

Predictive tools in the risk assessment of new proteins in GMOs: the case of Celiac Disease

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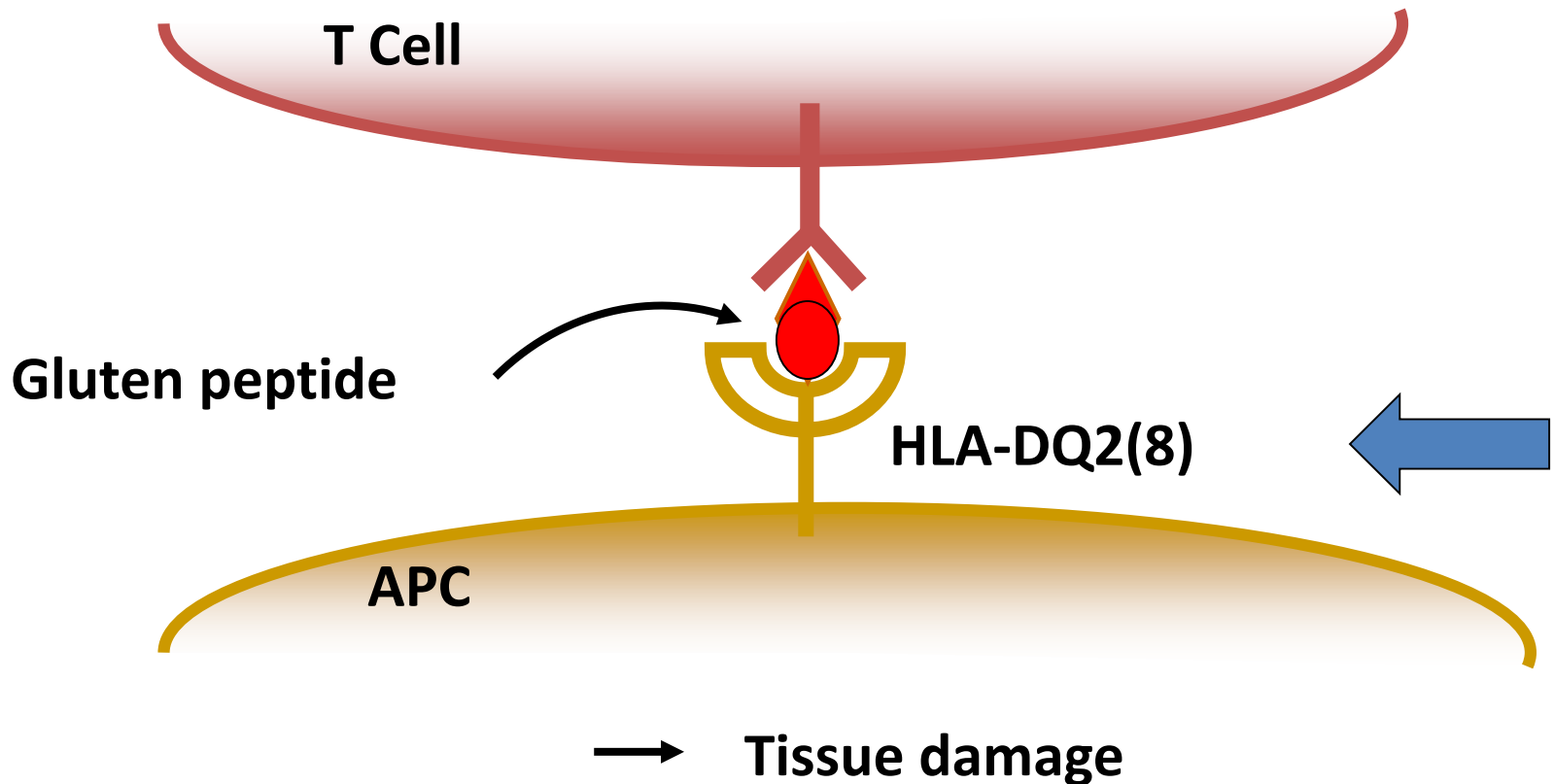
Celiac Disease Consortium

Gluten proteins in wheat

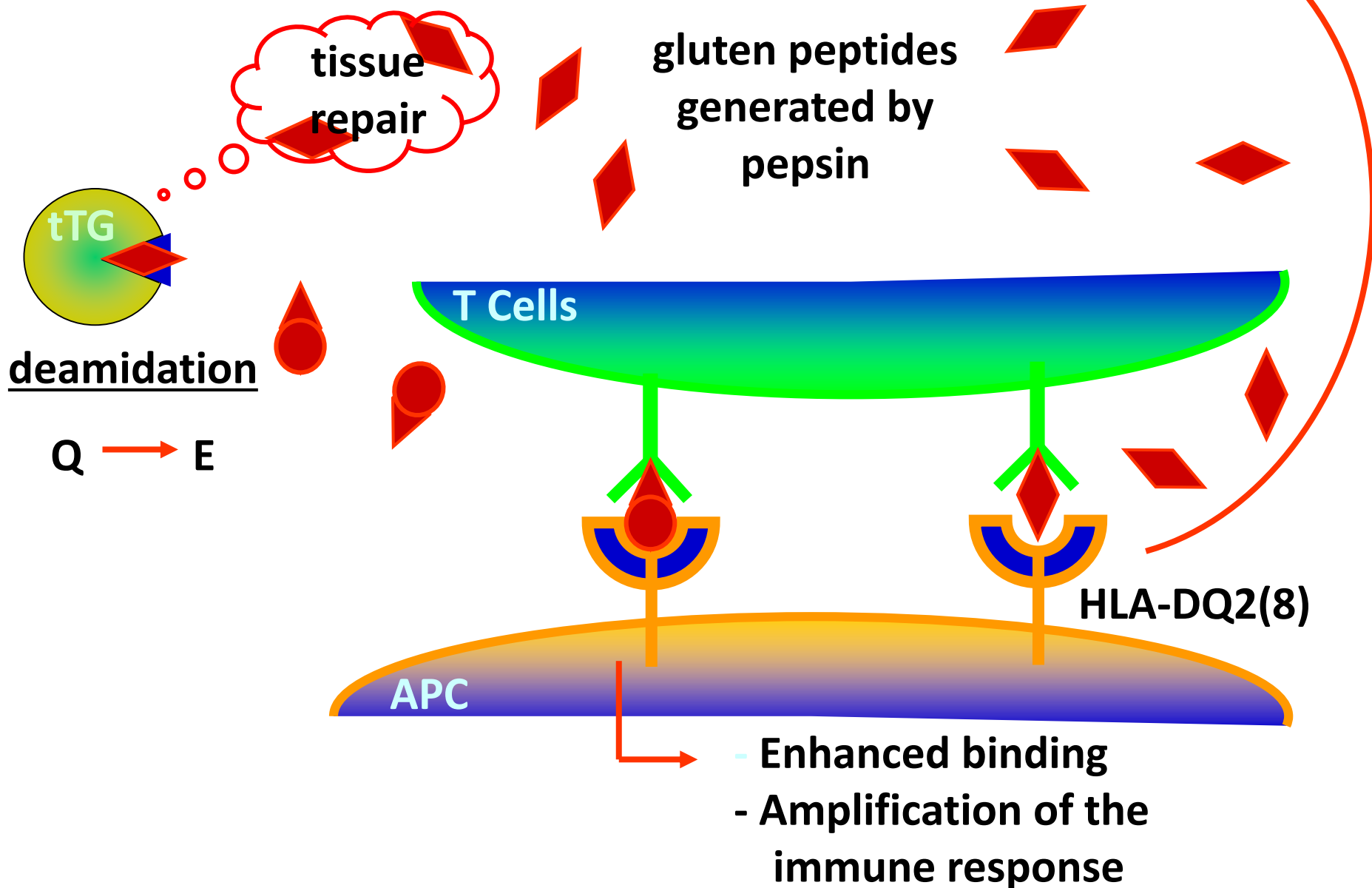
HLA-DQ2/8

T-cells

Gluten specific T cell response in the small intestine



Tissue damage: release of intracellular tTG



The specificity of tTG is determined by proline, the 2nd most abundant aa in gluten

Characteristic gluten sequences:

QP	no modification
QXP	yes
QXXP	no
QXPY or QXPF	yes

LGQQQPFPPQQPYPQPQPFPSQLPYLQLQPFPPQPQL

LGQEQPFPPEQPYPQPQPFPSELPYLQLQPFPPQPQL

Predict toxic gluten sequences?

	Gluten	Hordein	Secalin	Avenin	Tcells
	Wheat	Barley	Rye	Oats	
Search					
Algorithm	46	60	33	2	yes

Specificity of tissue transglutaminase explains cereal toxicity in celiac disease.

Vader, de Ru, van der Wal, Kooy, Benckhuijsen, Mearin, Drijfhout, van Veelen, and Koning. J. Exp. Med. 195: 643-649 (2002).

Identification of T cell stimulatory peptides in cereals

Gliadin (wheat) :

QLQPFPPQPLPYPPQ

PPPPQPLPY

PPQPLPYPPQ

Secalin (rye) :

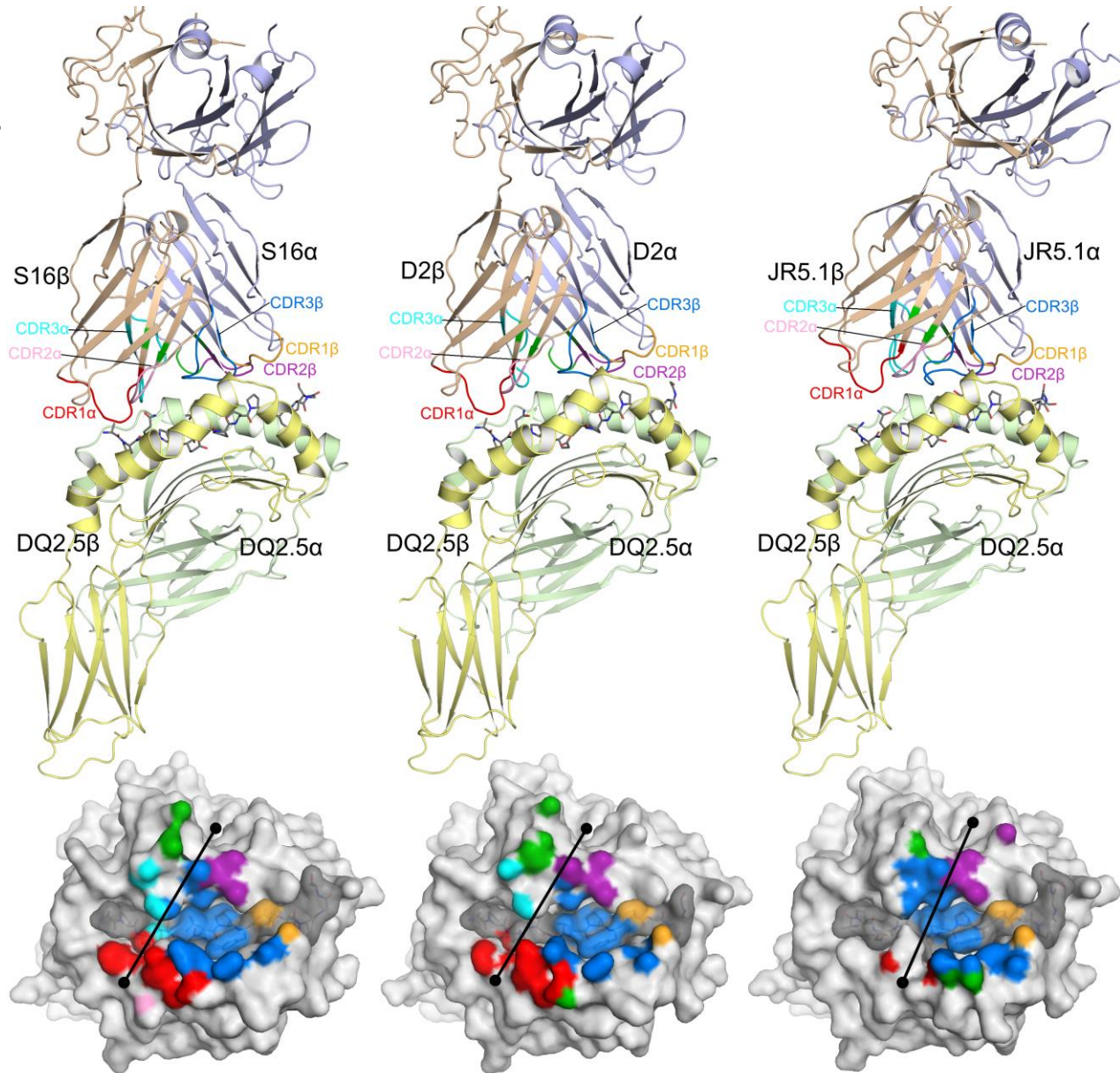
PPQPFPPPPQPFPPSQ

PPPPQPPQPF

PPPPQPFPPQ

DQ2-glia- α 2 recognition

TRAV26-01
TRBV7-02⁺



T cell receptor

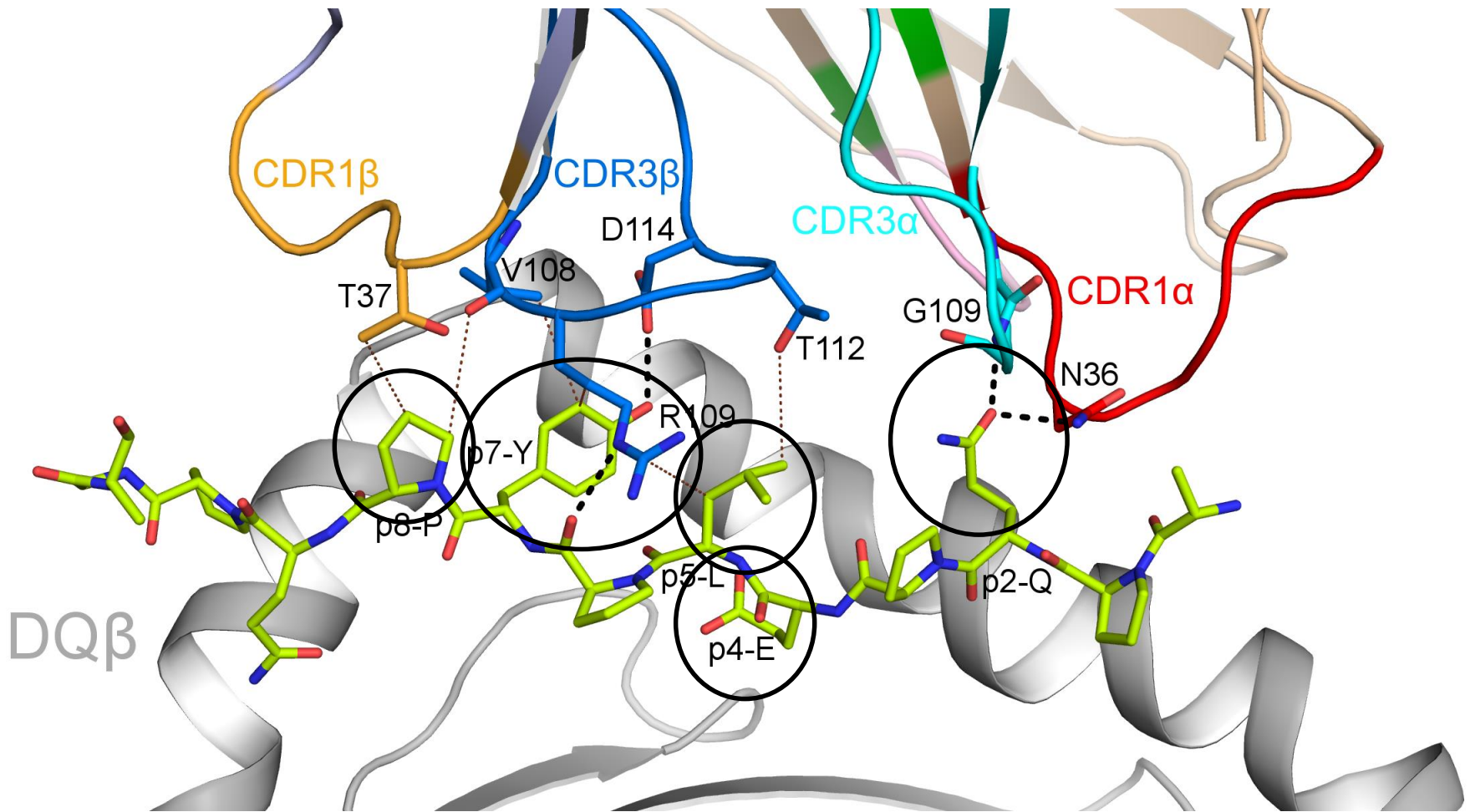
Peptide

DQ2

Conserved β -chain footprint

Petersen et al, NMSB 2014

DQ2-glia-a2 recognition: PQPQLPYPQ

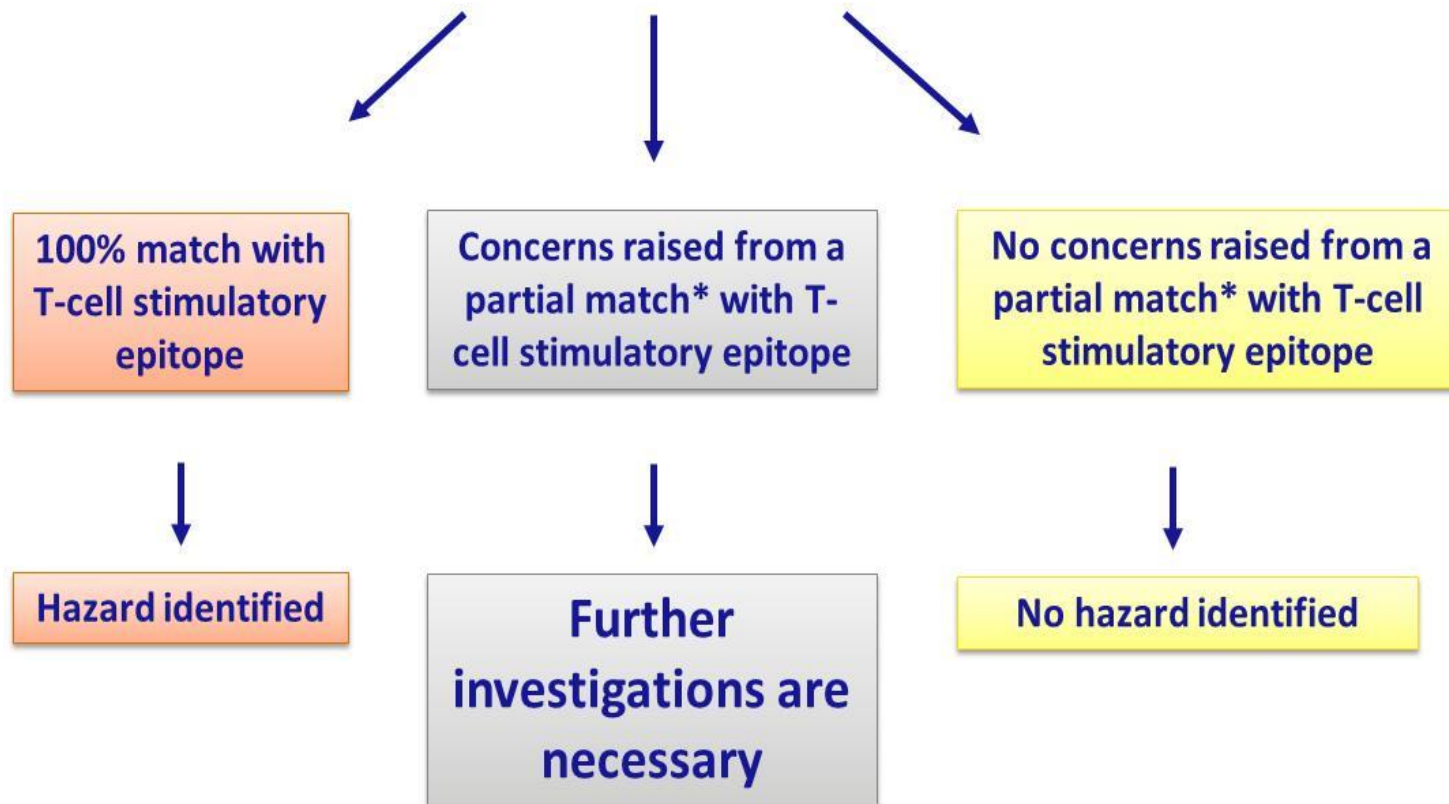


Bona fide toxicity of gluten for patients with celiac disease

- Well defined
- Mechanism underlying toxicity clear

RA of (novel) proteins: celiac disease

Fig 2. Search for sequence identity



*A partial match with a known T cell-stimulatory peptide raises concern because of the position and nature of the identical amino acids.

Celiac disease — DQ2 T-cell epitopes

DQ2 restricted epitopes

[Sollid et al., 2012. Immunogenetics, 64, 455-460](#)

Epitope	Motif	Reference
DQ2.5-glia- α 1a	P F P Q P Q L P Y	Arentz-Hansen et al. (2000)
DQ2.5-glia- α 1b	P Y P Q P Q L P Y	Arentz-Hansen et al. (2002)
DQ2.5-glia- α 2	P Q P Q L P Y P Q	Arentz-Hansen et al. (2000)
DQ2.5-glia- α 3	<div> E L P Y Q Q F F A S V E Q </div>	Vader et al. (2002b)
DQ2.5-glia- γ 1		Sjöström et al. (1998)
DQ2.5-glia- γ 2		Qiao et al. (2005), Vader et al. (2002b)
DQ2.5-glia- γ 3		Arentz-Hansen et al. (2002)
DQ2.5-glia- γ 4a		Arentz-Hansen et al. (2002)
DQ2.5-glia- γ 4b		Qiao et al. (2005)
DQ2.5-glia- γ 4c		Arentz-Hansen et al. (2002)
DQ2.5-glia- γ 4d		Qiao (unpublished)
DQ2.5-glia- γ 5		Arentz-Hansen et al. (2002)
DQ2.5-glia- ω 1		Tye-Din et al. (2010)
DQ2.5-glia- ω 2		Tye-Din et al. (2010)
DQ2.2-glut-L1		Vader et al. (2002b)
DQ2.5-glut-L2		Stepniak et al. (2005), Vader et al. (2002b)
DQ2.5-hor-1	<div> Q/E-X1-P-X2 </div>	Tye-Din et al. (2010), Vader et al. (2003)
DQ2.5-hor-2		Vader et al. (2003)
DQ2.5-sec-1		Tye-Din et al. (2010), Vader et al. (2003)
DQ2.5-sec-2	P Q P Q Q P F P Q	Vader et al. (2003)
DQ2.5-ave-1	P Y P E Q Q E P F	Arentz-Hansen et al. (2004), Vader et al. (2003)
DQ2.5-ave-1b	P Y P E Q Q Q P F	Arentz-Hansen et al. (2004), Vader et al. (2003)

Q-X-P-X

- PFPQPQLPY
- PQPQLPYPQ
- PXP in addition to QXPX is associated with the most immunogenic epitopes
- In contrast: PP is never found in T cell epitopes
- Positively charged amino acids in general diminish likelihood of DQ-binding and T cell recognition. Positive charge at p1, p4, p6, p7 and p9 bad for DQ-binding.

Celiac disease — DQ8 T-cell epitopes

[Sollid et al., 2012. Immunogenetics, 64, 455-460](#)

DQ8 restricted epitopes

Epitope	Motif	Reference
DQ8-glia- α 1	Q G S F Q P S Q Q	van de Wal et al. (1998b)
DQ8-glia- γ 1a	Q Q P Q Q P F P Q	Tollefsen et al. (2006)
DQ8-glia- γ 1b	Q Q P Q Q P Y P Q	Tollefsen et al. (2006)
DQ8-glut-H1	Q G Y Y P T S P Q	van de Wal et al. (1999)

Partial matches without the Q/E-X1-P-X2
to be investigated

Partial matches: Q/E-X1-P-X2 motif is present

PF**P**QP**Q**LPY and

AL**P**LT**Q**LPA

4 identical, two invisible, one conservative:
POTENTIAL HAZARD

PQP**Q**LPYPQ and

PLT**Q**LPASR

4 identical, one conservative BUT
Y > A, P > S and Q > R prohibit recognition:
NO HAZARD

Partial matches: Q/E-X1-P-X2 motif is NOT present

QGSFQPSQQ and

EGSIQAGQQ

5 identical, one conservative, one enhances
binding:

POTENTIAL HAZARD

QGSFQPSQQ and

QGLFSPSAQ

6 identical BUT

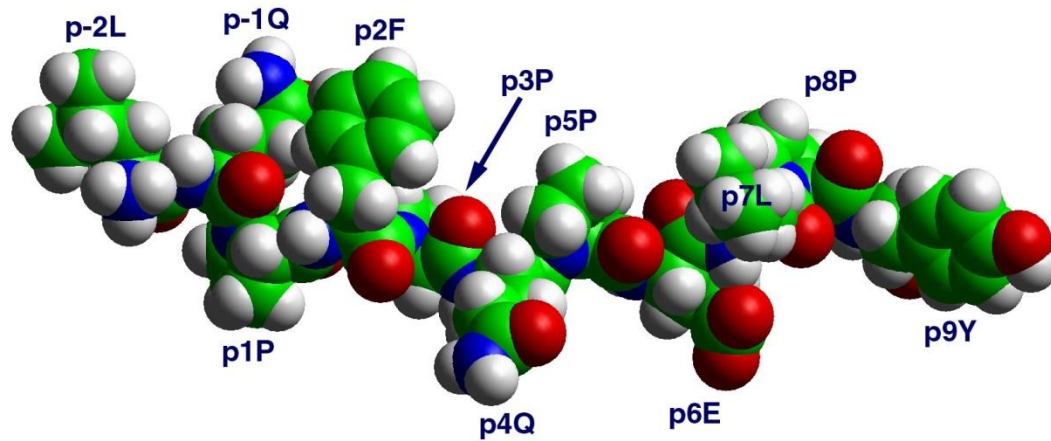
Critical T cell receptor contact residues differ:

NO HAZARD

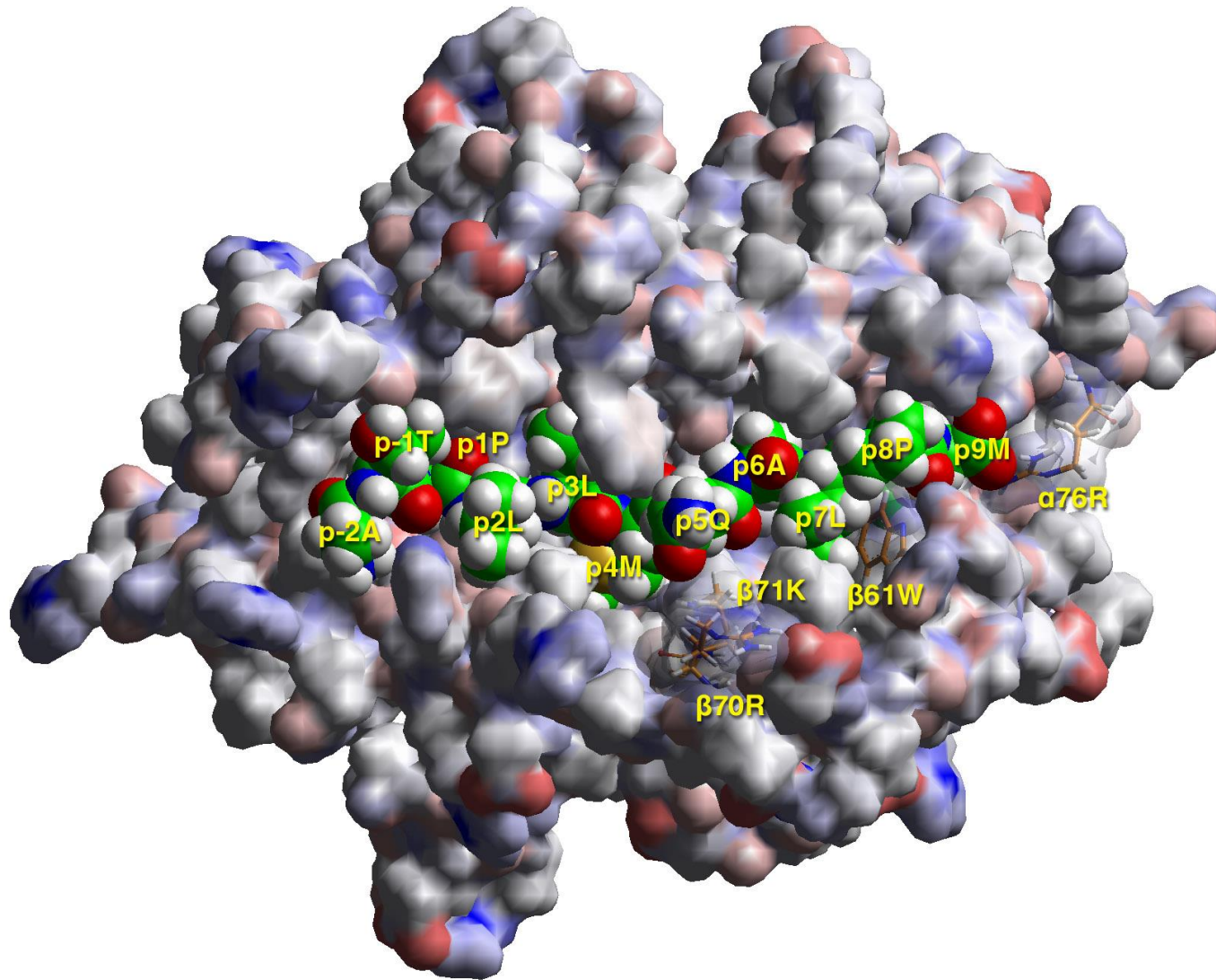
Peptide binding and Modelling

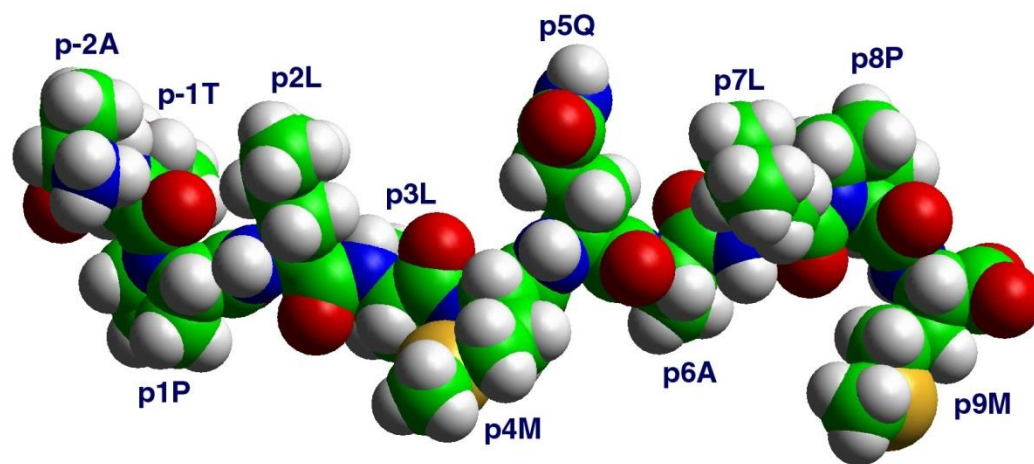
PFPQP ELPY

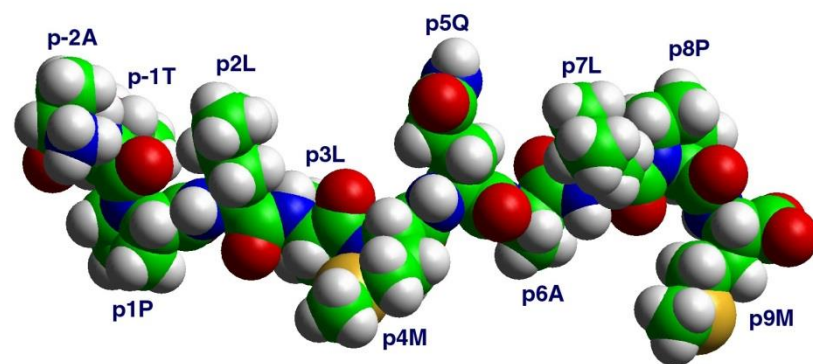
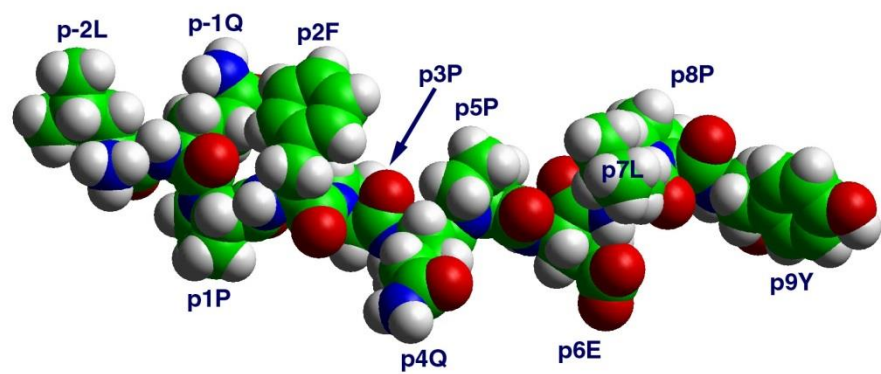
PLLMQ ALPM



PLLMQALPM







Molecular mimicry?

Cross-reactivity between microbial antigens and gluten epitopes?

Glia- α 1

PFPQPELPY

Bacterial peptide 1

6 matches

Bacterial peptide 2

5 matches

Glia- α 2

PQPELPYPQ

Bacterial peptide 3

7 matches

Bacterial peptide 4

5 matches

All have the Q/E-X1-P-X2 motif

Conclusion

Potential antigenicity can be predicted

AND YES

There are bacterial peptides that trigger gluten-specific T cells