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6. AUTHORS

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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.

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Philip Lukeman
UU	UU	UU	UU		19b. TELEPHONE NUMBER 718-990-2920

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RPPR Final Report
as of 14-Jun-2021

Agency Code: 21XD

Proposal Number: 69052LS **Agreement Number: W911NF-16-1-0178**

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DUNS Number: 073134744 EIN: 111630830
Report Date: 14-Jan-2020 Date Received: 06-Jun-2021
Final Report for Period Beginning 15-Apr-2016 and Ending 14-Oct-2019
Title: 8.1 Biochemistry: A Polyvalent, Allosteric Whole-Virus Binding Platform for Norovirus Detection
Begin Performance Period: 15-Apr-2016 **End Performance Period:** 14-Oct-2019
Report Term: 0-Other

Submitted By: Philip Lukeman Email: phil.lukeman@gmail.com
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 8 **STEM Participants:** 8

Major Goals: Goals: synthesis and optimization of a polyvalent DNA origami aptamer-displaying "Claw" that can be incorporated into a rapid, point-of-care, electrochemical Norovirus sensor. The proposal's aims are i) to screen various tethered/blocked claw designs to improve 1:1 capsid solution-phase binding efficiency and selectivity, ii) to functionalize surfaces with untethered claw and test binding for models of sensor construction, and iii) optimize claw aptamer recognition sites (before and after attachment to claw) to optimize binding strength and, ultimately, selectivity between different norovirus serotypes.

Accomplishments: Achievements across length of grant: Trained 8 new students.
Synthesized and tested claws – structures formed; however, studied Norovirus aptamers and observed no binding under physiological/sensing relevant conditions.
Pivoted to study Origami triangles as nanoscale polyvalent virus simulants.
Synthesized & validated triangle origami variants displaying: VLP binding – Electroactive Dyes – Surface attachment chemistry. Developed cells & electrodes that are large-surface-area gold 'chips'. Characterized electrode surfaces by AFM. Optimized reproducible attachment of triangles to electrodes; measured their electrochemical activity. Demonstrated electroactive dye attachment to electrode/triangle by strand displacement. Showed preliminary sensing of VLPs but supply issues limited this study. Performed fundamental study of conformational-change sensing using other triangles as virus simulats – successfully differentiated polyvalent from lower valent binding, making the first structure-switch electrochemical biosensor capable of recognizing virus-sized objects (and not their subunits).

Submitted to Angewandte Chemie; rejected in 4/2019 with request to do more modelling, we did so – engaging Profs Carlos Castro/Haijun Su + Grad Student Chao-min Huang (all at Ohio State) and performed major revisions. As of 31 October 2019 the revision was complete and about to be submitted.

Future work will focus on structure-activity relationships with DNA origami sensors.

Training Opportunities: Trained 8 new undergraduate students

Results Dissemination: Presented this work at FNANO conference in 2018; publication under revision at the end of the period of performance for Angewandte Chemie.

Honors and Awards: Nothing to Report

RPPR Final Report
as of 14-Jun-2021

Protocol Activity Status:

Technology Transfer: Nothing to Report

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Participant Type: PD/PI
Participant: Philip Stefan Lukeman
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
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National Academy Member: N

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Project Contribution:
National Academy Member: N

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Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Person Months Worked: 6.00 **Funding Support:**
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National Academy Member: N

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Project Contribution:
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Date Received: 29-Aug-2017 Completion Date: 9/9/16 3:19PM
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Authors: Ekaterina, Selivanovitch
Acknowledged Federal Support: **N**

Partners

,

All zero-hour consultants/advisors, provided technical advice and materials. Prof Kevin Plaxco, Chemistry, UC Santa

I certify that the information in the report is complete and accurate:
Signature: Philip Lukeman
Signature Date: 6/6/21 4:28PM

Scientific Progress and Accomplishments

Final Report

"8.1 Biochemistry: A Polyvalent, Allosteric Whole-Virus Binding Platform for Norovirus Detection"

Philip S Lukeman
Chemistry, St. John's University

Period of performance April 15-2016 to Oct 31-2019
Proposal Number: 69052-LS
Agreement Number: W911NF1610178

Abstract

Goals: synthesis and optimization of a polyvalent DNA origami aptamer-displaying "Claw" that can be incorporated into a rapid, point-of-care, electrochemical Norovirus sensor. The proposal's aims are i) to screen various tethered/blocked claw designs to improve 1:1 capsid solution-phase binding efficiency and selectivity, ii) to functionalize surfaces with untethered claw and test binding for models of sensor construction, and iii) optimize claw aptamer recognition sites (before and after attachment to claw) to optimize binding strength and, ultimately, selectivity between different norovirus serotypes.

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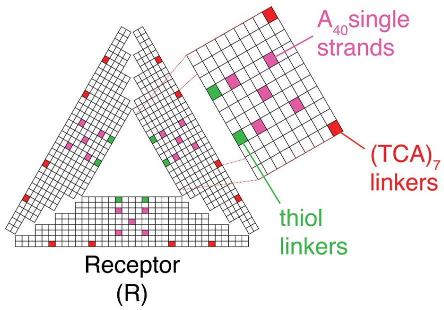
Submitted *to Angewandte Chemie*; rejected in 4/2019 with request to do more modelling, we did so – engaging Profs Carlos Castro/Haijun Su + Grad Student Chao-min Huang (all at Ohio State) and performed major revisions. As of 31 October 2019 the revision was complete and about to be submitted.

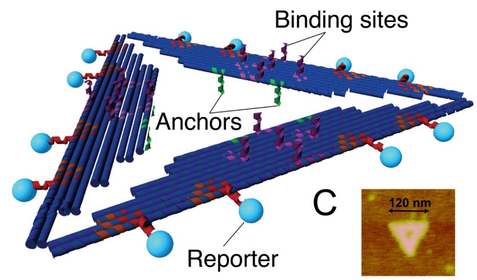
Future work will focus on structure-activity relationships with DNA origami sensors.

Editor's note (*after* period of performance): after this revision and further work supported by ARO grant W911NF-19-1-0326 the work was ultimately published in *Nanoscale*
(=ARO-supported undergraduate co-authors):.*
Arroyo-Currás N, Sadeia M, Ng AK*, Fyodorova Y*, Williams N*, Afif T*, Huang CM, Ogden N, Andresen Eguiluz RC, Su HJ, Castro CE, Plaxco KW, Lukeman PS* , 'An electrochemical biosensor exploiting binding- induced changes in electron transfer of electrode- attached DNA origami to detect hundred nanometer-scale targets'*, Nanoscale,* ***2020****, 12, 13907*

Visual summary of grant achievements.

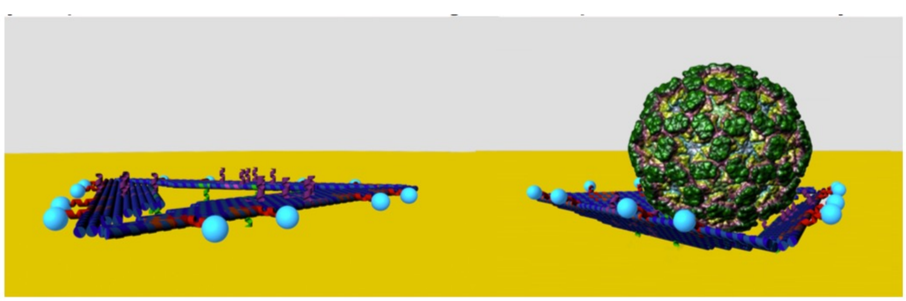
Design of triangle sensor





We call this molecule the "RECEPTOR"

Our initial model of our Receptor in action



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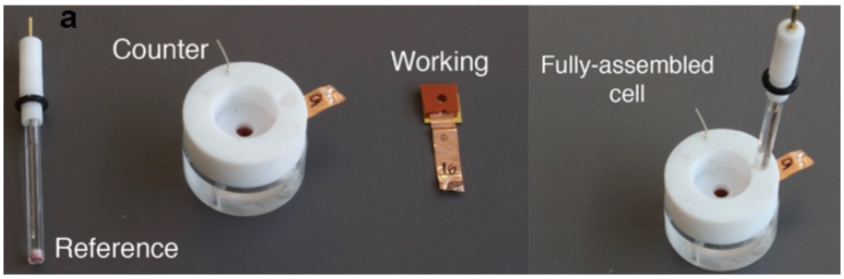
Developed

Origami Electrode attachment chemistry

Surface Passivation chemistry
(nonstandard monolayers)

Custom electrochemical cells



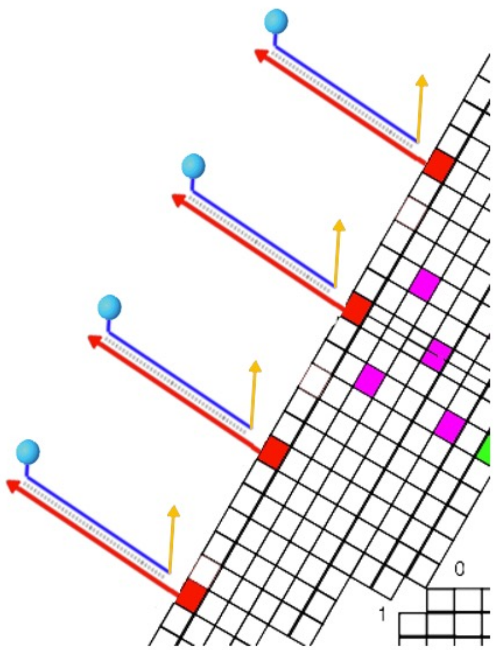
4

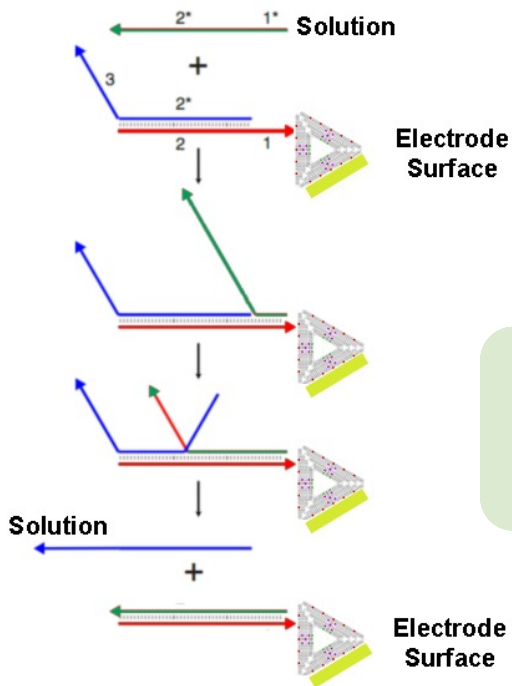
How do we know that origami adhered to the surface in the expected manner?

Is the Methylene blue still bound to the origami?

MB strands shown as
5' MB molecule, blue $(TCA)_7$ linkers
with toehold sequence in orange

Scaffold $(TGA)_7$ linkers shown in red

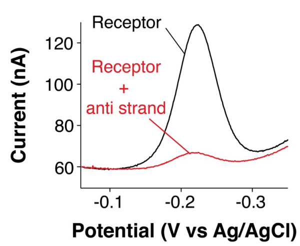


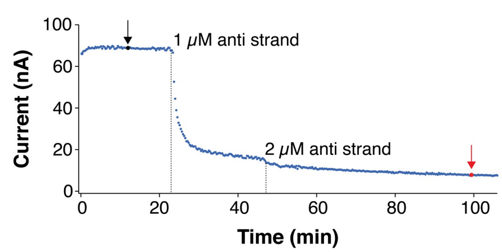


Toehold displacement of MB strands should **reduce current** if triangles are attached to surface as planned

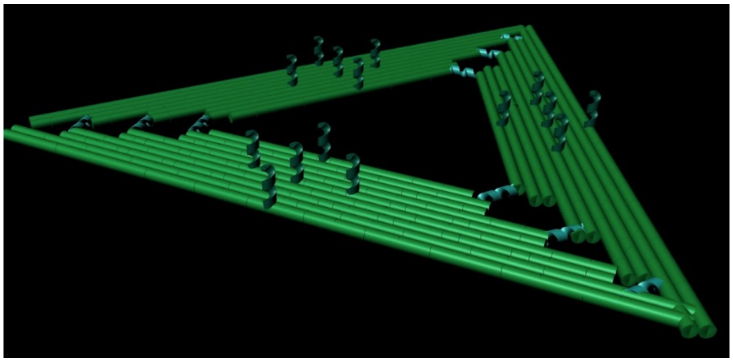
BLUE=MB strands
RED=SCAFFOLD STRANDS
GREEN=ANTI-STRAND

Displacement occurs as expected





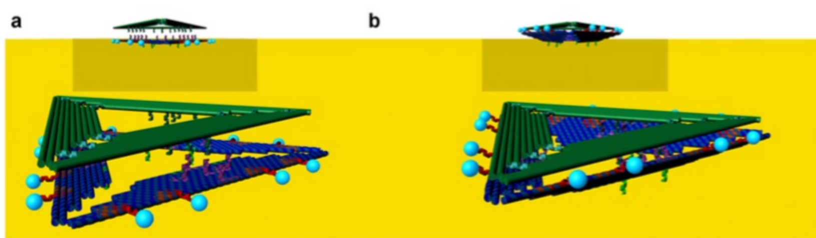
MS2 VLP sensing worked, but was prohibitive in terms of material.
What do we understand well, and can modify easily at this size scale
and can make a lot of in house?



How about another triangle?

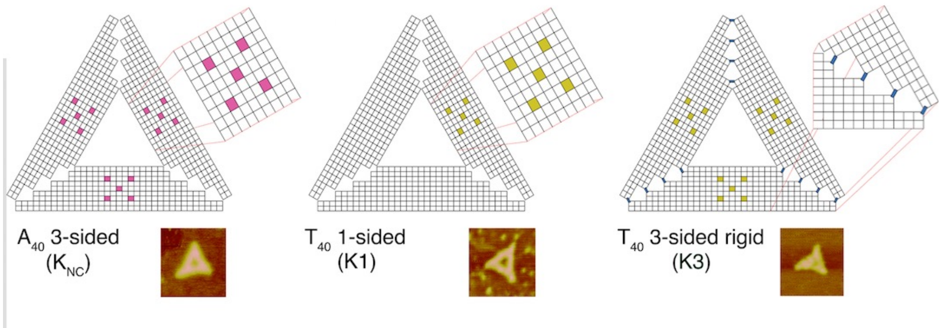
8

A new 'model' biosensor



Our new
Target is a rigid, 3-sided triangle "K3" which is "smaller", thereby causing the receptor's 3-sides to rigidify

What about controls?



NON-BINDING CONTROL

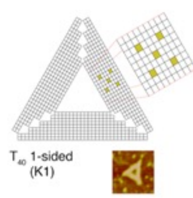
NON-POLYVALENT CONTROL

TARGET MOLECULE

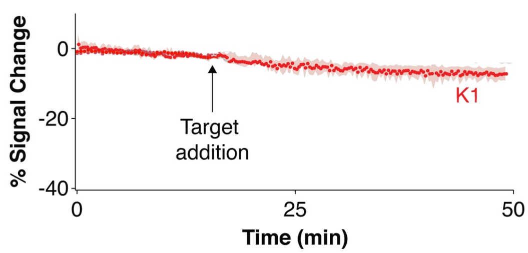
We showed, by gel electrophoresis these behaved with the receptor "as planned"

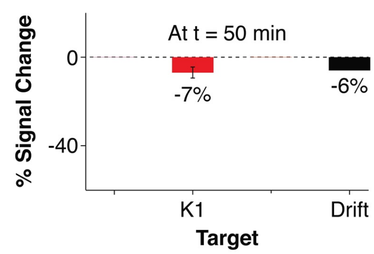
10

Single-sided triangle (non-polyvalent binding) gives similar change to sensor drift



NON-POLYVALENT CONTROL





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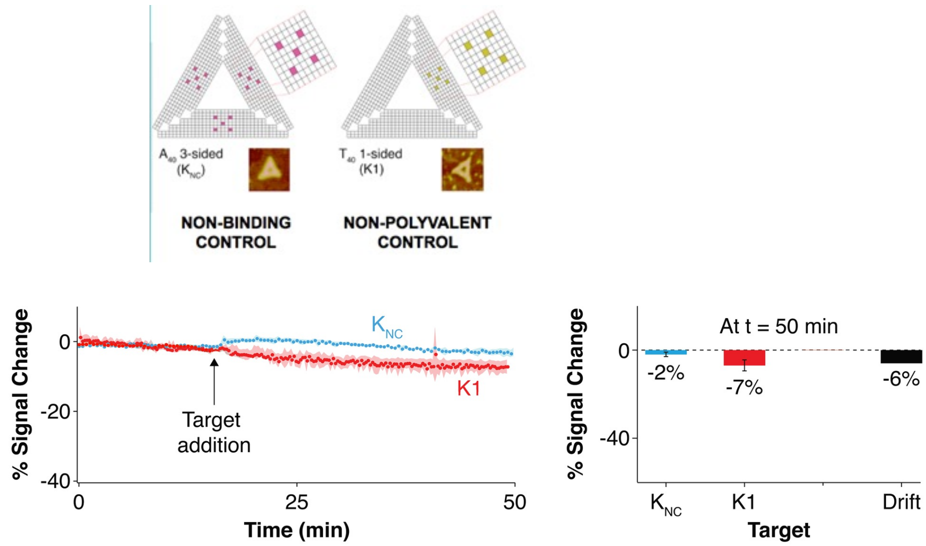
“Non-binding” control only gives small current change



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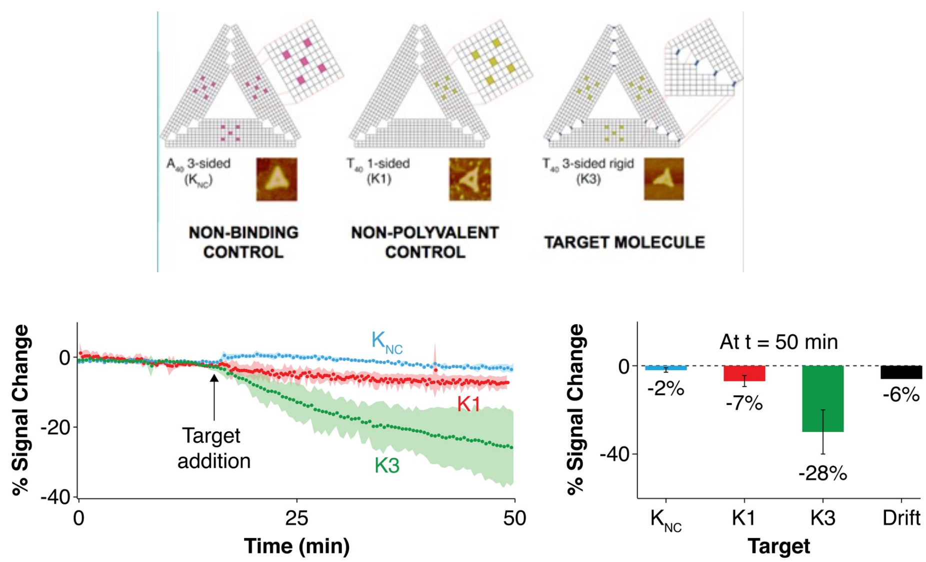
Polyvalent target yields **significant change** upon binding



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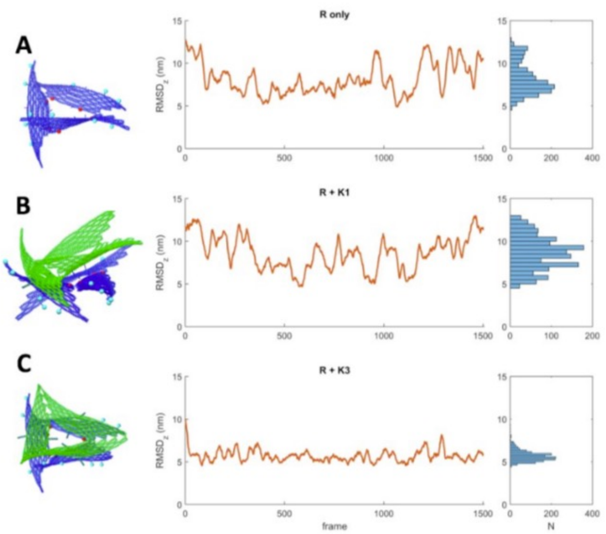
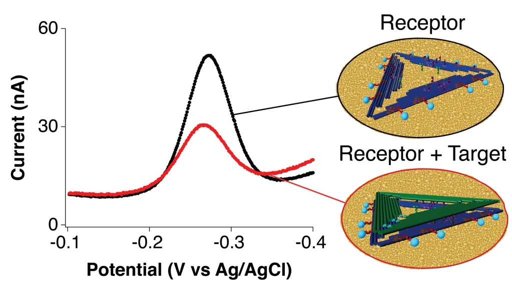


Fig. 4: OxDNA simulations of the Receptor show a decrease in redox reporter fluctuations upon binding K3; there is no significant fluctuation decrease upon binding of K1. The root mean square deviation(RMSD) in the z-direction, which is

Molecular Dynamics Simulations support signaling mechanism

First tunable origami electrochemical biosensor capable of distinguishing molecular targets with binding sites separated by > 40 nm



Future work : perform structure activity relationships on origami electrochemical biosensors
to inform the construction of other, clinically relevant sensors.