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Supplemental Material to:

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Structural analysis of a therapeutic monoclonal antibody dimer by hydroxyl radical footprinting

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Supplementary Files

Figure 1: Non-reduced CE-SDS profiles of non-reduced dimer and monomer samples

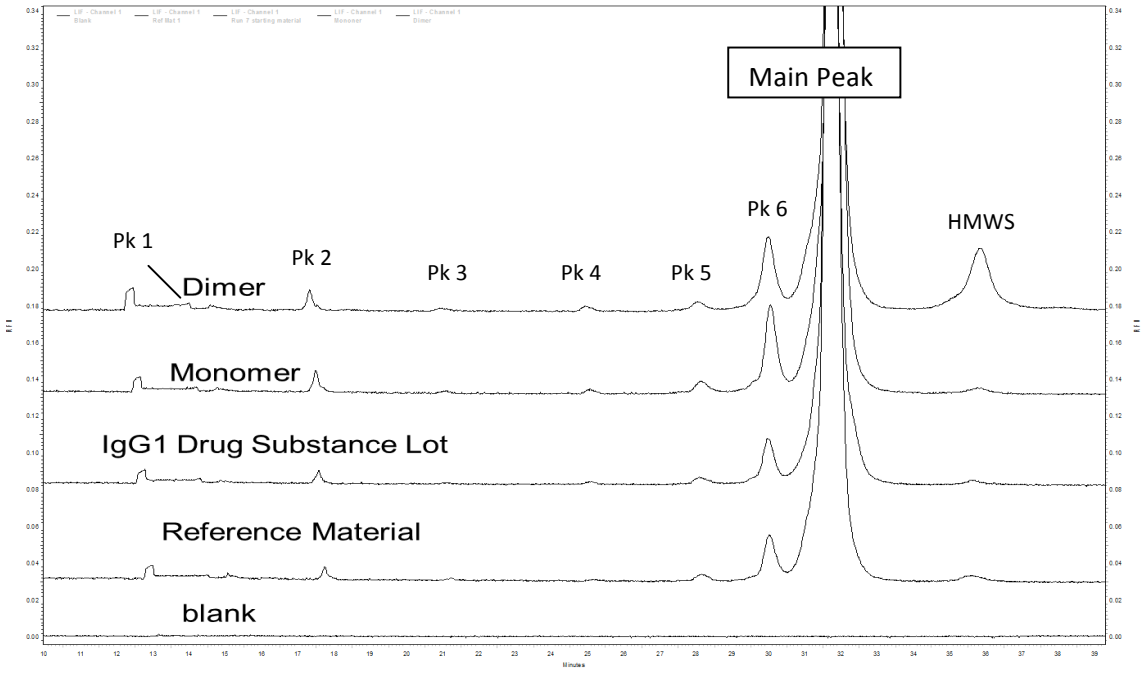
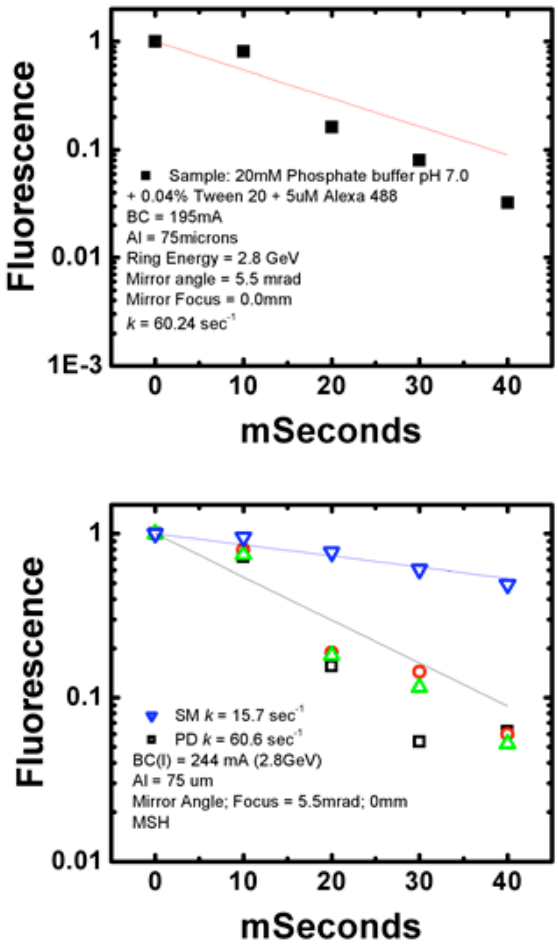


Table 1: Corrected peak areas (cpa) of non-reduced CE-SDS profiles

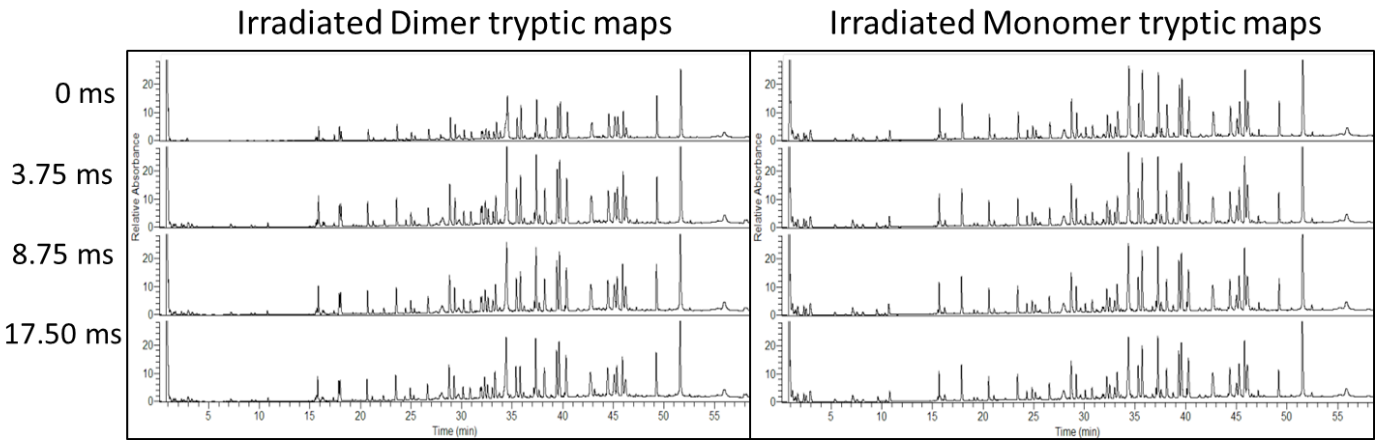
	%CPA							
Peak	1	2	3	4	5	6	Main peak	High molecular weight species
Ref Mat	0.4	0.7	0.2	0.2	0.6	1.0	93.3	0.5
IgG1 Drug Substance	0.2	1.0	0.2	0.3	0.7	4.4	92.9	0.4
Monomer	0.4	1.3	0.2	0.3	1.1	6.7	89.7	0.4
Dimer	0.5	1.3	0.4	0.4	0.7	6.7	82.6	4.5

Figure 2: Synchrotron dose response analysis of dimer and monomer with Alexa 488.



Fluorescence degradation dose response plots used to determine exposure conditions (e.g. BC or beam current, ring energy, mirror angle and focus, etc). Top panel shows the fluorescence dose response curve for 5 µM Alexa 488 in 20mM phosphate pH 7 and 0.04% PS20. Bottom panel shows the fluorescence dose response curves for the monomer (blue triangles), and the dimer (green, red, and black symbols for triplicate analysis) samples that have been diluted 10x with a dilution buffer (20mM phosphate). Rate constant (k) is 15.7 s^{-1} for the monomer, and 60.6 s^{-1} for the dimer.

Figure 3: UV Traces (214 nm) of Dimer and Monomer Tryptic Maps by RP-UHPLC

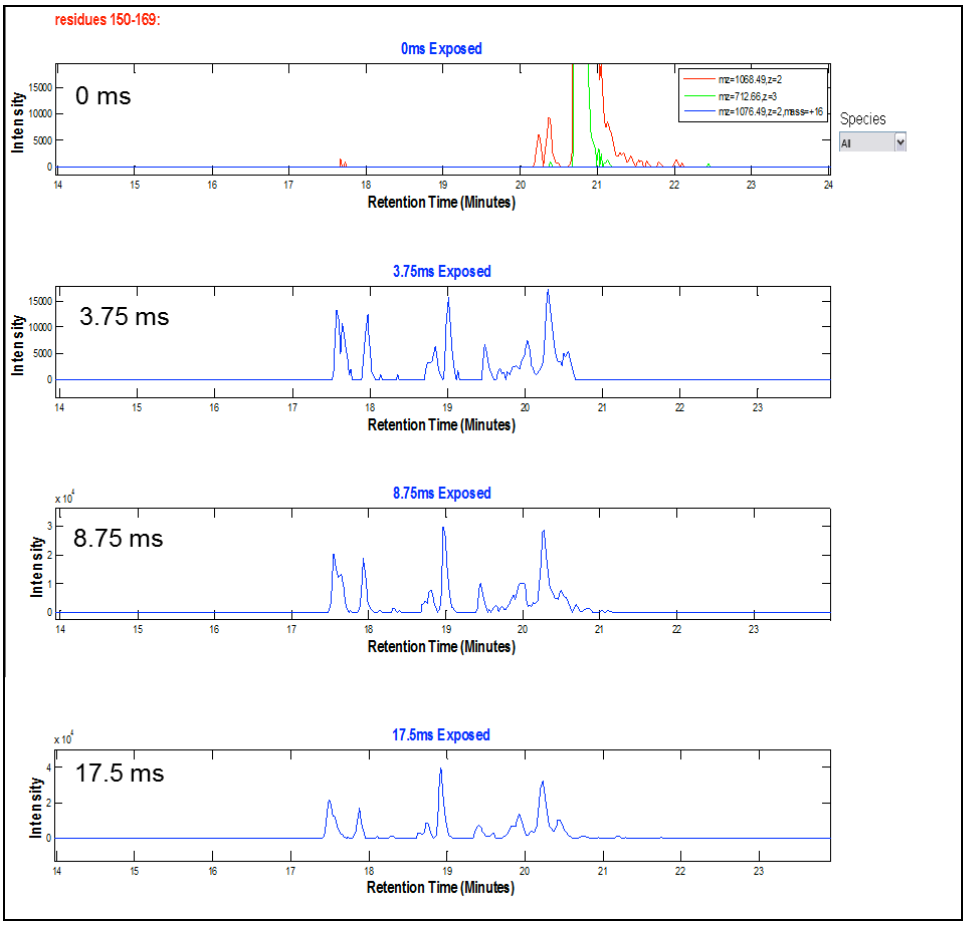


RP-UHPLC performed with an Agilent 1200 SL UHPLC equipped with an Agilent ZORBAX Rapid Resolution HT SB-C8, 2.1 × 150 mm, 1.8 µm column. Chromatograms are not normalized.

Note: Observed differences in the profiles between the dimer and monomer profiles at t=0 ms (control samples) were attributed to: very small peptides with ≤ 3 residues (peaks eluting from 2-11 minutes; these peptides were not used for structural assessment), known variable resolution regions where 2 peptides may sometimes appear as doublets or a singlet (18 min; this does not affect the ms results), and non-specific cleavage products (24 min; not used for structural assessment).

In addition, as noted in the main article, the native dimer sample contained increased amounts of pre-existing oxidized methionine residues prior to synchrotron irradiation. These would manifest as a priori peak height differences for both the oxidized peptides and its non-oxidized forms when comparing peptide map profiles of the dimer and monomer. These peak height differences between the profiles are observed at 32 min, 36 min, 45-46 min, and 49 min in the profiles; these do not affect the ms results, and merely reflect the structural differences between the dimer and monomer.

Figure 4A: Example EIC data output from ProtMapMS (LC 150-169 of Dimer)



Note: Red and green traces denote the EIC for the doubly and triply charged ions of the parent peptide. Blue traces are the EIC of the doubly charged +16 Da oxidized forms. The intensity of the oxidized forms increases as the radiolysis time increases.

Figure 4B: Oxidized residues identified in LC 150-169

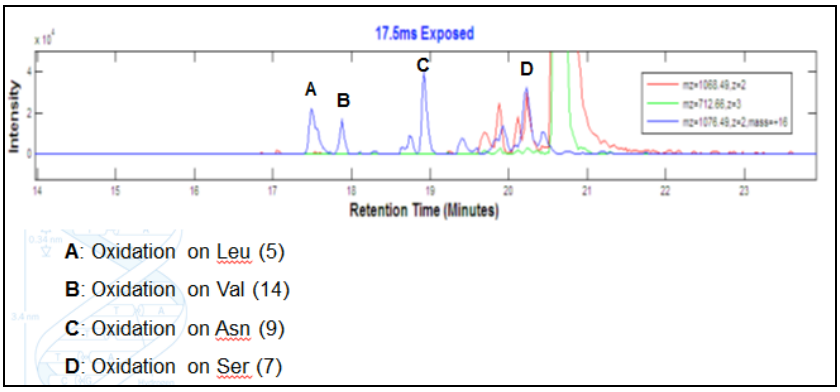
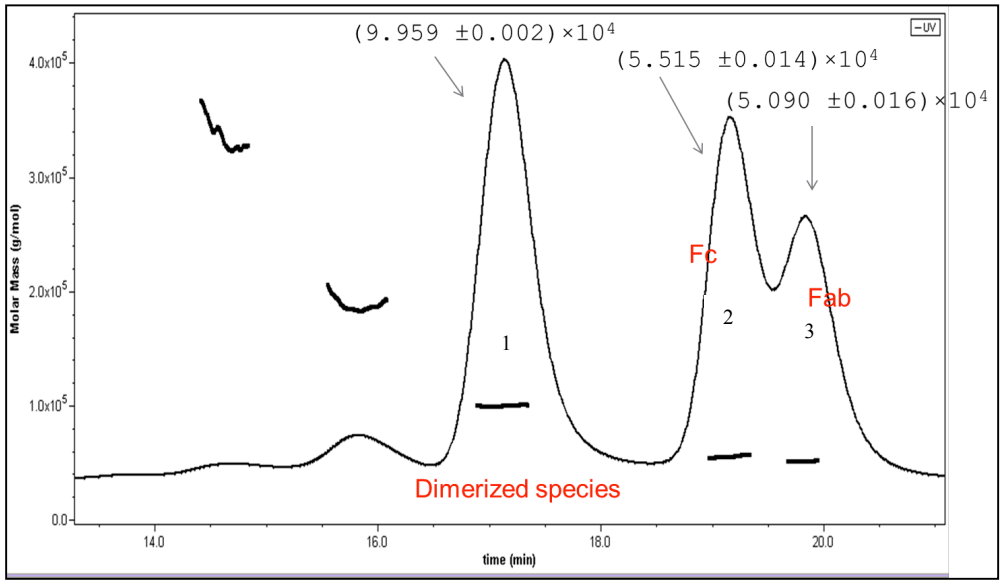
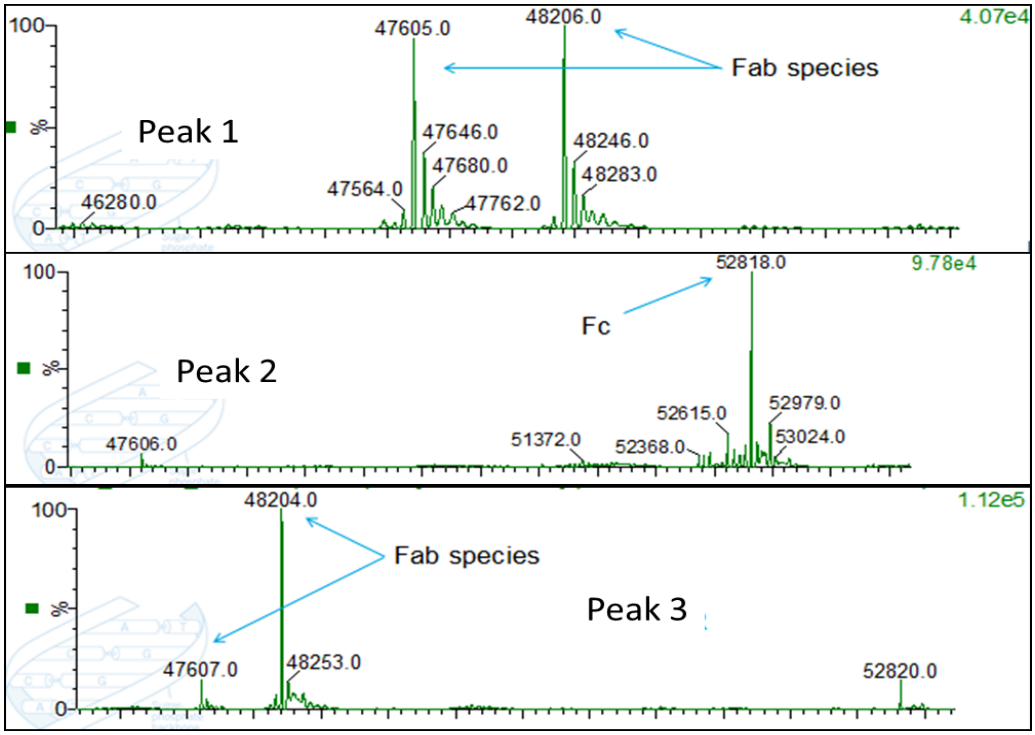


Figure 5A: SEC-MALLS of papain digested dimer



See Table 2

Figure 5B: LC-MS of papain-generated fragments separated by SEC



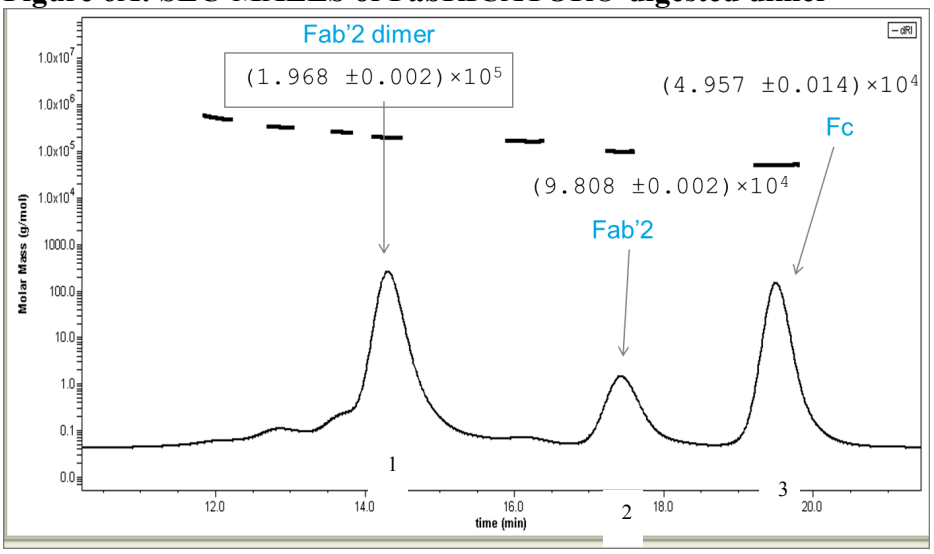
See Table 2

Table 2: Characterization data for papain-generated fragments

Papain-generated fragment monitored by SEC	Approximate molar mass by SEC-MALLS (g/mol)	Observed MW by LC-MS (Da)	Assigned SEC Peak Identity
Peak 1	100 x 10^3 g/mol	48206 Da, 47605 Da [a] (mass of Fab: LC + HC 1-230 or LC + HC 1-225)	Fab/Fab non-covalent dimer
Peak 2	55 x 10^3 g/mol	52818 Da (mass of intact disulfide-linked Fc)	Intact disulfide-linked Fc
Peak 3	51 x 10^3 g/mol	48204 Da, 47607 Da [a] (mass of Fab: LC + HC 1-230 or LC + HC 1-225)	Fab

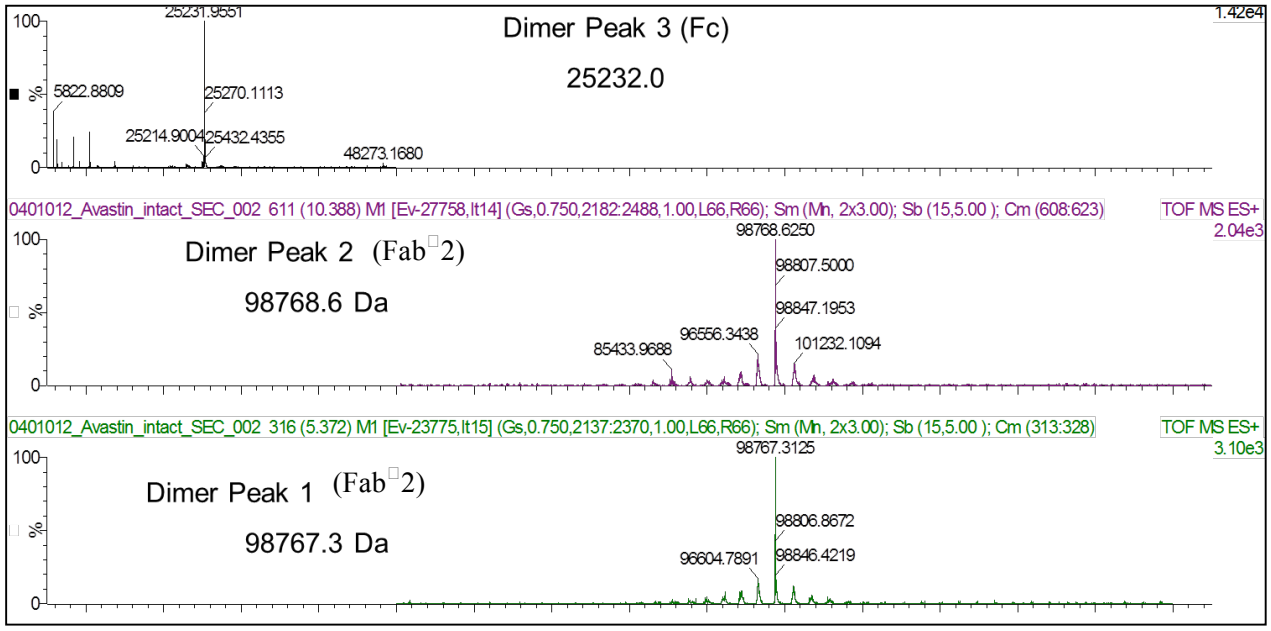
[a] Non-specific cleavage of the heavy chain hinge region by papain generates Fab species with two heavy chain fragments differentiated by five residues at their C-terminus. Either Fab form is presumed to be involved in the observed in-situ dimerization.

Figure 6A: SEC-MALLS of FabRICATOR®-digested dimer



See Table 3

Figure 6B: LC-MS of FabRICATOR®-generated fragments separated by SEC



See Table 3

Table 3: Characterization data for FabRICATOR®-generated fragments

FabRICATOR®-generated fragment monitored by SEC	Approximate molar mass by SEC-MALLS (g/mol)	Observed MW by LC-MS (Da)	Assigned SEC Peak Identity
Peak 1	200 x 10^3 g/mol	98767 Da (mass of Fab'2: Two disulfide-linked Fab arms)	Fab'2/Fab'2 non-covalent dimer
Peak 2	98 x 10^3 g/mol	98769 Da (mass of Fab'2: Two disulfide-linked Fab arms)	Fab'2
Peak 3	50 x 10^3 g/mol	25232 Da (mass of Fc fragment)	Fc/Fc non-covalent dimer

Table 4A: Summary of oxidized heavy chain tryptic peptide rate constants

Heavy Chain Tryptic Peptides	Dimer Rate Constant (k) in s^{-1}	Monomer Rate Constant (k) in s^{-1}
1-19	2.6	1.0
20-38	2.3	1.1
44-65	3.0	1.5
68-76	0.9	0.2
77-87	0.5	0.4
88-98	0.4	0.1
99-127[a]	-	-
128-139	0	0
140-153	0.4	0.1
154-216	14.9	4.9
229-254	6.3	1.9
255-261	56.5	9.1
262-280	2.2	1.2
281-294	3.4	1.3
299-307	1.8	0.7
308-323	1.5	0.6
333-340	1.6	0.6
351-361	1.0	0.4
362-366	19.6	3.1
367-376	0.4	0.2
377-398	4.5	1.7
399-415	0.7	0.4
423-445	58.0	9.0
446-452	1.3	0.4

Table 4B: Summary of oxidized light chain tryptic peptide rate constants

Light Chain Tryptic Peptides	Dimer Rate Constant (k)	Monomer Rate Constant (k)
1-18	3.3	4.1
19-42	3.3	3.6
46-61	3.3	1.4
62-103	9.1	4.6
109-126	0.5	1.2
127-142	0.4	0.3
146-149	1.2	0.4
150-169	1.9	1.0
170-183	0.6	0.3
191-207	3.7	2.2

[a] HC 99-127 showed inconsistent recovery of the parent peptide, leading to non-linear dose response plots. The rate constants for this peptide were not deemed reliable for structural mapping.