# Catch and Release: Designing Photocleavable Linkers for Attaching Antibodies to Activated Polymeric Surfaces

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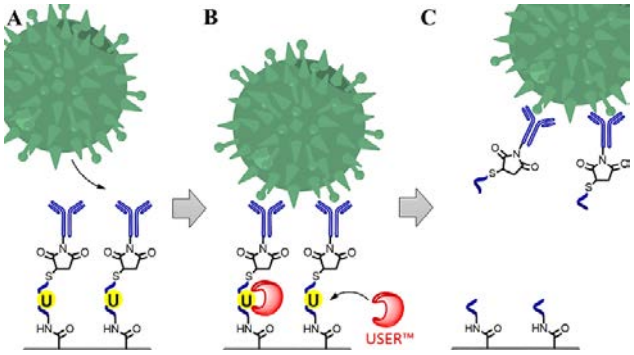
Our group has been developing new tools (microfluidics) to select cancer biomarkers from blood samples (*i.e.*, Liquid Biopsy). These tools use antibodies (Ab) covalently linked to a surface to select markers from blood. The ability to release these selected markers, such as circulating tumor cells (CTCs), 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 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 and exosomes, from surfaces with high efficiency (~90%) while maintaining cell viability (>85%); see Scheme 1.[1] The strategy utilizes single-stranded DNAs that link a capture Ab to the surfaces of a selection device. The DNA linkers contain an uracil residue that can be cleaved enzymatically.

In this REU project, we plan to investigate the use of oligonucleotide bifunctional linkers containing a unit that can be cleaved photolytically to release Ab-selected CTCs and exosomes. Bifunctional linkers can consist of single-stranded oligonucleotides of varying lengths and sequence content containing a primary amine at the 5' end and a thiol group at their 3' end. The substrate will be UV-activated to generate surface-confined carboxylic acids. Abs will then be reacted with a sulfo-NHS ester of succinimidyl trans-4 (maleimidylmethyl) cyclohexane-1-carboxylate (SMCC), yielding a maleimide-labeled Ab (SMCC-Ab). Once purified, the SMCC-Ab can be covalently attached to the reduced 3'-disulfide group (sulfhydryl) of a single-stranded oligonucleotide (ssDNA) linker immobilized to the activated surface using EDC/NHS coupling of the ssDNA linker's 5'-amino group to the surface-confined carboxylic acids (Fig. 1). The student will investigate the utility of this chemistry and use different oligonucleotide linker lengths and study their effects on CTC/exosome recovery. Using the optimized ssDNA linker, the REU student will investigate the ability to capture CTCs from clinical cancer blood samples and determine the efficiency of the photolytic release process and look at the effects of the UV cleavage reaction on the cancer cells.




**Scheme 1.** Cell selection and release assay. (A) mAbs immobilized to surfaces using oligonucleotide linkers containing a uracil residue are used for the positive selection of target cells. (B) Incubation of the selected cells and ssDNA linker with the USER™ enzyme system. (C) Removal of the uracil residue results in release of the selected cells.

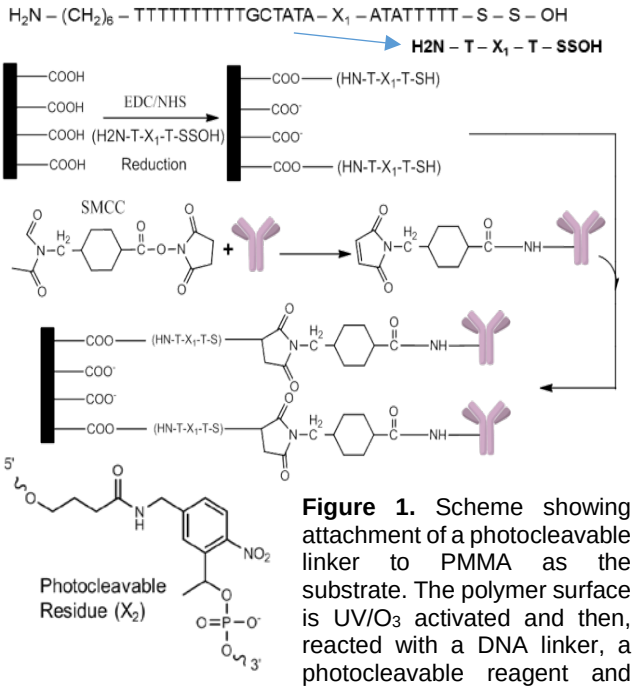



**Figure 1.** Scheme showing attachment of a photocleavable linker to PMMA as the substrate. The polymer surface is UV/$O_3$ activated and then, reacted with a DNA linker, a photocleavable reagent and finally, attachment to the mAb.

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.