

Reference list

Analytical size exclusion chromatography (SEC) with new-generation columns

The following is a list of recent journal publications where GE's Superdex™ Increase and Superose™ Increase columns have played an important role in the discoveries made.

Monoclonal antibodies:

1. Schmidt, P. M. *et al.* A robust robotic high-throughput antibody purification platform. *J. Chromatogr. A*, **1455**, 9–19 (2016). https://doi.org/10.1016/j.chroma.2016.05.076

Summary: In a high-throughput setup for purification of monoclonal antibodies, Superdex 200 Increase 5/150 GL column was used to analyze the purity of antibodies. With PBS as running buffer and at a flow rate of 0.4 mL/min, each analysis took only 10 min.

Membrane proteins:

 Liang, Y.-L. et al. Phase-plate cryo-EM structure of a class B GPCR-G-protein complex. Nature, 546, 118–123 (2017). http://www.nature.com/nature/journal/v546/n7656/full/nature22327.html

Summary: The paper describes the Cryo-EM structure of a membrane protein, the calcitonin receptor, a G protein-coupled receptor in complex with G proteins and peptide ligand. Superose 6 Increase 10/300 GL column was used to analyze the stability of the complex of calcitonin receptor with G proteins over 5 d with one analysis per 24 h.

Protein complexes:

3. Peng, M. *et al.* SZT2 dictates GATOR control of mTORC1 signaling. *Nature*, **543**, 433–437 (2017).http://www.nature.com/nature/journal/v543/n7645/full/nature21378.html

Summary: Using proteins from HMW Calibration Kit and a rat IgM (M_r 970 000) for calibration, Superose 6 Increase 10/300 GL column was used to analyze the purity of a protein complex involved in nutrient signaling and estimate its molecular weight (> M₂ 1 000 000).

4. Aydin, H. *et al.* Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex. *Nature*, **534**, 562–565 (2016). http://www.nature.com/nature/journal/v534/n7608/full/nature18595.html

Summary: The paper describes the structure of fertilization complex of human sperm protein IZUMO1 and egg receptor JUNO. Superdex 200 Increase 10/300 GL column was used in a size exclusion chromatography-multiangle light scattering (SEC-MALS) setup to study the oligomeric state of IZUMO1.

5. Kwon, S. C. *et al.* Structure of human DROSHA. *Cell*, **164 (1-2)**, 81–90 (2016). https://doi.org/10.1016/j.cell.2015.12.019

Summary: The paper describes the structure of an RNase DROSHA in complex with its cofactor DGCR8. Superdex 200 Increase 5/150 GL column was used in fluorescence detection size exclusion chromatography (FSEC) to study aggregation states of wild-type and mutants of DROSHA (fused with eYFP) in complex with the cofactor.

 Beljantseva, J. et al. Negative allosteric regulation of Enterococcus faecalis small alarmone synthetase RelQ by single-stranded RNA. Proc. Nat. Acad. Sci. 114 (14), 3726– 3731 (2017). http://www.pnas.org/content/114/14/3726

Summary: Alarmone synthetase RelQ is involved in regulation of intracellular environment in bacteria and tetramerization of RelQ is essential for the regulatory mechanism. Superdex 200 Increase 5/150 GL column was used to analyze the oligomeric state of wild-type and mutant of RelQ in the presence of substrates and mRNA.

Virus/virus-like particles:

7. Erez. Z. et al. Communication between viruses guides lysis-lysogeny decisions. Nature, 541, 488-493 (2017). http://www.nature.com/nature/journal/v541/n7638/full/ nature21049.html

Summary: The paper describes a study on communication between viruses where a specific peptide was utilized by the viruses as communication signal. A peptide receptor from the virus can adopt different oligomer states depending on the sequence of the peptide it binds, and thus transfer different signals. Using Superdex 200 Increase 10/300 GL column, the paper shows that the virus receptor adopts a monomer conformation when it binds a peptide with correct sequence and remains a dimer with peptides of other sequences.

8. Schoonen, L. et al. Stabilization of a virus-like particle and its application as a nanoreactor at physiological conditions. Biomacromol. 18 (11), 3492-3497 (2017). doi/10.1021/acs.biomac.7b00640

Summary: The paper describes a study on using viruslike particles (VLPs) as a tool in bionanotechnology. Cowpea chlorotic mottle virus (CCMV) can adopt different symmetry at different pH, resulting in virus capsids with different diameters. Superose 6 Increase 10/300 GL column was used to analyze the sizes of virus capsids at pH 5.0 and 7.5.

Glycoproteins:

9. Yanagihashi, Y. et al. Mouse macrophages show different requirements for phosphatidylserine receptor Tim4 in efferocytosis. Proc. Nat. Acad. Sci. 114 (33), 8800-8805 (2017). http://www.pnas.org/content/114/33/8800

Summary: Protein S (ProS) and growth arrest-specific 6 (Gas6) are two glycoproteins involved in efferocytosis (a process to remove dying/dead cells). The molecular masses of these two proteins were analyzed on a Superdex 200 Increase 3.2/300 GL column using blue dextran, ferritin, aldolase, and conalbumin as MW standards.

Food and beverage:

10. Raak, N. et al. Enzymatic cross-linking of casein facilitates gel structure weakening induced by overacidification. Food Biophys. **12**, 261–268 (2017). https://doi.org/10.1007/ s11483-017-9483-6

Summary: The paper describes the effect of cross-linking of casein, the major protein in milk, on gel structure. Superdex 200 Increase 10/300 GL column was used to monitor the oligomeric states of casein during cross-linking.

11. Kizzie-Hayford, N. et al. Enrichment of tigernut milk with microbial transglutaminase cross-linked protein improves the physico-chemical properties of the fermented system. LWT - Food Sci. Technol. 81, 226-232 (2017). https://doi. org/10.1016/j.lwt.2017.03.046

Summary: Fermentation of tiger nut milk that was supplemented with cross-linked proteins leads to improved physicochemical properties of the product, giving it more texture and greater consumer appeal. Superdex 200 Increase 10/300 GL column was used to monitor the cross-linking of proteins.

Receptors:

12. Tanji, H. et al. Autoinhibition and relief mechanism by the proteolytic processing of Toll-like receptor 8. Proc. Nat. Acad. Sci. 113 (11), 3012-3017 (2016). http://www.pnas. org/content/113/11/3012

Summary: The paper describes the study on Toll-like receptor 8 (TLR8), which is involved in immune response. Using Superdex 200 Increase 5/150 GL column, the authors show wild-type TLR8 elute as active dimers while TLR8 with the uncleaved Z-loop remains as inactive monomers.

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