Sequence-controlled RNA self-processing: computational design, biochemical analysis and visualization by AFM

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Abstract

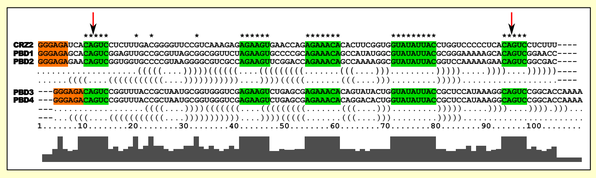
Naturally occurring small self-cleaving ribozymes such as the hairpin ribozyme can cleave and re-ligate target RNA backbones without the requirement for any protein machinery. Their self-cleaving property led to the construction of efficient regulators of gene expression, their self-ligation allows to form circular RNA or to join two RNA molecules. Using the Vienna RNA package, we designed four RNAs (PBD1-4) that incorporate the catalytic core of the hairpin ribozyme with the goal to produce RNA that predominantly circularizes or extends its own length (self-polymerizes) upon transcription. The designed ribozyme should first cleave off its 5' and 3' end to produce reactive ends and then ligate these ends to form concatemers or circular RNA. In vitro results confirm that all our designed ribozymes show variations of self-circularization and self-polymerization. Thereby, our results give important feedback for further design approaches that simulate the kinetics of self-processing systems in order to allow accurate prediction of ribozyme behavior.

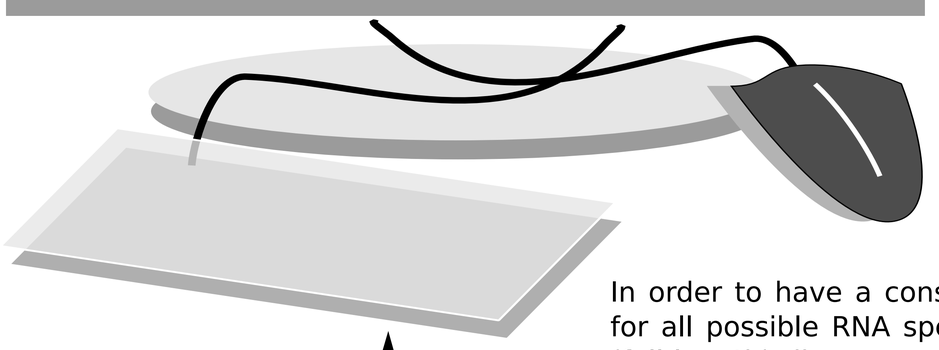
RNA design - Stefan Badelt

⇒ compute a set of candidate molecules (switch.pl)
⇒ maximize probabilities to form reactive conformations
⇒ differ between probabilites to form active monomers and dimers

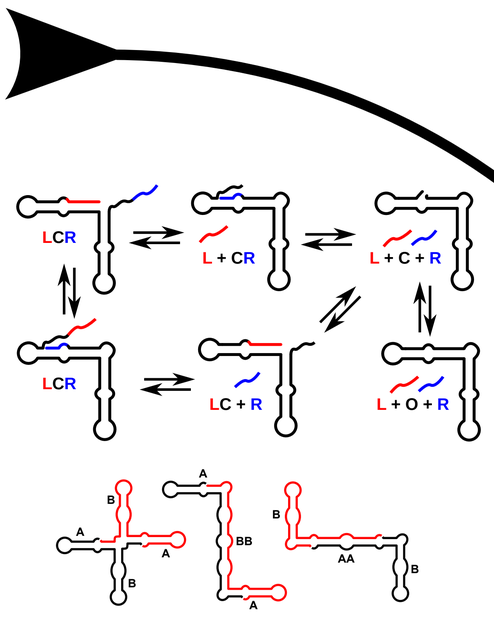
$$\kappa_1 = \left(\min \begin{cases} P(LCR^L), \\ P(CR^R) \end{cases} + \min \begin{cases} P(LCR^R), \\ P(LC^L) \end{cases} \right) \times e^{\frac{E(C^O)-E(O^C)}{kT}}$$

$$\kappa_2 = \frac{[LCR_{\text{dimer}}]_\theta}{[LCR_{\text{monomer}}]_\theta} \times \left(\min \begin{cases} P(LCR^L_{\text{dimer}}), \\ P(CR^R_{\text{dimer}}) \end{cases} + \min \begin{cases} P(LCR^R_{\text{dimer}}), \\ P(LC^L_{\text{dimer}}) \end{cases} \right) \times e^{\frac{E(C^{O_2}_{\text{dimer}})-E(O_2^{C_{\text{dimer}}})}{kT}}$$

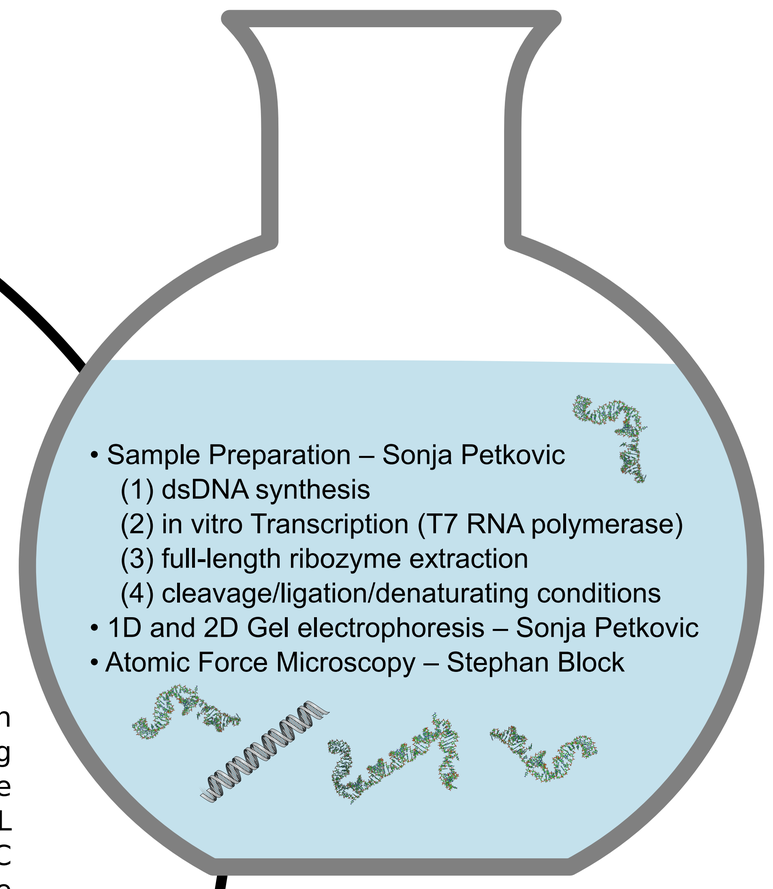




⇒ In order to have a consistent, length-independent annotation for all possible RNA species that can emerge from a starting (full-length) ribozyme, we introduce the following notation: We denote the 5'- and 3'-ends of the full-length molecule as L (left) and R (right), respectively, and the linear core as C (center). An initial ribozyme species therefore consists of three parts and can be abbreviated as “LCR” molecule. Additionally we introduce the term O for the circular version of C.



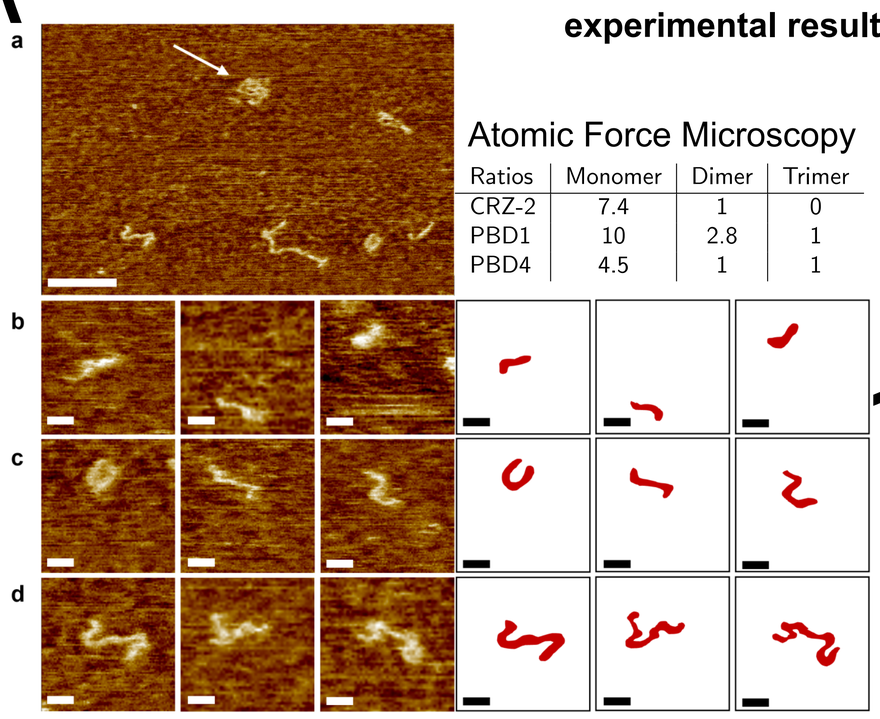
experimental setup



Objective

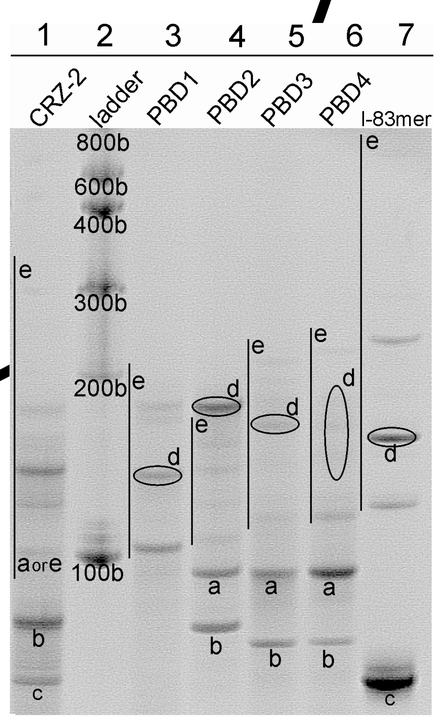
We compared five different self-processing ribozymes. CRZ-2 is based on the naturally occouring hairpin ribozyme sequence, PBD1-4 are optimized by base replacements in all non-conserved regions. The sequence optimization functions shown above were applied to design ribozymes that form predominantly circular monomers (PBD2, PBD4) or dimers (PBD1, PBD3).

experimental results



Atomic Force Microscopy

Ratios	Monomer	Dimer	Trimer
CRZ-2	7.4	1	0
PBD1	10	2.8	1
PBD4	4.5	1	1

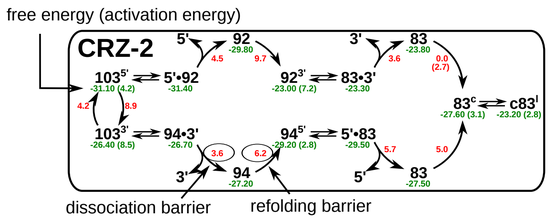


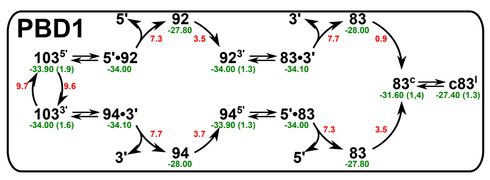
Results

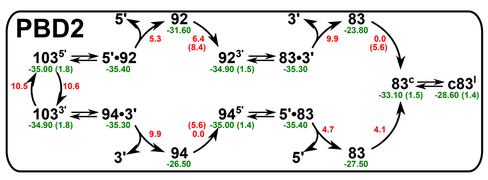
Denaturing PAGE shows a plethora of different reaction products: (a) full length ribozyme, (b) one end cleaved, (c) both ends cleaved, (d) circular monomer and (e) different kinds of multimeric species. Circular monomers were identified with 2D PAGE (not shown). Semi-denaturing AFM images were used to measure the length of single RNA molecules, enabling us to cluster them and characterize their self-polymerization tendency.

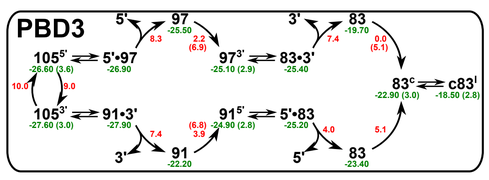
Discussion

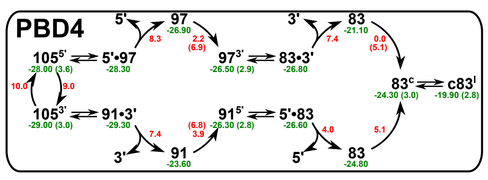
Comparing experimental results with theoretical prediction, shows agreement as well as contradiction. Designed molecules are generally more prone to self-polymerization, but PAGE shows increased amounts full-length molecules for PBD2-4. With a detailed analysis of the monomer cleavage cascade for each molecule we could show that kinetic traps which were not considered in our design approach can explain experimental findings and have to be considered in future design approaches.











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