Precisely integrating multiple different protein ligands into supramolecular assemblies

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Statement of Purpose: Supramolecular assemblies functionalized with folded protein ligands can provide biomaterials with unique biological or chemical properties when compared to assemblies displaying short, unfolded peptide ligands. However, the inability to integrate multiple different protein ligands at precisely defined molar ratios currently limits the full potential of these materials. Simple mixing of β-sheet fibrillizing domains terminated with different peptide ligands at the onset of assembly provides a facile route to supramolecular assemblies with modular and tunable ligand composition. These observations suggested that the same behavior should apply to mixtures of engineered fusion proteins having a β-sheet fibrillizing tail. To test this hypothesis, we created multiple different engineered fusion proteins having a β-sheet fibrillizing tail, herein referred to as a "βTail", and characterized their integration alone, or in combination, into nanofibers of synthetic β-sheet fibrillizing peptides. By eliminating the need to rely on short, unfolded peptide ligands, this approach is likely to provide biomaterials with unprecedented functionality that can be finely tuned according to the needs of specific applications, such as self-adjuvanting subunit vaccines.

Methods: The β-sheet fibrillizing peptides, QQKFQFQFEQQ (Q11), QQKFQFQFHQQ (HK-Q11), and FKFEFKFE (KFE8), were synthesized using a standard solid-phase Fmoc synthesis protocol. Origami B (DE3) E.coli having vectors encoding recombinant fusions of βTail or a non-folding βTail mutant (βTmut), a Ser-Gly linker, and Green Fluorescent Protein-UV (βTail-GFPuv), enhanced Green Fluorescent Protein (βTail-eGFP), or dsREDmonomer (βTail-dsRED) were used for expression. Following expression, E.coli was lysed into 1x PBS, and His-tagged βTail fusion proteins were purified from lysis buffer via metal-affinity chromatography. Neutral aqueous buffered solutions containing 1 mM peptide and 1 μM βTail-GFPuv, 1 μM βTmut-GFPuv, or 1 μM total of βTail-GFPuv/βTail-eGFP/βTail-dsRED were incubated overnight on a rocker table to induce microgel formation. Fluorescent microgels were visualized with an inverted epifluorescent microscope equipped with DAPI (for GFPuv), FITC (for eGFP) and Texas Red (for dsRED) filter cubes. Pseudocolor was applied using NIH ImageJ software. Total serum IgG from female C57BL/6 mice subcutaneously immunized with βTail-GFPuv either co-fibrillized with Q11 or without Q11 (dosing: 0.9 μmol day 0, 0.8 μmol day 31) was analyzed with ELISA.

Results: βTail-GFPuv and 1 mM Q11 co-assembled into fluorescent, micron-sized gels (Fig. 1A, left). On the other hand, incubation of a fusion of GFPuv having a non-folding βTail mutant with 1 mM Q11 provided micron-sized gels that were not fluorescent (Fig. 1A, right). βTail-GFPuv also efficiently integrated into nanofibers of HK-Q11 and KFE8 (Fig. 1B), two other synthetic, β-sheet fibrillizing peptides.

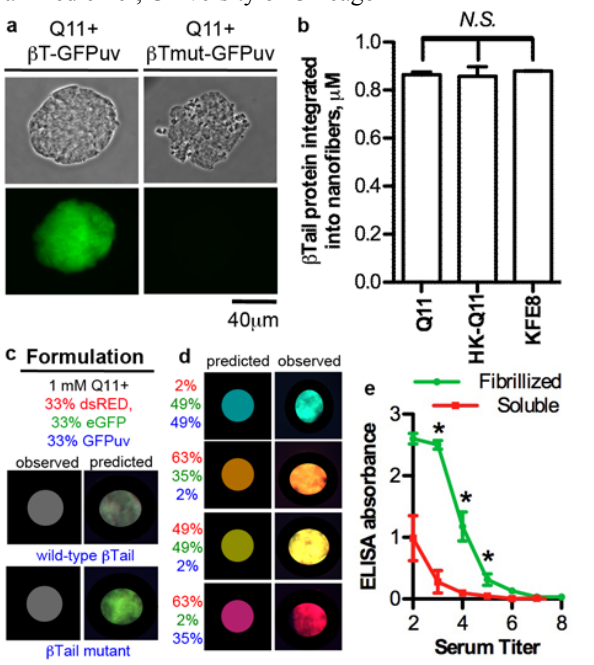

Figure 1: a) Bright-field and fluorescent images of 1 mM Q11 + 1 μM βTail-GFPuv or 1 μM mutated βTail-GFPuv microgels. b) βTail-GFPuv concentration integrated into 1 mM Q11, HK-Q11, or KFE8 nanofibers assembled in the presence of 1 μM βTail-GFP. c-d) Merged fluorescence images of 1 mM Q11 microgels formed in the presence of different fluorescent βTail proteins at varying mole ratios e) Anti-GFP total IgG in serum of C57BL/6 mice 7 weeks after primary immunization. (*$p<0.05$, T-test).

Merged fluorescence images of Q11 microgels formed in the presence of an equimolar mixture of βTail-GFPuv, βTail-eGFP, and βTail-dsRED were gray (Fig. 1C, top), which was consistent with the predicted color of an RGB image having 33% red, 33%green, and 33% blue pixels. In contrast, replacing βTail-GFPuv with a fusion having a non-folding βTail mutant resulted in fluorescent microgels that did not match the predicted color (Fig. 1C, bottom). Microgel color was precisely tunable by changing the mole ratio of βTail proteins in solution (Fig. 1D). Nanofibers having integrated βTail-GFPuv elicited robust anti-GFP serum IgG, whereas soluble βTail-GFPuv elicited weaker immune responses (Fig. 1E).

Conclusions: Fusion proteins having a β-sheet fibrillizing domain integrated into nanofibers of synthetic β-sheet fibrillizing peptides in a βTail-dependent manner. Multiple different βTail fusion proteins co-assembled into Q11 nanofibers, with each protein integrated at a precisely defined dose. Nanofibers with an integrated βTail fusion protein acted as self-adjuvanting vaccines. Together, these observations suggest that this versatile approach is likely to enable development of multi-antigen subunit vaccines with precisely tunable molecular composition.