

Improving separation of empty and full AAV capsids with a new multimodal column chemistry: CIMac PrimaS™ (AAV) beta

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INTRODUCTION

Separation of empty and full AAV capsids is important analytically as a means of monitoring the effects of different transfection strategies, cell culture conditions, lysis methods, sample preparation and purification methods. It is at least as important on a preparative level because it offers the possibility of removing empty capsids without ultracentrifugation. This poster introduces a new column for performing separation of empty and full capsids.

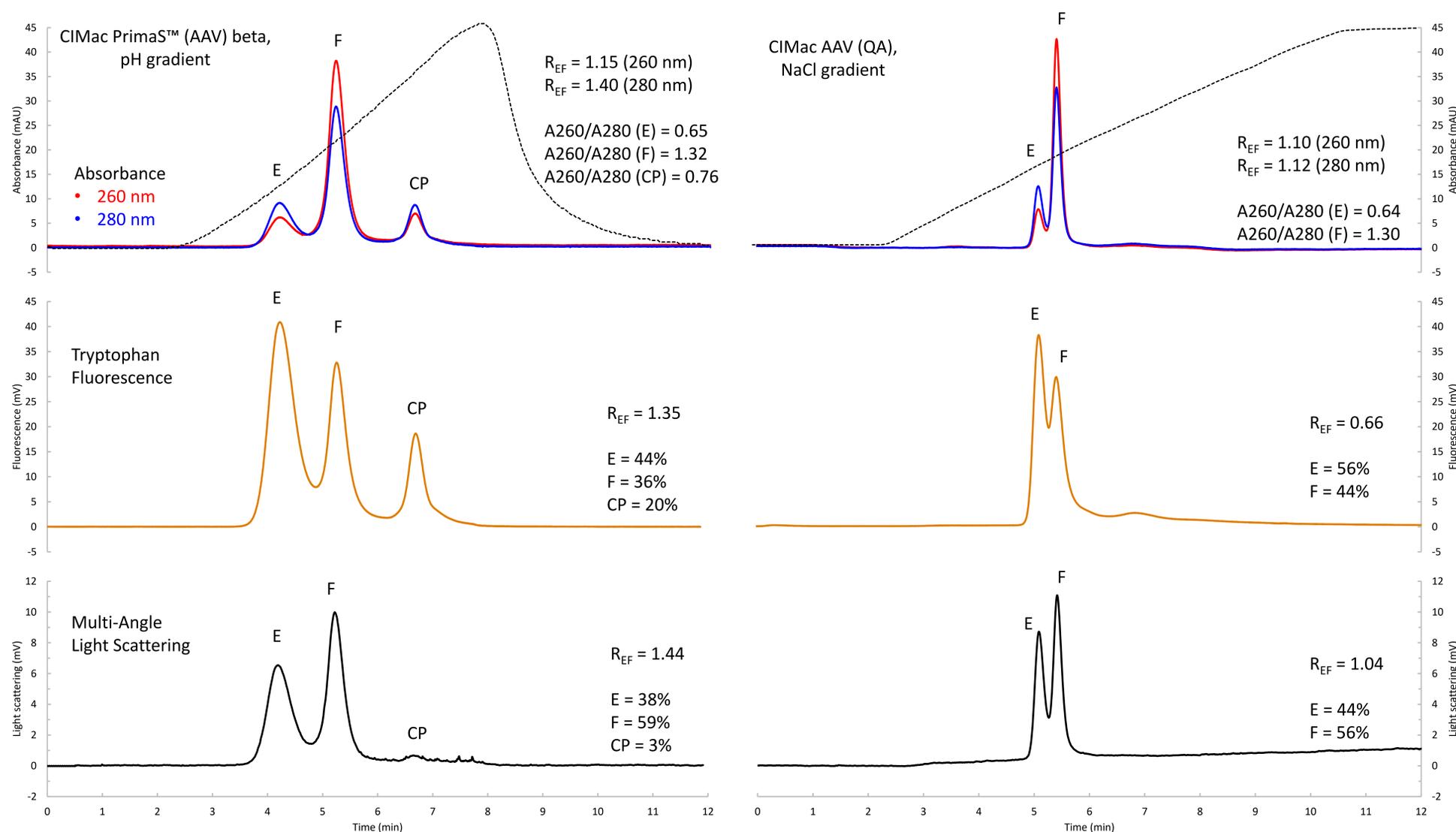
CIMac PrimaS™ (AAV) beta employs a new ion exchange-hydrogen bonding multimodal ligand that provides a new orthogonal option for separation of empty and full capsids. Gross selectivity is similar to strong (QA) anion exchangers eluted with salt gradients, but it generally provides better resolution. Its distinct separation mechanism is documented by the fact that it provides its best results when eluted with increasing pH gradients. This is directly opposite to QA exchangers where increasing pH causes capsids to bind more strongly.

CIMac PrimaS™ (AAV) beta can also be eluted with salt gradients and often provides better resolution than QA, but its best resolution is obtained with pH gradients. Separation of a free capsid protein (CP) occurs only with the pH elution format.

CIMac PrimaS™ (AAV) beta can be used instead of classical anion exchange, for example following capture by cation exchange chromatography or affinity. Its distinct separation mechanism also makes it possible to perform orthogonal separations in which it is combined with QA. The results illustrated below were obtained with AAV8.

The resolution between peaks was calculated with the standard equation: $R = 2 \frac{t_F - t_E}{w_F + w_E}$, where t_F and t_E are retention times and w_F and w_E are peak widths at baseline of full and empty AAV capsids.

RESULTS



Chromatographic conditions (CIMac PrimaS™ (AAV) beta column, pH gradient):

- Buffer A: 10 mM BTP + 10 mM TRIS + 2 mM MgCl₂ pH 8.0
- Buffer B: 10 mM BTP + 10 mM TRIS + 2 mM MgCl₂ pH 9.5

Chromatographic conditions (CIMac™ AAV (QA) column, NaCl gradient):

- Buffer A: 20 mM BTP + 2 mM MgCl₂ pH 9.0
- Buffer B: 20 mM BTP + 2 mM MgCl₂ + 400 mM NaCl pH 9.0

CONCLUSIONS

- ❖ CIMac PrimaS™ (AAV) beta is a new multimodal column for separation of empty and full AAV capsids.
- ❖ Its unique chemistry enables it to achieve superior separation using an easy 2-component increasing pH gradient.
- ❖ It can also be used with a simple salt gradient, as with strong anion exchangers like QA.
- ❖ CIMac PrimaS™ (AAV) beta can be used in combination with QA anion exchangers to provide orthogonal separations.