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A Novel Monovalent Cation-Stabilized Fold Mediates an Aptamer-Protein Complex

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RNA aptamers are structured single-stranded oligonucleotides selected to bind tightly and specifically to a broad spectrum of biomolecular targets. The structural stability and diverse functionality of aptamers have enabled their use as diagnostic tools, inhibitors and potential therapeutic agents. However, since very few attempts at solving atomic structures of protein-aptamer complexes have succeeded, surprisingly little is known about how aptamers specifically bind to selected regions on the surface of proteins and cells. We show that aptamers can be effectively minimized for structural analysis using chemical mapping to experimentally define the secondary structure and identify tertiary contacts within the RNA and with the target protein. Ribonuclease and SHAPE mapping were used to determine the correct predicted secondary structure of a high affinity aptamer (Lys1) selected against lysozyme (KD ~ 30 nM). A deletion variant, minE (KD ~ 20 nM), was engineered to delete a long, apparently unstructured region. The lysozyme-minE complex was determined by x-ray crystallography at a 2.0 Å resolution, yielding a seventh RNA aptamer-protein structure. Solution hydroxyl-radical footprinting confirms the binding interface observed in the crystal. Although the minE aptamer interacts with a positively charged face of lysozyme, the electrostatic contribution to the binding free energy is minimal. The minE aptamer was found to inhibit the function of lysozyme in the standard cell-wall hydrolysis assay – a surprising result since the aptamer binding site is quite far from the catalytic site, and no structural differences between free lysozyme and that in complex with the aptamer could be detected. The long term goal of this study is to develop a systematic approach to aptamer minimization and use solved structures to probe the mechanisms by which RNA aptamers bind their targets and regulate catalytic activity and/or cellular function.

Keywords: RNA aptamer, Lysozyme, Inhibition