

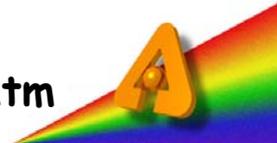


# *APS Strategic Planning Meeting*

**"Future Scientific Directions"** September 2 & 3, 2004 Fontana, Wisconsin

## ***Report from XOR Sectors***

*Gabrielle G. Long  
Experimental Facilities Division  
Advanced Photon Source*



# XOR Workshop - August 11 & 12, 2004

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- **Objective**

- Explore future scientific directions that XOR can develop at the APS to expand our scientific leadership in the next decade

- **Approach**

- Identify new opportunities for continued scientific discovery
  - *High scientific impact*
  - *Productive and satisfied user community*
- Identify new scientific programs to bring to the APS
- Examine existing beamline capabilities at the APS
  - *Unique capabilities*
  - *Effective sector utilization*

- **Report here on an outcome not covered by the 9 Workshops**

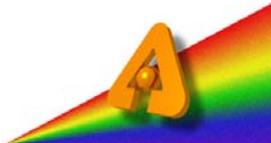
- Biological and biomedical applications of x-ray microscopy



# ***Biological and biomedical applications of x-ray microscopy***

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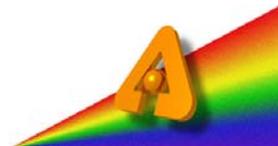
- **from XOR:** Stefan Vogt, Barry Lai, Jörg Maser, David Paterson, Francesco DeCarlo, Yong Chu, Zhounghou Cai, Daniel Legnini, Ian McNulty
- **from Northwestern University:** Gayle Woloschak, Tatjana Paunesku, Eileen Bigio
- **from Argonne National Laboratory:** David Glesne (Energy and Environmental Science and Technology)
- **from Duke University:** Peter Ingram
- **from Future Needs Workshops:**
  - Workshop on biological applications of x-ray microbeams (*Synchrotron Radiation News* **15** (2) 2002, p. 18)
  - Workshop on biological applications of x-ray microscopy and imaging (*Synchrotron Radiation News* **16** (5) 2003, p. 19)



# Experiments leading to scientific discovery

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- **Scanning X-ray fluorescence microscopy**
  - Trace elements / metals in biology & life sciences
    - *essential cofactors in proteins*
    - *linked to diseases*
    - *in therapeutic drugs*
    - *as intracellular labels*
- **Full field transmission imaging of live processes & tomography**
  - *biological discovery via real-time imaging*

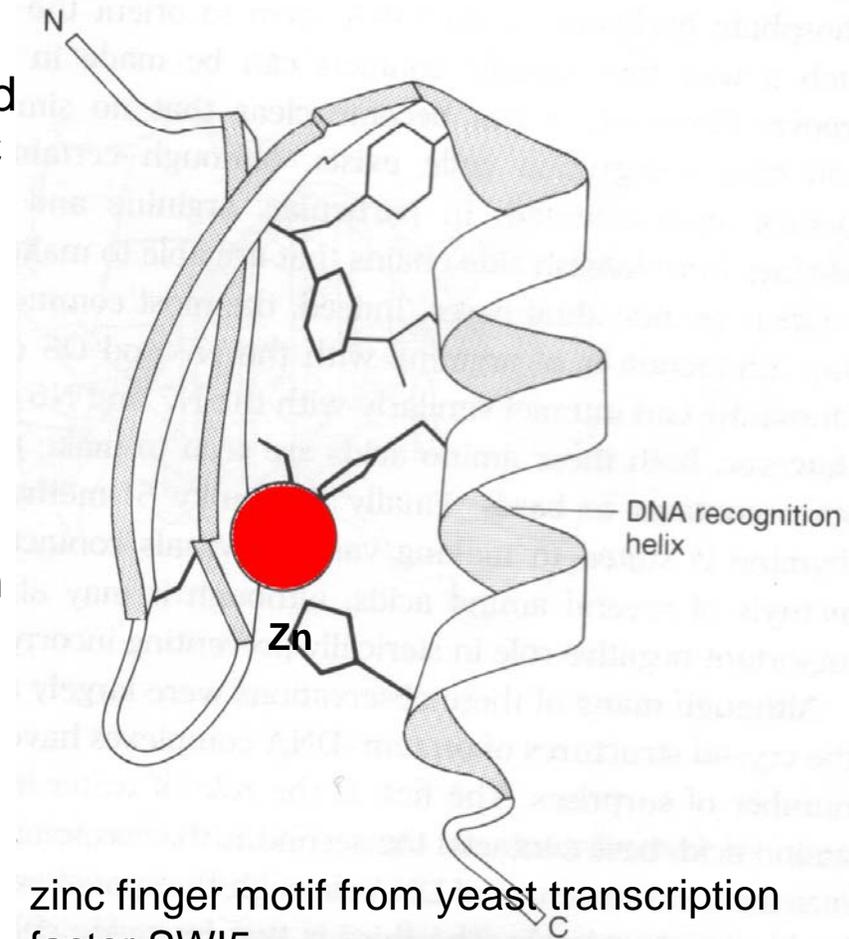


## Why study metals in cells ?

### **Metals: essential cofactors in 1/3 of proteins**

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- **estimated: 1/3 of all known proteins contain metal cofactors as integral, catalytic components. These proteins often have regulatory or catalyzing functions:**
  - Ca in calcium-binding proteins: second messenger pathways, e.g. Troponin C in muscle
  - Fe in Haemoglobin; and necessary in Chlorophyll synthesis
  - Cu binding chaperones (protein folding)
  - Zn in zinc finger proteins: transcription factors in the cell nucleus
- **At the same time: most essential trace metals toxic at higher concentrations (e.g., Cu, Se)**

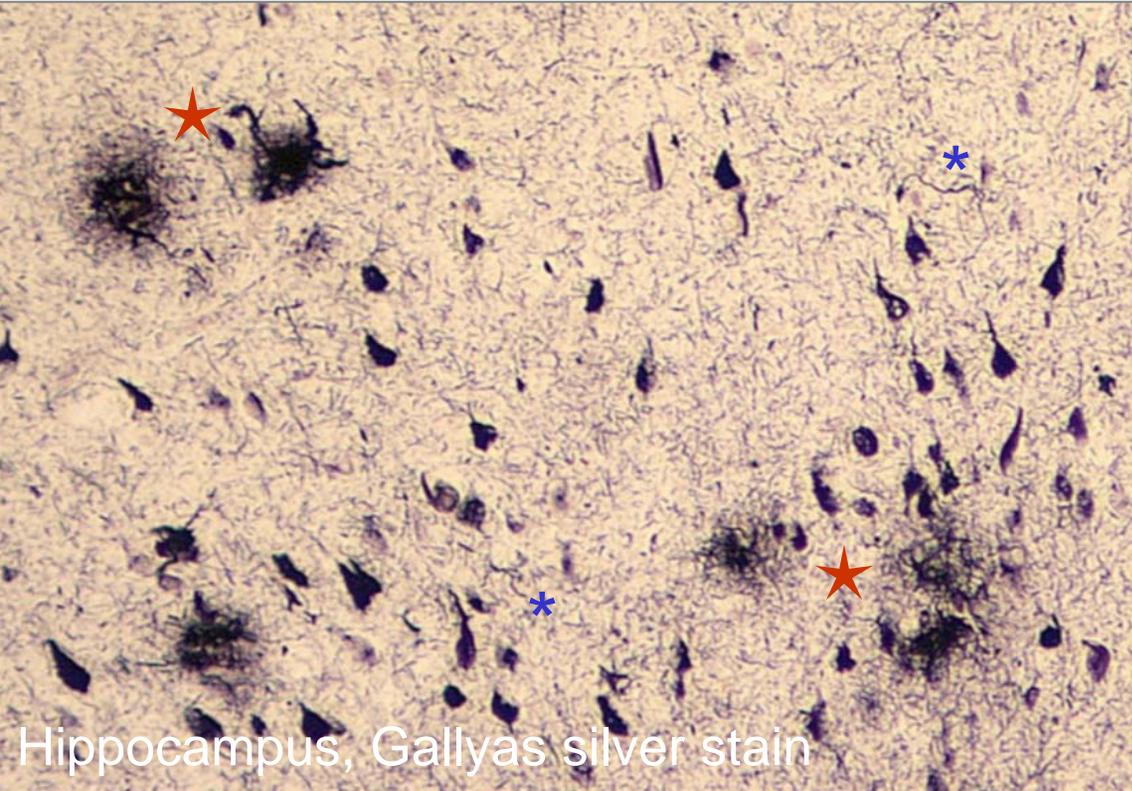


[from Encyclopedia of Molecular Biology]

## Why study metals in tissues ?

# Example: Alzheimer disease

## Neuritic plaques & neurofibrillary tangles • Alzheimer disease



Hippocampus, Gallyas silver stain

Al - blood/brain barrier

Fe/Cu - promote formation of free radicals

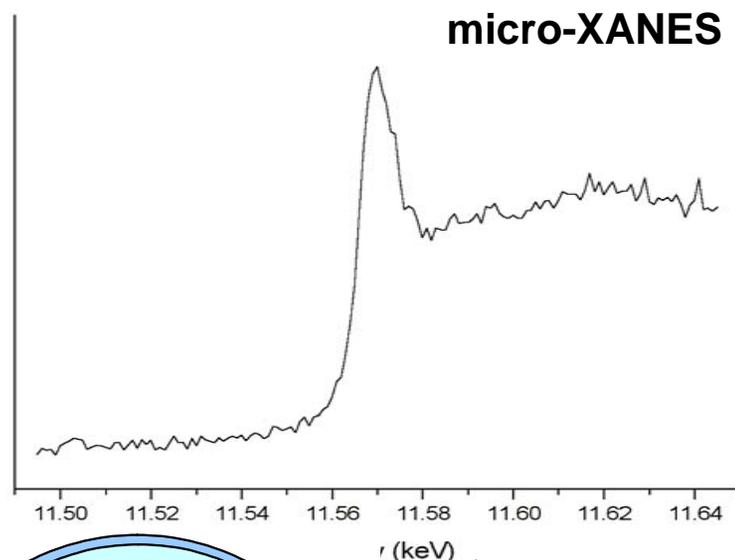
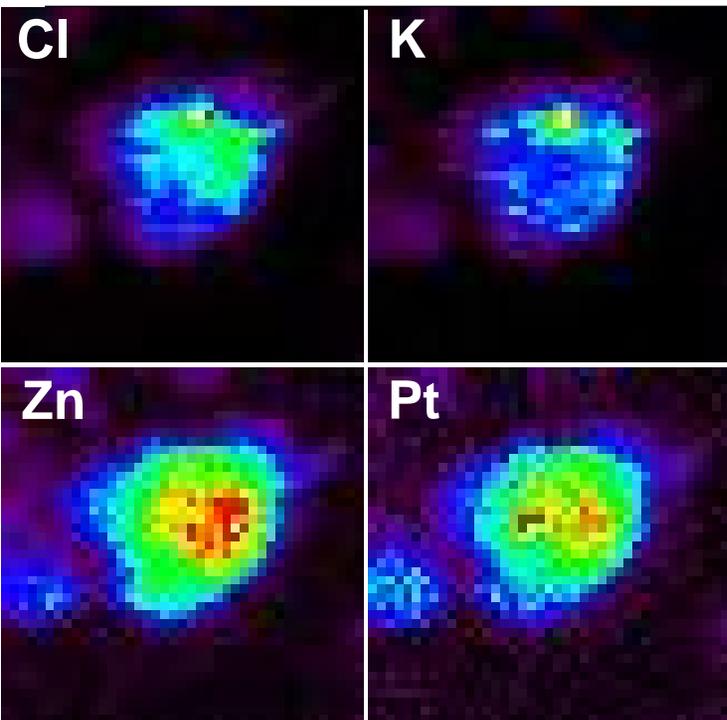
Zn - protective role

Cu/Zn - contribute to Abeta plaque formation

- Related to beta amyloid (Abeta<sub>42</sub>) protein/oligomers deposition
- Amyloid precursor protein regulates Cu homeostasis
- Pathology
  - Neurofibrillary tangles (*tau protein*) \*
  - Neuritic plaques (*tau & Abeta*) ★
- Metal-protein-attenuation therapy (Cu, Zn) improves cognition & lowers serum Abeta<sub>42</sub> (*Arch. Neurol.* 2003;60:1685-91)

Why study trace metals in life sciences ?

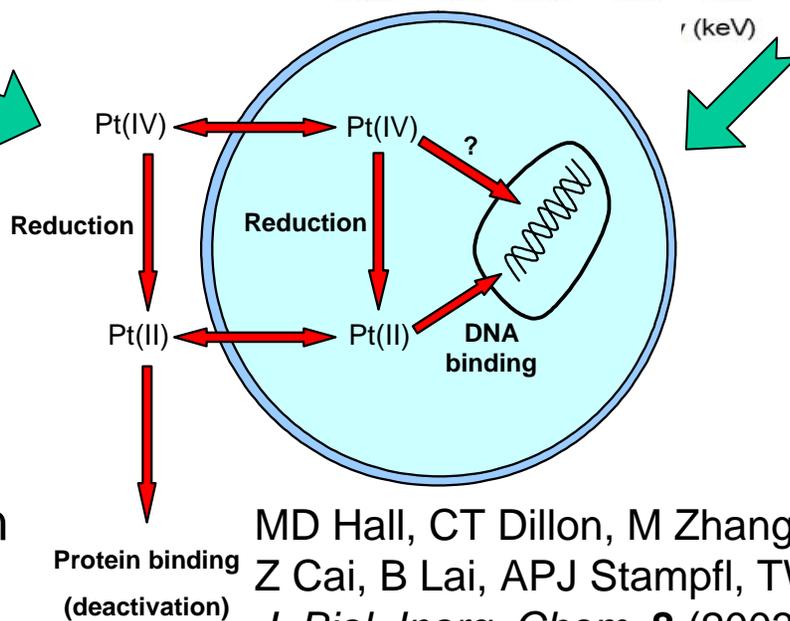
# to understand metabolic pathway of drugs



microscopy

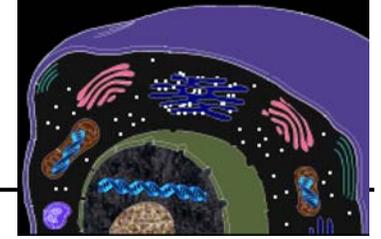
Pt(IV) complexes are more inert:

- less likelihood of deactivation
- potentially fewer side effects
- possibility of selective activation



MD Hall, CT Dillon, M Zhang, P Beale, Z Cai, B Lai, APJ Stampfl, TW Hambley  
*J. Biol. Inorg. Chem.* **8** (2003) 726-732

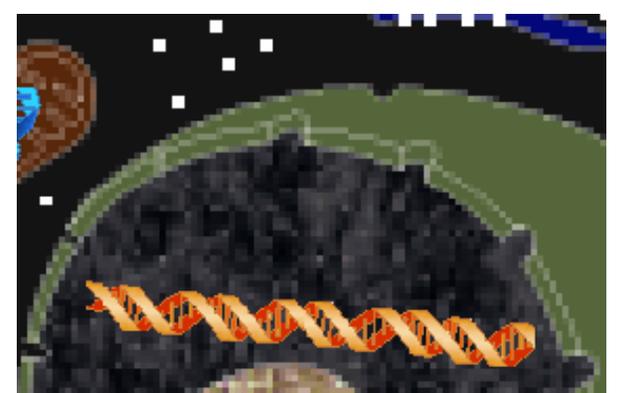
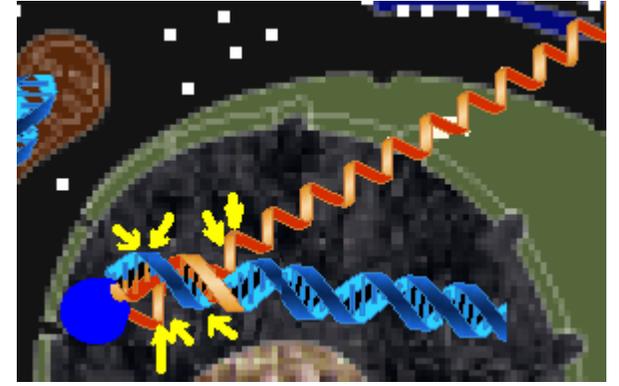
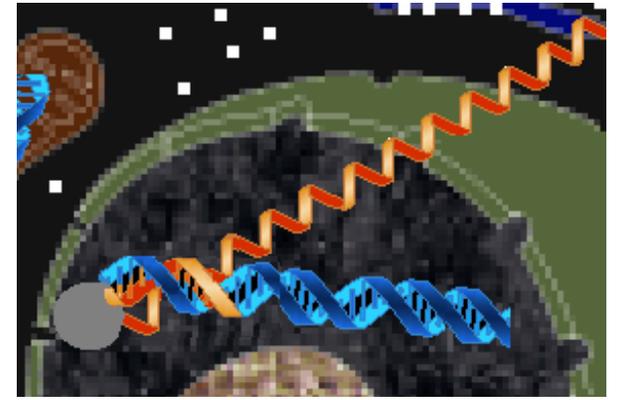
# Nanocomposites as tools for Gene therapy ?



Gene therapy:

Correct defective genes responsible for disease development

- mutated and dominant genes (e.g. oncogenes = genes that promote cancer)
  - introduce agent that can simply destroy gene
- mutated and recessive genes (e.g. tumor suppressor genes)
  - introduce agent that destroys gene and
  - replaces it with 'healthy' copy



See, for example: T Paunesku, T Rajh, G Wiederrecht, J Maser, S Vogt, N Stojicevic, N. Protic, B Lai, J Oryhon, M Thurnauer, G Woloschak, *Nature Materials* **2**, (2003) 343-346

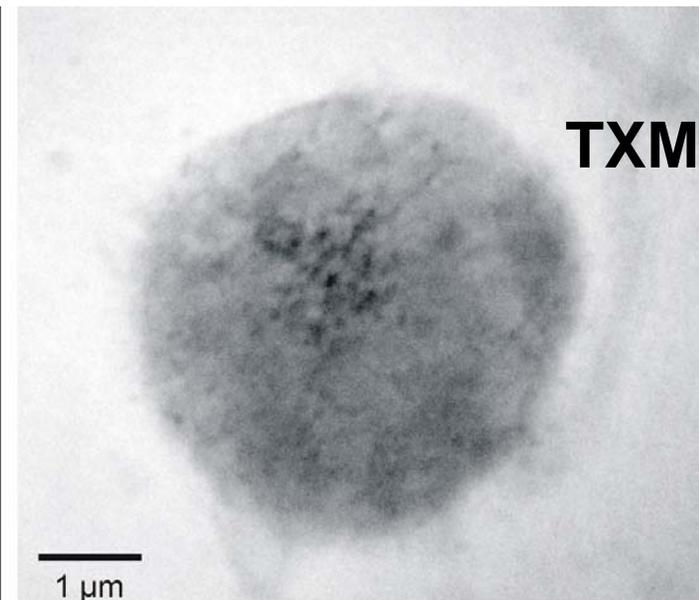
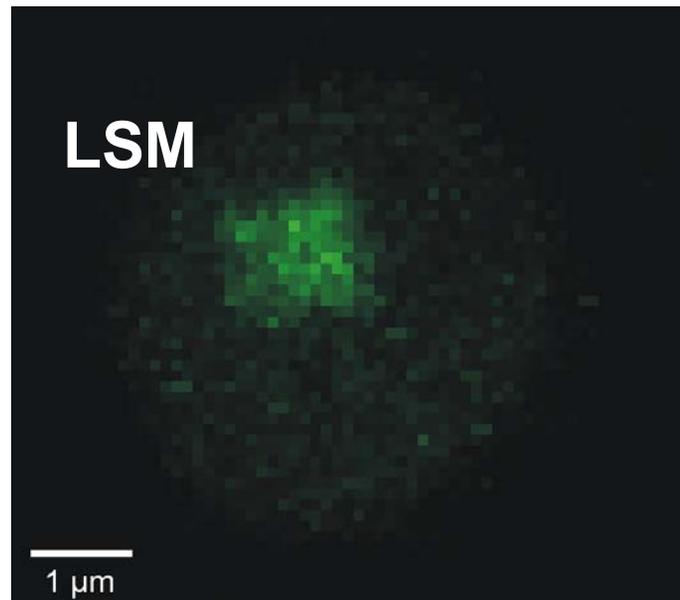
# 'metals' as intracellular labels for protein

- conjugate metal (e.g., Ti, V, ..) antibody & use immunolabelling to target protein of interest
- develop protein with a metal cofactor that can be stably expressed (similar to green fluorescent protein) to visualize specific proteins in cells (D. Glesne *et al*)
- → colocalize natural element distribution with specific protein(s)
- Future: custom intracellular tools, to manipulate proteins in the cell

*Drosophila melanogaster* cell,  
immuno-labelled  
against MSL-1 protein.

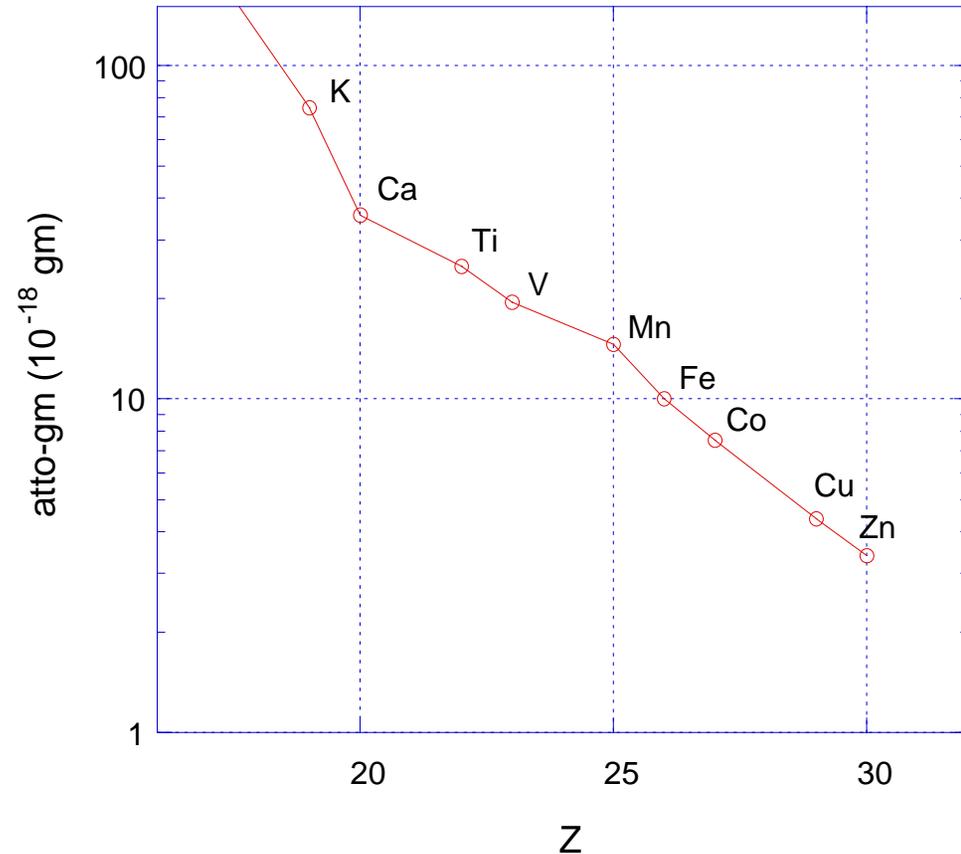
TXM micrograph  
acquired @ 0.5keV @  
BESSY,

Confocal laser scanning  
micrograph acquired  
with Zeiss LSM 510



# Why use x-ray-induced fluorescence to study trace metals?

- simultaneously map 15+ elements
- no dyes necessary
- very high sensitivity (< ppm)
- quantitative
- large penetration depth (> 100  $\mu\text{m}$ )
- chemical state mapping & micro-XANES



*Detection Limit for Transition Elements:  
for 1 s acquisition time, 0.2 x 0.2  $\mu\text{m}^2$   
spot,  $E = 10 \text{ keV}$ ,  $10^{10} \text{ ph/s}$*

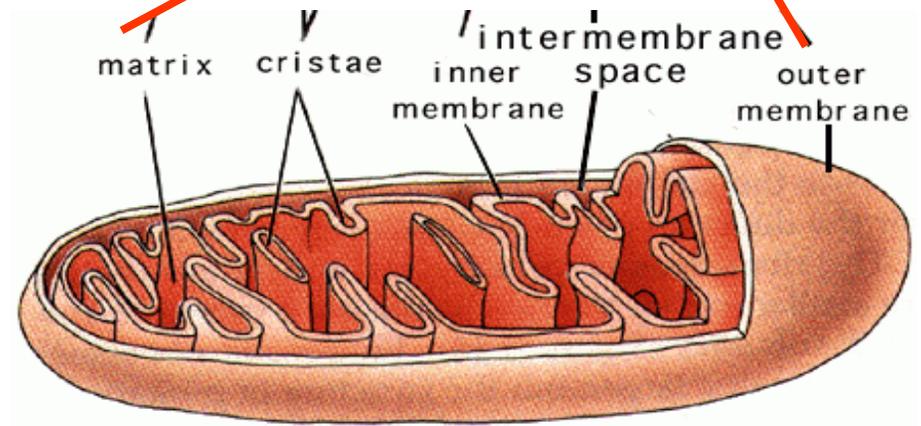
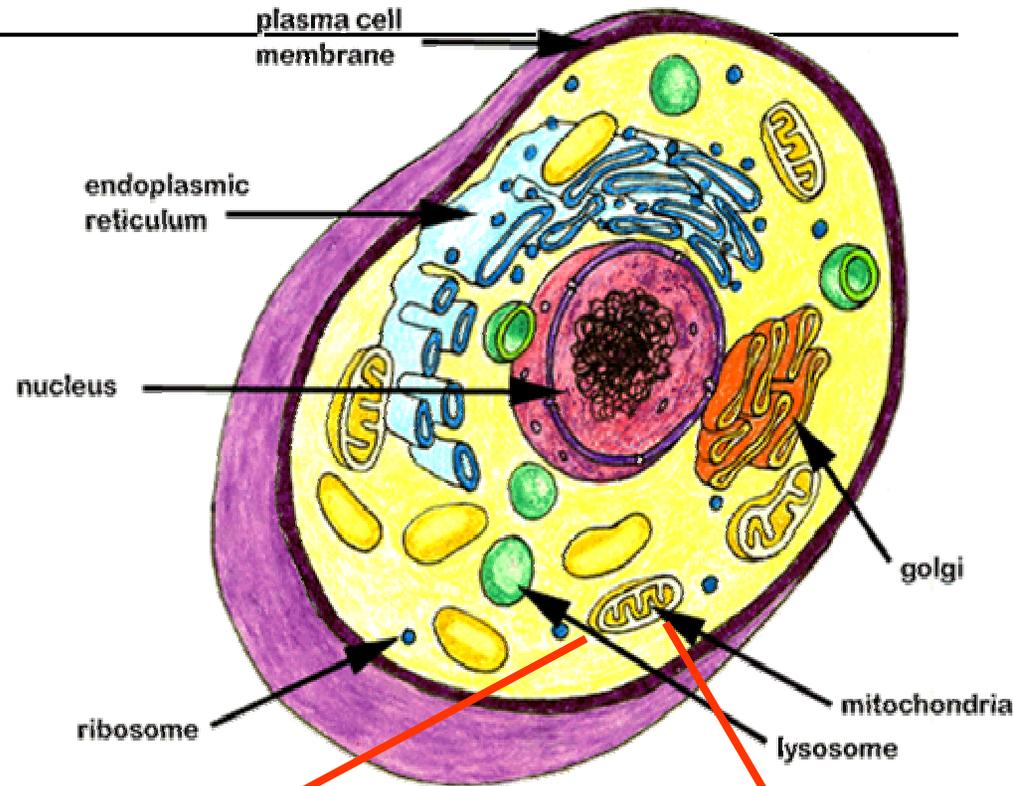
## Future requirements:

# increased spatial Resolution

### Typical sizes of cell organelles:

- nucleus: 2 - 5  $\mu\text{m}$
- mitochondrion: 0.5 x 2  $\mu\text{m}$  (cellular respiration), with substructure
- ribosome: 25 nm (protein synthesis from mRNA)
- chromatin fiber: 20 nm diameter (DNA double helix on histones)
- microtubuli: 20 nm diam. (cytoskeleton)
- membrane thickness: 8 nm

➔ need spatial resolution of < 20 nm



# *Outlook: desired facility*

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- **scanning: “bio-nanoprobe”**
  - < 20 nm spatial resolution, 1 - 4 keV and 4 - 30 keV
  - cryogenic preservation
  - micro-XANES (XAFS ?), @ < 20 nm resolution
  - X-ray fluorescence tomography
  - high throughput 100+ cells/day
  
- **full field: high speed imaging & tomography**
  - cryogenic preservation
  - contrast enhancement (phase, DEI)
  - high speed (1 ms ?)