A Dendritic DNA Nanostructure for Target DNA Detection

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Responsive and programmable DNA nanostructures have shown great promise as chemical detection systems. Here, we describe a DNA detection system employing the triggered self-assembly of a novel DNA dendritic nanostructure. Detection begins when a specific, single-stranded DNA sequence (target sequence, T) triggers a hybridization chain reaction (HCR)[1-3] between two, distinct DNA hairpins (α and β). Each hairpin opens and hybridizes the other, resulting in a branching nanostructure complex of alternating layers of α and β strands. In the absence of T, α and β hairpins are stable and remain in their poised, closed-hairpin form. In the presence of T, α hairpins are opened by toe-hold mediated strand-displacement[1]. Each opened α hairpin can subsequently open and hybridize 2 β hairpins. Likewise, each opened β hairpin can open and hybridize 2 α hairpins. Hence, each layer of the growing dentritic nanostructure complex can in principle accommodate an exponentially increasing number of cognate molecules, generating a high molecular weight nanostructure within seconds. Naked eye, colorimetric detection of the resulting dendritic nanostructure was achieved using a red-to-purple plasmonic color-shift in colloidal gold nanoparticles that were functionalized with DNA that could hybridize the surface of the nanostructure complex[4-7]. This HCR system has minimal sequence constraints, allowing reconfiguration for the detection of arbitrary target sequences. We also demonstrate the detection of unique sequence identifiers of HIV and *Chlamydia* pathogens.

Previous studies have demonstrated a triggered HCR where the product is a self-assembling, branched, DNA nanostructure[5]. In this case, the size of the nanostructure grows exponentially with the number of HCR species, permitting super-linear signal amplification. Here, we demonstrate a novel, DNA triggered HCR that forms a dendritic nanostructure that also grows exponentially, yet is composed of only two distinct DNA hairpin strands, α and β. The minimal complexity of this system results in few sequence constraints, permitting the accommodation, and detection, of arbitrary DNA sequences with minimal sequence reconfigurations.

The nucleotide sequences of α and β were designed by hand by first denoting generic sub-sequences that could accommodate the appropriate secondary structures of the closed hairpins conformations and the open conformations necessary for forming the dentritic nanostructure complex. Each strand forms a hairpin structure intended to have a bulged-loop protruding in the middle of the double-strand region and a single-stranded segment at the 5′ terminus. The analyte, a target DNA strand, T_1, is a single-strand DNA oligonucelotide

Detection begins when annealed α and β hairpins are combined (in equimolar amounts) and then initiated with the addition of T_1. In the first step of the hybridization cascade, T_1, hybridizes with the single-stranded sub-sequence of α, inducing strand displacement, resulting in an open-form bimolecular complex denoted T_1+α. In the second step of the hybridization cascade, the now single-stranded sub-sequences of α are available for hybridization to two copies of β, resulting in tetramolecular complex, T_1+α+2β, constituting the first layer of the nascent dendritic DNA nanostructure rooted by T_1+α.

β will, with some finite rate, spontaneously open and expose a single-stranded segment that binds two α molecules, yielding the new complex T_1+5α+2β, constituting the second layer of the growing dendritic nanostructure. Strand displacement, stem-loop openings, and hybridization of eight additional copies of β form the third layer of the nanostructure (complex T_1+5α+2β). Because each α and β strand can open and hybridize two additional strands, the dendritic nanostructure grows with kinetics that are, in principle, exponential. To analyze the HCR of T_1, α, and β, samples were run on native polyacrylamide gels (PAGE) where the hairpin structures, as well as the growing dentritic nanostructure could be visualized. The α and β reaction components were stable in isolation, but when all three components are present, product bands appear, indicating the formation of the dendritic nanostructure. The brightness and position of these product bands are highly variable, depending on the sequences and conditions, suggesting a diversity of nanostructures are formed.

The resulting dendritic nanostructure is predicted to have numerous exposed sub-sequences that can be exploited for the purposes of rapid and sensitive optical detection, obviating cumbersome analytical techniques such as native PAGE. Here, 15 nm colloidal gold nanoparticles (AuNP) were functionalized with both α-binding and β-binding strands. When combined, the dendritic nanostructure aggregates the DNA-AuNP, increasing their proximity and inducing a surface plasmon resonance that in turn produces a high-contrast macroscopic color change (from red to purple) visible to the naked eye.

Encouraged by these results, two new detection systems were designed based on sequences that are unique identifiers of the Human Immunodeficiency Virus[9] and *Chlamydia trachomati*[10]. Again, accurate colorimetric readouts for this augmented detection system were obtained, demonstrating the generality of the dendritic nanostructure HCR as a rapid and reliable diagnostic for arbitrary DNA analytes.

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