Computational design of a circular RNA with prion-like behavior

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Abstract

Sophisticated computational methods exist for the conformational design of RNA molecules and they have enabled the design of a multitude of functional RNA devices in the field of synthetic biology. Here we present for the first time an RNA design that mimics the behavior of prions, i.e. sequences capable of interaction triggered auto-catalytic replication of conformations. Our design was computed with the Vienna RNA package and is based on circular RNA that embeds domains amenable to inter-molecular "kissing interactions."

Introduction

During the last decade, the field of Synthetic Biology has impressively illustrated that nucleic acids and in particular RNA molecules are reliable materials for the design and implementation of functional circuits as well as nanoscale devices and objects (Guo, 2010; Khalil and Collins, 2010; Afonin et al., 2013; Ishikawa et al., 2013). The reasons for this success are grounded in the facts that for RNA (1) an experimentally measured energy model exists (Mathews, 2006) (2) regulation at the level of RNA molecules is faster than via the production of proteins and (3) design questions are readily expressed in the discrete framework of binary base-pairing than in continuous interactions between, e.g., the amino acids in proteins.

The "protein only hypothesis" of the scrapie agent (for a review see Aguzzi et al. (2008)) proposes that a prion protein, with an altered (misfolded) β-sheet-rich conformation, starts a catalytic cascade which uses the normally-folded prion proteins as a substrate, converting them to the misfolded form which can self-assemble into fibers. A high activation energy between the normal and the misfolded conformation prevents spontaneous conversion at detectable rates. The formation of a normal-misfolded heteromeric complex may lower the activation energy barrier to convert the normally-folded protein into a misfolded species. This conversion leads to further recruitment of normally-folded proteins in an auto-catalytic process. In essence, a single misfolded prion protein in a population of normally folded ones is enough to convert the whole population via auto-catalytic structure replication into an all misfolded protein population which self-assembles into long fibers.

This contribution was motivated by the question whether RNA molecules can be designed *in silico* to exhibit the aforementioned prion-like behavior. We show that it is indeed possible to design such an "RNA-prion"; whether the suggested sequence really shows the exponential refolding characteristics awaits experimental verification.

The RNA prion presented here is a 49nt long, circular RNA, designed as a bistable molecule. It thermodynamically favors one structure ($S1$) if present as a monomer and the other structure ($S2$) upon increase of its concentration. $S2$ is designed such that it forms two 10nt hairpins prone to form kissing interactions as reported for the HIV-DIS loop, the genomic RNA dimerization site (Ennifar et al., 2003), a highly conserved stem-loop sequence found in many retroviruses. Importantly, $S2$ should not only stabilize other $S2$ conformations at higher concentrations, but actively lower the energy barrier to refold $S1$ into $S2$.

We used the program `switch.pl` (Flamm et al., 2001) of the `ViennaRNA package` (Lorenz et al., 2011) to design many bistable molecules. On the sequence level we constrained the loop regions of conformation $S2$ to form a stable kissing interaction. The chosen sequences for interaction have similar free energy to the best kissing interaction examples shown in Weixlbaumer et al. (2004), but differ in point mutations to be compatible with structural constraints for $S1$. Importantly, in $S1$, the kissing interaction does not form a regular helix, but the strands are shifted relative to each other (see Figure 2). This asymmetric design enables $S2$ to open a shorter helical region that has a worse free energy than the subsequent formation of the kissing interaction.

Results

Out of 128 molecules that fulfilled the structural constraints, two showed a very large difference between the energy needed to open a kissing receptor strand and the free energy gain of the duplex interaction with $S2$. One of these molecules was chosen for detailed analysis and its switch-

ing behavior modeled as described below.

Figure 1 shows the equilibrium between the two structures as a function of RNA concentration. The relative concentrations of monomers $[M]$ and dimers $[D]$ can be computed by the equilibrium partition function. Let Z_M and Z_D be the equilibrium partition functions of the Monomer and two kissing Monomers (Dimer), respectively. We can compute Z_M using the McCaskill Algorithm (McCaskill, 1990) implemented in RNAfold of the Vienna RNA package (Lorenz et al., 2011). Z_D we compute as

$$Z_D = Z_{c1} \cdot Z_{c2} \cdot Z_{dup}, \tag{1}$$

with Z_{c1} and Z_{c2} denoting the partition functions of two monomers under the constraint that the first (c1) or second (c2) interaction region is unpaired and thus available for forming a inter-molecular (kissing) interaction. Z_{dup} is the partition function of the inter-molecular duplex formed between the two molecules. This model follows the assumption that dimerization can only involve an interaction between the strands of the kissing interaction.

Since we are interested in the conformations formed upon monomerization and dimerization, we divided the total partition function Z_M into three parts: Z_{S1}, Z_{S2} and Z_o. Z_{S1} and Z_{S2} contain all conformations constrained to form base-pairs that can only be formed in structure $S1$ or $S2$ respectively, whereas Z_o contains all other conformations, i.e. conformations that are not compatible with both constraints. Constraints are chosen such that (i) the helices formed by $S1$ and $S2$ are preserved and (ii) there are no structures fulfilling both constraints. We computed the relative concentration of structure 1 ($[S1]$) in Monomers and Dimers as:

$$[S1] = \frac{Z_{S1}}{Z_M} \cdot [M] + \left(\frac{Z_{S1+c1}}{Z_{c1}} + \frac{Z_{S1+c2}}{Z_{c2}} \right) \cdot [D] \tag{2}$$

where Z_{S1+c1} stands for a partition function that has both the constraint to fold into structure 1 ($S1$) and the constraint to be unpaired in interaction region 1 ($c1$). Relative concentrations of $S2$ were computed accordingly.

Next we computed refolding paths and thus the energy barrier for refolding between $S1$ and $S2$, either for a single RNA monomer or an RNA engaged in kissing interaction with another molecule. Due to the short length of the RNA, the problem of finding the best refolding path can be solved exactly, using the program `barriers` (Flamm et al., 2002).

Figure 2 shows the energy profiles resulting from these paths. Since $S1$ is the thermodynamically favored state in monomers, we show the refolding path from $S2$ (-10.70 kcal/mol) to $S1$ (-12.70 kcal/mol) in the top panel. The barrier of this refolding path is 16.70 kcal/mol, making a non-induced switching of conformations unlikely.

The bottom panel of Figure 2 shows the energy profile for a scenario where an inter-molecular interaction is first formed between one molecule in conformation $S1$ and a

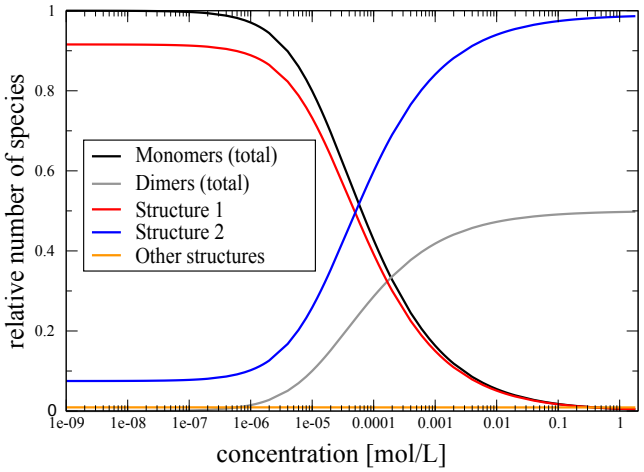

Figure 1: Conformational switching upon change of concentration. The transition from monomer to dimer conformations at around 10nM goes together with a switch from structure S1 to S2.

second in $S2$, followed by intra-molecular refolding of the first molecule from $S1$ into $S2$. $S2$ is now the favored conformation, since it is stabilized by the kissing interaction. In contrast, $S1$ is destabilized since one helix cannot be formed together with the inter-molecular duplex. Theoretically, there would be a second possible duplex interaction which requires $S1$ to open eight base-pairs in two helices, but since this interaction is not thermodynamically favored, it is not depicted in Figure 2.

According to Weixlbaumer et al. (2004), the energy of a 6nt kissing interaction can be computed from the energy of a regular RNA duplex with an additional bonus of -4.20 kcal/mol. For the initiation of the kissing interaction, we calculated the worst-case scenario, where all competing intra-molecular base-pairs of $S1$ have to open first and then the inter-molecular base-pairs can form. This results in an energy barrier of 6.40 kcal/mol and leads to a new local minimum conformation at -29.70 kcal/mol. For the intra-molecular refolding from $S1$ to $S2$, we compute the refolding path given that the kissing-strand is not available for intra-molecular base-pairing, resulting in a barrier of 13.60 kcal/mol. Note that the refolding path of $S1$ to $S2$ has to overcome the same barrier conformation as the pathway from $S2$ to $S1$, however, the kissing interaction destabilizes $S1$ and therefore lowers the relative energy barrier. A limitation of the energy evaluation is that the loop entropies of strands involved in the kissing interaction are ignored. The hairipin loop of $S2$ as well as the interior loop of $S1$ are evaluated as if they were unpaired and the energy contribution of the duplex (-12.20 kcal/mol) is added.

As an alternative approach, we modeled the kissing interaction as a regular intra-molecular helix. Both molecules are

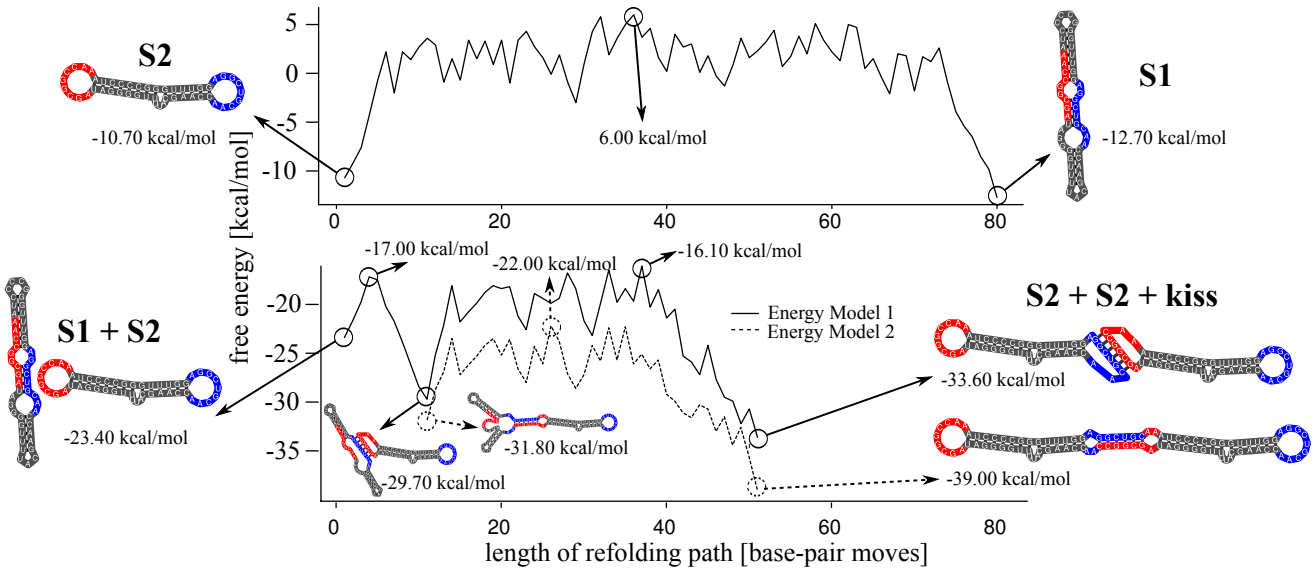

Figure 2: Energy profiles along the refolding path between structure $S1$ and $S2$. Top: Refolding of a monomer; bottom: refolding while interacting with a second molecule. Blue and red colored regions are designed to form inter-molecular base-pairing. The lower panel shows a comparison between two energy models that differ in the energy contribution of the loop regions involved in the inter-molecular pairing. In either case, the relative energy of refolding is lower than for the monomer.

cut after the 5'AA of the interacting loops and connected to the beginning of the respective other strand (see Figure 2, structures for Energy Model 2). Hence, we increment the degree of involved loop regions as if a regular helix was formed. The interior loop of $S1$ becomes a multi-loop and the hairpin loop of $S2$ becomes an interior loop. Computing the intra-molecular refolding with this altered energy model results in the same path, but with a lower energy-barrier of only 9.80 kcal/mol.

Conclusion

In this contribution we showed that the computational design of an RNA molecule which shows prion-like behavior is feasible. As in the original prion system, the "misfolded" conformation forms, via a kissing interaction, a hetero-dimeric complex with the native conformation. This interaction destabilizes the native conformation and triggers refolding into the "misfolded" conformation. The calculations show that the kissing interaction lowers the activation energy for refolding drastically. Furthermore, the "misfolded" conformation can oligomerize. Whether the molecule also behaves as expected in the wetlab is currently being checked by our experimental partners.

Acknowledgments

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References

Afonin, K. A., Lindsay, B., and Shapiro, B. A. (2013). Engineered RNA nanodesigns for applications in RNA nanotechnology. *RNA Nanotech*, 1:1–15.

Aguzzi, A., Sigurdson, C., and Heikenwaelder, M. (2008). Molecular mechanisms of prion pathogenesis. *Annu Rev Pathol Mech Dis*, 3:11–40.

Ennifar, E., Paillart, J.-C., Marquet, R., Ehresmann, B., Ehresmann, C., Dumas, P., and Walter, P. (2003). HIV-1 RNA dimerization initiation site is structurally similar to the ribosomal A site and binds aminoglycoside antibiotics. *J Biol Chem*, 278(4):2723–2730.

Flamm, C., Hofacker, I. L., Maurer-Stroh, S., Stadler, P. F., and Zehl, M. (2001). Design of multi-stable RNA molecules. *RNA*, 7:254–265.

Flamm, C., Hofacker, I. L., Stadler, P. F., and Wolfinger, M. T. (2002). Barrier trees of degenerate landscapes. *Z Phys Chem*, 216:155–173.

Guo, P. (2010). The emerging field of RNA nanotechnology. *Nature Nanotech*, 5:833–842.

Ishikawa, J., Furuta, H., and Ikawa, Y. (2013). RNA tectonics (tectoRNA) for RNA nanostructure design and its application in synthetic biology. *WIREs RNA*, 4:651–664.

Khalil, A. S. and Collins, J. J. (2010). Synthetic biology: applications come of age. *Nature Rev Gen*, 11:367379.

Lorenz, R., Bernhart, S. H., Höner zu Siederdissen, C., Tafer, H., Flamm, C., Stadler, P. F., and Hofacker, I. L. (2011). Vienna package 2.0. *Algorithms Mol Biol*, 6:26.

Mathews, D. H. (2006). Revolutions in RNA secondary structure prediction. *J Mol Biol*, (359):526–532.

McCaskill, J. S. (1990). The equilibrium partition function and base pair binding probabilities for RNA secondary structure. *Biopolymers*, 29:1105–1119.

Weixlbaumer, A., Werner, A., Flamm, C., Westhof, E., and Schröder, R. (2004). Determination of thermodynamic parameters for HIV-1 DIS type loop-loop kissing complexes. *Nucl Acids Res*, 32:5126–5133.