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Improved Signal-To-Noise Ratio by the 3D-Functionalization of Fully Zwitterionic Surface Coatings

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Abstract : False outcomes of diagnostic tests are a major concern in medical health care. To improve the reliability of surface-based diagnostic tests, it is of crucial importance to diminish background signals that arise from the non-specific binding of biomolecules, a process called fouling. The aim is to create surfaces that repel all biomolecules except the molecule of interest. This can be achieved by incorporating antifouling protein repellent coatings in between the sensor surface and it’s recognition elements (e.g. antibodies, sugars, aptamers). Zwitterionic polymer brushes are considered excellent antifouling materials, however, to be able to bind the molecule of interest, the polymer brushes have to be functionalized and so far this was only achieved at the expense of either antifouling or binding capacity. To overcome this limitation, we combined both features into one single monomer: a zwitterionic sulfobetaine, ensuring antifouling capabilities, equipped with a clickable azide moiety which allows for further functionalization. By copolymerizing this monomer together with a standard sulfobetaine, the number of azides (and with that the number of recognition elements) can be tuned depending on the application. First, the clickable azido-monomer was synthesized and characterized, followed by copolymerizing this monomer to yield functionalizable antifouling brushes. The brushes were fully characterized using surface characterization techniques like XPS, contact angle measurements, G-ATR-FTIR and XRR. As a proof of principle, the brushes were subsequently functionalized with biotin via strain-promoted alkyne azide click reactions, which yielded a fully zwitterionic biotin-containing 3D-functionalized coating. The sensing capacity was evaluated by reflectometry using avidin and fibrinogen containing protein solutions. The surfaces showed excellent antifouling properties as illustrated by the complete absence of non-specific fibrinogen binding, while at the same time clear responses were seen for the specific binding of avidin. A great increase in signal-to-noise ratio was observed, even when the amount of functional groups was lowered to 1%, compared to traditional modification of sulfobetaine brushes that rely on a 2D-approach in which only the top-layer can be functionalized. This study was performed on stoichiometric silicon nitride surfaces for future microring resonator based assays, however, this methodology can be transferred to other biosensor platforms which are currently being investigated. The approach presented herein enables a highly efficient strategy for selective binding with retained antifouling properties for improved signal-to-noise ratios in binding assays. The number of recognition units can be adjusted to a specific need, e.g. depending on the size of the analyte to be bound, widening the scope of these functionalizable surface coatings.

Keywords : antifouling, signal-to-noise ratio, surface functionalization, zwitterionic polymer brushes
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