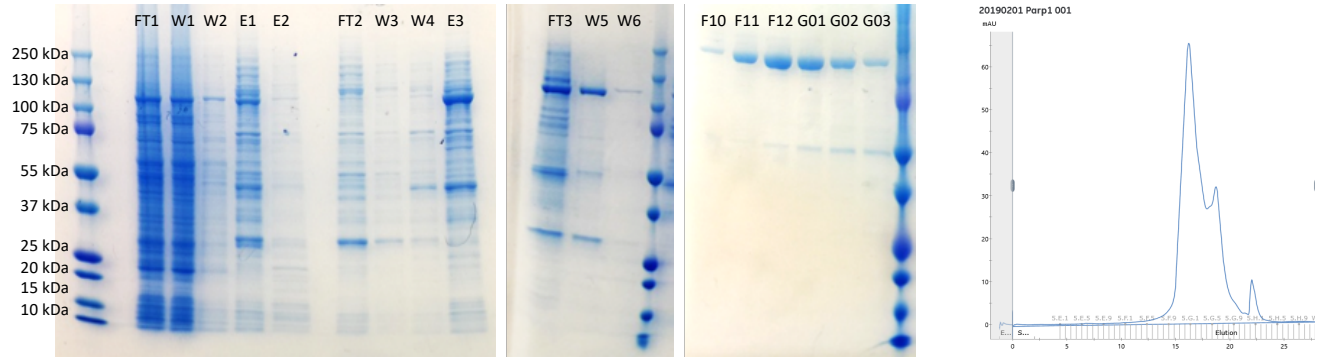
Project:
Investigating HTT parylation and PAR-binding in its putative role in DNA damage repair

Experiment:
Expression and purification of PARP1 from Sf9 Insect cells

Date completed:
2019/02/04

Rationale:
HTT may be parylated or recognize PAR motifs on other proteins involved in DNA damage repair. To test this hypothesis, the full-length PARP1 cDNA was cloned in to pFBOH-SBP-TEV-LIC, overexpressed in insect cell Sf9 culture and purified. The purified PARP1 protein will be shared with Dr. Tamara Maiuri for her future experiments on the link between HTT protein and parylation.

Experimental approach:
1 L Sf9 production of our TOC016-A03 full-length PARP1 construct (aa. 1-1014, UniProt ID P09874) was harvested. A standard PARP1 purification protocol was followed (see Pubmed 21870263). Briefly, cells were lysed and clarified lysate was purified through sequential heparin-affinity chromatography, Ni-affinity chromatography, ion-exchange and gel filtration. Gel filtration fractions containing pure PARP1 (F10-F12) were concentrated to ~2 mg/mL and flash frozen for long-term storage.



Superose6 gel filtration of purified PARP1 from Sf9 production and SDS-PAGE analysis of samples throughout the purification. FT1 – heparin flow through, W1 – heparin wash 0 M NaCl, W2 – heparin wash 0.2 M NaCl, E1 – heparin elution 0.6 M NaCl, E2 – heparin elution 1 M NaCl, FT2 – Ni flow through, W3 – Ni wash 5 mM imidazole, W4 – Ni wash 15 mM imidazole, E3 – Ni elution 300 mM imidazole, FT3 – DEAE IEX flow through, W5 – IEX wash 100 mM NaCl, W6 – IEX wash 200 mM NaCl, Gel filtration elution fractions - F10, F11, F12, G01, G02 and G03.

Conclusions:
PARP1 is not trivial to purify and the sample yield was low. Activity of the sample remains to be determined.

Next steps:
HTT parylation and interaction with PARP1 will be evaluated in the future using this purified sample.