



Membrane Protein Separation on Size Exclusion

Highlighted FACTS:

- We have highlighted the ability of Sepax's SRT, SRT-C and Nanofilm SEC phases to separate out a variety of different membrane proteins.
- Sepax' SEC columns can be run under a large variety of conditions, including those that use detergents such as: LDAO, Octyl glucoside, DDM and UDM (β -undecylmaltoside).
- Sepax's SEC columns are scalable for use in both analytical separations to preparative purifications.
- Sepax offers a variety of SEC pore sizes for membrane detergent complex separations.

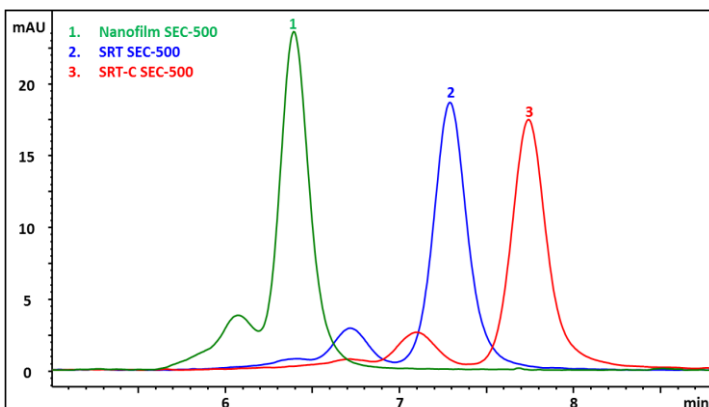
Order Information

201500-7830	Nanofilm SEC-500, 5 μ m, 450 A 7.8 x 300 mm
201500-4630	Nanofilm SEC-500, 5 μ m, 450 A 4.6 x 300 mm
201250-4630	Nanofilm SEC-250, 5 μ m, 250 A 4.6 x 300 mm
235500-4630	SRT-C SEC-500, 5 μ m, 500 A 4.6 x 300 mm
235500-7830	SRT-C SEC-500, 5 μ m, 500 A 7.8 x 300 mm
235300-7830	SRT-C SEC-300, 5 μ m, 500 A 7.8 x 300 mm
235300-4630	SRT-C SEC-300, 5 μ m, 500 A 4.6 x 300 mm
215500-4630	SRT SEC-500, 5 μ m, 500 A 4.6 x 300 mm
215500-7830	SRT SEC-500, 5 μ m, 500 A 7.8 x 300 mm
215300-4630	SRT SEC-300, 5 μ m, 500 A 4.6 x 300 mm

A larger pore size may be needed for separation of protein and detergent complex.

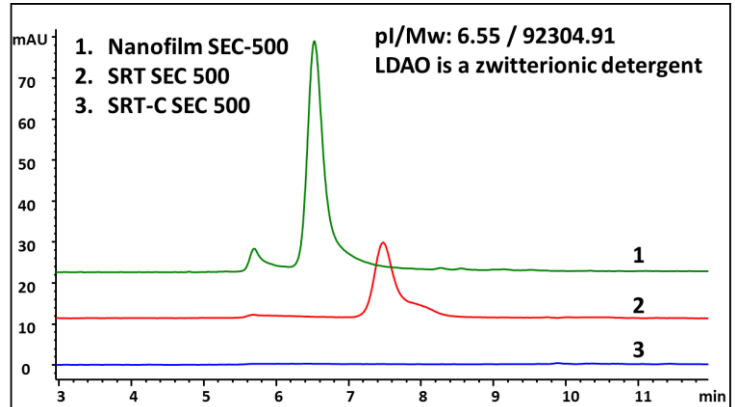
BSA (66 kDa) in 0.1% LDAO

Column: Nanofilm, SRT-C and SRT SEC-500 (all in 7.8 x 300 mm); Mobile phase: 10 mM Tris with 0.1% LDAO pH 8.0; Flow rate: 1 mL/min; Detection: UV 280 nm; Injection Volume: 5 μ L 2 mg/mL BSA in mobile phase



Photosynthetic Reaction Center in 0.1% LDAO

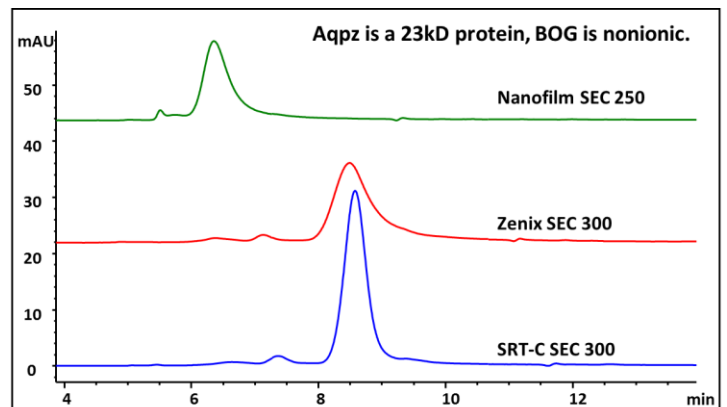
Column: Nanofilm, SRT-C and SRT SEC-500, (all in 7.8 x 300 mm); Mobile phase: 10 mM Tris with 0.1% LDAO pH 8.0; Flow rate: 1 mL/min; Detection: UV 280 nm; Injection Volume: 2 μ L 7 mg/mL RC in mobile phase



Nanofilm gives the best separation and recovery.

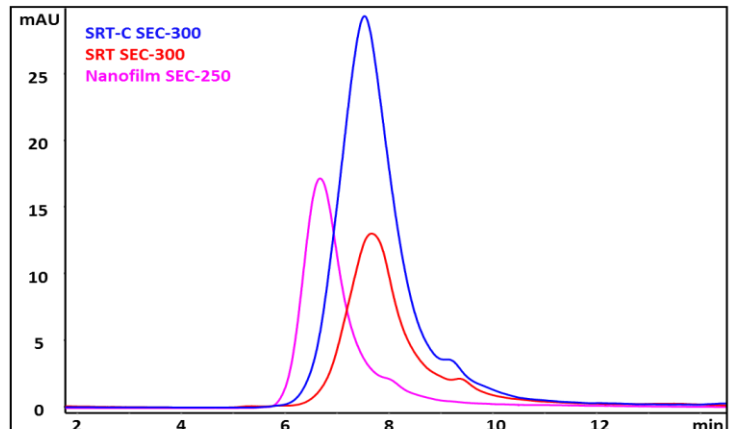
Membrane Protein Aqpz Separation in Octyl glucoside

Column: Nanofilm SEC-250, SRT-C and SRT SEC-300 (all in 7.8 x 300 mm); Mobile phase: 20 mM TrisHCl, pH 7.0, 190 mM NaCl, 10 mM KCl, 40 mM Octyl glucoside; Detection: 280nm; Flow: 1 mL/min; Injection: 2 μ L of 6 mg/mL



Bacterial K Channel (16 kDa homotetramer) in 0.261% DDM

Column: Nanofilm SEC-250, SRT-C and SRT SEC-300 (all in 4.6 x 300 mm); Mobile phase: 20 mM Tris, pH 7.5, 20 mM NaCl, 0.261% DDM; Detection: 280nm; Flow: 0.35 mL/min; AKTA FPLC system;



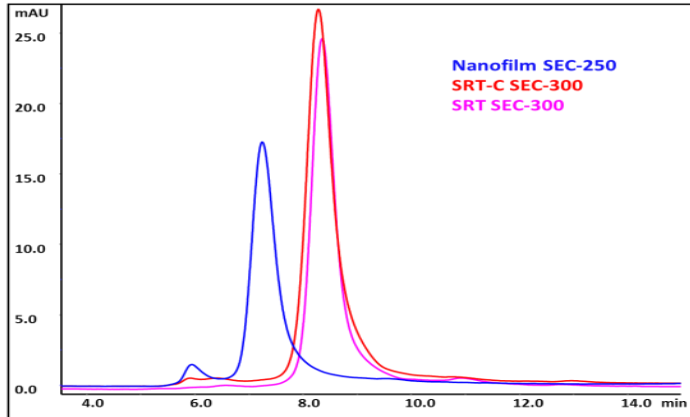
SRT-C gives the best separation with less secondary interaction, baseline separation with the high molecular weight proteins.



Membrane Protein Separation on Size Exclusion

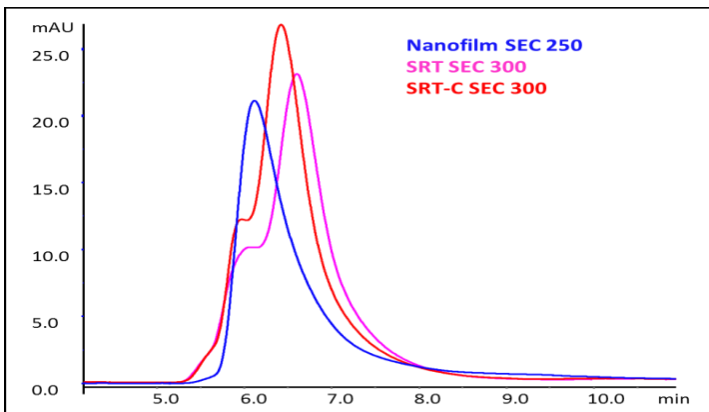
Aldolase (158 kDa) IN 0.261% DDM

Column: Nanofilm SEC-250, SRT and SRT-C SEC-300, (all in 4.6 x 300 mm);
Mobile phase: 20 mM Tris, pH 7.5, 20 mM NaCl, 0.261% DDM;
Detection: 280 nm; Flow rate: 0.35mL/min; AKTA FPLC system



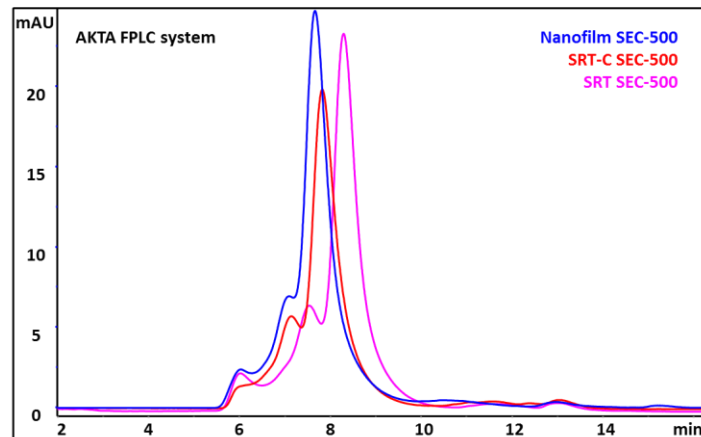
Ferritin (440 kDa) in 0.261% DDM

Column: Nanofilm SEC-250, SRT and SRT-C SEC-300, (all in 4.6 x 300 mm);
Mobile phase: 20 mM Tris, pH 7.5, 20 mM NaCl, 0.261% DDM (n-dodecyl-b-D-Maltoside); Detection: 280 nm; Flow rate: 0.35mL/min; AKTA FPLC system



Ferritin (440 kD) in 0.1% UDM

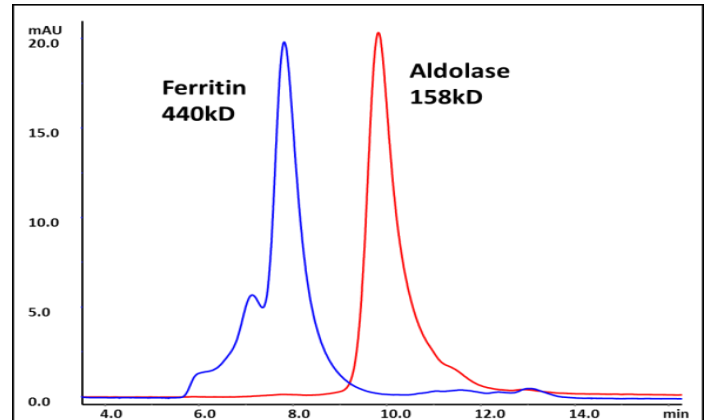
Column: Nanofilm SEC-500, SRT -C SEC-500, SRT SEC-500 (all 4.6 x 300 mm);
Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside); Flow rate: 0.35 mL/min; Detection: UV 280 nm;
Injection Volume: 100 μ L 0.5 mg/mL ferritin in mobile phase



SRT SEC-500 best separated the aggregates of Ferritin in the presence of detergent.

Ferritin/Aldolase on (SRT-C SEC-500) in 0.1% UDM

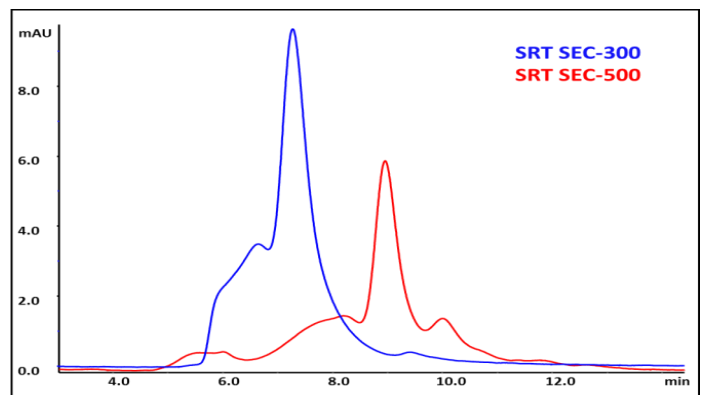
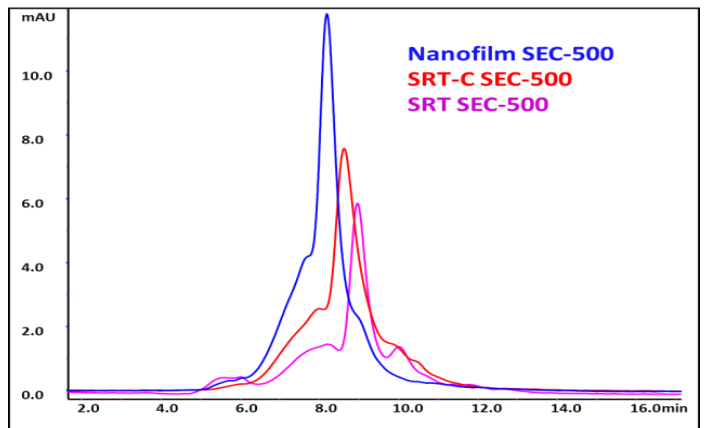
Column: SRT-C SEC-500, 4.6 x 300 mm



SRT-C SEC-500 is the best for Ferritin & Aldolase separation

Bacterial ABC Transporter, MsbA (65kDa homodimer) in 0.1% UDM

Column: Nanofilm SEC-500, SRT-C SEC-500, SRT SEC-500 and SEC-300 (all in 4.6 x 300mm); Mobile phase: 20 mM Tris, pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside); Detection: 280 nm; Flow rate: 0.35mL/min; AKTA FPLC system



SRT showed better separation and larger pore size 500 Å may help with separation of aggregates and monomer; however the recovery may be lower.

*Acknowledgement: Sung Lee at Scripps for the data