Purity Analysis of Bispecific Antibodies by Affinity Capillary Electrophoresis

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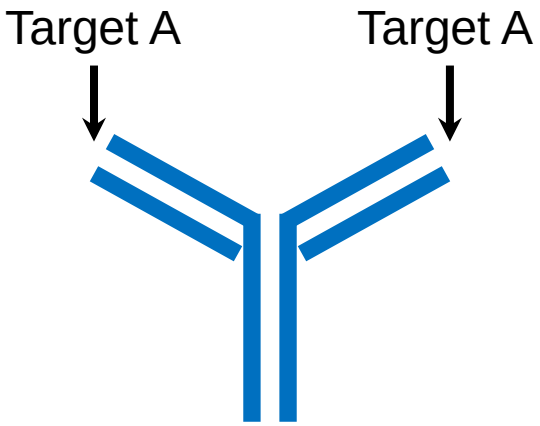
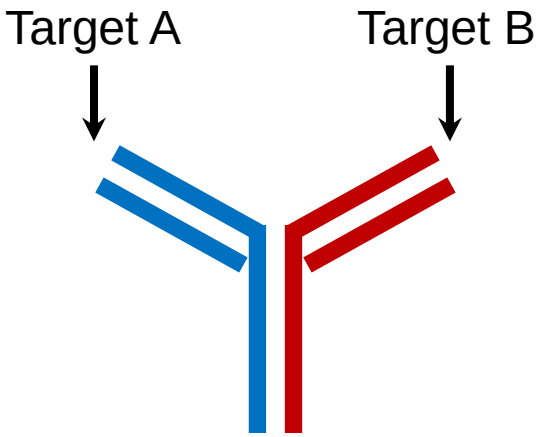
REGENERON

Bispecific vs. Monospecific Antibodies



Monospecific Antibody



Bispecific Antibody

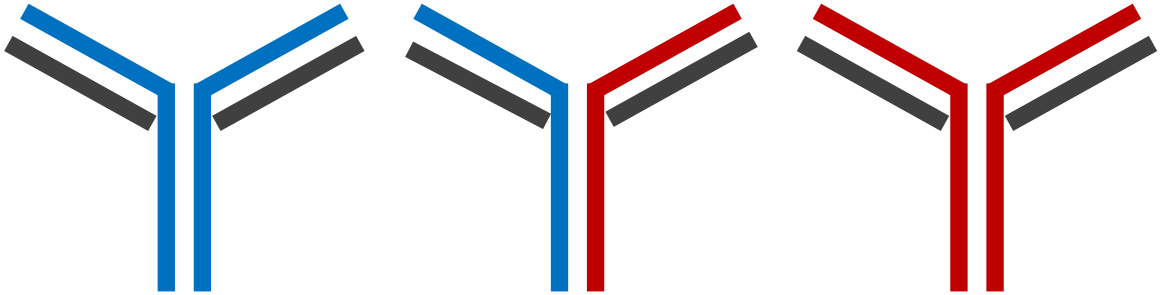
Production Challenge of Bispecific Antibodies: Undesirable Side Products

Co-expression of 2 different light chains and 2 different heavy chains:

→ *10 unique products*

Regeneron Bispecific format:

→ *3 predominant products*

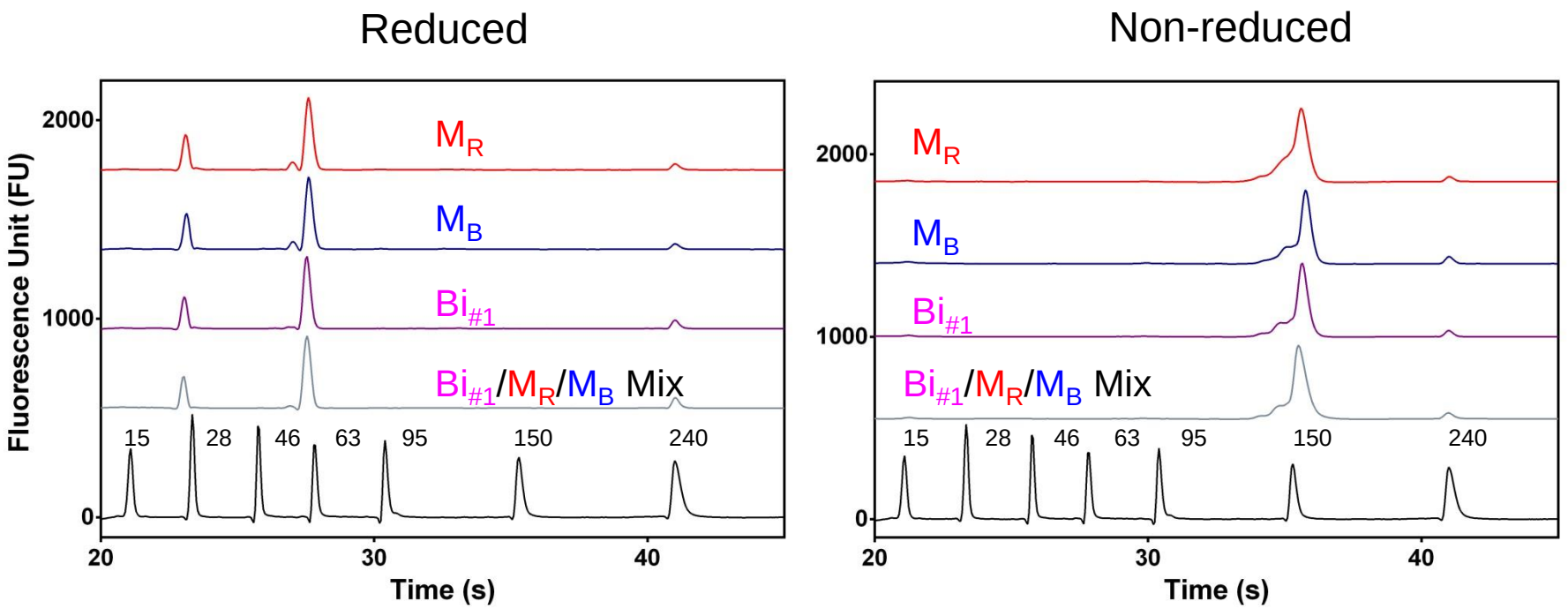


Blue monospecific (M_B)

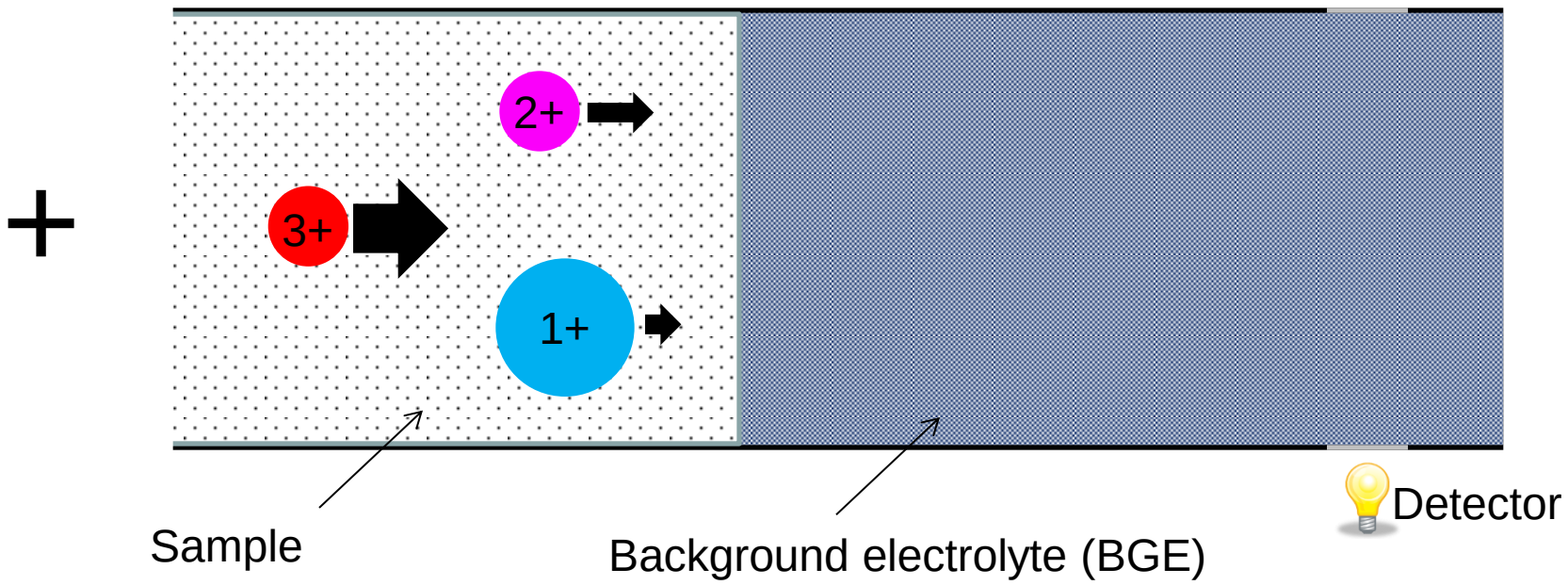
Bispecific (Bi)

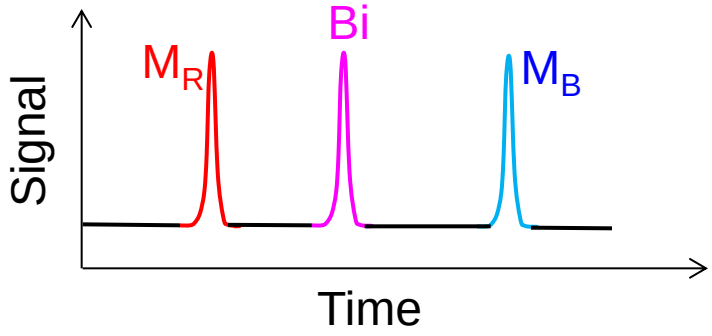
Red monospecific (M_R)

CE-SDS Separation Has Inadequate Resolution for Monospecific Side Products

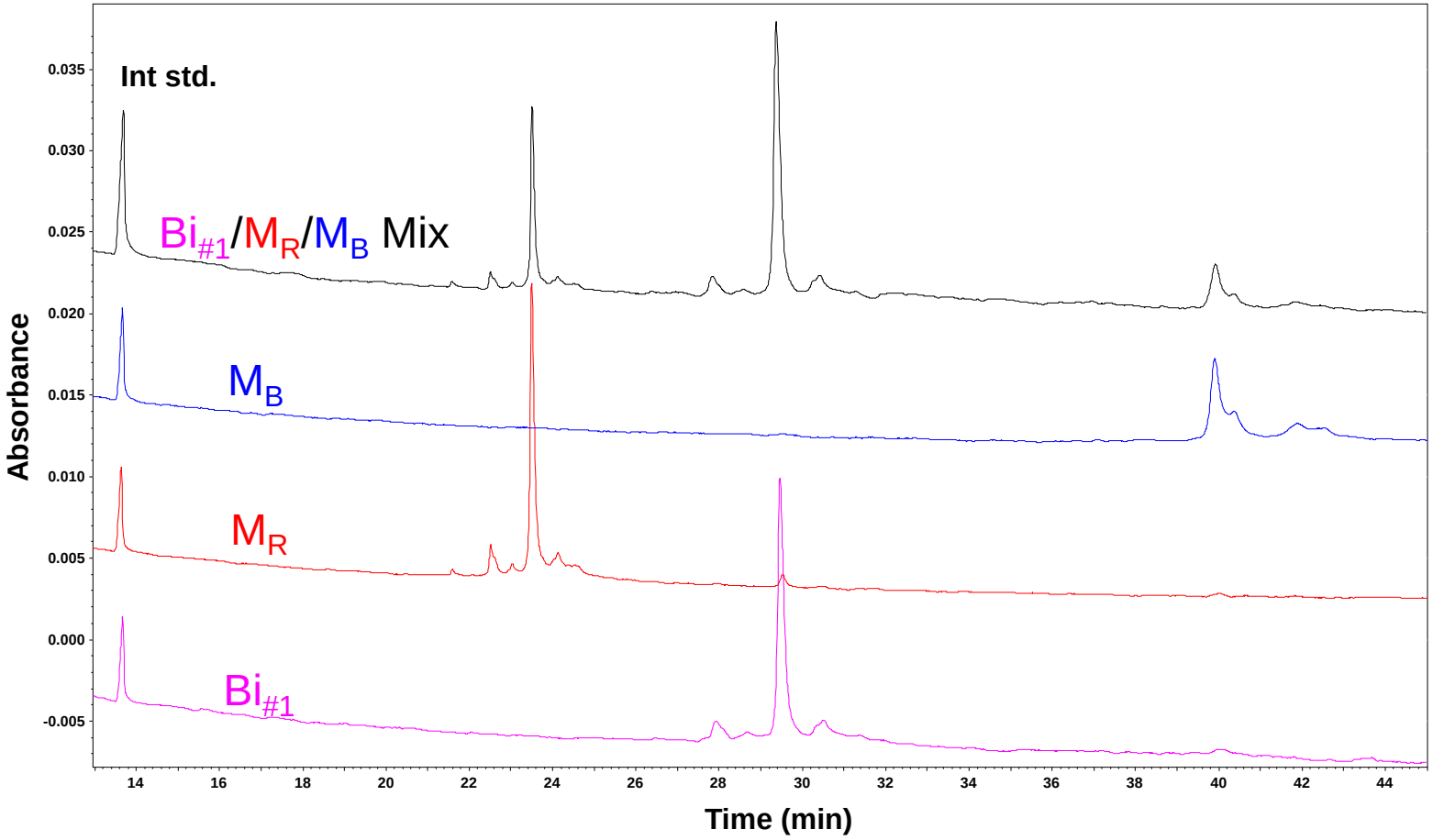


Capillary Zone Electrophoresis (CZE)

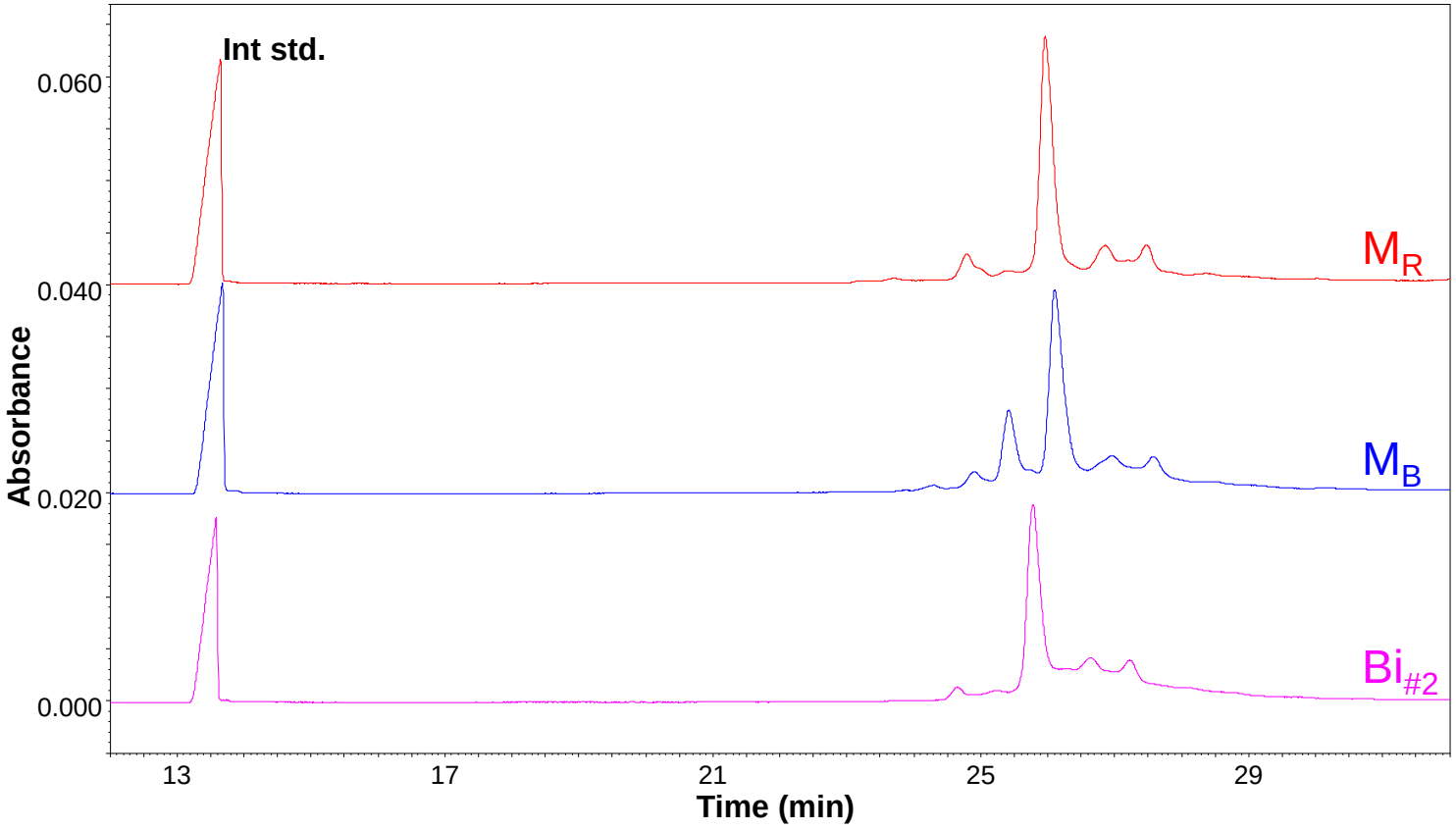




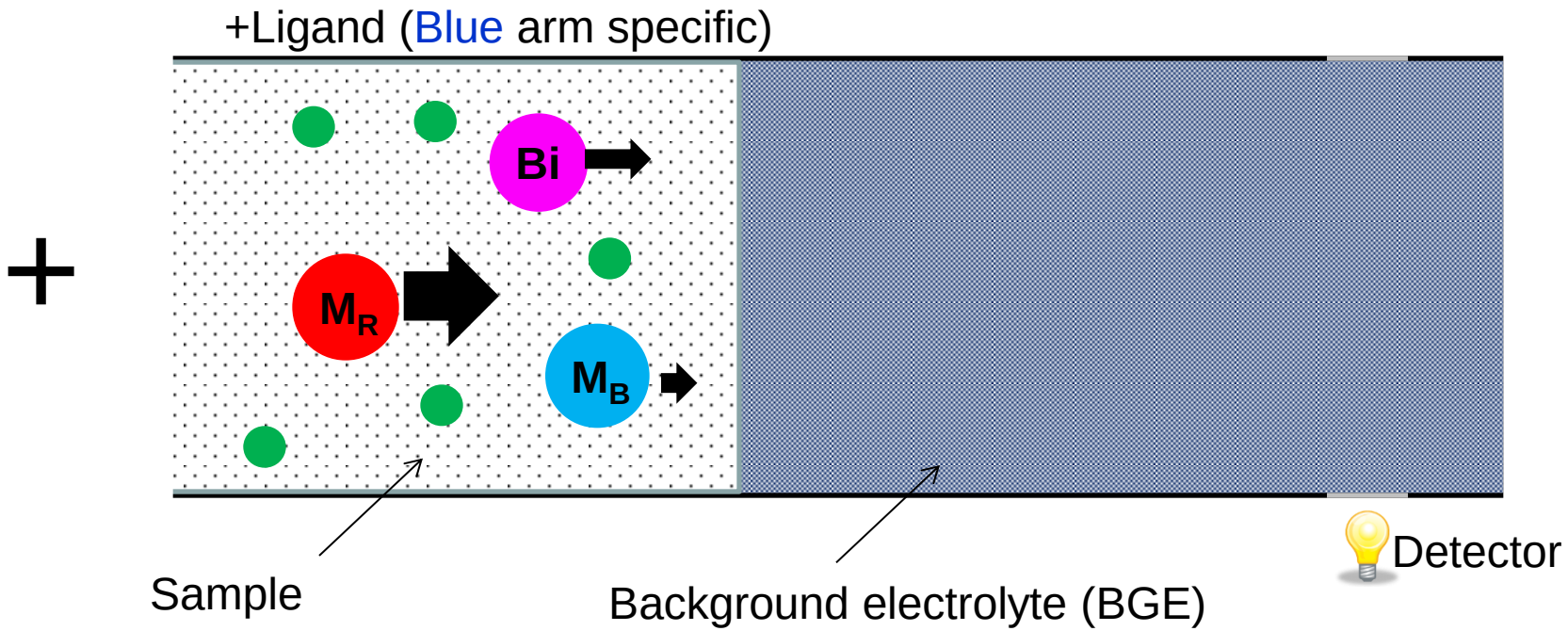
CZE Shows Clear Resolution of Bispecific From Monospecific Side-Products

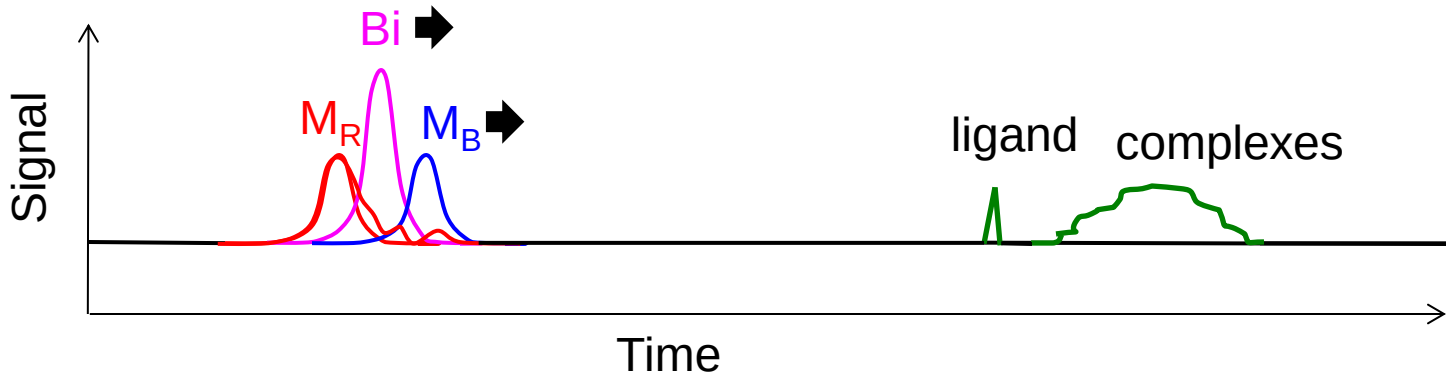


Potential CZE Problem: Incomplete Resolution Due to Charge/Mass Similarity Between the Three Products

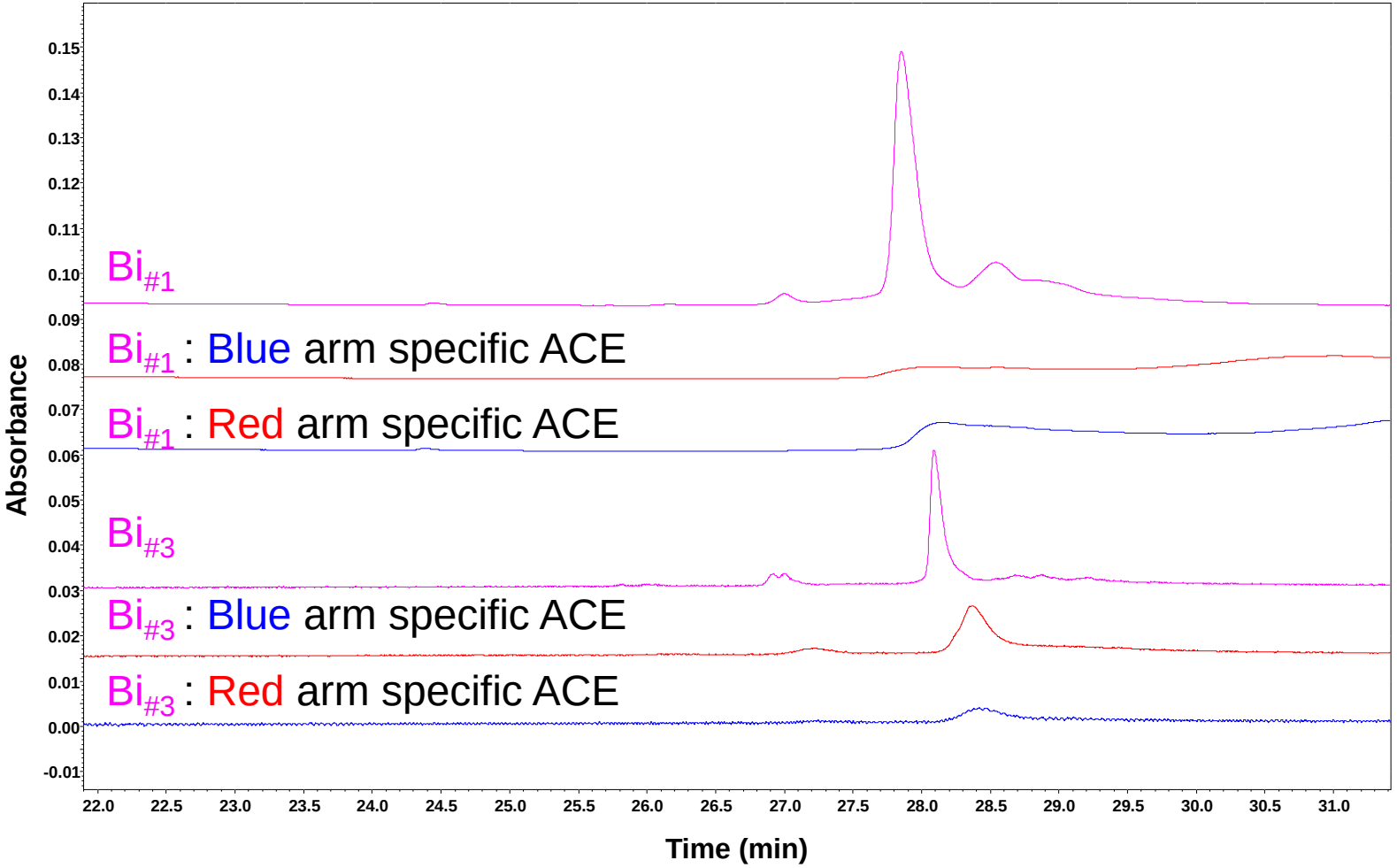


Affinity Capillary Electrophoresis (ACE)

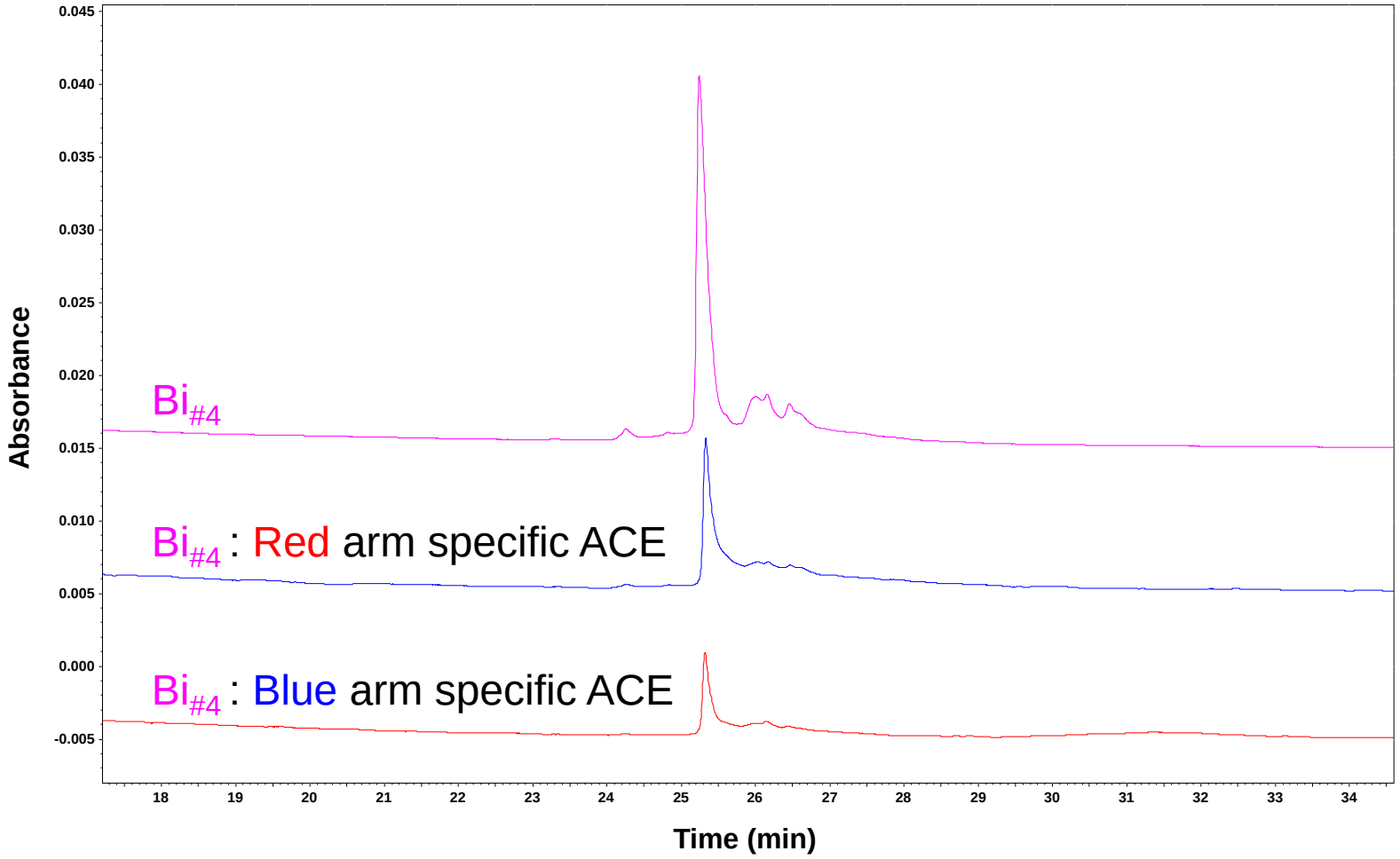




Unexpectedly High Levels of Residual Peaks in Some Bispecific ACE Analyses

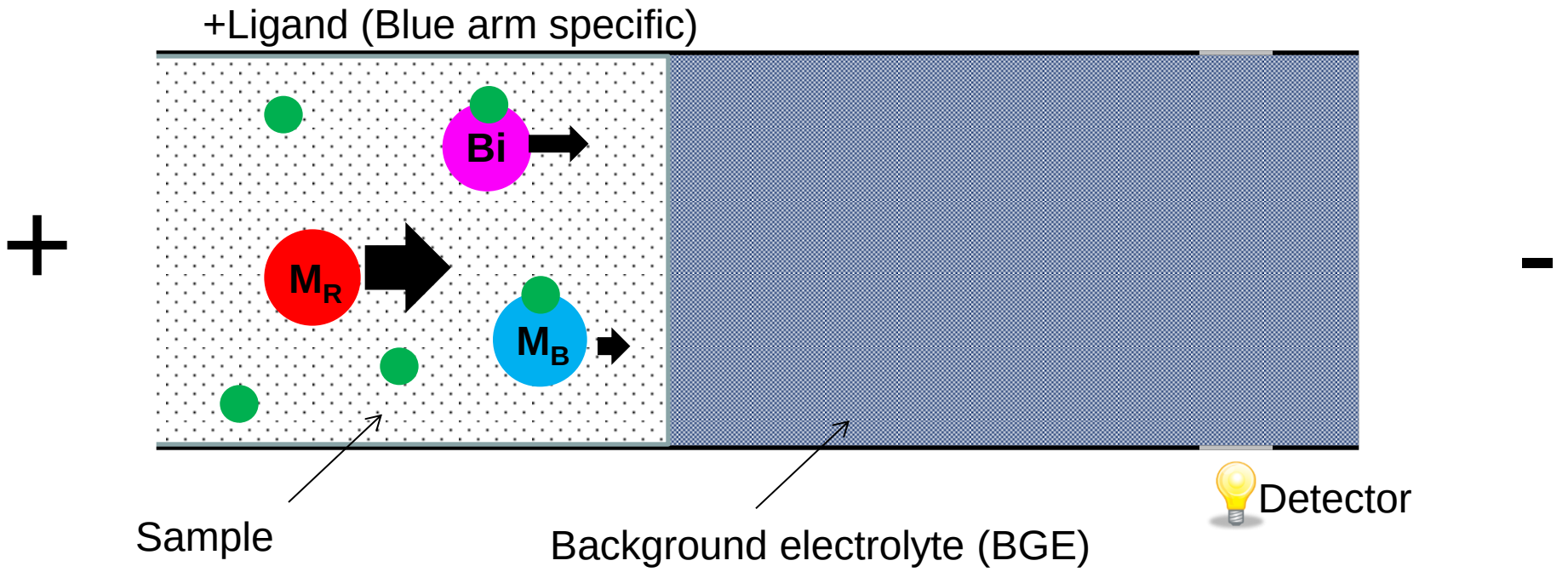


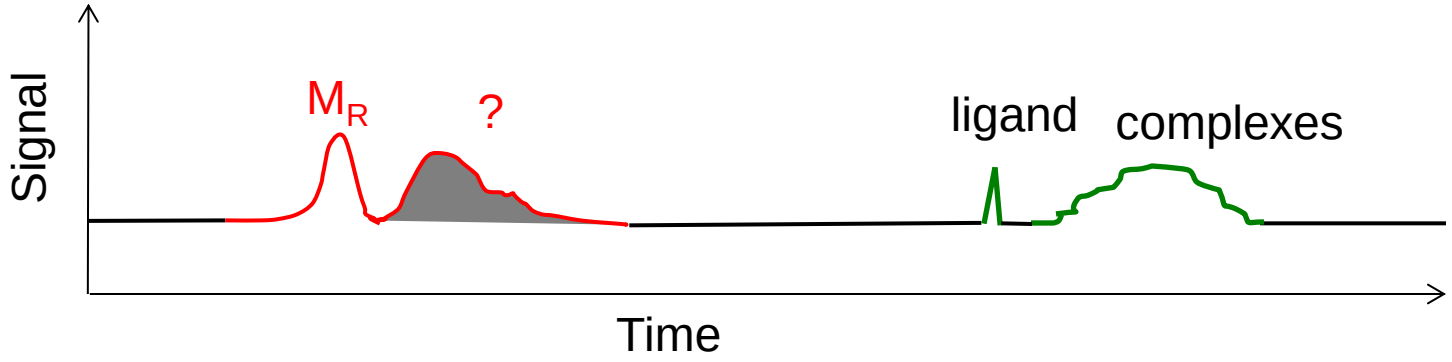
ACE Residual Peaks Show Similar Migration Times to the Bispecific Antibody



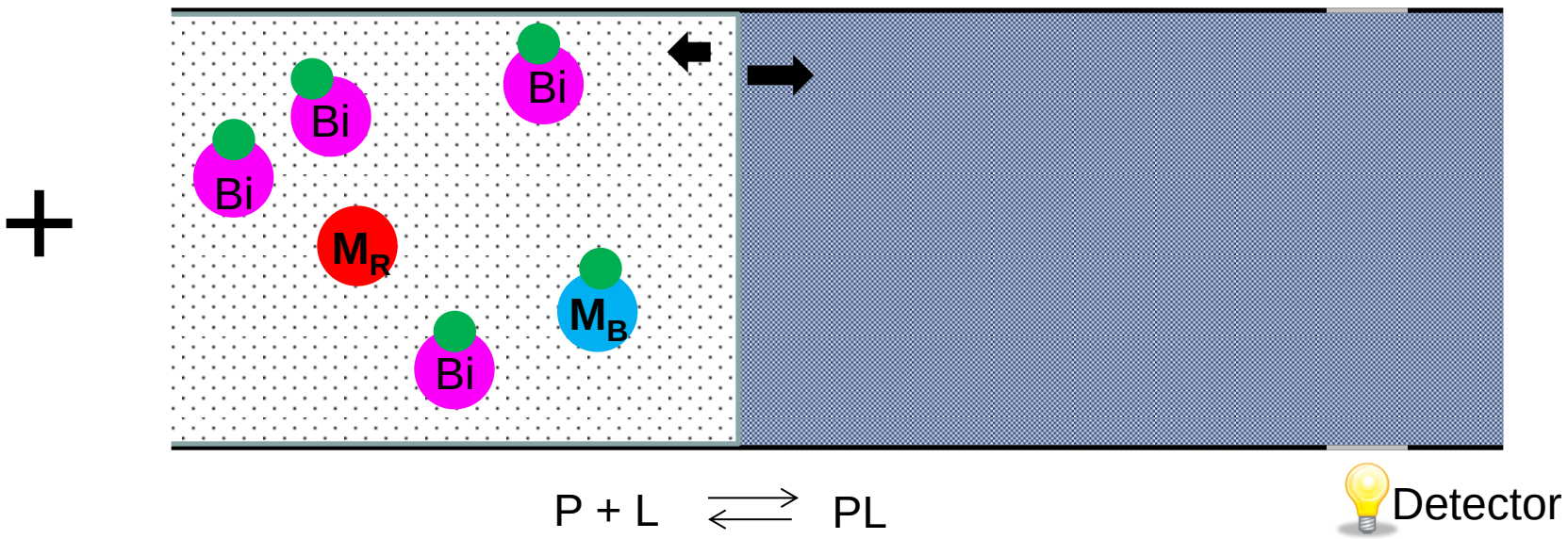
Affinity Capillary Electrophoresis (ACE) –

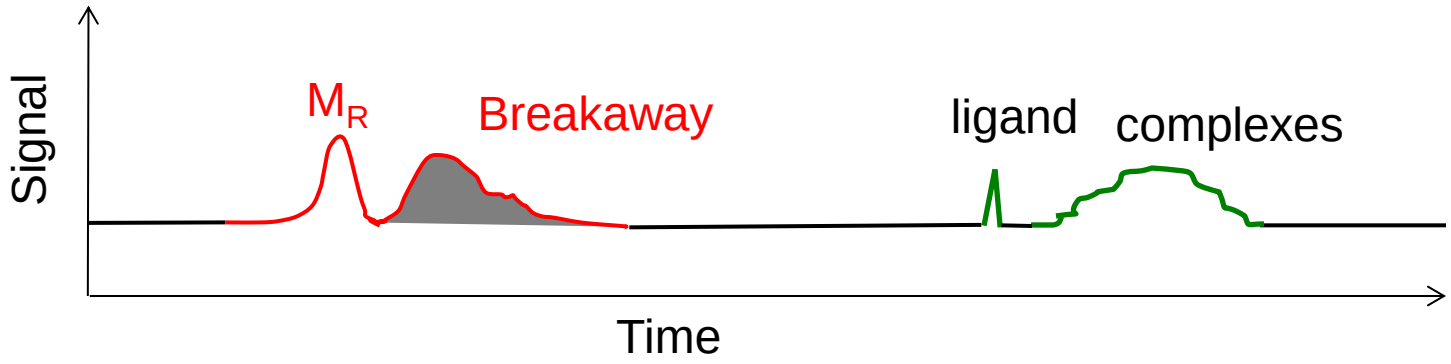
Residual Unbound Signal Despite Excess Ligand



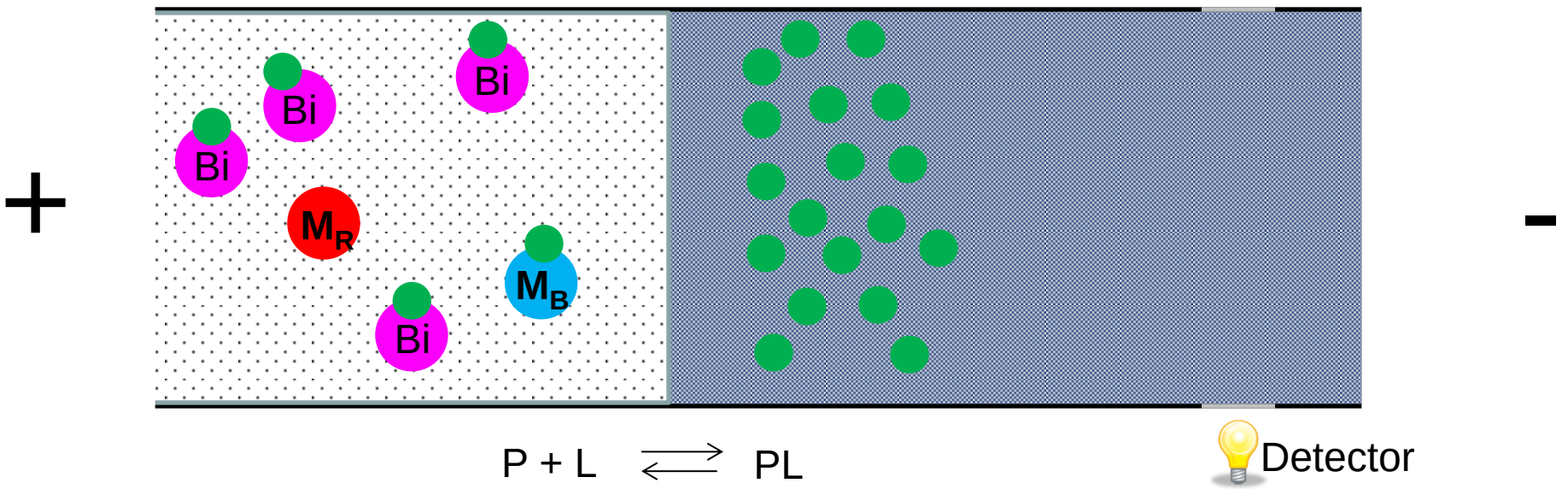


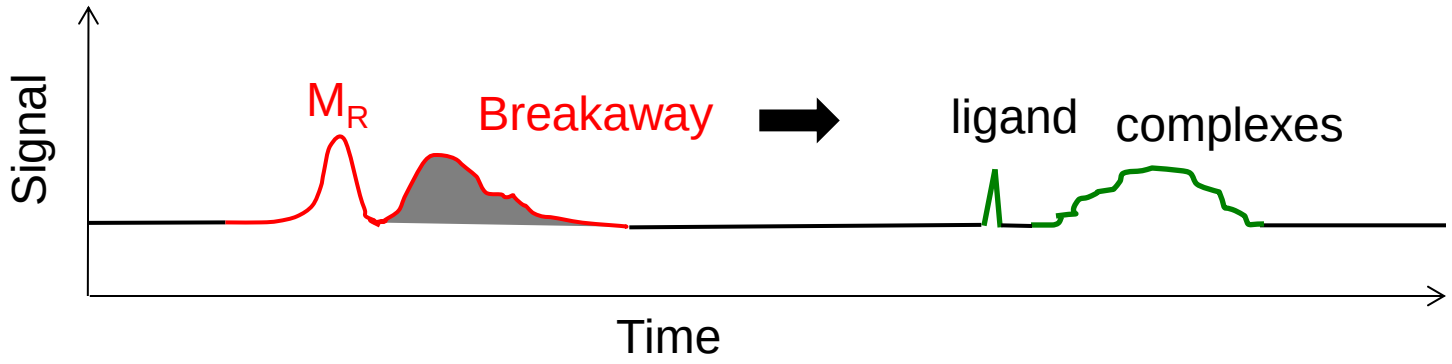
Hypothesis: A Breakaway Subpopulation Forms upon Start of Separation



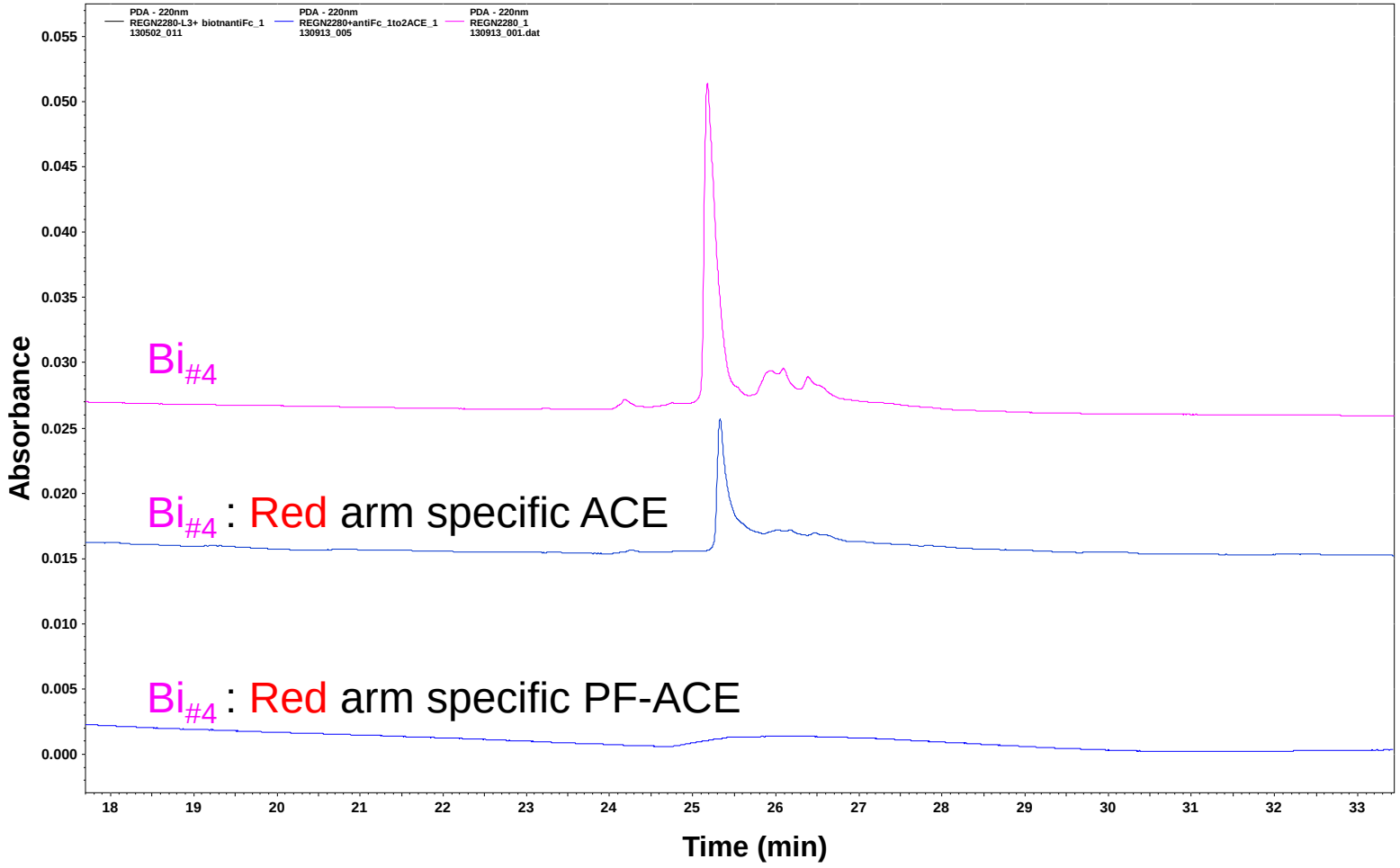


Partial Fill Affinity Capillary Electrophoresis (PF-ACE) – Trapping the Breakaway Subpopulation

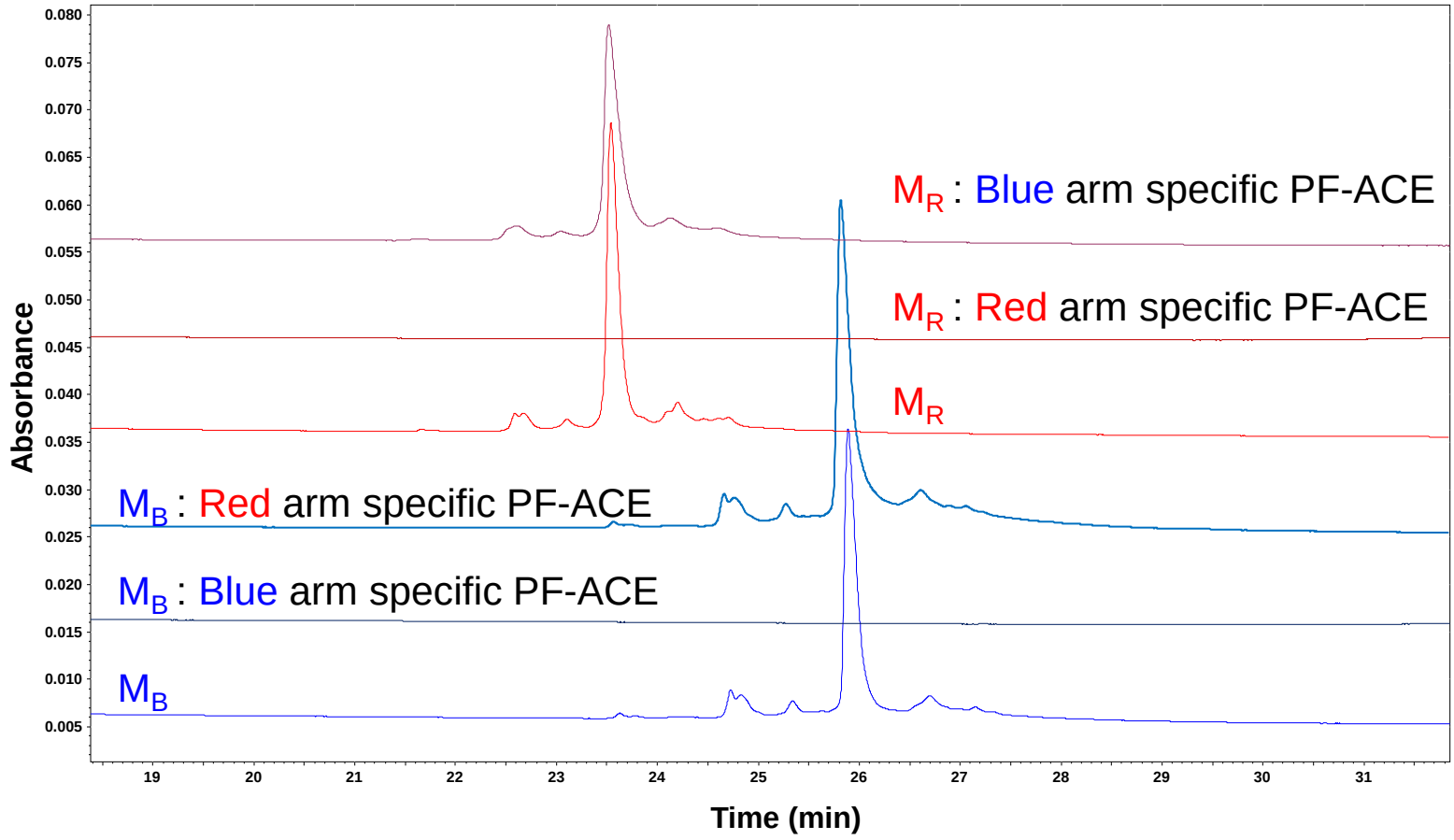




PF-ACE Completely Removes the Residual Peak Seen by ACE

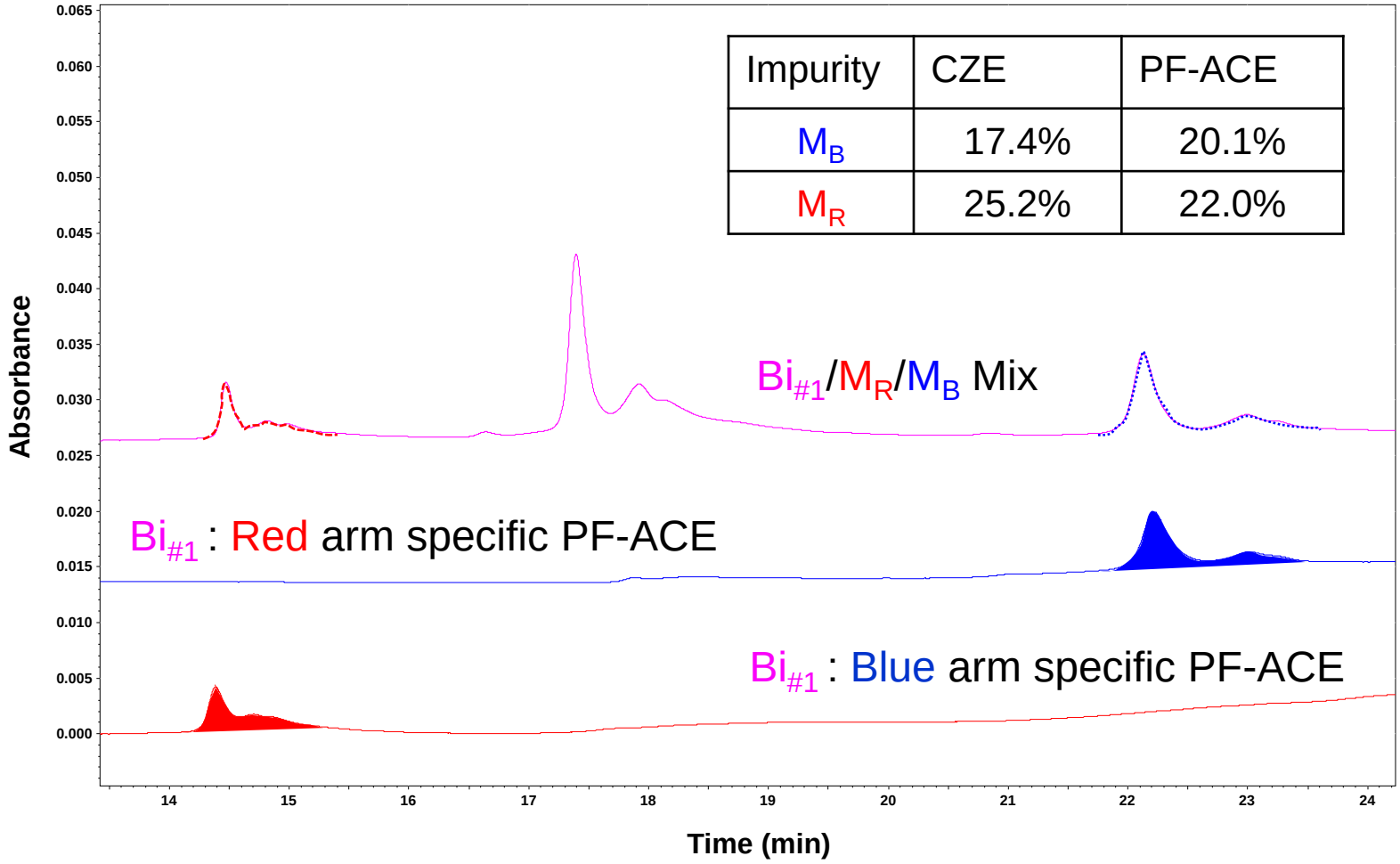


PF-ACE is Highly Specific in Mobility Shifts

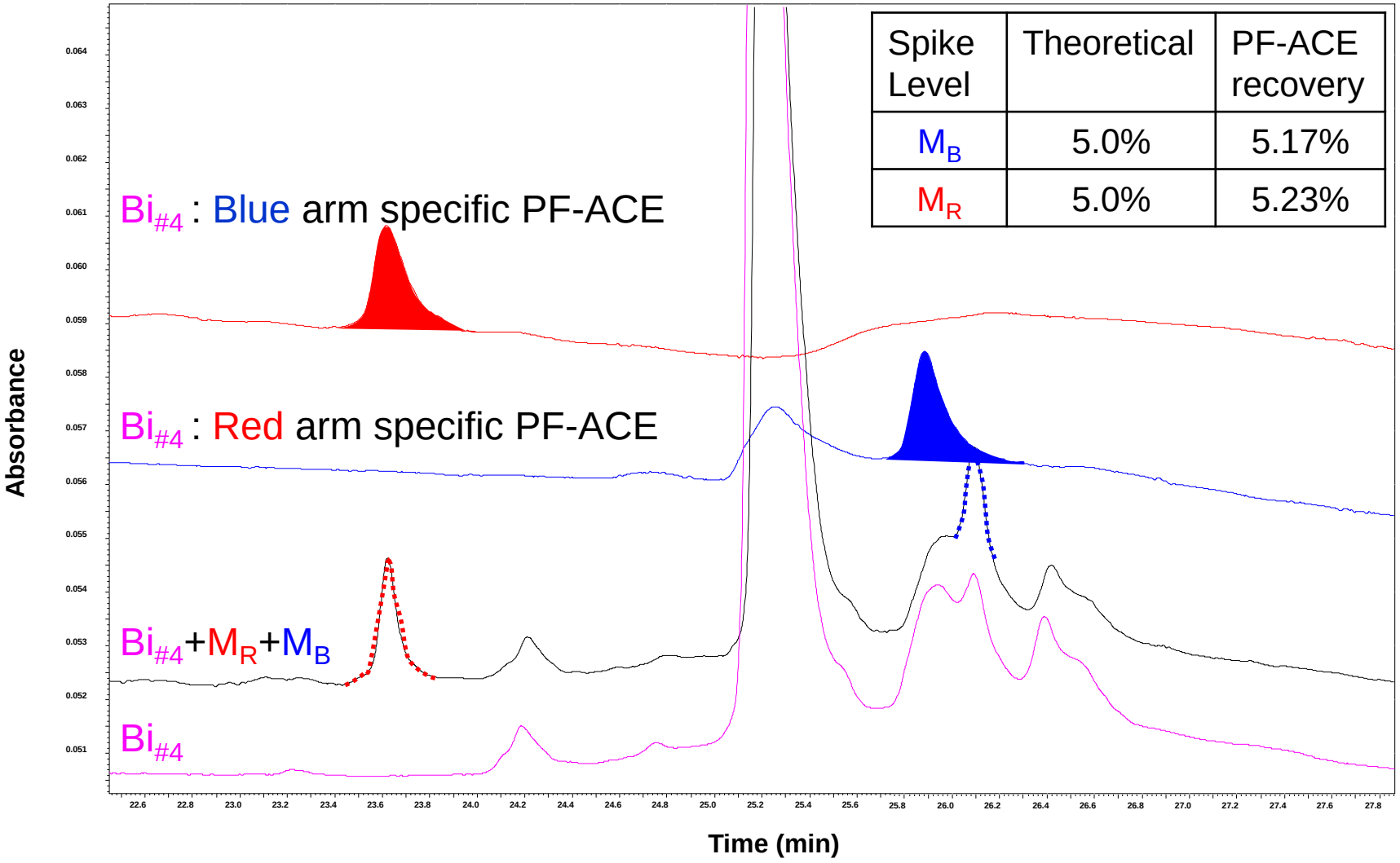


PF-ACE Is Comparable to CZE in Quantifying Monospecific Impurities

Impurity	CZE	PF-ACE
M_B	17.4%	20.1%
M_R	25.2%	22.0%



PF-ACE Shows Quantitative Spike Recovery



Spike Level	Theoretical	PF-ACE recovery
M_B	5.0%	5.17%
M_R	5.0%	5.23%

Conclusions

- Charge based separations, such as CZE, can resolve proteins with a high degree of sequence homology.
- Selective mobility shifts using affinity CE offers an alternative to mass spectrometry in providing peak identity.
- Dissociation of protein complexes can complicate affinity CE analysis. The PF-ACE approach adequately resolves this issue.

Acknowledgements

- Protein Chemistry
  - Bindiya Sanduja
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