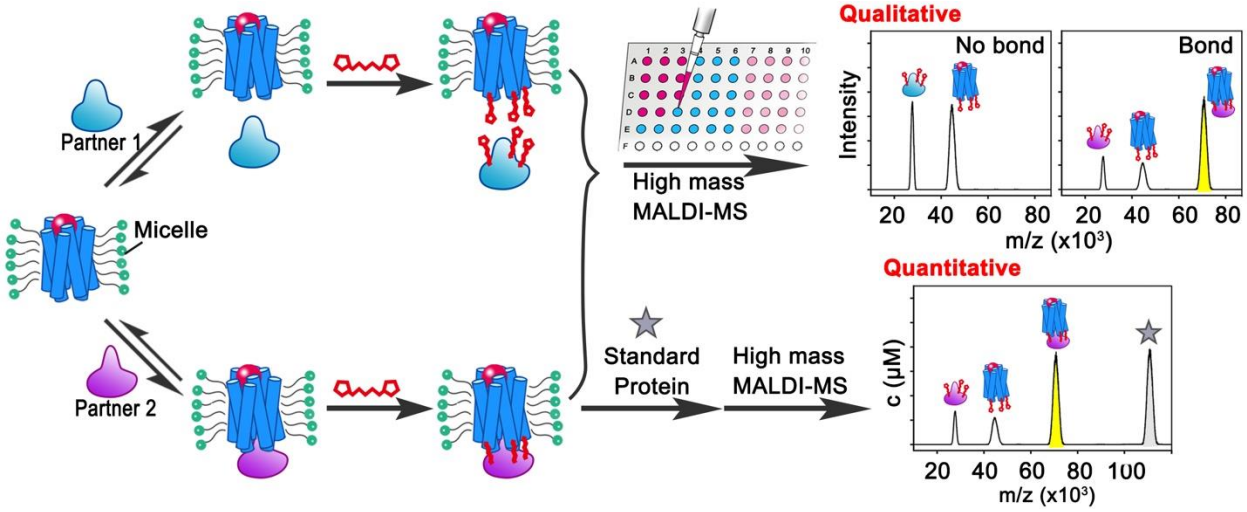
Mass Spectrometric detection of membrane-bound proteins (including GPCRs) and their noncovalent interaction partners

Membrane proteins, including G protein-coupled receptors (GPCRs), are important drug targets. Physiological effects are generally exerted by activating signaling cascades via allosteric activation by ligands. Although there are detailed structures of many membrane proteins, the effect of ligand on receptor selectivity for noncovalent interaction partners is still poorly understood.

We present a novel MS based method that combines chemical cross-linking of protein complexes with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and high-mass detection, which can report the effect of ligand binding by reading out the degree of membrane protein●partner protein complex formation. Using an internal standard, we are even able to measure the binding constant of these protein complexes using this methodology.

We assessed three class A GPCRs (rhodopsin, Rho; angiotensin II type I receptor, AT1R; and beta-1 adrenergic receptor, β1AR) and their selectivity to engineered, so-called "mini G" proteins upon ligand binding. We find that all tested apo GPCR can precouple to G_o protein, but that some ligands can bias receptor binding to a specific set of G proteins. This methodology also paves the way to high-throughput screening of possible GPCR targeting drugs.

A recent application to measuring the binding of SARS-CoV2-spike protein RBD●ACE2 receptor quantitatively, and the inhibition of this interaction by small molecule drugs will also be discussed.



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