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Dr. Jörg Standfuss :: Group Leader :: Laboratory of Biomolecular Research

Watching the release of a photopharmacological drug over fourteen orders of magnitude in time

Leaps meets Life Sciences, May 2023

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Human And Animal Locomotion Photographs

"Eadweard Muybridge, 1887"

Temporal snapshots allow the study of dynamic-function relationships

Time-resolved structural biology group

@ Laboratory of Biomolecular Research, PSI

Members: Hannah Glover, Robin Stipp, Peter Seidel, Georgii Khusainov, Thomas Mason, Melissa Carrillo, Matthias Mulder, Sina Hartmann, Quentin Bertrand, Yasushi Kondo, Fabienne Stierli, Michal Kepa, Tobias Weinert, Jörg Standfuss

Former Members: Max Wranik, Przemek Nogly, Antonia Furrer, Dan James, Petr Skopintsev, Demet Kekilli, Kathrin Jäger, Dardan Gashi, Steffen Brünle

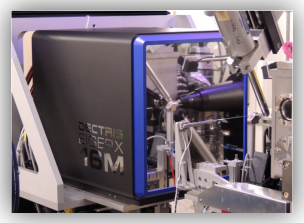
Mission statement:

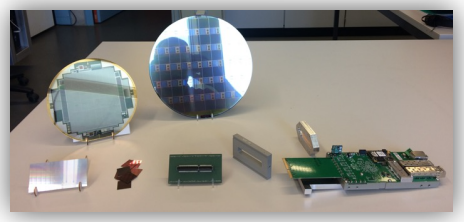
We include time as a fourth dimension in structural biology to resolve transient conformational states important for protein function.

“High-speed cameras” at the PSI

Swiss Light Source and Swiss X-ray Free Electron Laser

SLS (& SLS 2.0)

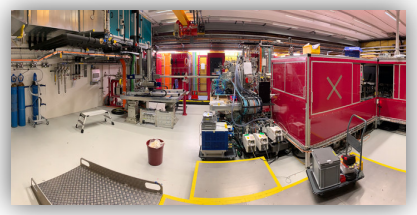


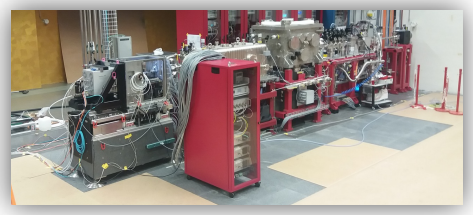


Macromolecular crystallography	X06SA PXI X10SA PXII X06DA PXIII SwissFEL MX	Macromolecular crystallography: MAD, SAD, large unit cells, small crystals, spectroscopy, screening, multi-axis go-niometer, ...

Collaboration: F. Dworkowski, M. Wang and co-workers

SwissFEL





1.experimental station **Alvra**
2.experimental station **Bernina**
3.experimental station **Cristallina**

C. Milne, J. Beale, C. Bacellar, and co-workers

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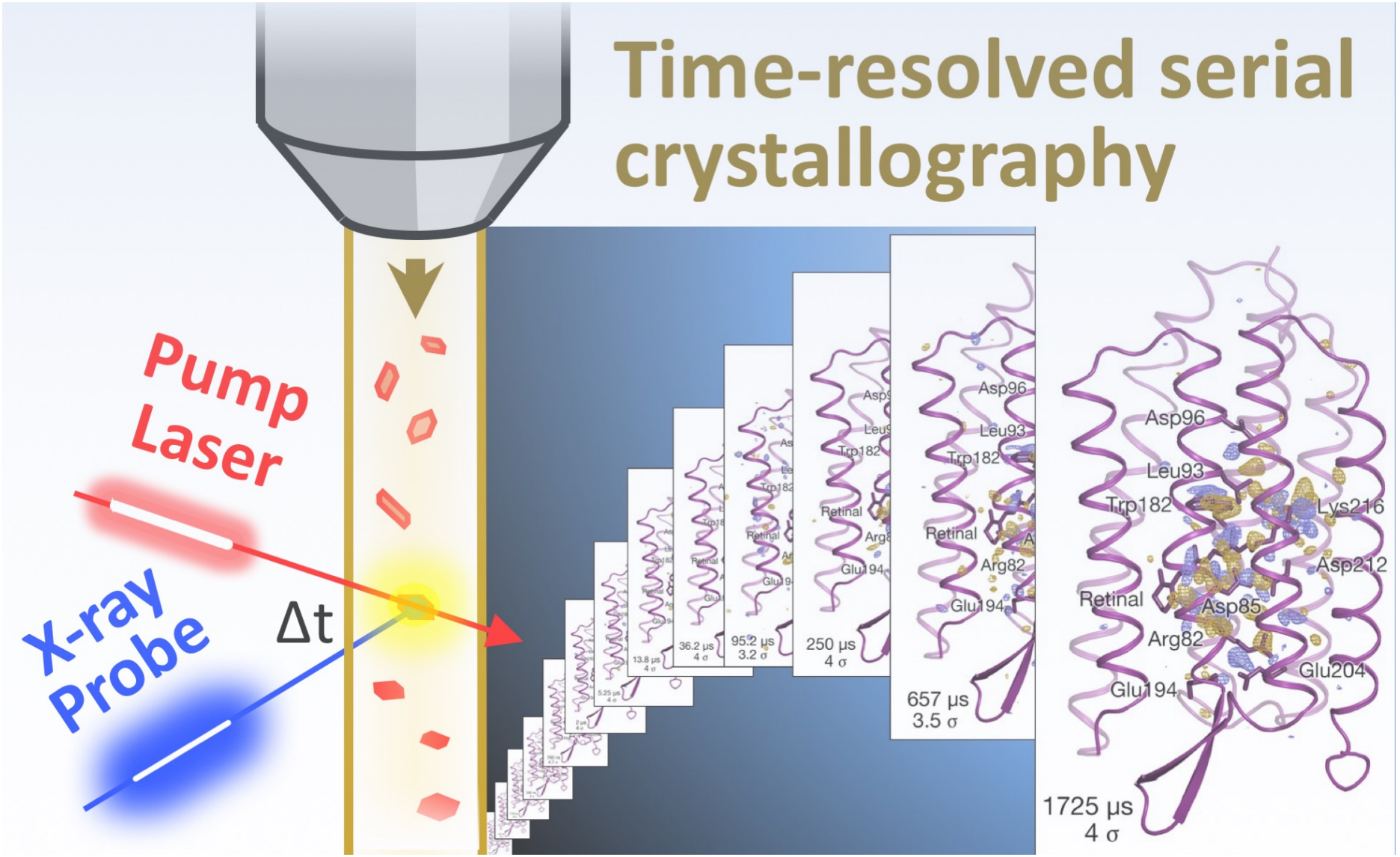
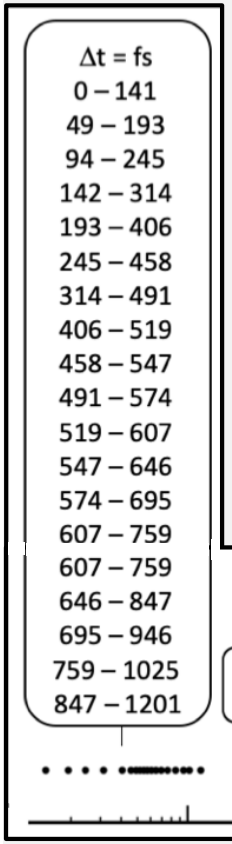
Bringing time-resolved measurements to the molecular scale

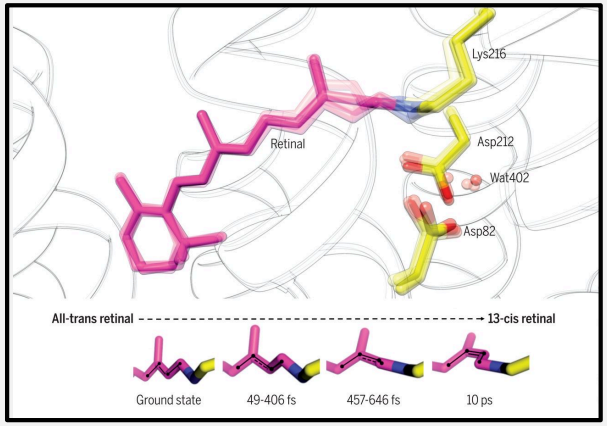


Standfuss, *Curr. Opin. Struc. Biol.*, 2019

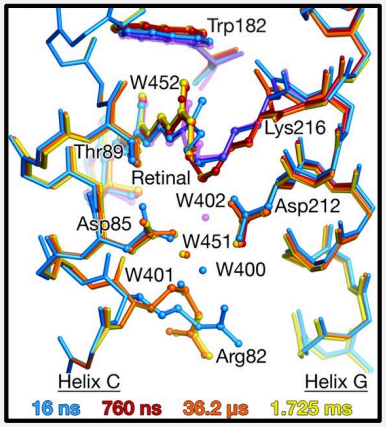
Pioneering works at synchrotrons and X-ray lasers

Learning and establishing technology abroad before bringing it to PSI

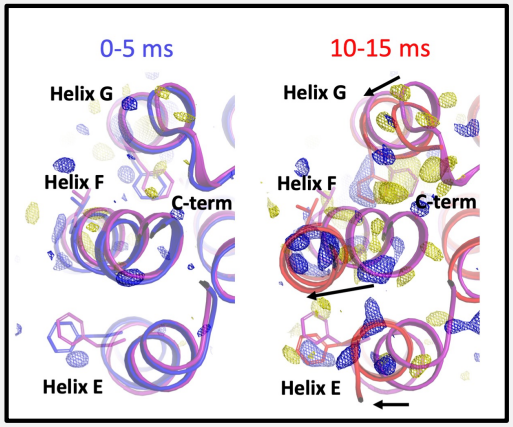




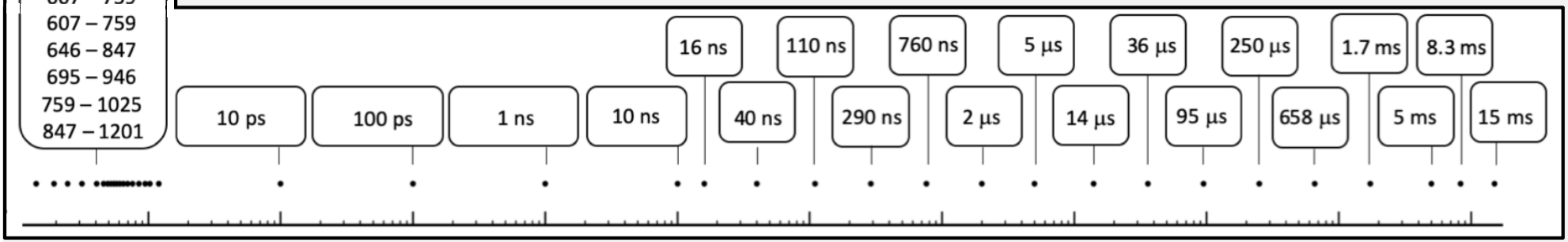
Nogly et al. 2018, Science



Nango et al. 2016, Science



Weinert et al. 2019, Science

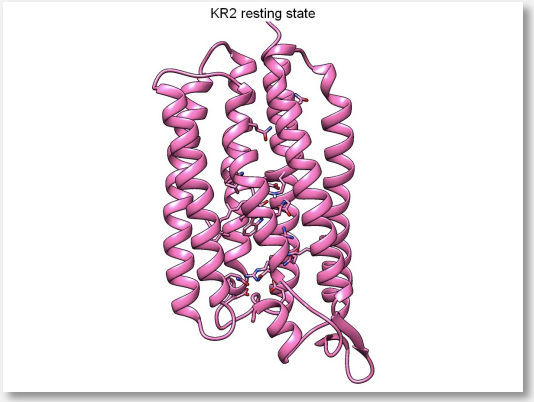


Light-driven proton pump bacteriorhodopsin

- Largest structural dynamics data resource available for any protein
- The (nearly) whole pumping cycle resolved from isomerization to proton release and re-uptake

First user experiments at SwissFEL

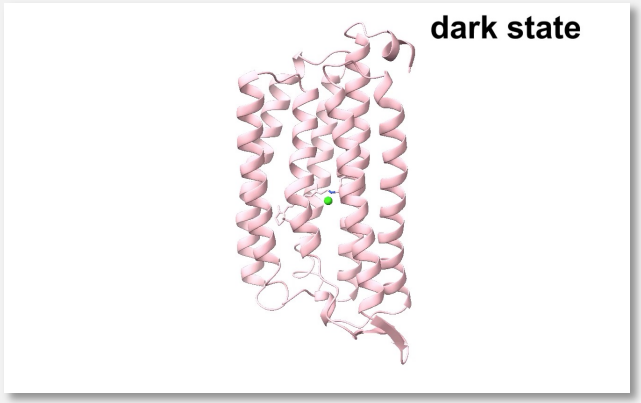
Rhodopsins pave the way into a dynamic future for structural biology



Skopintsev *et al.*, 2020, Nature

Sodium pumping rhodopsin

- Ten molecular snapshots of **sodium** transport **out of the cell**
- Next-generation **optogenetic tool**

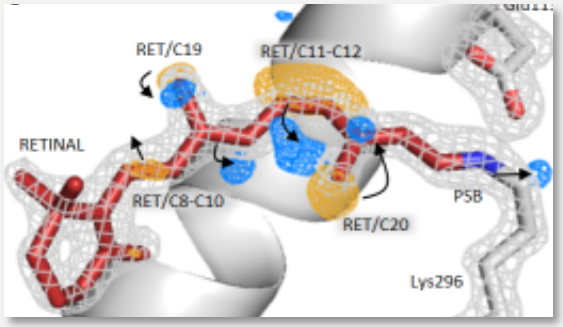


Mous *et al.*, 2022, Science

Chloride pumping rhodopsin

- SwissFEL and SLS resolves **chloride** transport **into the cell**
- Electrostatic gates **explain transport**

Nice seeing you Sandra!!



Gruhl *et al.*, 2023, Nature

Visual GPCR rhodopsin

- Molecular snapshots of the **early events in vision**
- **GPCR** activation

Great talk Valerie!!

Collaboration Nogly & Schertler

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Dynamics in Drug Discovery

Perhaps using the right kind of experiments these questions can be addressed

- **Binding and drug design**: Protein-ligand interactions are best described by dynamic induced-fit or conformational selection mechanisms and not a simplistic key-and-lock.
- **Binding kinetics**: Protein dynamics influence the rate at which a drug molecule binds to its target protein and the residence time of the drug at the binding site.
- **Allosteric regulation**: Protein dynamics are critical for transmitting allosteric signals from a drug binding site to distant sites affecting protein function.
- **Drug resistance**: Mutations can alter protein dynamics, reducing the effectiveness of a drug.
- **Mechanisms of drug action**: Conformational changes in receptors, transporters, channels or kinases can explain drug action in detail.

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X-ray lasers allow us to observe structural changes in a wide temporal window

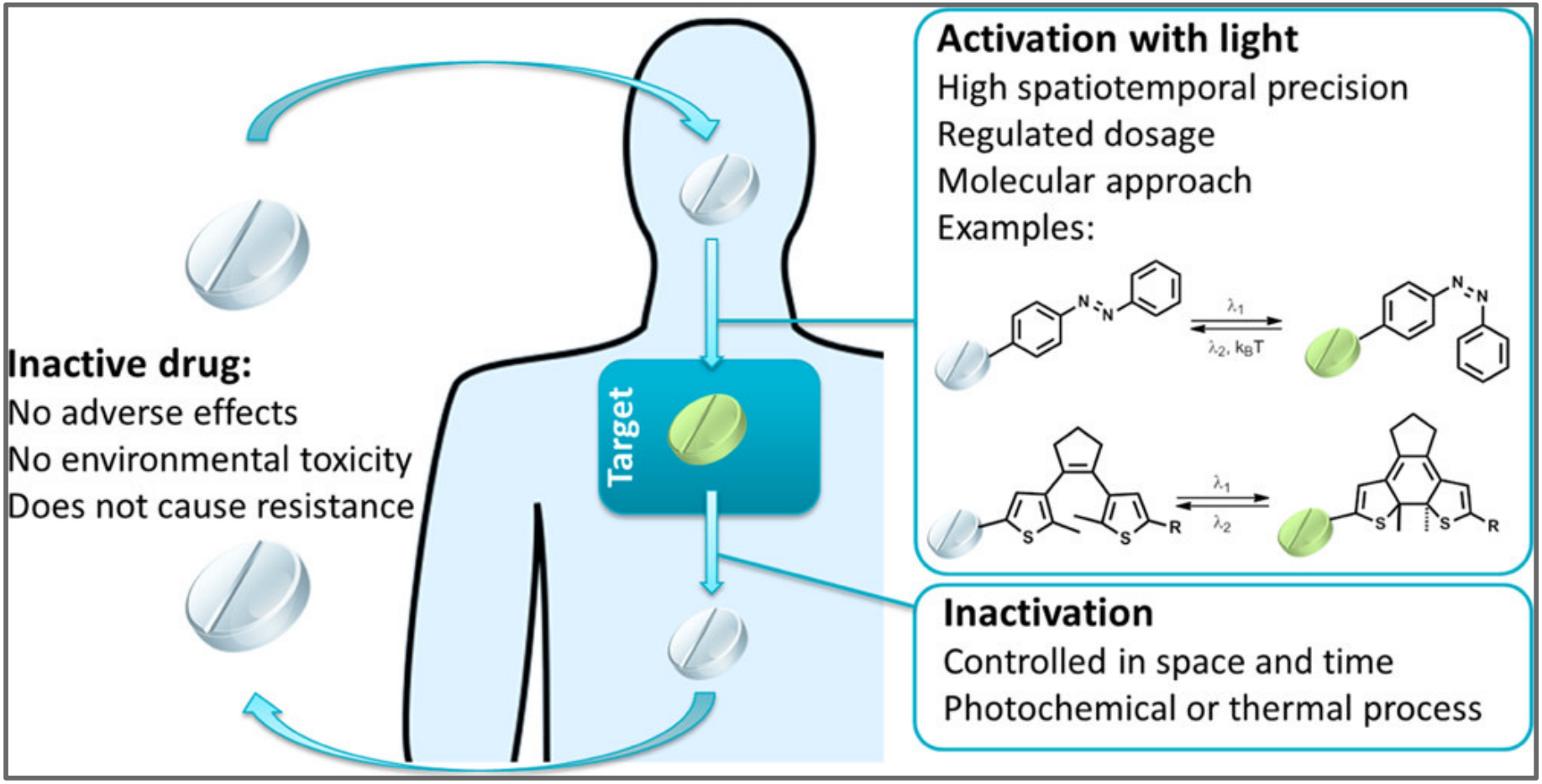
Blink of an eye	= 0.1 s
Photocycle	= 0.01 s
Retinal excitation	= 0.000 000 000 000 1 s

-> **But drug targets don't have photoswitches!**

…or do they?

The field of photopharmacology

Allows for remote spatial and temporal control of bioactivity by light

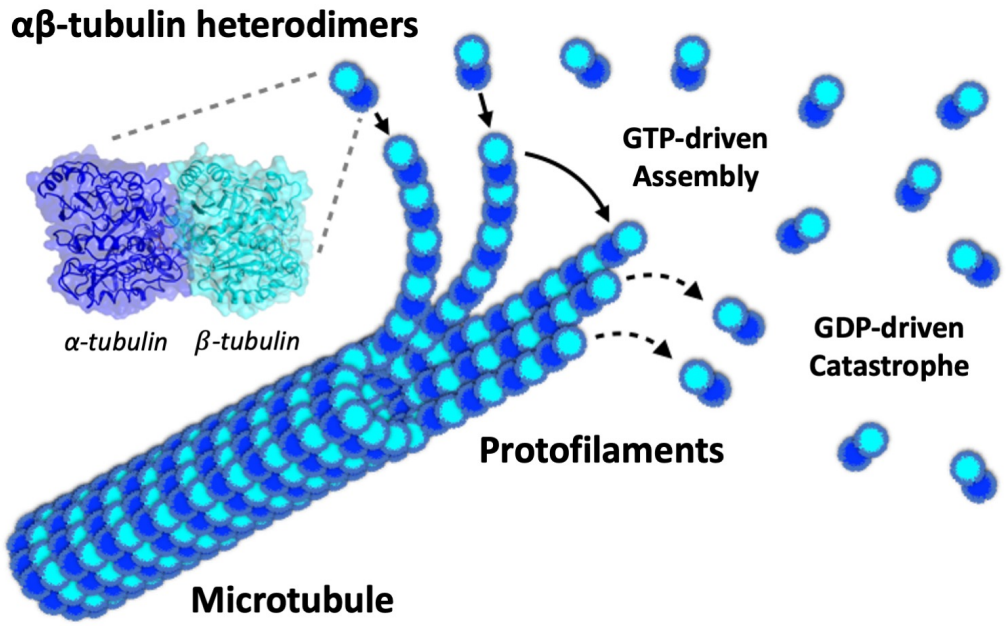


Taken from: W. Velema, W. Szymanski & B. Feringa (2014)

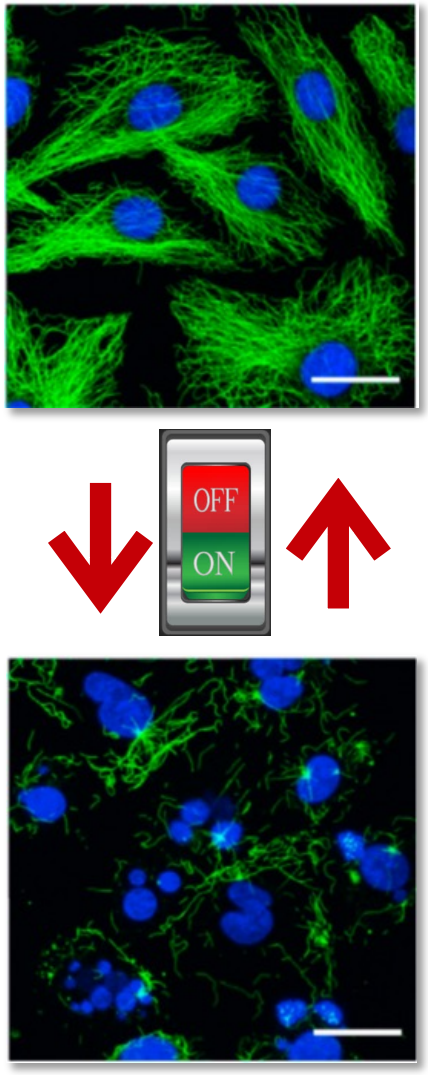
Photoswitchable Inhibitors of Cell Mitosis

The field of photopharmacology develops drugs activatable by light

Target of interest:



Photopharmacology:



Tubulin binding drugs:

- Kill cancer cells (Taxol chemotherapy)
- Reduce inflammation (Colchicine)
- Lower Covid-19 death rate (Sabizabulin)

Collaboration Steinmetz Group (PSI)

From: Borowiak *et al.*, *Cell*, 2015

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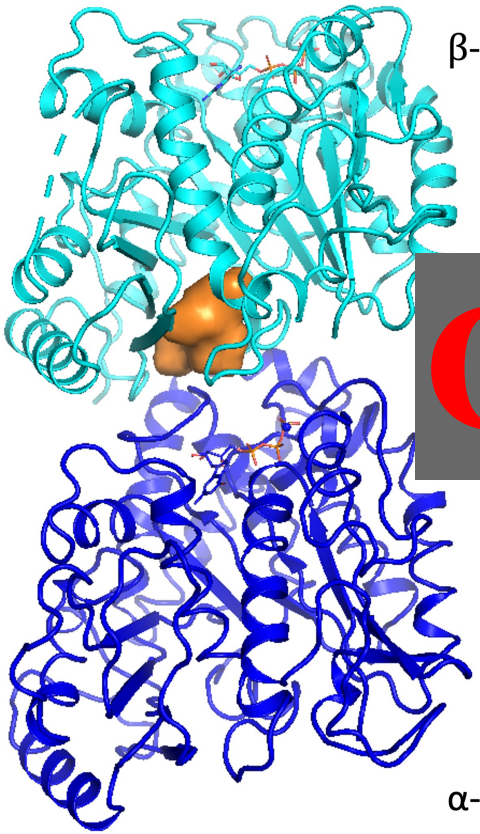
Microtubule dynamics: Colchicine binding site

Selection of small molecule drugs under development

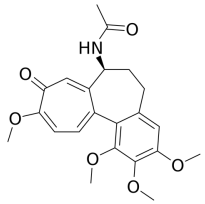
αβ-tubulin heterodimer



β-tubulin

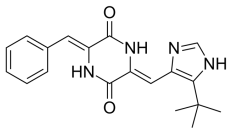
α-tubulin

How it started:



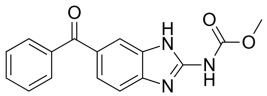
Colchicine
Gout pain
FDA approved

Where we go:

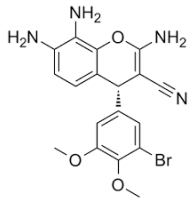


Plinabulin
Lung cancer
Clinical phase 3

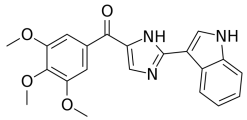
Off-target effects



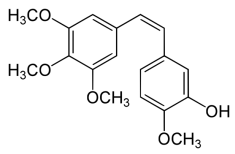
Mebendazole
Worm infections
FDA approved



Crolibulin
Thyroid cancer
Clinical phase 2



Sabizabulin
Covid-19
FDA approved



Combretastatin A4
Prostate cancer
Multiple clinical trials

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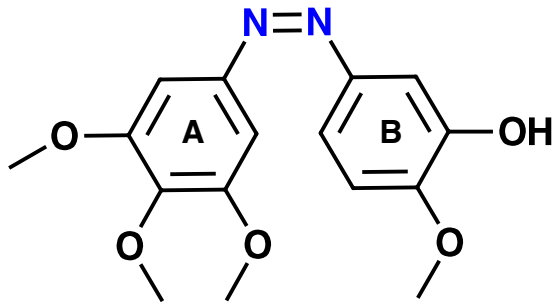
Prototypical azobenzene photoswitch

Azo-combretastatin A4, a photoswitchable anti-cancer compound

Natural source

Combretastatin A4 is a promising anti-cancer drug candidate from the bark of the South African bushwillow (a medical plant of the Zulu).

Photochemical affinity switch



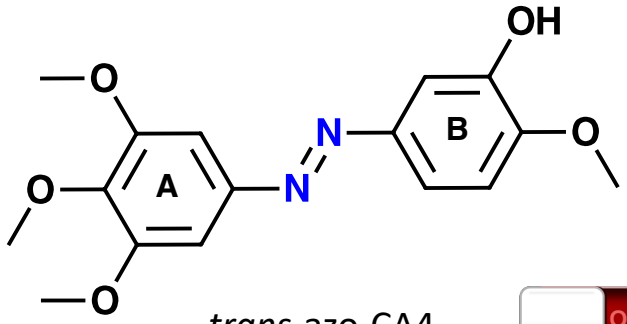
cis-azo-CA4

ON

Synthesis with azobenzene motif

λ= 450 to 530 nm

λ= 350 to 430 nm



trans-azo-CA4

OFF

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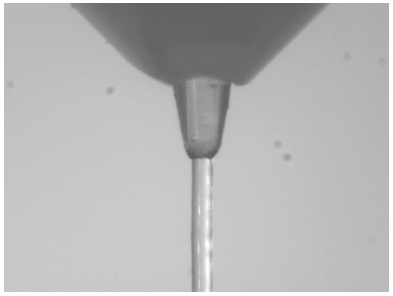
A couple of night shifts later

A big !!!THANK YOU!!! to everyone at SLS and SwissFEL

A few shifts at SLS

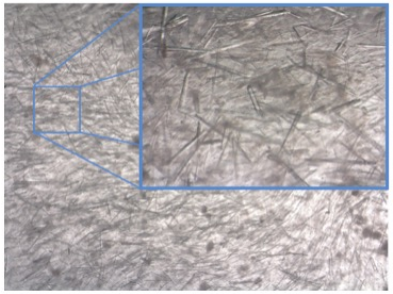
Crystal enthusiasts

HE Cellulose



A few shifts at SwissFEL

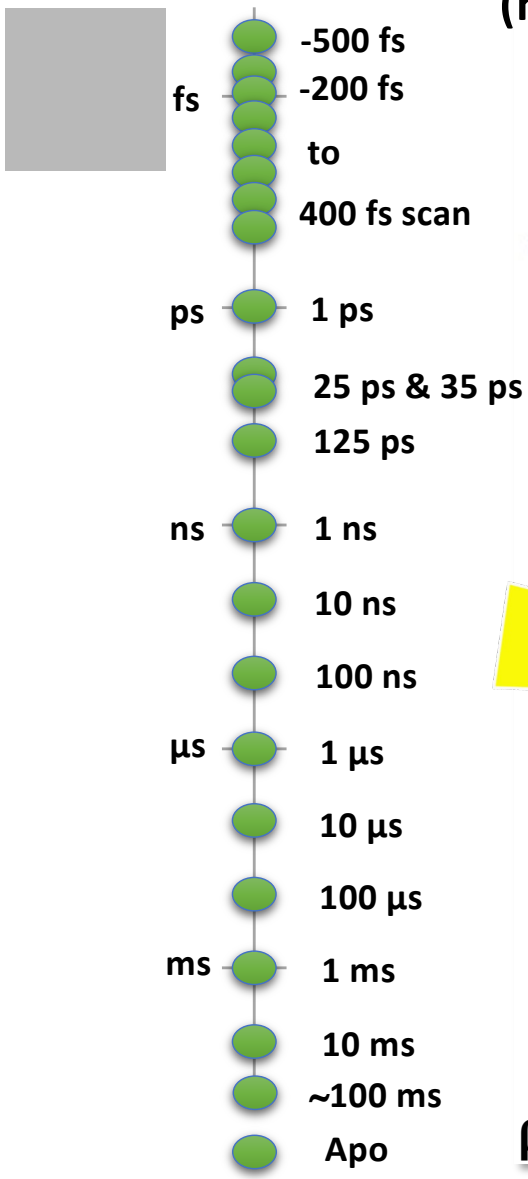
Microcrystals

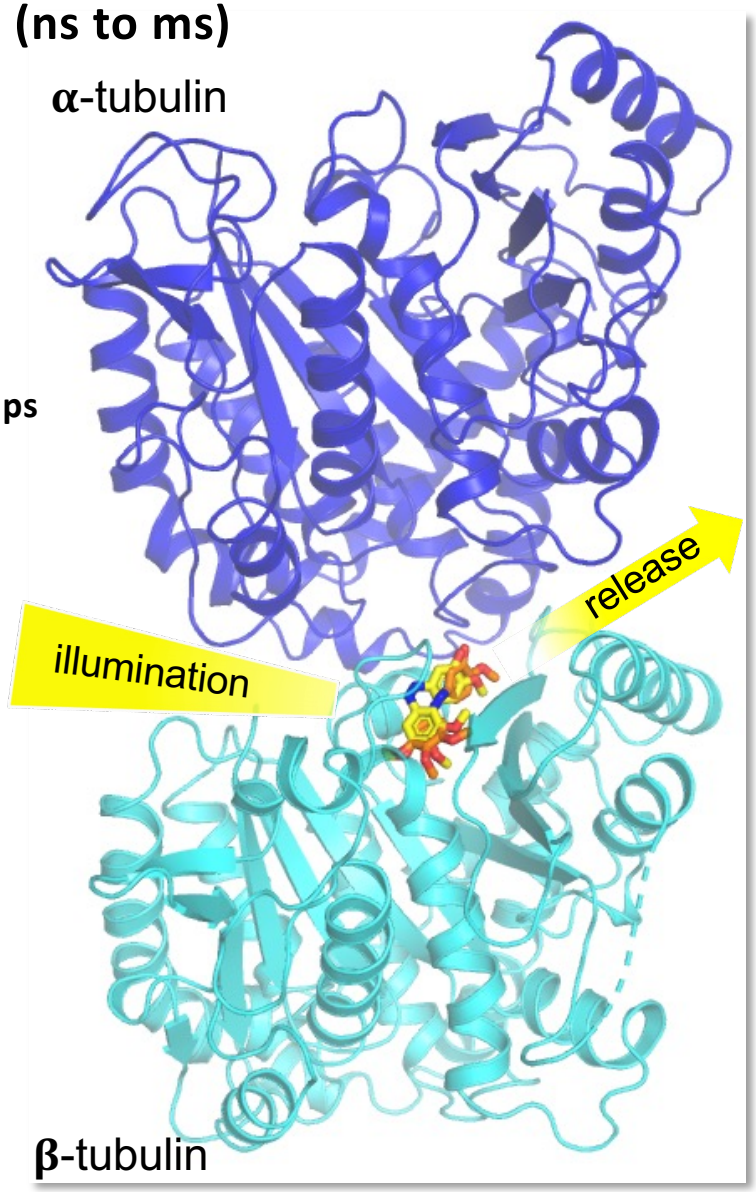


Molecular snapshots of drug release

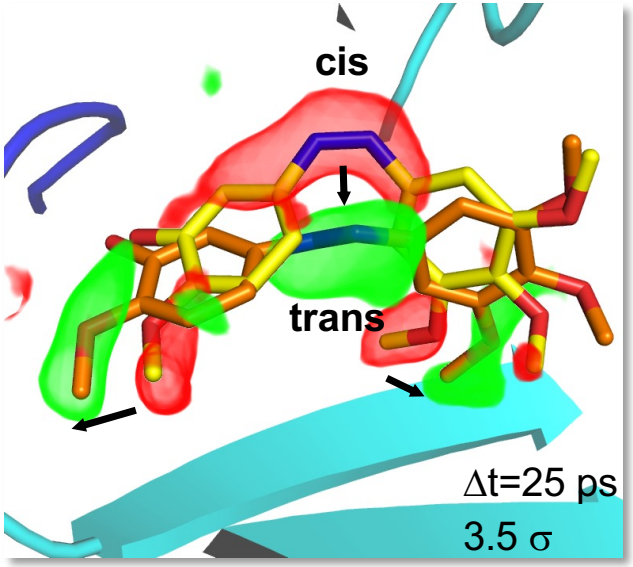
Using synthetic photoswitches for time-resolved crystallography

Mechanisms of Drug Action (ns to ms)





Azobenzene Photochemistry (fs to ps)



>2.000.000 patterns, 1.7 Å resolution

Ligand-protein interactions at **near-atomic resolution** over **fourteen orders** of magnitute in time

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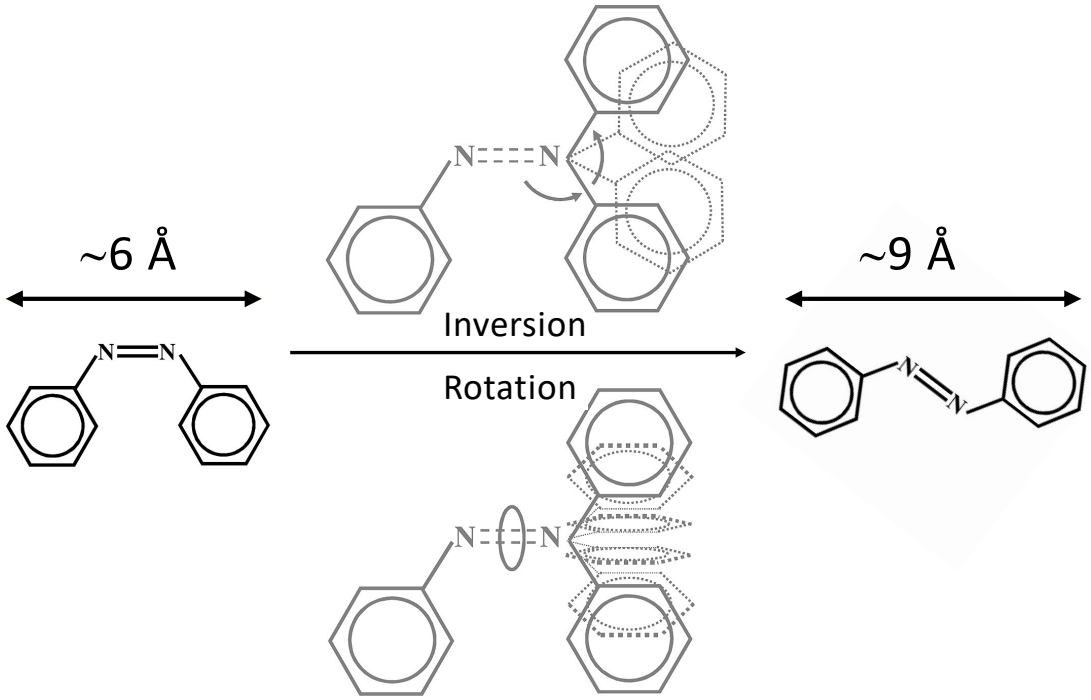
Photon energy is translated into mechanical energy via a transition in molecular shape!
… but the mechanisms remain controversial



From: Conti et al., *JACS*, 2008

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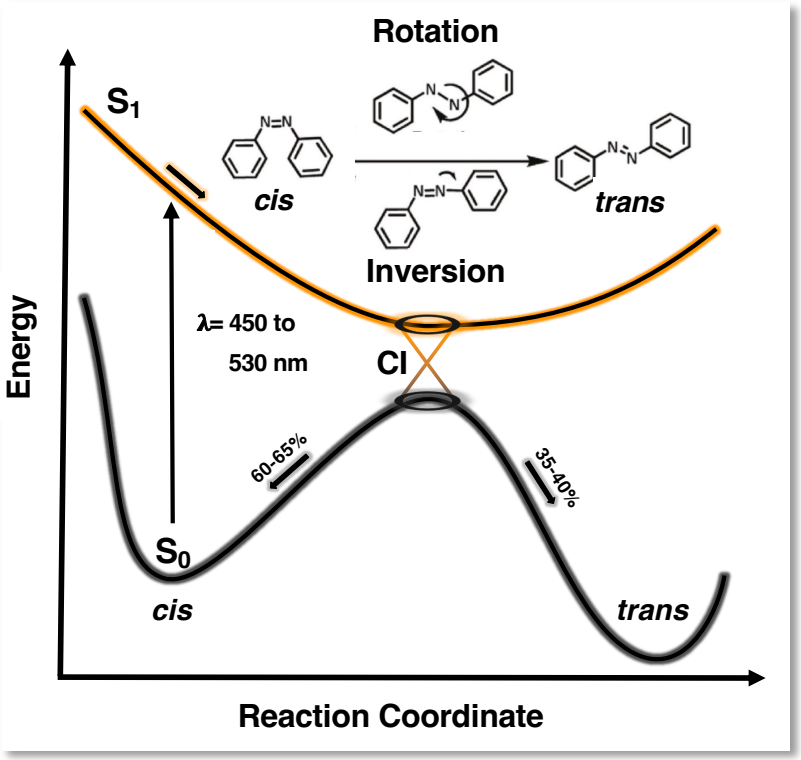
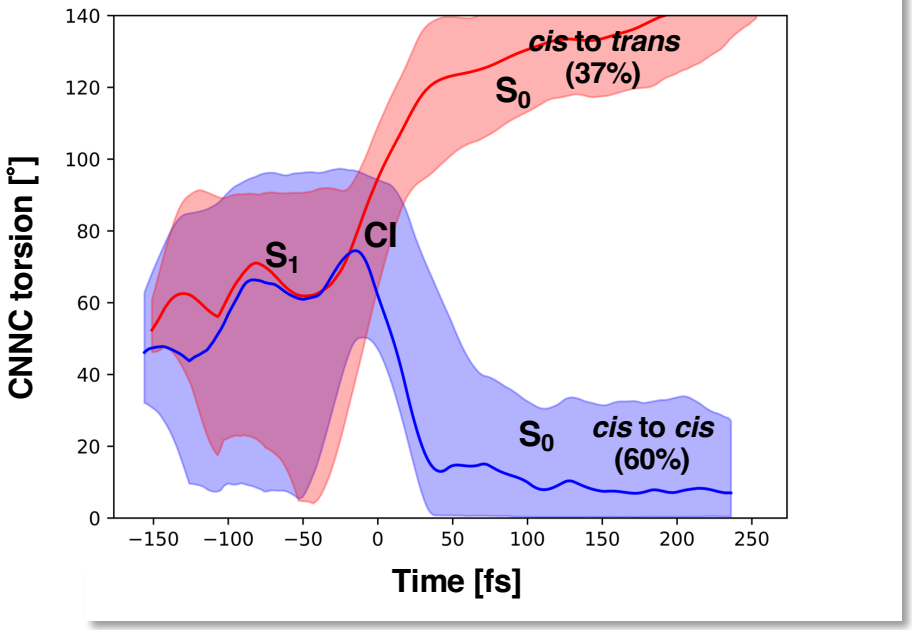
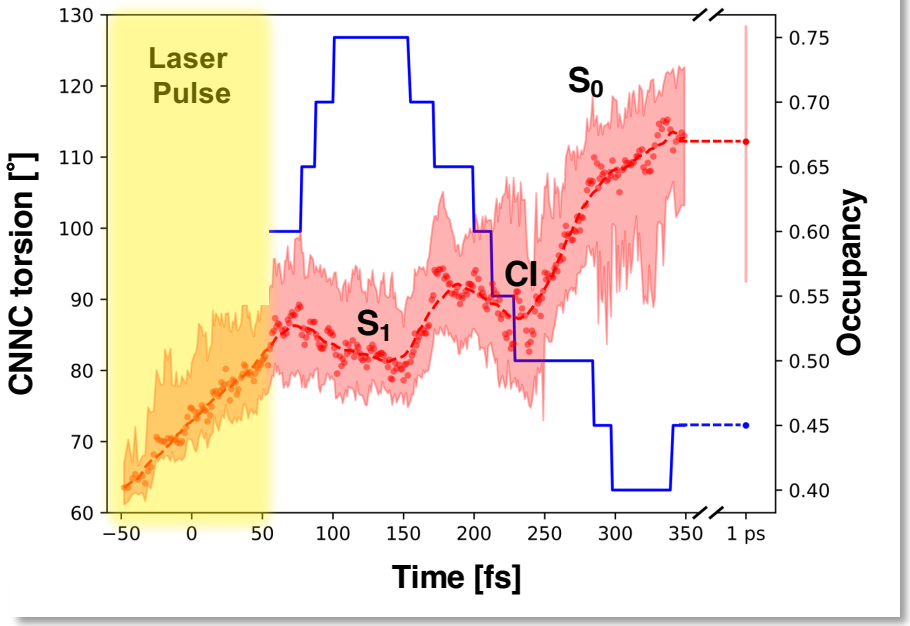
Potential Energy Diagram



Quantum Chemical Simulation



Femtosecond Structural Photochemistry



Collaboration Schapiro group
Hebrew University Jerusalem

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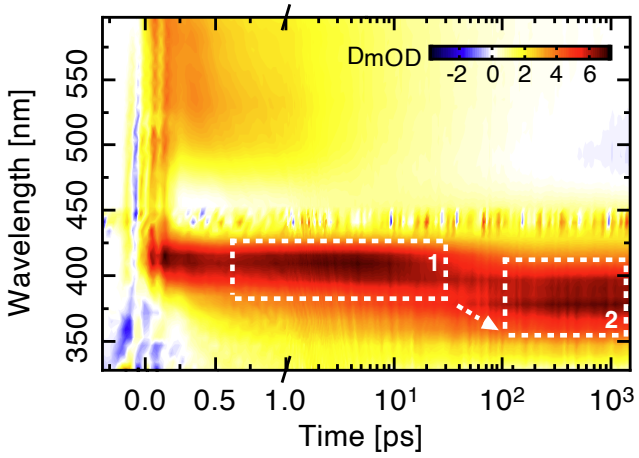
Ultrafast Photoreaction of a Molecular Switch

But the reaction is not finished within femtoseconds

Time-resolved absorption spectroscopy

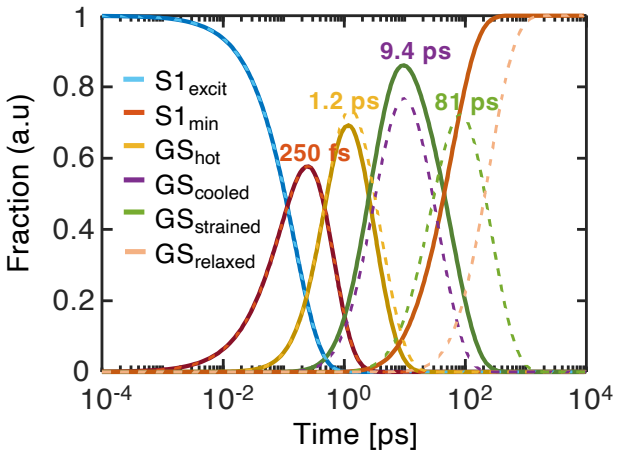
Azo-CA4 tubulin



Lifetimes

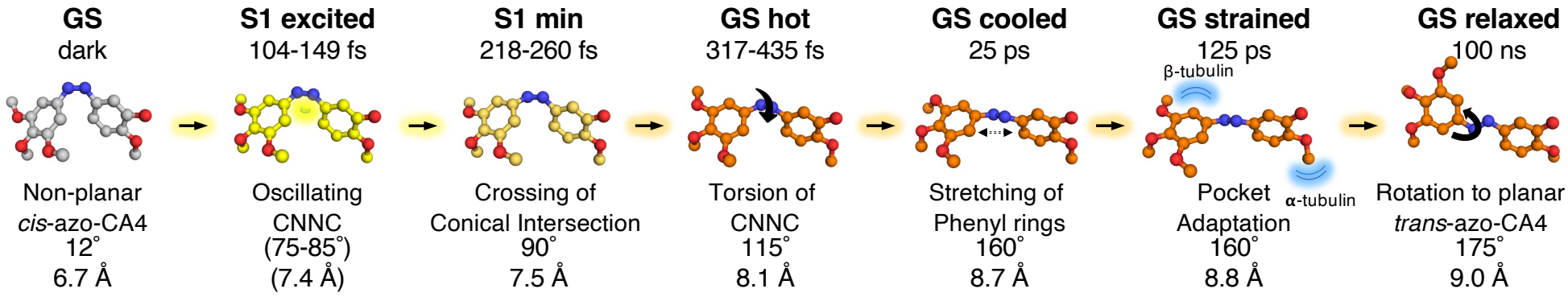
	Azo-CA4 solution	Azo-CA4 tubulin
τ_1	0.15	0.15
τ_2	0.45	0.45
τ_3	2.8	3.6
τ_4	X	35
τ_5	59	240

Populations



Collaboration Wachtveitl group, University of Frankfurt

Time-resolved structural biology



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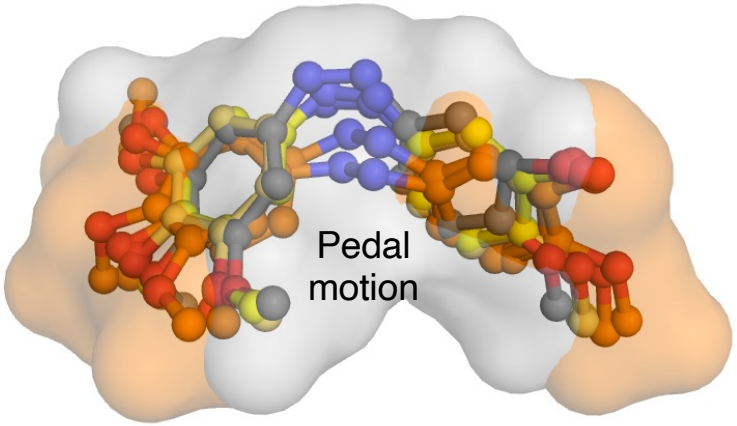
Ultrafast Photoreaction of a Molecular Switch

Two principal steps characterize the photoreaction

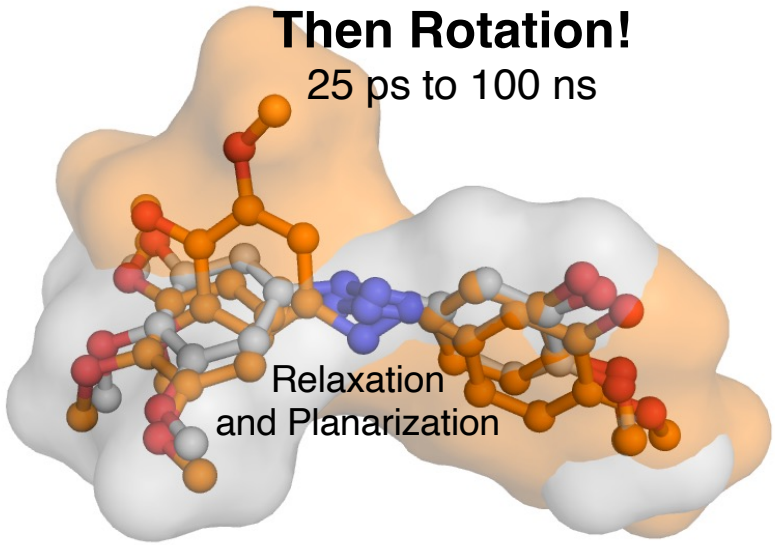
First Torsion!

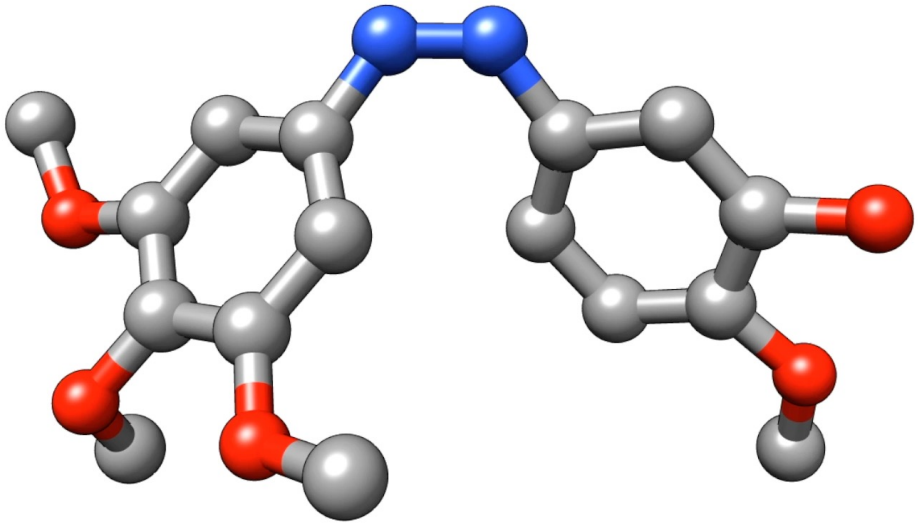
Dark to 25 ps



Then Rotation!

25 ps to 100 ns





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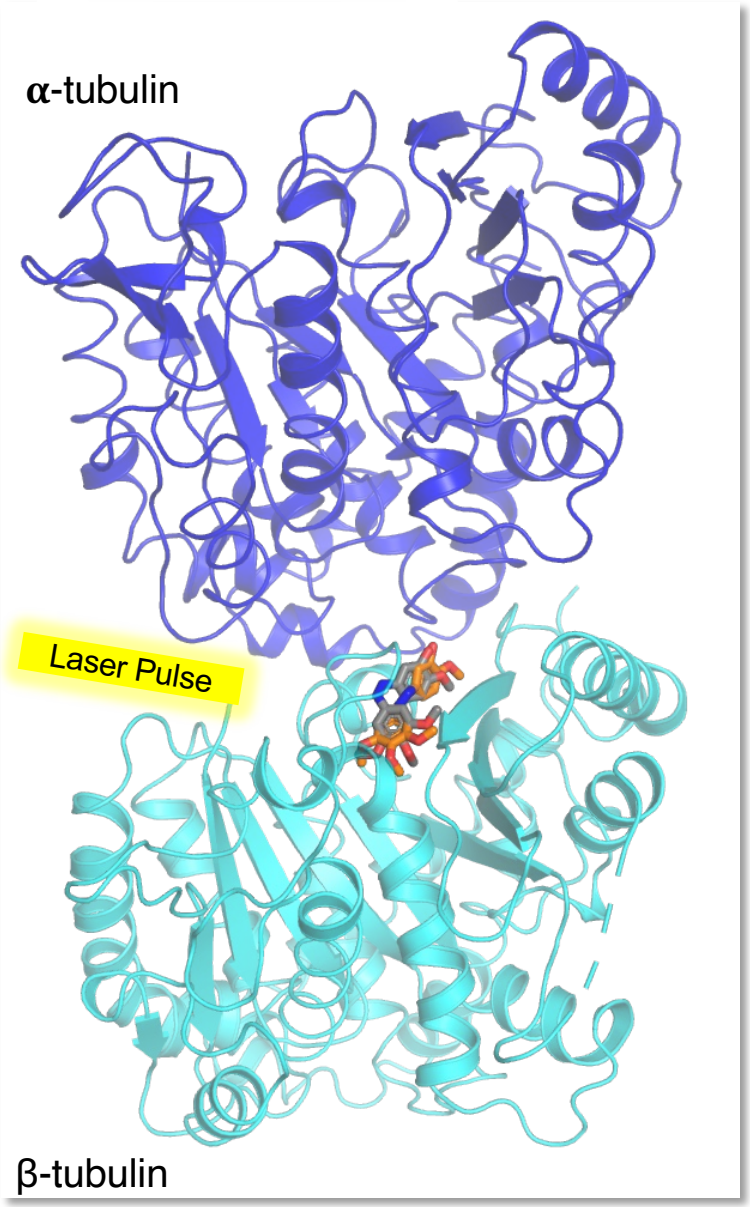
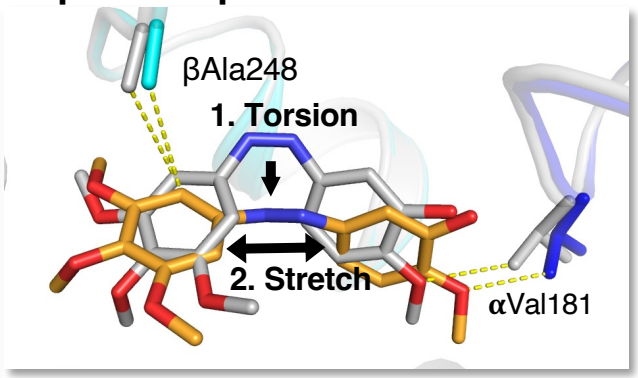
Photoreaction in context of tubulin

Protein environment constrains the early ligand response

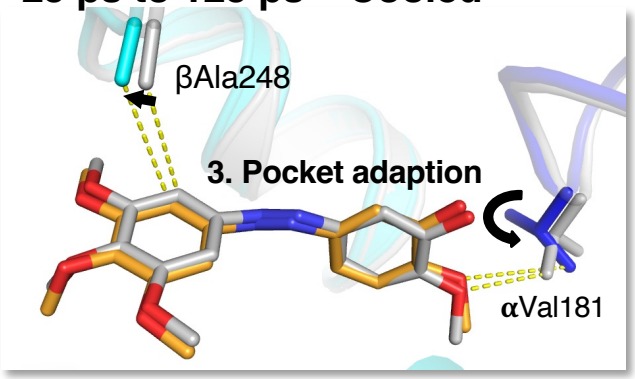
Tubulin heterodimer



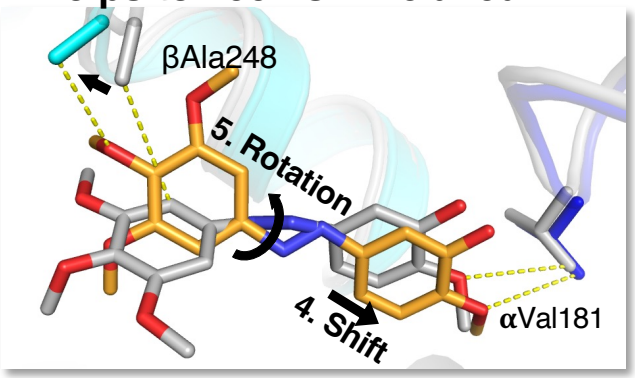
0 ps to 25 ps – Isomerized



25 ps to 125 ps – Cooled

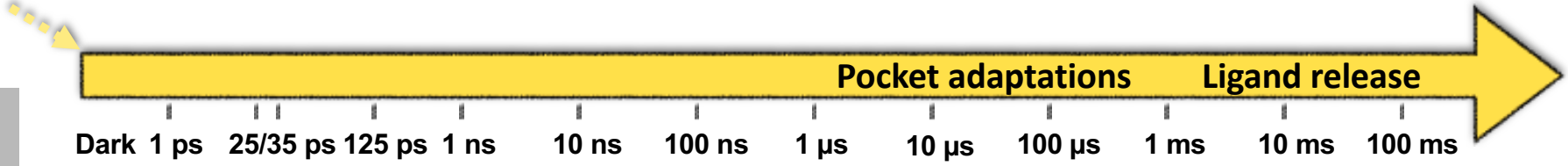


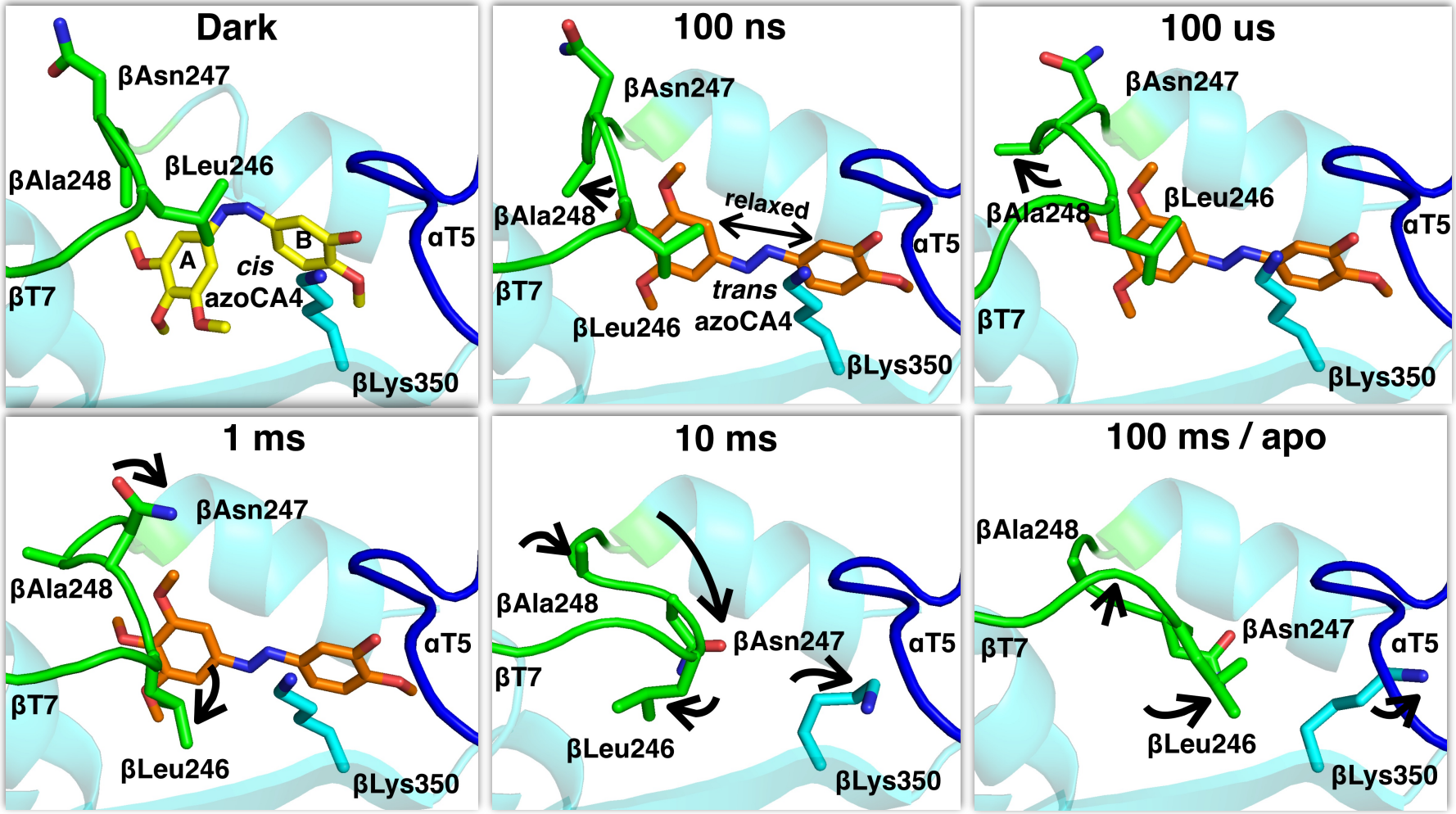
125 ps to 100 ns – Relaxed



Binding pocket adaptations in the microseconds

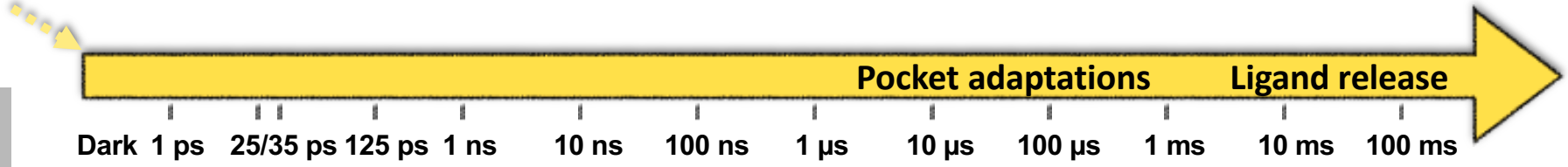
Movements of the βT7 gating loop lead to azo-CA4 release



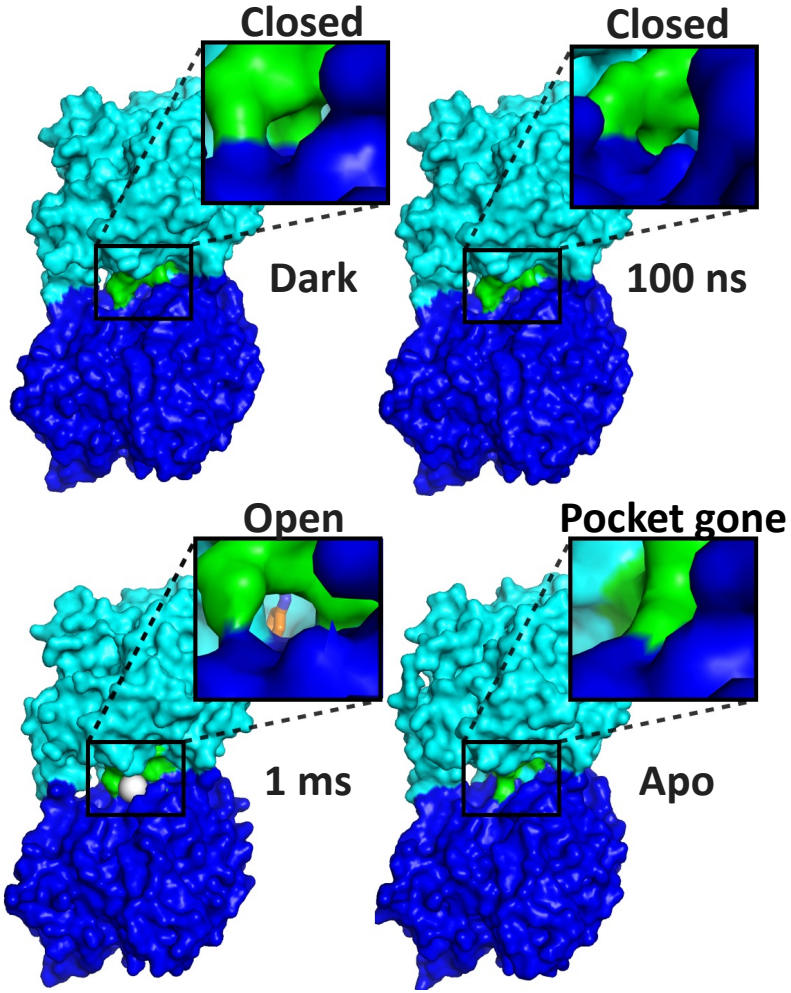


Molecular mechanism of ligand release

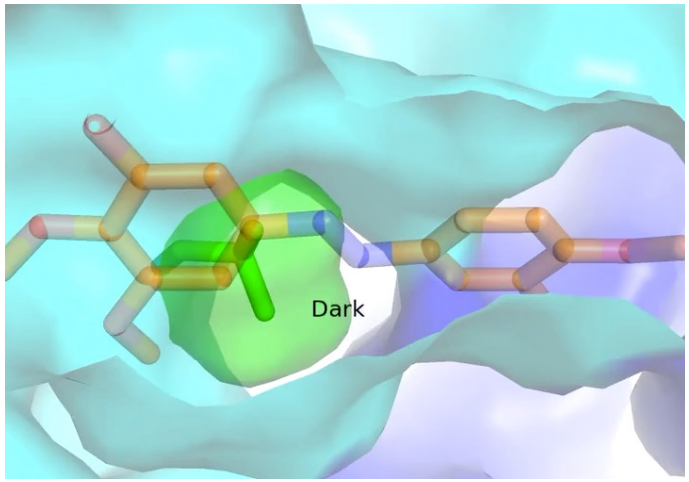
Rearrangements of β7 loop open ligand release pathway



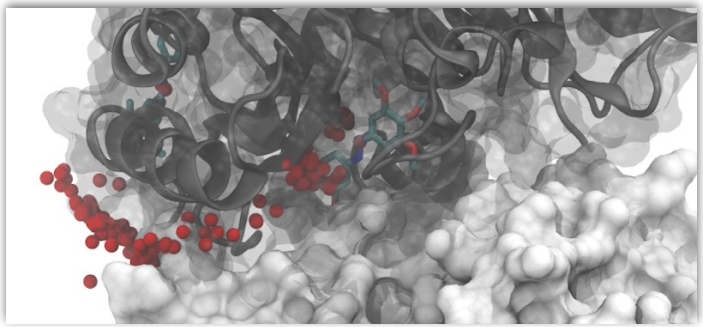
Release in the milliseconds



Role Leu246



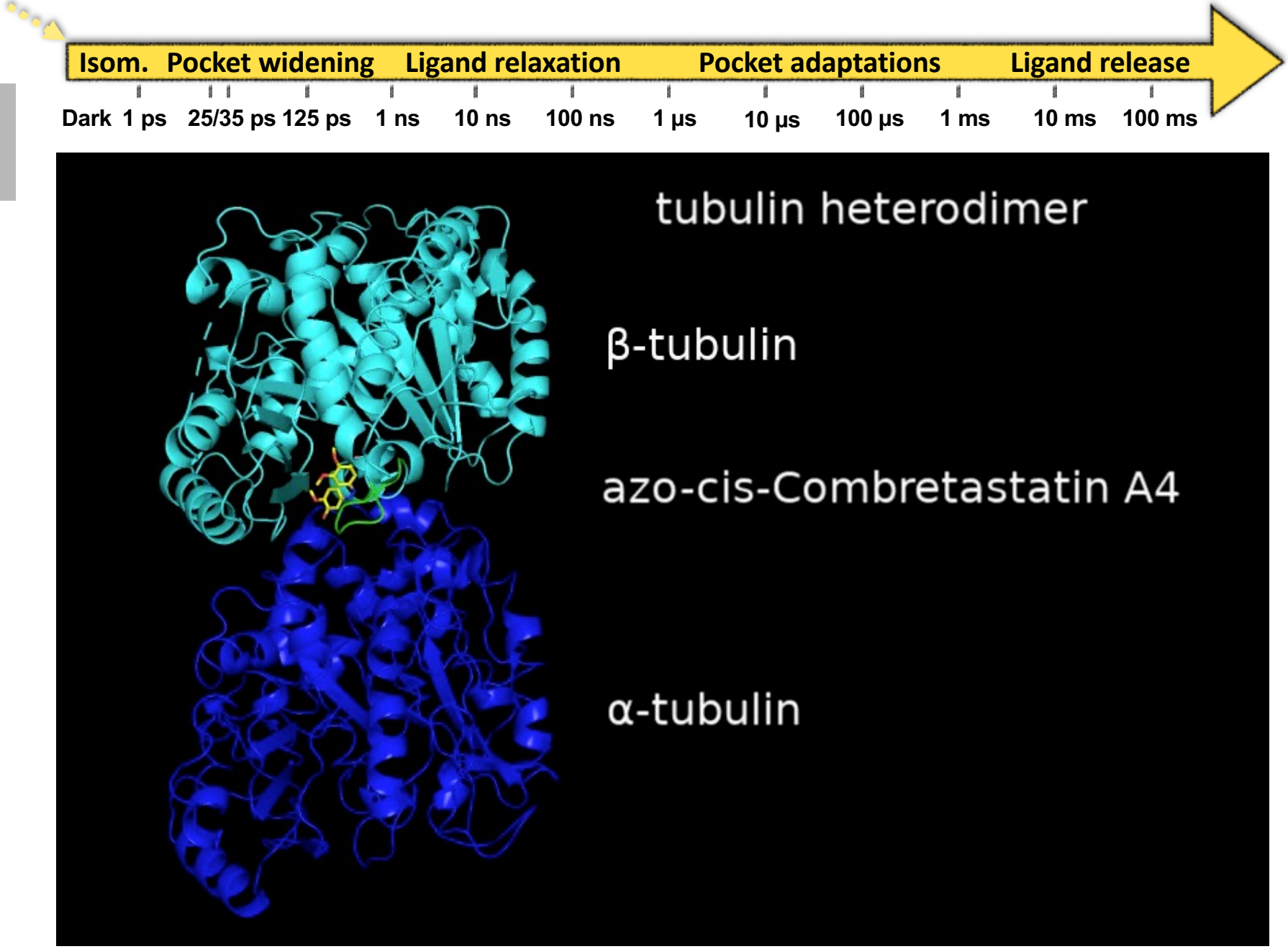
Molecular dynamic simulations



Simulations from Cavalli group, University of Bologna

Drug release “The Movie”

Dynamics within the colchicine site targeted by gout, cancer and covid-19 drugs



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But can we learn something about the molecular mechanisms of drug action?

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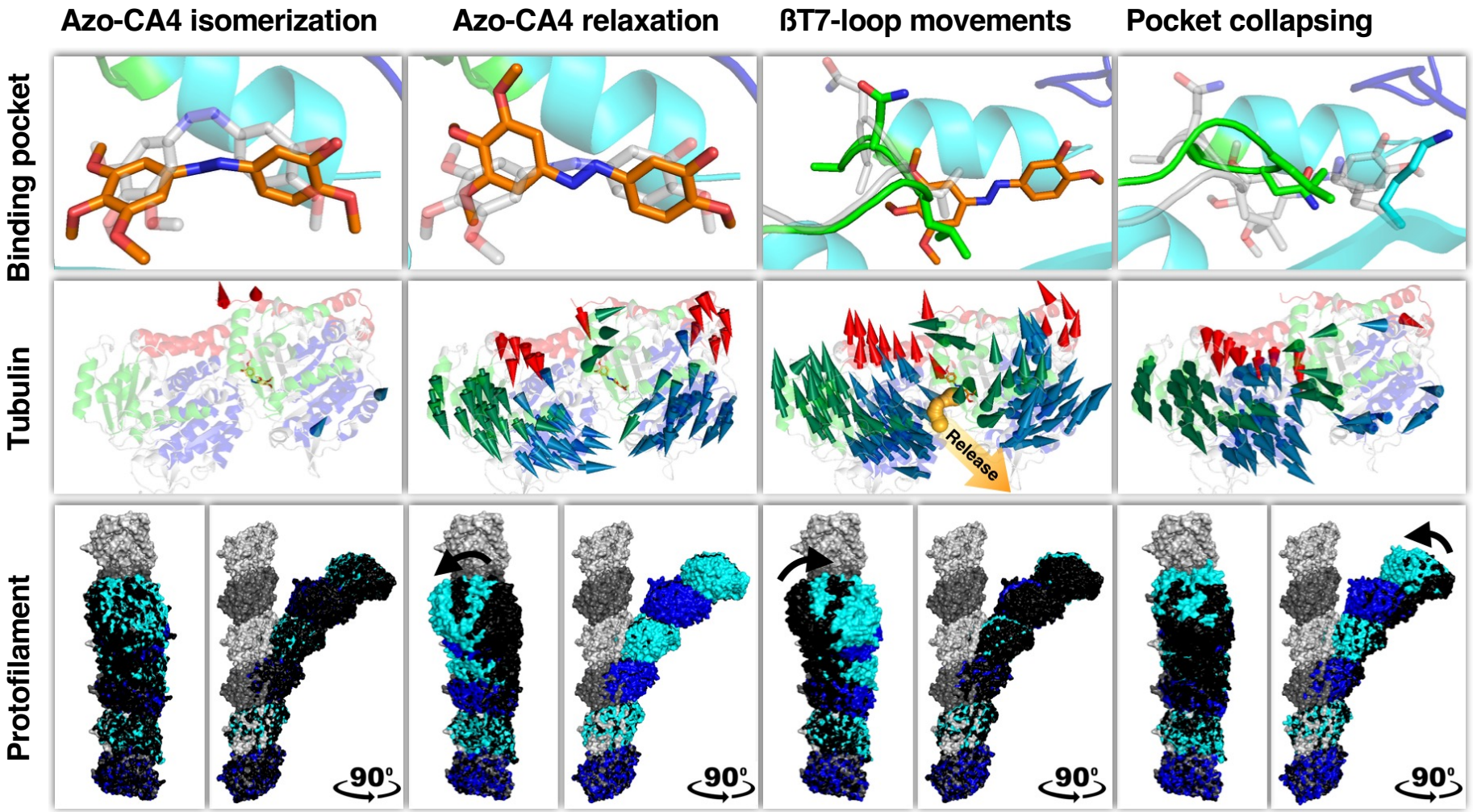
Global tubulin rearrangements

Local changes in the binding pocket have global impact on tubulin



Wranik et al., Nature Comm., 2023

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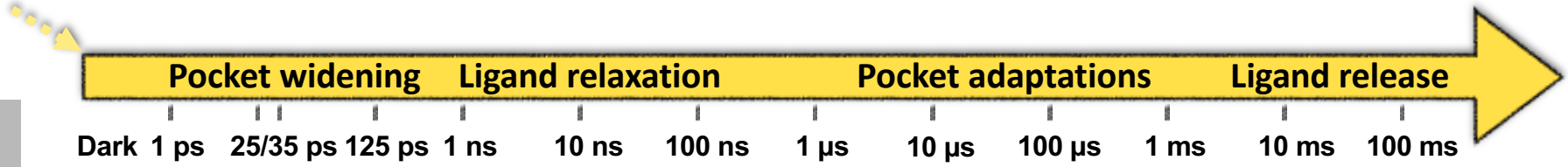
Why watch protein-drug interactions dynamics?



Insights for drug development approaches:

Photopharmacology

Expanding binding pocket

Metastable trans binding pose

Not a simple Key and Lock

→ Optimizing "residence time"

Intermediate states for simulations:

Verifying QM/MM of early photochemistry

Opening of ligand release pathway at 1 ms

→ Milliseconds out of reach Experimental verification

Methodological proof of concept:

Watch molecular motions in a wide range of targets with highest temporospatial resolution.

→ Diffusion based methods versatile, but slow

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Christmas Party, December 2022

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