Kinetic proofreading in a DNA strand displacement network

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The concept of kinetic proofreading was first introduced by Hopfield in his seminal article in 1974[1]. In many biosynthetic processes, the specificity of correct product formation is often orders of magnitude higher than that dictated by the free energy differences between correctly formed and incorrectly-formed complexes. Hopfield proposed a scheme called “kinetic proofreading”, where small free energy differences are utilised repeatedly over multiple steps in a reaction cycle to extract a large overall discrimination. A crucial component of the kinetic proofreading mechanism is the consumption of fuel to drive the reaction around the cycle; it is an inherently non-equilibrium (or kinetic) mechanism.

The challenge of discriminating between recognition domains is also ubiquitous in synthetic nanotechnology, whether in computational strand displacement cascades[2], tile assembly systems[3] or diagnostics-based applications[4]. Despite this similarity, kinetic proofreading has not, to our knowledge, previously been demonstrated in synthetic systems. Here we introduce a non-enzymatic DNA-based kinetic proofreading method that efficiently discriminates between very similar recognition domains via a fuel-consuming cycle.

We first characterise the synthetic proofreading mechanism in detail. We then demonstrate its application in a system in which sequence-specific dimers are catalytically produced from a pool of monomers with distinct recognition domains by molecular templates. The underlying templating reaction exploits a recently introduced motif called handhold mediated strand displacement (HMSD)[5].

Finally, we adapt the system to the clinically relevant challenge of distinguishing between single nucleotide polymorphisms (SNPs). The proofreading mechanism not only reduces the relative rate at which mutated strands trigger an output but also prevents the mutated strands from ever triggering a high response, even in the long-time limit. This highly desirable result arises because mutated strands are converted into stable waste complexes by the fuel-consuming cycles inherent to kinetic proofreading.

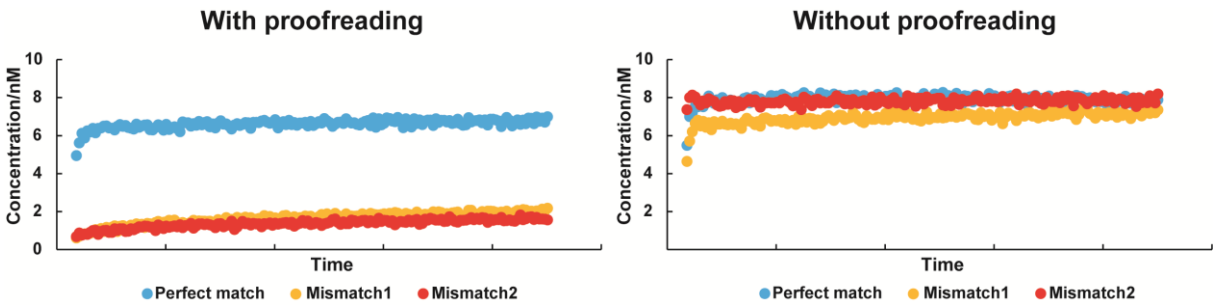

Fig. 1. An external reporter showing the formation of products with different monomers. When the proofreading fuel supply is present (left panel), only the monomer with a perfectly matching recognition domain is converted into the product in significant amounts, even in the long time limit. When there is no proofreading fuel (right panel), all the monomers form a similar amount of dimeric product.

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