CONTROL OF NANOMOLAR INTERACTION AND IN SITU ASSEMBLY OF PROTEINS IN FOUR DIMENSIONS BY LIGHT

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Light activation allows for complete control over conformation, function, interaction, and localization of molecules in time and space for in vitro and in vivo applications. With this aim, we have created a series of finely tuned photo-activatable multivalent trisNTA (PA-trisNTA) for in-situ labeling and assembly of His-tagged proteins [1].

Their design is based on the multivalent chelator head trisNTA connected through a peptidic linker to a histidine-tag sequence. The trisNTA is self-inactivated by the histidines in the presence of nickel ions due to the formation of an intramolecular complex. The activation is achieved by the inclusion of the photocleavable amino acid 3-amino-3-(2-nitrophenyl) propionic acid (Anp). Before illumination, outstanding self-inactivation prevents unspecific interactions, and after light-activation the protein interaction between PA-trisNTA towards His-tagged proteins is triggered over six orders of magnitude. The systematic decrease in the linker length from 11 to 5 amino acids formed a well-matched autoinhibited complex and the variation of position and number of Anp within the His-tag decreased its multivalency to prevented competition of the His-tag after photorelease. We also demonstrate the significant role of stereochemistry of Anp. The parameter optimization converged in the trisNTA-ACG-Anp(R)-G-H3-Anp(R)-H3, with superior autoinhibition and photoactivation properties.

PA-trisNTA functionalized hybrid hydrogels provided an excellent platform for in situ protein assembly with high x-y resolution by a photo-activating scanning laser (Figure 1). Extremely fast (ca. 140 µsµm⁻¹) photoactivation led to free-designed multiprotein arrays of different densities. This light-activatable lock-and-key facilitates biotechnological applications such as multi-protein arrays and triggering cellular pathways in time and space.

Figure 1. Protein assembly in PA-trisNTA functionalized hydrogel by mask patterning (red square) and in-situ 3D photo-activation using laser scanning microscopy (green ROI, snake).