INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **Vznik a charakterizace zlatých nanočástic v apoferritinu**

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Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

History of colloid gold

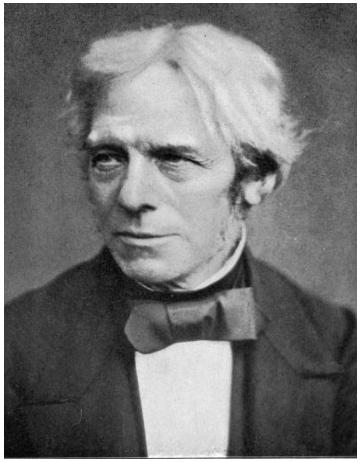
- Colouring of glass and ceramics

Lycurgus cup (5th to 4th century BC)

- Curative properties?

- Michael Faraday
  - first experiments with nanoparticles (1847)
  - reduction of an aqueous solution of $HAuCl_4$ by phosphorus in CS_2

Faraday's colloidal suspension of gold
(Faraday's museum, London)



Michael Faraday (1791-1867)

Synthesis of AuNP

- Most popular is reduction of HAuCl4.

  As reducing agents sodium or pottasium borohydride, hydrazine, ascorbic acid, dimethyl formamide… can be used.

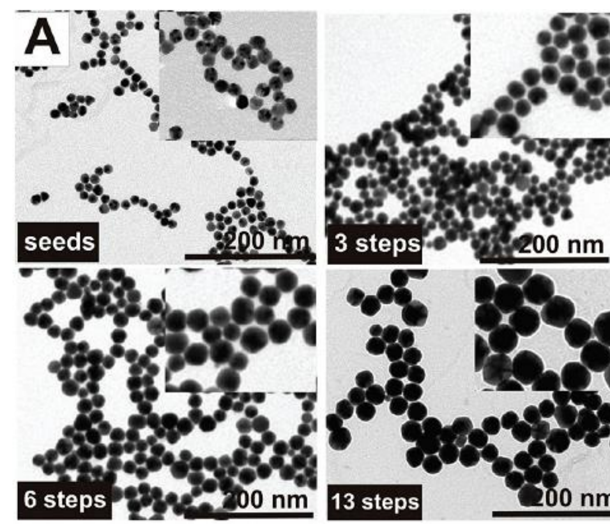
  Aggregates are stabilized by cystine, CS_2 , sodium citrate, nitrilotriacetate, 2-mercaptobenzimidazole, thiols and other sulfur ligands…

- Seeding growth for bigger particles.

- Microemulsion system

- Physical methods

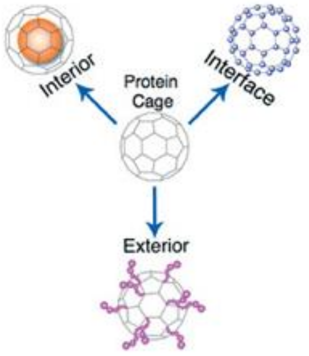
  metal-vapour synthesis, laser ablation, solvated metal atom dispersion (SMAD),



TEM images of Au seed particles and those obtained after different growth steps

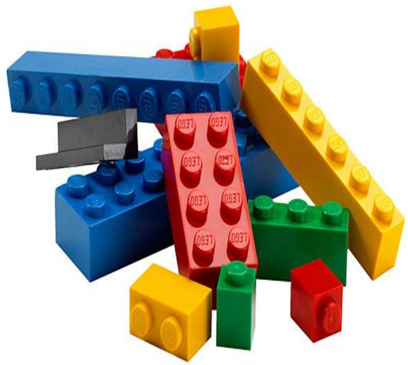
AND

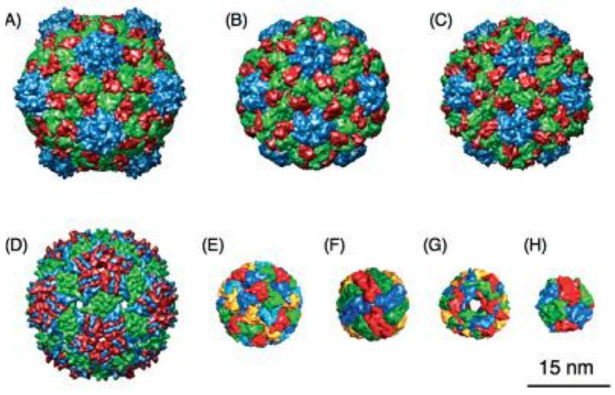
Schematic illustration of the three interfaces in a protein cage architecture available for chemical or genetic modification.



Protein cages

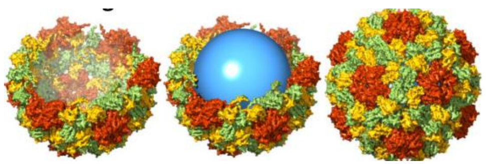
- well defined size and shape
- highly symmetrical architectures are based on helical, icosahedral, cubic, or tetrahedral symmetries
- container-like cage architectures have three chemically distinct interfaces (the interior surface, the exterior surface, and the interface between subunits) that can be genetically or chemically manipulated
- asemblies are responsive to pH and ionic strength





molecular lego → supramolecular assemblies

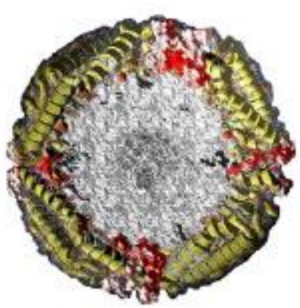
Space-filling images of protein-cage architectures.
A) Cowpea mosaic virus (31 nm in diameter)
B) Brome mosaic virus (28 nm)
C) Cowpea chlorotic mottle virus (28 nm)
D) MS2 bacteriophage (27 nm)
E) lumazine synthase (15 nm)
F) ferritin (12 nm)
G) small heat shock protein (12 nm)
H) DNA binding protein from starved cells (9 nm)



Encapsulation of nanoparticle in nanocage

Ferritines as protein cages

- In higher eukaryotes, ferritins are composed of 24 subunits (22 L and 2 H subunits) and are self assembled into a spherical cage (440 kDa).
- H-type subunit catalyzes the oxidation of Fe (II) to Fe (III) and is responsible for iron loading into ferritin, while the L-subunit lacks this activity, but promotes the nucleation inside the cage
- outer diameter of 12 nm and an inner cavity diameter of 8 nm
- 8 hydrophylic channels
- Fe_3O_4, Mn_3O_4, Co_3O_4,$Cr(OH)_3$, $Ni(OH)_3$, In_2O_3, FeS, CdS, CdSe, ZnS and other inorganic nanoparticle can be prepared within apo-ferritin (empty ferritine) under conditions of elevated temperature and pH
- and also metallic nanoparticles – Pd, Ag, CoPt, Au



A cross-section of the ferritin protein cage showing a full $Fe_2O_3{\cdot}H_2O$ mineral.

AuNP

- Absorbance and fluorescence AuNPs is much greater compared with bulk gold and can be tuned from the VIS to the NIR region by changing nanostructure size and morphology.
- Au NPs are chemically stable, non-toxic and easy to functionalize. DNA, enzymes, antibodies and some functional polymers can be easily conjugated with Au NPs without affecting their activities in most cases.
- are used in:

  - imaging

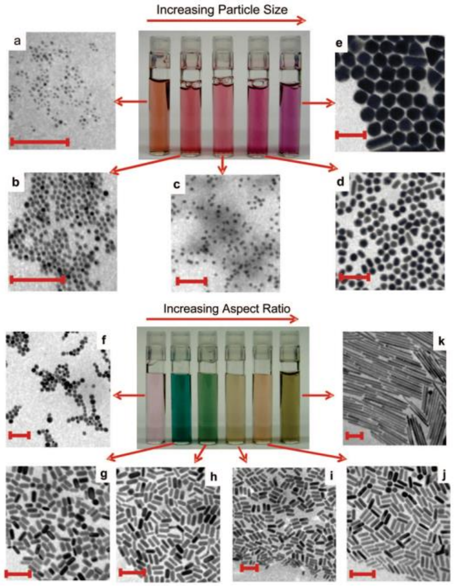
CT and MRI, fluorescence, SERS, photoacoustics

  - sensing

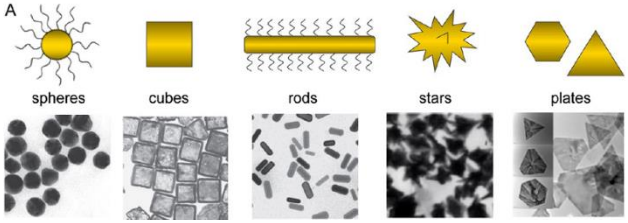
electrochemical, photoluminiscence, UV/VIS absorbtion responses

  - therapy

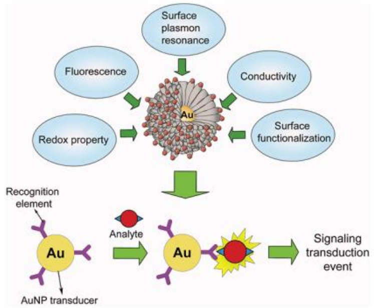
drug and NA delivery, photothermal therapy, radiotheraphy



Photographs of aqueous solutions of gold nanospheres (upper panels) and gold nanorods (lower panels) as a function of increasing dimensions. Corresponding transmission electron microscopy images of the nanoparticles are shown; all scale bars = 100 nm.



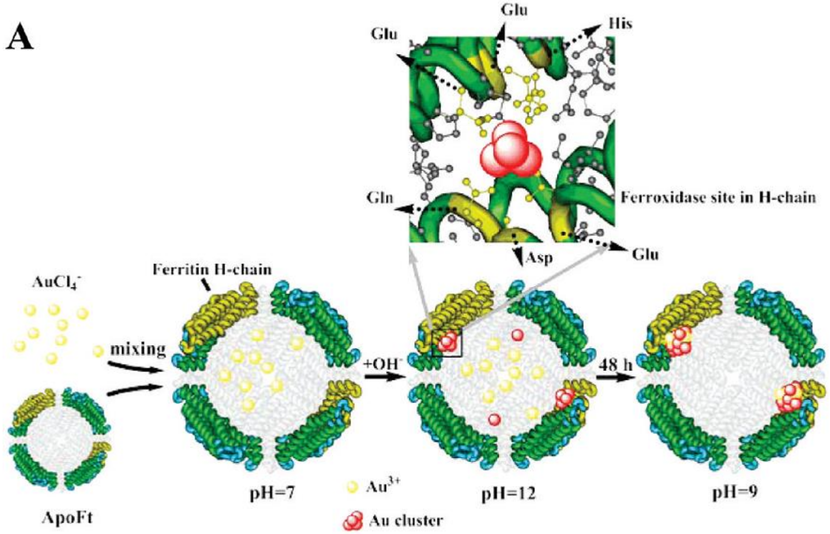
Examples of different gold nanostructures



Physical properties of AuNPs and schematic illustration of AuNP-based detection syste

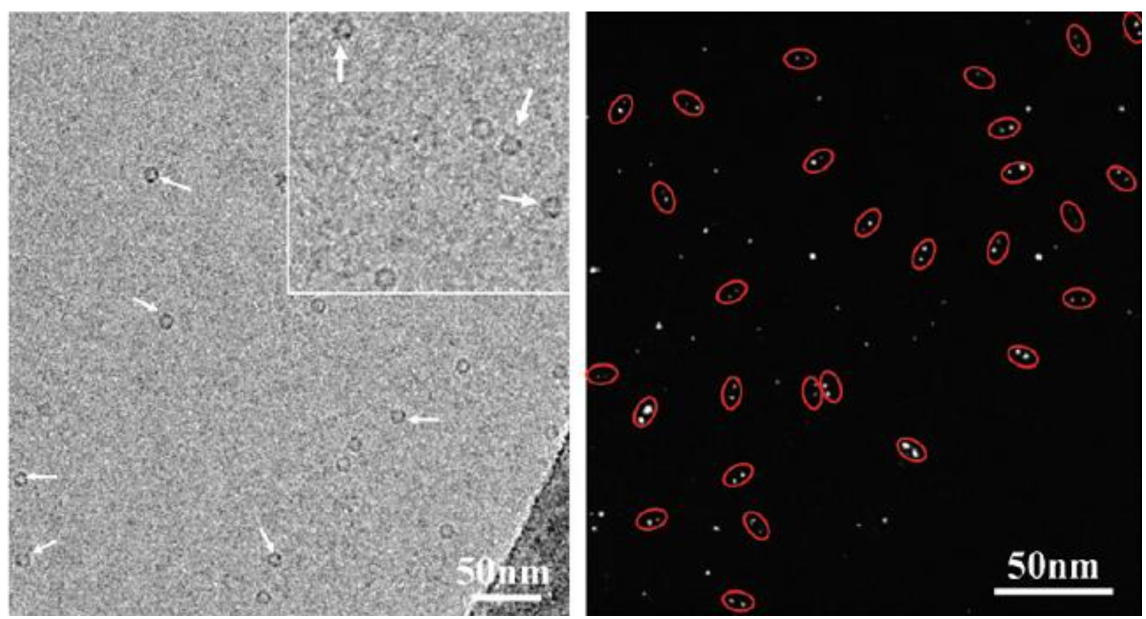
AuNP synthetised within ferritin

- Horse spleen apo-ferritine has two ferroxidaze centers.
- The ferroxidase center is composed of six amino acid residues.
  HISTIDINE, aspartic acid, glutamine and three glutamic acids
- Au^{3+} has strong binding afinity to the imidazole ring of the His residues
- a pair of Au clusters can be assembled in each ferritin
- pair of Au clusters within a ferritin shell may interact with each other, and the coupling between the Au clusters may enhance the fluorescence properties of the Au clusters
- We can observe the resonance energy transfer between two AuNPs.



Schematic illustration of the synthesis of AuFt.
The His residues at the ferroxidase centers play
an important role in the in situ Au cluster assembly

Characterization of far-red AuFt.
(a) Cryo-electron microscopy (Cryo-EM) image.
Paired Au clusters were observed within the ferritin nanoreactor (indic
by arrows).
(b) HAADF-STEM image of
far-red AuFt. There are about 40 paired Au clusters
(indicated by ovals) within every 8 nm zone.



Our aims

- ❑ Synthetise AuNPs with enhanced fluorescence in ferritine.

- ❑ Use it to in vivo imaging of cell culture.

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CZ.1.07/2.4.00/31.0023 NanoBioMetalNet

and you for attention

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