Week 37 - Overview

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Weekly summary:

During Week 37, only one day was spent in the lab because of other university responsibilities. A Bradford Assay was performed on purified ci2350-MBP and its double mutant (V263I and R215A), along with the empty pET-11a controls samples, all cultivated the previous week.

Main results of the week:

The purified samples had different protein concentrations. Some protein was expected in the empty pET-11a control cultures, due to non-specific binding of native *E. coli* proteins to the IMAC column. The concentrations were unexpectedly high: 0.266 mg/mL for uninduced pET-11a, and 0.926 mg/mL for induced pET-11a, which is higher than the induced ci2350-MBP double mutant (0.695 mg/mL). The IMAC column is the most likely cause, as it was clogging during purification, despite filtering the samples through 0.2 µm filters. We have also purified the ci2350-MBP samples before the empty pET-11a controls, which could have also contaminated pET-11a samples by carrying the protein on the column. If repeating the experiment, we will purify the empty pET-11a controls first, use a clean column, and reduce clogging risk by more thorough lysis. SDS-PAGE analysis of these purified samples will be done, to compare control and sample bands, and assess cross-contamination.

Samples	Absorbance - S1	Absorbance - S2	Absorbance - S3	Mean	St.dev	Concentration (mg/mL)
ci-MBP	0.496	0.512	0.568	0.525	0.037	1.247
ci-double mutant	0.3	0.304	0.344	0.316	0.024	0.695
pET (uninduced)	0.144	0.132	0.185	0.154	0.028	0.266
pET (induced)	0.363	0.42	0.428	0.404	0.035	0.926

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Responsibles: Rytis
Participants: Rytis, Hema

Daily overview of experiments

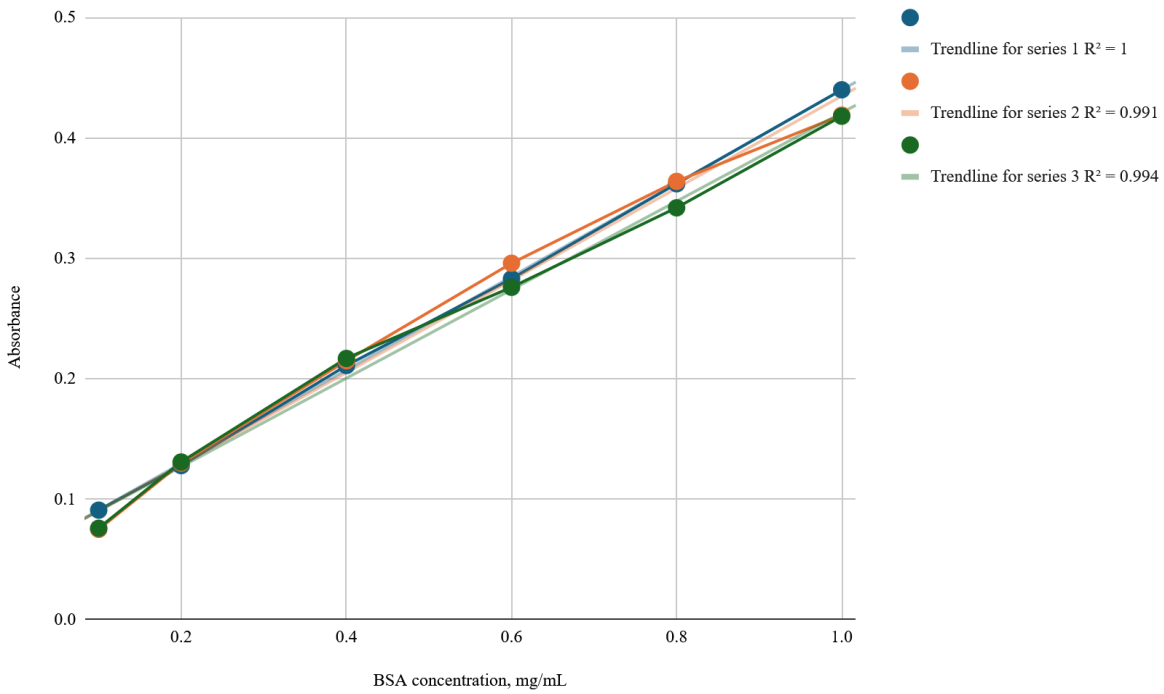
Protein content of 26/09/04 samples was measured using the Bradford method.

Activity	***Protocol File***	***Comments***
Bradford (BSA calibration curve + 09/04 samples)	Protein Quantificatio…	For Bradford reagent dilution, cell washing buffer was used.

Results and interpretation

Bradford assay

15 mL of 1 mg/mL BSA reagent was prepared, following which serial dilutions were made, to get 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL concentration samples.
Then Bradford assay was performed: 1 mL of Bradford reagent (diluted from 5x to 1x in cell wash buffer) + 20 µL BSA/protein samples. Triplicates were measured, data was put in Bradfords iGEM26.xlsx, where slope and intercept were calculated.



Slope (m)	0.379196347
Y-intercept (n)	0.05263744292

Triplicates of protein samples from 09/04 were measured the same way (1 mL of Bradford reagent + 20 µL of samples), mean was used to calculate their concentrations.

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