Using competitive selection and structurally informed pool design to select RNA aptamers with increased binding affinity.

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In vitro selection techniques (SELEX) have been used to identify many RNA and DNA sequences with specific, tight binding to target molecules. The best of these aptamer sequences have shown micromolar binding to small negatively charged molecules like ATP, and nanomolar binding to small neutral or positively charged molecules, and to more complex biomolecules like proteins. Aptamers are being pursued as therapeutic agents for many diseases, and also as diagnostic aids. The criteria for a diagnostic aptamer for a particular cancer, for instance, would be extremely tight binding coupled with exquisite selectivity for some molecular marker unique to that cancer. Thus, selection techniques that maximize the probability of finding an aptamer meeting these criteria are critical to the success of this diagnostic approach.

We report here the selection of RNA aptamers to GTP, using structurally informed pool design and a competitive selection process that enriches the population of very tight binders while simultaneously depleting the population of moderate binders. By including a stable hairpin into the middle of the pool, we increased by at least a factor of 10 the probability of finding certain types of aptamers. We used pre-elution with GTP to select against those aptamers with rapid off-rates. Long GTP soaks in the final elution stage ensured the enrichment of aptamers with slow off-rates. This combined approach resulted in at least five distinct classes of aptamers, several of which have dissociation constants of 25 – 50 nM, or about 25 times tighter than aptamers previously selected for the similar molecule ATP. These techniques should be useful in selecting for aptamers intended to diagnose cancers or other diseases.