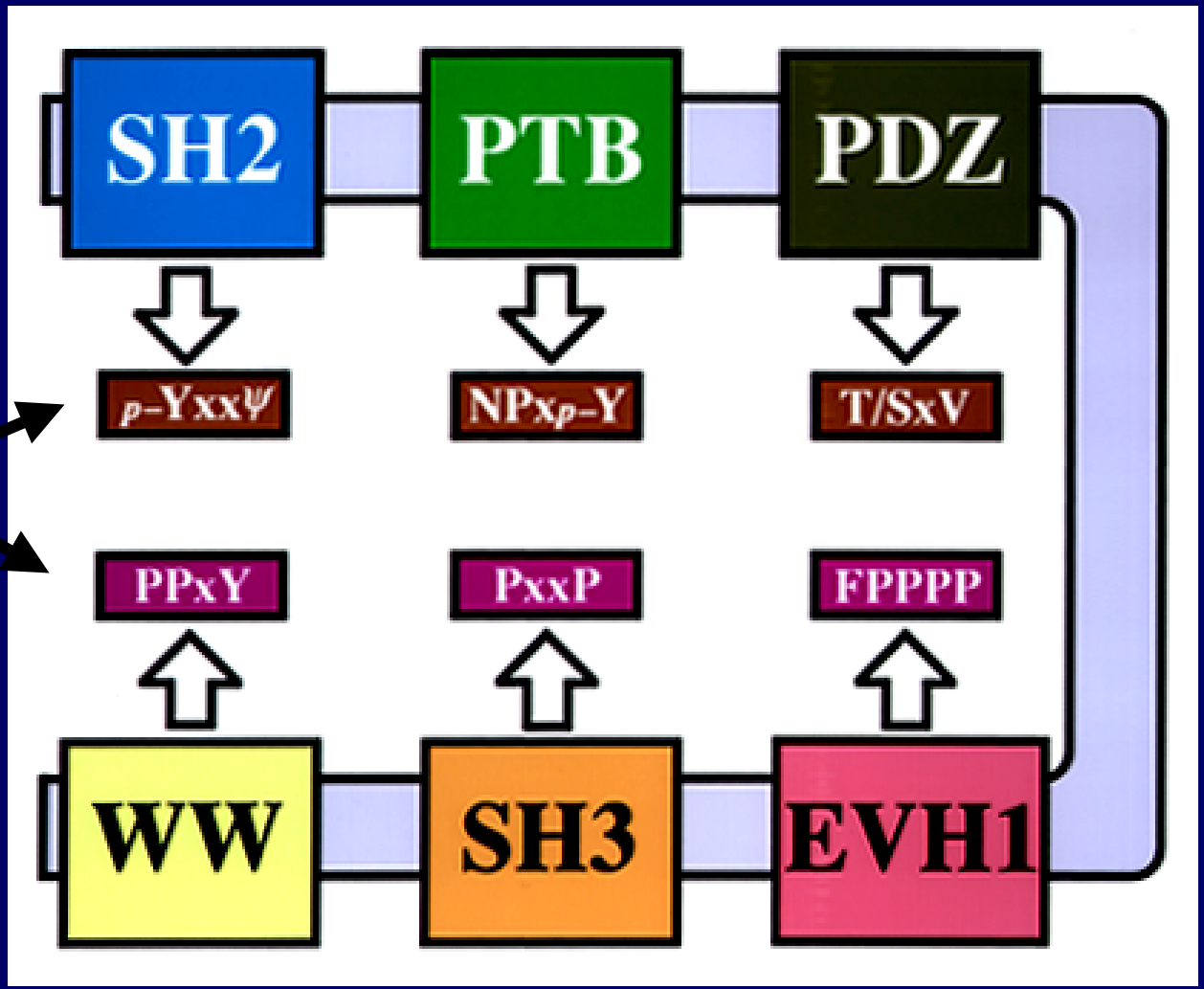
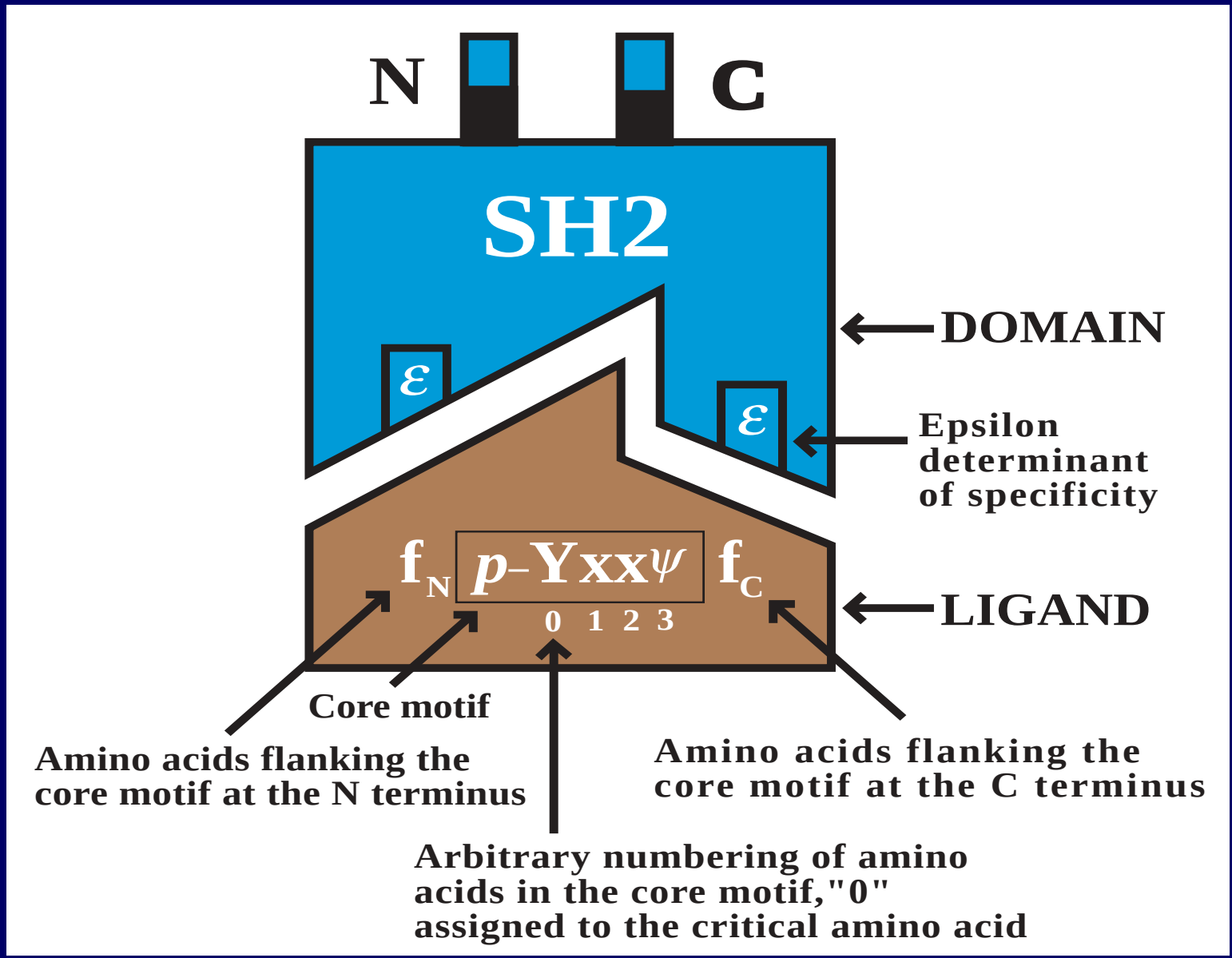
# PROTEIN DOMAINS AS BEADS ON A STRING

COGNATE MOTIFS



# SH2 DOMAIN AS A PARADIGM OF PROTEIN MODULES



# Domains and Diseases
## Mutations in Domains and Ligands

- Mutations in the SH2 domain of Bruton's tyrosine kinase cause a hereditary immunodeficiency, X-linked agammaglobulinemia - XLA (Mattsson et al., J. Immunol. 2000, 164, 4170)

- Point mutations in ENaC subunits cause Liddle's Syndrome of hypertension (Shimkets et al.,Cell, 1994, 79, 407)

# Domains and Diseases

## Changes in a Gene Product Affect the Domain or its Cognate Ligand Indirectly

- Huntington's disease is a neurodegenerative disease caused by mutated huntingtin gene with poly Q expansion in the huntingtin protein. Poly Q repeats are juxtaposed with polyprolines.
- Huntingtin with poly Q expansion interacts preferentially with WW domain-containing proteins involved in RNA processing and transcription (Holbert et al., PNAS, 2001, 98, 1811; Passani et al., Hum. Mol. Genet. 2000, 9, 2175)

# Domains and Diseases

## Mutations within a Gene Encoding Domain

- Mutations in SH2D1A (gene encoding SH2 domain protein 1A) cause X-linked lympho-proliferative syndrome (Duncan disease) characterized by extreme sensitivity to EBV (Coffey et al., Nature Genetics, 1998, 20, 129)

- Mutations in the SH3 domain-encoding gene, NPHP1, casue juvenile nephronophthisis, a cystic kidney disease (Hildebrant et al., Nature Genetics 1997, 17, 149)

# Domains and Diseases
# Interventions - Inhibitors

- Non-peptide inhibitor of the Src SH2 domain corrects osteoporosis in animal models by inhibiting osteoclast-mediated bone resorption (Sawer et al., PNAS 2000, 97, 9373)

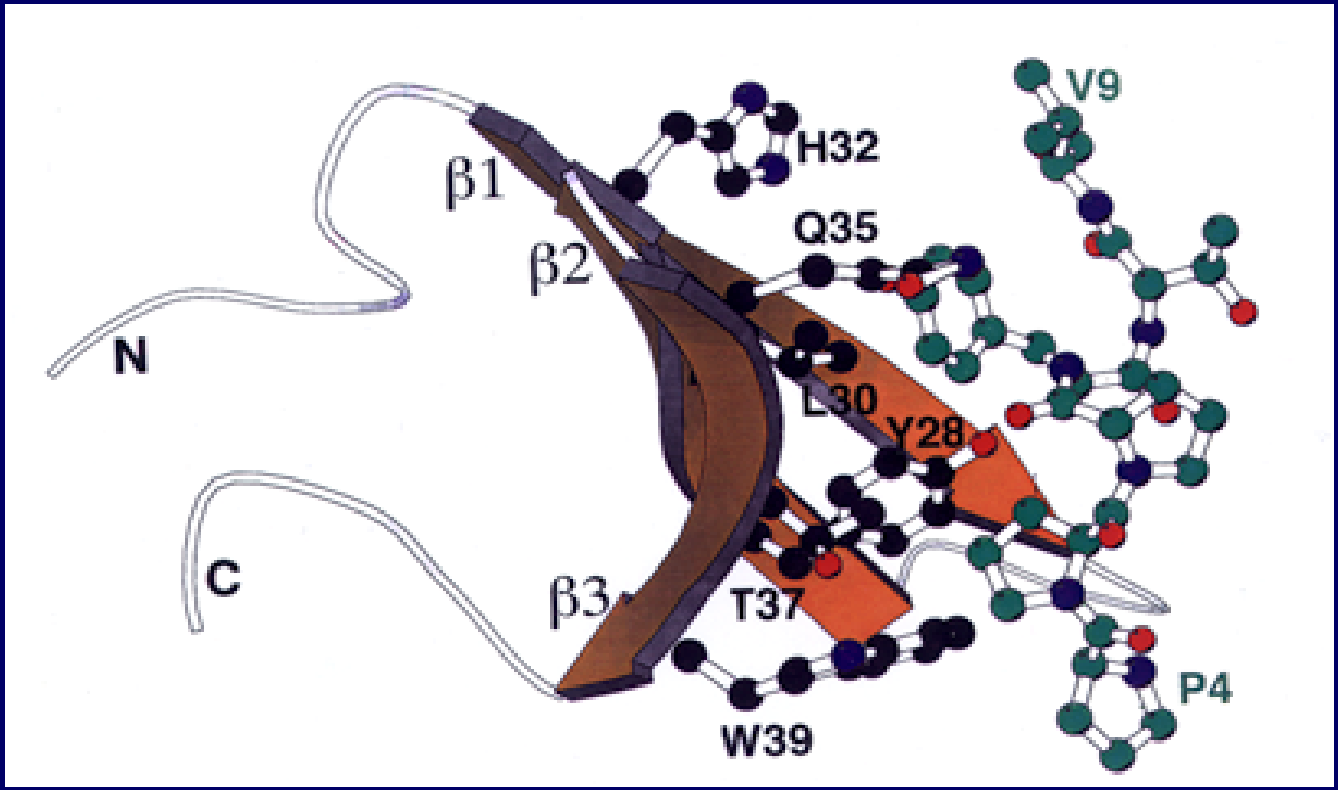
- FE65 with mutated WW domain inhibits A-beta peptide production in cell culture model (Sheinerman et al., unpublished, 2001)

# Domains and Diseases
# Tailoring New Domains

- Changing specificity of the SH2 domain of Src to that of Grb2 (Marengere et al., 1994, Nature, 369, 502)

- Changing specificity of the WW domain of YAP to that of FE65 (Espanel and Sudol, 1999, JBC, 274, 17284)

# STRUCTURE OF THE WW DOMAIN OF YAP IN COMPLEX WITH THE TARGET PEPTIDE




- Smallest globular domain
- $K_d$ of interaction =10-50 μM
- Phosphorylation of Y in PPPPPY motif disrupts the binding

# CONSENSUS SEQUENCE OF THE WW DOMAIN

**xLPtGWExxtttxGtxYYhNHxTtTTtWxtPtxxtxx**
**-------BBBBBB----BBBBBBBB---BBBBB----------**

**capital letters: conserved amino acids**
**h: hydrophobic**
**t: turn-like or polar**
**x: nonconserved amino acids**
**B: beta strand**

# MODULAR STRUCTURE OF PROTEINS CONTAINING THE WW DOMAIN

DYSTROPHIN/
UTROPHIN

RSP5

NEDD-4

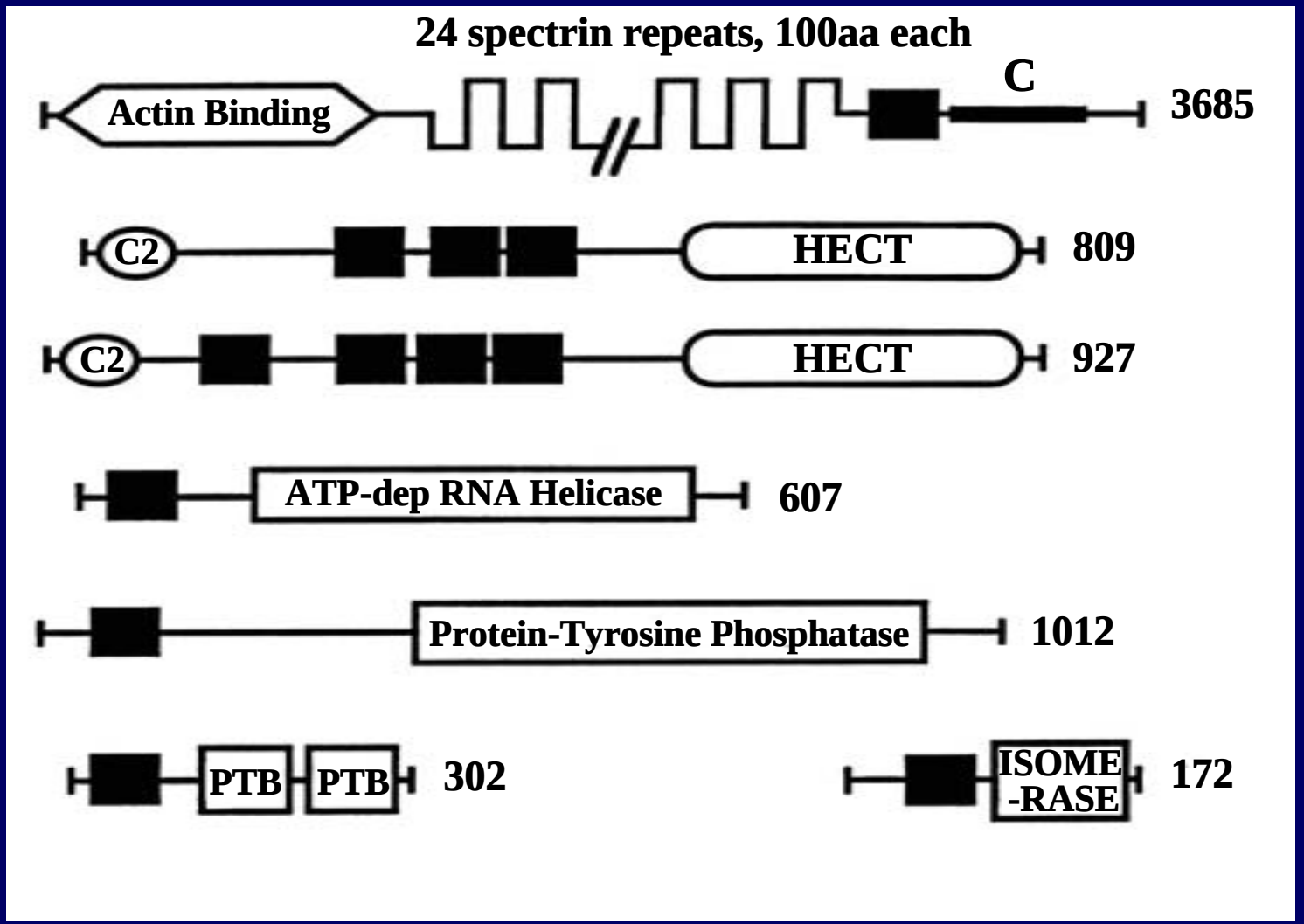
DB10

K010A

FE65

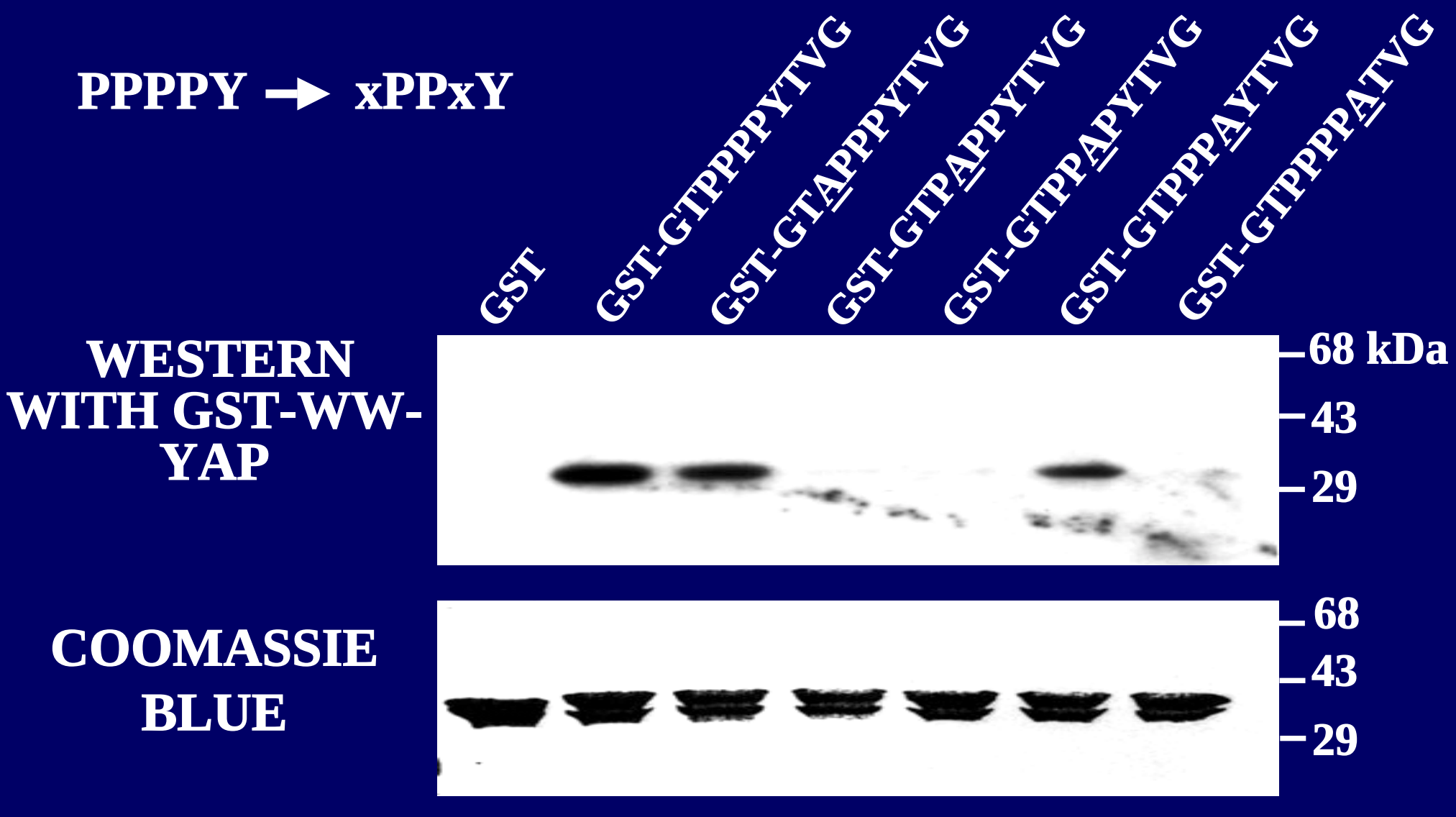
PIN1

ESS1

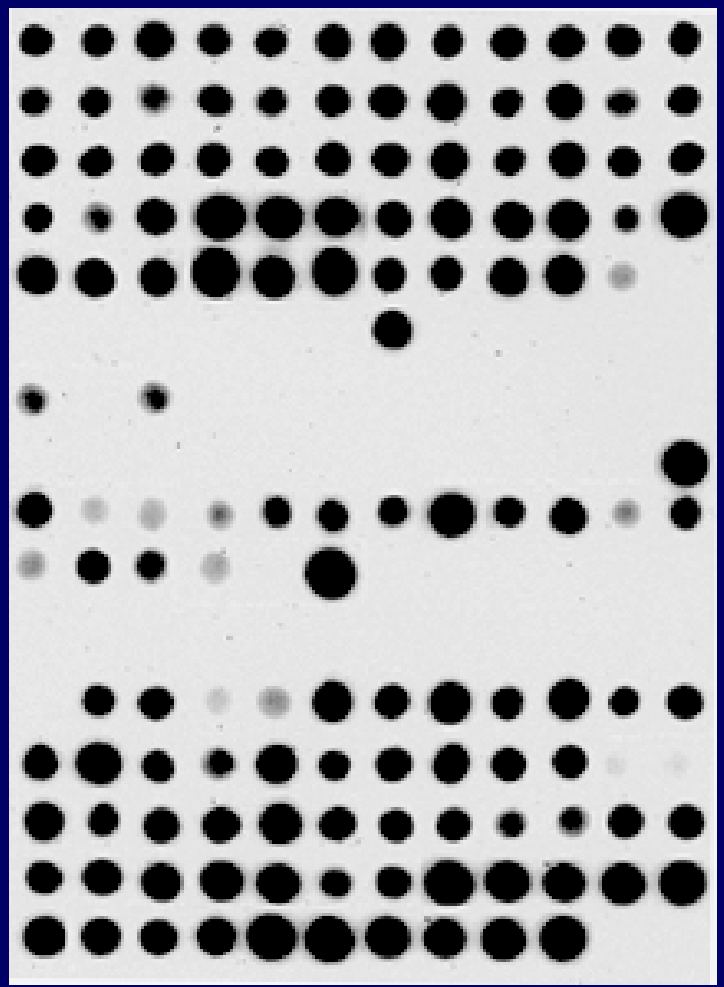


# ALANINE SCAN OF *GTPPPPYTVG* GIVES THE CONSENSUS SEQUENCE FOR LIGANDS TO THE WW DOMAIN OF YAP

**PPPPY → xPPxY**



# MUTATIONAL ANALYSIS OF THE TARGET PEPTIDE *GTPPPPYTVG* FOR THE WW DOMAIN OF YAP

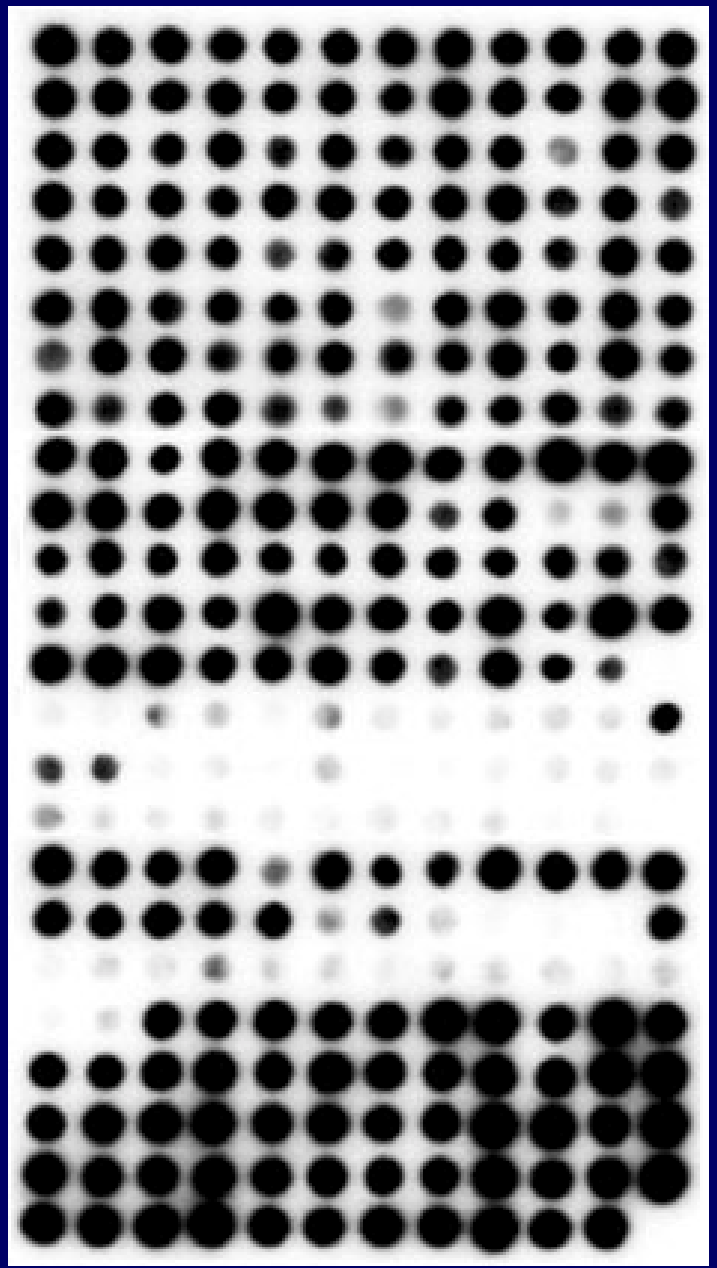


**Protein Footprint Corresponding to Central Prolines of the *PPxY* Core**

**Protein Footprint Corresponding to Terminal Tyrosine of the *PPxY* Core**

# MUTAGENESIS OF THE CARBOXY-TERMINAL END OF β-DYSTROGLYCAN

***KNMTPYRSPPPYVPP***



**Probe: Dystrophin-WW-CR-CT**

**Protein Footprint Corresponding to Central Prolines of the *PPxY* Core**

**Protein Footprint Corresponding to Terminal Tyrosine of the *PPxY* Core**

# Classification of WW domains

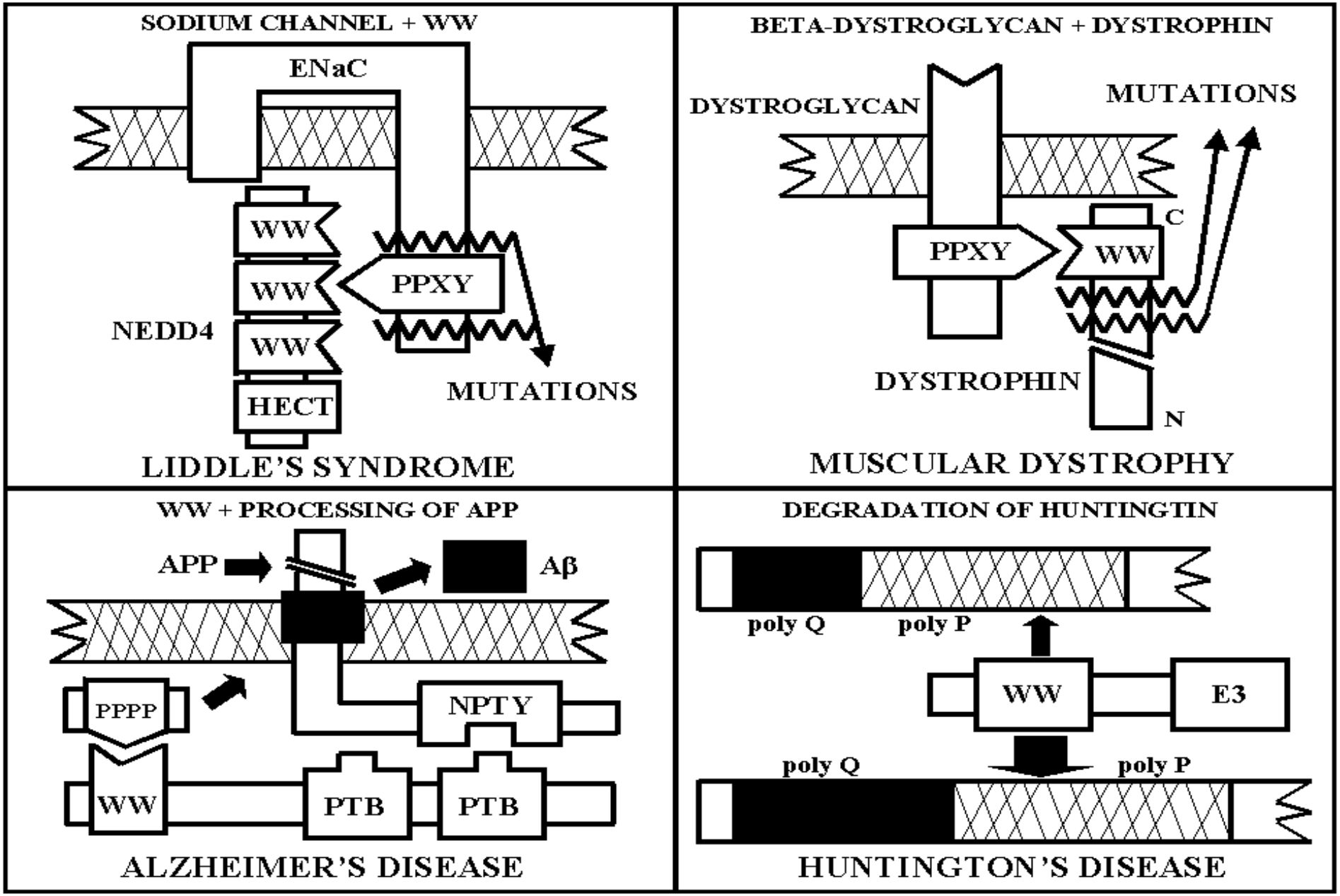
- Class I
- Class II
- Class III
- Class IV

- PPxY
- PPLP
- RPPP(R)
- p-S/TP

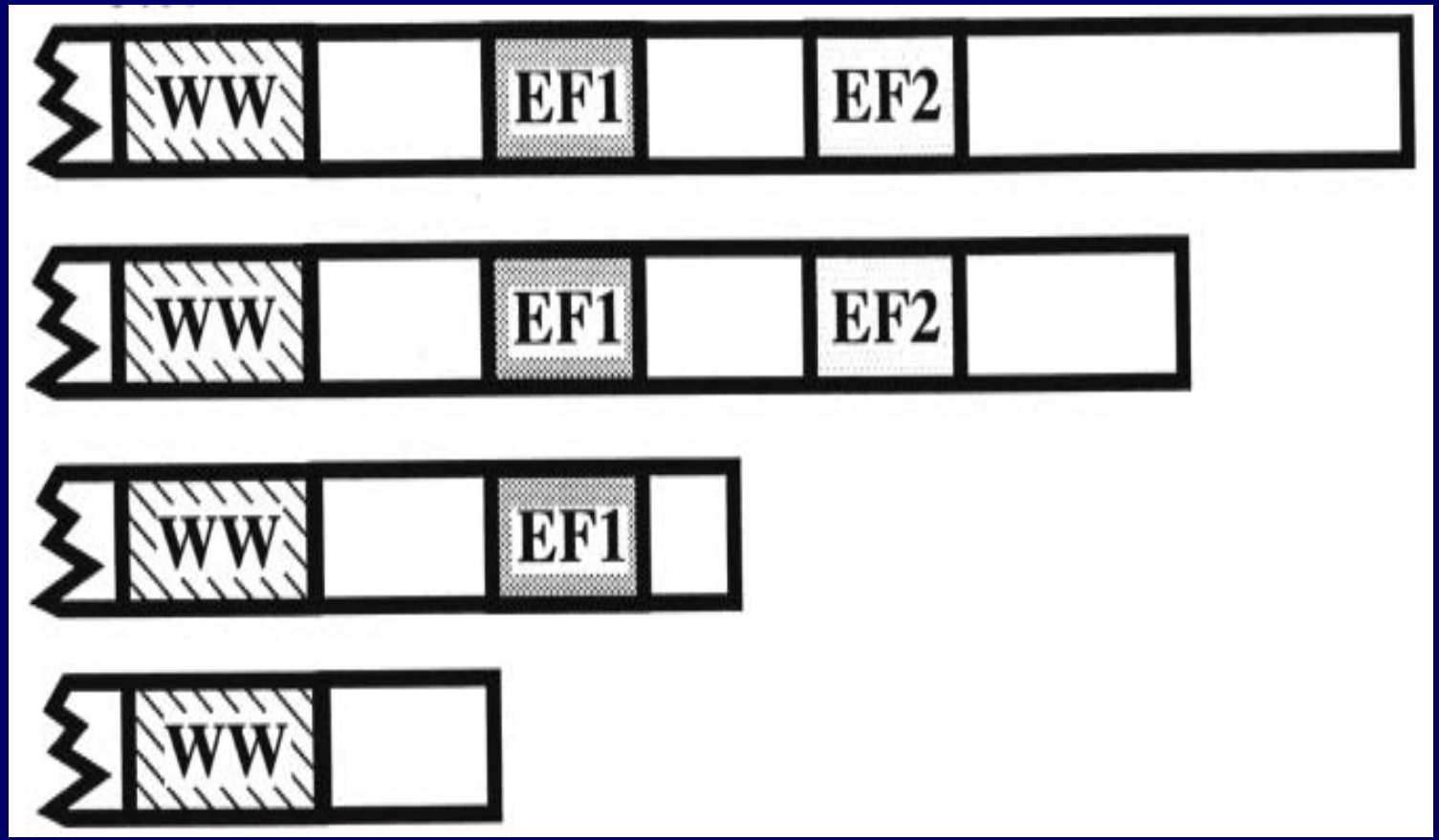
# WW DOMAIN PAGE ON THE WWW NETWORK

- ENTER ‘WW DOMAIN’ IN ANY SEARCH ENGINE
- CONTAINS UPDATES ON SEQUENCES, STRUCTURES, AND RELEVANT LITERATURE
- CONTAINS ORIGINAL ALGORITHM AND MANUAL ALIGNMENT
- CONTAINS AN AUTOMATIC ALIGNMENT

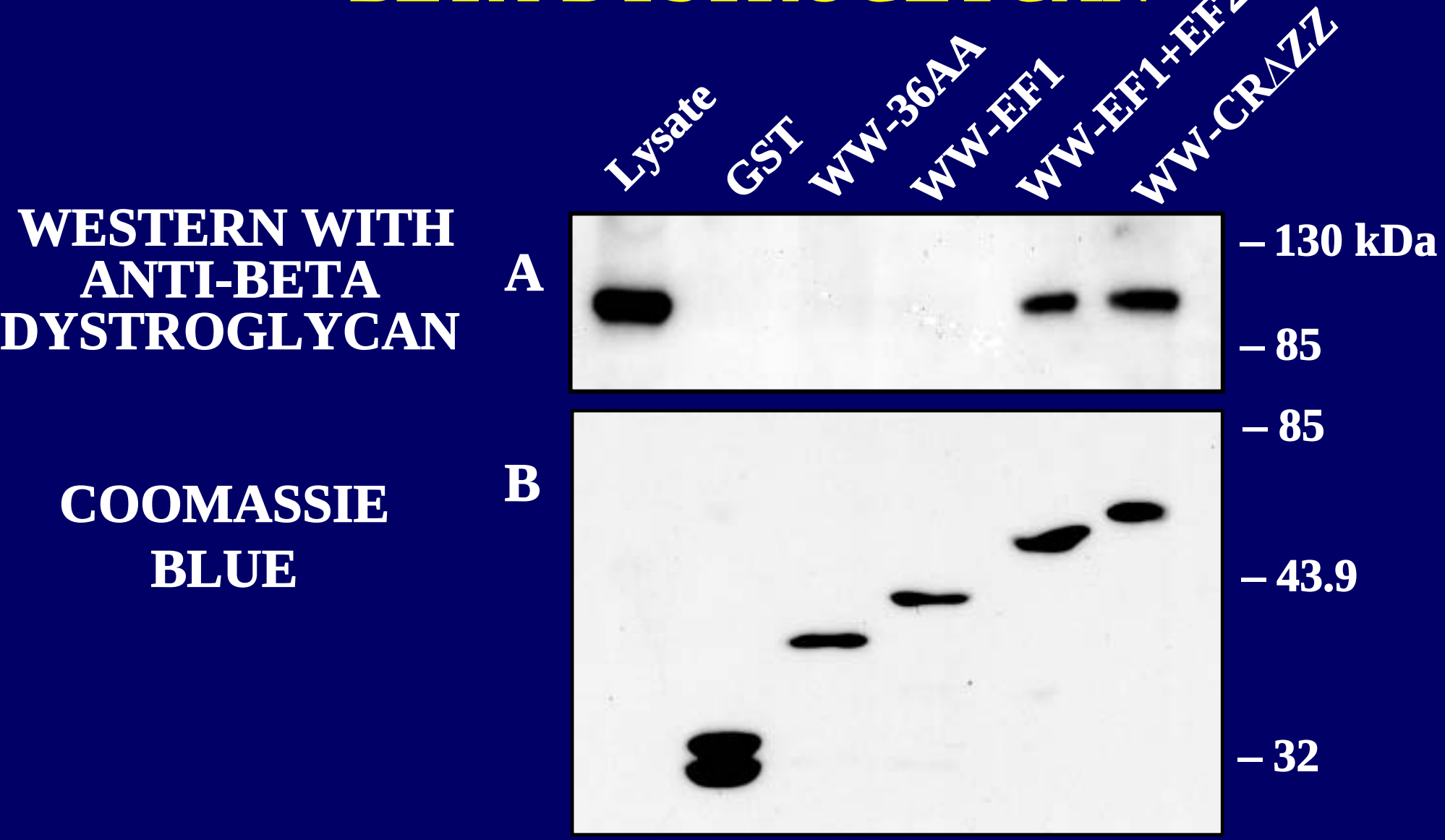
# WW DOMAINS AND HUMAN DISEASES



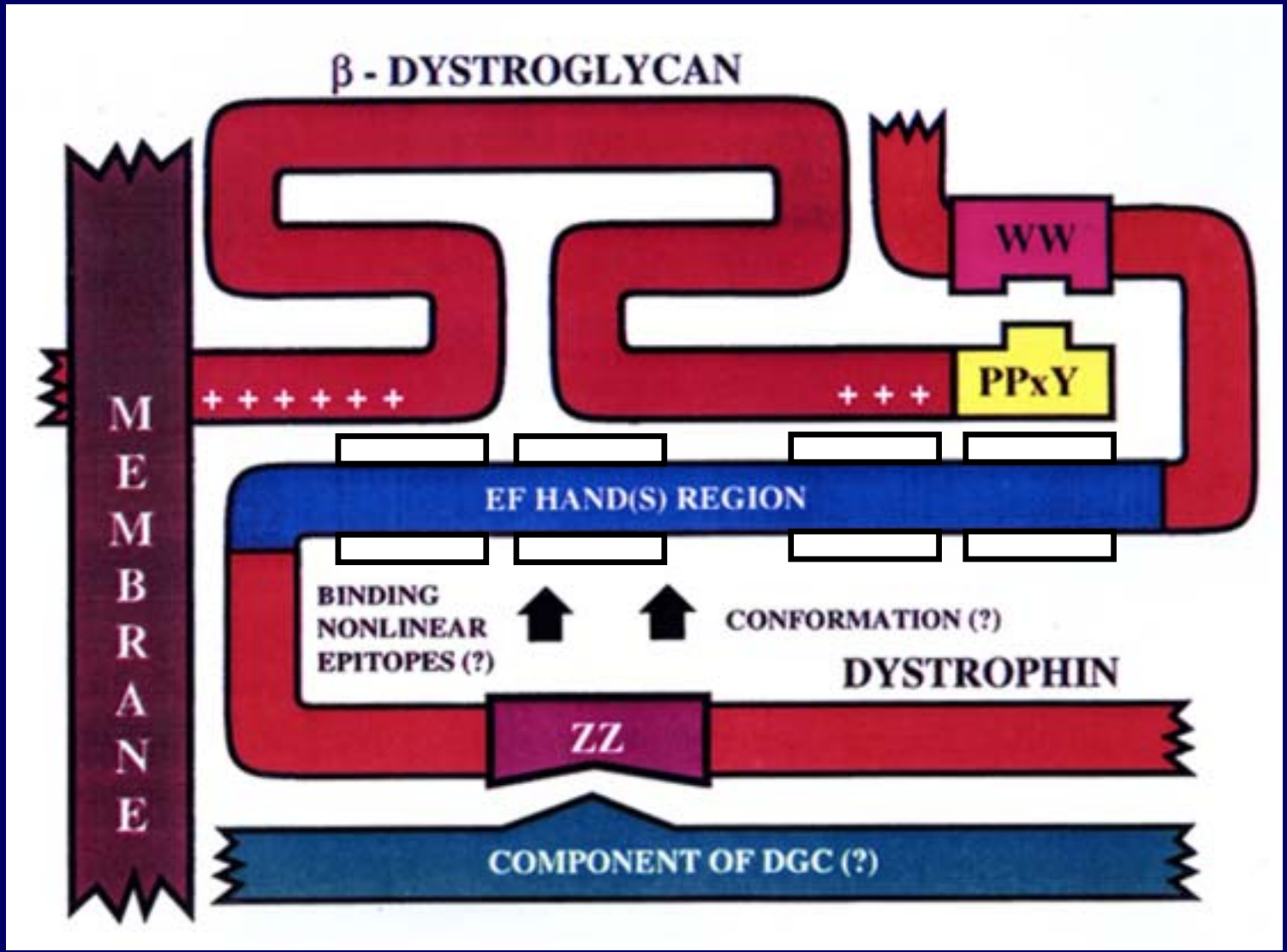
# DYSTROPHIN CONSTRUCTS USED TO MAP MINIMAL REGION REQUIRED FOR BINDING BETA-DYSTROGLYCAN



# WW DOMAIN AND TWO EF HANDS ARE REQUIRED FOR BINDING BETA-DYSTROGLYCAN

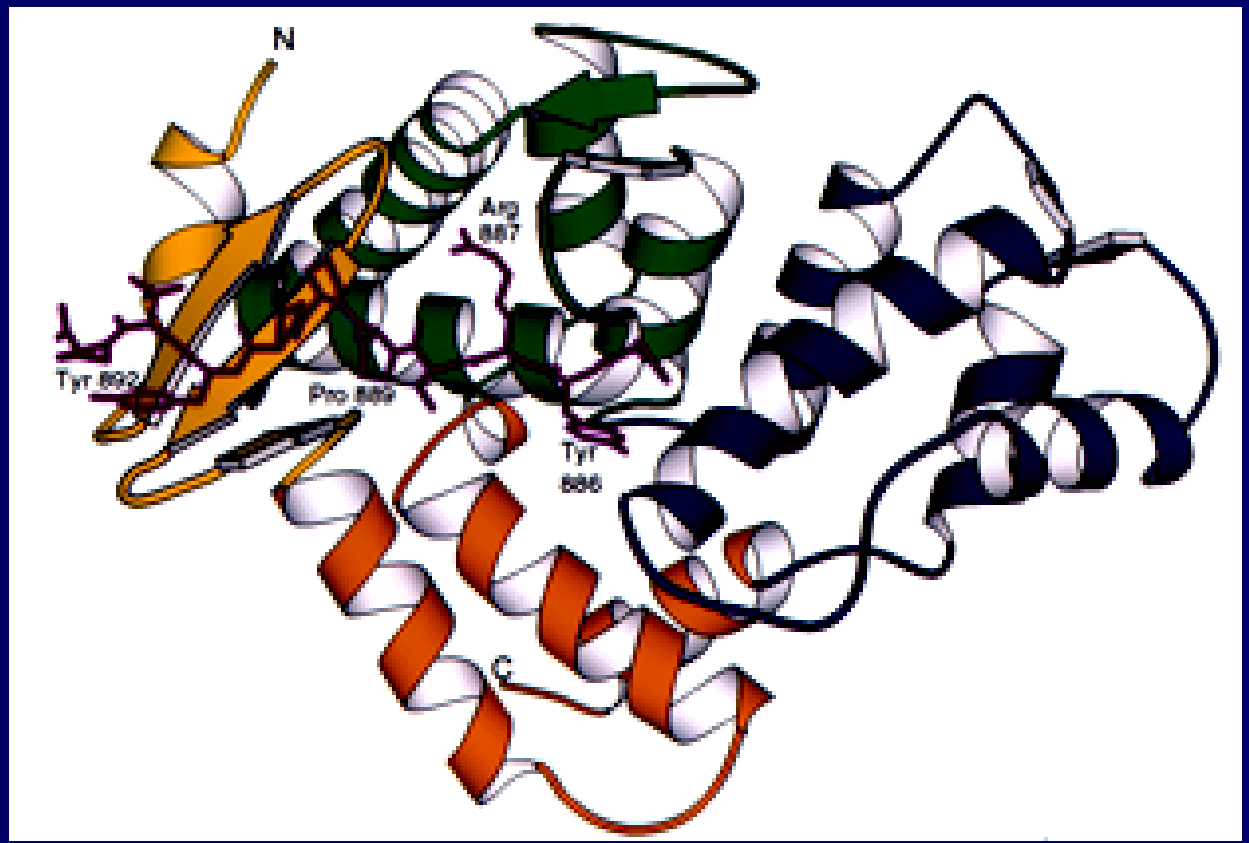


# INTERACTIONS BETWEEN DYSTROPHIN AND BETA-DYSTROGLYCAN

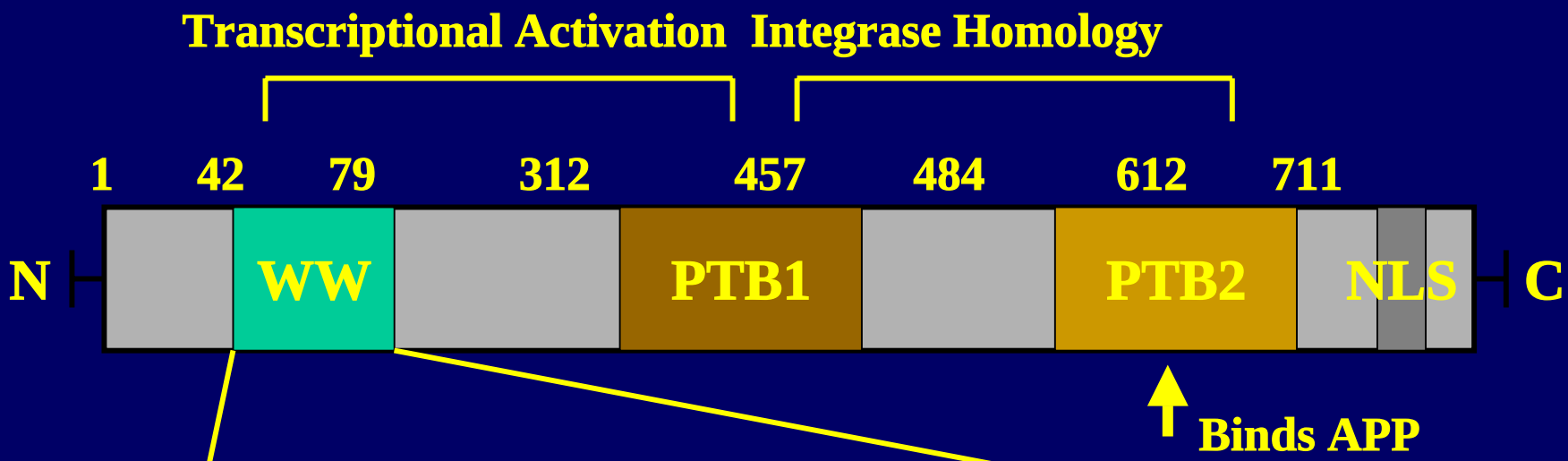



**WW Domain and EF Hands Constitute a Major Link**

# STRUCTURE OF THE WW DOMAIN OF DYSTROPHIN IN COMPLEX WITH THE BETA-DYSTROGLYCAN PEPTIDE



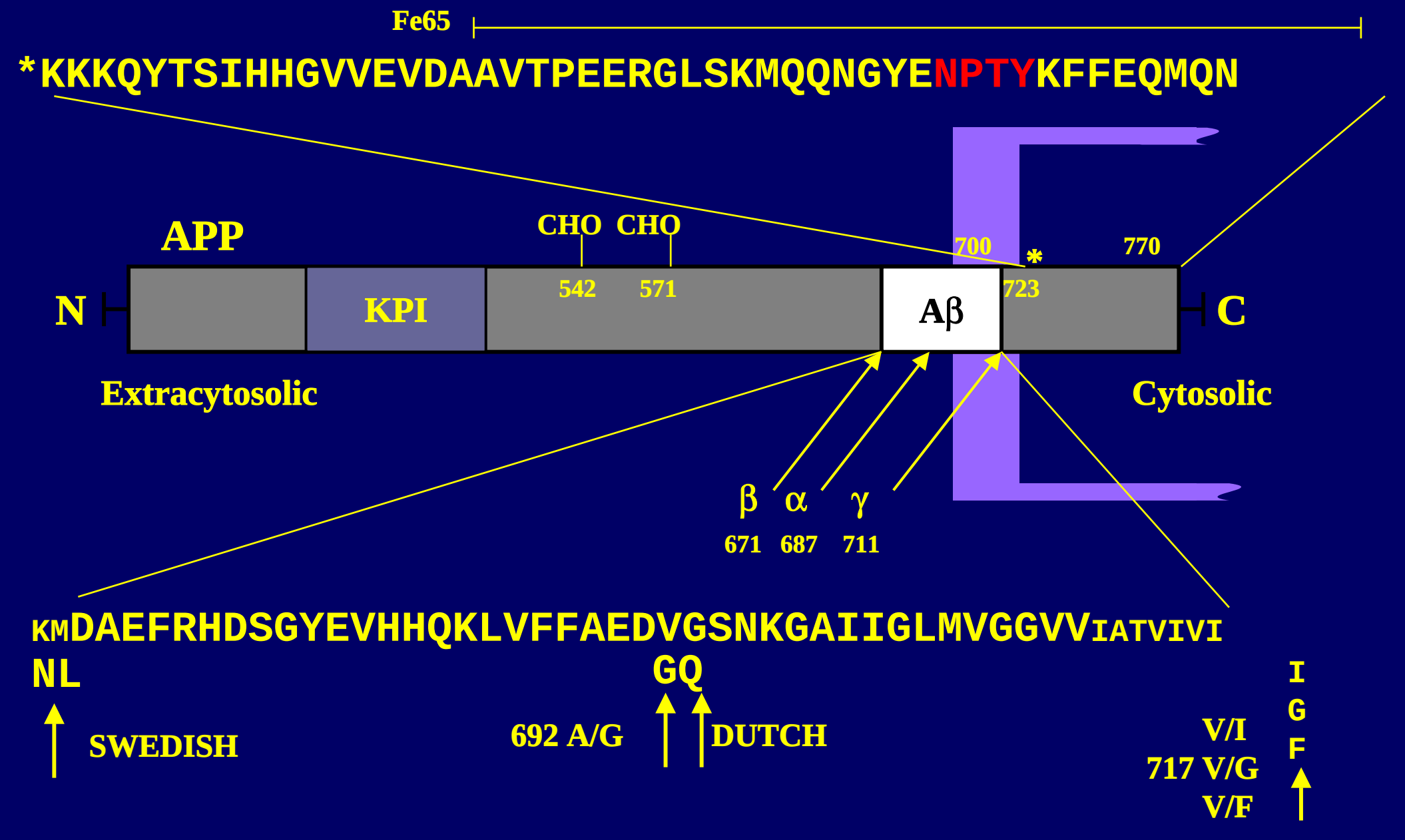
# Modular structure of the Fe65 adapter protein



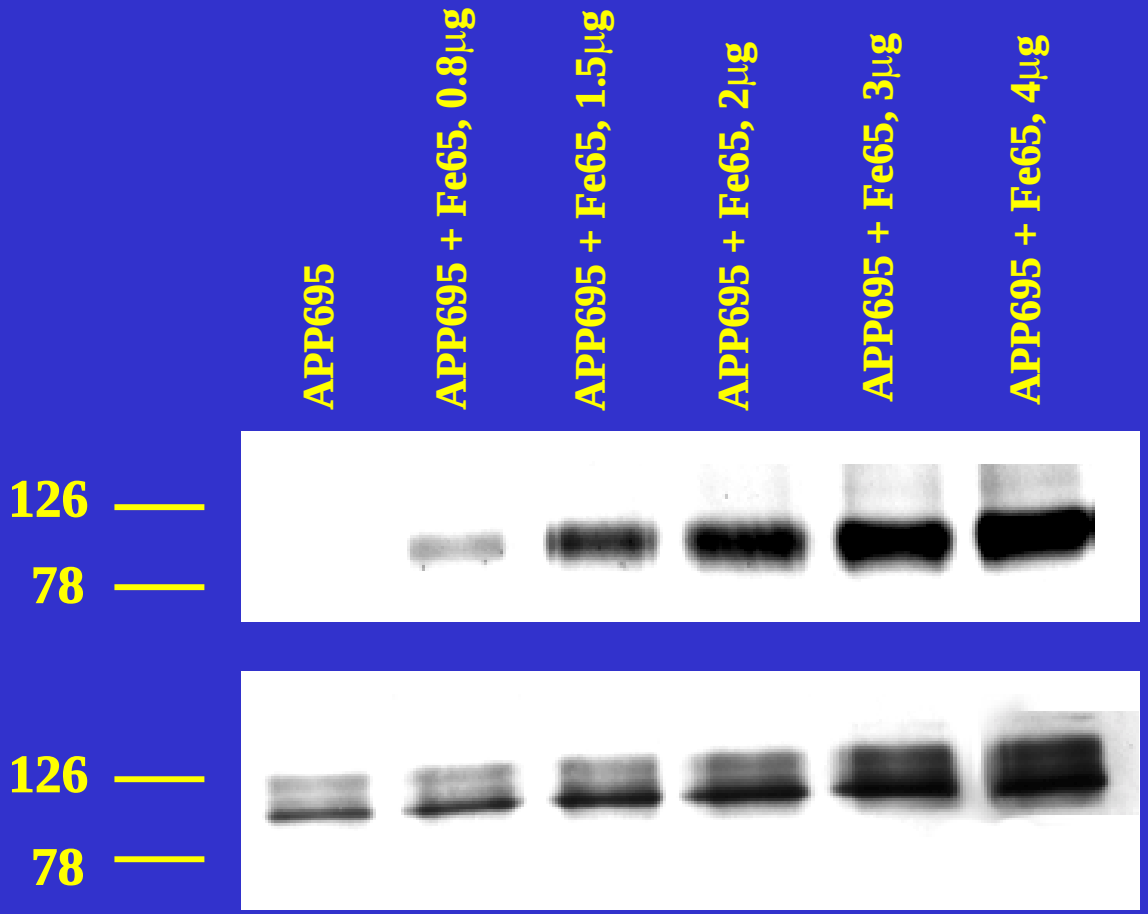

**Wild type:** DSLPAGWMRVQDTSGDYYWHIPTGTTQWEPPGRASPS

**Mutant:** ----------F----A----------

# Structure of APP



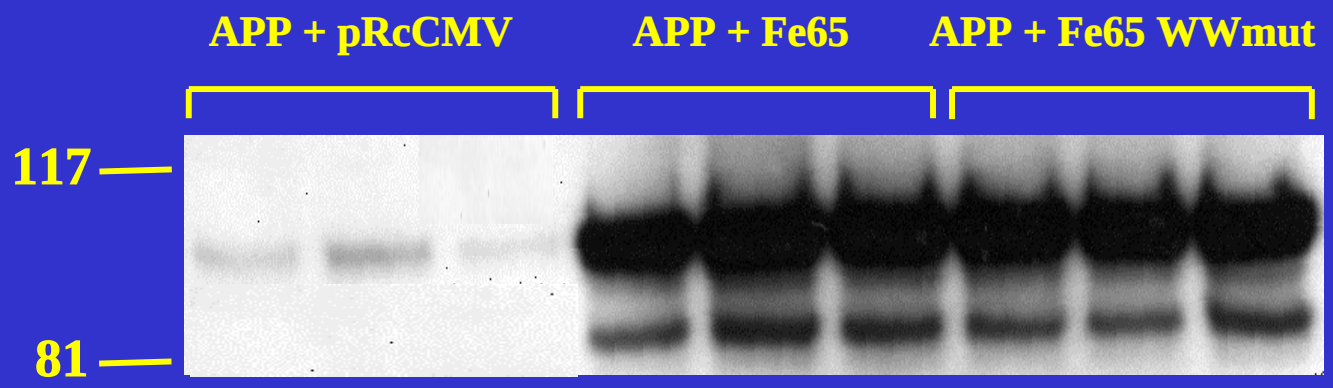
# Increase of cellular APP by Fe65 is concentration dependent




**WB:** anti-Fe65

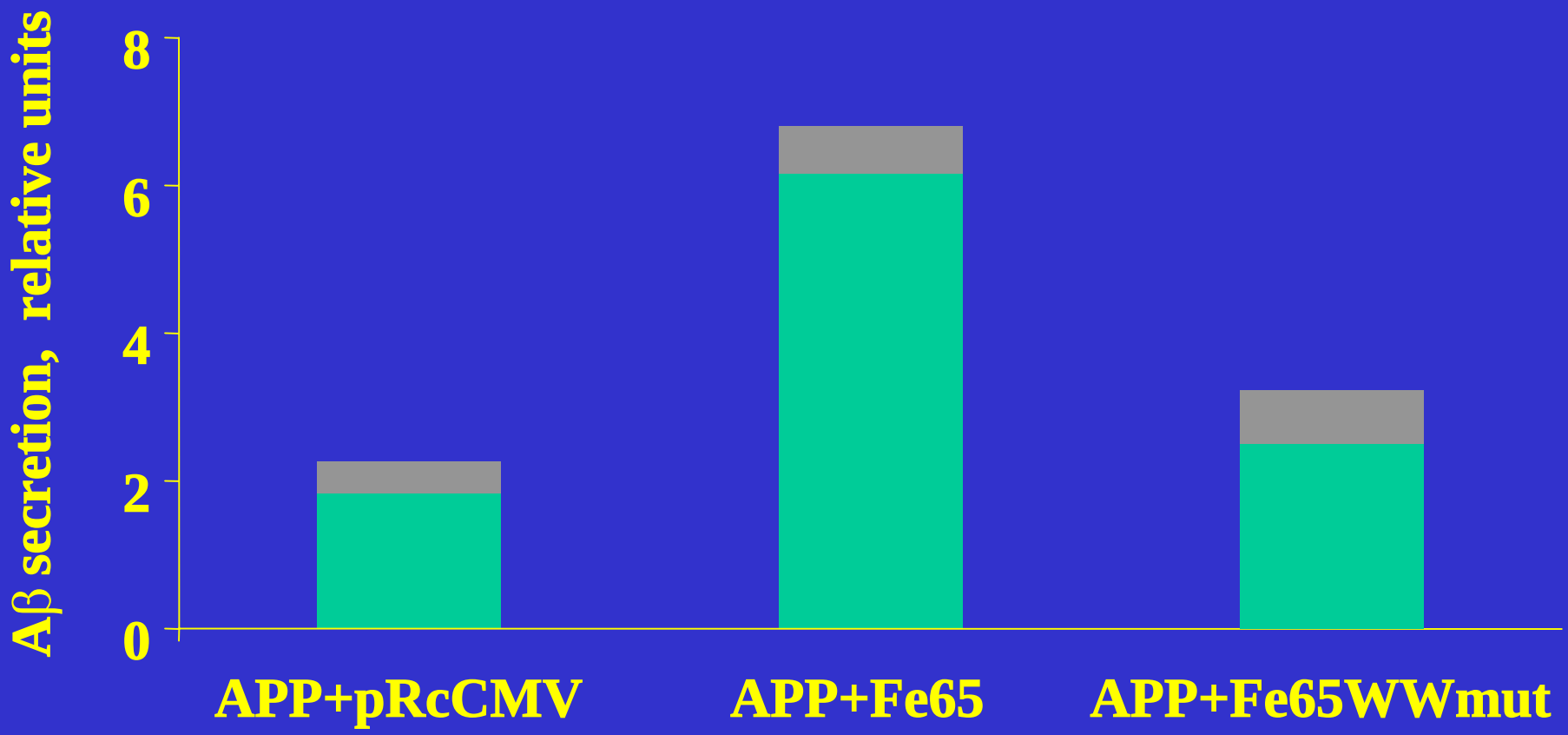
**WB:** anti-APP (369)

# Effect of Fe65 and Fe65 WW mutant on cellular APP



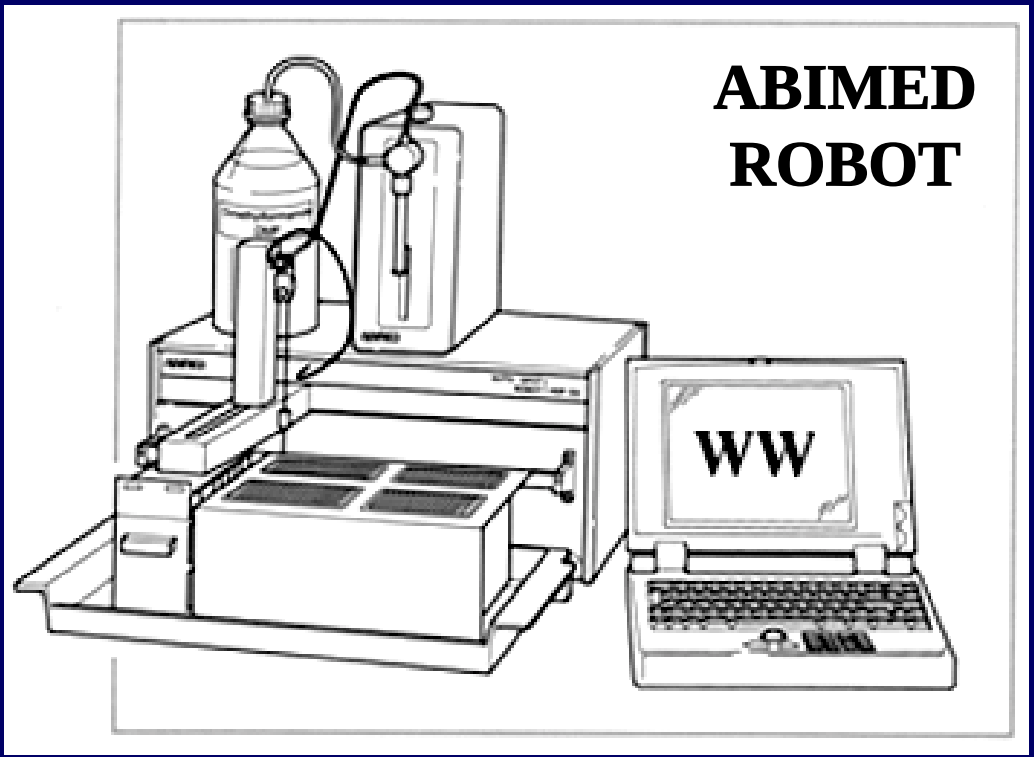

WB: anti-APP (W02)

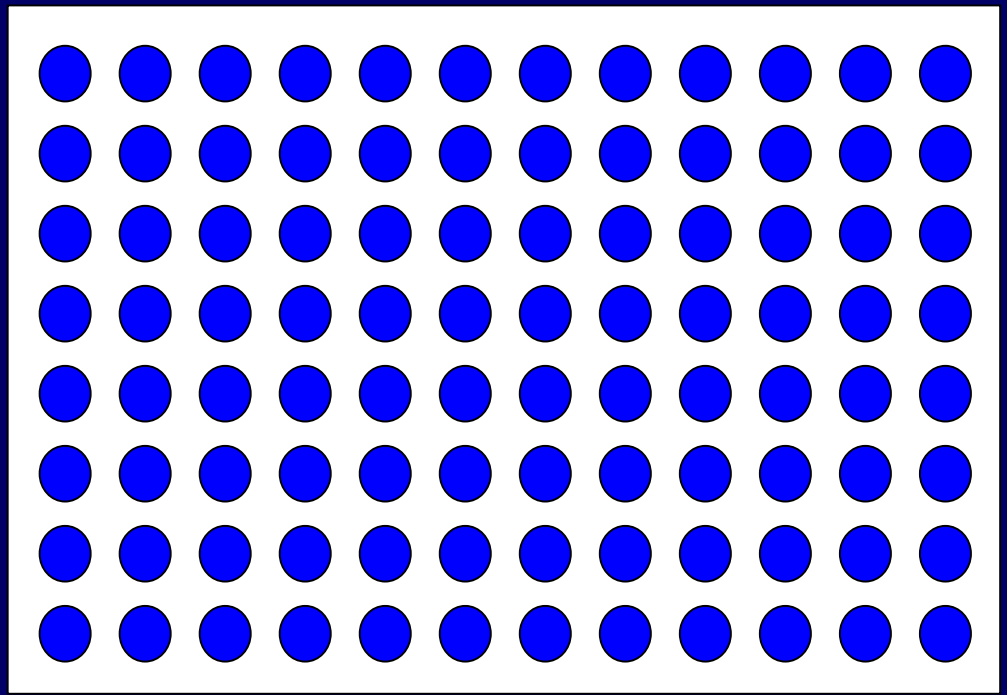
# Fe65 with the mutated WW domain does not increase Aβ secretion



# AUTOSPOTS ROBOT ASP 222

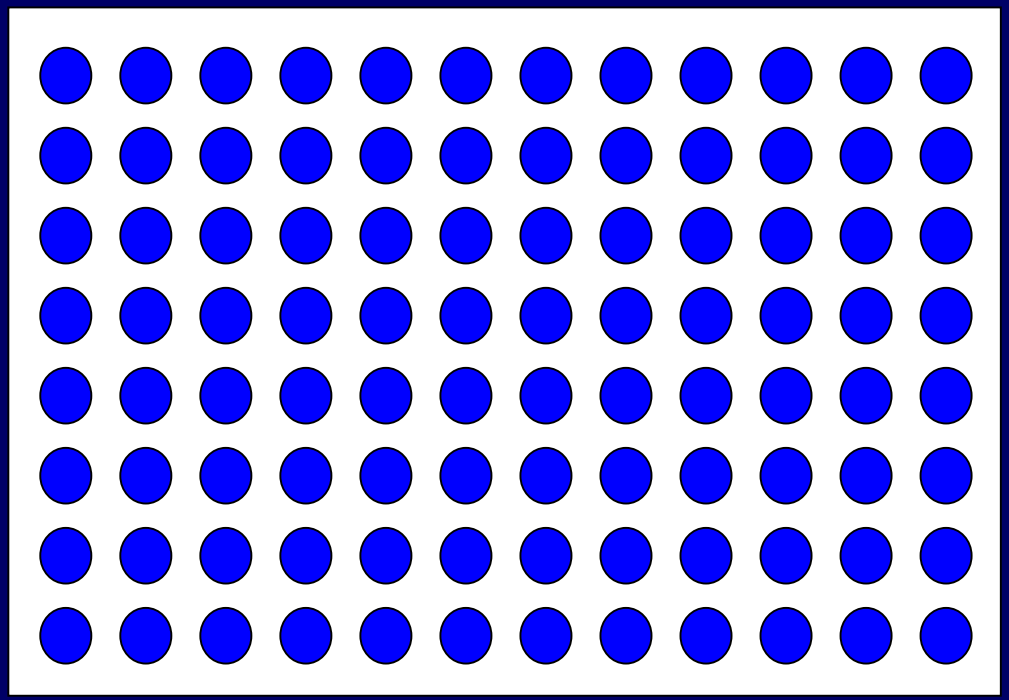
## AUTOMATED PEPTIDE SYNTHESIS ON MEMBRANES



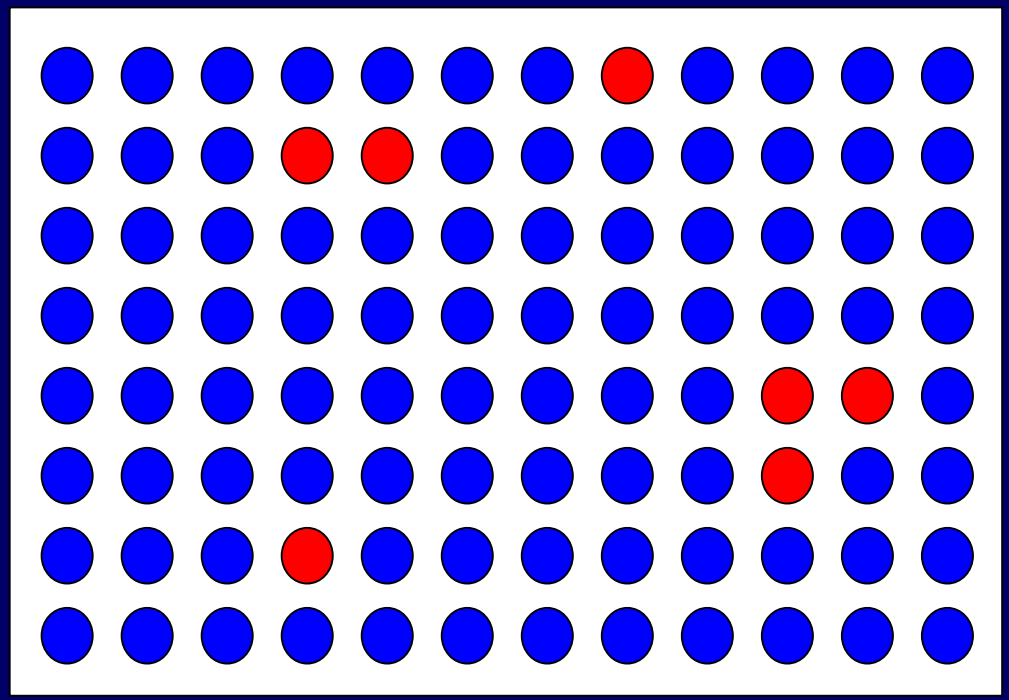



**Capable of generating 1600 polypeptides 400 per membrane within two weeks**

# HIGH-THROUGHPUT SCREEN WITH REPERTOIRES OF WW DOMAINS ON MEMBRANES



NORMAL

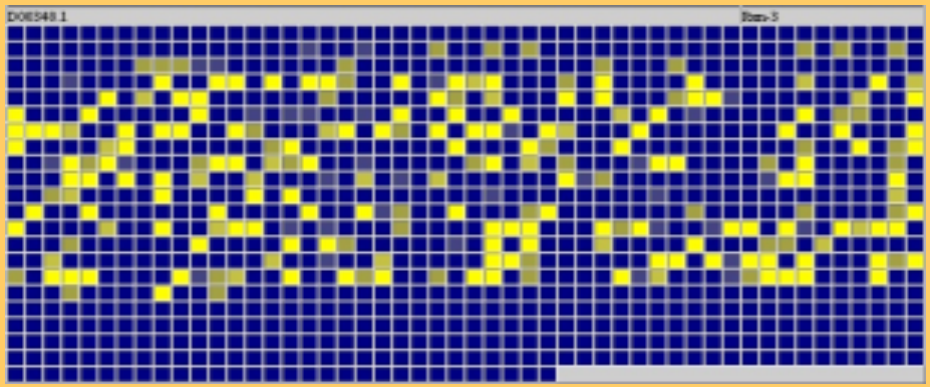


CANCER CELLS

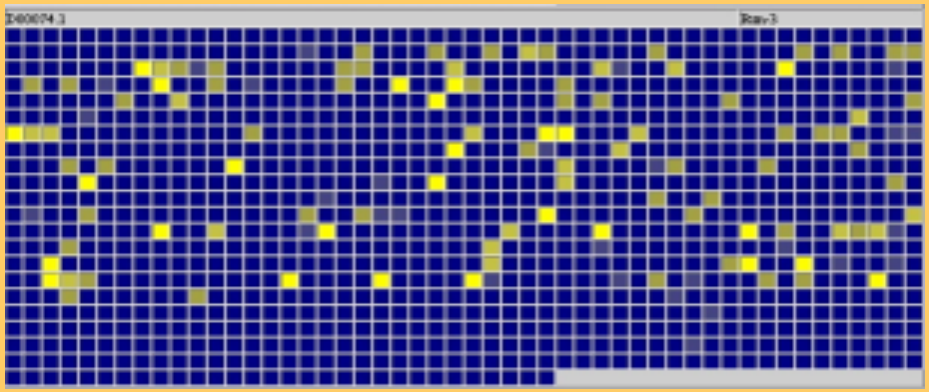
● indicates a robust change in binding

# Domain and Ligand Identification

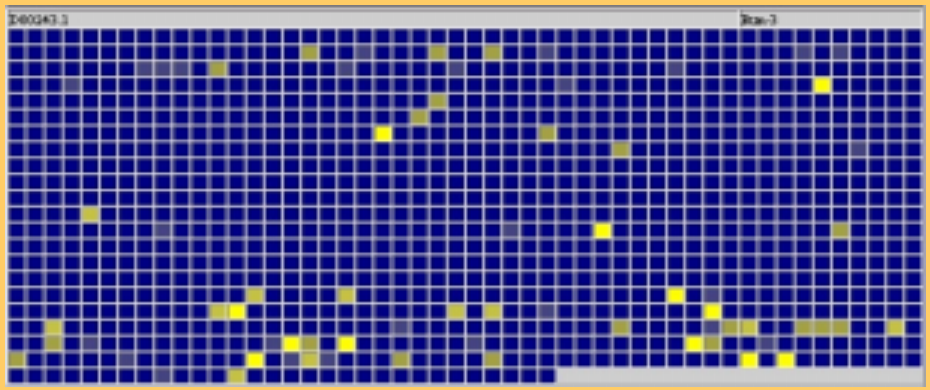
**W W Domain D00348**
sub-family 1



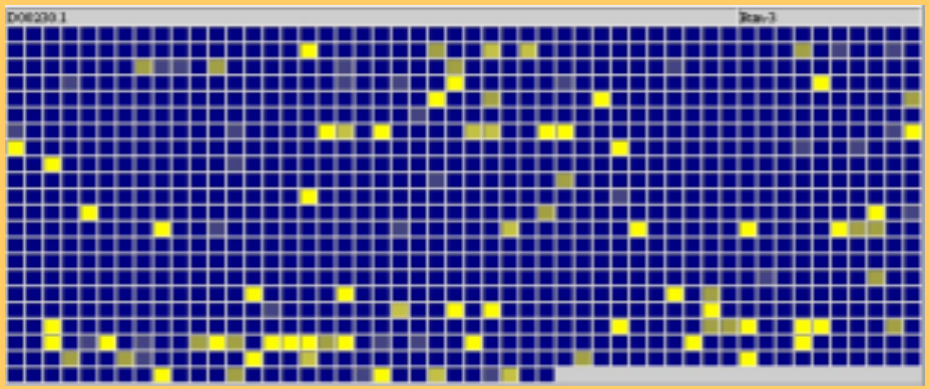
**W W Domain D00074**
sub-family 1



**Domain WW D00243**
sub-family 2



**W W Domain D00230**
sub-family ???



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