G-Protein-coupled receptors (GPCRs) embedded in native neuronal membranes receive signals from other neurons via molecular recognition of small-molecule neurotransmitters. Alterations in these chemical communication pathways between neurons have been associated with the causes and treatments of neurological and neuropsychiatric disorders. As a result, GPCR-ligand recognition has been investigated extensively. However, conventional assays using radiolabeled ligands to study GPCR-ligand complexes suffer from non-specific binding of protein targets to background matrices and laborious protocols to label each ligand for interrogating individual GPCRs in solution-phase assays. To address these challenges, our group employs self-assembled monolayers (SAMs) to tether small-molecule neurotransmitters to solid substrates for capturing protein receptors from solution as well as for selecting artificial receptors from combinatorial libraries. Surface chemistries are carefully controlled to reduce nonspecific biomolecule-substrate binding and to provide ample access for biomolecules to recognize surface-tethered neurotransmitters. We discovered that ligand conjugation chemistries employing ectopic functional groups are essential to preserving biomolecule recognition of native GPCRs.

We also developed a repertoire of patterning strategies to interrogate relative binding of biomolecules to ligand-functionalized and unfunctionalized regions of bioactive substrates. Microcontact insertion printing enables spacing tether molecules via stochastic insertion of tethers into SAM/substrate defects. Microfluidics enables generation of multiplexed substrates by spatially addressing multiple small-molecule probes. Chemical lift-off lithography relies on covalent interactions at the stamp/substrate interface to pattern surfaces via subtractive patterning. Since molecules are removed from surfaces, tether molecules can be backfilled into the negatively patterned regions on surfaces. Currently, we are optimizing surface functionalization strategies by investigating ligand conjugation to surface tethers under two conditions: pre-assembly conjugation vs. post-assembly conjugation. We find that the former showed consistent and improved binding to protein receptors compared to the latter. Our immediate goal is to use the optimized bioactive substrates to identify high-affinity receptors by screening nucleic acid combinatorial libraries. Once identified, these receptors will be used as molecular recognition elements in bioelectronic nanosensors to enable in vivo sensing of neurotransmitters at neural synapses.

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