About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

The role of oligosaccharides on structure and function of glycoprotein hormones: developing of agonists and antagonists

Prof. Fuad Fares

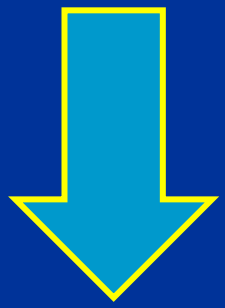
University of Haifa

Proteomics

Chicago

August 6th 2014

Structure-Function studies
Using site-directed mutagenesis
and gene transfer



Development of new analogs

Therapeutical Recombinant proteins

- 1978 Human Growth Hormone
- 1979 Human Insulin

The Problem

Most therapeutic proteins are <30 kD and hence:

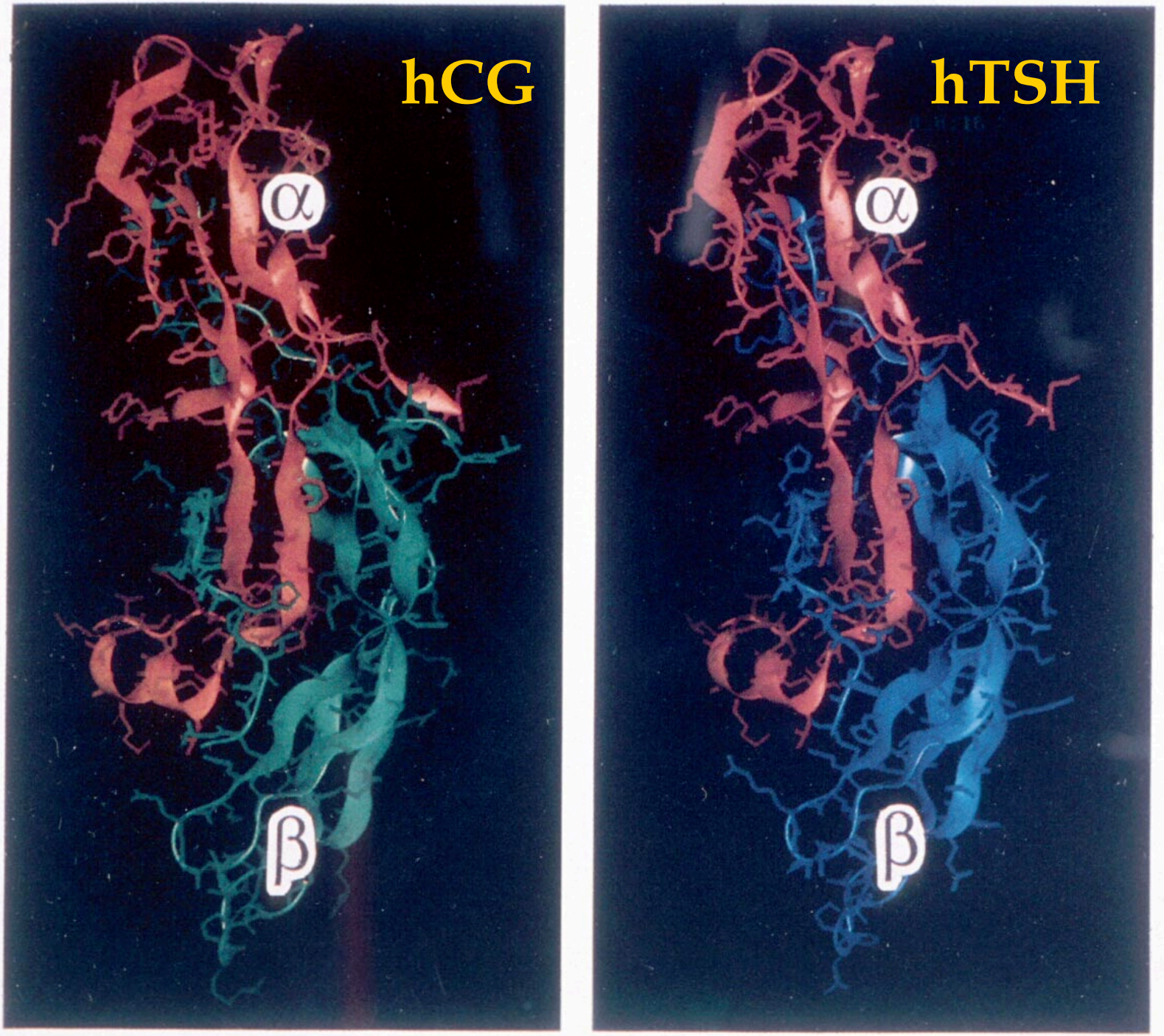
- Are filtered out quickly by the kidneys
- Are taken up by the liver and cleaved enzymatically
- Have to be injected frequently for optimal therapy
- Cause adverse effects due to peak dose injection

Success of Long-Lasting Proteins

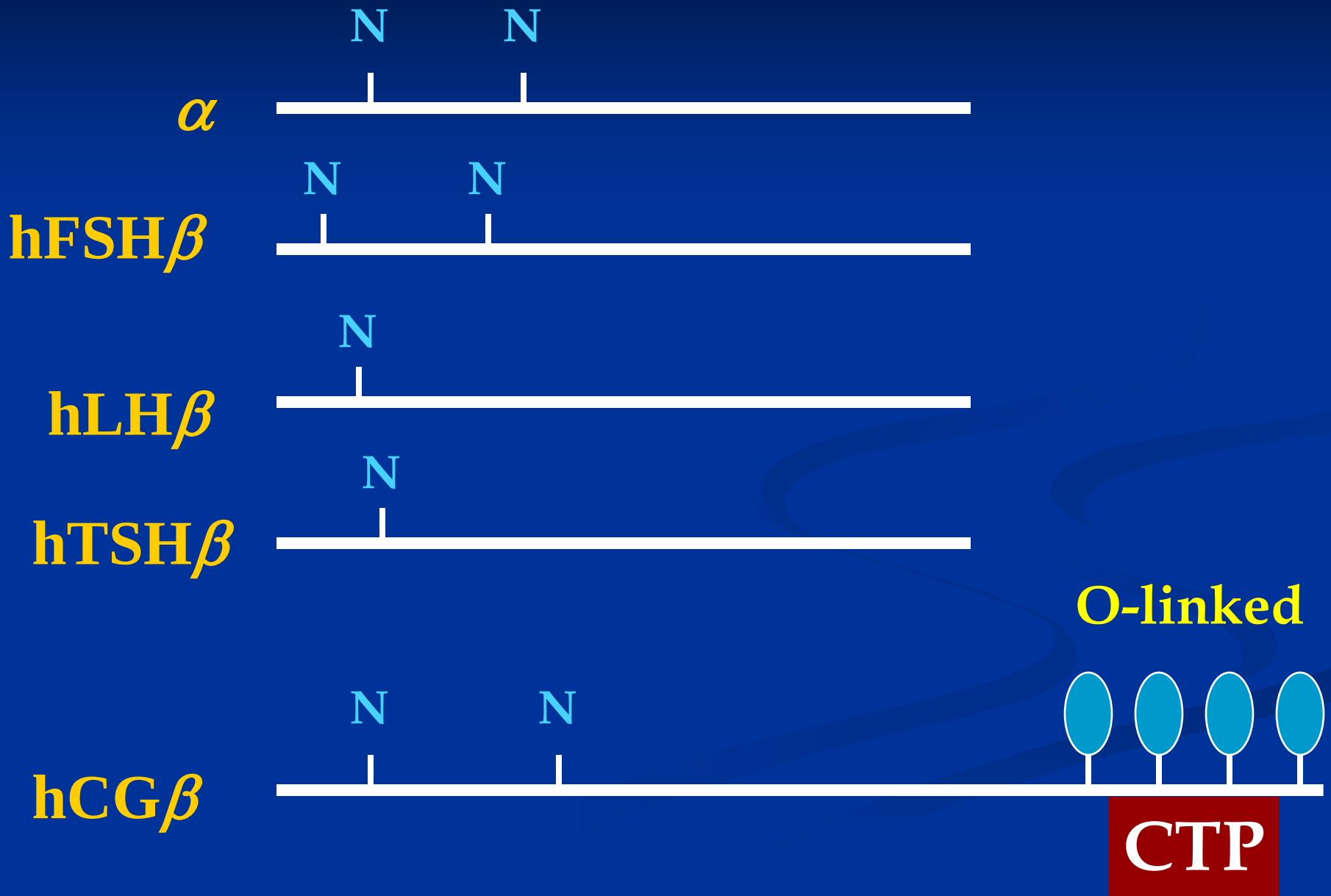
- PEGylation - Interferon α (SGP/Roche)
  - PEGIntron/Pegasys
  - $3.2 billion in sales in 2006
- PEGylation - GCSF (Amgen)
  - Neulasta
  - $2.5 billion in sales in 2006
- Hyper Glycosylation - EPO (Amgen)
  - Aranesp (DNA Modifications)
  - $3.9 billion in sales in 2006

Structure-Function of Glycoprotein Hormones

- FSH - Human Stimulating Hormone
- LH - Luteinizing Hormone
- hCG - Human Chorionic Gonadotropin
- TSH - Thyrotropin Hormone


hCG
α
β
hTSH
α
β

Glycoprotein Hormone Subunits



The Technology was Created By Nature During Evolution - the CTP "cassette"

Two fertility hormones

A

hCG – maintains pregnancy and requires long $T_{1/2}$

B

hLH – stimulates ovulation on a pulse mode requiring a short $T_{1/2}$

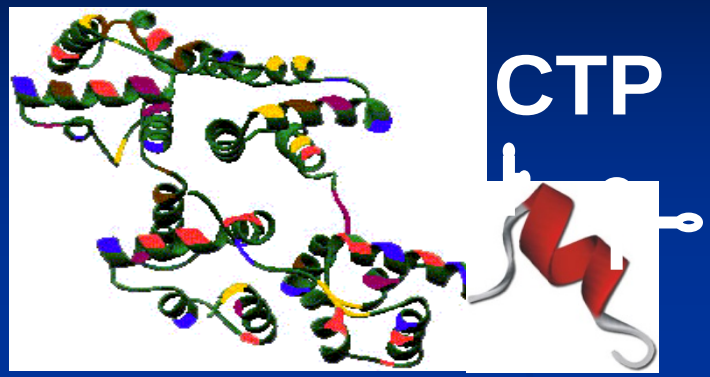
C

Amino acid sequence of hCG & hLH is almost identical

D

The 28 amino acid C-terminal peptide (CTP) of hCG with its 4 O-glycans does not exist in hLH

E

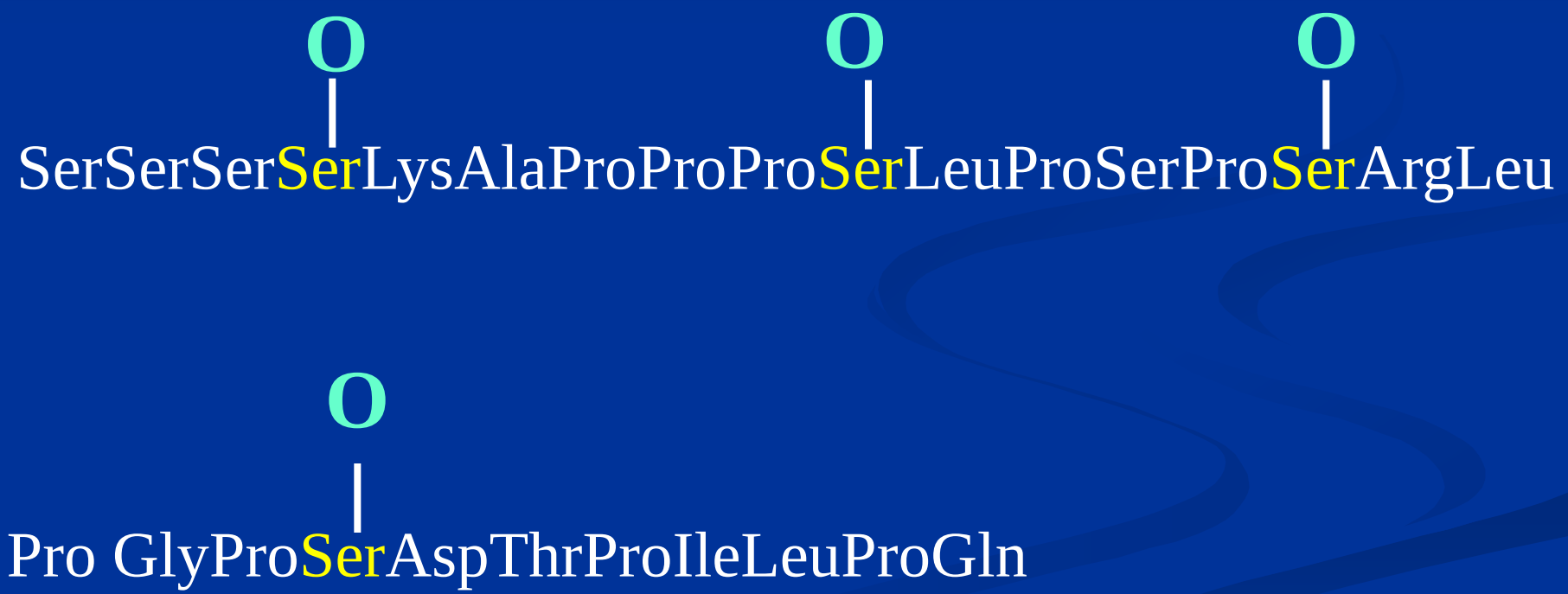


hCG

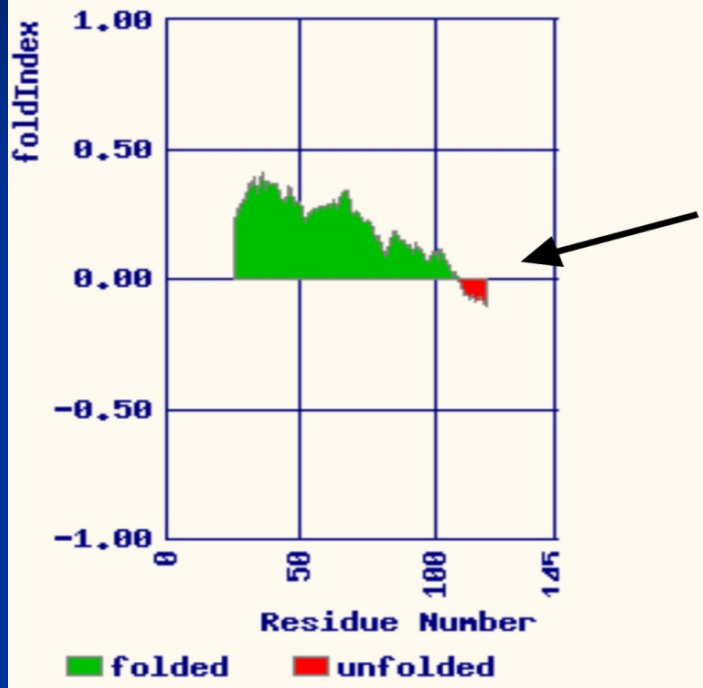
hLH

$T_{1/2}$ of hCG is ~5 times longer than $T_{1/2}$ of LH

CTP


O
O
O
SerSerSerSerLysAlaProProProSerLeuProSerProSerArgLeu
O
Pro GlyProSerAspThrProIleLeuProGln

Prediction of Folded and Unfolded Region of human chorionic gonadotropin (HCG) - chain B



CTP amino acid sequence is predicted to be unfolded

Summary:
Number Disordered Regions: 1
Longest Disordered Region: 37
Number Disordered Residues: 37
Predicted disorder segment: [109]-[145] lenght: 37 score: -0.06 ± 0.03

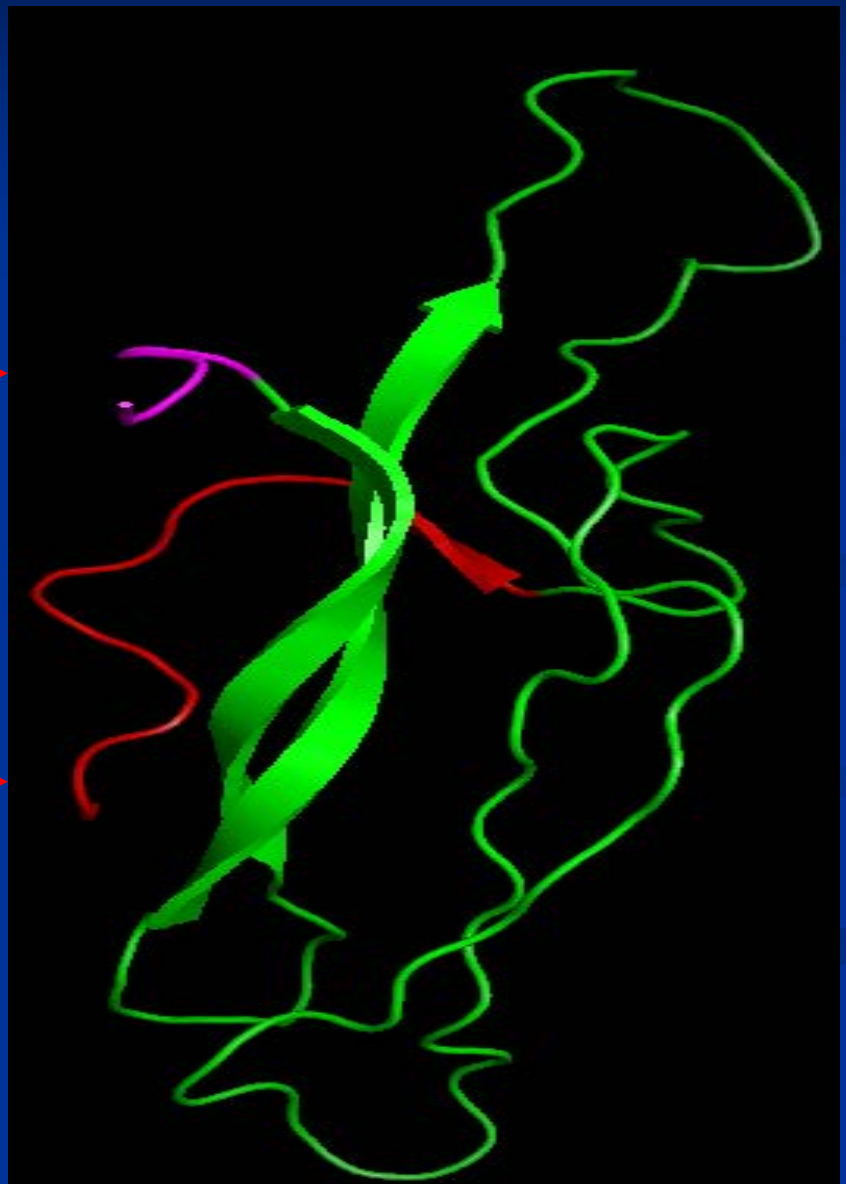
```
  1 SKEPLRPRCR PINATLAVEK EGCPVCITVN TTICAGYCPT MTRVLQGVLP
 51 ALPQVVCNYR DVRFESIRLP GCPRGVNPVV SYAVALSCQC ALCRRSTTDC
101 GGPKDHPLTC DDPRFQDSSS SKAPPPSLPS PSRLPGPSDT PILPQ
```

(Predicted disordered segment)

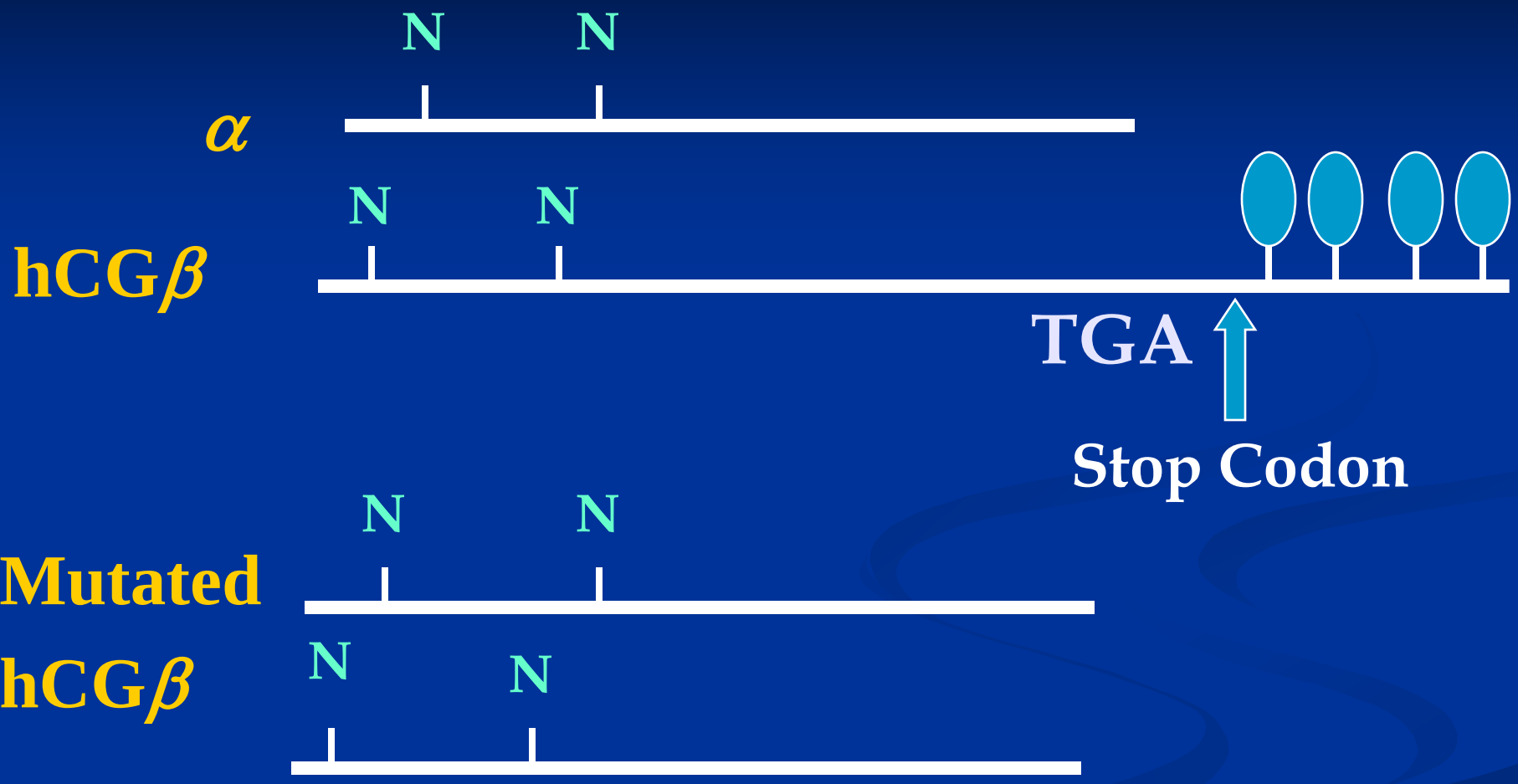
Crystal Structure of hGCβ showing long C-term lacking CTP

***N*-terminal**

CTP not seen in structure



The Role of CTP



Deletion of CTP from hCG

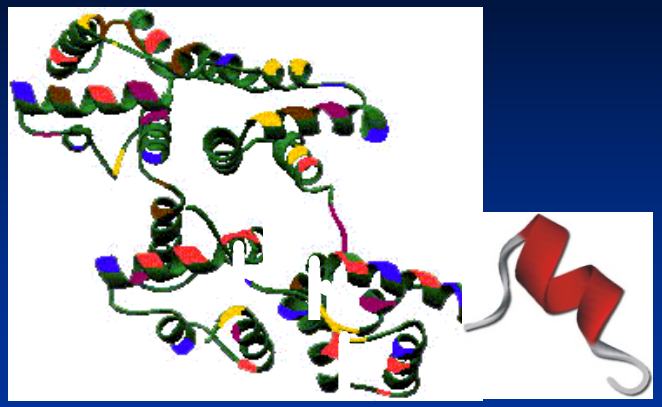
- No effect on the assembly of subunits

- No effect on receptor binding

- No effect on *in vitro* bioactivity

- Significantly decreased the bioactivity *in vivo*

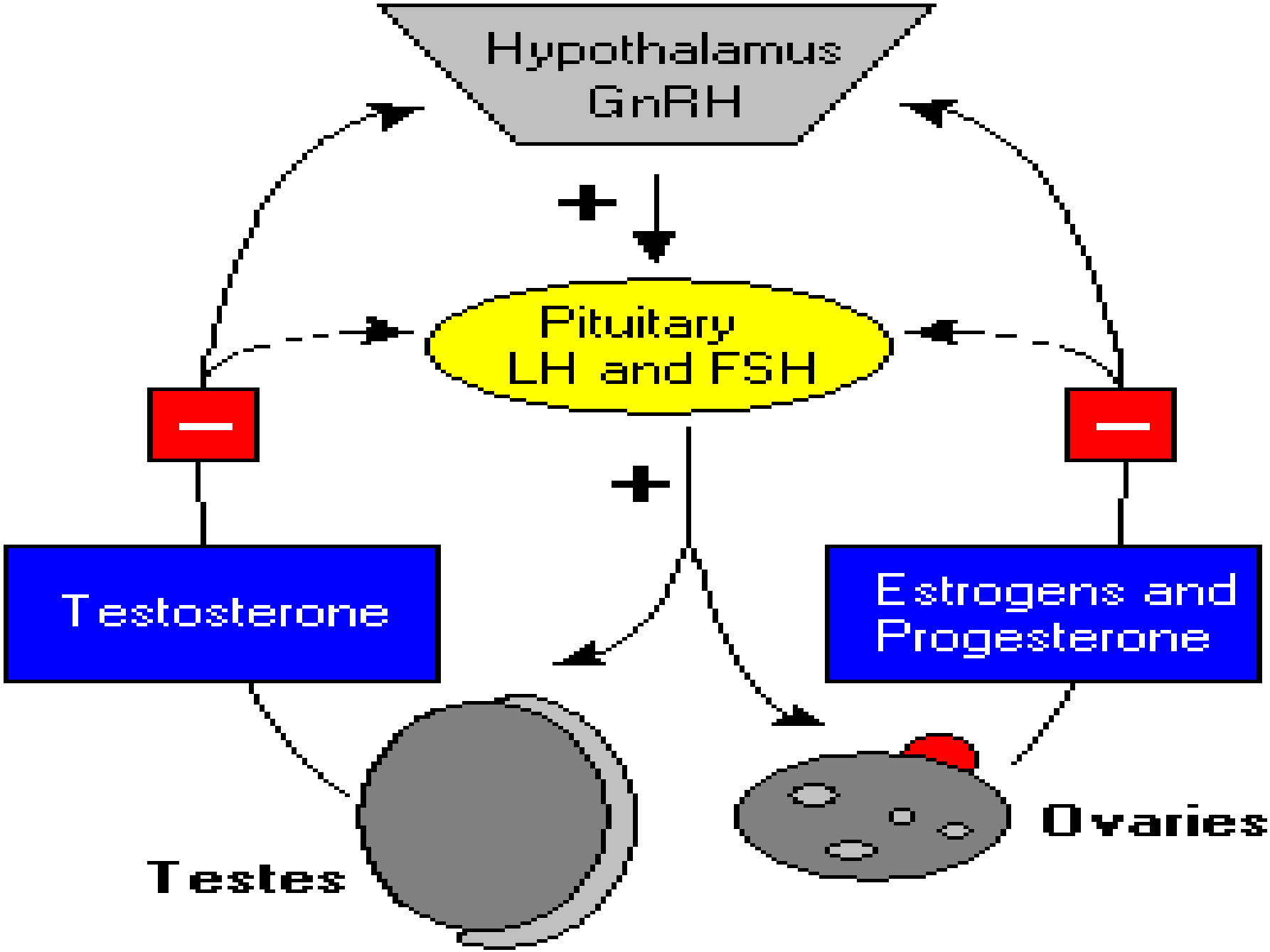
+

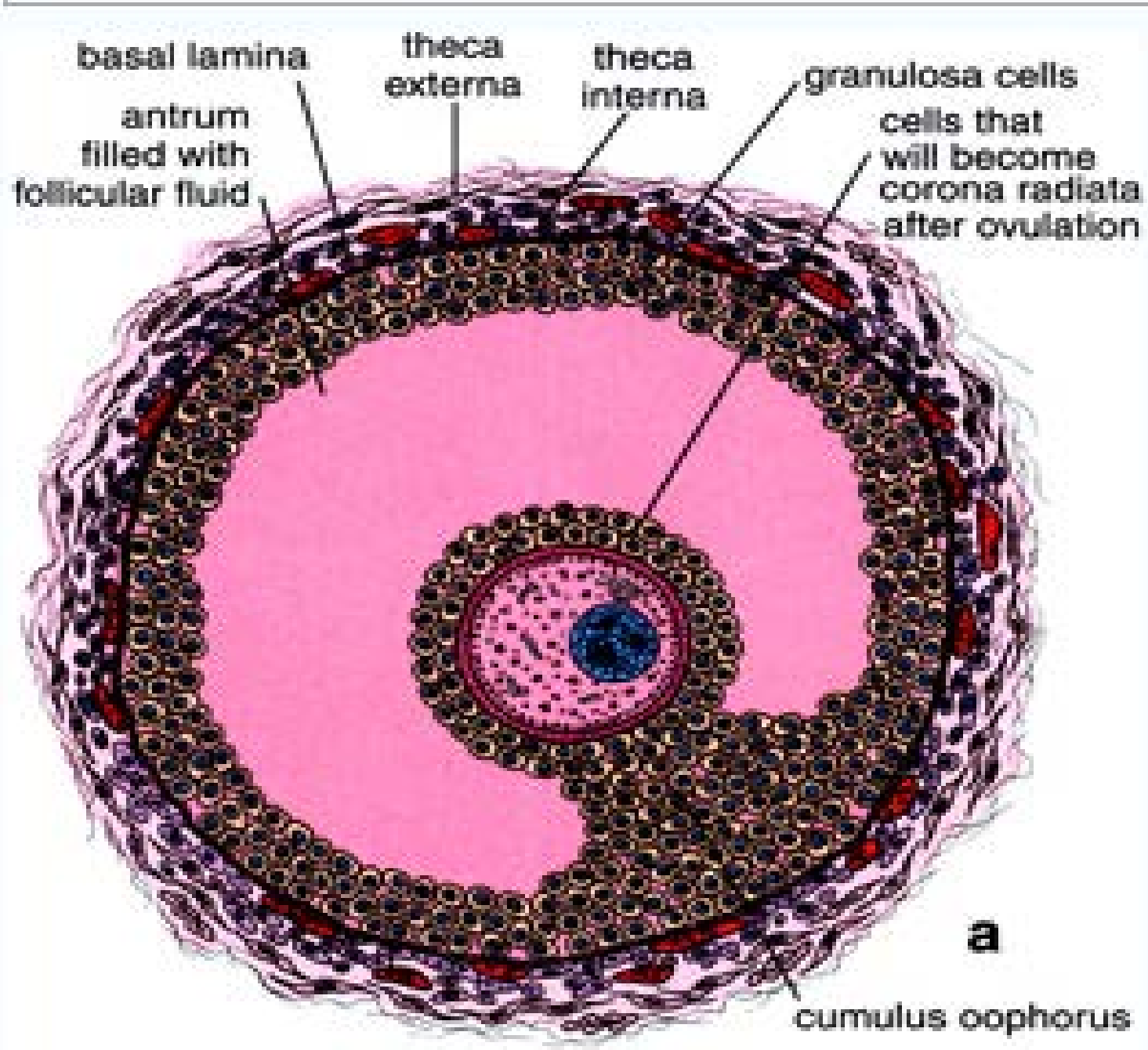


Protein CTP

Half- Life

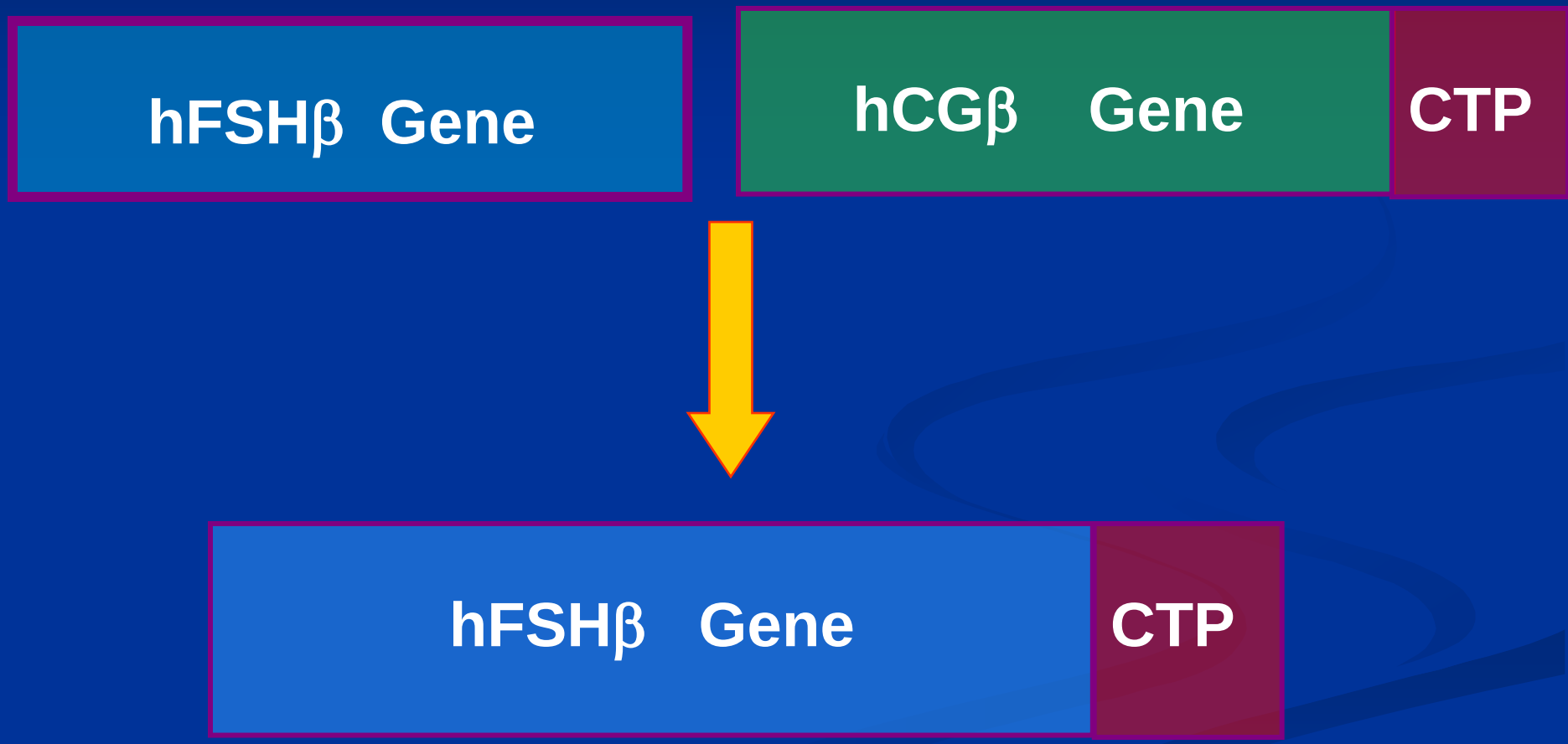
Designing New FSH Analog


Hypothalamus
GnRH
+
Pituitary
LH and FSH
−
−
+
Testosterone
Estrogens and
Progesterone
Testes
Ovaries

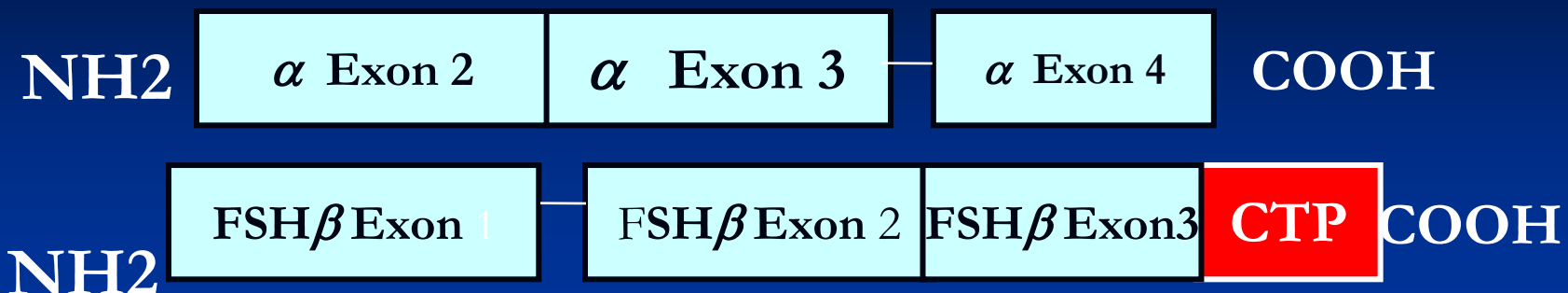


MATURE GRAAFIAN FOLLICLE

Designing New FSH Analog



hFSH - CTP



1- Assembly of the subunits

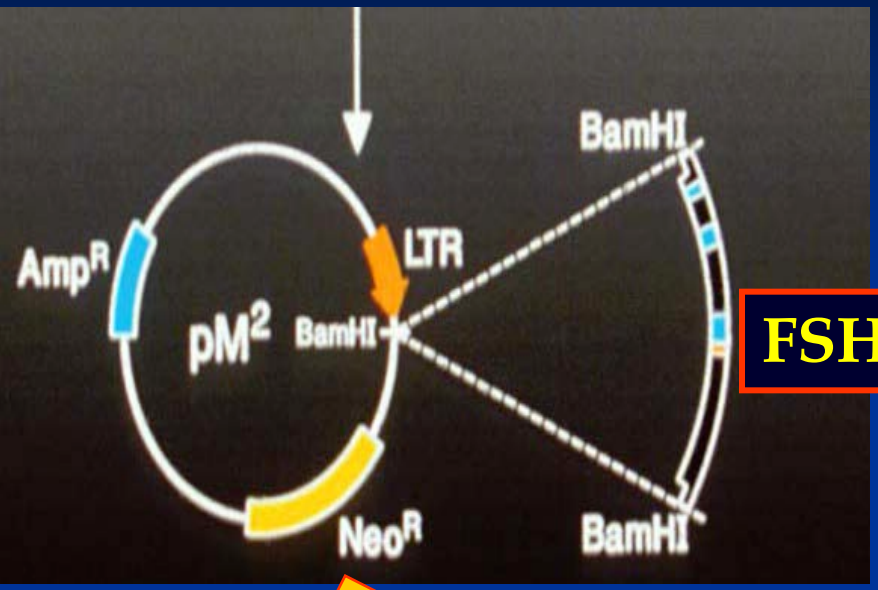
2- Binding to the receptor

3- *In vitro* Bioactivity

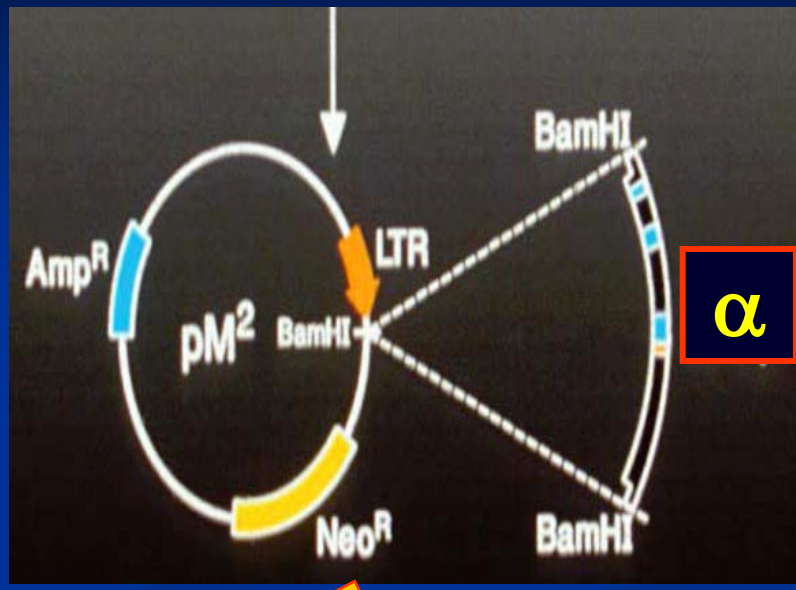
4- *In vivo* Bioactivity

5- Immunogenecity

Gene Expression



FSH β-CTP



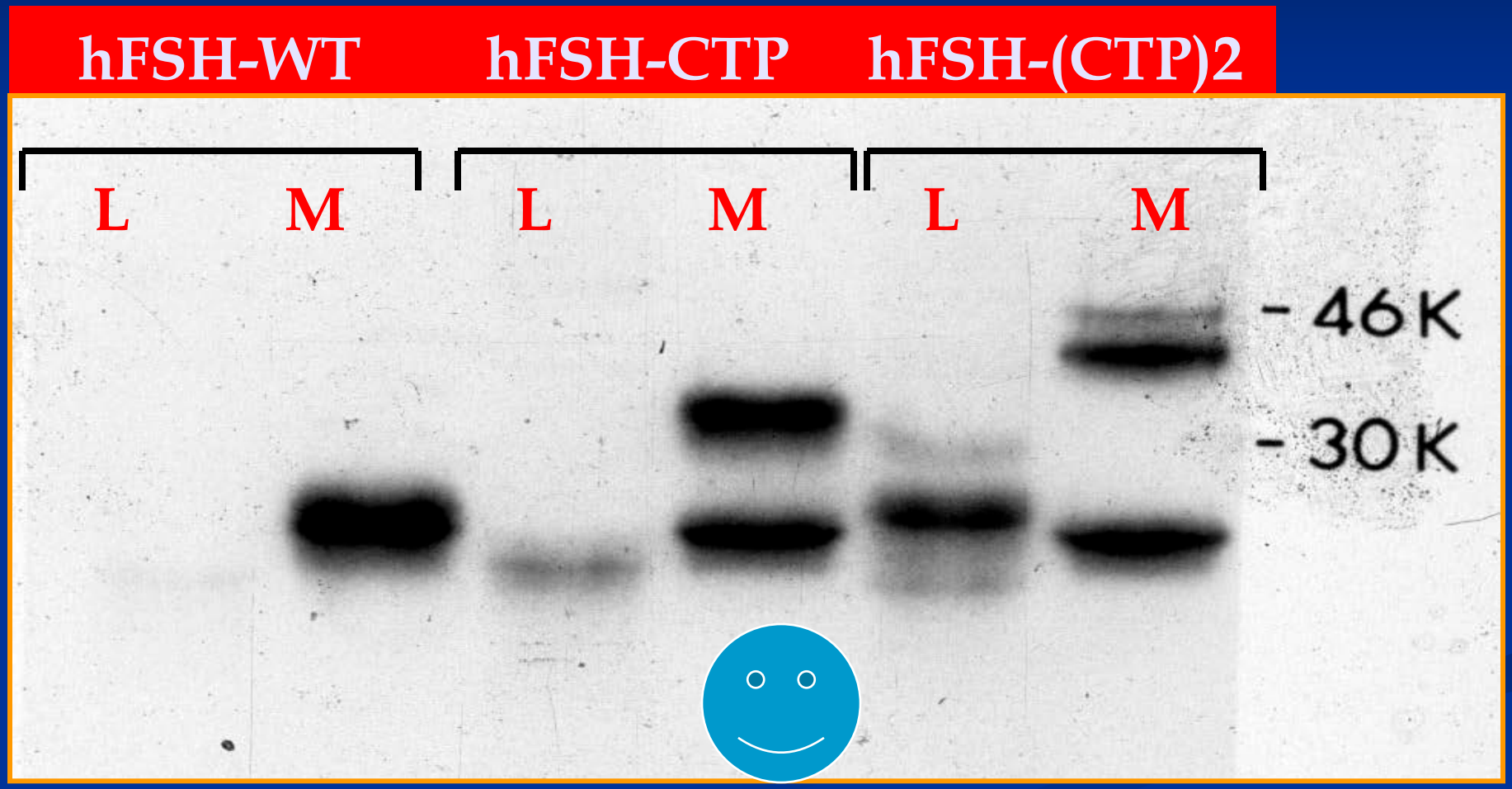
α

CHO

Transfection

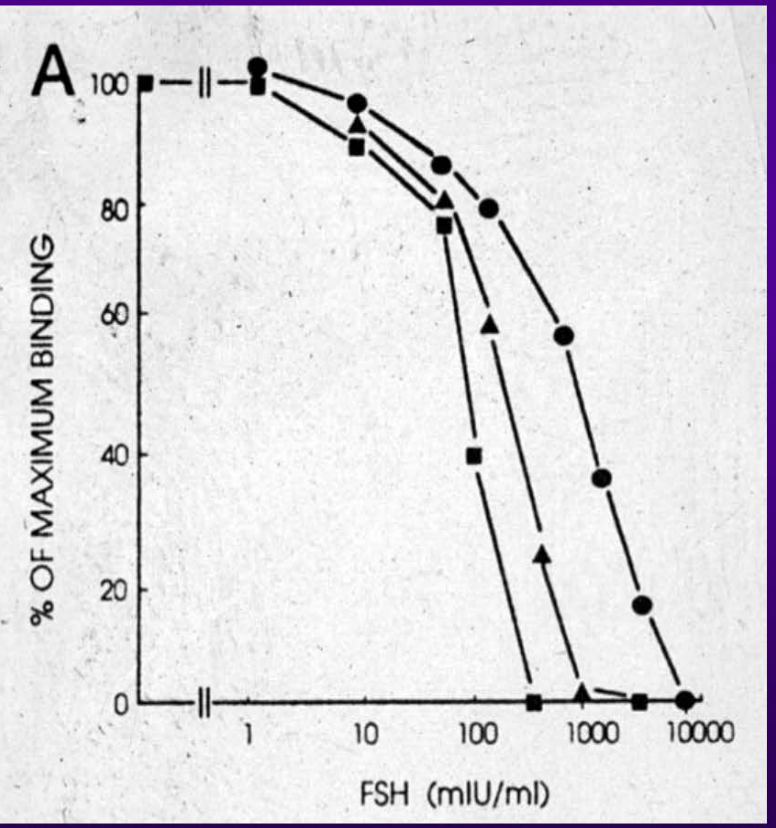
Stable Clone

Assembly of Subunits

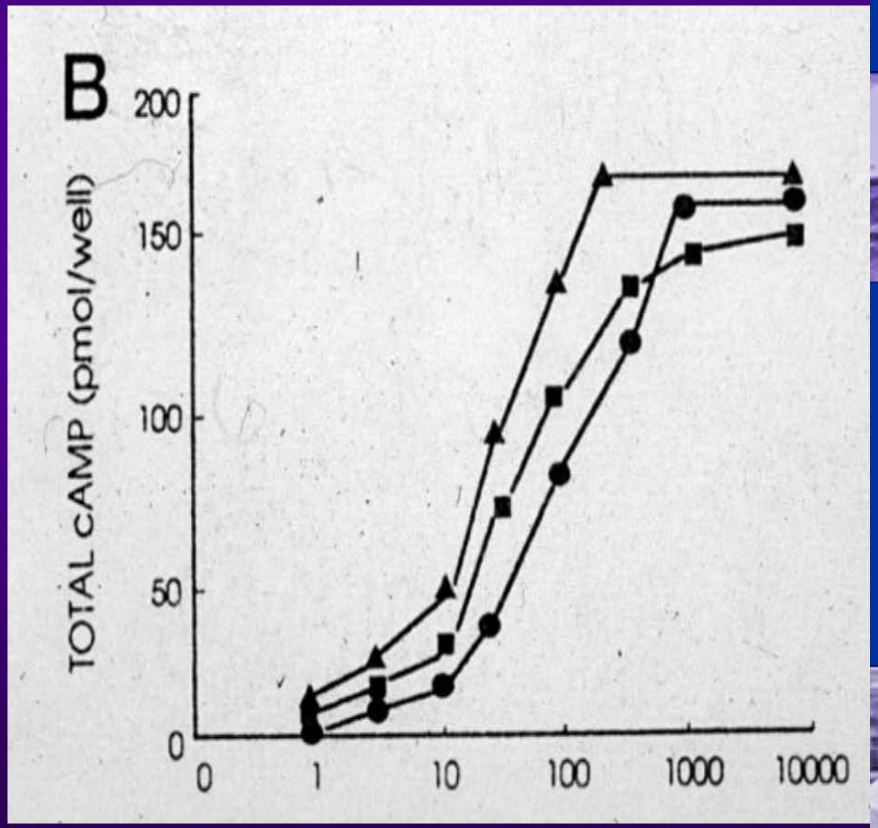


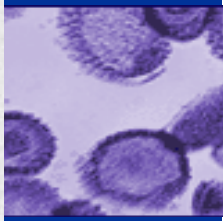
in vitro Studies

Receptor binding



Biological Activity

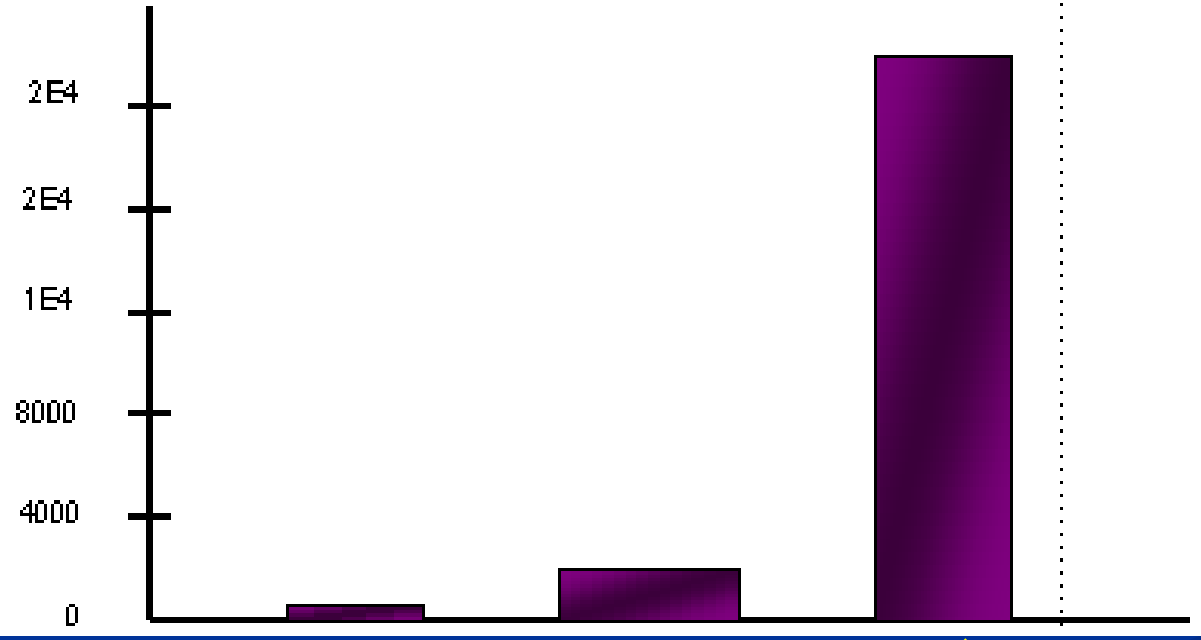


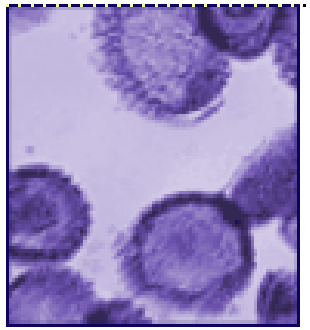


Biological Activity, *in vivo*

10 IU / 24h x 48h (IP)

Estrogen (pg/ml)





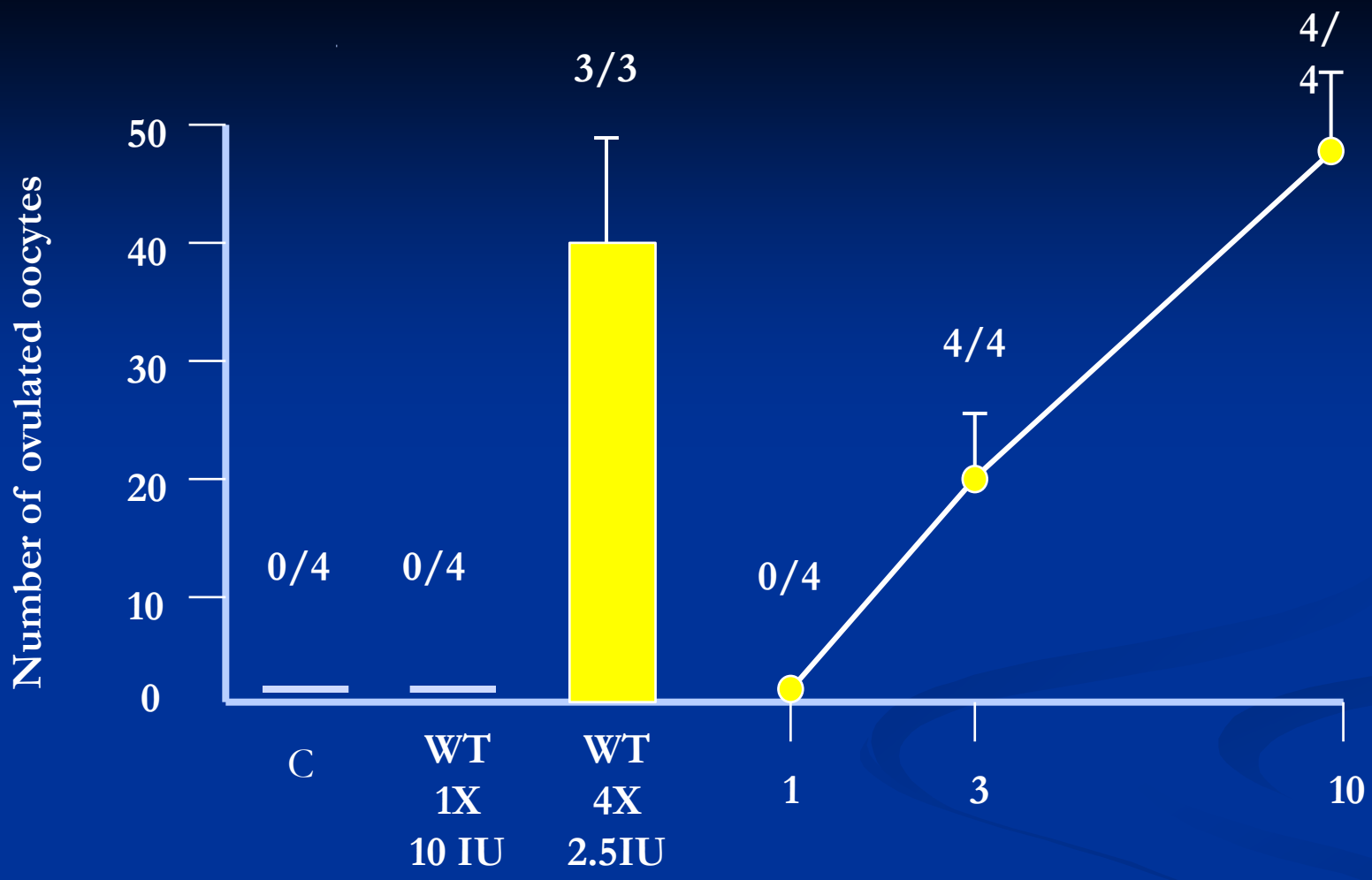
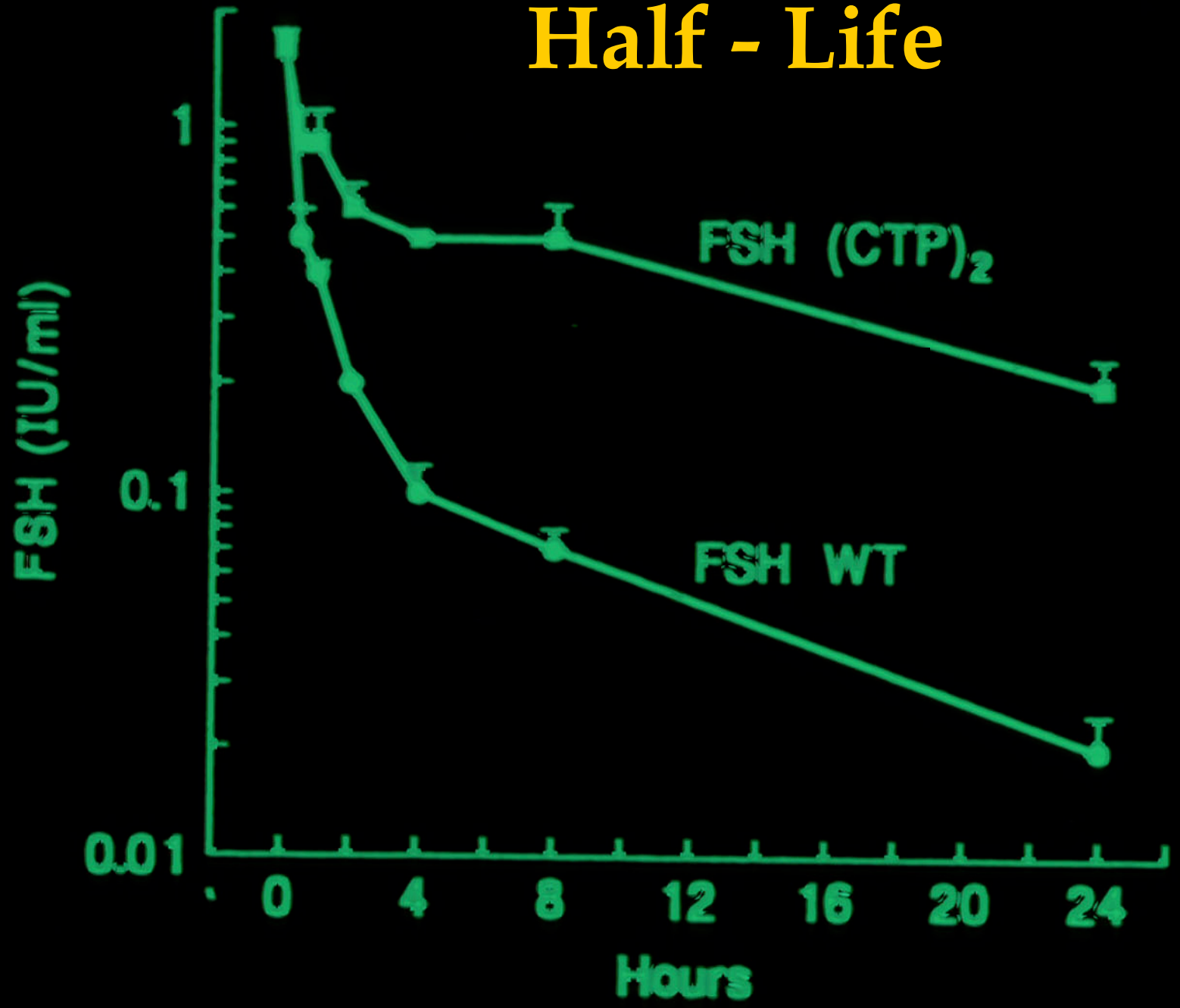
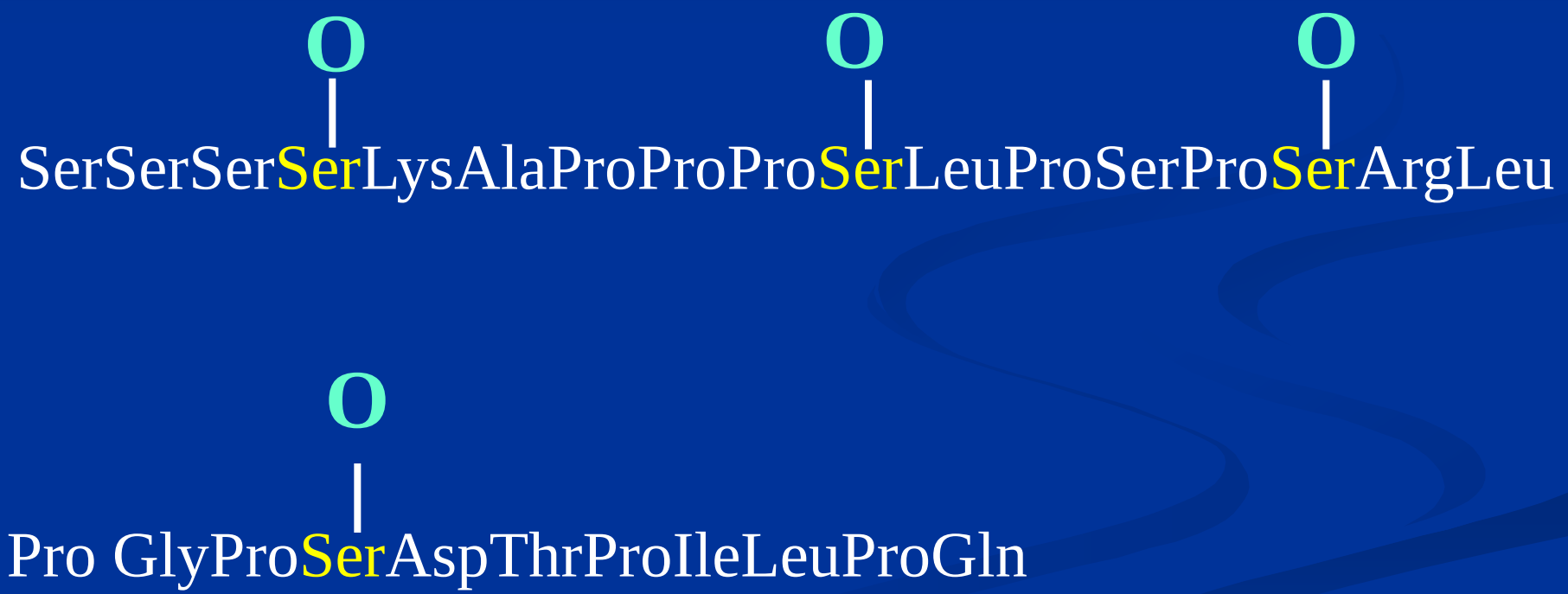


FIG. 4. Ability of a single ip injection of WT-FSH *vs.* FSH-CTP1 to increase ovulatory potential. Rats received a single ip injection of WT-FSH (1 × 10 IU) or 1, 3, or 10 IU FSH-CTP1, followed 52 h later by a high dose (5 μg) of hCG. The following morning, the oviducts were excised to count the numbers of ovulating ova. Some rats received four 2.5-IU injections at 12-h intervals before hCG (4 × 2.5 IU). Results are expressed as the mean number of ovulating oocytes per rat. The number of ovulating rats per total number of animals studied is presented as a ratio *above* each group. C, Controls.

Half - Life

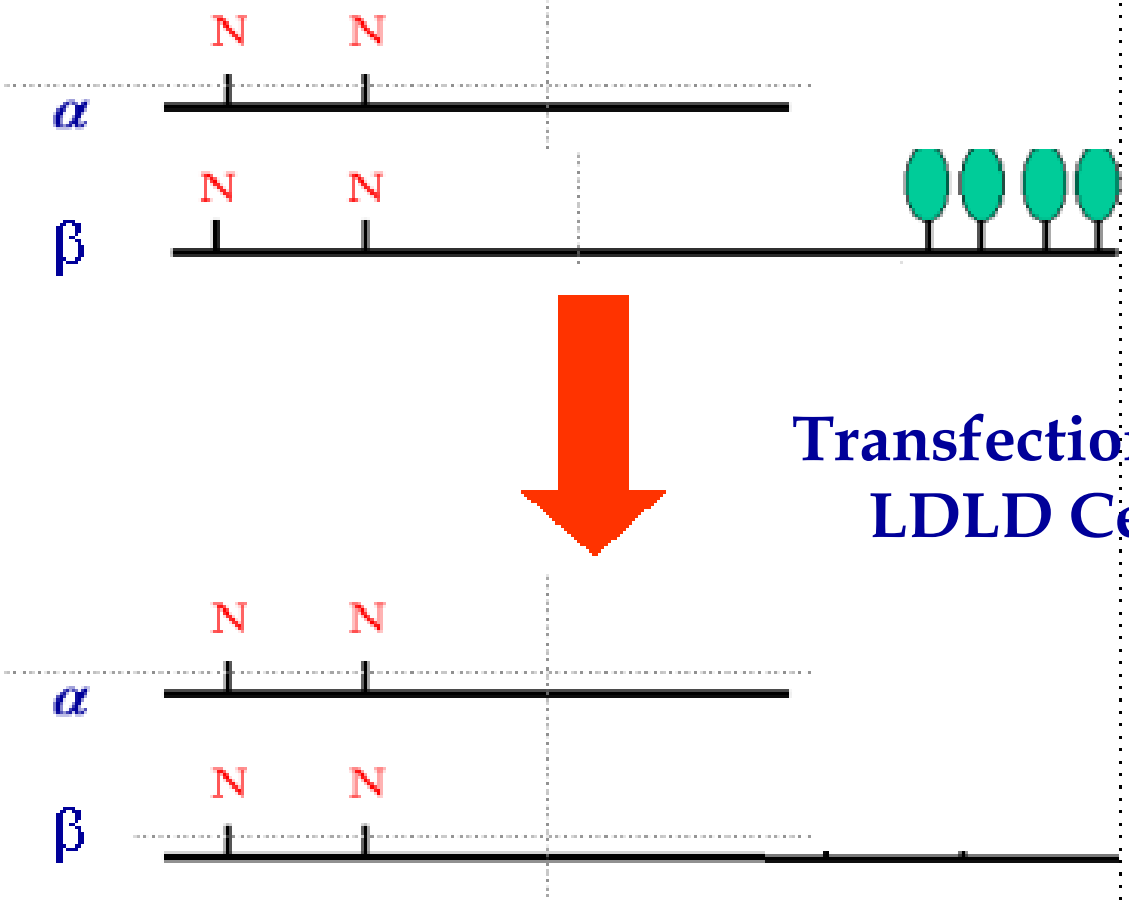


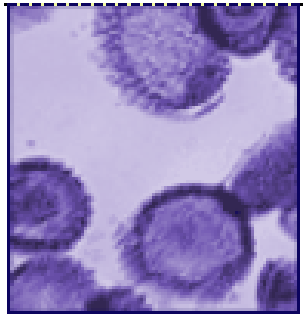
CTP


O
O
O
SerSerSerSerLysAlaProProProSerLeuProSerProSerArgLeu
O
Pro GlyProSerAspThrProIleLeuProGln

The role of O-linked Oligosaccharides

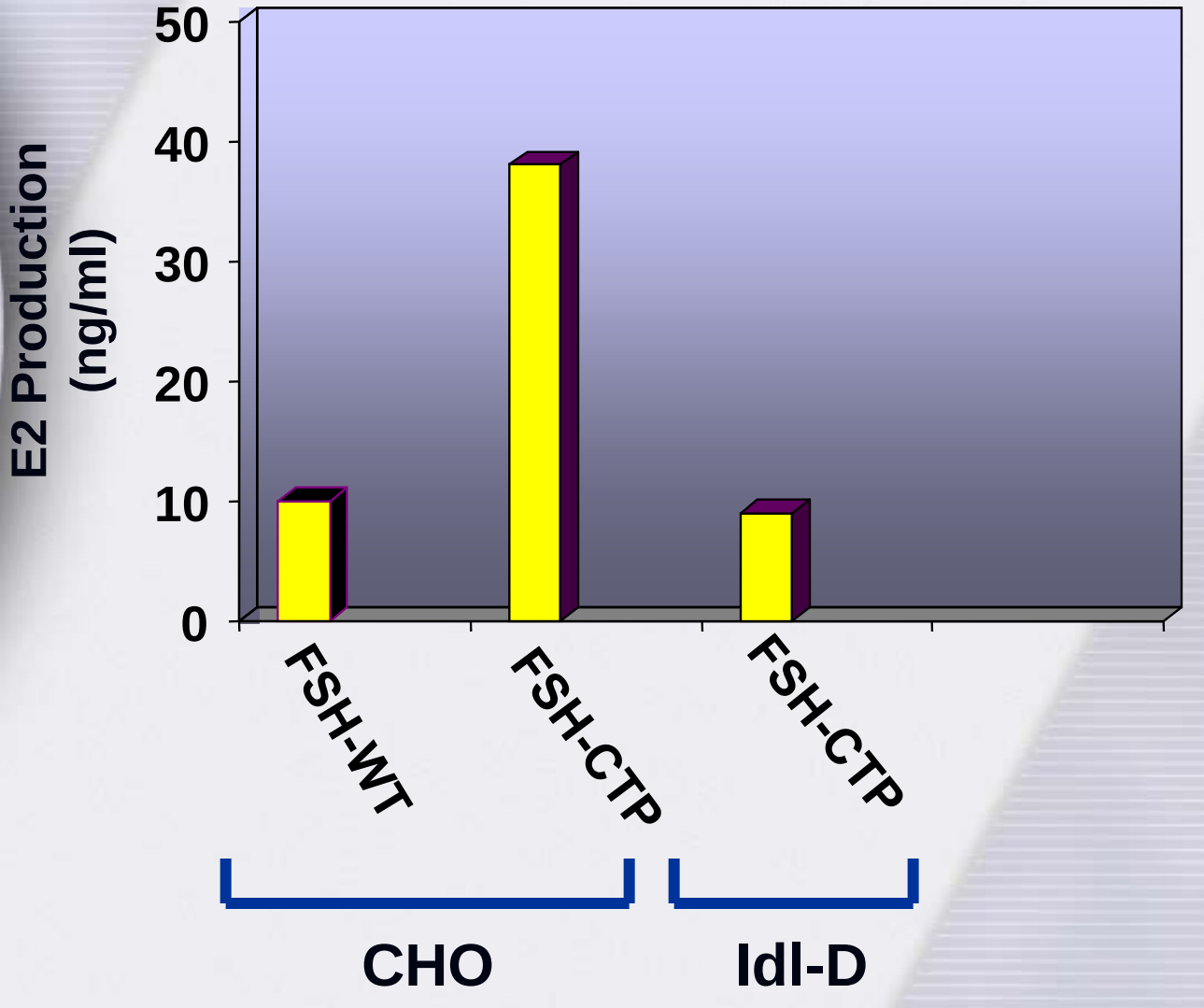
FSH -CTP





Transfection into LDLD Cells

Biological Activity



CTP


O
O
O
SerSerSerSerLysAlaProProProSerLeuProSerProSerArgLeu
O
Pro GlyProSerAspThrProIleLeuProGln

Human Studies

Organon - Merck

- FSH – CTP is effective in follicular stimulation
- FSH – CTP is safe
- FSH – CTP is not immunogenic

From Medscape Medical News > Alerts, Approvals and Safety Changes > International Approvals

EU Approves First Long-Acting Fertility Treatment

Yael Waknine

Authors and Disclosures

February 2, 2010 — The European Commission (EC) has approved ELONVA (FSH-CTP)

Merck Receives Positive Regulatory Opinion for European Marketing of Long-Acting CTP-Modified Fertility Treatment ELONVA

FSH – CTP

(ELONVA)

World – Wide Use

Start Up Company

CTP

"Enhancing the potency and longevity of highly valuable proteins"

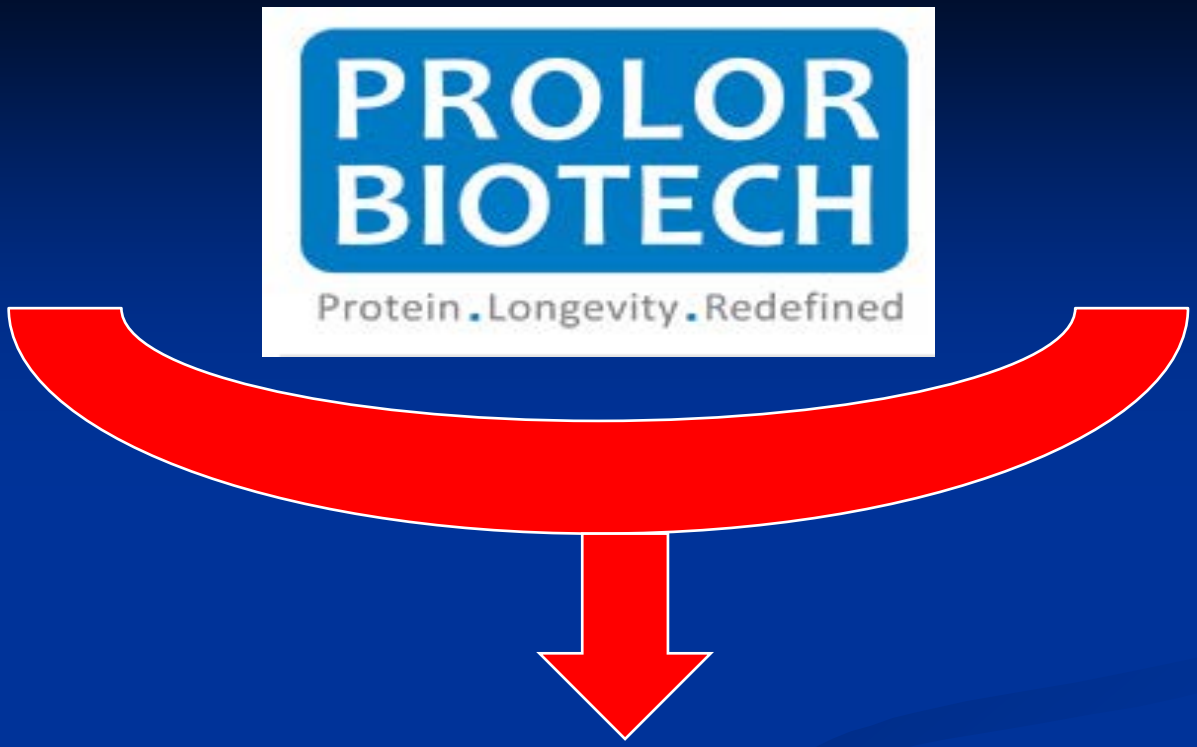
Start Up Company

PROLOR BIOTECH

Protein . Longevity . Redefined

Public Company

- NASDAQ, Stock Exchange, NY, USA.
- Tel-Aviv Stock Exchange, Tel-Aviv, Israel.



OPKO Health, Inc.

a multinational biopharmaceutical and diagnostics company

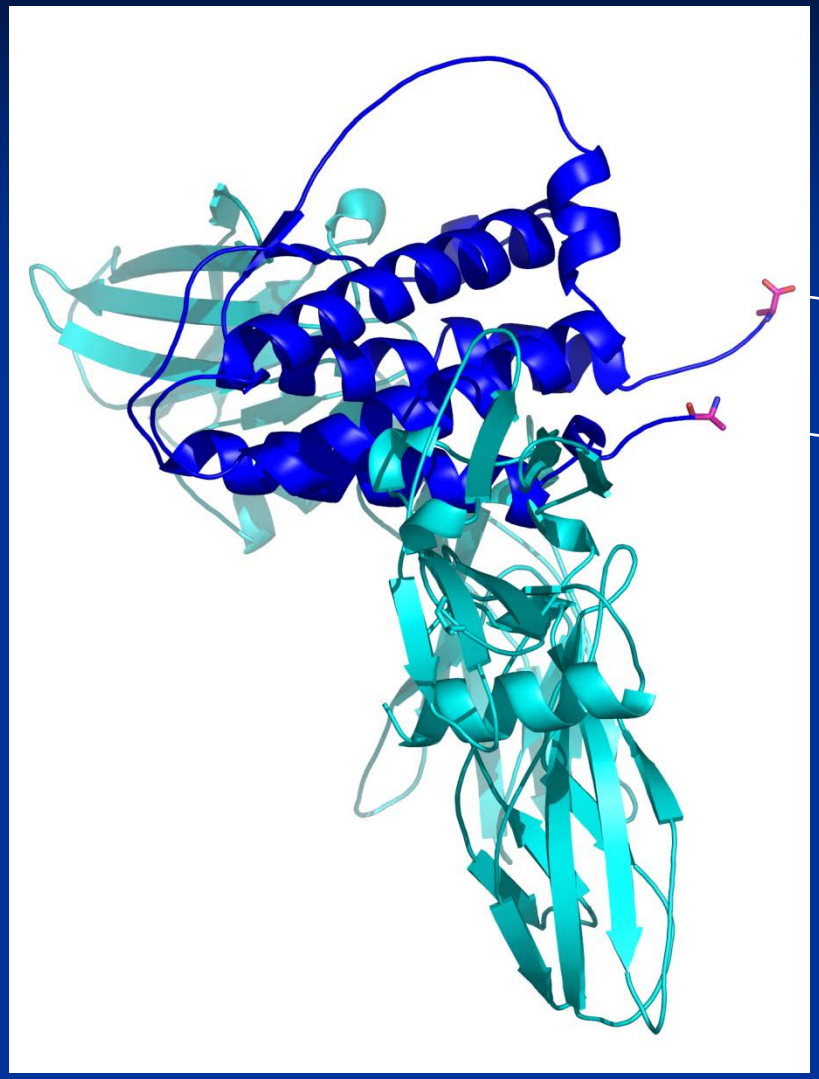
Designing Long Acting Proteins

- Erythropoietin
- Growth Hormone
- Interferon
- Factors, XI & VII
- Short Peptides

Erythropoietin (EPO)

The most common use is in people with anemia (low blood count) related to kidney dysfunction.

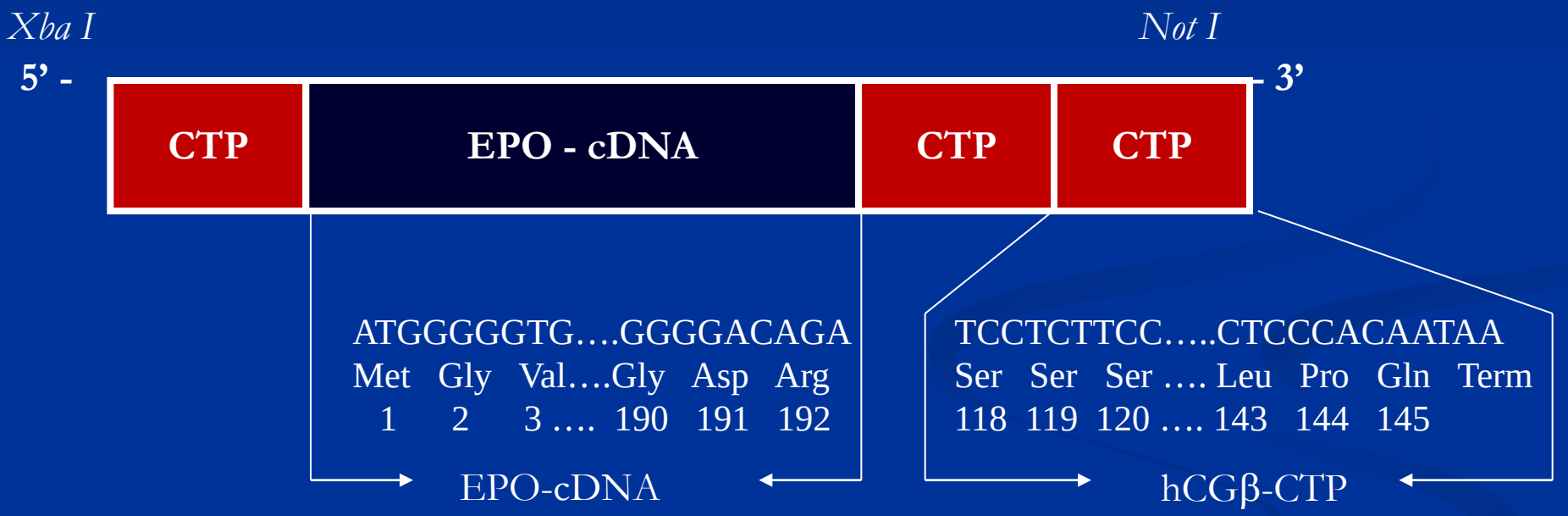
3 - D Structure Analysis



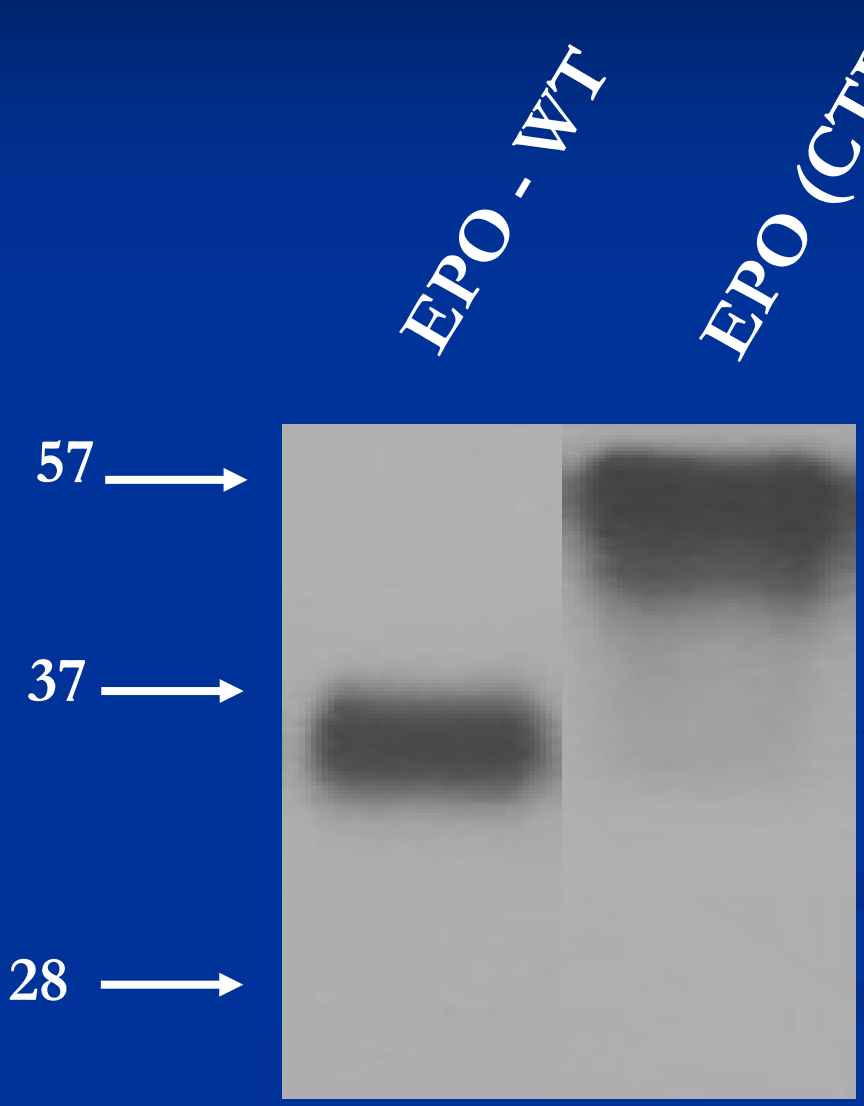
Human Erythropoietin α (blue) with its Receptors (cyan)

Conclusion:
Strands of both termini are fairly long and accessible.

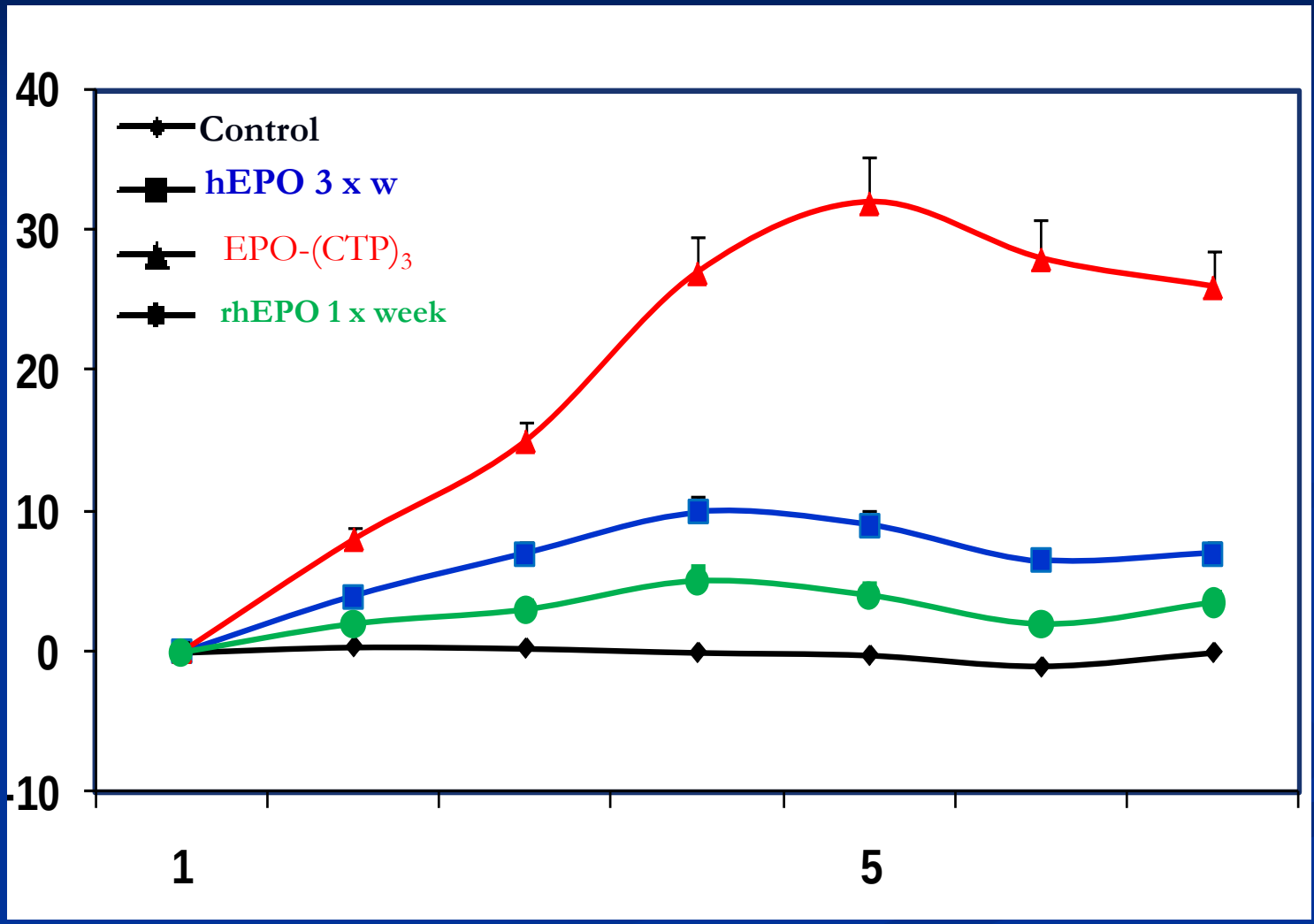
Human EPO-CTP3



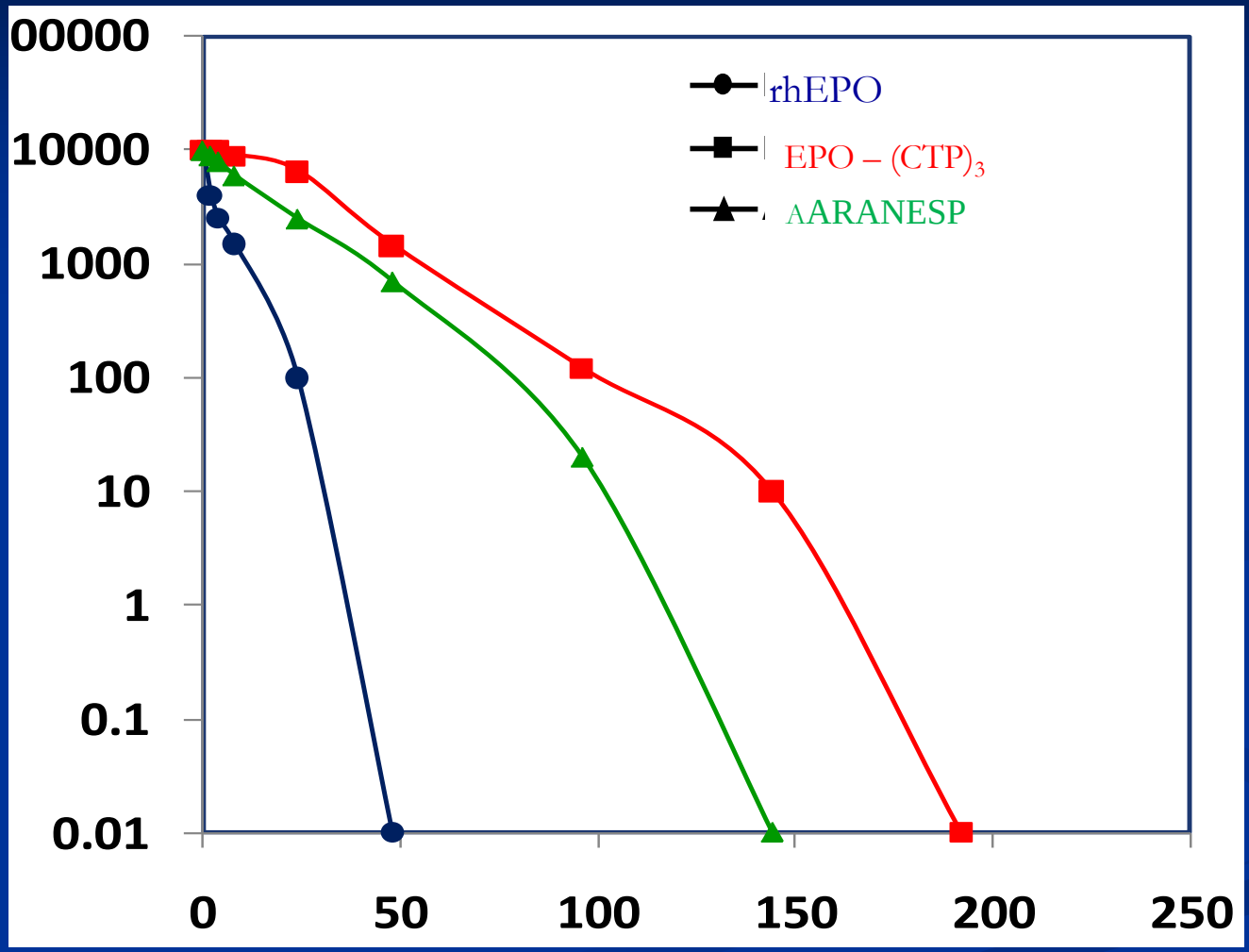
Human EPO-CTP3



Human EPO-CTP3

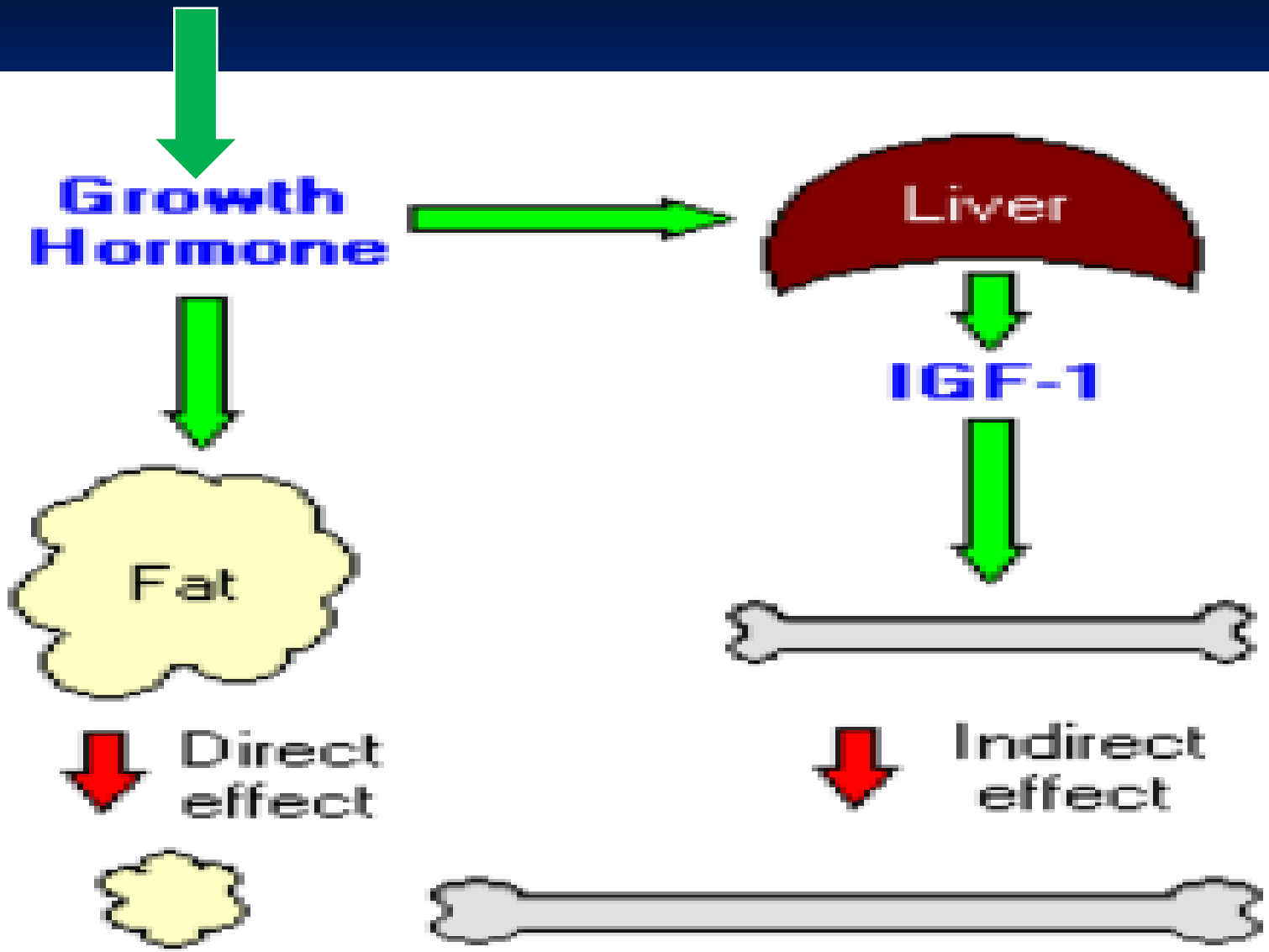


Human EPO-CTP3



Human Growth Hormone

Pituitary


Growth Hormone
Liver
IGF-1
Fat
Direct effect
Indirect effect

acquired gene: human growth hormone

Pharmaceutical and Biotechnological Uses of Growth Hormone

To treat children of pathologically short stature

3-D Structure Analysis

Human Growth Hormone (blue) with its Receptors (cyan)

Conclusion: Both termini pointing away from the receptors and are accessible

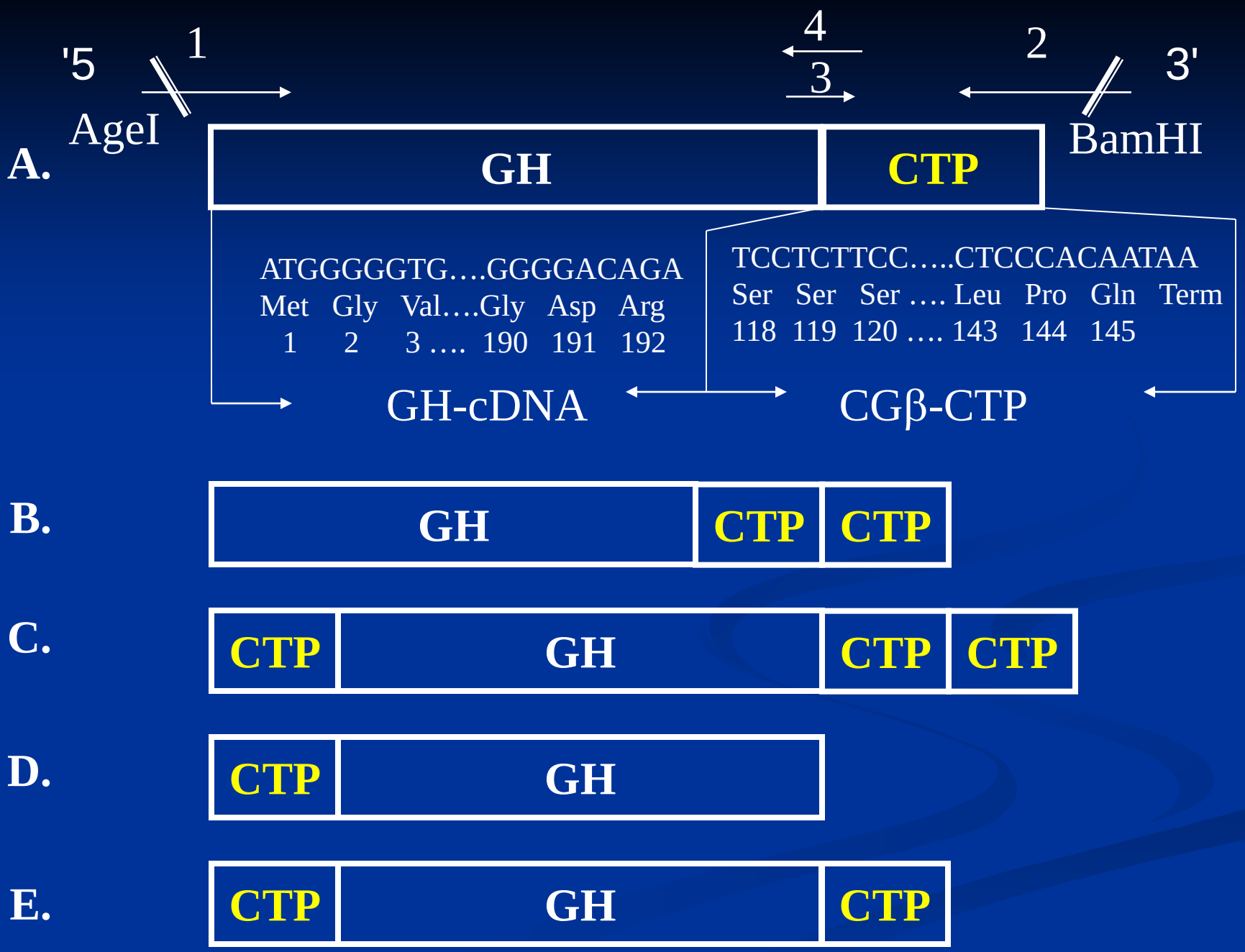
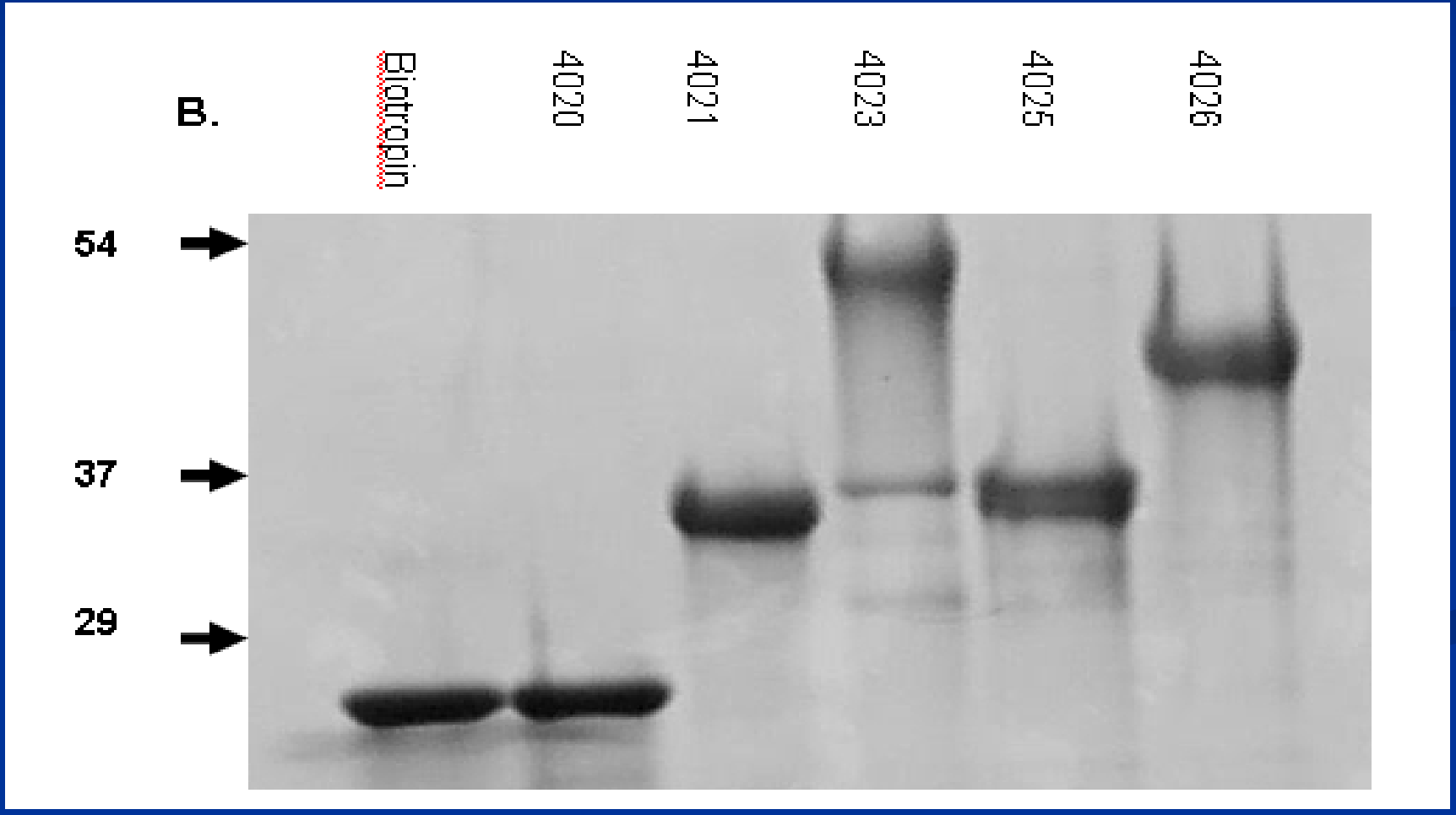
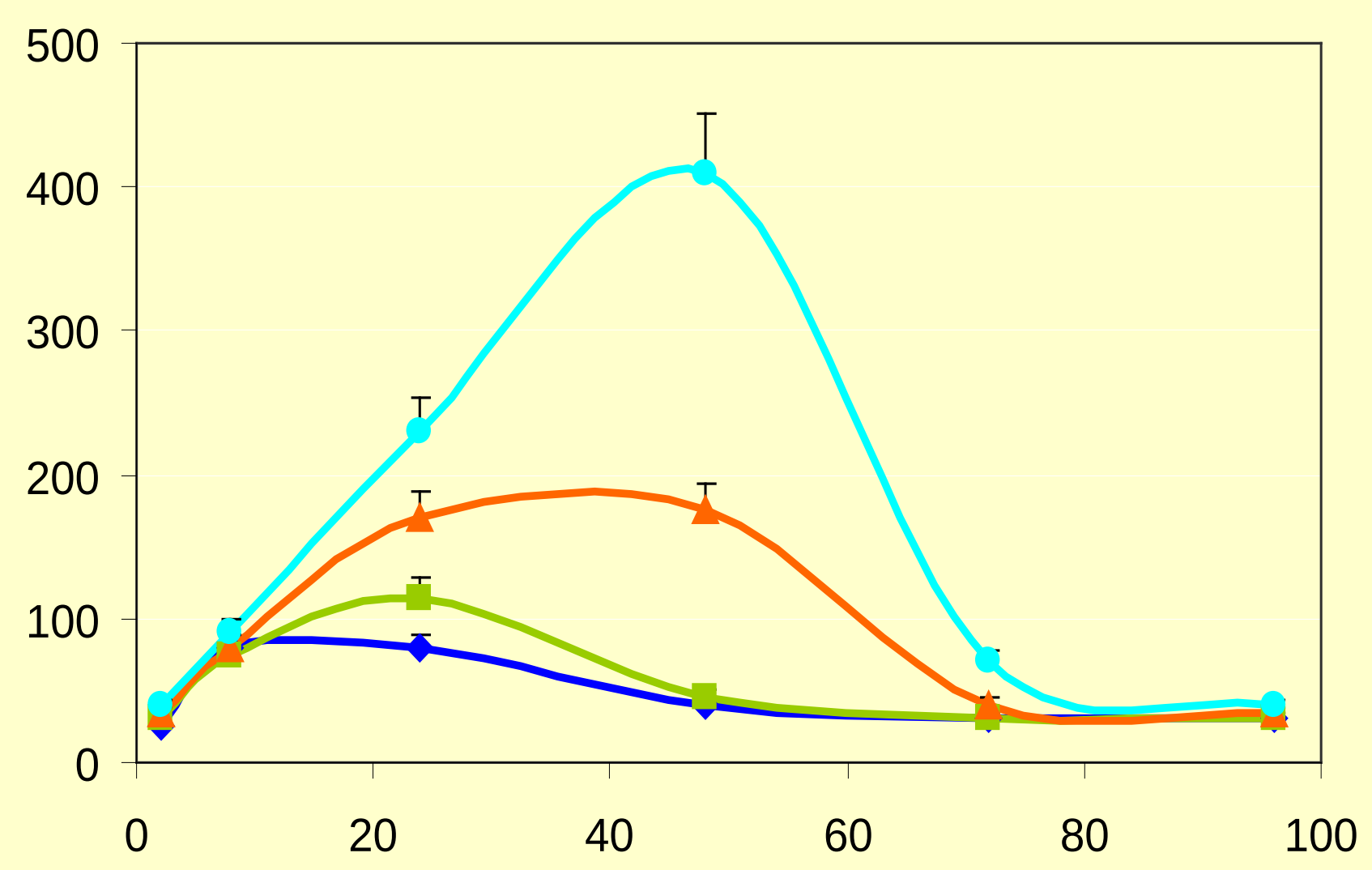

'5
1
4
3
2
3'
AgeI
BamHI
A.
GH
CTP
ATGGGGGTG….GGGGACAGA
Met Gly Val….Gly Asp Arg
1 2 3 …. 190 191 192
TCCTCTTCC…..CTCCCACAATAA
Ser Ser Ser …. Leu Pro Gln Term
118 119 120 …. 143 144 145
GH-cDNA
CGβ-CTP
B.
GH
CTP
CTP
C.
CTP
GH
CTP
CTP
D.
CTP
GH
E.
CTP
GH
CTP

Fig.1.

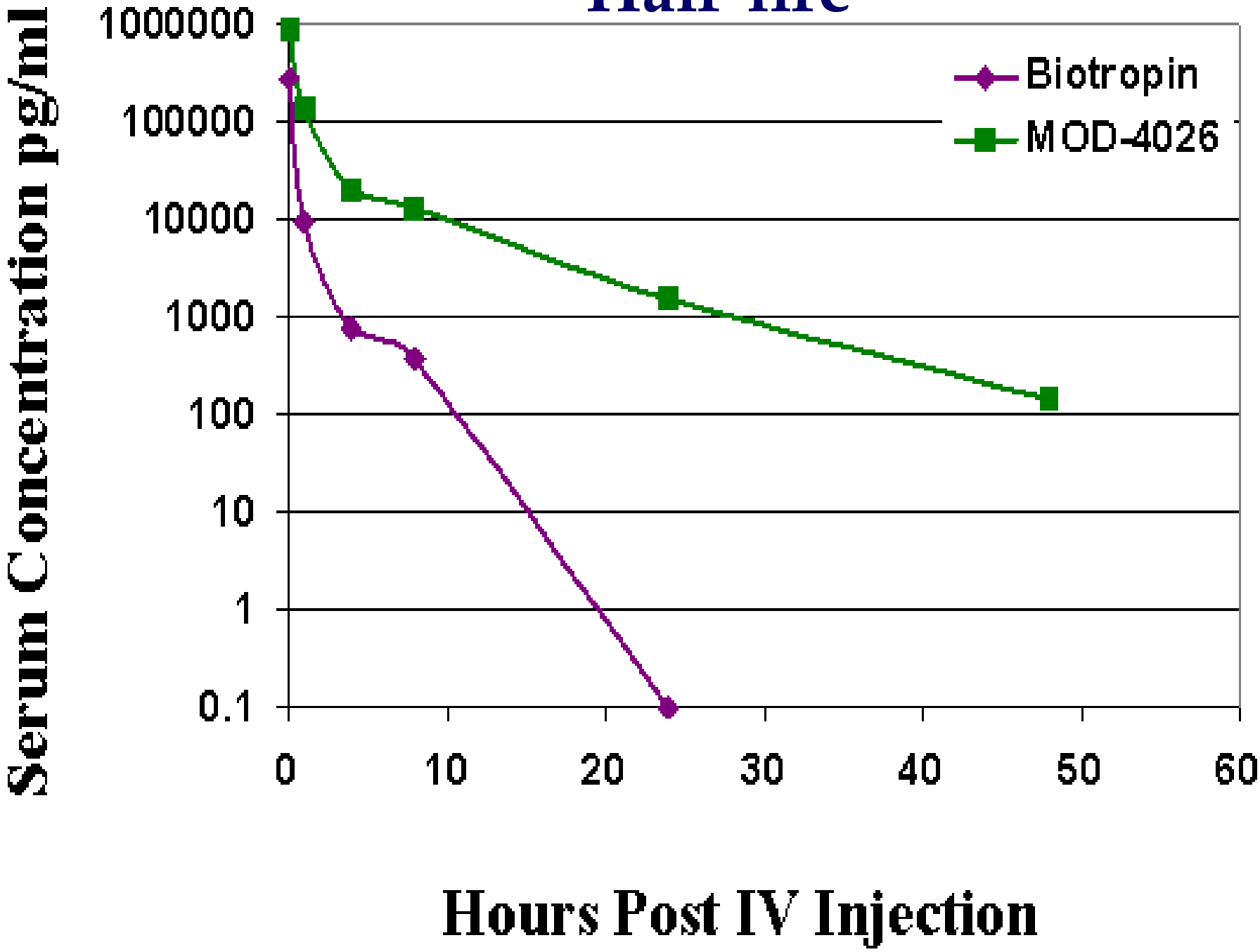
Secretion of GH Analogs from CHO cells



GH – (CTP)3


500
400
300
200
100
0
0
20
40
60
80
100

Half-life


Serum Concentration pg/ml
1000000
100000
10000
1000
100
10
1
0.1
0
10
20
30
40
50
60
Biotropin
MOD-4026
Hours Post IV Injection

GH – (CTP)3

- Expeiments in Rehsus Monkeys and human clinical trials phase I that GH-Long- acting is safe and not immunogenic

- GH-(CTP)3 is in human clinical trials phase III

Conclusions

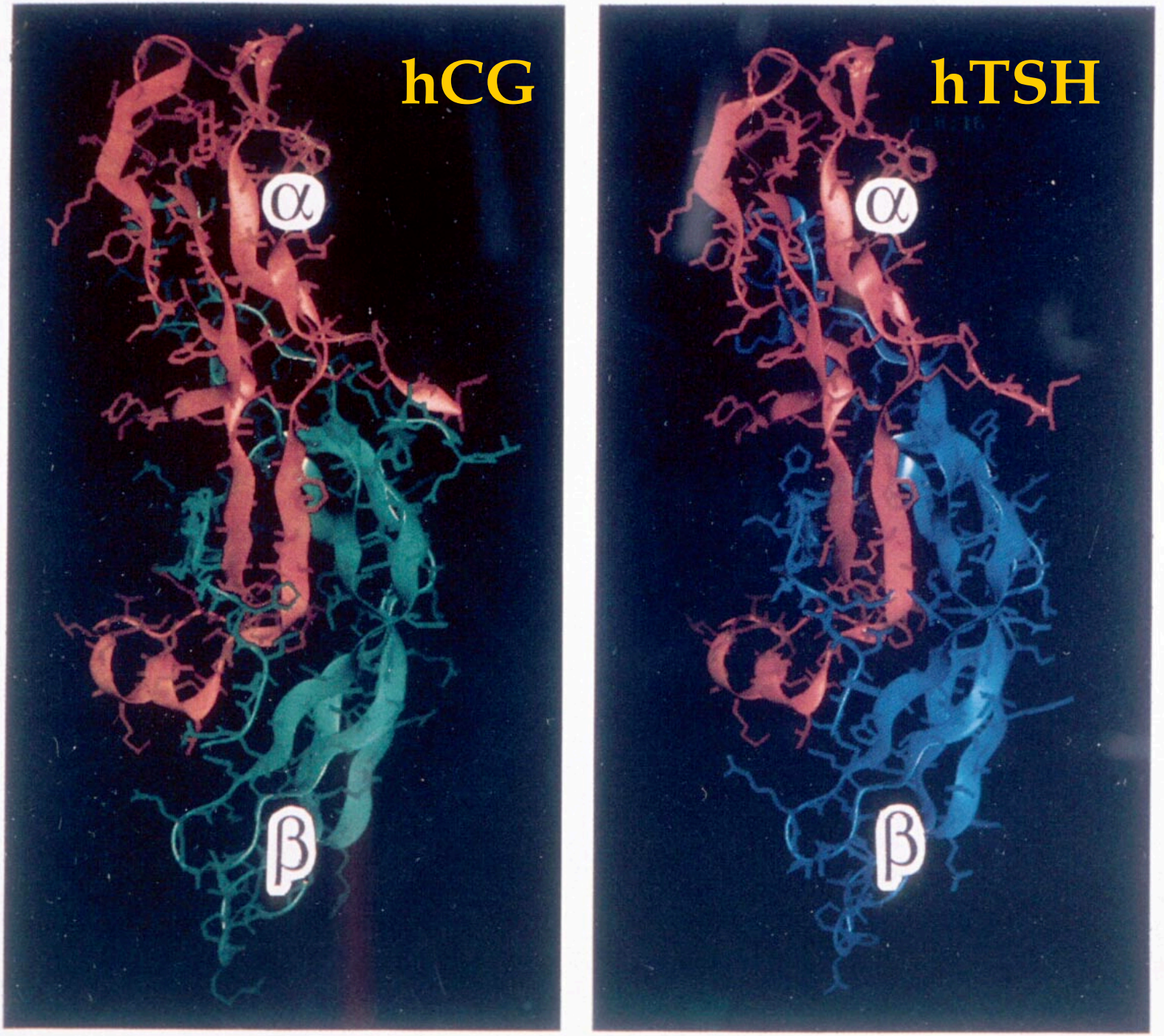
- Ligation of the CTP cassette gene bearing 4 O-linked Oligosaccharised chains to different proteins is an interesting strategy for increasing the *in vivo* half-life and *in vivo* bioactivity

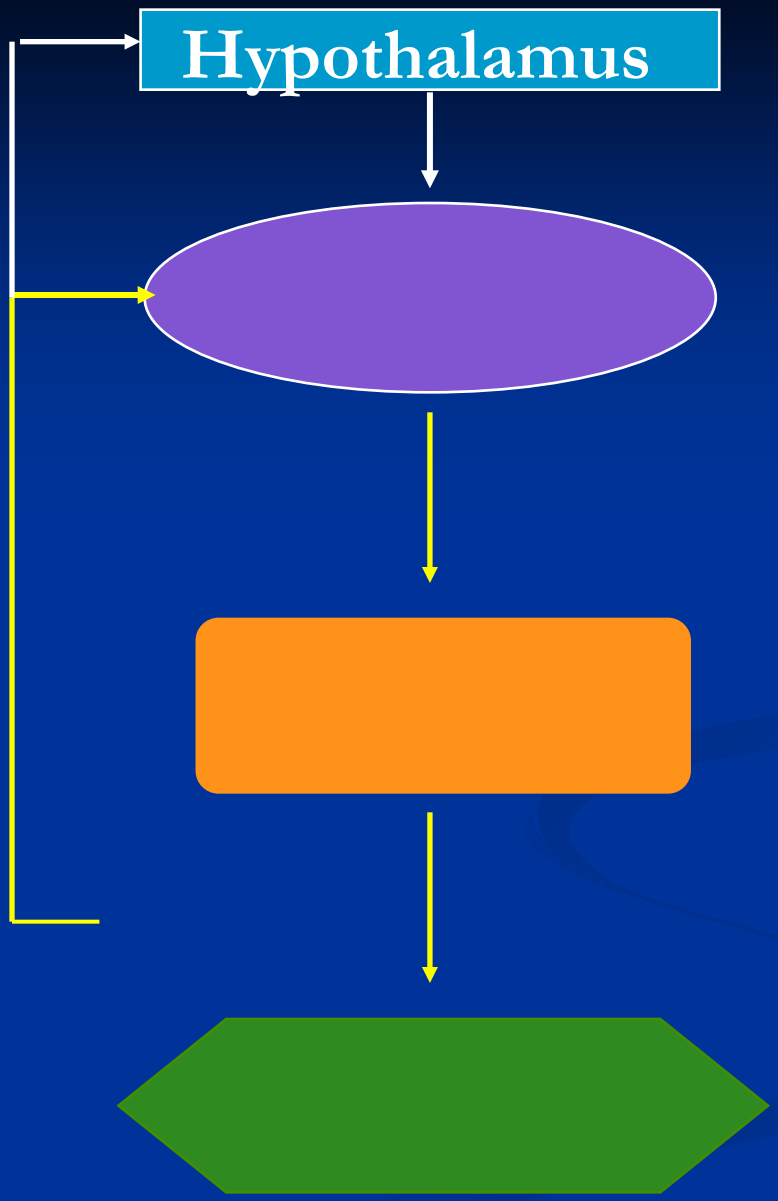
This may allow reducing :

A) Drug dose

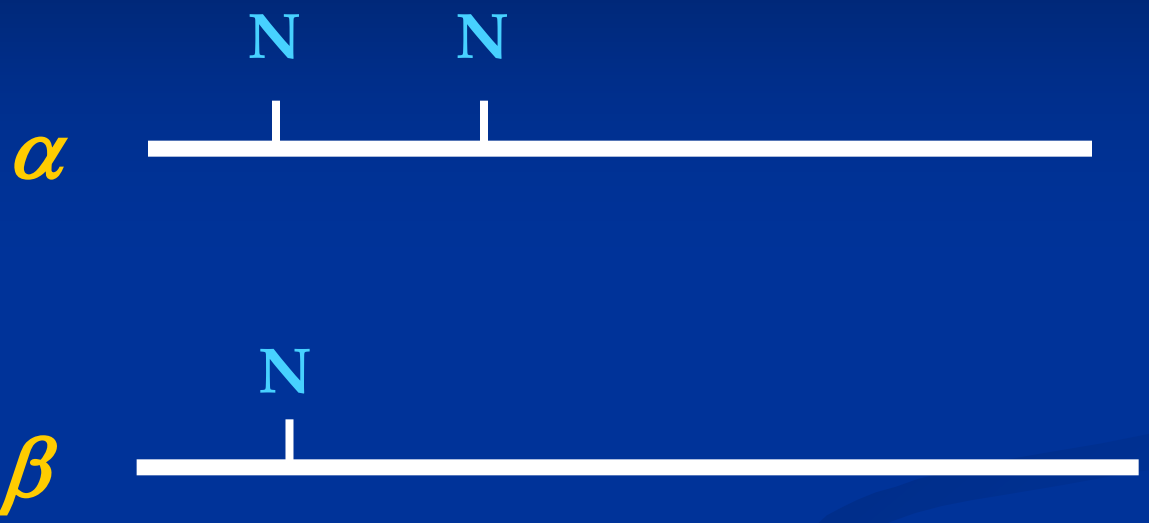
B) Number of injections

TSH Studies

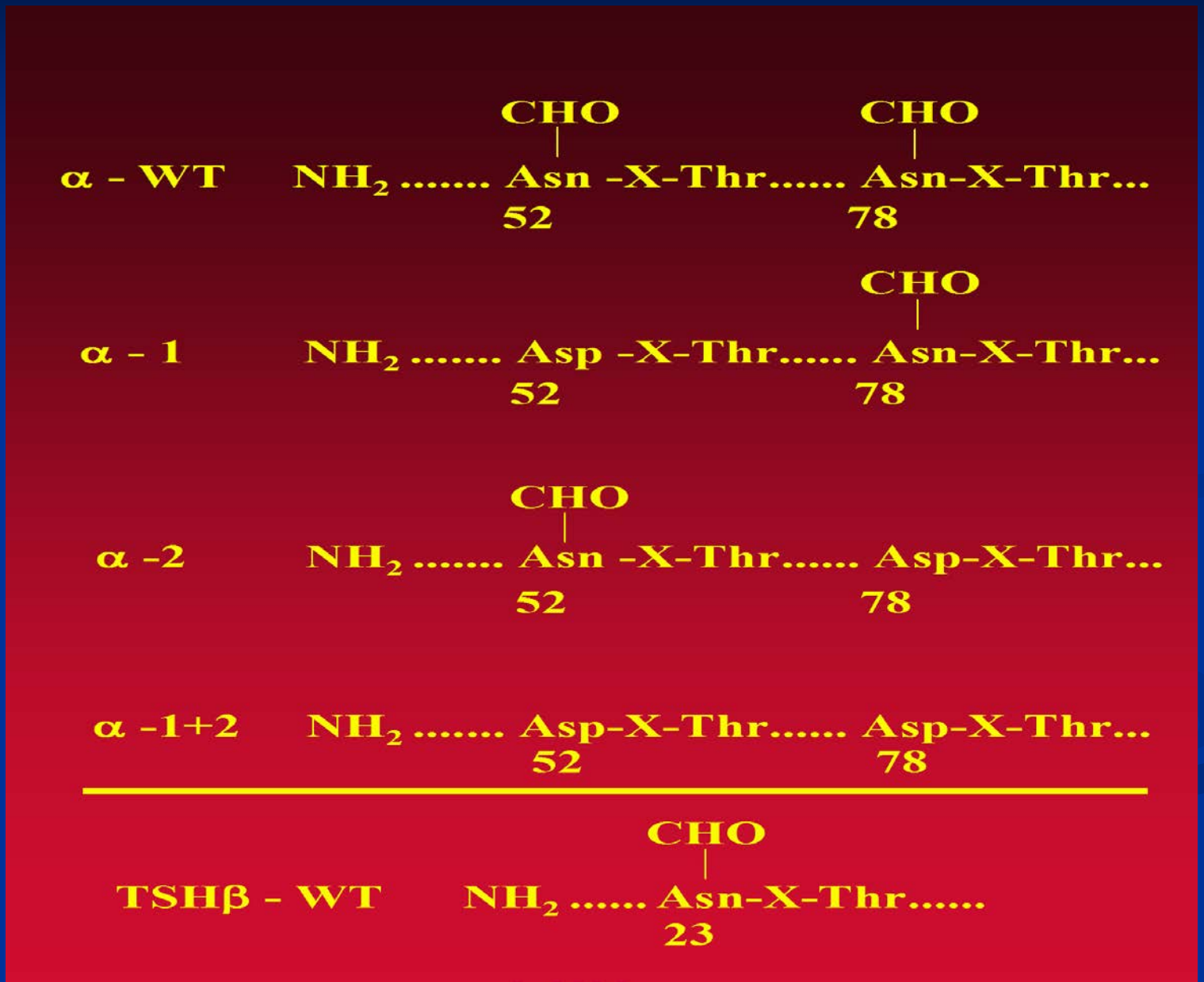

hCG
α
β
hTSH
α
β


Hypothalamus

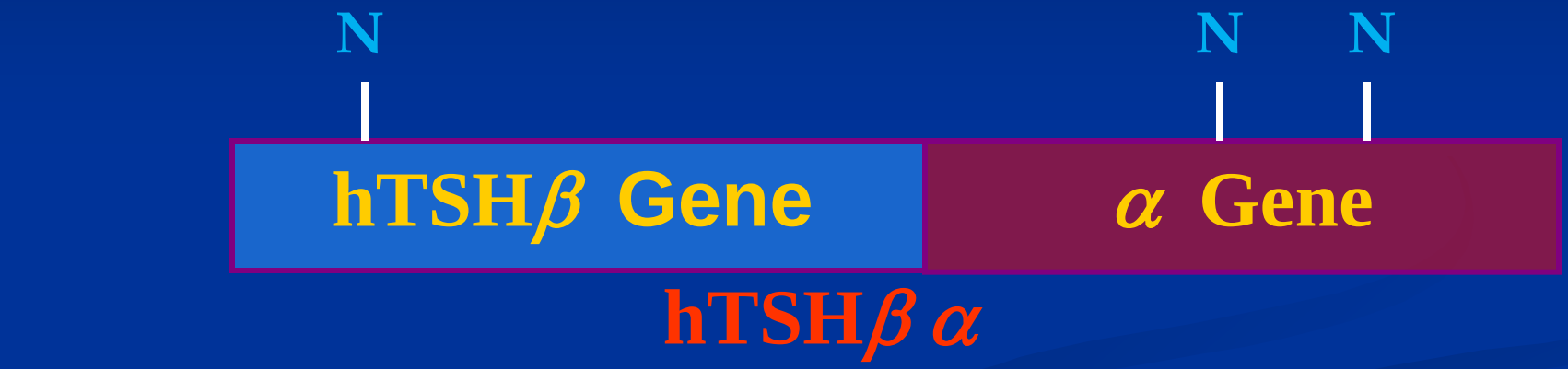
TSH Subunits

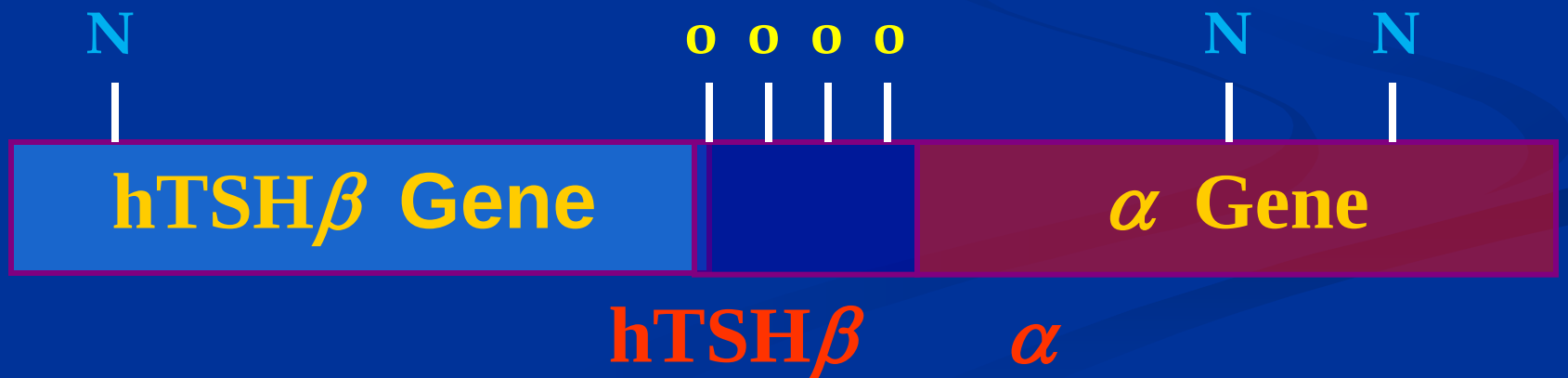


hTSH Variants



hTSH Single Chain

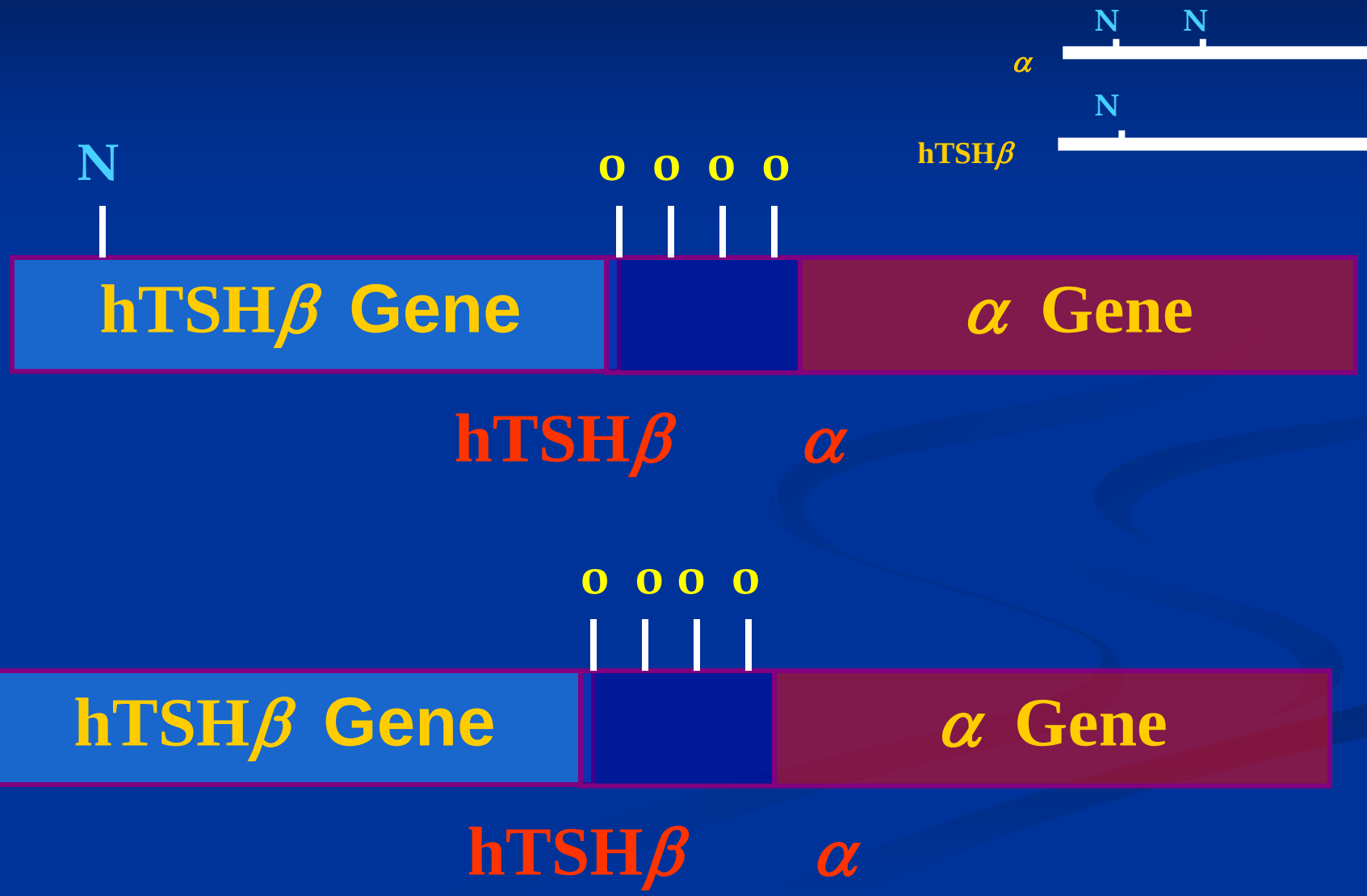




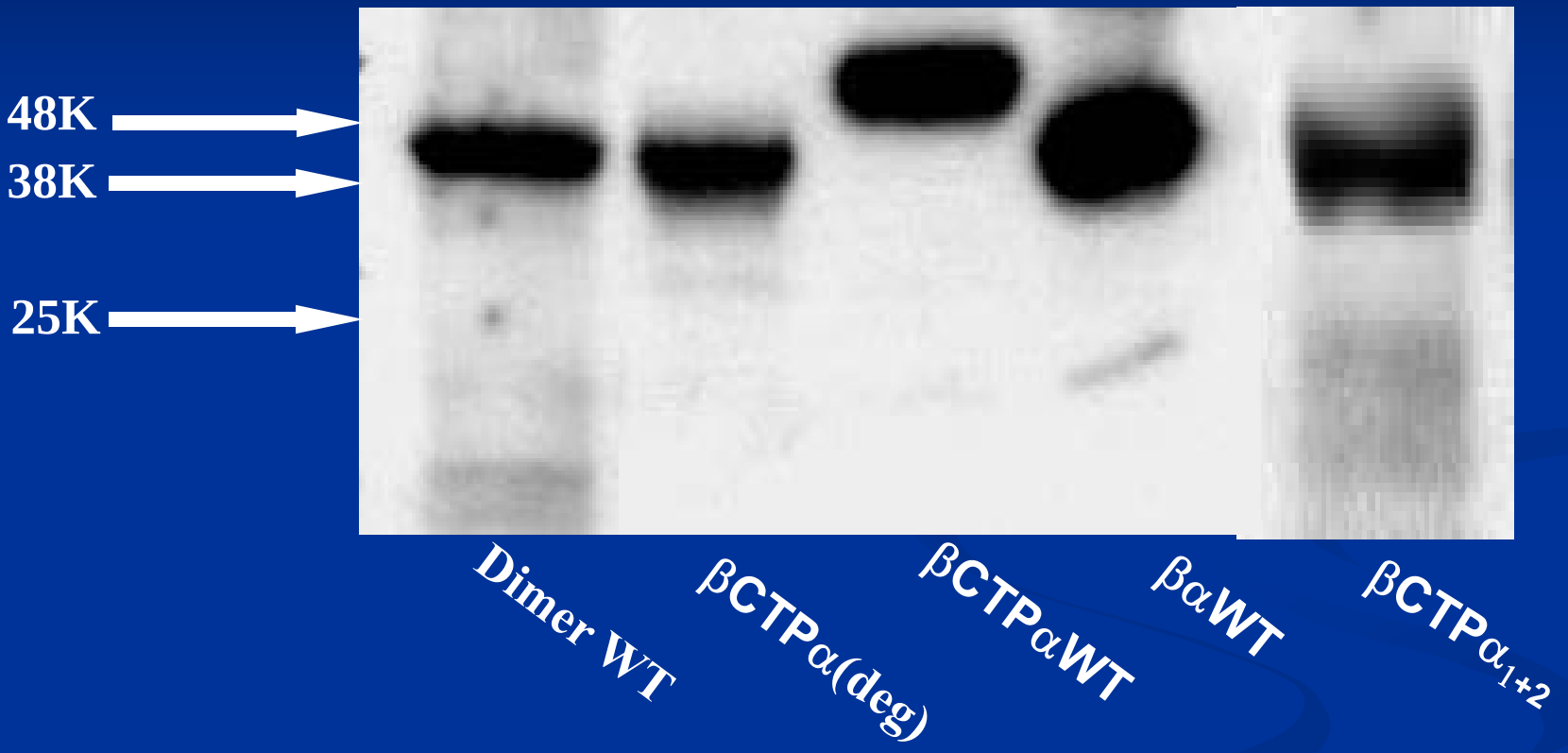
hTSH Single Chain

- Expressed in CHO cells
- Binds to TSH Receptor in high affinity as will as the TSH-WT
- Biologically active

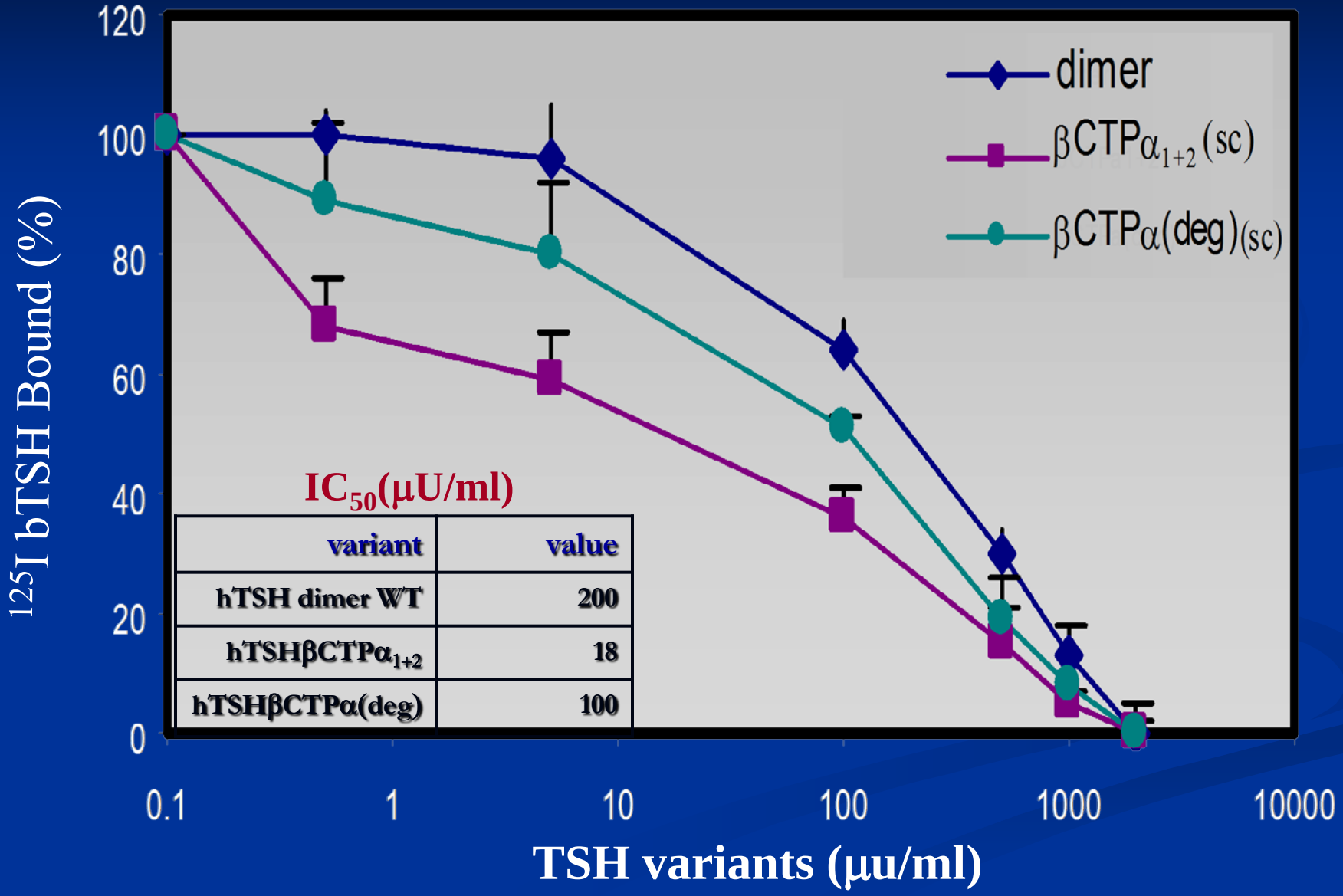
hTSH – Single Chain Variants



Secretion of TSH variants

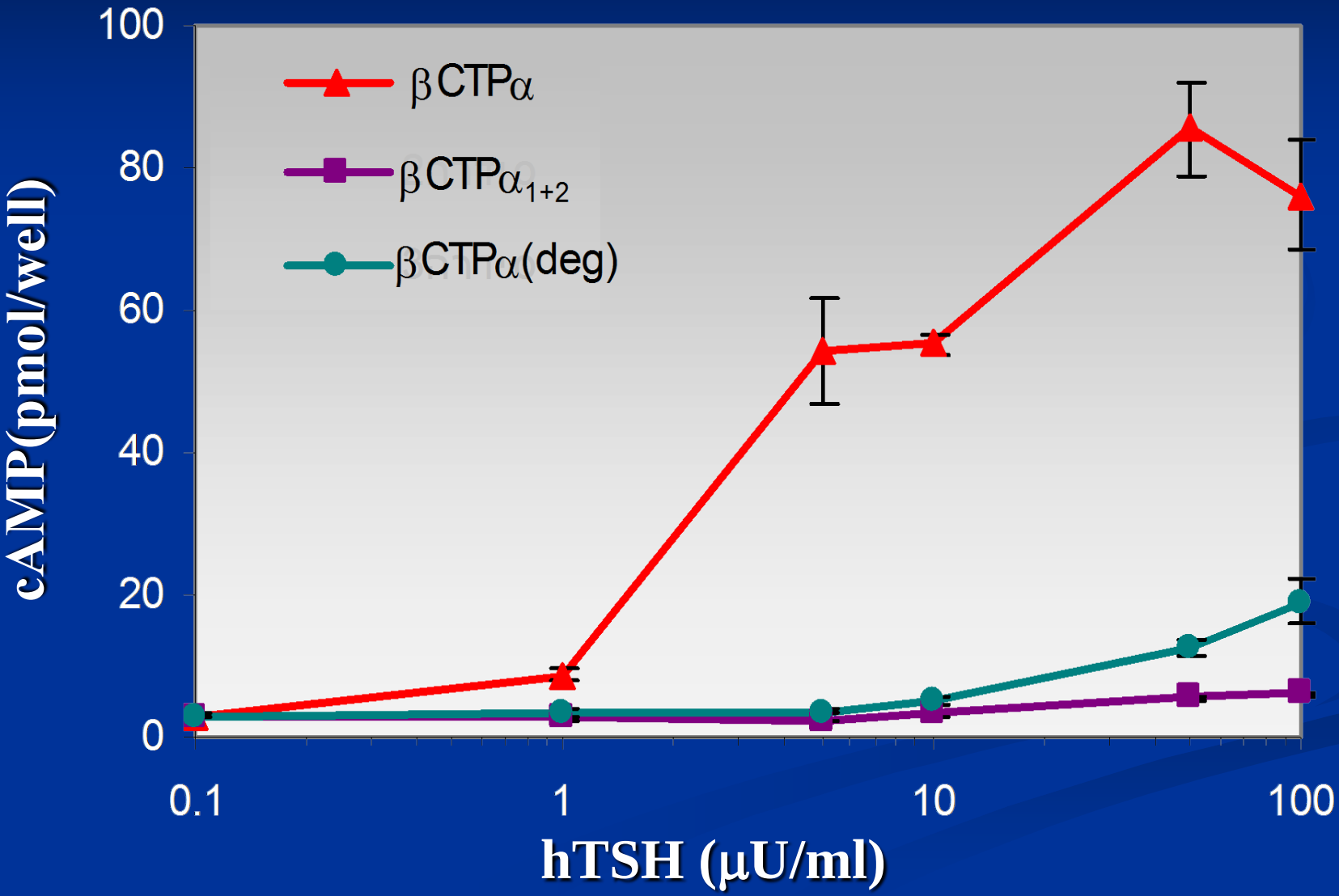


Receptor Binding TSH (Mutants)

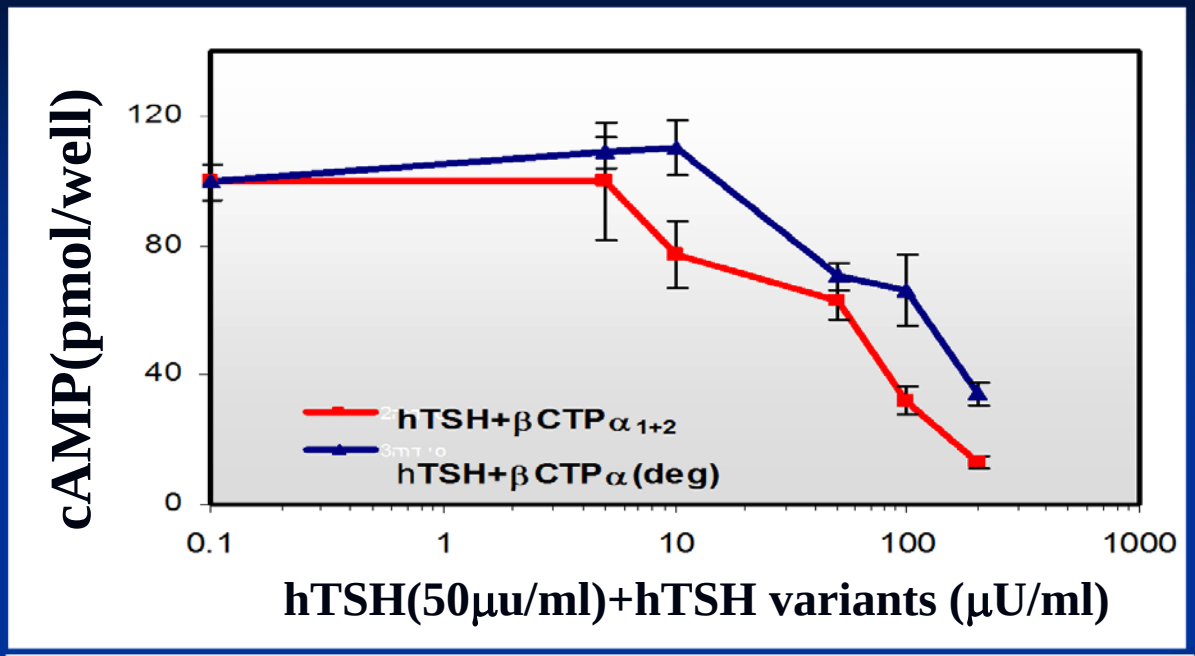


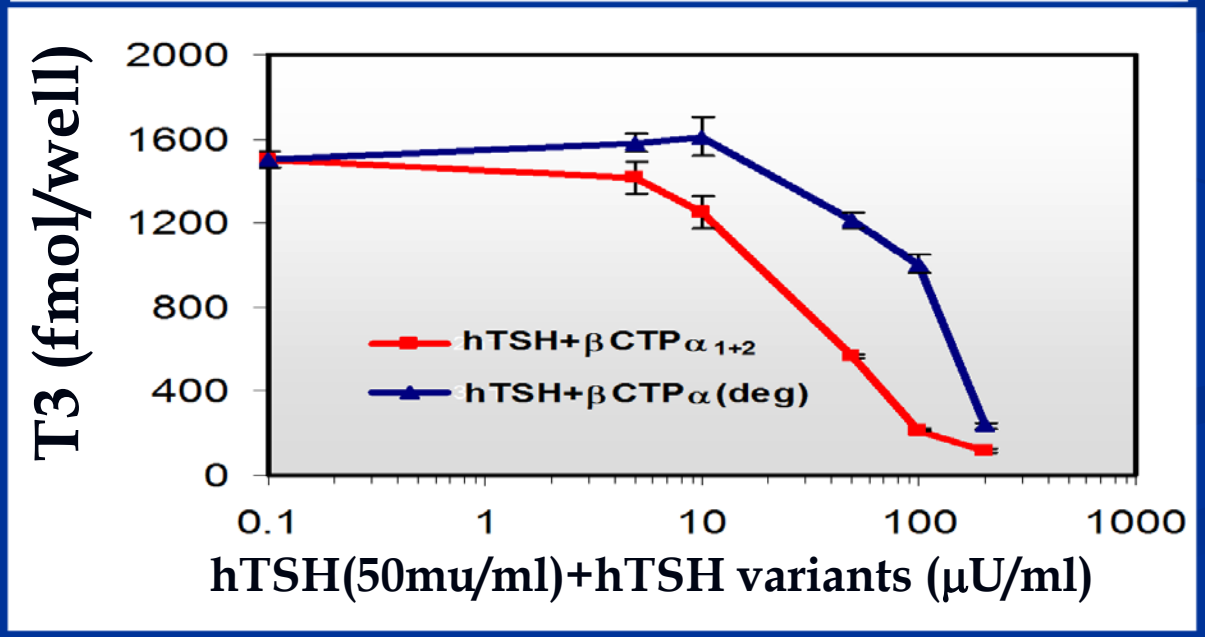
IC_{50}(μU/ml)

variant	value
hTSH dimer WT	200
hTSHβCTPα$_{1+2}$	18
hTSHβCTPα(deg)	100


100
80
60
40
20
0
βCTPα
βCTPα1+2
βCTPα(deg)
cAMP(pmol/well)
0.1
1
10
100
hTSH (μU/ml)

TSH Antagonist





Graves' Disease

Thyroid Stimulating Immunoglobolins

(TSI)

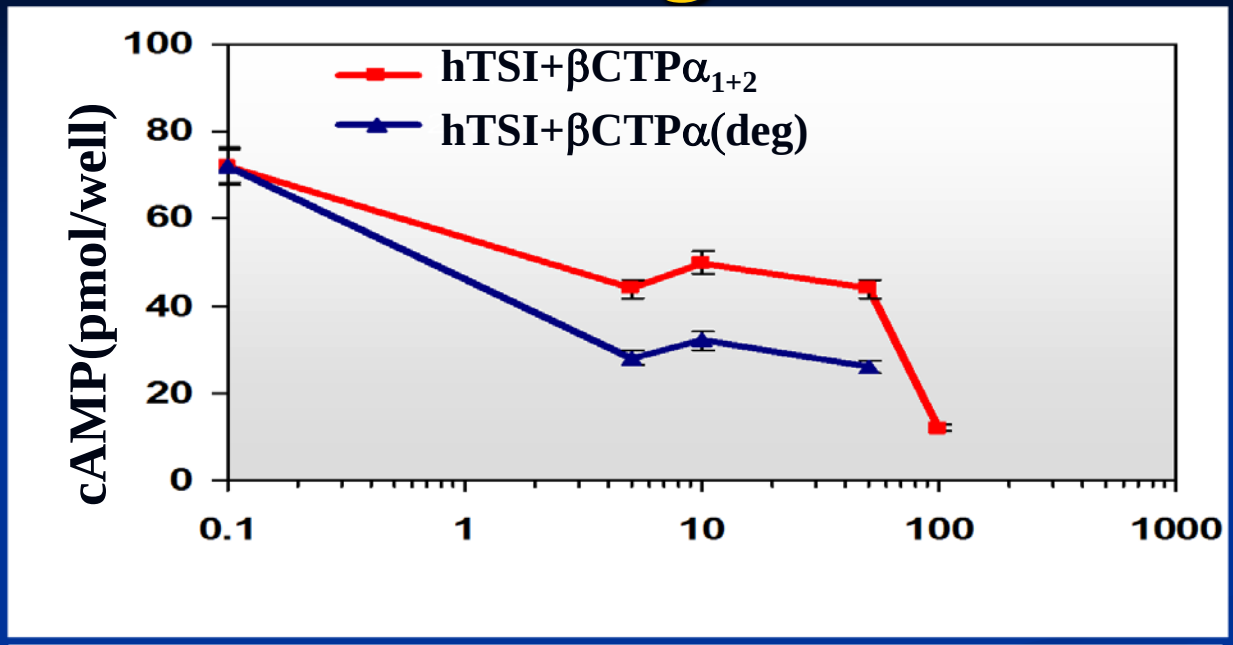
↓

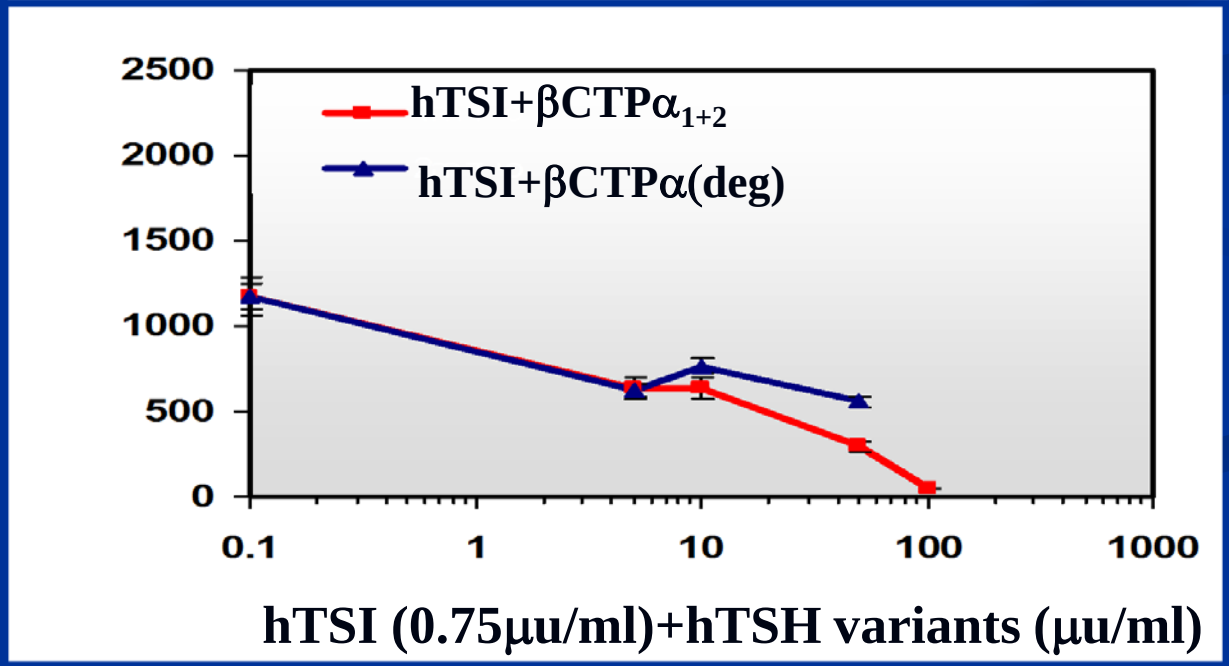
TSH Receptor

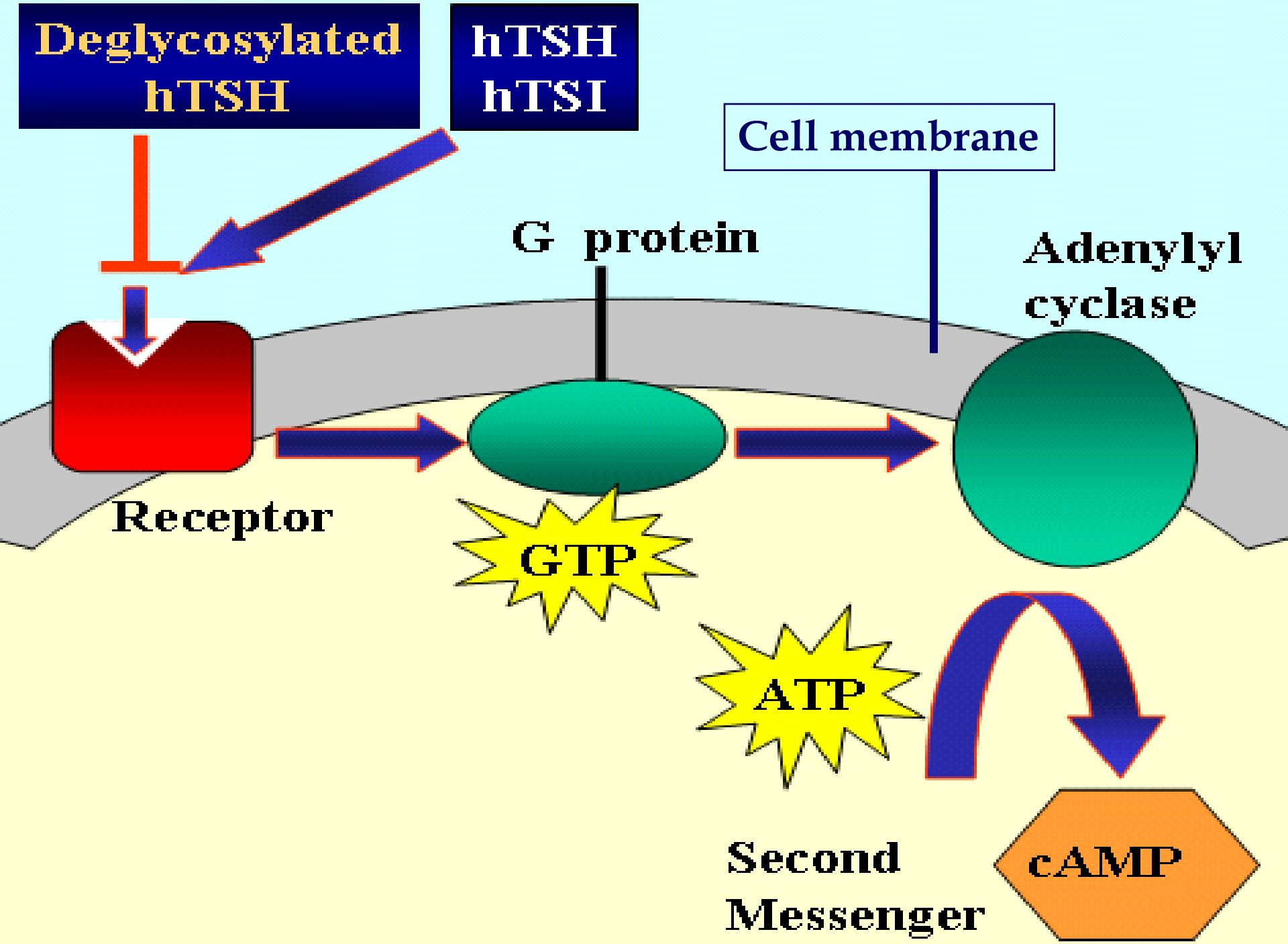
↓

Hyperthyroidism

TSI Antagonist






Deglycosylated hTSH
hTSH hTSI
Cell membrane
G protein
Adenylyl cyclase
Receptor
GTP
ATP
Second Messenger
cAMP

Conclusions

- Deletion of the *N*-linked oligosaccharides from TSH resulted in partial antagonists of TSH and TSI at the level of the receptor binding site.

- **TSH variants may offer a novel therapeutic strategy in the treatment of hyperthyroidism and Graves' disease.**

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