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Artificial repeat proteins evolved as habit modifiers and protein origami templates for the construction of gold-emitters hybrid nanostructures

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The construction of hybrid superstructures requires strategies to control the interfacial interactions. Precise recognition and high affinity are two intrinsic properties of natural proteins that can be exploited to drive the formation of hybrid assemblies. The challenge resides in the design and use of artificial proteins for material sciences purposes. For more than a decade, we have been constructing a family of billions of fully folded artificial proteins among which we are selecting specific members that display a high affinity and chemical robustness to create gold-nanoemitters superstructures by 3 possible strategies.

First, we demonstrate the *ab initio* design of a library of fully folded rigid and thermostable ankyrin-like artificial proteins (alpha-Repins) [1,2] and the evolutionary selection of high affinity protein pairs.[3] These proteins are grafted onto preformed gold nanoparticles or quantum dot with a controlled stoichiometry. The protein recognition and affinity drives the spontaneous formation fo hybrid assemblies upon mixing the two populations.[4,5] We will see how to control the topology of the assembly and the effect of the coupling on the optical properties of the hybrid.

Second, the evolutionary selection of alphaRep protein is applied to the discovery of proteins showing high affinity and specific binding for Au(111) crystal facets. These proteins are then used to control the morphology of gold nanocrystals by facet growth inhibition. [6] A seeded growth of gold nanocrystals in the presence of alpha-Rep reveals the exclusive formation of Au(111)-terminated nanostructures - icosahedrons, decahedrons and 2D nanoplates – in high yield, demonstrating the morphosynthetic efficiency of the selected proteins. Next, the protein-coated nanocrystals are coupled to active biomolecules conferring more functionality to the plasmonic nanocrystals such as biomolecule-driven self-assembly and surface confined catalysis. [6]

Finally, we are developing an alternative approach based on expanding the concept of DNA origami to proteins and so create shape-directing templates. We show how directed evolution of the alpha-Repin library leads to the selection of two artificial proteins that act as the supercoil unit and the staple to self-assemble into large helical superstructures. Their regioselective interactions are optimized to spontaneously form tubular protein origami superhelices as observed by cryoelectron microscopy. [7]

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[2] A. Urvoas, et al., *Trends in Biotechnology* **30**, 512(2012).
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[4] K. L. Gurunatha, K. L. *et al.*, *ACS Nano* **10**, 3176 (2016)
[5] M. Fernandez et al., *Nanoscale*, **12**, 4612 (2020)
[6] J. Prasad J., et al. *Nanoscale*, **11**, 17485 (2019)
[7] L. Moreaud et al., in press (2021)