2. Task

Elucidating the details of the binding mechanisms of functional biomolecules is crucial for understanding their structure-function relationship and the influence of different kinetic and thermodynamic parameters on their activity. The goal of this research project is therefore to examine the binding mechanisms of GTP aptamers and their target molecule by conducting the following experiments:

First, *in vitro* selection conditions will be optimized in order to isolate the desired high-affinity aptamers. A single *in vitro* selection experiment will then serve as the source of a well-selected population of aptamers performing a task with affinities spanning several orders of magnitude as the basis for a comprehensive characterization of aptamer binding mechanisms.

After generating mutagenized sequence libraries based on the minimal functional sequences of the aptamers, a doped re-selection will be performed to further optimize the structure of the aptamers. The mechanisms of interaction of the aptamers with GTP will then be studied in more detail by determining a number of different aptamer parameters: Equilibrium dissociation constants will be measured with GTP and several GTP analogs. Additional aptamer parameters such as free energy of secondary structure formation, fraction of correctly folded aptamer, number and intensity of contacts that the aptamer is making with GTP, and aptamer information content will then also be determined and compared in order to reveal the critical factors for aptamer interaction with GTP.

These investigations should provide a comprehensive profile of the binding mechanisms of GTP aptamers and their target to further deepen our understanding of aptamer specificity, target recognition, and the stability of the complex formed by an aptamer and its target.