AFRL-AFOSR-VA-TR-2023-0236

De Novo Biomachines

Beratan, David
DUKE UNIVERSITY
2200 W MAIN ST
DURHAM, NC, 27705
USA

12/29/2022
Final Technical Report

Air Force Research Laboratory
Air Force Office of Scientific Research
Arlington, Virginia 22203
Air Force Materiel Command

REPORT DOCUMENTATION PAGE

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED	
20221229	Final	START DATE 20190701	END DATE 20220630

4. TITLE AND SUBTITLE
De Novo Biomachines

5a. CONTRACT NUMBER	5b. GRANT NUMBER	5c. PROGRAM ELEMENT NUMBER
	FA9550-19-1-0331	61102F
5d. PROJECT NUMBER	**5e. TASK NUMBER**	**5f. WORK UNIT NUMBER**

6. AUTHOR(S)
David Beratan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
DUKE UNIVERSITY
2200 W MAIN ST
DURHAM, NC 27705
USA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)	11. SPONSOR/MONITOR'S REPORT NUMBER(S)
Air Force Office of Scientific Research 875 N. Randolph St. Room 3112 Arlington, VA 22203	AFRL/AFOSR RTB2	AFRL-AFOSR-VA-TR-2023-0236

12. DISTRIBUTION/AVAILABILITY STATEMENT
A Distribution Unlimited: PB Public Release

13. SUPPLEMENTARY NOTES

14. ABSTRACT
ABSTRACT:
The aim of this project is to design and synthesize bioinspired functional modules for information processing, to assemble these modules into functional elements, to characterize their performance, and to use theory to understand the observed function and to refine the performance. The project involves the close collaboration among theory, molecular and macromolecular design, synthesis, and state-of-the-art physical characterization methods that include fast and ultrafast dynamical experiments that interrogate charge and ion transport. The project will create functional assemblies based on de novo protein modules that will move charge across distances from nanometers to micrometers. The flow of electrons will trigger the polarization or depolarization of trans-membrane ionic concentration gradients. We will also explore the capture of mechanical energy to drive charge flow. The project will thus establish de novo modules (building blocks) that can be assembled into simple circuits that mimic the elementary processes of biological neural processing and may enable high- efficiency neurallike computation. The appeal of this approach is that it will define an enabling technology for the rapid manufacture of information processing networks (e.g., via printing of bioinspired de novo designed components) that circumvents the energetically wasteful need to maintain a host of ancillary cellular functions that do not directly contribute to neural computation tasks of interest.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	6

19a. NAME OF RESPONSIBLE PERSON		19b. PHONE NUMBER *(Include area code)*
PATRICK BRADSHAW	DISTRIBUTION A: Distribution approved for public release.	425-8492

REPORT DOCUMENTATION PAGE

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED	
		START DATE	END DATE
22 SEPT 2022	Final Report	1 JUL 2019	30 JUN 2022

4. TITLE AND SUBTITLE

De Novo Biomachines

5a. CONTRACT NUMBER	5b. GRANT NUMBER	5c. PROGRAM ELEMENT NUMBER
	FA9550-19-1-0331	
5d. PROJECT NUMBER	**5e. TASK NUMBER**	**5f. WORK UNIT NUMBER**

6. AUTHOR(S)

David N. Beratan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
Duke University, Office of Research Administration 2200 W. Main St. Ste 710 Durham, NC 27708-4677	

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)	11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFOSR 875 N. Randolph St., Rm 3112 Arlington, VA 22203-1954		

12. DISTRIBUTION/AVAILABILITY STATEMENT

for public release

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The aim of this project was to design and synthesize bioinspired functional modules for information processing, to assemble these modules into functional elements, to characterize their performance, and to use theory to understand the observed function and to refine the performance. The project involved the close collaboration among theory, molecular and macromolecular design, synthesis, and state-of-the-art physical characterization methods that include fast and ultrafast dynamical experiments that interrogate charge and ion transport. The project created functional assemblies based on de novo protein modules that will move charge across distances from nanometers to micrometers. The flow of electrons can trigger the polarization or depolarization of trans-membrane ionic concentration gradients. These studies can be used to further our understanding of how one may capture mechanical energy to drive charge flow. The project established de novo modules (building blocks) that can be assembled into simple circuits that mimic the elementary processes of biological neural processing and may enable high-efficiency neural-like computation. The appeal of this approach is that it defines an enabling technology for the rapid manufacture of information processing networks (e.g., via printing of bioinspired de novo designed components) that circumvents the energetically wasteful need to maintain a host of ancillary cellular functions that do not directly contribute to neural computation tasks of interest.

15. SUBJECT TERMS				
electron transfer, de novo proteins, redox cofactors, charge ratcheting				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
a. REPORT	b. ABSTRACT	C. THIS PAGE		
19a. NAME OF RESPONSIBLE PERSON			19b. PHONE NUMBER *(Include area code)*	
Dr. Patrick Bradshaw			(703)588-8492	

Distribution Statement
Cleared for public release.

Report Abstract
The aim of this project was to design and synthesize bioinspired functional modules for information processing, to assemble these modules into functional elements, to characterize their performance, and to use theory to understand the observed function and to refine the performance. The project involved the close collaboration among theory, molecular and macromolecular design, synthesis, and state-of-the-art physical characterization methods that include fast and ultrafast dynamical experiments that interrogate charge and ion transport. The project created functional assemblies based on de novo protein modules that will move charge across distances from nanometers to micrometers. The flow of electrons can trigger the polarization or depolarization of trans-membrane ionic concentration gradients. These studies can be used to further our understanding of how one may capture mechanical energy to drive charge flow. The project established de novo modules (building blocks) that can be assembled into simple circuits that mimic the elementary processes of biological neural processing and may enable high-efficiency neural-like computation. The appeal of this approach is that it defines an enabling technology for the rapid manufacture of information processing networks (e.g., via printing of bioinspired de novo designed components) that circumvents the energetically wasteful need to maintain a host of ancillary cellular functions that do not directly contribute to neural computation tasks of interest.

Technical Report

1. Accomplishments

Research Objectives

The computational design of cofactor-binding proteins represents a significant challenge that must be met to achieve the major aims of this proposal. During the project, the team built on its methodological breakthroughs to design proteins that aim to bind a donor-spacer-acceptor molecule, PZn-phenyl-NDI (where PZn represents a zinc porphyrin derivative and NDI represents a naphthalene diimide derivative). These designs used previously established methods to generate keystone interactions to the porphyrin and additionally aimed to engage several hydrogen-bonding groups in the NDI molecule. Proteins designed to hydrogen bond to NDI probe the effects of hydrogen bonding on the dynamics of charge separation and lifetime of the charge-separated state. We used our new computational approaches, guided by structural bioinformatics, to sample and score hydrogen bonds to the carbonyl groups of NDI. In addition, the designed protein scaffolds bind a molecule with extensively delocalized singlet states and polaron states, and our approach uses the EnFold method to design the binding of abiological cofactors. Designs to bind extended cofactors test our design capabilities and will allow for the control of electron flow. We continued to characterize these designs experimentally for expression, oligomerization state, stability, and cofactor binding. We have synthesized cofactors and confirmed their binding to the de novo proteins. The cofactor-bound proteins were interrogated spectroscopically by Therien's

group, and the corresponding photo-induced electron-transfer dynamics were examined theoretically in Beratan's group.

Research Accomplishments and Results Dissemination

In support of our objectives, covalently linked donor-bridge-acceptor system designs were developed. The functional abiological cofactors were bound by the de novo proteins developed by DeGrado. In these systems, optical absorption will trigger a photoinduced charge separation (CS) reaction in the protein environment. These designs take advantage of specific hydrogen bonding interactions that stabilize, to specific extents, the relative energies of the respective ground, electronically excited, and charge-separated states. Such CS reactions that deliver charges to specific protein sites can be coupled to next-generation designs that promote amino acid side chain rotamer configurational switching. This direction evolves proteins that replicate the functionality of stimulus-driven opening of a biological gated ion channel; work along these lines aims to realize a fundamental ratcheting paradigm in which an optical stimulus modulates the conformational landscape and biases the macromolecule toward populating a new functional conformation.

Understanding how the de novo protein environment influence the optical and electron transfer characteristics of light absorbing electron transfer active units represents a core theoretical challenge that we addressed in this project. We developed electronic structure models to describe how electric fields interacting with light absorbing molecules may be used to enhance the light absorbing properties of those chromophores. Theories of this kind were being used to model how the macromolecular environment impacts light absorption and redox characteristics of electron transfer active constructs. A key aim of this project was to enable ratcheted multi-step electron transfer in de novo constructs. We developed a first successful kinetic models to describe multi-step, ratcheted electron hopping fluxes at the nanoscale. These models establish the foundational principles for ratcheted electron transfer in the targeted de novo protein assemblies.

Trainees developed skills in computational protein design, using software such as Rosetta, as well as bioinformatics tools and kinetic network models developed in-house. They also gained training in protein expression, purification, and spectroscopic analysis of the designed proteins and cofactors. Structural analysis will provide training in X-ray crystallographic methods.

In addition, trainees were a part of regularly scheduled (biweekly) meetings among the Therien, Beratan, and DeGrado labs, where the team discussed progress toward the goals of the proposal. The trainees also discussed their results in their home departments through regular research group meetings and at other seminars. At times when travel is not restricted by pandemic concerns, all members of the research team participate in national and international research conferences, including meetings of the American Chemical Society, American Physical Society, Telluride Science Research Center, and Gordon Research Conferences. Additional training opportunities are

afforded by our teaching of interdisciplinary graduate courses focused on topics relevant to this project. For example, in Fall 2020, we taught an introductory hands-on course on quantum dynamics, and one of the graduate students involved in this project assisted in the development and deployment of novel instructional materials.

Five manuscripts, cited on the report web page, were published in peer reviewed journals during the project period. Research results are also documented in the Ph.D. theses of our graduate students. Our activities aim to develop strategies and theories to assist in the design of proteins that bind and tune the function of abiological cofactors, and that may have designed functionality.

Year three studies (the final project year) continued experimentally and theoretically to interrogate proteins (that were designed to bind the PZn-phenyl-NDI and PZn_3 species) for expression, oligomerization state, stability, and cofactor binding. After confirming cofactor binding, the cofactor-bound proteins are being studied spectroscopically by Therien's group and the DeGrado group is collecting structural data on the complexes. We will continue to use structural data and information from simulations to make refinements to the protein sequence, in order to influence the observable photoinduced electron-transfer dynamics.

We have elucidated de novo protein designs that bind donor-acceptor species that are expected to undergo photoinduced electron transfer and trigger pre-programmed structural changes. Light triggered dynamical events can be interrogated in these structures using time-resolved pump-probe transient optical methods. Next-generation designs would modulate thermodynamic driving forces for photoinduced charge separation and thermal charge recombination events; these designs will enable further fine tuning of the timescales over which protein structural regulation occurs. New functional protein designs can next be prepared that employ photoacids capable of photochemically liberating a proton within the protein interior, targeting a nearby amino acid side chain that serves as a proton acceptor; these latter designs will develop complimentary approaches by which light excitation gates protein structure. Efforts along these lines are tied closely to experimental work carried out in the DeGrado lab; photoinduced electron-transfer dynamics evinced by these systems can be modeled further in Beratan's group.

Importantly, theory, modeling, and simulation in Beratan's group has developed models of how thermal fluctuations influence electron transfer and of electron ratcheting, and the theory team has also pursued early protein design studies focused on flavin-binding proteins capable of carrying out multi-electron redox chemistry. As well, the theory team has completed studies of how electric fields impinging on molecular chromophores may be used to optimize the light-absorbing characteristics of the chromophores, enabling the design of next generation high-oscillator strength chromophores with applications to photoinduced electron transfer, organic electronics, and optoelectronics.

Impacts

On the discipline and other disciplines

In addition to the specific novel and functional compositions of matter described in our publications and in the above report, we developed novel computational approaches to enable the design of de novo proteins that bind (noncovalently) molecular species that carry out novel optical and electron transfer function. These methods are being used widely in our research groups to design next generation de novo structures that may carry out diverse electron transfer function, including charge ratcheting. There are payoffs to other disciplines as well. For example, we demonstrated that our computational approaches may be used to design de novo proteins that bind small molecules (drugs), suggesting a new paradigm of creating tailored "antidotes" to small molecule species.

On human resources, teaching, and our institutions

We used this project to motivate the development of novel undergraduate and graduate courses in biophysical chemistry and quantum dynamics. We also created a rich environment for training our graduate student and post-doctoral researchers in an interdisciplinary environment that includes, theory, modeling, simulation, spectroscopy, chemical synthesis, molecular biology, computational biology, physical biochemistry, and structural biochemistry. The direct impact of this project on the infrastructure of our institutions was limited. We suspect that the tools developed in this project will have long-term impact on the quality of life and national competitiveness, by enabling the design and synthesis of novel bio-inspired materials of potentially transformative value in energy science, sensing, and biomedicine.