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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 **"Hierarchical Self-Assembly Pathways of Polypeptoid Helices and Sheets"** by Mingfei Zhao (U. Chicago), Kacper Lachowski(U.W. Seattle), Sarah Alamdari (U.W. Seattle), Janani Sampath (PNNL), Peng Mu (PNNL), Chun-Long Chen (PNNL, U.W. Seattle), Lilo D. Pozzo (U.W. Seattle), Christopher J. Mundy (PNNL, U.W. Seattle), Jim Pfaendtner (U.W. Seattle, PNNL), and Andrew L. Ferguson (U. 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. Polypeptoids (poly-N-substituted glycines) are a class of highly tailorable synthetic peptidomic polymers with applications as drugs, antimicrobials, and catalysts that can be engineered to assemble into nanoaggregates including spheres, helices, tubes, and sheets. Amphiphilic diblock polypeptoids have been engineered to assemble high-aspect ratio 2D crystalline lattices with applications in catalysis, molecular separations, and photovoltaics. Assembly is induced by dissolving the peptoids in an organic solvent/water mixture and evaporating the organic phase to promote hydrophobic association, but the mechanistic pathways and thermodynamic/kinetic driving forces mediating assembly remain uncharacterized.

Key Results. In this work, we integrate all-atom molecular dynamics (MD) simulation, small angle x-ray scattering (SAXS), circular dichroism (CD), atomic force microscopy (AFM), and x-ray diffraction (XRD) to present an integrated computational/experimental study of the self-assembly of Nbrpe6Nc6 – an amphiphilic diblock polypeptoid comprising an NH_2 capped block of six hydrophobic N-((4-bromophenyl)ethyl)glycine residues conjugated to a hydrophilic 6-aminohexanal ($NH_3(CH_2)_5CO$) tail – in organic solvent/water mixtures. Our MD simulations predict a hierarchical assembly pathway by which monomers first assemble into disordered aggregates that self-order into 1D chiral helical rods that subsequently assemble 2D achiral crystalline nanosheets. Experimentally, we observe stable, free-floating 1D rods in mixed solvent using SAXS and CD, and 2D crystalline sheets in pure water using XRD and AFM. MD predictions of the relative thermodynamic stability of disordered aggregates, helical rods, and sheets as a function of the organic solvent concentration support a thermodynamically-controlled assembly process.

Significance. Our work integrates simulation and experiment to establish a previously unknown hierarchical polypeptoid assembly pathway wherein 0D monomers first assemble into disordered aggregates that ripen into 1D helical rods that subsequently form 2D crystalline nanosheets. This new understanding of the hierarchical assembly mechanisms and stable intermediaries presents new principles and guidance for the rational design of peptoid-based nanomaterials with potential biochemical, biomedical, and bioengineering applications including as drugs, antimicrobials, catalysts, and molecular sieves.

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 polypeptoid self-assembly. This new understanding establishes new mechanistic understanding and a thermodynamic basis for existing experimental evaporation-induced assembly protocols and identifies previously unknown solvent conditions for the stable fabrication of 1D helical rods. These principles offer new strategies for the supramolecular assembly of diverse peptoid sequences into 1D and 2D supramolecular aggregates.

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

Simulation.

Stephen Whitelam — Molecular Foundry, Lawrence Berkeley National Lab
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∘ *Expert in biomolecular simulation and molecular modeling of peptoid assembly.*

Monica Olvera de la Cruz — Materials Science and Engineering and Chemistry, Northwestern University
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∘ *Expert in molecular modeling and simulation of peptides, polymers, and materials assembly.*

Arthi Jayaraman — Chemical and Biomolecular Engineering, University of Delaware
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∘ *Expert in theory and simulation of biomolecular assembly and molecular materials design.*

Experiment.

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∘ *Expert in biomolecular and polymeric assembly and characterization.*

Rachel Segalman — Chemical Engineering, UC Santa Barbara
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∘ *Expert in biological and polymeric materials assembly.*

Ron Zuckermann — The Molecular Foundry, Lawrence Berkeley National Laboratory
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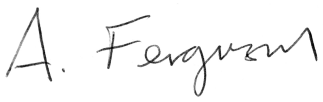
∘ *Pioneer of peptoid science. Expert in peptoid synthesis and characterization.*

We look forward to hearing from you.

Yours Sincerely



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