

# About OMICS Group

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# 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.

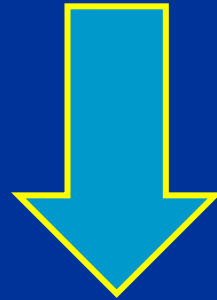
# The role of oligosaccharides on structure and function of glycoprotein hormones: developing of agonists and antagonists



*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  $\alpha$  (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

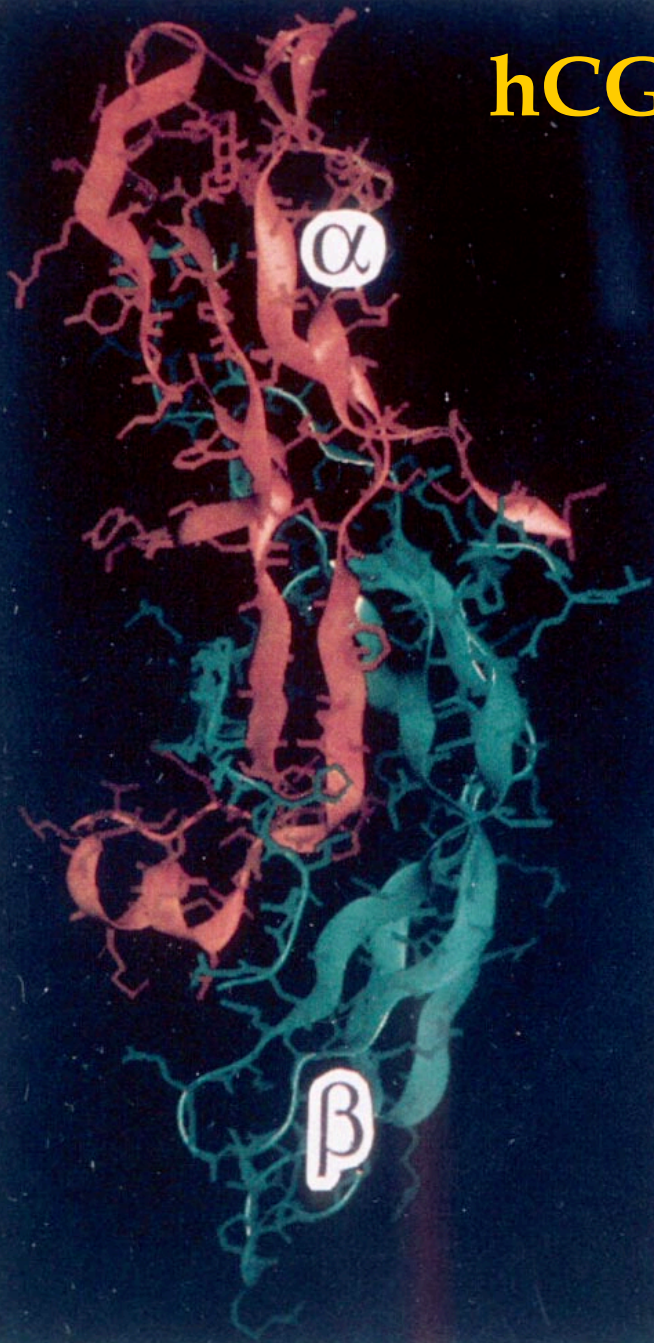


# Structure-Function of Glycoprotein Hormones

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



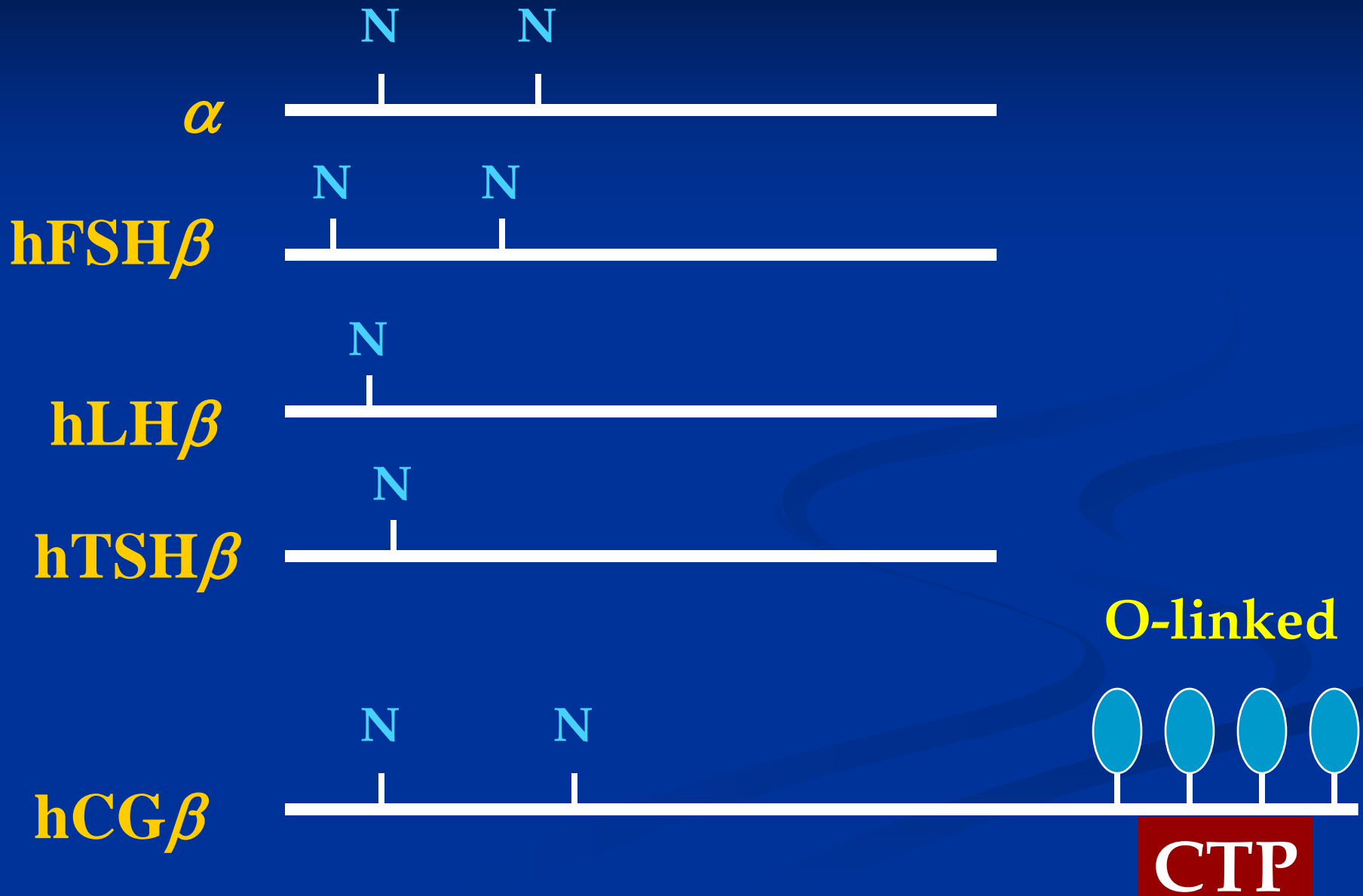
**hCG**



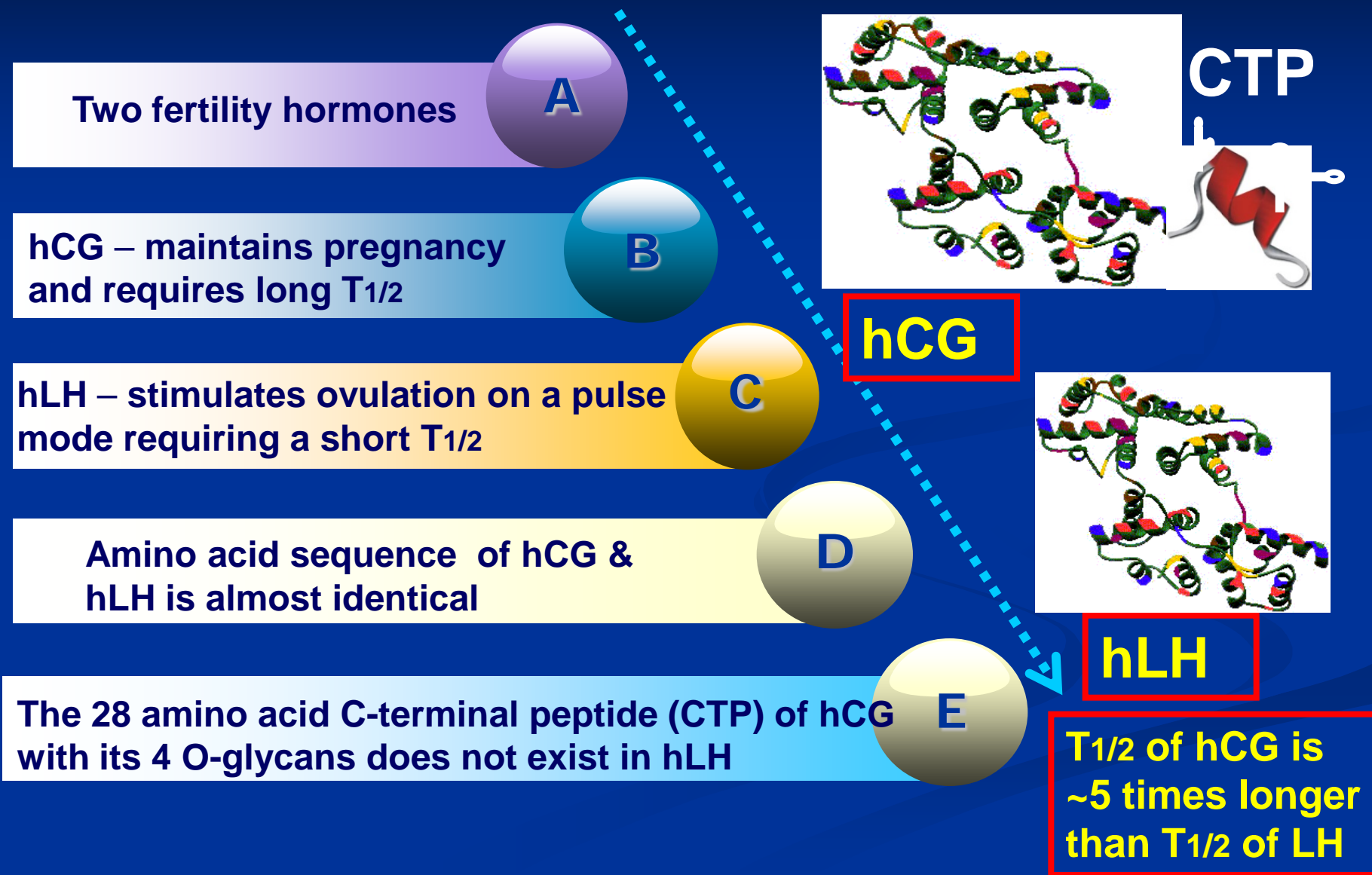
**hTSH**



# Glycoprotein Hormone Subunits



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

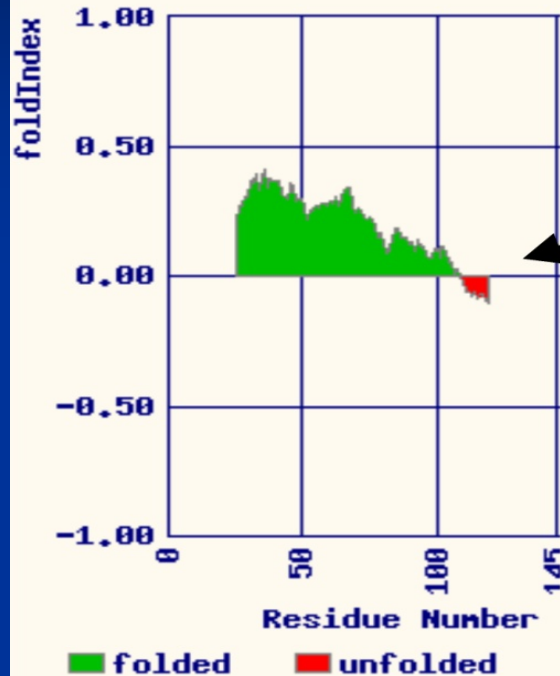


# CTP

0 0 0  
| | |  
SerSerSerSerLysAlaProProProSerLeuProSerProSerArgLeu

0  
|  
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] length: 37 score:  $-0.06 \pm 0.03$

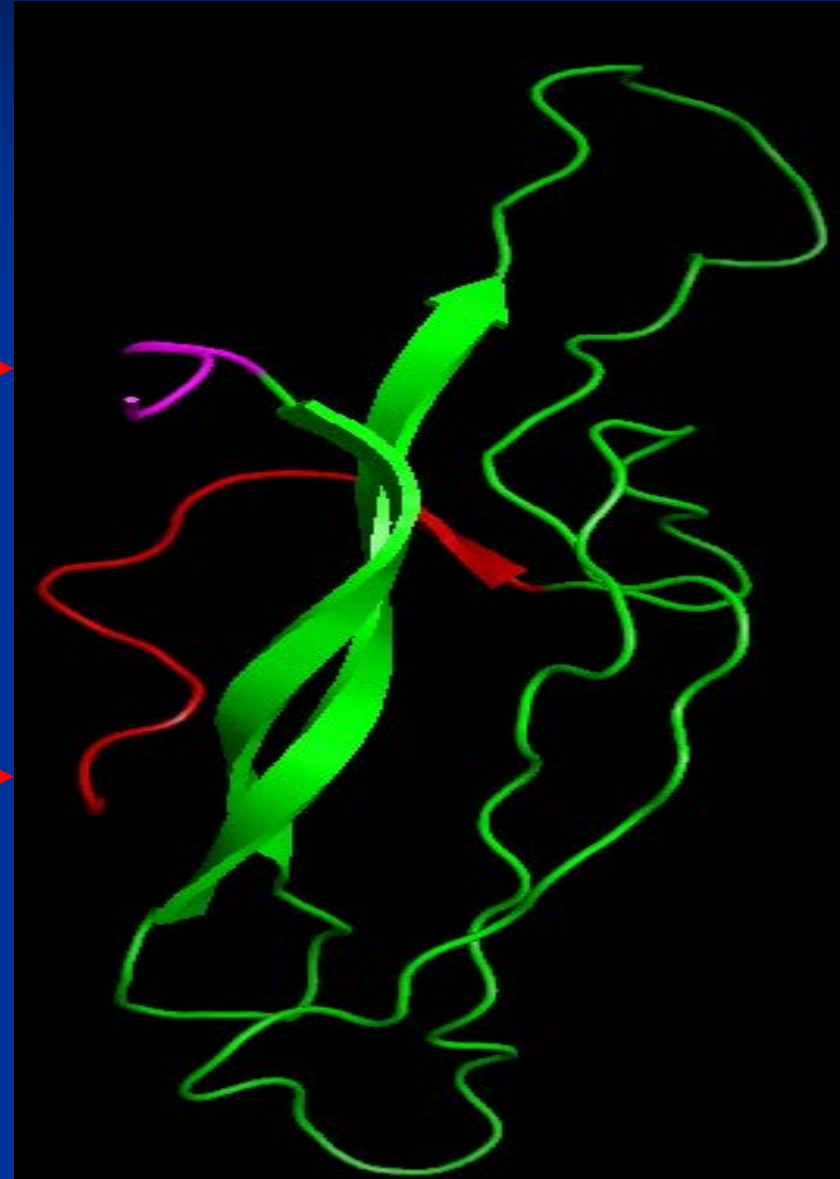
```
1  SKEPLRPCR PINATLAVEK EGCPVCITVN TTICAGYCPT MTRVLQGVLP
51 ALPQVVCNYR DVRFESIRLP GCPRGVNPVV SYAVALSCQC ALCRRSTTDC
101 GGPKDHLPTC DDPRFQDSSS SKAPPPSLPS PSRLPGPSDT PILPQ
```

( Predicted disordered segment )

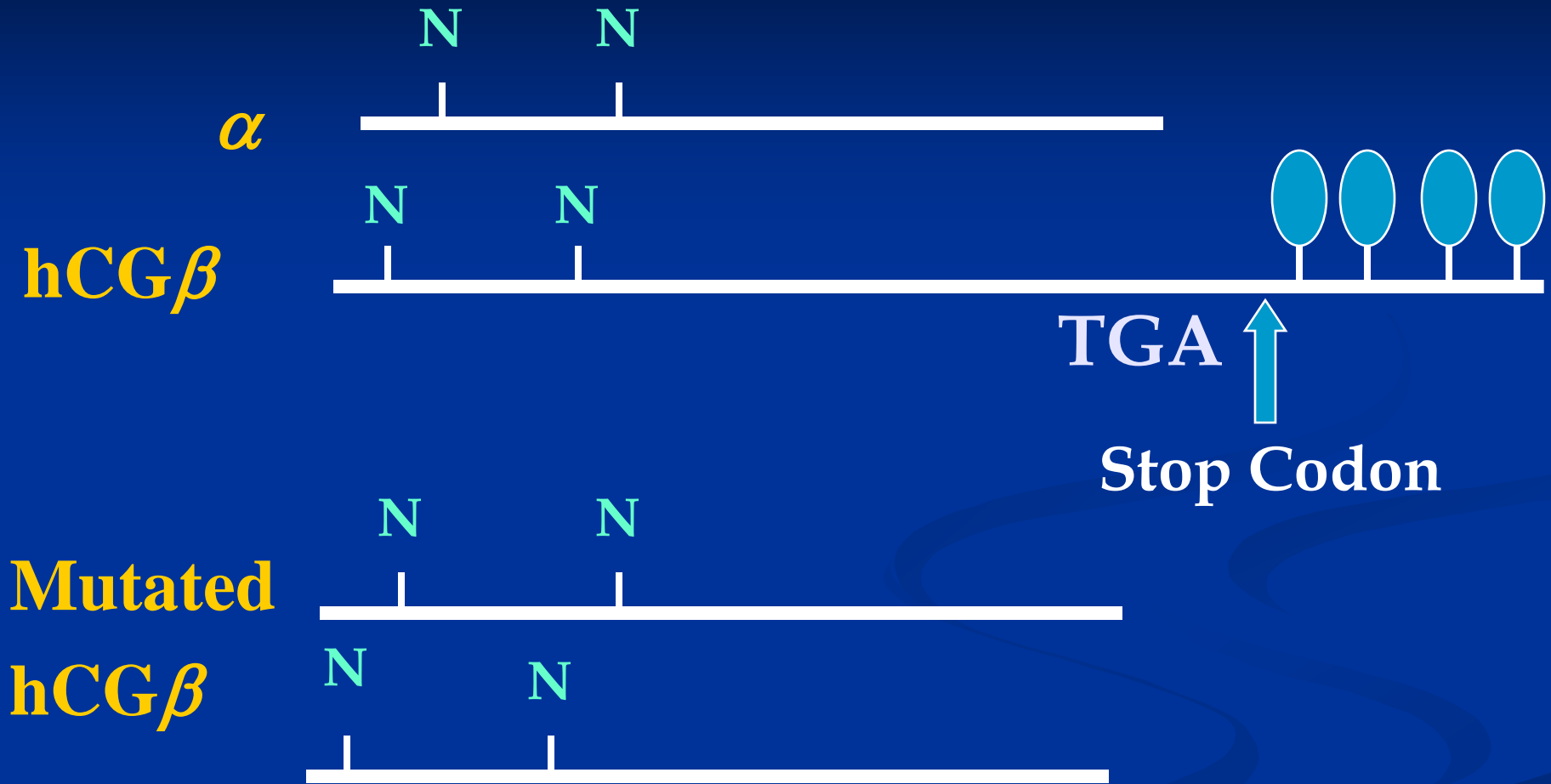
# Crystal Structure of hGC $\beta$ showing long C-term lacking CTP

**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*



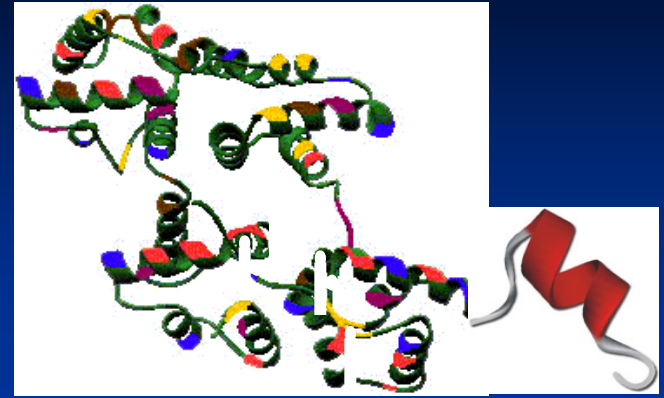


Protein

+

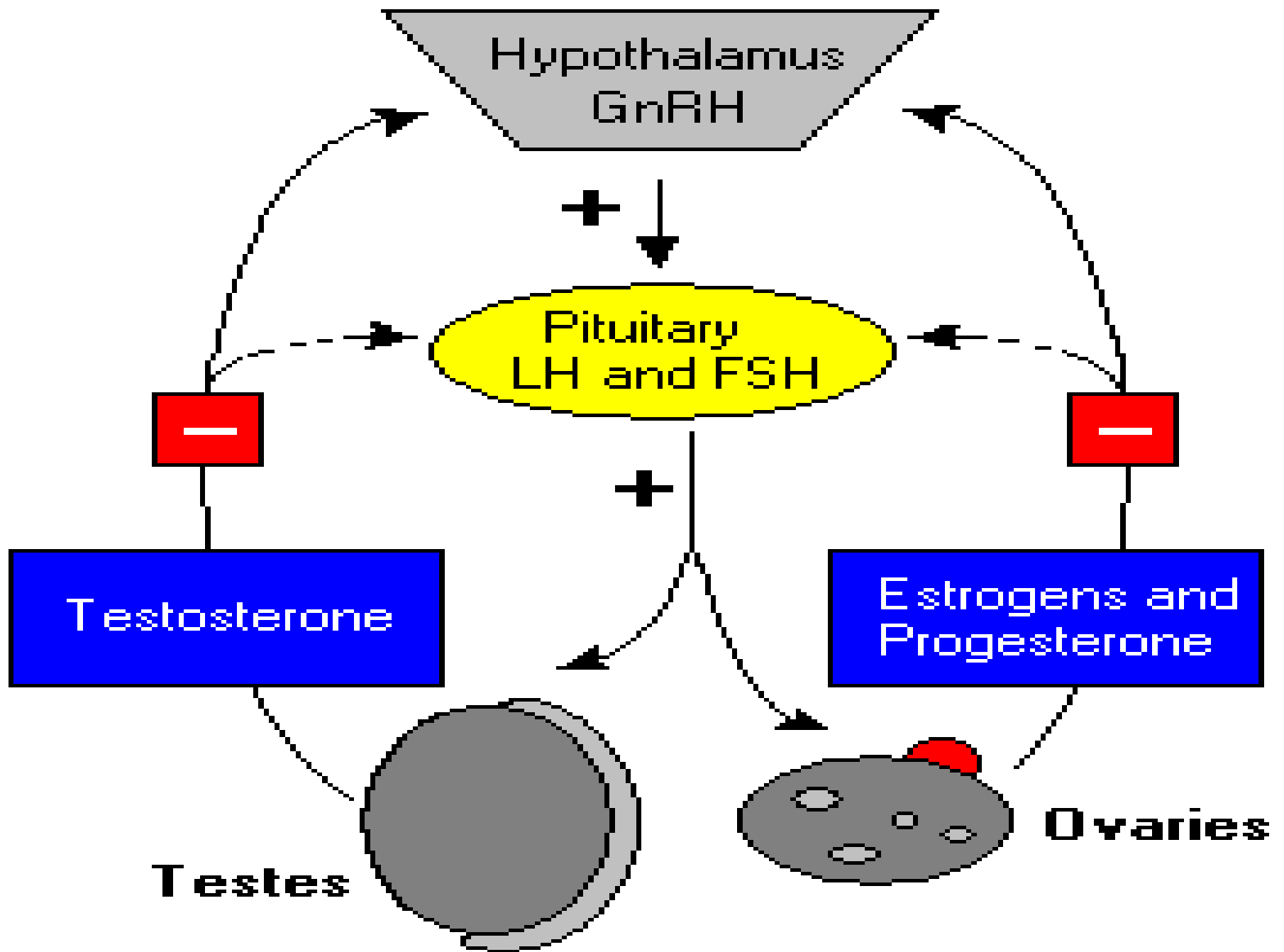


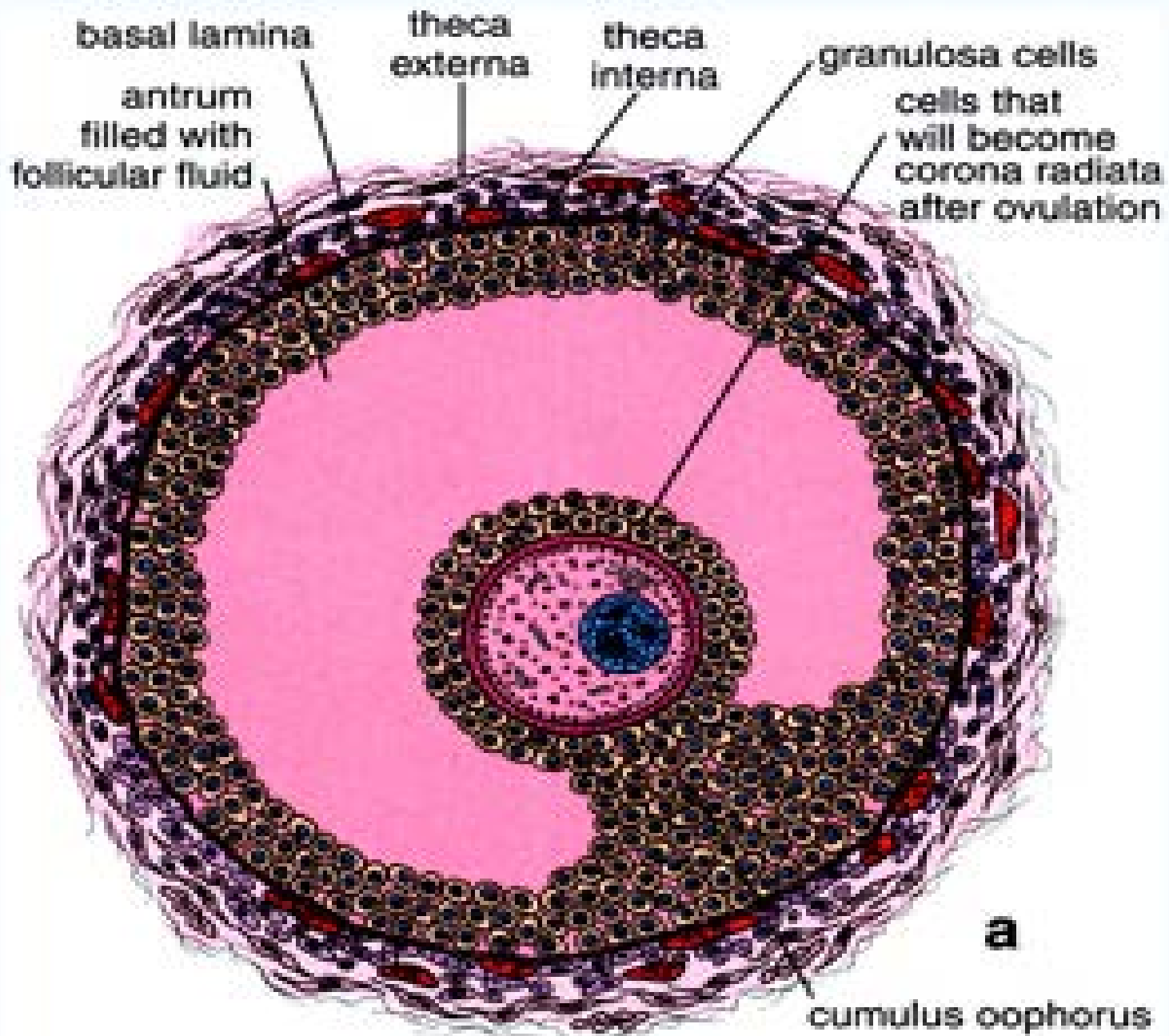
CTP



Half- Life

# Designing New FSH Analog





**MATURE GRAAFIAN FOLLICLE**

# Designing New FSH Analog

hFSH $\beta$  Gene

hCG $\beta$  Gene

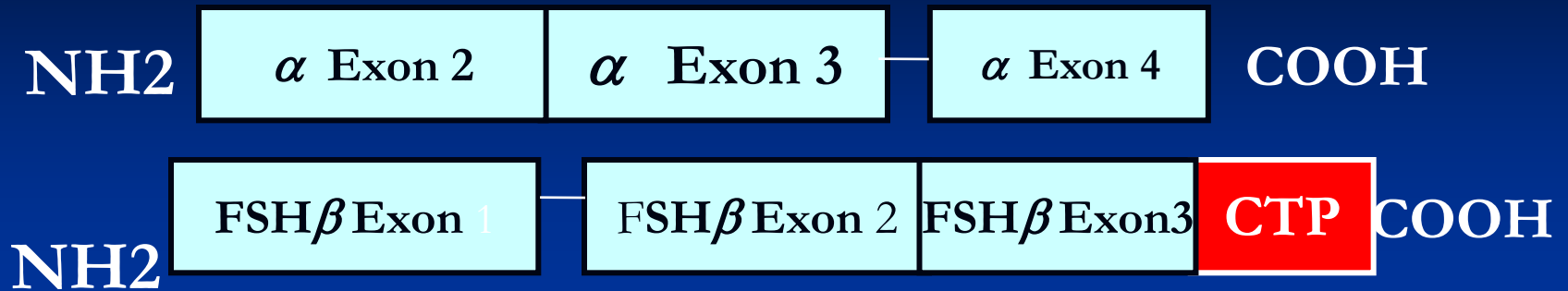
CTP



hFSH $\beta$  Gene

CTP

# hFSH - CTP



1- Assembly of the subunits

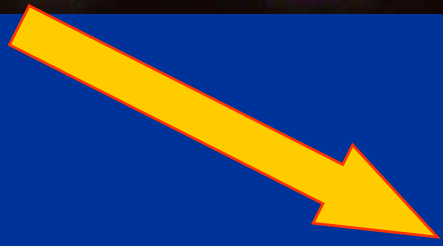
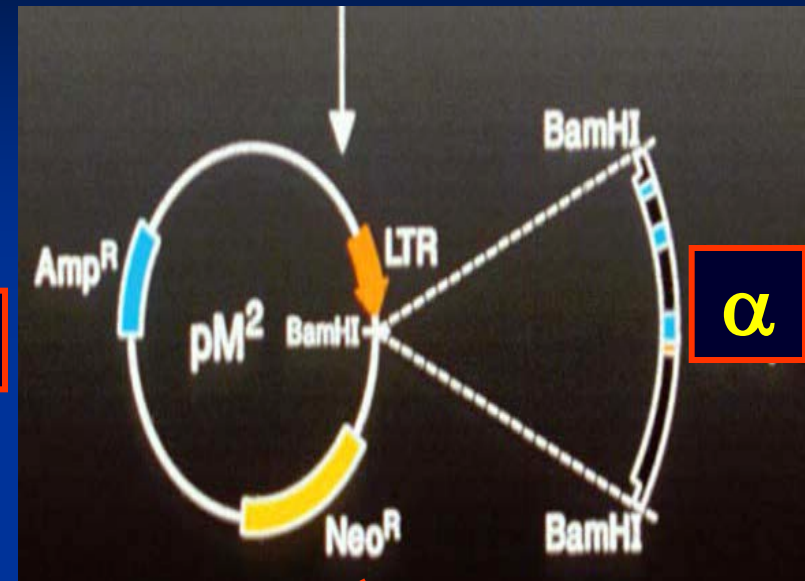
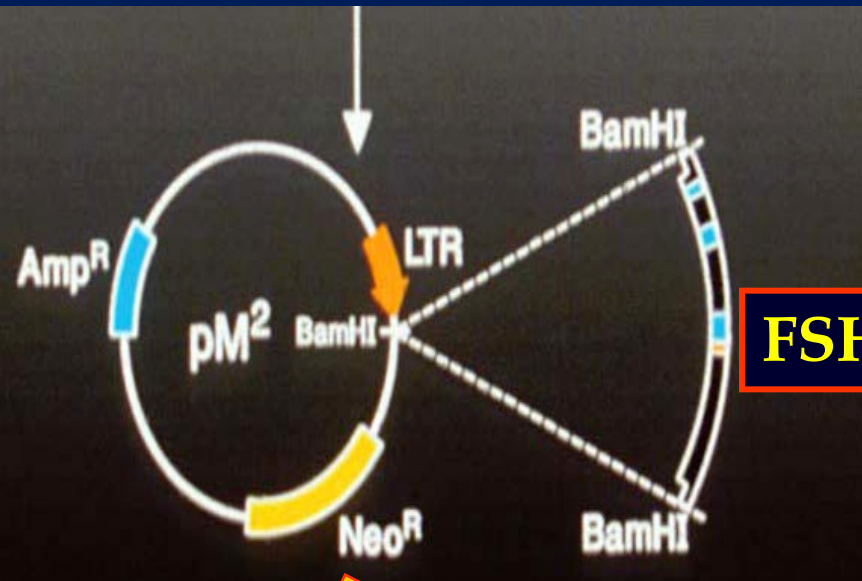
2- Binding to the receptor

3- *In vitro* Bioactivity

4- *In vivo* Bioactivity

5- Immunogenecity

# Gene Expression



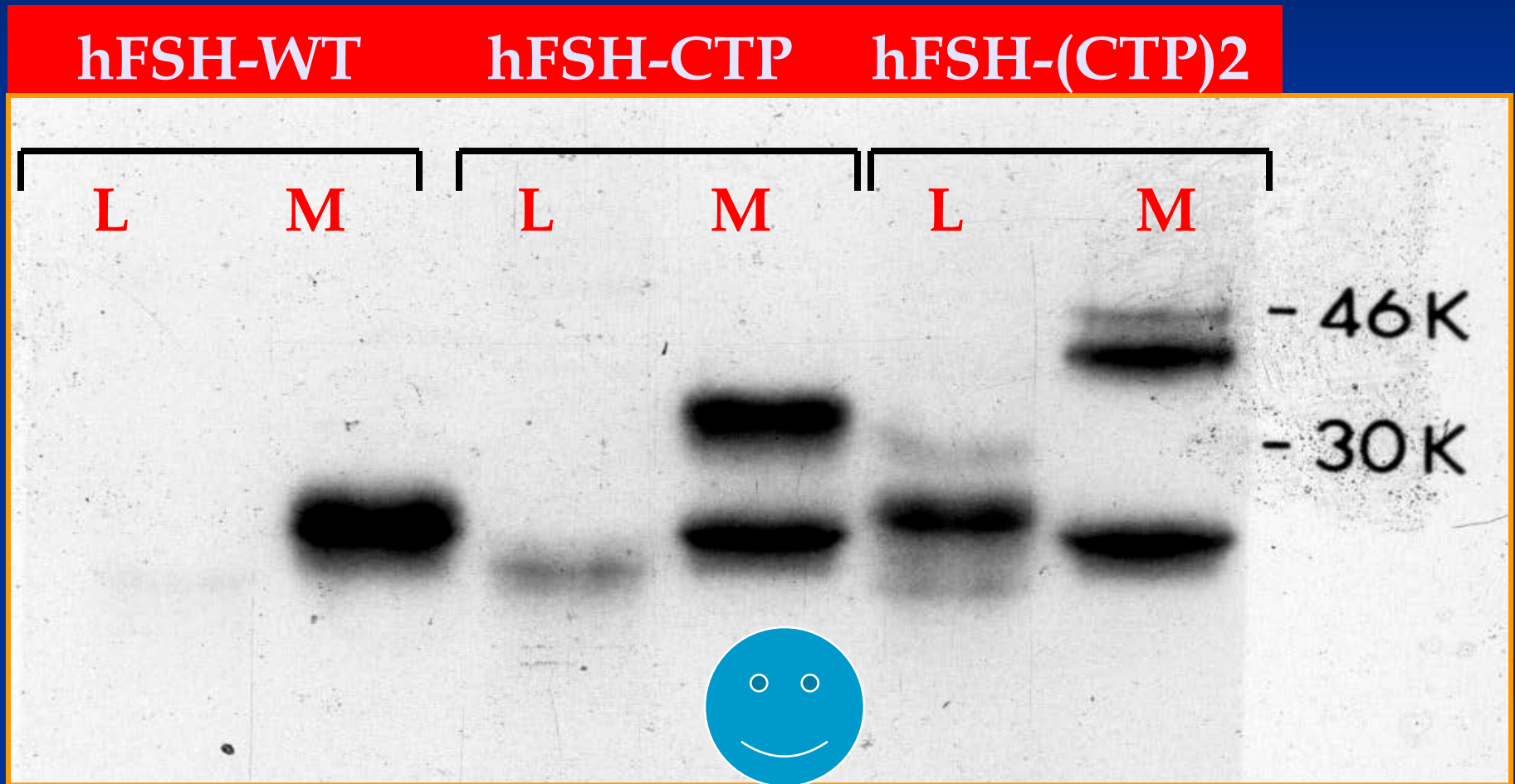
**CHO**

**Transfection**



**Stable Clone**

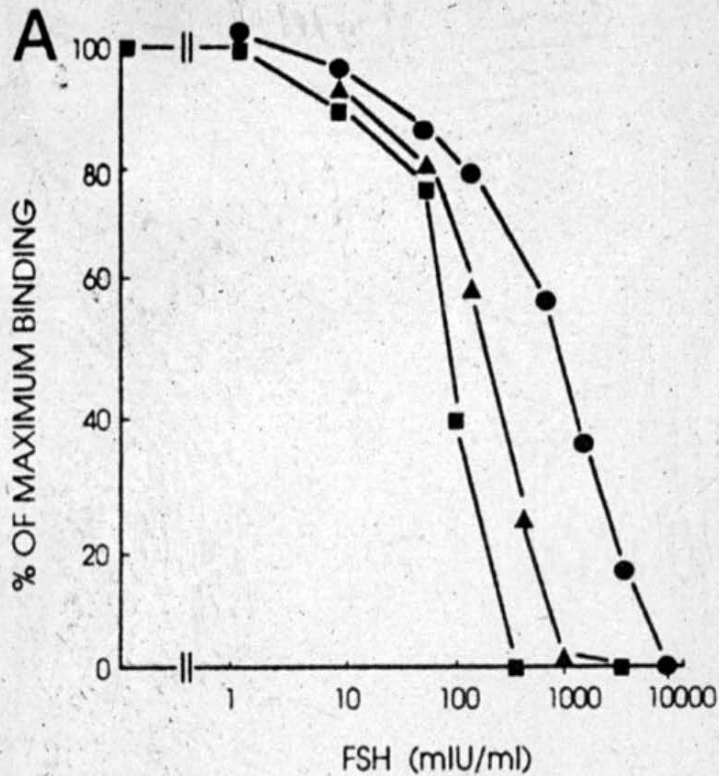
# Assembly of Subunits



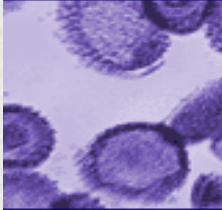
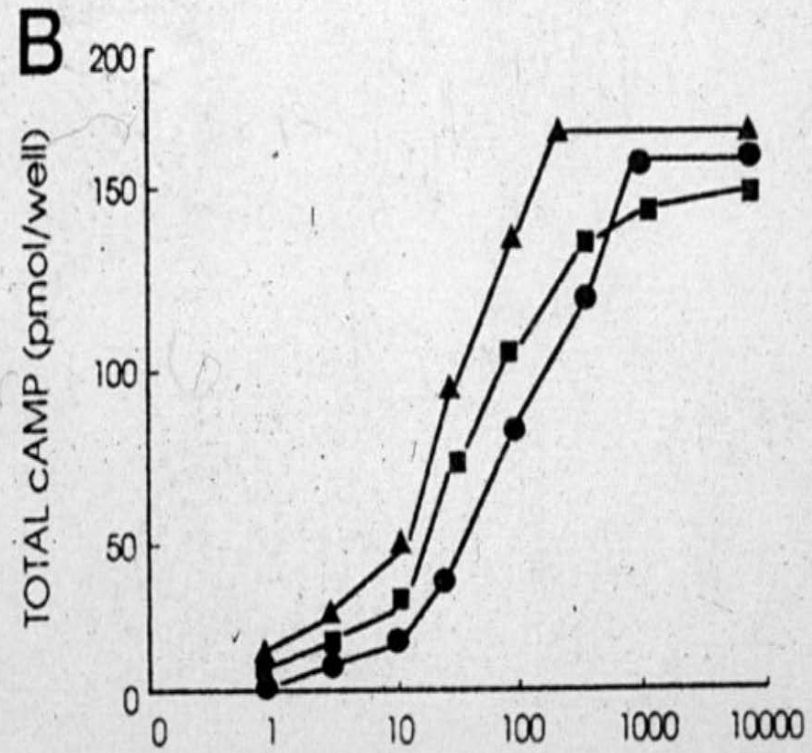


# *in vitro Studies*

## Receptor binding



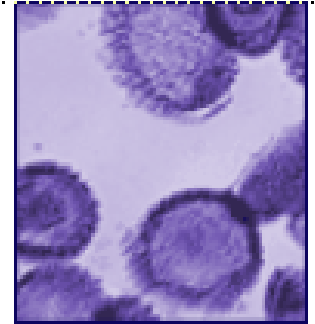
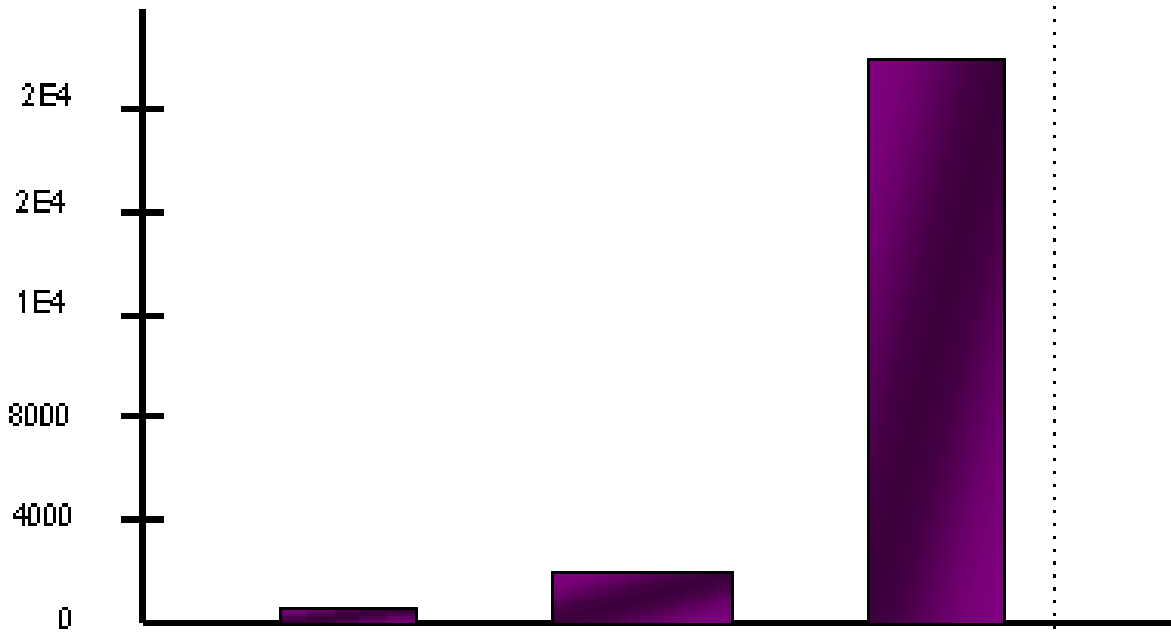
## Biological Activity



# Biological Activity, *in vivo*

10 IU / 24h x 48h (IP)

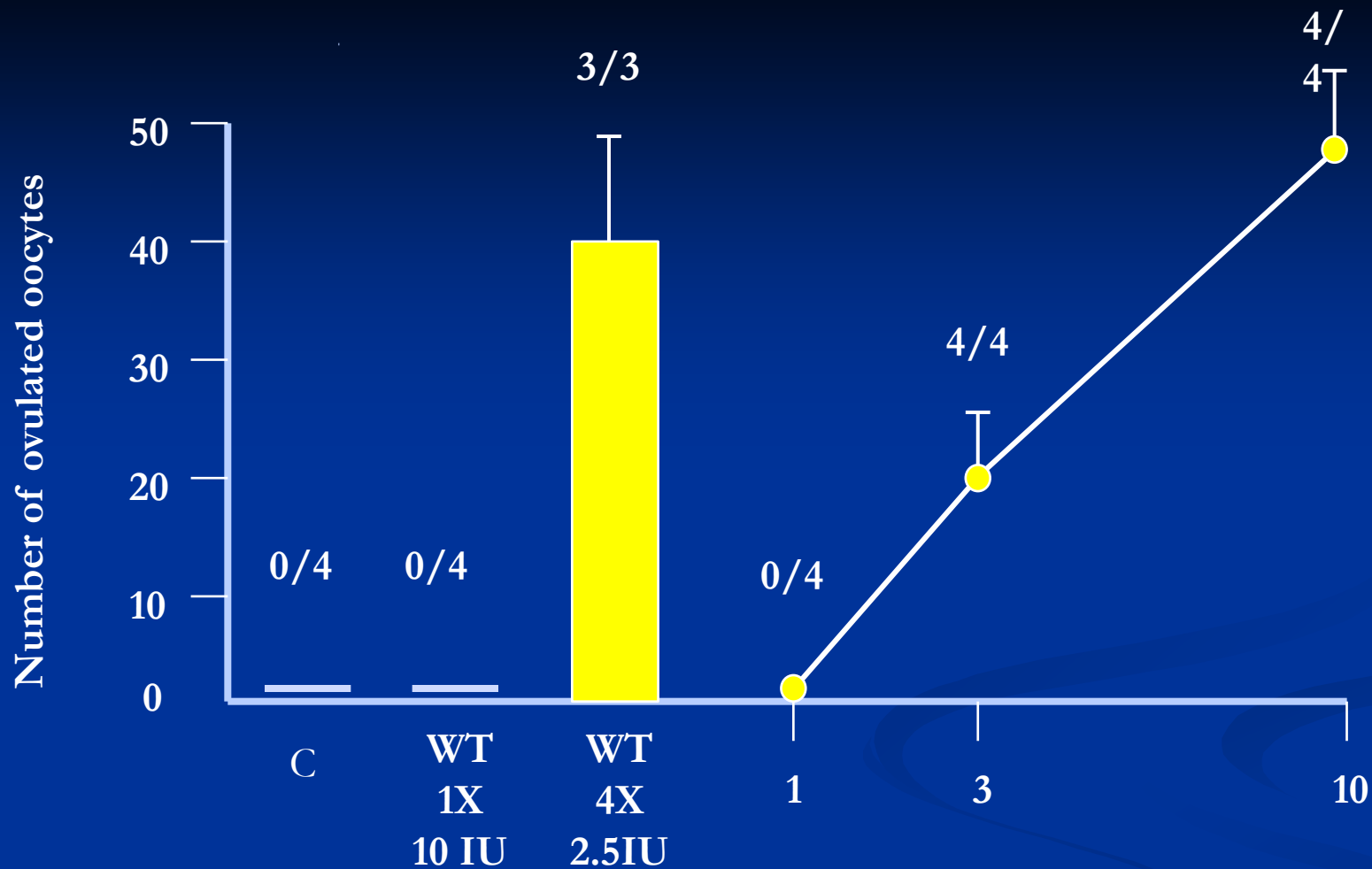
Estrogen (pg/ml)



Control

FSH-WT

FSH-CTP



**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

SerSerSerSerLysAlaProProProSerLeuProSerProSerArgLeu

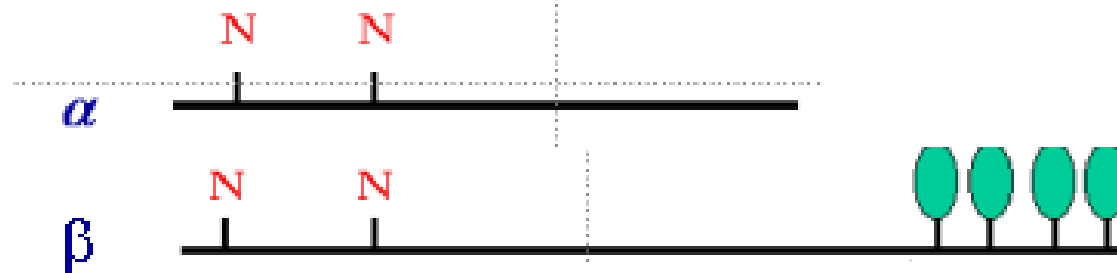
Diagram showing a sequence of amino acids with three oxygen atoms (O) positioned above the 4th, 7th, and 10th positions (Ser, Ser, Ser, Ser, Lys, Ala, Pro, Pro, Pro, Ser, Leu, Pro, Ser, Pro, Ser, Arg, Leu). Vertical lines connect the oxygen atoms to the 4th, 7th, and 10th amino acids. The 4th, 7th, and 10th amino acids (Ser, Ser, Ser) are highlighted in yellow.

Pro GlyProSerAspThrProIleLeuProGln

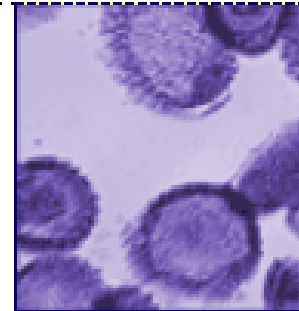
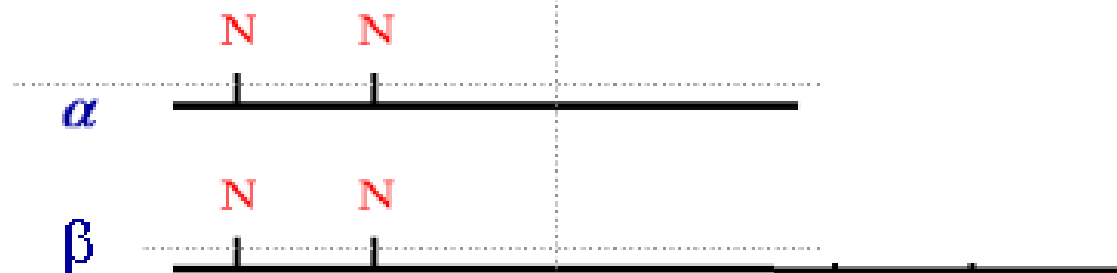
Diagram showing a sequence of amino acids with one oxygen atom (O) positioned above the 4th position (Ser). A vertical line connects the oxygen atom to the 4th amino acid. The 4th amino acid (Ser) is highlighted in yellow.

# The role of O-linked Oligosaccharides

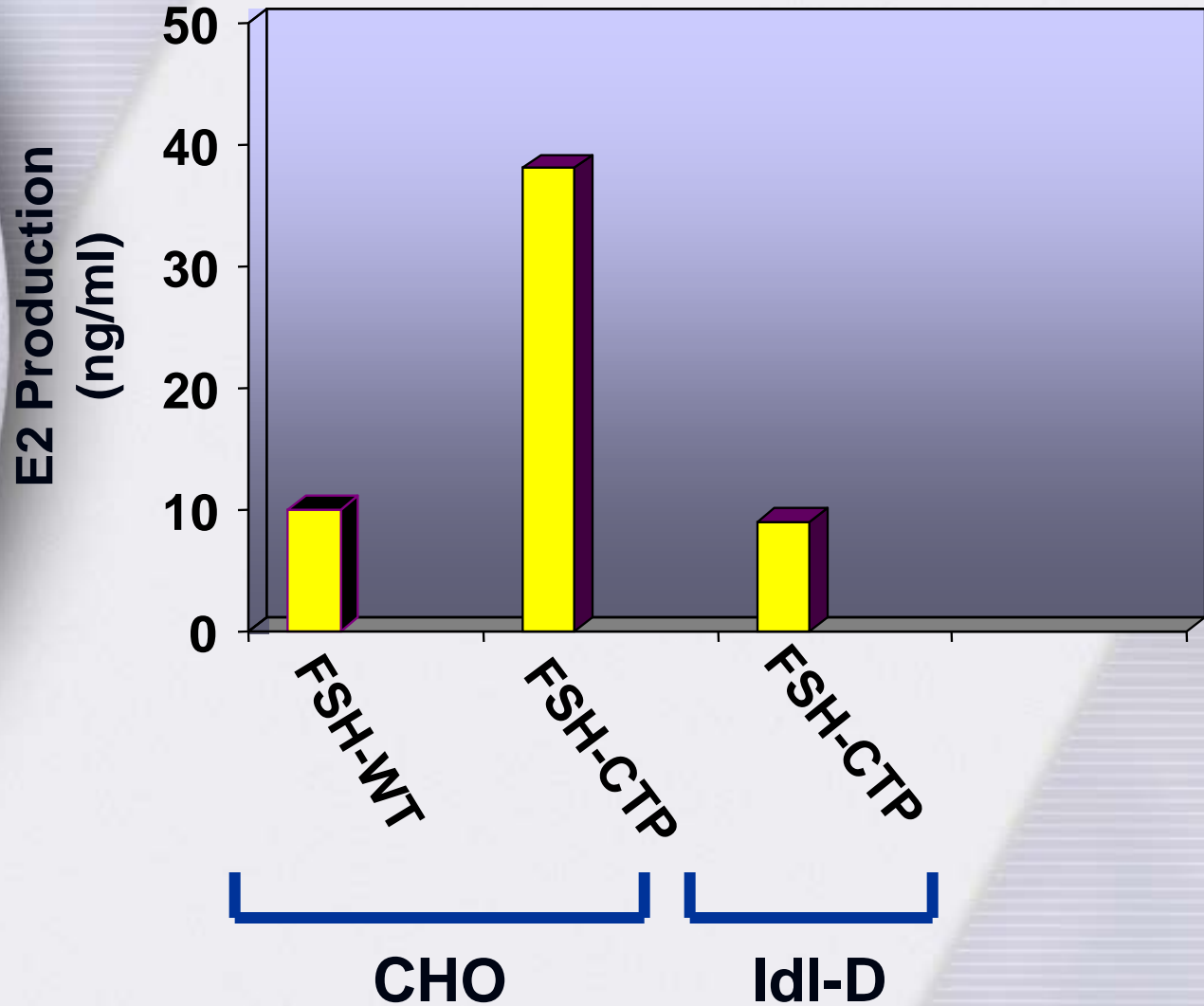
## FSH -CTP



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**

## 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

The logo for ModiGene is centered on a white rectangular background. The word "Modi" is in a black, sans-serif font. The letter "g" is a large, stylized purple character with a thick stroke. The word "ene" is in a black, sans-serif font, matching "Modi". The background of the slide features a complex, 3D molecular structure with red and purple lines and spheres, suggesting a scientific or biotech theme.

ModiGene

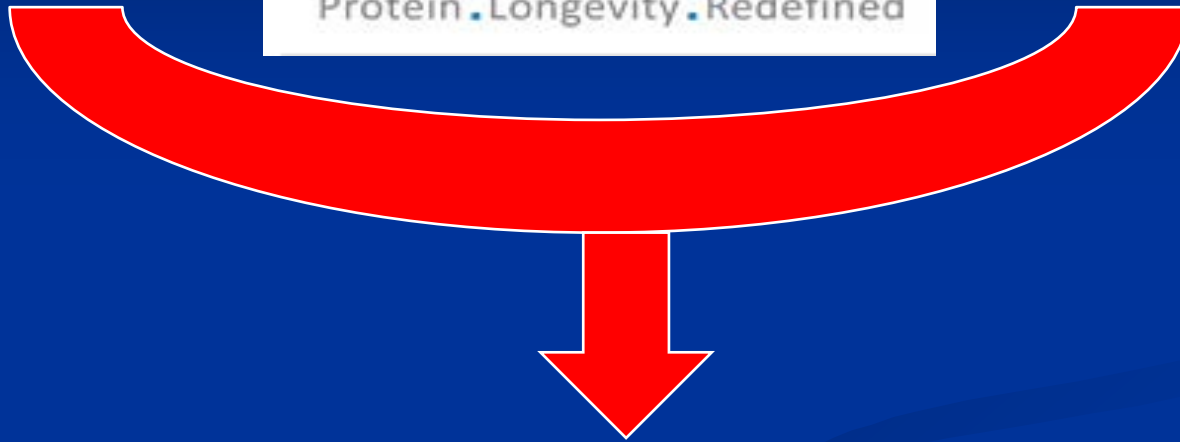
**“Enhancing the potency and longevity  
of highly valuable proteins”**

# Start Up Company



## 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

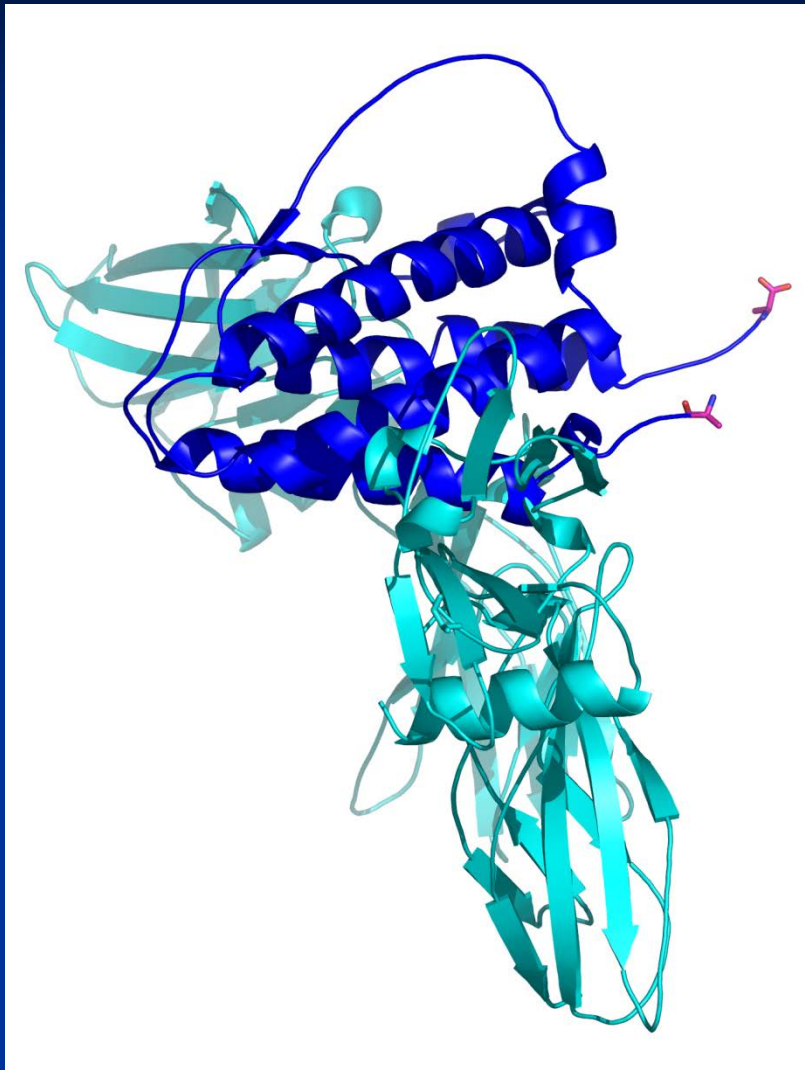
- Erythropoietin
- Growth Hormone
- Interferon
- Factors, XI & VII
- Short Peptides

# *Erythropoietin (EPO)*

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



# 3 - D Structure Analysis



C-term

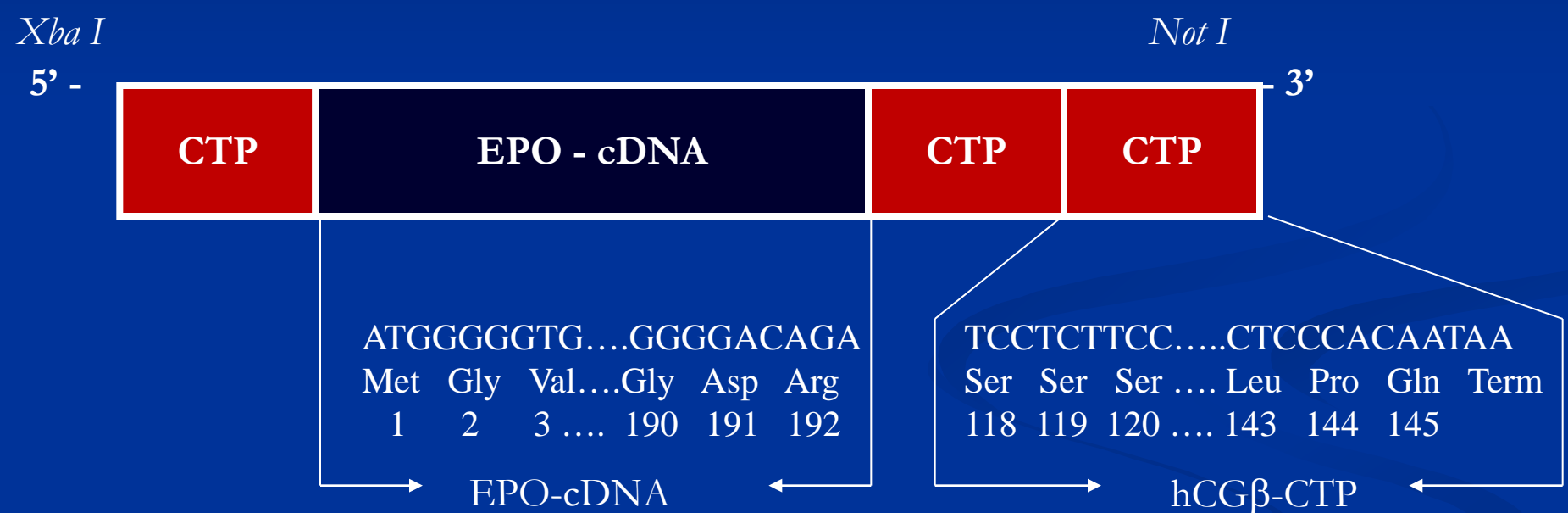
N-term

**Human  
Erythropoietin  $\alpha$   
(blue) with its  
Receptors (cyan)**

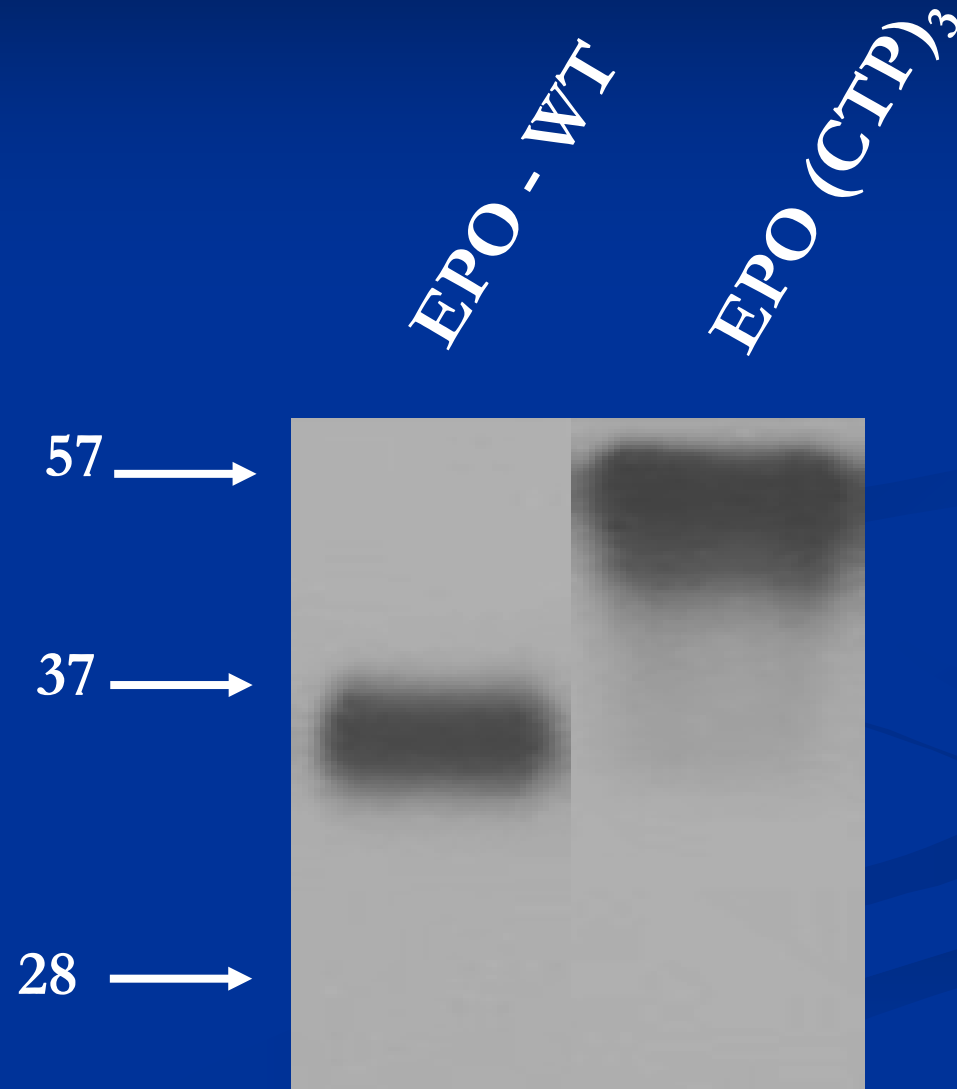
**Conclusion:**

**Strands of both termini are fairly long and accessible.**

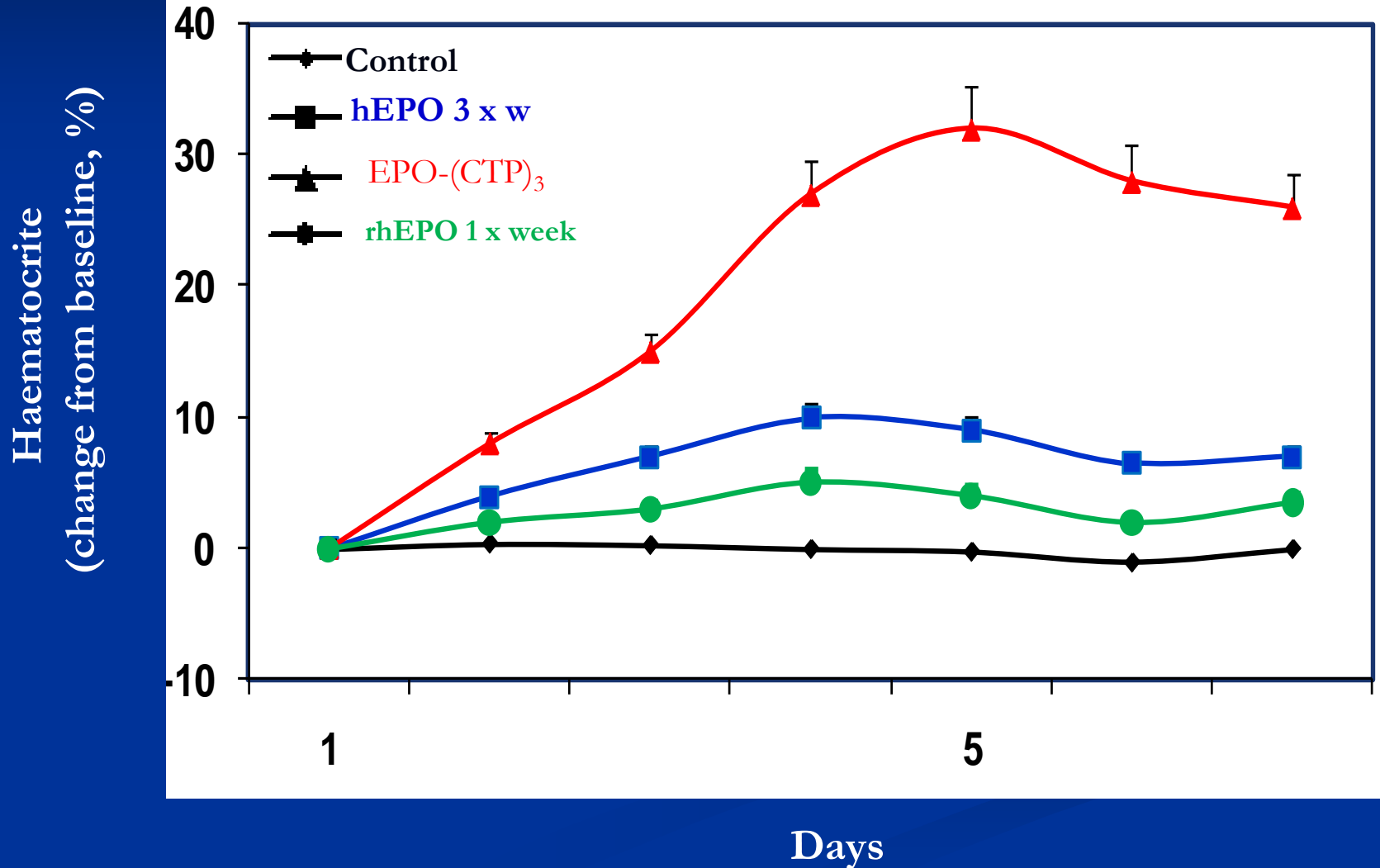
# Human EPO-CTP3



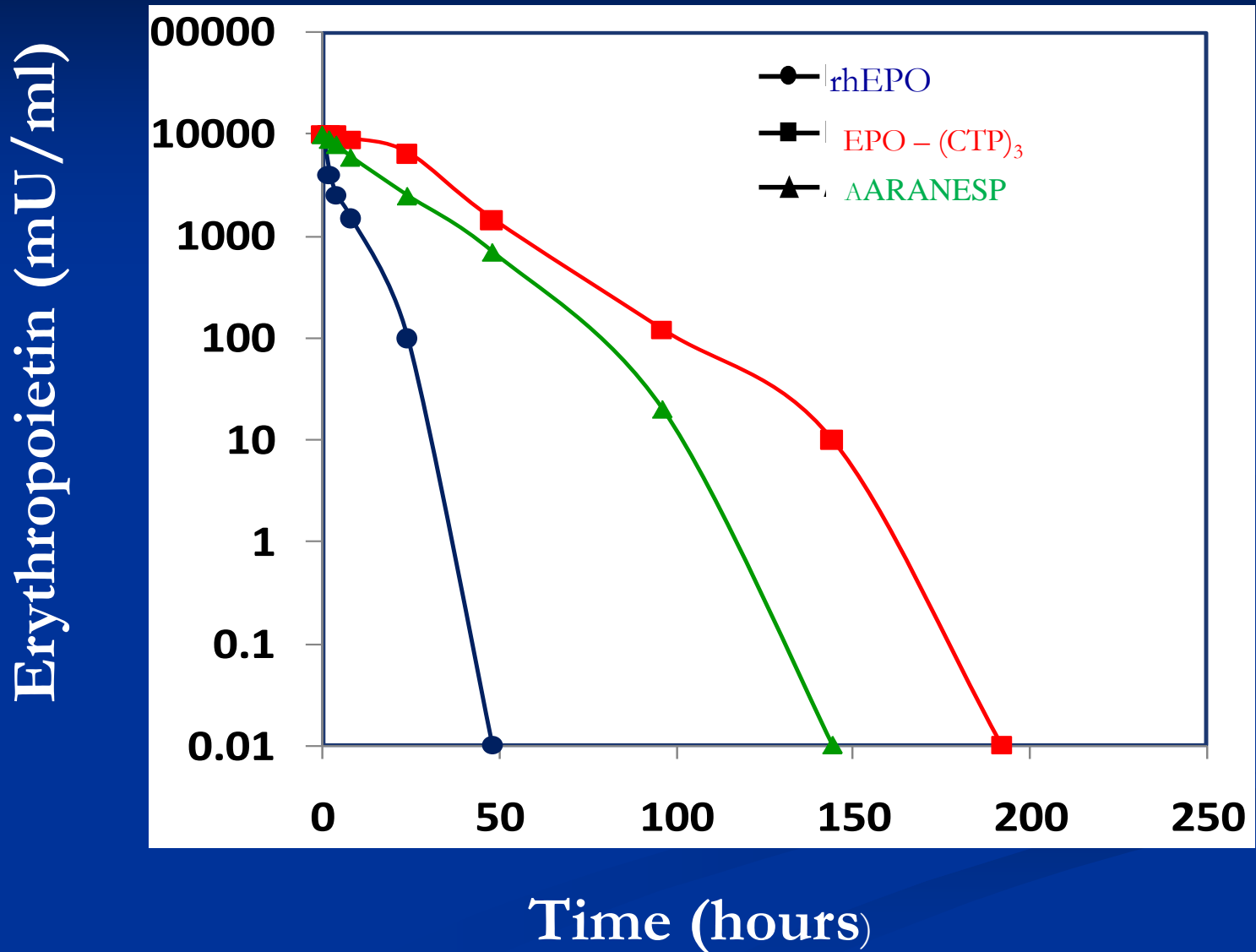
# Human EPO-CTP3



# Human EPO-CTP3

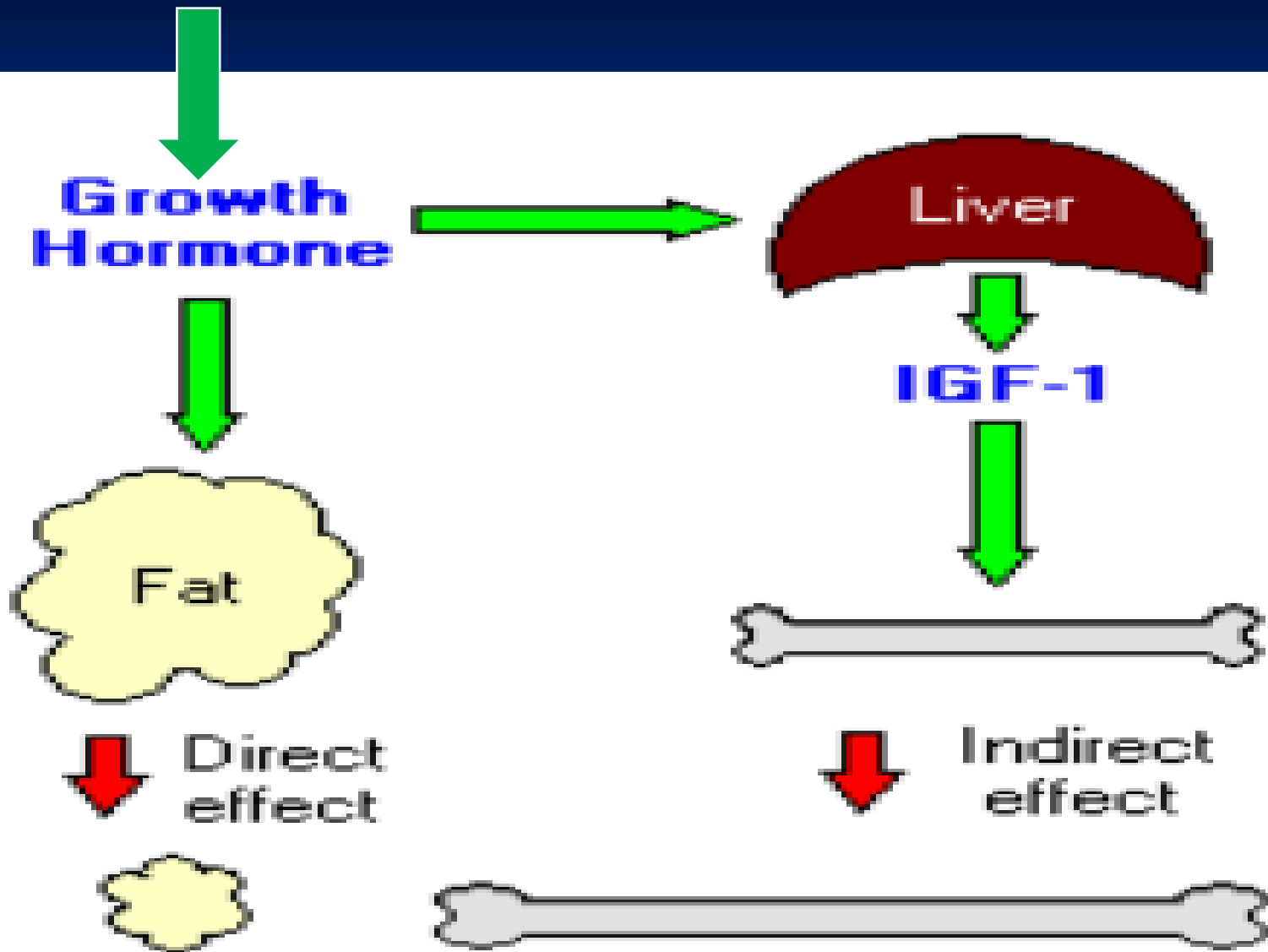


# Human EPO-CTP3



# Human Growth Hormone

# Pituitary





acquired gene: human growth hormone

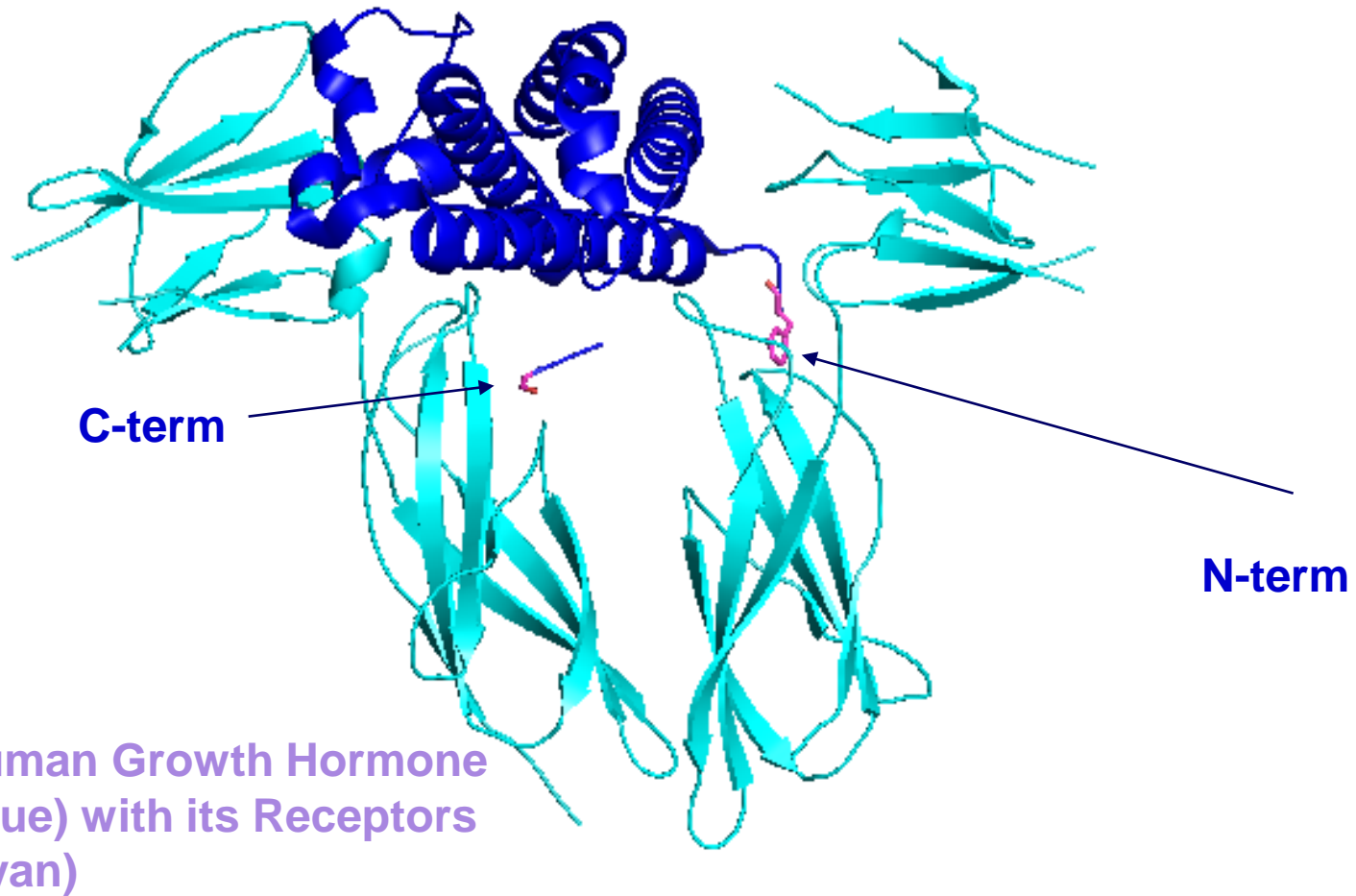




# Pharmaceutical and Biotechnological Uses of Growth Hormone

To treat children of pathologically  
short stature

# 3-D Structure Analysis



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

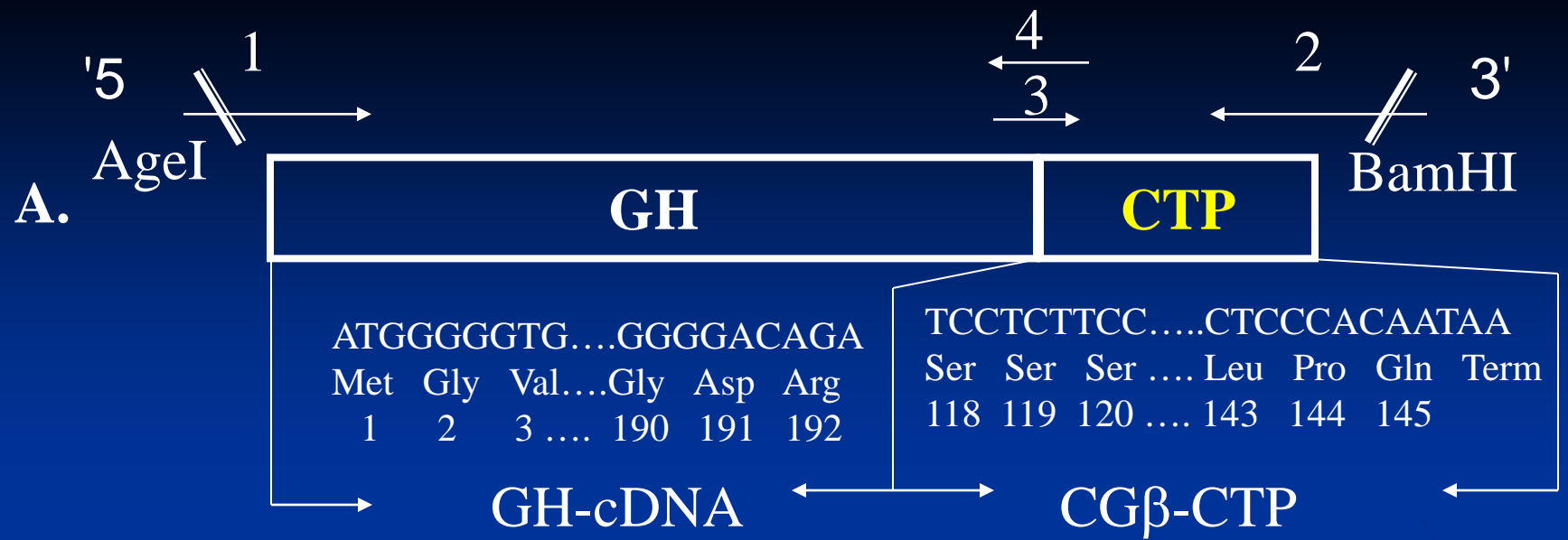
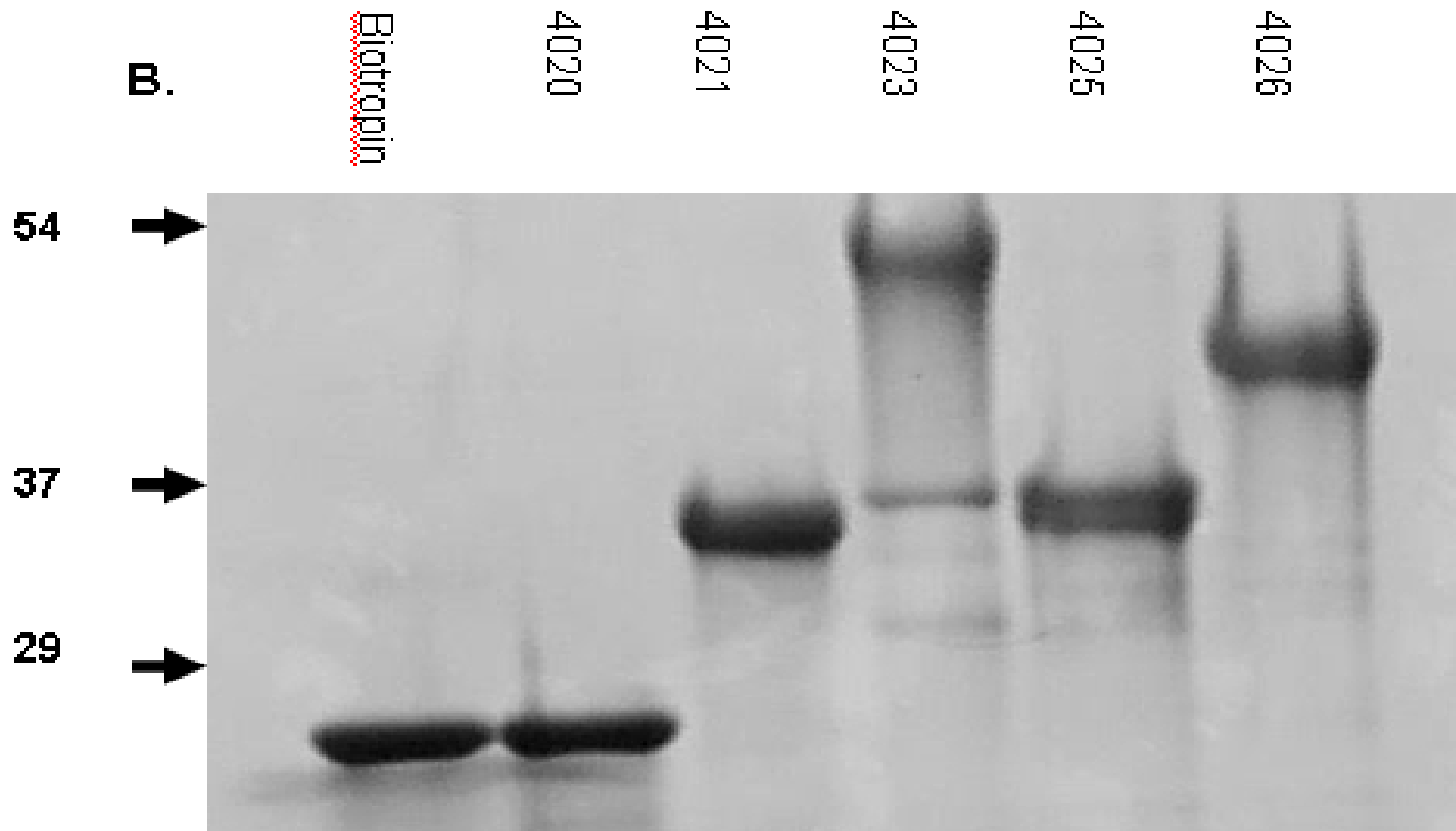


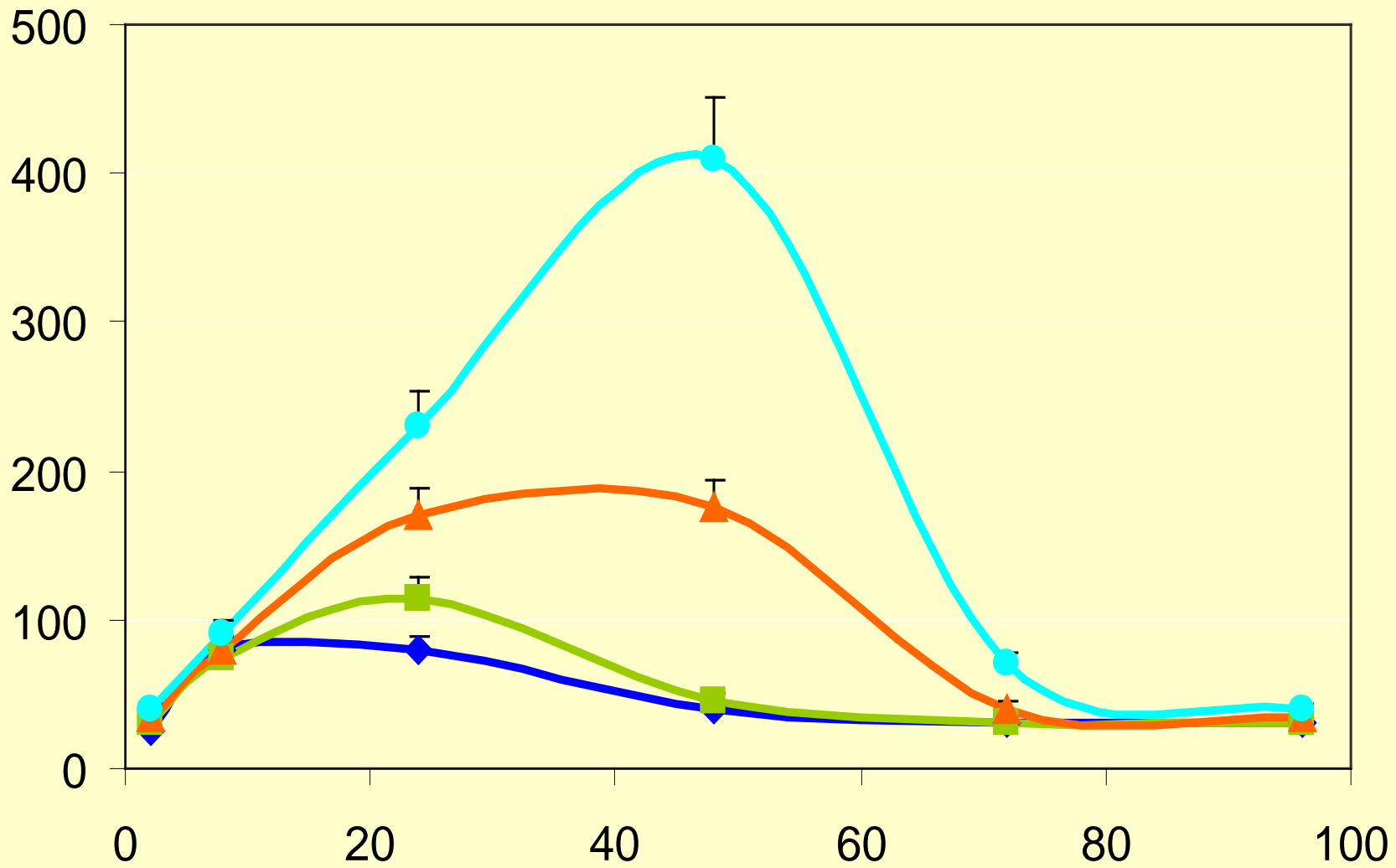
Fig.1.

# Secretion of GH Analogs from CHO cells

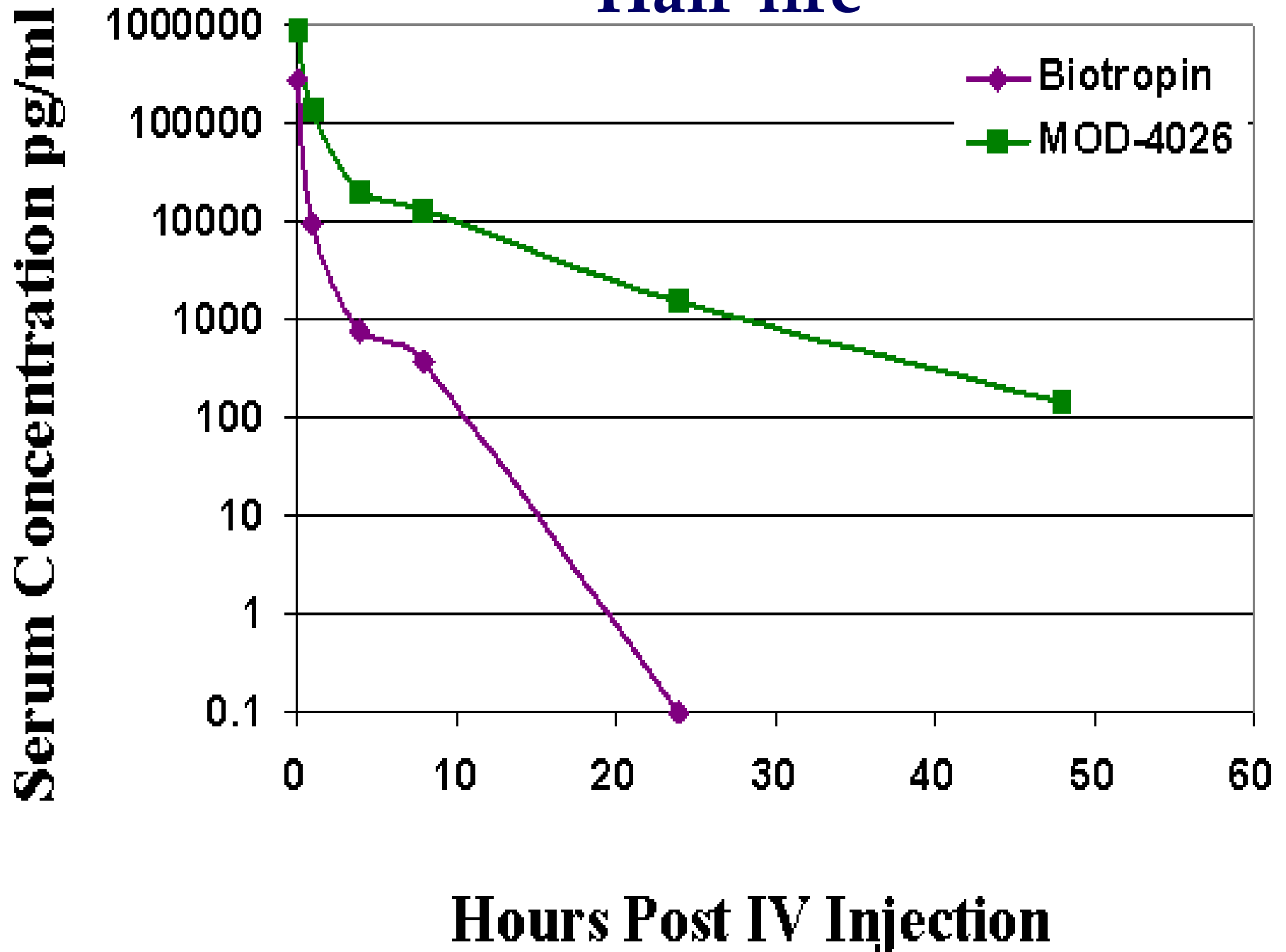


**GH – (CTP)3**

<b>CTP</b>	<b>GH</b>	<b>CTP</b>	<b>CTP</b>
------------	-----------	------------	------------



# Half-life



# GH – (CTP)<sub>3</sub>

- Experiments in Rhesus Monkeys and human clinical trials phase I that GH-Long-acting is safe and not immunogenic
- GH-(CTP)<sub>3</sub> 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

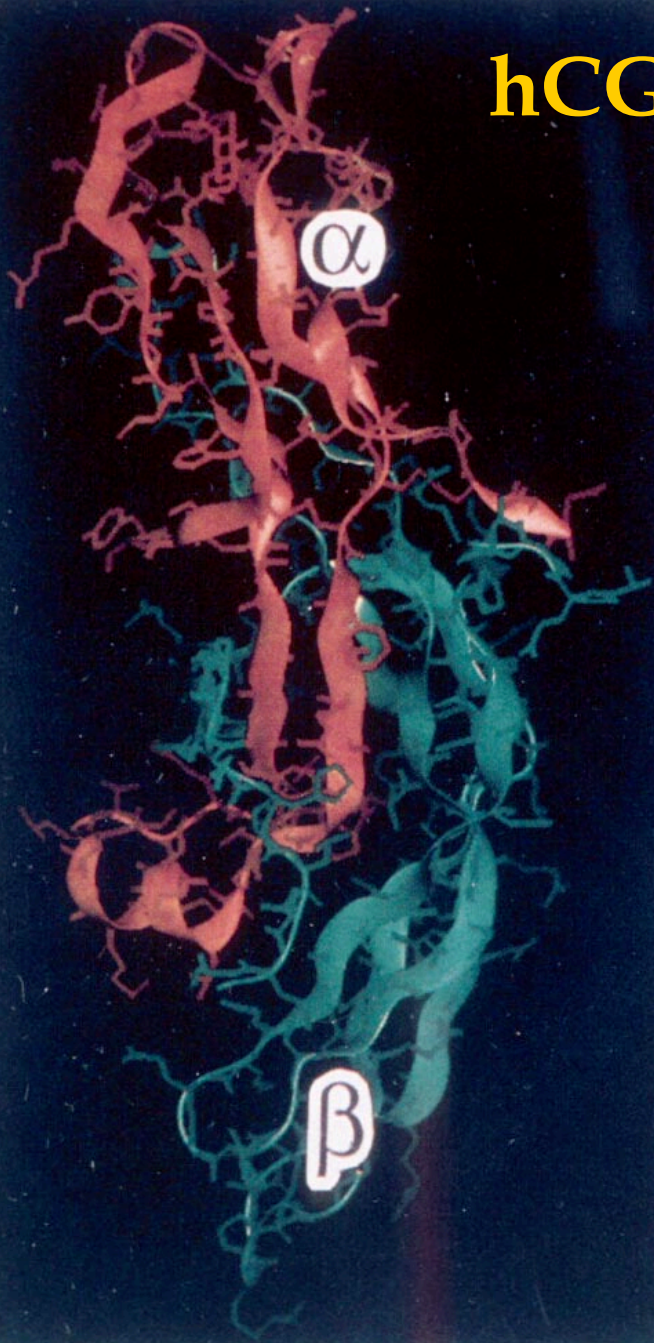
**This may allow reducing :**

**A) Drug dose**

**B) Number of injections**

# TSH Studies

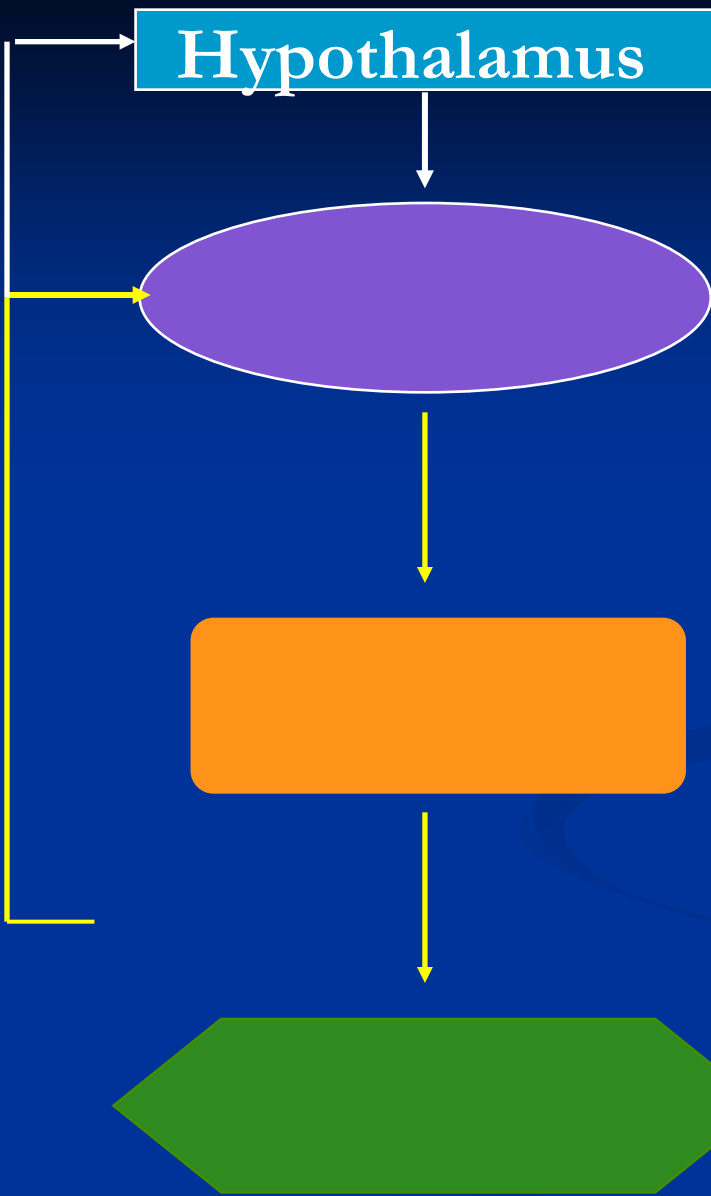
**hCG**



**hTSH**



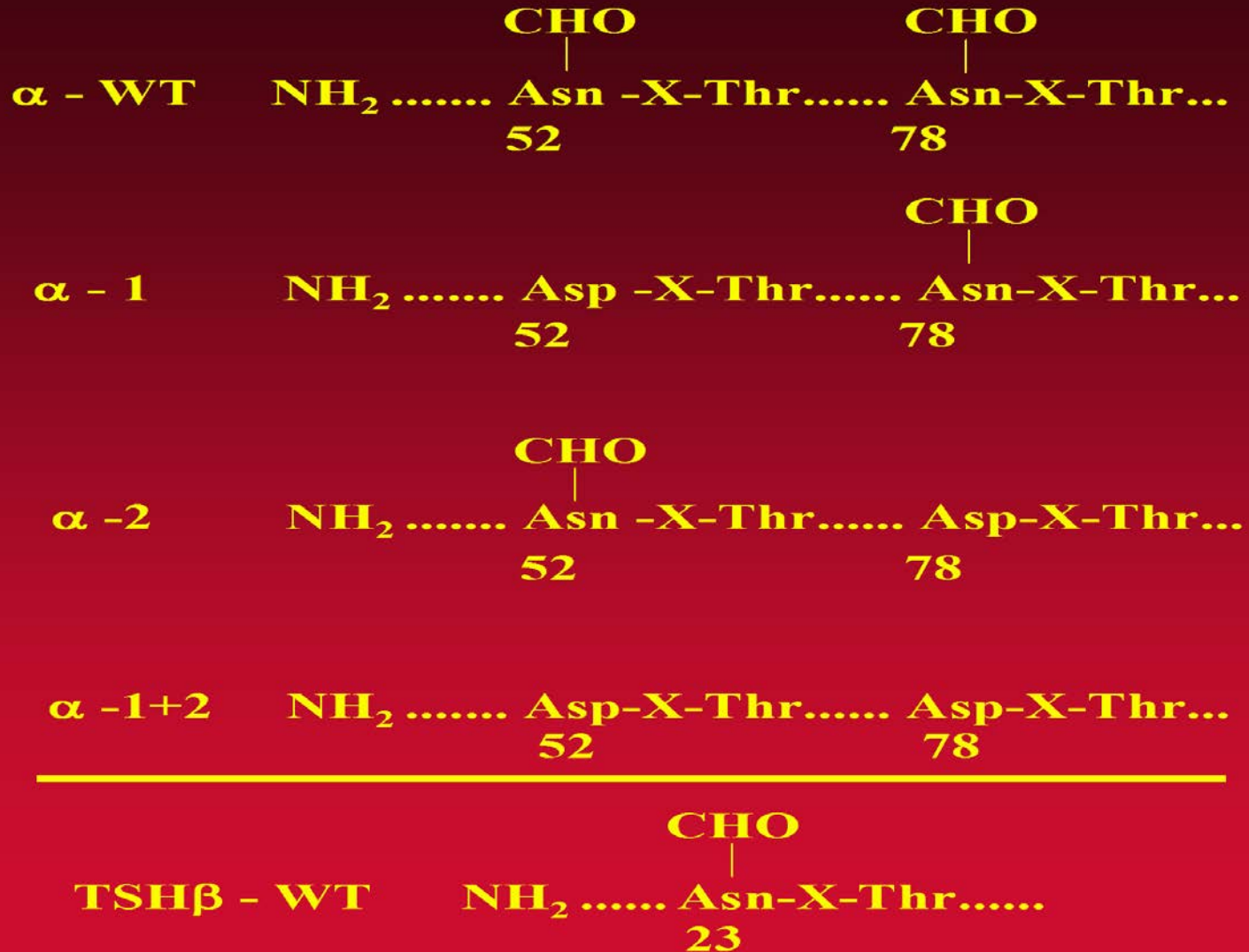
Hypothalamus



# TSH Subunits



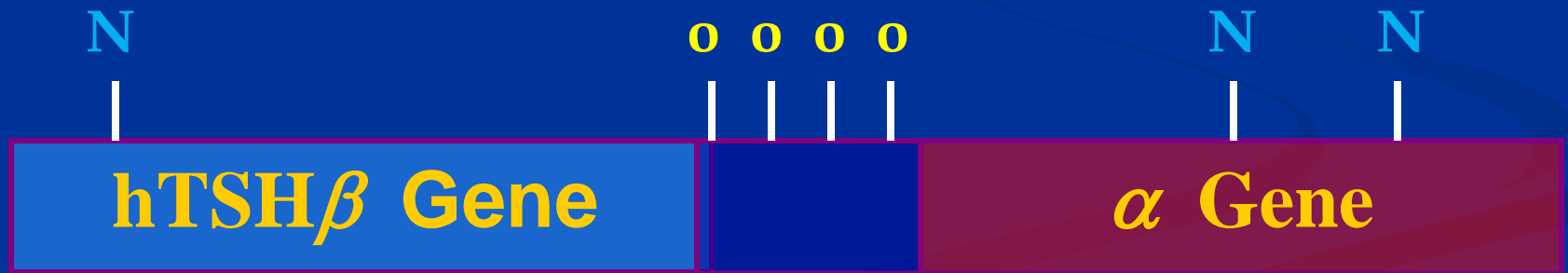
# hTSH Variants



# hTSH Single Chain



*hTSH $\beta$   $\alpha$*



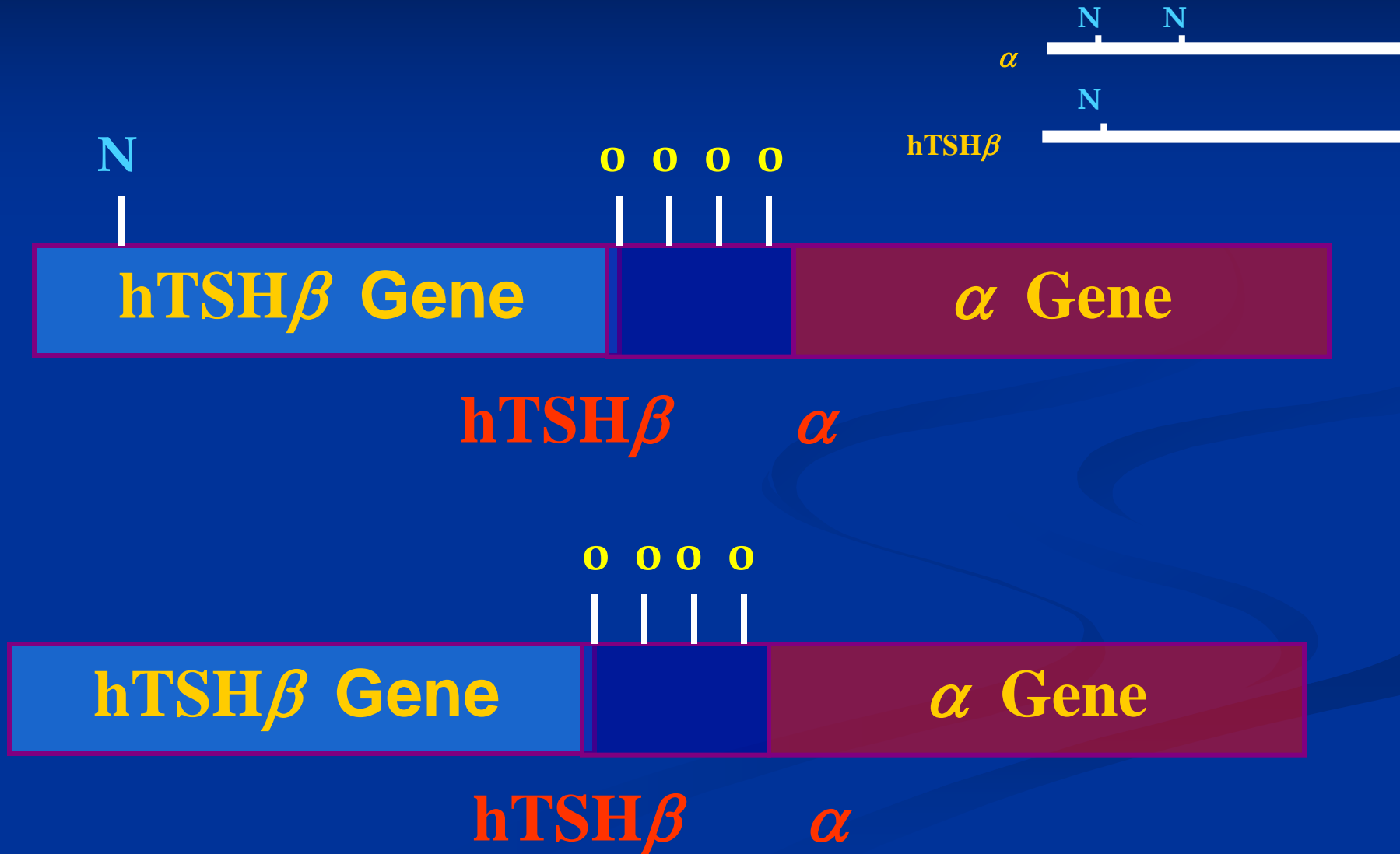
*hTSH $\beta$   $\alpha$*

# hTSH Single Chain

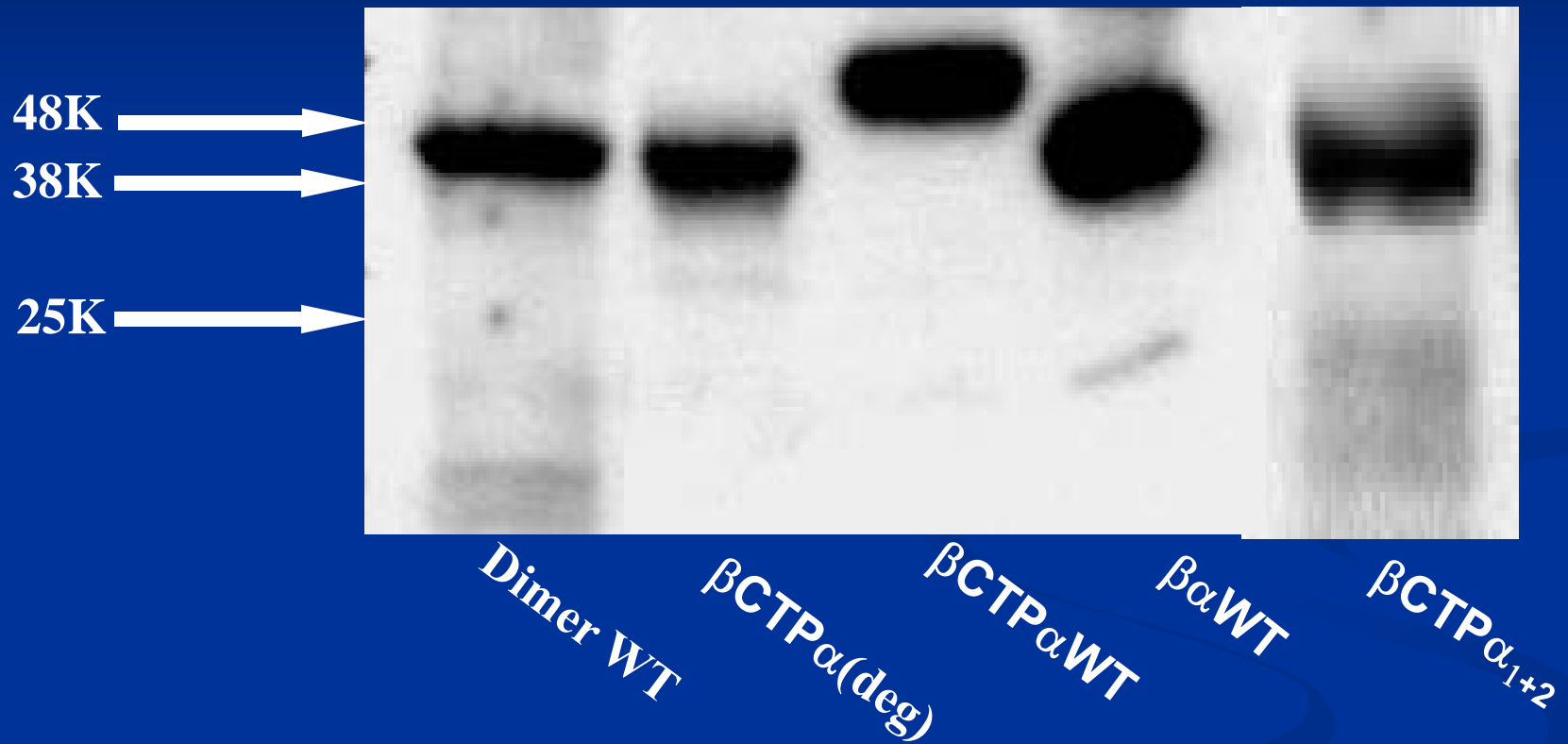
- ◆ **Expressed in CHO cells**
- ◆ **Binds to TSH Receptor in high affinity as well as the TSH-WT**
- ◆ **Biologically active**



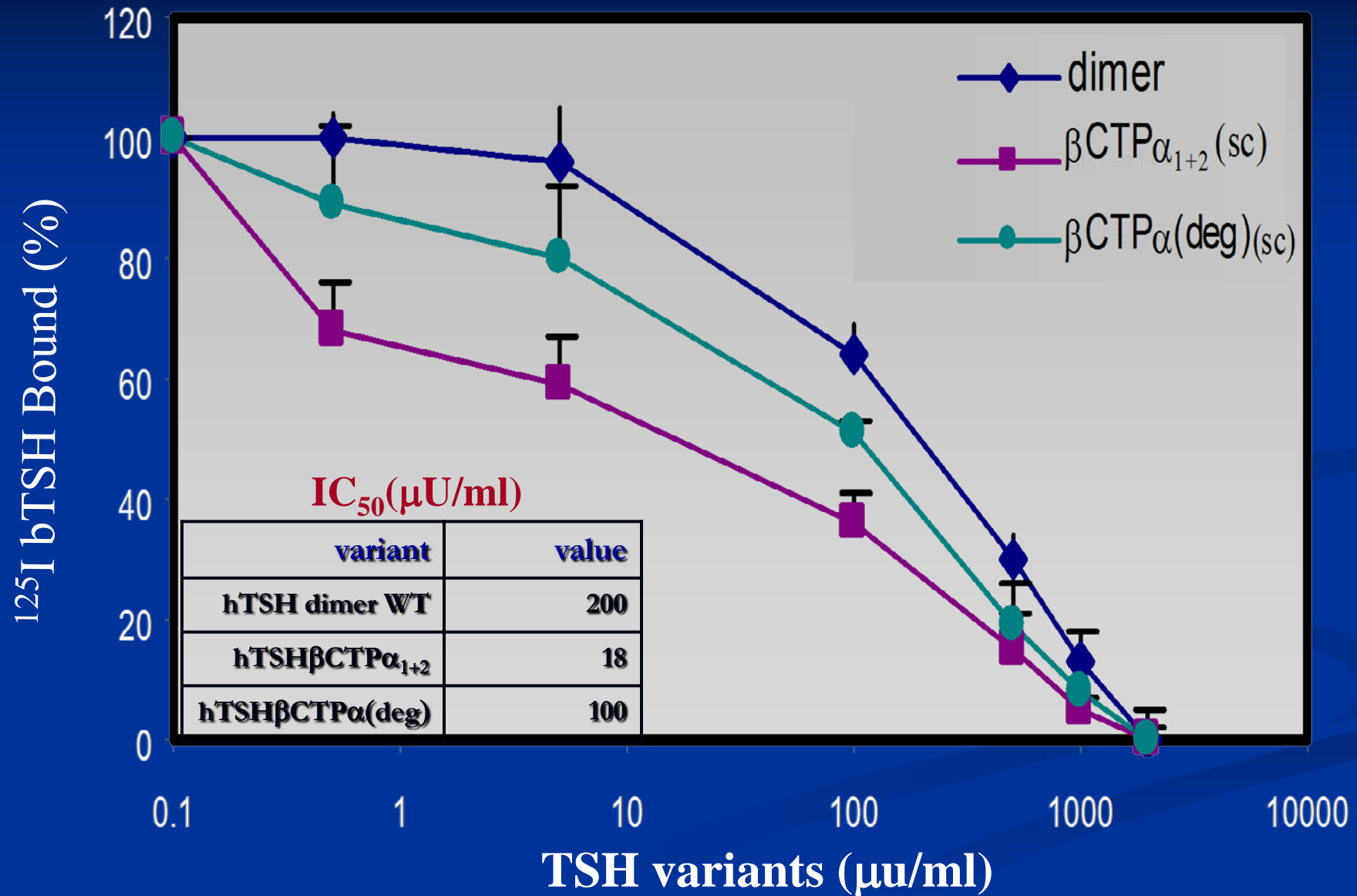
# *hTSH – Single Chain Variants*

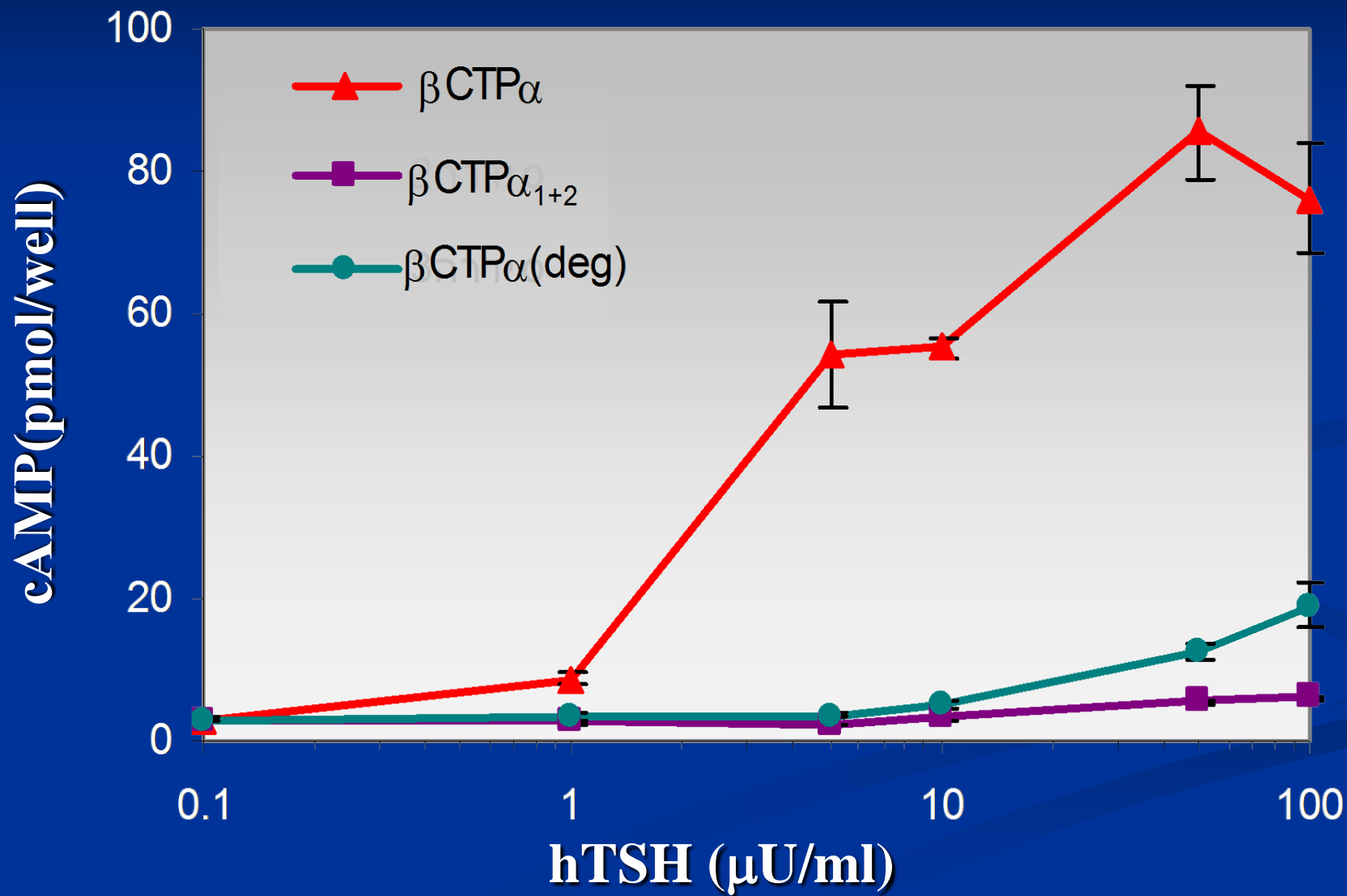


# Secretion of TSH variants

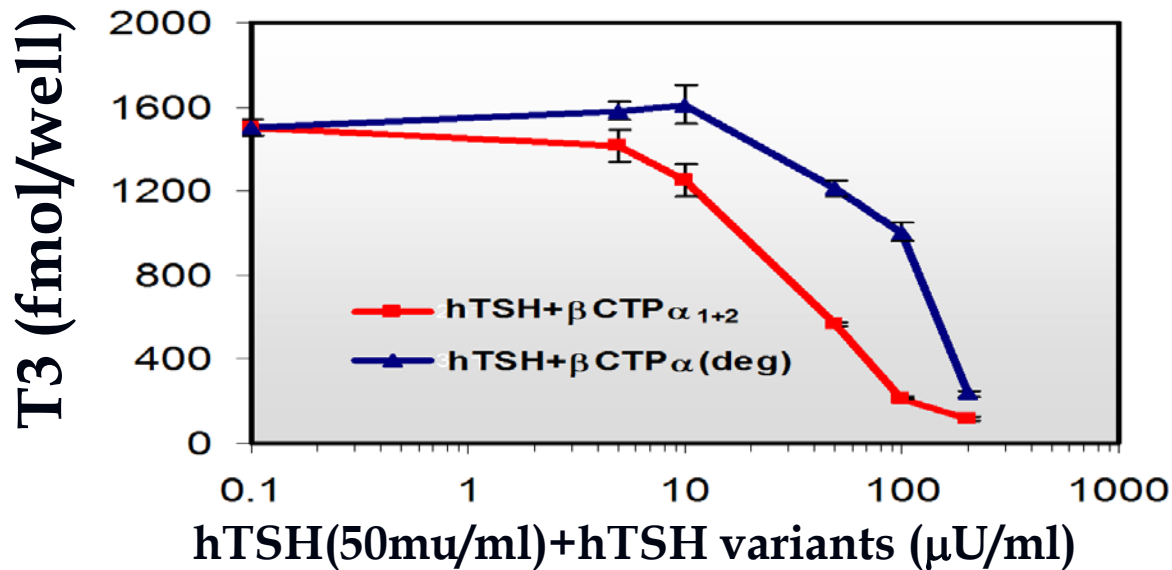
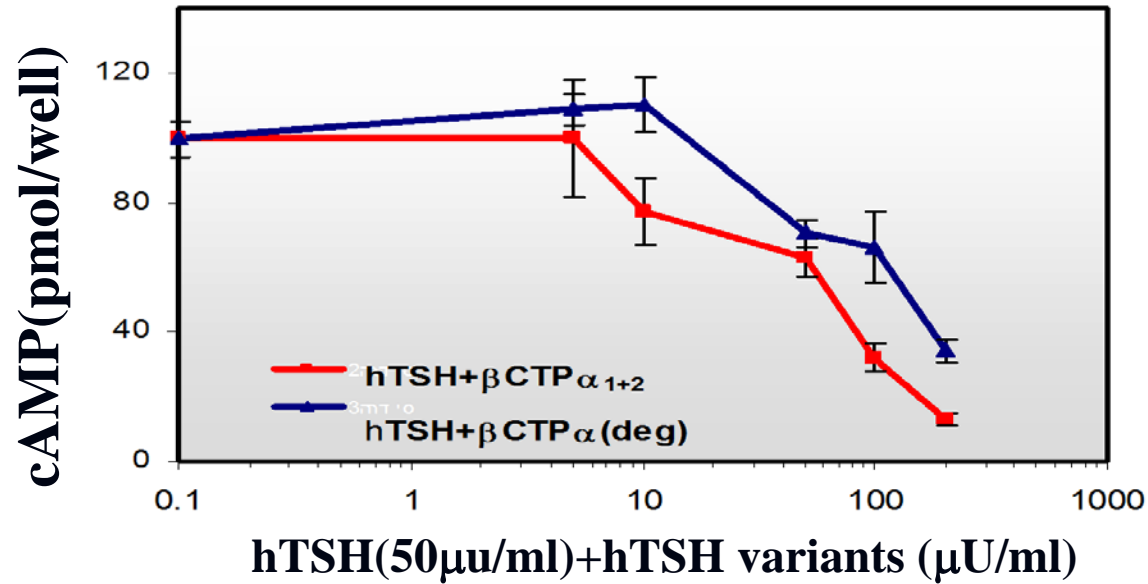


# Receptor Binding TSH (Mutants)





# TSH Antagonist



# Graves' Disease

Thyroid Stimulating Immunoglobolins  
(TSI)



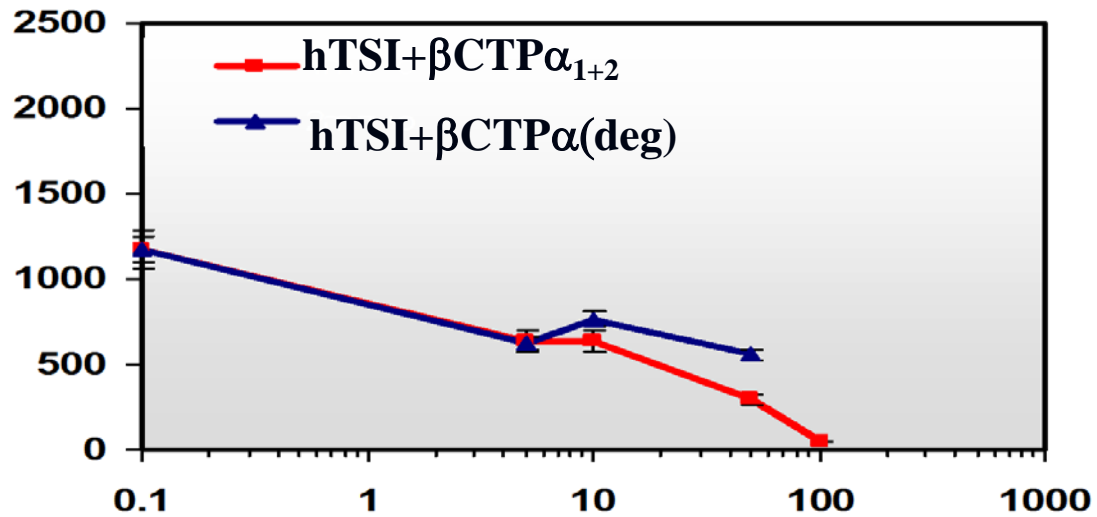
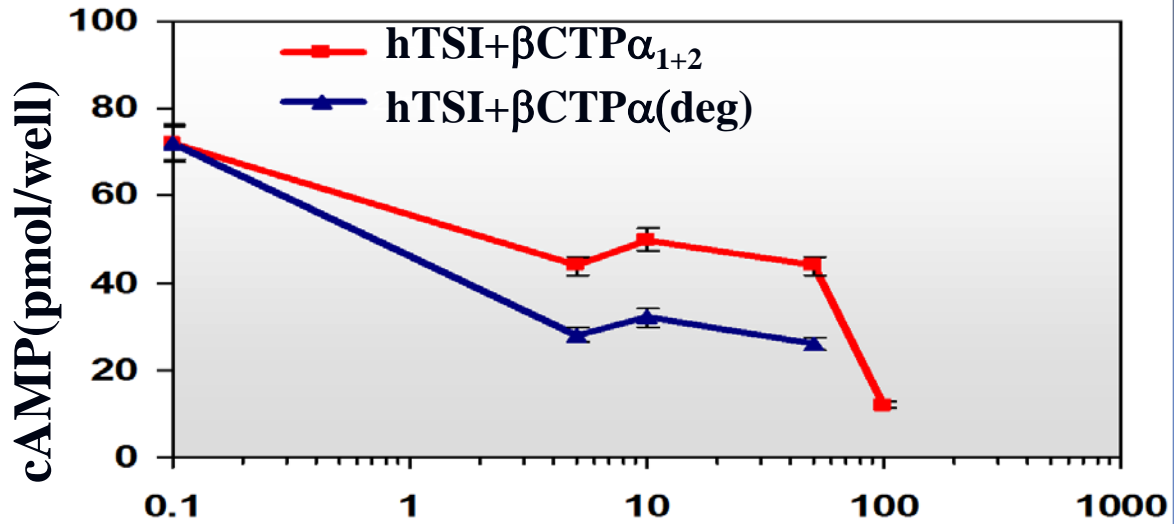
TSH Receptor



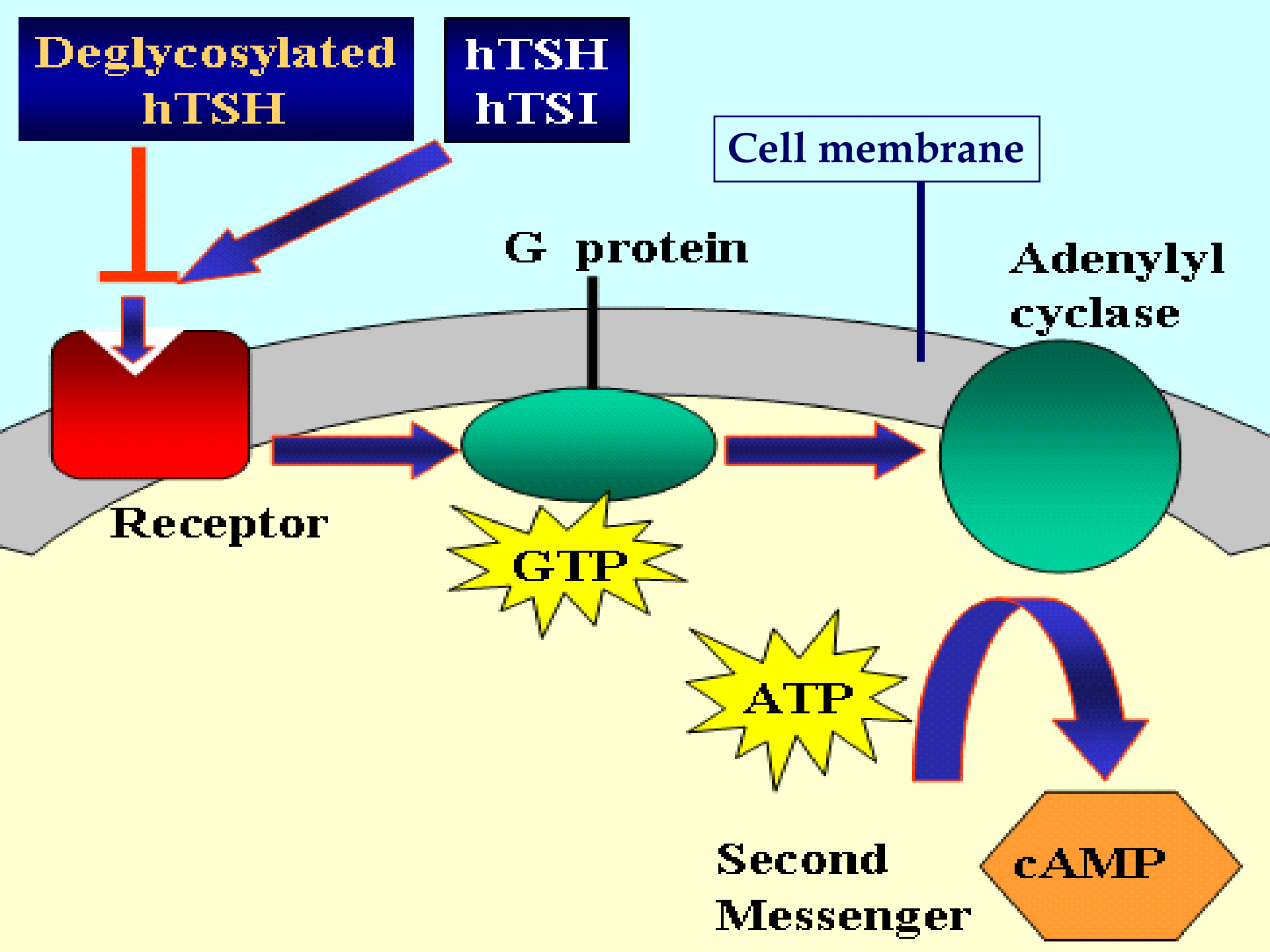
Hyperthyroidism



# TSI Antagonist



hTTSI (0.75μu/ml)+hTSH variants (μu/ml)





# *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.**

**Prof. Zaki Kraiem**

**Prof. Irving Boime**

**Prof. Aaron Hsueh**

**Dr. Naeil Azam**

**Dr. Avri Havron**

**Orit Sadeh**

**Dr. Eyal Fima**

**Flonia Levi**

**Rinat Alenberg**

**Mr. Shai Novik**

**Dr. Sharif Ganim**

**Dr. Taleb Hajoj**

# Clinical Advisory Panels

## World Known Opinion Leaders

### ■ hGH

- Ron Rosenfeld, MD, Stanford
- Barry Sherman, MD, Genentech, BiPar
- Zvi Zadik, MD, Hebrew University

### ■ EPO

- Allen Nissenson, MD, UCLA
- Anatole Besarab, MD, Henry Ford, Detroit

### ■ Interferon-beta

- William Mobley, MD, Stanford
- Hillel Panitch, MD, Vermont
- Ron Milo, MD, Israel

- National Institutes of Health (NIH)
- The Rockefeller Foundation
- United States - Israel Binational Science Foundation (BSF)
- Israel Science Foundation (ISF)
- The Israel Ministry of Science
- The Israel Ministry of Industry and Trade
- Private Investors



Haifa

Thank You for your attention

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International

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