342e Overcoming Mass Transport Limitations in Plasmon Resonance Biosensor

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Due to the small dimensions of flow cells in commercial BIAcore biosensor chips, only laminar fluid flow occurs in the microchannels of these sensors. As a result, the main transport of analytes to the ligand-immobilized chip surface where the binding reactions occur is facilited by molecular diffusion.Although shorter diffusion pathways are believed to enhance the efficient delivery of analytes to the surface-immobilized ligand binding sites, complicated folding structure and proportionately small binding site area in the proteins result in decreased efficiency of the reactions between ligand and analyte, especially in the microchannels. Consequently the surface plasmon resonance is unlikely to give a signal reflecting the true binding kinetics.

In this study, we used a multi-step approach that immobilizes a specific capturing molecule (anti-GST antibody) that binds to GST (glutathione S- transferase) moiety of the fusion protein carrying an ectoplasmic loop of erythrocyte band 3 protein termed GST5ABC. This approach generally helps detect the protein interactions between interacting partners with fewer binding sites encoded by less than 40 amino acids. Once the GST moiety is captured, the remaining portion of the fusion protein is exposed thus increasing which will increase the interaction opportunity of binding sites between captured protein, GST5ABC, and the analyte binding protein termed TrxMSP19. The TrxMSP19 is a recombinant fusion of the malaria parasite surface protein. This approach not only overcomes the mass transport limitation due to fewer binding sites, but also avoids the avidity effects of GST.

In our study, the calculation of the binding kinetic constants was performed at 5 ul/min, 30 ul/min, and 50 ul/min flow rates of TrxMSP19 analyte. The overall association constants and values of ligand binding capacity increase as the flow rate is increased. Finally, the temperature increase did not result in any significant difference in the overall association constants but the ligand binding capacity appears to be enlarged.