Engineering Synthetic Splicing Ribozyme Systems

Reusable components, such as logic gates and code libraries, simplify
the design and implementation of electronic circuits and computer
programs. The engineering of biological systems would benefit also
from reusable components. I show the utility of splicing ribozymes for
the biological engineer. Ribozymes allow the engineer to manipulate
existing biological systems and to program self-modifying RNA
systems. In addition, splicing ribozymes are easy to engineer,
malleable, modular, and scalable.

I used the model ribozyme from Tetrahymena to explore the principles
behind engineering biological splicing systems in vivo. I show that
the core ribozyme is modular and functions properly in many different
contexts. Simple base pairing rules and computational RNA folding can
predict splicing efficiency in bacterial cells. To test our
understanding of the ribozyme, I generated synthetic ribozymes by
manipulating the primary sequence while maintaining the secondary
structure. Results indicate that our biochemical understanding of the
ribozyme is accurate enough to support engineering.

Splicing ribozymes can form core components in an all-RNA logic
system. I developed biological transzystors, switches analogous to
electrical transistors. Transzystors can use any trans-RNA as input
and any RNA as output, allowing the genetic reading of RNA levels. I
also show the ribozyme can write RNA using the trans-splicing
reaction. Trans-splicing provides an easy mechanism to hook into an
existing biological system and patch its operation. The generality of
these ribozymes for a wide set of applications makes them promising
tools for synthetic biology.