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DEVELOPING NANO-REACTION CHAMBER TO PROBE TRANSIENT MACROMOLECULAR COMPLEXES

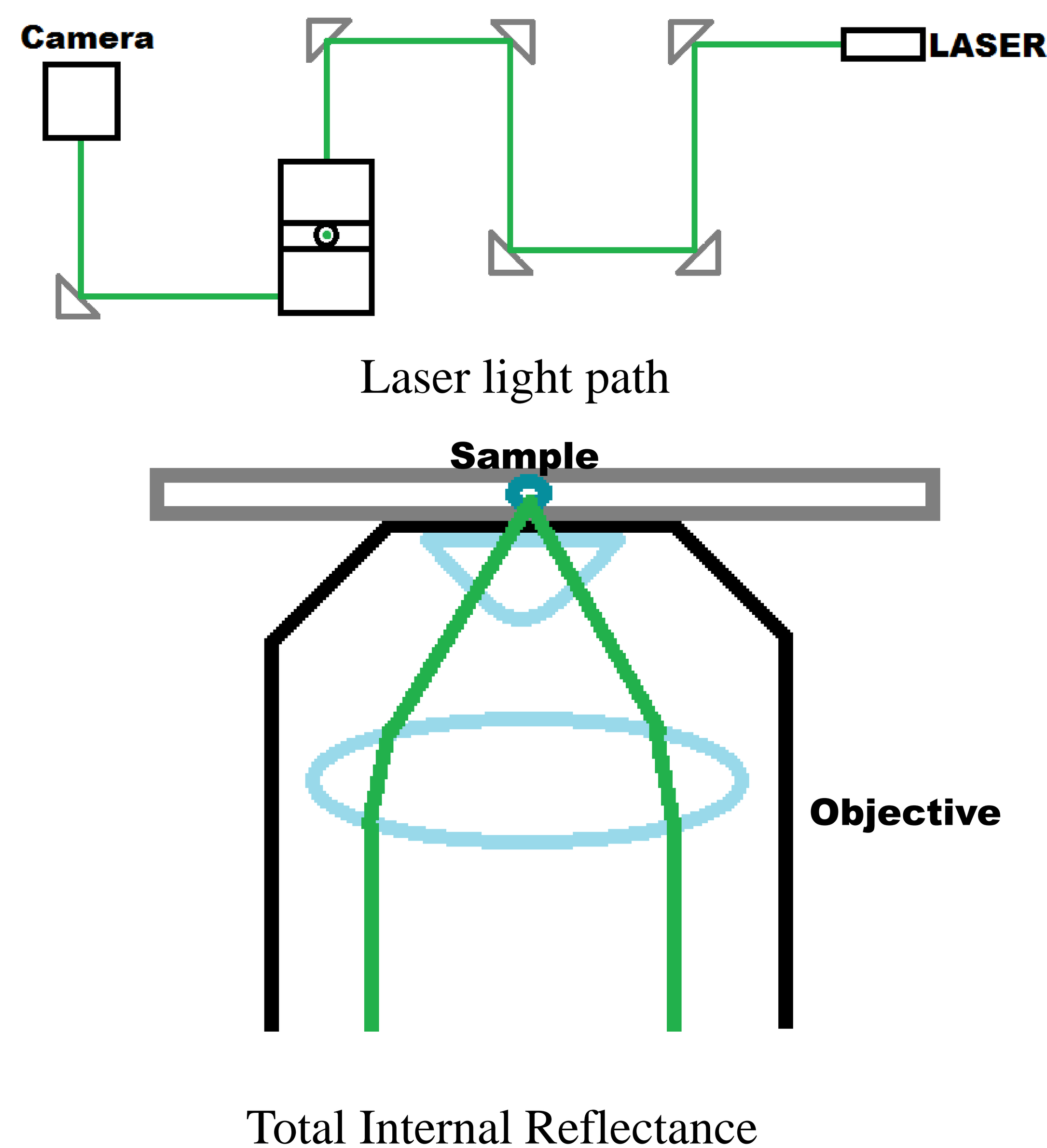
Blake Ford*, Noah Robinson, Vanessa Nwaiwu, and Minjoung Kyoung
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
**Department of Chemistry, Stevenson University, 1525 Greenspring Valley Road, Stevenson, MD 21153*

Abstract

This project is focused on developing a novel method to examine weak, short-lived, protein-protein interactions in macromolecular complexes. A broad variety of signaling pathways, like those related to disease, contain transient protein complexes. To study these interactions, we will trap molecules in vesicle "nano-chambers" and monitor them using a single molecule fluorescence microscope constructed in-house. Vesicles are nano-scale fluid filled sacs composed of a spherical lipid membrane separating the outside from the in. We designed vesicle pairs to fuse, thereby mixing the contents stored inside to allow for controlled small volume mixing experiments. This new nano-reaction chamber approach is motivated by the need to overcome limits of conventional methodologies to study transient protein-protein interactions. The small volume of ~100nm diameter nano-vesicle allows for higher effective concentrations of the reactants mimicking the physiological conditions found in cells. By engineering a system where single molecules are trapped and probed in one location, it will be possible to examine not only equilibrium concentrations, but the dynamics of a specific protein-protein interaction.

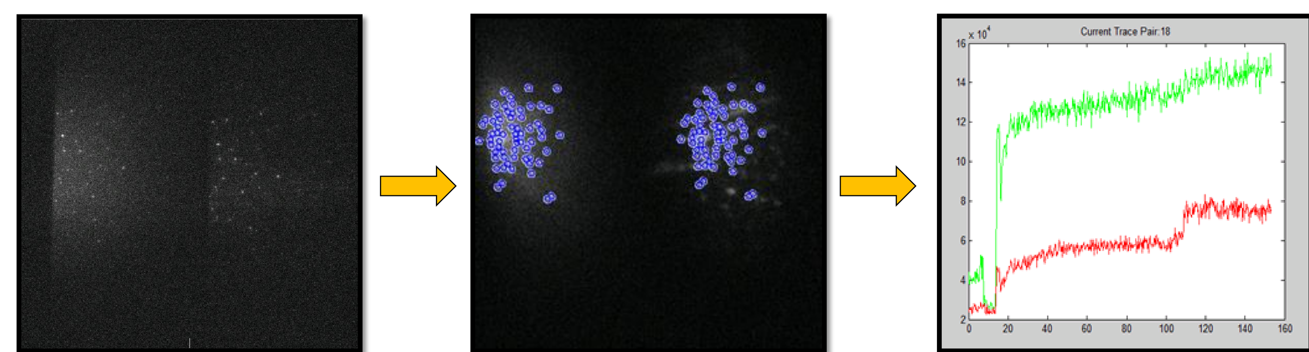
We strive to prove that our system is capable of multiple rounds of controlled fusion, which would allow for sequential addition of multiple components to the reaction chamber under observation. Here, we present an explanation of the data analysis software used, proof of first step of fusion, and the development of sequential fusion. Moving forward, once sequential fusion has been proven to occur, the project will then progress towards examining specific protein-protein interactions involved in signaling pathways present in cancer development.

Microscope Set-Up



Laser light path

Total Internal Reflectance



Matlab program converts raw real-time videos into information about individual nano-chambers

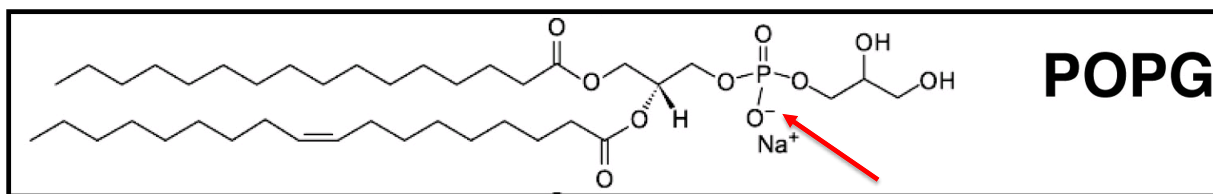
Each video contains about 1000 individual images. Matlab code takes 1 image and identifies signals based on a 2D Gaussian, marking locations in red and green channels

For each location a data set, or trace, is created for signal in the green and red channels

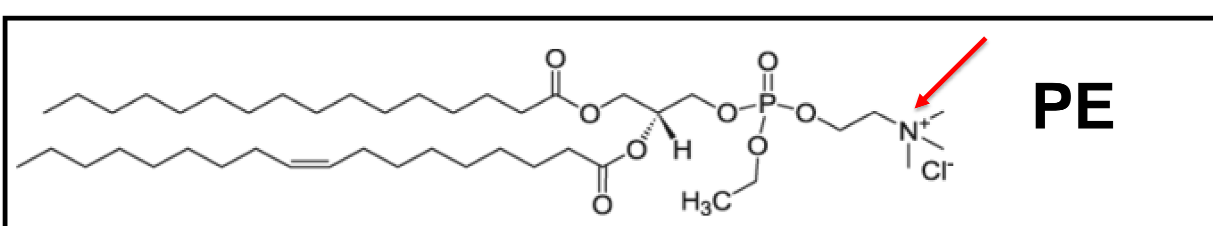
Data traces are then examined for convincing signals and evidence of fusion events in the effort of showing optimized conditions for the working system.

Charge-Charge Fusion

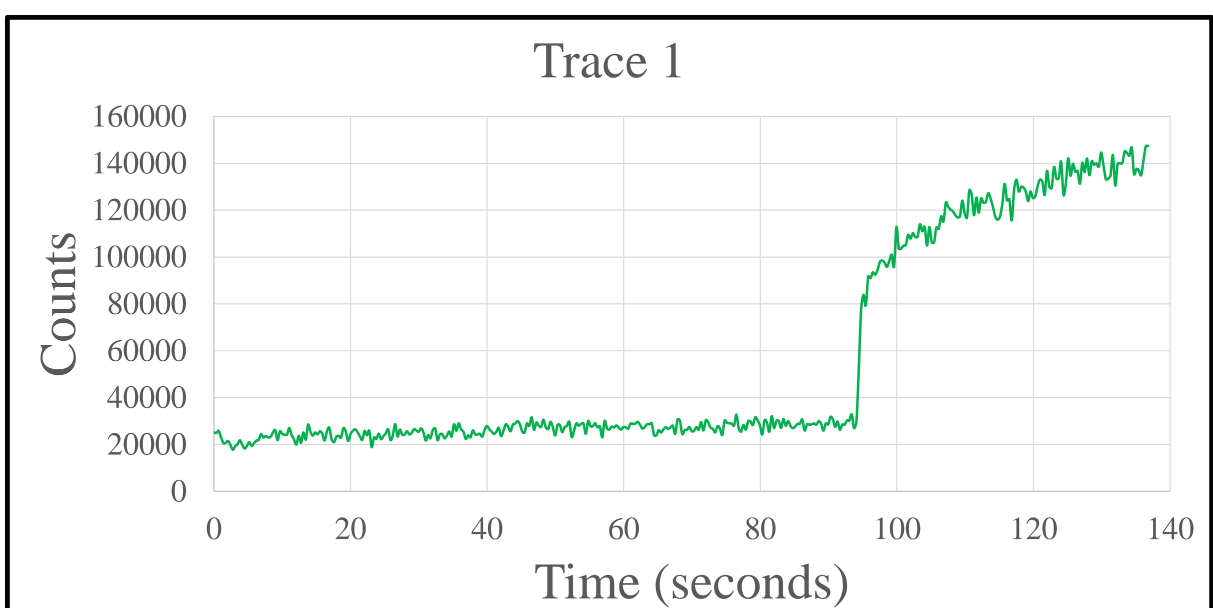
Conditions	Dye	Charge
Vesicles Attached to Surface	None	55% PE (+)
Vesicles in Solution	100 mM Sulforhodamine	60% POPG (-)

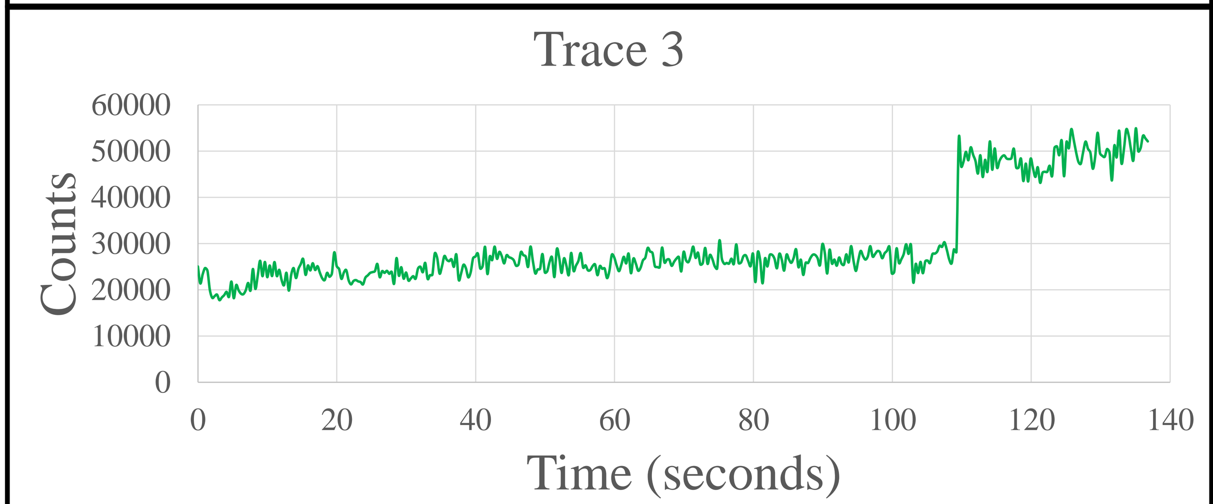


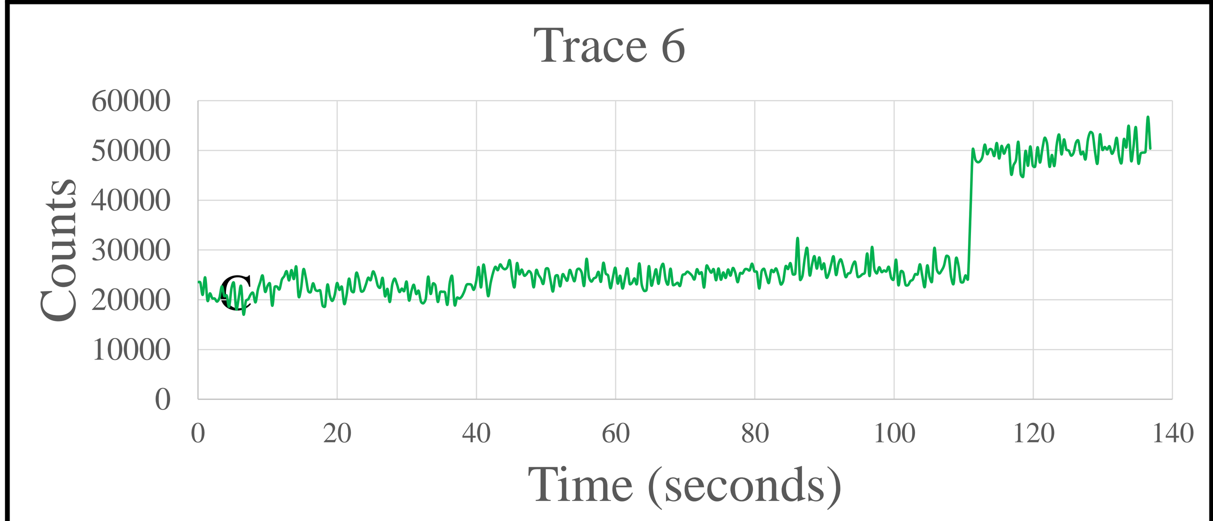
Structure of 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphor-(1'-rac-glycerol) (POPG) showing negatively charged headgroup



Structure of 1-palmitoyl-2-oleoyl-sn-glycerol-3-ethylphospocholine (PE) showing postively charged headgroup

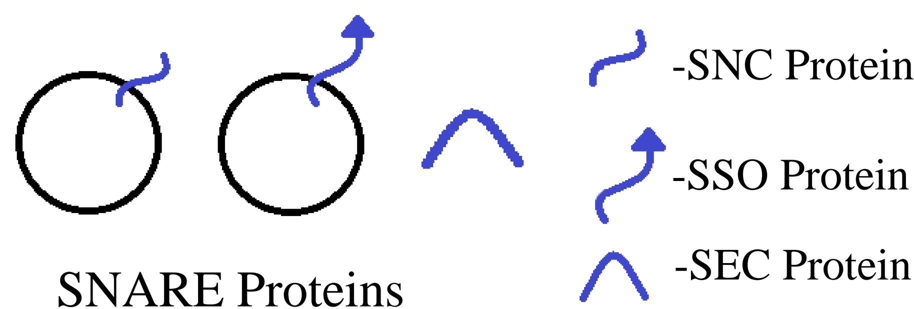






Single localization traces from charged vesicles containing only dye showing fusion events

Protein Mediated Fusion



The three SNARE membrane proteins come together to form a tight helix, pulling vesicles close and causing fusion

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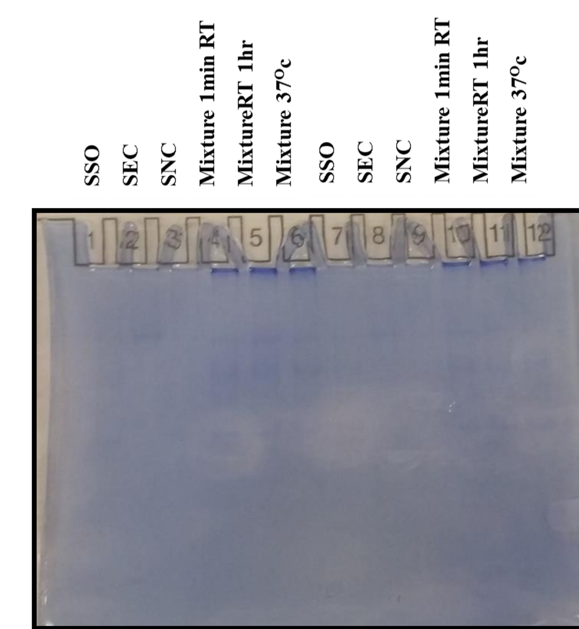
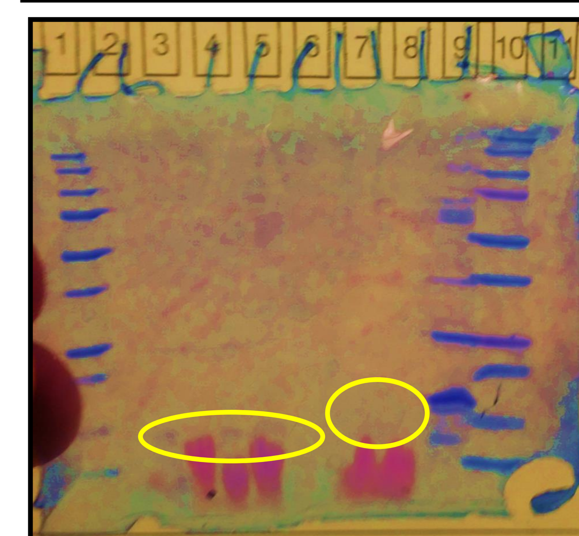
We wish to acknowledge Mathew Law and Rishi Raval, UMBC computer science undergraduates for developing Matlab programs for acquiring and analyzing imaging data. The project is supported by the National Science Foundation Research Experience for Undergraduates (REU) Award No. CHE-1460653.

Gels



- Native Gel electrophoresis analysis of proteins
- Here we see complex or aggregate forms, regardless of time
- There are bands seen mixing lanes also corresponding to individual constituents
- This indicates the proteins are active



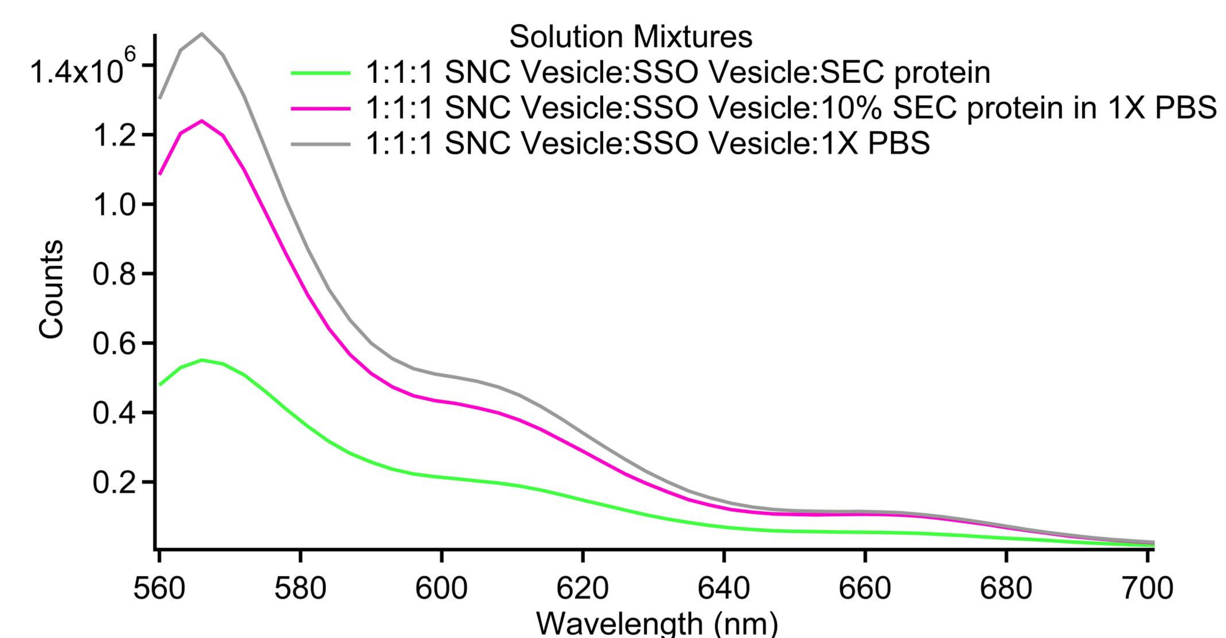
Without Trypsin   With Trypsin

Image modifications made to highlight difference. Very visible to eye, very difficult to capture.

- SDS Gel Electrophoresis to test incorporation of SNARE proteins into vesicles membranes.
- Protein bands visible in the vesicle lanes without Trypsin.
- No such bands present in the trials with trypsin.
- Indicates the membrane SNARE proteins are incorporated into the membrane of the vesicles

Bulk Assay

Lipid Composition of Vesicles		
43%	Phosphatidylcholine	(POPC)
40%	Cholesterol	
15%	Phosphatidylserine	(POPS)
2%	Dye (DiI or DiD for SNC and SSO containing vesicles, respectively)	



Notice decrease in DiI signal(max peak at 570nm) with the presence of SEC SNARE protein requires for SNARE mediated fusion. This indicates DiI(FRET donor) in close proximity to DiD(FRET acceptor)

- We interpret this close proximity as fusion

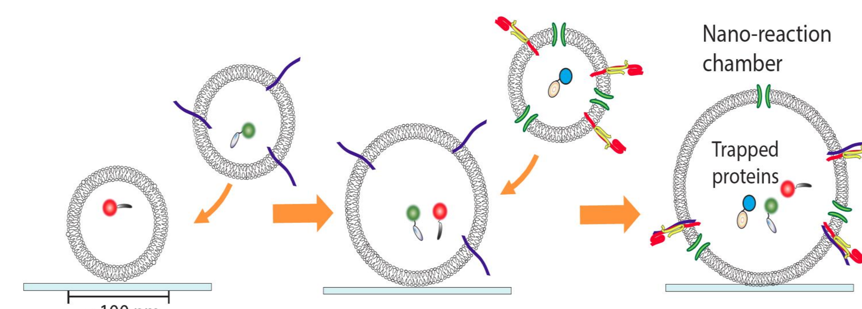
Future Goals

Proof of Principle

Show fusion of vesicles with SNARE proteins incorporated
Conduct two step fusion experiments using both fusion processes with dye markers for fusion

Proof of Concept

Incorporate a test model of interacting proteins to show this system can deliver contents to the nano-chamber and interaction can be monitored



A nano-chamber is used to trap and immobilize molecules. Sequential fusion allows for the addition of various components for the researcher to observe