# Internalization of Anticancer Gold(I) Complexes in Human H Ferritin to Improve Drug Selectivity

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To date, the major challenges in the anticancer therapy are the improvement of drug's efficacy and the lowering of the off-target toxicity. Because of that, in the last years, a lot of new metal based chemotherapeutic drugs has been developed and studied together with some "repurposed" drugs (originally indicated for different diseases) with the aim to ameliorate or increase the chemotherapeutic performances.

The internalization of anticancer metal-based drugs in a protein nanocage plays an important role in the development of new strategies to enhance the efficacy and selectivity of metal compounds against cancer cells.

Ferritin, with its 24-mer nanocage structure, is a well-known system used for the drug delivery [1]. In this work, a recombinant homopolymeric H ferritin (HuHf) [2] was used; each H subunit contains several cysteines residues that represent a favourable binding site for the gold complexes.

ESI MS is a powerful investigative tool to analyse the reactions of metallodrugs with proteins and describe the resulting adducts.

Accordingly, the ESI-MS approach has been applied to a small panel of experimental and clinically established anticancer gold (I) compounds i.e. Auranofin, Aurothiomalate, Au(NHC)Cl and the corresponding bis-carbene complex [Au(NHC)2]PF6 (where NHC is a N-heterocyclic carbene ligand).

The analysis of the obtained mass spectra allowed to reveal the formation of adducts between gold complexes and HuHf and to characterize the interactions occurring between the various gold compounds and a single ferritin subunit.

Another important task was the quantification of the internalized gold in the ferritin 24-mer nanocage through ICP-OES; finally, to evaluate the cytotoxic effect of the inclusion complexes towards the A2780 ovarian cancer cells, the pharmacological activities (IC50) of the free drug and the complexed drug were compared.

[1] Monti DM, Ferraro G, Merlino A. Ferritin-based anticancer metallodrug delivery: Crystallographic, analytical and cytotoxicity studies. Nanomedicine. 2019, 101997.

[2] Massai L, Ciambellotti S, Cosottini L, Messori L, Turano P, Pratesi A. Direct detection of iron clusters in L ferritins through ESI-MS experiments. Dalton Trans. 2021, 50(45):16464-16467.