Design and manufacturing of nanobiosensors suitable for protein detection

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Abstract

Different types of click chemistry reactions have been proposed and used for the functionalization of surfaces and materials, and covalent attachment of organic molecules. In the present work, we present and compare two different catalyst-free click approaches, namely azide-alkyne and thiol-alkyne click chemistry, for the generation of nanobiosensors suitable for protein detection. For this purpose, we first functionalized the surface of glass with dibenzocyclooctyne-acid (DBCO-acid), a cyclooctyne with a carboxyl group. Then, the DBCO-terminated surfaces were functionalized with different fluorescent and non-fluorescent azide and thiol inks via microchannel cantilevers spotting (µCP) (Figure 1). Click reactions were performed at different temperatures and times and the optimum conditions of 37 °C/20 min and 37 °C/40 min were found for azide-alkyne and thiol-alkyne reactions, respectively. Although, due to no need for catalysts or additional additives, mild reaction conditions, and high reaction rate, both routes worked reliable for surface functionalization, the protein binding experiments revealed that using a thiol-alkyne route will obtain the highest surface density of molecular immobilization in such spotting approaches. The obtained achievements and results from the protein binding experiments with streptavidin proved the potential for application of these microarrays in manufacturing nanobiosensors for protein detection and other biomedical/biological applications (Figure 2).

Keywords—*Biotin, Click chemistry, Nanobiosensor, Streptavidin*

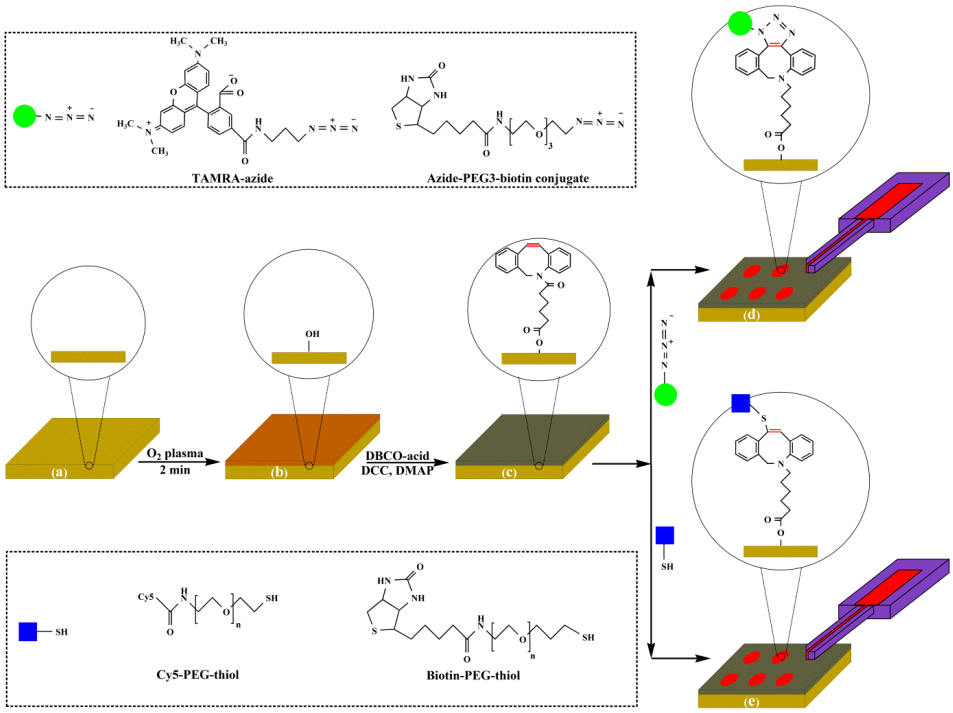


Figure 1. Comparison between azide-alkyne and thiol-alkyne click reactions: (a) bare glass; (b) hydroxyl-terminated glass; (c) DBCO-terminated glass; (d) treatment of the DBCO-terminated surface with TAMRA-azide and azide-PEG3-biotin; (e) treatment of the DBCO-terminated surface with Cy5-PEG-thiol and biotin-PEG-thiol.

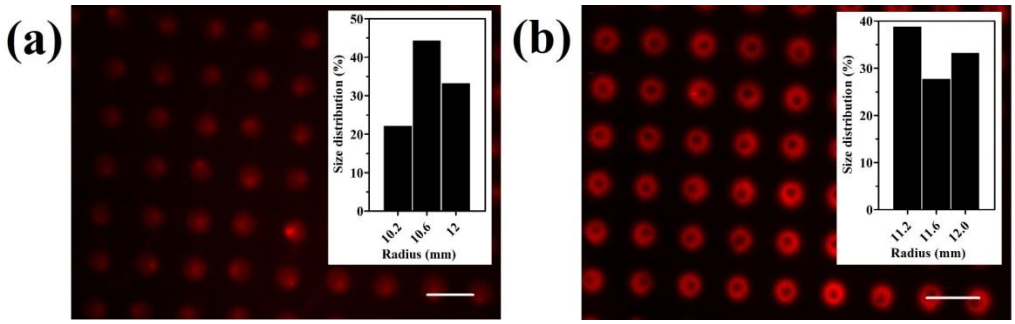


Figure 2. Fluorescence microscope images of microarrays of (a) azide-PEG3-biotin conjugate immobilized on the DBCO-terminated glass after incubating with streptavidin-Cy3, click reaction time = 20 min, click reaction temperature = 37 ˚C; (b) biotin-PEG-thiol immobilized on the DBCO-terminated glass after incubating with streptavidin-Cy3, click reaction time = 40 min, click reaction temperature = 37 ˚C. The insets show the size distribution of the spots. Scale bars equal 50 µm.

Professional Biography

S.M. Mahdi Dadfar has completed his Bachelor and Master of Science at Tehran University and Shiraz University, respectively, both top-tier universities in Iran. He is doing his PhD at Karlsruhe Institute of Technology (KIT), Institute of Nanotechnology (INT) under supervision of PD Dr. Dr. Michael Hirtz and Prof. Dr. Annie Powell. His PhD project is about “functionalized diamond optomechanical circuits for infrared spectroscopy and site-specific gas sensing applications”. He is a Member of “Iran’s National Elites Foundation” (The highest prestige and professional nation foundation for supporting elites). Mahdi Dadfar

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