Figure 1: Lists of preferred residues

A: List of the preferred CDR positions, of which all positions or a subset should be diversified to form the two binding regions in the most preferred dual targeting antibodies (all numbering according to Kabat)

Group "Lib1" = VL24, VL25, VL26, VL27, VL28#, VL29##, VL93, VL94, VL95§, VL95a§, VL95b§, VH58, VH59, VH60, VH61, VH62, VH63, VH64, VH65
Group "Lib2" = VH26*, VH27*, VH28*, VH29*, VH30*, VH31, VH32, VH94**, VH96-VH99, VH102, VL49**, VL53, VL54, VL55, VL56

This residue having number 28 in the Kabat nomenclature assumes that CDR-L1 length is 11; if the length is 10, then the residue number is 29; if the length is 12 or more, then the residue number is 27a.
This residue having number 29 in the Kabat nomenclature assumes that CDR-L1 length is 11; if the length is 10, then the residue number is 30; if the length is 12, then the residue number is 28; if the length is 13 or more, then the residue number is 27b.
$ Only in case of libraries comprising Vlambda light chains
* These residues are not included in the Kabat definition of CDR residues (Kabat et al., 1991), but they are included in the Chothia definition of CDR residues (Chothia et al., 1992), and they are included in our definition of CDR residues.
** These residues are not included in the Kabat definition of CDR residues (Kabat et al., 1991), but they are included in the Contact definition of CDR residues (MacCallum et al., 1996), and they are included in our definition of CDR residues.

B: List of preferred enhancing positions that can be diversified in some library designs to enhance the properties of the two binding regions in the novel dual targeting antibodies (all numbering according to Kabat)

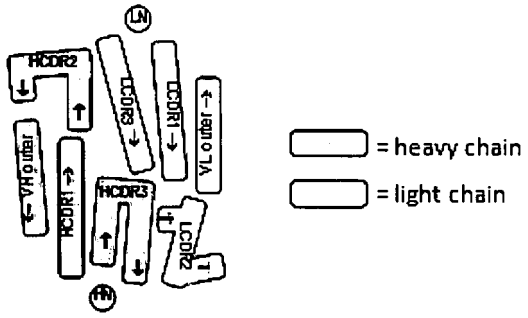
Group "Lib1E" = VL1, VL2, VL3, VL69, VL70, VL100, VH46
Group "Lib2E" = VH1, VH2, VH3, VH25, VH76, VH105, VL45, VL57, VL58

C: List of preferred CDR positions, of which all positions or a subset is preferably left invariant or subjected to restricted diversification in both Lib1 and Lib2 library designs, to create an antibody core region that allows the two binding regions comprising Lib1 and Lib2 residues to behave independently (all numbering according to Kabat)

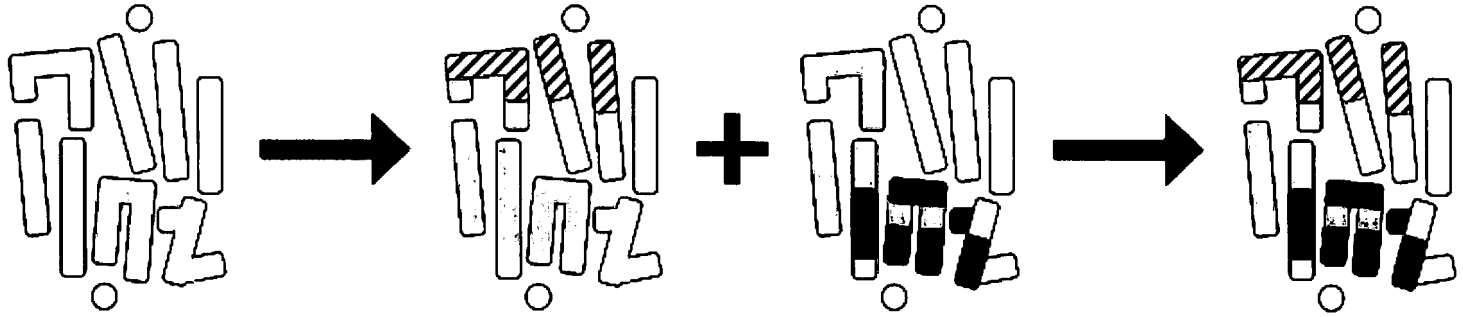
VL residues = VL32, VL33, VL34, VL50, VL51, VL52, VL89, VL90, VL91, VL96
VH residues = VH33, VH34, VH35, VH50, VH51, VH52, VH52a, VH52b if present, VH52c if present, VH53, VH54, VH55, VH57, VH95, and the residue directly before VH101 (which can have variable numbering depending on HCDR3 length)

Figure 2: Novel discovery process

A) Schematic illustration of a heterodimeric VH-VL antibody variable domain (top view):



B) Method of Discovery:



C) Residue Definitions:

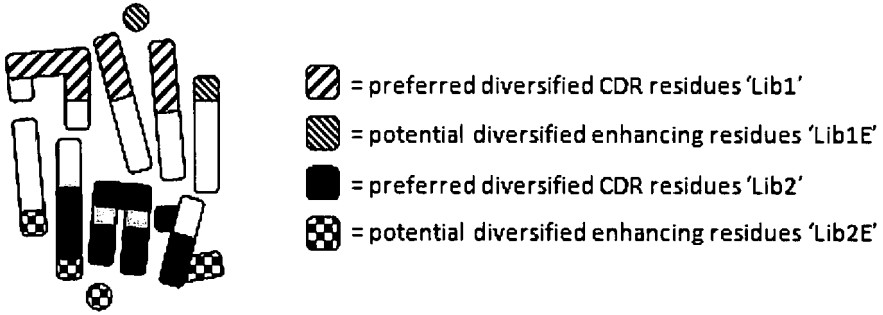


Figure 3: Library Designs

X = diversified position

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                    10        20        30        40        50        60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Dummy 1 VK          DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
Lib D1L1 VK         XXQMTQSPSSLSASVGDRVTITCRXXXXXSSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGXXFTLTISSLQPEDFATYYCQQYSXLPYTFGQGTKVEIKR
Lib D1L2 VK         XXXMTQSPSSLSASVGDRVTITCRXXXXXSSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGXXFTLTISSLQPEDFATYYCQQYSXXPYTFGQGTKVEIKR

                    10        20        30        40        50        60        70        80        90       100       110
                    ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
Dummy 1 VH          EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
Lib D1L1 VH         EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTXYXXXXXGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
Lib D1L2 VH         EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLXWIGQISGSGGSTXYXXXXXGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS

                    10        20        30        40        50        60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Dummy 1 VK          DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
Lib D1H1 VK         DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPXLLIXAASXLXXXXPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
Lib D1H2 VK         DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPXLLIXAASXLXXXXPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
Lib D1H3 VK         DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIXAASXLXXXXPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR

                    10        20        30        40        50        60        70        80        90       100       110
                    ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
Dummy 1 VH          EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
Lib D1H1 VH         XXQLVESGGGLVQPGGSLRLSCAAXGFXFSXYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKXTLYLQMNSLRAEDTAVYYCAXDSGYFDXWGQGTLVTVSS
Lib D1H2 VH         XXXLVESGGGLVQPGGSLRLSCAAXGXXXSXYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKXTLYLQMNSLRAEDTAVYYCARDSGYFDXWGQGTLVTVSS
Lib D1H3 VH         XXQLVESGGGLVQPGGSLRLSCAASGXXXSXXAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDXGXFDXWGQGTLVTVSS

                              20        30        40        50        60        70        80        90       100
                    ....|.........|....|....|..ab..|....|....|....|....|....|....|....|....|....|....|....|ab....|....|..
Dummy 2 VL          SSVLTQPPSVSGAPGQRVTISCSGSSSNIGSNTVSWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVL
Lib D2L1 VL         XXVLTQPPSVSGAPGQRVTISCSGXXXNIGXNTVSWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSKSGXSASLAITGLQSEDEADYYCAAWDXXXXGYVFGTGTKVTVL

                    10        20        30        40        50        60        70        80        90              110
                    ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|.........|....|...
Dummy 2 VH          EVQLVESGGGLVQPGGSLRLSCAASGFTFSSNYMSWVRQAPGKGLEWVGTISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSSGYFDYWGQGTLVTVSS
Lib D2L1 VH         EVQLVESGGGLVQPGGSLRLSCAASGFTFSSNYMSWVRQAPGKGLEWVGTISGSGGSTXYXXXXXGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSSGYFDYWGQGTLVTVSS

                              20        30        40        50        60        70        80        90       100
                    ....|.........|....|....|..ab..|....|....|....|....|....|....|....|....|....|....|....|ab....|....|..
Dummy 2 VL          SSVLTQPPSVSGAPGQRVTISCSGSSSNIGSNTVSWYQQLPGTAPKLLIYGNNNRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVL
Lib D2H1 VL         SSVLTQPPSVSGAPGQRVTISCSGSSSNIGSNTVSWYQQLPGTAPKLLIXGNNXRXXXXPDRFSGSKSGTSASLAITGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVL

                    10        20        30        40        50        60        70        80        90       100       110
                    ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
Dummy 2 VH          EVQLVESGGGLVQPGGSLRLSCAASGFTFSSNYMSWVRQAPGKGLEWVGTISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSSGYFDYWGQGTLVTVSS
Lib D2H1 VH         XXQLVESGGGLVQPGGSLRLSCAASGXXXSXXYMSWVRQAPGKGLEWVGTISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAXTXXGXFDXWGQGTLVTVSS
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Figure 4: Examples of bi-specific antibodies

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                10        20        30        40        50        60        70        80        90        100
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Dummy 1 VK      DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
LG1 VK          GIQMTQSPSSLSASVGDRVTITCRARWYISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGKDFTLTISSLQPEDFATYYCQQYSELPYTFGQGTKVEIKR
HM2 VK          DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIFAASRLILNVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
DT3 VK          GIQMTQSPSSLSASVGDRVTITCRARWYISSYLAWYQQKPGKAPKLLIFAASRLILNVPSRFSGSGSGKDFTLTISSLQPEDFATYYCQQYSELPYTFGQGTKVEIKR

                10        20        30        40        50        60        70        80                  90        100       110
                ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
Dummy 1 VH     EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
LG1 VH          EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTWYNHDIKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
HM2 VH          WVQLVESGGGLVQPGGSLRLSCAARGFPFSHYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDWWGQGTLVTVSS
DT3 VH          WVQLVESGGGLVQPGGSLRLSCAARGFPFSHYAMSWIRQAPGKGLEWIGQISGSGGSTWYNHDIKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDWWGQGTLVTVSS

                10        20        30        40        50        60        70        80        90        100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Dummy 1 VK      DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
IL6P VK         NIQKTQSPSSLSASVGDRVTITCRALWHISSYLAWYQQKPGKAPKLLIYAASSLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSGLPYTFGQGTKVEIKR
VEGFP VK        DTQMTQSPSSLSASVGDRVTITCRASQSISSYLAWYQQKPGKAPKLLIYAASHLHYGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSLPYTFGQGTKVEIKR
VH6L VK         NIQKTQSPSSLSASVGDRVTITCRALWHISSYLAWYQQKPGKAPKLLIYAASHLHYGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSGLPYTFGQGTKVEIKR

                10        20        30        40        50        60        70        80                  90        100       110
                ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
Dummy 1 VH     EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
IL6P VH         EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWIGQISGSGGSTVYNYNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
VEGFP VH        YYQLVESGGGLVQPGGSLRLSCAADGFLFSGYAMSWIRQAPGKGLEWIGQISGSGGSTYYNDNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDTGYFDHWGQGTLVTVSS
VH6L VH         YYQLVESGGGLVQPGGSLRLSCAADGFLFSGYAMSWIRQAPGKGLEWIGQISGSGGSTVYNYNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDTGYFDHWGQGTLVTVSS

                10        20        30        40        50        60        70        80        90        100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
GH6L VK         ATQRTQSPSSLSASVGDRVTITCRALWHISSYLAWYQQKPGKAPKLLIYAASWLYWDVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSGLPYTFGQGTKVEIKR

                10        20        30        40        50        60        70        80                  90        100       110
                ....|....|....|....|....|....|....|....|....|..a..|....|....|....|....|....|..abc..|....|....|...|....|....|...
GH6L VH         WVQLVESGGGLVQPGGSLRLSCAAKGALFSTYAMSWIRQAPGKGLEWIGQISGSGGSTVYNYNVLGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDSGYFDIWGQGTLVTVSS
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Figure 5: ELISA data of anti-MBP anti-GST dual targeting Fab clone DT3, using Dummy 1 Fab and irrelevant targets HEL and VEGF as controls

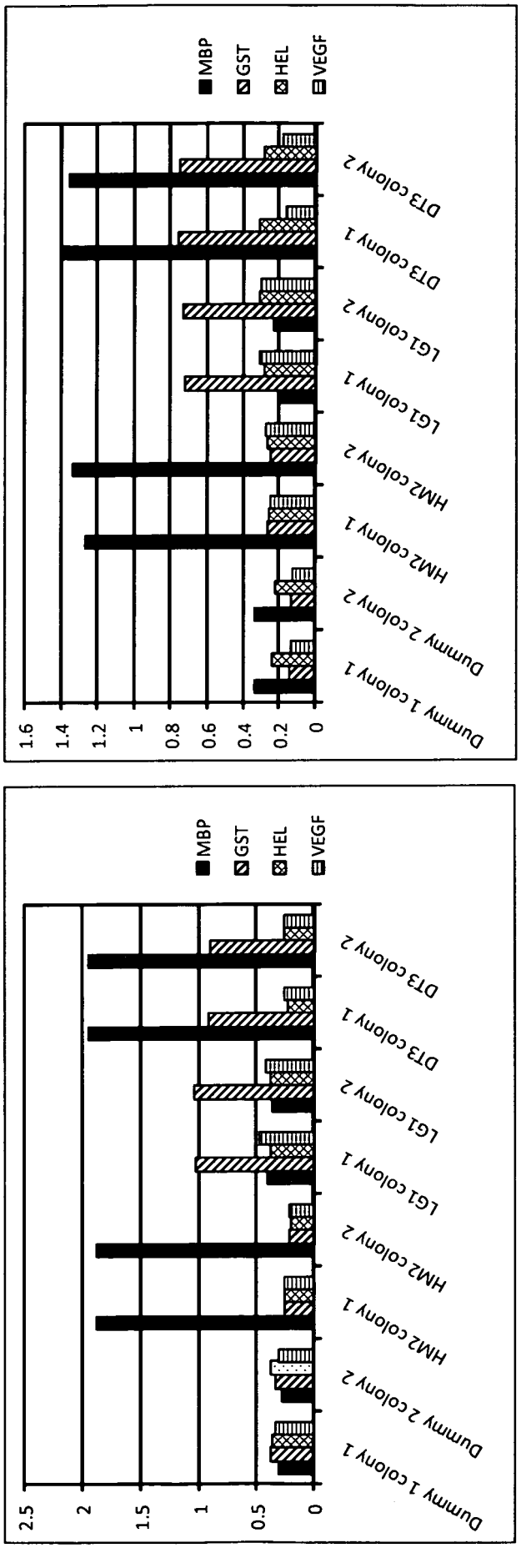


Figure 6: Biacore™ data of anti-MBP / anti-GST dual targeting Fab clone DT3

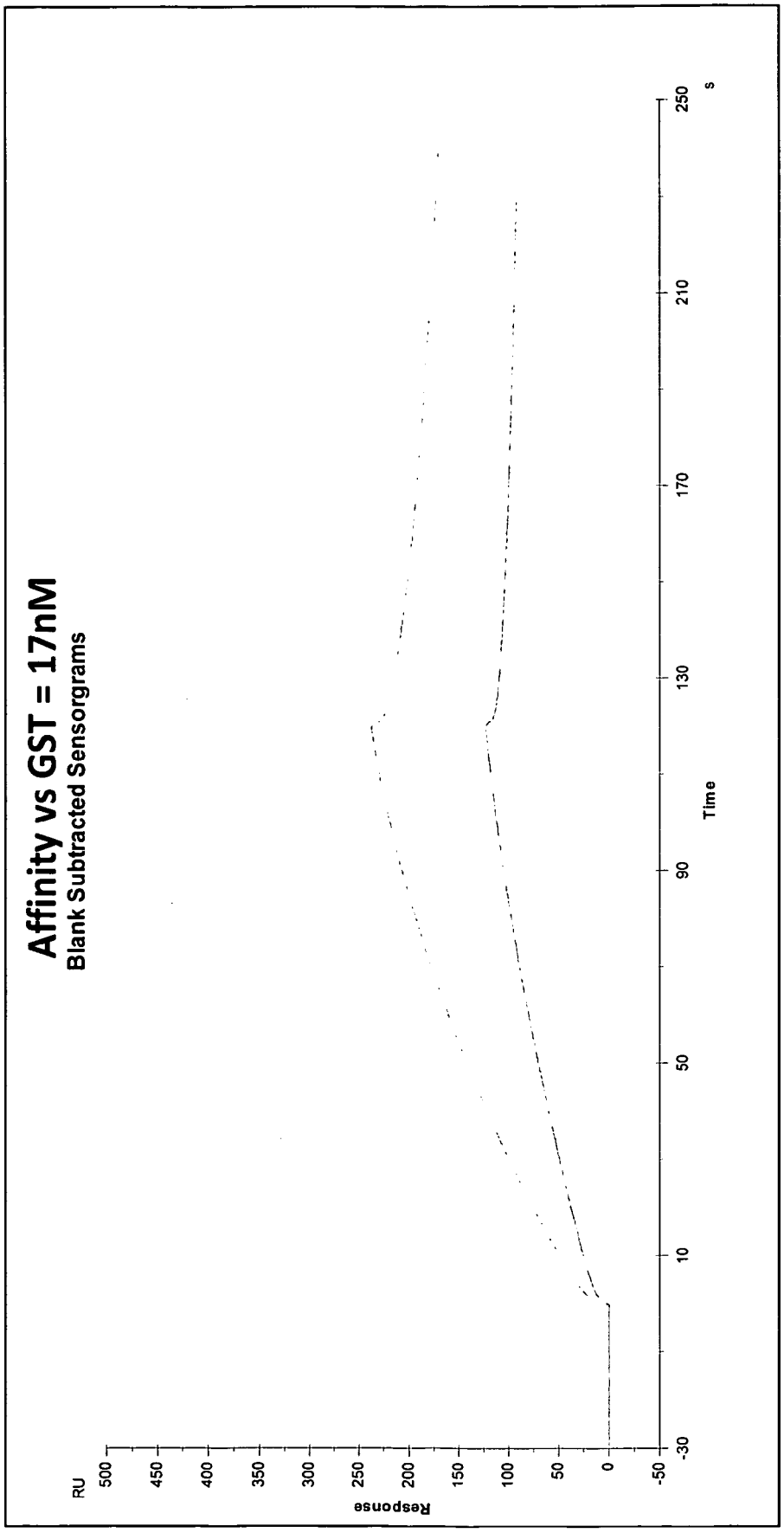


Figure 6: Biacore™ data of anti-MBP / anti-GST dual targeting Fab clone DT3 (contd.)

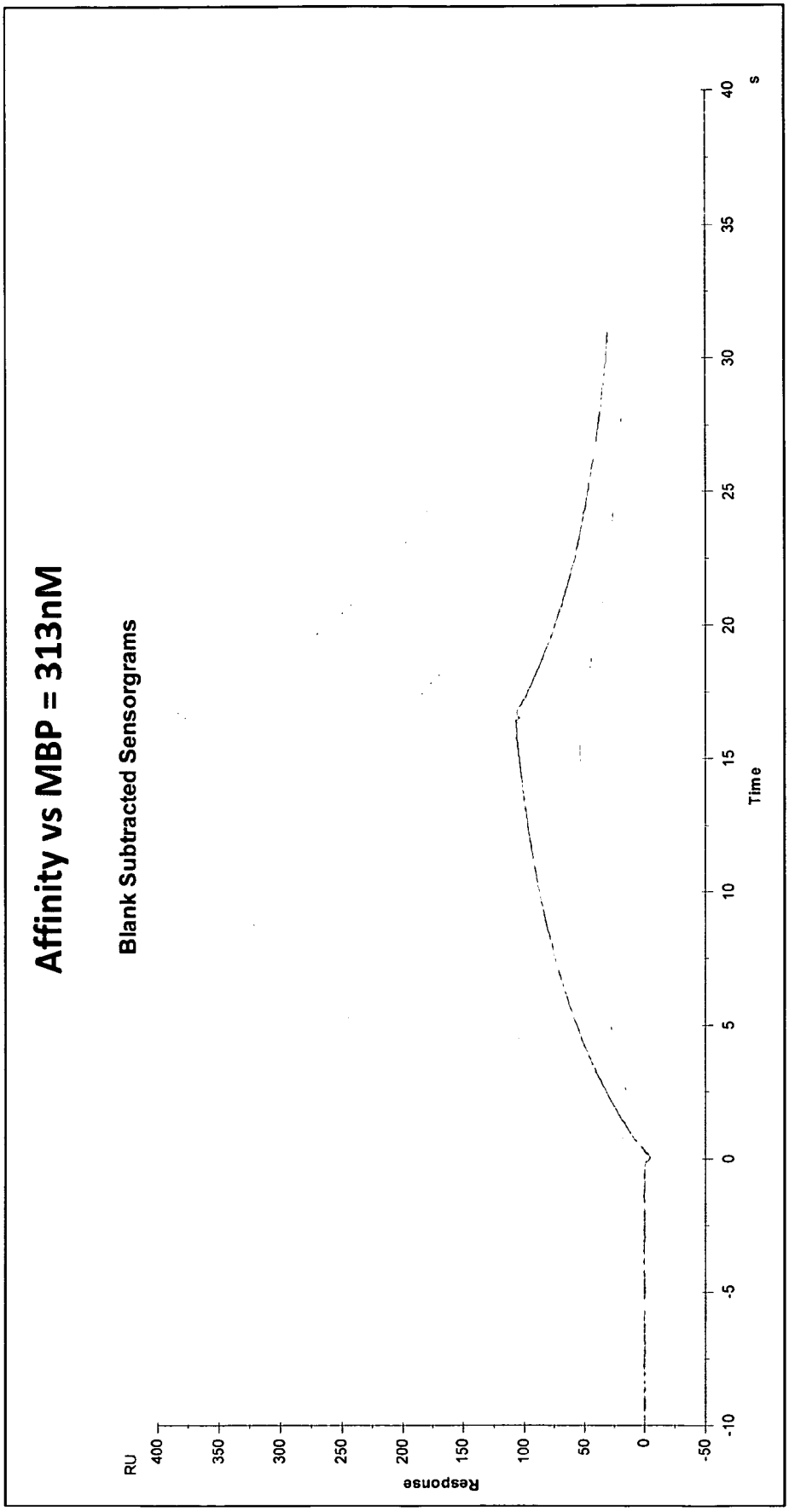


Figure 7: Biacore™ analysis of parental and bi-specific antibodies against VEGF and IL6

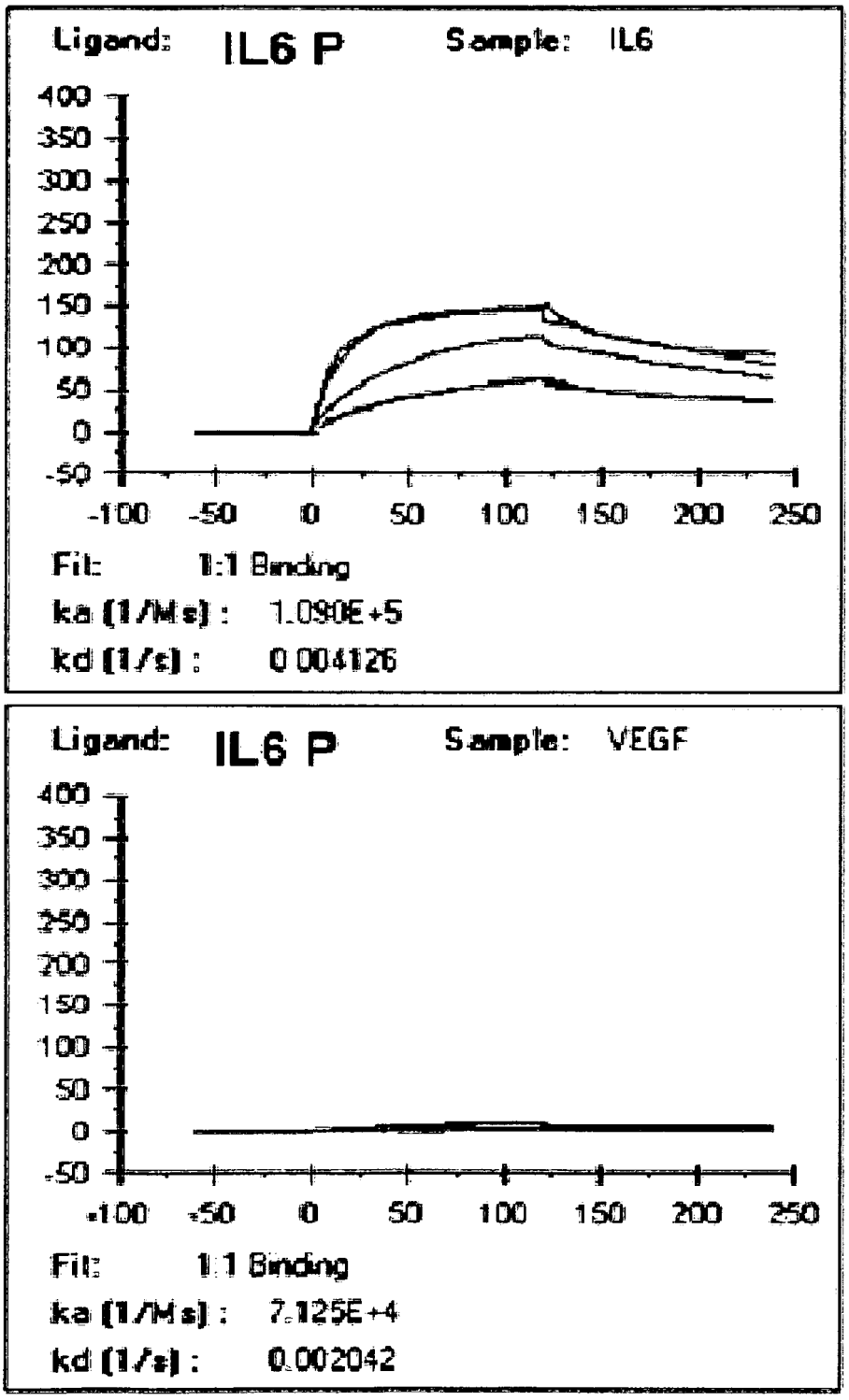
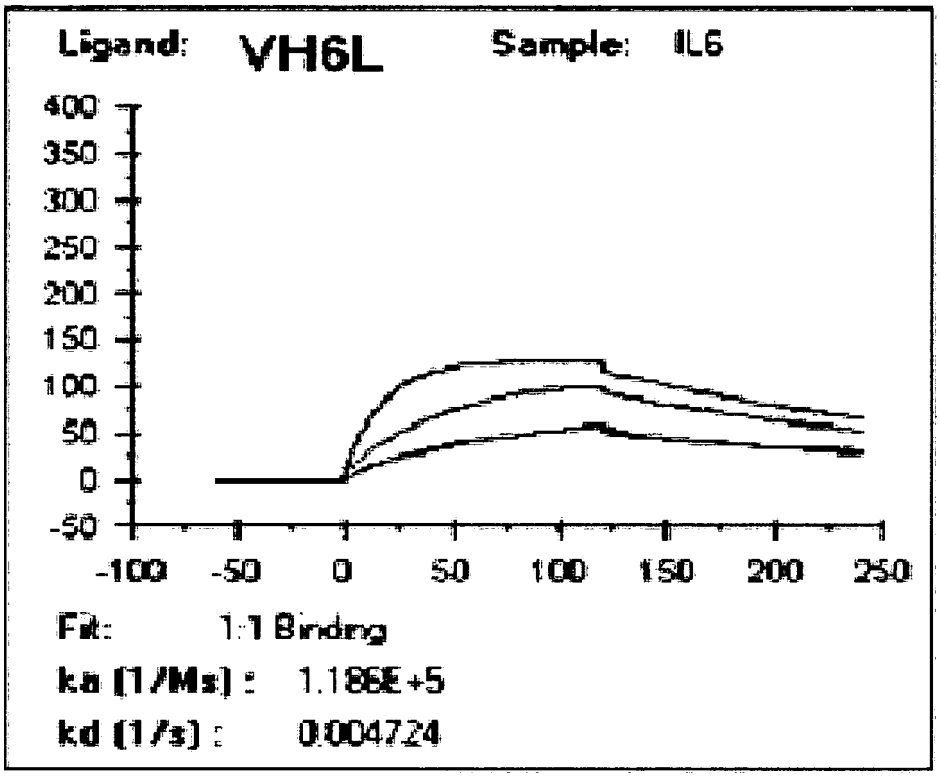


Figure 7: Biacore™ analysis of parental and bi-specific antibodies against VEGF and IL6 (contd.)



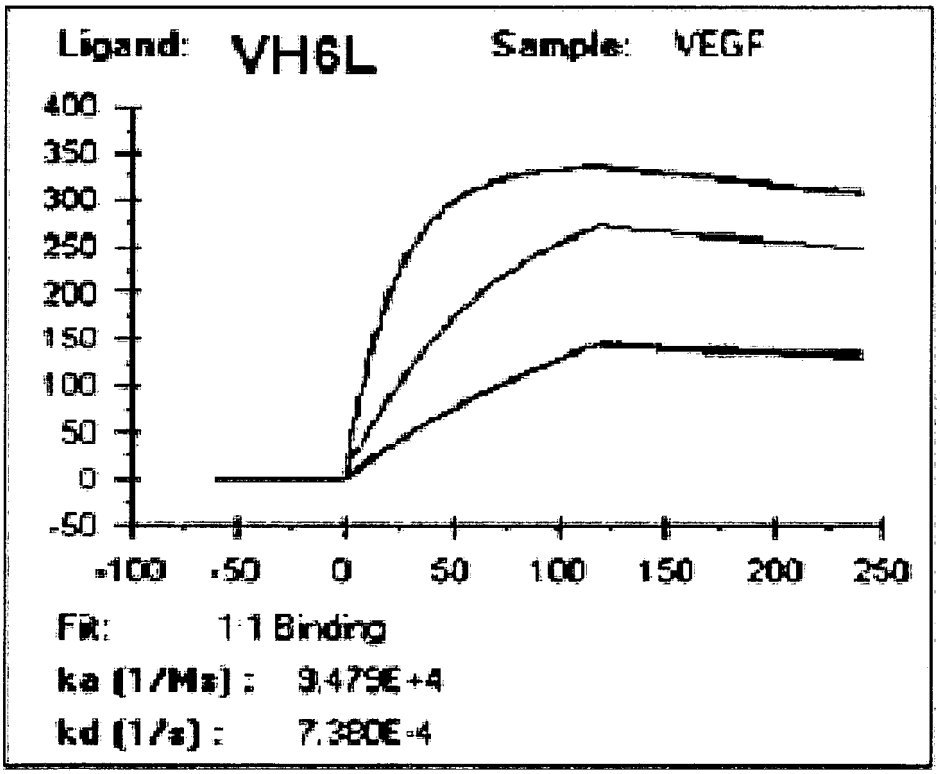


Figure 7: Biacore™ analysis of parental and bi-specific antibodies against VEGF and IL6 (contd.)

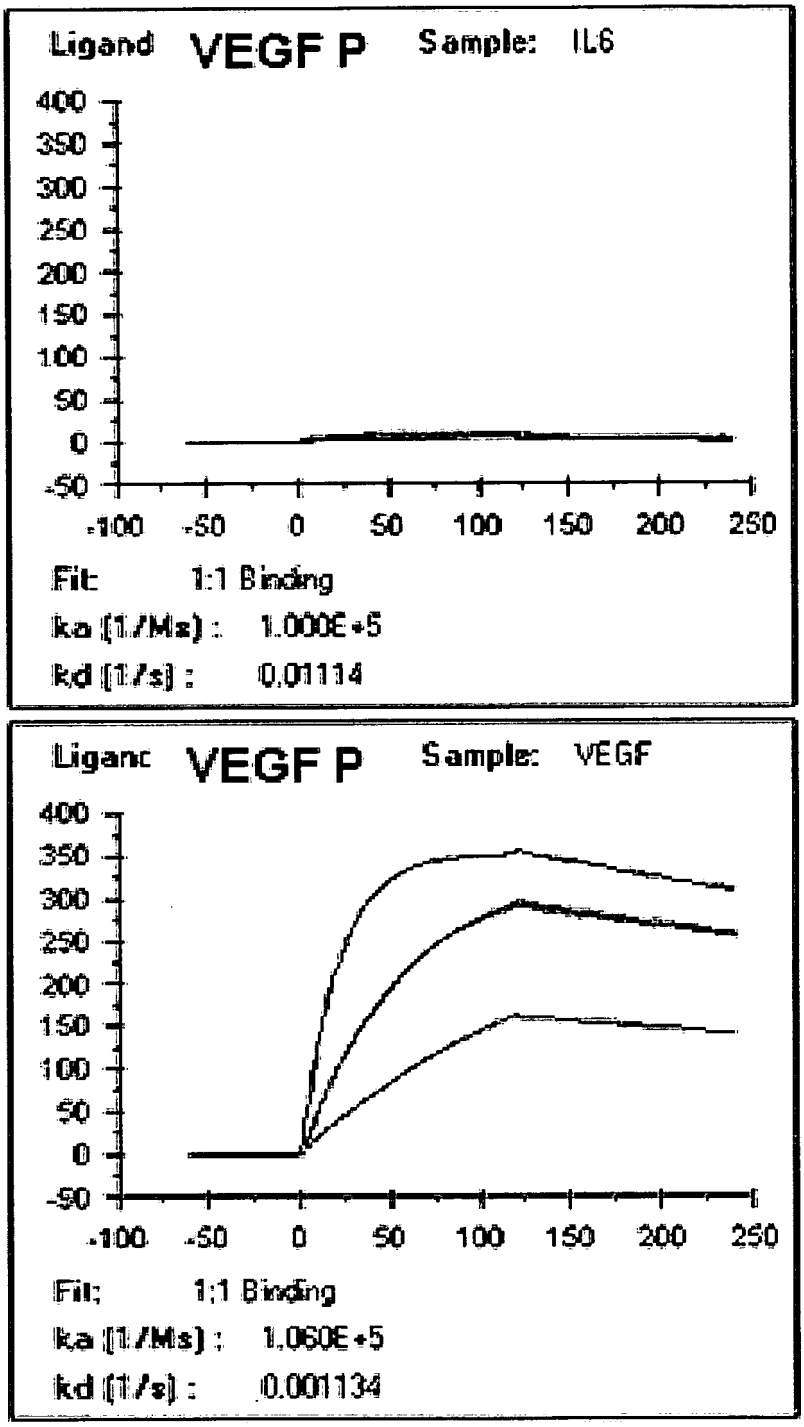


Figure 8:

A: Co-binding of GMCSF + Antibody + IL6

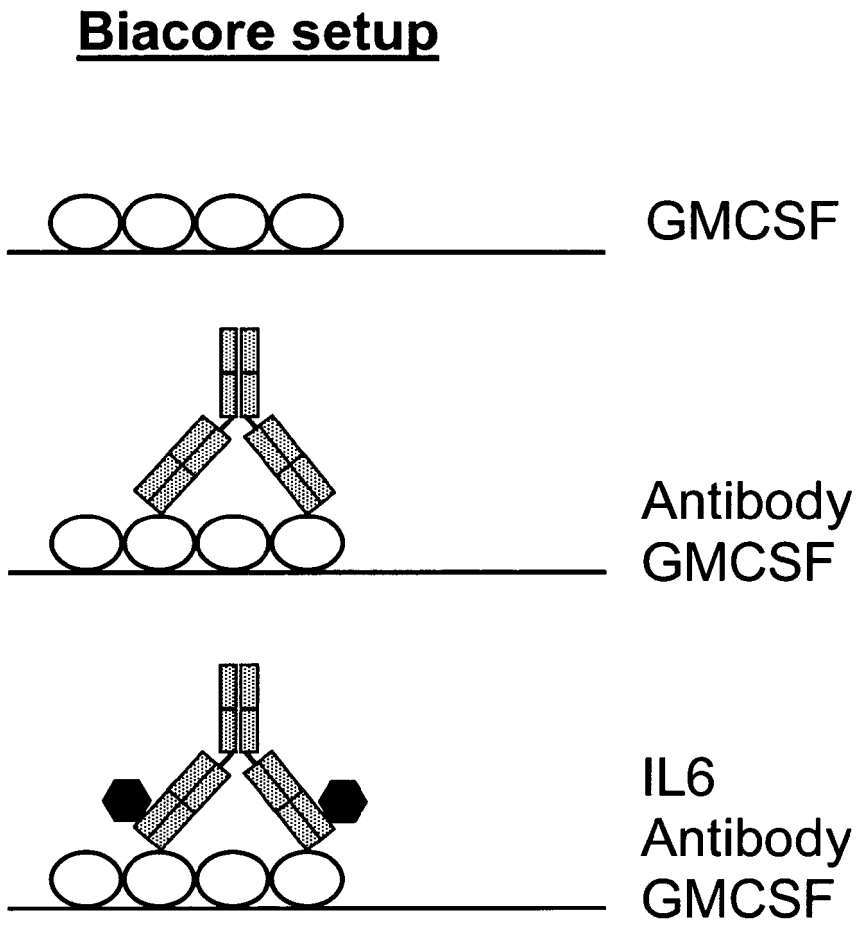


Fig. 8 A: Co-binding of GMCSF + Antibody + IL6 (contd.)

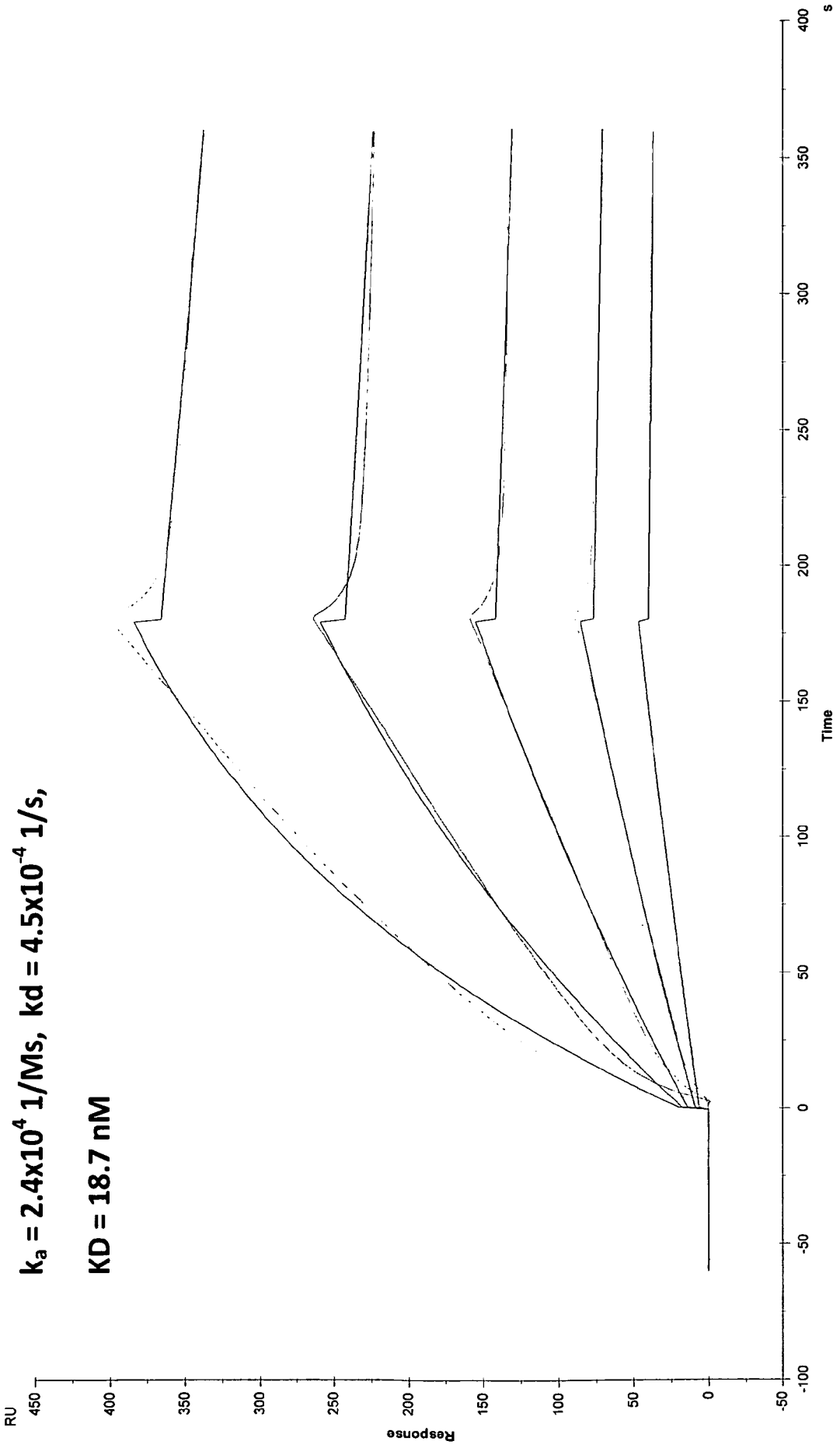


Figure 8:

B: Co-binding of anti-LC + Antibody + IL6

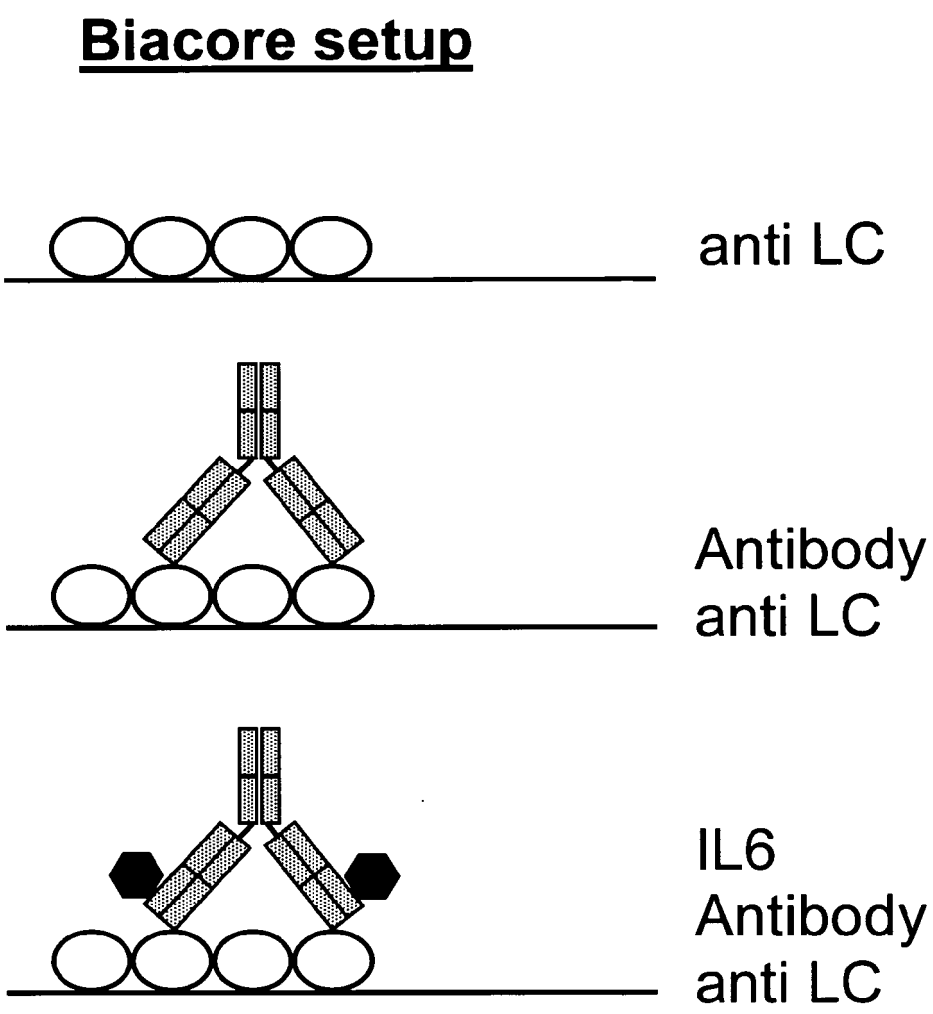


Fig. 8 B: Co-binding of anti-LC + Antibody + IL6 (contd.)

$k_a = 5.7x10^4$ 1/Ms, kd = $1.7x10^{-3}$ 1/s,

KD = 30.4 nM

