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Research review paper

Computational design of allosteric ribozymes as molecular biosensors

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ABSTRACT

Nucleic acids have proven to be a very suitable medium for engineering various nanostructures and devices. While synthetic DNAs are commonly used for self-assembly of nanostructures and devices *in vitro*, functional RNAs, such as ribozymes, are employed both *in vitro* and *in vivo*. Allosteric ribozymes have applications in molecular computing, biosensoring, high-throughput screening arrays, exogenous control of gene expression, and others. They switch on and off their catalytic function as a result of a conformational change induced by ligand binding. Designer ribozymes are engineered to respond to different effectors by *in vitro* selection, rational and computational design methods. Here, I present diverse computational methods for designing allosteric ribozymes with various logic functions that sense oligonucleotides or small molecules. These methods yield the desired ribozyme sequences within minutes in contrast to the *in vitro* selection methods, which require weeks. Methods for synthesis and biochemical testing of ribozymes are also discussed.

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Introduction

Nanobiotechnology aims to engineer a variety of functional systems at a molecular scale applying biomolecules. The biomolecular manufacturing brings new classes of problems and unfamiliar research areas that are at the core of the emerging fields of nanotechnology and synthetic biology (Lienert et al., 2014). The progress achieved in nucleic acid engineering during the past 24 years proved that the nucleic acids are a very suitable medium for engineering various nanostructures and devices (Ellington and Szostak, 1990; Penchovsky, 2012b; Penchovsky and Breaker, 2005). The nucleic acid engineering offers some significant advantages over organic chemistry-based nanotechnology. The main advantage of making structures and devices with nucleic acids is the possibility of applying many established engineering methods in conjunction with various tools of molecular biology and nucleic acid

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chemistry (Green et al., 2012). We can easily detect, amplify, modify, and store nucleic acids, which make nanobiotechnology a very promising research area. Applying these well-established tools, we can engineer synthetic RNA and DNA molecules for various applications. The major goal of RNA-based nanotechnology is to employ the functional diversity of RNA in many versatile *in vitro* and *in vivo* applications (Afonin et al., 2011; Prenninger et al., 2006). Such applications include self-assembly of nanostructures (Nasalean et al., 2006), molecular computing (Benenson, 2012), biosensoring (Seetharaman et al., 2001), reporter systems in high-throughput screening arrays for drug discovery (Blount et al., 2006; Penchovsky and Stoilova, 2013) and in microfluidic devices, exogenous control of gene expression (Culler et al., 2010; Klauser and Hartig, 2013; Leisner et al., 2012; Neupert and Bock, 2009; Penchovsky, 2012a; Win and Smolke, 2008), and others.

Synthetic DNAs are commonly used for self-assembly of nanostructures (Winfree, 2000; Winfree et al., 1998) and devices for various *in vitro* applications (Stojanovic and Stefanovic, 2003a,b). Artificial RNAs are employed as molecular sensors (Liu et al., 2009) not only *in vitro* but also in different *in vivo* strategies for exiguous control of gene expression in pro and eukaryotes. The major goal of RNA-based nanotechnology is to employ the functional diversity of RNA in many versatile *in vitro* and *in vivo* applications. RNA sensors can be designed to work as simple logic gates as well as integrated computing circuits. Therefore, different RNA engineering approaches are becoming of interest for many researchers working within the fields of nanobiotechnology (Guo et al., 2012) and RNA synthetic biology (Isaacs et al., 2006).

Many synthetic RNA sensors are based on ribozymes. The ribozymes (ribonucleic acid enzyme) are catalytic RNA molecules found in all domains of life. One of the catalytically active RNAs, found in all domains of life, is known as the hammerhead ribozyme. In nature, the hammerhead ribozymes (HHRs) cleave themselves at a specific position (Flores et al., 2001; Scott et al., 1996). Various versions of the HHRs are used for engineering allosteric sensors. In fact, most of the designer ribozymes are engineered to work as allosteric enzymes. They consist of ligand(s)-binding allosteric domain and a catalytic center. The allosteric ribozymes switch on or turn off as a result of ligand binding. They can sense the presence of various effectors, including different small molecules, RNA and DNA oligomers, proteins and others. Allosteric RNA sensors can be designed to work like simple logic gates as well as integrated computing circuits (Penchovsky, 2012b).

Our ability to easily engineer functional RNA molecules with desired properties is an essential condition for the practical success of RNA-based nanotechnology and synthetic biology (Penchovsky, 2014). There are three distinct types of methods for obtaining allosteric ribozymes, include *in vitro* selection (Piganeau et al., 2001), rational design (Liang and Smolke, 2012; Tang and Breaker, 1997), and computational selection (Penchovsky, 2013; Penchovsky and Breaker, 2005). In this review, I discuss the main computational approaches for designing allosteric ribozymes that sense the presence of oligonucleotides or small molecules. The computational methods for designing allosteric ribozymes discussed throughout this paper have any advantage over *in vitro* selection and rational design methods. While the *in vitro* selection methods (Ellington and Szostak, 1990; Joyce, 2007) require weeks and even months of experimental time, the computational methods can test hundreds of thousands different sequences in the timescale of minutes using partition function for RNA folding (McCaskill, 1990) in conjunction with a random search algorithm (Penchovsky and Ackermann, 2003). They produce accurately RNA sequences with desired properties in over 90% of the oligonucleotide sensing ribozymes. The current paper mainly reviews four research publications for computational design of allosteric ribozymes that sense random (Penchovsky and Breaker, 2005) or predefined oligonucleotides (Penchovsky and Kostova, 2013), small molecules (Penchovsky, 2013), or the length of their target RNAs (Penchovsky, 2012b).

The designer ribozymes have various Boolean logic functions, including YES, AND, OR, and NOT. In addition, they can be engineered to sense the length of external target RNAs and to serve as integrated computing circuits. The allosteric ribozymes are based either on the minimal or on the extended versions of the HHR, which have different kinetics of cleavage. The ribozyme designs using the minimal version are intended for *in vitro* applications at high concentration of Mg^{2+} (10 mM). The riboswitches based on the extended version are for *in vivo* usage because their high-speed kinetics of cleavage at physiologically relevant concentration of Mg^{2+} (1 mM) as discussed in the following sections.

Hammerhead ribozymes — structure, function, and variants

All RNA sensors, discussed throughout this paper, are variants of HHRs (Fig. 1). The HHRs are small self-cleaving RNAs. Their secondary structure diagrams resemble the form of a hammerhead shark from where they got their name. The secondary structure of the HHR has three stems and unpaired nucleotides among them. The cleavage site is indicated by the arrowhead (Fig. 1). The HHRs were, firstly, discovered in two classes of plant virus-like RNAs such as satellite RNAs and viroids (McKay, 1996). Nowadays, it is established that they are widely dispersed within many forms of life. In nature, the self-cleavage of the HHRs is part of a rolling circle replication mechanism. They form conserved three-dimensional structures that require the presence of Mg^{2+} or other divalent metals. Note that the presence of divalent metals is necessary for the formation of the right three-dimensional structure of the HHR (Scott, 1997). The divalent metals do not participate in the catalytic reaction of the HHR.

In nature, the HHRs are cis-acting RNAs that are not able to function under multiple turnover conditions in contrast to protein enzymes. However, HHRs can be engineered to work *in vitro* as trans-acting ribozymes cleaving external substrate RNAs under multiple turnover conditions (Penchovsky, 2012b). The HHRs execute a rather simple chemical reaction that results in the breakage of RNA at the cleavage-site nucleotide. RNA cleavage mechanism involves an isomerization that consists of rearrangement of the linking phosphodiester bond. It is the same reaction, chemically, that occurs with random base-mediated RNA degradation, except that it is highly site-specific and the rate is accelerated by 10,000-fold or more (Flores et al., 2001). Therefore, the pH-value of the reaction solution should be over 8 to achieve the highest rate of cleavage.

The hammerhead sequence, which is essential for its catalytic function is called the minimal version of the HHR (Fig. 1A). These HHRs require a rather high concentration of Mg^{2+} (10 mM) to react their highest catalytic rate of 1 molecule per minute. We use the minimal version of HHR for designing RNA sensors for *in vitro* applications only. In contrast, the extended version of the HHR from the human parasite *Schistosoma mansoni* (Osborne et al., 2005) requires a rather low concentration of Mg^{2+} (1 mM) to react its highest catalytic rate of 10 molecules per minute (Fig. 1C). Note that 1 mM is also a physiologically relevant concentration of Mg^{2+}. Therefore, we employ the extended version of HHR for designing RNA sensors for *in vivo* applications. These differences derive from the additional canonical and non-canonical interactions between the nucleotides in the bulge loop of the stem I, and those in the loop of stem II, which are present only in the extended versions of the HHR (Fig. 1C). These 3D interactions are established by X-ray crystallographic analysis (Shepotinovskaya and Uhlenbeck, 2008). These additional intersections help the extended versions of the HHR to adapt easily the 3D structure, which is catalytically active in lower concentration of Mg^{2+} than that of the minimal version of the HHR. In fact, the Mg^{2+} connects stems, I and II, which have to get close together in the 3D structure of the HHR to trigger the catalytic function of the ribozyme.

We have modified the minimal version of the HHR by introducing an allosteric domain(s) in stem II (Fig. 1B). These ribozymes work at high concentration of Mg^{2+} and, therefore, are designed for *in vitro* applications only. In contrast, all allosteric ribozymes, which are engineered for *in vivo* applications used the extended version HHR from *S. mansoni* as a