DLS or Dynamic Light Scattering is an assay that measures light refracting off of a sample in solution to determine the overall size of particles within.

A beam of light is passed through a sample in a cuvette and “snapshots” are taken at many instants over a period of time. Particles entering the beam of light obstruct passage. The larger the particle, the longer it takes for it to stop obstructing the light, and the time it takes for this obstruction to clear is correlated with time by observing small changes between the instantaneous snapshots taken by the DLS machine. Algorithms then further correlate these changes to create spectrums that relate size, percentage of particles constituting a sample, and particle count.

Materials:

1) Sample to be measured - AT LEAST 35 uL
2) Helma Analytics Suprasil #105.251.006 – Q5 3mm Path Length Quartz Cuvette
3) Malvern Instruments Zetasizer - DLS Instrument.
4) diH_2O
5) 70% Ethanol
6) Vacuum Cuvette Cleaner

DLS Instrument Settings:

- Measurements taken in triplicate (3X)
- Equilibrate samples for 2 minutes at 25C
- Q5 3mm Path Length Cuvette preset selected.
- 1.435 Protein Preset

Protocol:

1) Wash Helma Analytics Quartz Cuvette (2) using vacuum cuvette cleaner. First with diH_2O followed by 70% ethanol. Continue to vacuum to dry interior.
2) Ensure Malvern Instruments Zetasizer is on and accompanying program is open.
3) Load 35 uL of sample into cuvette, place into Malvern Zetasizer DLS instrument, close lid.
4) Begin readings using established instrument settings.
5) Readings complete automatically, either remove sample by pipette and keep if desired or discard.
6) Wash cuvette using vacuum cleaner. Flush with diH_2O followed by 70% ethanol. Dry by vacuuming until ethanol no longer visible.
7) Repeat from step 3 for all desired samples.

Data Analysis:

1) Average each set of 3 measurements per sample for all samples. Include Size, Intensity, and Volume data columns.
2) Graph each sample’s Volume data against its Size data and Intensity against its Size to generate % Volume and % Intensity graphs.

3) Interpret presence and absence of peaks as successful or unsuccessful assembly formation depending on project design.

Disassembly / De-phosphorylation removes the phosphate group initially added onto the Atrazine A subunit by the ABCAM SRC Kinase effectively eliminating the impetus for subunit assembly. As a result treatment of assembly with YOP Phosphatase enables phosphorylation based control of assembly formation.

Material:
- Previously formed Atrazine A+C subunit assembly.
- YOP Phosphatase

Protocol:
1) Add 3 uL of YOP at 200kU/mL (1.6mg/mL) to Atrazine A+C assembly.
2) Rotate overnight at room temperature (25C).

Assembly Formation is dependent on mixing Atrazine subunits together in specific ratios conducive to the formation of fractaline assemblies. The process is heavily dependent on the level of phosphorylation of the A subunit and slightly time dependent. Several conditions have been tested and confirmed as forming assemblies assuming sufficient levels of phosphorylation.

Assembly Conditions:
- Concentration of subunits are usually determined before the experiment begins. Normally, concentration of individual subunits are double the final desired concentration.
- Equal volumes of Atrazine A and C subunits at predetermined concentration are used to form assembly. The final volume as a result is twice the volume of its parts therefore halving the individual concentrations.

Materials:
- Phosphorylated or Non-phosphorylated Atrazine A subunit proteins at predetermined concentration.
- Atrazine C subunit protein at predetermined concentration
- diH_2O

Protocol:
1) Dilute Atrazine C subunit protein with diH_2O if necessary to reach desired initial concentration.
2) Mix equal volumes of Phosphorylated Atrazine A and Atrazine C.
3) Incubate at 4C for 2 hours.

Phosphorylation of Atrazine Subunits adds a phosphate group to a free tyrosine on the protein using ABCAM SRC Kinase and ATP. This modification enables fractaline formation of Atrazine subunit protein assemblies.

This is a standardized protocol used in nearly all experiments requiring phosphorylated Atrazine A. Following this protocol will produce phosphorylated protein at 6 uM concentration and assembly at 3uM AtzA and 2uM AtzC. This can be adjusted to fit the need of the experimenter.

Materials:

- Atrazine A subunit Protein - any model.
- $MgCl_2$
- $MnCl_2$
- DTT
- ATP
- ABCAM SRC Kinase
- MOPS
- Glycerol-2-Phosphate
- EGTA
- EDTA
- diH_2O

Protocol:

1) Mix protein to be phosphorylated with listed materials such that the following conditions are achieved in the desired final volume. diH_2O can be added to reach desired volume.
    a) 6 uM AtzA subunit Protein
    b) 4 mM $MgCl_2$
    c) 2.5 mM $MnCl_2$
    d) .25 mM DTT
    e) 1 mM ATP
    f) 5 mM MOPS
    g) 2.5 mM Glycerol-2-Phosphate
    h) 1 mM EGTA
    i) 400 nM EDTA
2) Add 200 ng of ABCAM SRC Kinase to samples to be phosphorylated. Kinase can be excluded from samples to produce negative controls for phosphorylation and assembly formation.
3) Rotate for 7 hours or overnight at room temperature (25C).
4) Store at 4C after incubation until phosphorylated protein is needed.