Co-transcriptionally encoded RNA strand displacement circuits:
Towards continuous *in vivo* nucleic acid computing

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The programmable and predictable control of DNA strand displacement reactions make them a powerful tool for engineering diverse chemical information processing circuits. Chemical reaction networks based on DNA strand displacement have demonstrated complex digital computation, molecular pattern recognition, and signal cascades and amplifiers. However, current DNA strand displacement circuits are single-use and are susceptible to degradation in biological samples or living cells[1]. This limits their ability to detect and process nucleic acid inputs in many practical settings. Here, we present co-transcriptionally encoded RNA strand displacement (ctRSD) circuits[2], in which DNA strand displacement components are mapped to RNA transcripts that self-assemble into circuits during transcription. Key to ctRSD circuit design is the ability to co-transcriptionally fold kinetically trapped RNA gates, which allows circuit components to be produced together without significant cross reaction (Figure A). Prototyping *in vitro*, we have tested >100 unique ctRSD gate sequences and identified robust design heuristics for engineering modular and composable ctRSD components analogous to those in DSD circuits. Further, we present a library of alternative and/or orthogonal gate design options to meet diverse applications. To demonstrate the programmability of ctRSD circuits, we develop OR, AND, and signal amplification elements, and integrate these elements into larger signaling cascades composed of up to four layers[2]. Importantly, ctRSD circuit kinetics quantitatively match the predictions of a simple model of coupled transcription and strand displacement, enabling model-driven circuit design (Figure B). We envision our robust ctRSD circuit design will allow the existing information processing capabilities of DNA computing to be readily adopted for new sensing and diagnostic applications in biological samples. Ultimately, these circuits could be genetically encoded inside living cells for continuous, real-time nucleic acid computation *in vivo*.

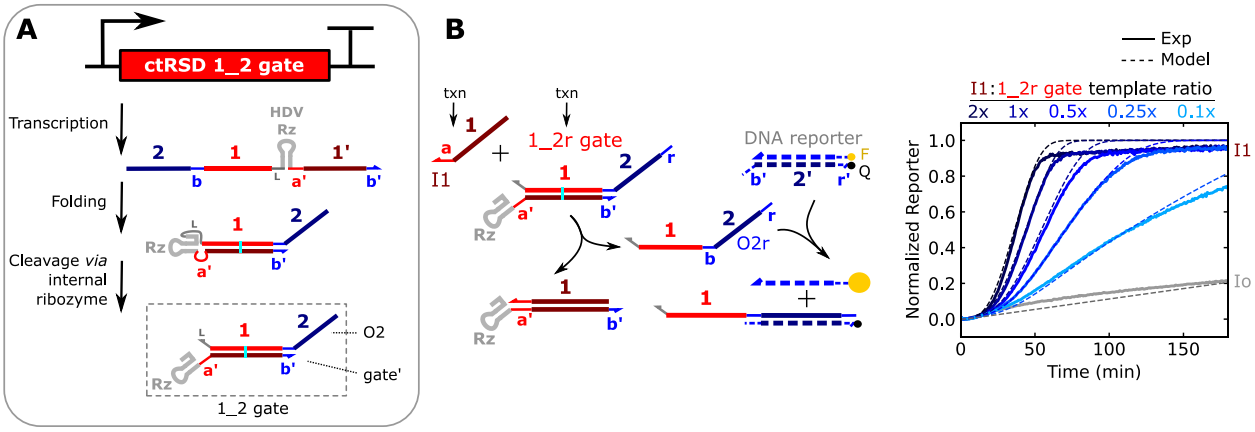

Figure: (A) ctRSD gates independently fold and self-cleave *via* an internal ribozyme (Rz) during transcription (**B**) Experimental and simulated ctRSD circuit kinetics. The rate of reaction can be controlled by varying the relative ratio of input to gate template.

References

1. Chen, Y.-J., Groves, B., Muscat, R. A. & Seelig, G. DNA nanotechnology from the test tube to the cell. *Nat. Nanotechnol.* **10**, 748–760 (2015).
2. Schaffter, S. W. & Strychalski, E. A. Co-transcriptional RNA strand displacement circuits. *bioRxiv* 2021.07.20.450530 (2021) doi:10.1101/2021.07.20.450530.