REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
08-06-2018	Final Report	20-Sep-2014 - 19-Mar-2015

4. TITLE AND SUBTITLE
Final Report: In Search of Biomimetic Catalysts: Exploring Sequence-Reactivity Space with Unnatural Oligomeric Backbones

5a. CONTRACT NUMBER
W911NF-14-1-0313

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Wisconsin - Madison 1.00
The Board of Regents of the University of Wisconsin S
Suite 6401
Madison, WI 53715 -1218

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
66095-CH-DRP.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Samuel Gellman
UU	UU	UU	UU		19b. TELEPHONE NUMBER 608-262-3303

RPPR Final Report
as of 08-Jun-2018

Agency Code:

Proposal Number: 66095CHDRP **Agreement Number: W911NF-14-1-0313**
INVESTIGATOR(S):

Name: Samuel H Gellman Ph.D.
Email: gellman@chem.wisc.edu
Phone Number: 6082623303
Principal: Y

Organization: **University of Wisconsin - Madison**
Address: The Board of Regents of the University of Wisconsin Sys, Madison, WI 537151218
Country: USA
DUNS Number: 161202122 EIN: 396006492
Report Date: 19-Jun-2015 Date Received: 08-Jun-2018
Final Report for Period Beginning 20-Sep-2014 and Ending 19-Mar-2015
Title: In Search of Biomimetic Catalysts: Exploring Sequence-Reactivity Space with Unnatural Oligomeric Backbones
Begin Performance Period: 20-Sep-2014 **End Performance Period:** 19-Mar-2015
Report Term: 0-Other
Submitted By: Samuel Gellman Email: gellman@chem.wisc.edu
Phone: (608) 262-3303
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: **STEM Participants:**

Major Goals: Please see attached report.

Accomplishments: Please see attached report.

Training Opportunities: The participants had the opportunity to master diverse synthetic and analytical skills, and to develop collaboration skills.

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Sam Gellman
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: Y
Other Collaborators:

Participant Type: Co-Investigator
Participant: David Walt
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:

International Travel:
National Academy Member: Y
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Melissa Macdonald
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

FINAL REPORT:
In Search of Biomimetic Catalysts: Exploring Sequence-Reactivity Space with Unnatural Oligomeric Backbones

Long-term Goal

Our long-term goal has been and remains to identify hetero-oligomers with unnatural backbones and specific sequences that function as highly effective catalysts. We hypothesize that such oligomers will display discrete folding patterns (tertiary structure). The nature of the reaction catalyzed is not important at this early stage of the program, as long as the reaction is challenging from the chemical perspective. We seek high catalytic activity, >1000-fold above the background rate in aqueous solution, at neutral pH and room temperature, for reactions such as transamidation, amide hydrolysis, or unactivated ester hydrolysis. In addition, we have sought to establish an analytical strategy that allows detection of weakly active catalysts on the single-molecule level. Such a method would facilitate the evaluation of enormous oligomer diversity in our search of catalytic activity. The novel exploratory experiments pursued under the auspices of this short-term grant represent early steps toward the ultimate goal of identifying unprecedented types of biomimetic catalysts.

Motivation

All highly effective catalysts found in living systems on earth are folded polymers with specific and irregular subunit sequences, either poly-L-α-peptides or poly-D-ribonucleotides. Thus, biology seems to tell us that polymeric skeletons capable of adopting discrete conformations are particularly well suited, perhaps uniquely so, for achieving high catalytic activities. However, from a chemical perspective it is logical to predict that polymeric backbones other than those of L-α-peptides and D-ribonucleotides would be competent as molecular scaffolds to host catalytic active sites. We seek to explore this prediction.

In light of the apparent dependence of high-activity biopolymer catalysis on a specific sequence and a discrete folding pattern, we hypothesize that unnatural polymeric catalysts will have to display these characteristics. This hypothesis represents a very high barrier to experimental action, because current understanding of the folding of unnatural backbones is limited to a relatively small number of systems, such as β-peptides, γ-peptides, aryl oligoamides, phenylene ethynylene oligomers, and peptoids.[1] Moreover, even for the most carefully studied of these "foldamer" families, conformational control is presently limited to secondary structure (helices, sheets or reverse turns). In contrast, biopolymer catalysis seems to require backbone ordering at the level of tertiary structure.

Design of new and highly effective protein or RNA catalysts represents an extremely important goal and has been avidly pursued, but rational approaches are not yet within reach because our mechanistic understanding enzyme or ribozyme function remains incomplete. Since relationships between sequence and structure are much better understood for proteins and RNA than for any unnatural foldamer, pursuit of a rational strategy is unlikely to be productive for development of highly active catalysts based on unnatural foldamer backbones.

Artificial screening and selection methods offer an alternative to rational design for generating new protein- or RNA-based catalysts. The most effective of these methods take advantage of several features that are unique to biopolymers, including: (1) the biosynthetic machinery, which provides access to large libraries; (2) the self-replicating nature of viruses or single-celled organisms, which enables amplification after active species have been selected; and (3) the ability to encode protein or RNA sequences in DNA, which enables facile identification of active sequences after selection. Unfortunately, these powerful tools are not available for hetero-oligomers with unnatural backbones.

There has been very little effort so far to identify oligomeric catalysts with unnatural backbones, presumably because there is widespread recognition of the challenges and limitations outlined above. This

grant funded first steps in our unprecedented (and high-risk) effort to identify synthetic and analytical strategies that could ultimately enable us to discovery biopolymer-mimetic catalysts with unnatural backbones.

Achievements and ongoing challenges

The Wisconsin team has pursued two approaches for generating large collections of oligomeric chains with unnatural backbones. One approach involves ring-opening polymerization of β-lactam mixtures, which produces diverse pools of nylon-3 (poly-β-peptide) chains (Figure 1).

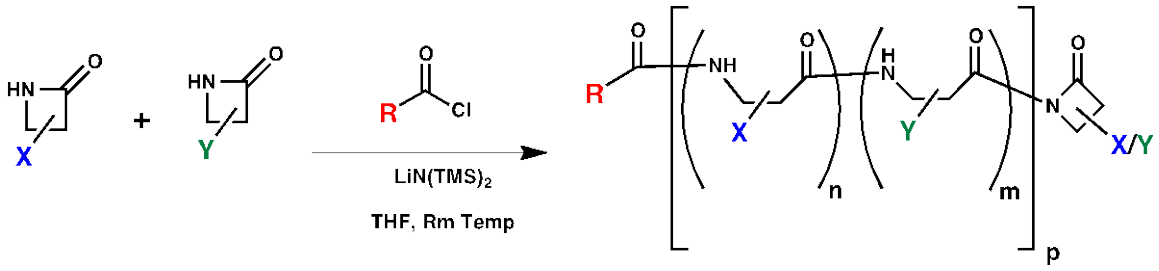

Figure 1. β-Lactam Polymerization.

A second approach for generating oligomer chain pools involves an unconventional implementation of solid-phase synthesis (Figure 2). We have come to favor this latter approach, and we have generated many pools of α/β-peptides (i.e., oligomers that contain both α-amino acid and β-amino acid residues) in this way.

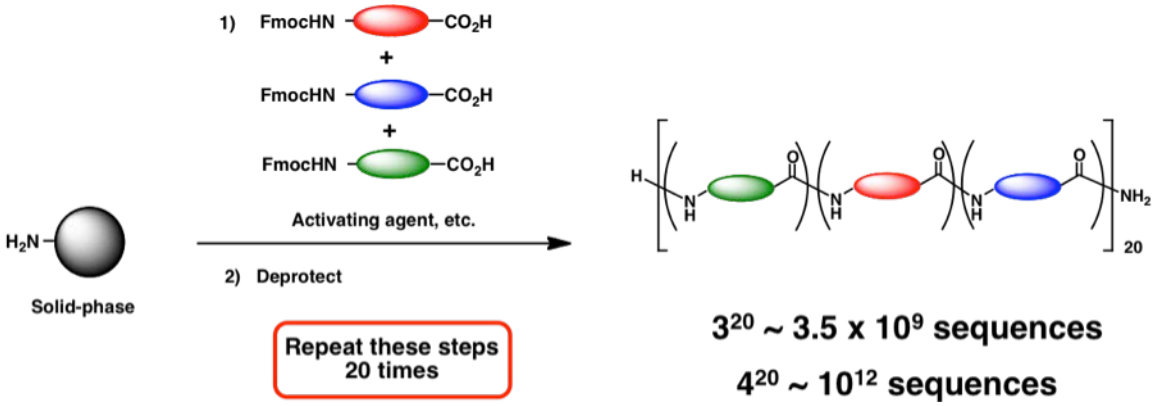

Figure 2. Synthesis of oligomer pools via solid-phase methodology.

The choice of reaction(s) to be used in our search for novel catalysts is crucial. The reaction must lead to a large change in fluorescence in order to enable detection at the single-molecule level, via the techniques employed by the Tufts team (discussed below). Thus, the starting material should display minimal fluorescence, but the product should be highly fluorescent. In addition, the reaction should not be so "easy" that catalysis is readily achieve with small molecules (e.g., hydrolysis of activated esters), nor so "difficult" that the prospects of catalyst discovery are infinitesimal (e.g., hydrolysis of peptide bonds or other simple amides). We identified the intramolecular transamidation reaction shown in Figure 3 as a promising test reaction. Under the aqueous buffer conditions we employ, the rate constant for spontaneous cyclization is ~10^{-5} per second.

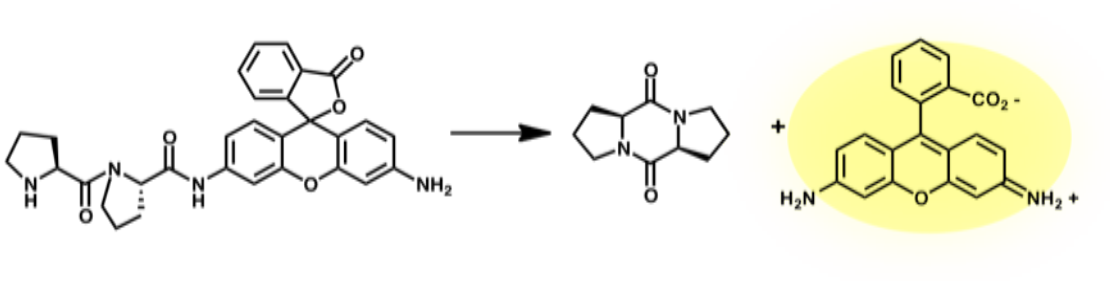

Figure 3. Fluorogenic intramolecular transamidation reaction.

Our experimental approach hinges upon unique analytical capabilities developed by the Tufts team. The Walt group can create an array of ~50,000 femtoliter-scale "reaction vessels" that can be individually monitored for the growth of fluorescence. In previous efforts, these researchers have used this capability to study enzymatic reactions at the single-molecule level (Figure 4). This approach requires a fluorogenic substrate.

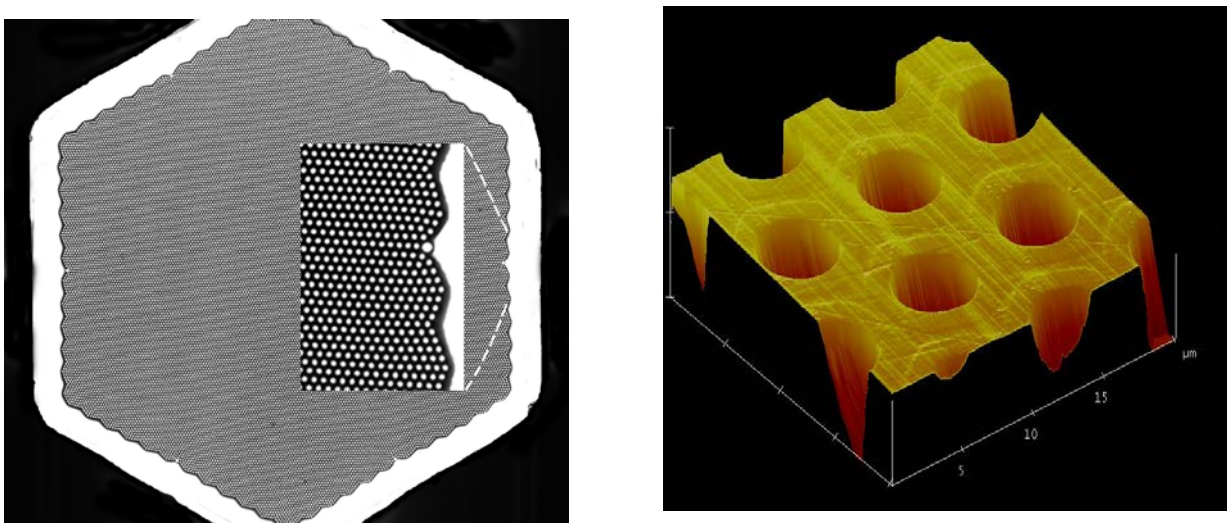

Figure 4. Images of the femtoliter arrays created by the Tufts team. Each well in the array has a volume of ~46 fL, and each well can be independently monitored for growth of fluorescence.

Our experimental approach involves generating large pools of oligomers via the methods illustrated in Figures 1 and 2. A pool is assessed by generating a solution containing the pool in the μM concentration range, based on the average molecular weight of the pool members, along with the fluorogenic substrate (e.g., the dipeptide shown in Figure 3). This solution is then distributed into the wells of the femtoliter array, and the wells are monitored for a growth in fluorescence. If a pool contains a few oligomers that are effective catalysts, then a small number of wells should display increasing fluorescence. We hypothesize that if such a signature were observed, then we could work toward identifying the sequences of active catalysts by "biased" resynthesis of the pool in ways that skew the population toward certain subsets, and comparisons of activities among different sub-pools with distinct sequence biases.

As we took initial steps to implement this plan, we identified an unanticipated problem arising from sensitivity limits of the femtoliter array. The enzymes previously studied by the Walt laboratory are highly

active catalysts, but our exploratory efforts require that we be able to detect catalysts that have only marginal activity. (We hypothesize that activity could be improved by rational or selection-based methods, once we had initial "hits" as starting points.) The Walt laboratory's standard format for conducting femtoliter-array measurements have involved mechanical sealing of the individual wells in the array, but this approach allows monitoring for only ~0.5 hr (after that evaporation becomes problematic). Going forward, we are exploring new array formats that allow sealing with an oil layer, which enables monitoring over longer periods. This improvement should make the approach more sensitive to weak catalysts.

Another technical challenge arises from the substrate: to the extent that the substrate displays intrinsic fluorescence (before hydrolysis), we lose sensitivity to the hydrolytic event. Our dipeptide substrates (Figure 3) display this problem. Recently, however, we have begun to examine an unactivated ester as a hydrolysis substrate (Figure 5). This substrate was provided to us be Dr. Luke Lavis of Janelia Farms. The difference in fluorescence between the ester and hydrolysis projects is much larger than for the dipeptide, which has encouraged us to screen for ester hydrolysis catalysts.

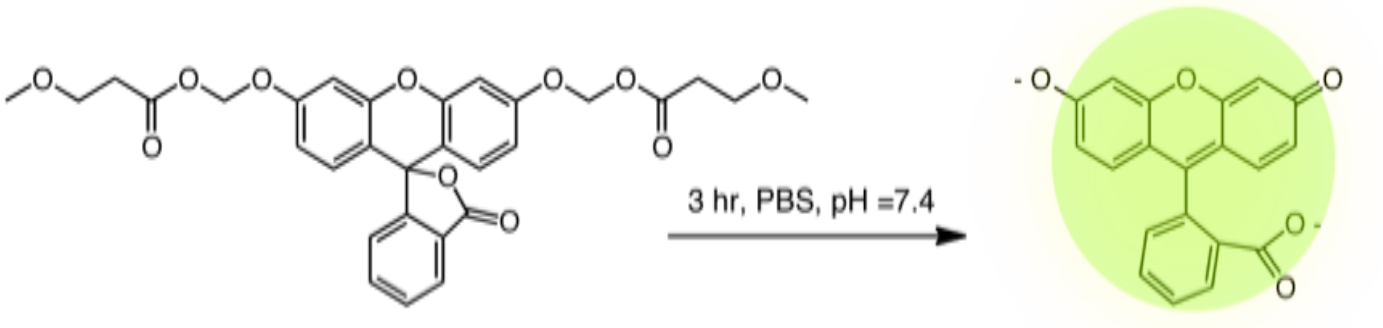

Figure 5. Fluorogenic ester hydrolysis reaction.

The UW-Tufts team is continuing to generate new oligomer pools and to evaluate them with the femtoliter array technique to determine whether any contain active catalysts of dipeptide cyclization or ester hydrolysis. It is not yet clear whether we have identified pools that contain active catalysts, because the sensitivity and capabilities of our analytical strategy are not yet fully established.