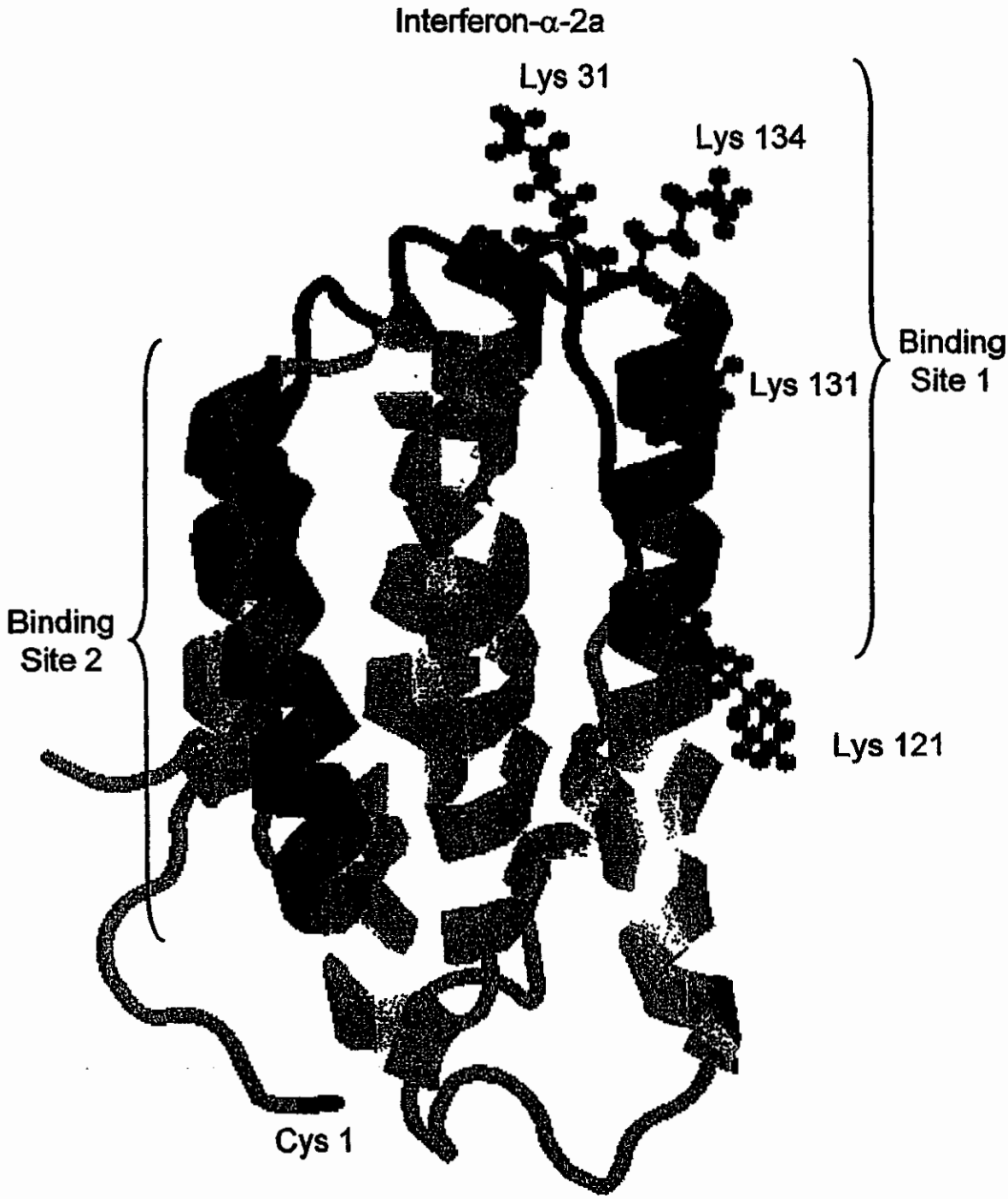

Interferon-α-2a
Lys 31
Lys 134
Lys 131
Binding Site 1
Binding Site 2
Lys 121
Cys 1

FIG. 1a

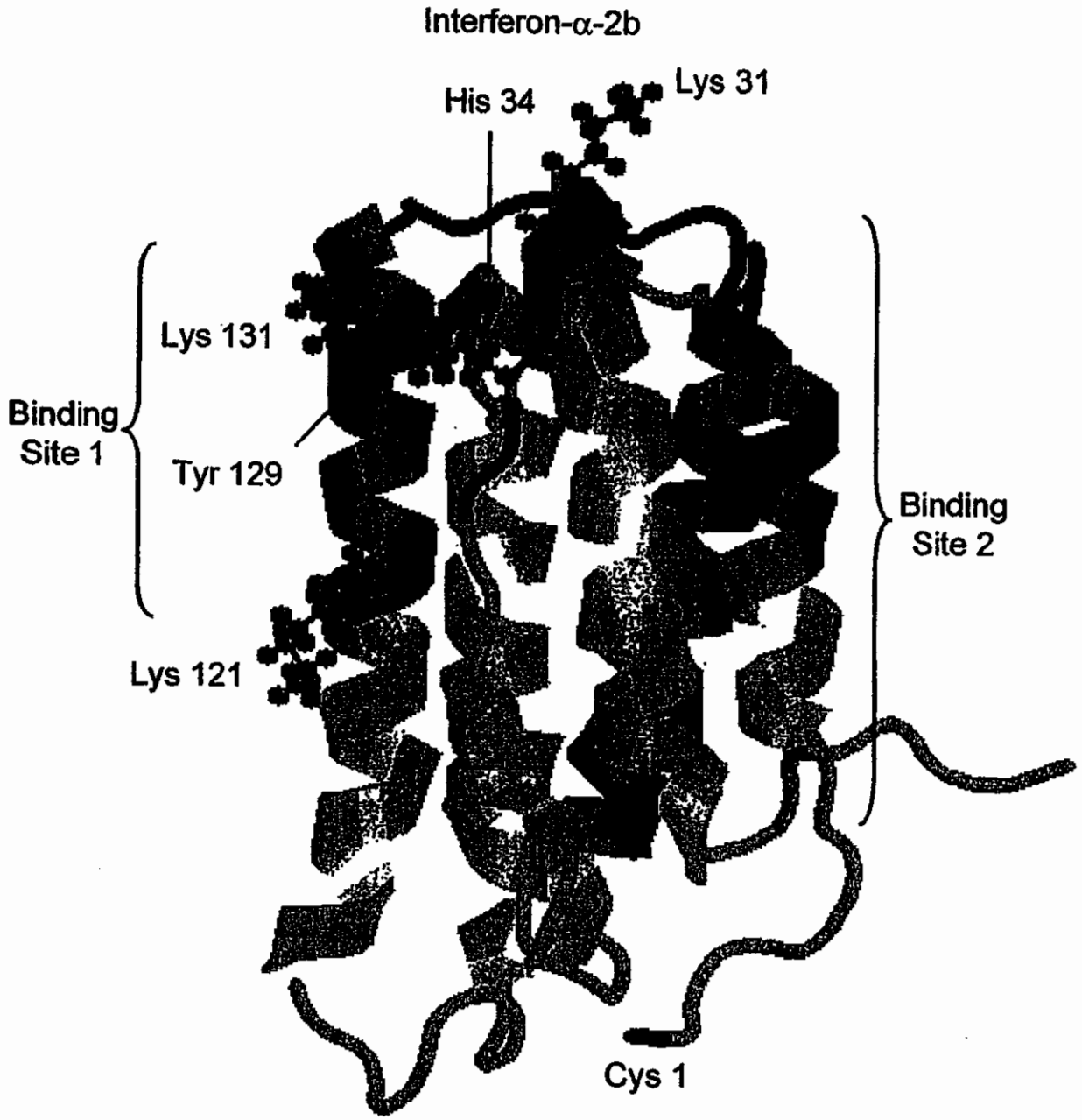

Interferon-α-2b
His 34
Lys 31
Lys 131
Binding Site 1
Tyr 129
Binding Site 2
Lys 121
Cys 1

FIG.1b

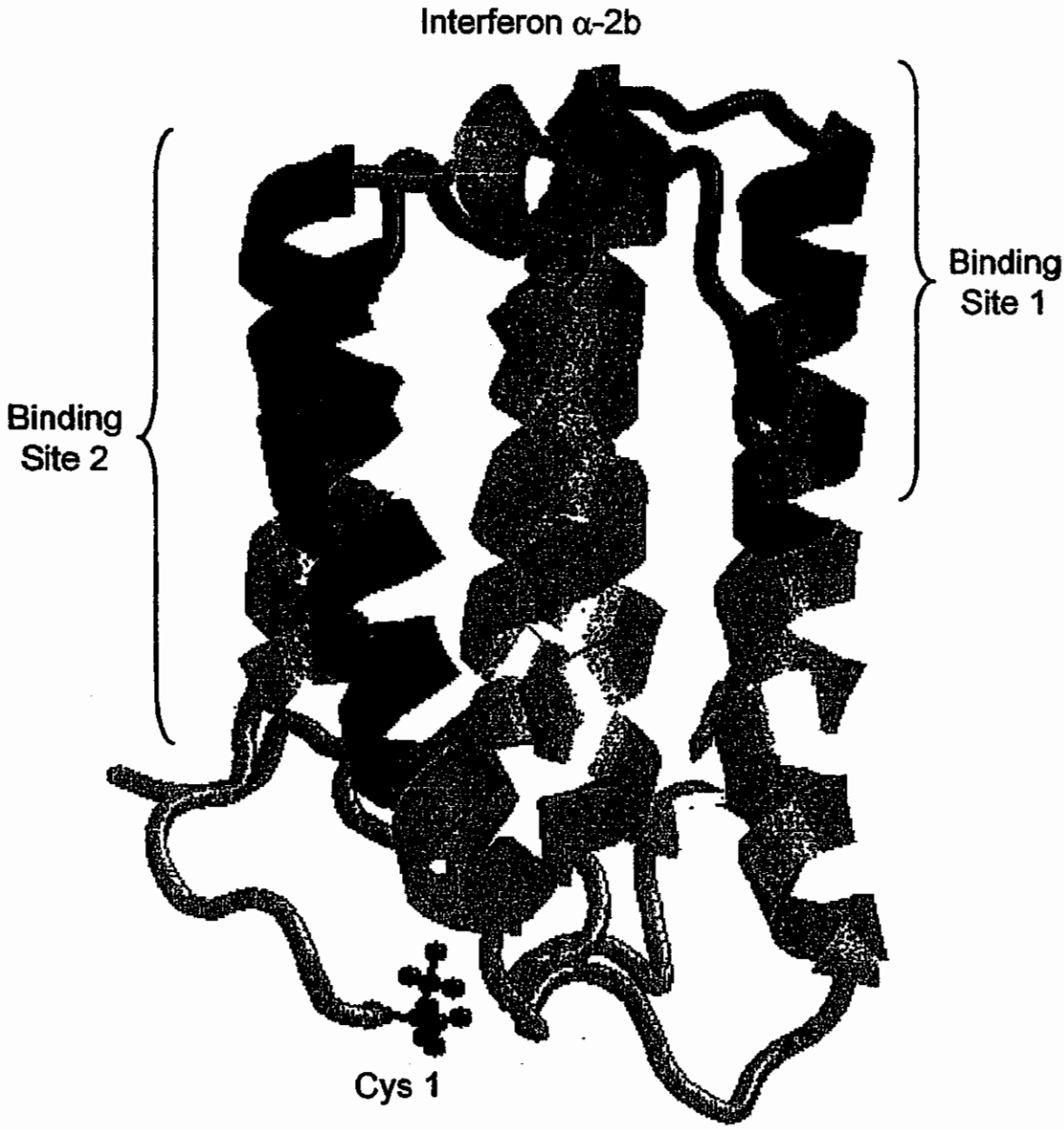


FIG. 1c

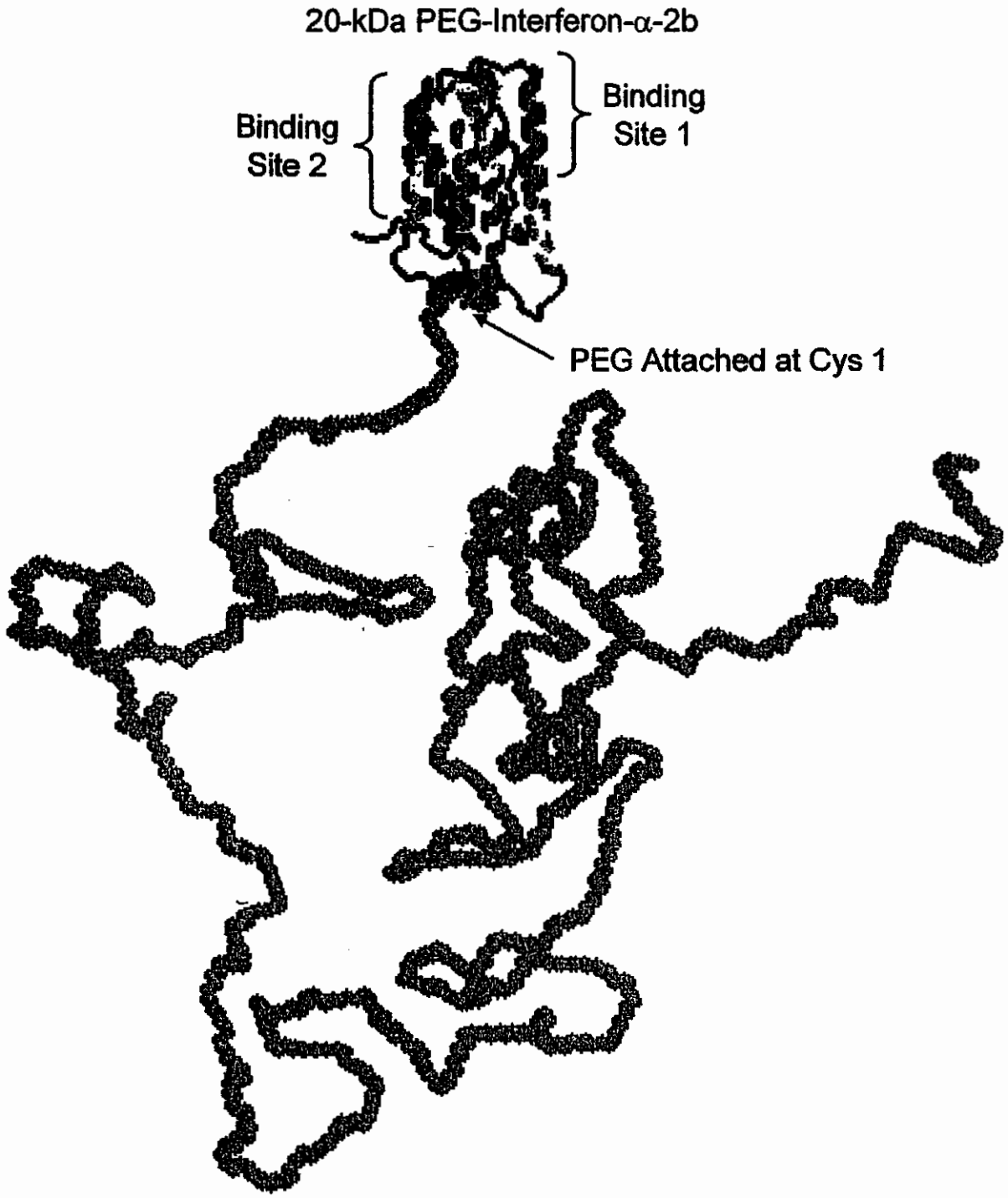

20-kDa PEG-Interferon-α-2b
Binding Site 2
Binding Site 1
PEG Attached at Cys 1

FIG. 1d

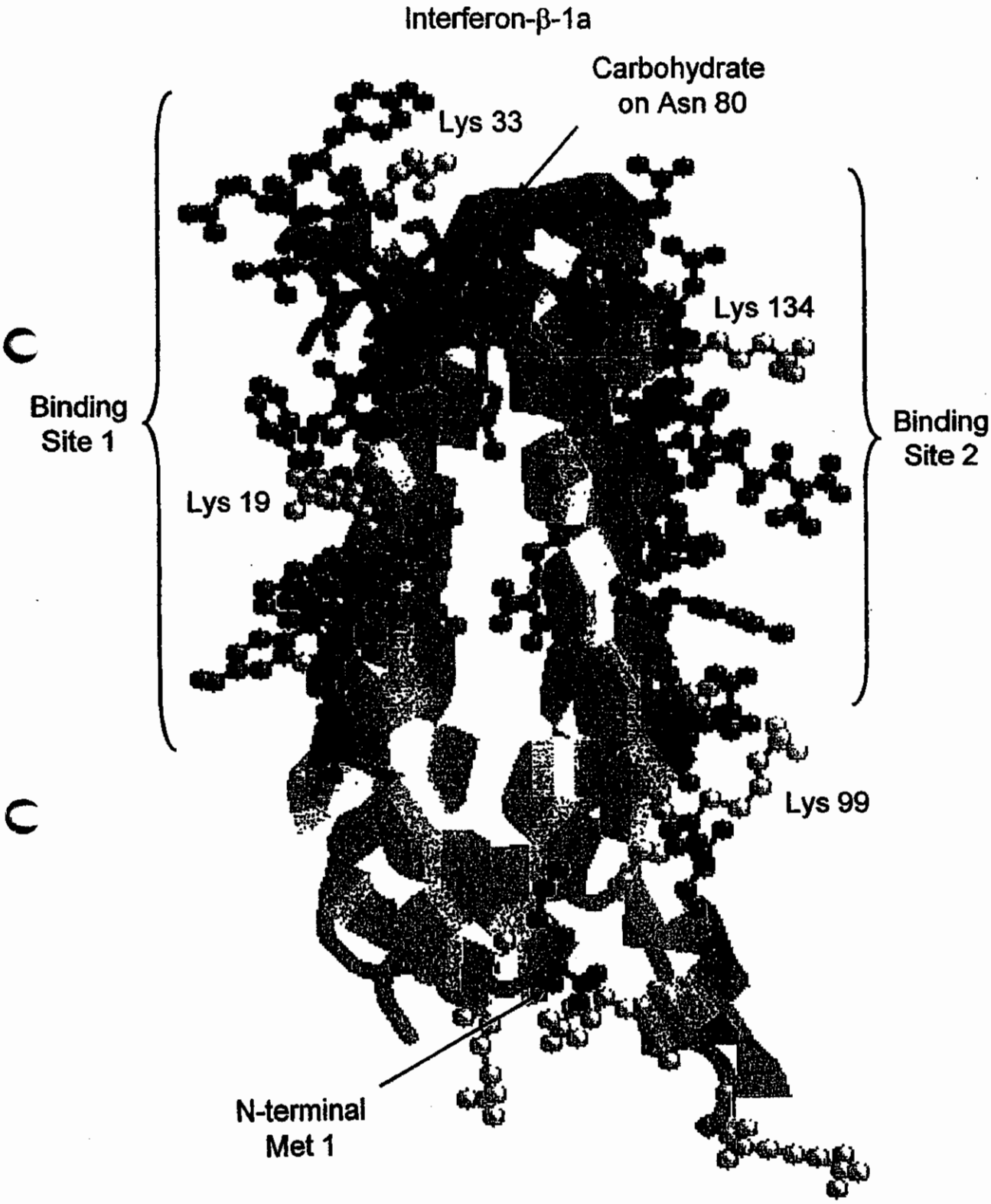

Interferon-β-1a
Carbohydrate on Asn 80
Lys 33
Lys 134
Binding Site 1
Binding Site 2
Lys 19
Lys 99
N-terminal Met 1

FIG.2

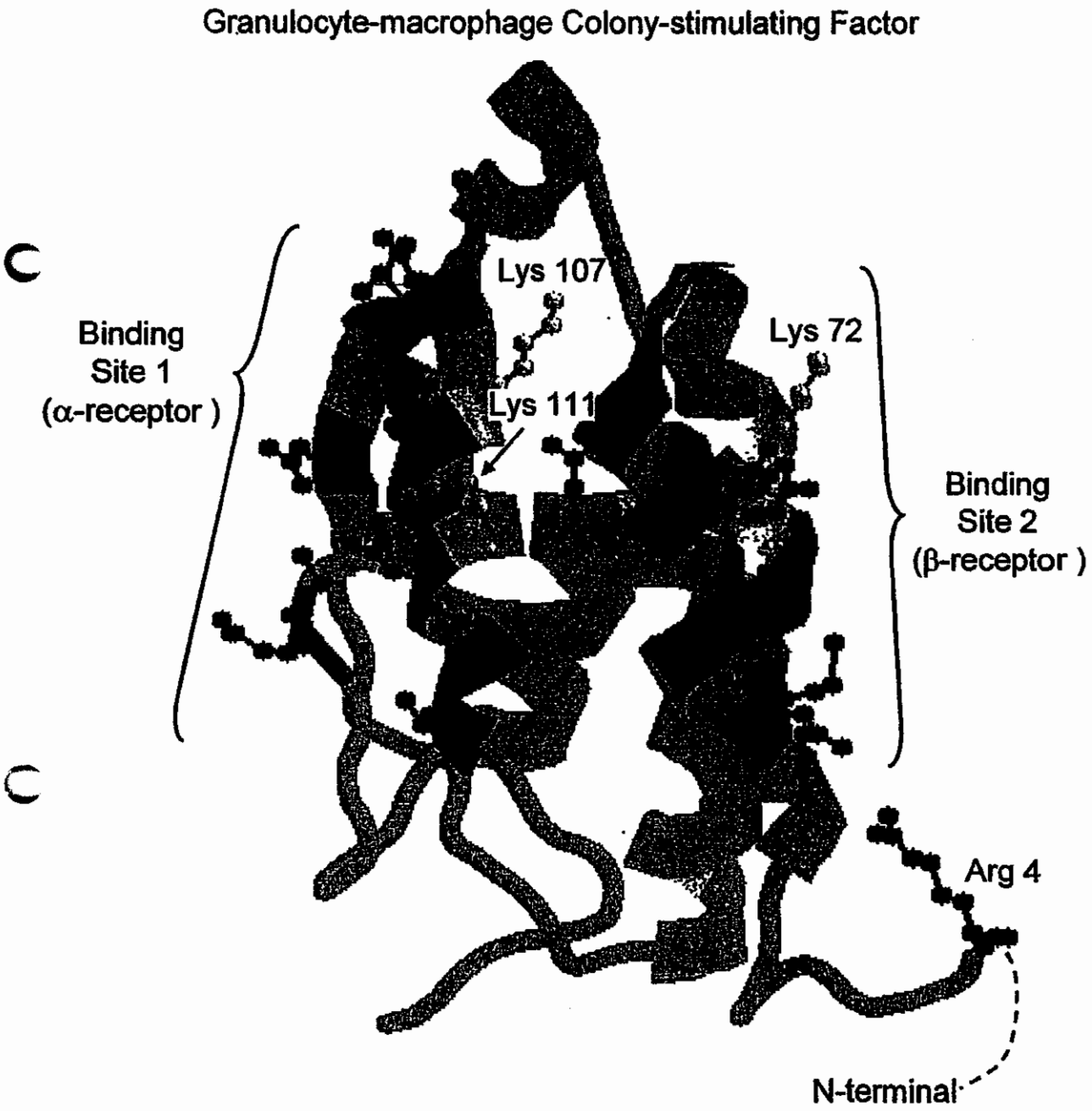

Granulocyte-macrophage Colony-stimulating Factor
Lys 107
Lys 72
Lys 111
Binding
Site 1
(α-receptor)
Binding
Site 2
(β-receptor)
Arg 4
N-terminal

FIG.3

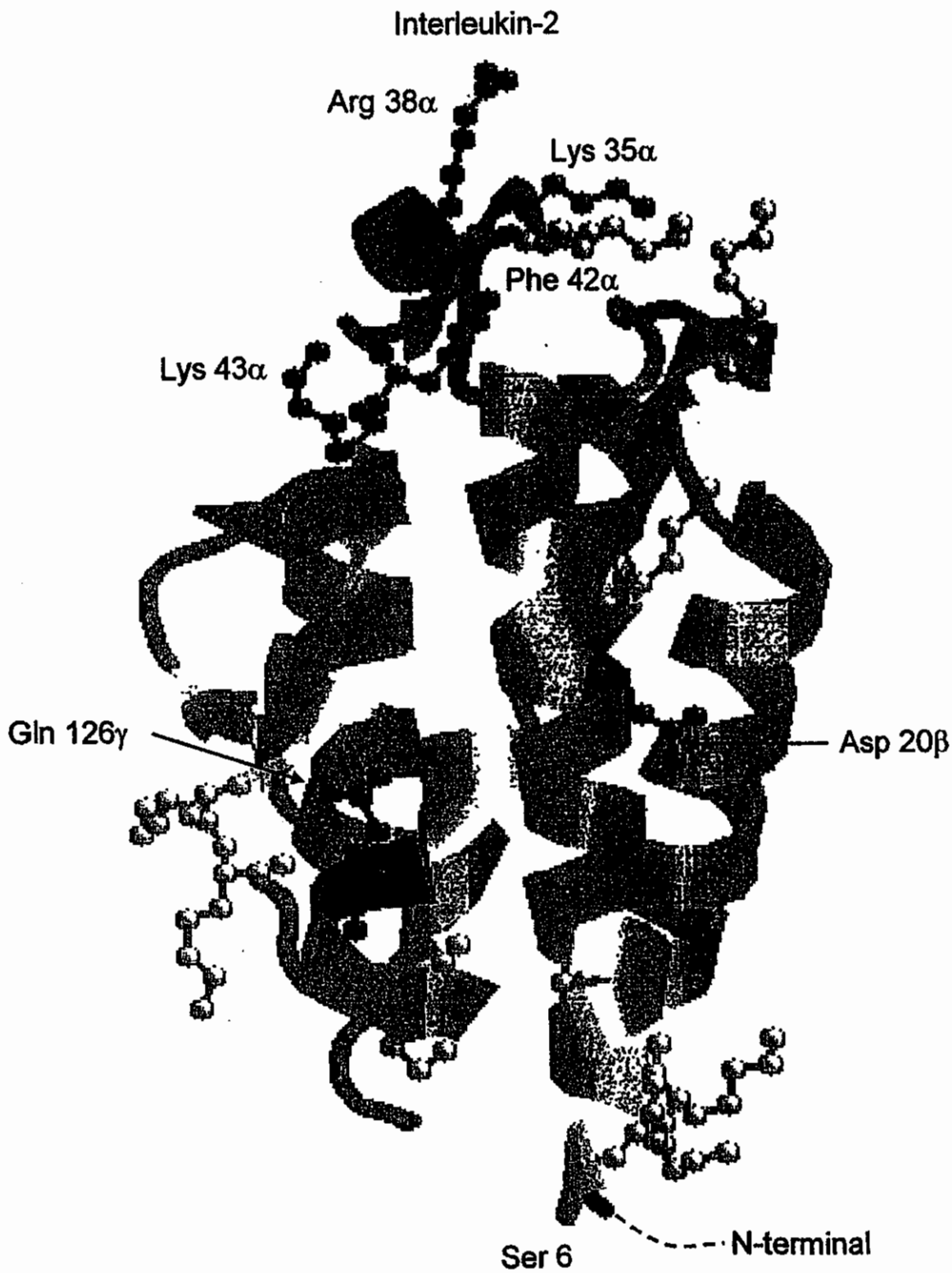

Interleukin-2
Arg 38α
Lys 35α
Phe 42α
Lys 43α
Gln 126γ
Asp 20β
Ser 6
N-terminal

FIG.4

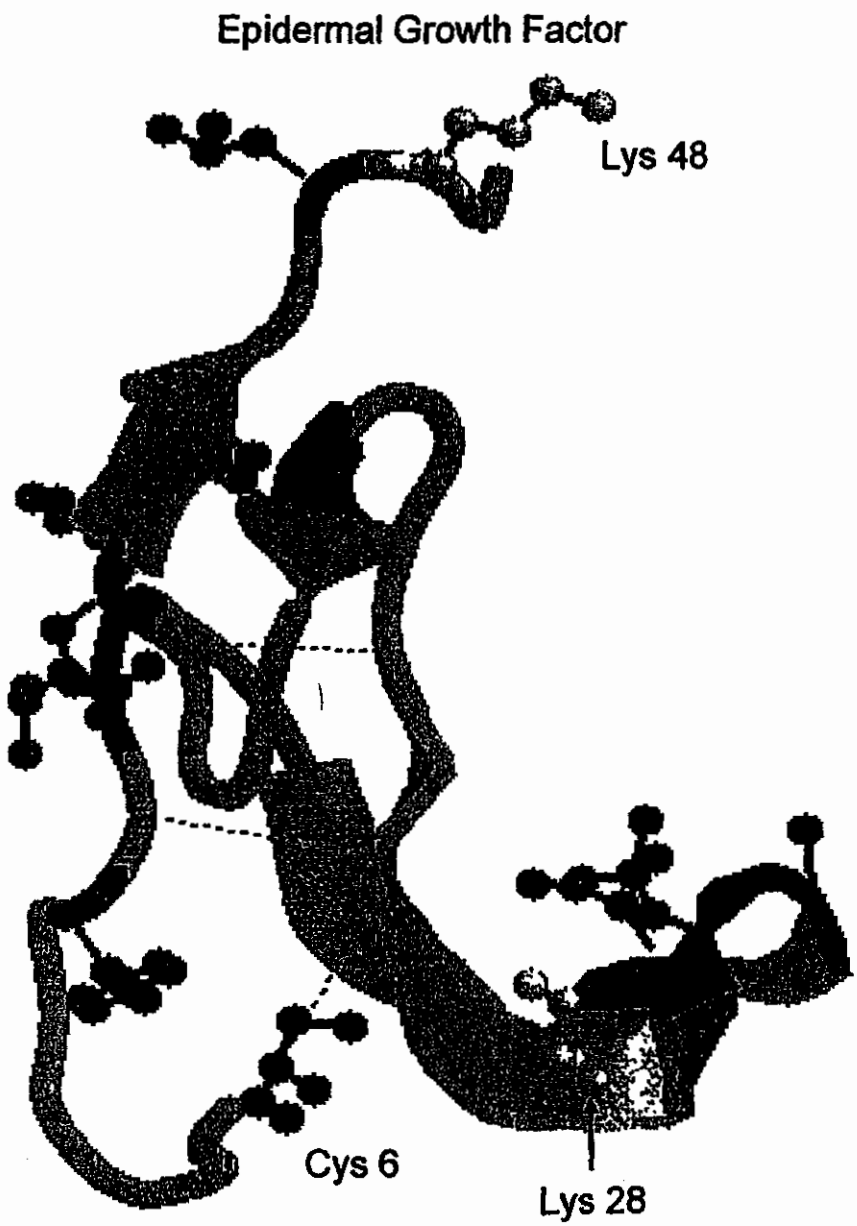


FIG.5

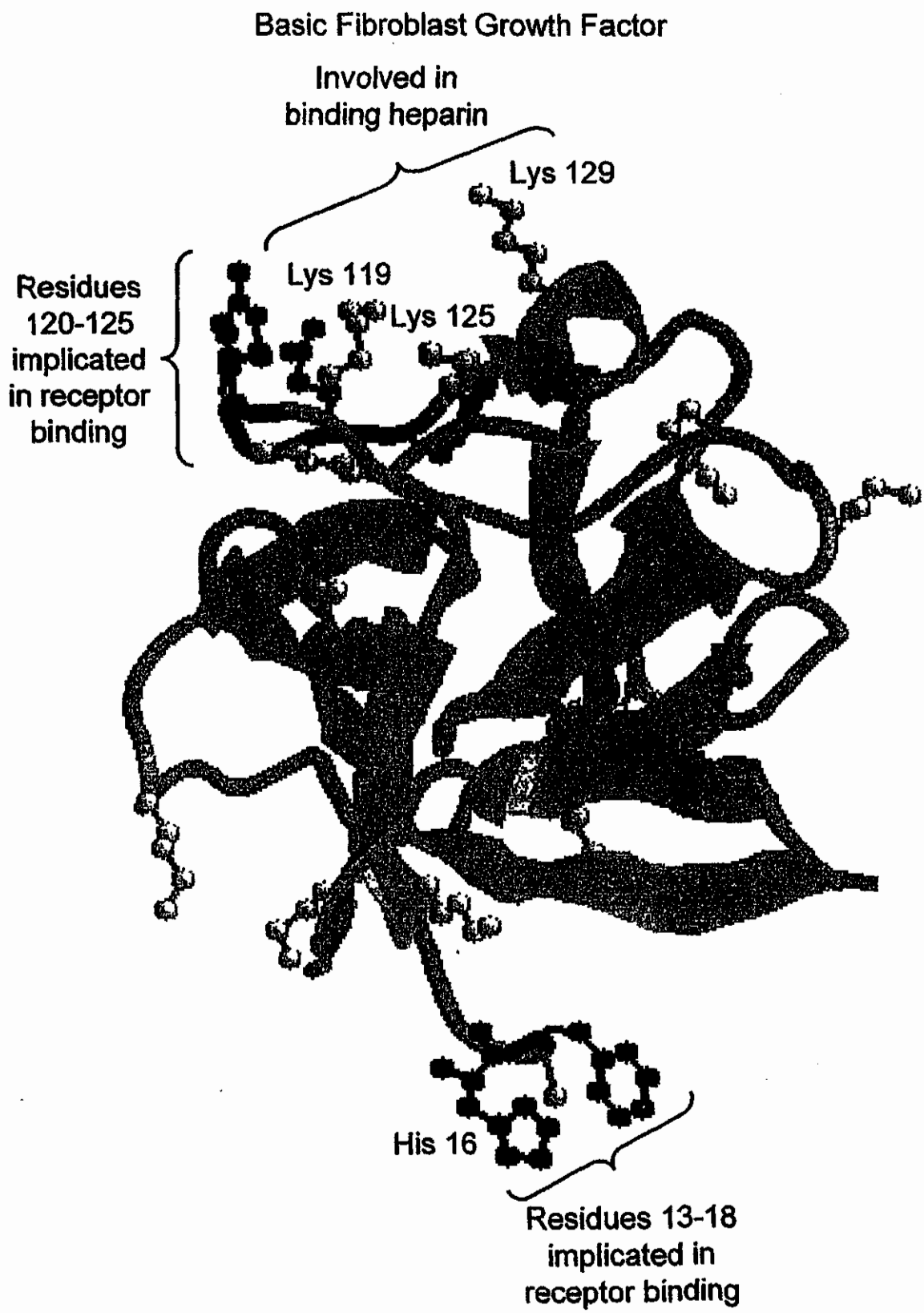

Basic Fibroblast Growth Factor
Involved in binding heparin
Lys 129
Lys 119
Lys 125
Residues 120-125 implicated in receptor binding
His 16
Residues 13-18 implicated in receptor binding

FIG.6

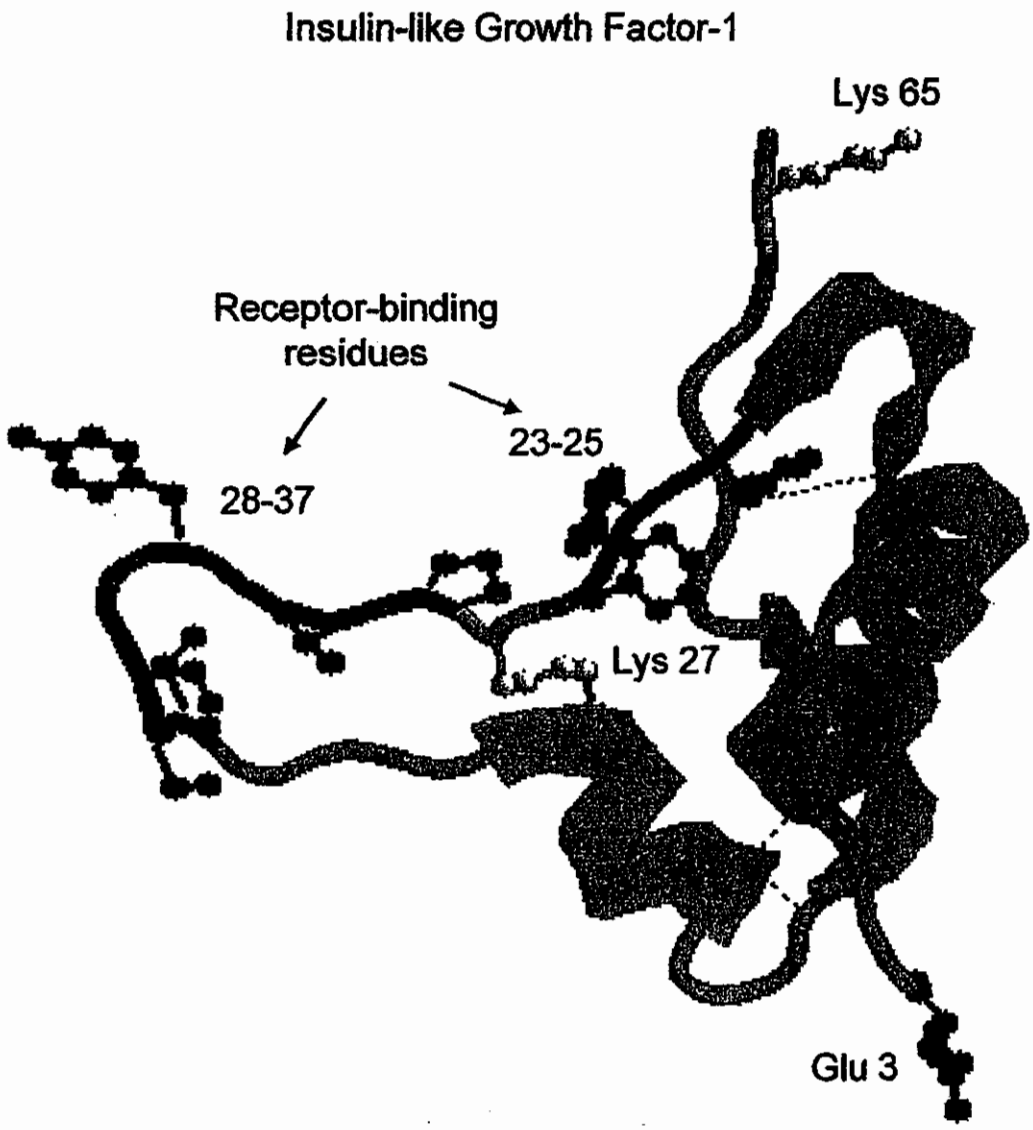

Insulin-like Growth Factor-1
Lys 65
Receptor-binding residues
23-25
28-37
Lys 27
Glu 3

FIG.7

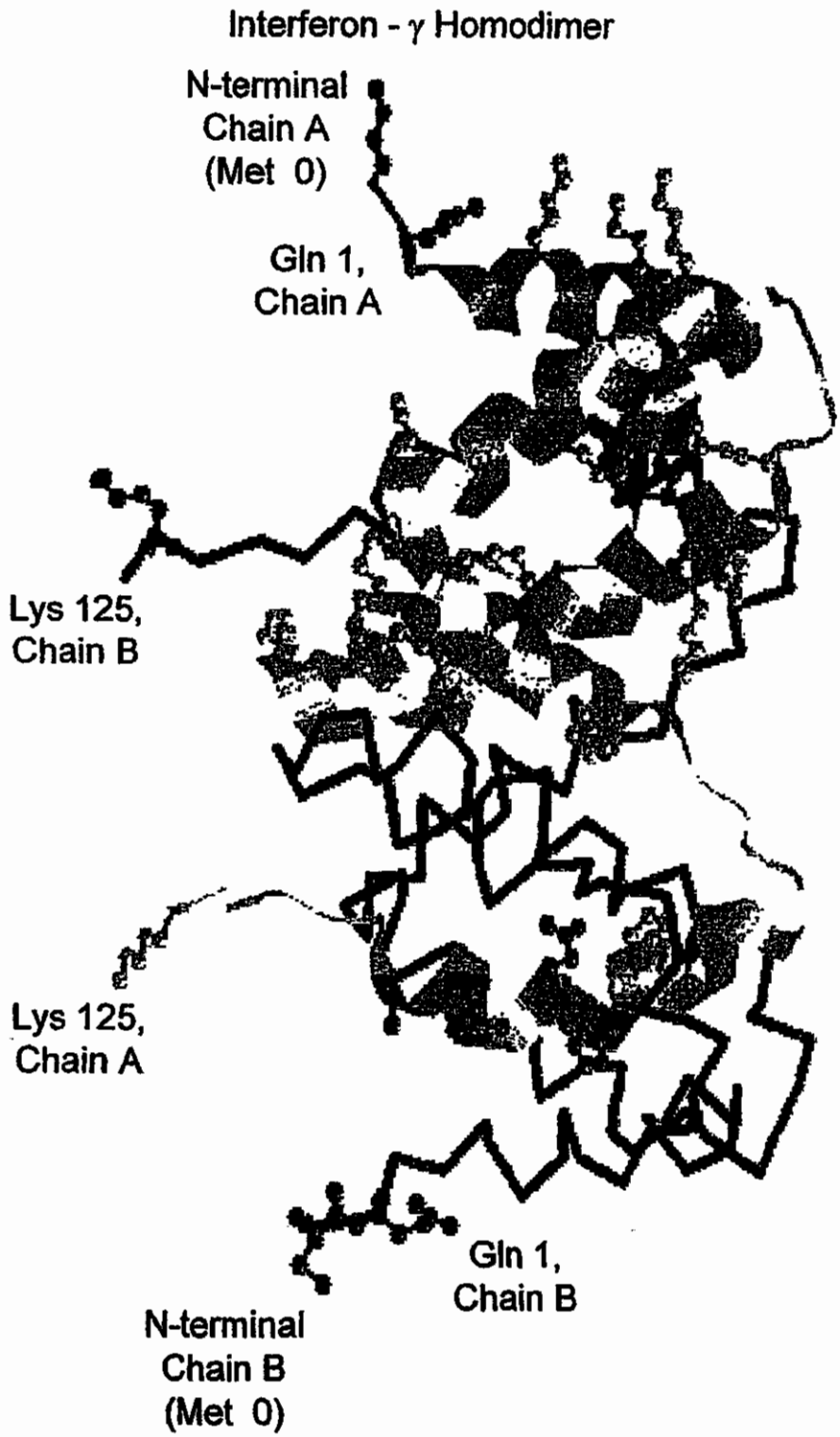

Interferon - γ Homodimer
N-terminal
Chain A
(Met 0)
Gln 1,
Chain A
Lys 125,
Chain B
Lys 125,
Chain A
Gln 1,
Chain B
N-terminal
Chain B
(Met 0)

FIG.8

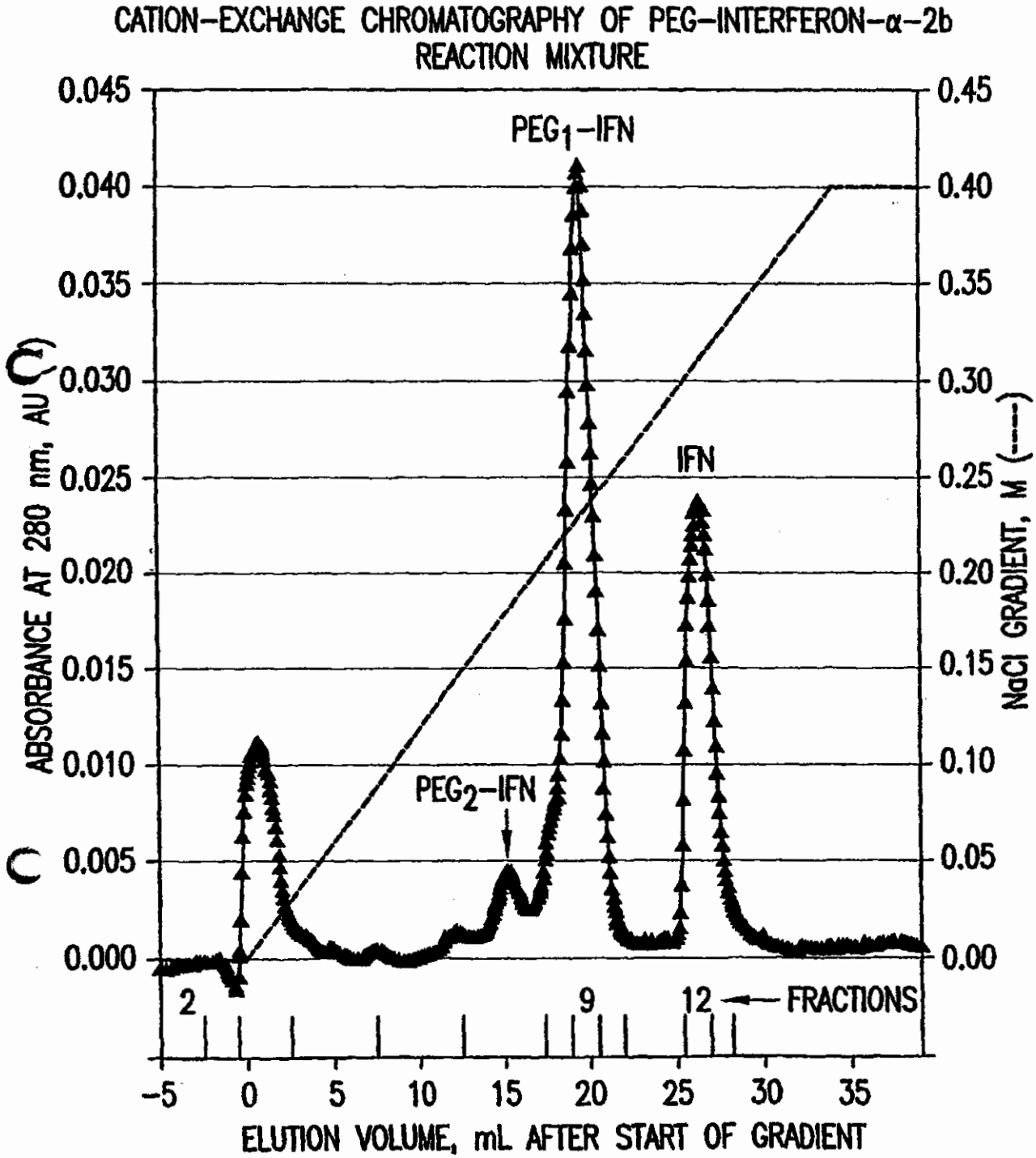

CATION-EXCHANGE CHROMATOGRAPHY OF PEG-INTERFERON-α-2b REACTION MIXTURE
PEG1-IFN
IFN
PEG2-IFN
2
9
12
FRACTIONS
ABSORBANCE AT 280 nm, AU
NaCl GRADIENT, M (----)
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-5
0
5
10
15
20
25
30
35
ELUTION VOLUME, mL AFTER START OF GRADIENT

FIG.9

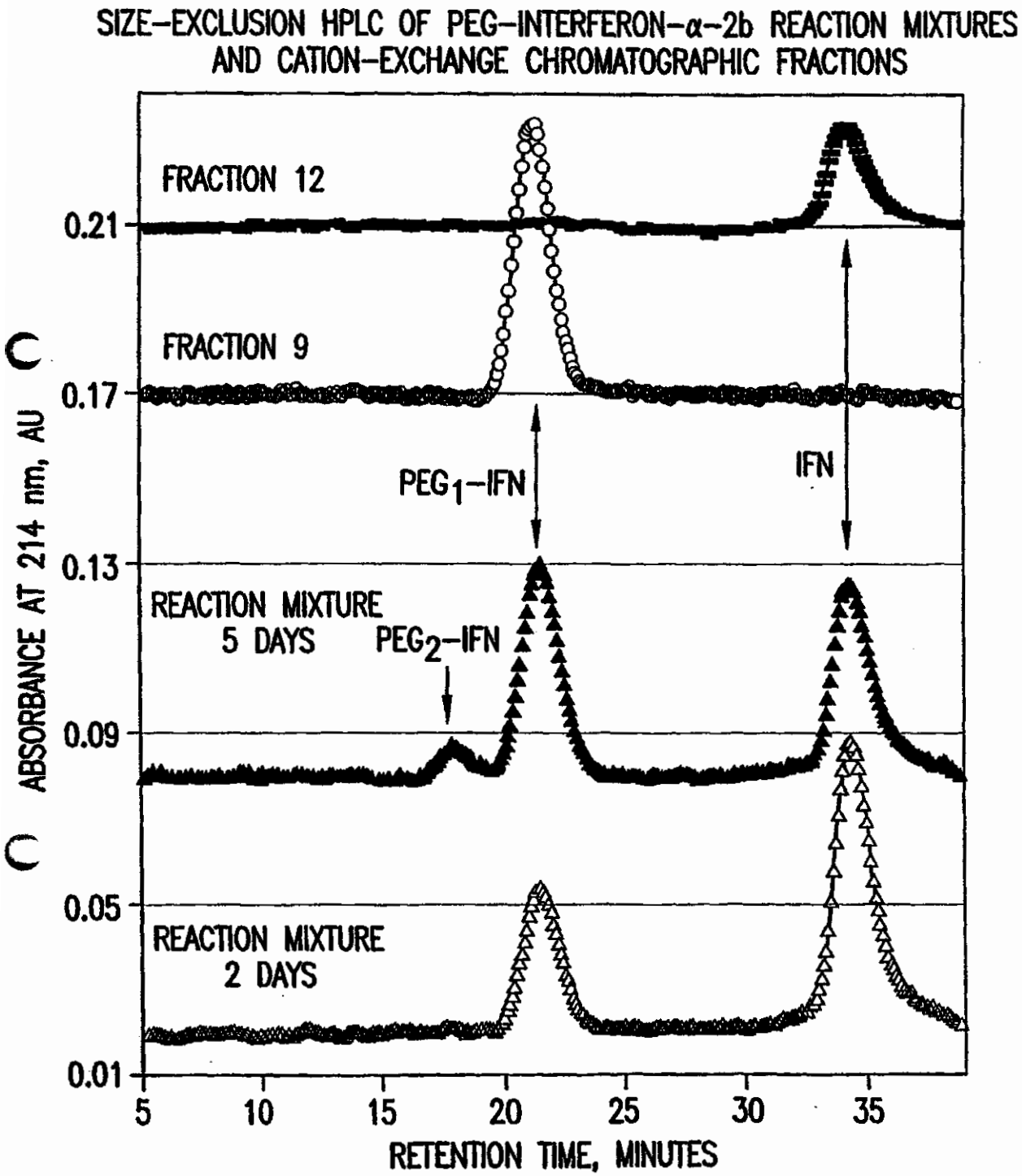

SIZE-EXCLUSION HPLC OF PEG-INTERFERON-α-2b REACTION MIXTURES AND CATION-EXCHANGE CHROMATOGRAPHIC FRACTIONS
FRACTION 12
FRACTION 9
PEG1-IFN
IFN
REACTION MIXTURE 5 DAYS
PEG2-IFN
REACTION MIXTURE 2 DAYS
ABSORBANCE AT 214 nm, AU
0.21
0.17
0.13
0.09
0.05
0.01
5
10
15
20
25
30
35
RETENTION TIME, MINUTES

FIG.10

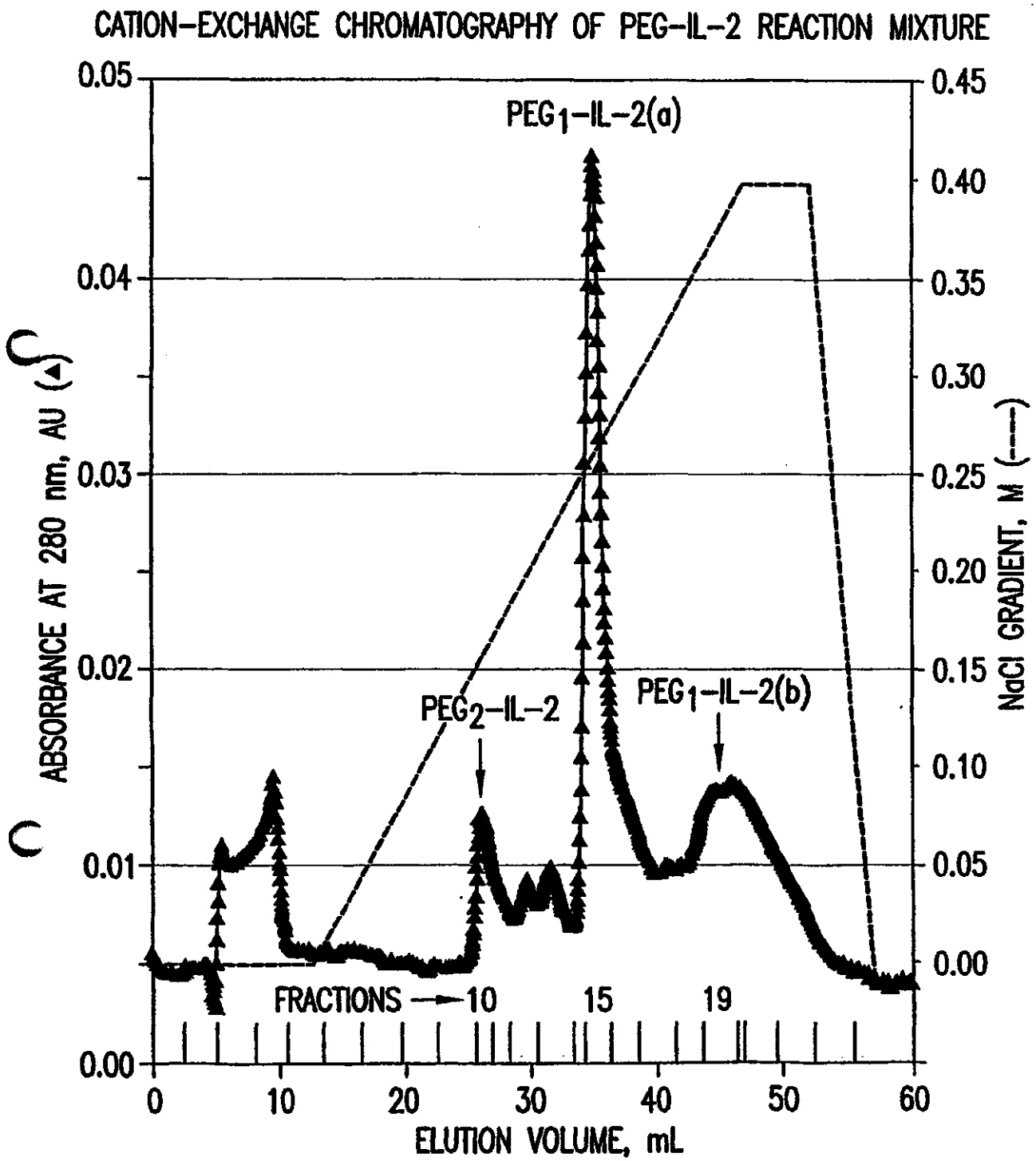

CATION-EXCHANGE CHROMATOGRAPHY OF PEG-IL-2 REACTION MIXTURE
PEG1-IL-2(a)
PEG2-IL-2
PEG1-IL-2(b)
FRACTIONS
10
15
19
ABSORBANCE AT 280 nm, AU (▲)
NaCl GRADIENT, M (----)
ELUTION VOLUME, mL
0.05
0.04
0.03
0.02
0.01
0.00
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
60

FIG.11

SIZE-EXCLUSION HPLC OF PEG-INTERLEUKIN-2 REACTION MIXTURE AND CATION-EXCHANGE CHROMATOGRAPHIC FRACTIONS

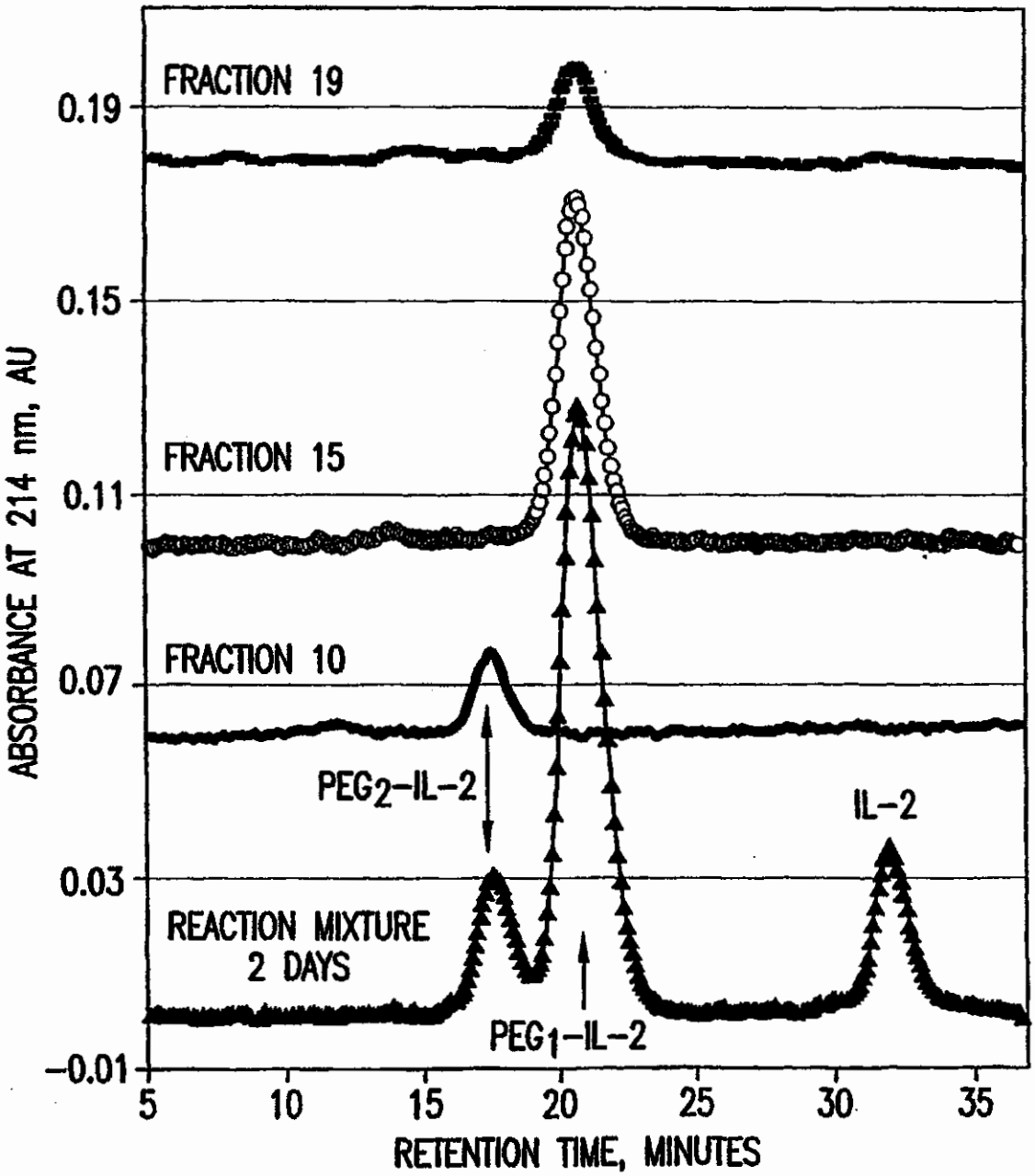


FIG.12

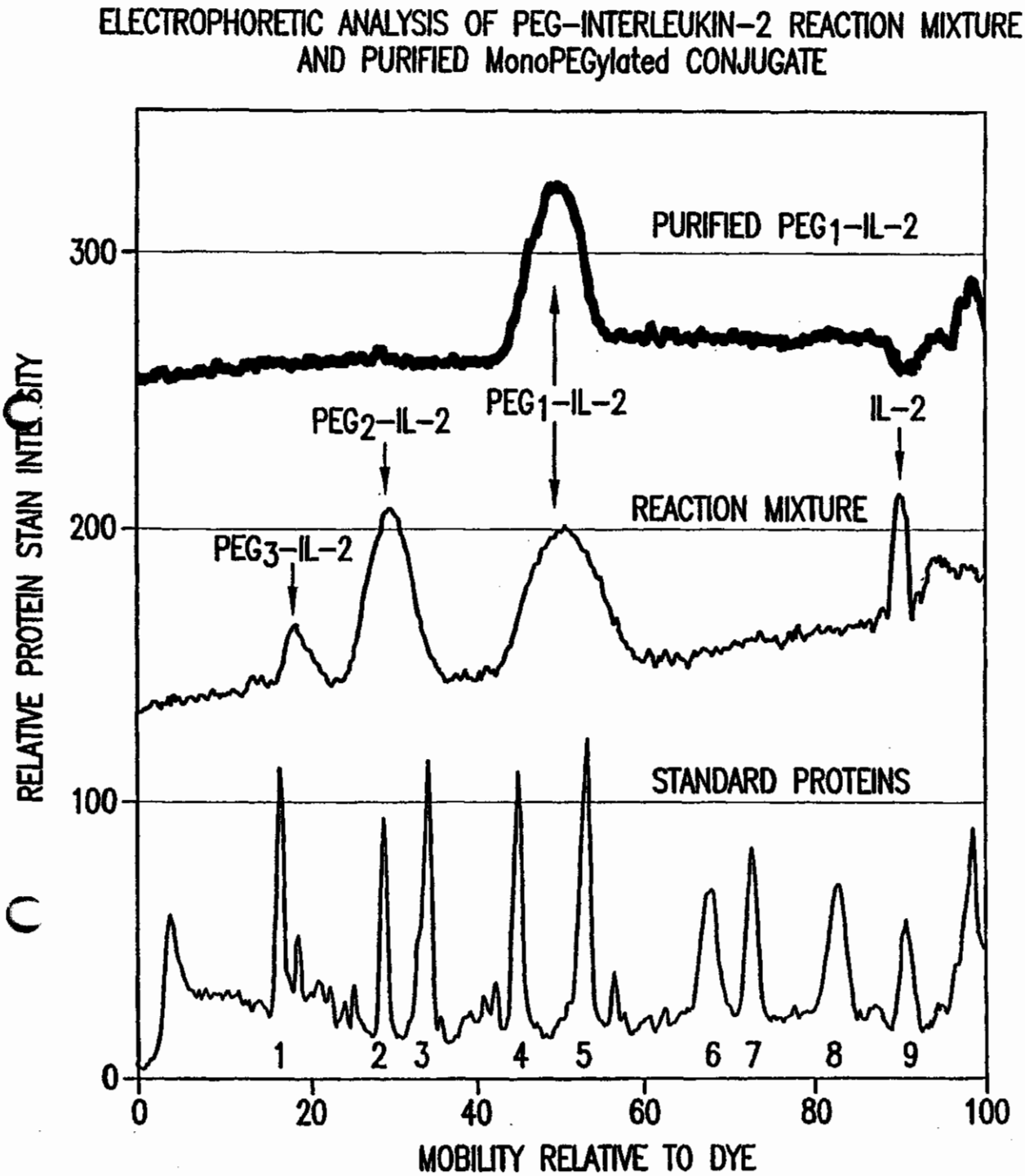

ELECTROPHORETIC ANALYSIS OF PEG–INTERLEUKIN–2 REACTION MIXTURE AND PURIFIED MonoPEGylated CONJUGATE
PURIFIED PEG1–IL–2
PEG2–IL–2
PEG1–IL–2
IL–2
REACTION MIXTURE
PEG3–IL–2
STANDARD PROTEINS
RELATIVE PROTEIN STAIN INTENSITY
300
200
100
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
MOBILITY RELATIVE TO DYE

FIG.13

SEQUENCE LISTING

<110> Mountain View Pharmaceuticals, Inc.
3475-S Edison Way
Menlo Park, California 94025
United States of America

<120> POLYMER CONJUGATES OF CYTOKINES, CHEMOKINES, GROWTH FACTORS, POLYPEPTIDE HORMONES AND ANTAGONISTS THEREOF WITH PRESERVED RECEPTOR-BINDING ACTIVITY

<130> 2057.006PC02/JAG/BJD

<140> (To be assigned)
<141> 2003-12-23

<150> US 60/479,914
<151> 2003-06-20

<150> US 60/436,020
<151> 2002-12-26

<160> 9

<170> FastSEQ for Windows Version 4.0

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<211> 165
<212> PRT
<213> Homo sapiens

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1				5					10					15	
Leu	Leu	Ala	Gln	Met	Arg	Lys	Ile	Ser	Leu	Phe	Ser	Cys	Leu	Lys	Asp
			20					25					30		
Arg	His	Asp	Phe	Gly	Phe	Pro	Gln	Glu	Glu	Phe	Gly	Asn	Gln	Phe	Gln
		35					40					45			
Lys	Ala	Glu	Thr	Ile	Pro	Val	Leu	His	Glu	Met	Ile	Gln	Gln	Ile	Phe
	50					55					60				
Asn	Leu	Phe	Ser	Thr	Lys	Asp	Ser	Ser	Ala	Ala	Trp	Asp	Glu	Thr	Leu
65					70					75					80
Leu	Asp	Lys	Phe	Tyr	Thr	Glu	Leu	Tyr	Gln	Gln	Leu	Asn	Asp	Leu	Glu
				85					90					95	
Ala	Cys	Val	Ile	Gln	Gly	Val	Gly	Val	Thr	Glu	Thr	Pro	Leu	Met	Lys
			100					105					110		
Glu	Asp	Ser	Ile	Leu	Ala	Val	Arg	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu
		115					120					125			
Tyr	Leu	Lys	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp	Glu	Val	Val	Arg
	130					135					140				

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160
Leu Arg Ser Lys Glu
165

<210> 2
<211> 165
<212> PRT
<213> Homo sapiens

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1 5 10 15
Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30
Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45
Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95
Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110
Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125
Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140
Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160
Leu Arg Ser Lys Glu
165

<210> 3
<211> 166
<212> PRT
<213> Homo sapiens

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Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15
Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45
Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95
His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110
Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160
Thr Gly Tyr Leu Arg Asn
165

<210> 4
<211> 143
<212> PRT
<213> Homo sapiens

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Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys Tyr Phe Asn
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Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe Leu Gly Ile
20 25 30
Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met Gln Ser Gln
35 40 45
Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys Asp Asp Gln
50 55 60
Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met Asn Val Lys
65 70 75 80
Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu Lys Leu Thr
85 90 95
Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala Ile His Glu
100 105 110

Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys Thr Gly Lys
115 120 125
Arg Lys Arg Ser Gln Met Leu Phe Arg Gly Arg Arg Ala Ser Gln
130 135 140

<210> 5
<211> 127
<212> PRT
<213> Homo sapiens

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Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr
20 25 30
Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp
35 40 45
Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln
50 55 60
Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met
65 70 75 80
Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser Cys
85 90 95
Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp
100 105 110
Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu
115 120 125

<210> 6
<211> 133
<212> PRT
<213> Homo sapiens

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1 5 10 15
Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30
Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
35 40 45

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Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
    50                  55                  60
Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65                  70                  75                  80
Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                85                  90                  95
Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110
Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125
Ile Ser Thr Leu Thr
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<213> Homo sapiens

<400> 7

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Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
            20                  25                  30
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
        35                  40                  45
Trp Trp Glu Leu Arg
    50
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<210> 8
<211> 146
<212> PRT
<213> Homo sapiens

<400> 8

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Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His
1               5                   10                  15
Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu
            20                  25                  30
Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp
        35                  40                  45
```

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Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser
    50                  55                  60
Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly
65                  70                  75                  80
Arg Leu Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu
                85                  90                  95
Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Thr
            100                 105                 110
Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser
        115                 120                 125
Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala
    130                 135                 140
Lys Ser
145
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<210> 9
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<400> 9

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1               5                   10                  15
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30
Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45
Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60
Lys Pro Ala Lys Ser Ala
65                  70
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