1 MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR

101 IVRGTQLFED NYALAVLDNG DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA LTLIDTNRSR ACHPCSPMCK

201 GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP

301 YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF

401 ETLEEITGYL YISAWPDSLP DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV PWDQLFRNPH QALLHTANRP

501 EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC

601 PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAE (SEQ ID NO: 13)

FIG._1A

7C2	aa	22-53	(31 RESIDUES)
7F3	aa	22-53	(31 RESIDUES)
2C4	aa	22-584	(562 RESIDUES)
7D3	aa	22-584	(562 RESIDUES)
3E8	aa	512-625	(113 RESIDUES)
4D5	aa	529-625	(96 RESIDUES)
2H11	aa	529-645	(116 RESIDUES)
3H4	aa	541-599	(58 RESIDUES)

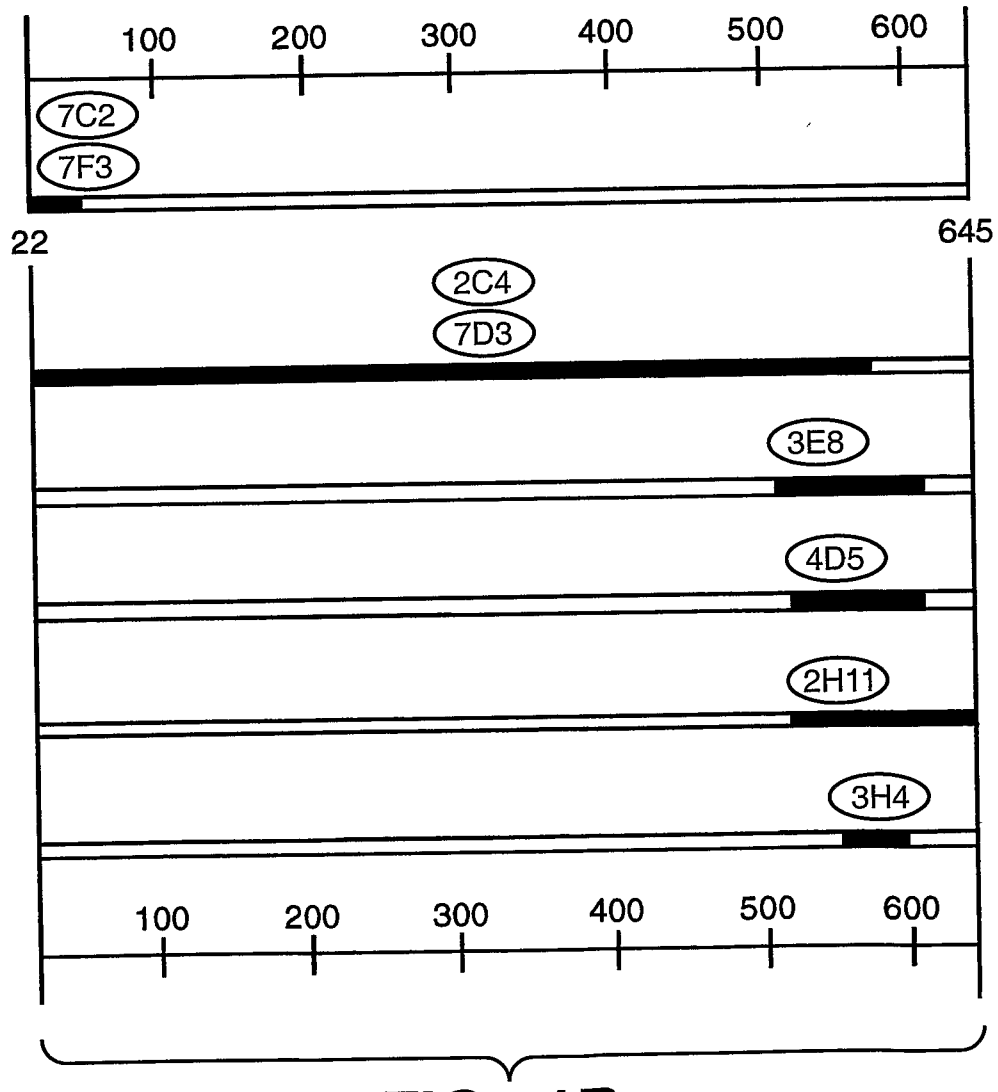


FIG._1B

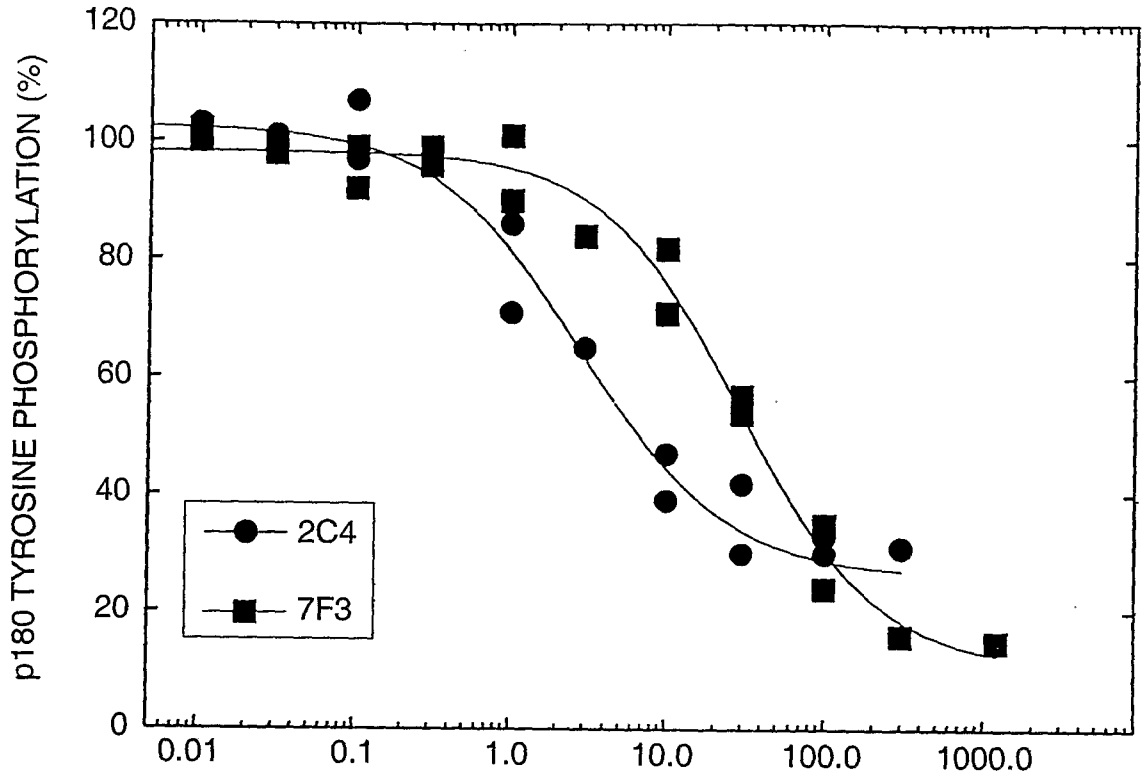

p180 TYROSINE PHOSPHORYLATION (%)
120
100
80
60
40
20
0
0.01
0.1
1.0
10.0
100.0
1000.0
mAb, nM
2C4
7F3

FIG._2A

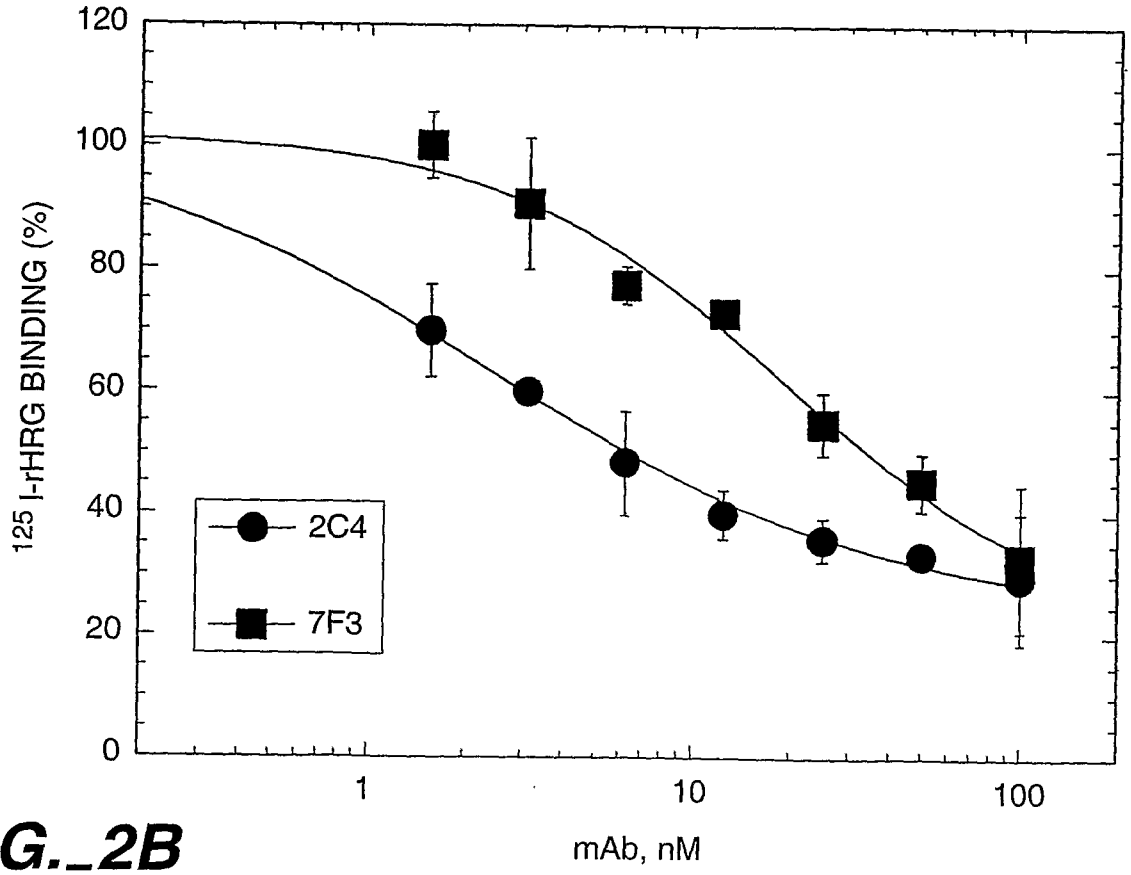

125 I-rHRG BINDING (%)
120
100
80
60
40
20
0
1
10
100
mAb, nM
2C4
7F3

FIG._2B

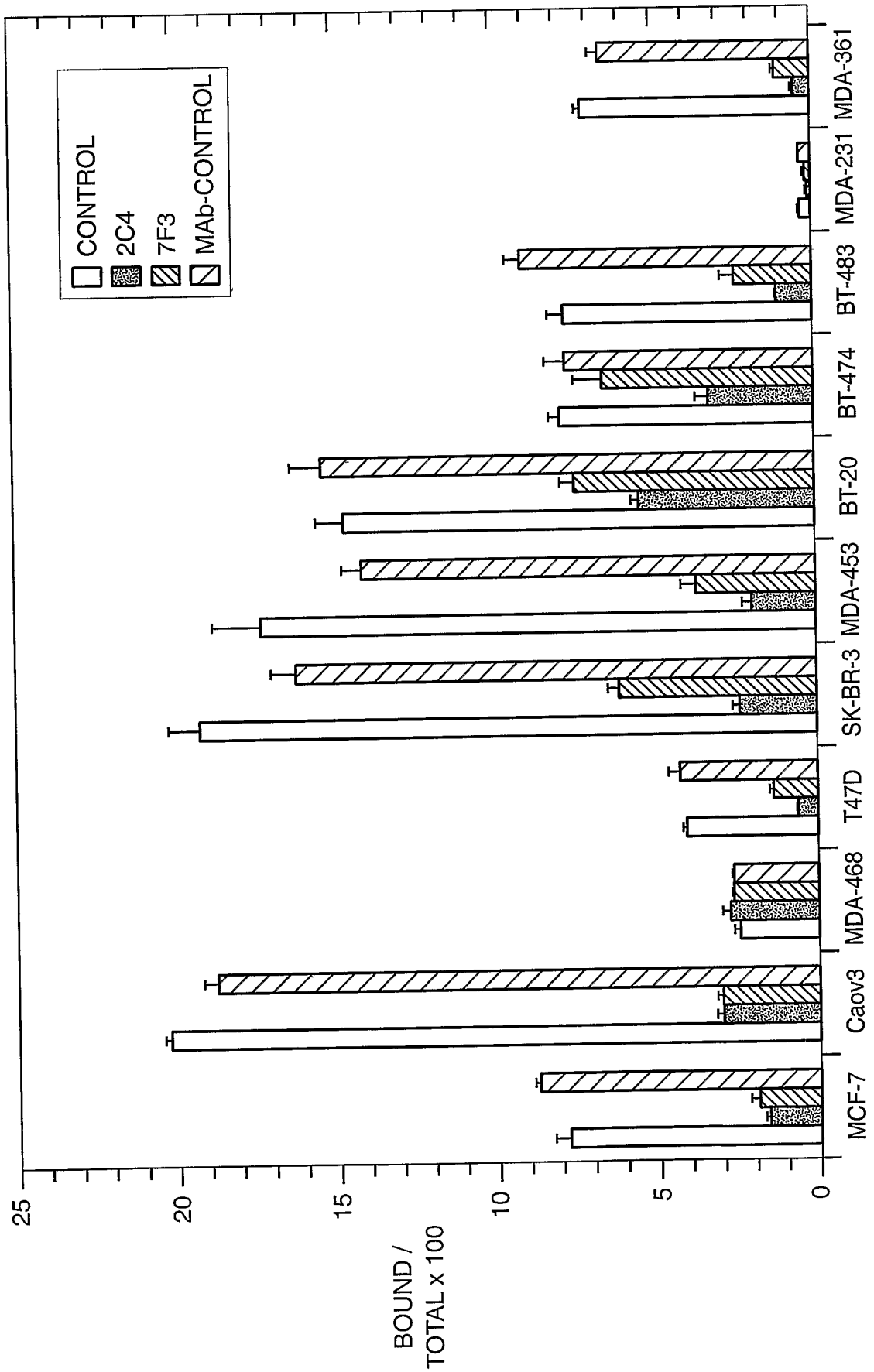

CONTROL
2C4
7F3
MAb-CONTROL
BOUND / TOTAL x 100
0
5
10
15
20
25
MCF-7
Caov3
MDA-468
T47D
SK-BR-3
MDA-453
BT-20
BT-474
BT-483
MDA-231
MDA-361

FIG._3

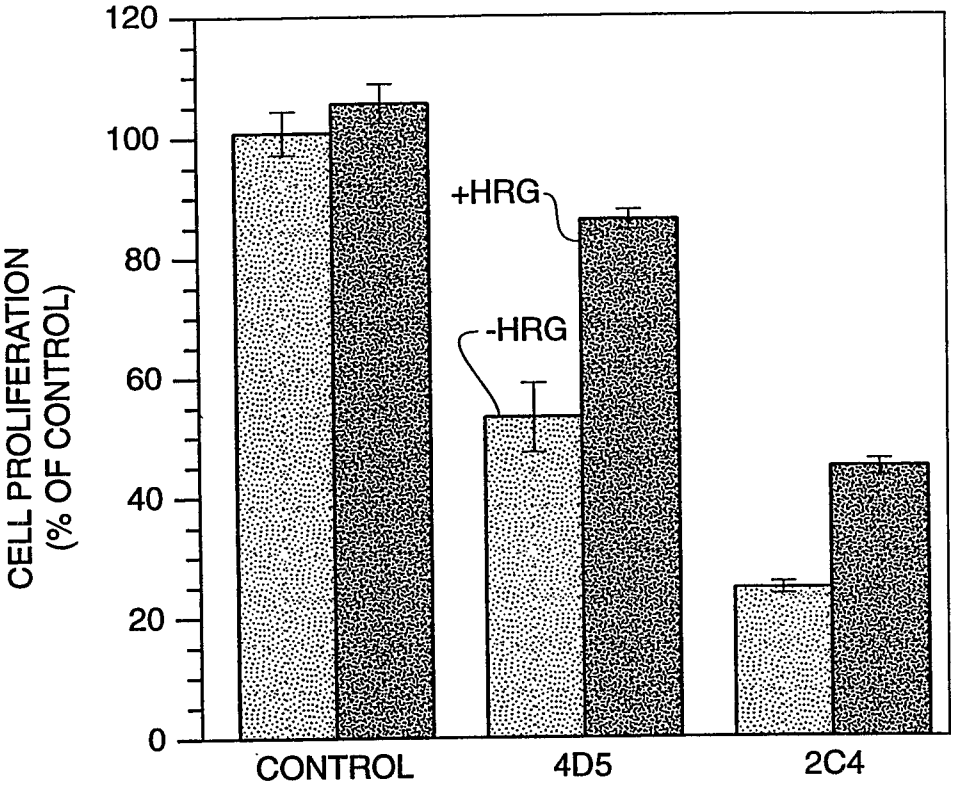

120
100
80
60
40
20
0
CELL PROLIFERATION
(% OF CONTROL)
+HRG
-HRG
CONTROL
4D5
2C4

FIG._4A

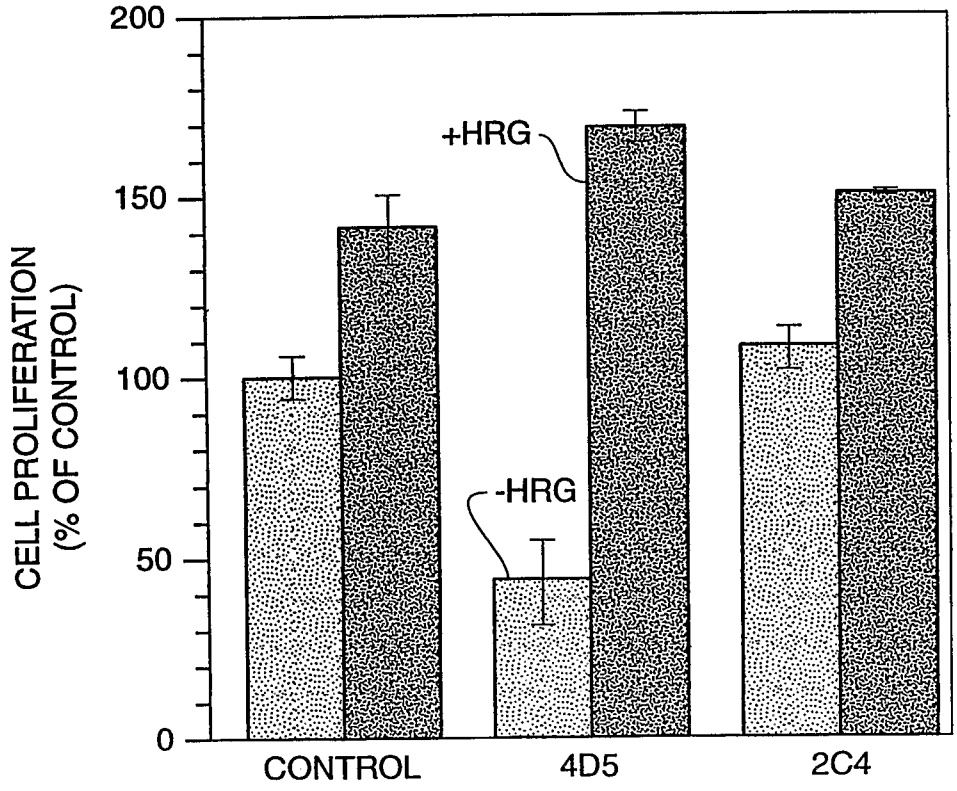

200
150
100
50
0
CELL PROLIFERATION
(% OF CONTROL)
+HRG
-HRG
CONTROL
4D5
2C4

FIG._4B

FIG._5B
SK-BR-3
HIGH HER2
IP: αHER2
WB: αHER3
175 –
83 –
HRG:
– + – + – + – +
CONTROL
2C4
HERCEPTIN®
αEGFR

FIG._5A
MCF7
LOW / NORMAL HER2
IP: αHER2
WB: αHER3
175 –
83 –
HRG:
– + – + – + – +
CONTROL
2C4
HERCEPTIN®
αEGFR

FIG._10
EGF
TGFα
HRG
2C4
HERCEPTIN®

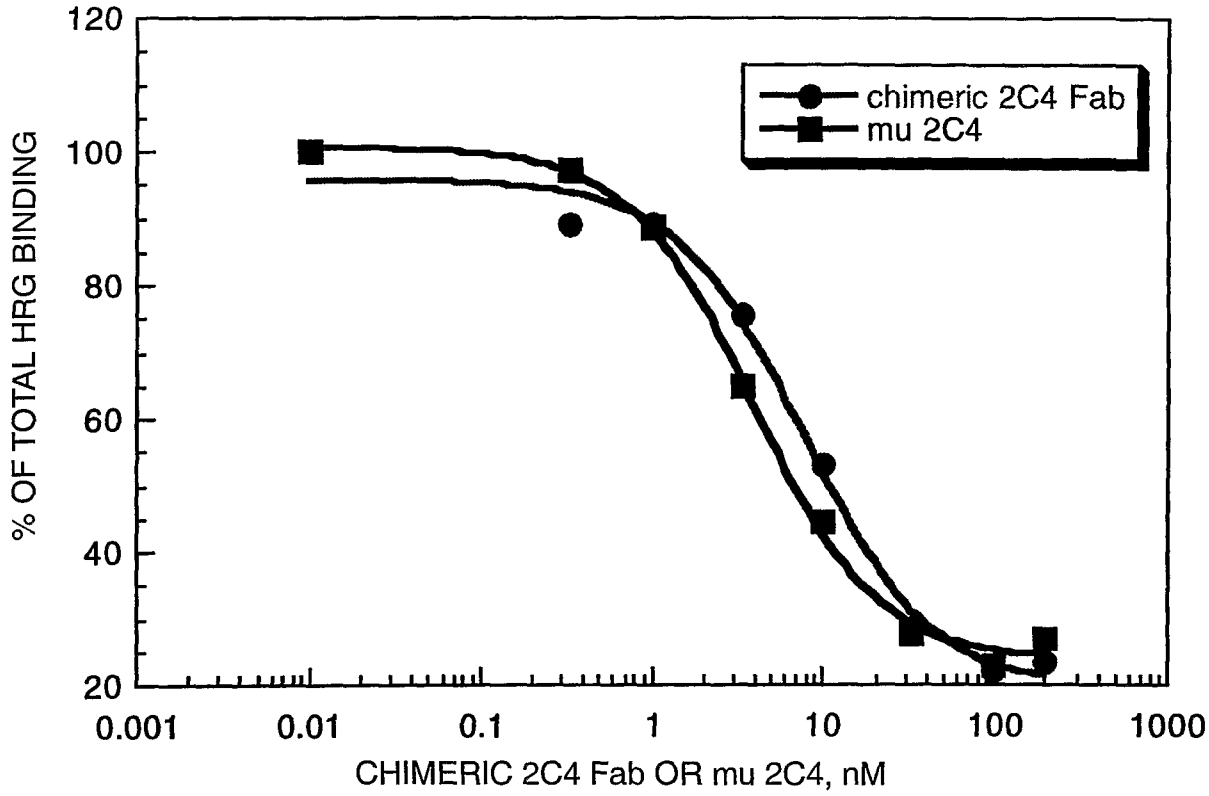

120
100
80
60
40
20
% OF TOTAL HRG BINDING
chimeric 2C4 Fab
mu 2C4
0.001
0.01
0.1
1
10
100
1000
CHIMERIC 2C4 Fab OR mu 2C4, nM

FIG._6A

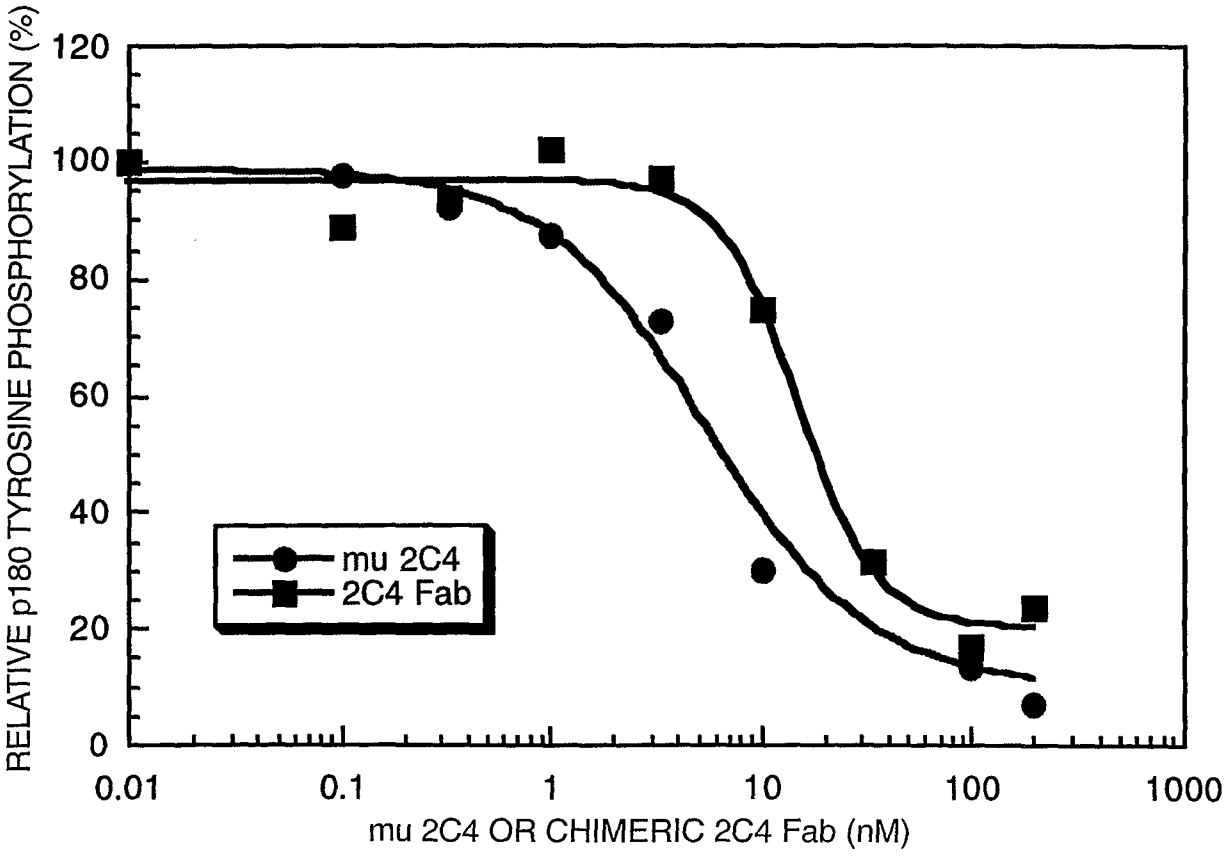

120
100
80
60
40
20
0
RELATIVE p180 TYROSINE PHOSPHORYLATION (%)
mu 2C4
2C4 Fab
0.01
0.1
1
10
100
1000
mu 2C4 OR CHIMERIC 2C4 Fab (nM)

FIG._6B

VARIABLE LIGHT

```
                10        20          30          40
2C4     DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
         **    **** *      *                     *
574     DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                  *   ** ***
hum κI  DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                50          60         70         80
2C4     GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
          **                  *  *         *    * *
574     GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                   * *****
hum κI  GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                90         100
2C4     EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
           * *                 *   *
574     EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                     *** *
hum κI  EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

FIG._7A

VARIABLE HEAVY

```
                10        20          30          40
2C4     EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
           **  **  *  * ***  *                    * *
574     EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                     ** * *
hum III EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                50 a      60          70         80
2C4     HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                    *** *    **** *
574     PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                   ****** *** ****       * * *
hum III PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                abc      90         100ab        110
2C4     ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS (SEQ ID NO:2)
        ***  **                                  **
574     QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                           ********
hum III QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

FIG._7B

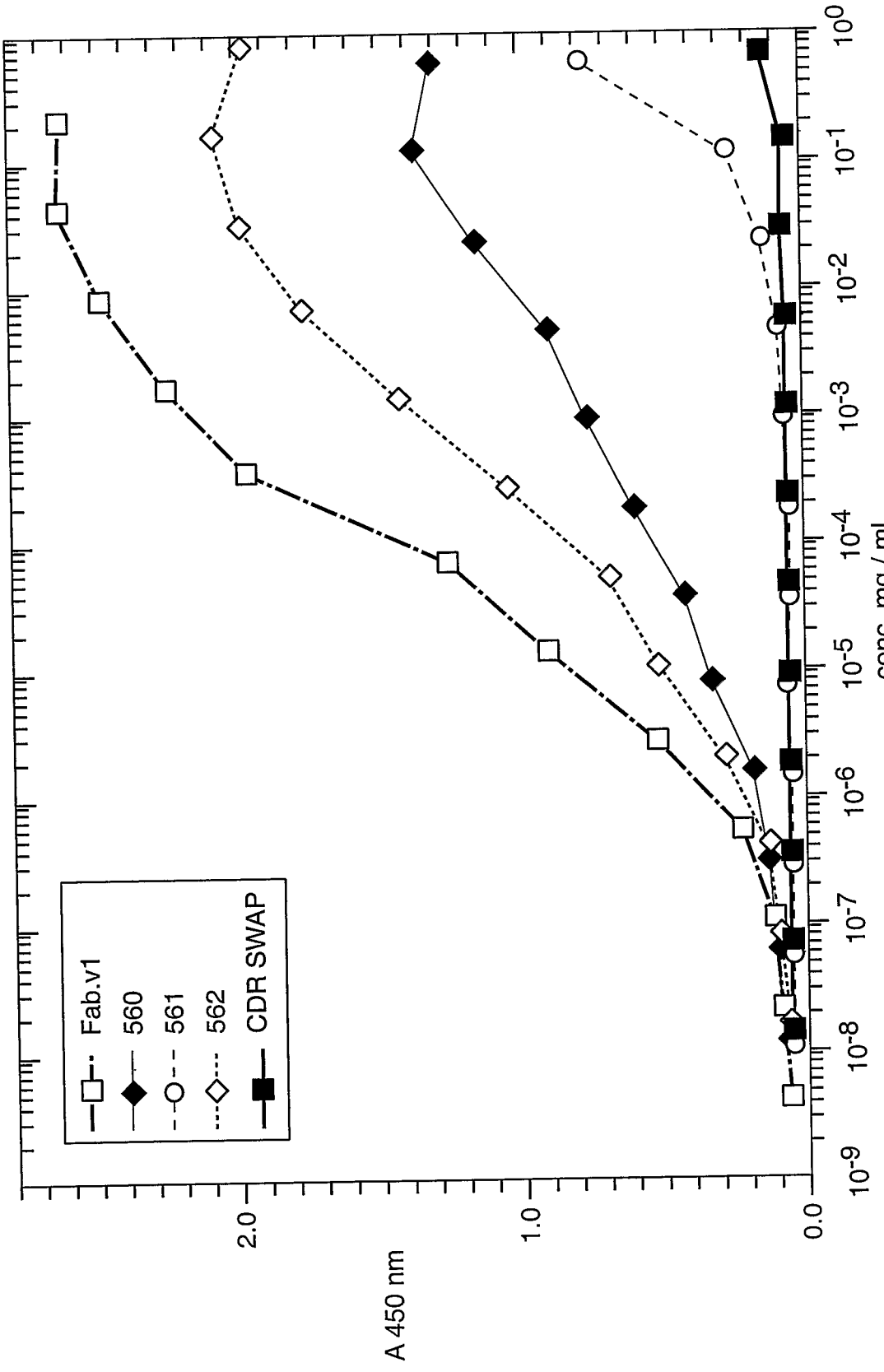

A 450 nm
0.0
1.0
2.0
conc, mg / ml
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Fab.v1
560
561
562
CDR SWAP

FIG._8A

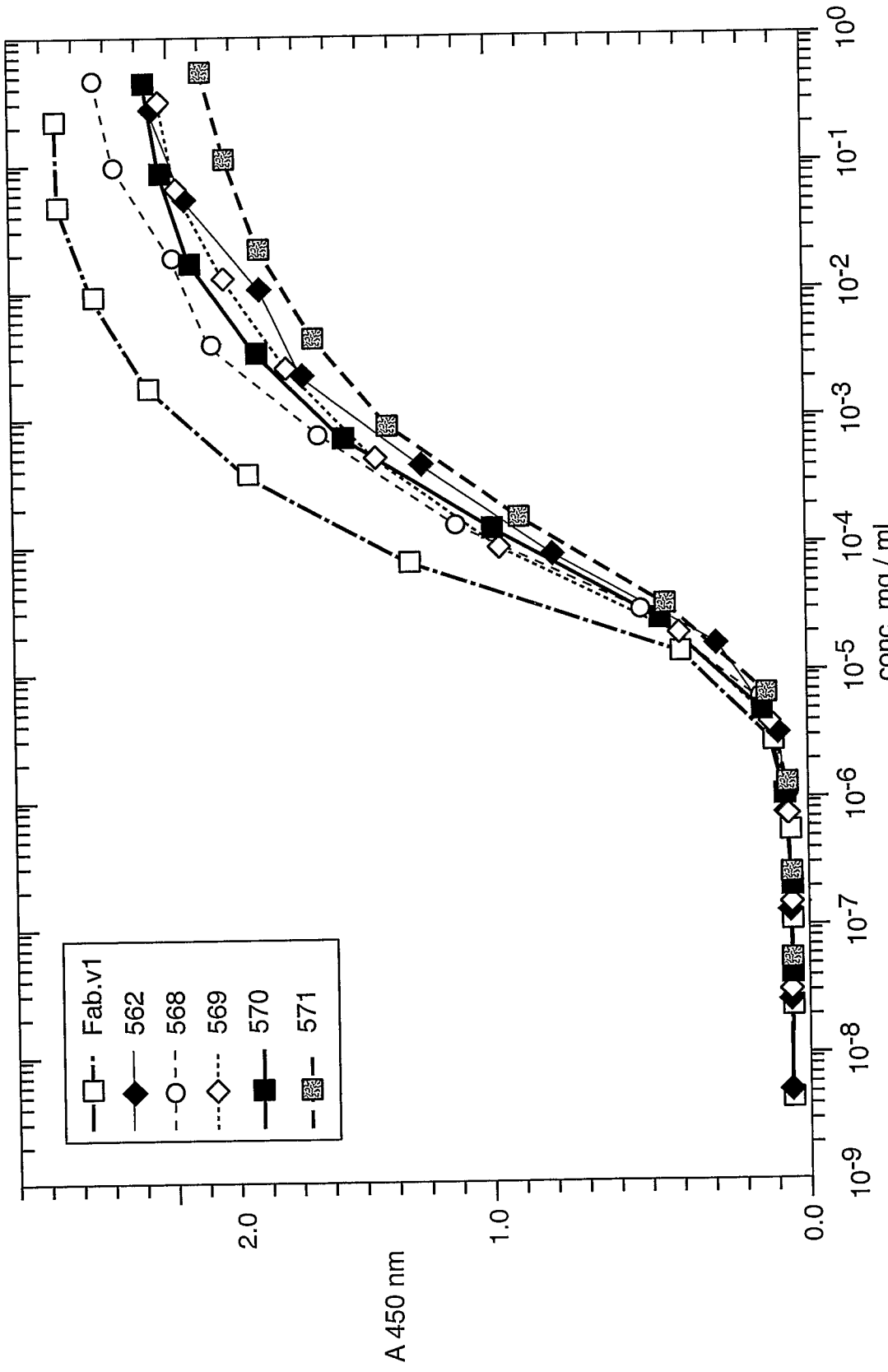

A 450 nm
0.0
1.0
2.0
conc, mg / ml
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Fab.v1
562
568
569
570
571

FIG._8B

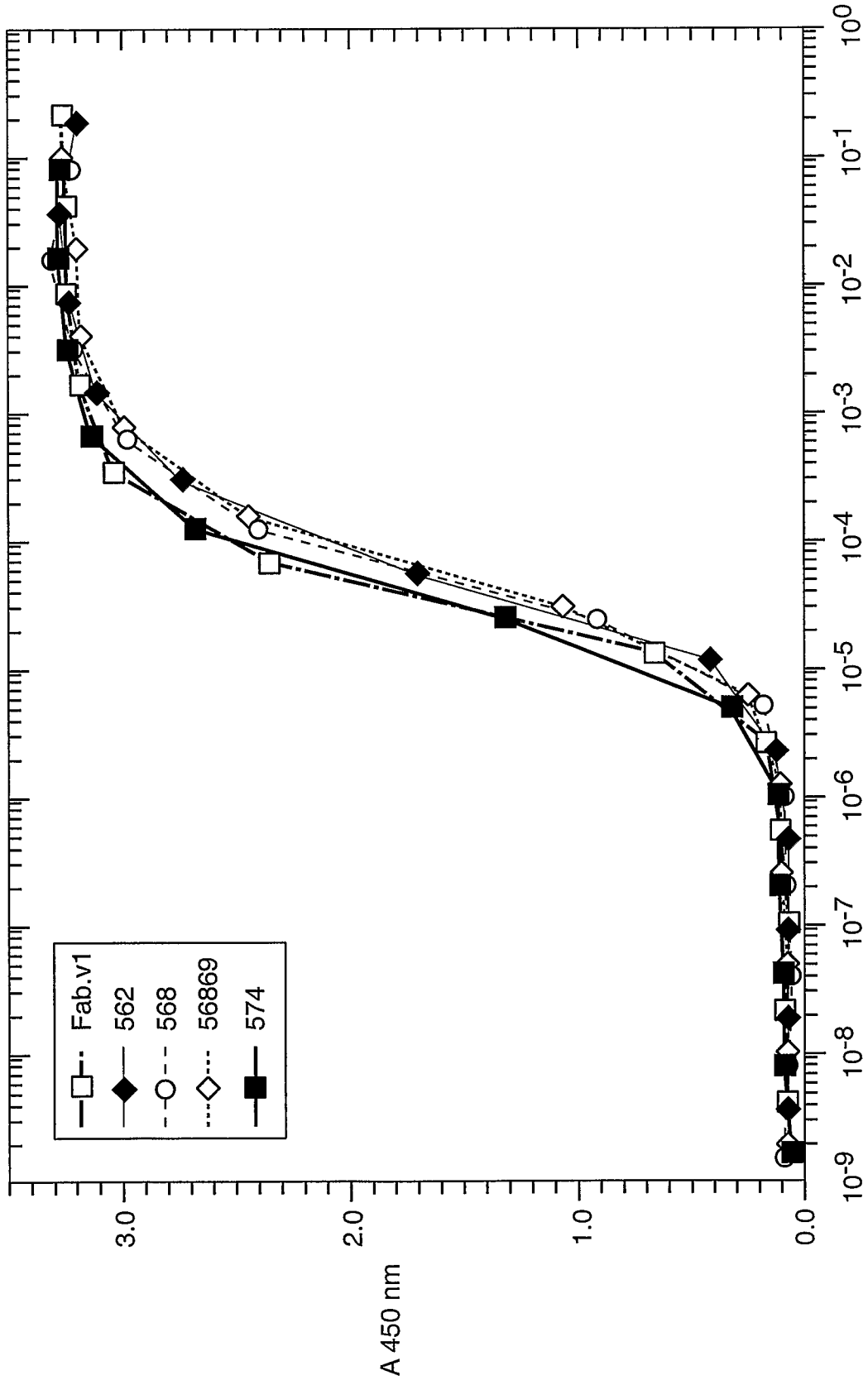

A 450 nm
3.0
2.0
1.0
0.0
conc, mg / ml
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Fab.v1
562
568
56869
574

FIG._8C

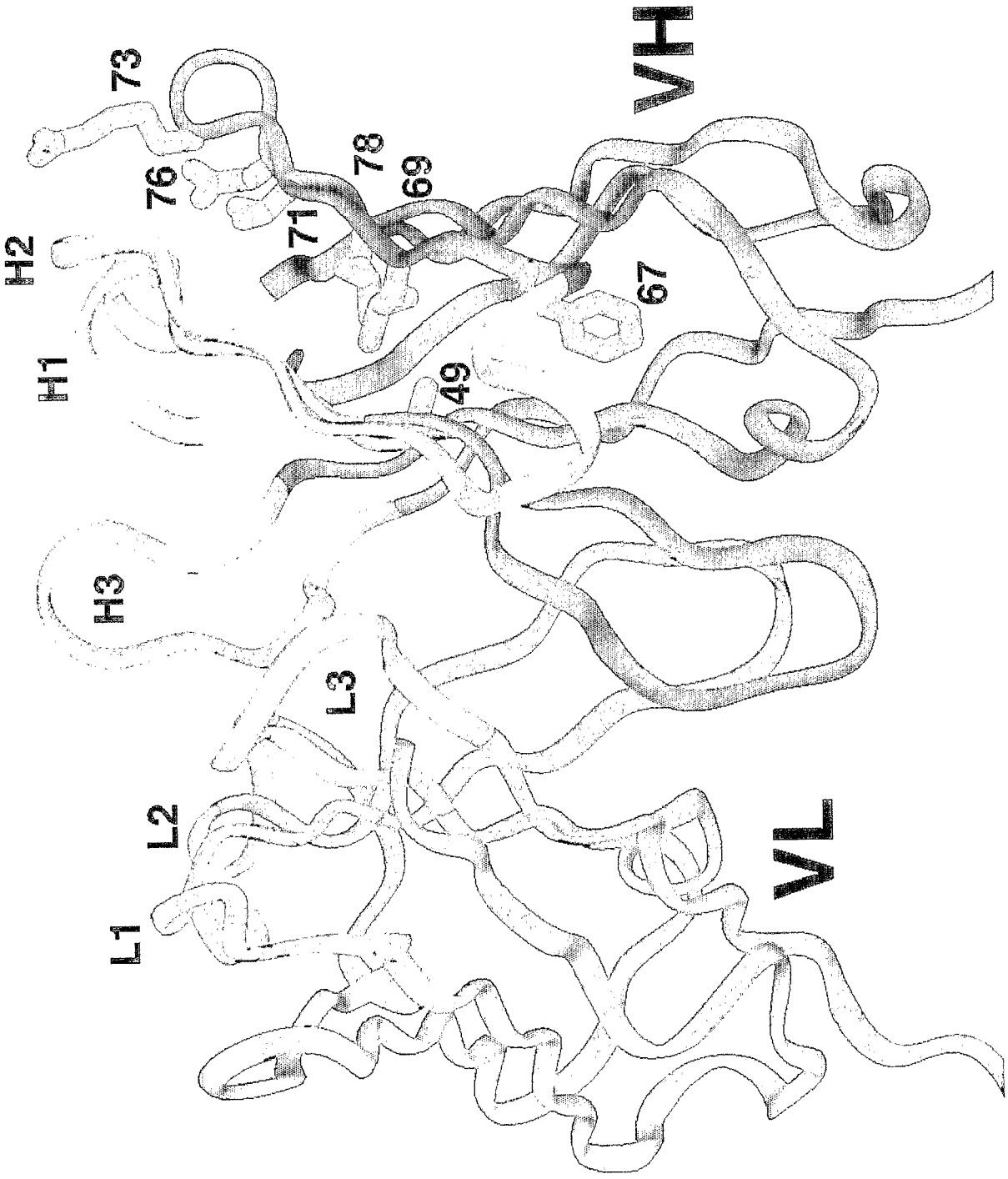


FIG._9

LIGHT CHAIN

```
1   D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K
46  L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q
91  H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L
136 L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T
181 L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG._11A

HEAVY CHAIN

```
1                               15                              30                              45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L

46                              60                              75                              90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D

91                              105                             120                             135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S

136                             150                             165                             180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

181                             195                             210                             225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K

226                             240                             255                             270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S

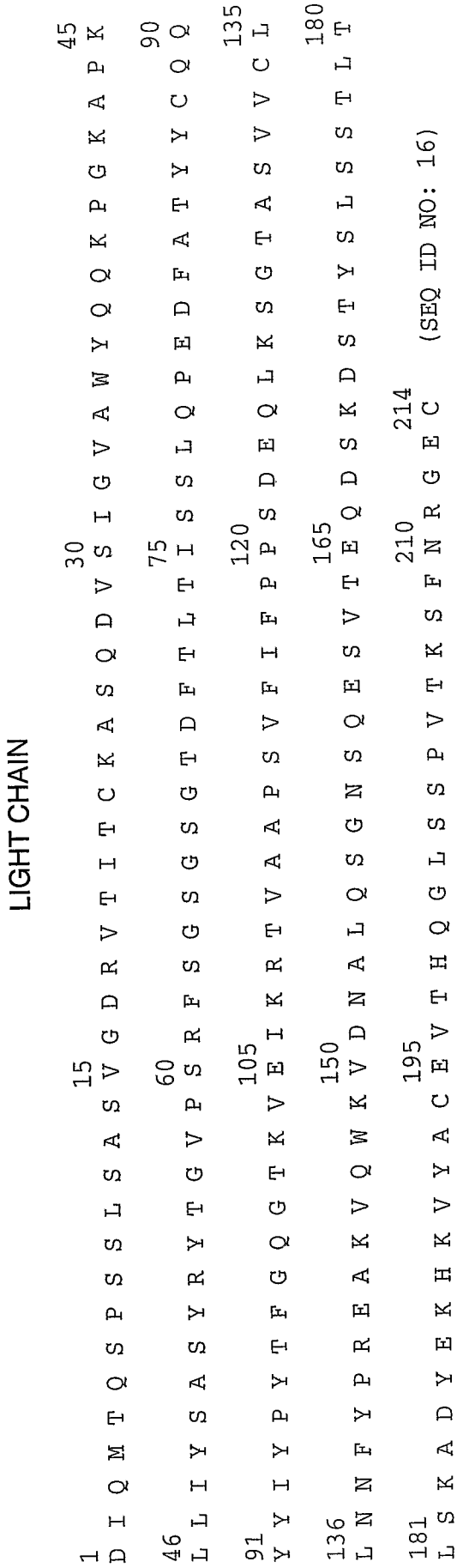
271                             285                             300                             315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D

316                             330                             345                             360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E

361                             375                             390                             405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

406                             420                             435                             449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
```

FIG._11B



(SEQ ID NO: 16)

FIG._12A

HEAVY CHAIN

```
1                               15                              30                              45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T D Y T M D W V R Q A P G K G L

46                              60                              75                              90
E W V A D V N P N S G G S I Y N Q R F K G R F T L S V D R S K N T L Y L Q M N S L R A E D

91                              105                             120                             135
T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S K

136                             150                             165                             180
S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G

181                             195                             210                             225
L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K T

226                             240                             255                             270
H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H

271                             285                             300                             315
E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W

316                             330                             345                             360
L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M

361                             375                             390                             405
T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S

406                             420                             435                             449
F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
```

(SEQ ID NO: 17)

FIG._12B

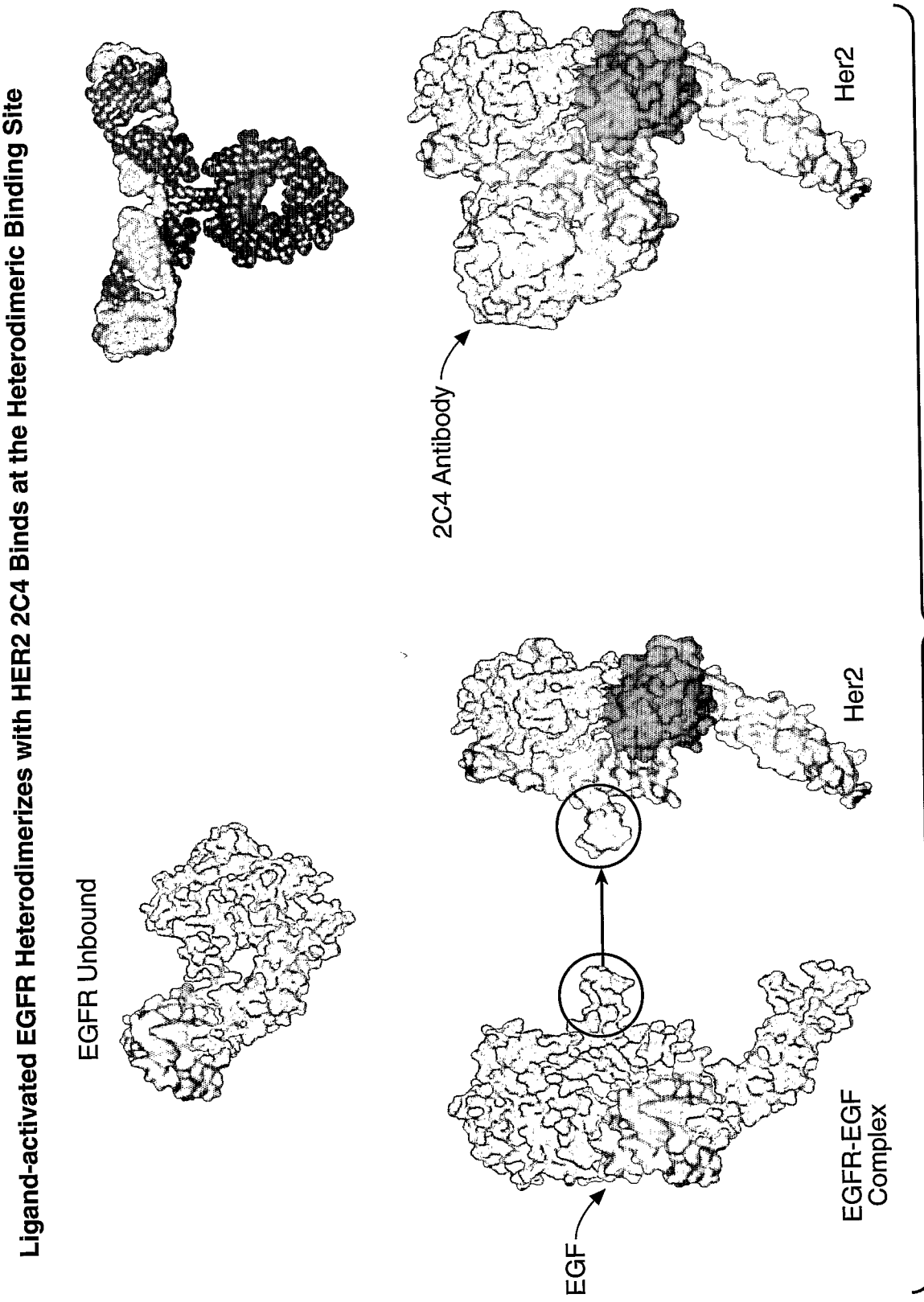

Ligand-activated EGFR Heterodimerizes with HER2 2C4 Binds at the Heterodimeric Binding Site
EGFR Unbound
2C4 Antibody
Her2
Her2
EGF
EGFR-EGF Complex

FIG._13

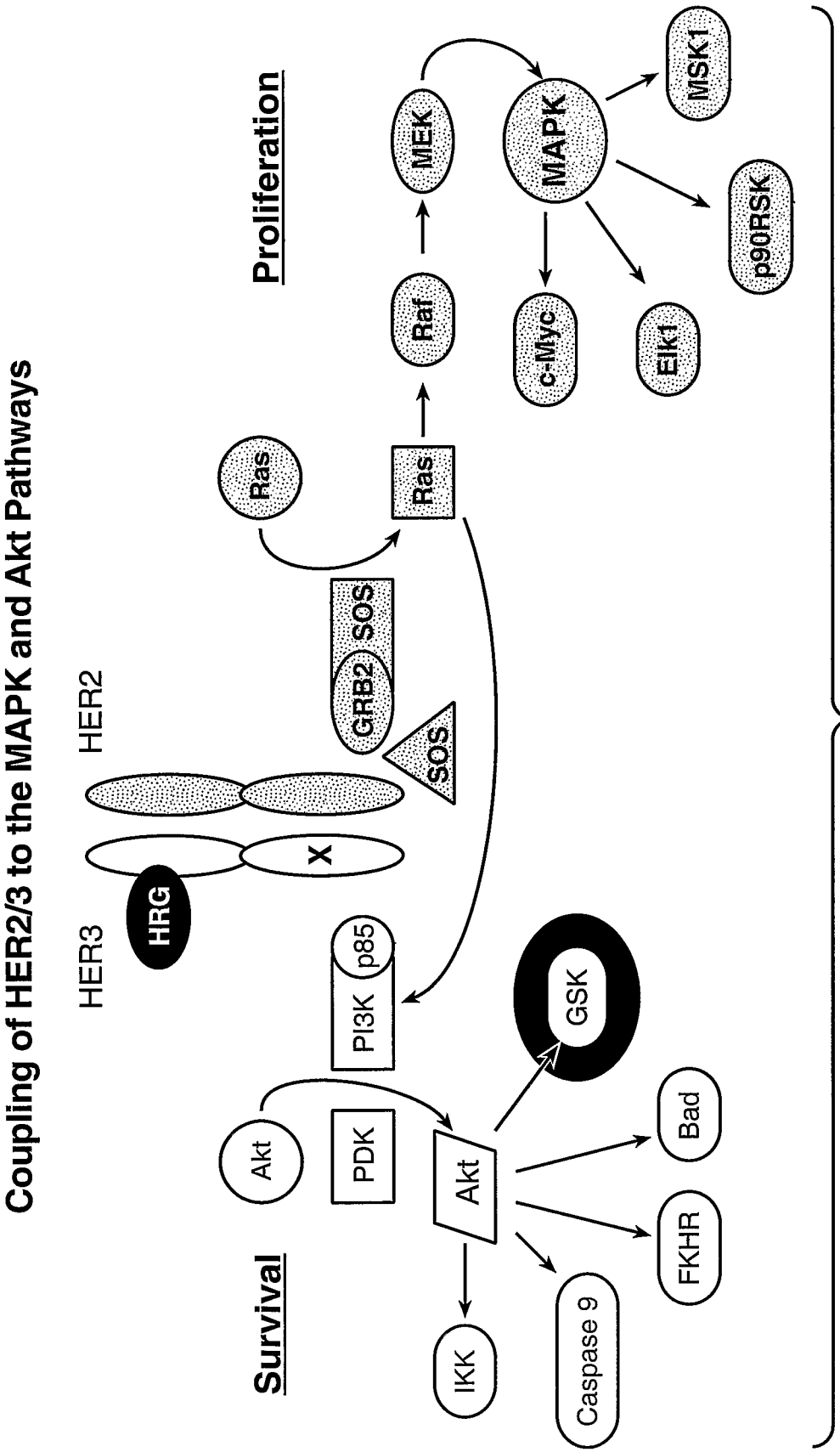
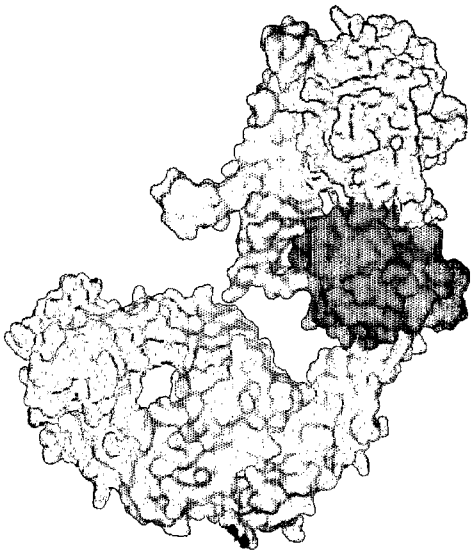

Coupling of HER2/3 to the MAPK and Akt Pathways
Proliferation
Survival
HER3
HER2
HRG
X
Ras
GRB2
SOS
SOS
Ras
Raf
MEK
MAPK
MSK1
p90RSK
c-Myc
Elk1
PI3K
p85
PDK
Akt
Akt
GSK
Bad
FKHR
IKK
Caspase 9

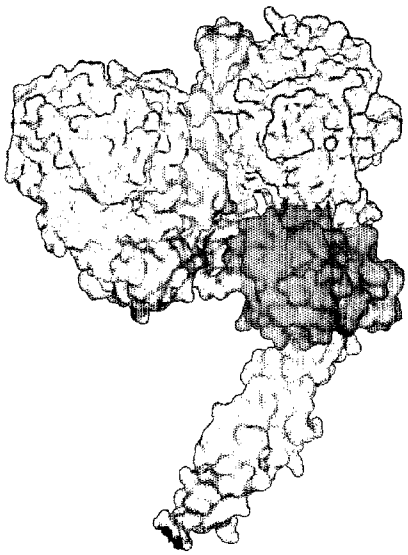
FIG._14

Trastuzumab
Herceptin



- Binds in IV near JM.
- Protects against receptor shedding
- Moderately affects receptor down-modulation
- Slight effect on HER2's role as a coreceptor

Pertuzumab
Omnitarg



- Binds in II at dimerization interface
- Does not prevent receptor shedding
- Moderately affects receptor down-modulation
- Major effect on HER2's role as a coreceptor

FIG._15

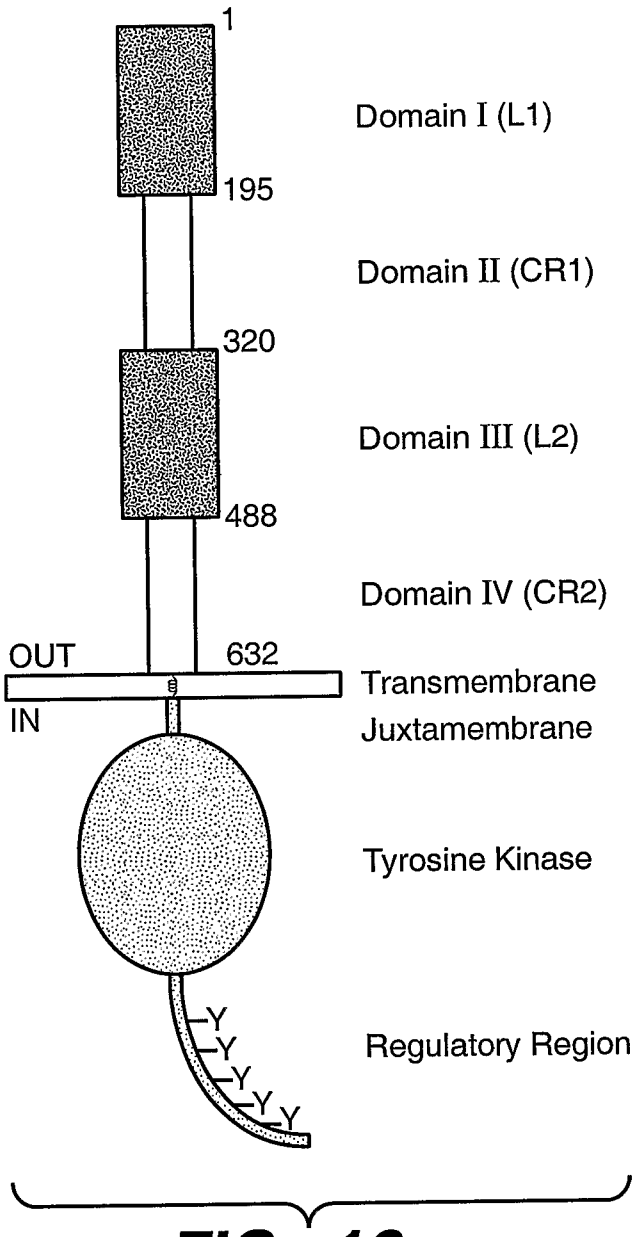

1
195
320
488
632
OUT
IN
Domain I (L1)
Domain II (CR1)
Domain III (L2)
Domain IV (CR2)
Transmembrane
Juxtamembrane
Tyrosine Kinase
Regulatory Region
Y
Y
Y
Y
Y

FIG._16