

# Advances in infinite binding of proteins to targets

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Engineering the permanent formation of a receptor-ligand complex has a number of potential applications in chemistry and biology, including targeted medical imaging and therapy. These systems can be prepared by a combination of protein engineering and synthetic chemistry, for example using the site-directed incorporation of nucleophiles at the periphery of an antibody's binding site, paired with the chemical design of a weakly electrophilic ligand, to produce a receptor/ligand pair that associates efficiently and permanently. An exemplary system involving metal-DOTA complexes shows that this approach can lead to the straightforward production of infinite binding ligand-protein pairs beginning from weakly binding starting materials. In contrast to combinatorial strategies for strong binding, which seek binding sites with the best complementarity to a single structure, infinite binding of a set of structurally related ligands — such as a set of probe molecules — can be easily achieved. A greater challenge is engineering a tumor-binding single-chain antibody (scFv) to permanently attach to its protein target. We will describe progress toward this goal. Supported by NIH research grants CA016861 and CA098207, and NIH Shared Instrumentation Grant RR014701.

We have engineered a single-cysteine mutant of the rare earth-DOTA binding antibody 2D12.5, at a site proximal to the protein's binding site, so that weakly electrophilic metal complexes may bind and form permanent linkages. At 37 °C, pH 7.5, the complexes of (S)-2-(4-acrylamidobenzyl)-DOTA with all the rare earths, plus other metals including indium and copper, bind permanently to the mutant antibody, in yields that correlate with their relative binding affinities.

