595g Photopolymerization for Signal Amplification in the Detection of Biomolecular Recognition Events

Hadley D. Sikes, Ryan Hansen, Robert Jenison, Kathy Rowlen, and Christopher N. Bowman

Molecular recognition events such as antibody-antigen interactions and the hybridization of complementary nucleic acid sequences are increasingly used in clinical settings to aid in the diagnosis of disease. As the number of events to be detected decreases, target and/or signal amplification becomes necessary, and the difficulty and cost of detection increases. We have demonstrated photopolymerization as a new method of low-cost, rapid signal amplification that results in large gains and is suitable for point-of-care and field-portable diagnostic applications. The method relies on the localization of a number of photoinitiator molecules on a surface only in areas where molecular recognition has taken place. In the presence of an appropriate monomer solution and low-intensity, long-wave UV light, polymer films that are up to 10 microns thick and easily visible by eye can be grown from the surface-bound initiators. Macrophotoinitiators comprised of a water-soluble backbone with ~100 pendant photoinitiator substituents and 1 or 2 pendant recognition substituents (streptavidin) were synthesized and characterized; these macromolecules were used to localize initiators on surfaces as a result of molecular recognition. Monomer formulations and polymerization conditions such as the intensity and duration of initiating light were studied in order to maximize the amount of polymer formed on the surface. The method was demonstrated on microarrays on glass and silicon, and on the surface of polymer microfluidic devices. In order to quantify sensitivity, a chip containing an array of biotinylated oligonucleotides that decreased in concentration over five orders of magnitude was prepared. A visible amount of polymer grew from spots (d=600 microns) that had as few as ~1000 possible recognition events. Photopolymerization was compared side by side with enzymatic amplification methods that are used in commercially available optical immunoassays; photopolymerization proved to be more sensitive by at least two orders of magnitude.