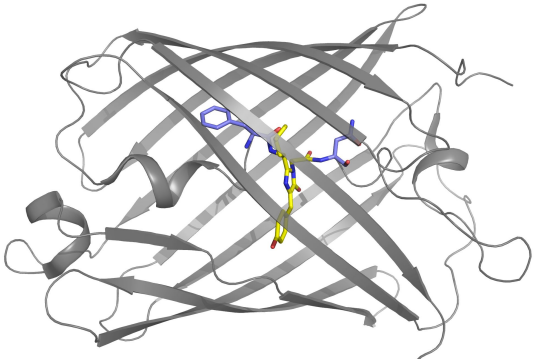
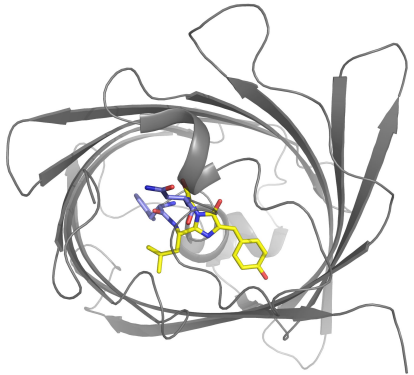
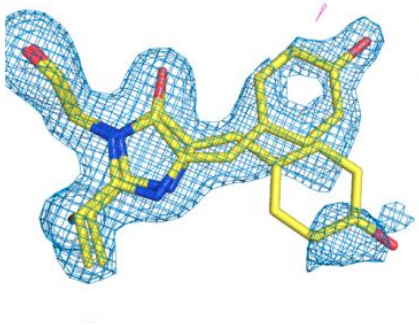
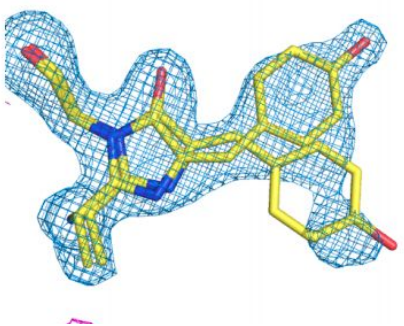
- Chromophore Omit maps at 3.5 σ



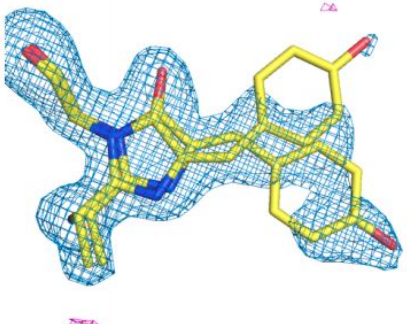




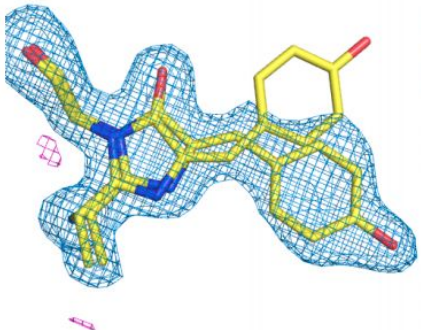
0 mJ/mm^2



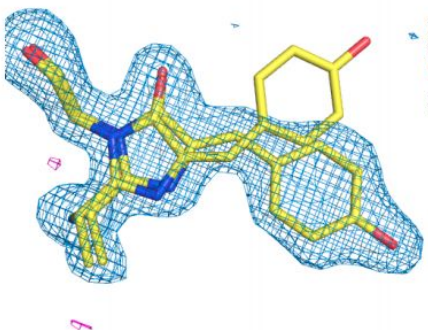
0.1 mJ/mm^2



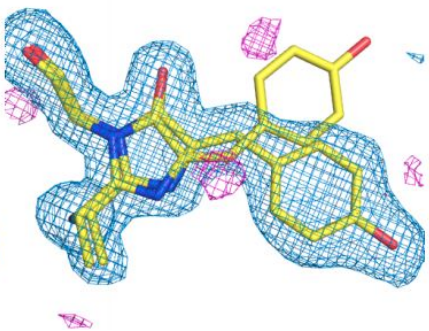
0.53 mJ/mm^2



1.78 mJ/mm^2



6.74 mJ/mm^2



14.42 mJ/mm^2

Fixed Target Serial Crystallography

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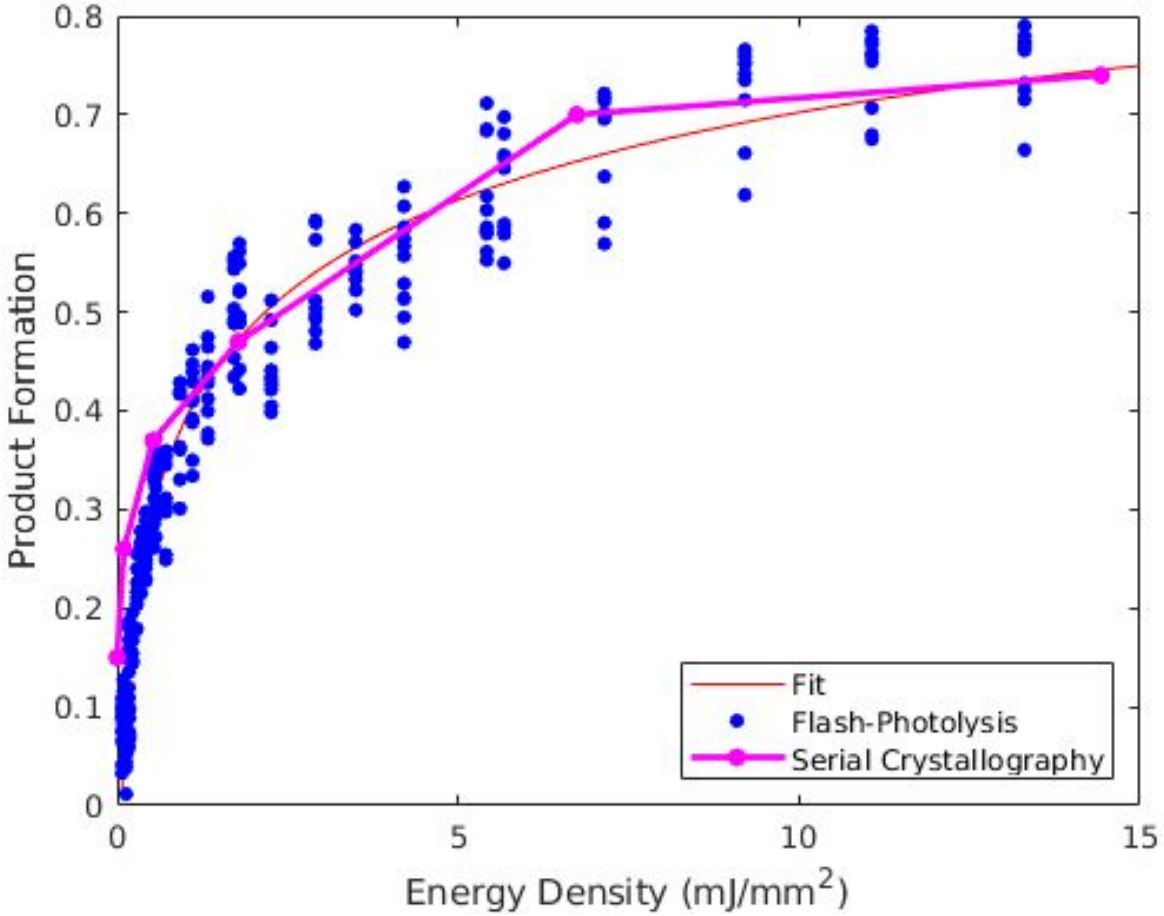

Conclusions & Thanks

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- Demonstrated time-resolved serial crystallography can resolve small molecular motions
- Show dynamics can be resolved to high resolution (1.70 Å) using serial crystallography at synchrotrons
- Clear demonstration dependence on occupancy for energy density

ICL:
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