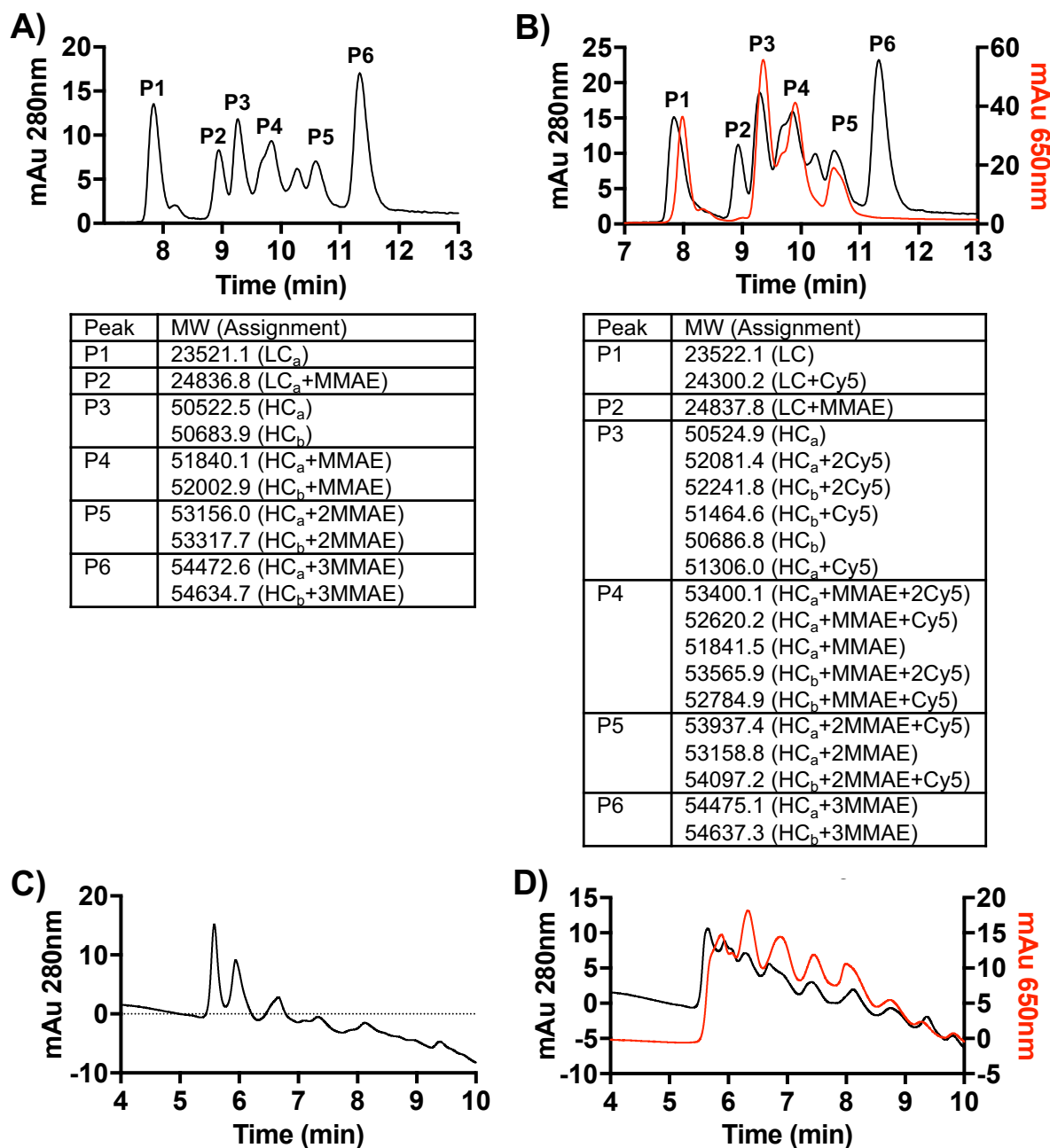
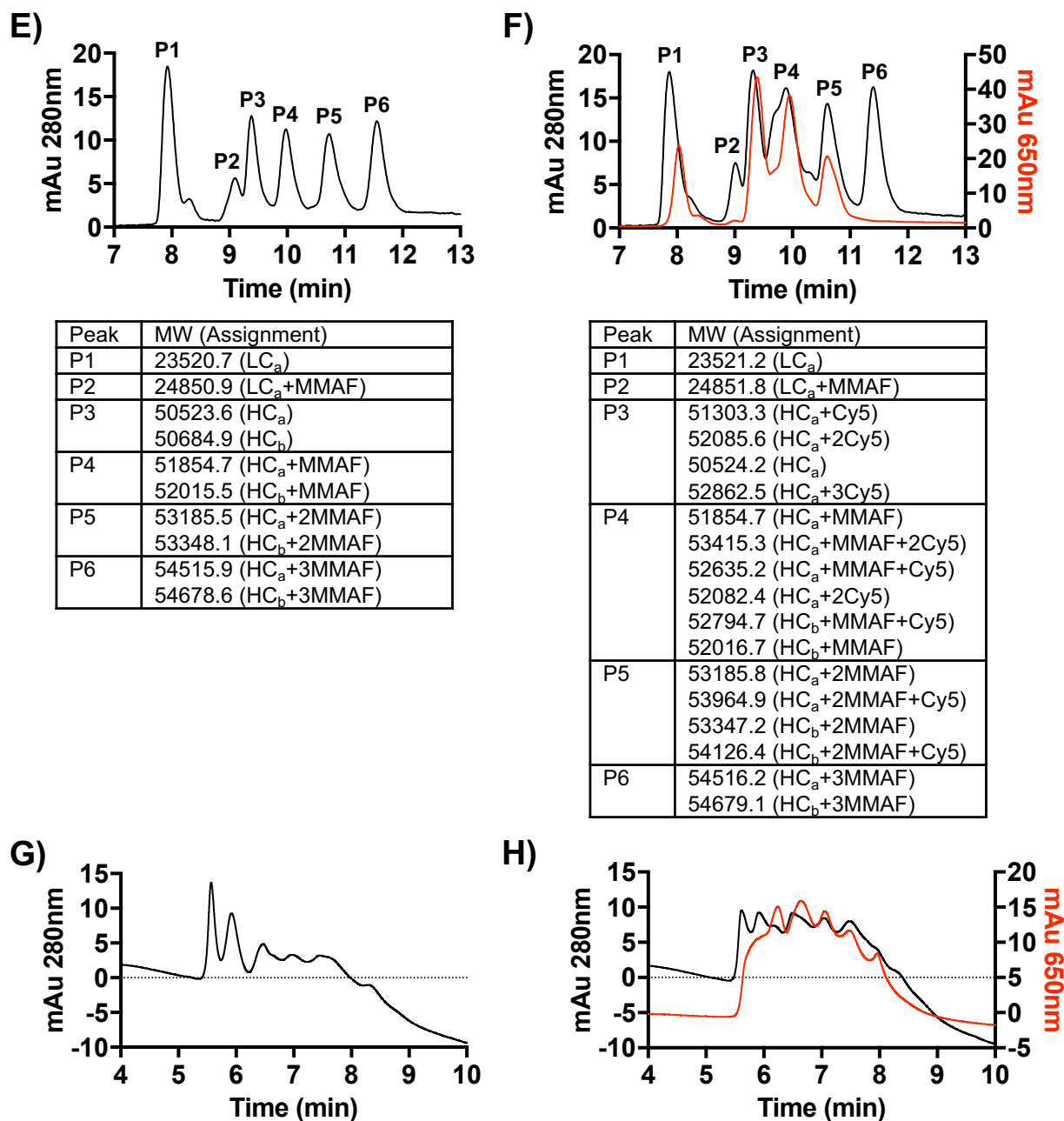


Supplementary Figure 1



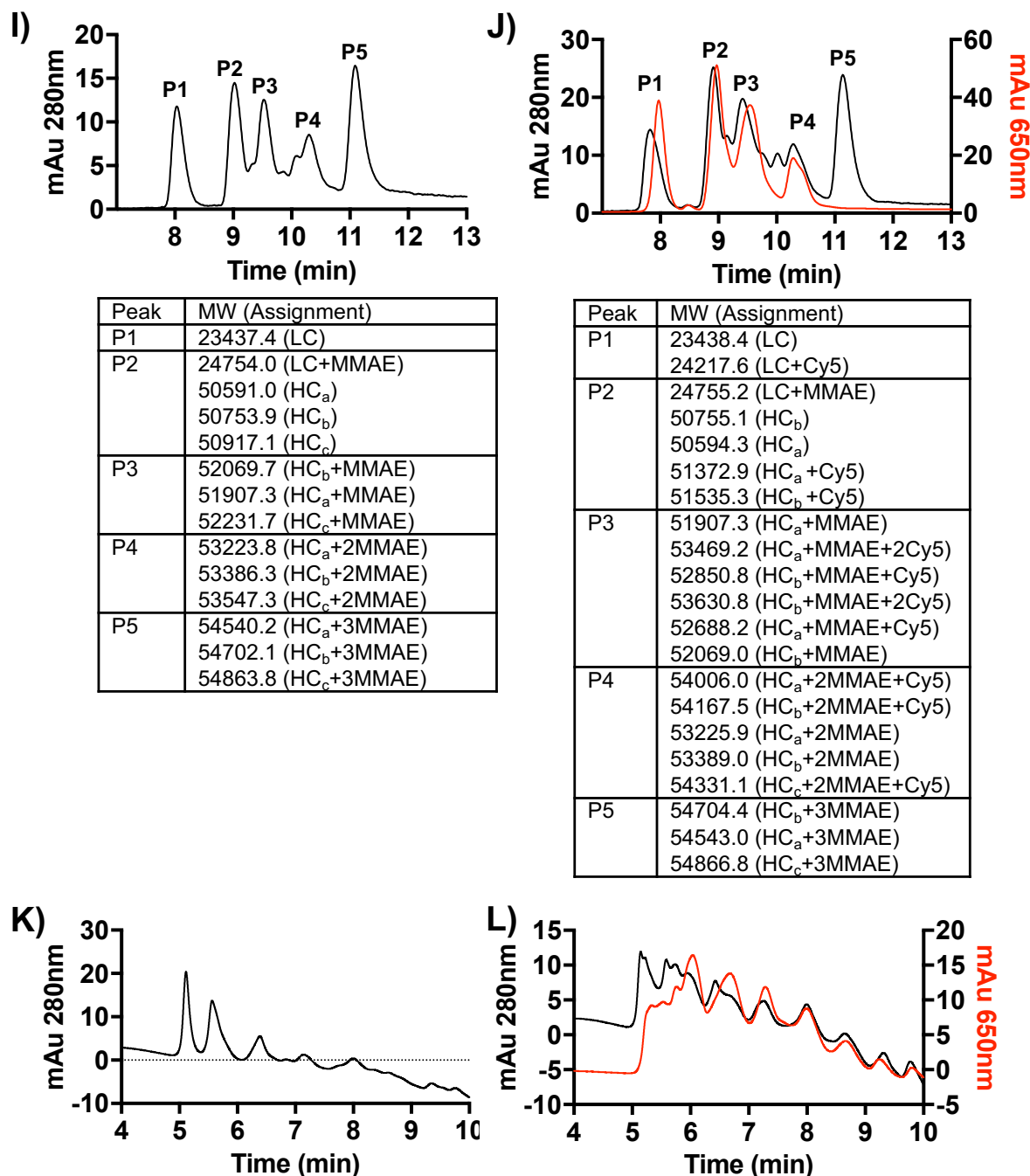
Supplementary Figure 1: Pertuzumab conjugation to MMAE and Cy5. A, B) Reverse-phase protein chromatograms and electro-spray mass spectrometry analysis of pertuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAE (**A**) and 2 equivalents of Cy5-maleimide (**B**). Samples gel filtered and concentrated. Pertuzumab light chain MW is 23522.6 and major heavy chain glycoforms MW are 50527.1 and 50688.5 with batch variability. Expected addition in mass from MC-VC-MMAE and Cy5-maleimide is 1316.6 and 779.9 respectively. **C, D)** Hydrophobic interaction chromatograms of pertuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAE (**C**) and then 2 equivalents of Cy5-maleimide (**D**), followed by gel filtration and concentration.

Supplementary Figure 1 (Cont)



Supplementary Figure 1: Pertuzumab conjugation to MMAF and Cy5. E, F) Reverse-phase protein chromatograms and electro-spray mass spectroscopy analysis of pertuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAF (**E**) and 2 equivalents of Cy5-maleimide (**F**). Samples gel filtered and concentrated. Pertuzumab light chain MW is 23522.6 and major heavy chain glycoforms MW are 50527.1 and 50688.5 with batch variability. Expected addition in mass from MC-VC-MMAF and Cy5-maleimide is 1330.6 and 779.9 respectively. **G, H)** Hydrophobic interaction chromatograms of pertuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAF (**G**) and then 2 equivalents of Cy5-maleimide (**H**), followed by gel filtration and concentration.

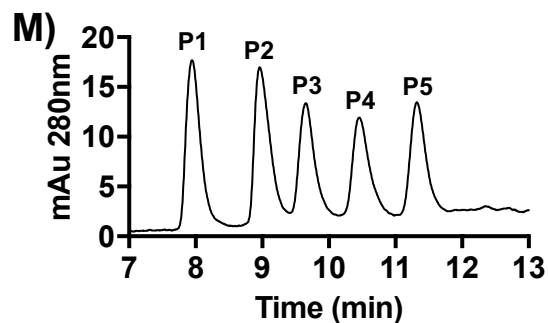
Supplementary Figure 1 (Cont)



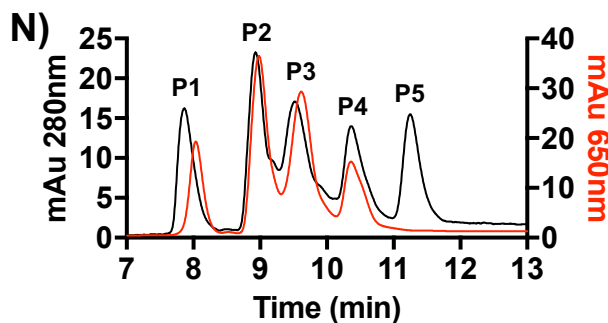
Supplementary Figure 1: Trastuzumab conjugation to MMAE and Cy5. I, J)

Reverse-phase protein chromatograms and electro-spray mass spectroscopy analysis of trastuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAE (**I**) and 2 equivalents of Cy5-maleimide (**J**). Samples gel filtered and concentrated. Trastuzumab light chain MW is 23438.9 and major heavy chain glycoforms MW are 50594.2, 50756.1 and 50918.9 with batch variability. Expected additional mass from MC-VC-MMAE and Cy5-maleimide is 1316.6 and 779.9 respectively. **K, L**) Hydrophobic interaction chromatograms of trastuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAE (**K**) and then 2 equivalents of Cy5-maleimide (**L**), followed by gel filtration and concentration.

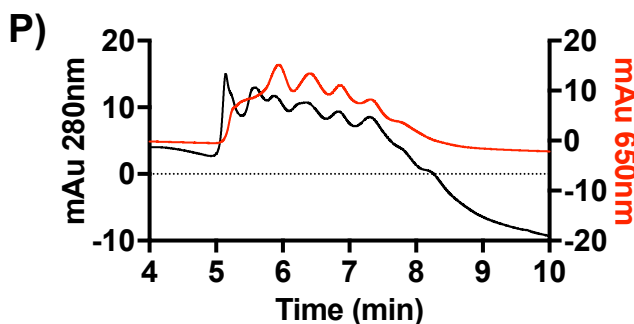
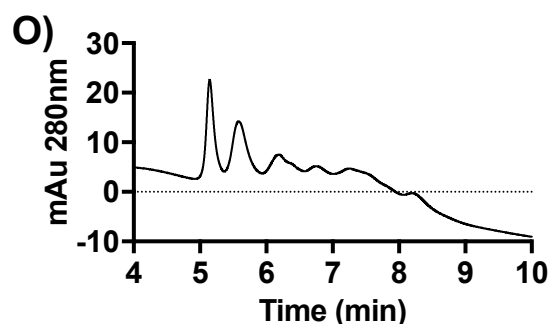
Supplementary Figure 1 (Cont)



Peak	MW (Assignment)
P1	23438.1 (LC)
P2	24766.7 (LC+MMAF) 50592.1 (HC _a) 50917.4 (HC _c)
P3	52084.7 (HC _b +MMAF) 51923.0 (HC _a +MMAF) 52246.7 (HC _c +MMAF)
P4	53253.5 (HC _a +2MMAF) 53415.4 (HC _b +2MMAF) 53577.1 (HC _c +2MMAF)
P5	54583.7 (HC _a +3MMAF) 54745.7 (HC _b +3MMAF) 54907.3 (HC _c +3MMAF)



Peak	MW (Assignment)
P1	23438.3 (LC) 242187.4 (LC+Cy5)
P2	24768.7 (LC+MMAF) 50591.1 (HC _a) 50757.2 (HC _b) 51373.6 (HC _a +Cy5) 51531.4 (HC _b +Cy5)
P3	51922.4 (HC _a +MMAF) 52701.1 (HC _a +MMAF+Cy5) 52863.4 (HC _b +MMAF+Cy5) 53482.7 (HC _a +MMAF+2Cy5) 53026.6 (HC _c +MMAF+Cy5)
P4	53254.1 (HC _c +3Cy5) 53416.2 (HC _b +2MMAF) 54194.9 (HC _b +2MMAF+Cy5) 53576.1 (HC _c +2MMAF) 54032.9 (HC _a +2MMAF+Cy5) 54356.0 (HC _c +2MMAF+Cy5)
P5	54584.8 (HC _a +3MMAF) 54746.8 (HC _b +3MMAF) 54908.5 (HC _c +3MMAF)



Supplementary Figure 1: Trastuzumab conjugation to MMAF and Cy5. **M, N)**

Reverse-phase protein chromatograms and electro-spray mass spectroscopy analysis of trastuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAF (**M**) and 2 equivalents of Cy5-maleimide (**N**). Samples gel filtered and concentrated. Trastuzumab light chain MW is 23438.9 and major heavy chain glycoforms MW are 50594.2, 50756.1 and 50918.9 with batch variability. Expected addition in mass from MC-VC-MMAF and Cy5-maleimide is 1330.6 and 779.9 respectively. (**O, P**) Hydrophobic interaction chromatograms of trastuzumab after partial reduction with 4 equivalents of TCEP and subsequent reaction with 4 equivalents of MC-VC-PABC-MMAF (**O**) and then 2 equivalents of Cy5-maleimide (**P**), followed by gel filtration and concentration.