IMPACT OF PHYSICOCHEMICAL PROPERTIES ON PROTEIN ALLERGENICITY: PLANT VERSUS ANIMAL ALLERGENS

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FOOD ALLERGY

Why do some proteins act as allergens, while others do not?

What are the differences among proteins that increase their intrinsic allergenic potential?

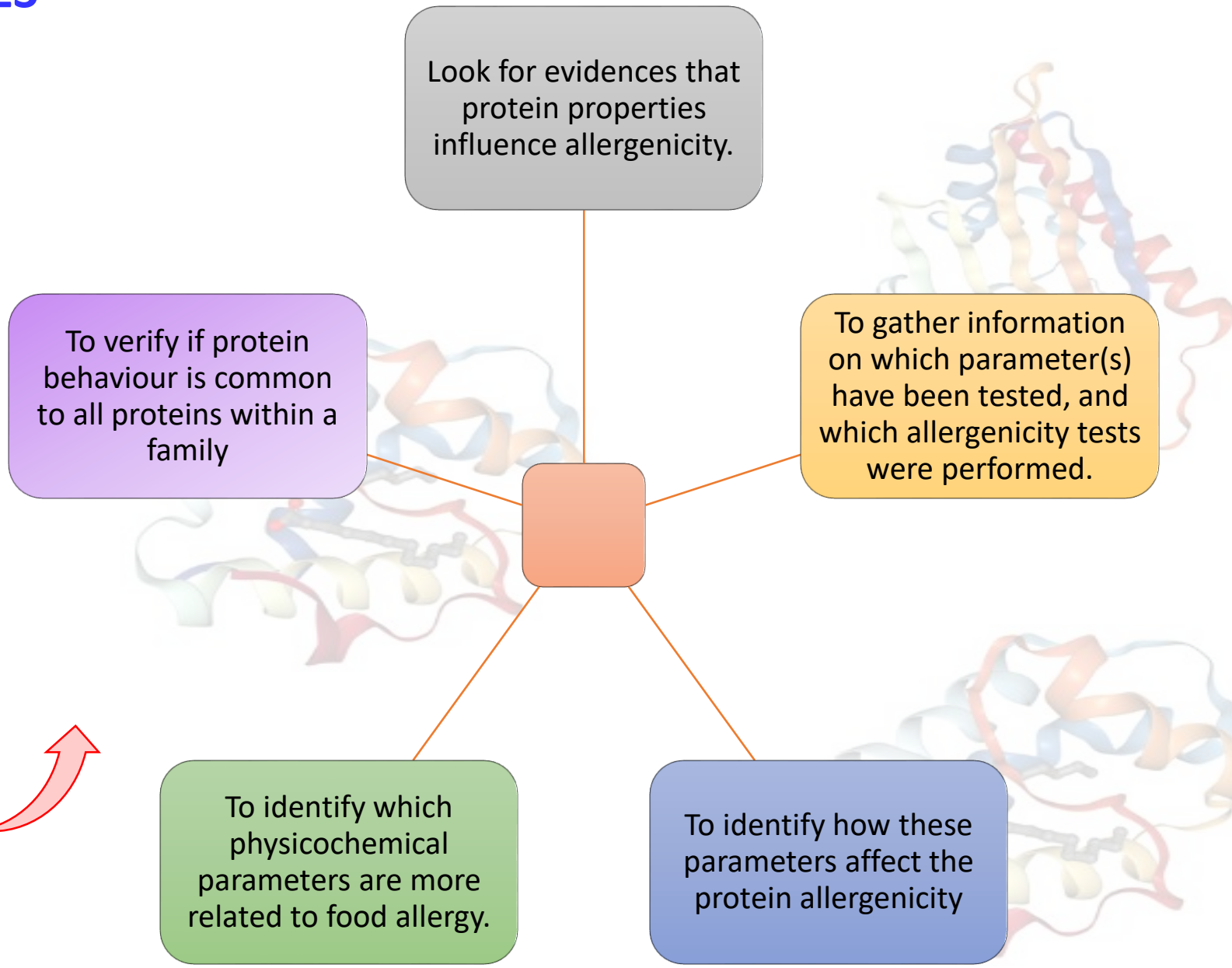
Which factors drive sustained tolerance to foods and food proteins?

Which immunological events intervene in tolerance breakdown, leading to sensitisation, and most likely to subsequent food allergy?

WHAT MAKES A PROTEIN INTO AN ALLERGEN?

Costa et al. (2020) Are physicochemical properties shaping the allergenic potency of plant allergens? Clin Rev Allergy Immunol. https://doi.org/10.1007/s12016-020-08810-9
Costa et al. (2021) Are physicochemical properties shaping the allergenic potency of animal allergens? Clin Rev Allergy Immunol (in press). https://doi.org/10.1007/s12016-020-08826-1

LOOKING FOR EVIDENCES



Are physicochemical properties shaping protein allergenicity??

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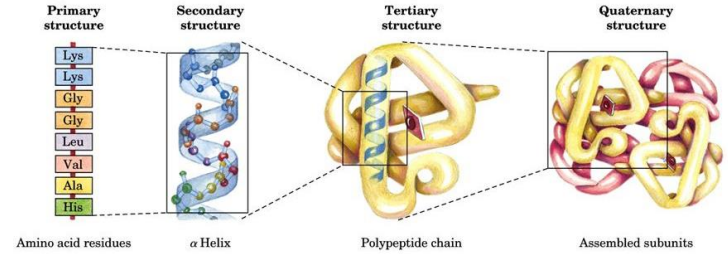
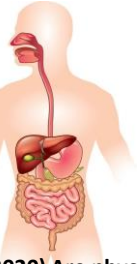
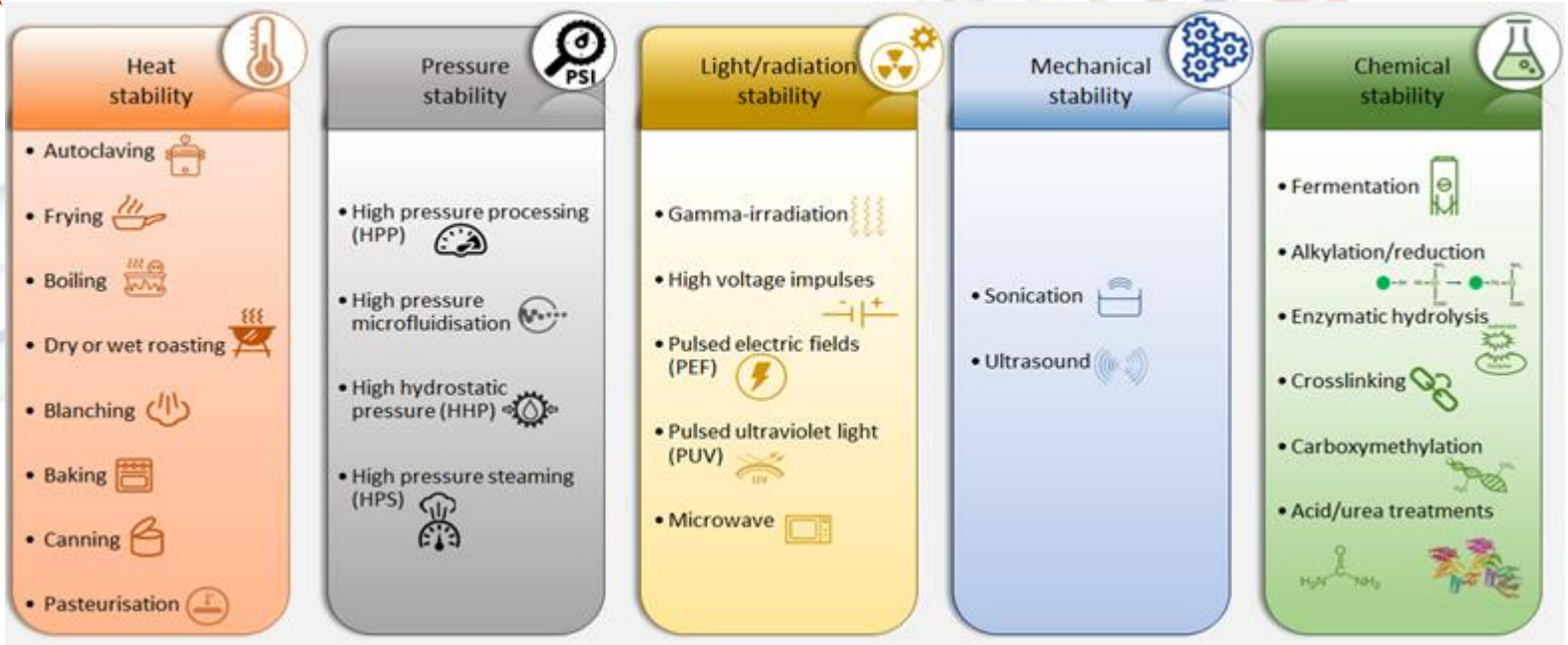
PHYSICOCHEMICAL PROPERTIES

Which physicochemical parameters are important to determine protein allergenicity?



- Pressure stability
- Abundance
- Heat stability
- Glycosylation
- Light stability
- Mechanical stability
- Hydroxylation
- Chemical stability
- Phosphorylation
- Aggregation
- Acetylation
- Glycation
- Protein structure
- Ligand binding
- Digestibility
- Lipid binding
- Lipid interaction





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FOOD ALLERGENS

AllFam
Database of allergen families

Allergens belong to a restricted number of protein families

Search AllFam [Help]

Go!

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Department of Pathophysiology

Medical University of Vienna > AllFam > Browse

AllFam Allergen Family List

Search options:

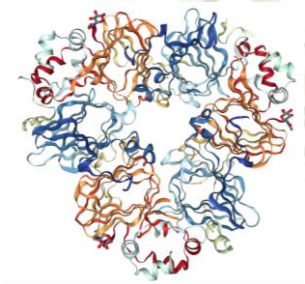
Sources: All

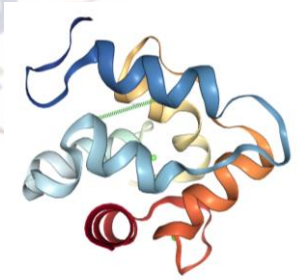
Routes of exposure: All

Include only IUIS approved allergens.

Update list

151 allergen families found.
959 of **1042** allergens from all sources with all routes of expos





ALLERGEN NOMENCLATURE
WHO/IUIS Allergen Nomenclature Sub-Committee
Financial contribution from IUIS, EAACI, and AAAAI organizations

Home | Search | Tree View | Publications | Carbohydrate Epitopes | Executive Committee | Submission Form | Log In

Search The Database

By Allergen Name (Three letter genus submit)

By allergen source (common or scientific name)
[a space and submit gives the list of sources]

Major Taxonomic Group: All

Order: All

Limit Search To: All allergens | food allergens | airborne allergens | contact allergens | injection allergens | unknown

Biochemical Name

Submit

Browse — Display all allergenes in the database

Search Results: 1037

http://www.meduniwien.ac.at/allfam/browse.php
http://www.allergen.org/

SELECTED FOOD ALLERGENS

Search options:

Sources: Plants

Routes of exposure: Ingestion

☐ Include only IUIS approved allergens.

Update list

45 allergen families found.
233 of **251** allergens from plants with ingestion as route of exposure classified into them.

	ID	Protein family name	Number of allergens
1	AF050	Prolamin superfamily	75
2	AF045	Cupin	36
3	AF051	Profilin	26
4	AF069	Bet v 1 family	21
5	AF060	Thaumatin-like protein	10

~63%

Allergens belong to a restricted number of protein families

Search options:

Sources: Animals

Routes of exposure: Ingestion

☐ Include only IUIS approved allergens.

Update list

24 allergen families found.
144 of **153** allergens from animals with ingestion as route of exposure classified into them.

	ID	Protein family name	Number of allergens
1	AF054	Tropomyosin	50
2	AF007	EF hand family	46
3	AF049	ATP:guanido phosphotransferase	7
4	AF065	Alpha/beta casein	4
5	AF031	Enolase	4

~70%

http://www.meduniwien.ac.at/allfam/browse.php

SELECTED PROTEIN FAMILIES

PLANT FAMILIES

CUPIN

- Legumins
- Vicilins

PROLAMIN

- 2S albumins
- nsLTP
- Cereal α-amylase
- Cereal prolamins

PROFILINS

PR-10

ANIMAL FAMILIES

TROPOMYOSINS

PARVALBUMINS

ARGININE KINASES

CASEINS

MINOR FAMILIES

- Glycoside hydrolase family 22 clan
- Transferrins
- Lipocalins
- Ovomucoids
- Serpins

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ASSAYS FOR ALLERGENICITY TESTING

	Specific serum screening			Cellular in vitro or ex vivo assays			In vivo assays			
Families	Immunoblot/ dot blot	ELISA	RAST/EAST/ ImmunoCAP	Basophil activation test	RBL mediator release assay	T-cell proliferation	Murine IgE response	Murine anaphylaxis	Human Skin prick tests*	Human Food challenges**
2S albumins	√	√	√	√	√	√	√	√	√	√
nsLTP	√	√	√	√	√	NR	NR	NR	√	√
ATI	√	√	NR	√	NR	√	√	NR	√	NR
Cereal prolamins	√	√	√	√	√	√	√	√	√	√
Profilins	√	√	√	NR	√	NR	NR	NR	√	√
Legumins	√	√	NR	NR	√	√	NR	NR	√	NR
Vicilins	√	√	√	√	√	√	NR	NR	√	√
PR-10	√	√	√	√	√	√	NR	NR	√	√
Tropomyosins	√	√	NR	√	√	√	√	√	√	NR
Parvalbumins	√	√	√	√	√	NR	√	NR	√	NR
Arginine kinase	√	√	NR	NR	√	√	√	√	NR	NR
Caseins	√	√	√	√	√	√	NR	NR	√	√
Serum albumins	√	√	√	NR	NR	√	NR	√	√	√
Glycoside Hydrolase	√	√	NR	√	√	√	√	√	√	√
Transferrins	√	√	NR	NR	NR	√	√	√	NR	NR
Lipocalins	√	√	NR	√	√	√	√	√	√	√
Ovomucoids	√	√	NR	√	√	√	√	√	NR	√
Serpins	√	√	NR	√	√	√	√	√	NR	√

√, confirmation of tests performed as reported on literature; NR, no evidence found in the literature; *, Human SPT were performed mainly with pure protein, although pure food extracts were also used. **, Food challenges are normally performed using pure food extracts or entire food (either alone or hidden within a prepared matrix), respectively.

In general, for the same allergen or set of allergens there is a high variability in the results from different assays

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	Impact on IgE-binding capacity	Supporting evidence/Main concerns (PLANT ALLERGENS)
ABUNDANCE (allergen content in relation to total protein)	High	Potent allergens are often highly abundant.
BIOLOGICAL FUNCTION	High	Potent allergens display biological functions as capacity, transport and defence.
PTM		
Glycosylation	Limited	Increases allergenicity, most likely IgE-binding regions containing N-glycans. Information mostly limited to vicilins
Hydroxylation	Limited	Increase the allergenic potential of Ara h 2 (limited to 2S albumins)
Phosphorylation	-	Not reported
LIPID-BINDING	High	Lipid binding stabilises protein structure, increasing resistance to proteolysis and processing.
PROTEIN STRUCTURE		
Loss of 2D	High	Decreases allergenicity of most plant allergens. Loss of structural integrity. Valid for conformational epitopes
Loss of S-S bonds	High	Decreases allergenicity of most plant allergens. Loss of structural integrity. Valid for conformational epitopes
GLYCATION	Low or inconclusive	Depending on the protein family, glycation decreases, maintain or increase allergenicity
AGGREGATION	Low or inconclusive	Depending on the protein family, aggregation decreases, maintain or increase allergenicity
HEAT STABILITY	High	Potent allergens are heat stable. Fails to explain potent heat-labile allergens (e.g. profilins, PR-10 proteins)
PRESSURE STABILITY	Limited	Potent allergens are pressure stable, but in vivo evidence has hardly been studied. Maintain protein integrity.
LIGHT/RADIATION STABILITY	High	Potent allergens are light/radiation stable. Maintain protein integrity.
MECHANICAL STABILITY	Low	Most allergens are stable to mechanical processing. Maintain protein integrity.
CHEMICAL STABILITY		
Changes in protein structure	High	Maintain or reduce the IgE-binding capacity. Limited information to nsLTP and legumins
Changes in protein size (fragmentation)	High	Maintain or reduce the IgE-binding capacity of 2S albumins, ATI, legumins, vicilins, profilins and PR-10 families. Fragmentation of protein into peptides. Loss of protein primary structure.
Changes in protein size/structure	High	Enhance and maintain the IgE-binding capacity. Limited information to cereal prolamins and 2S albumins
DIGESTIBILITY		
Pepsin resistance	Low or inconclusive	Fails to explain potent pepsin-labile allergens (e.g. Ara h 3, Gly m 6)
Trypsin/chymotrypsin resistance	High	Most allergens are resistant to trypsin/chymotrypsin activities.
Lipid interaction	High	Presence of lipids protects allergens from proteolysis. Maintain protein integrity.

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	Impact on IgE-binding capacity	Supporting evidence/Main concerns (ANIMAL ALLERGENS)
ABUNDANCE	Low	Low abundant as well as high abundant proteins are known as potent allergens, e.g. tropomyosins (low), caseins (high abundant).
BIOLOGICAL FUNCTION	High	Potent allergens display biological functions as storage, regulation, transport, and defence.
PTM		
Glycosylation	Low	Contradictory effects are found for potent allergens. Information is limited to tropomyosins, arginine kinases, caseins, ovomucoids.
Acetylation	Limited	Increase the IgE-binding capacity of parvalbumins. Information limited to parvalbumins
Phosphorylation	Limited	Phosphorylation increases IgE-binding capacity. Information limited to caseins and serpins
LIPID-BINDING	Limited	Reduces allergenicity. Information limited to Bos d 5 (lipocalins).
LIGAND-BINDING	Low	Contradictory effects are found for different potent allergens. Information limited to parvalbumins, caseins, transferrins, lipocalins.
PROTEIN STRUCTURE		
Loss of 2D/Loss of S-S bonds	Low	Contradictory effects. Loss of structural stability decrease (destruction of conformational epitopes) or maintain/increase (unmasking hidden linear epitopes) IgE-binding capacity.
GLYCATION	Low/inconclusive	Chemical changes (formation of advanced glycation products) can decrease, maintain, or increase IgE-binding capacity (depending on protein family or within the same family). Data missing for transferrins and serum albumins.
AGGREGATION	Low/inconclusive	Structural changes (formation of aggregates and potentially new conformational epitopes) can decrease, maintain, or increase IgE-binding capacity. Data missing for transferrins.
HEAT STABILITY	Low	Heat stable allergens are potent allergens. Fails to explain potent heat-labile allergens (e.g. arginine kinase, lipocalins)
PRESSURE STABILITY	Low	Pressure alone has a limited effect on allergens. Maintain protein integrity. Data missing for arginine kinases and transferrins.
LIGHT/RADIATION STABILITY	High	Light/radiation stable proteins are potent allergens. High doses of radiation decrease IgE-binding capacity (promotes unfolding).
MECHANICAL STABILITY	Low	Most allergens are stable to mechanical processing, preserving their IgE-binding capacity. Maintain protein integrity. Data missing for caseins, transferrins, and ovomucoids.
CHEMICAL STABILITY		
Changes in protein structure	High	Reduce the IgE-binding capacity.
Changes in protein integrity (fragmentation)	High	Reduce/mitigate the IgE-binding capacity. Loss of protein primary structure.
DIGESTIBILITY		
Pepsin resistance	Low/inconclusive	Fails to explain potent pepsin-labile allergens (e.g. some members of tropomyosins)
Trypsin/chymotrypsin resistance	High	Most allergens are labile to trypsin/chymotrypsin activities.
Lipid interaction	High	Presence of lipids protects allergens from proteolysis. Maintain protein integrity.

MAIN CONCLUSIONS

- ✓ Each property has **DIFFERENT IMPACT** depending on the protein family or even on the allergen itself.
- ✓ Independently on the effect of each property, they all converge to a **COMMON OUTCOME**, which concerns **PROTEIN INTEGRITY**.
- ✓ **GLYCOSYLATION IS NOT AN UNIVERSAL TRAIT OF ALLERGENS**.
- ✓ **HEAT STABILITY and PROTEOLYTIC RESISTANCE ARE NOT ALWAYS A SYNONYM OF INCREASED PROTEIN ALLERGENICITY**.
- ✓ With the increasing number of proteins that has been identified and classified as food allergens, there are **several important allergens that do not fit into the general classification of allergens (ex: potent heat-labile allergens, potent pepsin-labile allergens).**
- ✓ **Properties affecting PROTEIN INTEGRITY and COMPOSITION can be correlated with the ELICITATION CAPACITY** of certain allergens, but **WHAT RENDERS A PROTEIN TO BE ALLERGENIC IN THE FIRST PLACE** and **WHICH PROPERTIES MIGHT IMPACT SENSITISATION ARE STILL QUITE UNCLEAR**.
- ✓ Several physicochemical properties shape the allergenicity of proteins, although at different extents. **THE LEVEL IS NOT THE SAME AMONG PLANT OR ANIMAL ALLERGENS**.

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	PLANT ALLERGENS	ANIMAL ALLERGENS
PROPERTIES	IMPACT ON IGE-BINDING CAPACITY	
ABUNDANCE	High	Low
BIOLOGICAL FUNCTION	High	High
PTM		
Glycosylation	Limited	Low
Acetylation	-	Limited
Hydroxylation	Limited	-
Phosphorylation	-	Limited
LIPID-BINDING	High	Limited
LIGAND-BINDING	-	Low
PROTEIN STRUCTURE		
Loss of 2D	High	Low
Loss of S-S bonds	High	Low
GLYCATION	Low or inconclusive	Low or inconclusive
AGGREGATION	Low or inconclusive	Low or inconclusive
HEAT STABILITY	High	Low
PRESSURE STABILITY	Limited	Low
LIGHT/RADIATION STABILITY	High	High
MECHANICAL STABILITY	Low	Low
CHEMICAL STABILITY		
Changes in protein structure	High	High
Changes in protein integrity (fragmentation)	High	High
Changes in protein size/structure	High	-
DIGESTIBILITY		
Pepsin resistance	Low or inconclusive	Low or inconclusive
Trypsin/chymotrypsin resistance	High	High
Lipid interaction	High	High

Can we use parameters associated with protein stability for allergenicity prediction???

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CRITICAL OBSERVATIONS/GAPS

- ✓ Using this information for **ALLERGENICITY PREDICTION OF NOVEL PROTEINS REMAINS A FUTURE CHALLENGE**.
- ✓ **HARMONISATION OF PROTOCOLS** is much needed.
- ✓ **RING TRIALS** – data from interlaboratory analysis is **practically inexistent** (could help clarify if the contradictory effects observed for specific allergens are real or if they result from cumulative differences in protocols.
- ✓ **COMPARATIVE STUDIES** – **allergens vs non-allergens** – are also needed.
- ✓ **Weight of evidence** from different assays to test allergenicity is quite different (serological vs cellular/in vitro vs in vivo assays).
- ✓ Studies about the **IMPACT OF PROCESSING *IN VIVO* IN HUMANS** are very scarce but much needed.
- ✓ Work on single allergens, processed under lab conditions may help to better characterize allergens at molecular level but this setting might not fully reflect in vivo reality.
- ✓ Possible solution: would be to **CREATE A LINK USING ALLERGY ANIMAL MODELS**.

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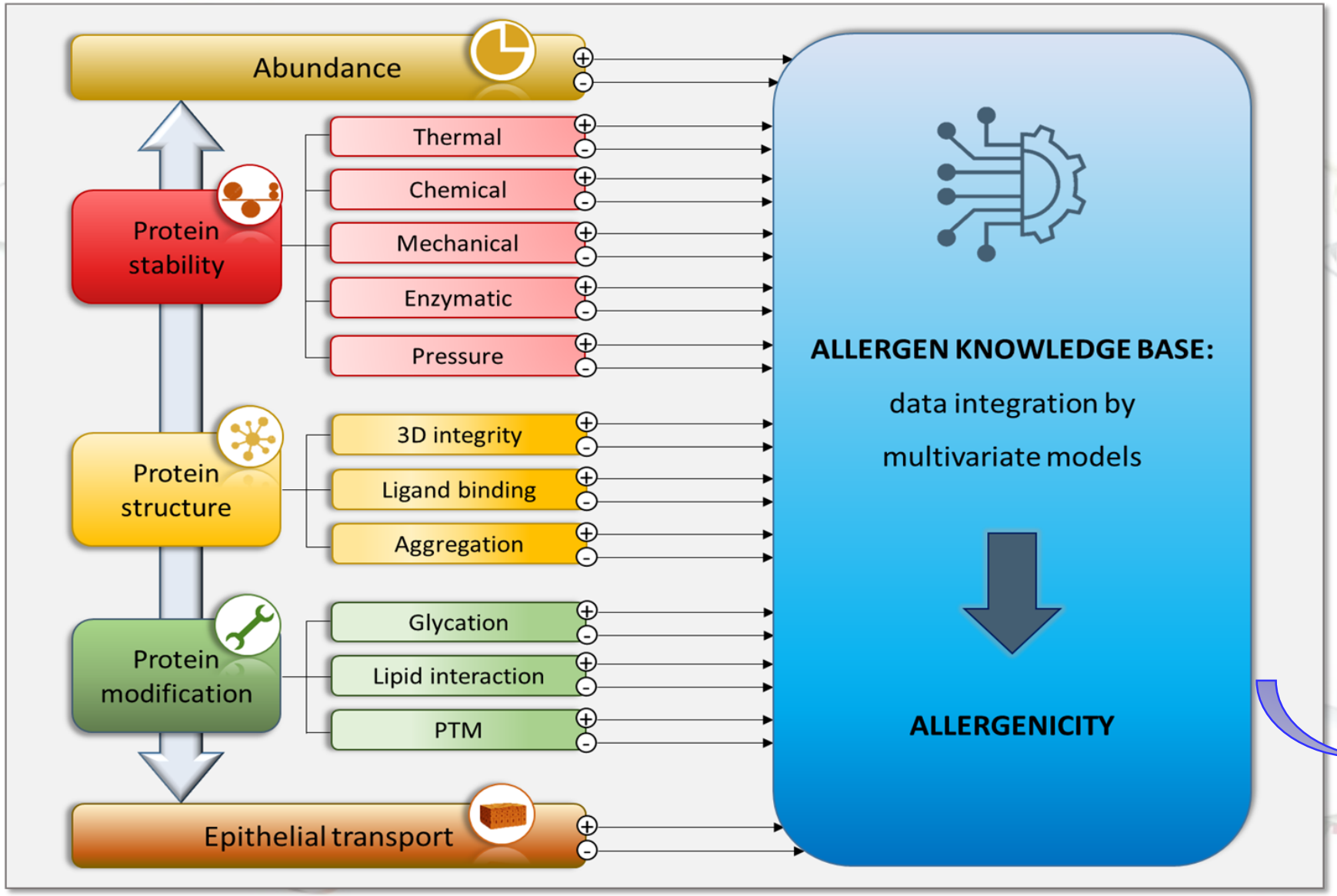
FUTURE?



DATA INTEGRATION COULD GIVE A BROADER PICTURE ON HOW THE COMPLETE SET OF PROPERTIES SHAPE PROTEIN ALLERGENICITY.

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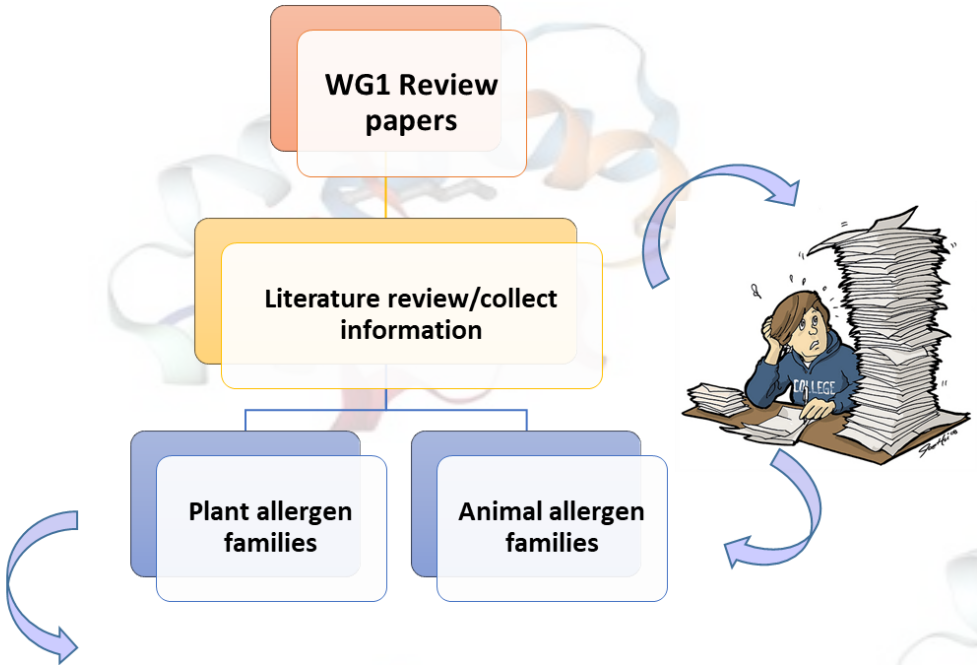

Clinical Reviews in Allergy & Immunology
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Are Physicochemical Properties Shaping the Allergenic Potency of Animal Allergens?

Joana Costa[1] · Caterina Villa[1] · Kitty Verhoeckx[2] · Tanja Cirkovic-Velickovic[3,4,5] · Denise Schrama[6] · Paola Roncada[7] · Pedro M. Rodrigues[6] · Cristian Piras[8,9] · Laura Martín-Pedraza[10] · Linda Monaci[11] · Elena Molina[12] · Gabriel Mazzucchelli[13] · Isabel Mafra[1] · Roberta Lupi[14] · Daniel Lozano-Ojalvo[15] · Colette Larré[14] · Julia Klueber[16,17] · Eva Gelencser[18] · Cristina Bueno-Diaz[19] · Araceli Diaz-Perales[20] · Sara Benedé[12] · Simona Lucia Bavaro[11,21] · Annette Kuehn[16] · Karin Hoffmann-Sommergruber[22] · Thomas Holzhauser[23]

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Are Physicochemical Properties Shaping the Allergenic Potency of Plant Allergens?

Joana Costa[1] · Simona Lucia Bavaro[2,3] · Sara Benedé[4] · Araceli Diaz-Perales[5] · Cristina Bueno-Diaz[6] · Eva Gelencser[7] · Julia Klueber[8,9] · Colette Larré[10] · Daniel Lozano-Ojalvo[11] · Roberta Lupi[10] · Isabel Mafra[1] · Gabriel Mazzucchelli[12] · Elena Molina[4] · Linda Monaci[2] · Laura Martín-Pedraza[13] · Cristian Piras[14,15] · Pedro M. Rodrigues[16] · Paola Roncada[17] · Denise Schrama[16] · Tanja Cirkovic-Velickovic[18,19,20] · Kitty Verhoeckx[21] · Caterina Villa[1] · Annette Kuehn[8] · Karin Hoffmann-Sommergruber[22] · Thomas Holzhauser[23]

Check for updates

ImpARAS

GMO Workshop on allergenicity assessment

Acknowledgments: This work was supported by COST Action FA1402 (IMPARAS) with the involvement of several of its WG1 members. J. Costa thanks FCT for funding through program DL 57/2016 – Norma transitória (SFRH/BPD/102404/2014).

THANKS FOR YOUR ATTENTION