Fluorogen-Activating Proteins as Retention Modules for Local Deposition of Antibodies

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Statement of Purpose: The goal of this work was to characterize a biomaterial system to localize and retain antibodies for sustained release *in vivo*. A bifunctional protein, named dL5_EAK, was constructed to possess the fluorogen-activating protein dL5 and the self-assembling peptide (SAP) AEAEAKAKAEAEAKAK (hereafter "EAK") [1]. dL5 is a single chain variable antibody fragment (scFv) light chain homodimer that has a specific affinity for malachite green (MG), a fluorogen. When bound in dL5, MG emits strong fluorescence [2]. We have previously shown that fibrils formed by self-assembly of EAK can accommodate peptides containing ionic complementary sequences [1, 3-6]. The hypothesis of this work was that EAK membranes containing dL5_EAK can display and localize IgG molecules via protein A/G conjugated with malachite green (pAG^{MG}).

Methods: dL5_EAK was expressed in Escherichia Coli using pET21 plasmid vector. Protein A/G was conjugated with MG isothiocyanate in basic buffers and purified using Sepharose resins. Assembly of dL5_EAK with pAG^{MG} in EAK membranes was confirmed using confocal microscopy. Localization of a near infrared dye conjugated antibody (IgG^{800}) in vivo was demonstrated in female BALB/c mice using optical imaging.

Results: The unique design hinges on engineering of a fluorogen-FAP into the SAP fibrillar networks in which the bioaffinity sites can be monitored. The fluorogen MG was conjugated to pAG as the linker between the dL5 domain in the peptidic fibrils and Fc of IgG molecules. We determined that intermixing EAK16-II with dL5_EAK at 400: 1 molar ratio resulted in 94% of the latter captured. As such, experiments described in the study were conducted with SAPs mixed at this ratio unless indicated otherwise.

The binding affinity of pAG^{MG} to dL5_EAK in the coacervates was determined based on MG fluorescence. SAPs mixed with varying concentrations of pAG^{MG} were incubated in phosphate buffered saline in assay plates and measured for fluorescence without washing steps because no signal above background was expected from MG outside of dL5. Titration of pAG^{MG} in dL5_EAK coacervates revealed the binding constant K_d = 90.9 nM. dL5_EAK and pAG^{MG} was kept constant at 1:1 molar ratio in subsequent experiments described herein. Addition of pAG^{MG} to EAK16-II did not yield fluorescence above the background. These data show that specific fluorescence generated from pAG^{MG} and dL5_EAK.

Deposition of a near infrared dye labeled antibody (IgG^{800}) in vivo (footpad) was demonstrated in mice using optical imaging and the retention is at least 3 days. SAPs dissolved in deionized water assemble into coacervates upon injection in vivo. To determine efficiency of the process in vivo, material components that included IgG^{800} were injected subcutaneously into footpad of mice and monitored optically. The data indicate that the gelation occurred rapidly in physiological environment and that IgG^{800} were retained with the coacervates at the injection site. Compared to injection of IgG^{800}, the dL5_EAK/EAK16-II/pAG^{MG} coacervates rendered superior retention of the antibody at the site of injection, reflected by the stronger fluorescent signal at the 800nm channel (Fig. 1). Strong MG fluorescence was also found localized at the injection site (red; 700 nm channel) of the complete system but not in the absence of pAG^{MG} (data not shown).

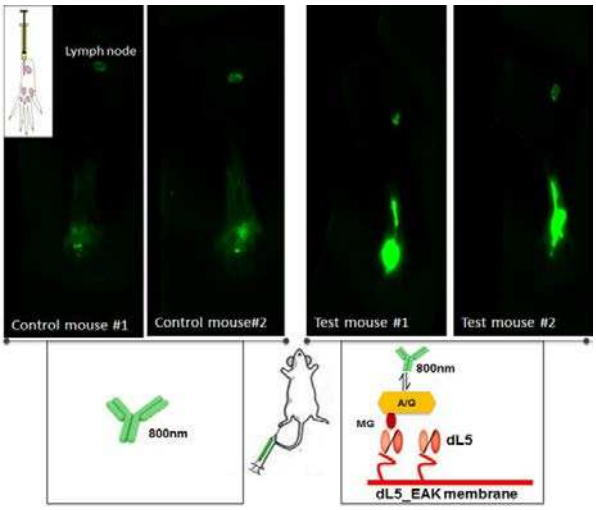

Figure 1 In vivo imaging of dye-labeled IgG injected with or without SAP materials

Conclusions: Introducing fibril-assembling capability transformed dL5 into a module through which *in vivo* applications can be developed. The self-assembling system of proteins can be formulated as injectable to render local disposition of therapeutic antibodies. Fluorescence generated from the MG-dL5 binding allows monitoring the physical integrity of the system.

References:

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