Total electron scattering

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a.o. based on: Latychevskaia T, Abrahams JP (2019) “Inelastic scattering and solvent scattering reduce dynamical diffraction in biological crystals” *Acta Crystallogr.* **B75**, 523–531

Protein electron crystallography: theory *vs.* practice

electron nano-crystallography will not work for 3D protein crystals

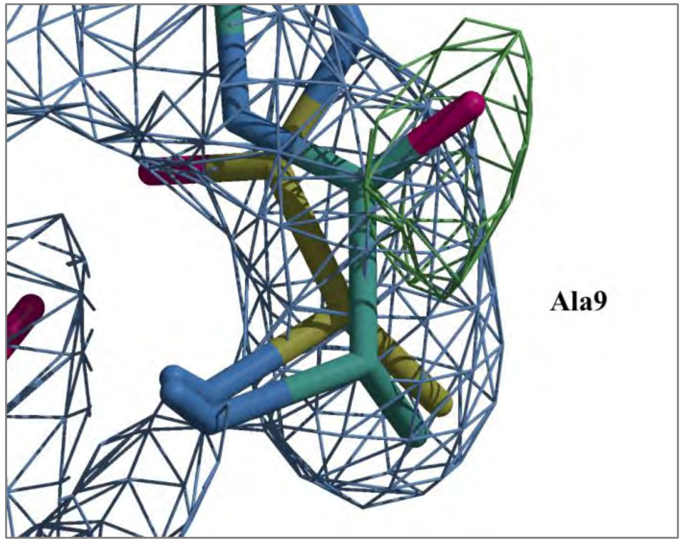
"Simulations show that **dynamical scattering** in 3D protein crystals is **too high** for structure determination."

Glaeser, R.M. & Downing, K.H. (1993) *Ultramicroscopy* **52**, 478-486, DOI: 10.1016/0304-3991(93)90064-5

Subramanian, G., Basu, S., Liu, H.G., Zuo, J.M. & Spence, J.C.H. (2015) *Ultramicroscopy* **148**, 87-93, DOI: 10.1016/j.ultramic.2014.08.013

*Solvent and **inelastic scattering** were not considered*

Protein structures were solved by electron nano-crystallography



Electron diffraction of a single 100 nm lysozyme crystal reveals a peptide flip in a rare lysozyme polymorph*.

*Clabbers MTB, van Genderen E, Wan W, Wiegers EL, Gruene T, Abrahams JP (2017) "Protein structure determination by electron diffraction using a single three-dimensional nanocrystal" Acta Crystallogr. D73 738-748

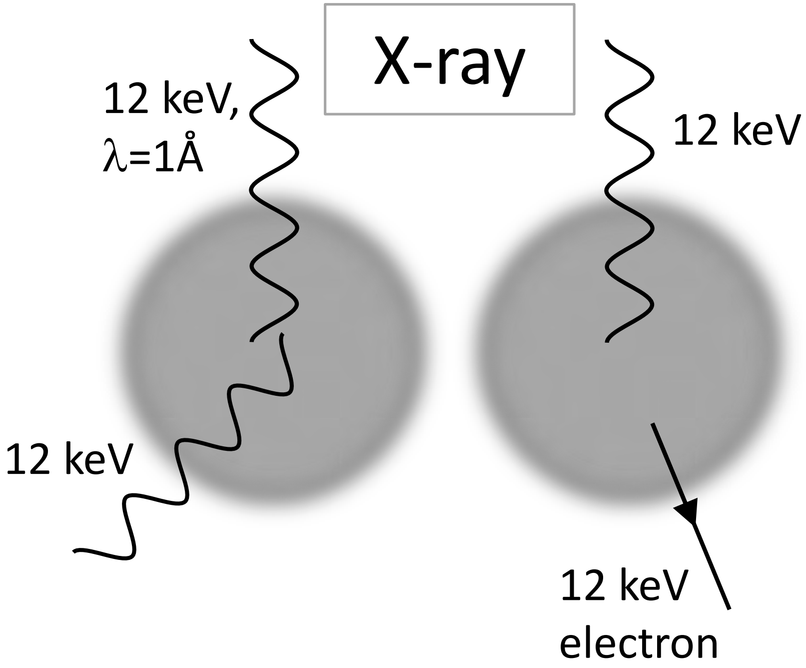
Learning objectives

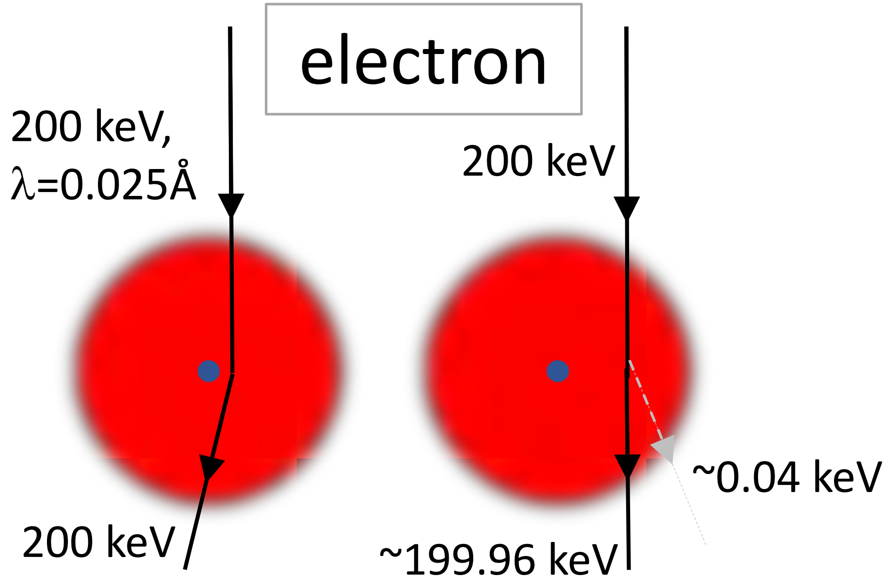
- Improved understanding of:
  - Physics of diffraction: electrons *vs.* X-rays
  - Elastic electron diffraction
  - Inelastic/plasmon electron scattering
  - Dynamical electron diffraction
  - Interplay of elastic & inelastic electron scattering: total scattering

Electrons *vs* X-rays in organic matter

- Electrons interact 100’000 times more strongly with organic matter.
- Penetration depth; 50% scattered in organic matter after:
  - 200 keV electrons: 50 nm
  - 12 keV X-rays: 16 mm
- Relative to heavier elements, H-atoms scatter e^- more effectively than X-rays:
  - $p_{X-ray}(Z) \propto \sim Z^2$
  - $p_{electron}(Z) \propto \sim Z^{4/3}$

Scattering of X-rays and electrons





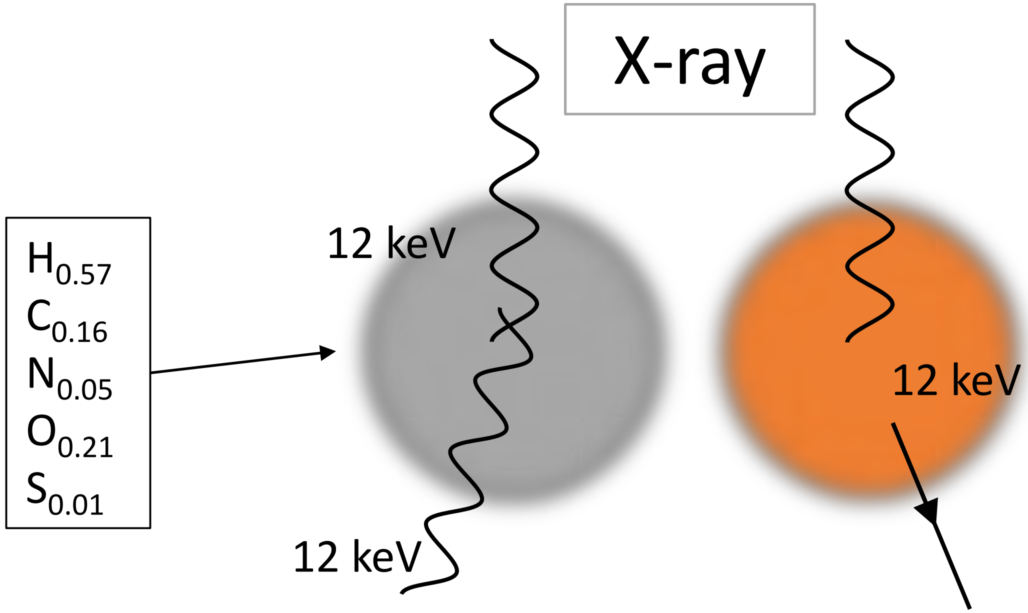
Heavy elements suffer relatively more from radiation damage by X-rays:

$$\frac{p_{elastic}}{p_{inelastic}}(Z) \propto \sim Z^{-1}$$

Light elements suffer relatively more from radiation damage by electrons:

$$\frac{p_{elastic}}{p_{inelastic}}(Z) \propto \sim Z$$

Scattering probabilities of X-ray & e$^-$ by an 'average' hydrated protein atom ($Z \approx 3.7$)



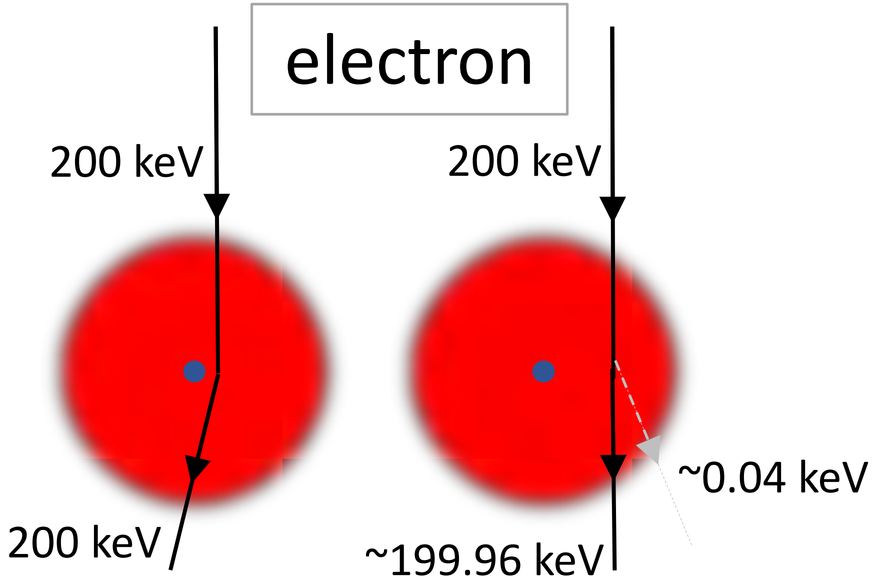
Scattering probabilitiy / nm:

Inelastic: $P_{i,Xray}$ = ~3.9 10^{-7}

Elastic: $P_{e,Xray}$ = ~2.5 10^{-8}

ratio:

$P_{i,Xray} / P_{e,Xray}$ = ~15



Scattering probabilitiy / nm:

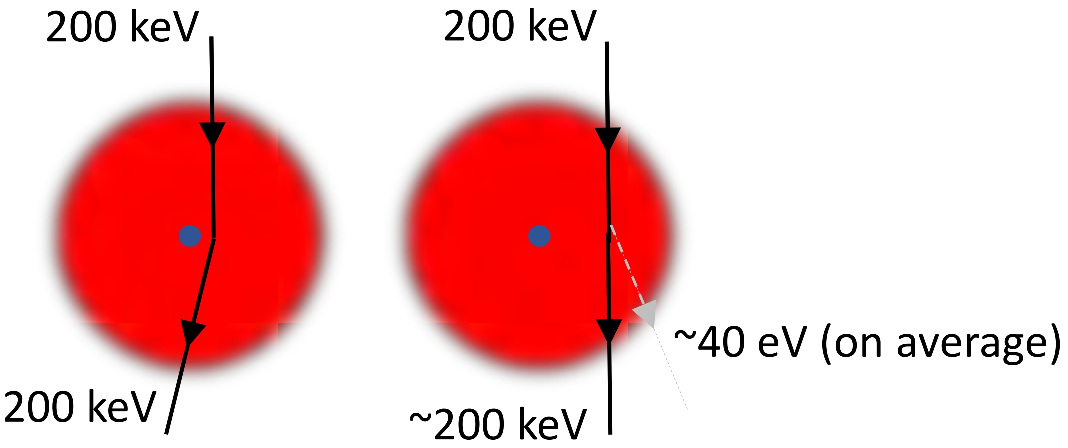
Inelastic: $P_{i,electr}$ = ~1.1 10^{-2}

Elastic: $P_{e,electr}$ = ~2.7 10^{-3}

ratio:

$P_{i,electr} / P_{e,electr}$ = ~4

The elastic & inelastic electron scattering



For organic samples:

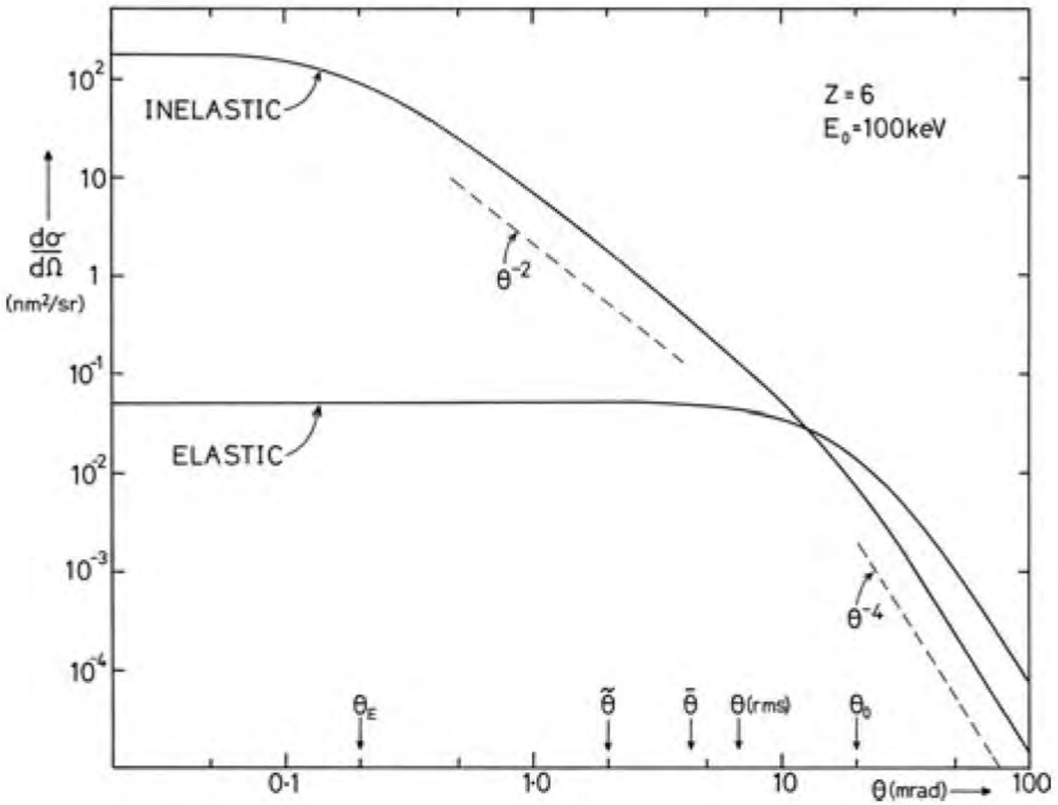
Elastic / nuclear:	Inelastic
$P_e = \pm 0.2$	$P_i = \pm 0.8$

In organic matter, only 1 in 5 scattering events is elastic!

Deposited energy can vary and is used in EELS for determining elemental composition.

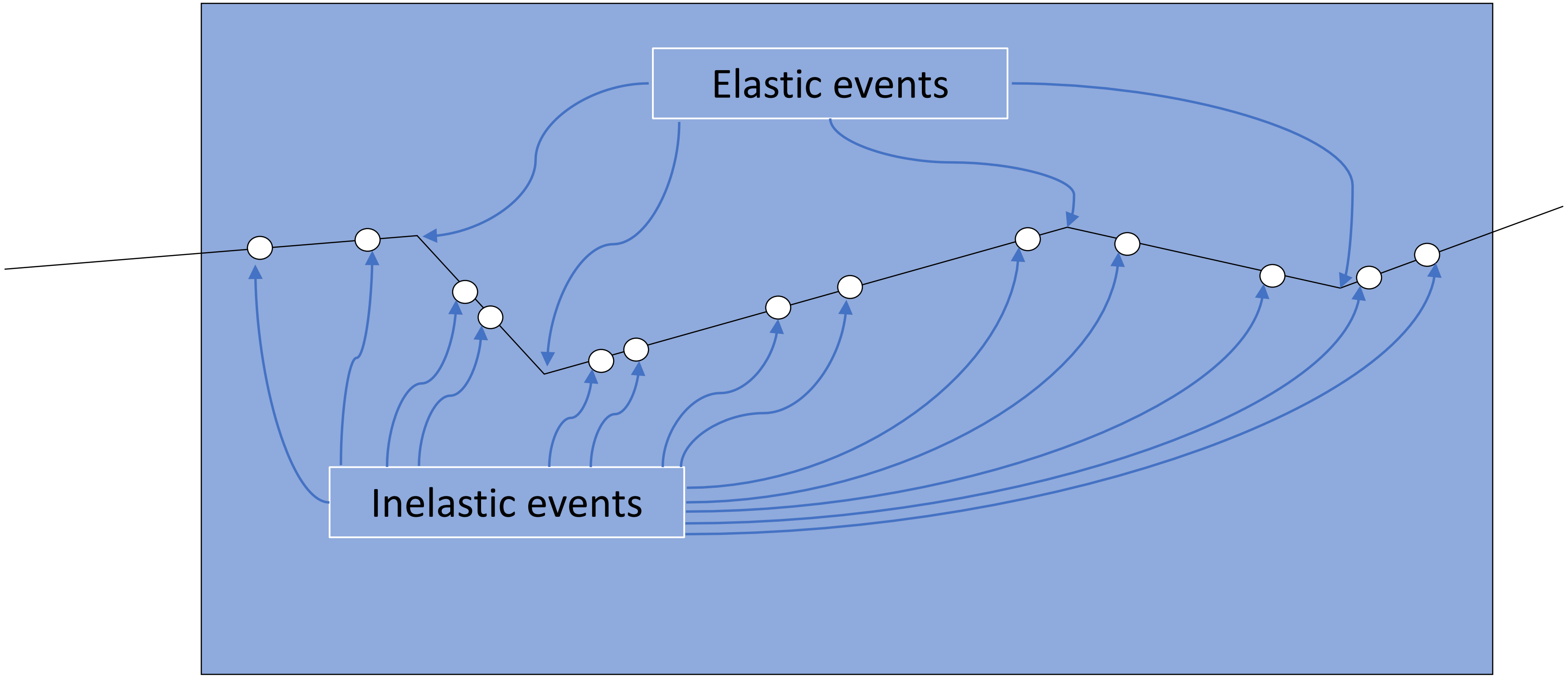
Elastic and inelastic scattering angles

Fig. 3.7 Angular dependence of the differential cross sections for elastic and inelastic scattering of 100-keV electrons from a carbon atom, calculated using the Lenz model (Eqs. (3.50), (3.7), and (3.15)). Shown along the horizontal axis are (*from left to right*) the characteristic, median, mean, root-mean-square and effective cutoff angles for total inelastic scattering, evaluated using Eqs. (3.53), (3.54), (3.55), and (3.56)

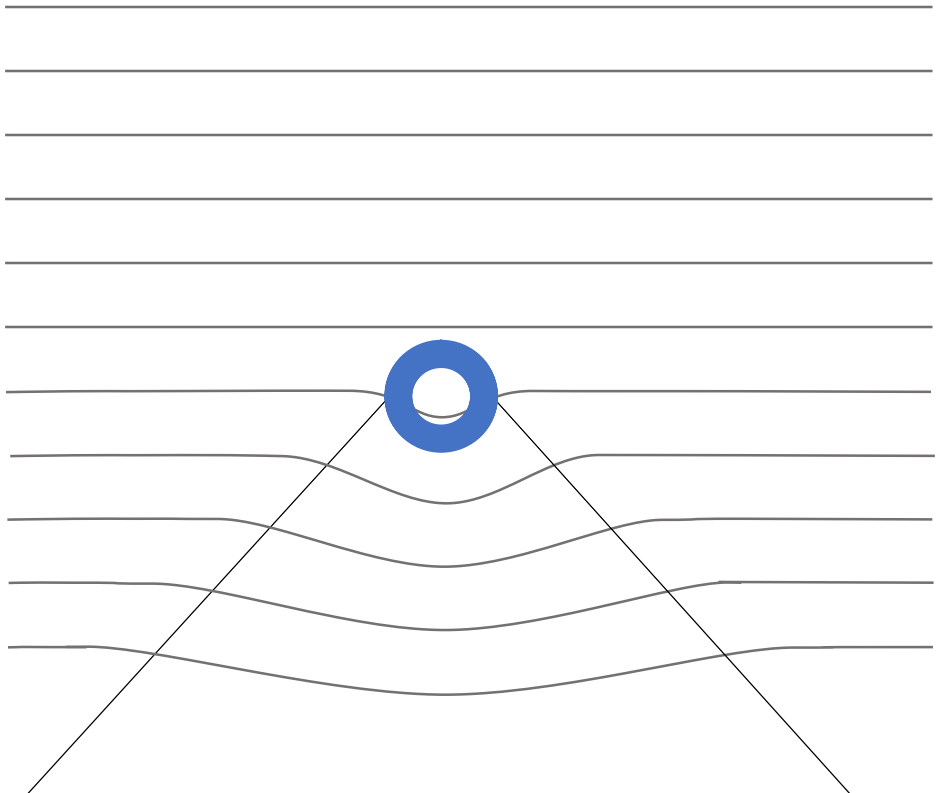


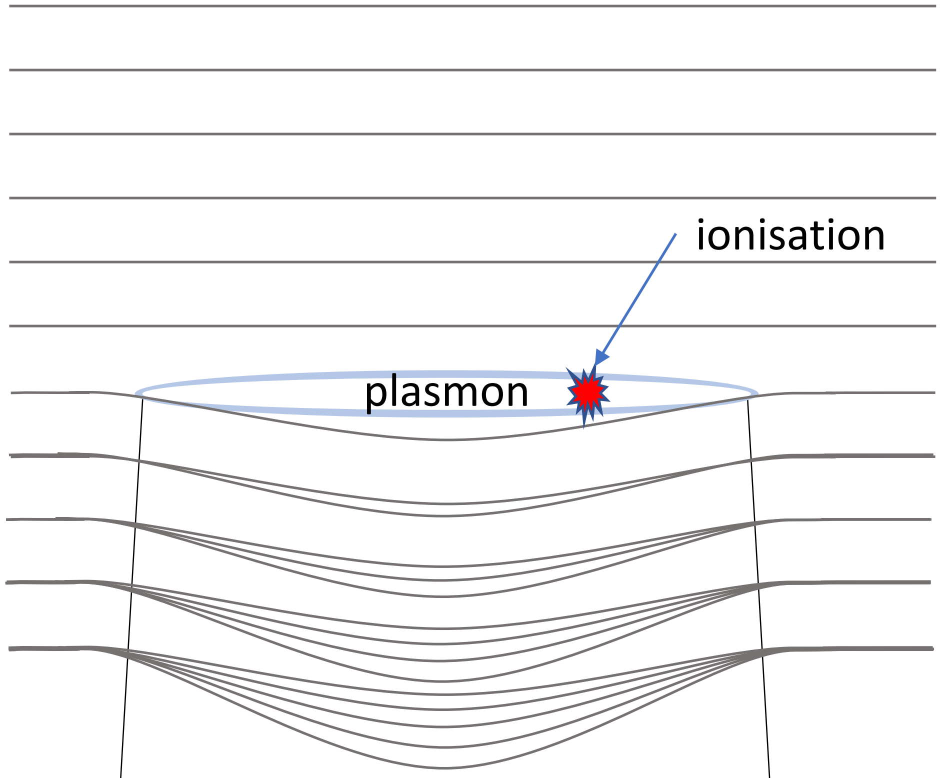
Egerton, R.E "Electron energy loss spectroscopy in the electron microscope - 3rd Ed.", Springer (2011), p 125

Multiple scattering of an electron (as a particle)



Elastic & inelastic electron scattering wave functions





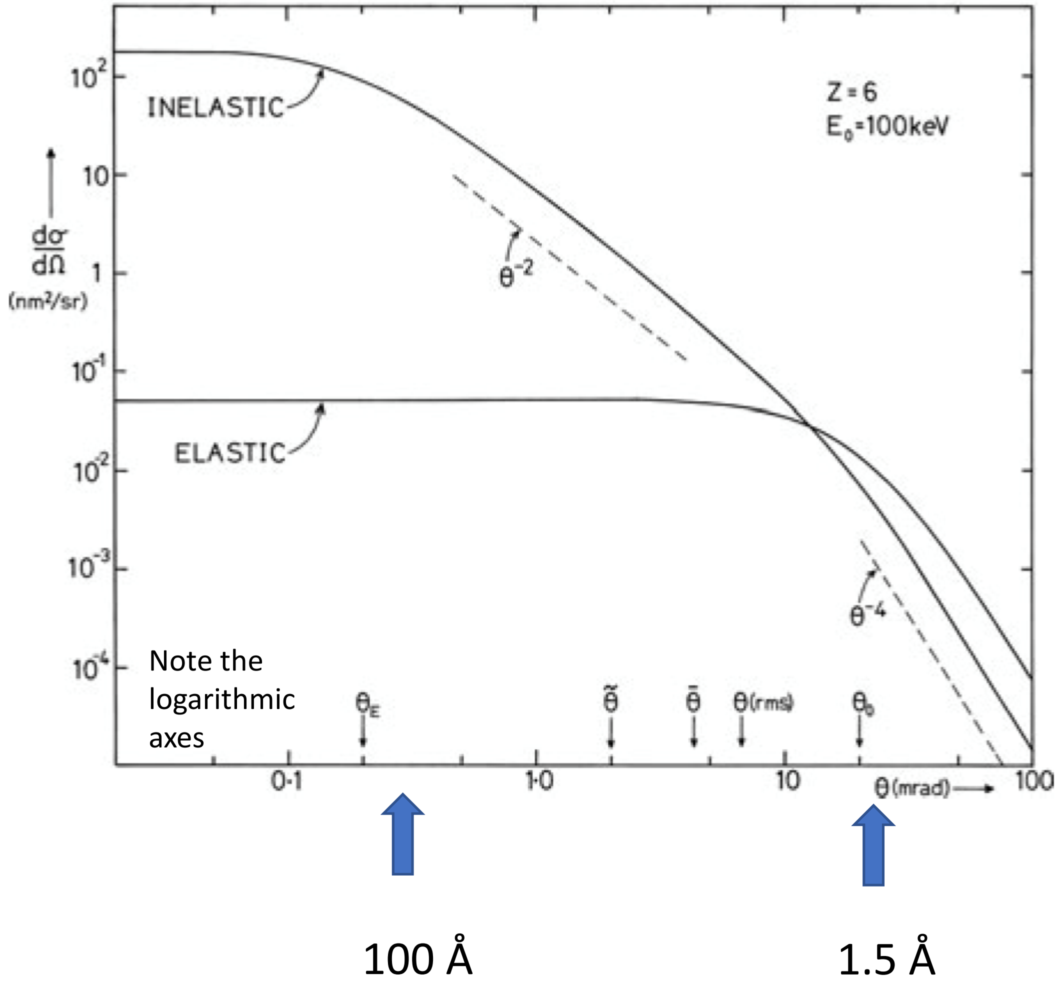
Elastic scattering:

- No energy deposition
- Wide scattering angle
- Coherent wave function

Inelastic scattering:

- Variable amount of energy deposition causes local damage
- Narrow scattering angle
- Wavelength dispersion, incoherent wave function

How big is a plasmon?



The scattered wave is determined by the Fourier transform of the object.

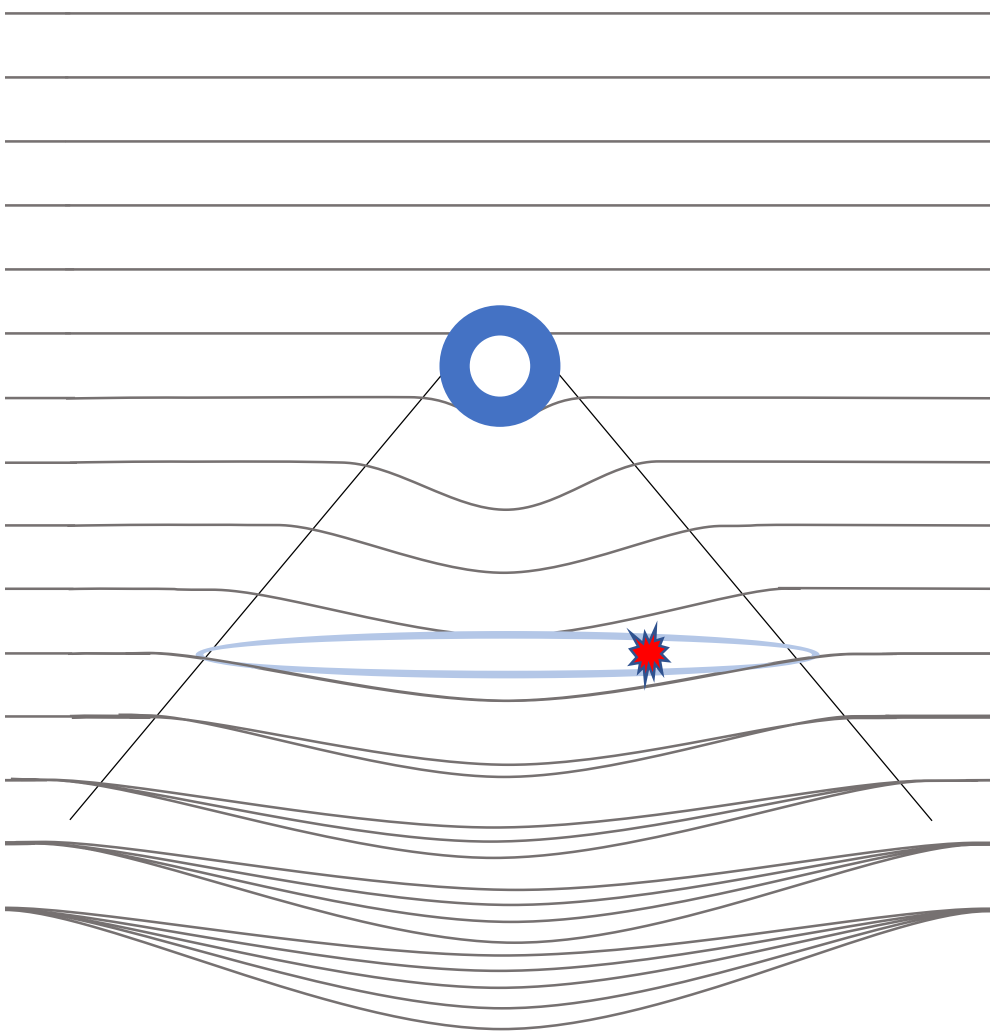
If the object is Gaussian (⌀ = α), its FT has a width α^{-1}.

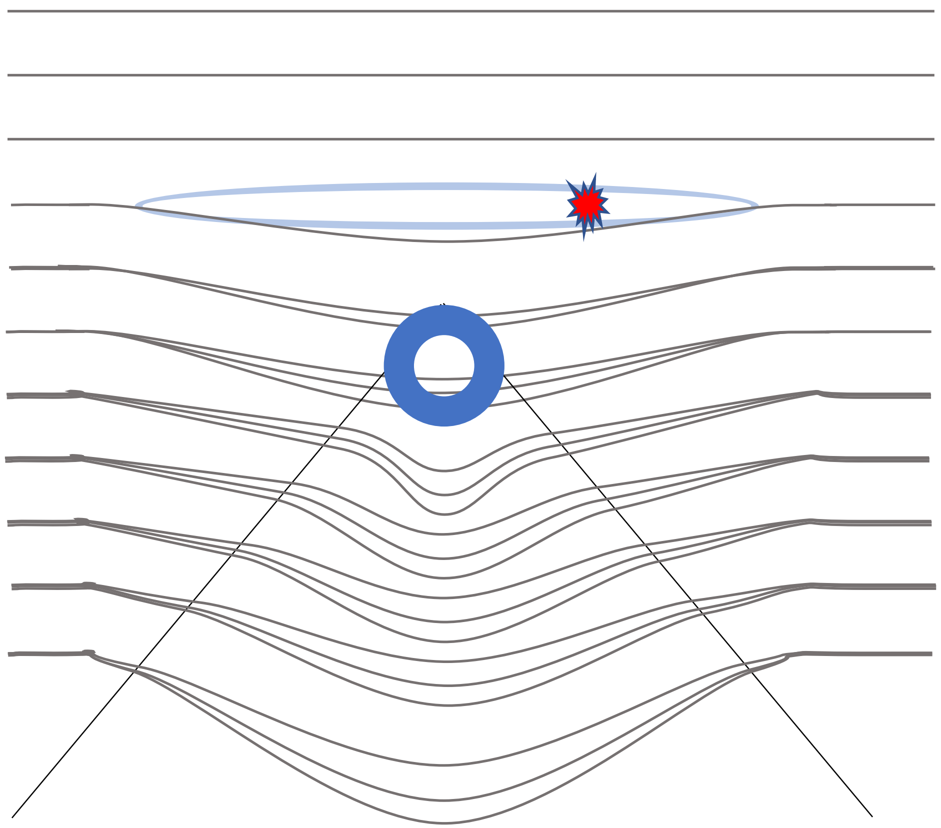
Atoms (⌀ ≈ 1Å) scatter elastically.

Plasmons scatter inelastically.

Plasmon diameter in carbon is ~100Å.

Multiple scattering (wave interpretation)

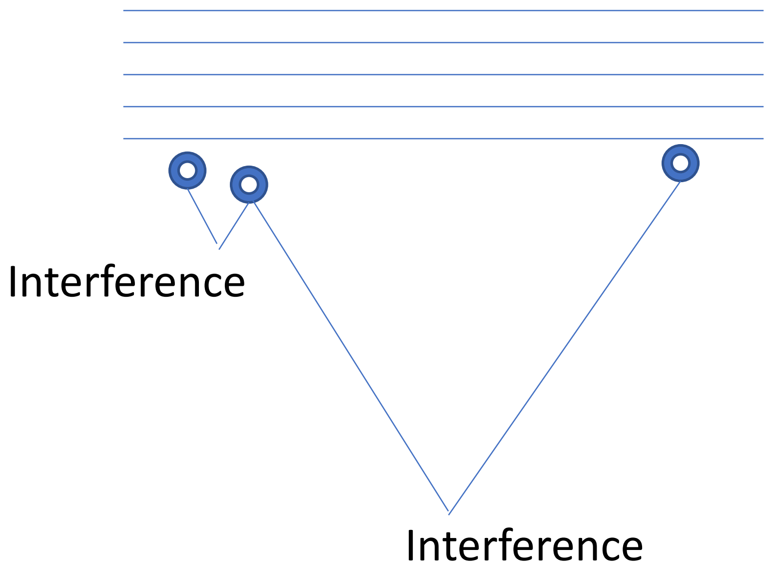




Multiple elastic/inelastic scattering produces an incoherent wave function

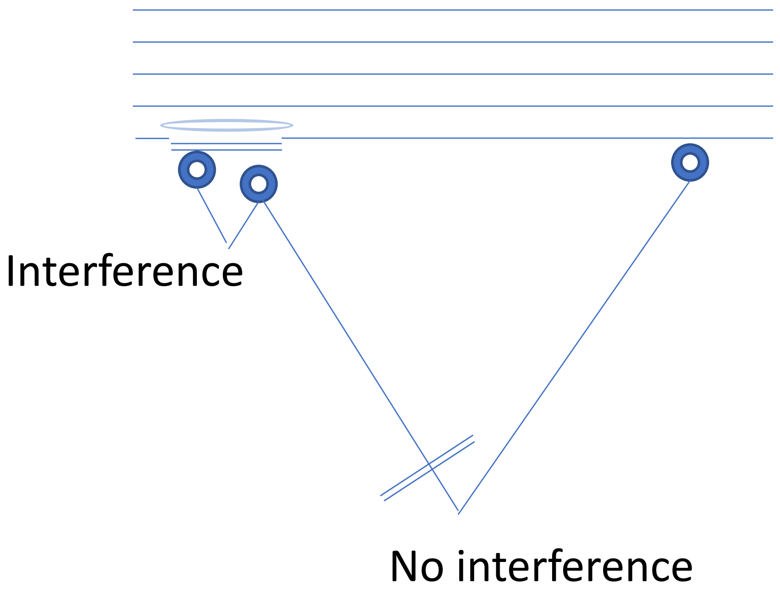
Inelastic scattering fragments the wave function

All elastic



Local & global structure both induce interference.

Elastic & inelastic



Only local structure induces interference. Global structure does not.

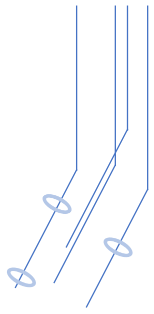
Summarizing…

- Inelastic (plasmon) scattering dominates in organic samples.
- Electrons lose coherence by inelastic scattering.
- Inelastic scattering hardly disperses the wave function.
- Inelastic scattering fragments the wave function.
- Spatial coherence loss is determined by the size of the plasmon.
- In organic samples, plasmon size is in the 10 nm range.
- Chromatic coherence loss due to wavelength dispersion is determined by variable energy deposition (the type of radiation damage).
- This deteriorates the diffraction signal of features larger than the plasmon size.

Reduction of dynamical diffraction in crystals with large unit cells

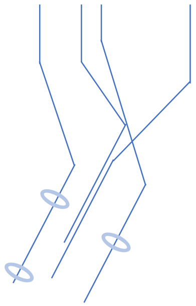
Kinematic diffraction is determined by considering all paths for which:

The first interaction is elastic
(Any subsequent interactions are inelastic)



Dynamic diffraction for all paths for which:

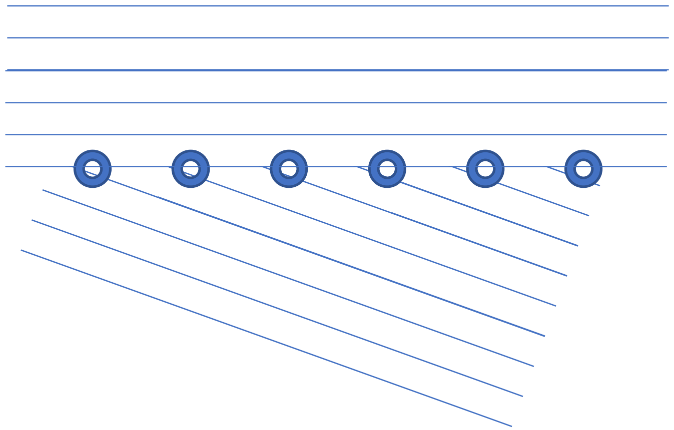
The first *n* interactions are elastic ($n > 1$)
(Any subsequent interactions are inelastic)



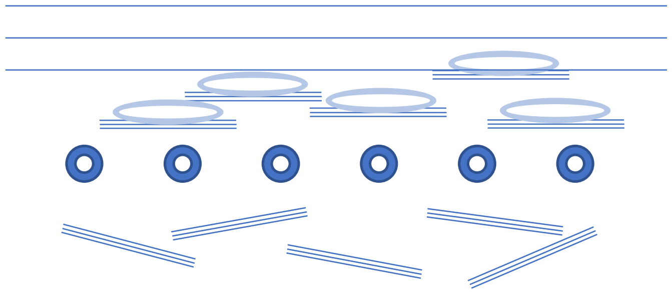
In organic samples, the probability of an inelastic event is higher than of an elastic event.
Therefore, there are more paths* where the initial elastic event is followed by an inelastic event,
compared to paths where it is followed by an elastic event.

*If you want to know why this matters, google for 'Feynman path integral'

Inelastic scattering increases background between Bragg peaks



Kinematic scattering of a coherent electron wave



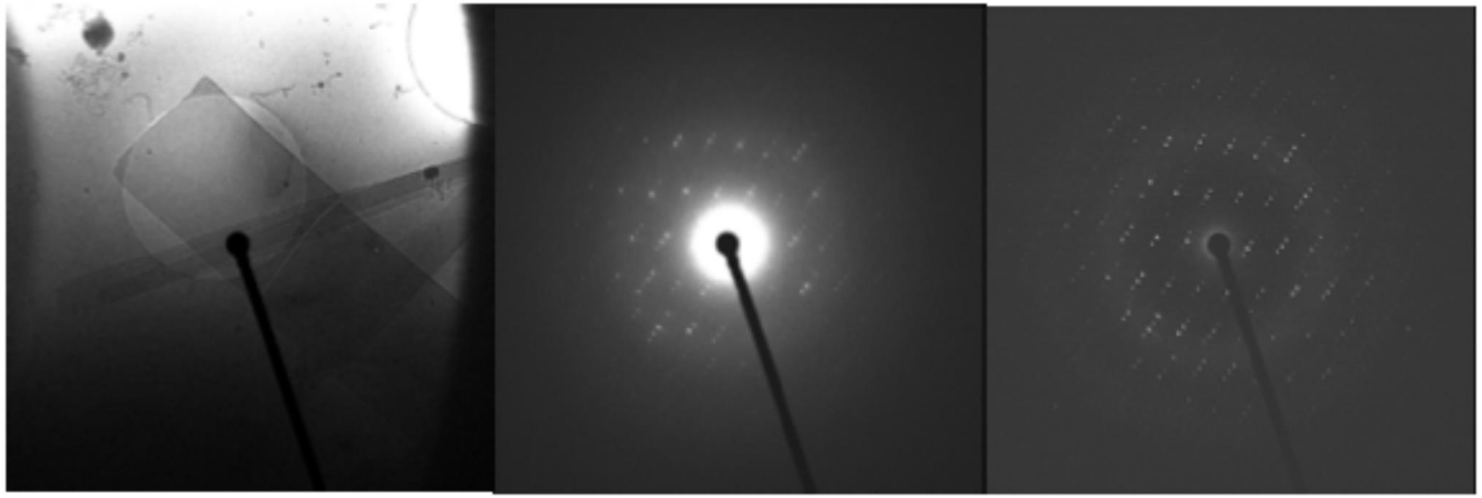
Diffuse scattering of a plasmon fragmented electron wave

When the plasmon size is smaller than the size of the unit cell, elastically diffracted wave fragments will not add up coherently in the Bragg angles.

The diffracted signal will therefore be diffuse.

Reduction of background due to energy filtering was indeed observed

Without filtering Zero loss mode



First acquisition Second acquisition

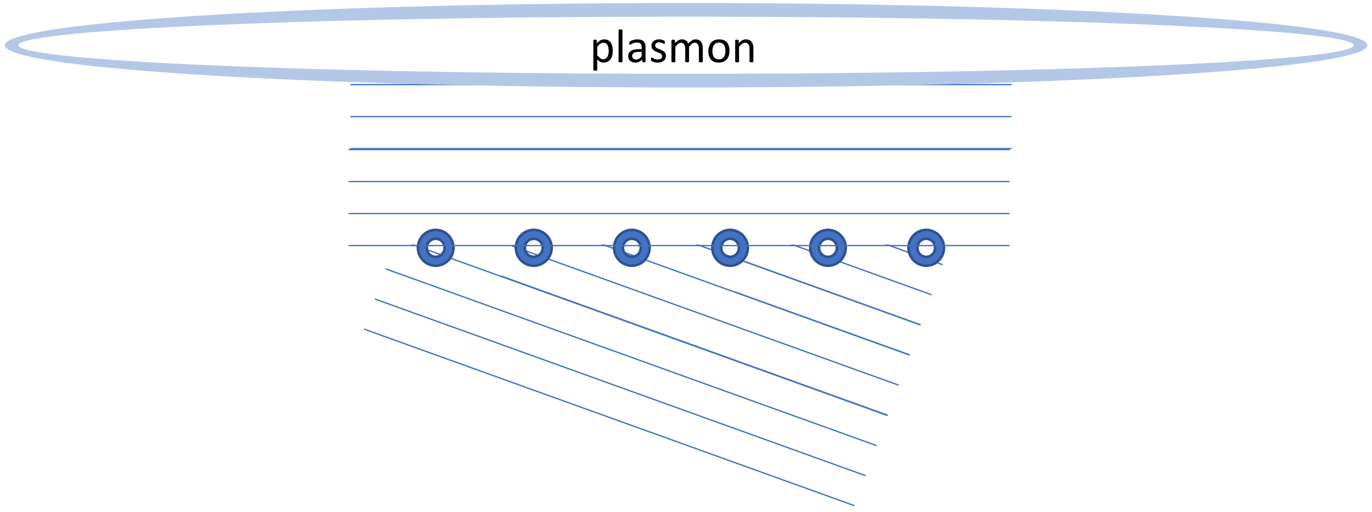
Recorded on a JEOL 2200FS, Lysozyme micro-crystals

Courtesy Patrick Bron & Francois Hoh, CBS Montpellier

Consequences of multiple elastic / inelastic scattering in protein crystals

- Dynamical scattering is reduced
- Background between Bragg peaks is increased

Electron diffraction of crystals with a unit cell smaller than a plasmon (and single molecules)



Effects of inelastic scattering on the observed diffraction is much smaller!

Conclusions

- Energy filtering reduces background but increases the dynamical diffraction signal for protein crystals.
- The more inelastic scattering, relative to elastic scattering, the lower the dynamical signal. So for materials containing heavier atoms, this effect is much less pronounced.
- The larger the size of the unit cell, relative to the size of the plasmon, the lower the dynamic component.
- The calculated crystal thickness based on the dynamical signal, underestimates the physical thickness in organic samples.