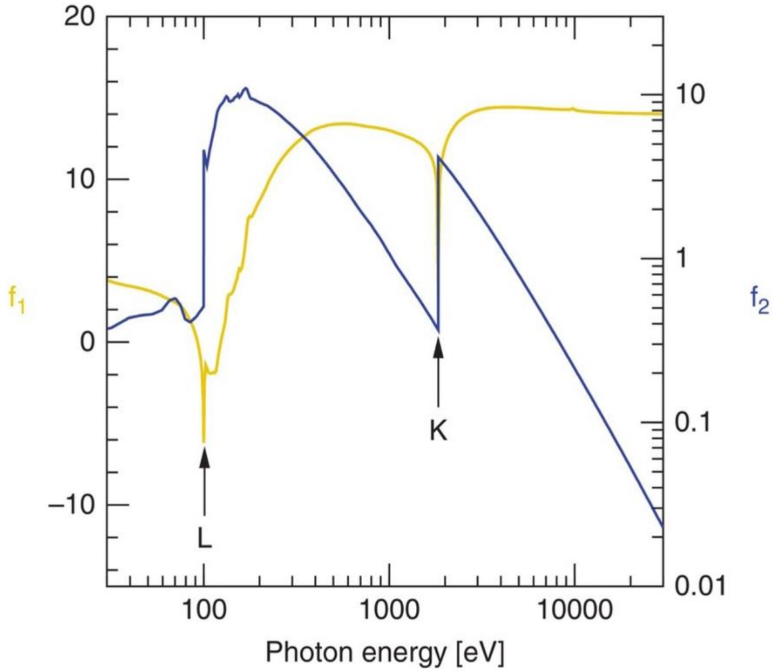
Structural Analysis
Part III - X-ray tools

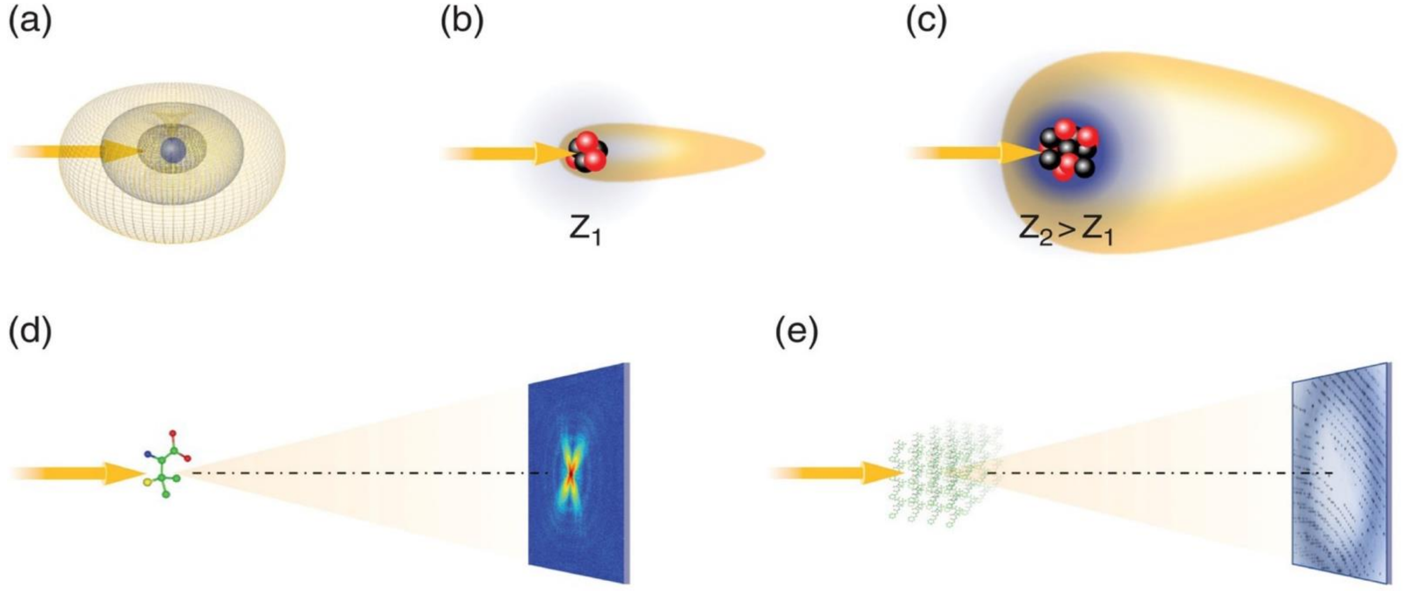
Session 4:

X-ray diffraction applications
X-ray spectroscopy

Reminder about previous weeks

Atomic scattering factors again

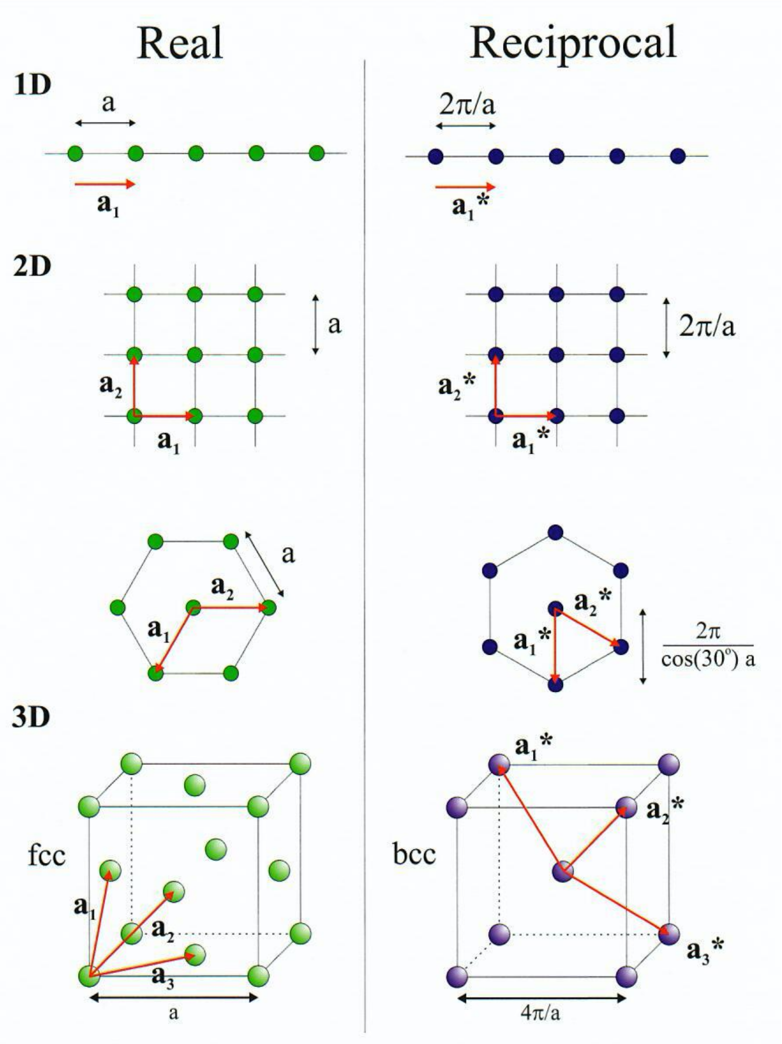




Z for $Q \to 0$

$$\boxed{f(Q,h\nu) = \underbrace{f^0(Q) + f'(h\nu)}_{\text{"scattering"}} + \underbrace{i f''(h\nu)}_{\text{absorption}}}$$

For each lattice a reciprocal lattice can be defined



From physics

→ for each lattice there exist a so-called reciprocal lattice

→ the reciprocal lattice ~~th~~ is the Fourier transform of the direct lattic

→ reciprocal lattice is defined

$$\vec{G} = h\vec{a}_1^* + k\vec{a}_2^* + l\vec{a}_3^*$$

$$|\vec{G}| = \frac{2\pi}{d_{hkl}}$$

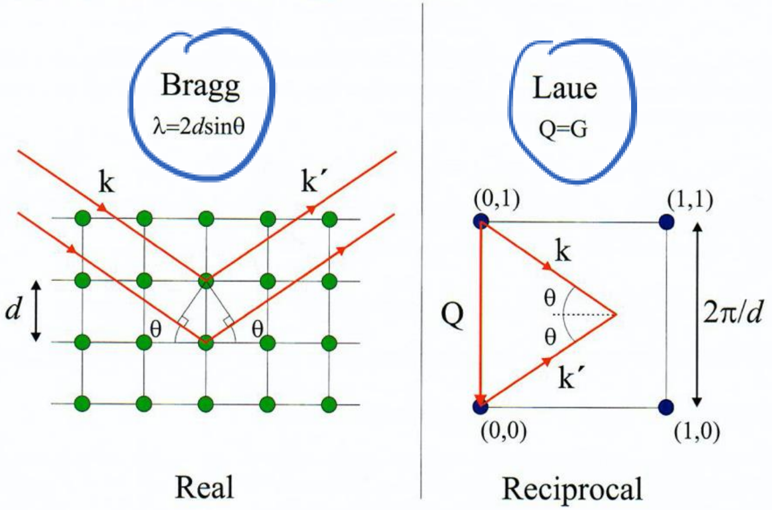
$\vec{G}$ → perpendicular on planes

$h\,k\,l$ → Miller indices

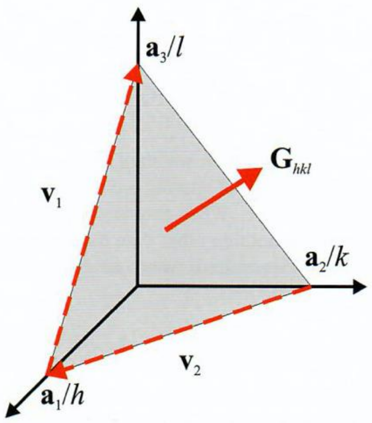
Bargg and Laue conditions

Real Inverse

(a) Equivalence of Bragg and Laue



(b) Miller indices and reciprocal lattice vectors



The Nobel Prize in Physics 1914

Photo from the Nobel Foundation archive.

Max von Laue

Prize share: 1/1

In reciprocal space one can show that for

$$\boxed{\vec{Q} = \vec{G}}$$

constructive interference is obtained

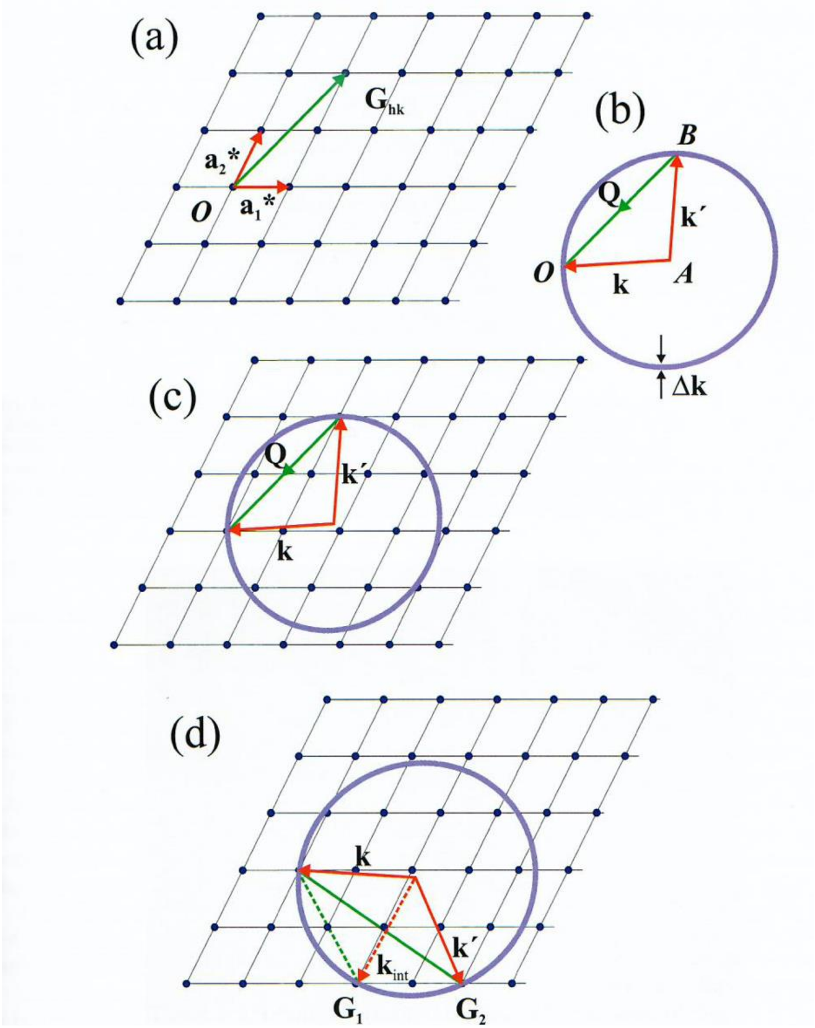
$\vec{G}$ → perpendicular to lattice planes

$|\vec{G}| = \frac{2\pi}{d_{hkl}}$ → lattice spacing of hkl planes

The Ewald Sphere

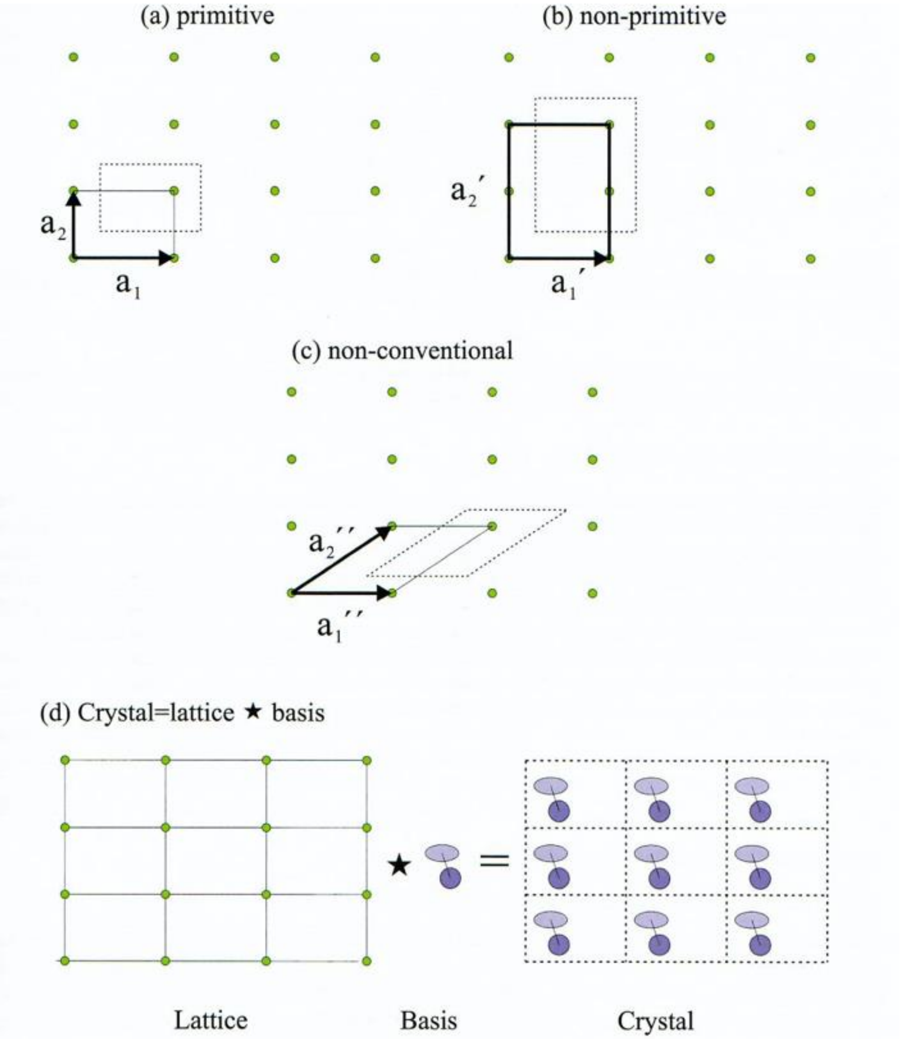
→ Elegant geometric construction of relationship

- $\vec{Q} = \vec{k} - \vec{k}'$ → wave vector $\vec{k}$
  → diffraction angle
  → in reciprocal lattice
- Laue condition $\vec{Q} = \vec{G}_{hkl}$
- Create sphere (in 2D: circle) with radius k
- Move sphere on origin O
- Monochromatic light can be scattered constructively ("signal") whenever sphere overlaps with reciprocal lattice point as here $\vec{Q} = \vec{G}$



More on Bragg peaks

A crystal is defined by ist lattice and basis



Convolution Lattice ⊗ Base

Remember: diffraction ⟷ Fourier transform

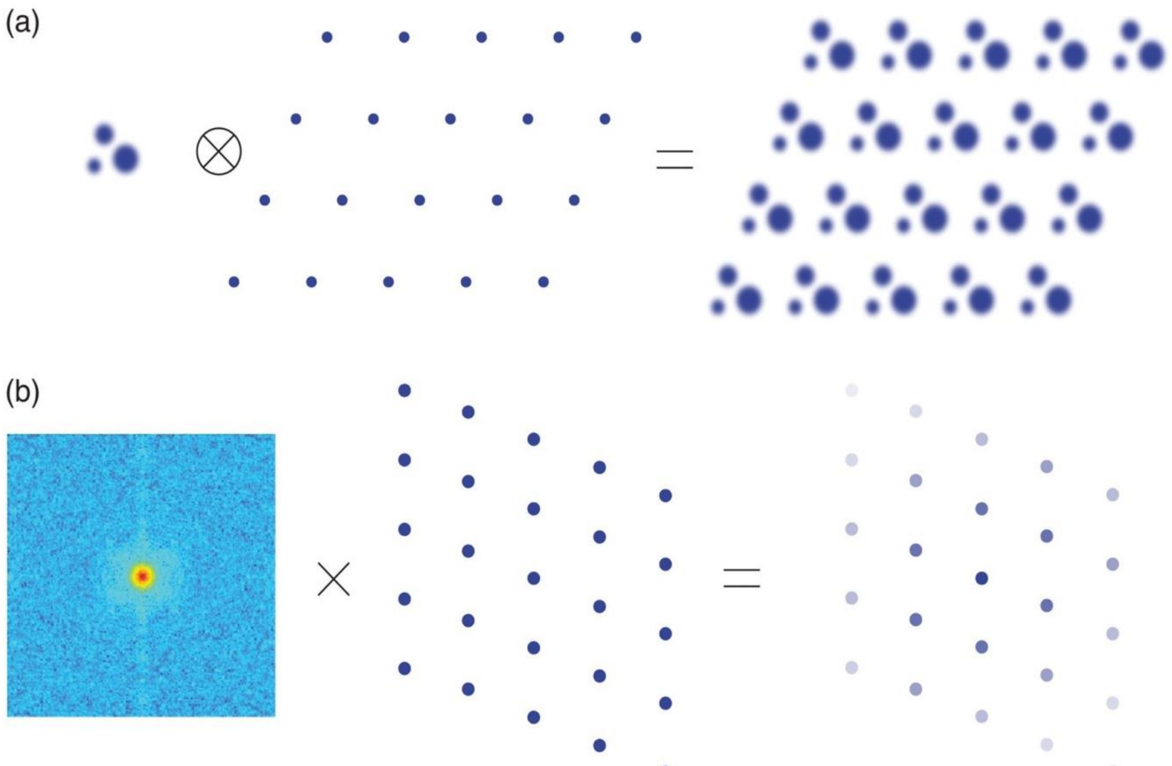
Diffraction: FT

$$FT(f \otimes g) = FT(f) \cdot FT(g)$$

or

$$FT(\text{crystal}) = FT(L \otimes B) = \underset{\substack{\downarrow \\ \text{physics, solved}}}{FT(L)} \cdot \underset{\substack{\downarrow \\ \text{sample}}}{FT(B)}$$

The structure factor



Decomposition of scattering

$$F_Q^{\text{crystal}} = \sum_e^{\text{all atoms}} f_e(Q)\, e^{iQr}$$

structure factor

scattering vector

Leave calculations to physicists

$$F_{\text{crystal}} = \underbrace{\sum e^{iQR_n}}_{\text{Lattice}} \cdot \underbrace{\sum f_i(Q)\, e^{iQr}}_{\text{Base}}$$

Geometry info | Structure factor

Other way to look at it:
Crystal in real space – Convolution of Lattice ⊗ Base
in diffraction – FT(Lattice) $\cdot$ FT(Base)

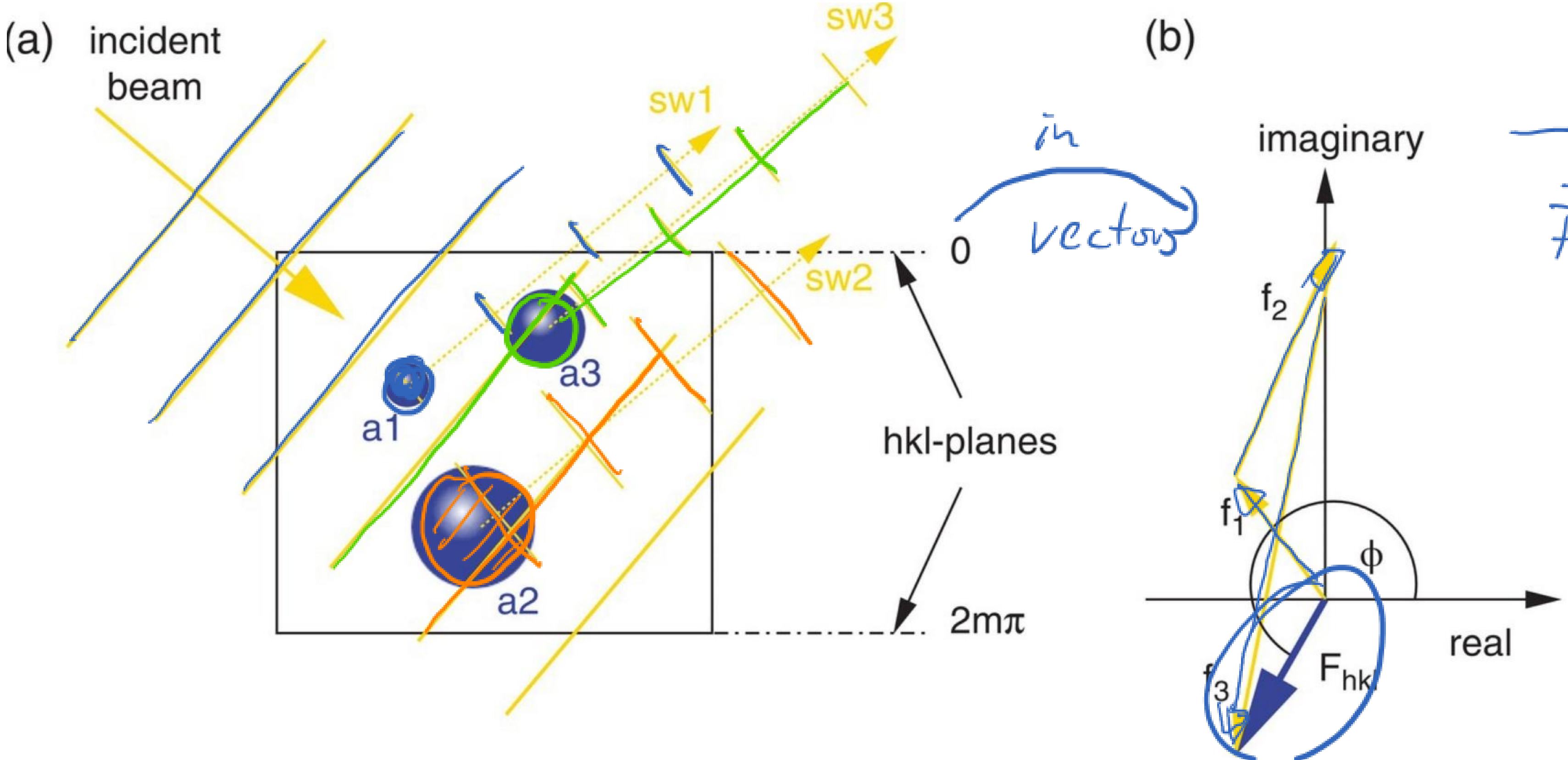
The structure factor

→ incoming X-rays scattered at atoms #1 – #3

→ how does it reflect the Bragg intensities?

⇒ Need to consider amplitude & phase

⇒ Define structure factor $F_{hkl} = \sum_i f_i \underbrace{\exp[-i2\pi(hx_i+ky_i+lz_i)]}_{\text{position in unit cell}}$



in vectors

$$\underbrace{\vec{F} = \vec{f}_1 + \vec{f}_2 + \vec{f}_3}_{\text{structure factor of unit cell}}$$

Allowed and forbidden reflections

→ There exist systematic absences due to the symmetry of the unit cell

→ "forbidden" reflections

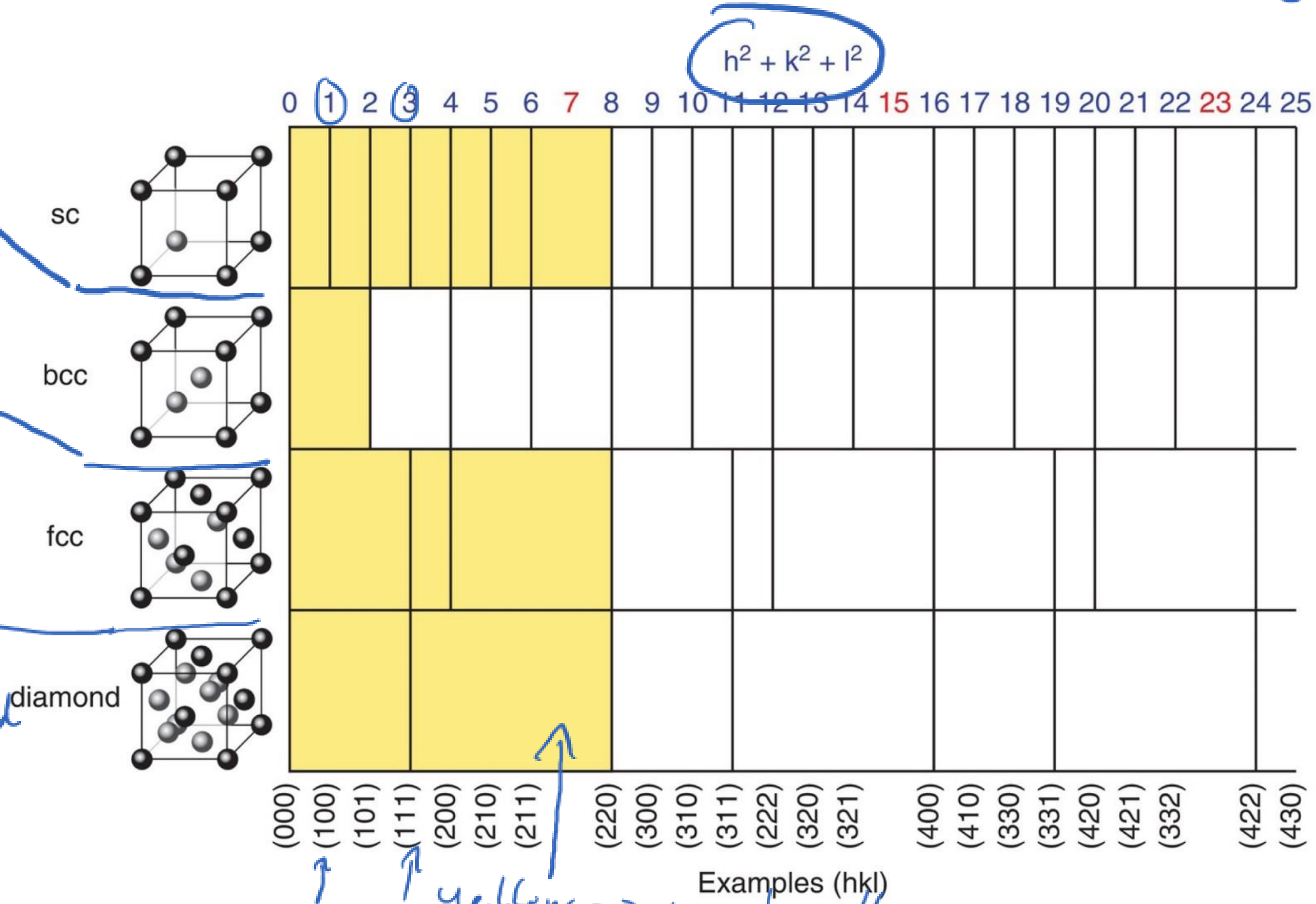
→ Can help to classify the lattice

all allowed

h+k+l = odd missing

odd/even mixed missing

more complicated



yellow → repeat pattern

Note:
this highlights the shortcomings of Bragg formalism
⇓
Not just simple reflections but it requires detailed knowledge of position & scattering strength of all atoms

The phase problem

→ So far: Bragg reflections and crystal symmetries (forbidden reflections)

→ Convolution theorem: separate lattice & structure factor

→ Structure factor contains information about atomic positions and relative distances in unit cell

Problem

→ calculated x-ray diffraction intensities are easy

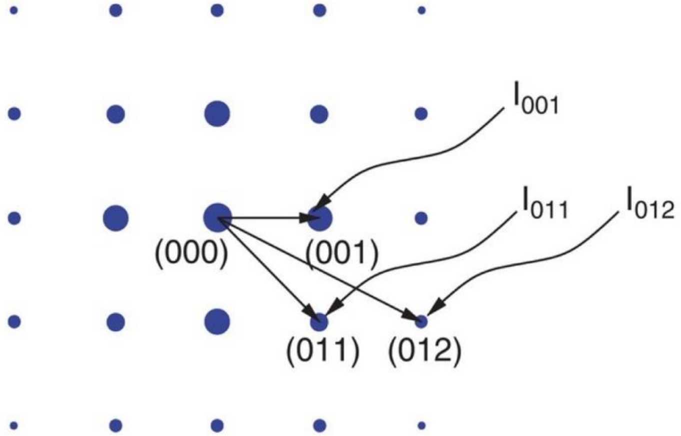
→ but we need to do the inverse

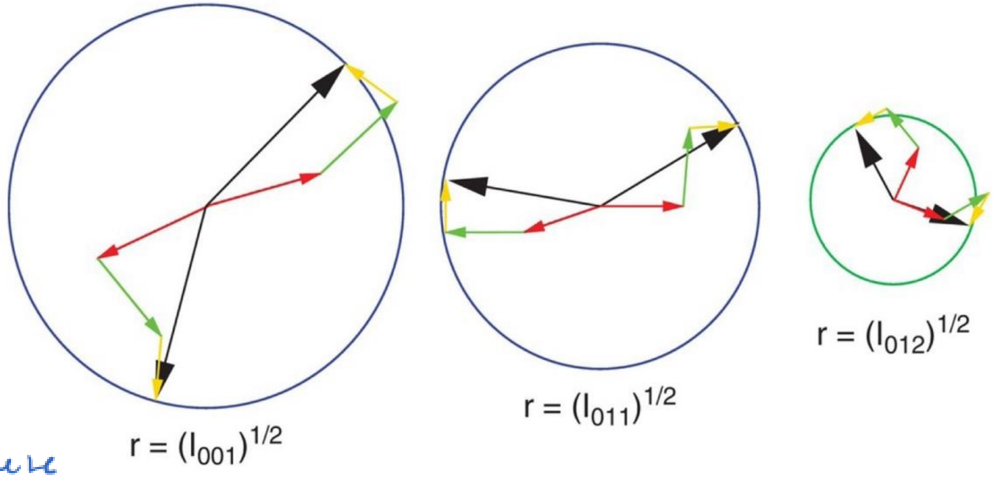
$$f(Q) = FT(\rho_{(r)})$$

$$FT^{-1}(f(Q)) = \rho_{(r)}$$

↳ we need phase

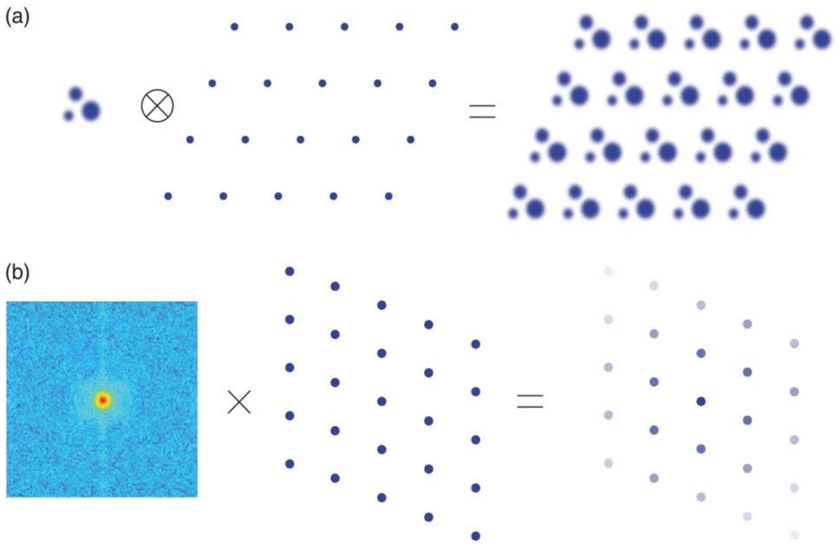
↳ but we measure intensities $I = |A|^2$ → phases lost!





Solving a crystal structure

Base ⊗ Lattice = Crystal



Molecular pattern   Bragg pattern = diffraction pattern

FT(Base) × FT (Lattice) = FT (Crystal)

Envelope   Peaks   Data

But ... Phase is lost (remember?!)

Problem

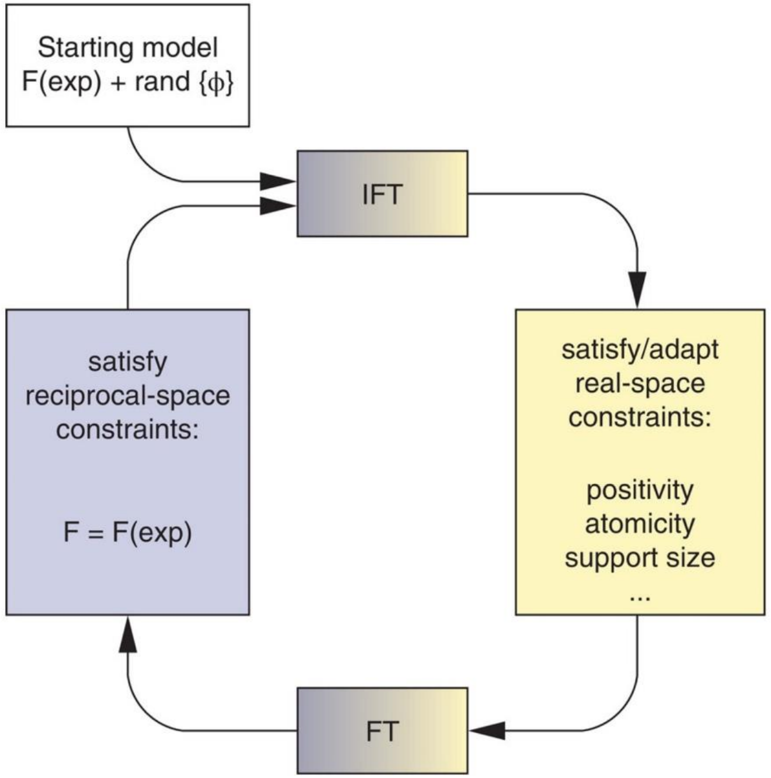
$A = A_0 \exp[\ldots \phi]$

phase needed for FT

Measurement

$I = |A^2| \Rightarrow$ Phase lost !

Reconstruct:



Starting model
F(exp) + rand {ϕ}

IFT

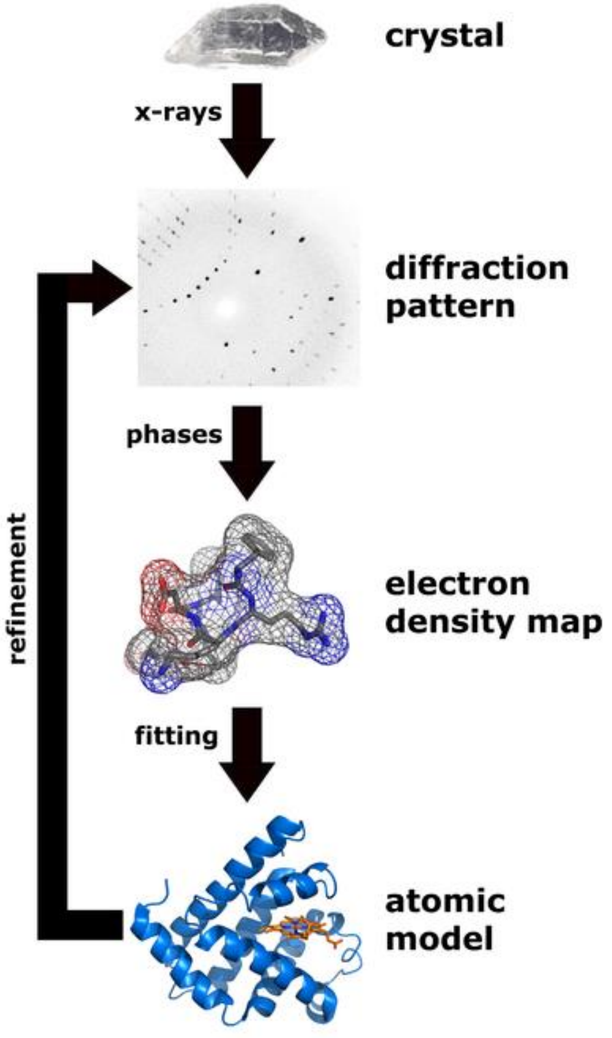
satisfy/adapt
real-space
constraints:

positivity
atomicity
support size
...

FT

satisfy
reciprocal-space
constraints:

F = F(exp)



crystal

x-rays

diffraction pattern

phases

electron density map

fitting

atomic model

refinement

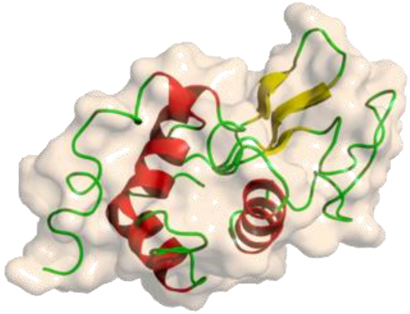
A few examples and case studies

Remember last week example for diffaction of lysozyme crystal

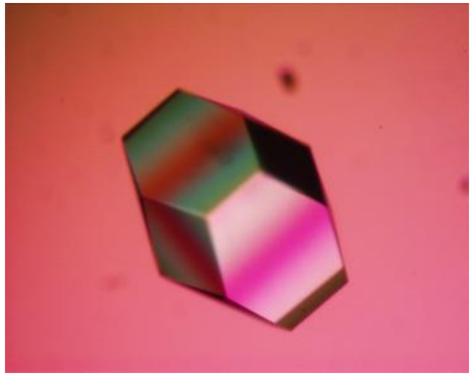
Lysozym (Protein, Immunsystem)

Stochiometric formula
$C_{125}H_{196}N_{40}O_{36}S_2$

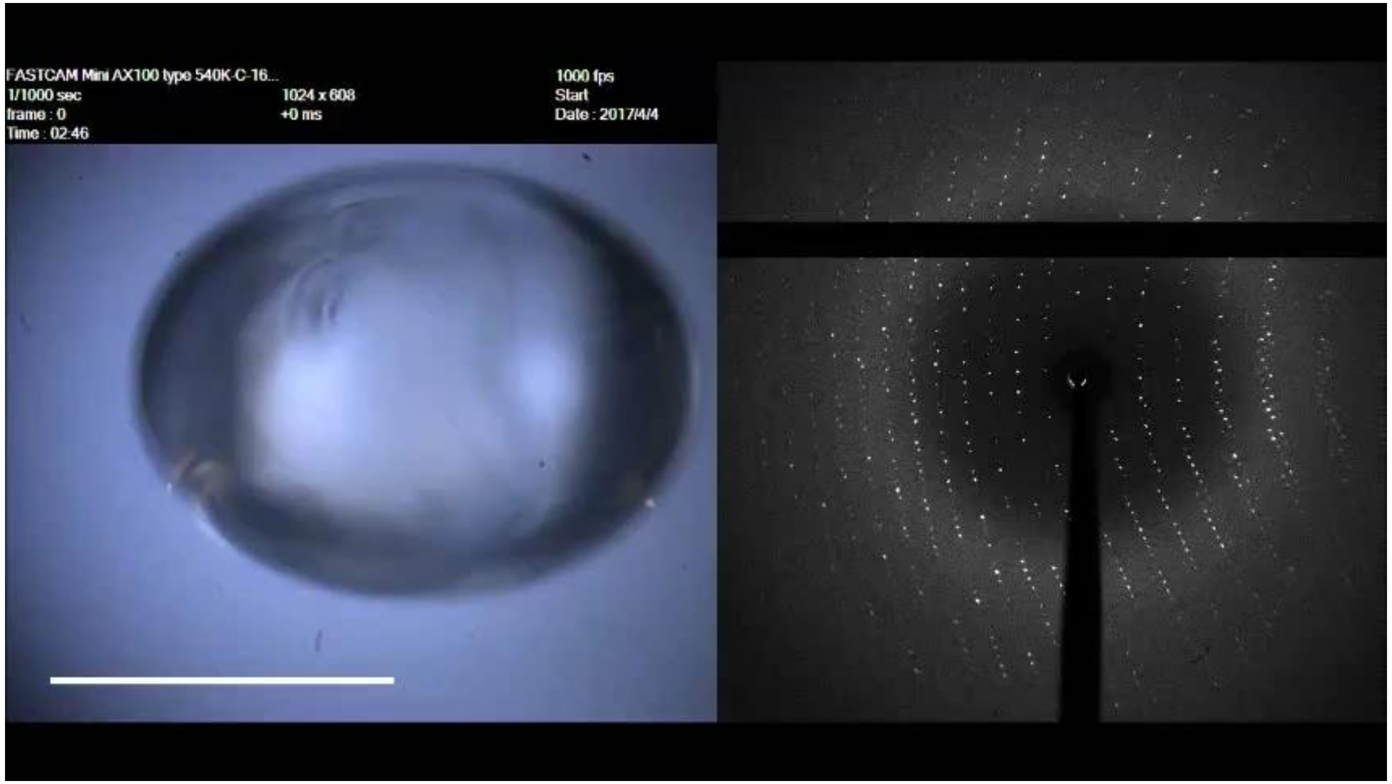
Structure



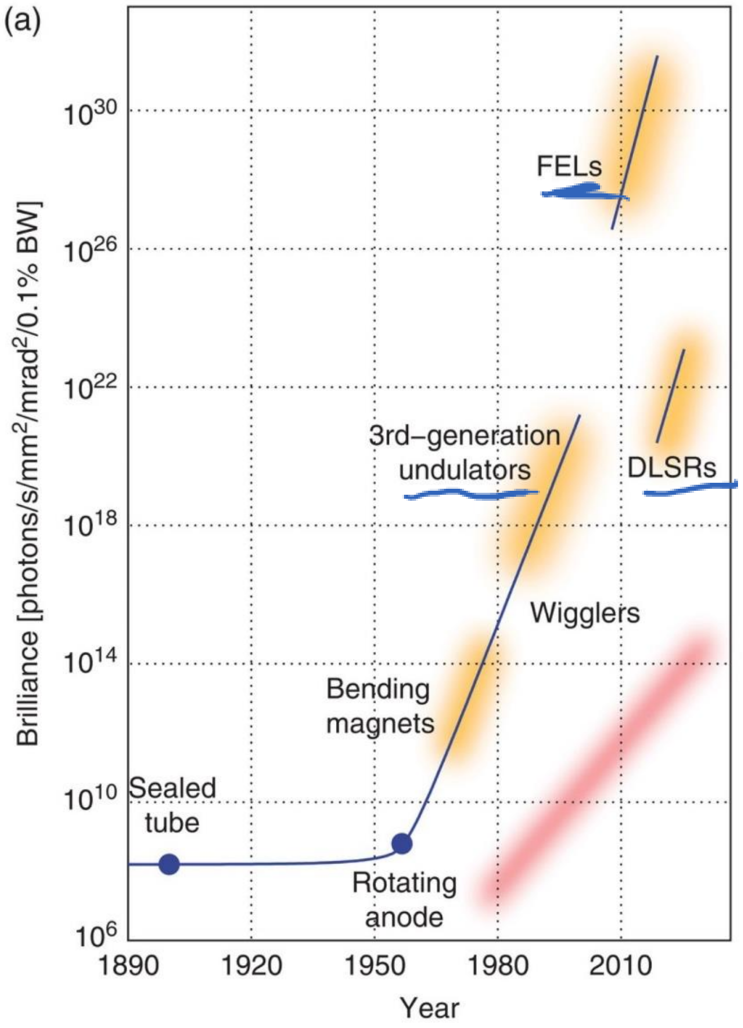
Crystal



Experiment at the Swiss Light Source



Each X-ray source allows characteristic experiments. „Output“ varies by >20 orders of magnitude

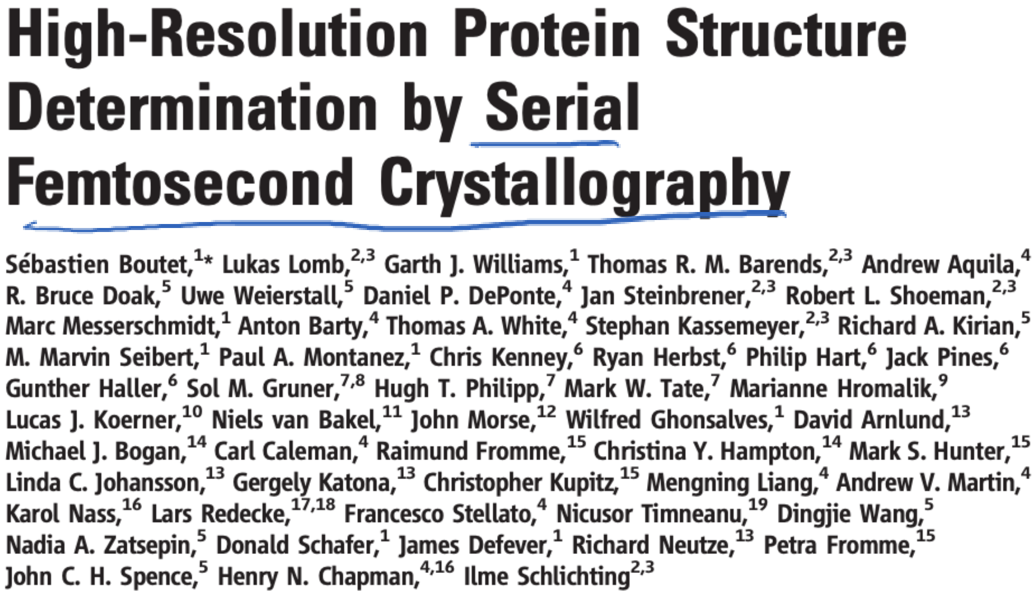


Swiss FEL
XFEL

SLS
S.R.

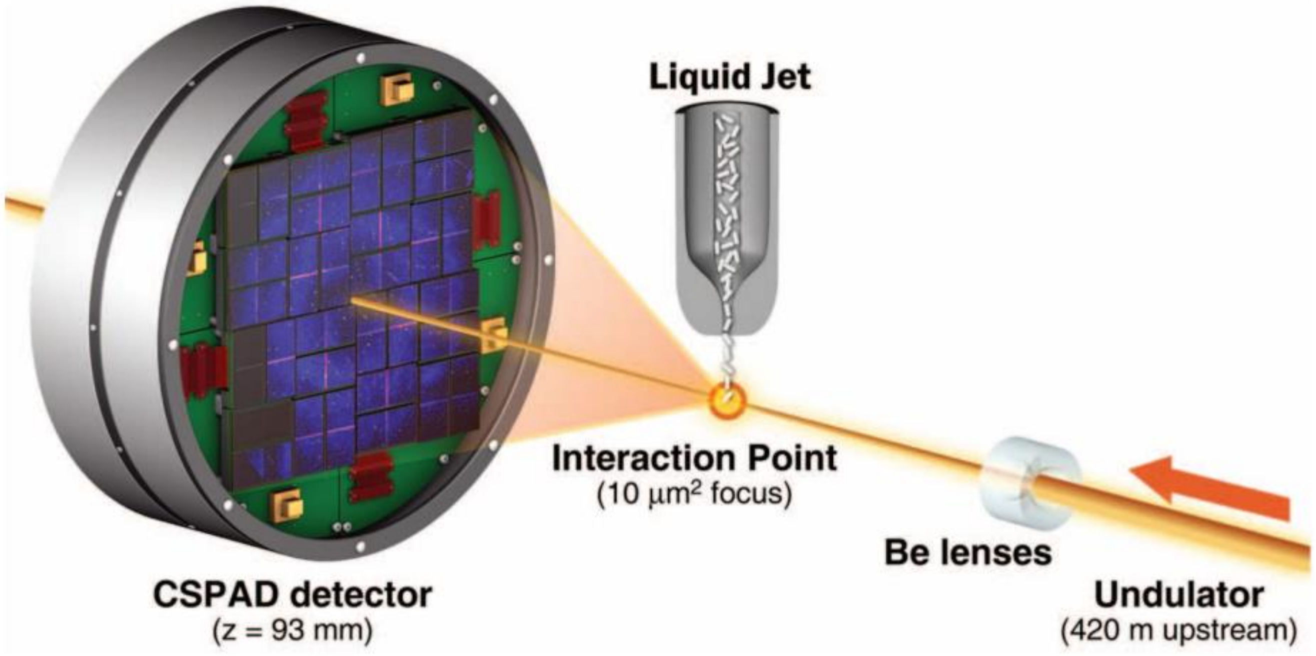
X-ray diffraction patterns from micro (nano) crystals

Science 337, page 363 (2012)



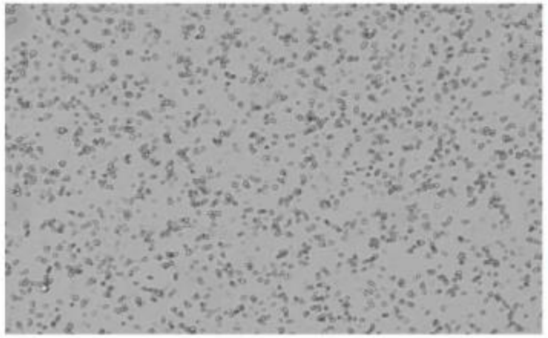
High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography

Sébastien Boutet,[1]* Lukas Lomb,[2,3] Garth J. Williams,[1] Thomas R. M. Barends,[2,3] Andrew Aquila,[4] R. Bruce Doak,[5] Uwe Weierstall,[5] Daniel P. DePonte,[4] Jan Steinbrener,[2,3] Robert L. Shoeman,[2,3] Marc Messerschmidt,[1] Anton Barty,[4] Thomas A. White,[4] Stephan Kassemeyer,[2,3] Richard A. Kirian,[5] M. Marvin Seibert,[1] Paul A. Montanez,[1] Chris Kenney,[6] Ryan Herbst,[6] Philip Hart,[6] Jack Pines,[6] Gunther Haller,[6] Sol M. Gruner,[7,8] Hugh T. Philipp,[7] Mark W. Tate,[7] Marianne Hromalik,[9] Lucas J. Koerner,[10] Niels van Bakel,[11] John Morse,[12] Wilfred Ghonsalves,[1] David Arnlund,[13] Michael J. Bogan,[14] Carl Caleman,[4] Raimund Fromme,[15] Christina Y. Hampton,[14] Mark S. Hunter,[15] Linda C. Johansson,[13] Gergely Katona,[13] Christopher Kupitz,[15] Mengning Liang,[4] Andrew V. Martin,[4] Karol Nass,[16] Lars Redecke,[17,18] Francesco Stellato,[4] Nicusor Timneanu,[19] Dingjie Wang,[5] Nadia A. Zatsepin,[5] Donald Schafer,[1] James Defever,[1] Richard Neutze,[13] Petra Fromme,[15] John C. H. Spence,[5] Henry N. Chapman,[4,16] Ilme Schlichting[2,3]

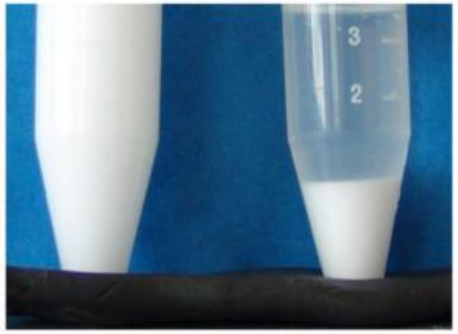


→ new XFEL application

B



C



Time-resolved x-ray diffraction

nature

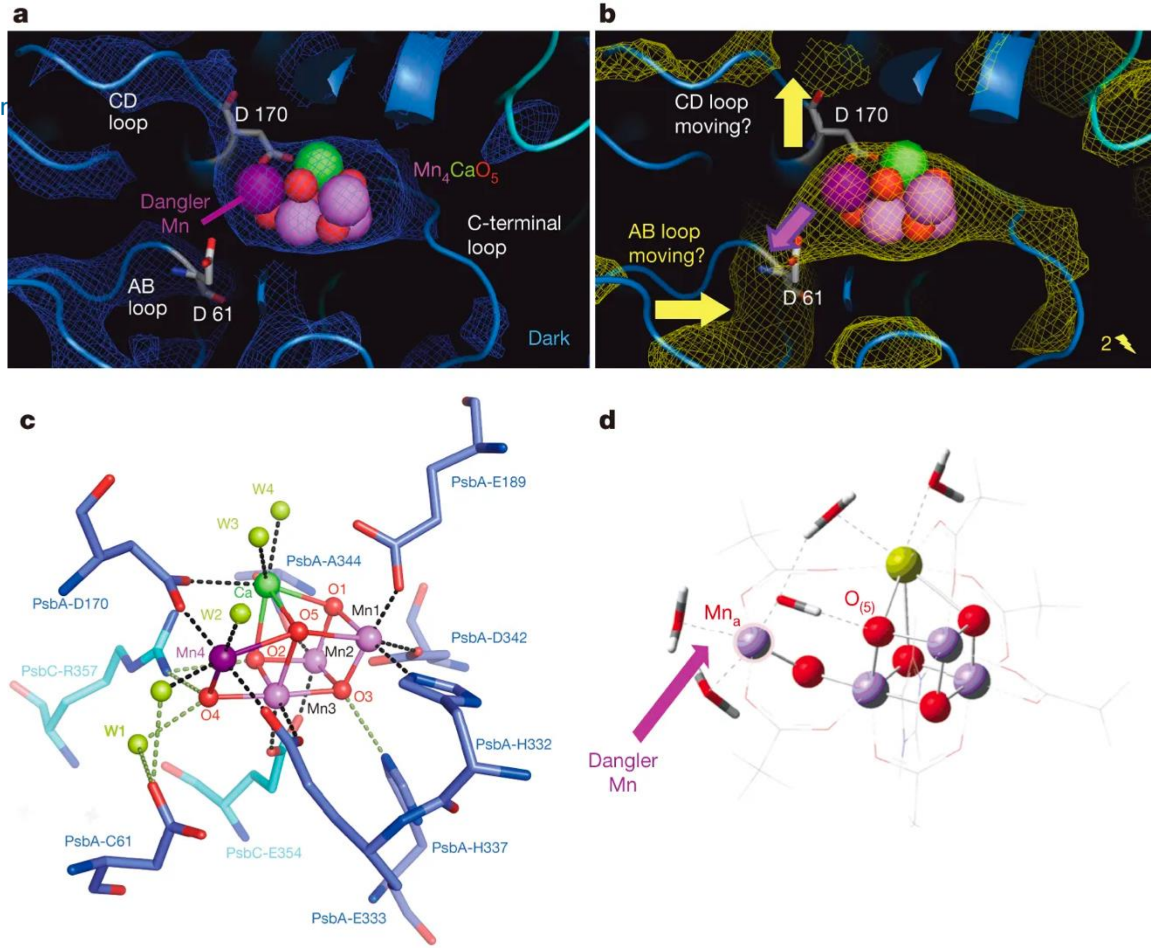
Letter | Published: 09 July 2014

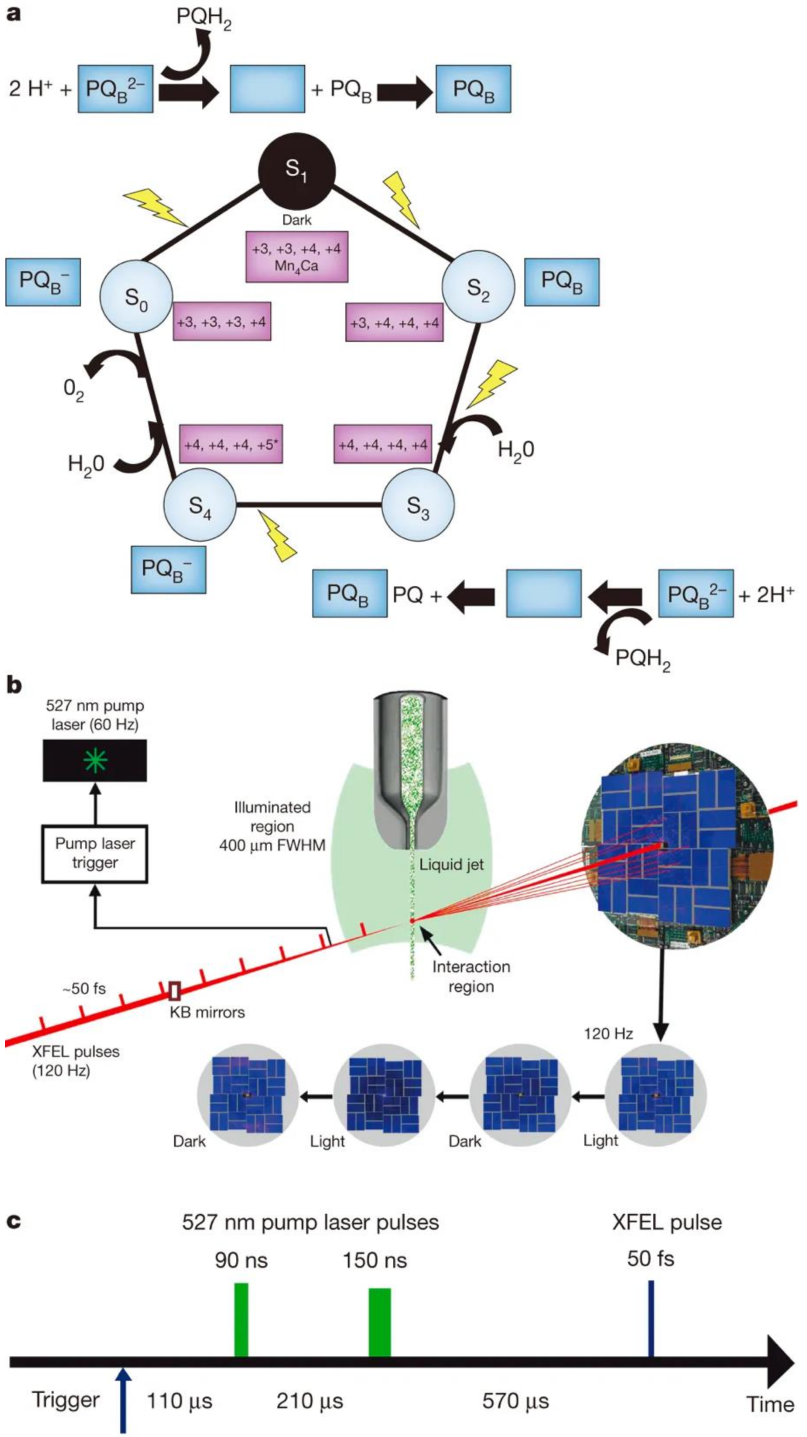
Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser

Christopher Kupitz, Shibom Basu, […] Petra Fro

Nature **513**, 261–265(2014) | Cite this article

XFEL:
from structure
to function





Recent results from SwissFEL

Article

Femtosecond-to-millisecond structural changes in a light-driven sodium pump

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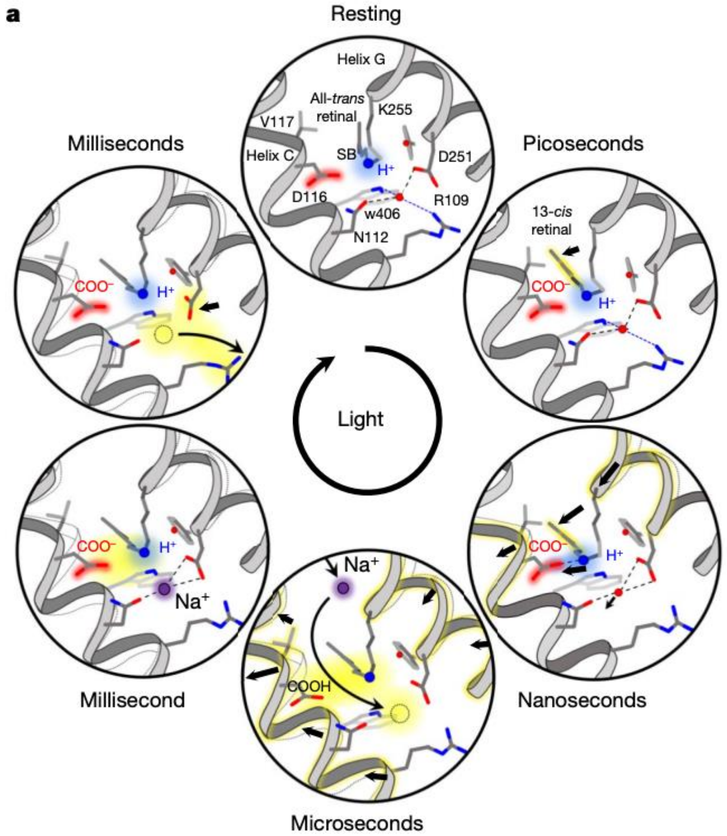
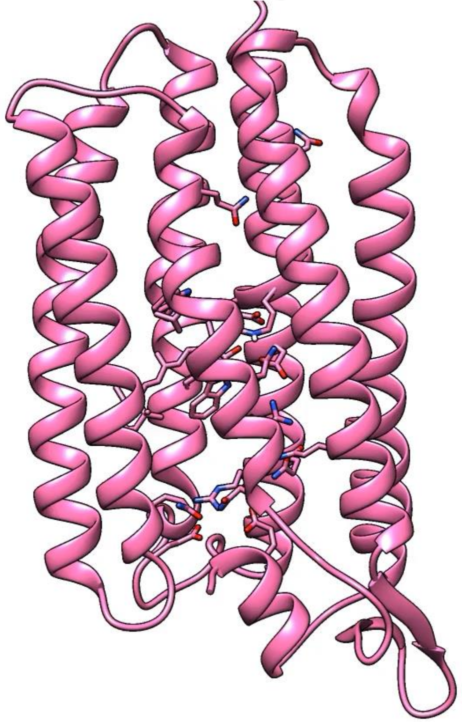
Petr Skopintsev[1], David Ehrenberg[2,13], Tobias Weinert[1,13], Daniel James[1], Rajiv K. Kar[3], Philip J. M. Johnson[4], Dmitry Ozerov[5], Antonia Furrer[1], Isabelle Martiel[6], Florian Dworkowski[6], Karol Nass[6,7], Gregor Knopp[7], Claudio Cirelli[7], Christopher Arrell[8], Dardan Gashi[1,7], Sandra Mous[9], Maximilian Wranik[1], Thomas Gruhl[1], Demet Kekilli[1], Steffen Brünle[1], Xavier Deupi[1,10], Gebhard F. X. Schertler[1,11], Roger M. Benoit[1,12], Valerie Panneels[1], Przemyslaw Nogly[9], Igor Schapiro[3], Christopher Milne[7], Joachim Heberle[2] & Jörg Standfuss[1]✉

Light-driven sodium pumps actively transport small cations across cellular membranes[1]. These pumps are used by microorganisms to convert light into membrane potential and have become useful optogenetic tools with applications in neuroscience. Although the resting state structures of the prototypical sodium pump *Krokinobacter eikastus* rhodopsin 2 (KR2) have been solved[2,3], it is unclear how structural alterations over time allow sodium to be translocated against a concentration gradient. Here, using the Swiss X-ray Free Electron Laser[4], we have collected serial crystallographic data at ten pump–probe delays from femtoseconds to milliseconds. High-resolution structural snapshots throughout the KR2 photocycle show how retinal isomerization is completed on the femtosecond timescale and changes the local structure of the binding pocket in the early nanoseconds. Subsequent rearrangements and deprotonation of the retinal Schiff base open an electrostatic gate in microseconds. Structural and spectroscopic data, in combination with quantum chemical calculations, indicate that a sodium ion binds transiently close to the retinal within one millisecond. In the last structural intermediate, at 20 milliseconds after activation, we identified a potential second sodium-binding site close to the extracellular exit. These results provide direct molecular insight into the dynamics of active cation transport across biological membranes.

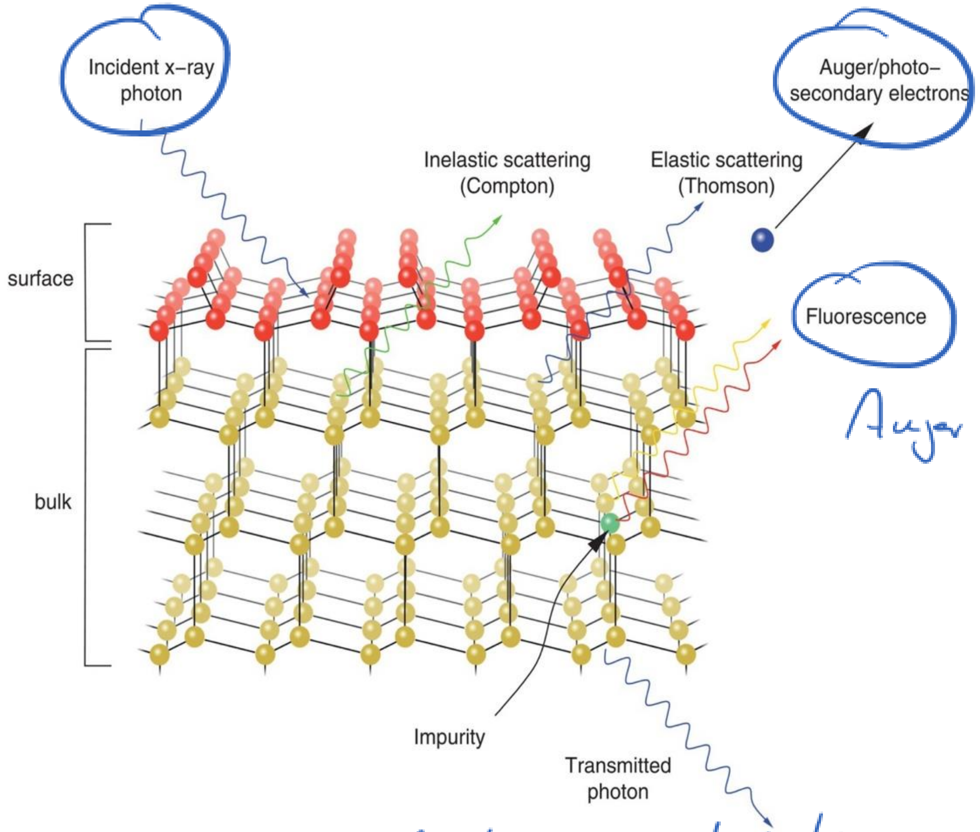


KR2 resting state



Some notes on X-ray Spectroscopy

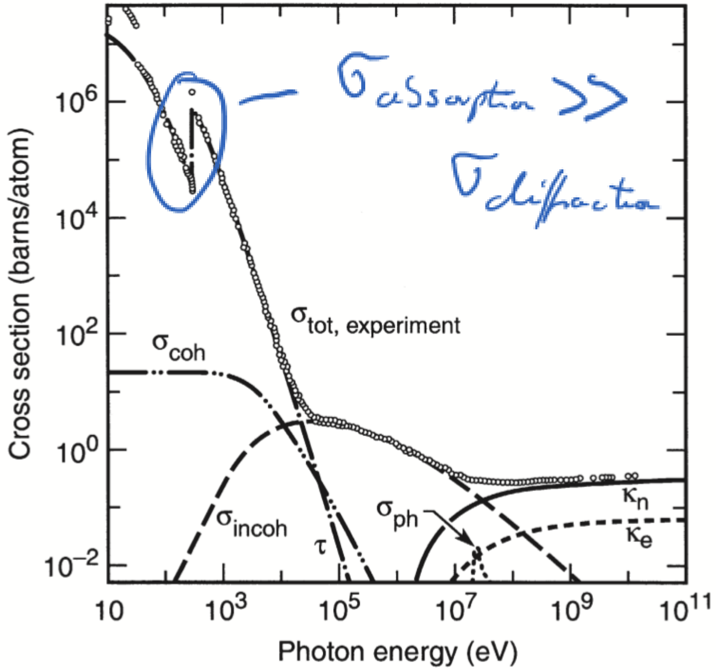
Remember: interaction of x-rays with matter



Auger

decay channels

Cross sections for x-ray - matter interactions

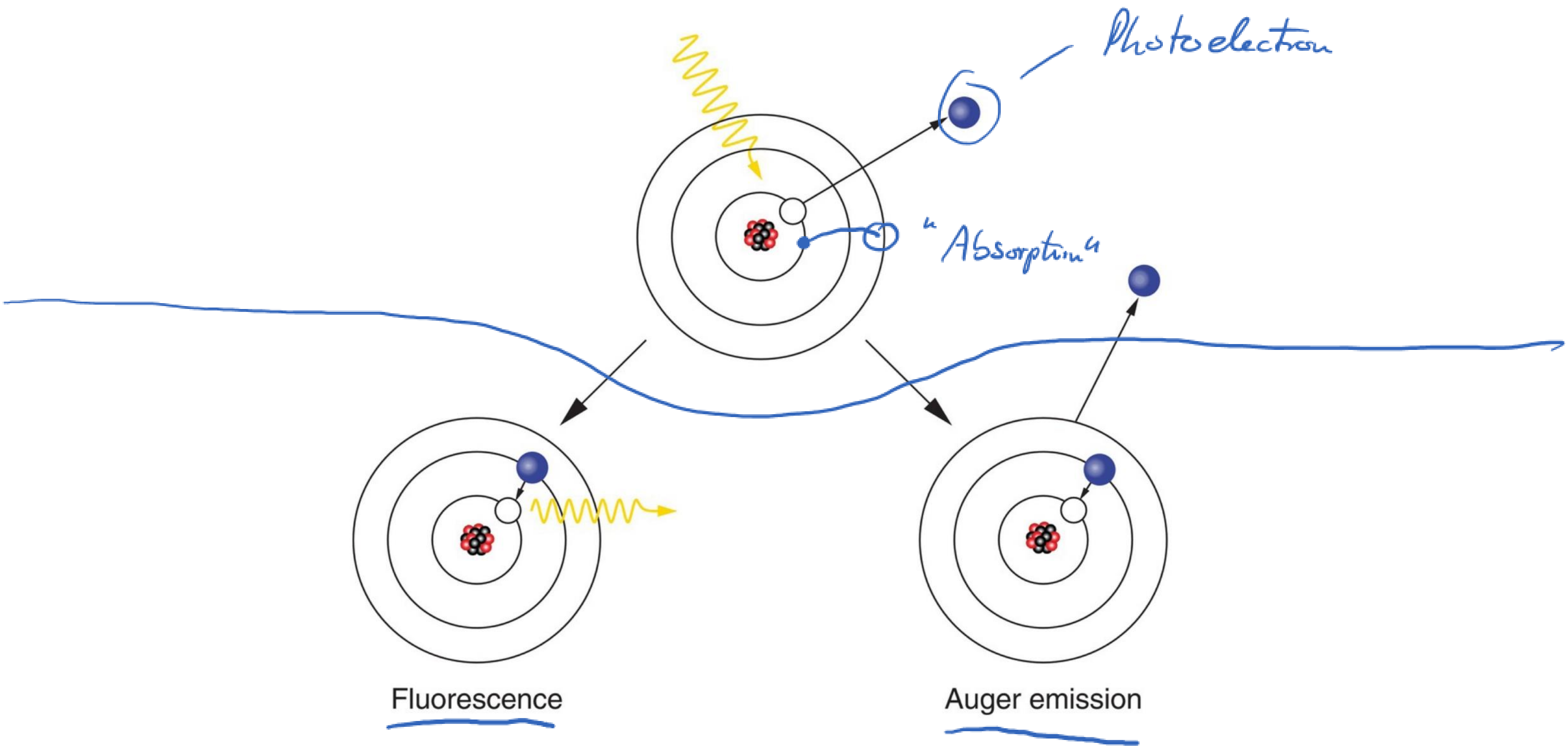


$\sigma_{\text{absorption}} \gg \sigma_{\text{diffraction}}$

Fig. 3-1. Total photon cross section σ_{tot} in carbon, as a

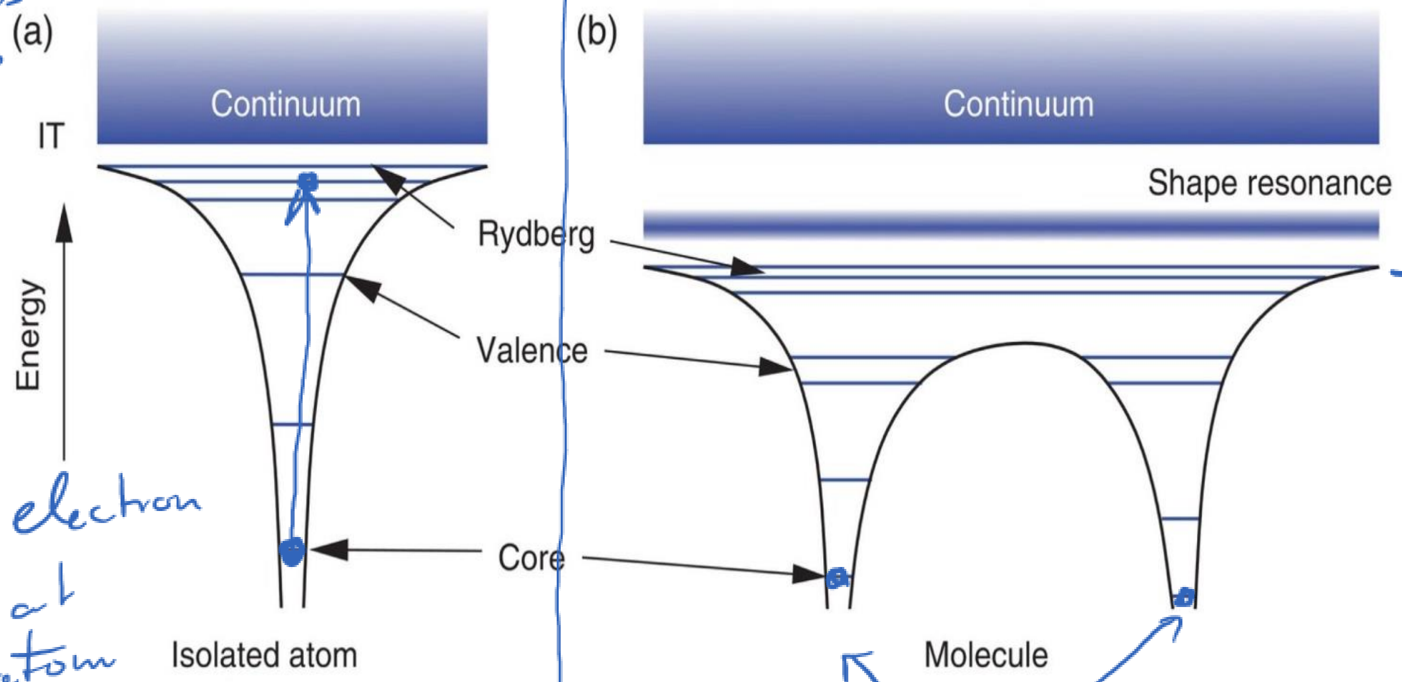
$\Rightarrow$ Obtain electronic structure information through observation of electrons or photons

Photoionization / absorption → Remember session 1 !



Atoms vs molecules → Core level electron spectroscopy

empty states, unoccupied levels, Rydberg states



Start: Core-level electron localized at specific atom

→ element specific!

different energies for different elements

in molecules, subtle differences in core-level binding energies reflect changes in the bond configuration

↓

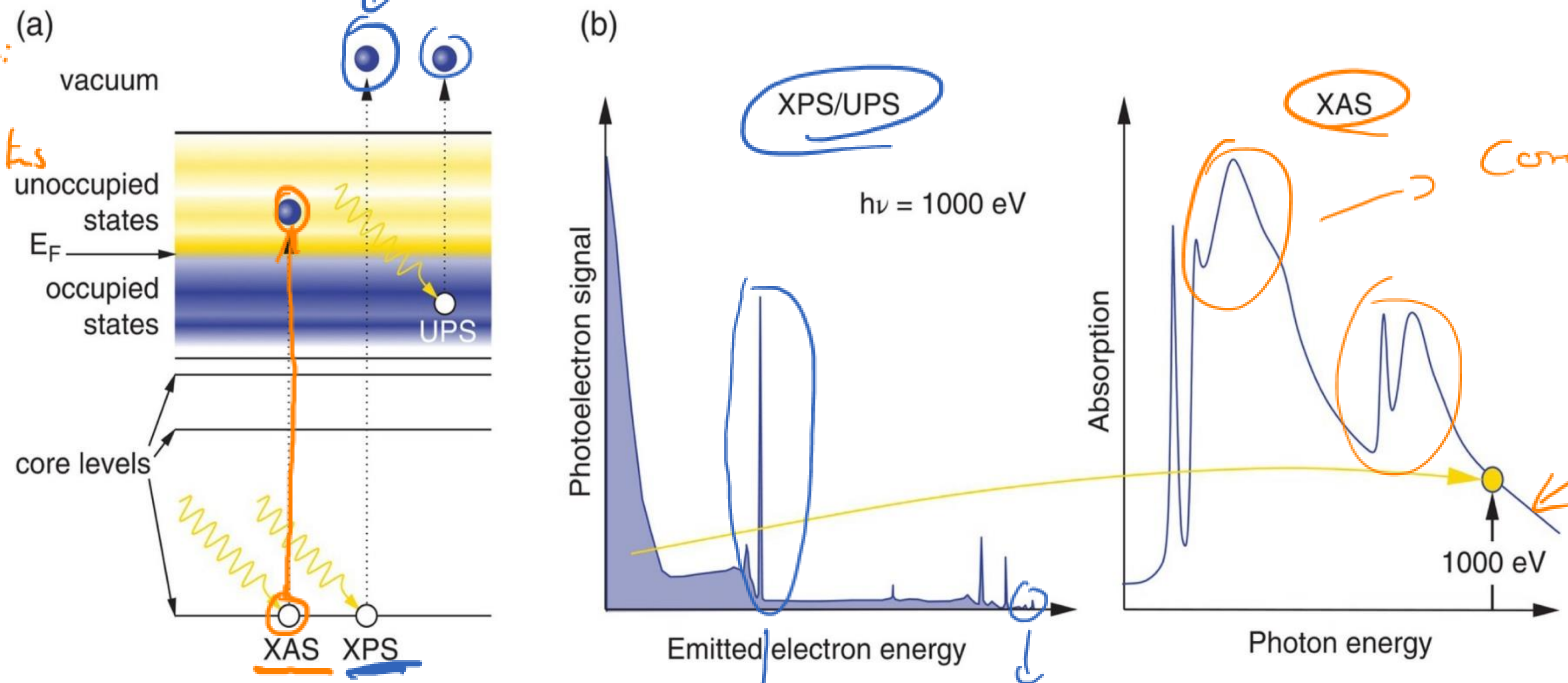
Chemical shifts!

Absorption vs Photoemission

→ hand waving argument:
depends on where you put your electron

Photoemission / photo electron spectroscopy → Ekin } → electron densities / energies

X-ray absorption:
unoccupied states
↓
probe local electronic structure
↓
empty states
↓
binding



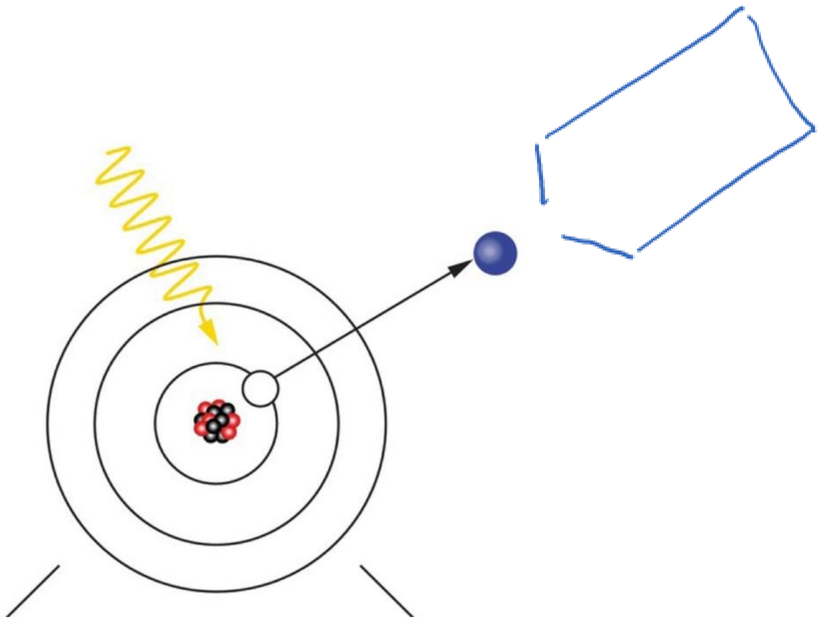
→ Core levels at resonances

Lambert de Beer laws away from resonance

↓
Core level

↓
valence states

Photoemission:



Electron analyzer

↳ Measure kinetic energy

$$M + h\nu \rightarrow M^+ + e^-$$

$$E_{kin} = \underbrace{h\nu - E_B}_{\text{"Einstein"}} - e\phi$$

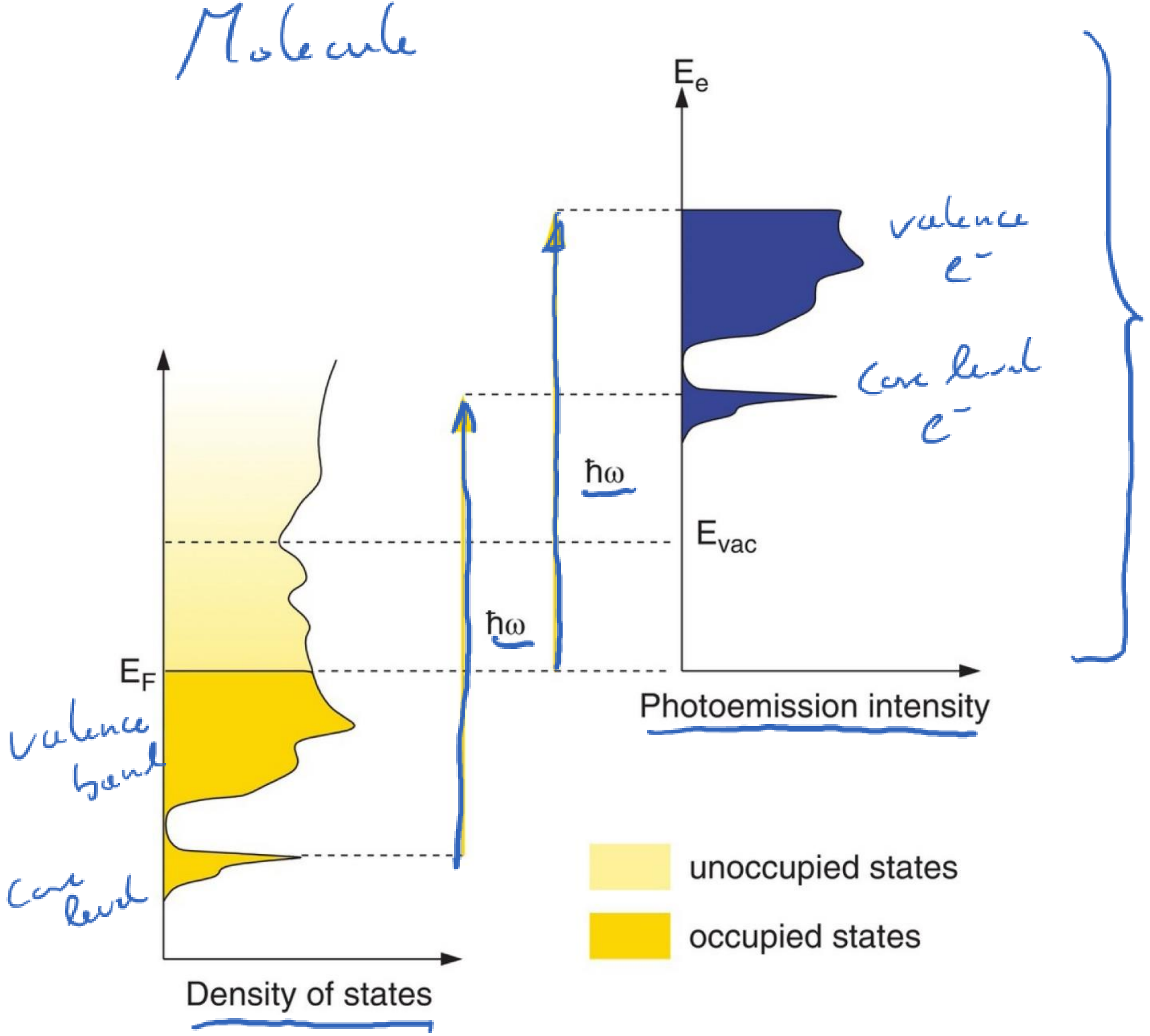
Note put e^- into continuum, here all states are infinitely close

↓

all transitions allowed

Photoelectrons reflect occupied density of states

Molecule



The interpretation of photoelectron Spectroscopy (PES/XPS) is that for a fixed $h\nu$ the photoemission intensity reflects the density of occupied states.

Ionization energies of elements → Session 1

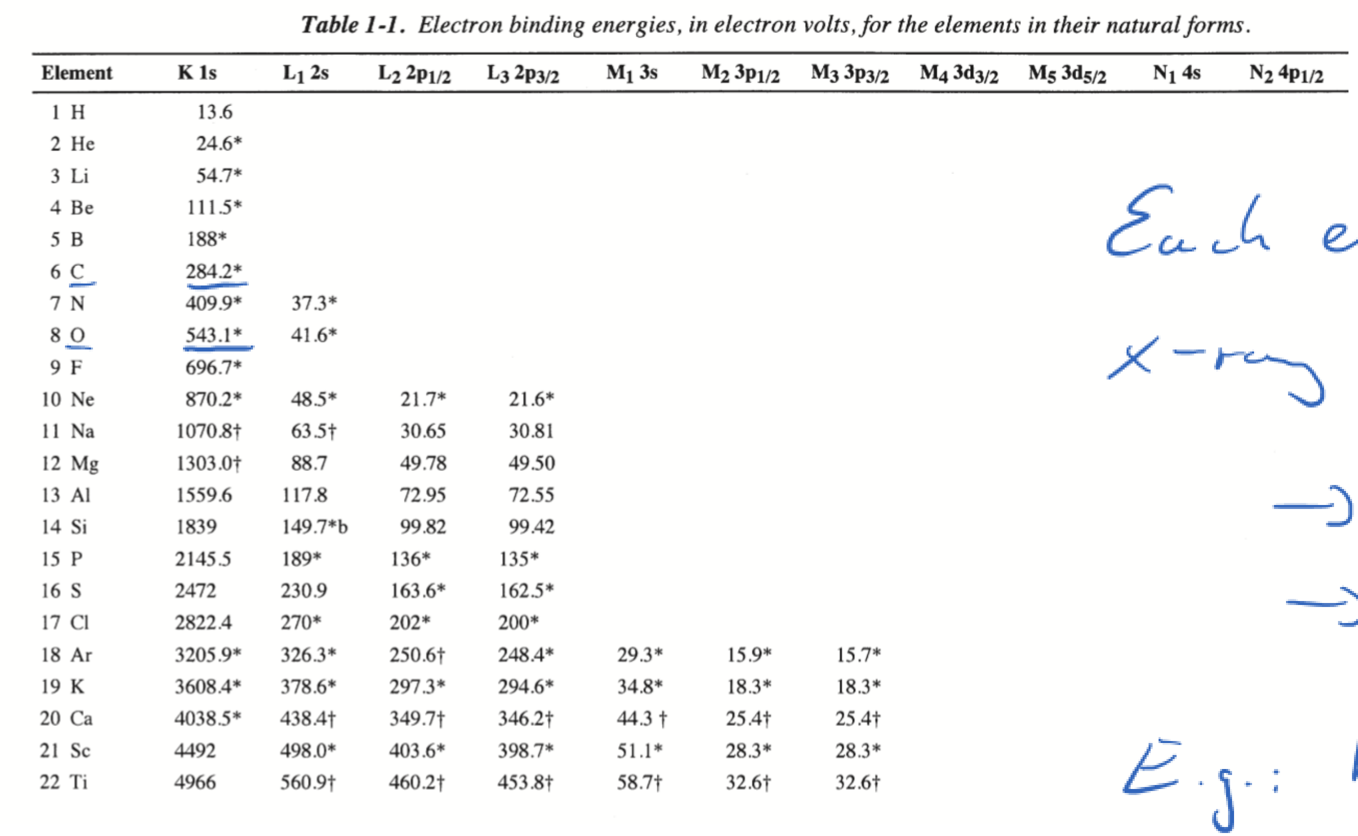


Table 1-1. Electron binding energies, in electron volts, for the elements in their natural forms.

Element	K 1s	L_1 2s	L_2 $2p_{1/2}$	L_3 $2p_{3/2}$	M_1 3s	M_2 $3p_{1/2}$	M_3 $3p_{3/2}$	M_4 $3d_{3/2}$	M_5 $3d_{5/2}$	N_1 4s	N_2 $4p_{1/2}$
1 H	13.6										
2 He	24.6*										
3 Li	54.7*										
4 Be	111.5*										
5 B	188*										
6 C	284.2*										
7 N	409.9*	37.3*									
8 O	543.1*	41.6*									
9 F	696.7*										
10 Ne	870.2*	48.5*	21.7*	21.6*							
11 Na	1070.8†	63.5†	30.65	30.81							
12 Mg	1303.0†	88.7	49.78	49.50							
13 Al	1559.6	117.8	72.95	72.55							
14 Si	1839	149.7*b	99.82	99.42							
15 P	2145.5	189*	136*	135*							
16 S	2472	230.9	163.6*	162.5*							
17 Cl	2822.4	270*	202*	200*							
18 Ar	3205.9*	326.3*	250.6†	248.4*	29.3*	15.9*	15.7*				
19 K	3608.4*	378.6*	297.3*	294.6*	34.8*	18.3*	18.3*				
20 Ca	4038.5*	438.4†	349.7†	346.2†	44.3 †	25.4†	25.4†				
21 Sc	4492	498.0*	403.6*	398.7*	51.1*	28.3*	28.3*				
22 Ti	4966	560.9†	460.2†	453.8†	58.7†	32.6†	32.6†				

Each element has a characteristic x-ray energy / electronic configuration

→ binding energies

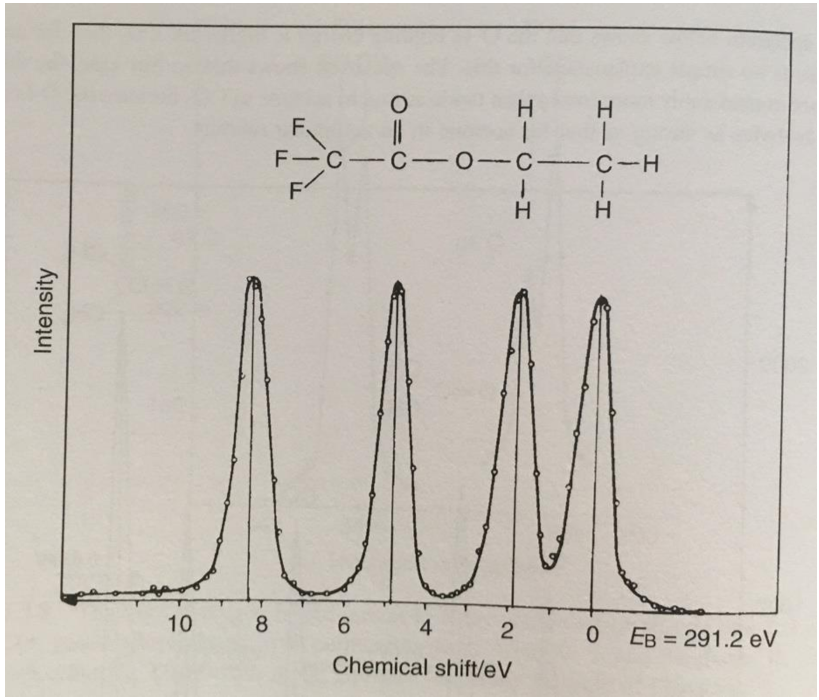
→ use for chemical analysis

E.g.: $h\nu = 700$ eV, CO molecules

peaks at 160 eV, 420 eV

$$\begin{tikzcd} 160\text{ eV} \arrow[d] & 420\text{ eV} \arrow[d] \\ O & C \end{tikzcd}$$

Chemical shifts



High-resolution PES can yield information about the bond configuration/chemical environment of the target atom

Different oxidation states lead to a characteristic shift of the core-level binding energies → Chemical shift

The Nobel Prize in Physics 1981

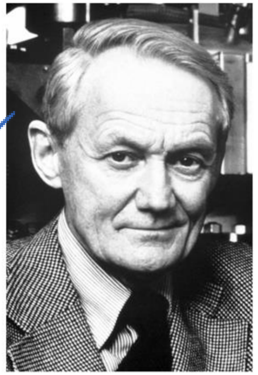
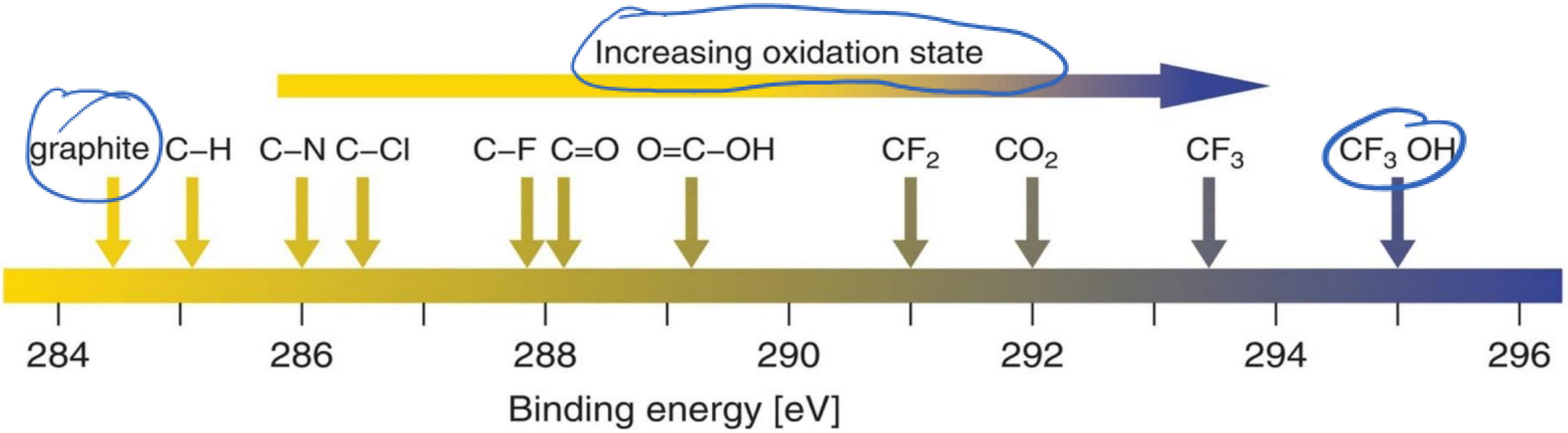


Photo from the Nobel Foundation archive.

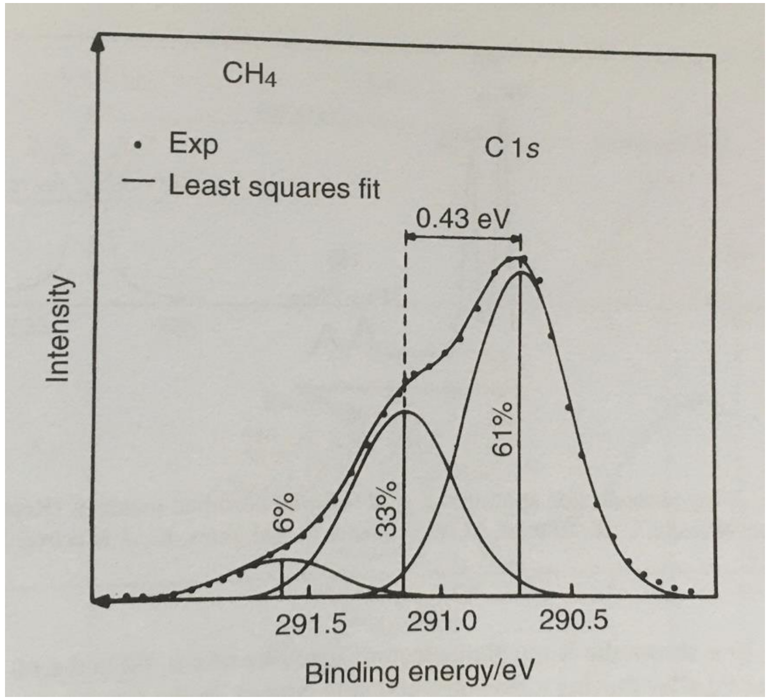
Kai M. Siegbahn

Prize share: 1/2

Generally: higher oxidation states/electronegativ. lead to large chem shifts.



Vibrational information in photoelectron spectroscopy

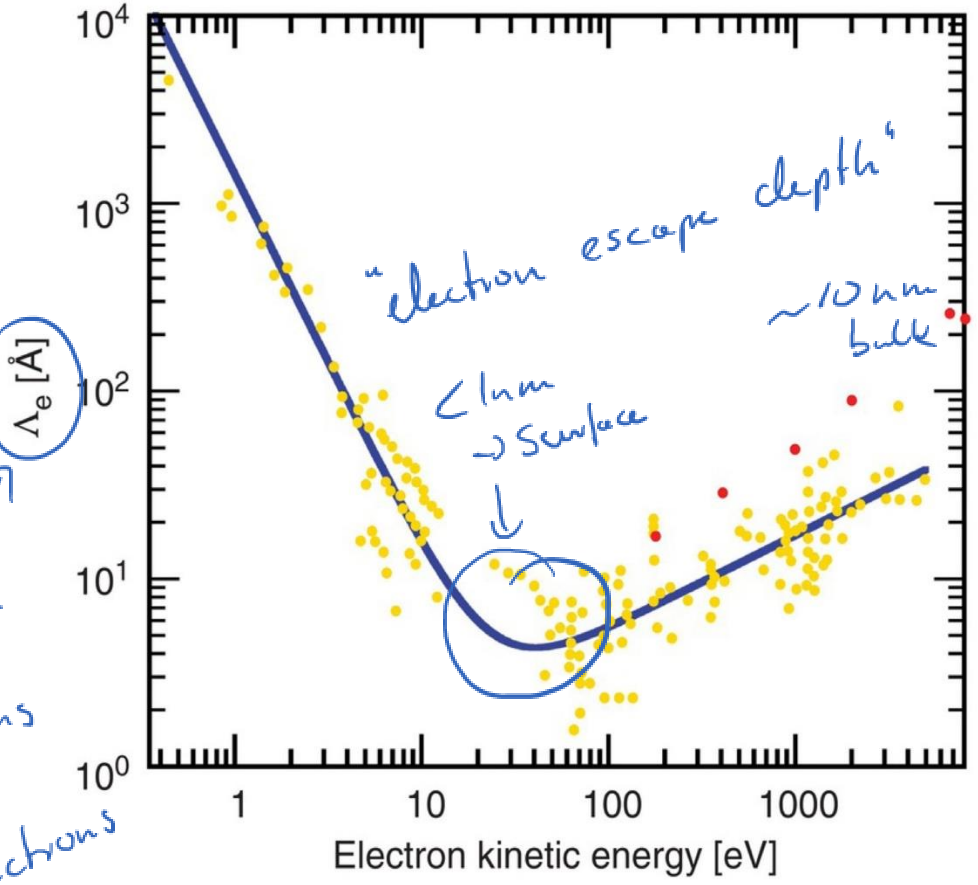


Brief comment

Core level binding energies

can also reflect vibrational levels

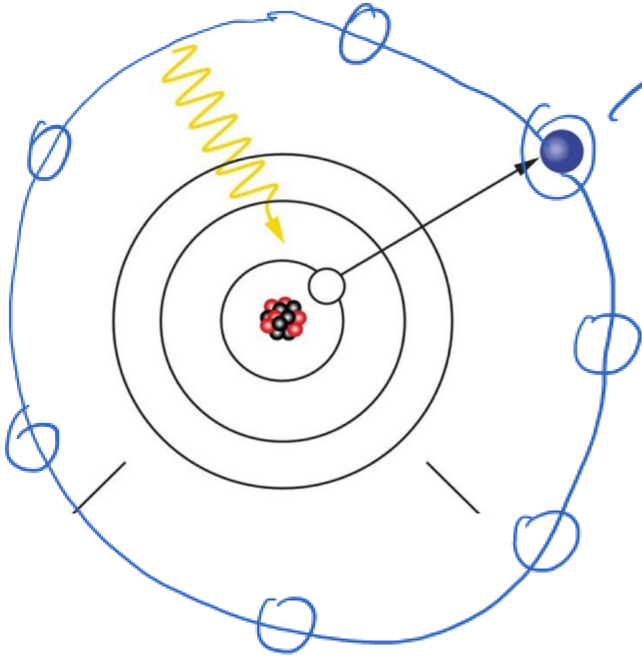
The „universal curve of photoemission“



mean free paths for electrons

- → (photo) electrons are scattered in any material
- → electrons have a large scattering cross section
- → escape depth depends on their kinetic energy

X-ray absorption XAS



→ promote electron into specific empty state

→ highly sensitive to (dipole) selection rule $\ell \pm 1$

→ Fermi golden rule

$$P_{ij} = \frac{2\pi}{\hbar} \left| \langle f | H | i \rangle \right|^2 \mathcal{E}$$

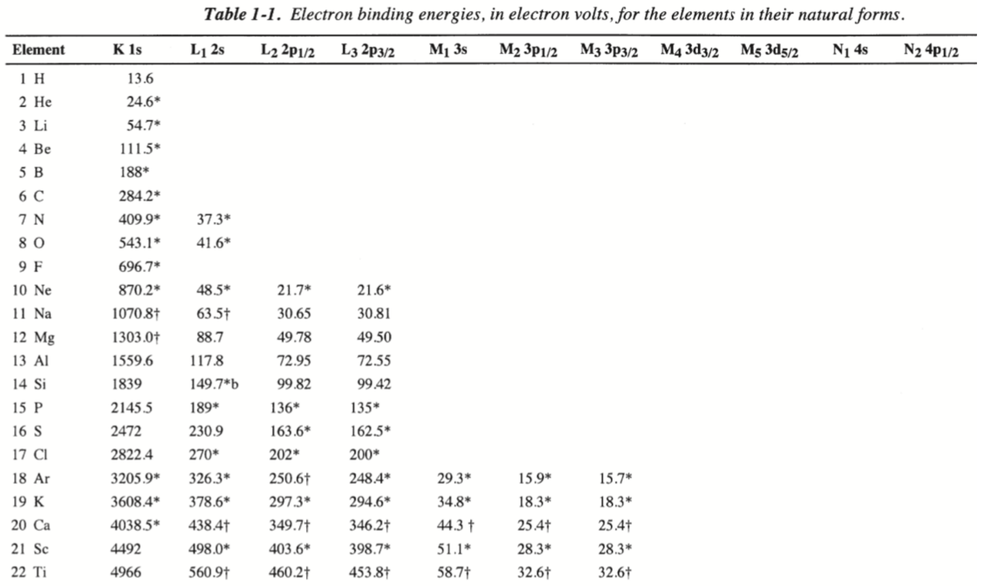
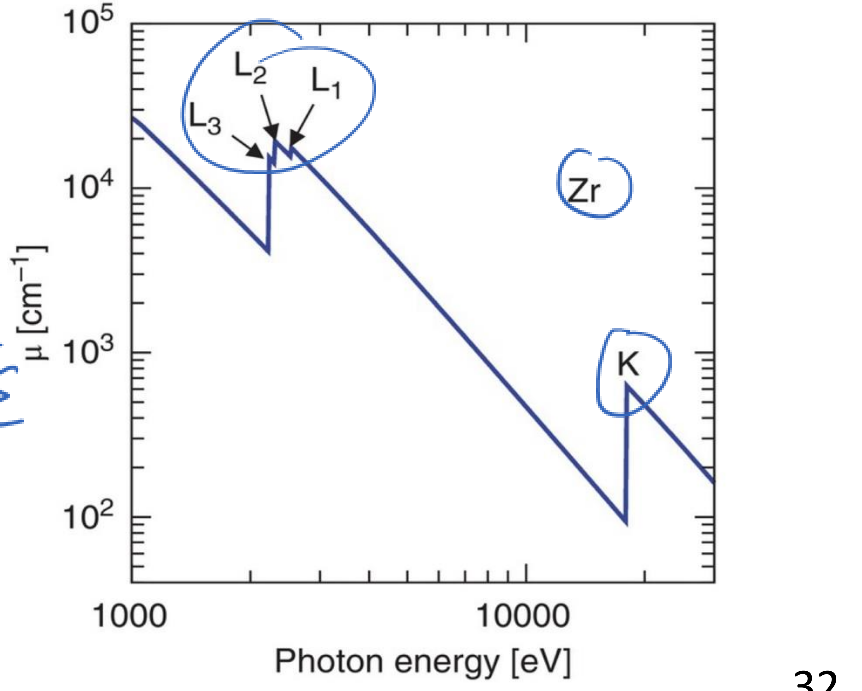


Table 1-1. Electron binding energies, in electron volts, for the elements in their natural forms.

Element	K 1s	L_1 2s	L_2 $2p_{1/2}$	L_3 $2p_{3/2}$	M_1 3s	M_2 $3p_{1/2}$	M_3 $3p_{3/2}$	M_4 $3d_{3/2}$	M_5 $3d_{5/2}$	N_1 4s	N_2 $4p_{1/2}$
1 H	13.6										
2 He	24.6*										
3 Li	54.7*										
4 Be	111.5*										
5 B	188*										
6 C	284.2*										
7 N	409.9*	37.3*									
8 O	543.1*	41.6*									
9 F	696.7*										
10 Ne	870.2*	48.5*	21.7*	21.6*							
11 Na	1070.8†	63.5†	30.65	30.81							
12 Mg	1303.0†	88.7	49.78	49.50							
13 Al	1559.6	117.8	72.95	72.55							
14 Si	1839	149.7*b	99.82	99.42							
15 P	2145.5	189*	136*	135*							
16 S	2472	230.9	163.6*	162.5*							
17 Cl	2822.4	270*	202*	200*							
18 Ar	3205.9*	326.3*	250.6†	248.4*	29.3*	15.9*	15.7*				
19 K	3608.4*	378.6*	297.3*	294.6*	34.8*	18.3*	18.3*				
20 Ca	4038.5*	438.4†	349.7†	346.2†	44.3 †	25.4†	25.4†				
21 Sc	4492	498.0*	403.6*	398.7*	51.1*	28.3*	28.3*				
22 Ti	4966	560.9†	460.2†	453.8†	58.7†	32.6†	32.6†				

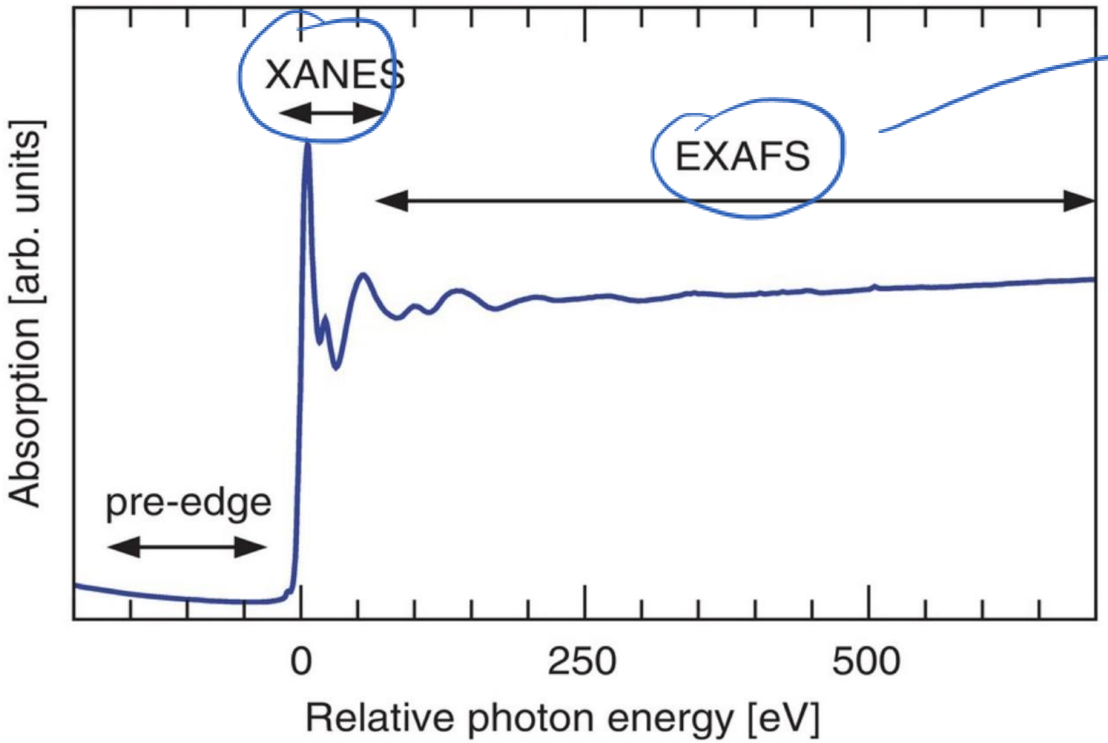


Distinct regions of X-ray absorption

XANES

X-ray absorption near edge structure

↳ sensitive to empty states (LUMO) of the local environment

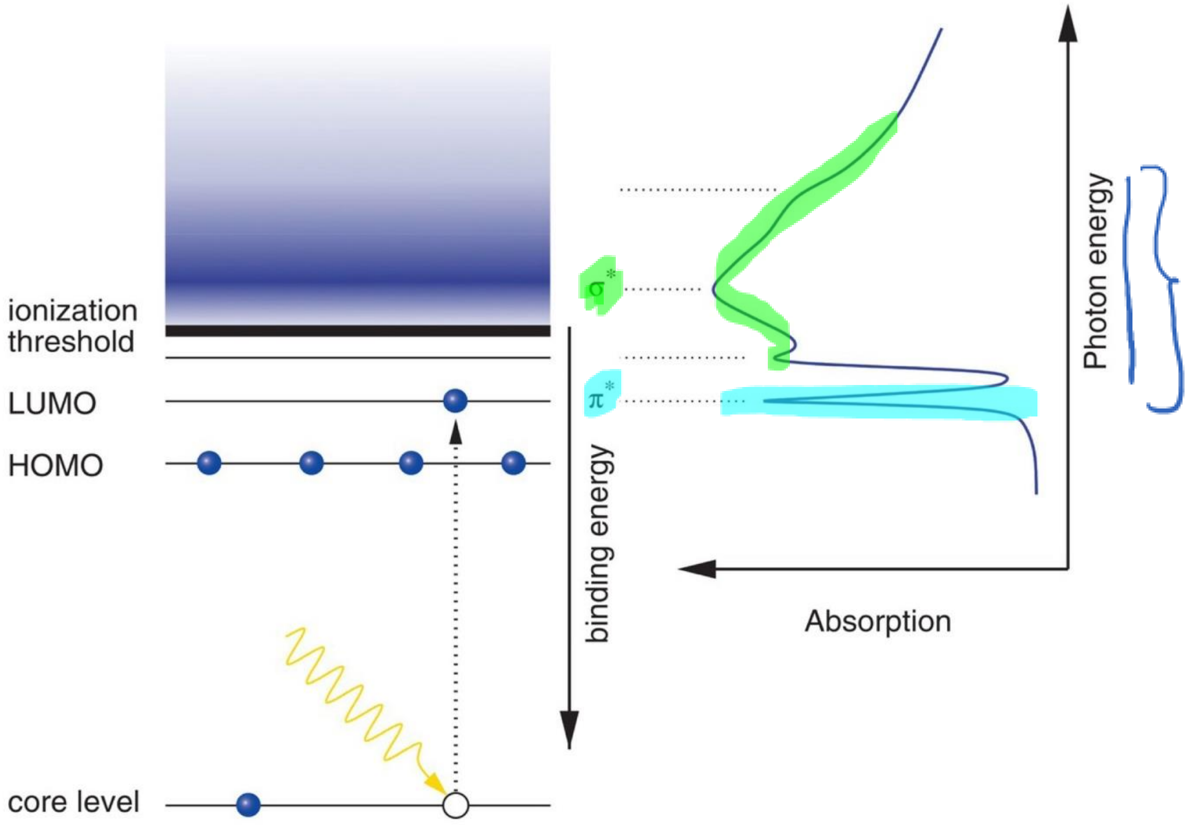


EXAFS

extended X-ray absorption fine structure

↳ oscillations contain info about nearest neighbor structure

X-ray Absorption Near Edge Structure (XANES)



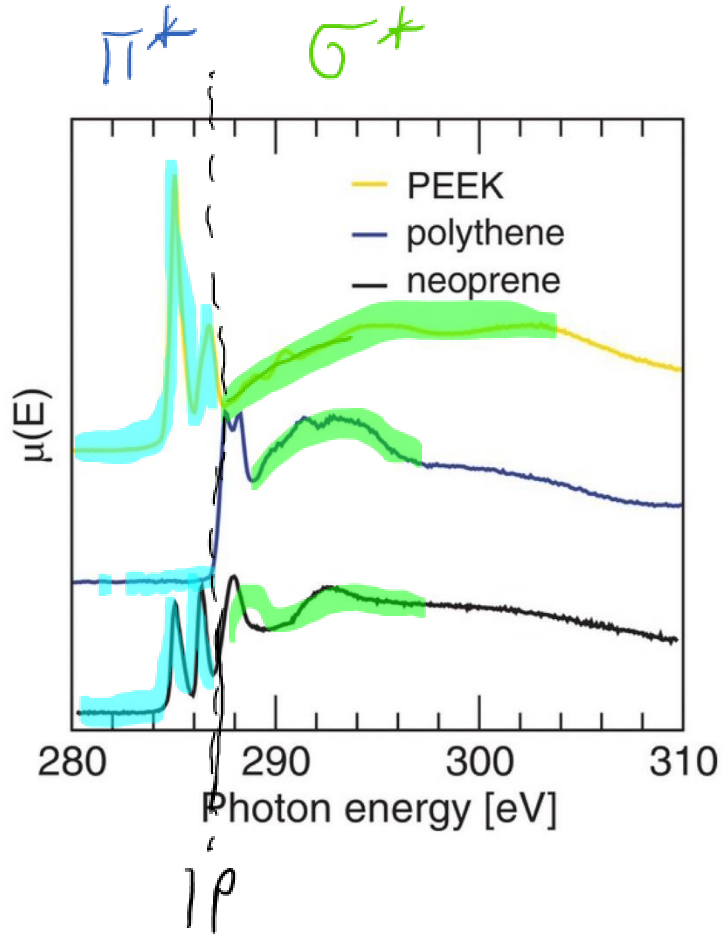
→ probe unoccupied states

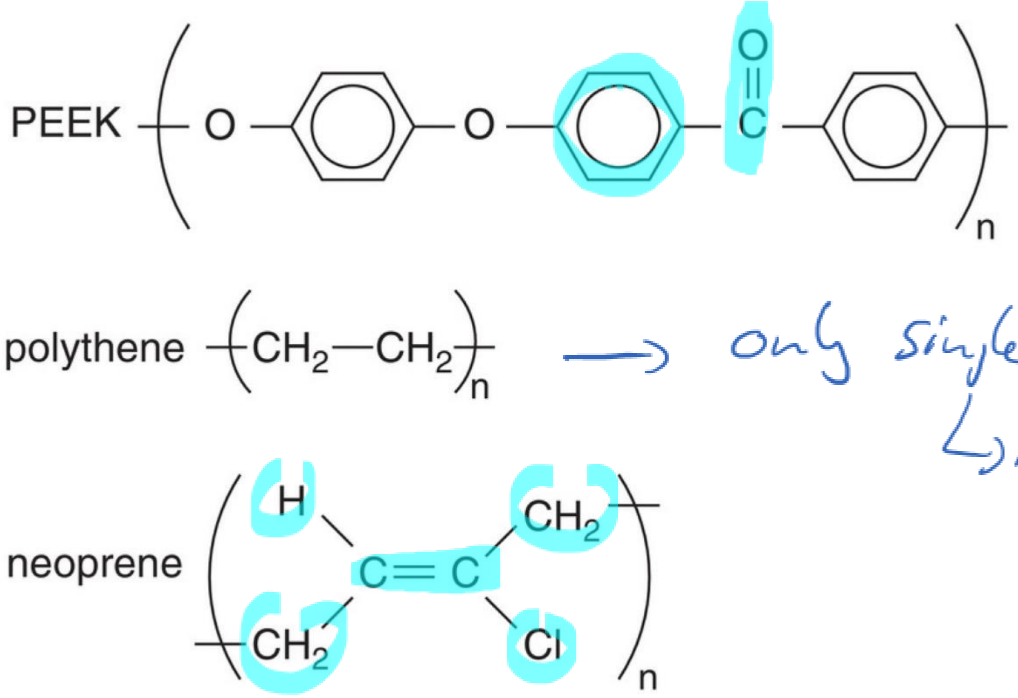
→ transitions are described by Fermi golden rule

→ Info about LUMO

XANES spectra yield detailed information about bonding structure

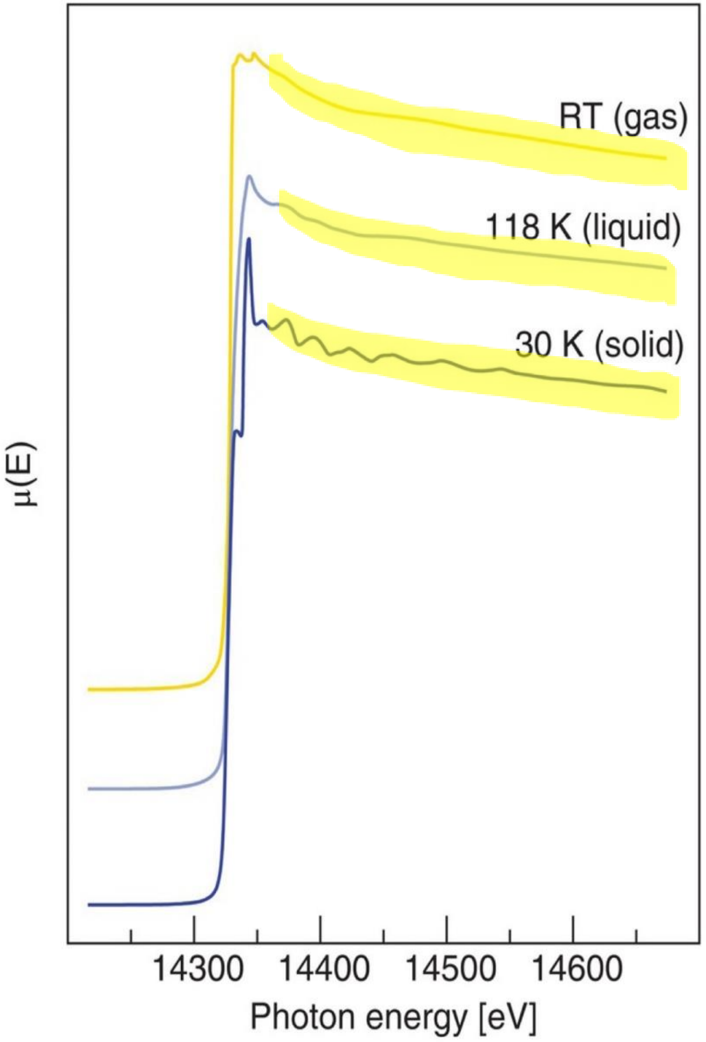
absorption → local binding config!





→ only single bonds σ^*
↳ no π^*!

Extended X-ray Absorption Fine Structure (EXAFS) contains information about nearest neighbors



Wiggles → nearest neighbor information

⇒ three different phases have distinct differences in the absorption spectra that are related to the structure

EXAFS and nearest neighbors

Remember

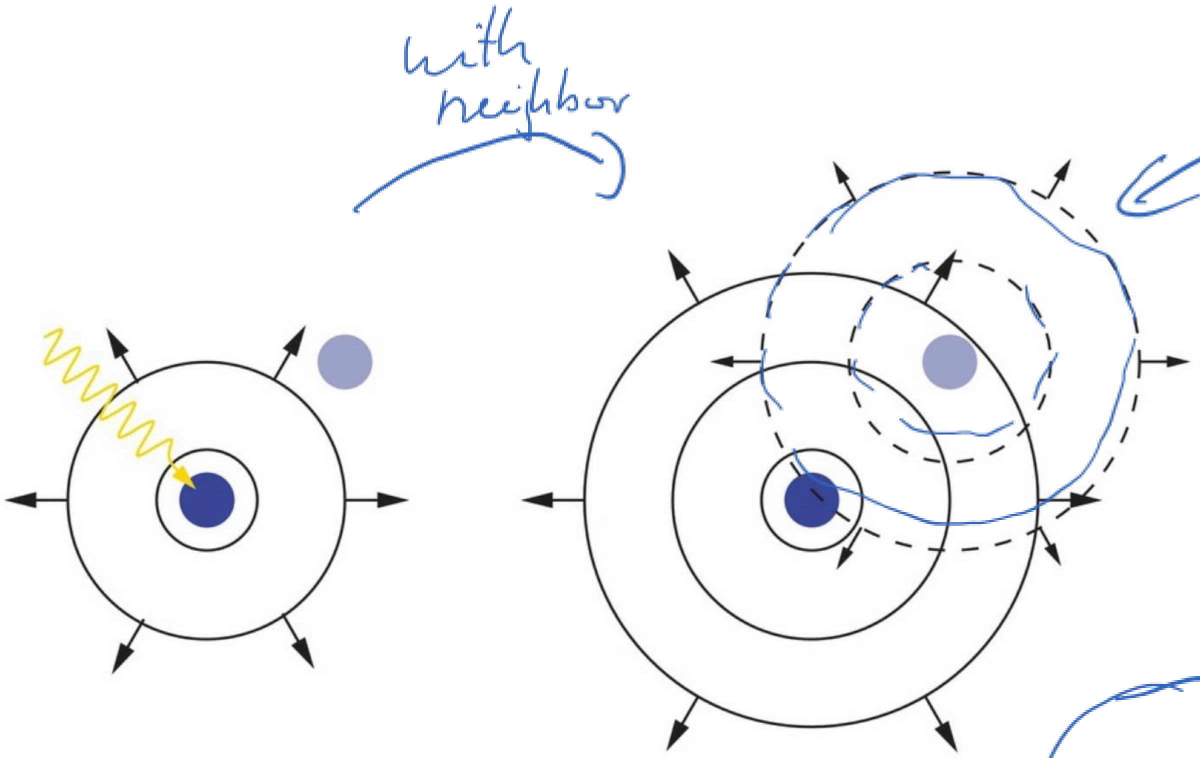
particle-wave dualism

↓

electron with E_{kin} has an associated wavelength

$$\lambda = h/mc$$

$\Rightarrow$ can scatter like a wave!



Can scatter

↓

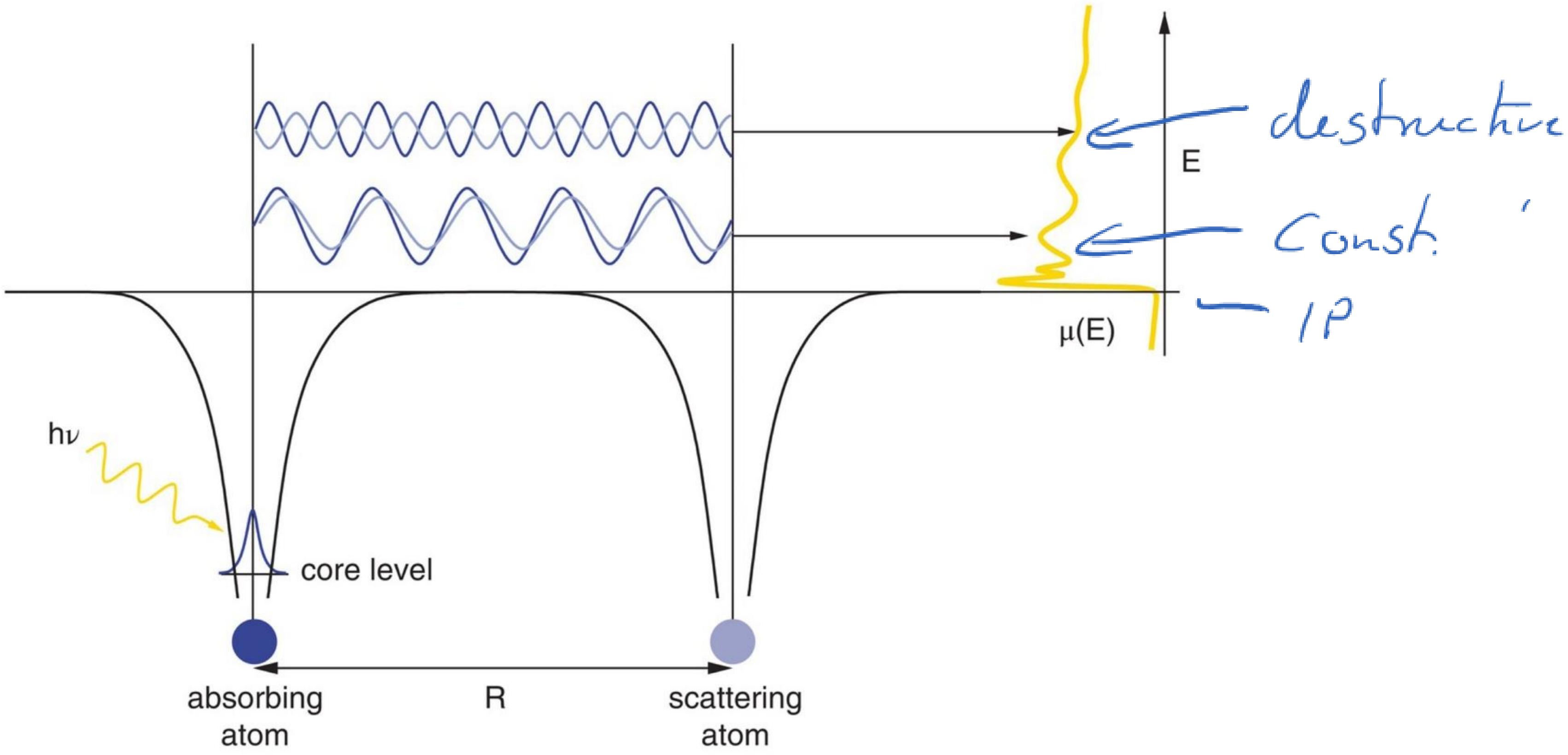
Interference phenomena

↓

wiggles

outgoing electron can scatter of neighboring atoms

EXAFS interference examples

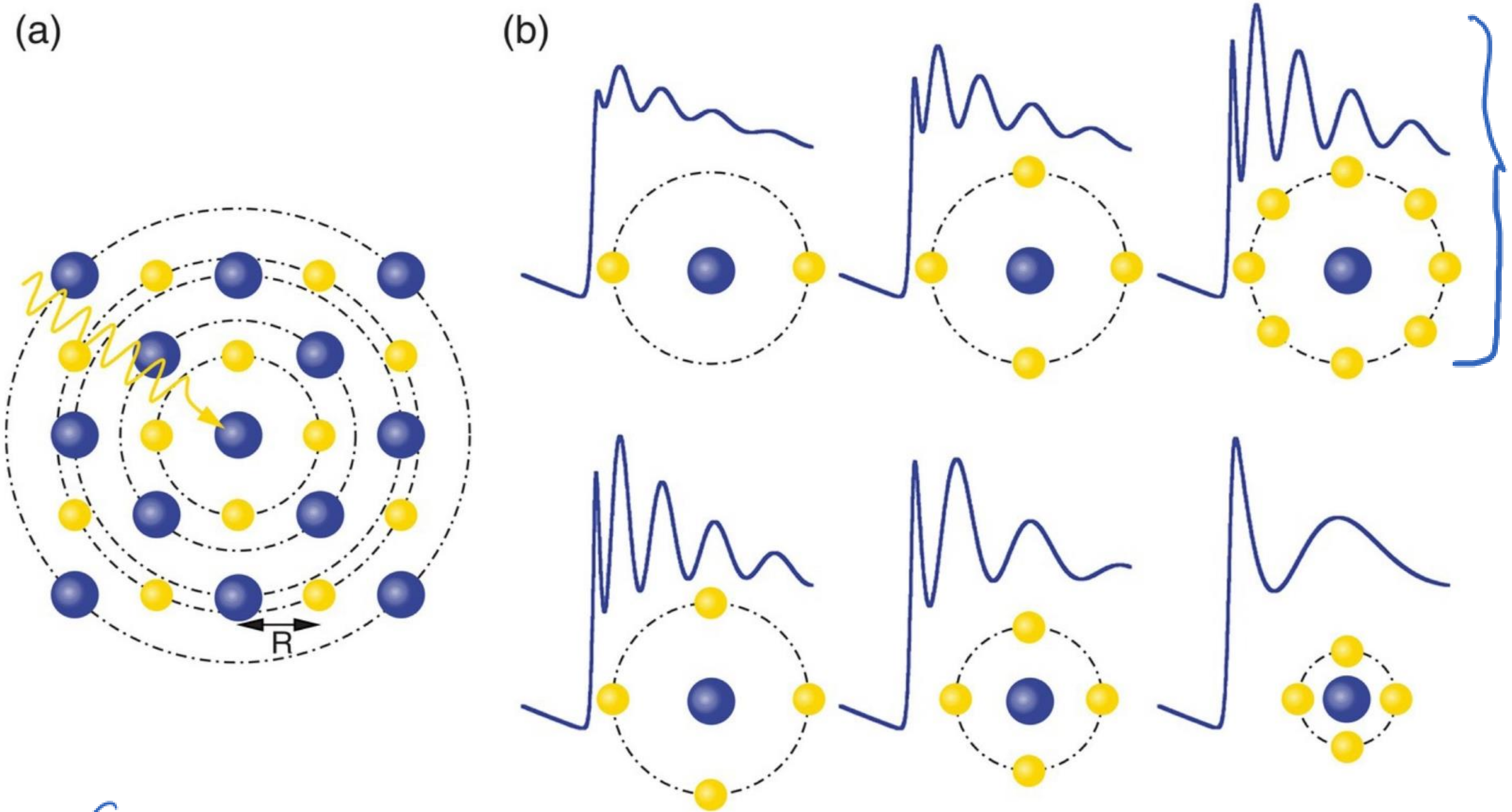


destructive

Const.

IP

Characteristic features in EXAFS → rules of thumb



oscillation signal

- increases with number of neighbors

- depends on shell radius

Generally theoretical modelling / comparisson to references needed for interpretation

The end