APLICACIONS BIOMÈDIQUES DE LA LLUM DE SINCROTRÓ

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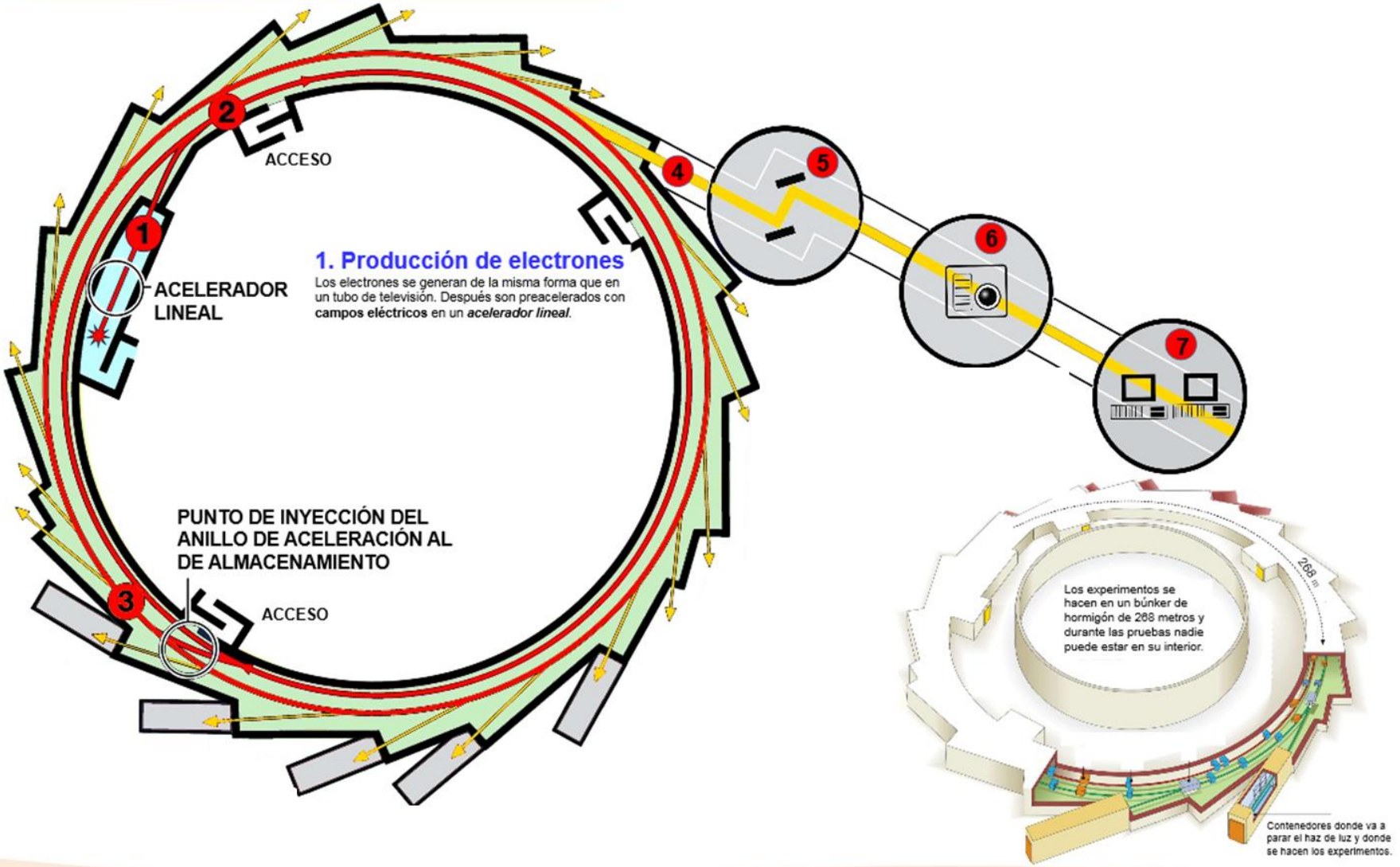
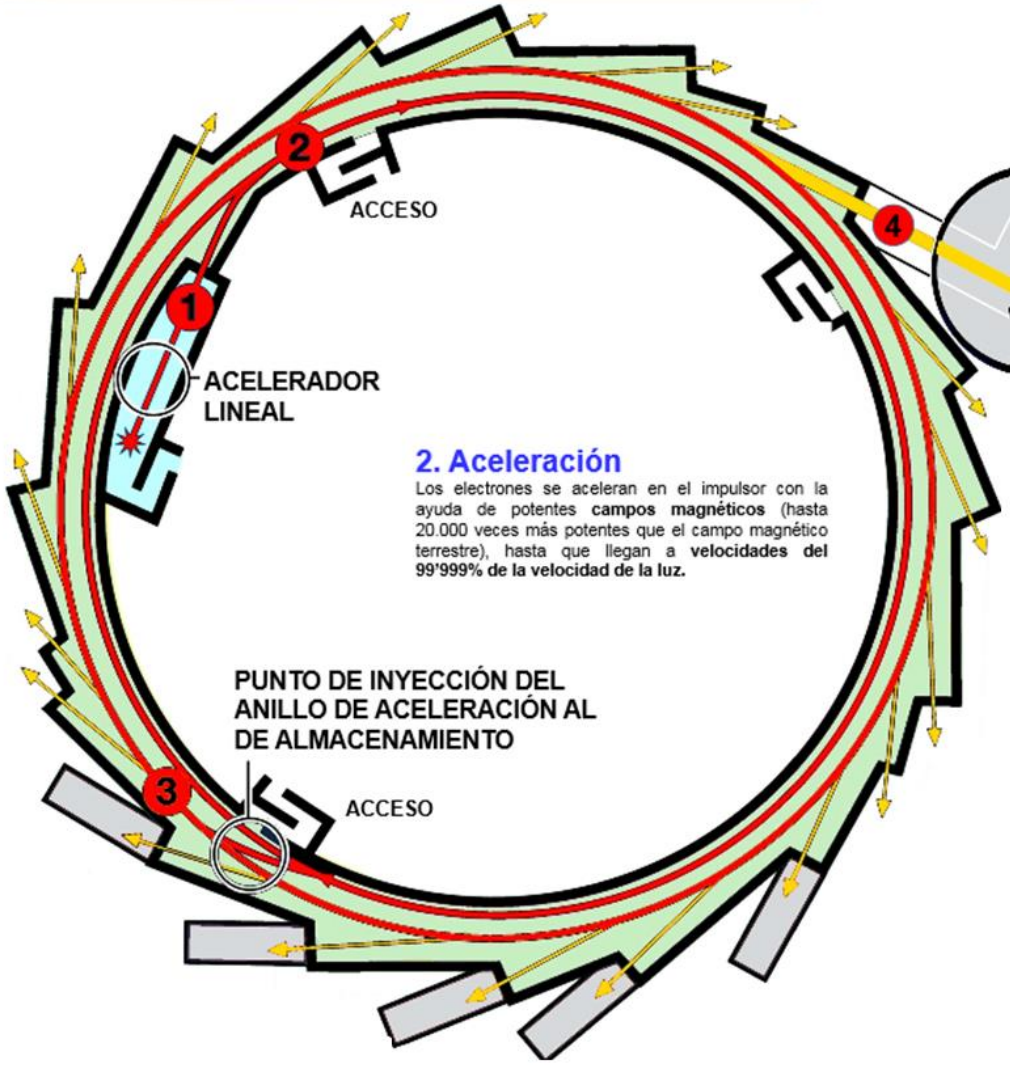
Postodoc at Infrared Beamline (MIRAS)
ALBA Synchrotron

1. Com funciona un sincrotró
2. MIRAS: Microscopia d'Infraroig
    1. Microscopia dInfraroig
    2. Estudis d'Infraroig en la malaltia d'Alzheimer
    3. Altres Exemples
3. MISTRAL: Tomografia de rajos X
4. CLAES: Absorció de rajos X
5. NCD I Xaloc: Difracció de Rajos X
6. Demanar beamtime

¿Cómo funciona un sincrotrón?

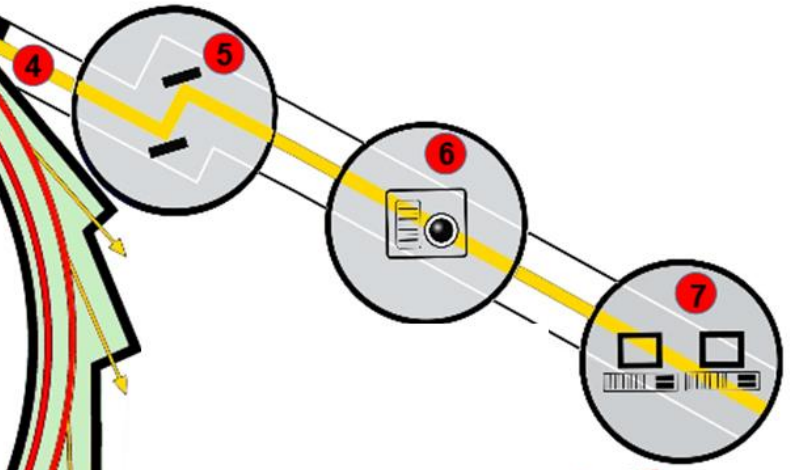


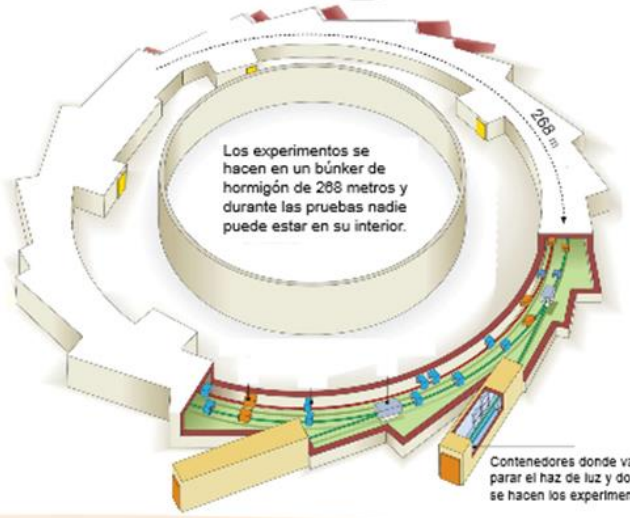
¿Cómo funciona un sincrotrón?



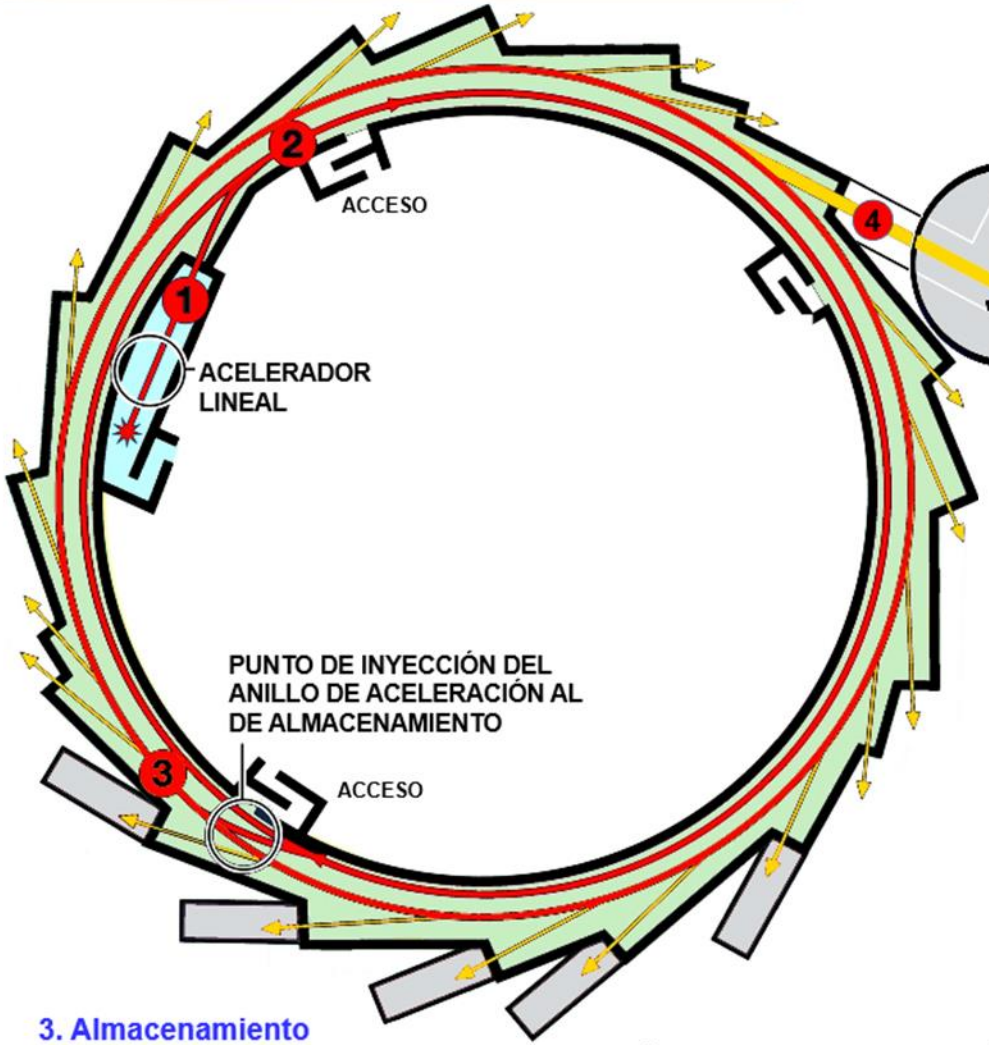
2. Aceleración

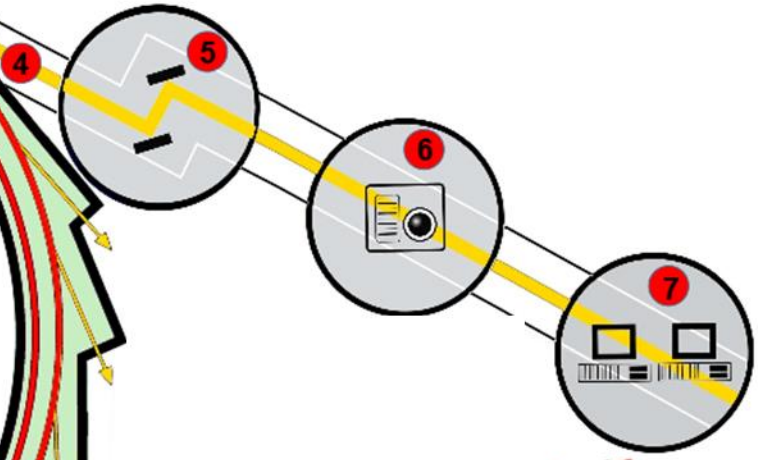
Los electrones se aceleran en el impulsor con la ayuda de potentes **campos magnéticos** (hasta 20.000 veces más potentes que el campo magnético terrestre), hasta que llegan a **velocidades del 99'999% de la velocidad de la luz.**

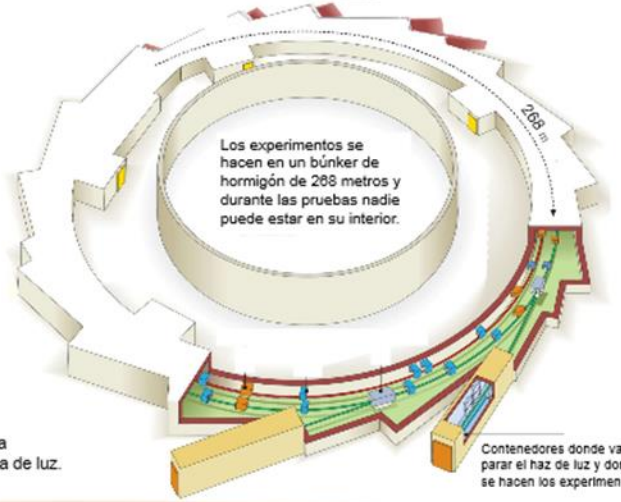




¿Cómo funciona un sincrotrón?

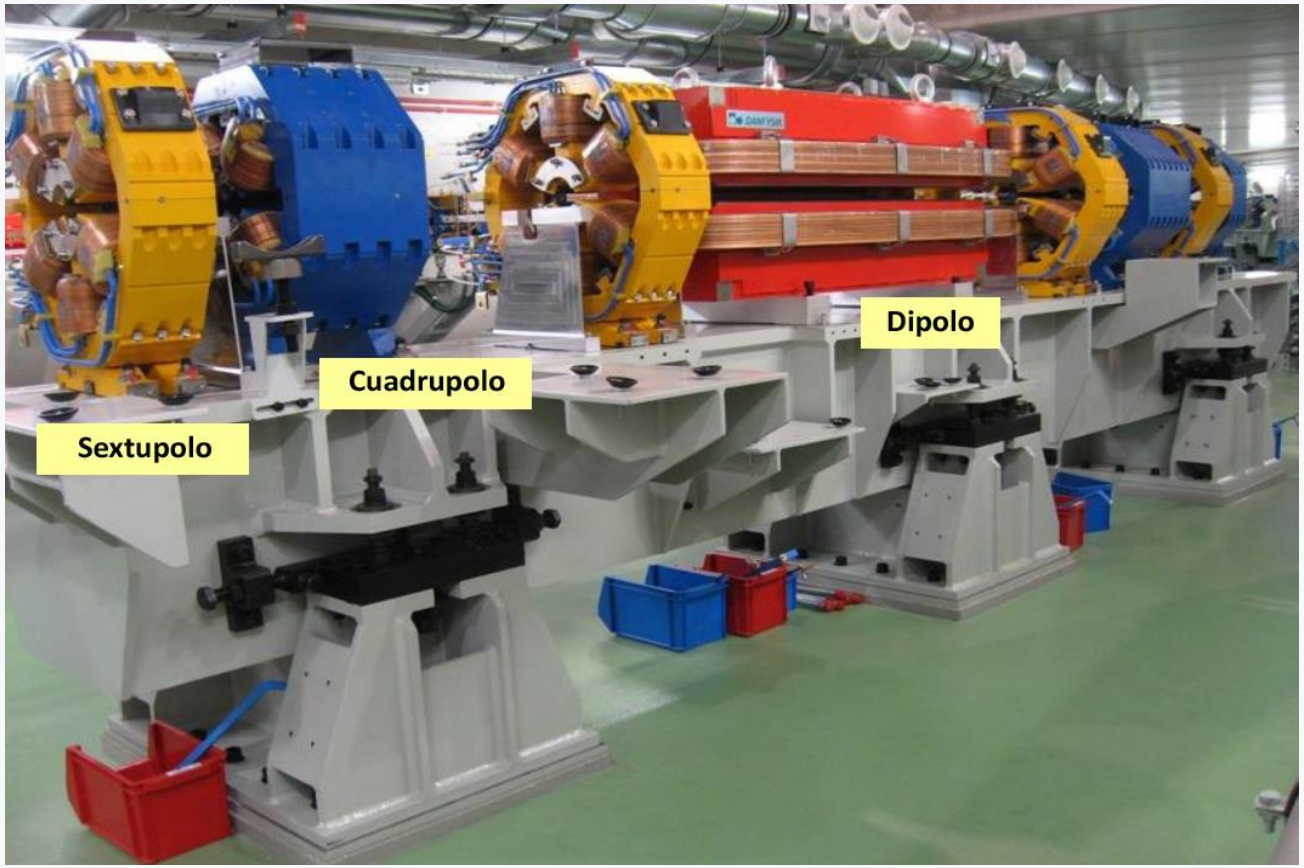




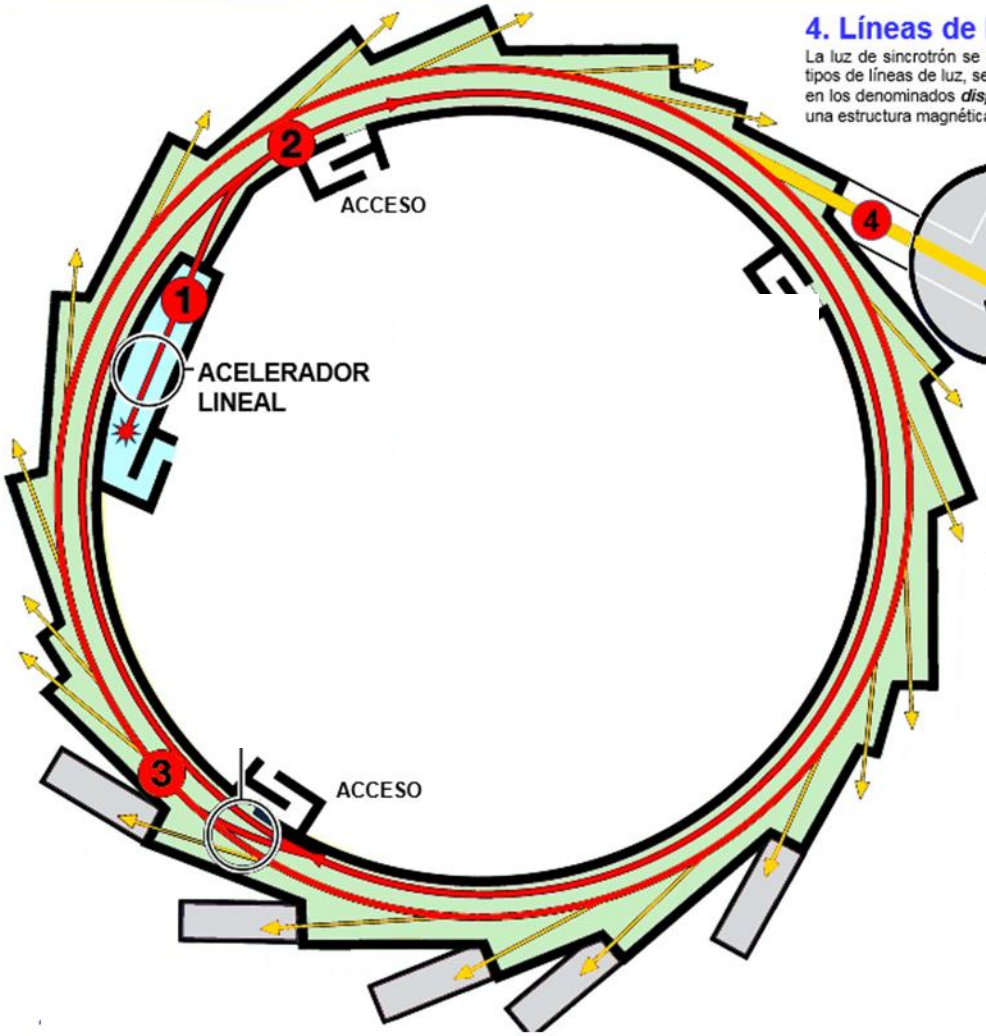


3. Almacenamiento

Los electrones se inyectan en el anillo de almacenamiento, donde mantienen una **órbita circular** canalizados por **campos magnéticos** muy fuertes. La velocidad se mantiene constante con **campos eléctricos** alimentados por **radiofrecuencia**, compensando la energía que se pierde al emitirse en forma de luz.


Dipolo
Cuadrupolo
Sextupolo

¿Cómo funciona un sincrotrón?



4. Líneas de luz

La luz de sincrotrón se propaga hasta las *líneas de luz*, colocadas tangencialmente al acelerador. Hay dos tipos de líneas de luz, según si usan los rayos X generados en los **imanes curvadores**, o bien los generados en los denominados ***dispositivos de inserción***, que hacen ondular la trayectoria de los electrones mediante una estructura magnética periódica.

5. Acondicionamiento

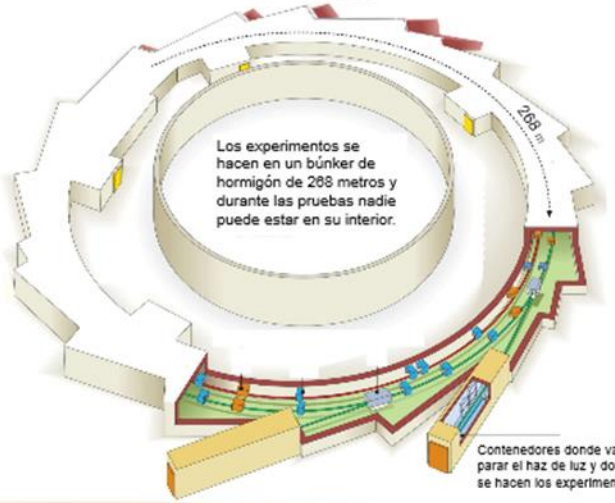
La luz emitida pasa por una cabina óptica, donde se **selecciona** una determinada energía o **longitud de onda** con un **monocromador**. Los fotones de luz de sincrotrón **se enfocan** y se canalizan hacia la muestra usando **espejos** de rayos X.

7. Análisis de datos

En la cabina de control, las **computadoras** controlan todo el proceso de medición, y recogen los datos mesurados por los detectores. Los datos son extraídos, procesados y preparados para el **análisis e interpretación de los científicos** responsables de los experimentos.

6. Detección

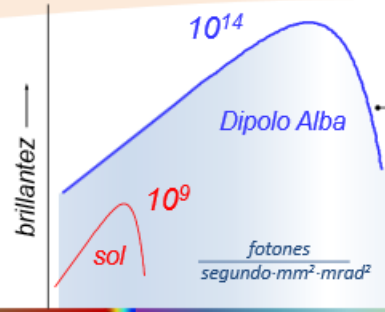
En la cabina experimental, se coloca la muestra y un sistema de detección **recoge la luz reflejada, difractada o transmitida**. Hay muchos tipos de detectores, que están especializados para cada tipo particular de experimento.



Contenedores donde va a parar el haz de luz y donde se hacen los experimentos.

La fuente de luz ALBA

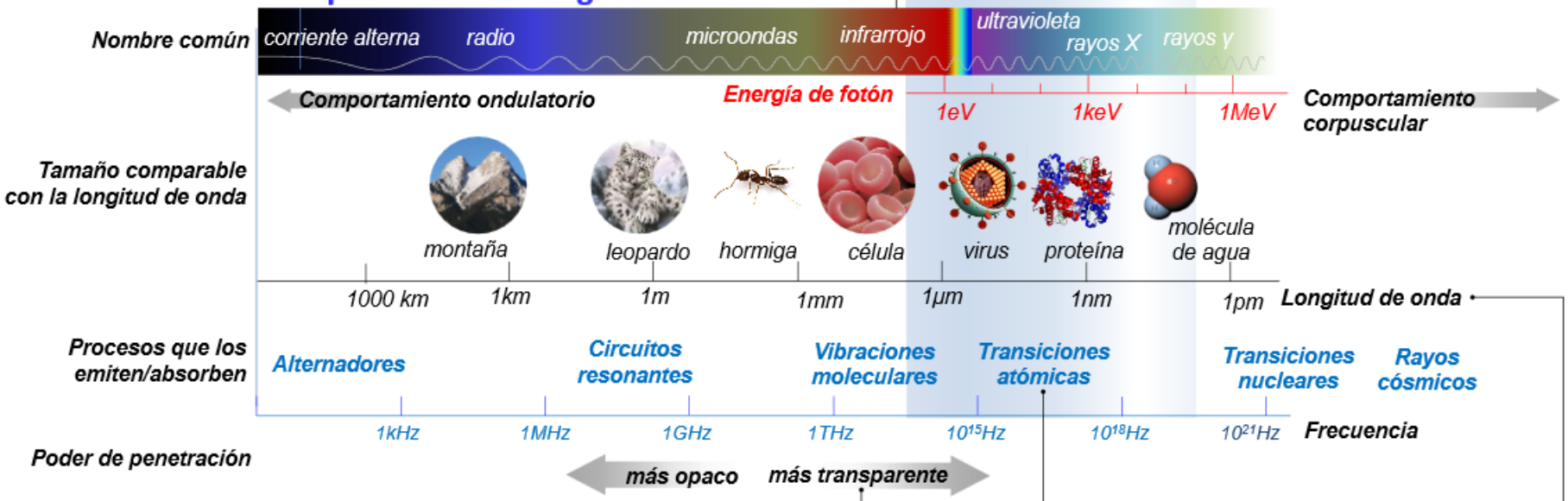
Los **rayos X** son **ondas electromagnéticas**, como la luz visible, aunque tienen **longitud de onda** mucho más **corta**, o sea, una **frecuencia** más **alta** y **más energía** por cada fotón. Sus propiedades les hacen muy útiles para **estudiar la materia**.



Brillantez

Los rayos X interactúan débilmente con la materia. Así que los experimentos requieren muchos fotones.

El espectro electromagnético



Poder de penetración

Los rayos X interactúan débilmente con la materia. Lo hacen principalmente cuando están en resonancia con alguna transición atómica. El resto de elementos son transparentes. Esto permite **observar el interior** de los objetos.

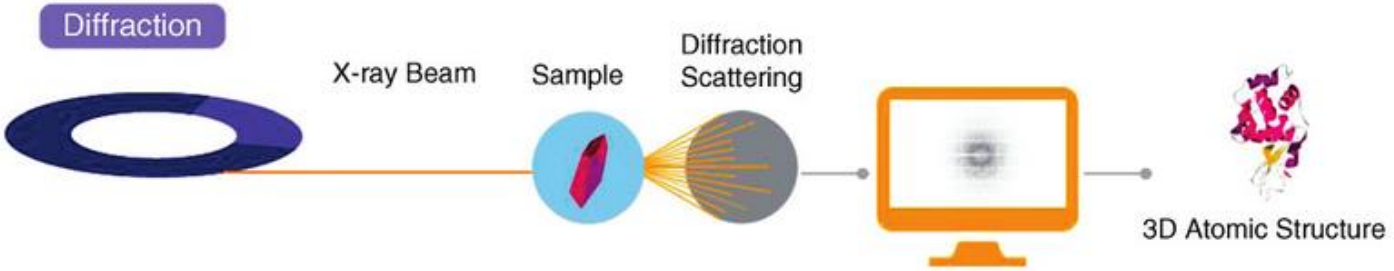
Transiciones atómicas

La energía de los fotones de rayos X corresponde a las transiciones de los electrones entre los niveles atómicos, para la mayoría de materiales sólidos.
Estos niveles determinan las **propiedades físicas y químicas** de los materiales, que se pueden estudiar con experimentos de **espectroscopia**.

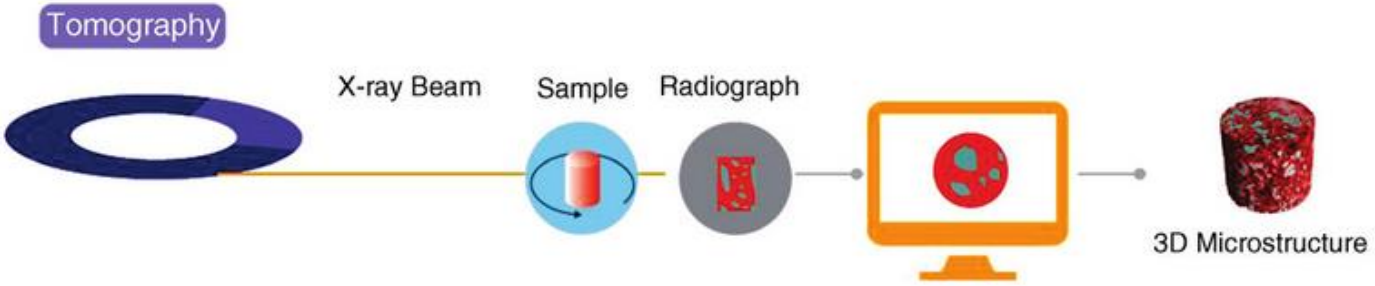
Longitud de onda muy corta

La longitud de onda de los rayos X es comparable al tamaño de los átomos. La cual cosa permite observar su estructura mediante experimentos de **difracción**.

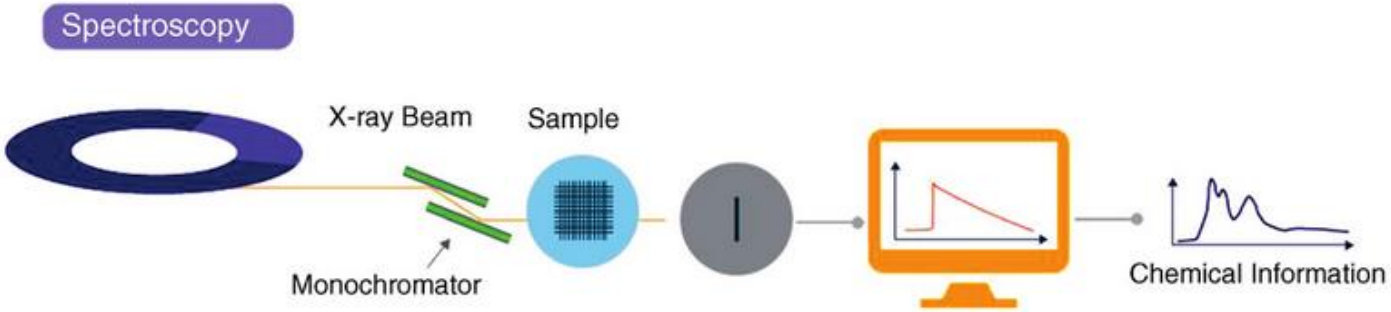
Interacció de la llum amb la matèria



XALOC: Línia de cristalografia
NCD: Difracció no cristal·lina



MISTRAL: Tomografia de rajos X

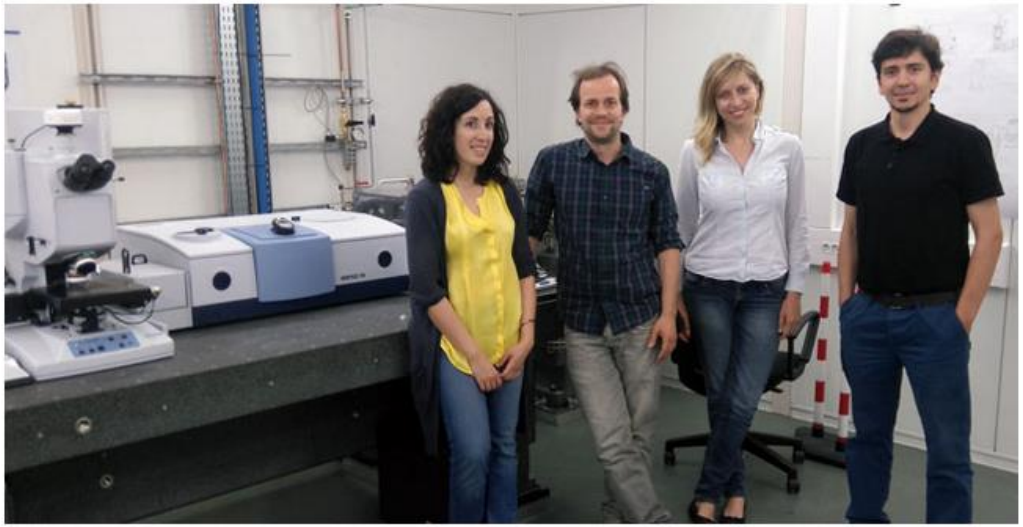


CLAES: Espectroscopia d'absorció de rajos X
MIRAS: Microscopiqa d'IR

MIRAS

BEAMLINE TEL.: (+34) 93 592 4001

BEAMLINE SCIENTISTS



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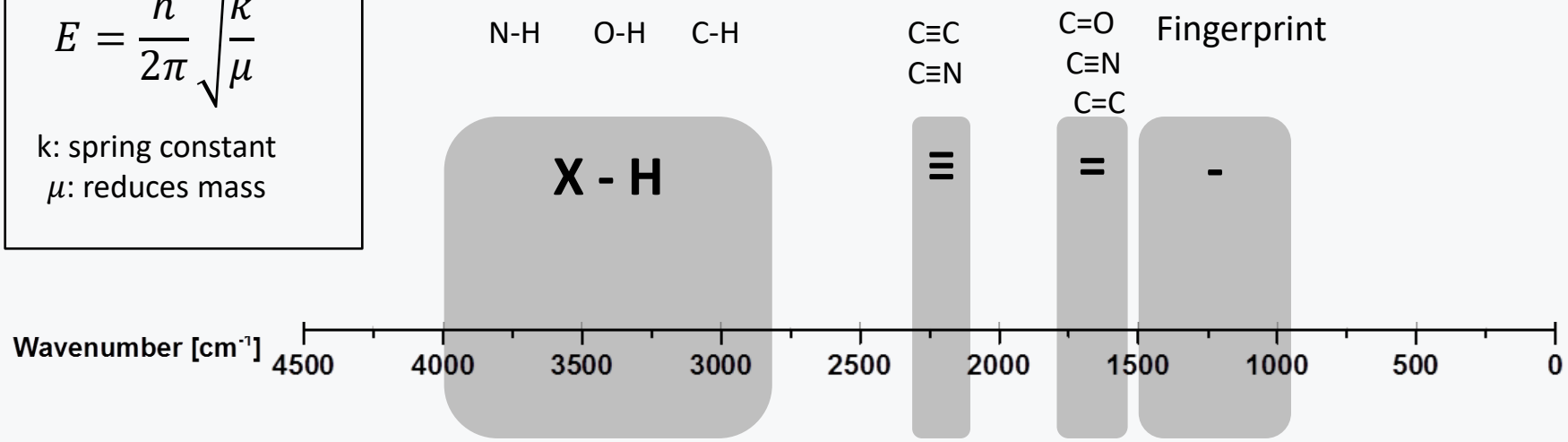
Chemical bonds – *springs & mass*

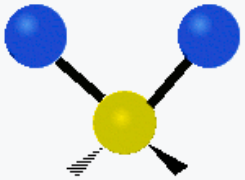
$$E = \frac{h}{2\pi}\sqrt{\frac{k}{\mu}}$$

k: spring constant
μ: reduces mass

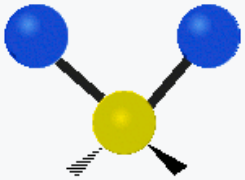


Vibrational modes:

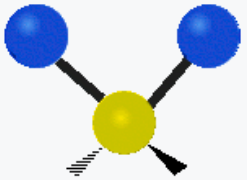
Symmetric stretching



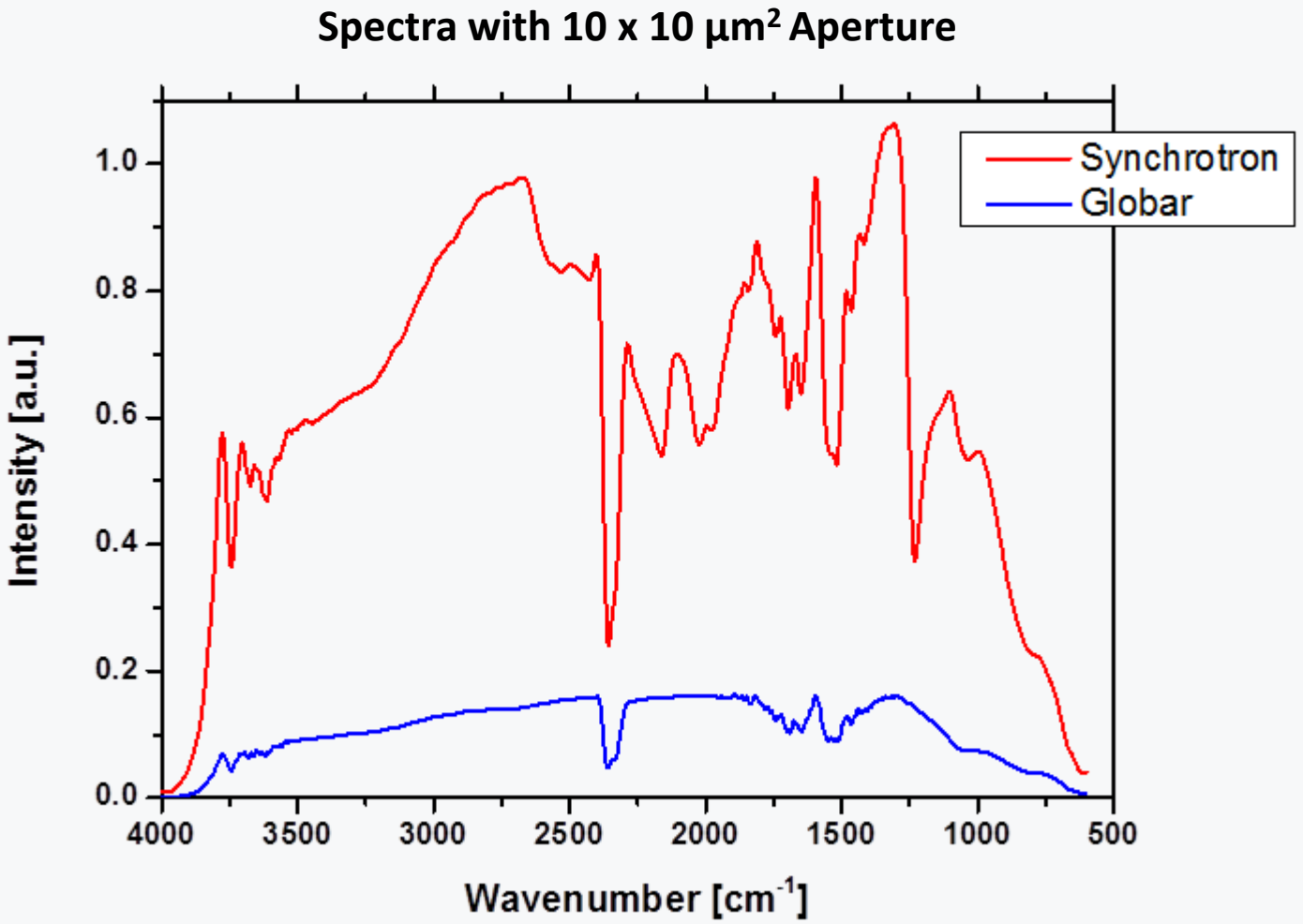
Antisymmetric stretching



Scissoring

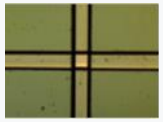


Martin Kreuzer

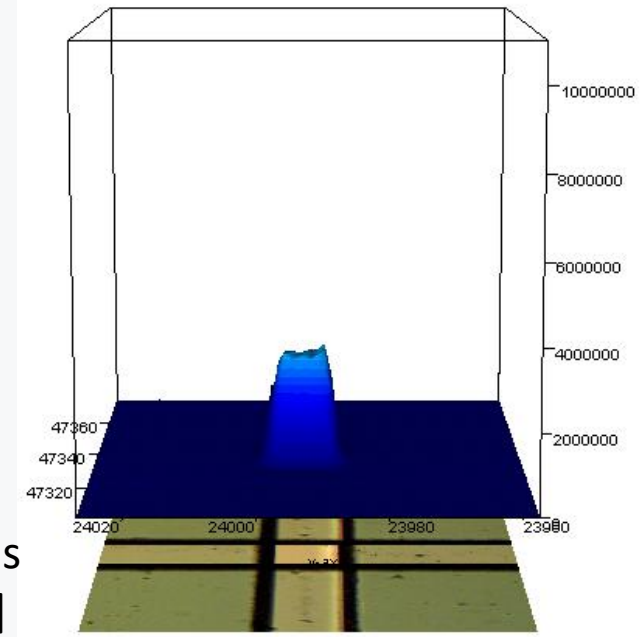

Spectra with 10 x 10 µm² Aperture
Synchrotron
Globar
Intensity [a.u.]
1.0
0.8
0.6
0.4
0.2
0.0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber [cm⁻¹]

Globar vs Synchrotron light source

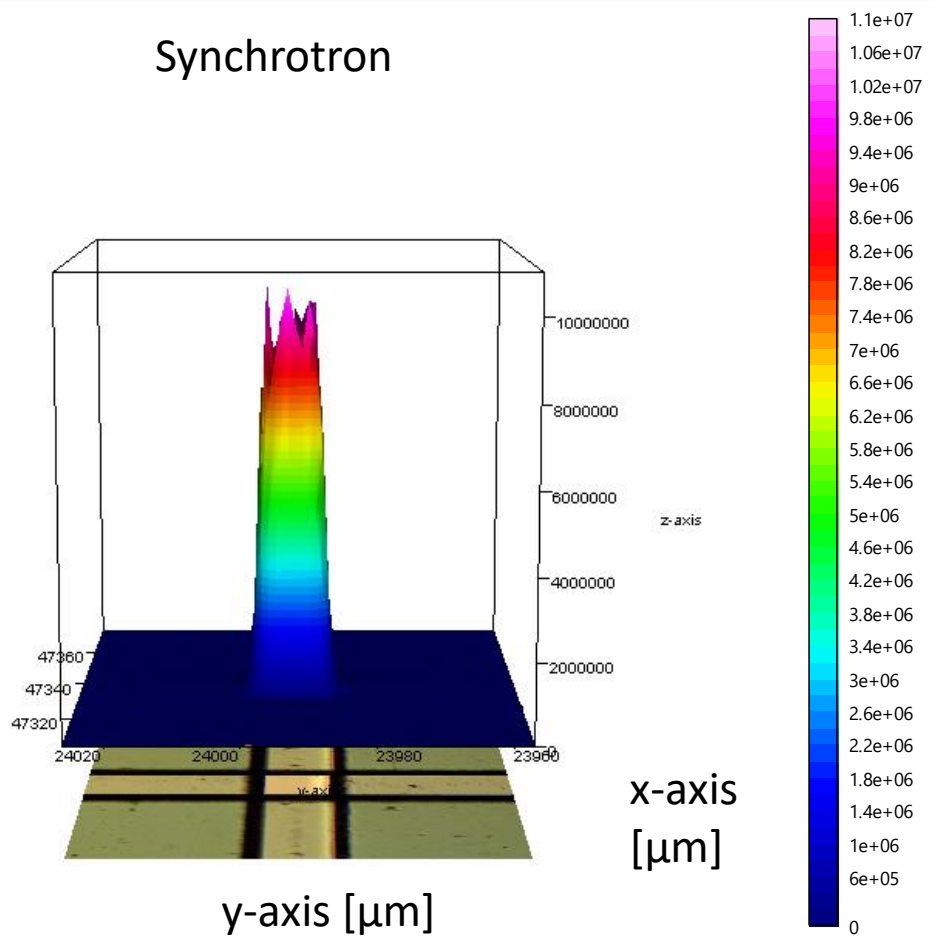
10 x 10 µm^2 Aperture:



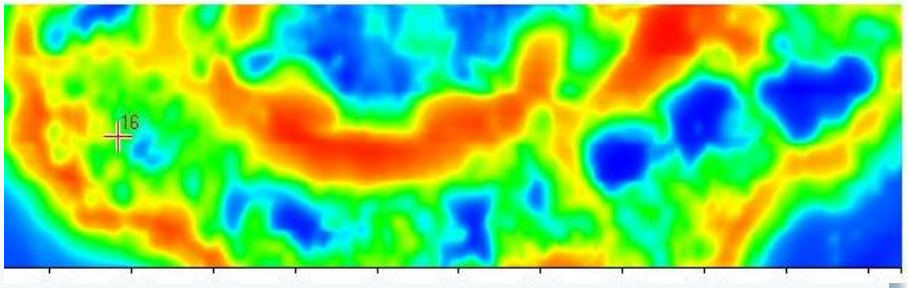
GLOBAR

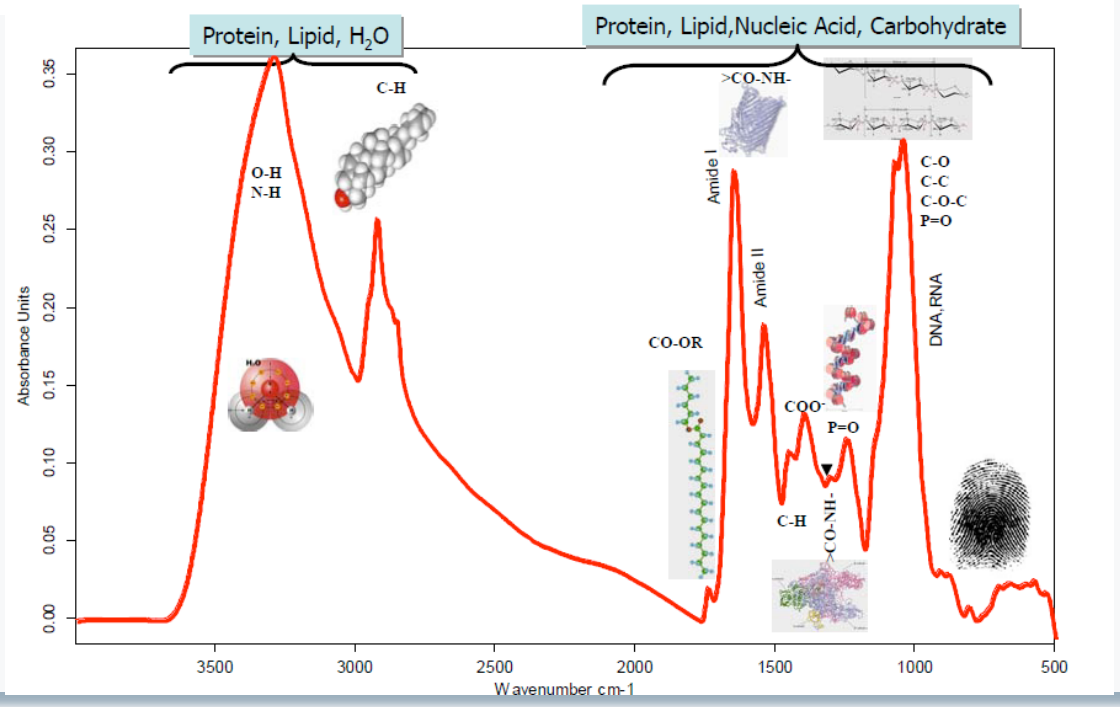


Synchrotron

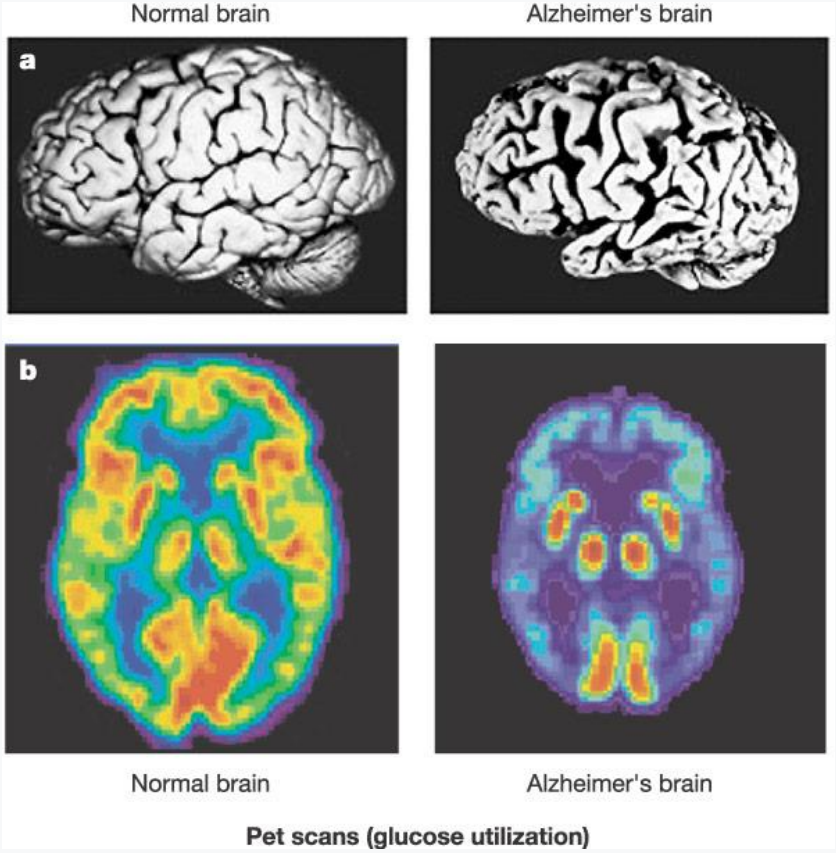



MCT DETECTOR
FPA DETECTOR
ENTRANCE OF THE SYNCHROTRON LIGHT
MATCHING UNIT
SAMPLE
HYPERION 3000 MICROSCOPE
VERTEX 70 SPECTROMETER


16


Protein, Lipid, H2O
Protein, Lipid,Nucleic Acid, Carbohydrate
C-H
O-H
N-H
>CO-NH-
Amide I
Amide II
C-O
C-C
C-O-C
P=O
DNA,RNA
CO-OR
COO-
P=O
C-H
CO-NH-
Absorbance Units
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

Alzheimer Disease



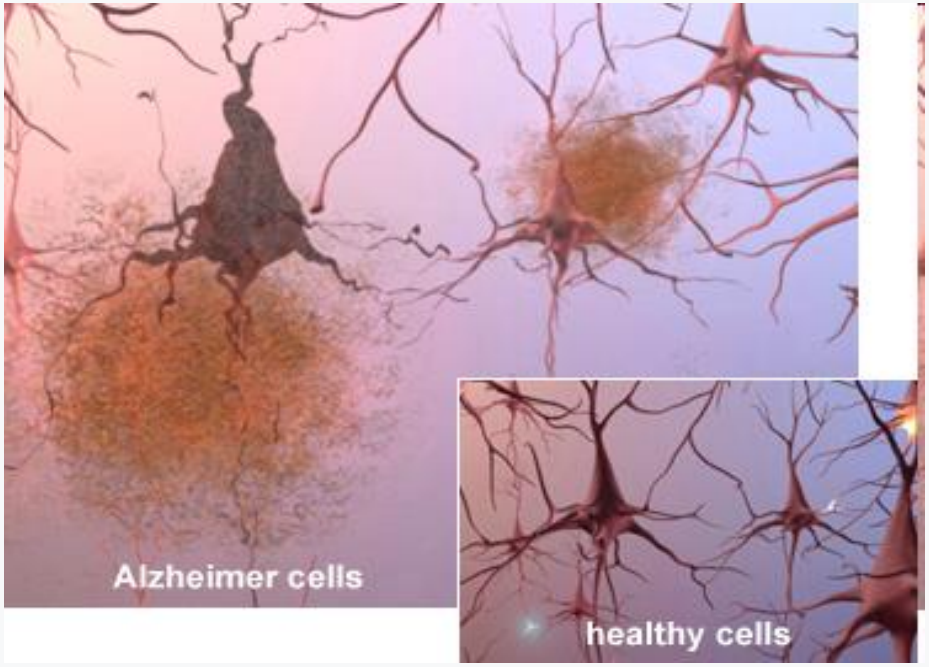
Pet scans (glucose utilization)

Cortex shrivels up:
Damaging areas involved in thinking, planning and remembering.

Severe Hippocampus shrinkage
Damaging areas play a key role in formation of new memories.

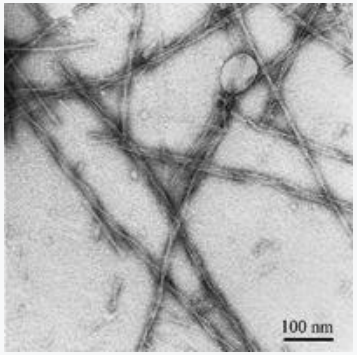
Ventricles grow larger

Amyloid peptides



Presence of amyloid plaques in the affected regions.

Plaques present in Alzheimer Disease brain

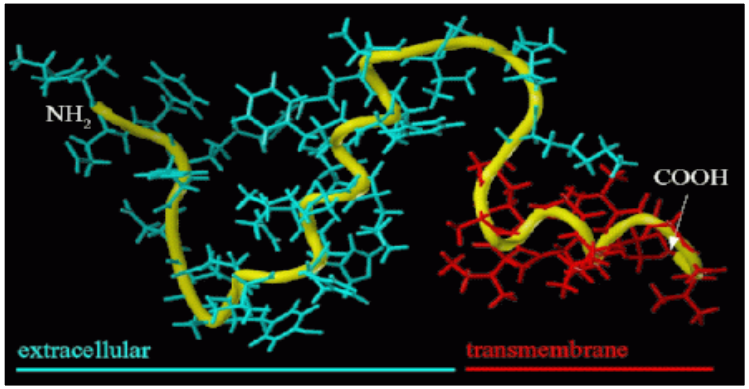


Fibers

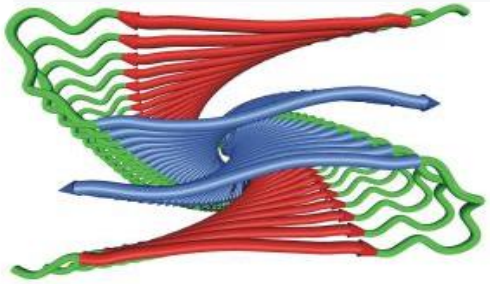
APP and Aβ(1-40)

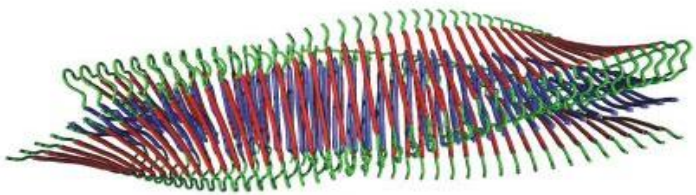
…EVKM AEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIVITLVMLKK…

Aβ(1-40)

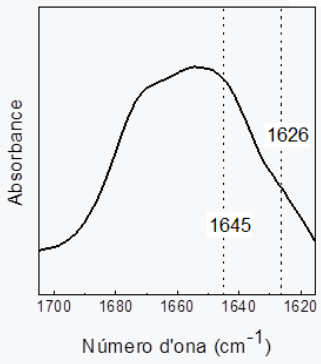


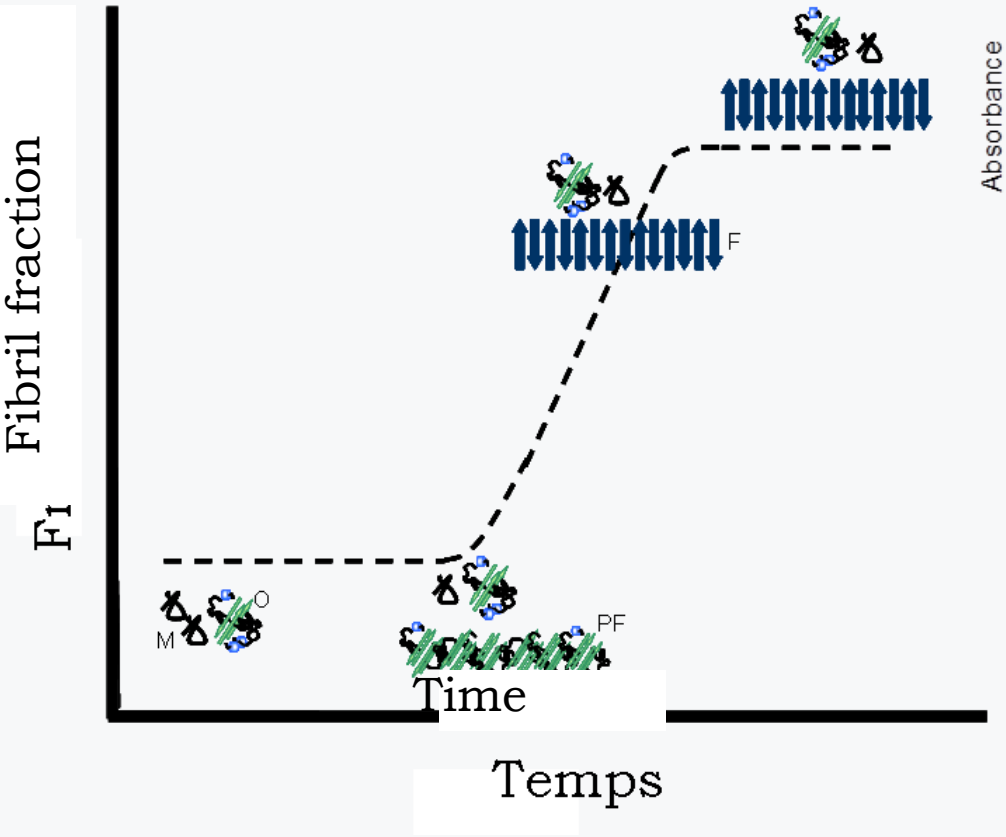
Sticht, H. et al. (1995) Structure of amyloid Aβ-(1-40)-peptide of Alzheimer's disease, *Eur J Biochem. 233*, 293-8.

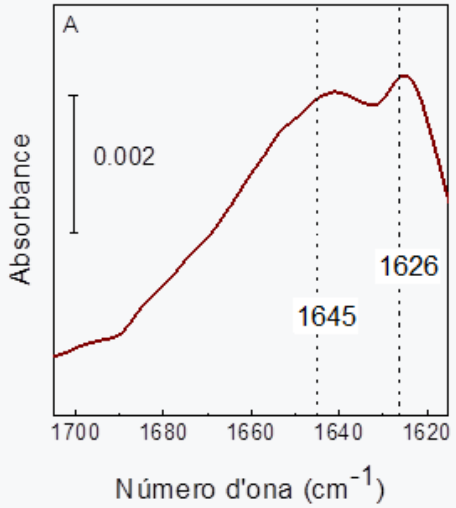




β structure

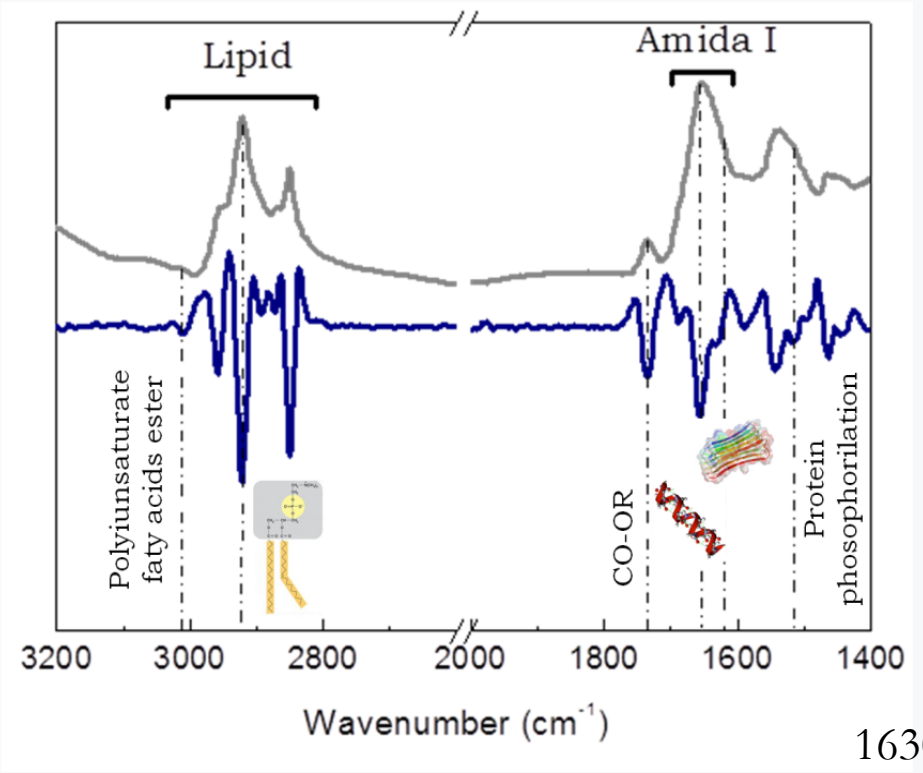


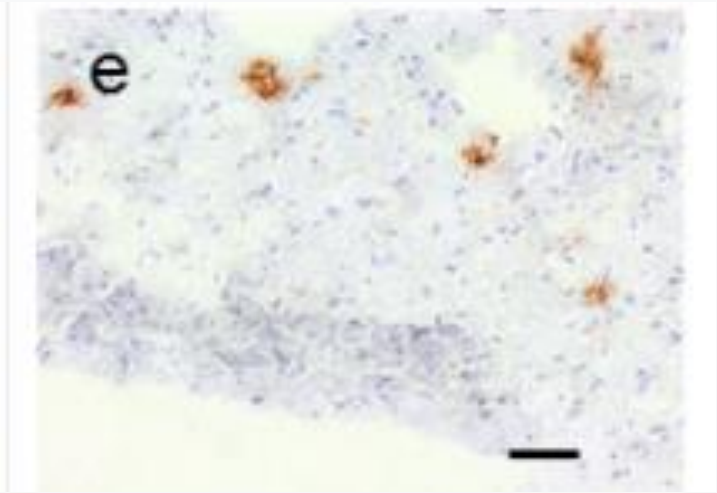




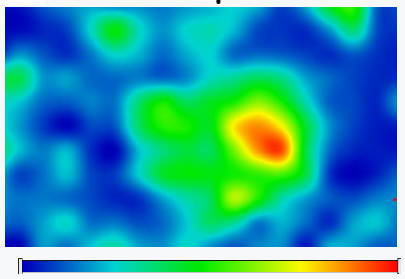
Benseny-Cases *N, Cócera M, Cladera J.*
Biochem Biophys Res Commun. 2007 Oct 5;361(4):916-21.

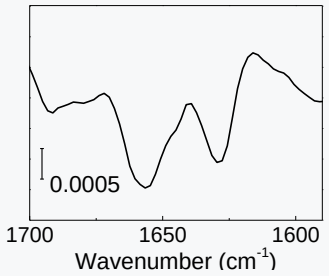
Amyloid plaques in situ

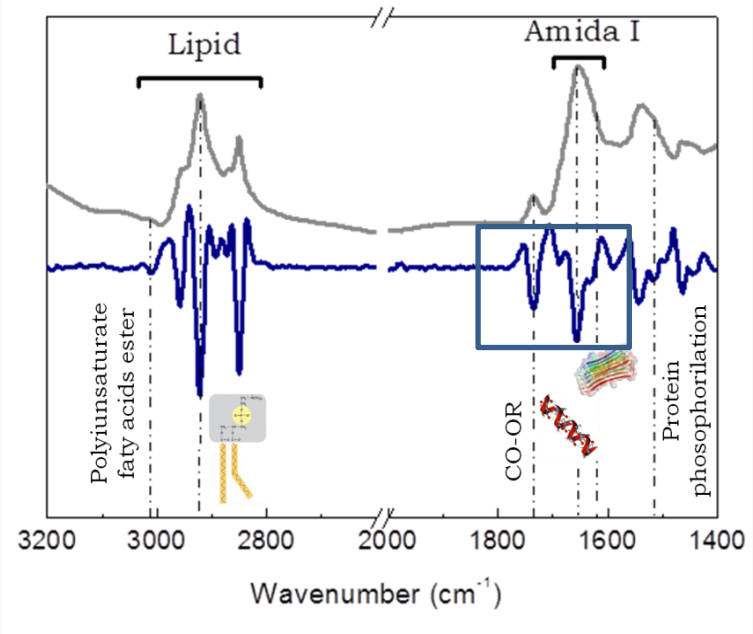




1630/1650: β structure/α helices

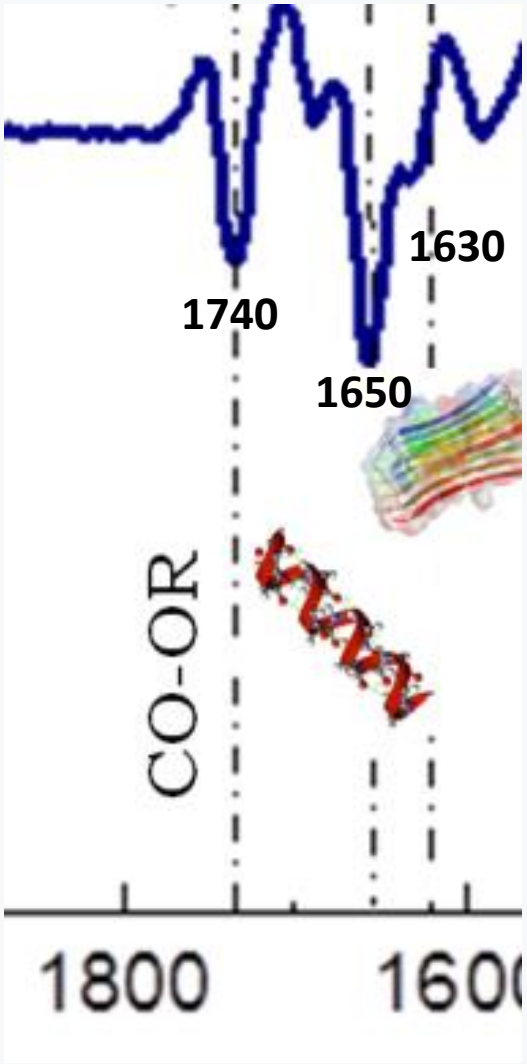




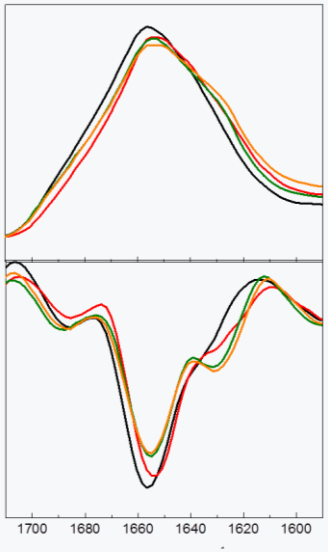


Protein Aggregation Ratio: 1630/1650

Lipid Oxidation Ratio: 1740/total lipid



Oxidation co-localizes with plaques

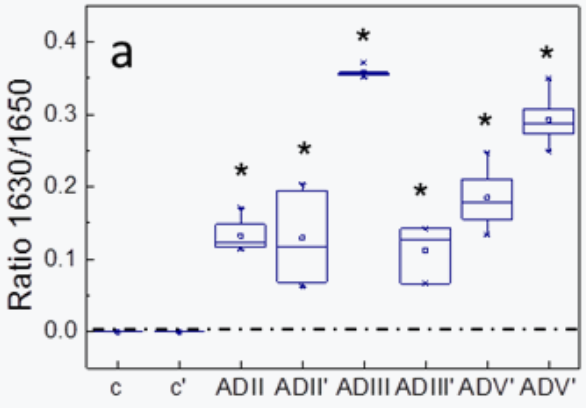


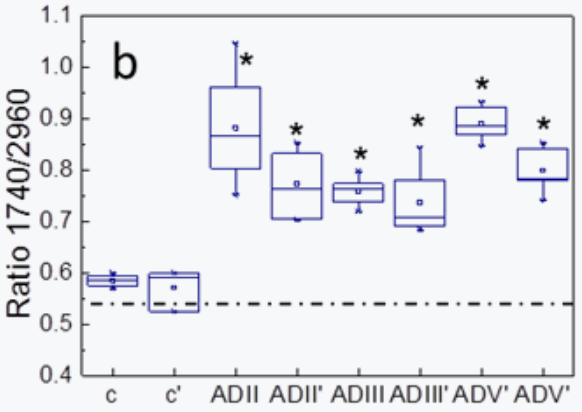
β-sheet content

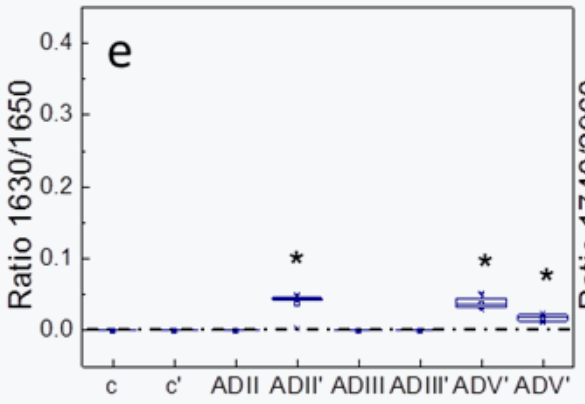
C-OOH (Lipid Oxidation)

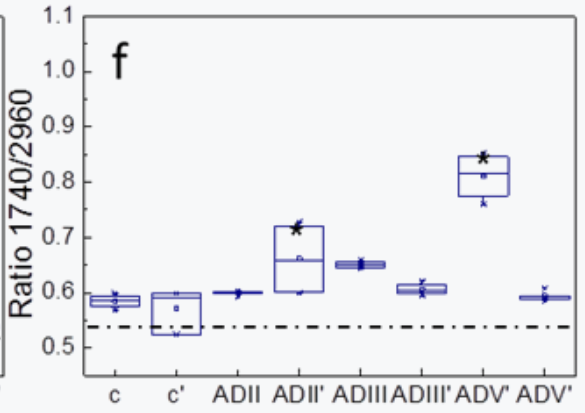
Plaques

Tissue outside the plaques

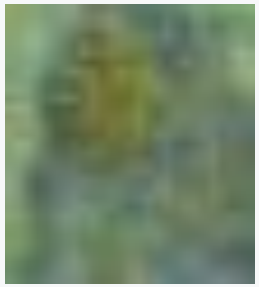




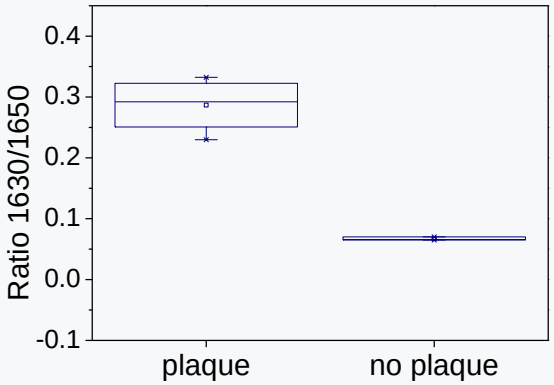




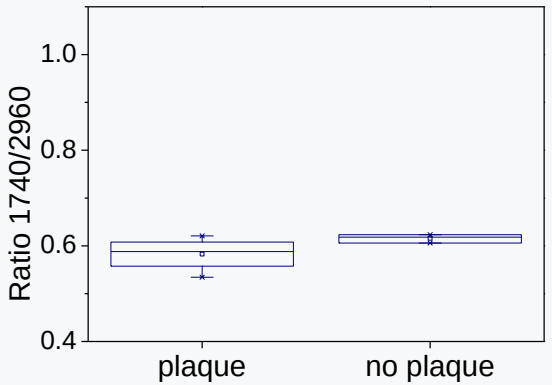
Brain with Plaques but without Alzheimer Disease symptoms



β-sheet content



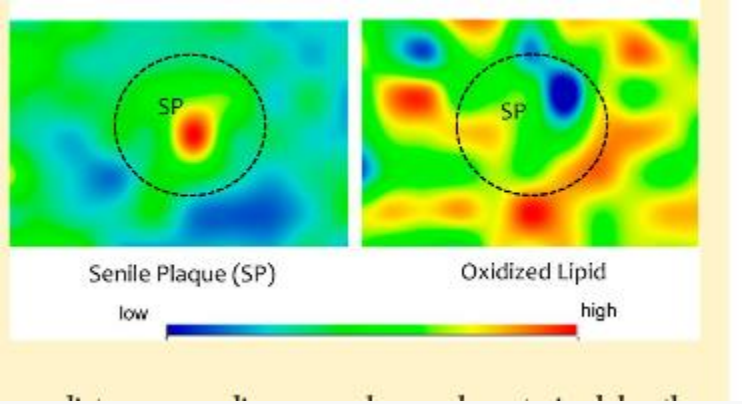
C-OOH (Lipid Oxidation)



No co-localization of oxidation and plaques

Main Conclusions

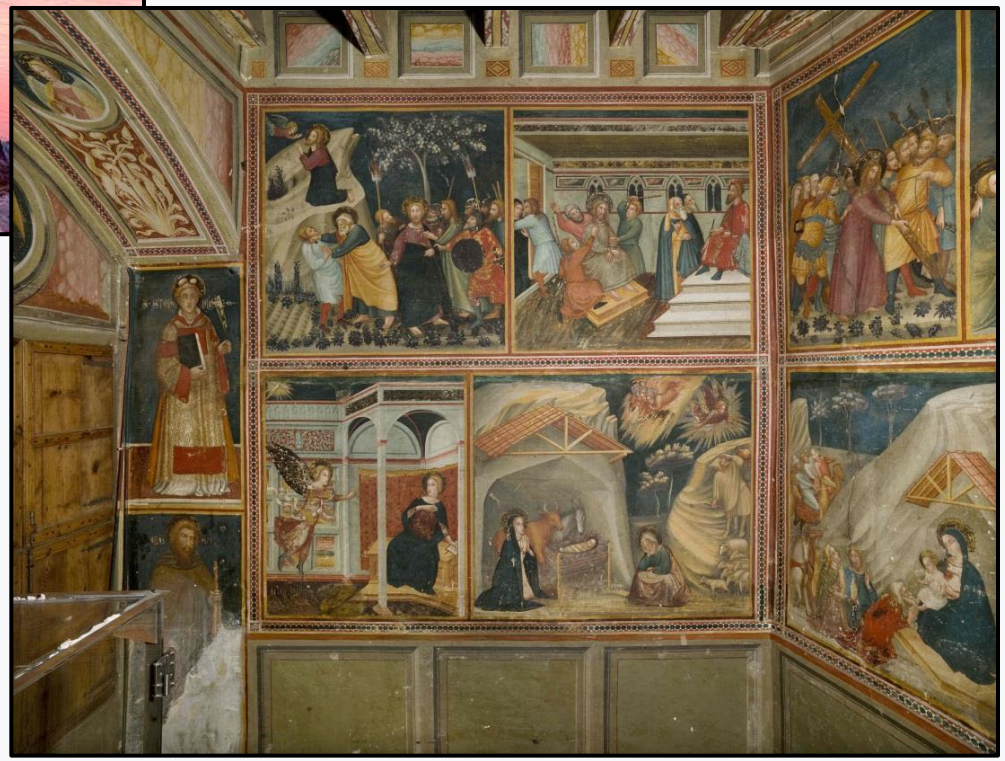
- Lipids in the plaques and surroundings are oxidized.



- Control brains, with plaques but without symptoms of Alzheimer, show no oxidation.

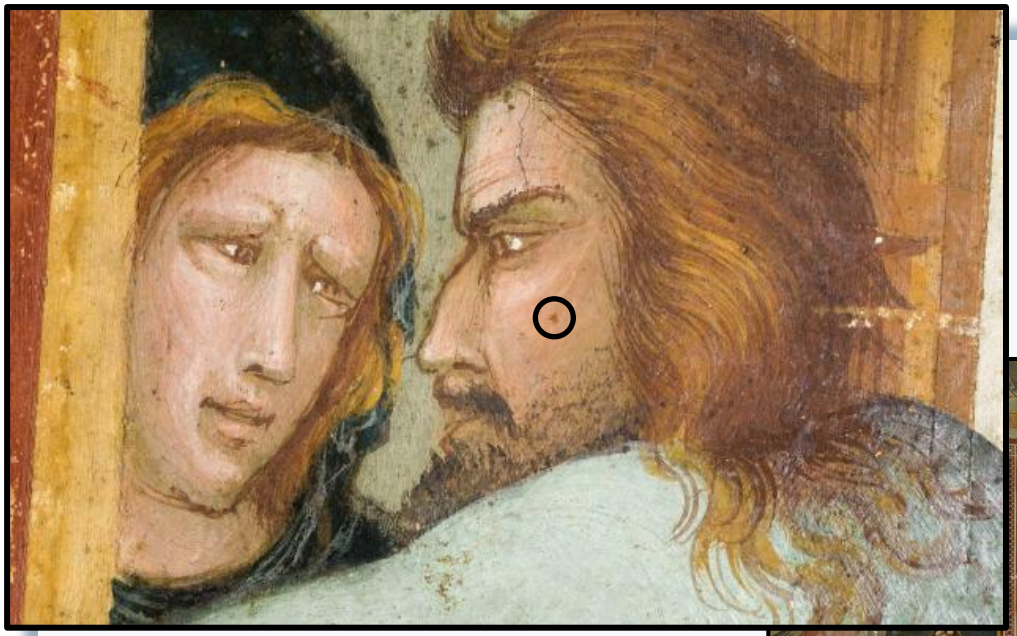
Example: cultural heritage – paintings

Sitges in the Barcelona area
Photo by Rich2012



Painting by Ferrer Bassa (1346)
Saint Michael's Chapel in Barcelona

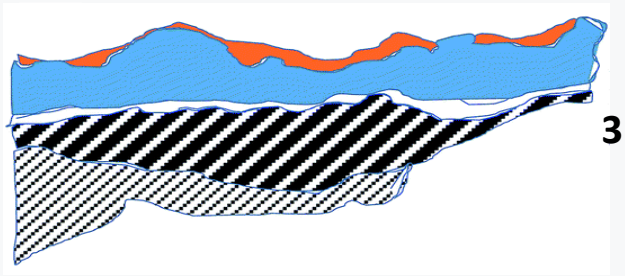
Example: cultural heritage – paintings



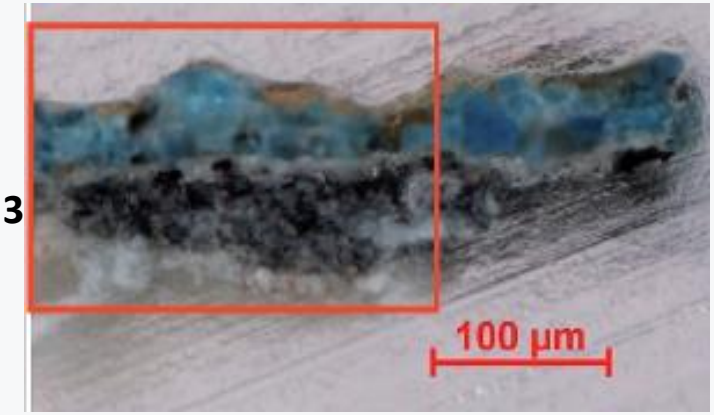
How to refurbish/restore the paintings?
What are the origins of the dark spots?

Painting by Ferrer Bassa (1346)
Saint Michael's Chapel in Barcelona

Example: cultural heritage – paintings



1 fungi layer 1
2 blue azurite 2
3 calcium carbonate 3
4 substrate 4



- The salts are directly related to the reaction compounds secreted by fungis.
- Black marks result from fungi attacks.
- The dark colour observed is due to the presence of melanin secreted by the fungi.
- Melanin is water-soluble, and therefore, can be easily removed.
- This treatment has been successfully applied in the restoration wall paintings.

Anal. Methods, 2016,**8**, 1637-1645

Example: Drug Delivery in Dermatology

Drug

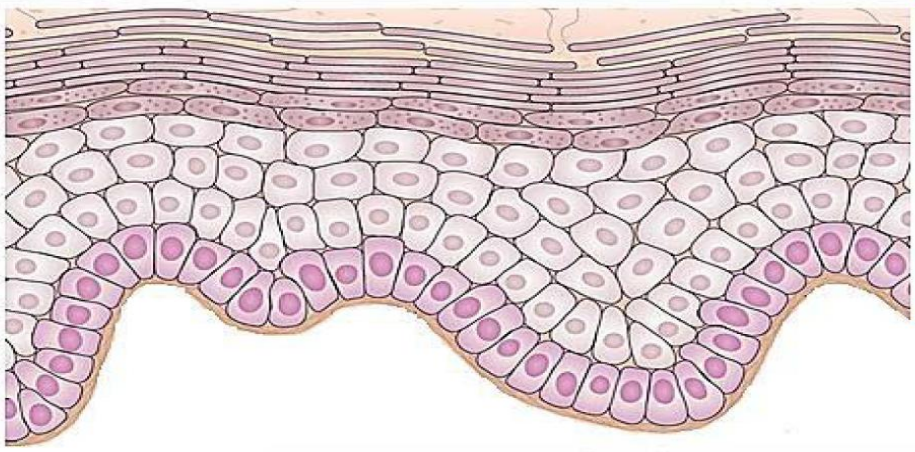
~20nm

Stratum corneum

Stratum granulosum

Stratum spinosum

Stratum basale



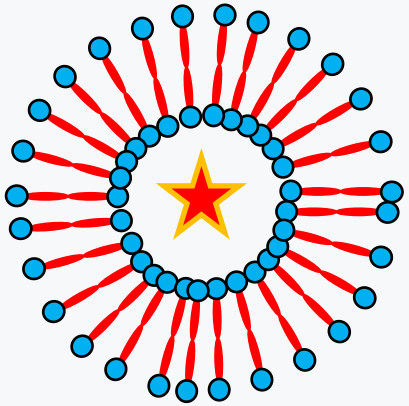
Epidermis

Cross section of the outer layers of the skin.

J Drug Discov Develop and Deliv. 2014;1(2): 8.

Example: Drug Delivery in Dermatology

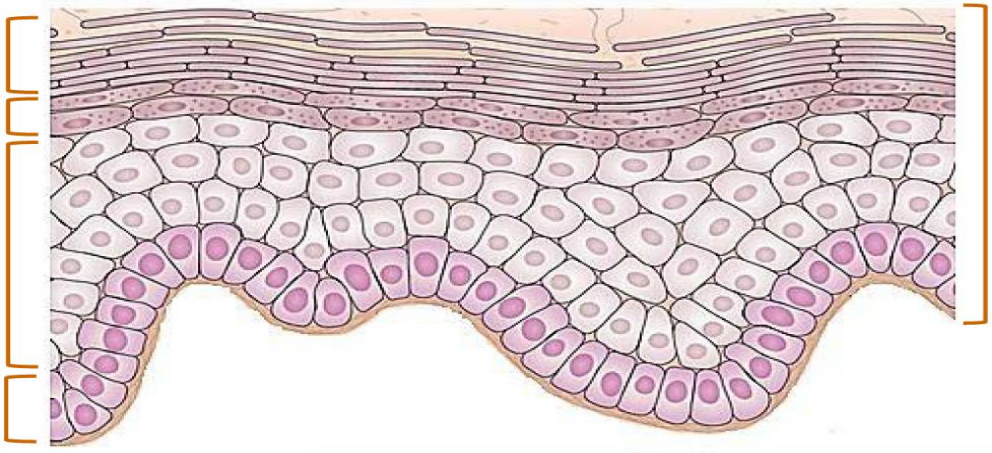
Drug
in lipid vesicle



~20nm

~200nm

Stratum corneum
Stratum granulosum
Stratum spinosum
Stratum basale



Epidermis

Cross section of the outer layers of the skin.

J Drug Discov Develop and Deliv. 2014;1(2): 8.

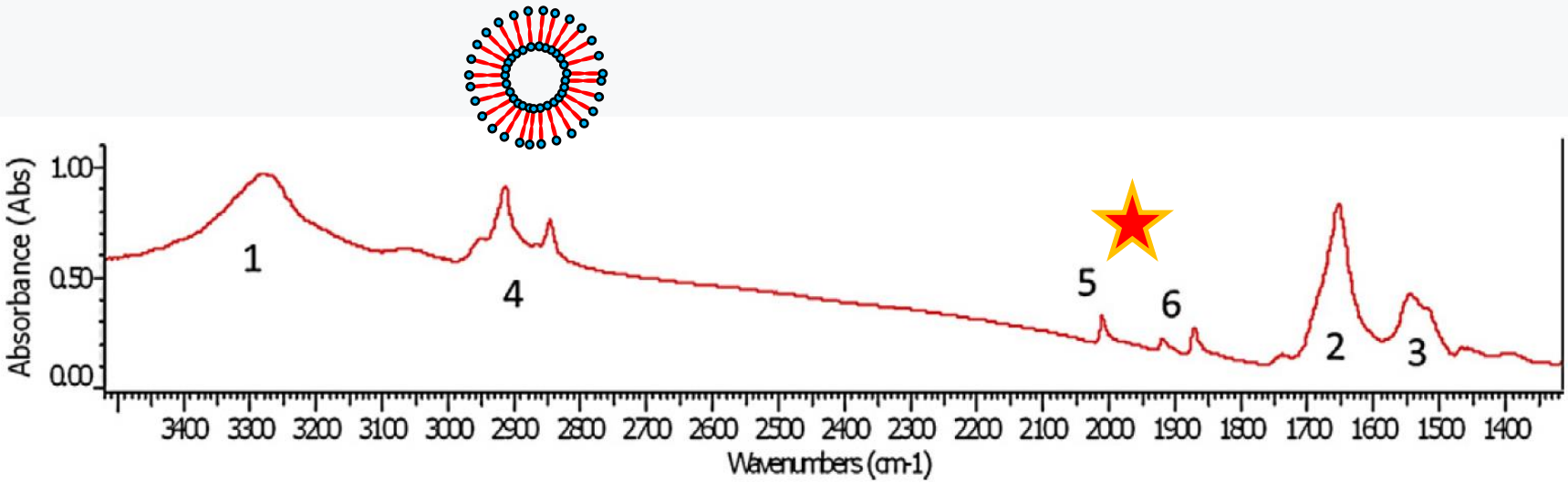
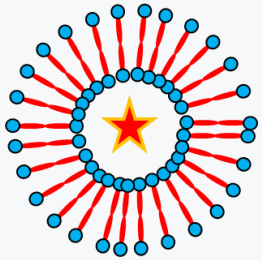
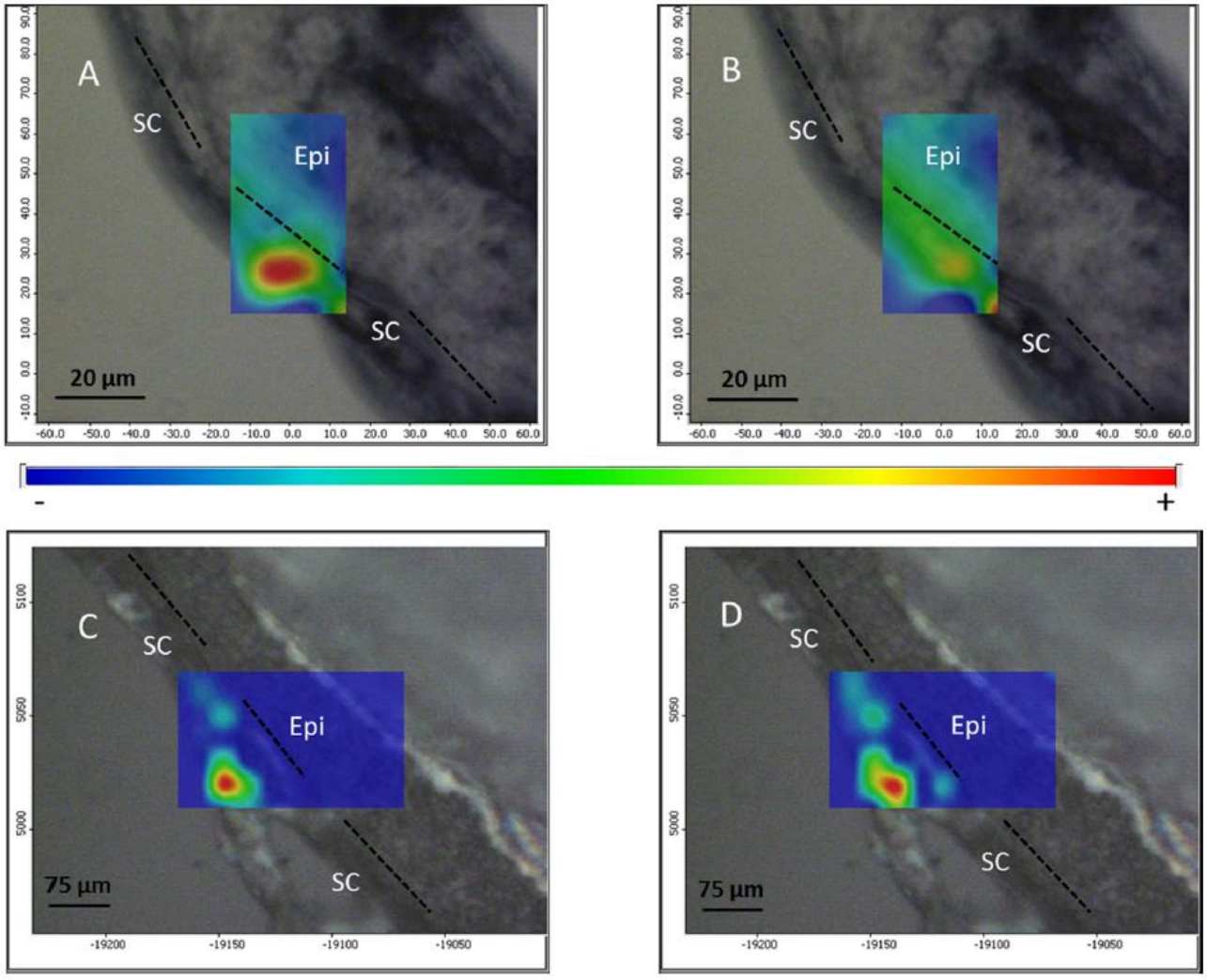


Fig. 3. IR spectra of skin sample treated with bicosomes incorporating $C_{12}Re(CO)_3$ at 1% for 24 h and at room temperature. (1) NH vibration of polypeptides and proteins of the skin, (2) CO vibration of proteins, (3) CN vibration of proteins, (4) CH_3 and CH_2 stretching vibration of skin and bicosome lipids and (5, 6) symmetric and asymmetric stretching vibrations of $C_{12}Re(CO)_3$ (A_1 and E respectively).

Colloids and Surfaces B: Biointerfaces 131 (2015) 102–107

Example: Drug Delivery in Dermatology





Colloids and Surfaces B: Biointerfaces 131 (2015) 102–107

MISTRAL:Soft Xray Transmision Microscopy

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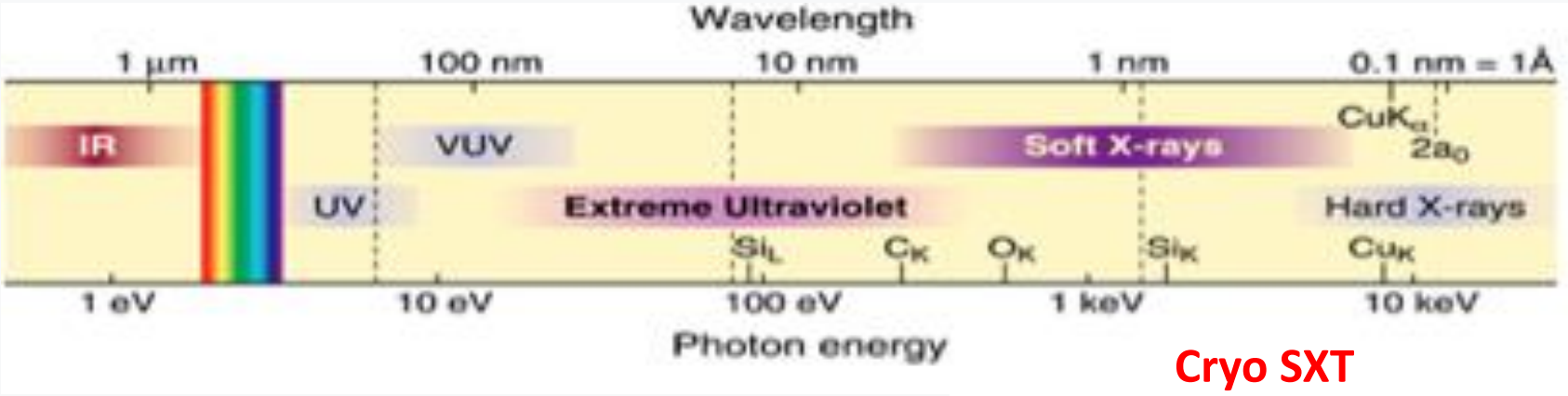
Soft Xray Transmision Microscopy

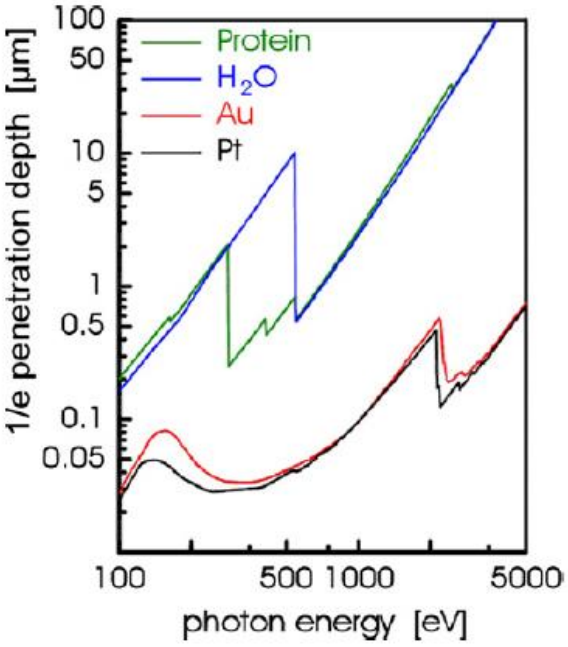


Tomography of vitrified samples

Working at water window 520eV:
natural contrast

Ø cryo system (sample < 130K -148C) –
Frozen Hydrated state

Cryo SXT



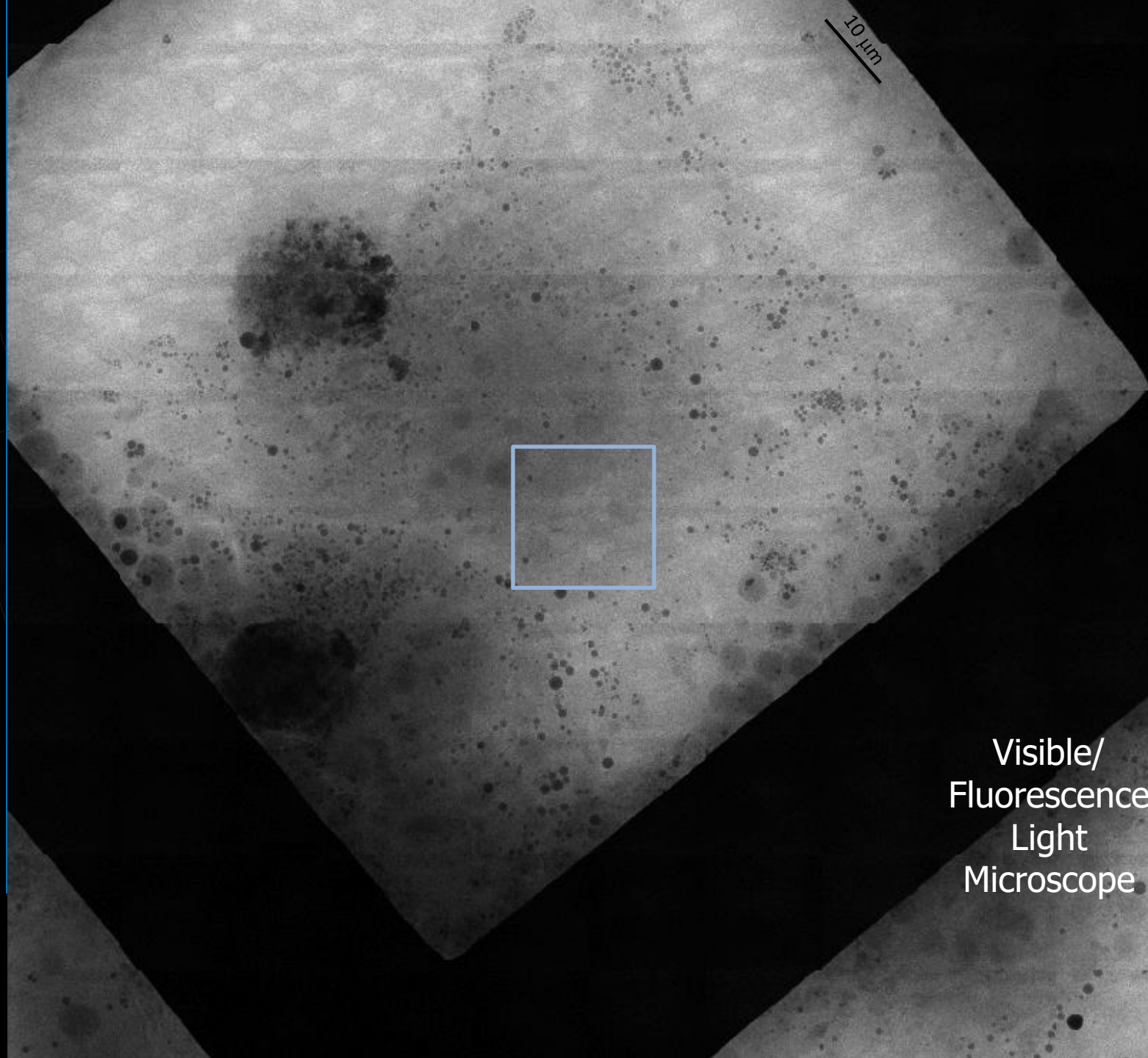
Select the cell

Transmission X-Ray Microscope

Select the region of interest

Tomogram -70, +70

Reconstruction



Visible/ Fluorescence Light Microscope

Hepatocyte control cells

- Mitochondria
- Endoplasmic Reticulum
- Cytoskeleton
- Nucleus
- Nuclear Envelope
- Mitochondrial Cristae

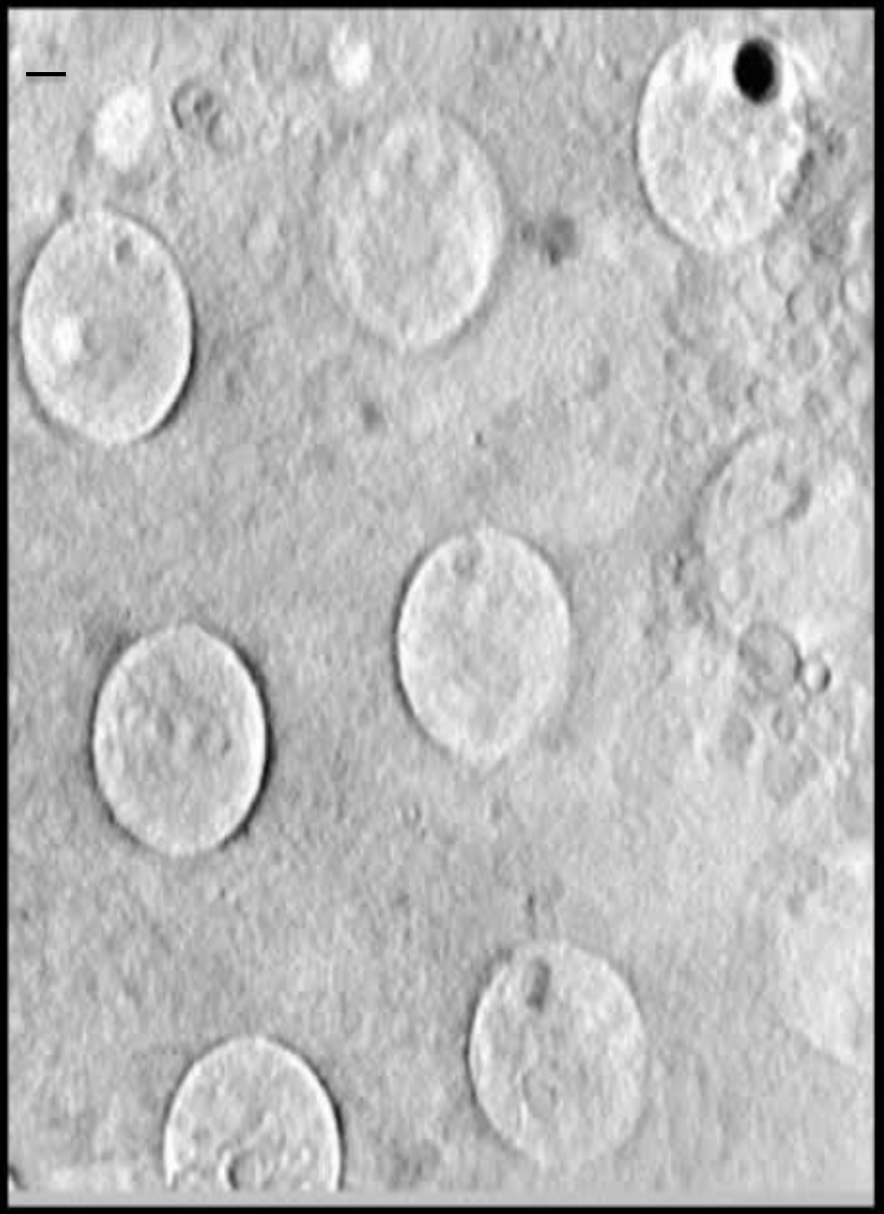
A whole cell

Natural contrast

Without any addition of chemical compounds for fixation or staining

Without artifacts

Structural Changes In Cells Imaged by Soft X-ray Cryo-Tomography During Hepatitis C Virus Infection.
Pérez-Berná AJ et al, ACS Nano. 2016 Jun 28



Accumulation of vesicles

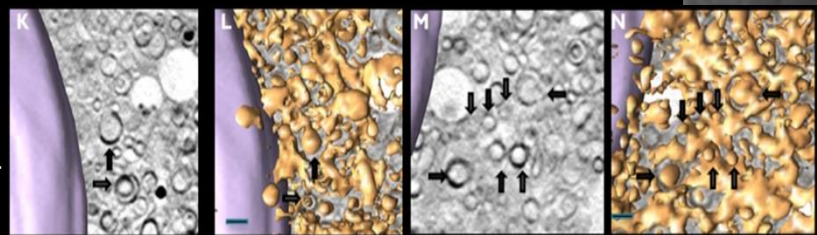
Membranous web

Membranous vesicles

Membranous vesicles are tubes in different orientation
3D map

Neck-like structures connecting endoplasmic reticulum extrusions to the tubular network

Close Compartments
for RNA replication
Functional RNA replicase complexes



Structural Changes In Cells Imaged by Soft X-ray Cryo-Tomography During Hepatitis C Virus Infection.
Pérez-Berná AJ et al, ACS Nano. 2016 Jun 28

CLAES:X-ray absorption spectroscopy

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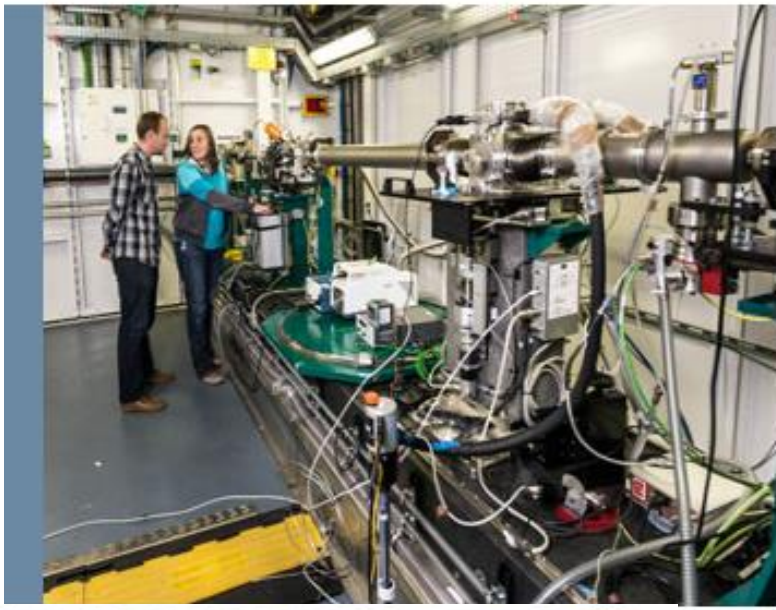
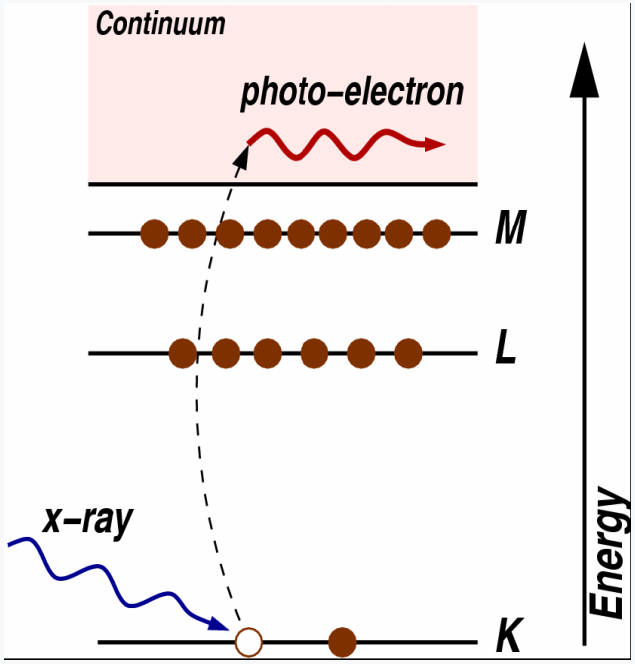


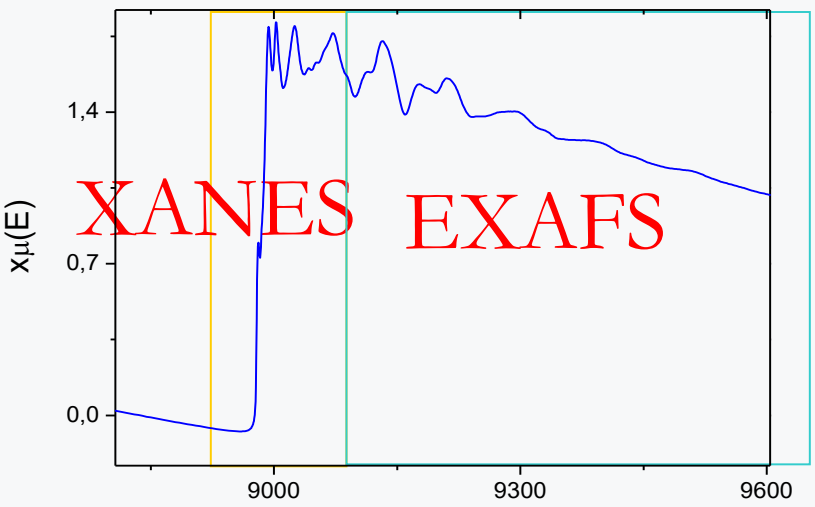
X-ray absorption spectroscopy

- When an x-ray is absorbed by an atom, its energy is transferred to a core-level electron (*K*, *L*, or *M* shell) resulting in its ejection from the atom.
- The atom is left in an *excited state* with an empty electronic level (a *core-hole*).
- Any excess energy from the x-ray is given to the ejected *photoelectron*.



XAS is an element selective technique

S K-edge ~2400 eV	Cu K-edge ~9000 eV	Ag K-edge ~25500 eV
S L-edges ~160-230 eV	Cu L-edges ~930 eV	Ag L-edges ~3500 eV



Cu K edge (8979 eV)

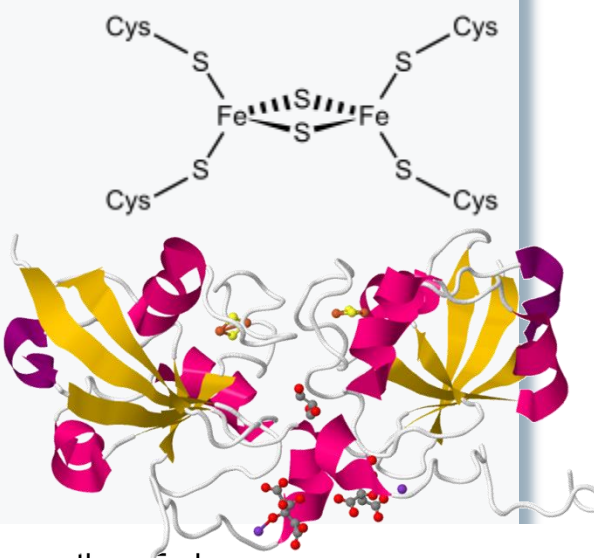
The ejected photoelectron is backscattered by the neighboring atoms.

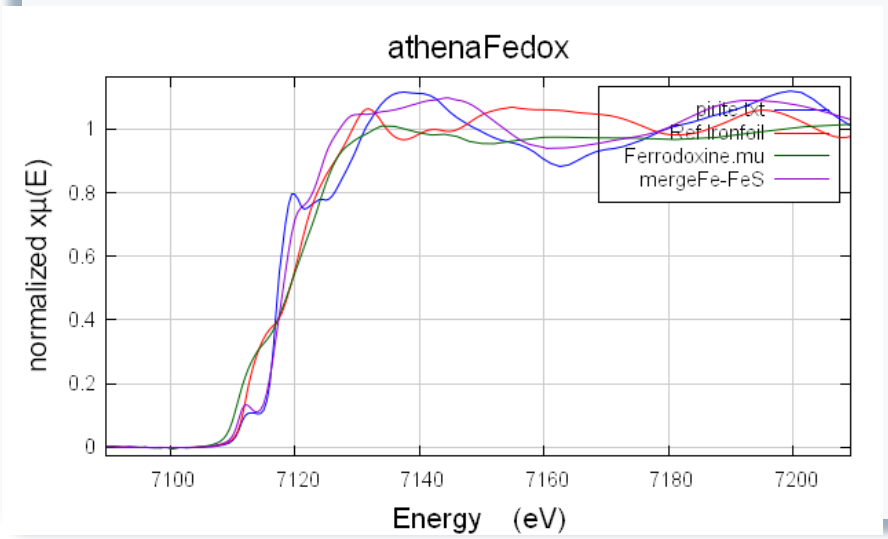
- local structure
- frequencies ~ bond distances

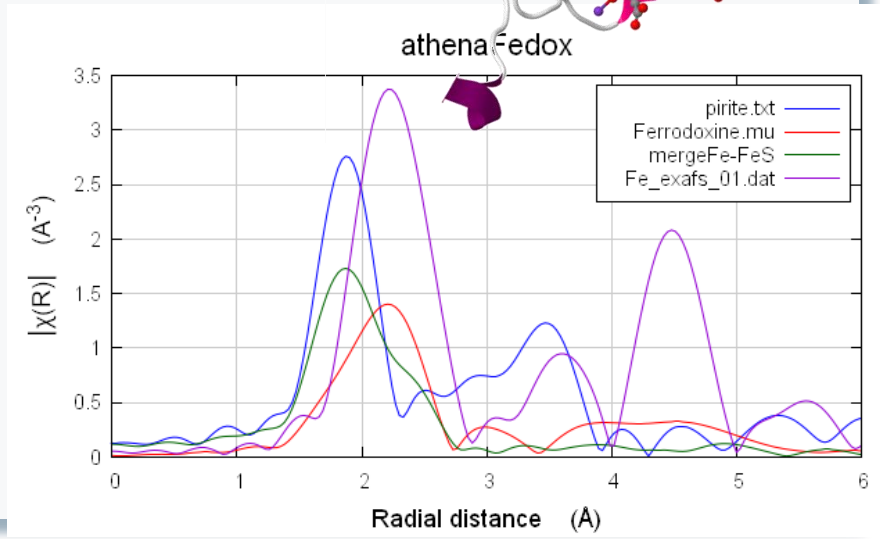
An example studied in Claess: the spinach ferrodoxine

In collaboration with prof P. Postorino, University of Roma Sapienza

Ferredoxins are iron-sulfur proteins that mediate electron transfer in a range of metabolic reactions. Ferredoxin is involved in both cyclic and non-cyclic photophosphorylation reactions of photosynthesis. In non-cyclic photophosphorylation, ferredoxin is the last electron acceptor thus reducing the enzyme $NADP^+$ reductase. These biological "capacitors" can accept or discharge electrons, with the effect of a change in the oxidation state of the iron atoms between +2 and +3.



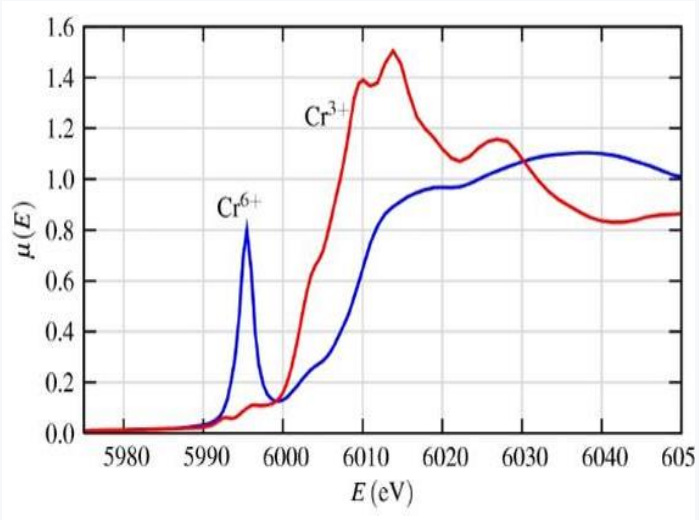




Am. Min. 95, 200 (2010)

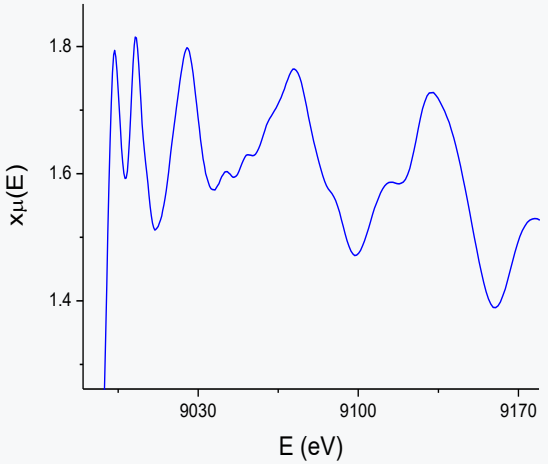
XANES

- Oxidation state
- Unoccupied electronic states
- Spin state
- Local structure
- direct information about bond angles.



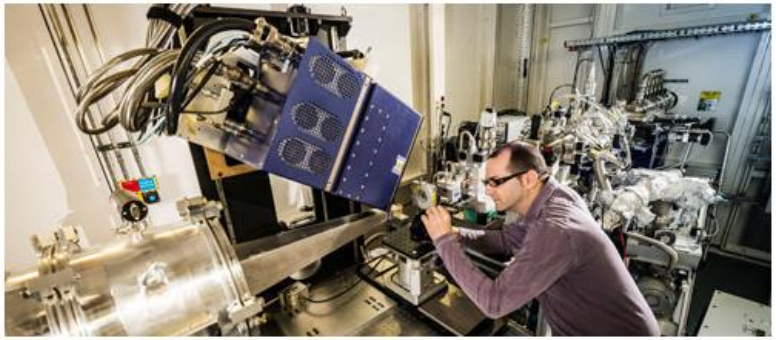
EXAFS

- Bond distances
- Coordination number
- Static and dynamic disorder



Am. Min. 95, 200 (2010)

NCD: Non cristalline difraction
Xaloc: Cristalline Difraction



NCD

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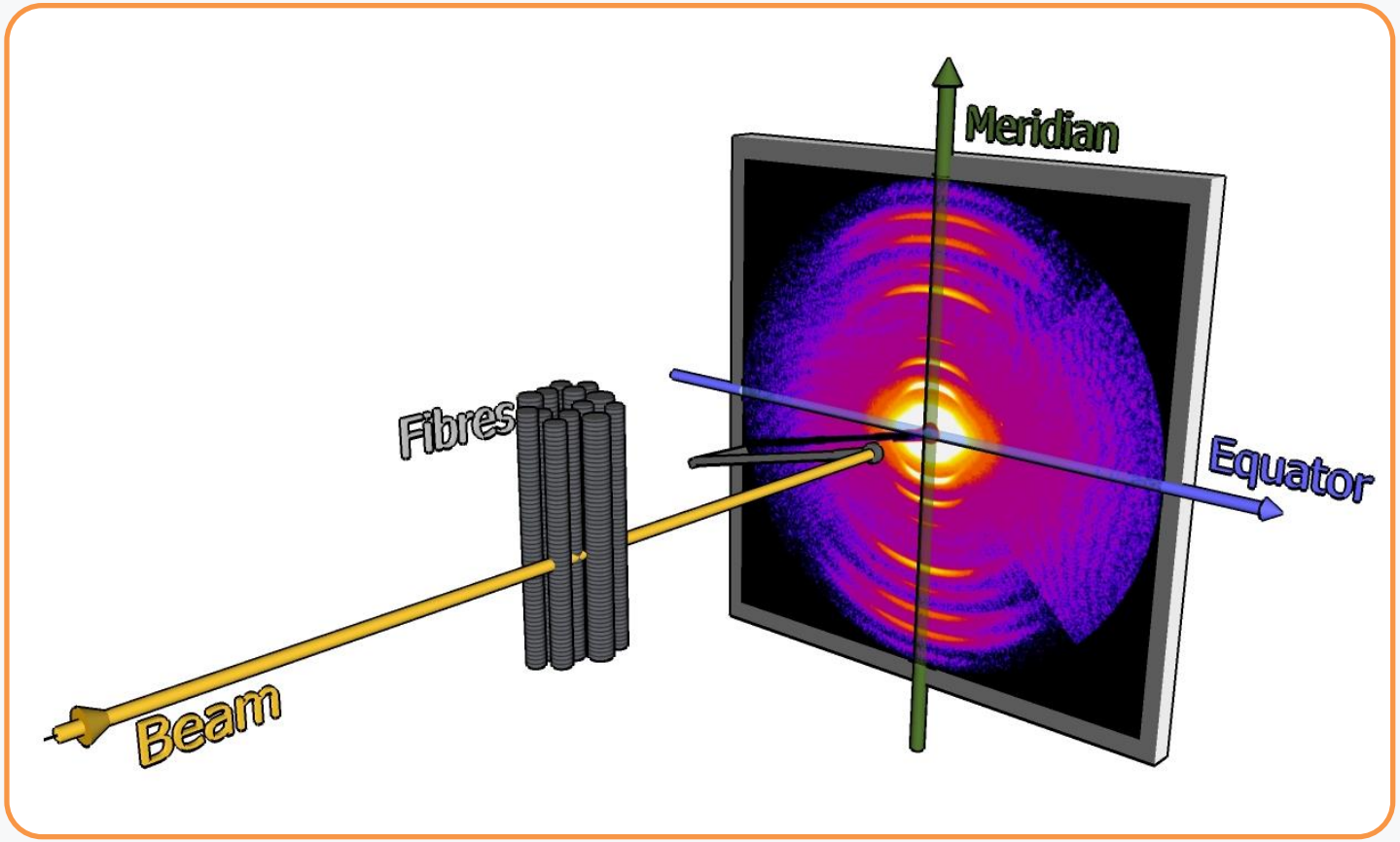
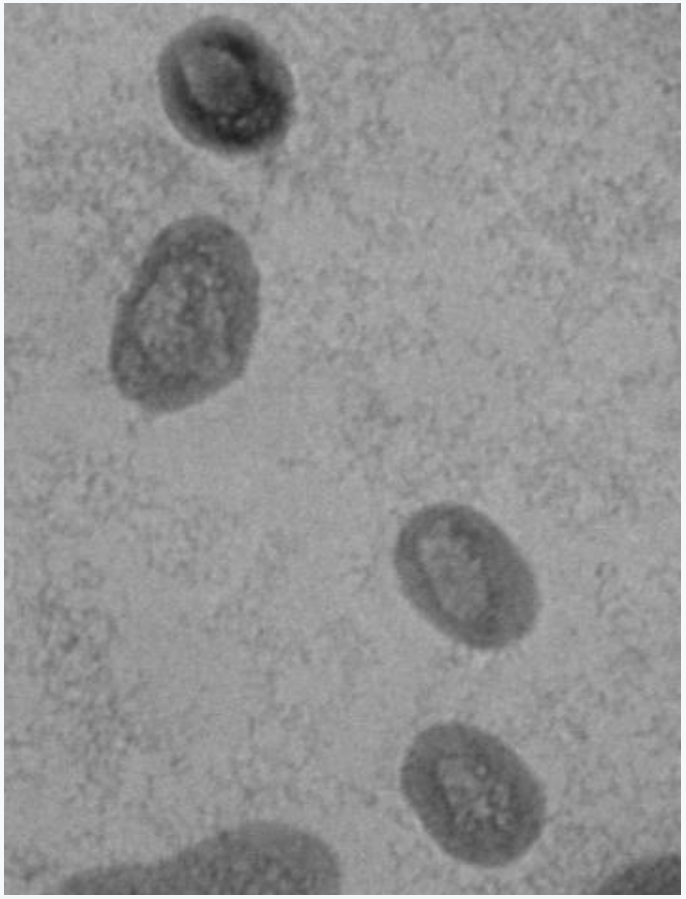
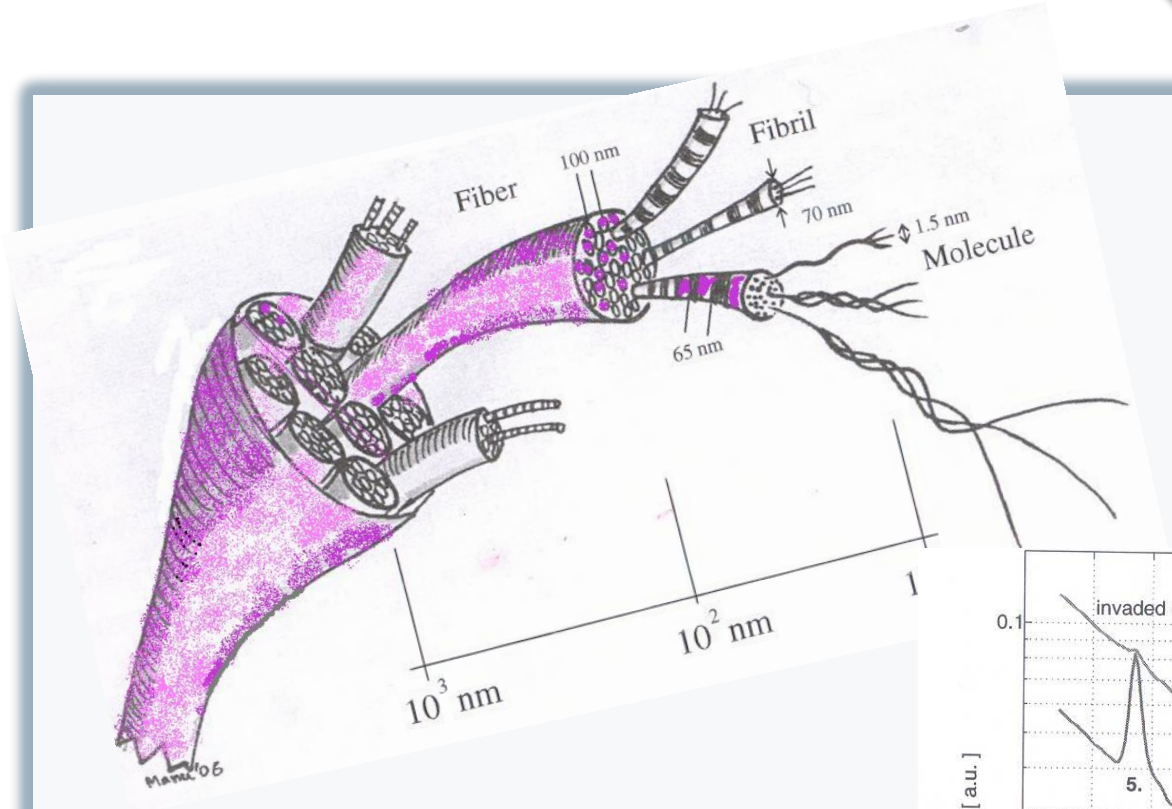

Meridian
Fibres
Equator
Beam

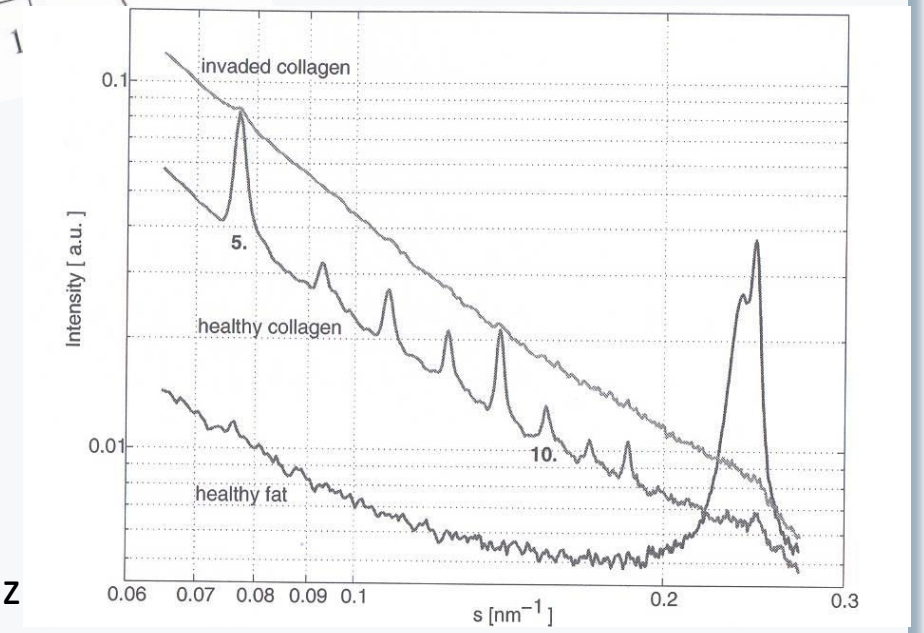
Liposomes

Multilamelar
Unilamelars

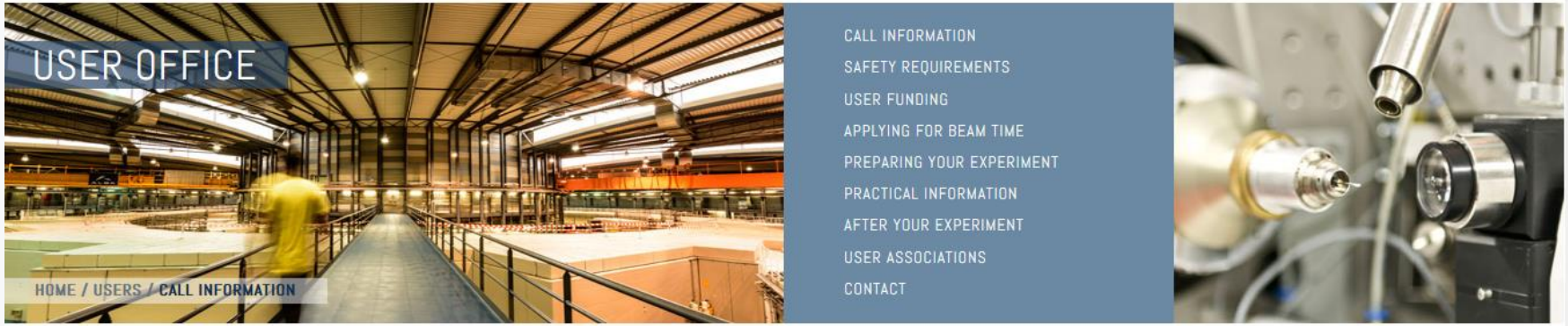


SAXS Material no cristal·lí





Manuel Fernandez



2 CONVOCATÒRIES
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- Setembre