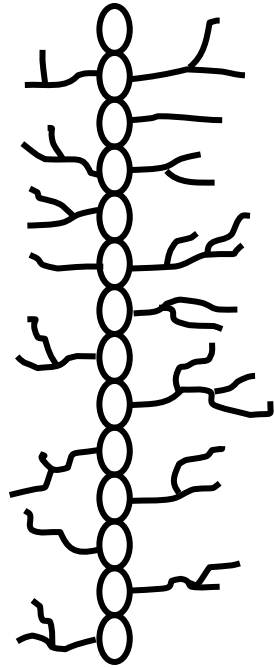


Example 5

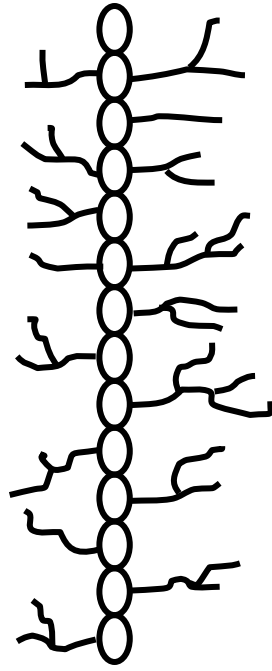
Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1
Using synthetic Peptides and MUC-1 specific Antibodies

MUC1	¹ PDTRPAPGSTAPPAHGVTS ²⁰	Gendler et al. 1988
MUC2	¹ PTTTPITTTTTVTPPTGTQT ²³	Gum et al. 1989
MUC3	¹ HSTPSFTSSITTTETTS ¹⁷	Gum et al. 1990
MUC4	¹ TSSASTGHATPLPVD ¹⁶	Porchet et al. 1991
MUC5	¹ TTSTTSAP ⁸	Meerzaman et al. 1994
MUC6	169 amino acids	Toribara et al. 1993
MUC7	¹ TTAAPPTPSATTPAPPSSSAPPE ²³	Bobek et al. 1993
MUC8	¹ TSCRPLQEGTRV ¹³	Shankar et al. 1994

Normal tissue



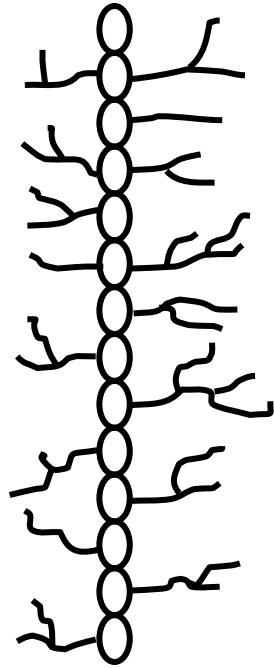
Normal tissue



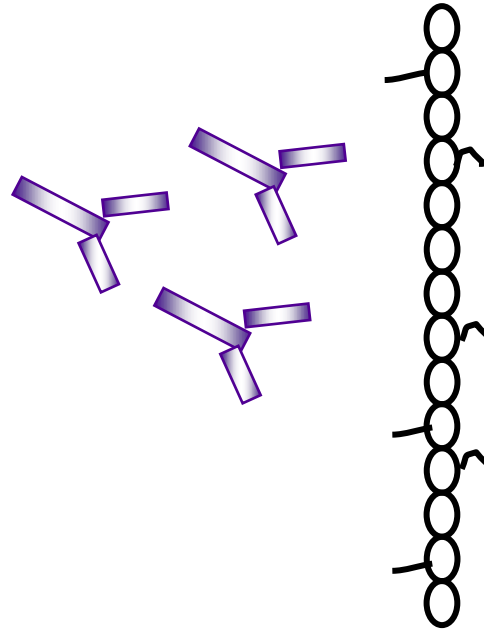
Tumour tissue



Normal tissue



Tumour tissue



Autoantibodies



Tumour diagnosis/immunotherapy

Example 5

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1
Using synthetic Peptides and MUC-1 specific Antibodies

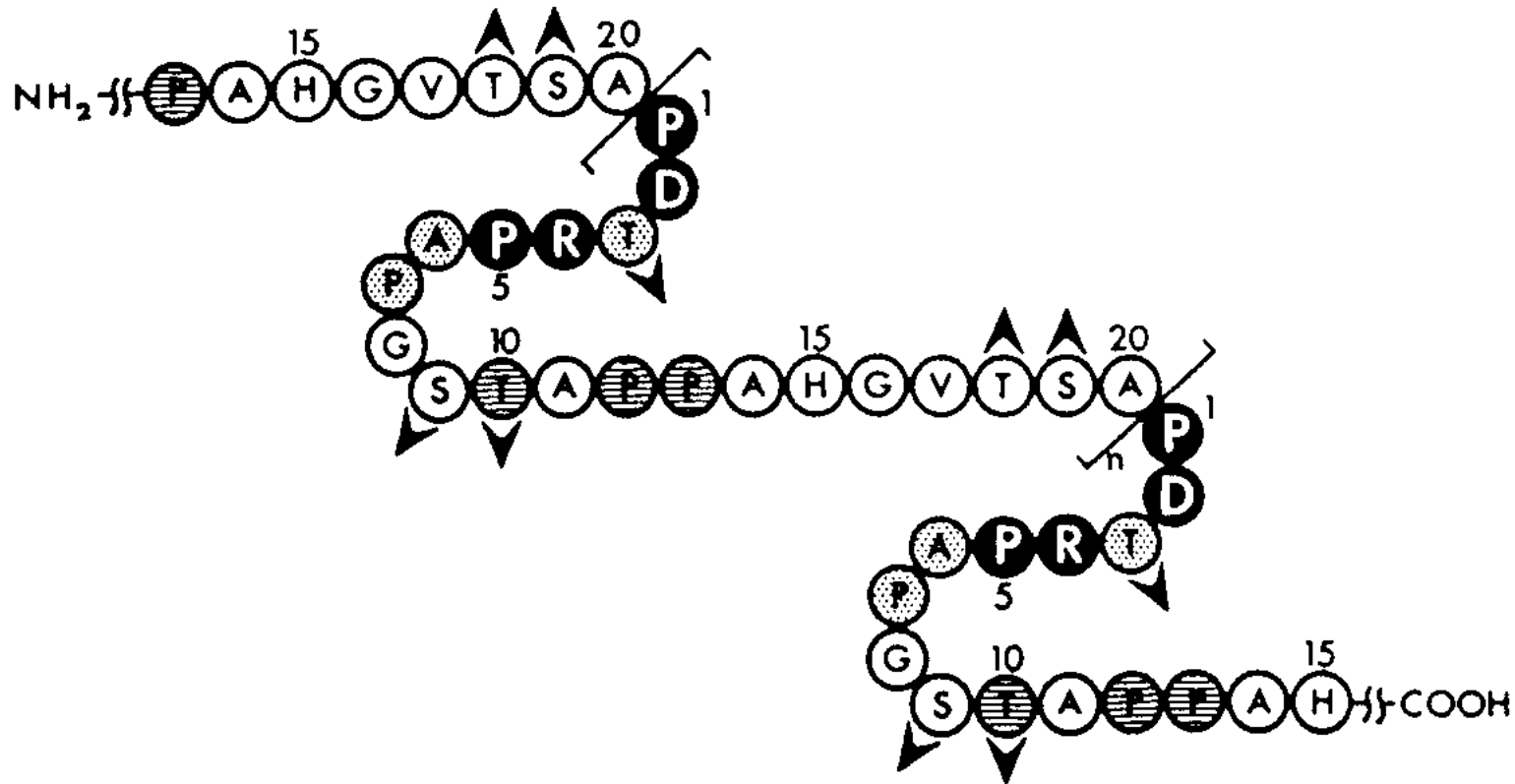


Fig. 1. Diagrammatic representation of the predicted secondary structure of the mucin polypeptide core. Turn tetrapeptides are denoted by chain reversals. The hydrophilicity values for each amino acid, averaged over a window of seven amino acid residues, are represented as follows: (●) ≥ 1.4 ; $1.4 > (\text{◐}) \geq 0.7$; $0.7 > (\text{◑}) \geq 0.5$; (○) < 0.5 . Arrows indicate potential glycosylation sites.

Example 5

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1 Using synthetic Peptides and MUC-1 specific Antibodies

Phase I

2. step

Synthesis of overlapping heptapeptides covering the repeat of an antigenic 20 amino acid sequence of MUC-1

Method: Solid phase synthesis of peptides on polyethylene pin support.
(Peptides were not removed from the pin during testing.)

3. step

Binding studies with purified MUC-1 specific antibodies on immobilized synthetic overlapping peptides.

Method:

- a. Incubation of peptides with antibody [HMFG-1].
- b. Incubation with peroxidase-labeled rabbit anti-mouse Ig.
- c. Development of colour reaction by the addition of substrate solution (azino-di-3-ethyl-benzothiazoline-sulphonate) and hydrogen peroxidase.
- d. Reading of absorbance at $\lambda = 405$ nm.

Example 5

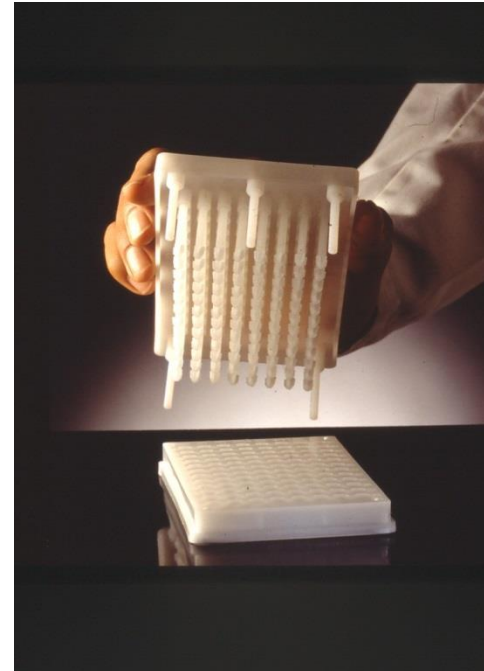
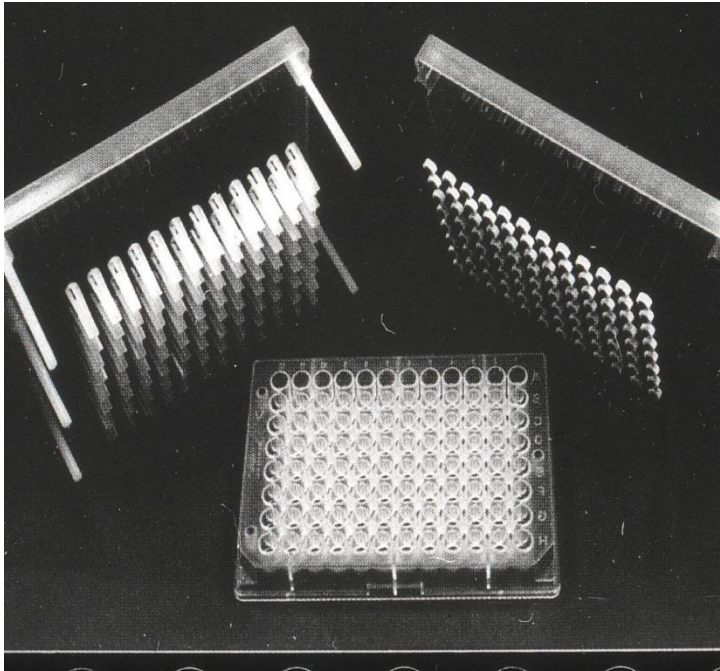
Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1 Using synthetic Peptides and MUC-1 specific Antibodies

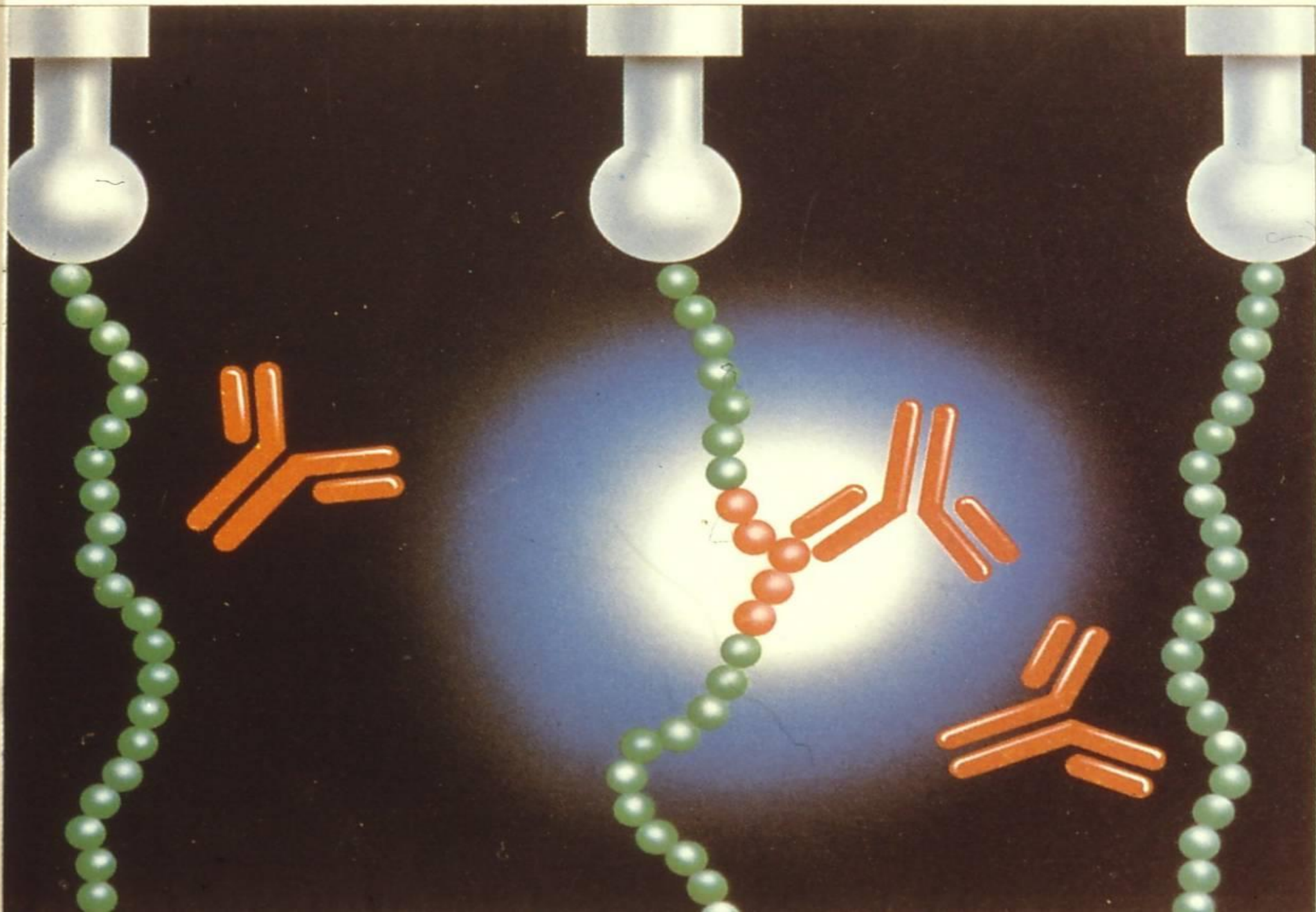
Phase I

2. step

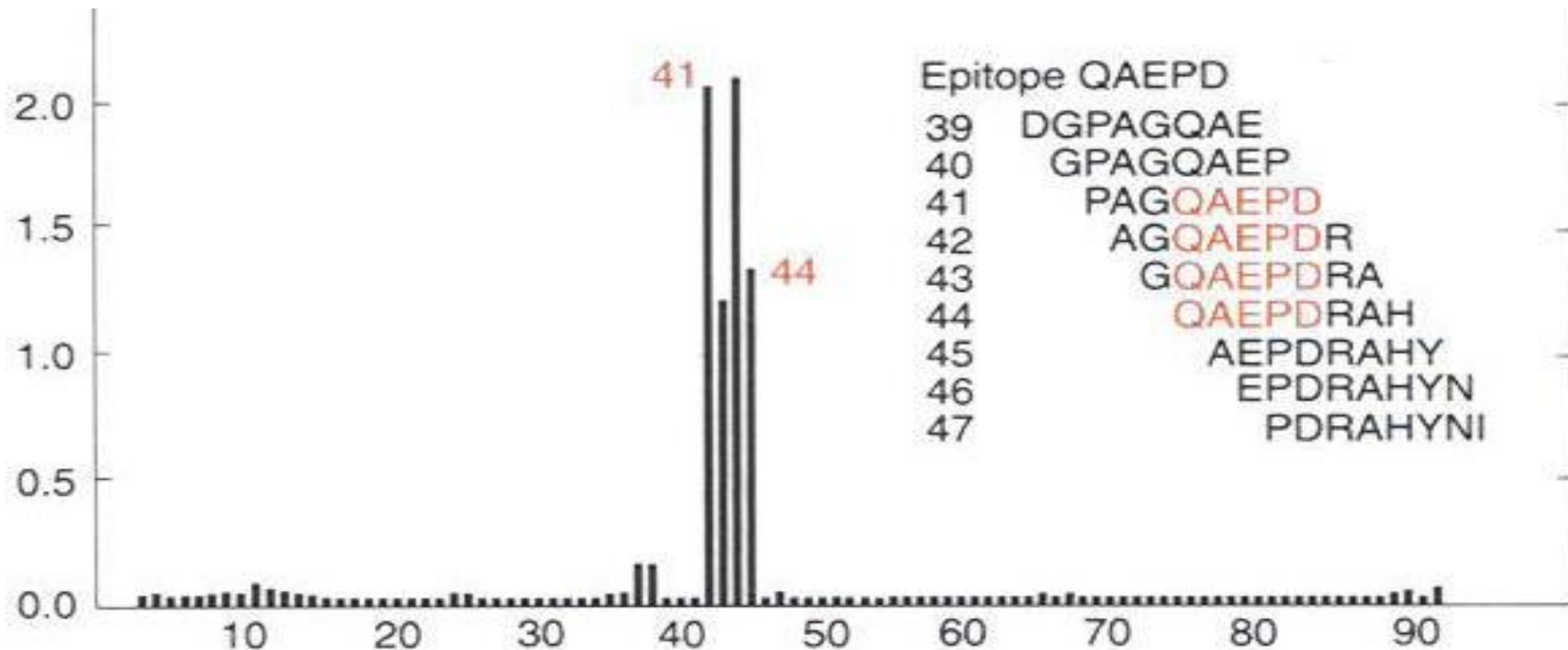
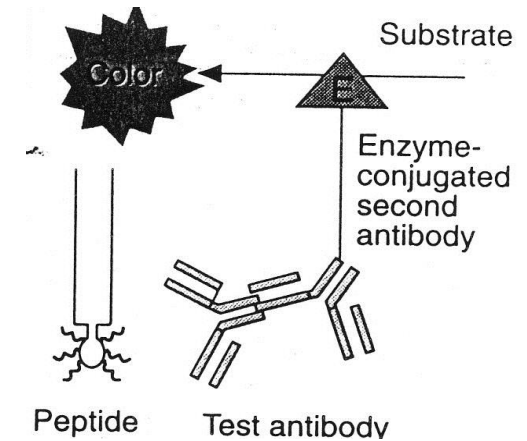
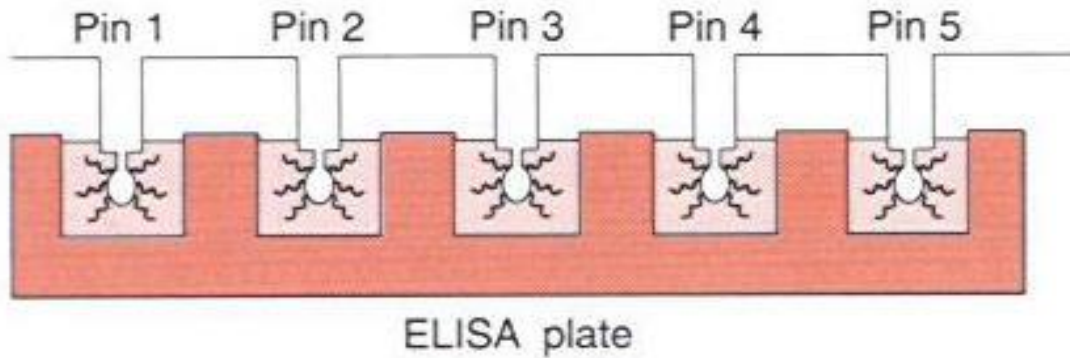
Synthesis of overlapping heptapeptides covering the repeat of an antigenic 20 amino acid sequence of MUC-1

Method: Solid phase synthesis of peptides on polyethylene pin support.
(Peptides were not removed from the pin during testing.)





Binding studies: Identification of linear antibody epitopes by monoclonal antibodies



Example 5

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1 Using synthetic Peptides and MUC-1 specific Antibodies

Peptide	¹ PPAHGVSTSAPDTRPAPGSTA ²¹	Elisa (A ₄₀₅)
1	PPAHGVS	0.00
2	PAHGVST	0.08
3	AHGVSTS	0.04
4	HGVSTSA	0.03
5	GVSTSAP	0.00
6	VSTSAPD	0.02
7	STSAPDT	0.01
8	<u>TSAPDTR</u>	0.73
9	<u>SAPDTRP</u>	0.94
10	<u>APDTRPA</u>	1.09
11	<u>PDTRPAP</u>	0.63
12	DTRPAPG	0.02
13	TRPAPGS	0.08
14	RPAPGST	0.03
15	PAPGSTA	0.03
16	APGSTAP	0.02

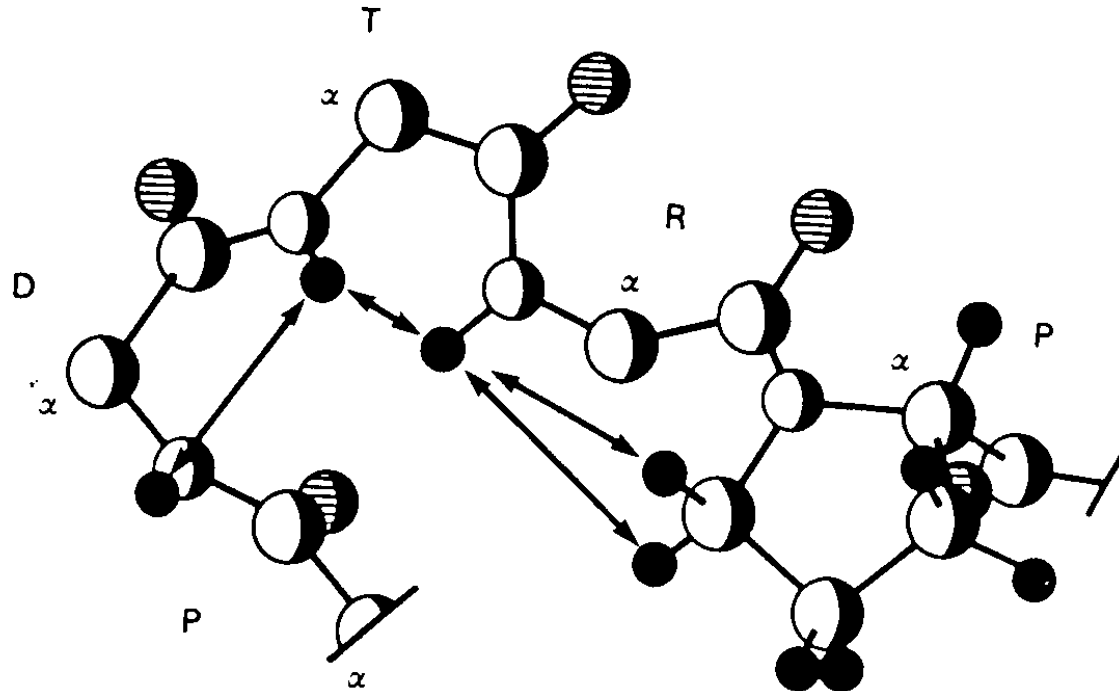
Observation:

Mucin specific MoAb, HMFG-1 binds to heptapeptides containing PDTR sequence

Example 5

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-1 Using synthetic Peptides and MUC-1 specific Antibodies

Determination of 3D structure of epitope region containing PDTR sequence.
Methods: 2D NMR (^1H HOHAHA)



Reference:

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

Example 6

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-2 Using synthetic Peptides and MUC-2 specific Antibodies

- MUC-2:** high molecular mass, MUC-2 gene related glycoprotein, associated with human colon cancer, primary structure is known
- Antibodies:** mouse monoclonal antibodies recognizing MUC-2 glycoprotein
- Interaction:** Antibody - antigen interactions
- Aim:** Identification of epitopes recognized by anti-MUC-2 antibodies

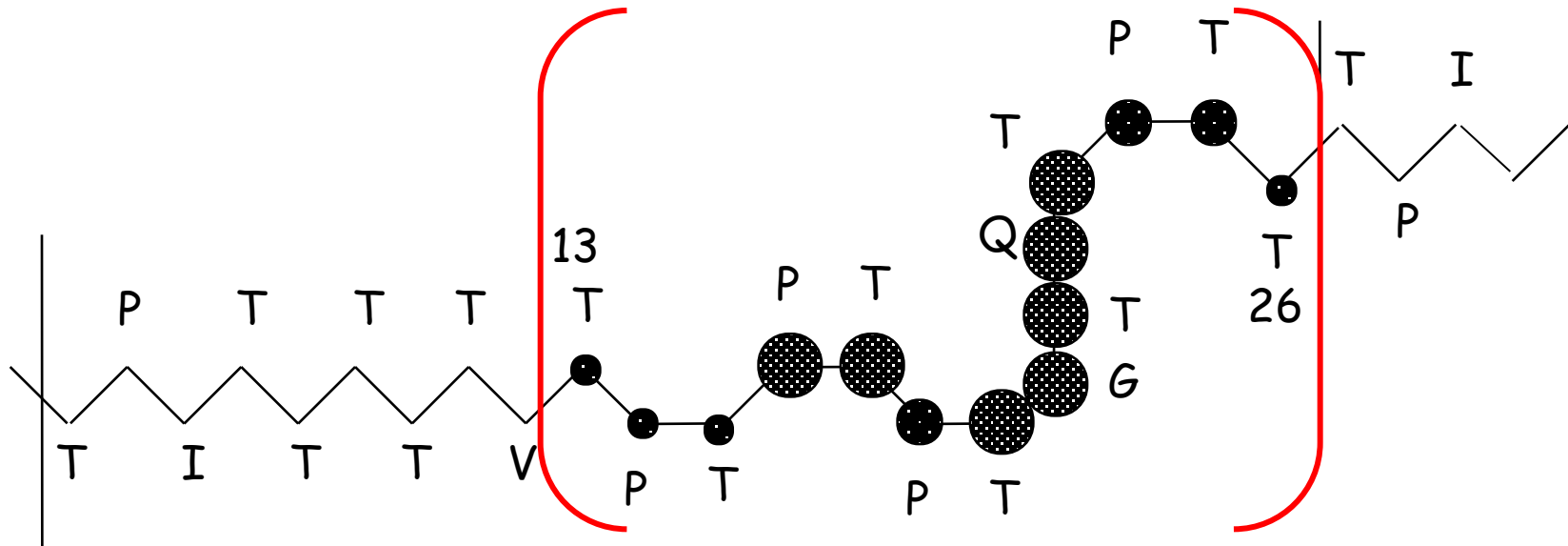
Phase I

1. step Analysis of the primary structure of MUC-1 glycoprotein.
- Method:** Prediction of B-cell epitopes using various algorithms searching for
- hydrophilic region and
 - β -turn secondary structure

Mucin glycoproteins

MUC1	¹ PDTRPAPGSTAPPAHGV TSA ²⁰	Gendler et al. 1988
MUC2	¹ PTTTPITTTTTVTPTPTGTQT ²³	Gum et al. 1989
MUC3	¹ HSTPSFTSSITTTETTS ¹⁷	Gum et al. 1990
MUC4	¹ TSSASTGHATPLPVT D ¹⁶	Porchet et al. 1991
MUC5	¹ TTSTTSAP ⁸	Meerzaman et al. 1994
MUC6	169 amino acids	Toribara et al. 1993
MUC7	¹ TTAAPPTPSATTPAPPSSSAPPE ²³	Bobek et al. 1993
MUC8	¹ TSCRPLQEGTRV ¹³	Shankar et al. 1994

Predicted secondary structure of MUC2 repeat motif



predicted antibody epitope region

MUC2 protein specific monoclonal antibodies
MAb 994 and MAb 996

Immunogen: KLH-[K¹²VTPPTPTGTQTPT²⁵]

Immunohistochemistry:
Recognition of human colon tumour tissue.

Mouse IgG1

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

Example 6

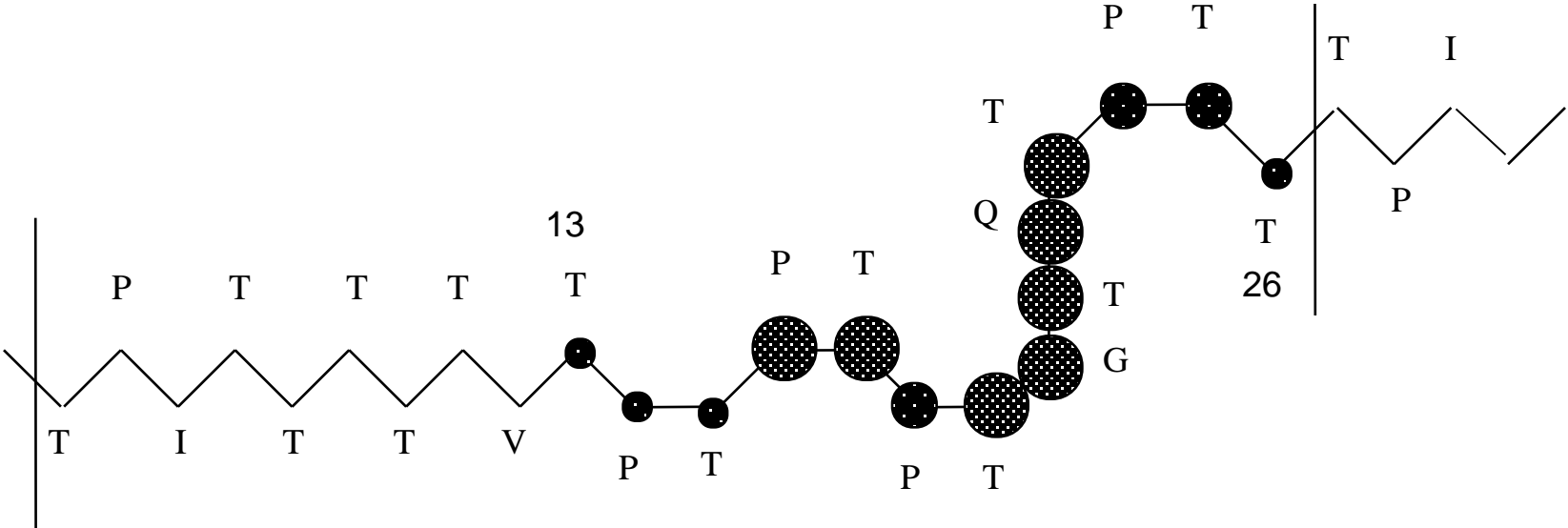
Localization of Immunogenic Determinants (Epitopes) of
human epithelial mucin glycoprotein, MUC-2
Using synthetic Peptides and MUC-2 specific Antibodies

Identification of MAbs 994 epitope

Multiple epitopes: $^{21}\text{TQTPT}^{25}$

$^{19}\text{TGTQT}^{23}$

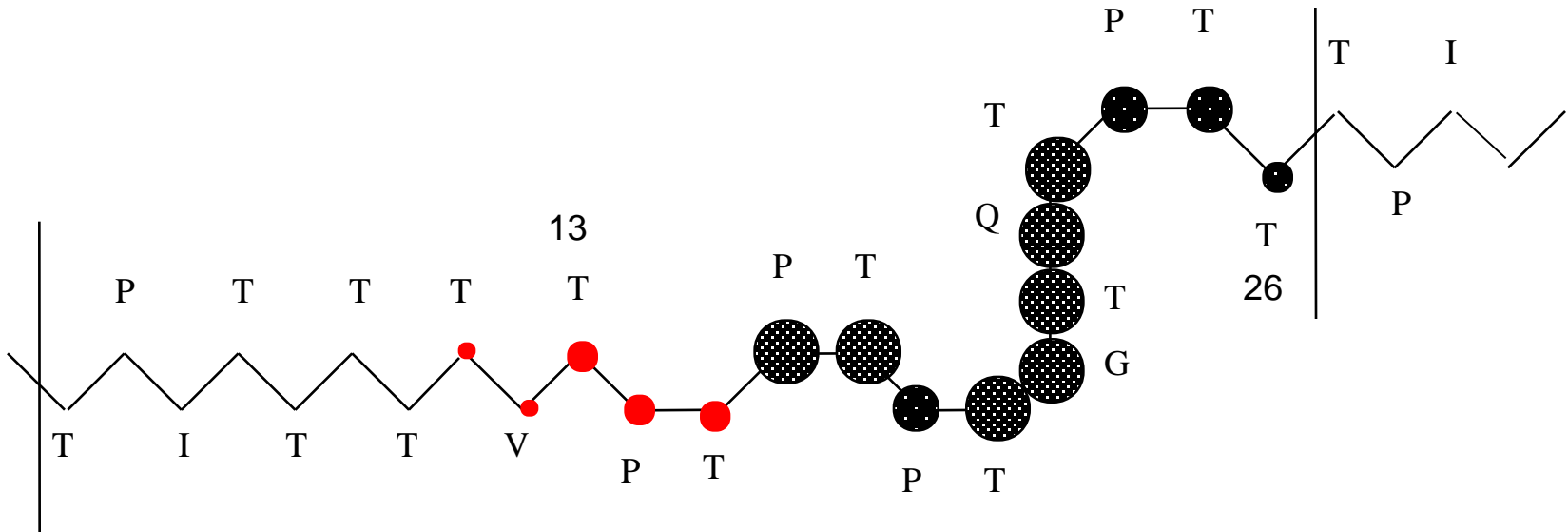
$^{13}\text{TPTPT}^{17}$



Conclusion

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

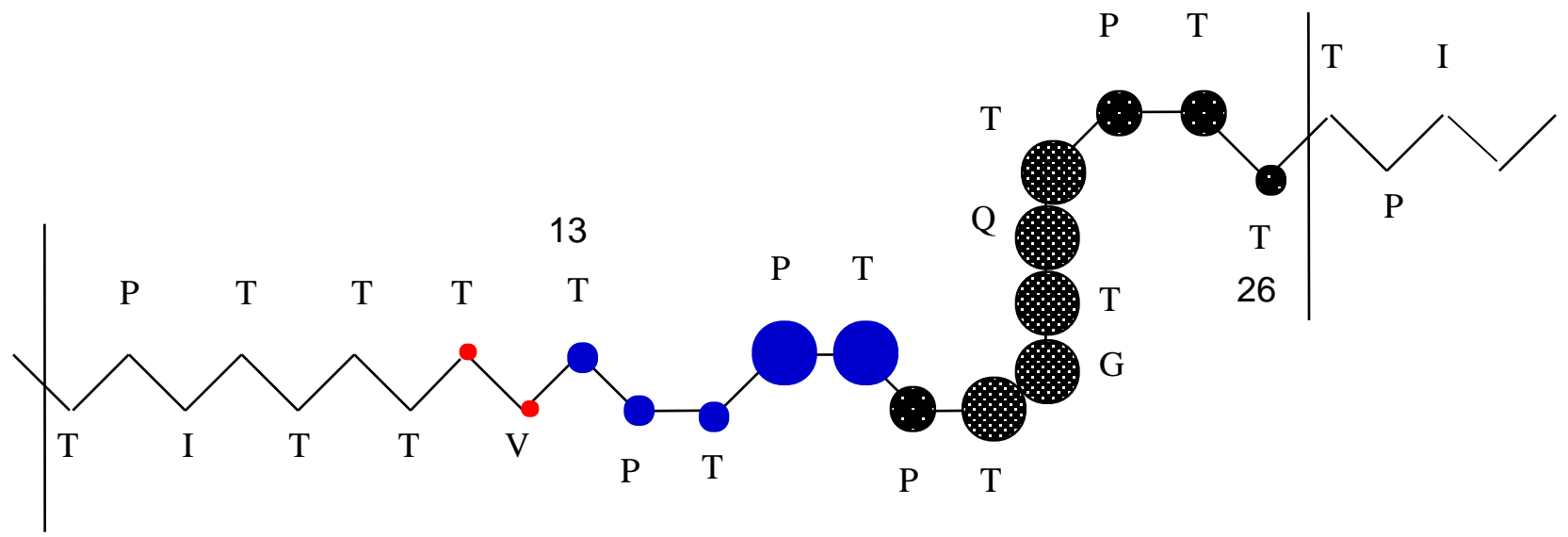
Common motif:
TXTXT



Conclusion

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

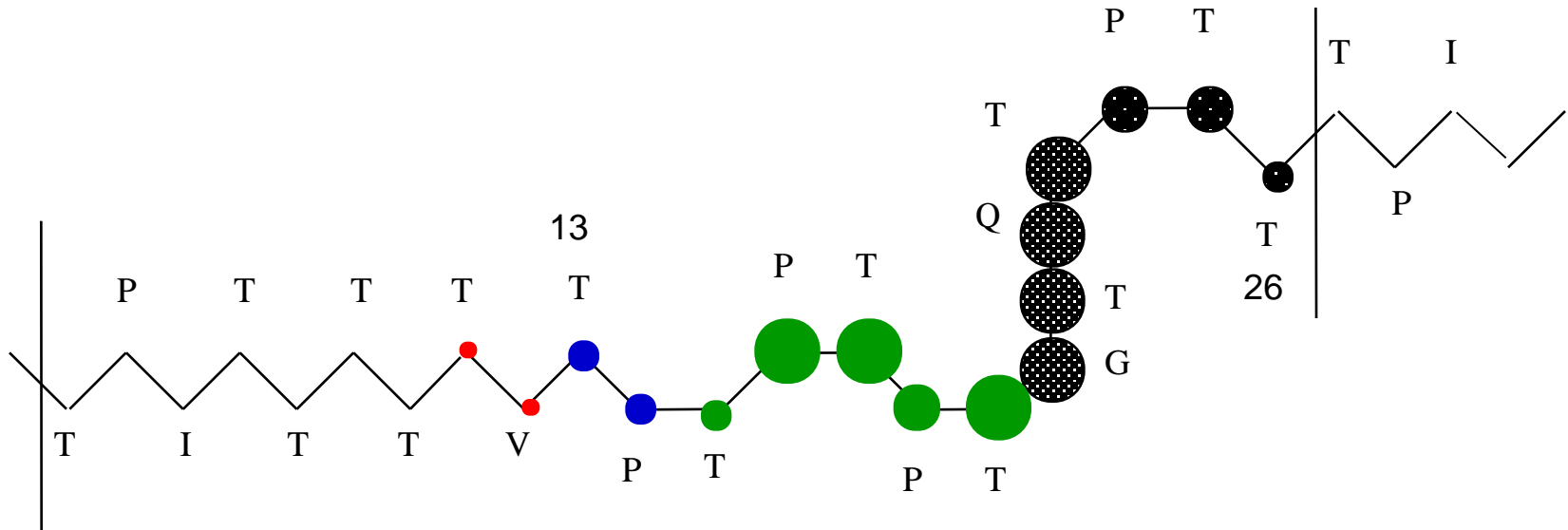
Common motif:
TXTXT



Conclusion

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

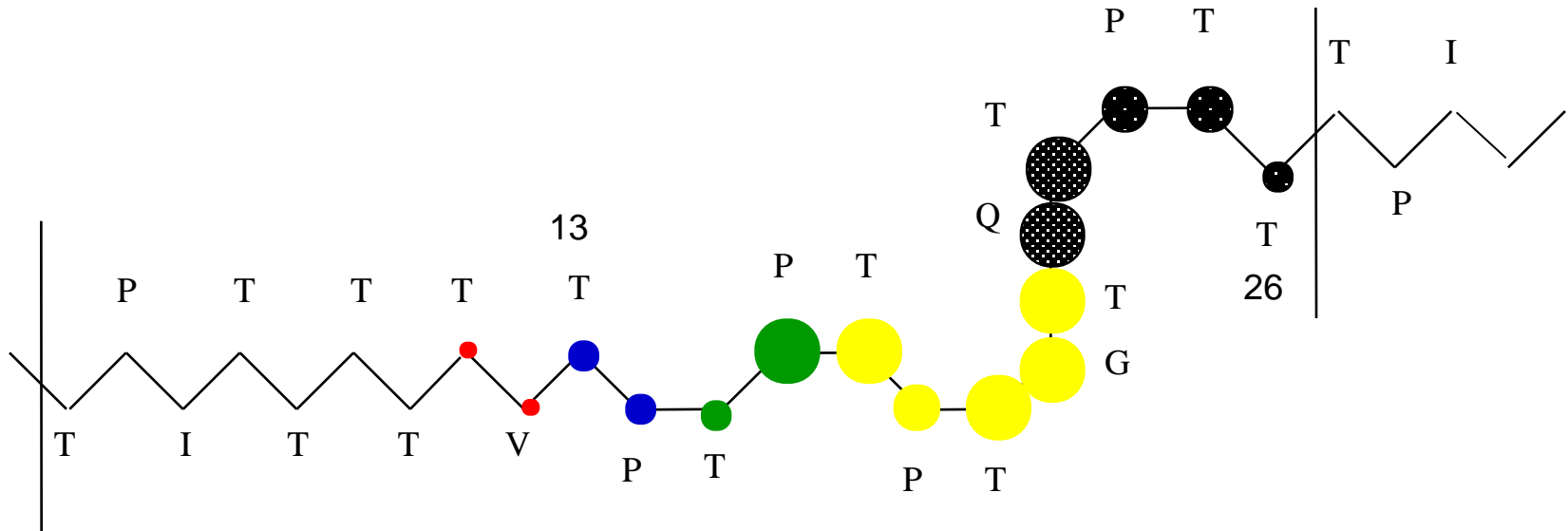
Common motif:
TXTXT



Conclusion

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

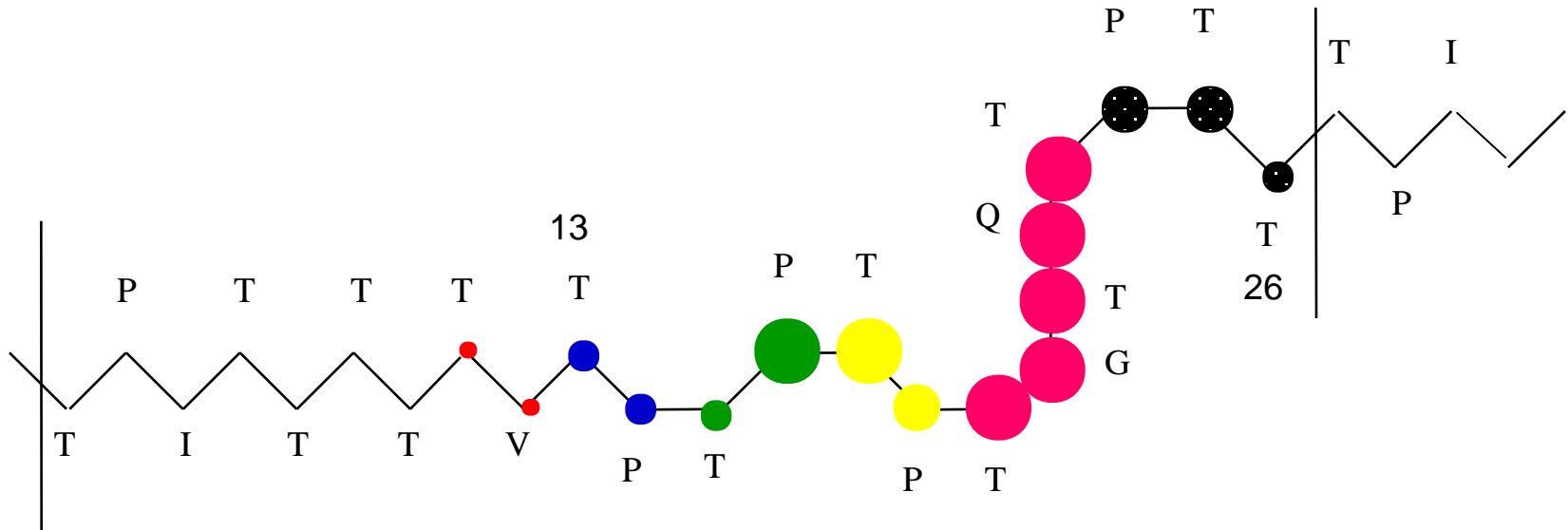
Common motif:
TXTXT



Conclusion

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

Common motif:
TXTXT

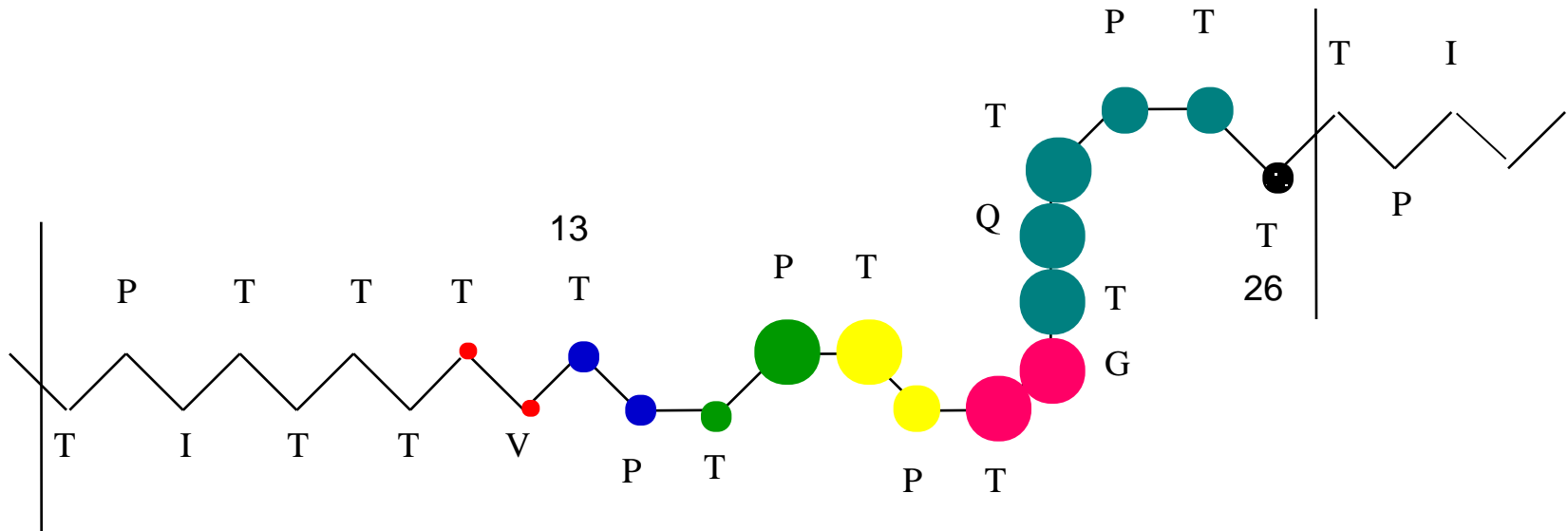


Uray et al. Arch. Biochem. Biophys. (2003)

Conclusion

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

Common motif:
TXTXT



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

Example 6

Localization of Immunogenic Determinants (Epitopes) of
human epithelial mucin glycoprotein, MUC-2
Using synthetic Peptides and MUC-2 specific Antibodies

2. step Which one (s)?

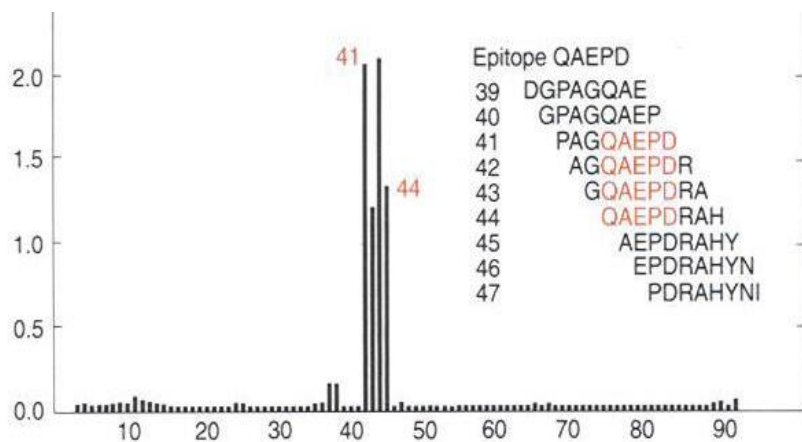
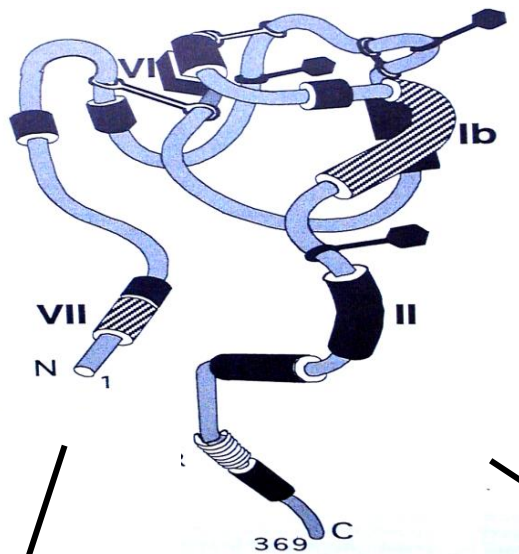
Method:

a. Synthesis and characterization of
combinatorial peptide library

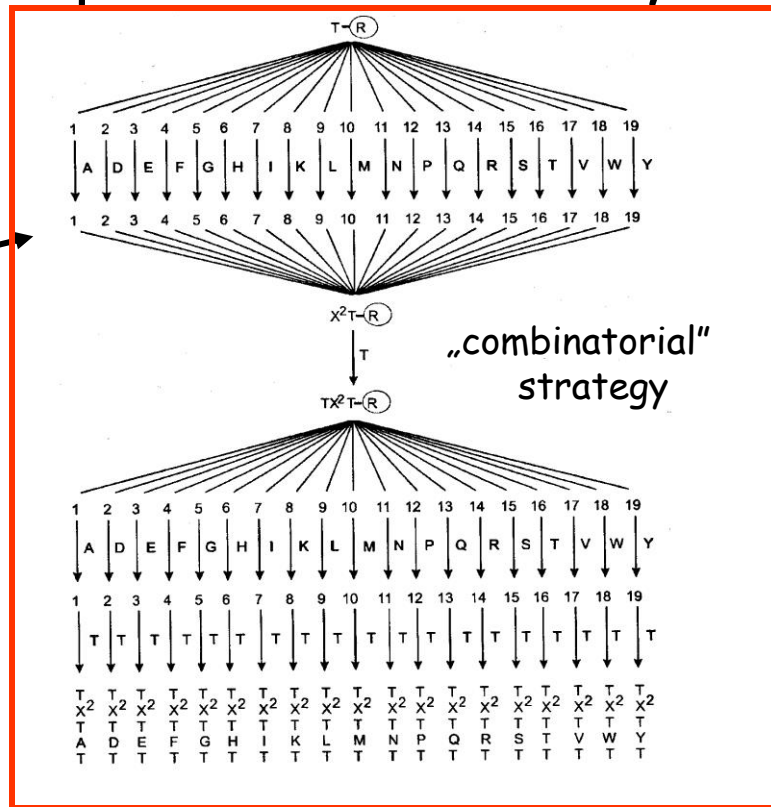
b. Binding assay with epitope specific monoclonal antibody

Identification of short sequences responsible for activity

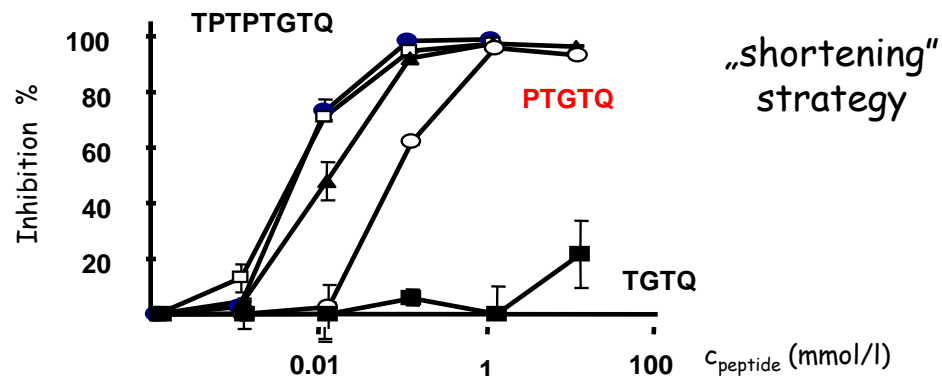
„predicted“



„overlapping“ strategy

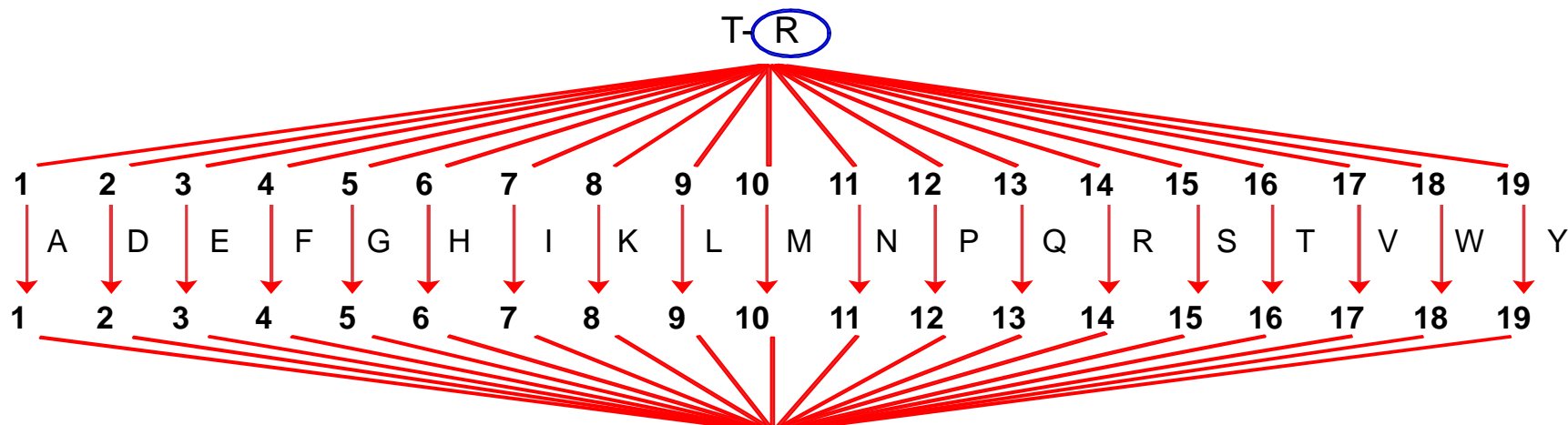


„combinatorial“ strategy



„shortening“ strategy

Combinatorial synthesis of TX¹TX²T peptide library

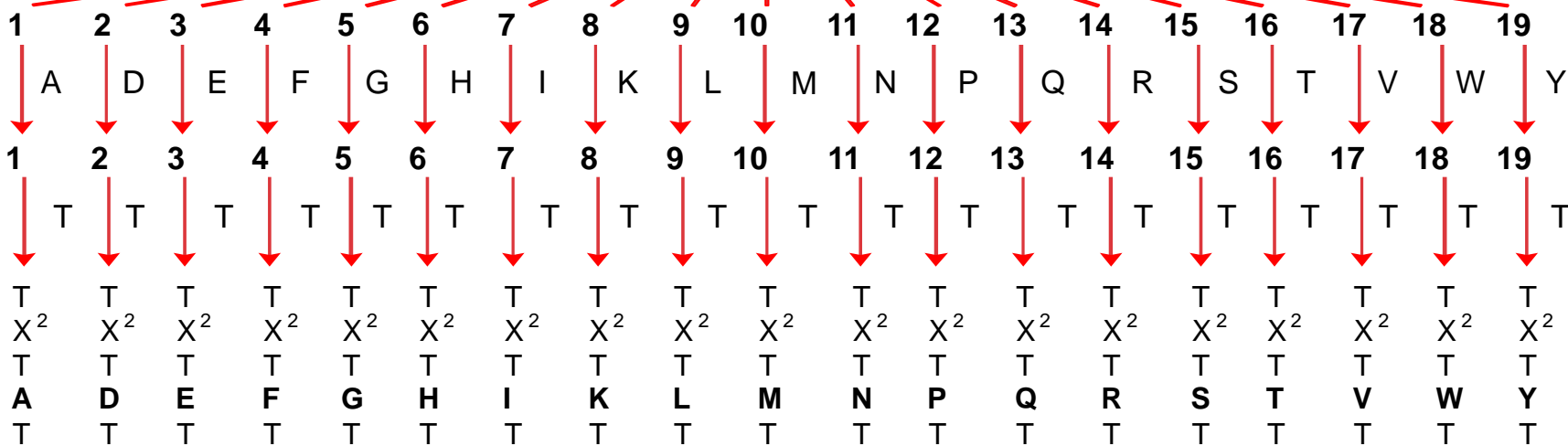


X²T-R (with R circled)

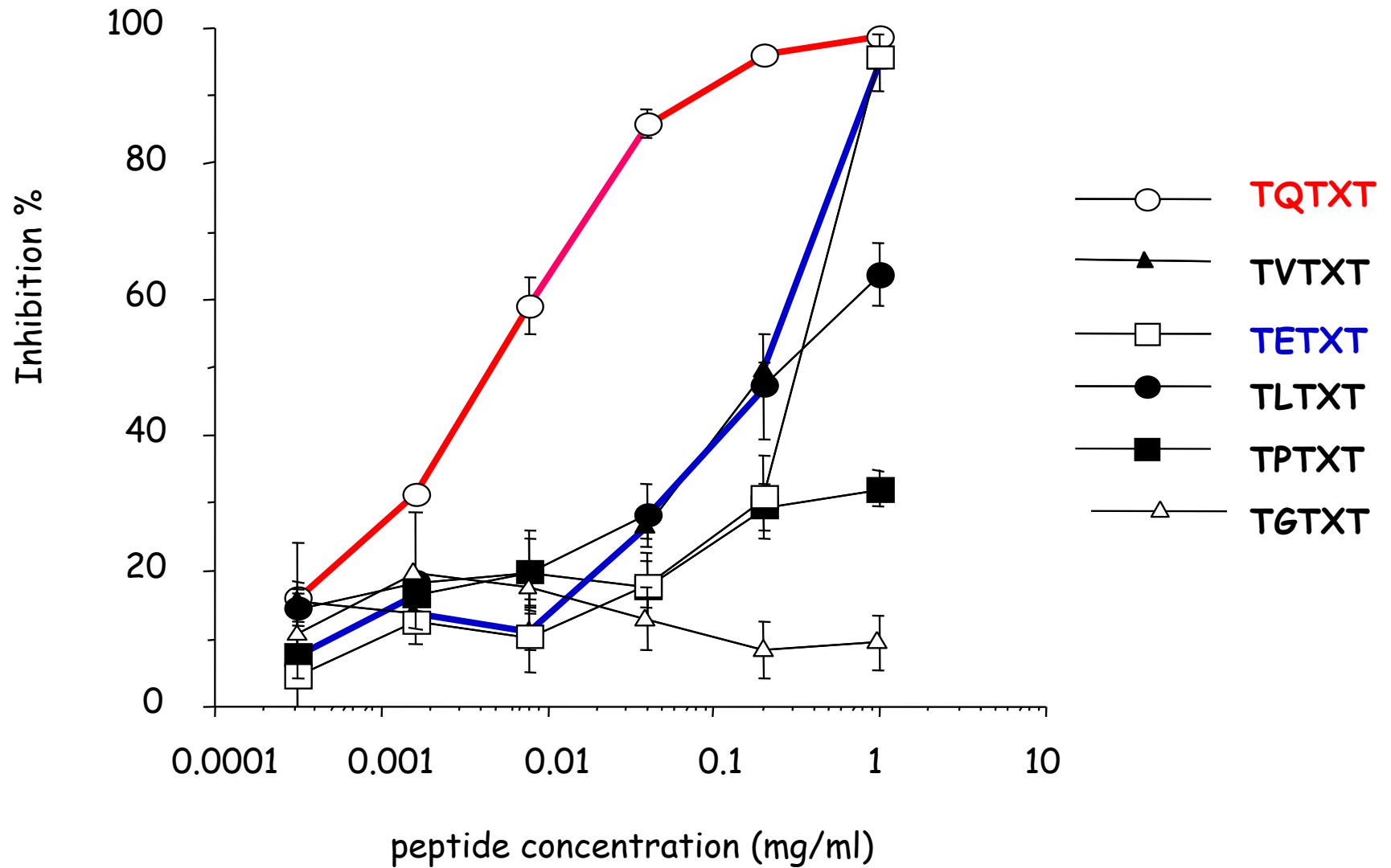
T

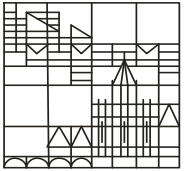
TX²T-R (with R circled)

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

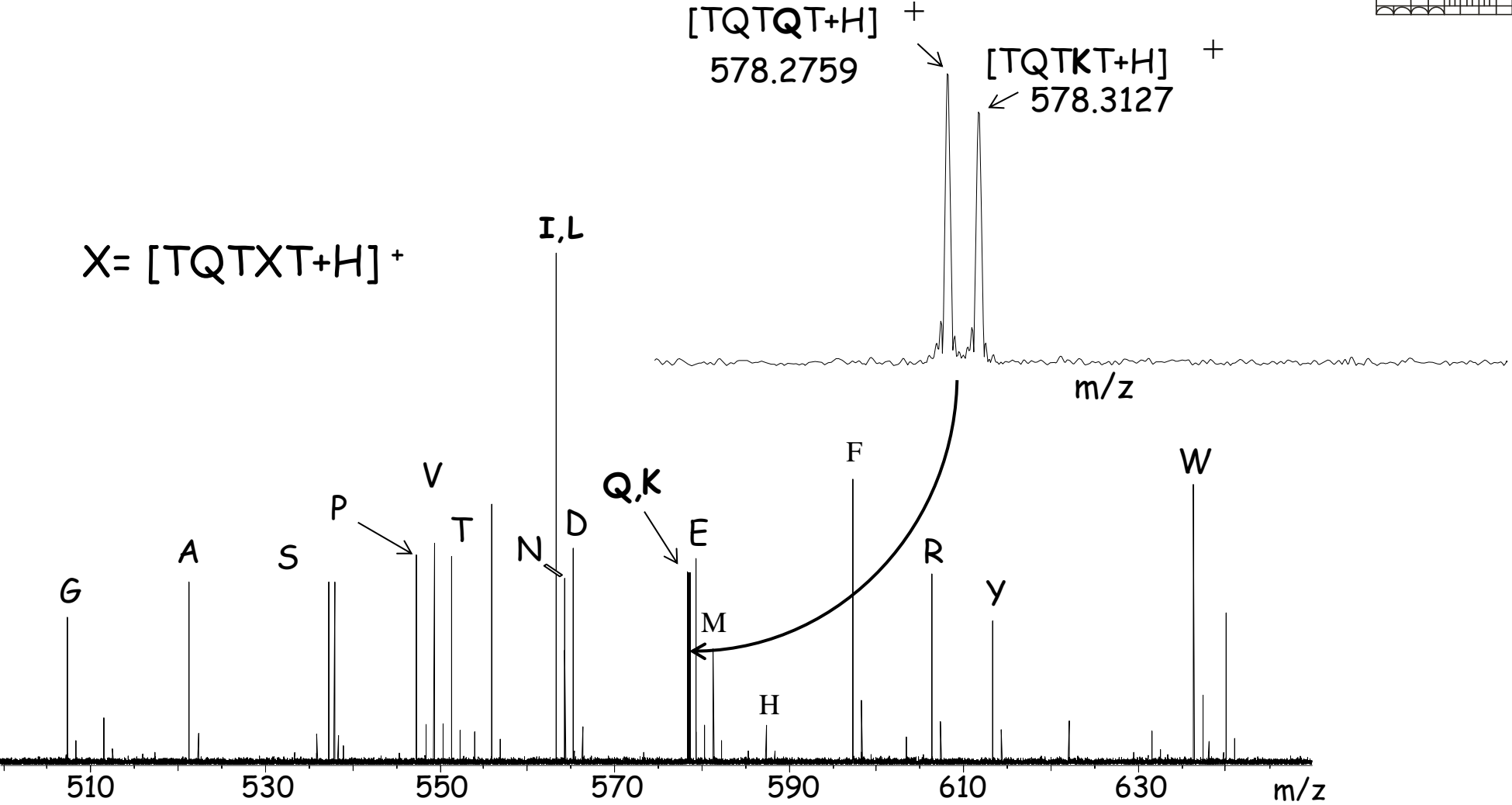


Binding of Mab 994 to TX¹TX²T peptide mixtures



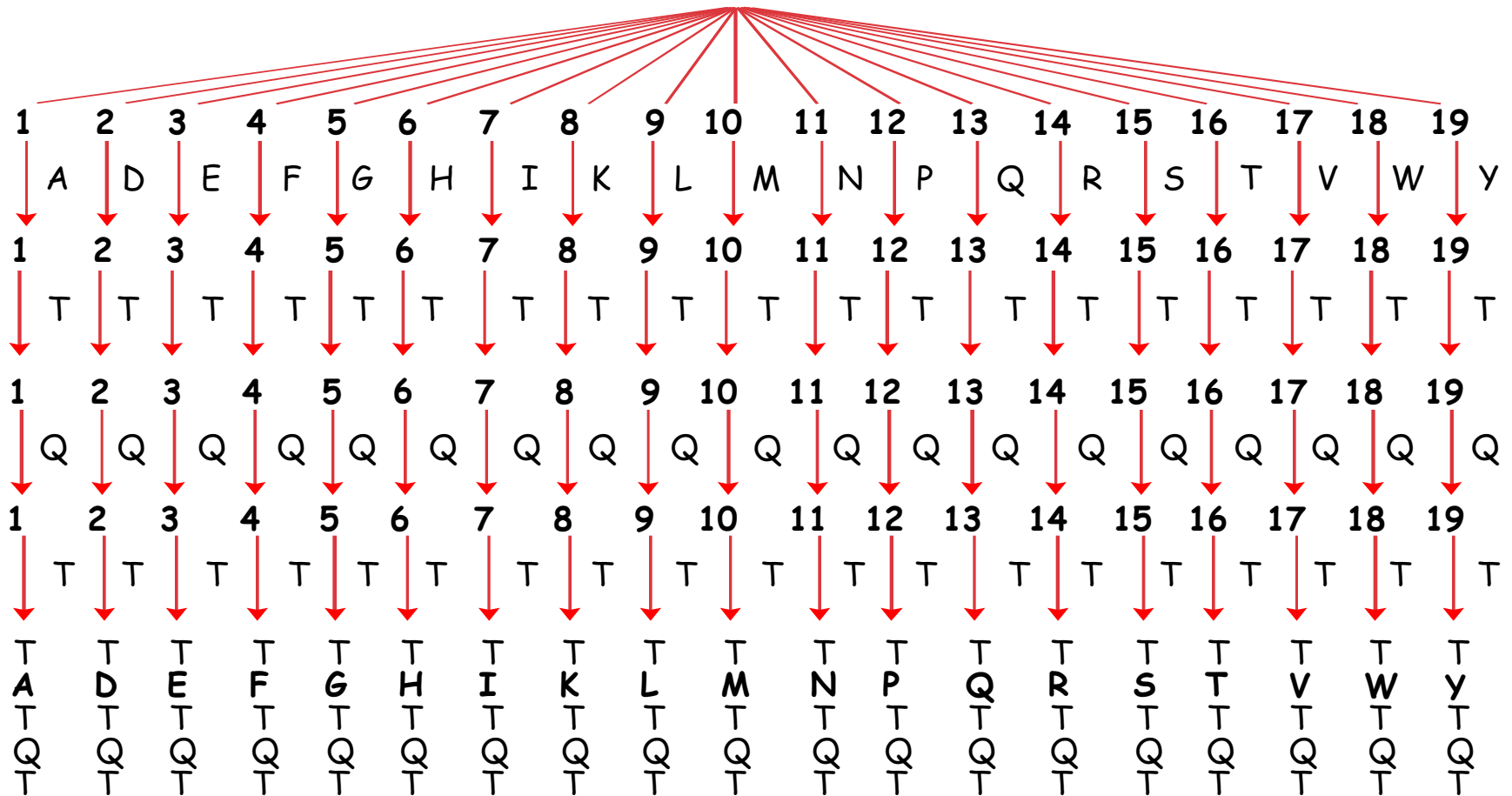


ESI-FTICR spectrum of TQTX²T peptide library

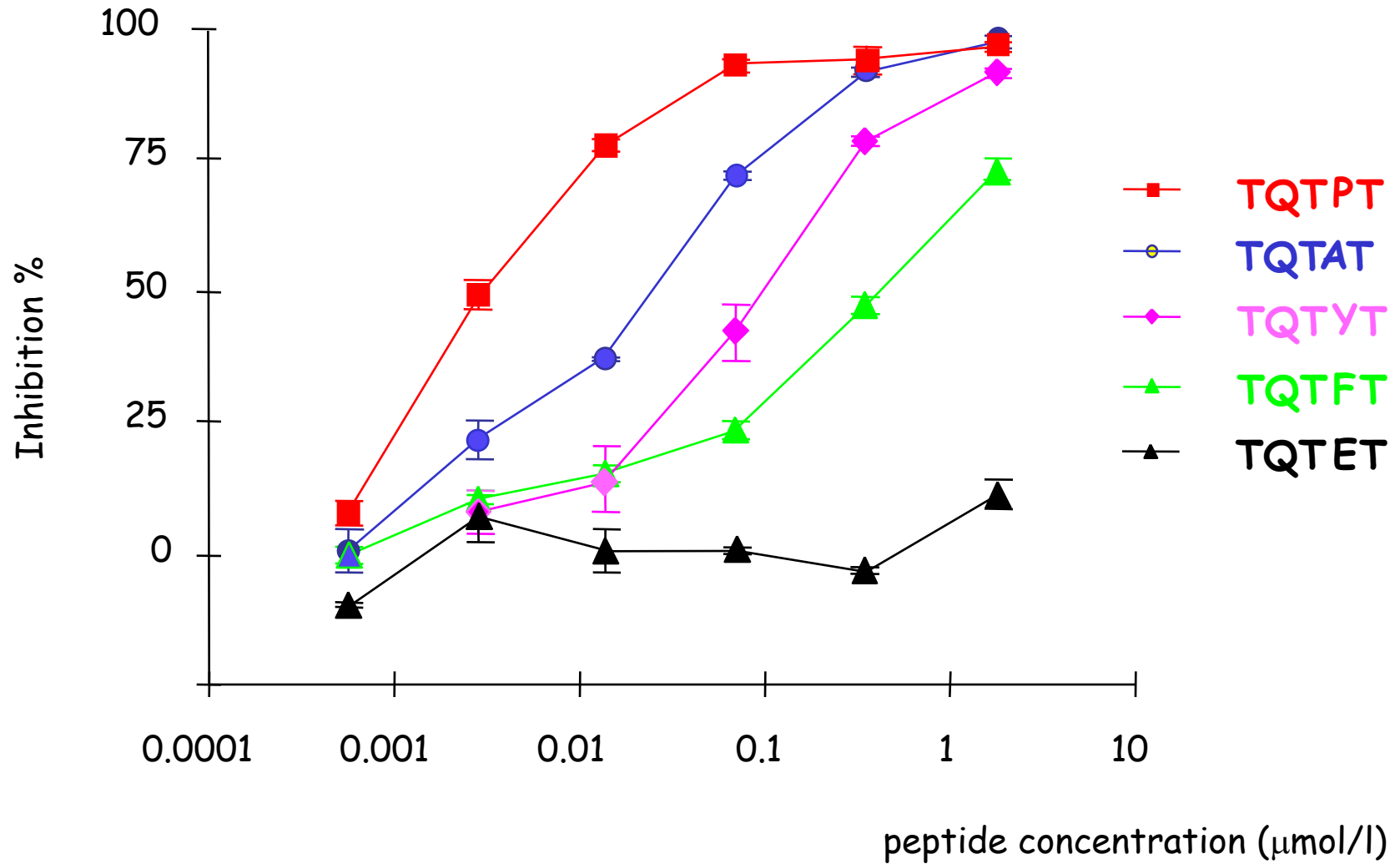


Parallel synthesis of TQTX²T peptide sub-library

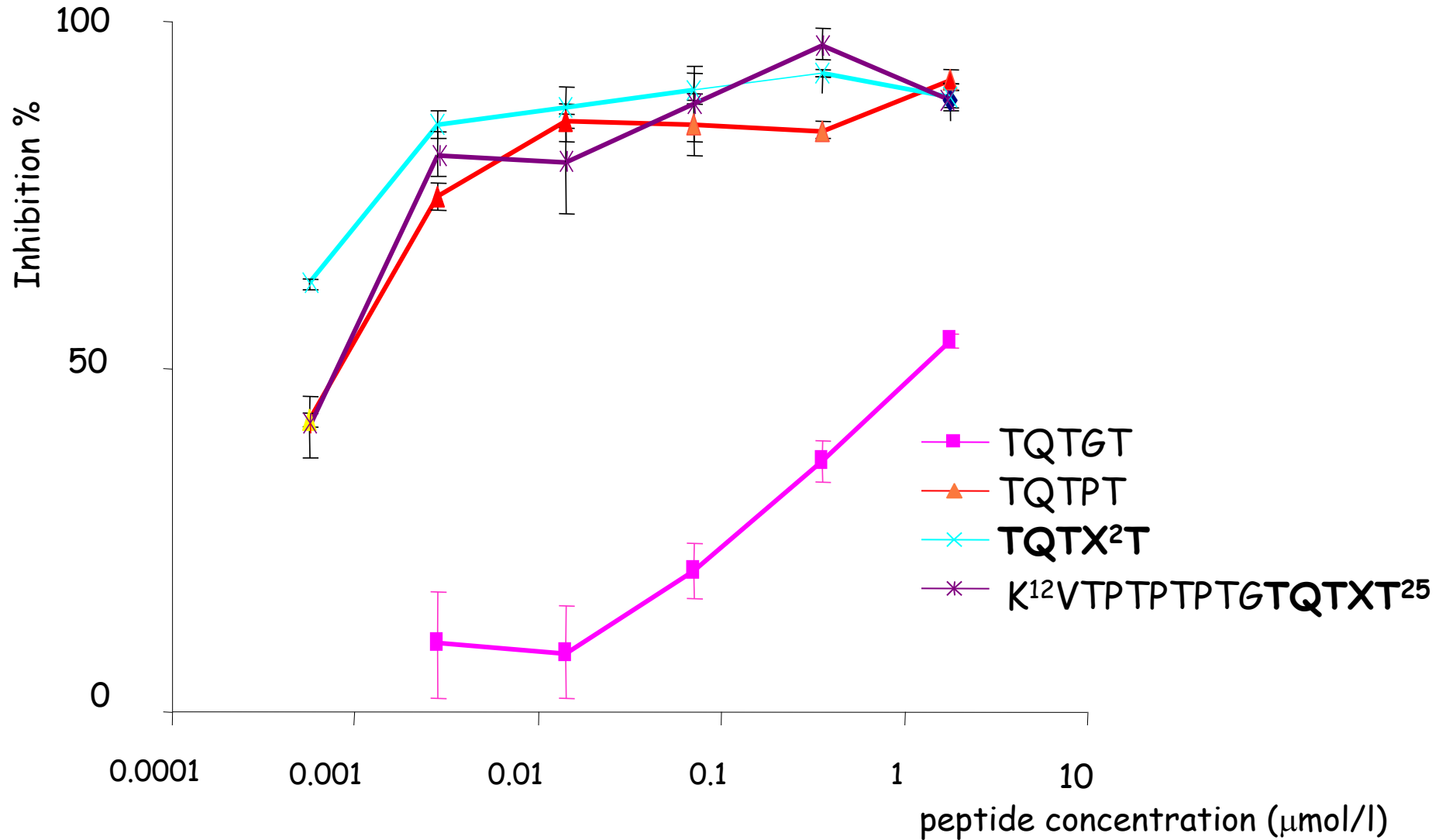
T-**R**



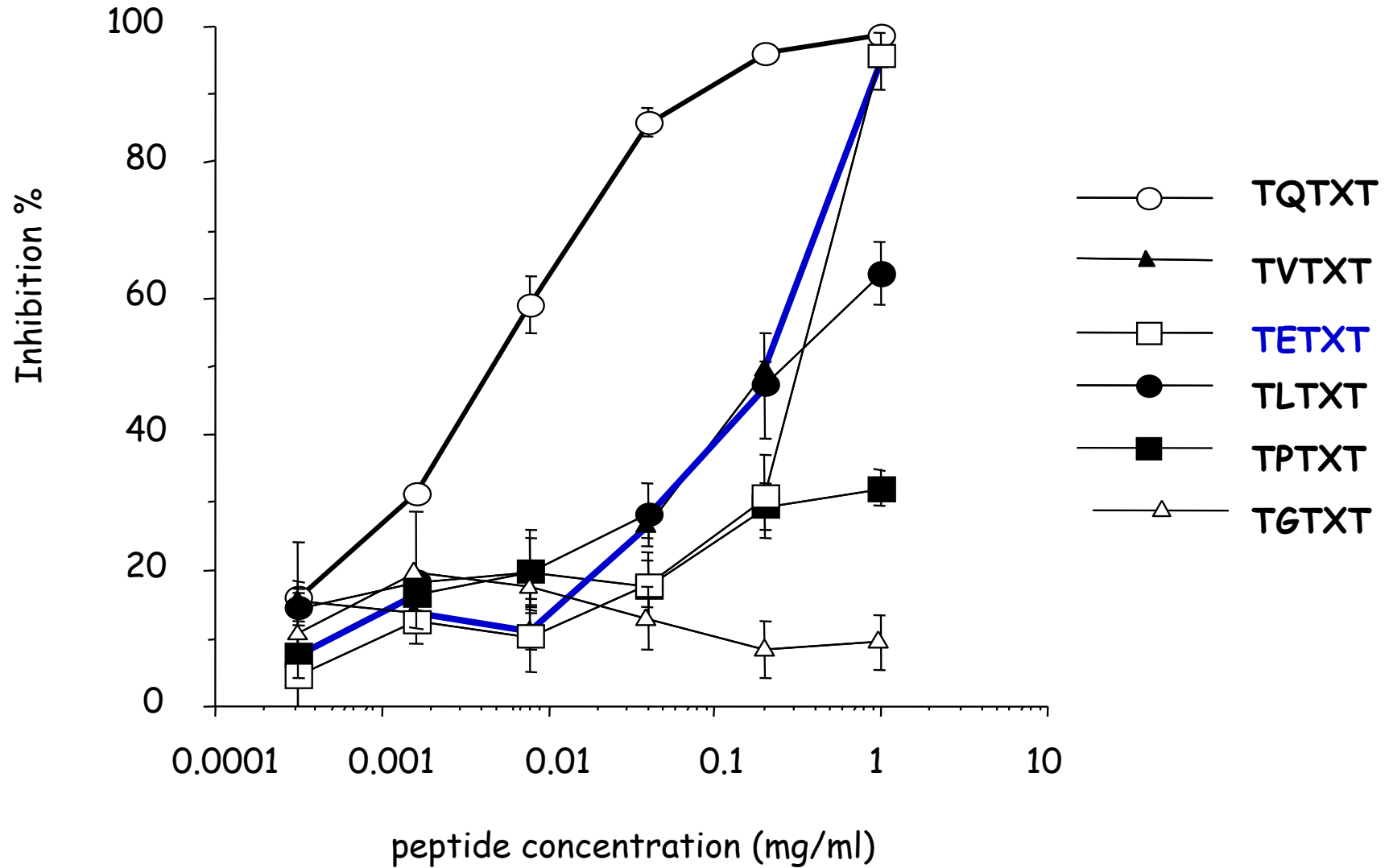
Binding of Mab 994 to TQTX²T peptides



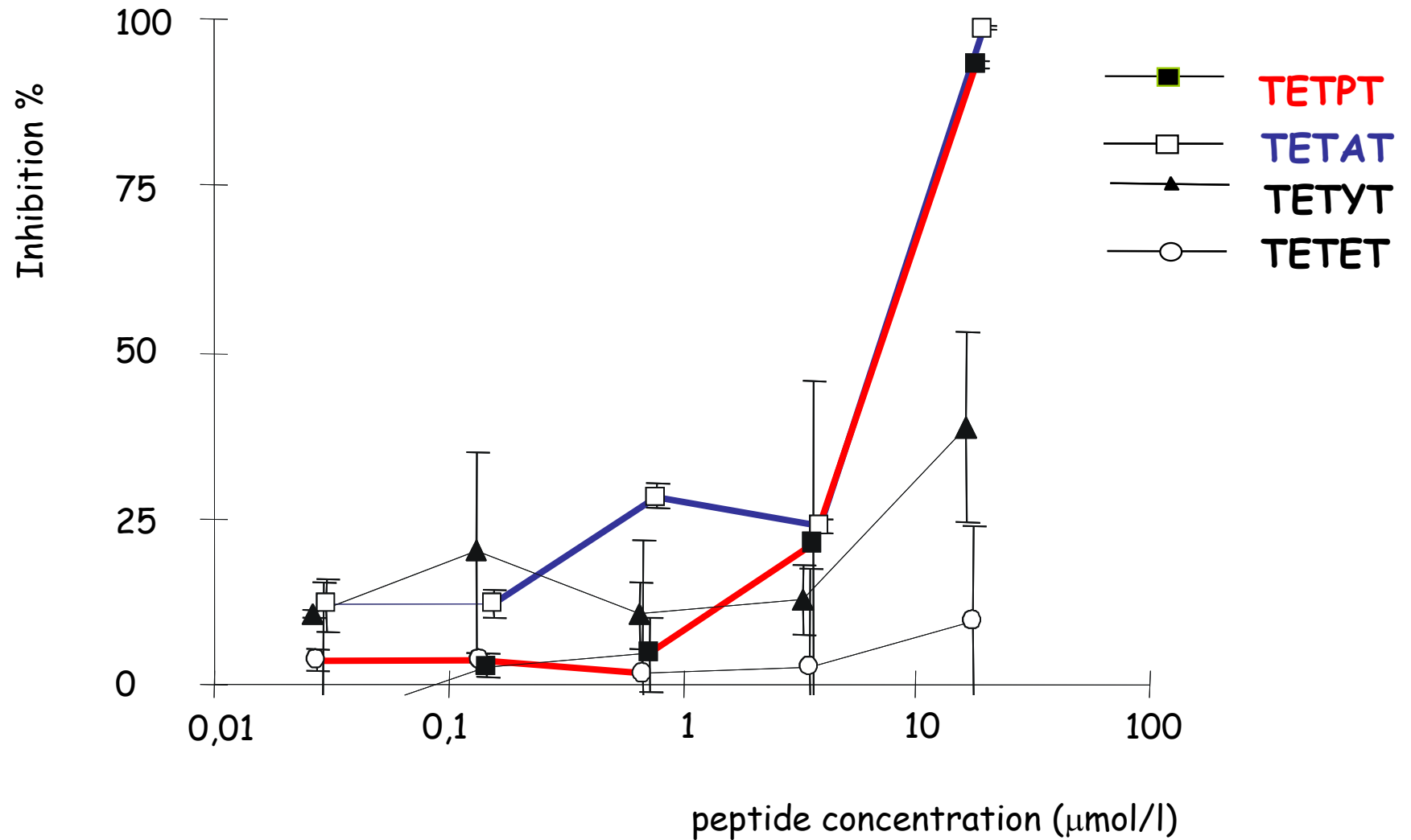
Binding of Mab 994 to $K^{12}VTPPTPTGTQTX^{25}$ peptide mixtures



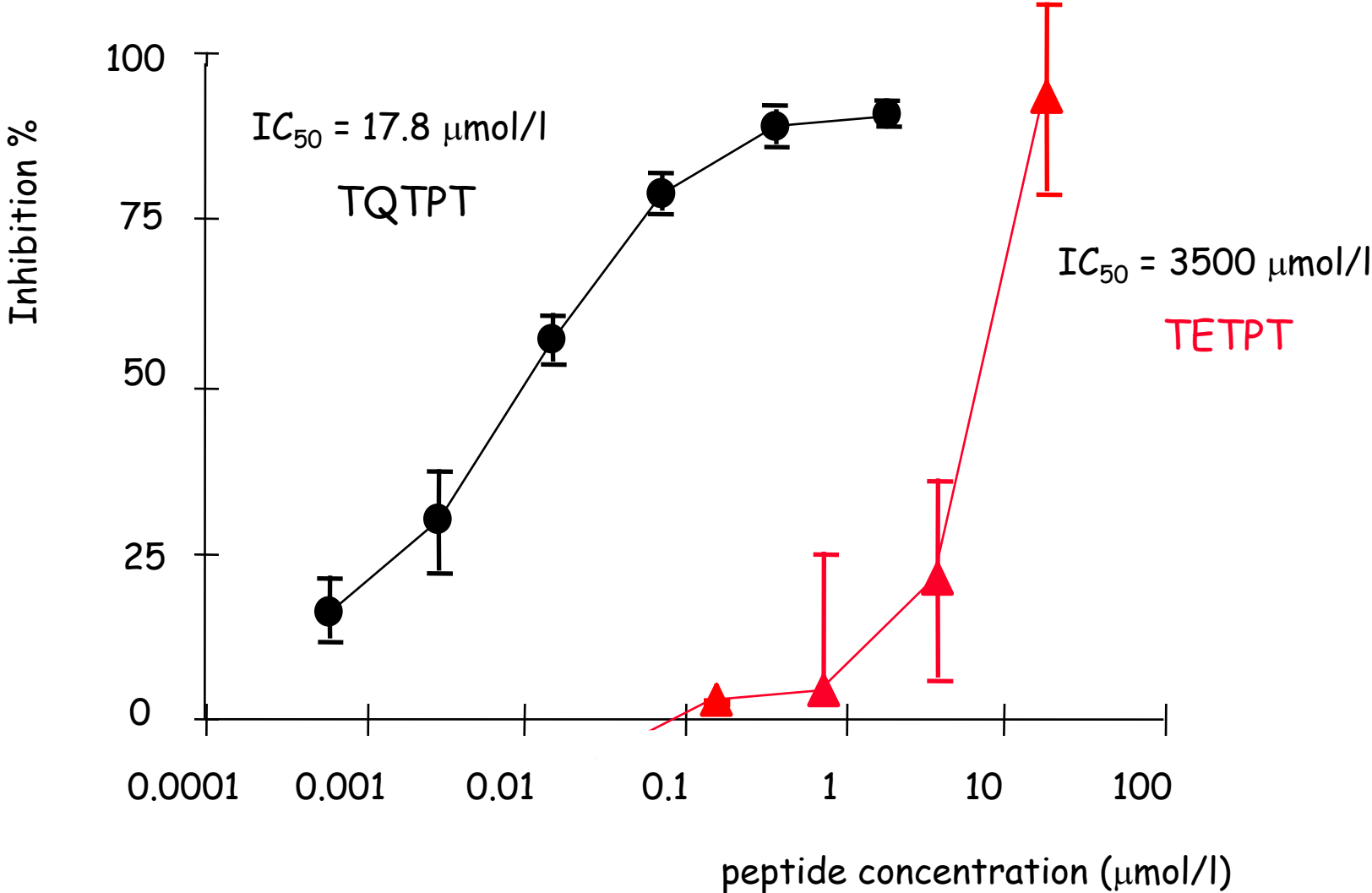
Binding of Mab 994 to TX¹TX²T peptide mixtures



Binding of Mab 994 to TETXT peptides



Comparison of Mab 994 binding to TQTPT and TETXT peptides

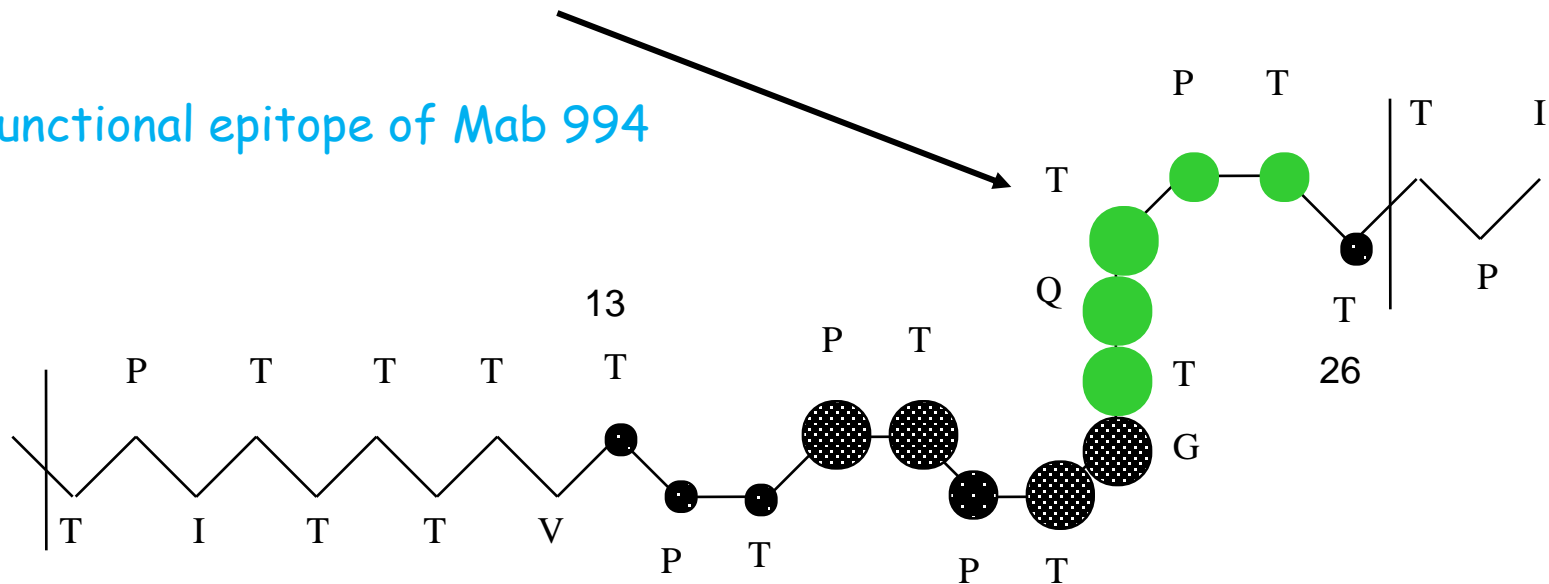


Example 6

Localization of Immunogenic Determinants (Epitopes) of human epithelial mucin glycoprotein, MUC-2
Using synthetic Peptides and MUC-2 specific Antibodies

ELISA: **TQTPT** > TQTAT > TQTYT > TQTWT > TQTFT > TQTST
(IC₅₀: **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)

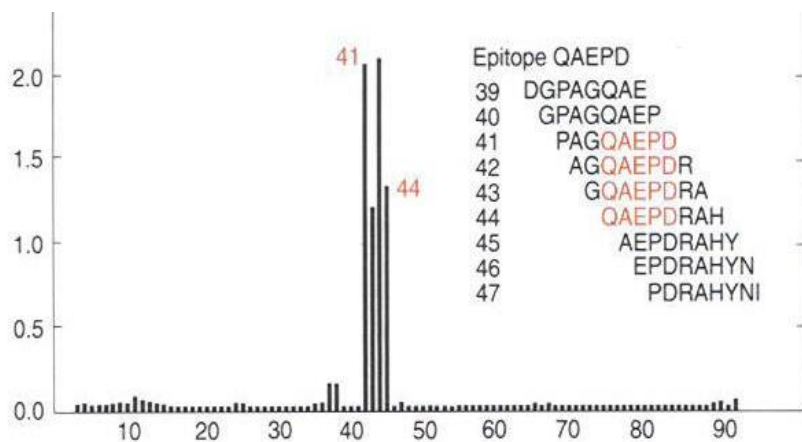
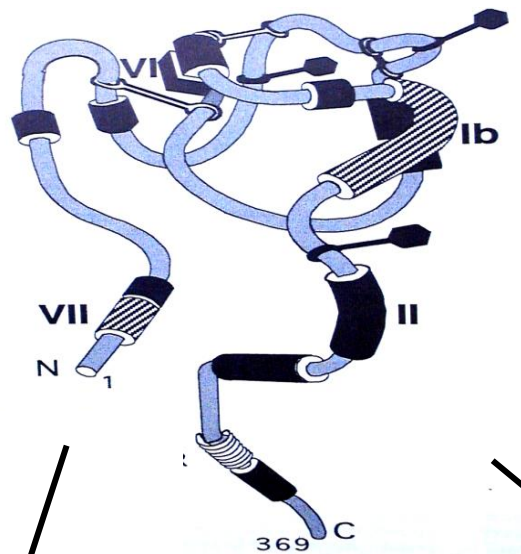
Example 7

Localization of Domain Responsible for Hemolytic Activity of Melittin

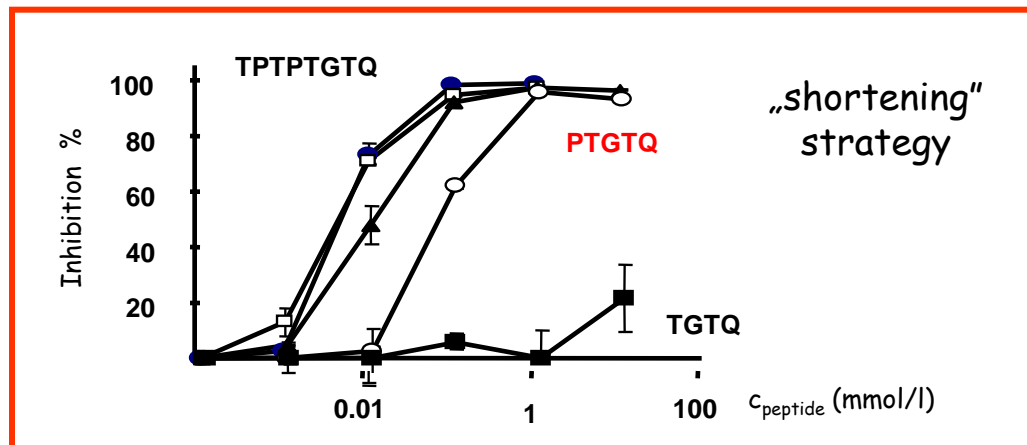
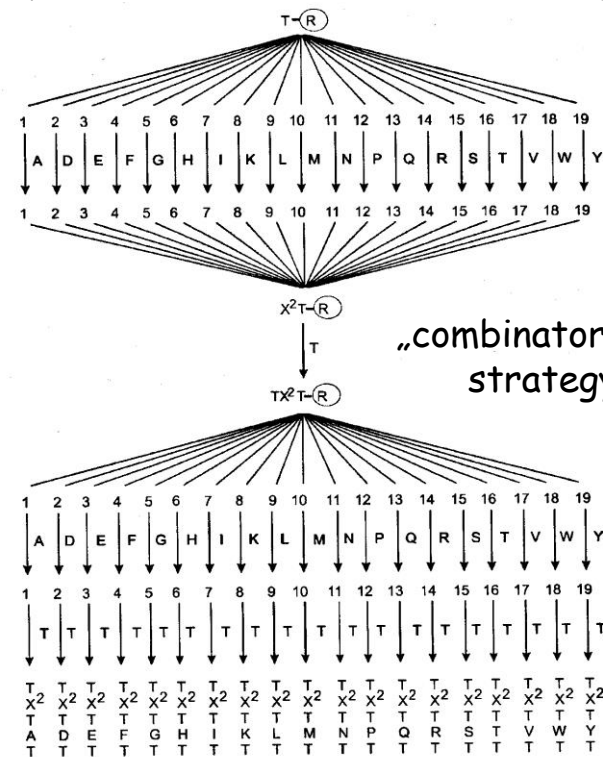
- Melittin:** Amphipathic 26-residue peptide
primary and 3D structure are known
- Target:** Human red blood cells (RBC)
 1.8×10^7 binding sites per erythrocyte
 $K_d = 10^{-7} - 3 \times 10^{-8} \text{ M}$
- Aim:** Identification of the smallest fragment,
which can induce hemolysis at melittin level

Identification of short sequences responsible for activity

„predicted“



„overlapping“ strategy



Example 7

Localization of Domain Responsible for Hemolytic Activity of Melittin

1. step Peptide synthesis

Residue	Amino acid sequence
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ – amide
2 – 26	IGAVLKVLTTGLPALISWIKRKRQQ – amide
3 – 36	GAVLKVLTTGLPALISWIKRKRQQ – amide
4 – 26	AVLKVLTTGLPALISWIKRKRQQ – amide
5 – 26	VLKVLTTGLPALISWIKRKRQQ – amide
6 – 26	LKVLTTGLPALISWIKRKRQQ – amide
1 – 25	GIGAVLKVLTTGLPALISWIKRKRQ – amide
1 – 24	GIGAVLKVLTTGLPALISWIKRKR – amide
1 – 23	GIGAVLKVLTTGLPALISWIKRK – amide
1 – 22	GIGAVLKVLTTGLPALISWIKR – amide
1 – 21	GIGAVLKVLTTGLPALISWIK – amide
1 – 20	GIGAVLKVLTTGLPALISWI – amide

Example 7

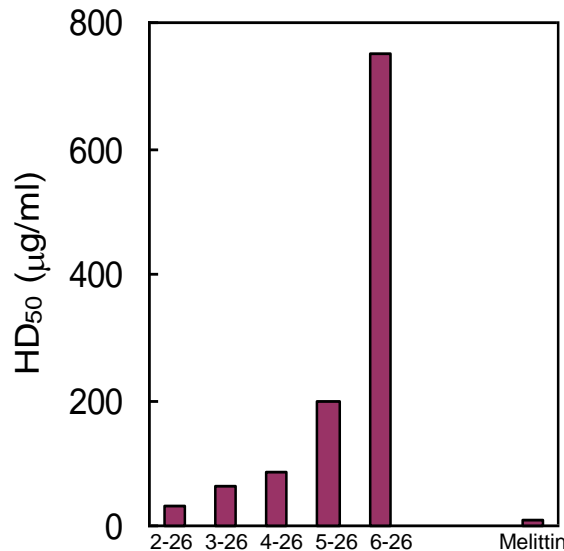
Localization of Domain Responsible for Hemolytic Activity of Melittin

2. step Binding studies with human red blood cells. Hemolytic assay

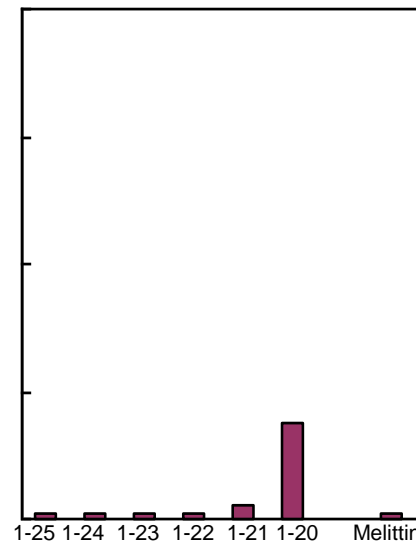
Method:

- Incubation of peptides at equimolar ratio with washed and counted RBC for 1h at 37 °C
- Centrifugation.
- Measurement of absorbance at $\lambda = 414 \text{ nm}$ $e = 14.7 \times 10^{-4}$.

N Truncation



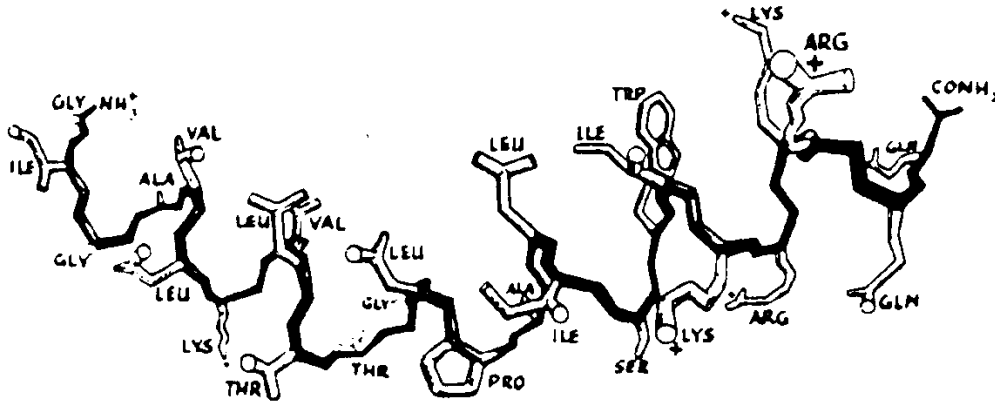
C Truncation



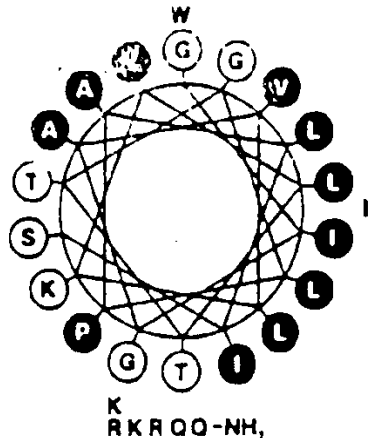
Observation 1:
The critical residues
for hemolytic activity of melittin
are between amino acid 2 and 22

Example 7

Localization of Domain Responsible for Hemolytic Activity of Melittin



The conformation of monomeric subunits of melittin from the crystalline tetramer.



Observation 2:
The removal of amino acid residues from the N-terminal affect the amphipathic alpha-helix.

Helical wheel representations of the conformation