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a. REPORT	b. ABSTRACT	c. THIS PAGE			Sharon Glotzer
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RPPR Final Report
as of 08-May-2019

Agency Code:

Proposal Number: 66675LS **Agreement Number: W911NF-15-1-0185**
INVESTIGATOR(S):

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DUNS Number: 073133571 EIN: 386006309
Report Date: 14-Dec-2018 Date Received: 25-Mar-2019
Final Report for Period Beginning 15-May-2015 and Ending 14-Sep-2018
Title: Design of Protein Biomaterials Through Tailored Shape and Packing Strategies of Patchy Particles
Begin Performance Period: 15-May-2015 **End Performance Period:** 14-Sep-2018
Report Term: 0-Other
Submitted By: Sharon Glotzer Email: sglotzer@umich.edu
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 0 **STEM Participants:** 3

Major Goals: Under this effort, PI's Glotzer and Ellington aim to apply their understanding of the assembly of model colloids to the design of a biomaterial with defined functionality. They will apply advanced understanding of shape, packing, and assembly of hard shapes and patchy (anisotropically interacting) nanoparticles (Glotzer) to create protein-based aggregates (Ellington). Using fluorescent proteins as a model system, we will predict specific surface substitutions that will lead to thermodynamically stable packings that can be engineered and validated using biophysical methods.

In the first reporting period the Ellington group observed the assembly of well-defined, symmetric protein 16mers (termed protomers) from oppositely charged green fluorescent protein (GFP) derivates. We were able to rationalize the stability of the observed structures using a novel computational model parameterizing the protein shape and attractive patches. In the second reporting period the main objective was to predict the self-assembly of those structures from minimal models in computer simulation, before these are realized in the lab. One of the first steps will be to test whether our model is capable of predicting the effect of mutations in order to disrupt oligomer formation. Corresponding mutagenesis experiments will be carried out in the Ellington group. We will investigate whether it is possible to predict the pathway to protomer formation via coarse grained Molecular Dynamics and/or Monte Carlo simulations of charged monomers. Predicting pathways of self-assembly for realistic models of biomolecules into higher order structures represents a major challenge in the field and would enable us to explore the predictive design of biomaterials from protein building blocks with defined functionality.

A purely computational goal of this grant is to elucidate pathways to protein crystal formation. After completing the study of nucleation pathways in the present funding period, we will, in the time ahead, focus on crystal shape prediction from patchy shape models. The goal will be to predict the three-dimensional habit (such as needle-like, bipyramidal etc.) of the crystal using large-scale simulations of 10,000s of proteins simultaneously using the patchy shape model. Elucidating both protein crystallization pathways and crystal shape is of high relevance for drug discovery and manufacturing.

Accomplishments: (please see uploaded PDF for correct formatting of special characters)

GFP Protomer formation:
The study on protomer formation involved an intense collaboration between the Ellington, Taylor and Glotzer groups that led to submission of a manuscript in late 2017. At that time the protomer structure was only available at 15 Å resolution (from negative stain EM) and the limited resolution made it conceivable for several hypothesized protomer arrangements to be stable. We tested several candidate structures using computer simulations and found

that none of them were preferred. In order to address reviewer questions, the detailed microstructure of the protomer was determined by obtaining a 3.4Å cryo-EM electron density map of the protomer (done by the Taylor group).

We iterated the computational model based upon the higher resolution cryo-EM data and found that we were able to achieve a much more stable structure from simulation. Moreover, the detailed protomer structure depended on a different set of contacts than any of the earlier candidate structures, showing that our model was right in not identifying any of the earlier arrangements as stable. A substantially revised manuscript has been published in Nature Chemistry [DOI: 10.1038/s41557-018-0196-3], and was featured on the cover.

Although the model only confirmed the stability of a structure that had been established experimentally, the simulations do suggest that our computational model is indeed able to discriminate between stable and unstable structures. We hope therefore, to build on this achievement and extend the predictive capabilities of the model even further for de-novo structure prediction.

Protein crystallization:
We performed large-scale simulations of rubredoxin as a model protein crystal former. Our simulations on the Titan supercomputer allowed us to gain a detailed view of the crystallization pathway using a patchy shape model of the protein, which crystallizes into the experimentally observed orthorhombic P212121 structure. We find that the presence of competing, non-native attractive interactions between patches produces a metastable fluid-fluid transition, in the vicinity of which the crystal forms. We were able to obtain evidence for stable and metastable phases as minima of the free energy using advanced sampling methods (well-tempered Metadynamics) and thermodynamic integration. In general, we confirm that it is unfavorable for the protein to crystallize inside the metastable two-phase region, and we observe optimal crystal yield in the metastability gap between the fluid-fluid and the fluid-solid coexistence line. The critical temperature of the metastable fluid-fluid transition is controlled by the strength of non-specific interactions between the monomers, which confirms previous findings. A new finding is that the proteins crystallize according to classical nucleation and growth with a low barrier, and this provides a counter-example to previous predictions that rely on a non-classical, two-step pathway. Our findings show how shape and patchiness together determine the crystallization pathway. Even though they currently apply to a single type of protein molecule only, they significantly go beyond existing models, which are mainly based on isotropic interactions and/or isotropic shape. The research will be ongoing in the follow-on grant, including generalization to other proteins, and a manuscript is in preparation.

Training Opportunities: Postdoctoral Fellow Jens Glaser presented research at professional society meetings and gave invited talks at Rensselear Polytechnic Institute, the University of Georgia, Athens, and the 4th annual MVAPICH User Group meeting at Ohio State University. Additionally he organized an invited session on “Biological Materials Self-Assembly” at the 2017 APS March Meeting in New Orleans.

Glaser also mentored graduate students Luis Y. Rivera-Rivera and Vyas Ramasubramani on research projects supporting this effort.

The postdoc and graduate students on the project have presented results at national society meetings; all students have presented research in front of large audiences.

Results Dissemination:
Glaser delivered several technical presentations at national meetings:
ACS Colloids, State College, 2018, “Necessity of non-specific interactions for protein self-assembly” (talk)
OLCF User Meeting, Oak Ridge, 2018, “Nucleation of Protein Crystals and Strong Scaling on Summit” (poster)
APS March Meeting, Los Angeles, 2018, “Metadynamics study of protein crystal nucleation and growth”, (talk)
AIChE Annual Meeting, Minneapolis, 2017, “Self-Assembly of Open Structures Using Depletion” (talk)
AIChE Annual Meeting, Minneapolis, 2017, “Self-Assembly of Proteins: The Role of Shape and Specific Interaction” (talk)
APS March Meeting, New Orleans, 2017, “The role of shape vs. patches in protein crystallization” (talk)

RPPR Final Report
as of 08-May-2019

Honors and Awards: Sharon C. Glotzer:

• 2019 Aneesur Rahman Prize for Computational Physics, American Physical Society. Citation: “For innovative molecular dynamics simulations of the self-assembly of variously shaped particles which opened up new directions in soft matter and materials science research.”
• 2018 Nanoscale Science and Engineering Forum Award, American Institute of Chemical Engineers.
• Materials Communications Lecture Award, Materials Research Society, 2017.
• Materials Research Society, elected Fellow 2017.
• 2016 Alpha Chi Sigma Award for Chemical Engineering Research, American Institute of Chemical Engineers. Citation: “For elucidating thermodynamic principles of assembly in nanoscale and colloidal matter, and discovering the unexpected role of shape, entropy, and interaction patchiness for materials design.
• American Institute of Chemical Engineers, elected Fellow 2016.
• Royal Society of Chemistry, named Fellow 2016.

Named or Otherwise Distinguished Lectures during the reporting period

1. Jennifer Mills Lecture, Kalamazoo, MI, October, 2018.
2. Distinguished Lecture in Theoretical and Computational Chemistry, Department of Chemistry and Biochemistry, UC San Diego, May 8, 2018.
3. 2018 Ashton Cary Lecturer, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, April 2018.
4. The Keith E. Gubbins Lecture Series, Department of Chemical and Biomolecular Engineering, North Carolina State University, February 2018.
5. Student Selected Annual Seminar Speaker, Department of Chemical and Biological Engineering, Princeton University, December 2017.
6. Distinguished Seminar Speaker, Department of Chemical Engineering, University of Illinois at Chicago, September 2017.
7. Sackler Lecture, Tel Aviv University, May 2017.
8. Racheff Lecture, Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, April 2017.
9. Student Selected Seminar Speaker, Departments of Chemistry and Biochemistry, Indiana University, April 2017.
10. Barnett F. Dodge Distinguished Lecture in Chemical Engineering, Yale, February 2017.
11. Closs Lecture, Chemistry Department, University of Chicago, January 2017.
12. Carolyn and Charles Knobler Lecture, Physical Chemistry Division, University of California - Los Angeles, Los Angeles CA, May 2016.
13. Eleventh Annual Richard S.H. Mah Lectures on Modeling and Computation in Chemical and Biological Engineering, Northwestern University, Evanston, IL, January 2016.

Protocol Activity Status:

Technology Transfer: We ran the simulations for the protomer paper on the NSF XSEDE supercomputer Stampede 2, at UT Austin. Protein crystal simulations of rubredoxin were carried out with our 2017 INCITE Allocation (100,000,000 core hours) on the Titan super computer at the Oak Ridge National Laboratory Leadership Computing Facility (OLCF). Additionally, we applied for and were granted a renewal of our INCITE allocation for compute time on the Summit and Titan supercomputer in 2019 (395,000 node hours total). As part of this, we were awarded an Early Science Project on Summit (200,000 node hours) for “Large-Scales Simulation of Biological Crystallization”, with the aim to find the Wulff shape (or crystal habit) of protein crystal structures.

Preliminary simulations were also run on Stanford xStream Supercomputer, 2016-2017 for: “GPU-enabled simulations of the hydrophobic effect in biological self-assembly”

PARTICIPANTS:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Jens S Glaser
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:

International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: PD/PI
Participant: Sharon C. Glotzer
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: Y
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Vyas Ramasubramani
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Luis Y. Rivera-Rivera
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Contract Number: W911NF1510185

Title: Design of Protein Biomaterials Through Tailored Shape and Packing Strategies of Patchy Particles

PI: Sharon C. Glotzer

Major Goals:

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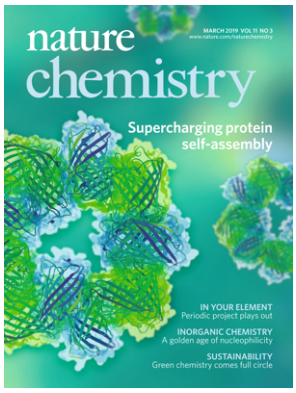
Accomplished under Goals:

GFP Protomer formation:



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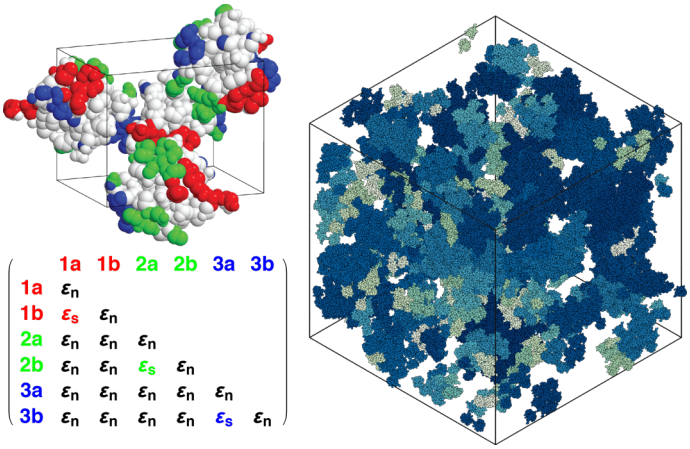

Left: Computational patchy shape model for the directed self-assembly of rubredoxin. *Top left*: experimental crystal unit cell with four proteins in orthorhombic $P2_12_12_1$ symmetry from PDB entry 1BRF, with unique interfaces on every protein colored in red, green, and blue. Light shaded, non-interface areas are purely repulsive. *Bottom left:* Matrix of interaction parameters between interfaces.

Right: Snapshot of a simulation of 8,192 proteins with crystallites highlighted by different shades, showing homogeneous nucleation.