8. Analytical Systems for the Binding Interaction of Structurally Defined Oligosaccharides with Proteins/Cells: Use of Surface Plasmon Resonance (SPR) or Gold Nano-Particles (GNP)

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We developed an advanced method for the immobilization of oligosaccharides onto a gold-coated chip for surface plasmon resonance (SPR). Linker compounds containing intra-molecular cyclic disulfide were first designed. Oligosaccharides were then reacted with linker compounds using an optimized reductive amination reaction to afford ligand-conjugates. An attachment of the solution of each ligand-conjugate with a gold coated SPR chip formed spontaneous S (in the ligand-conjugate) – Au (chip) binding to prepare oligosaccharide immobilized chips (Sugar chips). Using sugar chips, oligosaccharide – protein interactions were systematically evaluated without labeling of protein. Sugar chips were further applied for SPR/MS (MALDI-TOF/MS) tandem analysis toward the identification of unknown target

To establish an on-site analytical tool, the above immobilization method was applied to gold nano particle (GNP). For example, a ligand-conjugate containing  $\alpha$ -D-glucopyranoside was reacted with GNP, followed by dialysis to prepare  $\alpha$ -D-glucopyranoside immobilized GNP (Glc $\alpha$ -GNP). The color of Glc $\alpha$ -GNP colloid solution (in PBS) was purple, showing plasmon absorption at 520 nm. When Glc $\alpha$ -GNP was incubated with Con A, the colloid solution became colorless as nano-particles were quickly aggregated. The change was detected visually. The aggregate was dissolved by adding excess D-glucose, and the Con A was quantitatively recovered, offering a quick purification of protein.