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Prof. Erick Carreira
Editor-in-Chief, *The Journal of the American Chemical Society*

Dear Prof. Carreira

We are pleased to submit a manuscript for consideration for publication in the *The Journal of the American Chemical Society* an original research **Article** entitled **"Determining sequence-dependent DNA oligonucleotide hybridization and dehybridization mechanisms using coarse-grained molecular simulation, Markov state models, and infrared spectroscopy"** by Michael S. Jones, Brennan Ashwood, Andrei Tokmakoff, and Andrew L. Ferguson, all from the University of Chicago. This article has not been published previously and is not under consideration in any other journal. All authors have seen and approved the submission of the manuscript.

Background. A robust understanding of the sequence-dependent thermodynamics of DNA hybridization has enabled rapid advances in DNA nanotechnology. A fundamental understanding of the sequence-dependent kinetics and mechanisms of hybridization and dehybridization remains comparatively underdeveloped. Although previous experimental and computational studies have identified deviations from an "all-or-nothing" hybridization/dehybridization model, few studies have integrated these approaches in order to evaluate and quantify sequence-dependent dynamics.

Key Results. In this work, we establish new understanding of the sequence-dependent hybridization/dehybridization kinetics and mechanism within a family of self-complementary pairs of 10-mer DNA oligomers by integrating coarse-grained molecular simulation, machine learning, data-driven inference of long-time kinetic models, and experimental temperature-jump infrared spectroscopy. We conducted more than 1 ms of coarse-grained molecular dynamics simulations and employed deep learning techniques to construct high-resolution Markov state models as predictive and interpretable models of the sequence-dependent dynamics. Our results reveal that the specific placement of interrupting G:C pairs within an otherwise repetitive AT sequence can have a profound impact on the kinetic pathways and mechanisms for association and dissociation of the DNA duplex.

Significance. Our results establish new understanding of the dynamical richness of sequence-dependent kinetics and mechanisms of DNA hybridization/dehybridization, offer quantitative models of their long-time kinetics, present a molecular basis with which to understand experimental temperature jump data, and furnish foundational design rules by which to rationally engineer the kinetics and pathways of DNA association and dissociation in burgeoning DNA nanotechnology applications.

Scope. This work fits within the scope of *The Journal of the American Chemical Society* in that it reports new fundamental physical chemical understanding of the molecular pathways and mechanisms of sequence-dependent DNA hybridization/dehybridization. These sequence-dependent mechanisms are of both fundamental interest in biophysical and biochemical processes and of increasing importance in burgeoning DNA nanotechnology applications reliant on quantitative understanding of the sequence-dependent kinetic

pathways and rates.

Reviewers. We propose the following scientists as well qualified to review this work.

Simulation.

Joshua Lequieu — Chemical and Biological Engineering, Drexel University
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∘ *Expert in molecular dynamics simulations of nucleic acids.*

Experiment.

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Yours Sincerely

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