Surface Binding and Channel Transport of Gold(I) Compounds in Human H-Ferritin

G. Di Paco[1*], A. Giachetti[2], A. Rosato [1], P. Turano[1]

1 University of Florence, Department of Chemistry "Ugo Schiff", via della Lastruccia 3, 50019, Sesto Fiorentino, Italy

2 Consorzio Interuniversitario di Risonanze Magnetiche di Metallo Proteine, Via Sacconi 6, 50019 Sesto Fiorentino, Italy.

Human H-ferritin (HuHf) is a robust protein nanocage widely explored as a platform for targeted drug delivery. A distinctive advantage of HuHf as drug carrier is the enhanced cellular uptake of the cargo molecule in cancer cells that overexpress the Tfr1 receptor. Drugs can interact with HuHf with two main modalities: by forming adducts at the protein surface or by entering the HuHf cavity. The latter process can be spontaneous or require *in vitro* disassembly/reassembly of the nanocage.

In the case of gold(I)-based drugs, surface binding to the solvent-exposed C90/C102 cysteine pair generates structurally intact gold(I)–protein complexes [1]. Distinct ^{19}F-NMR signals from a nearby 5-fluorotryptophan reveal local conformational rearrangements upon gold coordination [2]. A particularly complex system is chlorido(1-butyl-3-methyl-imidazole-2-ylidene)gold(I) (AuNHC), which forms gold(I) complexes and at the same time enters the ferritin cavity, as suggested by structural and mass spectrometry data. The structural and energetic characteristics of the diffusion process through the C3 channel that leads to the cavity have not been yet characterized at the molecular level.

Here, we combine classical molecular dynamics, umbrella sampling, and ab initio parameterization to investigate the two complementary loading modes of gold(I)-based compounds with HuHf: covalent surface binding of Auranofin (AF) and Aurothiomalate (AuTM) to the C90/C102 pair [1,2], and passive transport of AuNHC and AuTM through the C3 channel [3].

Surface-binding simulations confirm stable coordination of AF and AuTM to the C90/C102 pair without perturbing the 24-mer quaternary structure, consistent with experimental mass spectrometry and spectroscopic data. The simulations also explain the distinct ^{19}F-NMR chemical shifts observed upon gold coordination by revealing how binding modulates the orientation and solvent exposure of the nearby 5-fluorotryptophan residue (W93).

Channel transport simulations provide a structural and a possible energetic landscape for the previously hypothesized translocation of AuNHC through the C3 pores. The small, cationic Au(NHC) spontaneously traverses the channel in multiple trajectories, whereas the bulkier, anionic AuTM is excluded and diffuses into solvent. Umbrella sampling simulations confirm a favorable free-energy profile scale for AuNHC translocation and a significant energy barrier for AuTM, demonstrating a clear charge–size selectivity mechanism.

Overall, these results show that HuHf supports stable surface conjugation whereas its C3 channel imposes selective constraints on molecular transport. We demonstrated how the integration of different types of MD simulation provides mechanistic insight into the interaction between gold(I)-

based drugs and HuHf. This provides a useful computational strategy supporting nanocarrier design for gold therapeutics.

[1] Cosottini, Lucrezia, et al. "Unlocking the Power of Human Ferritin: Enhanced Drug Delivery of Aurothiomalate in A2780 Ovarian Cancer Cells." Angewandte Chemie International Edition 63.40 (2024): e202410791.

[2] Ghini, Veronica, et al. "19 F NMR as a tool to probe drug binding and structural dynamics in ferritin-based nanocarriers." Materials Advances 6.18 (2025): 6337-6344.

[3] Di Paco G. et al. "Energetic Barriers to Gold(I) Complex Translocation Through the C3 Channel of Human Ferritin", in preparation

Financial support from the projects Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC, Acronym “ITACA.SB”