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March 24, 2020

SEC-MALS: USP5 and Ubiquitin

Objective: To determine the stoichiometry of binding of monoubiquitin (Ubq) to the full-length wildtype USP5 and R221A mutant protein by size exclusion chromatography-multiple angle light scattering (SEC-MALS) to elucidate the binding properties of native ubiquitin for future cell-based and activity studies.

Method & Results:

Samples were prepared by Mandeep Mann. Dr. Rachel Harding carried out the MALS experiment.

1 mL of 0.5 mg/mL of USP5$^{1-835}$ (SDC075-B03)/ USP5$^{R221A\ 1-835}$ (TOC023A06) ± 5x molar excess of Ub$^{1-76}$ (25 µM) was prepared in 50 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP. Sample was injected though a Superose6 10/300 GL column (GE Healthcare Life Sciences) at 0.4 mL/min followed by a Dawn Heleos-II light scattering detector (Wyatt Technologies). Molecular mass calculations were performed using ASTRA 6 assuming a dn/dc of 0.1850 mL/g (Wyatt Technologies). The data was plotted using GraphPad Prism 8.2.0.

Please see attached data files:

- Astra6 reports (MALS_WT.pdf/MALS_WT_Ubq.pdf/MALS_R221A.pdf/MALS_R221A_Ubq.pdf)
- Raw data (MALS_WT.csv/MALS_WT_Ubq.csv/MALS_R221A.csv/MALS_R221A_Ubq.csv)
- GraphPad files (MALS_WT.pzfx/MALS_R221A.pzfx)

The results are summarized in Table 1 and depicted in Figures 1, 2 and 3.

Table 1. Summary of SEC-MALS

	Peak Elution Volume (mL)	Mn (g/mol)	Mv (g/mol)	Mw (g/mol)	Mz (g/mol)	Mp (g/mol)	Polydispersity (Mw/Mn)	Peak Molecular Weight (kDa)
WT USP5	17.4	1.004 x 10^5 (±1.426%)	n/a	1.083 x 10^5 (± 3.154%)	2.182 x 10^5 (± 58.989%)	9.970 x 10^4 (±0.271%)	1.1	99.7
WT USP5 + Ubq	17.4	4.397 x 10^4 (±2.031%)	n/a	9.223 x 10^4 (±2.378%)	1.614 x 10^5 (±27.249%)	1.068 x 10^5 (±0.338%)	2.1	106.8
R221A USP5	17.5	1.051 x 10^5 (±0.561%)	n/a	1.058 x 10^5 (±0.672%)	1.078 x 10^5 (±2.322%)	1.064 x 10^5 (±0.321%)	1.0	106.4
R221A USP5 + Ubq	17.3	1.051 x 10^5 (±0.681%)	n/a	1.056 x10^5 (±0.839%)	1.064 x 10^5 (±2.348%)	1.059 x 10^5 (±0.351%)	1.0	105.9

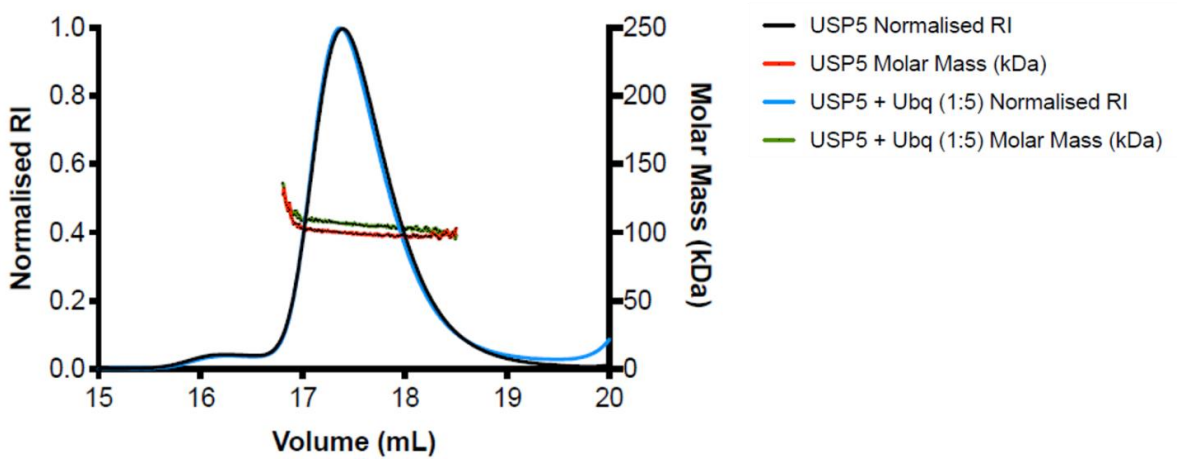

Figure 1. SEC-MALS of wildtype USP5 only (black) and wildtype USP5 + Ubq (blue)

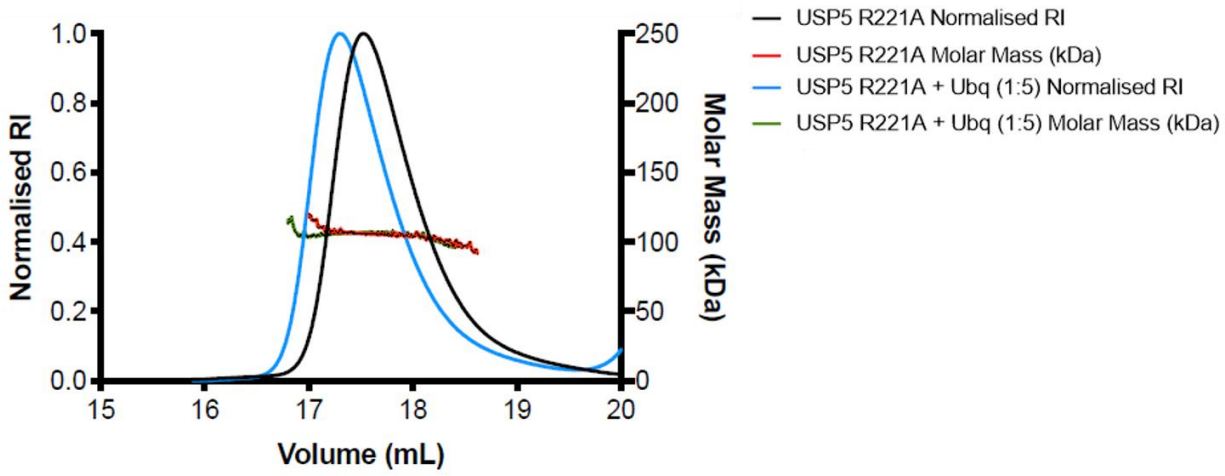

Figure 2. SEC-MALS of R221A mutant USP5 only (black) and R221A USP5 + Ubq (blue)

Conclusions & Future Directions

WT USP5 is fairly monodispersed (Mw/Mn= 1.1) and has a molecular weight of approximately 99 kDa, similar to the expected molecular weight of the protein which is 95.3 k Da. WT USP5 + Ubq elutes at the same volume as WT USP5 (17.4 mL) and has a molecular weight of approximately 107 kDa. This corresponds to 1:1 binding of ubiquitin (MW= 8.6 kDa) to USP5.

R221A USP5 has an approximate molecular weight of 106 kDa, which is higher than the expected molecular weight of the protein (MW= 99.6 kDa). R221A + Ubq also has an approximate molecular weight of 106 kDa. The mutant R221A ± Ubq are not significantly different and without orthogonal experiments, it is difficult to interpret what the MALS data means. SPR assay results confirm ubiquitin binds to R221A USP5 (K_D= 38 ± 3 µM) albeit 100-fold weaker than the WT (K_D= 0.3 ± 0.1 µM). Interestingly, there was a shift in the elution volume of the R221A + Ubq compared to R221A, where the R221A + Ubq elutes approximately 0.2 mL before R221A USP5. Perhaps, there is a conformational shift

of the R221A USP5 in the presence of ubiquitin. Large conformational remodeling of full-length USP5 was previously suggested[1].

Next, we will use dynamic light scattering (DLS) and small angle X-ray scattering (SAXS) to better understand ubiquitin binding to various USP5 constructs, including R221A.

[1]*Biochemistry* 2012, 51, 6, 1188-1198