Calixcrown Deriviative as a Versatile Linker for Biochips with High Sensitvity and Oriented Immobilzation

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Protein microarrays have recently had an explosion of interest due to advances in proteomics, robotics, microelectronics, and bioinformatics. One important factor in fabricating protein microarray is to immobilize proteins without losing their activity on a solid phase. To keep them functional, it is necessary to immobilize proteins in a way that preserve their folded structural integrity. In this study, we compared the sensitivity and the specificity of the linker molecules with other protein attachment agents on glass slides using a prostate-specific antigen and its antibodies as a model system. The calixcrown-coated protein chip showed a superior sensitivity and a much lower detection limit than those chips prepared by other methods. We showed that the coupling mechanism of proteins to the Calixcrown is mediated by the ionized amine group of capture proteins. When we tested the capability of Calixcrown to immobilize antibody molecules, it appeared that Calixcrown makes arrangement of antibody be more regular with the vertical orientation than the covalent-bond agent. We also observed that the Calixcrown chip could be used for the diagnostic application with clinical samples from prostate cancer and HIV patients. Finally, we applied the Calixcrown chip using an antibody microarray to identify up- or down-regulated proteins in specific tissue and detected several up- or down-regulated proteins from a rat liver by administering toxin. Thus, the Calixcrown chip can be used as a powerful tool with a wide range of applications, including protein-protein interaction, protein-DNA interaction, and an enzyme activity assay.