



New serological biomarkers of celiac disease: The neo-epitope tTg/mTg story

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Human Transglutaminase Family

The TG family

TG1: keratinocyte transglutaminase

TG2: tissue transglutaminase

TG3: epidermal transglutaminase

TG4: prostate transglutaminase

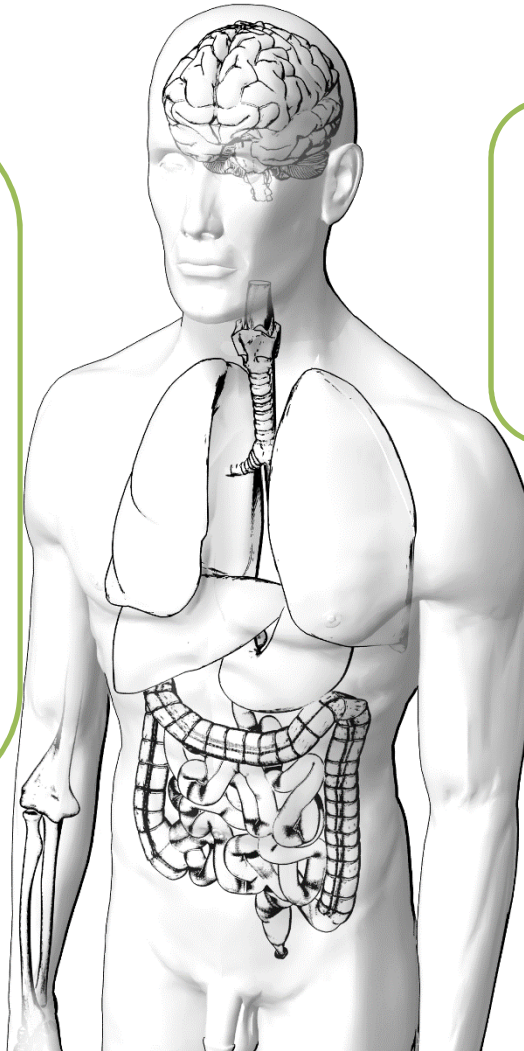
TG5: transglutaminase 5

TG6: neuronal transglutaminase

TG7: transglutaminase 7

FXIII: blood coagulation factor XIII

B4.2: inactive structural protein



Functions

Post translational
modifier of proteins:

- Cross-linking
- Deamidation

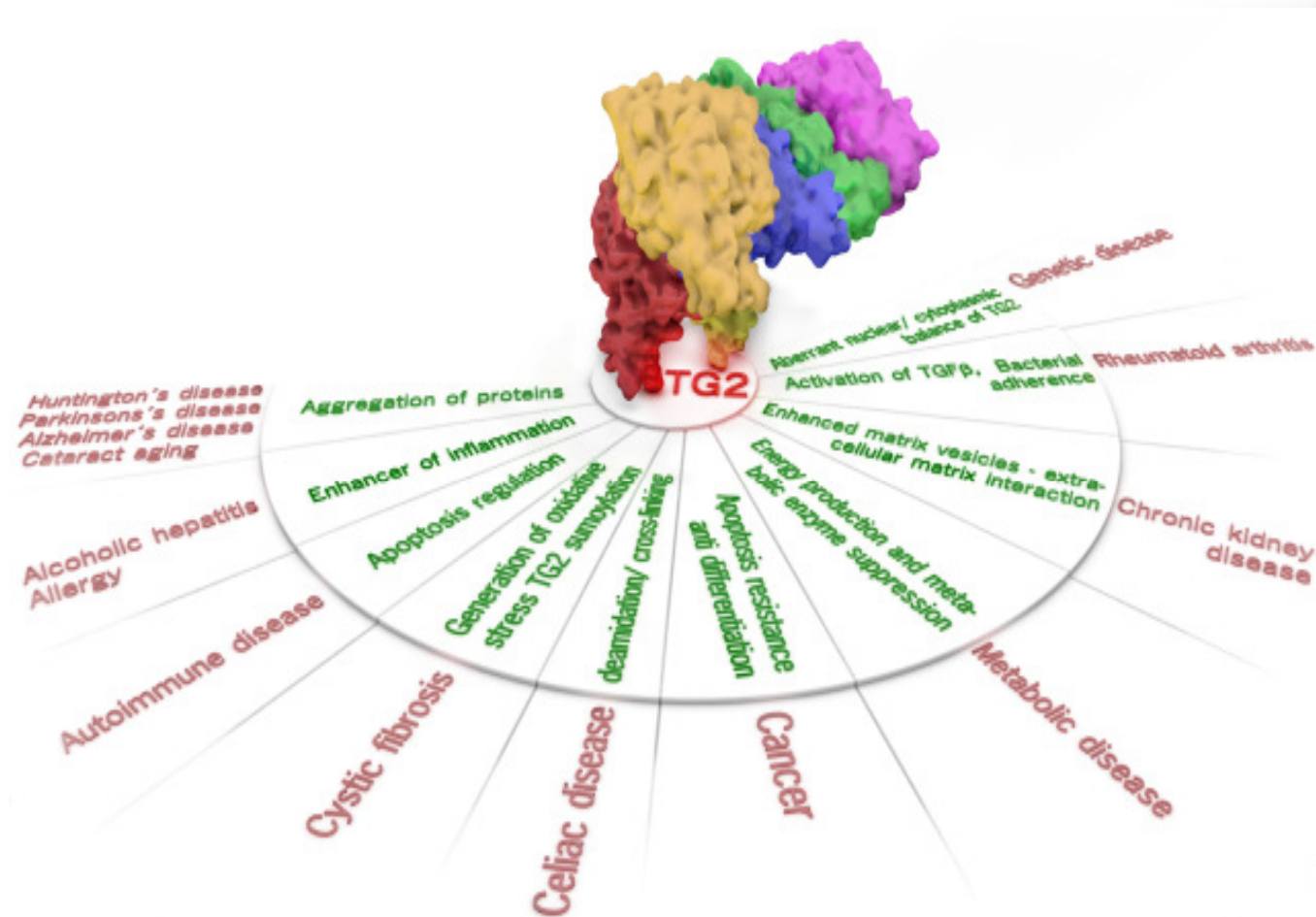
Autoimmune Diseases

Celiac Disease

Dermatitis Herpetiformis

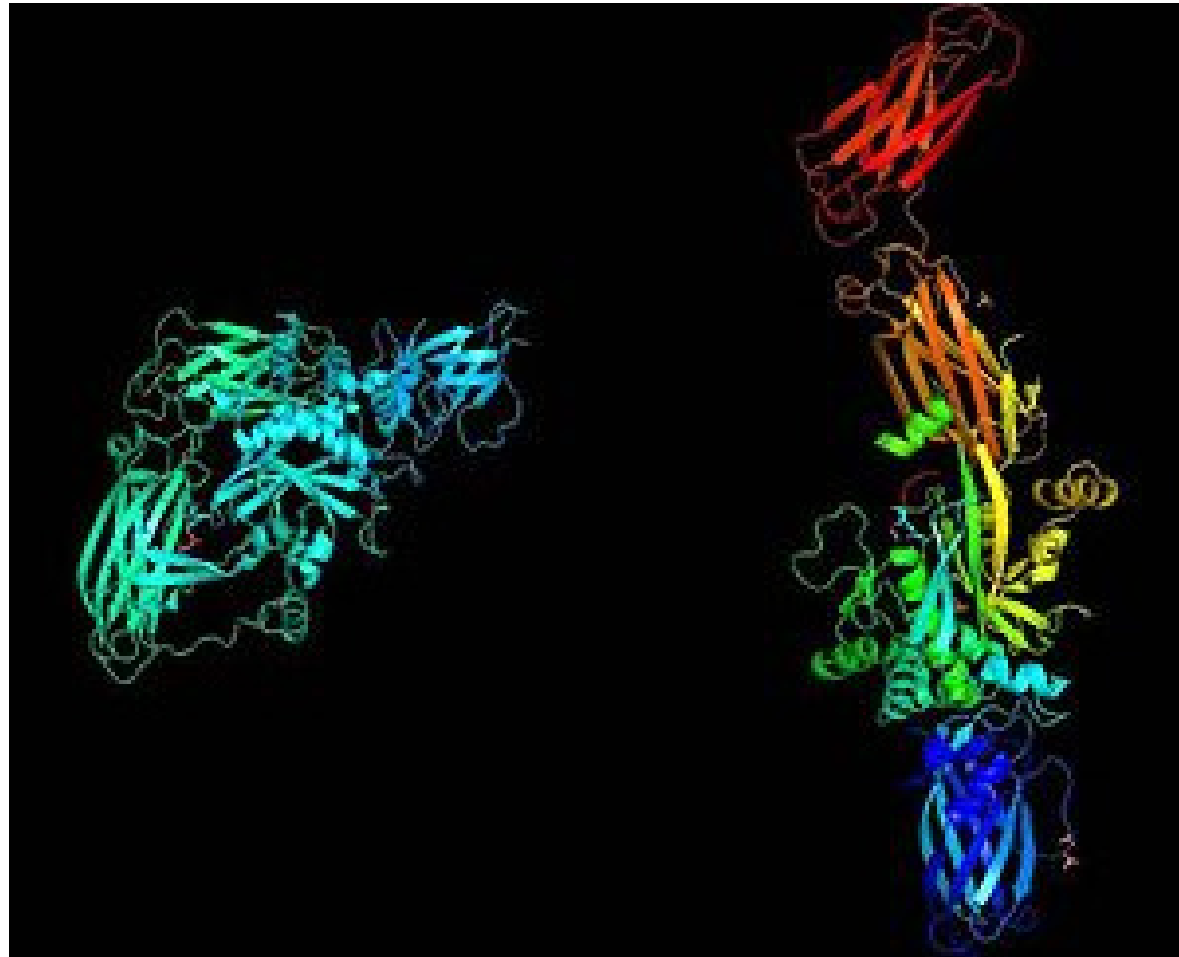
Gluten Ataxia

Tissue Transglutaminase (TG2) :central role in biological processes and diseases

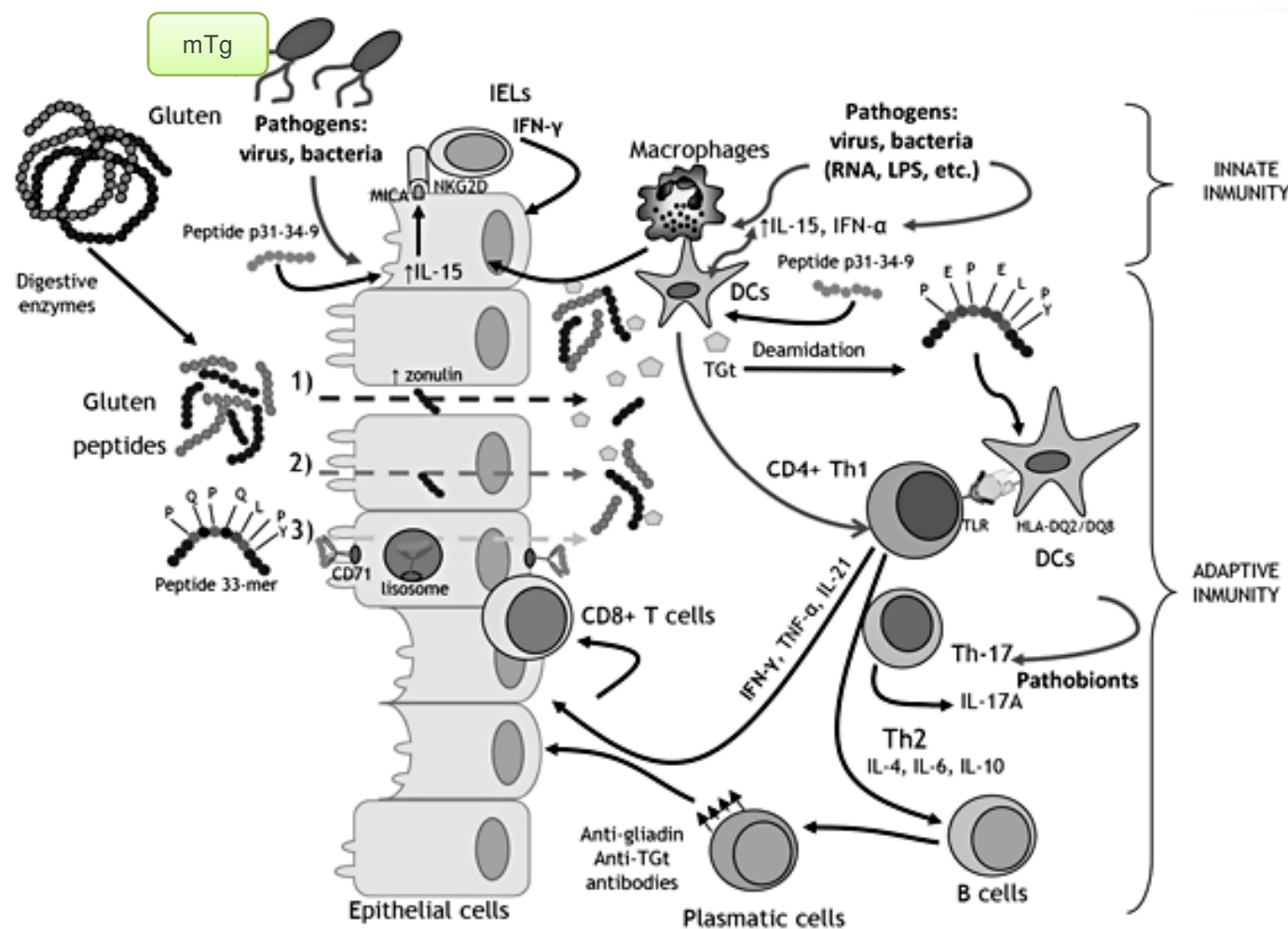


Lerner A, Neidhöfer S, Matthias T. **Transglutaminase 2 and anti transglutaminase 2 autoantibodies in celiac disease and beyond: Part A: TG2 double-edged sword: gut and extraintestinal involvement.** Immunome Research, 2015;11:101-105.

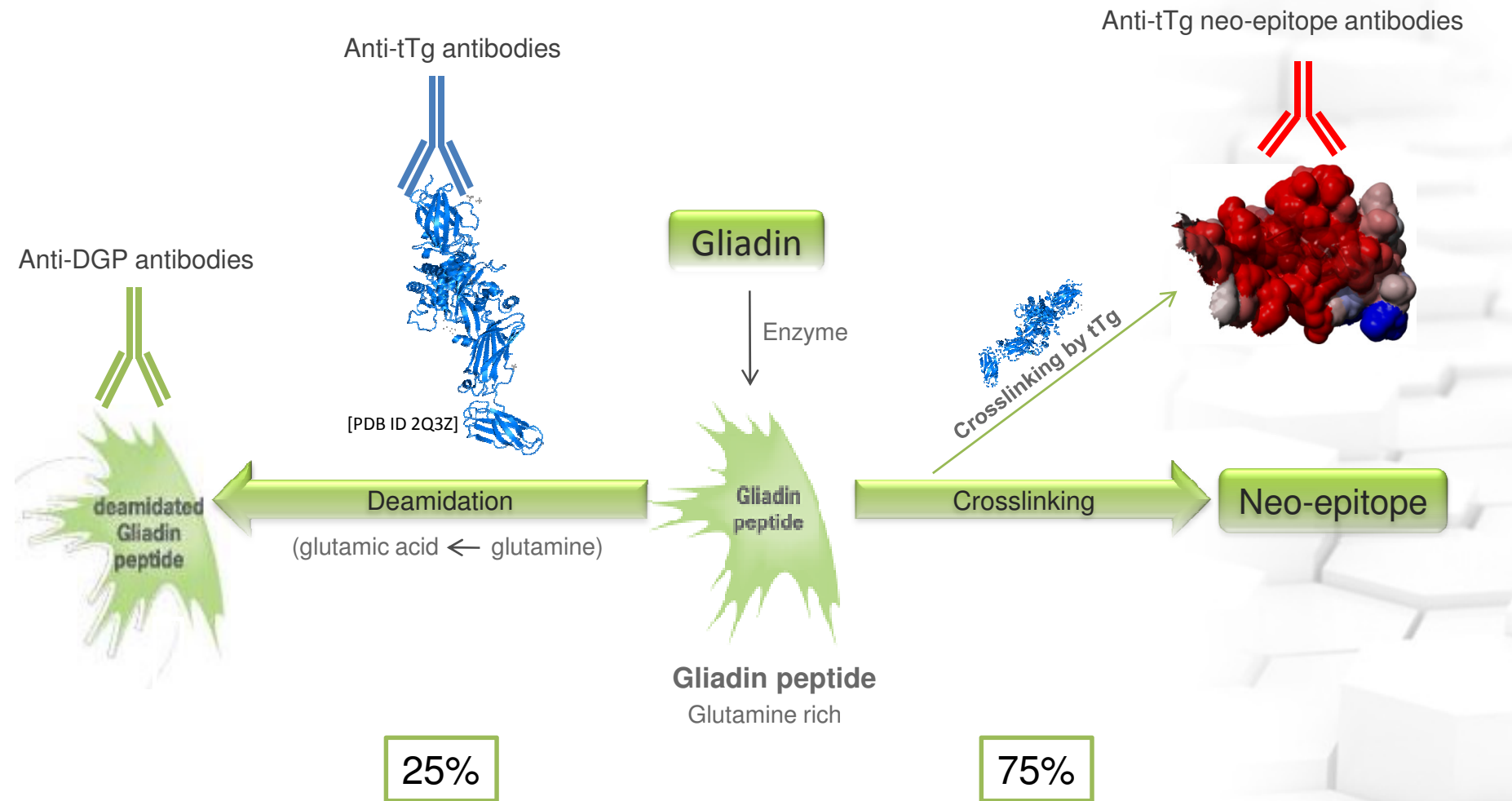
Compact (inactive) and extended (active) conformations of tTG



Schematic representation of the proposed model for CD pathogenesis



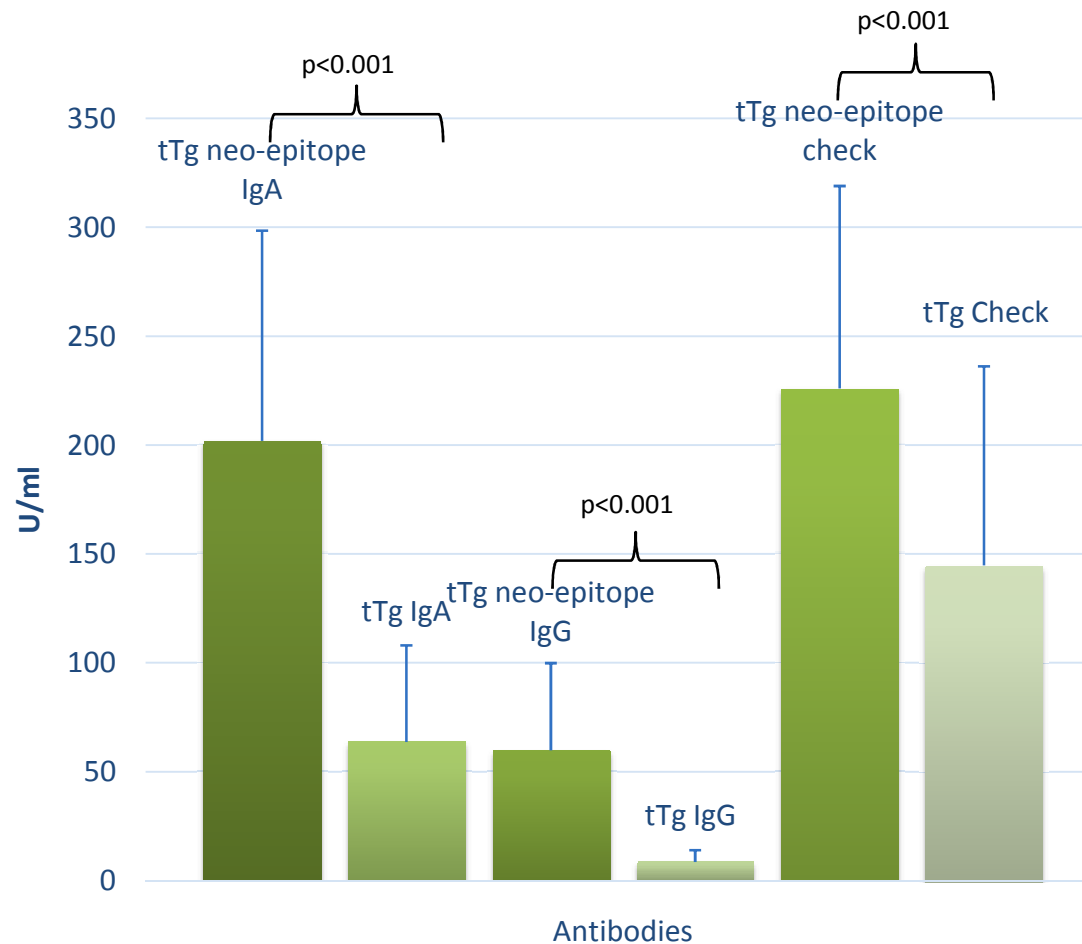
Antibodies in Celiac Disease



Courtesy of Dr. Christian Meesters, AESKU.KIPP Institute 2012//

A. Lerner et al., Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable than anti-tTg for the diagnosis of pediatric celiac disease (Journal of Immunological Methods 429 (2016) 15-20)

Overall immunoreactivity in PCD patients



A significantly higher OD activity was detected for tTg neo-epitope IgA, IgG and IgA+IgG than for tTg

Diagnostic power of neo-epitopes

n=198 pediatric samples of an actual study

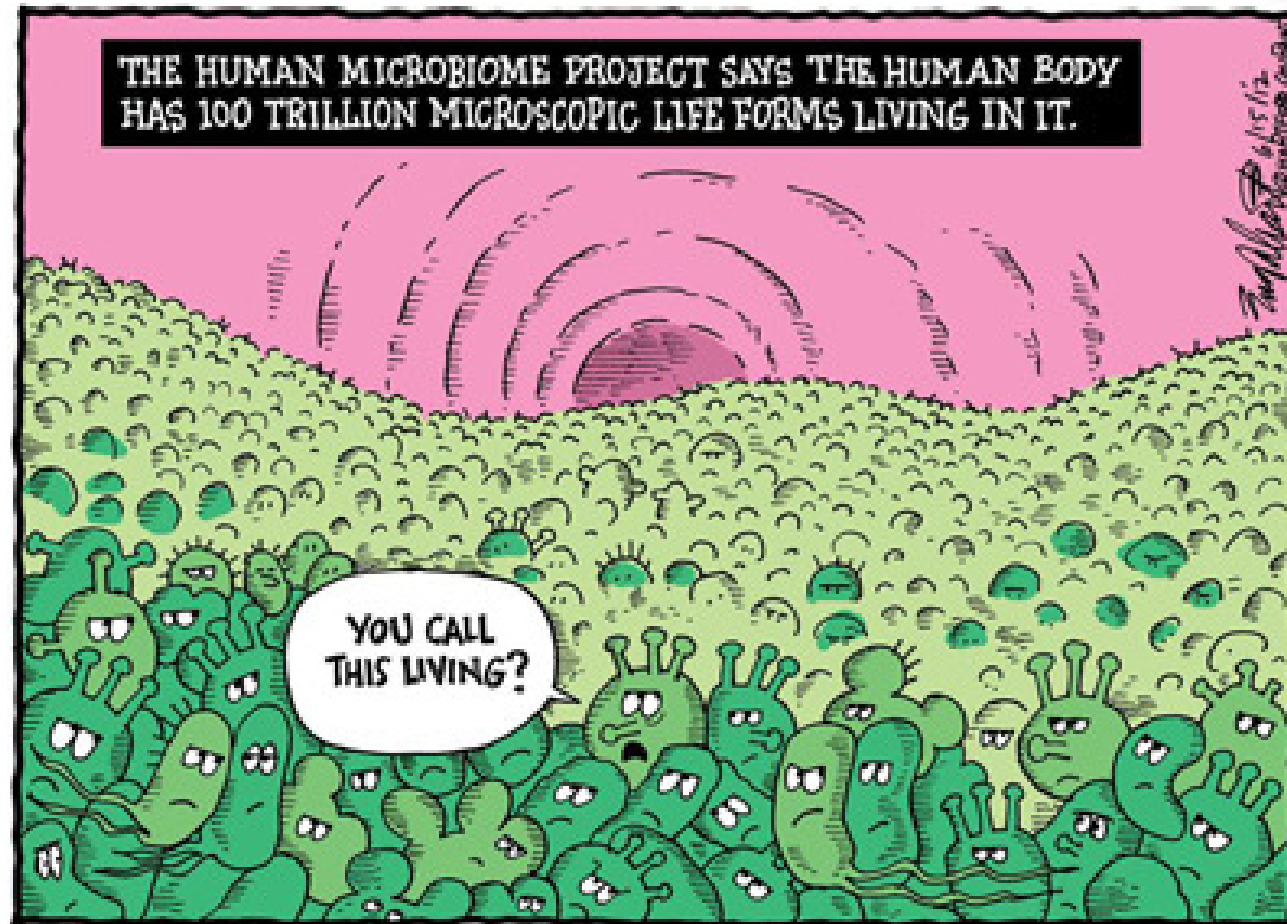
ELISA	sensitivity	specificity	AUC	Correlation	P
hTG2 IgG	12.12	98.99	0.56	.0.2601	<0.001
hTG2 IgA	58.59	98.99	0.79	0.4692	<0.001
tTg neo IgG	77.78	97.98	0.88	0.5334	<0.001
hTG2 Check	79.8	98.99	0.89	0.6093	<0.001
tTg neo IgA	86.87	98.99	0.93	0.6165	<0.001
tTg neo check	97.98	100	0.99	0.6492	<0.001
EMA IgA	90.2	94.1	0.94	0.8098	<0.001
EMA IgG	55.7	99.3	0.78	0.5996	<0.001

➤ *tTg showed the highest sensitivity in its combined version IgA + IgG (Check)*

The genetic environmental balance in autoimmunity

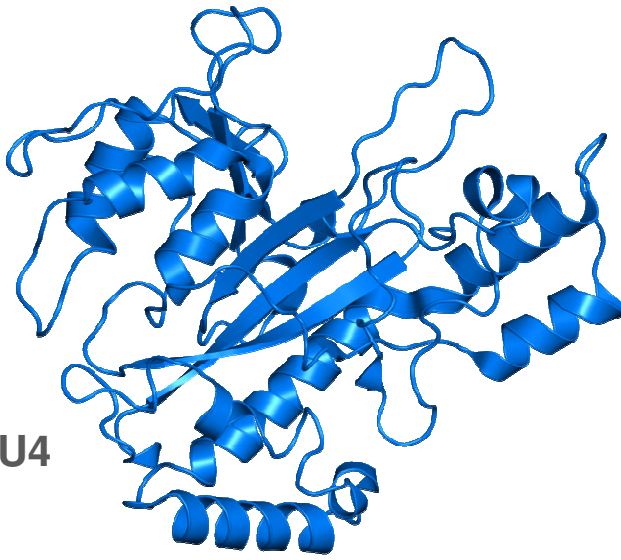
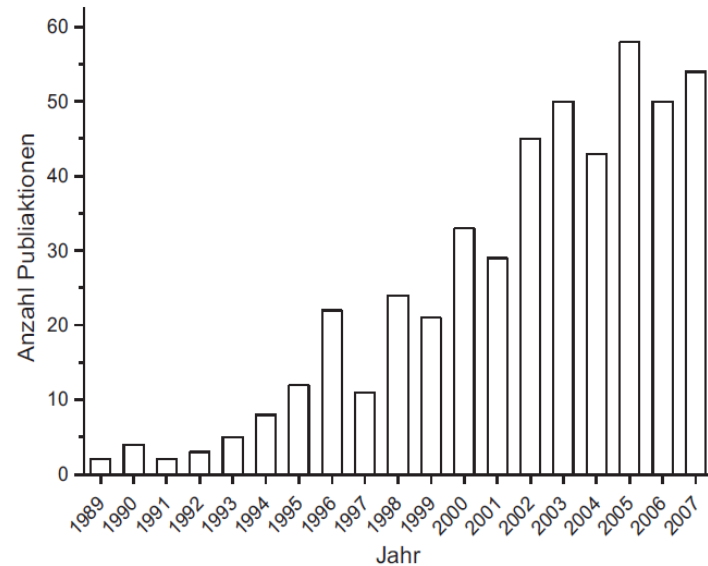


The human microbiome



Microbiological transglutaminase (mTg)

PDB ID: 1IU4



Increase of publications about ``microbial transglutaminase`` after discovery in 1989.

- Molecular weight: **38 kDa**
- Most of the identified mTgs derived from *Streptomyces* (ubiquitary living soil microorganisms)
- Comparable enzymatic activity as tTg
- Difference to tTg:
 - Ca^{2+} independent protein
 - Lower substrate specificity

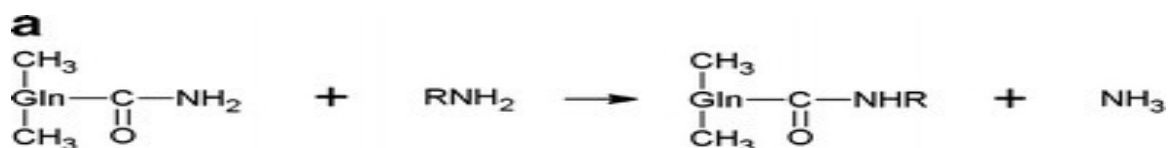
Intestinal enzymatic events that post translationally modify proteins

Peptides crosslinking,
de/amination/deamidation,
de/phosphorylation,
a/deacetylation,
de/tyrosination,
de/glutamylolation,
de/glycylation,
ubiquitination,
palmitoylation,
glycosylation,
galactosylation,
arginylation,
methylation,
citrullination,
sumoylation
carbamylation
Ect.....

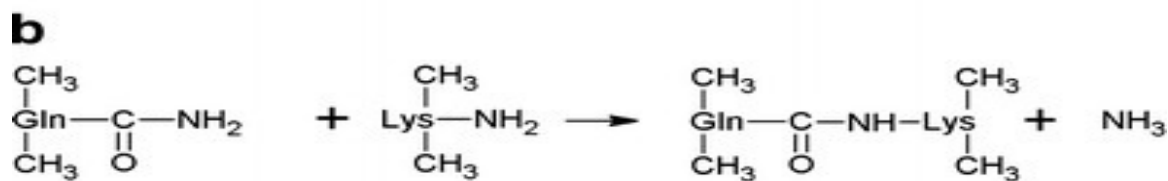
Lerner A et al. **Dysbiosis may trigger autoimmune diseases via inappropriate posttranslational modification of host proteins.** Frontiers in Microbiology, 2016. // Lerner A et al. **Intestinal dysbiotic transglutaminases are potential environmental drivers of systemic autoimmunogenesis.** Submitted, Frontiers in Microbiology, 2016.

Reactions catalyzed by transglutaminase

The reactions catalysed by transglutaminase include:



A. acyl-transfer reaction;



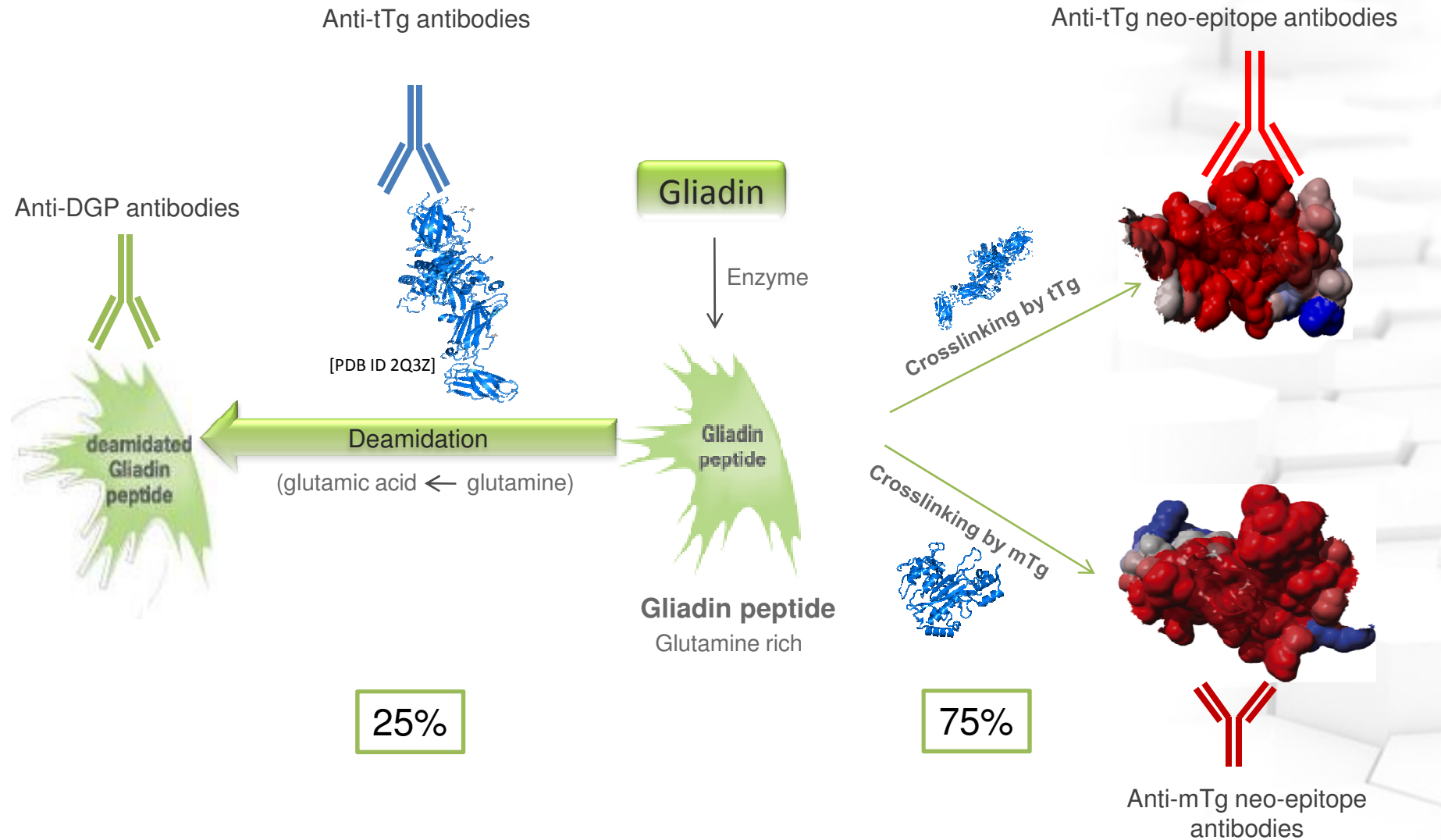
B. cross-linking reaction between Gln and Lys residues of proteins or peptides.



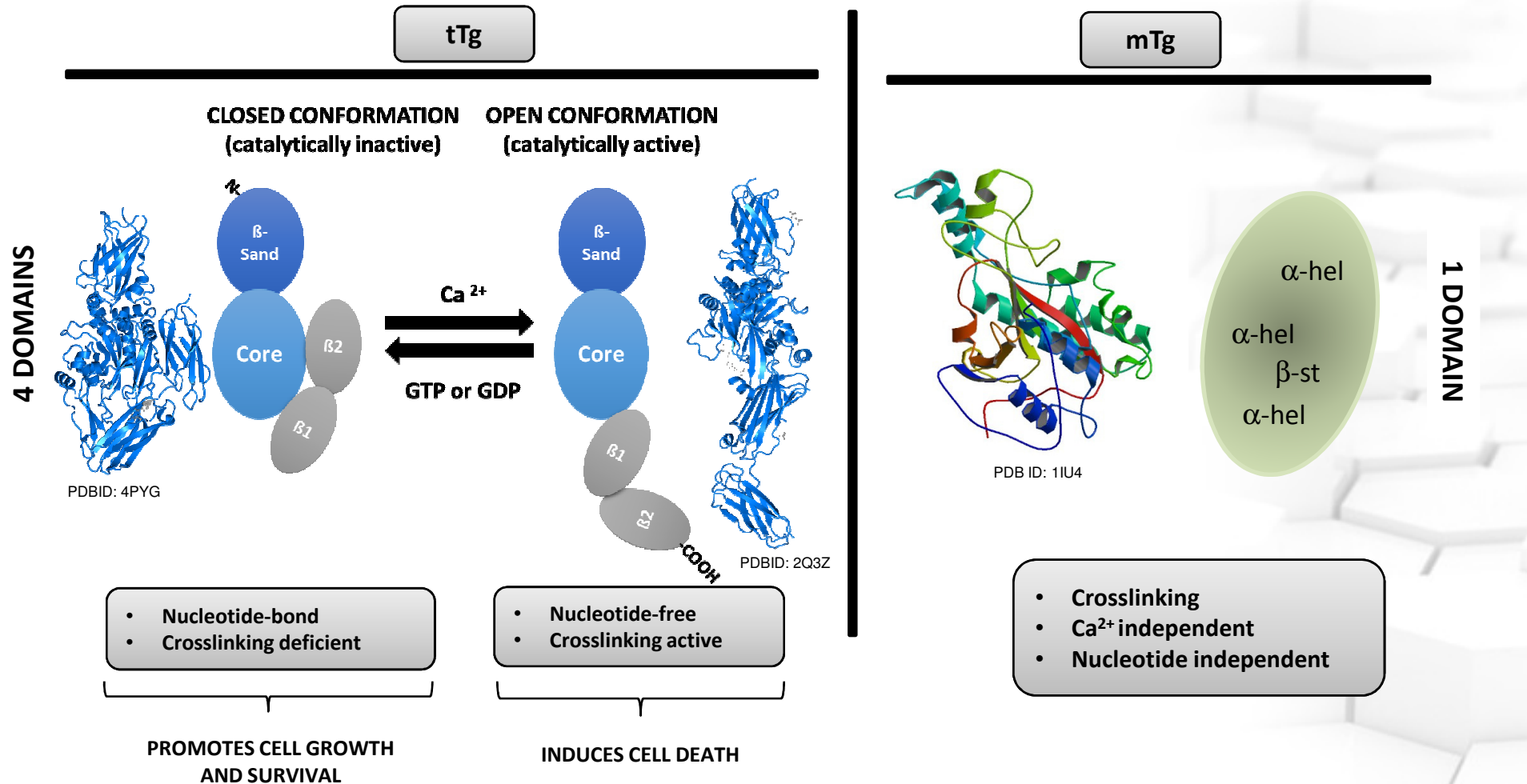
C. deamidation

A schematic presentation of the interplay between gliadin, tTg and mTg enzymes and their corresponding complexes and specific antibodies.

A Lerner, P Jeremias, S Neidhöfer, T Matthias. Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable than anti-tTg for the diagnosis of pediatric celiac disease. **J Immunol Methods**. 2016;429:15-20.



The different conformational states of tTg and mTg and their effects on cells



Application of mTgase on food processing

Source	Product	Effect	Reference
Meat	Restructured meat	Restructured meat texture and appearance, increased hardness	Kuraishi et al. (1997); Motoki and Seguro (1998); Trespalacios and Pla (2007)
Fish	Fish paste, restructured product	Increased hardness	Téllez-Luis et al. (2002)
Milk	Cream, deserts, milk drinks, dressings	Improved quality and texture	Lauber et al. (2000); Şanlı et al. (2011)
Casein	Cross linked protein	Allergenicity reduction	Lauber et al. (2000); Ozer et al. (2007)
Wheat	Baked foods	Improved texture and high volume	Gerrard et al. (2001)
Gelatin	Sweet foods	Low calorie foods with good texture and elasticity	Giosafatto et al. (2012)

Folia Microbiol (Praha). 2014; 59(3): 241–250.

Microbial transglutaminase and its application in the food industry. A review Marek Kieliszek, Anna Misiewicz.

Mahta Mirzaei et al. 2011
// M. Griffin et al. 2002



Cross-linking of Casein
Increase of gelation



Cross-linking of Gluten Proteins
Positive influence of dough characteristics

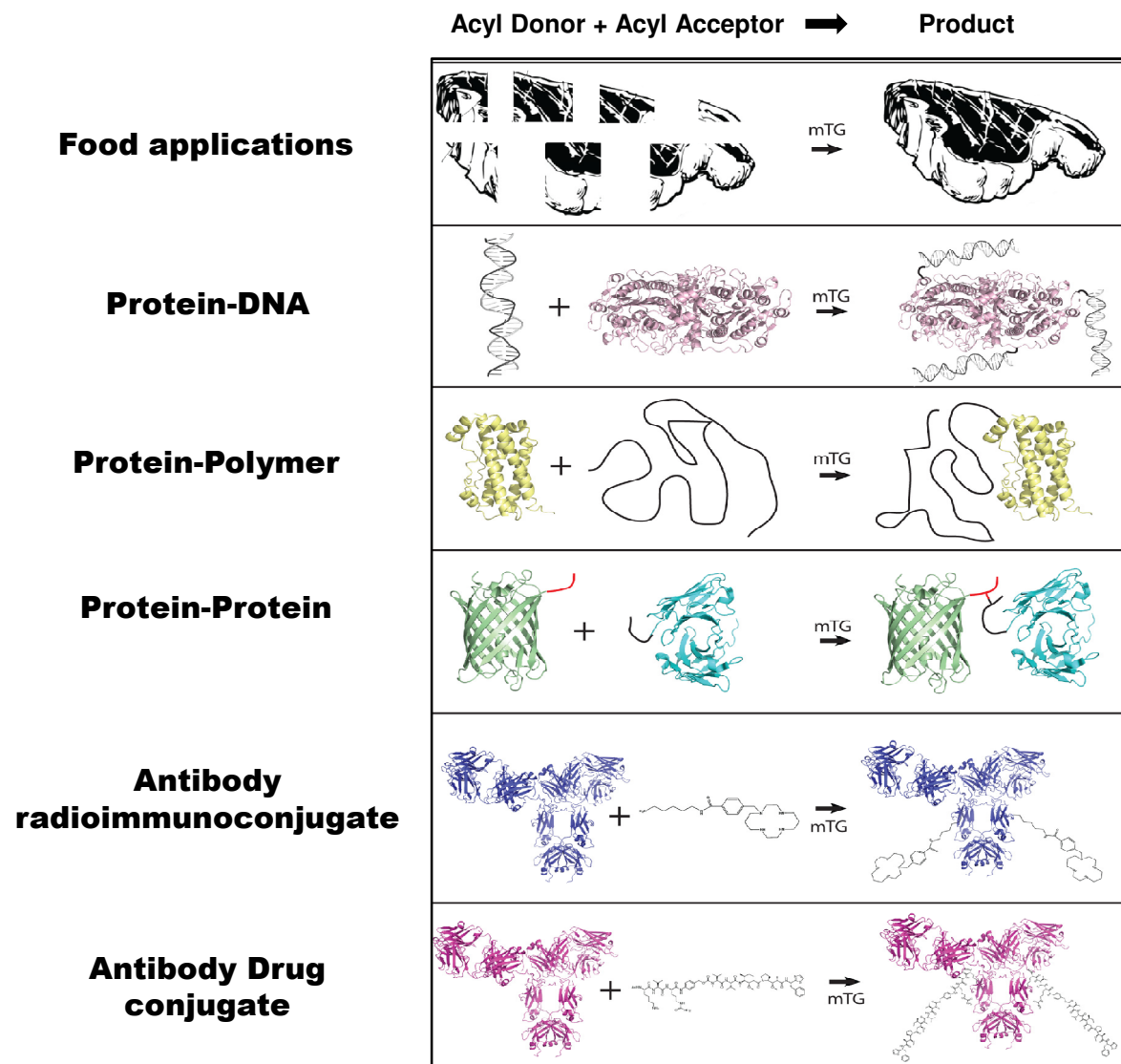


Myosin & Actin Cross-linking
Enhancement of texture, elasticity & persistency

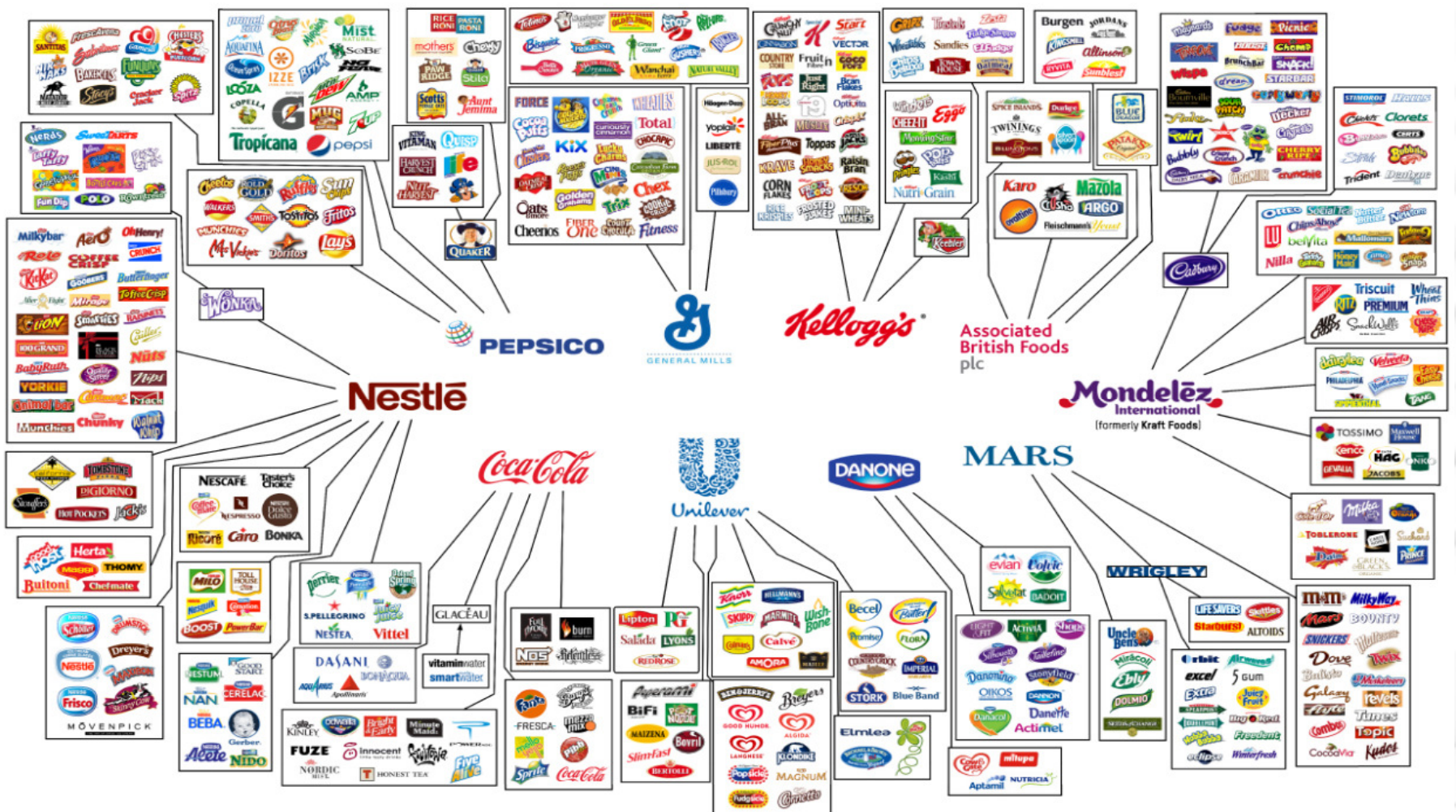


Surimi fabrication
Enhancement of elasticity & persistency

Examples of mTg application versatility



The change in modern nutrition!



Change in nutrition responsible for increasing CD numbers?

U.S. FOOD CONSUMPTION AS A % OF CALORIES

PLANT FOOD:

Vegetables, Fruits, Legumes,
Nuts & Seeds, Whole Grains

Fiber is only found in plant foods.

NOTE: Up to half of this category may be processed, for example: almonds in candy bars, apples in apple pies or spinach in frozen spinach soufflé, and of course these would not be healthy choices. The focus should be on whole unprocessed vegetables, fruits, legumes, nuts and seeds and whole grains.

ANIMAL FOOD:

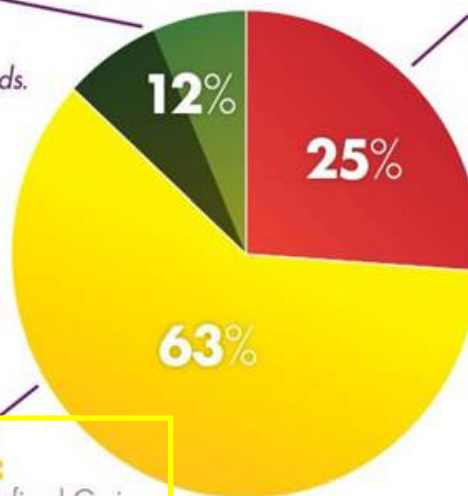
Meat, Dairy, Eggs, Fish, Seafood
Cholesterol is only found in animal foods. Animal foods are the **PRIMARY** source of saturated fat.

GUIDE TO HEALTHY EATING:

Much easier to understand than the USDA Food Pyramid, with no food industry influence.

Eat **LESS** from the animal and processed food groups and **MORE** whole foods from the plant food group.

In general, food from the animal and processed food group contribute to disease, while **WHOLE** foods from the plant group contribute to good health.



PROCESSED FOOD:

Added Fats & Oils, Sugars, Refined Grains

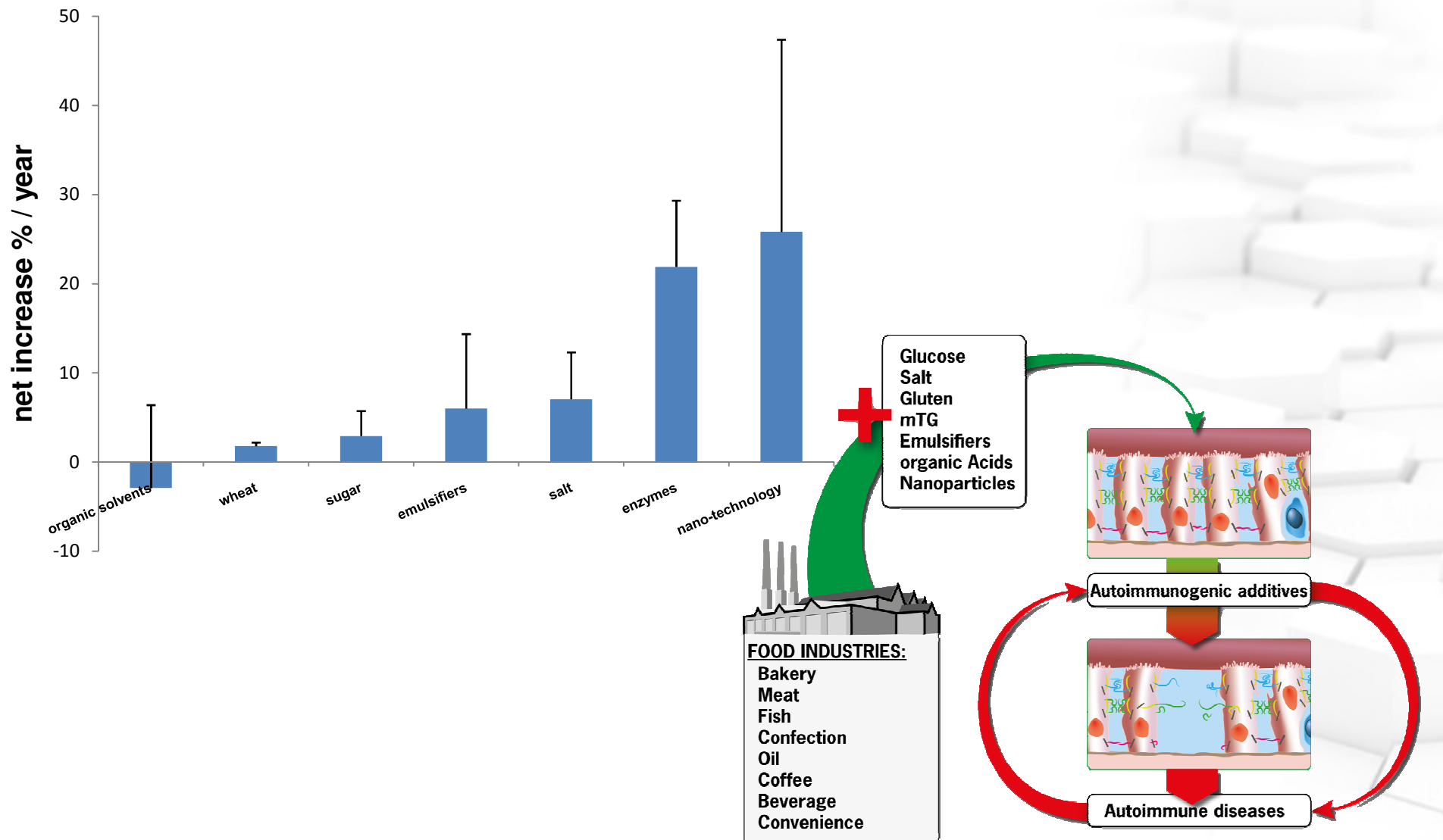
Source: USDA Economic Research Service, 2009; www.ers.usda.gov/publications/EIB333; www.ers.usda.gov/Data/FoodConsumption/FoodGuideIndex.htm#calories

New York Coalition for Healthy School Food * www.healthyschoolfood.org

Special thanks to Joel Fuhrman, MD, author of *Disease Proof Your Child: Feeding Kids Right* * Graphics by MichelleBanda.com

© 2009, New York Coalition for Healthy School Food

Change in nutrition responsible for the increasing numbers of ADs?

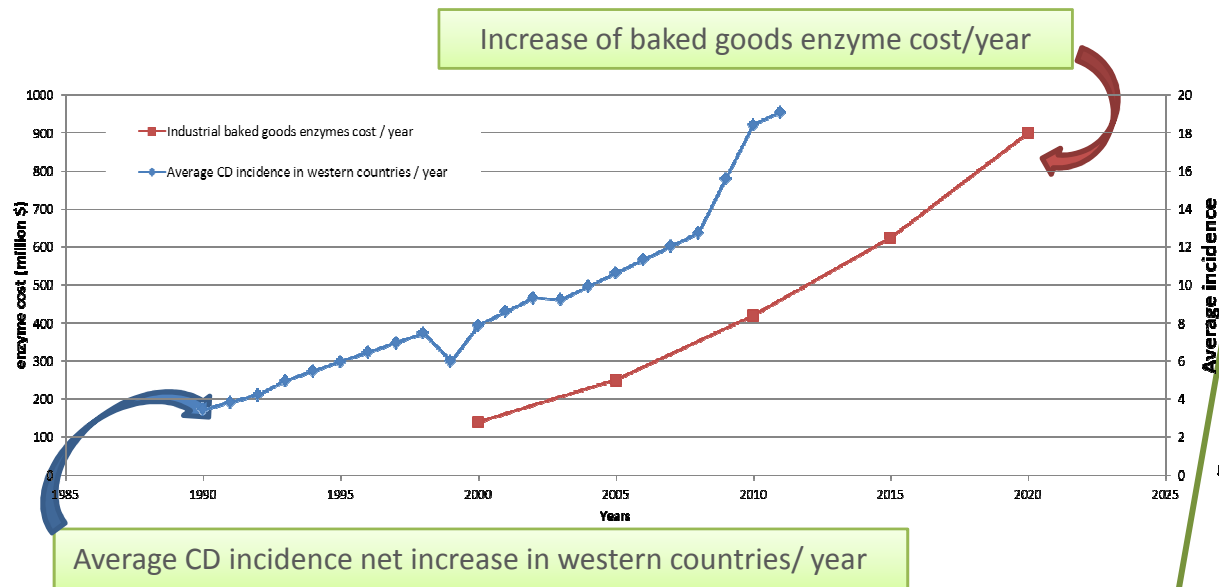
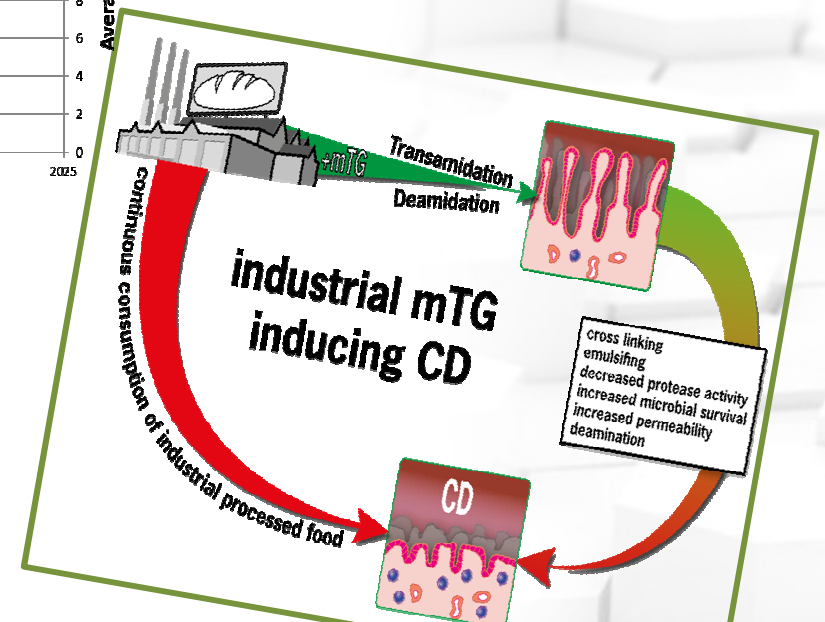


Lerner A, Matthias T. **Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease.** Autoimmun Rev. 2015;14:479-89

Mechanisms of food additives to increase intestinal permeability

Food additives	Net % increase/year	Mechanism of TJ integrity breaching
Glucose	2.9±2.8	Change in distribution of ZO-1, claudin-1, E cadherin. Perijunctional cytoskeleton condensation.
Salt	7.1±5.3	Increased phosphorylation of myosin light chain, contraction of the perijunctional actomyosin ring, loss of function of claudin 2 and 15.
Organic solvents	-2.9±9.3	Alterations in TJ proteins, dissociates the PTP1B-E-cadherin-beta-catenin complex
Emulsifiers	6±8.4	P-glycoprotein inhibition, decrease the hydrophobicity of the mucus layer, actine disbandment and structural separation of TJ, change the distribution of ZO-1 and actine.
Gluten	1.8±0.4	Rearrangement of the cytoskeleton through the zonulin pathway, reduces F-actin content, interaction between occludin and Zo-1 is compromised, zonulin release is leading to PKC-mediated cytoskeleton reorganization, zonulin release by binding to the CXCR3 receptor in intestinal cells, in a MYD88-dependent pathway and subsequent transactivation of EGFR by PAR2.
Microbial transglutaminase	21.9±7.4	Increases luminal microbial load, Hypothesis: cross-linking TJ proteins, imitating emulsifiers and nanoparticles functions.
Nanoparticles	25.8±21.5	Redistribution of ZO-1 TJ proteins, clustering of integrin $\alpha(V)\beta(3)$ along the cell border, F-actin reorganization and claudin 4 down regulation, open epithelial TJ via C-Jun N-terminal kinase-dependent pathway, TJ electrostatic interactions

Microbial transglutaminase & Celiac Disease



Increased consumption of food industry mTg may explain the surge in CD incidence

Raw and processed food antibodies

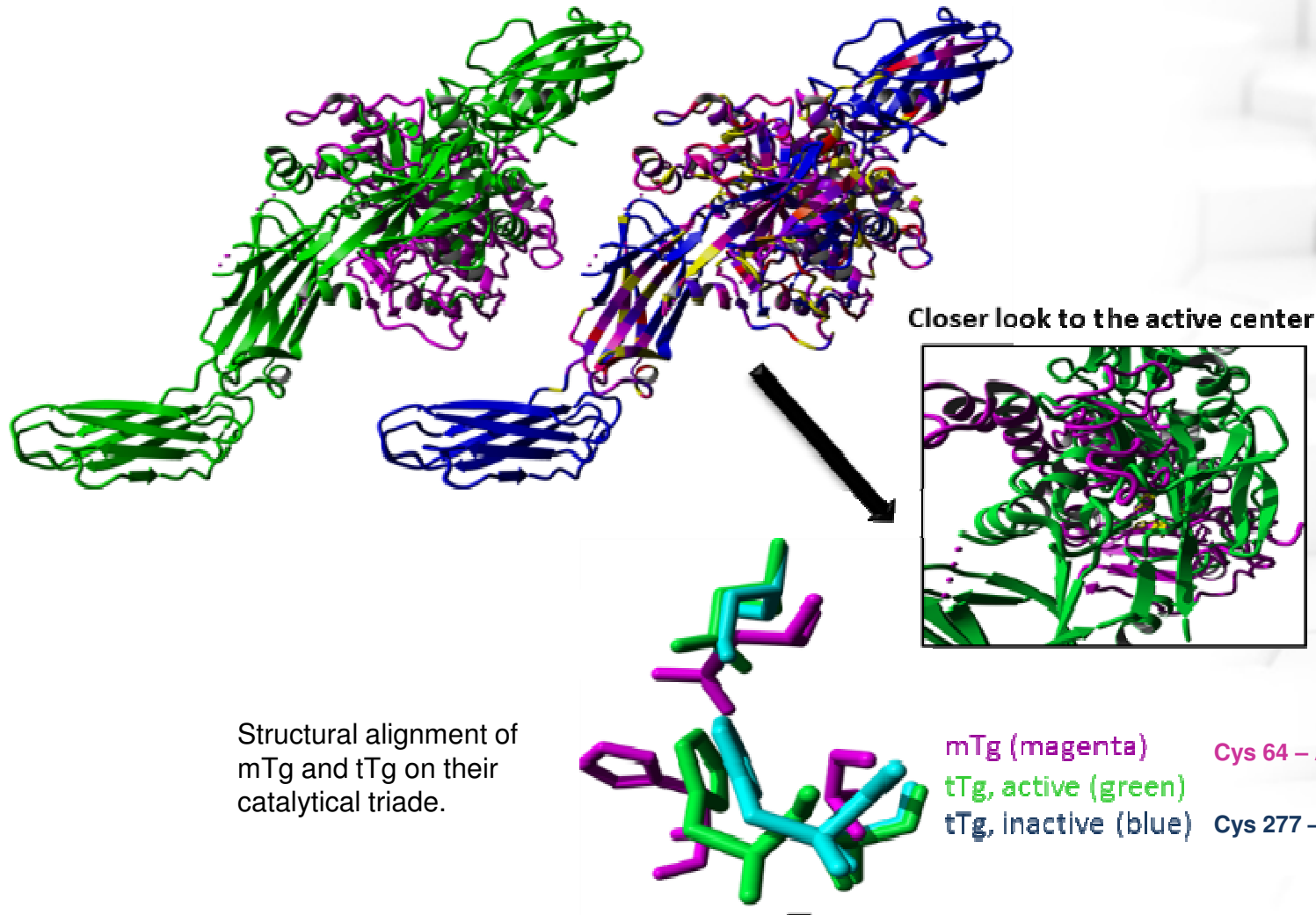
Levels of IgG, IgA, IgM antibodies (ELISA units) against raw and processed food antigens

Processed/raw	IgG		IgA		IgM	
	processed	Raw	processed	Raw	processed	Raw
Doughnut	118		130		42	
wheat		68		85		32
barley		10		6		6
yeast		14		10		6
soy		18		16		6
Cake	138		134		58	
wheat		66		84		32
egg		12		10		4
corn		10		8		10
milk		34		54		18
Cereal	124		138		54	
wheat		64		84		30
oat		60		42		20
corn		10		8		6
rice		8		6		4
barley		9		7		6
milk		34		52		12

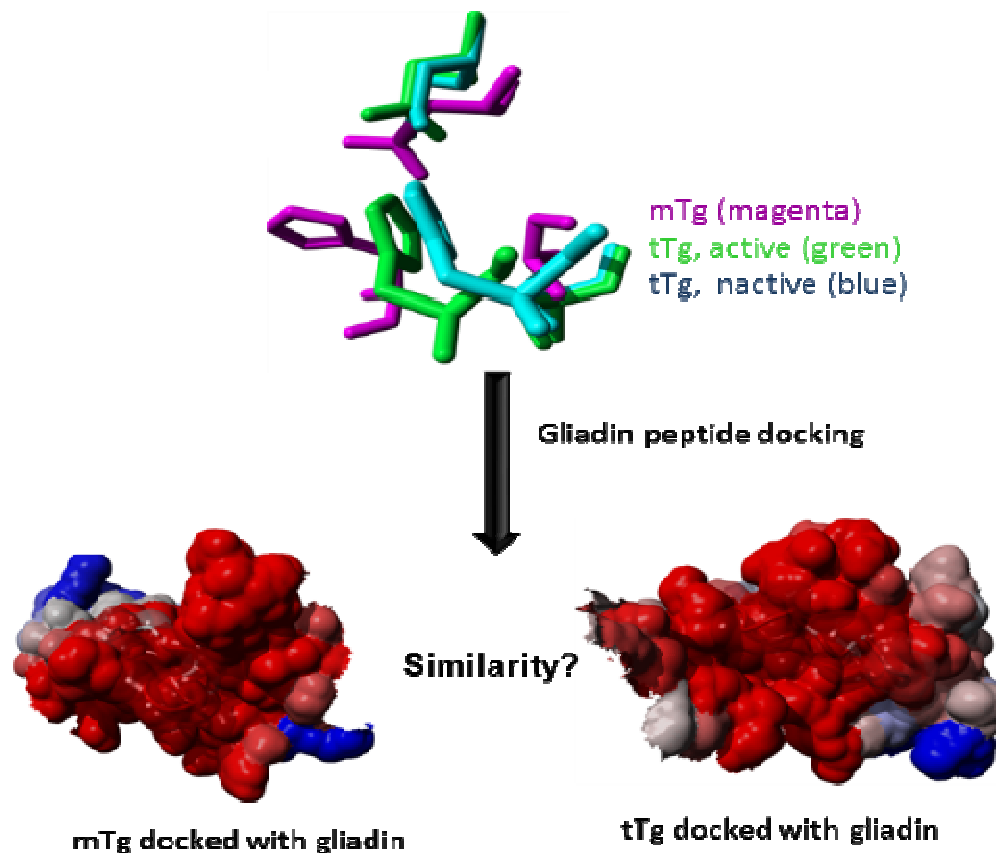
Similarity between mTg and tTg?

3D structure alignment of mTg and tTg

Yellow=conserved segments.

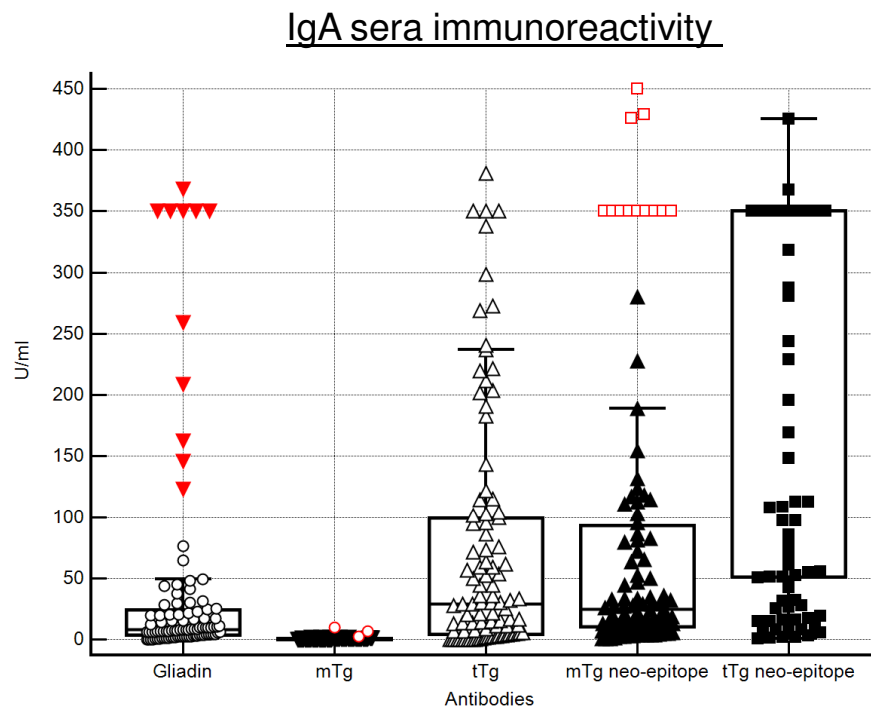
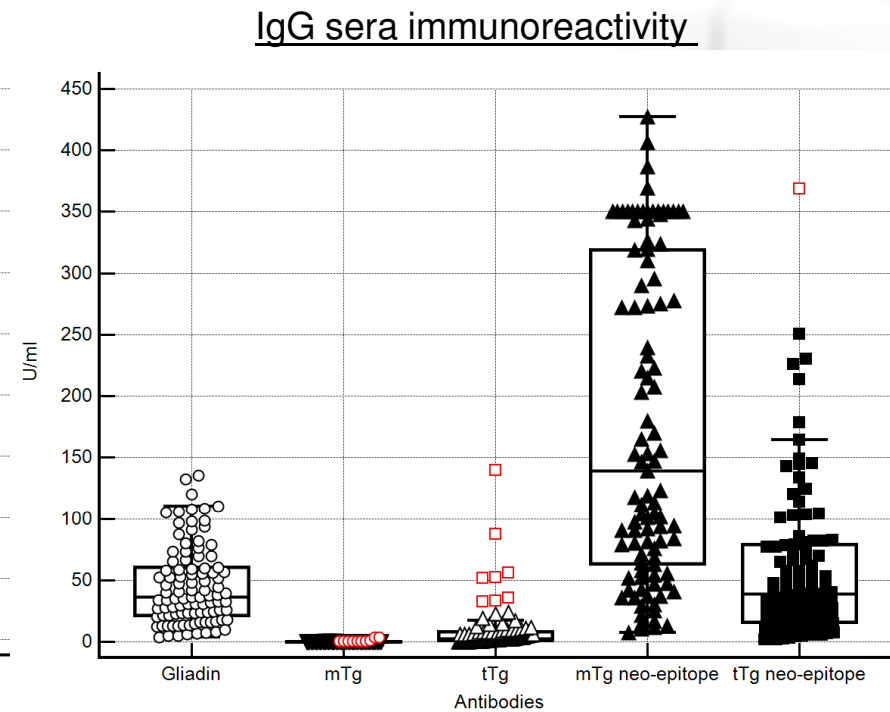


Similarity between mTg and tTg?



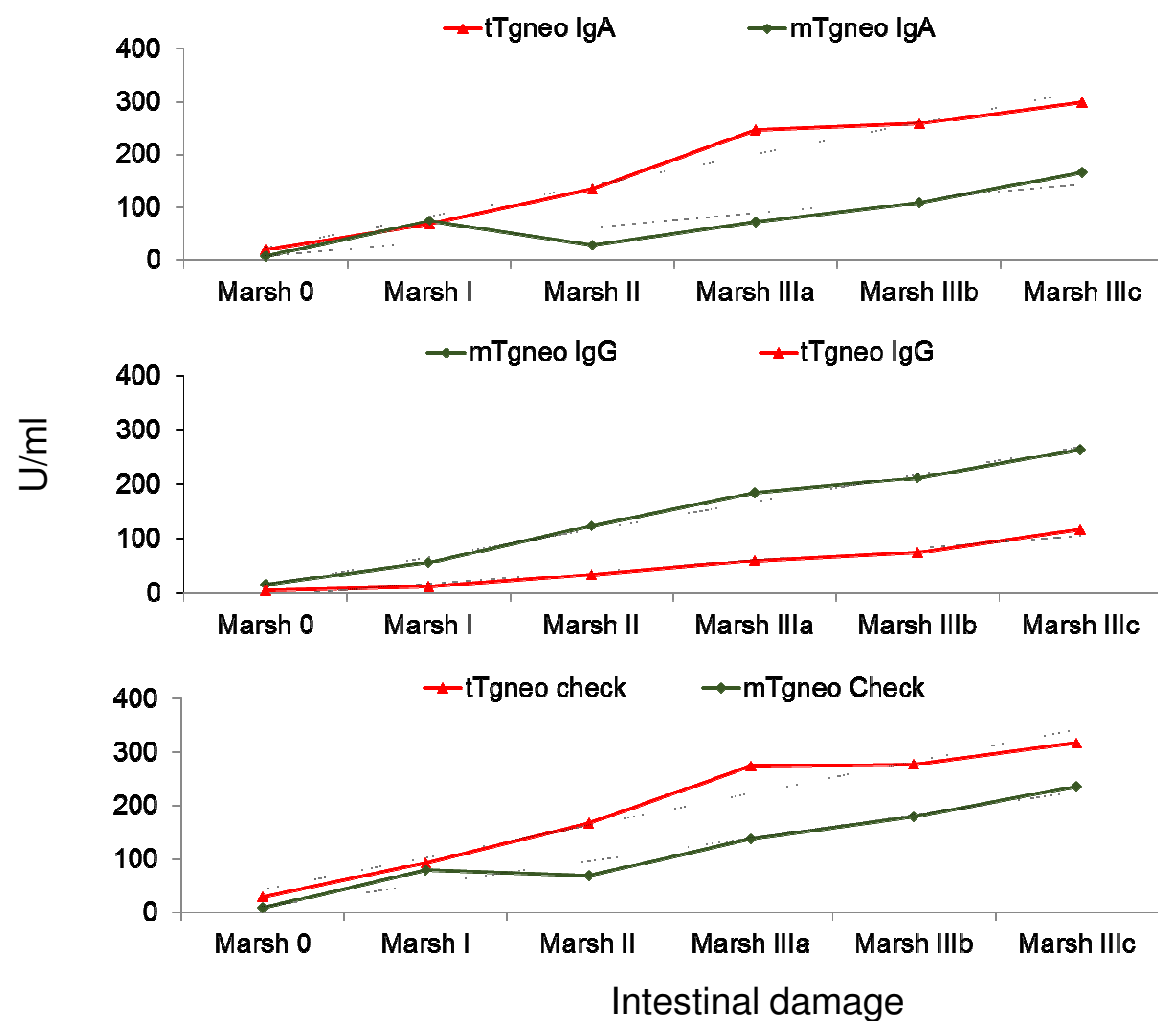
Docking of gliadin peptide (PDB-ID: 1NNA) with mTg (left side) and tTg (right side). The arisen complexes got stained among their electrostatic profile; red = negative charges.

Sera immunoreactivity of the neo-epitopes



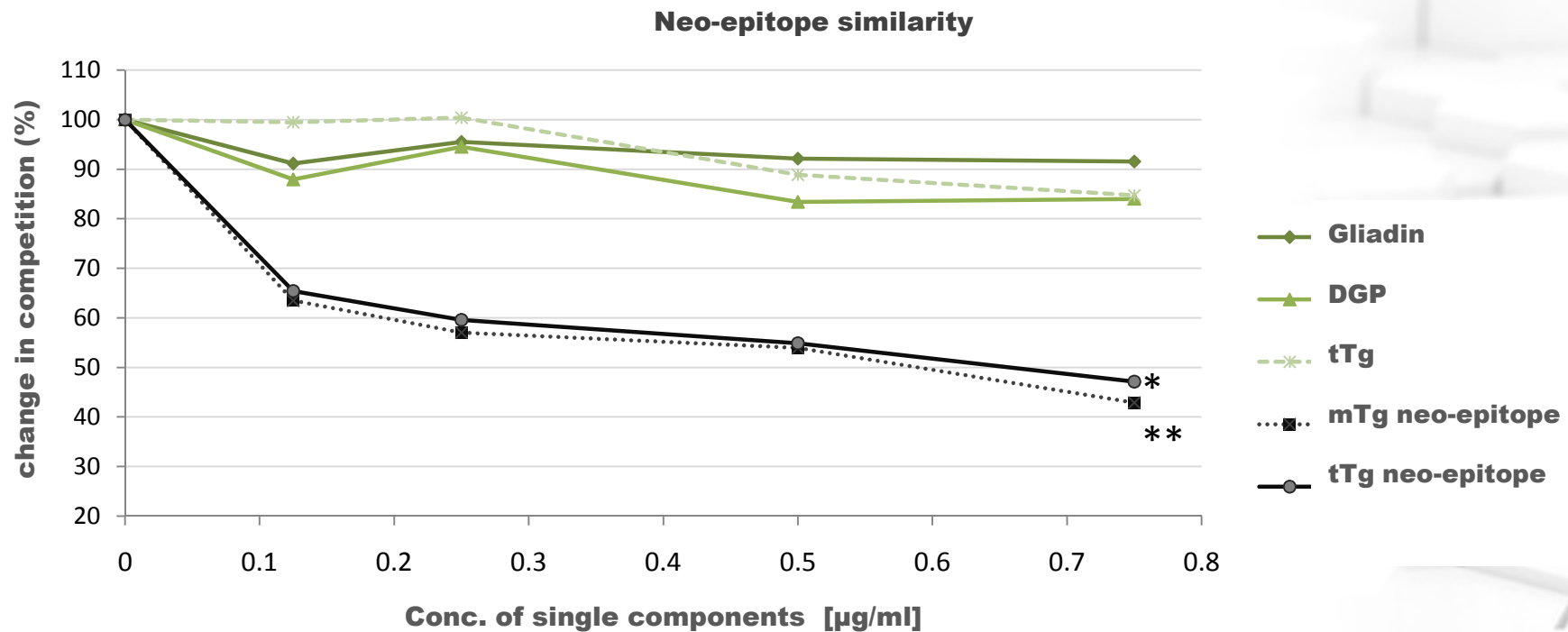
Immunoreactivity of the mTg/ tTg neo-epitope anti IgA and IgG and the single compounds mTg, tTg and gliadin (not complexed to gliadin complexes) anti IgA and IgG.

Antibodies' activity in relation to the degree of intestinal damage in CD



Antibody	Correlation coefficient r	P
tTg neo check	0.6492	<0.0001
tTg neo IgA	0.6165	<0.0001
mTg neo IgG	0.5633	<0.0001
tTg neo IgG	0.5334	<0.0001
mTg neo check	0.5127	<0.0001
mTg neo IgA	0.3018	0.003

Competition ELISA (human IgA)



Competition to the “IgA neo-epitope” showed a decrease in response if transglutaminases complexed with gliadin peptides were added

* $p < 0.05$ // ** $p < 0.005$

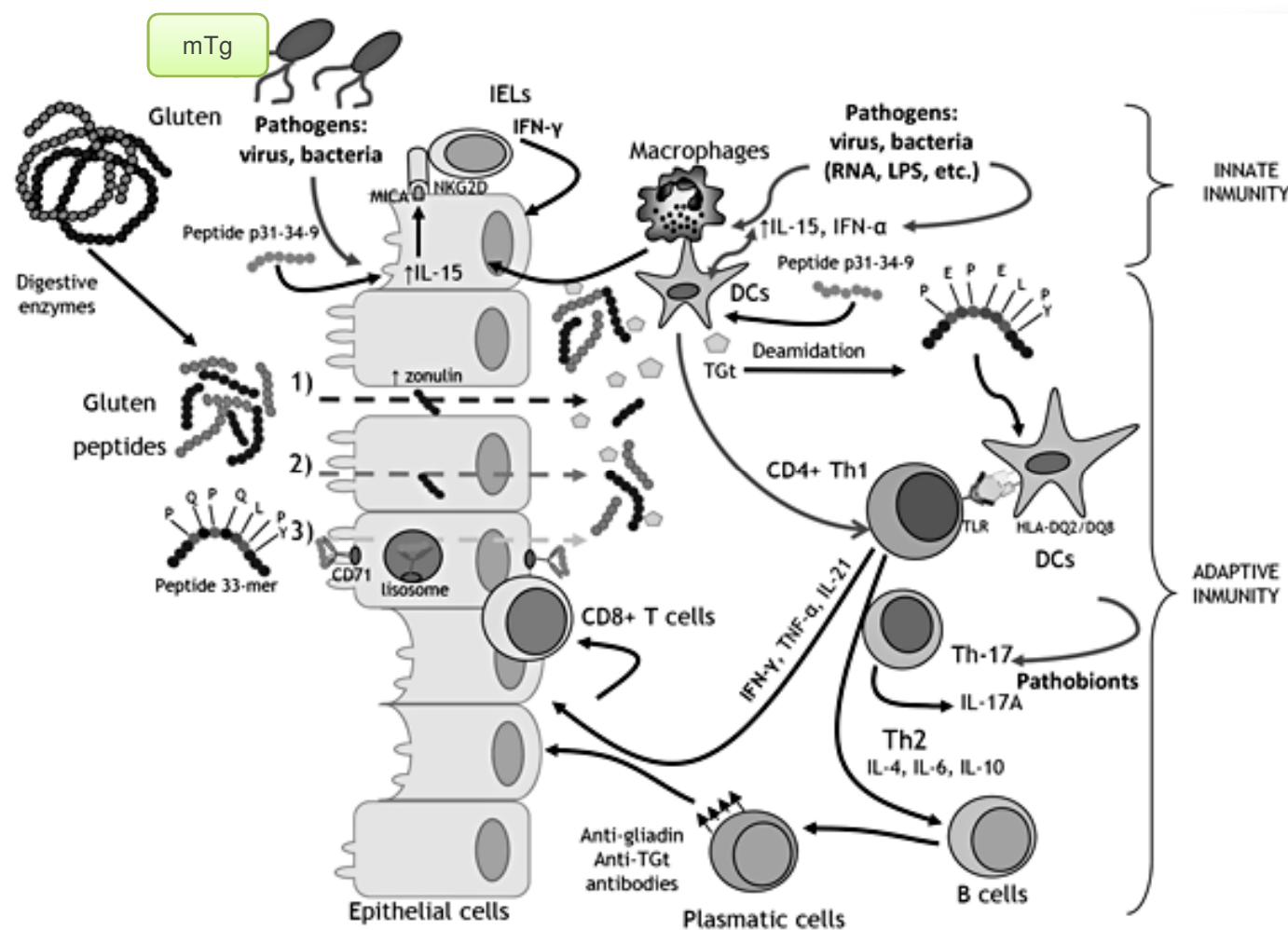
Diagnostic power of neo-epitopes

n=198 pediatric samples (CD=99 , N=99)

ELISA	sensitivity	specificity	AUC
mTg neo IgA	64.65	98.99	0.82
tTg neo IgG	77.78	98.0	0.88
mTg neo Check	90.91	87.88	0.89
hTG2 Check	79.8	98.99	0.89
tTg neo IgA	88.89	98.99	0.94
mTg neo IgG	94.96	93.94	0.95
tTg neo check	97.98	98.99	0.98

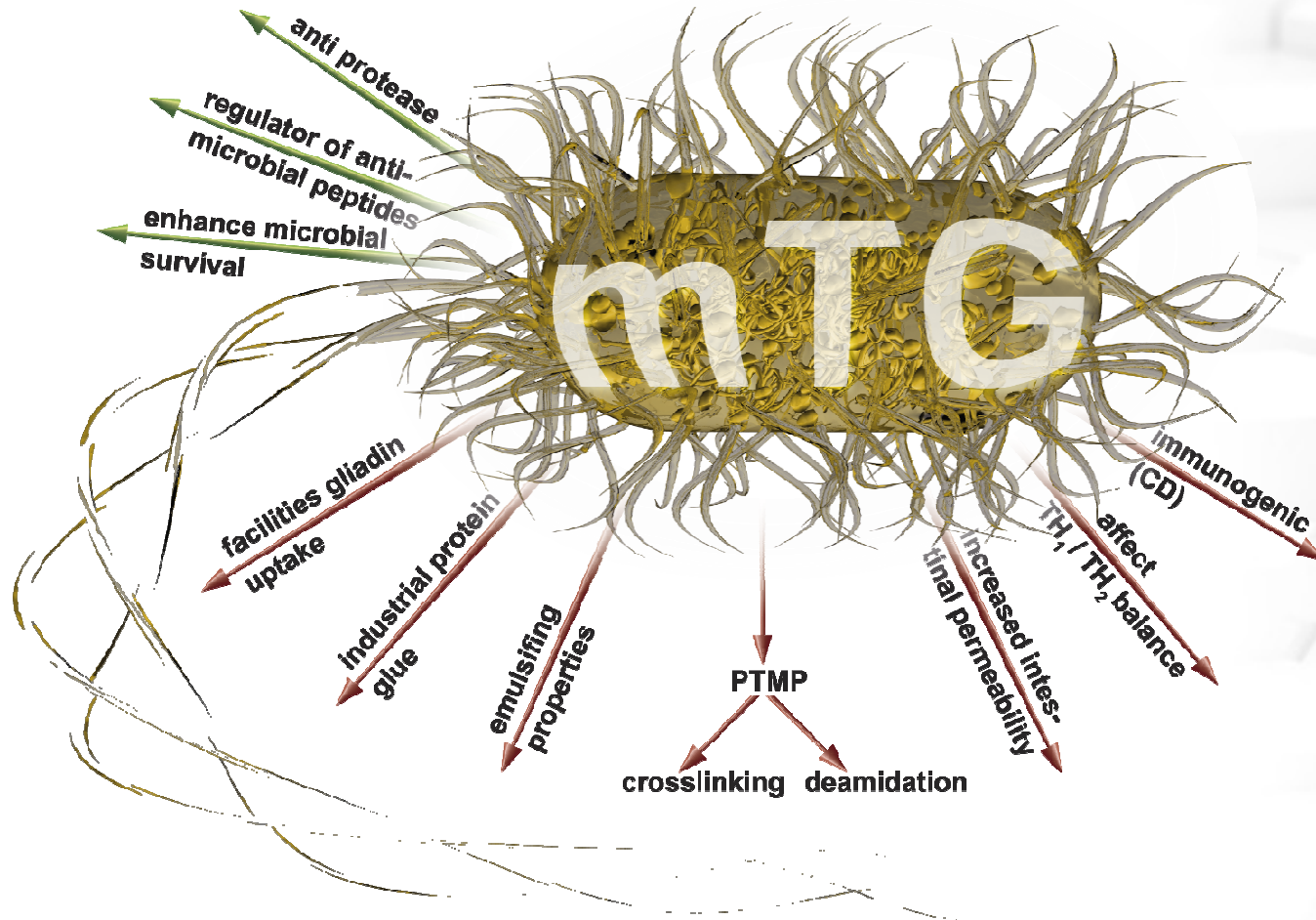
- mTg neo-epitope IgG had higher sensitivity than tTg neo-epitope IgG
- tTg neo-epitope showed the highest sensitivity in its combined version IgA + IgG (Check)

Schematic representation of the proposed model for CD pathogenesis



Effects of mTg that might drive autoimmunity

Luminal (green) and systemic (red) effects of the mTg that might drive autoimmunity



Intestinal dysbiotic transglutaminases are potential environmental drivers of systemic autoimmunogenesis
Aaron Lerner, Rustam Aminov, Torsten Matthias. Frontiers in Microbiology, 2016

Effects of mTg that might drive autoimmunity

It is tempting to estimate the **amount and activity of mTg** that reside in the **human gut** during daily life. Three sources can be envisioned: from the **industrial processed food**, from the ingestion of **probiotic** and from the **micro/dysbiota luminal residents**. Based on the following observations, a hypothetical estimate can be suggested:

1. Each kilogram of food treated with microbial transglutaminase contains about **50–100mg** of microbial transglutaminase.
 2. Examples of the mean yield of bacteria used by the industries for mTg production is *Streptoverticillium* sp 1.43 unit/ml, ***Streptoverticillium mobaraense*** (ajinomoto “wild type”) **1.25unit/ml** or 22 unit/mg and *Escherichia coli* 19.55 unit/mg.
 3. No information to my knowledge, is available, on the amount of tTg secreted in the human gut lumen, however **Tgase activity is depicted** in the human jejunum.
 4. As a result of the increasing use of microbial transglutaminase in the food industry, the common Western diet now contains large amounts of microbial transglutaminase, with the **maximum daily intake ranging up to 15 mg**.
 5. Assuming additive effect of a daily intake of **10⁸ viable** human originated **probiota** (*Bifidobacterium bifidum/longum/adolescentis/infantis*) , as probiotics, that secrete mTg to the **100 trillion** bacteria constituting the human microbiome, with their mTg load, one can reach a **substantial continuous mTg** activity residing constantly in the human intestinal lumen.
- Interestingly enough, mTg crosslinking can be used as a new probiotic carrier for enhanced gastrointestinal transit and storage.

Conclusions

- **tTg is the autoantigen of CD** and plays a **pivotal role in PTM** of the gliadin and CD autoimmunogenesis
- tTg is **essential for human** and mTg for **bacterial survival**
- **mTg & tTg are capable to deamidate and crosslink proteins, including gliadin**
- mTg is Ca^{+2} independent, less substrate specific, secreted by the luminal microbes and is a heavily used industrial food additive.
- **CD patients are immunoreactive to the neo-epitopes mTg and tTg.** Both antibodies represents **new bio-markers** for CD diagnosis.

True/false, reality/imagination interplay

mTg parameters	Industrial producers	Processed food products	Human effects
Activity	None	Yes	Intestinal human
Toxic	No	?	?
Immunogenic	No	Yes	Yes
Allergic	No	Yes	Yes
pathogenic	no	?	?

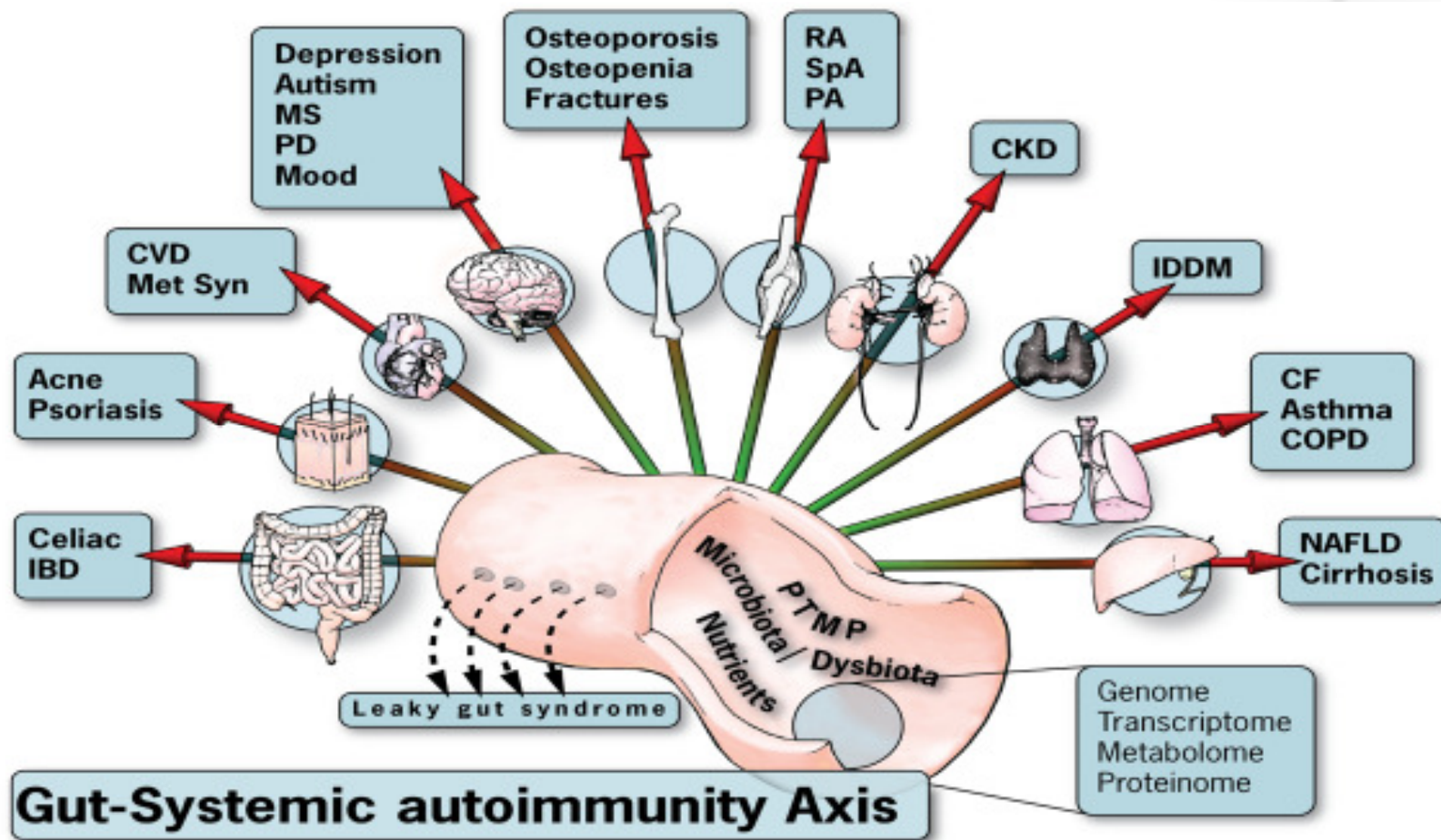
Environmental and pathophysiological aspects involving tTg & mTg in CD



Lerner A, Matthias T. **Possible association between celiac disease and bacterial transglutaminase in food processing: a hypothesis.** Nutr Rev. 2015;73:544-552.



Gut-Systemic autoimmunity Axis

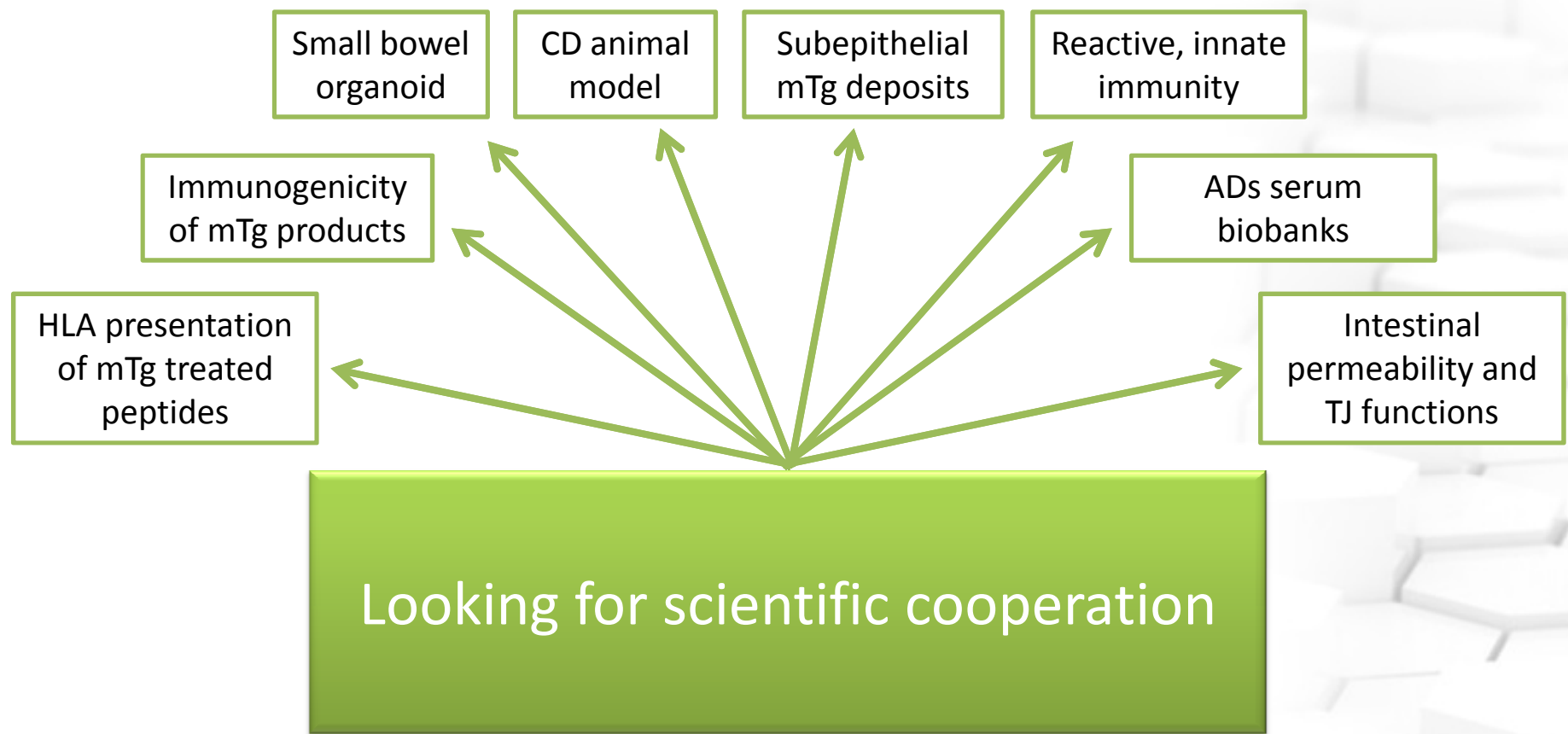


Lerner A et al. **Rheumatoid arthritis-celiac disease relationship: joints get that gut feeling.** Autoimm Rev. 2015;14:1038-47.

Lerner A et al. **Gut- bone cross talks and implications in celiac disease.** Internat J of Celiac disease 2016;4:19-23

Lerner A et al. **The gut-gut axis:// Cohabitation of celiac, Crohn's disease and IgA deficiency.** Internat J Celiac Dis. 2016;4:68-70.

Lerner A et al. **GUT-the Trojan horse in remote organs' autoimmunity.** Journal of Clinical & Cellular Immunology. 2016;7:401.



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THANKS TO

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Haifa, Israel***

mTg-neo-epitope check diagnostic performance in CD and other ADs, compare to controls

Diseases	# Tested	# positive AESKULISA
Celiac Disease	99	97 (97.9%)
Celiac Disease (GFD)	5	0 (0%)
Disease control (total)	1037	87 (8.4%)
IBD	52	7 (13.5%)
Rheumatoid Arthritis	575	54 (9.4%)
Vasculitis	58	5 (8.6%)
Adult Healthy donors	264	7 (2.7%)
Pediatric Healthy Donors	88	14 (15.9%)

mTg-neo-epitope check diagnostic performance

AESKULISA mTg-neo-epitope check			Pos	Neg	Total
	Pos		97	2	99
	Neg		87	950	1037
	Total		184	952	1136

Area under the Curve: **0.95**

Sensitivity: 97.98 %

Specificity: 91.61 %

- The mTg-gliadin complexes were separated by **size fractionation**.
- Defolding and digesting experiments were performed to prove the **complex formation** as well as the **stability** of the immunopotent epitopes after treatment of the complexes.
- The ELISA antigenic complexes are not linear and the **3D confirmation** carry the antigenic epitopes.