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Eric T. Kool, Niaz Banaei

5d. PROJECT NUMBER

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5f. WORK UNIT NUMBER

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Stanford University
3160 Porter Drive
Suite 100
Stanford, CA 94304 -8445

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14. ABSTRACT
This project was aimed at studying and developing polyfluorophores on a DNA backbone for sensitivity to the environment; ultimately, to sense organic bacterial metabolites in the air. Overall, we made substantial progress in synthesizing new classes of fluorescent DNA monomers and DNA polyfluors and in finding new ways to put them on solid supports. We synthesized and studied a new class of DNA fluorophores based on aggregation-induced emission, which we hypothesized might be particularly environmentally sensitive. Moreover, during our studies we identified a new type of dye that undergoes unusual photobleaching with color changes, which may lead to new

15. SUBJECT TERMS

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Eric Kool
UU	UU	UU	UU		19b. TELEPHONE NUMBER 650-724-4741

Report Title

Final Report: Interaction of Bacterial Metabolites with Fluorescent Synthetic DNA

ABSTRACT

This project was aimed at studying and developing polyfluorophores on a DNA backbone for sensitivity to the environment; ultimately, to sense organic bacterial metabolites in the air. Overall, we made substantial progress in synthesizing new classes of fluorescent DNA monomers and DNA polyfluors and in finding new ways to put them on solid supports. We synthesized and studied a new class of DNA fluorophores based on aggregation-induced emission, which we hypothesized might be particularly environmentally sensitive. Moreover, during our studies we identified a new type of dye that undergoes unusual photobleaching with color changes, which may lead to new tools for biology and microscopy. Finally, very recently we completed a 1296-member library of DNA-polyfluorophores, and we hope that it will be able to distinguish organic compounds in the air. Although the project ran out of time before we could begin to test this library against bacterial colonies, the work has allowed us to discover novel DNA-dye structures and novel mechanisms of sensing and switching. We hope to test our recently completed ODF library with bacterial metabolites in the future.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Plenary Speaker, "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", FB3: Annual International Meeting (Fluorescent Biomolecules and Their Building Blocks), San Diego, CA, August 6-9, 2014.

Invited Speaker, "Oligomeric DNA Fluorophores for Labeling and Chemosensing", Waitt Advanced Biophotonics Center 2nd Annual Symposium, Salk Institute, La Jolla, CA, Sept 26, 2014.

Invited talk: Department of Chemistry, University of Alberta (Edmonton), "Designer DNA Bases: Fluorescent Probes for Molecules and Mechanisms in Biology", Sept 8, 2014.

Invited talk: Department of Chemistry, University of Florida (Gainesville, FL), "Designer DNA Bases: Fluorescent Probes for Molecules and Mechanisms in Biology", Sept 15, 2014.

Invited talk: Department of Chemistry, University of Zurich (Zurich, Switzerland), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", January 12, 2015.

Invited talk: Department of Chemistry, University of Basel (Basel, Switzerland), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", January 13, 2015.

Invited talk: Department of Chemistry, University of Bern (Bern, Switzerland), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", January 14, 2015.

Invited talk: Department of Chemistry, University of Geneva (Geneva, Switzerland), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", January 15, 2015.

Invited Speaker, "The New DNA", Personalized Medicine World Conference 2015, Mountain View, CA, Jan 28, 2015.

Keynote Address: "Designer DNA Bases: Probing Molecules and Mechanisms in Biology," National Award Symposium, 249th American Chemical Society National Meeting, Denver, March 22, 2015.

Invited talk: Department of Chemistry, University of North Carolina (Chapel Hill, NC), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", April 16, 2015.

Department of Chemistry, Miami University (Oxford, OH), "Designer DNA Bases: Probing Molecules and Mechanisms in Biology", September 17, 2015.

Invited talk: "Small Molecule Strategies for Mapping RNA Structure and Sequence," 251st American Chemical Society National Meeting, San Diego, March 16, 2016.

Keynote Address: "Fluorescent Sensors Built from DNA", FB3: Annual International Meeting (Fluorescent Biomolecules and Their Building Blocks), Tianjin, China, July 13-17, 2016.

Invited talk: "Designer DNA Bases with Biological Function", 252nd American Chemical Society National Meeting, Philadelphia, August 21, 2016.

Number of Presentations: 15.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

P.I received the American Chemical Society's Breslow Award for Achievement in Biomimetic Chemistry
P.I. received Dean's Award for Distinguished Teaching, Stanford University.

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Roy Ke Min Chan	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Eric T. Kool, Ph.D.	0.00	
Niaz Banaei, M.D.	0.08	
FTE Equivalent:	**0.08**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Rajiv L. Gaur	0.39
FTE Equivalent:	**0.39**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

None to report.

Scientific Progress and Accomplishments (Final Report)

Abstract of project: Bacteria release molecules into the growth medium and air as they grow. Here we hypothesize that different bacteria can be distinguished by the distinct mixtures of metabolites that they release. Our preliminary research has shown that short synthetic DNA-polyfluorophores can be highly sensitive to organic molecules in the air and to enzymes in solution, and thus can act as sensors of such bacterial products. In this project we are studying how these unusual DNA-like molecules interact, in the molecular and photophysical sense, with bacterial products in the air. Experiments with systematically varied fluorophores will help determine what structural features of the DNA-polyfluorophores are most responsible for selective signals, and what features lead to the largest changes in fluorescence. We will also study how these natural products and the corresponding responses vary among different bacterial strains. The project is collaborative, involving the P.I.'s laboratory in the Chemistry Department and the co-P.I.'s clinical microbiology laboratory at Stanford University Medical Center. The basic information gained from these experiments will ultimately enable the design of rapid and inexpensive sensors that identify bacteria in the field.

Overview of progress. This project was aimed at studying and developing polyfluorophores on a DNA backbone for sensitivity to the environment; ultimately, to sense organic bacterial metabolites in the air. Overall, we made substantial progress in synthesizing new classes of fluorescent DNA monomers and DNA polyfluors and in finding new ways to put them on solid supports. We synthesized and studied a new class of DNA fluorophores based on aggregation-induced emission, which we hypothesized might be particularly environmentally sensitive. Moreover, during our studies we identified a new type of dye that undergoes unusual photobleaching with color changes, which may lead to new tools for biology and microscopy. Finally, very recently we completed a 1296-member library of DNA-polyfluorophores, and we hope that it will be able to distinguish organic compounds in the air. Although the project ran out of time before we could begin to test this library against bacterial colonies, the work has allowed us to discover novel DNA-dye structures and novel mechanisms of sensing and switching. We hope to test our recently completed ODF library with bacterial metabolites in the future.

Research Aim 1. Study of the structural and mechanistic origins of sensitivity.

This research aim received a large share of our focus during this project. We succeeded in testing the effect of ODF size on sensitivity, and also evaluated new ways to associate ODFs on solid supports, as described in interim reports. Much of our effort was focused on synthesis of several new and structurally interesting fluorescent deoxyriboside monomers for incorporation into ODFs. Structures developed and synthesized during this project are given in Fig. 1.

In year 3 we discovered a truly unusual phenomenon involving the ODF dye having the sequence SSVV, where S is a nonfluorescent spacer and V is a phenylethynyl pyrene (PEP) dye (last entry, Fig. 1). Remarkably, we found that whereas the V monomer is stable, the VV dimer photobleaches in seconds in a UV spectrometer. In addition, rather than simply becoming dark (as usually happens with photobleaching), this SSVV dye changes color, from green to blue (Fig. 2a). Mechanistic studies were carried out that point to an unprecedented mechanism of oxygen

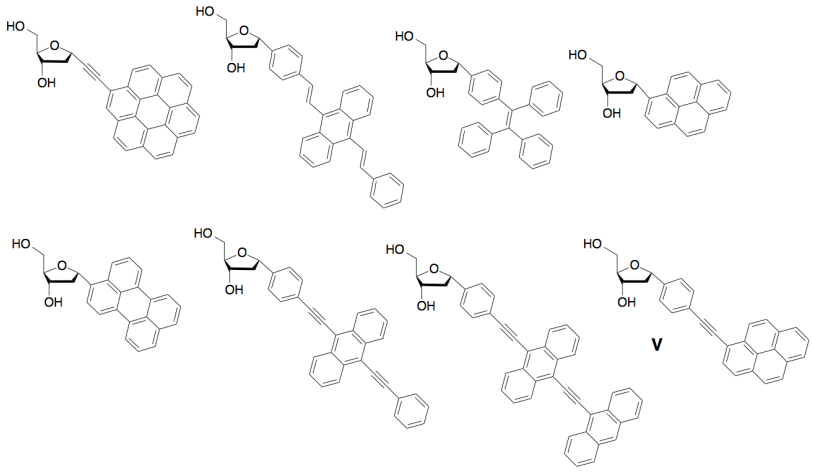

Figure 1. Synthetic fluorescent nucleoside monomers prepared during this project. Most are new and not yet published.

reaction with the alkynes of adjacent dyes (Fig. 2c). A paper describing this is near submission (see below). We believe that this dye could be useful in studies of membrane diffusion, in superresolution imaging, and in quantitative measurements of light exposure.

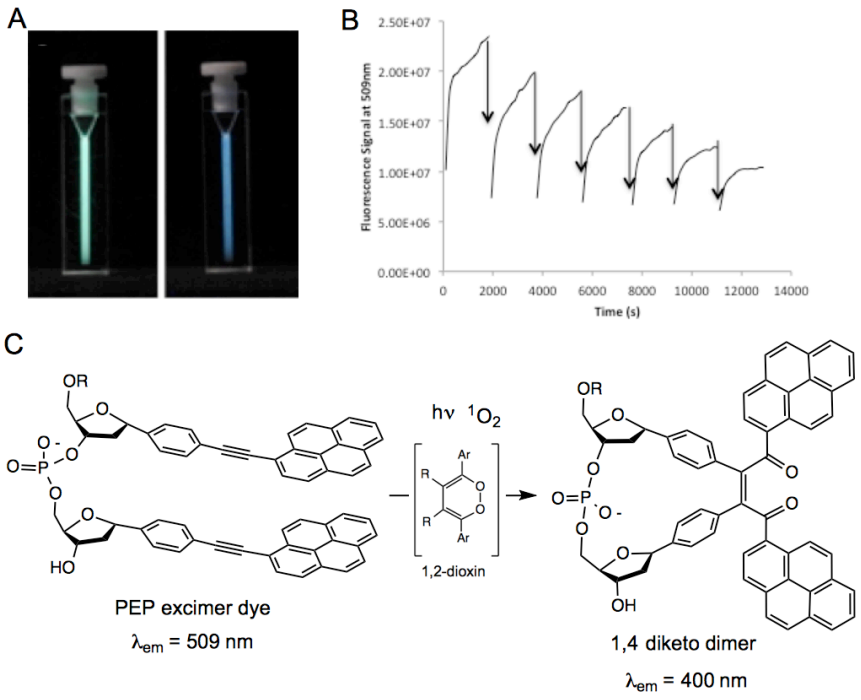

Figure 2. Color change photobleaching of the synthetic alkyne dye dimer SSVV. (A) Color change from green to blue. (B) Cycles of fluorescence recovery after photobleaching (FRAP). (C) Proposed novel mechanism of singlet oxygen addition leading to loss of excimer signal.

Beyond the reported one, two additional papers citing this ARO support are nearly completed or are in revision; although they are not yet available for reporting, their authors and titles are listed below. Additional publications will likely also arise further in the future as we screen our ODF library for sensing responses.

Ke Min Chan, Dominik Koelmel, Shenliang Wang, Eric T. Kool, Unusual Color-change Photobleaching of an Alkyne-substituted Excimer Fluorophore, *TBA* **2017**, to be submitted October 2016.

Wang Xu, Ke Min Chan, Eric T. Kool, Fluorescent nucleobases for study of DNA and RNA, *Nature Chemistry* **2016**, in revision.

Research Aim 2. Mechanisms of interactions of metabolites with ODFs printed on paper. Due to our discovery of the unusual color-change photobleaching mechanism, and our discovered need to synthesize a complete new ODF library (see below), we shifted our efforts away from this aim so that we could focus on completing publishable studies from Aims 1 and 3. It is worth noting that a recent PhD thesis published by a group member (H. Kwon, 2016) concluded that paper-based ODF sensors yielded lower sensitivity than those on PEG-polystyrene beads, so we will focus future efforts on that latter type of substrate.

Research Aim 3. Synthesis of ODF sensors on beads and study of fluorescence changes with bacterial metabolites. As originally proposed, we planned to start this study with bacteria using a previously prepared ODF library. However, subsequent testing revealed that our existing library was no longer reliably decodable, presumably due to air or light degradation. Unfortunately, this meant that those previous ODFs on beads could not be useful in this project. Thus it was necessary to prepare an entirely new library. While time consuming, we felt that this was also an opportunity to make fluorophores that were more sensitive to their environment, and we did make strong progress on that front with the new fluorescent monomers characterized in Aim 1.

Given that an ODF library was not yet available (until now) to test against bacteria, the Banaei lab focused their efforts on developing methods for optical sensing of pathogenic bacterial strains, using a previously published colorimetric sensor library approach (Carey et al, JACS 2011, 133, 7571). To this end, the Banaei lab studied organisms grown on agar and in broth culture. In these experiments pigment-based sensors were embedded inside agar plates or blood culture bottles, the media was spiked with pathogenic bacteria and sensors were used to measure the bacterial volatiles in culture headspace. The methods and expertise for culturing and headspace analysis developed during this work can be applicable for future fluorescence (ODF) studies with bacterial metabolites.

To advance the goals of Aim 3, the Kool lab proceeded to synthesize a new ODF library on PEG-polystyrene microbeads containing 1296 different sequences. This was completed recently, near the end of year 3 of the project. It shows an appealingly broad range of fluorescence emission using a single-wavelength excitation (Fig. 3). We plan to test the sensitivity of these ODFs to organic species in the air and to other physicochemical stimuli as well.

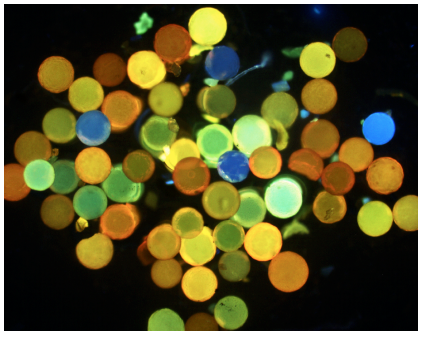

Figure 3. Fluorescence image of new ODF library on polystyrene microbeads (140 microns) completed as part of this project. Excitation 365 nm; emission ranges from violet-blue to orange.

Outlook. Our development of new fluorescent nucleoside monomers will aid in many future studies of sensors. Moreover, our recent completion of a new ODF library will lead to multiple sensing studies of varied chemical species (including bacteria) and physical stimuli. Finally, our discovery of a novel color-change dye may well have important uses in biology and possibly in other fields as well.