Binding kinetics and biological recognition of nanobodies functionalized on graphene derivatives for immunosensing applications

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The development of nanobodies for therapeutic and diagnostic applications has attracted the attention of a number of biomedical research groups due to their smaller size and ease of production on a large scale. They can selectively bind to a specific antigen like the conventional antibody but the size of nanobody molecule is much smaller, only at about 12–15 kDa while that of a common antibody is approximately 150–160 kDa. They are considered effective protein receptors because of their single antigen-binding site and fewer possible orientations compared to the conventional antibody, however, their bio-interface functionalization on solid platforms is still challenging. In this study, we have explored methods to functionalize nanobodies and graphene derivatives based on physical adsorption, amine crosslinking and streptavidin (SA)-biotin interaction. The graphene-based solid supports require functionalization with molecular receptors to promote specific binding to the target molecules. We have previously reported an effective method to obtain a film of denatured BSA (dBSA) on reduced graphene oxide (rGO) and its application in immunosensing [1,2]. We have also demonstrated the development and use of graphene-coated (G-) quartz crystal microbalance (QCM) sensor chips to study interactions between biomolecules and graphene surfaces [1], [3,4] and the application of G-QCM in clinical diagnostics [2]. The nanobody against lysozyme was used as a model protein pair. The QCM-D technique was used to monitor the adsorption of the nanobody on different surfaces, its biomolecular recognition and selectivity against other proteins and animal serum. rGO-biotBSA-SA was used as a sensing platform in this study (Figure 1). The kinetics dissociation (KD) factor was determined from the QCM-D results and compared with those from Surface Plasmon Resonance (SPR) for validation. This sensing surface exhibited good specificity and high sensitivity toward the target, with a detection limit of 0.5 µg/ml, and capable of detecting an analyte in serum media. The KD value obtained from this sensing platform is 545 nM which is comparable to the standard value from the SPR technique, validating its feasibility to be used as the basis of a highly selective and reliable immunosensing device.

References

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Figures

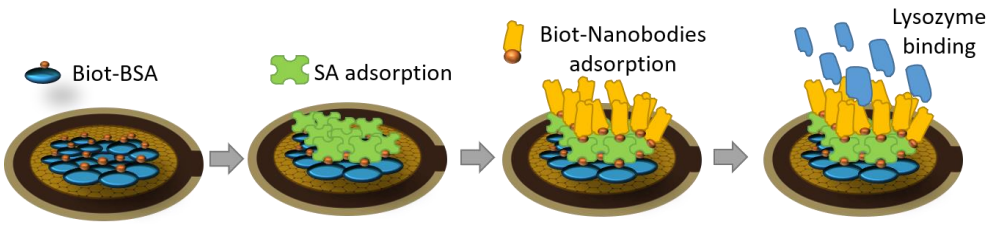

Figure 1: *Sensing surface preparation for quantifying lysozyme in cow serum. The gold electrode from a QCM crystal is coated with a thin layer of GO then thermally reduced. Injection sequences are shown.*