

Supplementary Information for

Engineering peptide inhibitors of the HFE-Transferrin Receptor 1 Complex

Daniela Goncalves Monteiro¹, Gautam Rishi² Declan M. Gorman¹, Guillaume Burnet¹, Randy Aliyanto¹, K. Johan Rosengren¹, David M. Frazer³, V. Nathan Subramaniam^{2,*} and Richard J. Clark^{1,*}

¹ The University of Queensland, School of Biomedical Sciences, Brisbane, QLD 4072, Australia

² School of Biomedical Sciences, Centre for Genomics and Personalised Health, Queensland University of Technology, Brisbane, QLD 4059, Australia

³ QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia

*Correspondence: richard.clark@uq.edu.au (RJC); Nathan.subramaniam@qut.edu.au (VNS)

Contents:

- **Figure S1.** Analytical HPLC trace of TfR1 h1 with attached ESI-MS spectra. – page 2
- **Figure S2.** Analytical HPLC trace of TfR1 h3 with attached ESI-MS spectra. – page 3
- **Figure S3.** Analytical HPLC trace of TfR1 h1h3 with attached ESI-MS spectra. – page 4
- **Figure S4.** Analytical HPLC trace of TfR1 negative control with attached ESI-MS spectra. – page 5
- **Figure S5.** Analytical HPLC trace of HFE α 1 with attached ESI-MS spectra. – page 6
- **Figure S6.** Analytical HPLC trace of HFE α 2 with attached ESI-MS spectra. – page 7
- **Figure S7.** Analytical HPLC trace of HFE α 1 α 2 with attached ESI-MS spectra. – page 8
- **Figure S8.** Analytical HPLC trace of HFE α 1 α 2 staple 1 with attached ESI-MS spectra. – page 9
- **Figure S9.** Analytical HPLC trace of HFE α 1 α 2 staple 2 with attached ESI-MS spectra. – page 10
- **Figure S10.** Analytical HPLC trace of HFE α 1 α 2 double staple with attached ESI-MS spectra. – page 11
- **Figure S11.** Analytical HPLC trace of HFE negative control with attached ESI-MS spectra. – page 12
- **Figure S12.** Analytical HPLC trace of HFE α 1 α 2 scrambled sequence with attached ESI-MS spectra. – page 13

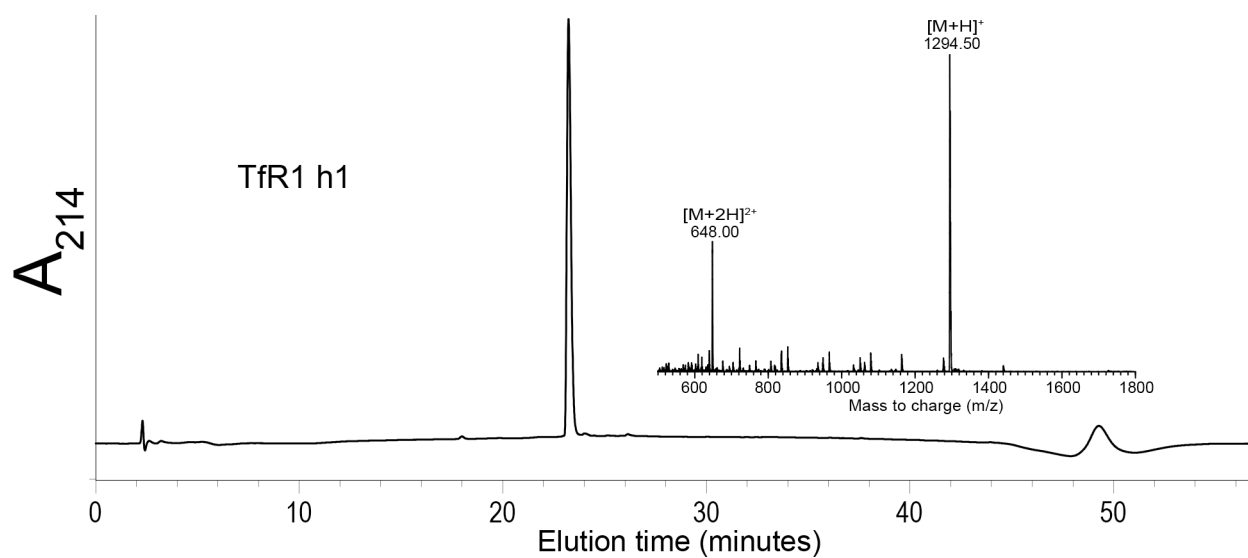


Figure S1. Analytical HPLC trace of TfR1 h1 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

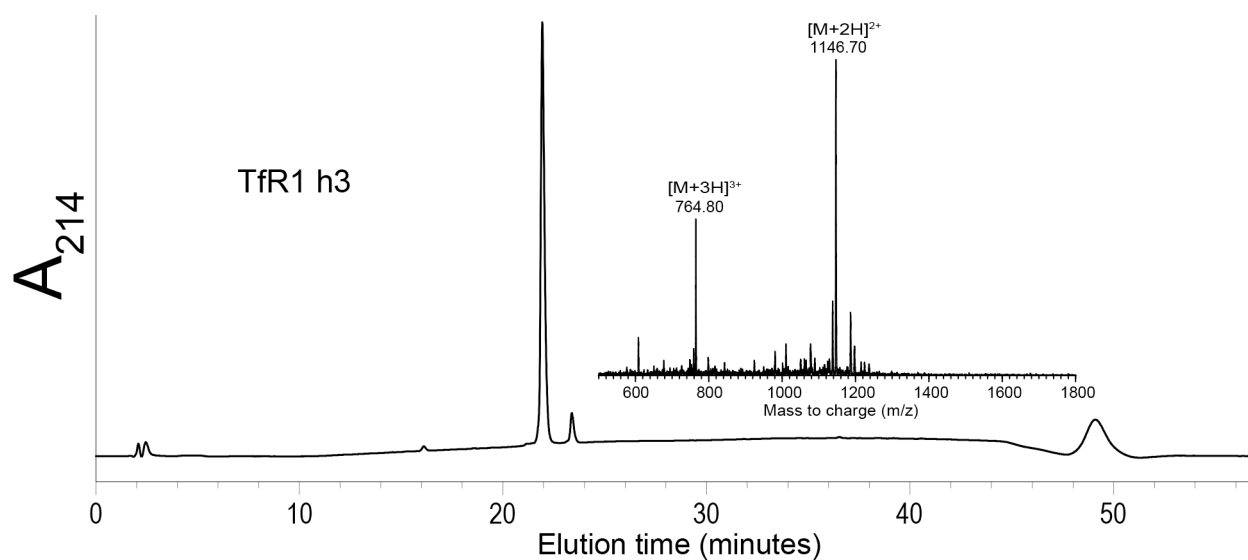


Figure S2. Analytical HPLC trace of TfR1 h3 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

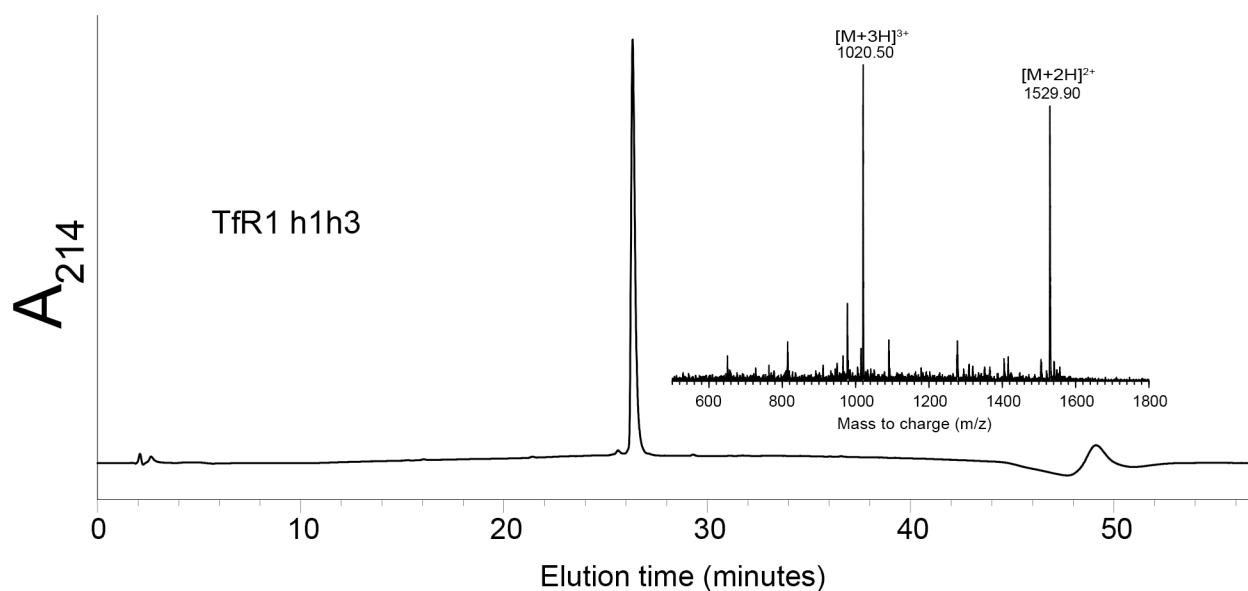


Figure S3. Analytical HPLC trace of TfR1 h1h3 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

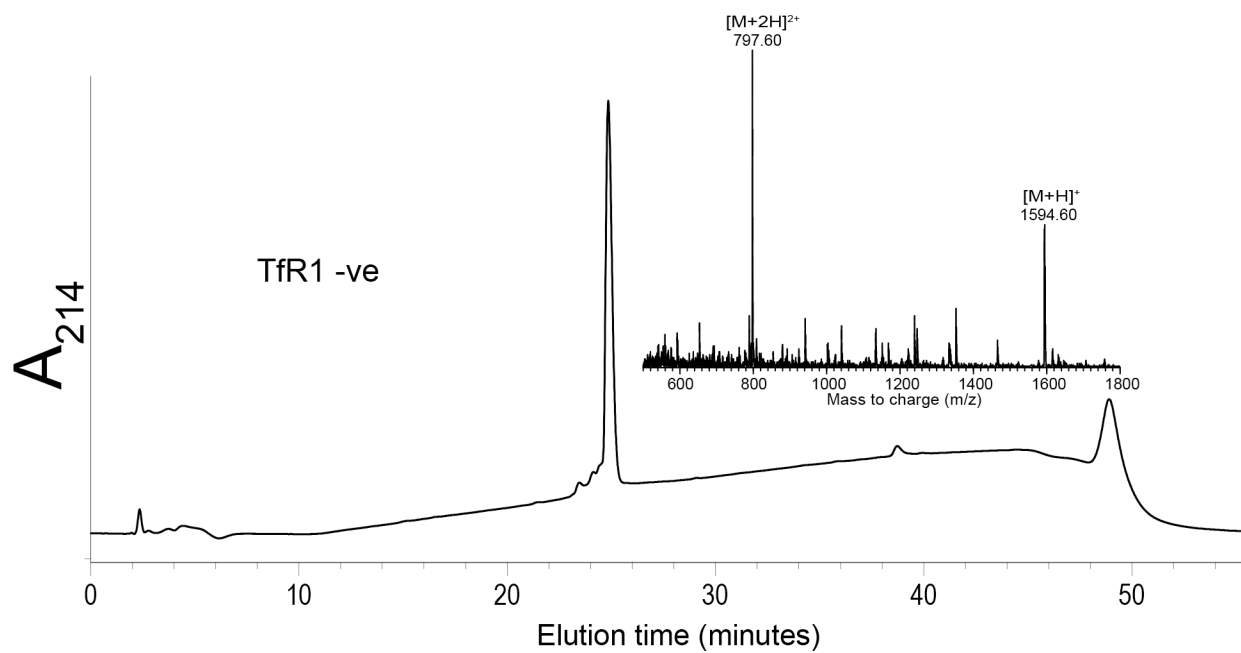


Figure S4. Analytical HPLC trace of TfR1 negative control with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

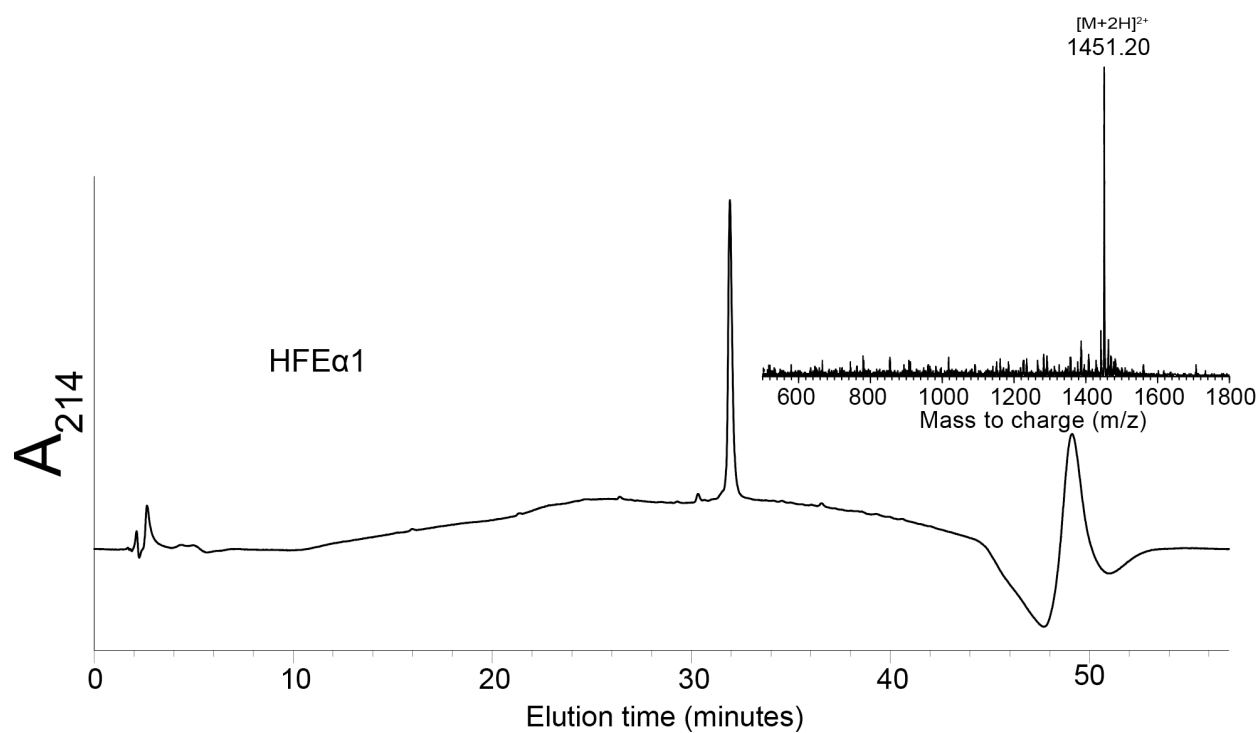


Figure S5. Analytical HPLC trace of HFE α 1 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

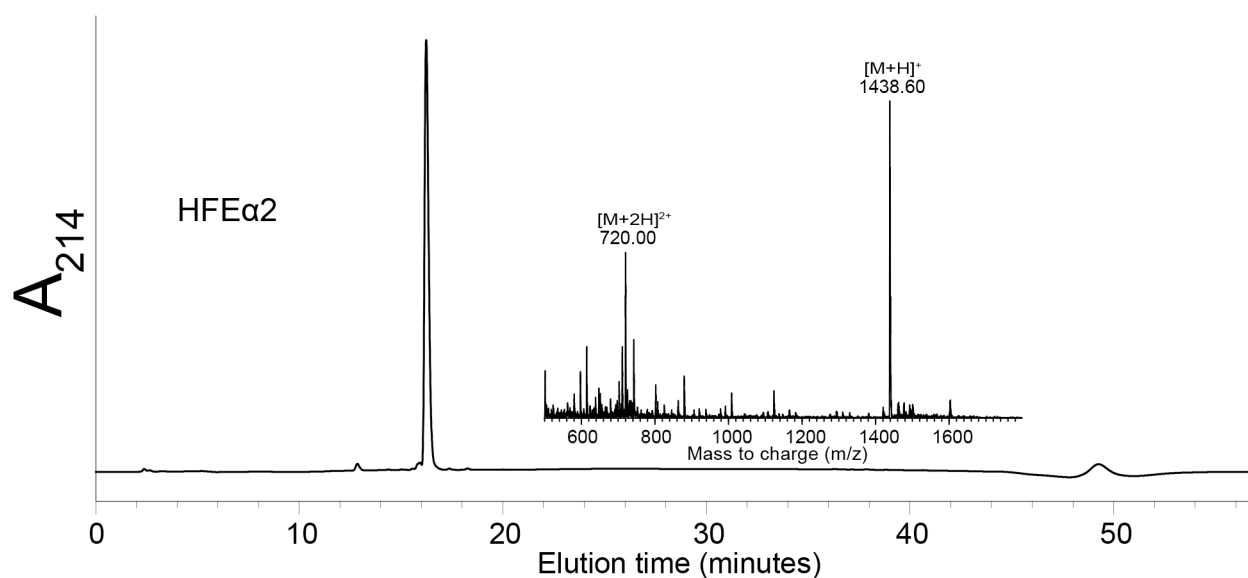


Figure S6. Analytical HPLC trace of HFE α 2 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

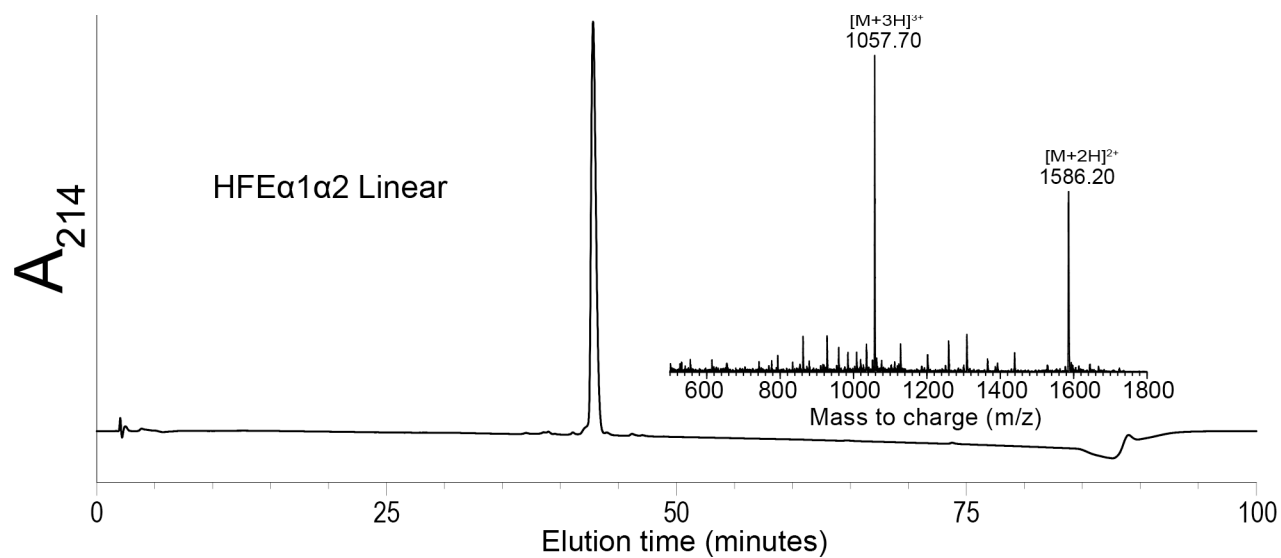


Figure S7. Analytical HPLC trace of HFE $\alpha 1\alpha 2$ with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 1% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

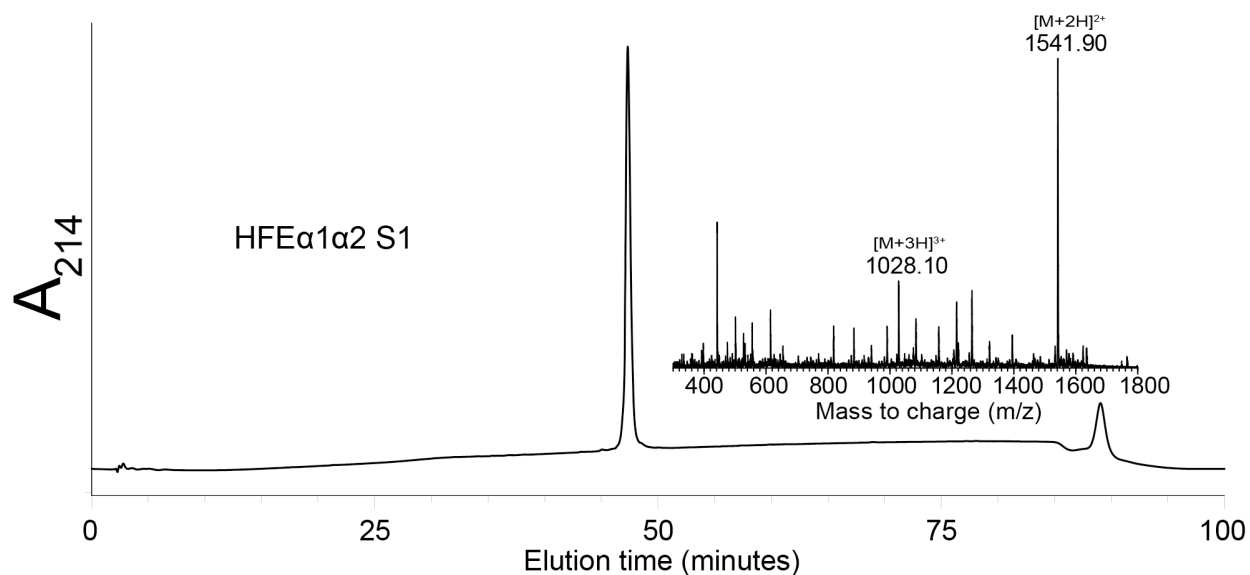


Figure S8. Analytical HPLC trace of HFE $\alpha 1\alpha 2$ staple 1 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 1% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

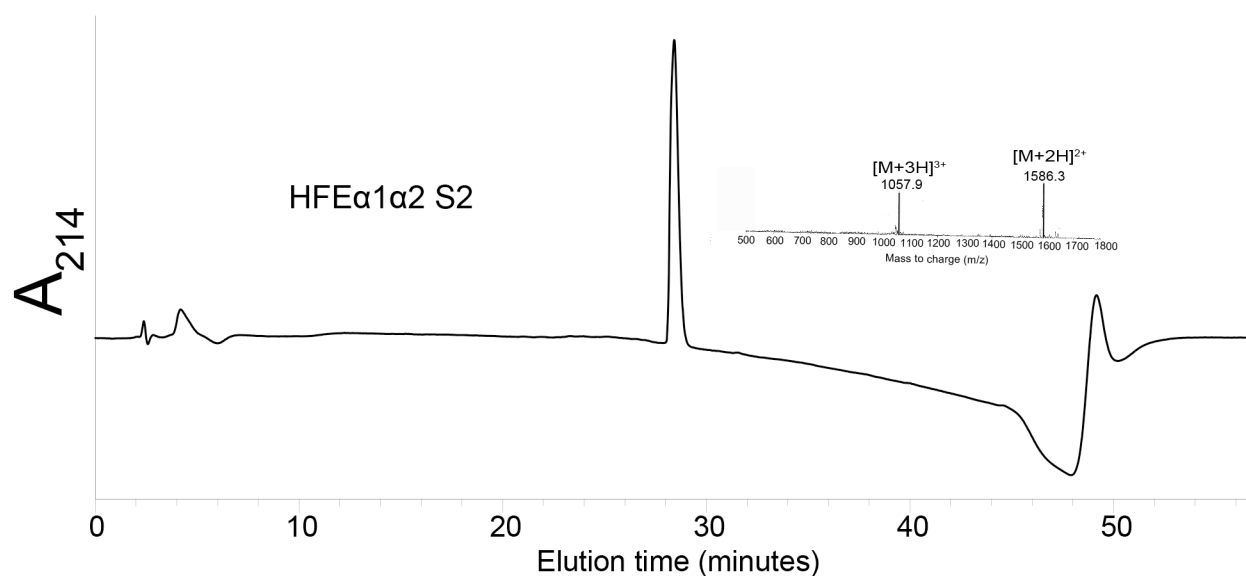


Figure S9. Analytical HPLC trace of HFE $\alpha 1\alpha 2$ staple 2 with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

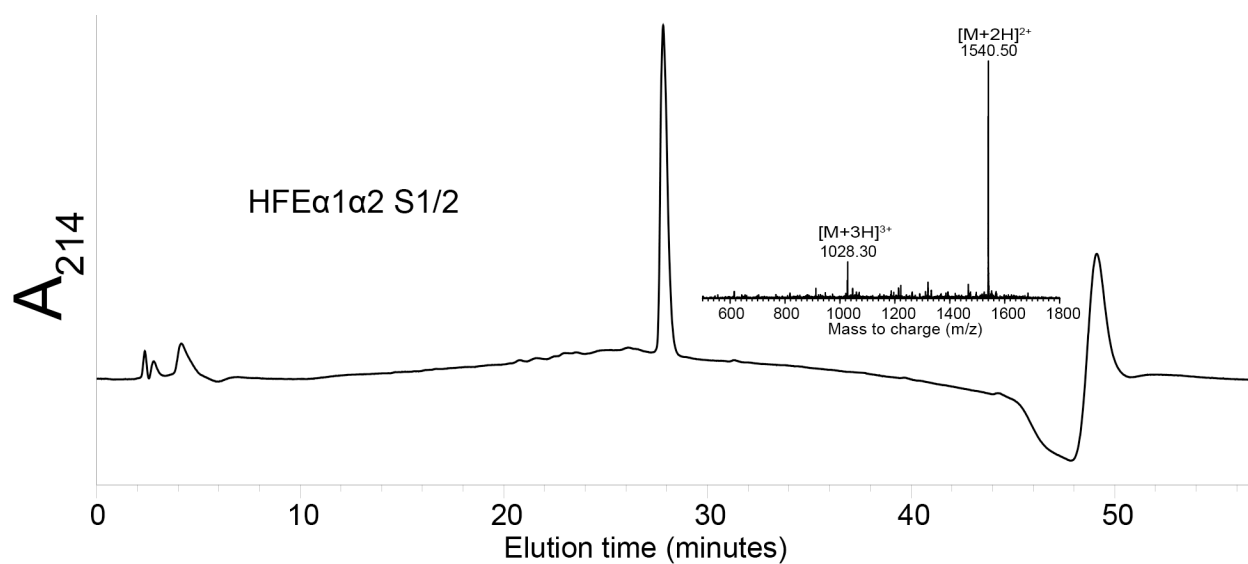


Figure S10. Analytical HPLC trace of HFE α 1 α 2 double staple with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

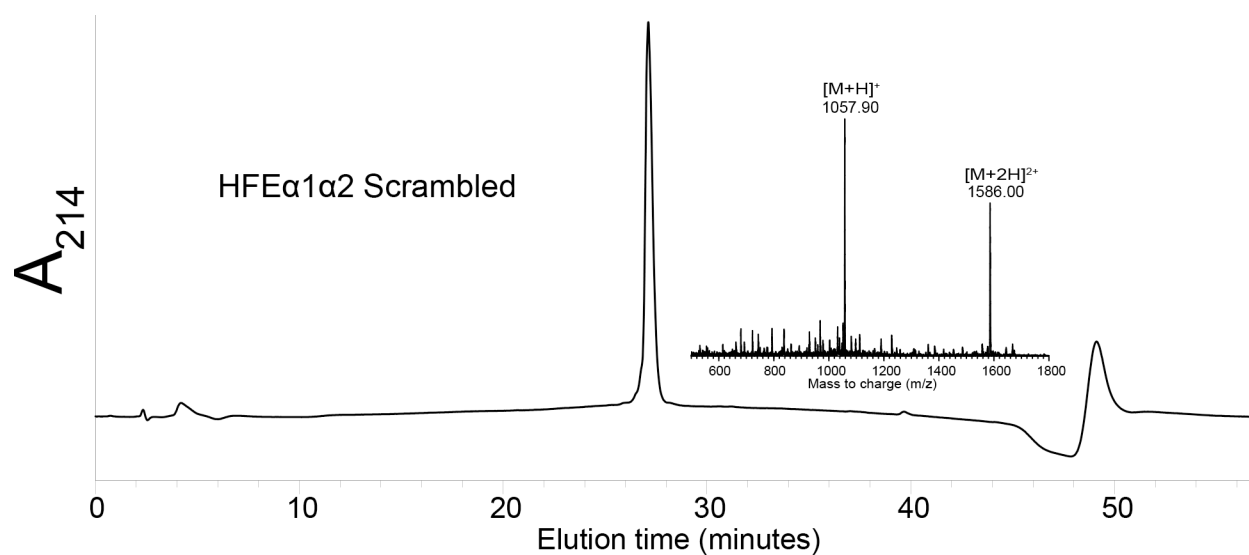


Figure S11. Analytical HPLC trace of HFE $\alpha 1\alpha 2$ scrambled sequence with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.

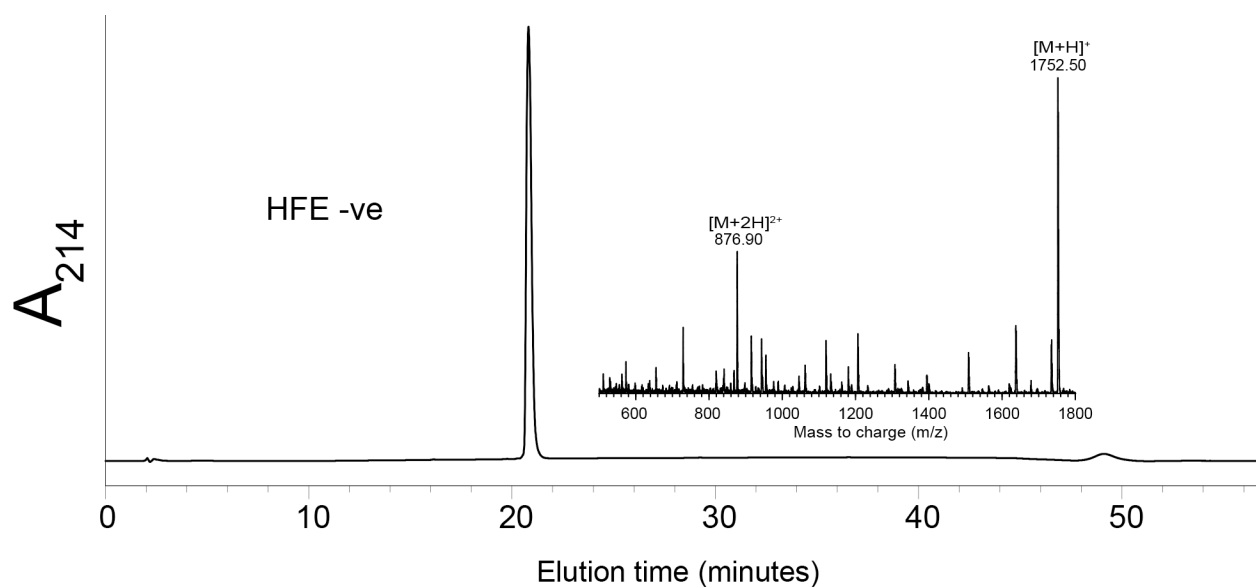


Figure S12. Analytical HPLC trace of HFE negative control with attached ESI-MS spectra. Analytical HPLC was run at an increasing gradient of 2% buffer B (90% ACN, 0.05% TFA) in buffer A (0.05% TFA) per minute using an Agilent, 300Å, 5 μ m, 150 x 2.1 mm C18 column.