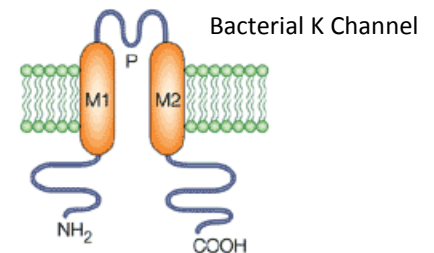


Membrane Protein Separations by SEC

Katherine McLaughlin
Sepax Technologies, Inc.



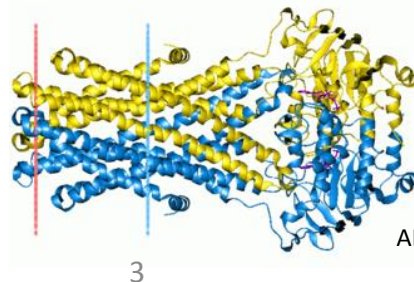
Outline

- Membrane Protein Introduction
- Sepax SEC Phases
 - SRT[®]
 - SRT[®]-C
 - Nanofilm[®]
- Standard proteins with detergent separation by SEC - BSA, Aldolase, Ferritin.
- Examples of “real world” membrane protein/detergent complex SEC separation.
- Conclusion



What are Membrane Proteins?

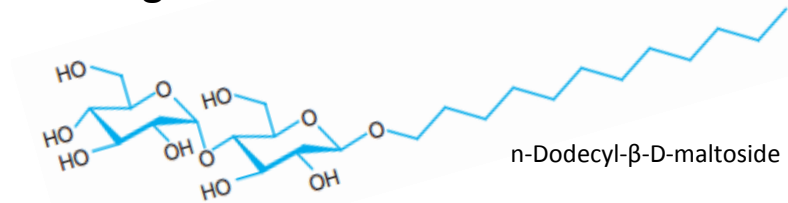
- Membrane proteins are large biomolecules that sit in the lipid bilayer of cells. The function of a membrane protein is to help the cell communicate through the lipid bilayer and with its outside environment.
- Types of membrane proteins:
 - a) **Integral membrane proteins** are permanently attached to the membrane. They can only be separated from the biological membranes using detergents, nonpolar solvents, or denaturing agents
 - b) **Peripheral membrane proteins** are attached to either the lipid bilayer or to integral proteins and can be removed under mild conditions.
- Membrane proteins are targets of about 50% of all small molecule drugs. In order to design better drugs it is necessary to understand the molecular structure that these drugs bind to.
- Another importance of the research into membrane proteins is that a number of diseases are caused by their mutation. Depression and schizophrenia are the result of mutations in iron channels and Cystic fibrosis is a mutation in an ABC transporter protein.



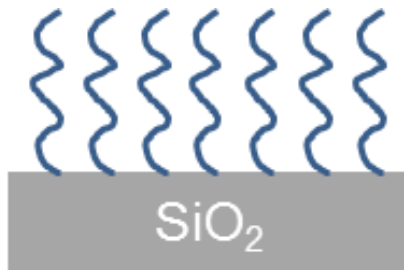
ABC Transporter, MsbA

Membrane Protein/Detergent Complexes

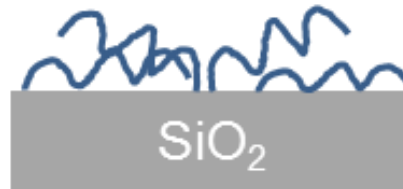
- The type of detergent chosen depends on several different factors including the CMC, solubility needs of the complex, downstream analyses needed, etc.
- There are four different categories of detergents:
 - Mild non-ionic detergents
 - Bile Salts
 - Mild amphoteric detergents
 - Denaturing ionic detergents
- The membrane protein/detergent complex behaves larger than the protein would itself.
- The large size and the hydrophobic properties of membrane protein/detergent complexes bring some difficulties when trying to separate them.



SEC Phases



SRT and Zenix SEC
Phase Stand-up
Monolayer



SRT-C and Zenix-C
SEC Phase Lay-down
Monolayer



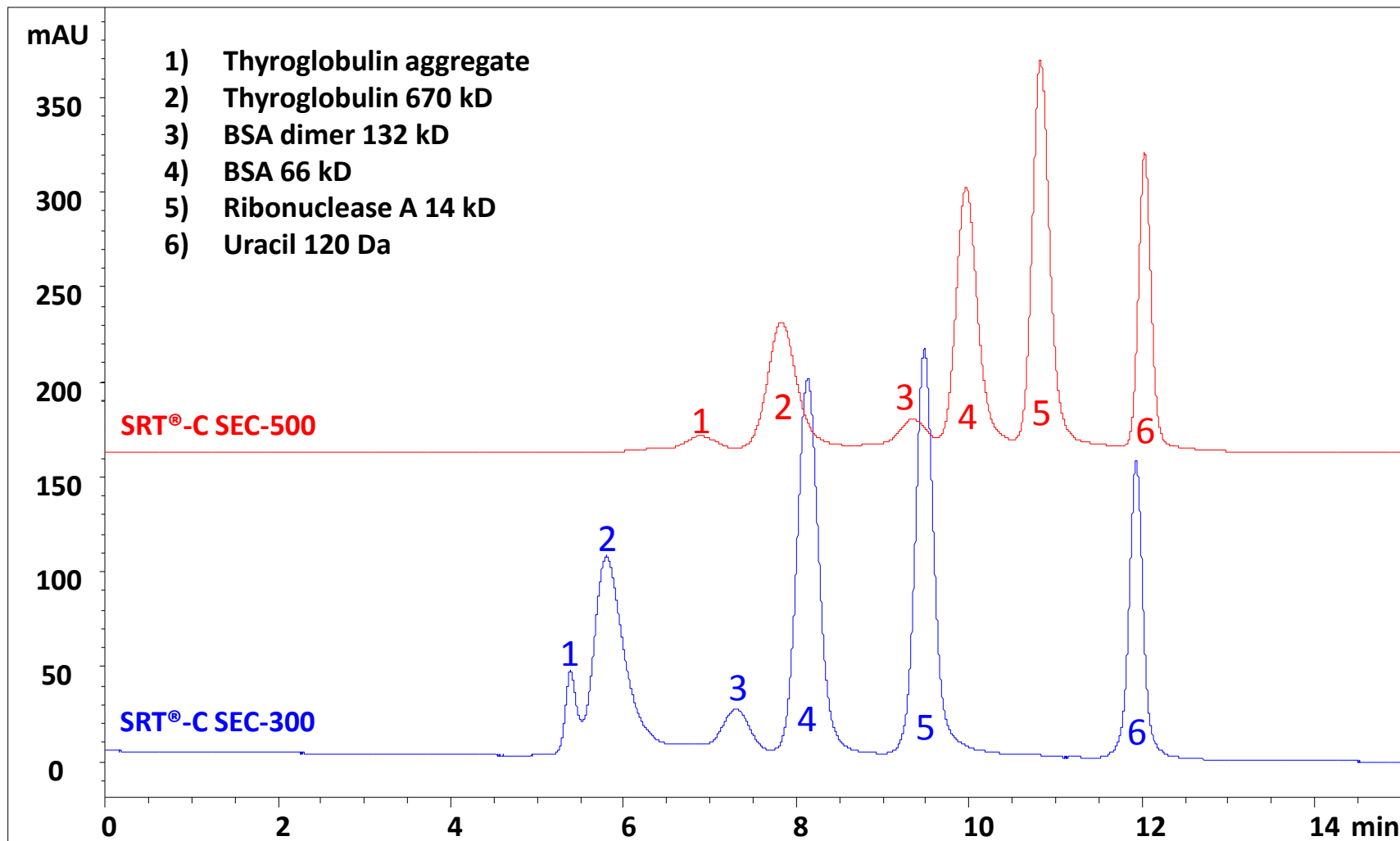
Nanofilm SEC
Hydrophilic nm
Thin Film

Protein standard separation on SRT-C

Column: SRT®-C SEC (5 μm, 300 and 500 Å, 7.8 x 300 mm each)

Mobile phase: 150 mM Phosphate buffer, pH 7.0; Flow rate: 1.0 mL/min;

Detector: UV 214 nm; Column temperature: 25 °C; Injection volume: 5 μL



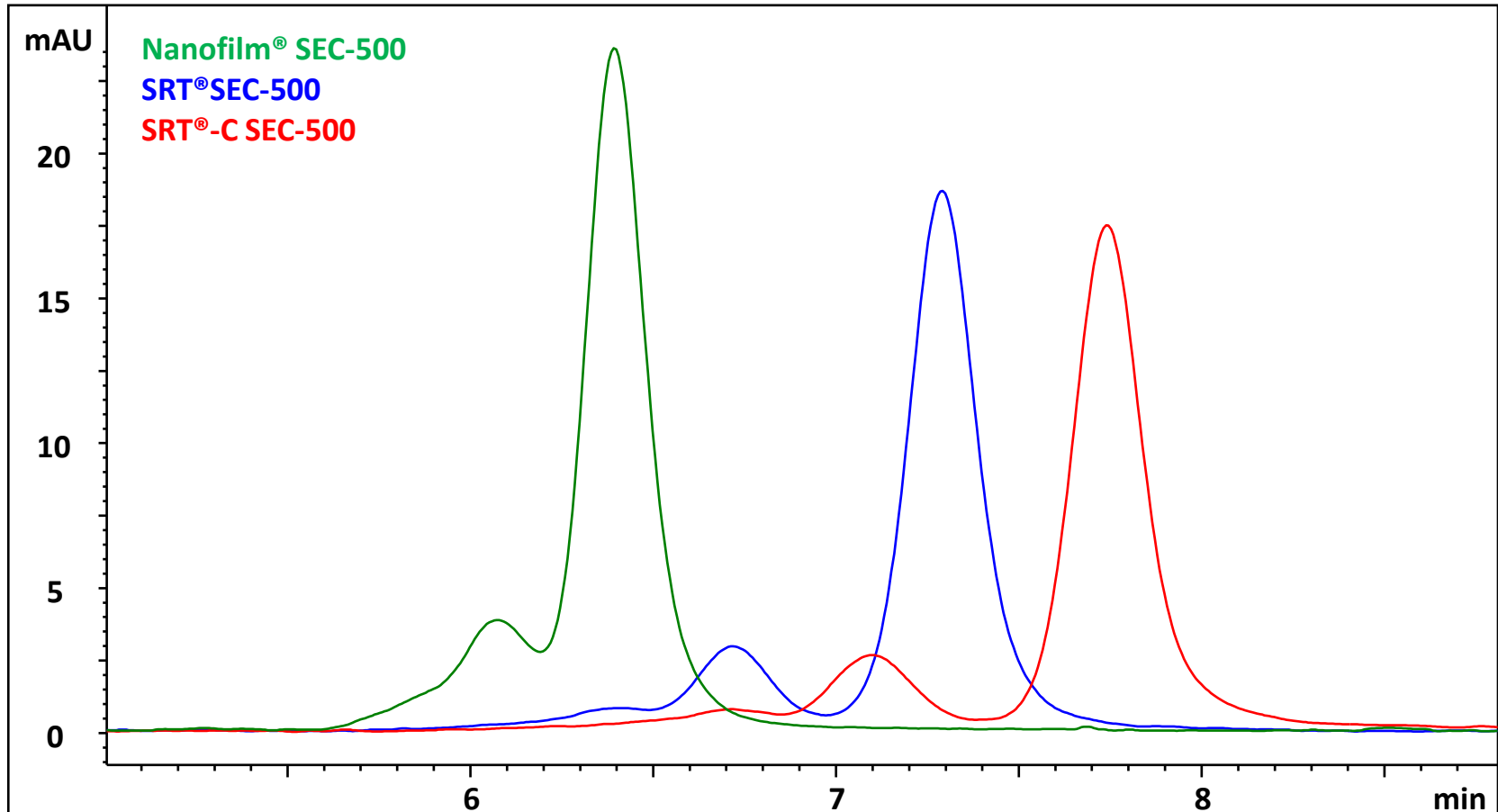
BSA (66 kD) in 0.1% LDAO

Column: Nanofilm® SEC-500, SRT®-C SEC-500 and SRT® SEC-500 (all 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 µL 2 mg/mL BSA in mobile phase

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



Ferritin and Aldolase

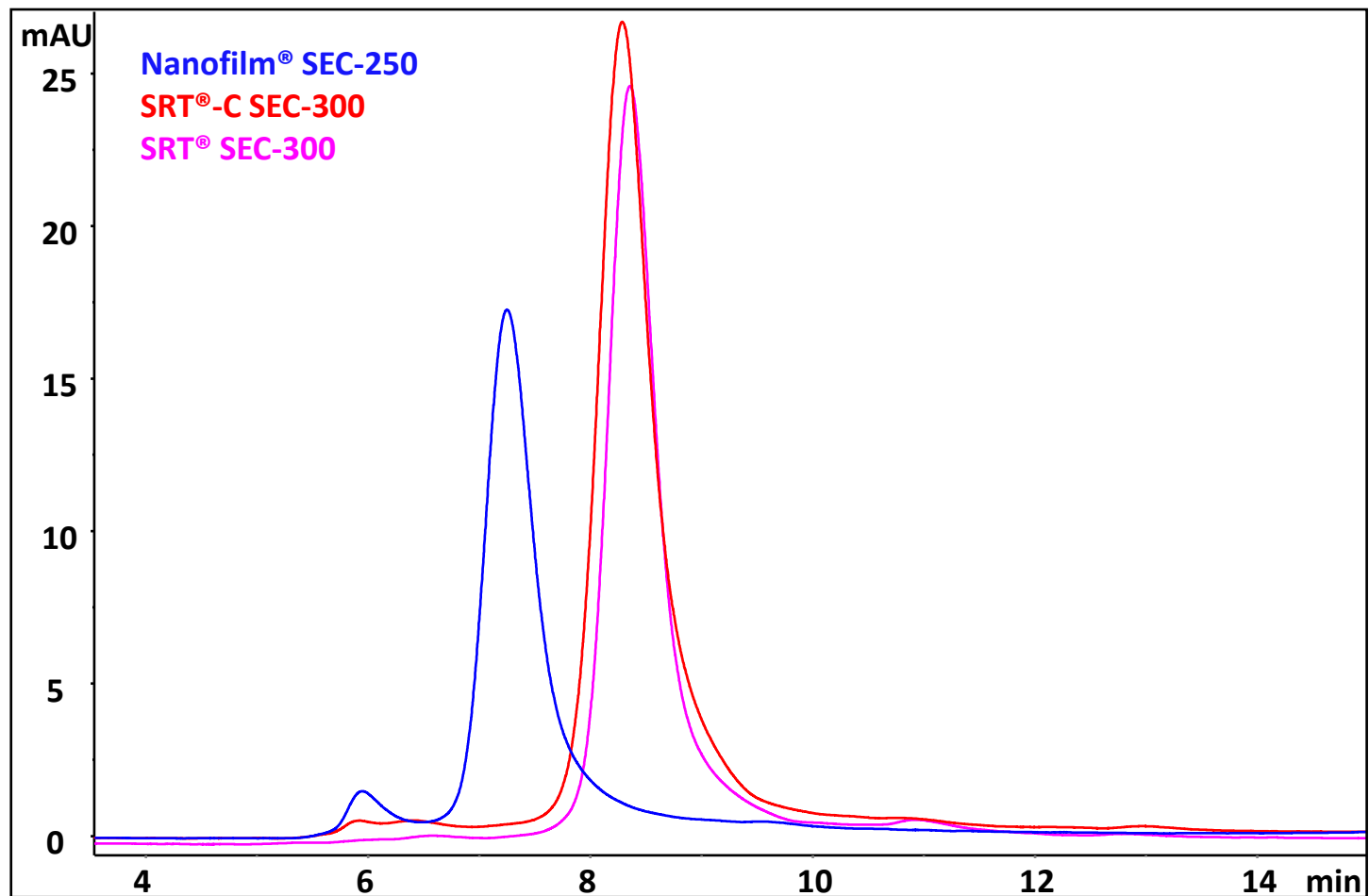


Aldolase (158 kD) in 0.261% DDM

Column dimension: 4.6 x 300 mm (all),

Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.261% DDM (n-dodecyl-b-D-Maltoside),

Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system



Acknowledgement: Sung Lee at Scripps for the runs in the following slides.

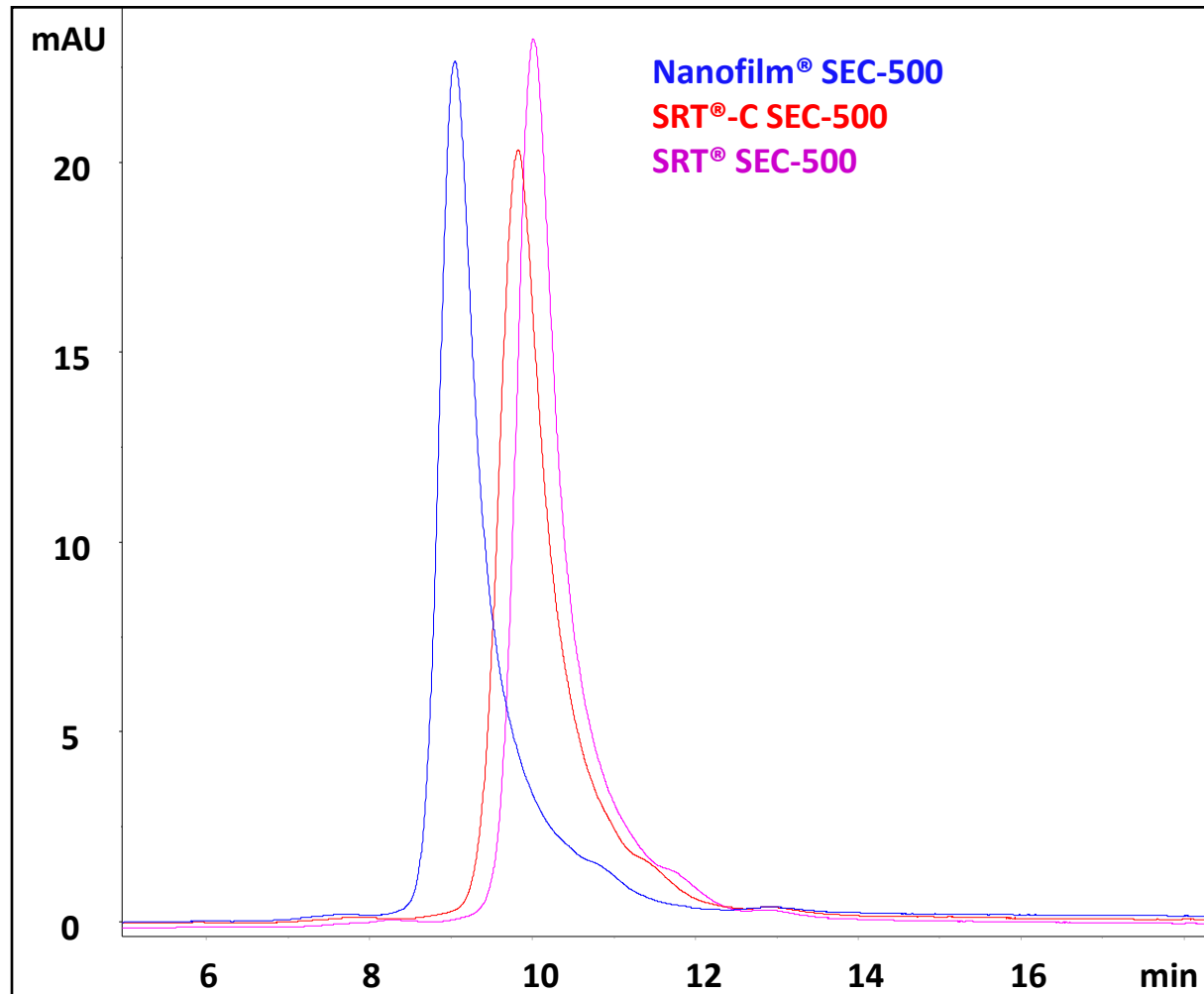


Aldolase (158 kD) in 0.1% UDM

Column dimension: 4.6 x 300 mm (all),

Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside),

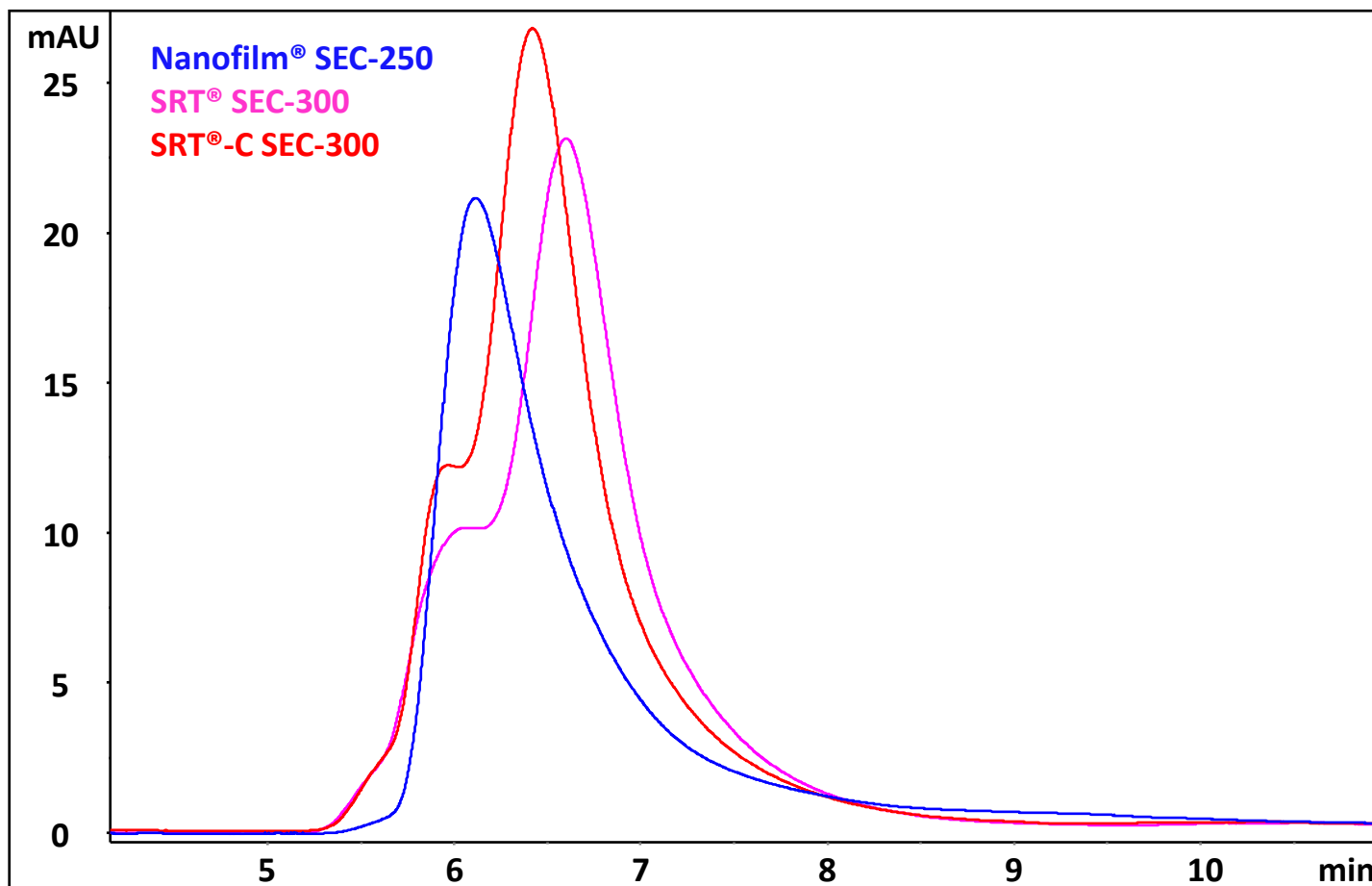
Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system



Ferritin (440 kD) in 0.261% DDM

Column dimension: 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: 0.35 mL/min; AKTA FPLC system

Larger Pore size 500 Å may help with the separation of aggregates and monomers.



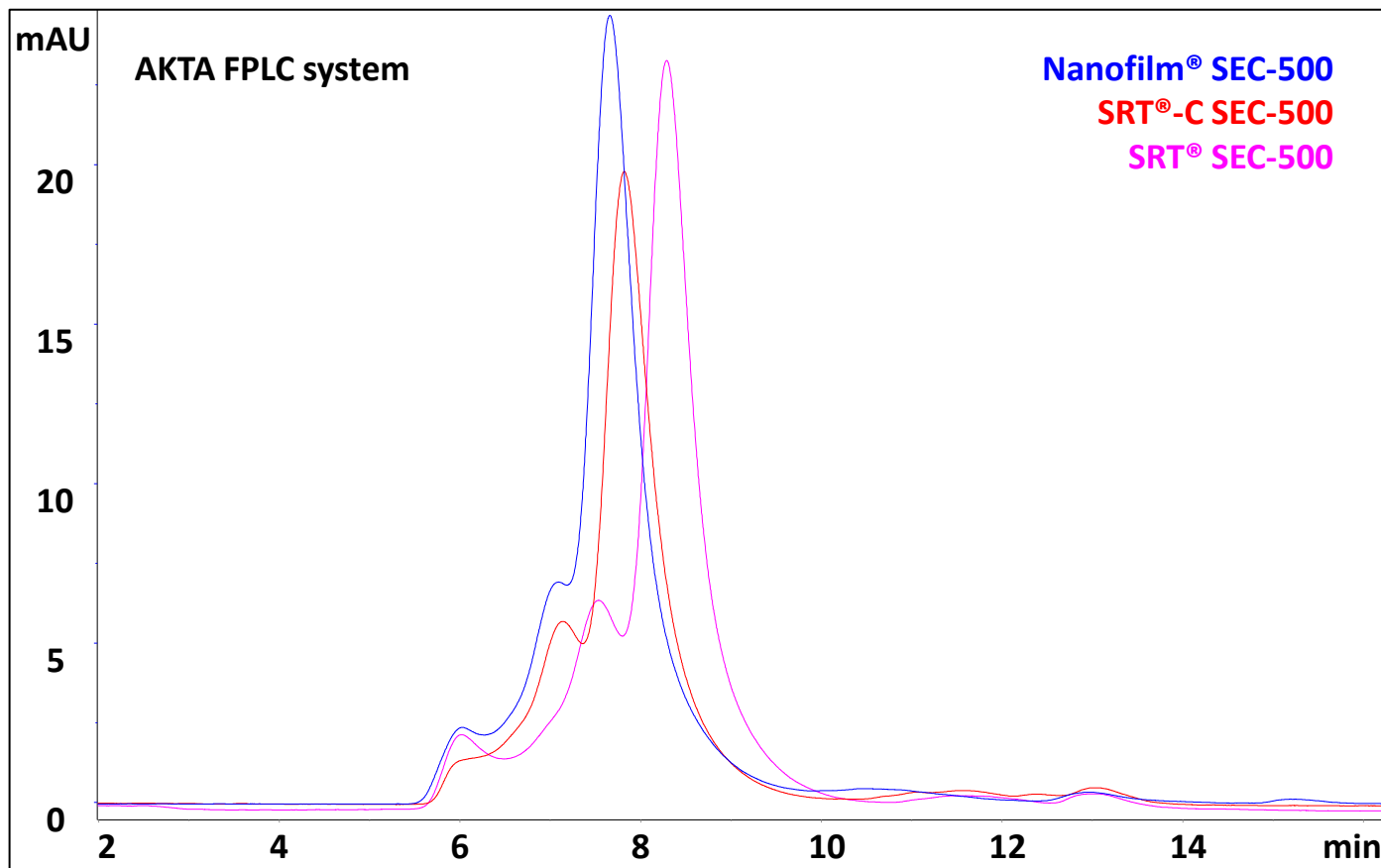
Ferritin (440 kD) in 0.1% UDM

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.

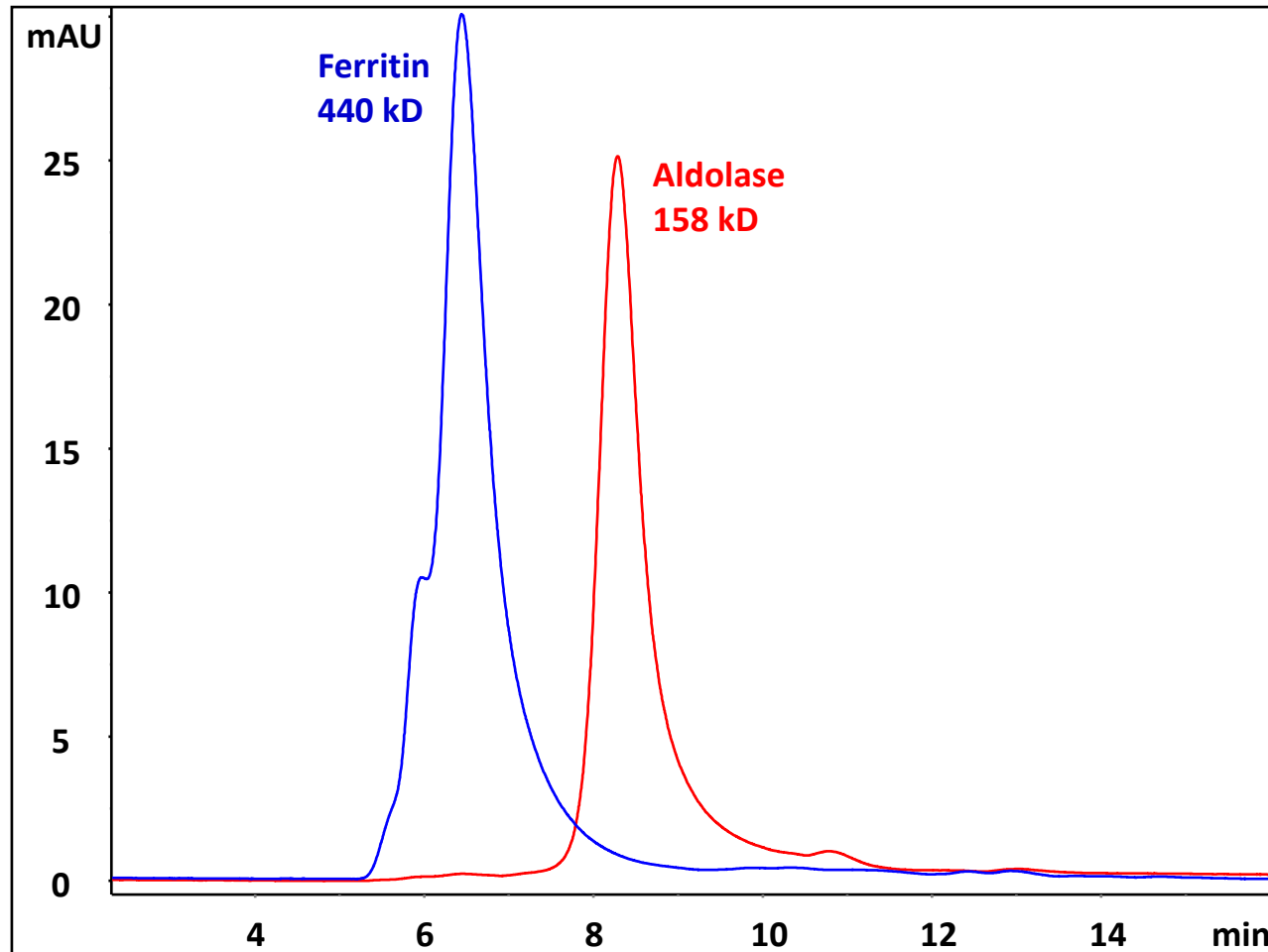


Ferritin and Aldolase on SRT[®]-C SEC-300 in 0.261% DDM

Column dimension: SRT[®]-C SEC-300 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: 0.35 mL/min; AKTA FPLC system



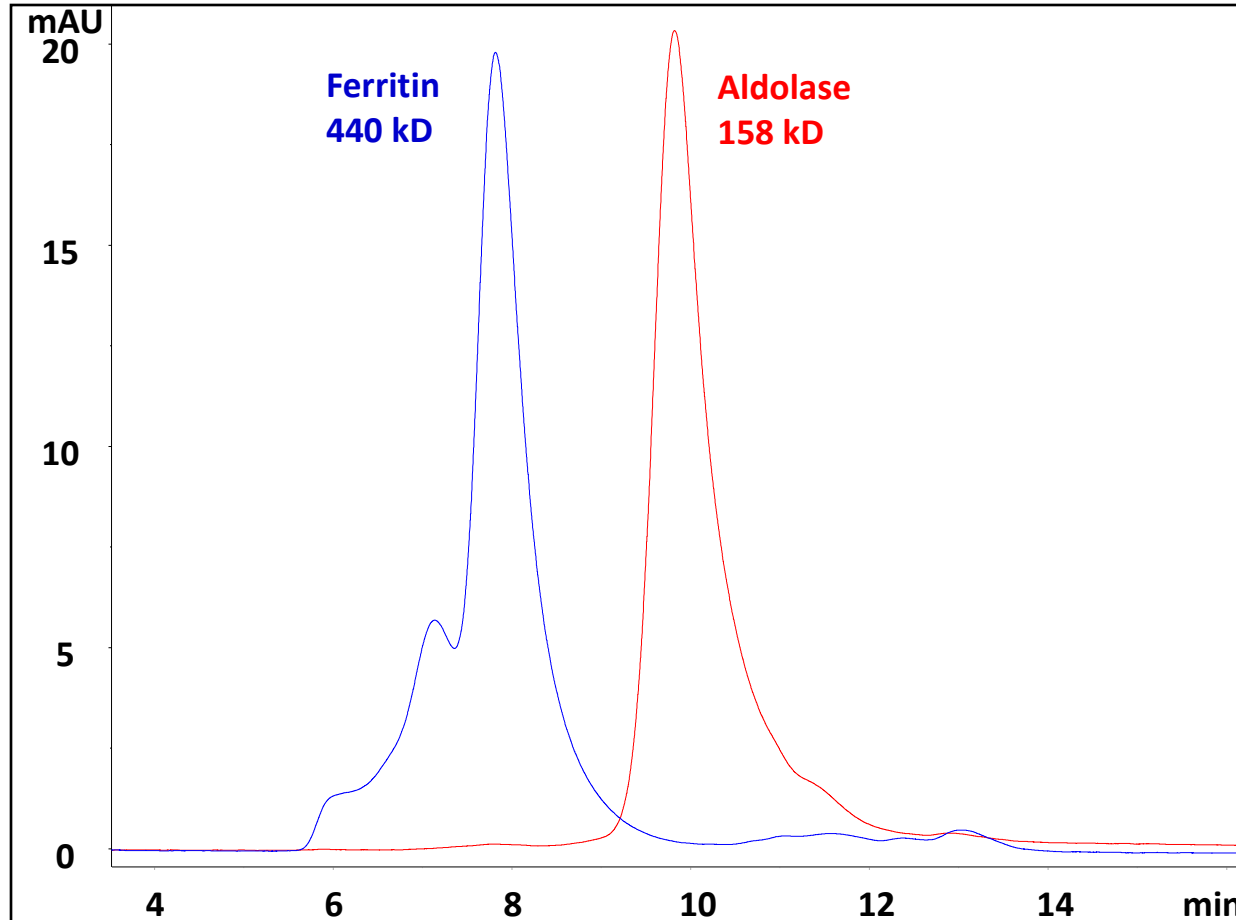
Ferritin and Aldolase on SRT[®]-C SEC-500 in 0.1% UDM

Column dimension: SRT[®]-C SEC-500 4.6 x 300 mm;

Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside);

Detection: 280 nm; Flow rate: 0.35 mL/min; AKTA FPLC system

SRT[®]-C SEC-500 is best for the separation of Ferritin and aldolase.



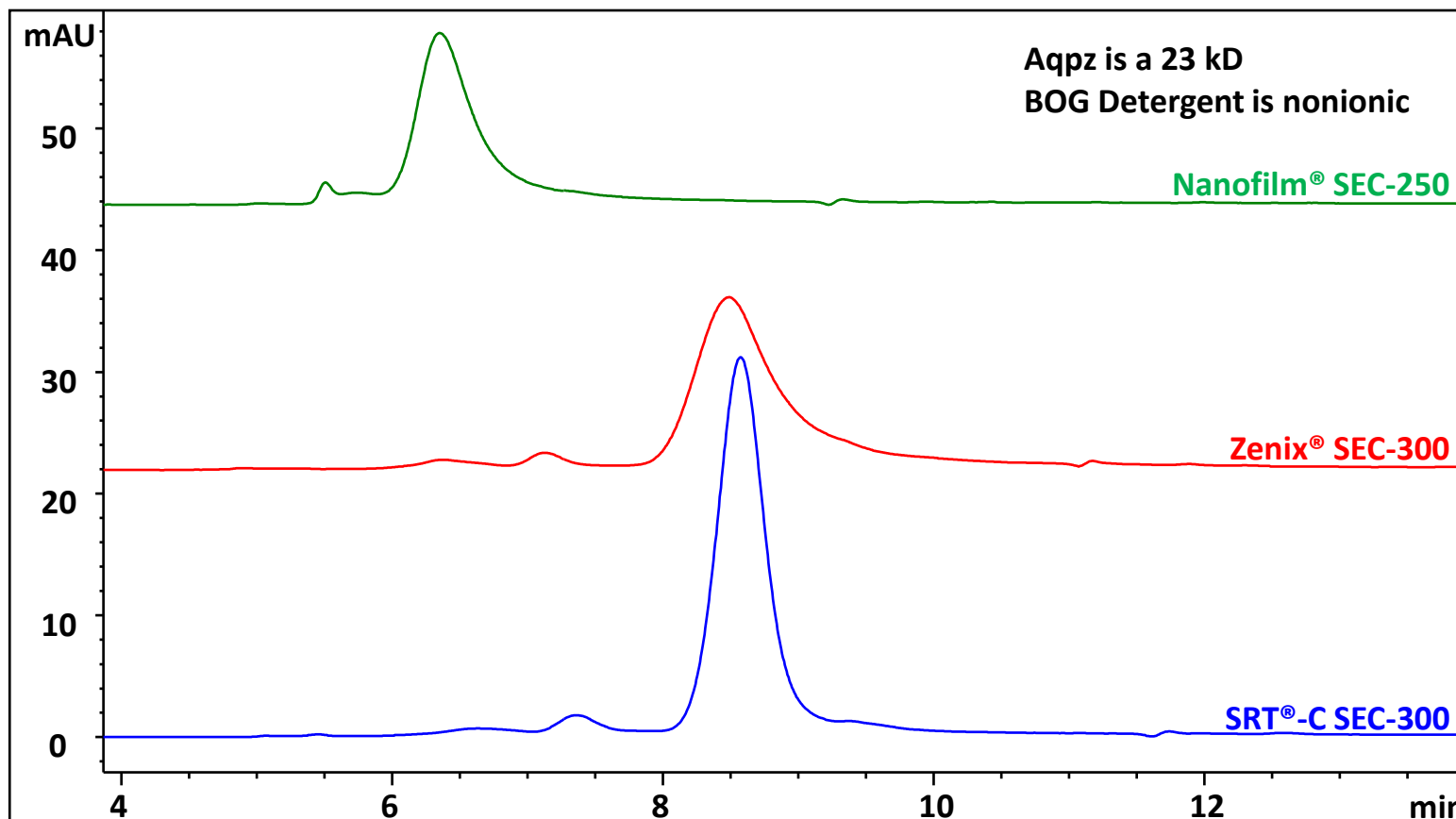
Membrane Proteins



Membrane protein Aqpz separation in Octyl glucoside

Column: Nanofilm® SEC-250, Zenix® SEC-300 and SRT®-C SEC-300 (all 7.8 x 300 mm),
Mobile phase: 20 mM tris-HCl pH 7.0, 190 mM NaCl, 10 mM KCl, 40 mM Octyl glucoside,
Detection: UV 280 nm, Flow: 1 mL/min, Injection: 2 µL of 6 mg/mL Aqpz

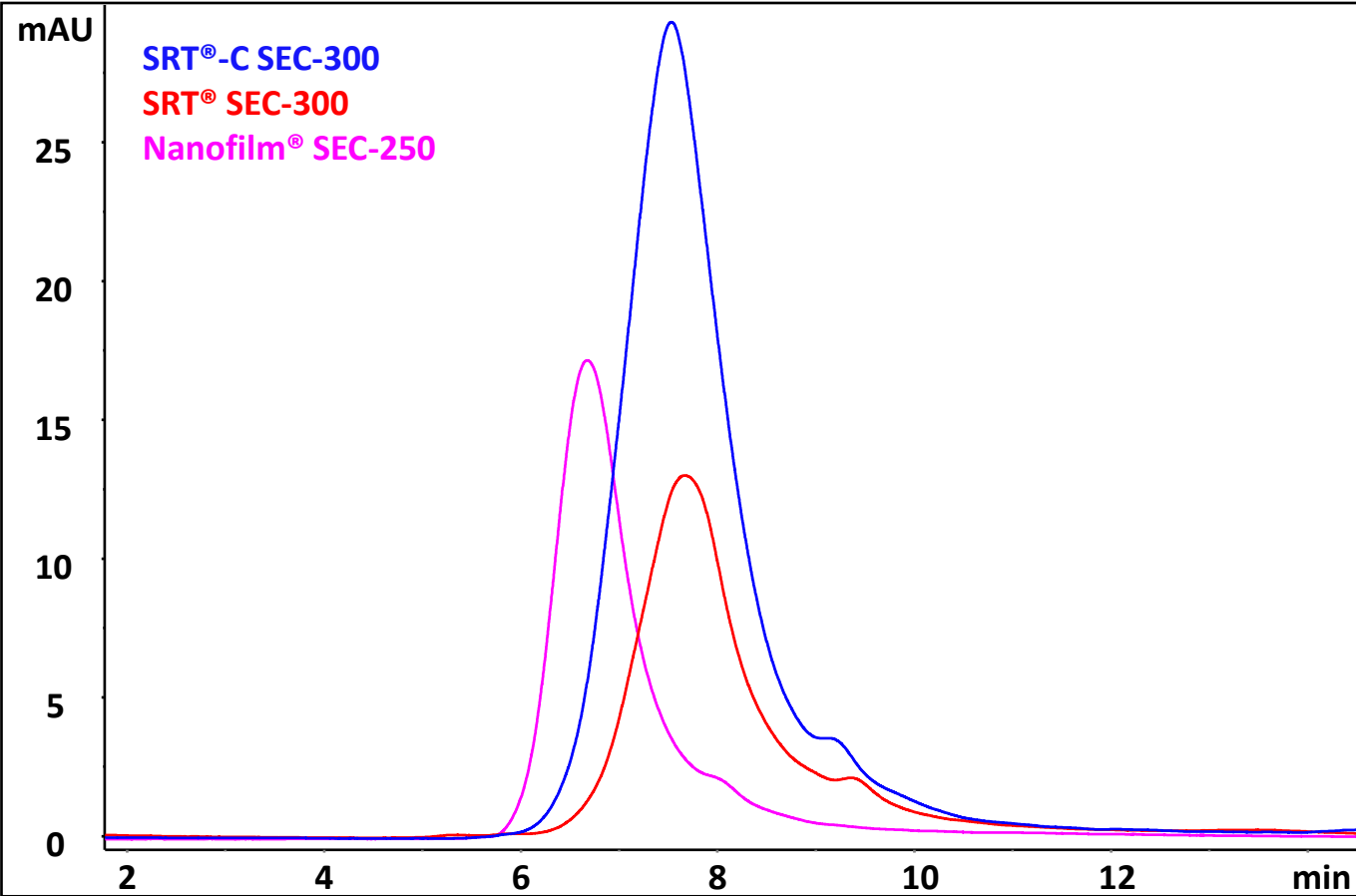
SRT®-C SEC-300 gave the best separation with less secondary interactions and baseline separation of the high molecular weight proteins.



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

Column dimension: 4.6 x 300 mm, Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.261% DDM, Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system

SRT®-C SEC-300 has the best recovery for Bacterial K Channel in the presence of detergents.



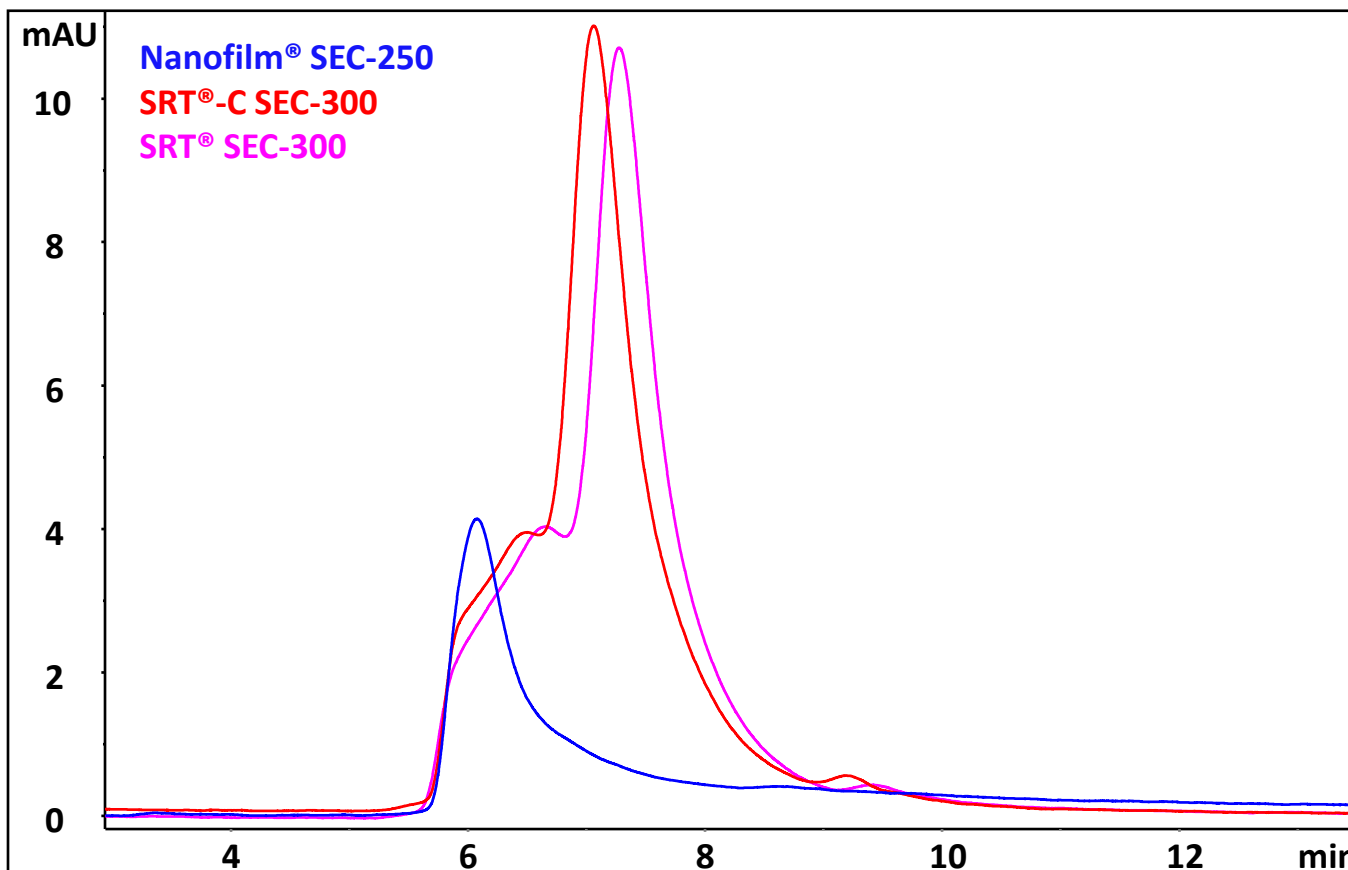
Acknowledgement: Sung Lee at Scripps for the runs in the following slides.



Bacterial ABC transporter, MsbA (65 kD homodimer) in 0.1% UDM

Column dimension: 4.6 x 300 mm, Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside), Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system

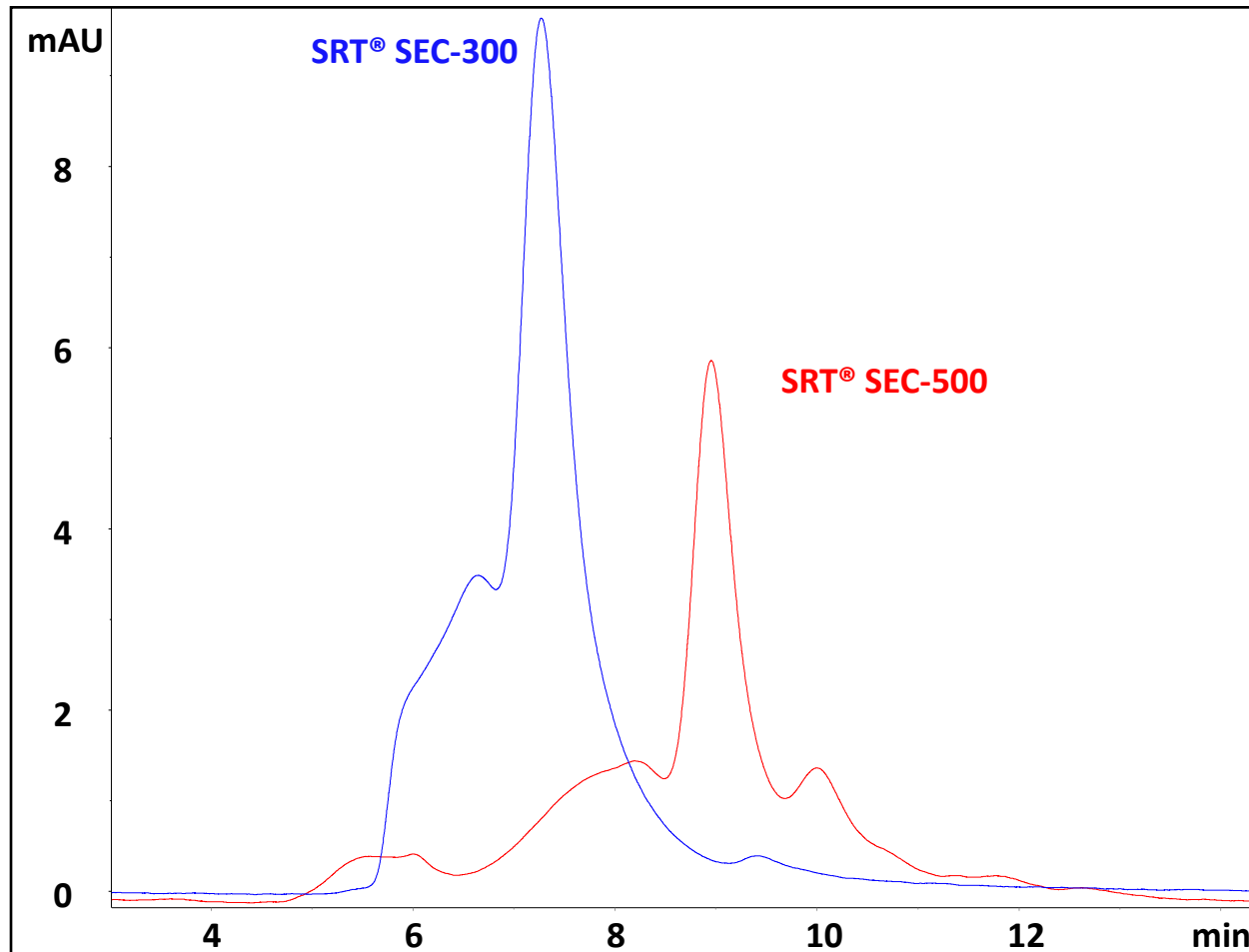
Larger Pore size 500 Å may help with the separation of aggregates and monomers.



MsbA (65 kD homodimer) in 0.1% UDM

Column dimension: 4.6 x 300 mm, Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.1% UDM (β -undecylmaltoside), Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system

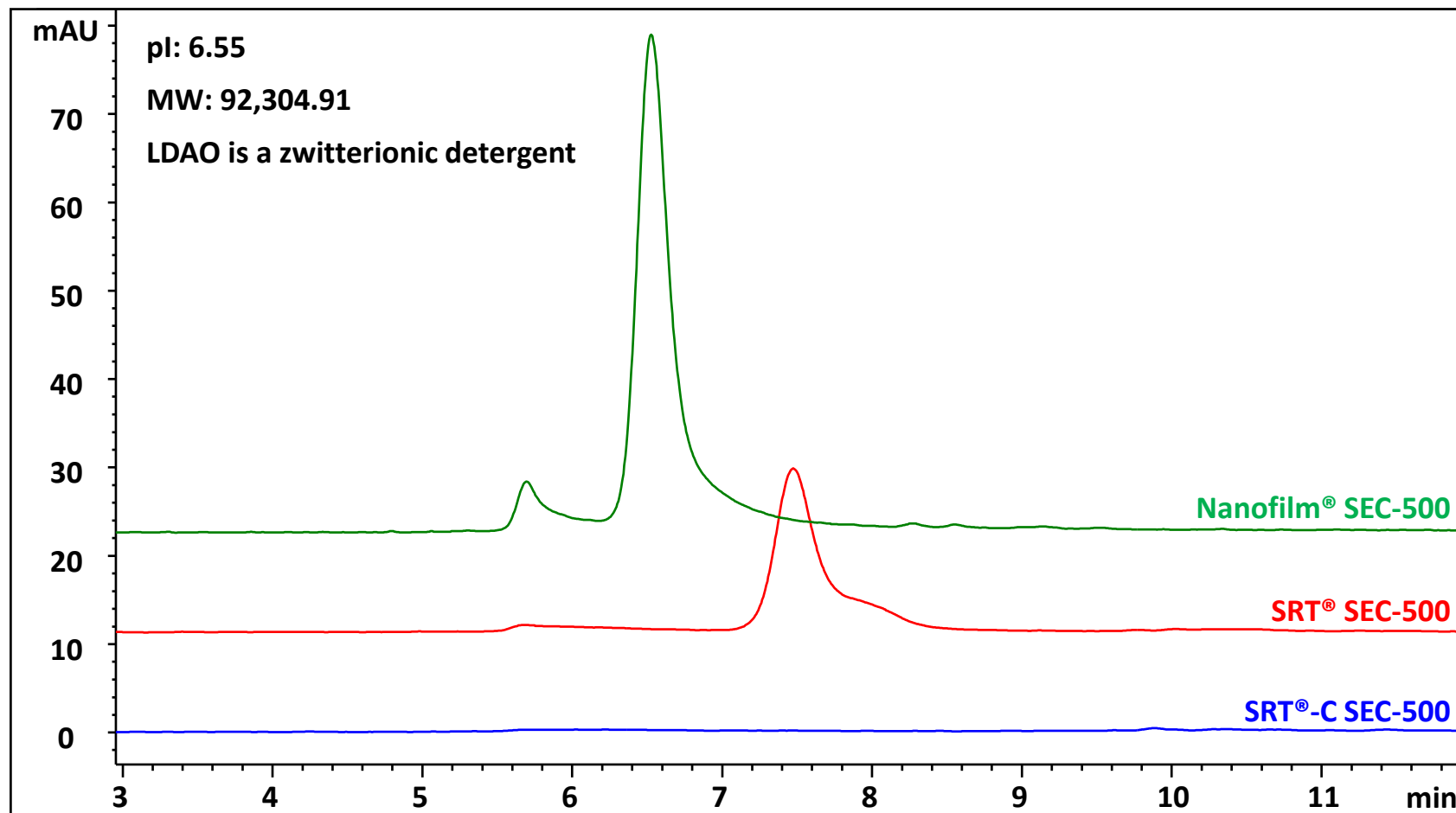
SRT® SEC-500 showed better separation.



Photosynthetic Reaction Center in 0.1% LDAO

Column: Nanofilm® SEC-500, SRT®-C SEC-500, SRT® SEC-500 (all 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 µL 7 mg/mL RC in mobile phase

Nanofilm® SEC-500 provided the best separation and recovery.



Acknowledgement: University of Delaware Chemical Engineering Dept. for use of this sample.

Conclusion

- Sepax SEC offers three different phase chemistries for different membrane protein/detergent complex separations. Due to the unique features of membrane protein/detergent complexes, one of the phases may provide best resolution/recovery.
- Due to the different hydrodynamic sizes of these complexes, a larger pore size may need to be evaluated to achieve the best HMW aggregate and monomer separation.
- Sepax SEC columns can be used for detergent screening and analytical high resolution separation for specific membrane proteins. These SEC columns can scale up to larger particle sizes such as 10 μm as well as larger column sizes at 10 mm and 21.2 mm I.D. for membrane protein purifications in protein crystallography processes.



Product information

Column	Dimension	Part Number
Nanofilm SEC-500, 5um, 450 A	10 x 300 mm	201500-10030
	7.8 x 300 mm	201500-7830
	4.6 x 300 mm	201500-4630
Nanofilm SEC-250, 5um, 250 A	10 x 300 mm	201250-10030
	7.8 x 300 mm	201250-7830
	4.6 x 300 mm	201250-4630
SRT-C SEC-500, 5um, 500 A	10 x 300 mm	235500-10030
	7.8 x 300 mm	235500-7830
	4.6 x 300 mm	235500-4630
SRT-C SEC-300, 5um, 300 A	10 x 300 mm	235300-10030
	7.8 x 300 mm	235300-7830
	4.6 x 300 mm	235300-4630
SRT SEC-500, 5um, 500 A	10 x 300 mm	215500-10030
	7.8 x 300 mm	215500-7830
	4.6 x 300 mm	215500-4630
SRT SEC-300, 5um, 300 A	10 x 300 mm	215300-10030
	7.8 x 300 mm	215300-7830
	4.6 x 300 mm	215300-4630
SRT-10 SEC-500, 10um, 500 A	10 x 300 mm	225500-10030
SRT-10 SEC-300, 10um, 300 A	10 x 300 mm	225300-10030
SRT-10C SEC-500, 10um, 500 A	10 x 300 mm	239500-10030
SRT-10C SEC-300, 10um, 300 A	10 x 300 mm	239300-10030

Find More Product Information at: <http://www.sepax-tech.com/products.php>

Technical Support: call (877) SEPAX-US and press 3 or email techsupport@sepax-tech.com

Sales: call (302) 366-1101 or email sales@sepax-tech.com

