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Genetically targeted fluorogenic macromolecules for subcellular imaging and cellular perturbation

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ABSTRACT

The alteration of cellular functions by anchoring macromolecules to specified organelles may reveal a new area of therapeutic potential and clinical treatment. In this work, a unique phenotype was evoked by influencing cellular behavior through the modification of subcellular structures with genetically targetable macromolecules. These fluorogen-functionalized polymers, prepared *via* controlled radical polymerization, were capable of exclusively decorating actin, cytoplasmic, or nuclear compartments of living cells expressing localized fluorgen-activating proteins. The macromolecular fluorogens were optimized by establishing critical polymer architecture-biophysical property relationships which impacted binding rates, binding affinities, and the level of internalization. Specific labeling of subcellular structures was realized at nanomolar concentrations of polymer, in the absence of membrane permeabilization or transduction domains, and fluorogen-modified polymers were found to bind to protein intact after delivery to the cytosol. Cellular motility was found to be dependent on binding of macromolecular fluorogens to actin structures causing rapid cellular ruffling without migration.

1. Introduction

The modification of cells and proteins with macromolecules has shown significant clinical impact and therapeutic potential [1]. Poly(ethylene glycol) modifications, *i.e.* PEGylation, can inhibit the immune response to foreign proteins and cells, improve biomolecule solubility, increase protein stability and circulation lifetime, and prevent rejection of heterologous transplanted cells [1c]. Protein-polymer hybrids have been used in numerous applications, including uses as siRNA delivery vehicles, sensitive tags for fluorescence or mass-based detection, and as high-dose drug and radionuclide carriers [1d,2]. The effectiveness of these approaches and their ability to alter biological function in living systems is contingent upon the degree of specificity to which these hybrid molecules can be delivered to their target organism or subcellular structure.

Targeting and anchoring of macromolecules onto subcellular structures and organelles has been an ongoing scientific challenge and represents a new frontier in polymer-based engineering of biological systems [3]. To date, many effective approaches exist to target macromolecules within the body and to enhance their uptake by specific cell types using various “passive” and “active” targeting ligands [3a]. Macromolecule conjugated proteins, including antibodies and fragments [4], affibodies [5], nanobodies [6], and recombinant endogenous proteins [7] have been effectively used for cellular targeting within tumors and brain tissues. Macromolecules coupled to peptides, sugars, and folate molecules [8], as well as nucleic acid-type targeting ligands (*e.g.* aptamers) [9] have enabled receptor mediated targeting and uptake in specific cells *in-vivo*. In spite of these accomplishments, successful navigation of complex *in vivo* environments remains elusive, and targeting of macromolecular structures has been limited to cell-specific targets and cytosolic internalization. To our knowledge, targeting of polymeric materials to specific intracellular structures or subcellular

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organelles is not yet possible [3c].

Fluorogen-activating proteins (FAPs) and their cognate small molecule fluorogens provide a powerful imaging tool uniquely capable of homing small molecule probes to programmed locations within living cells. Fluorogen molecules, when in the presence of FAPs, specifically and non-covalently bind with strong host-guest binding affinities of *ca.* <1 nM accompanied by an incipient fluorescent activity [10]. In their unbound state, fluorogen molecules are fluorescently inactive which circumvents non-specific background signals and simplifies labeling procedures. A variety of fluorogen molecules have been developed based on unrigidized dyes including thiazole orange [11], dimethylindole red [12], and malachite green (MG) [10]. Fluorogen molecules can also be coupled with other augmenting molecules to provide supplemental imaging elements (*i.e.* "tandem-dyes") [13], stimuli-responsive diagnostic properties [14], or to enhance probe performance [15]. Among the aforementioned fluorogens, MG based fluorogens have been demonstrated to have excellent binding affinities to FAPs in the pM concentration range and to function within living cells [16]. To extend the functionality of this fluoromodule technology, we envisioned that this FAP-fluorogen tool could serve as a self-reporting molecular anchoring system to position macromolecular structures within cells (Fig. 1A–B). Similar to polymer-modified proteins *in vitro* and *in vivo*, the properties of cellular proteins fused with FAPs could be altered upon polymer binding thereby providing dials to influence cellular behavior (Fig. 1C).

Here, we present the use of a genetically encoded FAP combined with a new macromolecular platform to achieve specific and predefined targeting of the same polymeric materials to distinct subcellular structures within living cells (Fig. 1). Site-specific targeting was unequivocally demonstrated *via* live-cell imaging using stable cell lines expressing FAP-fusion constructs within actin, cytoplasmic, and nuclear compartments. Genetically targetable polymers, based on a fluorogen functionalized initiator and a controlled radical polymerization process, have nanomolar FAP binding affinities and cell membrane permeability under physiological conditions (Fig. 1A–B). Modification of the actin cytoskeleton *via* polymer binding shows pronounced effects on macroscopic cellular ruffling (Fig. 1C). To our knowledge, this work represents the first instance of a genetically targetable macromolecule that can be specifically delivered to subcellular compartments, visualized, and which perturbs cellular behavior. This approach can reveal details about subcellular targeting of polymeric materials and drug carriers, improve targeting and design of diagnostic and therapeutic biomaterials, and can provide a means to manipulate cellular behavior and function for applications in fundamental and synthetic biology.

2. Results and discussion

Prior to evaluating whether the FAP-fluorogen platform could be utilized as a macromolecular homing device, imaging agent, and cellular modification tool; the influence of polymer architecture on fluoromodule properties was first investigated. A series of narrow dispersity fluorogen-functionalized polymers was prepared to determine the effect of side-chain length and polymer backbone length on their biophysical properties when complexed with dL5** protein, *i.e.* the FAP. In order to establish these critical polymer structure-biophysical property relationships, controlled radical polymerization was employed to produce well-defined macromolecules from an assortment of monomers having predetermined number-average molecular weights (M_n) with narrow molar-mass dispersity values ($Đ_M \leq 1.3$) [17]. In other literature accounts, step-growth polymerization was used to synthesize conjugated polymers having broad dispersity values ($Đ_M \geq 1.8$) for live cell imaging; however, these systems lacked the required

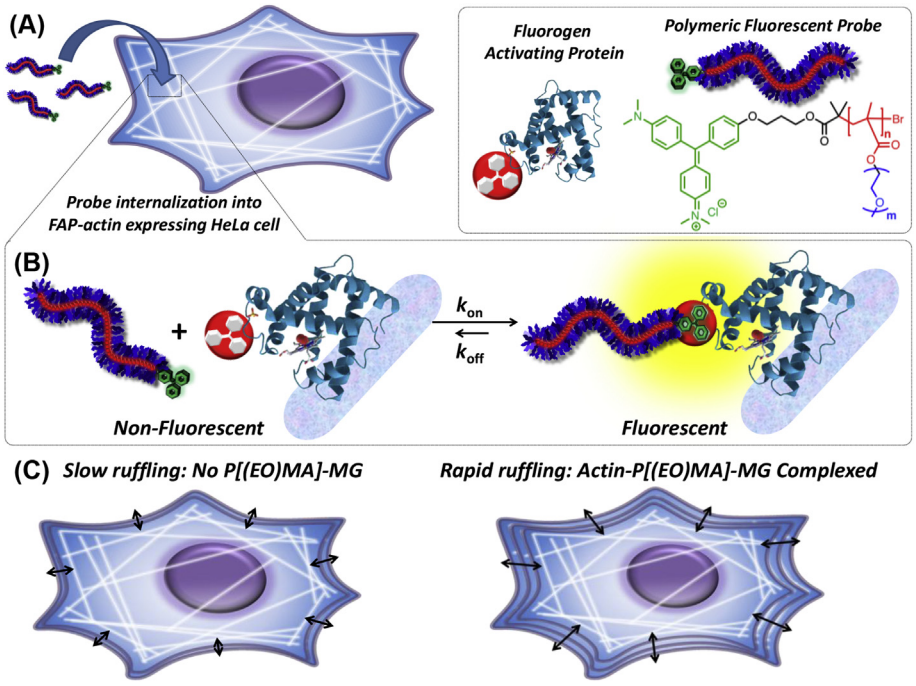

Fig. 1. (A) Illustration depicting internalization of a polymer fluorogen into a genetically targetable actin-modified HeLa cell. Legend and schematic breakdown of the polymer fluorogen consisting of MG (green), methacrylate backbone (red) with a DP of "n" repeat units, and ethylene oxide (EO) side-chains (blue) with "m" repeat units. Subsequent data in Fig. 3 and 4 are color-coded for clarity, where red figures indicated backbone DP variations and blue EO side-chain variations. (B) Polymeric fluorescent probe disassociated and complexed with a FAP representing its non-fluorescent and fluorescent states, respectively. (C) Illustration of binding polymer fluorogens to FAP-fused actin and their effect on cellular ruffling behavior. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)