Ligand-dependent Aptamer Design

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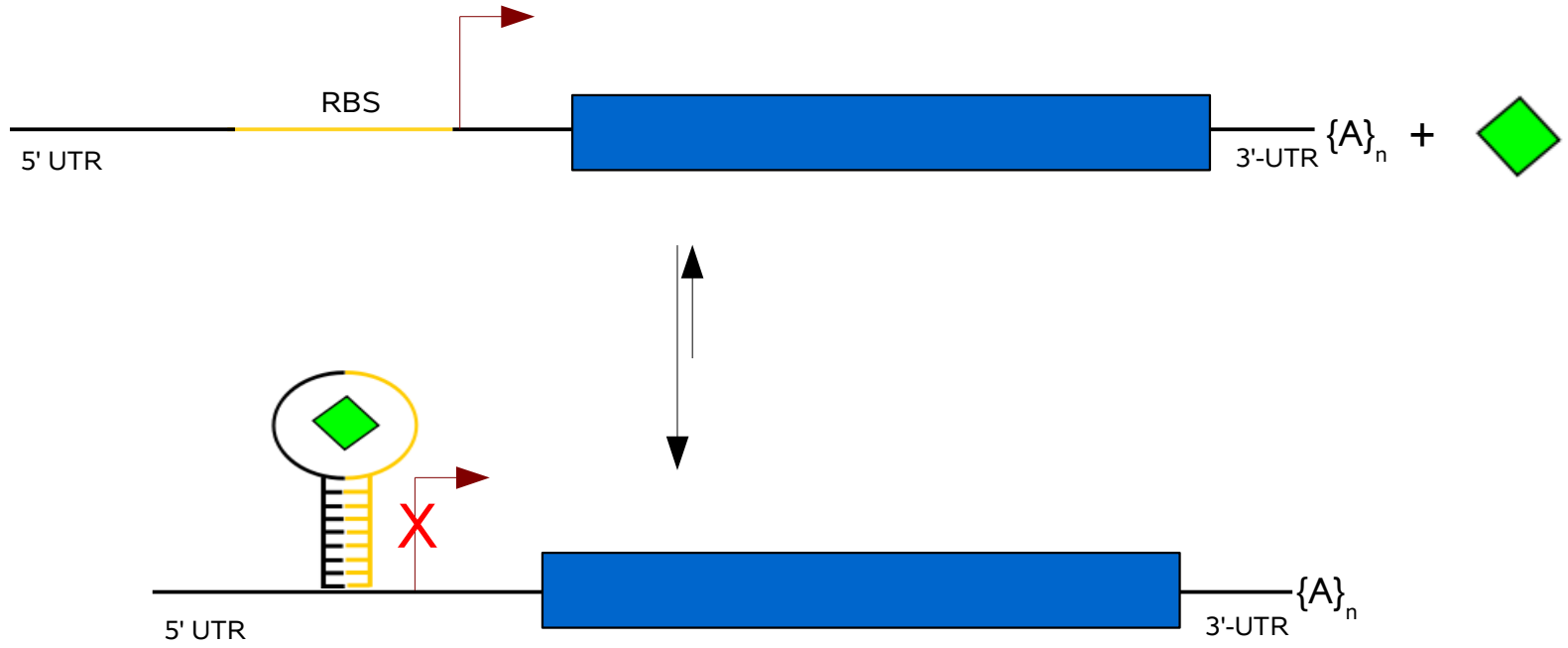
TBI Winterseminar 2011
Bled

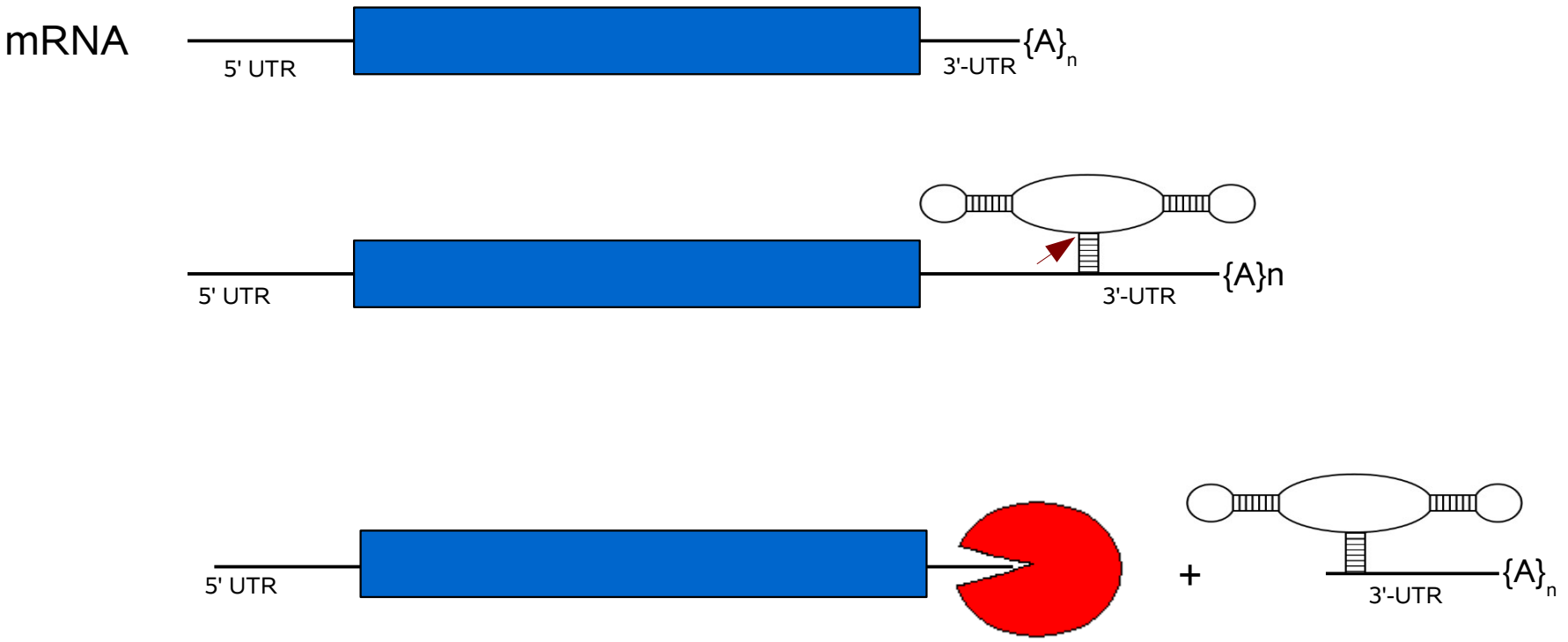
Outline

- Aptamer structures and constructs
- Conformational analysis of RNA
- Sampling
- Ligands
- Virtual Screening / Docking / MD-simulation

RBS-Aptamer Construct



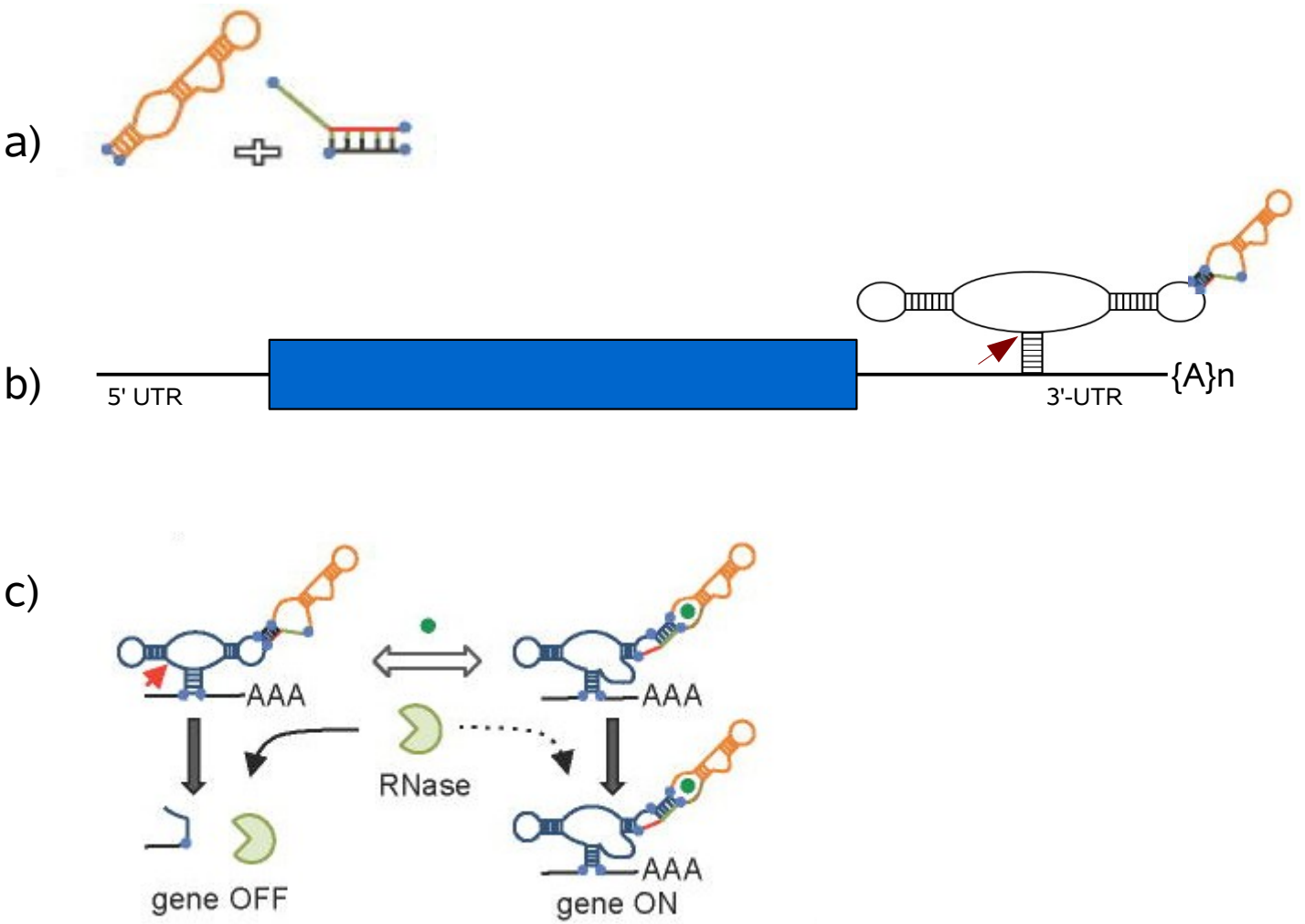
Aptamer Ribozyme Construct I



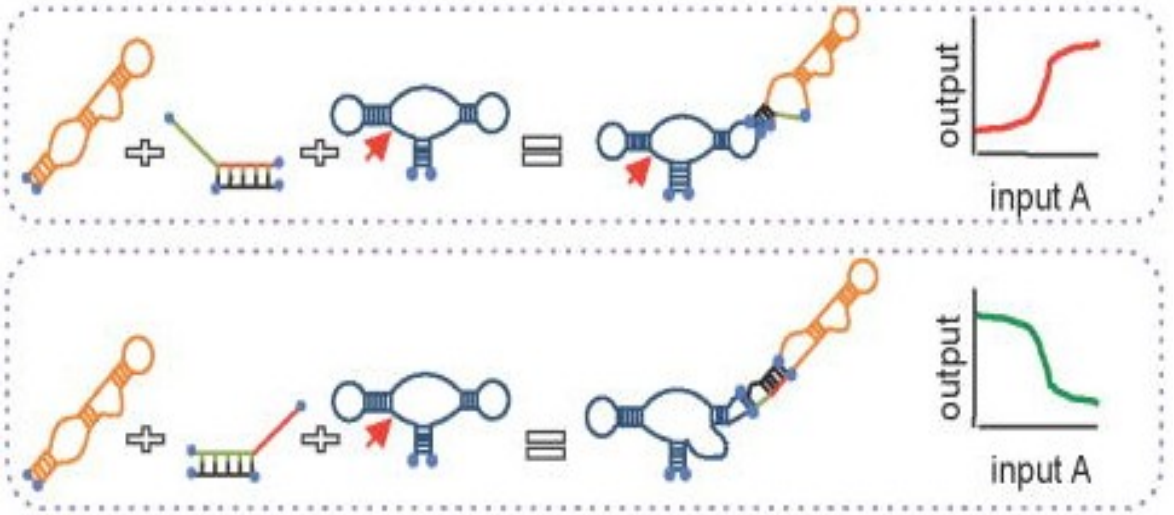
Beisel CL, Smolke CD **Design Principles for Riboswitch Function**. PLoS Comput Biol;(2009);5(4)

Win MN, Liang JC, Smolke CD. **Frameworks for programming biological function through RNA parts and devices**. Chem Biol;(2009);16(3)

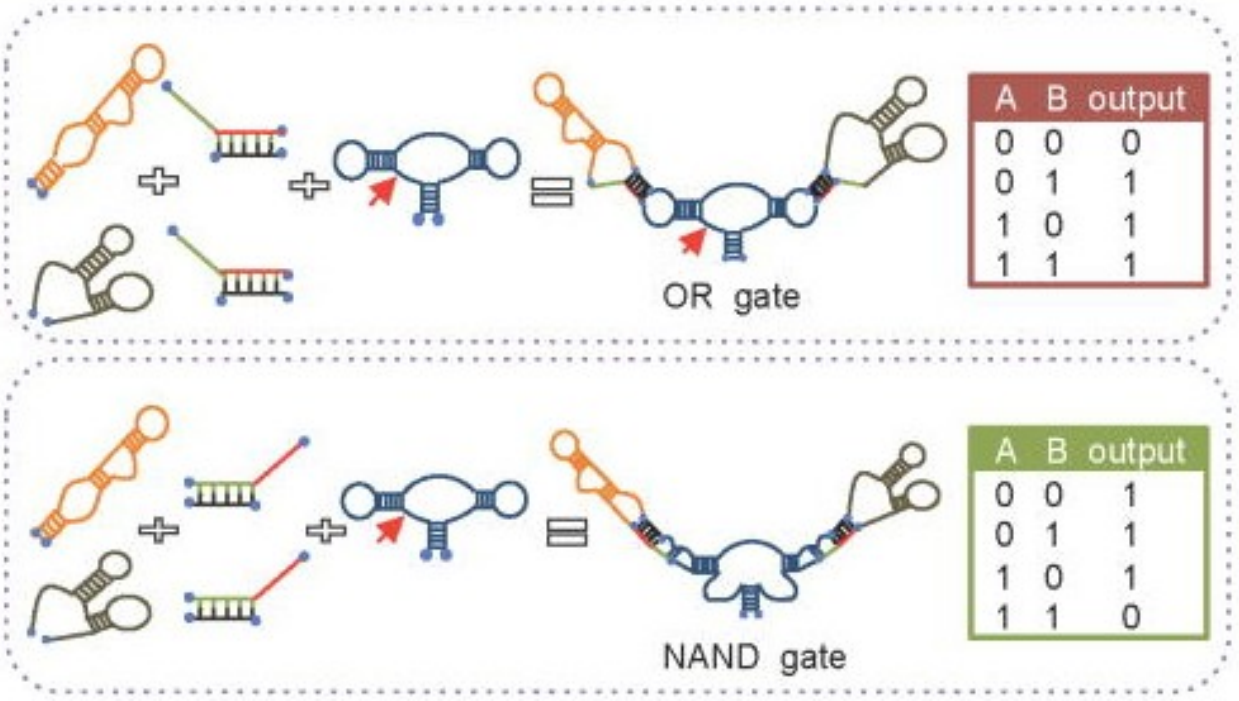
Aptamer-Linker-Ribozyme Construct II



Aptamer-Linker-Ribozyme Construct III

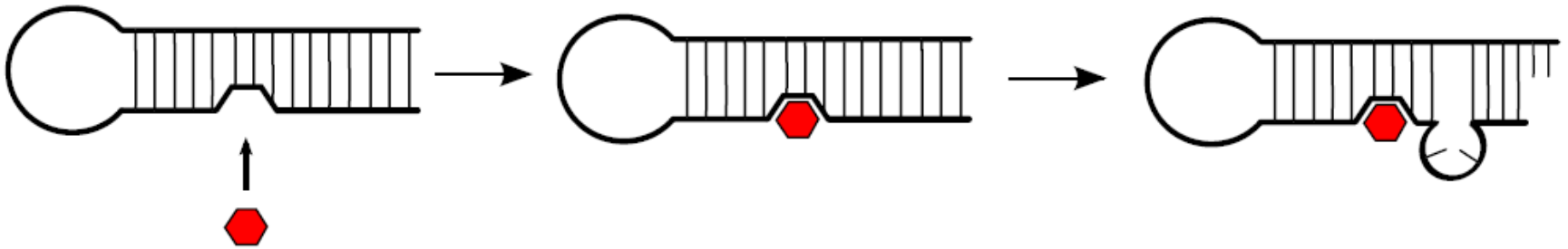


Aptamer-Linker-Ribozyme Construct IV



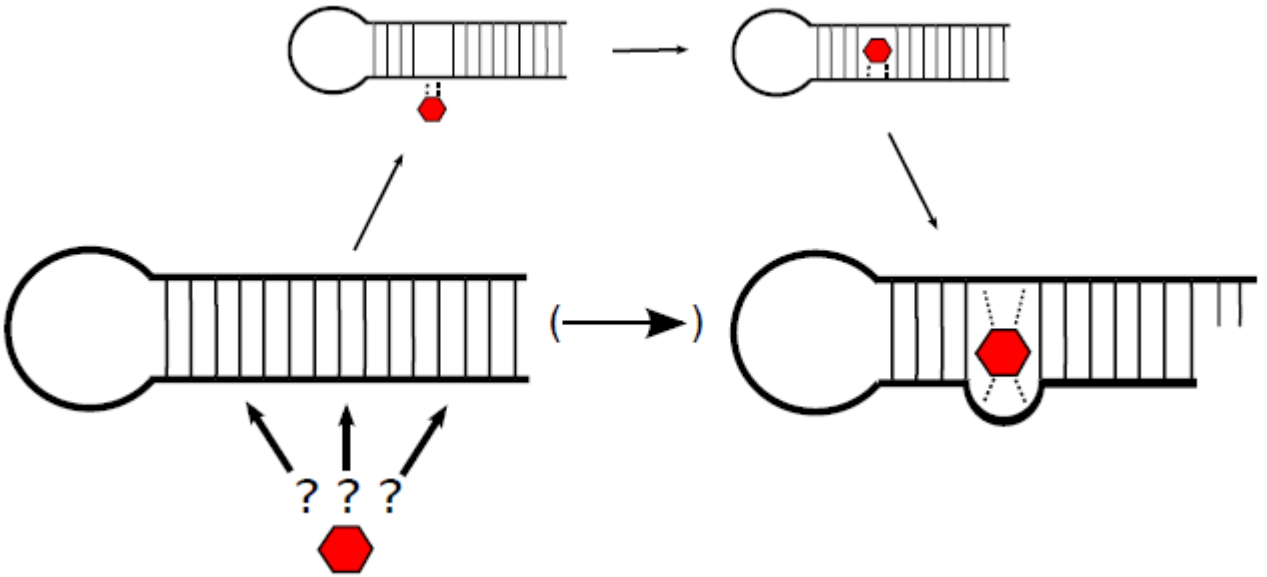
Aptamer Structures

- **Aptamer-Structure with a „binding pocket“**



Aptamer Structures

- „Induced fitting“ of a ligand into interaction site



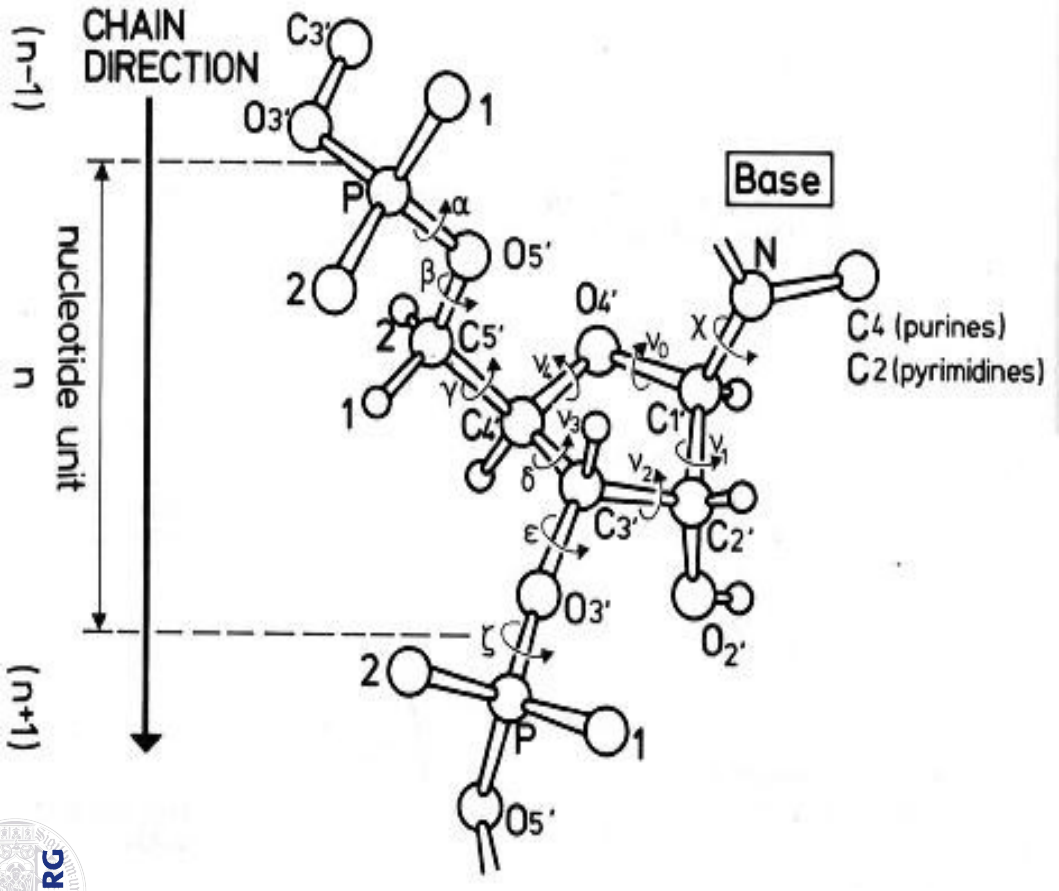
Aptamer Structures

- Macromolecules with the ability to undergo confomational change on:
  - ligand/structure - binding
  - temperature (ds dehybridization $\leftrightarrow$ hybridization)
  - salt-concentrations?

- ~3400 Aptamer structures derived from **SELEX** method
  (**S**ystematic **E**volution of **L**igands by **Ex**ponential Enrichment)
- SELEX method is restricted to aptamer size of n ~ 100 nt
- SELEX method tends to generate „simple“ structures (mostly 1J, 2J and 3J)
- Cost intensive

Conformational Analysis

- **Basis : Conformational information of RNA**
  - ~1900 structures containing „RNA“ from the PDB
    - Extraction of RNA atoms, semi-automatic chain curation



Single nt measurement:

- Intra: bondlengths, bondangles and torsion angles, sugar phase angle and base orientation within a single nt
  ~1067010 data points (37D)

- Inter: same, but also values ranging into the adjacent nt
  ~1047630 data points (43D)

Outlook:

Concideration of context:

- Measuring 3nt or 4nt as fragments
- Base orientation and distance description

Conformational Analysis

Finding „Building Blocks“

Mono nt:

- Reduce the single sample process by drawing together groups of variables
  - Parts of the backbone
  - Sugar
  - Base

Oligo nt

- Get a sense of the context of the neighboring nt, to be able to sample nt with information about the neighborhood, or previously sampled nt
- Statistical overview of the base orientation of the „next“, resp „previous“ nt

Sampling

Ahead:

- „raw“ structure sampling {building blocks, atoms}
  - size exclusion of vdW-radii
- Refinement by adding all atoms, which were neglected in the first round (e.g. Hs, OPs)
- Evaluation of the structure by a force-field (e.g. AMBER-ff or CHARM-ff)
  - $E(S) = E_{bonds} + E_{angles} + E_{torsions} + E_{vdW} + E_{coulomb} + ...$
- Using swarm optimization or other optimization algorithms to
  - min (E(S))
- -> Generate single structures or a library/libraries of potential aptamer structures

Ligands – collecting datasets

- **Design of new ligands following de-novo drug design principles**
  - Use a „working“ RNA aptamer structure for the design of a collection of potential compunds
- **Prepare libraries of already existing ligands**
  - Use collections of compunds, which are namely bio-available but show no bio-active function yet (ligand-recycling)
  - Use collections of compounds, which already have a functional assignment

Virtual Screening (VS) / Docking

- **Find a aptamer structure, which binds to a certain molecule**
  - Using a collection of known or newly sampled aptamer structures and test all against a single ligand
- **Find a ligand which is able to bind to a certain aptamer**
  - Using a collection of wanted ligands, which should be taken into consideration and test a single aptamer structure against all ligands
  -
- **Difficulty: Aptamer structure performes conformational change**
  - Problem: Induced fitting of the ligand into the aptamer structure

MD - simulation

- Determination/ identification of „stable“ and working aptamer structures
- Validation of VS/Docking experiments

→Derivation of binding mechanisms
→Derivation of mechanisms for conformational changes

Thank you!