Catch and Release: Designing Photocleavable Linkers for Attaching Antibodies (Abs) to Activated Polymeric Surfaces

Steven A. Soper, PhD

Abstract

Our group has been developing new tools (microfluidics) to select cancer biomarkers from blood samples *(i.e.,* Liquid Biopsy). These tools use Abs covalently linked to a surface to select markers from blood. The ability to release these selected markers, such as circulating tumor cells (CTCs), and cancer-associated nanoscale vesicles (exosomes) from the capture surfaces containing Abs without perturbing the targets' morphology, viability, and molecular content has been a major challenge. In spite of the challenge, compelling applications would result from the ability to capture and release the selected markers, such as securing molecular information for basic discovery and/or molecular diagnostics. We have reported a strategy to enzymatically release affinity-selected cells, such as CTCs, from surfaces with high efficiency (~90%) while maintaining cell viability (>85%).[1] The strategy utilizes single-stranded DNAs that link a capture Ab to the surfaces of a selection device. The DNA linkers contain a uracil residue that can be cleaved enzymatically. In this project, we plan to investigate the use of photocleavable (PC) linkers instead of using enzymatically cleavable linkers.

Overall Goal of Project

The PC linker employs a 7-(diethyl amino)coumaryl-4-methyl derivative (**Fig. 1**) that contains: (i) a central coumarin group that cleaves at the *meta* carbon, breaking the linker upon photoexposure; (ii) a primary amine with a short, 2-unit PEG spacer for EDC/NHS coupling to microfluidic devices; and (iii) a -COOH group for subsequent EDC/NHS activation and Ab immobilization. Rather than the more common o-nitrobenzyl PC group that cleaves upon UV exposure, which can damage cells and genetic material by reactive oxygen species or photon absorption, the coumarin PC group cleaves via non-invasive blue light (400-450 nm). Further, the linker's good cleavage quantum efficiency (0.25) coupled with inexpensive, high power LEDs (Light Emitting Diodes) outputting light (2×10^{18} photons/s) in excess of linker molecules on a microfluidic device (10^{11}-10^{14}) enables rapid (<1 min) biomarker release without any labile or costly reagents, thereby enabling time-sensitive clinical applications. Additional benefits are achieved by simplifying the immobilization chemistry for the PC linker and affinity-selection Abs, which both use EDC/NHS coupling and does not require Ab modifications that incur Ab losses during purification.

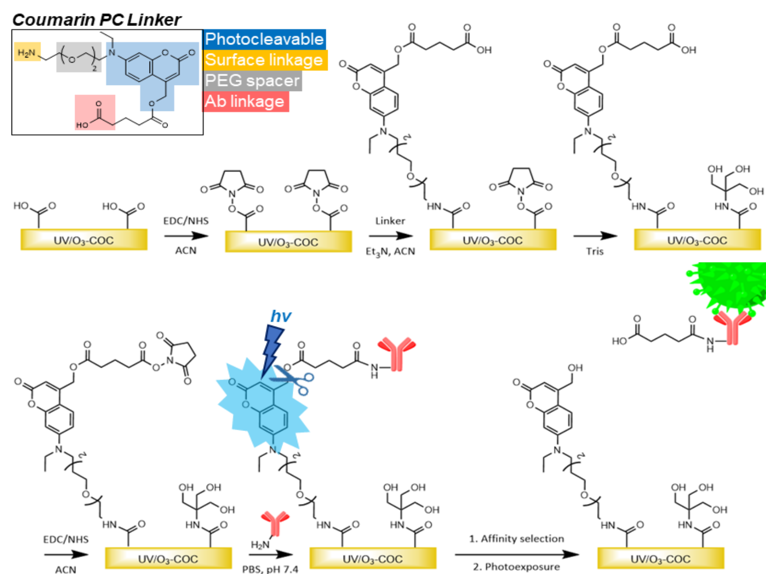

Figure 1. Design and reaction scheme of the heterobifunctional, PC linker. The linker's terminal primary amine attaches to COOH groups on UV/O_3-activated COC devices via EDC/NHS coupling. Any remaining, free NHS esters are quenched with tris buffer. The PC linker's COOH group is then activated with EDC/NHS reagents for antibody coupling, yielding a covalent linkage of the affinity-selection Ab to the surface through the PC linker. After affinity selection, isolated biomarkers (EVs or CTCs) are released by exposing the linker to blue light (400-450 nm), thereby cleaving the coumarin derivative at the *meta* carbon via a carbocation intermediate. Note the EDC/NHS reaction for immobilizing the PC linker is conducted in anhydrous solvent (acetonitrile – ACN) to avoid hydrolysis of the NHS ester, which could yield free COOH groups on the surface to which Abs could attach but not release by photoexposure.

The REU student will work with a graduate student in the Soper group to optimize the photocleaving efficiency of the PC linker using different LED intensities, capture cancer exosomes from cancer patient samples secured from the KU Medical Center and perform molecular analyses on their RNA content; looking for diagnostic mutations. The skills the REU student will garner from this project include microfluidics for clinical diagnostics, surface chemistry, and photochemistry. Instrumentation used in this study includes fabrication of plastic microfluidic devices using injection molding, DNA/RNA sequencers, microscopy, and FTIR.

1. Capture and Enzymatic Release of Circulating Tumor Cells, Soumya Nair, Joshua Jackson, Maggie Witek , V. Bae-Jump, P.A. Gehrig, W.Z. Wysham, P.M. Armistead, P. Voorhees and S.A. Soper, *Chemical Communications* 51 (2015) 3266-3269.