

1 **Antibody Recognition of the Pandemic H1N1 Influenza**

2 **Hemagglutinin Receptor Binding Site**

3

4 Running title: Pandemic H1N1 Recognition by Human Antibody 5J8

5

6 **Supplemental Material**

7 **Sequences of HA proteins used in binding studies.** The sequences listed below represent the
8 full-length ORF as cloned in the baculovirus transfer vector. Most of the N-terminal signal
9 peptide (MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSAFA) is presumably
10 removed during secretion, leaving four non-native residues (ADPG) at the N-terminus of HA1.
11 The C-terminal BirA biotinylation site, thrombin cleavage site, trimerization domain, and His₆
12 tag are retained on all proteins.

13
14 **>A/South Carolina/1/1918 (H1N1)**

15 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSAFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLD
16 SHNGKLCCLKGIAPLQLGKCNIAGWLLGNPECDLLLTASSWSYIVETSNSENGTCYPGDFIDYEELREQLSSVSSFE
17 KFEIFPKTSSWPNHETTKGVTAACSYAGASSFYRNLLWLTKKGSSYPKLSKSYVNNKGKEVLVLWGVHHPPTGTDQQ
18 SLYQNADAYVSVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTITFEATGNLIAPWYAFALNRGSGSII
19 TSDAPVHDCNTKCTPHGAINSSLPFQNIHPVTIGCEPKYVRSTKLRMATGLRNIPSIQSRGLFGAIAAGFIEGGWTG
20 MIDGWYGYHHQNEQGSYAADQKSTQNAIDGITNKVNSVIEKMNTQFTAVGKEFNLERRIENLNKKVDDGFLDIWT
21 YNAELLVLLLENERTLDFHDSNVRNLYEKVKSQKNNAKEIGNGCFEFYHKCDDACMESVRNGTYDYPKYSEESKLN
22 EEIDGVSGGGGLNDIFEAQKIEWHERLVPRGSPGSGYIPEAPRDGQAYVRKDGGEWVLLSTFLGHHHHHH

23
24 **>A/WSN/1933 (H1N1)**

25 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSAFAADPGDTICIGYHANNSTDTVDTIFEKNVAVTHSVNLLD
26 RHNGKLCCLKGIAPLQLGKCNIAGWLLGNPECDLLPARSWSYIVETPNSENGACYPGDFIDYEELREQLSSVSSLE
27 RFEIFPKESSWPNHFTFNGVTVSCHRKSSFYRNLLWLTKKGDSPKLTNSYVNNKGKEVLVLWGVHHPSSSDEQQS
28 LYSNGNAYVSVASSNRRFTPEIAARPKVKDQHGMRNYYWTLLEPGDTITFEATGNLIAPWYAFALS RGFESGII
29 SNASMHECNTKCTPQGSINSNLPFQNIHPVTIGCEPKYVRSTKLRMVTGLRNIPSIQYRGLFGAIAAGFIEGGWTGM
30 IDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNLEKR MENLNKKVDDGFLDIWTY
31 NAELLVLLLENERTLDFHDLNVKNLYEKVKSQKNNAKEIGNGCFEFYHKCDNECMESVRNGTYDYPKYSEESKLNRE
32 KIDGVSGGGGLNDIFEAQKIEWHERLVPRGSPGSGYIPEAPRDGQAYVRKDGGEWVLLSTFLGHHHHHH

33
34 **>A/Puerto Rico/8/1934 (H1N1)**

35 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSAFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLD
36 SHNGKLCRLKGIAPLQLGKCNIAGWLLGNPECDPLL PVRWSYIVETPNSENGICYPGDFIDYEELREQLSSVSSFE
37 RFEIFPKESSWPNHNTNGVTAACSHGKSSFYRNLLWLTEKEGSYPKLNKSYVNNKGKEVLVLWGIHHPNSKEQQN
38 LYQENAYVSVVTSNRRFTPEIAERP KVRDQAGRMNYYWTLKPGDTITFEANGNLIAPMYAFALS RGFESGII
39 SNASMHECNTKCTPLGAINSSLPYQNIHPVTIGCEPKYVRS AKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTGM
40 IDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNTVIEKMNIQFTAVGKEFNKLEKR MENLNKKVDDGFLDIWTY
41 NAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNAKEIGNGCFEFYHKCDNECMESVRNGTYDYPKYSEESKLNRE
42 KVDGVSGGGGLNDIFEAQKIEWHERLVPRGSPGSGYIPEAPRDGQAYVRKDGGEWVLLSTFLGHHHHHH

43
44 **>A/AA/Marton/1943 (H1N1)**

45 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSAFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLD
46 SHNGKLCRLKGIAPLQLGKCNIAGWILGNPECESL LRSWSYIVETPNSENGTCYPGDFIDYEELREQLSSVSSFE
47 RFEIFSKESSWPKHNTTRGVTAACSHAGKSSFYRNLLWLTEKDGSPN LNNSYVNNKGKEVLVLWGVHHPNSNIKDQ
48 TLYQKENAYVSVSSNRRFTPEIAERP KVRGQAGRMNYYWTLKPGDTIMFEANGNLIAPWYAFALS RGFESGII
49 TSNASMHECDTKCTPQGAINSSLPFQNIHPVTIGCEPKYVRSTKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTG
50 MIDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNLEKR MENLNKKVDDGFLDIWT
51 YNAELLVLLLENERTLDFHDSNVKNLYEKVKNQLRNNAKEIGNGCFEFYHKCNNECMESVKNGTYDYPKYSEESKLN
52 EKIDSGGGGLNDIFEAQKIEWHERLVPRGSPGSGYIPEAPRDGQAYVRKDGGEWVLLSTFLGHHHHHH

53
54 **>A/duck/Alberta/345/1976 (H1N1)**

55 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICVGYHANNSTDTVDTVLEKNVTVTHSVNLLED
56 SHNGKLCRLKGIAPLQLGKCNVAGWLLGNPECDLLLTANSWSYIIETSNSENGTCYPGEFIDYEELREQLSSISSFE
57 KFEIFPKASSWPNHETTKGVTAACSYSGASSFYRNLLWITKKGTSYPKLSKSYTNNKGKEVLVLWGVHHPSPVSEQQ
58 SLYQNADAYVSVGSSKYNRRFAPEIAARPEVRGQAGRMNYWTLLDQGDITTFEATGNLIAPWYAFALNKGSDSGII
59 TSDAPVHNCDCRQTPHGFALNSSLPFQNVHPITIGECPKYVKSTKLRMATGLRNVPSIQSRGLFGAIAAGFIEGGWTG
60 MIDGWYGYHHQNEQGSYAADQKSTQNAIDGITNKVNSVIEKMNTQFTAVGKEFNLERRIENLNKKVDDGFLDVT
61 YNAELLVLLLENERTLDFHDSNVRNLYEKVKSQLRNNAKEIGNGCFEFYHKCDECEMESVKNGTYDYPKYSEESKLN
62 EEIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

63
64 **>A/USSR/90/1977 (H1N1)**

65 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLED
66 SHNGKLCRLKGIAPLQLGKCNVAGWLLGNPECESLFSKKSWSYIAETPNSENGTCYPGYFADYEELREQLSSVSSFE
67 RFEIFPKERSWPKHNVTRGVTASCSSHGKSSFYRNLLWLTEKNGSYPNLSKSYVNNKEKEVLVLWGVHHPSPNIEDQK
68 TIYRKENAYVSVVSSNYNRRFTPEIAERPQVRGQAGRINYYWTLLLEPGDTIIFEANGNLIAPWHAFALNRGFGSGII
69 TSNASMDECDTKCQTPQGAINSSLPFQNIHPVTIGECPKYVVRSTKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTG
70 MIDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLEKRMENTLNKKVDDGFLDIWT
71 YNAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNNAKEIGNGCFEFYHKCNNECEMESVKNGTYDYPKYSEESKLN
72 EKIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

73
74 **>A/Singapore/6/1986 (H1N1)**

75 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLED
76 SHNGKLCRLKGIAPLQLGNCIAGWILGNPECESLFSKKSWSYIAETPNSENGTCYPGYFADYEELREQLSSVSSFE
77 RFEIFPKESSWPNHTVTGVTASCSSHGKRSSFYRNLLWLTKKNGSYPNLSKSYVNNKEKEVLVLWGVHHPSPNIGDQR
78 AIYHTENAYVSVVSSHYNRRFTPEIAKRPKVRDQEGRINYYWTLLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGII
79 TSNASMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVVRSTKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTG
80 MIDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLEKRMENTLNKKVDDGFLDIWT
81 YNAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNNAKEIGNGCFEFYHKCNNECEMESVKNGTYDYPKYSEESKLN
82 EKIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

83
84 **>A/Texas/36/1991 (H1N1)**

85 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLED
86 SHNGKLCRLKGIAPLQLGNCVAGWILGNPKCESLFSKESWSYIAETPNPENGTCPYGYFADYEELREQLSSVSSFE
87 RFEIFPKESSWPNHTVTGVTASCSSHGKSSFYRNLLWLTEKNGLYPNLSKSYVNNKEKEVLVLWGVHHPSPNIRDQR
88 AIYHTENAYVSVVSSHYSRRTPEIAKRPKVRDQEGRINYYWTLLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGII
89 TSNASMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVVRSTKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTG
90 MIDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLEKRMENTLNKKVDDGFLDIWT
91 YNAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNNAKEIGNGCFEFYHKCNNECEMESVKNGTYDYPKYSEESKLN
92 GKIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

93
94 **>A/Beijing/262/1995 (H1N1)**

95 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLED
96 SHNGKLCRLKGIAPLQLGNCVAGWILGNPECESLISKESWSYIVETPNPENGTCPYGYFADYEELREQLSSVSSFE
97 RFEIFPKESSWPNHTVTGVTASCSSHGKSSFYRNLLWLTEKNGLYPNLSNSYVNNKEKEVLVLWGVHHPSPNIGVQRA
98 IYHTENAYVSVVSSHYSRRTPEIAKRPKVRDQEGRINYYWTLLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIIT
99 SNAPMNECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVVRSTKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTGM
100 MDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLEKRMENTLNKKVDDGFLDIWTY
101 NAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNNAKEIGNGCFEFYHKCNNECEMESVKNGTYDYPKYSEESKLNRE
102 KIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

103
104 **>A/New Caledonia/20/1999 (H1N1)**

105 MVLVNQSHQGFNKEHTSKMVSAIVLYVLLAAAAHSFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLED
106 SHNGKLCRLKGIAPLQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCPYGYFADYEELREQLSSVSSFE
107 RFEIFPKESSWPNHTVTGVSASCSSHGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGVHHPSPNIGNQRA
108 LYHTENAYVSVVSSHYSRRTPEIAKRPKVRDQEGRINYYWTLLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIIT
109 SNAPMNECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSALRMTGLRNIPSIQSRGLFGAIAAGFIEGGWTGM
110 VDGWYGYHHQNEQGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLEKRMENTLNKKVDDGFLDIWTY
111 NAELLVLLLENERTLDFHDSNVKNLYEKVKSQKNNNAKEIGNGCFEFYHKCNNECEMESVKNGTYDYPKYSEESKLNRE
112 KIDSGGGGLNDIFEAQKIEWHERLVPGRSPGSGYIPEAPRDGQAYVRKDGWVLLSTFLGHHHHHH

113
114 **>A/Solomon Islands/3/2006 (H1N1)**
115 MVLVNQSHQGFNKEHTSKMVSIAIVLYVLLAAAAHSAFAADPGDTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLLED
116 SHNGKLCRLKGIAPLQLGNCVAGWILGNPECELLISRESWSYIVEKPNPENGTCYPGHFADYEELREQLSSVSSFE
117 RFEIFPKESSWPNHTTTGVSASC SHNGESSFYKNLLWLTGKNGLYPNLSKSYANNKEKEVLVLWGVHHPNIGDQRA
118 LYHKENAYVSVVSSHYSRKFTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPRYAFALSRGFGSGIIN
119 SNAPMDECDKACQTPQGAINSSLPFQNVHPVTIGECPKYVRSAKLRMVTGLRNIPSIQSRGLFGAIAAGFIEGGWTGM
120 VDGWYGYHHQNEQSGSYAADQKSTQNAINGITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDDGFIDIWY
121 NAE LLV LLENERTLDFHDSNVKNLYEKVKSQ LKNNAKE I GNGCFEFYHKCNDECMESVKNGTYDYPKYSEESKLNRE
122 KIDSGGGGLNDIFEAQKIEWHERLVP RGS PGSGYIPEAPRDGQAYVRKDG EWVLLSTFLGHHHHHH
123
124 **>A/California/04/2009 HA2 E47G (H1N1)**
125 MVLVNQSHQGFNKEHTSKMVSIAIVLYVLLAAAAHSAFAADPGDTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLED
126 KHNGKLCRLRGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFE
127 RFEIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQ
128 SLYQNADTYV FVGSSRYSKFKKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGII
129 ISDTPVHDCNTTCTPKGAIN TSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAAGFIEGGWTG
130 MVDGWYGYHHQNEQSGSYAADLKSTQNAIDGITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWT
131 YNAELLV LLENERTLDYHDSNVKNLYEKVRSQ LKNNAKE I GNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNR
132 EEIDSGGGGLNDIFEAQKIEWHERLVP RGS PGSGYIPEAPRDGQAYVRKDG EWVLLSTFLGHHHHHH

133 **K_d determination.** The experimental binding curves for each Fab or IgG for fitting k_{on} and k_{off}
134 for K_d determination are reported below.

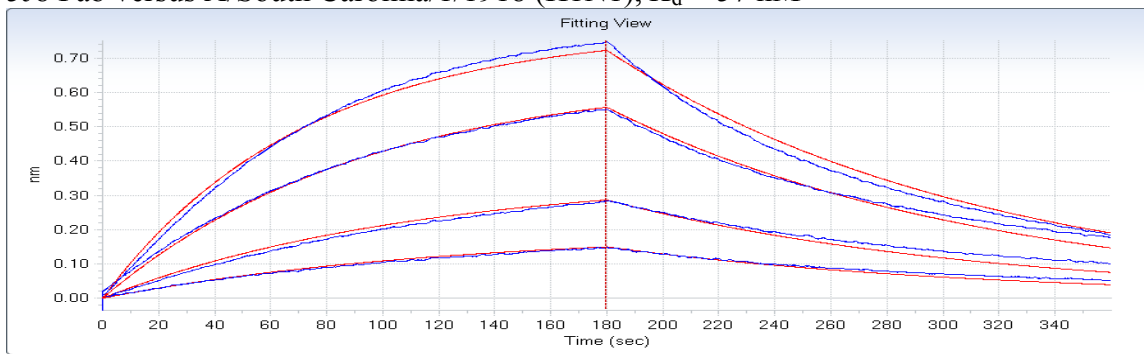
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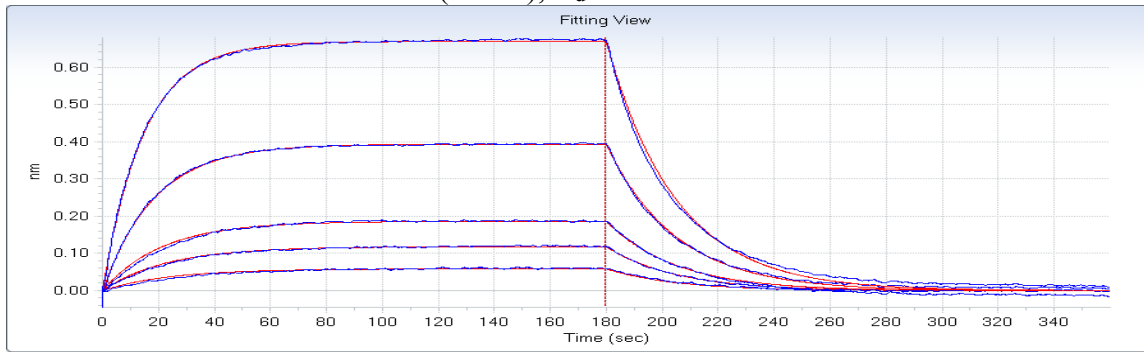
139 5J8 Fab versus A/South Carolina/1/1918 (H1N1), $K_d = 57$ nM



140

141

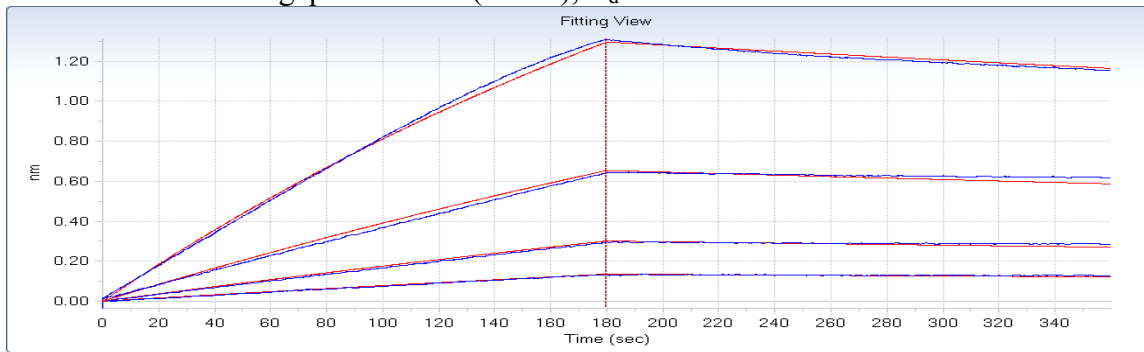
142 5J8 Fab versus A/USSR/90/1977 (H1N1), $K_d = 590$ nM



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144

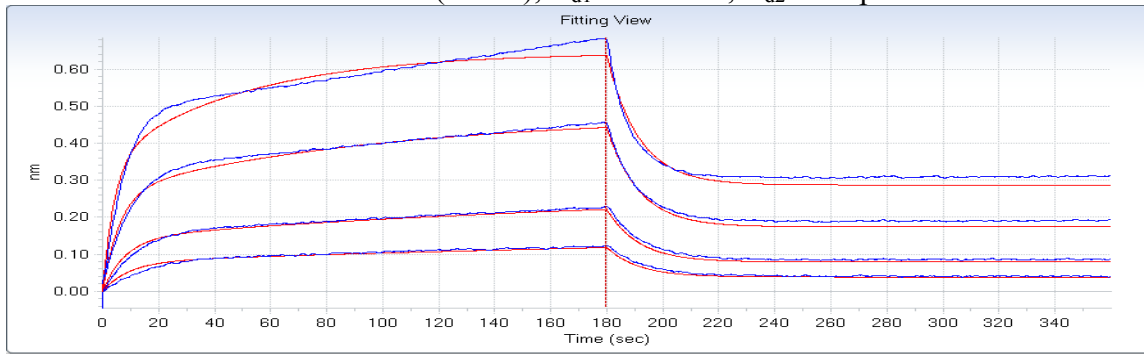
145 5J8 Fab versus A/Singapore/6/1986 (H1N1), $K_d = 5.7$ nM



146

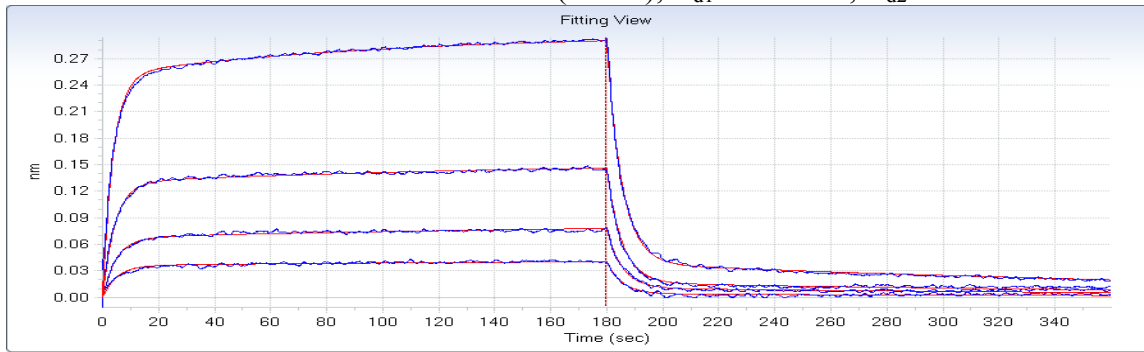
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148 5J8 Fab versus A/Texas/36/1991 (H1N1), $K_{d1} = 120$ nM, $K_{d2} = 46$ pM



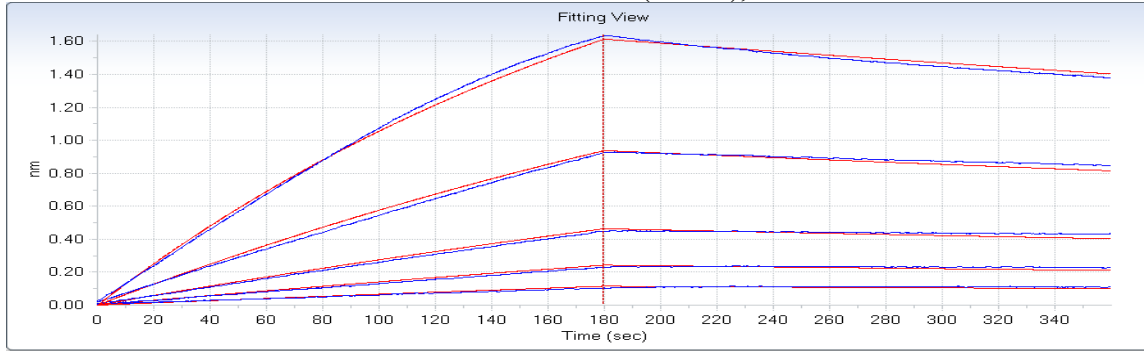
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151 5J8 Fab versus A/Solomon Islands/3/2006 (H1N1), $K_{d1} = 930$ nM, $K_{d2} = 240$ nM



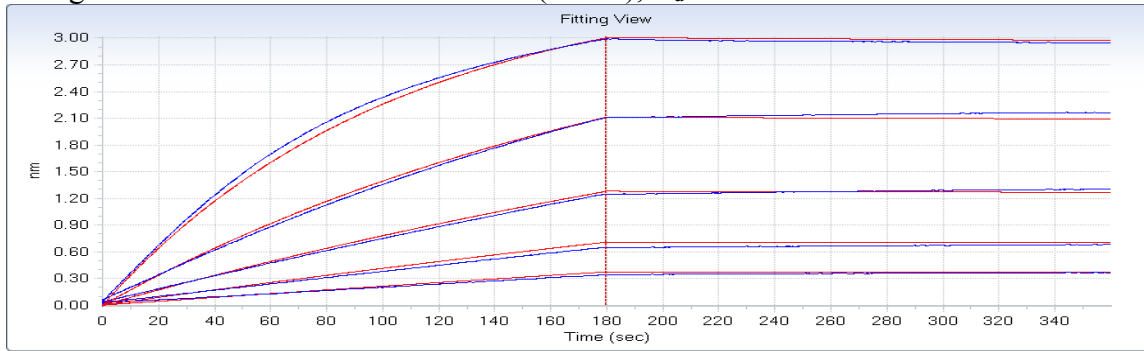
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153

154 5J8 Fab versus A/California/04/2009 HA2 E47G (H1N1), $K_d = 10$ nM



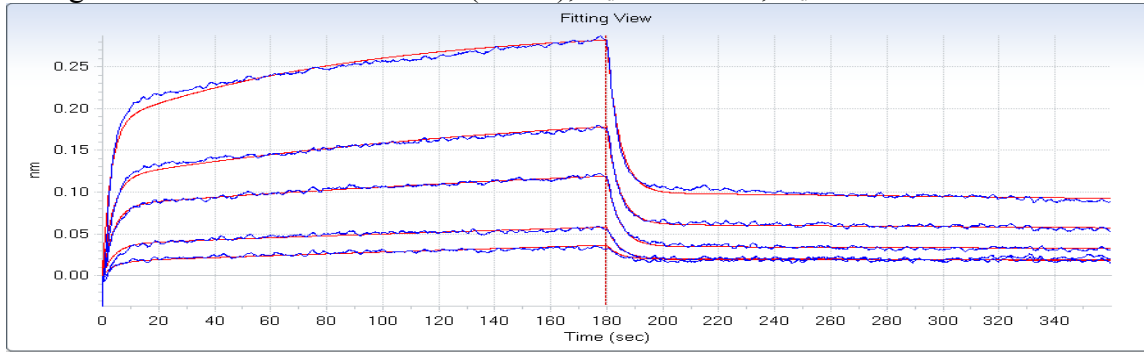
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157 5J8 IgG versus A/South Carolina/1/1918 (H1N1), $K_d = 0.30$ nM



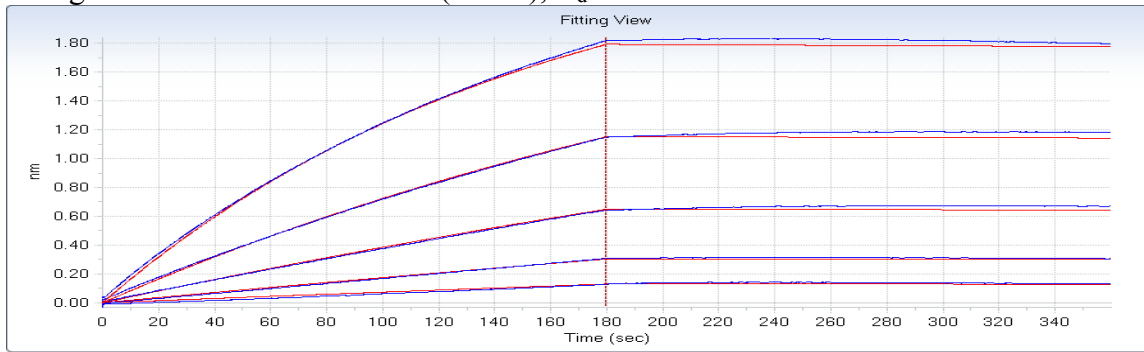
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160 5J8 IgG versus A/AA/Marton/1943 (H1N1), $K_{d1} = 950$ nM, $K_{d2} = 17$ nM



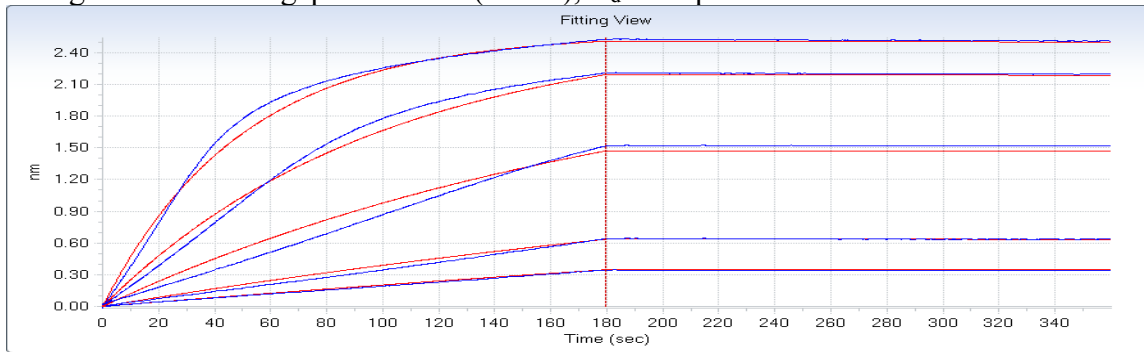
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162

163 5J8 IgG versus A/USSR/90/1977 (H1N1), $K_d = 0.40$ nM



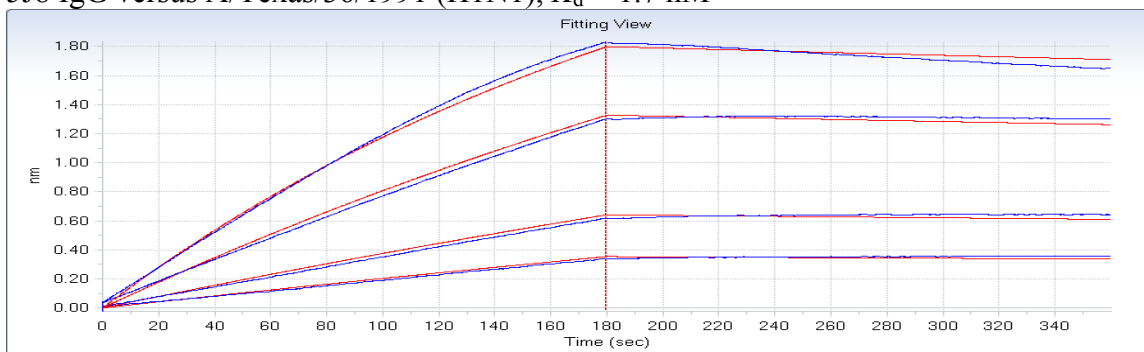
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5J8 IgG versus A/Singapore/6/1986 (H1N1), $K_d = 51$ pM



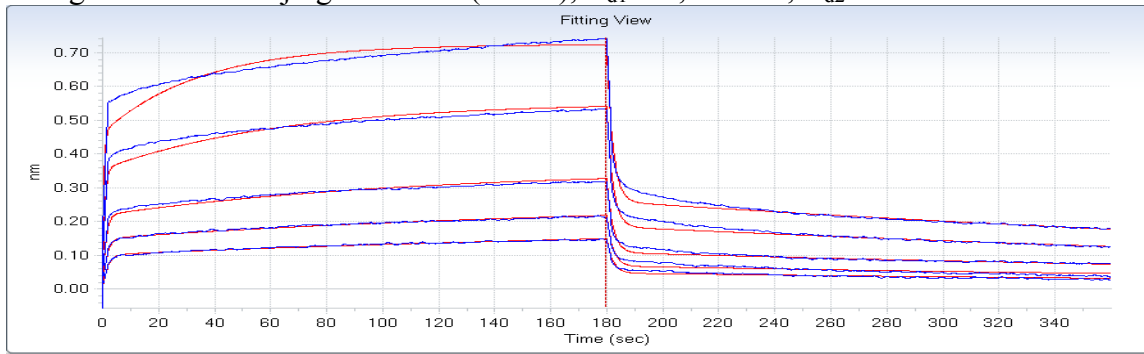
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5J8 IgG versus A/Texas/36/1991 (H1N1), $K_d = 1.7$ nM



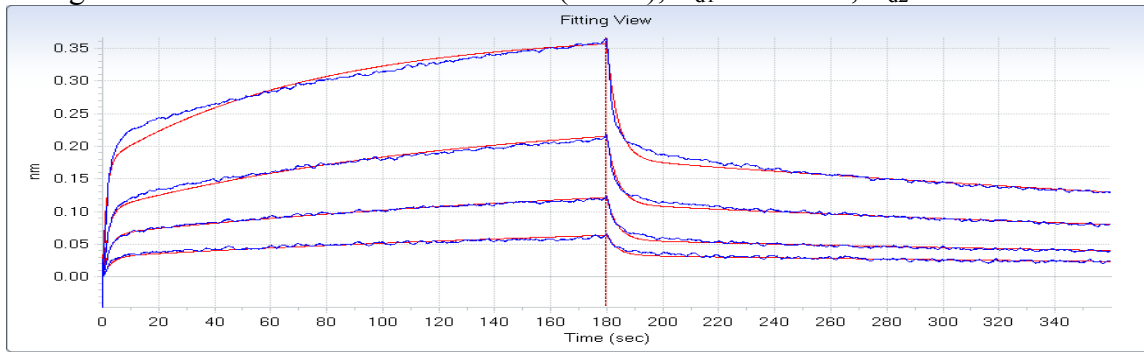
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172 5J8 IgG versus A/Beijing/262/1995 (H1N1), $K_{d1} = 1,350$ nM, $K_{d2} = 400$ nM



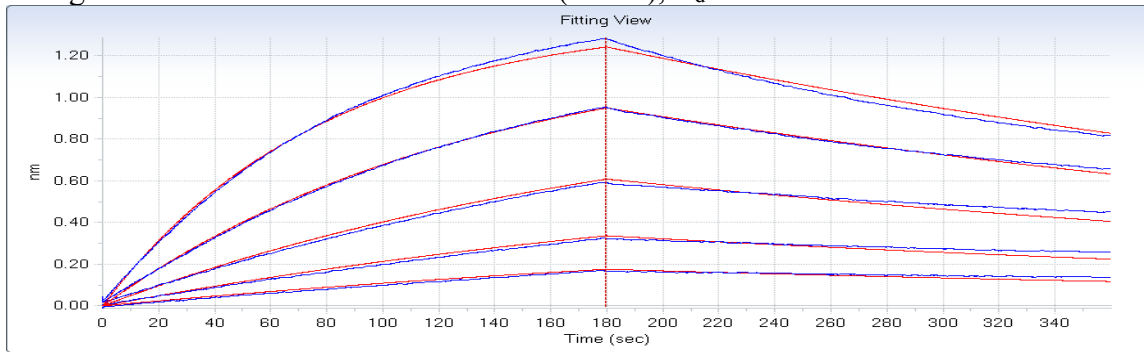
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175 5J8 IgG versus A/New Caledonia/20/1999 (H1N1), $K_{d1} = 220$ nM, $K_{d2} = 63$ nM



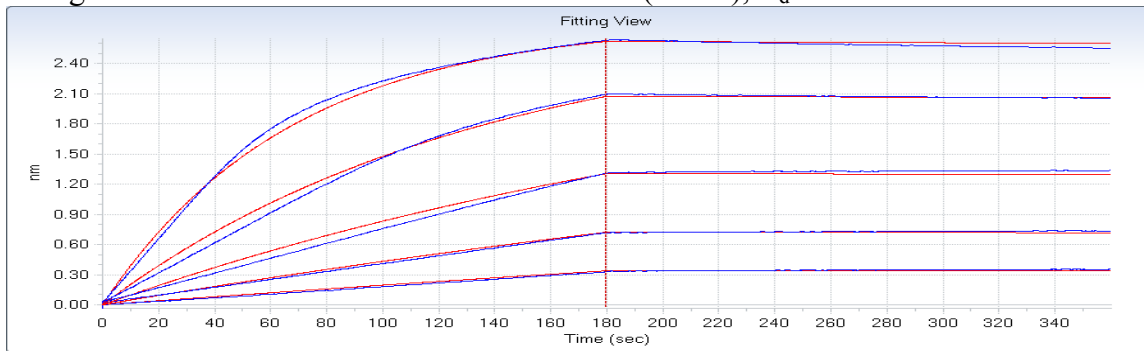
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178 5J8 IgG versus A/Solomon Islands/3/2006 (H1N1), $K_d = 11$ nM



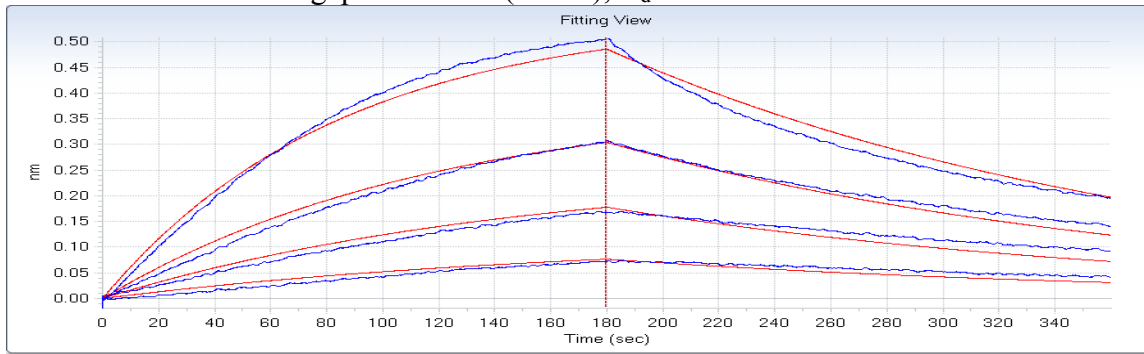
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181 5J8 IgG versus A/California/04/2009 HA2 E47G (H1N1), $K_d = 0.15$ nM



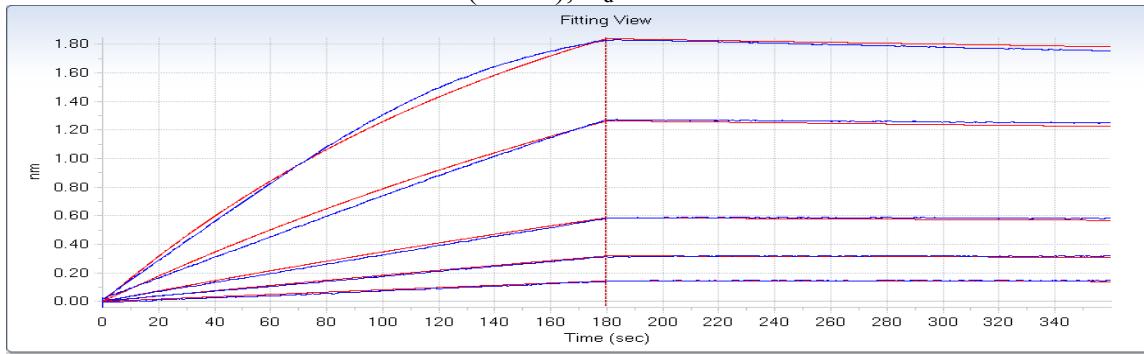
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184 CH65 Fab versus A/Singapore/6/1986 (H1N1), $K_d = 18$ nM



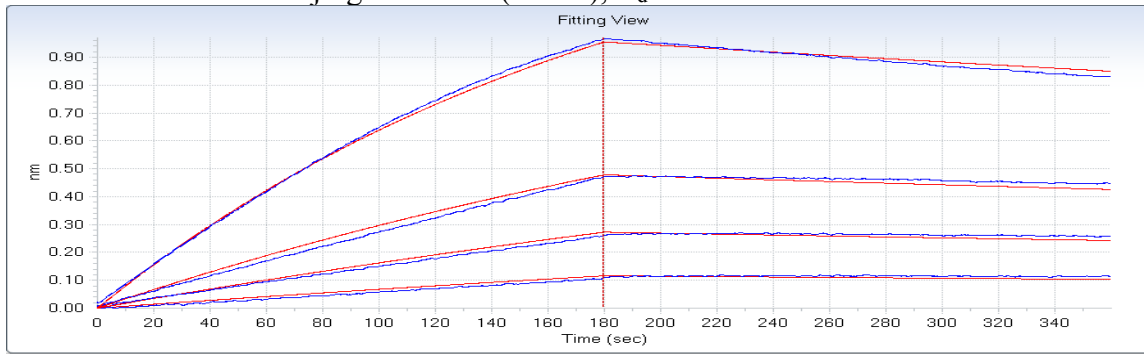
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186

187 CH65 Fab versus A/Texas/36/1991 (H1N1), $K_d = 1.5$ nM



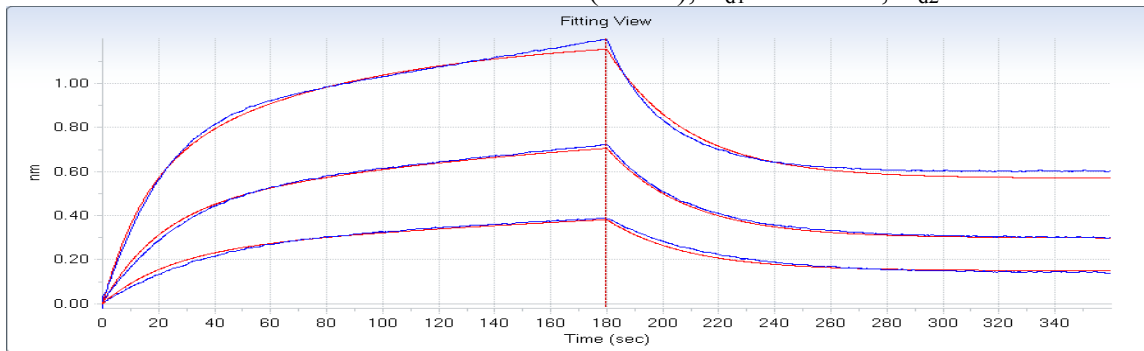
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189 CH65 Fab versus A/Beijing/262/1995 (H1N1), $K_d = 3.5$ nM



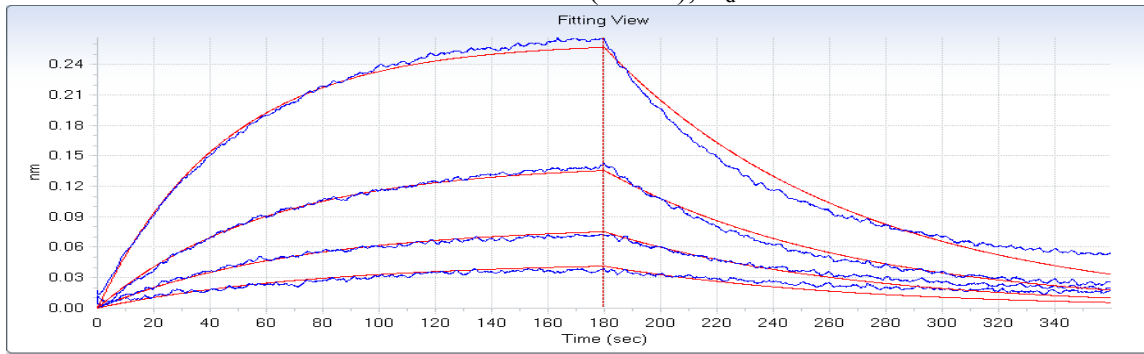
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192 CH65 Fab versus A/New Caledonia/20/1999 (H1N1), $K_{d1} = 110$ nM, $K_{d2} = 0.28$ nM



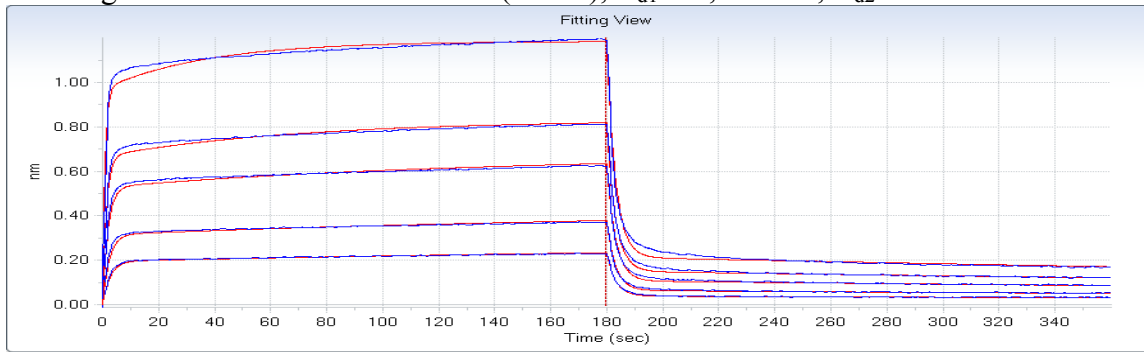
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195 CH65 Fab versus A/Solomon Islands/3/2006 (H1N1), $K_d = 26$ nM



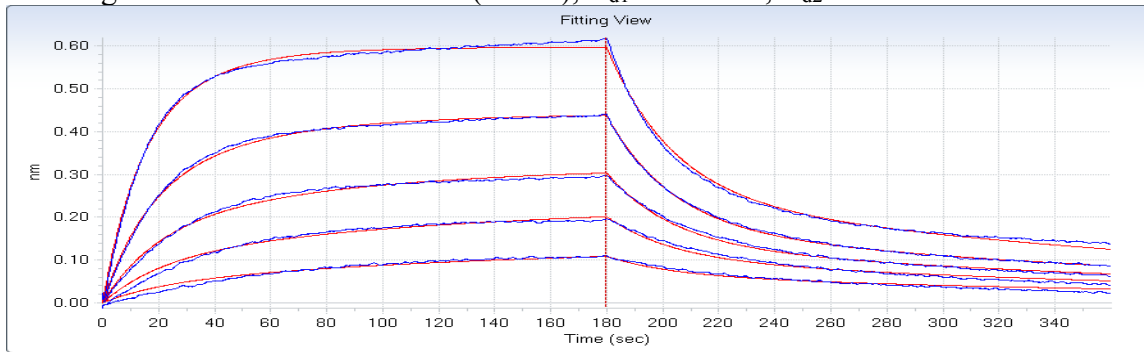
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198 CH65 IgG versus A/AA/Marton/1943 (H1N1), $K_{d1} = 2,000$ nM, $K_{d2} = 240$ nM



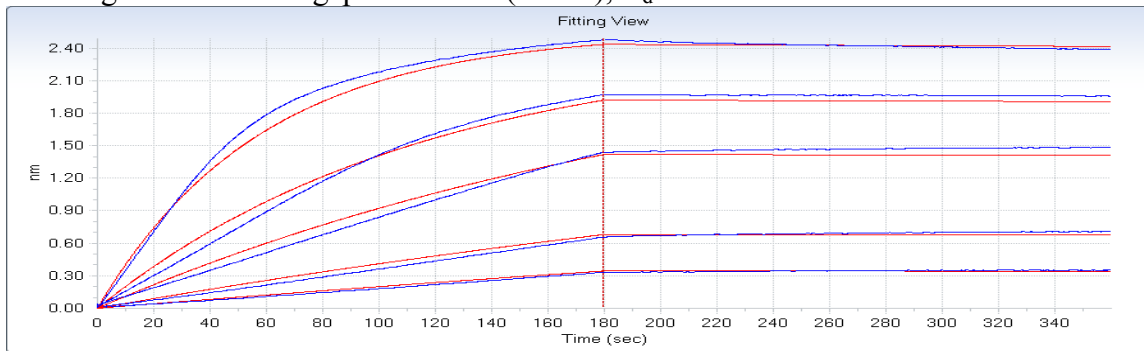
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201 CH65 IgG versus A/USSR/90/1977 (H1N1), $K_{d1} = 230$ nM, $K_{d2} = 23$ nM



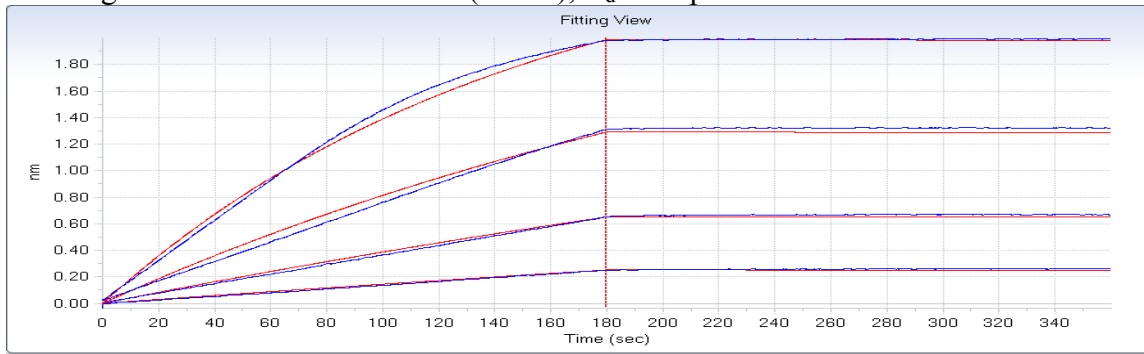
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204 CH65 IgG versus A/Singapore/6/1986 (H1N1), $K_d = 0.13$ nM



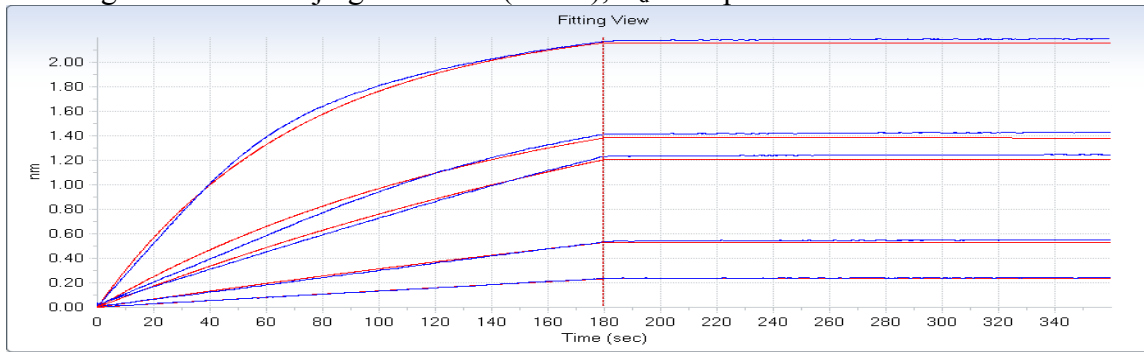
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206

207 CH65 IgG versus A/Texas/36/1991 (H1N1), $K_d = 76$ pM



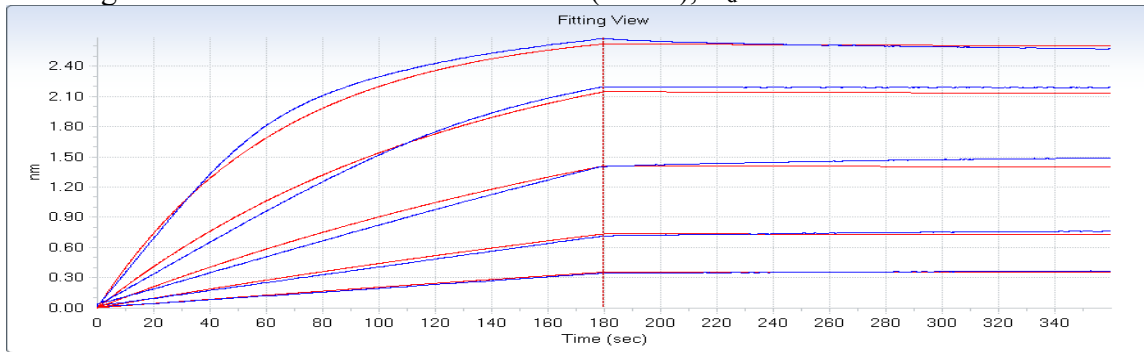
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209

210 CH65 IgG versus A/Beijing/262/1995 (H1N1), $K_d = 23$ pM



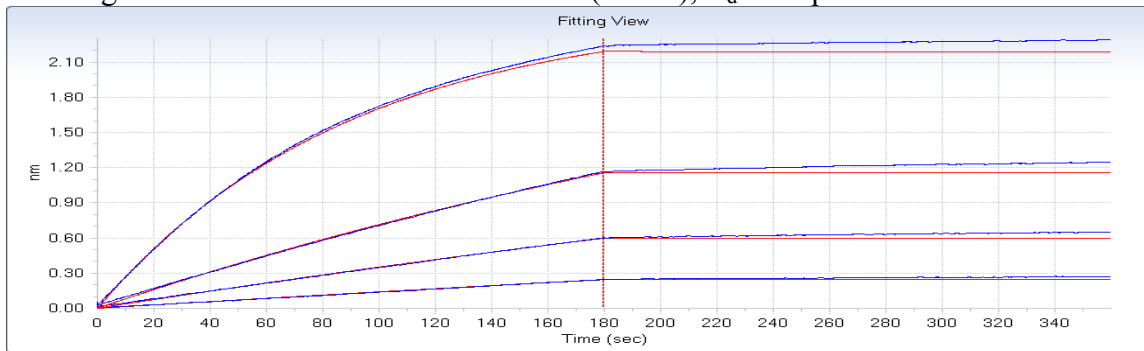
211
212

213 CH65 IgG versus A/New Caledonia/20/1999 (H1N1), $K_d = 0.15$ nM



214
215

216 CH65 IgG versus A/Solomon Islands/3/2006 (H1N1), $K_d = 40$ pM



217