Initial Steps Towards Sequence Parameterized Simulations of DNA-PAINT

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Abstract:

DNA-PAINT is a powerful and flexible implementation of Stochastic Reconstruction Microscopy (STORM), a super resolution technique that enables researchers to produce images with subresolution accuracy[1,2]. In its most rudimentary implementation, this imaging system requires two DNA strands: a fluorophore containing imager strand and a docking strand which is anchored to a substrate of interest and is complimentary to the imager strand. The strands are designed in such a manner that they spontaneously hybridize and dehybridize. In the seminal DNA-PAINT publication, it was demonstrated that the rate of detected localizations is directly related to the concentration of the imager strand and independent of the length of the hybridization[3]. These rates of localizations in turn determine the 'on-time' of a localization which is an important parameter to control in order to avoid overlaps.

Currently, Picasso is the primary DNA-PAINT simulator that allows one to input custom kinetic parameters such as k_{on} and dark time[2]. While important parameters to be sure, we hypothesize that these parameters can be computed from the sequences that are to be used as the imager and the docking strands when the problem is articulated in a statistical mechanical framework: What is the probability of observing the micro-state in which the imager and docking strand are hybridized?

The Boltzmann distribution is a powerful tool when computing macroscale thermodynamic parameters of chemical systems from its molecular components. Certain formulations of the distribution use three parameters: the number of lattice sites and ligands denoted as Ω and L respectively, and the free energy of a microstate ΔG[4]. The ΔG of hybridization can be computed using the NUPACK software, while Ω and L can be set by the user[5–7]. In systems such as DNA-PAINT, $\Omega >> L$ as the concentration of the imager strand is dilute. The Boltzmann distribution parameterized by these three parameters can output a probability that in turn parameterizes a Monte Carlo model that simulates an observed localization of the imager strand.

Our initial simulations using this sequence informed framework demonstrate that the frequency of localizations and consecutive localizations, indicated by a broad peak in the time-intensity trace diagram, is directly proportional to L when the sequences are complimentary to one another. This is consistent with expected experimental results as

STORM necessitates a trace amount of the fluorescent molecule to promote sparse localizations in order to prevent overlap of adjacent signals.

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