SUPPORTING INFORMATION

Title: The Role of Structural Flexibility in Hydrocarbon Stapled Peptides Designed to Block Viral Infection via Human ACE2 Mimicry

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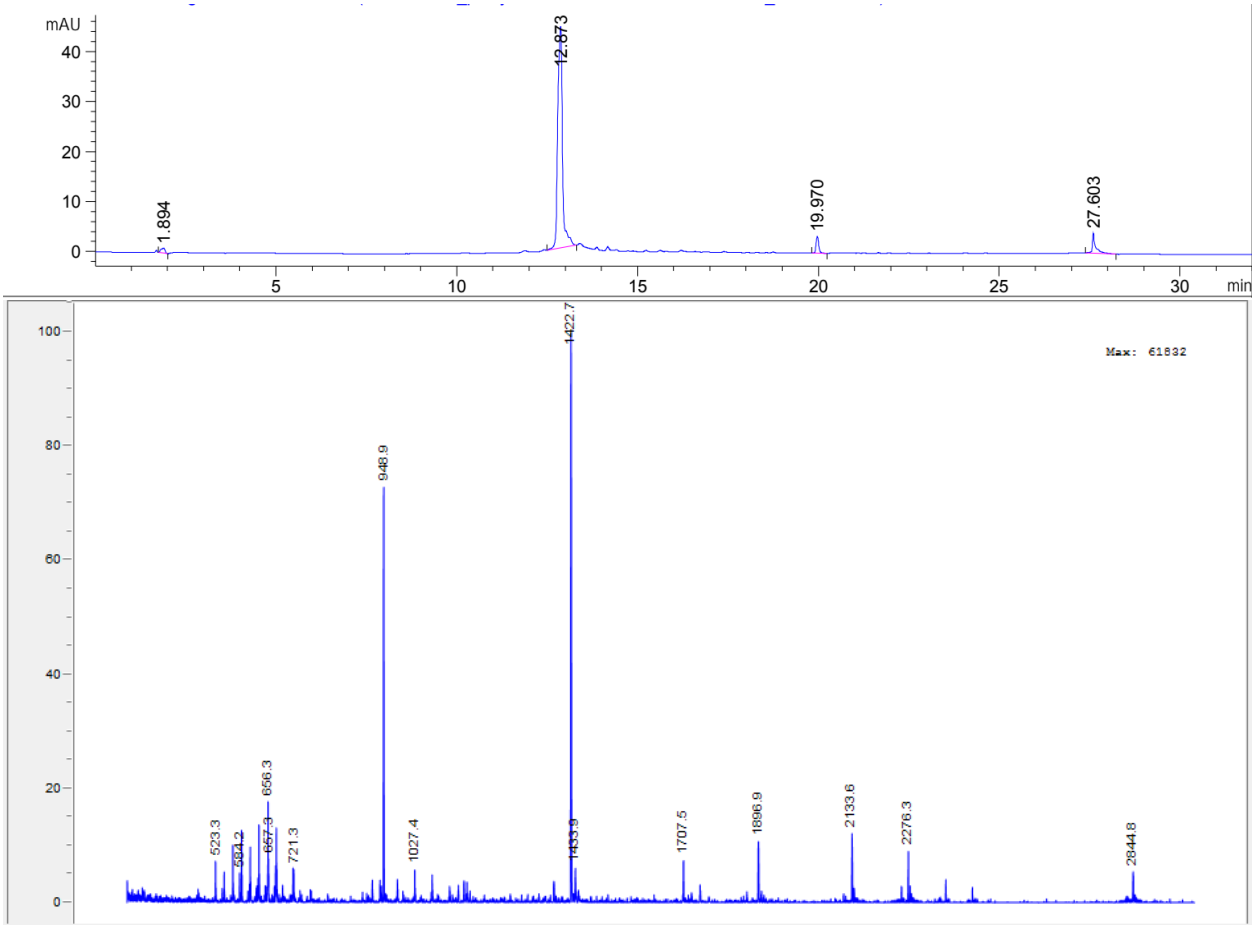
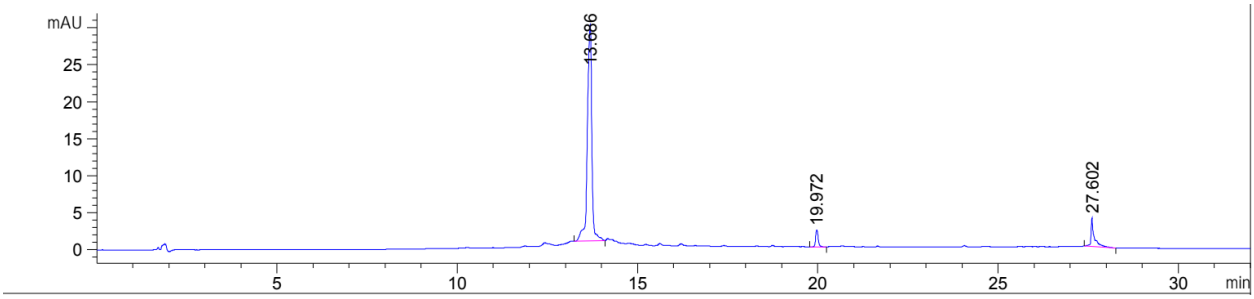
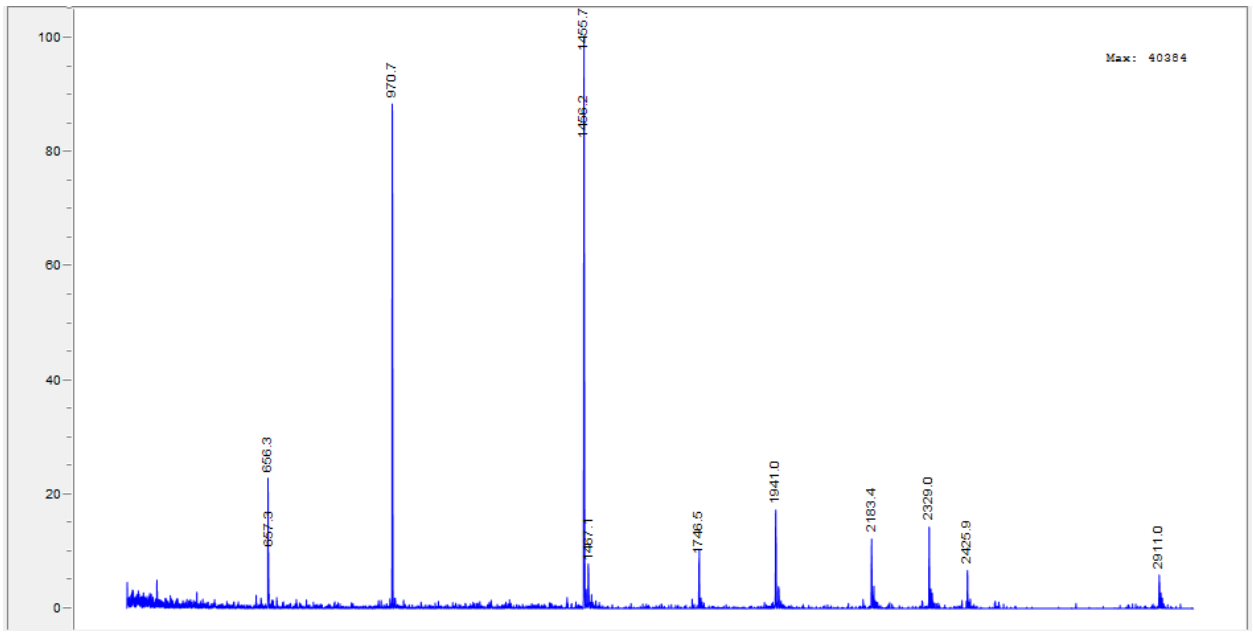
(a) ACE2(21-43), MW = 2844 g/mol.

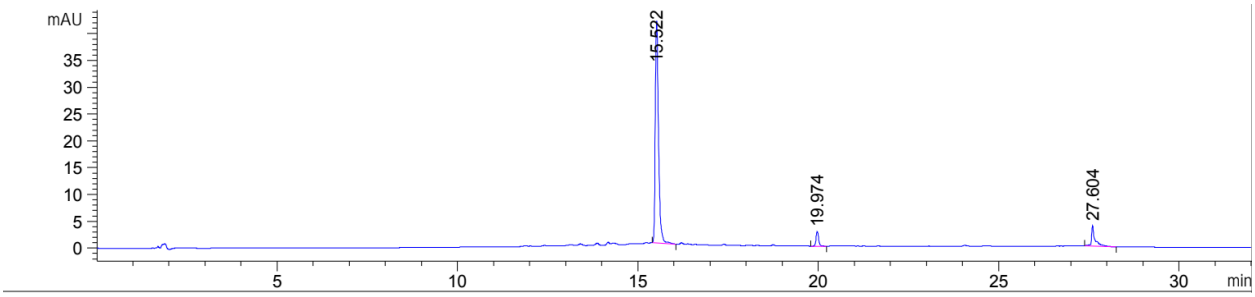


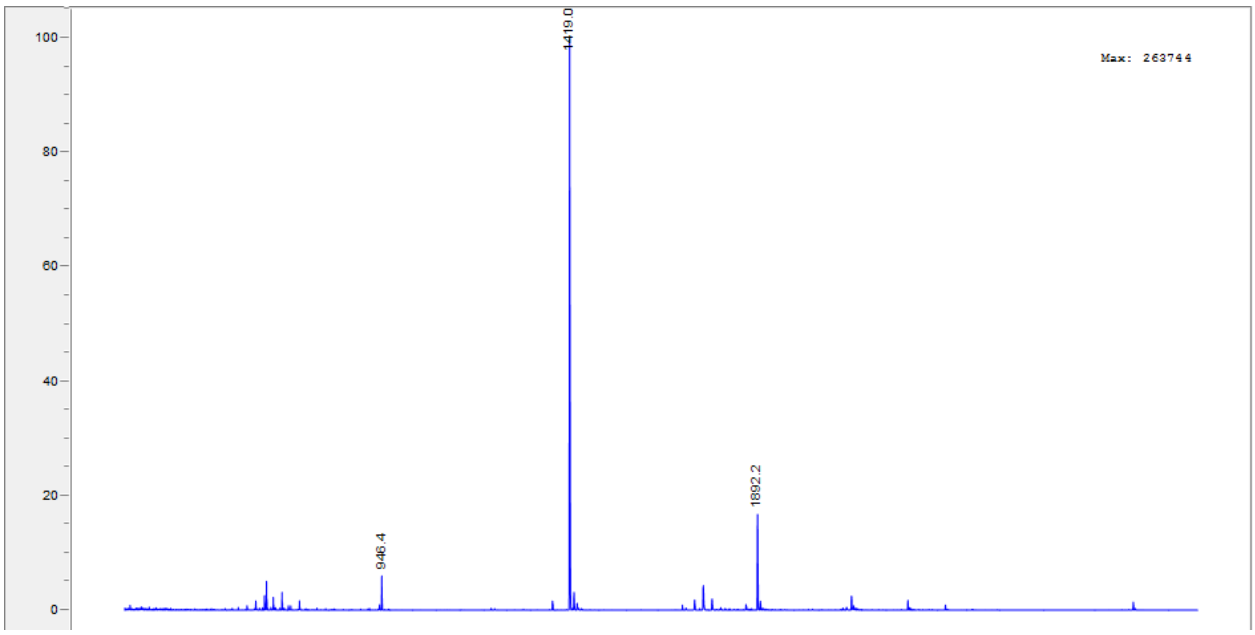
(b) sSAH-ACE2-1, MW = 2910 g/mol.



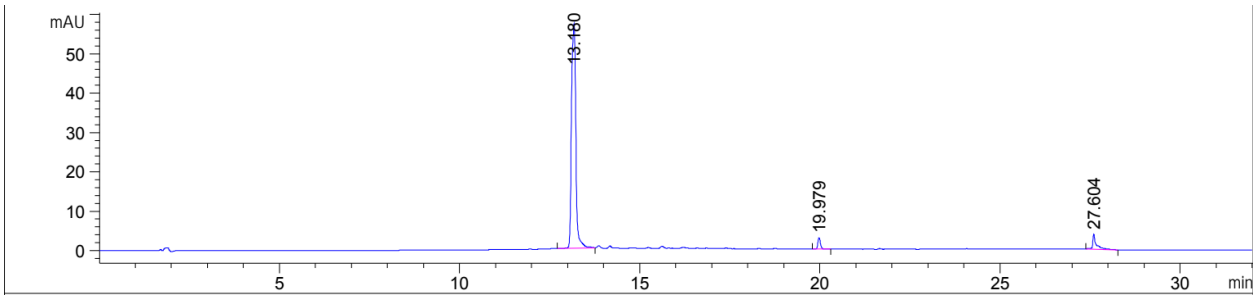


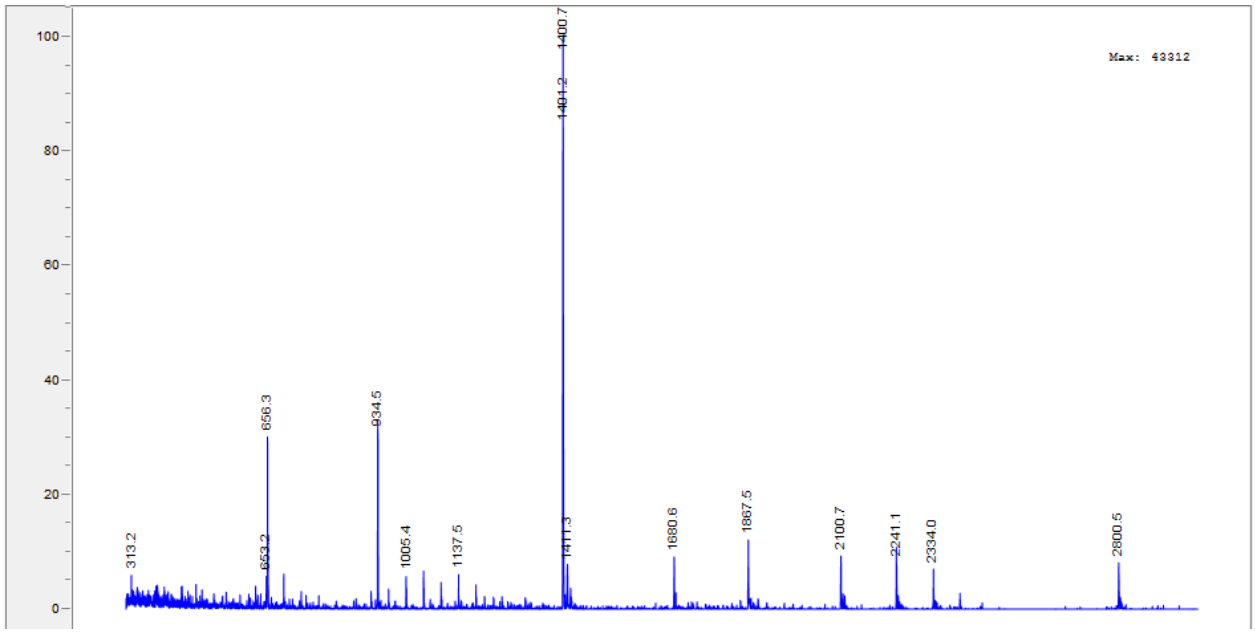
(c) sSAH-ACE2-2, MW = 2837 g/mol.



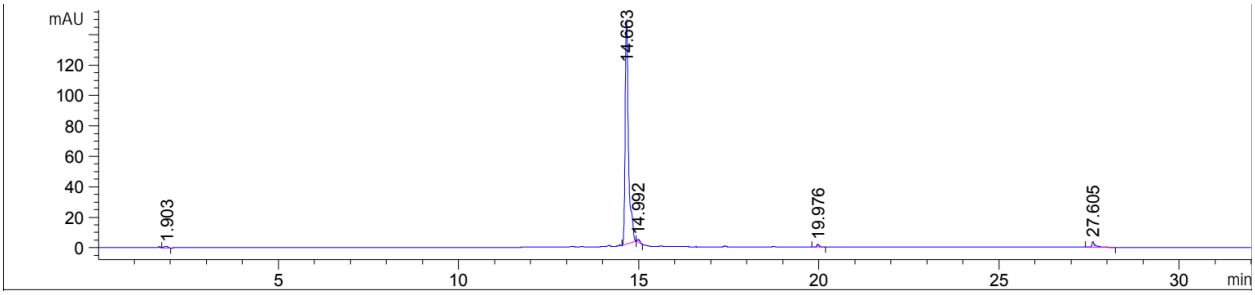


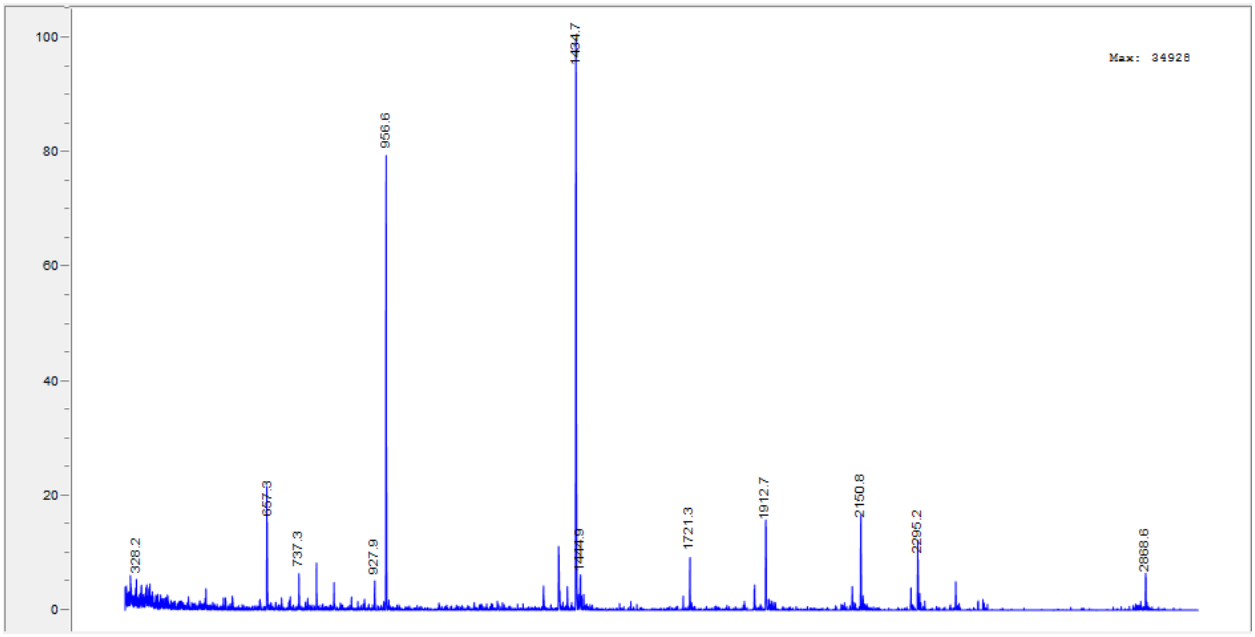
(d) sSAH-ACE2-3, MW = 2800 g/mol.



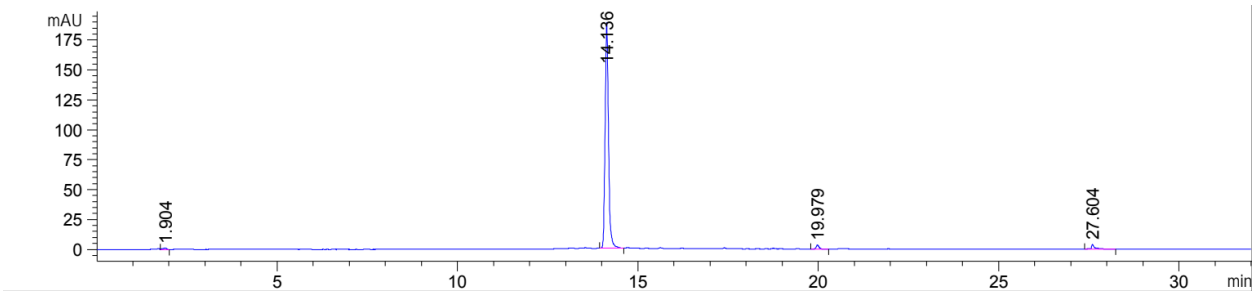


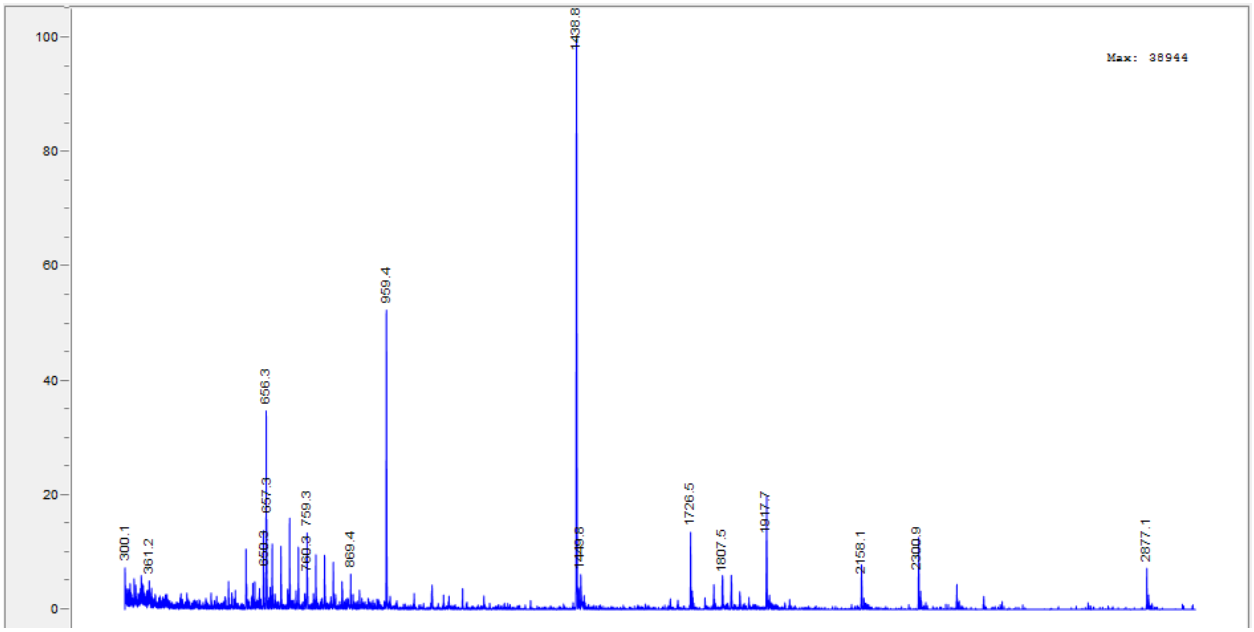
(e) sSAH-ACE2-4, MW = 2867 g/mol.



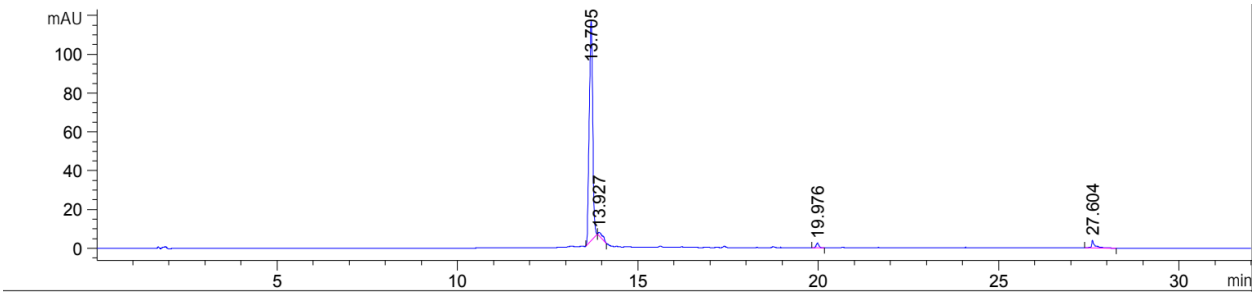


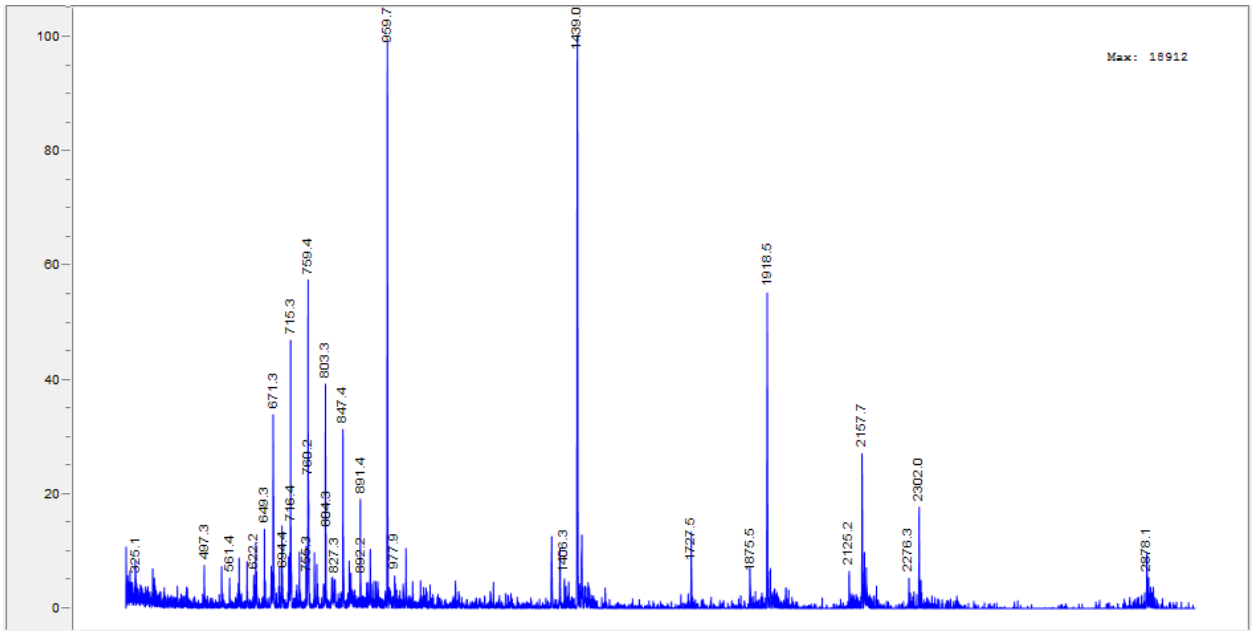
(f) sSAH-ACE2-5, MW = 2876 g/mol.



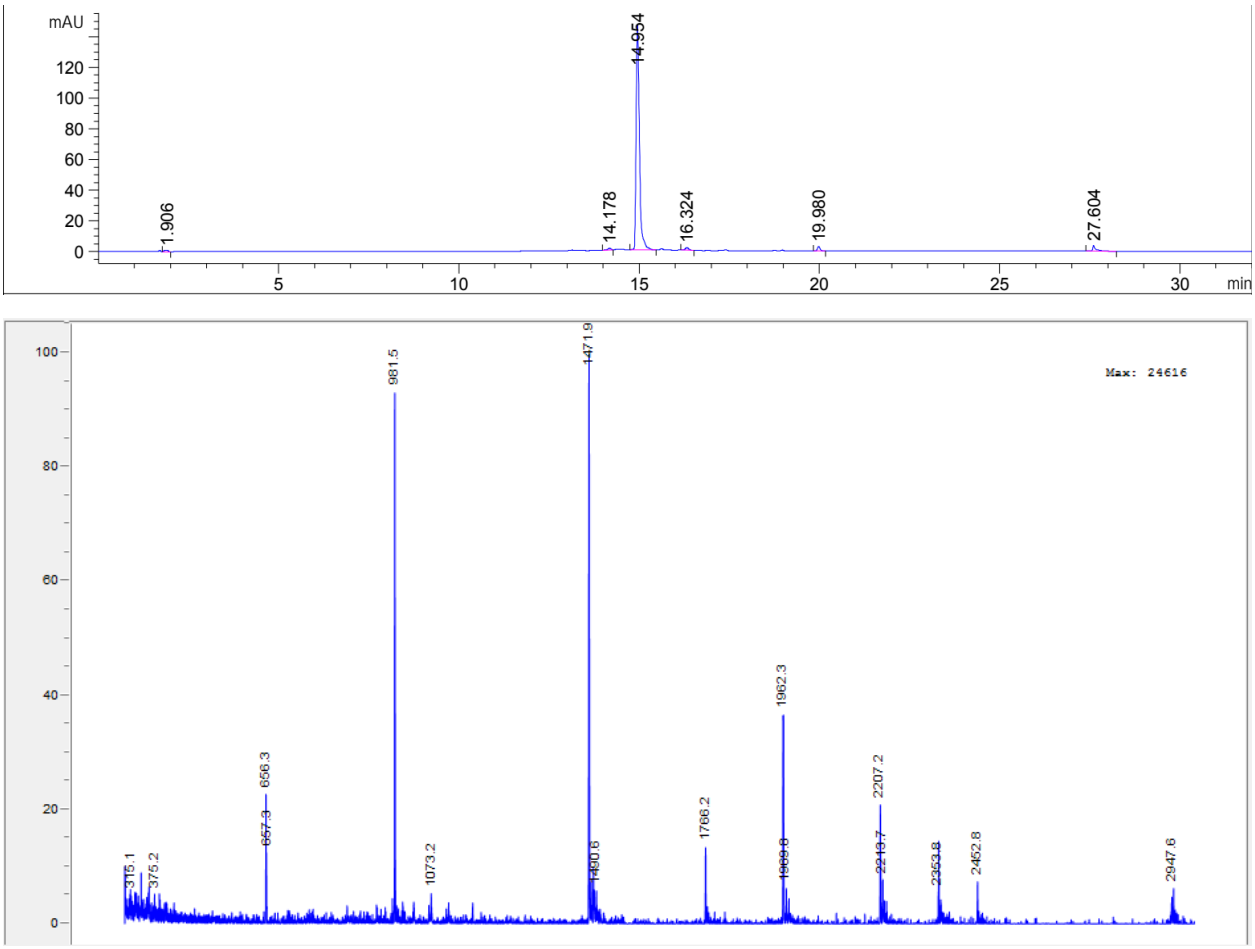


(g) sSAH-ACE2-6, MW = 2876 g/mol.

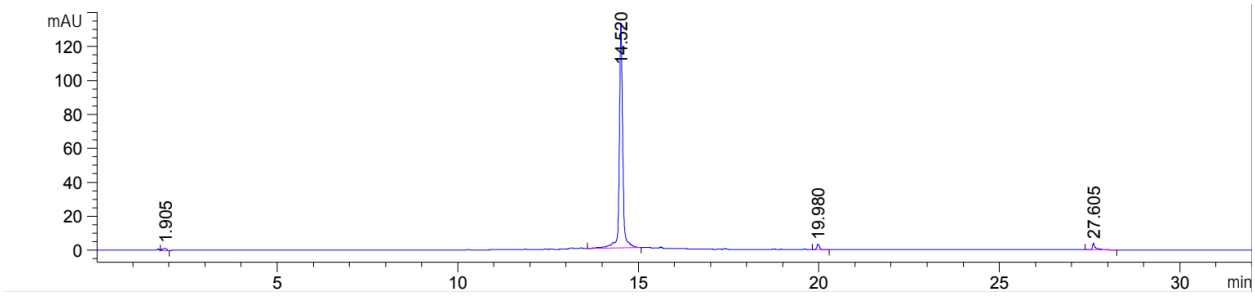


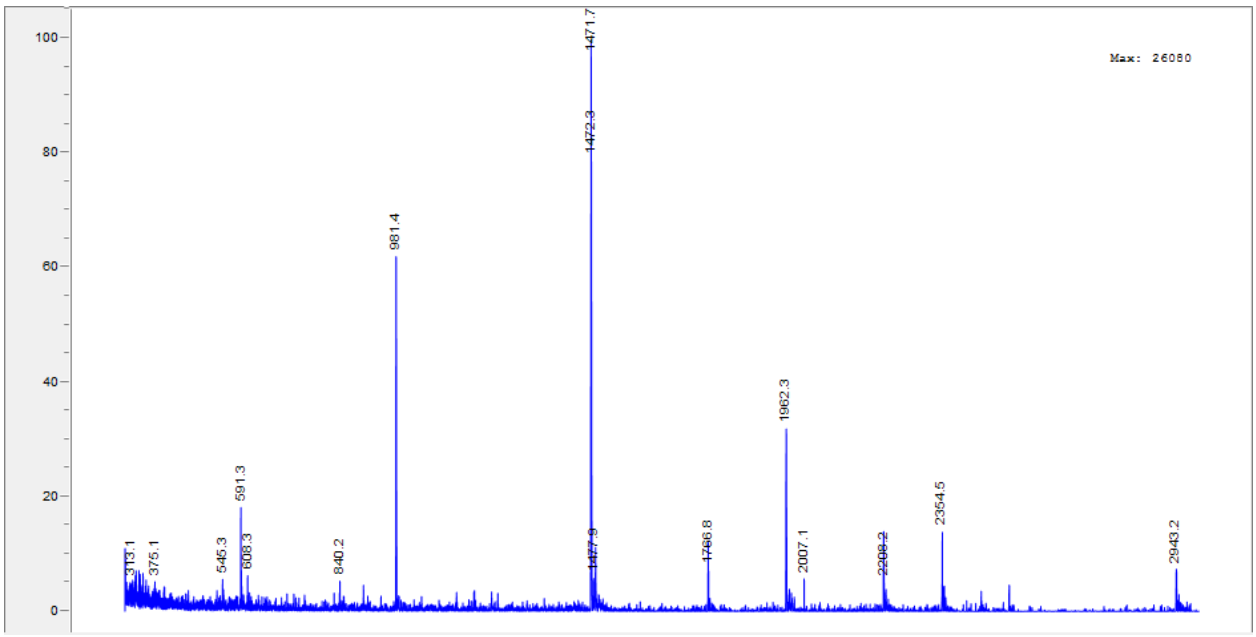


(h) dSAH-ACE2-7, MW = 2942 g/mol.

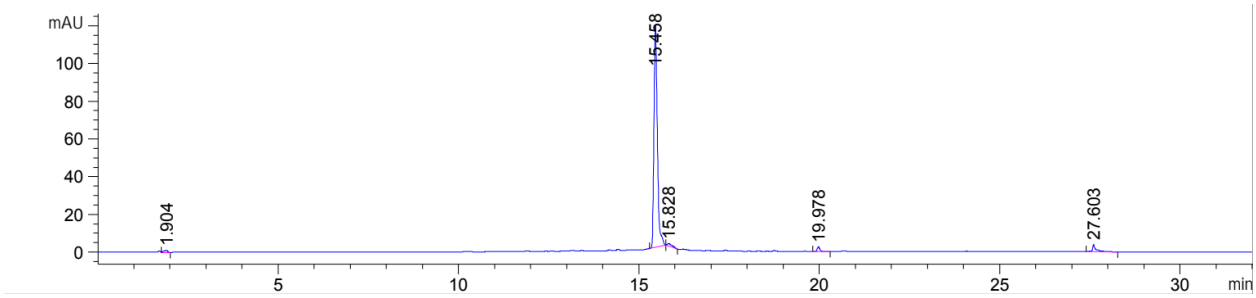


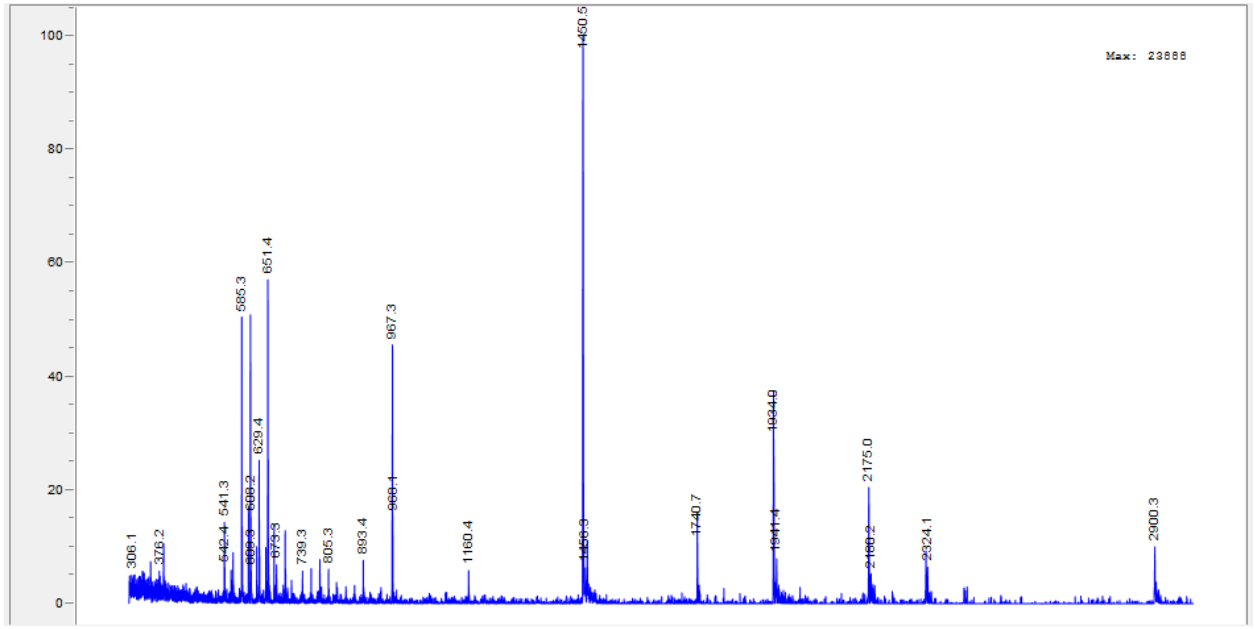
(i) dSAH-ACE2-8, MW = 2942 g/mol.



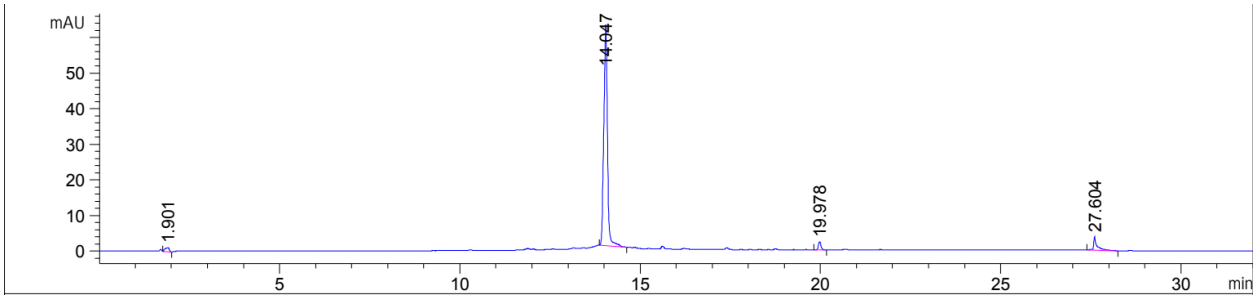


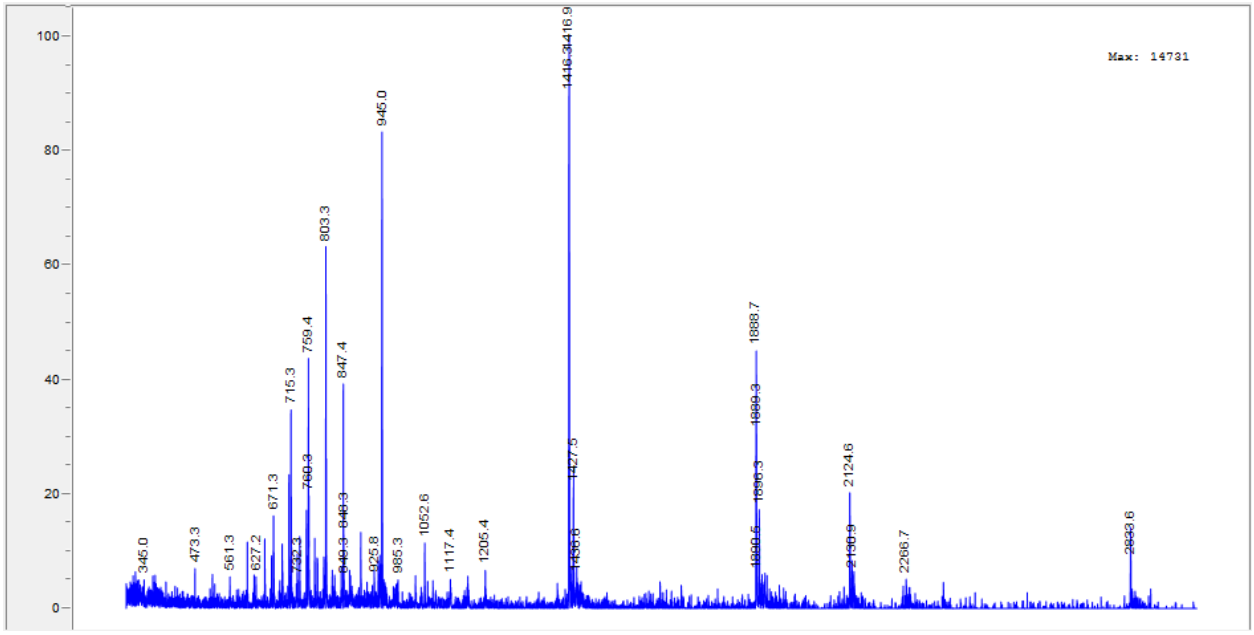
(j) dSAH-ACE2-9, MW = 2899 g/mol.



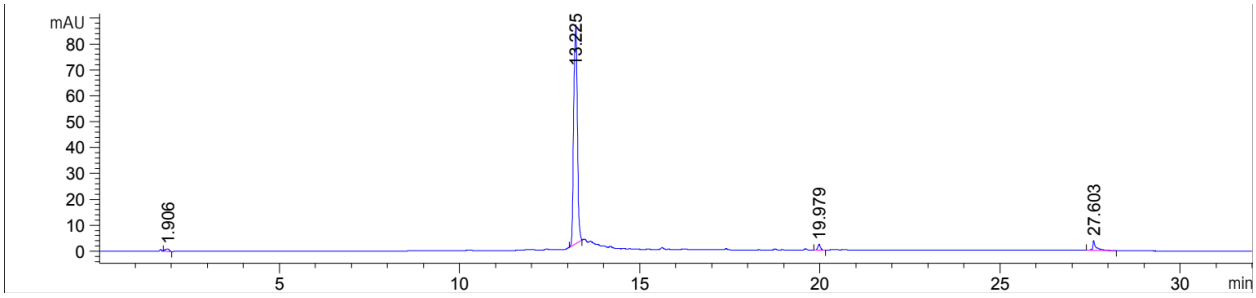


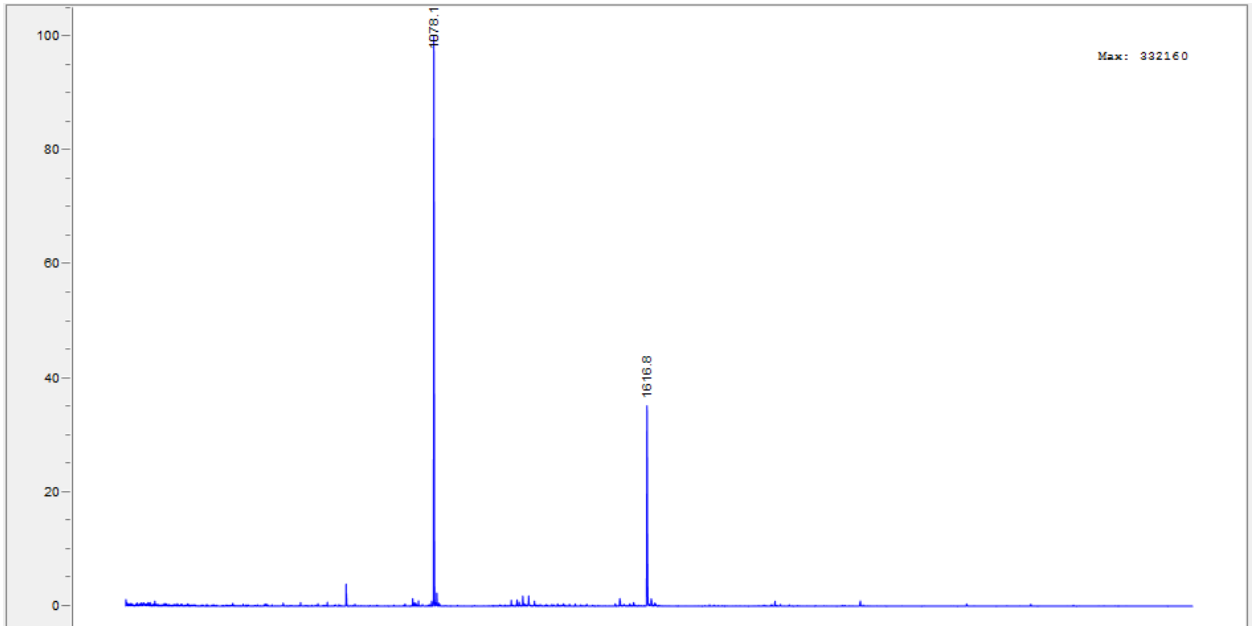
(k) dSAH-ACE2-10, MW = 2832 g/mol.



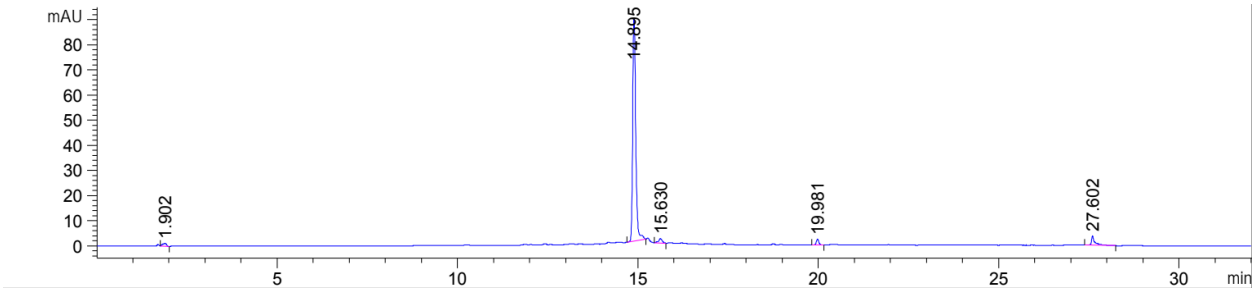


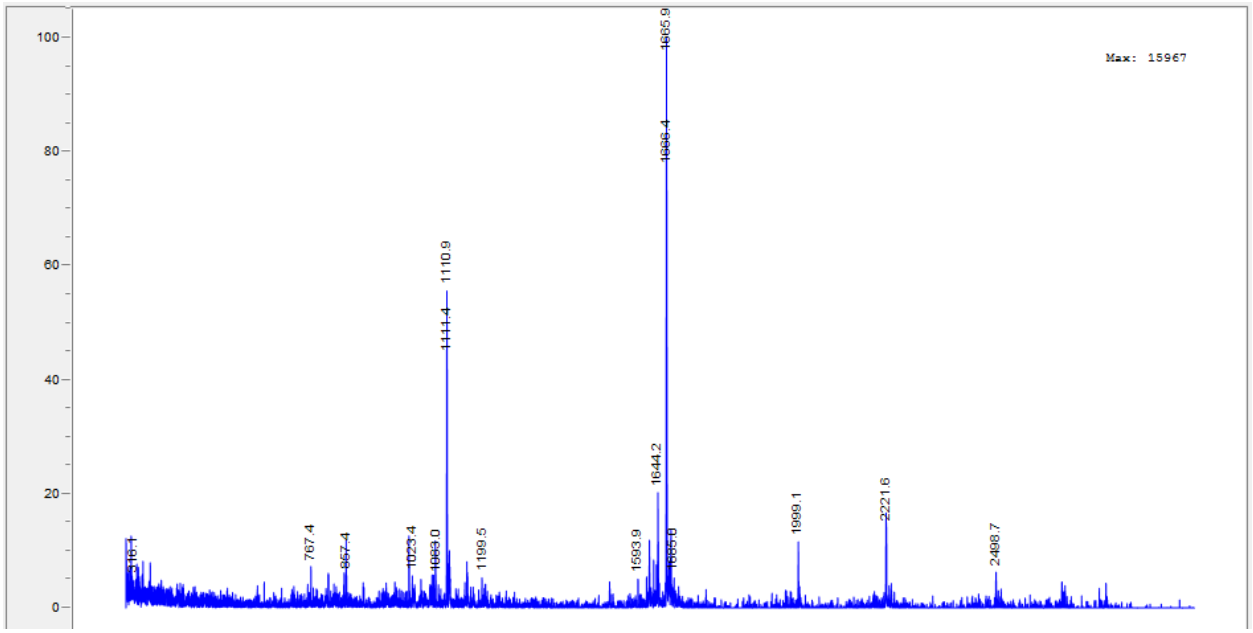
(l) ACE2(19-45), MW = 3232 g/mol.



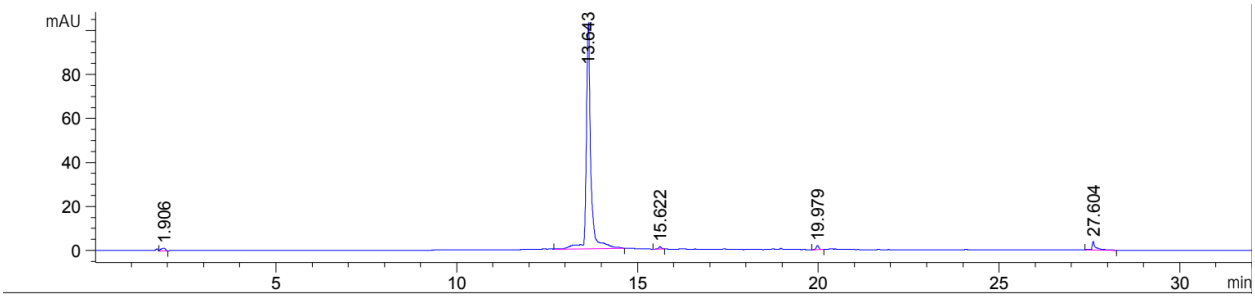


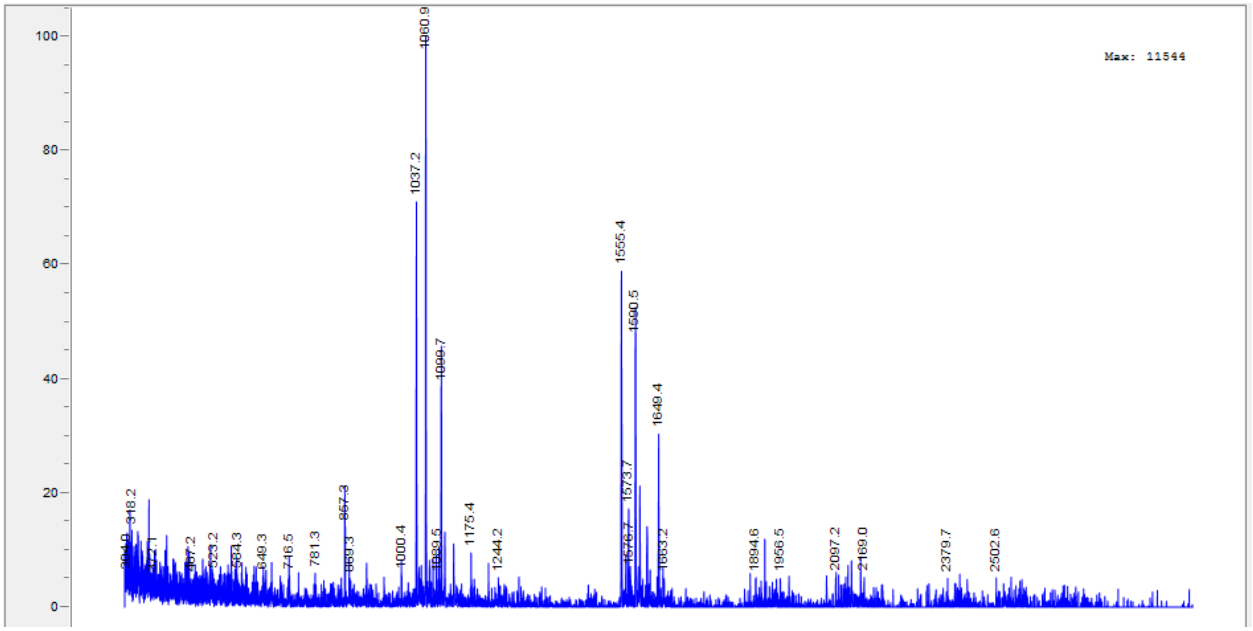
(m) dSAH-ACE2-11, MW = 3330 g/mol.



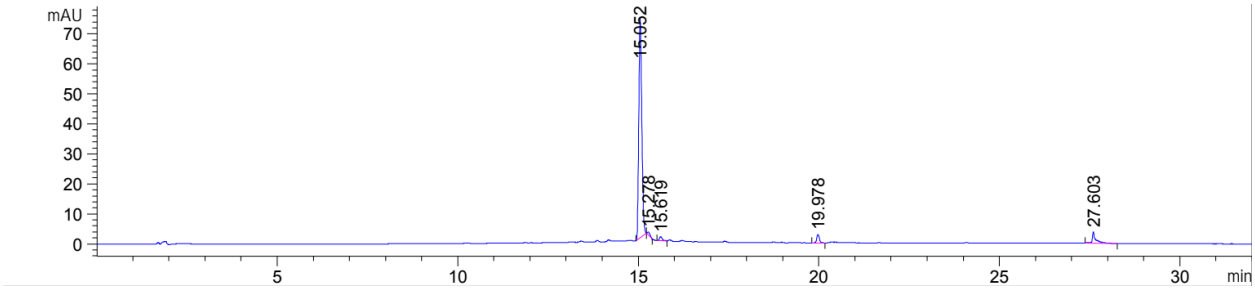


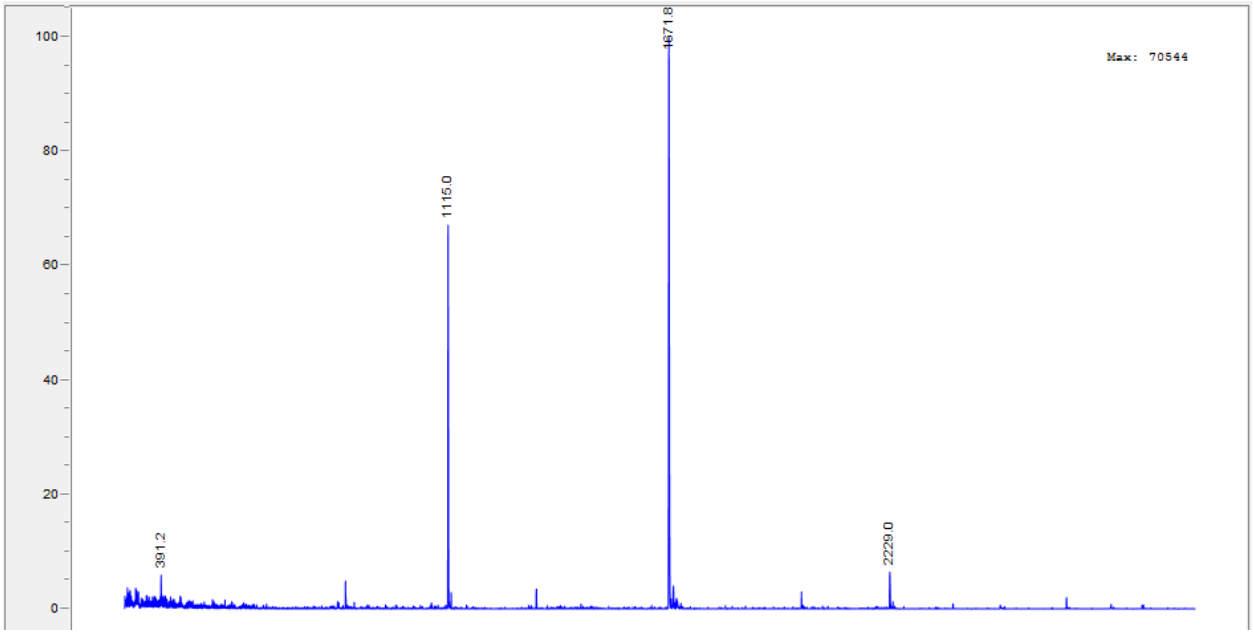
(n) dSAH-ACE2-12, MW = 3297 g/mol.



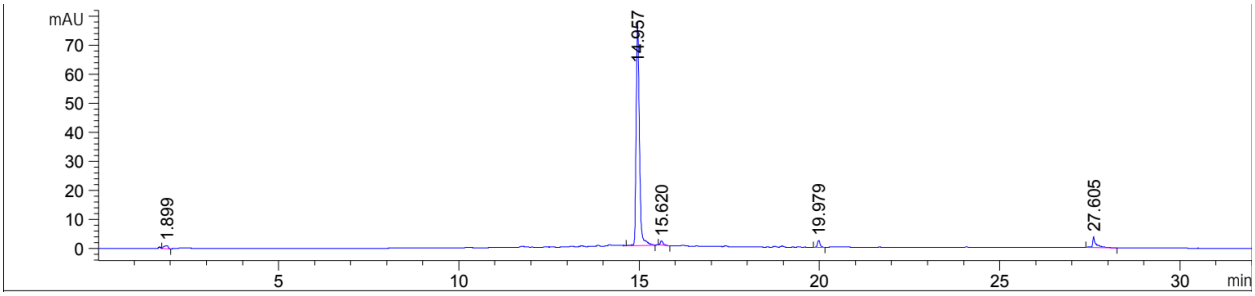


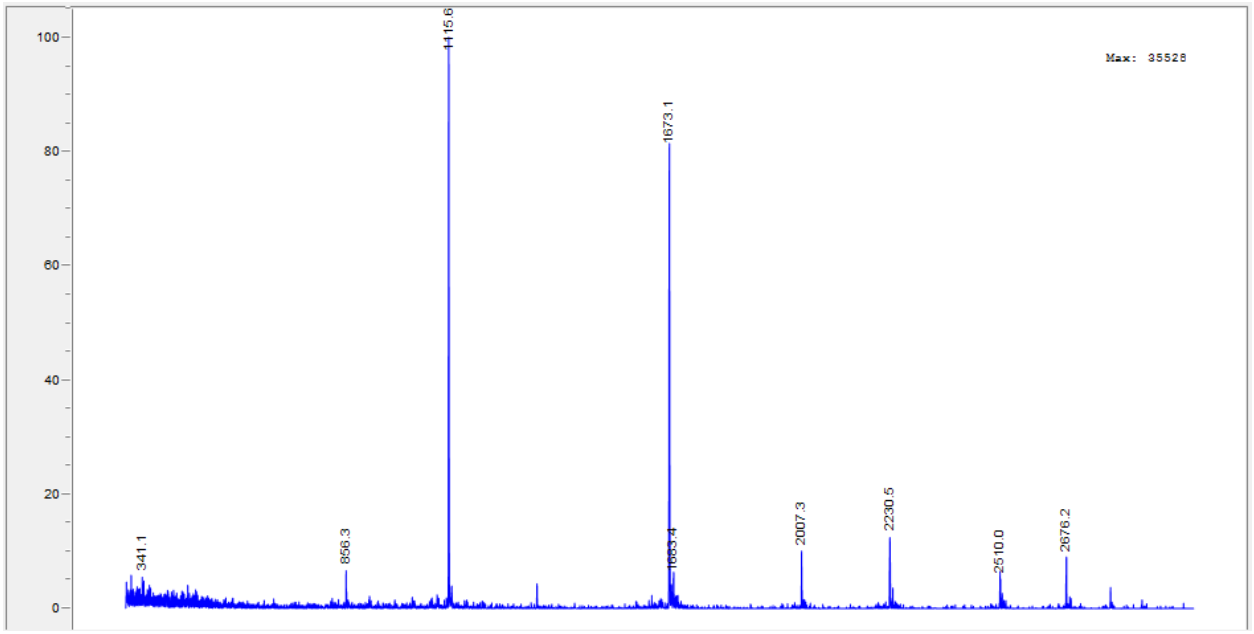
(o) dSAH-ACE2-13, MW = 3342 g/mol.



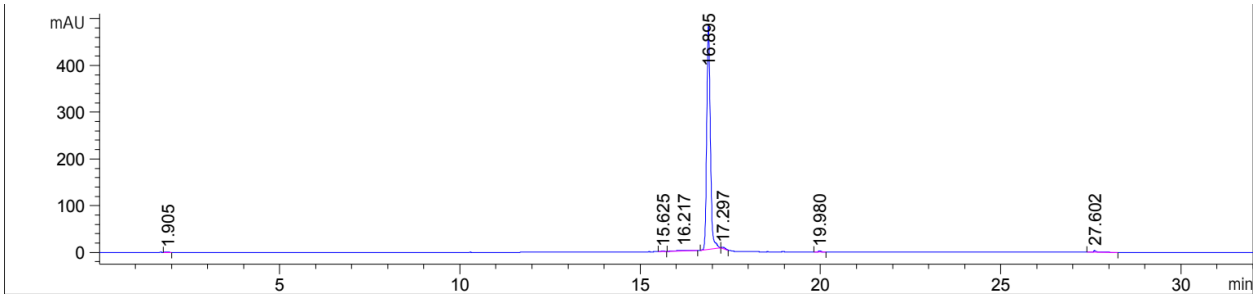


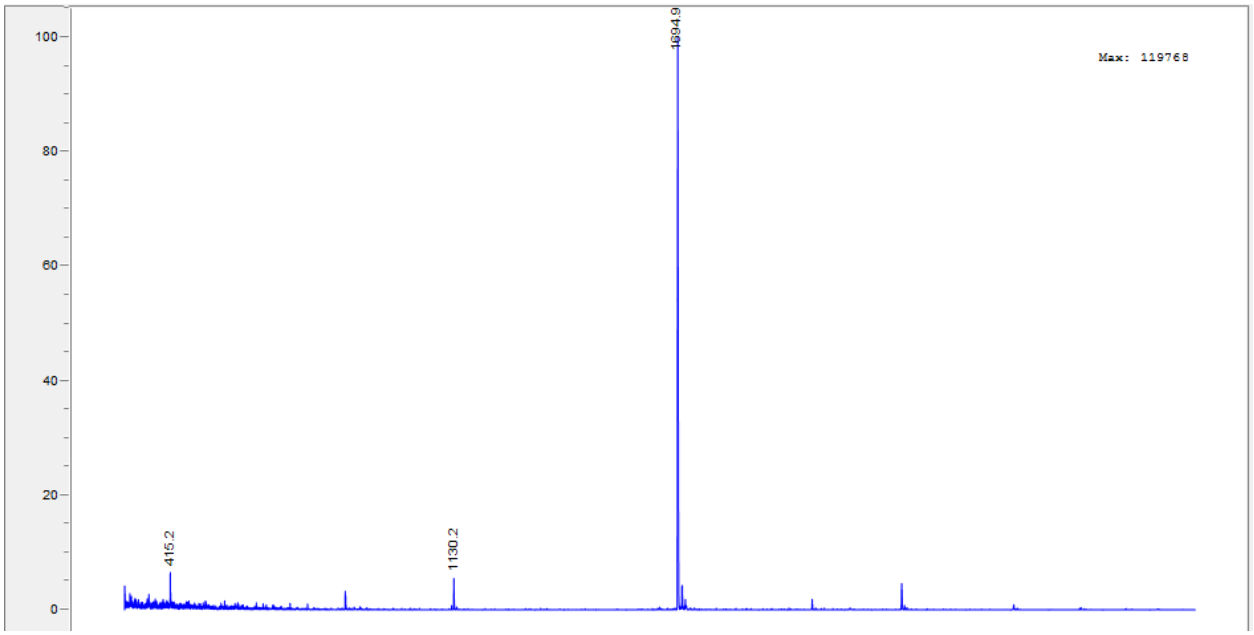
(p) dSAH-ACE2-14, MW = 3344 g/mol.



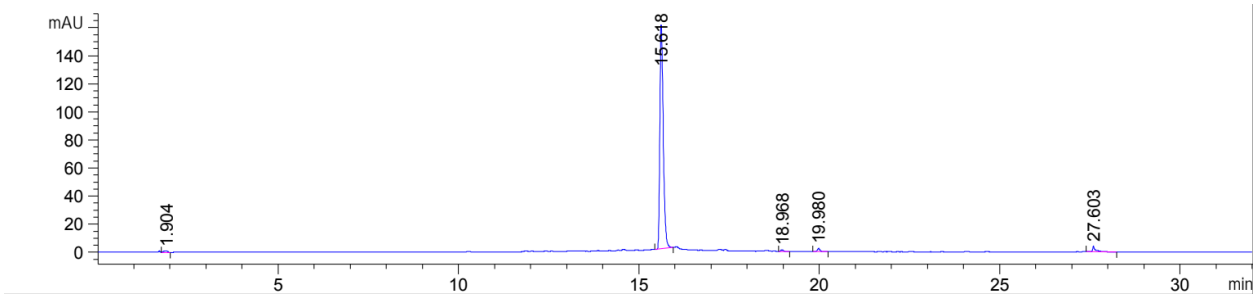


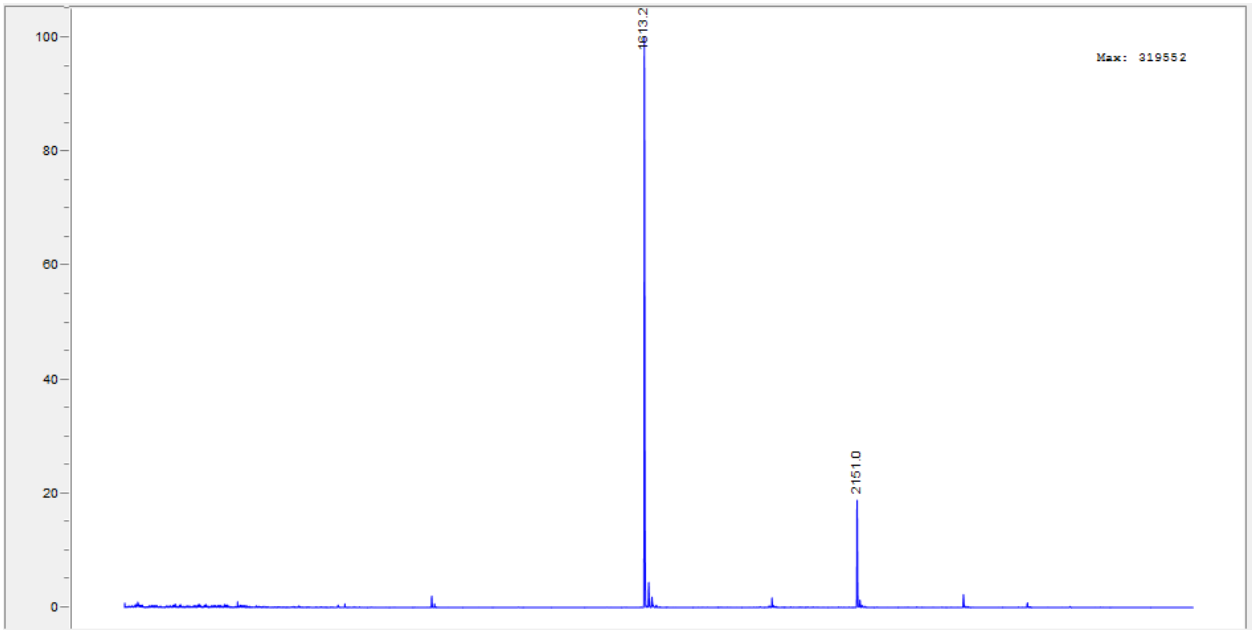
(q) dSAH-ACE2-15, MW = 3388 g/mol.



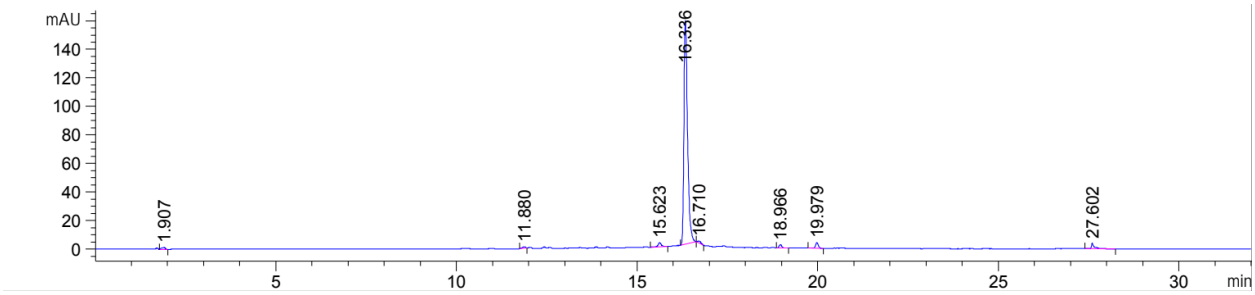


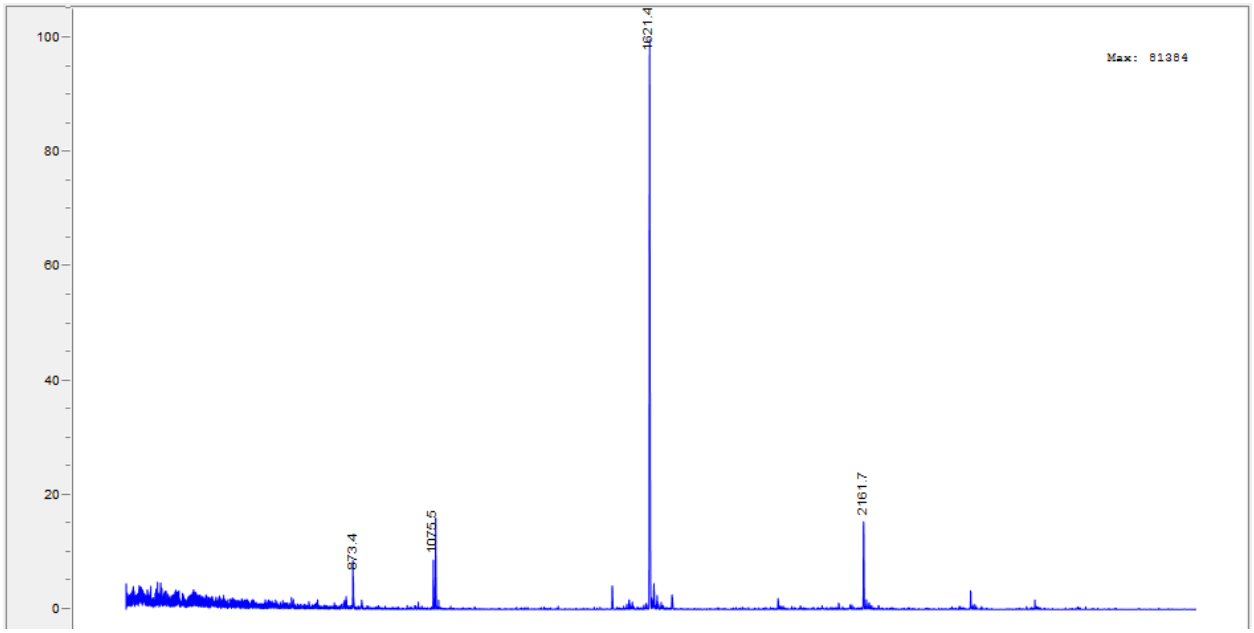
(r) sSAH-ACE2-16, MW = 3225 g/mol.



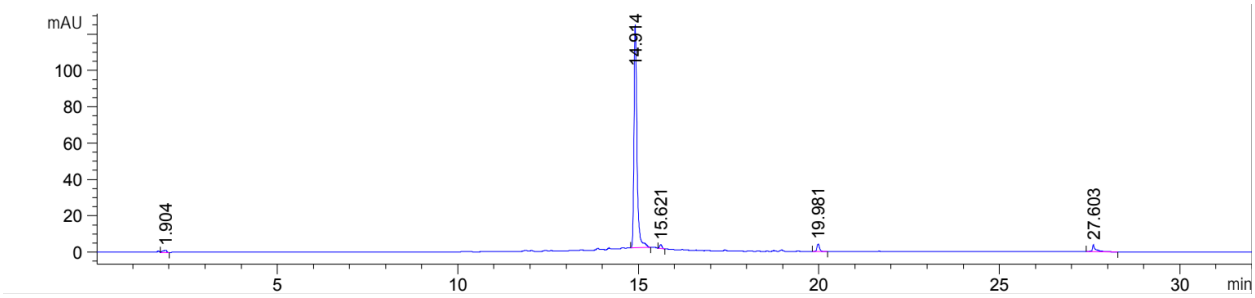


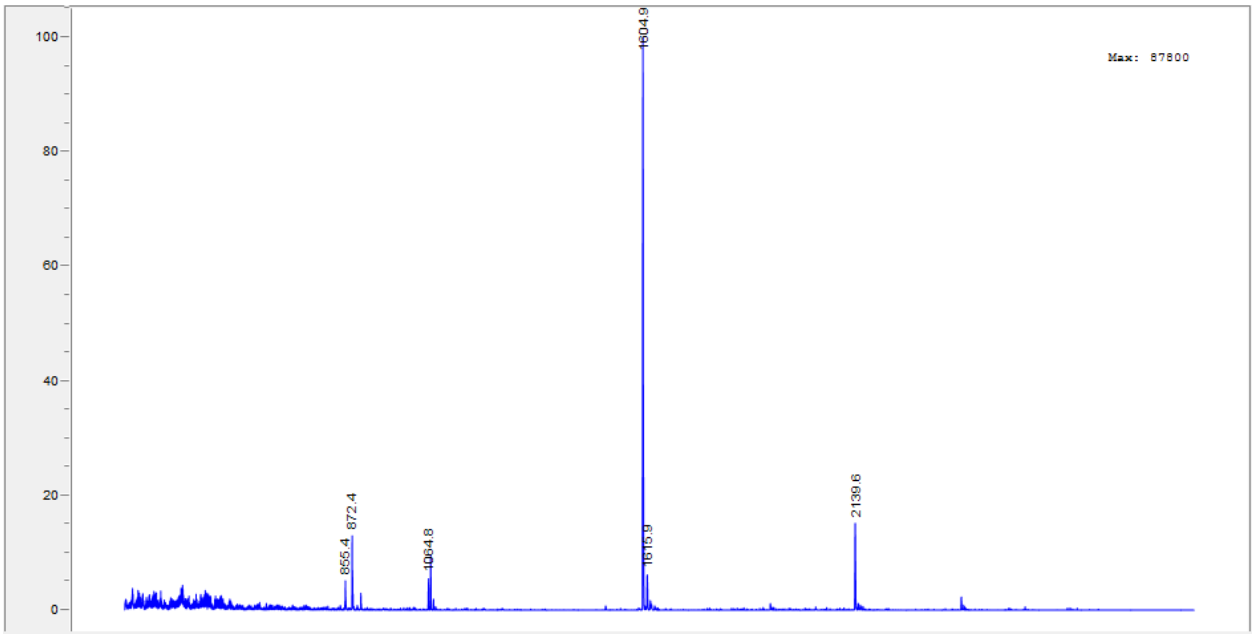
(s) dSAH-ACE2-17, MW = 3241 g/mol.



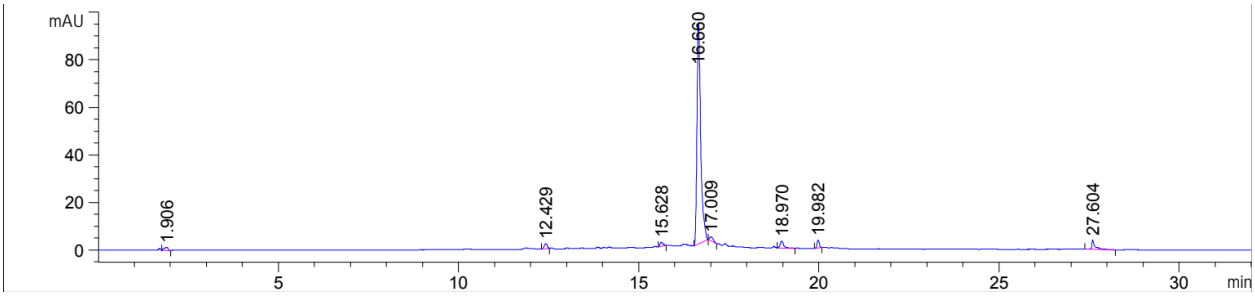


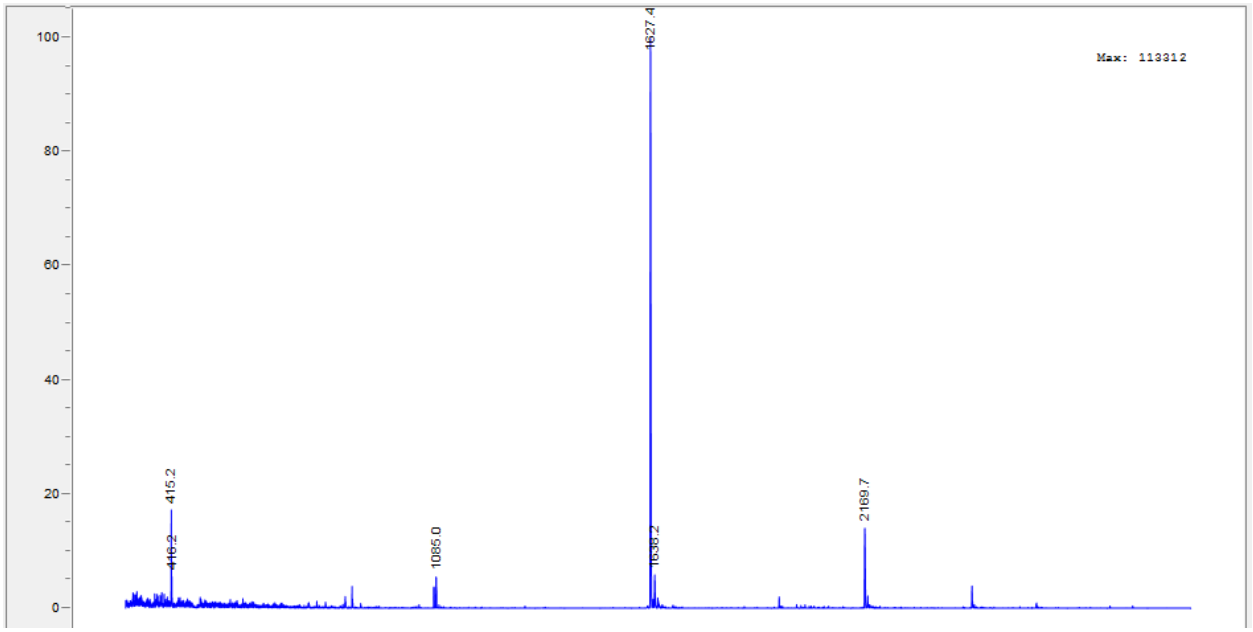
(t) dSAH-ACE2-18, MW = 3208 g/mol.



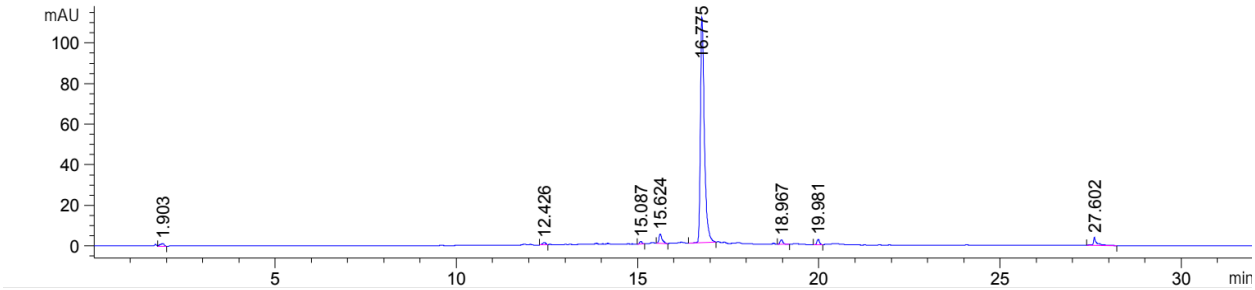


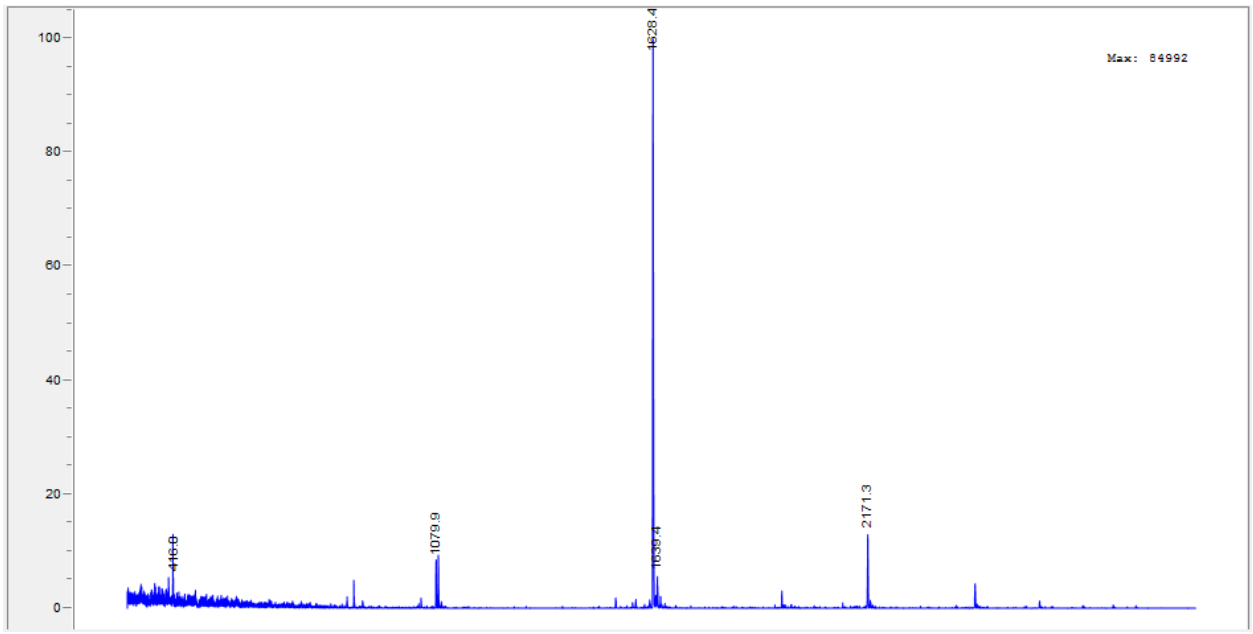
(u) dSAH-ACE2-19, MW = 3253 g/mol.





(v) dSAH-ACE2-20, MW = 3255 g/mol.





(w) dSAH-ACE2-21, MW = 3299 g/mol.

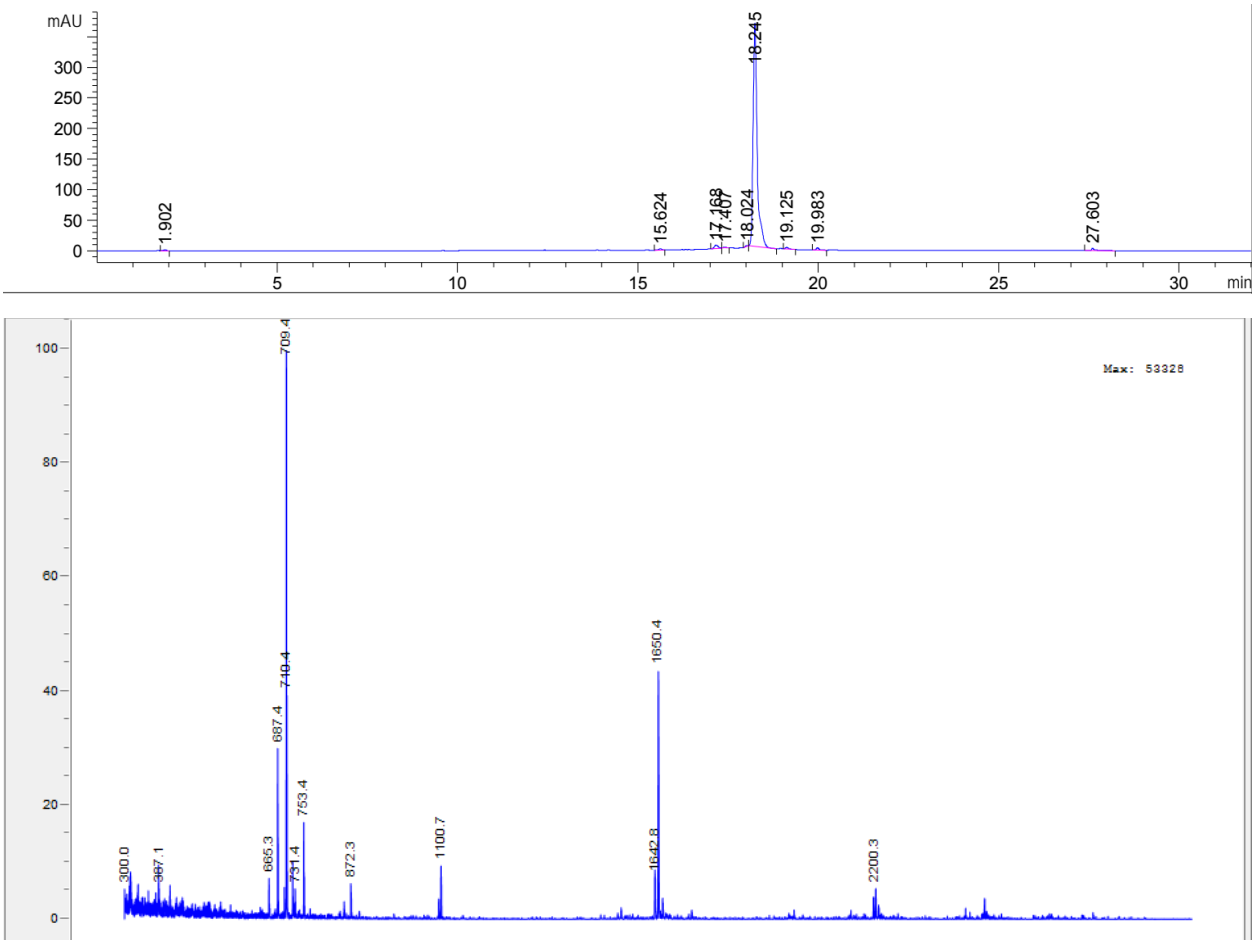
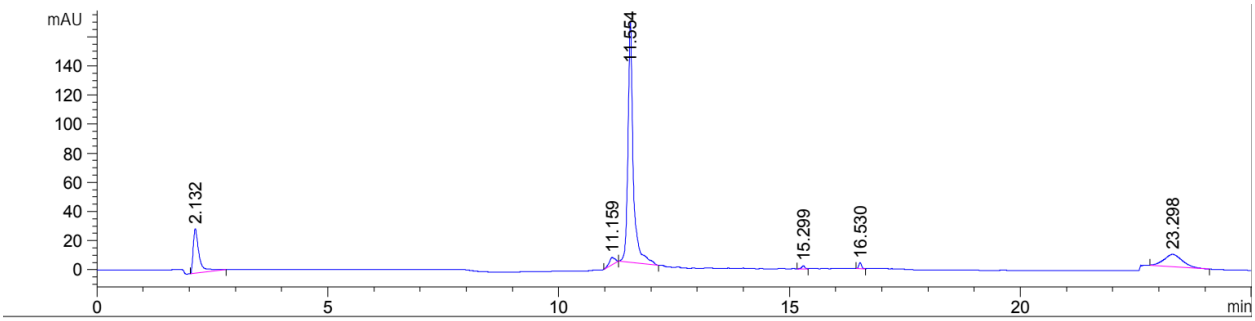
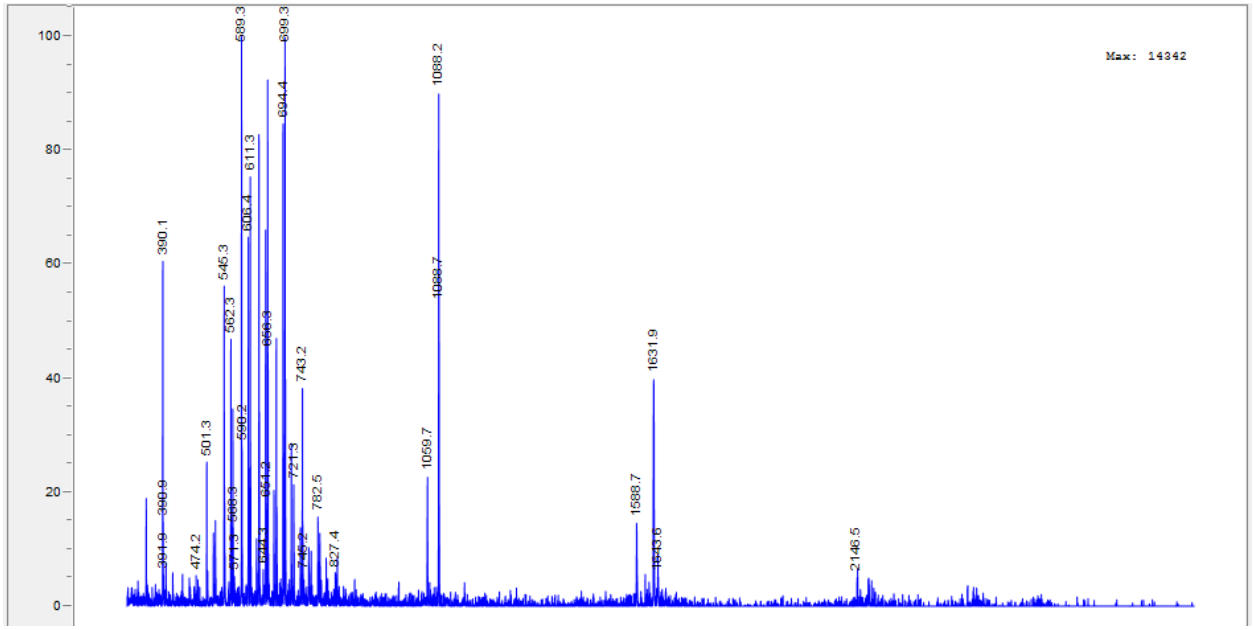


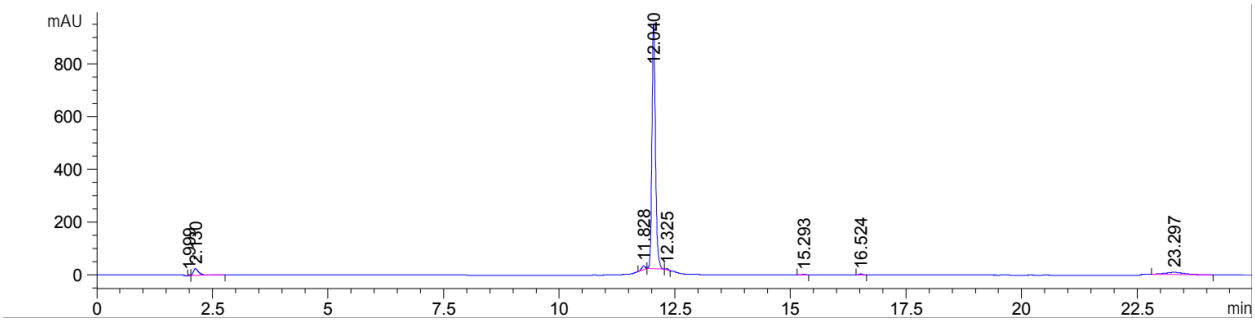
Figure S1. LC-UV280nm trace and corresponding ESI mass spectrum of acetylated hydrocarbon-stapled and unstapled peptides.

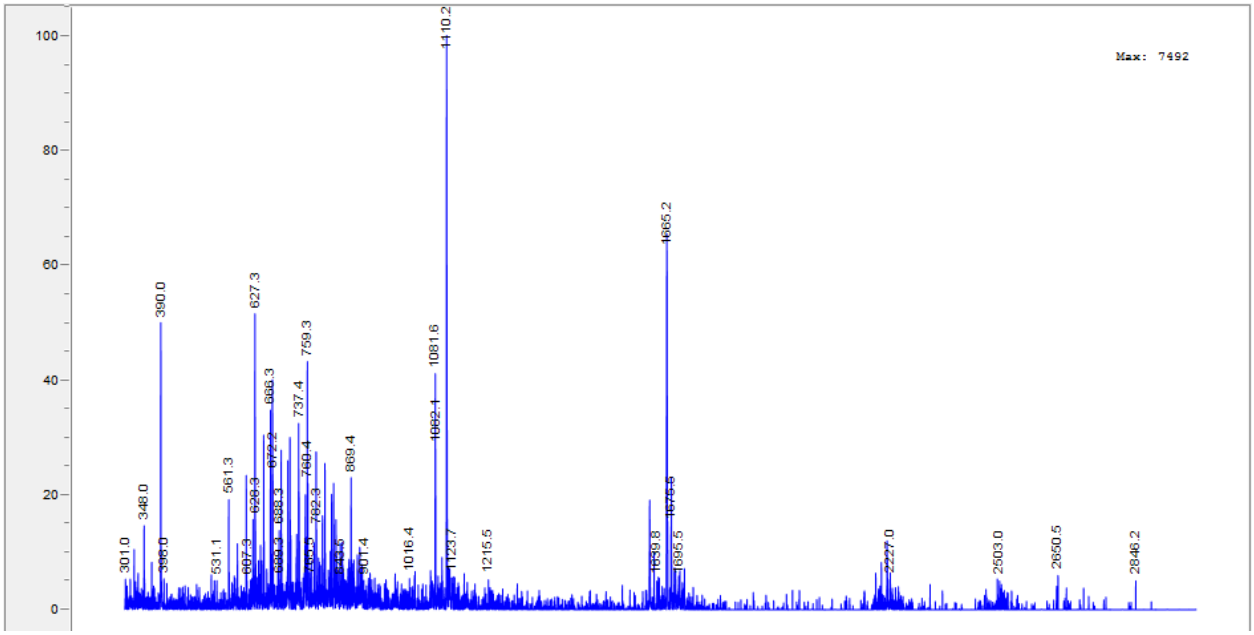
(a) ACE2(21-43), MW = 3262 g/mol.



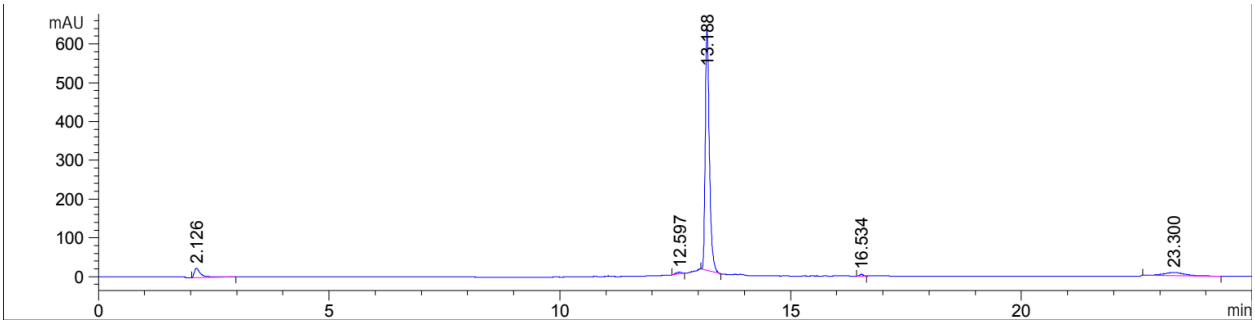


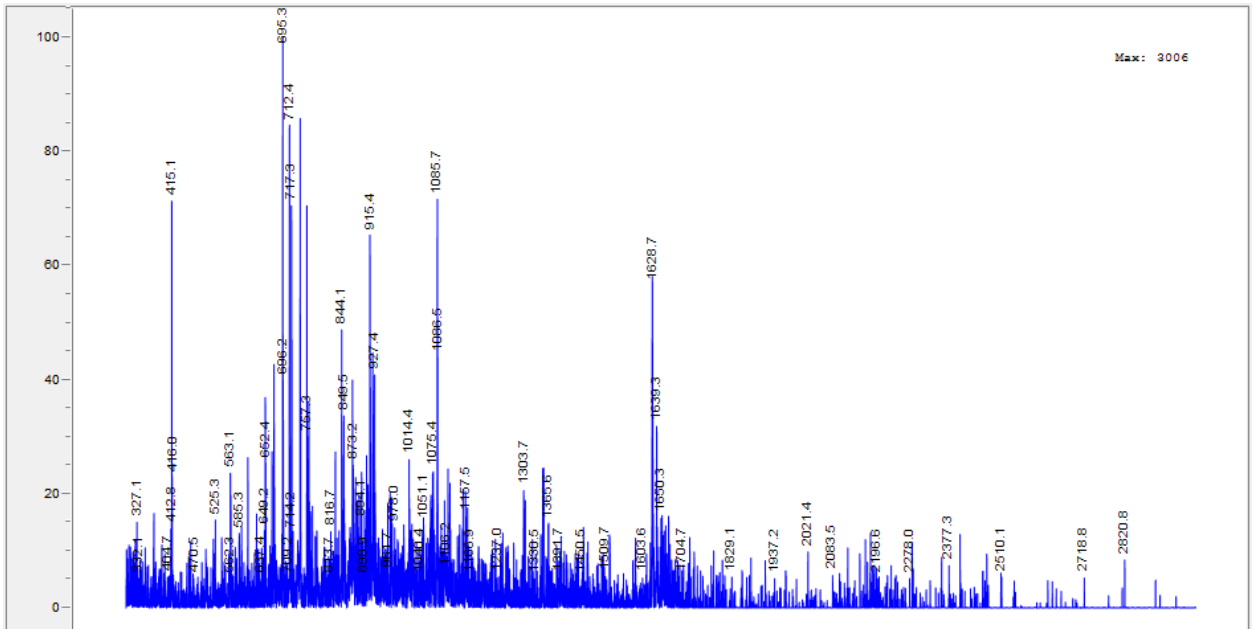
(b) sSAH-ACE2-1, MW = 3328 g/mol.



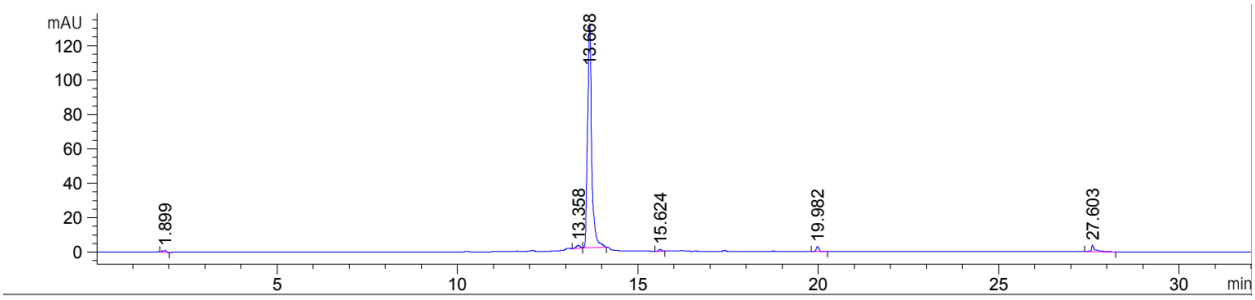


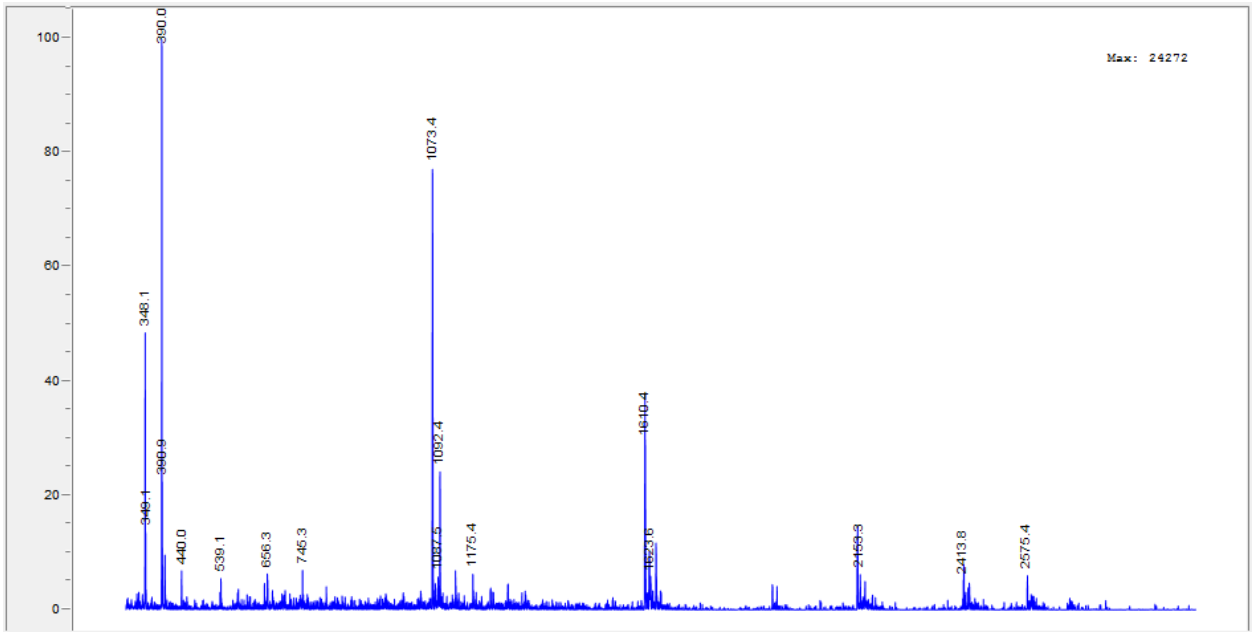
(c) sSAH-ACE2-2, MW = 3255 g/mol.



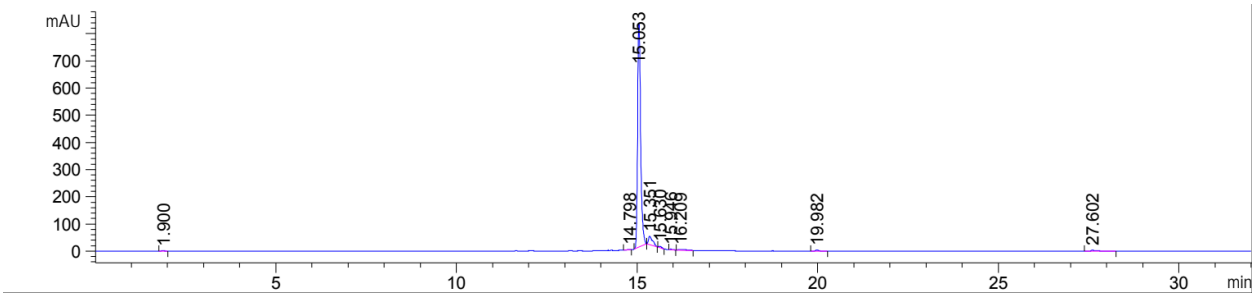


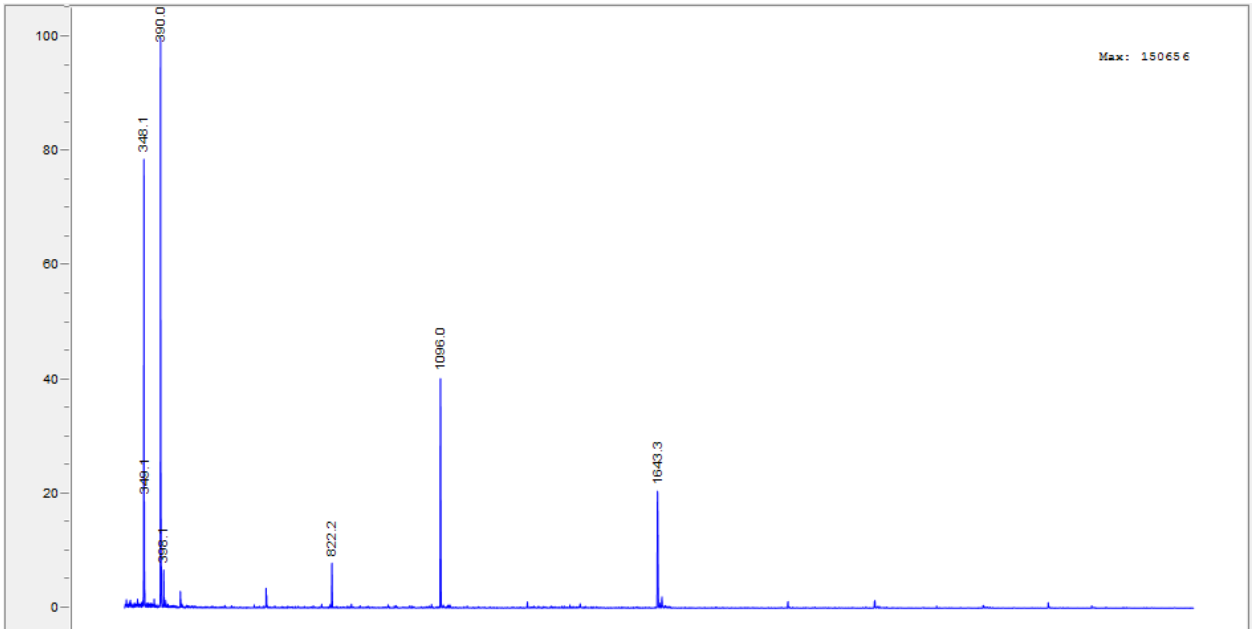
(d) sSAH-ACE2-3, MW = 3218 g/mol.



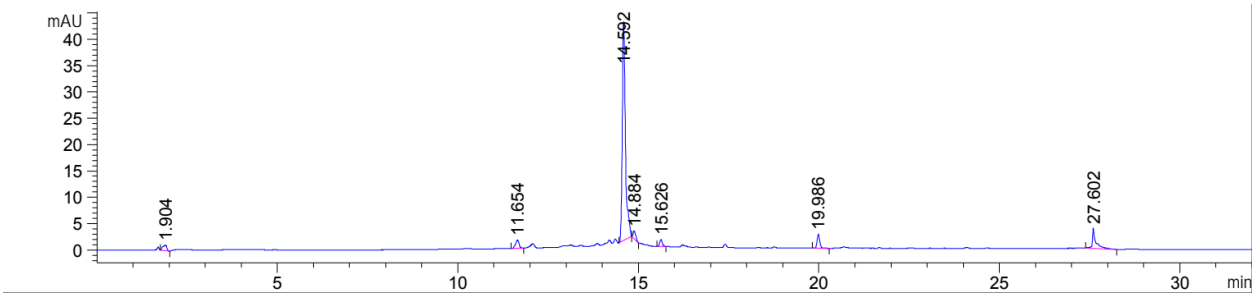


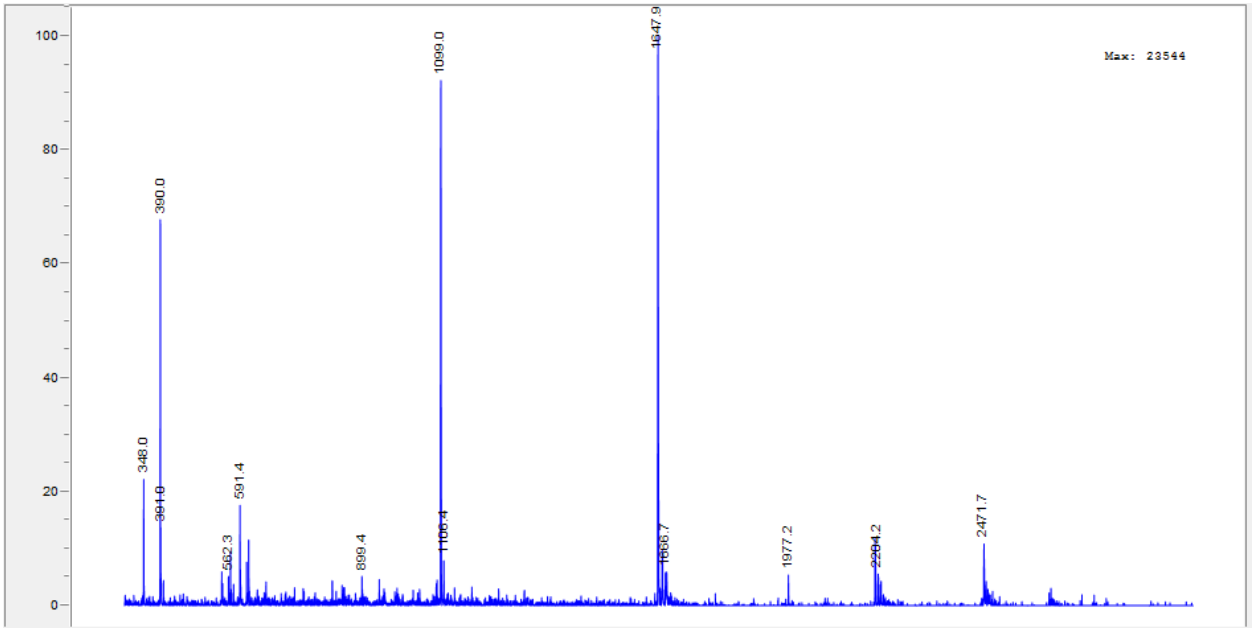
(e) sSAH-ACE2-4, MW = 3285 g/mol.



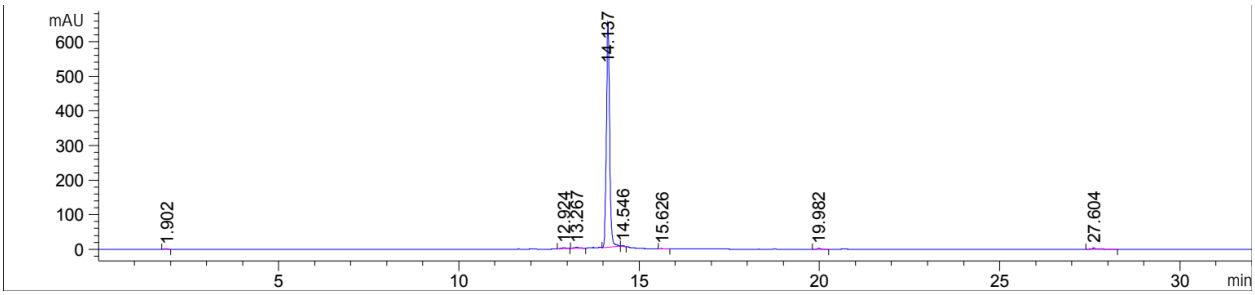


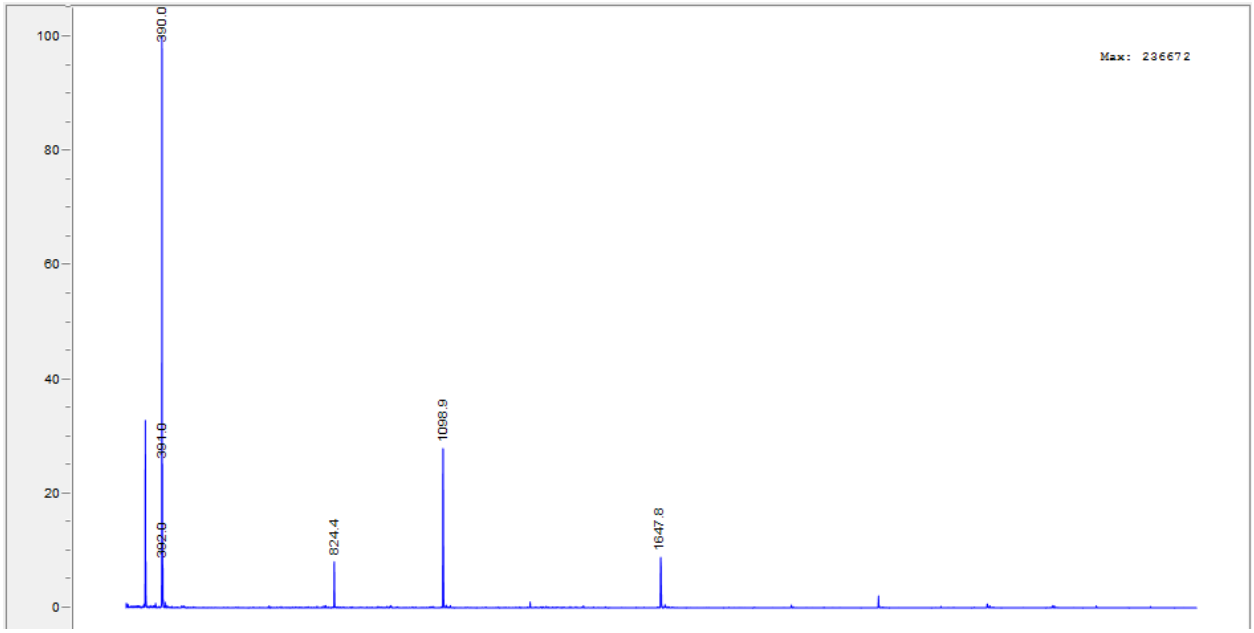
(f) sSAH-ACE2-5, MW = 3294 g/mol.



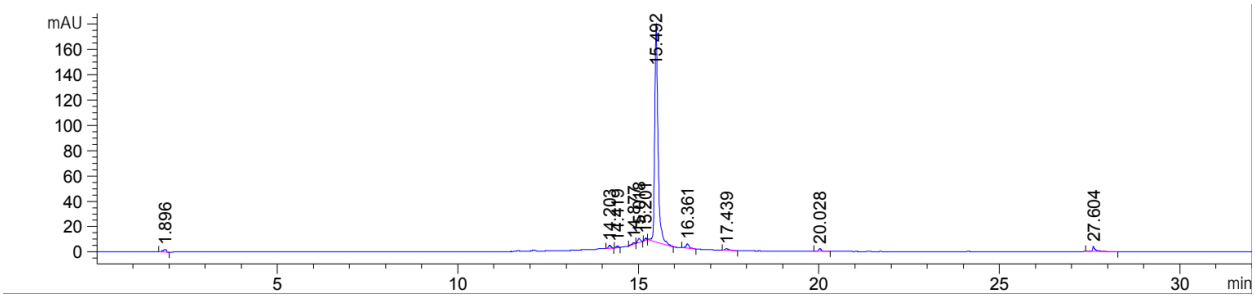


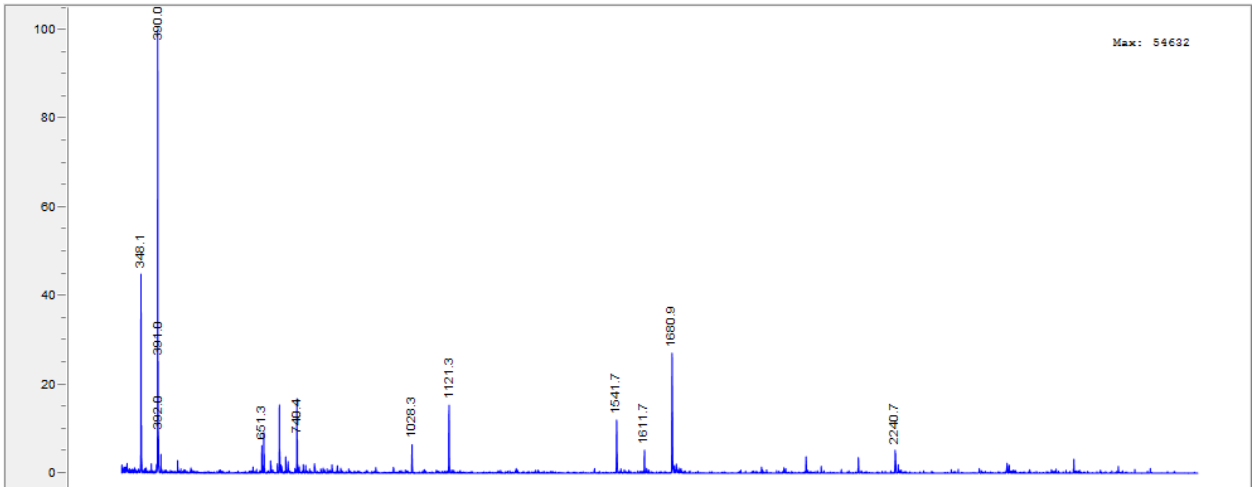
(g) sSAH-ACE2-6, MW = 3294 g/mol.



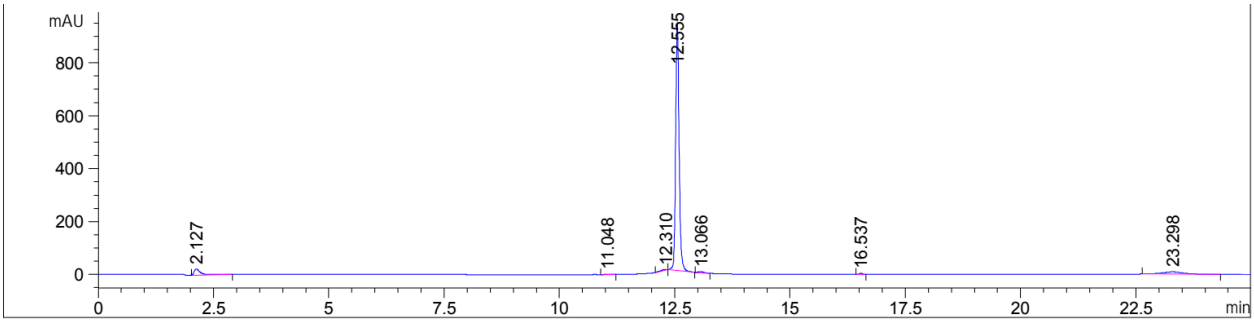


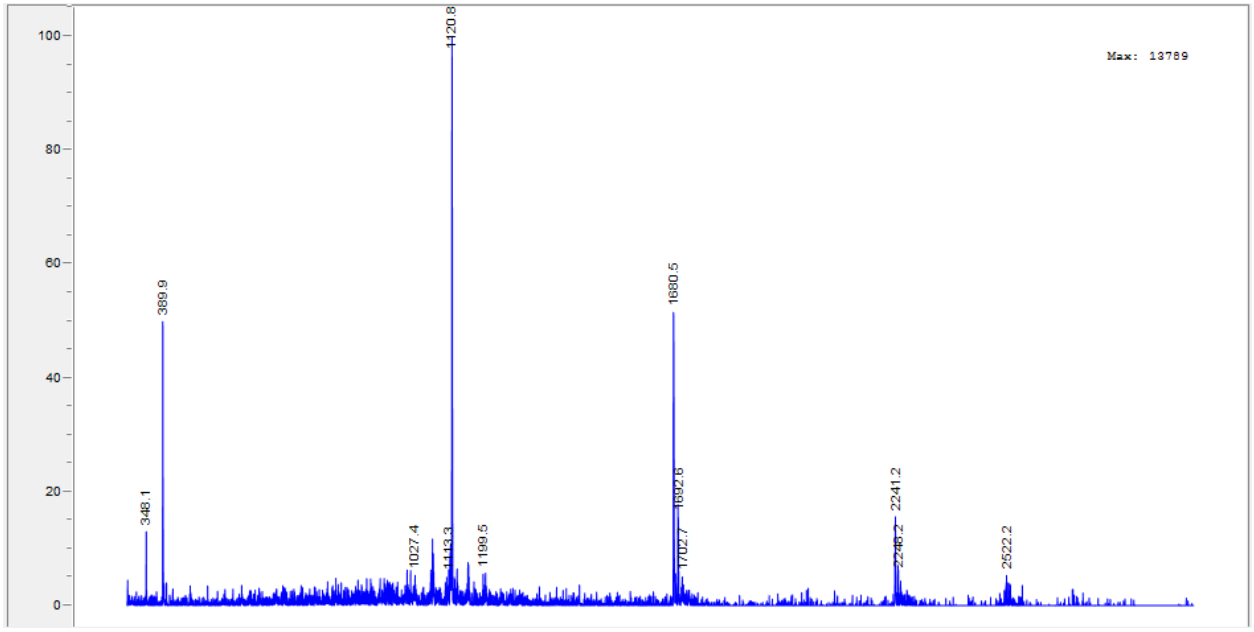
(h) sSAH-ACE2-7, MW = 3360 g/mol.



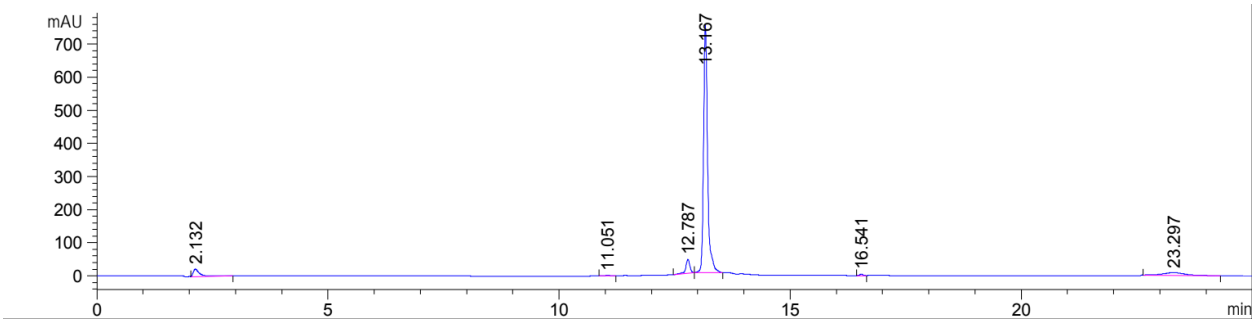


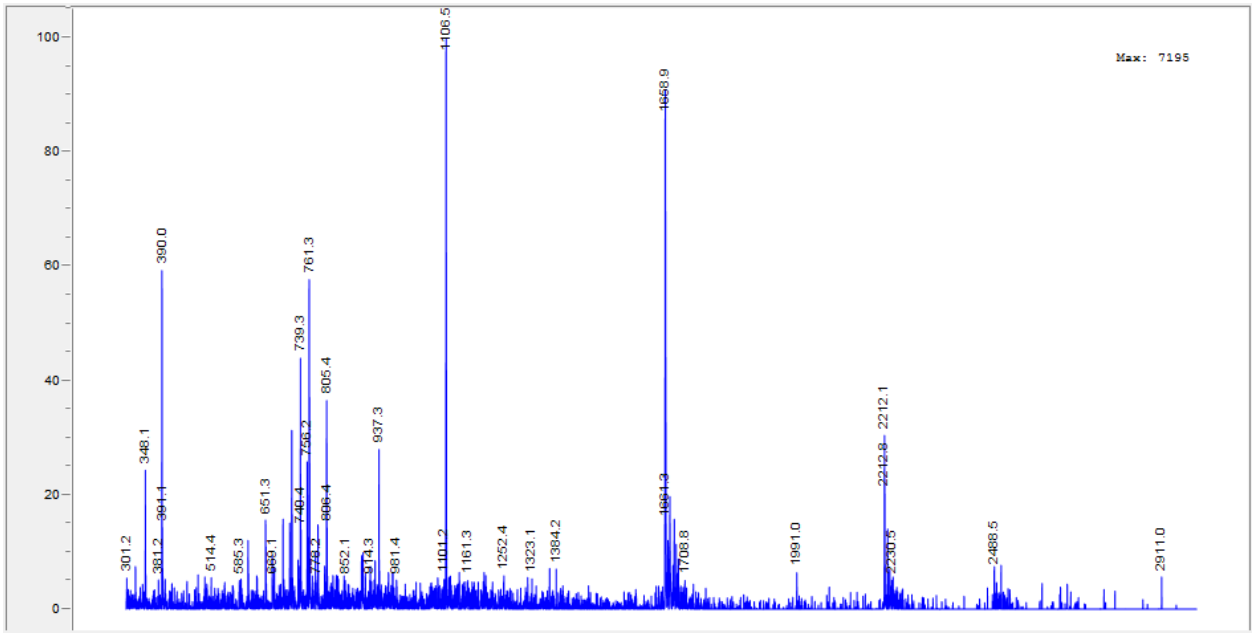
(i) dSAH-ACE2-8, MW = 3360 g/mol.



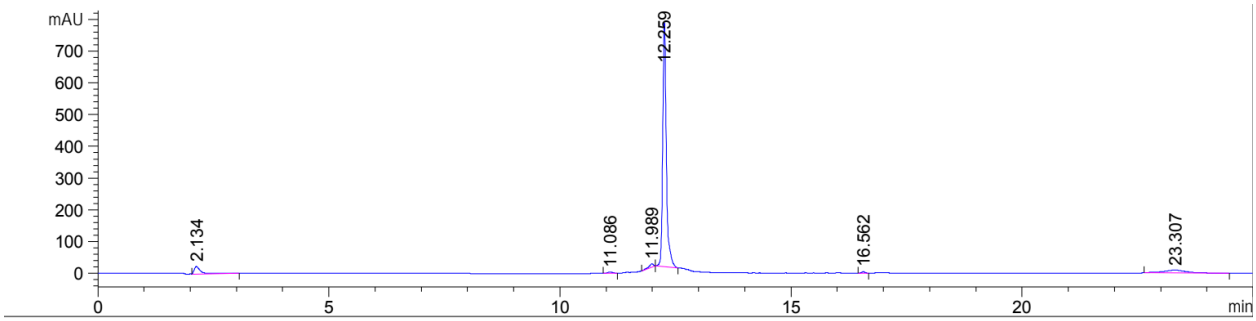


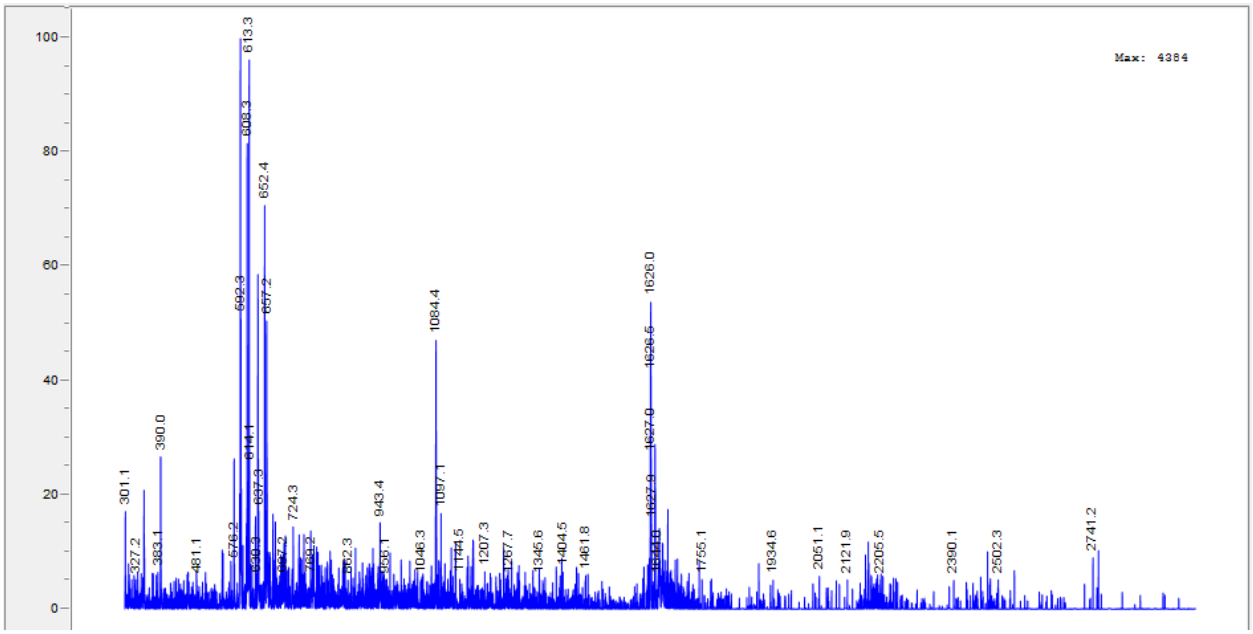
(j) dSAH-ACE2-9, MW = 3317 g/mol.



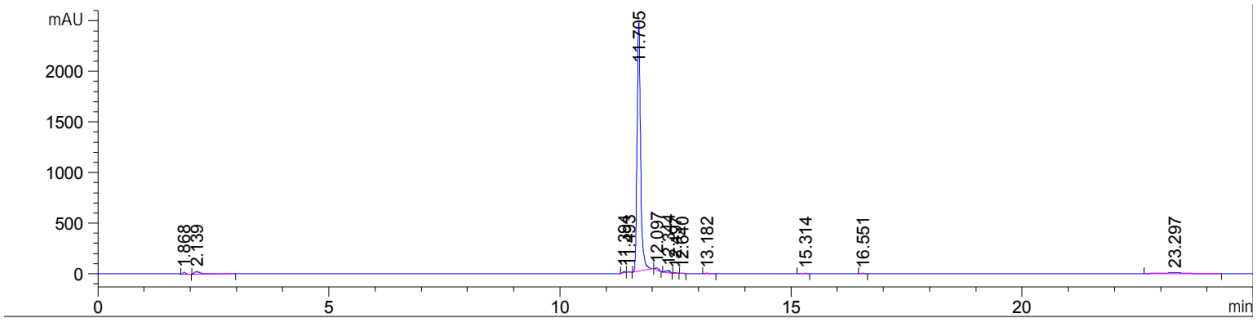


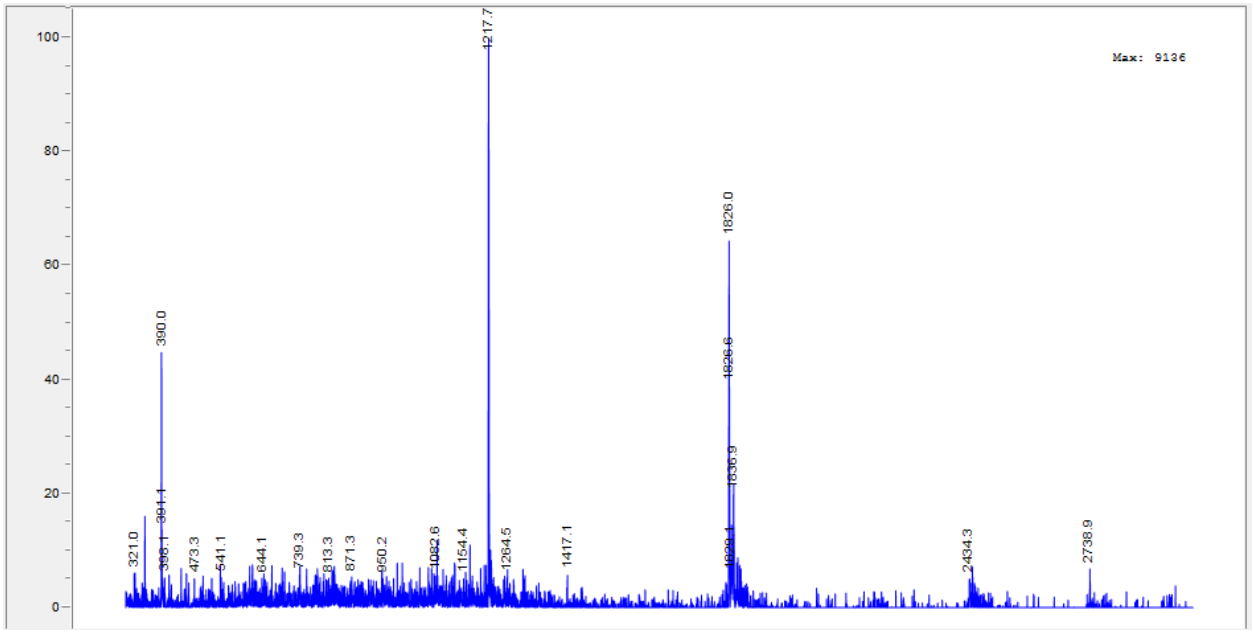
(k) dSAH-ACE2-10, MW = 3250 g/mol.



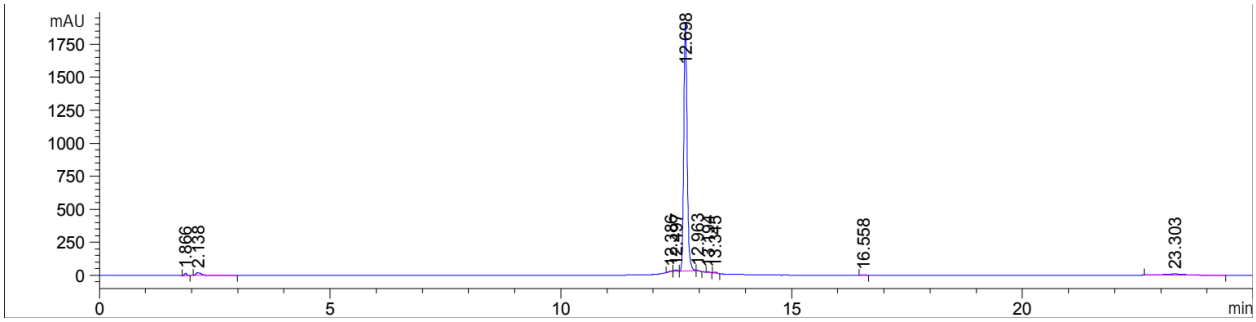


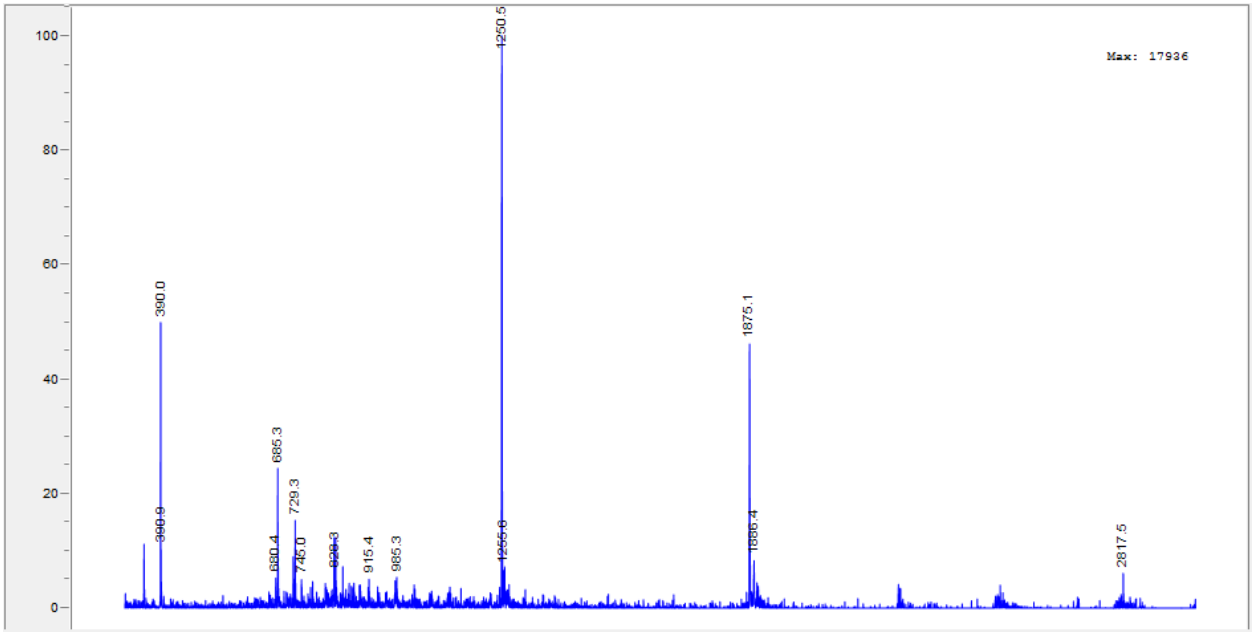
(l) ACE2(19-45), MW = 3650 g/mol.



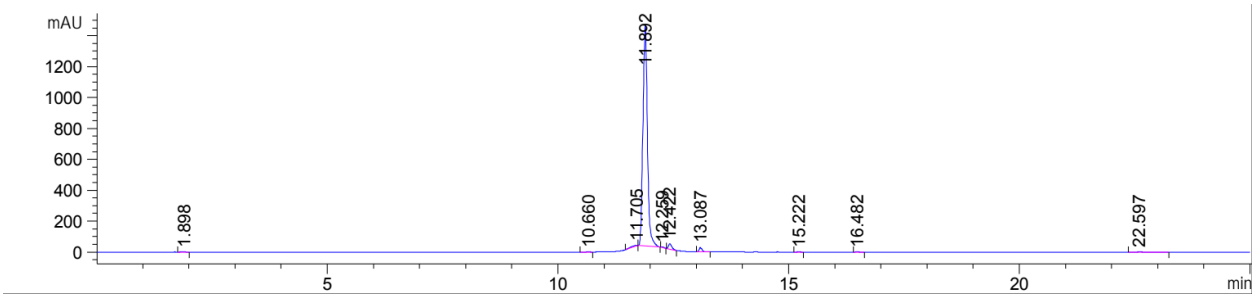


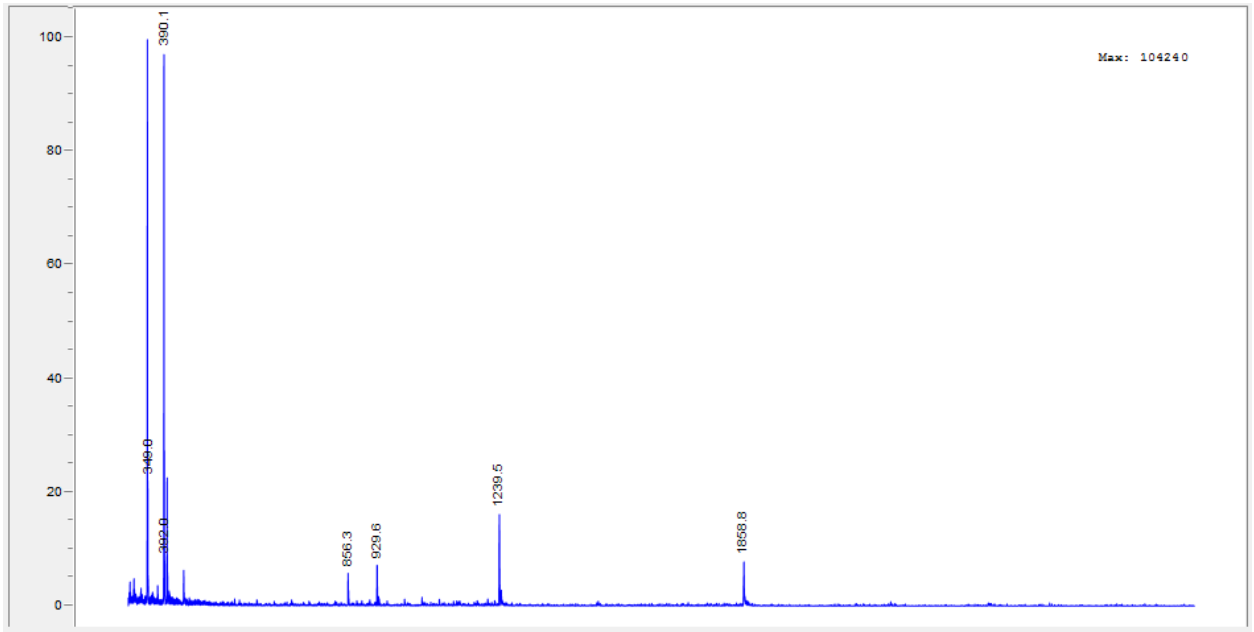
(m) dSAH-ACE2-11, MW = 3748 g/mol.



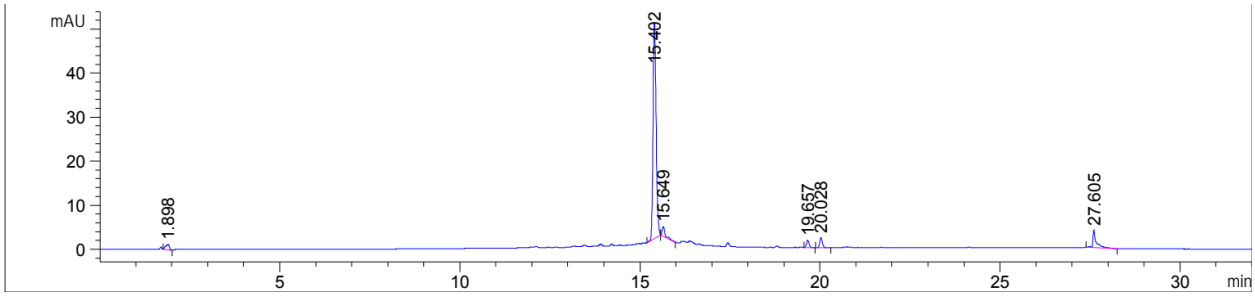


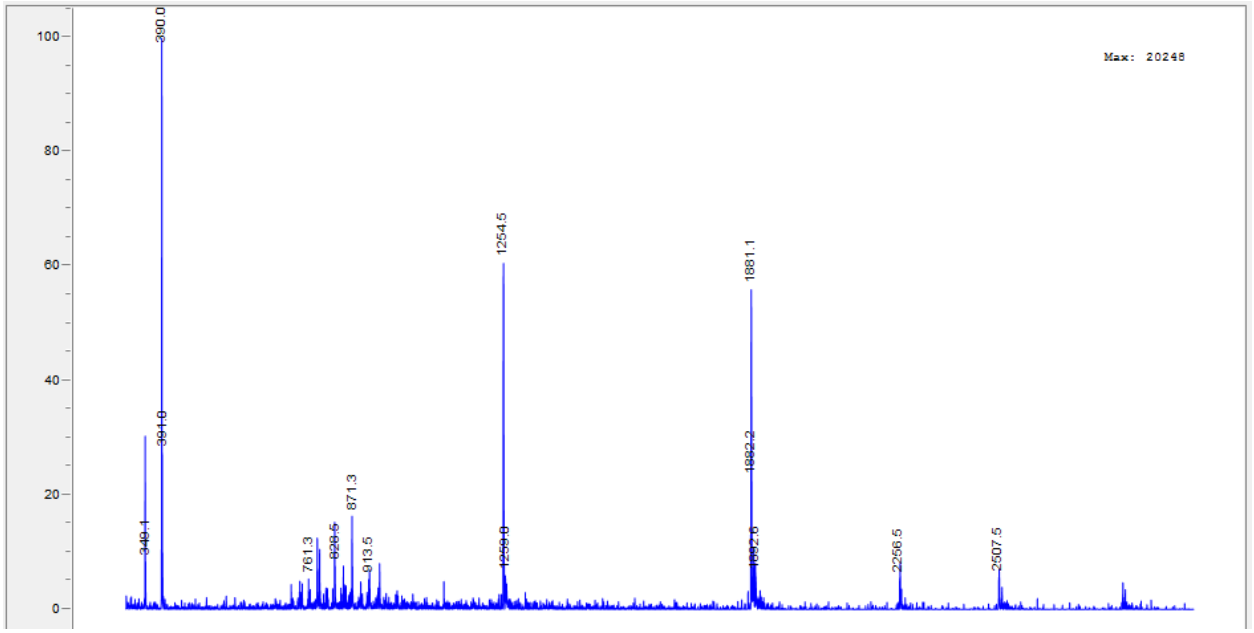
(n) dSAH-ACE2-12, MW = 3715 g/mol.



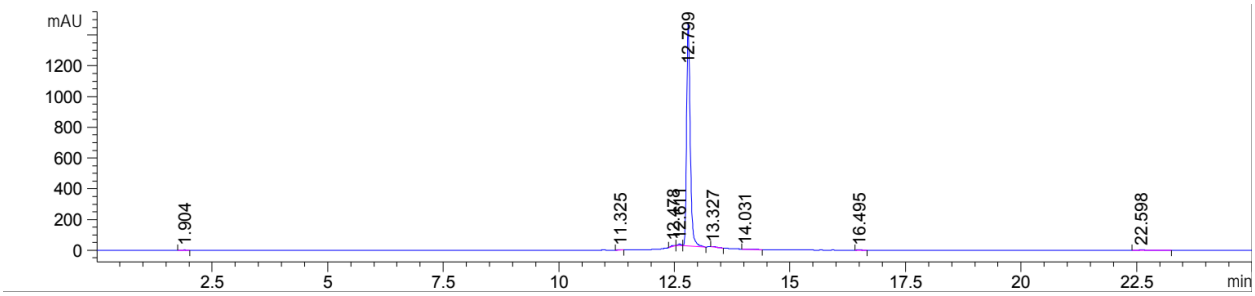


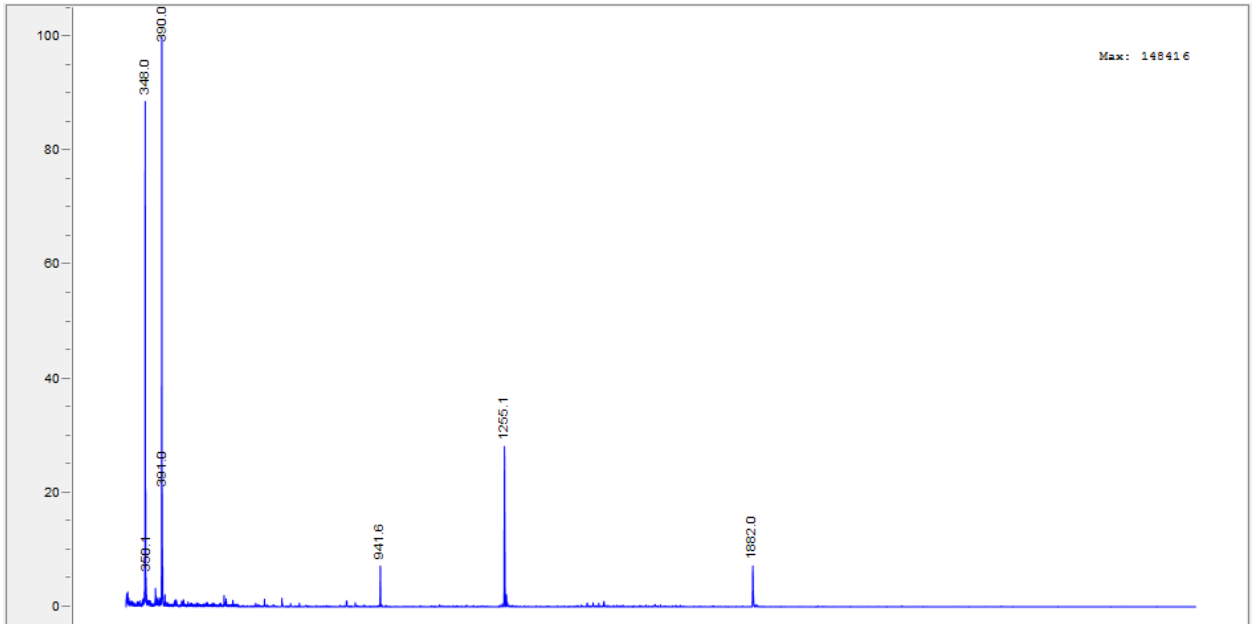
(o) dSAH-ACE2-13, MW = 3760 g/mol.



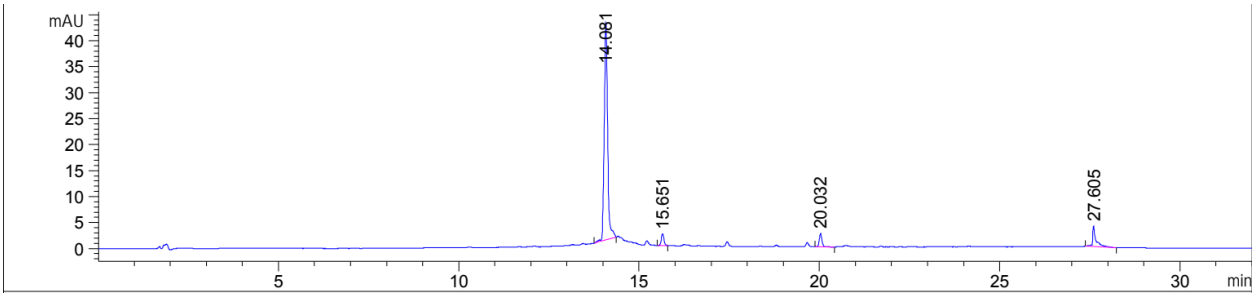


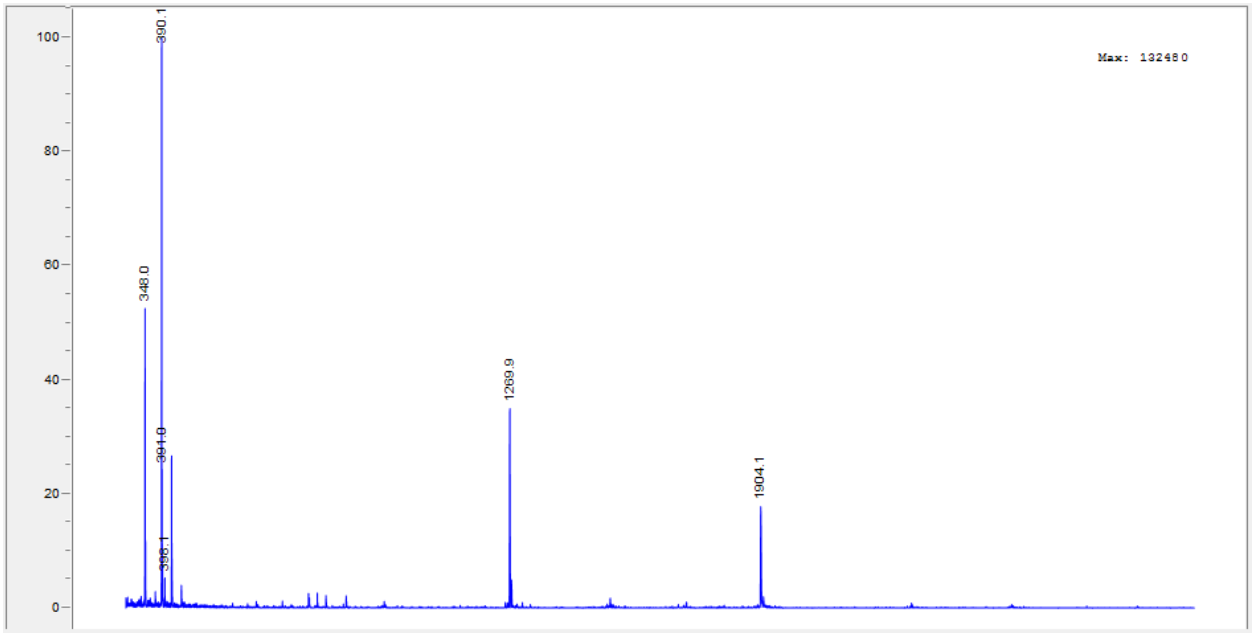
(p) dSAH-ACE2-14, MW = 3762 g/mol.



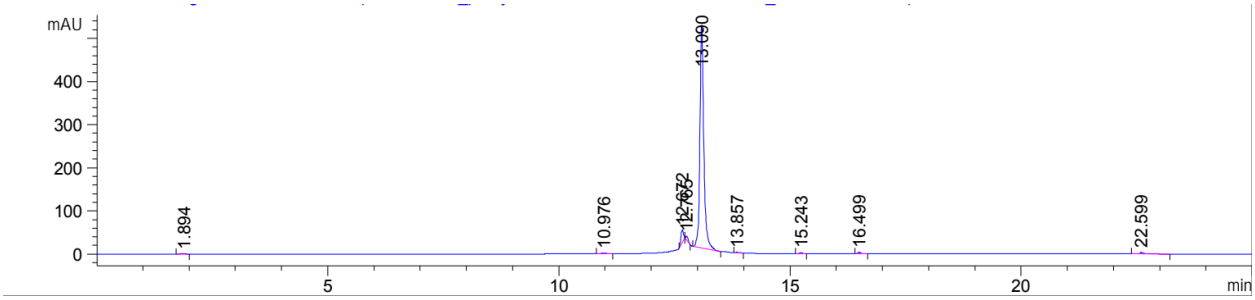


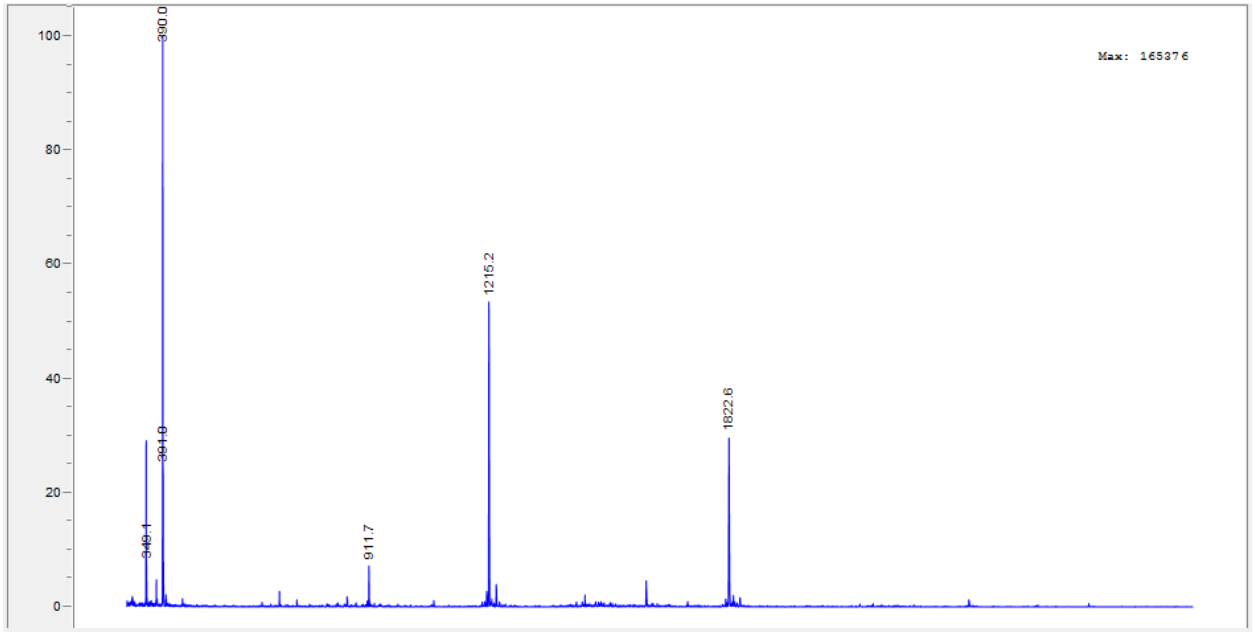
(q) dSAH-ACE2-15, MW = 3806 g/mol.



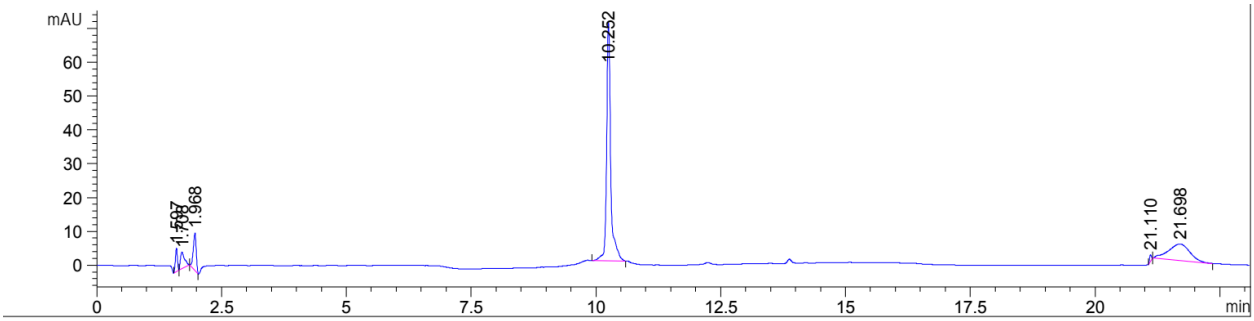


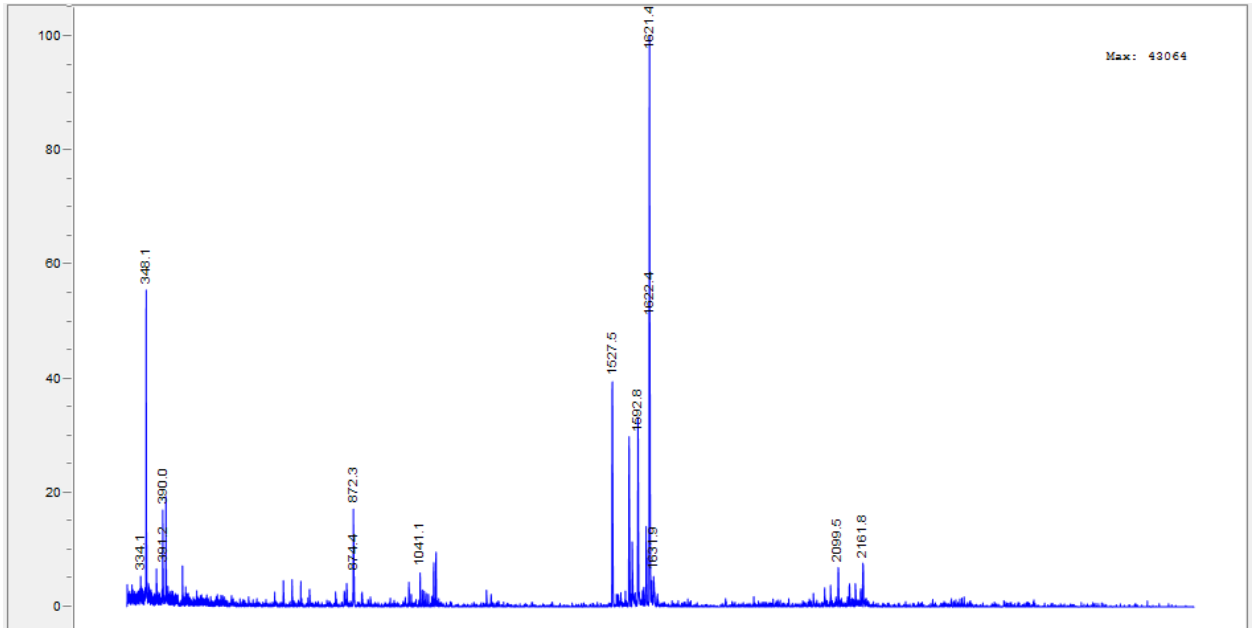
(r) sSAH-ACE2-16, MW = 3643 g/mol.



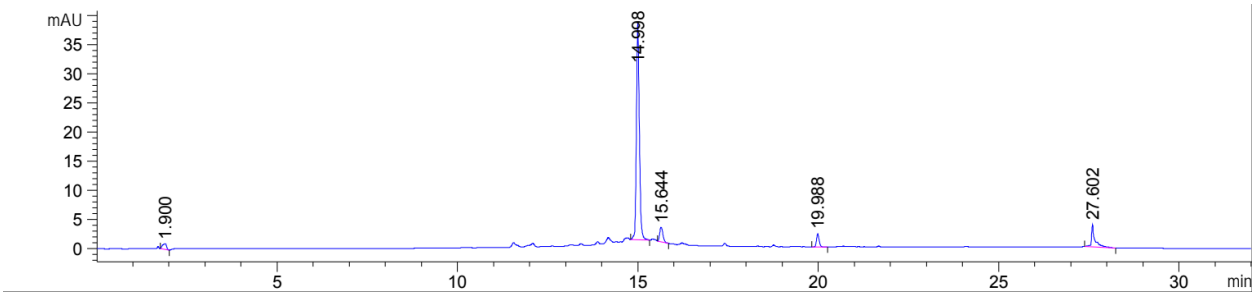


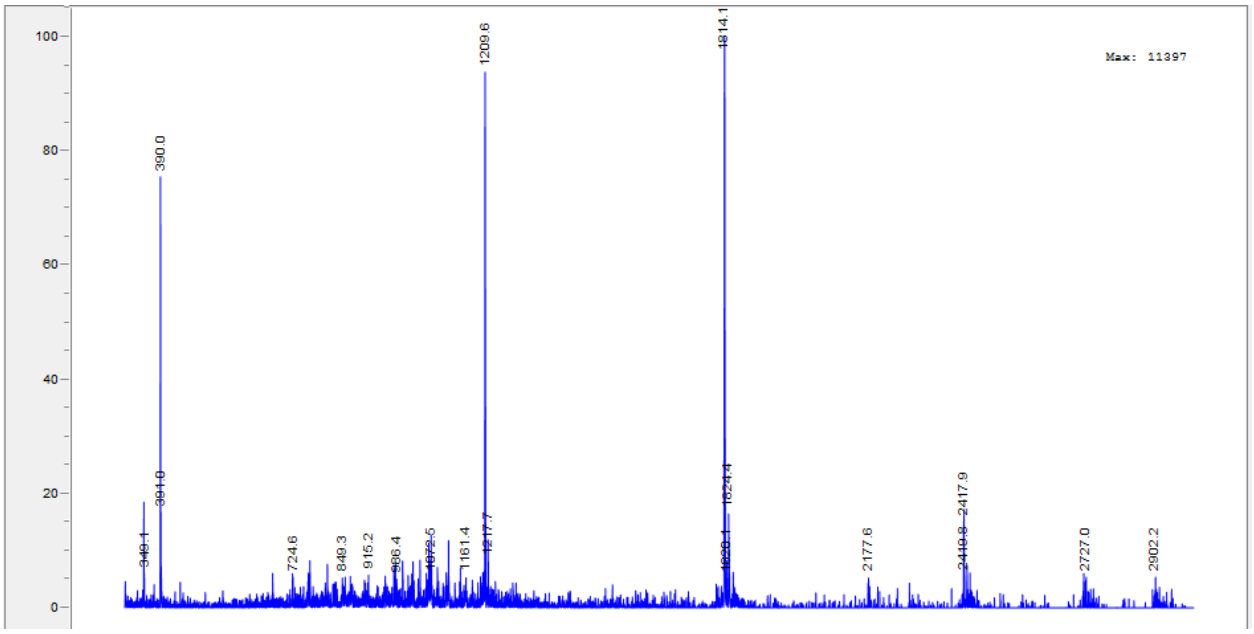
(s) dSAH-ACE2-17, MW = 3659 g/mol.



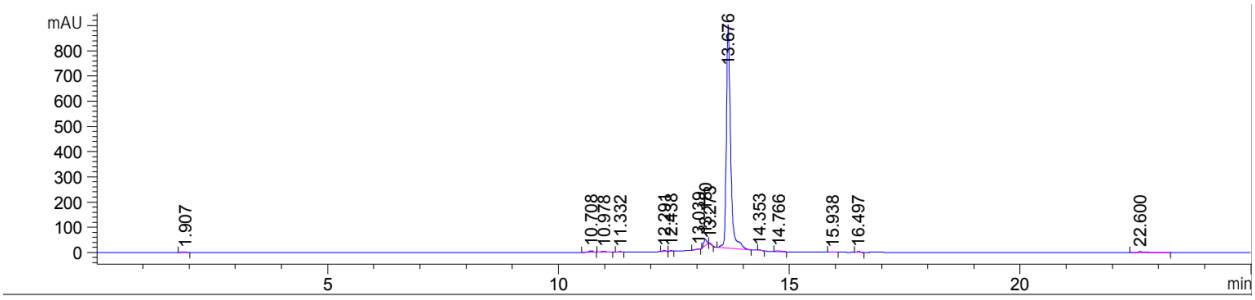


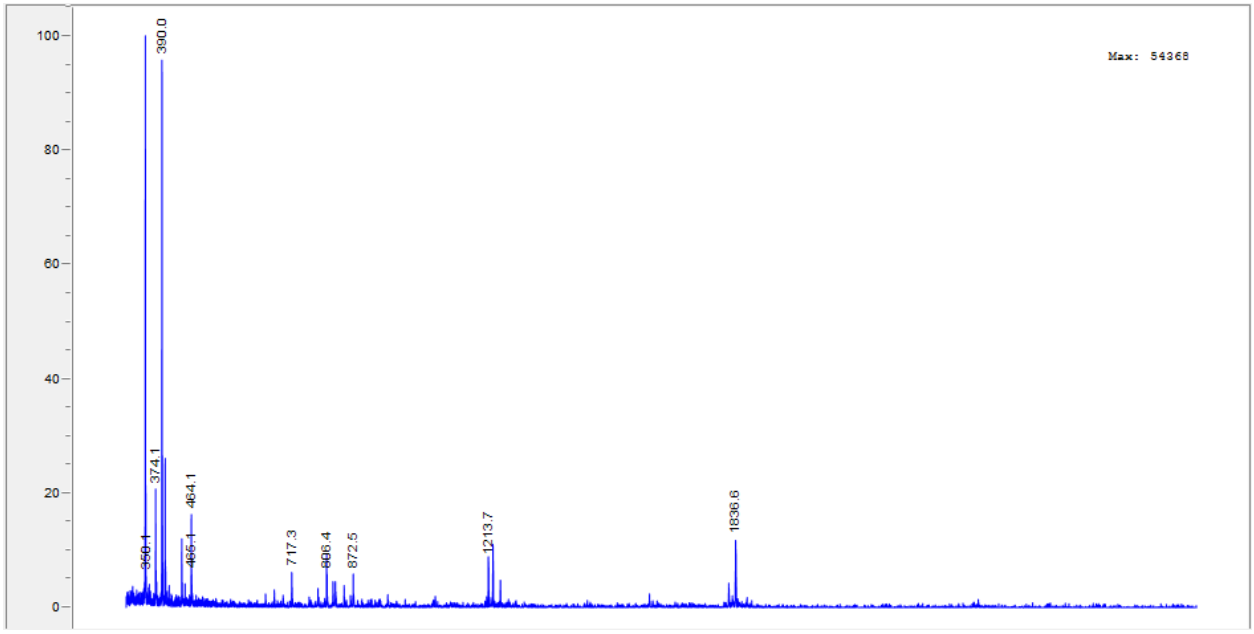
(t) dSAH-ACE2-18, MW = 3626 g/mol.



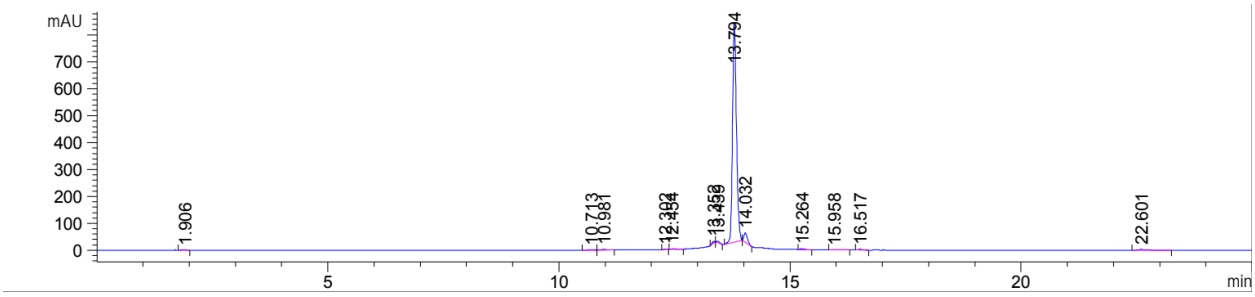


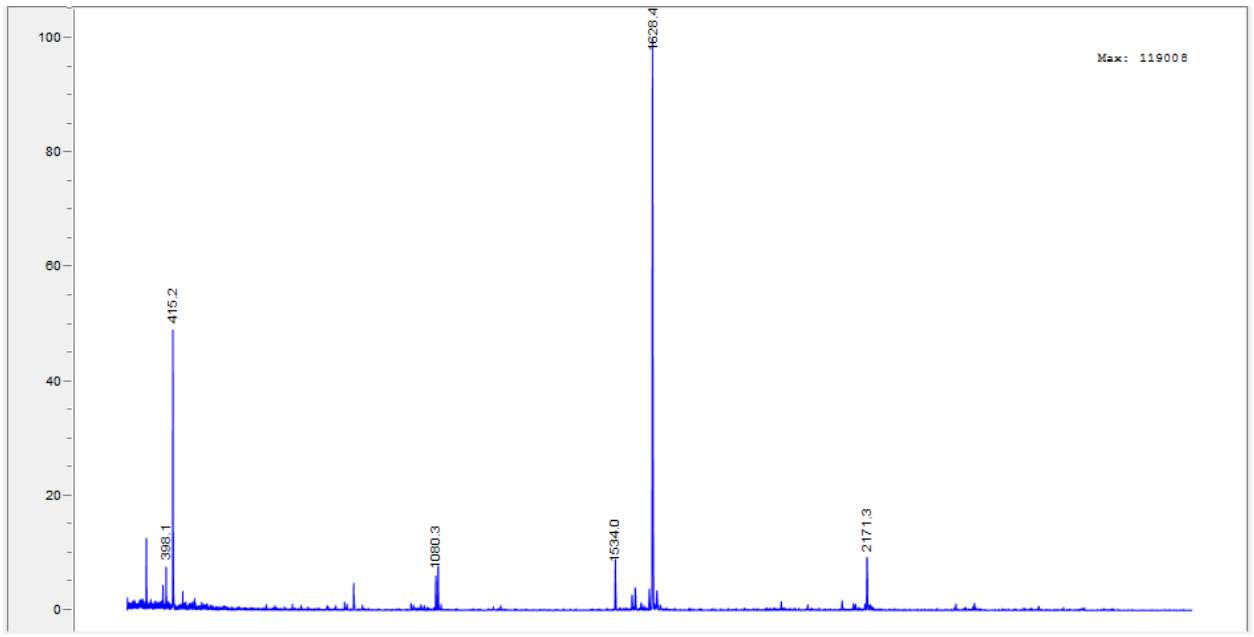
(u) dSAH-ACE2-19, MW = 3671 g/mol.



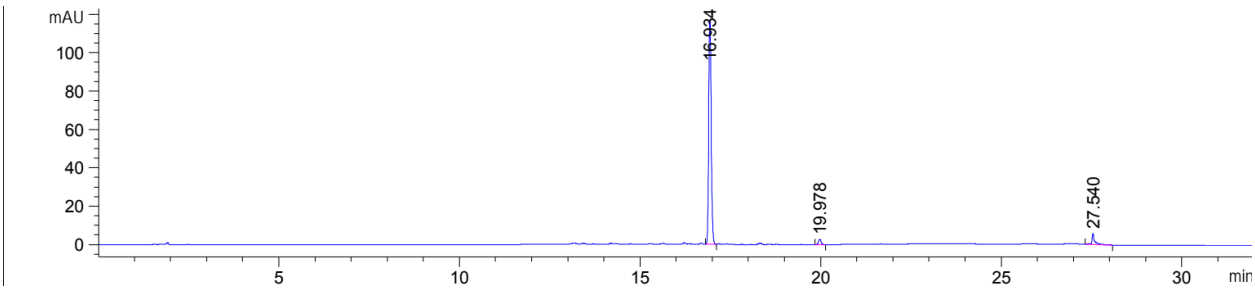


(v) dSAH-ACE2-20, MW = 3673 g/mol.





(w) dSAH-ACE2-21, MW = 3717 g/mol.



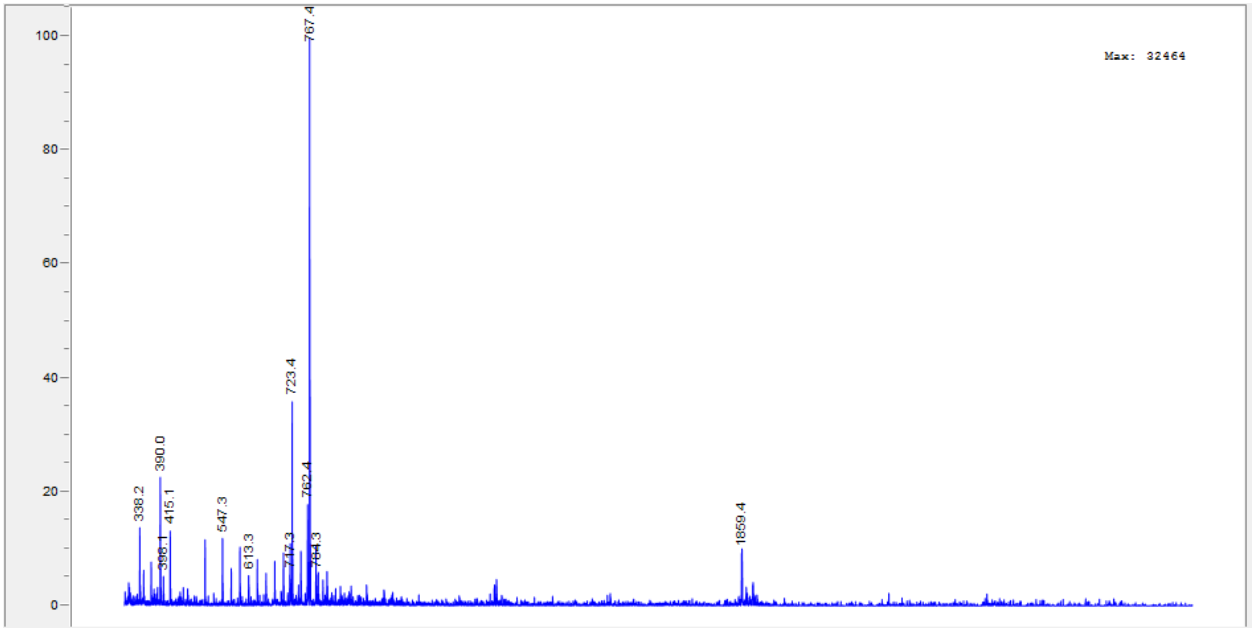
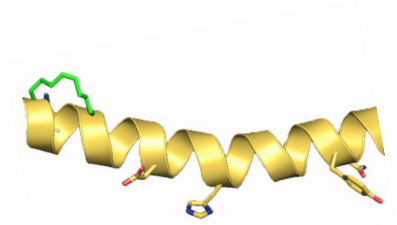
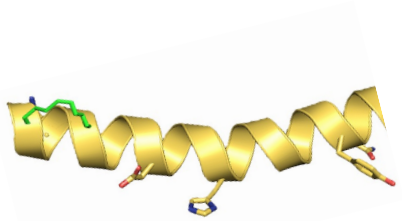


Figure S2. LC-UV280nm trace and corresponding ESI mass spectrum of FITClyated hydrocarbon-stapled and unstapled peptides.

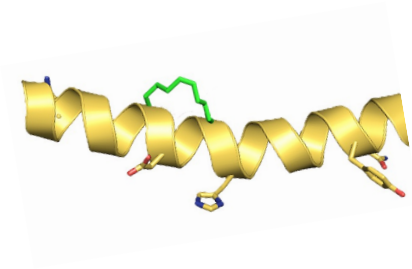
(a)



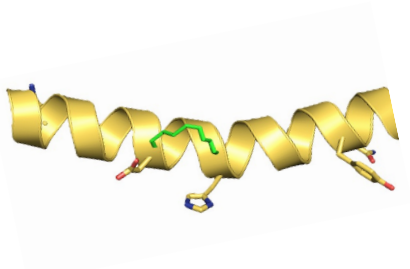
sSAH-ACE2-1



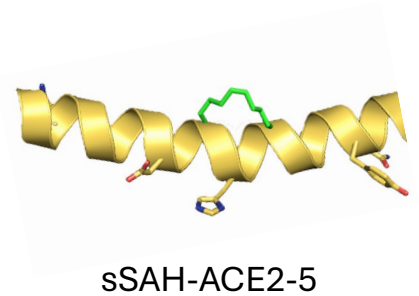
sSAH-ACE2-2



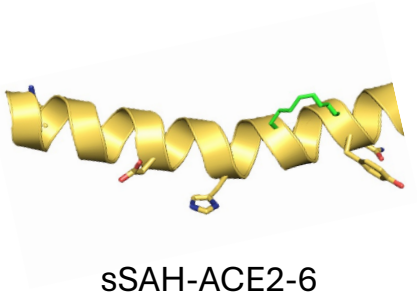
sSAH-ACE2-3



sSAH-ACE2-4



sSAH-ACE2-5



sSAH-ACE2-6

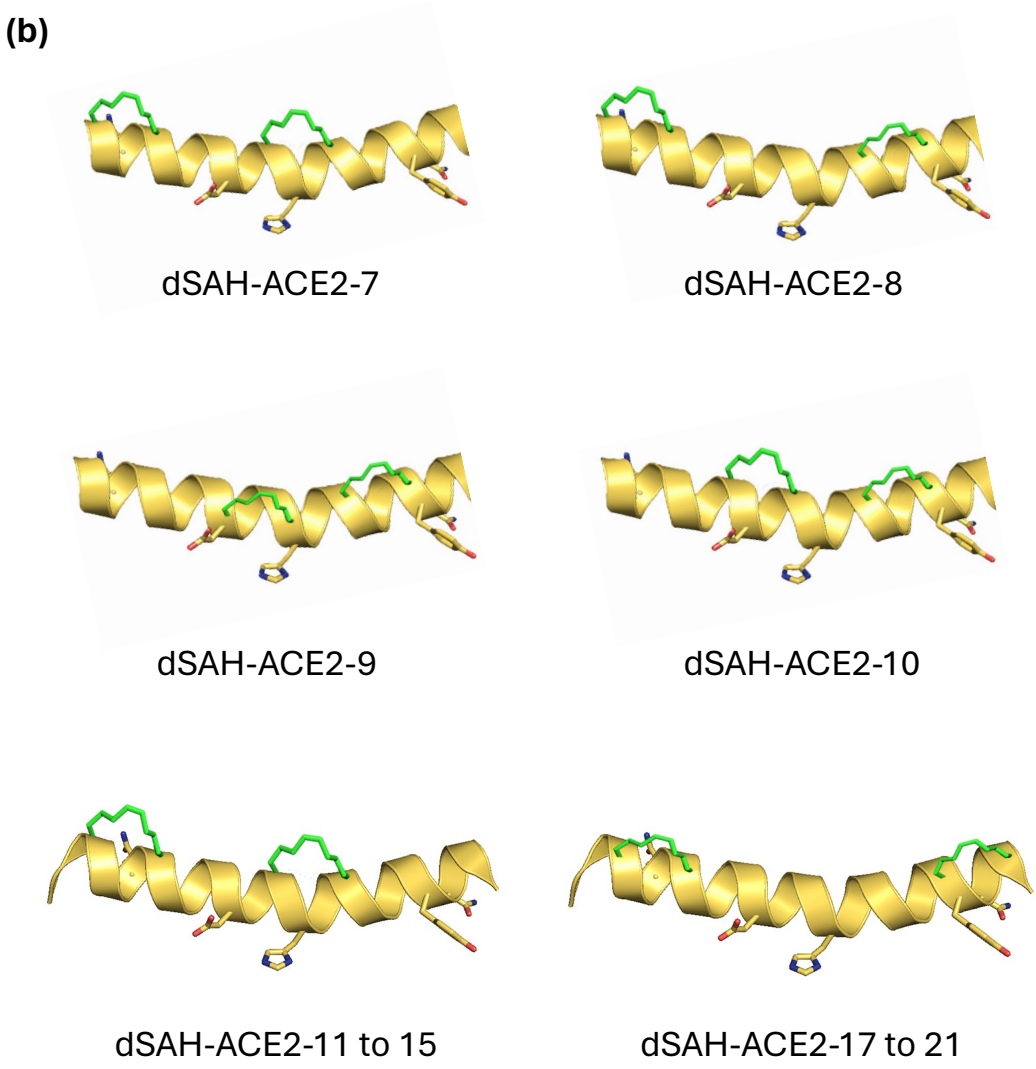
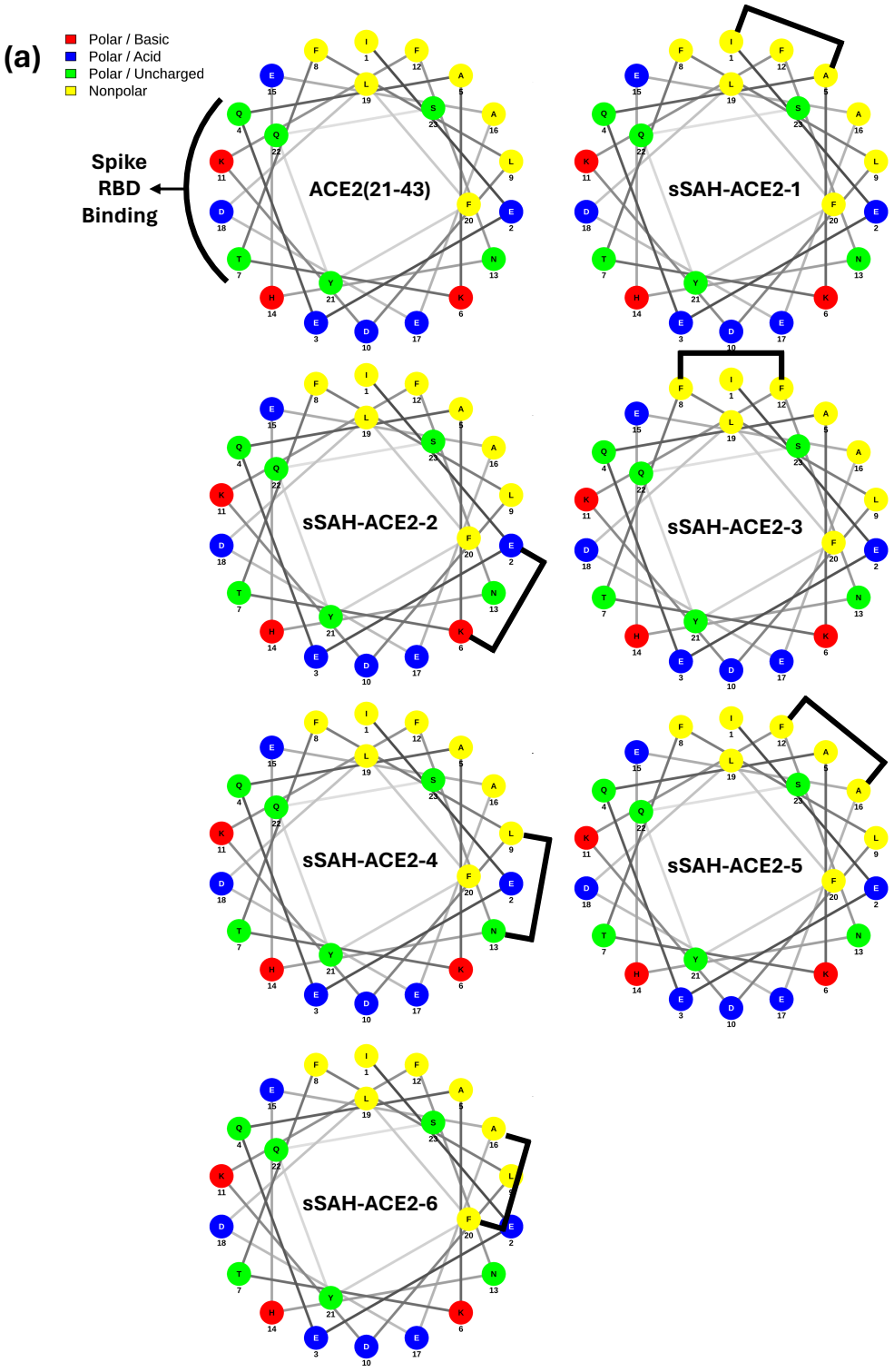


Figure S3. Position of hydrocarbon staples screened in SAH-ACE2 design. Staples were designed to face away from the RBD:ACE2 interface and were positioned N-terminally, C-terminally, or centrally.


(a)
Polar / Basic
Polar / Acid
Polar / Uncharged
Nonpolar
Spike
RBD
Binding
ACE2(21-43)
sSAH-ACE2-1
sSAH-ACE2-2
sSAH-ACE2-3
sSAH-ACE2-4
sSAH-ACE2-5
sSAH-ACE2-6

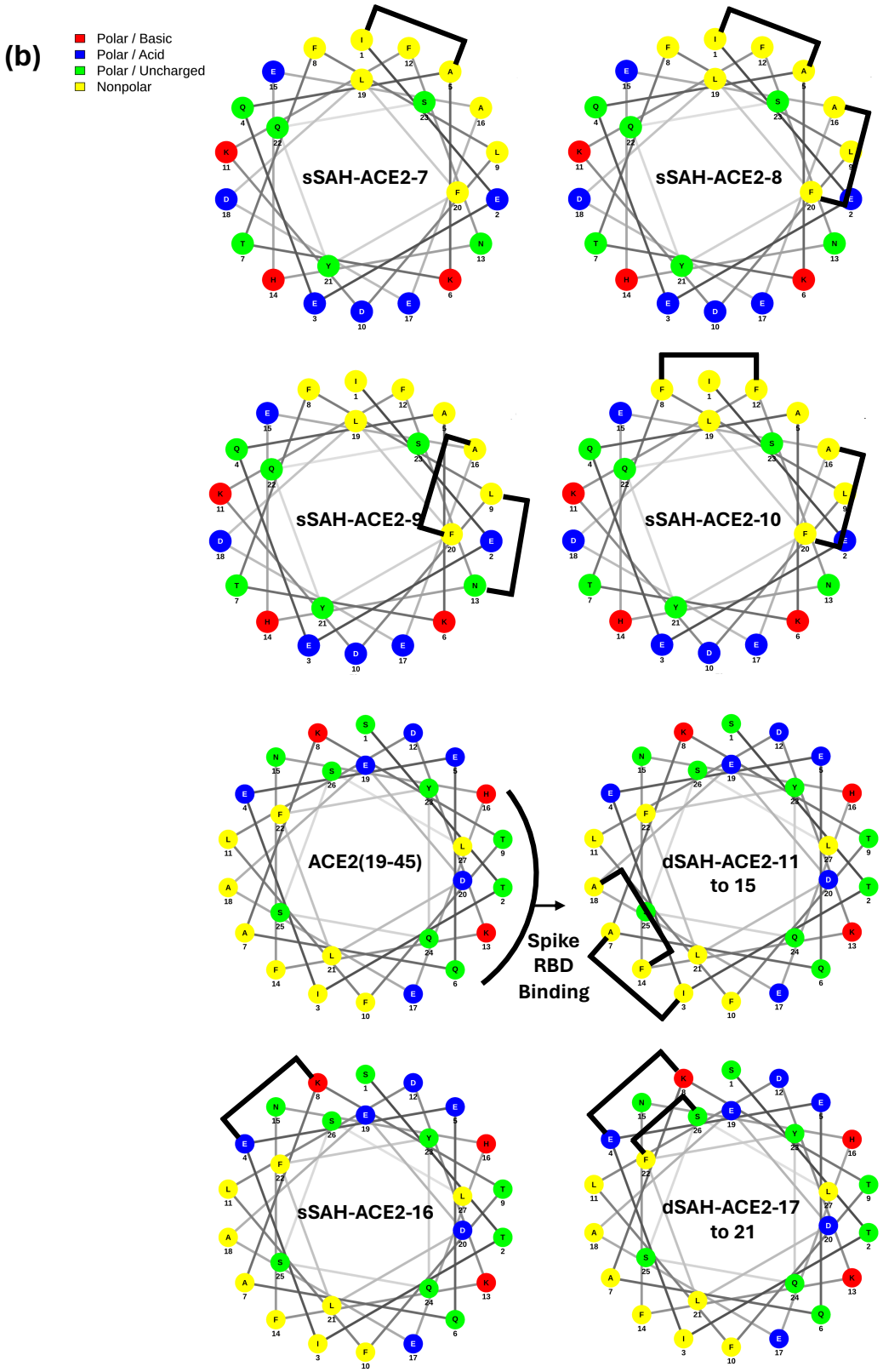
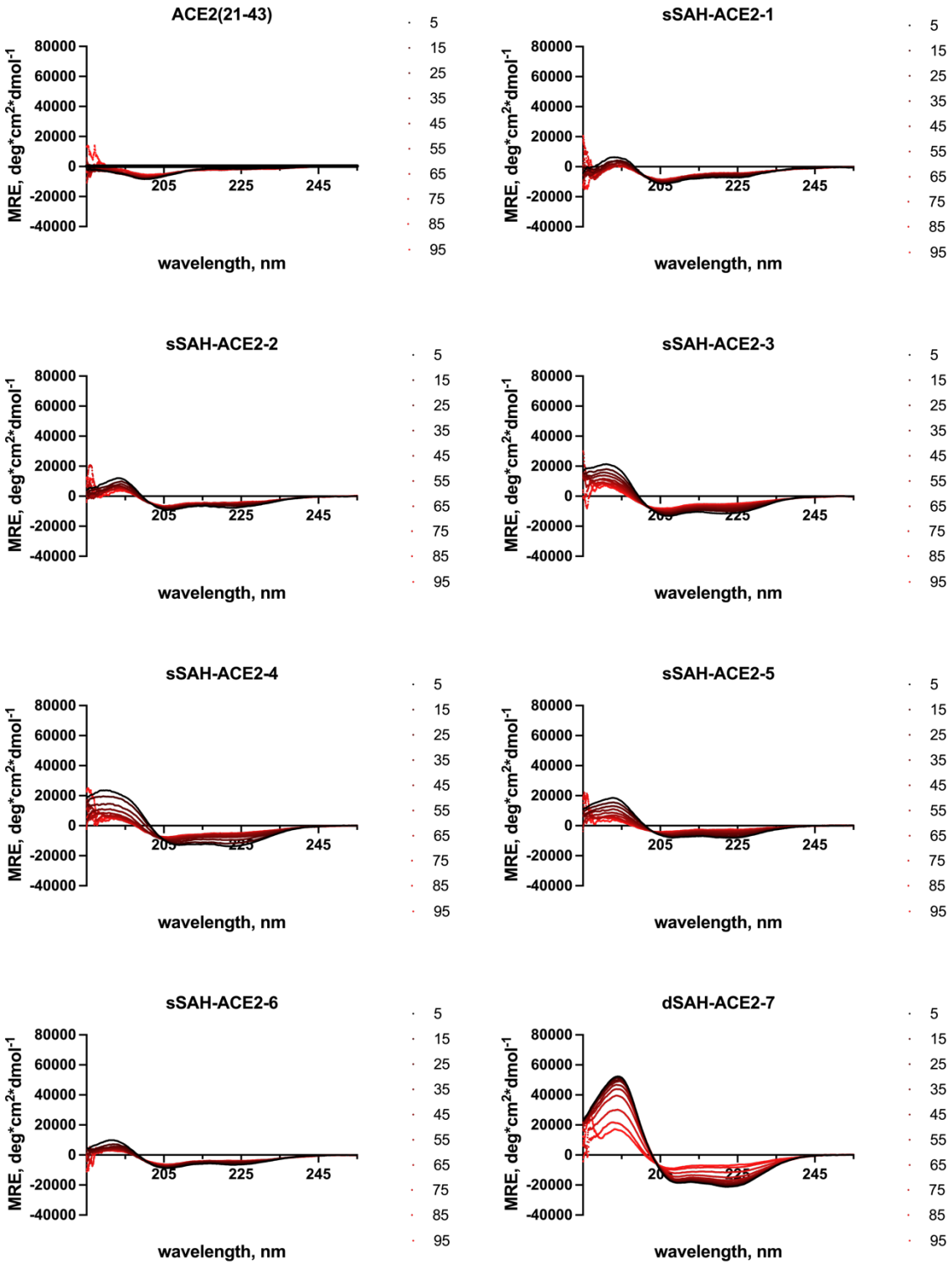
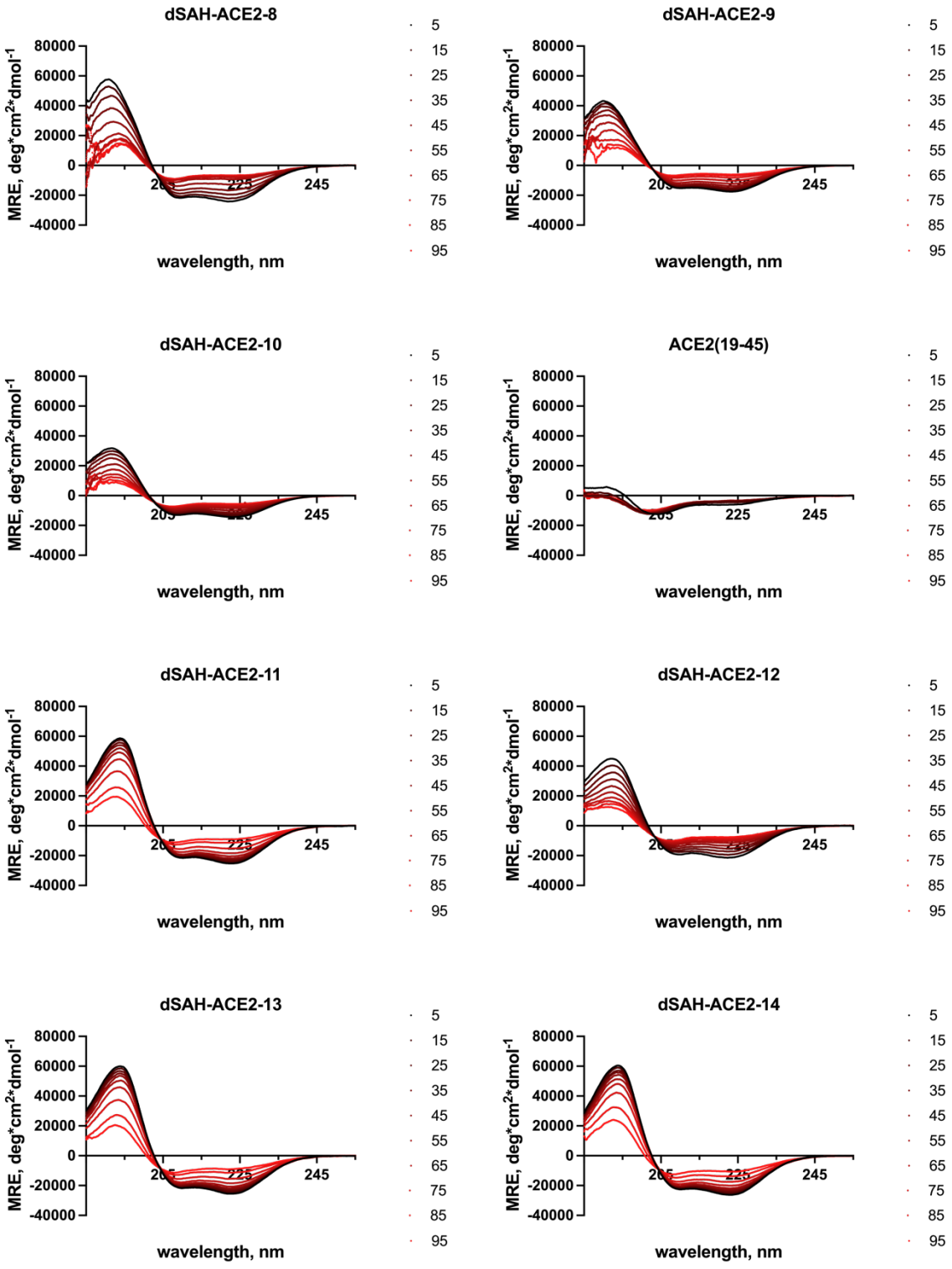


Figure S4. Visualization of staple positions of SAHs targeting SARS-CoV-2 spike protein in relation to the RBD:ACE2 interface. Helical wheel plots indicate the relative position of amino

acids looking down the barrel of the helix. Positively charged amino acids (red), negatively charged amino acids (blue), polar yet non-charged amino acids (green), and hydrophobic amino acids (yellow) are indicated. The more polar side of the helix constitutes the majority of the residues on the interacting face (black half circle). Helical wheel plots for screened single- and double-stapled SAHs are shown with staple positions indicated with a black “staple”. Staples are placed either opposite the interacting face to avoid steric hinderance.


ACE2(21-43)
sSAH-ACE2-1
sSAH-ACE2-2
sSAH-ACE2-3
sSAH-ACE2-4
sSAH-ACE2-5
sSAH-ACE2-6
dSAH-ACE2-7
MRE, deg*cm2*dmol-1
80000
60000
40000
20000
0
-20000
-40000
205
225
245
wavelength, nm
5
15
25
35
45
55
65
75
85
95


dSAH-ACE2-8
dSAH-ACE2-9
dSAH-ACE2-10
ACE2(19-45)
dSAH-ACE2-11
dSAH-ACE2-12
dSAH-ACE2-13
dSAH-ACE2-14
MRE, deg*cm2*dmol-1
80000
60000
40000
20000
0
-20000
-40000
225
245
wavelength, nm
5
15
25
35
45
55
65
75
85
95

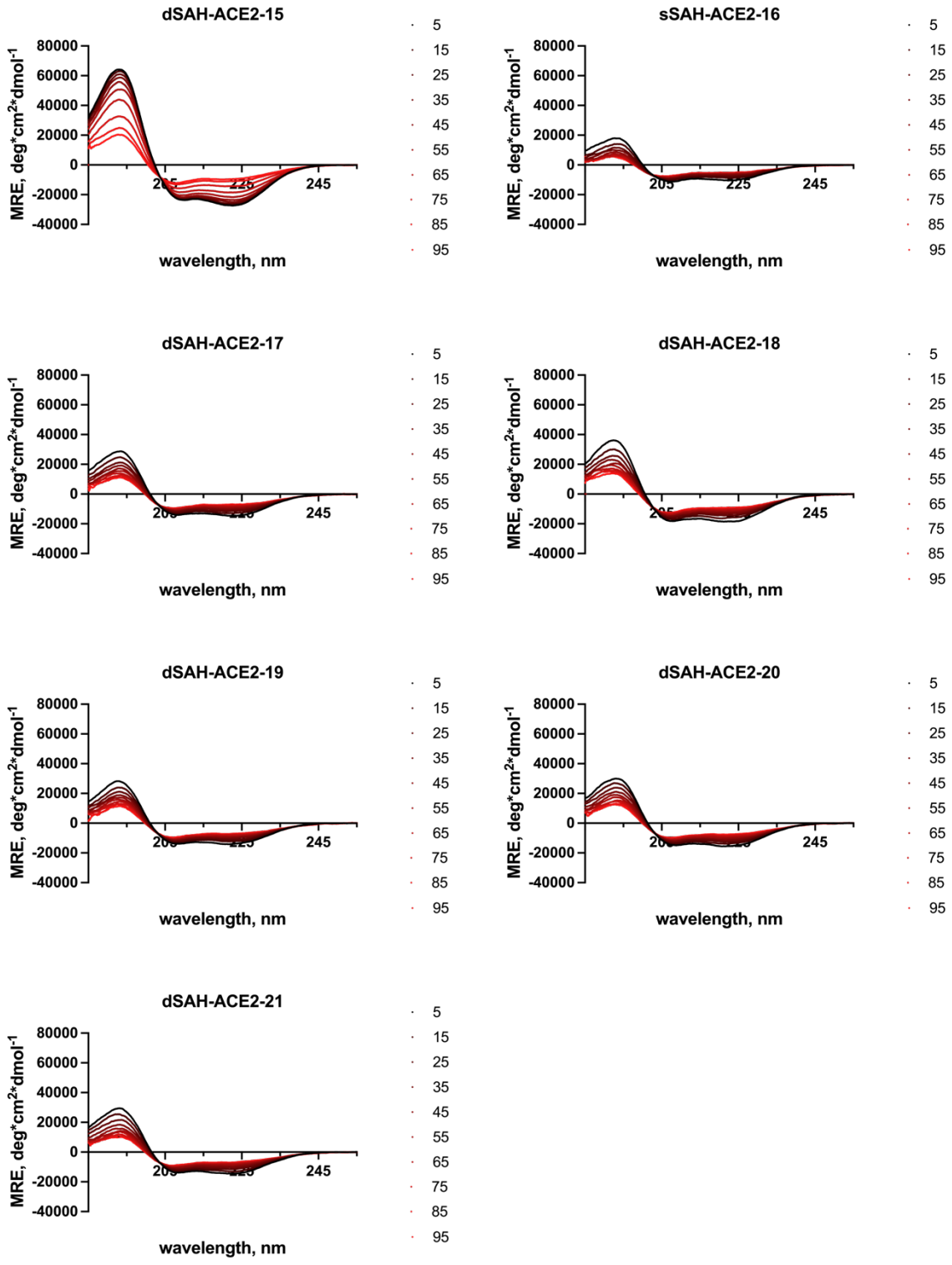


Figure S5. Circular dichroism (CD) of acetylated peptides at various temperatures (5-95° C).

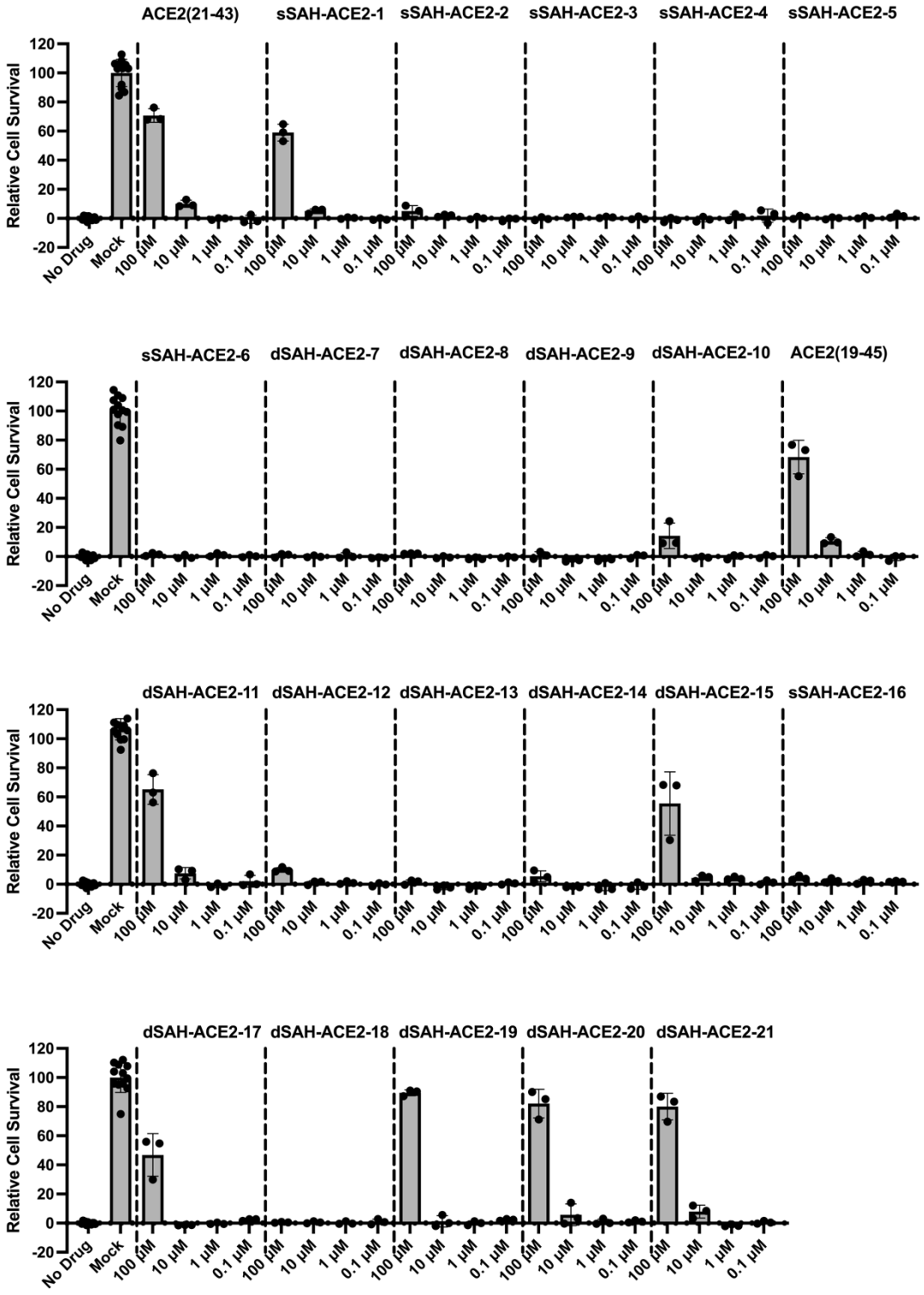


Figure S6. Authentic SARS-CoV-2 neutralization data for all SAHP ACE2 peptides.