Clusterin purification from HEK293E cells

Buffers

Binding buffer: 20 mM Na acetate pH 5.0.

Denaturing buffer: 20 mM Na acetate pH 5.0, 6 M urea.

Elution buffer: 20 mM Na acetate pH 5.0, 500mM NaCl.

Size exclusion chromatography buffer: 20 mM Na acetate pH 5.0, 100 mM NaCl, 1 mM EDTA.

Clusterin expression

1.- Express Clusterin (Clu) in HEK293E cells cultured in FreeStyle 293 Expression Medium (Thermo Fisher Scientific, 12338018) for 4 days.

Note: This protocol was optimized using HEK293E cells stably expressing Clu-Strep tag (pB-T-PAF-CluStrep), however Clu without any affinity tag can be purified following this method since the binding of the fusion protein Clu-Strep to the Strep-Tactin column was too weak for purification and the method was then optimized for purification without any affinity tag by cation exchange chromatography followed by size exclusion chromatography.

2.- Centrifuge culture and keep conditioned medium.

3.- Dialyze conditioned medium overnight in 20 mM Na acetate pH 5.0 (volume ratio <1:100).

4.- If some precipitates are observed, remove by centrifugation.

Cation exchange chromatography

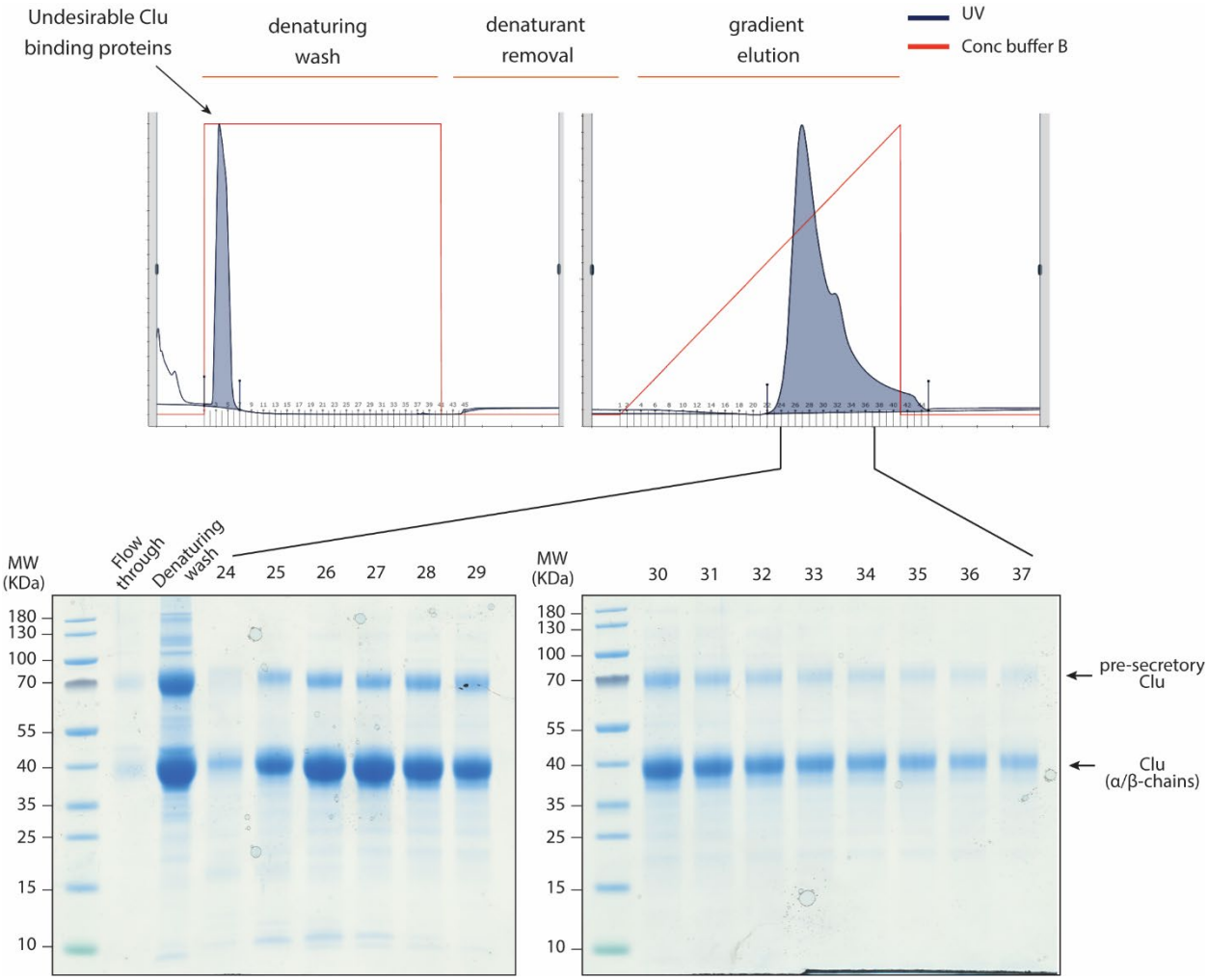
5.- Load dialyzed conditioned medium into a HiTrap SP XL cation exchange column previously equilibrated with 20 mM Na acetate pH 5.0. Wash with 20 mM Na acetate pH 5.0.

6.- Wash the column with 10 column volumes (CV) of denaturing buffer (20 mM Na acetate pH 5.0, 6 M urea) to remove undesired proteins bound to Clu.

7.- Wash the column with 5 CVs 20 mM Na acetate pH 5.0.

8.- Elute Clu with a 0-500 mM linear NaCl gradient in 20 mM Na acetate pH 5.0.

9.- Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.



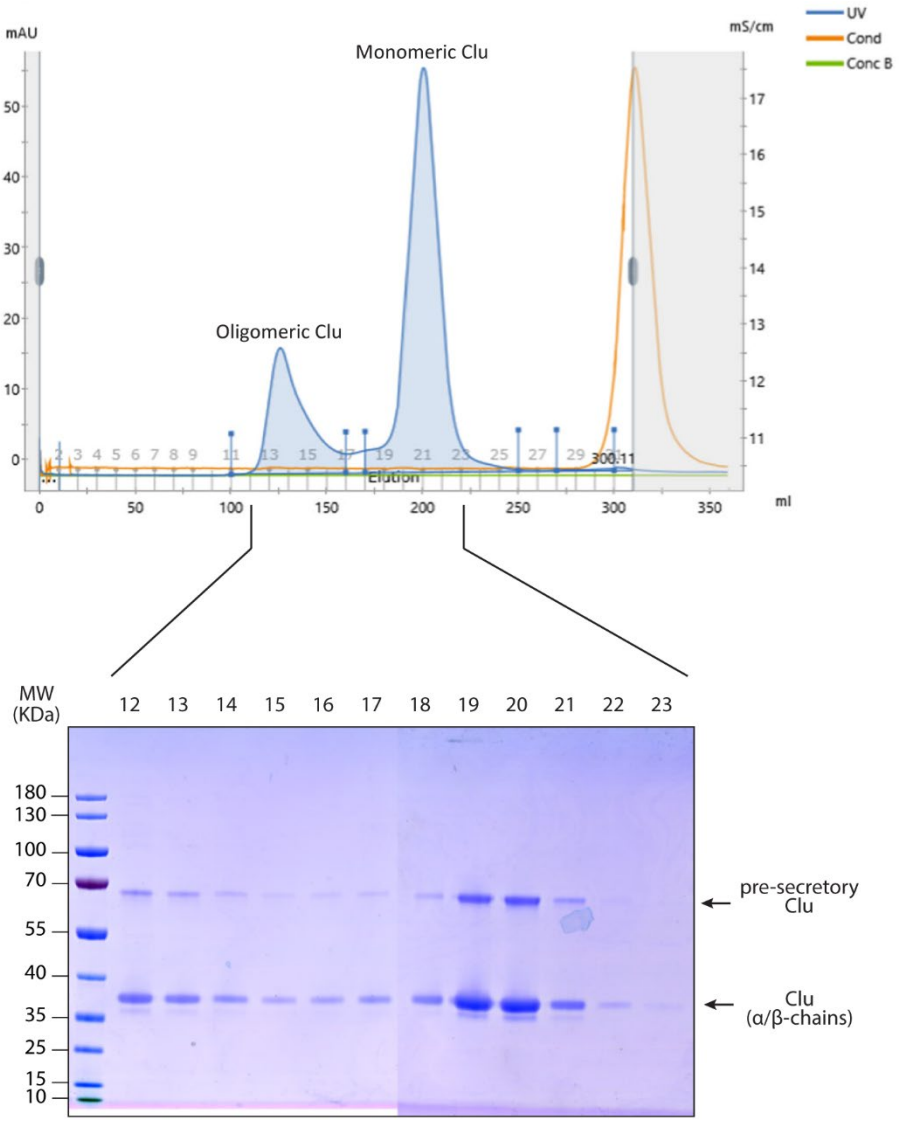
Note: Denaturing wash can be omitted if not many contaminants are observed in the conditioned media. A small percentage of Clu in the conditioned media is not cleaved at the furin-like protease cleavage site (pre-secretory Clu), probably due to its high expression level. Clus is

highly glycosylated and migrates in the denaturing SDS-PAGE gel at around 40 kDa (α and β chains, not resolved) or around 70 kDa (pre-secretory, uncleaved Clu)

Size exclusion chromatography

10.- Load Clu-containing fractions into a Superdex-200 previously equilibrated 20 mM Na acetate pH 5.0, 100 mM NaCl, 1 mM EDTA.

11.- Analyze eluted fraction by SDS-PAGE and Coomassie blue staining. Clu oligomers are in equilibrium with monomeric Clu so all peaks containing Clu can be pooled.



Note: Oligomeric state of Clu is pH dependent. At pH 5.0 mainly monomeric Clu is eluted.

12.- Concentrate Clu-containing fractions using a Vivaspin ultracentrifugation unit 10,000 MWCO or similar until reach desired concentration.

13.- Aliquot and flash-freeze purified Clu in liquid nitrogen for storage at −80 °C.

Note: Approximate yield: from 250 ml of conditioned media around 12 mg of pure Clu are obtained. Clu purified from HEK293E cells present a comparable glycosylation pattern as Clu purified from plasma (Biovendor R&D, RD172034100). In order to obtain sharp bands of the α and β Clu chains in the SDS-PAGE, deglycosylation can be performed with PNGase F.