

Systematic characterisation of RNA/DNA hybrid toehold exchange kinetics

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Nucleic acid strand displacement represents the core molecular reaction of dynamic nucleic acid nanotechnology. Extensive characterisation of DNA/DNA strand displacement kinetics has enabled the development of increasingly complex molecular reaction networks with controllable kinetics [1], facilitating wide-ranging applications including molecular computation and biosensing [2,3].

While most *de novo* molecular systems have focussed on the use of DNA as a building material, recent years have seen increased interest in exploiting RNA within these systems [4]. RNA exhibits numerous advantages over DNA including a broader range of structures that can be adopted and potential catalytic functionalities [4], enabling novel design capabilities within these dynamic systems. Moreover, owing to these unique properties, RNA holds a critical role in a range of *in vivo* processes, such as gene regulation, structural scaffolding and catalysis [5]. Importantly, given RNA is so fundamentally embedded in intracellular processes, the initiation or progression of disease is often associated with dysregulation of RNA making it a crucial biomarker for many diseases. Consequently, we have seen increased development of strand displacement-based nanodevices which either directly utilise or interface with RNA [6,7]. Nevertheless, a systematic characterisation of RNA/DNA hybrid strand displacement kinetics has yet to be performed, with major holes in the fundamental understanding of this reaction mechanism.

In this work we develop a continuous-time Markov chain model with the potential to predict the kinetics of RNA/DNA strand displacement. We present initial results of RNA/DNA hybrid strand displacement kinetics. This experimental data in conjunction with our model has the potential to reveal the detailed mechanism behind RNA/DNA hybrid strand displacement, as well as to inform the rational design of novel RNA/DNA hybrid reaction networks with optimised reaction kinetics.

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