Molecular Science: Part 2
Molecular structure and dynamics

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CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

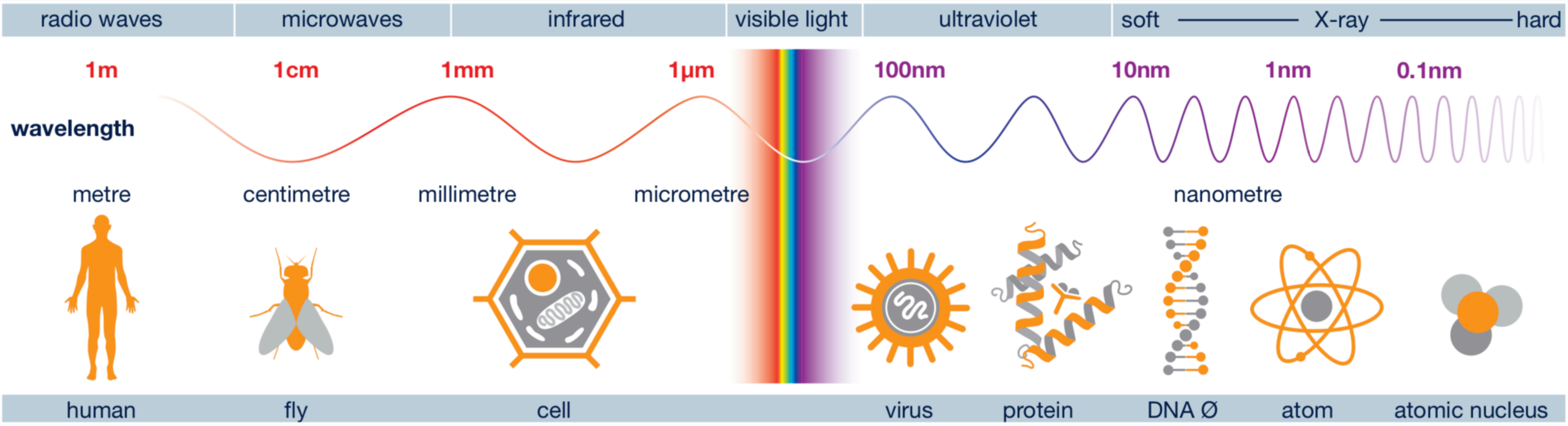
UHH Universität Hamburg DER FORSCHUNG | DER LEHRE | DER BILDUNG

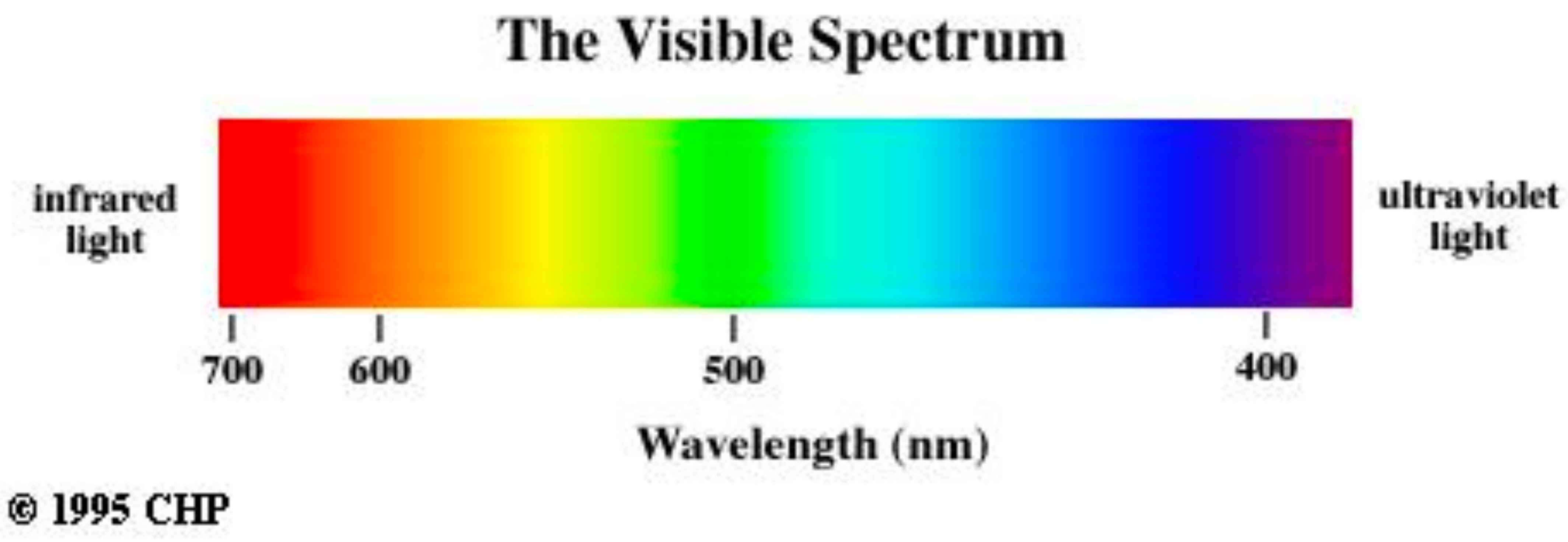
CFEL SCIENCE Controlled Molecule Imaging

erc COMotion European Research Council Established by the European Commission

DESY

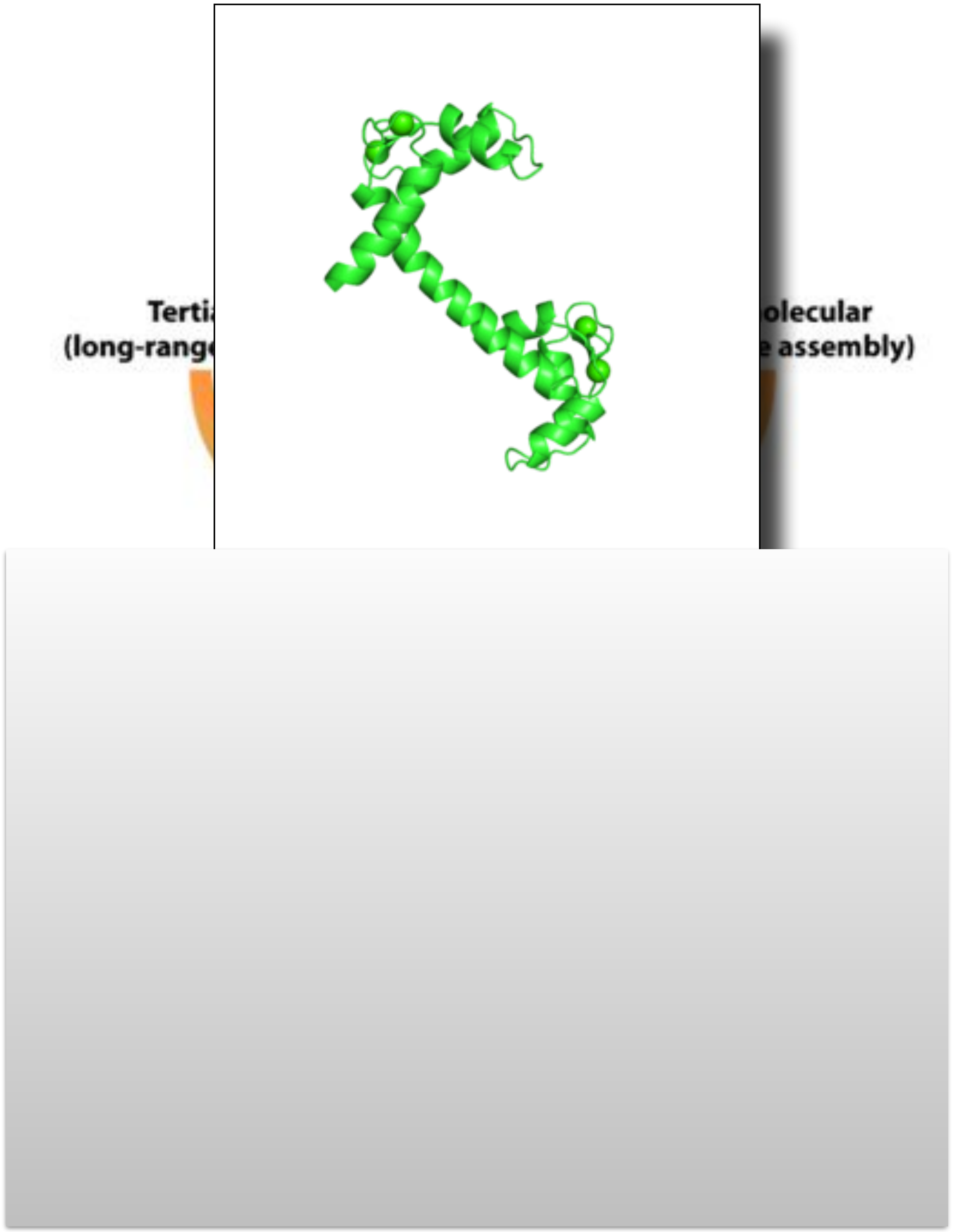
Length scale

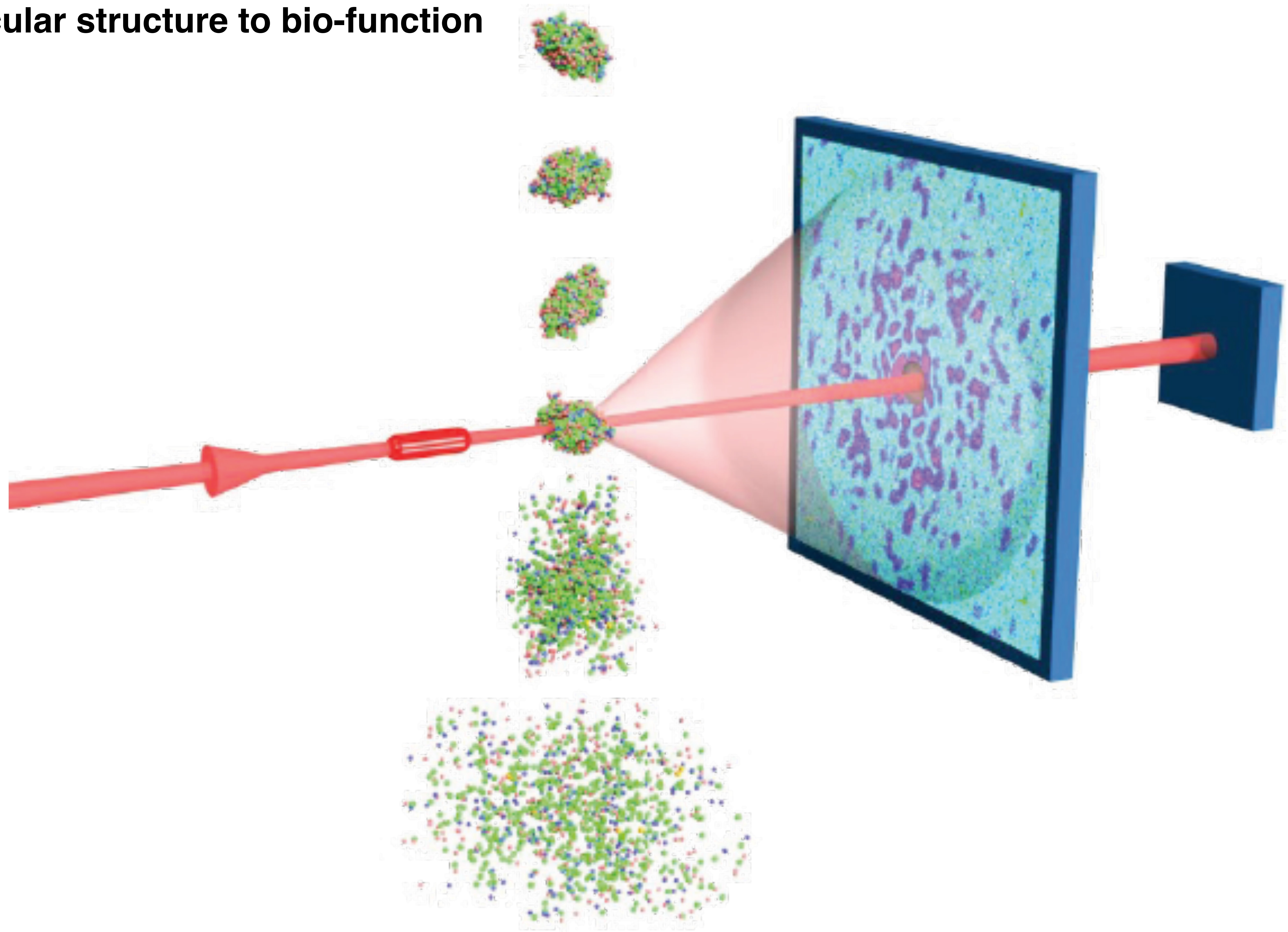




Structure & Function

Moving beyond the static picture; relate molecular structure to bio-function

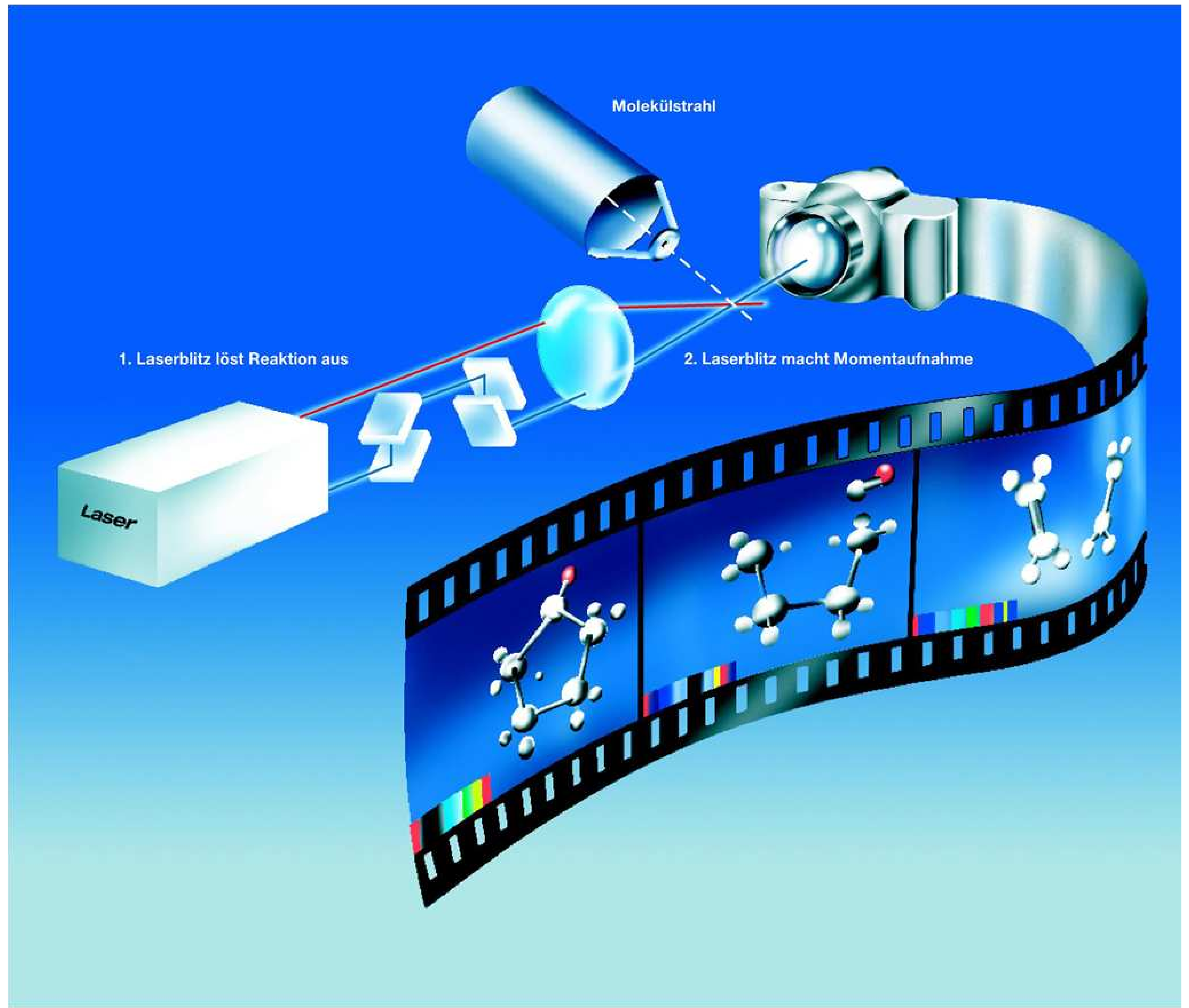


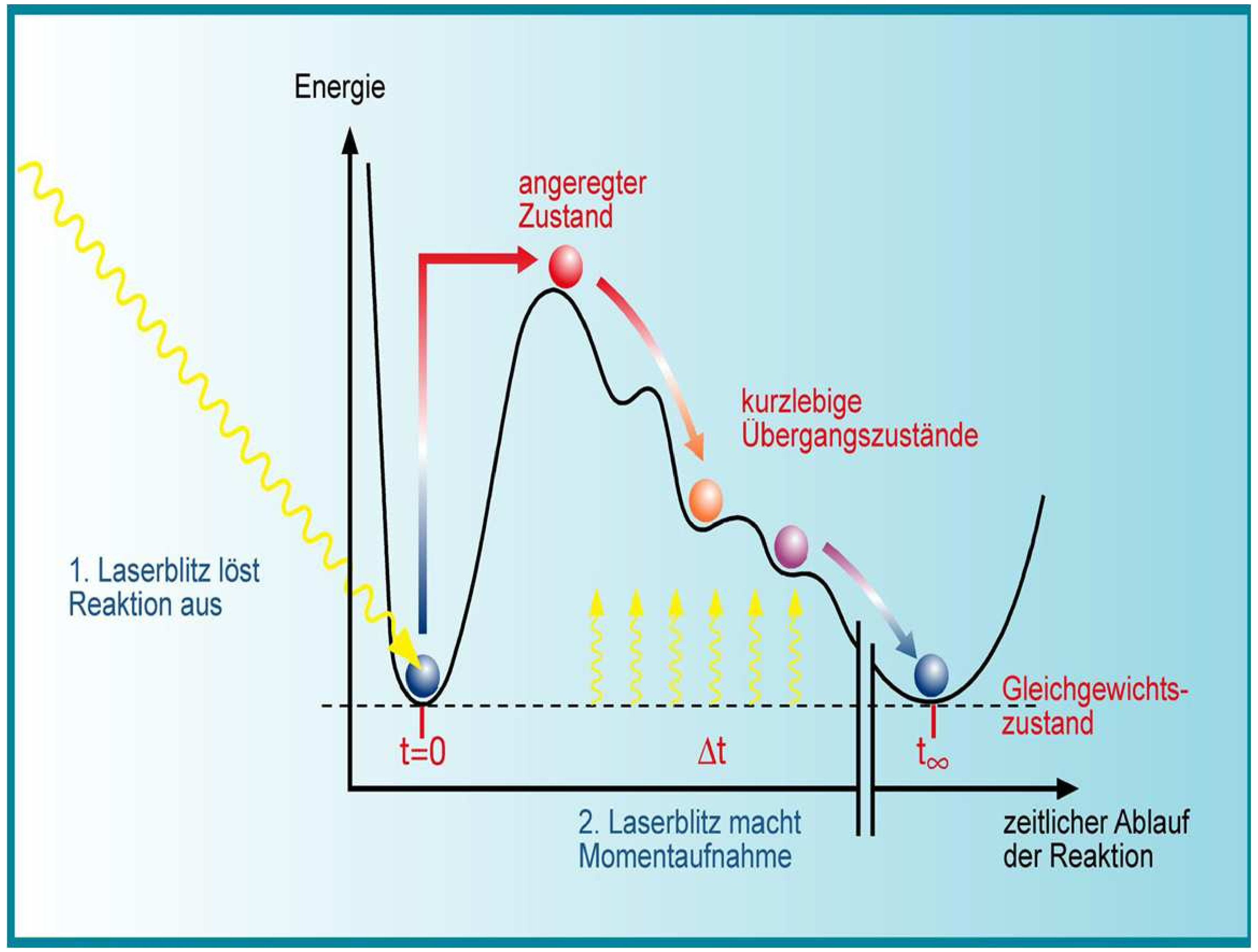


Barty, Küpper, Chapman, *Ann. Rev. Phys. Chem.* **64**, 415–435 (2013)
Chang, Horke, Trippel, Küpper, *Int. Rev. Phys. Chem.* **34**, 557–590 (2015); arXiv:1505.05632 [physics]

Watching chemistry
with atomic spatial (100 pm) and temporal (10 fs) resolution

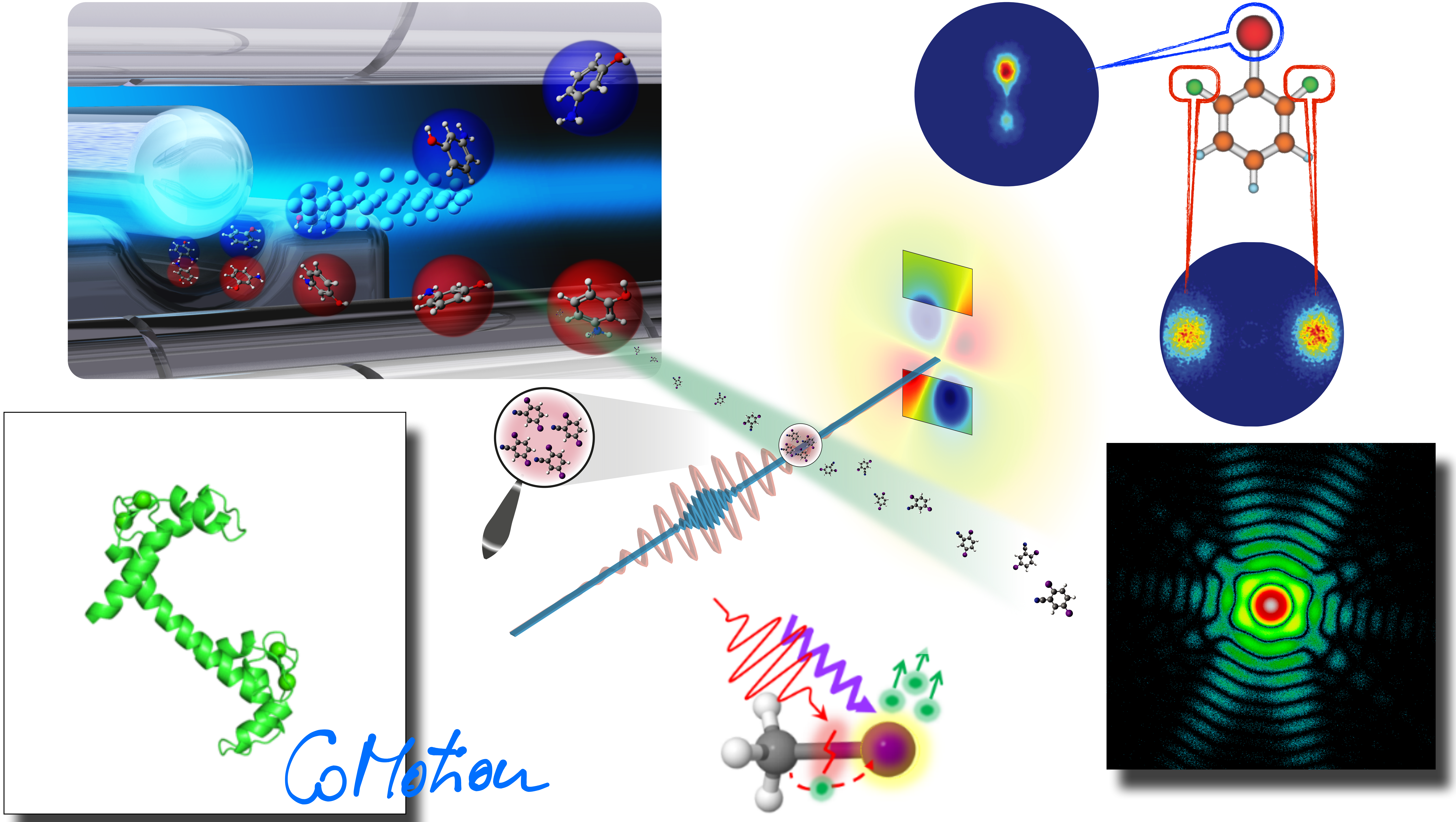
- Imaging chemical reactions of single molecules



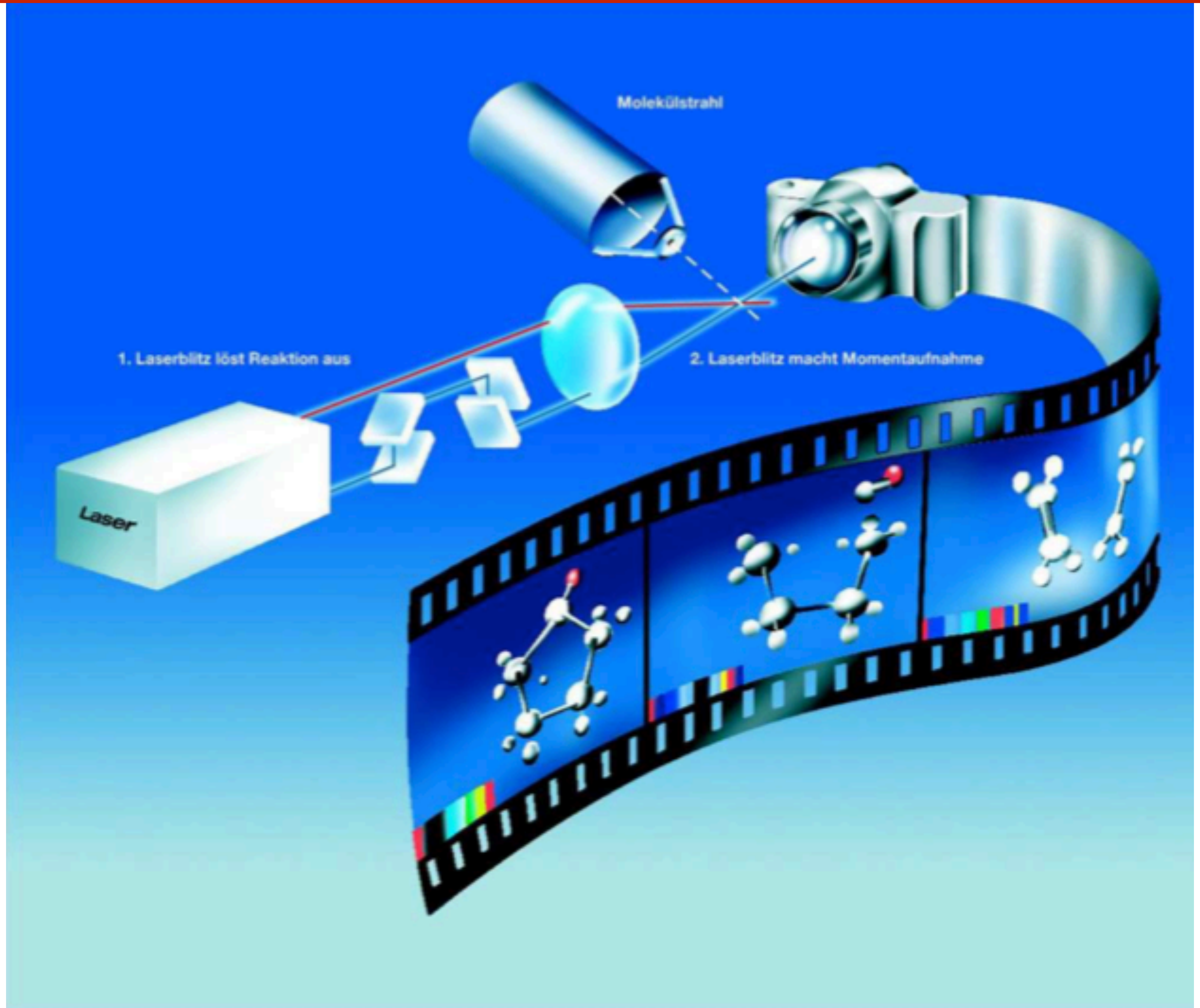


with atomic resolution in real time

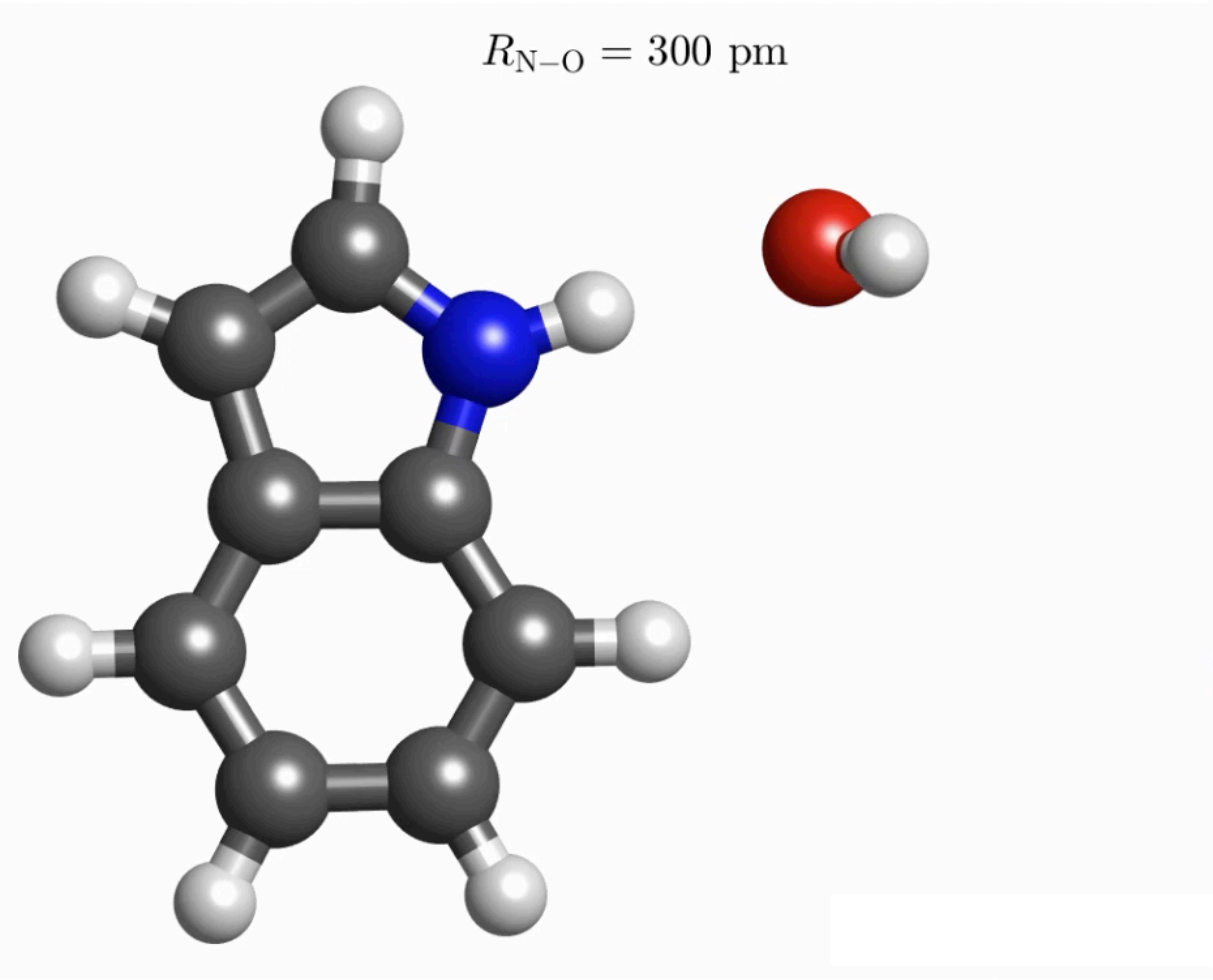
Controlled Molecule Imaging
Toward a microscopic understanding of molecules at work

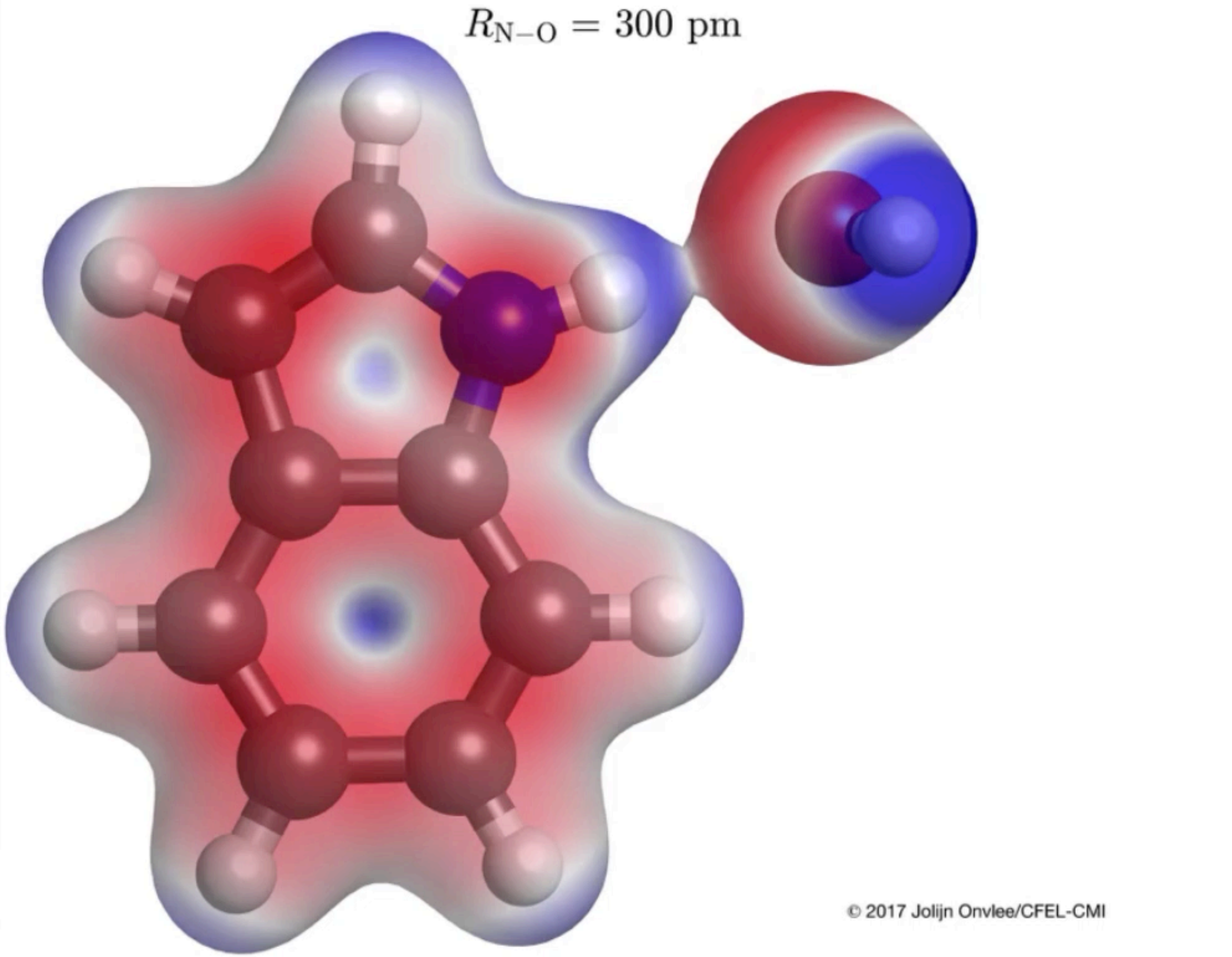


Atomic-resolution imaging of ultrafast chemical dynamics

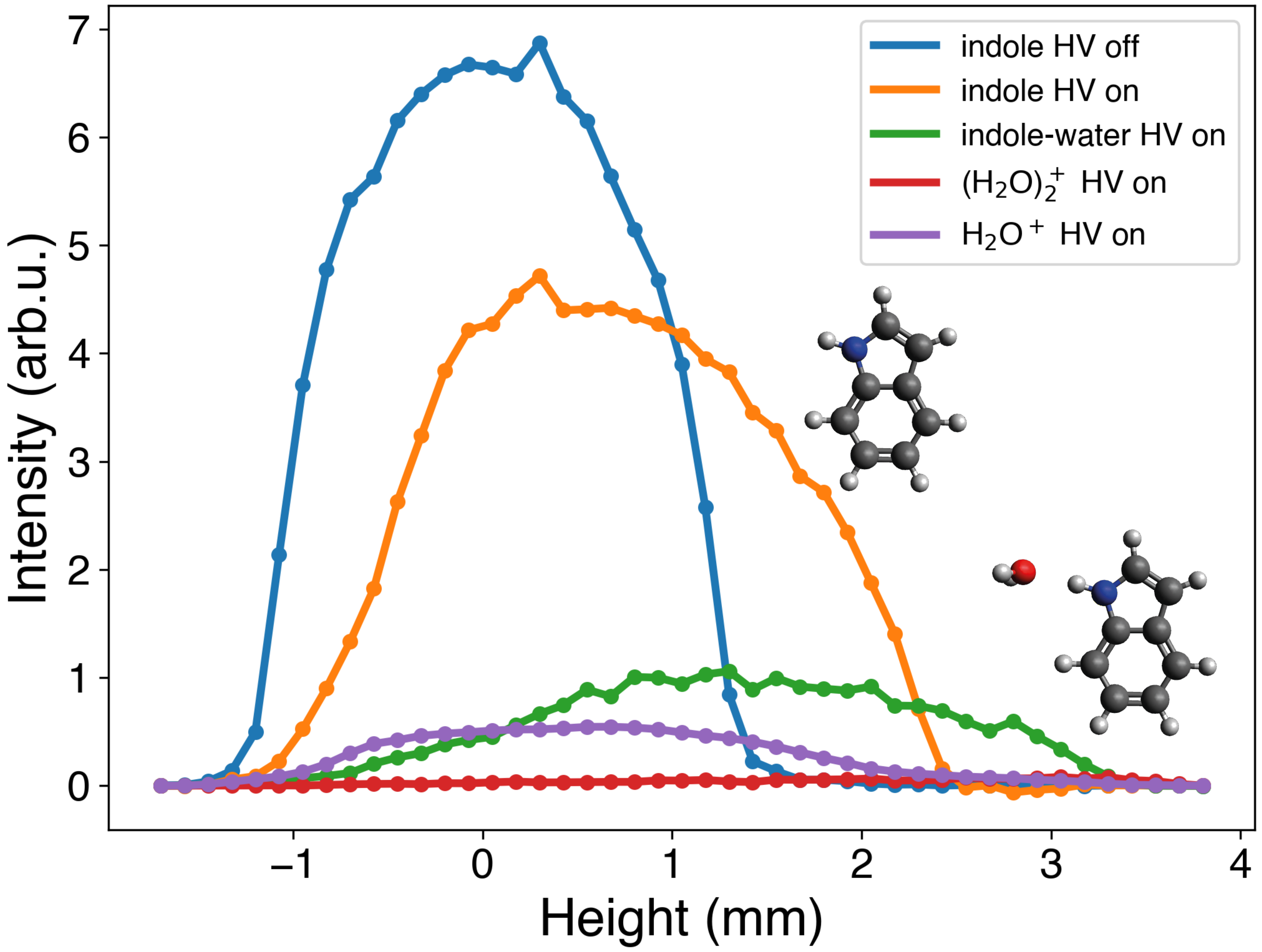


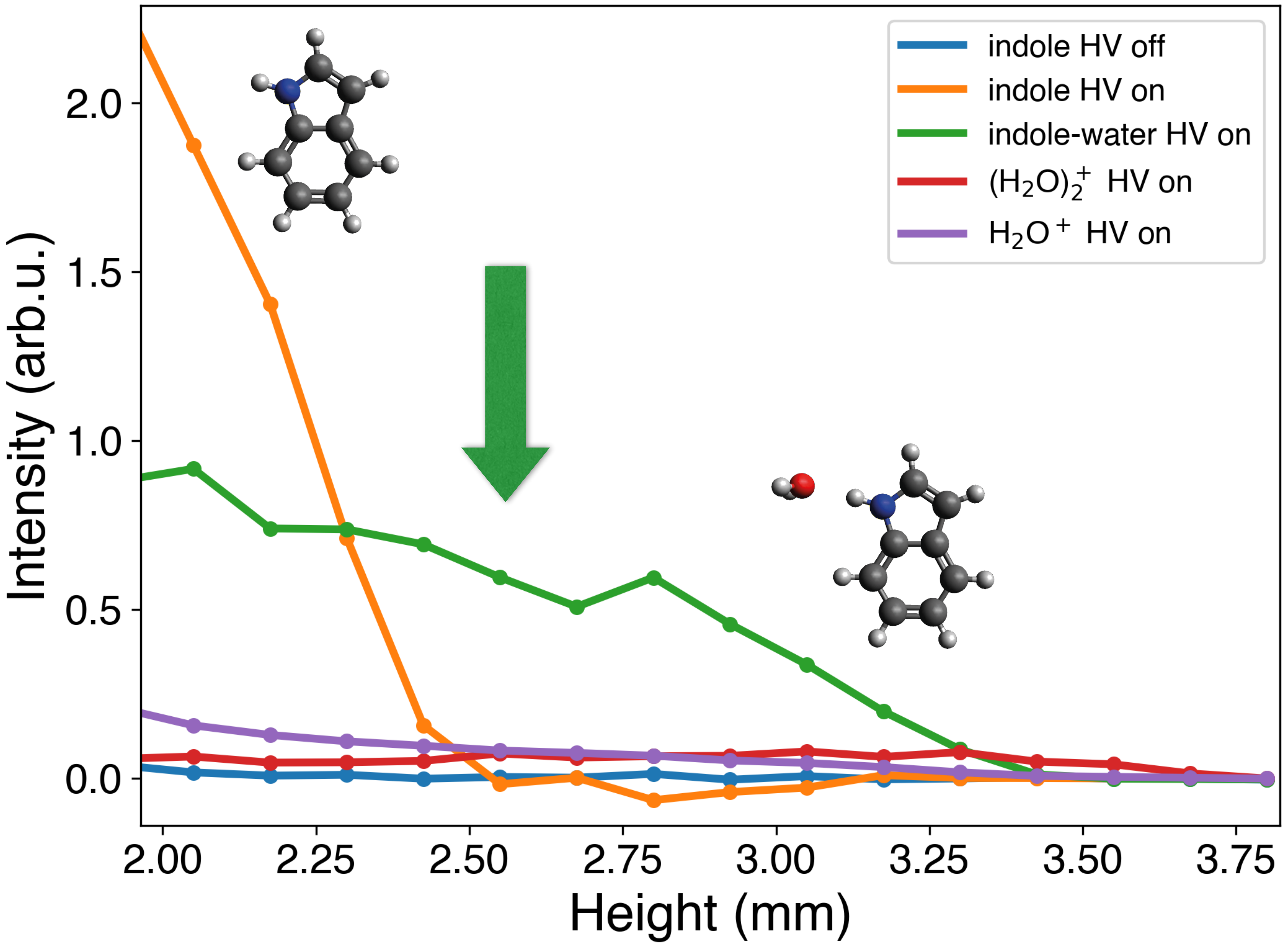
The ‘Quantum Molecular Movie’





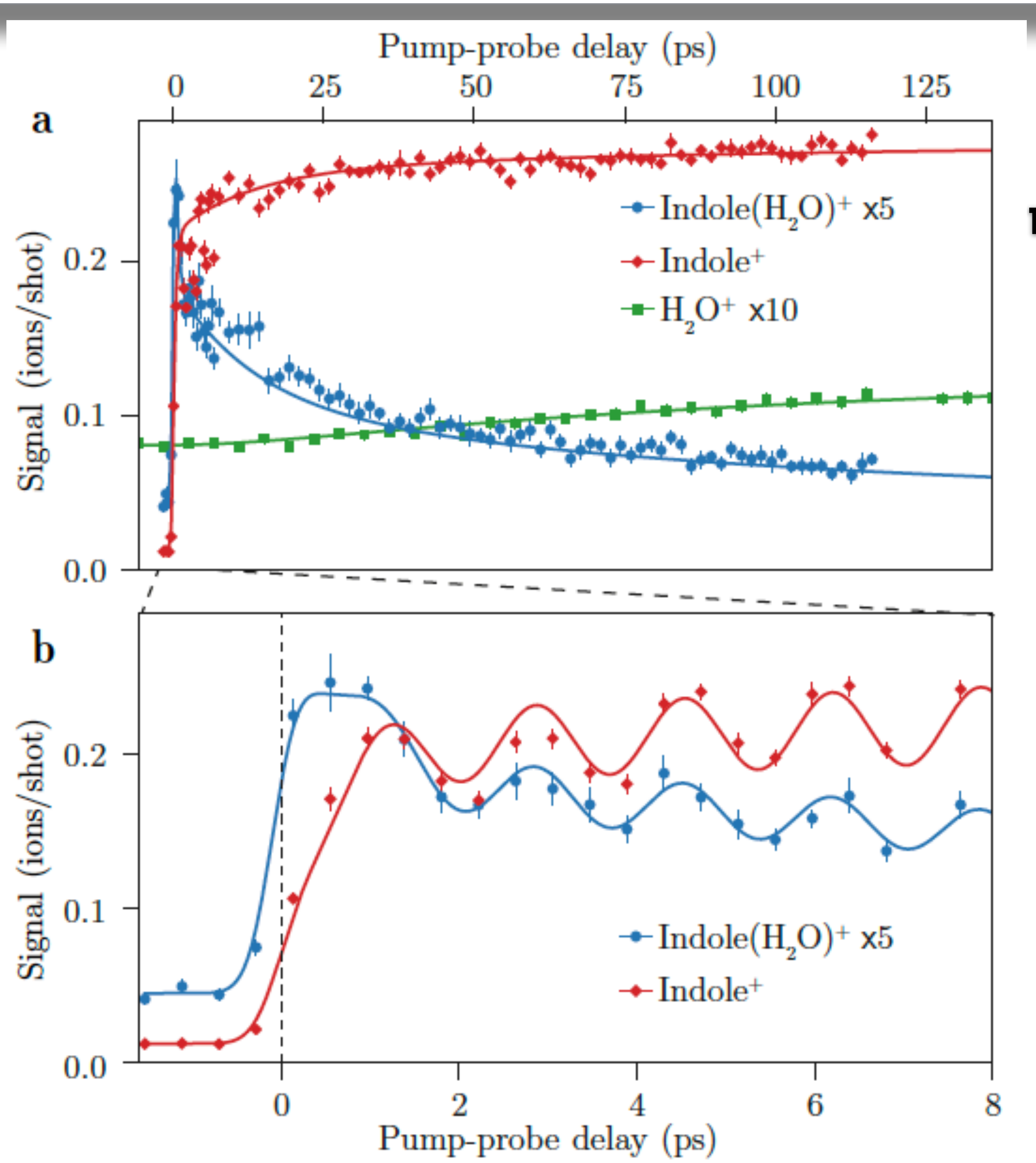
Spatially separated indole-water dimer

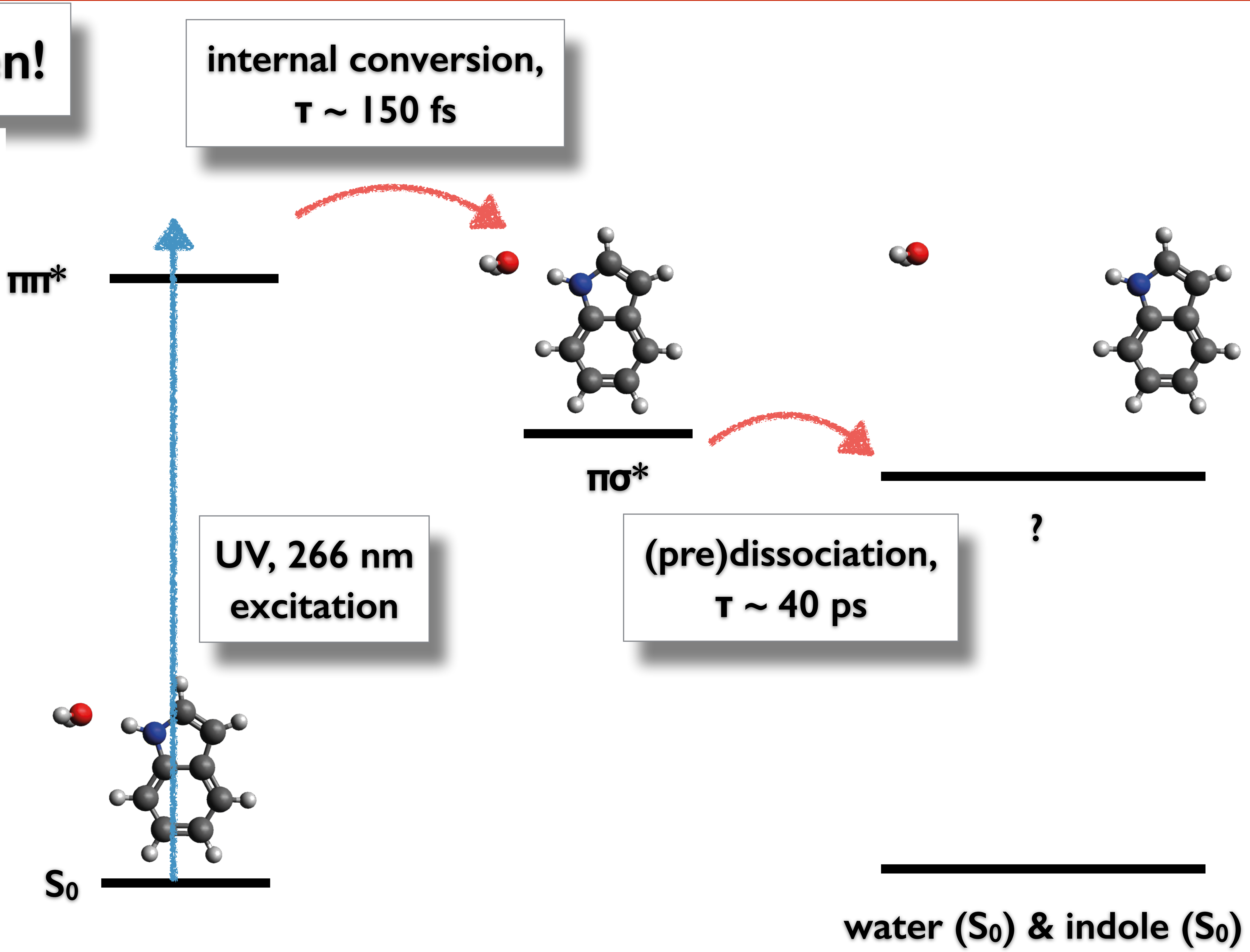




What could be happening?
Ion-imaging of ultrafast dynamics in pure indole-water-dimer sample

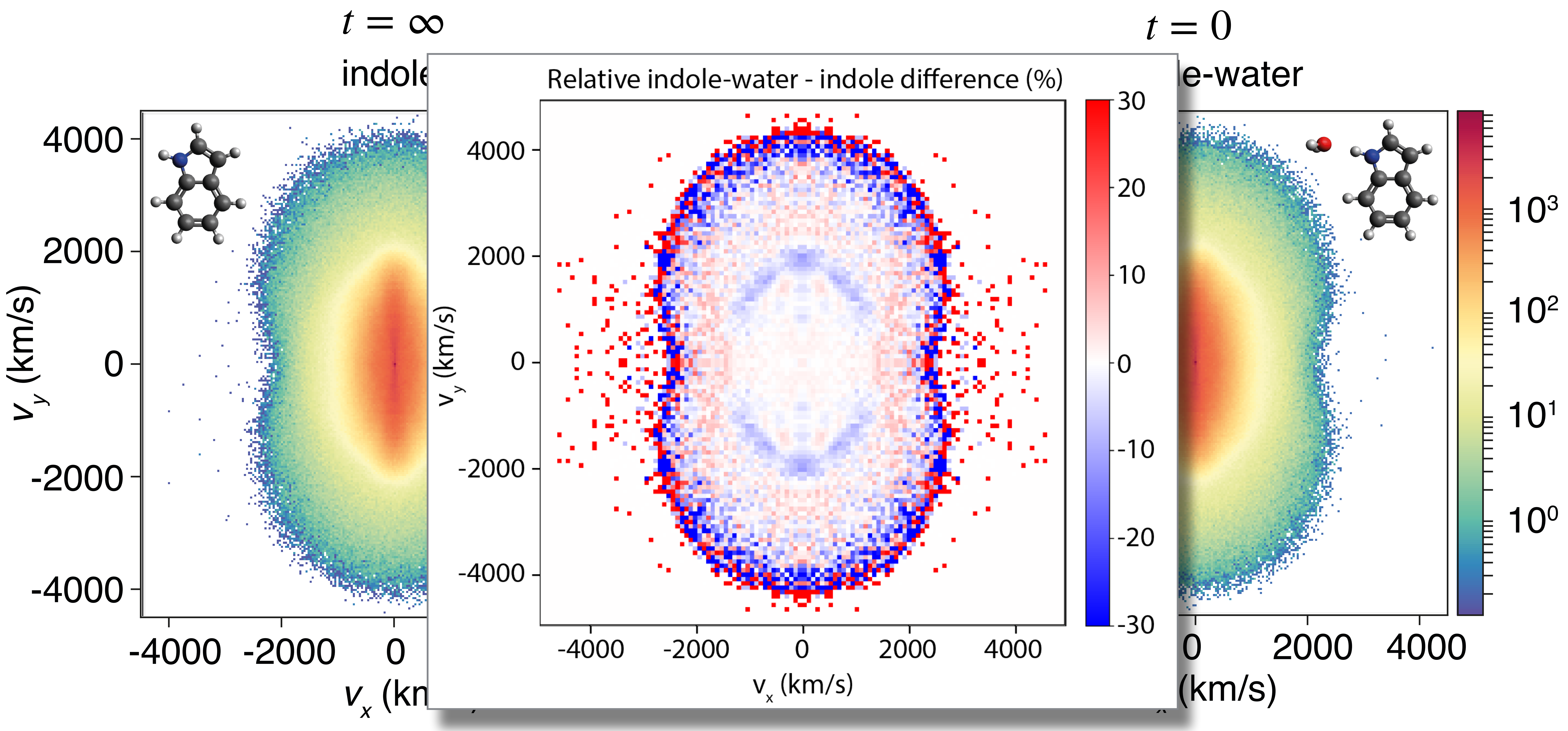
Water acts as molecular sunscreen!





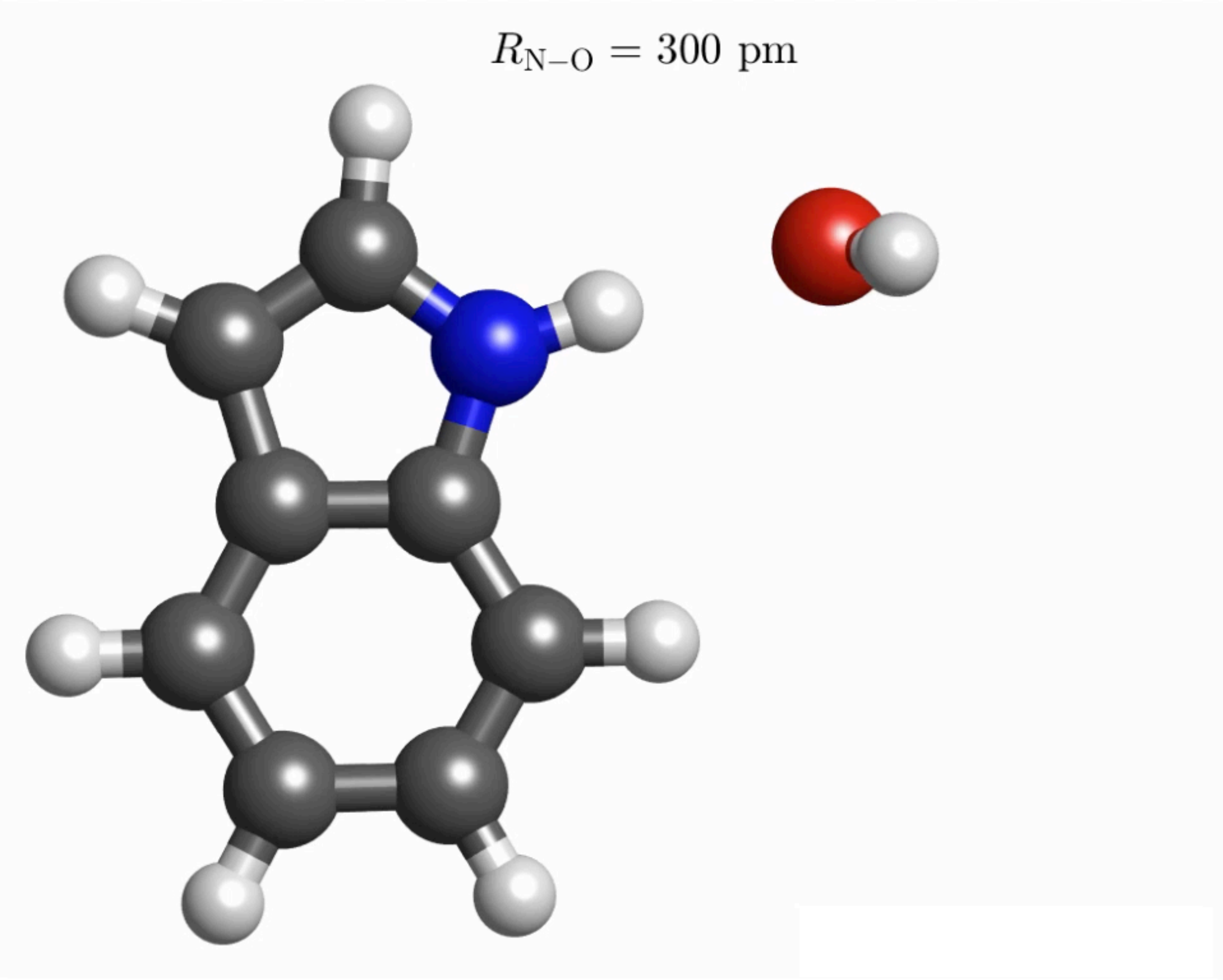
cf. Sobolewski, Domcke, Computational studies of the photophysics of hydrogen-bonded molecular systems, *J. Phys. Chem*. A **111**, 11725 (2007)

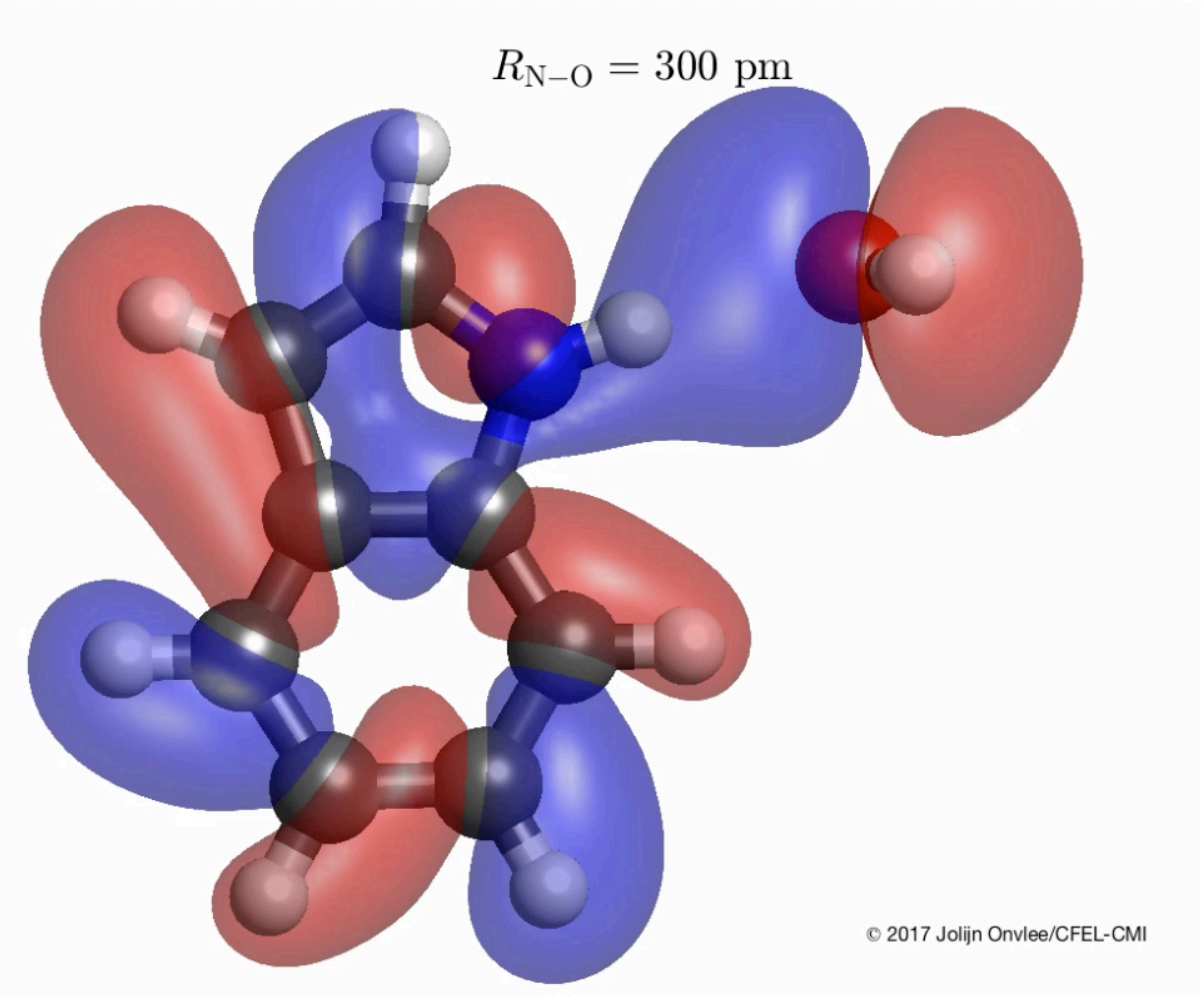
Laser-induced electron diffraction of indole & indole-water



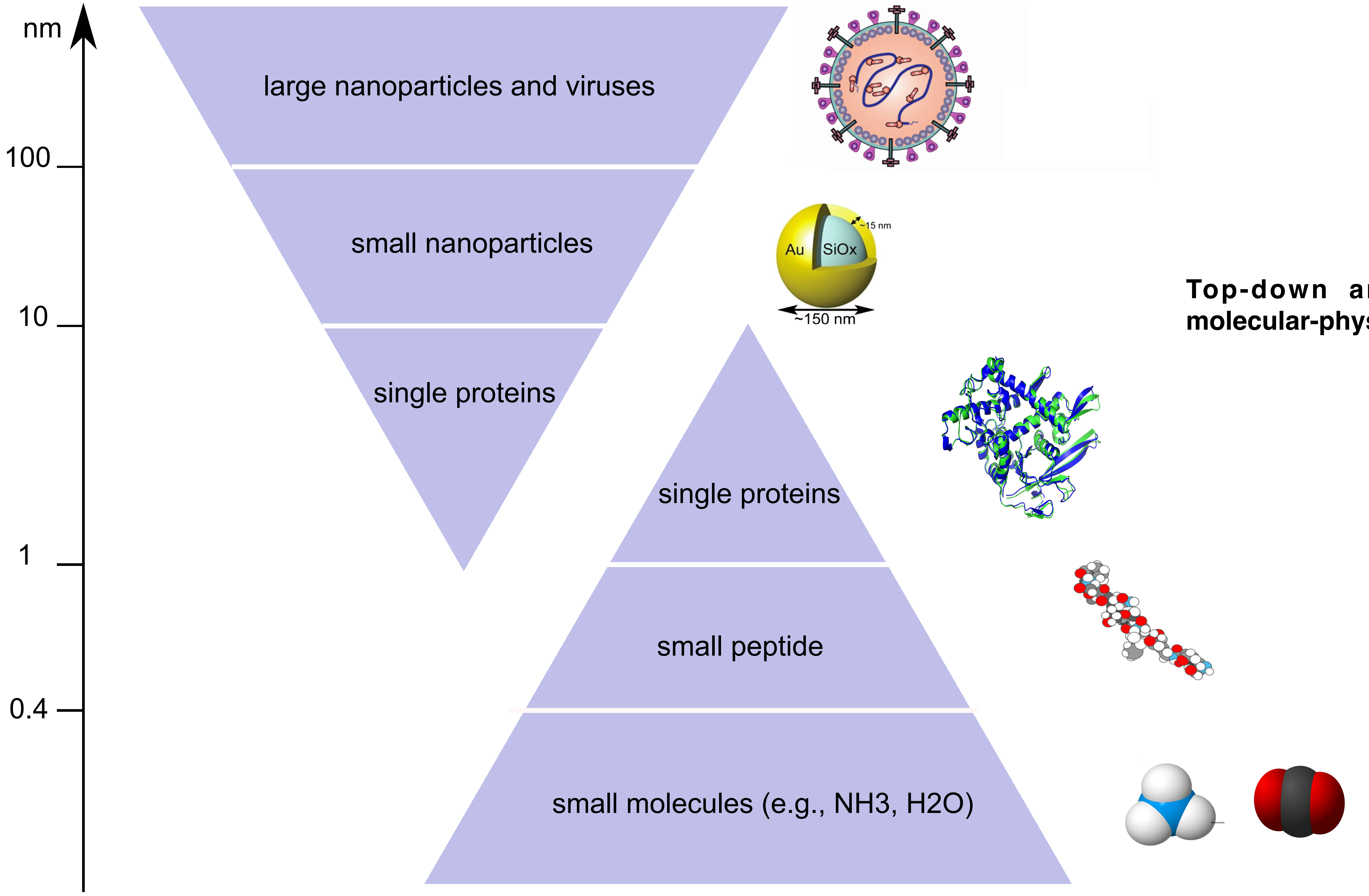
λ = 1300 nm, $I \approx 8\times10^{13}$ W/cm, $U_p \approx 13$ eV

The ‘Quantum Molecular Movie’





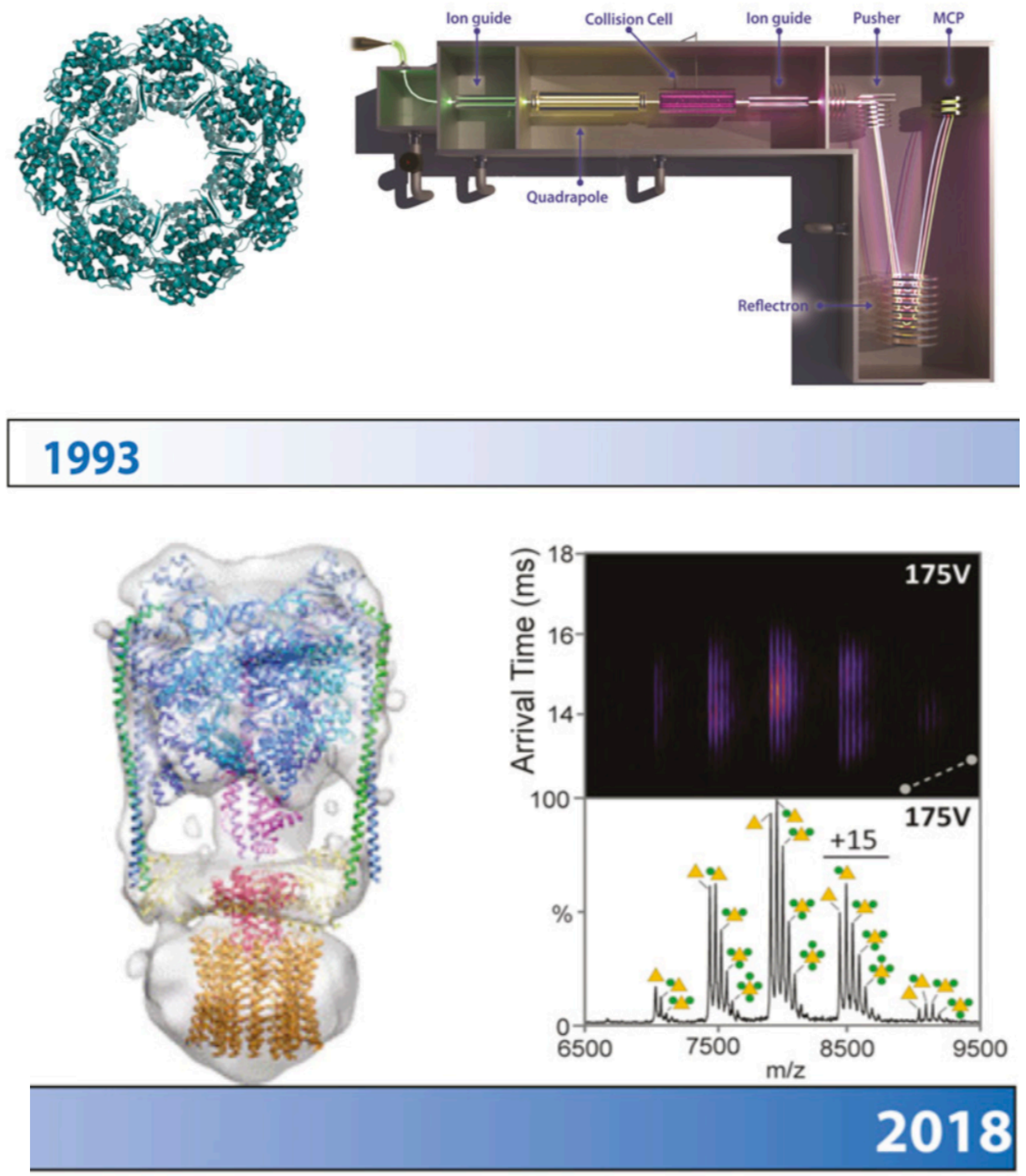
Structure and dynamics of large biomolecules and nanoparticles



Top-down and bottom-up approaches toward molecular-physics studies of proteins.

Tools

Mass



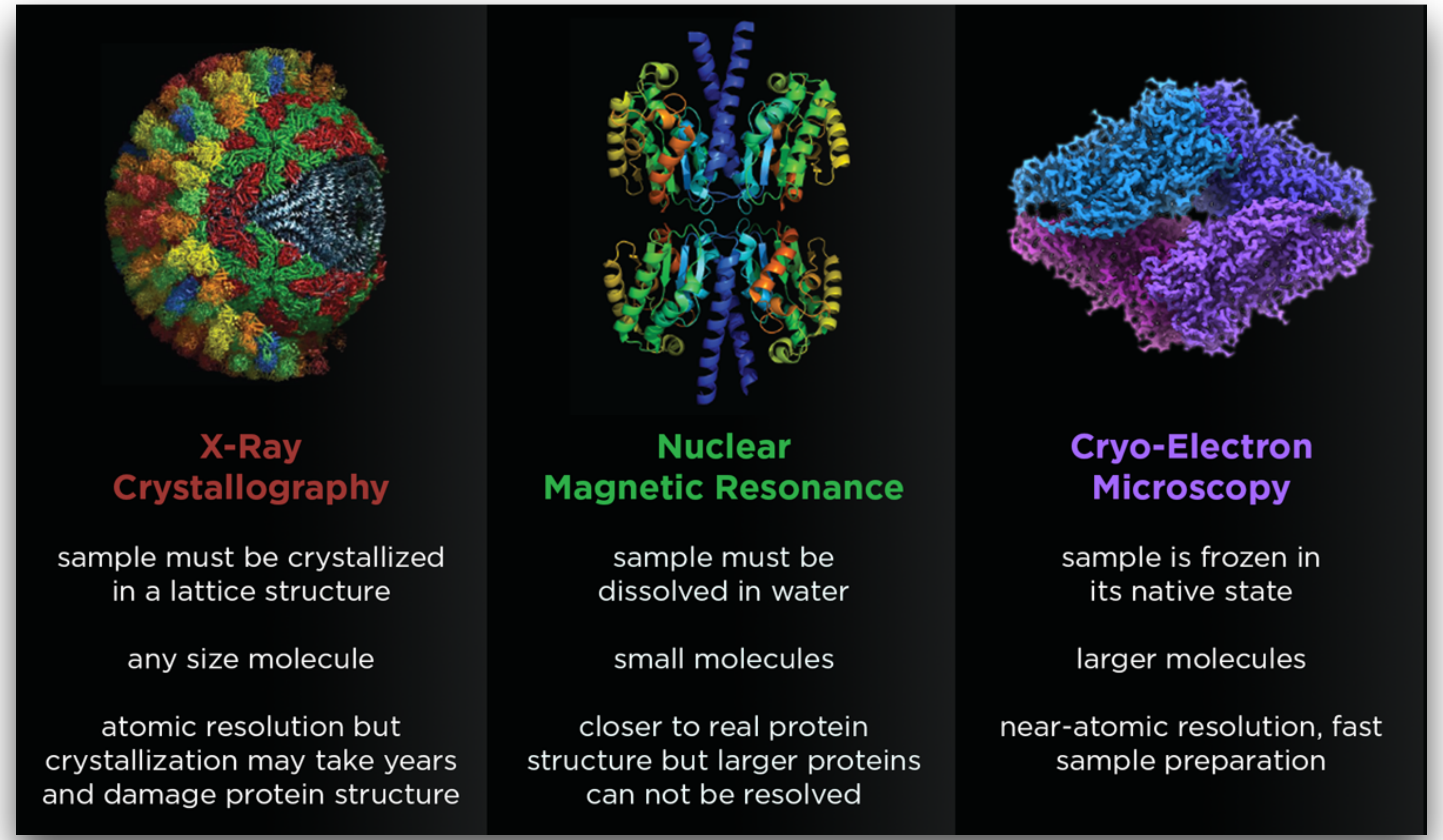
Robinson *PNAS* **2019**

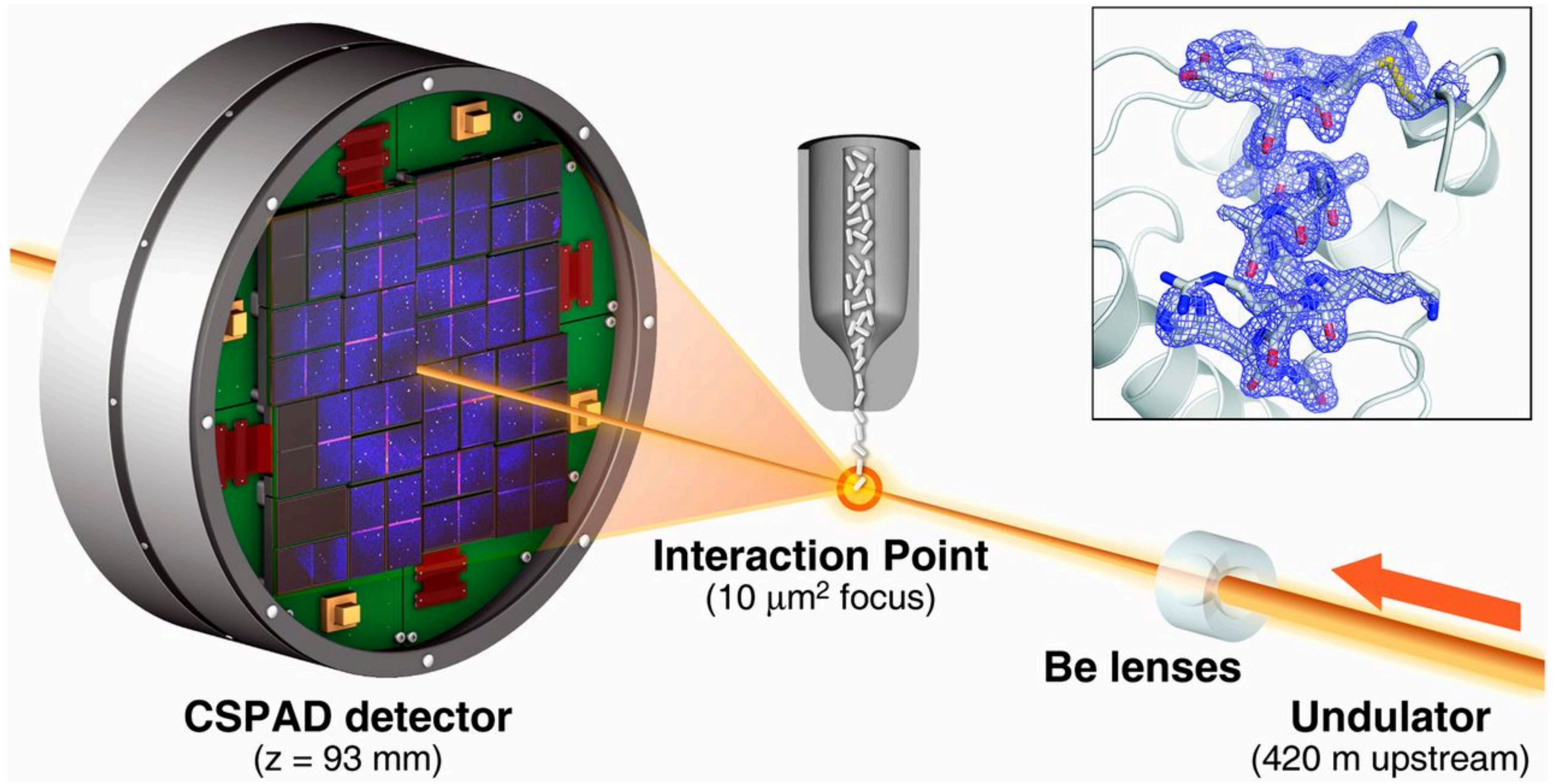
Size

- Single particle microscopy techniques.
- Scanning mobility particle sizer.

https://www.mi.fu-berlin.de/biocomputing/index.html

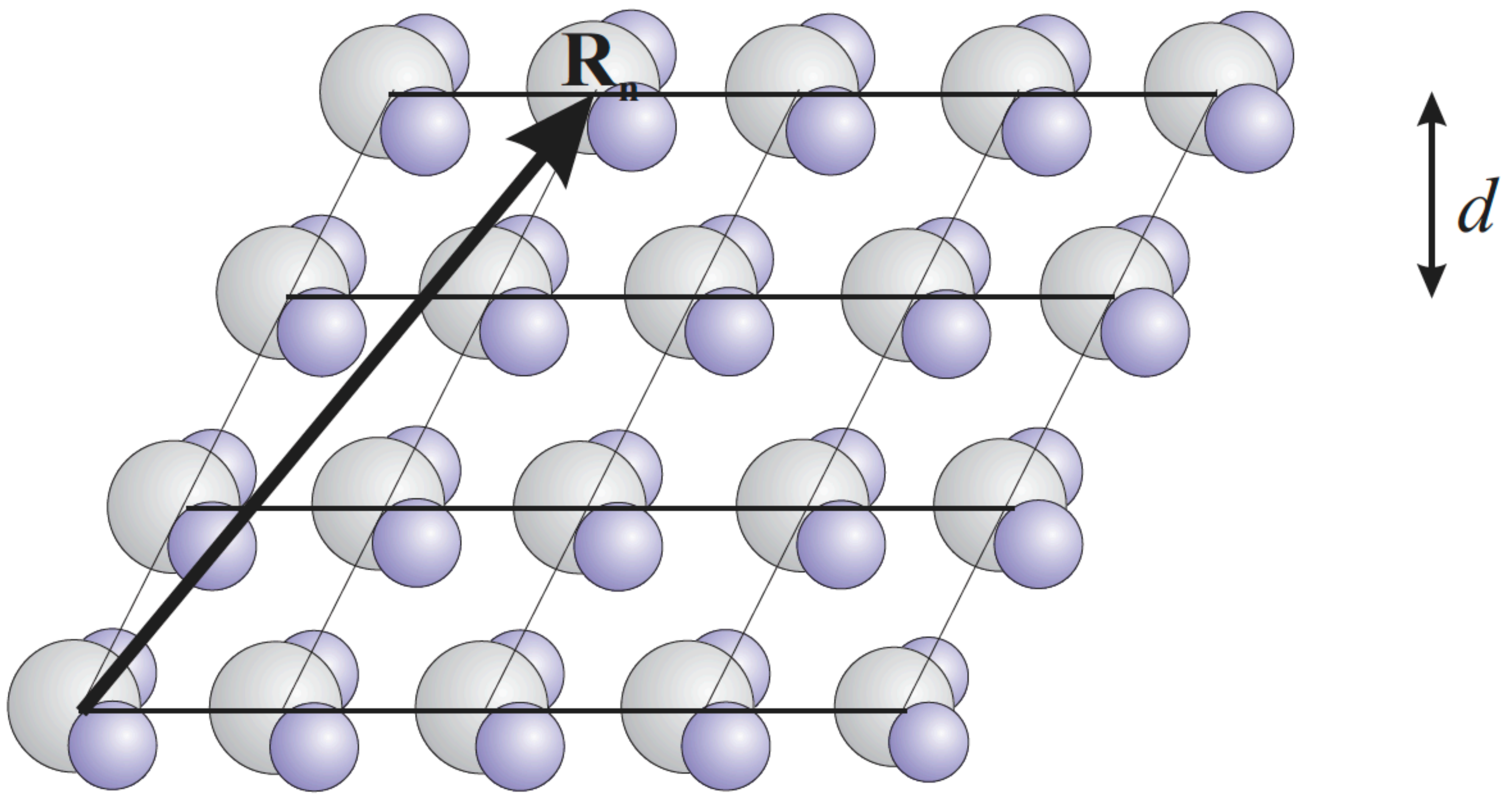
Structure





Gruner *PNAS* **2014**

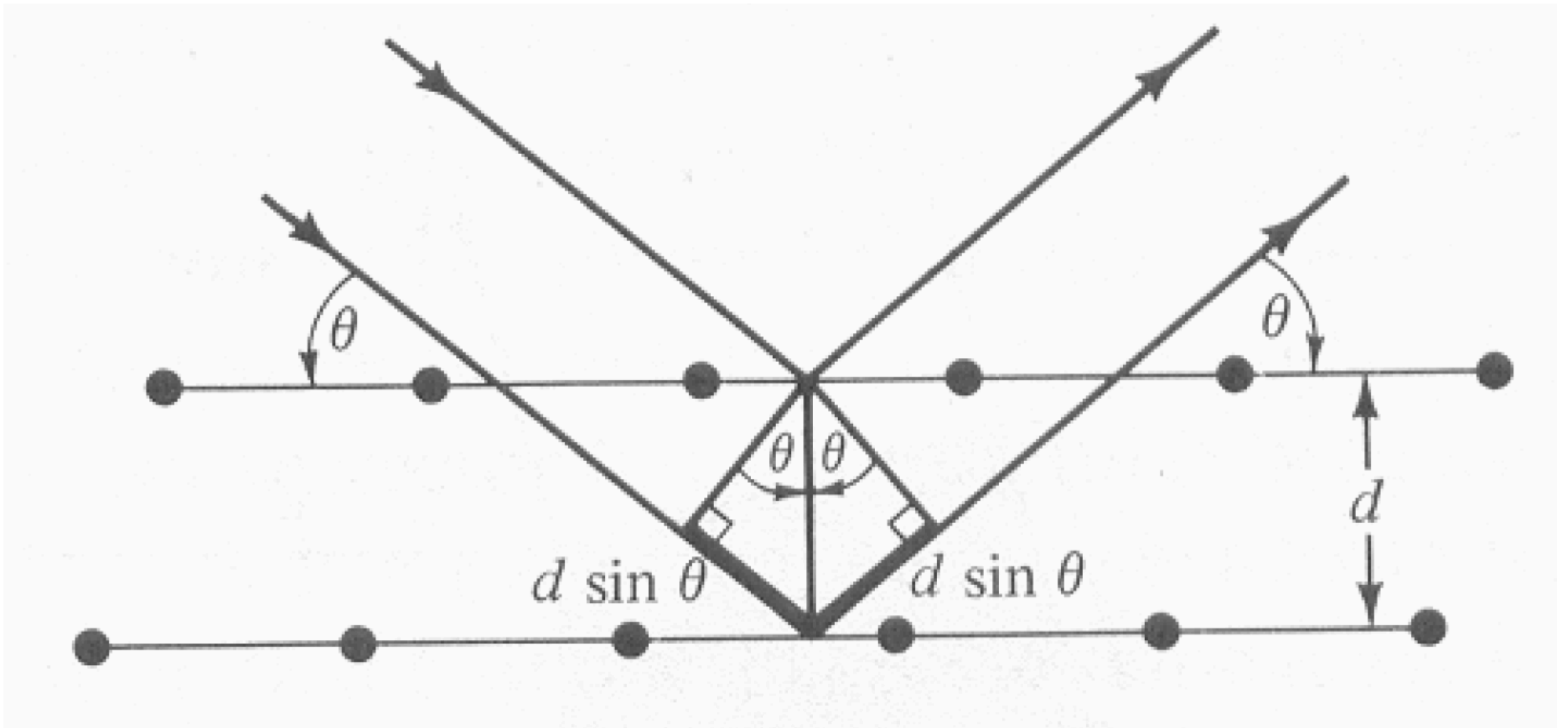
X-ray crystallography



Defining property of a crystalline material is that it is periodic in space
scattering of X-rays from a crystal lattice,
Bragg's law

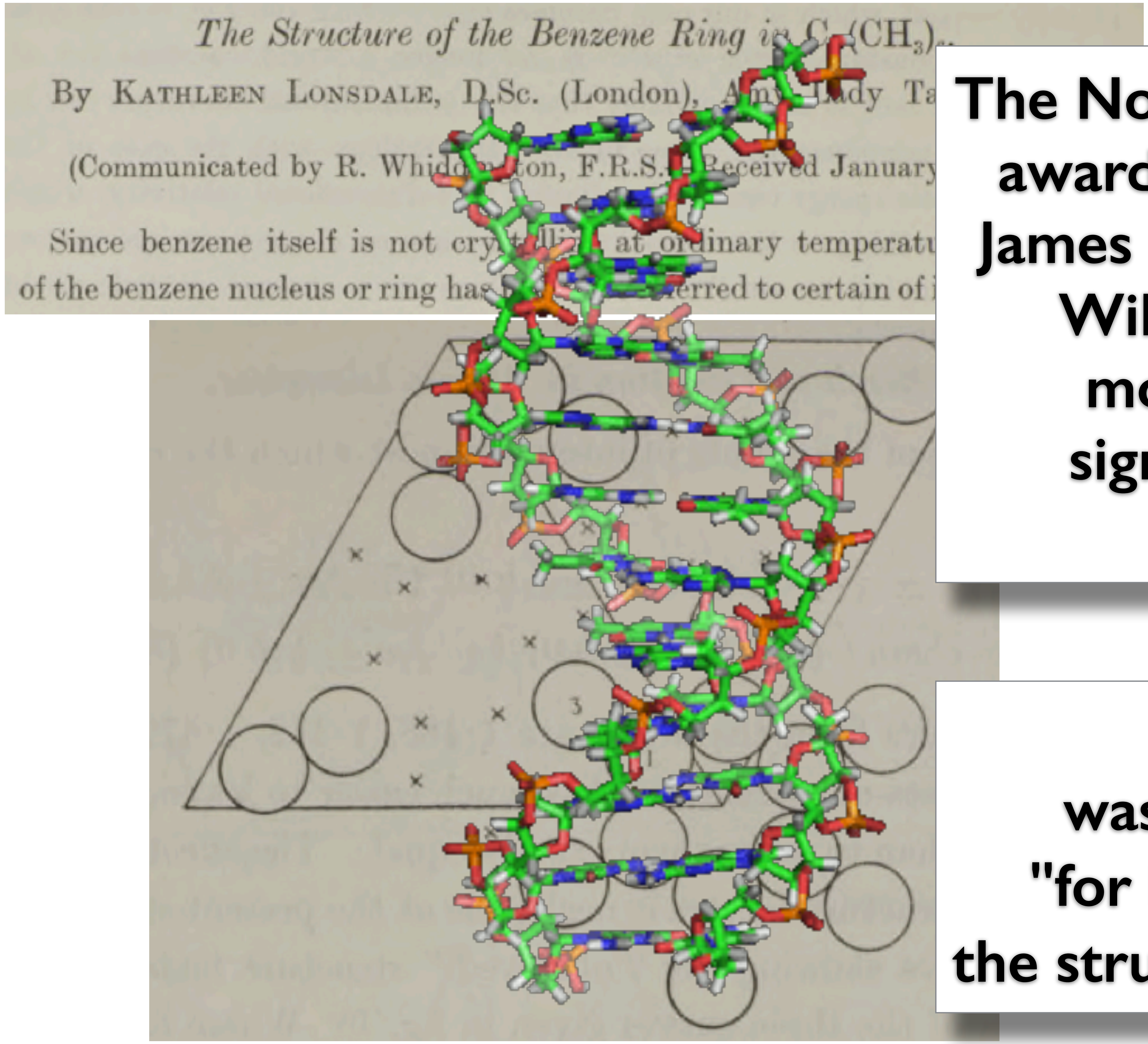
$$m\lambda = 2d\sin\theta$$

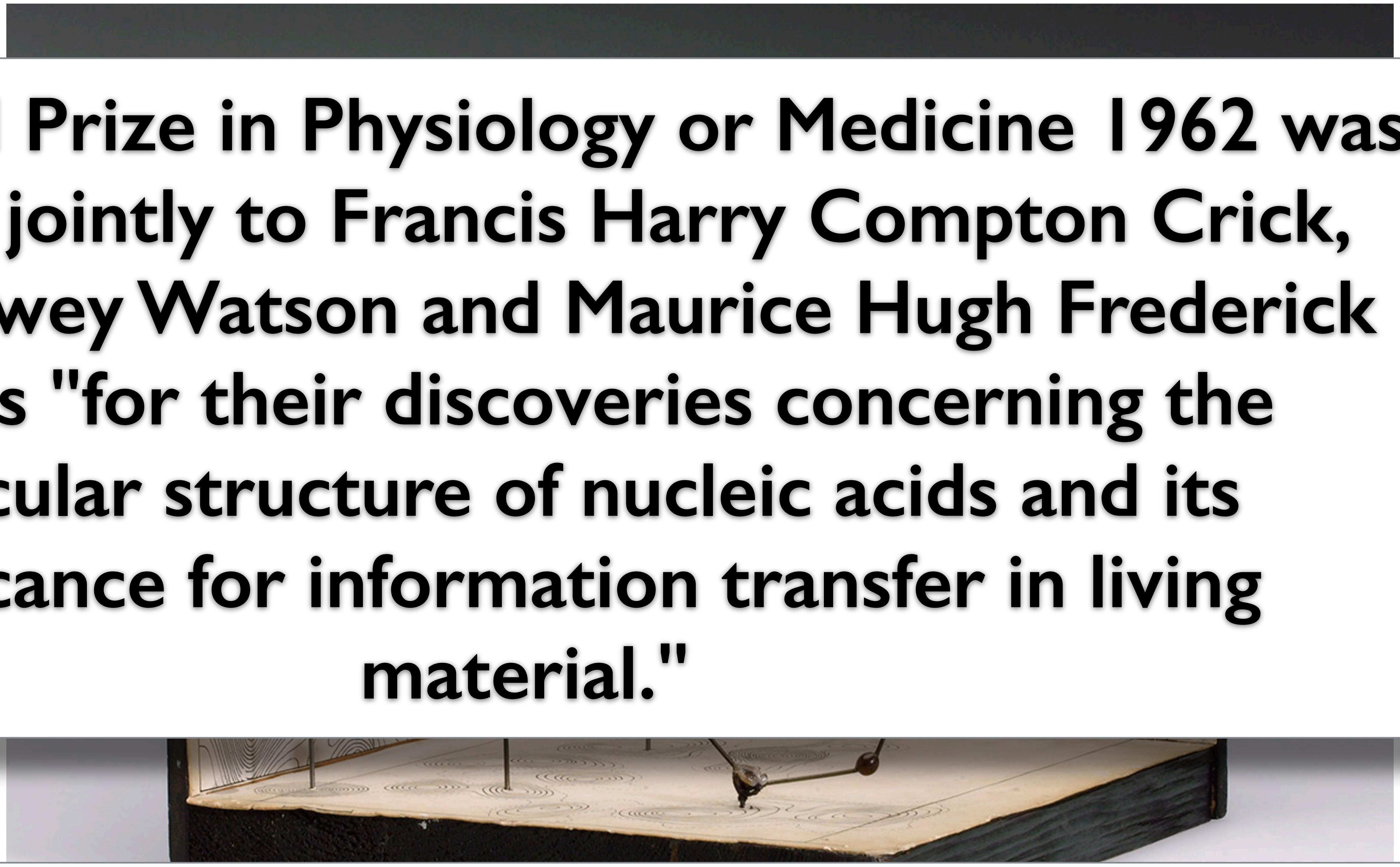
Path difference between two rays reflected from adjoining planes



This is the condition for the constructive interference of waves which have an angle of incidence θ to a set of lattice planes a distance d apart.

Crystallography: structures of molecules





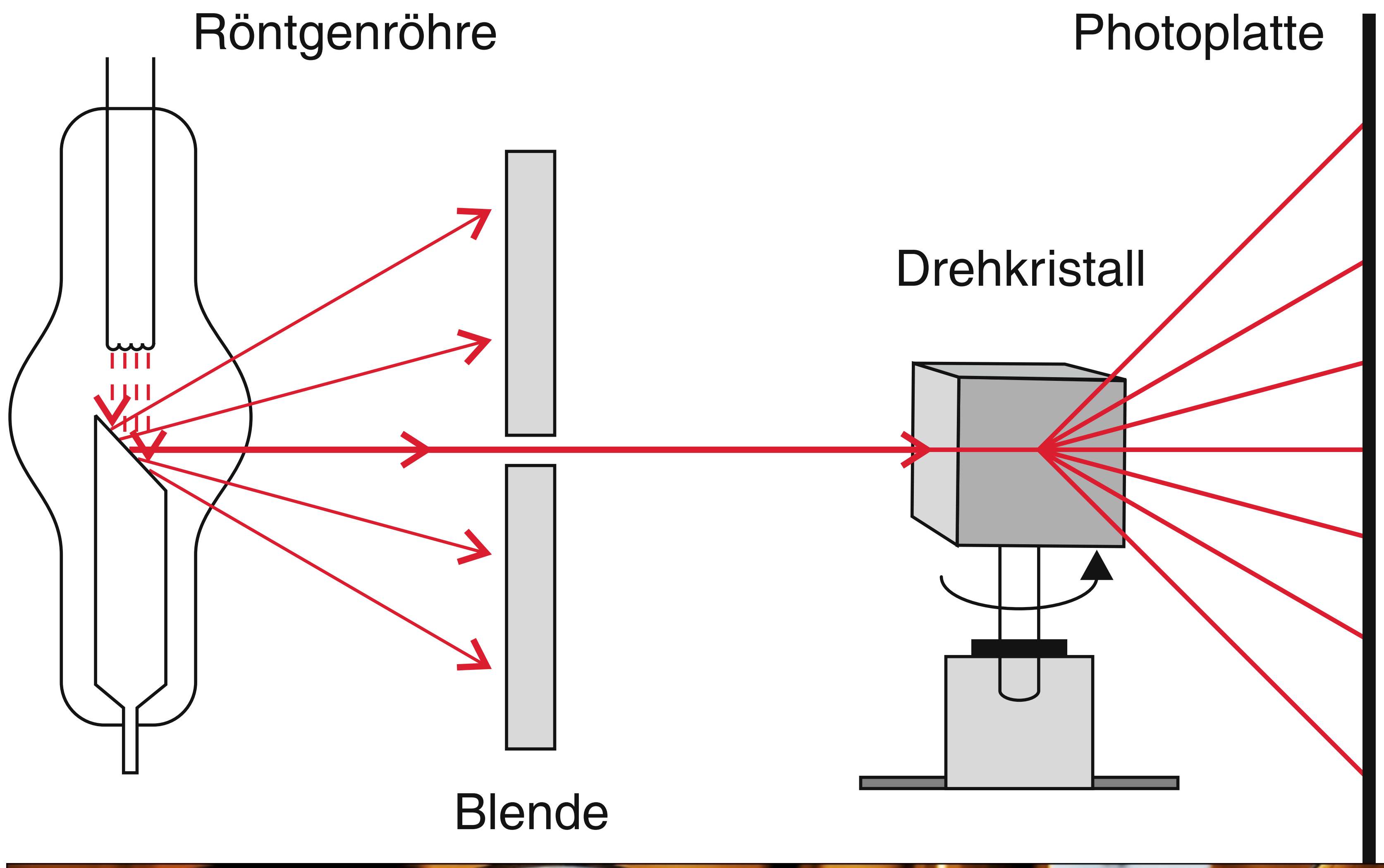
The Nobel Prize in Physiology or Medicine 1962 was awarded jointly to Francis Harry Compton Crick, James Dewey Watson and Maurice Hugh Frederick Wilkins "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material."

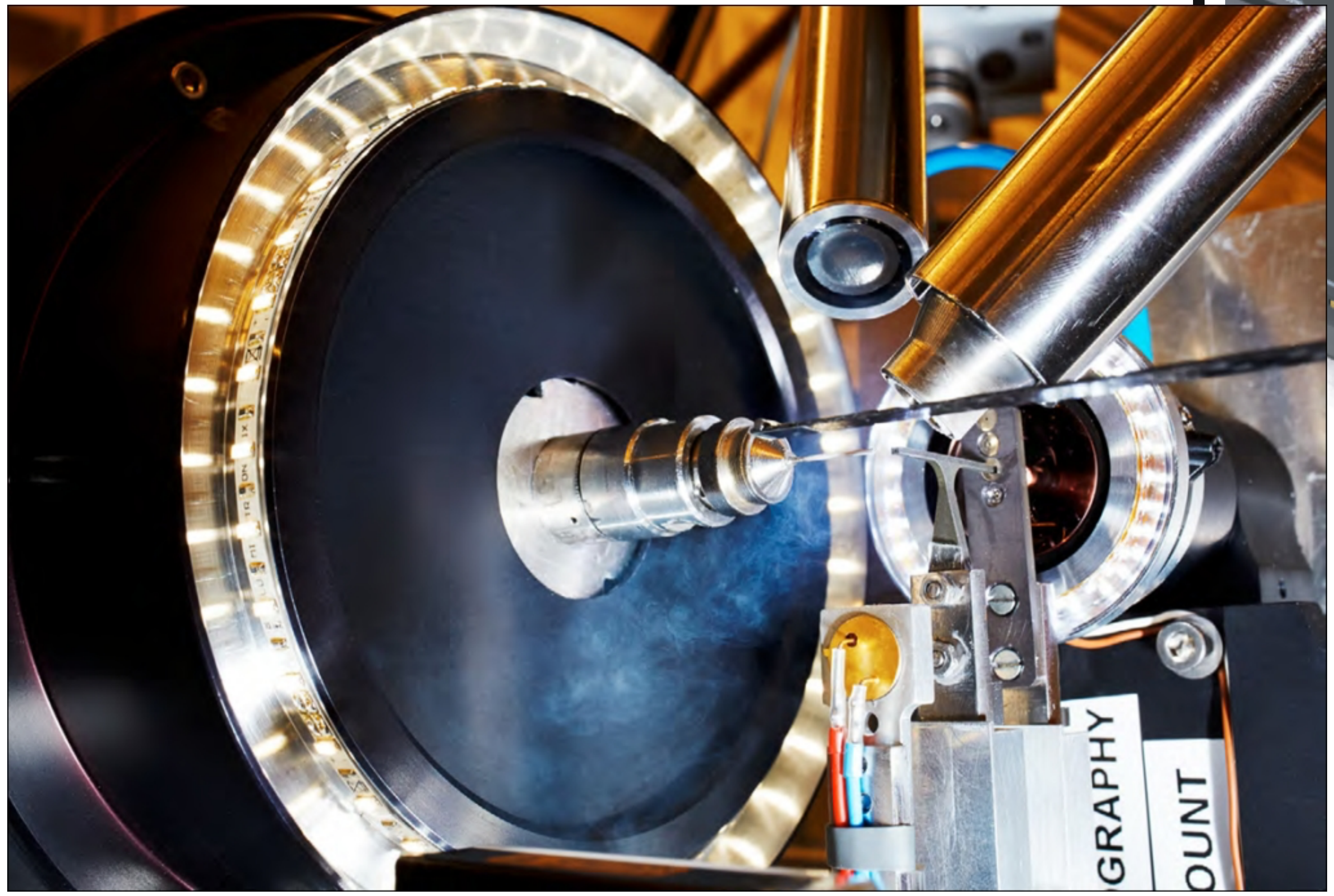
The Nobel Prize in Chemistry 1964 was awarded to Dorothy Crowfoot Hodgkin "for her determinations by X-ray techniques of the structures of important biochemical substances."

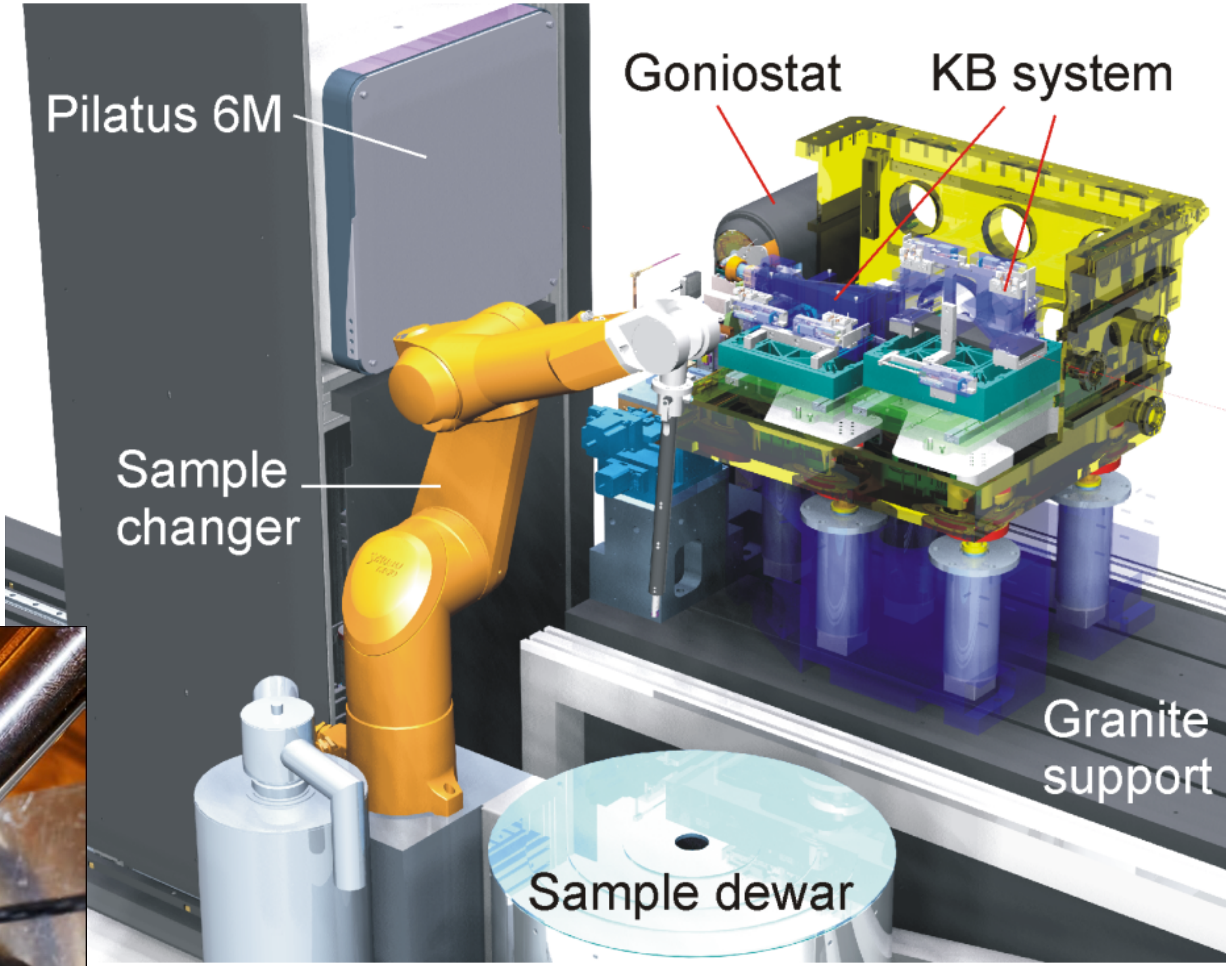
Lonsdale, Proceedings of the Royal Society of London A **123**, 494 (1929)

Crowfoot, Bunn, Rogers-Low, Turner-Jones in Clarke, Johnson, Robinson, (eds.) “Chemistry of Penicillin” Princeton University Press, pp. 310–67 (1949)

Todays X-ray crystallography

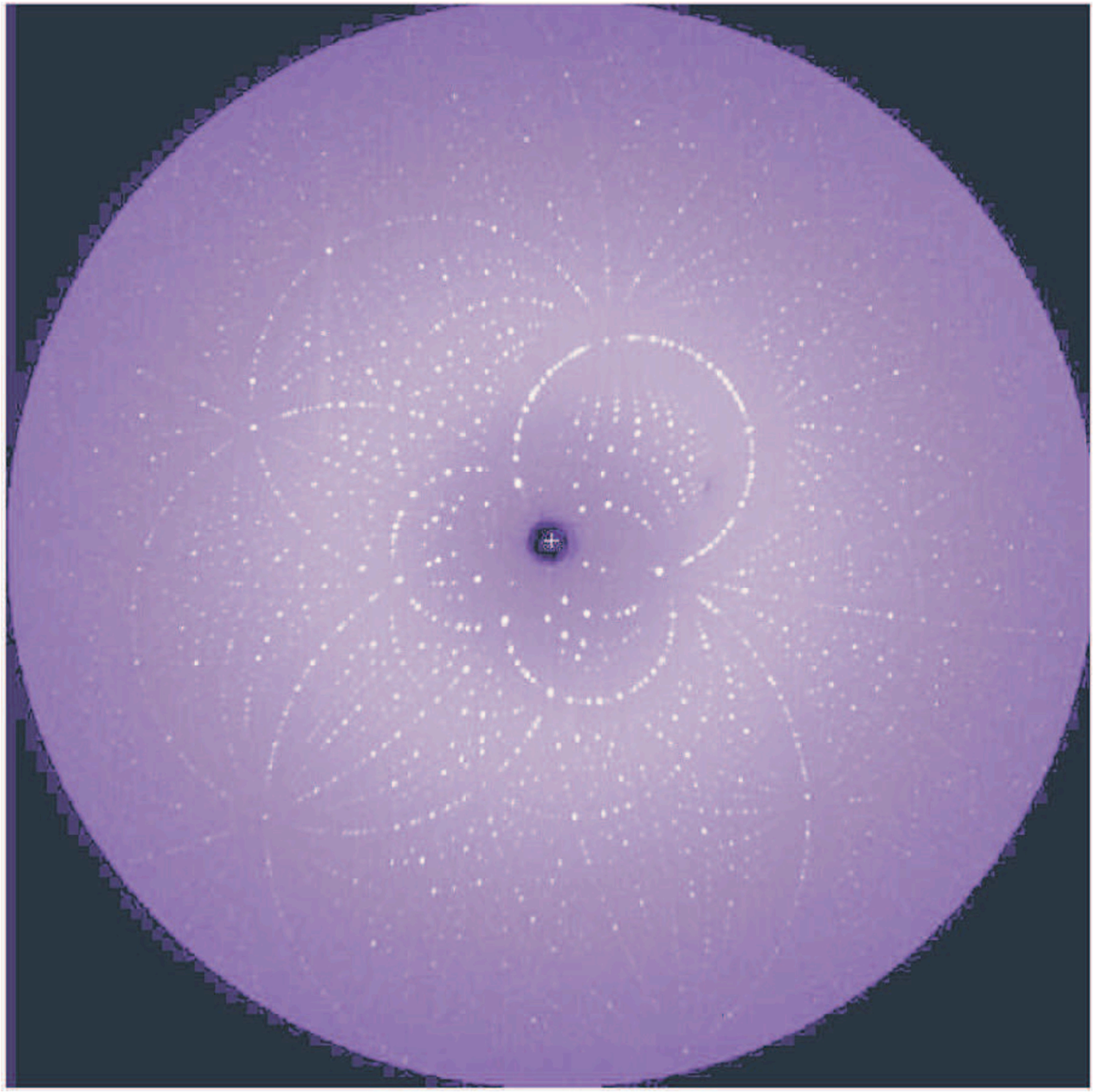






Beamline P11 @ Petra3, DESY

Todays X-ray crystallography



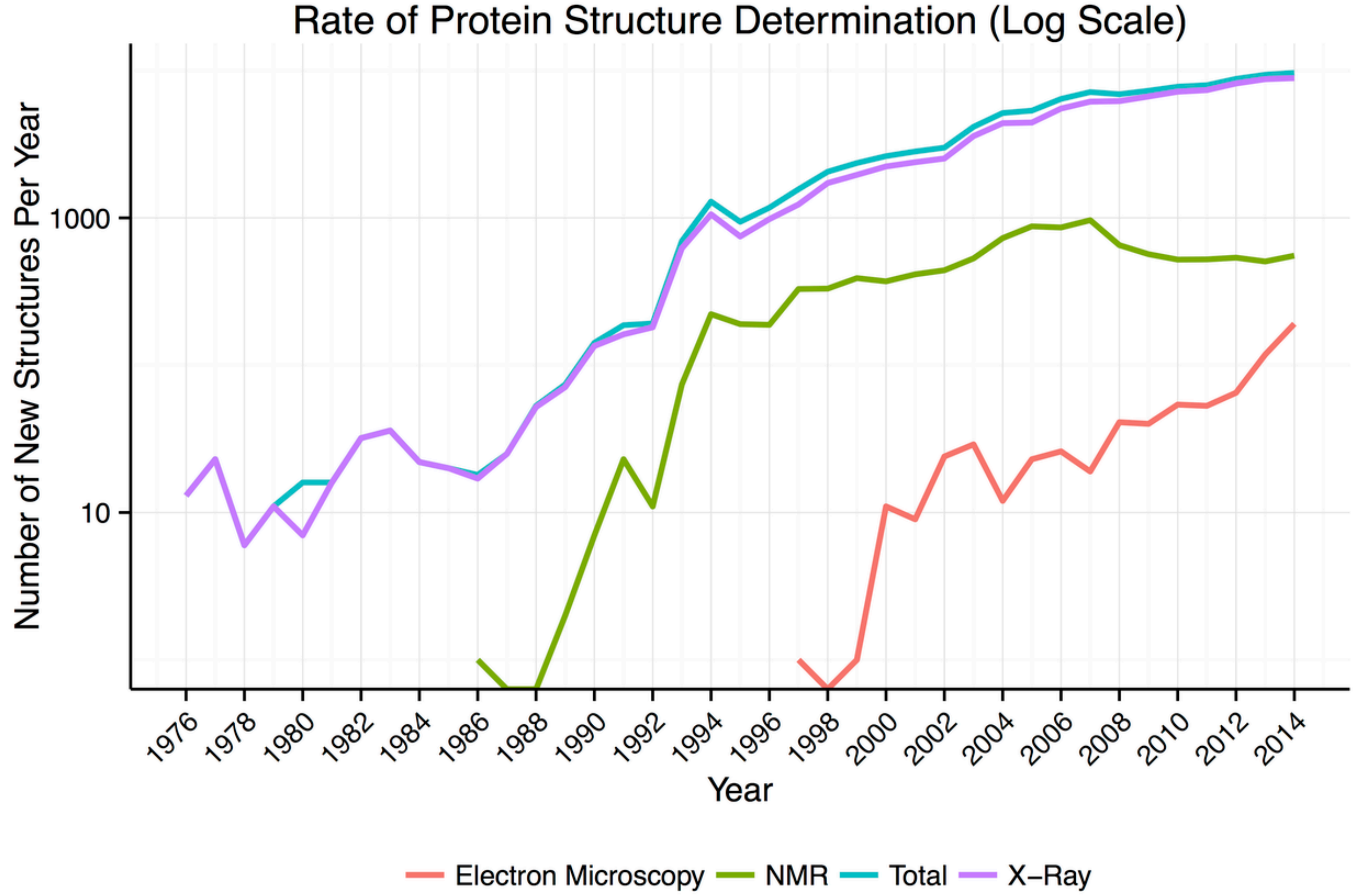
Laue diffraction from photoactive yellow protein, 10 exposures, ~3700 reflections

One can solve protein structures to <2Å resolution from crystals <1 mm in size.

Todays X-ray crystallography

Protein Data Bank statistics (as of March 2017)

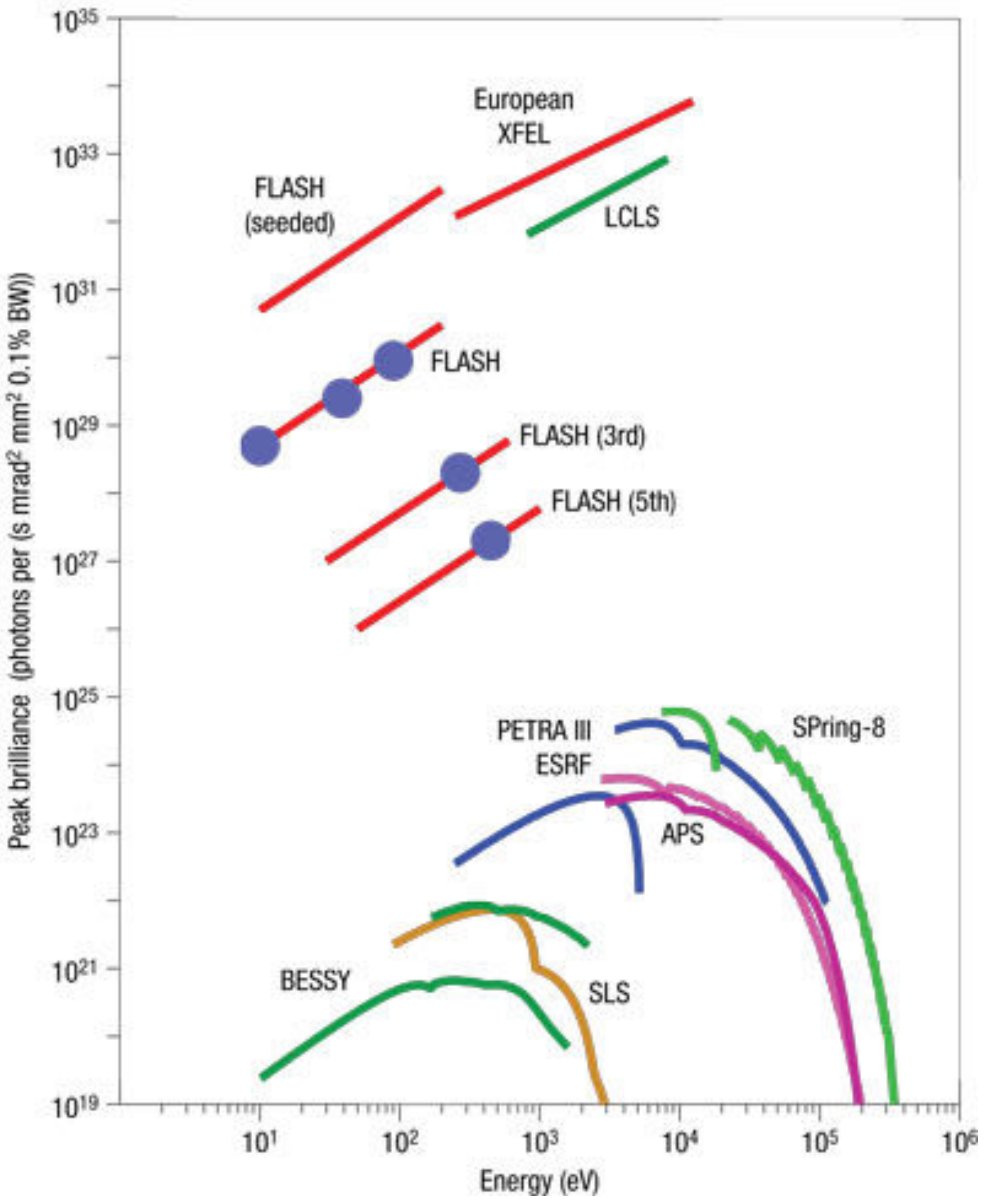
Experimental Method	Proteins	Nucleic Acids	Protein/Nucleic Acid complexes	Other	Total
X-ray diffraction	106595	1820	5471	4	113890
NMR	10296	1190	241	8	11735
Electron microscopy	1021	30	367	0	1418
Hybrid	99	3	2	1	105
Other	181	4	6	13	204
Total:	118192	3047	6087	26	**127352**



<u>But there are a few things crystallography cannot do / struggles with:</u>

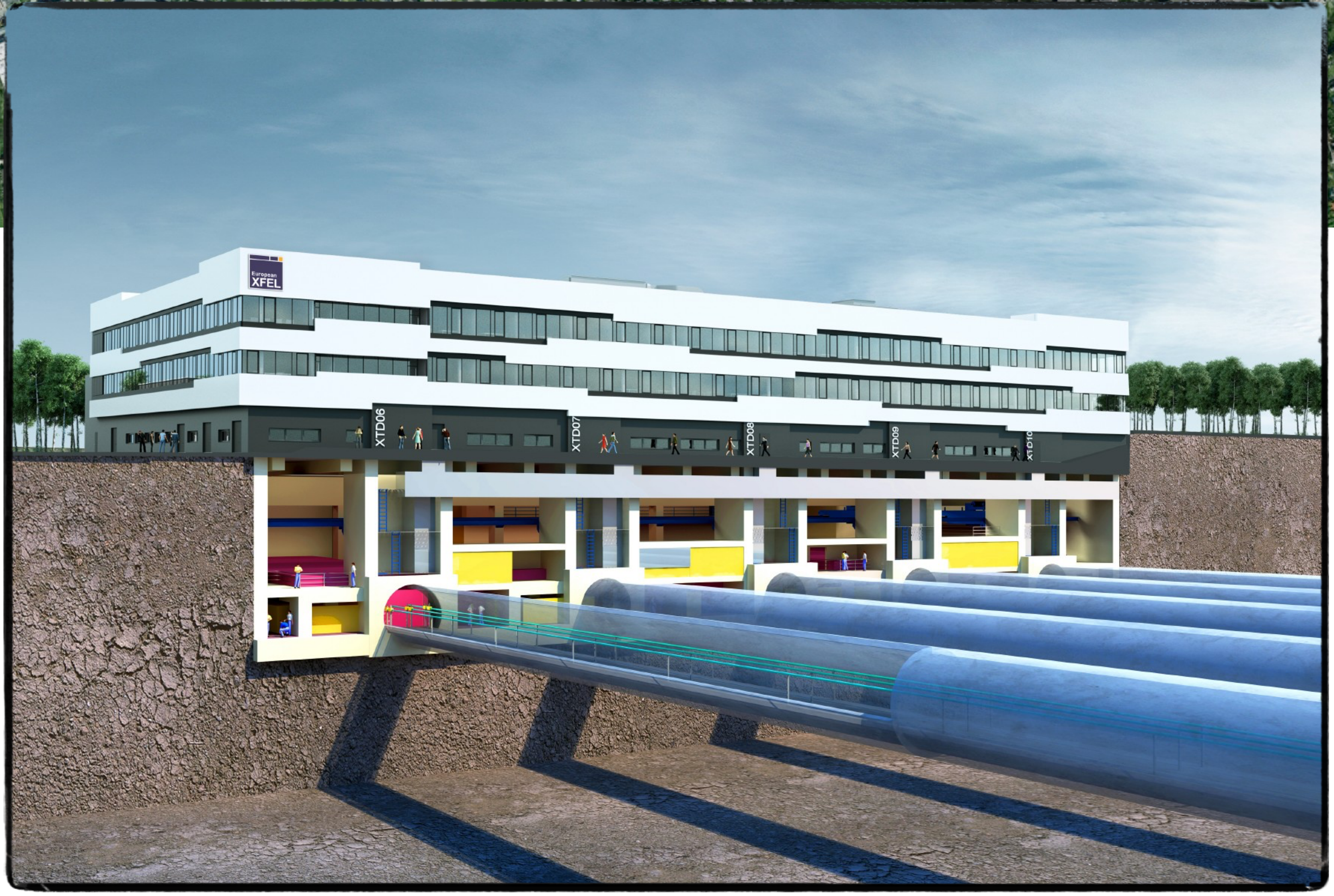
- Not every molecule (esp. proteins) crystallises, especially into such comparatively large crystals!
- Radiation damage - the same crystal is bombarded for many exposures with hard x-rays that can damage the structure
- ultrafast dynamics (except some special cases)

X-ray sources and the FEL revolution

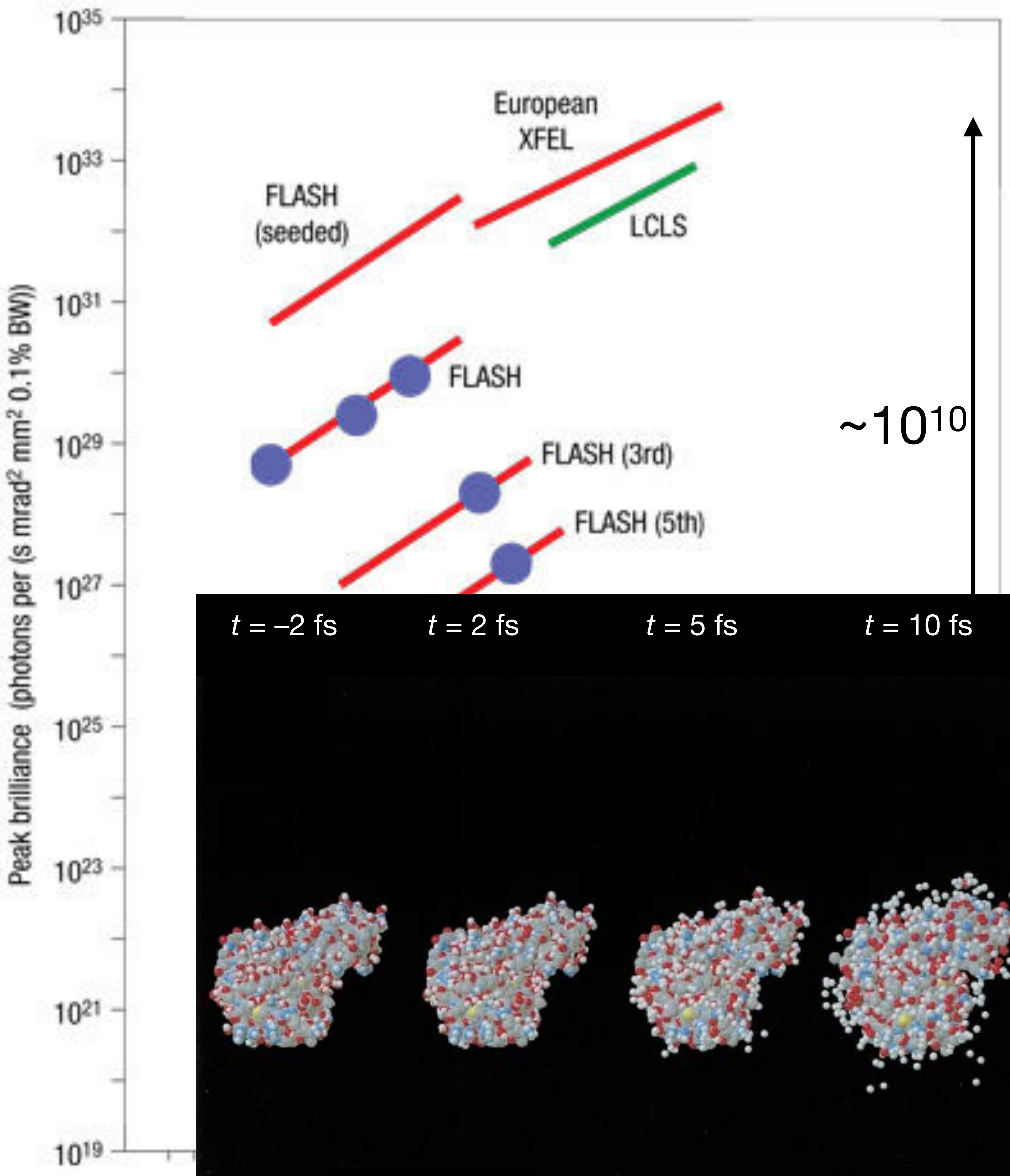


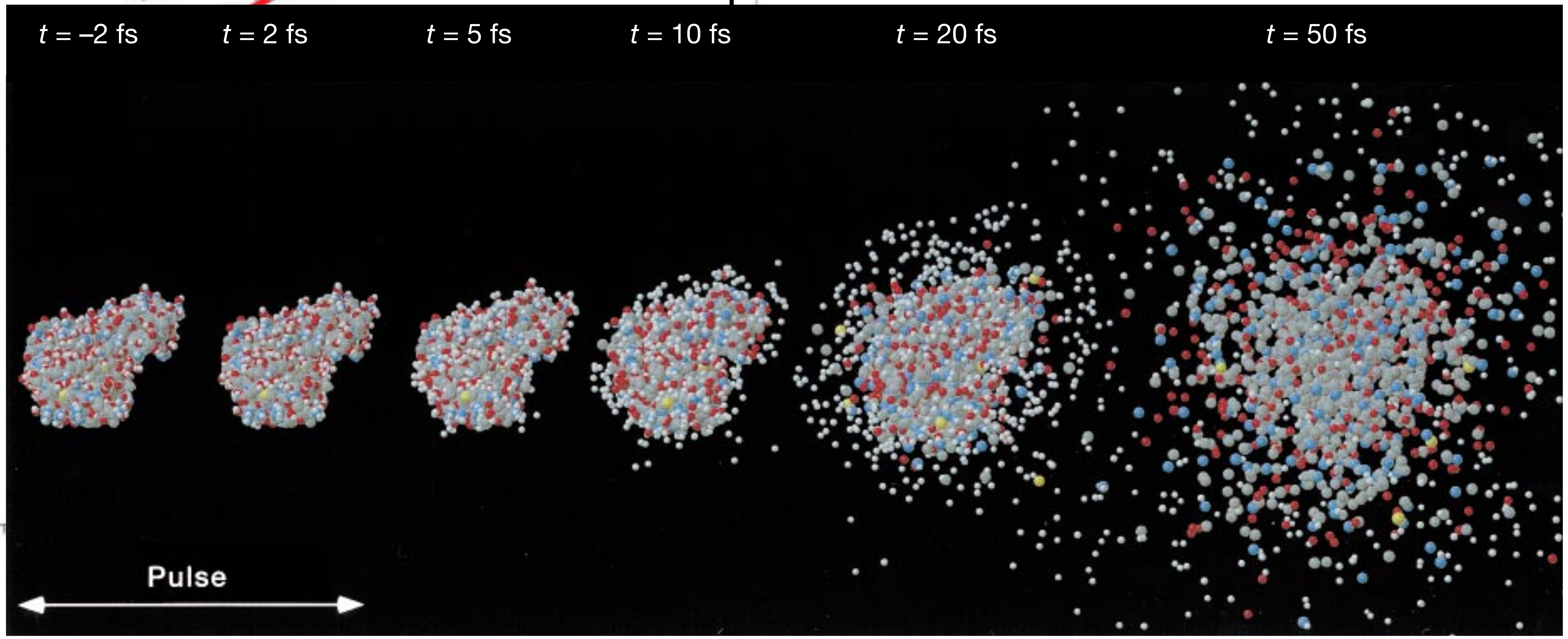
The European XFEL

Max. electron energy	17.5 GeV
Wavelength	0.05 - 4.7 nm
Pulse duration	< 100 fs
Photons / pulse	10^{12}
Peak brilliance	5×10^{33}
Pulses / second	27.000,000



X-ray sources and the FEL revolution





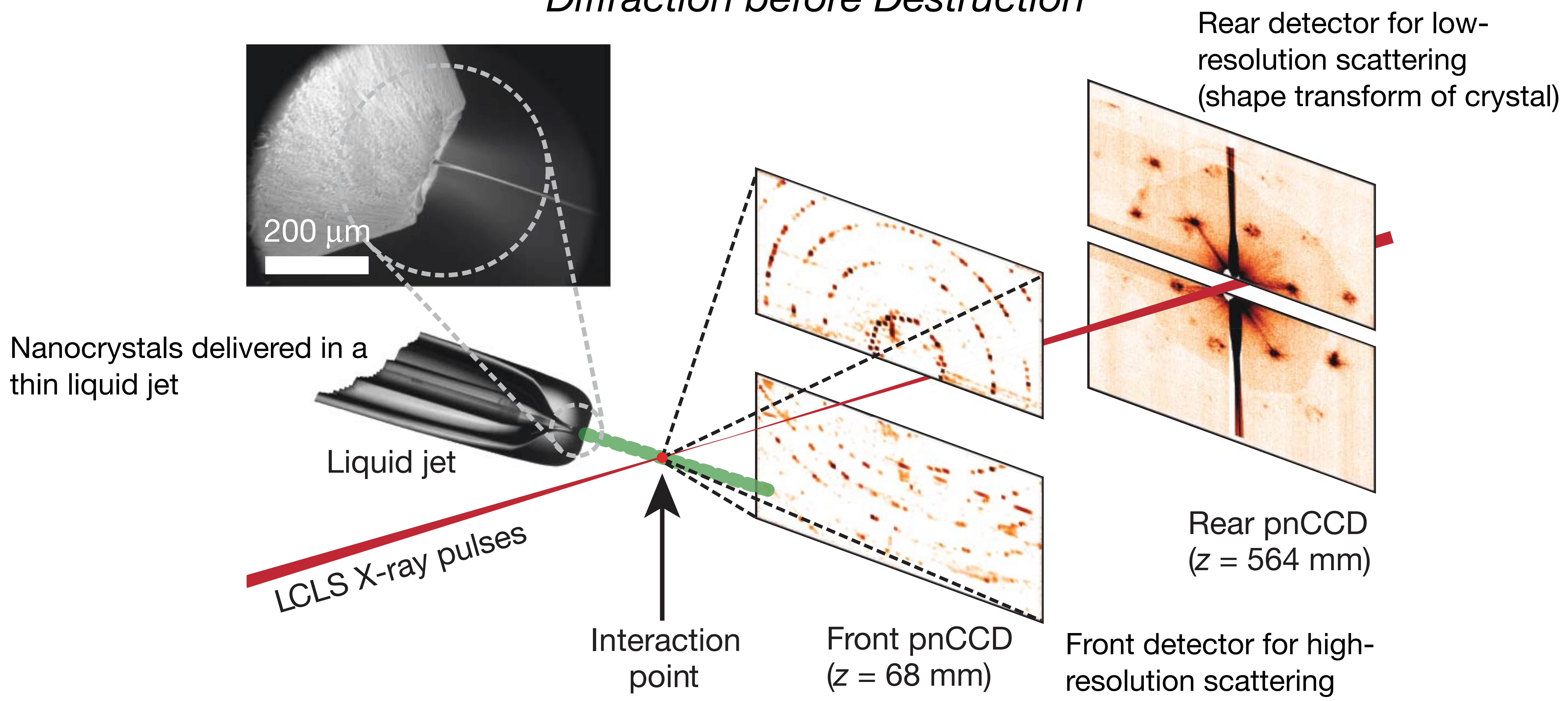
- More incoming photons → more scattered photons
- Do not require the huge amplification from large crystals anymore
- Very short pulses enable time-resolved studies (Note: synchrotrons typically have ~100 ps pulses)
- Higher coherence beams for novel quantum-optics experiments

- BUT: the huge intensity destroys samples - in a single shot!

Serial femtosecond crystallography (SFX)

- The much higher x-ray intensities allow the use of much smaller crystals (100s of nm)
- Sample destruction issue is solved by providing a new (but identical) crystal every shot
  - Every crystal only exposed to one x-ray pulse
  - If the x-ray pulse is short enough, the diffraction image is recorded before the sample gets destroyed by the high intensity pulse

"Diffraction before Destruction"



Chapman et al., Nature 470, 73 (2011)

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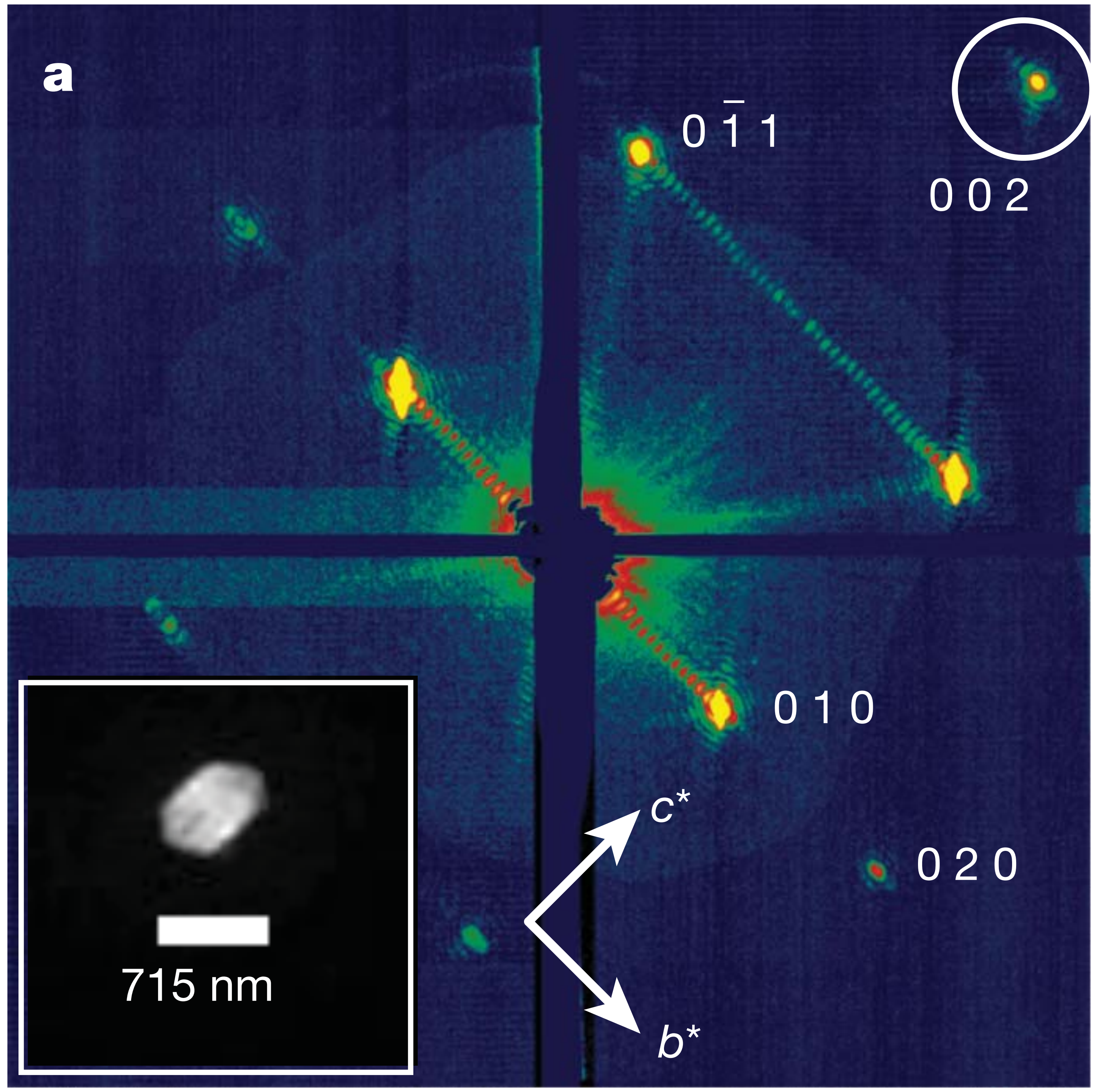
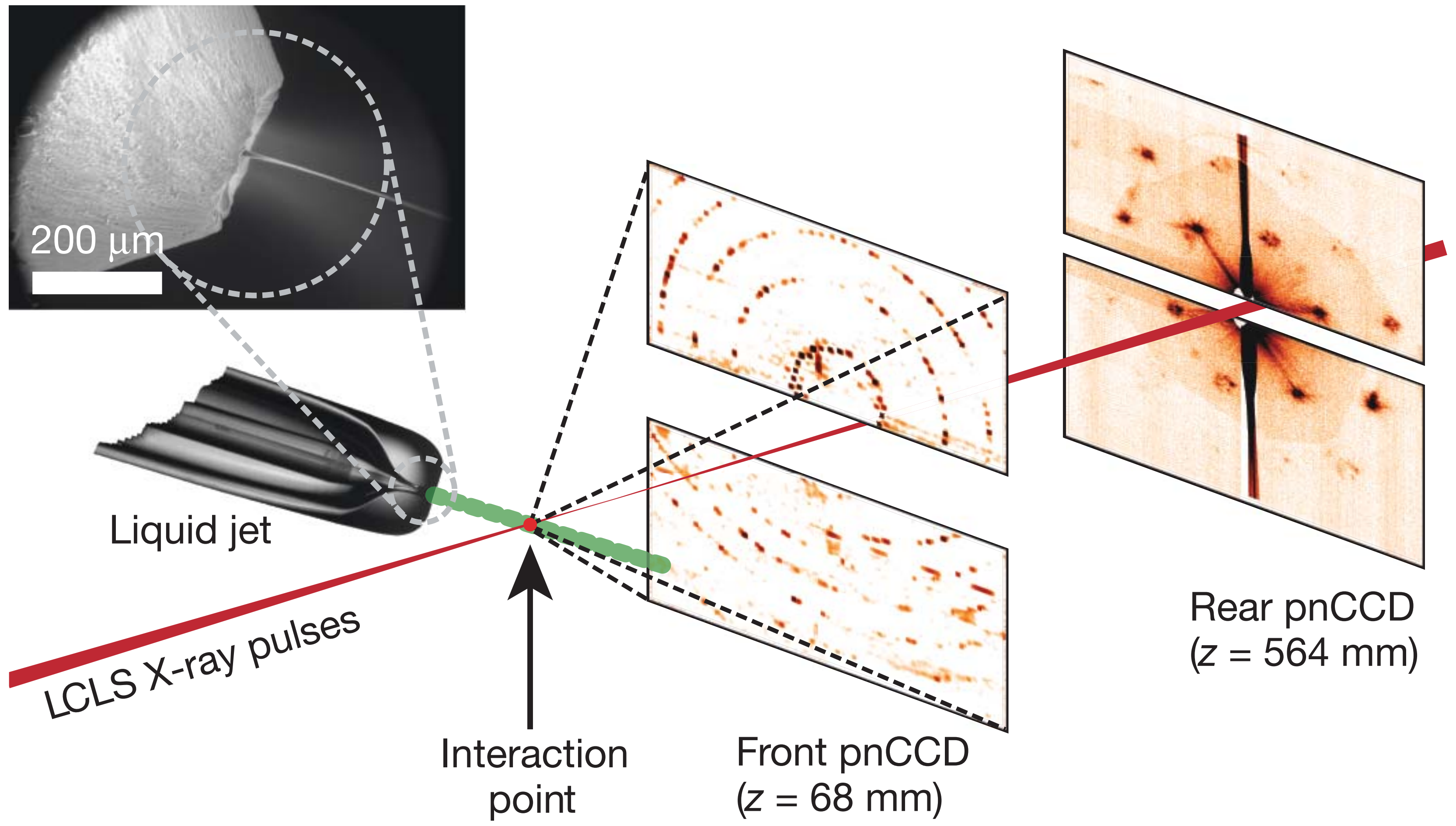
low-resolution data





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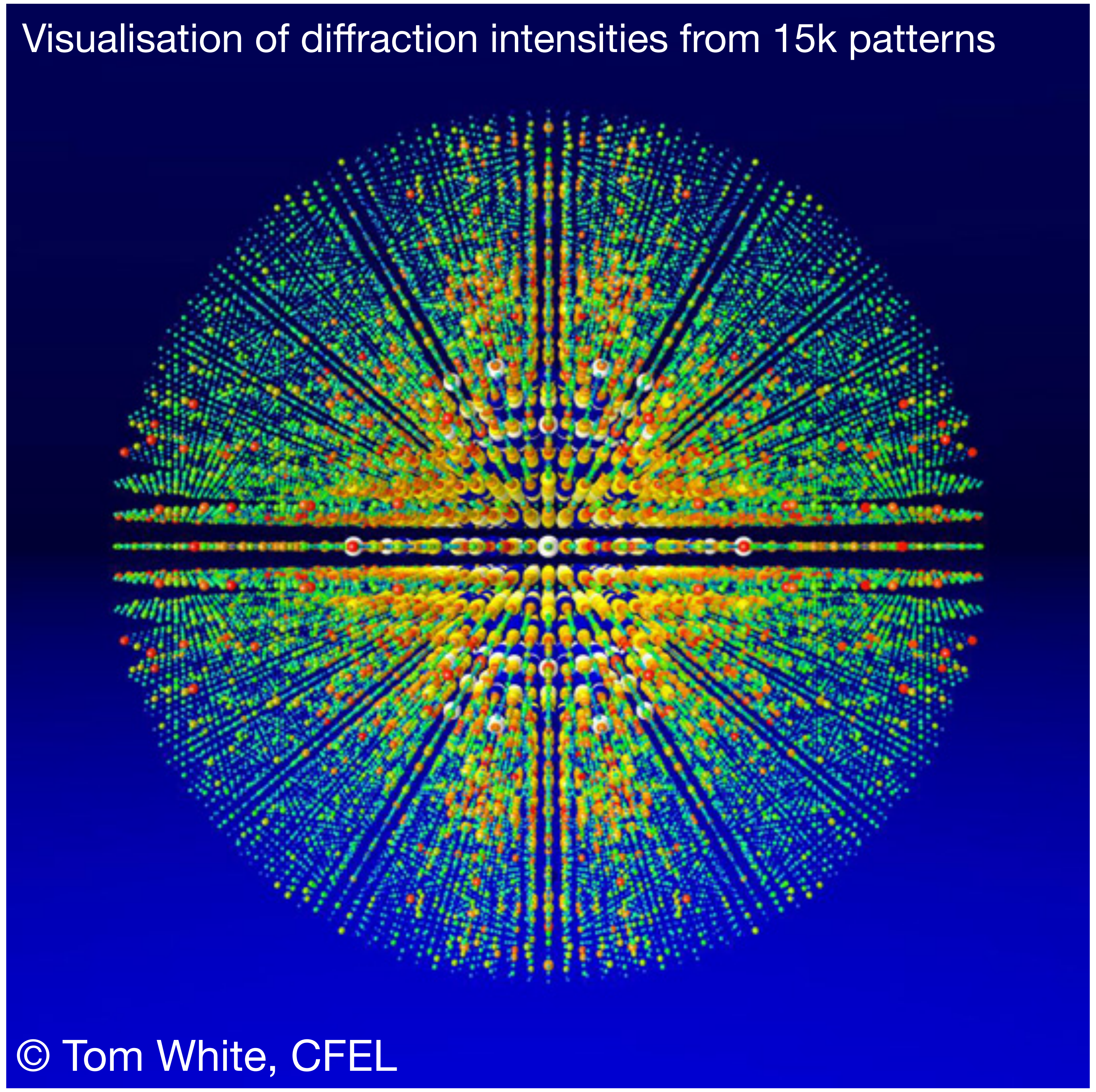
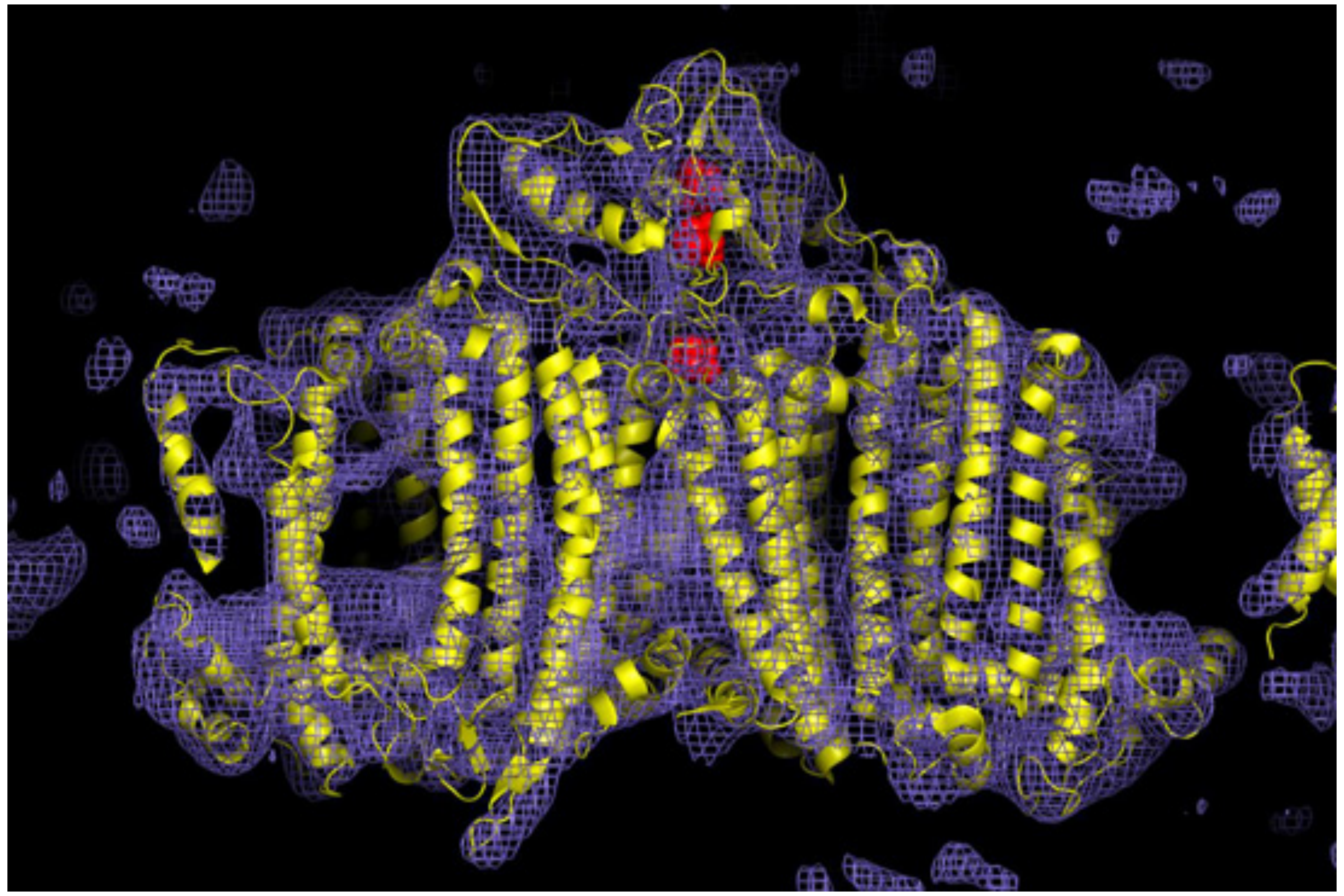




Reconstructed molecular structure
(Photosystem I)

Chapman et al., Nature 470, 73 (2011)

Coherent diffractive imaging CDI (Single particle imaging SPI)

- SFX allows structure determination of many systems that previously could not be studied
- But it still relies on (albeit very small) crystals…can we image isolated molecules/particles?

Chapman et al., Nature 470, 73 (2011)

Coherent diffractive imaging CDI (Single particle imaging SPI)

For 3D reconstruction we need images from many orientations of an identical object!

→ **many identical objects in known orientations**

- Image a single object (molecule, nanoparticle) at a time
- Recover the orientation from the recorded pattern afterwards
- Sample different orientations “randomly”

Pro:

- no need for alignment

Con:

- orientation recovery not trivial (might require a-priori knowledge)
- orientation recovery requires a certain number of scattered photons in each diffraction pattern

- Image an ensemble of identical objects at a time
- Make sure all object are aligned in a known way
- Change alignment of objects to sample orientation space

Pro:

- Can have scattering from many objects
- No need for difficult orientation recovery

Con:

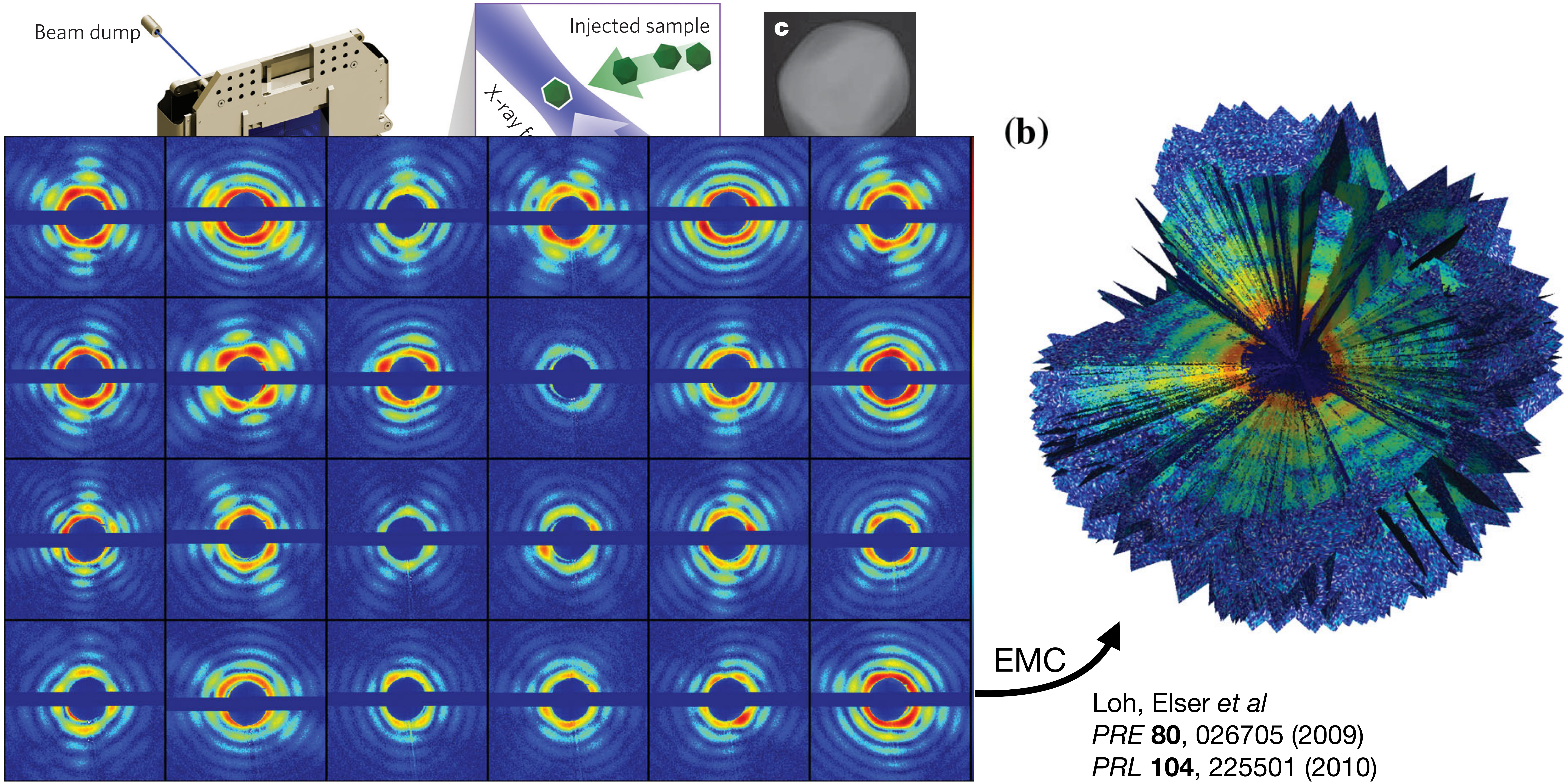
- Need to align all objects in a well-defined and controlled fashion

Barty, Küpper, Chapman; Annu. Rev. Phys. Chem. 64, 415 (2013)

Single particle imaging (SPI)

For 3D reconstruction we need images from many orientations of an identical object!

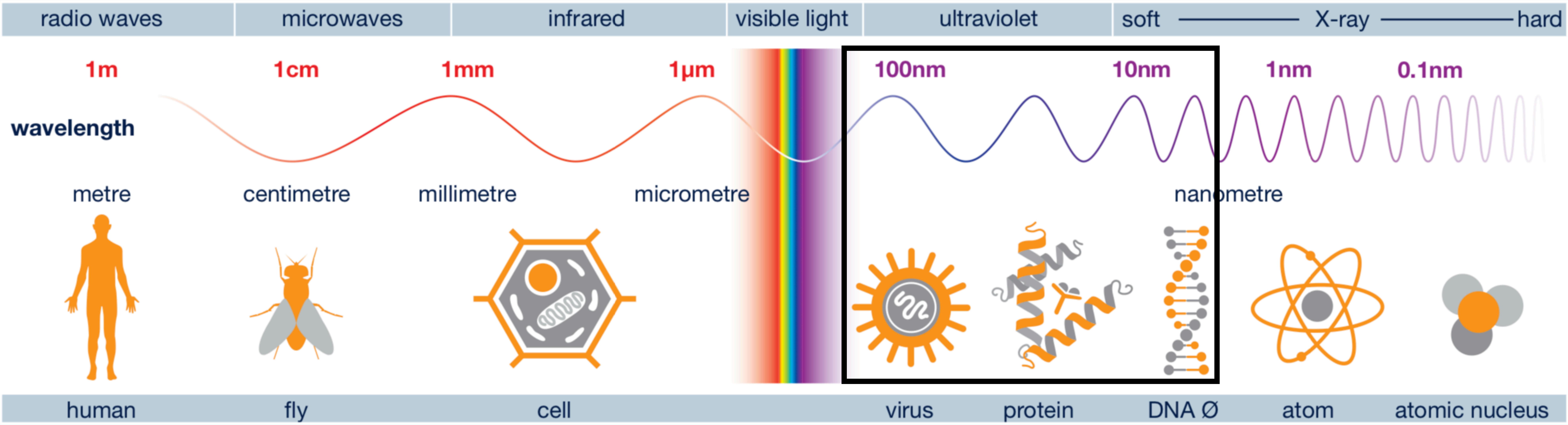
→ **many identical objects in known orientations**



Loh, Elser *et al*
PRE **80**, 026705 (2009)
PRL **104**, 225501 (2010)

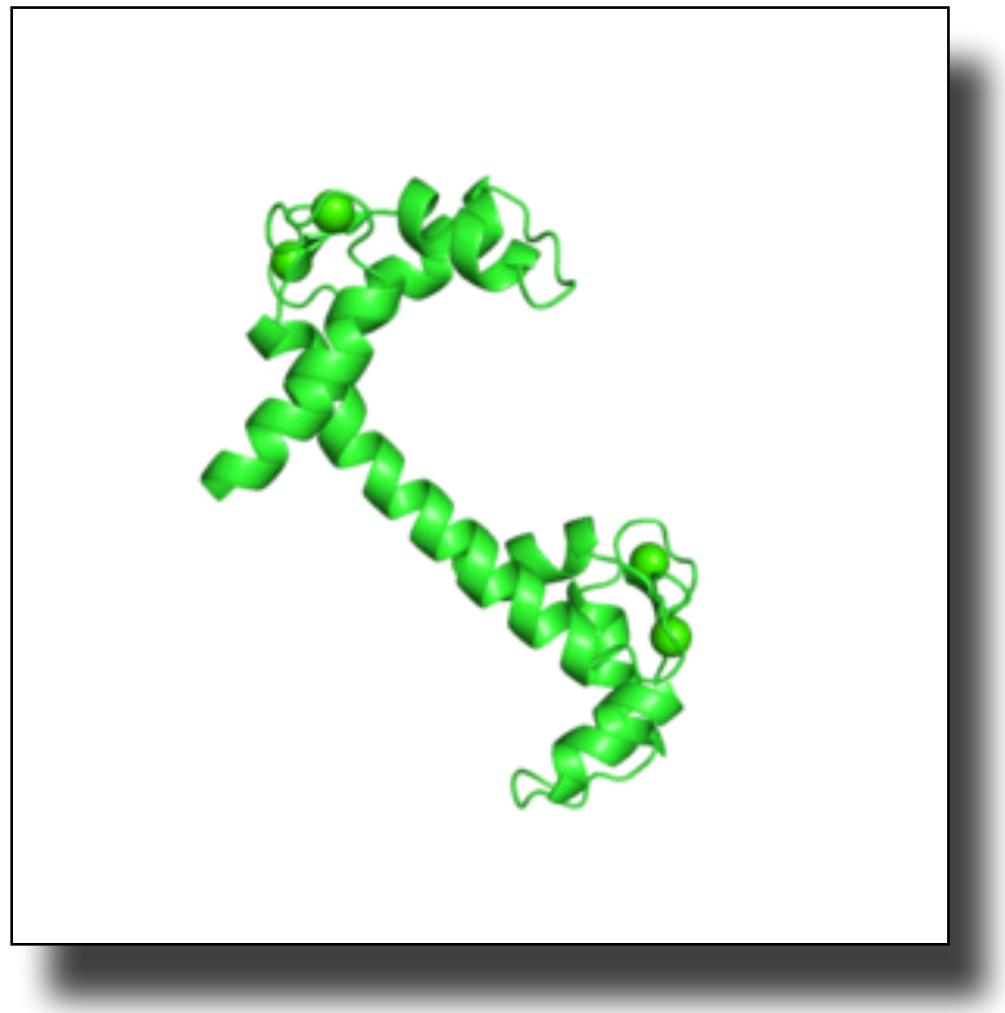
Seibert et al, Nature 470, 78 (2011)
Ekeberg et al, PRL 114, 098102 (2015)

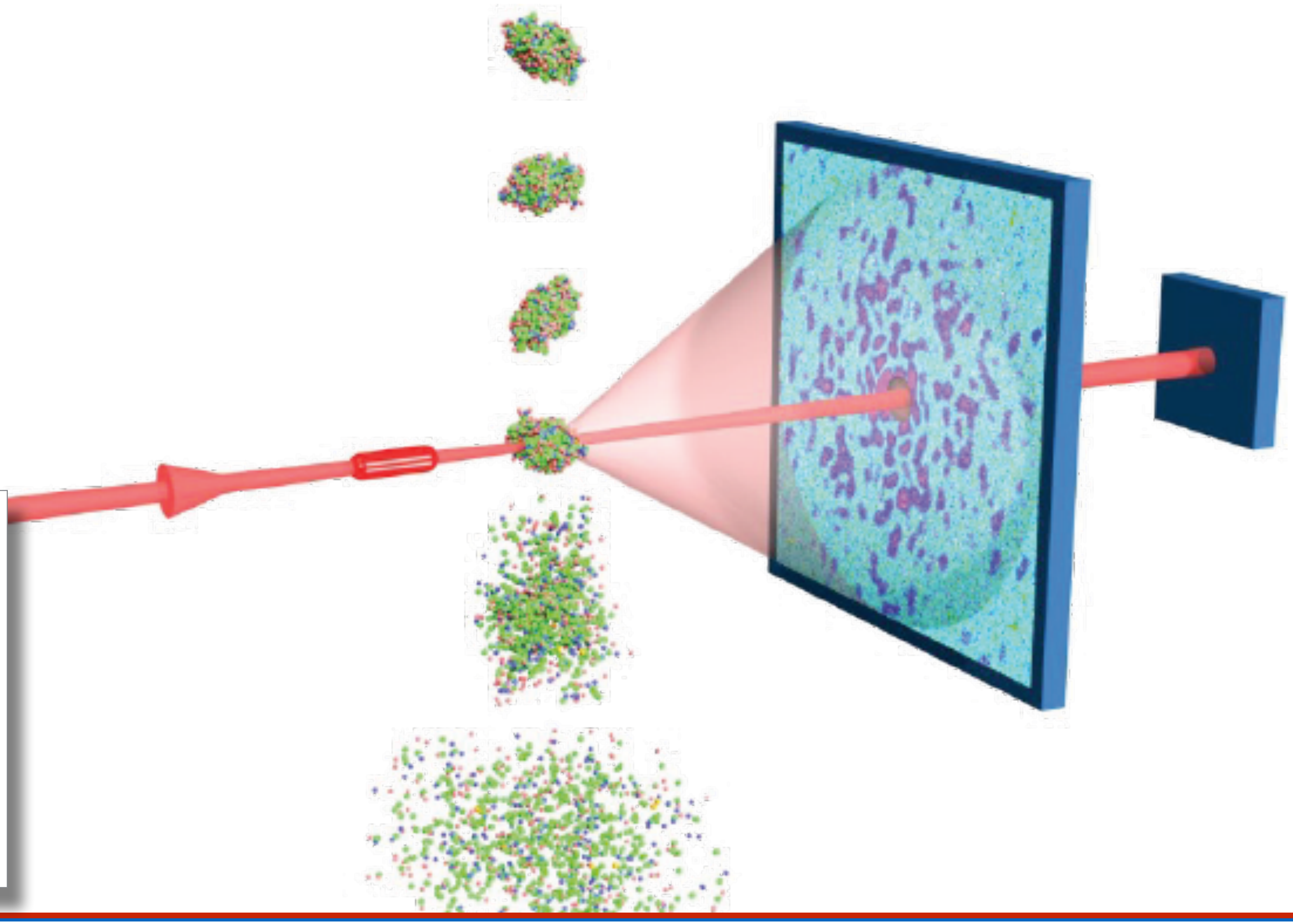
ALS: towards single proteins



Top down approach

Atomic-resolution coherent-x-ray-diffractive single-particle imaging

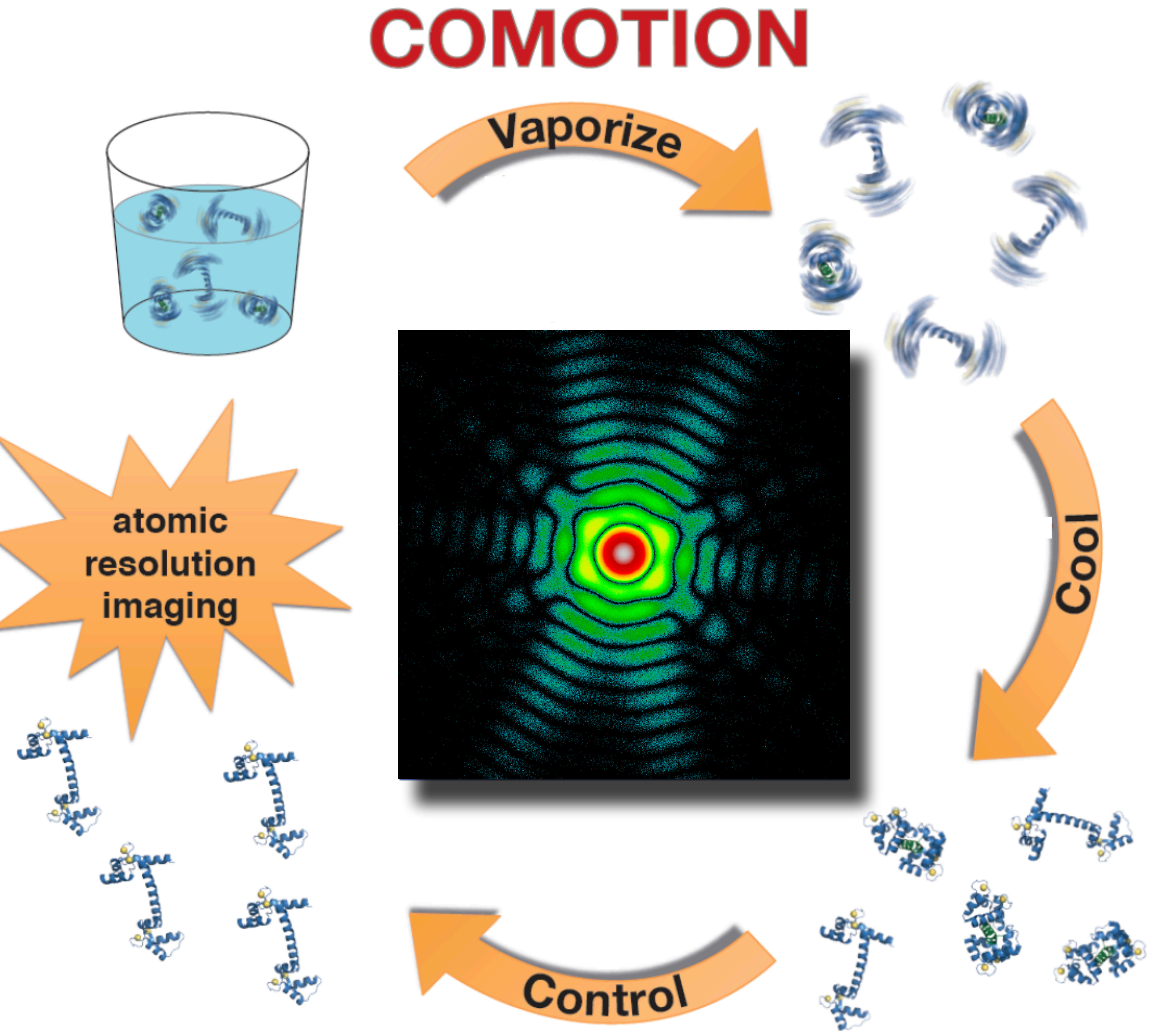




Sample conditions
- in vacuum
- identical
- intact, 'native'
- high density
- synchronized to XFEL pulses

Barty, Küpper, Chapman, *Ann. Rev. Phys. Chem.* **64**, 415–435 (2013)
Chang, Horke, Trippel, Küpper, *Int. Rev. Phys. Chem.* **34**, 557–590 (2015); arXiv:1505.05632 [physics]

Our approach towards optimal sample



- Efficient creation of isolated nano-objects.
- Control techniques to optimize the particle beam.
- Shock-freezing using cryogenic cooling techniques.
- Where we are going…..

Aerosolization techniques

Gas-focused liquid jet

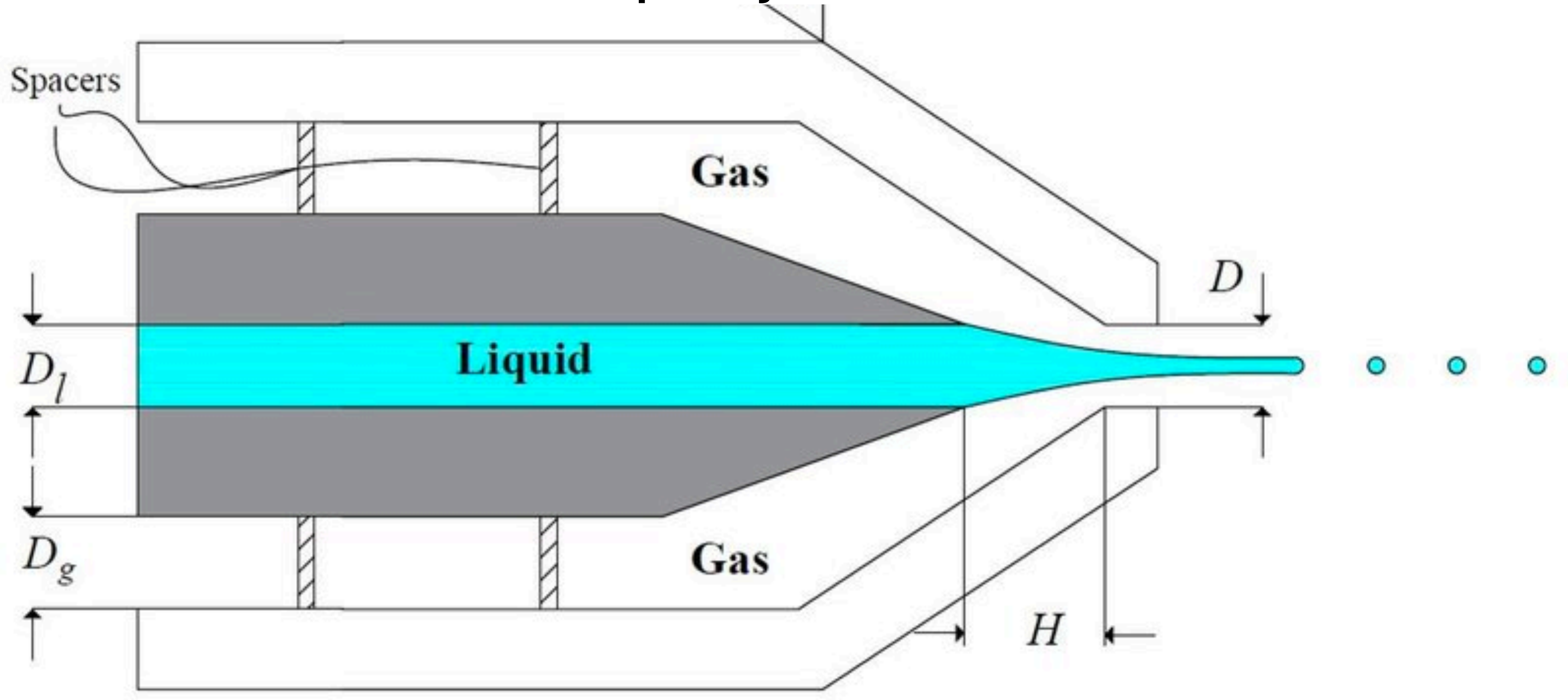
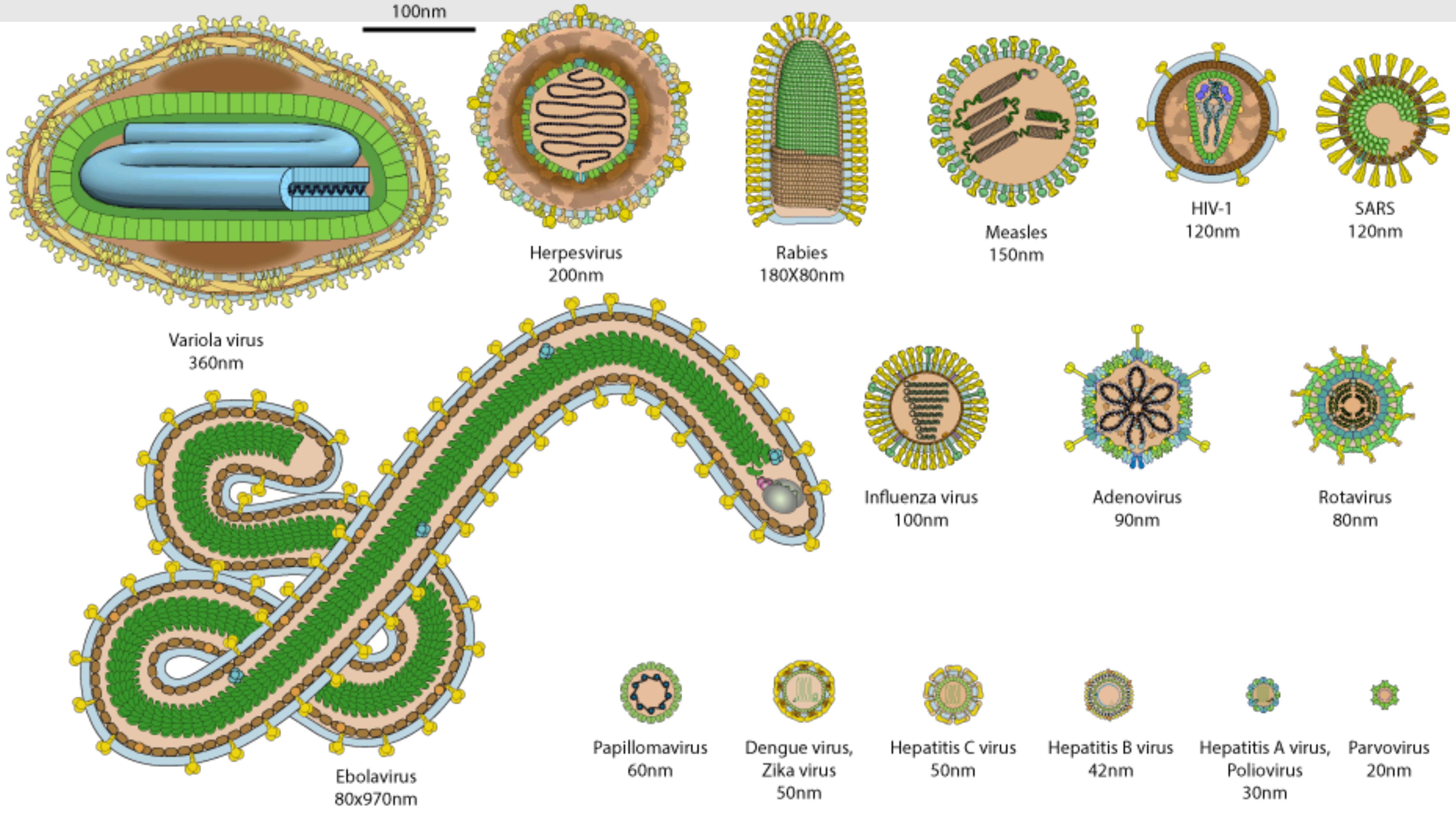
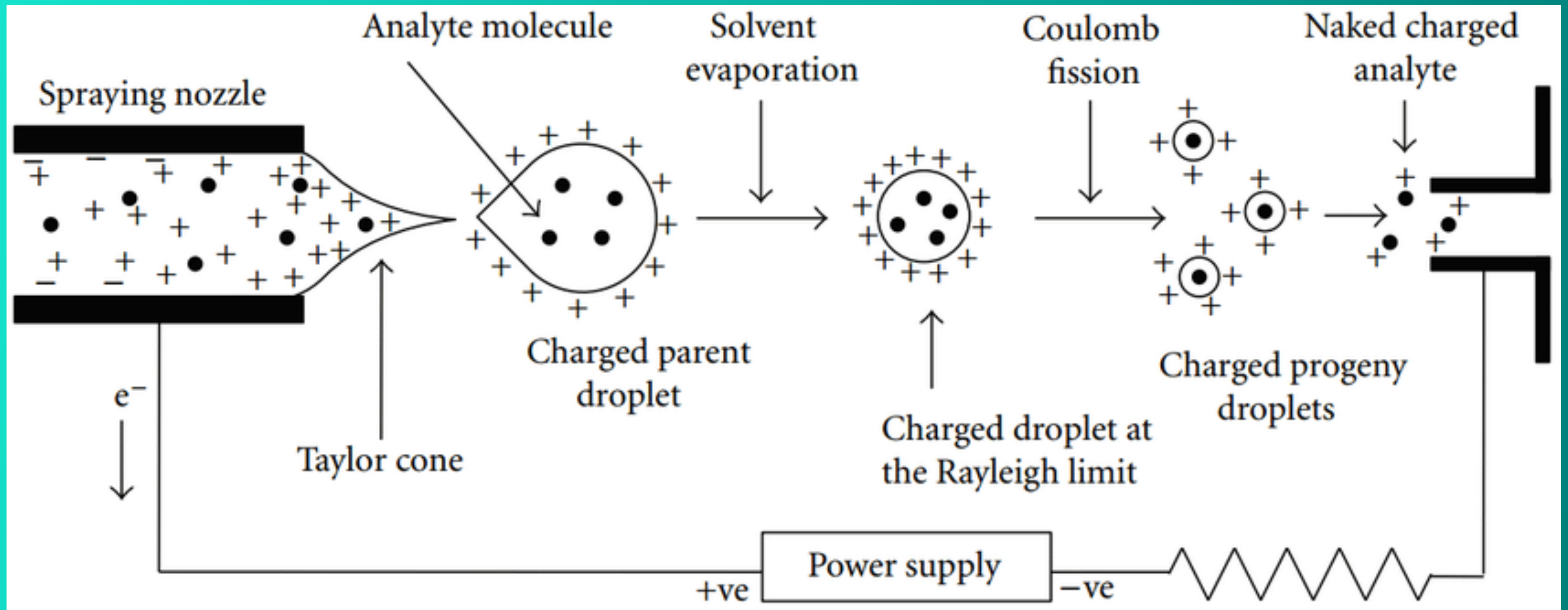


Table 1. Aerosolization parameters. Characteristic parameters for sample aerosolization with ES and a GDVN assuming an average droplet occupancy of 1.

	Sample flow rate	Droplet size	Sample concentration	Particle rate
ES	0.06 μl/min	150 nm	5×10^{14}/ml	5.7×10^{8}/s
GDVN	2 μl/min	1000 nm	2×10^{12}/ml	0.6×10^{8}/s

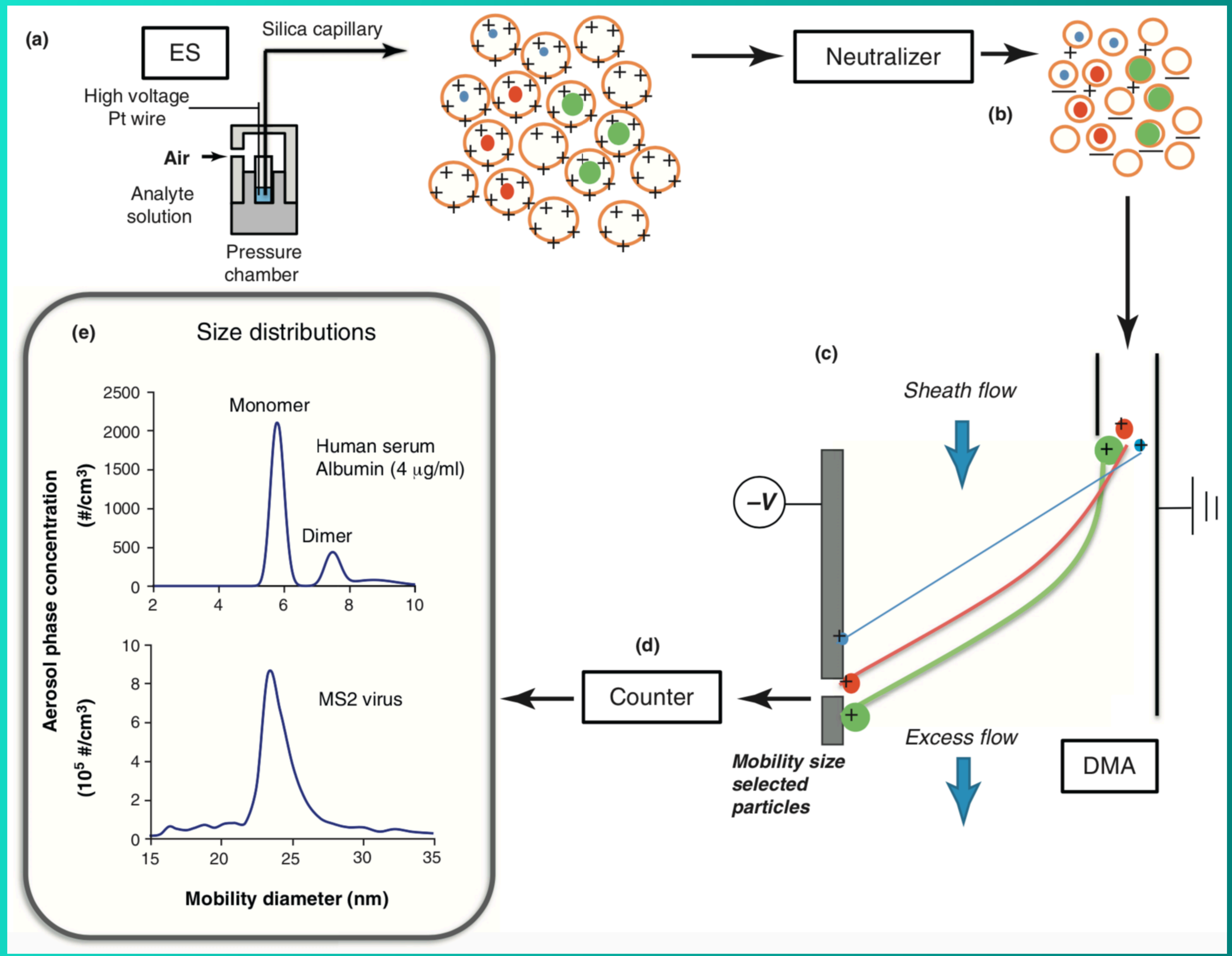


Electrospray



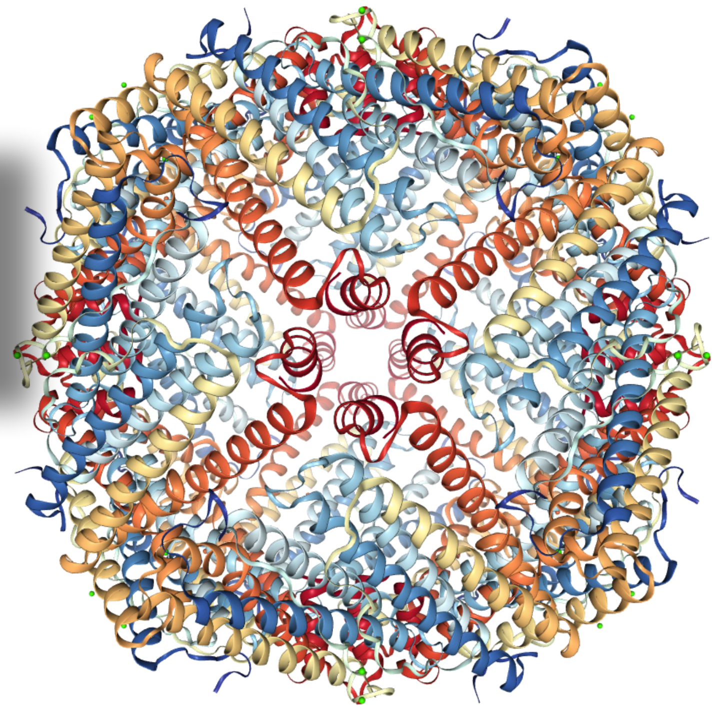
Beyerlein et al., *Rev. Sci. Instrum.*, **86**, 125104 (2015) Bielecki et al., *Science Advances*, **5**, 5 (2019)

Differential Mobility Analyser (DMA) and particle counter



ALS: towards single proteins

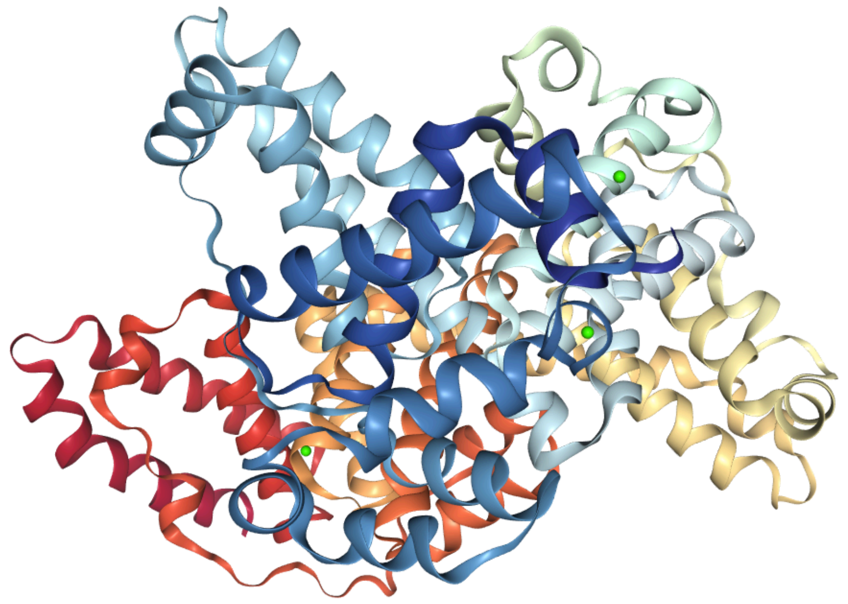
Ferritin
(OD ~ 13 nm)



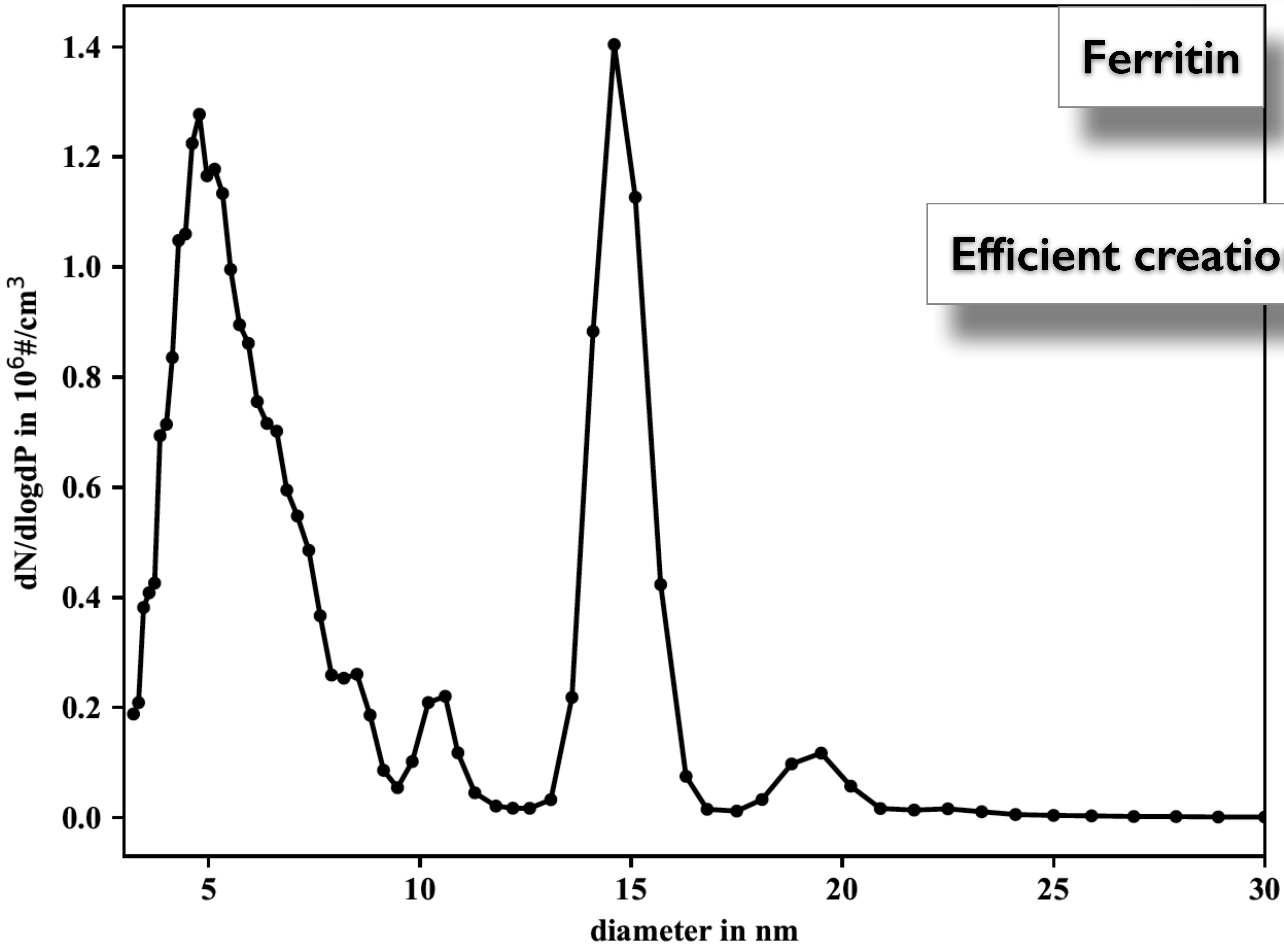
Electrospray

Differential Mobility Analyser (DMA)

Condensation Particle Counter (CPC)

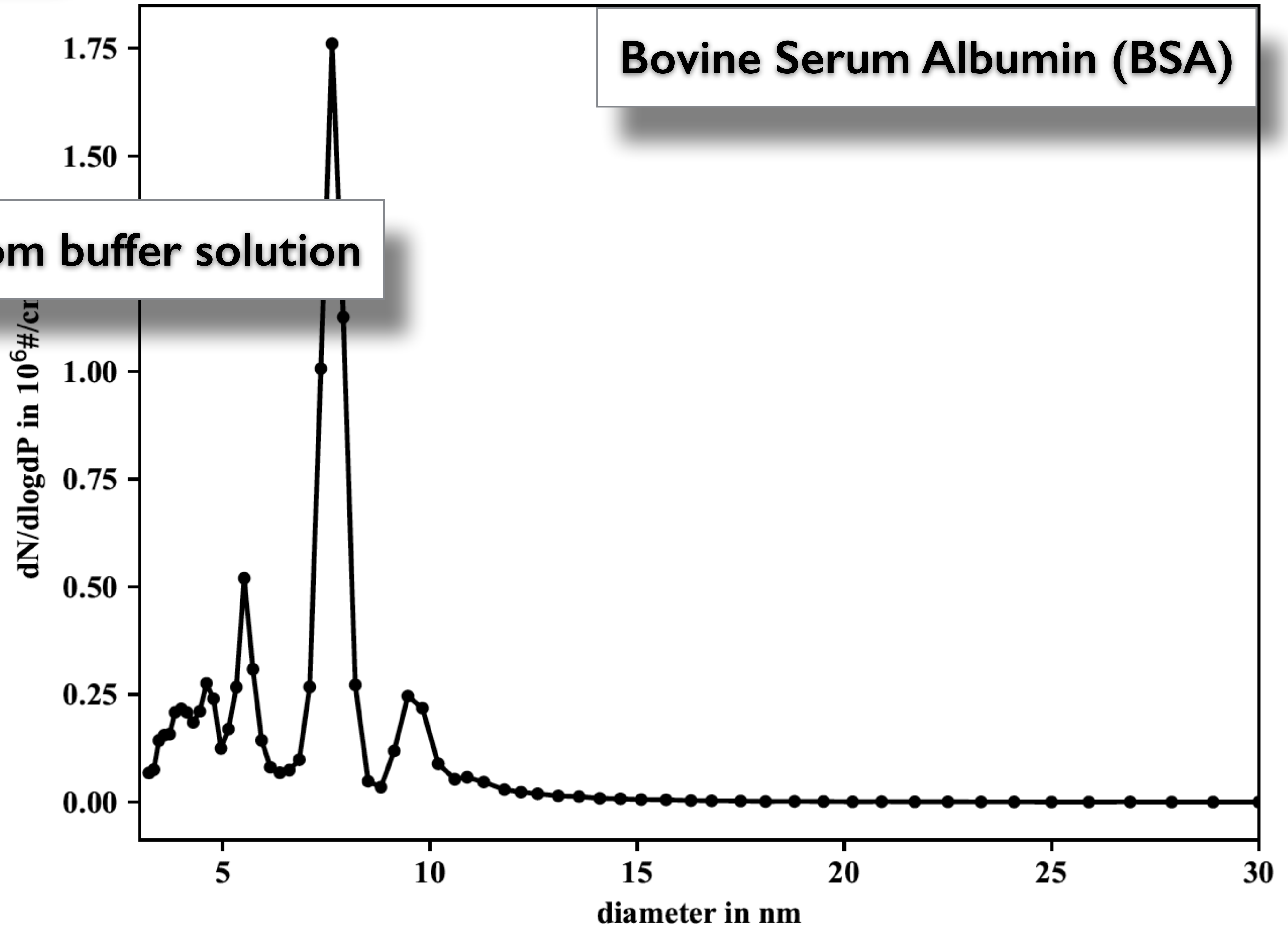


BSA
(OD ~ 7 nm)



Efficient creation of isolated proteins directly from buffer solution

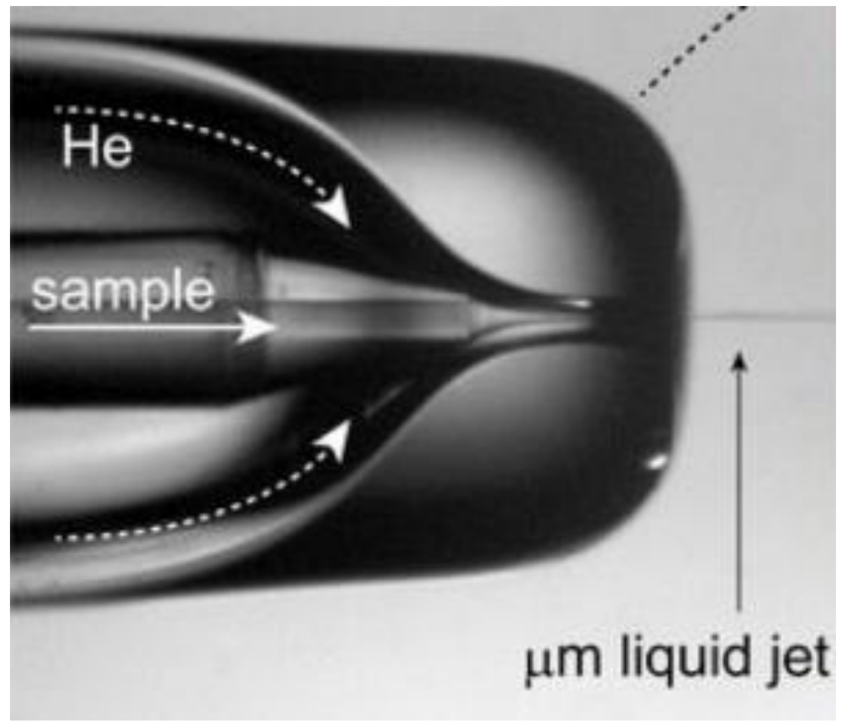
hydrodynamic focusing

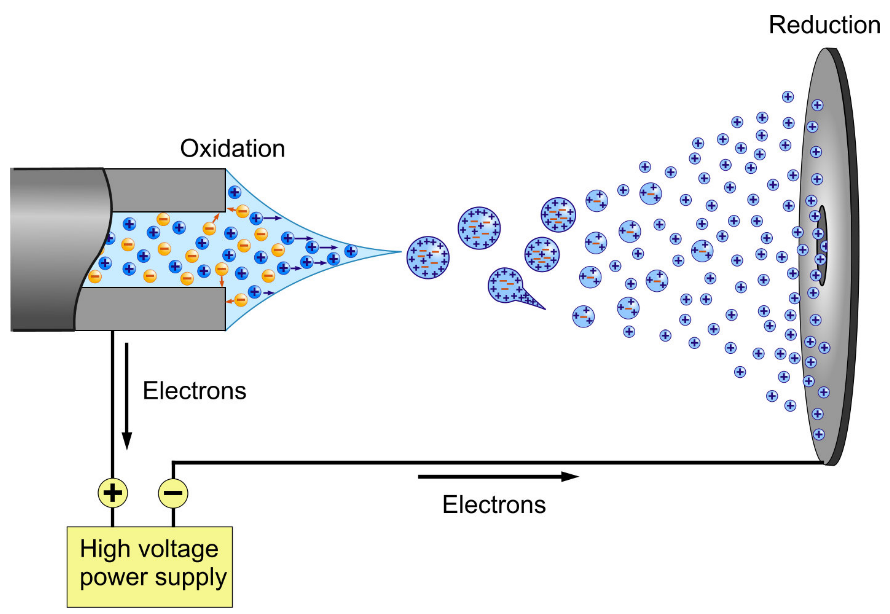


Worbs, Lübke, Estillore, Samanta, Küpper, *in preparation*

Aerosolization and particle beam formation

Aerosolisation



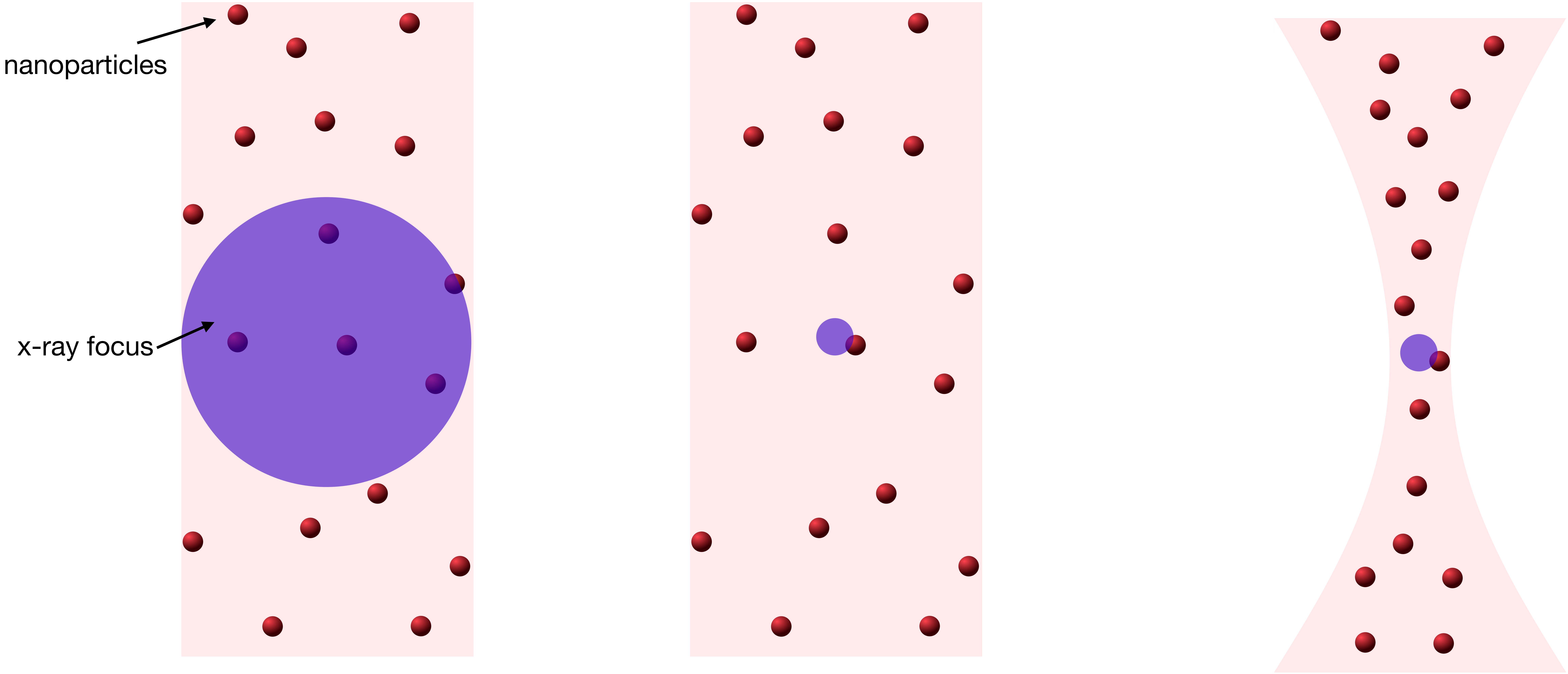


Injection

1 µm x-ray focus

Beyerlein, Adriano, Heymann, Kirian, Konska, Wilde, Chapman, Bajt, *Rev. Sci. Instrum.* **86**, 125104 (2015)
Roth, Awel, Horke, Küpper, *J. Aerosol. Sci.* **124**, 17-29 (2018) arxiv:1712.01795 [physics.flu-dyn]

Particle beam formation: Importance



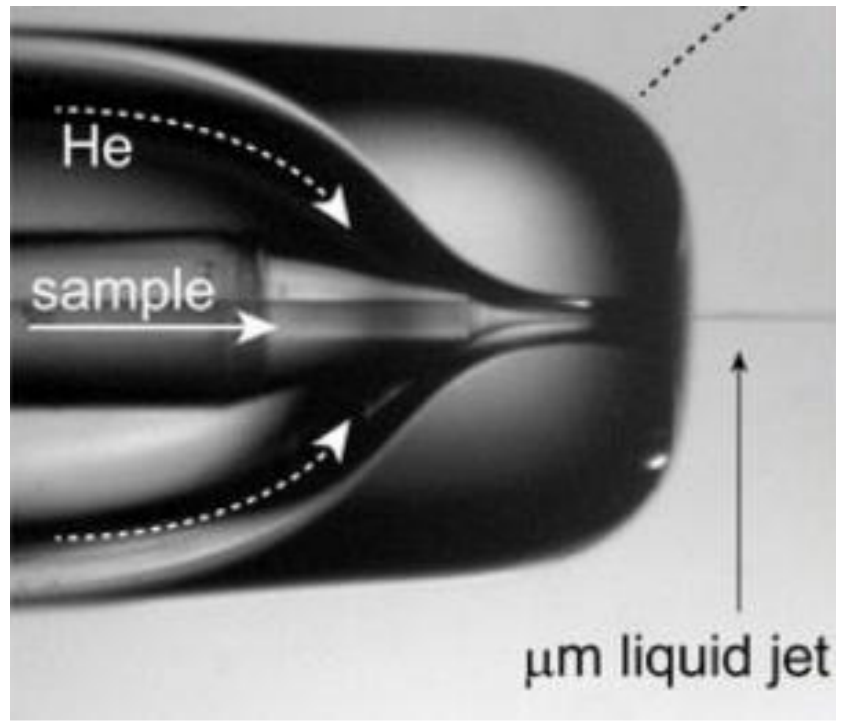
Smaller x-ray focus
100 nm

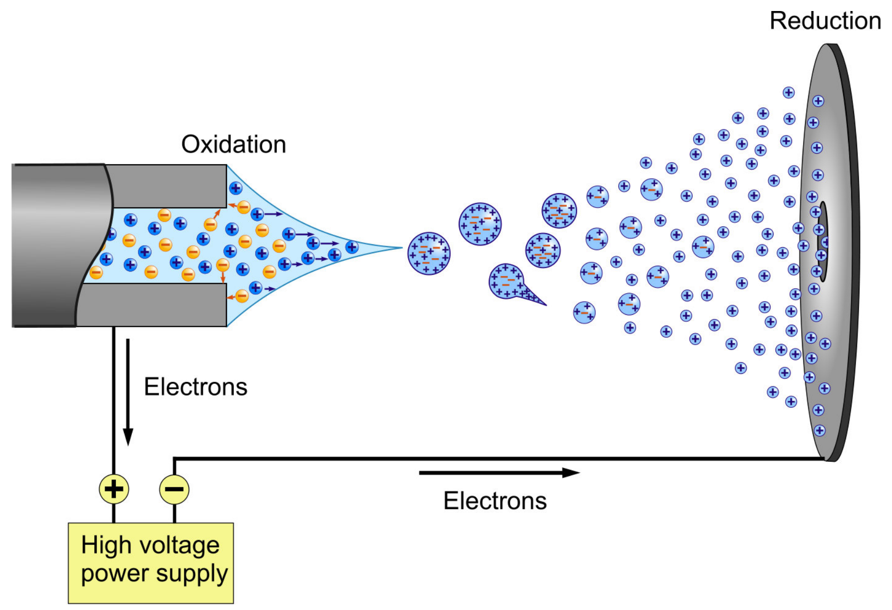
Need: smaller particle beam

Beyerlein, Adriano, Heymann, Kirian, Konska, Wilde, Chapman, Bajt, *Rev. Sci. Instrum.* **86**, 125104 (2015)
Roth, Awel, Horke, Küpper, *J. Aerosol. Sci.* **124**, 17-29 (2018) arxiv:1712.01795 [physics.flu-dyn]

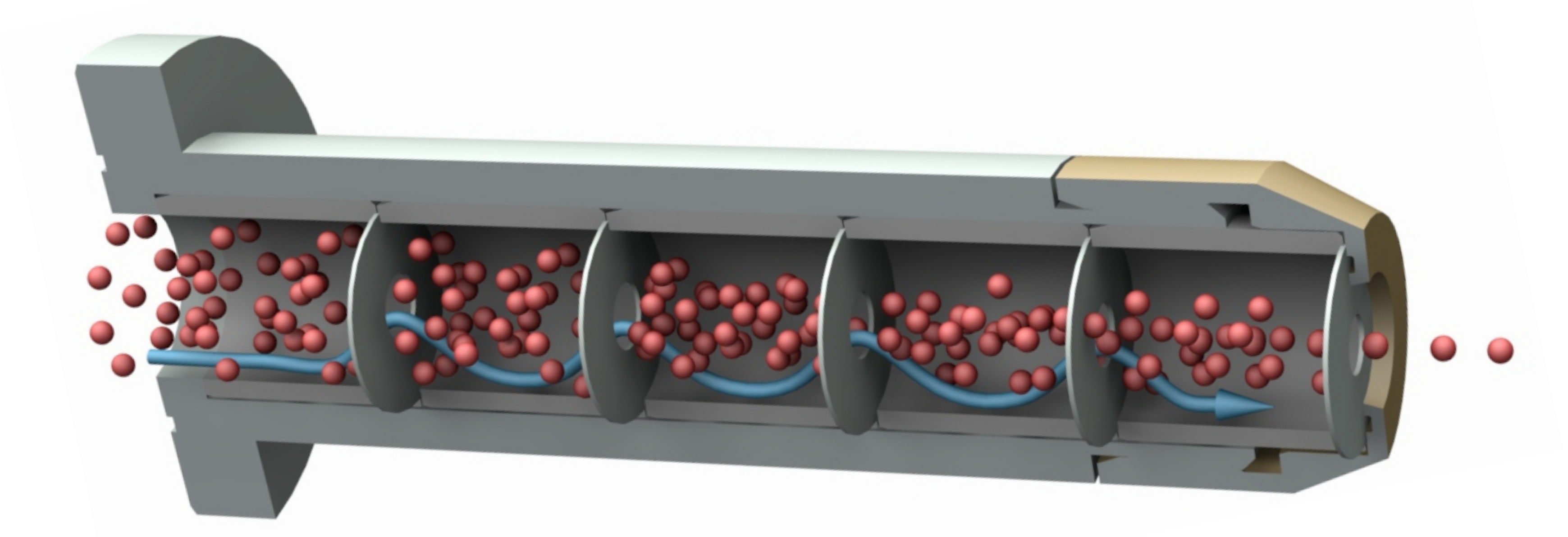
Aerodynamic-lens-injectors for generating particle beams

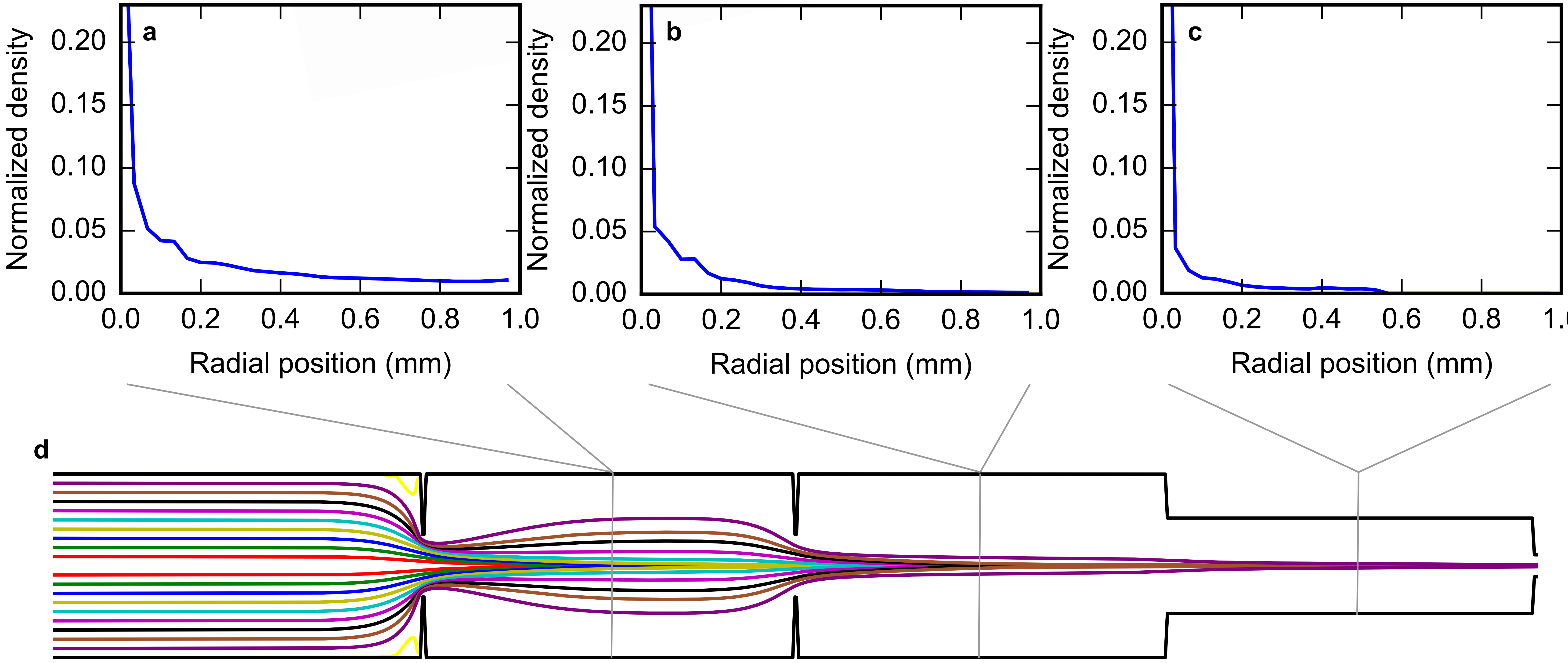
Aerosolisation





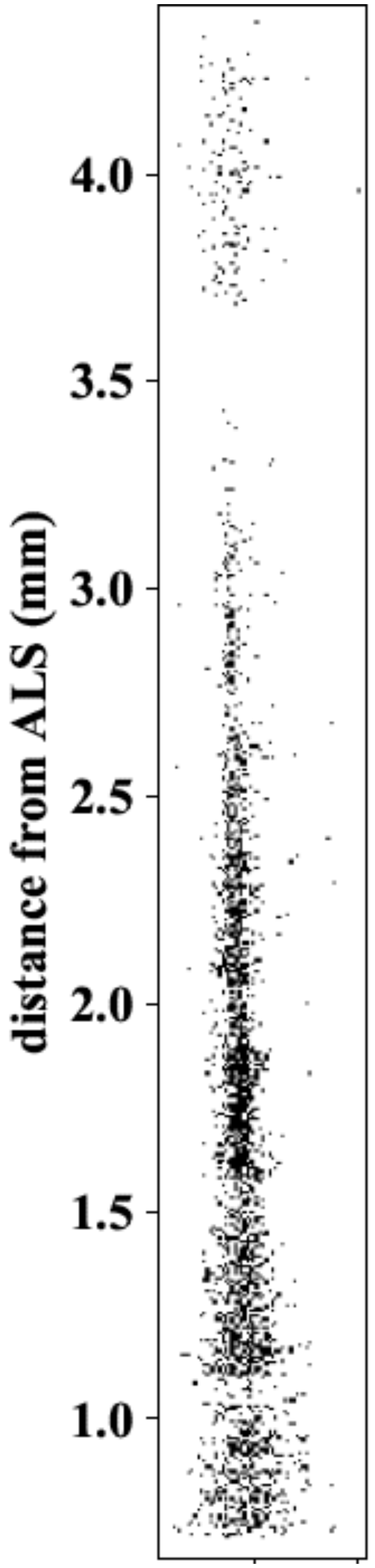
Injection

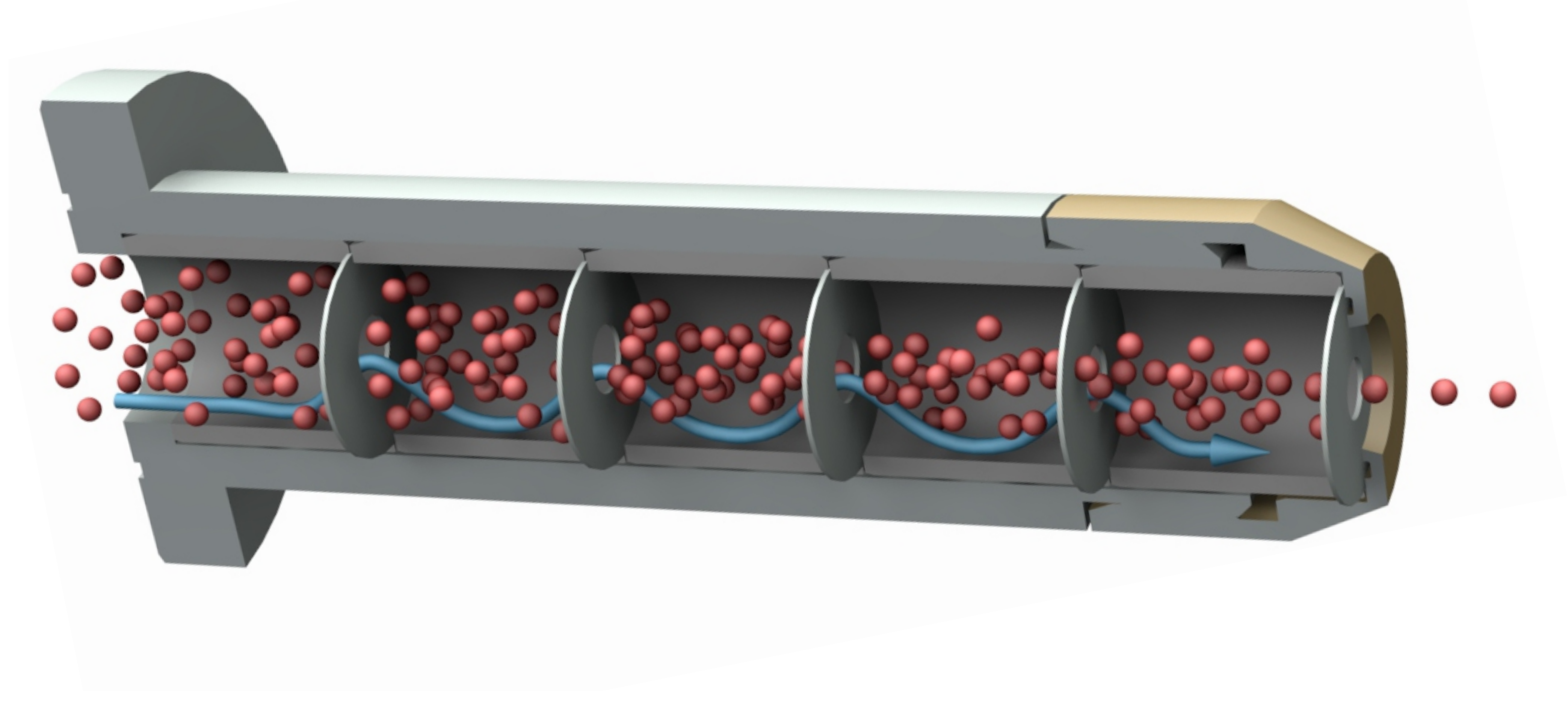




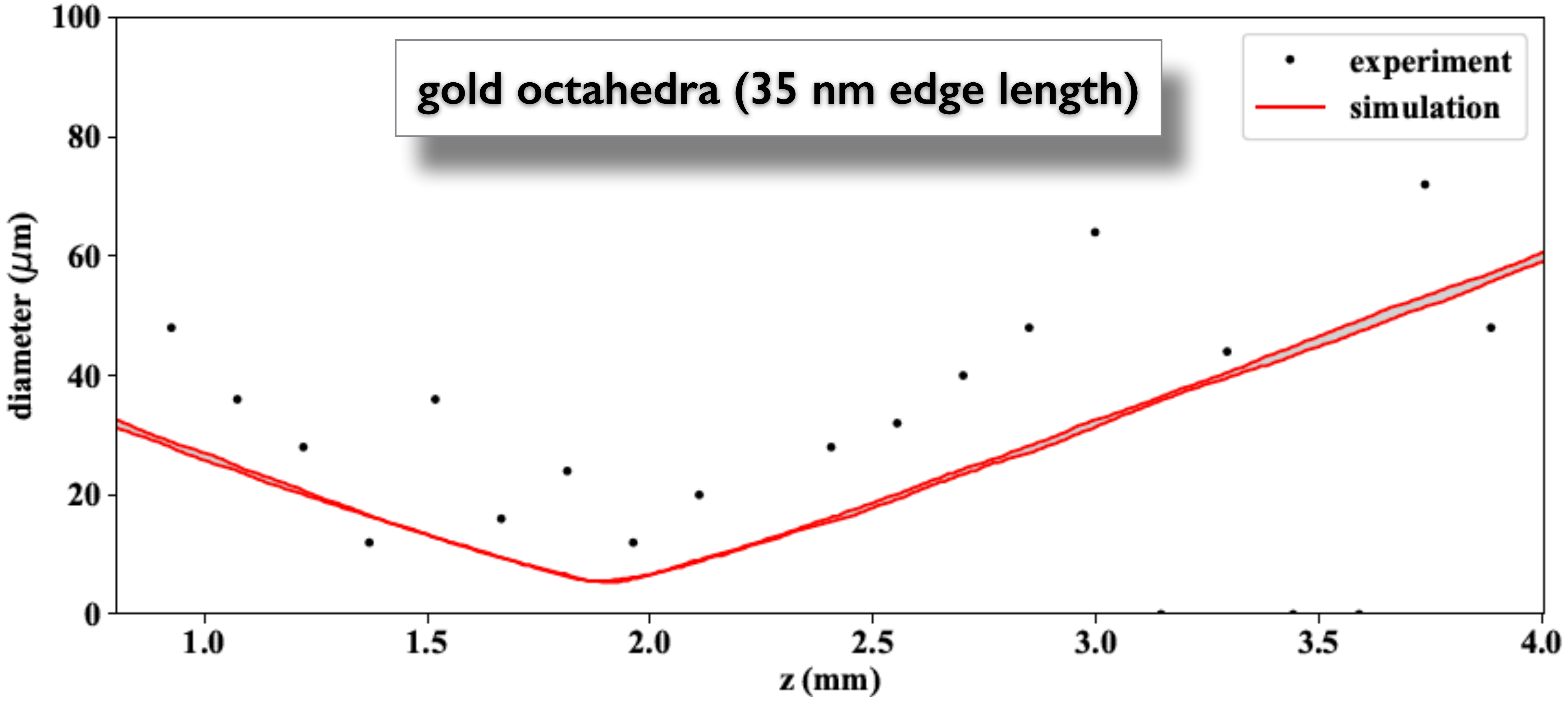
Beyerlein, Adriano, Heymann, Kirian, Konska, Wilde, Chapman, Bajt, *Rev. Sci. Instrum.* **86**, 125104 (2015)
Roth, Awel, Horke, Küpper, *J. Aerosol. Sci.* **124**, 17-29 (2018) arxiv:1712.01795 [physics.flu-dyn]

ALS with gold simulation and experimental comparison



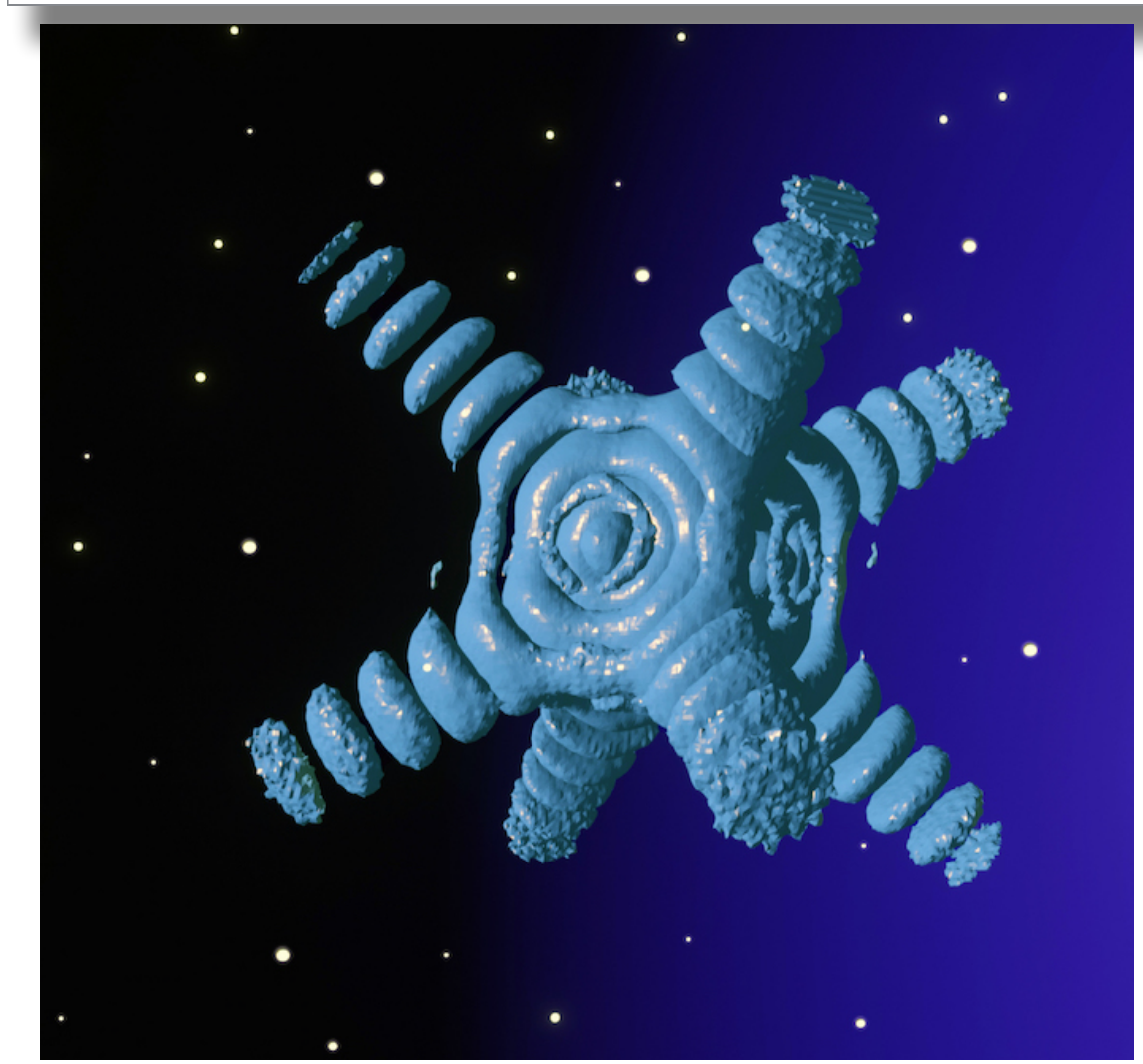


gold octahedra (35 nm edge length)



First experiment with the optimised lens

10 million diffraction patterns (Eu-XFEL, P2160)

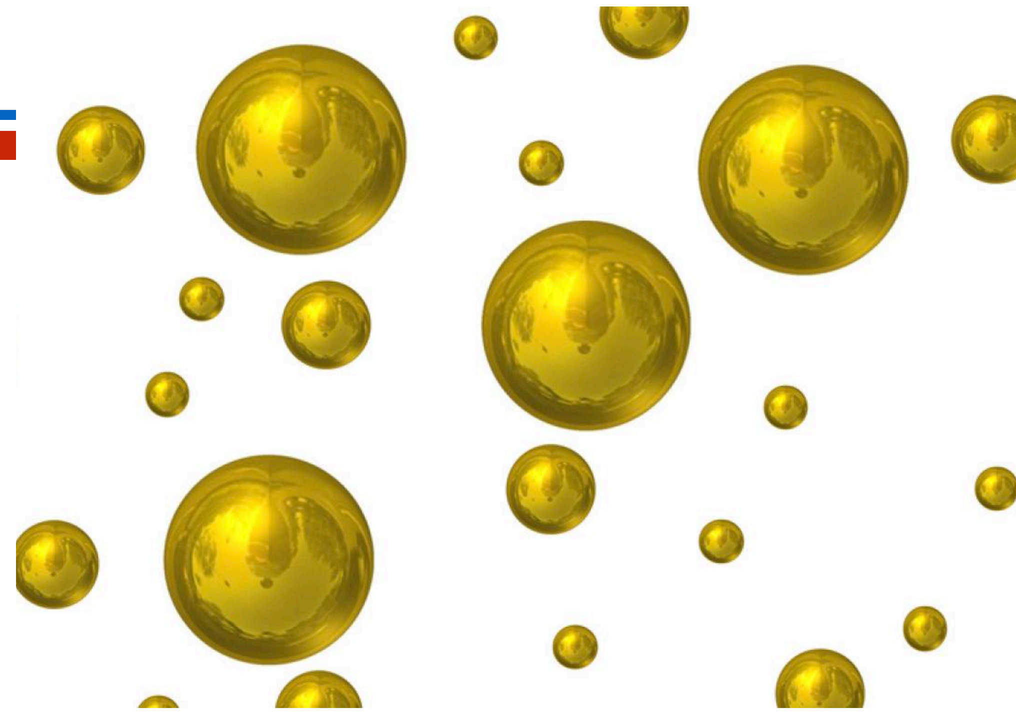


3D diffraction pattern of octahedral nanoparticles

Optica Vol. 8, **Issue 1**, pp. 15-23 (2021)

Worbs, Lübke, Estillore, Samanta, Küpper, *in preparation*

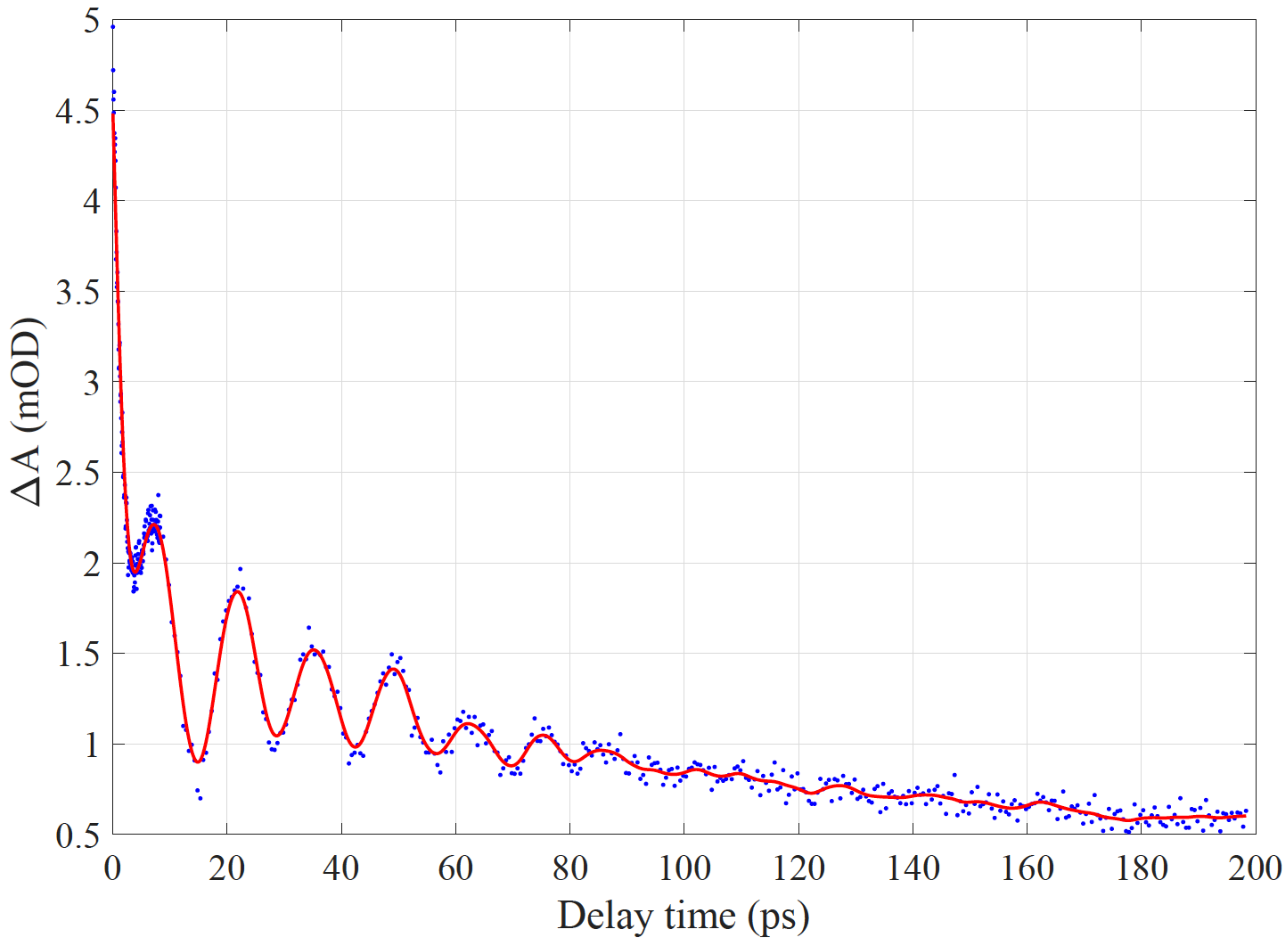
Pump-probe experiments



Proposed experiment:

Recording structural dynamics of electronically excited gold nanoparticles (AuNPs)

- coherent plasmon excitation followed by electron-electron scattering and electron-phonon coupling
- energy is dissipated into different phonon modes and a symmetric breathing mode on ps timescale
- the breathing mode modulates the particle size by ~1%
- monitored by optical transient absorption spectroscopy (in solution)
- the breathing mode frequency is size dependent
- the plasmon damping rate depends on the local environment.

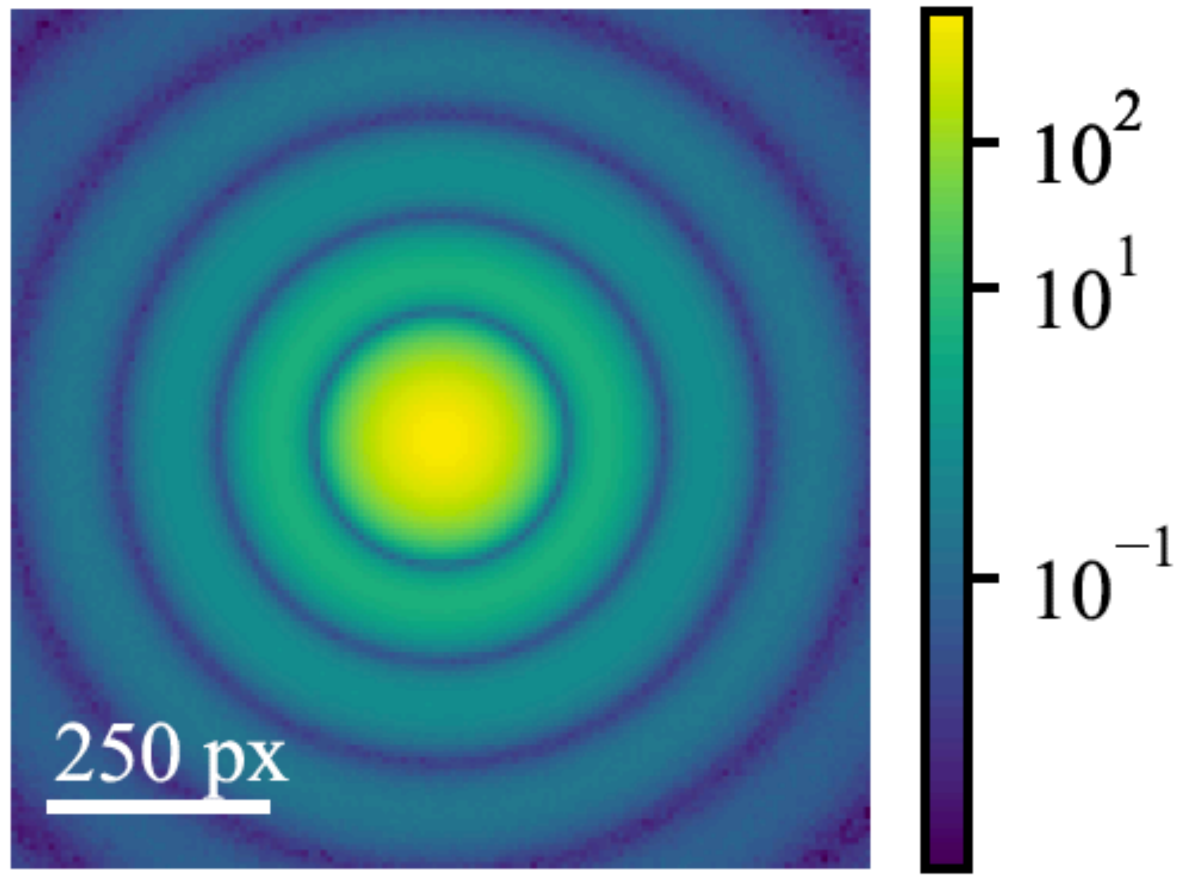


Direct imaging of this structural dynamics: **Optical-pump (400 nm) x-ray-probe (4.5 nm)**

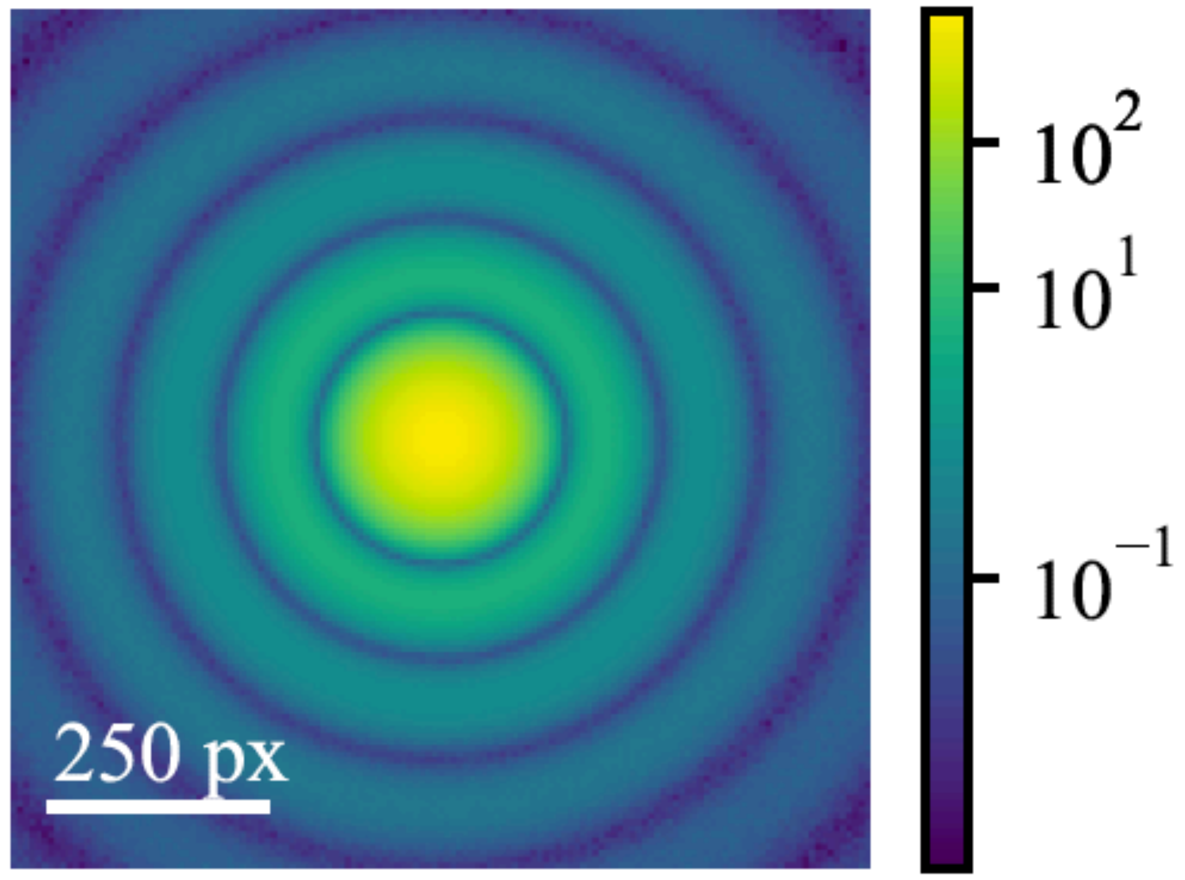
Recording structural dynamics of electronically excited gold nanoparticles

simulated diffraction patterns for 40nm AuNP

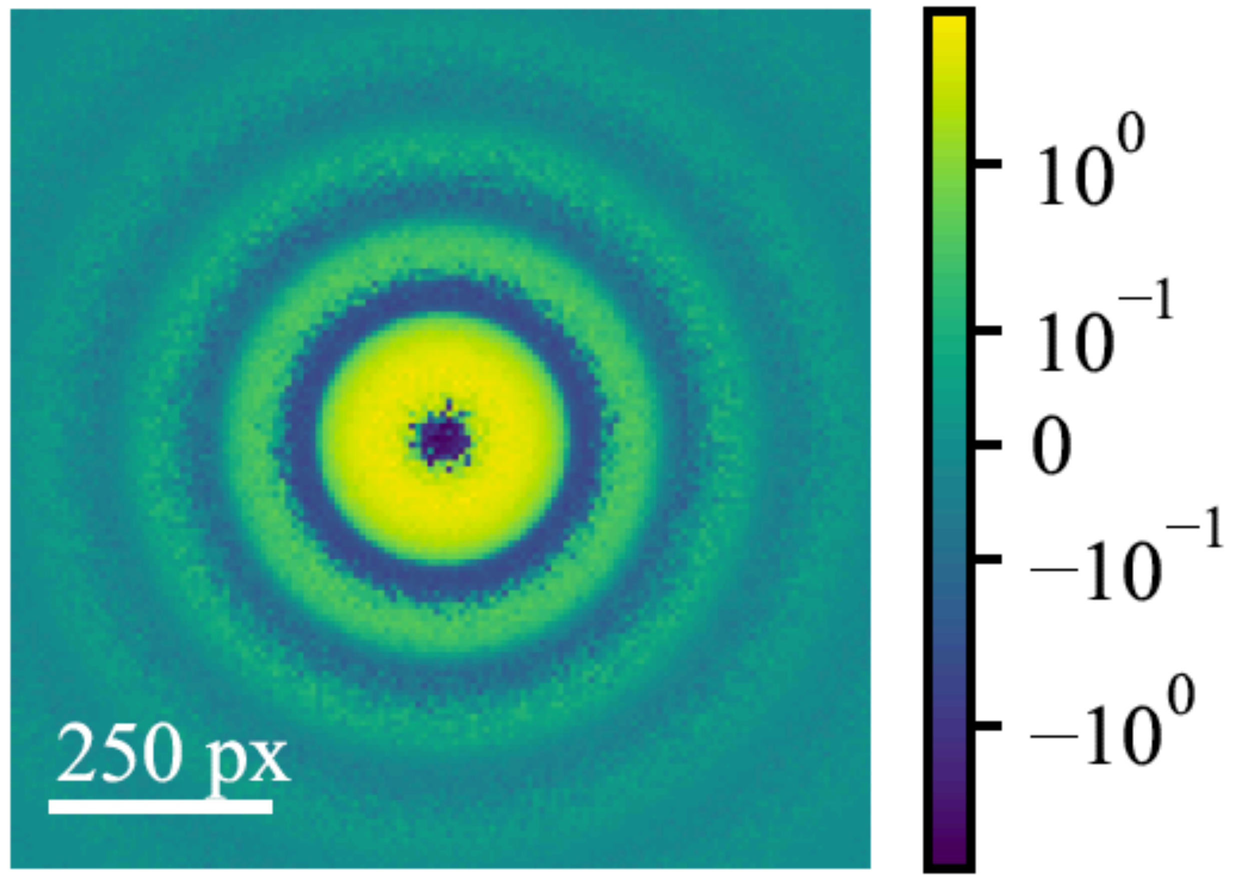
Static sample (40 nm)

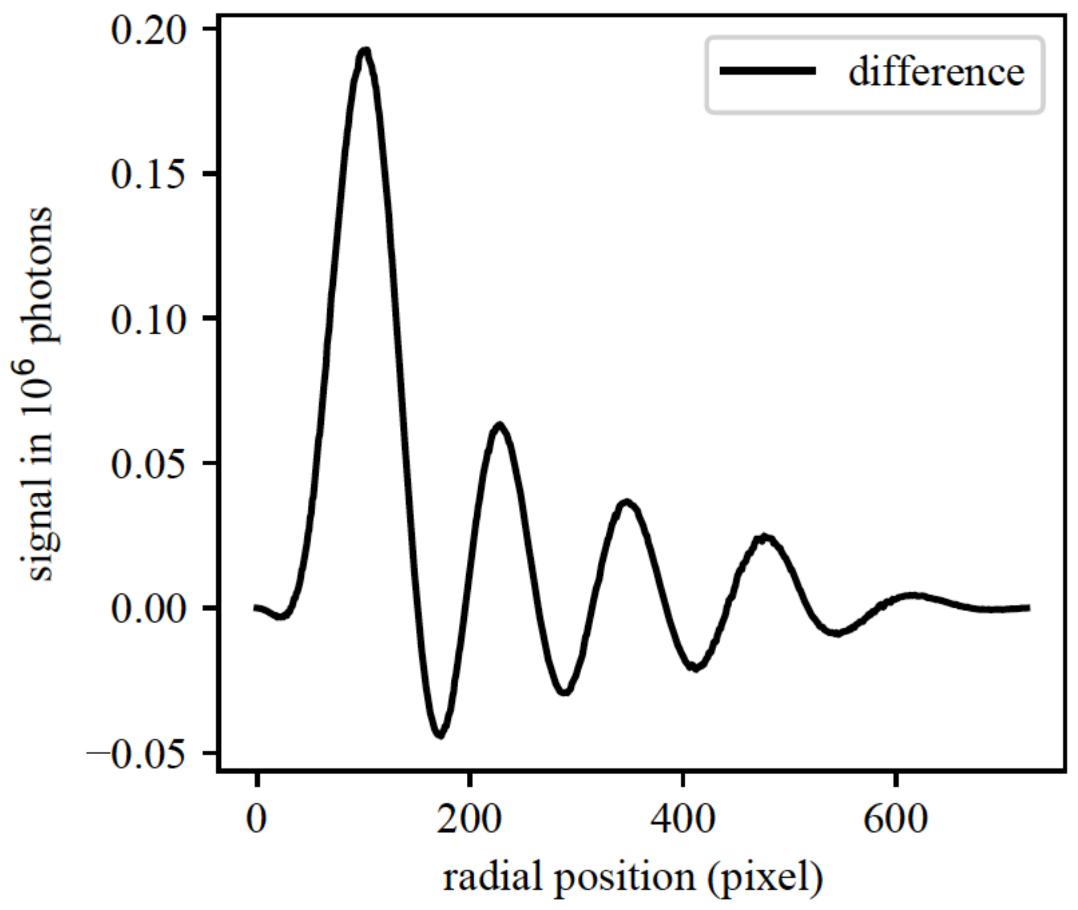


Excited sample (40.4 nm)



Difference image

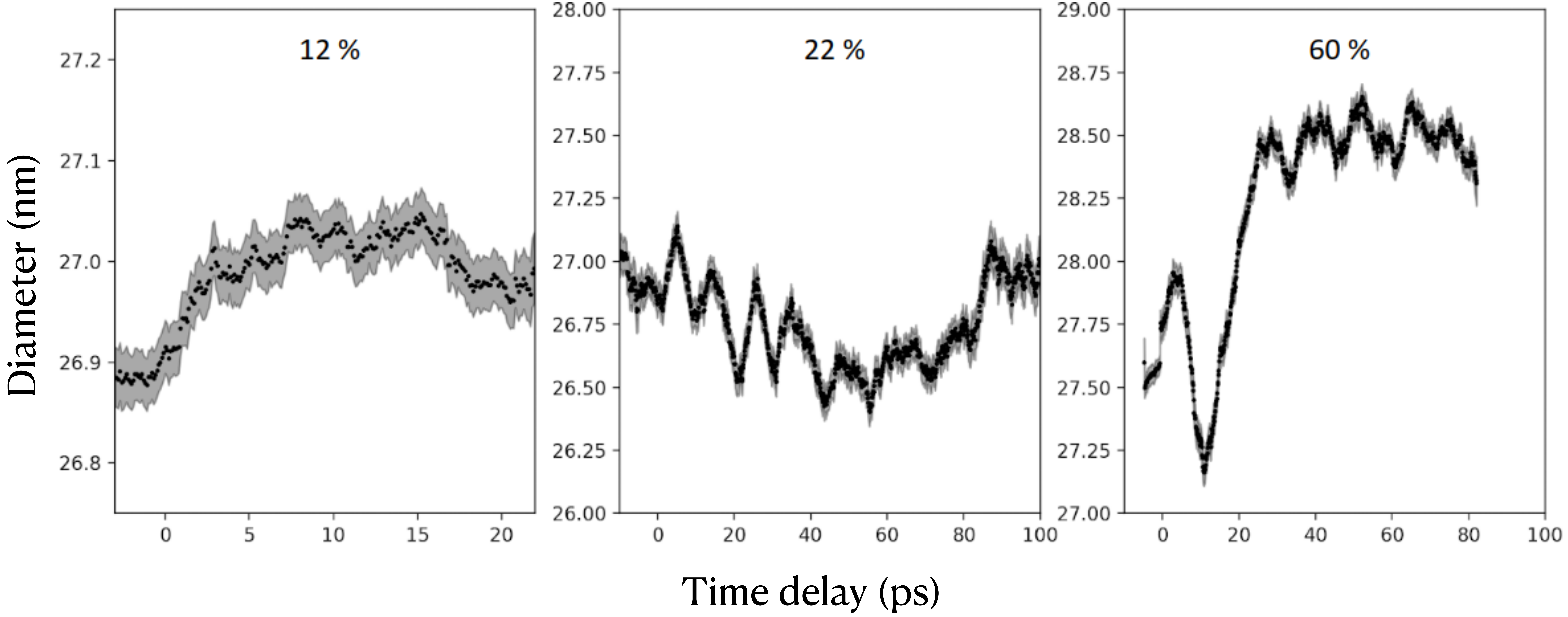




Goal: Record molecular movie by changing the pump-probe delay

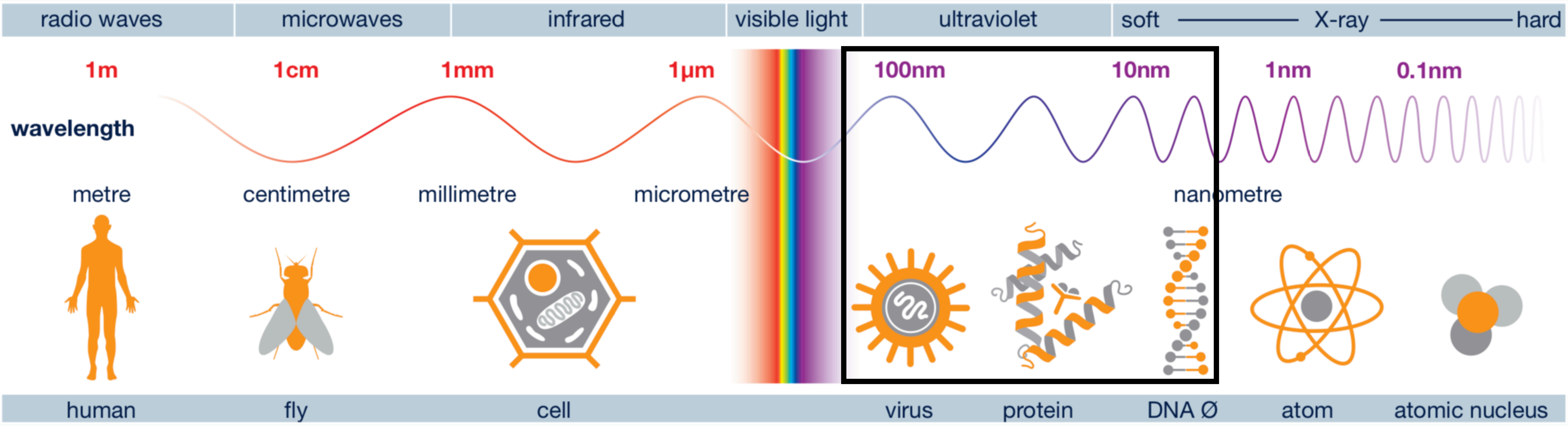
Recording structural dynamics of electronically excited gold nanoparticles

Direct imaging of this structural dynamics: **Optical-pump (400 nm) x-ray-probe (4.5 nm)**



At different pump laser intensity

ALS: towards single proteins

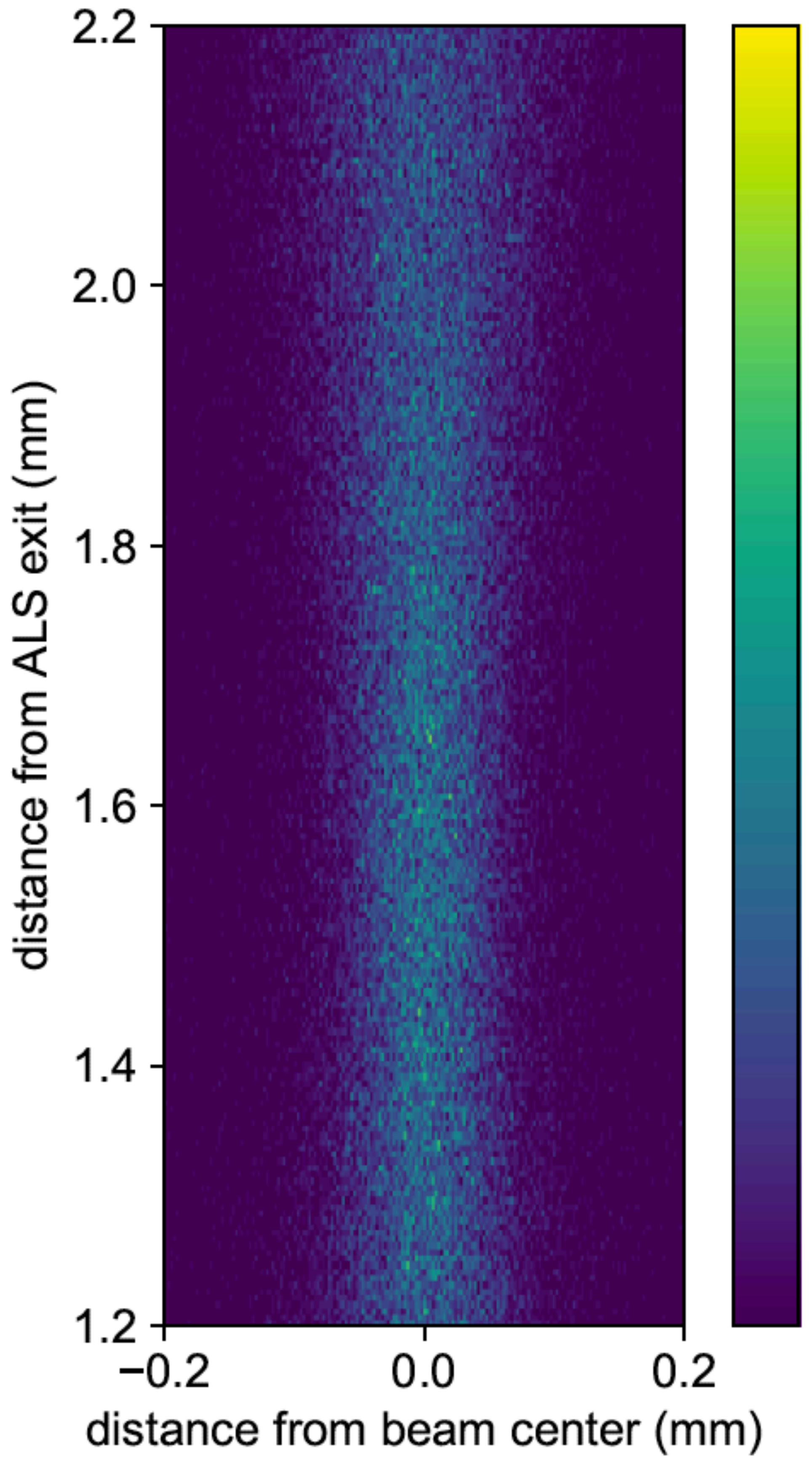


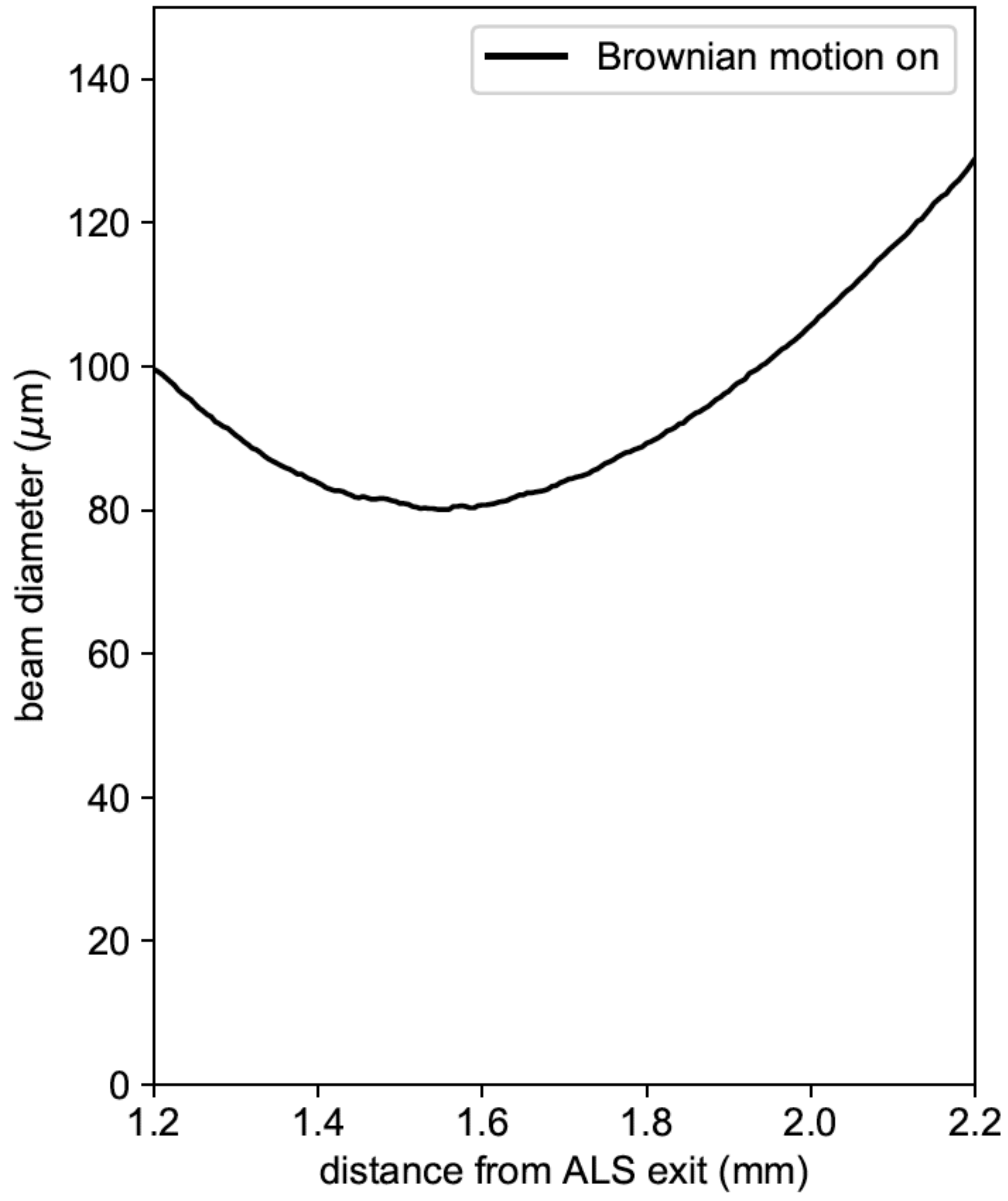
Top down approach

ALS: towards single proteins

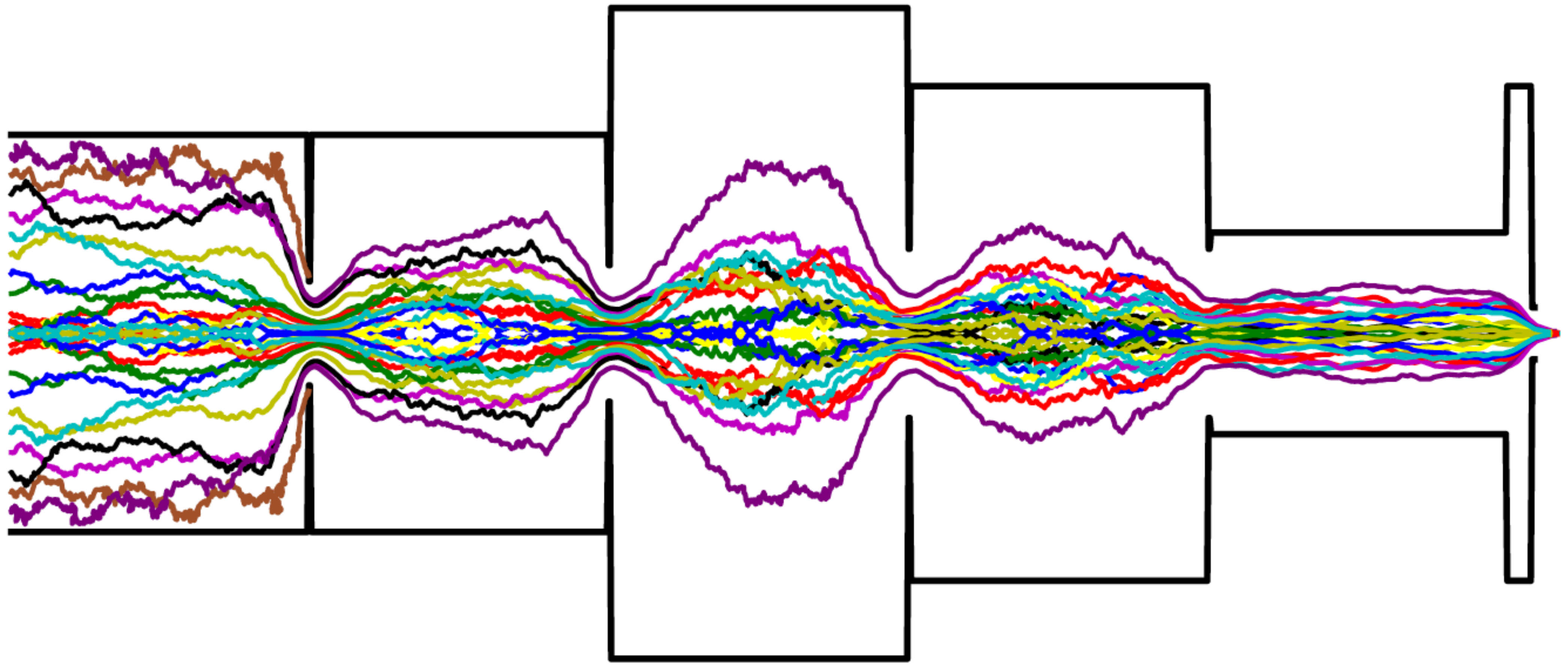
Focusing plot for 20 nm polystyrene spheres in optimised ALS

particle beam diameter ~ 80 um





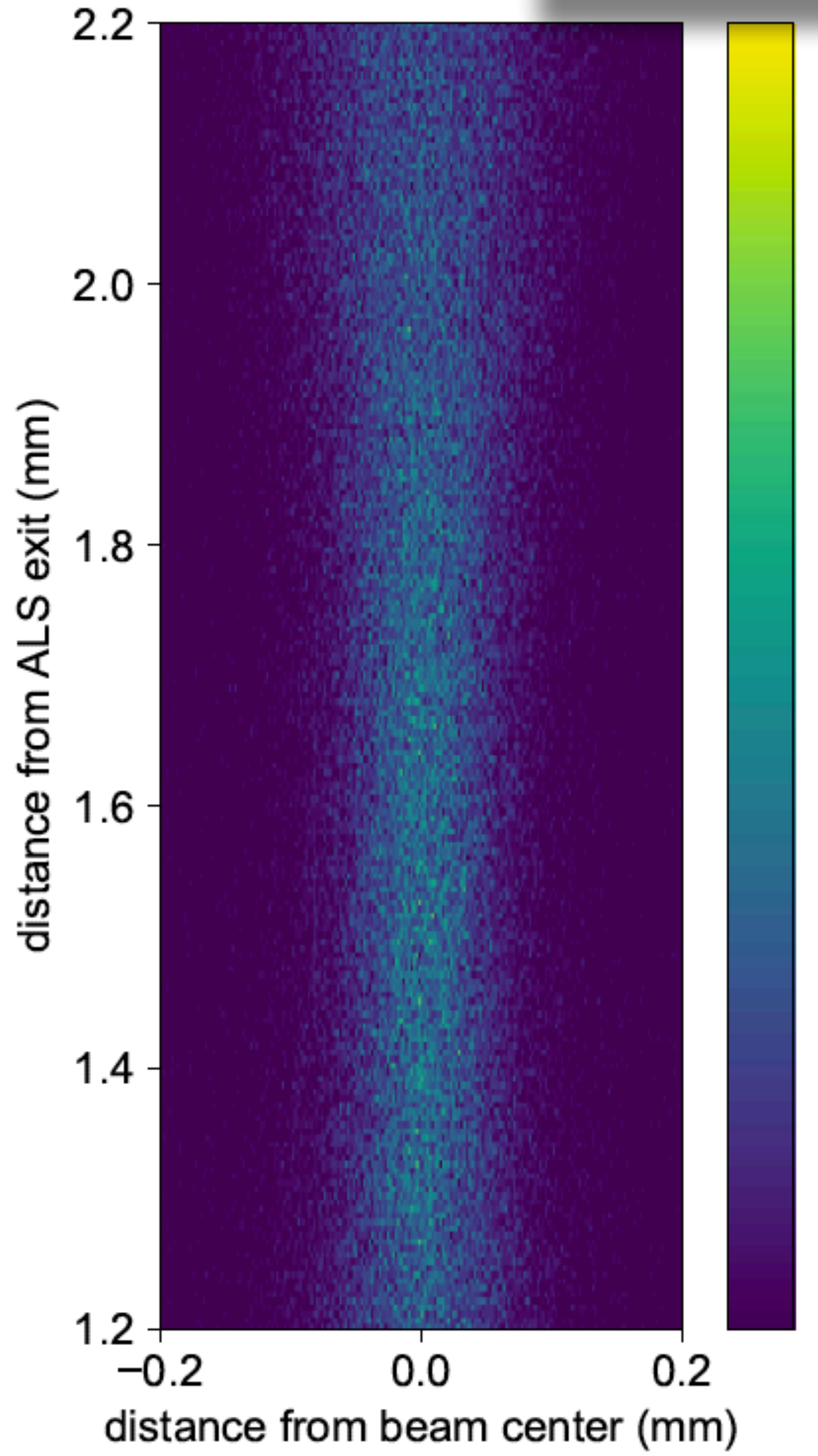
Brownian motion

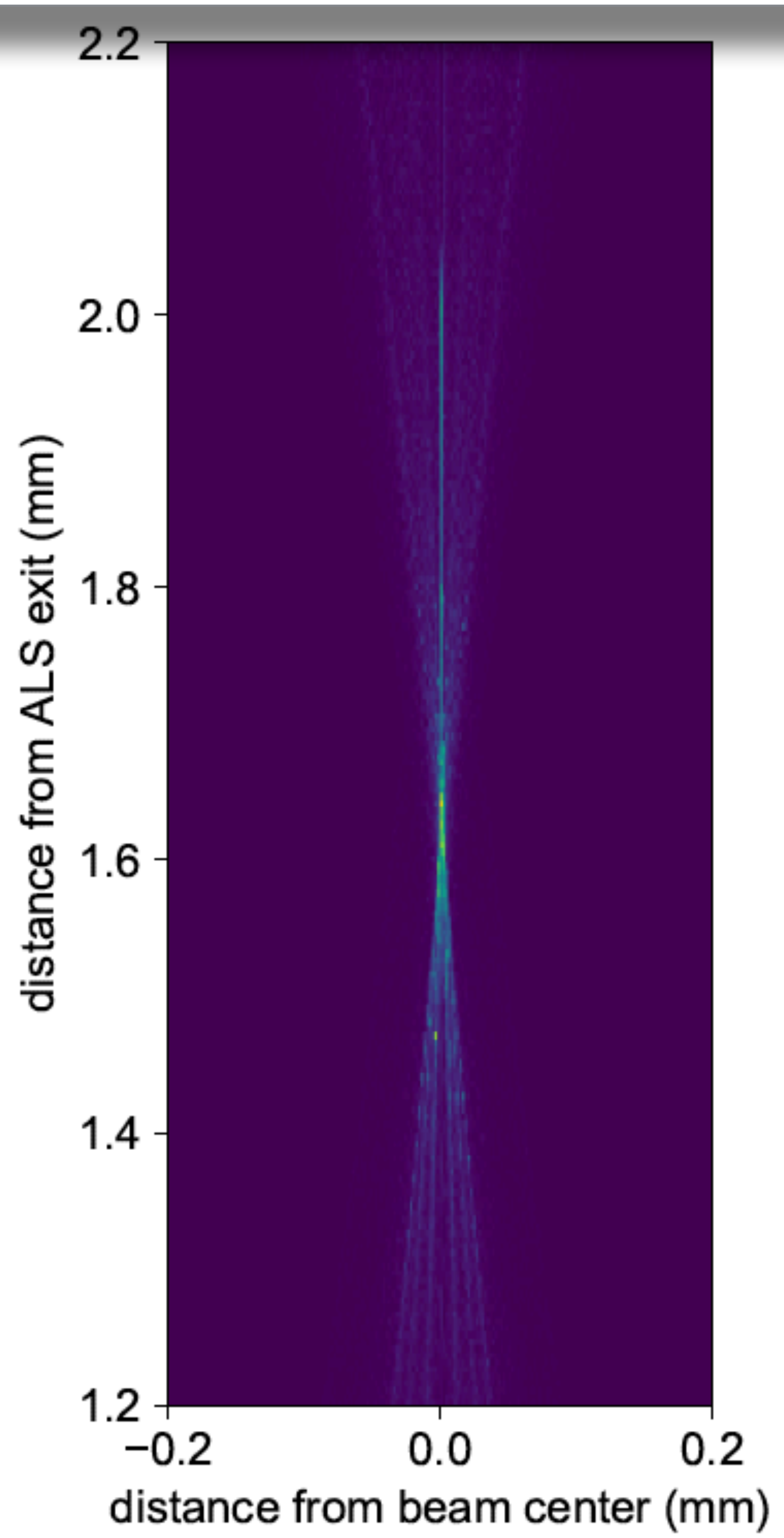


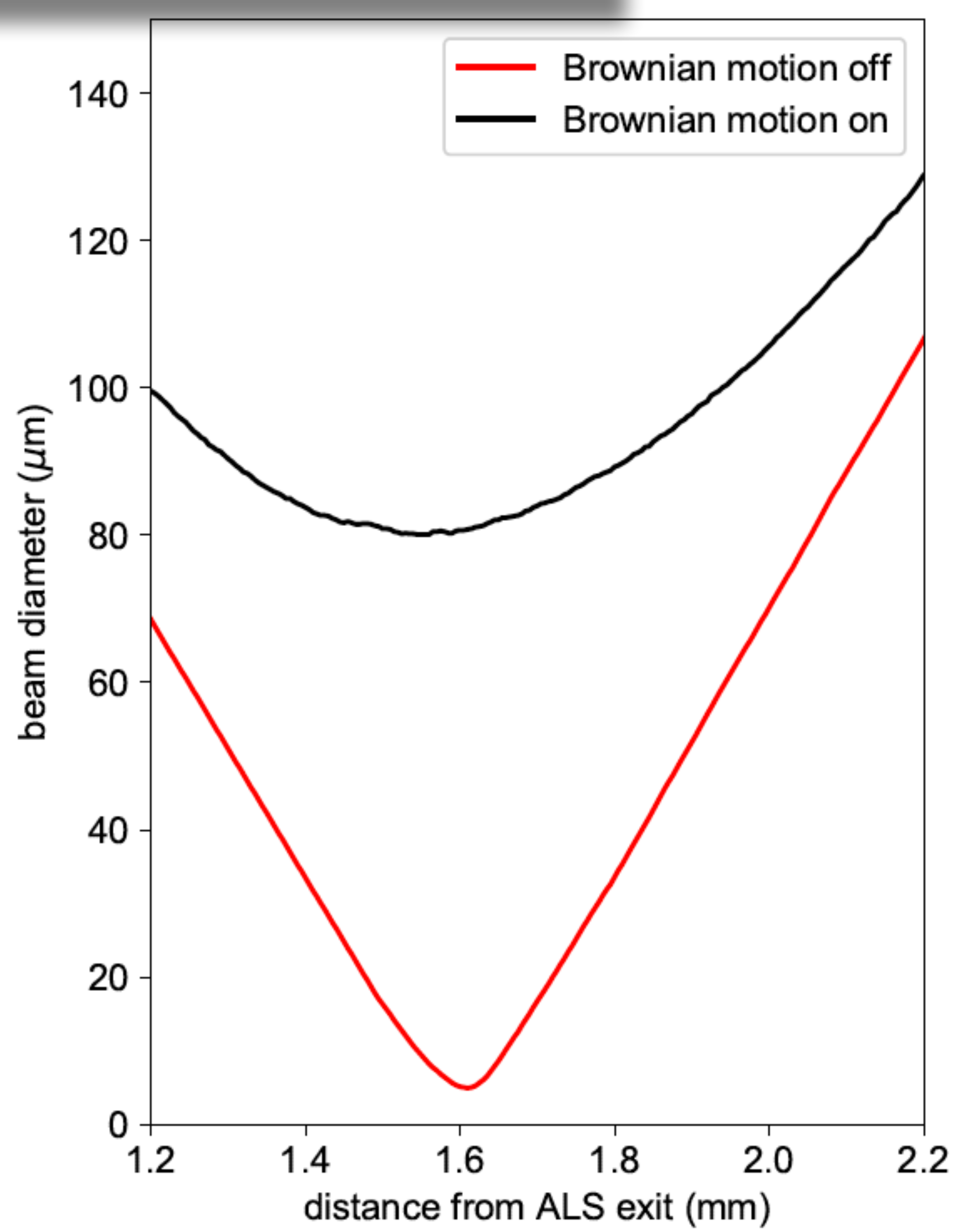
$$F_{\mathrm{b}} = m_{\mathrm{p}} \vec{G} \sqrt{\frac{\pi S_0}{\Delta t}} \quad \text{with} \quad S_0 = \frac{216 \mu k T}{\pi^2 d_{\mathrm{p}}^5 \rho_{\mathrm{p}}^2 C_{\mathrm{c}}}$$

Problem of focussing small particles, effect of Brownian motion

Focusing plot for 20 nm polystyrene spheres in optimised ALS with and without Brownian motion



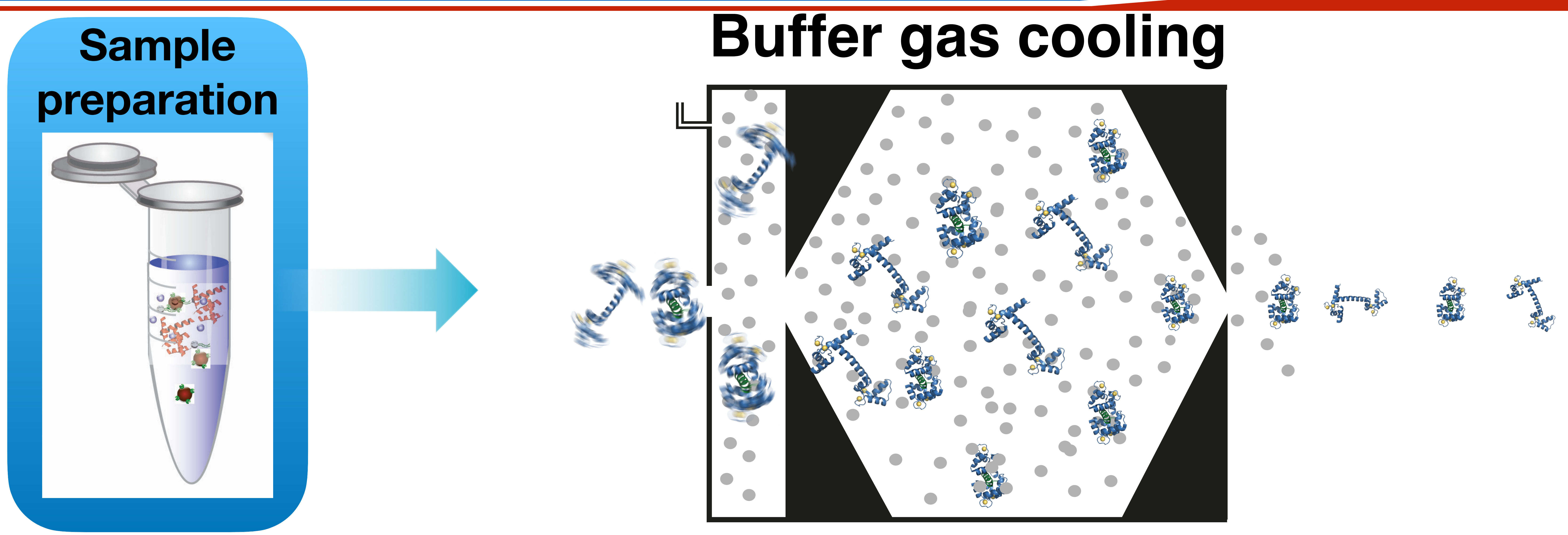




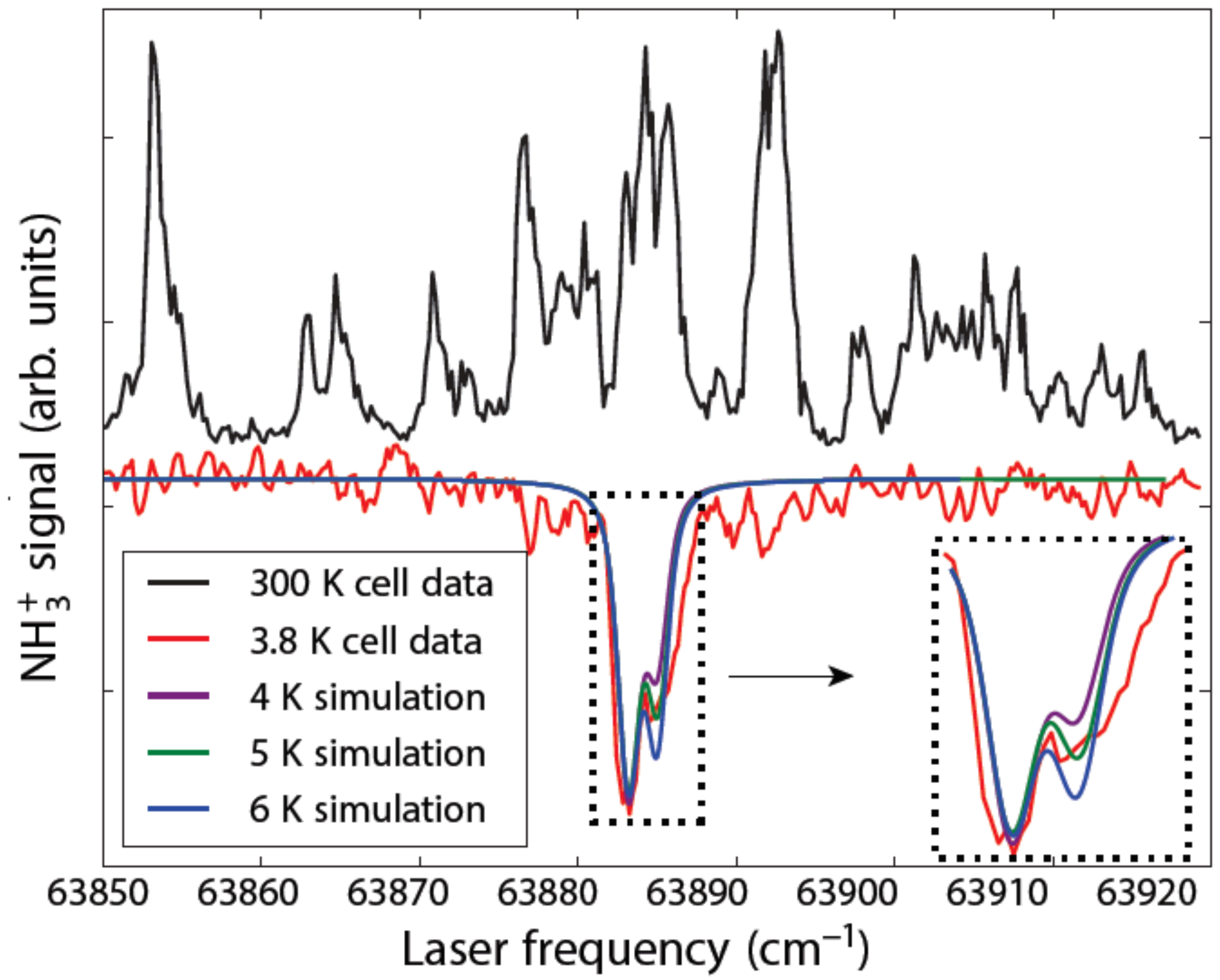
How we can reduce the Brownian motion??

Cryogenic buffer gas cooling

Cryogenic buffer gas cooling



Temperature Characterization
Multiphoton ionisation of NH_3

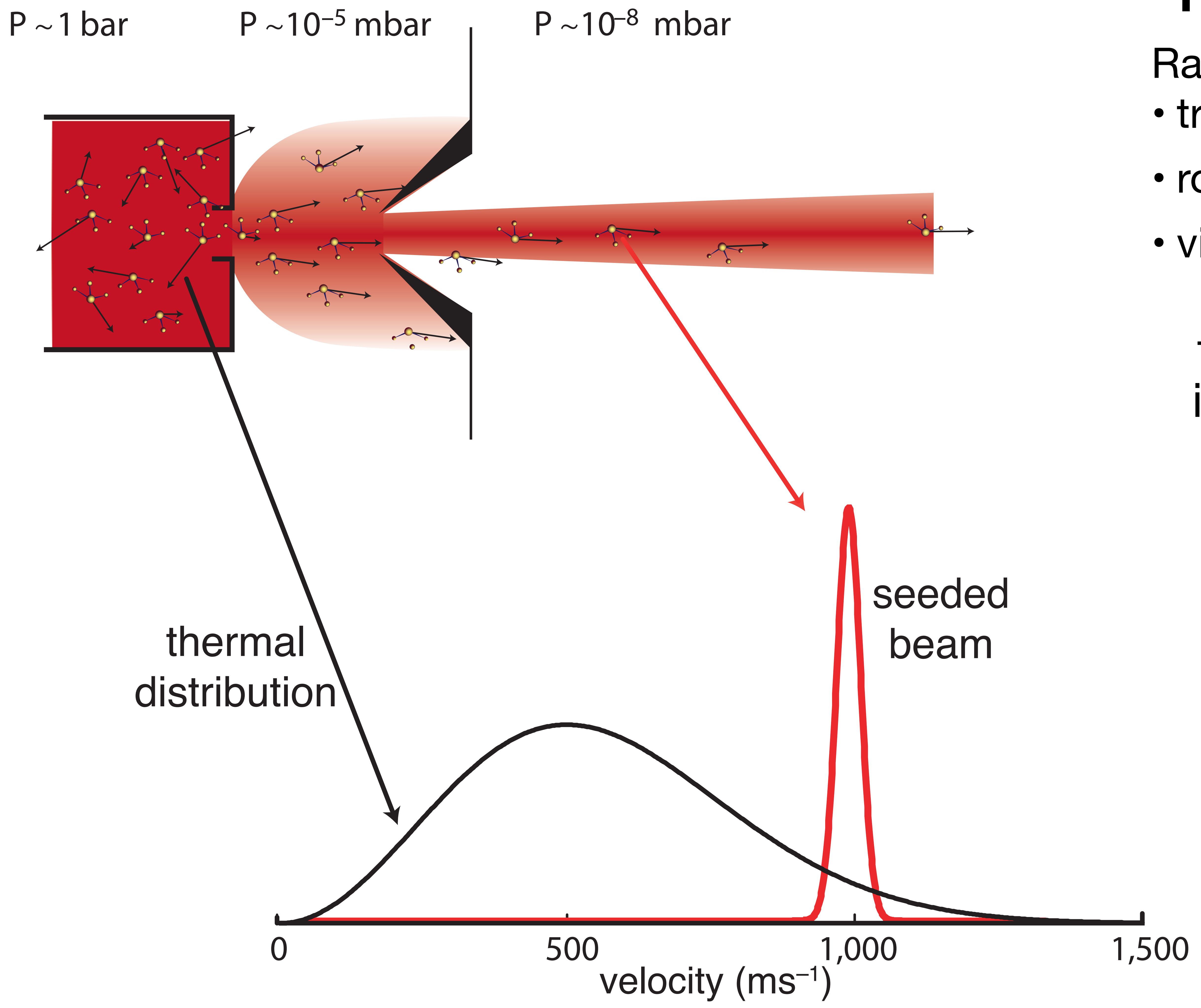


Buffer gas cooling of bio-nanoparticles:

1. Fast freezing of nanoparticles preserving their native state.
2. Better utilisation of control techniques
    - Better hydrodynamic focusing/beam formation (high density)
    - Conformer seperation/electrostatic deflection techniques
    - Laser-induced alignment
3. Time resolved measurements,
    - Better starting point!
    - Trapping reaction intermediates!

Singh, Samanta, Roth, Gusa, Ossenbrüggen, Rubinsky, Horke, Küpper, *Phys. Rev.* A **97**, 032704 (2018)

Similarity to supersonic expansion molecular beams



Properties of supersonic expansions

Rapid cooling of:
- translation ← directly cooled through collisions in the expansion
- rotation ← cooled through coupling to the translations
- vibration ← cooled through coupling to the translations

→Convert random thermal energy into directed (forward) motion

for ideal gas: $$v_{\max} = \sqrt{\frac{5RT_0}{m}}$$

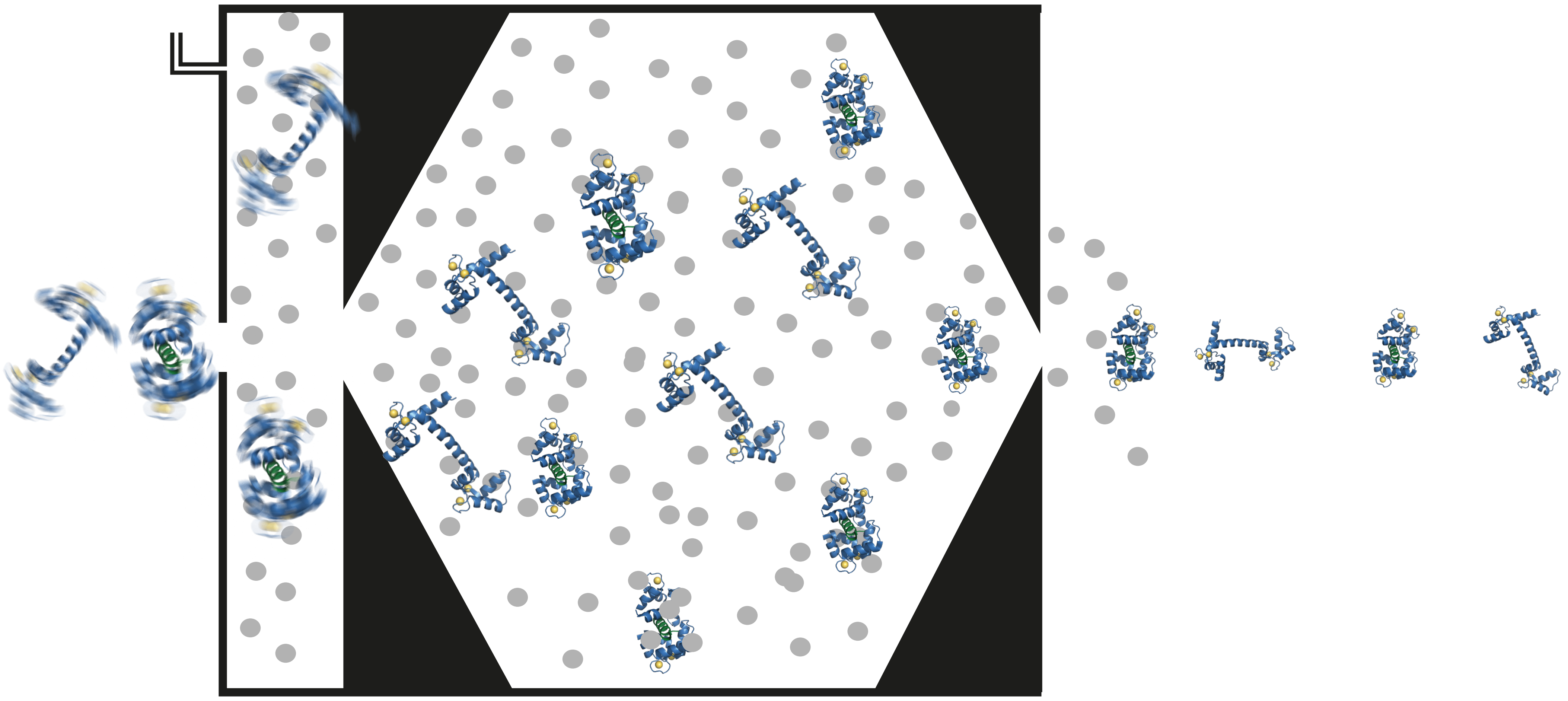
Typical achieved temperatures:
- translation ~ 0.1 K
- rotation ~ 1 K
- vibration < 50 K

tells you something about typical coupling strengths…

e.g., van de Meerakker, Bethlem, Meijer, *Nature Phys*. **4**, 595 (2008)

Similarity to supersonic expansion molecular beams

Buffer gas cooling



Rapid cooling of:
- translation
- rotation
- vibration

directly cooled through collisions in the expansion

cooled through coupling to the translations

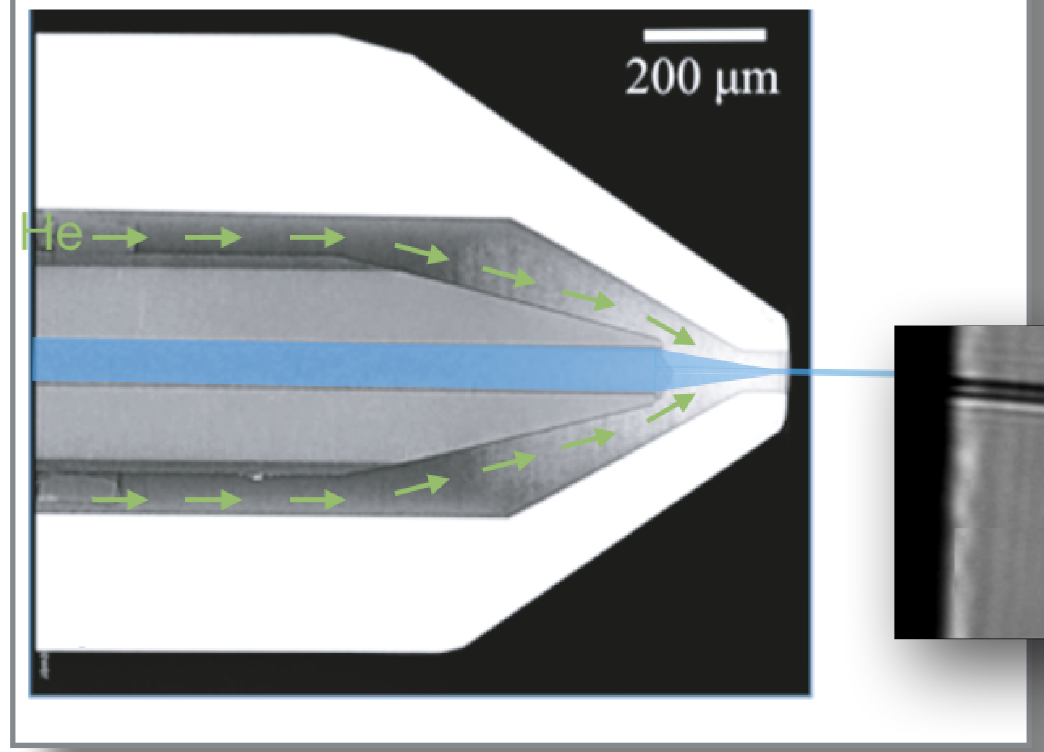
<u>Typical achieved temperatures:</u>
<u>(temperature of helium, 4 K)</u>
- translation ~ 4 K
- rotation ~ 4 K
- vibration ~ 4 K

main factor cell length (number of effective collision)

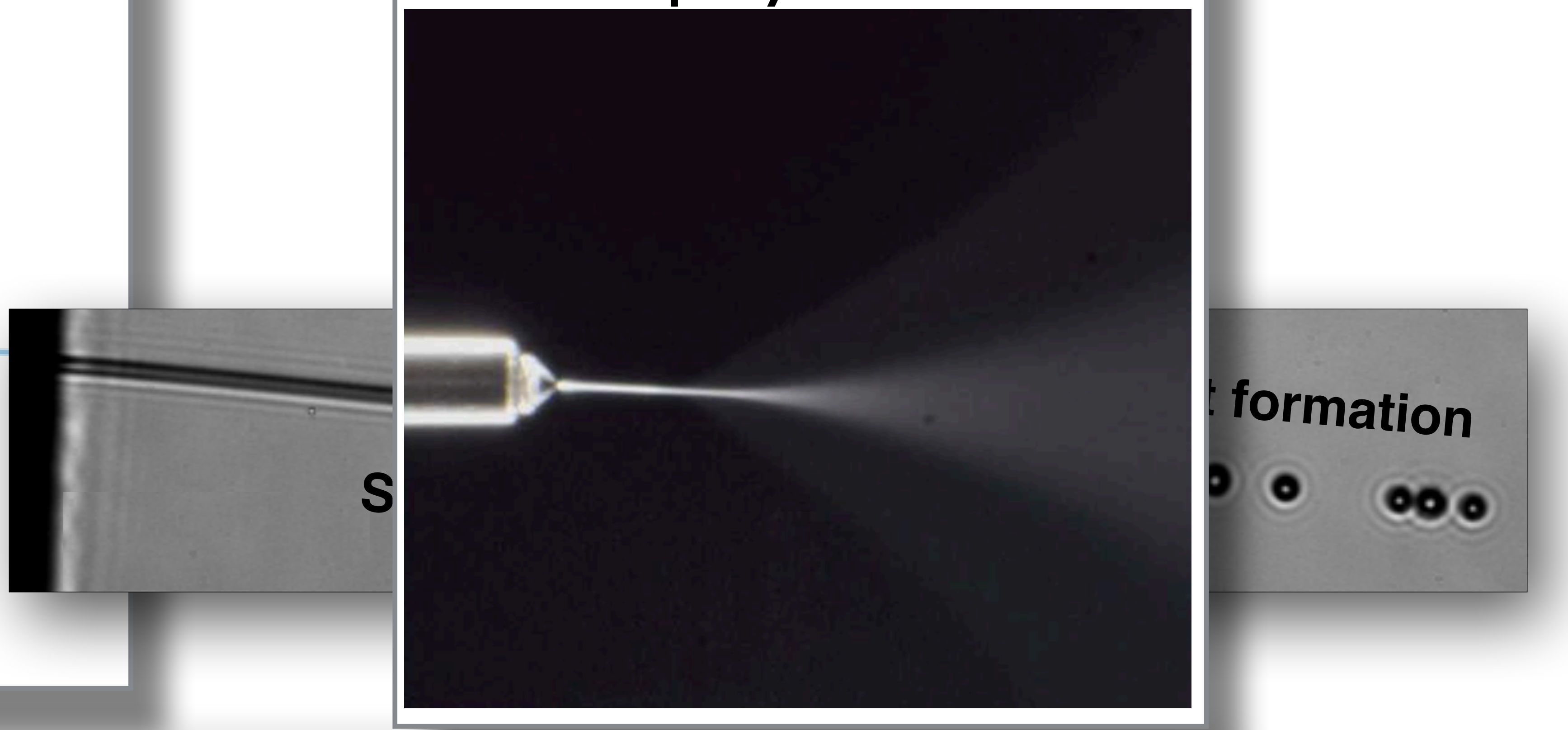
e.g., van de Meerakker, Bethlem, Meijer, *Nature Phys*. **4**, 595 (2008)

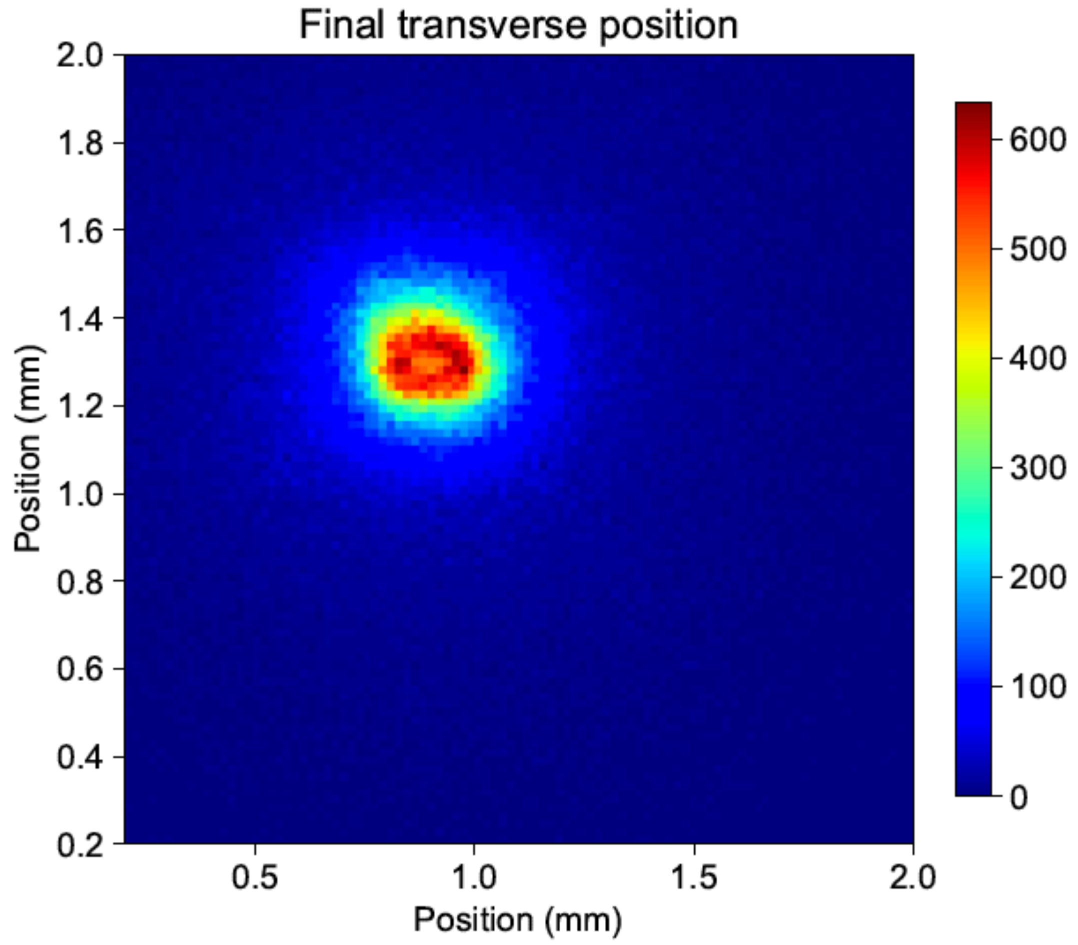
Cryogenically shock-frozen focused and selected bio-nano-particles
Experimental setup

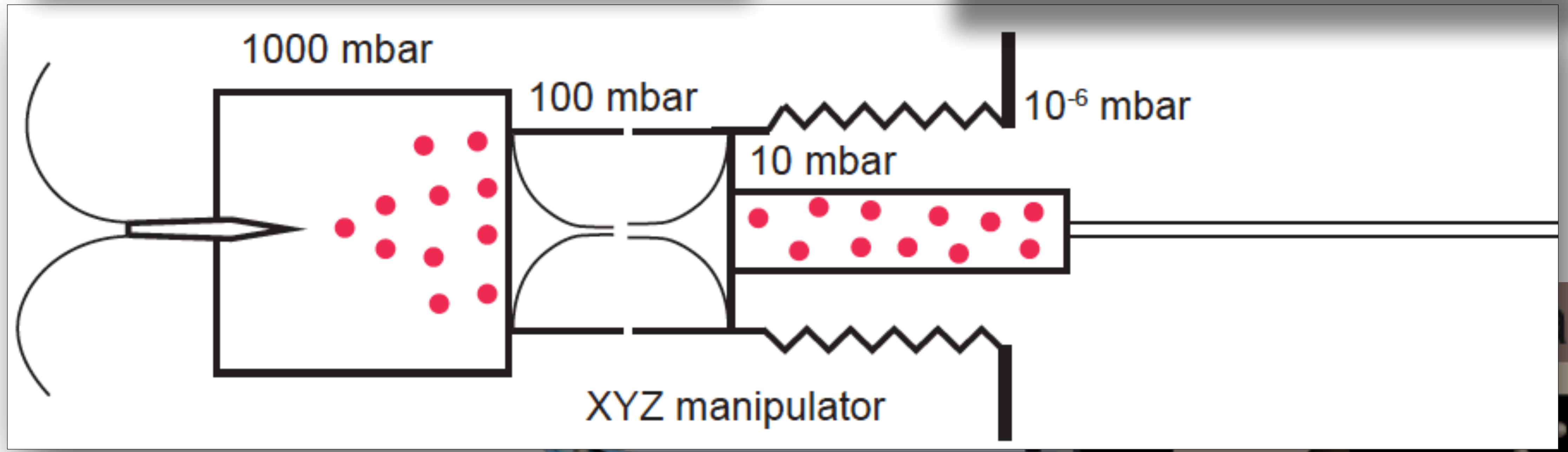
Gas-dynamics virtual nozzle



Electrospray

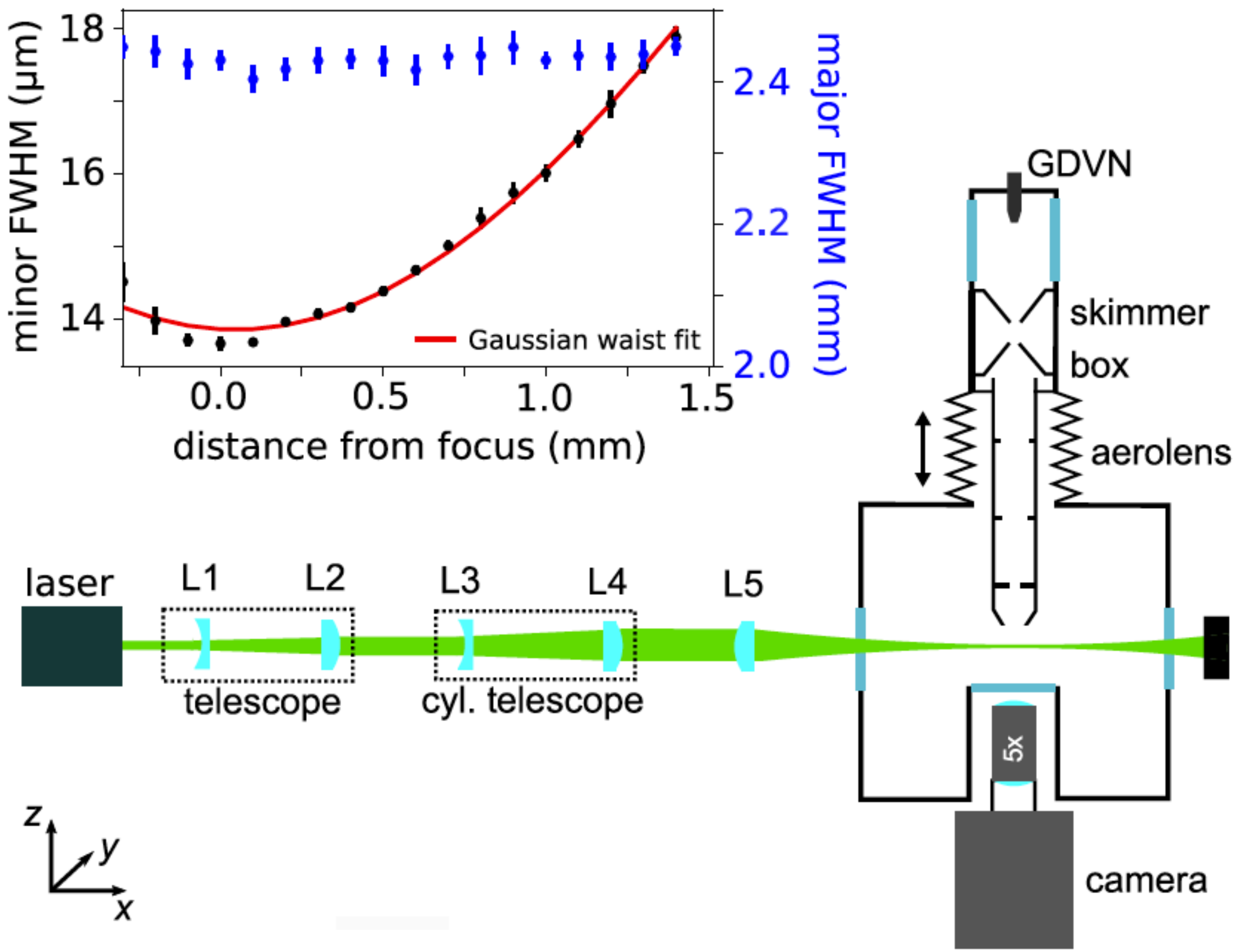






Samanta, Amin, Estillore, Roth, Worbs, Horke, Küpper, arXiv:1910.12606 [physics.bio-ph]
Worbs, Lübke, Roth, Samanta, Horke, Küpper, *Opt. Express* **27**, 36580-36586 (2019) arXiv:1909.08922 [physics.optics]

Light-sheet imaging for the recording of transverse absolute density distributions of gas-phase particle-beams from nanoparticle injectors



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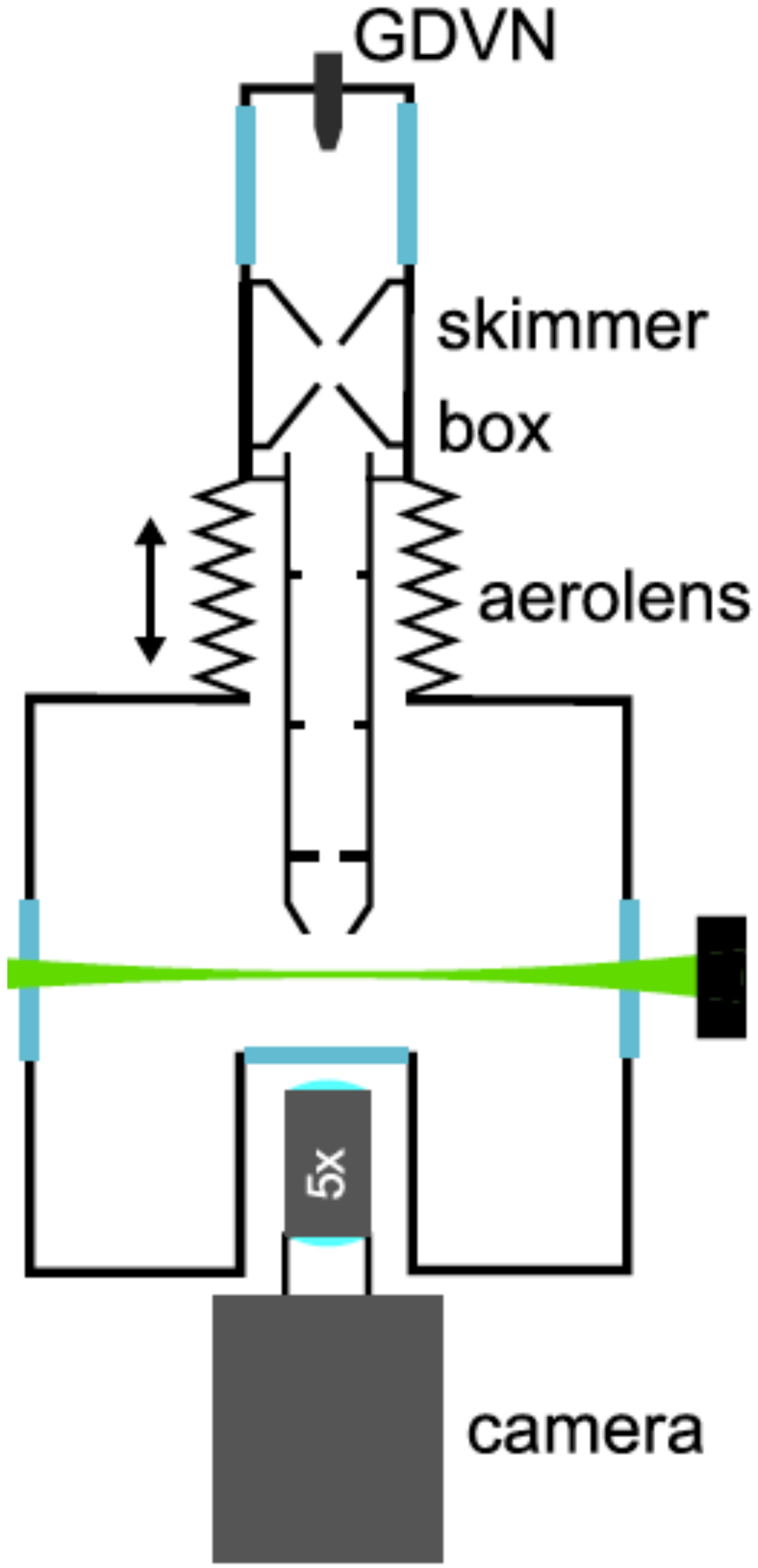

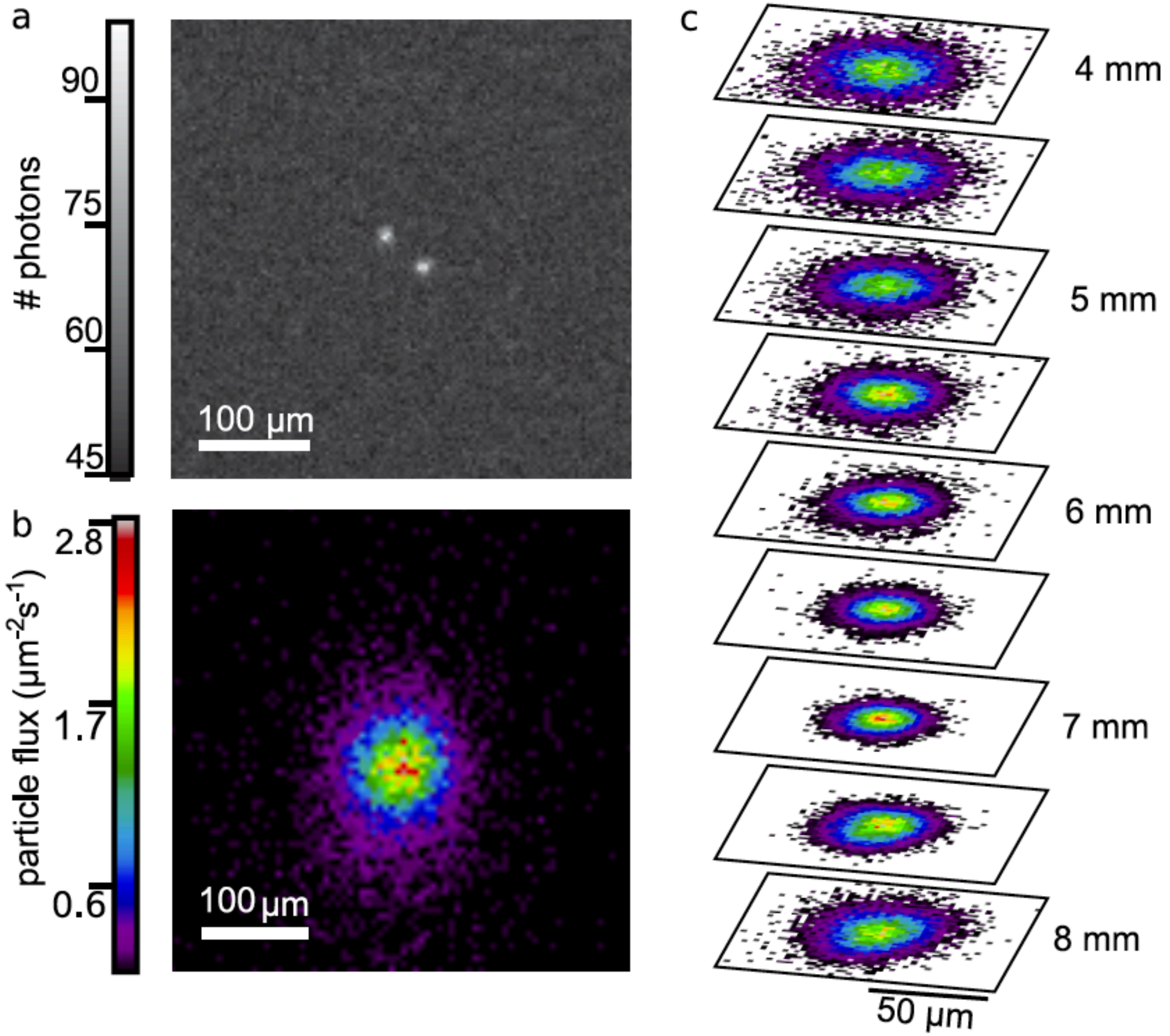

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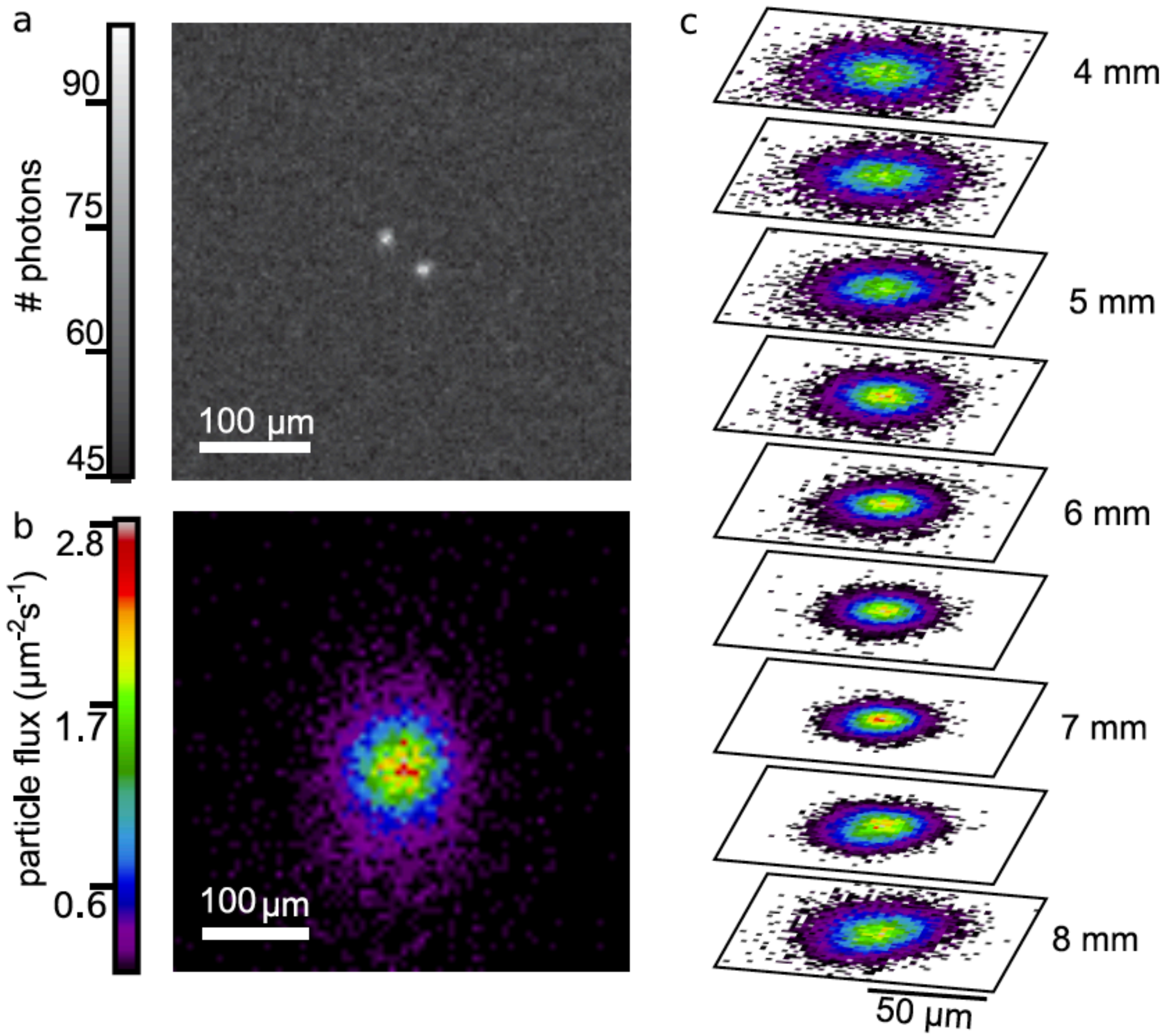
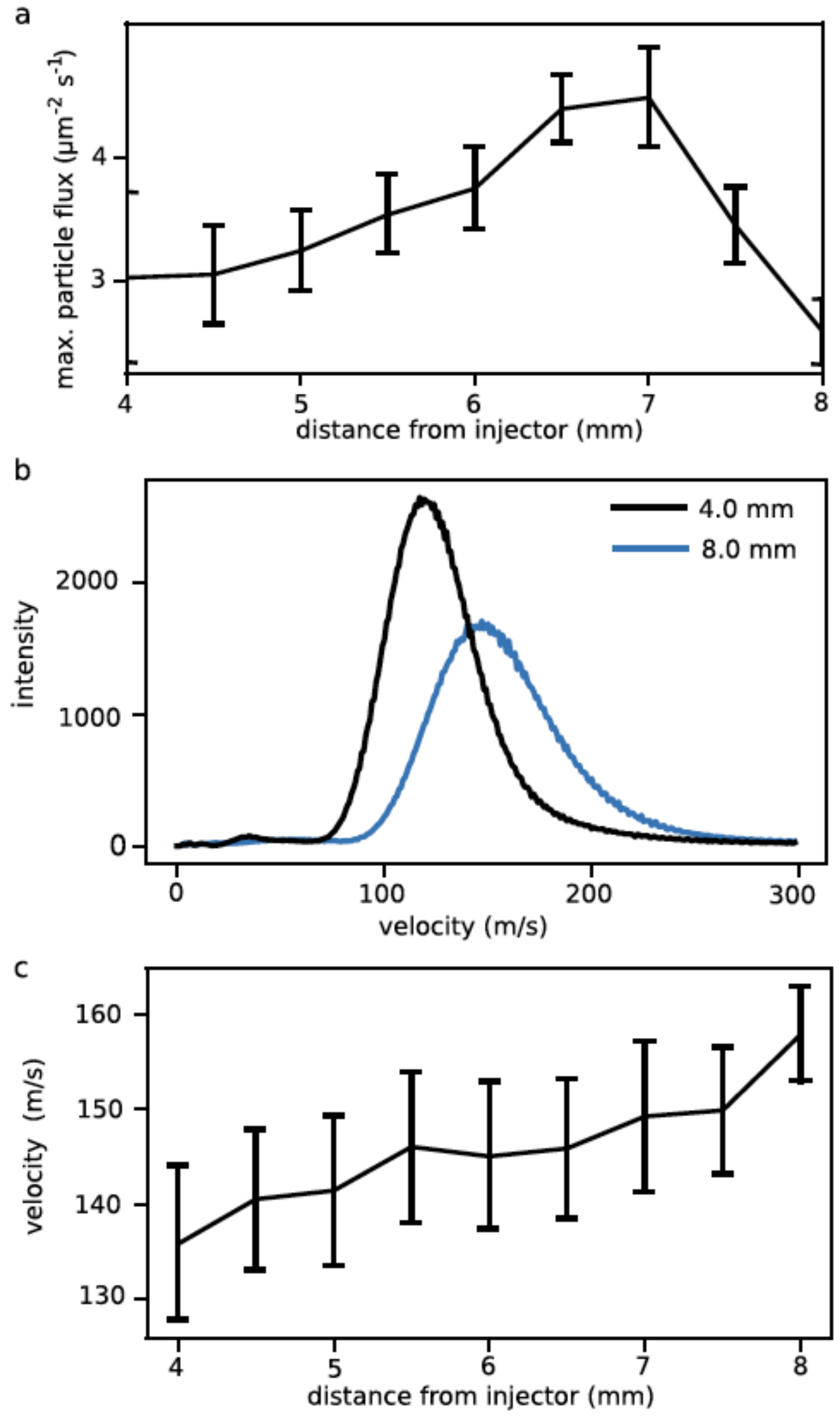
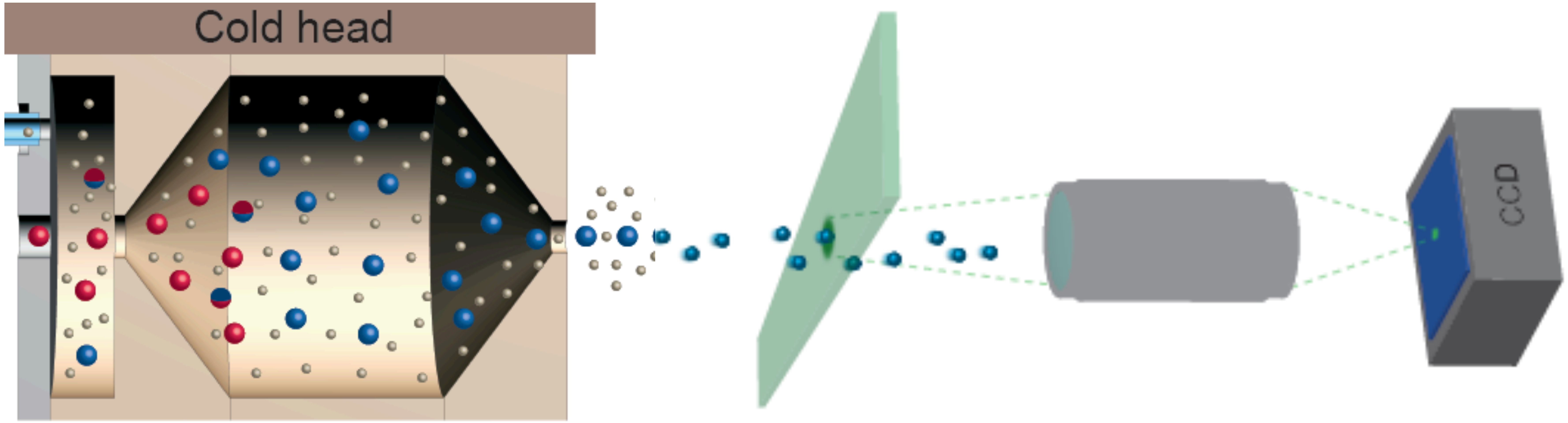




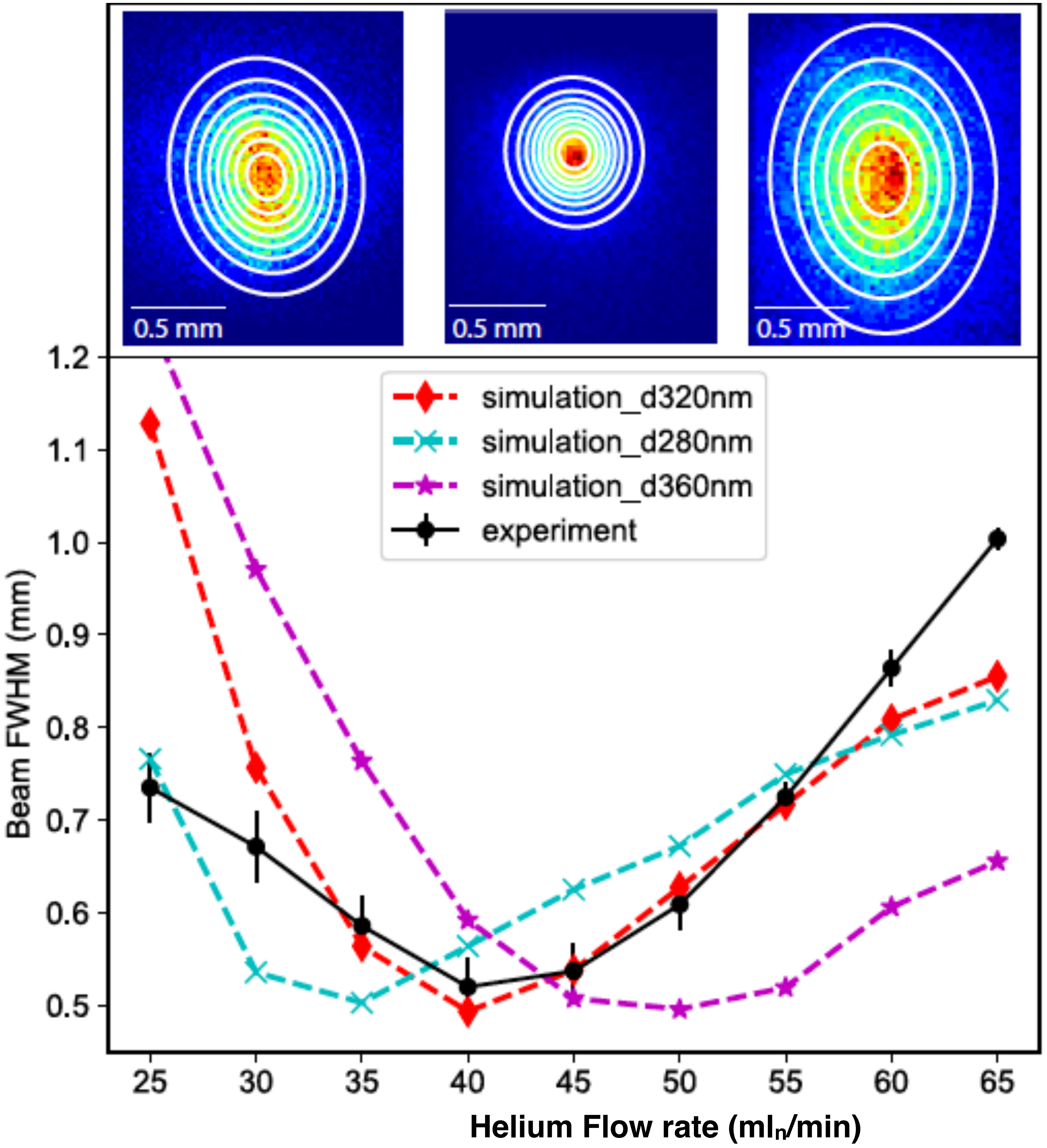
Worbs, Lübke, Roth, Samanta, Horke, Küpper, *Opt. Express* **27**, 36580-36586 (2019) arXiv:1909.08922 [physics.optics]

Cryogenically shock-frozen, focused, and selected bio-nano-particles — polystyrene spheres —



Samanta, Amin, Estillore, Roth, Worbs, Horke, Küpper, arXiv:1910.12606 [physics.bio-ph]

Cryogenically shock-frozen focused and selected bio-nano-particles Granulovirus occlusion body

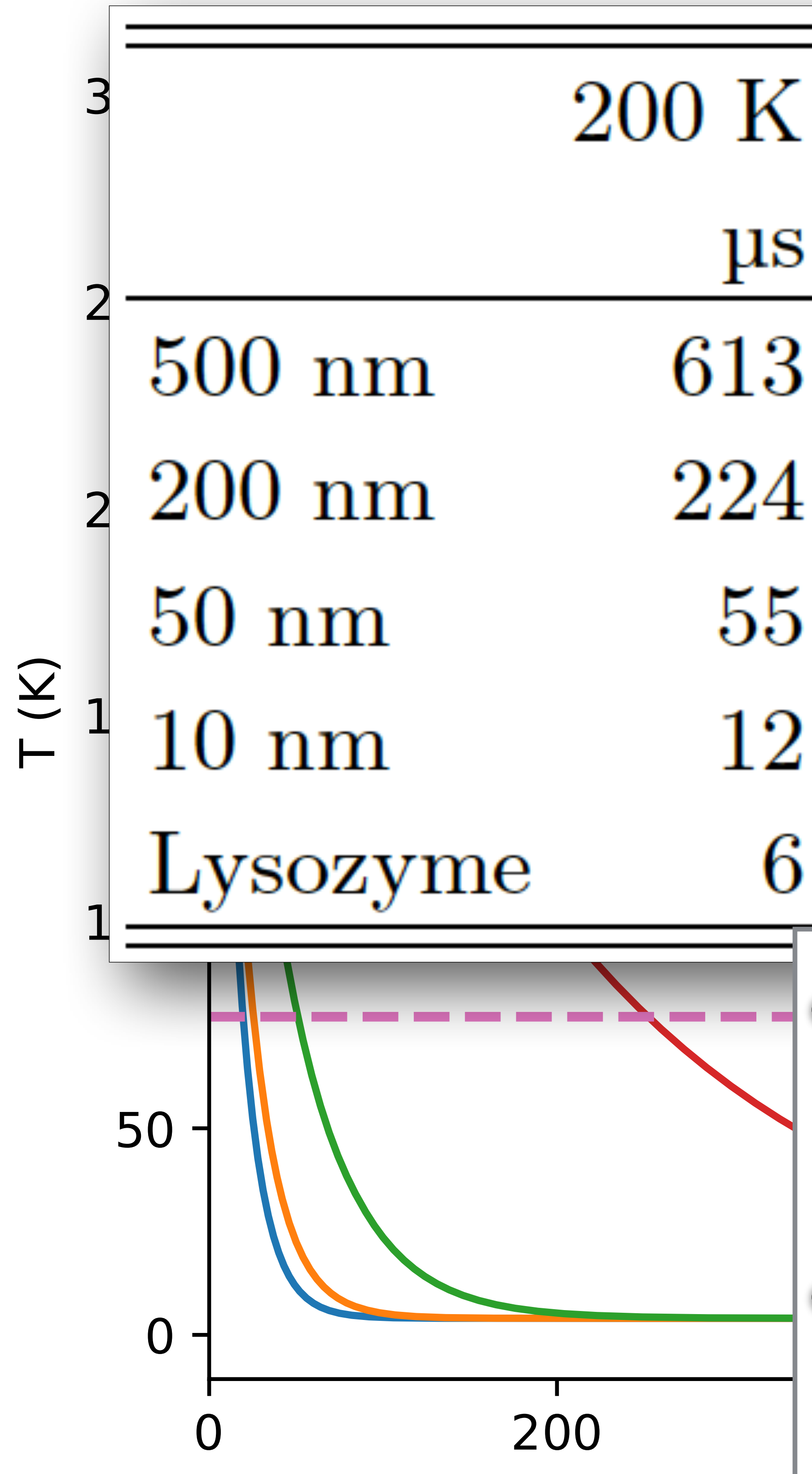


265 nm × 265 nm × 445 nm

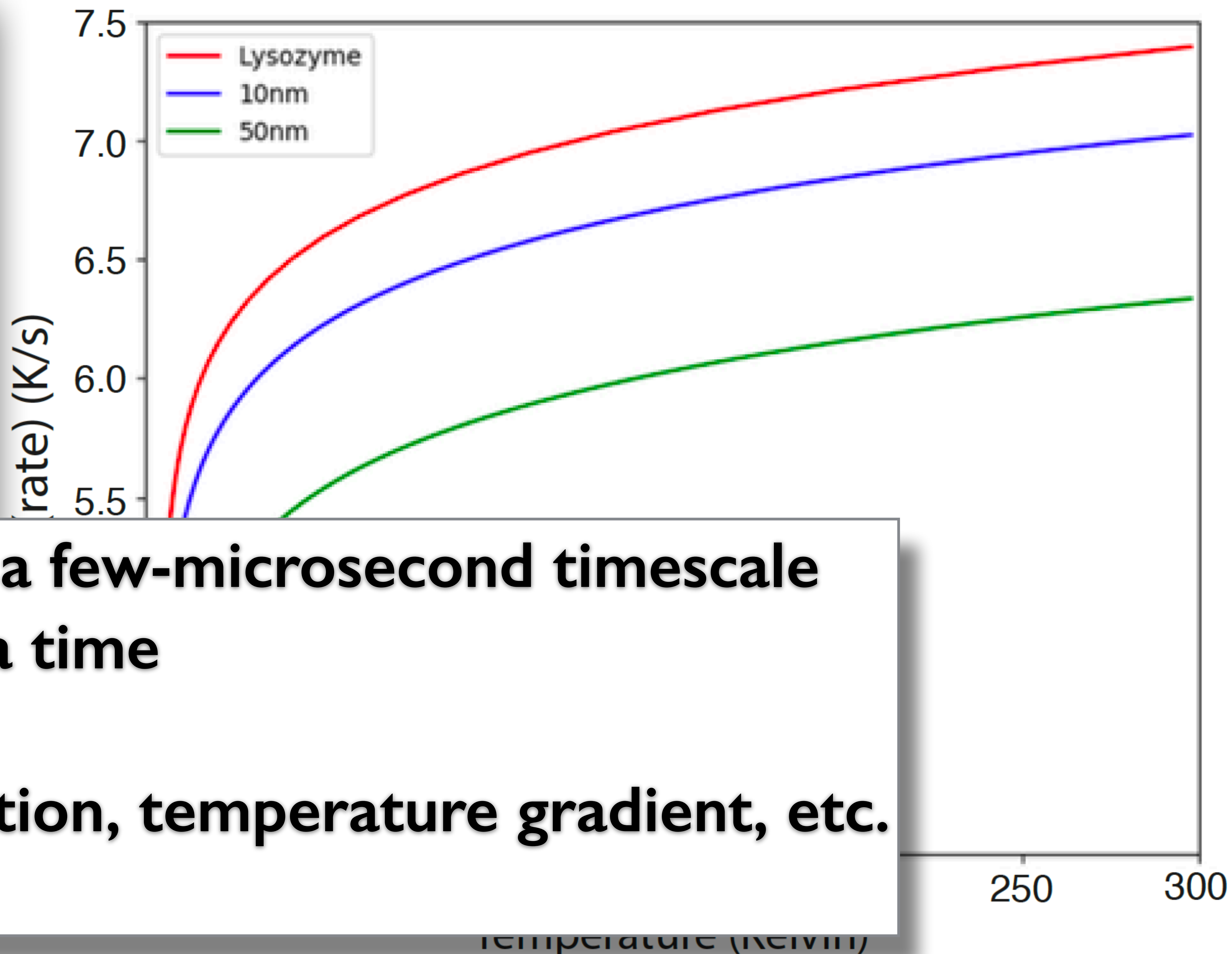
Analysis yields hydrodynamic diameter of 320±20 nm

Cryogenically shock-frozen focused and selected bio-nano-particles
Cooling rates

Newton's law of cooling: $T_t = T_{\mathrm{He}} + (T_0 - T_{\mathrm{He}})e^{-hA/C}$ with $\begin{cases} A \text{ area} \\ C \text{ heat capacity} \end{cases}$



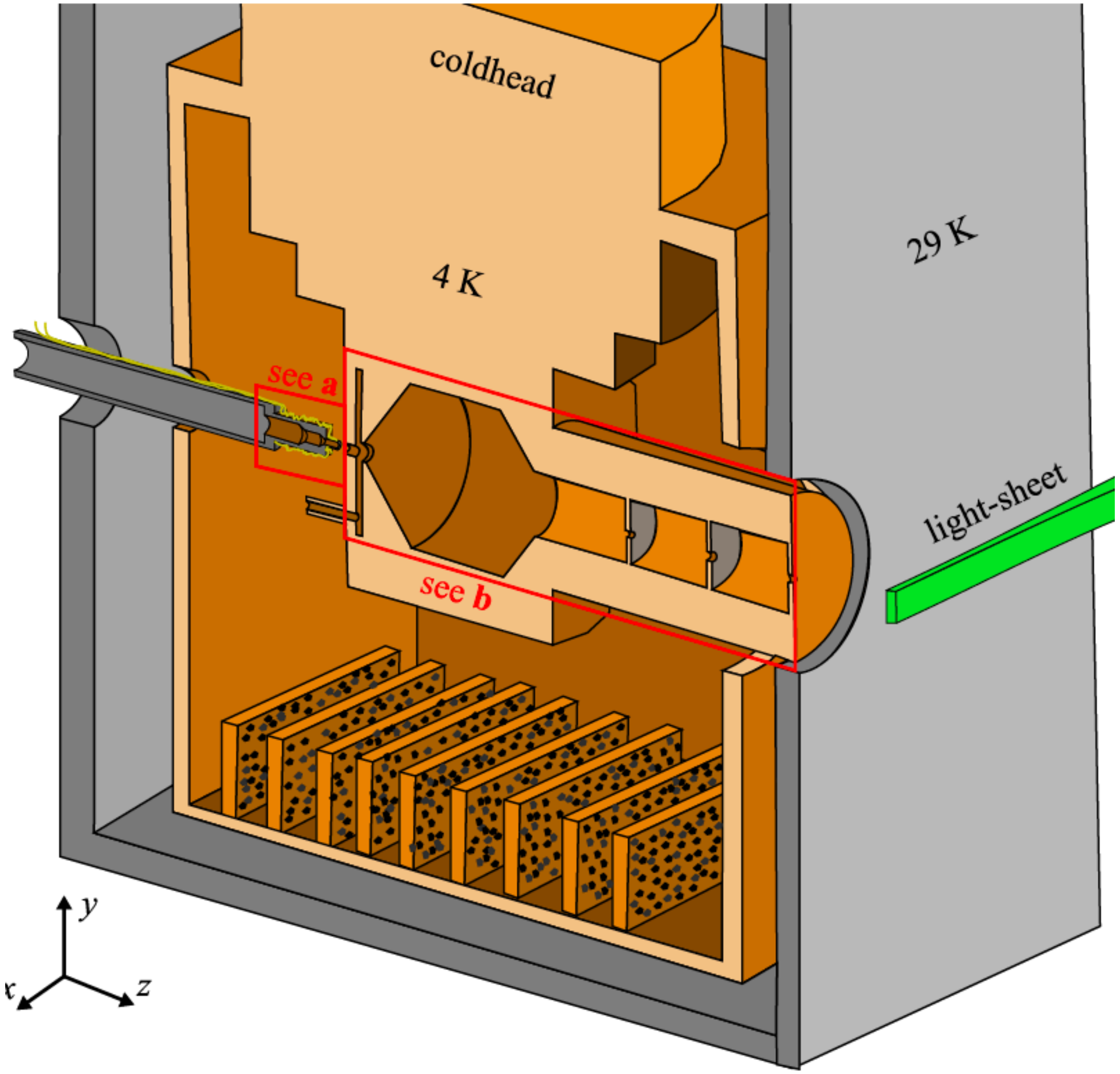
	200 K µs	133 K µs	77 K µs	10 K µs	Cooling-Rate (K/s)
500 nm	613	1409	2467	12000	1.8×10^5
200 nm	224	476	821	3007	4.9×10^5
50 nm	55	110	185	539	2.2×10^6
10 nm	12	23	37	103	1.1×10^7
Lysozyme	6	10	16	40	2.6×10^7

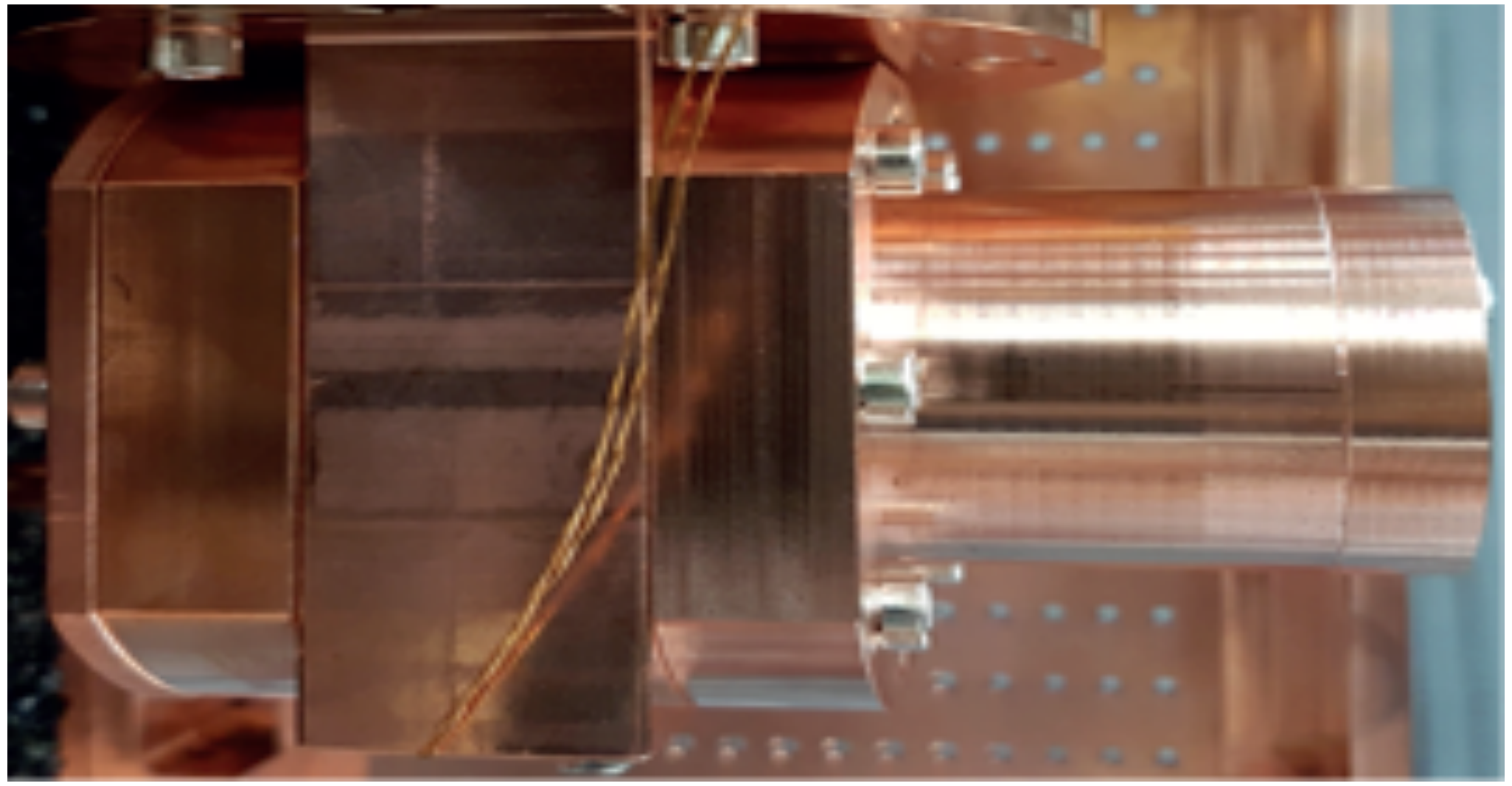


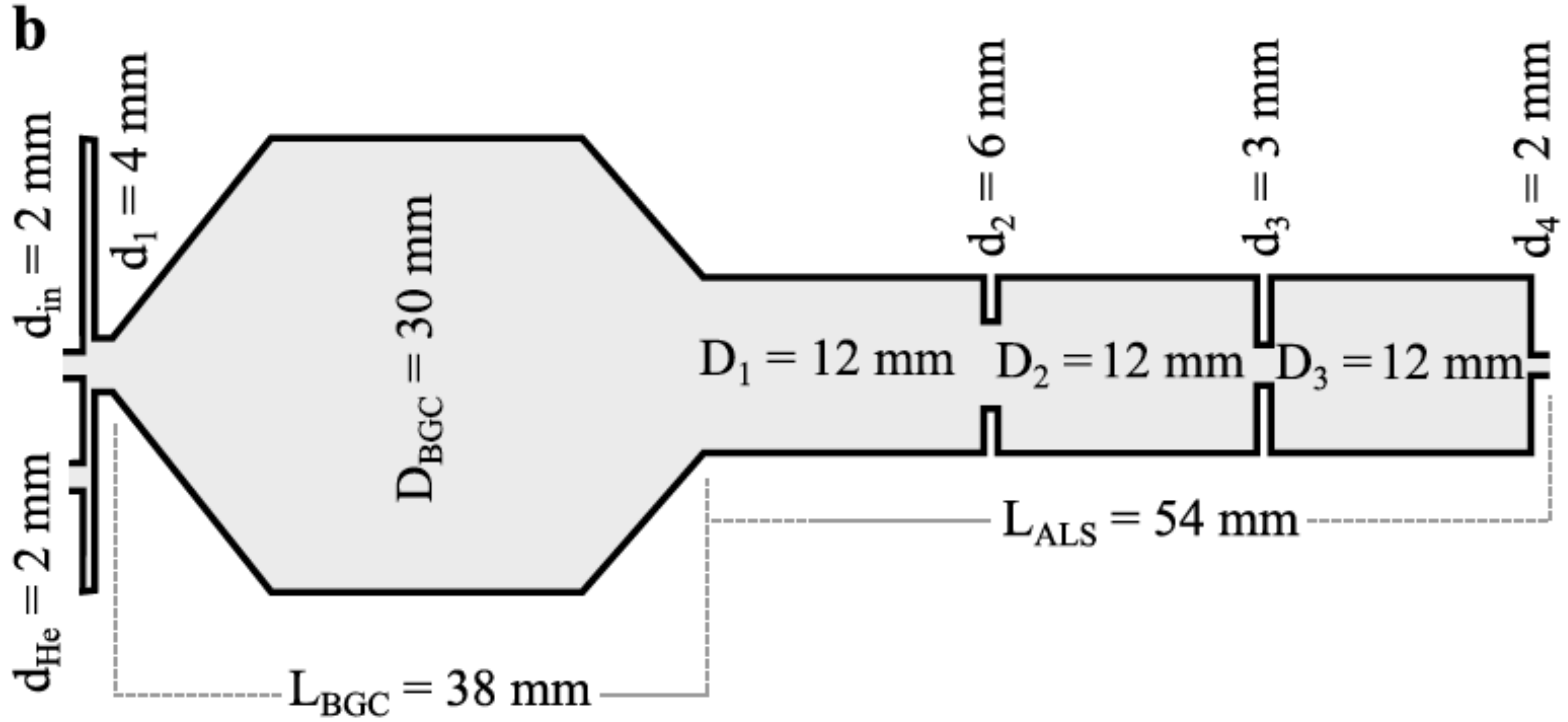
- **Shock freezing occurs on a few-microsecond timescale**
  - **every single particle at a time**
- **No external parameters:**
  - **layer thickness, distribution, temperature gradient, etc. cf. plunge freezing**

cf. Shoemaker, Ando, *Biochemistry* **57**, 277 (2018)

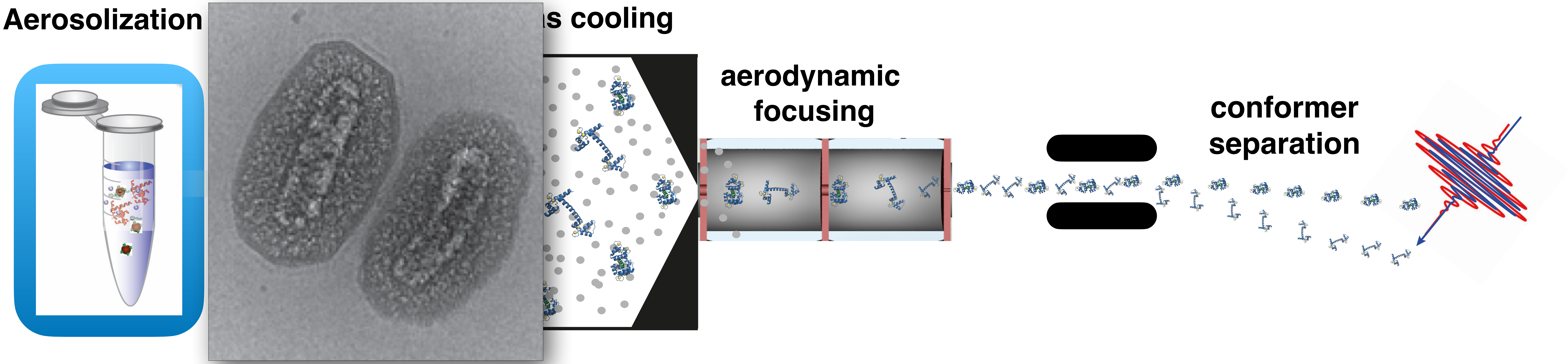
Optimizing the shape of the buffer-gas-cell

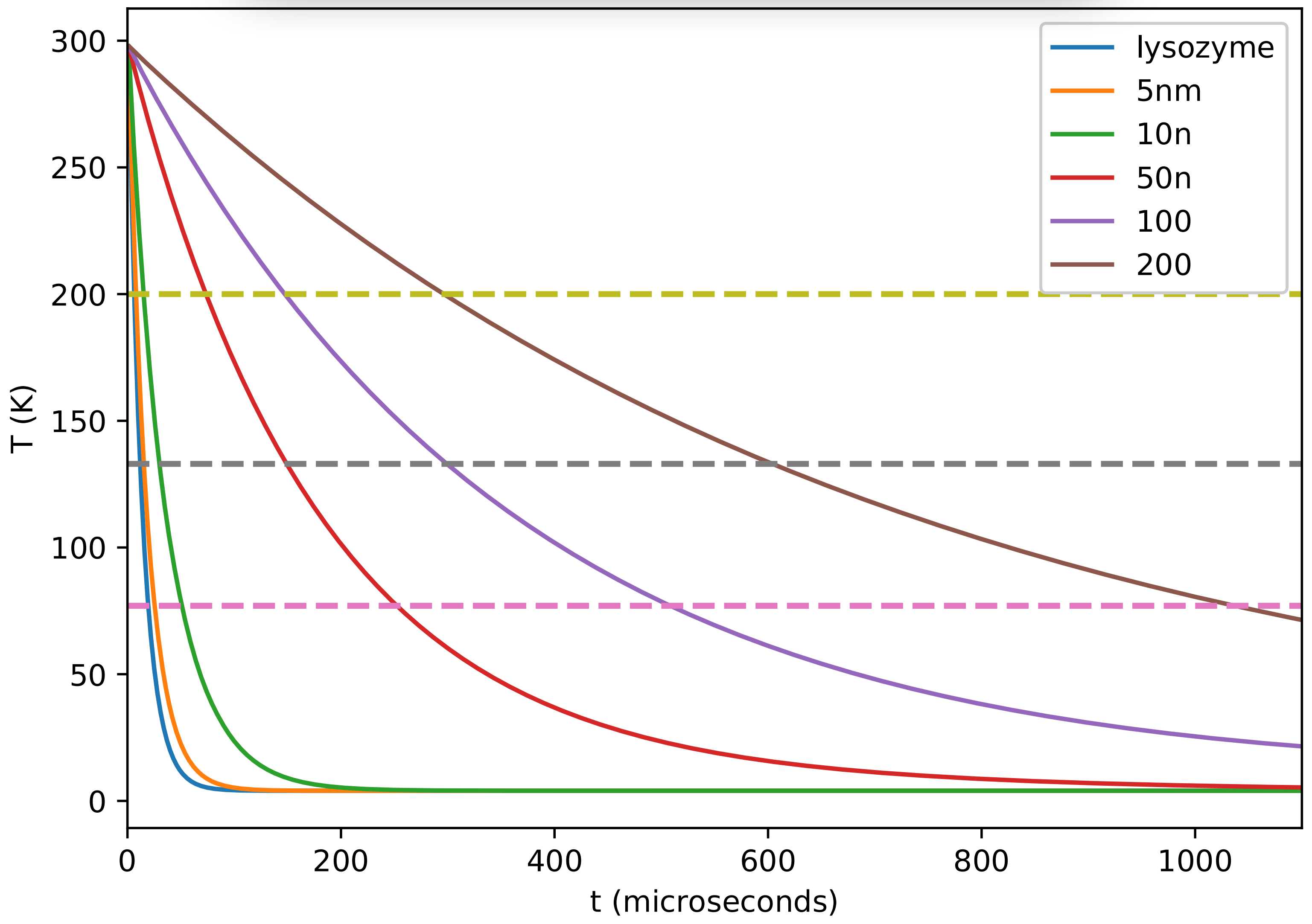


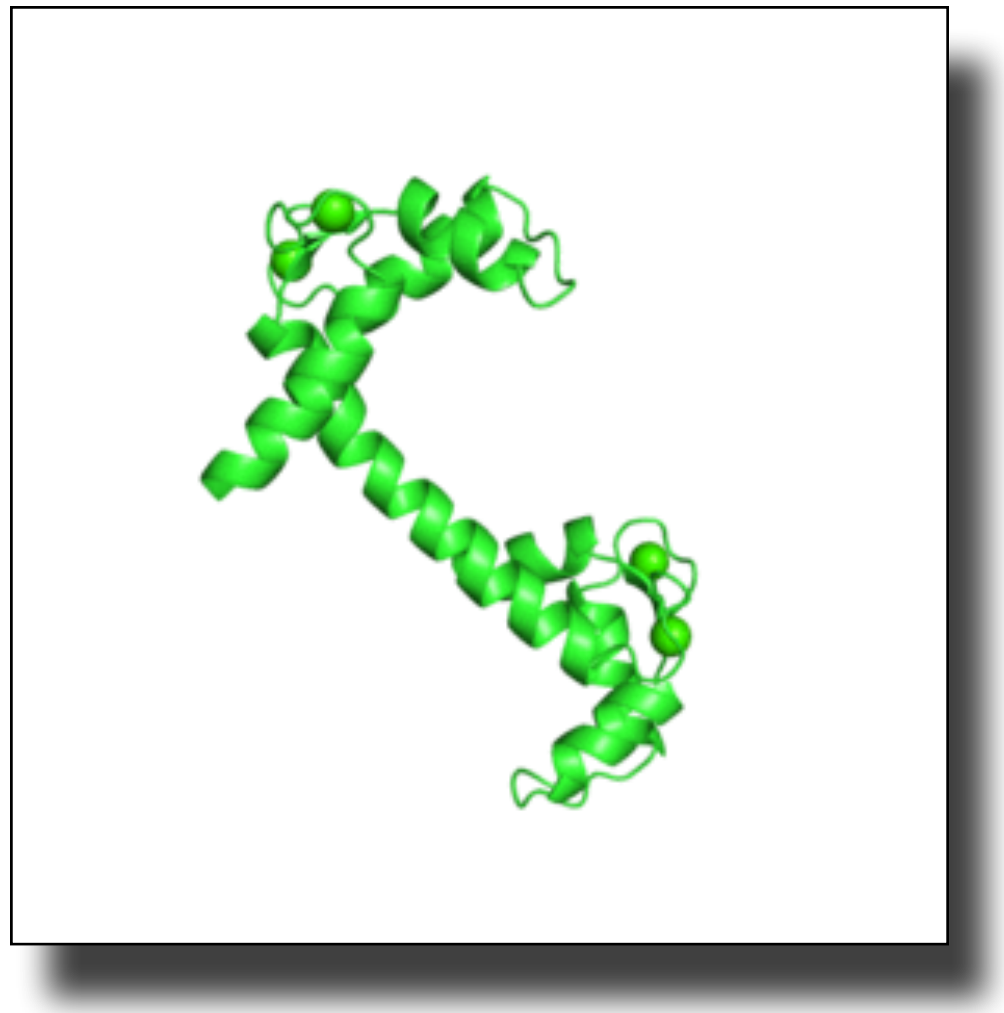


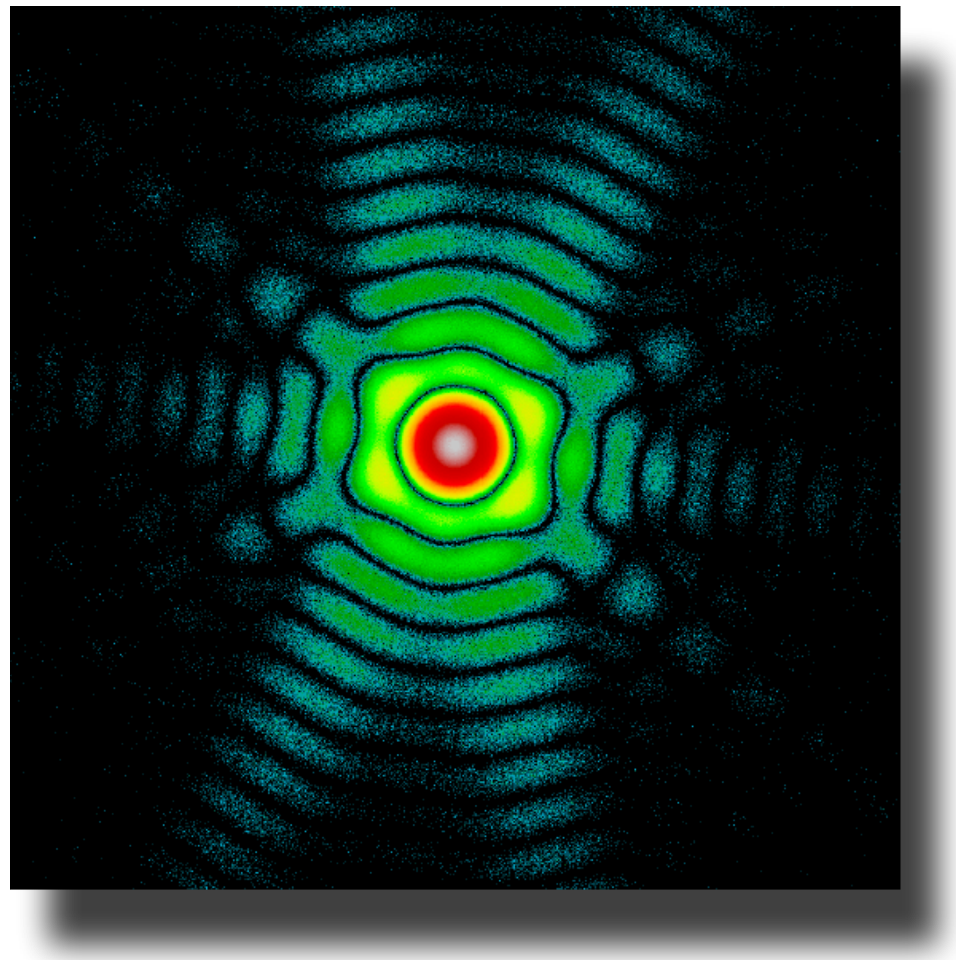


Conclusions & outlook









separation (small molecules): Chang, Horke, Trippel, Küpper, *Int. Rev. Phys. Chem.* **34**, 557–590 (2015); arXiv:1505.05632 [physics]