BioScience in the Light Beam

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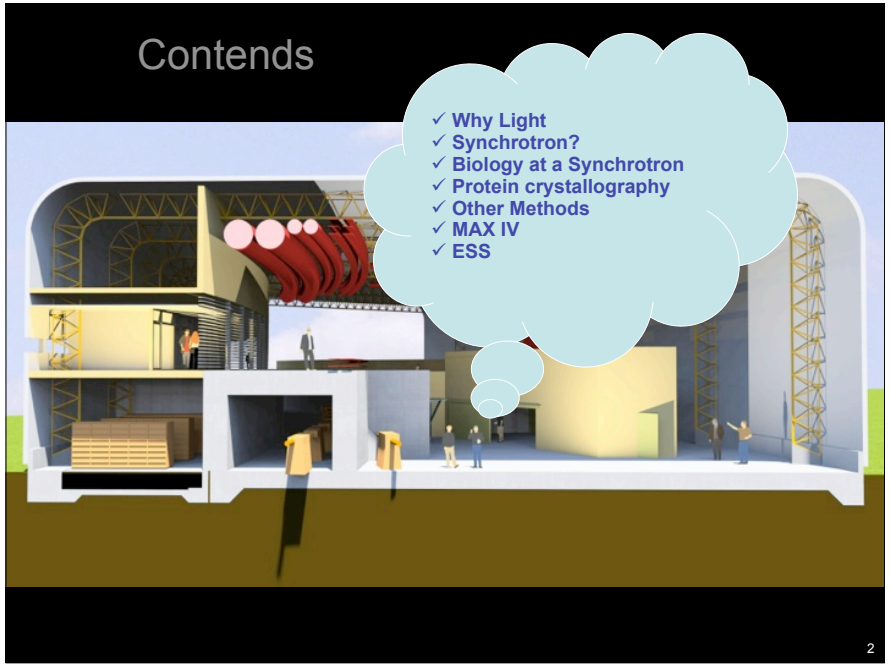
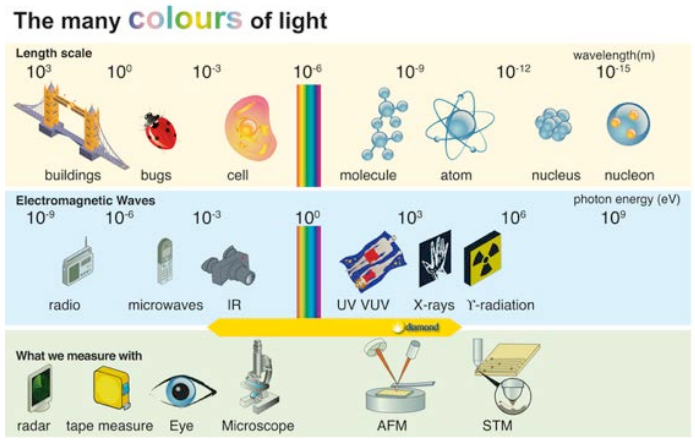
Contends



Why use light?



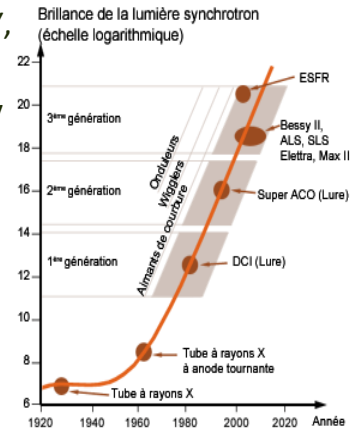
Length of the light-wave should have the same size as object to be studied.

Light generator: synchrotron

- A synchrotron is an accelerator of electrons. The electrons are maintained in a circular ring by magnetic field and produce light tangentially to their trajectory.
- The light is used to study different systems; applications from medicine to hard physics.

Synchrotrons

- 1st generation: *parasitic* on high-energy physics operations (DESY, SPEAR, NINA, VEPP)
- 2nd generation: *Dedicated* X-ray sources (SRS, CHESS, NSLS, Photon Factory)
- 3rd generation: High Brilliance, use of *insertion elements*: undulators and wigglers (ESRF, APS, MAX2, SLS, DIAMOND, SOLEIL)
- 4th generation: Free electron Lasers

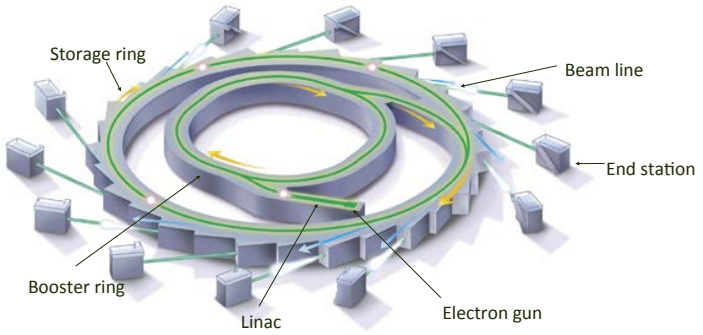


Properties of synchrotron light

- **High brightness:** synchrotron light is extremely intense and highly collimated.
- **Wide energy spectrum:** light is emitted with energies ranging from infrared light to hard, energetic (short wavelength) X-rays.
- **Tunable:** through sophisticated monochromators and insertion devices, it is possible to obtain an intense beam of any selected wavelength.
- **Highly polarised:** the synchrotron emits highly polarised radiation, which can be linear, circular or elliptical.

Synchrotrons in the world

Typical modern synchrotron

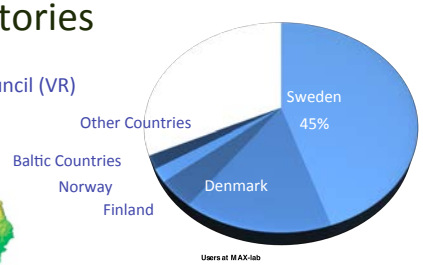


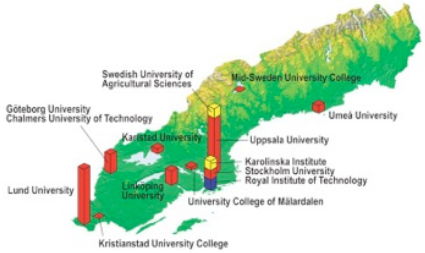
Schematic picture of Boomerang
Australian synchrotron

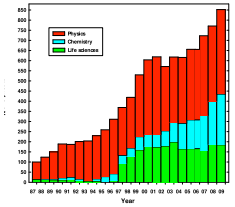
MAX-lab, One Out of Two Swedish National Laboratories

- Host: Lund University

  Operated by: LU & Swedish Research Council (VR)
- National laboratory – "open access"

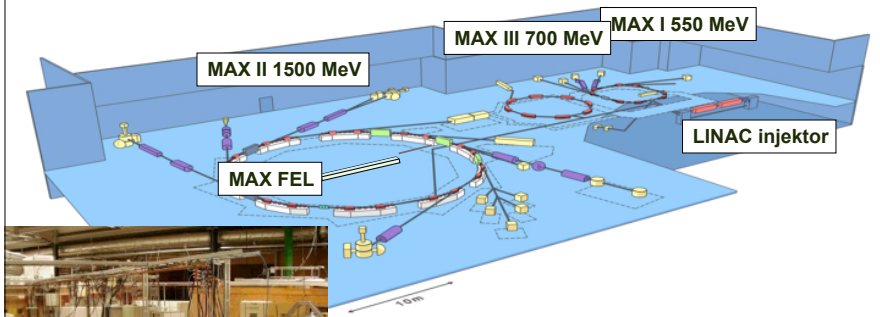


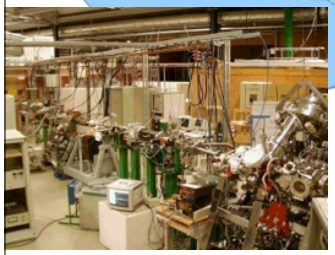




+ Commercial users

MAX-lab Today – A National Laboratory for Synchrotron Radiation Based Science

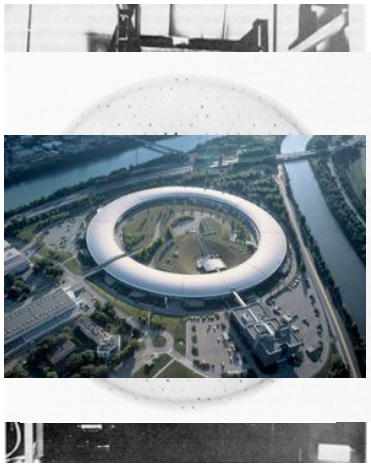




1985 MAX I – Synchrotron Radiation & Nuclear Physics
1997 MAX II – Synchrotron Radiation – higher energies
2008 MAX III – SR – lower energies & Prototype for MAX IV
2009 MAX-FEL – Test Facility for Free Electron Laser
(EUROFEL – IRUVX)

Some history on biology at synchrotrons

- Natural Source for "synchrotron radiation": Space
- First synchrotron radiation,1947
- X-ray spectra, 1963 at Frascati synchrotron
- 1971, first X-ray-diffraction experiments at DESY (Rosenbaum & Holmes)
- 1975: First protein diffraction at SPEAR
- During 70's MAD, EXAFS, SAXS, DNA Fiber diffraction pioneered
- At the end of the 1980's ESRF and APS proposed



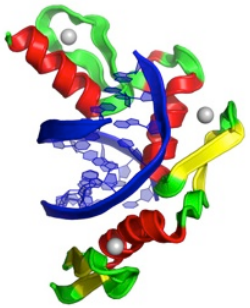
Biology at synchrotrons, why

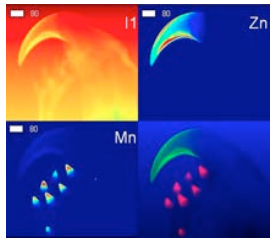
- Plusses:
- High intensity
- Small focus
- Higher flux at the sample
- Range of wavelengths available
- Other resources available (experts, lasers, labs etc.)

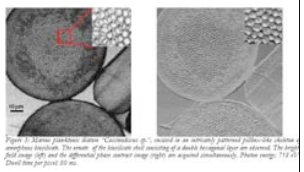
- Minusses
- Samples get destroyed: Radiation damage
- One has to travel to the synchrotrons

Biology at Synchrotrons

1. Diffraction and Scattering studies: Protein Crystallography and S/WAXS.
2. Spectroscopy: EXAFS, XANES, XRF, IR/UV, CD
3. Imaging and Tomography



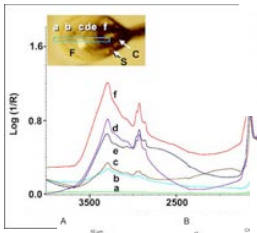


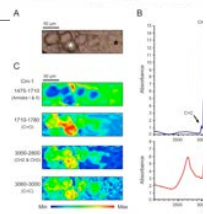


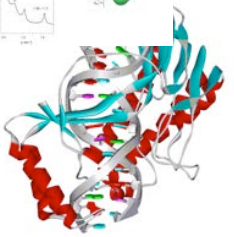
Biology at Synchrotrons

What fields:

-Structural Biology
-Biophysics
-Enzymology
-Biochemistry
-Biotechnology
-Radiation Biology
-Physiology
-Paleontology
and more







Study proteins: Why?

Transport of oxygen

Hair and skin

Receptors

Digestion

Defense against infections

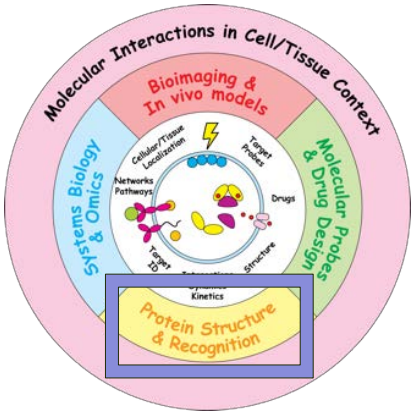
Muscles

Growth and development

And much more….

Front line Biological Sciences

What competences and techniques do we need?

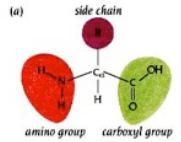


Methods to study 3D structures.

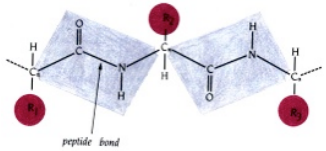
1. Electron microscopy.
   No size limit, but particles need to be big. Medium resolution
2. NMR. >40 000 Da, high solubility
3. X-ray Crystallography. Crystals!
No size limit, high resolution.
4. Modelling
   Extensively used but of "limited" value.

Proteins are polymers

Proteins are formed by a chain of repeating molecules. One such molecule is called an amino-acid. There are 20 types of amino-acids but they have all a common backbone or main-chain:



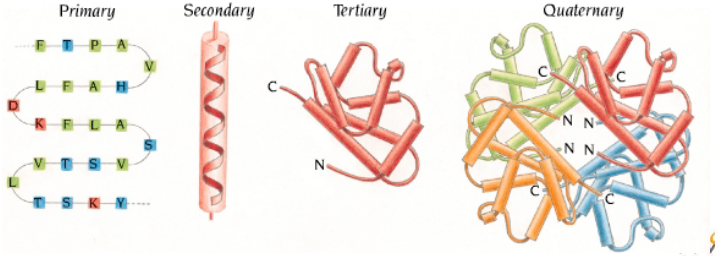
The protein chain is formed by linking the amino-acids together. The linkage is called the peptide bond:



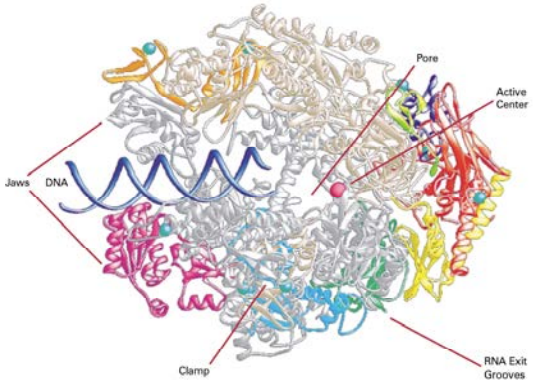
The chain of amino-acids linked to each other by peptide bonds is also called: polypeptide chain.

Structure in four dimensions:

Primary Structure Amino acid sequence.
Secondary Structure Local regular structure: α-helices and β-sheets.
Tertiary Structure Packing of secundary structure into one or several compact globular domains
Quaternary Structure The arrangement of several folded chains together: multimeric proteins



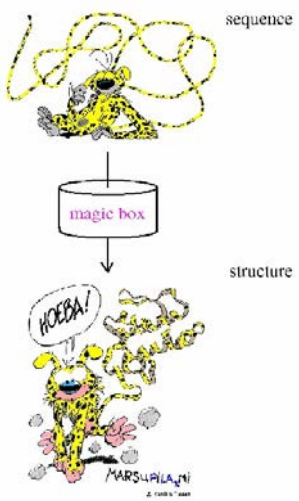
Why study structure?



1. FUNCTION IS STRUCTURE!

Structure of RNA polymerase: clues of how it reads DNA and makes RNA

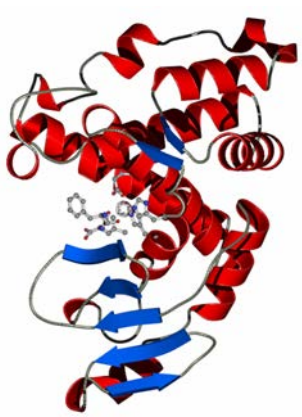
2. Protein folding problem



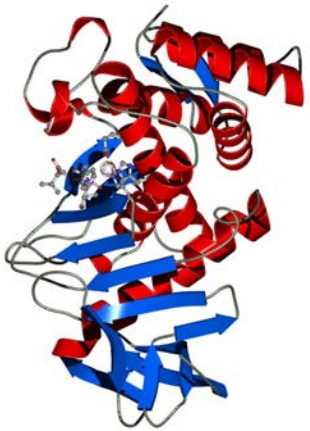
We still do not fully understand why a specific sequence folds into a specific structure.

Until then we have to determine structures.

3 Find unexpected functional relationships.

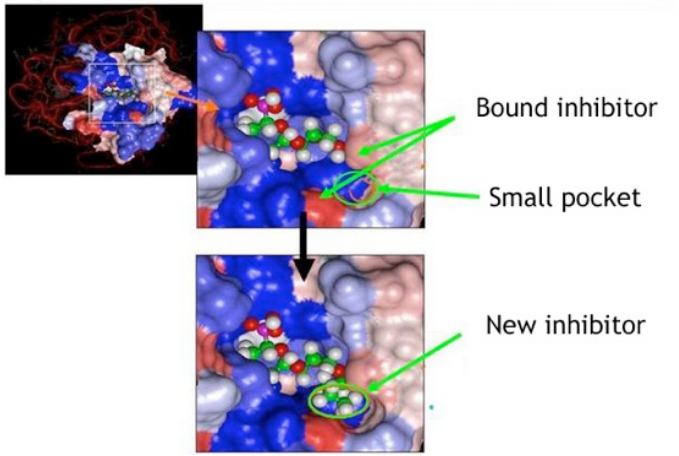


LTA_4 hydrolase

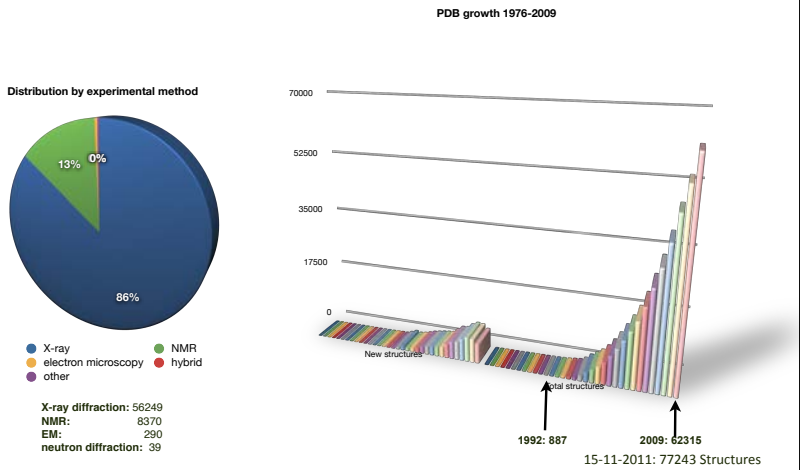


Thermolysin

4. Structure aided drug design



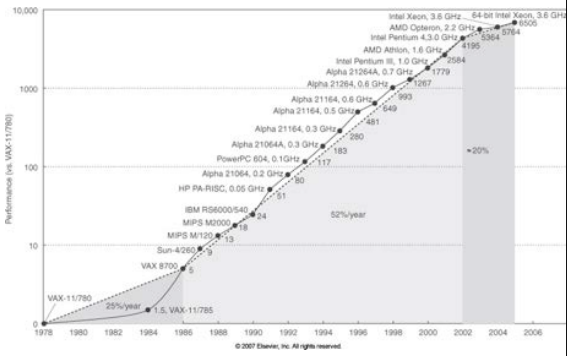
Growth in 3D structural information 1976–2009



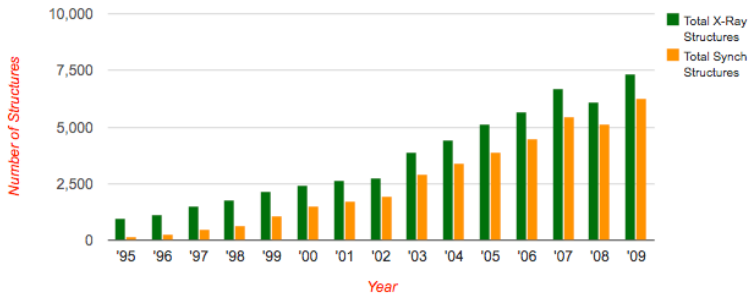
Medium resolution methods such as SAXS/SANS (and Imaging) are strongly upcoming methods in Structural Biology

Reasons explosion

1. Molecular Biology developments
2. Computer developments
3. Synchrotrons



Datasets collected at synchrotrons



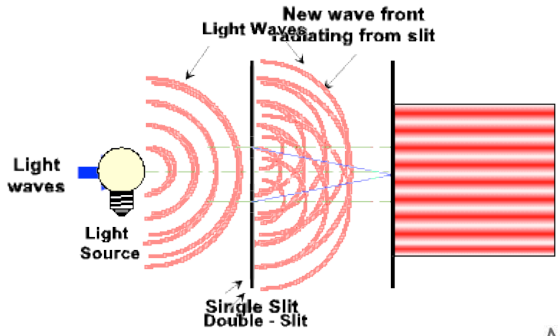
Propagation of waves

If a light wave hits a single slit, a new wave front will spread out from this point.

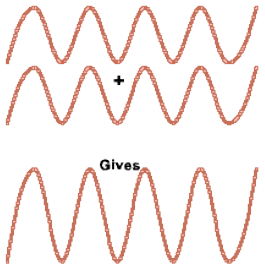
The slit has to have approximately the same width as the wavelength of the light that hits it.

If several slits are close together, the resulting waves overlap with each other.

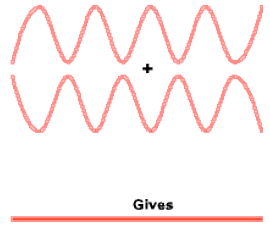
A pattern of amplification and cancellation is formed, called interference



Constructive interference leads to enhanced amplitudes.



Destructive interference leads to decreased amplitudes.



X-ray diffraction

To be able to see certain details we need light with approximately the same wavelength as what we want to see.

Atomic structure has details with distances on the order of 1-2 Å ($1Å=10^{-7}m$)

Hard X-ray radiation has wavelengths around 1Å.

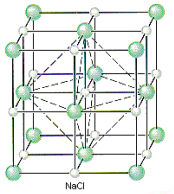
Electrons and neutrons have similar wavelengths and can also be used for diffraction and scattering experiments.

Why crystals?

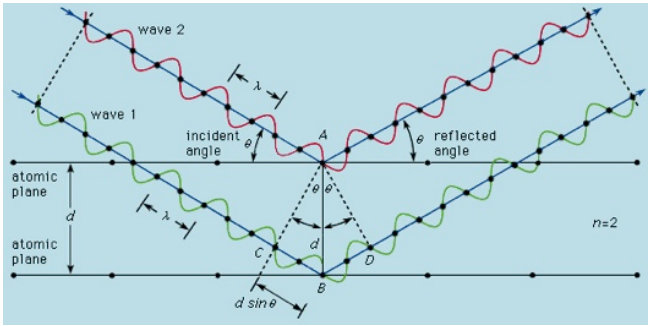
One molecule scatters X-rays poorly.
Many molecules are needed.
If they are organized in a crystal it simplifies work tremendously.

Bragg (father and son, Nobel price 1915) realized that the atomic structure of a molecule could be established using X-ray diffraction on crystals

X-ray diffraction can be related to microscopy.



Basis for structure determination by diffraction: Bragg's law



For constructive diffraction waves need to be in phase (λ) so
CB + BD = n (λ) ==> $2d\sin\theta = n(\lambda)$
θ is called the glancing angle
Measured reflex = F(hkl)

Electron density

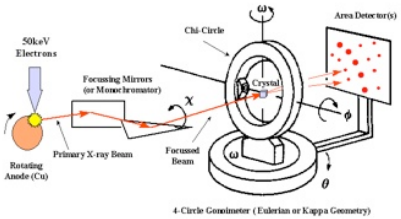
If measure all reflections from all planes F(hkl) we can calculate the electron density distribution:

$$\rho(r) = 1/V \sum F_{hkl} \exp(-2\pi i(hx+ky+lz))$$

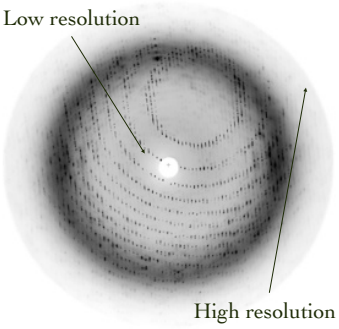
V = volume of the unit cell

This is a **Fourier** synthesis.

How does this relate to experiment?



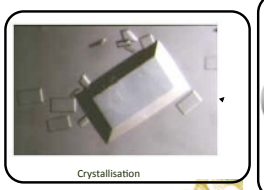
Crystal rotated by about 1°
Many pictures collected to get full "reciprocal space"

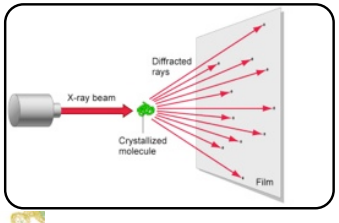


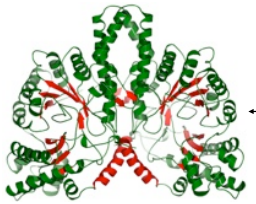
Several hundred planes went through the diffracting position during 1°
Each one has unique indices h,k,l

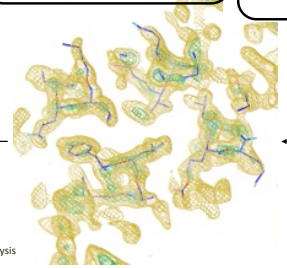
Macromolecular crystallography 101

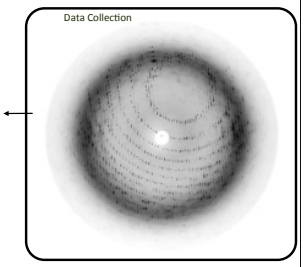

Protein Expression & Purification


Crystallisation




Structure Solution & Analysis





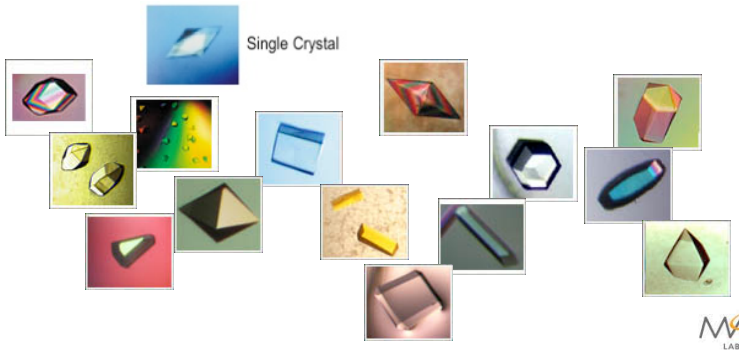
>90% of all high-resolution data collection for publication is is nowadays done at **synchrotrons**

06/27/12

Steps in determining a protein structure by crystallography

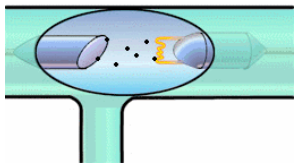
1. Express and purify the protein.
   10mg in non-denaturing conditions.

2. Crystallize the protein.



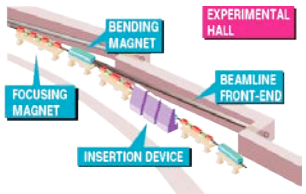
Steps in determining a protein structure by crystallography

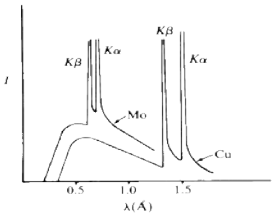
3. Data collection



Two main sources:

Rotating anode; CuK_α 1.54Å
Synchrotron; higher energy, wavelengths between 0.2 - 2Å





CuK_α, λ = 1.5418Å

Importance of synchrotron radiation

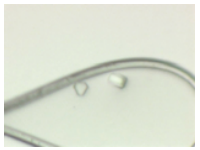
High intensity SR improves resolution limits

Highly intense beams with low divergence allow the study of smaller samples and larger complexes

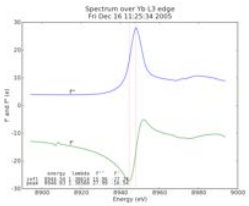
Difficult targets (e.g. membrane proteins) may give only microcrystals

Accurately tuneable SR is essential for modern methods to solve the "phase problem", e.g. multiple wavelength anomalous dispersion

The high-throughput methods require intense radiation



10 µm crystals



Typical anomalous edge

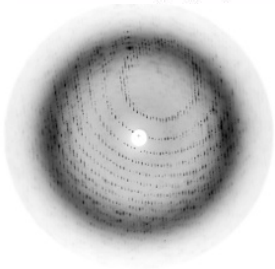
MAX IV LABORATORY

Steps in determining a protein structure by crystallography

4) Solve the phase problem

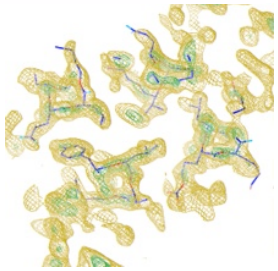
$$\rho_{(x,y,z)} = \frac{1}{V}\sum_h\sum_k\sum_l F_{(h,k,l)} \exp[-2\pi \cdot i(hx+ky+lz)]$$

$$\rho_{(x,y,z)} = \frac{1}{V}\sum_h\sum_k\sum_l |F_{(h,k,l)}| \exp[-2\pi \cdot i(hx+ky+lz-\alpha_{(h,k,l)})]$$



Diffraction pattern:

Not straight-forward!



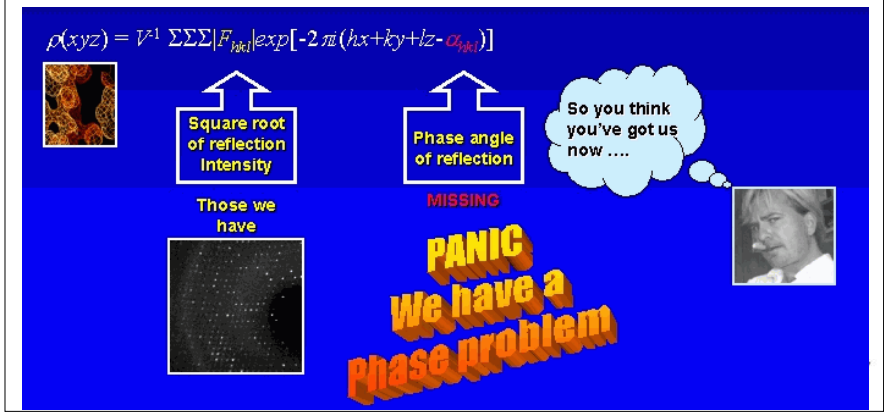
Electron density

MAX IV LABORATORY

What is the phase problem?

$$\rho_{(x,y,z)} = \frac{1}{V}\sum_h\sum_k\sum_l F_{(h,k,l)} \exp[-2\pi \cdot i(hx+ky+lz)]$$

$$\rho_{(x,y,z)} = \frac{1}{V}\sum_h\sum_k\sum_l |F_{(h,k,l)}| \exp[-2\pi \cdot i(hx+ky+lz-\alpha_{(h,k,l)})]$$



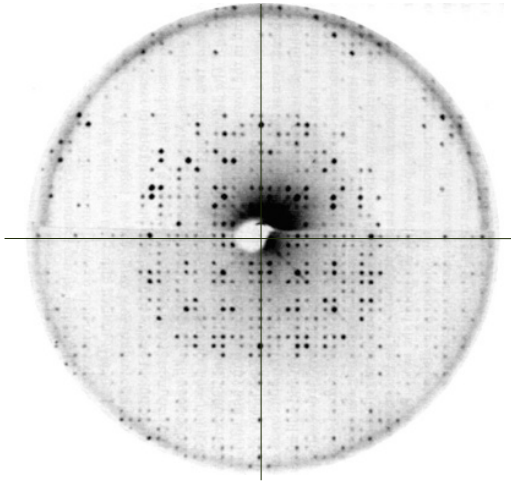
How to overcome the phase problem?

Different methods to overcome the phase problem:
-Patterson methods
-Direct methods

In protein crystallography:
-Multiple isomorphous replacement(MIR)
-Single or Multiple anomalous dispersion measurements (SAD or MAD)
-Molecular Replacement (MR)

MAX IV LABORATORY

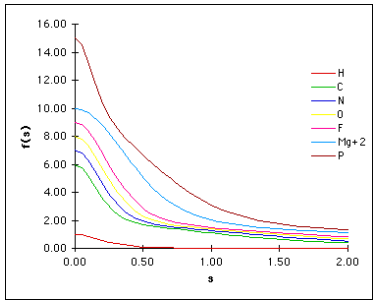
How do “heavy atoms” affect the observed diffraction?



“native”

“derivative”

What is anomalous dispersion?



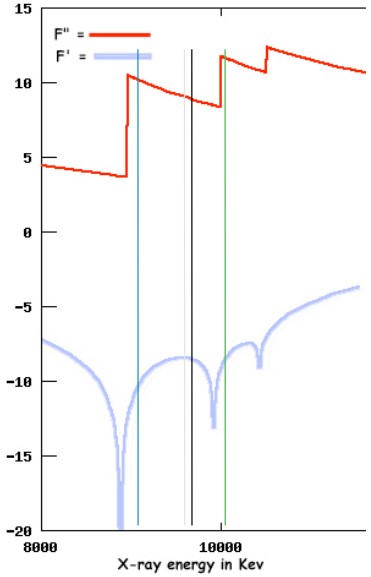
Each element has its own scattering factor f.
Scattering factors ~ amount of electrons/resolution

$$f^{o}(\sin\theta/\lambda) = \sum_{i=1}^{4} a_i \cdot e^{-b_i(\sin\theta/\lambda)^2} + c$$

Near absorption edges of elements f gets modified:

$$f = f^{o} + f' + i \cdot f''$$

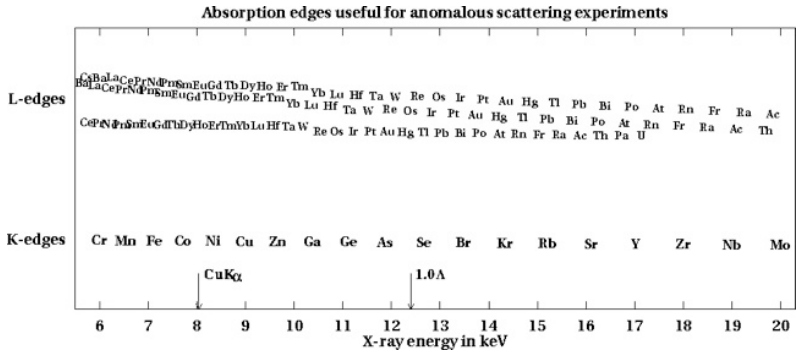
Multiple Anomalous Dispersion measurements



Data is collected around an absorption edge for a heavy atom (Fe, Cu, Se) in the protein.
3 or 4 λ:s at points where difference between f’ and f’’ are biggest!
Instead of collecting data on several different derivative soaked crystal, collect data from the same crystal at several different wavelengths.

This can only be done reliably on a dedicated beam line at a synchrotron.

Which edges can be found surrounding 1Å ?

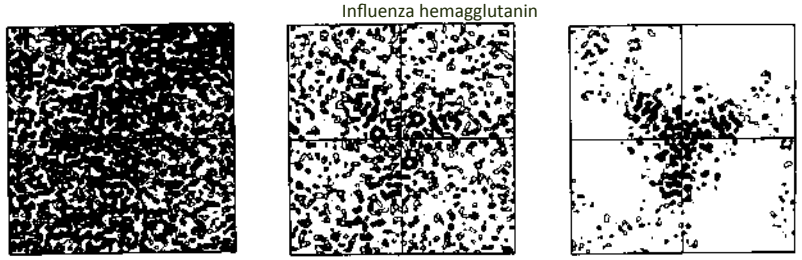


Steps in determining a protein structure by crystallography

5. Improve electron density (phase angles)

Local symmetry averaging (e.g. viruses), Solvent flattening. The solvent regions (more than 50% of the crystal) should have a flat density.

Influenza hemagglutanin



Experimental map: single heavy atom derivative

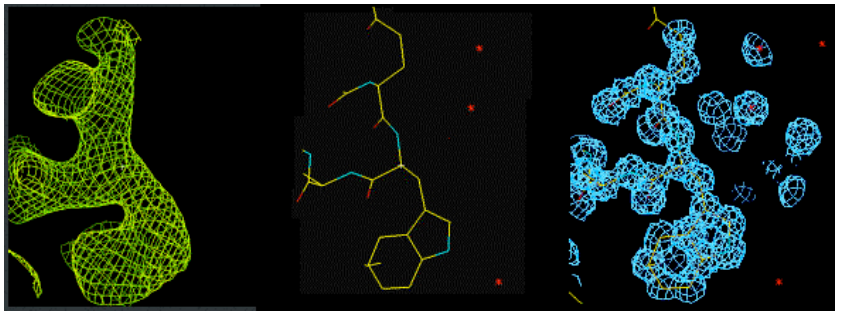
One cycle of averaging

Eleven cycles of averaging

Steps in determining a protein structure by crystallography

6. Interpret the electron density.

This is now done with more and more automatic methods.

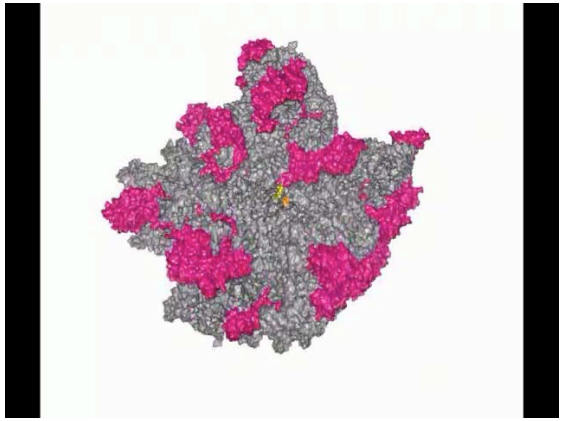


Electron density map

Model

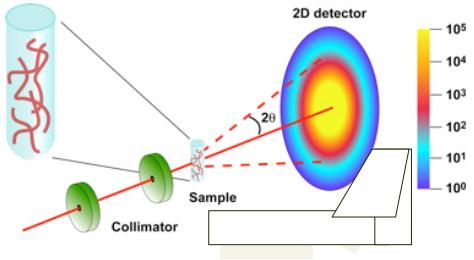
After refinement

2009 Nobel price for chemistry

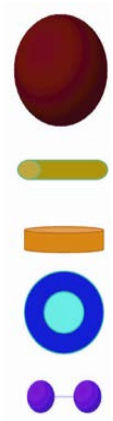


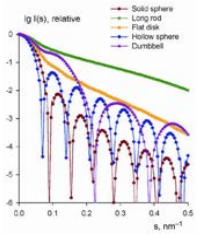
Small angle scattering of X-rays (SAXS)

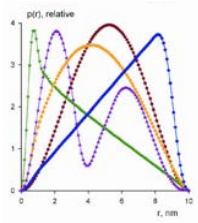
- Used since the 1960's to obtain low resolution structural information in the absence of crystals.
- Possible to obtain information on domain organisation and detailed modeling of macromolecular complexes using rigid body refinement.



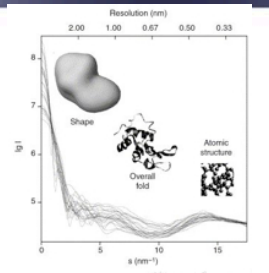
SAXS – Basic Principles



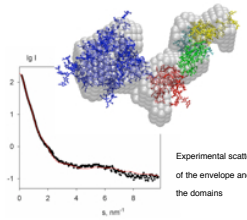




Specific shapes will give rise to specific scattering profiles. These can be used to get size and shape information



X-ray solution scattering curves computed from atomic models of 25 proteins with masses between 10–300 kDa.



Experimental scattering curve for a protein, an ab initio model of the envelope and a best fit using X-ray crystal structures of the domains

What can we do with SAXS (and SANS)?

- Model proteins and complexes that are impossible to crystallise
- Model disordered parts of high resolution crystal structures
- Confirm that the crystal structure of a flexible protein is the same as in solution

Advantages

- solution technique
- minimal sample preparation required
- ideal for studying dynamic processes and complexes
- rapid data collection

Disadvantages

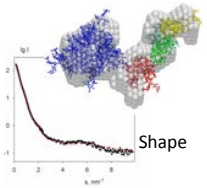
- low resolution structural information (10–20 Å)
- difficulty of interpreting 1D scattering curves as 3D models
- most powerful in combination with external information (crystal structures, NMR constraints etc.)

SAXS requires *highly focused synchrotron X-ray beams*

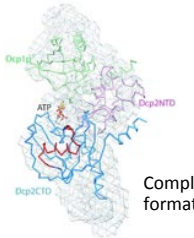
Ideal for MAX-IV!

MAX-lab

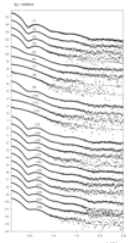
What can one do more with SAXS



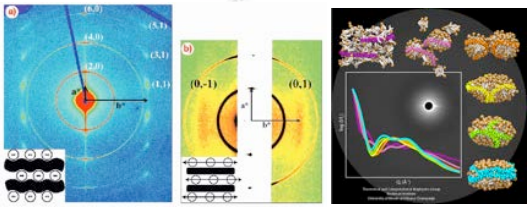
Shape



Complex formation



Kinetics

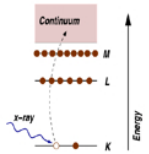


Membrane/micelle formation and organisation

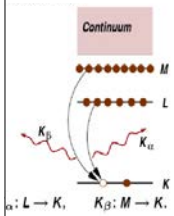
X-ray Photon Absorption Spectroscopy

XANES = X-ray Absorption Near-Edge Spectroscopy
EXAFS = Extended X-ray Absorption Fine Structure

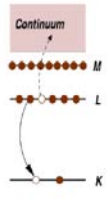
XAFS = X-ray Absorption Fine Structure



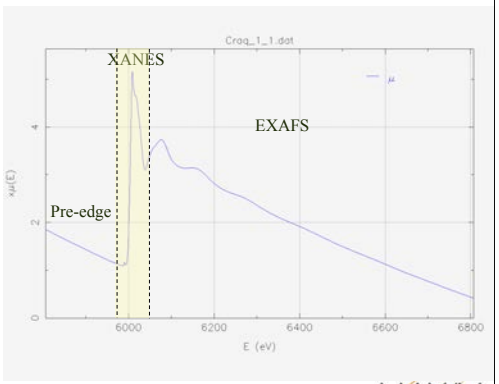
Absorption



Fluorescence



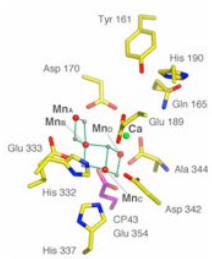
Auger effect



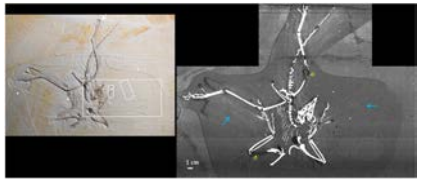
The specific energies are hight depending on the environment of the atom studied.

Spectroscopy:XAS (EXAFS, XANES), XRF

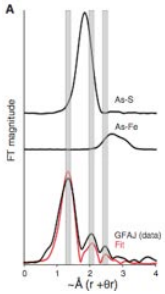
- Elegant probe for element speciation in biological and environmental samples. Probes can be very small: microns.
- Element-specific structural information.
- Detailed information on metal centers in proteins.

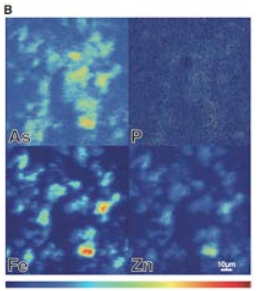


Jano et al., Science 2006



Bergmann U et al. PNAS 2010;107:9060-9065





Wolfe-Simon et al., Science 2010

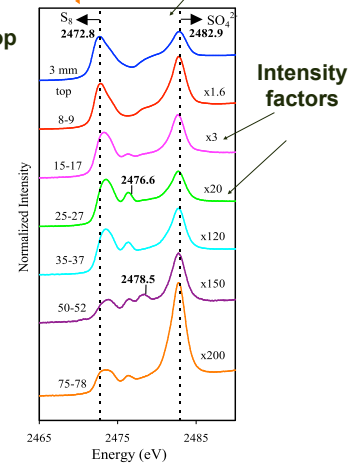
What is happening with the Vasa ship?

Elemental sulfur + thiols
R-SH 2473 eV

Sulfate 2483 eV

Sulfur XANES spectra from core C1 of the

Core top

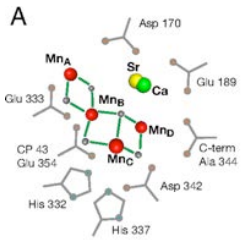


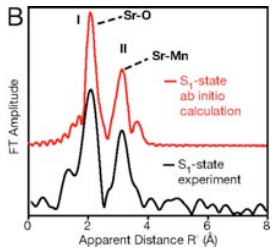
Intensity factors

Nature **2002**, *415*, 893-897
M. Sandström *et al.*

EXAFS looking at metallocenters in proteins

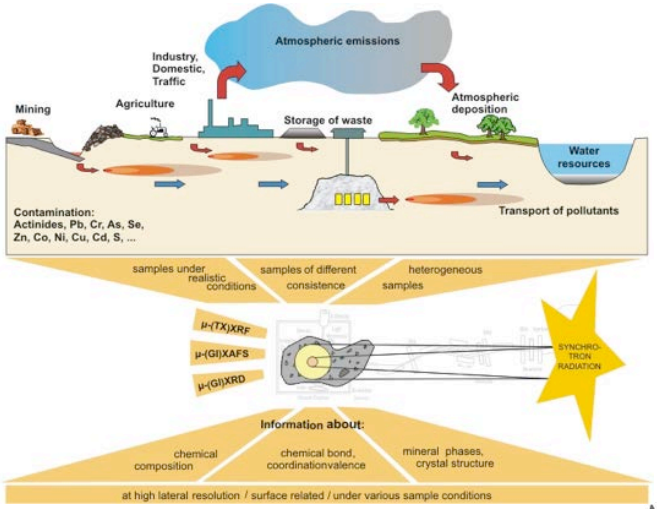
- Metallocenters in proteins are often very important for the function they perform
- Often involved in catalysis, especially transition metals
- EXAFS probes a specific element
- can reveals the oxidation states
- give detailed information of the metal centre coordination (distances precise within 0.01 Å).
- can be done on crystals but also in solution



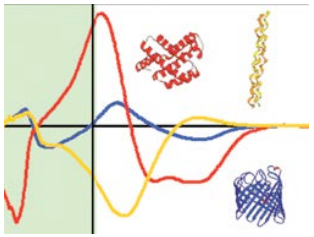


Metal centre of PS-II studied with combination of X-ray diffraction and EXAFS

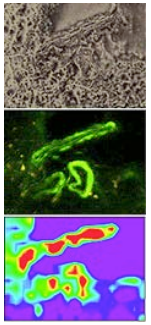
XAS: Environmental Sciences

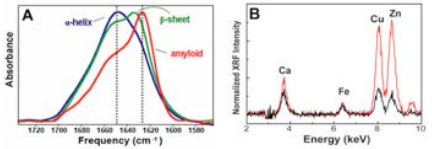


Other Spectroscopy



Circular dichroism --> secondary structure, folding





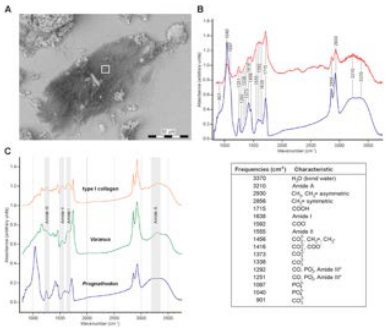
IR/UV -->Real-Time Biochemistry of Living Cells

(Brain tissue in Alzheimer's) Miller et al., 2006 J Struct. Biol.

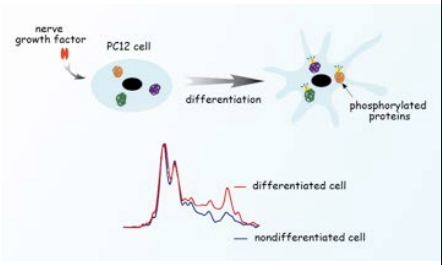
IR

In infrared spectroscopy molecular vibrations can be measured , since infrared radiation is absorbed specifically by chemical bonds.

Since peptide bonds are very specific they create an IR fingerprint for specific structural features (e.g. helix, strands, etc). Conformation changes or protein modifications can be measured, e.g. phosphorylation. Fingerprints can also indicate presence of certain molecules



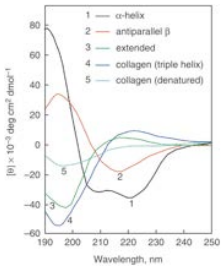
Lindgren et al., 2011, Plos One

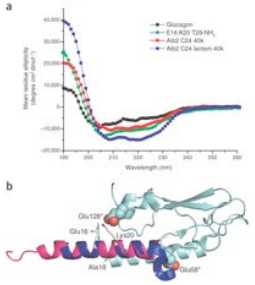


Chen et al., 2012 Anal. Chem.

Circular Dichroism

- Chiral molecules absorb left and right polarised light differently.
- This is what is being exploited in Circular Dichroism, in the UV, secondary structures of proteins can be probed.
- SR light gives access to lower wavelengths and better signal to noise.
- Used to study secondary structure contend of proteins, folding, stability, kinetics etc

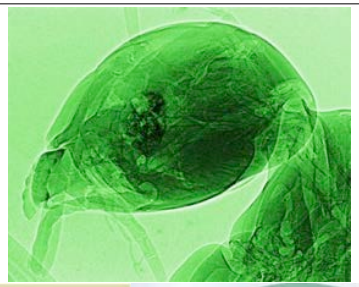


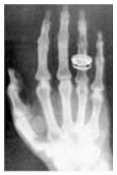


Day et al., 2009 Nat. Chem. Biol.

Cellular and Organism Imaging

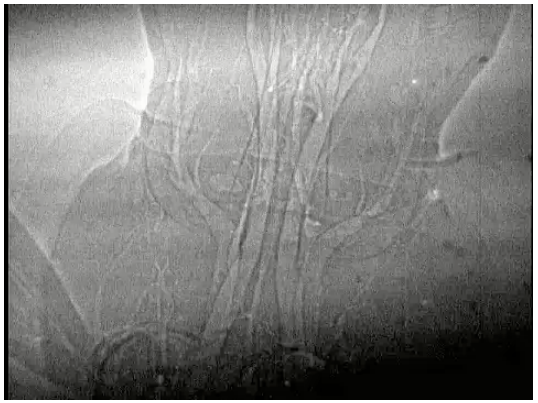
- Microscopy
- Tomography



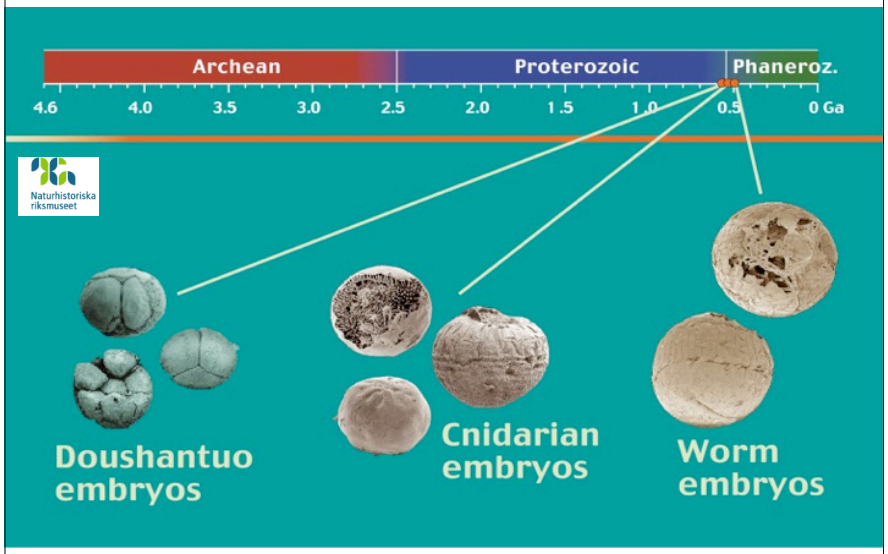


Imaging at synchrotrons

- Possibility to study live animals (insects) to look physiological processes, morphology of organs, penetrating the opaque shell.
- Processes like breathing, feeding, locomotion and more have been studied

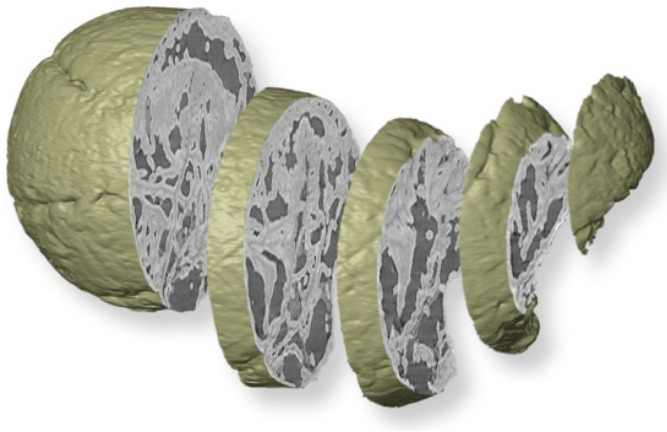


Westneat et al. , Science 299 (5606) 558-560.



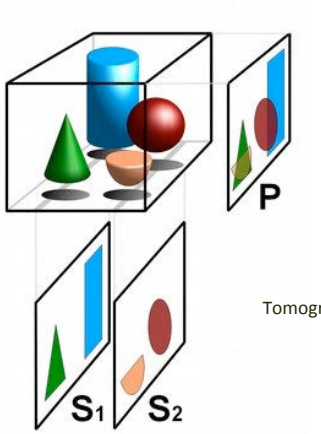
Earliest embryos of animals presenting initial diversification of metazaons - petrified

... the greatest invention since sliced bread!!!

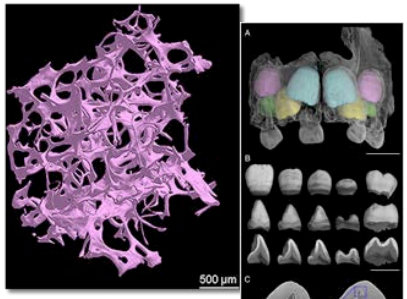


Naturhistoriska riksmuseet

Tomography



Scanning object with waves, reconstruct in a computer



Tomography image of osteoporotic bone

Aging of Neandertalers studied with micro-CT scans ,PNAS, 2010

MAX IV – What has happened?

2002 – 2006	2006 – 2008	2009
Dialogue with the user community First funding (KAW) Conceptual Design Report (CDR)	Evaluation(s) Redesign(s) Continued dialogue with user community	Research Government-Bill 27th of April MoU between Research Council, Vinova, LU & Region. Secured funds for a "start version" of MAX IV.

MAX IV will happen?

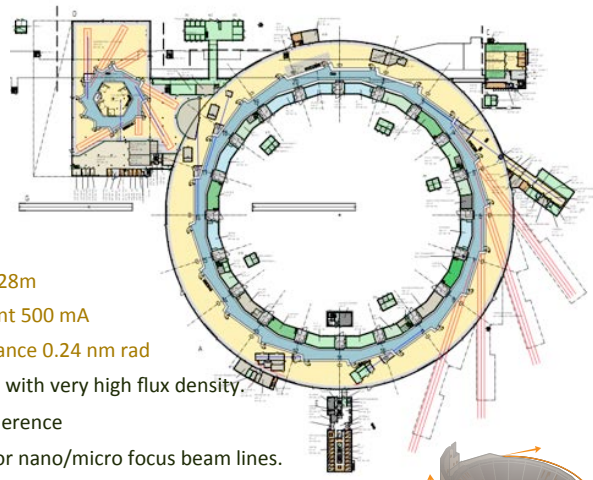
2009	2010	2010-16	2015-16
City plan for the MAX IV area. Procurement procedure building company	Detailed building First orders 22 Nov Ground Breaking Ceremony	2011 spring construction starts 2012 Linac 2013 Building (rings) 2015 1st beam	Start version of MAX IV in operation

Max IV

MAX IV – Unique Design

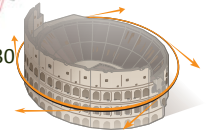
3 GeV ring	20 straight sections (0.24 nmrad) 528 m in circumference
1.5 GeV ring	12 straight sections (5.6 nmrad) 96 m in circumference (≈ MAX II)
3 GeV linac	Injections + Short Pulse Facility (partly underground)

MAX IV

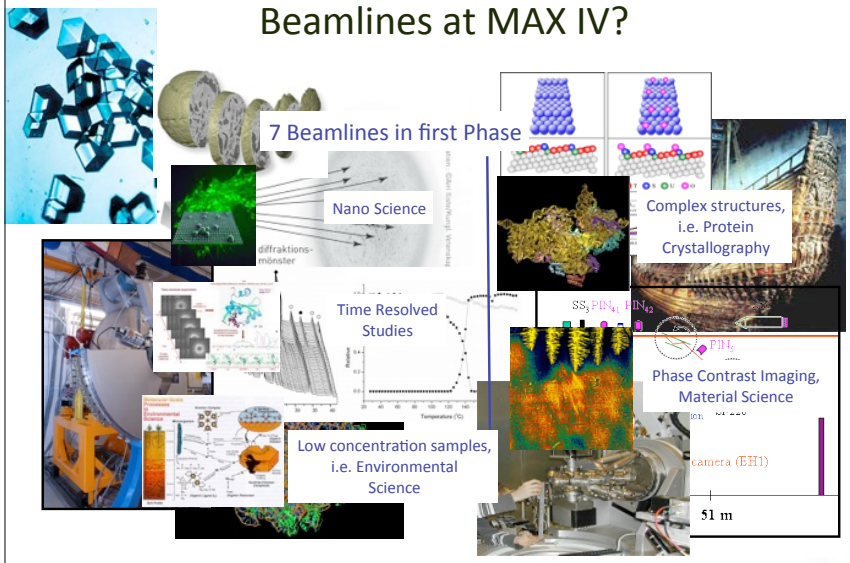


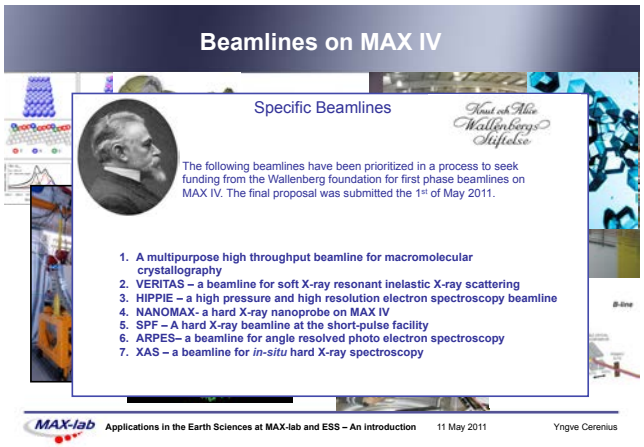
- energy 3 GeV
- circumference 528m
- circulating current 500 mA
- horizontal emittance 0.24 nm rad

- Very parallel beam with very high flux density.
- High degree of coherence
- Optimum source for nano/micro focus beam lines.
- 19 beamlines at 3 GeV, 9 beamlines at 1.5 GeV and 2 at the SPF: 30 in total.
- 7 beamlines financed through KAW and Swedish Universities
- Strategic plan for 80% of beamlines made, see www.maxlab.lu.se



Beamlines at MAX IV?

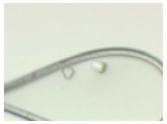




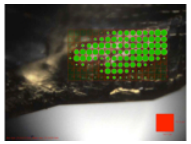
Needs and Possibilities for

For macromolecular crystallography- MAX IV Laboratories

- More brilliant, more focused beam for micro-crystals (e.g. membrane proteins)
  - MAX-II >150 µM; MAX-IV < 1 µM
  - Beam size and focus of MAX IV will be the best in the world!
- Challenging studies
  - Large protein complexes - small crystals, large unit cells
  - Membrane proteins - small crystals
- Automation and high throughput
  - few minutes vs. several hours per data set
  - rapid data collection for kinetic studies
  - up to 1000 samples per day?
- Combination with spectroscopic methods (UV/vis, EXAFS etc.)



10 µm crystals



For macromolecular crystallography- ESS

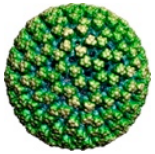
- Higher Intensity source with detectors such that work on moderate unit cells will be possible

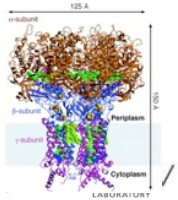
For solution studies

- A dedicated SAXS beam line
- A dedicated SANS beam line

Future

- Imaging beam lines

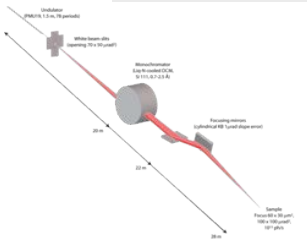




Plans for structural biology at MAX IV

- Macromolecular crystallography
- Two beamlines proposed, both tuneable wavelength and with *in situ* spectroscopy
- BIOMAX (First Phase)
  - **High throughput, highly automated beamline**
  - Beam size at crystal 20–100 µm
  - Tried and tested technology
  - **Operational and reliable from day 1**
  - Sample changer robotics
  - Remote access
- MX-2
  - **Microfocus beamline**
  - Cutting edge technology
  - Beam size at crystal < 1–5 µm

preliminary design for MX-1



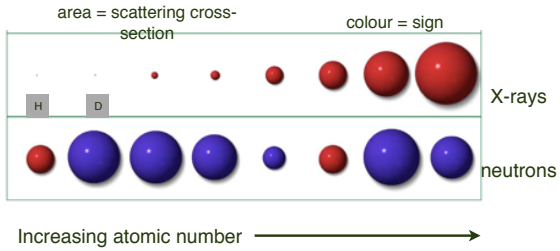
www.bioxtas.org

Small-angle X-ray scattering

- A SAXS beamline (both bio and non-bio)
- Reduced sample volumes (from 100s of µl to sub-µl using microfluidics

- In a longer perspective: Biological/Medical Imaging

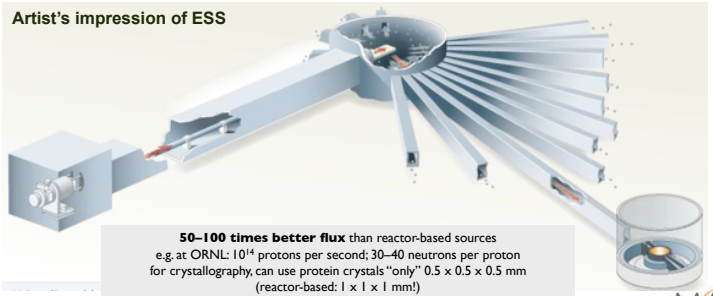
Neutrons and X-rays are complementary



- X-rays interact with the electron clouds around atoms
- Strength of interaction depends on the no. of electrons
- Neutrons interact with the nuclei
- No linear relationship with atomic number
- Neutrons also have a magnetic moment
- Neutrons are non-destructive

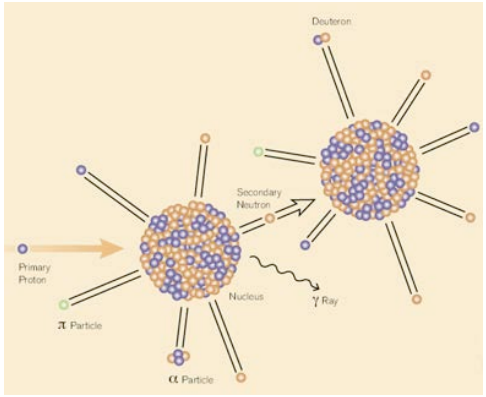
Why spallation sources?

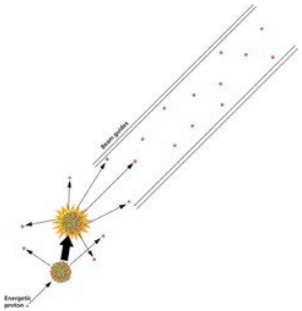
- The traditional source of neutrons is a nuclear reactor
- Very large and weak beam compared to X-rays
- An diffraction experiment that takes 5-10 minutes with X-rays at the ESRF could take several days with neutrons at the nearby ILL.
- Only three other spallation sources in the world
  - Spallation Neutron Source (Oak Ridge National Laboratory, Kentucky, USA)
  - Los Alamos National Laboratory
  - J-PARC (Ibaraki, Japan)



50–100 times better flux than reactor-based sources
e.g. at ORNL: 10^{14} protons per second; 30–40 neutrons per proton
for crystallography, can use protein crystals "only" 0.5 x 0.5 x 0.5 mm
(reactor-based: 1 x 1 x 1 mm!)

Neutron Spallation





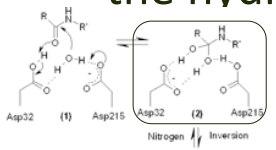
A high velocity proton is accelerated into a heavy particle (e.g. Hg nucleus).
A number of spallation particles is produced amongst which neutrons

Biology at a Neutron Source

- Many techniques same as those that are used at a synchrotron

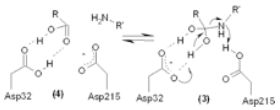
- Crystallography
- Small Angle Scattering
- Reflectometry
- Spectroscopy
- Imaging

Diffraction: Neutrons help us see the hydrogen atoms



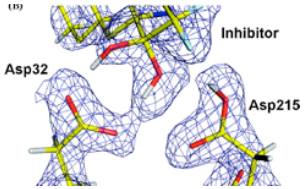
proposed reaction mechanism for aspartic proteases

transition state stabilised by negative charge on Asp32



studies on human aldose reductase:
0.66 Å X-ray map: 54% of H visible
2.* Å neutron map: **% of H visible

perdeuteration is very important



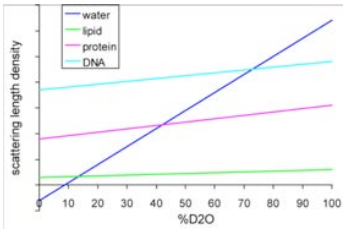
endothiapepsin complexed with gem-diol transition-state mimicking inhibitor

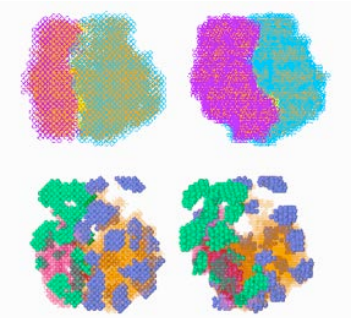
Neutron map at 2.0 Å resolution reveals the protonation states

An X-ray map at 1.0 Å resolution did not reveal any protons

Coates et al. (2008), *JACS* **130**, 7235

Scattering:Neutrons give contrast





- By playing with the level of D2O/deuteration of components in an experiment, specific components can be highlighted or blanked out.
- Especially in studies of complexes this can be a big advantage.

The future is bright……