

Effectiveness of Large-Pore Sulfated Chromatography Media in Viral Vector Purification: Cellufine MLP DexS



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Abstract
JNC has developed a novel large-pore cellulose bead, “Cellufine MLP.” These beads are composed of highly crosslinked cellulose with large through-pore structures that enable intraparticle convective transport. As a result, even large targets such as viral particles can fully access the interior of the beads. Cellufine MLP DexS is a pseudo-affinity medium in which the surface of the MLP beads is modified with dextran sulfate, which has affinity for enveloped viruses. We have found that this affinity medium is suitable for viral vector purification. In this poster, we provide detailed insights into the development of MLP and explore its potential applications in lentiviral vector and AAV vector purification processes.

1 Introduction to Cellufine MLP

Bead structure

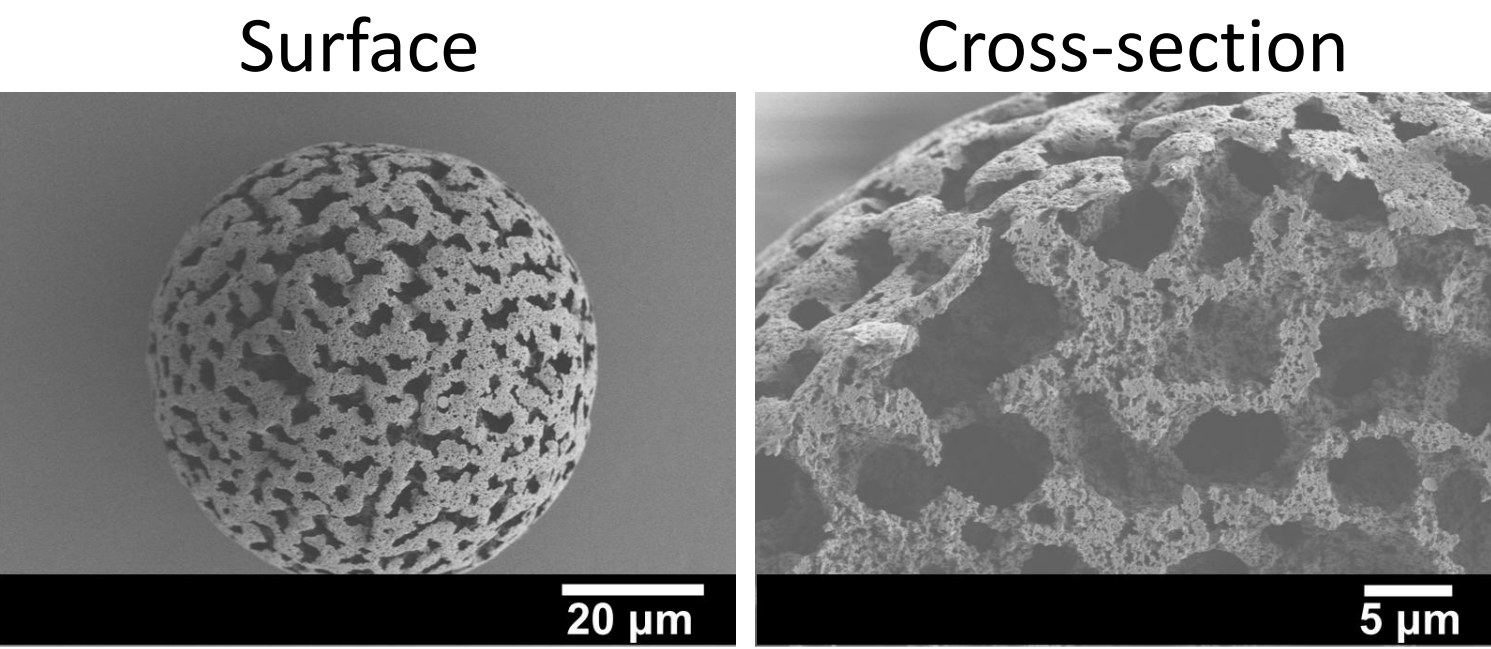


Fig. 1. SEM Morphology of Cellufine MLP
The through-pores of Cellufine MLP were estimated to have a mode pore radius of 1.5 μm using mercury porosimetry [1]. The average particle diameter of MLP DexS is approximately 90 μm.

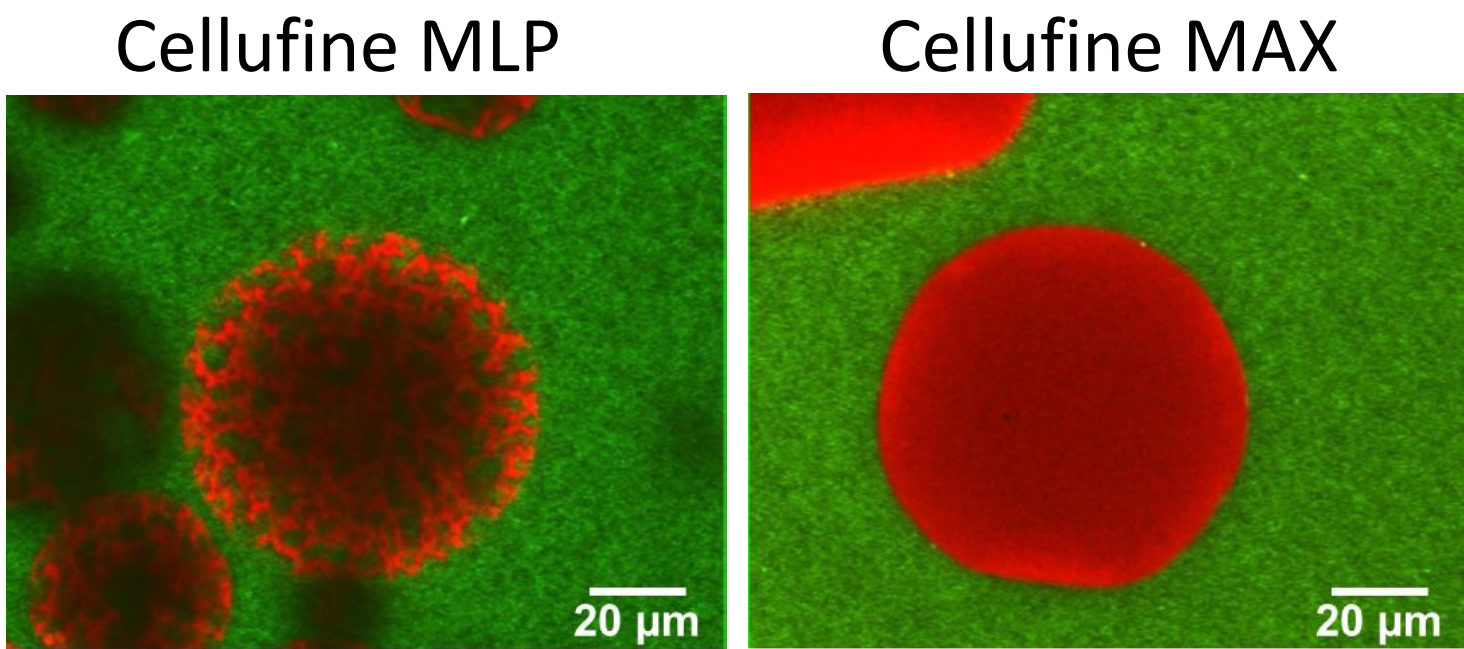
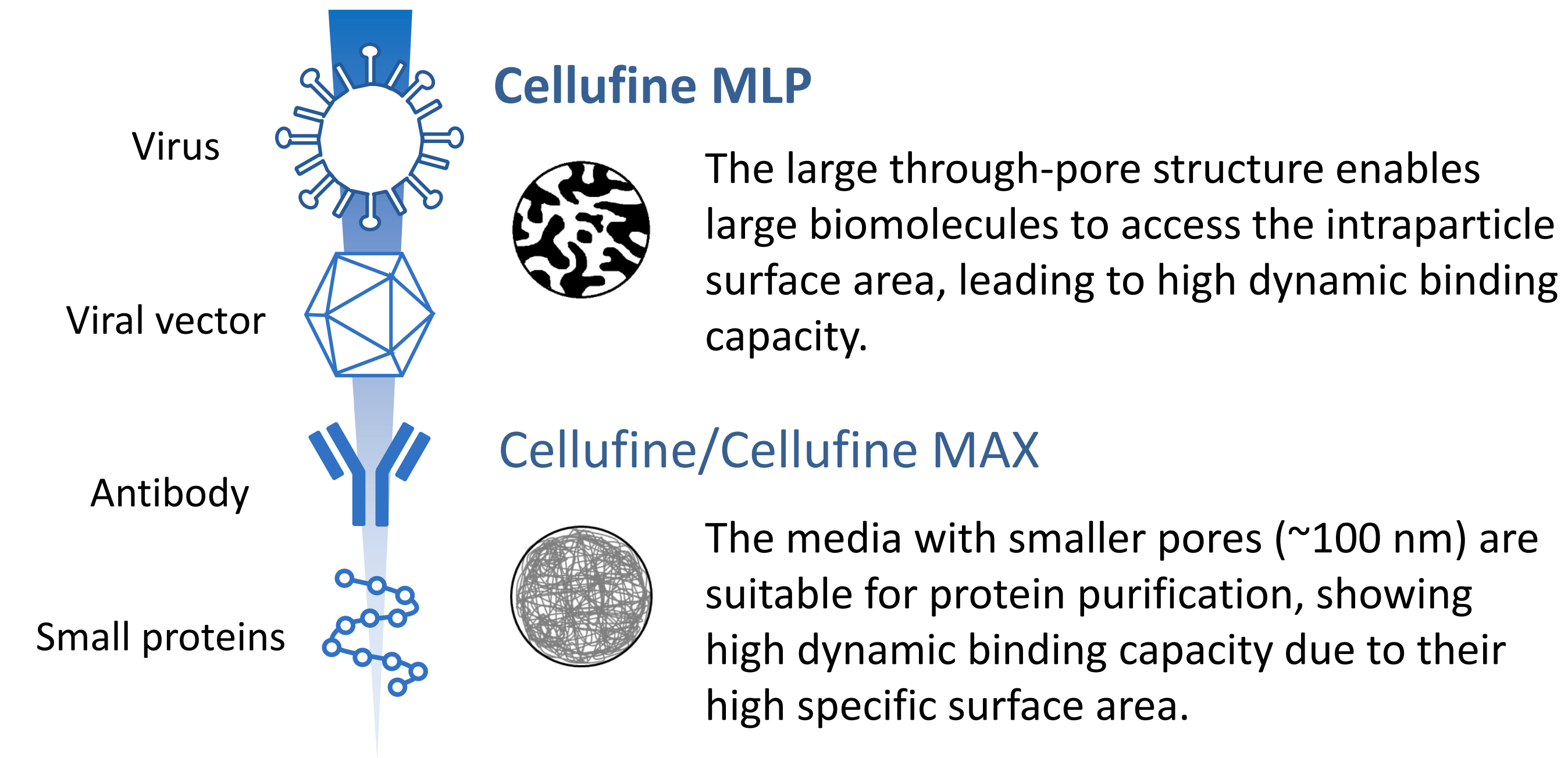


Fig. 2. CLSM observation of Cellufine MLP
Green fluorescent nanoparticles (100 nm) can diffuse into the intraparticle area of Cellufine MLP.

Target biomolecules for Cellufine MLP



2 Comparison of Lentivirus Purification

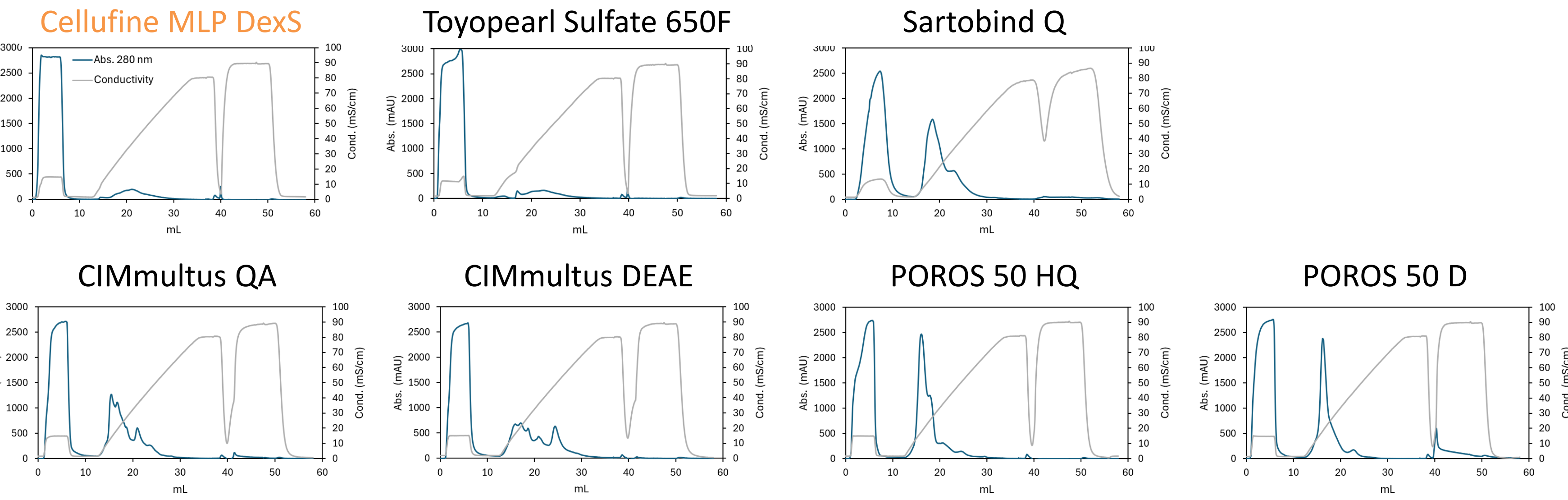


Fig. 3. Chromatograms of lentivirus purification using different chromatography media
Column : Super Edge™ (ID 6.7 mm x 30 mm) 1.06 mL
Flow rate : 1.06 mL/min (residence time: 1 min)
Load : Lentivirus supernatant from HEK293T cell culture
Equilibration buffer : 20 mM Tris buffer, pH7.5
Elution buffer : 20 mM Tris buffer, pH7.5 + 1 M NaCl

Resin	qPCR Recovery (%)	Protein removal (%)	DNA removal (%)
Cellufine MLP DexS	85.2	93.6	73.3
Toyopearl Sulfate 650F	49.2	94.1	80.3
Sartobind Q	47.2	85.0	64.4
CIMmultus QA	22.4	70.1	58.5
CIMmultus DEAE	19.1	72.6	50.7
POROS 50 HQ	70.7	75.3	56.1
POROS 50 D	7.54	47.6	60.1

✓ Cellufine MLP DexS showed high recovery of lentiviral vectors and efficient impurity removal compared with commercially available sulfated and AEX media.

3 Purification of AAV2 (Capture step)

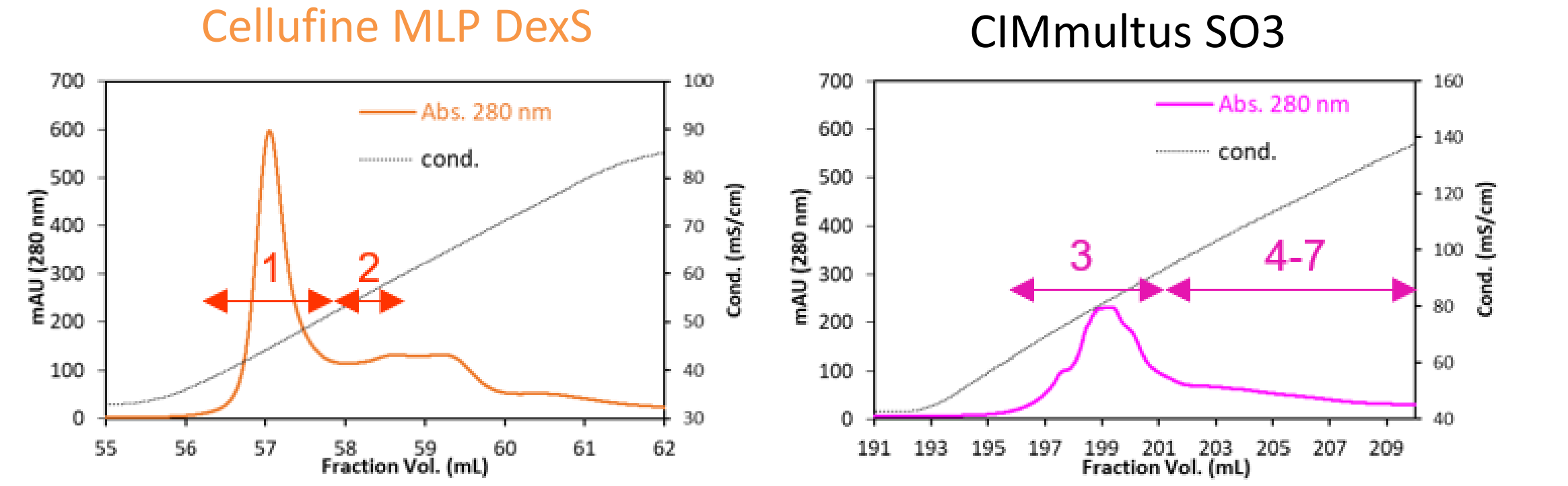


Fig. 4. Chromatograms for AAV2 purification in the capture step

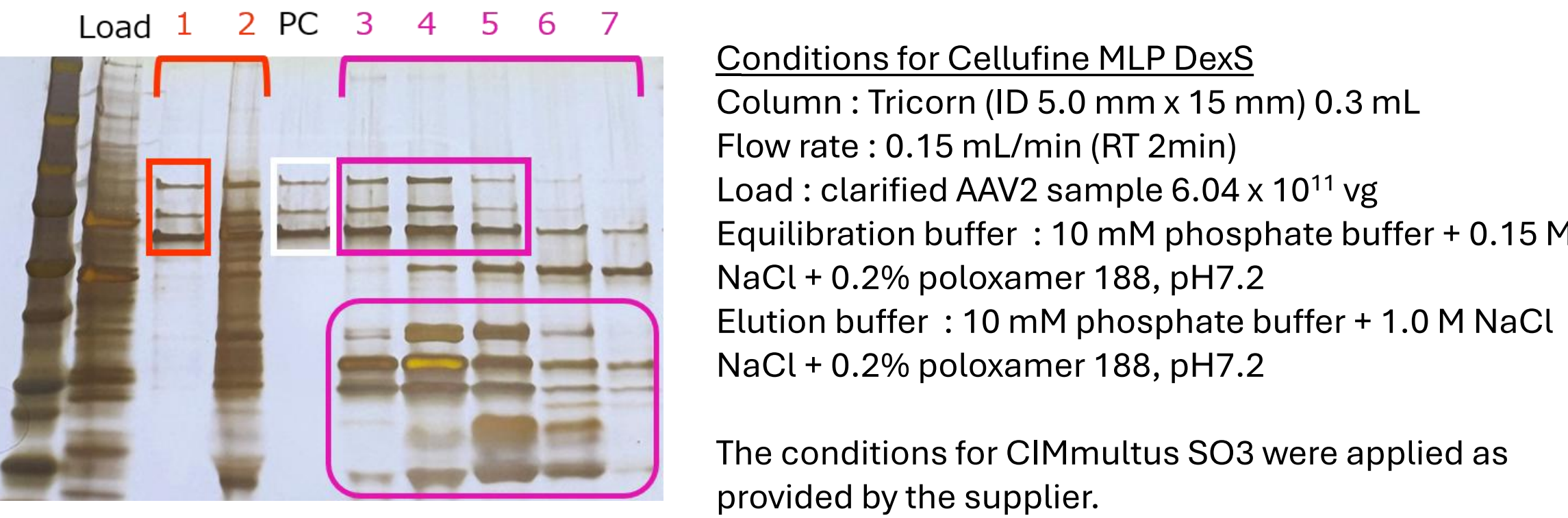


Fig. 5. SDS-PAGE (silver stain) analysis of elution fractions

	Buffer pH condition	Recovery rate (%)
Cellufine MLP DexS	pH 7.2	89
CIMmultus SO3	pH 3.5	9-22

✓ AAV2 was purified with high recovery (89%) under neutral pH conditions (pH 7.2) using Cellufine MLP DexS.

4 Conclusion & Key takeaways

- ✓ Cellufine MLP is novel large-pore cellulose-based beads that are suitable for large biomolecules such as viral vectors.
- ✓ Cellufine MLP DexS has the potential to enable an efficient capture step in viral vector purification processes.

Reference
[1] K. Kadoi, E. Iwamoto, T. Nakama, Fabrication and characterization of a cellulose monolith-like particle for virus purification, *Biochem Eng J.* 192 (2023) 108849.

