UnaG: Transformation and Extraction

Transforming the Plasmid:
Same-day-made competent cells using the protocol detailed in the "Synthetic Biology Handbook" by the Forster Lab [1] were used to provide maximum transformation efficiency.

Extraction of UnaG:
The protocol for the extraction of our integral membrane protein from the transformed BL21 cells proceeded as follows: Note that this was done for both iGEM 2016 cells (nicknamed "bad" and our repositioned start codon graced with the moniker "good").

Bilirubin solution:
A chloroform solution of 2.6mM bilirubin was made. When a "bilirubin test" was conducted, 30 uL of this solution was added to a solution suspected to contain our UnaG protein. The tubes were then placed under a UV light to see if any fluorescence occurred. The final concentration of bilirubin in all test solutions was 78uM.

Materials:
Lysis buffer: PBS solution with 1mM EDTA, 5% glycerol, and 20mM Tris, pH7.4
Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4, 5% glycerol
PBS, 1mM EDTA, 5% glycerol, 20mM Tris, pH7.4
Binding/Washing Buffer = 0.5 M NaCl, 2 EDTA-free tablets, 10 % glycerol, 20mM sodium phosphate, 1% Triton x100, pH 7.4 (400 mL total)
Binding/washing buffer with 1% triton x-100 by weight

Cells were centrifuged at 4000 g 25 minutes at 4 degrees Celsius and then resuspended in Lysis buffer. Cells were lysed using cell disruption with a french press. The now lysed cells were then centrifuged again at at 4000 g 25 minutes at 4 degrees Celsius. The pellet was resuspended in 20mL binding/washing buffer with 1% triton x-100. The solution was incubated on ice for one hour before another round of centrifugation at the same temperature and speed. After centrifugation the supernatant should contain the protein of interest. Bilirubin tests were conducted on both solutions of the pellet and supernatant to observe any fluorescence under a UV light.

Affinity chromatography was then performed on both "good" and "bad" solutions using prepacked "His-Gravitrap" Columns from GE Healthcare. The protocol for use was performed according to GE healthcare's specifications, with modified binding/washing/elution buffers. After affinity chromatography, the resulting elutants were tested for fluorescence with a bilirubin test.

References

[1] Liljeruhm J, Gullberg E, Forster A. (2014). Synthetic Biology: *A Lab Manual.* Uppsala University, Sweden