Modular design of RNA origami scaffolds and devices

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Single-stranded RNA origami is an architecture enabling the design of genetically expressible RNA scaffolds that assemble cotranscriptionally. Through the addition of RNA aptamer modules, such scaffolds can control the relative position and orientations of small molecules and proteins in three dimensions (3D). The design of large and complex RNA origami has been limited by a lack of modeling and design tools. Here we expand the complexity of RNA origami architecture, and provide software to automate the 3D modeling of structures and thermodynamic design of sequences. This enables the scale and diversity of RNA origami scaffolds to be greatly extended, as exemplified by a series of 14 different shapes of increasing size and complexity. Nanometer-scale positioning of small-molecule and protein binding modules is demonstrated using fluorescence resonance energy transfer (FRET) for both light-up aptamers and fluorescent proteins. The RNA origami FRET systems are further used to design and characterize a set of 6 dynamic devices that switch conformation in response to small molecule, RNA and protein inputs. Finally, FRET output is used to demonstrate scaffolding of fluorescent proteins on RNA origami scaffolds when expressed in *E. coli* cells. This study opens the door for the modular design of functional and switchable RNA scaffolds with potential to control molecular systems in the cell ranging from signaling pathways to enzyme cascades.