

6. Summary

Insights into natural evolution can be gained from *in vitro* selection studies using RNA molecules (aptamers) that interact with high affinity with their target molecules. Developing an understanding of their binding mechanisms is critical for elucidating their structure-activity relationship.

In order to examine the binding mechanisms of RNA aptamers, optimal conditions for *in vitro* selection were first devised for isolating high-affinity aptamers. Eleven GTP aptamers with affinities spanning three orders of magnitude served as a model system for binding studies. These were isolated by a single *in vitro* selection experiment. Their structures were further optimized by conducting doped re-selections. Equilibrium dissociation constants were then determined by spinfiltration, methods were developed to determine the fraction of aptamer RNA that is correctly folded, and the informational complexity of each aptamer was calculated using information content as a parameter.

Studies of aptamer binding mechanisms with 23 GTP analogs showed that aptamers interacted strongly with the nucleobase region of GTP. Weaker contacts with the sugar region were observed, and only a few aptamers interacted with the phosphate region. Neither the number of contacts that an aptamer makes with its target nor the strength of the interactions appeared to have a major effect on aptamer performance. Aptamer size, free energy of secondary structure formation, RNA fraction correctly folded and information content are correlated however and thus crucial for achieving high-affinity binding.

By examining a variety of different aptamer parameters, important insights could be gained into aptamer binding mechanisms. Results of these experiments confirmed that the stability of the correctly folded aptamer and of the complex formed with GTP are the key factors for aptamer affinity.