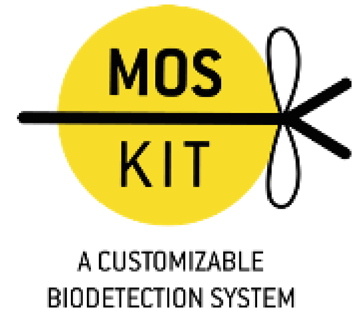
iGEM Pasteur Paris 2016
Protocols



SILIFICATION & ASSAY

Aim: verify that the C2 protein makes silica precipitate by catalyzing the sol/gel process and quantify the silification efficiency of the C2 protein.

Protocol:

- **Silification:**

- in a 2ml Eppendorf tube, put:
  - 22.4µl of pure TEOS
  - 1.0ml of 1mM HCl
- incubate at room temperature for 15min, under stirring.
- add 300µl of the C2 protein.

Use a mixture without C2 protein as a control for kinetics.
Use a mixture with a non specific protein (such as BSA) as a negative control.
Use a mixture with only the C2 protein and 1ml of 1mM HCl to check that the C2 protein does not precipitate in these acidic conditions.

- incubate at room temperature for 2 hours, under stirring. A silica gel appears.
- centrifuge the tube at 13,200rpm for 15min.
- recover the supernatant in 1.5ml Eppendorf tube (for the assay).
- store the pellet at 4°C to make a patch (see the “patch compression” protocol).

- **Assay:**

- in a 2ml Eppendorf tube, put:
  - 22.4µl of pure TEOS
  - 1.0ml of 1mM HCl
- incubate at room temperature for 15min, under stirring.

N.B.: this stock solution contains 208µg/ml of silicic acid.

- perform several dilutions of this stock solution.
- in each tube, add 100µl of ammonium molybdate 10%wt. The mixture turns yellow.
- incubate at room temperature for 15min under stirring.
- for each dilution tube, measure absorbance at 420nm.
- establish the standard curve by plotting absorbance vs silicic acid mass.

- in a 1.5ml Eppendorf tube, put 1ml of the supernatant got from silification.
- add 100µl of ammonium molybdate 10%wt. The mixture turns yellow.
- incubate at room temperature for 15min, under stirring.
- measure absorbance at 420nm.
- thanks to the standard curve, determine the concentration of silicic acid left in the supernatant after silification.