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Ferritin-based anticancer metallodrug delivery: encapsulation of Arsenoplatin-1 within the ferritin nanocage

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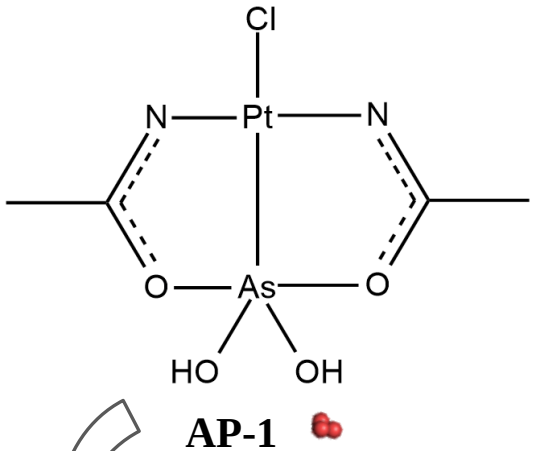
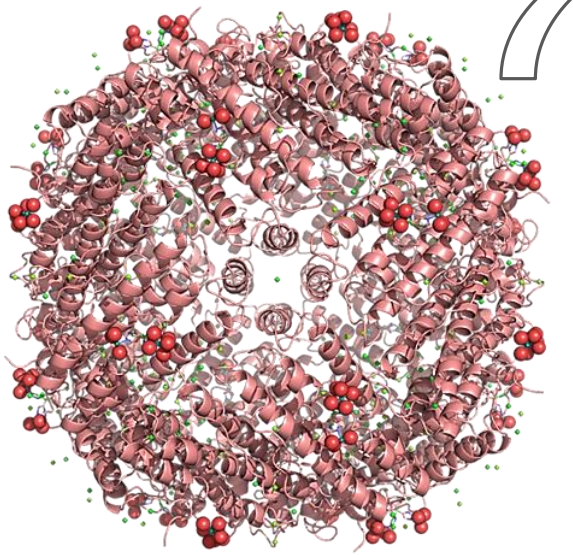
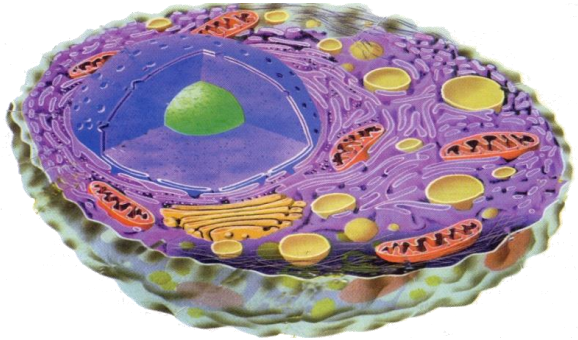
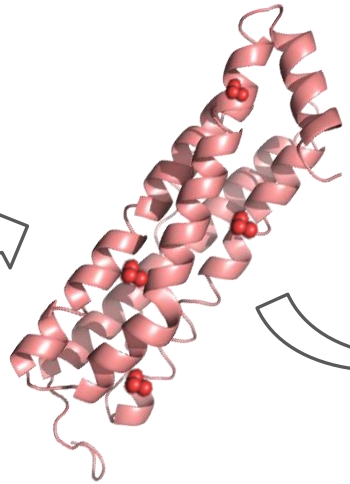
Ferritin-based anticancer metallodrug delivery: encapsulation of Arsenoplatin-1 within the ferritin nanocage



AP-1

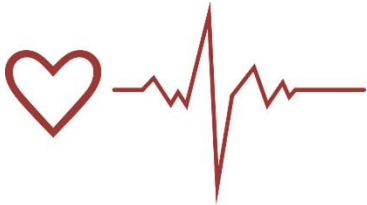






cancer cells →

normal cells →

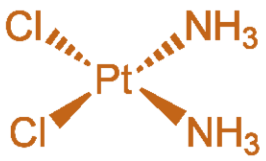


Abstract:

An ideal drug delivery system is characterized by high stability, biocompatibility and selective delivery of molecules to target cells. Molecules already present in the body, like proteins, are safer for the immune defence mechanism. Ferritins (Fts) are proteins that self-assemble into hollow cage-like structures, already used to encapsulate molecules for optical imaging, catalysis and photodynamic therapy. Here, Ft was chosen to encapsulate **Arsenoplatin-1** (**AP-1**), the prototype of a novel class of metallodrugs containing a $PtAs(OH)_2$ core. **AP-1-encapsulated Ft** was prepared following the alkaline pH procedure: Ft cage is disassembled at basic pH and then reassembled in the presence of the drug by raising the pH at a neutral value. In this way, the metallodrug is trapped inside the Ft core. UV-Vis spectroscopy and ICP-OES measurements confirm the encapsulation and allow to evaluate the exact amount of **AP-1** in the nanocage. The X-ray structure of **AP-1-encapsulated Ft** reveals that an **AP-1** fragment without the chloride ligand binds the protein. The biological activity of **AP-1-loaded Ft** was tested on cancer cell lines and compared to that observed in normal ones. The results indicate that, even if reducing the overall drug toxicity, the presence of the cage improves **AP-1** selectivity for cancer cells.

Keywords: drug delivery; biocompatible; protein; nanocarrier; metallodrug

Cisplatin



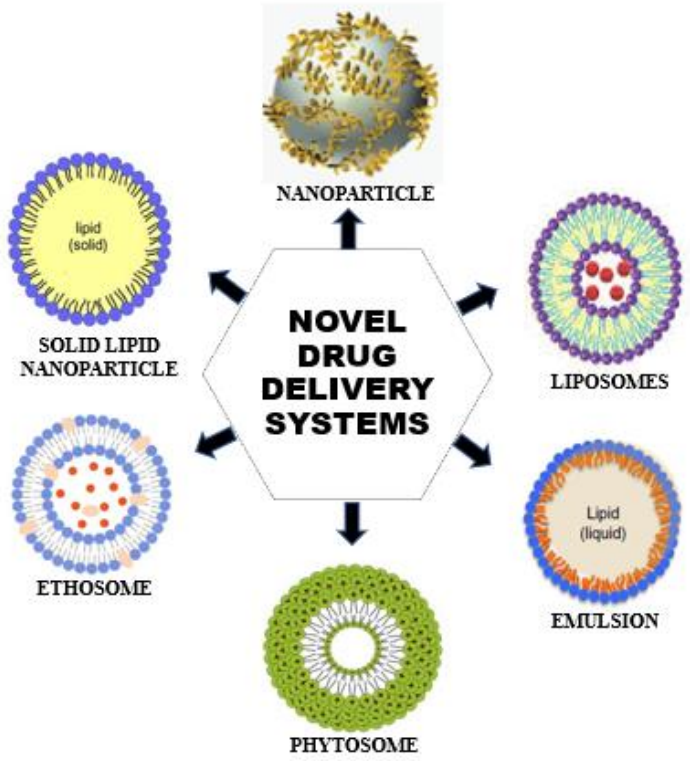
Rosenberg B. Nature (1965) 205, 698

Metal compounds have an important place in the clinical practice

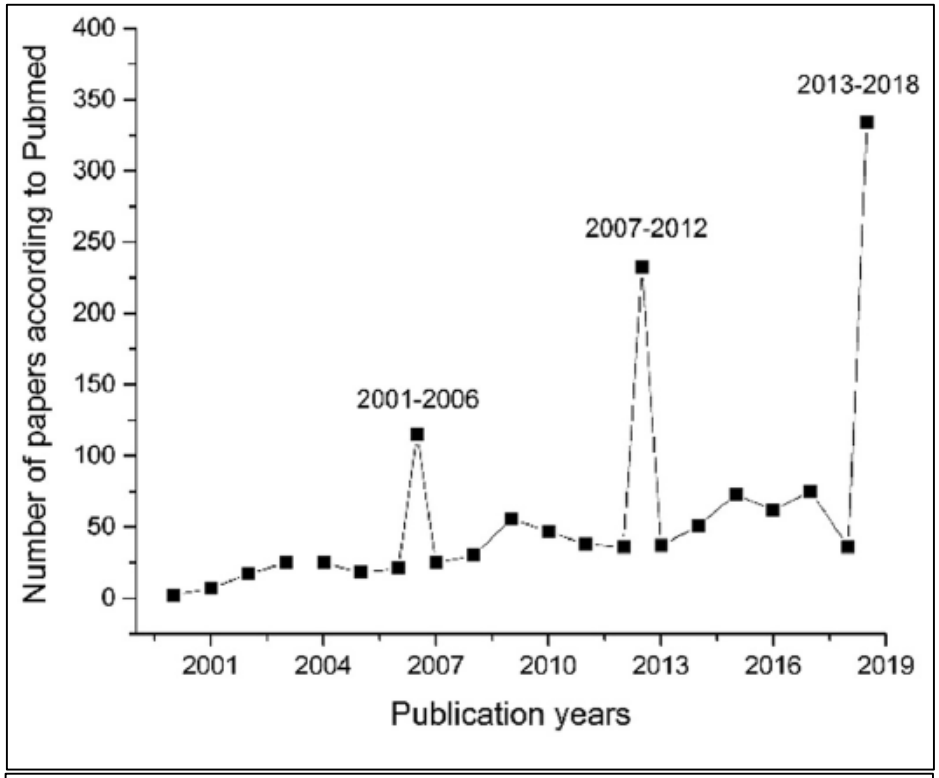
They are used as drugs to treat several human diseases (carcinomas, lymphomas, infection control, anti-inflammatory, diabetes, and neurological disorders).

Several side effects: general toxicity and drug resistance

- ✓ alternative metal centers
- ✓ change of oxidation state and coordination geometry
- ✓ ligand modification
- ✓ development of polynuclear systems
- ✓ ***drug delivery/targeting protocols***

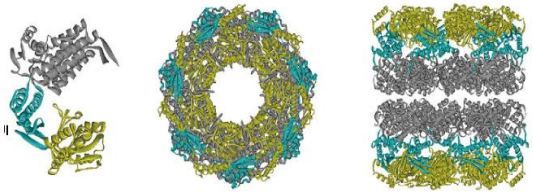
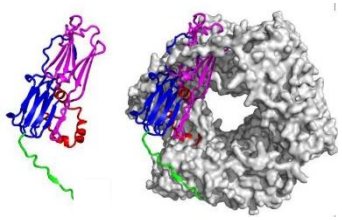
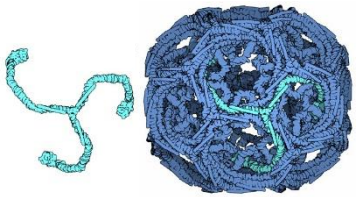
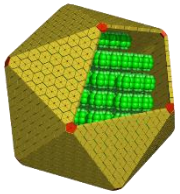
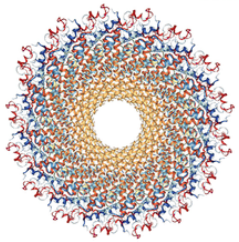


Proteins as delivery systems for anticancer drugs

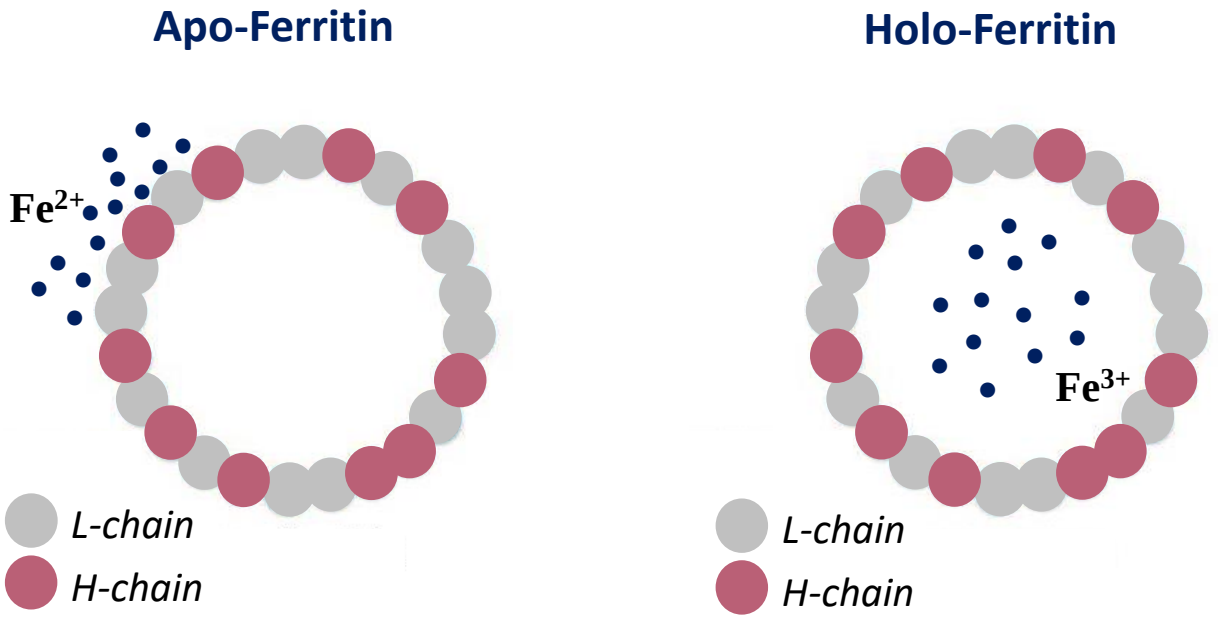


A graph on the growing interest of researchers in the field of "proteins as delivery systems for anticancer drugs" from 2000 to 2018 (data from Pubmed).

Monti, D.M. *et al.* Nanomedicine: NBM (2019) 20, 101997

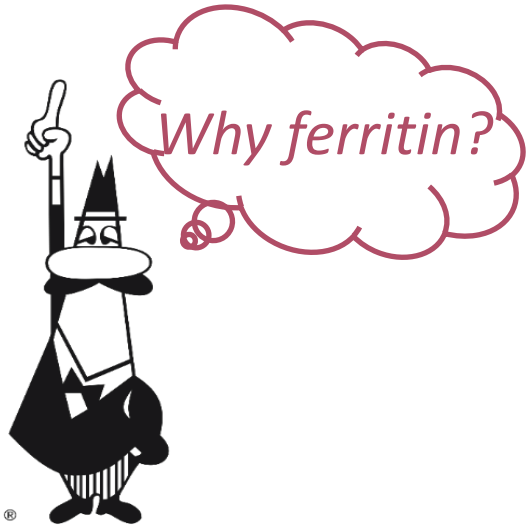
* chaperonines 
* heat shock proteins 
* clathrin 
* carboxysomes 
* mosaic viruses 

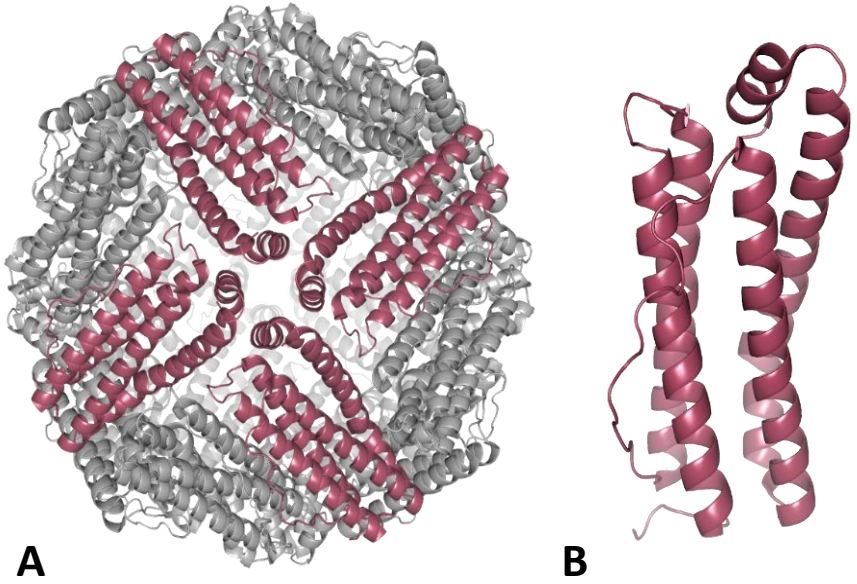
Ferritin: a protein nanocage for iron store and mineralization



- 24-mer cage with octahedral symmetry
- outer diameter ~120 Å - inner diameter ~80 Å
- 2 type of chains: H-chain (heavy) + L-chain (light)
- iron sequestration → detoxification and cellular reserve

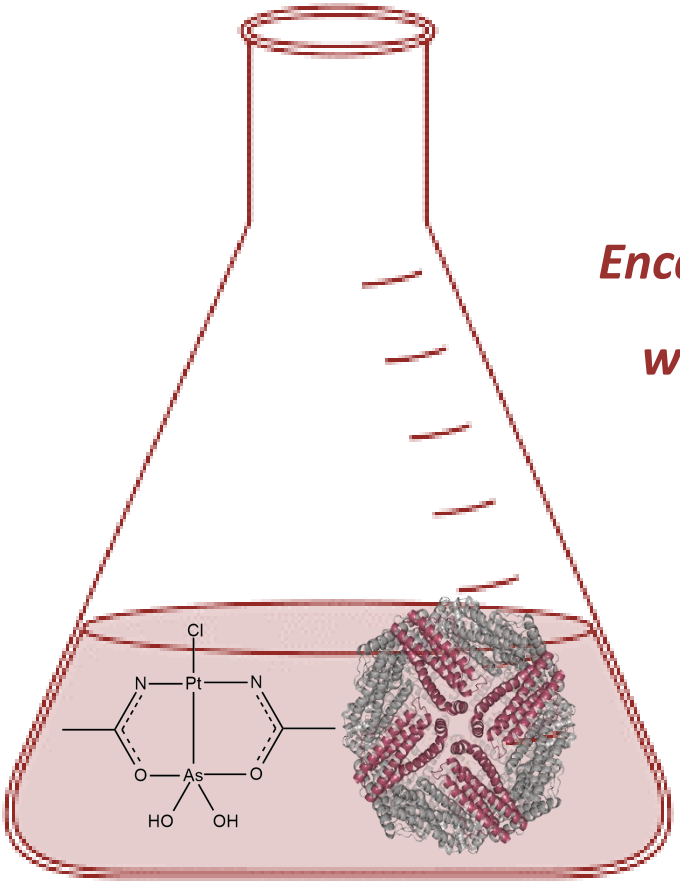
Monti, D.M. *et al.* Nanomedicine: NBM (2019) 20, 101997





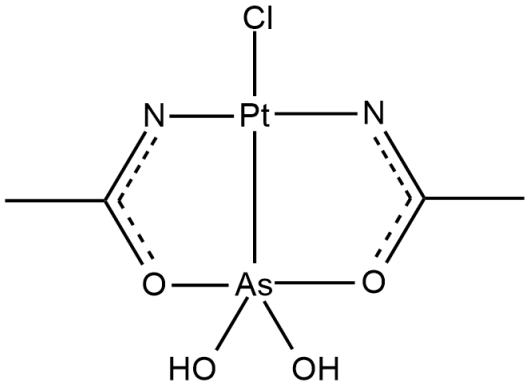
Cartoon representation of the structure of the globular ferritin (A) and its monomeric form (B)

- biocompatible
- non-immunogenic
- stable and soluble in the bloodstream
- amenable to both genetic and chemical functionalization
- could lead to longer circulation half-life and to better tumor accumulation rates
- **recognized by receptors over-expressed on cancer cells surface**



Encapsulation of Arsenoplatin-1 within the ferritin nanocage

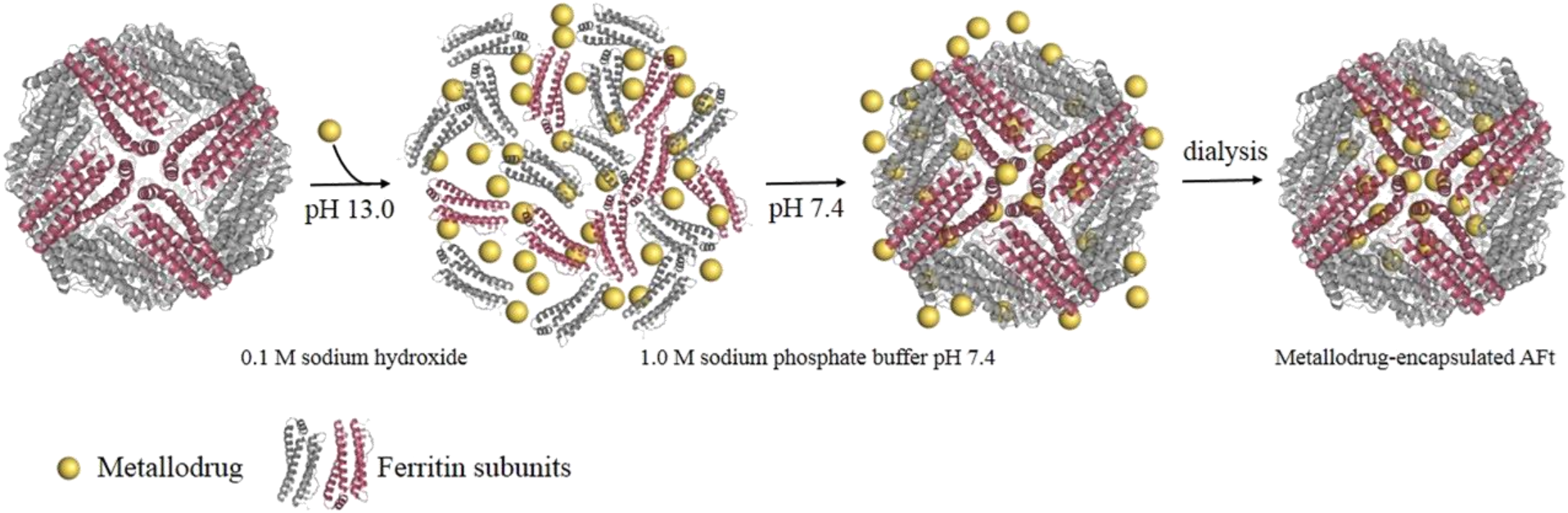
Arsenoplatin-1 (AP-1)



- Complexes containing a $PtAs(OH)_2$ core with a square planar Pt(II) and a five coordinate As(III) geometry
- Stable in solution
- With chemical bonding, ligand substitution chemistry and biological activities distinct from the parent compounds cisplatin and arsenic trioxide
- With a promising activity in drug-resistant cancer cell lines

Miodragović Đ.U. *et al.* **Angew Chem** Int Ed Engl (2013) 52, 10749.

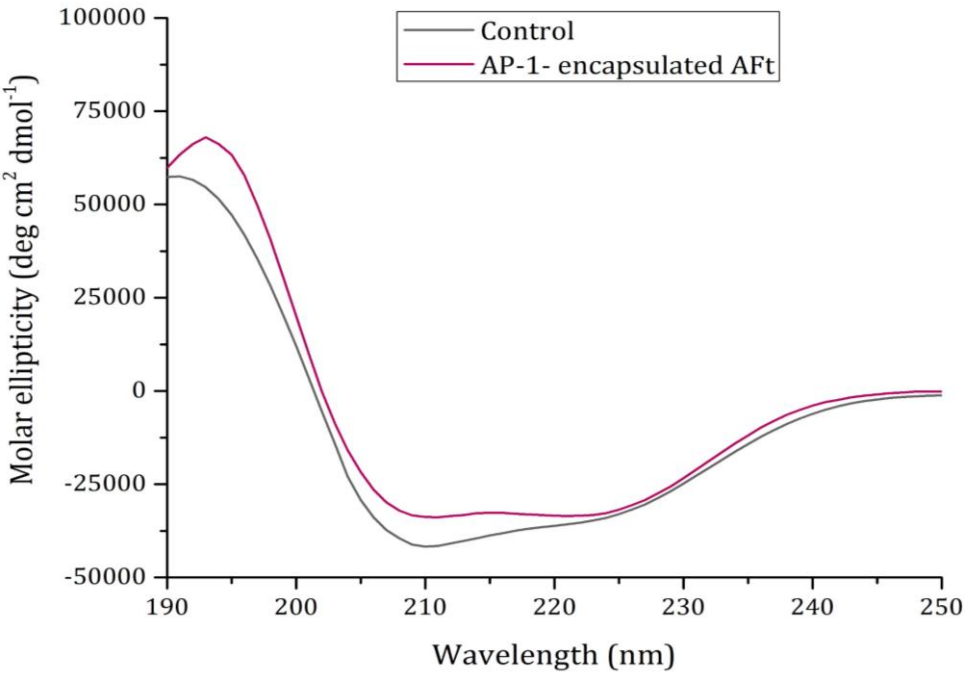
Alkaline pH encapsulation protocol



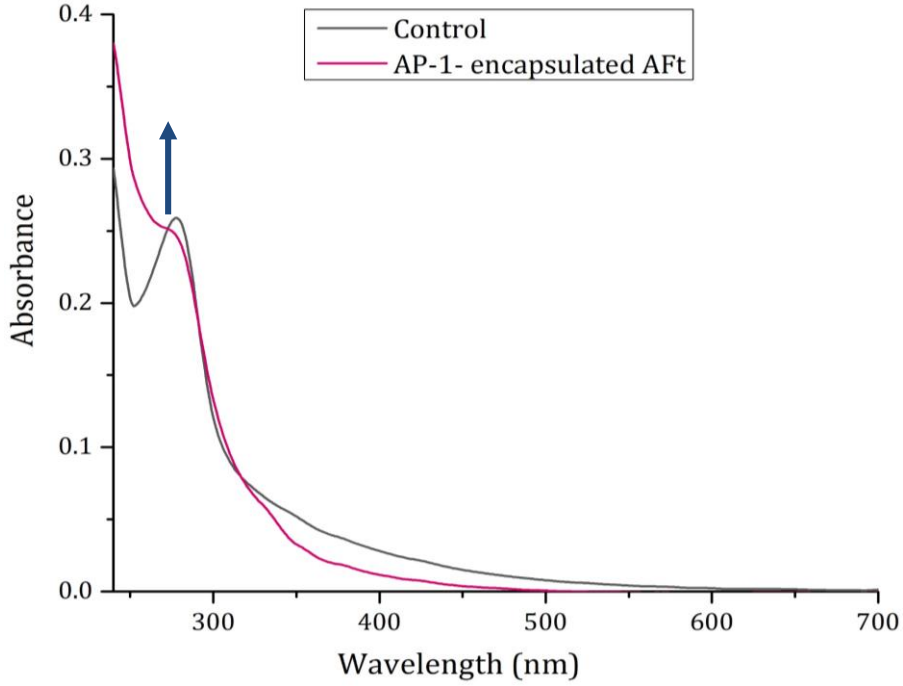
- The ferritin cage is disassembled at alkaline pH (13.0) in the presence of 0.1 M NaOH
- AP-1 is added to the ferritin solution and the reaction mixture is incubated for 1h at room temperature under stirring
- After the incubation time, the pH is restored to 7.4 by adding 1.0 M sodium phosphate buffer
- The AP-1-encapsulated ferritin is then extensively dialyzed and stored at 4°C

Pontillo, N. *et al.* **Chem Commun** (2016) 52, 4136

Far UV-CD spectroscopy



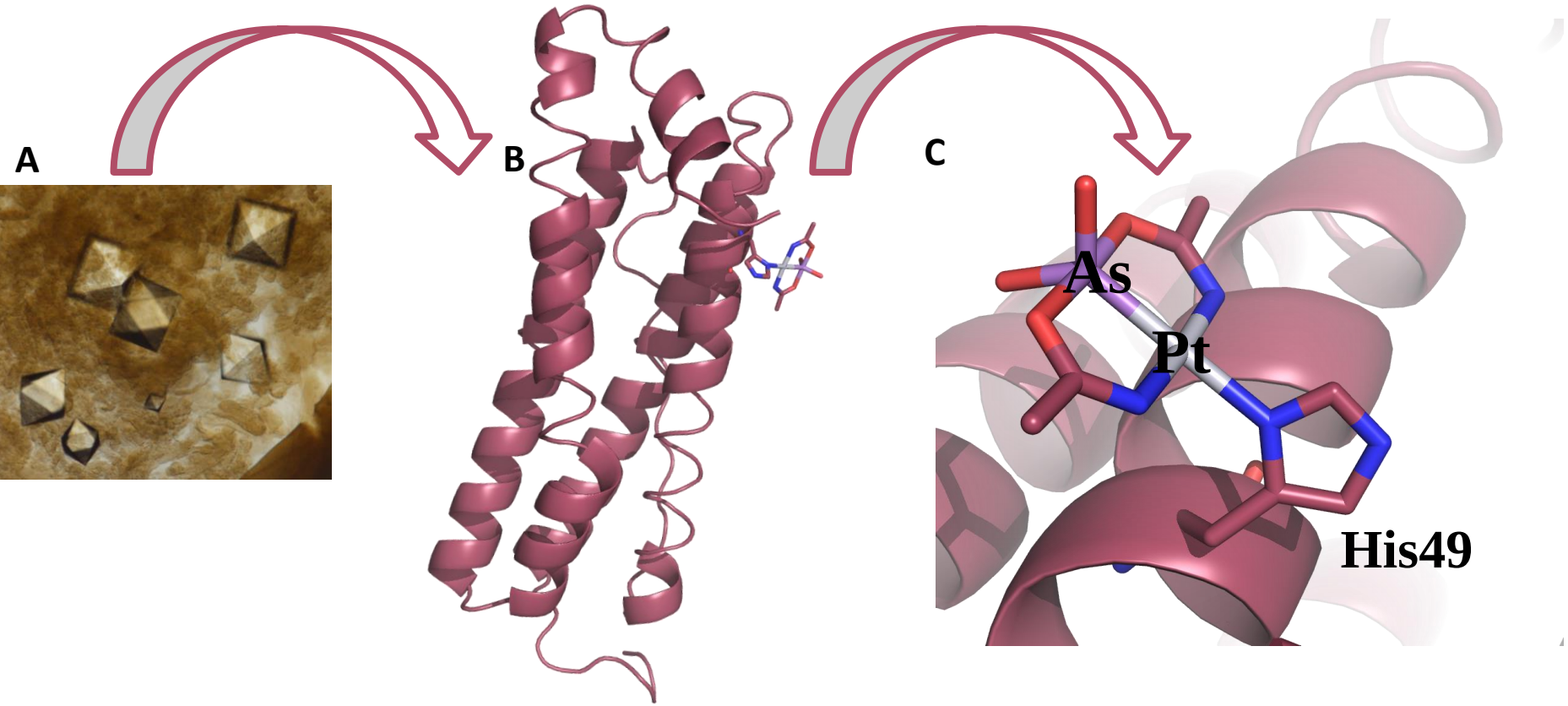
UV-Vis absorption spectroscopy



ICP- OES measurements

AP-1 molecules/cage	AP-1 molecules/single chain
600 – 700	25.0 – 29.2

X-ray crystallography



(**A**) Crystals of AP-1-encapsulated AFt.

(**B**) Cartoon representation of the structure of a single ferritin chain of **AP-1-encapsulated AFt**.

(**C**) Details of the AP-1 binding site.

Biological activity

IC_{50} values obtained after 48 h of incubation with AP-1 and AP-1-encapsulated AFt

	48 h incubation	
	AP-1 (μM)	AFt-AP-1 (μM/AP-1)
HaCaT (human keratinocyte cells)	2.60 ± 0.26	10.91 ± 0.73
A431 (human epidermoid carcinoma)	4.04 ± 0.92	4.66 ± 0.29
Balb/c 3T3 (immortalized murine fibroblast)	3.60 ± 0.10	27.80 ± 0.14
Svt2 (immortalized murine fibroblast transformed with SV40 virus)	3.87 ± 1.01	11.64 ± 0.29

Conclusions

A well-characterized hetero-bimetallic compound of potential medicinal interest, AP-1, has been selected and encapsulated within the ferritin nanocage.

The drug-loaded nanocarrier has been investigated:

- ✓ **From a biophysical point of view**, analysing the conformation of the nanocomposite and solving the X-ray structure. These data have allowed highlighting the nature of the interactions of AP-1 with the nanocarrier.

- ✓ **From an analytical point of view**, defining the exact amount of metal that is encapsulated in the nanocage, and the protein-metallodrug stoichiometry.

- ✓ **From a biological point of view**, testing the cytotoxic effects of the nanocomposite, in comparison to the free drug, on cancer and non-cancer cell lines to assess the possible target-selectivity.

Conclusions

- The AFt nanocage re-assembles in the presence of the drug and is able to trap the drug inside the cage.

- The structure and the electrostatic potential of **the outer surface of AFt are basically not affected by the presence of the drug**. The AP-1-AFt nanocomposite retains the chemical-physical features of the native protein, even upon drug encapsulation, confirming that this system can be used as a suitable nanocarrier.

- In the drug-loaded ferritin, **the binding site is located on the inner surface of the cage**. An AP-1 moiety binds the side chain of His49, but there is a significant amount of metal compound in the bulk. These molecules could be the active species, which are responsible for the activity of the nanocomposite.

Conclusions

- **Ligand-free ferritin is non-toxic** either for normal or for cancer cell lines, confirming the biocompatibility of this nanocarrier.

- **The drug-loaded nanocarrier shows moderate selectivity**, since it kills tumor cell lines at a lower concentration than that needed to kill normal cell lines.

- **The cytotoxicity of the drug-encapsulated AFt depends mainly on the intrinsic properties of the encapsulated compound**, rather than on the structure of the ferritin obtained upon encapsulation.

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Prof. Luigi Messori

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