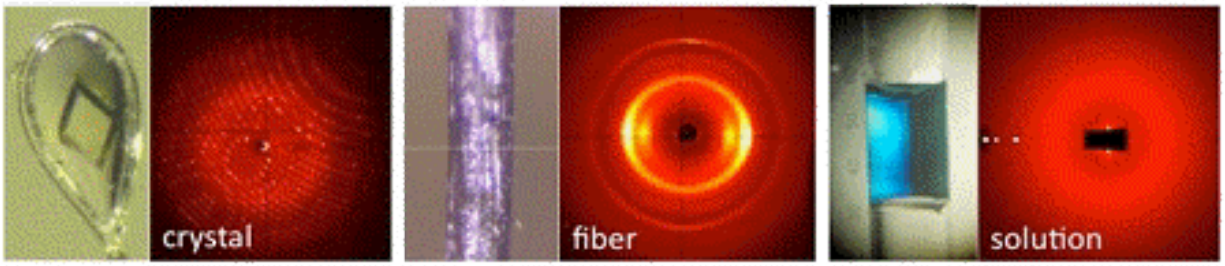
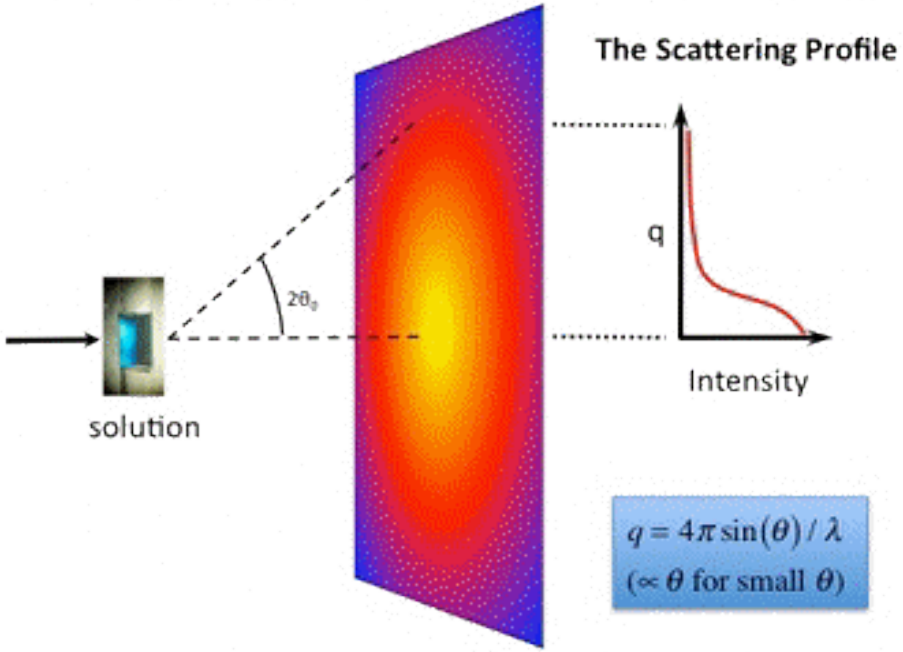
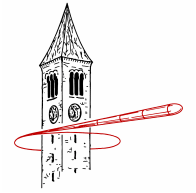
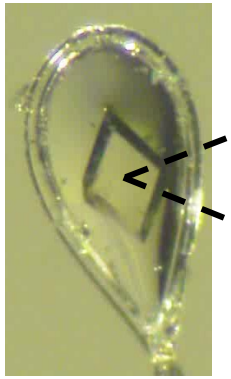
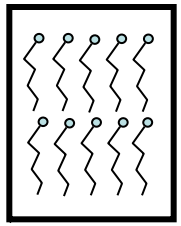
BioSAS Overview and Applications



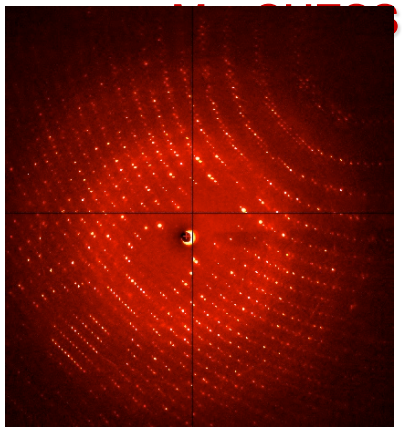


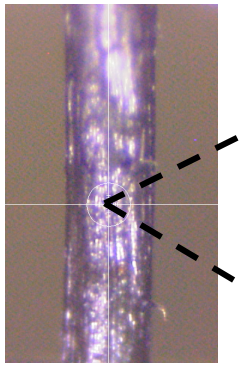


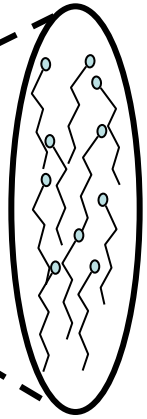




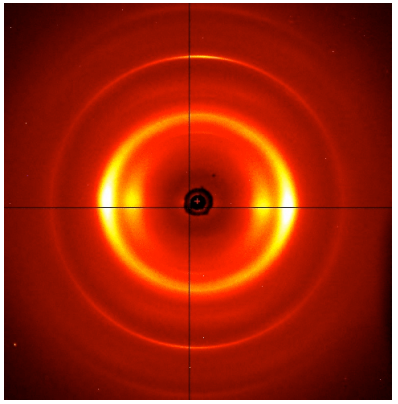
crystal

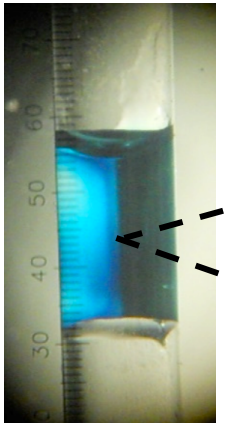


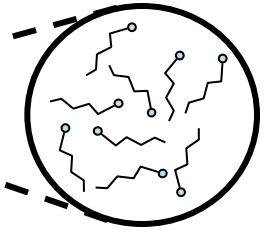




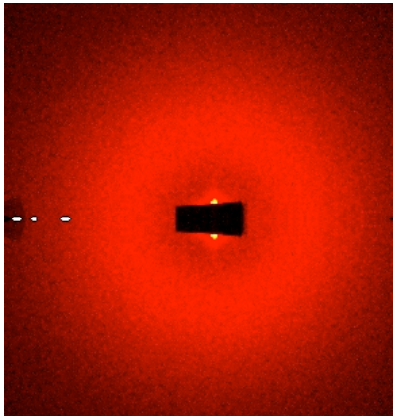
fiber

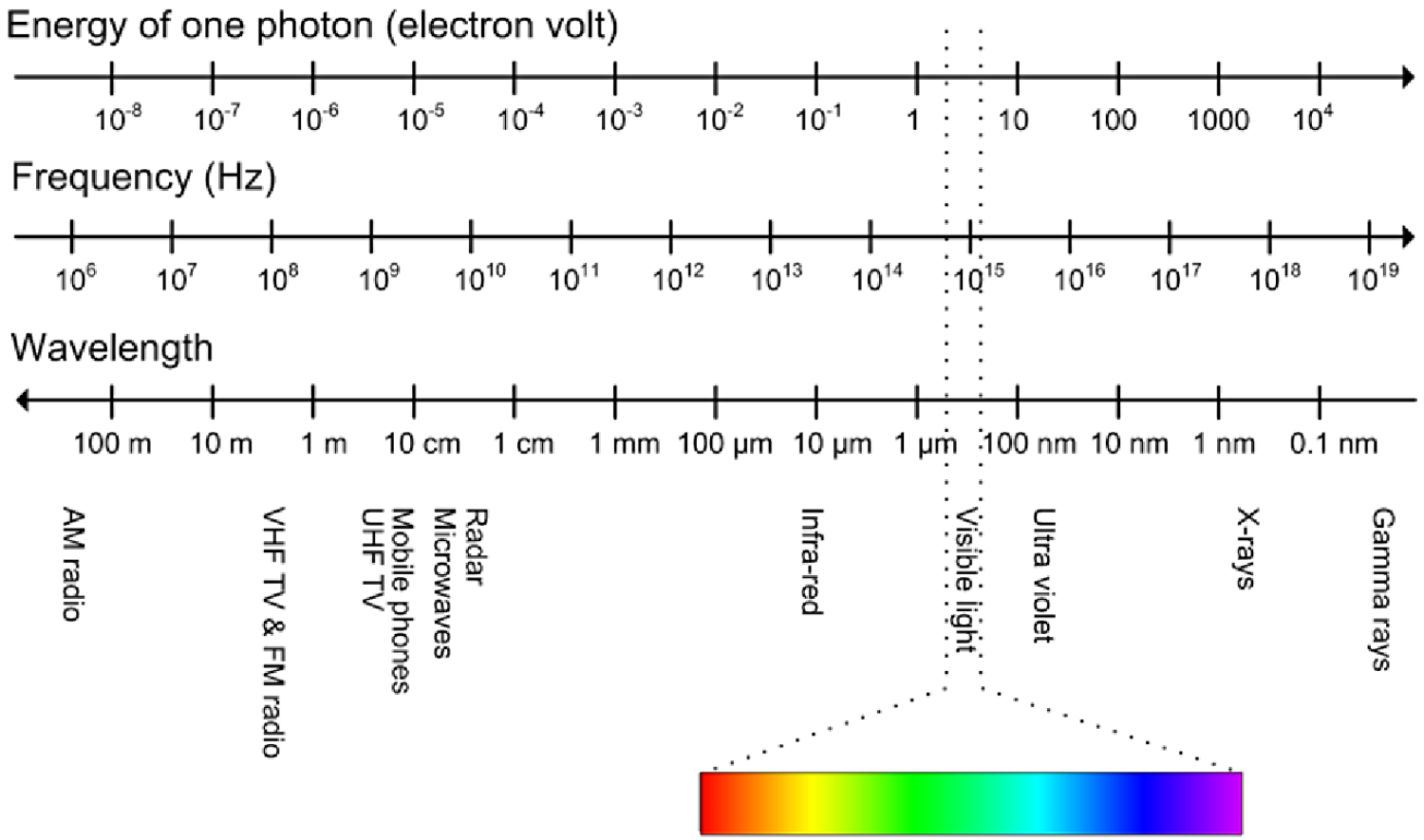






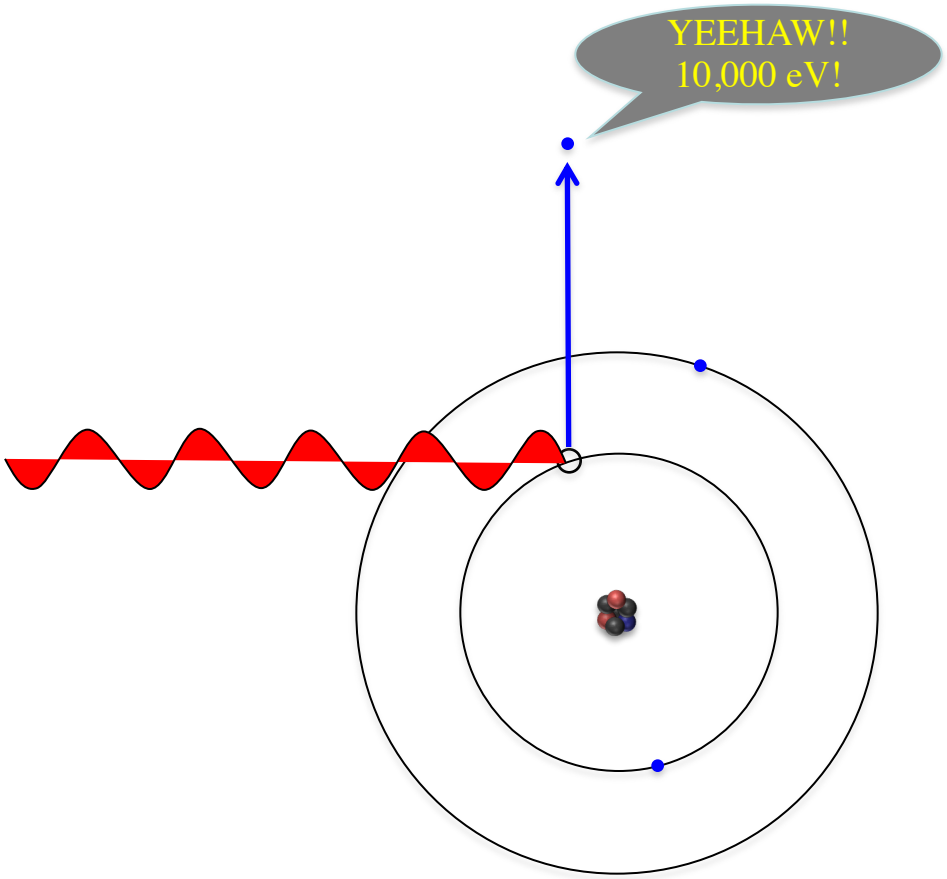
solution





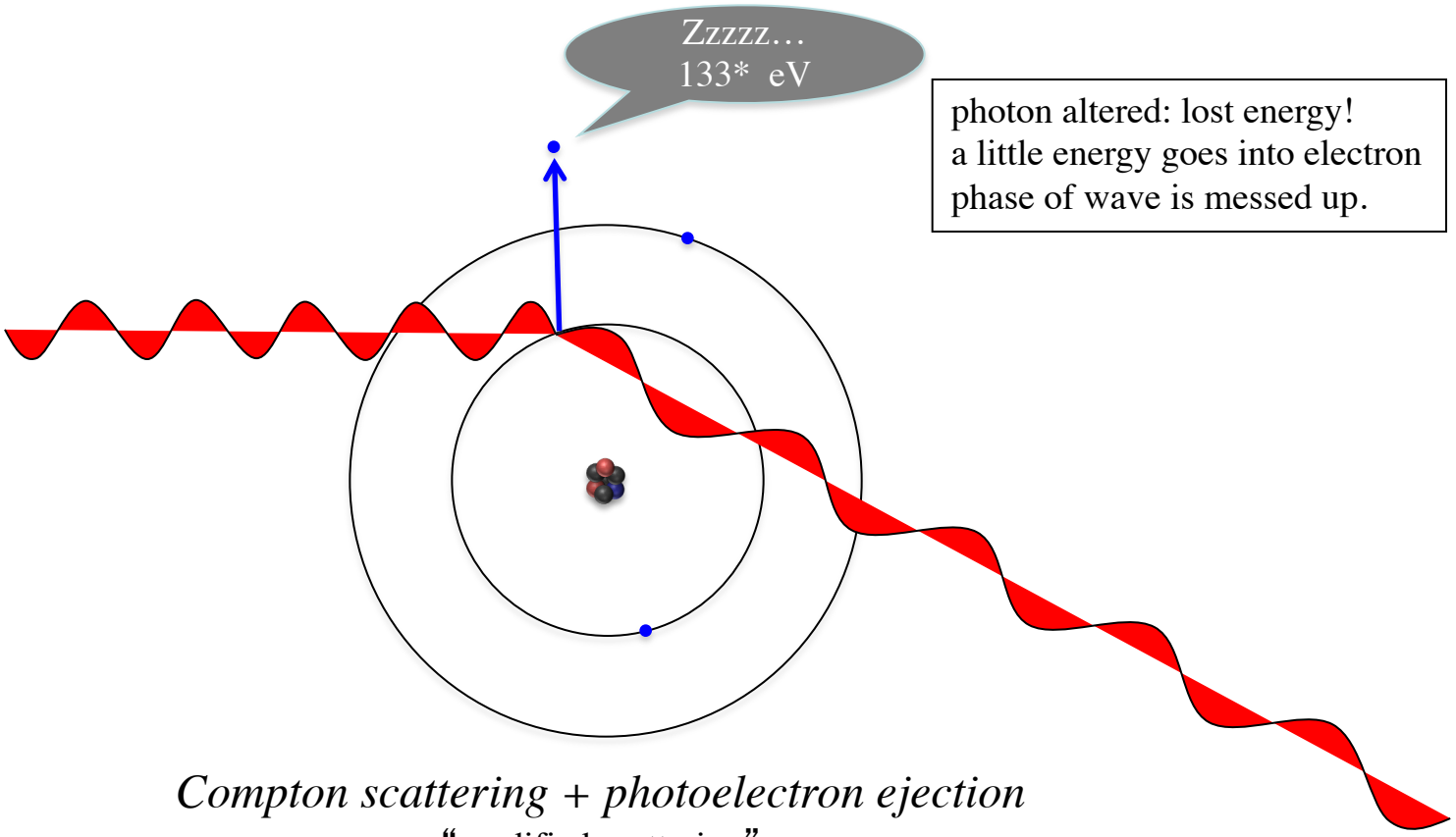
Useful: Energy (keV) = 12.4/wavelength (Angstroms)

* From http://what-when-how.com/wp-content/uploads/2012/07/tmp26dc54.png



Photon vanishes!
All energy goes into electron

Photoabsorption ➔ photoelectron ejection ➔ fluorescence

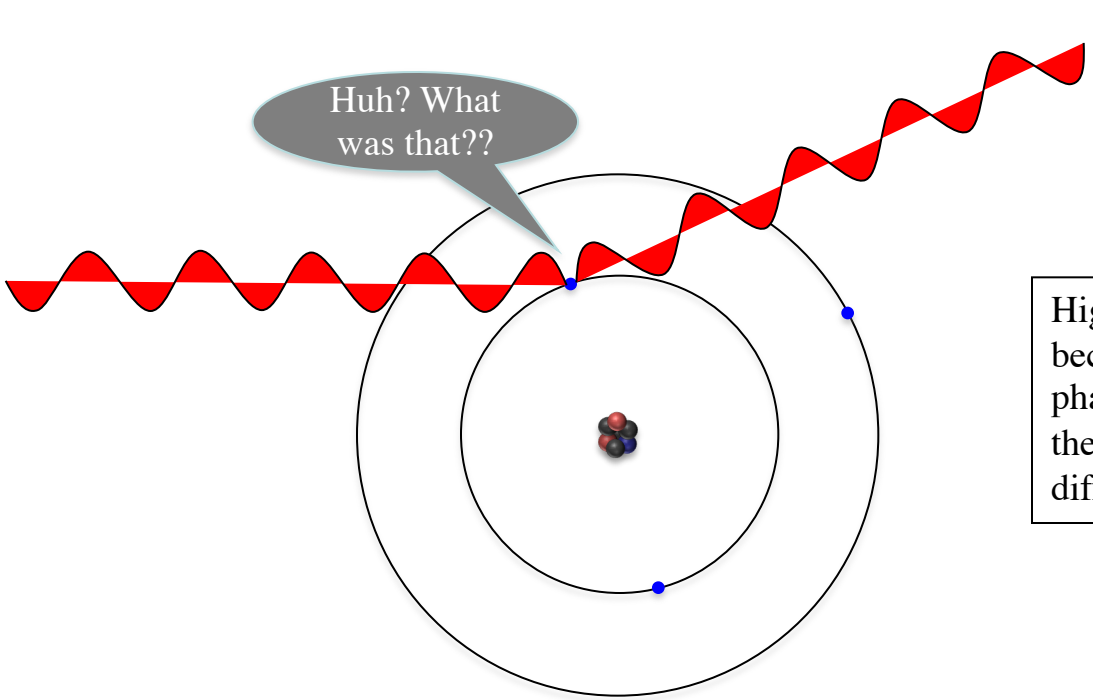


photon altered: lost energy!
a little energy goes into electron
phase of wave is messed up.

Compton scattering + photoelectron ejection

"modified scattering"

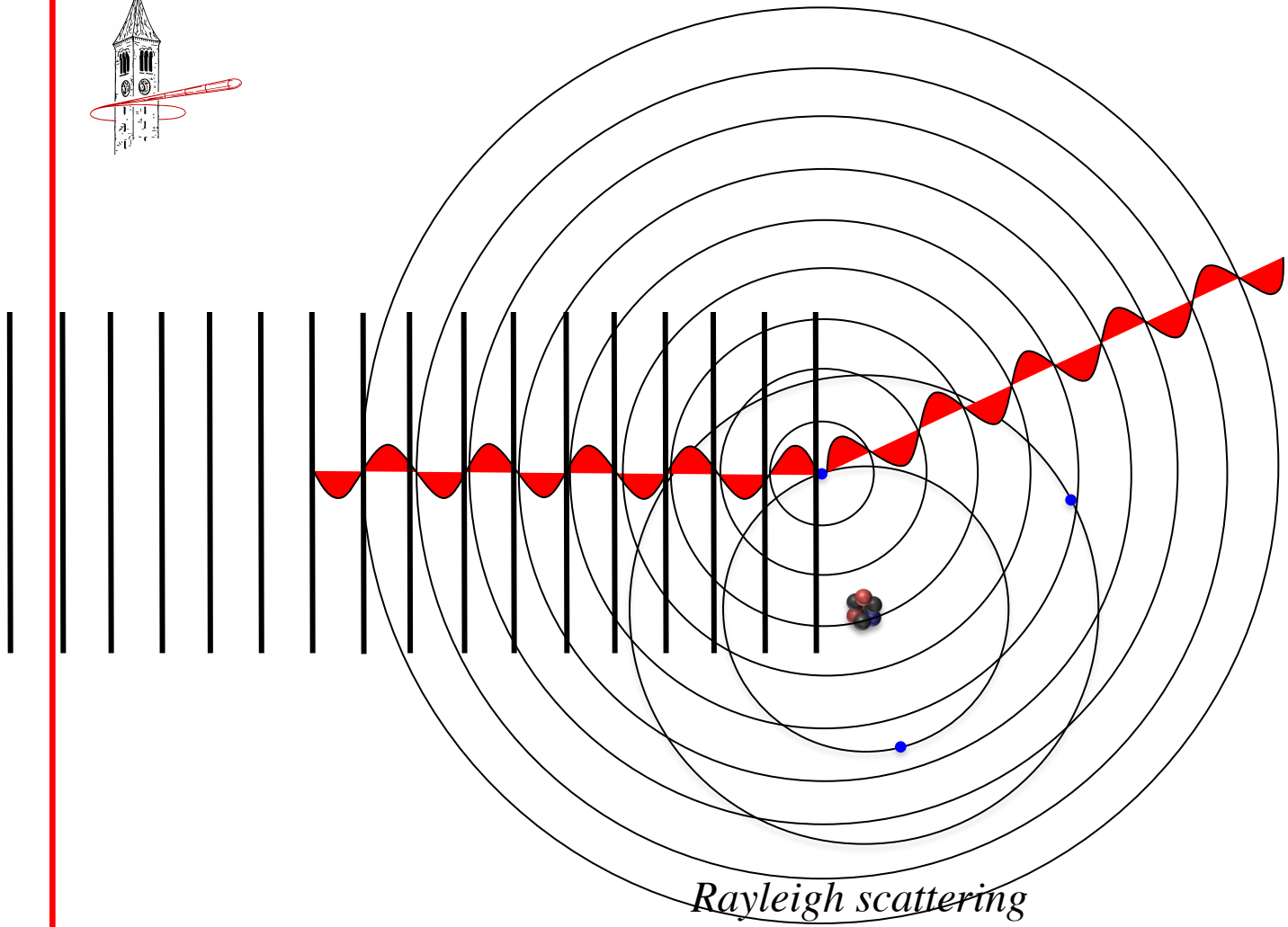
* Compton ΔE @ 90° = 188 eV
Lithium 1s binding = 54.7 eV
188 eV – 54 eV = 133 eV



Highly useful scattering because the wave's phase change is always the same: you can get diffraction!

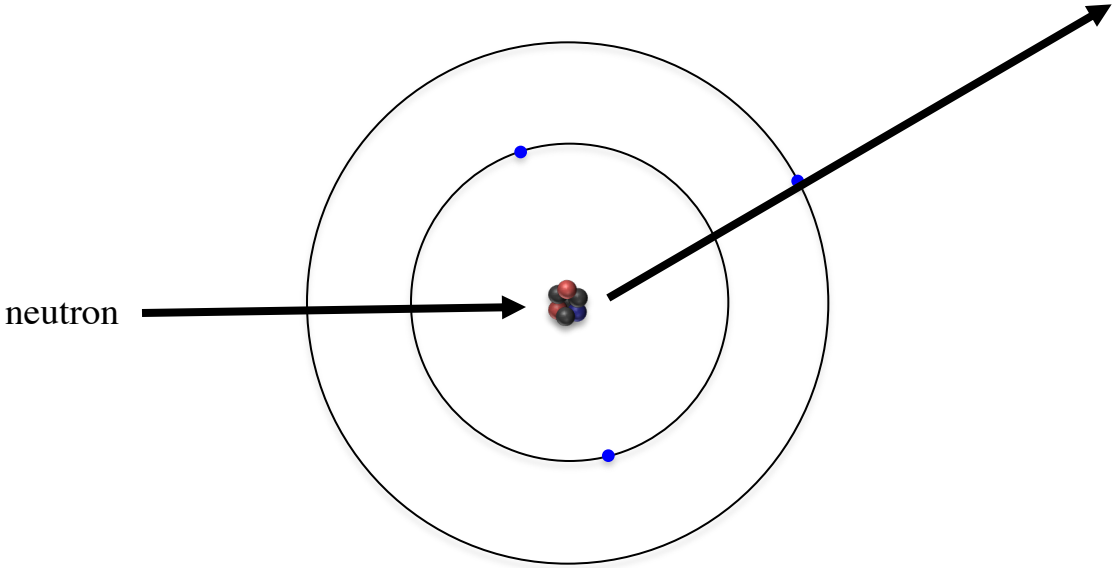
Rayleigh scattering
"unmodified scattering"

Rayleigh scattering = bound electron
Thomson scattering = free electron
To hard X-rays, everything looks like a free electron anyhow!



Rayleigh scattering

"unmodified scattering"



Scattering of **photons** by the nucleus does happen, it's just far too weak to observe.
Neutrons, on the other hand, are scattered by the nucleus very effectively: SANS!

**when photon Energy > 1 MeV: photonuclear effects, pair production, Delbrück scattering etc.*

Energy and X-ray Damage

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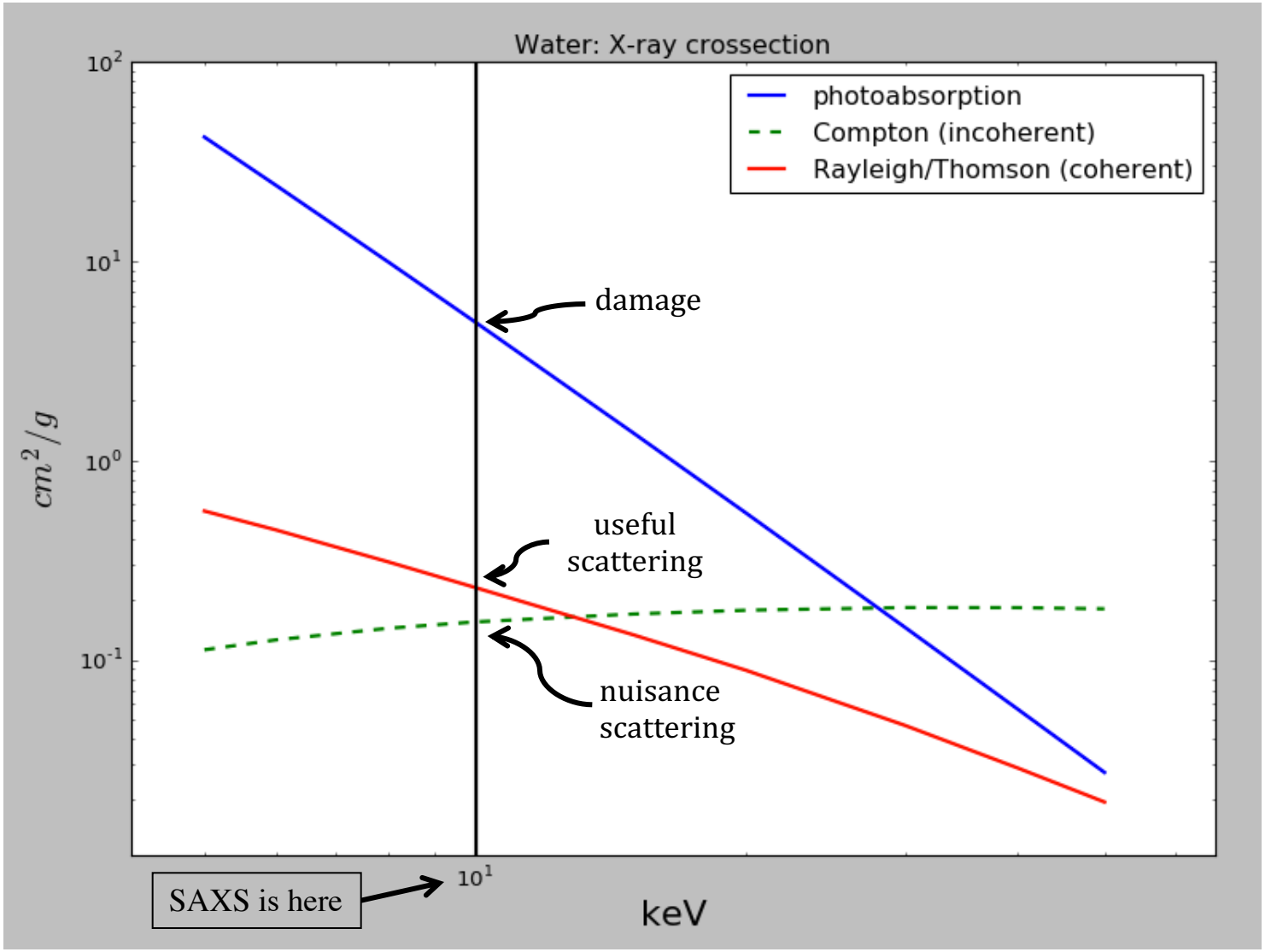

The sad reality of biology with X-rays

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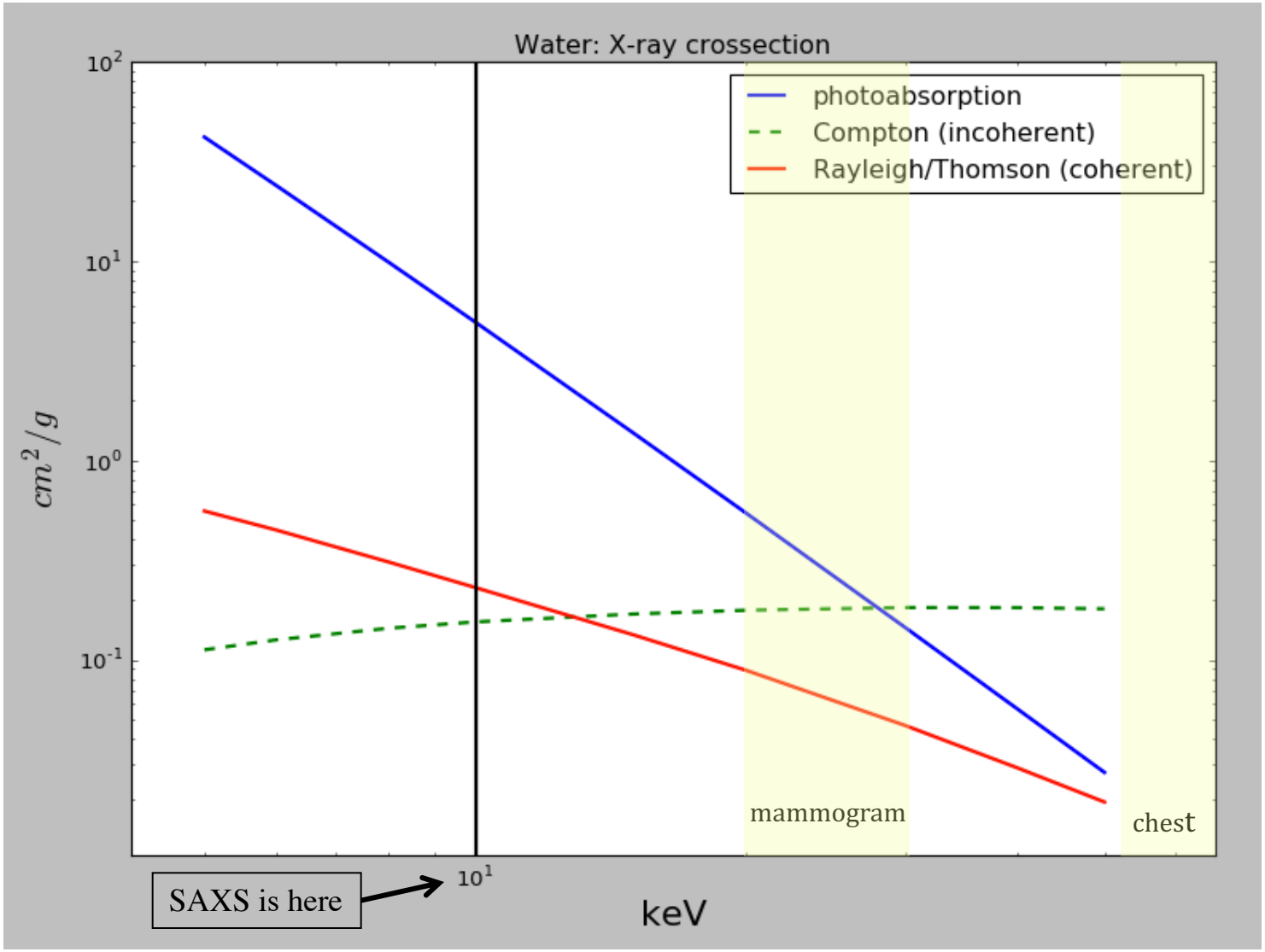

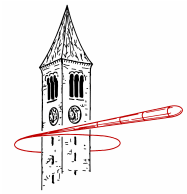

Diffraction from a single molecule

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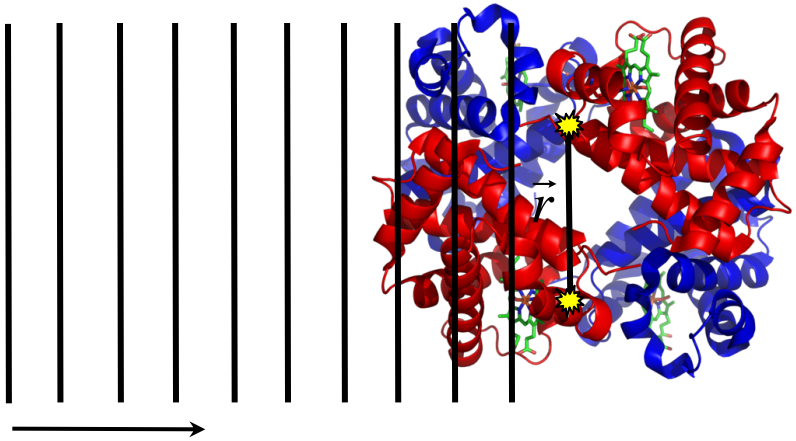
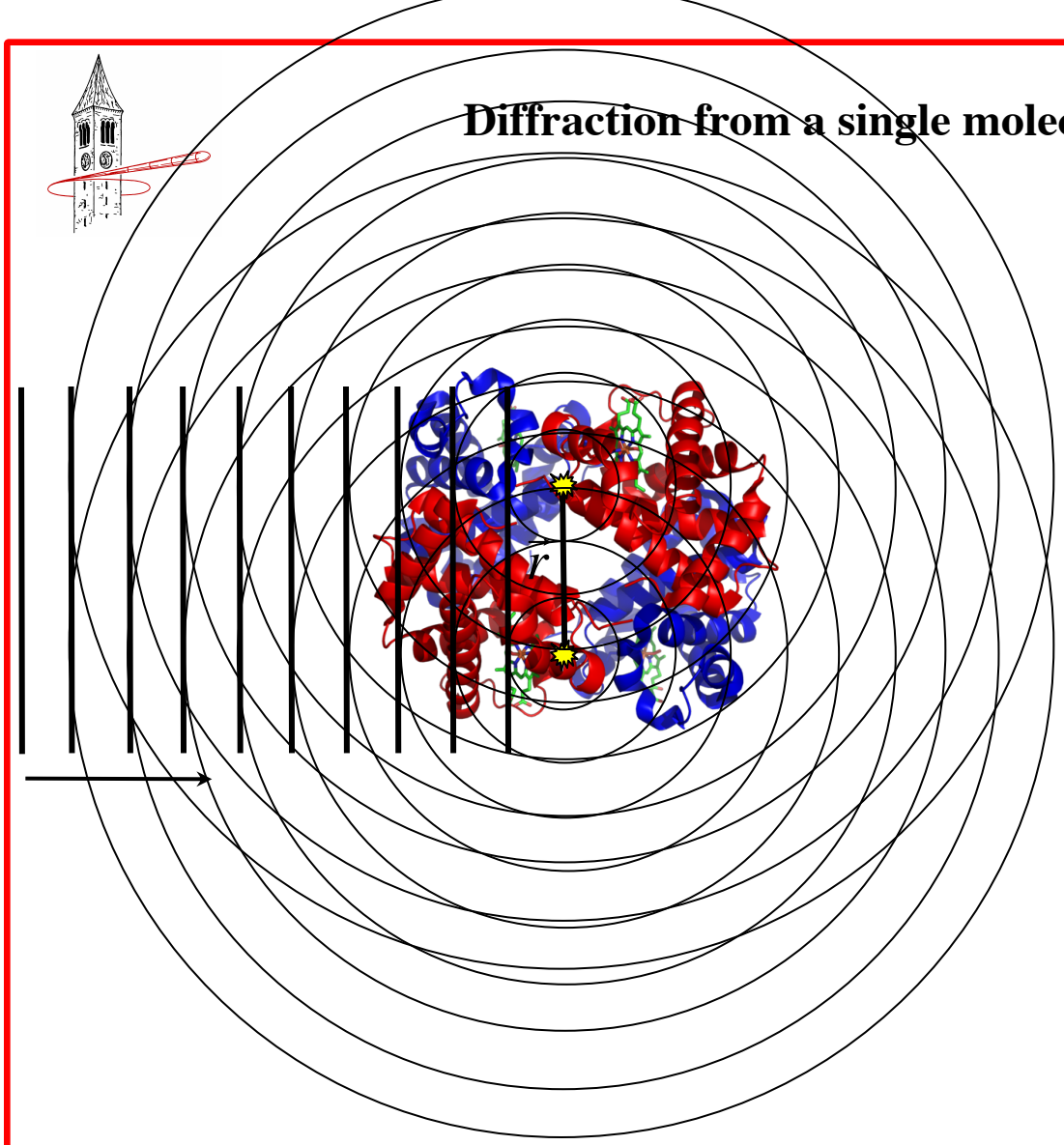


Diffraction from a single molecule



Diffraction from a single molecule

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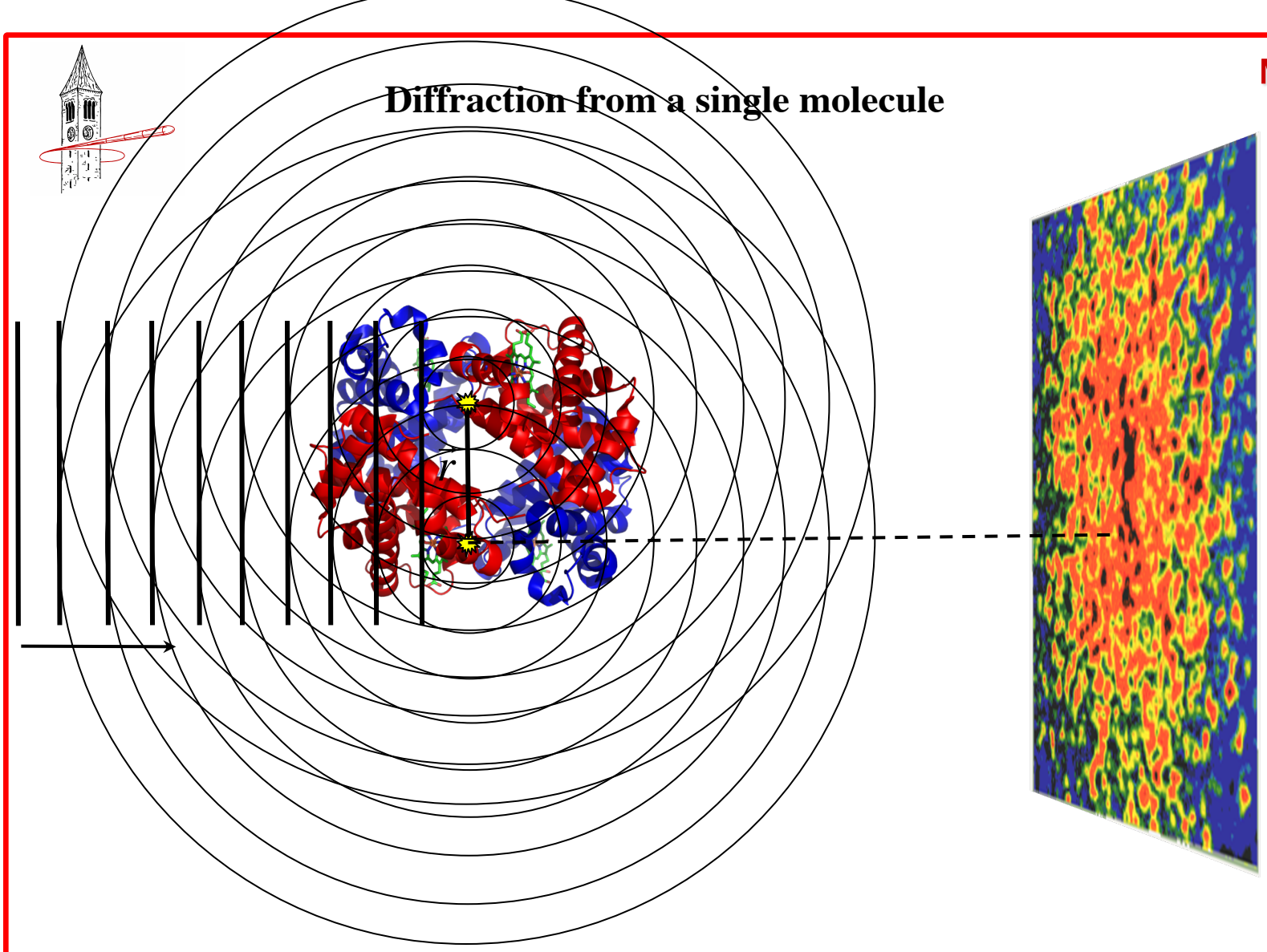

Diffraction from a single molecule

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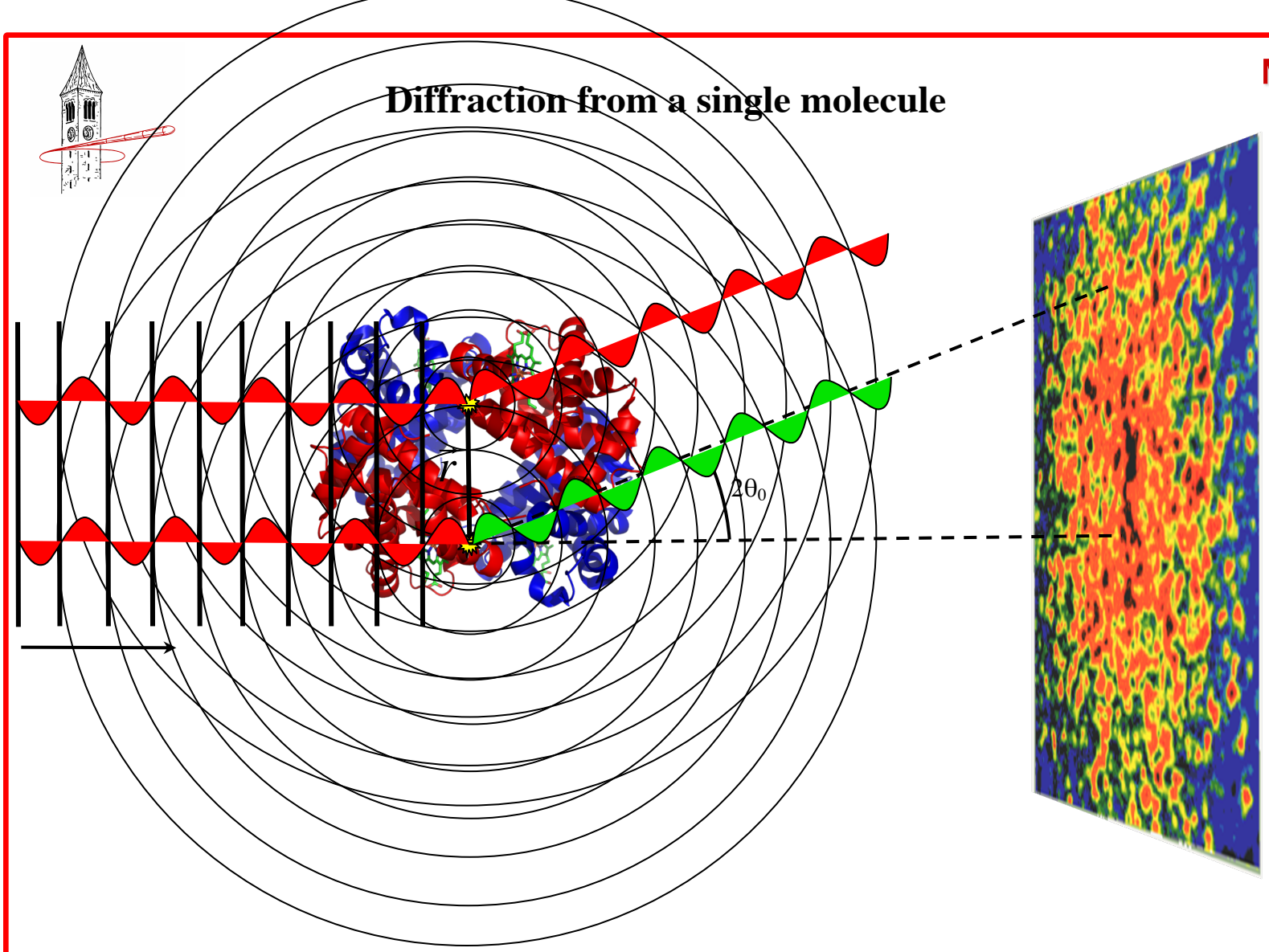
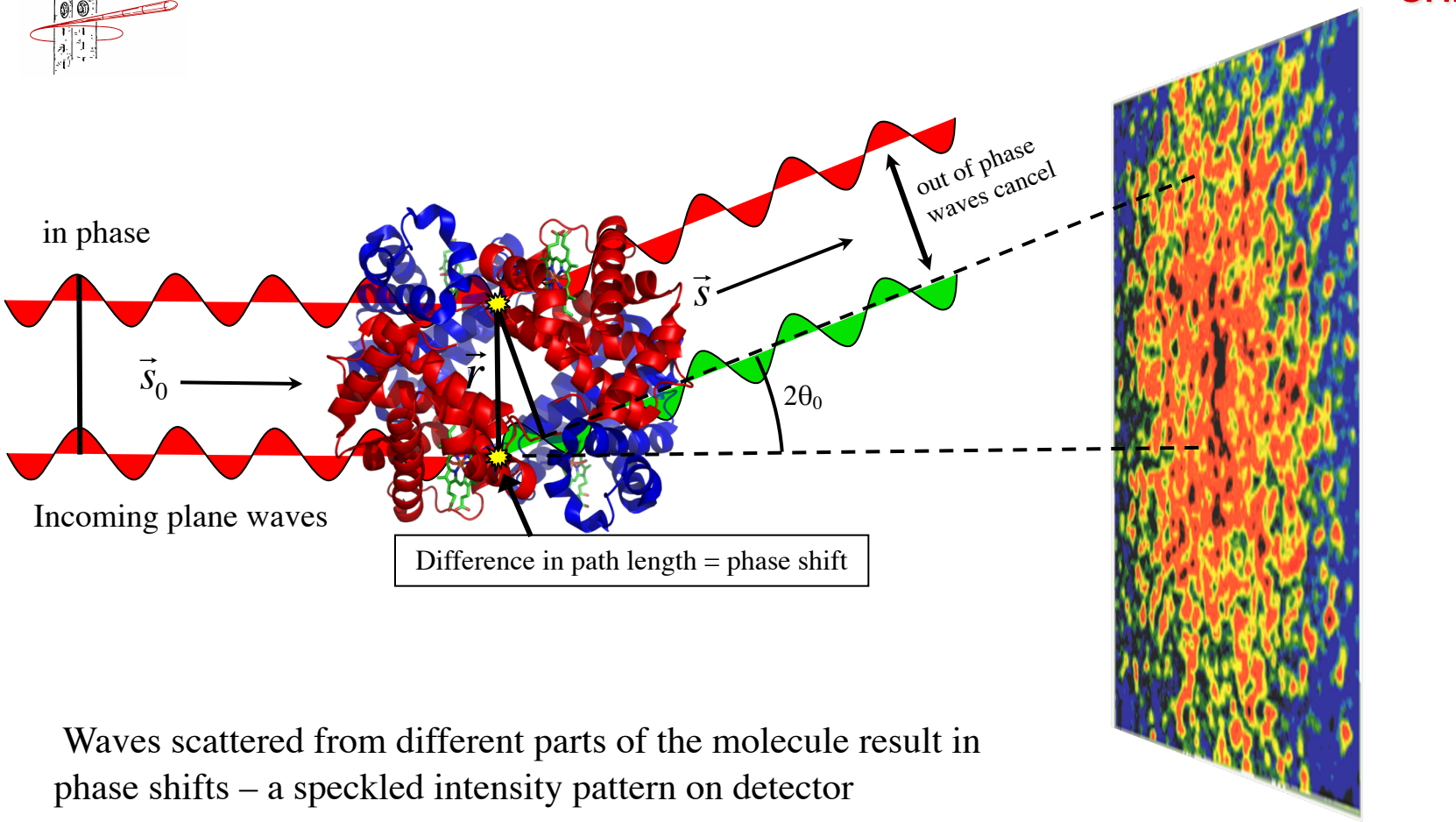


Diffraction from a single molecule



Waves scattered from different parts of the molecule result in phase shifts – a speckled intensity pattern on detector

Far-field diffraction pattern

Diffraction from a rotationally-averaged molecule

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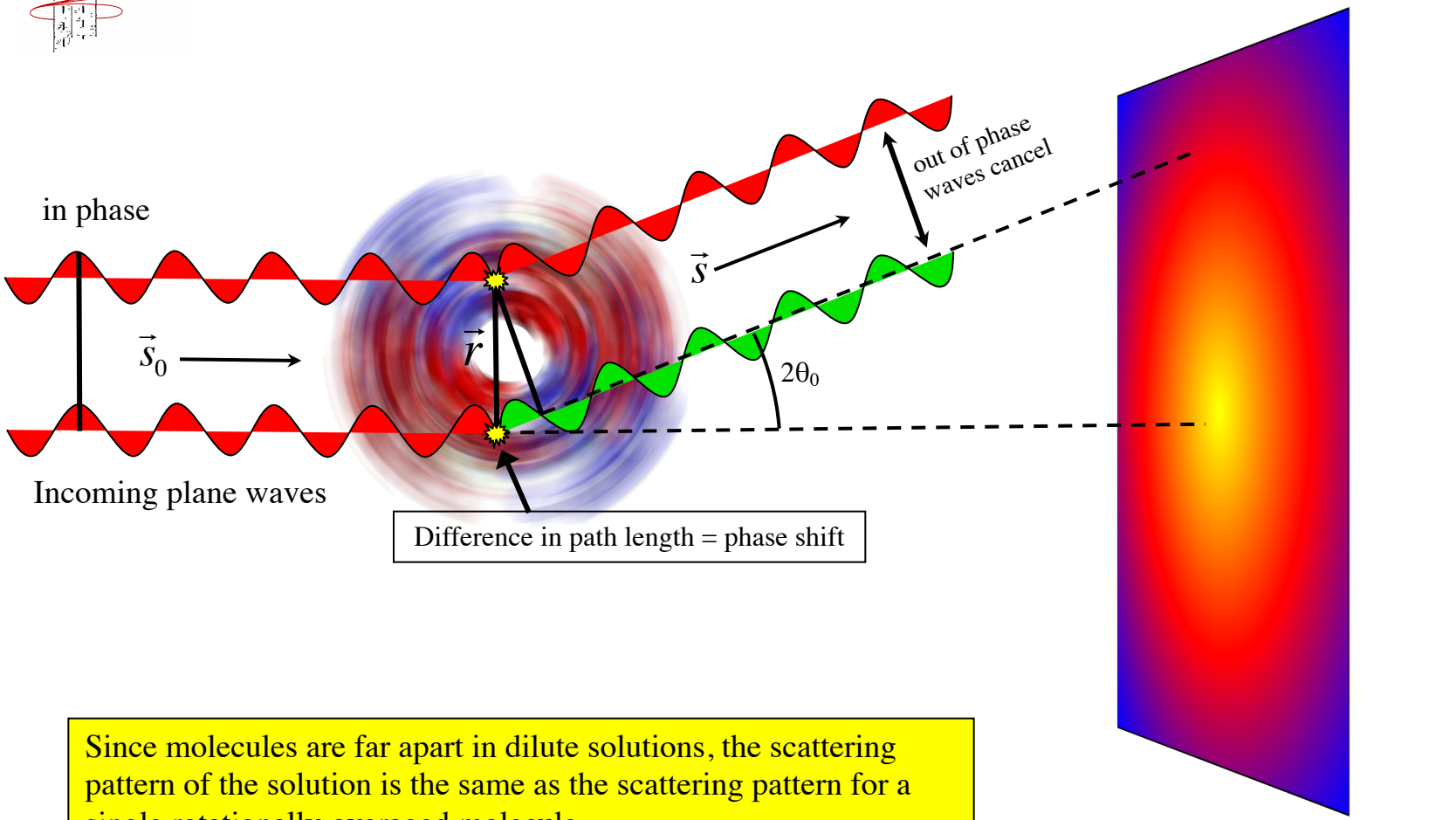

Since molecules are far apart in dilute solutions, the scattering pattern of the solution is the same as the scattering pattern for a single rotationally-averaged molecule.

Far-field diffraction pattern

The Scattering Profile

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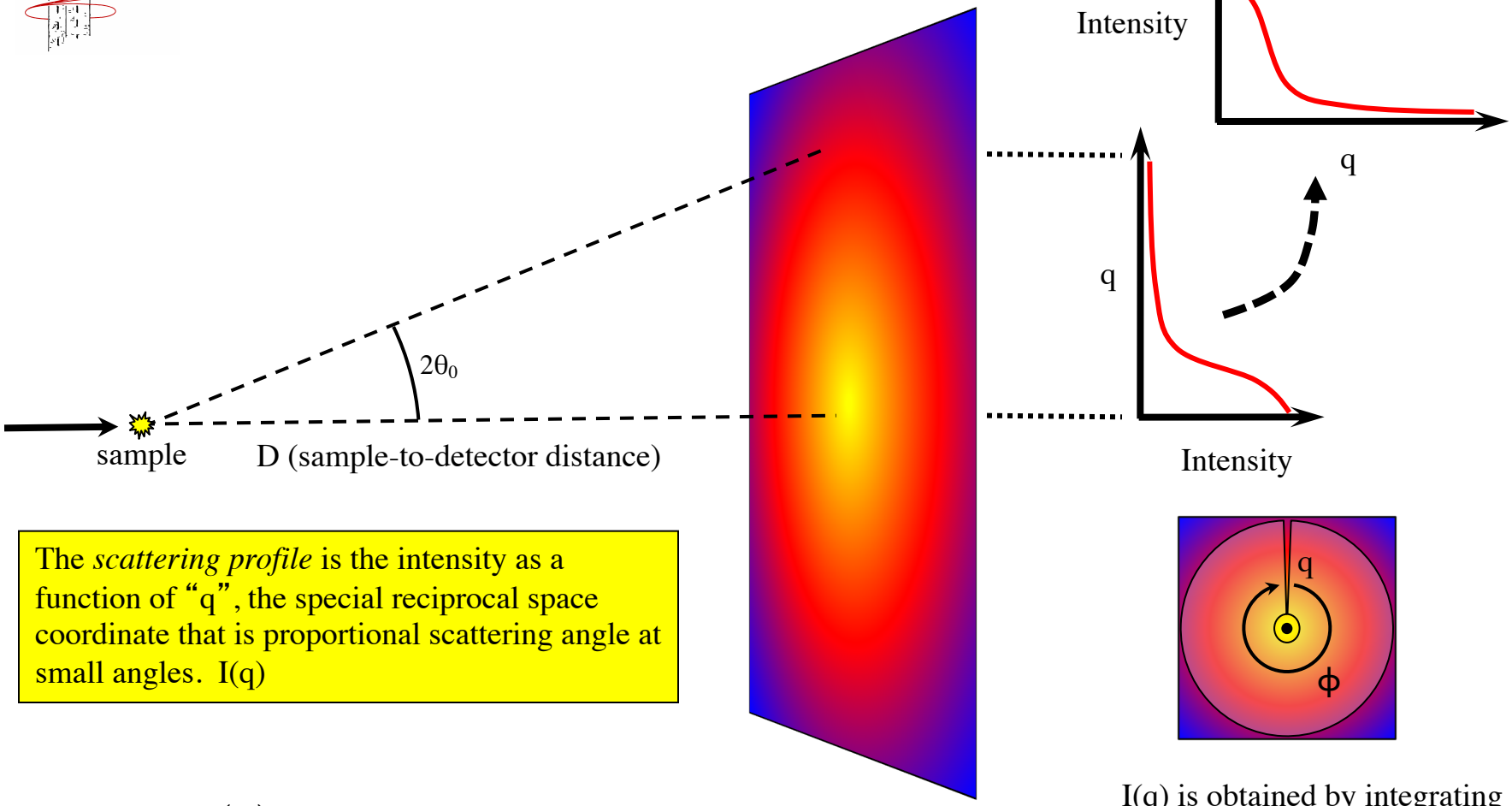
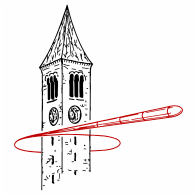


The *scattering profile* is the intensity as a function of "q", the special reciprocal space coordinate that is proportional scattering angle at small angles. I(q)

$$q = 4\pi \sin(\theta) / \lambda \quad (\propto \theta \text{ for small } \theta)$$

Far-field diffraction pattern

I(q) is obtained by integrating around the circle. For detectors, the standard deviation of signal σ(q) is also calculated.



Most important variable in SAXS: **q, s,** *or* … **h ??**

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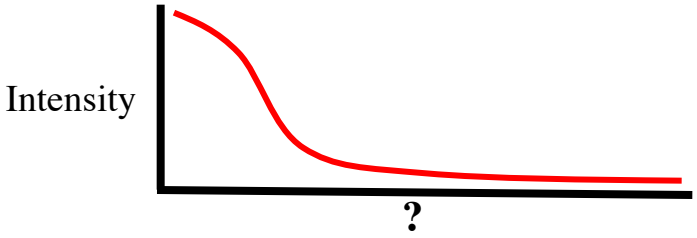
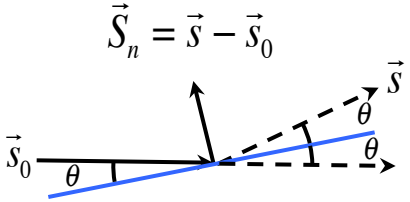




Can be viewed as light S_0 reflecting off a fictitious mirror plane. Normal vector to plane, $\vec{S}_n$, is the momentum transferred.

$$\left\|\vec{S}\right\|^2 = \left(\vec{s} - \vec{s}_0\right)\bullet\left(\vec{s} - \vec{s}_0\right) = 2 - 2\cos(2\theta) = (2\sin(\theta))^2 \,^*$$

$$\frac{2\pi\left\|\vec{S}_n\right\|}{\lambda} = \frac{4\pi\sin(\theta)}{\lambda} \quad = \textit{Oscillations of radiation (in radians) per unit length}$$

Sometimes called ***momentum transfer***. Can have units of $Å^{-1}$, or sometimes nm^{-1}.

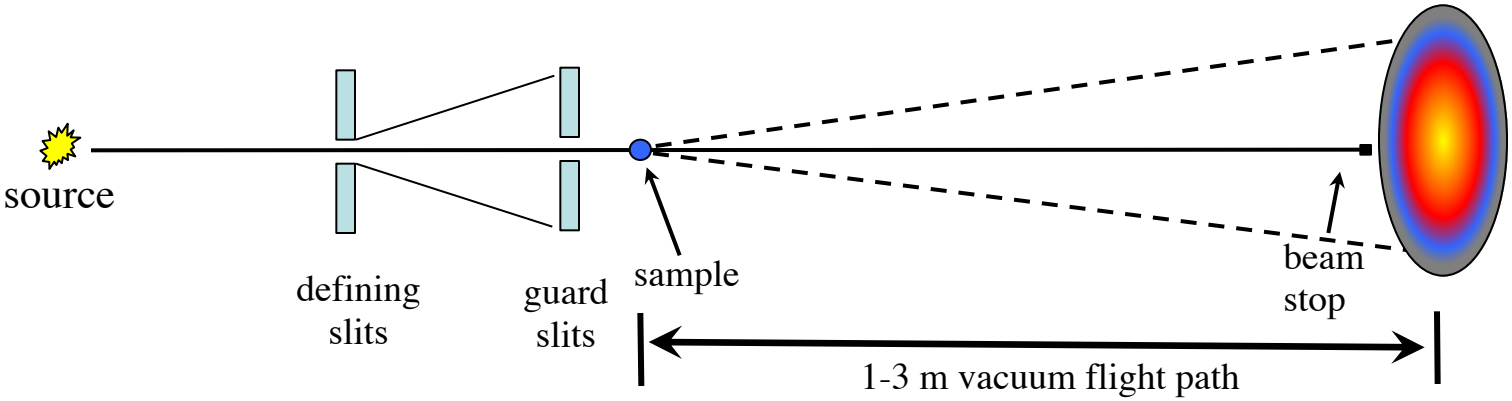
$h = 4\pi \sin(\theta)/\lambda$ [Guinier & Fournet (1955); Glatter & Kratky (1982)]
$s = 4\pi \sin(\theta)/\lambda$ [Feigin & Svergun (1987)]
$q = 4\pi \sin(\theta)/\lambda$ [Putnam, Hammel, Hura & Tainer (2007); Jacques & Trewhella (2010)]

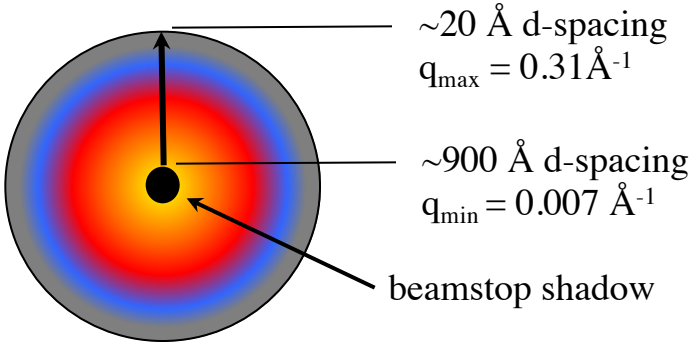
Sometimes you will see $s = 2\sin(\theta)/\lambda$ because 1/s = "d-spacing" (resolution in crystallography).

* $\|S\| = \|S_0\| = 1$ $\quad \vec{S}\bullet\vec{S}_0 = \cos(2\theta)$ $\quad 1 - \cos(2\theta) = 2\sin^2(\theta)$ *(double-angle formula)*

Simplified diagram of SAXS setup

typically 7-12 keV (1.7 Å - 1.0 Å). - practical high-energy limit ~20 keV

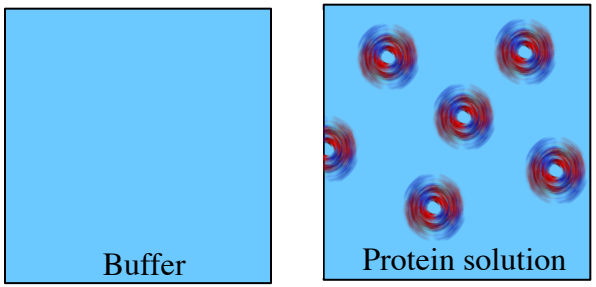




As you get lower in scattering angle (smaller q), the background scatter gets VERY high – this ultimately limits how low you can go and how large of molecules you can study.

$q = 4\pi \, Sin(\theta)/\lambda$

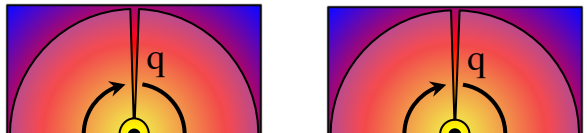
Real protein signal is obtained by buffer subtraction



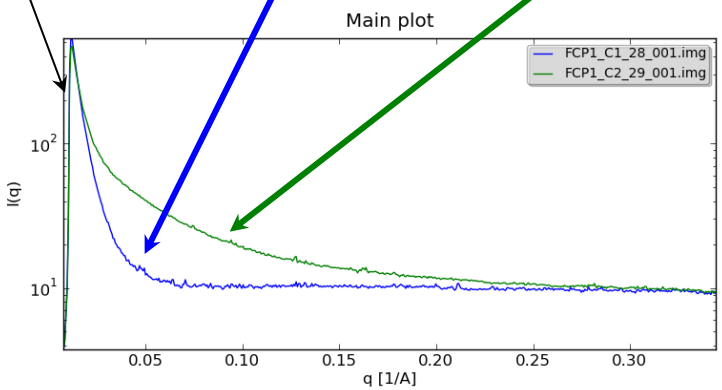
$$I_{protein} = I_{solution} - \alpha I_{buffer}$$

Scattering pattern for protein in vacuum is obtained by subtracting separate images normalized to the same exposure. The normalization constant α can be obtained in several ways:

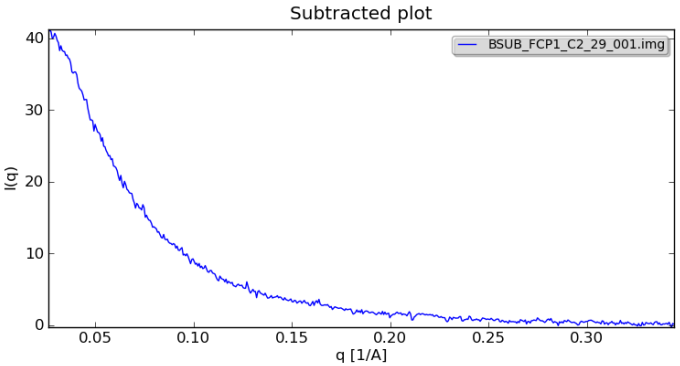
1. Assume no beam decay: α = 1
2. Average before and after buffers
3. Scale so that tails of protein and buffer meet
4. Use transparent beamstop to integrate direct beam
5. **Integrate beamstop diode readings**



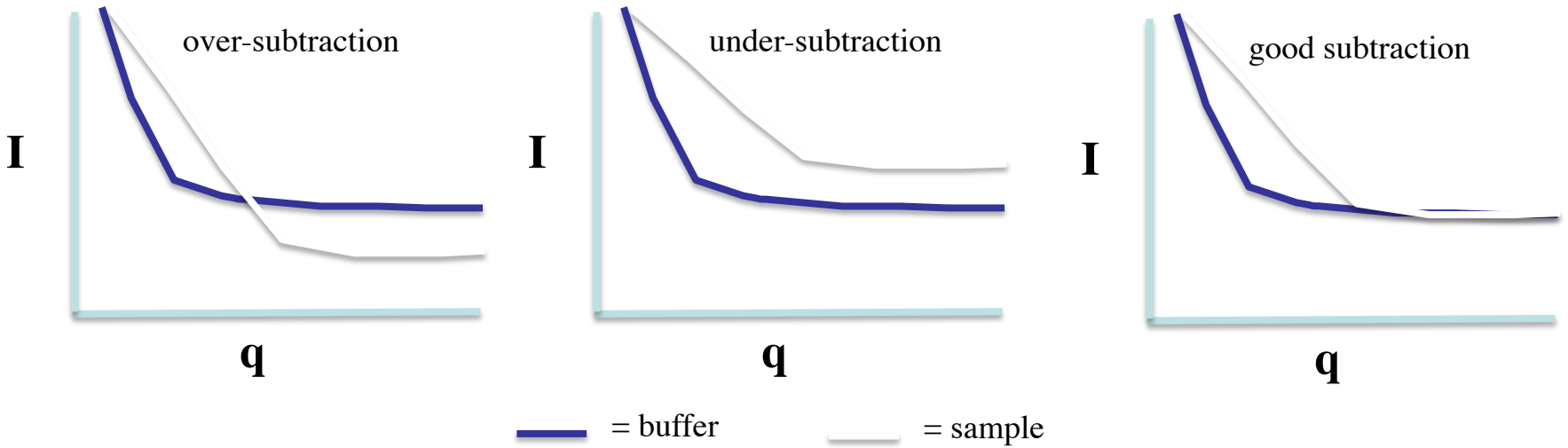
beamstop shadow



difference



Perfect "buffer match" is everything in BioSAXS



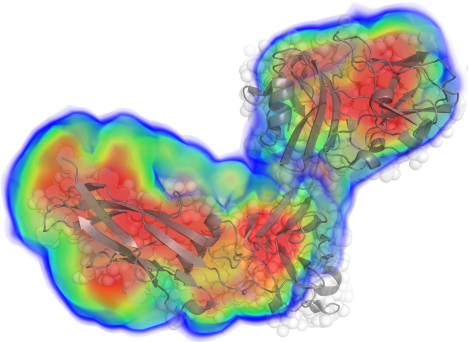
Small changes in concentration of salts and additives can result in change of baseline scattering level

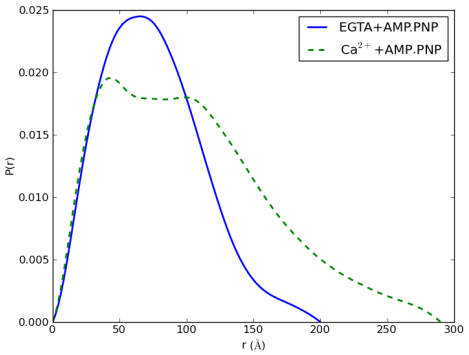
How to get matching buffer:

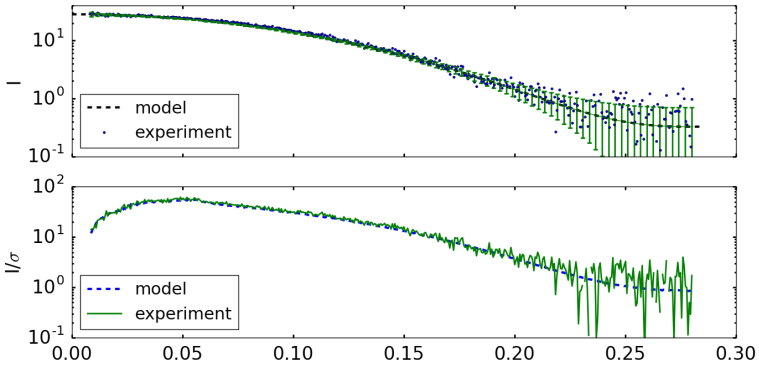
1. Use buffer from a size-exclusion chromatography run
2. Change to known buffer using centrifugal concentrator
3. Change buffer using dialysis
4. Use a "desalting" spin column

BioSAS tells you about how biomolecules behave in solution!

- quantifying **flexibility**, **disorder**, and **unfolding** in biomolecules.
- tracking of **time-resolved** structural changes (sub-milliseconds and longer)
- study of molecular crowding and **high-concentration** samples
- determination of **conformational changes** induced by binding ligands etc.
- characterizing the **ensembles** of conformations **in solution**.
- measurement of molecular weight, radius of gyration, and maximum length
- identification of physiological **oligomeric states**
- determination of structural **stability limits**
- **verification** of proposed molecular **models**
- **assembly of complexes** from known domain structures (pseudoatomic)
- calculation of true low-resolution **electron density** *in solution*.

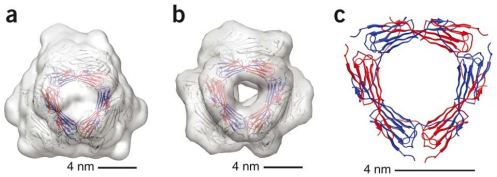






Specific Applications of BioSAXS

- combining NMR with SAXS to build oligomers from monomers
- **are some parts of the protein extended or disordered in solution?**
- comparing Lit vs. dark states of photoactive proteins
- comparing ligand-induced conformational changes
- adding and refining loops to homology models
- determining spatial distributions of domains connected by flexible linkers
- modeling changes in protein interaction with salt concentration and cation type
- determine fractions of monomer and dimer with change in ionic strength/additives
- categorizing discrete folded and unfolded states
- monitoring changes in protein stability with additives (stabilization due to binding)
- combining computational docking with SAXS data to improve hit rate
- building pseudoatomic models from known fragments and homology models



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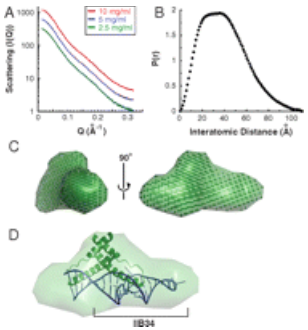

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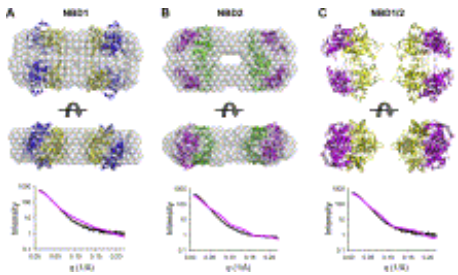
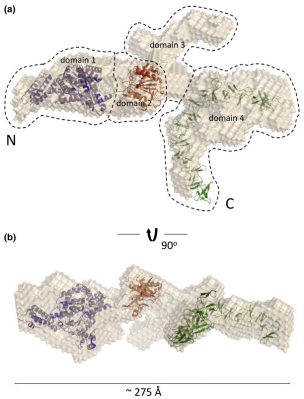


Park S, et al. J Struct Biol 2010 Feb: 169(2):243-51



Albesa-Jove D, et al. J Mol Biol. 2010 Mar 12;396(5):1260-70.

Sample characteristic

- Purity (monodispersity)
- Oligomeric State
- Shape/anisometry
- Flexibility Folded/Non-folded
- Interparticle Interactions
- Conformation

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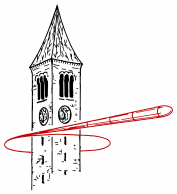
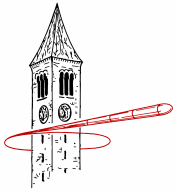


Sample characteristic	Basic parameter/data
Purity (monodispersity)	Guinier plot
Oligomeric State	Particle volume, Molecular Mass
Shape/anisometry	Envelope, Rg, P(r) function
Flexibility Folded/Non-folded	Porod exponent, Kratky plot
Interparticle Interactions	Concentration Series
Conformation	I(q)

Based on H.D.T. Mertens, D.I. Svergun / Journal of Structural Biology 172 (2010) 128–141



Sample characteristic	Basic parameter/data	Program/method
Purity (monodispersity)	Guinier plot	Mixture analysis OLIGOMER, SVD, EFA
Oligomeric State	Particle volume, Molecular Mass	Docking: CORAL, FoXSDock
Shape/anisometry	Envelope, Rg, P(r) function	Ab initio modelling DAMMIF, DENSS
Flexibility Folded/Non-folded	Porod exponent, Kratky plot	Mult. conformations SCATTER, EOM
Interparticle Interactions	Concentration Series	Interaction potentials
Conformation	I(q)	Compare to model Crysol, FoxS

Based on H.D.T. Mertens, D.I. Svergun / Journal of Structural Biology 172 (2010) 128–141

Example of SAXS titration and model validation

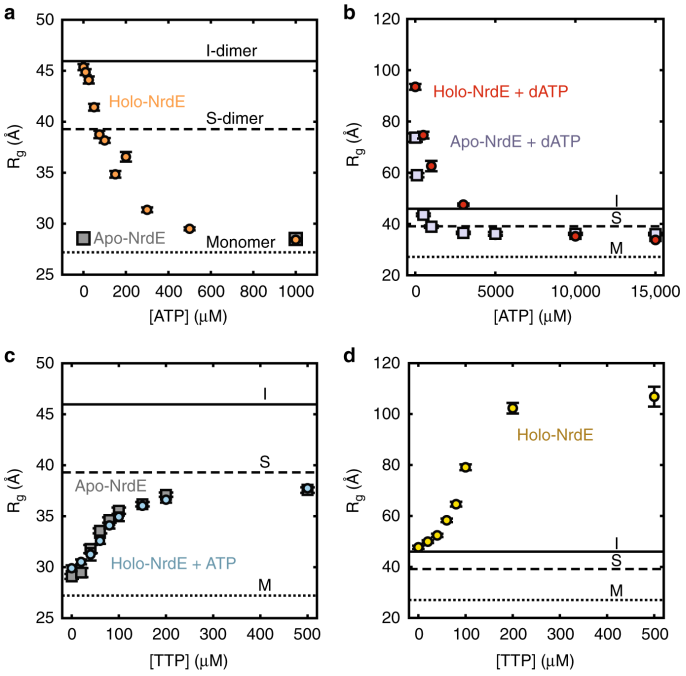
MacCHESS
CHESS

Thomas, W.C., Brooks, F.P., Burnim, A.A. *et al*. Convergent allostery in ribonucleotide reductase. *Nat Commun* **10,** 2653 (2019). https://doi.org/10.1038/s41467-019-10568-4

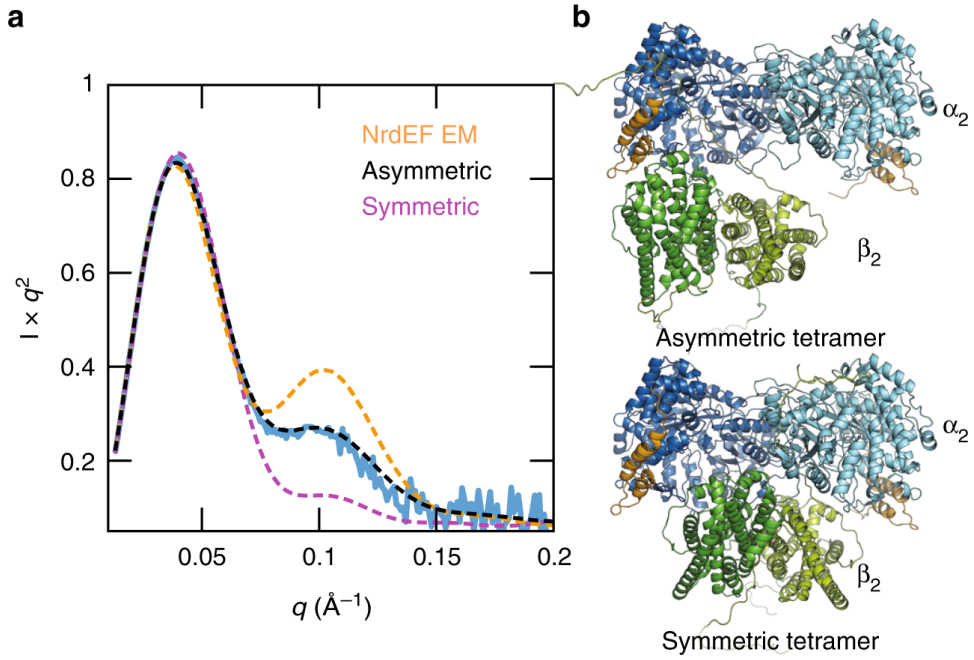
Study combines:
- SAXS
- cryoEM
- crystallography

"Reversible interconversion of six unique structures ... conformational gymnastics necessary for RNR activity"

Watching Rg of a complex change while titrating in ATP:



Comparing models to data:



Example of how basic SAXS data complement a larger study

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CHESS

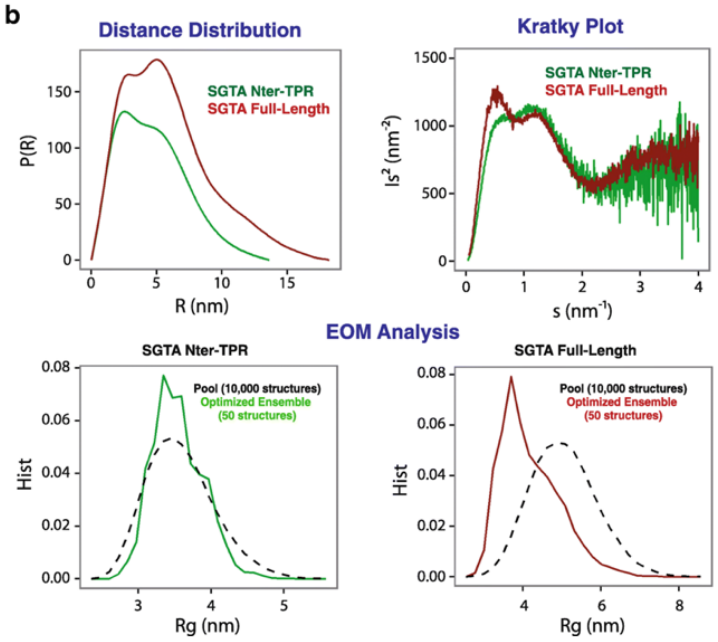
BMC Biol. 2018 Jul 11;16(1):76.

Structural complexity of the co-chaperone SGTA: a conserved C-terminal region is implicated in dimerization and substrate quality control.

Martínez-Lumbreras S[1], Krysztofinska EM[1], Thapaliya A[1], Spilotros A[2], Matak-Vinkovic D[3], Salvadori E[4,5], Roboti P[6], Nyathi Y[6,7], Muench JH[1], Roessler MM[4], Svergun DI[2], High S[6], Isaacson RL[8].

- Confirmed dimeric state
- P(r) function confirms domains with 5 nm separation
- P(r) also confirms full-length protein is more compact than truncated
- Kratky indicates moderate flexibility
- EOM also shows how full-length construct is more compact

SAXS combined with
- Native mass spectrometry (shows dimer in solution)
- NMR
- EPR spectroscopy (DEER)
- DLS
- CD

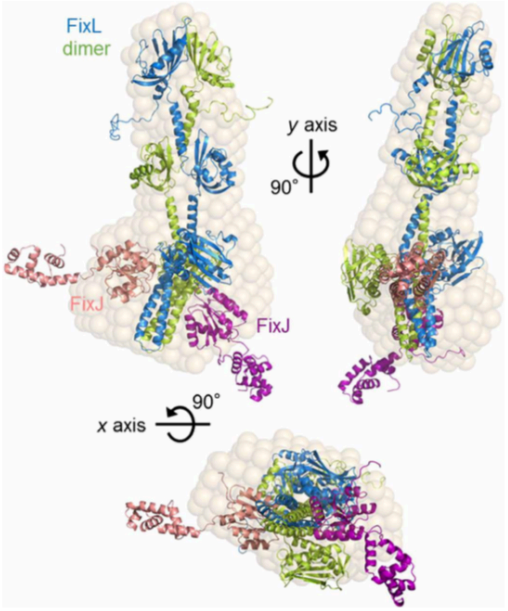


Example of sophisticated pseudoatomic homology model building and refinement oxygen-sensing FixL-FixJ

(Wright et al. Sci. Signal. 11 10 April 2018)

Data from:

- crystallography
- SEC-SAXS
- Existing fragments from PDB
- homology modeling
- Validation of part of model via SAXS on truncated protein



Software:

- JPred, Coils (which parts are helices vs coils)
- PEP-FOLD generate models of linkers
- Torsion angle MD in CNS to refine positions of domains and loops
- SWISS-MODEL to generate homology model
- HADDOCK to refine and dock domains
- pyDockSAXS and FoXS-Dock for placement of domains
- FTDock/Crysol and PatchDock/FoXS