In vivo post-translational modifications

alkyl
acyl (O-,N-,S-)
- N-terminal,
- Lys,
- Ser, Thr

amide
- C-terminal

phosphoric acid ester (Ser, Thr, Tyr)
sulphonic acid ester
glycosylation
- O- in Golgi (Ser, Thr)
- N- in RER (Asn)

nitrosation
desamidation
decarboxylation
Arg desamination,
- citrullination (Arg -> citrullin)

hydroxylation (Pro, Lys)
oxidation
gamma-carboxylation (e.g. Glu)
beta-elimination (e.g. Thr ->alkene)

Cleavage of peptide bond
- N-terminals Met or fMet
- signalpeptide
- precursor activation (proinzuline → inzuline)

Disulphid bond formation

Isomerisation (Pro)

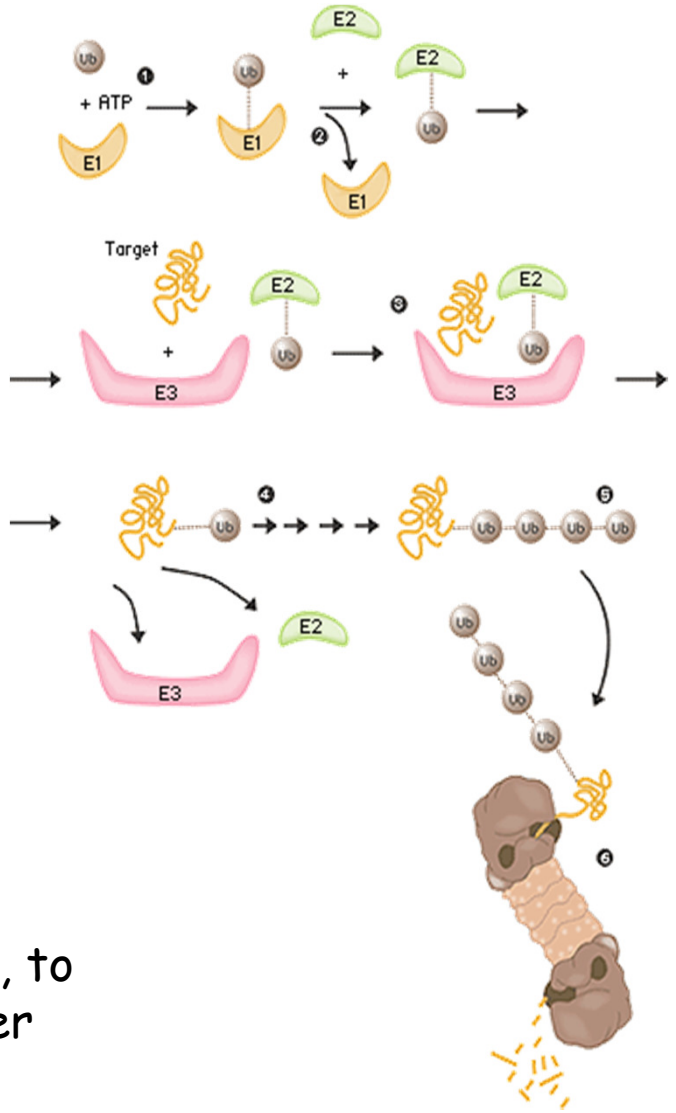
Coupling of nucleotide (e.g. flavine)

Coupling of protein/peptide :
- sumoylation (SUMO protein)
- ubiquitination (ubiquitin)
- neddylation (Nedd)

Ubiquitination

KUNGL. VETENSKAPSAKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

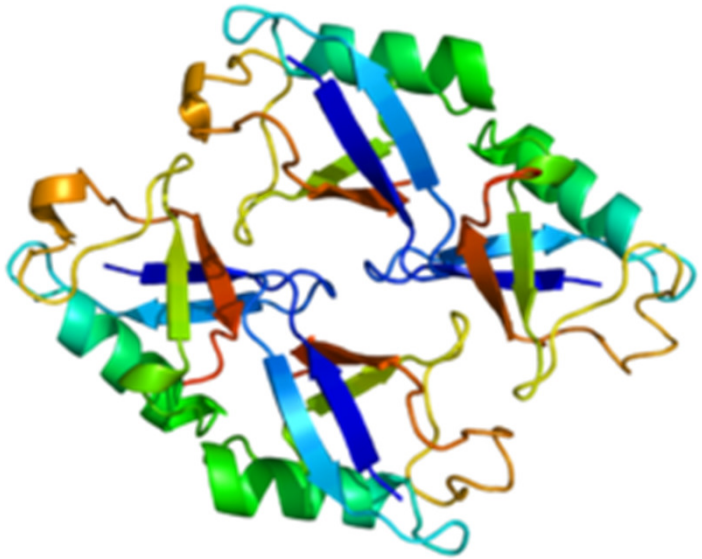
Nobel Prize in Chemistry, 6 October 2004
A. Ciechanover, A. Hershko, I. Rose

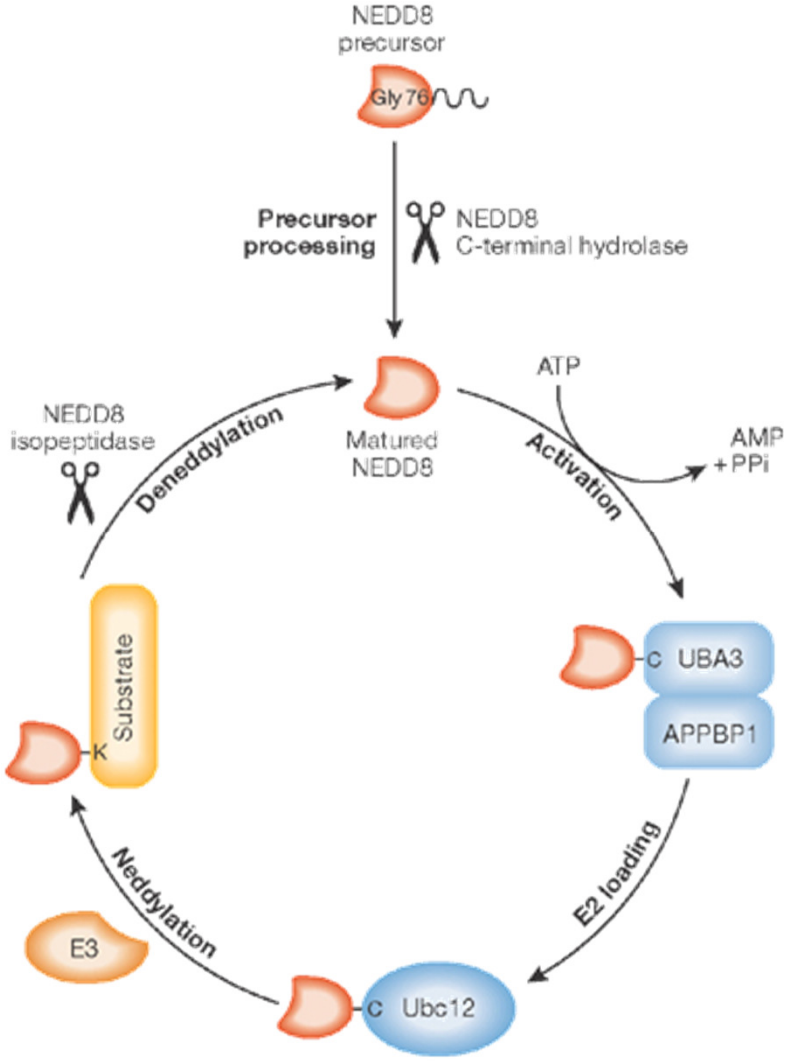


- Ubiquitin: protein (76 amino acids, 8.5 kDa)
- Almost all tissues of eukaryotic organisms
- It can signal the degradation of the attached protein by transporting to proteasome.
- Isopeptide linkage (4)
- Enzymes involved:
  - E1 (ub activation)
  - E2 (ub conjugation to ε-amino group of Lys, to thiol of Cys by thioester, to OH of Thr/Ser by ester)
  - E3 (ub ligation)

NEDDylation
(Neural-precursor-cell-expressed developmentally down-regulated 8)

- Function:
  activation/regulation of ubiquitin

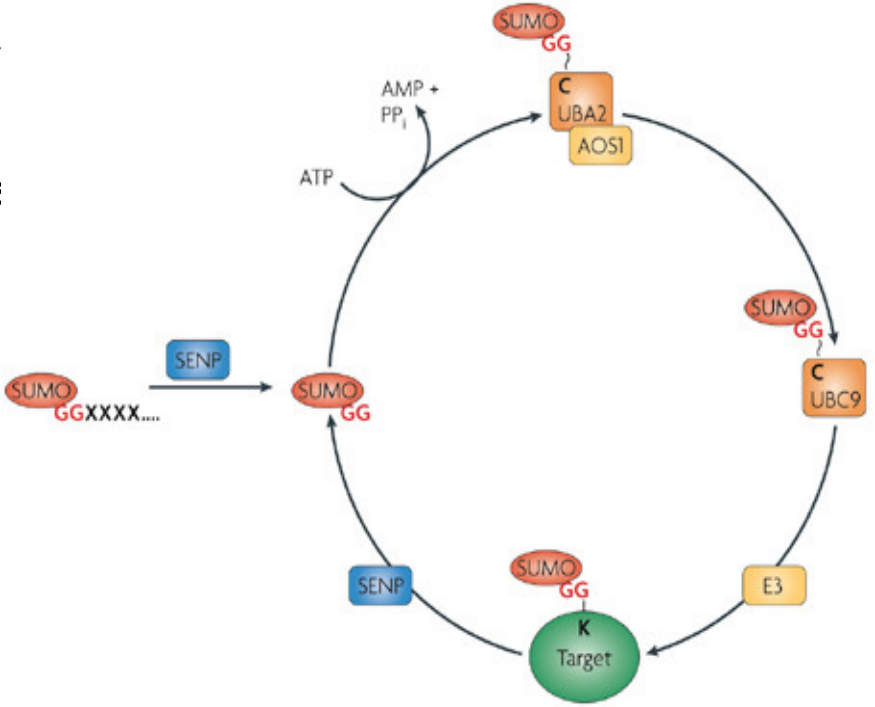




SUMOylation
(Small Ubiquitin-like Modifier)

Involved in nuclear-cytosolic transport,
transcriptional regulation,
apoptosis
protein stability,
but, not in degradation

- SUMO proteins: 100 aa. , 12 kDa, 4 isoforms
- Post-translational modification
- Aktivation: cleavage of 4 residues at the C-terminal
- Attachment to target protein by using three enzymes .



Nature Reviews | Molecular Cell Biology

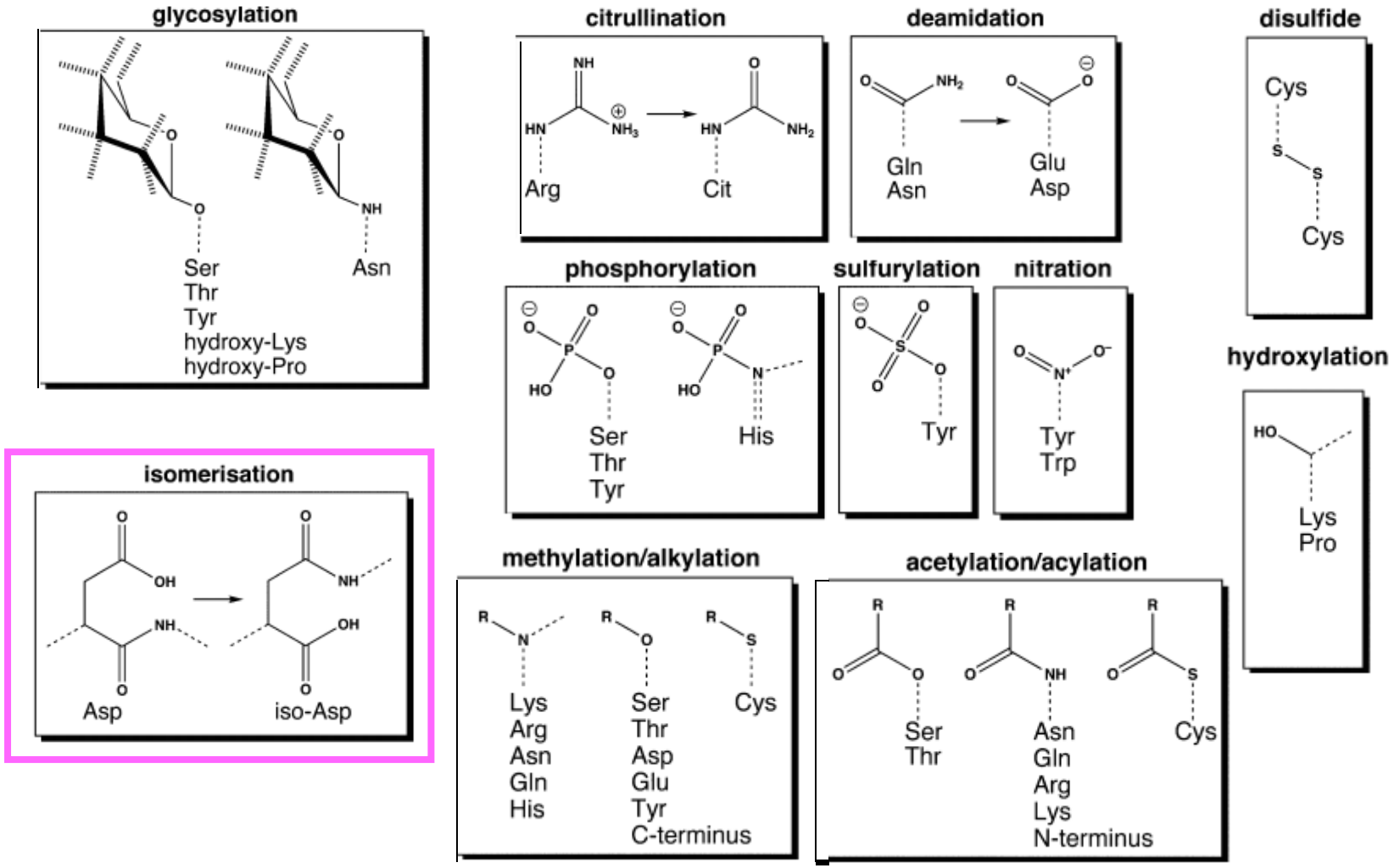
R. Geiss-Friedlander & F. Melchior
Nature Rev. Mol. Cell Biol. 8, 947-956 (2007)

Post-translational modification of proteins in the context of immune recognition and related diseases

Modification	Autoantigen	Disease
Acetylation	Myelin basic protein	Multiple sclerosis
Citrullination	Collagen type II Myelin basic protein	RA Multiple sclerosis
Deamidation	Insulin	Type I diabetes
Glycosylation	Insulin proceptor Collagen type II Thyrotropin receptor Myelin oligodendrocyte glycoprotein Mucin glycoprotein (MUC2)	Diabetes RA Graves disease MS Colon carcinoma
Isoaspartylation	snRNP	Systemic lupus erythematosus
Lipoylation	PDC-E2	Primary biliary cirrhosus
Phosphorylation	Myelin basic protein	Multiple sclerosis
Methylation	Sm, D1,D3	Systemic lupus erythematosus
Transglutamination	Histone H2	Systemic lupus erythematosus
Tyrosine nitration	Mitochondrial proteins	Experimental autoimmune uveitis

Posttranslational modification of Auto-antigens p.41
in "Autoantibodies" Eds. Y. Shoenfeld, M. E. Gershwin, P.- L. Meroni, pp. 838, (2007) Elsevier

Post-translational modification: influence on immune recognition



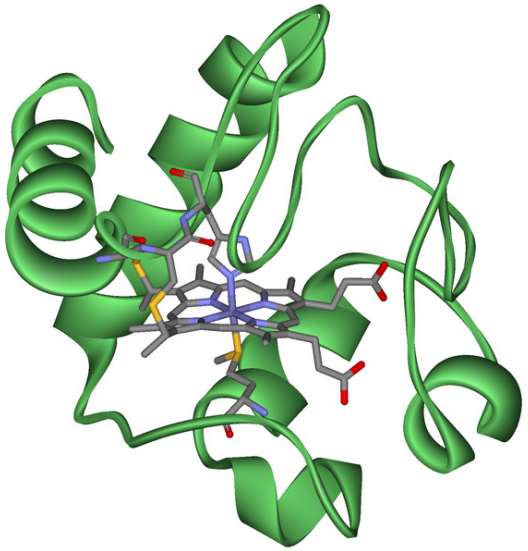
Petersen, J., et al. J. Mol.Med. 87: 1045-1051 (2009)

The effect of post-translational modification on immune recognition: Isomerisation of Asp to β-Asp

T cell response

peptide 94-104 from murine cytochrome c

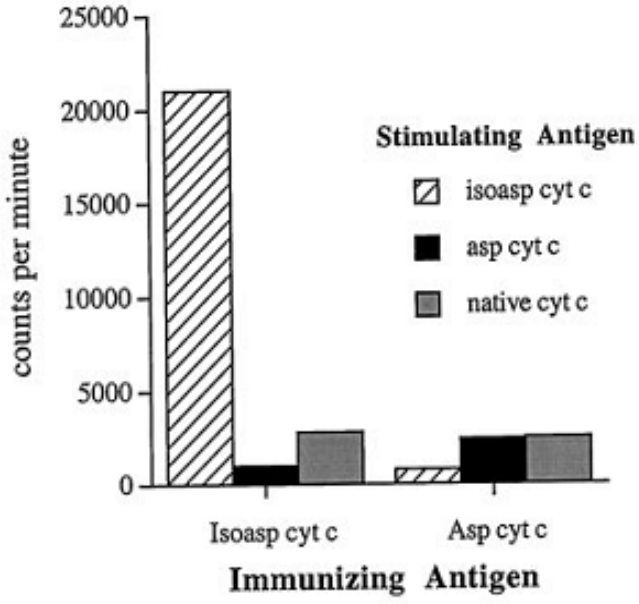
^{94}ERADLIAYLKKATNE^{104} → ^{94}ERAisoDLIAYLKKATNE^{104}



structure of horse heart cytochrome c (PDB:1HRC)

Assay:

a) B10.A mice immunized with 100 μg peptide 94-104 with CFA
b) after 10 days cell suspension from lymph nodes
c) antigen stimulation with peptide a, b or full protein
d) [^{3}H]thymidine incorporation assay

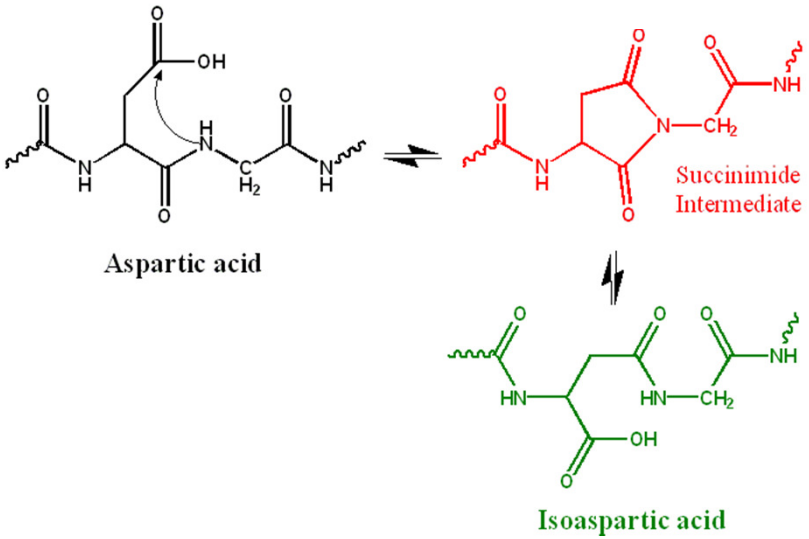


T cells respond to immunization with isoAsp self-peptide and fail to respond Asp self-peptide

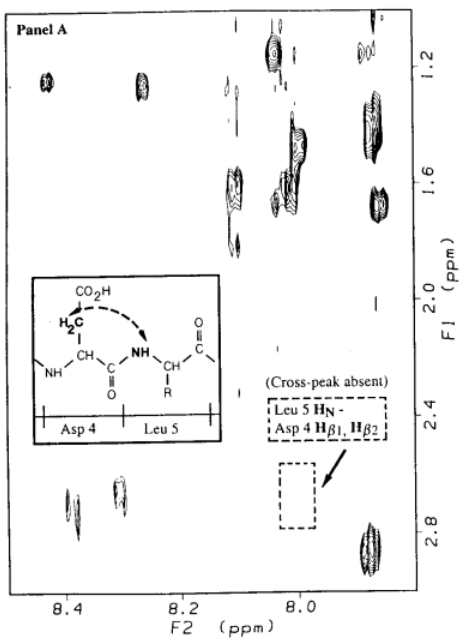
Mamula, M.J. et al. J. Biol.Chem. 274: 22321-22327 (1999)

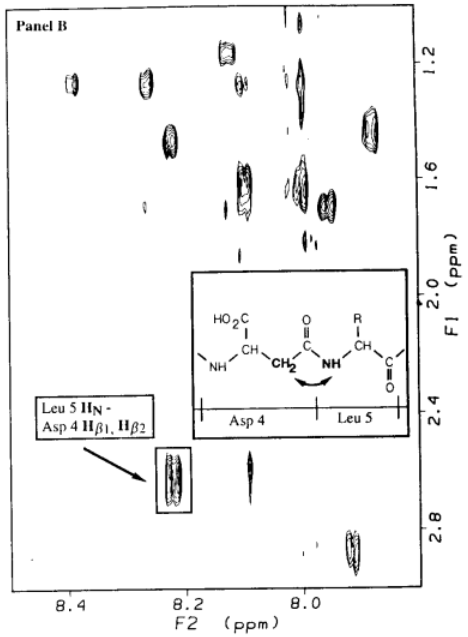
Post-translational modification: influence on immune recognition

peptide 94-104 from murine cytochrome c



NMR analysis
of immunogenic/non-immunogenic self-peptide





^{94}ERADLIAYLKKATNE^{104}

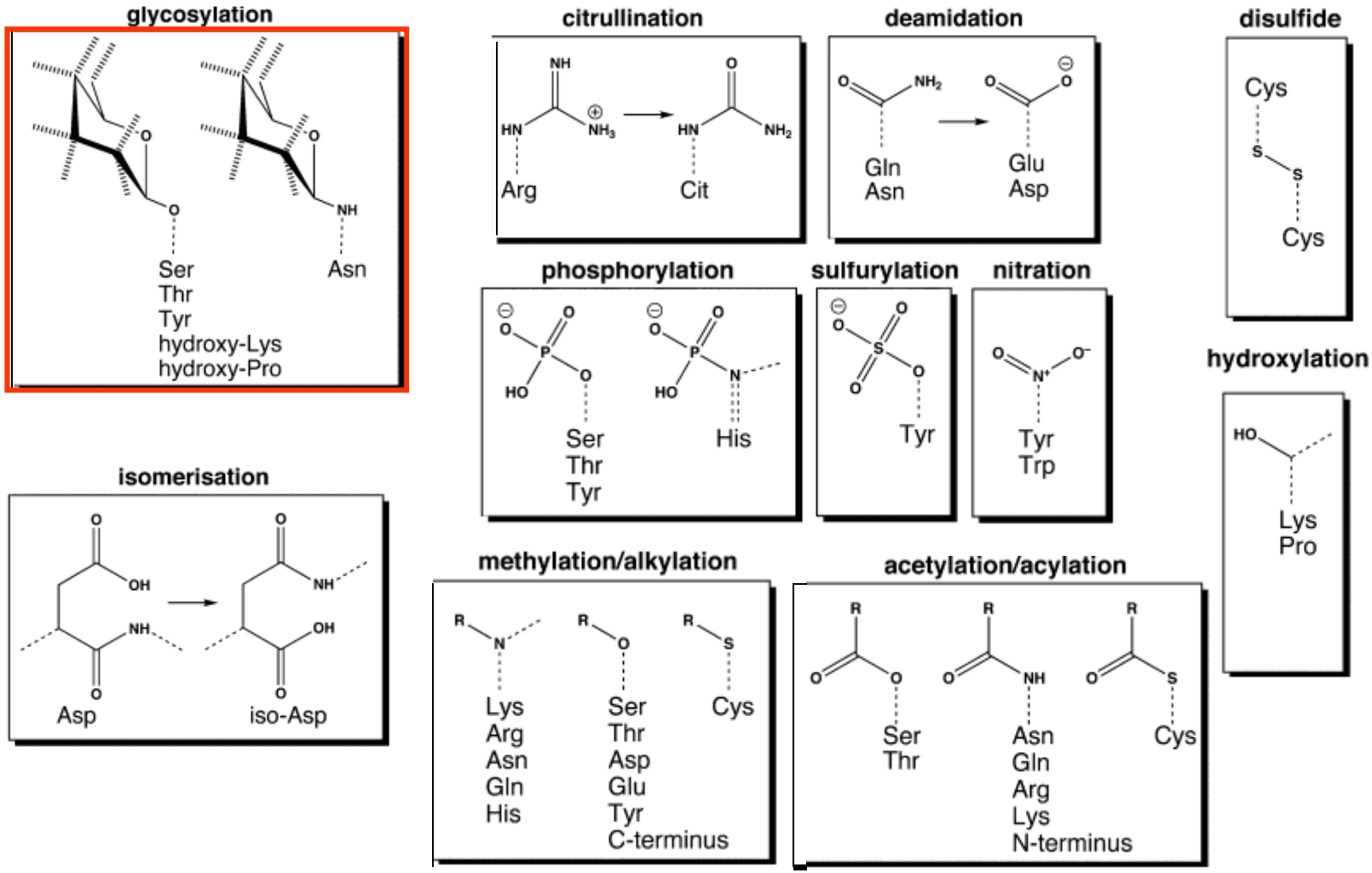
^{94}ERAisoDLIAYLKKATNE^{104}

Mamula, M.J. et al. J. Biol.Chem. 274: 22321-22327 (1999)

Example 7

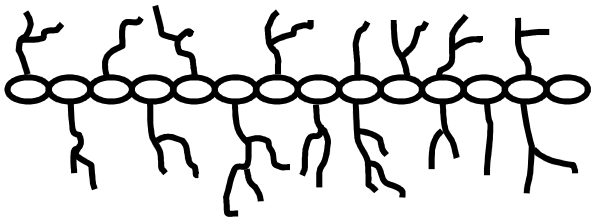
Localization of glycosylation site on a B-cell epitope of mucin-2 glycoprotein. Effect on antibody recognition

Glycosylation

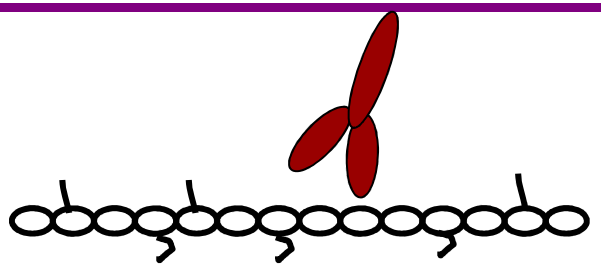


Petersen, J., et al. J. Mol.Med. 87: 1045-1051 (2009)

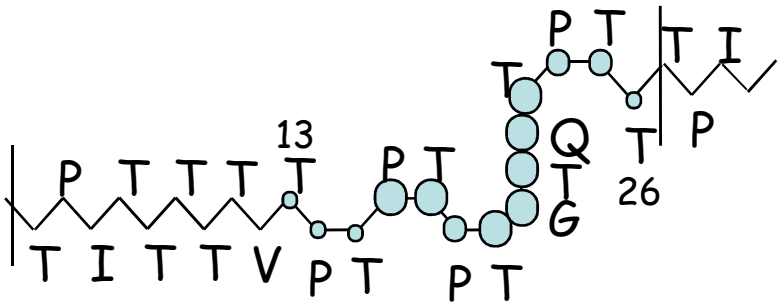
Identification of antibody epitope of mucin-2

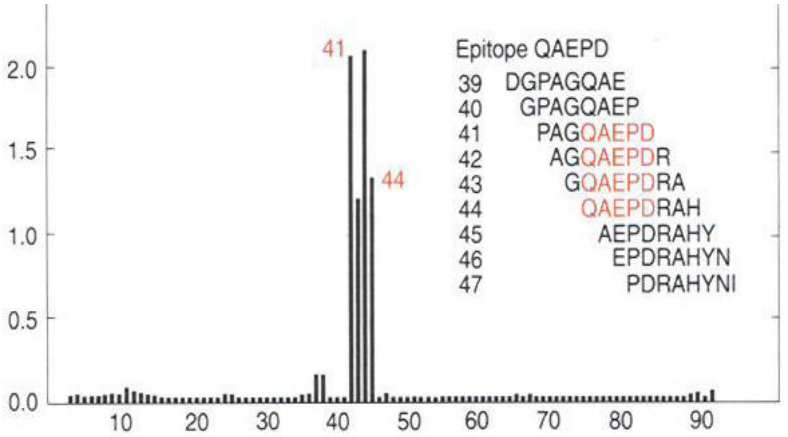


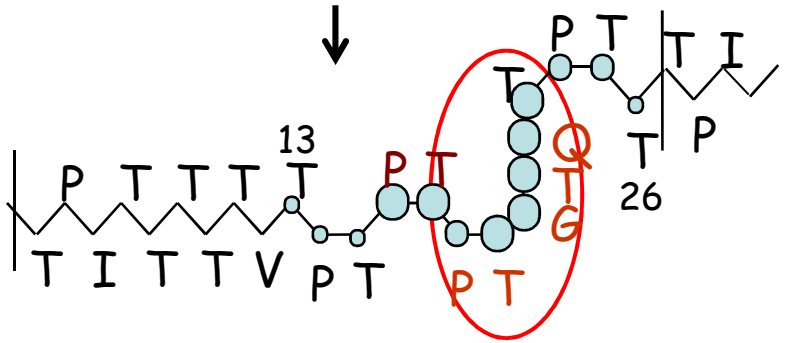
Healthy tissue

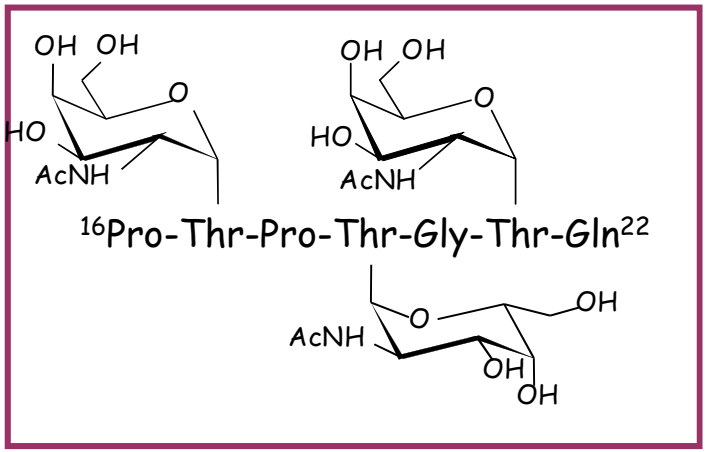


Tumor tissue

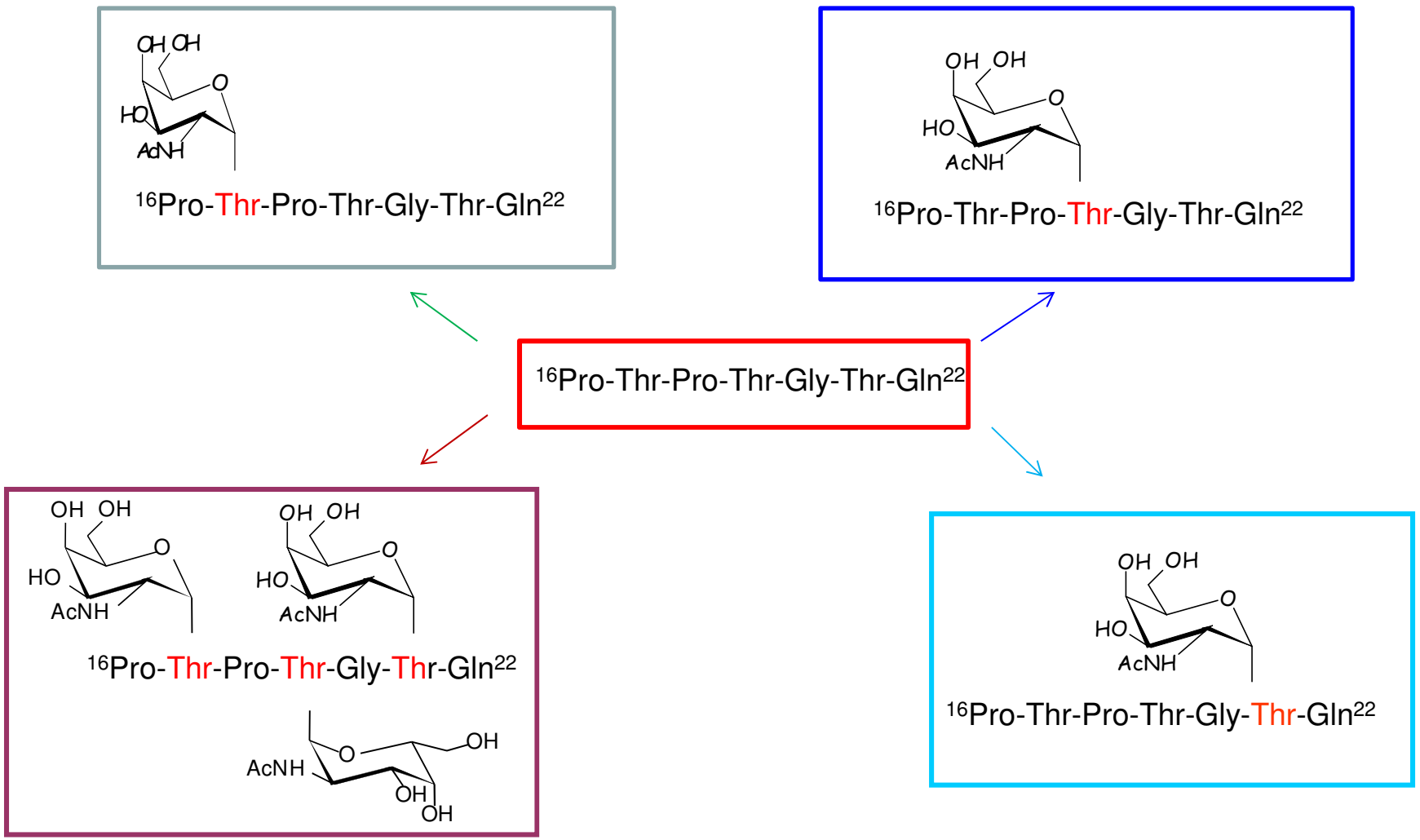






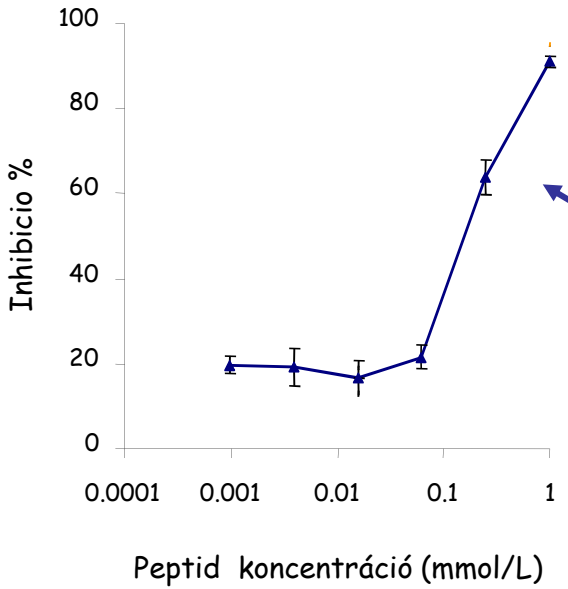


The effect of carbohydrate moiety on MoAb binding



Uray, K., Mizuno, M. et al. Biopolymers 102: 390-395 (2014)

The effect of carbohydrate moiety on MoAb binding

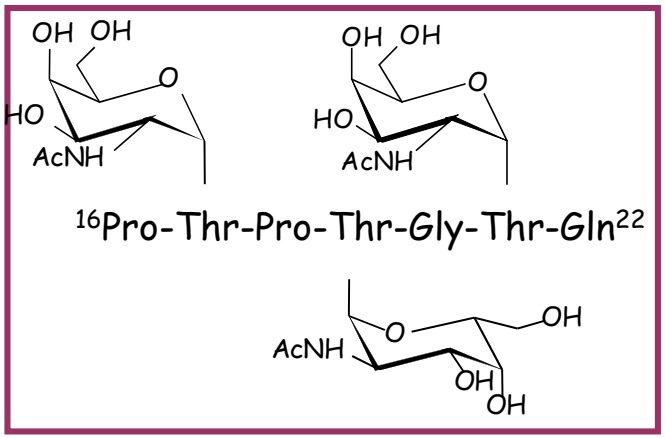


^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

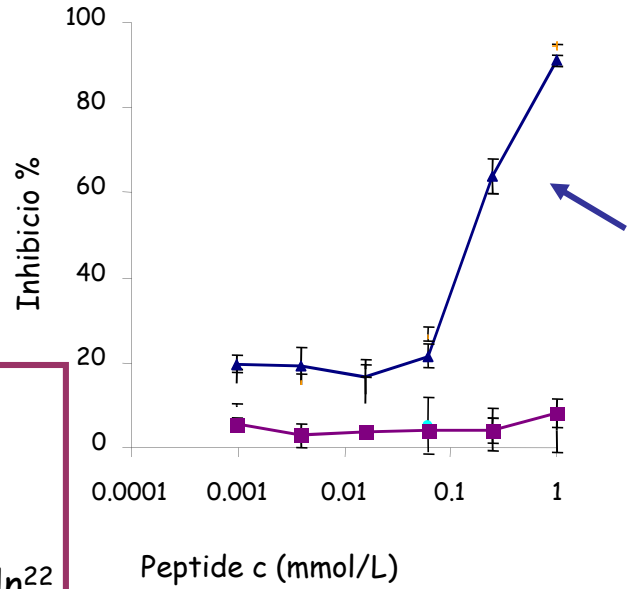
IC_{50} (μM) = 39

MAb 996: Anti-BSA-[K^{12}VTPTPTPTGTQTPT^{25}]

The effect of carbohydrate moiety on MoAb binding



IC_{50} (μM) = > 1000



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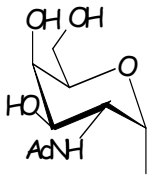
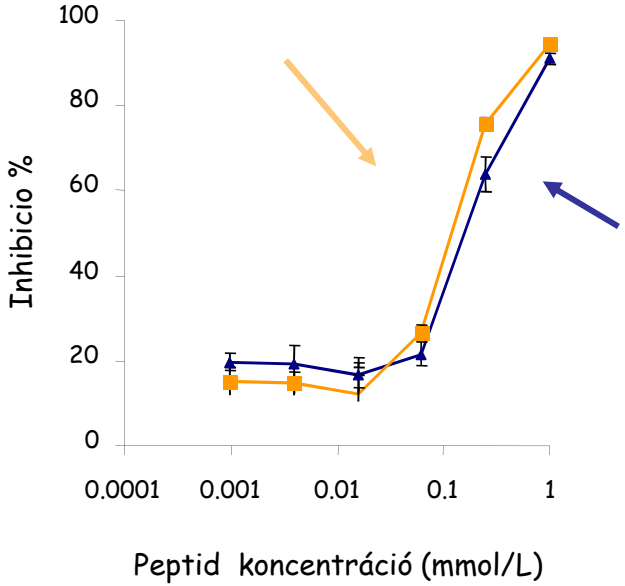


^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = 25



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IC_{50} (μM) = 39

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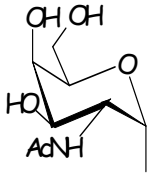
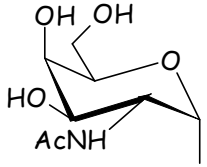


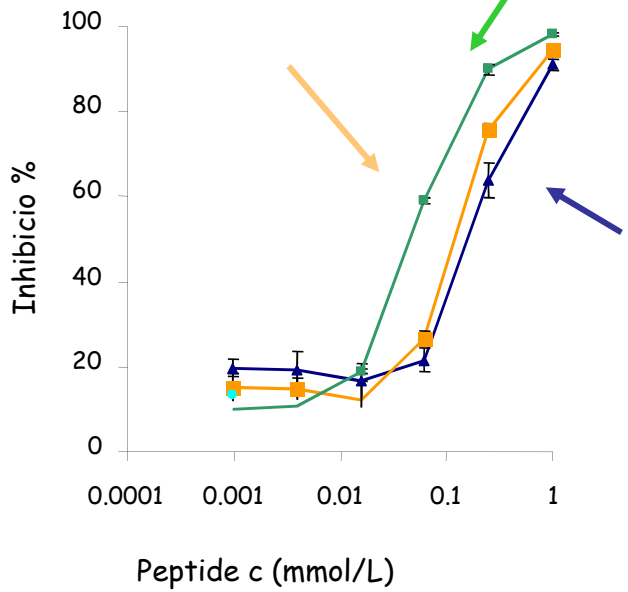
^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = 25



IC_{50} (μM) = 6.4

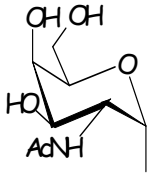
^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}



^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = 39

The effect of carbohydrate moiety on MoAb binding



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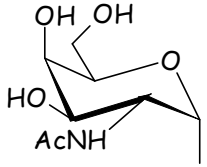
IC_{50} (μM) = 25



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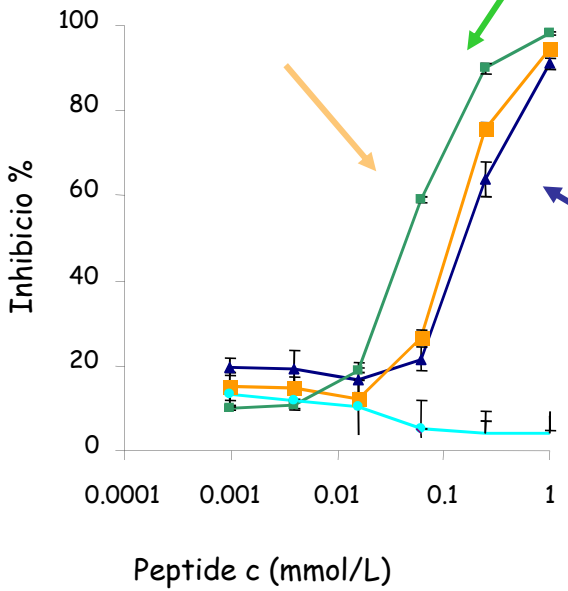
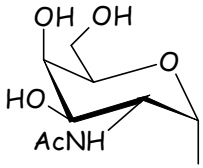


^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

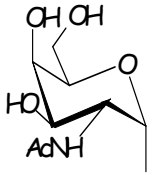
IC_{50} (μM) = 39



^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

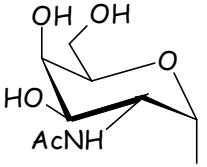
IC_{50} (μM) = > 1000

The effect of carbohydrate moiety on MoAb binding



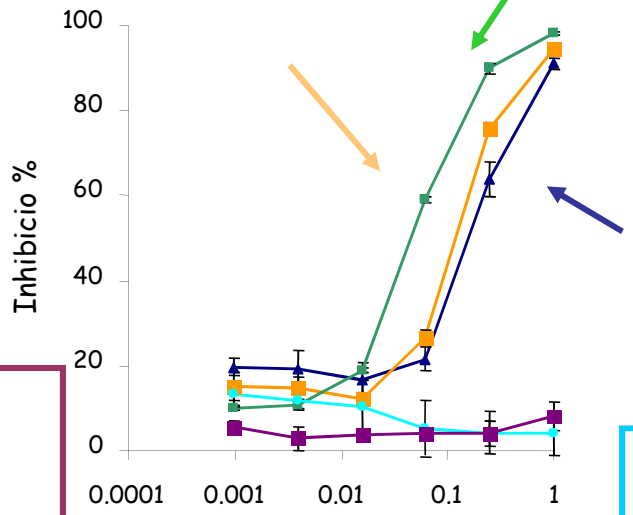
^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = 25



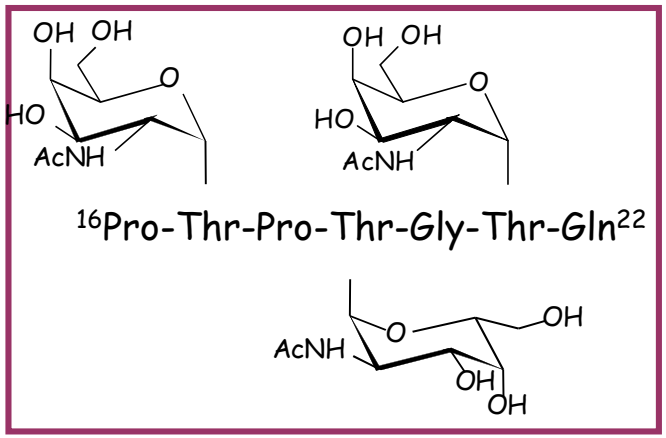
IC_{50} (μM) = 6.4

^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}



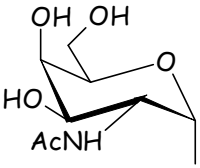
^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = 39



^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

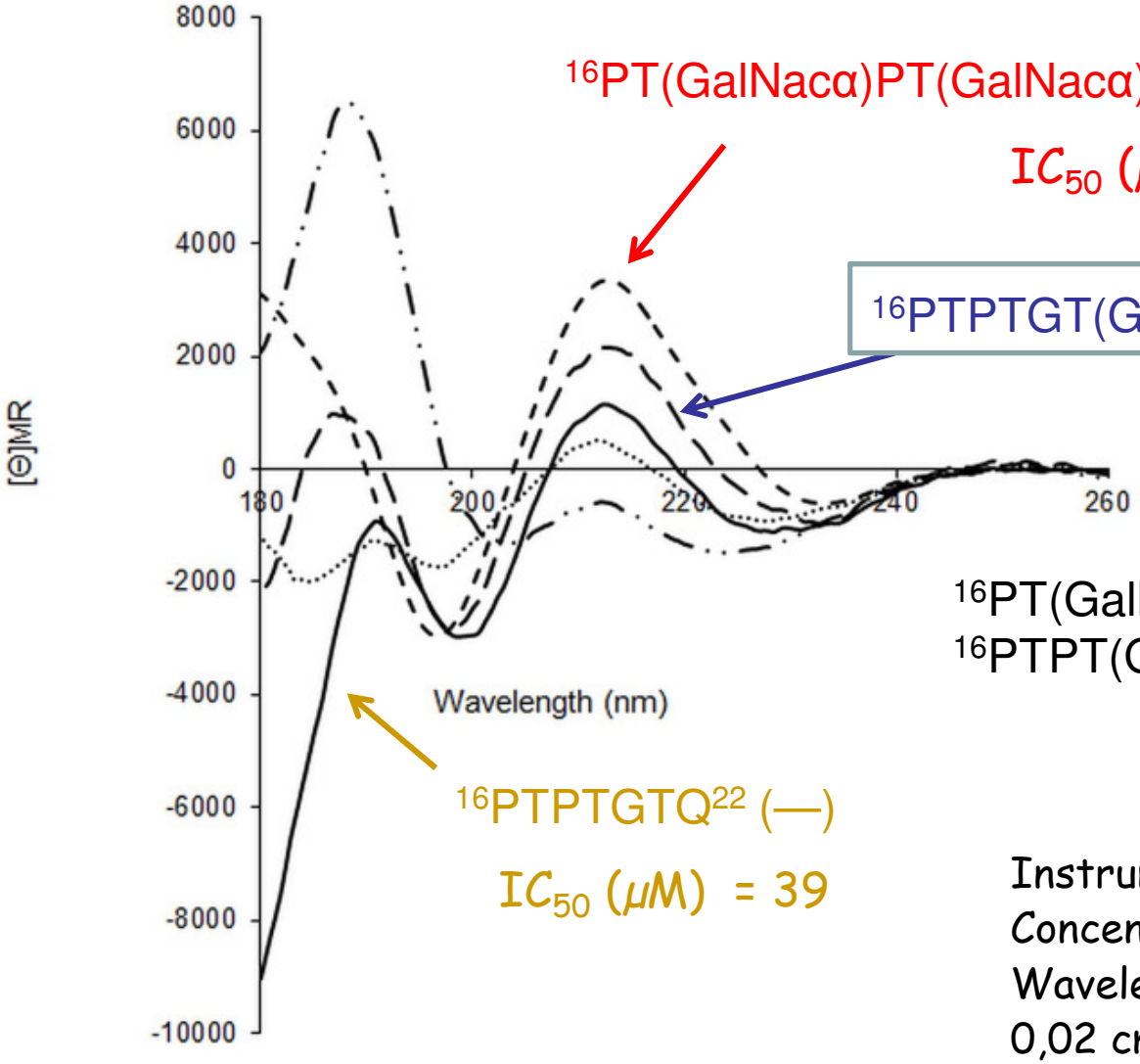
IC_{50} (μM) = > 1000



^{16}Pro-Thr-Pro-Thr-Gly-Thr-Gln^{22}

IC_{50} (μM) = > 1000

The CD spectra of peptides in TFE and MoAb binding



^{16}PT(GalNacα)PT(GalNacα)GT(GalNacα)Q^{22} (- -).

IC_{50} (μM) > 1000

^{16}PTPTGT(GalNacα)Q^{22} (– –)

IC_{50} (μM) > 1000

^{16}PT(GalNacα)PTGTQ^{22} (….)

^{16}PTPT(GalNacα)GTQ^{22} (– . .)

^{16}PTPTGTQ^{22} (—)

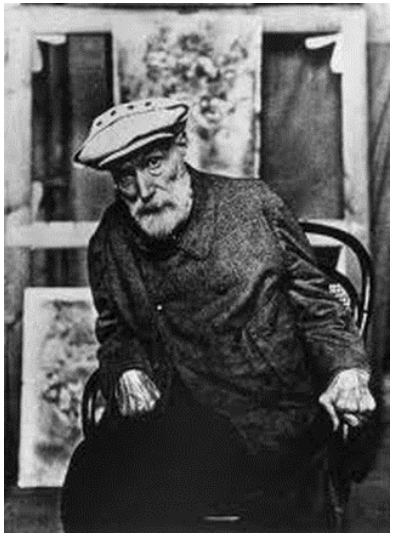
IC_{50} (μM) = 39

Instrument: Jasco-810
Concentration: 0,5 mg/ml
Wavelength: λ=180-300 nm
0,02 cm quartz cuvette

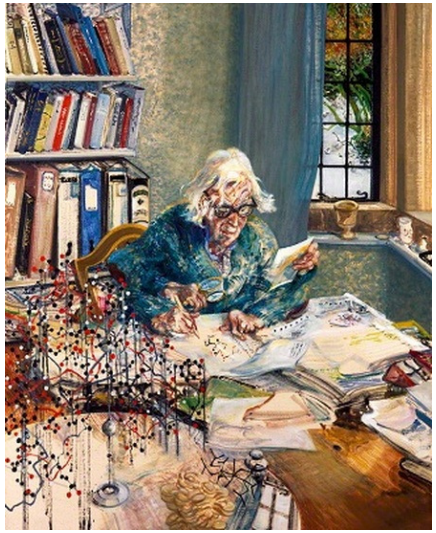
Example 8

Localization of citrullination site(s) on a B-cell epitope of filaggrin.
Effect on antibody recognition

Rheumatoid arthritis

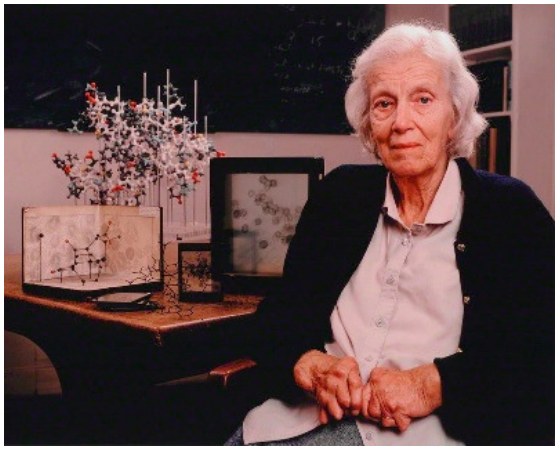


Pierre Auguste Renoir (1841 - 1919)



Dorothy C. Hodgkin (1910-1994)
Nobel dij (1964)

Raoul Dufy (1877-1953)



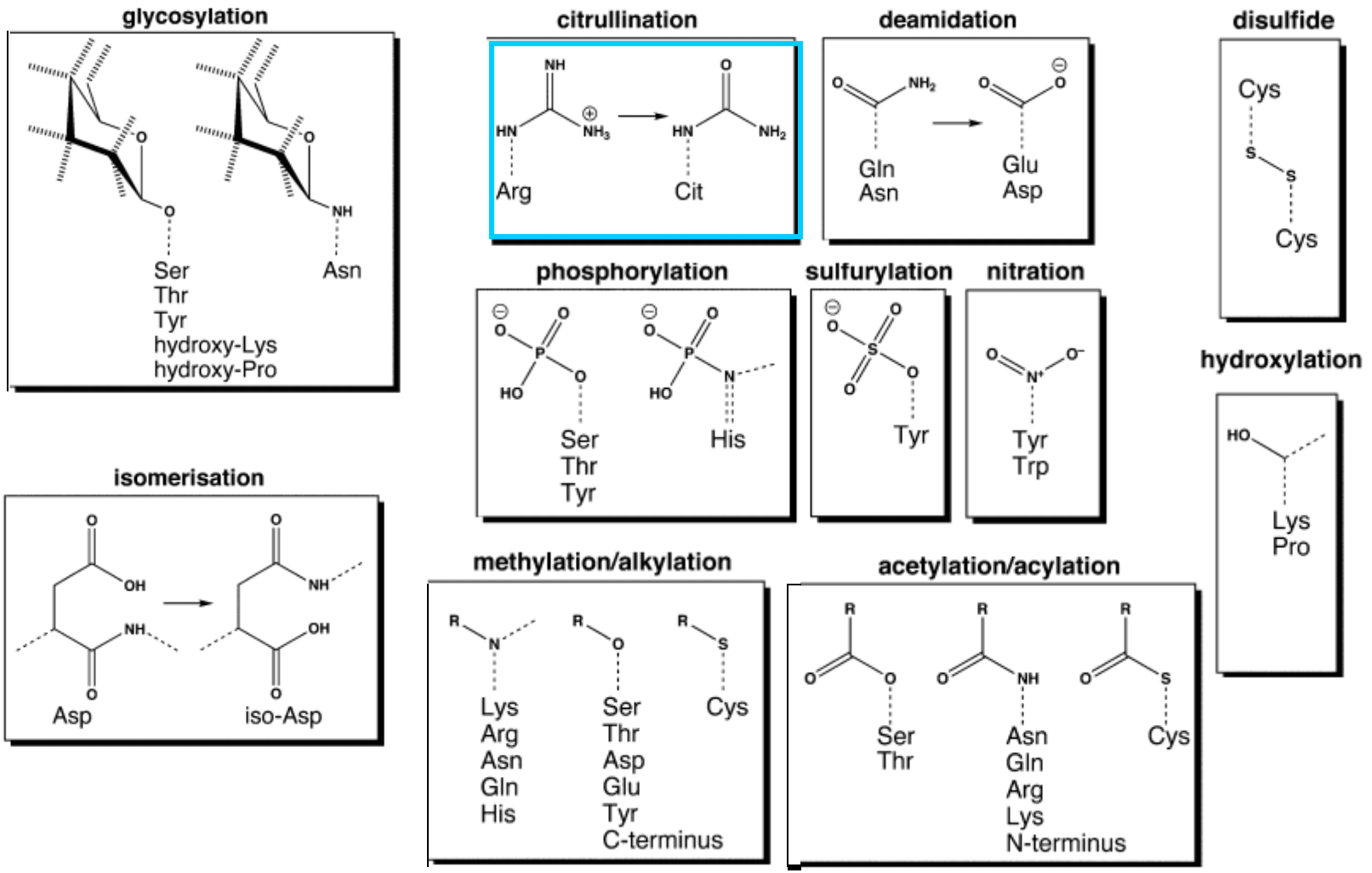
http://wlcollins.weebly.com/the-art-of-rheumatoid-arthritis.html

Post-translational modification: Immune recognition related diseases

Modification	Autoantigen	Disease
Acetylation	Myelin basic protein	Multiple sclerosis
Citrullination	Collagen type II Myelin basic protein	RA Multiple sclerosis
Deamidation	Insulin	Type I diabetes
Glycosylation	Insulin proceptor Collagen type II Thyrotropin receptor Myelin oligodendrocyte glycoprotein	Diabetes RA Graves disease MS
Isoaspartylation	snRNP	Systemic lupus erythematosus
Lipoylation	PDC-E2	Primary biliary cirrhosus
Phosphorylation	Myelin basic protein	Multiple sclerosis
Methylation	Sm, D1,D3	Systemic lupus erythematosus
Transglutamination	Histone H2	Systemic lupus erythematosus
Tyrosine nitration	Mitochondrial proteins	Experimental autoimmune uveitis

Posttranslational modification of Auto-antigens p.41
in "Autoantibodies" Eds. Y. Shoenfeld, M. E. Gershwin, P.- L. Meroni, pp. 838, (2007) Elsevier

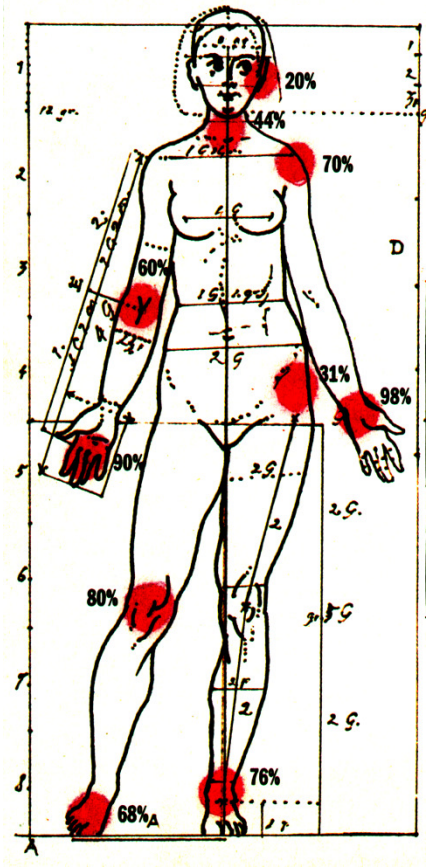
Citrullination

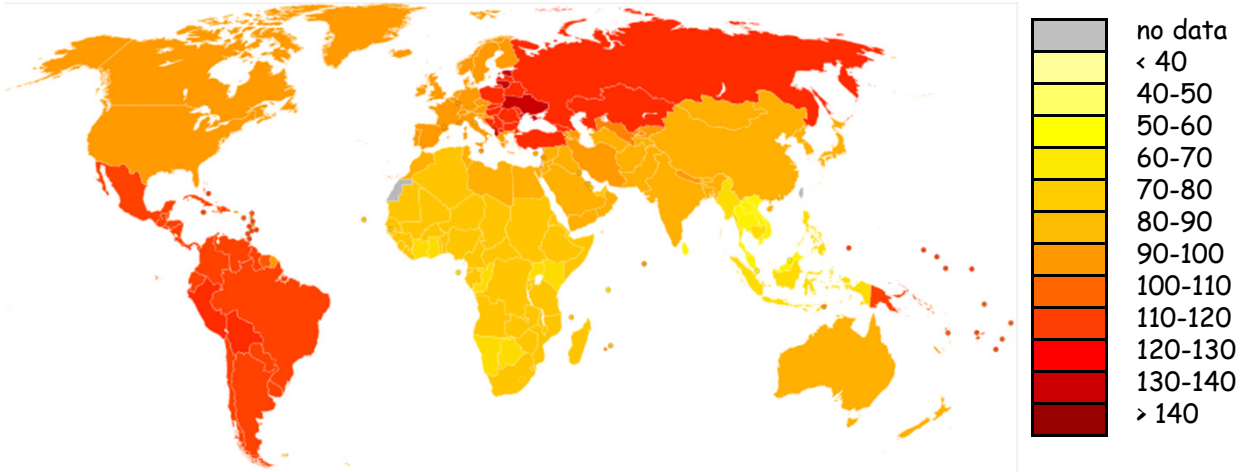


Petersen, J., et al. J. Mol.Med. 87: 1045-1051 (2009)

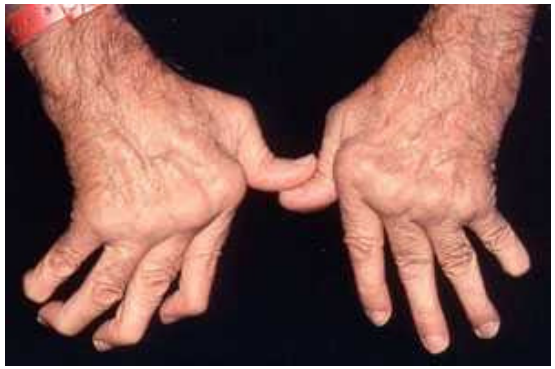
Rheumatoid Arthritis

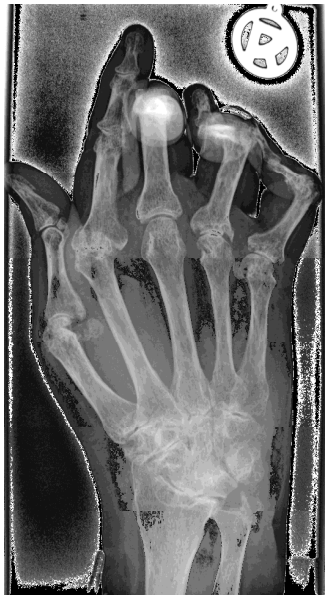
- chronic, systemic inflammatory disorder
- systemic autoimmune disease
- attacks synovial joints
- hyperplasia of synovial cells,
- excess synovial fluid,





http://en.wikipedia.org/wiki/File:Rheumatoid_arthritis_world_map_-_DALY_-_WHO2004.svg

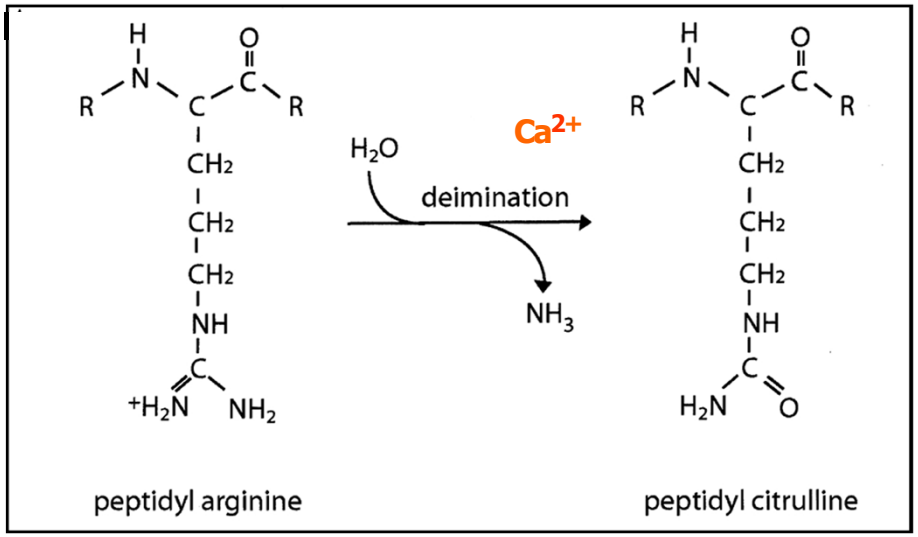


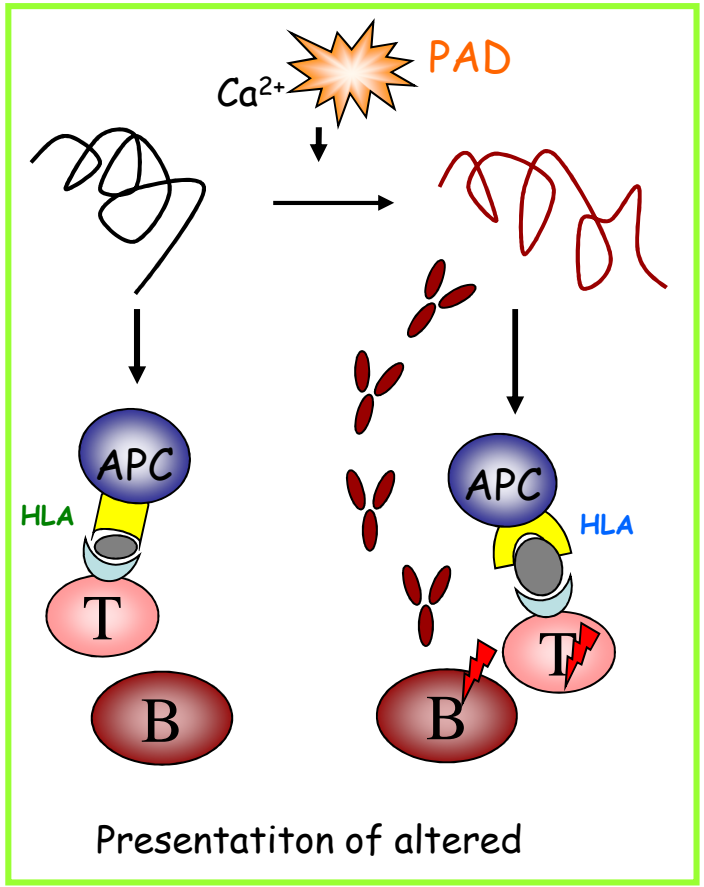


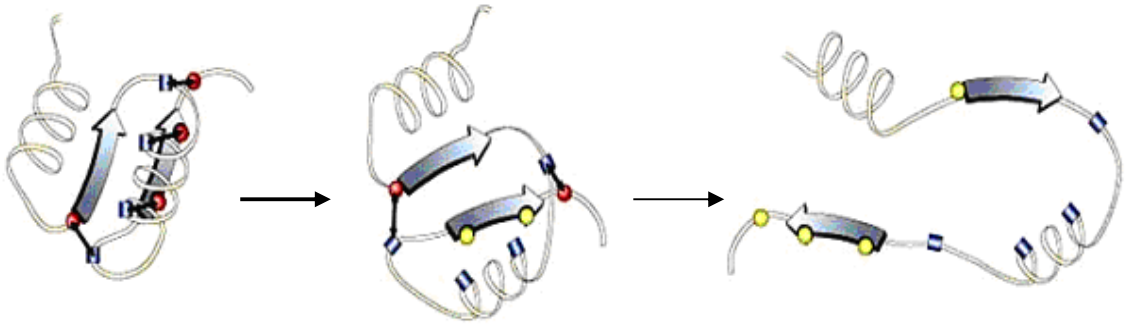
Epidemiology

- 1% of the world's population
- women : man = 3:1
- most frequent ages 40 – 50

The effect of post-translational modification on immune recognition:change in 3D stucture of proteins







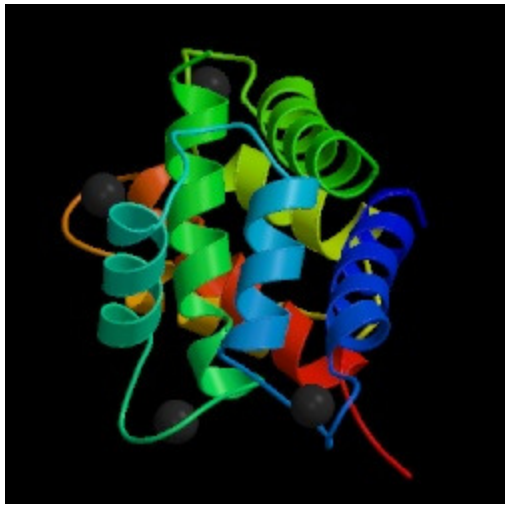
Asp/Glu Arg → citrullin

Yamada, R. et al. *Bioscience* 10: 54-64 (2005)
Yamada, R. *Autoimmunity Reviews* 4: 201-206 (2005)

The effect of post-translational modification on immune recognition: proteins involved

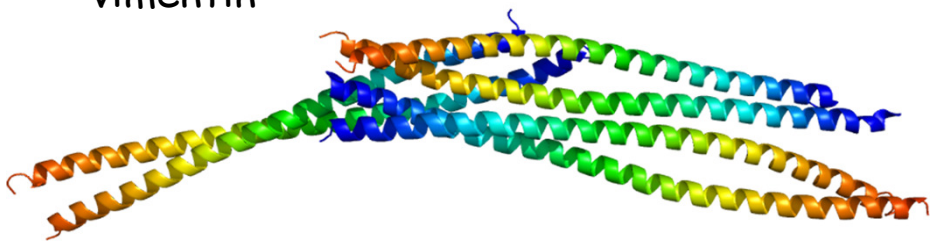
Disease	Modification	Antigen modified
RA	Hydroxylation Glycosylation Oxidation Citrullination	Type II collagen Fillagrin Fibrin Vimentin
	Glycosylation	IgG
SLE	Phosphorylation Deamidation Mannose modification Methylation Oxidation	Multiple snRNP D, H2B Multiple SM D1, D3 Cardiolipin, ox LDL, C1q, calreticulin

Eggleton, P. et al. Rheumatology 47: 567-571 (2008)

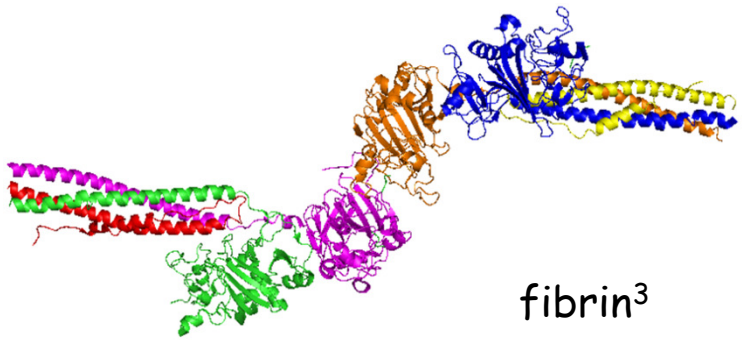


filaggrin[1]

vimentin[2]



Based on PyMOL rendering of PDB 1gk4



fibrin[3]

1 Sebbag, M. et al. *Clin. Invest.* 95: 2672-2679 (1995)
2 Vossenaar, E.R. et al. *Arthritis Res. Ther.* 6(2): 86-89 (2004)
3 Masson-Bessiere, C. et.al. *J. Immun.* 166: 4177-4184 (2001)

Crystal Structure of Fibrin from *Petromyzon marinus*, 1n73
http://www.proteopedia.com/wiki/index.php/Image:1n73b.png

Aims

1. Identification of minimal and optimal antibody epitope of partially deimidated filaggrin by synthetic peptides based on 306-324 sequence using multi-pin approach and serum samples from diseased individuals.

2. Introduction of biotin label for soluble epitope peptide

3. Analyze
   - the effect epitope size and orientation on antibody recognition,
   - the effect the presence and position of biotin on solution conformation,
   - RA specificity in serum samples as compared with that of SLE and healthy individuals using the optimized peptide epitope by direct ELISA.

2-88

Filaggrin (filament aggregating protein)

(FILA_HUMAN), http://swissmodel.expasy.org/

profilaggrin: 4061 AA, 435170 Da

10-12 filaggrin unit, 324 AA → SHQESTRGRSRGRSGRSGS *

MSTLLENIFA IINLFKQYSK KDKNTDTLSK KELKELLEKE FRQILKNPDD PDMVDVFMDH LDIDHNKKID FTEFLLMVFK LAQAYYESTR KENLPISGHK HRKHSHHDKH EDNKQEENKE NRKRPSSLER
RNNRKGNKGR SKSPRETGGK RHESSSEKKE RKGYSPTHRE EEYGKNHHNS SKKEKNKTEN TRLGDNRKRL SERLEEKEDN EEGVYDYENT GRMTQKWIQS GHIATYYTIQ DEAYDTTDSL
LEENKIYERS RSSDGKSSSQ VNRSRHENTS QVPLQESRTR KRRGSRVSQD RDSEGHSEDS ERHSGSASRN HHGSAWEQSR DGSRHPRSHD EDRASHGHSA DSSRQSGTRH AETSSRGQTA
SSHEQARSSP GERHGSGHQQ SADSSRHSAT GRGQASSAVS DRGHRGSSGS QASDSEGHSE NSDTQSVSGH GKAGLRQQSH QESTRGRSGE RSGRSGSSLY QVSTHEQPDS AHGRTGTSTG
GRQGSHHEQA RDSSRHSASQ EGQDTIRGHP GSSRGGRQGS HHEQSVNRSG HSGSHHSHTT SQGRSDASHG QSGSRSASRQ TRNEEQSGDG TRHSGSRHHE ASSQADSSRH SQVGQGQSSG
PRTSRNQGSS VSQDSDSQGH SEDSERWSGS ASRNHHGSAQ EQSRDGSRHP RSHHEDRAGH GHSADSSRKS GTRHTQNSSS GQAASSHEQA RSSAGERHGS RHQLQSADSS RHSGTGHGQA
SSAVRDSGHR GSSGSQATDS EGHSEDSDTQ SVSGHGQAGH HQQSHQESAR DRSGERSRRS GSFLYQVSTH KQSESSHGWT GPSTGVRQGS HHEQARDNSR HSASQDGQDT IRGHPGSSRR
GRQGSHHEQS VDRSGHSGSH HSHTTSQGRS DASRGQSGSR SASRTTRNEE QSRDGSRHSG SRHHEASSHA DISRHSQAGQ GQSEGSRTSR RQGSSVSQDS DSEGHSEDSE RWSGSASRNH
RGSAQEQSRH GSRHPRSHHE DRAGHGHSAD SSRQSGTPHA ETSSGGQAAS SHEQARSSPG ERHGSRHQQS ADSSRHSGIP RRQASSAVRD SGHWGSSGSQ ASDSEGHSEE SDTQSVSGHG
QDGPHQQSHQ ESARDWSGGR SGRSGSFIYQ VSTHEQSESA HGRTRTSTGR RQGSHHEQAR DSSRHSASQE GQDTIRAHPG SRRGGRQGSH HEQSVDRSGH SGSHHSHTTS QGRSDASHGQ
SGSRSASRQT RKDKQSGDGS RHSGSRHHEA ASWADSSRHS QVGQEQSSGS RTSRHQGSSV SQDSDSERHS DDSERLSGSA SRNHHGSSRE QSRDGSRHPG FHQEDRASHG HSADSSRQSG
THHTESSSHG QAVSSHEQAR SSPGERHGSR HQQSADSSRH SGIGHRQASS AVRDSGHRGS SGSQVTNSEG HSEDSDTQSV SAHGQAGPHQ QSHKESARGQ SGESSGRSRS FLYQVSSHEQ
SESTHGQTAP STGGRQGSRH EQARNSSRHS ASQDGQDTIR GHPGSSRGGR QGSYHEQSVD RSGHSGYHHS HTTPQGRSDA SHGQSGPRSA SRQTRNEEQS GDGSRHSGSR HHEPSTRAGS
SRHSQVGQGE SAGSKTSRRQ GSSVSQDRDS EGHSEDSERR SESASRNHYG SAREQSRHGS RNPRSHQEDR ASHGHSAESS RQSGTRHAET SSGGQAASSQ EQARSSPGER HGSRHQQSAD
SSTDSGTGRR QDSSVVGDSG NRGSSGSQAS DSEGHSEESD TQSVSAHGQA GPHQQSHQES TRGQSGERSG RSGSFLYQVS THEQSESAHG RTGPSTGGRQ RSRHEQARDS SRHSASQEGQ
DTIRGHPGSS RGGRQGSHYE QSVDSSGHSG SHHSHTTSQE RSDVSRGQSG SRSVSRQTRN EKQSGDGSRH SGSRHHEASS RADSSRHSQV GQGQSSGPRT SRNQGSSVSQ DSDSQGHSED
SERWSGSASR NHLGSAWEQS RDGSRHPGSH HEDRAGHGHS ADSSRQSGTR HTESSSRGQA ASSHEQARSS AGERHGSHHQ LQSADSSRHS GIGHGQASSA VRDSGHRGYS GSQASDSEGH
SEDSDTQSVS AQGKAGPHQQ SHKESARGQS GESSGRSGSF LYQVSTHEQS ESTHGQSAPS TGGRQGSHYD QAQDSSRHSA SQEGQDTIRG HPGPSRGGRQ GSHQEQSVDR SGHSGSHHSH
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GHGHSAESSR QSGTHHAENS SGGQAASSHE QARSSAGERH GSHHQQSADS SRHSGIGHGQ ASSAVRDSGH RGSSGSQASD SEGHSEDSDT QSVSAHGQAG PHQQSHQEST RGRSAGRSGR
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GSRHHEASSR ADSSGHSQVG QGQSEGPRTS RNWGSSFSQD SDSQGHSEDS ERWSGSASRN HHGSAQEQLR DGSRHPRSHQ EDRAGHGHSA DSSRQSGTRH TQTSSGGQAA SSHEQARSSA
GERHGSHHQQ SADSSRHSGI GHGQASSAVR DSGHRGYSGS QASDNEGHSE DSDTQSVSAH GQAGSHQQSH QESARGRSGE TSGHSGSFLY QVSTHEQSES SHGWTGPSTR GRQGSRHEQA
QDSSRHSASQ DGQDTIRGHP GSSRGGRQGY HHEHSVDSSG HSGSHHSHTT SQGRSDASRG QSGSRSASRT TRNEEQSGDG SRHSGSRHHE ASTHADISRH SQAVQGQSEG SRRSRRQGSS
VSQDSDSEGH SEDSERWSGS ASRNHHGSAQ EQLRDGSRHP RSHQEDRAGH GHSADSSRQS GTRHTQTSSG GQAASSHEQA RSSAGERHGS HHQQSADSSR HSGIGHGQAS SAVRDSGHRG
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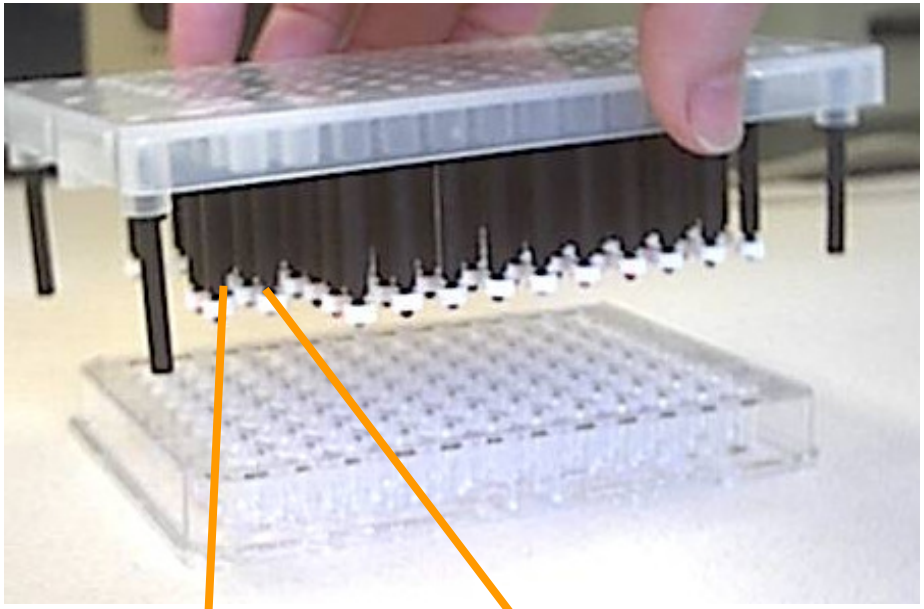
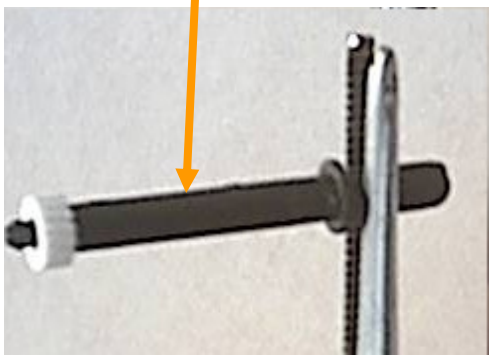
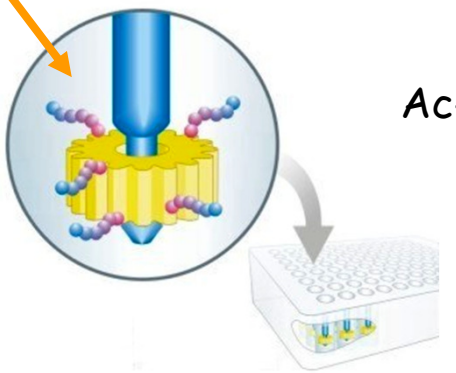
SARDRSGGRS GRSGSFLYQV STHEQSESAH GRTRTSTGRR QGSHHEQARD SSRHSASQEG QDTIRGHPGS SRRGRQGSHY EQSVDRSGHS GSHHSHTTSQ GRSDASRGQS GSRSASRQTR
NDEQSGDGSR HSWSHHHEAS TQADSSRHSQ SGQGQSAGPR TSRNQGSSVS QDSDSQGHSE DSERWSGSAS RNHRGSAQEQ SRDGSRHPTS HHEDRAGHGH SAESSRQSGT HHAENSSGGQ
AASSHEQARS SAGERHGSHH QQSADSSRHS GIGHGQASSA VRDSGHRGSS GSQASDSEGH SEDSDTQSVS AHGQAGPHQQ SHQESTRGRS RGRSGRSGSF LYQVSTHEQS ESAHGRAGPS
TGGRQGSRHE QARDSSRHSA SQEGQDTIRG HPGSRRGGRQ GSYHEQSVDR SGHSGSHHSH TTSQGRSDAS HGQSGSRSAS RETRNEEQSG DGSRHSGSRH HEASTQADSS RHSQSGQGES
AGSRRSRRQG SSVSQDSDSE AYPEDSERRS ESASRNHHGS SREQSRDGSR HPGSSHRDTA SHVQSSPVQS DSSTAKEHGH FSSLSQDSAY HSGIQSRGSP HSSSSYHYQS EGTERQKGQS HGSVS

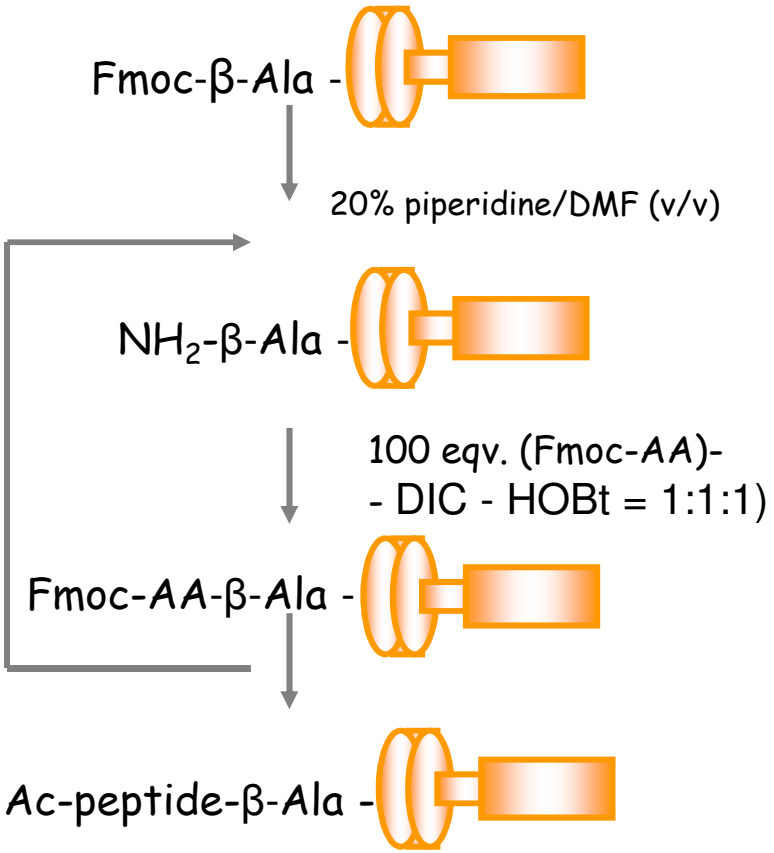
* Schellekens, G.A. et al. *J. Clin. Invest.* 101, 273-281 (1998)

Search for minimal/optimal epitope: Multi-pin approach



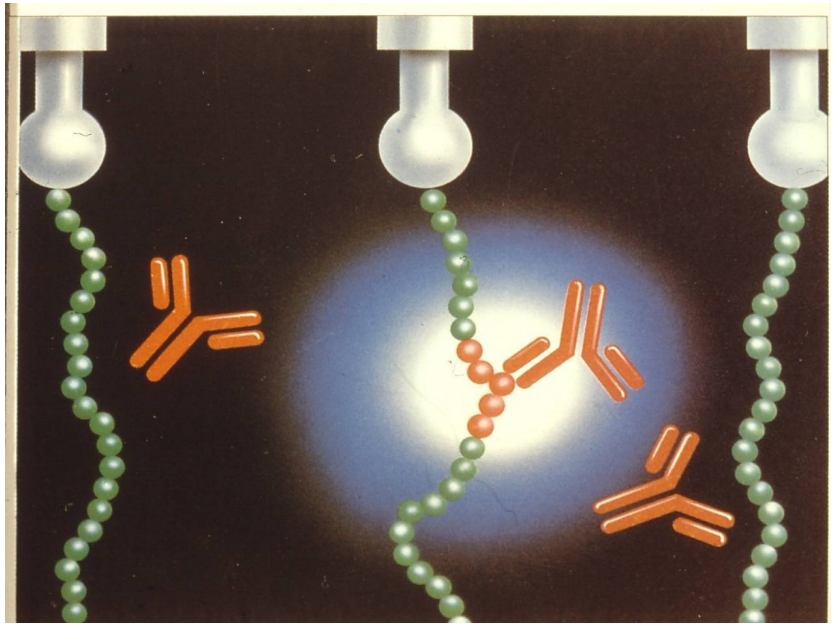


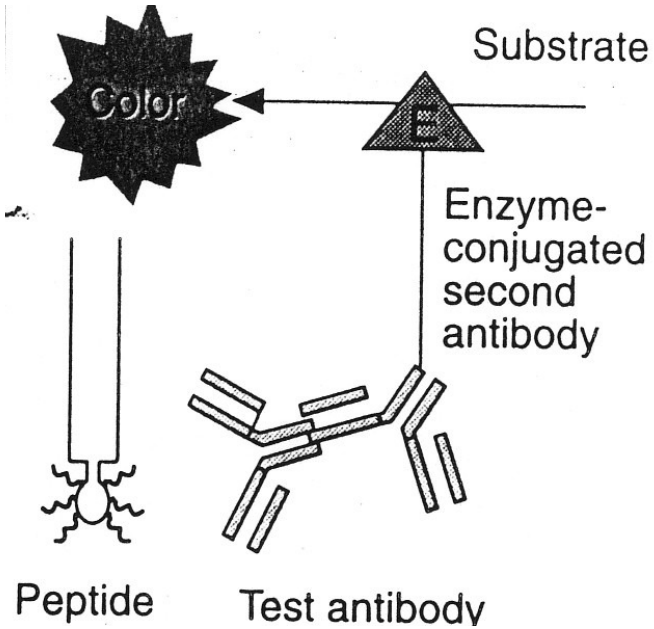


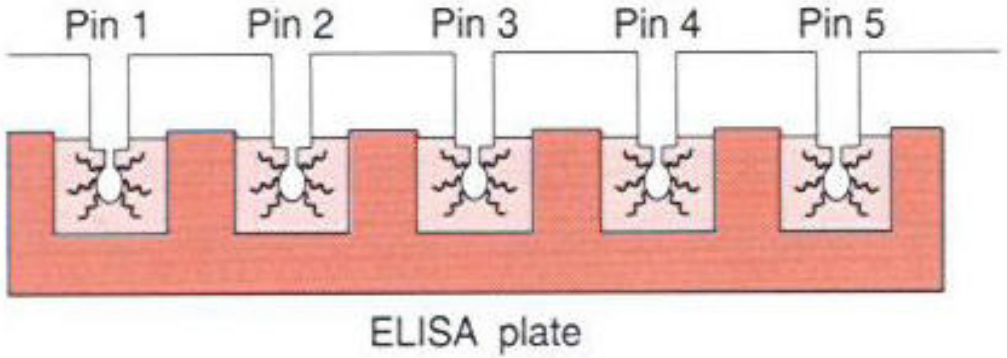


Deprotection
1) 20 % piperidine/DMF
2) TFA/tioanisole/anisole/water/EDT =
= 82,5 : 5 : 5 : 5 : 2,5 (v/v/v/v/v)

Identification of linear antibody epitopes







In vitro analysis of antibody recognition

- Peptides - C-terminally pin-bound oligopeptides
  - free peptides labelled with biotin at the N-terminal
  - free peptides labelled with biotin at the C-terminal
- Serum samples:
  from healthy CCP positive individuals,
  from diseased CCP positive RA individuals,
  from healthy CCP negative individuals,

- Synovial fluid samples:

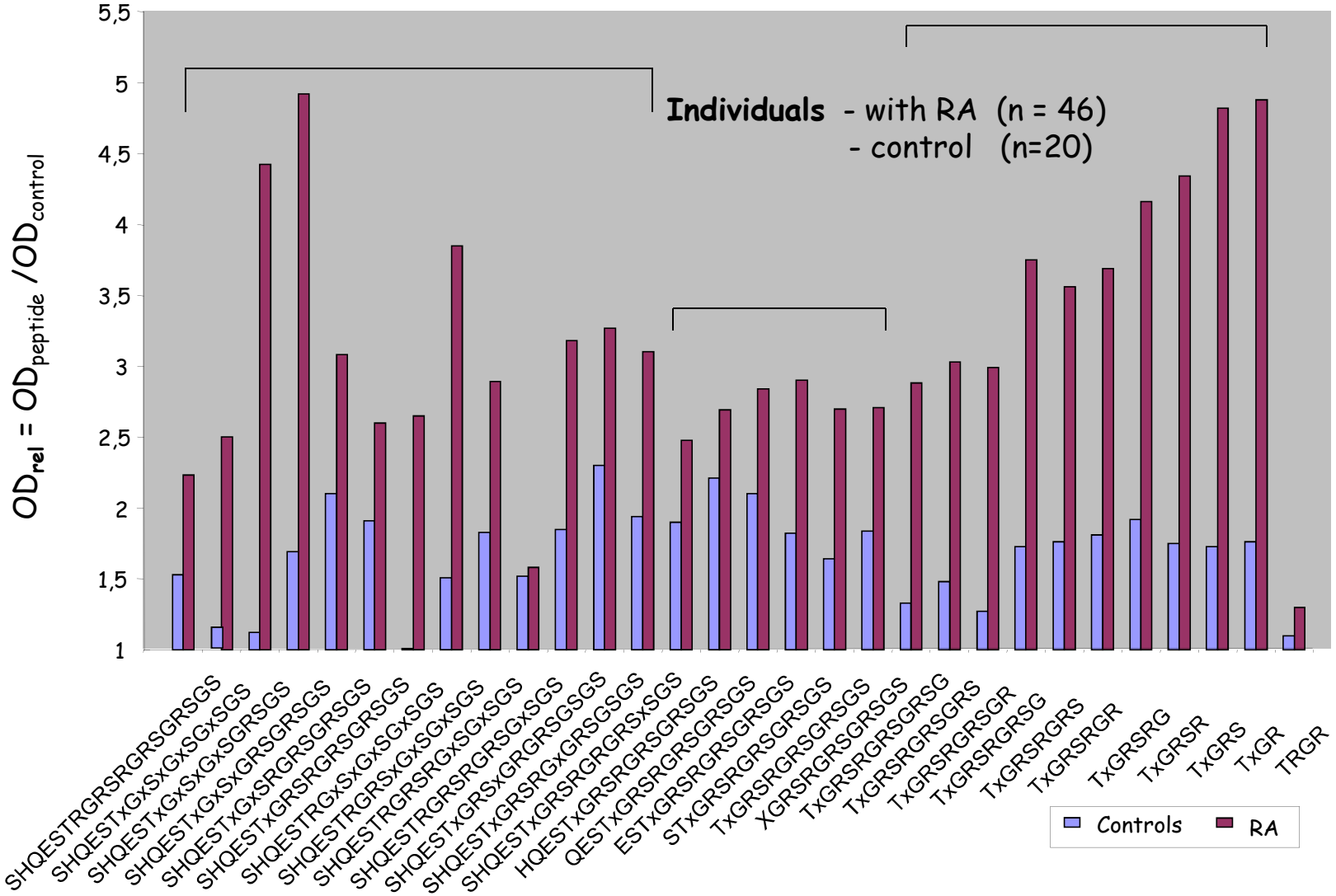
- direct ELISA

Search for minimal/optimal epitope

19-mer analogues	N-terminal truncation of peptide Cit[312] (306-324)	C-terminal truncation of peptide 311-324
[306]SHQESTRGRSRGRSGRSGS[324]	[306]SHQESTXGRSRGRSGRSGS[324]	[311]TXGRSRGRSGRSGS[324]
[306]SHQESTXGXSXGXSGXSGS[324]	[307]HQESTXGRSRGRSGRSGS[324]	[311]TXGRSRGRSGRSG[323]
[306]SHQESTXGXSXGXSGRSGS[324]	[308]QESTXGRSRGRSGRSGS[324]	[311]TXGRSRGRSGRS[322]
[306]SHQESTXGXSXGRSGRSGS[324]	[309]ESTXGRSRGRSGRSGS[324]	[311]TXGRSRGRSGR[321]
[306]SHQESTXGXSRGRSGRSGS[324]	[310]STXGRSRGRSGRSGS[324]	[311]TXGRSRGRSG[320]
[306]SHQESTXGRSRGRSGRSGS[324]	[311]TXGRSRGRSGRSGS[324]	[311]TXGRSRGRS[319]
[306]SHQESTRGXSXGXSGXSGS[324]	[312]XGRSRGRSGRSGS[324]	[311]TXGRSRGR[318]
[306]SHQESTRGRSXGXSGXSGS[324]		[311]TXGRSRG[317]
[306]SHQESTRGRSRGXSGXSGS[324]		[311]TXGRSR[316]
[306]SHQESTRGRSRGRSGXSGS[324]	Control peptides	[311]TXGRS[315]
[306]SHQESTXGXSRGRSGRSGS[324]	PLAQGGGGGG	[311]TXGR[314]
[306]SHQESTXGRSXGRSGRSGS[324]	GLAQGGGGGG	[311]TRGR[314]
[306]SHQESTXGRSRGRSGXSGS[324]		

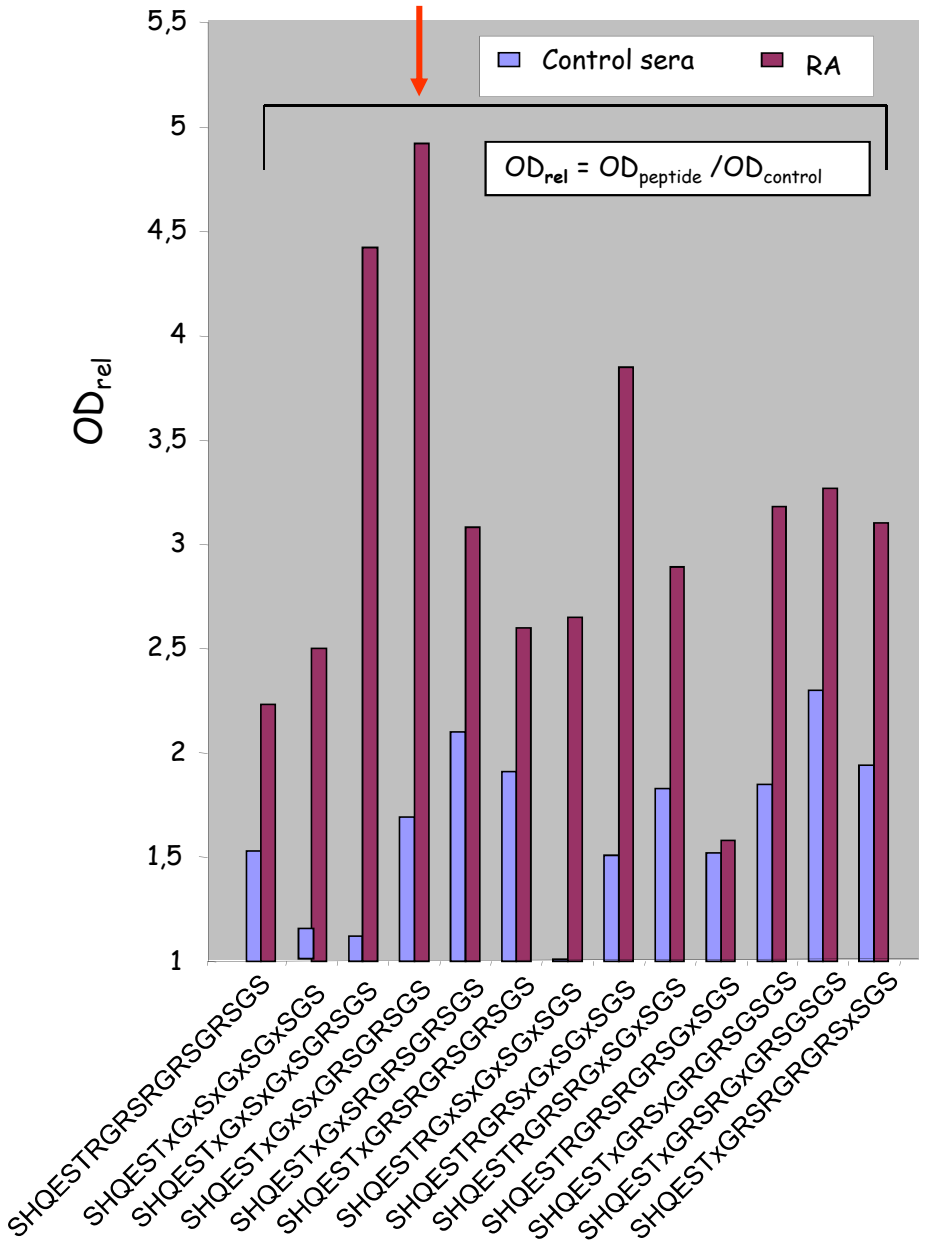
(X=citrullin)

Search for minimal/optimal epitope



Magyar, A. et al. in *Peptides 2000, Proc. 26th European Peptide Symposium* (Ed.: Martinez, J., Fehrentz, J-A.) EDK, Paris, France, 679-680 (2001)

Analogues 19-mer peptides: Critical Cit residue(s)



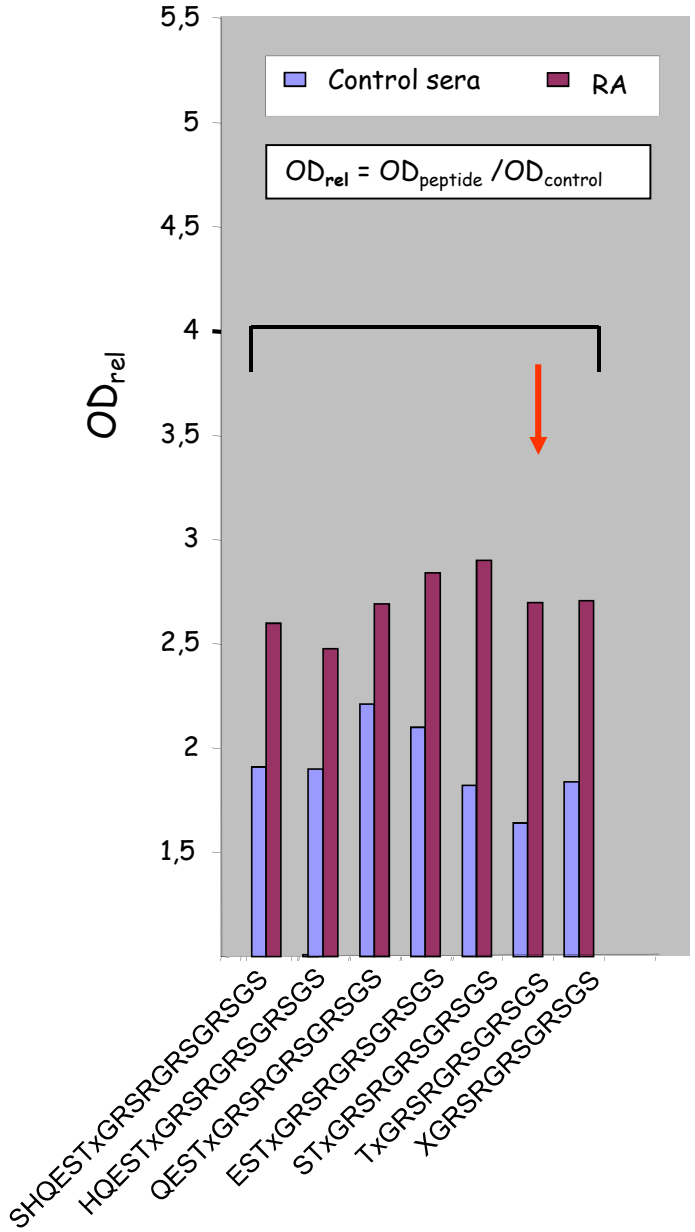
19-mer analogues
^{306}SHQESTRGRSRGRSGRSGS^{324} *
^{306}SHQESTXGXSXGXSGXSGS^{324}
^{306}SHQESTXGXSXGXSGRSGS^{324}
^{306}SHQESTXGXSXGRSGRSGS^{324}
^{306}SHQESTXGXSRGRSGRSGS^{324}
^{306}SHQESTXGRSRGRSGRSGS^{324}
^{306}SHQESTRGXSXGXSGXSGS^{324}
^{306}SHQESTRGRSXGXSGXSGS^{324}
^{306}SHQESTRGRSRGXSGXSGS^{324}
^{306}SHQESTRGRSRGRSGXSGS^{324}
^{306}SHQESTXGXSRGRSGRSGS^{324}
^{306}SHQESTXGRSXGRSGRSGS^{324}
^{306}SHQESTXGRSRGRSGXSGS^{324}

(X = citrullin)

*based on Hu-profilaggrin cDNS aa 306-324

Schellekens G.A. et al., *J. Clin. Invest.*, 101: 273-281 (1998)

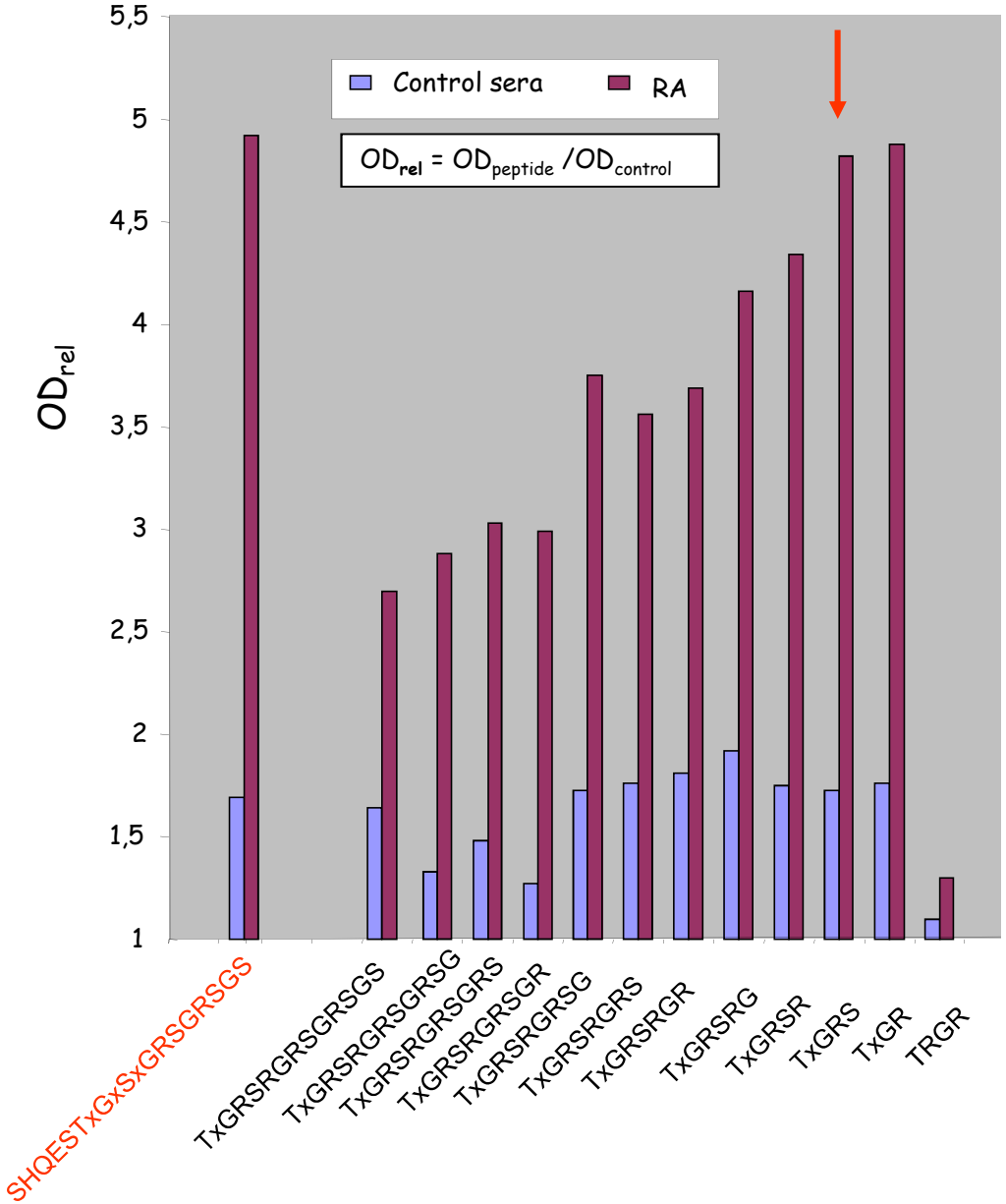
N-terminal truncation of peptide Cit^{312} (306-324)



N-terminal truncation of peptide Cit^{312} (306-324)
^{306}SHQESTXGRSRGRSGRSGS^{324}
^{307}HQESTXGRSRGRSGRSGS^{324}
^{308}QESTXGRSRGRSGRSGS^{324}
^{309}ESTXGRSRGRSGRSGS^{324}
^{310}STXGRSRGRSGRSGS^{324}
^{311}TXGRSRGRSGRSGS^{324}
^{312}XGRSRGRSGRSGS^{324}

(X = citrullin)

C-terminal truncation of peptide Cit^{312} (311-324)

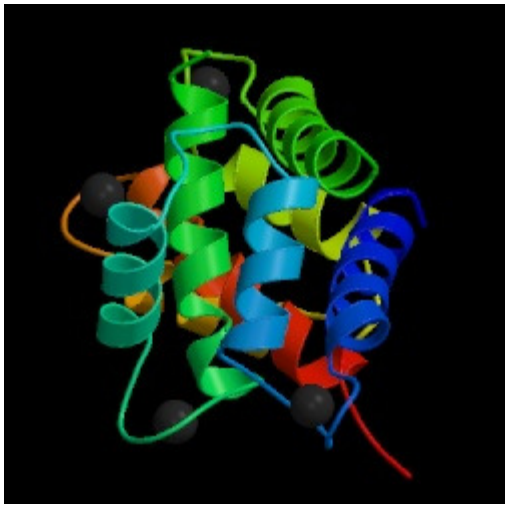


C-terminal truncation of peptide 311-324
^{311}TXGRSRGRSGRSGS^{324}
^{311}TXGRSRGRSGRSG^{323}
^{311}TXGRSRGRSGRS^{322}
^{311}TXGRSRGRSGR^{321}
^{311}TXGRSRGRSG^{320}
^{311}TXGRSRGRS^{319}
^{311}TXGRSRGR^{318}
^{311}TXGRSRG^{317}
^{311}TXGRSR^{316}
^{311}TXGRS^{315}
^{311}TXGR^{314}
^{311}TRGR^{314}

(X = citrullin)

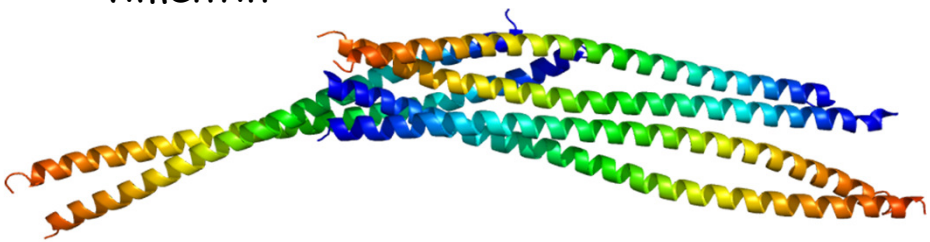
The effect of post-translational modification on immune recognition: epitope peptide

Vimentin	^{65}SAVRARSSVPGVRK^{77}
Fibrin α	^{34}GPRVVRHQSACKDS^{48}
Fibrin β	^{60}RPAPPPISSGGYRAR^{74}
Filaggrin (5-mer)	^{311}TRGRS^{315}
Filaggrin (19-mer)	^{311}SHQESTRGRSRGRSGRSGS^{326}



filaggrin

vimentin



fibrin [1]

[1] Iobagiu C., Magyar, A. et al. *J. Autoimmunity* 37: 263-272 (2011)

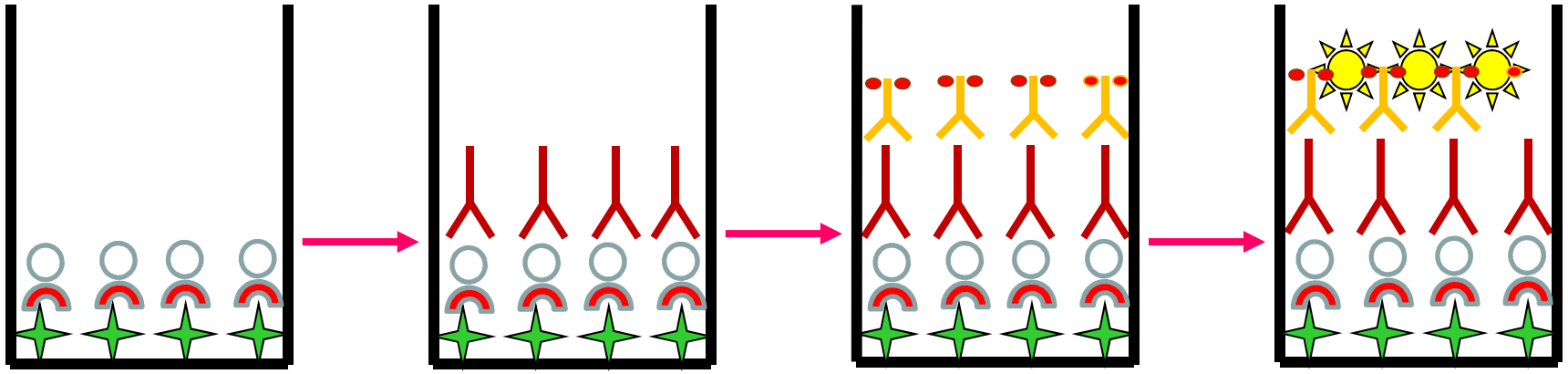
Aims

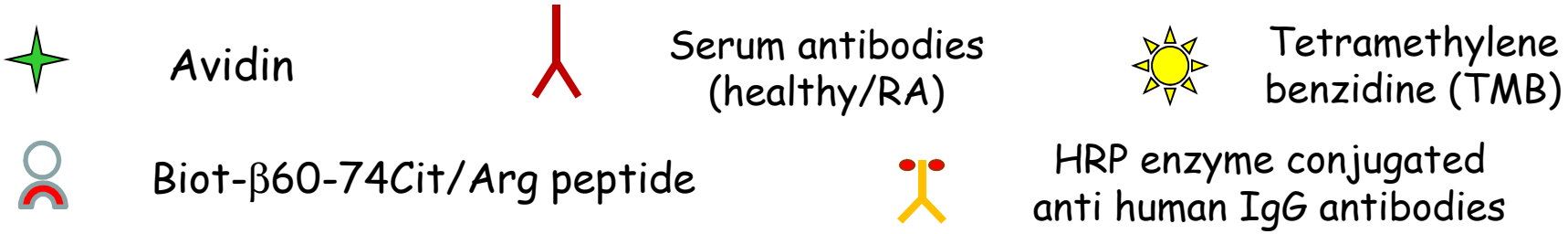
1. Identification of minimal and optimal antibody epitope of partially deimidated filaggrin by synthetic peptides based on 306-324 sequence using multi-pin approach and serum samples from diseased individuals.

2. Introduction of biotin label for soluble epitope peptide

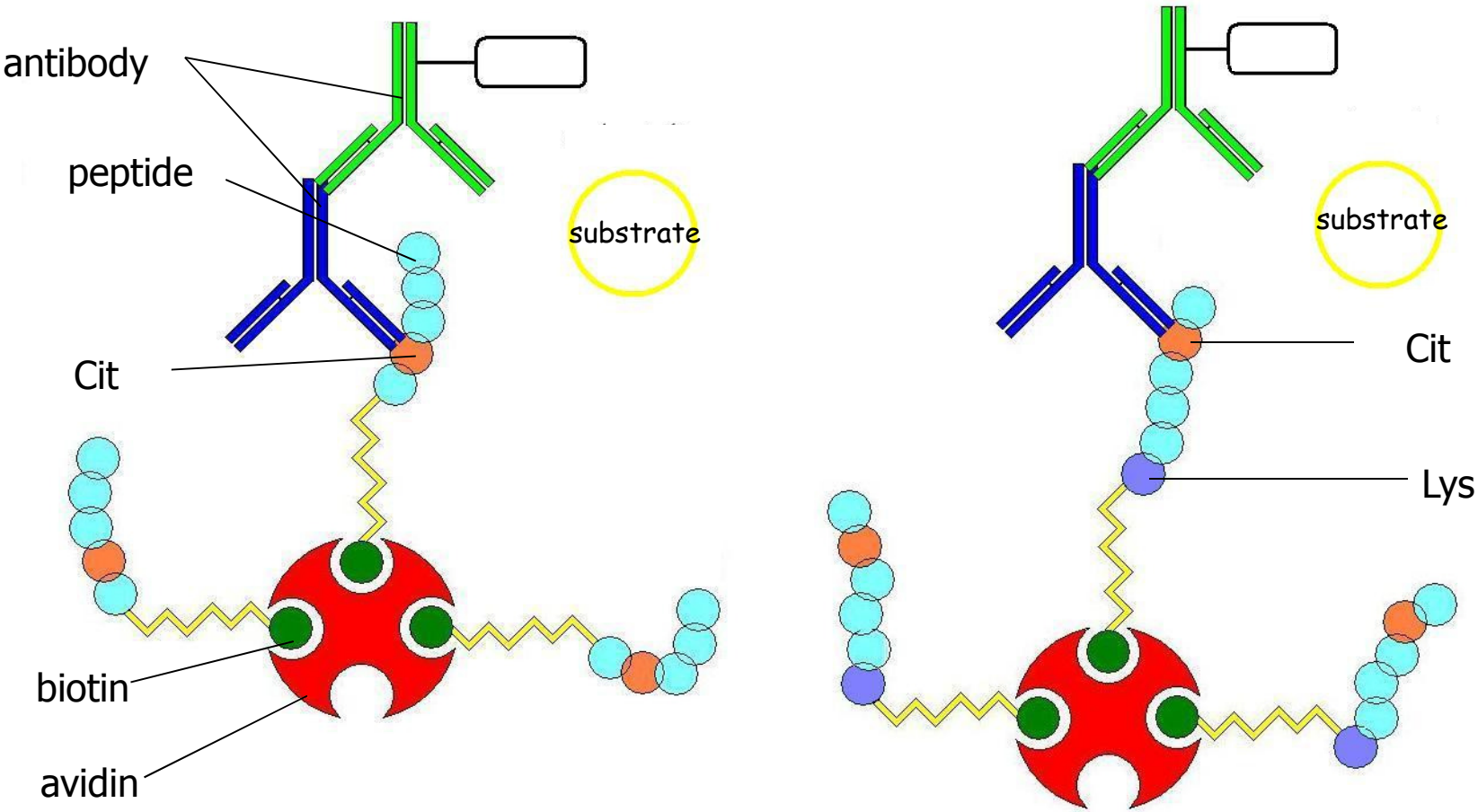
3. Analyze
   - the effect epitope size and orientation on antibody recognition,
   - the effect the presence and position of biotin on solution conformation,
   - RA specificity in serum samples as compared with that of SLE and healthy individuals using the optimized peptide epitope by direct ELISA.

Analysis of antibody binding to biot-β60-74Cit/Arg peptide by direct ELISA





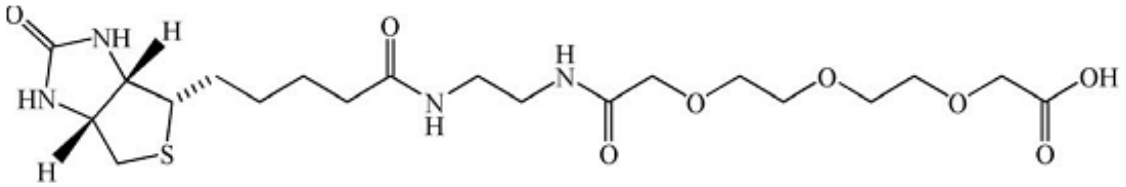
The effect of epitope orientation:the position of biotin



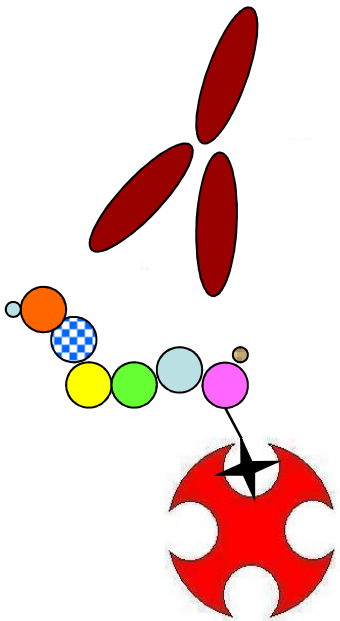
N-terminal

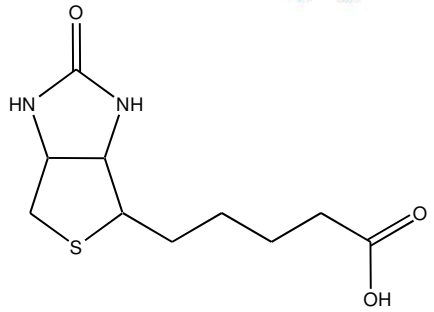
C-terminal

The effect of epitope accessibility: linkers

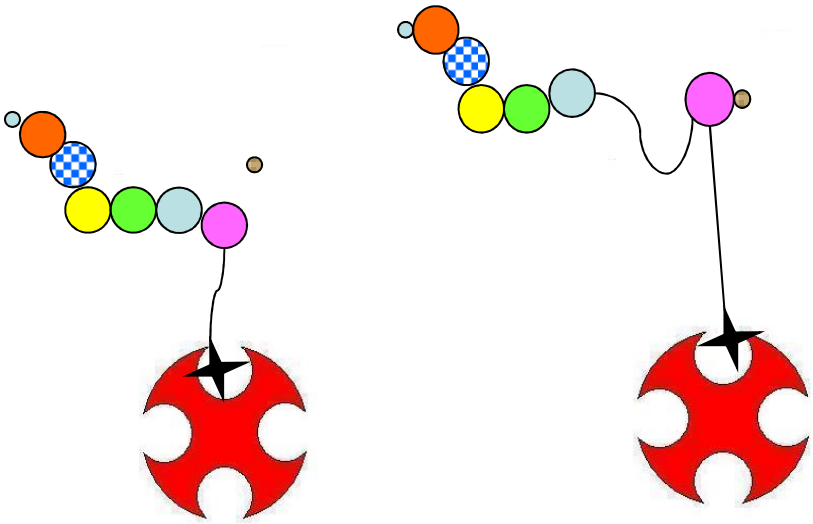


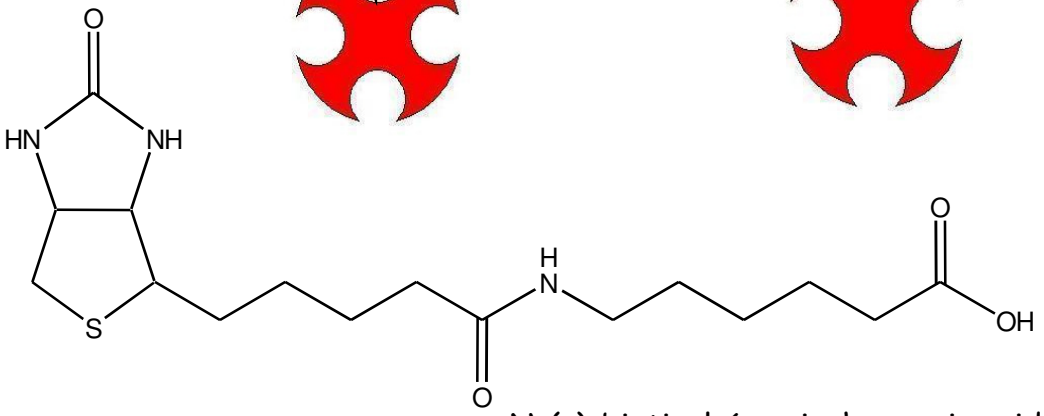
Biotinyl-4,7,10-trioxa-1,13-tridecandiamino-succinic acid





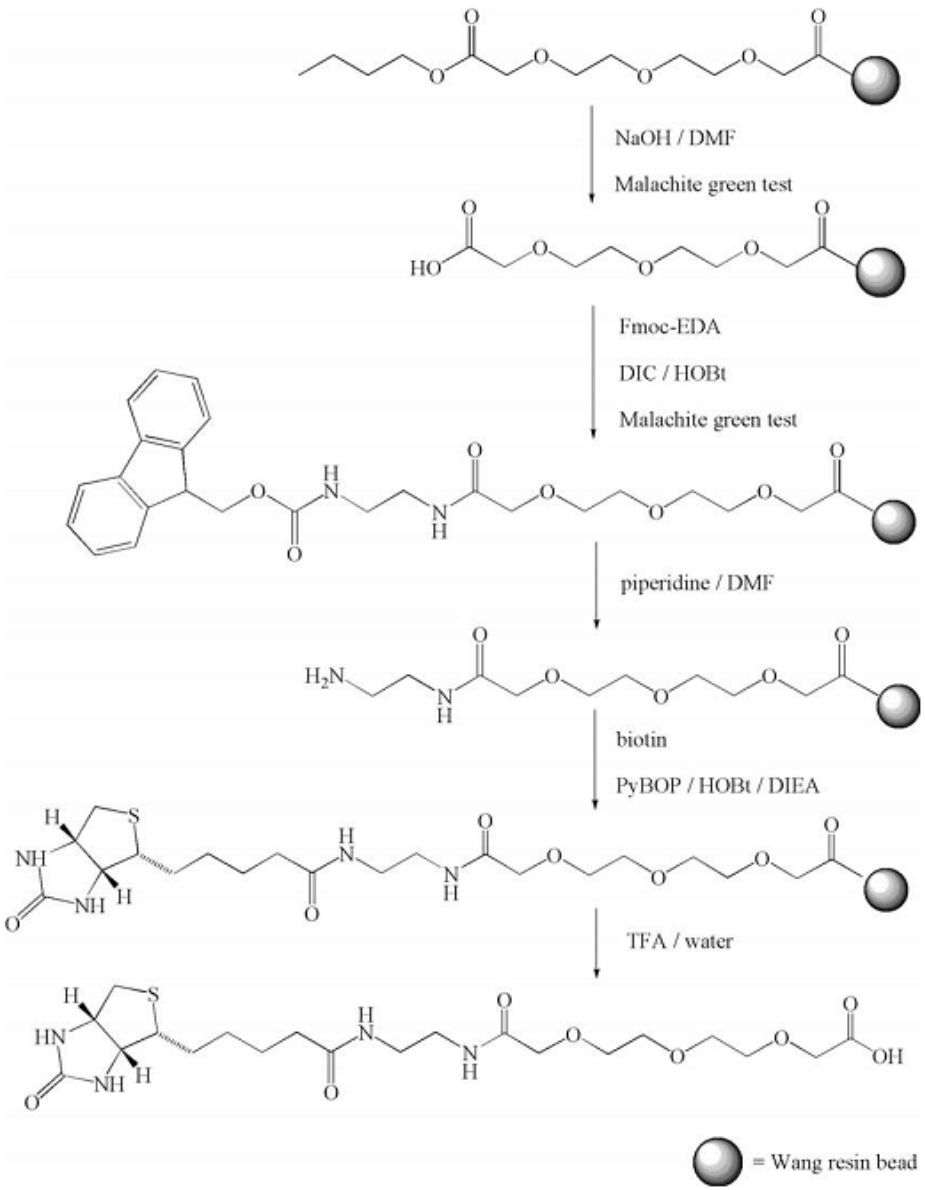
(+)-biotin





N-(+)-biotinyl-6-aminohexanoic acid

Synthesis of biotinyl-4,7,10-trioxa-1,13-tridecandiamino-succinic acid



Bartos, Á. et al.
Biopolymers 92: 110-115 (2009)
Bartos, Á. et al.
Tetrahedron Letters 50: 2661-2663 (2009)

Aims

1. Identification of minimal and optimal antibody epitope of partially deimidated filaggrin by synthetic peptides based on 306-324 sequence using multi-pin approach and serum samples from diseased individuals.

2. Introduction of biotin label for soluble epitope peptide

3. Analyze
   - the effect epitope size and orientation on antibody recognition,
   - the effect the presence and position of biotin on solution conformation,
   - RA specificity in serum samples as compared with that of SLE and healthy individuals using the optimized peptide epitope by direct ELISA.

Analysis of serum samples

Samples:
263 RA patients with established disease,
46 CCP negative, non-RA patients with other autoimmune diseases
18 patients with systemic lupus erythematosus
152 age-matched healthy controls

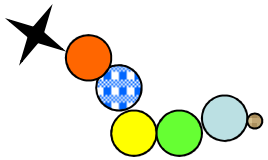
The diagnosis of the disease was established on the basis of the revised ACR/EULAR classification criteria. [1]

The baseline data of RA patients:
32 men/176 women; age: 58,4 +/– 14,3 years;
rheumatoid factor (RF) +/–: 127/30; CCP2 +/–: 157/27; MCV +/–: 164/25;
disease duration: 9.8 +/– 9,4 years.

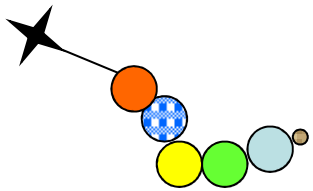
Statistical analysis: ANOVA, compared with Pearson's correlation analysis

1. Aletaha, D., Neogi ,T., Silman, A.J. et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 69:1580–8 (2010).

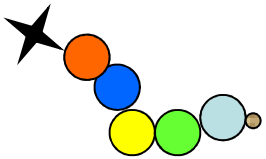
Synthesis of 5-mer epitope peptide with *N*- terminal biotin



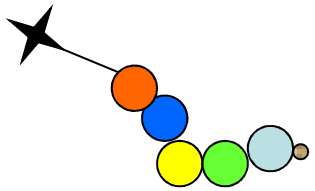
biotinyl-TXGRS-NH_2



biotinyl-6-aminohexanoyl-TXGRS-NH_2



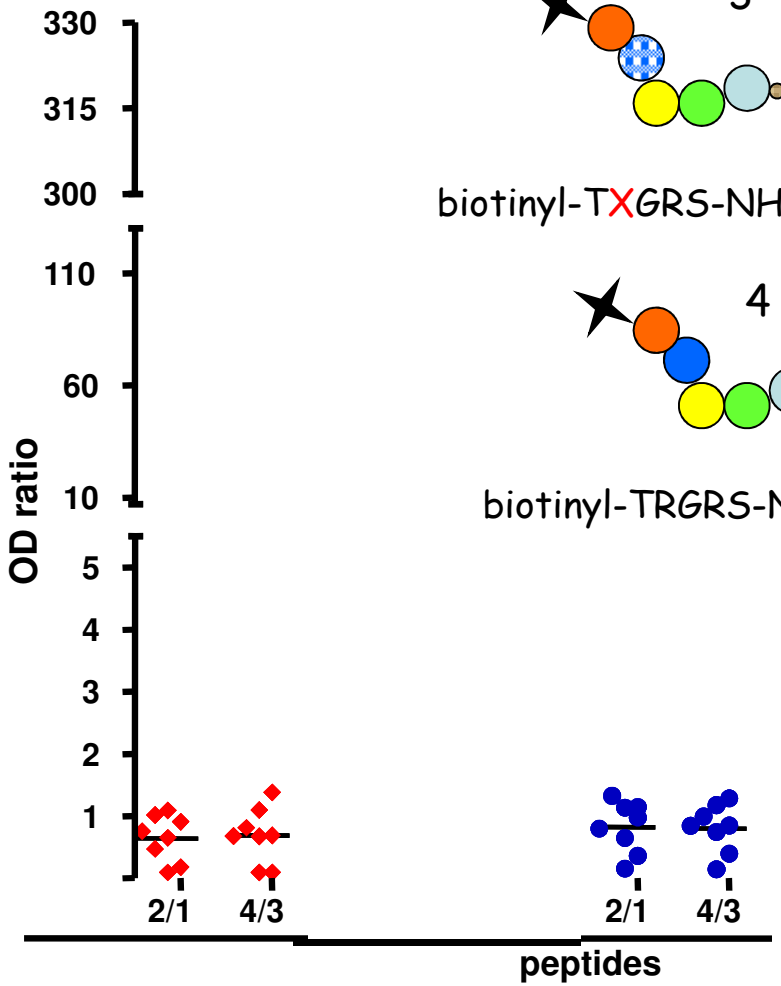
biotinyl-TRGRS-NH_2



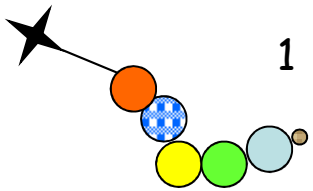
biotinyl-6-aminohexanoyl-TRGRS-NH_2

Compound	M_{av} (calc)	M_{av} (meas)	R_t (min)
biotinyl-TRGRS-NH_2	802,9	802,8	14,23
biotinyl-TXGRS-NH_2	803,9	803,7	14,12
biotinyl-6-aminohexanoyl-TRGRS-NH_2	914,1	913,9	17,07
biotinyl-6-aminohexanoyl-TXGRS-NH_2	915,1	914,9	16,50

Antibody recognition of 5-mer epitope peptides with *N*- terminal biotin

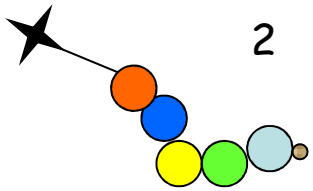


biotinyl-TXGRS-NH_2



biotinyl-6-aminohexanoyl-TXGRS-NH_2

biotinyl-TRGRS-NH_2



biotinyl-6-aminohexanoyl-TRGRS-NH_2

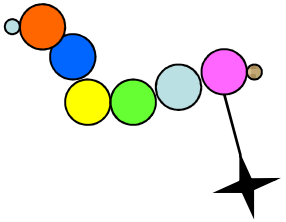
- Sera from healthy individuals
- CCP^+ (RA) sera

OD ratio = $OD_{Cit\ peptide}$ / $OD_{unmodified\ peptide}$

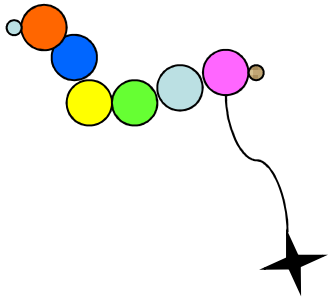
The presence of *N*-terminal biotin prevents recognition.

Babos, F. et al. Bioconjugate Chemistry 24: 817-827 (2013)

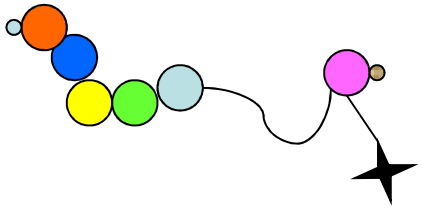
Synthesis of 5-mer epitope peptides with *C*-terminal biotin



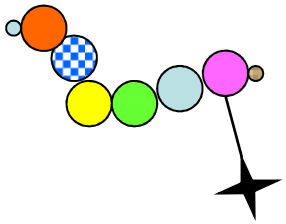
Ac-TRGRSK(biotinyl-hexanoyl)-NH_2



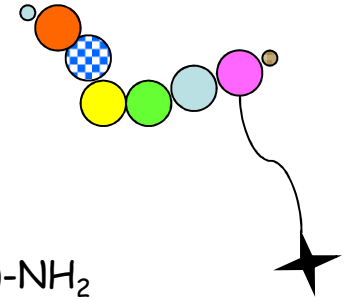
Ac-TRGRS-Ttds-K(biotinyl-hexanoyl)-NH_2



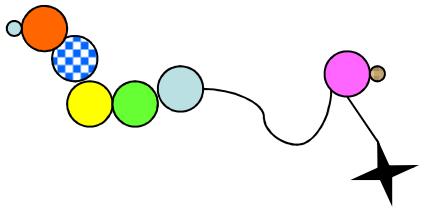
Ac-TRGRSK(biotinyl-Ttds)-NH_2



Ac-TXGRSK(biotinyl-hexanoyl)-NH_2

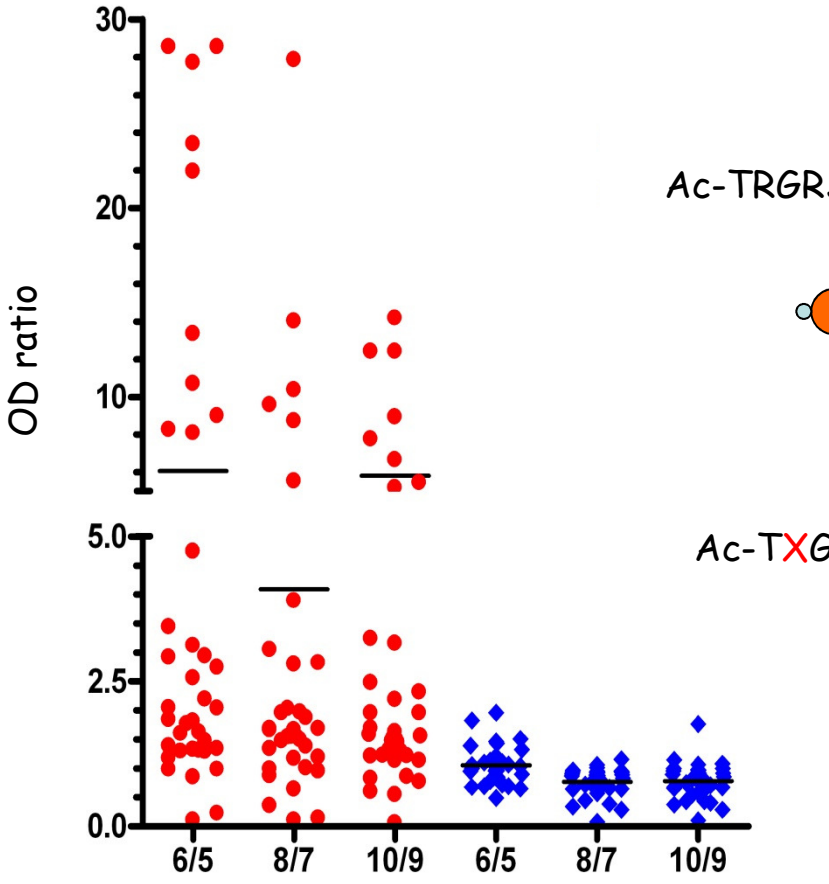


Ac-TXGRS-Ttds-K(biotinyl-hexanoyl)-NH_2

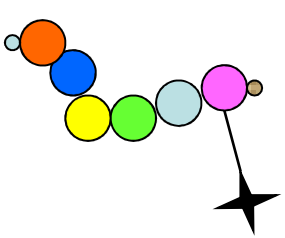


Ac-TXGRSK(biotinyl-Ttds)-NH_2

Antibody recognition of 5-mer epitope peptides with *C*- terminal biotin

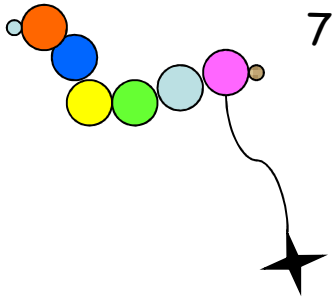


OD ratio = $OD_{Cit\ peptide}$ / $OD_{unmidified\ peptide}$



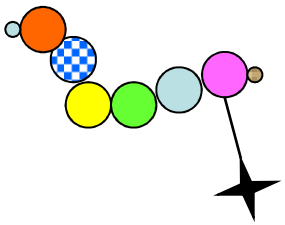
5

Ac-TRGRSK(biotinyl-hexanoyl)-NH_2



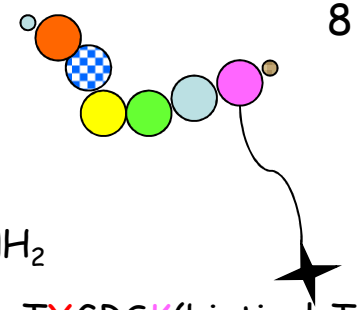
7

Ac-TRGRSK(biotinyl-Ttds)-NH_2



6

Ac-TXGRSK(biotinyl-hexanoyl)-NH_2



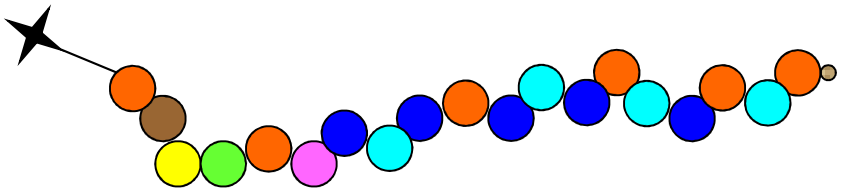
8

Ac-TXGRSK(biotinyl-Ttds)-NH_2

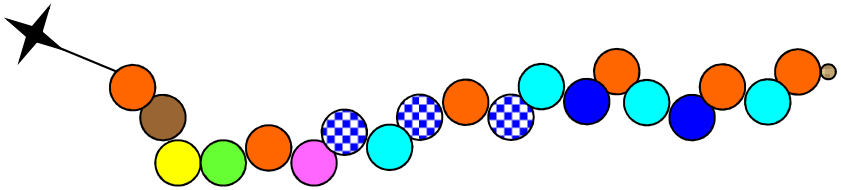
- CCP+ (RA) sera
- Sera from healthy individuals

The presence of biotin at the C-terminal of the 5-mer peptides has no effect on specific recognition by sera from patients.

Synthesis of 19-mer epitope peptide (^{306}SHQESTRGRSRGRSGRSGS^{324}) with *N*-terminal biotin



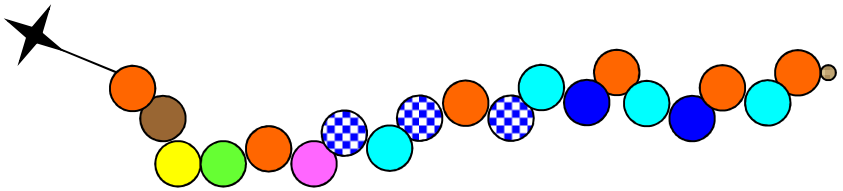
biotinyl-6-aminohexanoyl-SHQESTRGRSRGRSGRSGS-NH_2



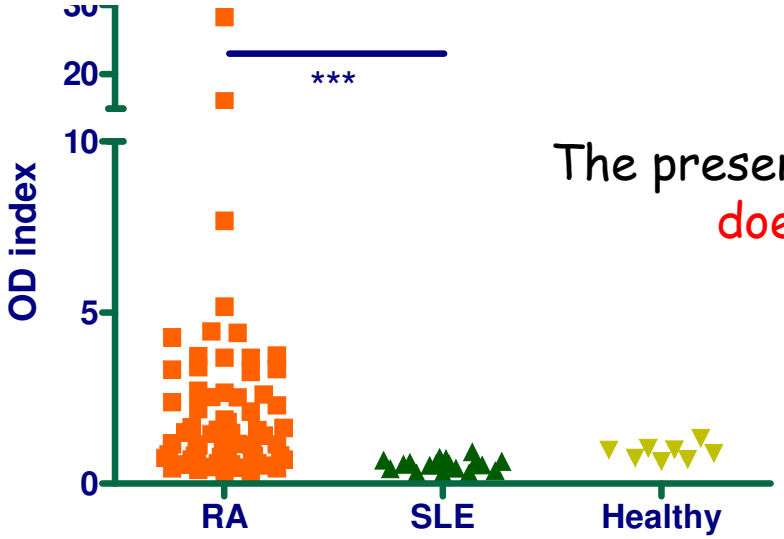
biotinyl-6-aminohexanoyl-SHQESTXGXSXGRSGRSGS-NH_2

Compounds	M_{av} (calc)	M_{av} (meas)	R_t (min)
biotinyl-6-aminohexanoyl-SHQESTRGRSRGRSGRSGS-NH_2	2383,6	2383,8	13,27
biotinyl-6-aminohexanoyl-SHQESTXGXSXGRSGRSGS-NH_2	2386,6	2386,7	12,95

Antibody recognition of 19-mer epitope peptide with *N*- terminal biotin by RA, SLE and healthy samples



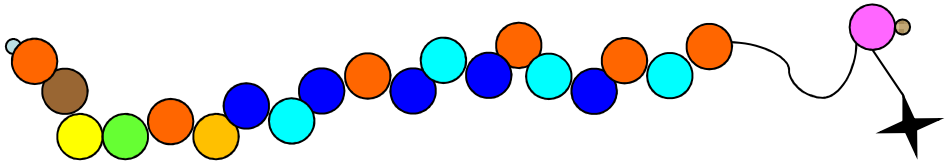
biotinyl-6-aminohexanoyl-SHQESTXGXSXGRSGRSGS-NH_2



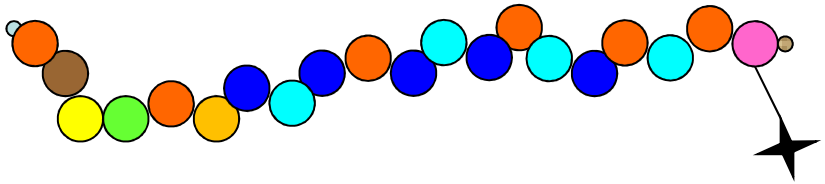
The presence of biotin at *N*-terminal biotin does not prevent recognition.

RA vs SLE	P < 0.001
RA vs Healthy	P > 0.05
SLE vs Healthy	P > 0.05

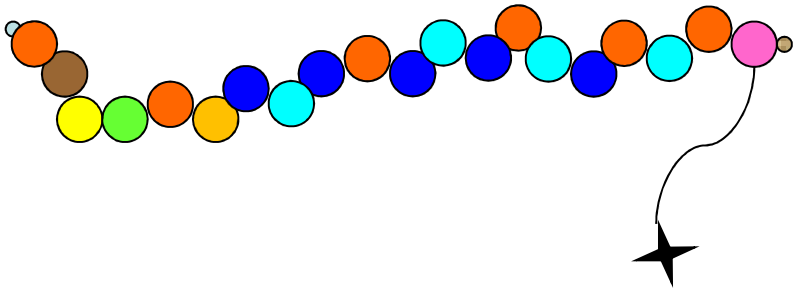
Synthesis of 19-mer epitope peptide (^{306}SHQESTRGRSRGRSGRSGS^{324}) with *C*-terminal biotin



Ac-SHQESTRGRSRGRSGRSGS-Ttds-K(biotinyl-6-aminohexanoyl)-NH_2



Ac-SHQESTRGRSRGRSGRSGSK(biotinyl-6-aminohexanoyl)-NH_2



Ac-SHQESTRGRSRGRSGRSGSK(biotinyl-Ttds)-NH_2

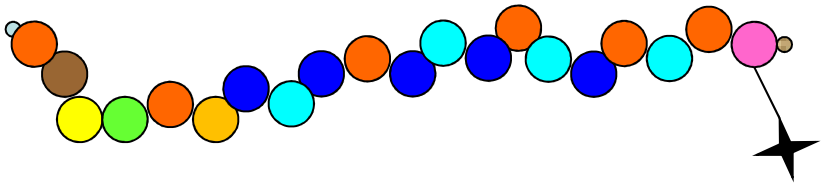
Characteristics of 19-mer epitope peptide (^{306}SHQESTRGRSRGRSGRSGS^{324}) with *C*-terminal biotin

Compound	M_{av} (calc)	M_{av} (meas)	R_t (min)
Ac-SHQESTRGRSRGRSGRSGSK(biotinyl-aminohexanoyl)-NH_2	2553,8	2553,8	14,23
Ac-SHQESTXGXSXGRSGRSGSK(biotinyl-aminohexanoyl)-NH_2	2556,8	2556,9	13,73
Ac-SHQESTRGRSRGRSGRSGSK(biotinyl-Ttds)-NH_2	2743,3	2743,4	15,25
Ac-SHQESTXGXSXGRSGRSGSK(biotinyl-Ttds)-NH_2	2746,3	2746,5	14,90
Ac-SHQESTRGRSRGRSGRSGS-Ttds-K(biotinyl-aminohexanoyl)-NH_2	2856,4	2856,5	17,65
Ac-SHQESTXGXSXGRSGRSGS-Ttds-K(biotinyl-aminohexanoyl)-NH_2	2859,4	2859,5	17,35

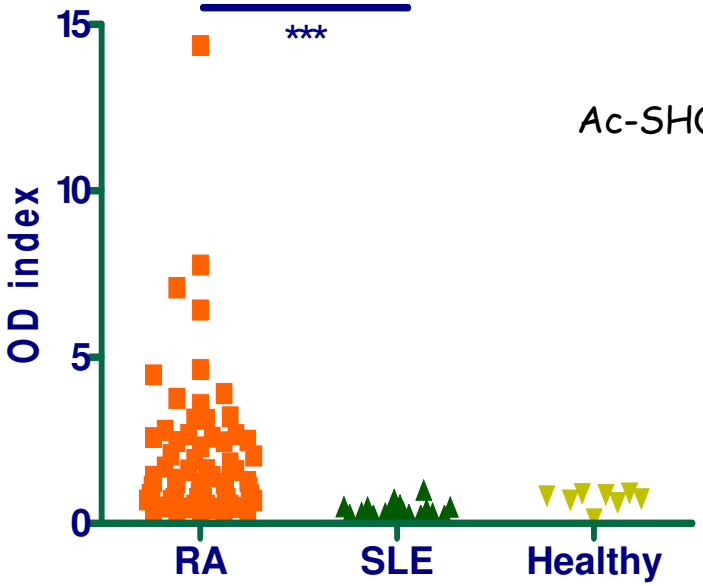
HPLC: KNAUER, Synergi MAX-RP, C12, 250 x 4mm, 5µm silica, 100 Å column, 5%B – 95 % B, 50 min, eluent A: 0,1% TFA/water (V/V); eluent B: 0,1% TFA/acetonitrile-water (80:20 V/V)

MS: Esquire 3000+

Antibody recognition of 19-mer epitope peptide with *C*- terminal biotin by RA, SLE and healthy samples



Ac-SHQESTXGXSXRSGRSGSK(biotinyl-6-aminohexanoyl)-NH_2

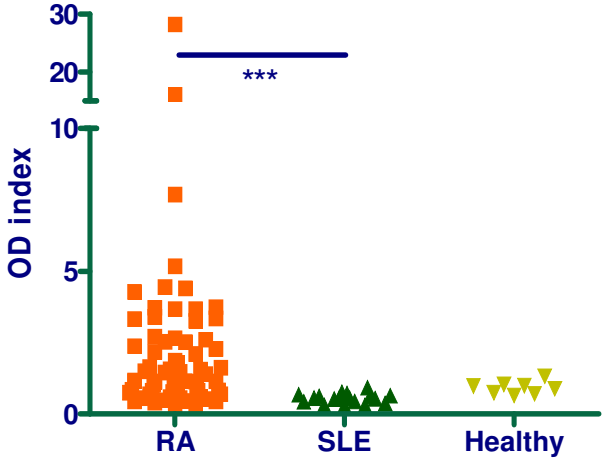


RA vs SLE	P < 0.001
RA vs Healthy	P > 0.05
SLE vs Healthy	P > 0.05

The peptide with *C*-terminal biotin is recognized.

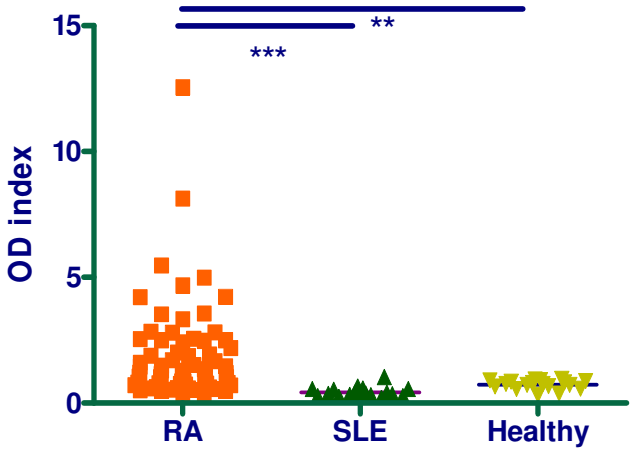
Antibody recognition of 19-mer epitope peptide with *N*- or *C*- terminal biotin

biotinyl-6-aminohexanoyl-
-SHQESTXGXSXGRSGRSGS-NH_2



RA vs SLE	P < 0.001
RA vs Healthy	P > 0.05
SLE vs Healthy	P > 0.05

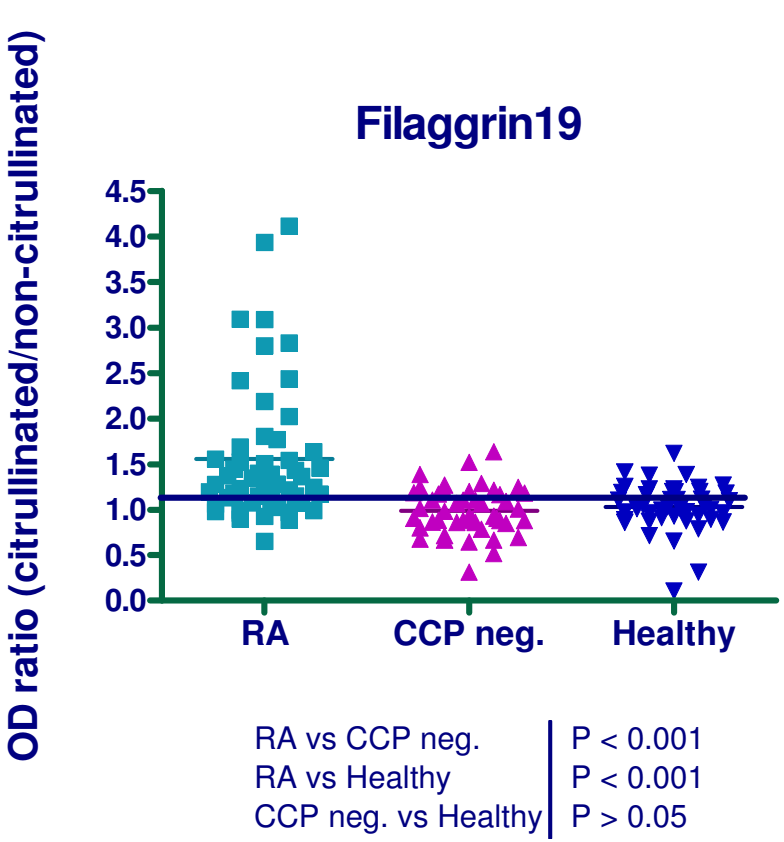
Ac-SHQESTXGXSXGRSGRSGSK
(biotinyl-aminohexanoyl)-NH_2

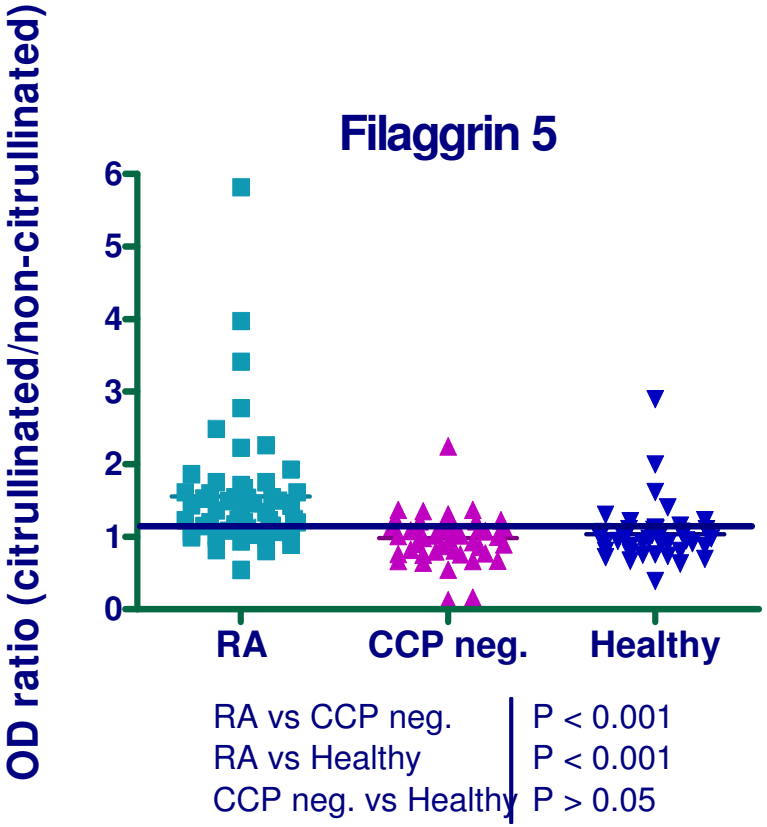


RA vs SLE	P < 0.001
RA vs Healthy	P < 0.01
SLE vs Healthy	P < 0.05

Both C- and N-terminal biotinylated 19-mer epitope peptides are recognized by RA sera samples

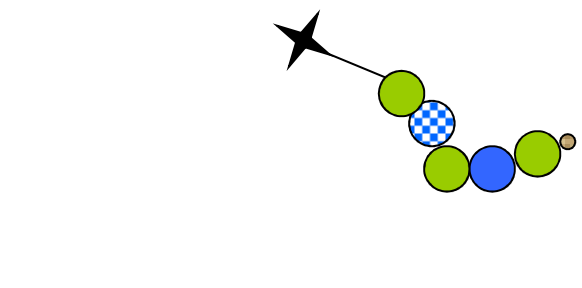
Comparison of antibody recognition of the 5-mer and the 19-mer epitope peptide

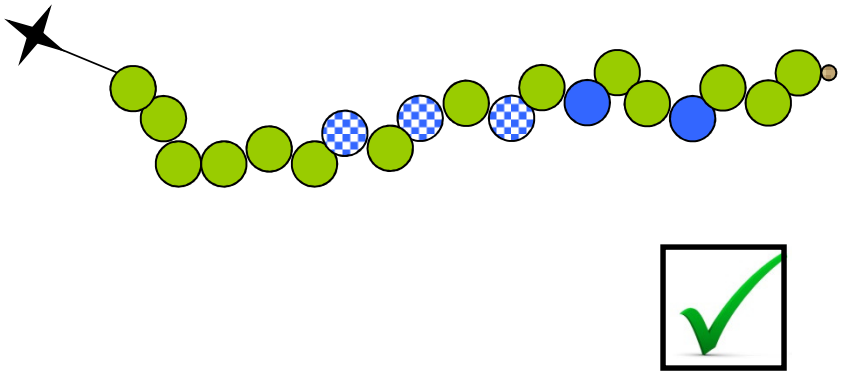




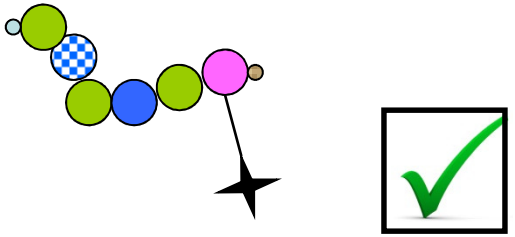
Short summary

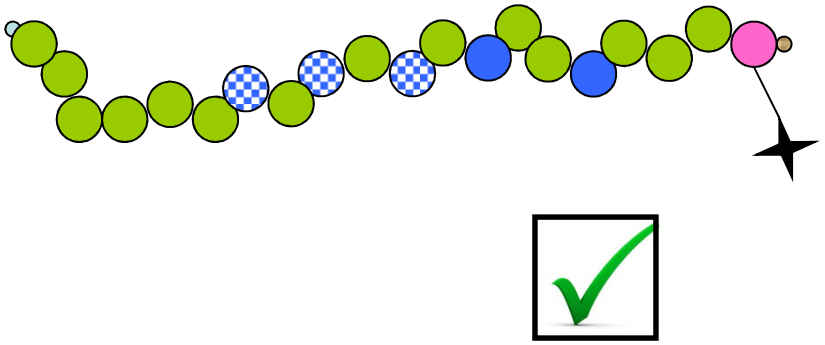
N-terminal biotinylation





C-terminal biotinylation





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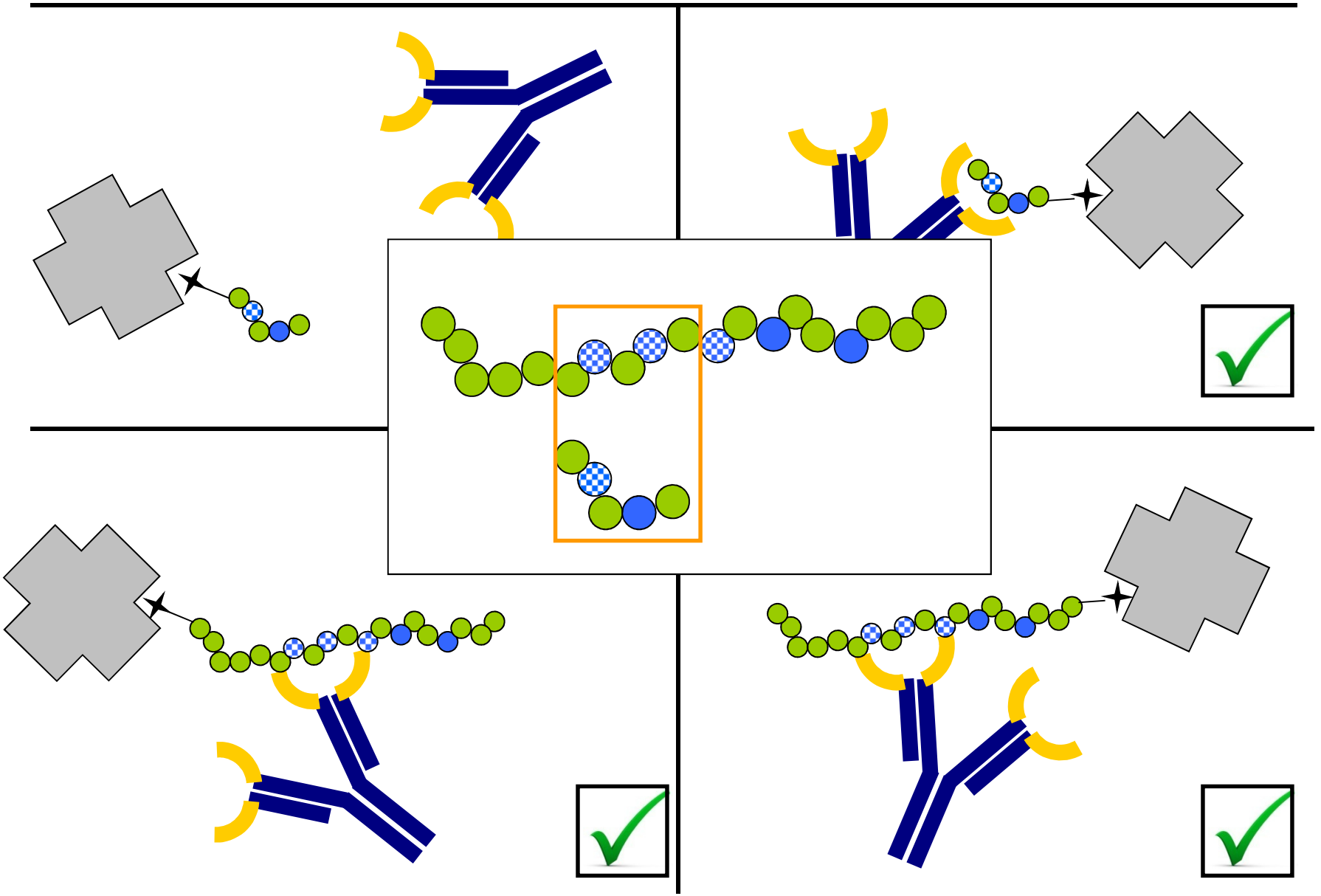
The position of the epitope core within the epitope region influence the antibody recognition



Conclusions

1. An epitope region (19-mer) and an epitope core (5-mer) were identified.

2. Introduction of biotin to the *N*-terminal of the 5-mer resulted in no binding. The presence of biotin at the C-teminal of the 5-mer had no effect on binding.

3. However, the presence of biotin at *N*- or *C*-terminal of the 19-mer has no effect on epitope recognition by serum antibodies.

4. The 5-mer as well as the 19-mer citrullinated peptides have shown a significantly higher reactivity with CCP^+ RA sera as compared to healthy controls, CCP^- serum samples.