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

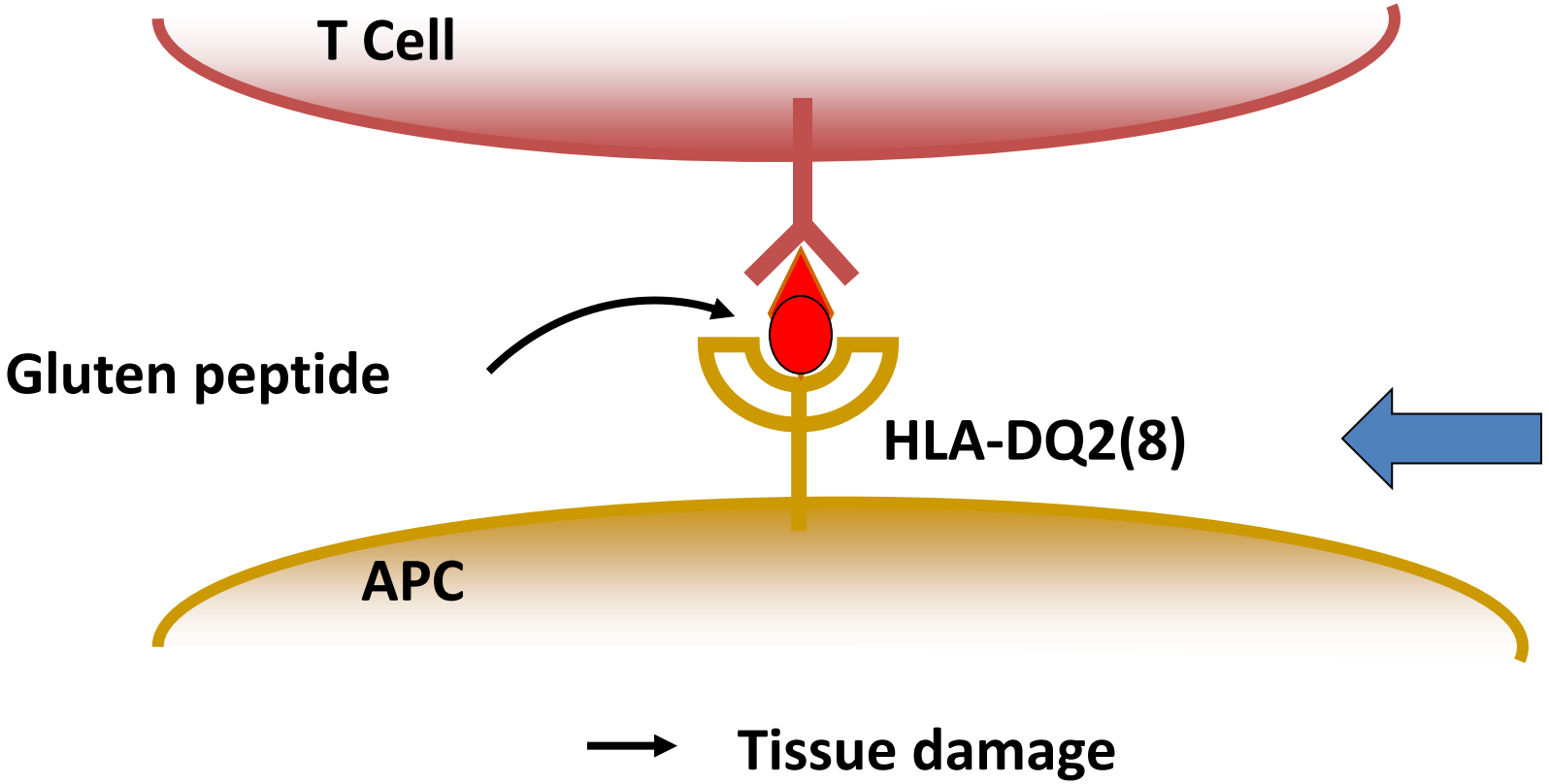
Frits Koning
LUMC, Leiden, The Netherlands
Celiac Disease Consortium

Gluten proteins in wheat

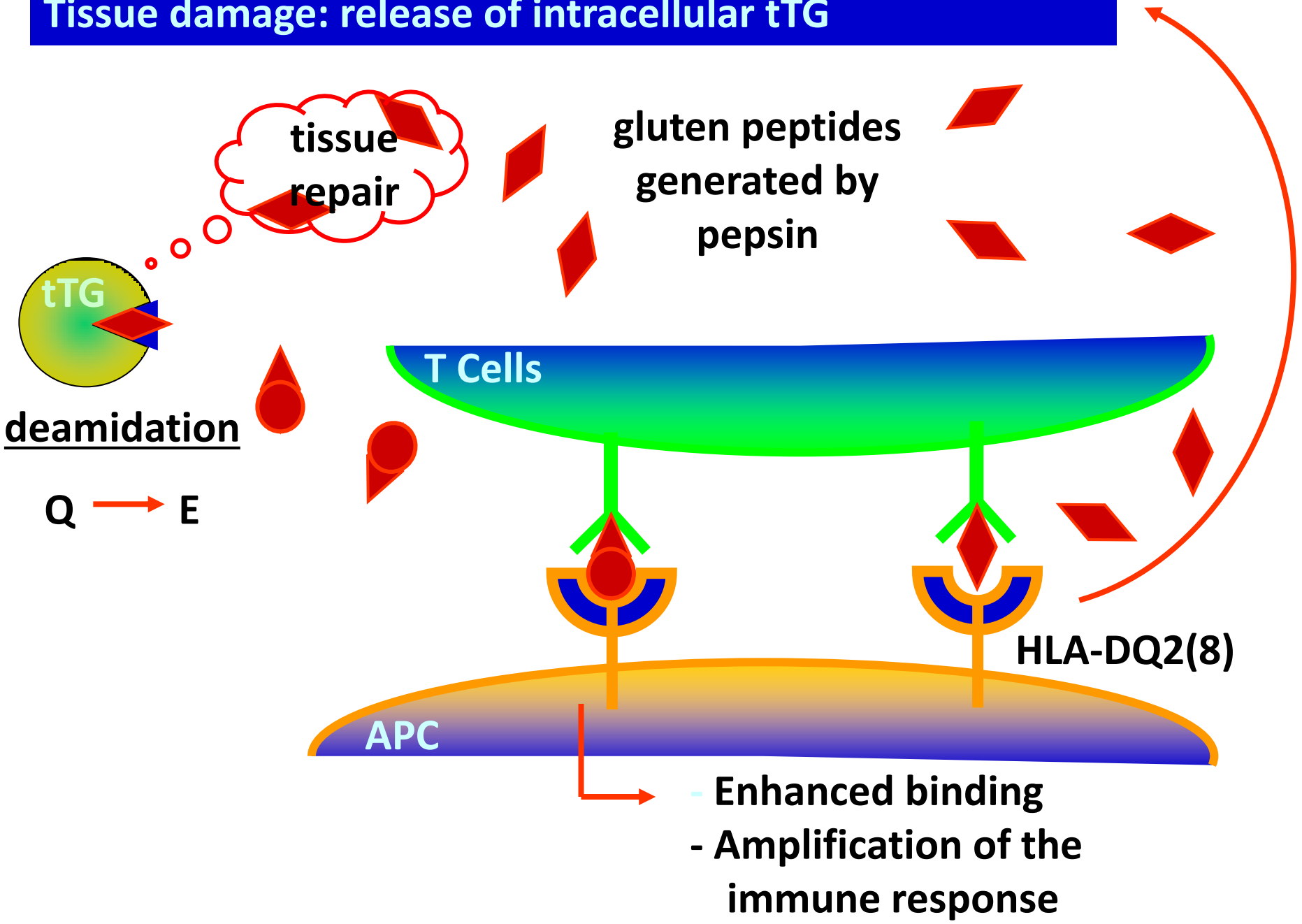
HLA-DQ2/8

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

LGQQQPFPPQQPYPQPQPFPSQLPYLQLQPFPQPQL

LGQEQPFPPEQPYPQPQPFPSELPYLQLQPFPQPQL

Predict toxic gluten sequences?

	Gluten Wheat	Hordein Barley	Secalin Rye	Avenin Oats	Tcells
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).

LUMC

Identification of T cell stimulatory peptides in cereals

***Gliadin* (wheat):**

QLQPFPQPQLPYPQPQ

PFPQPQLPY

PQPQLPYPQ

***Secalin* (rye):**

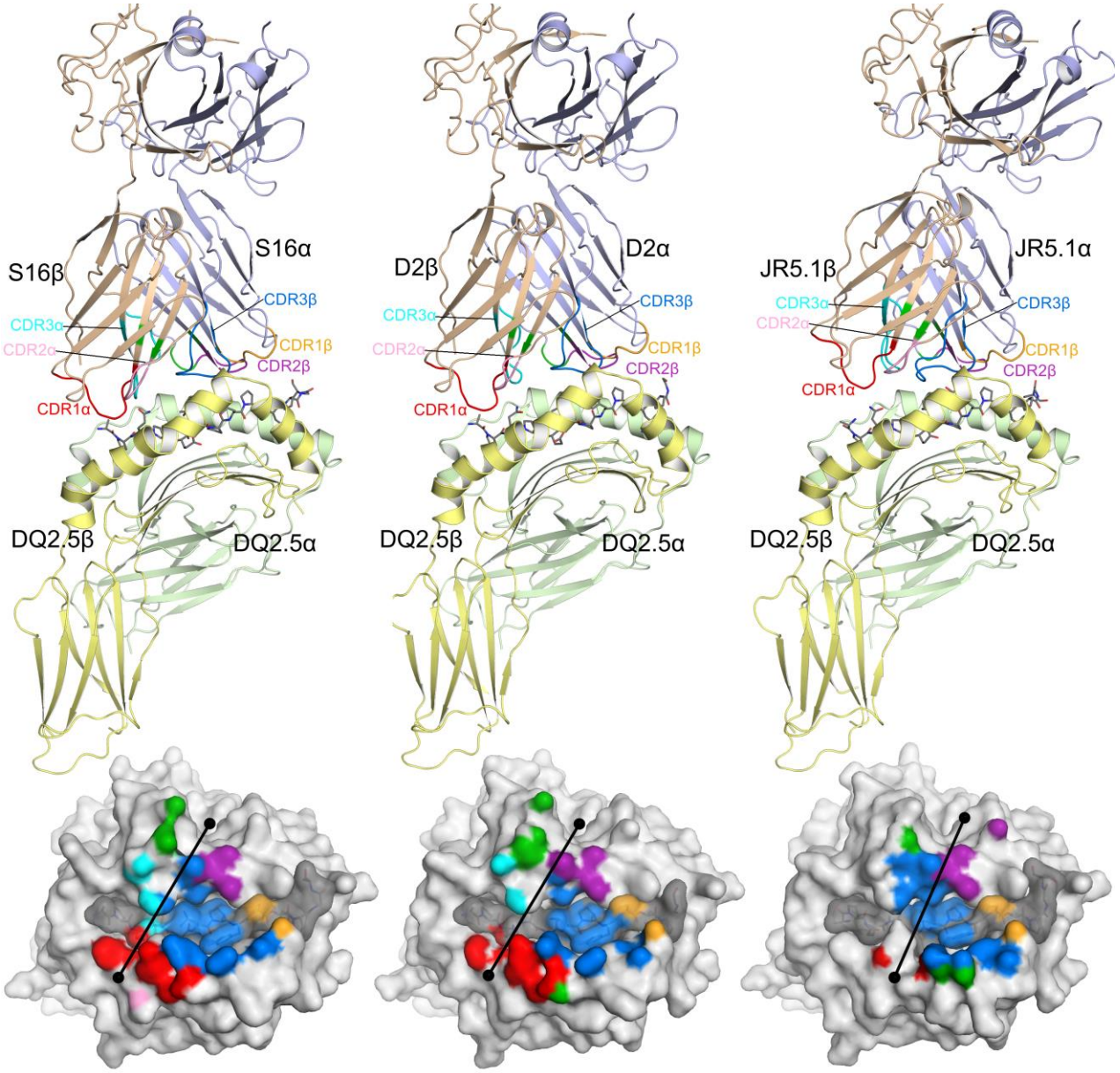
PQQPFPQPQQPFPQSQ

PFPQPQQPF

PQPQQPFPQ

DQ2-glia-α2 recognition

TRAV26-01
TRBV7-02⁺



T cell receptor

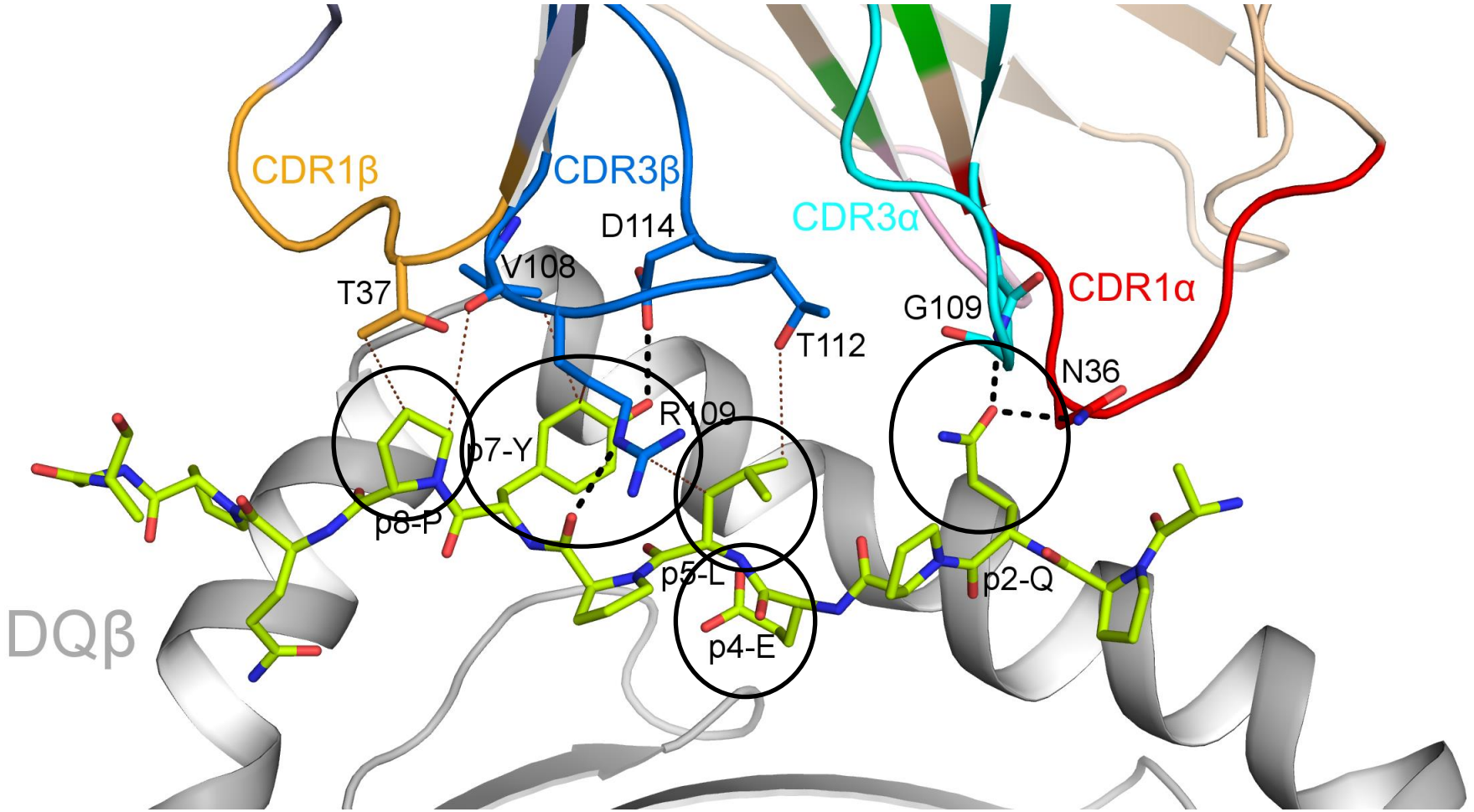
Peptide

DQ2

Conserved β-chain footprint

Petersen et al, NMSB 2014

DQ2-glia-a2 recognition: PQPQLPYPQ

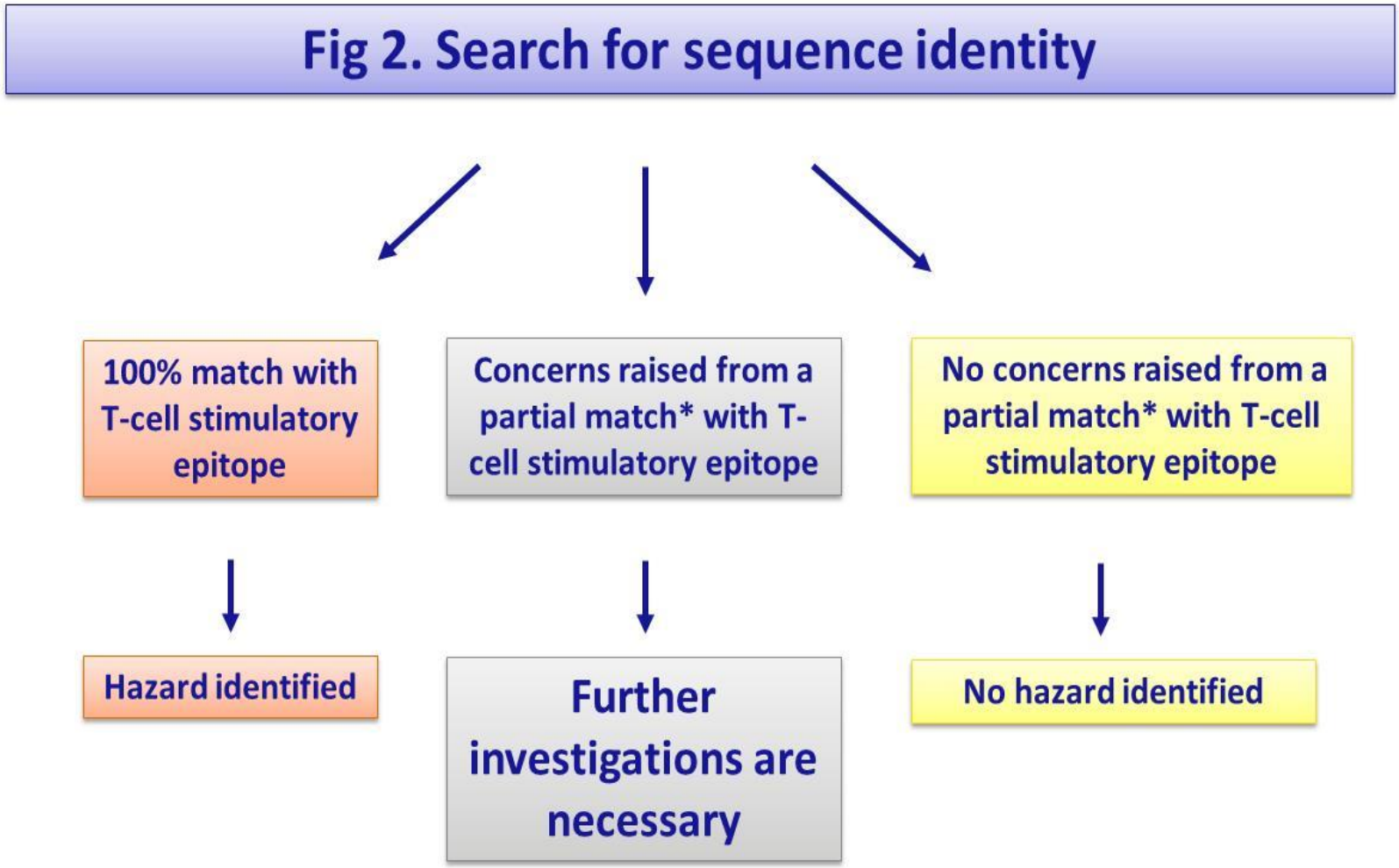


Broughton Immunity 2012; Petersen et al, NMSB 2014; Petersen JI 2015

Bona fide toxicity of gluten for patients with celiac disease

- Well defined
- Mechanism underlying toxicity clear

RA of (novel) proteins: celiac disease



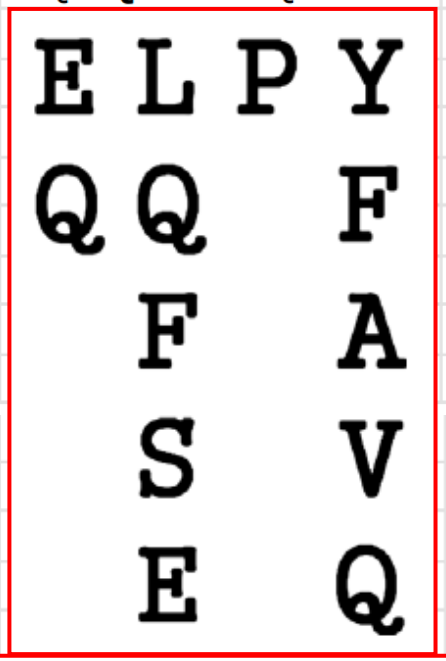
*A partial match with a known T cell-stimulatory peptide raises concern because of the position and nature of the identical amino acids.

Celiac disease — DQ2 T-cell epitopes

DQ2 restricted epitopes

Sollid et al., 2012. Immunogenetics, 64, 455-460

Epitope	Motif	Reference
DQ2.5-glia-α1a	P F P Q P **Q L P Y**	Arentz-Hansen et al. (2000)
DQ2.5-glia-α1b	P Y P Q P **Q L P Y**	Arentz-Hansen et al. (2002)
DQ2.5-glia-α2	P Q P **Q L P Y** P Q	Arentz-Hansen et al. (2000)
DQ2.5-glia-α3		Vader et al. (2002b)
DQ2.5-glia-γ1		Sjöström et al. (1998)
DQ2.5-glia-γ2		Qiao et al. (2005), Vader et al. (2002b)
DQ2.5-glia-γ3		Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4a		Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4b		Qiao et al. (2005)
DQ2.5-glia-γ4c		Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4d		Qiao (unpublished)
DQ2.5-glia-γ5		Arentz-Hansen et al. (2002)
DQ2.5-glia-ω1		Tye-Din et al. (2010)
DQ2.5-glia-ω2		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		ye-Din et al. (2010), Vader et al. (2003)
DQ2.5-hor-2		ader et al. (2003)
DQ2.5-sec-1		ye-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/E-X1-P-X2

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-α1	**Q** G S F Q P S Q **Q**	van de Wal et al. (1998b)
DQ8-glia-γ1a	**Q** Q P Q Q P F P **Q**	Tollefsen et al. (2006)
DQ8-glia-γ1b	**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

PFPQPQLPY and

ALPLTQLPA

4 identical, two invisible, one conservative:

POTENTIAL HAZARD

PQPQLPYPQ and

PLTQLPASR

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

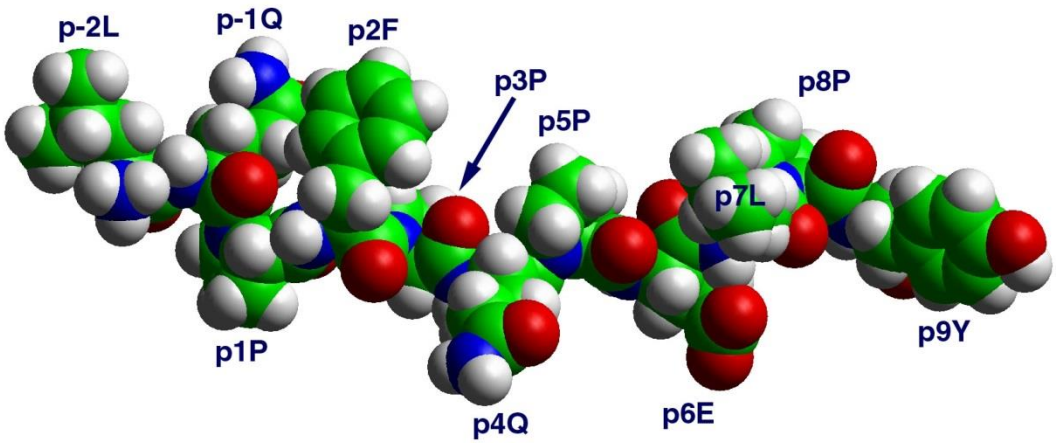
Critical T cell receptor contact residues differ:

NO HAZARD

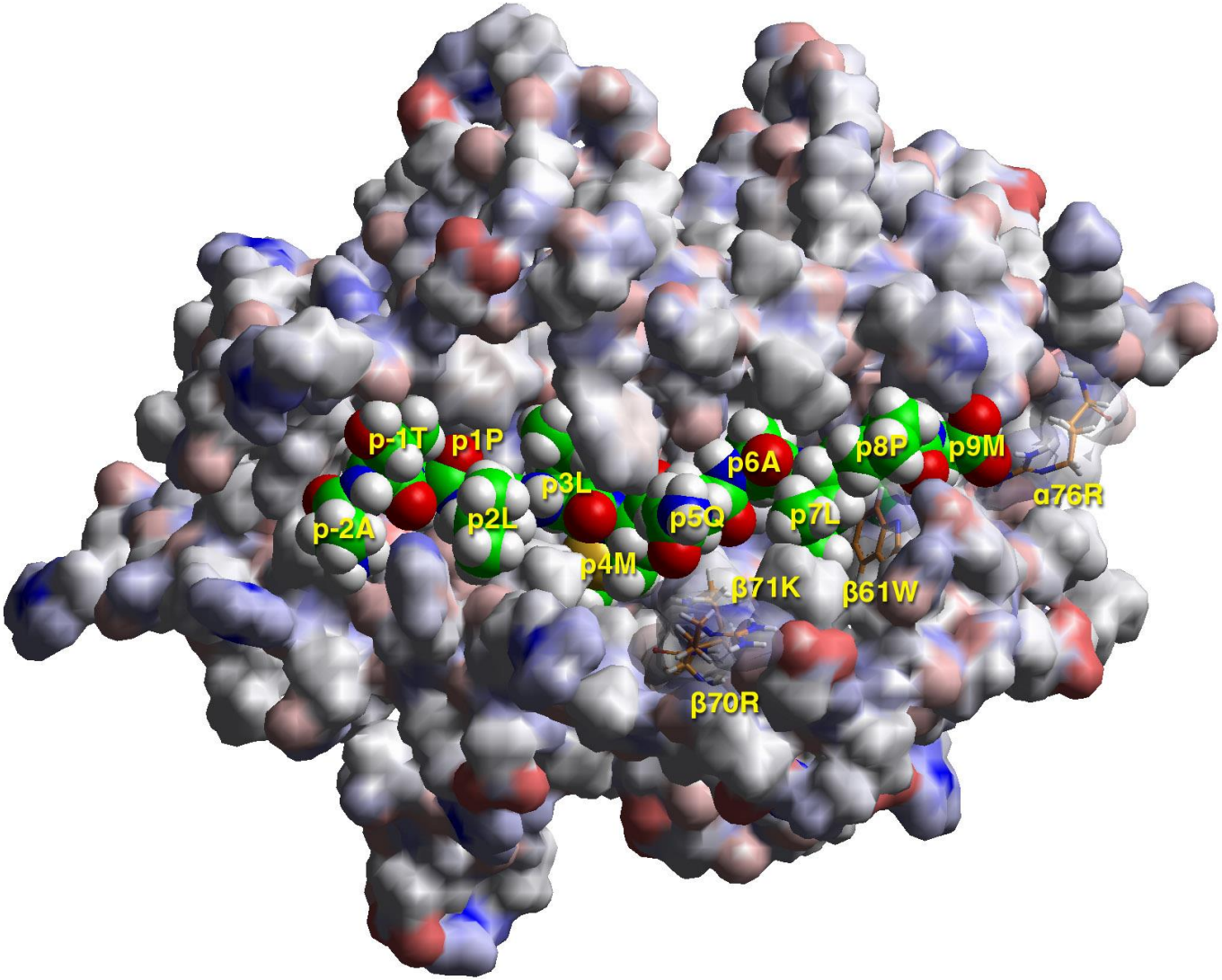
Peptide binding and Modelling

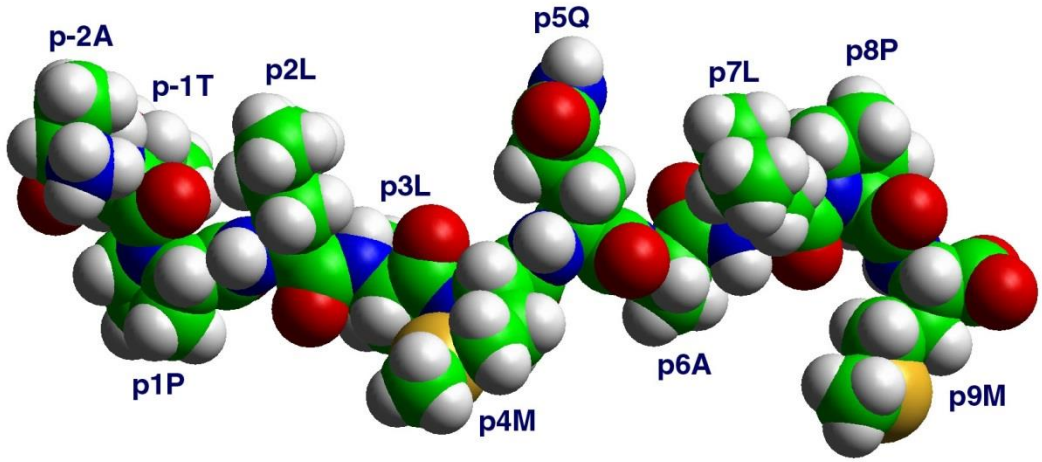
PFPQP ELPY

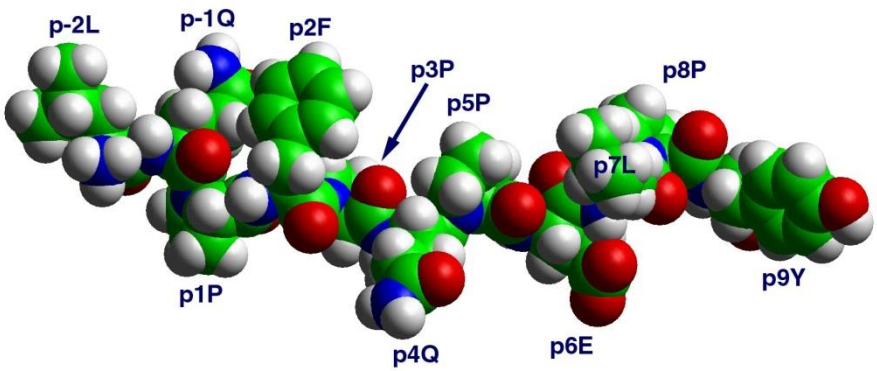
PLLMQ ALPM

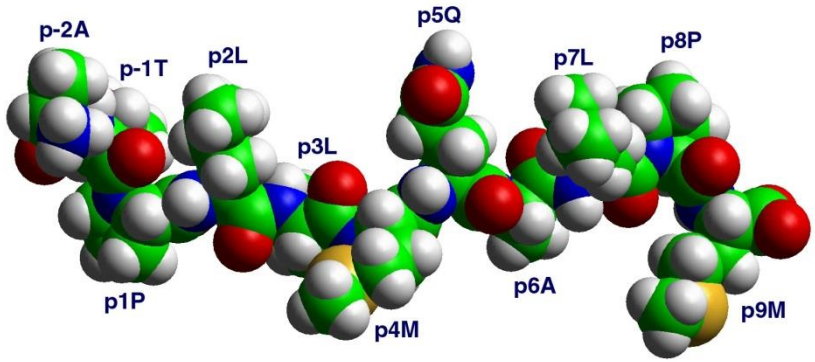


PLLMQALPM




p-2A
p-1T
p2L
p5Q
p8P
p7L
p3L
p1P
p4M
p6A
p9M


p-2L
p-1Q
p2F
p3P
p5P
p8P
p7L
p1P
p4Q
p6E
p9Y


p-2A
p-1T
p2L
p5Q
p3L
p7L
p8P
p1P
p4M
p6A
p9M

Conclusion

Potential antigenicity can be predicted