STATEMENT OF WORK – 10/16/2017
PROPOSED START DATE Aug 01, 2018

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Site 2: Rensselaer Polytechnic Institute
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Specific Aim 1: Optimization of SL spacing /size in SL-DNA designs. (1-24 months)	**Time**	**Site 1**	**Site 2**
Major Task 1: Design, synthesis and characterization of different SL-DNA conjugates (all modified and unmodified DNA oligonucleotides are obtained from IDTDNA Inc. 6SL- and 3SL- ligands are obtained from Sigma-Aldrich. Formation of all SL-DNAs will be done through a thermal annealing. Gel electrophoresis, AFM imaging, and LC-MS will be used for the characterization of SL-DNAs).	Months		
(a) dsDNA-S3-G4 dendrimer arrays with variable inter-dendrimer particle spacing.	5	Dr. Wang	Dr. Dordick
(b) SL-DNAs (built on finitely sized DNA fishnets) with variable intra- and inter-triangle-SL ligand spacing, and with variable dimensions.	9	Dr. Wang (Dr. Linhardt's group will provide assistance in LC-MS based characterization)	
(c) SL-DNAs (built on large size DNA fishnets) with the optimal SL ligand spacing configured by SPR analysis of SL-DNAs in (b).	4		
(d) SL-DNAs (built on finitely sized DNA origami 2D-sheet).	6		
Milestone Achieved: A library of DNA based multivalent SL conjugates that carry variable SL ligand spacing and sizes will be obtained.	1-24		
Major Task 2: SPR analysis of the interaction between SL-DNAs and inactive IFV to obtain the optimal SL ligand spacing/ dimension (if applicable) for each SL-DNA; TEM imaging analysis to confirm the interaction with IFVs (Inactive H1N1 (A/California/04/2009) will be used. It is available and will be obtained from IRR or ATCC. Note these analyses are expected to be carried out with intervals upon the actual availability of SL-DNAs prepared in Task 1, and			

finished within 24 months).			
(a) dsDNA-S3-G4 dendrimer arrays.	6		Dr. Dordick (Drs. Kwon and Zhang will provide support and assistance in SPR and TEM analyses)
(b) SL-DNAs (built on finitely sized DNA fishnets). The timeline is projected with intervals upon the timely availability of the SL-DNAs.	12-24		
Milestone Achieved: Optimal SL ligand spacing and SL-DNAs dimension (if applicable) of each SL-DNA design will be obtained. The interaction with IFV particle will be confirmed by TEM imaging analysis.	1-24		
Specific Aim 2: *In vitro* antiviral assays of SL-DNAs (Inactive IFVs, H1N1 (A/California/04/2009), H5N1 (A/Indonesia/05/2005), H7N9 (A/Shanghai/1/2013), and H3N2 (A/Wyoming/3/2003) are available and will be obtained from IRR or ATCC. MDCK, A549 and Beas-2B cell lines are from ATCC. Note these analyses will be carried out with intervals starting from month 13 upon the availability of the optimized SL-DNAs prepared in Aim 1).	Month (13-36)		
(a) IFV infection inhibition by SL-DNAs: using micro-neutralization (MN) assay (involving MDCK cells) that will be quantified by ELISA using an anti-NP murine antibody and an anti-mouse goat antibody–HRP conjugate (Millipore).	6		Dr. Dordick (Dr. Kwon will provide supervision and assistance in all these assays)
(b) Hydrolysis of SL-ligands of SL-DNAs by NA of IFVs: using LC-MS to monitor the hydrolysis levels.	6		
(c) Standard plaque assay to test SL-DNAs inhibition of multicycle viral replication.	6		
(d) *In vitro* SL-DNAs cytotoxicity test: using MTT dye reduction and propidium iodide assay (involving A549 and Beas-2B cells)	6		

Milestone Achieved: IFV inhibition profiles (e.g., antiviral efficiencies and toxicities) of the SL-DNAs will be obtained as preparation for follow-on clinical applications in IFV prevention and therapies (next research phase).	13-36		
Specific Aim 3: Development of a DNA origami nanotube (NT)-based IFV sensor (At this stage, inactive IFVs, H1N1 (A/California/04/2009), H7N9 (A/Shanghai/1/2013), and H7N1 (A/rhea/North Carolina/39482/93) are used. They are available and will be obtained from IRR or ATCC.)	Months (13-36)		
Major Task 1: Design, synthesis and characterization of SL-DNA-NTs (all modified and unmodified DNA oligonucleotides are obtained from IDTDNA. 6SL- and 3SL- ligands are obtained from Sigma-Aldrich. Formation of all SL-DNA-NTs will be done through a thermal annealing. Gel electrophoresis, AFM imaging, and LC-MS will be used for the characterization of SL-DNA-NTs).	9	Dr. Wang (Dr. Linhardt's group will provide assistance in LC-MS based characterization)	
Milestone Achieved: A library of SL-DNA-NTs containing variable SL spacing (for SPR analysis below) and ssDNA loop lengths (for loop optimization below).	13-22		
Major Task 2: SL-DNA-NTs optimization.			
(a) Inter-triangle-SL spacing optimization: using SPR analysis and TEM imaging.	6		Dr. Dordick (Drs. Kwon and Zhang will provide assistance in all these assays)
(b) ssDNA loop size optimization: measuring fluorescence signal using microplate reader.	3	Dr. Wang	
Milestone Achieved: Optimal SL-DNA-NT sensor structure will be obtained.	13-30		
Major Task 3: IFV sensor demonstration.	6		
Milestone Achieved: Detection limit, a standard curve and a demonstration of sensing different types of IFV strains will be achieved.	13-36	Dr. Wang	Dr. Dordick