

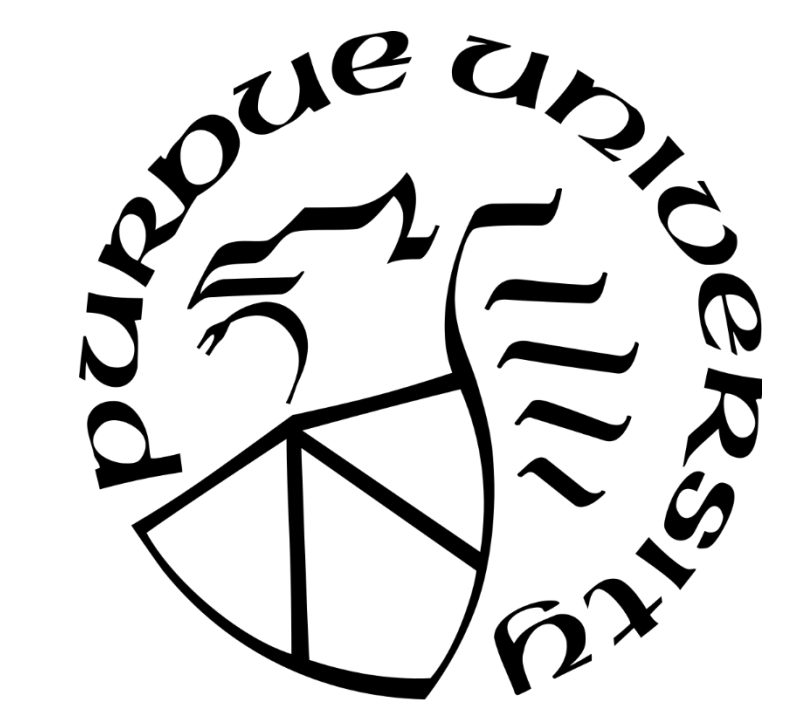
High Resolution Chromatography Coupled with Mass Spectrometry for Therapeutic IgG1, IgG2, and IgG4



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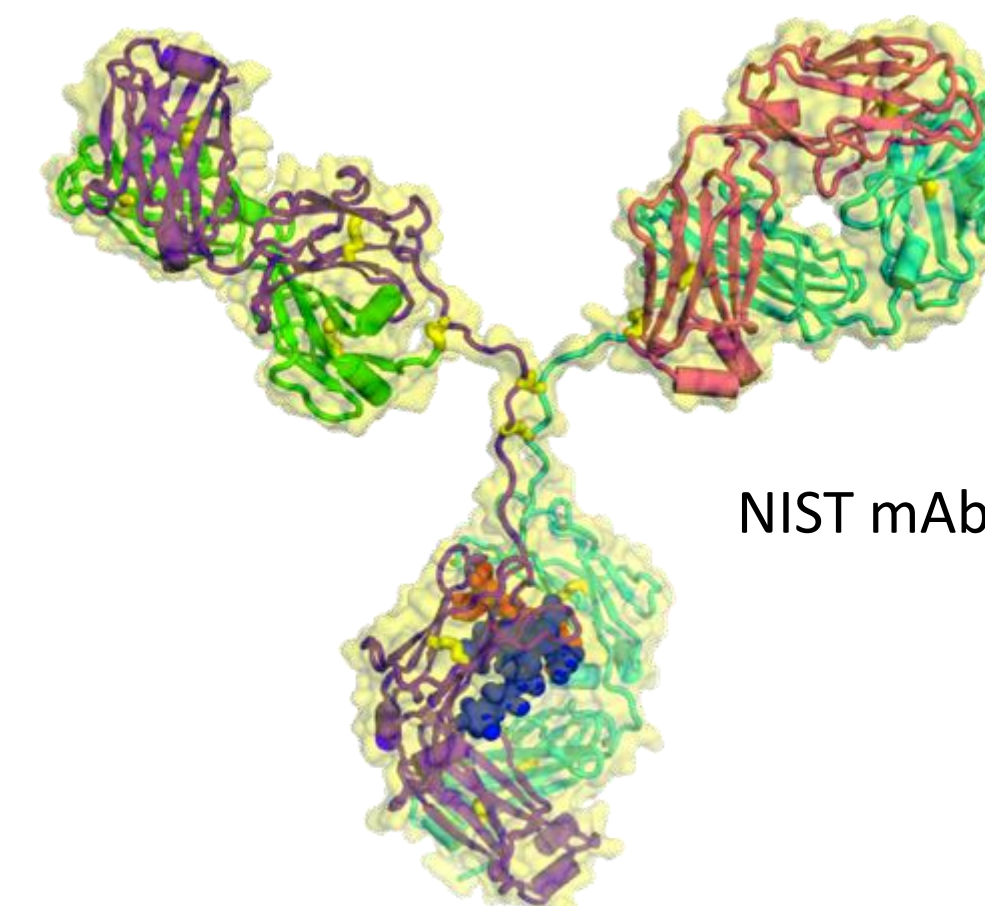
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Objectives

LCMS of monoclonal antibodies, ADCs, and other protein drugs requires new ideas in column design rather than adapting columns from the realm of small-molecule drugs.

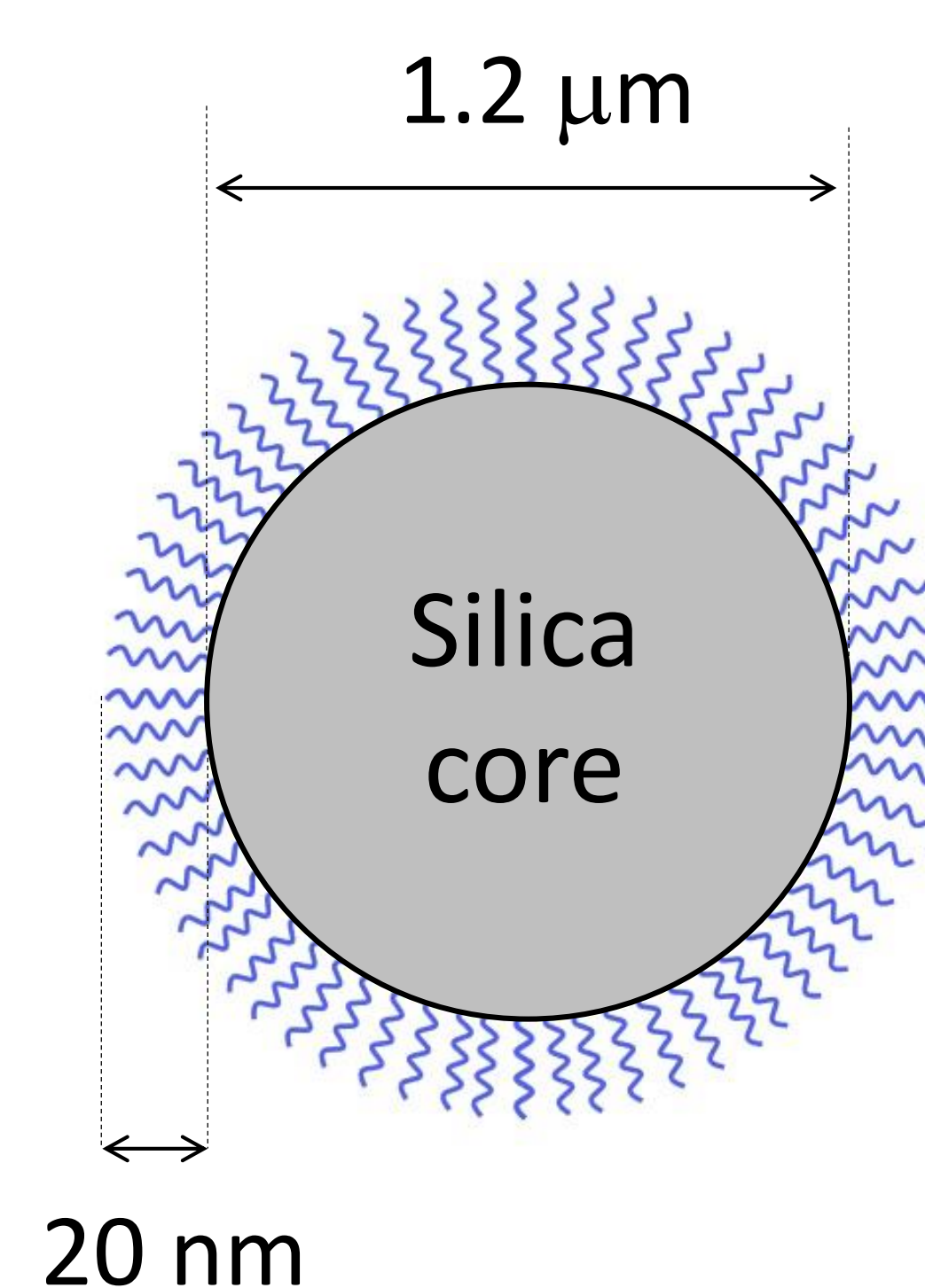


The design investigated is a core of solid silica and a shell of dense polymer brush for attaining high resolution in liquid chromatography and high sensitivity in mass spectrometry for protein drugs.

The design is tested for its ability to separate without trifluoroacetic acid (TFA) since TFA suppresses ionization and forms ion pairs with proteins. This is especially a problem with mAbs and ADCs. The polymer layer provides a distance between silanols and the proteins to avoid tailing. The polymer contains multiple functional groups that introduce more selectivity.

The design is demonstrated here to give unprecedented LC resolution and MS sensitivity in RPLC of IgG1 and IgG2. Comparable large advantages are obtained for IgG4 and reduced ADCs. The design is being extended to HIC, HILIC and IEX.

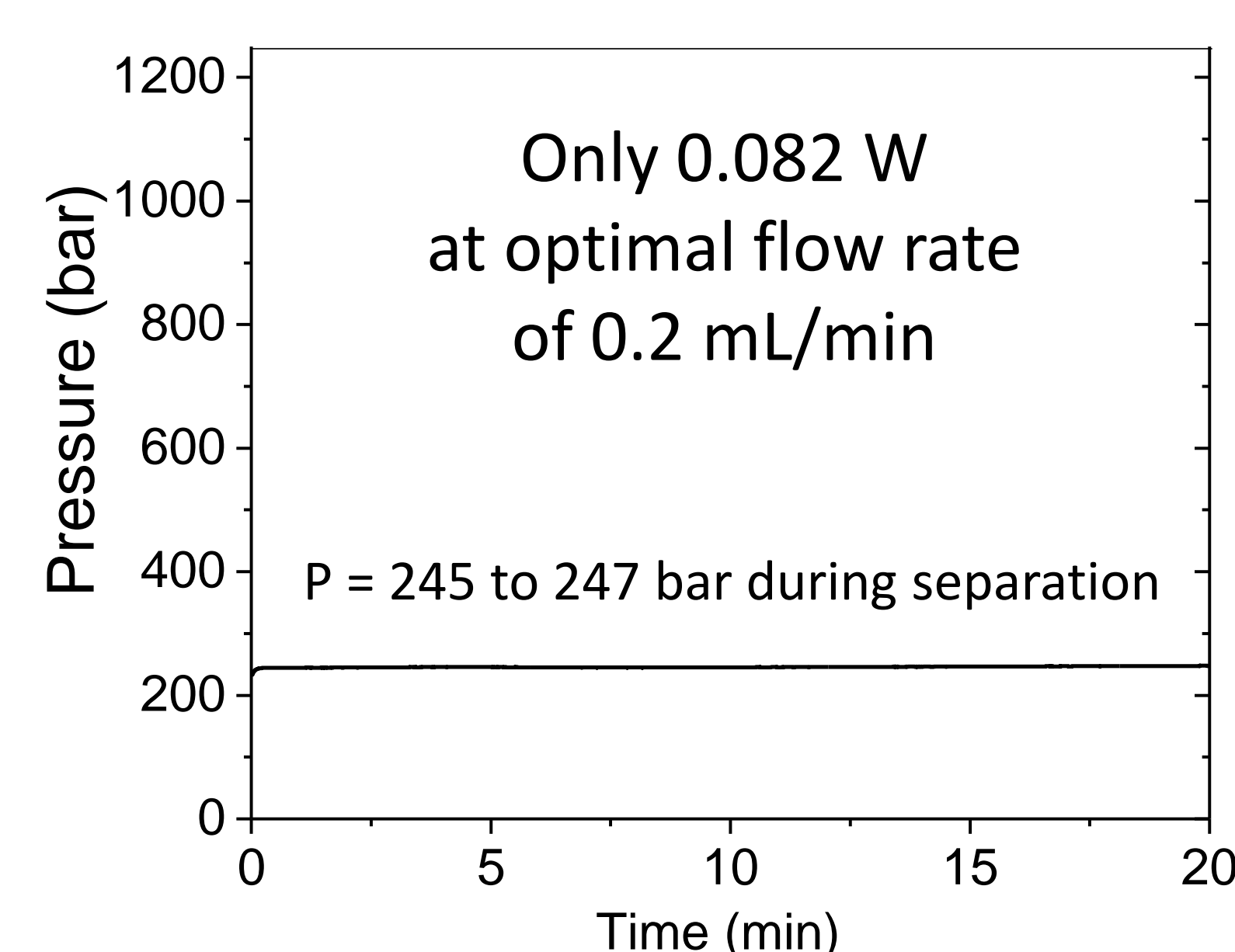
Polymer-shell technology



Unique benefits for proteins

- Screens surface charges to reduce tailing
- Sterically prevents protein-silica contact
- Envelops protein to increase selectivity

Low back-pressure avoids frictional heating

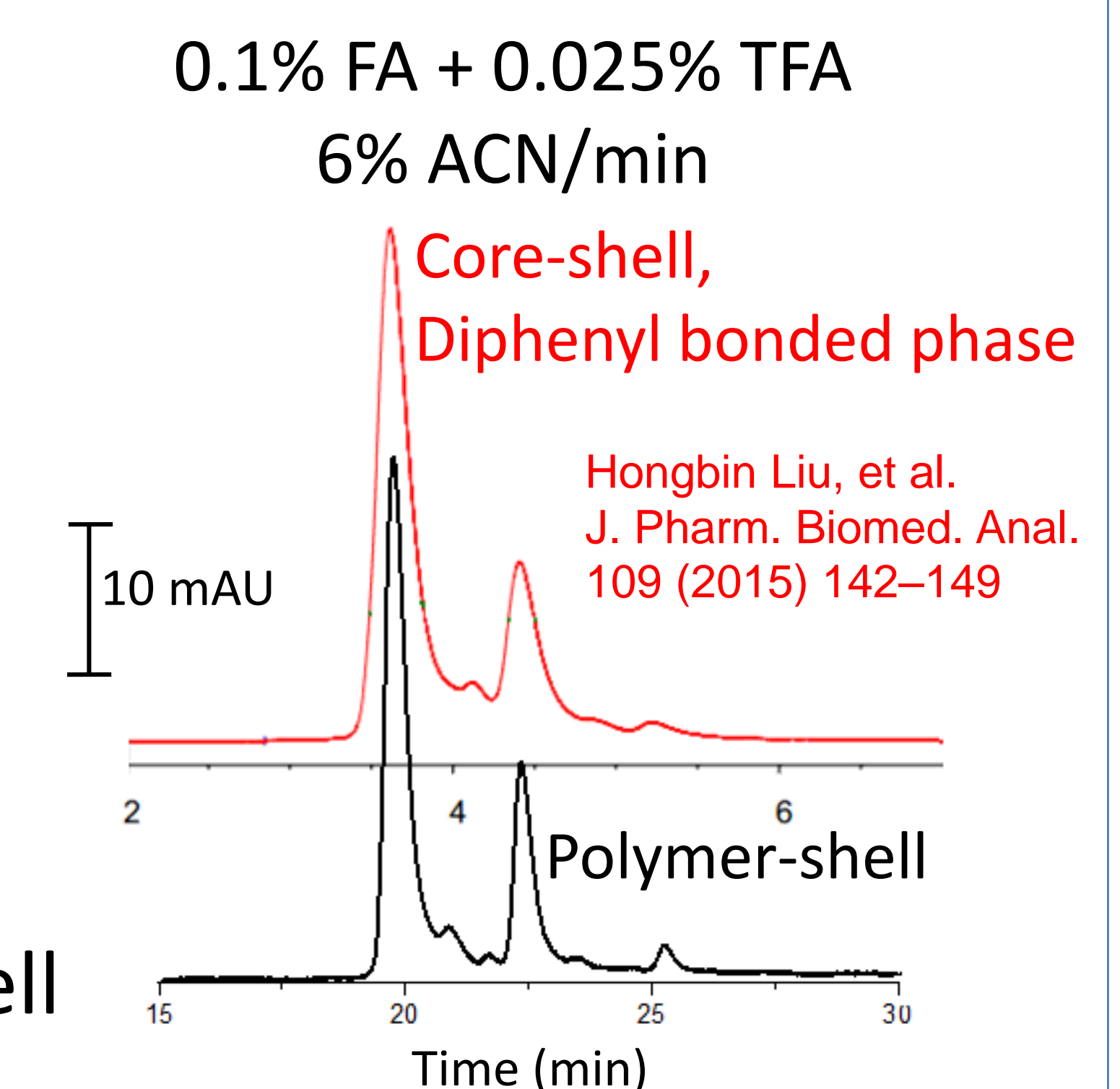


2.1 mm x 50 mm

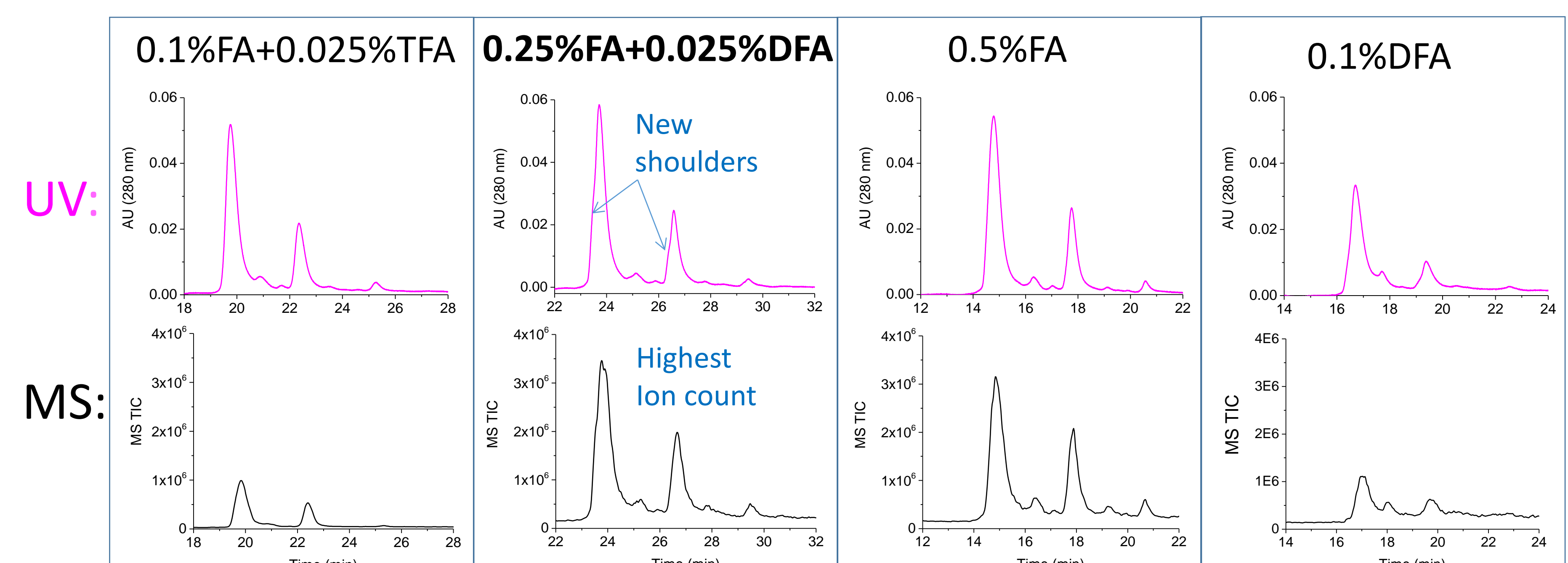
RPLC-MS of IgG1

1. Comparison with published results shows that resolution for free thiols is higher for the polymer shell column using MS-compatible mobile phase.

- Peaks are further apart for the same gradient, showing the higher selectivity.
- New peaks are resolved by polymer shell column for the same Genentech IgG1.



2. Study of both MS sensitivity and LC resolution reveals that 0.25% FA+0.025%DFA gives best LC resolution and best MS sensitivity:

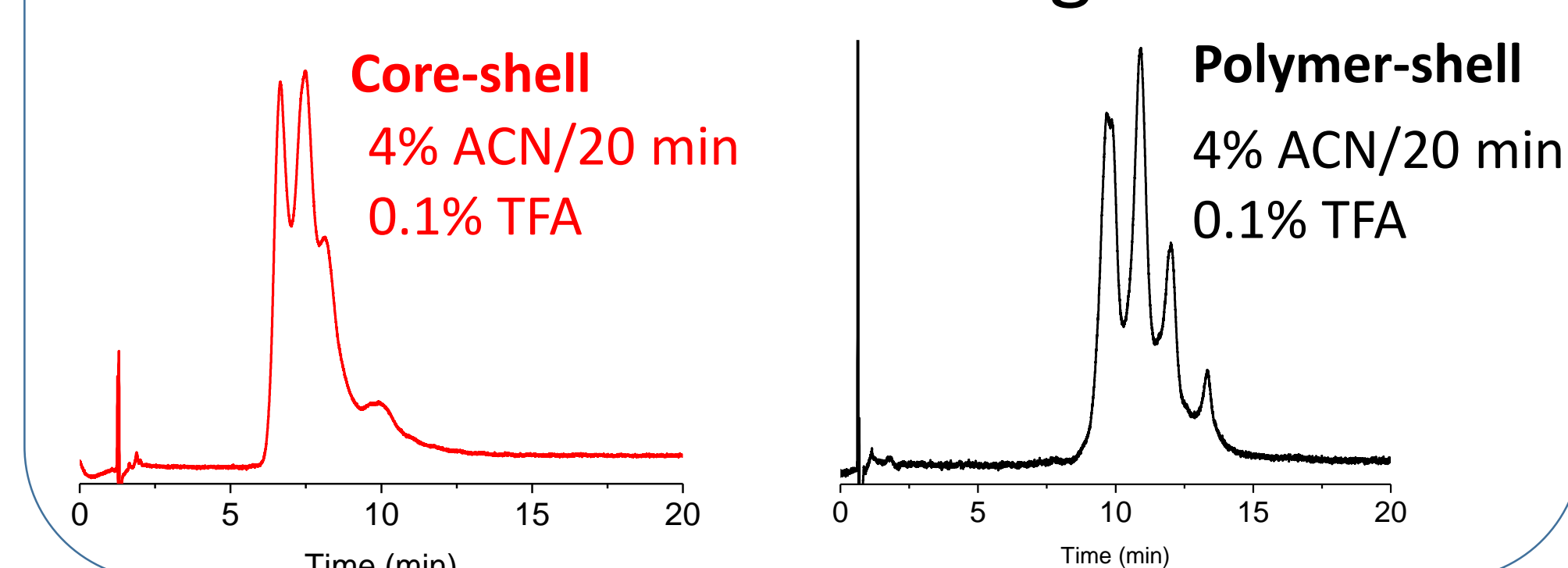


Gradient was 4% ACN/20 min, with varying starting point.

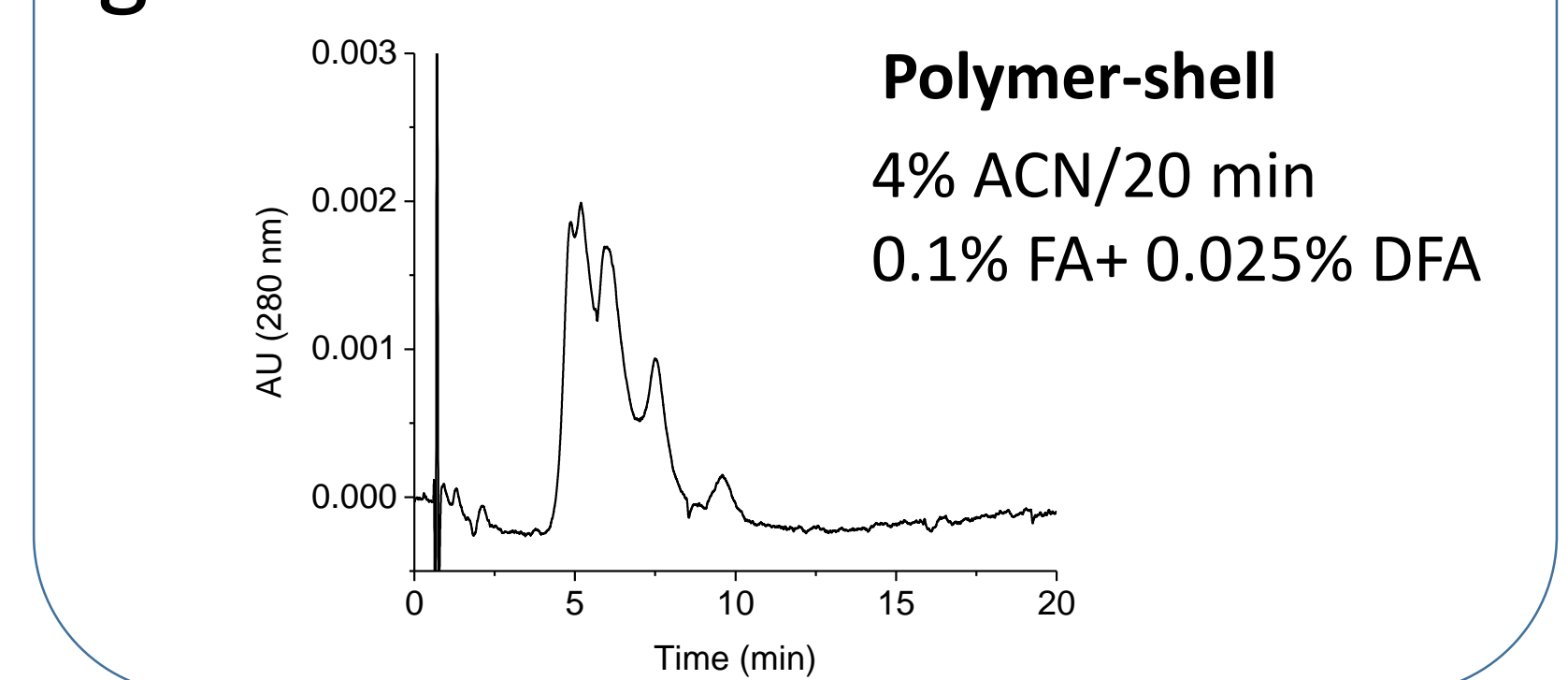
RPLC-MS of IgG2

Resolution of disulfide isoforms in IgG2 is higher for polymer shell column, and MS-compatible mobile phase gives high resolution.

a) Polymer shell gives higher resolution than core-shell for Pfizer IgG2.



b) MS-compatible mobile phase gives little sacrifice in resolution.



mAbs courtesy of Pfizer, Genentech

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