

Název: Apoferritin structures for cytostatics transport
and tools for their visualization

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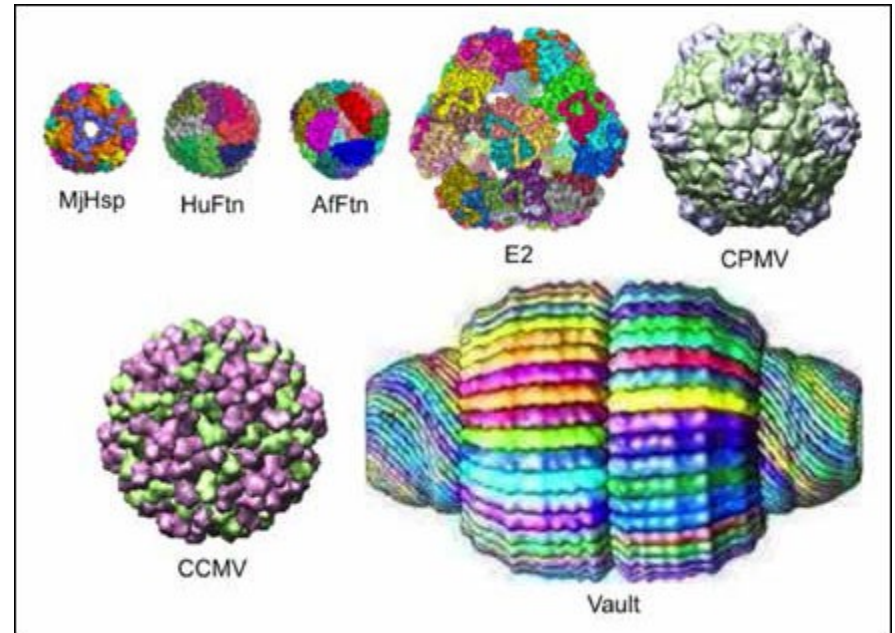
Datum: 13.9.2013

- (Apo)ferritin
- Preparation of apoferritin
- Using of apoferritin
- Encapsulation of doxorubicin to apoferritin structure
- Doxorubicin
- Results
- Summary

Protein nanocages

- **Natural barrels**
- **homo or heterooligomers**
- **conditions-related stability**
- **self-assembly**

- **viral capsids**
 - Cowpea mosaic virus (CPMV)
 - Cowpea chlorotic mottle virus (CCMV)
- **chaperonins**
 - GroEL from *Thermococcus thermophilus*
- **heat shock proteins**
 - *Methanococcus janashii* heat shock protein (MjHsp)
- **multienzyme complexes**
 - *Geobacillus stearothermophilus* E2 protein of pyruvate dehydrogenase multienzyme complex
- **vaults**
 - major vault protein
- **ferritins**
 - eucaryotic (HuFtn)
 - procaryotic (AfFtn)



Ferritins

- **Ferritin - intracellular iron storage and transport protein**

- Binds Fe^{2+} , stored as Fe^{3+}
- up to 4000 Fe atoms per cage
- The heavy chain of ferritin subunits possesses ferroxidase activity, this involves the conversion of iron from the Fe^{2+} (soluble) to Fe^{3+} (insoluble) forms



- In almost all living organisms
- **Cage-like structure**

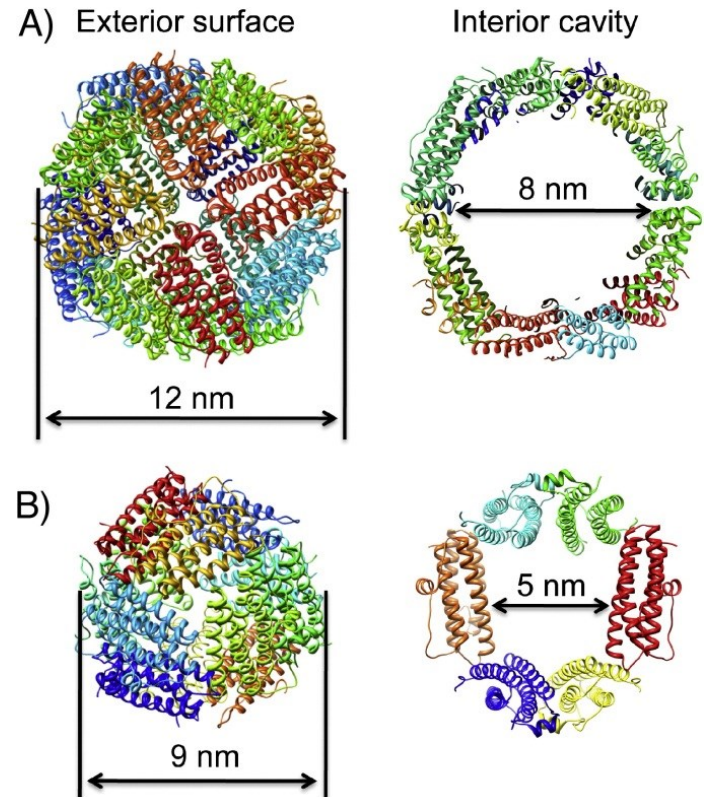
- **Maxiferritin**

- 24-mer
- 480 kDa
- diameter of inner cavity 8 nm

- **Miniferritin**

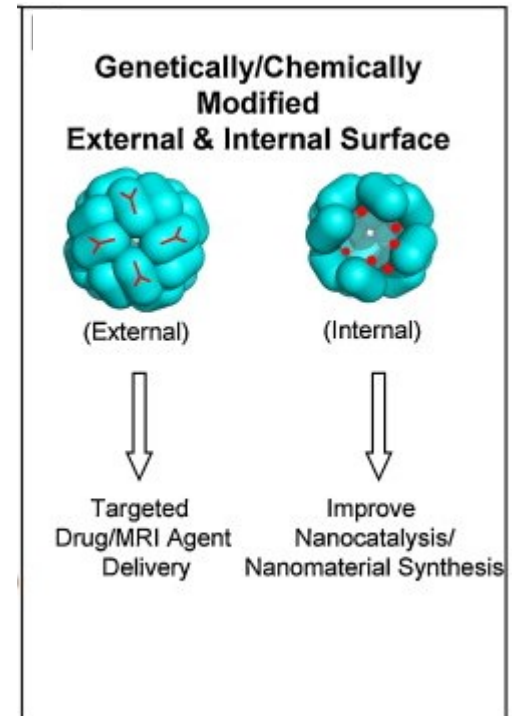
- 12-mer
- 240 kDa
- diameter of inner cavity 8 nm

- Synthesis of proteins with iron cofactors
- Antioxidants
- **Iron removal \rightarrow apoferritin**



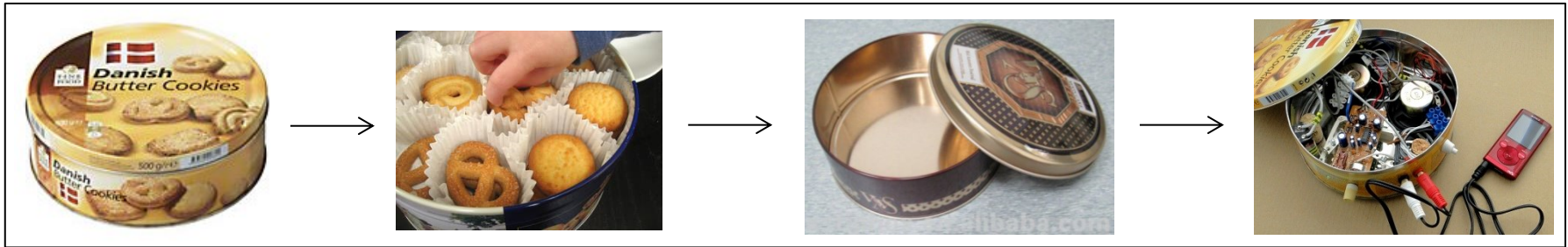
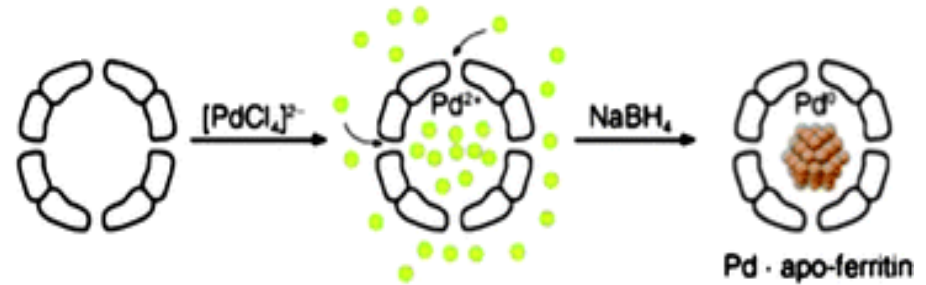
(Apo)ferritins in nanotechnology

- **Natural reactors**
 - first used as templates for FeS nanoparticles synthesis in 1991
- **Natural metal-binding sites**
- **Under *in-vitro* conditions also other heavy metals can be incorporated**
 - Fe, Au, Pd, Rh, Pt, Ni, Cr, Cd, Ti, Tb, Co, Cu, Zn
- **pH-dependent subunits stability**
 - pH < 3 – dissociation
 - pH > 5 re-association
- **Self-assembling**
- **Hydrophobic/hydrophilic channels**
 - reagents transfer
- **Stable to relatively harsh conditions**
 - 80°C, 6M urea, 6M guanidine, 1% SDS at pH = 7
- **Oxidoreductase activity**
- **Engineering of the properties**
 - surface modification
 - inner cavity modification
 - additional metal-binding sites
 - channels permeability
 - stability
 - ferritins from thermophilic microorganisms



Preparation of apoferritin – loaded molecules:

- Preparation of apoferritin:
 - Iron removal
 - Repeated reduction/dialysis
 - Using of hydrophilic channels



- Apoferritin loading with molecules:
 - diffusion
 - hydrophobic/hydrophilic channels
 - re-assembly
 - pH-directed dis-assembling and re-assembling of apoferritin subunits

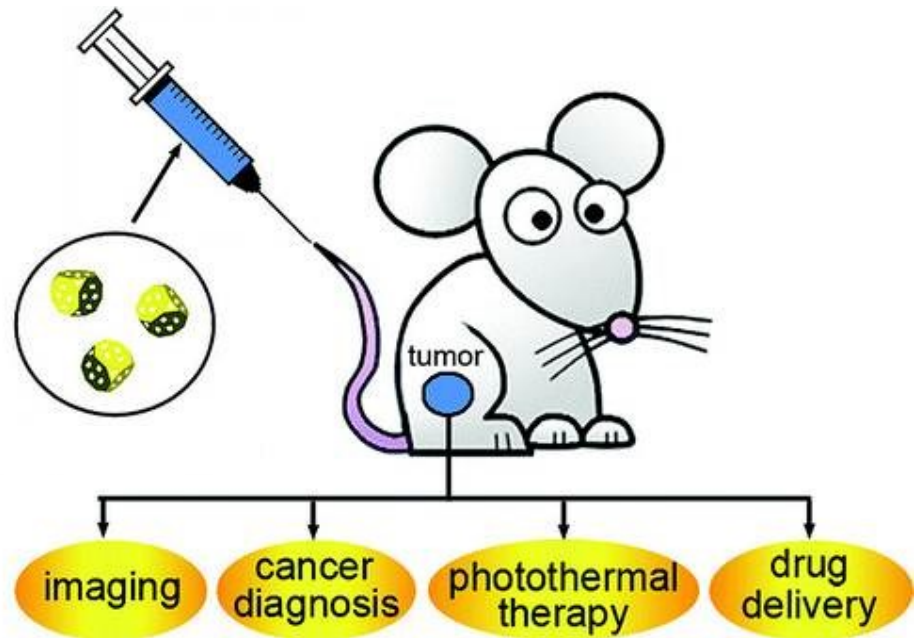


Using of apoferritin cages

- **Nanotransporters**
 - targeted drug delivery
- **Nanoreactors**
- **Catalysis**
- **Nanoparticles synthesis**
 - uniform size
- **Nanoelectronics**
- **Probes**
 - *in vivo* imaging
 - bioassays
 - fluorescence detection
 - electrochemical detection

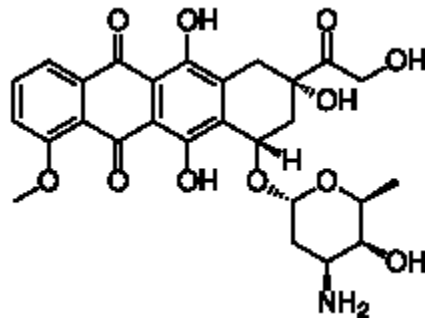
Drugs encapsulation

- Specific delivery to target cells
- Increase of the therapeutic effect
- Decrease of the side effects
- Lower dosing
- Monitoring of the drugs distribution
- Encapsulation in
 - Liposomes
 - Micelles
 - Protein cages
 - Inorganic cages
 - Organic carriers



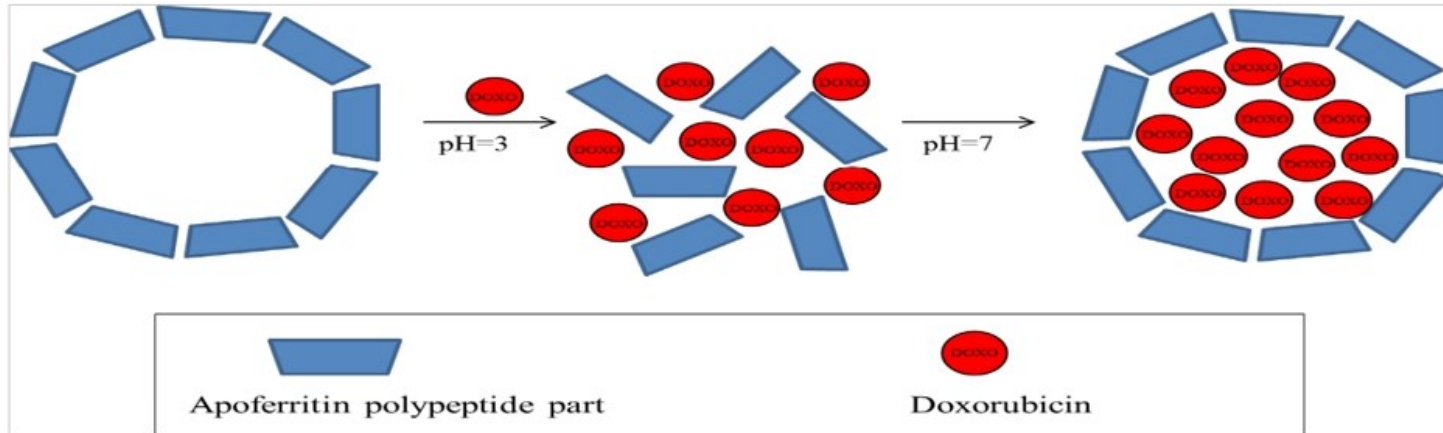
Doxorubicin

- **Anticancer drug**
- **anthracycline antibiotic** derived from daunomycin (*Streptomyces* spp.), brand names:
 - Adriamycin, Rubex
 - liposome-encapsulated forms: Doxil, Caelyx, Myocet, Lipodox
- **Severe side effects:** cardiotoxicity, neutropenia, alopecia, skin injury
 - *red devil, red death*
- **Mechanism of action:**
 - DNA intercalator
 - Inhibition of macromolecular synthesis (DNA-gyrase)
- **Autofluorescence**
- **Electrochemical activity**
- *antimalarial effect* (inhibition of plasmapepsin II, a protein unique for *P. falciparum*)



Encapsulation of doxorubicin to apoferritin structure:

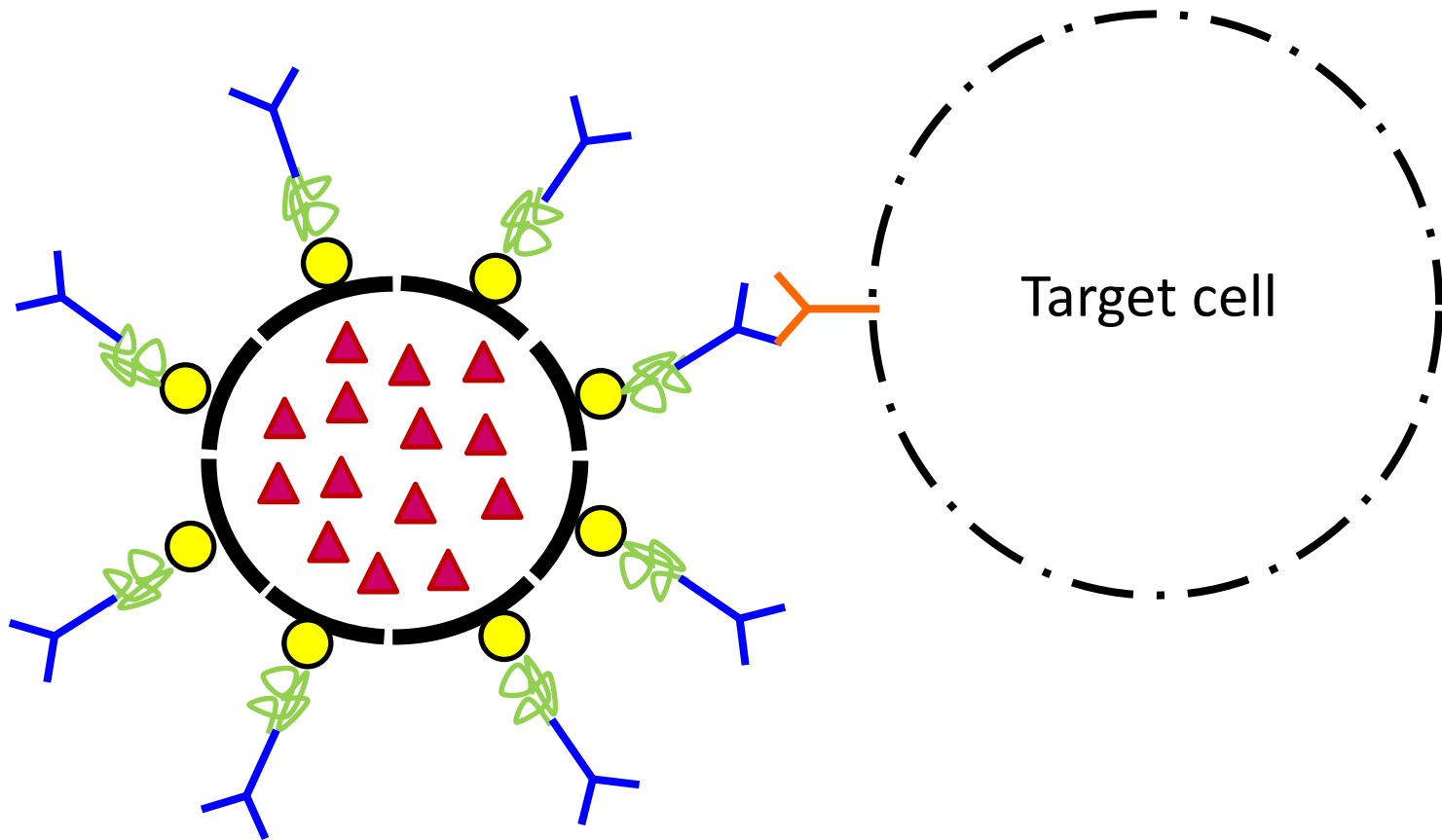
- hypoxia – typical for cancer cells, leads to decrease of intracellular pH value below 3
- physiological intracellular pH - neutral
- pH-dependent release of doxorubicin
- surface modification – targeted delivery to cancer cells



Encapsulation of doxorubicin to apoferritin nanostructures - methods used in our laboratories

- **electrochemical methods**
- **electromigration methods**
 - gel electrophoresis
 - capillary electrophoresis
- **fluorescence spectroscopy**
- **immunomethods**

Does it work?



Apoferritin



Doxorubicin



Gold nanoparticles



HWR peptide



Human IgG



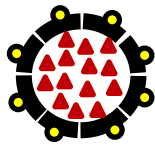
Goat anti-human IgG

Gel electrophoresis

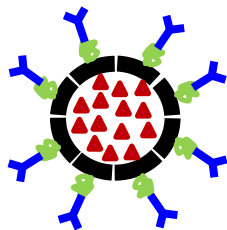
- Assembly of apoferritin subunits
- apoferritin conjugation with antibodies
- Co-localization of proteins and doxorubicin
- Agarose electrophoresis
- Native polyacrylamide electrophoresis



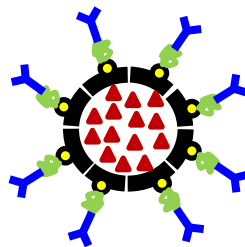
1. ApoDox
#



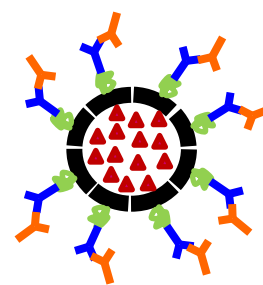
2. ApoDox + nanoAu
#



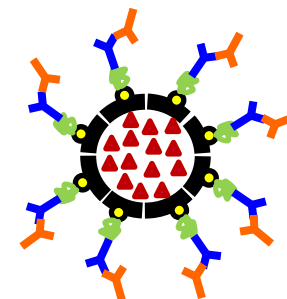
3. ApoDox + HWR + IgG
#



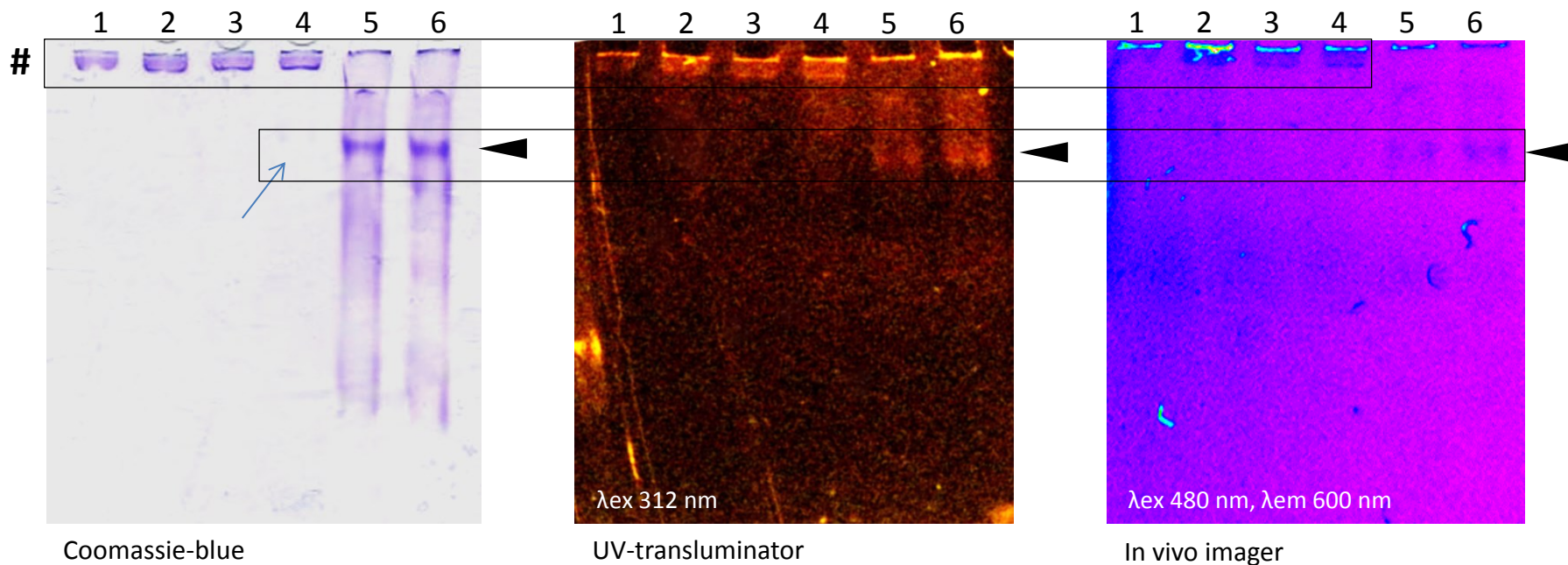
4. ApoDox + nanoAu + HWR + IgG
▲



5. ApoDox + HWR + IgG + antiIgG
▲

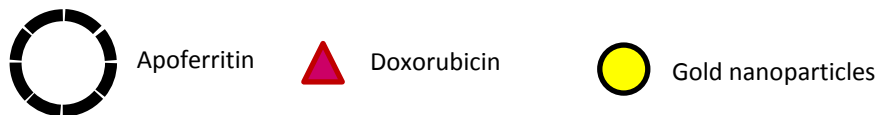
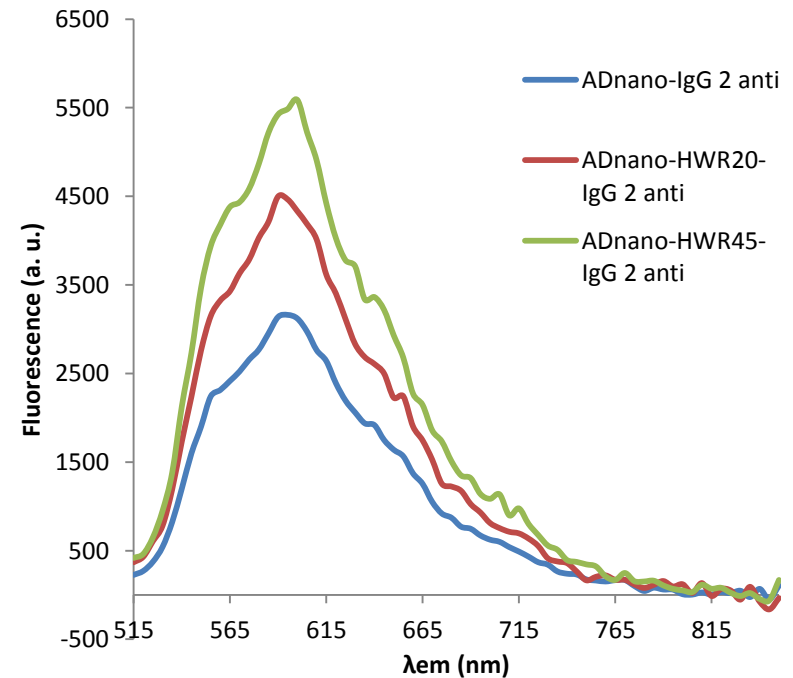
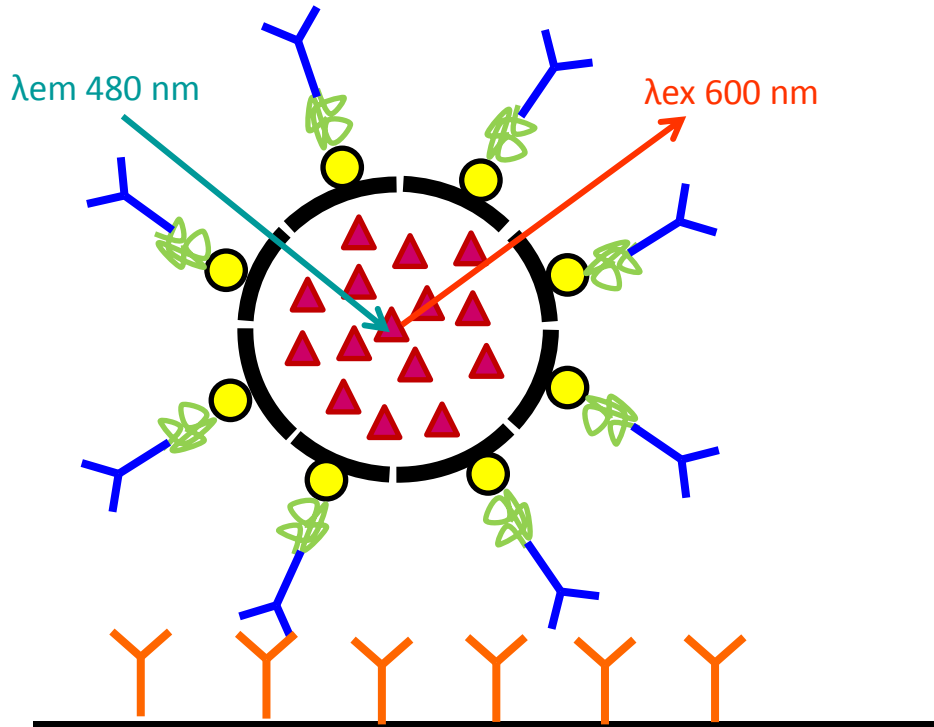


6. ApoDox nanoAu + HWR + IgG + antiIgG
▲



Immunomethods

- Correct function of the nanostructure
- ELISA
- Measurement of doxorubicin fluorescence



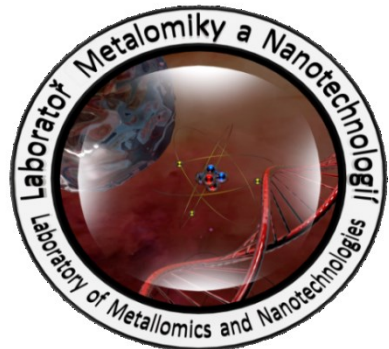
Summary

- Apoferritin is suitable for doxorubicin encapsulation
- Apoferritin encapsulation offers many possibilities for further improvement of nanostructures function
- IgG were successfully immobilized onto apoferritin surface
- The complex has affinity to anti-IgG antibodies
- Insights for future: real cancer cells, real antigen

Thank you for your attention!

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