Controlling self-assembling and tumor cell-targeting of protein-only nanoparticles through modular protein engineering

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Abstract [1]

The use of proteins as drug delivery systems is gaining interest in nanomedicine, especially when dealing with targeted therapies which require specific interaction and penetration into target cells [2]. By genetic fusion, proteins with multiple biological activities can be synthetized as single-chain polypeptides. This way, just a unique step of recombinant production is needed to obtain the final modular protein, in which each domain plays a determinant and distinct role [3]. In this context, the combination of both cationic peptides and polyhistidines into a protein structure has been proven as a universal self-assembling protein platform [4]. The paradigmatic example of this is the modular protein T22-GFP-H6, in which the combination of T22 (a potent ligand of the tumoral marker CXCR4) and H6 promote the formation of protein-only nanostructures around 11 nm (above renal filtration cut-off) which selectively bind and internalize CXCR4$^+$ tumor cells *in vitro* and *in vivo* [5]. This makes T22-GFP-H6 and its related fusions appealing vehicles for selective drug delivery. In here, we study the relevance of T22 and H6 positioning inside the modular structure by performing a morphological and functional characterization of alternative fusion proteins in which T22 and H6 have been rearranged in different locations **(Figure 1)**. The results show that swapping modules crucially impacts on protein functionalities. For instance, we prove that positioning T22 at the amino terminus and H6 at the carboxy terminus is crucial for promoting protein self-assembly as oligomers (ten protein units each nanoparticle), whereas the alternative dispositions result in hydrodynamic sizes compatible with those from GFP monomers or dimers. Moreover, T22 must be placed in the amino terminus for allowing CXCR4$^+$ cell binding and specific internalization. Besides this, it has been demonstrated that the ability to oligomerize as regular protein nanoparticles increases cell penetrability in a cooperative way. When T22 is located at the N-terminus, proteins (irrespectively of being assembled as oligomers or not) enter CXCR4$^+$ cells by endocytosis, showing a punctuated pattern of fluorescence with perinuclear distribution in confocal images **(Figure 2)**. In contrast, when T22 is located in the C-terminus, proteins tend to accumulate in CXCR4$^+$ cell membranes, lacking the ability to internalize them **(Figure 2)**. Taken together, all these data teaches how to design smart multifunctional protein nanocarriers with feasible applications in targeted drug delivery, not simply for the treatment of CXCR4$^+$ human cancers but also for the development of pharmaceuticals against other diseases in which CXCR4 is a relevant homing marker, such as HIV.

References

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Figures

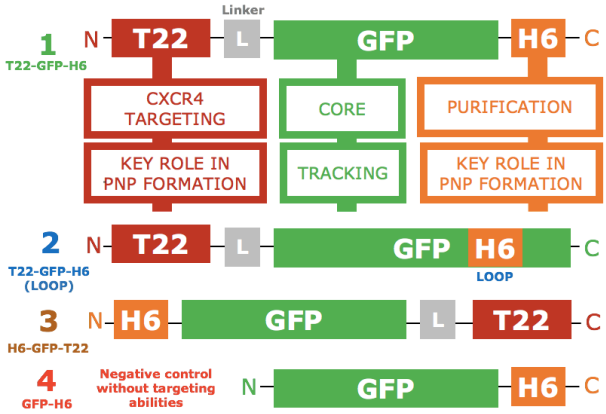

Figure 1. Modular proteins with alternative arrangements of T22, GFP and H6. Each module owns two key functions in protein functionality.

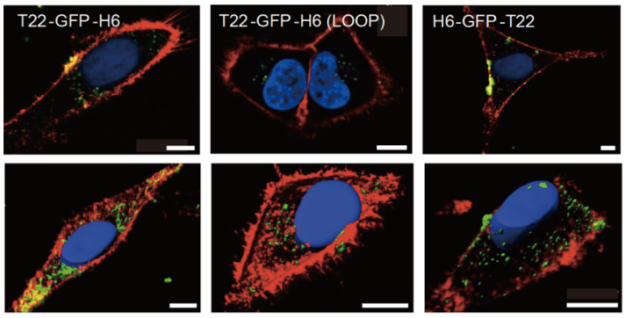

Figure 2. Intracellular location of proteins in protein-exposed cells. Red signal indicates labelled membranes, blue signal labelled nuclear nucleic acids and green signal is the natural green fluorescence of proteins. 2D confocal images and 3D reconstructions. Bars indicate 10^4 nm. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Science China Materials, Voltà-Durán E, Cano-Garrido O, Serna N et al. Controlling self-assembling and tumor cell-targeting through modular protein engineering (2019), https://doi.org/10.1007/s40843-019-9582-9 ©.