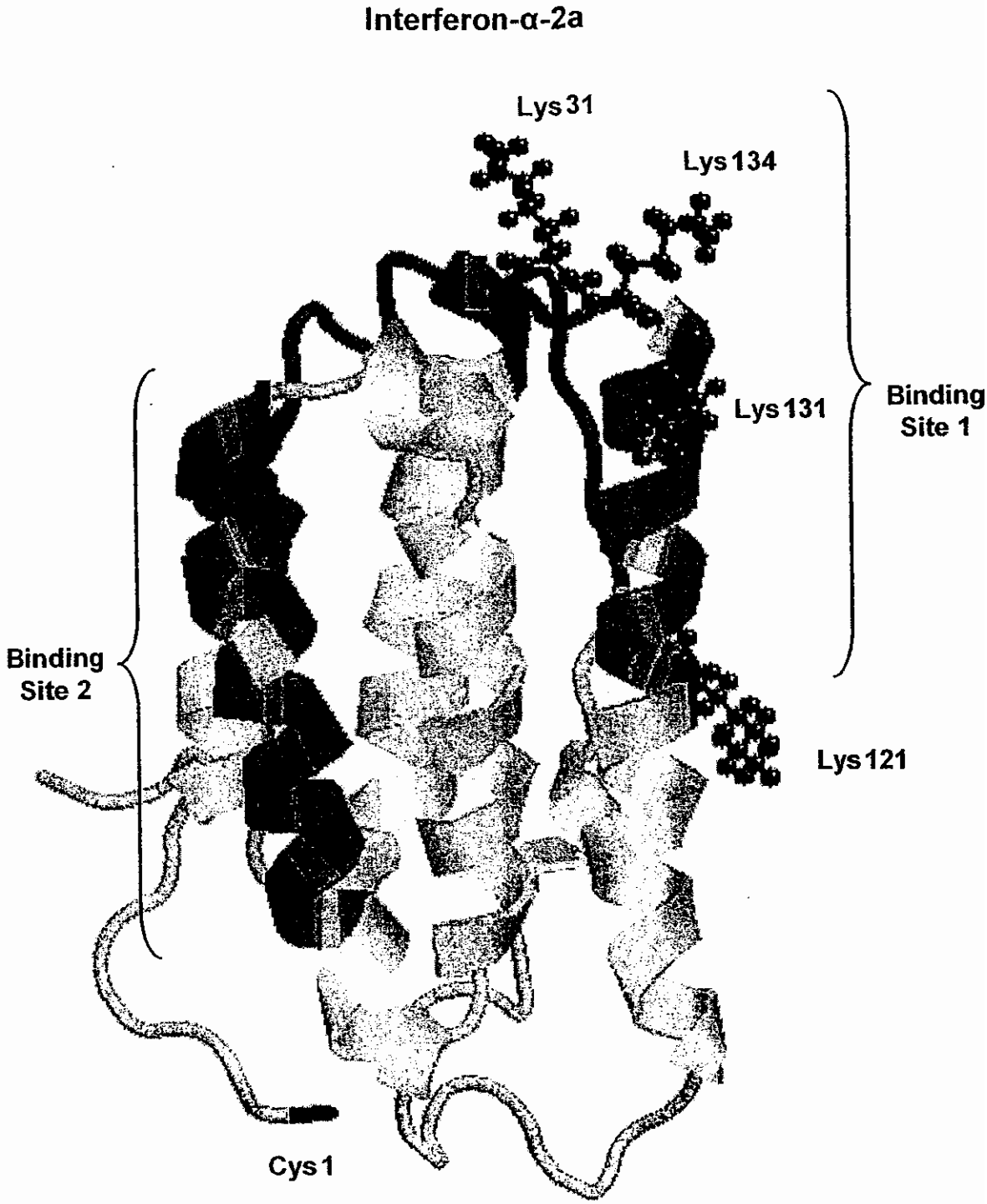

Interferon-α-2a
Lys 31
Lys 134
Lys 131
Binding Site 1
Binding Site 2
Lys 121
Cys 1

Figure 1a

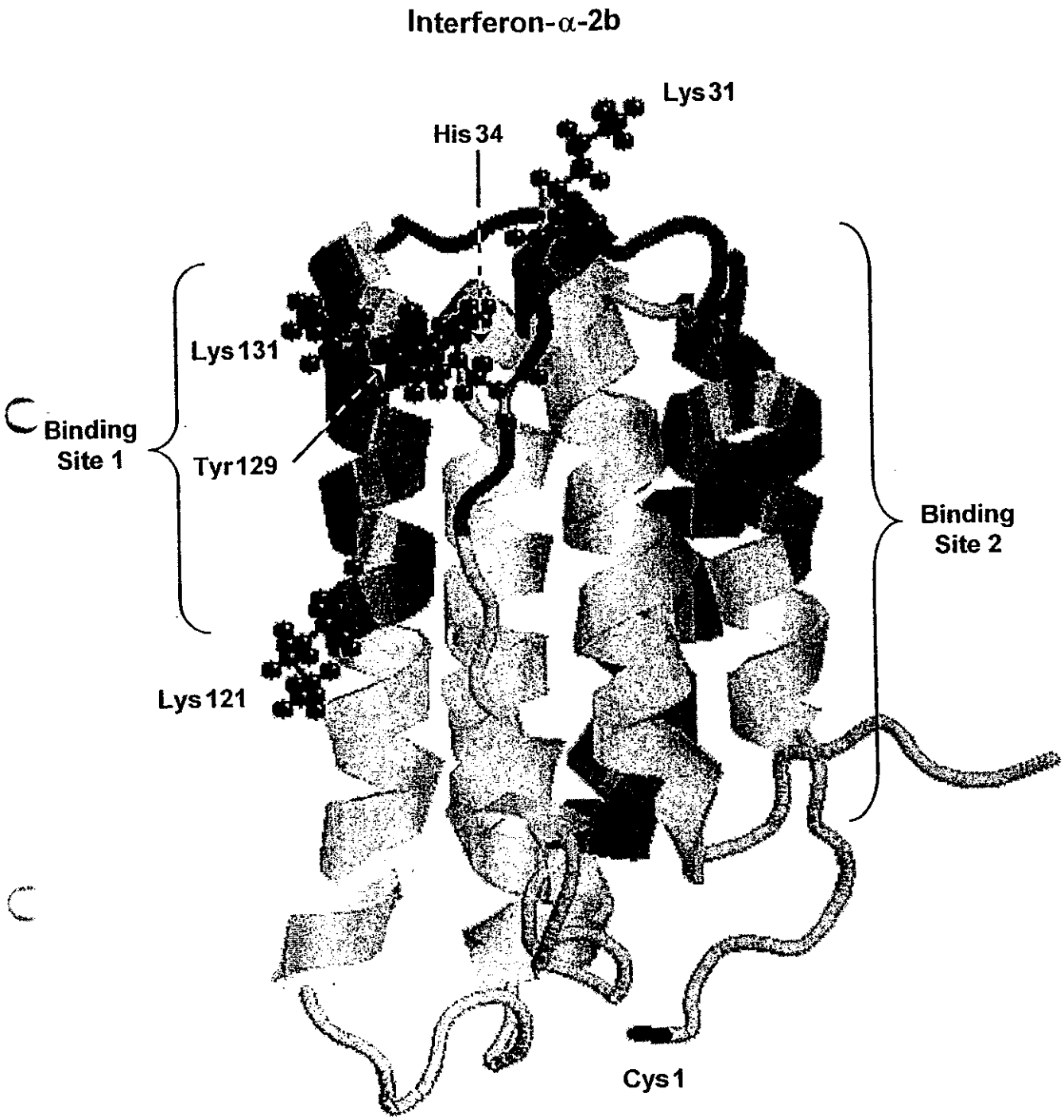

Interferon-α-2b
Lys 31
His 34
Lys 131
Binding Site 1
Tyr 129
Binding Site 2
Lys 121
Cys 1

Figure 1b

Interferon α-2b

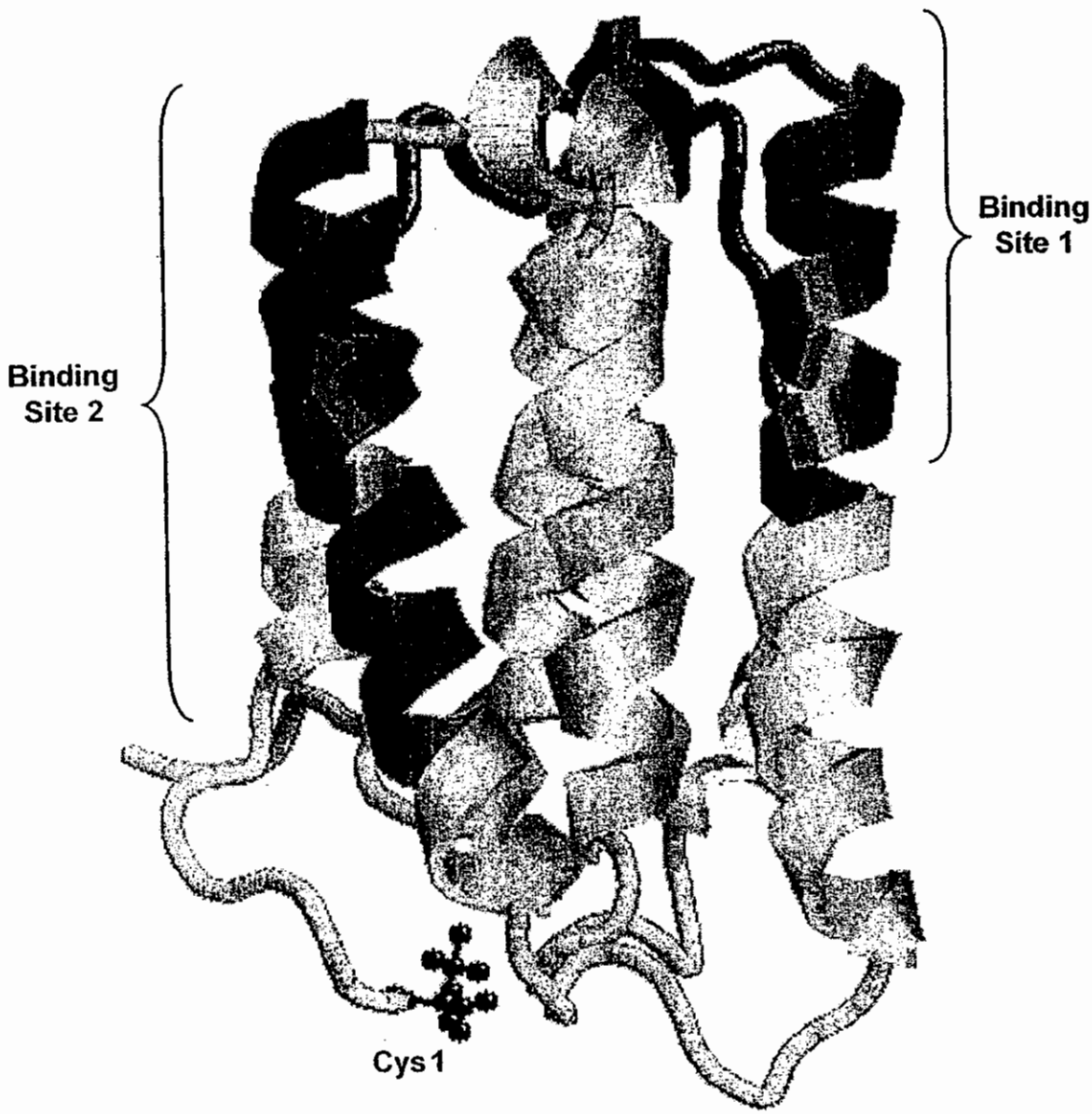


Figure 1c

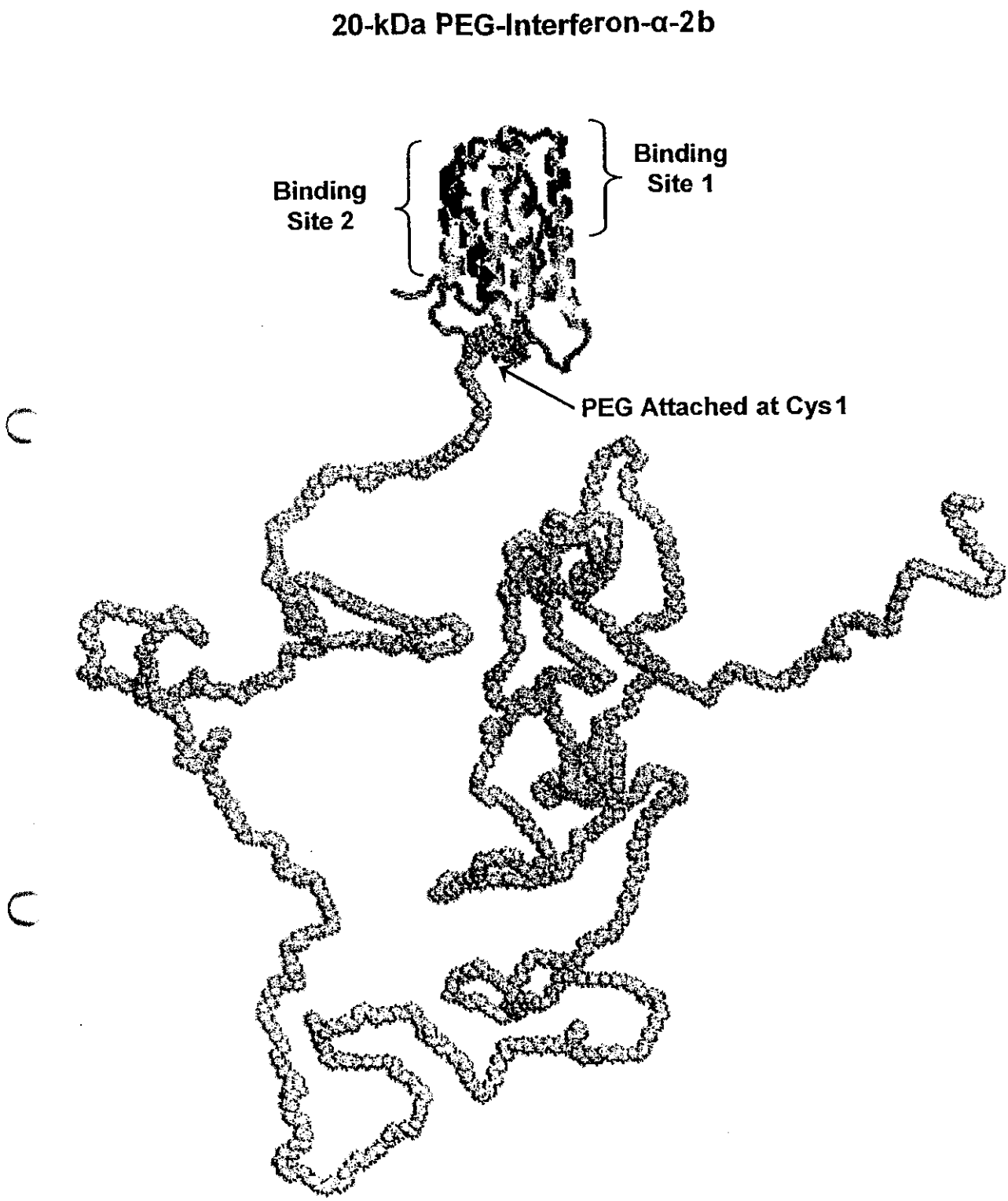

20-kDa PEG-Interferon-α-2b
Binding Site 2
Binding Site 1
PEG Attached at Cys1

Figure 1d

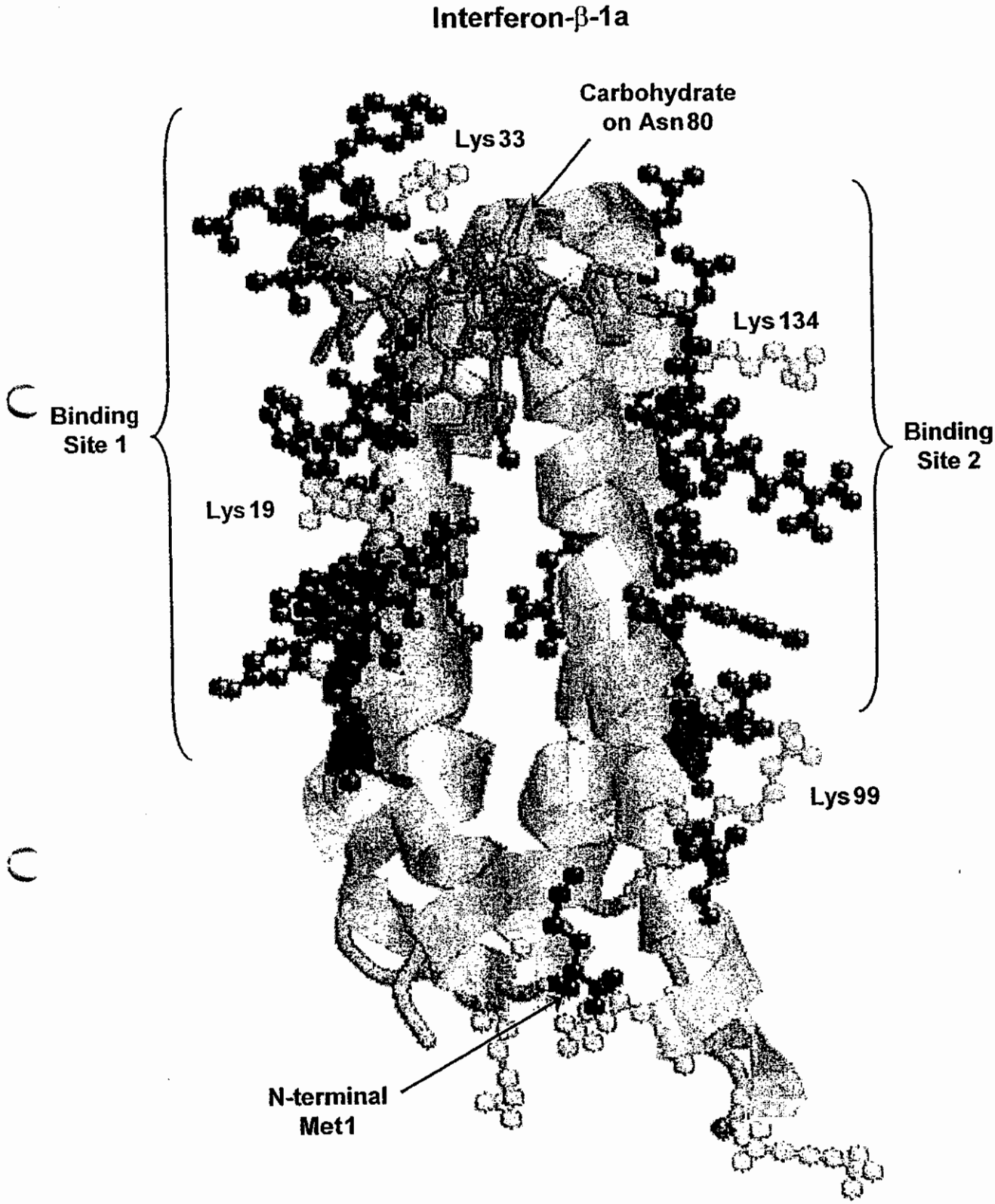

Interferon-β-1a
Carbohydrate on Asn80
Lys 33
Lys 134
Binding Site 1
Binding Site 2
Lys 19
Lys 99
N-terminal Met1

Figure 2

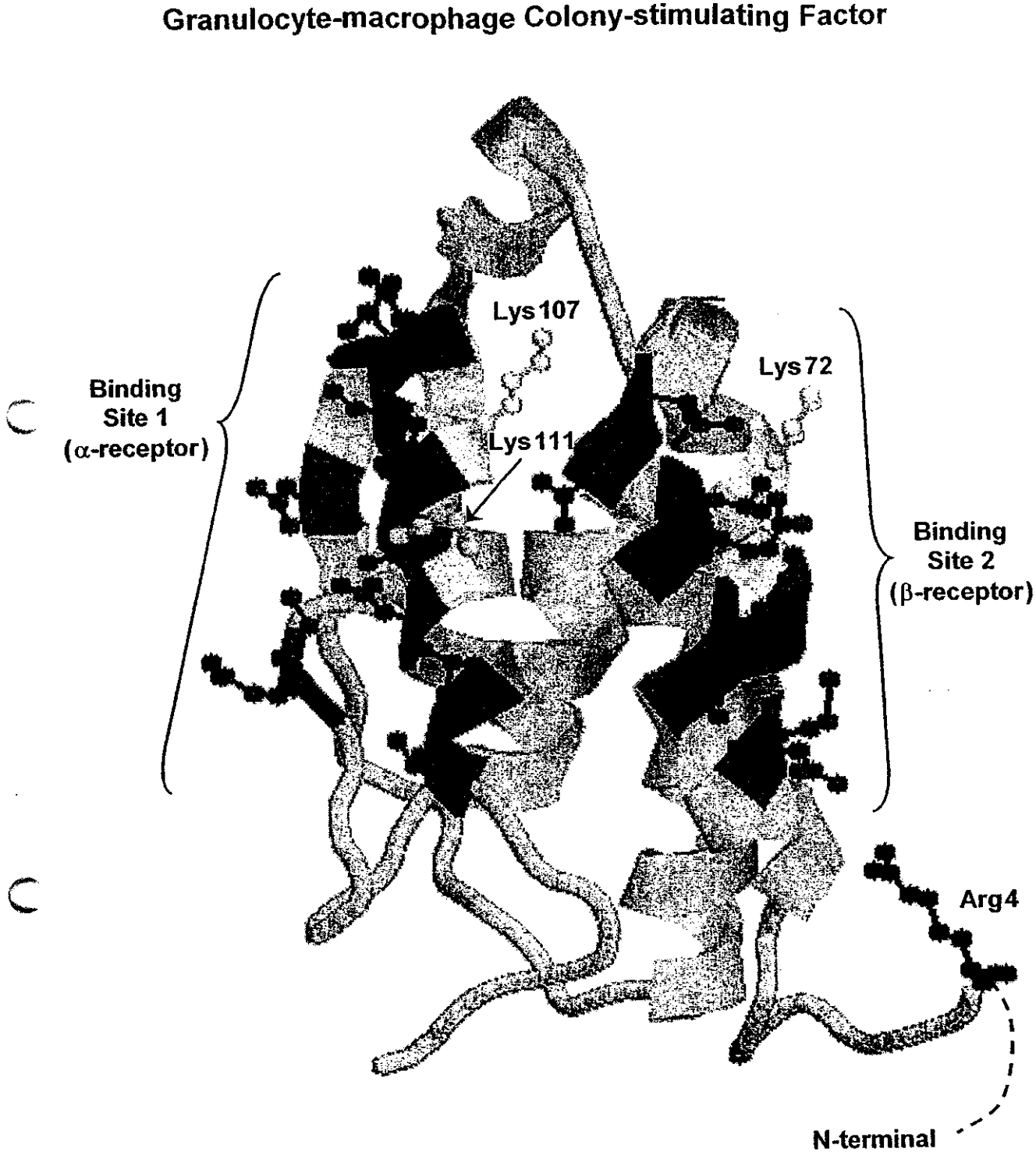

Granulocyte-macrophage Colony-stimulating Factor
Lys107
Lys72
Lys111
Binding Site 1 (α-receptor)
Binding Site 2 (β-receptor)
Arg4
N-terminal

Figure 3

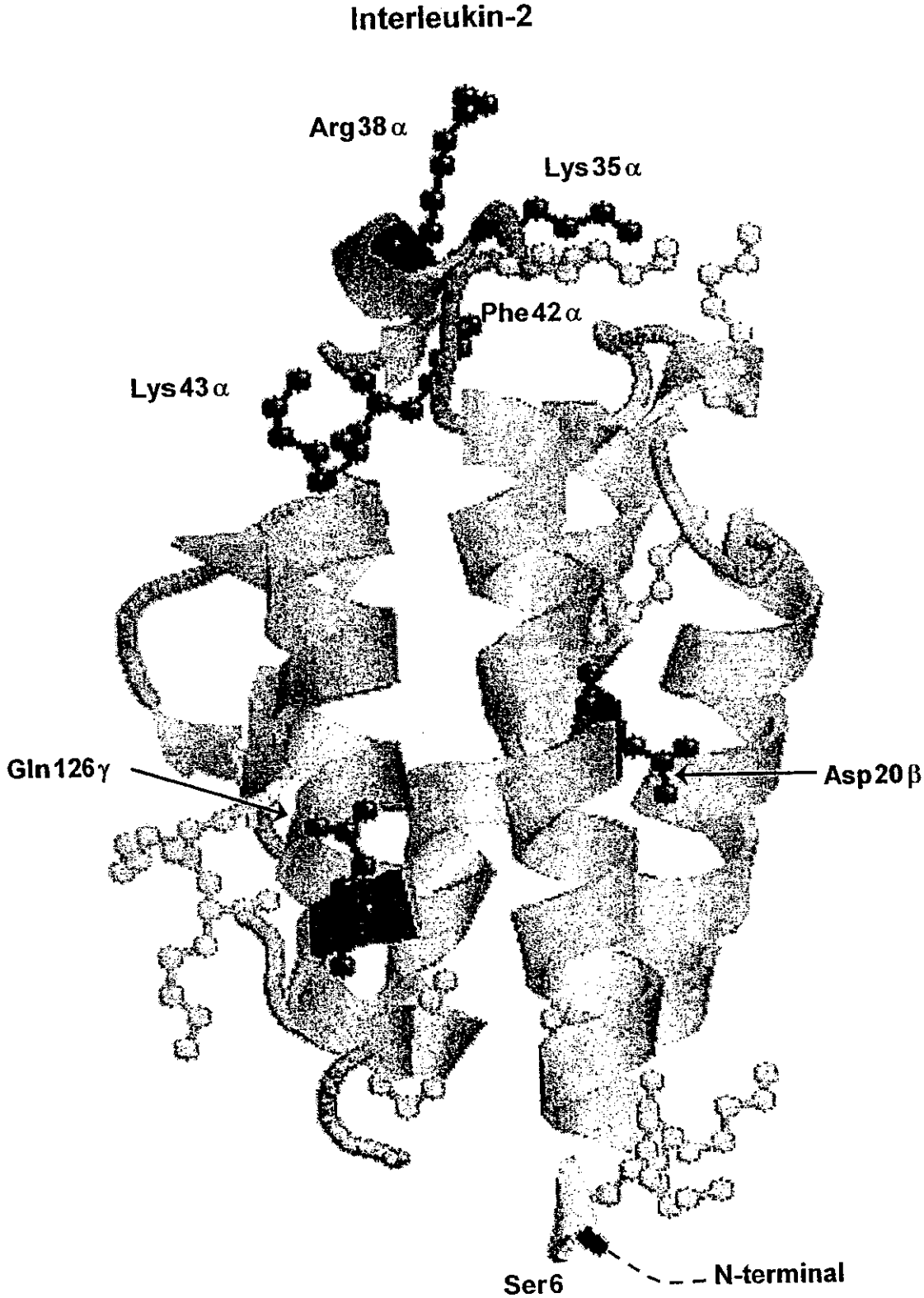

Interleukin-2
Arg38α
Lys35α
Phe42α
Lys43α
Gln126γ
Asp20β
Ser6
N-terminal

Figure 4

Epidermal Growth Factor

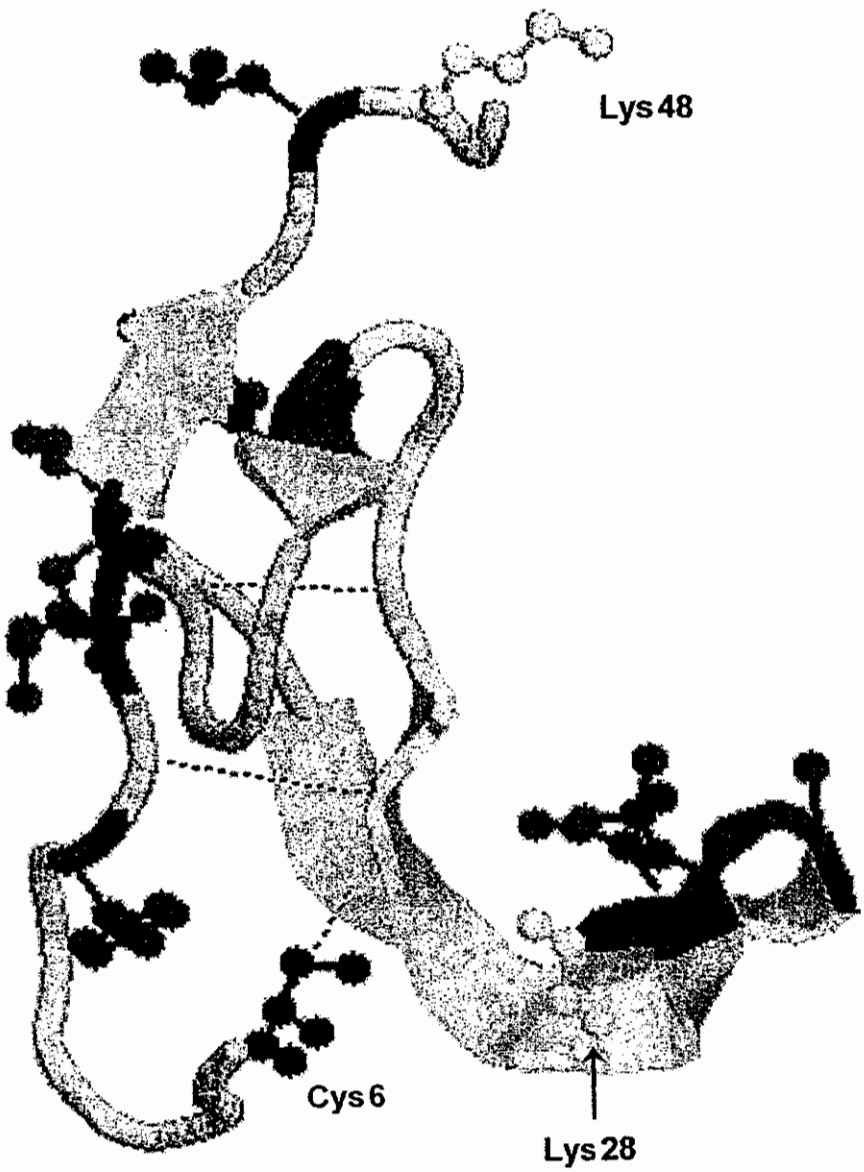


Figure 5

Basic Fibroblast Growth Factor

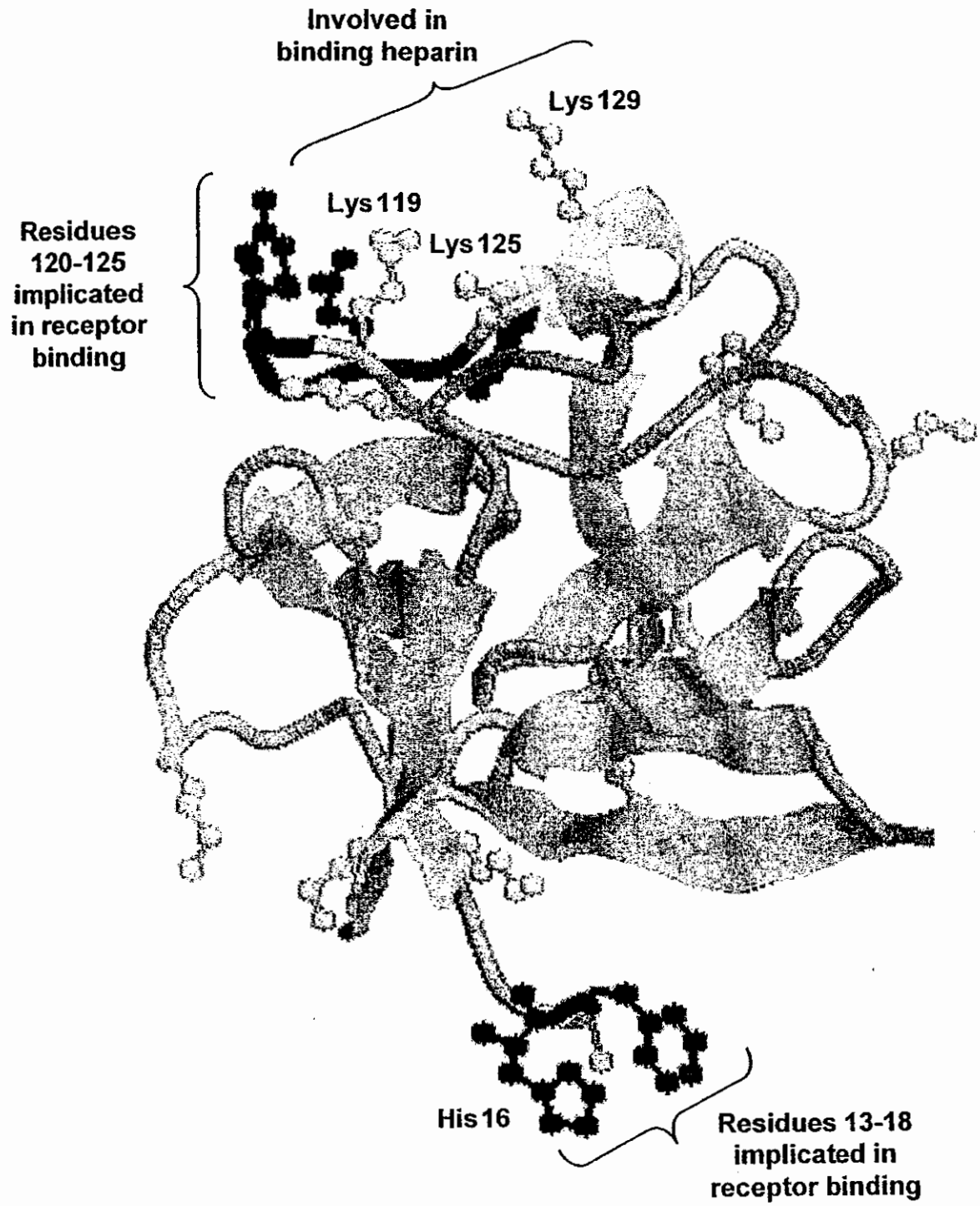


Figure 6

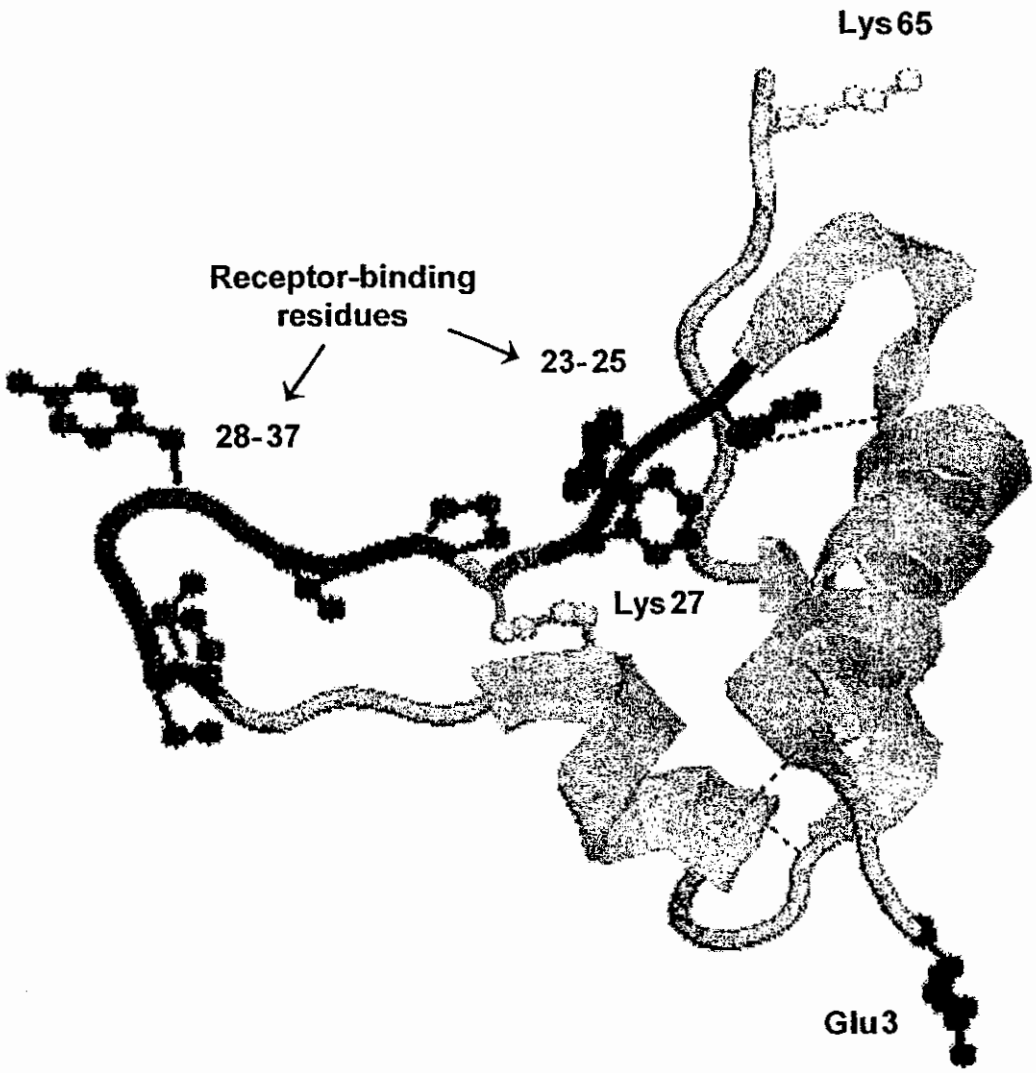

Insulin-like Growth Factor-1
Lys65
Receptor-binding residues
23-25
28-37
Lys27
Glu3

Figure 7

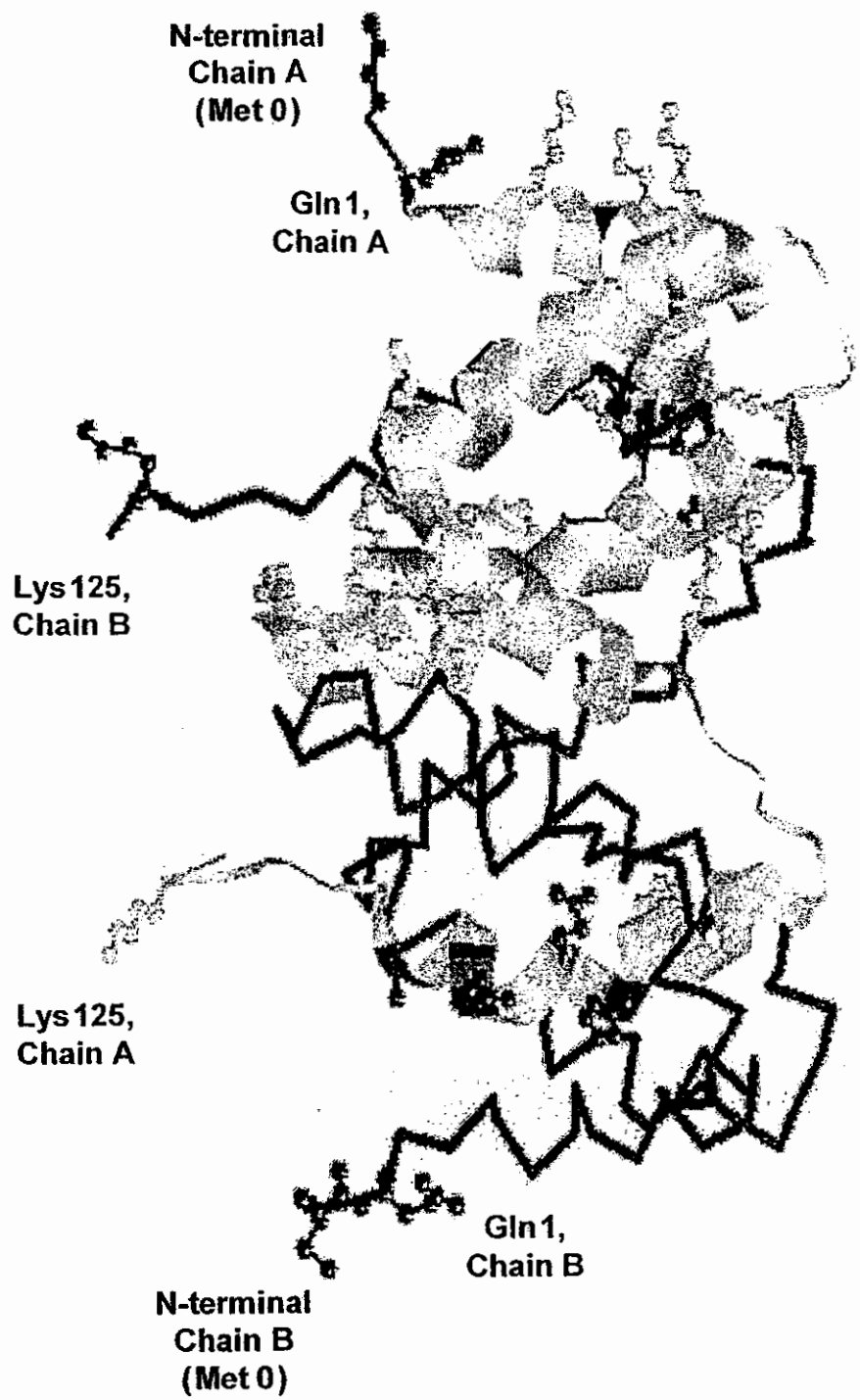

Interferon-γ Homodimer
N-terminal
Chain A
(Met 0)
Gln 1,
Chain A
Lys 125,
Chain B
Lys 125,
Chain A
Gln 1,
Chain B
N-terminal
Chain B
(Met 0)

Figure 8

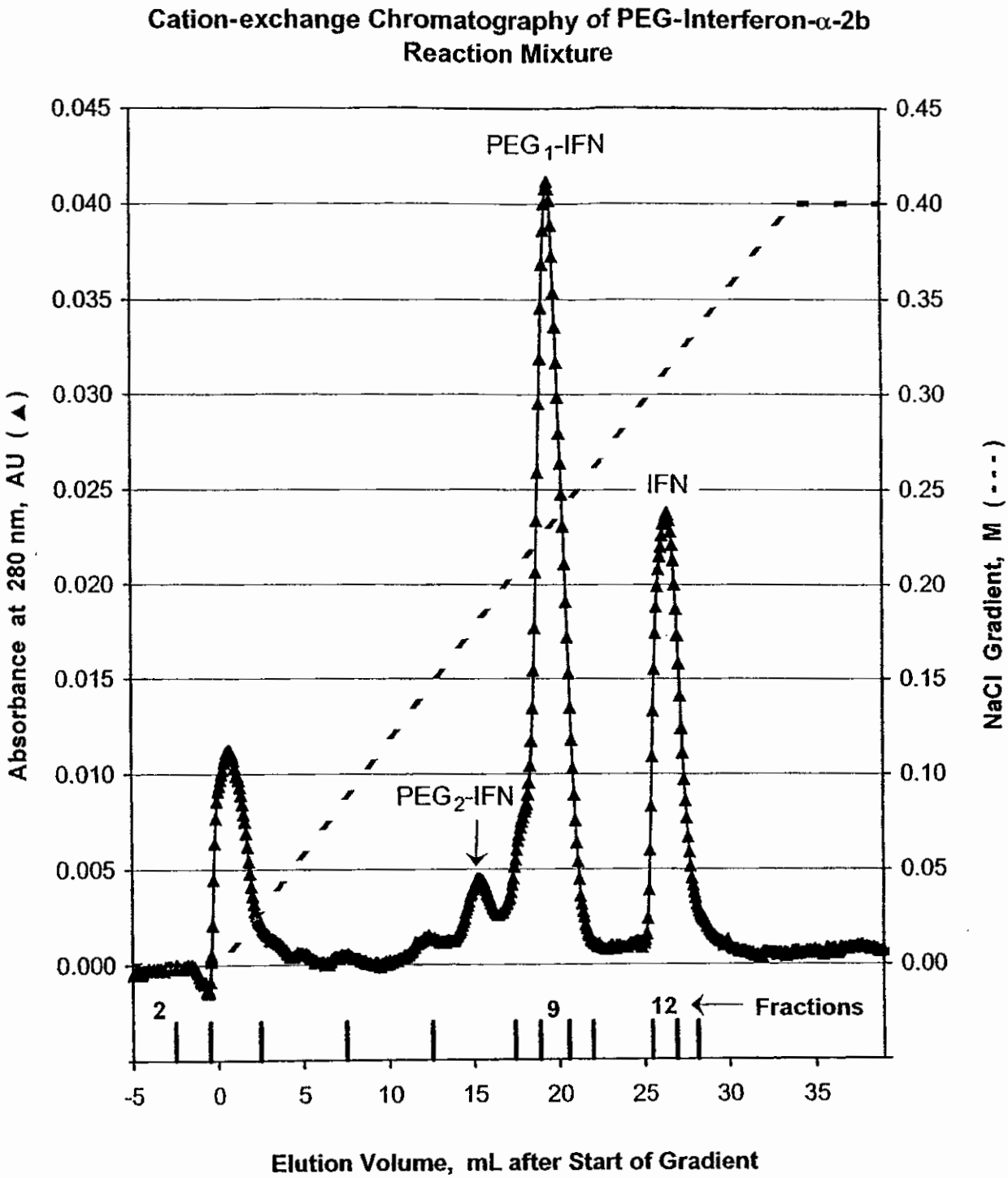

Cation-exchange Chromatography of PEG-Interferon-α-2b
Reaction Mixture
PEG1-IFN
IFN
PEG2-IFN
2
9
12
Fractions
Absorbance at 280 nm, AU (▲)
NaCl Gradient, M (- - -)
Elution Volume, mL after Start of Gradient
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-5
0
5
10
15
20
25
30
35

Figure 9

Size-exclusion HPLC of PEG-Interferon-α-2b Reaction Mixtures and Cation-exchange Chromatographic Fractions

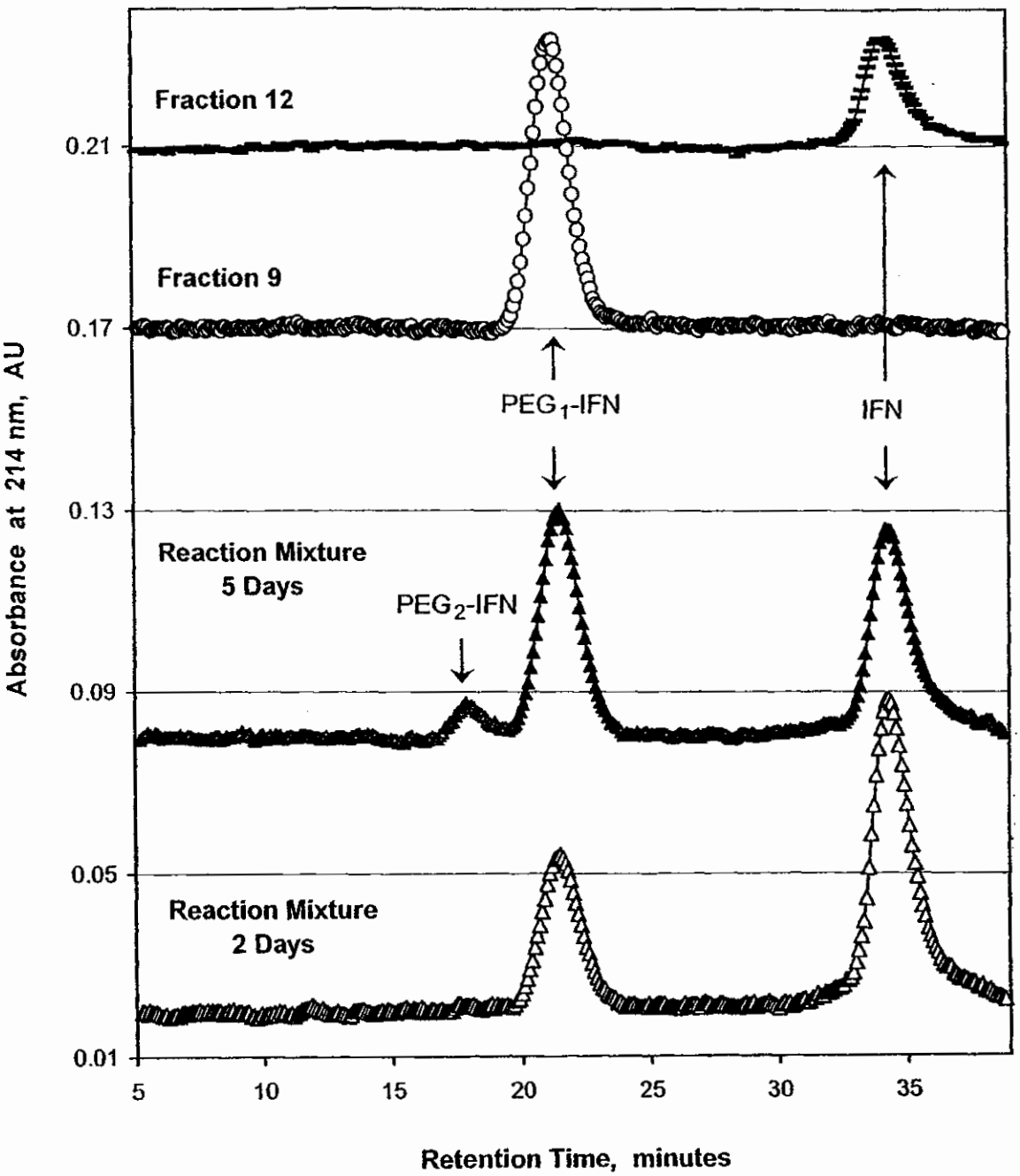


Figure 10

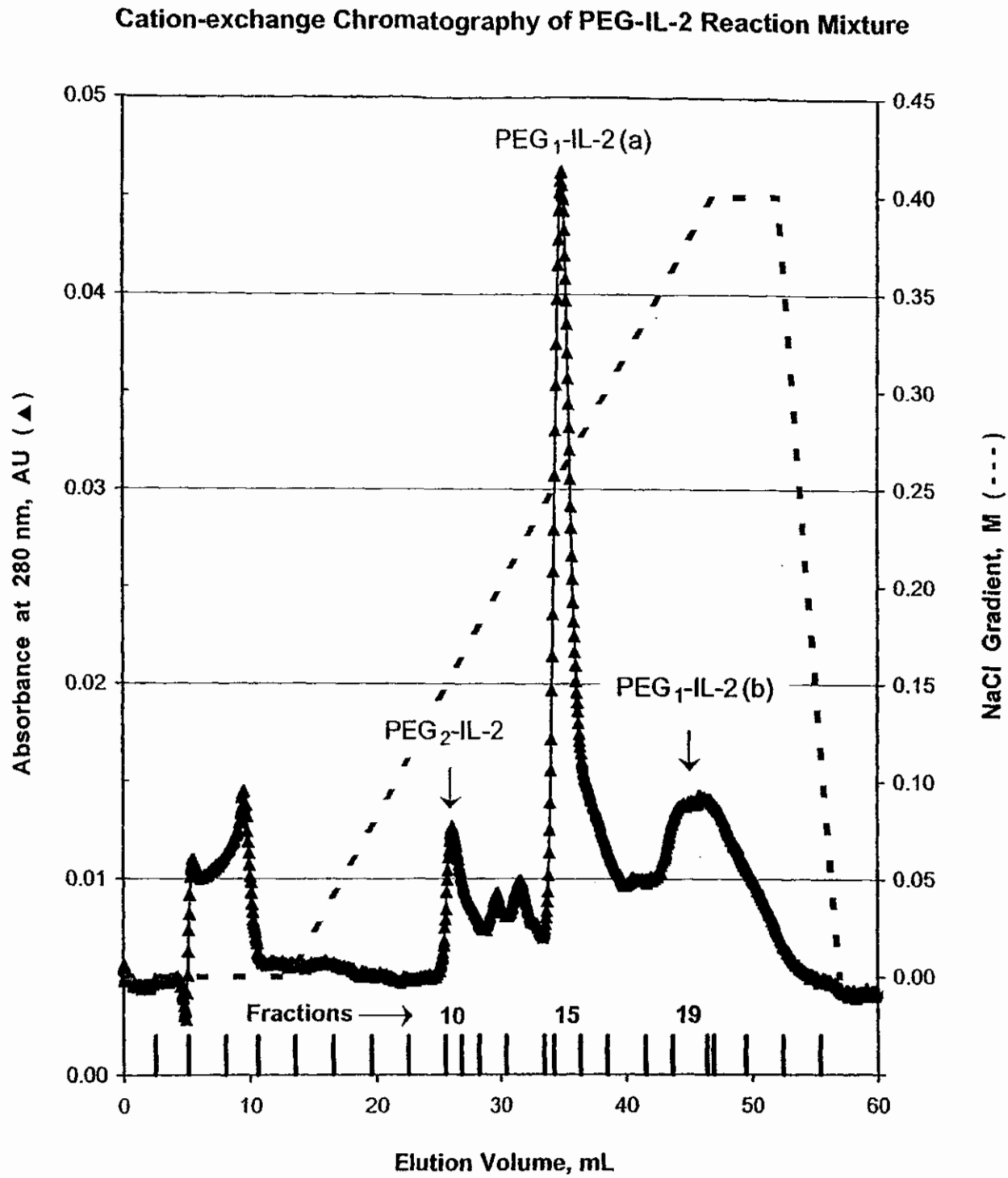

Cation-exchange Chromatography of PEG-IL-2 Reaction Mixture
PEG1-IL-2 (a)
PEG2-IL-2
PEG1-IL-2 (b)
Fractions
10
15
19
Absorbance at 280 nm, AU (▲)
NaCl Gradient, M (- - -)
Elution Volume, mL
0.05
0.04
0.03
0.02
0.01
0.00
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
60

Figure 11

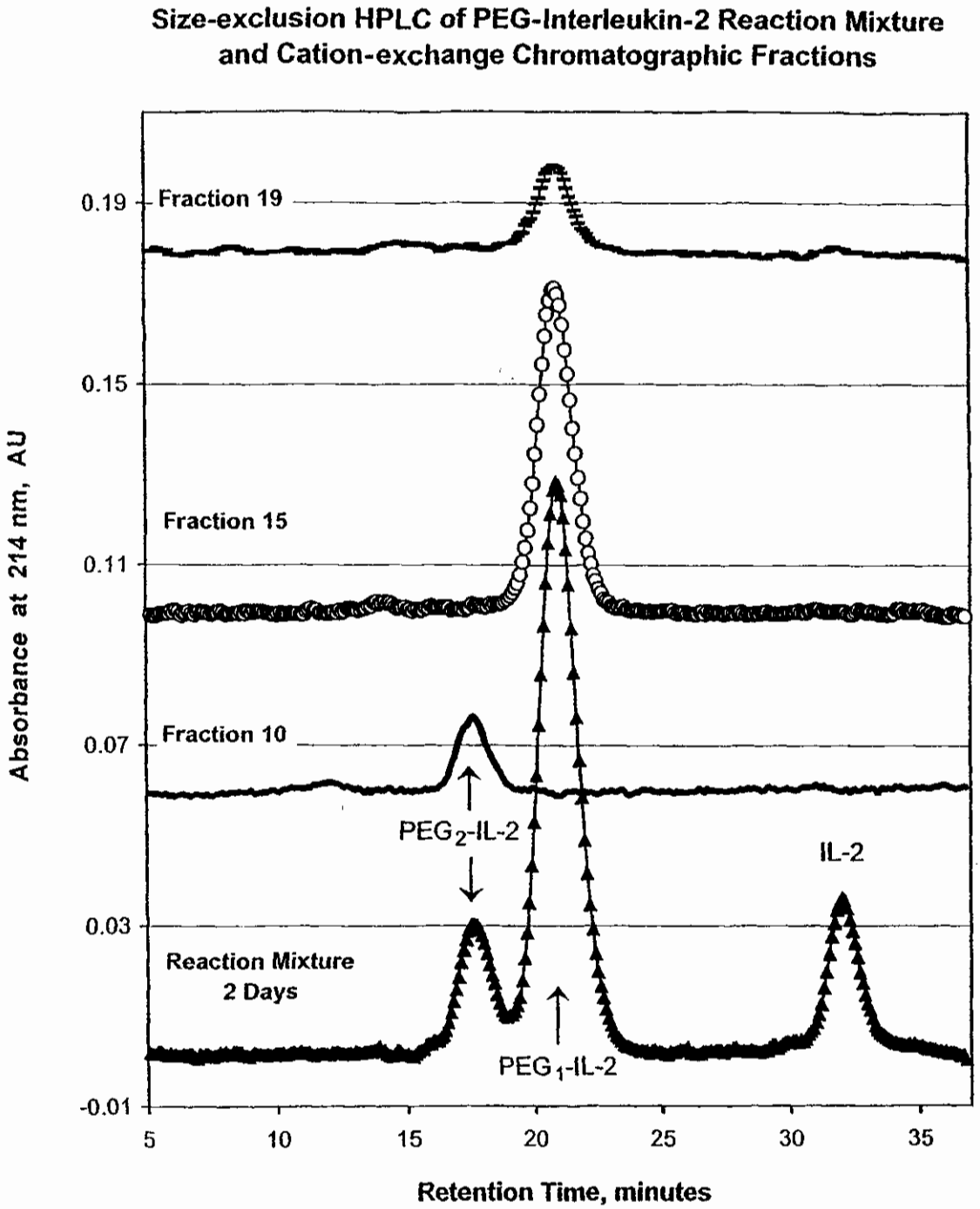

Size-exclusion HPLC of PEG-Interleukin-2 Reaction Mixture
and Cation-exchange Chromatographic Fractions
Fraction 19
Fraction 15
Fraction 10
Reaction Mixture
2 Days
PEG2-IL-2
PEG1-IL-2
IL-2
Absorbance at 214 nm, AU
0.19
0.15
0.11
0.07
0.03
-0.01
5
10
15
20
25
30
35
Retention Time, minutes

Figure 12

Electrophoretic Analysis of PEG-Interleukin-2 Reaction Mixture and Purified MonoPEGylated Conjugate

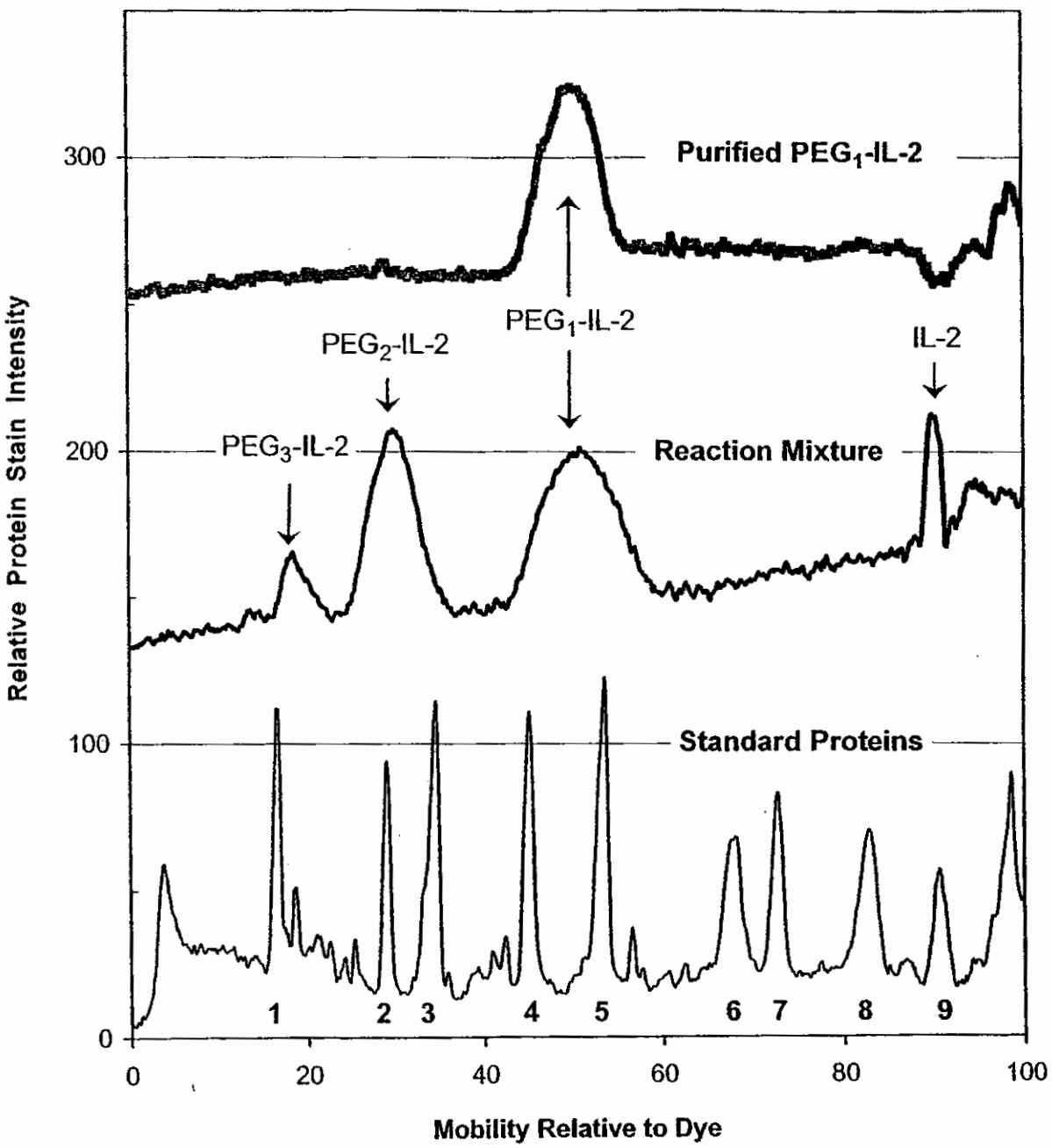


Figure 13

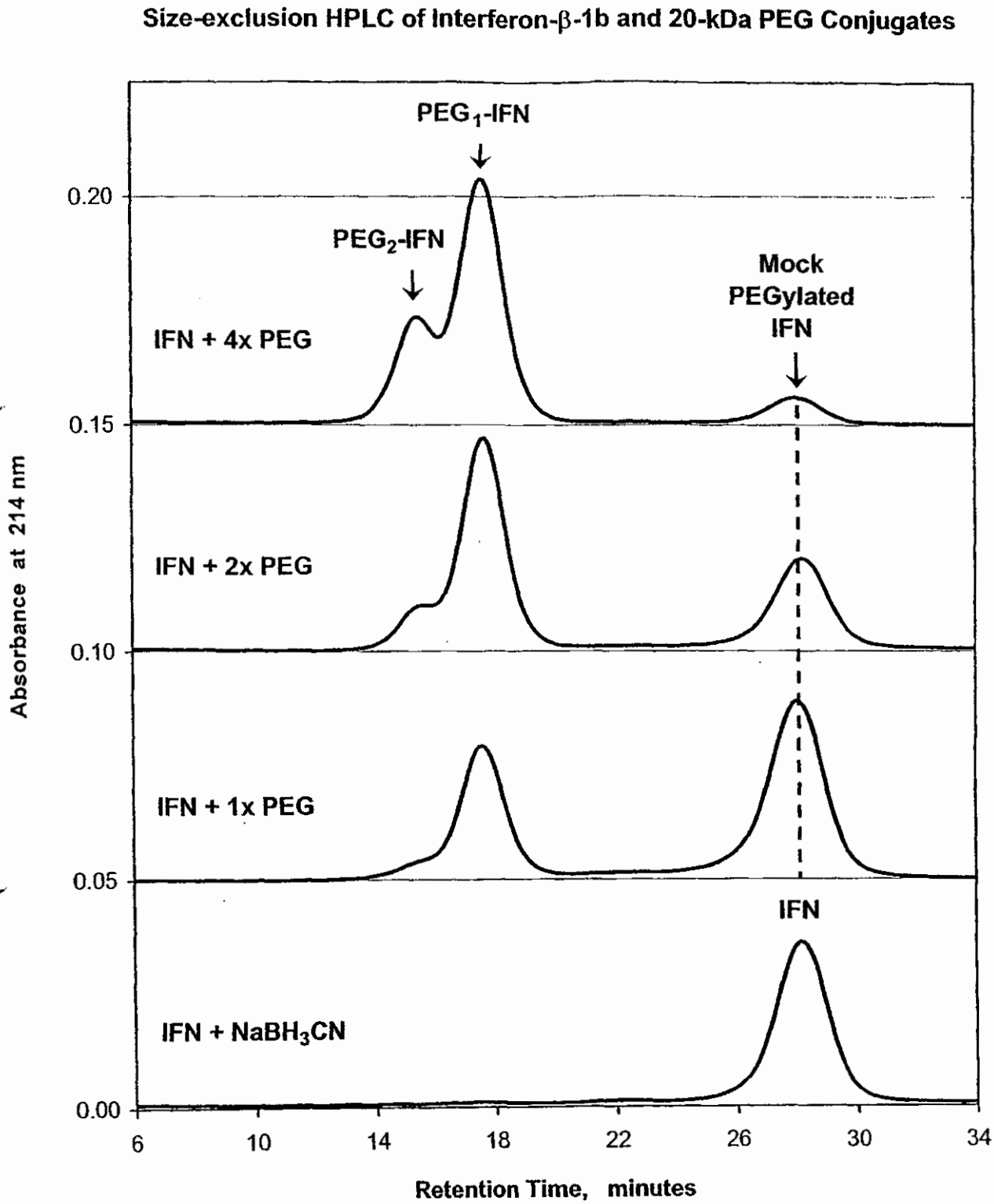

Size-exclusion HPLC of Interferon-β-1b and 20-kDa PEG Conjugates
PEG1-IFN
PEG2-IFN
Mock PEGylated IFN
IFN + 4x PEG
IFN + 2x PEG
IFN + 1x PEG
IFN
IFN + NaBH3CN
Absorbance at 214 nm
0.20
0.15
0.10
0.05
0.00
6
10
14
18
22
26
30
34
Retention Time, minutes

Figure 14

Size-exclusion HPLC of PEG_1-Interferon-β-1b Synthesized at pH 6.4 +/- SDS or NaCl, before and after Oxidative Cleavage with 3 mM $NaIO_4$

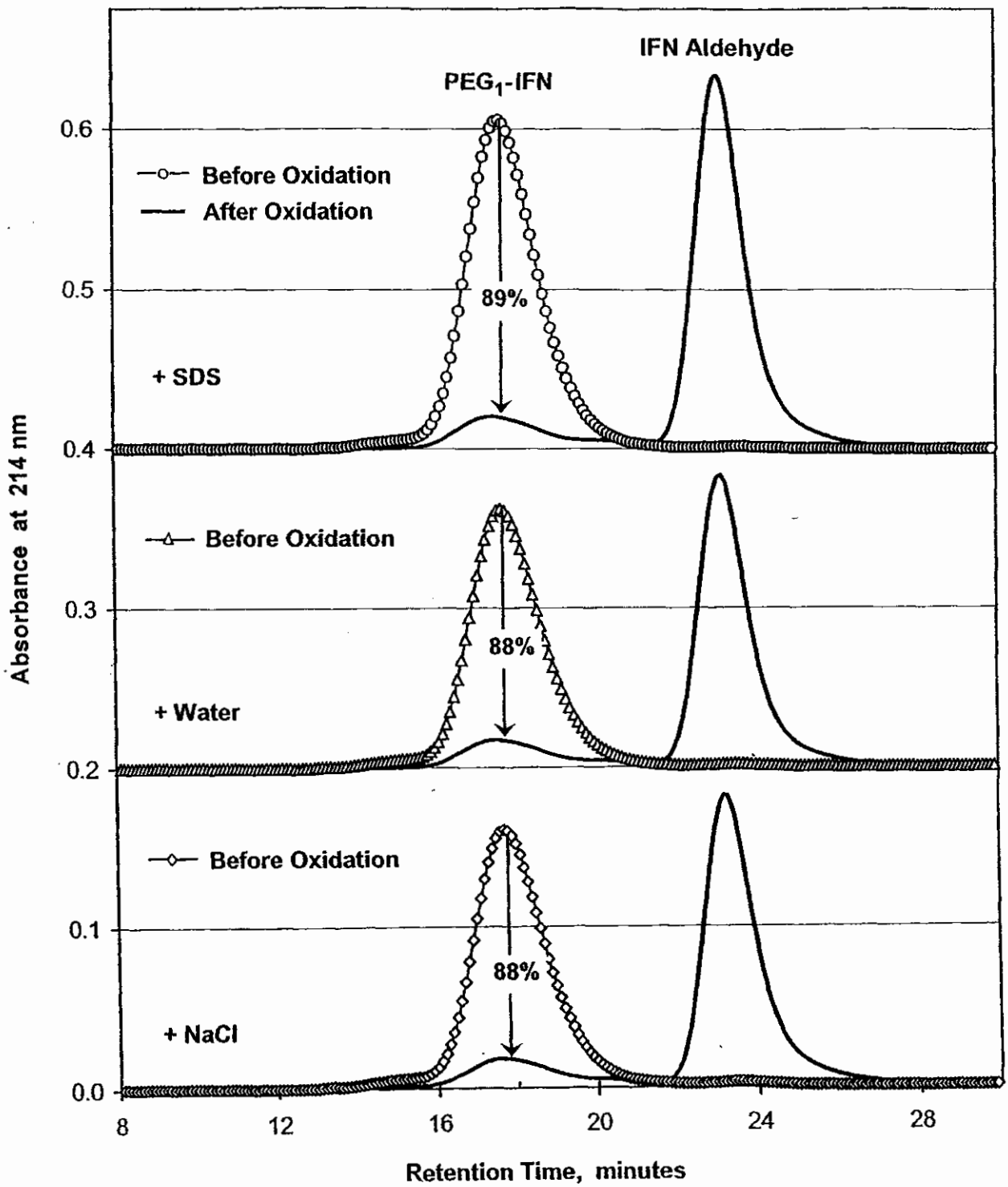


Figure 15

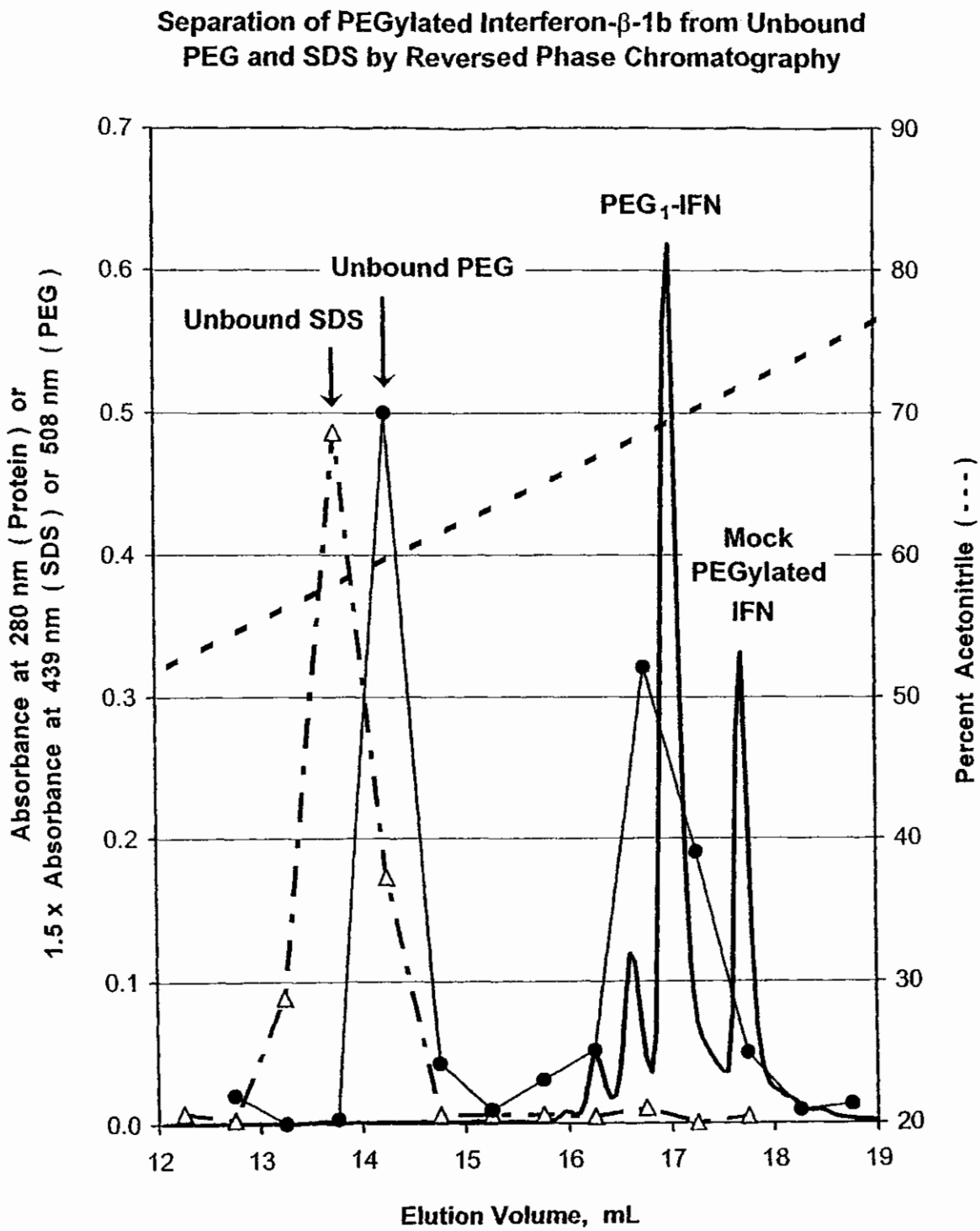

Separation of PEGylated Interferon-β-1b from Unbound PEG and SDS by Reversed Phase Chromatography
PEG1-IFN
Unbound PEG
Unbound SDS
Mock PEGylated IFN
Absorbance at 280 nm (Protein) or 1.5 x Absorbance at 439 nm (SDS) or 508 nm (PEG)
Percent Acetonitrile (- - -)
Elution Volume, mL
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
20
30
40
50
60
70
80
90
12
13
14
15
16
17
18
19

Figure 16

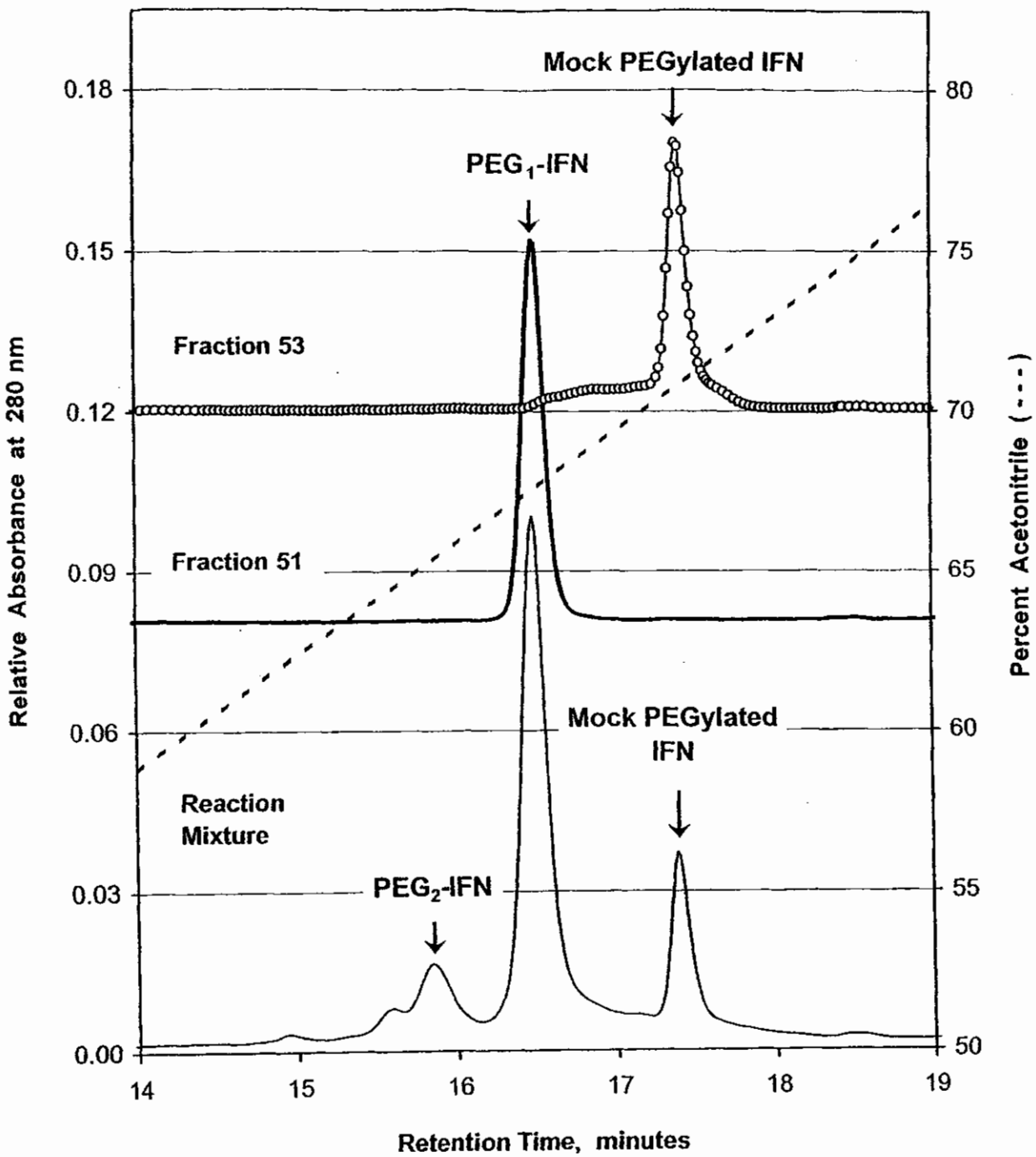

Purification of MonoPEGylated IFN-β-1b by Reversed Phase HPLC
Mock PEGylated IFN
PEG1-IFN
Fraction 53
Fraction 51
Mock PEGylated IFN
Reaction Mixture
PEG2-IFN
Relative Absorbance at 280 nm
Percent Acetonitrile (- - -)
Retention Time, minutes
0.18
0.15
0.12
0.09
0.06
0.03
0.00
80
75
70
65
60
55
50
14
15
16
17
18
19

Figure 17

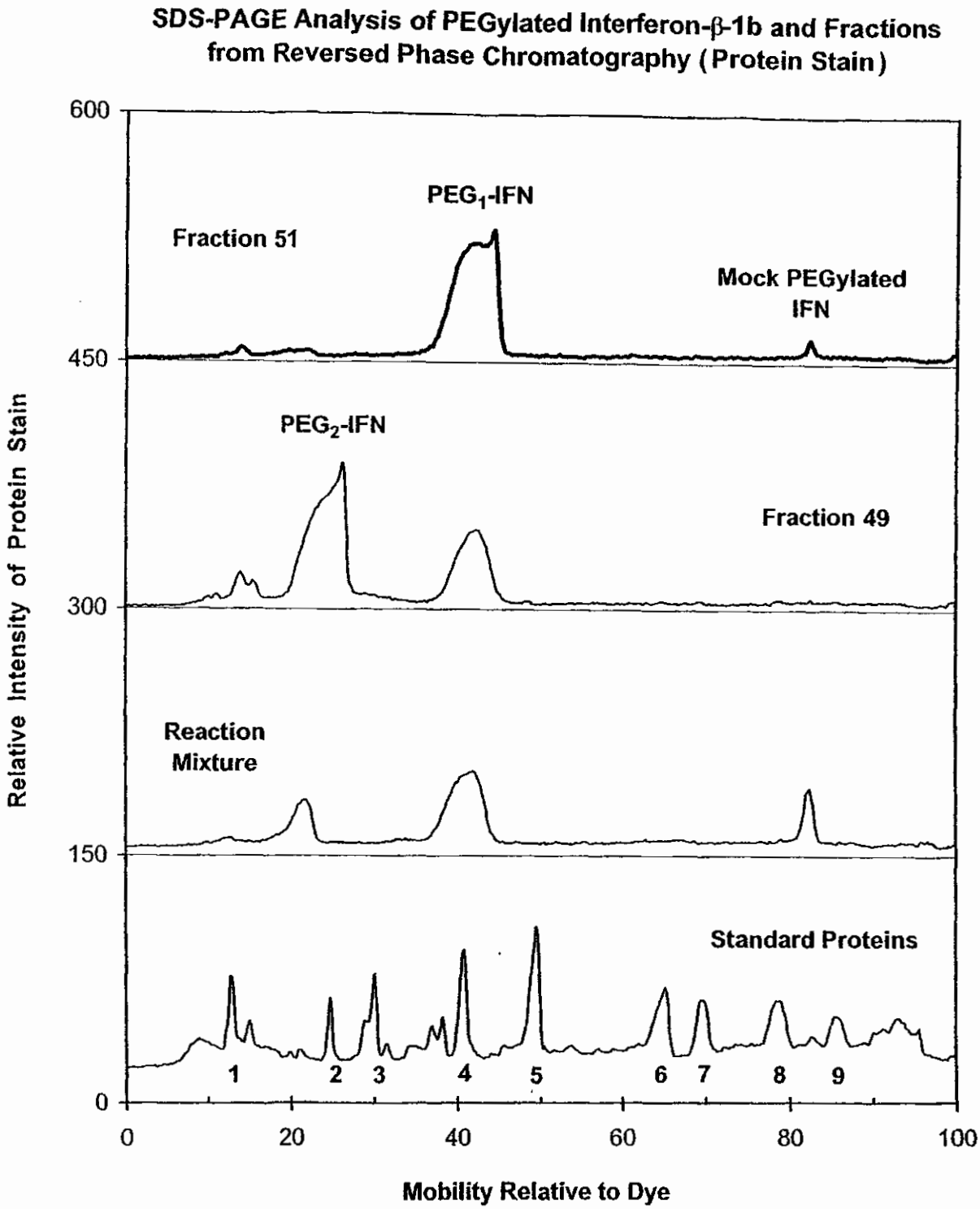

SDS-PAGE Analysis of PEGylated Interferon-β-1b and Fractions from Reversed Phase Chromatography (Protein Stain)
Relative Intensity of Protein Stain
600
450
300
150
0
PEG1-IFN
Fraction 51
Mock PEGylated IFN
PEG2-IFN
Fraction 49
Reaction Mixture
Standard Proteins
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
Mobility Relative to Dye

Figure 18

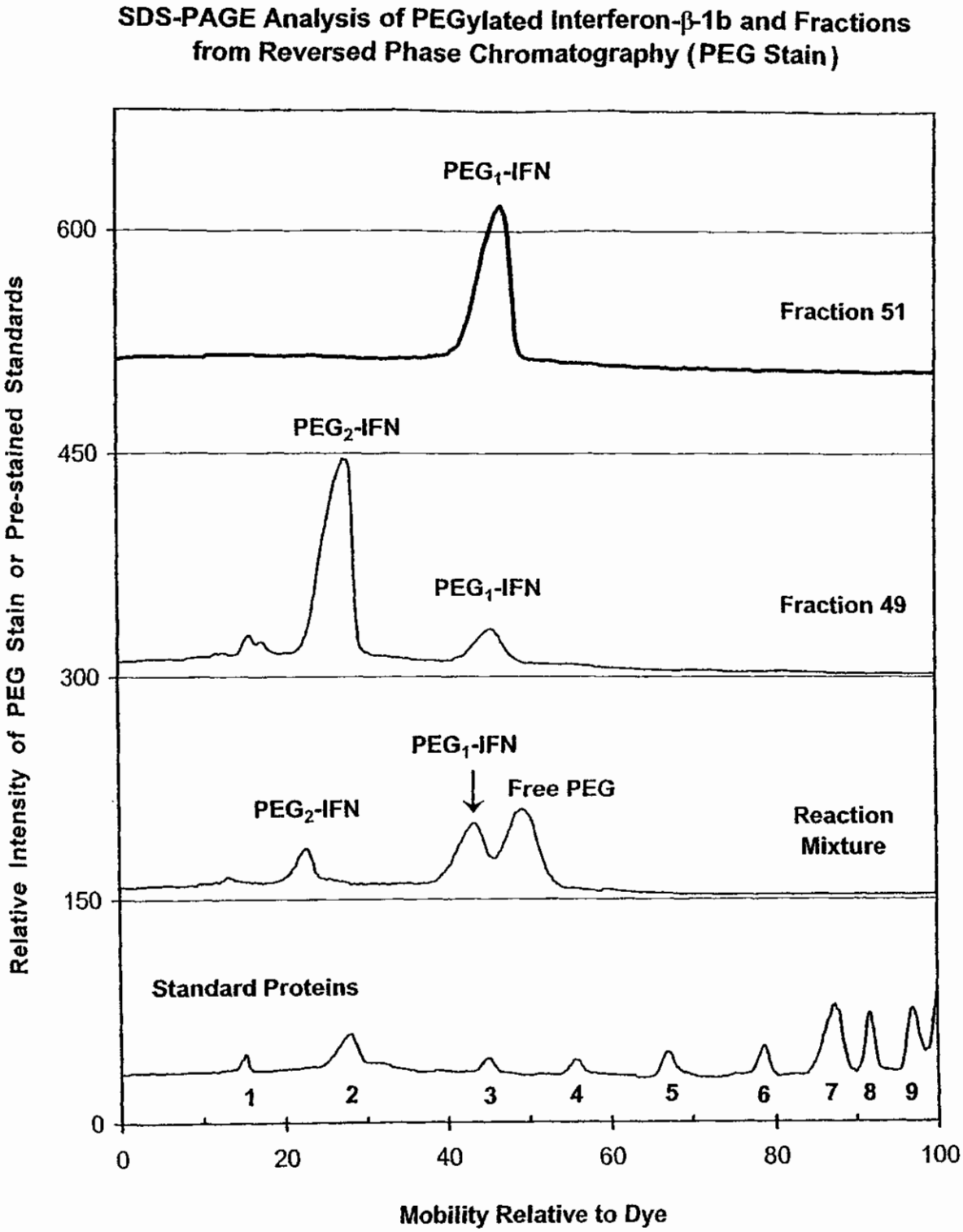

SDS-PAGE Analysis of PEGylated Interferon-β-1b and Fractions from Reversed Phase Chromatography (PEG Stain)
Relative Intensity of PEG Stain or Pre-stained Standards
600
450
300
150
0
PEG1-IFN
Fraction 51
PEG2-IFN
PEG1-IFN
Fraction 49
PEG1-IFN
Free PEG
PEG2-IFN
Reaction Mixture
Standard Proteins
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
Mobility Relative to Dye

Figure 19

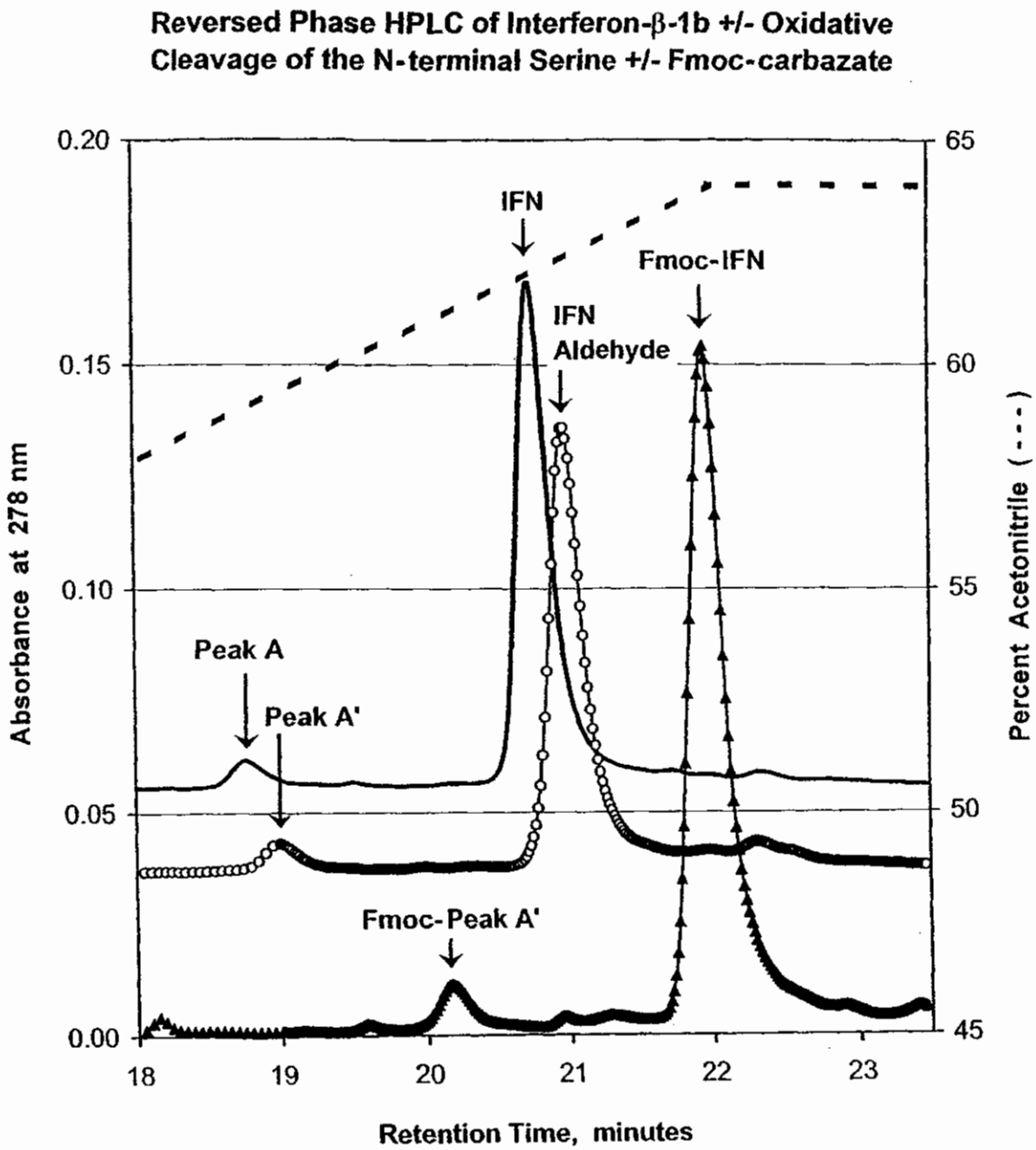

Reversed Phase HPLC of Interferon-β-1b +/- Oxidative Cleavage of the N-terminal Serine +/- Fmoc-carbazate
0.20
0.15
0.10
0.05
0.00
65
60
55
50
45
IFN
Fmoc-IFN
IFN Aldehyde
Peak A
Peak A'
Fmoc-Peak A'
18
19
20
21
22
23
Absorbance at 278 nm
Percent Acetonitrile (- - -)
Retention Time, minutes

Figure 20

Time Course of Interferon-β-1b Oxidization before Reaction with PEG-carbazate, Shown by Size-exclusion HPLC

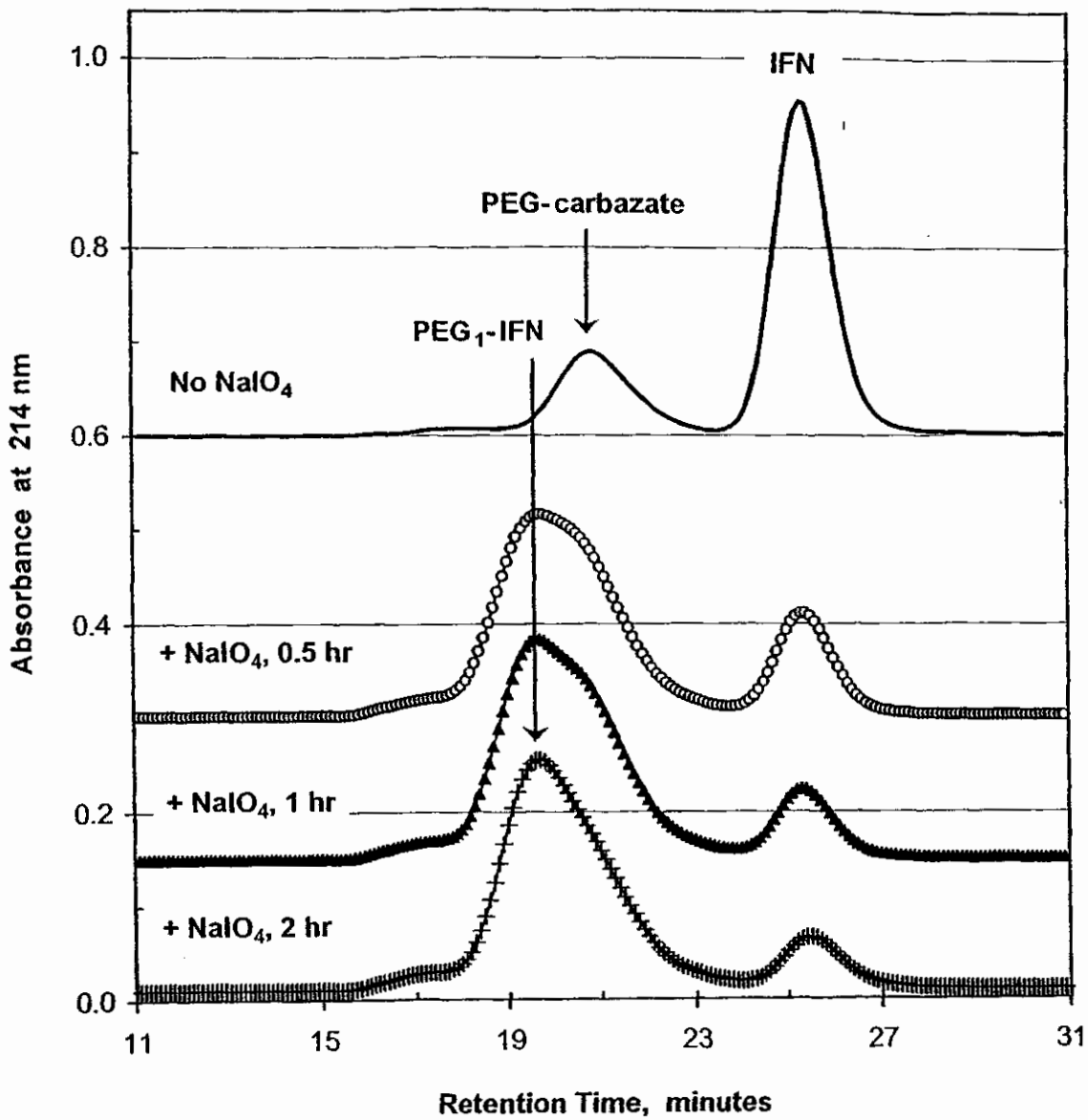


Figure 21

Daudi Cell Growth Inhibition by Untreated IFN-β-1b and by Reversed Phase HPLC Fractions Containing Mock PEGylated or MonoPEGylated IFN-β-1b

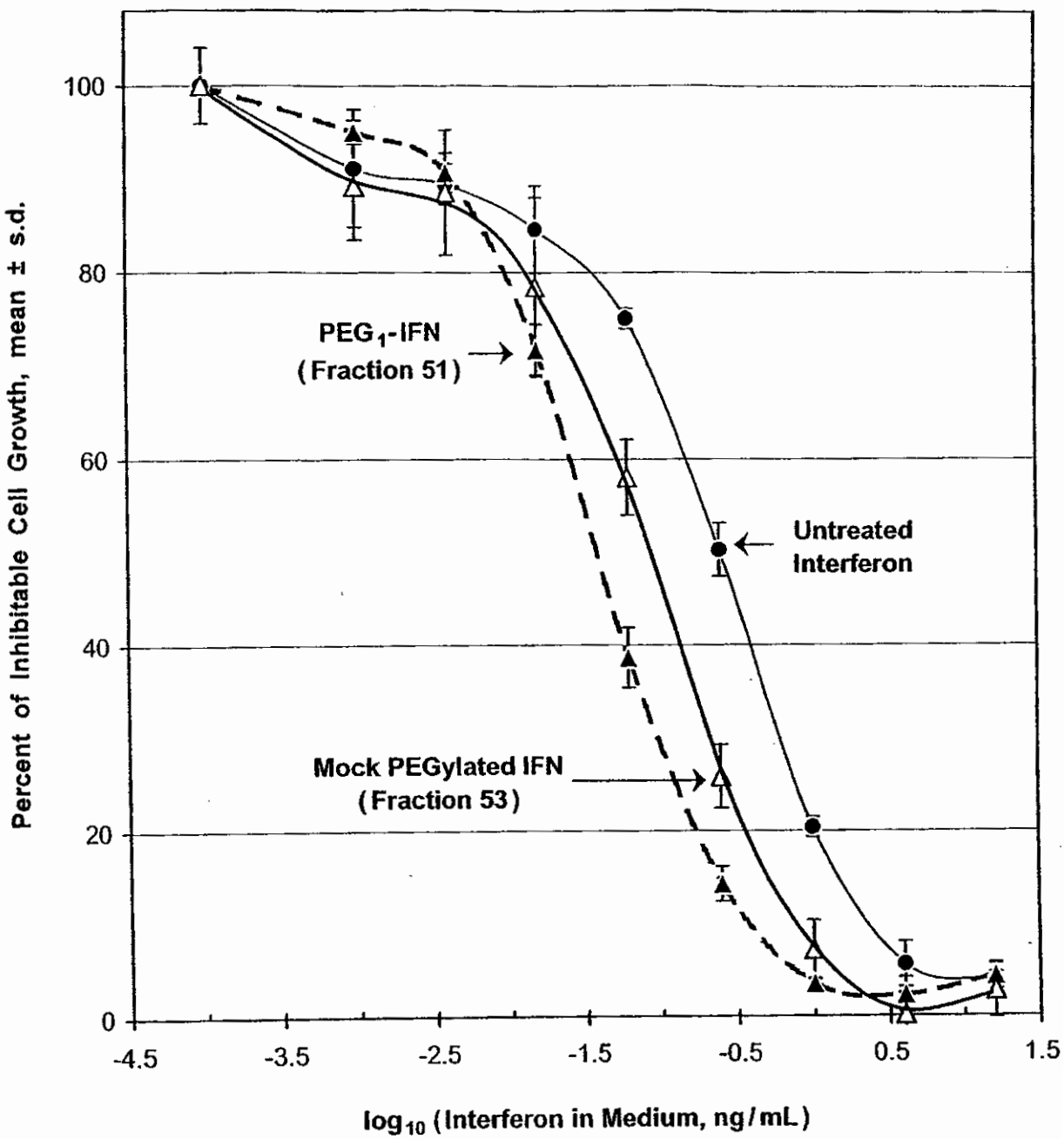


Figure 22

SEQUENCE LISTING

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

<120> POLYMER CONJUGATES OF INTERFERON-BETA WITH ENHANCED BIOLOGICAL POTENCY

<130> 2057.012PC01/JAG/BJD

<140> (To be assigned)
<141> (herewith)

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

<150> US 60/479,913
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<150> US 60/436,020
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			20					25				30			
Asp	Arg	Met	Asn	Phe	Asp	Ile	Pro	Glu	Glu	Ile	Lys	Gln	Leu	Gln	Gln
		35					40				45				
Phe	Gln	Lys	Glu	Asp	Ala	Ala	Leu	Thr	Ile	Tyr	Glu	Met	Leu	Gln	Asn
	50					55					60				
Ile	Phe	Ala	Ile	Phe	Arg	Gln	Asp	Ser	Ser	Ser	Thr	Gly	Trp	Asn	Glu
65					70					75					80
Thr	Ile	Val	Glu	Asn	Leu	Leu	Ala	Asn	Val	Tyr	His	Gln	Ile	Asn	His
				85					90					95	
Leu	Lys	Thr	Val	Leu	Glu	Glu	Lys	Leu	Glu	Lys	Glu	Asp	Phe	Thr	Arg
			100					105				110			
Gly	Lys	Leu	Met	Ser	Ser	Leu	His	Leu	Lys	Arg	Tyr	Tyr	Gly	Arg	Ile
		115					120				125				
Leu	His	Tyr	Leu	Lys	Ala	Lys	Glu	Tyr	Ser	His	Cys	Ala	Trp	Thr	Ile
	130					135					140				

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Gly Tyr Leu Arg Asn
165

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<212> PRT
<213> Homo sapiens

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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
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Thr Gly Tyr Leu Arg Asn
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