

Structural Optimization of Dendritic DNA Self-Assembly

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Hybridization Chain Reaction* (HCR) is a bottom-up signal amplification technique that has been proposed during the use of DNA target recognition experiments. Its recursive self-assembly reaction is an intriguing alternative to conventional Polymerase Chain Reaction (PCR) based signal amplification which is both power and time inefficient. Given the appealing characteristics of HCR we have chosen to try to optimize its structure from the point of view of false positive reduction, signal amplification and structural integrity. In this paper we present three modifications to the original recursive dendritic structures that were previously used in HCR experiments.

The *first modification* reduces the false positive rate by creating a specific external toehold for one of the hairpins. This hairpin will systematically interact with only the initiator strand during the target recognition phase of the reaction and thus, the toehold length may be optimized to maximize signal-to-noise ratio without negatively affecting the HCR. In addition, the concentration of this hairpin will establish a tree-to-branching ratio which is related to the expected number of growing trees within a reaction versus the expected amount of branching within a tree. This allows one to predict the average amplification within a tree. The *second modification* helps achieve exponential amplification beginning at the first stage of the reaction and creates more equally stable hairpin structures before a HCR begins. Exponential amplification is achieved by creating two amplification binding sites immediately following the target recognition phase. Each of these sites will then be amplified exponentially throughout the HCR. More equally stable hairpins, or those with similar melting temperatures, will help ensure that the different types of hairpins will have similar reaction rates regardless of environmental conditions. With the proper hairpins, poor tree growth will occur at the consequence of the entire system as opposed to an individual component. Clearly, systematic failure is a feature which is easier to detect experimentally than a single component failure. Thus, error rates associated with environmental conditions are more stable and error recognition can be deciphered more readily. The *third modification* alleviates an identified potential structural issue while minimizing the required number of extra distinct hairpins. The prevention of this structure requires four additional hairpins, but if this structure arises merely once in a single dendritic tree it would cut the tree's potential signal amplification in half.

The three modifications proposed would increase the number of hairpins required for the HCR from three to nine. However, we believe we have created a more methodically correct reaction (as illustrated in Figure 1) which optimizes proper hybridization while reducing false-positive noise often associated with self-assembly experiments. The next step is to develop the necessary computer design applications to create these equally stable hairpins which will lead to more predictable, repeatable and quantitative HCR experiments.

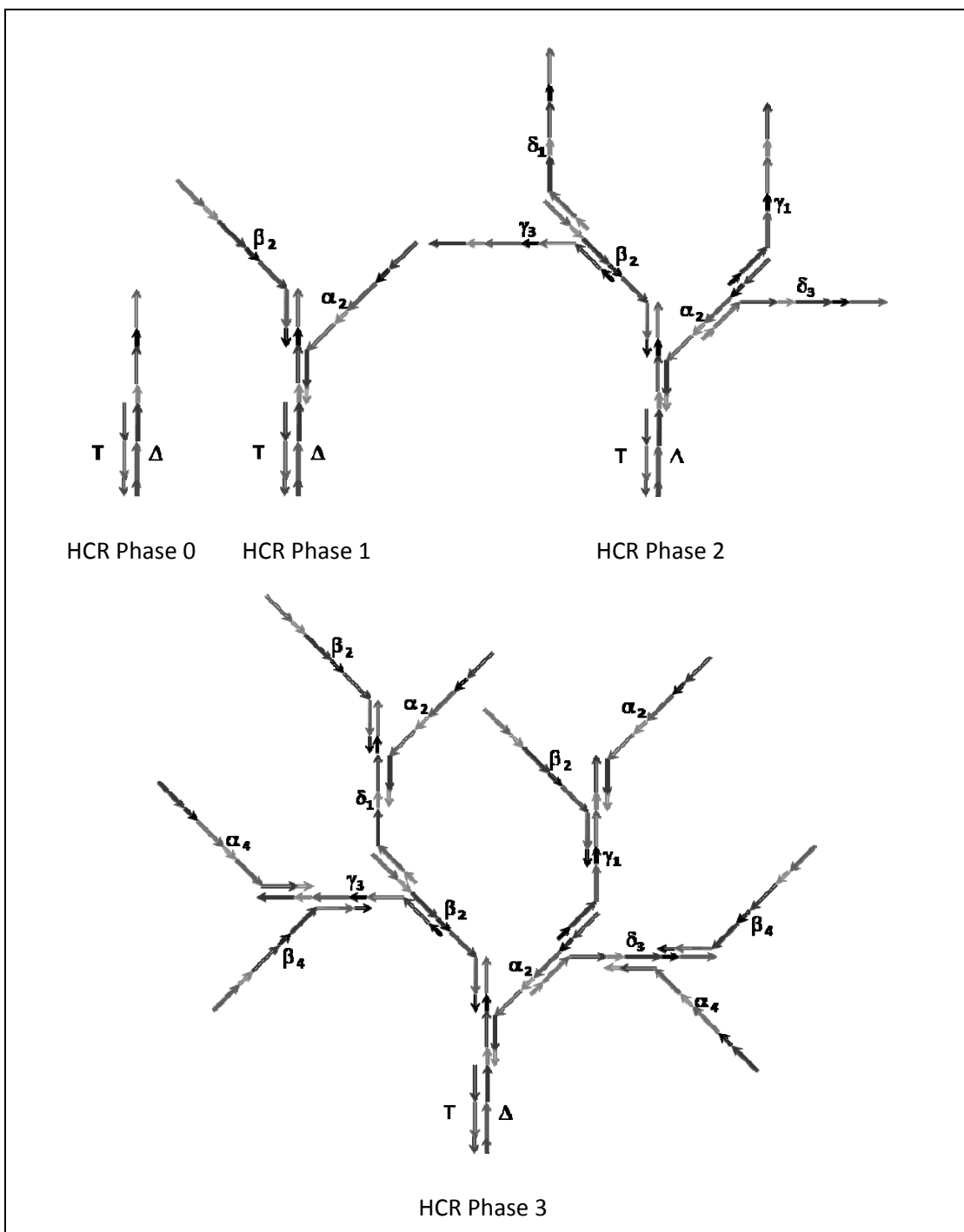


Figure 1: Example of our Hybridization Chain Reaction

employing nine optimized hairpin sequences.

* Reference: Dirks, R. M. & Pierce, N. A.. Triggered amplification by hybridization chain reaction. Proc. Natl Acad. Sci. USA 101, 15275-15278 (2004).