Biofabrication of Self-assembling Protein Nanomaterials through Histidine-templated Cysteine Coupling

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Nanoscale protein materials have gained significant importance in the fields of biotechnology and biomedicine due to their diverse applications in catalysis, drug delivery, and tissue engineering [1-3]. Protein oligomerization can be achieved through various engineering methods, such as the use of divalent cations to target histidine-rich regions [4], however, the success of cation-histidine binding is contingent upon factors such as protein structure, media composition, and the presence of chelating agents [5].

Therefore, we have developed a robust, cross-linker-free, and versatile platform for oligomerization, centered around histidine-templated cysteine coupling: a H6-derived His-Cys tag (H3C). This innovative hybrid peptide tag enables the spontaneous and efficient self-assembly of proteins into covalently bound nanoparticles. The resulting nanostructures are stabilized by the formation of disulfide bridges and can be readily disassembled using reducing agents, but not affected by chelating agents.

Moreover, the incorporation of cysteine residues into the tag does not compromise the metal-binding capabilities of the histidine residues. This particularity allows to keep the His-associated properties of one-step IMAC-based protein purification as well as the cation induced formation of higher-order microparticulate materials [6], serving as nanoparticle-releasing depots for protein delivery.

The dual interaction modes exhibited by the engineered H3C tag, along with the structural robustness and stability of the resulting nanoparticles make the biofabrication approach presented here broadly applicable for advancing in the development of novel therapeutic protein materials.

References

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Figures

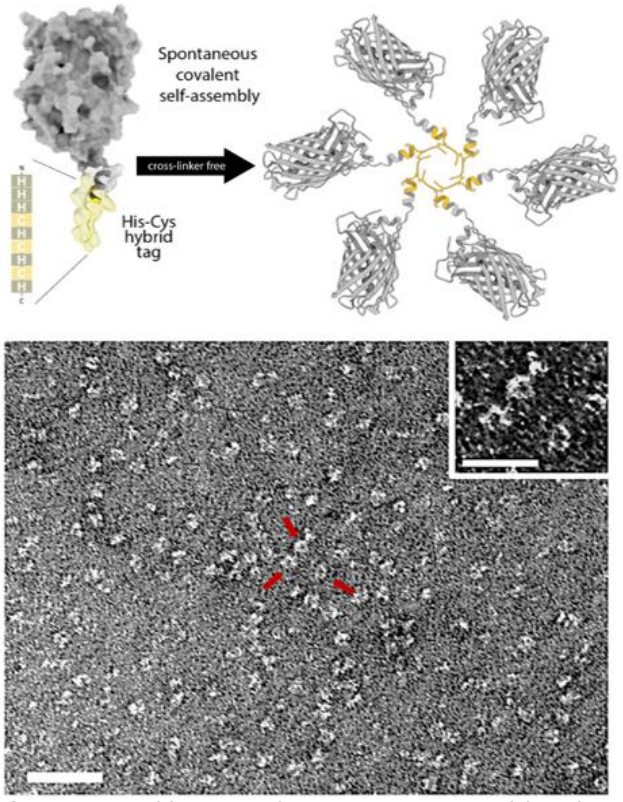

Figure 1. Architecture of GFP-H3C nanoparticles (Cys interaction in yellow) and it's morphometric analysis by transmission electron microscopy (TEM).

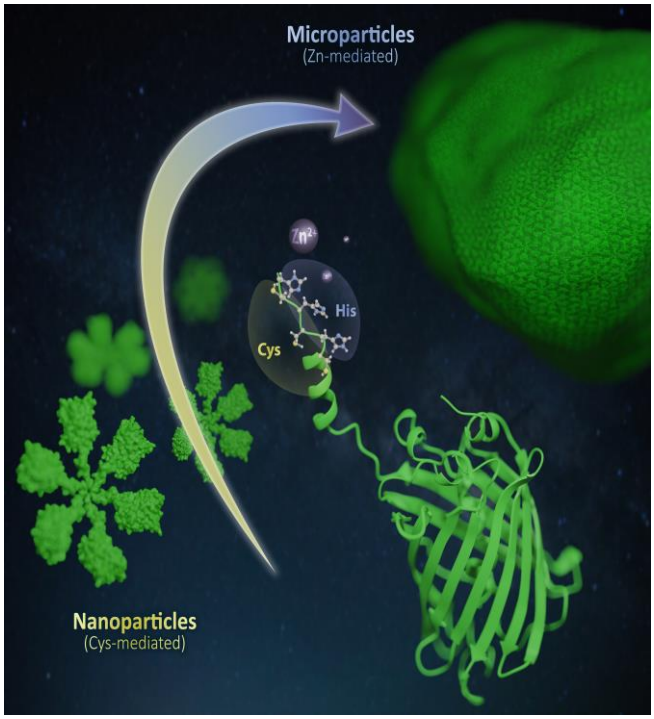

Figure 2. Versatility of the H3C tag. Formation of covalent hexametric nanoparticles through the histidine templated cysteine coupling process. The use of cations can then induce supramolecular organization into microparticle depots.