Antigenic structure of proteins: peptide epitopes

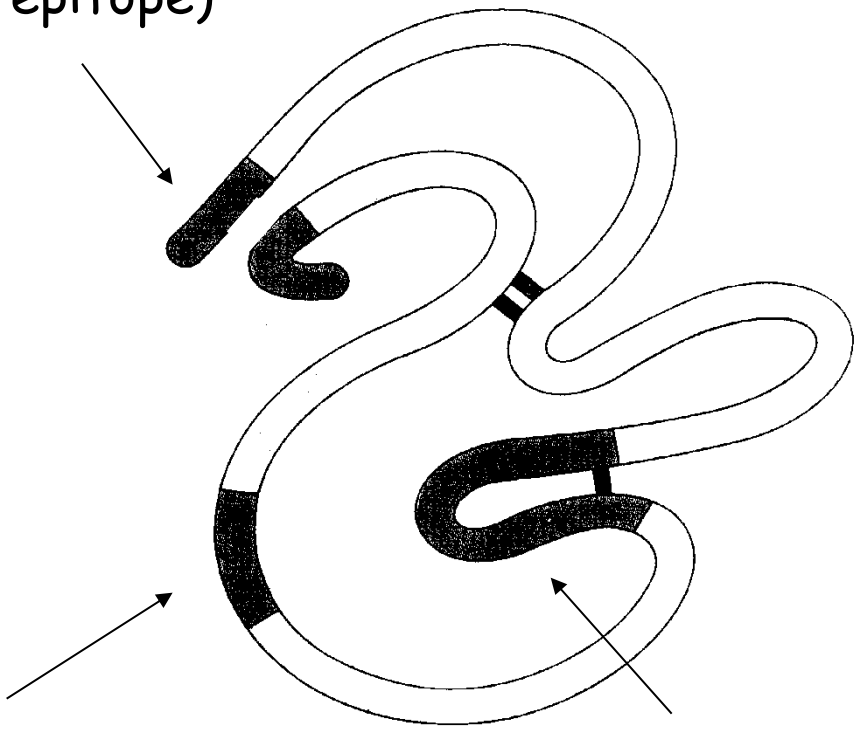
Ferenc Hudecz [1,2]

[1] Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University,

[2] Department of Organic Chemistry, Institute of Chemistry, Eötvös L. University,

Protein epitopes

Topographic, non-continuous
(antibody epitope)



Linear, sequential
(antibody epitope, T-cell epitope)

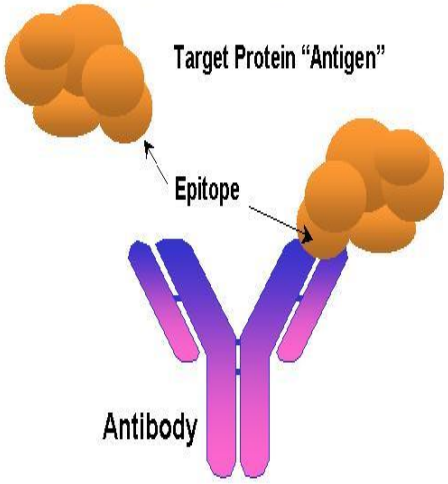
Topographic, continuous
(antibody epitope)

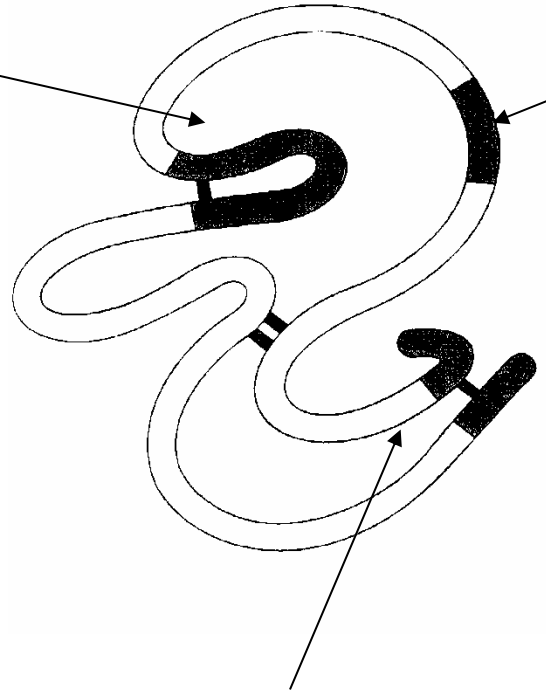
B- and T-cell epitope recognition

Antigen structure - peptide epitopes – epitope recognition

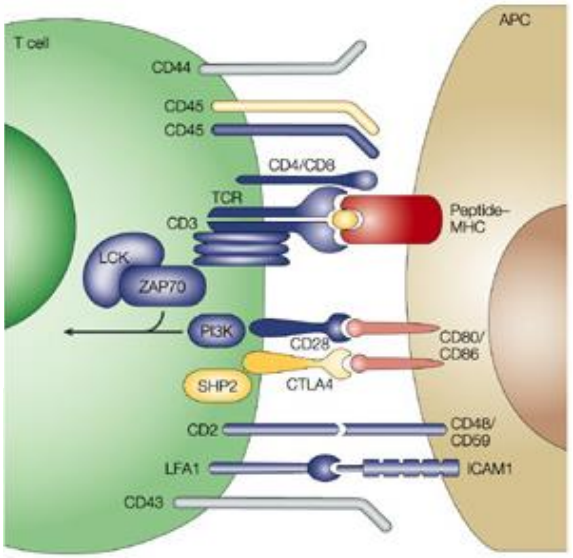
Topographic, non-continuous
(antibody epitope)

Linear, sequential
(antibody or T-cell epitope)

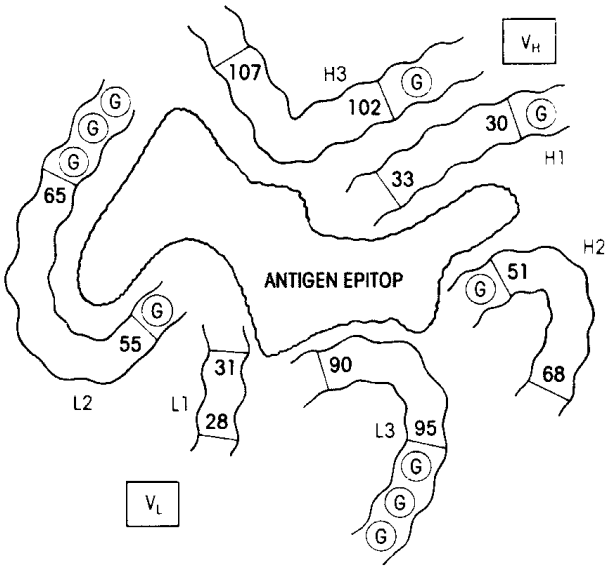




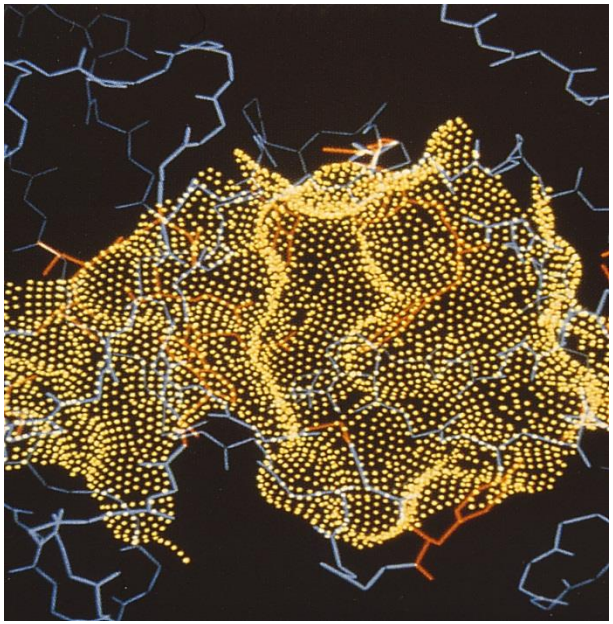
Topographic, continuous
(antibody epitope)

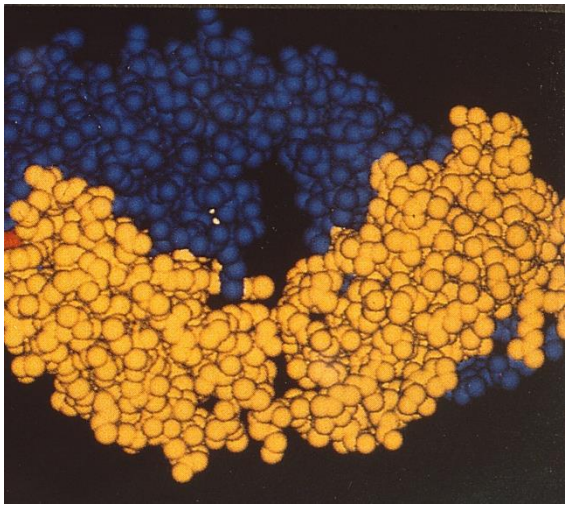


Antibody – epitope interaction



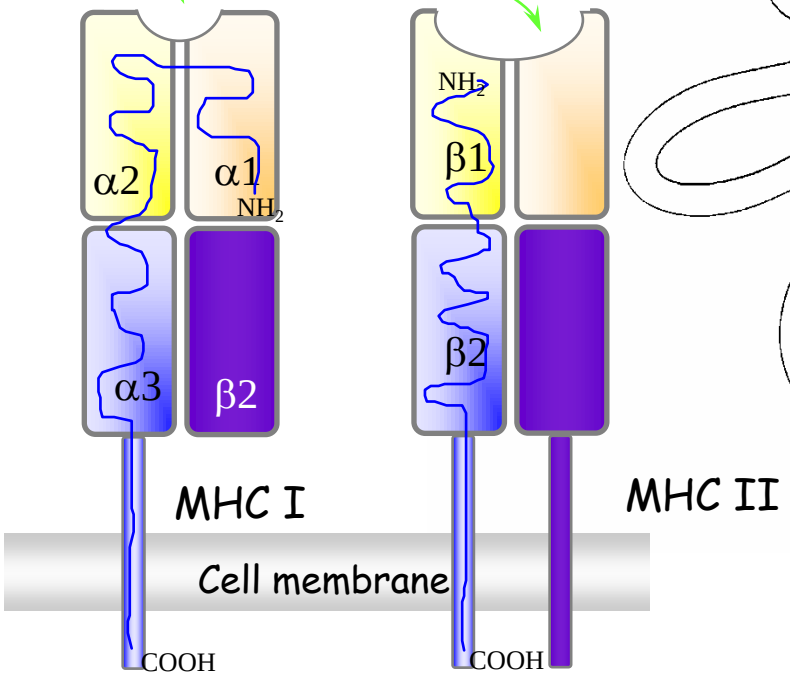
Chothia et al. Nature 342, 877, 1989

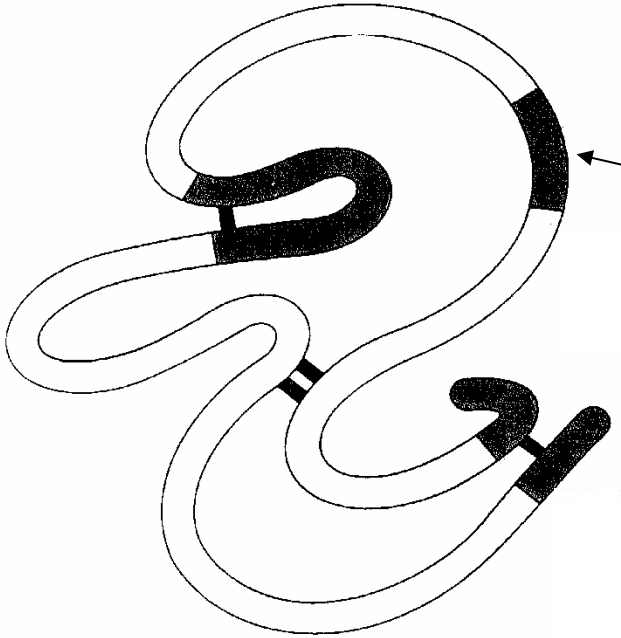




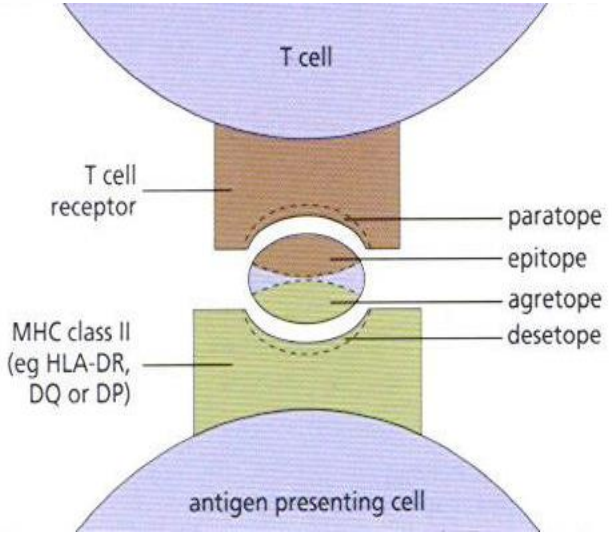
T- cell epitope recognition

binding site





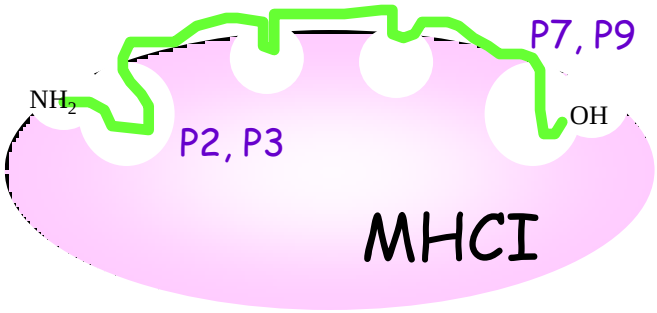
Linear, sequential
(T-cell epitope)



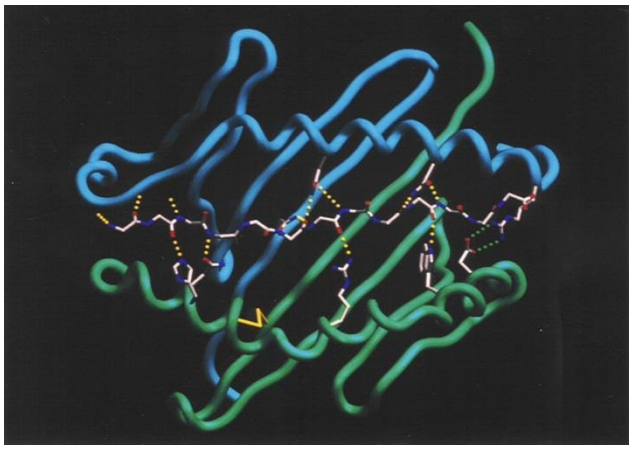
1 polymorf α-chain
β-mikroglobulin

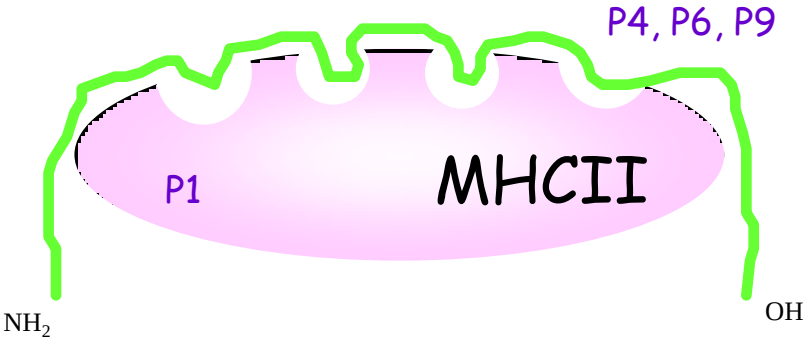
1 polymorf α-chain
1 polymorf β- chain

T- cell epitope recognition

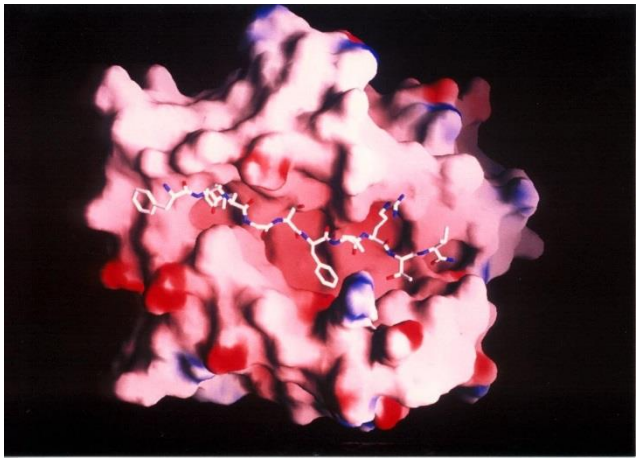


MHC I : 8 - 10 amino acid

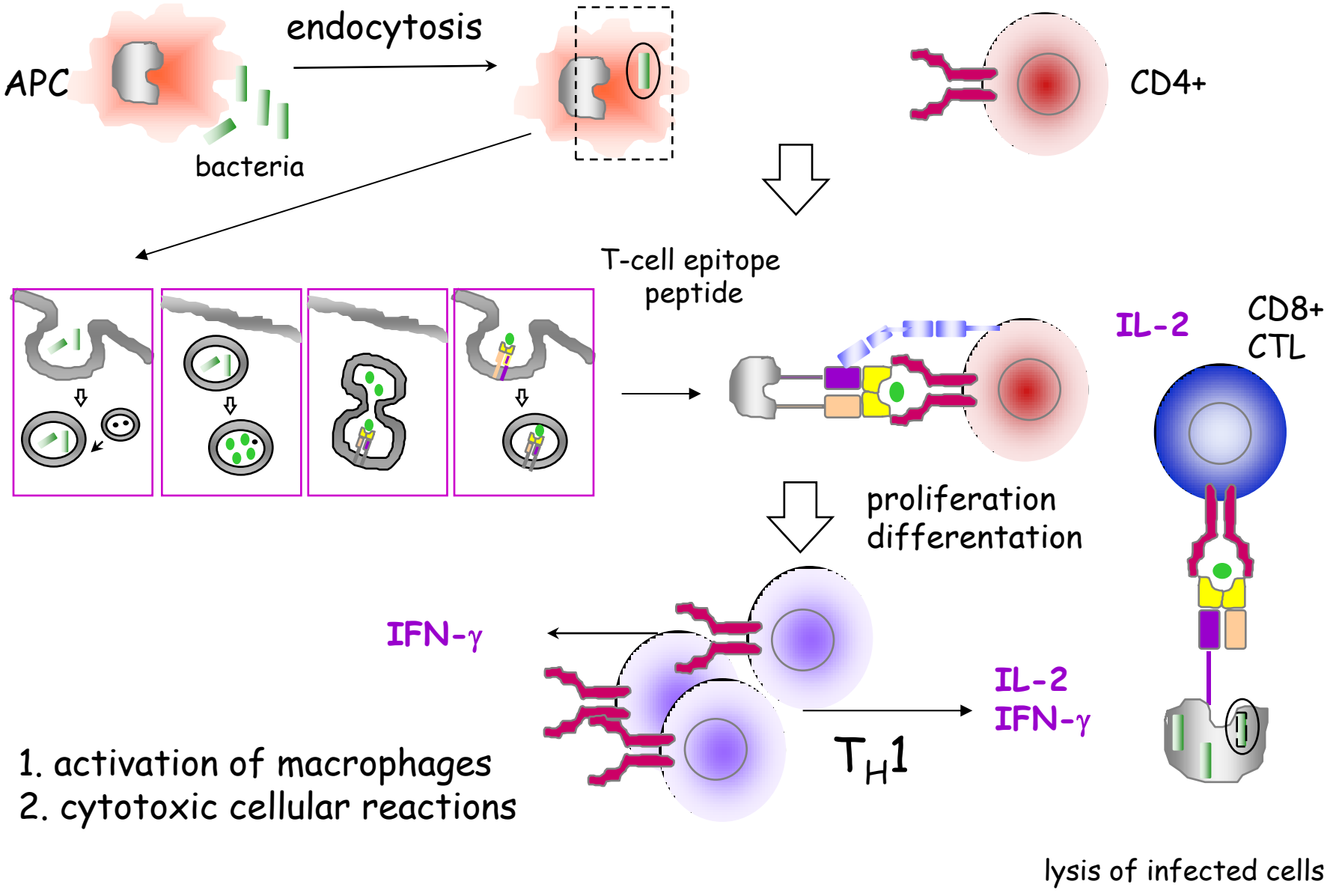




MHC II : 13 - 23 amino acid

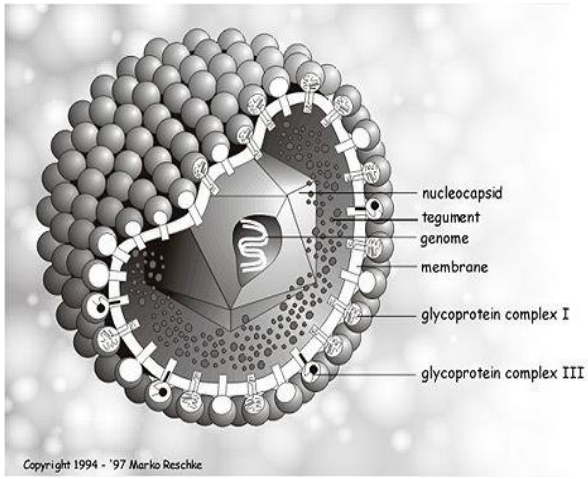


Development of immune response against T-cell epitopes



Identification of protein epitopes

Identification of protein epitopes

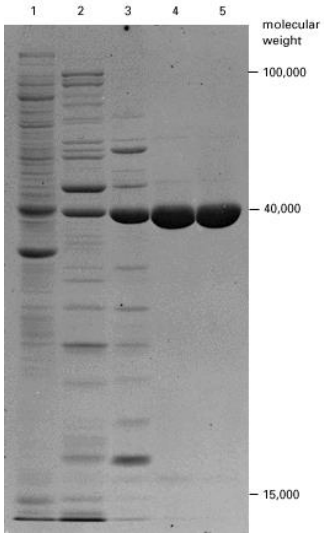


virus

Víral envelope proteins (mixture)

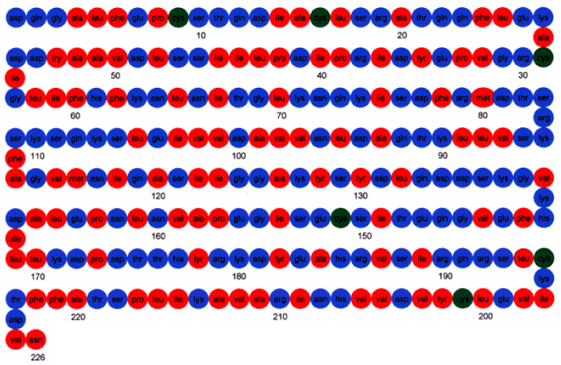
1. Affinity chromatography
2. Gradient centrifugation

Immunoprecipitation (Gelelectrophoresis)

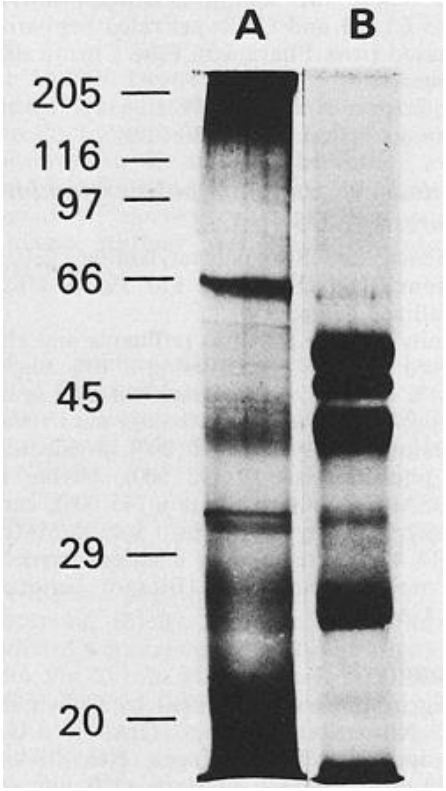


Immunodominant protein component(s)

Sequencing



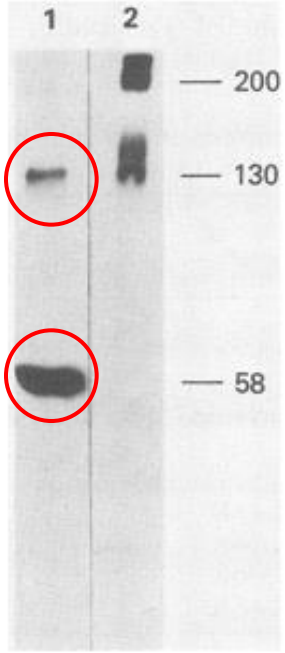
Amino acid sequence



Vaccine, Vol. 3, September 1985

Preparation of highly purified human cytomegalovirus envelope antigen

Ferenc Hudecz*, Eva Gonczol and Stanley A. Plotkin

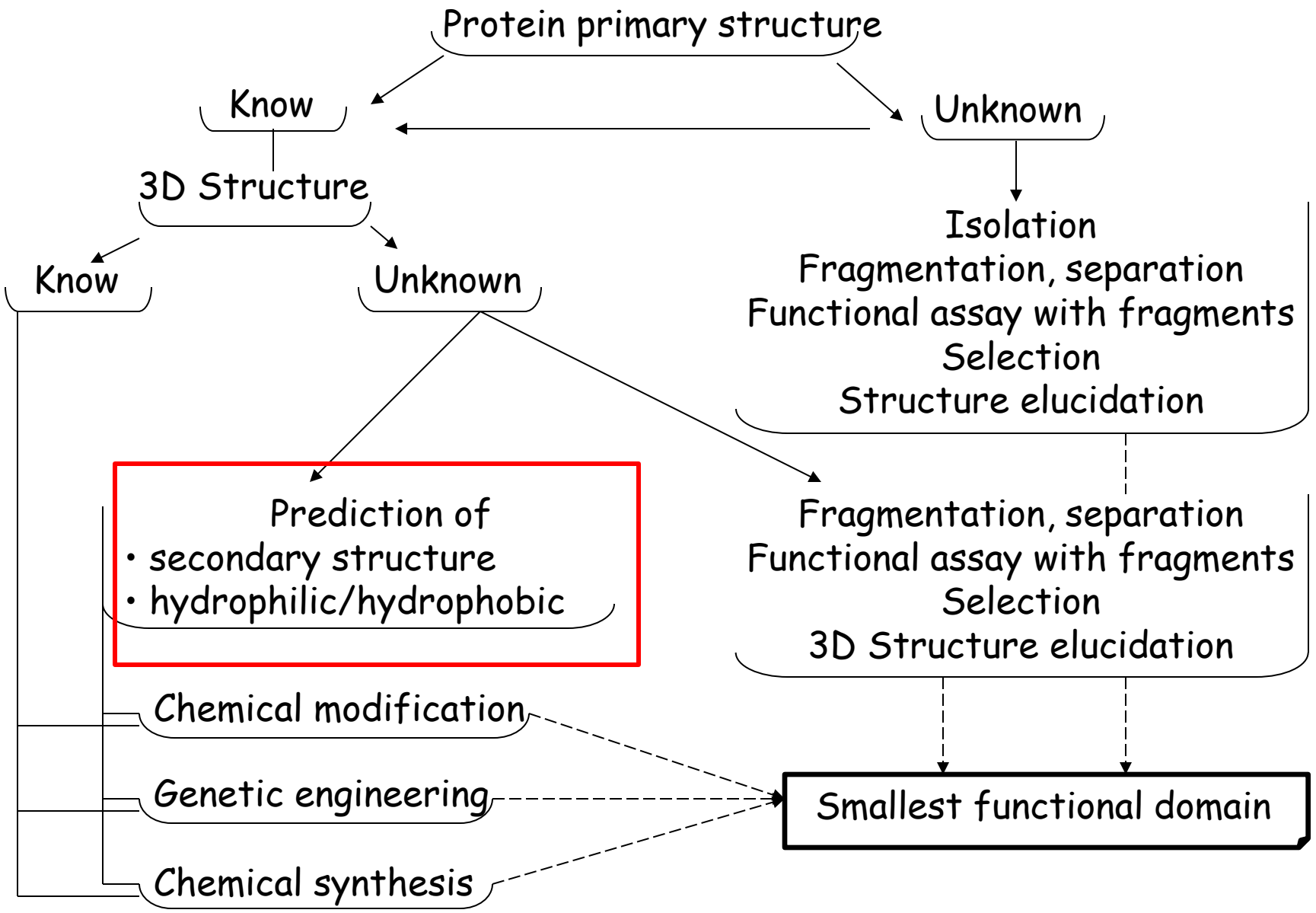


JOURNAL OF VIROLOGY, May 1986, p. 661–664

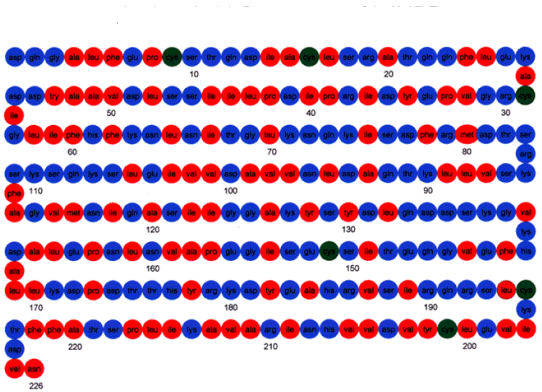
Immune Responses to Isolated Human Cytomegalovirus Envelope Proteins

EVA GÖNCZÖL,* FERENC HUDECZ, JOHN IANACONE, BERNHARD DIETZSCHOLD, STUART STARR, AND STANLEY A. PLOTKIN

Approaches for the localisation of epitope(s)

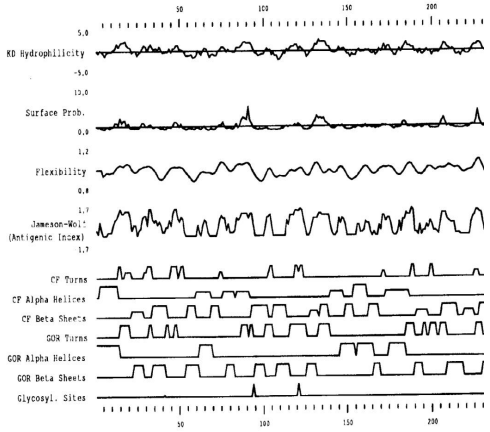


Identification of peptide epitopes – theoretical methods

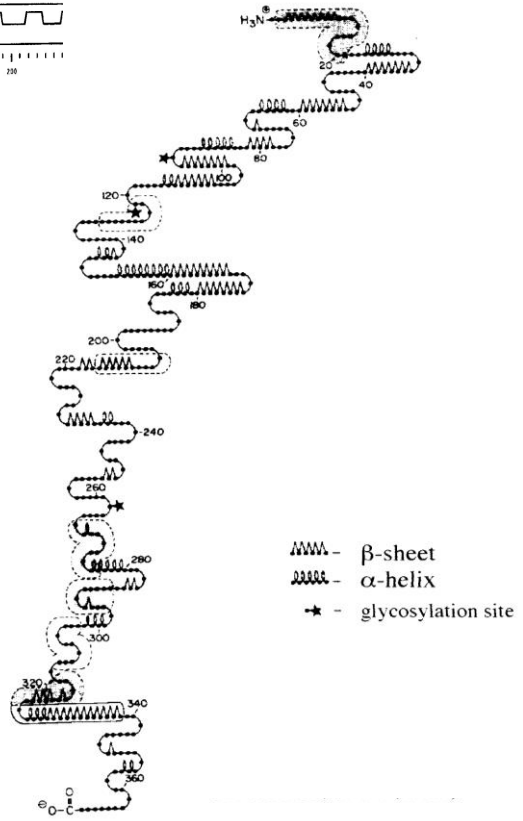


protein

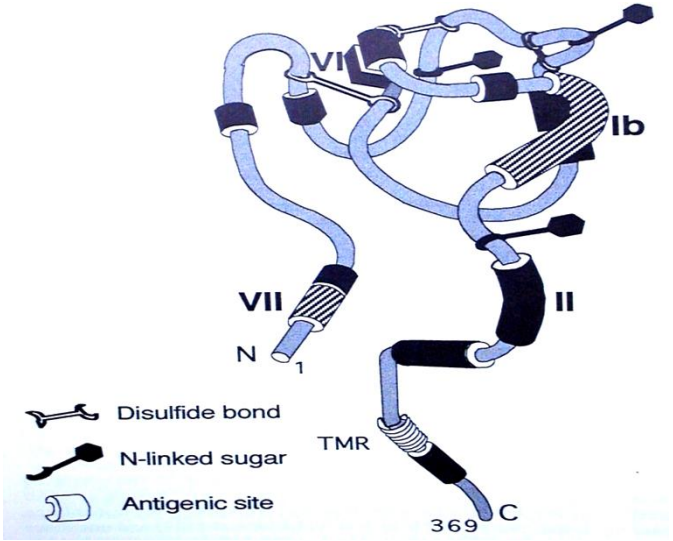
3D structure prediction



Hydrofilicity prediction

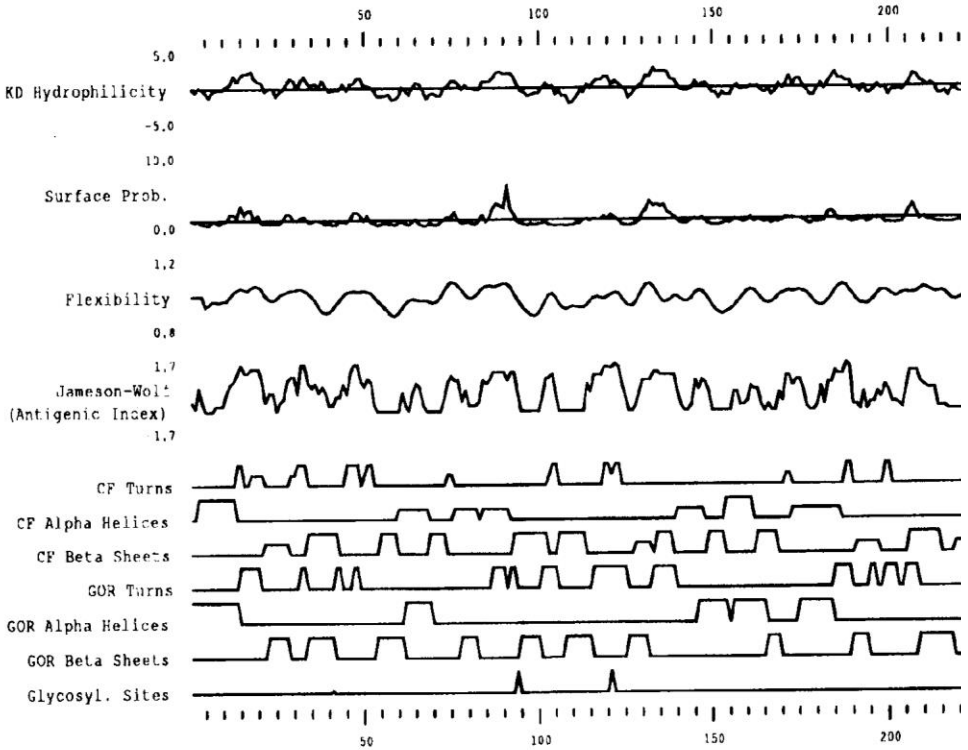


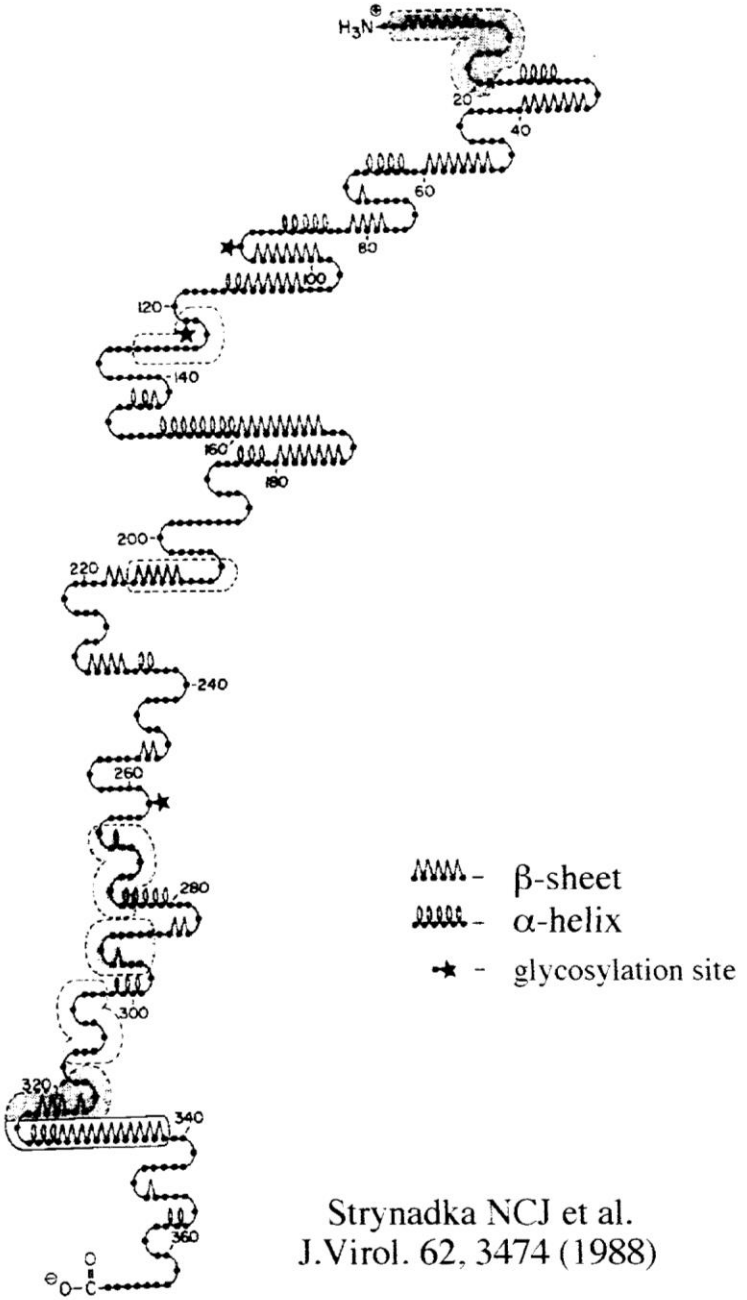
modelling



epitope „map"

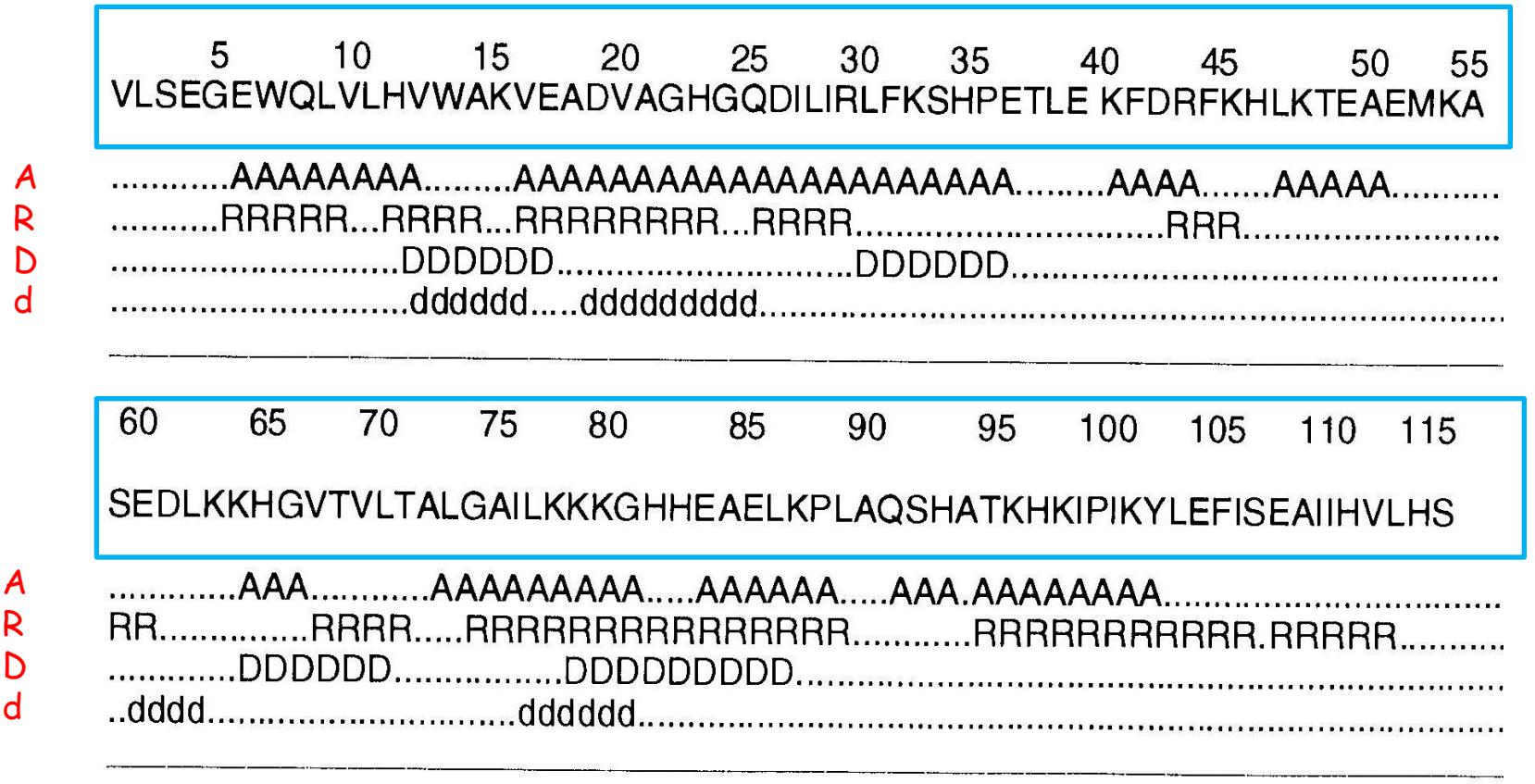
Prediction of B-cell epitope





Strynadka NCJ et al.
J.Virol. 62, 3474 (1988)

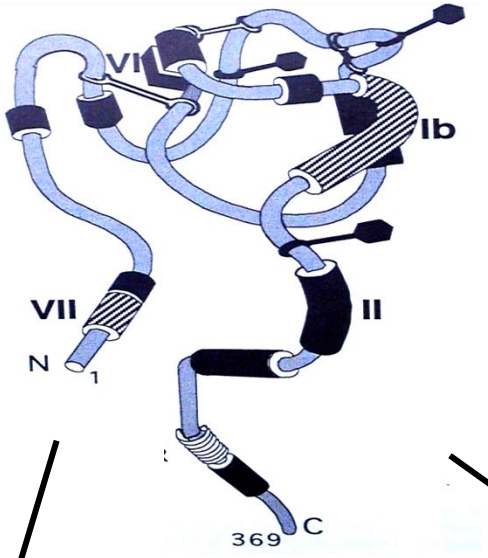
Prediction of T-cell epitopes

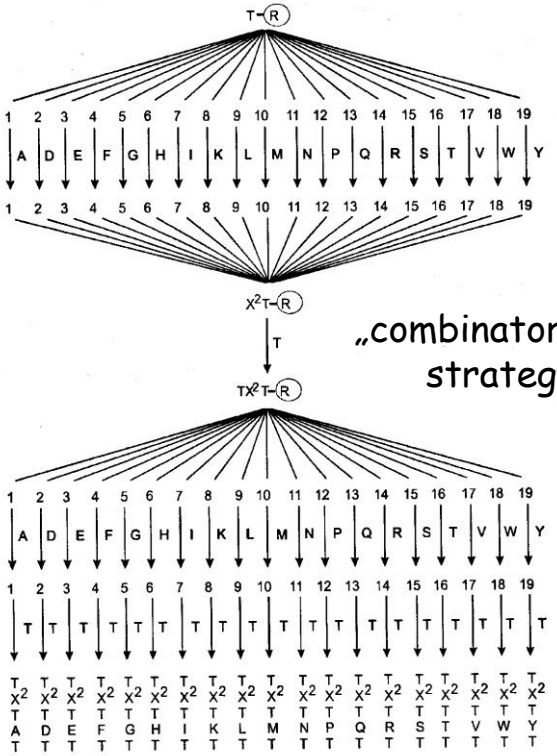


A, amphipathic helix, R, Rothbard motif, D, IAd motif, d, IEd motif

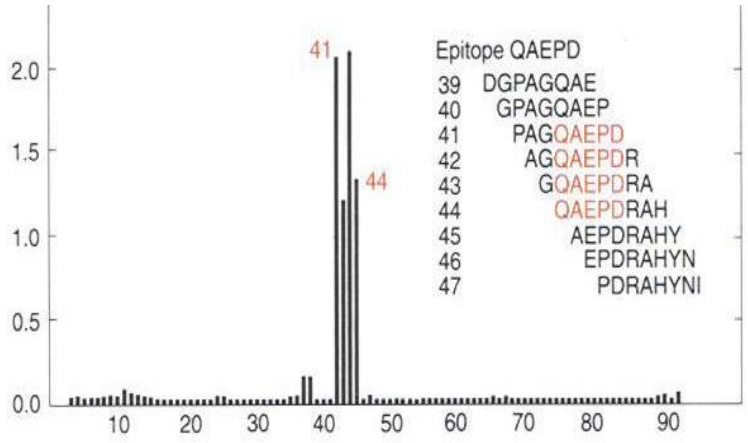
Identification of epitope sequences

„predicted"

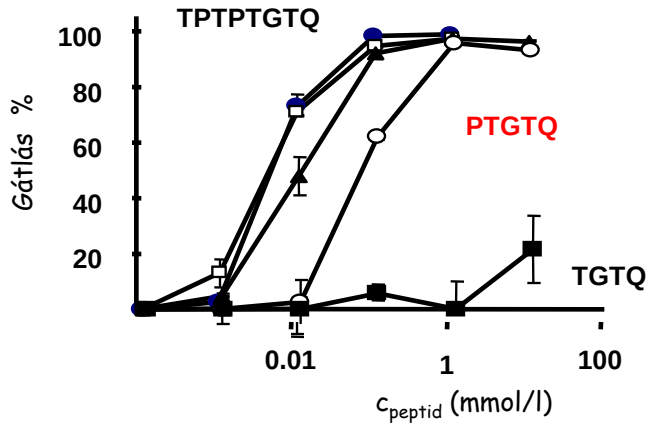




„combinatorial" strategy



„overlapping" strategy



„shortening" strategy

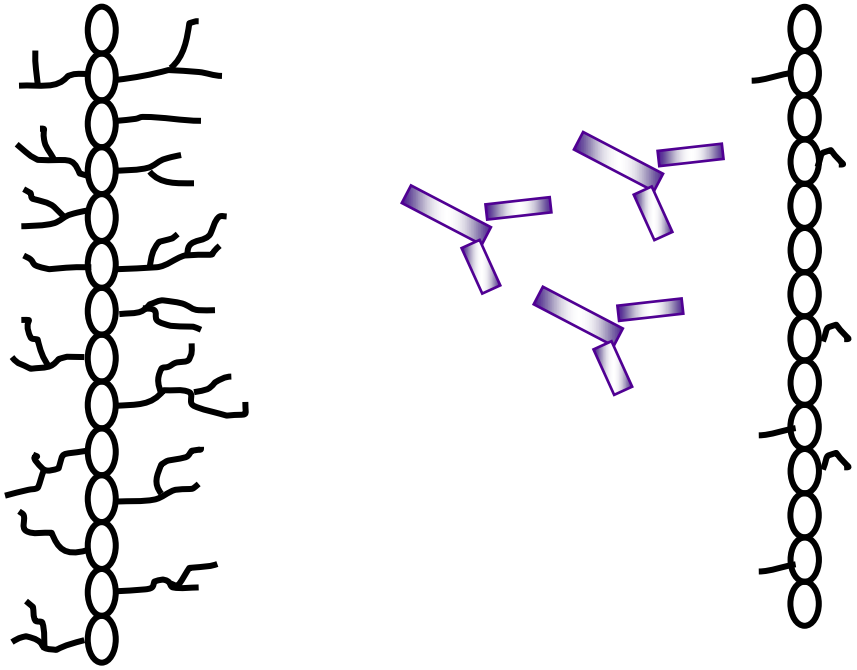
TPTPTGTQ
PTPTGTQ
TPTGTQ
PTGTQ
TGTQ

„Overlapping" strategy

Epitopes of human epithelial mucin glycoprotein, MUC-1 using synthetic peptides and MUC-1 specific antibodies

Normal tissue

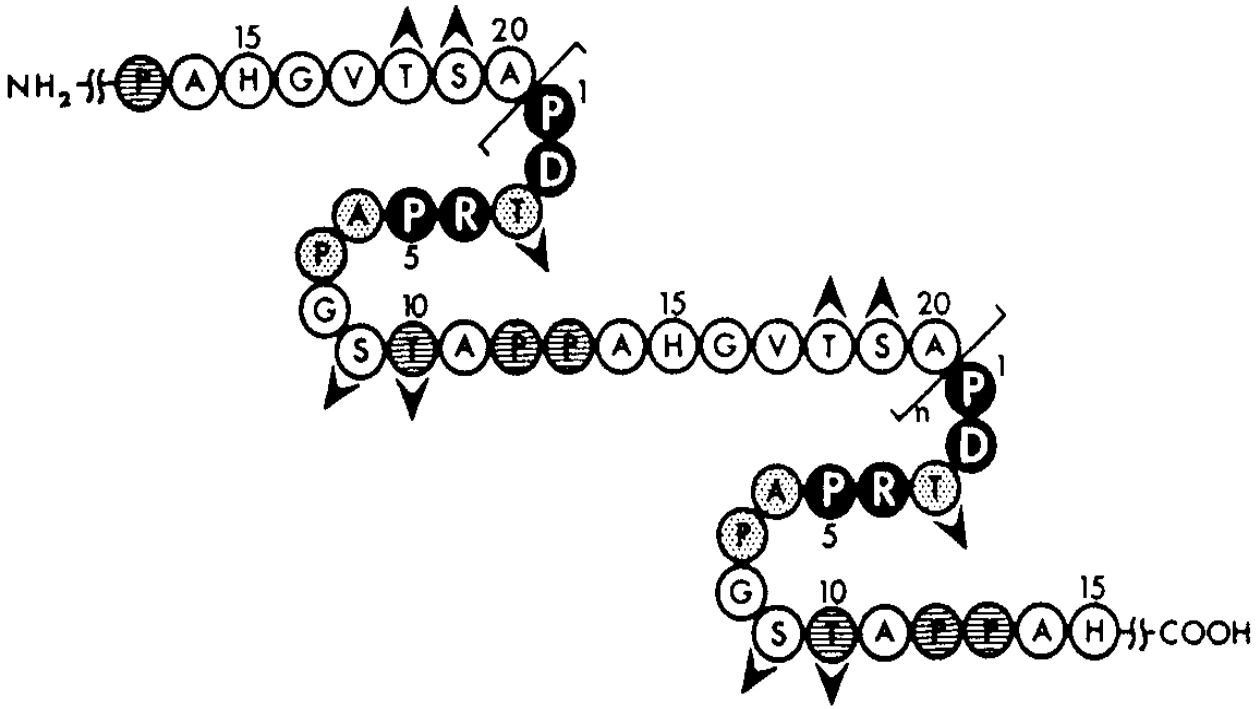
Tumour tissue



Autoantibodies → Tumour diagnosis/immunotherapy

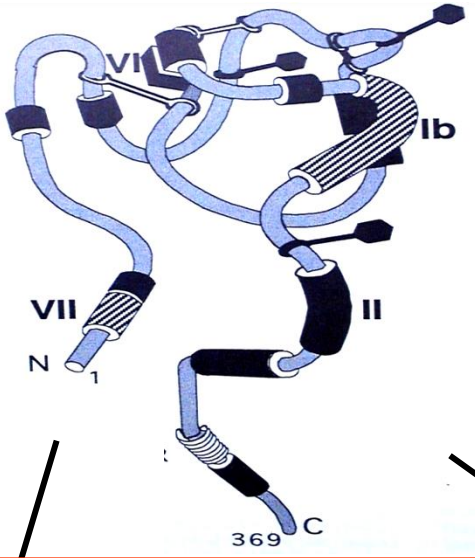
MUC-1: repeat unit – prediction analysis

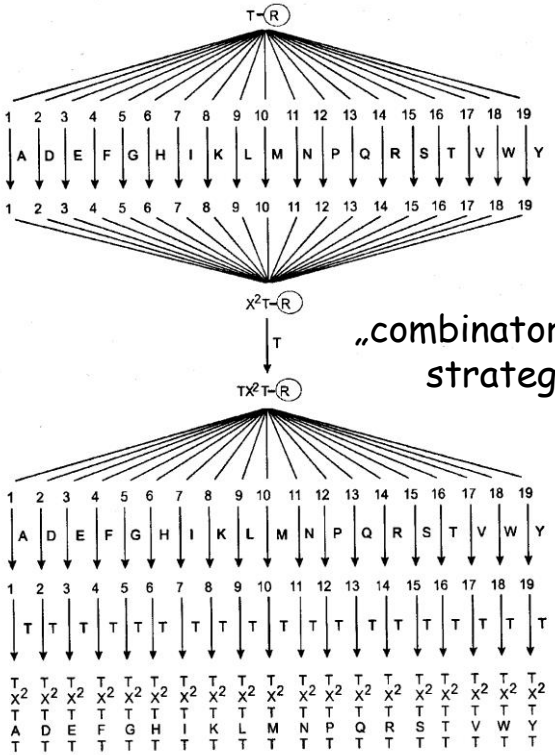
MUC1	^{1}PDTRPAPGSTAPPAHGVTSA^{20}	Gendler et al. 1988
MUC2	^{4}TPITTTTTTVTPTPTPTGTQTPTT^{26}	Gum et al. 1989
MUC3	^{1}HSTPSFTSSITTTETTS^{17}	Gum et al. 1990



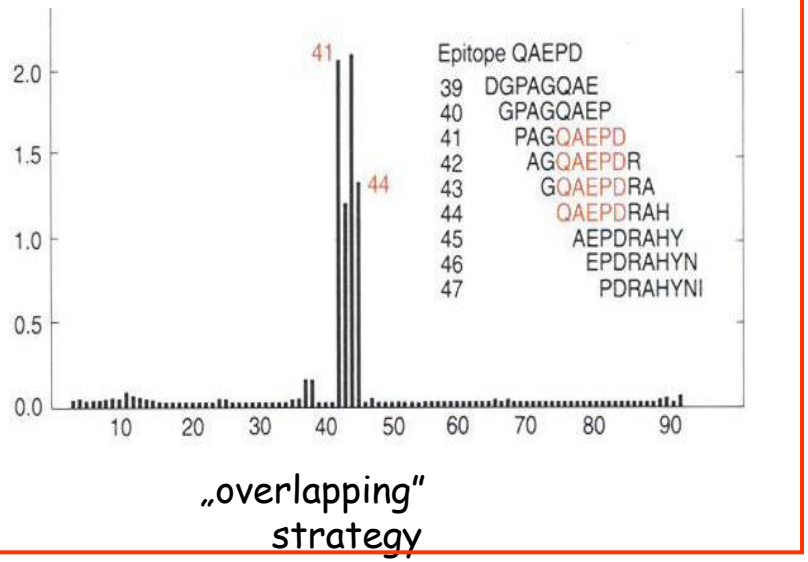
Identification of epitope sequences

„predicted"

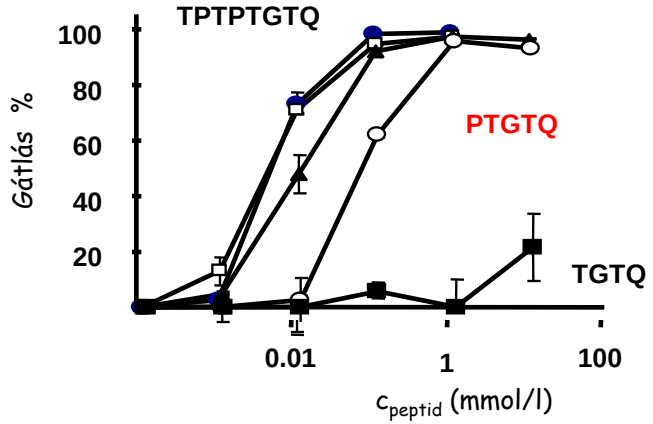




„combinatorial" strategy

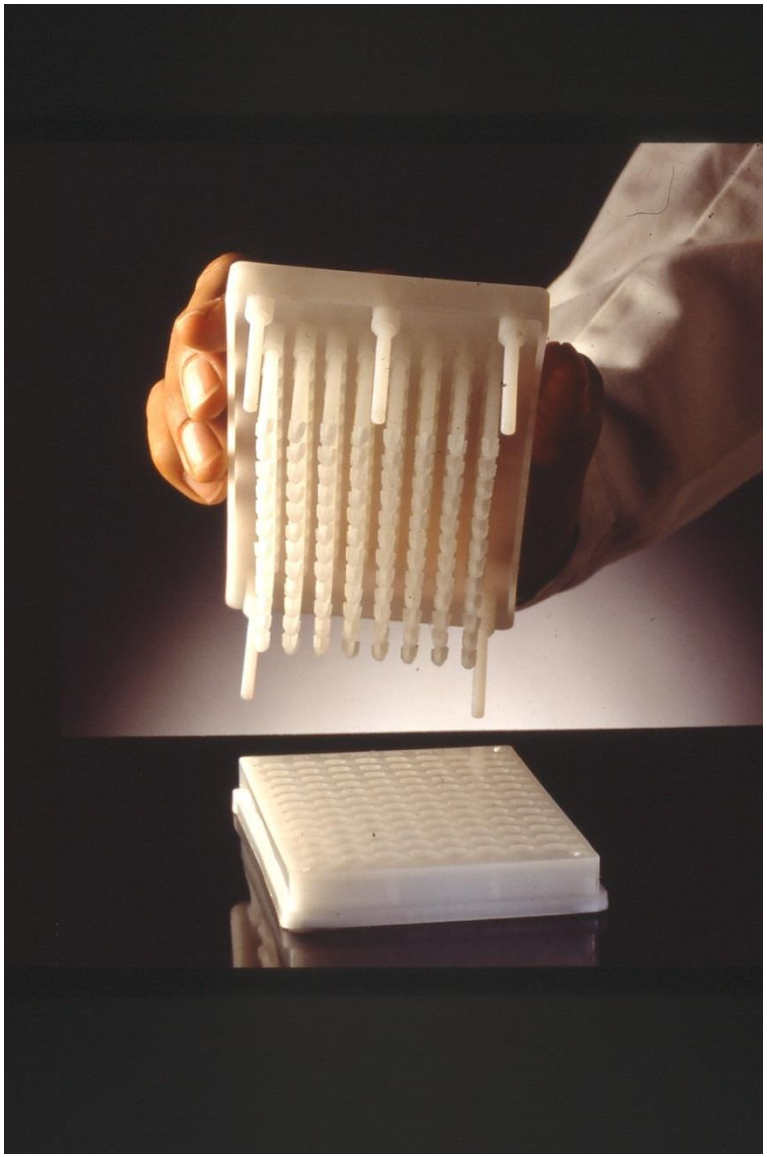


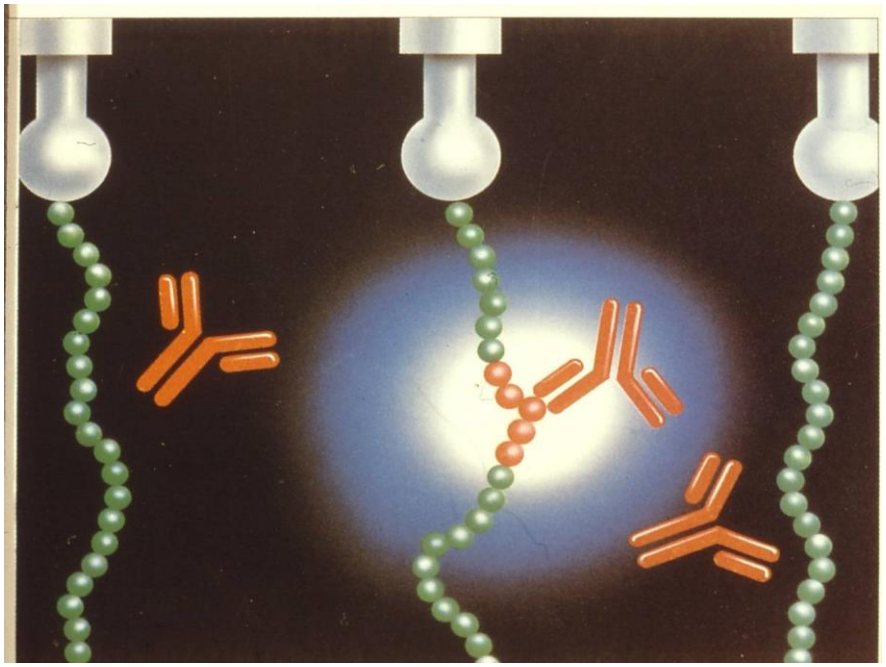
„overlapping" strategy

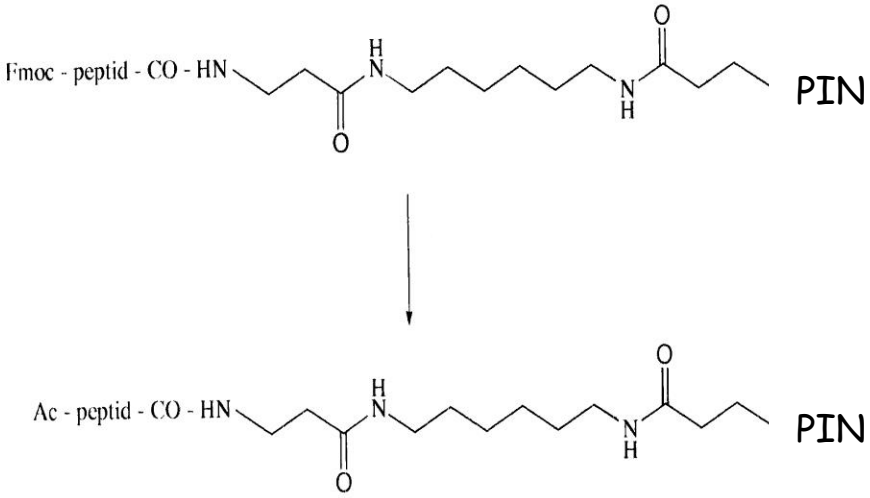


„shortening" strategy

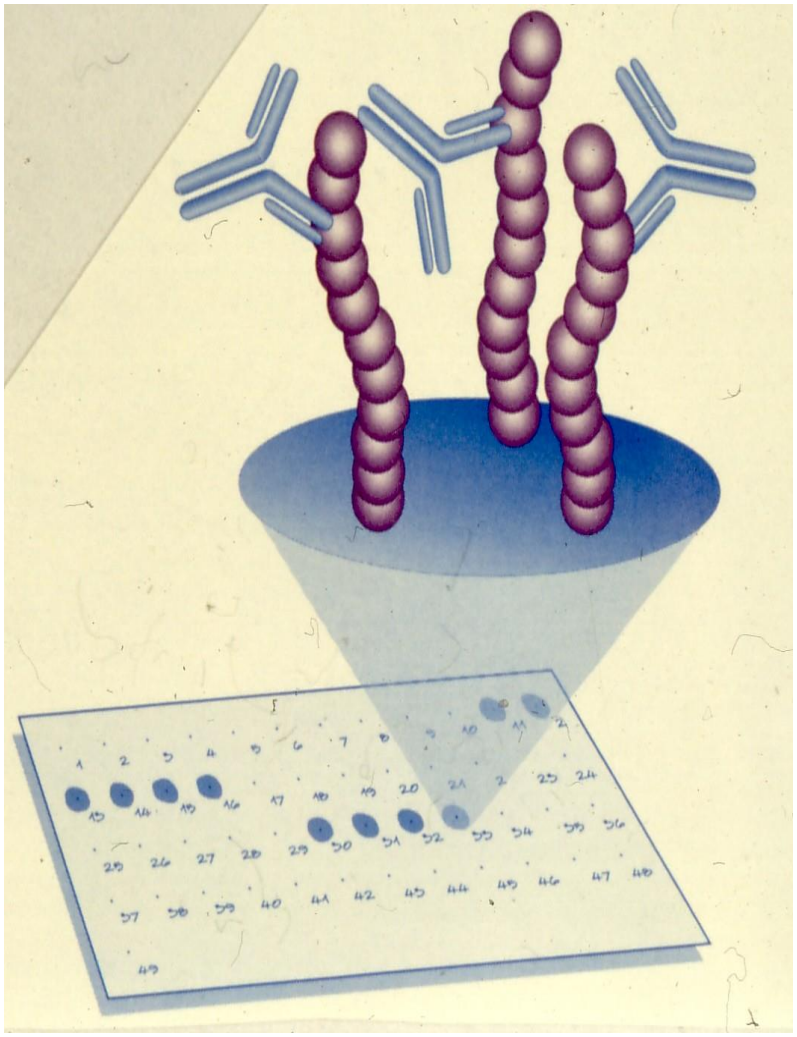
TPTPTGTQ
PTPTGTQ
TPTGTQ
PTGTQ
TGTQ

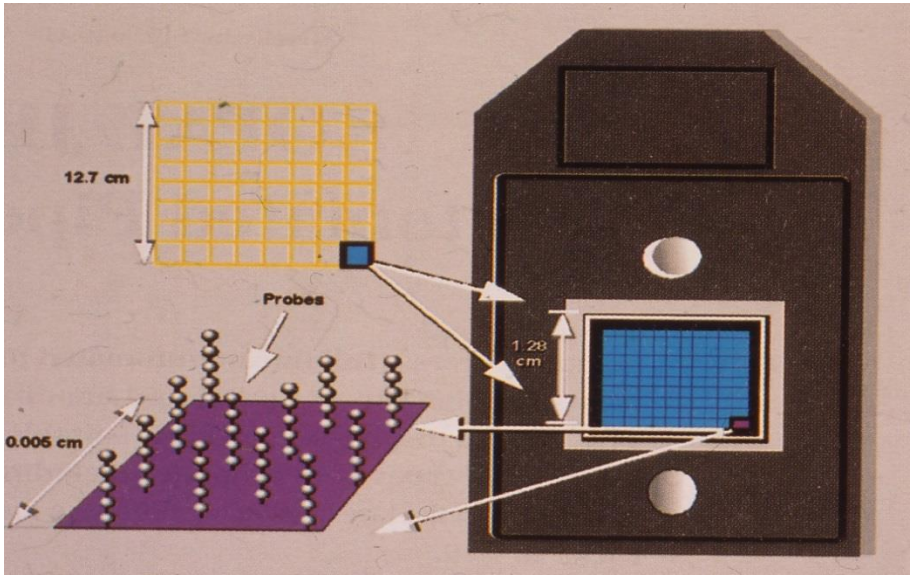




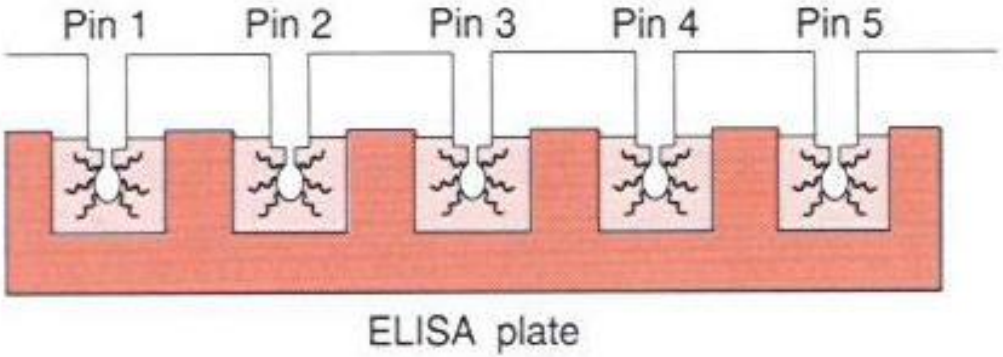

Fmoc - peptid - CO - HN
H
N
O
N
H
O
PIN
Ac - peptid - CO - HN
H
N
O
N
H
O
PIN

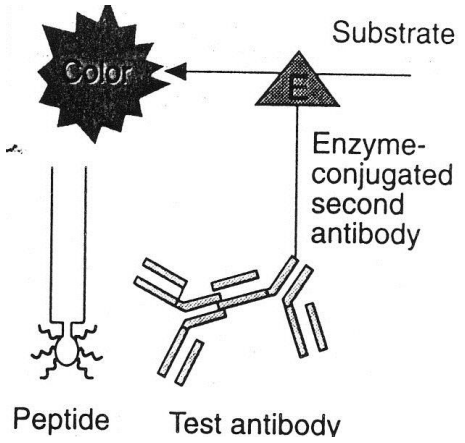
SPOT/chip peptide synthesis

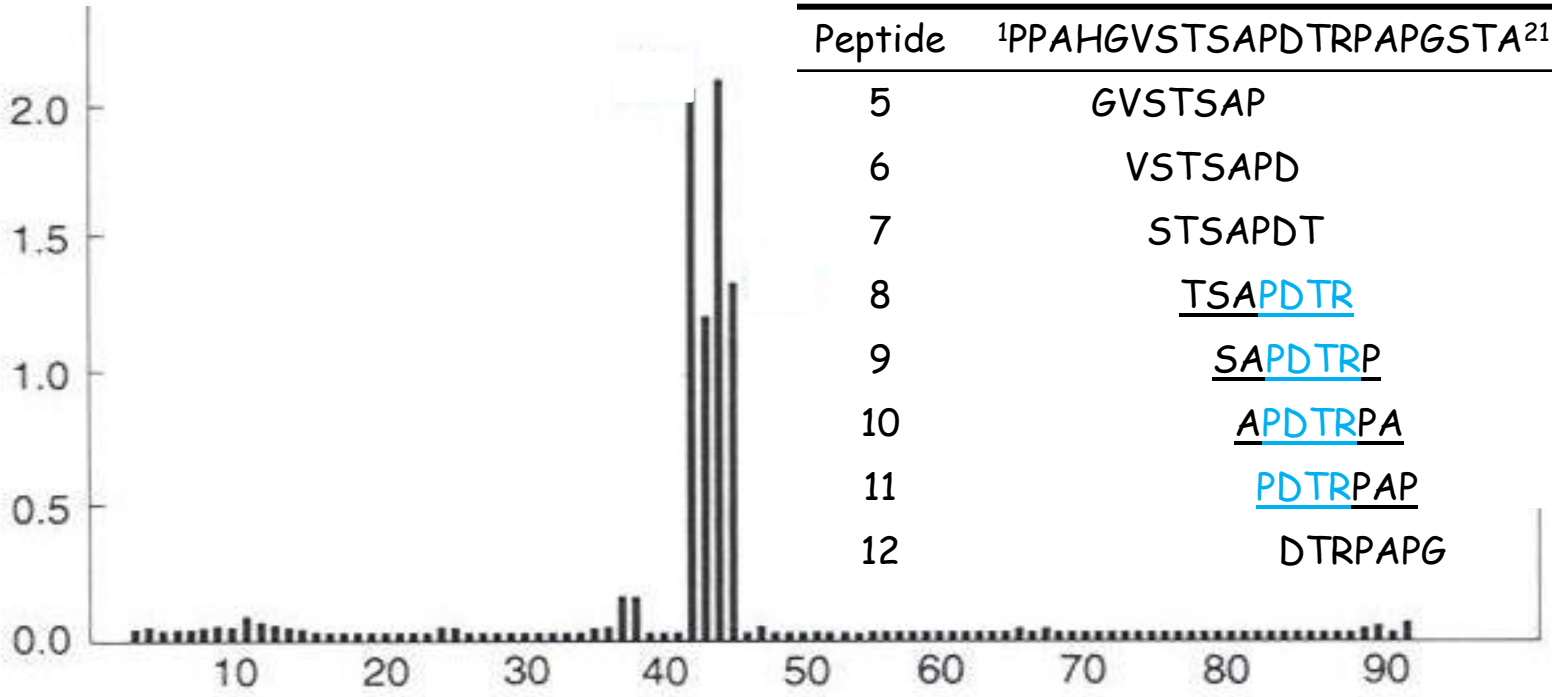




Binding studies: Identification of linear antibody epitopes by monoclonal antibodies

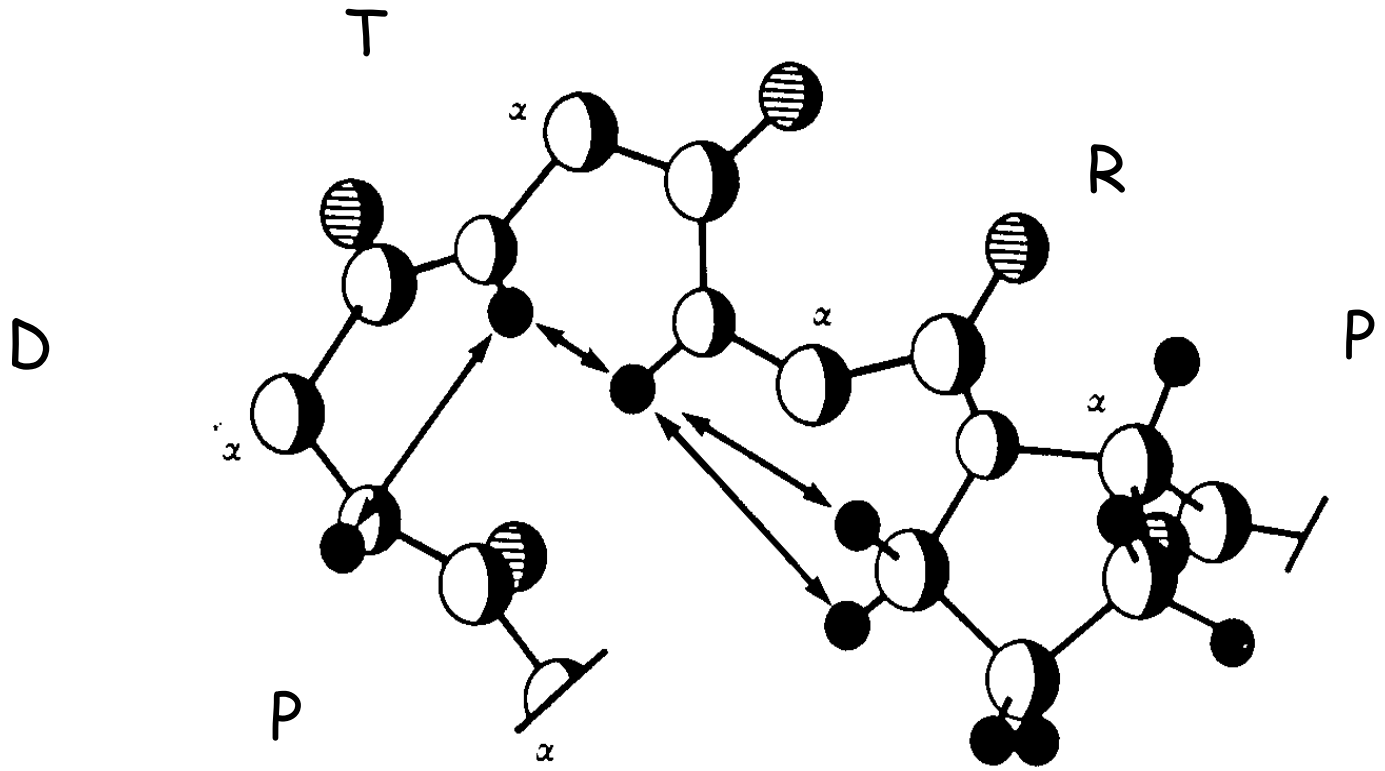






Peptide	^{1}PPAHGVSTSAPDTRPAPGSTA^{21}	Elisa (A_{405})
5	GVSTSAP	0.00
6	VSTSAPD	0.02
7	STSAPDT	0.01
8	TSAPDTR	0.73
9	SAPDTRP	0.94
10	APDTRPA	1.09
11	PDTRPAP	0.63
12	DTRPAPG	0.02

3D structure of epitope region containing PDTR sequence by 2D NMR



M.R. Price, F. Hudecz et al. Mol. Immunol. 62: 795 (1990)
S.J.B. Tendler Biochem. J. 267: 733 (1990)

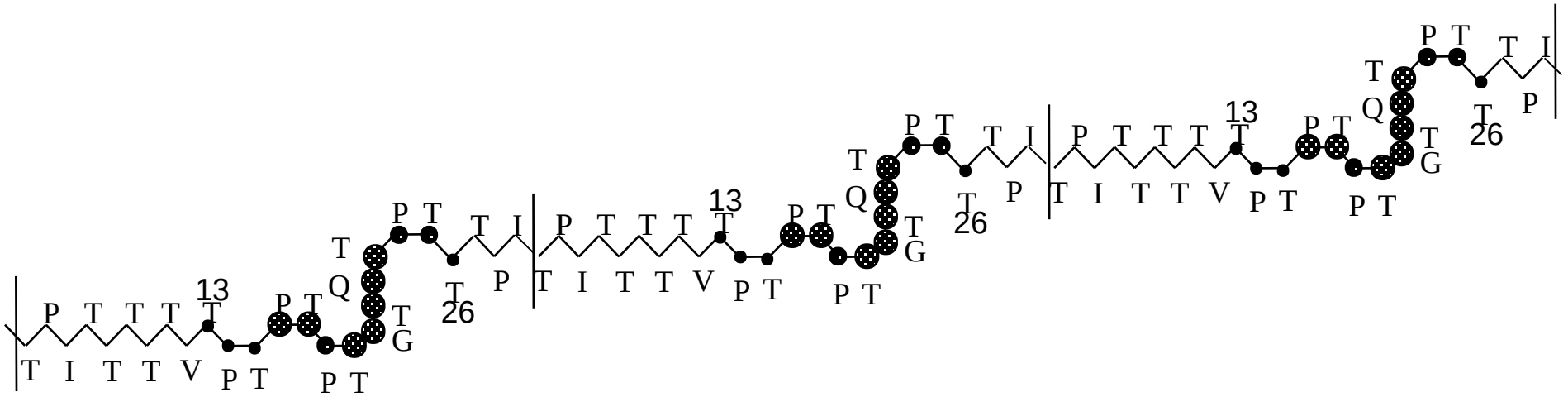
Combinatorial strategy

MUC-2: repeat unit – prediction analysis

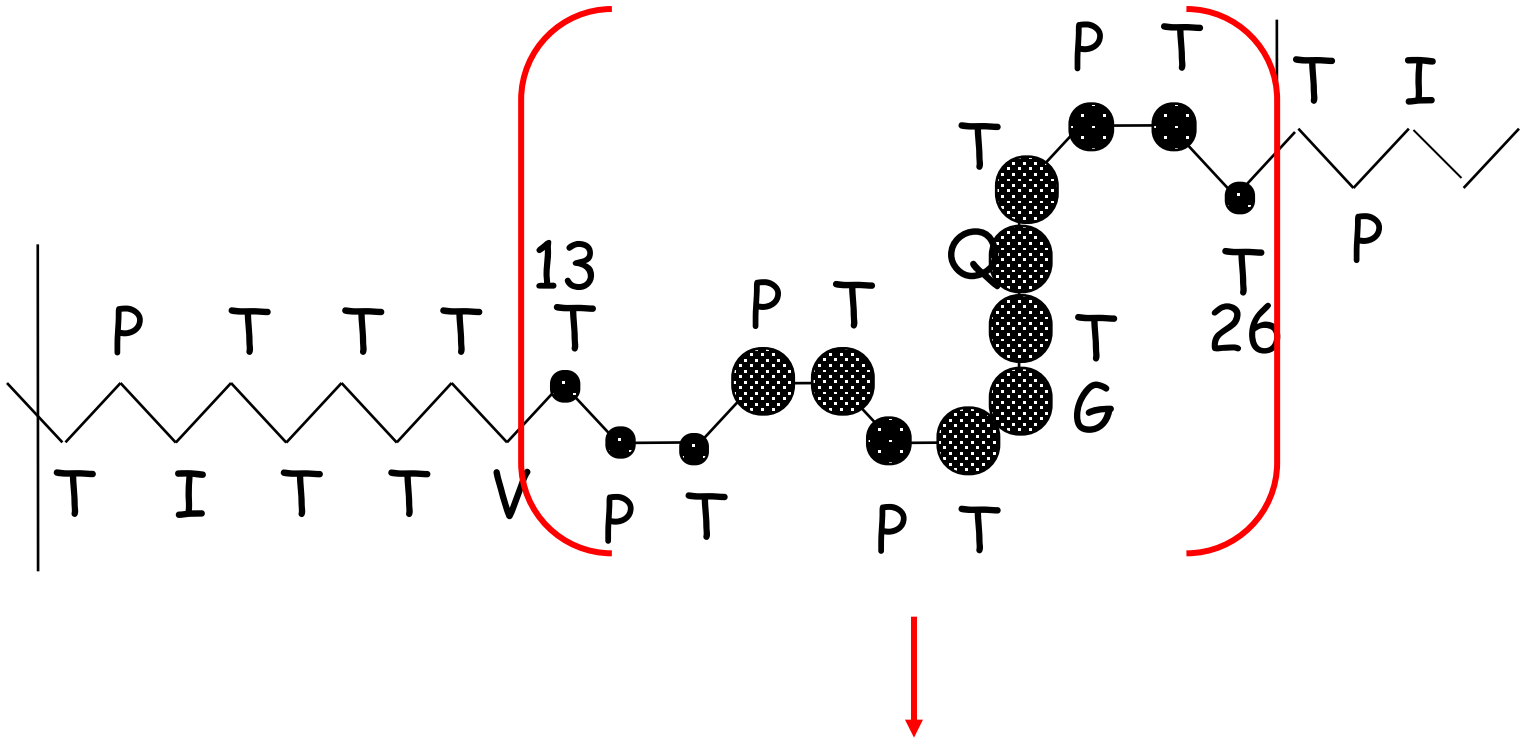
MUC1 ^{1}PDTRPAPGSTAPPAHGVTSA^{20} Gendler et al. 1988

MUC2 ^{4}TPITTTTTTVTPTPTPTGTQTPTT^{26} Gum et al. 1989

MUC3 ^{1}HSTPSFTSSITTTETTS^{17} Gum et al. 1990



Predicted secondary structure of MUC2 repeat motif

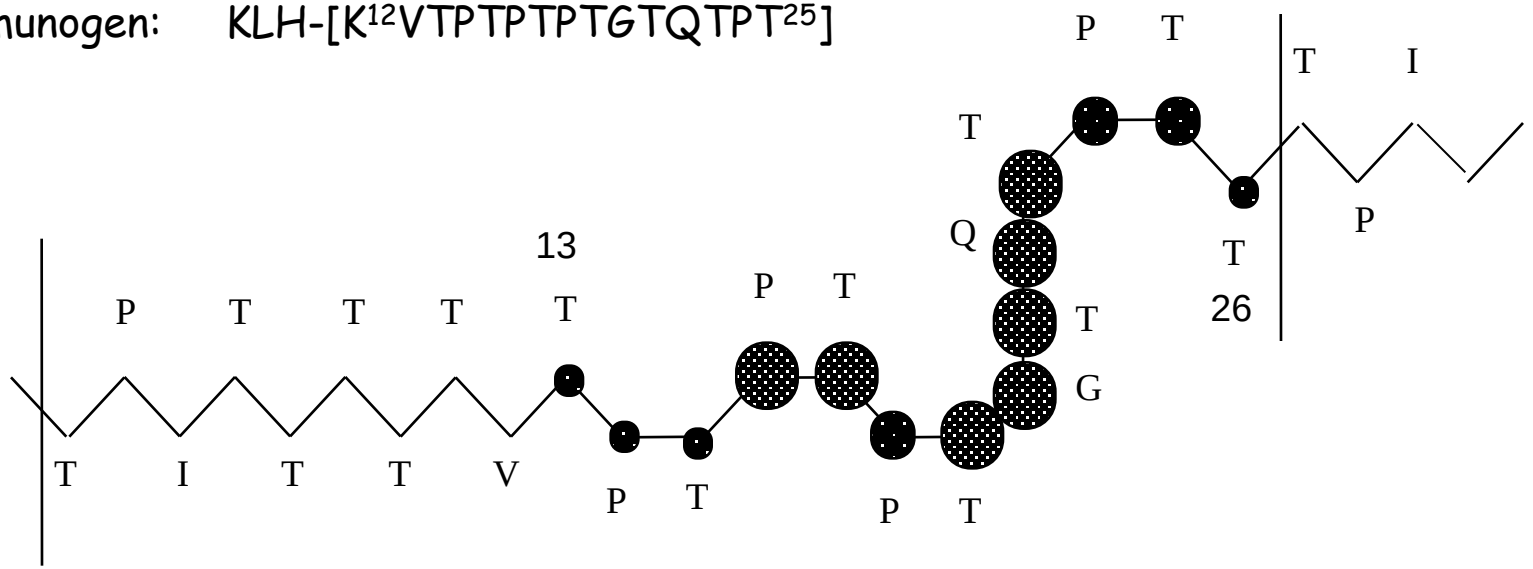


predicted antibody epitope region

Conclusion

Multiple epitopes: ^{21}TQTPT^{25}
^{19}TGTQT^{23}
^{13}TPTPT^{17}

Immunogen: KLH-[K^{12}VTPTPTPTGTQTPT^{25}]



Immunohistochemistry: Recognition of human colon tumour tissue by mouse IgG1 (Mab 994)

Durrant et al. Eur. J. Cancer (1994)

Conclusion

Multiple epitopes:

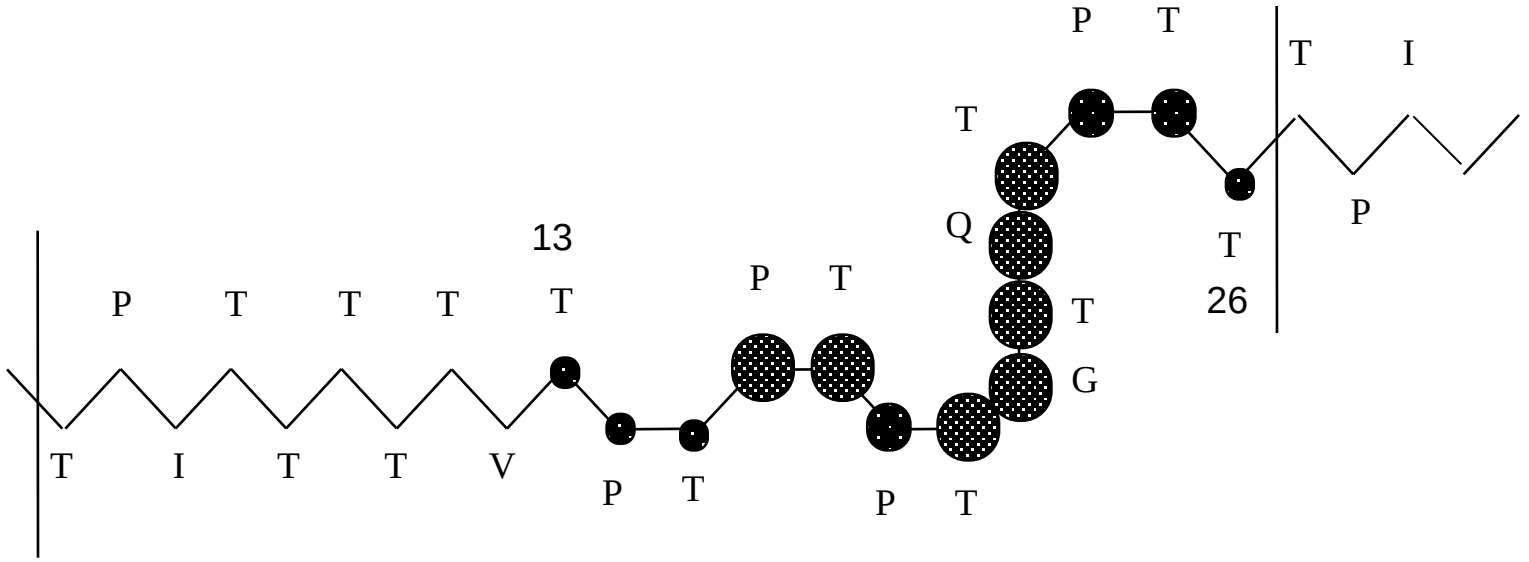
^{21}TQTPT^{25}

^{19}TGTQT^{23}

^{13}TPTPT^{17}

Common motif:

TXTXT



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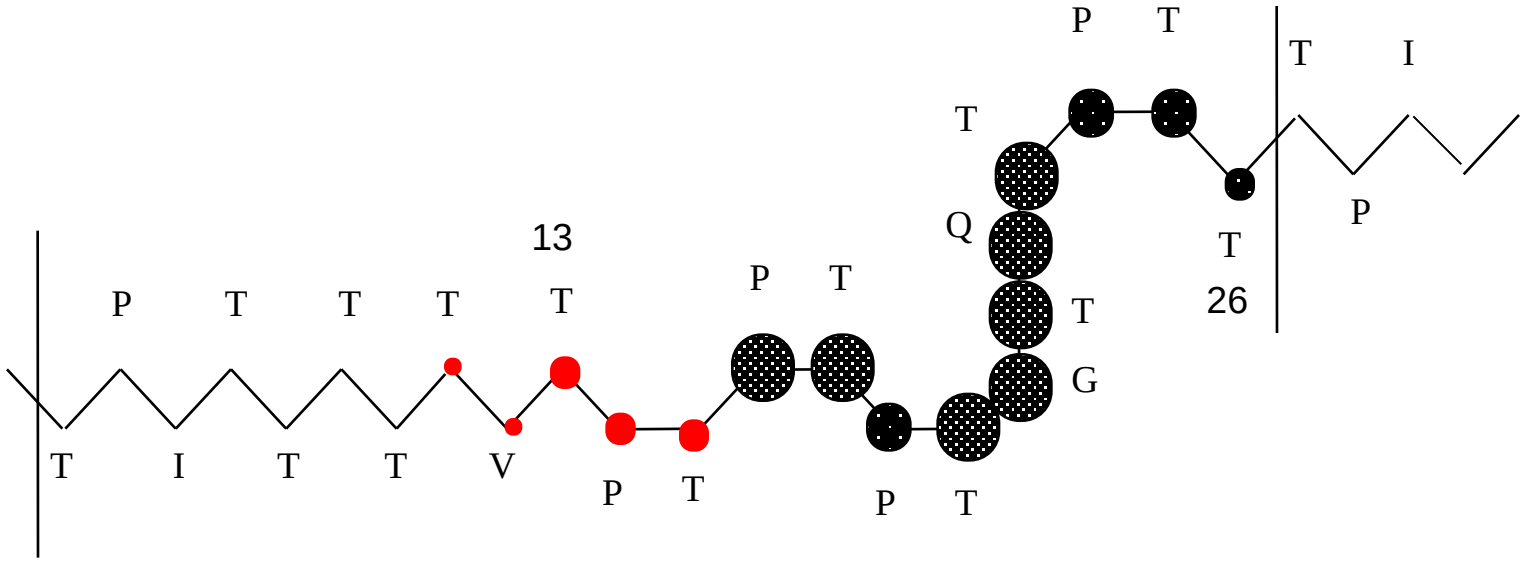

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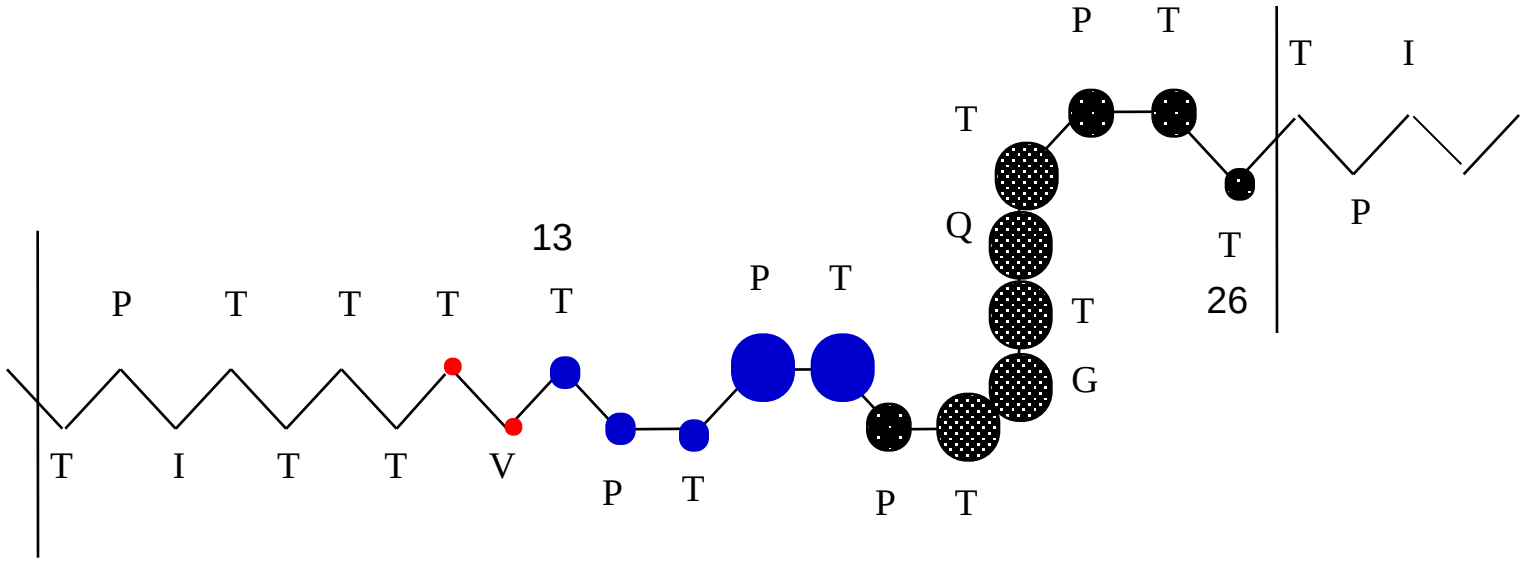

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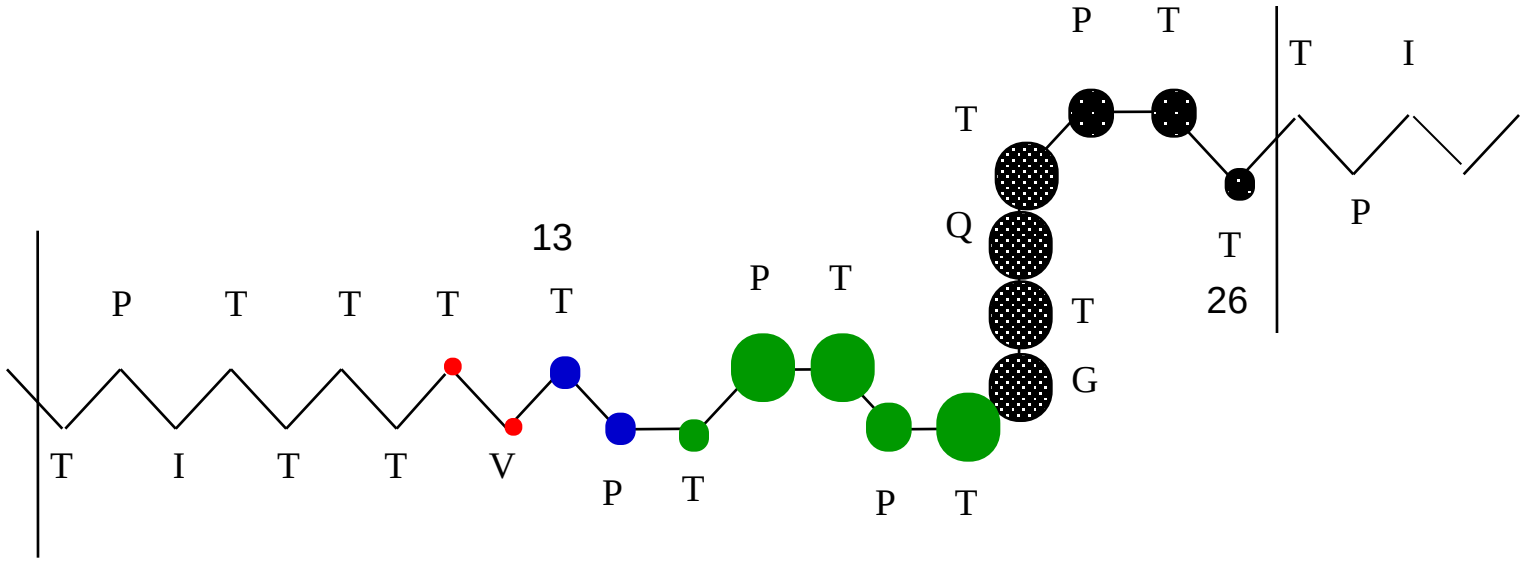

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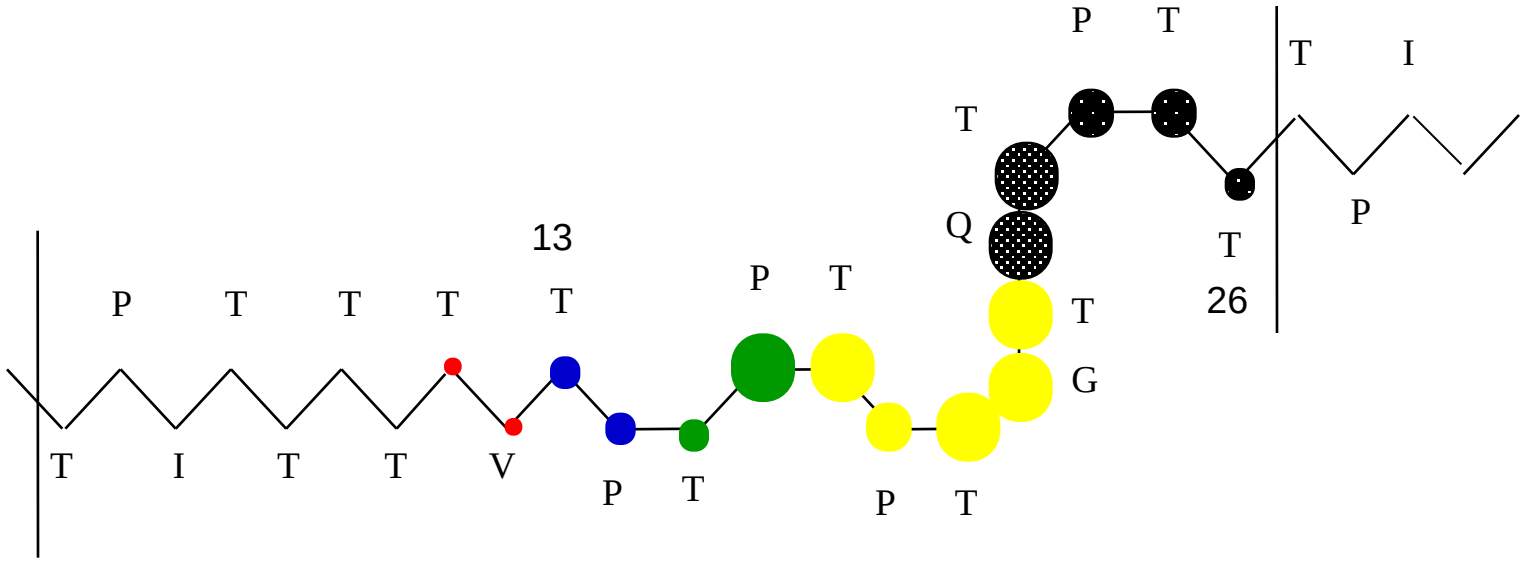

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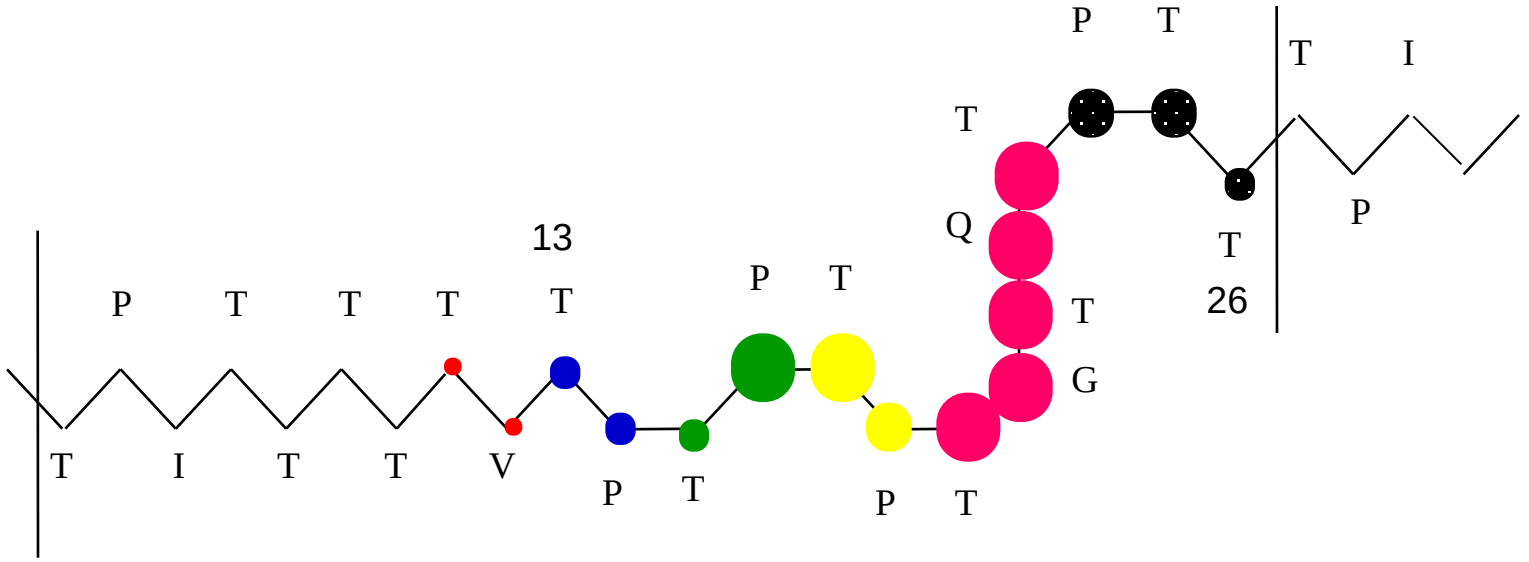
Common motif: TXTXT



Uray et al. Arch. Biochem. Biophys. (2003)

Conclusion

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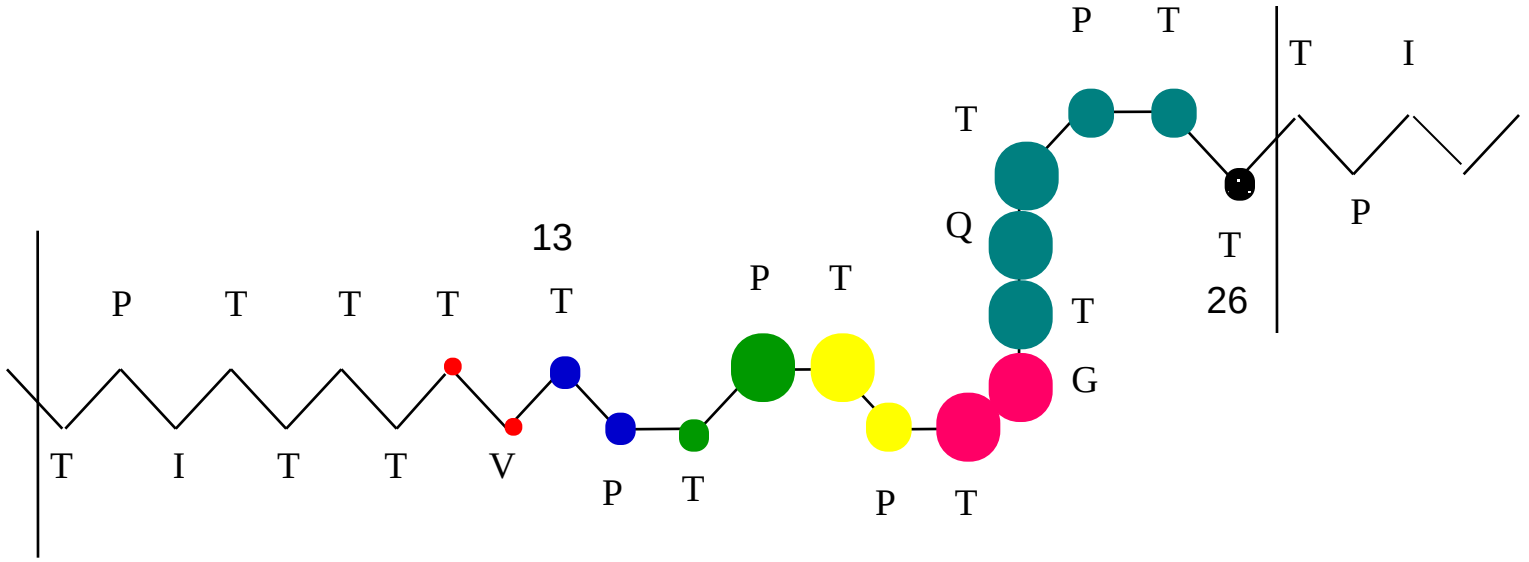
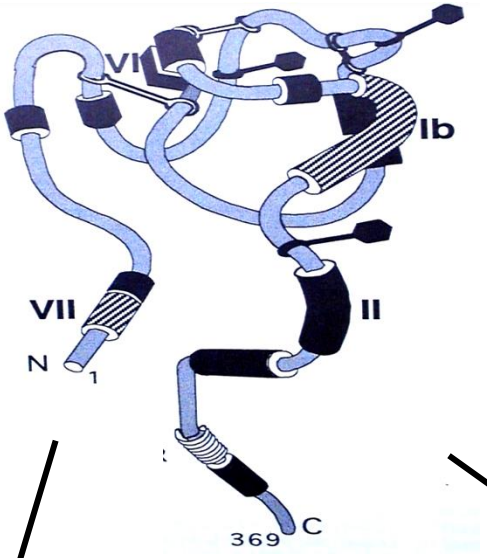
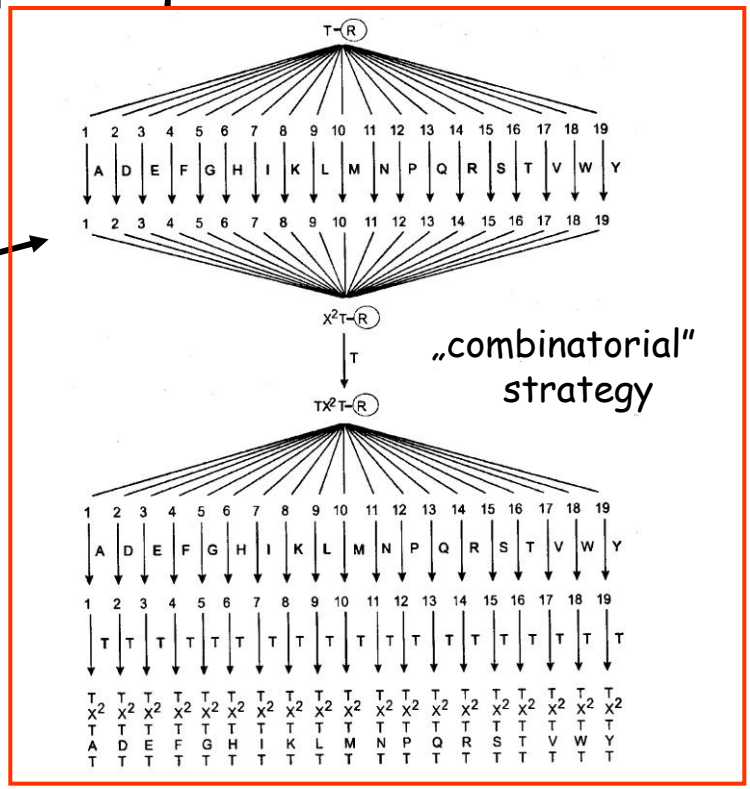


Uray et al. Arch. Biochem. Biophys. (2003)

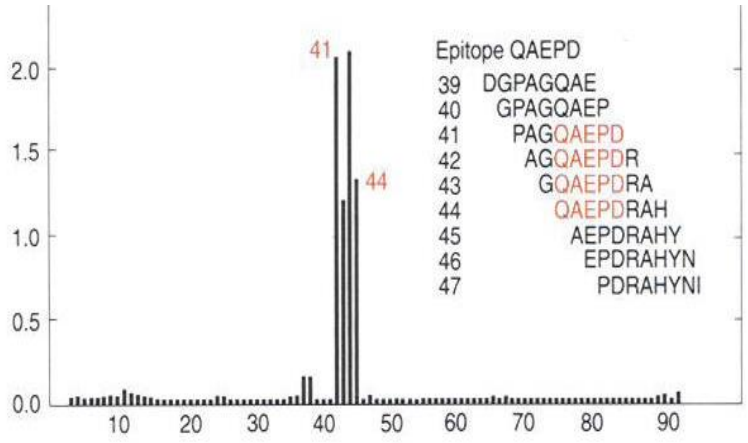
Identification of epitope sequences

„predicted"

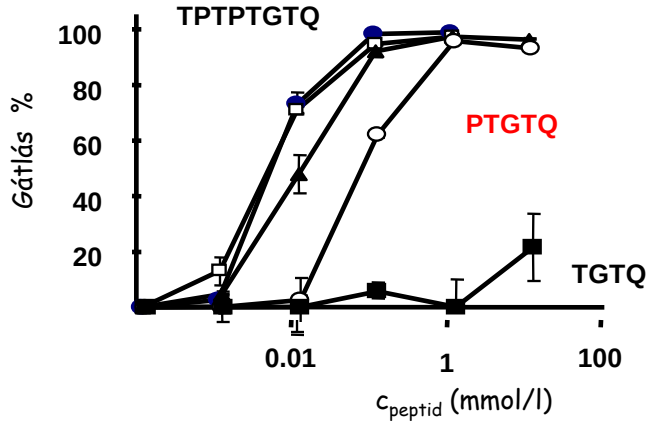




„combinatorial" strategy



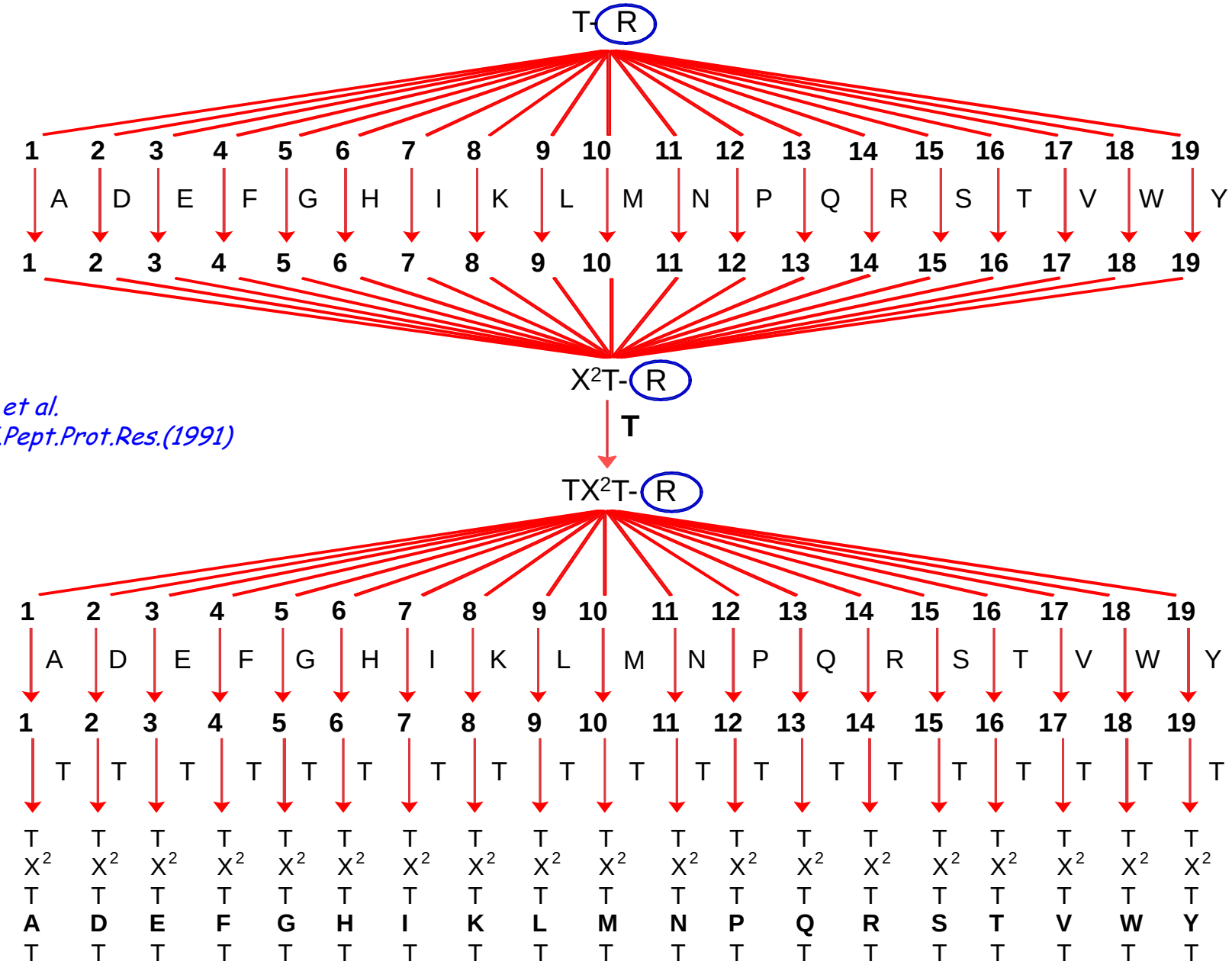
„overlapping" strategy



„shortening" strategy

TPTPTGTQ
PTPTGTQ
TPTGTQ
PTGTQ
TGTQ

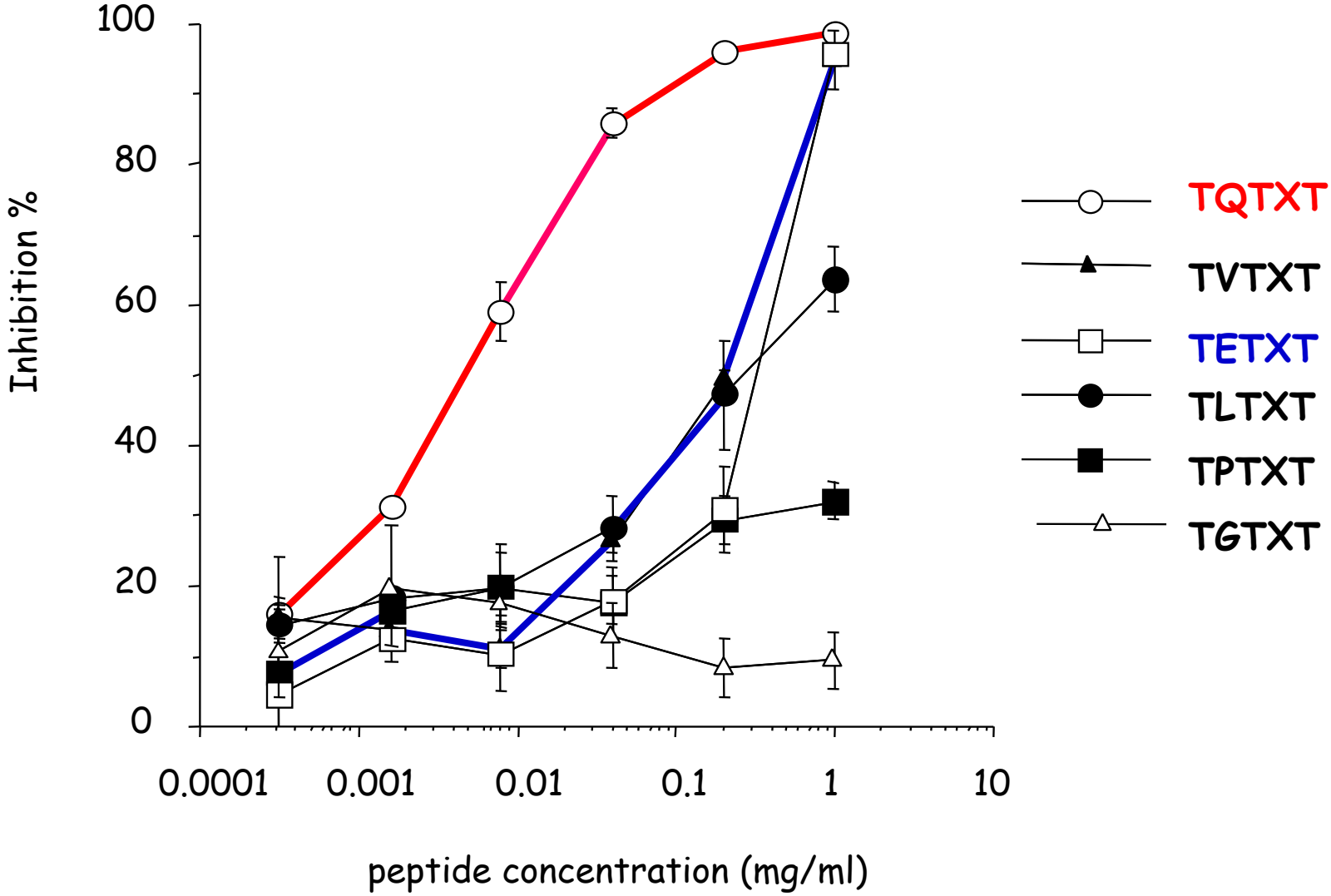
Combinatorial synthesis of TX^1TX^2T peptide library



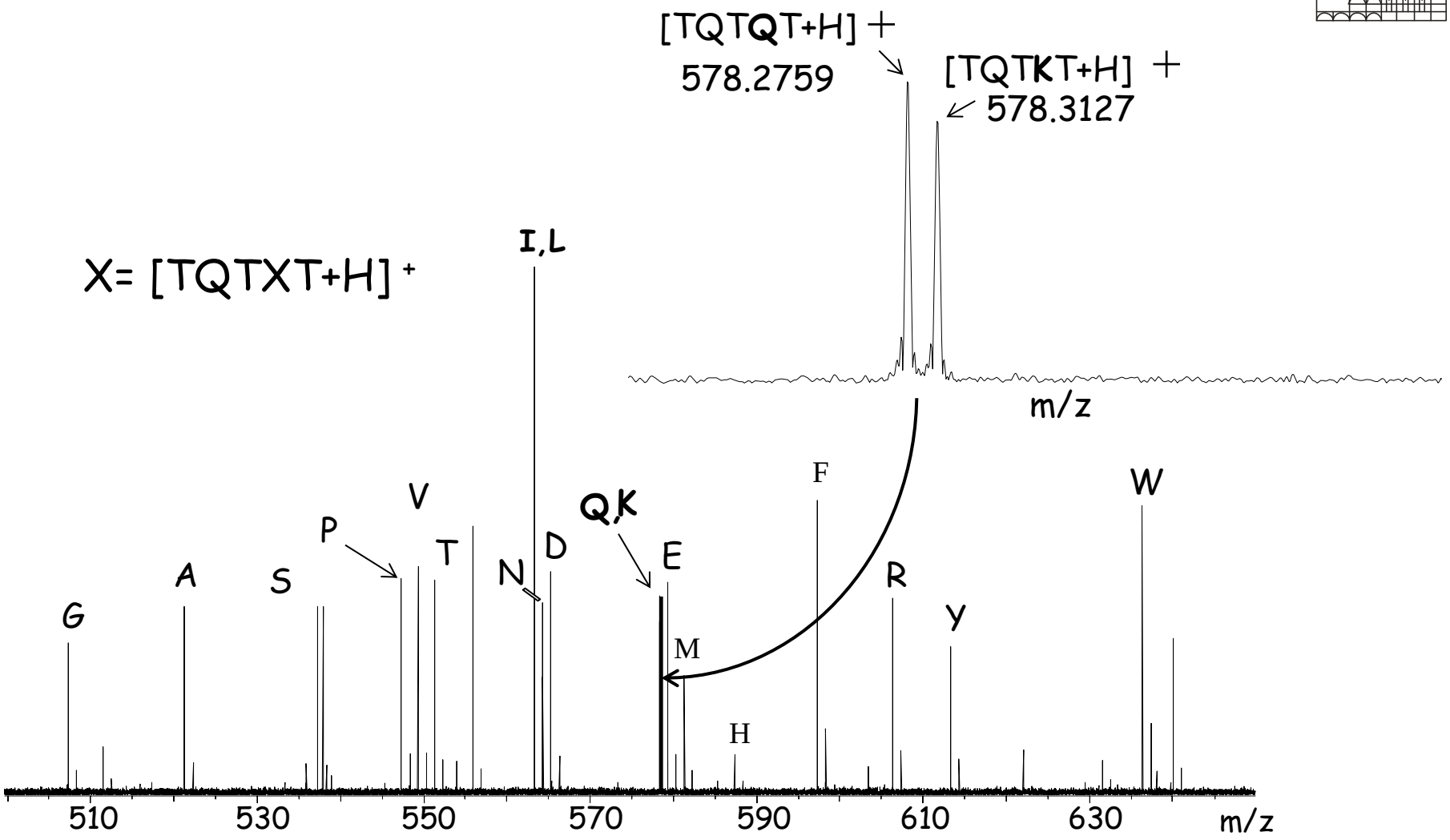
Furka et al.
Int. J.Pept.Prot.Res.(1991)

Binding of Mab 994 to TX^1TX^2T peptide mixtures

with 19 x 19 components

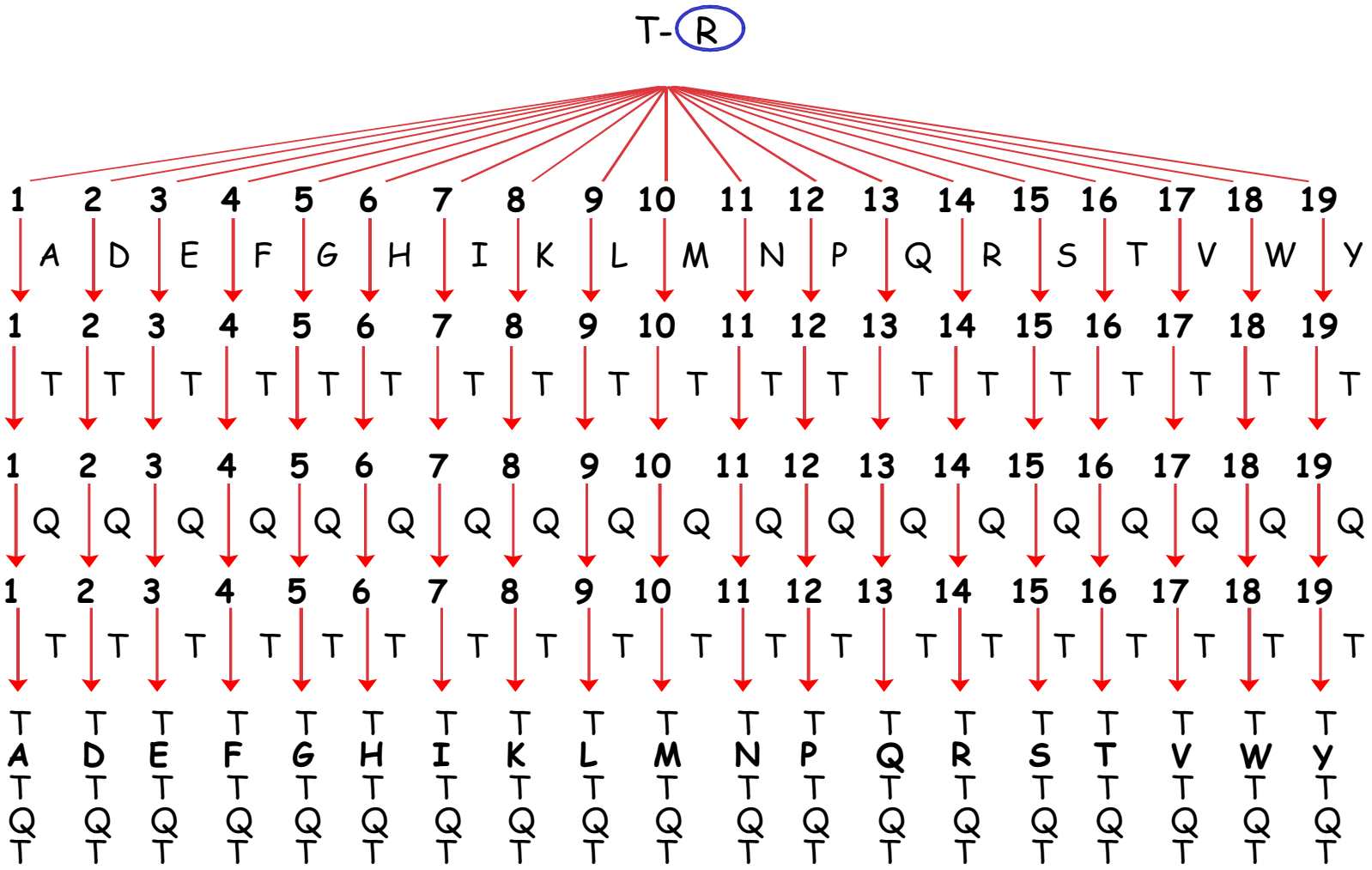


ESI-FTICR spectrum of TQTX²T peptide library



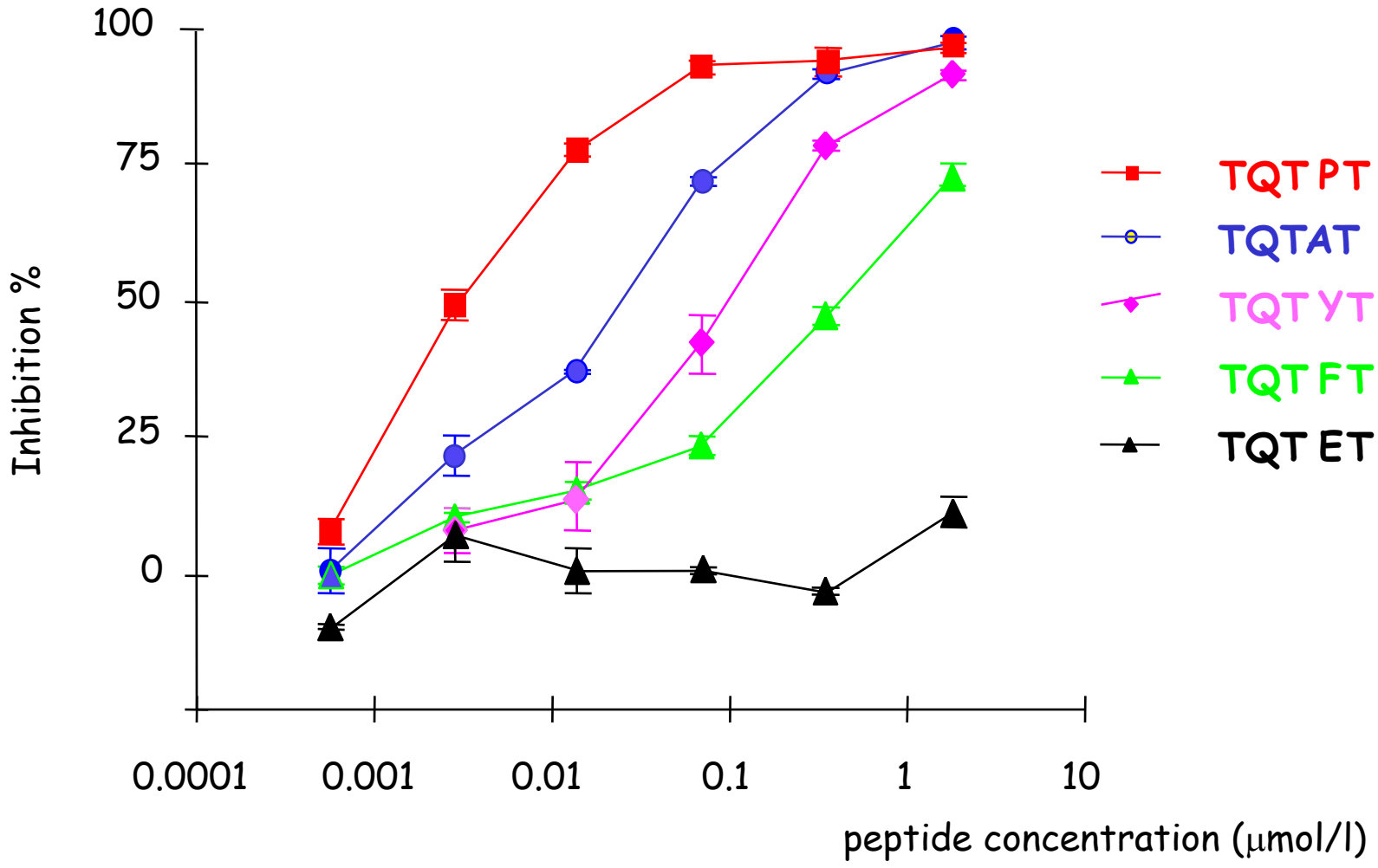
Windberg et al. Rapid Comm.Mass Spectrometry (2003)

Paralell synthesis of TQTX²T peptide sub-library

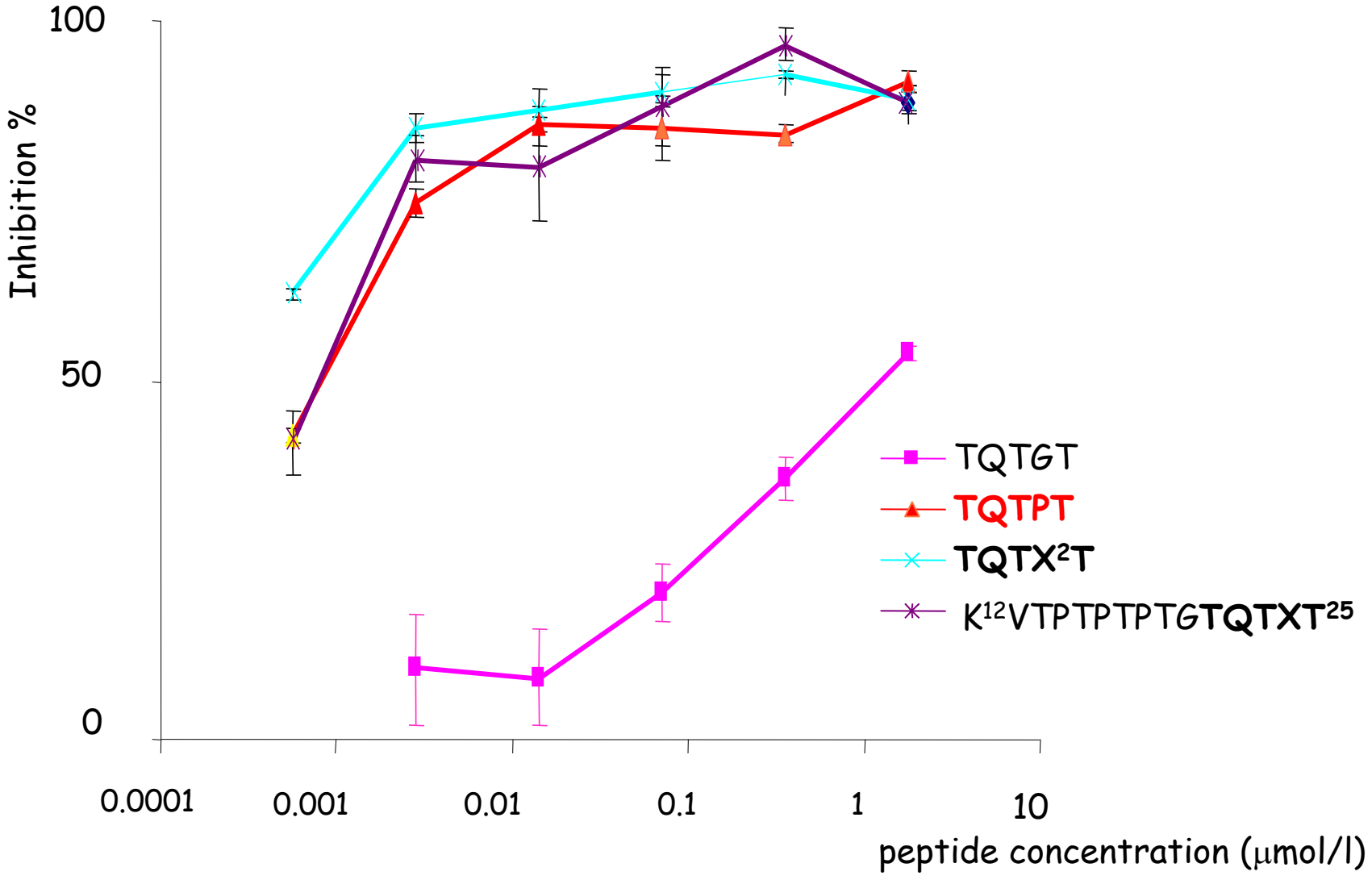


Binding of Mab 994 to TQTX²T peptides

with 19 peptides

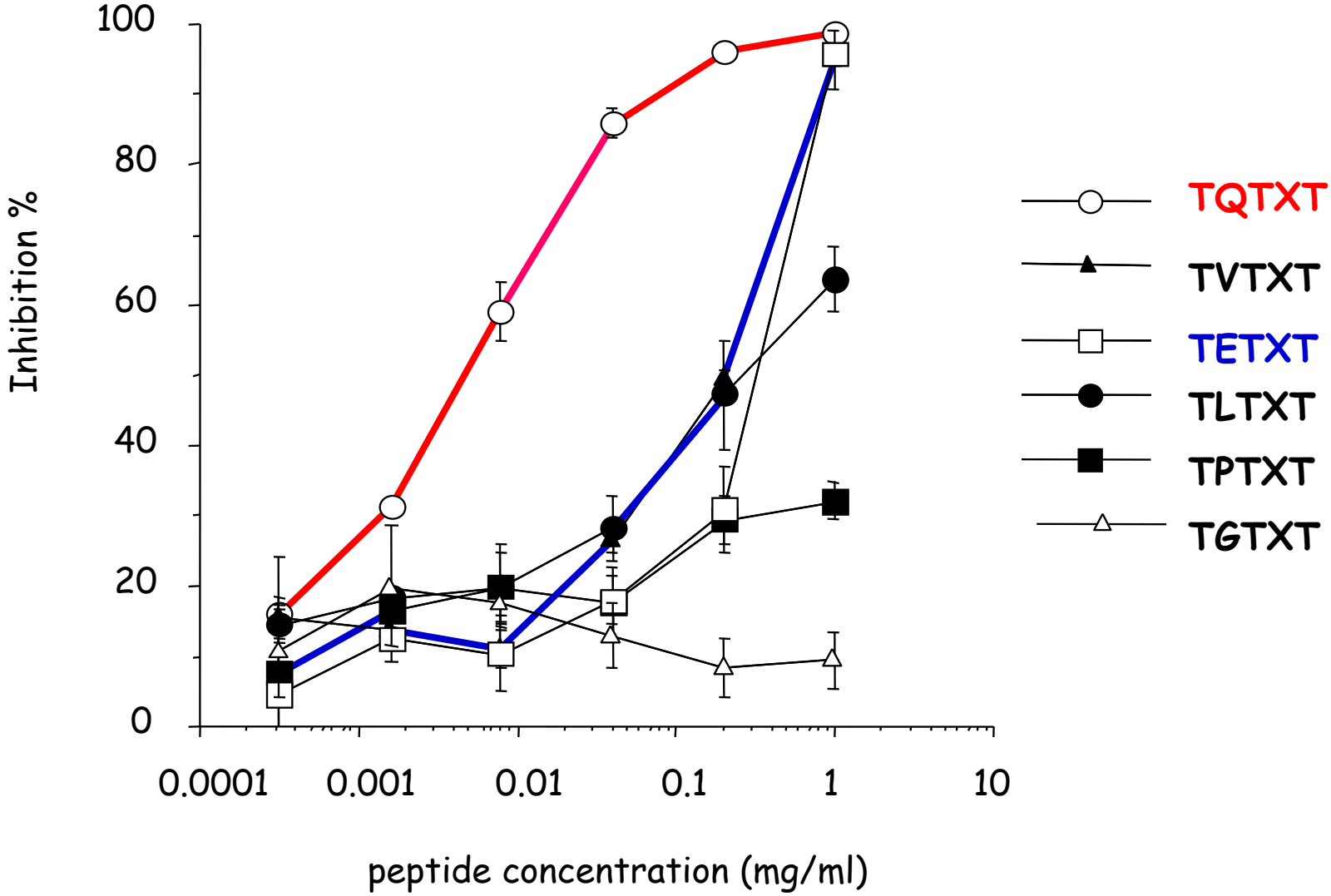


Binding of Mab 994 to $K^{12}VTPTPTPTG\mathbf{TQTXT}^{25}$ peptide mixtures



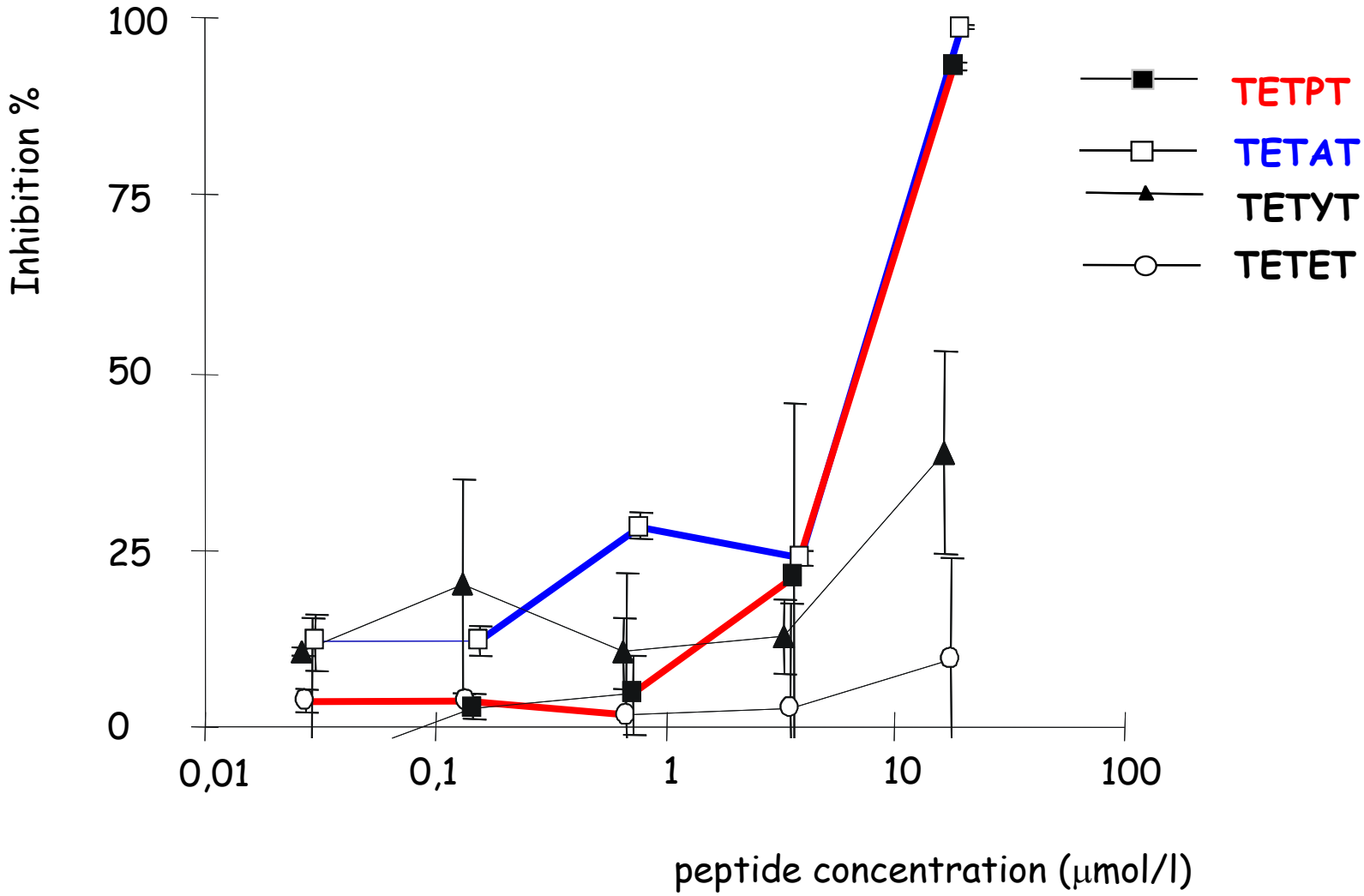
Binding of Mab 994 to TX^1TX^2T peptide mixtures

with 19 x 19 components

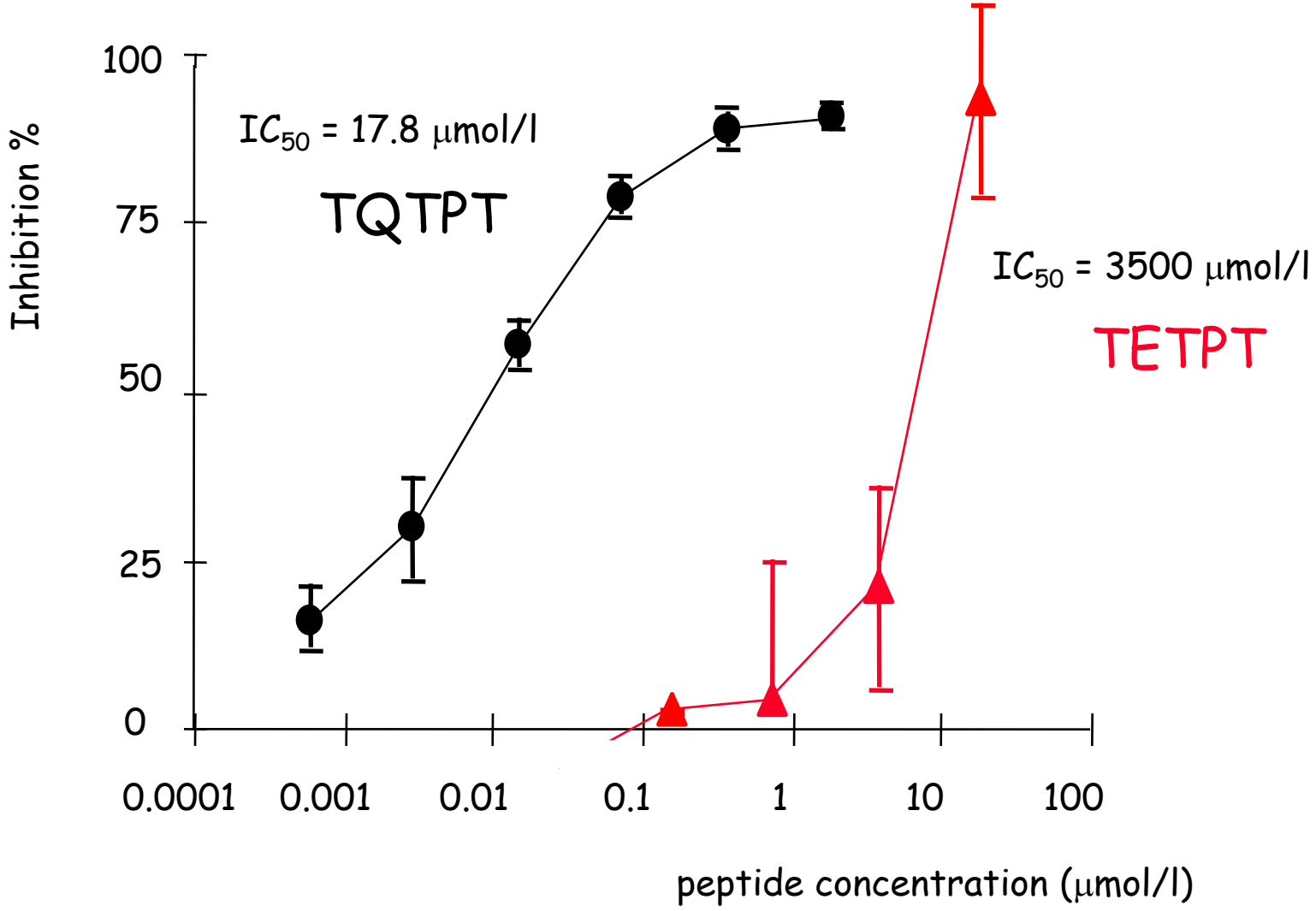


Binding of Mab 994 to TETXT peptides

with 19 peptides



Comparison of Mab 994 binding to TQTPT and TETXT peptides

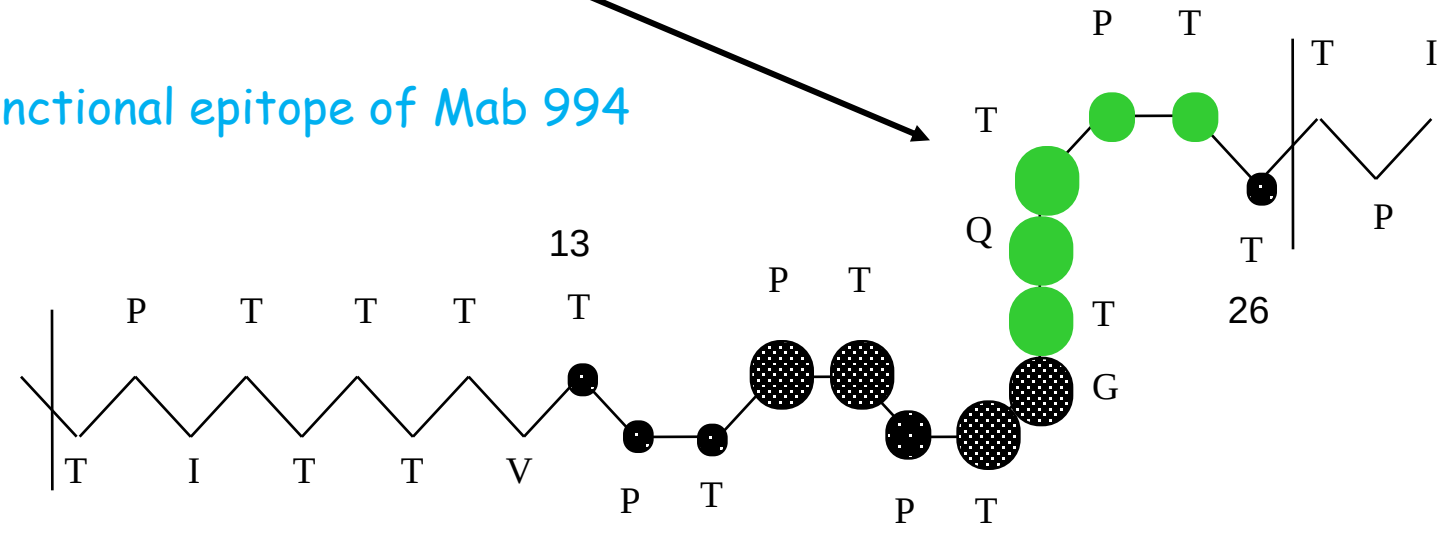


MUC-2: epitope hierarchy of the repeat unit

ELISA: TQTPT > TQTAT > TQTYT > TQTWT > TQTFT > TQTST

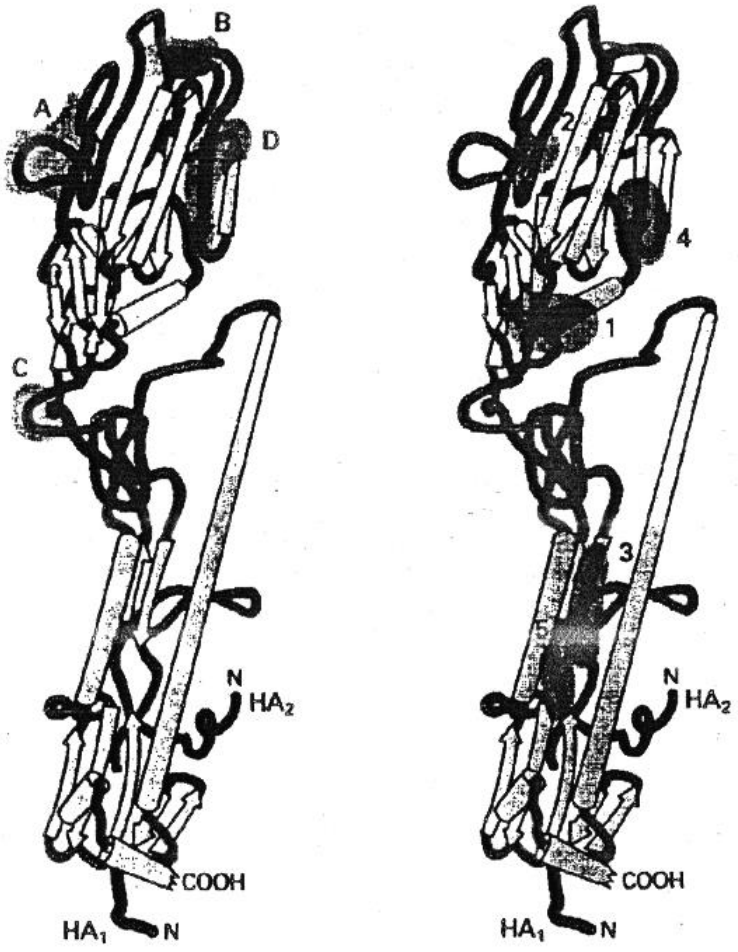
(IC_{50} : 3.4 < 14.2 < 39.8 < 70.0 < 88.0 < 208 μmol/l)

Functional epitope of Mab 994



Windberg et al. J. Peptide Science (2004)

Antigenic structure of influenza vírus hemagglutinin protein

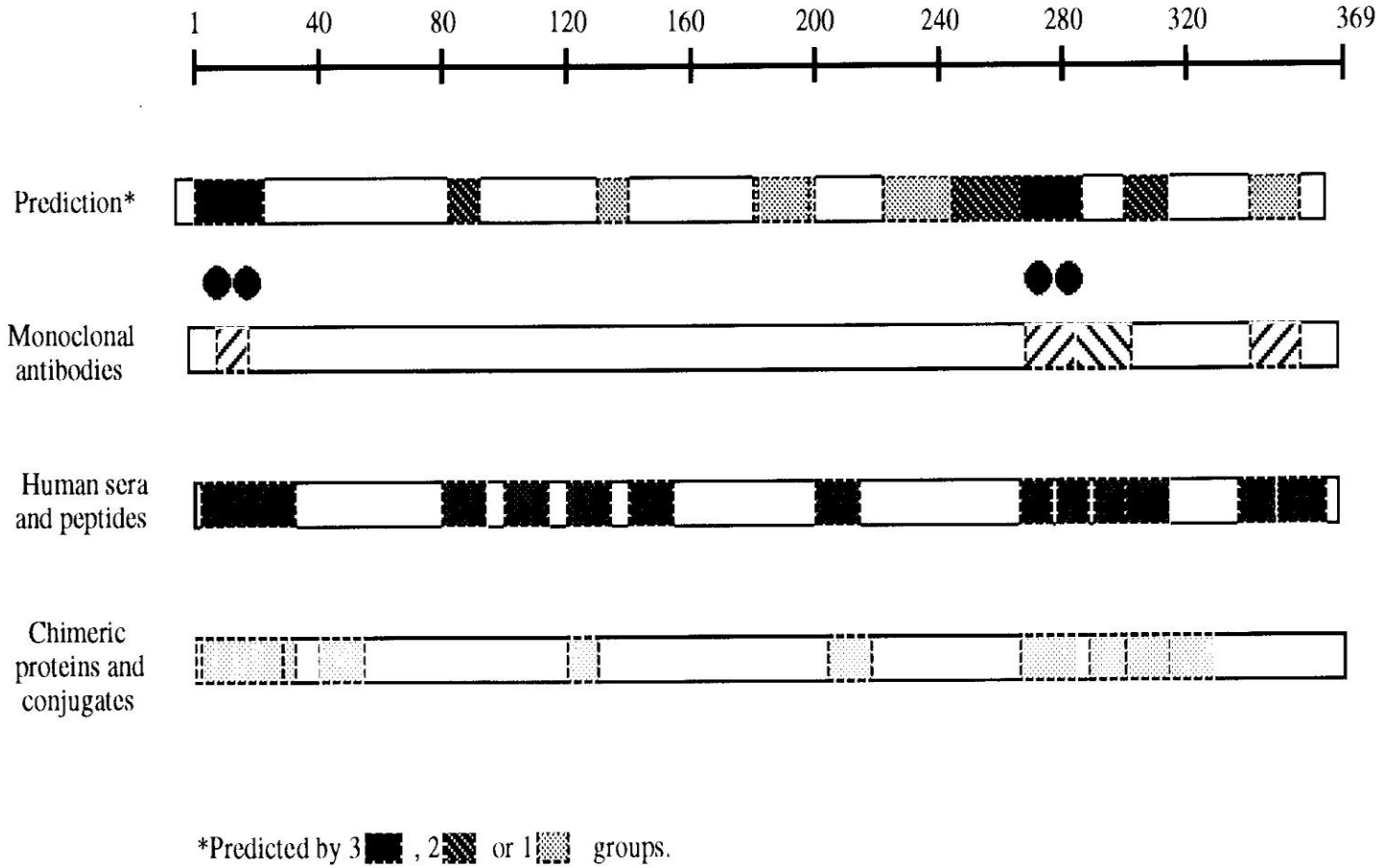


B-cell epitopes

T-cell epitopes

Erdei Anna, Sármay Gabriella, Prechl József (2012)
http://www.tankonyvtar.hu/hu/tartalom/tamop425/2011_0001_524_Immunologia/ch17.html

Comparative analysis



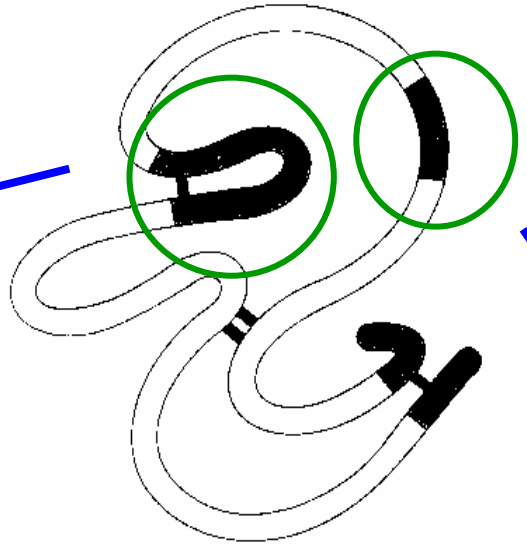
In: Synthetic peptides in the search for B-and T-cell epitopes. (Ed. Rajnavölgyi, É.) 1994, R.G.Landes Company, Austin, pp. 157-169.

Structural modification of protein epitopes

Modification of epitope structure: why and how?

- Improve immune recognition
- Protection against enzymatic degradation

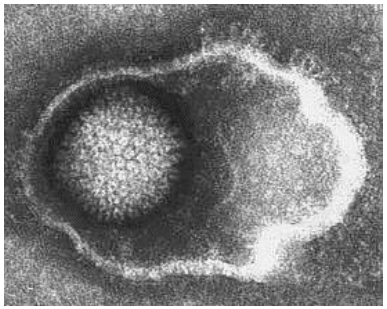
„Flanking" regions



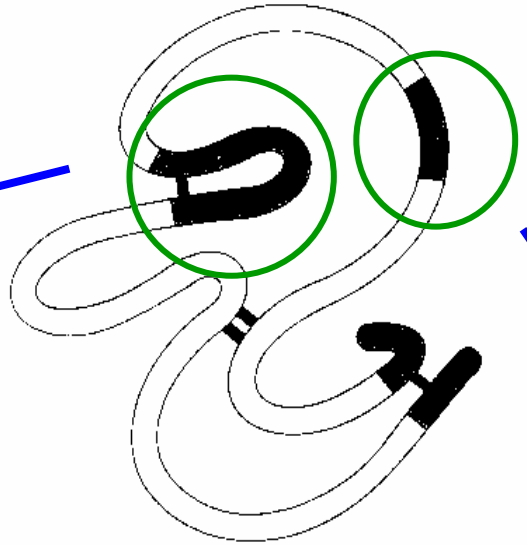
„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

Multiplication
- polymerisation
- conjugation

Modification of B-cell epitopes



HSV gD epitope cyclisation



„Flanking" regions

„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

Multiplication
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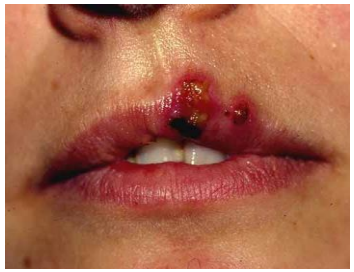
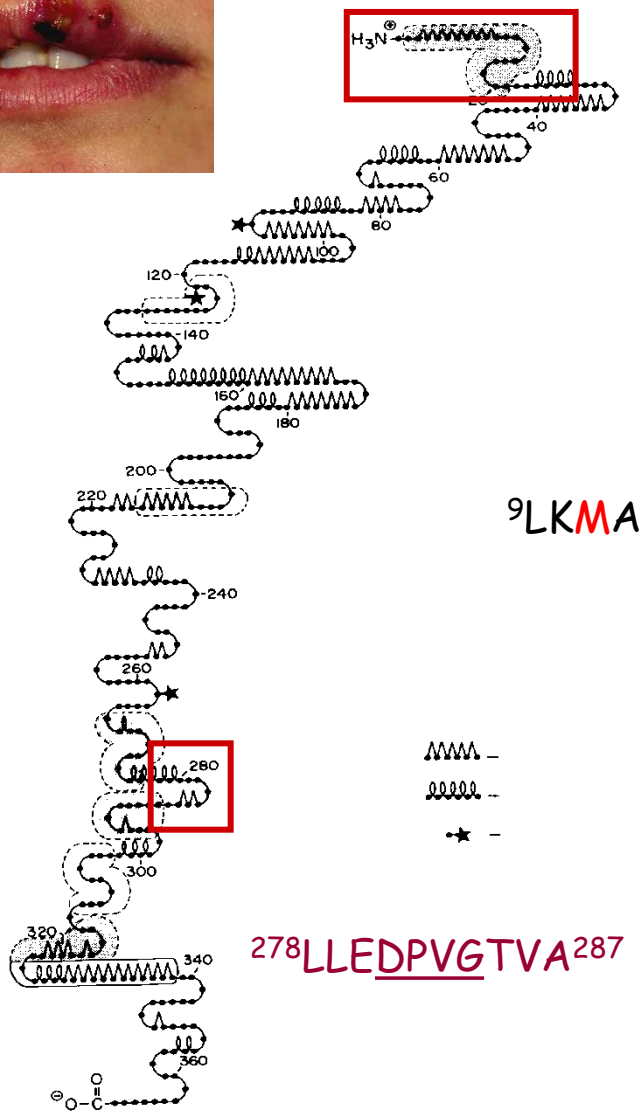
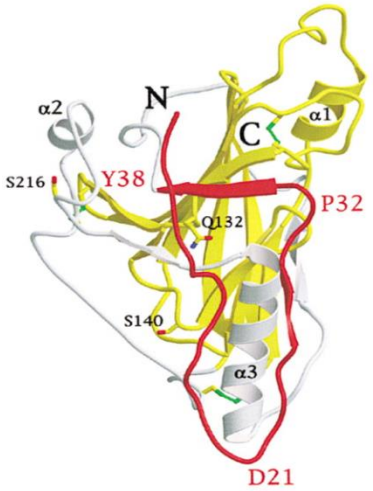
- conjugation

Identification of HSV gD-1 epitopes

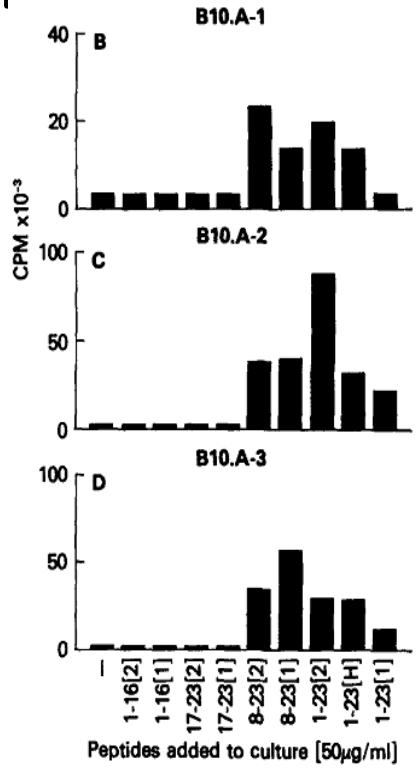




^{278}LLEDPVGTVA^{287}



^{9}LKMADPNRFRGKDL^{22}

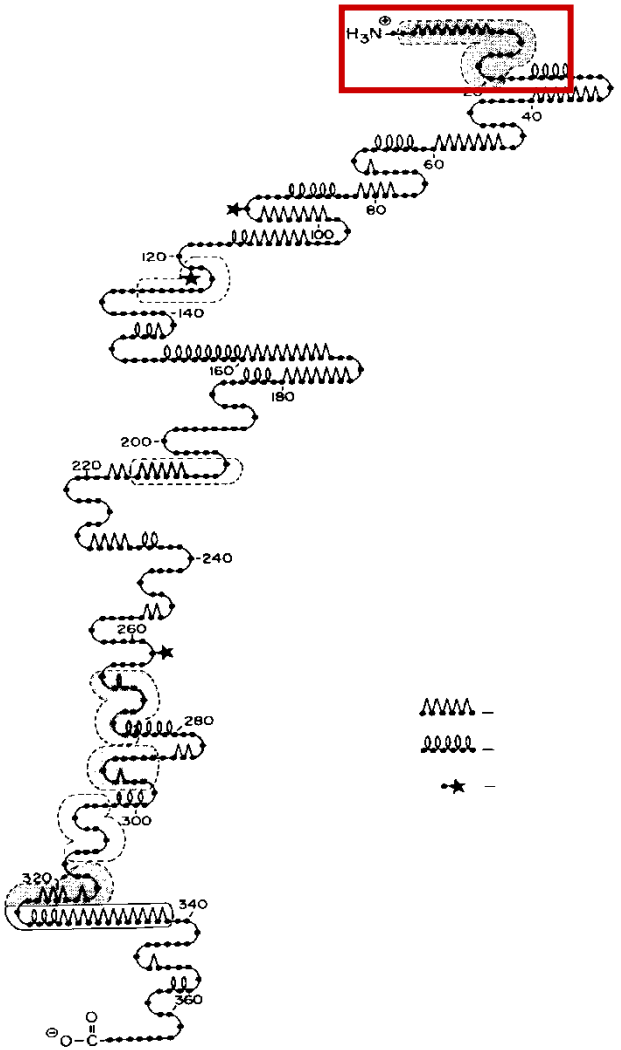


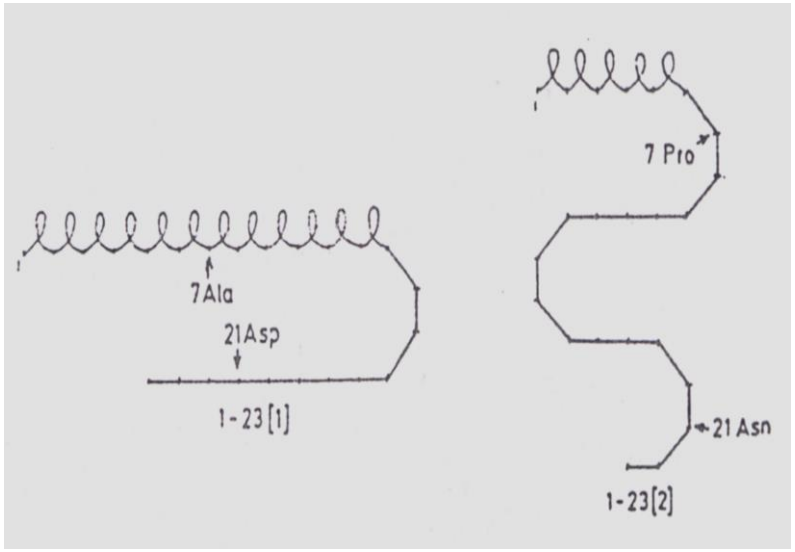
Peptide[a]	Amino acid sequence[b]																			
1–23[1]	K	Y	A	L	A	D	A	S	L	K	M	A	D	P	N	R	F	R	G	K	D	L	P
1–23[2]	K	Y	A	L	A	D	P	S	L	K	M	A	D	P	N	R	F	R	G	K	N	L	P
1–23[H]	K	Y	A	L	A	D	P	S	L	K	M	A	D	P	N	R	F	R	G	K	D	L	P
1–16[1]	K	Y	A	L	A	D	A	S	L	K	M	A	D	P	N	R							
1–16[2]	K	Y	A	L	A	D	P	S	L	K	M	A	D	P	N	R							
3–23[H]			A	L	A	D	P	S	L	K	M	A	D	P	N	R	F	R	G	K	D	L	P
8–23[1]								S	L	K	M	A	D	P	N	R	F	R	G	K	D	L	P
8–23[2]								S	L	K	M	A	D	P	N	R	F	R	G	K	N	L	P
12–23[1]											M	A	D	P	N	R	F	R	G	K	D	L	P
13–23[2]												A	D	P	N	R	F	R	G	K	N	L	P
17–23[1]																R	F	R	G	K	D	L	P
17–23[2]																R	F	R	G	K	N	L	P

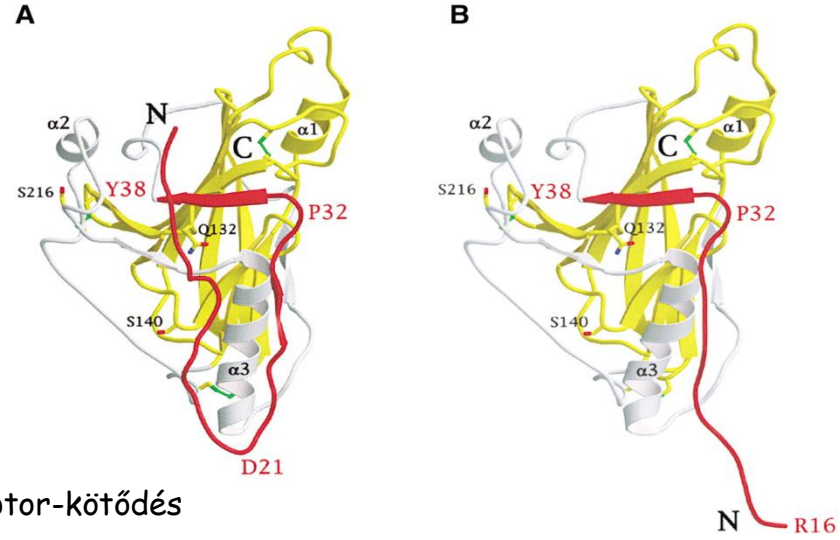
Epitope cyclization:

effect of the design on antibody recognition

HSV gD-1 cyclic epitope



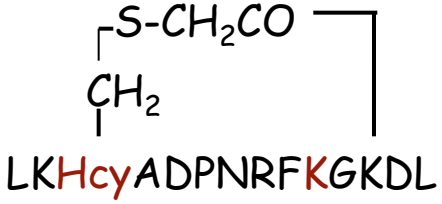


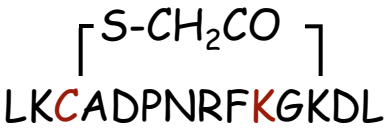


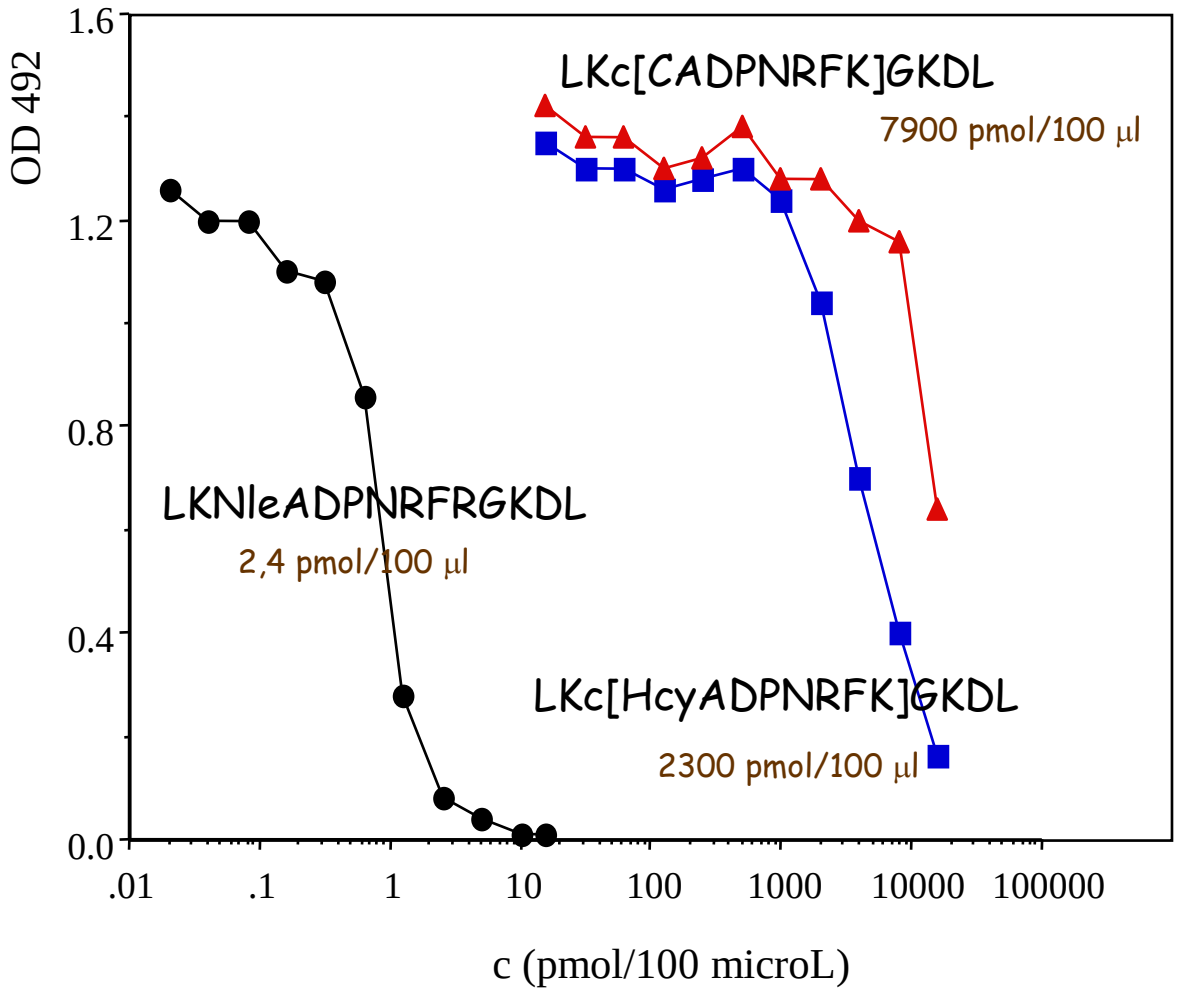
Receptor-kötődés

HSV gD-1 cyclic epitope: the loss of recognition

^{9}LKMADPNRFRGKDL^{22}

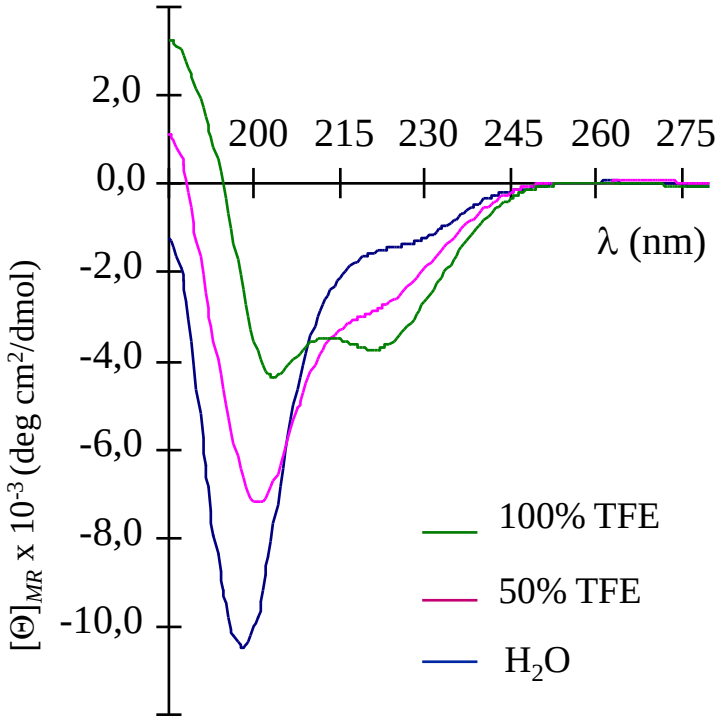




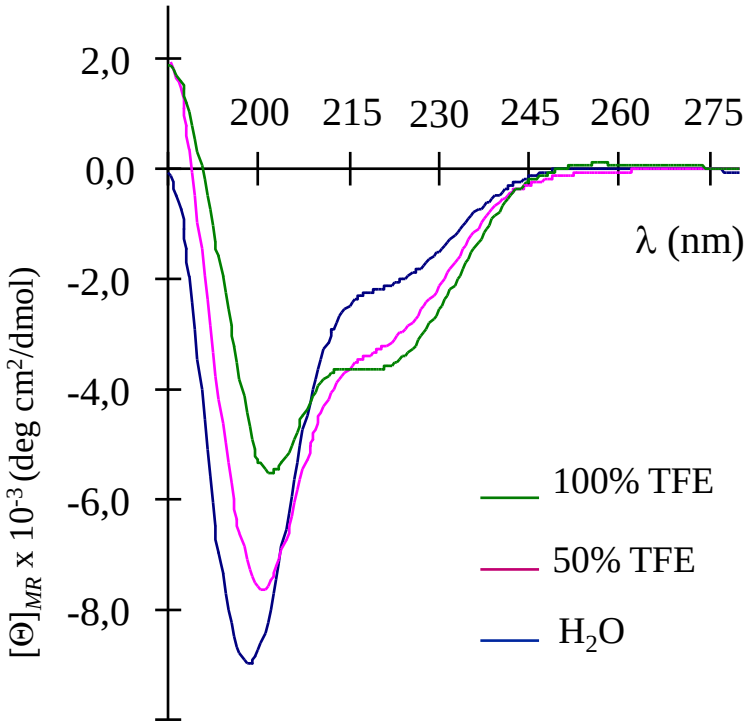


OD= 1.00 (λ = 492 nm)

CD spectra of cyclic peptides of 9-22 gD

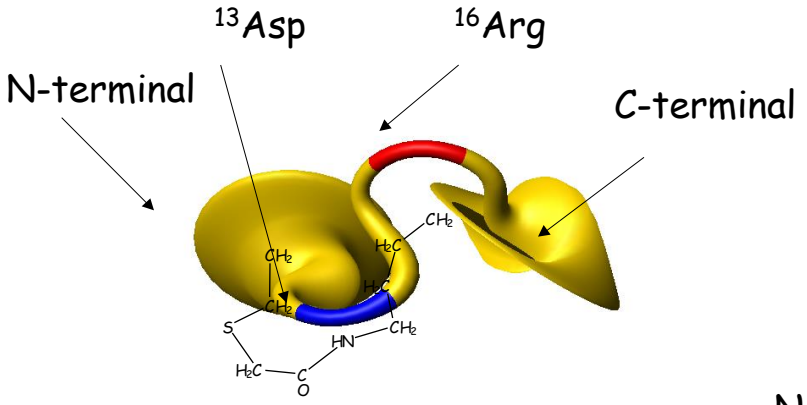


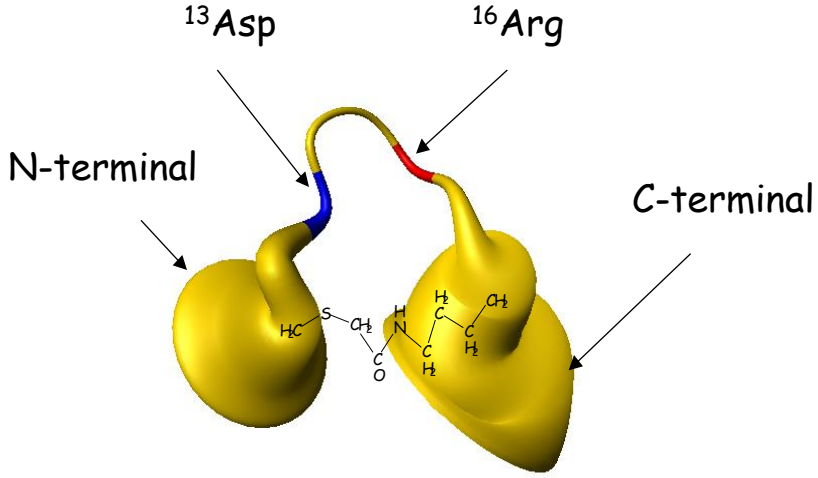
LKc[HcyADPNRFK]GKDL

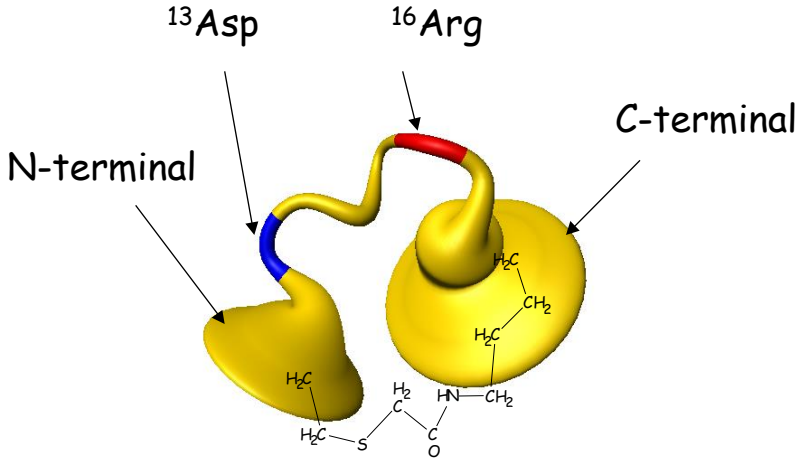


LKc[CADPNRFK]GKDL

Solution structure of the cyclopeptides







Epitope cyclization:

effect of linkage type on enzymatic stability

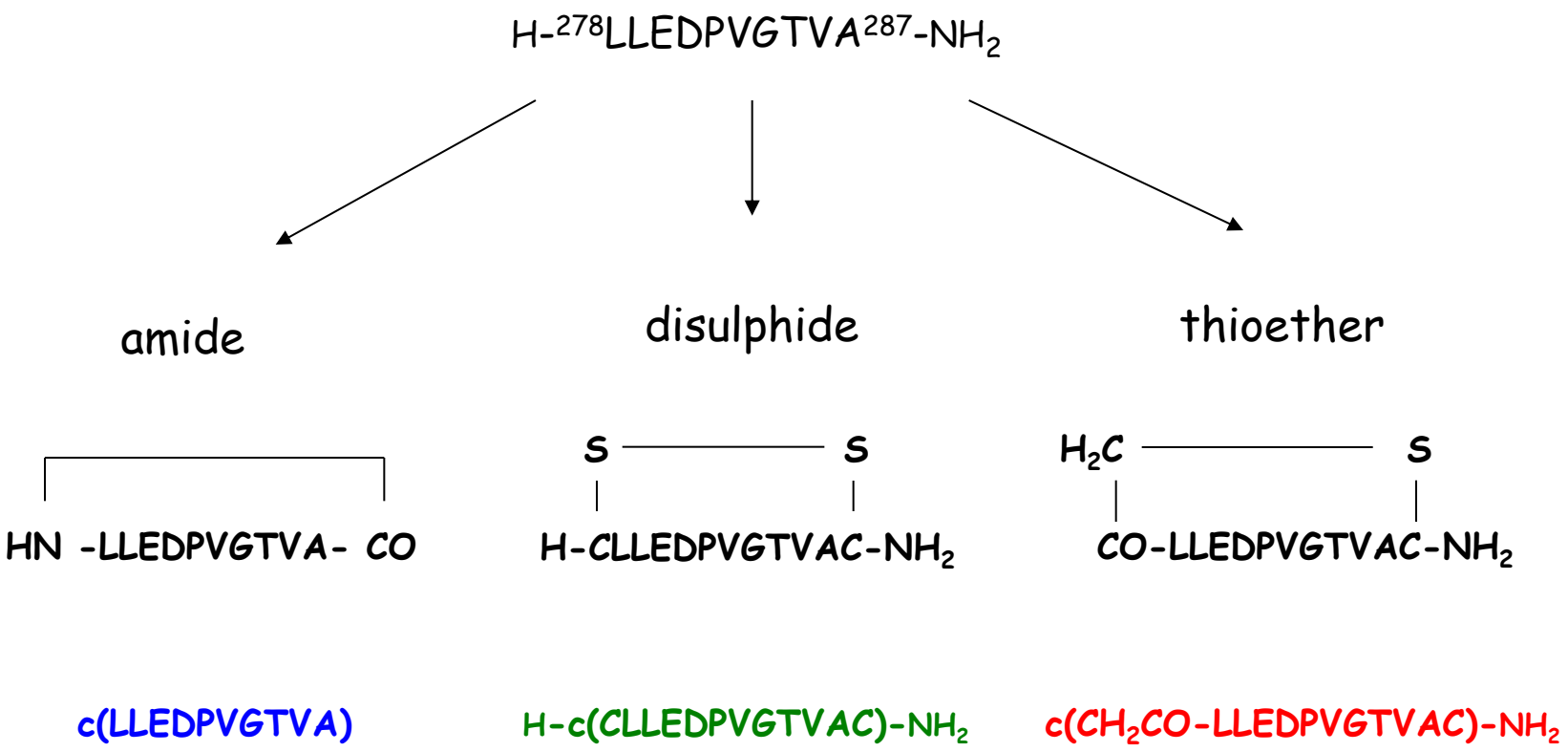
Predicted secondary structure of gD

Epitope peptide:

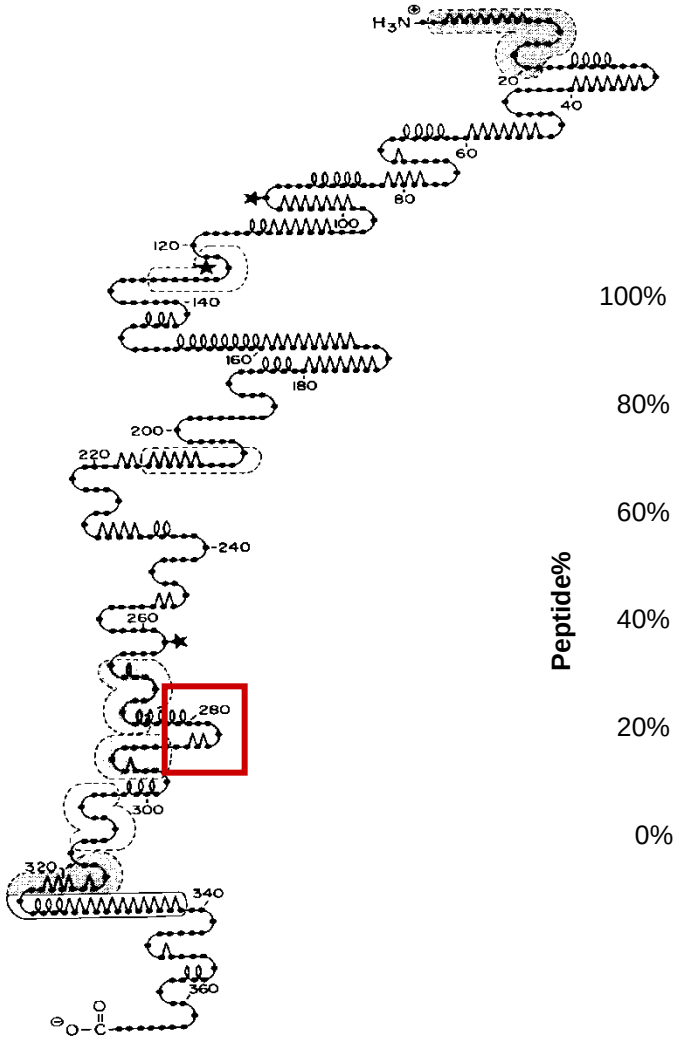
^{278}LLEDPVGTVA^{287}

DPVG is a core epitope

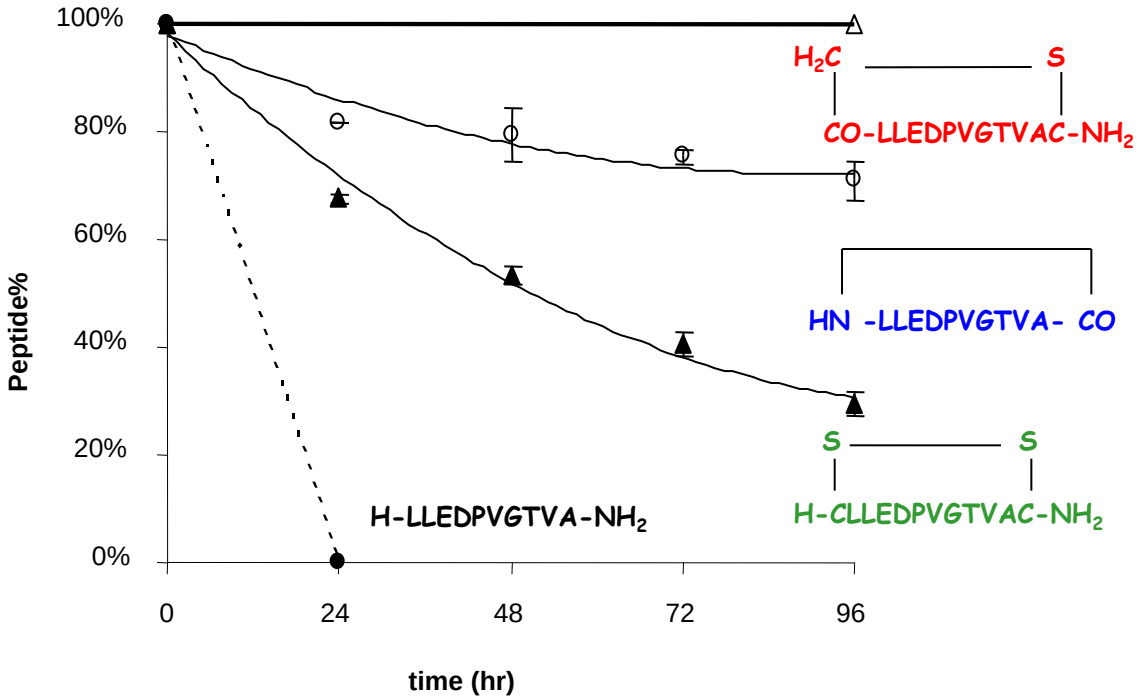
Cyclic peptides derived from 278-287 sequence of HSV gD



Increased enzymatic stability

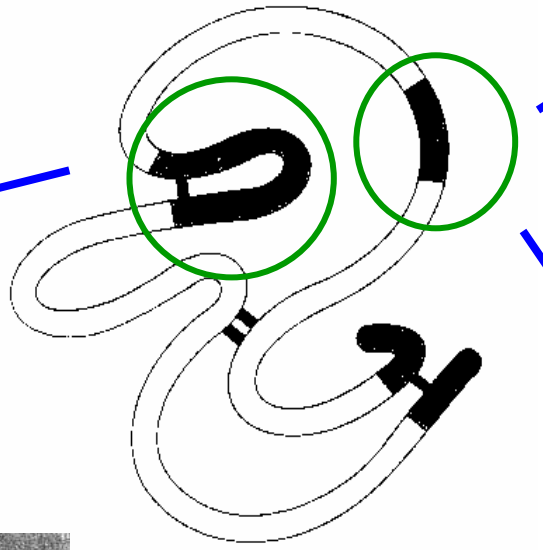


- Tioether linkage
- Amide linkage
- Disulfid linkage

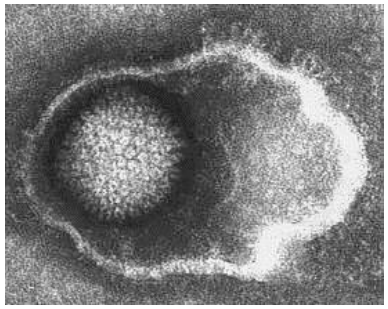


Stabilisation/optimalisation of epitope recognition

„Flanking" regions



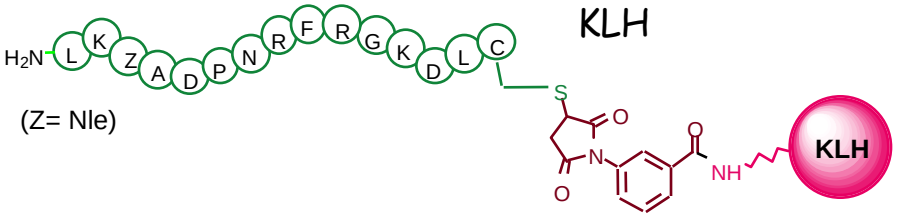
„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

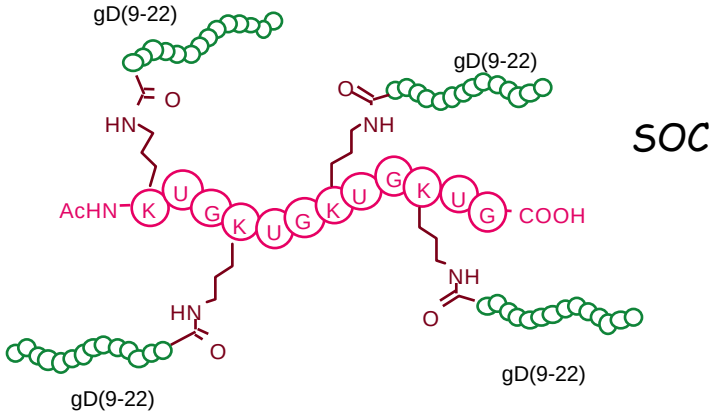


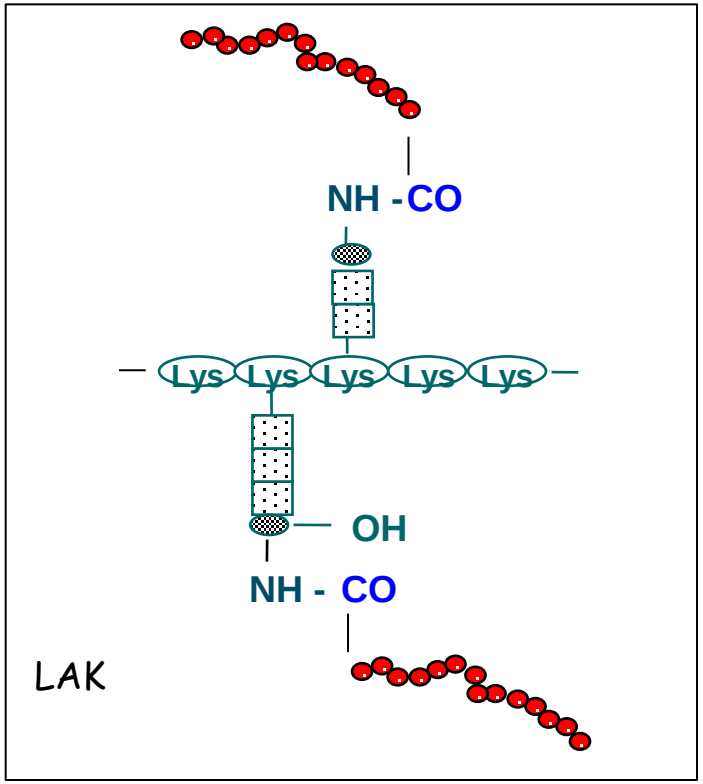
HSV gD epitope multiplication

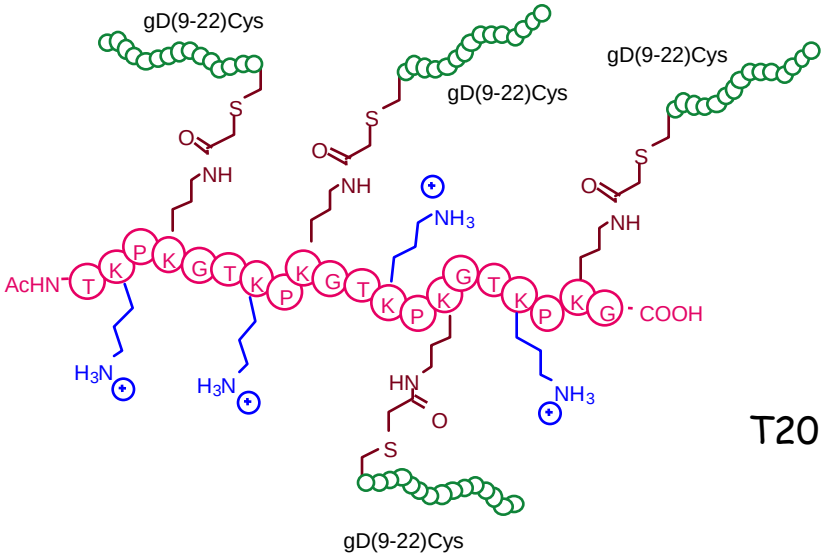
Multiplication
- polymerisation
- conjugation

Multivalent epitope - conjugates





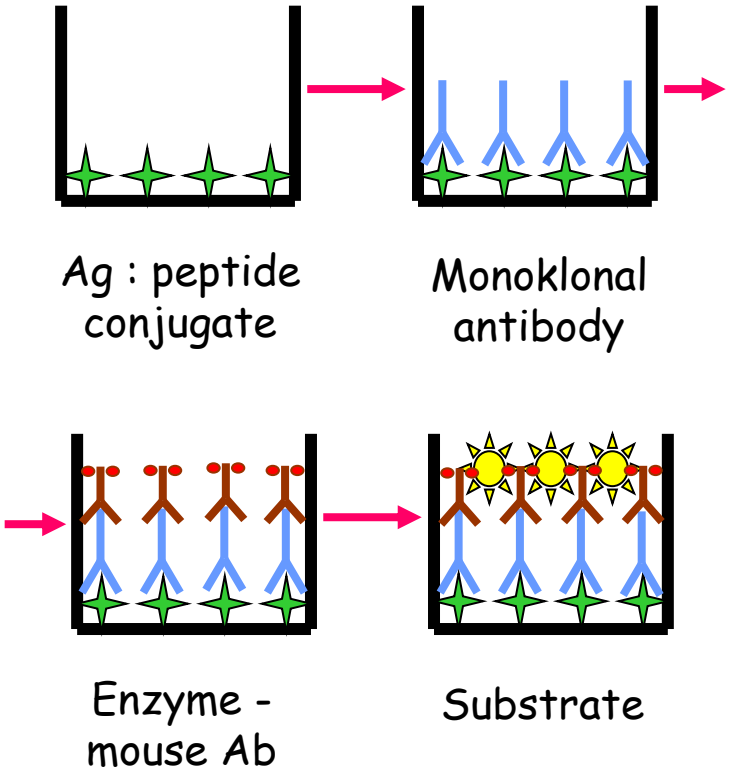


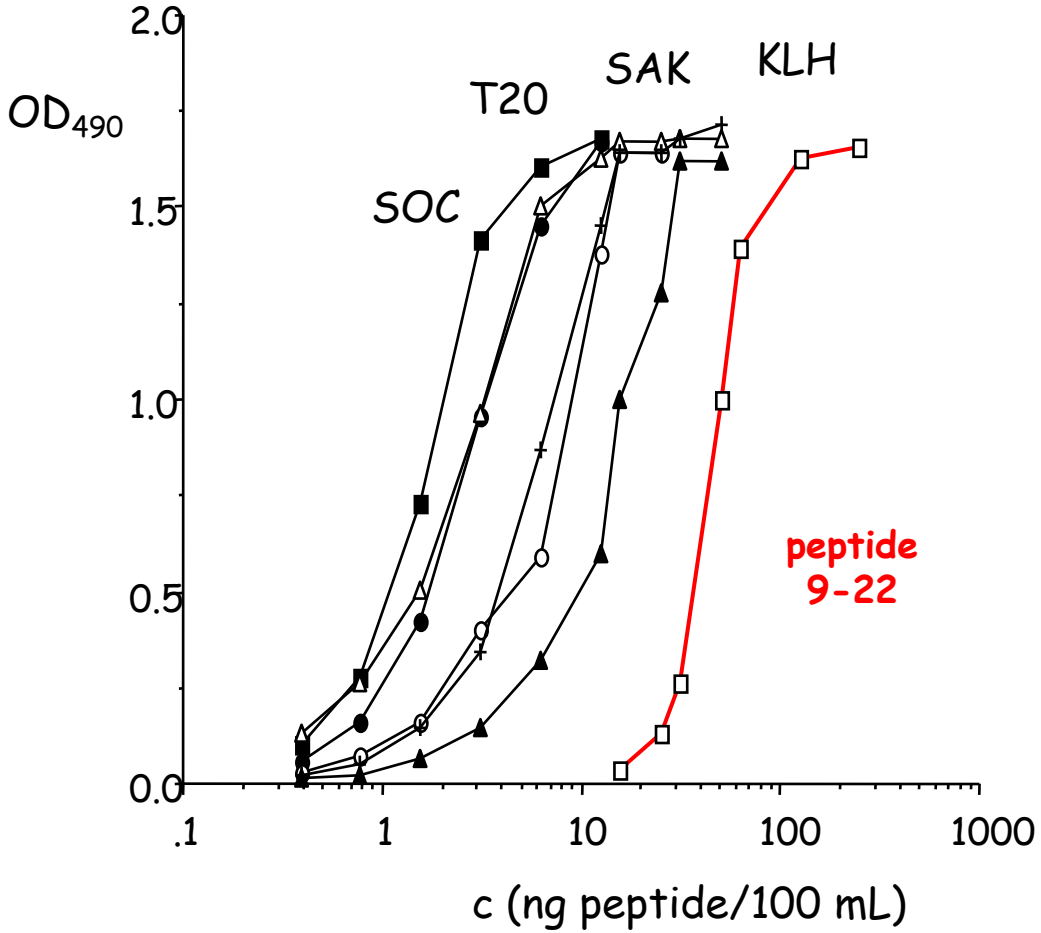


Epitope multiplication:

effect of the carrier component on antibody recognition

Antibody binding of HSV gD epitope (9-22) conjugates
(direct ELISA)

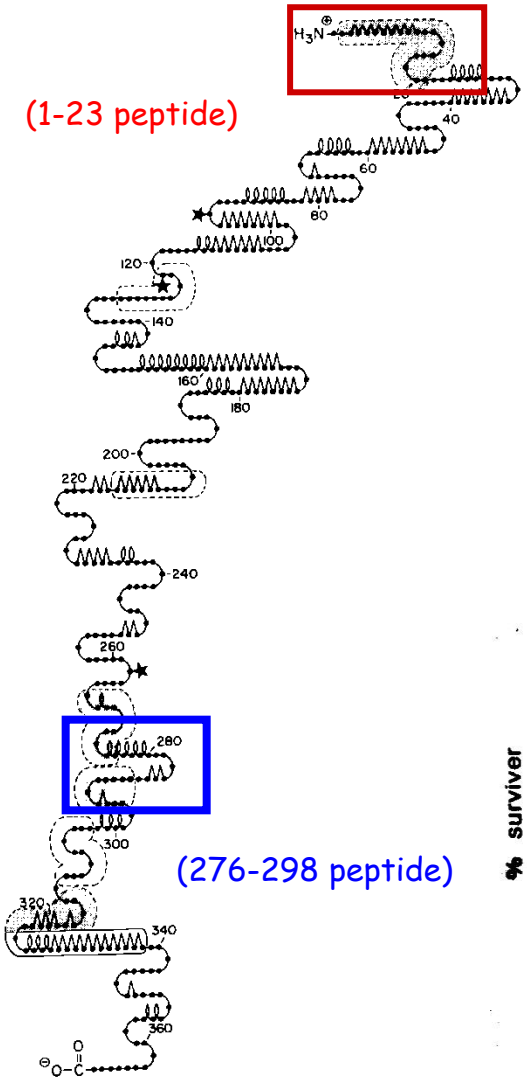




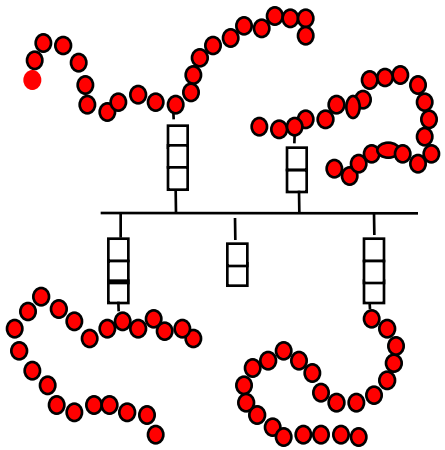
Epitope multiplication:

effect of carrier component on immune protection

Protection against lethal infection by HSV-1

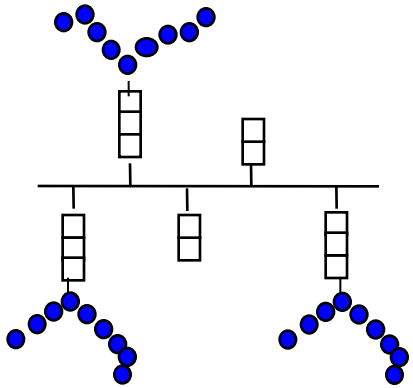


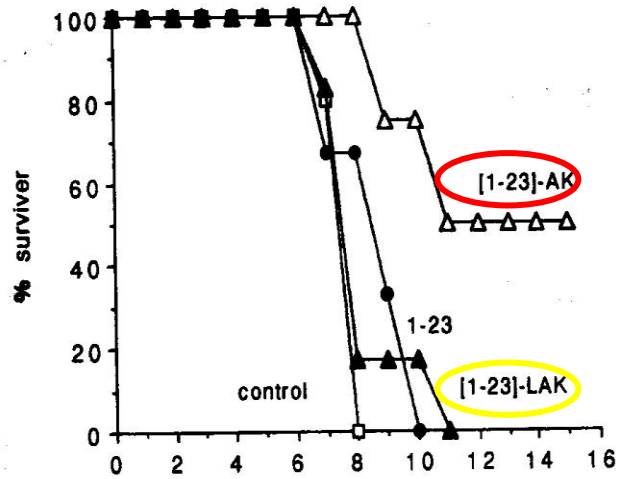
(1-23 peptide)

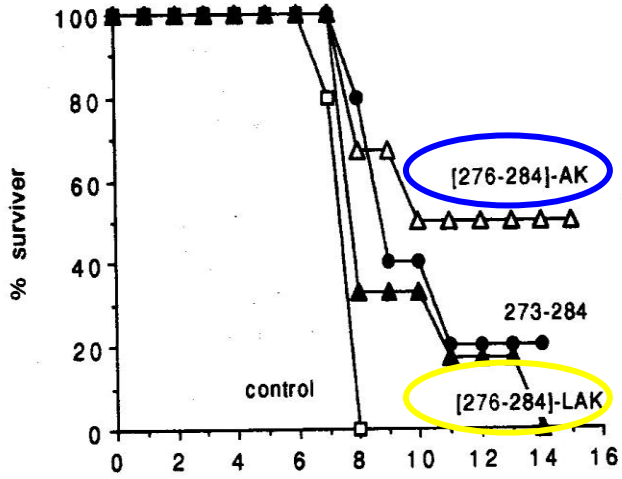


AK vs LAK

(276-284 peptide)



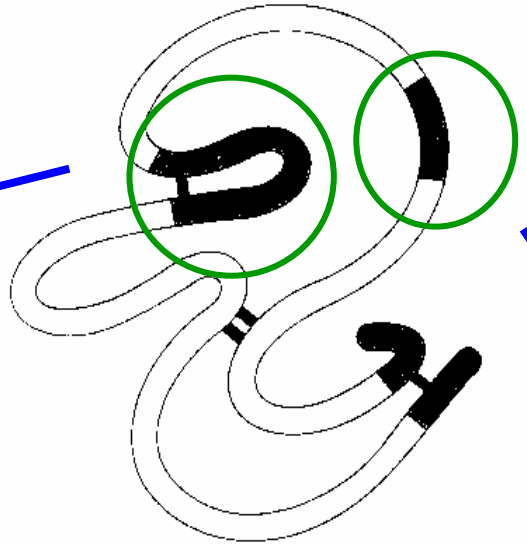




Balb/c mice, survival after lethal infection

Stabilisation/optimalisation of epitope recognition

„Flanking" regions



MUC2 epitope modification

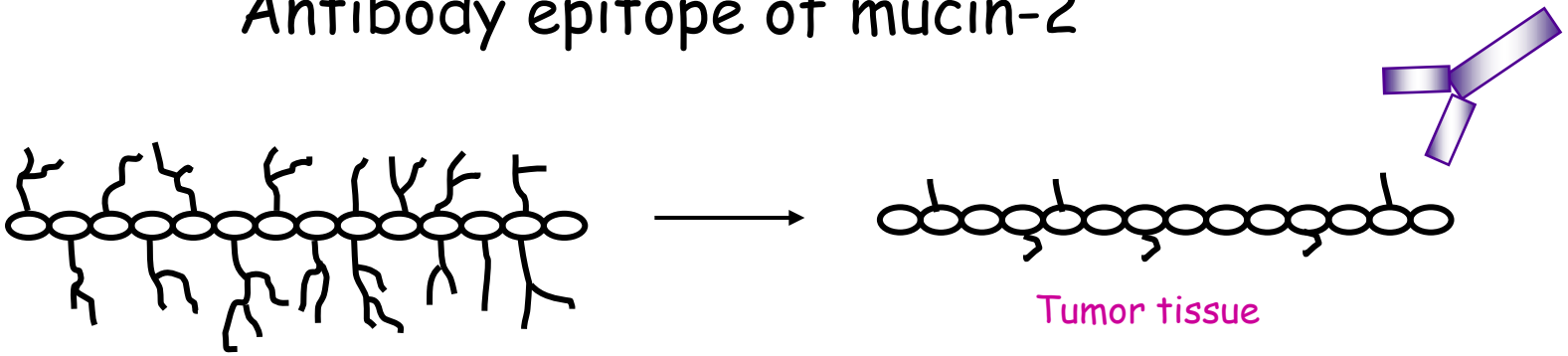
„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

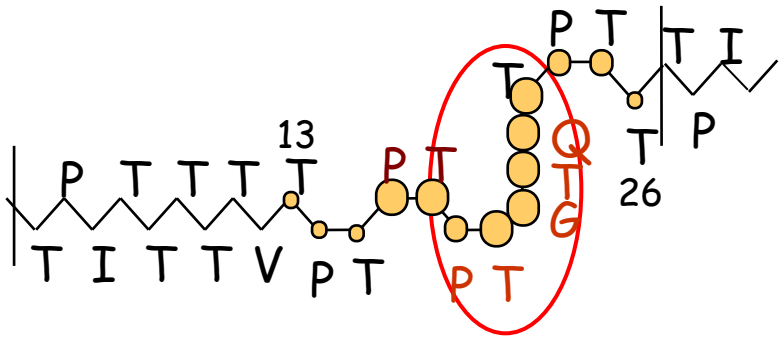
Multiplication
- polymerisation
- conjugation

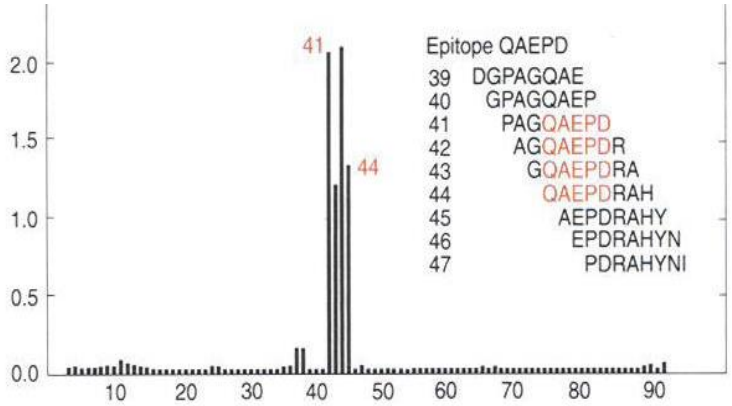
Flank substitution:

effect of D- amino acid(s)
on enzymatic stability and antibody binding

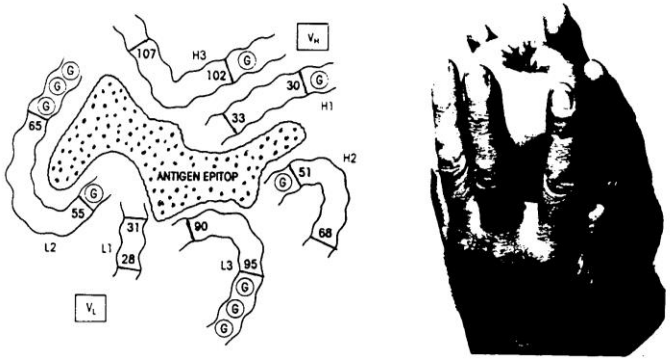
Antibody epitope of mucin-2



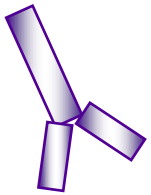




^{1}TPITTTTTVTPTPTPTGTQTPTT^{26}



The concept: epitope „core" and „flanking" regions



mAb 996

…TPT PTGTQ TPT….

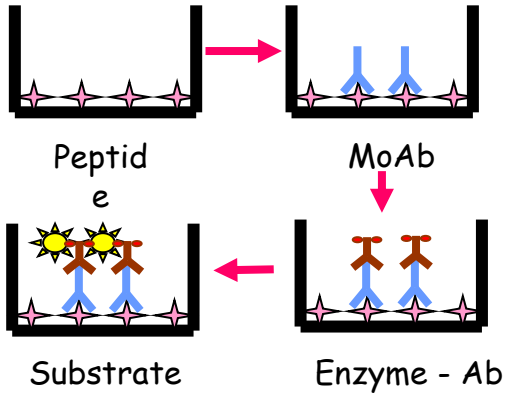
N-terminal epitope „core" C-terminal

1. Synthesis

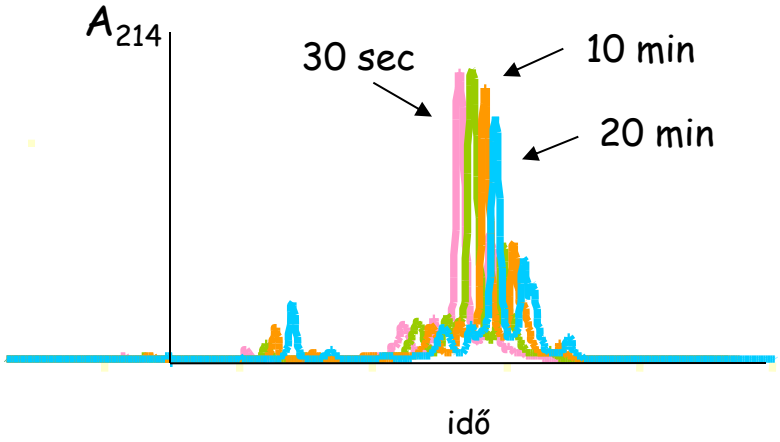
TPTPTGTQTPT
TPTPTGTQ tpt
tpt PTGTQTPT

tpt PTGTQTPt
tpt PTGTQTpt
tpt PTGTQ tpt
tpTPTGTQ tpt
tPTPTGTQ tpt

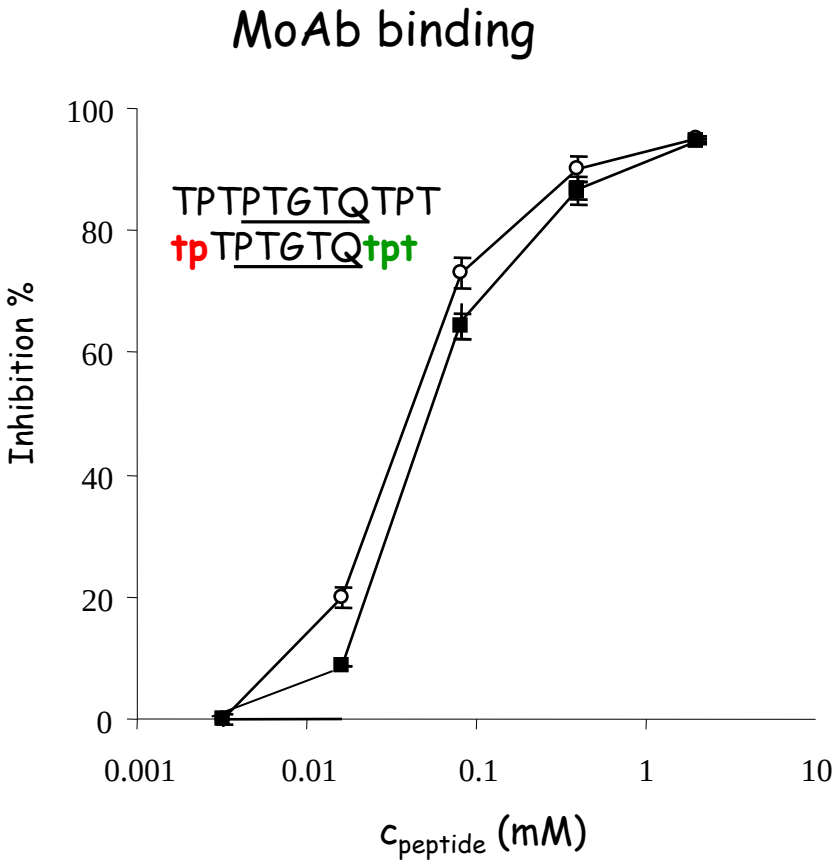
2. Antibody binding



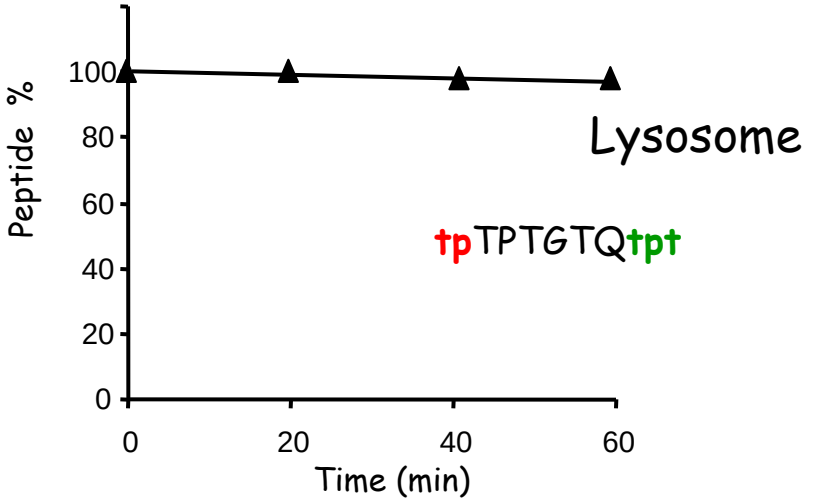
3. Enzyme stability
   - human serum
   - lysosome preparation

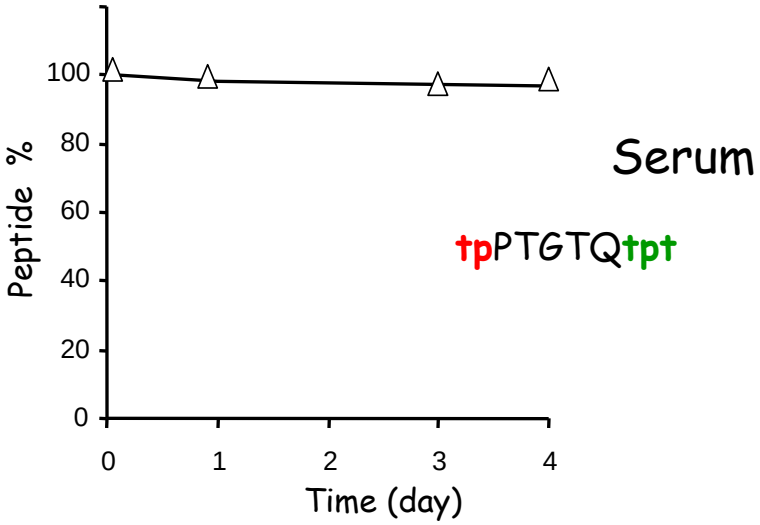


Modification in the „flanking" region: binding and enzyme resistance

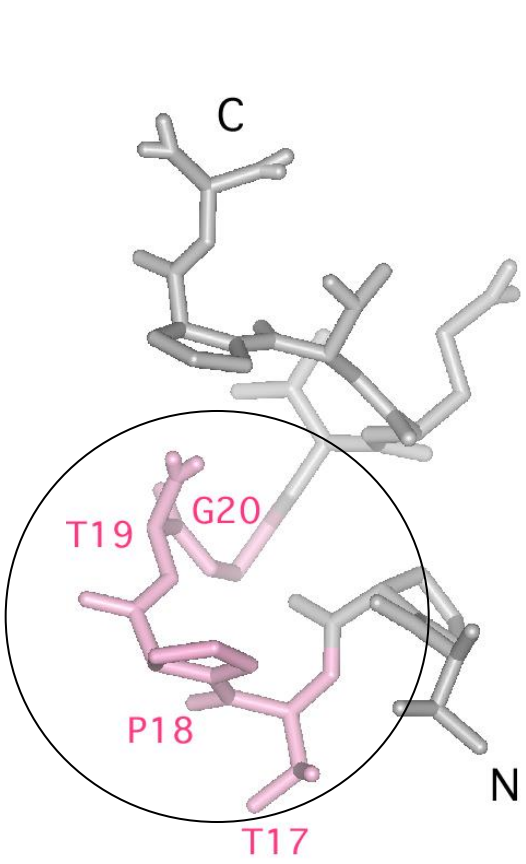


tpTPTGTQtpt

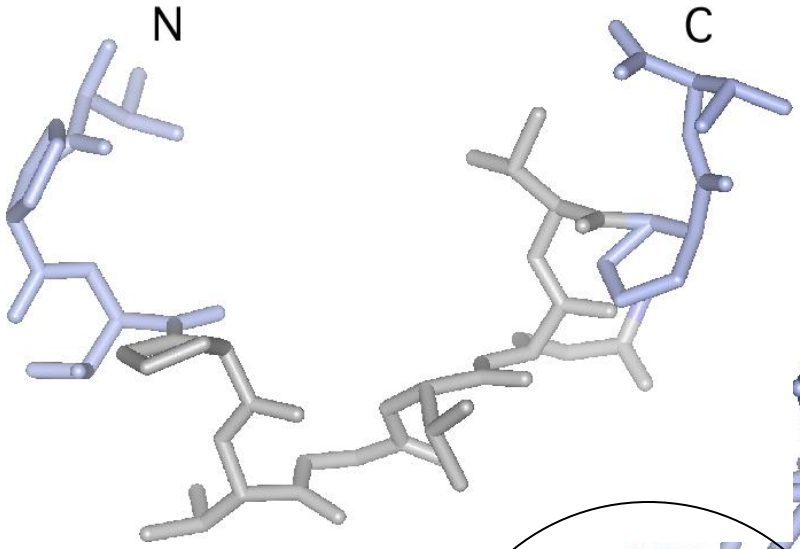




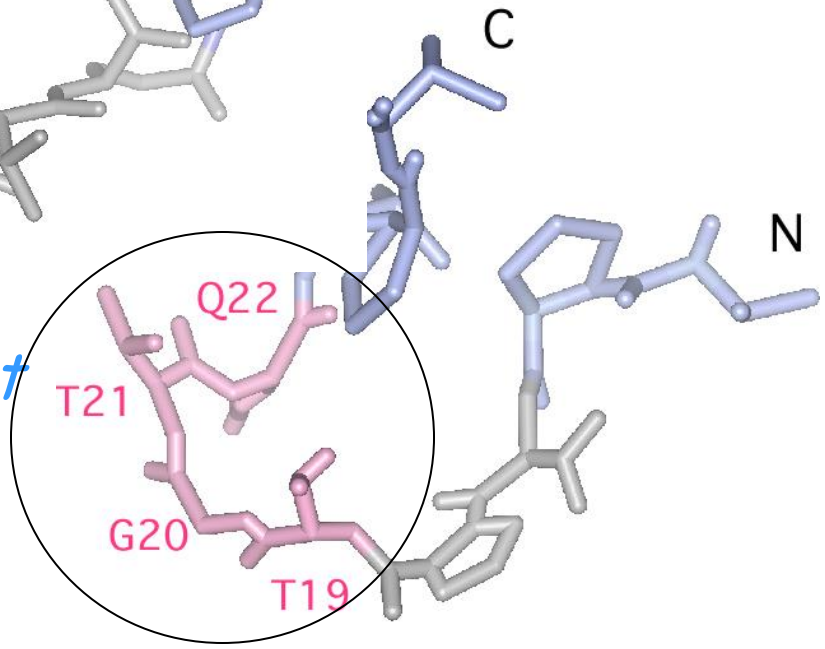
Secondary structure of D-amino acid substituted peptides
(based on NMR)



TPTPTGTQTPT



*tpt*PTGTQT*pt*



*tp*TPTGTQ*tpt*

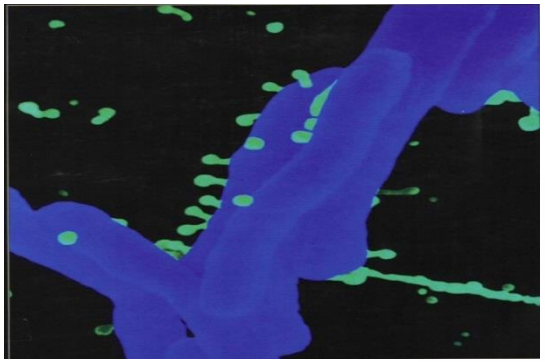
Conclusions

1) The influence of the flanking amino acids could be „sensed" and utilized for antigen design.

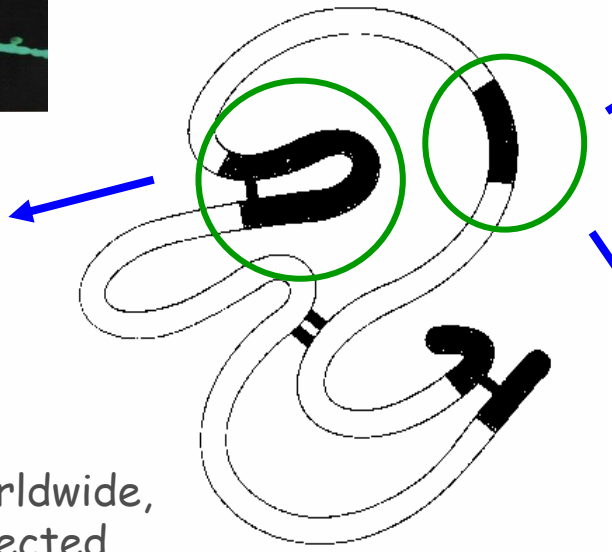
2) Epitope binding (antigenicity) as well as enzyme stability could be optimized by changes in the flanking sequences by
   - non-native, but proteogenic amino acid
   - D-amino acid substitutions

3) Epitope peptide stable in human serum as well as in lysosome with preserved antibody binding was identified.

Stabilisation/optimalisation of T-cell epitope recognition



„Flanking" regions



Tuberculion epitope modification

„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

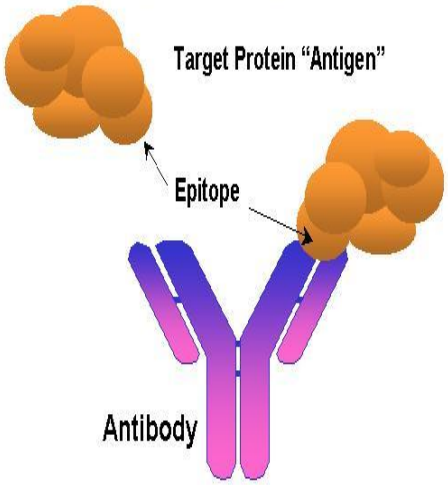
- a leading infectious disease worldwide,
- 2 billion people are latently infected,
- 8 million new TB cases per year (increasing),
- 2 million deaths per year,
- increase of (multi)drug-resistant TB,
- 50 million people infected with drug-resistant TB,
- AIDS

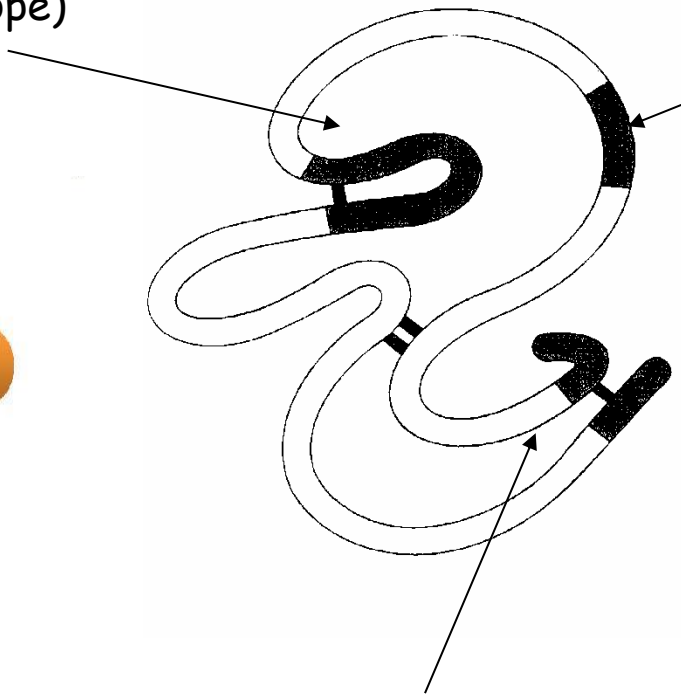
Multiplication
- polymerisation
- conjugation

Antigen structure - peptide epitopes – epitope recognition

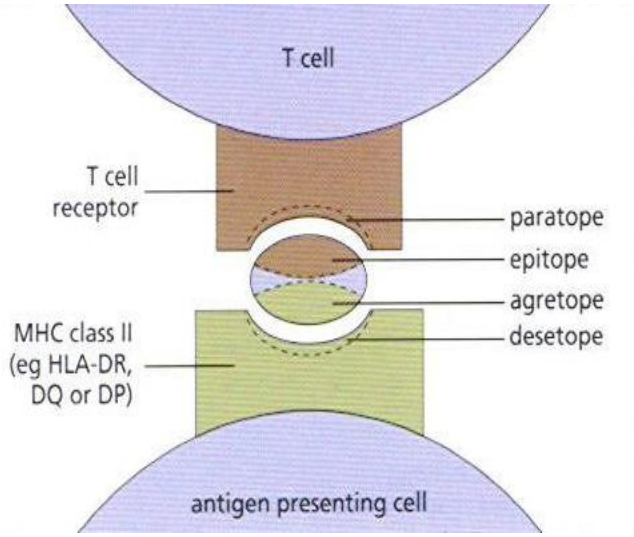
Topographic, non-continuous
(antibody epitope)

Linear, sequential
(antibody or T cell epitope)

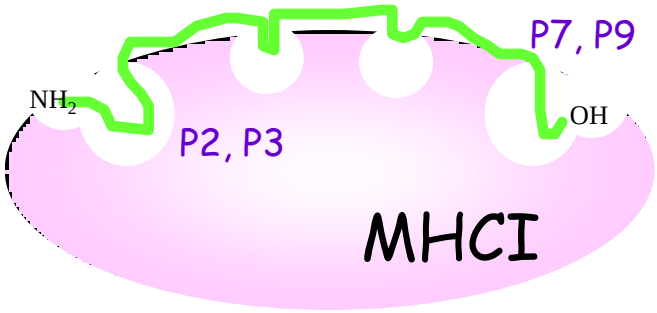




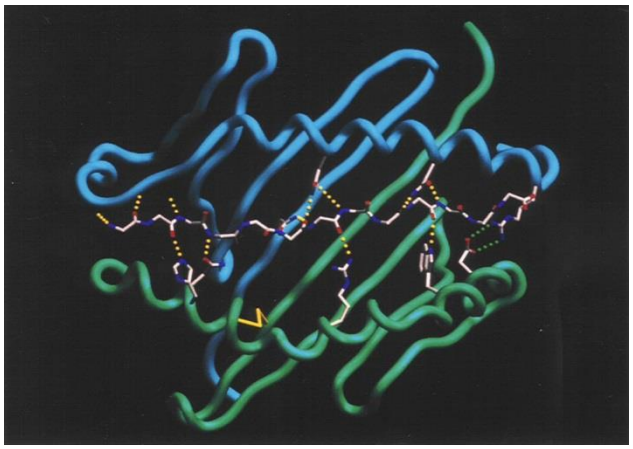
Topographic, continuous
(antibody epitope)

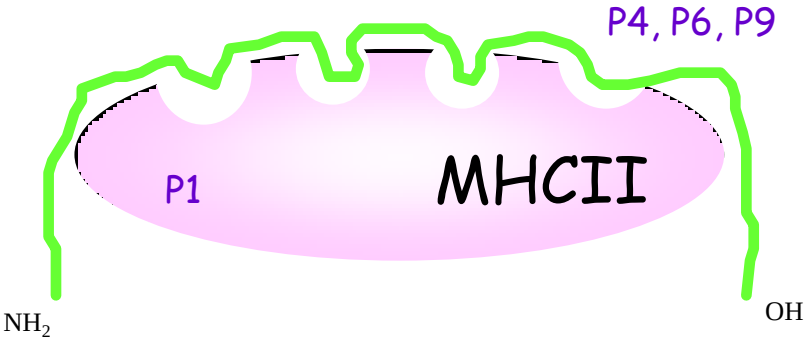


T- cell epitope recognition

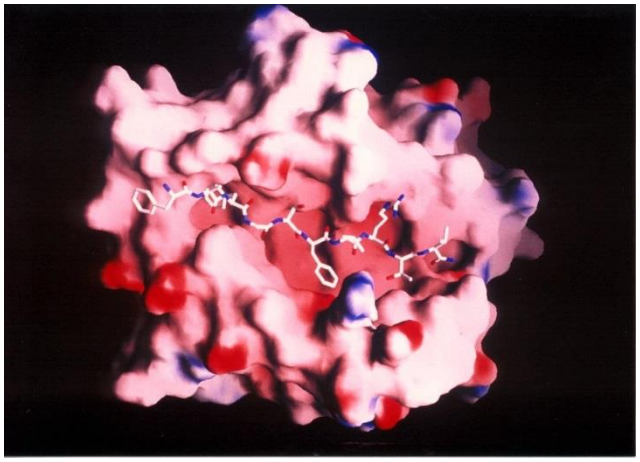


MHC I : 8 - 10 amino acid

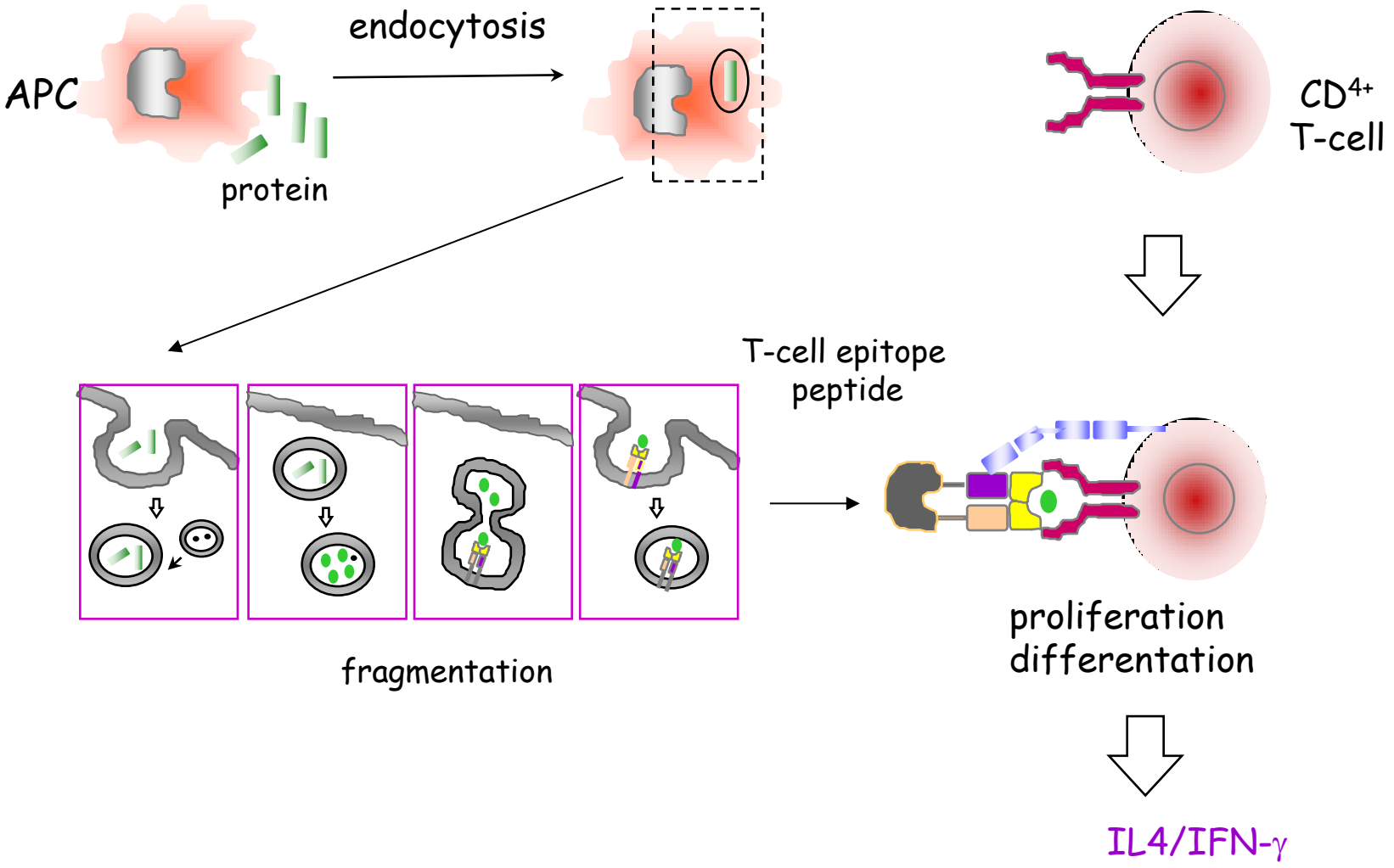




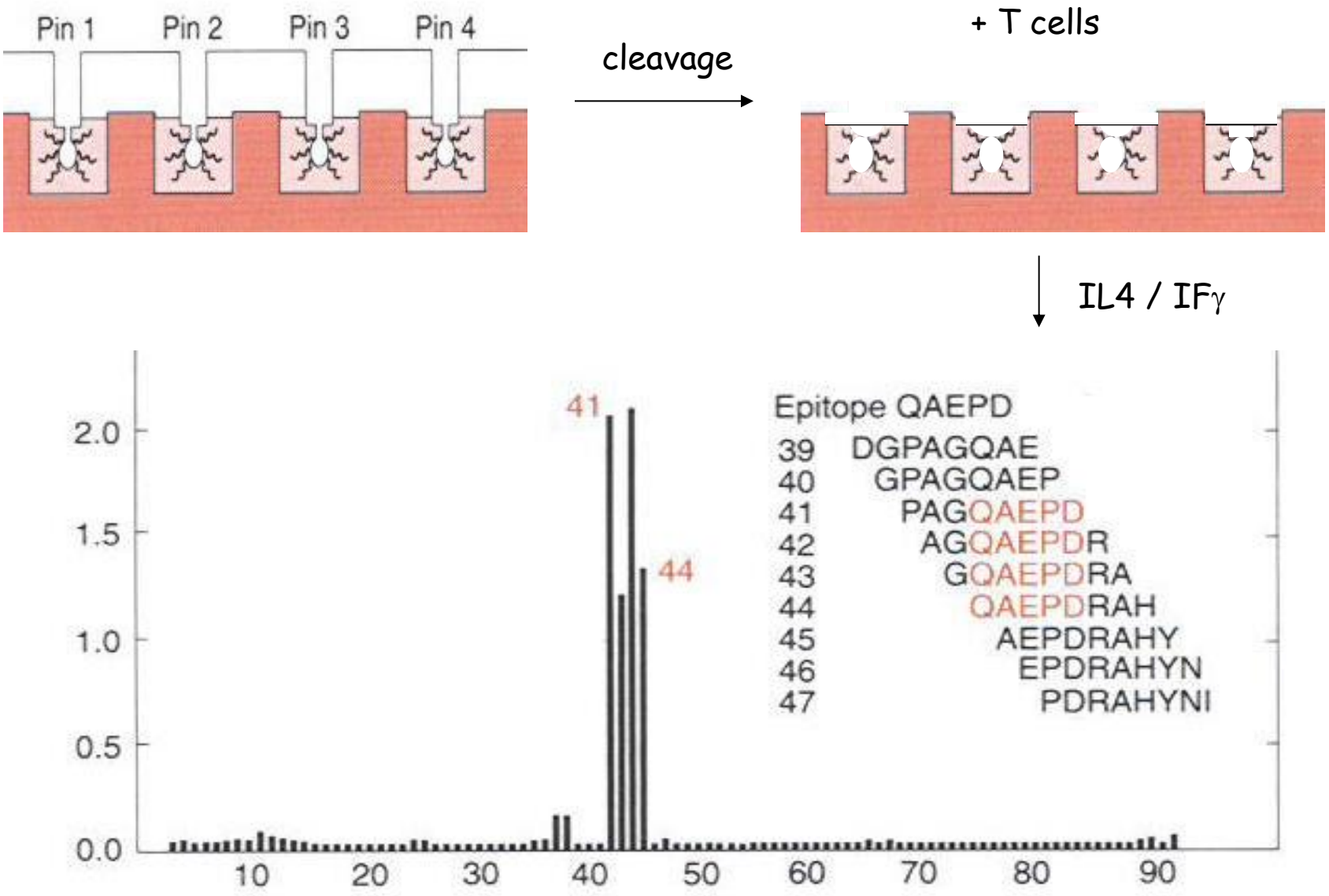
MHC II : 13 - 23 amino acid

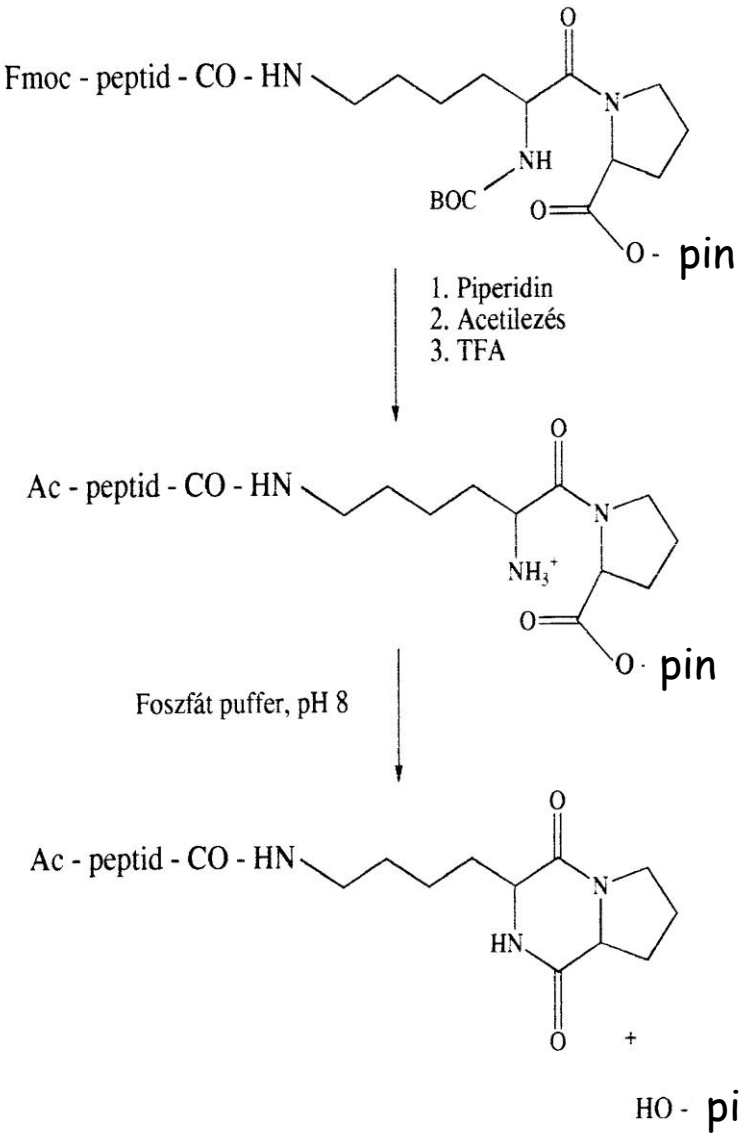


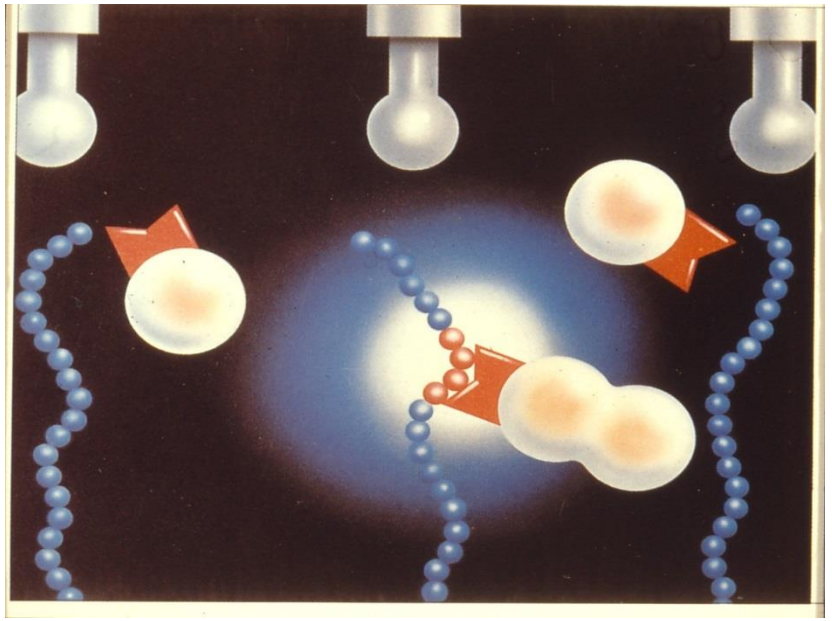
Development of immune response against T-cell epitopes



T- cell epitope identification

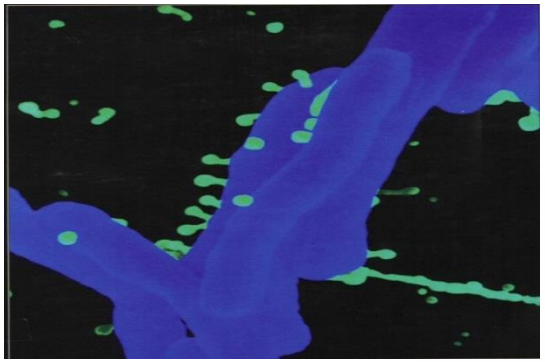



Fmoc - peptid - CO - HN
O
N
NH
BOC
O
O - pin
1. Piperidin
2. Acetilezés
3. TFA
Ac - peptid - CO - HN
O
N
NH3+
O
O - pin
Foszfát puffer, pH 8
Ac - peptid - CO - HN
O
N
HN
O
+
HO - pin

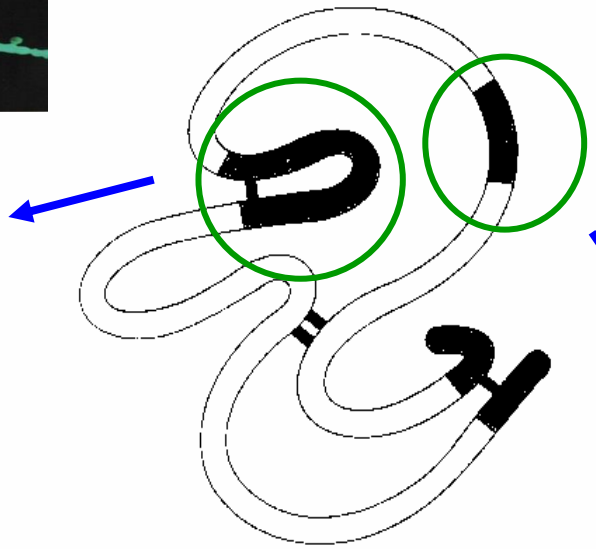


Epitope regions of 38 kDa of *M.tuberculosis*

Stabilisation/optimalisation of T-cell epitope recognition



„Flanking" regions



Tuberculion epitope modification

„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

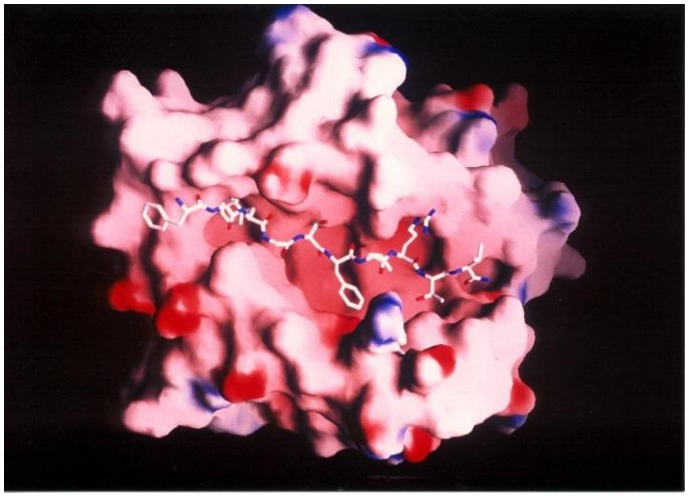
Multiplication
- polymerisation
- conjugation

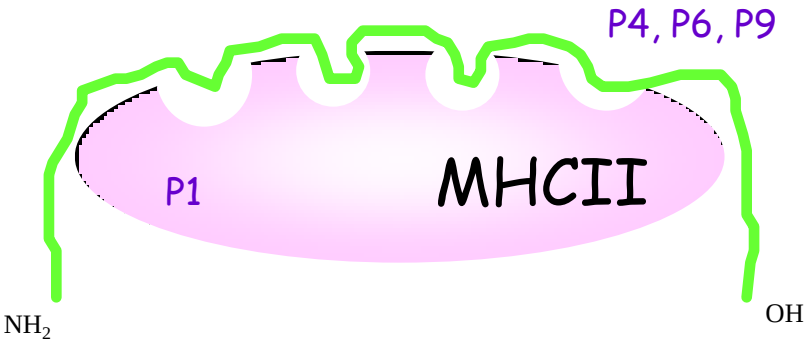
The effect of „flanking" regions of a 75-81 peptide epitope specific T-cell response

^{65} FNLWGPAFHERYPNVTITA ^{83}

^{75} RYPNVTI ^{81}

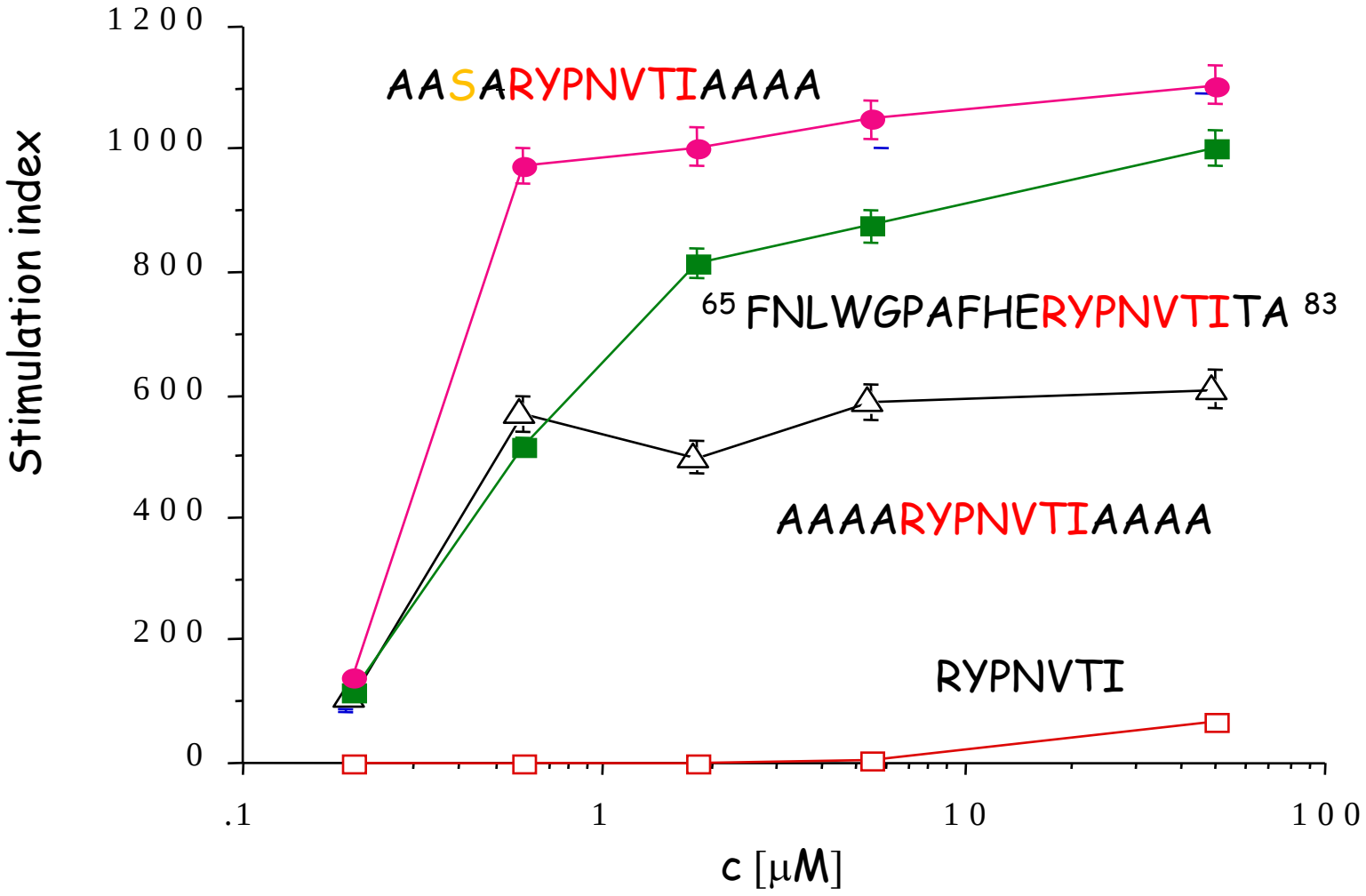
AARYPNVTIAA
AAARYPNVTIAAA
AAAARYPNVTIAAAA
AASARYPNVTIAAAA



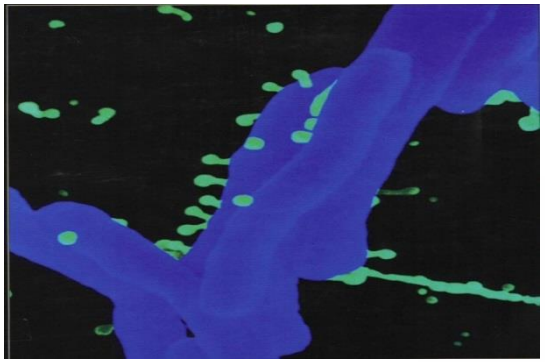


MHC II : 13 - 23 amino acids

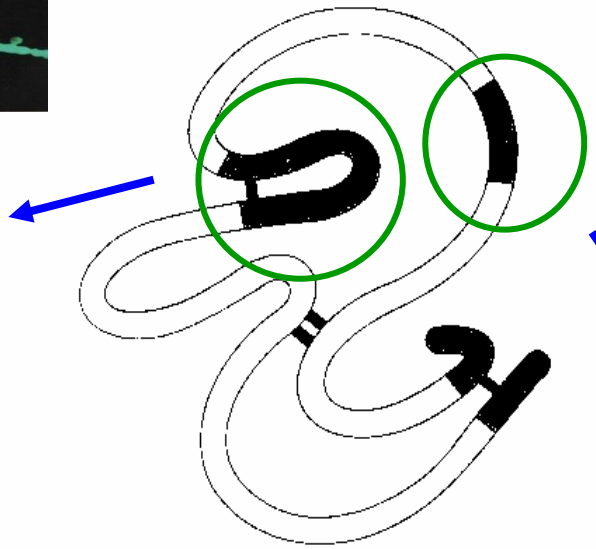
The effect of „flanking" regions of a 75-81 peptide epitope specific hybridoma T-cell response



Stabilisation/optimalisation of T-cell epitope recognition



„Flanking" regions

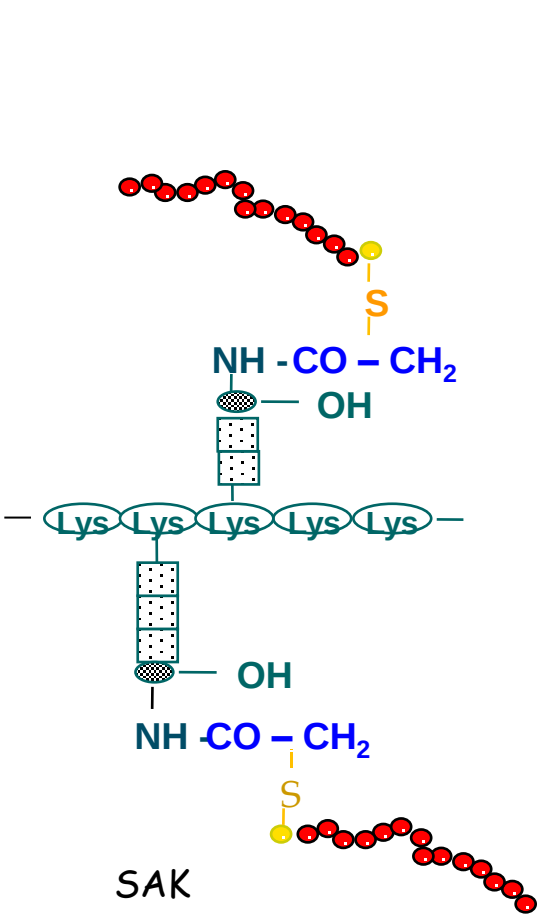


Tuberculion epitope modification

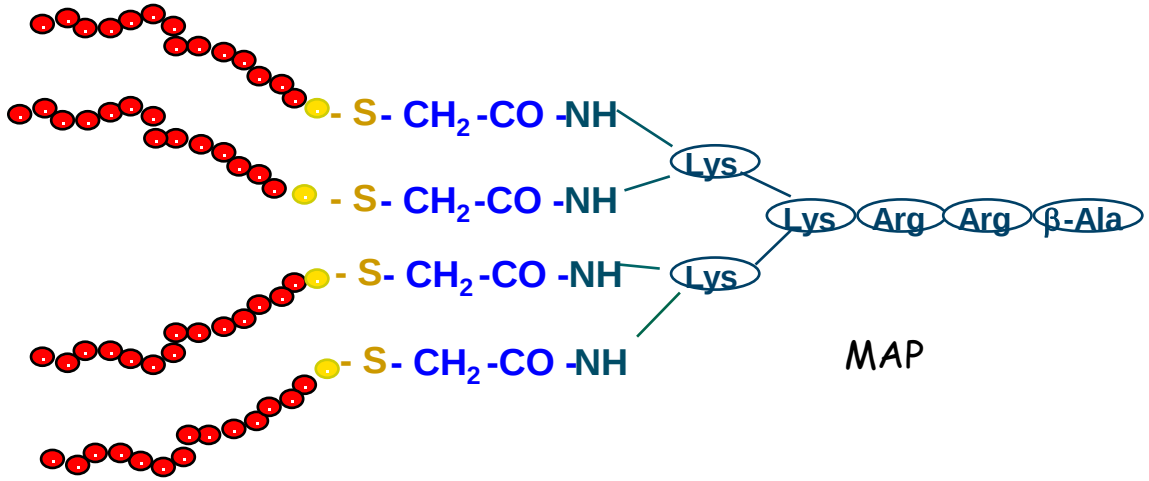
„Restricted" conformation
- cyclopeptides
- „scaffold" chimera

Multiplication
- polymerisation
- conjugation

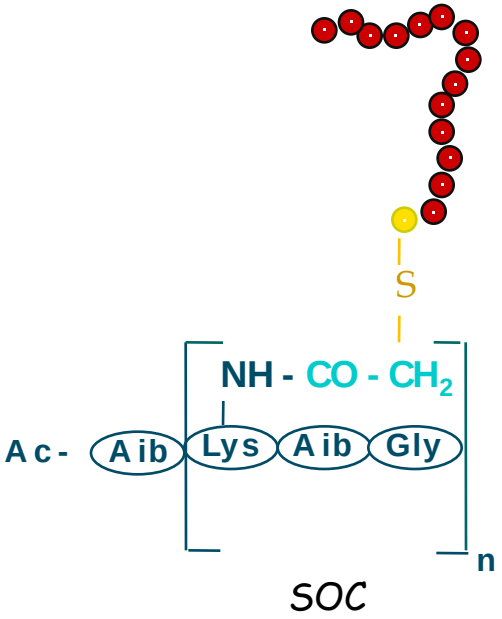
Multivalent epitope - conjugates



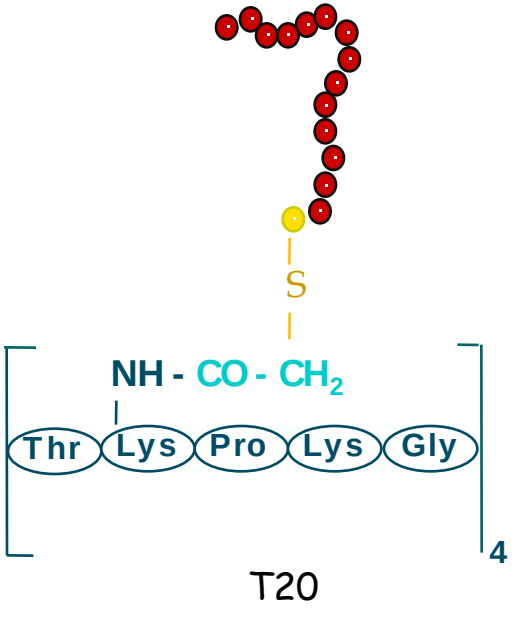
SAK



MAP

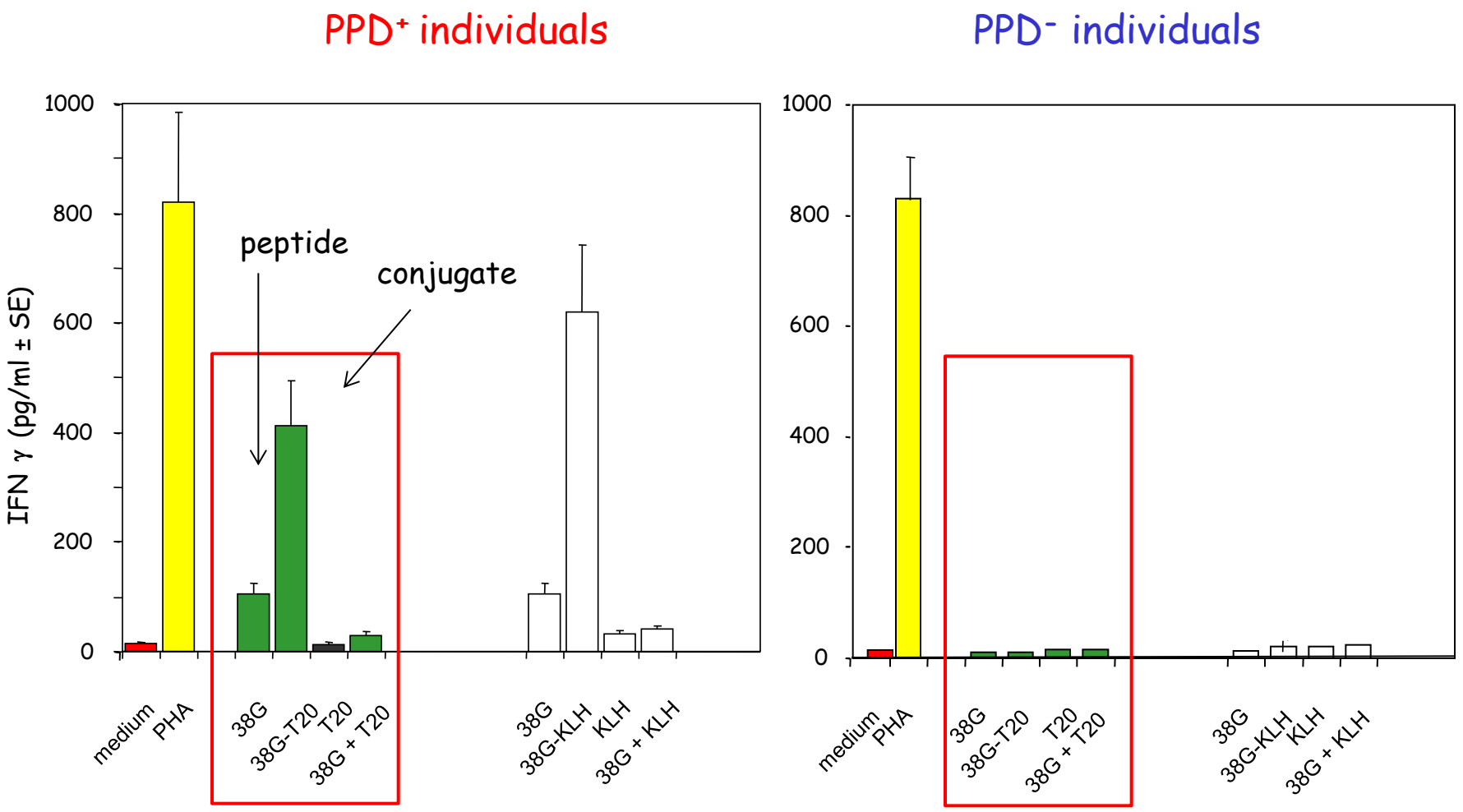


SOC

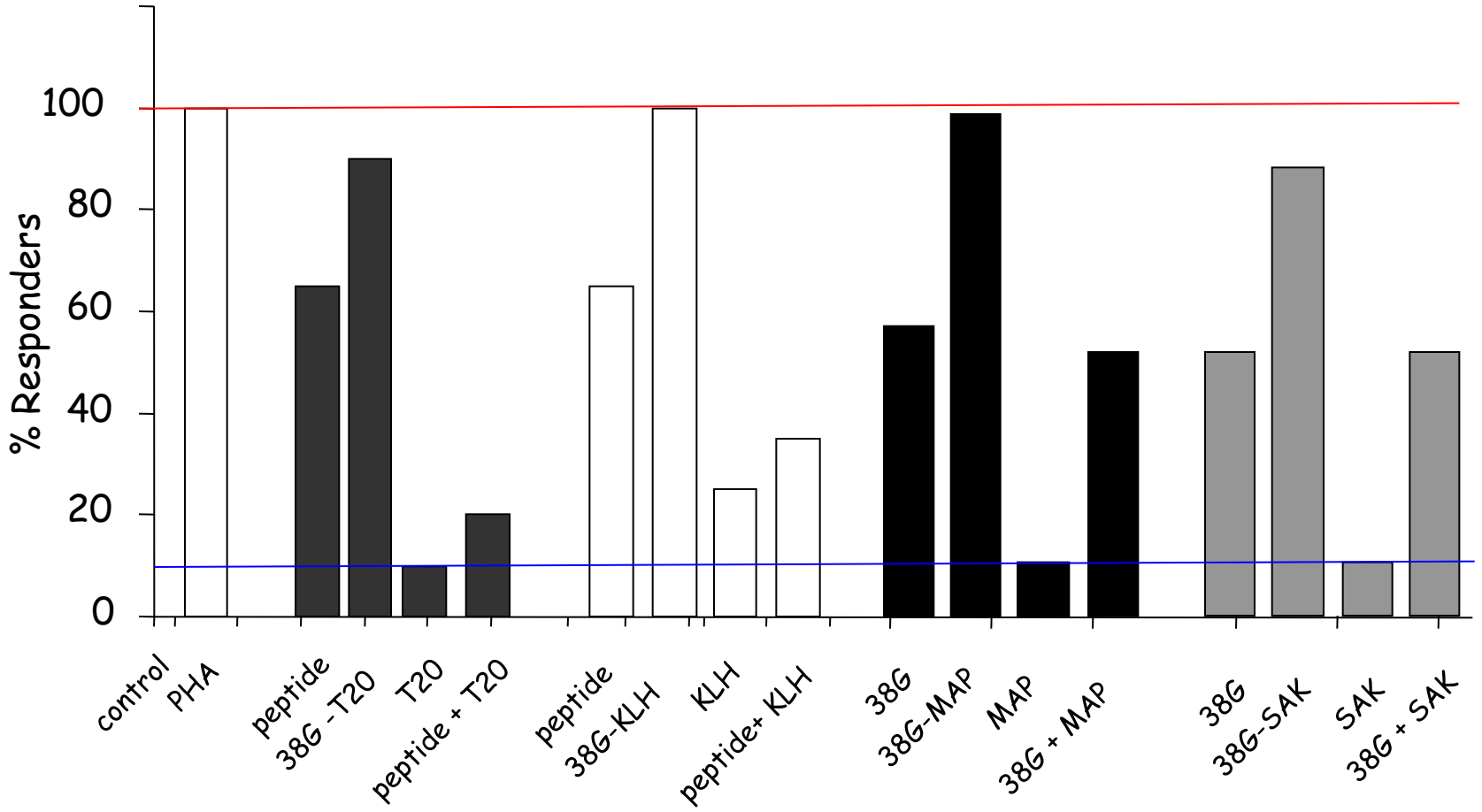


T20

IFNγ production by PBMC from healthy PPD⁺/PPD⁻ subjects stimulated with ^{350}DQVHFQPLPPAVV^{362}-conjugates



Response by PBMC from healthy PPD+ subjects stimulated with ^{350}DQVHFQPLPPAVV^{362}-conjugates



Conclusions

1. Conjugation to macromolecules enhances specific T cell response against an epitope from M.tuberculosis protein in PPD+ healthy individuals.

2. Conjugation to macromolecules has no effect on specific T cell response against epitope from M.tuberculosis protein in PPD- healthy individuals.

Summary: Target proteins

Mucin 1, mucin 2
glycoproteins

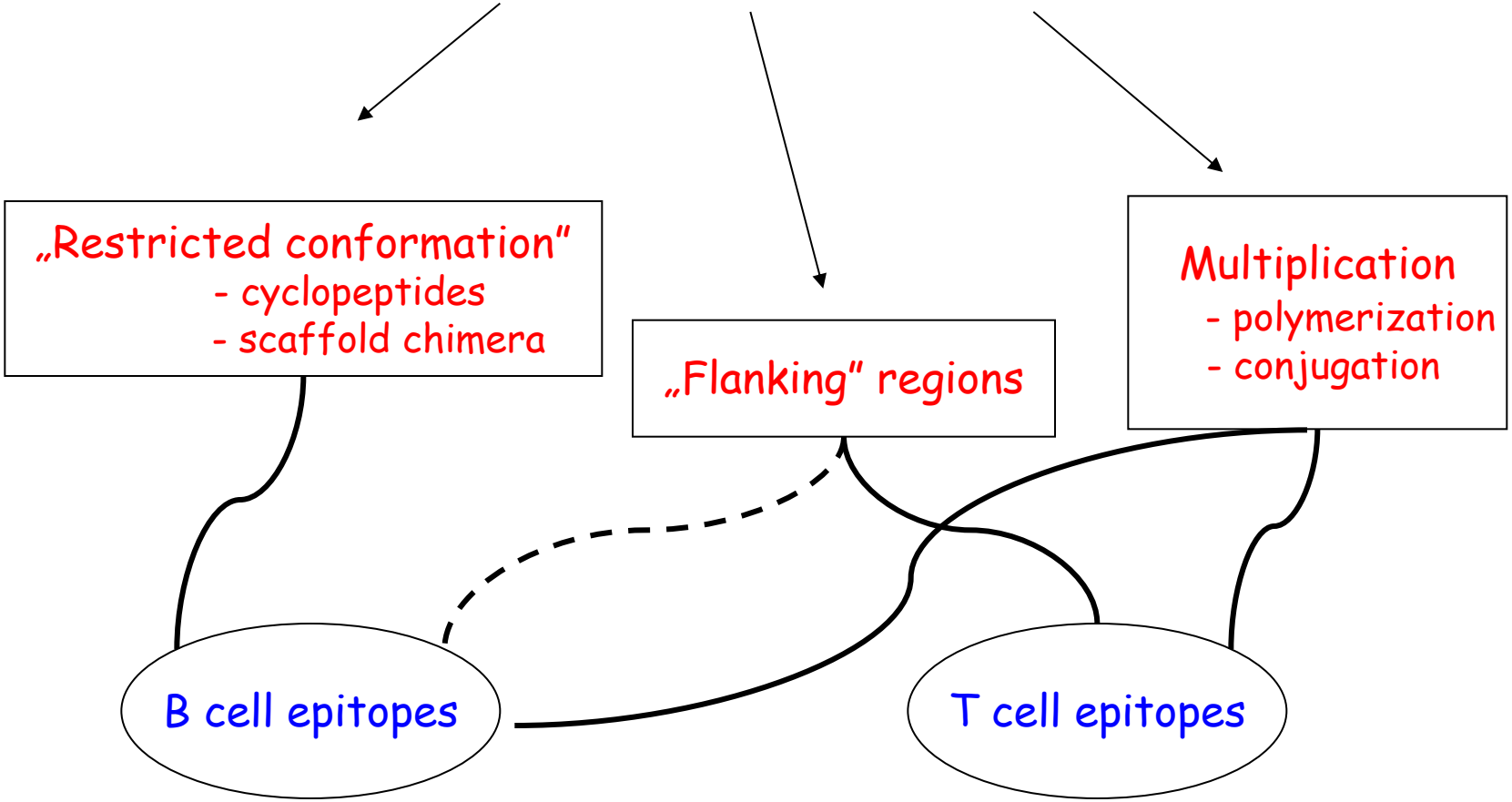
Tuberculin proteins
HSV gD glycoprotein

Filaggrin, fibrin
Dezmoglein 1 amd 3

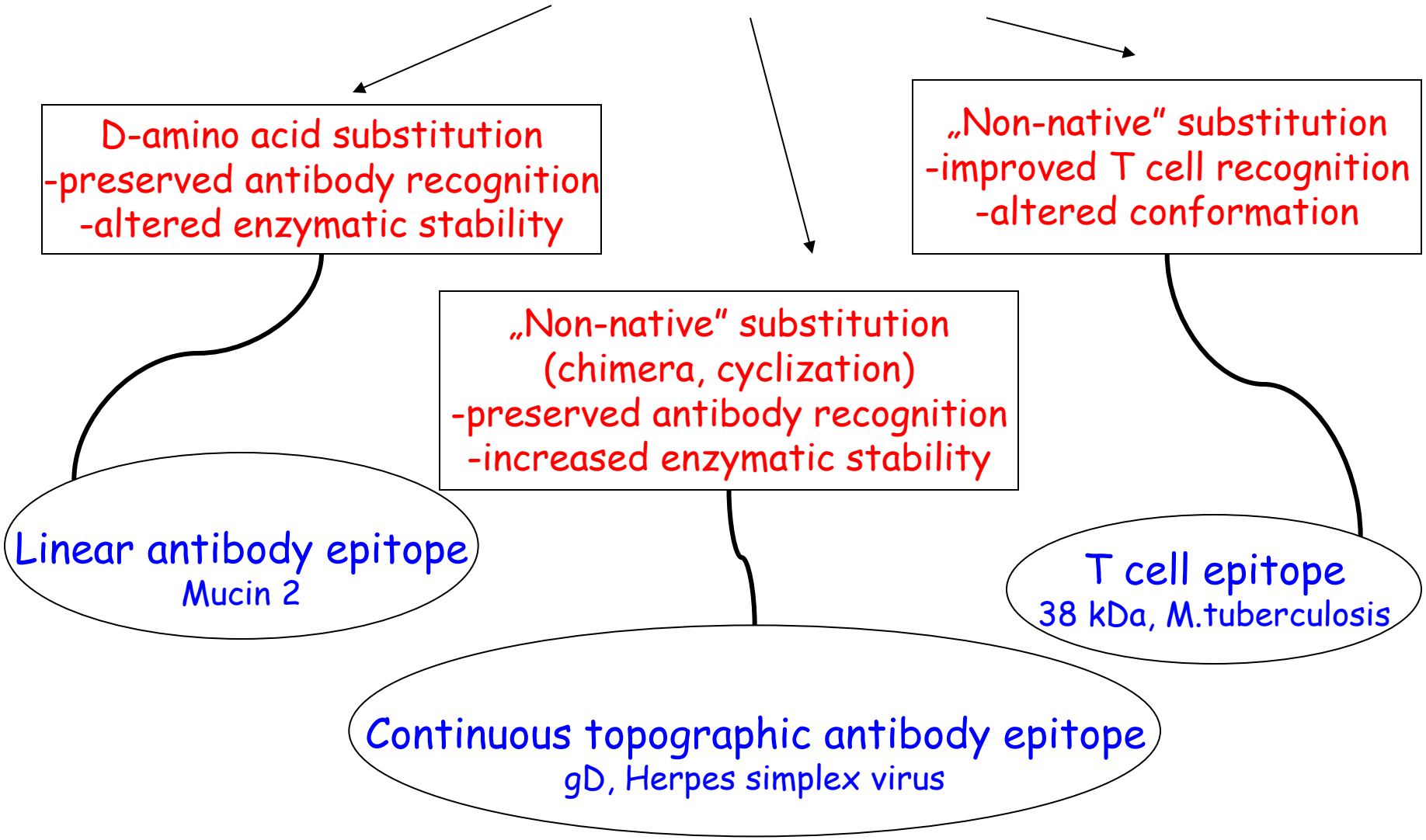
Beta amyloid

J. Med. Chemistry 51: 1150-1161 (2008)
Biopolymers. 19: 94-104 (2008)

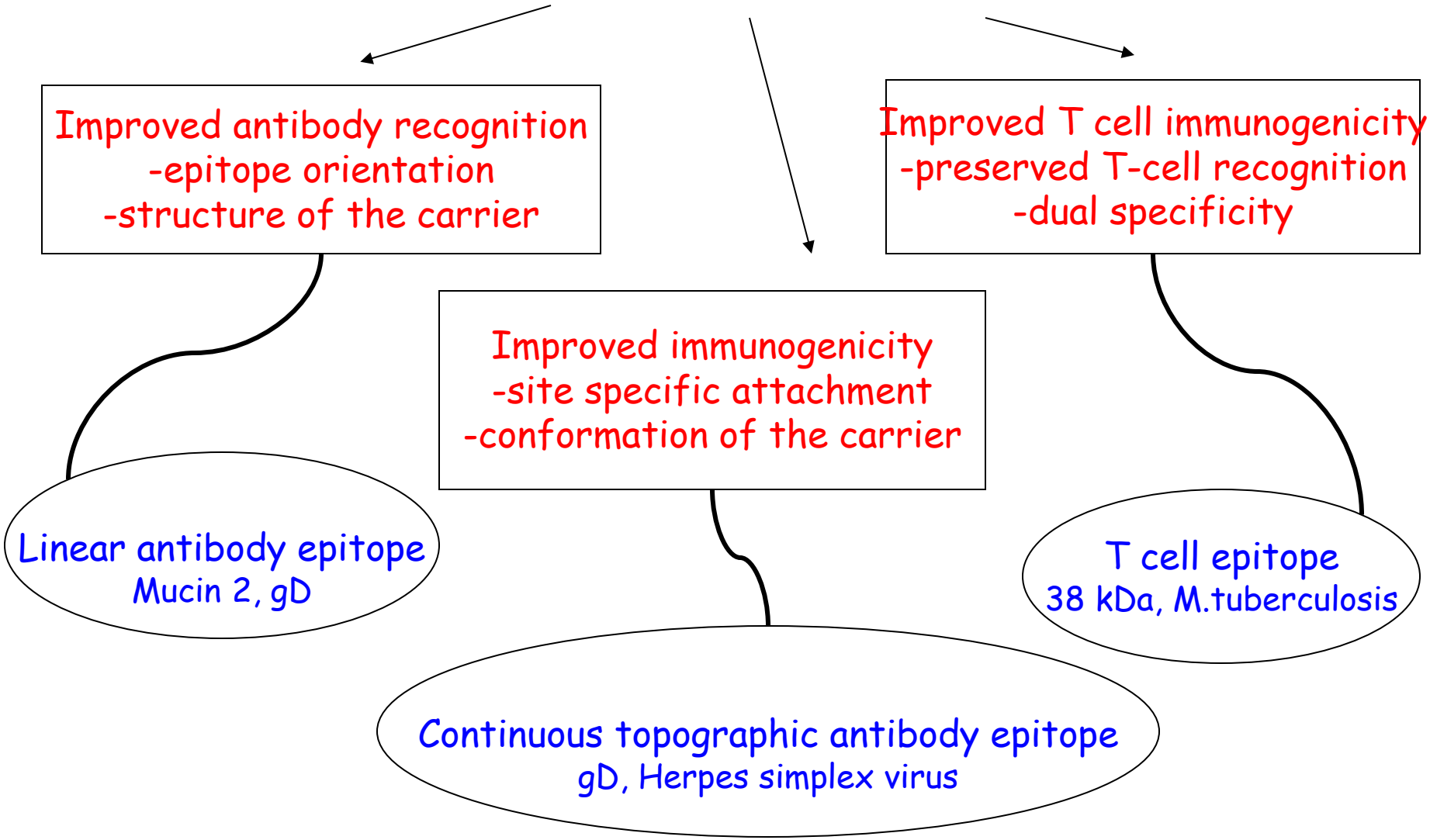
Structural manipulation of epitope recognition, enzyme stability, immunogenicity



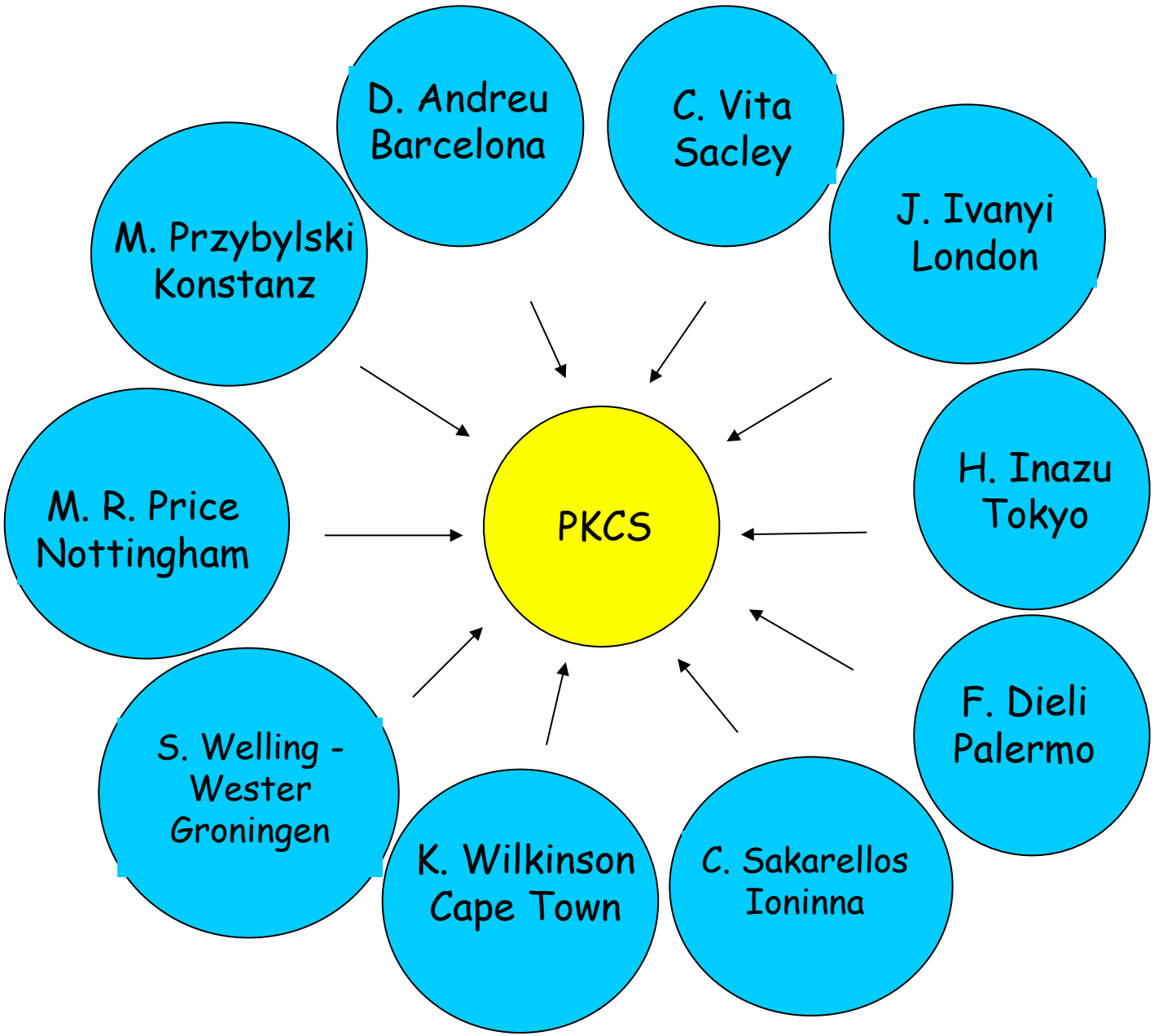
Modification in the flanking regions of the epitope



Multiplication by epitope conjugation to carrier



Partners


D. Andreu
Barcelona
C. Vita
Sacley
J. Ivanyi
London
M. Przybylski
Konstanz
M. R. Price
Nottingham
PKCS
H. Inazu
Tokyo
S. Welling -
Wester
Groningen
F. Dieli
Palermo
K. Wilkinson
Cape Town
C. Sakarellos
Ioninna

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DIRECTORATE-GENERAL
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COST CHEMISTRY WORKING GROUP
Peptide based synthetic antigens against infectious diseases

D.Krikorian, V. Tsikaris, C. Sakarellos
University of Ioannina, Greece

D.Andreu
University Pompeu Fabra, Barcelona, Spain

N. Caccomo, F. Dieli,
University of Palermo, Italy

S.Welling-Wester, M. Feijbrief
University of Groningen, The Netherlands

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