Pediatric RPAC Educational Day (Feb. 16, 2000)

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- Dept of Biomedical Sciences, College of Osteopathic Medicine, Ohio University

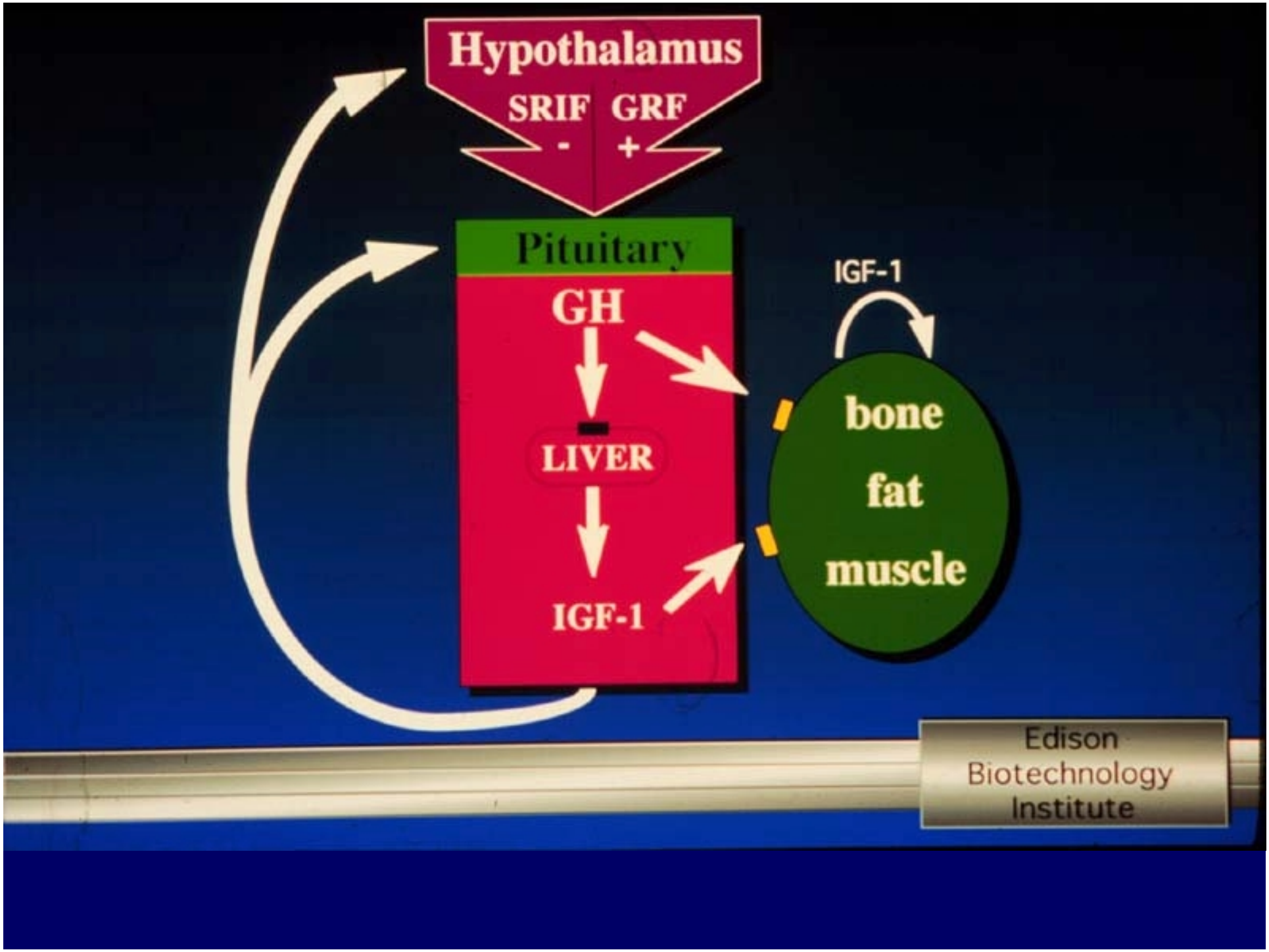
- **Growth Hormone Action: From Mouse to Man**

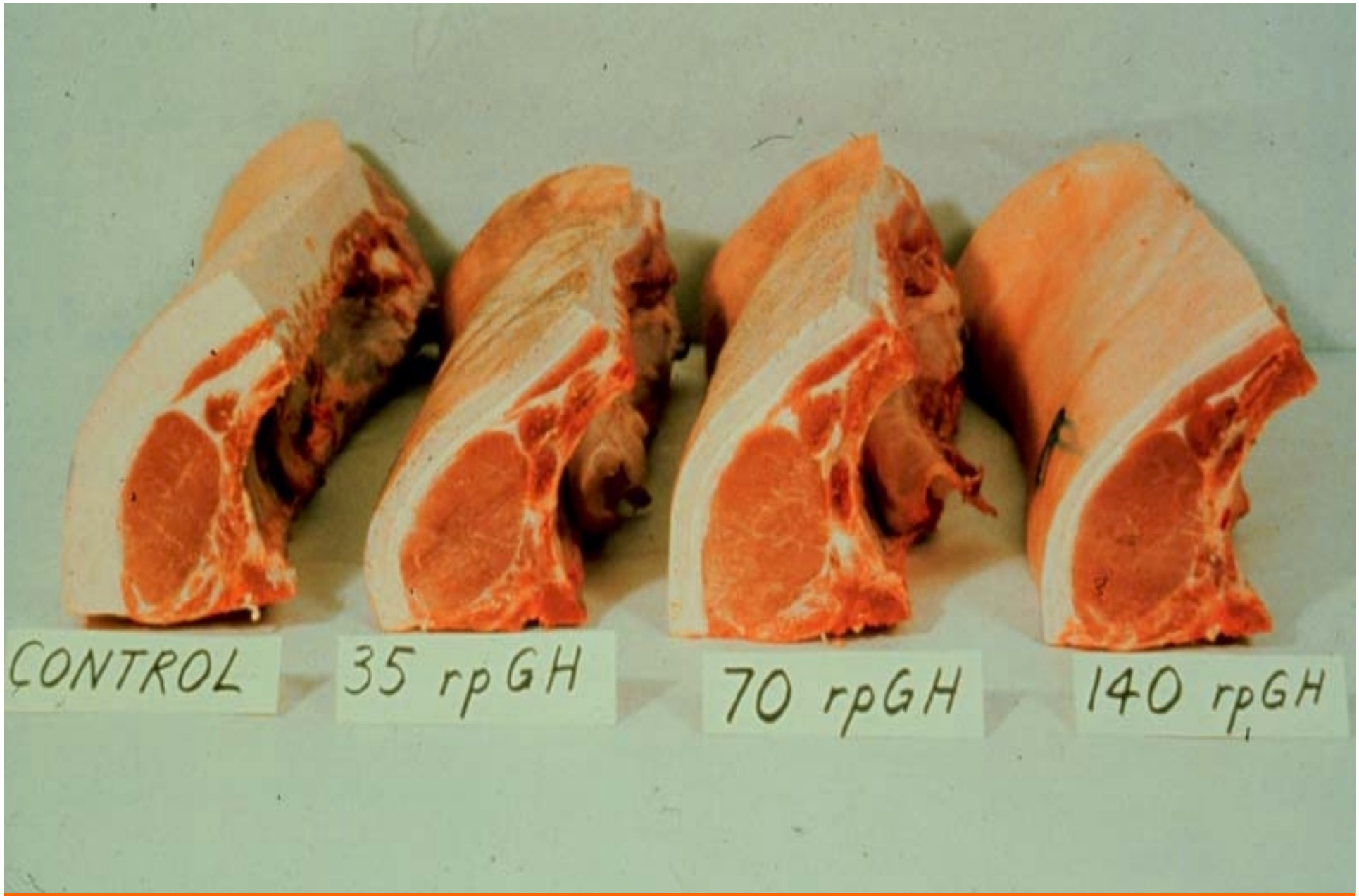
Growth Hormone Structure/function studies

- "Change the structure and assay the alteration in function"

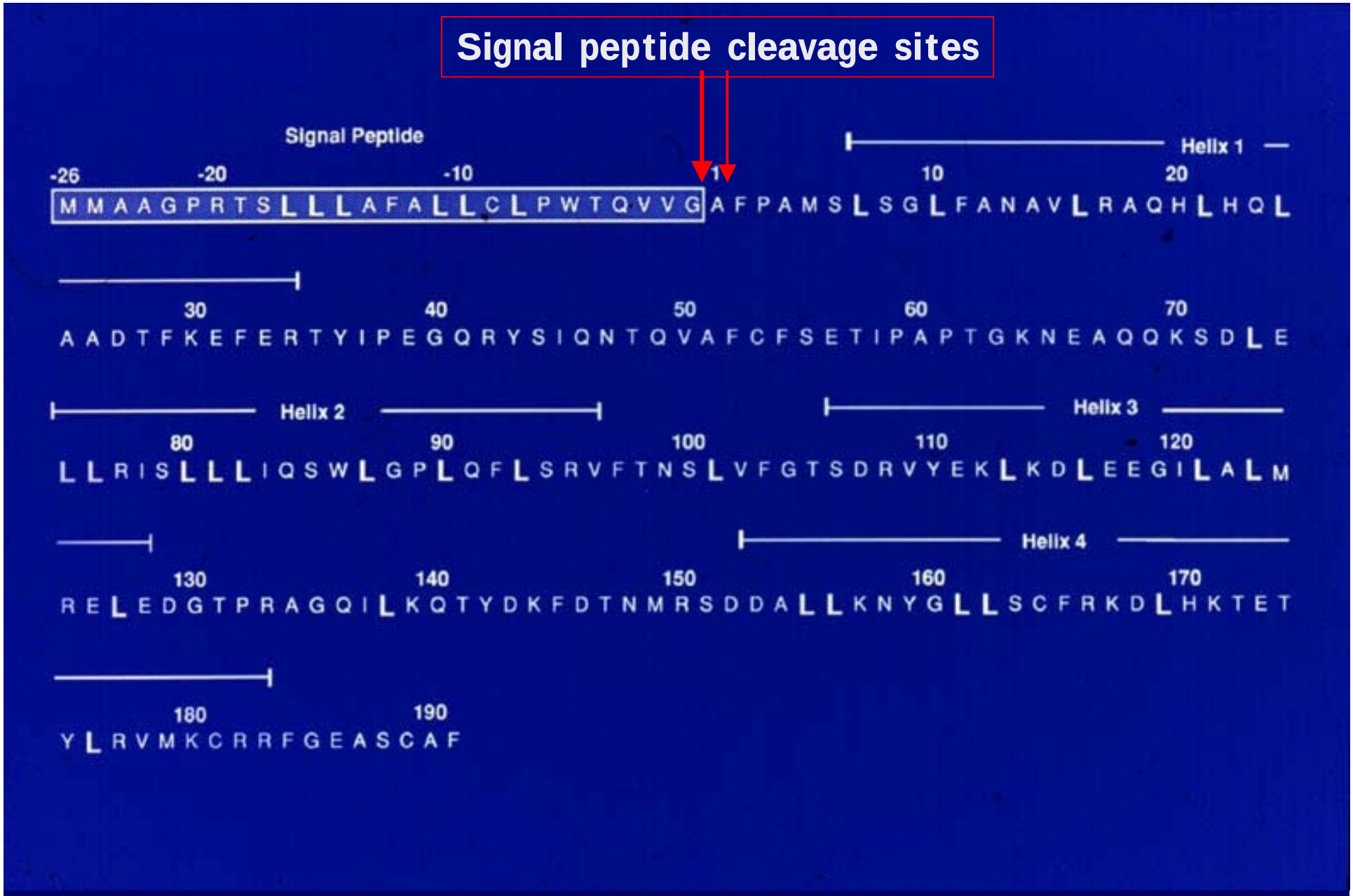
Growth Hormone Biological Activities

- Growth
- Lactation
- Metabolism
  - Bone
  - Fat
  - Muscle
  - Liver
  - Kidney
  - Others

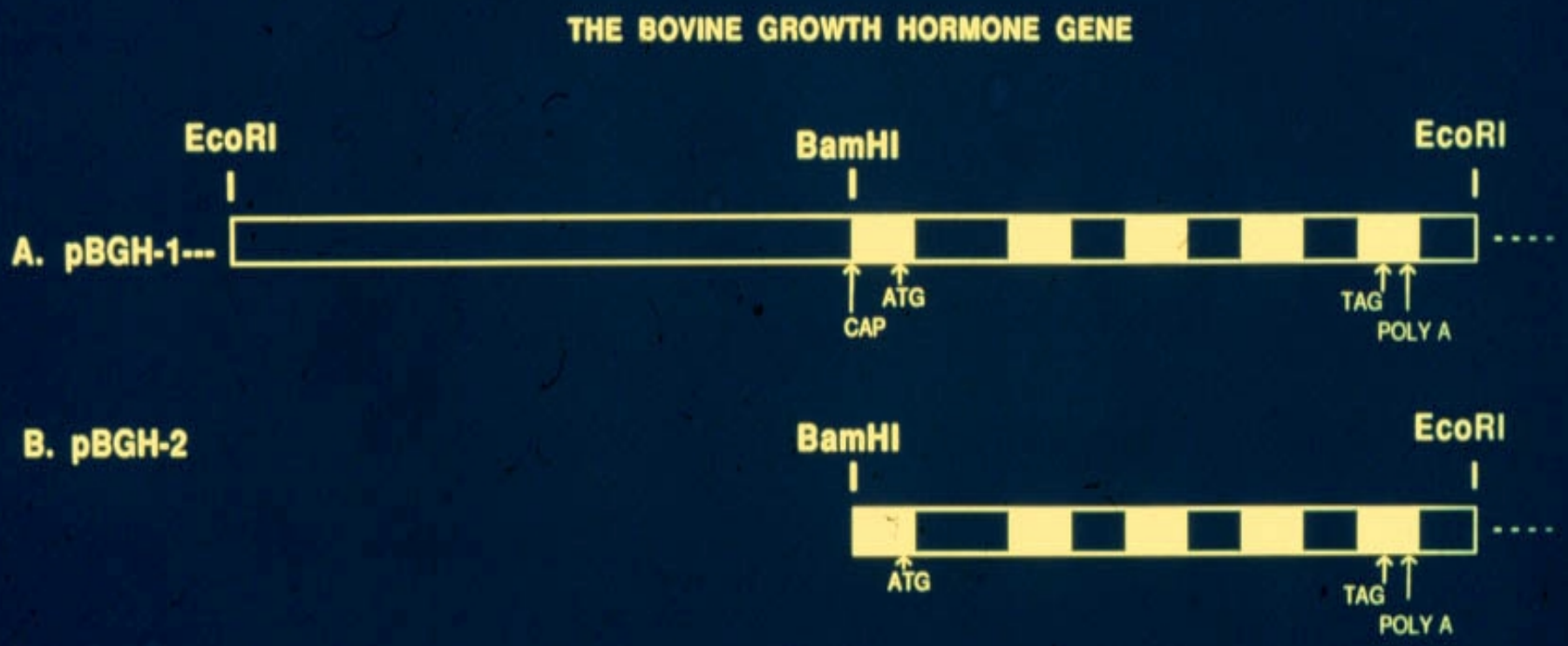

Hypothalamus
SRIF GRF
- +
Pituitary
GH
LIVER
IGF-1
IGF-1
bone
fat
muscle
Edison
Biotechnology
Institute



Dose dependent decrease in fat, and increase in muscle and bone!!!

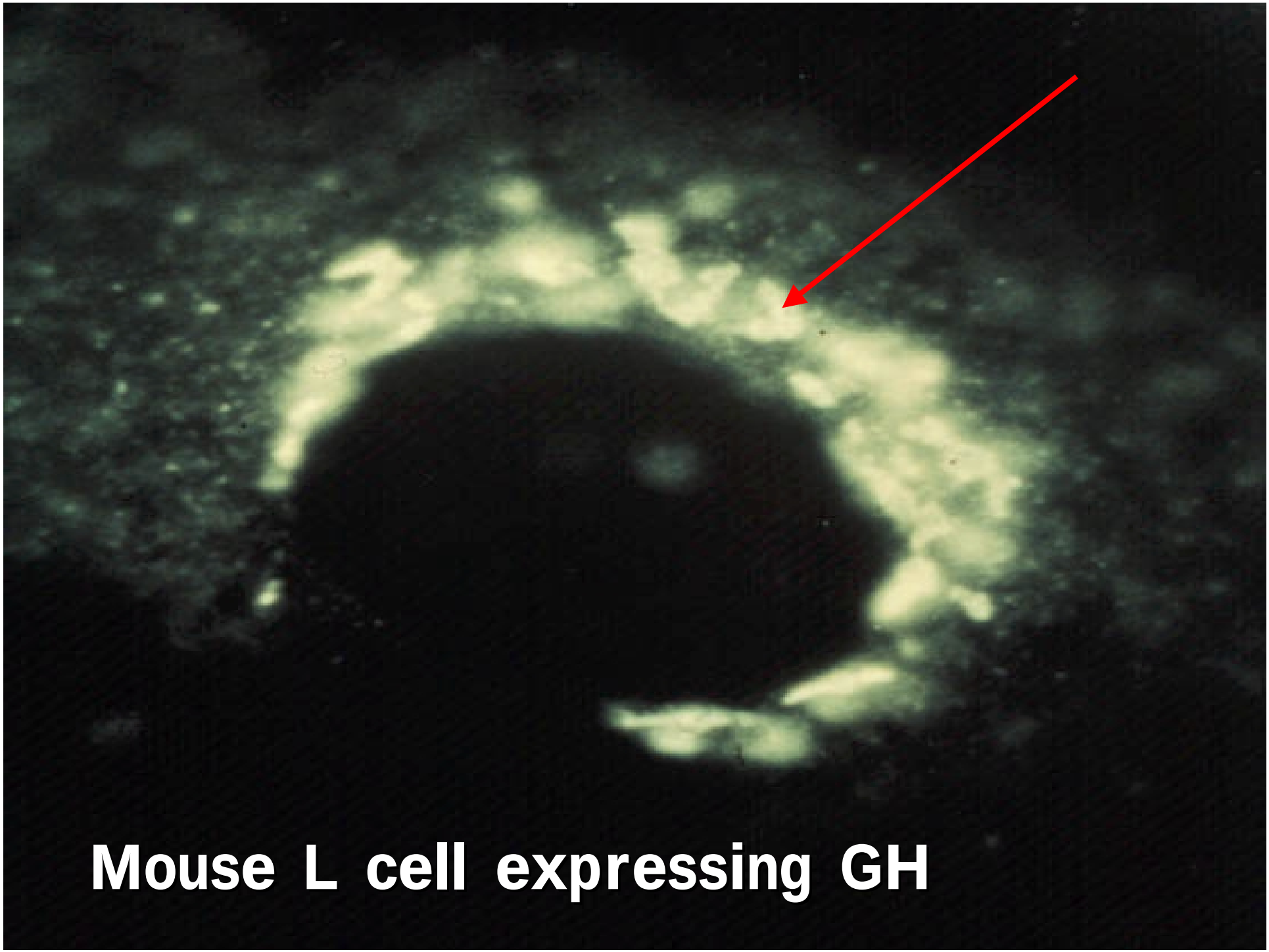


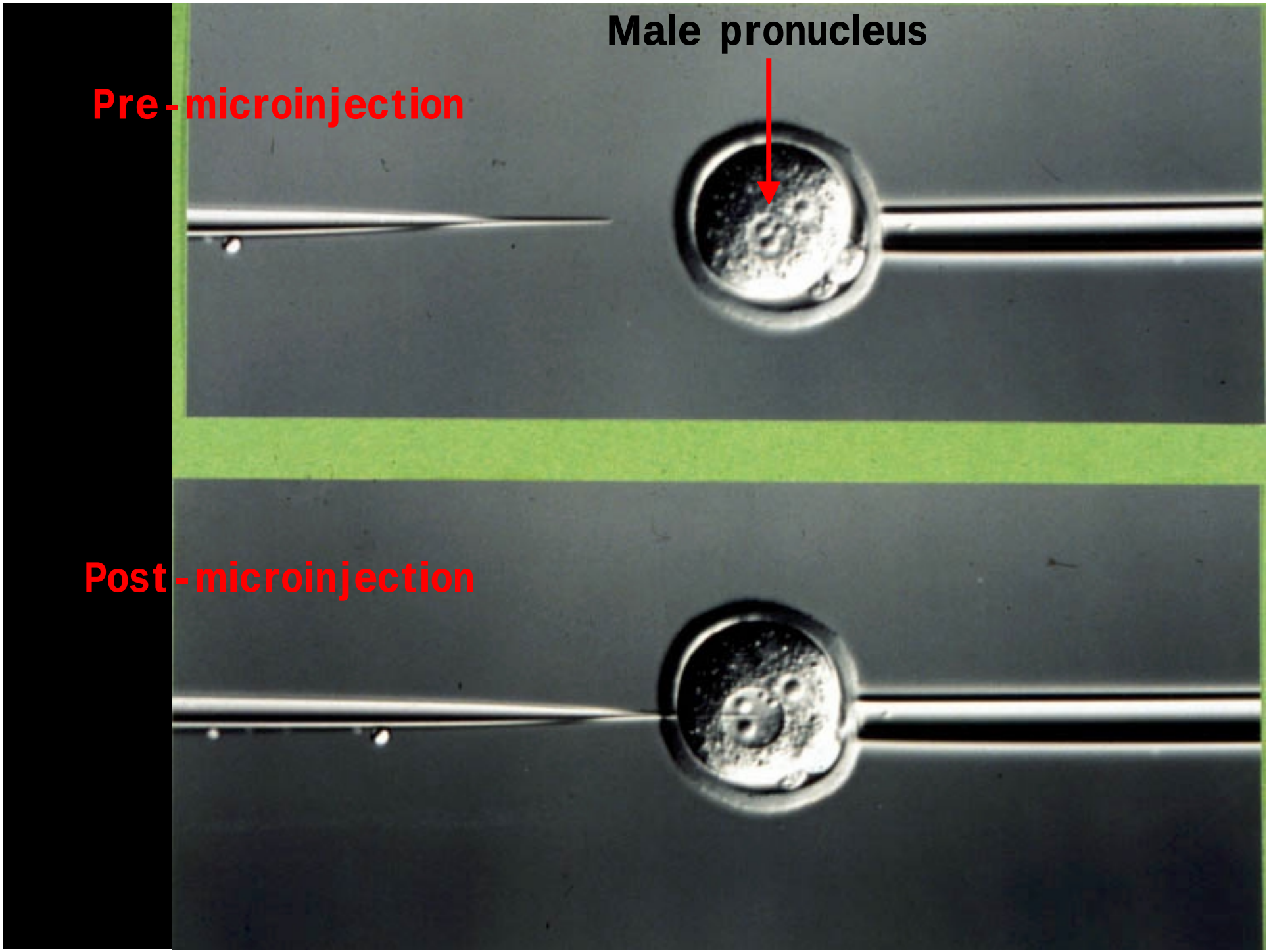
Amino acid sequence of bGH with four α-helices indicated



GH Genes

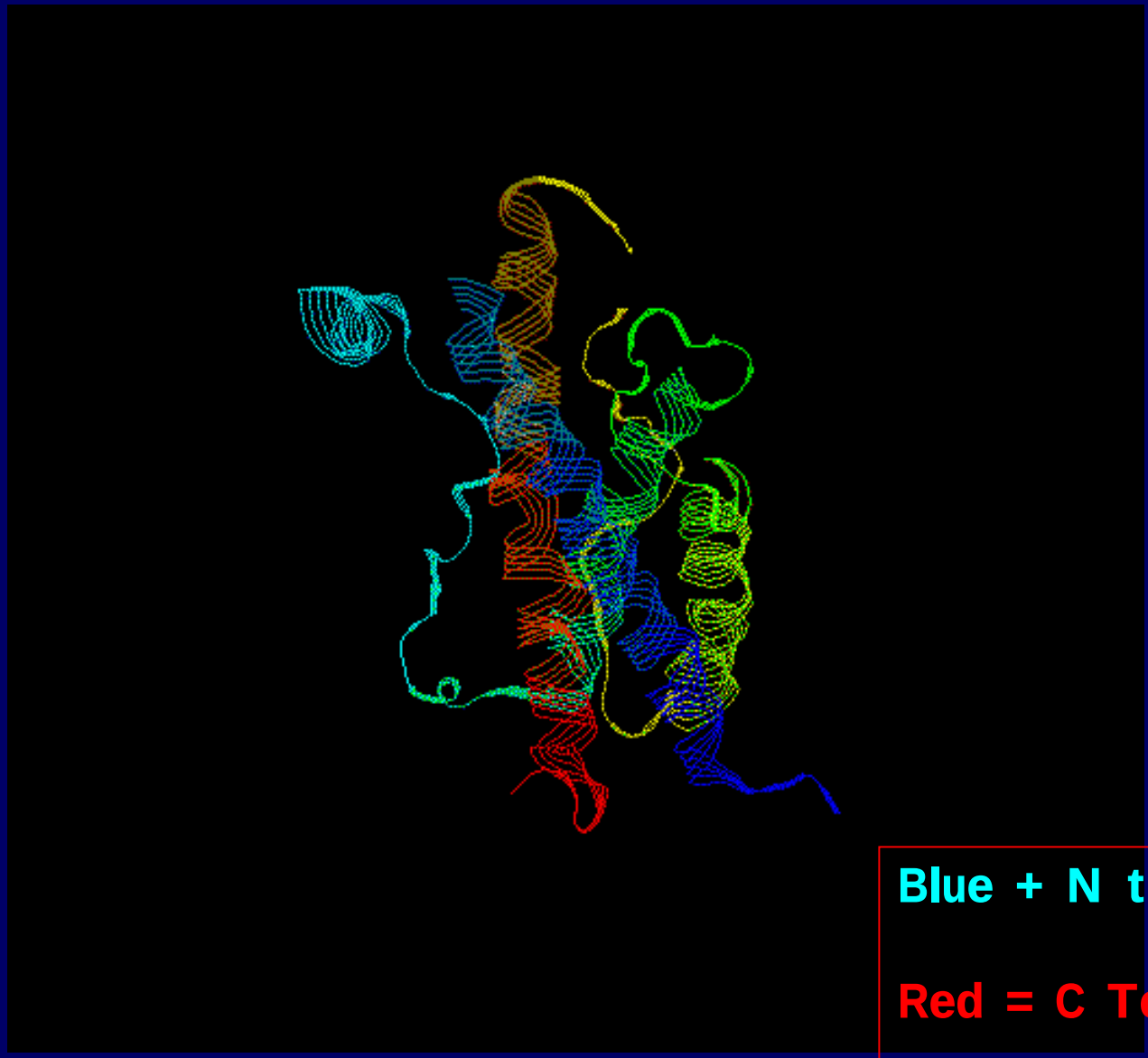
One can insert GH genes into cultured mouse cells or fertilized mouse eggs


Mouse L cell expressing GH


Male pronucleus
Pre-microinjection
Post-microinjection


GH trangenic mice

GH Crystal Structure



2.5 Angstrom Resolution

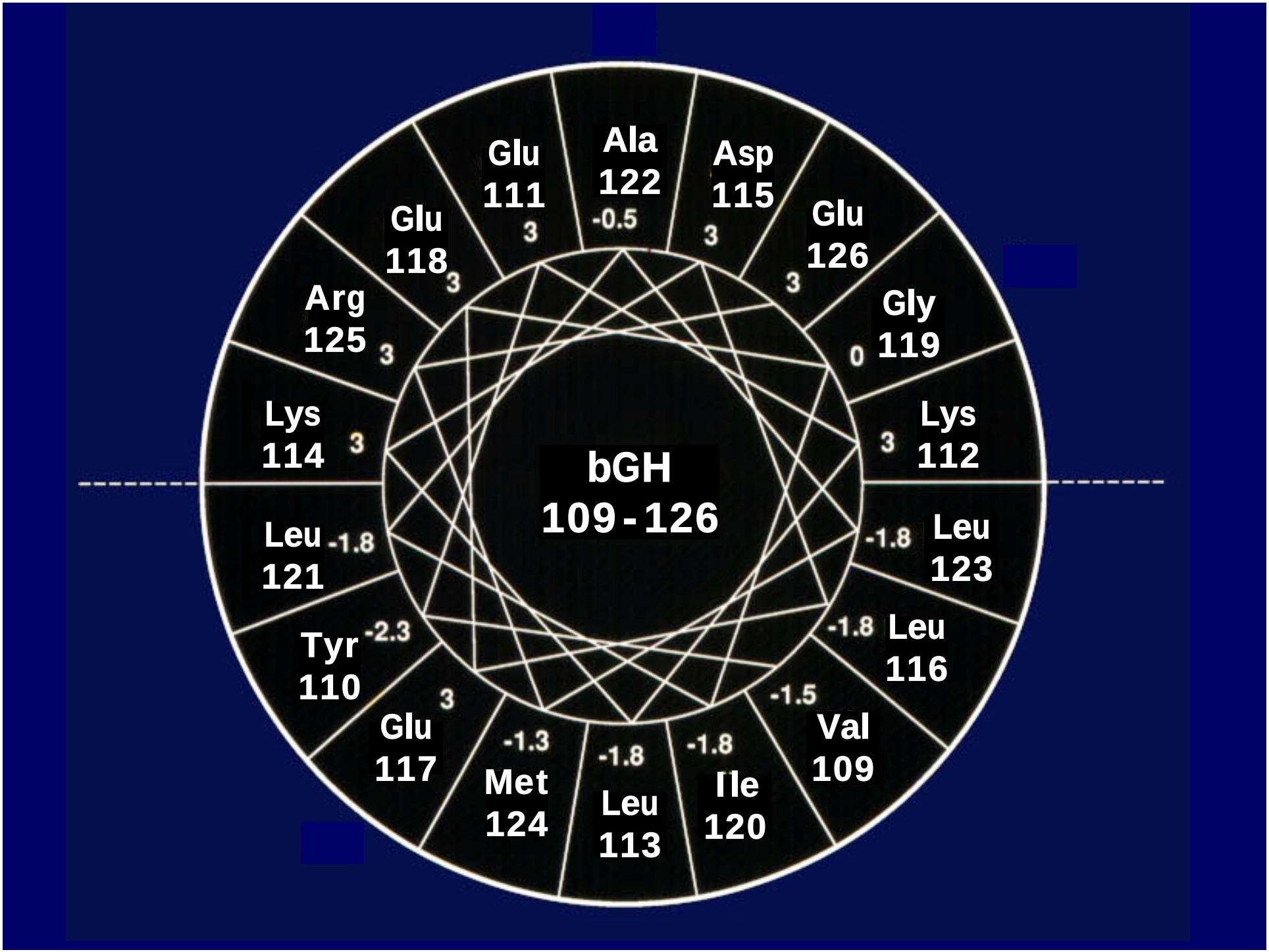
Blue + N terminus

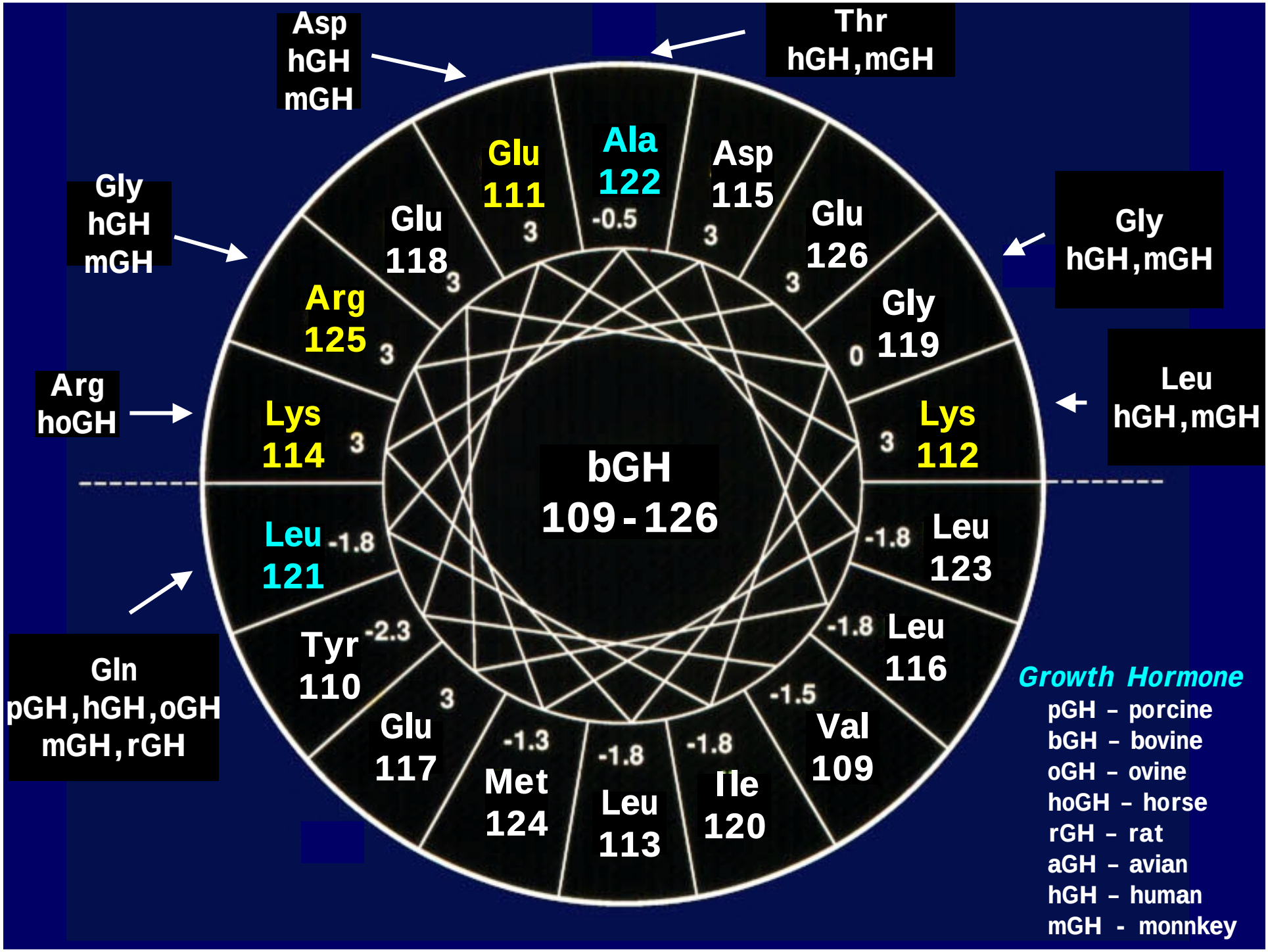
Red = C Terminus

Light green = helix 3

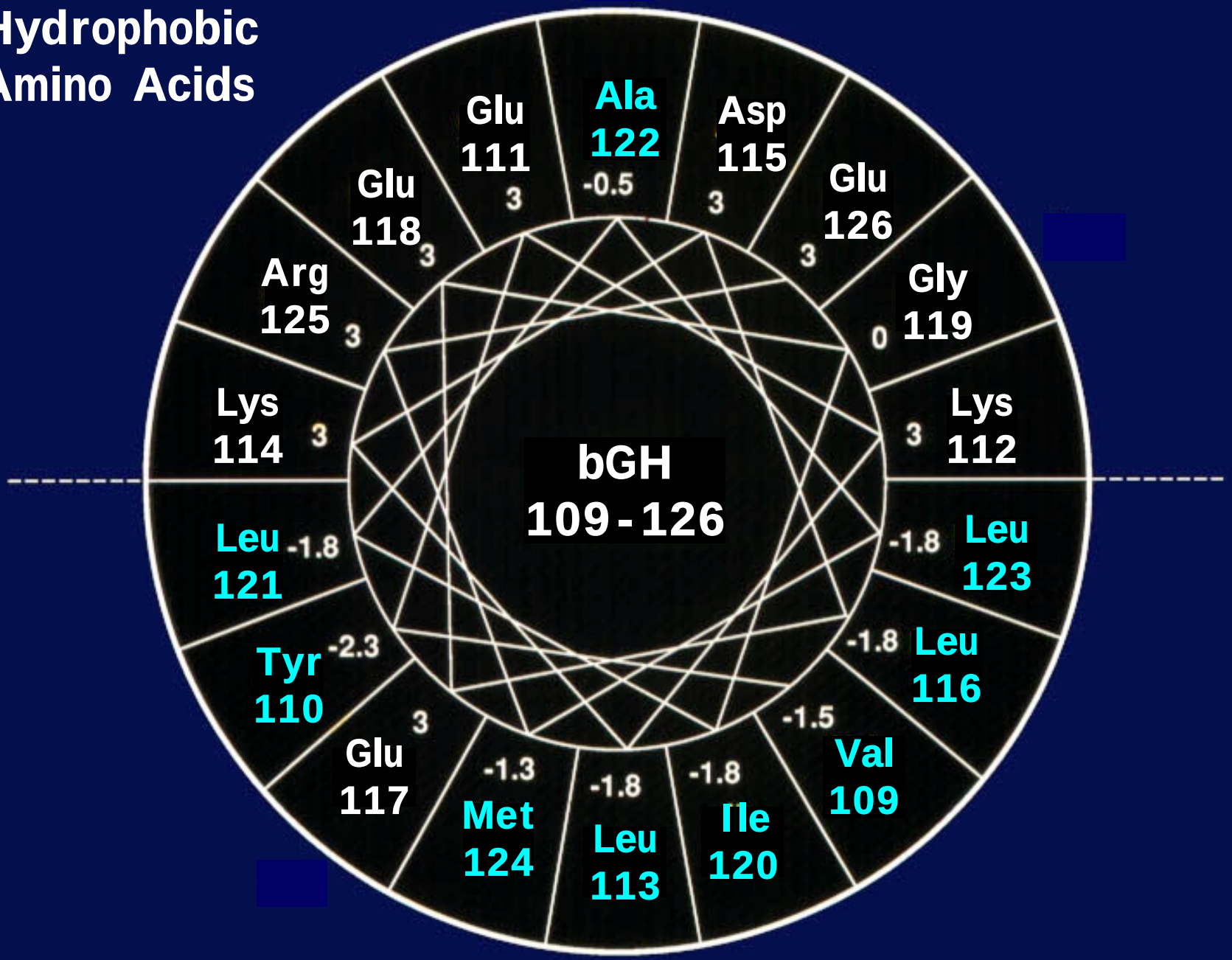
Experimental Protocol

- In vitro mutagenesis of GH gene or cDNA
- Oligonucleotide sequencing of mutations
- Expression of mutated DNA in mamalian cell culture
- Purification of GH analog
- Receptor Binding studies
- GH Responsive cell lines
  - Preadipocytes
  - Engineered GHR cell lines
  - NB_2
  - IM9
- Production of transgenic animals
  - Growth parameters
  - Morphometrics
  - Endocrine and physiological studies
  - Histological Studies
- Animal models

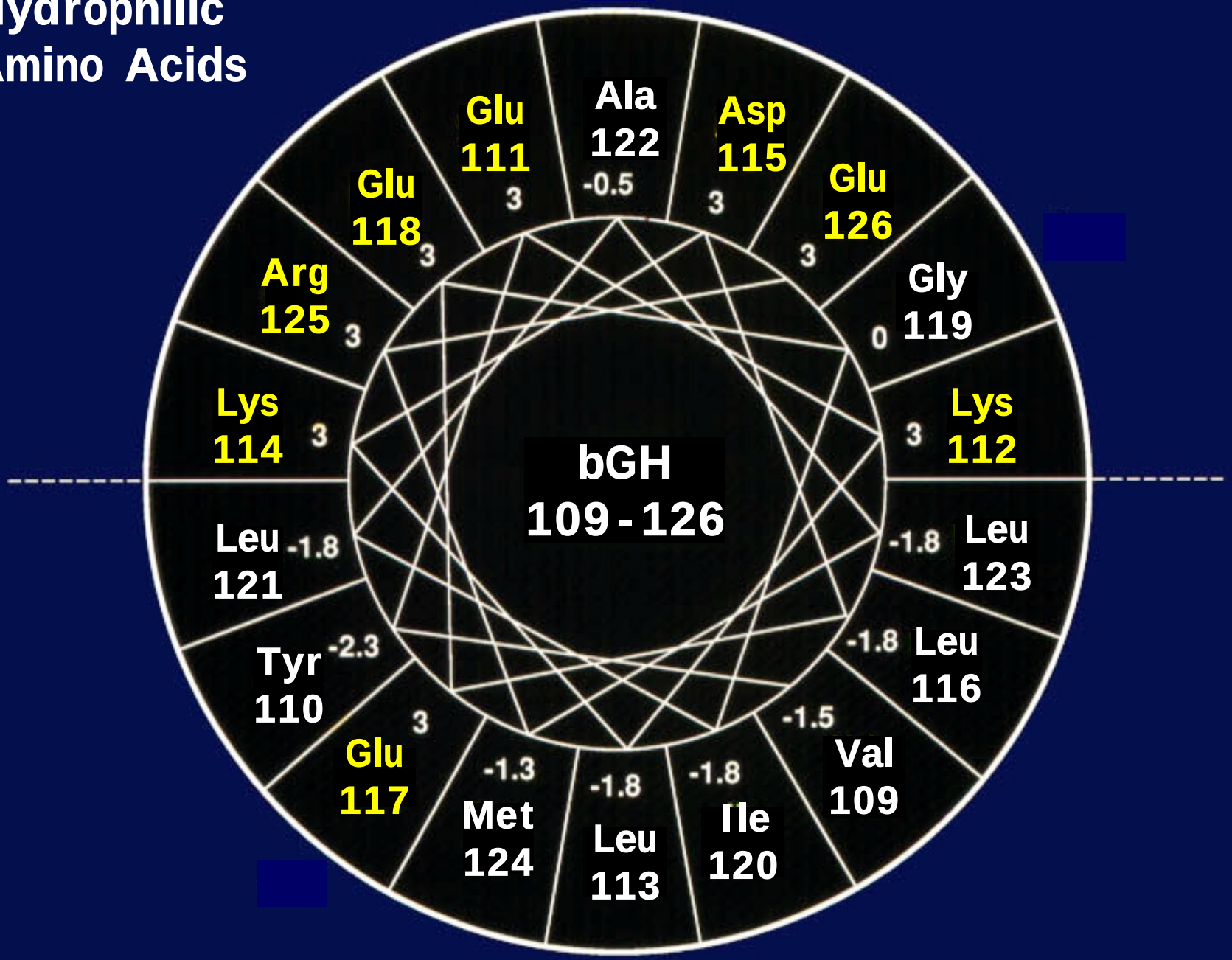

bGH
109 - 126
Ala
122
-0.5
Asp
115
3
Glu
126
3
Gly
119
0
Lys
112
3
Leu
123
-1.8
Leu
116
-1.8
Val
109
-1.5
Ile
120
-1.8
Leu
113
-1.8
Met
124
-1.3
Glu
117
3
Tyr
110
-2.3
Leu
121
-1.8
Lys
114
3
Arg
125
3
Glu
118
3
Glu
111
3


Asp
hGH
mGH
Thr
hGH,mGH
Gly
hGH
mGH
Gly
hGH,mGH
Arg
hoGH
Leu
hGH,mGH
Gln
pGH,hGH,oGH
mGH,rGH
Glu
111
3
Ala
122
-0.5
Asp
115
3
Glu
118
3
Glu
126
3
Arg
125
3
Gly
119
0
Lys
114
3
Lys
112
3
bGH
109-126
Leu
121
-1.8
Leu
123
-1.8
Tyr
110
-2.3
Leu
116
-1.8
Glu
117
3
Val
109
-1.5
Met
124
-1.3
Leu
113
-1.8
Ile
120
-1.8
Growth Hormone
pGH – porcine
bGH – bovine
oGH – ovine
hoGH – horse
rGH – rat
aGH – avian
hGH – human
mGH - monnkey

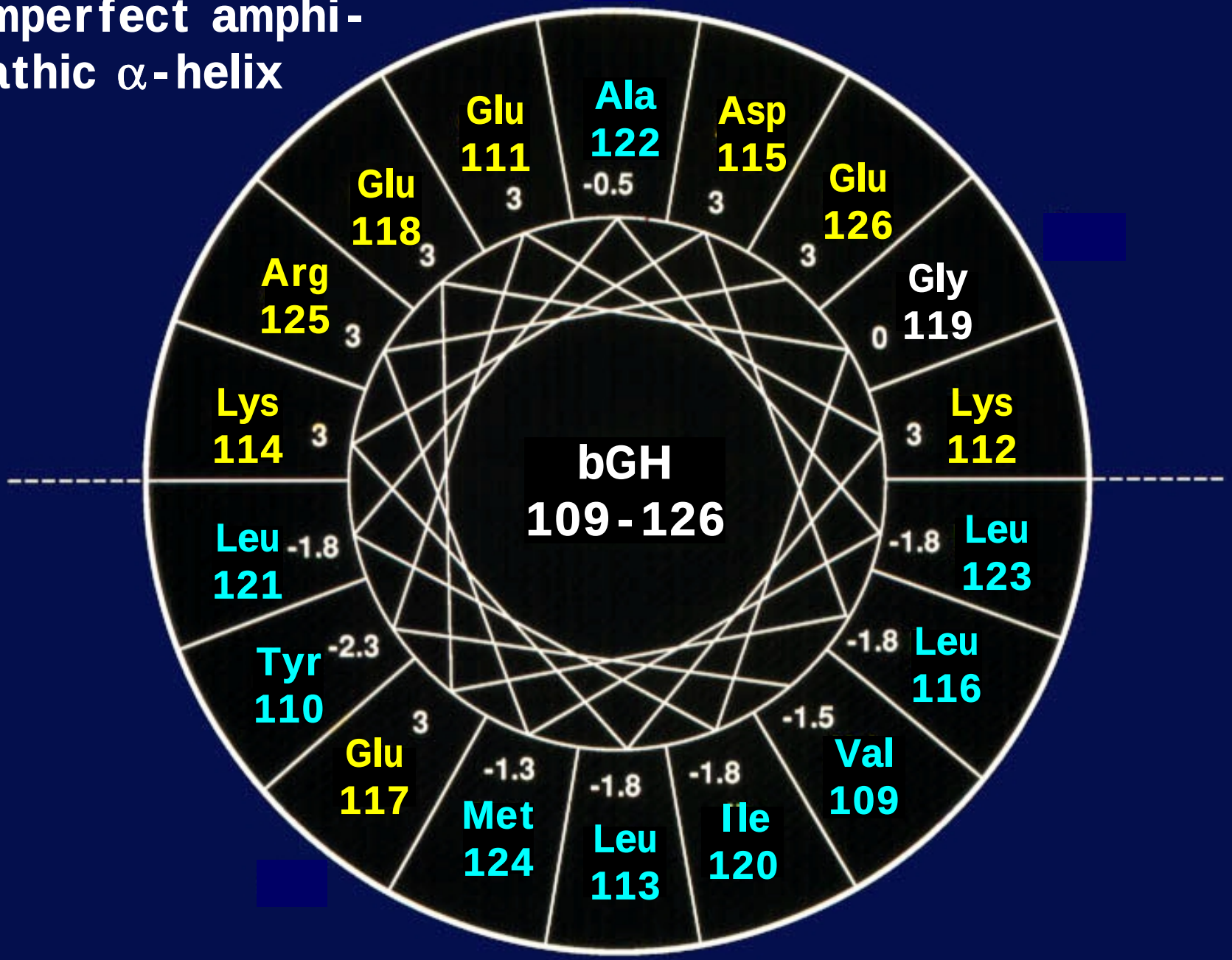
Hydrophobic Amino Acids



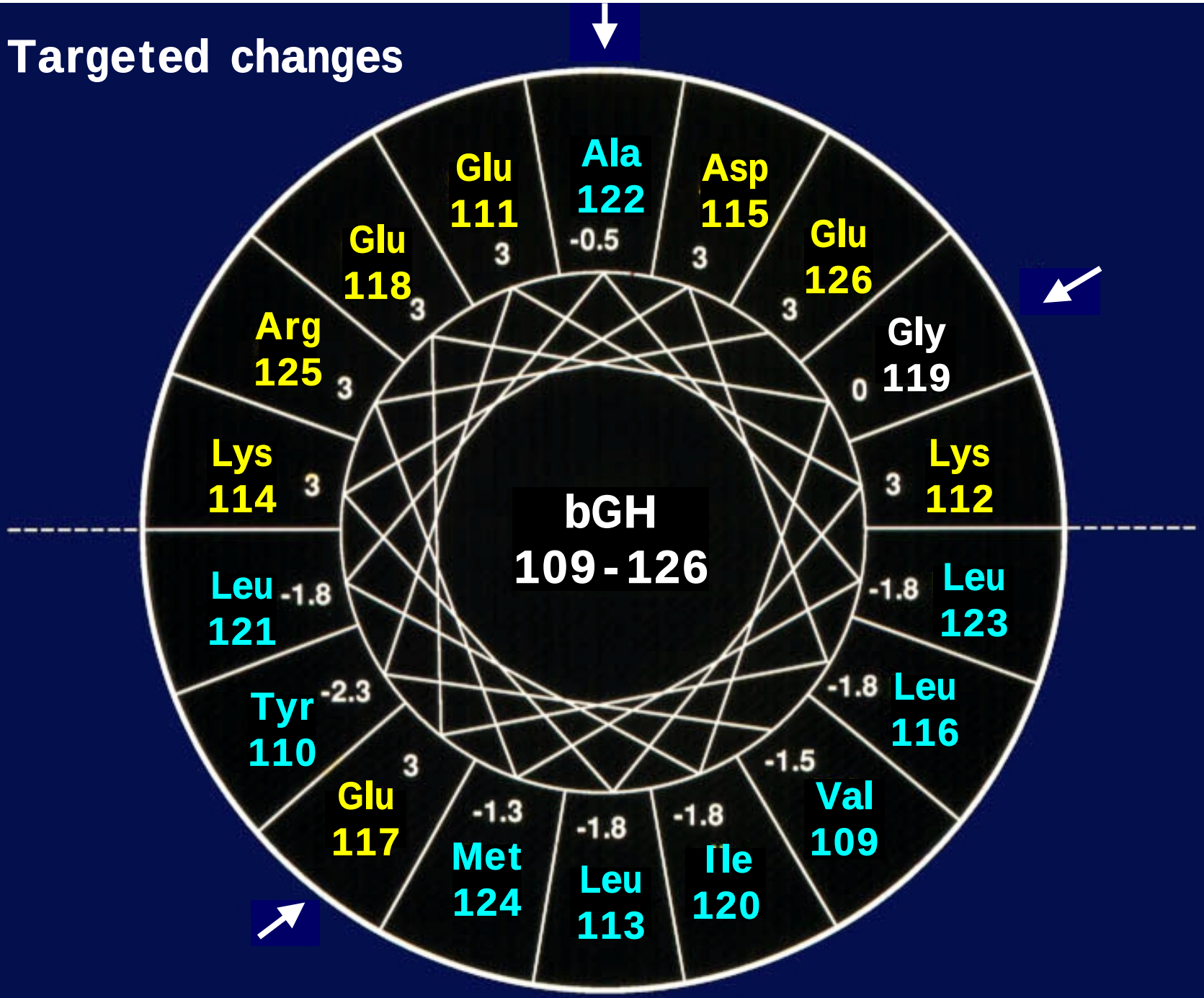
Hydrophilic Amino Acids

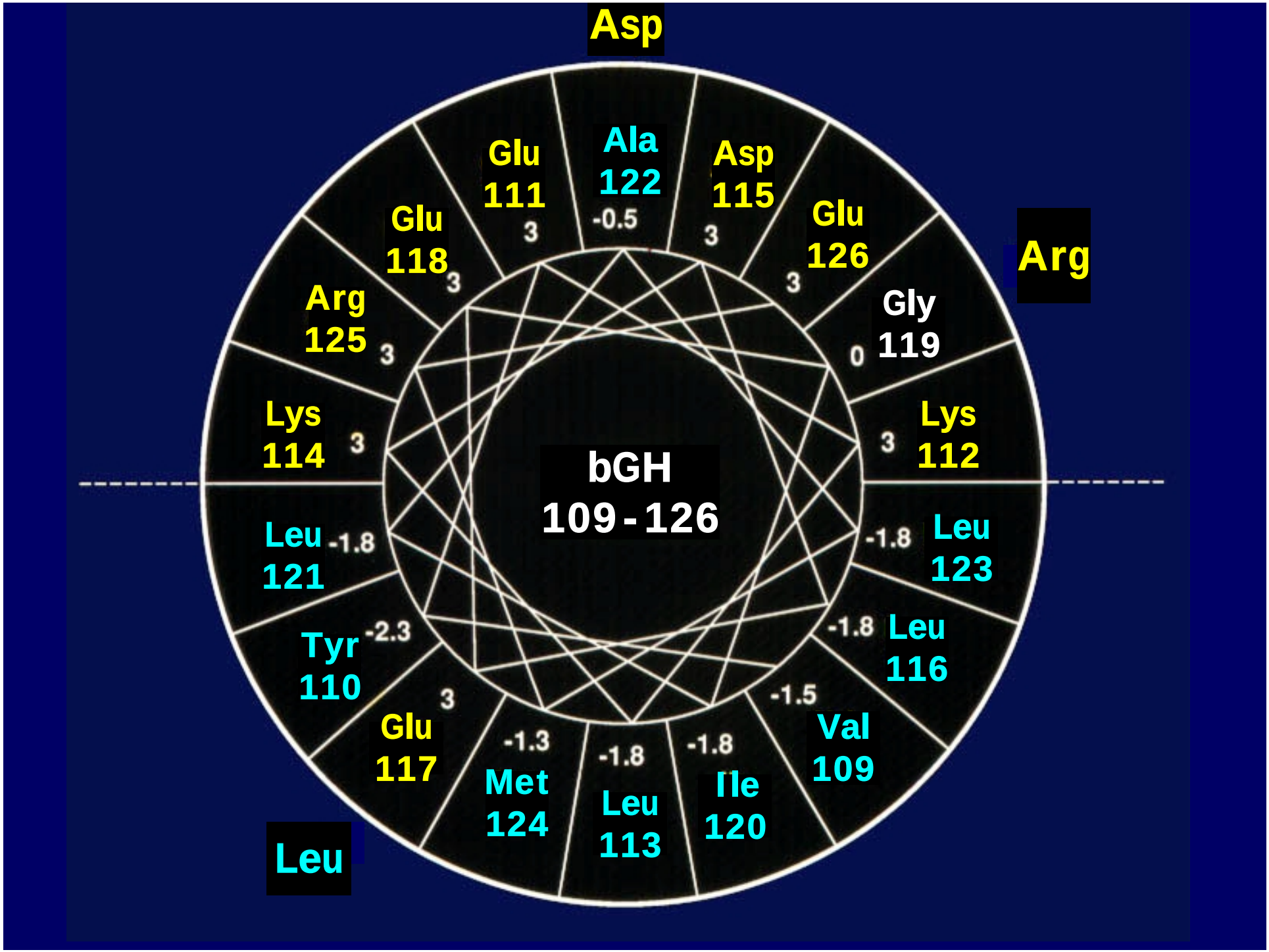


Imperfect amphipathic α-helix

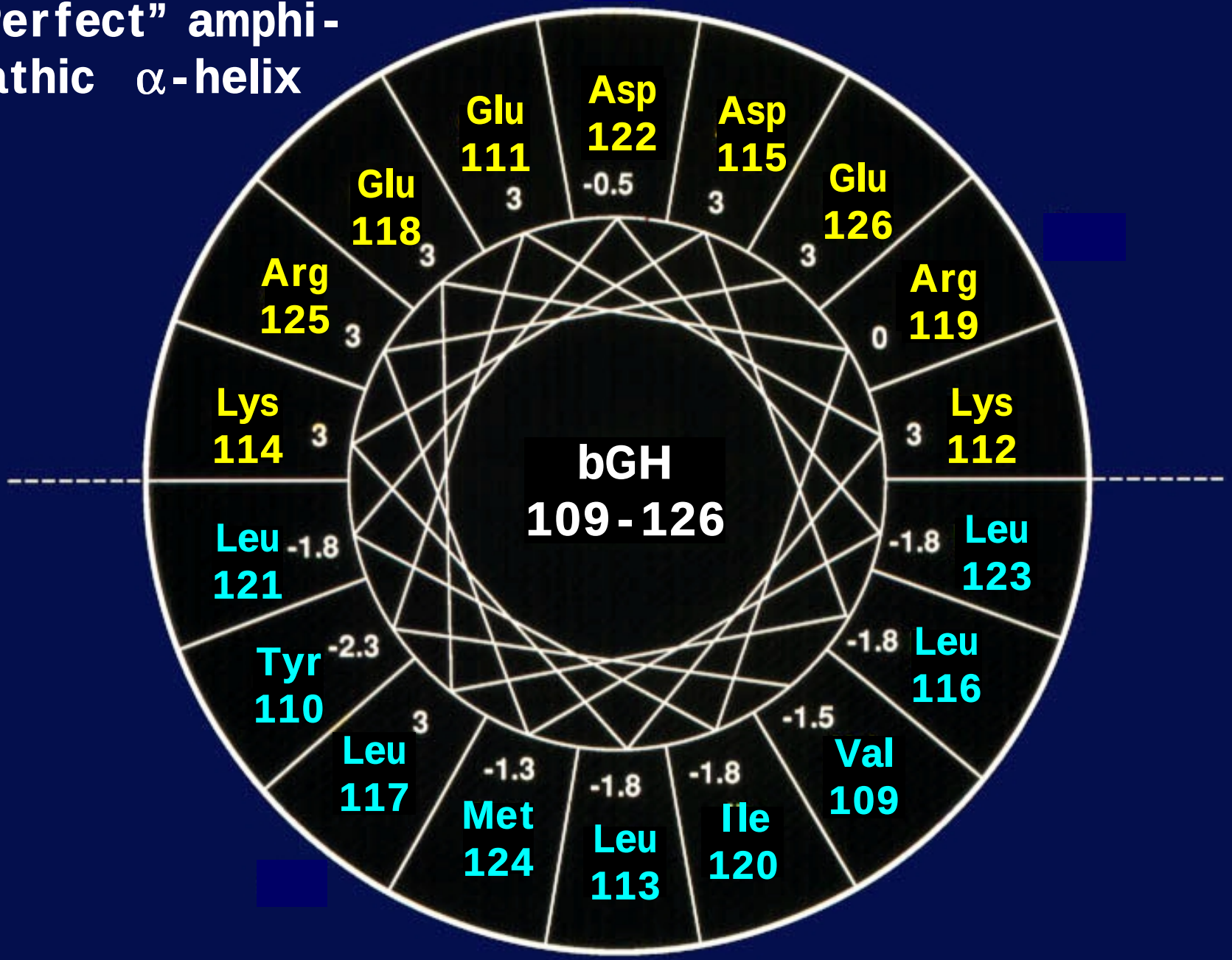


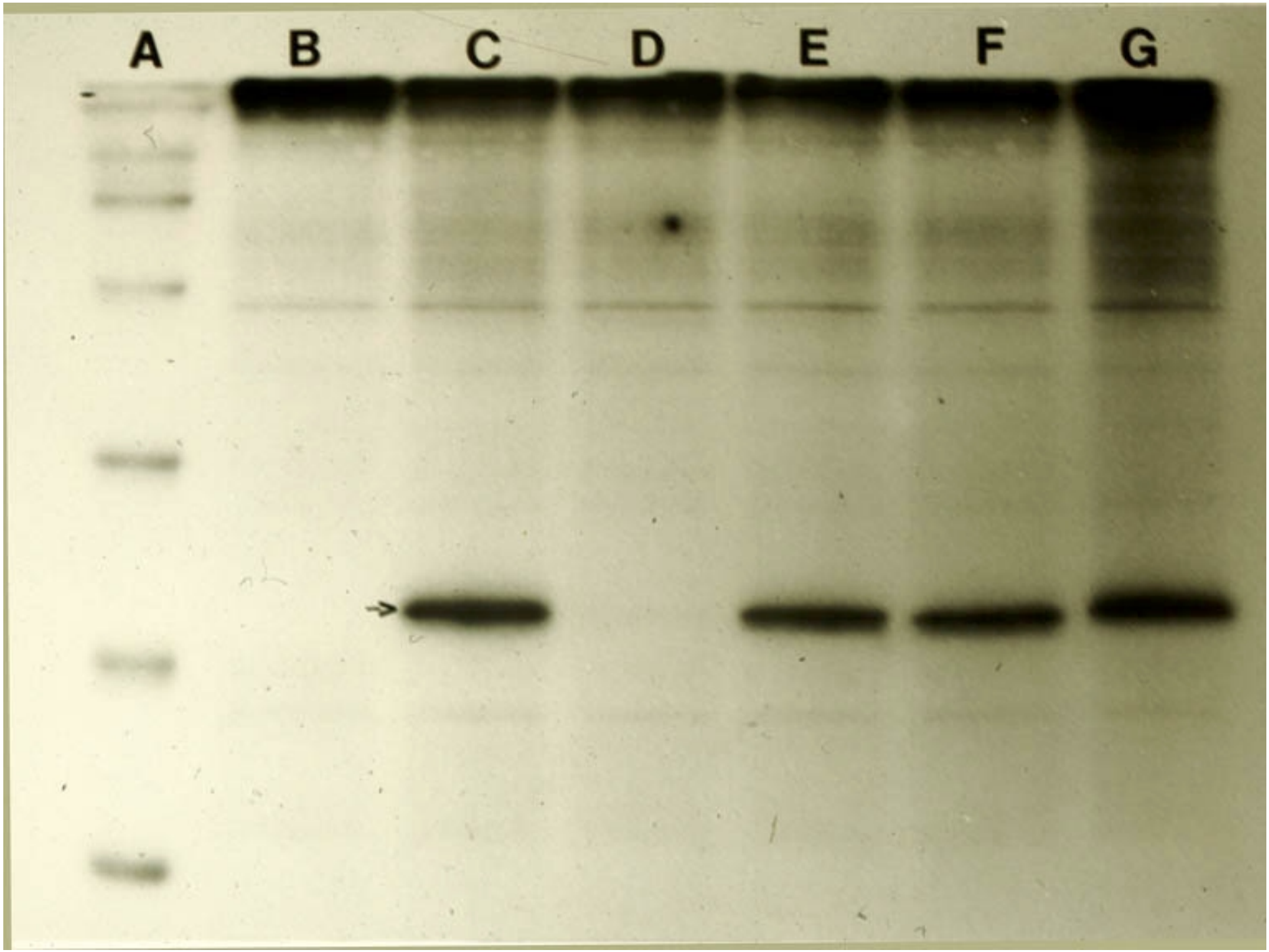
Targeted changes

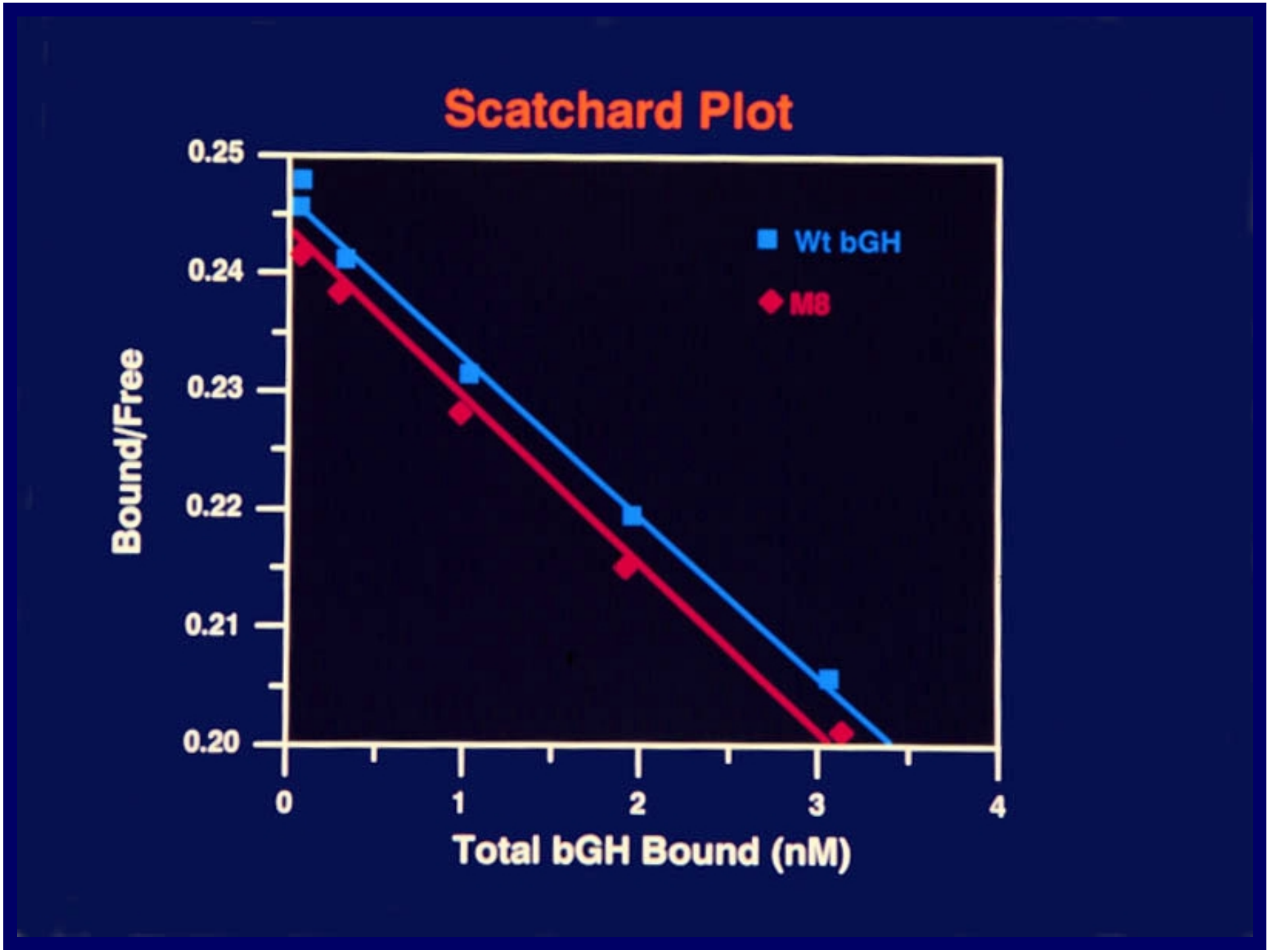


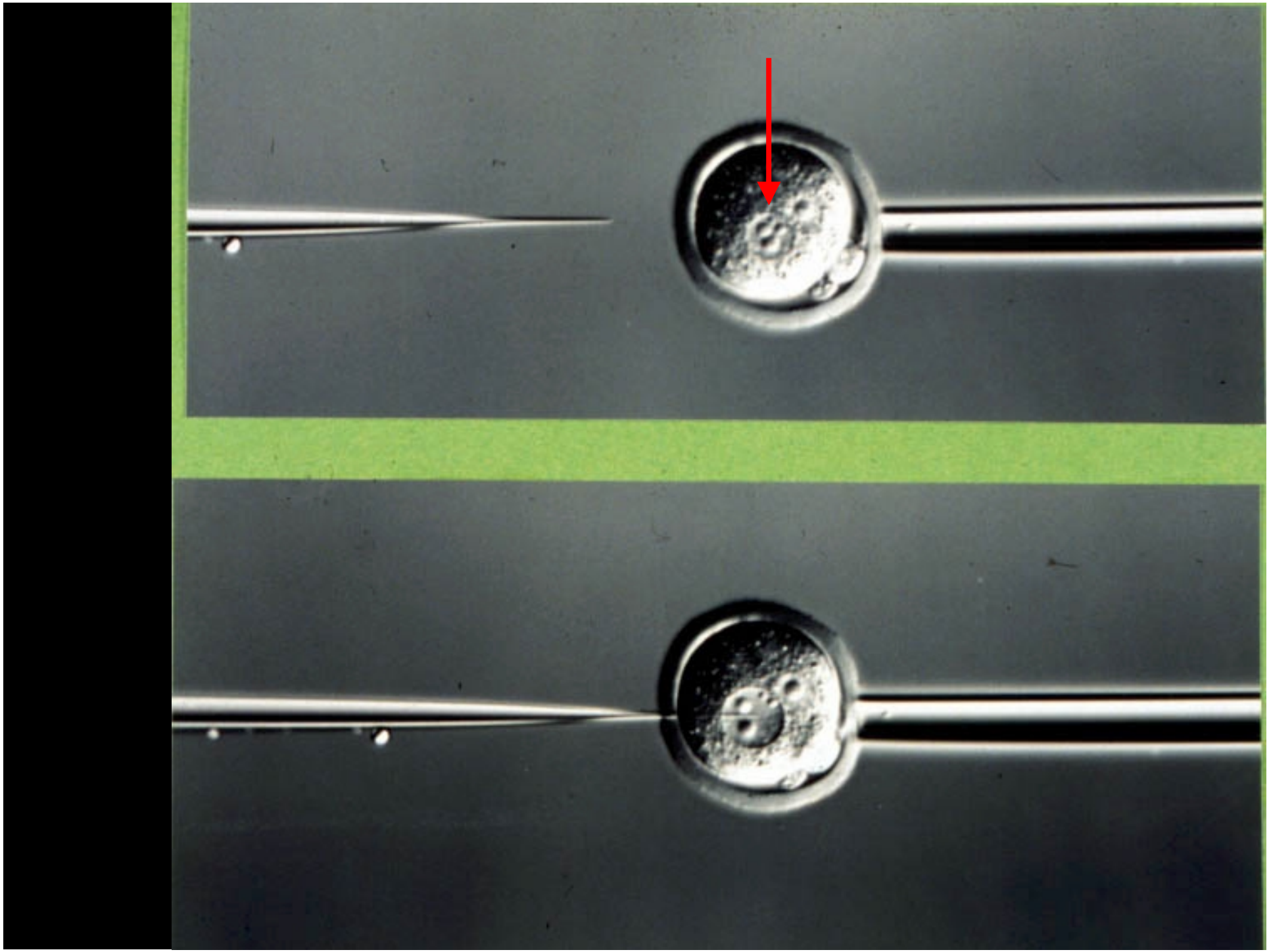

Asp
Glu 111 3
Ala 122 -0.5
Asp 115 3
Glu 126 3
Arg
Glu 118 3
Arg 125 3
Gly 119 0
Lys 114 3
Lys 112 3
bGH 109-126
Leu 121 -1.8
Leu 123 -1.8
Tyr 110 -2.3
Leu 116 -1.8
Glu 117 3
Met 124 -1.3
Leu 113 -1.8
Ile 120 -1.8
Val 109 -1.5
Leu

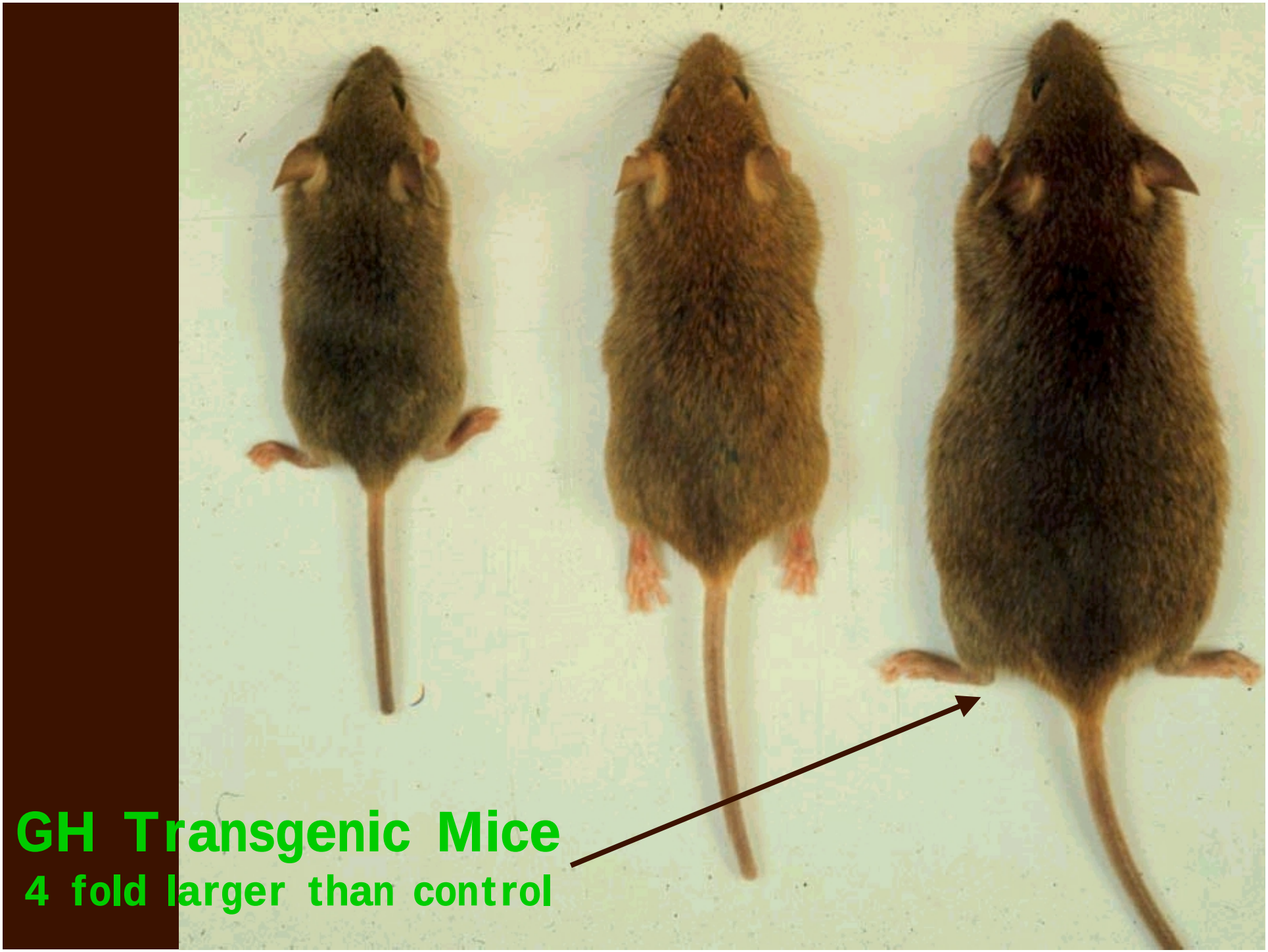
"Perfect" amphipathic α-helix



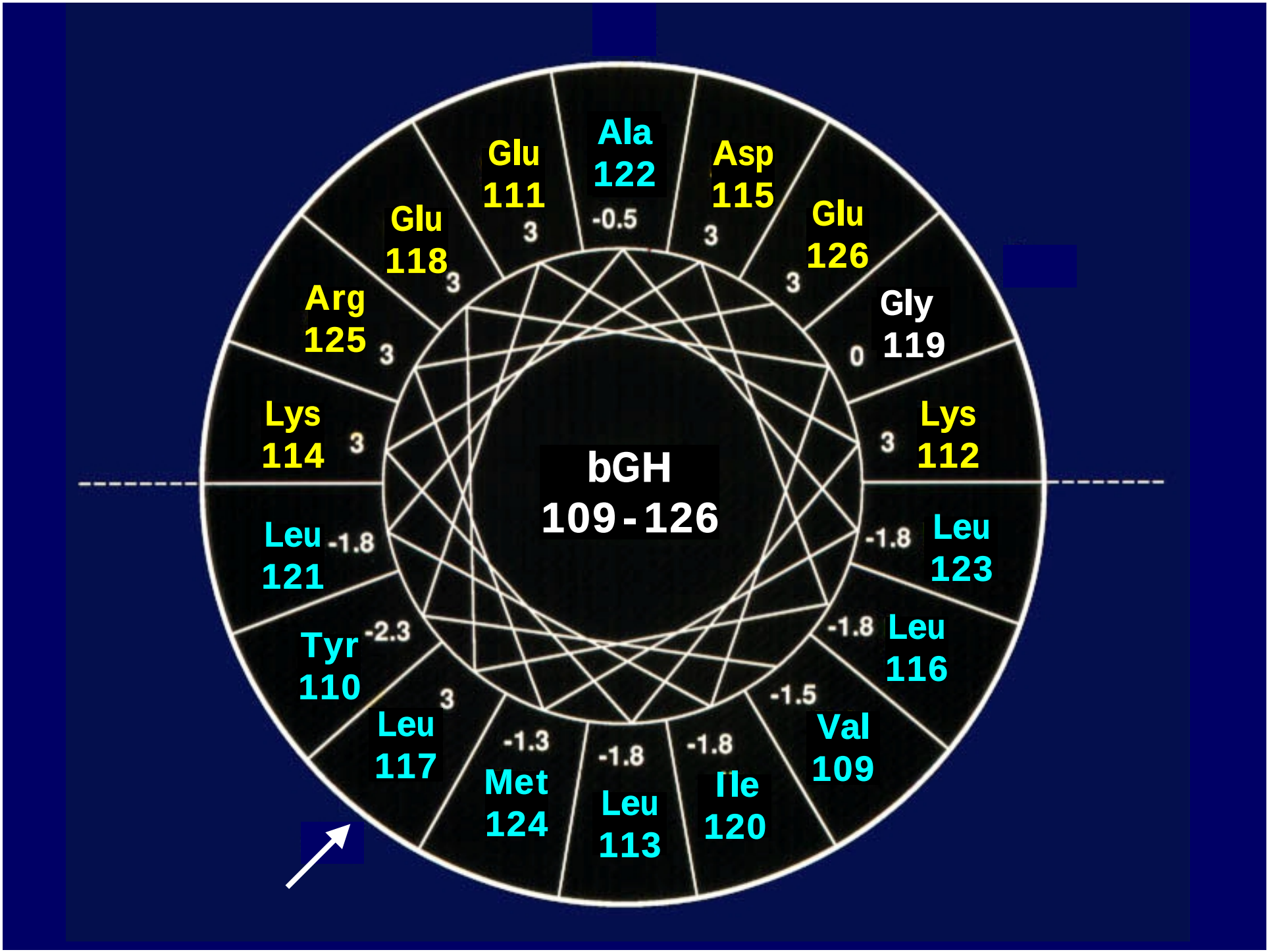

A
B
C
D
E
F
G

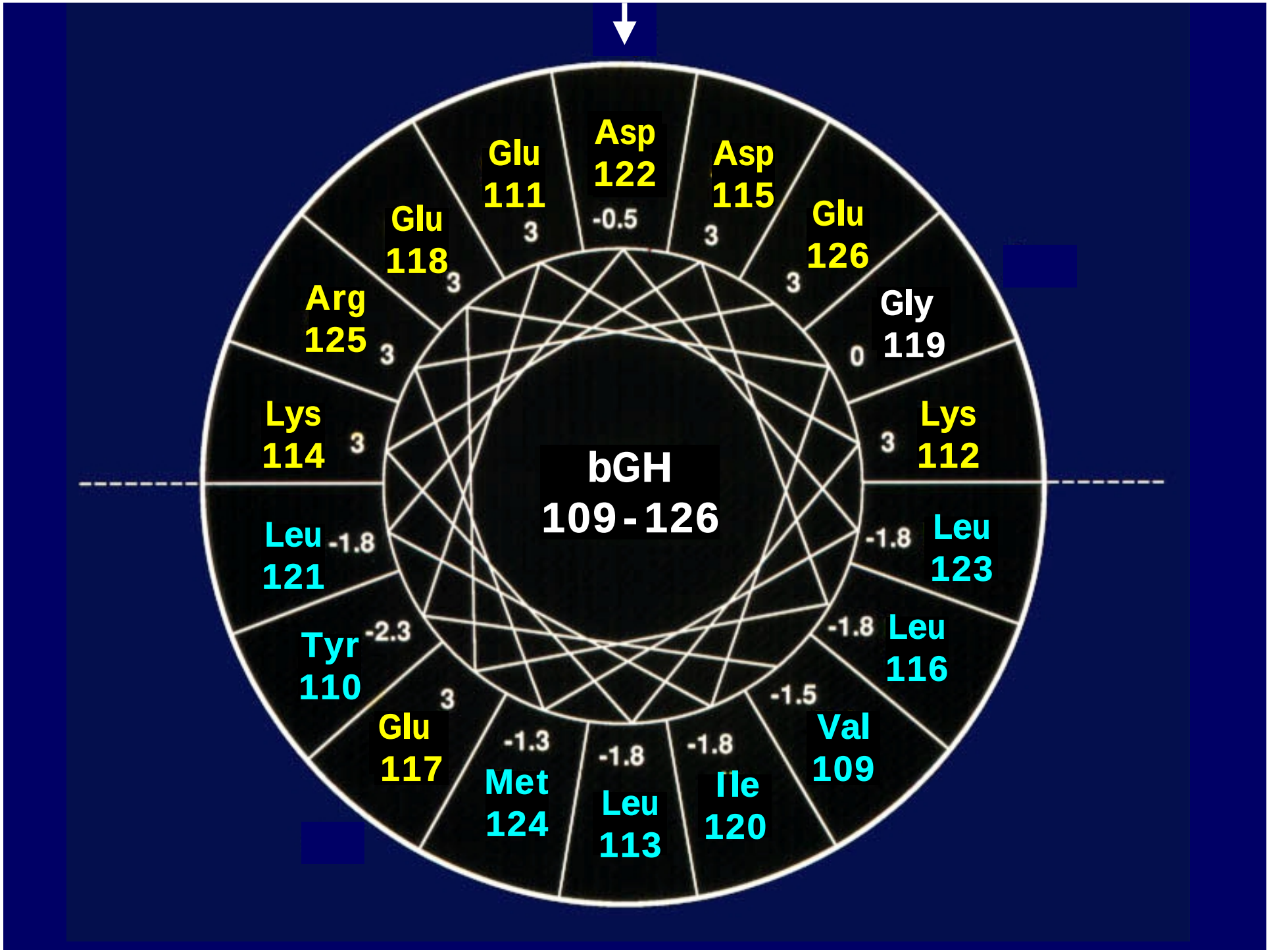

Scatchard Plot
0.25
0.24
0.23
0.22
0.21
0.20
Bound/Free
Wt bGH
M8
0
1
2
3
4
Total bGH Bound (nM)

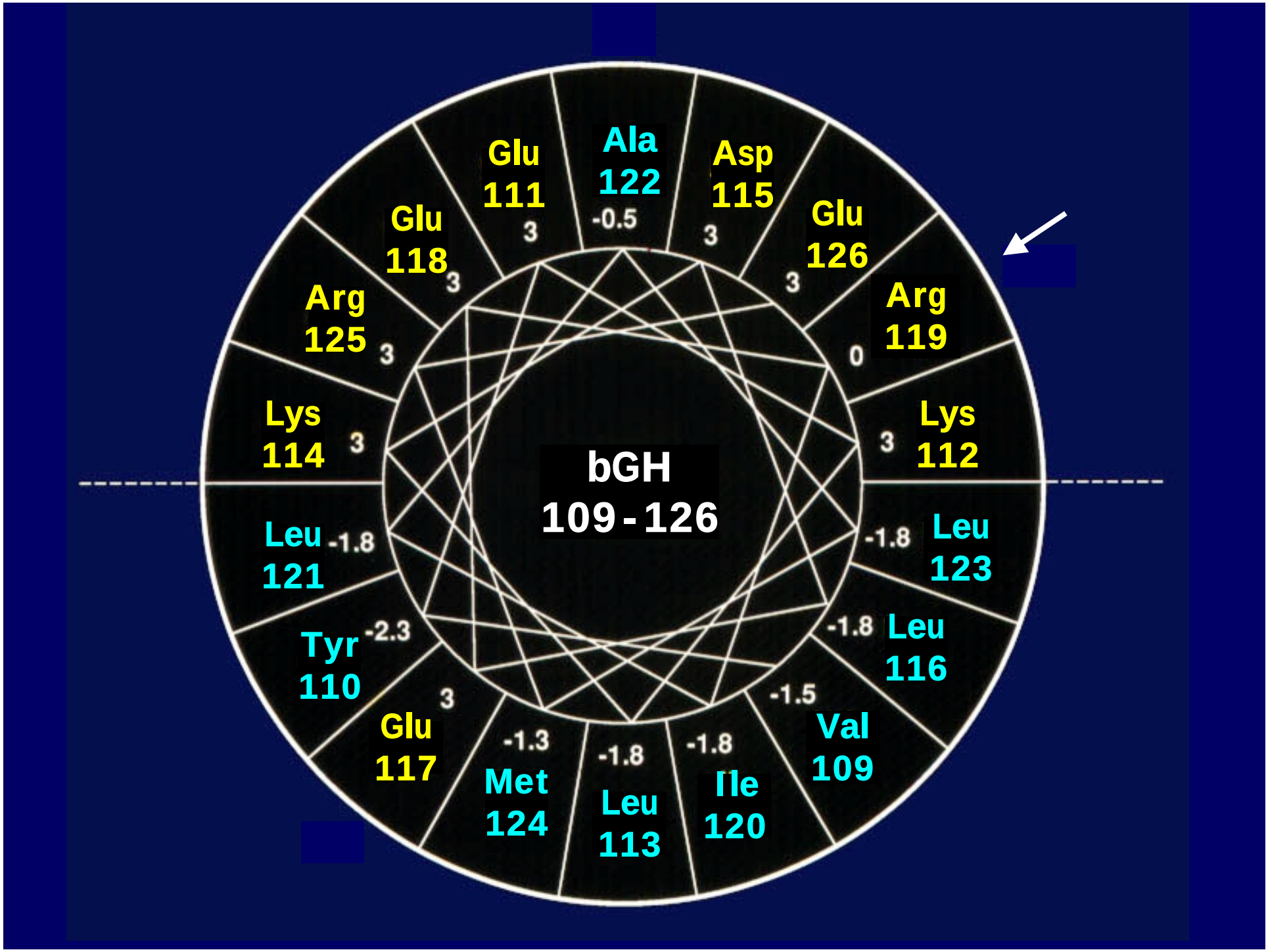


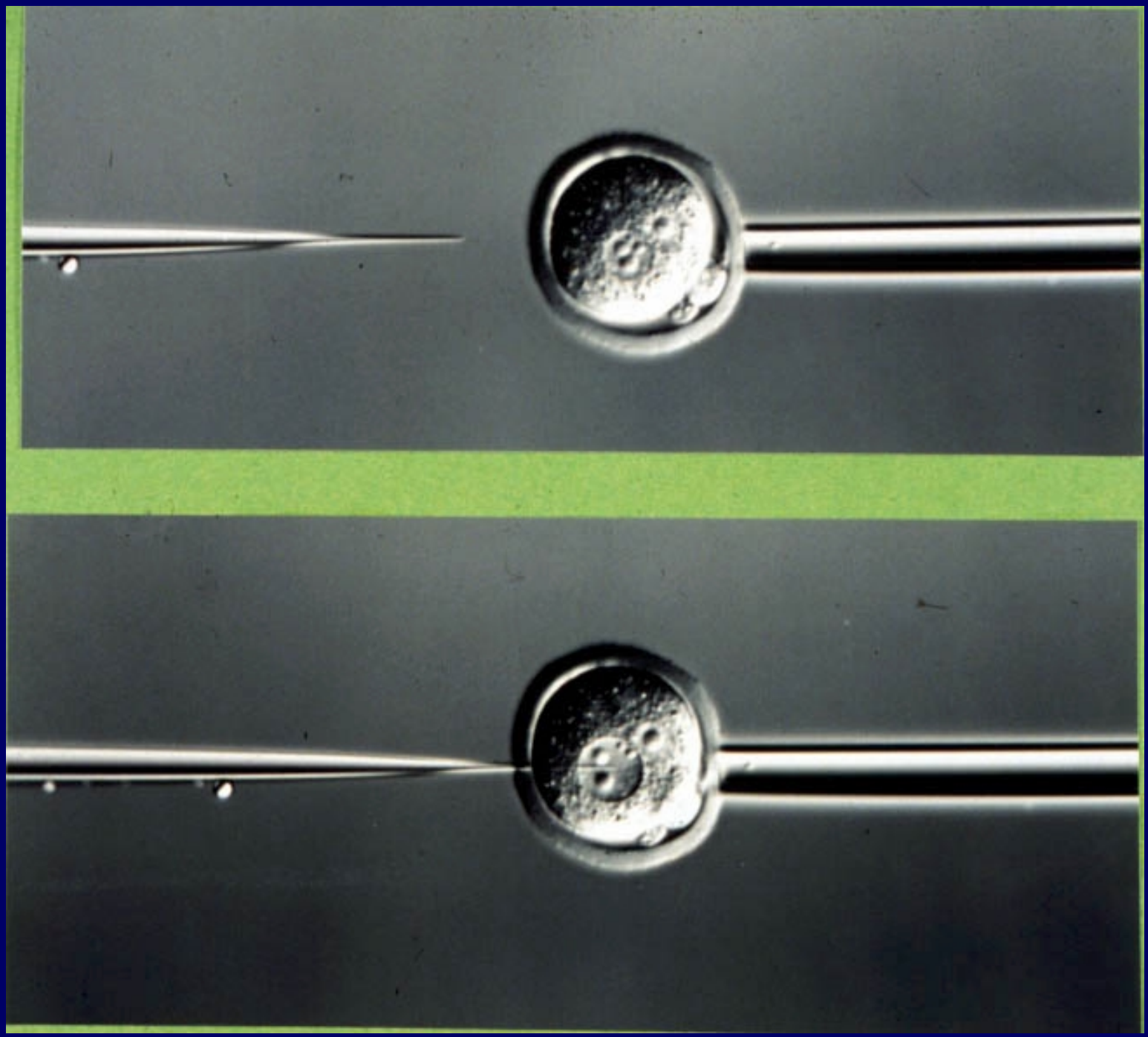

GH Transgenic Mice
4 fold larger than control


GH M8 Transgenic Mouse
2 fold smaller than control


bGH
109 - 126
Ala
122
-0.5
Asp
115
3
Glu
126
3
Gly
119
0
Lys
112
3
Leu
123
-1.8
Leu
116
-1.8
Val
109
-1.5
Ile
120
-1.8
Leu
113
-1.8
Met
124
-1.3
Leu
117
3
Tyr
110
-2.3
Leu
121
-1.8
Lys
114
3
Arg
125
3
Glu
118
3
Glu
111
3

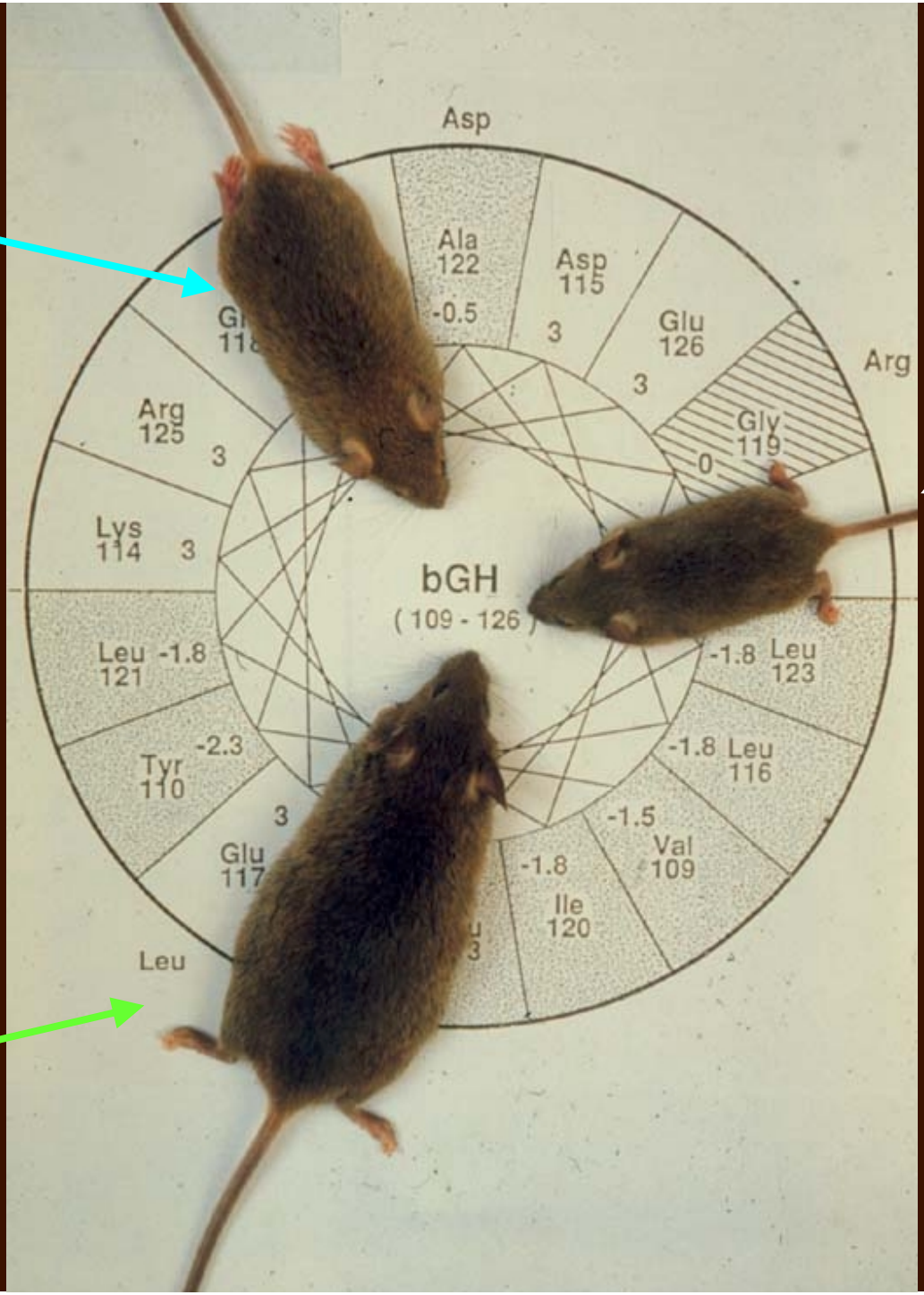

bGH
109 - 126
Asp
122
-0.5
Asp
115
3
Glu
126
3
Gly
119
0
Lys
112
3
Leu
123
-1.8
Leu
116
-1.8
Val
109
-1.5
Ile
120
-1.8
Leu
113
-1.8
Met
124
-1.3
Glu
117
3
Tyr
110
-2.3
Leu
121
-1.8
Lys
114
3
Arg
125
3
Glu
118
3
Glu
111
3


bGH
109 - 126
Glu 111 3
Ala 122 -0.5
Asp 115 3
Glu 126 3
Arg 119 0
Lys 112 3
Leu 123 -1.8
Leu 116 -1.8
Val 109 -1.5
Ile 120 -1.8
Leu 113 -1.8
Met 124 -1.3
Glu 117 3
Tyr 110 -2.3
Leu 121 -1.8
Lys 114 3
Arg 125 3
Glu 118 3



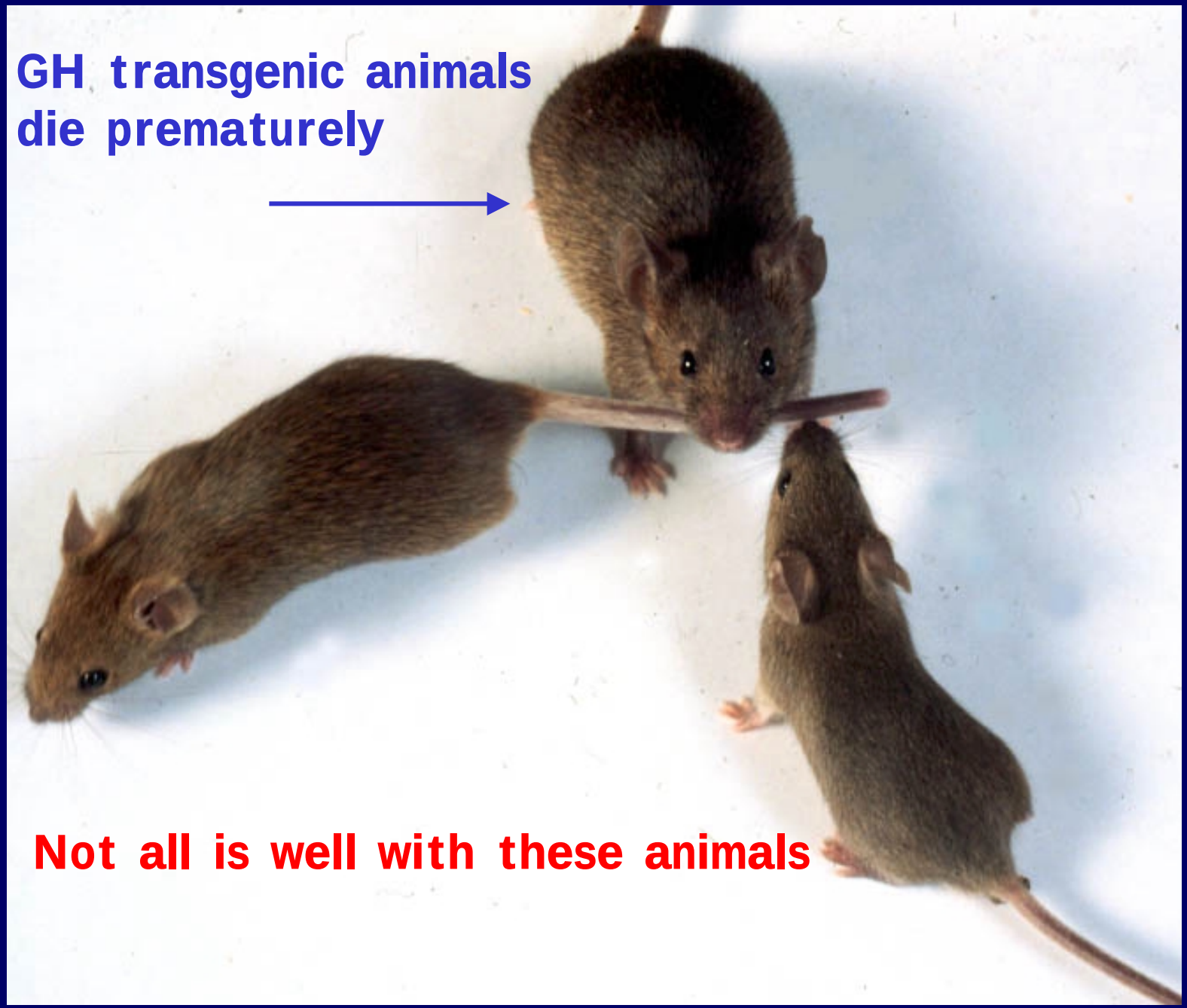
Normal

Giant



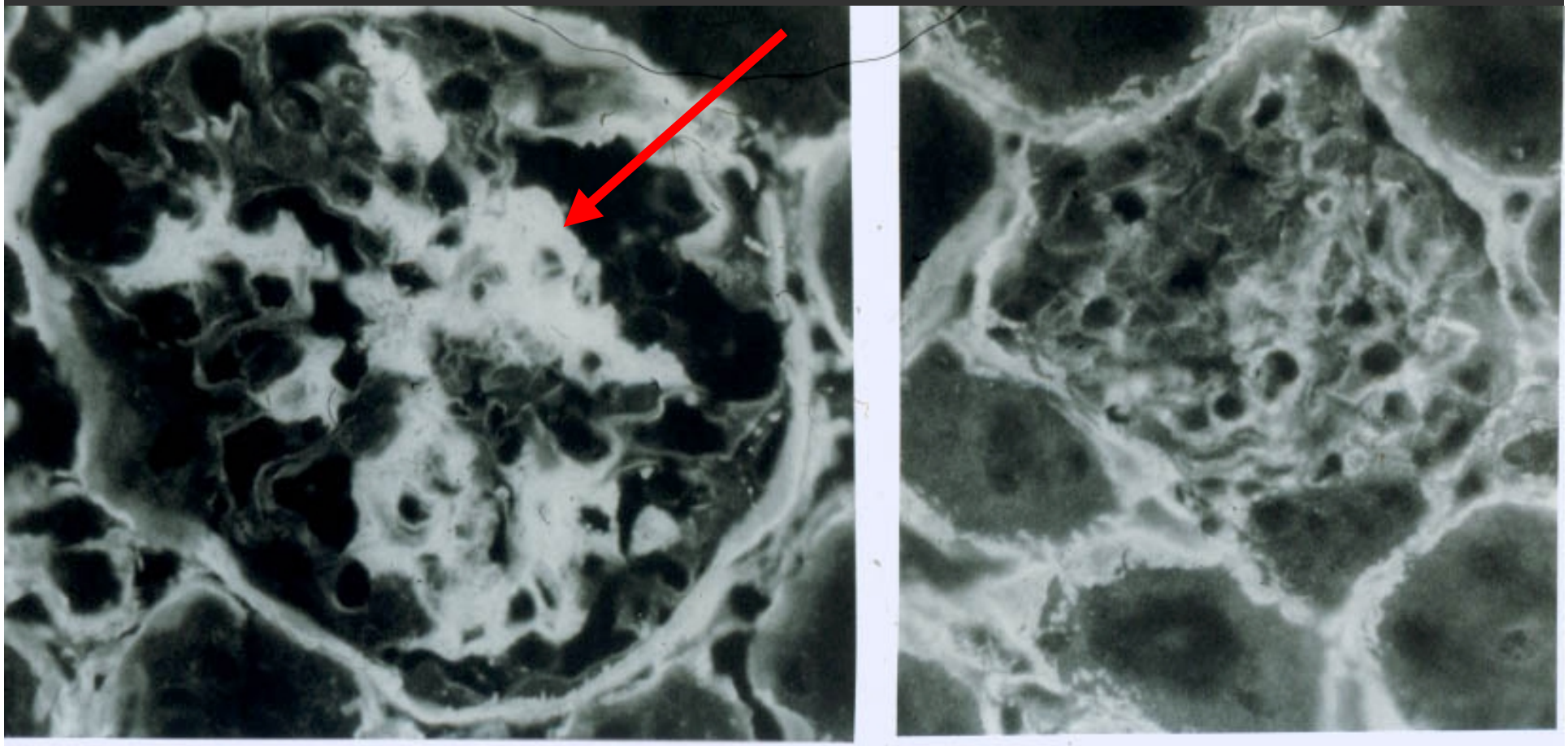
Dwarf

One amino acid change, ie. glycine to arginine results in a growth inhibitor or **“growth hormone antagonist”**


GH transgenic animals
die prematurely
Not all is well with these animals

GH Transgenic Mice

Control Mice



COLLIV Antiserum

Glomerulosclerosis – scarring of the glomerulus

Back to structure/function!!!

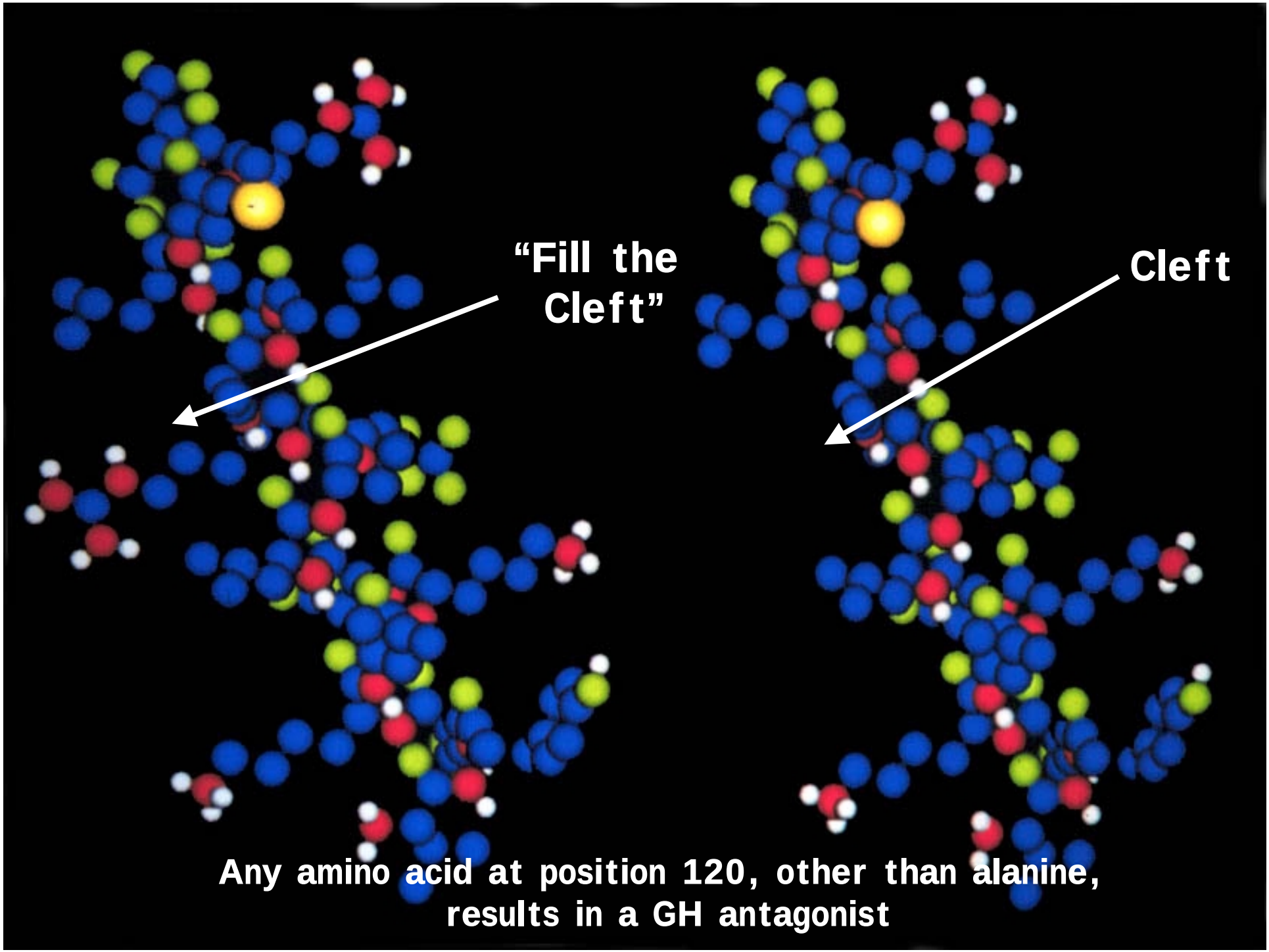
Helix 3

bGH (aa 109-126)

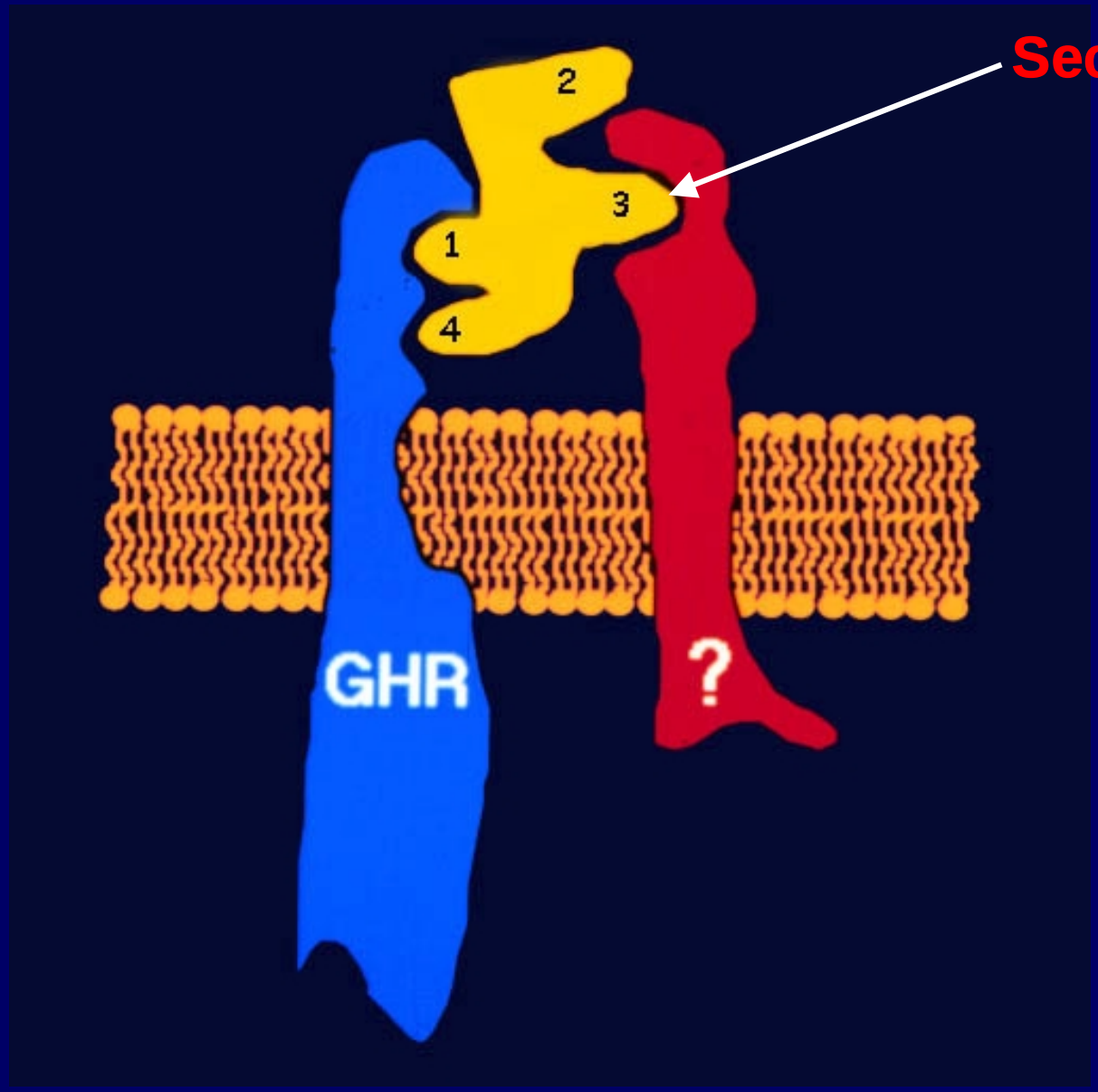
109
Val Tyr Glu Lys Leu Lys Asp Leu Glu

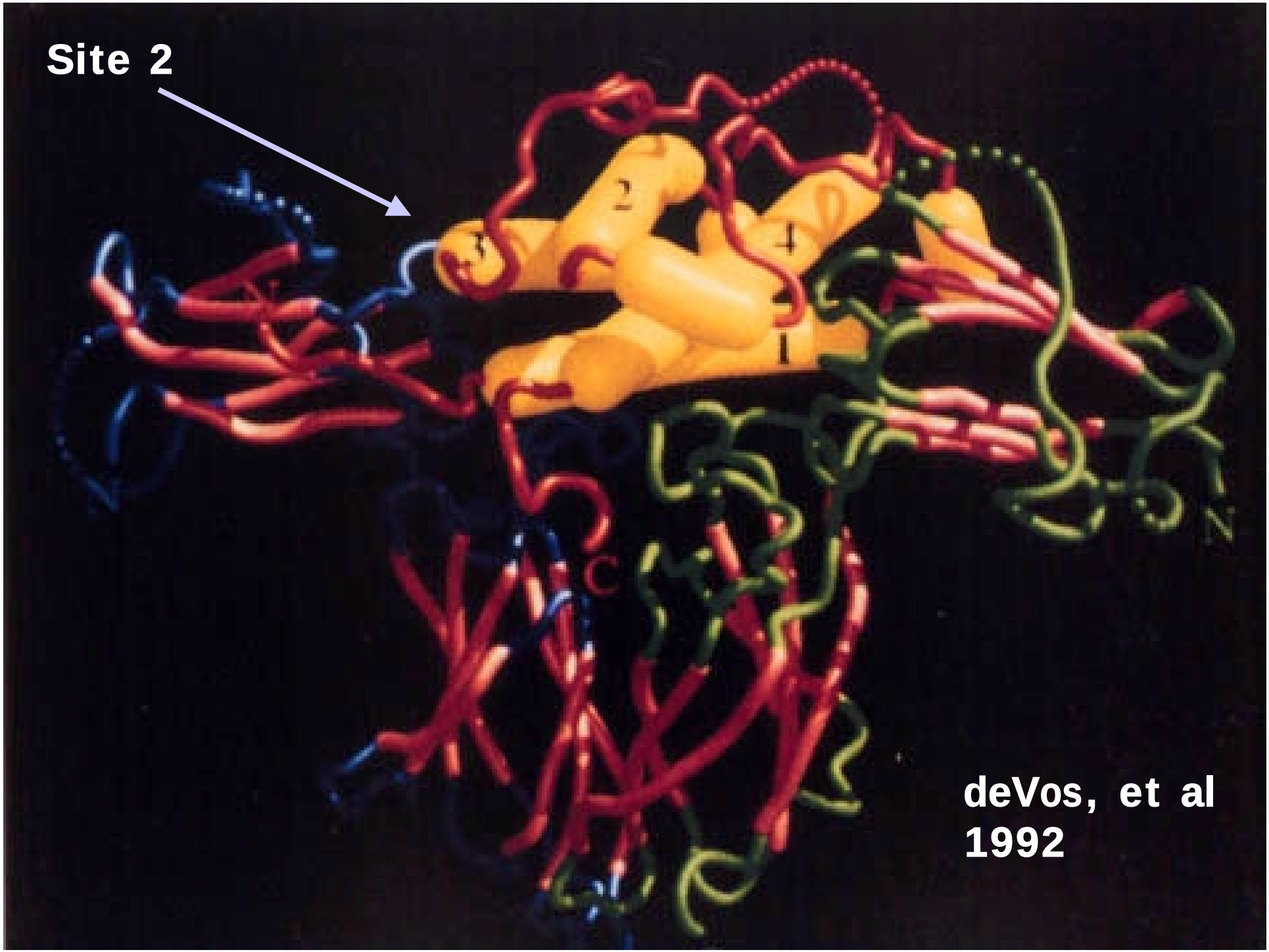
119 122 126
Glu Gly Ile Leu Ala Leu Met Arg Glu

Gly 119 and Ala 122 – one helical turn apart!!!!!!!

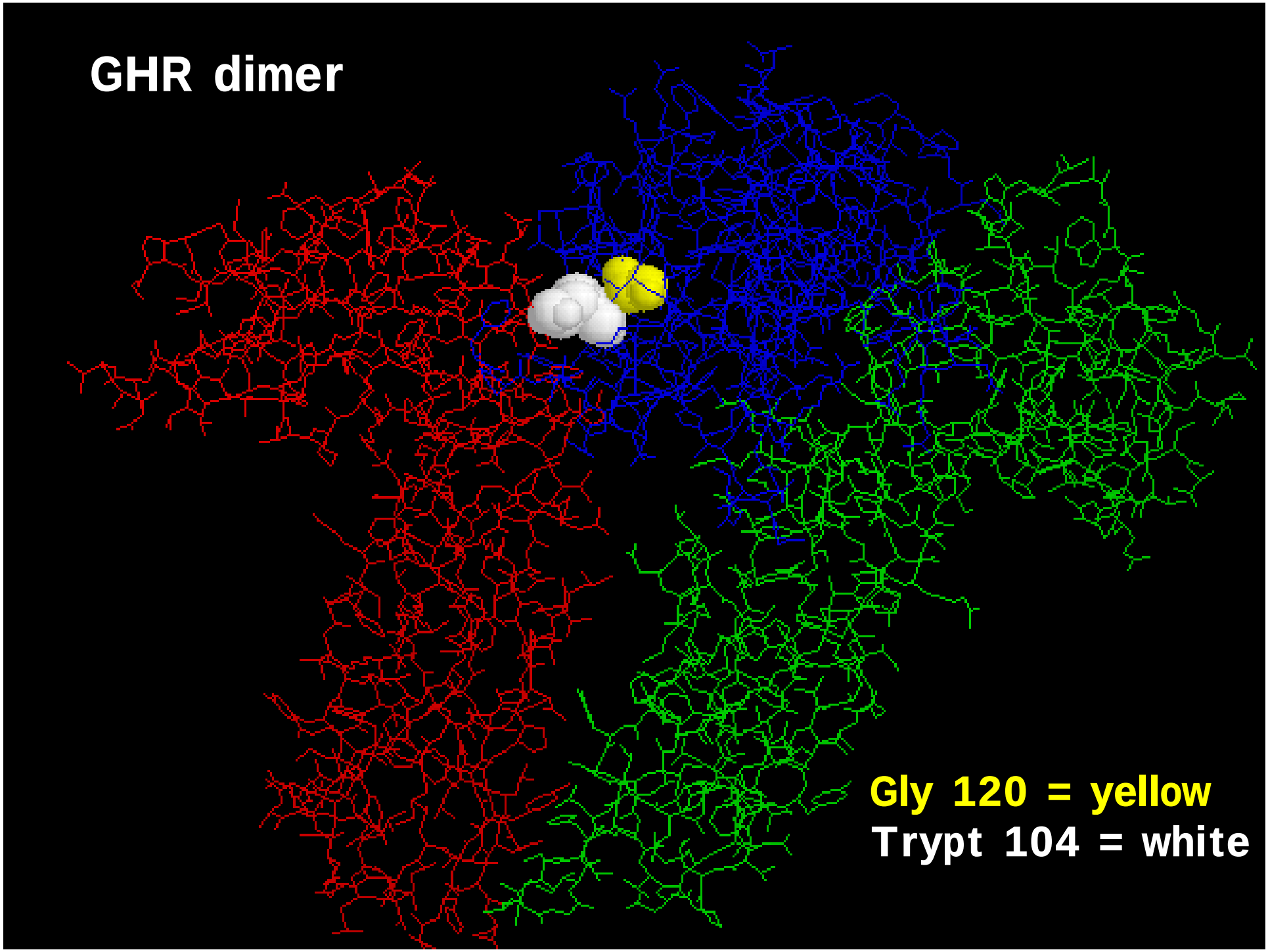

"Fill the Cleft"
Cleft
Any amino acid at position 120, other than alanine, results in a GH antagonist

Second target model of GH action - 1990




Site 2
2
3
4
1
C
N
deVos, et al
1992

GHR dimer

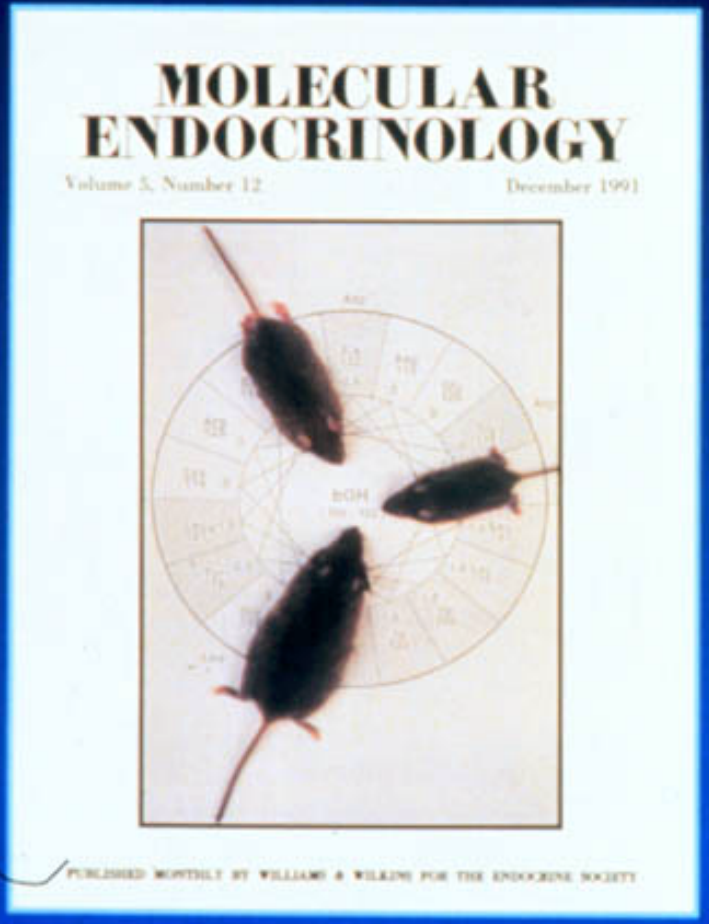


Clinical uses of a Growth Hormone Antagonists

- Acromegaly
- Diabetes
- Cancer

Growth Hormone Antagonists (GHAs)

sensus



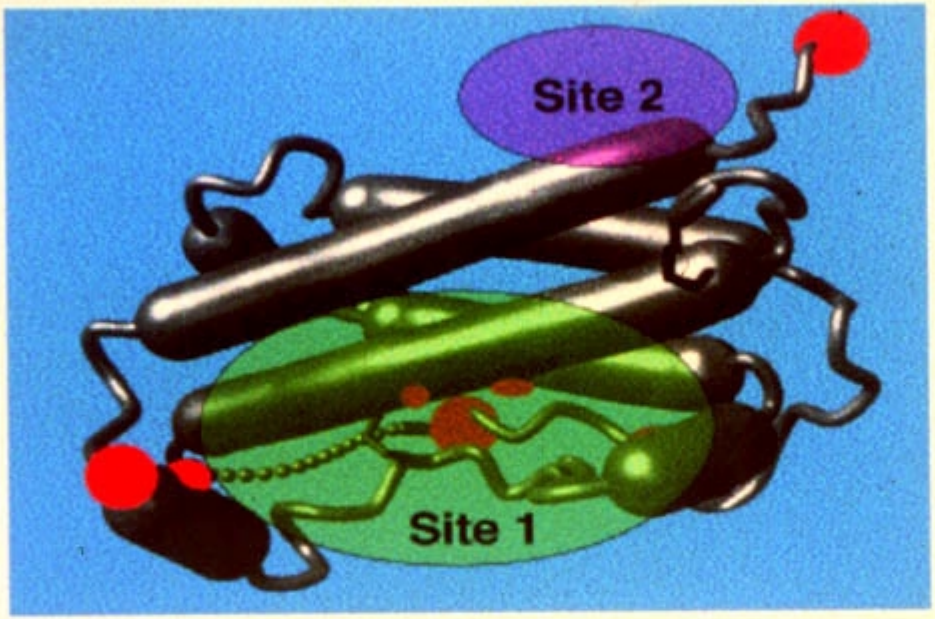
Acromegaly

- Increased GH levels due to pituitary gland tumor
- High circulating levels of IGF-1

Potential Problems with a growth hormone antagonist in the clinic

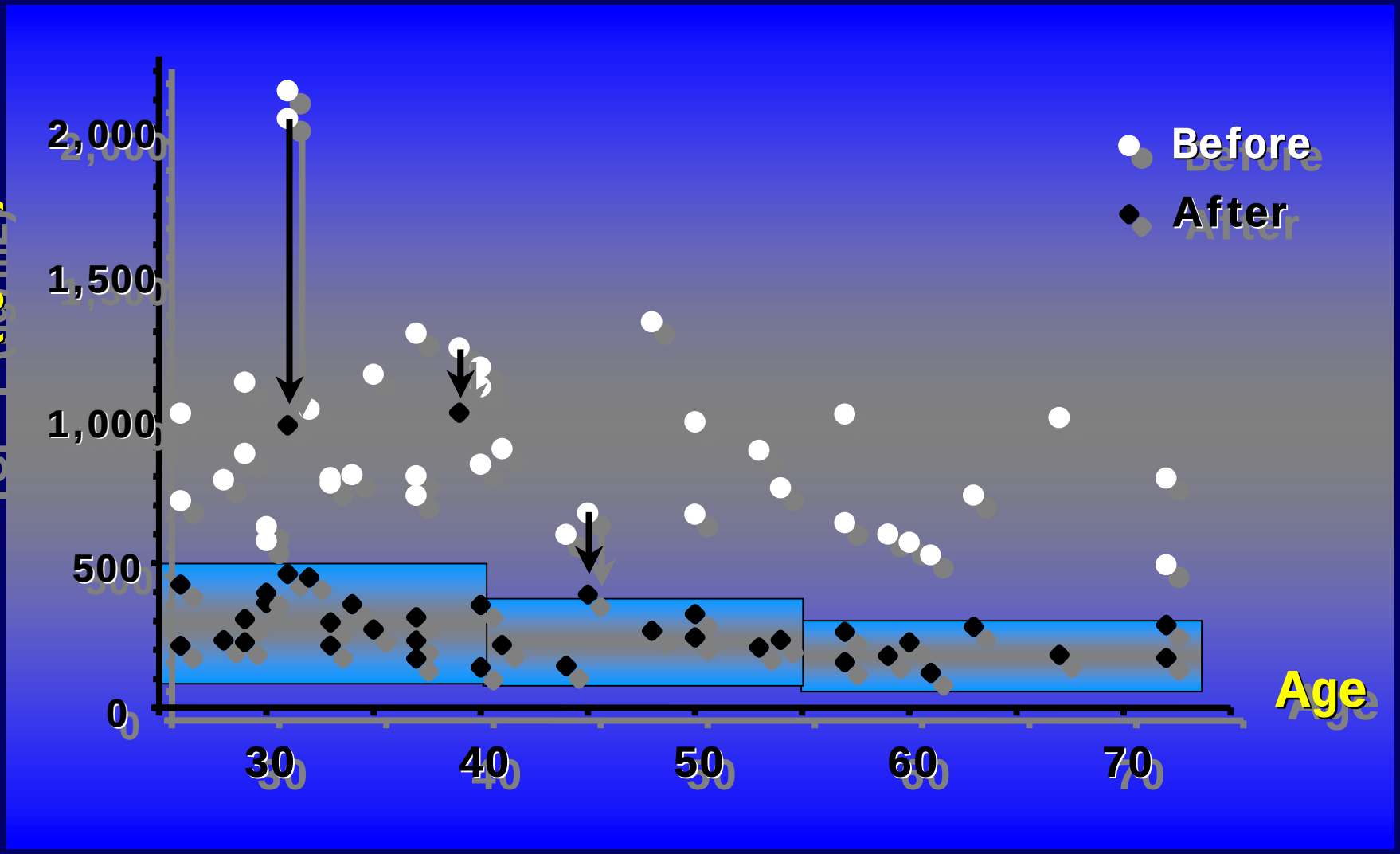
- Short half-life
- Antibody formation

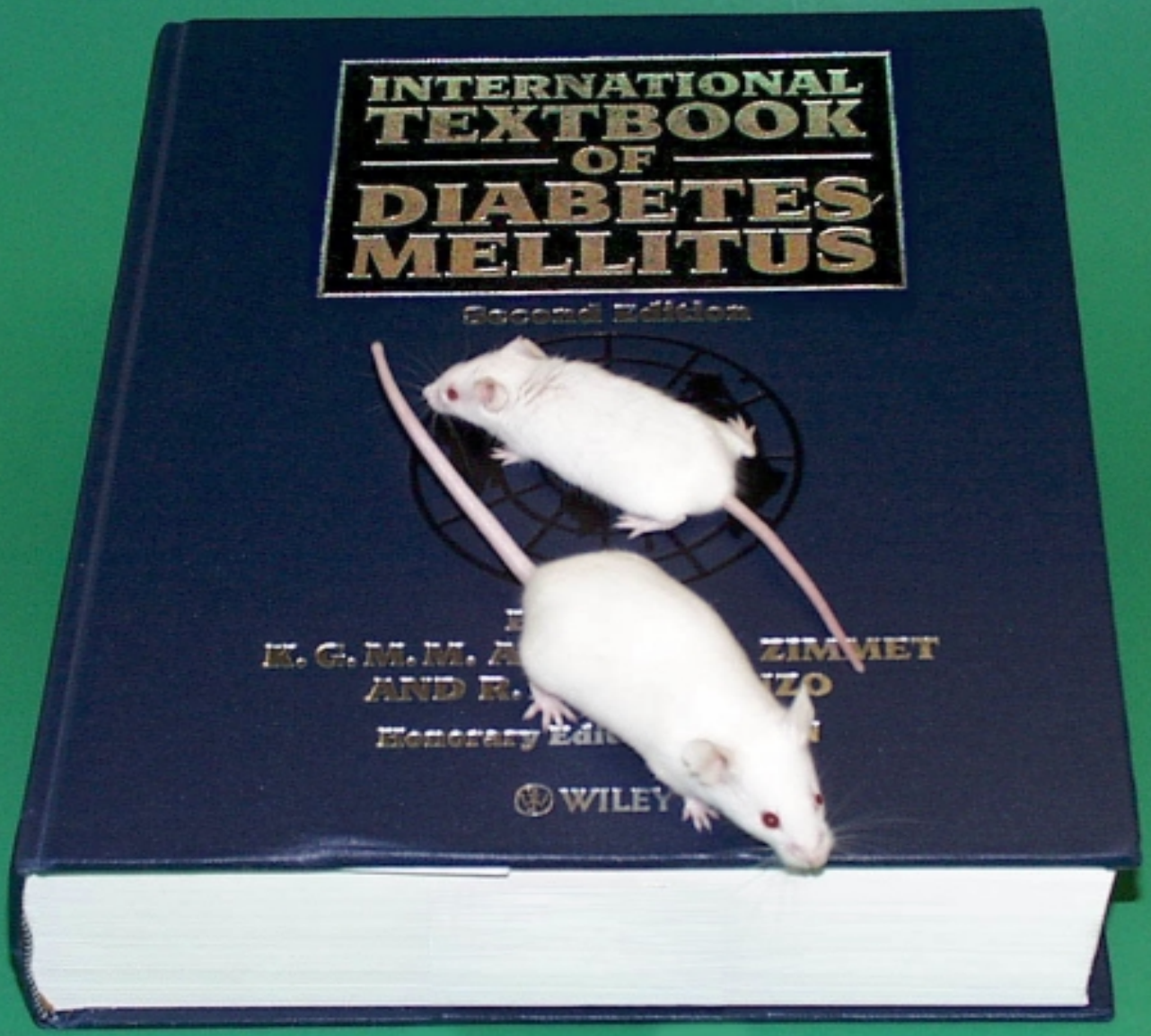
Rational design of B2036-PEG



Site 1	Site 2	Pegylation
8 mutations	1 mutation	Increases half-life
Increases binding to 1st GHR	Prevents binding to 2nd GHR	

Individual IGF-I levels before and after B 2036-PEG

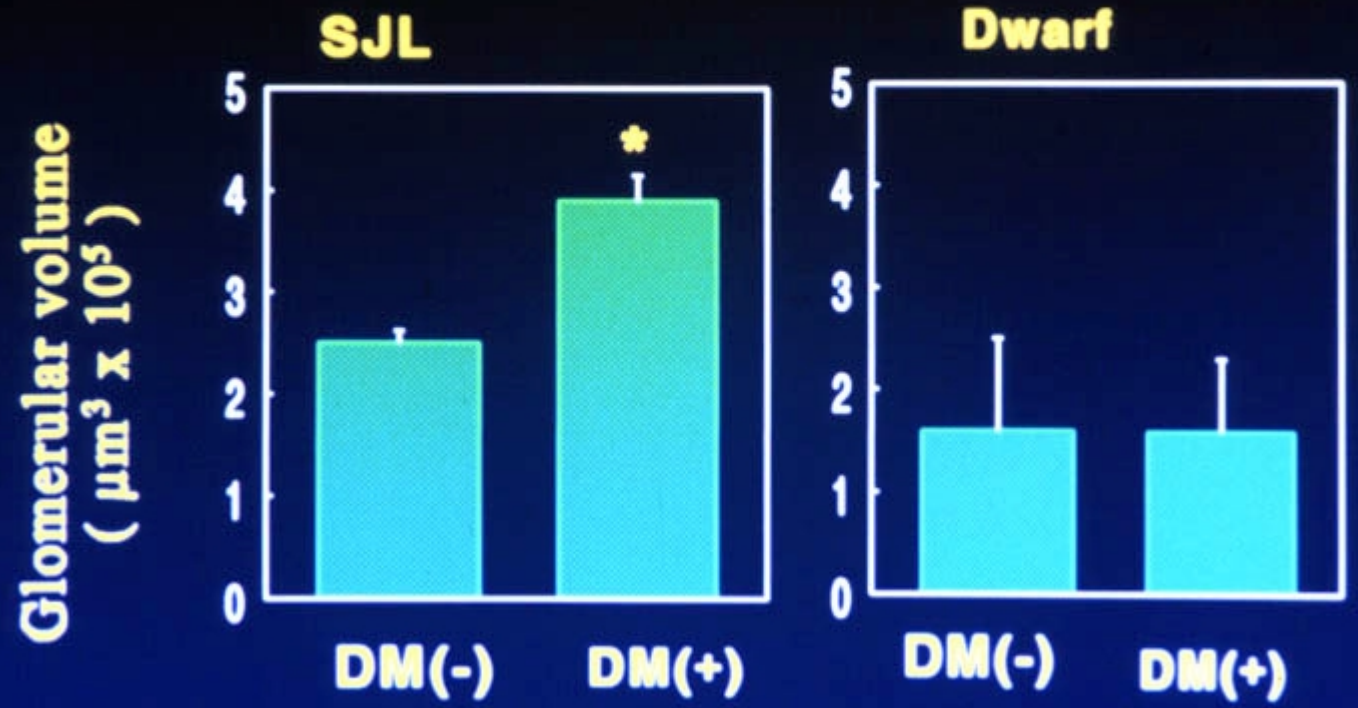




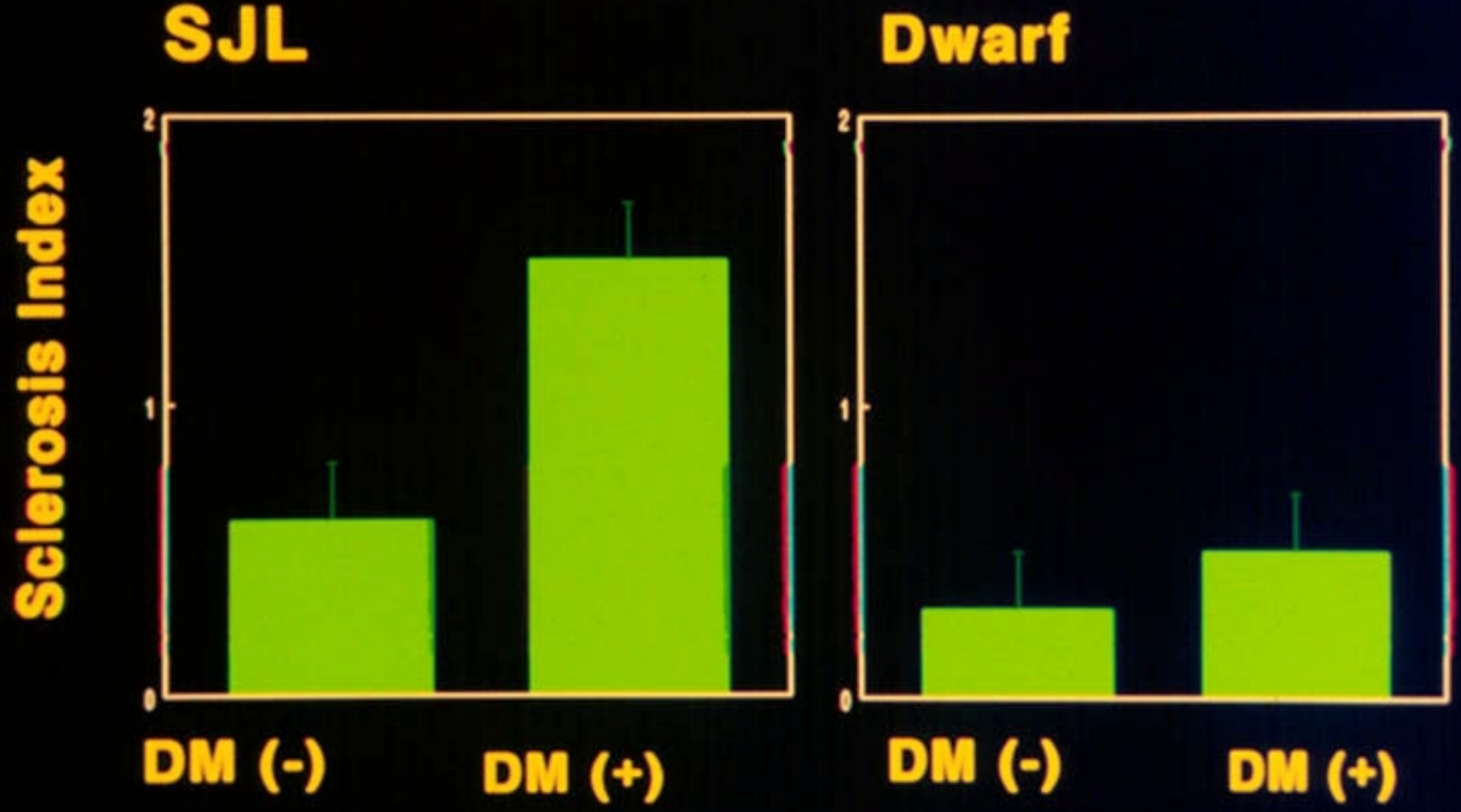
GH Antagonists and Diabetes Induced End Organ Damage

Kidneys and Eyes

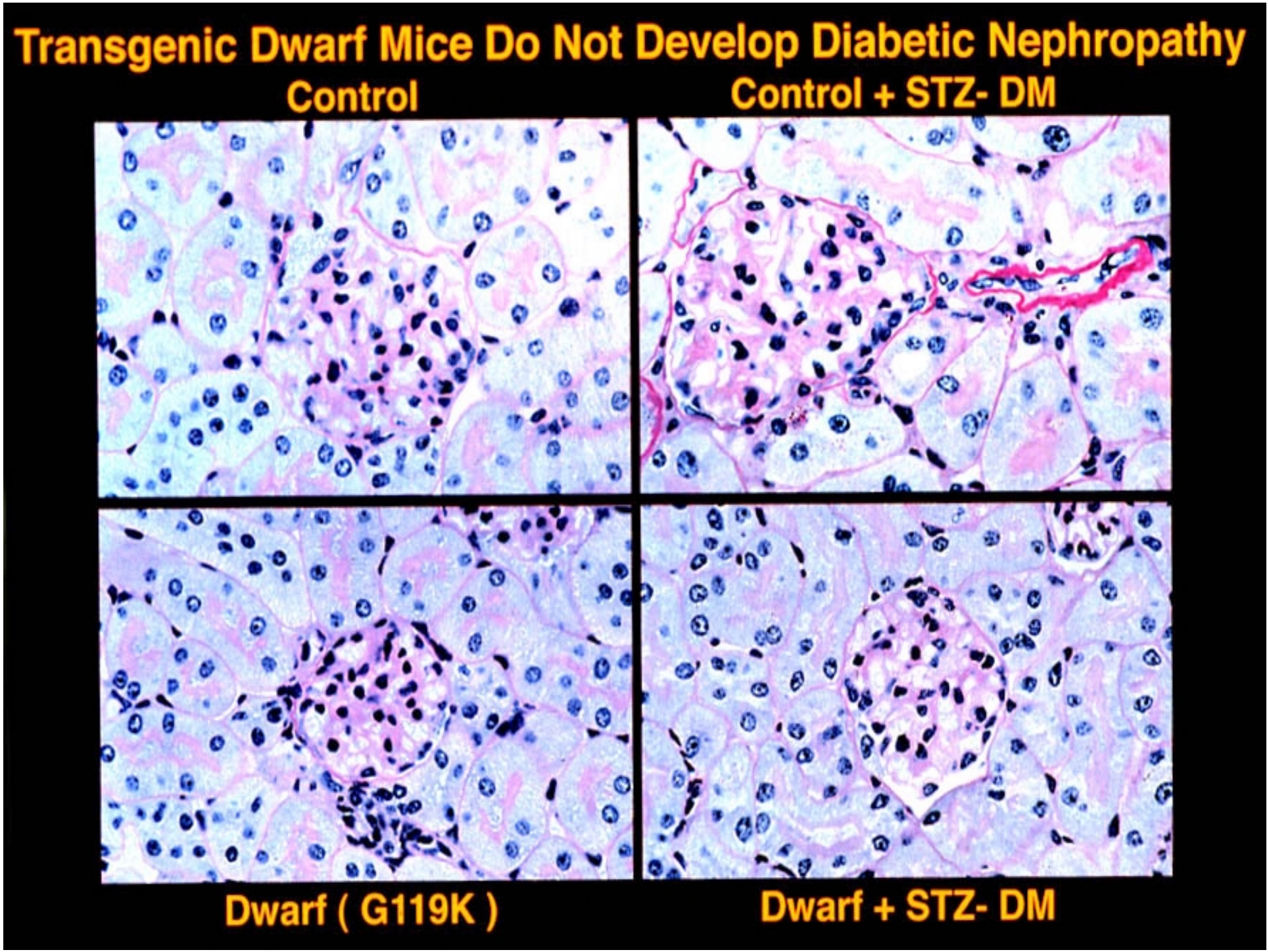
Dwarf Mice are Protected from Glomerular Hypertrophy

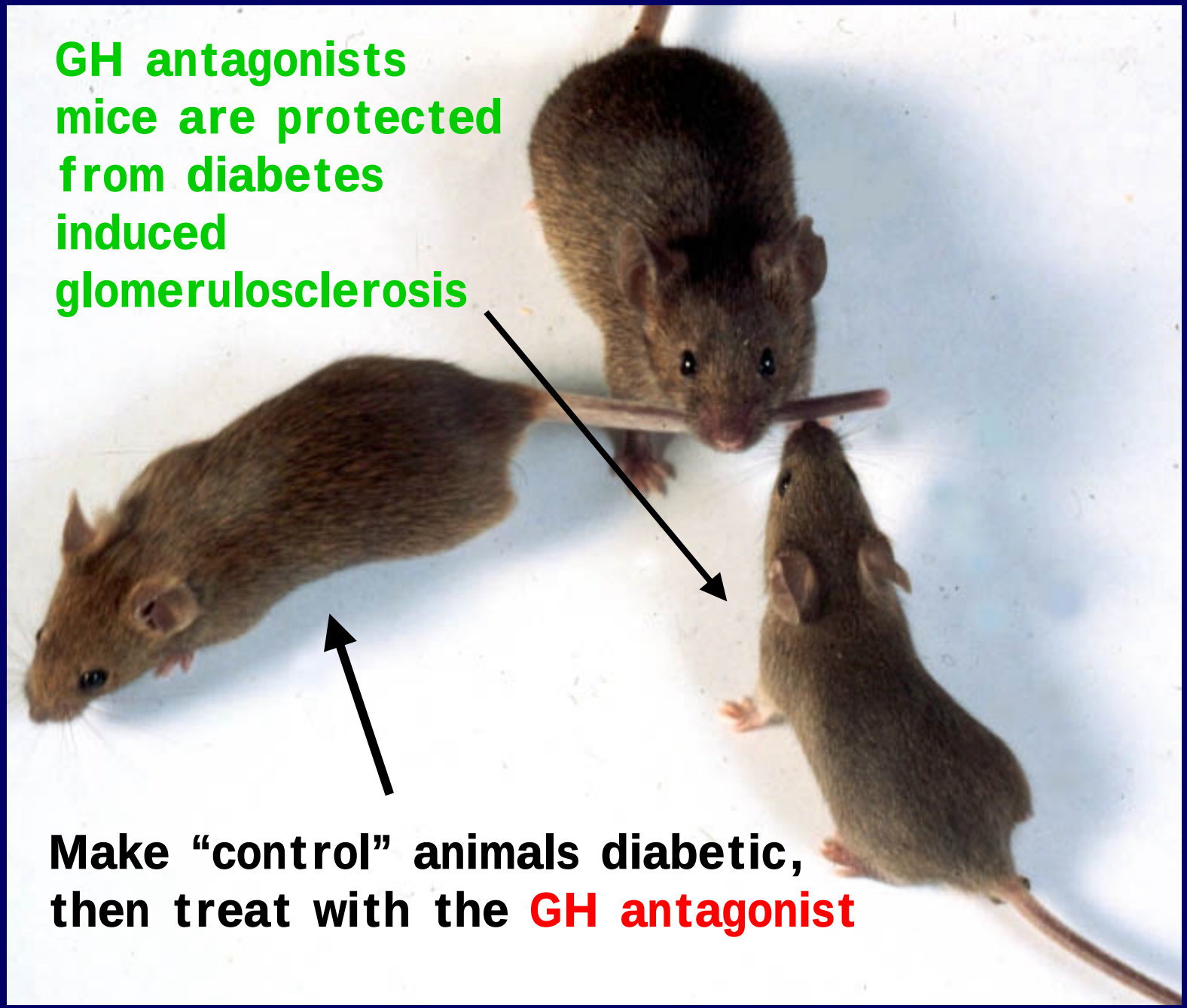


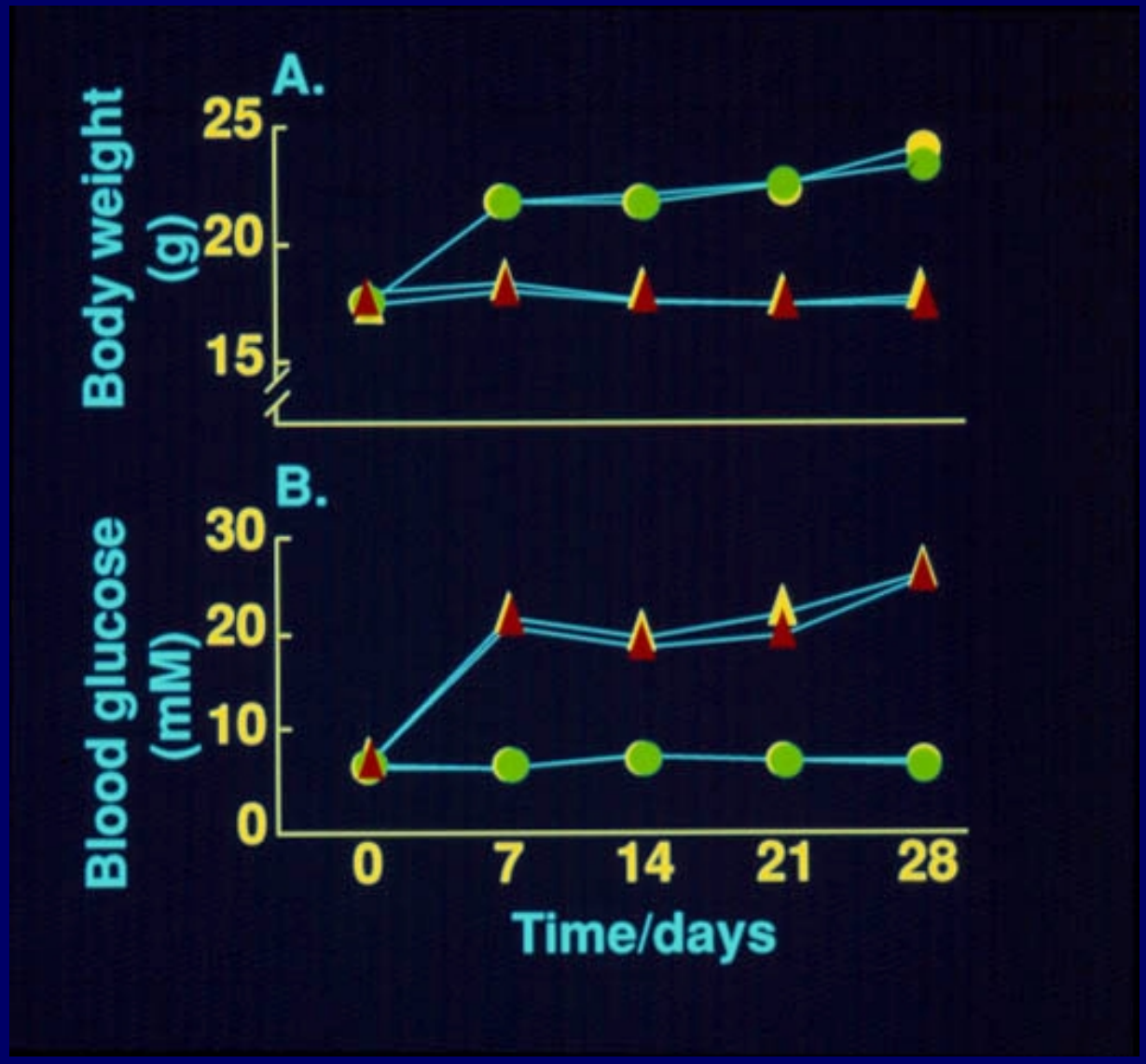
Dwarf Mice Are Protected From Diabetic Glomerulosclerosis

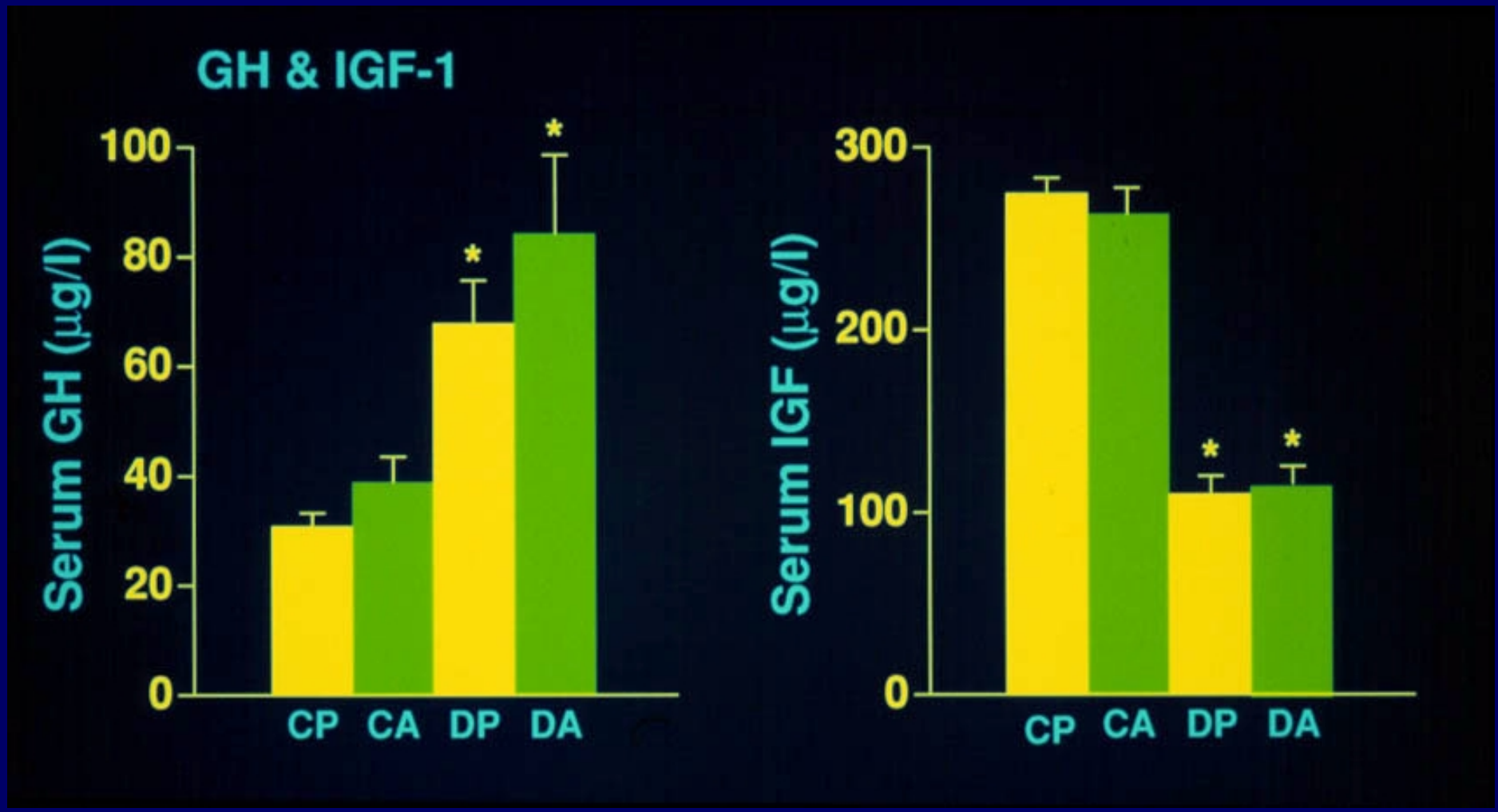


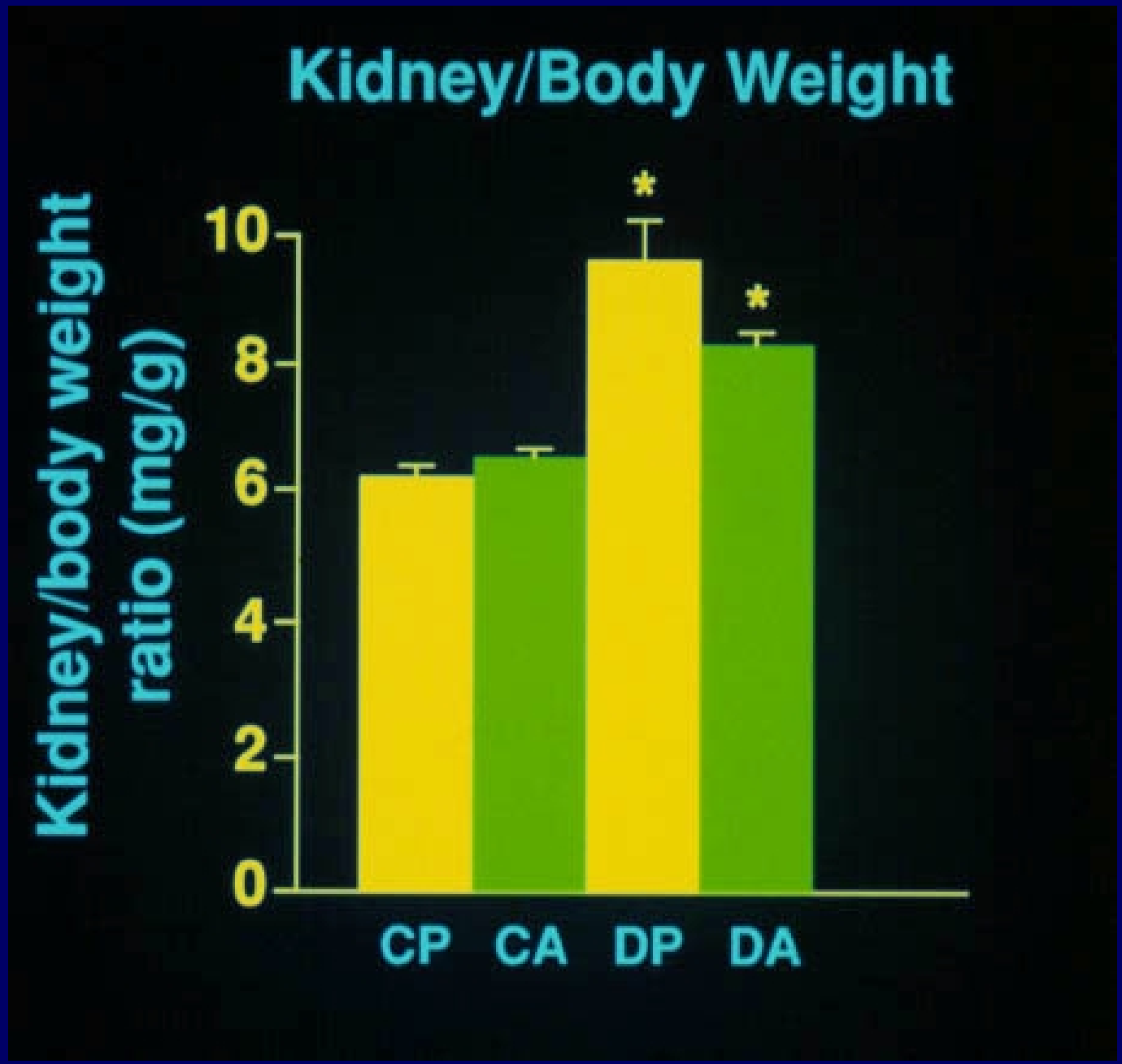
Transgenic Dwarf Mice Do Not Develop Diabetic Nephropathy

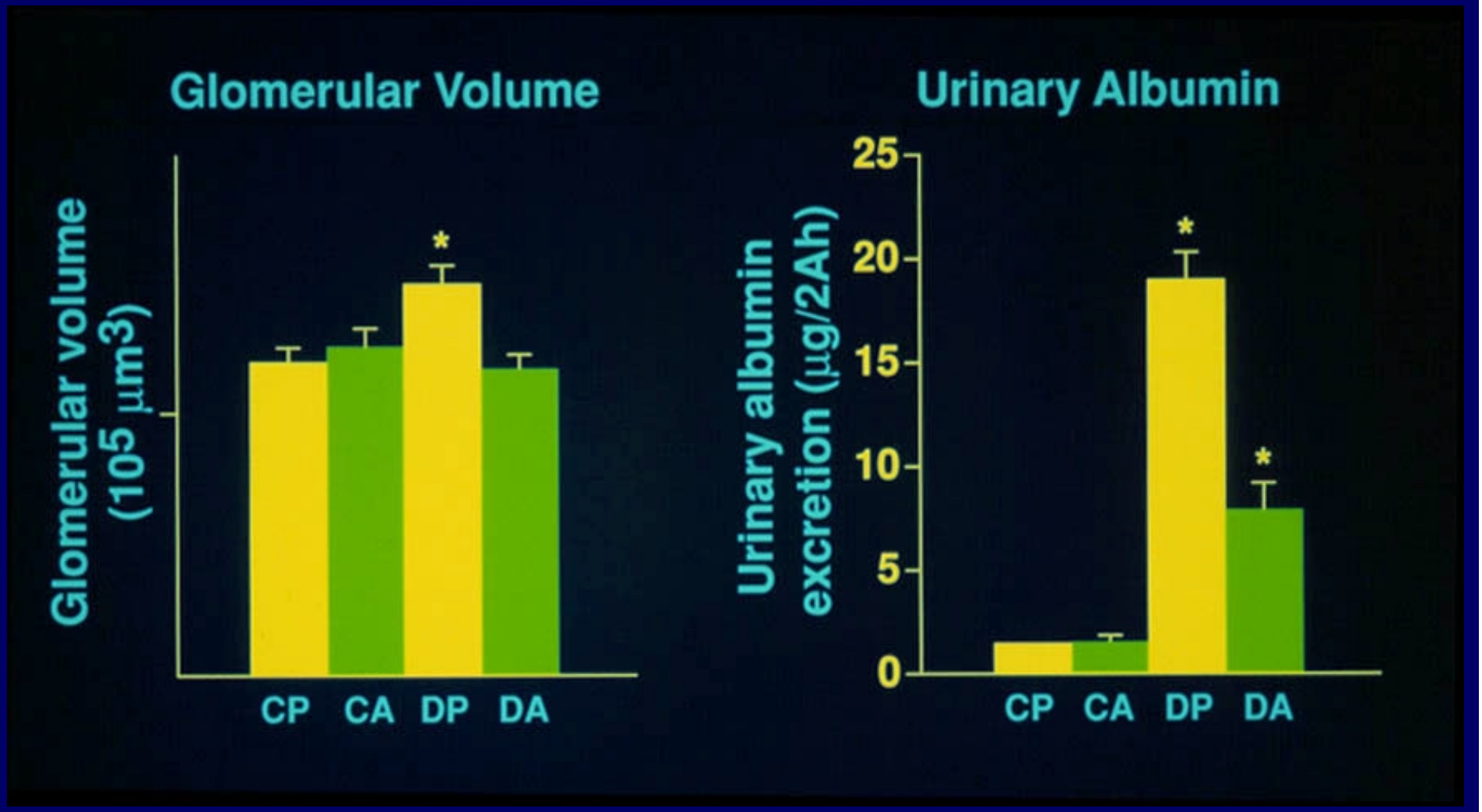



GH antagonists
mice are protected
from diabetes
induced
glomerulosclerosis
Make “control” animals diabetic,
then treat with the GH antagonist


A.
Body weight (g)
25
20
15
B.
Blood glucose (mM)
30
20
10
0
0
7
14
21
28
Time/days

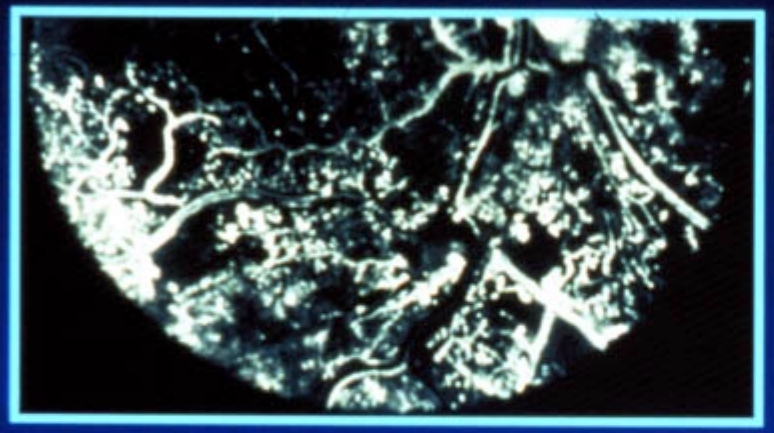

GH & IGF-1
Serum GH (µg/l)
100
80
60
40
20
0
CP CA DP DA
*
*
Serum IGF (µg/l)
300
200
100
0
CP CA DP DA
*
*

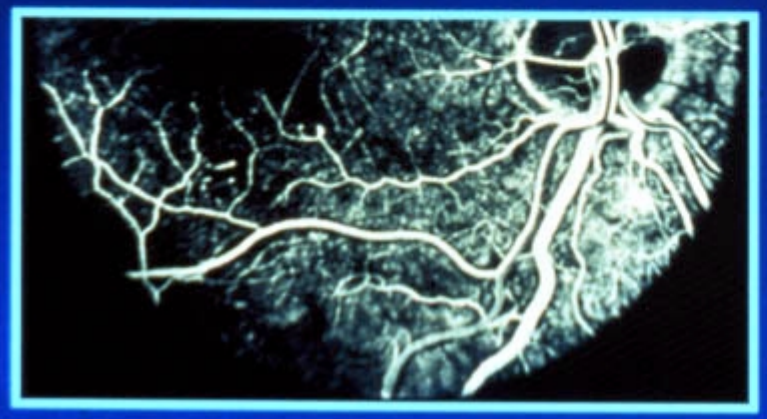

Kidney/Body Weight
Kidney/body weight ratio (mg/g)
10
8
6
4
2
0
*
*
CP
CA
DP
DA


Glomerular Volume
Glomerular volume (10^5 µm^3)
*
CP
CA
DP
DA
Urinary Albumin
Urinary albumin excretion (µg/2Ah)
25
20
15
10
5
0
*
*
CP
CA
DP
DA

Retinopathy - excess hGH action

sensus

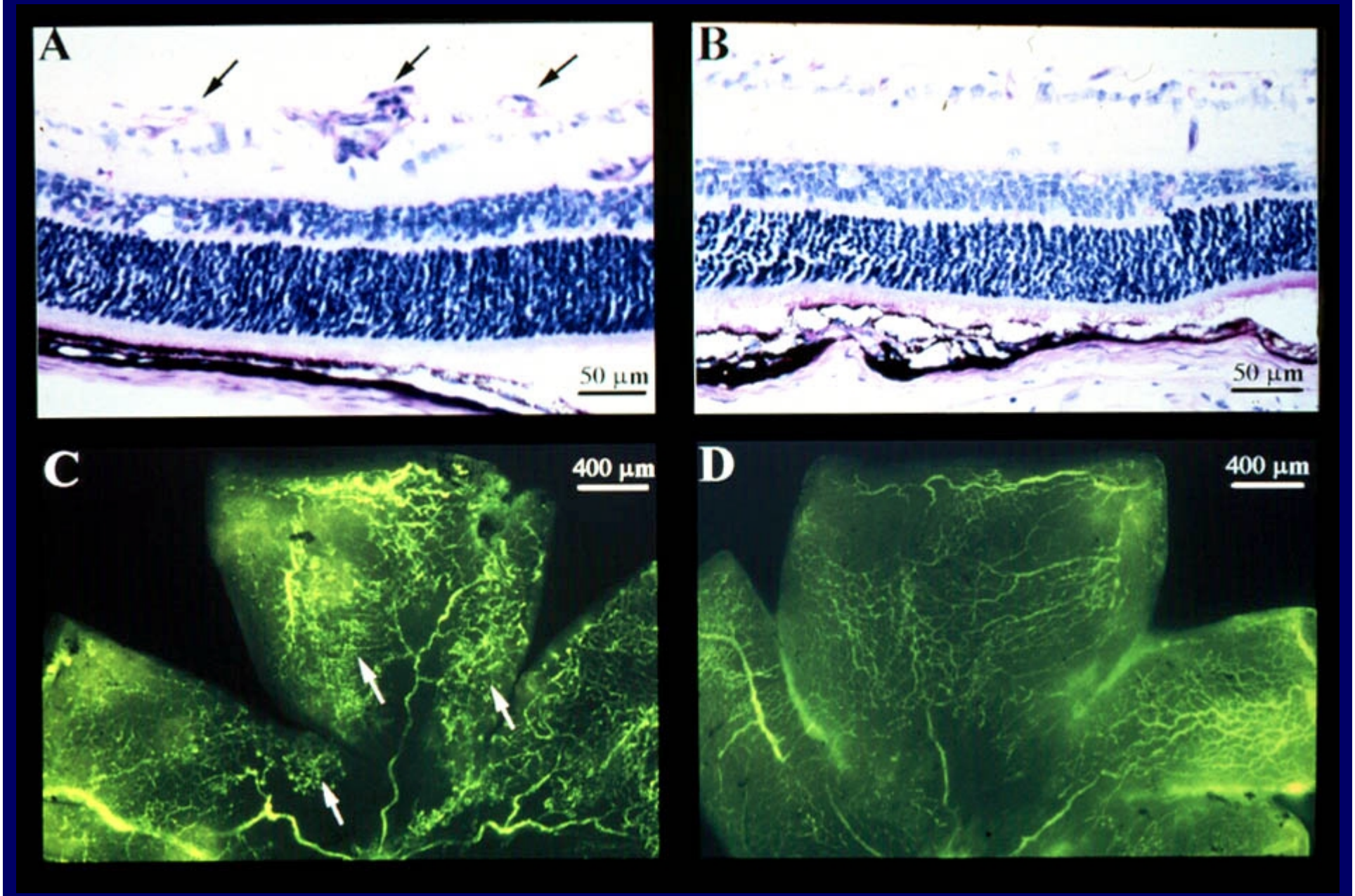




- Neovascularization, microaneurysms, fluid leakage, blindness
- Frequently associated with diabetes and prematurity (most common cause of blindness in U.S. and Europe)
- Laser photocoagulation only therapy
- Linkage to excess hGH?

GH and Retinal Neovascularization

- GH, GH antagonists, and controls
- Expose 7 day old mice to ***75% oxygen***
  - ***induces*** retinal vaso-obliteration
  - return to room air at day 12
  - check for retinal neovascularization through day 17
  - Extensive neovascularization occurs in **100%** of wild type mice


A
50 µm
B
50 µm
C
400 µm
D
400 µm

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USDA

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