Membrane for Rapid Immunoaffinity Purifications and Processing

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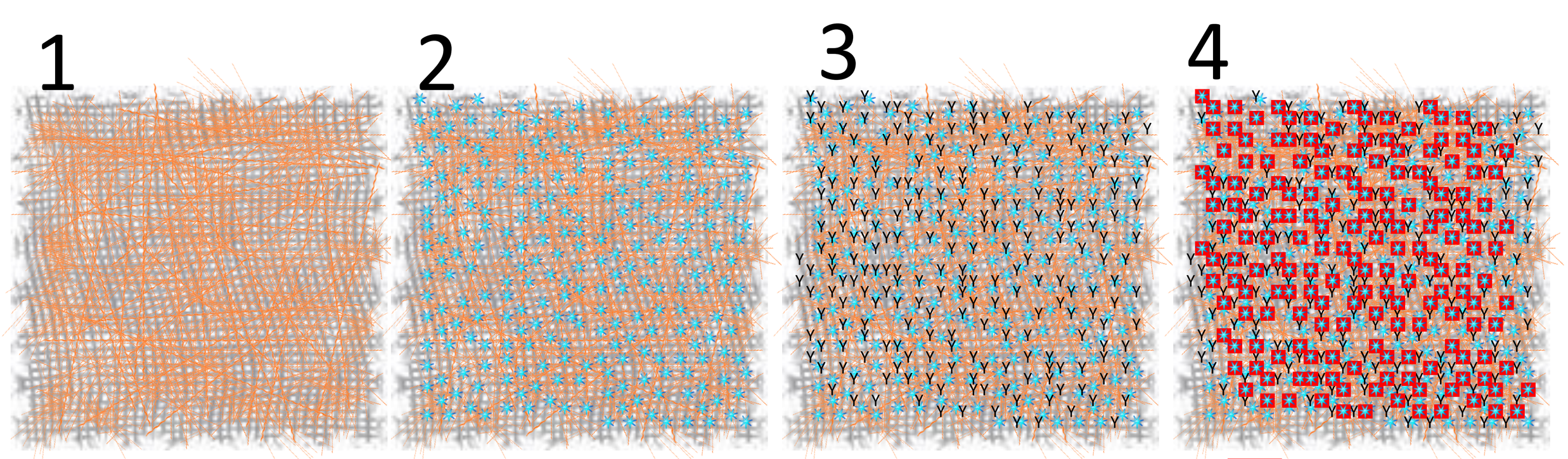
Introduction:
Described here-in is the construction of a membrane that uses covalently linked Protein A to capture antibodies, in either a specific and non-specific manner, for use in immunoaffinity purification schemes as well as in the study of the heterogeneity of immunoglobulin preparations. The objective of this work is to enable an inexpensive and rapid technology for immunoassay type experiments as well as for immunoglobulin heterogeneity analysis to be performed with MALDI-MS detection.

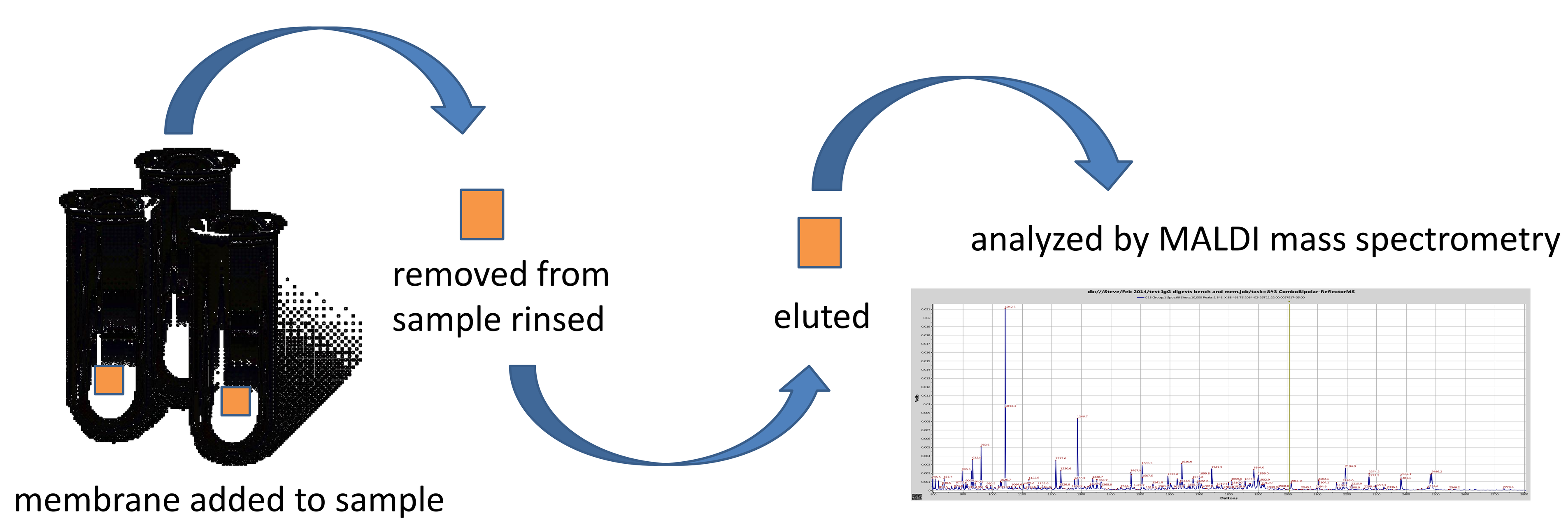
Methodology:



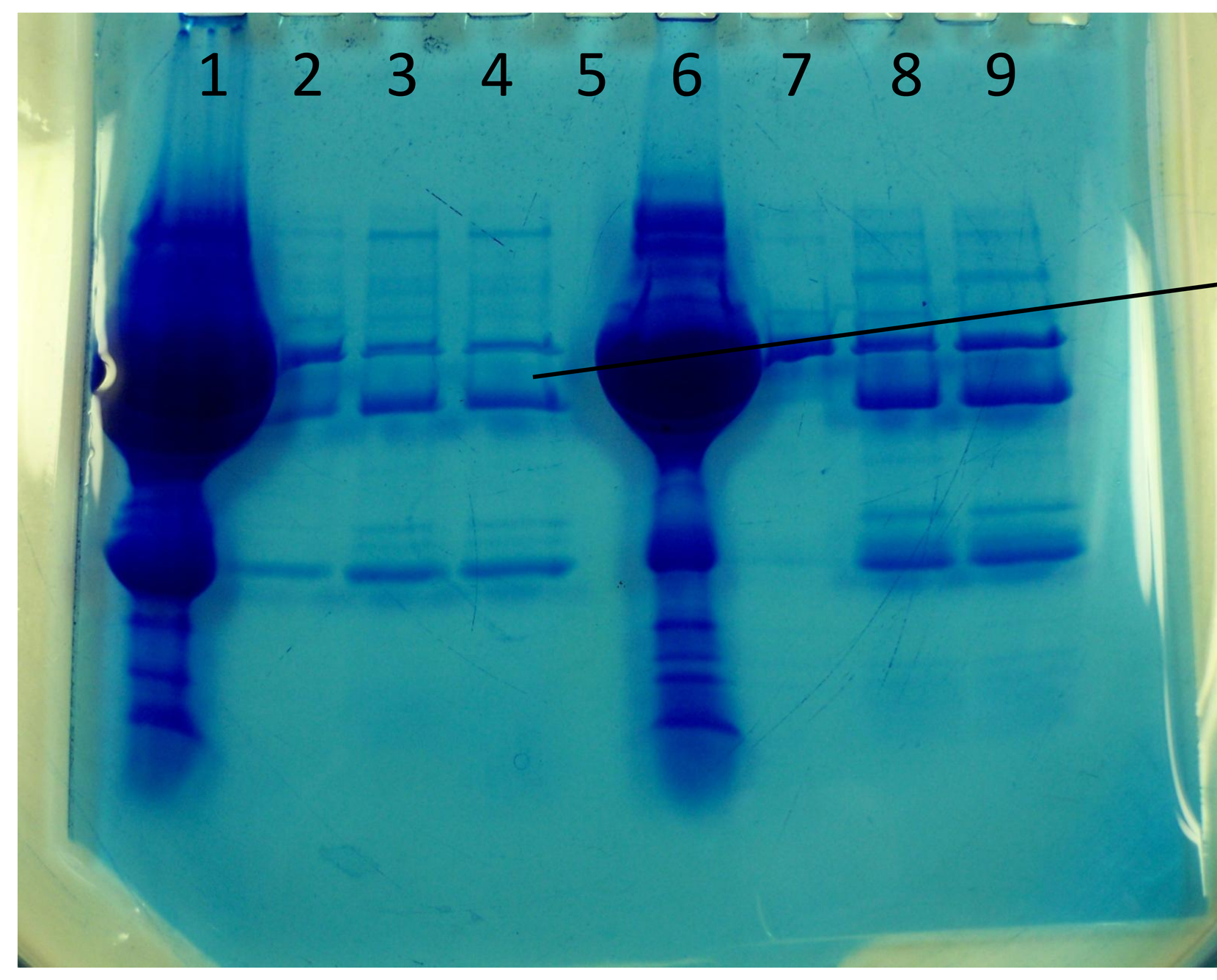
= Protein A Y = antibody = antigen

1)Membrane is functionalized to provide covalent capture of protein
2) Protein A is covalently linked to membrane
3) Protein A containing membrane used to bind antibodies
-bound antibodies can be eluted for analysis
-bound antibodies can be cross linked to Protein A to provide specific antigen capture
4) Targeted antigen captured and eluted for analysis

= membrane section ~ 5mm^2

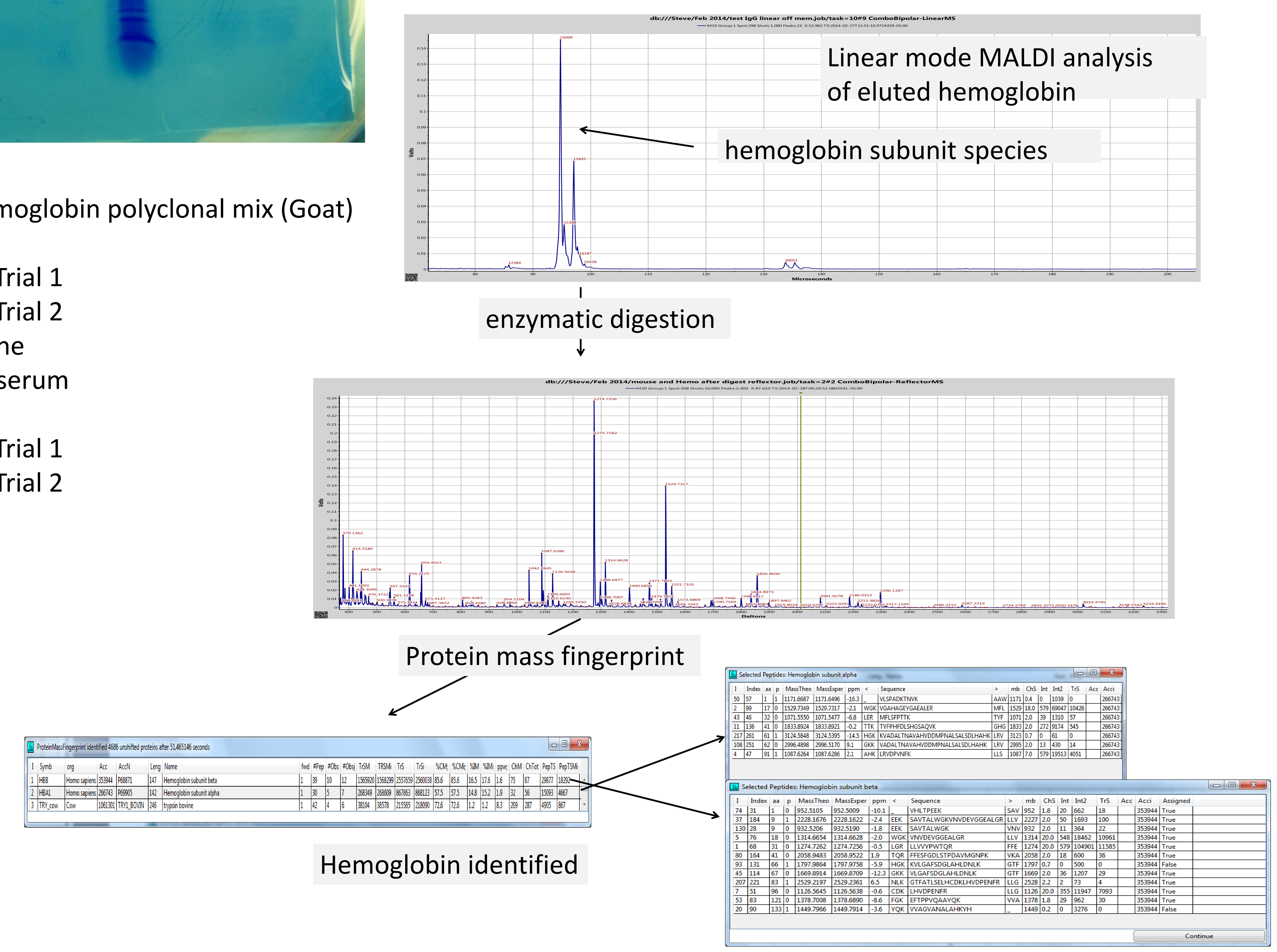


2: Capture of Hemoglobin for human plasma using cross-linked polyclonal anti-hemoglobin mix (Goat)



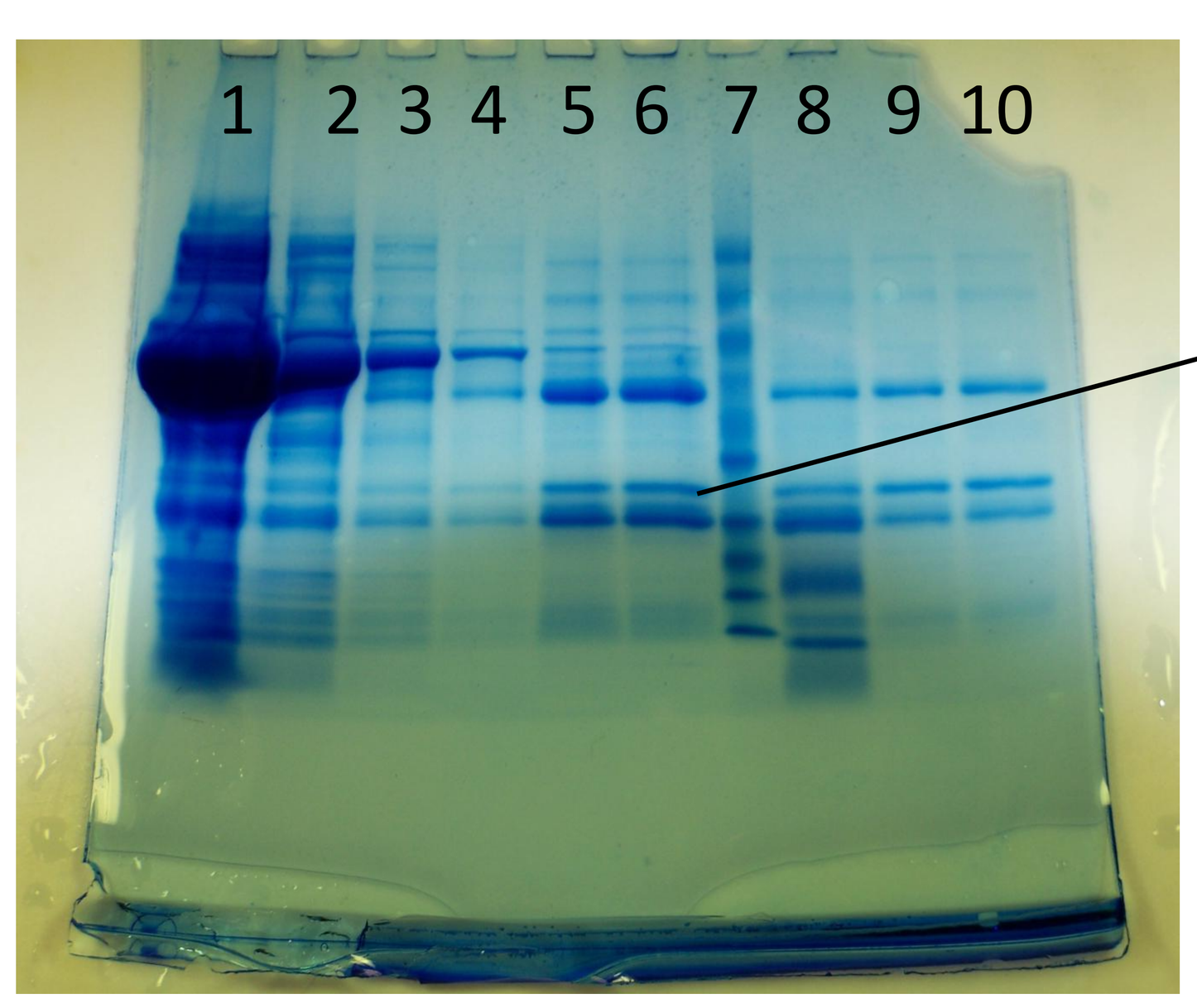
Gel lanes
1 anti –hemoglobin polyclonal mix (Goat)
2) Rinse 3
3) Elution Trial 1
4) Elution Trial 2
5) Blank lane
6) Human serum
7) Rinse 3
8) Elution Trial 1
9) Elution Trial 2

1)Protein A membrane used to capture anti-hemoglobin antibody
-ab19184 Abcam Cambridge, MA
2) Antibody covalently linked to Protein A using dimethylpimelimidate
3) 5 mm section of membrane placed in blood plasma sample
4) Membrane removed, rinsed, eluted and analyzed by MALDI

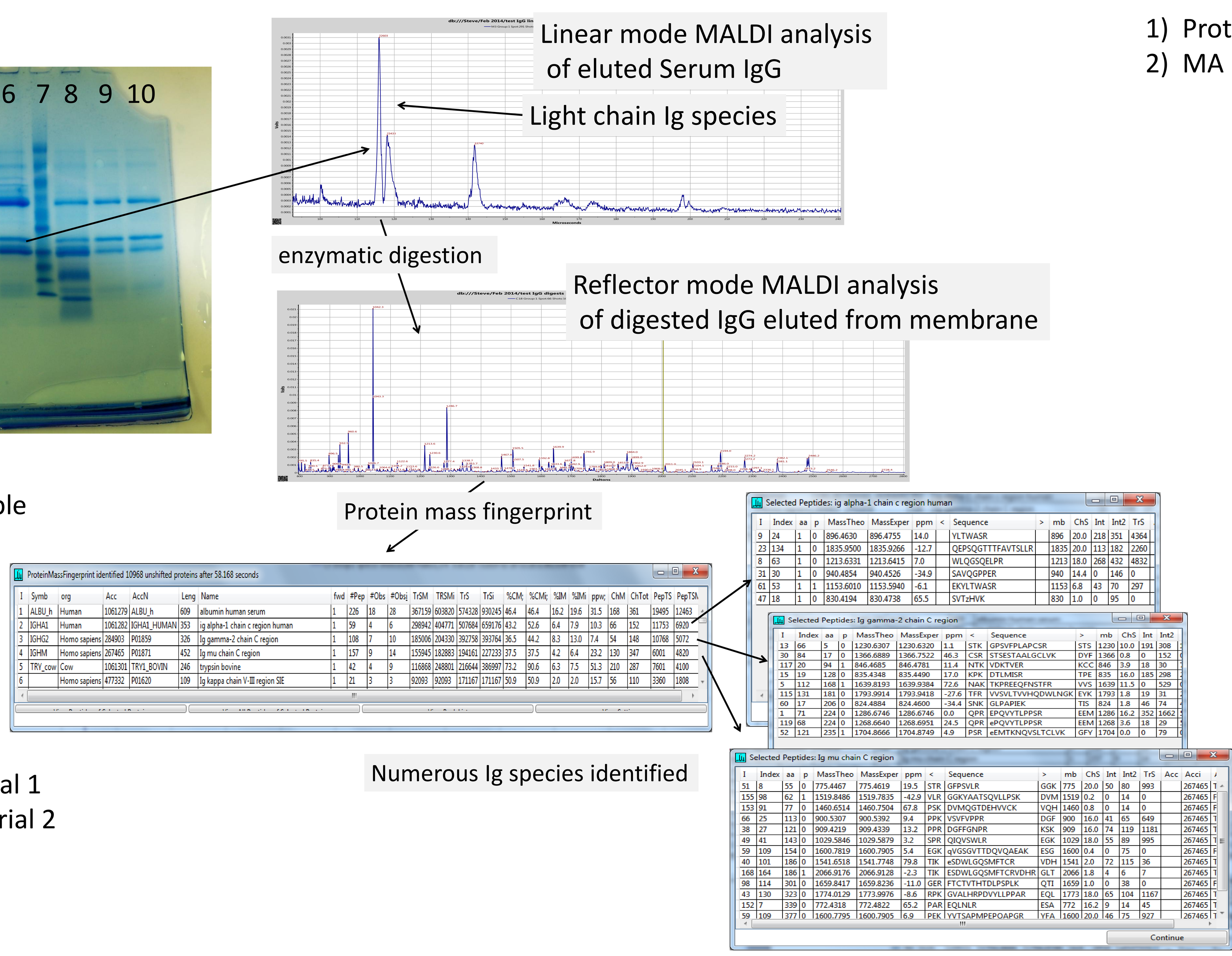


Results:

1: Capture of antibody population for pooled human serum sample of IgG standard mixture

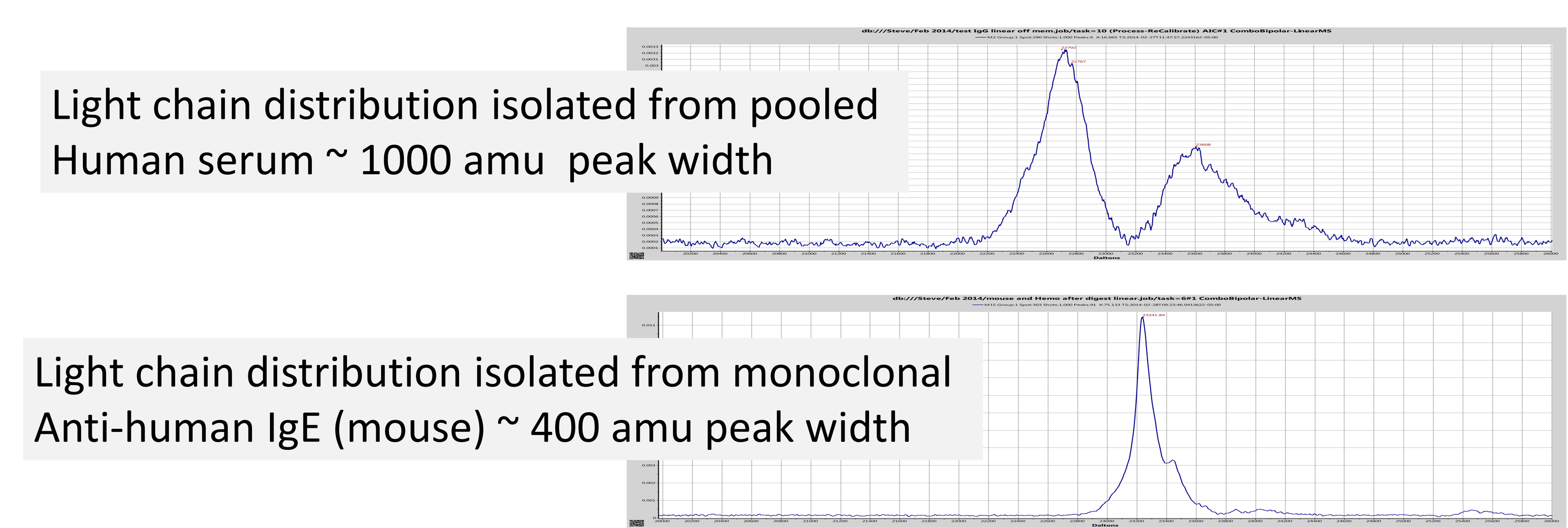


Gel lanes
1 parent serum sample
2) Rinse 1
3) Rinse 2
4) Rinse 3
5) Elution Trial 1
6) Elution Trial 2
7) MW Stds
8) IgG std
9) IgG std capture Trial 1
10) IgG std capture Trial 2



3: Analysis of antibody populations

1) Protein A membrane used to capture antibody population for sample
2) MA LDI analysis and antibody light chain profile used to gauge for hyper-expression of species



Conclusions:
Successful design and construction of membrane with covalently linked Protein A. Membrane can be used in a variety of different schemes for performing immunoaffinity purifications
For studying targeted antigen(s) and antibody populations