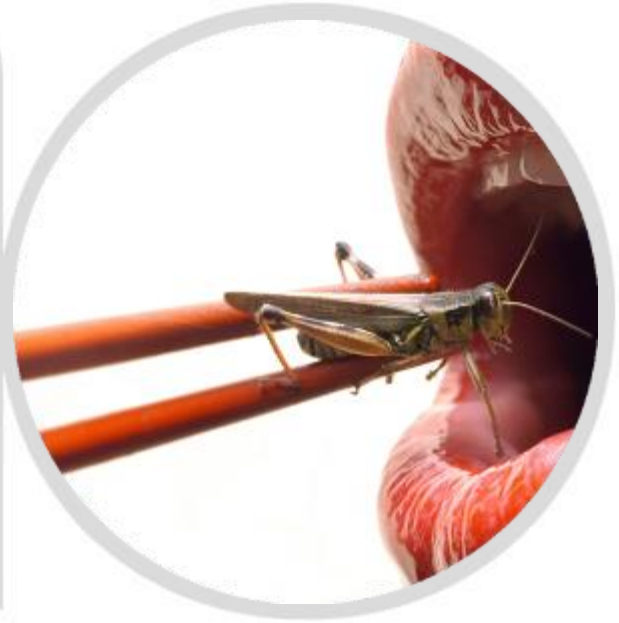
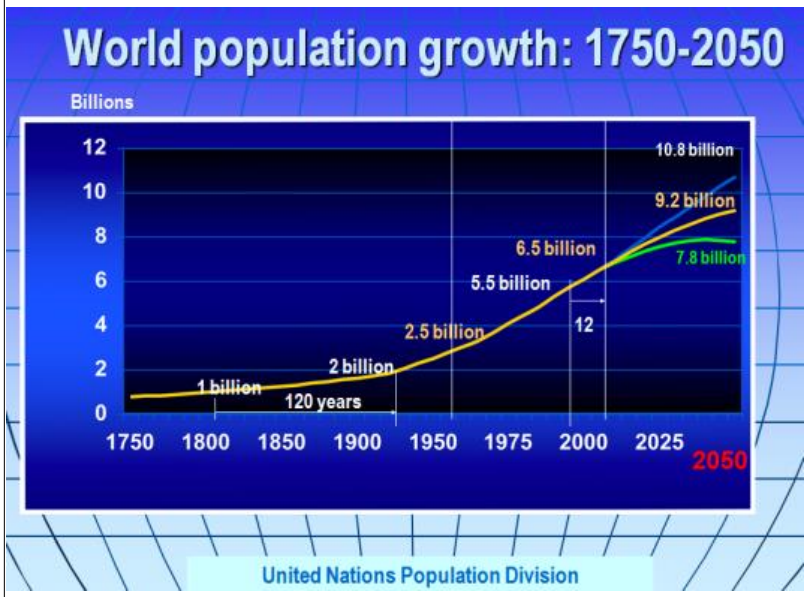
Allergenicity risk assessment of novel proteins in food:

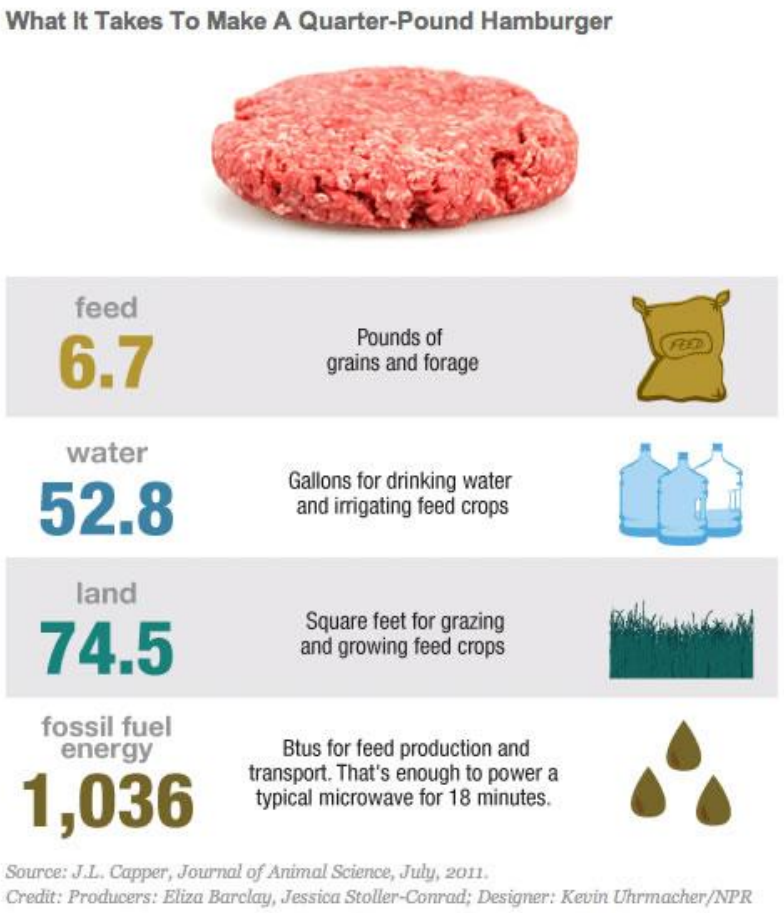
Case study and future improvements



Kitty Verhoeckx

Background





Sustainable food production >
Alternative protein sources >

General food law

(EC regulation No 258/97 and EU recommendation 97/618)

- The law requires that safety is assured for all food ingredients placed on the market.
- Responsibility of the producers

- Novel food law: Comprehensive food safety assessment for novel foods introduced after 1997

Nutritional

Nutrition Facts

Serving Size 172 g

Amount Per Serving	
Calories 200	Calories from Fat 8
	% Daily Value*
Total Fat 1g	1%
Saturated Fat 0g	1%
Trans Fat	
Cholesterol 0mg	0%
Sodium 7mg	0%
Total Carbohydrate 36g	12%
Dietary Fiber 11g	45%
Sugars 6g	
Protein 13g	

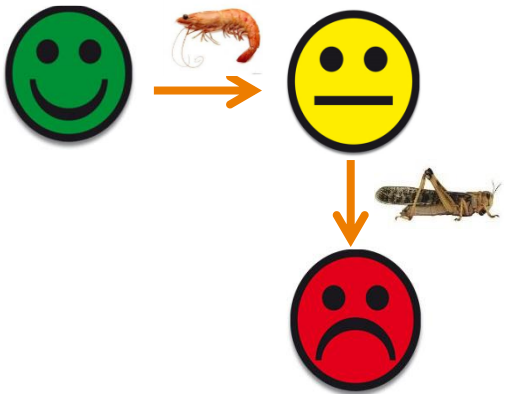
Microbial

Toxicological

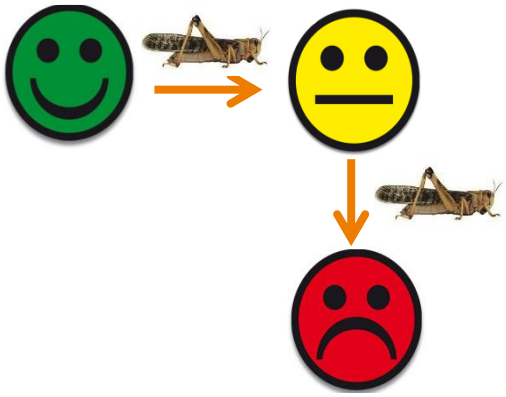
Allergenic

Allergy risk assessment strategy

- Is the novel protein able to elicit an allergic reaction in a food allergic population (cross reactivity)?
- Is the novel protein able to induce a new allergy (sensitization)?



cross reactivity



new allergy

Current strategy (EFSA/GMO)

- Weight of evidence approach

efsa
European Food Safety Authority

Source of gene

No reports on allergy → Allergy common

Sequence alignment

< 35% identity over 80 aa → > 70% identity over full length

IgE tests

No specific IgE → Specific IgE to allergen

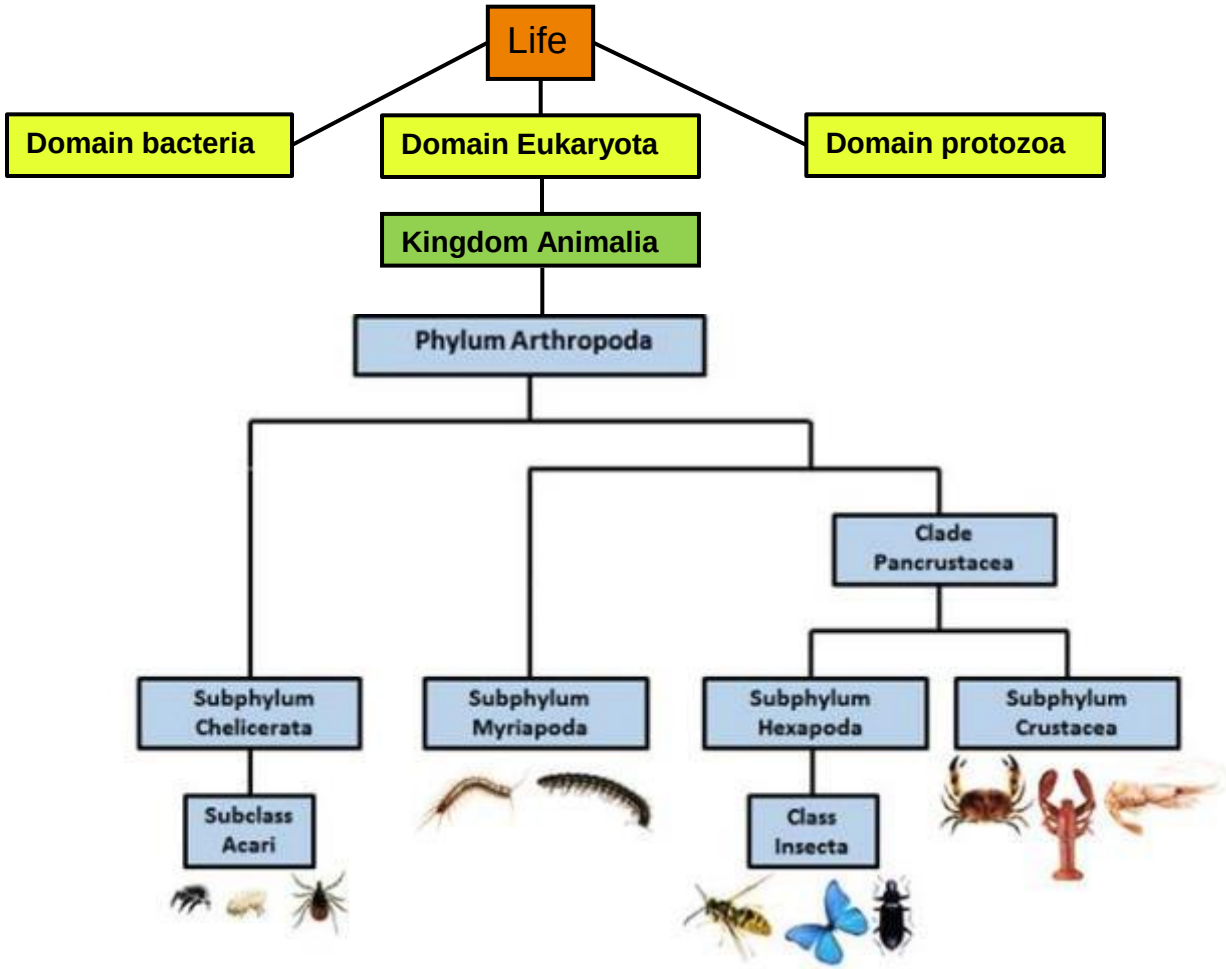
Pepsin digestion

Digested > 90% in < 2 min → Stable for 60 min

Yellow Mealworm (Tenebrio molitor)

- ✓ Larval stage of the Yellow mealworm beetle
- ✓ Originally produced as feed for animals such as fish, reptiles and birds.
- ✓ Commercially available for human consumption (Australia, UK, NL and Belgium)

Mealworm related to shrimp and house dust mite

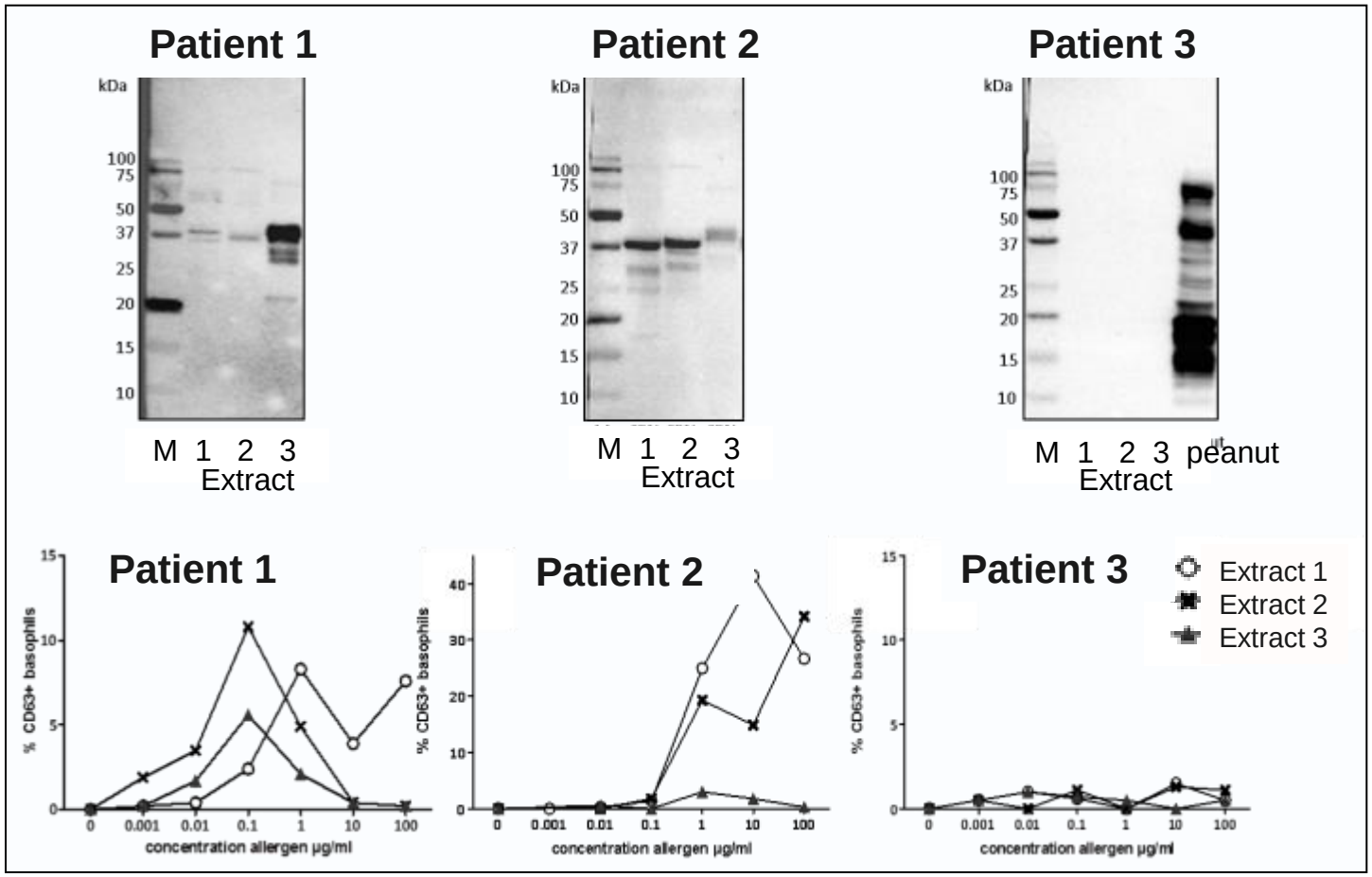


Verhoeckx & van Broekhoven Food and Chemical Toxicology (2014)

sIgE from shrimp allergic patients react with mealworm proteins

Immunoblot

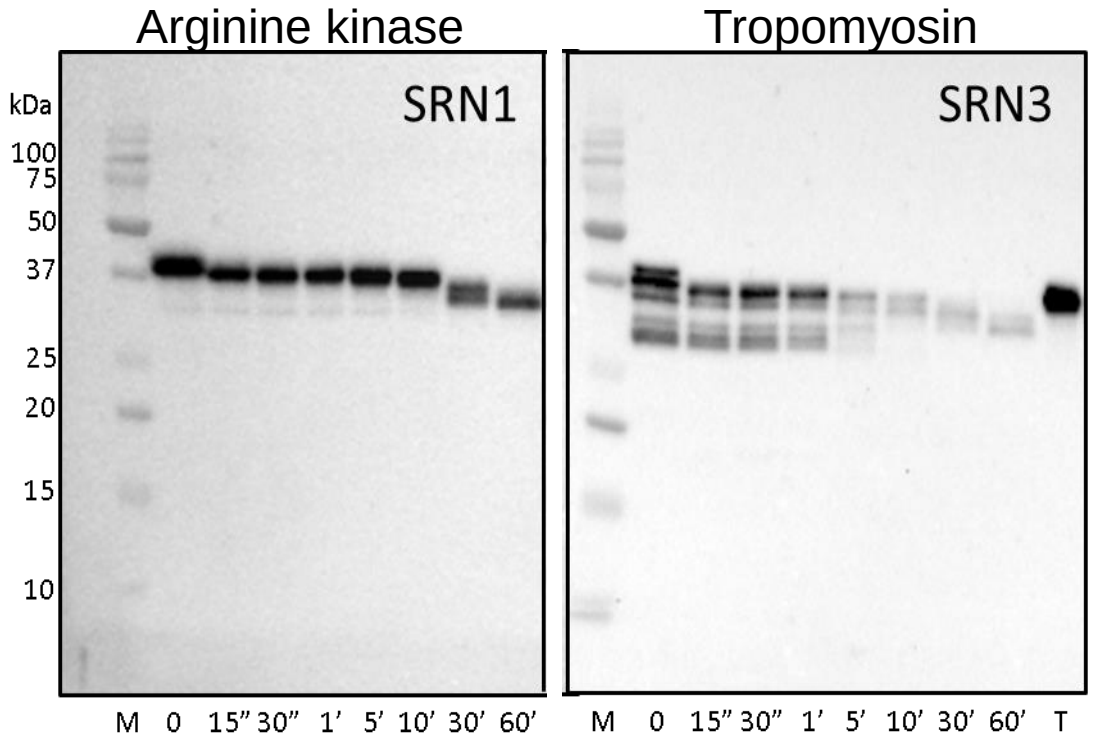
Basophil activation



Verhoeckx & van Broekhoven Food and Chemical Toxicology (2014)

Tropomyosin and arginine kinase are mildly stable in pepsin resistance test

Immunoblot



SRN1 Soluble proteins (tris)
SRN3 Difficult to solubilize proteins (ureum)

Verhoeckx & van Broekhoven Food and Chemical Toxicology (2014)

Universitair Medisch Centrum Utrecht

TNO innovation for life

New protein (source)

History of human exposure (work/food)

Relationship: Taxonomy, Homology

Identification proteins in source

Information on usage: raw, matrix, processing

Research material: Extract(s), GMP

Research protocol, METC

Cross reactivity

New allergies & Cross reactivity

New allergies

Cross reactivity
Sera known allergy with new protein

- Immuno-blot
- ELISA
- CAP/ISAC

Identification proteins

Functional Cross reactivity
Sera known allergy with new protein

Basophil activation test

Skin prick test

Food challenge

Sensitising potency

History Sensitisation
Screening (interview and/or serology)

- Working population
- Targeted people with symptoms

No history
TNO®RAPT
(under development)

Identification reactive proteins in source (potential new allergens)

Intrinsic properties

- **Digestion**
- **Physical chemical**
- **Biological**

Cross reaction
Sera new allergy with known allergen

- *In vitro*
- *In vivo*

New allergy
Sera new allergy with new protein

- *In vitro*
- *In vivo*


Universitair Medisch Centrum Utrecht

TNO innovation for life

Conclusions cross reactivity

- All shrimp allergic patients (n=15) were sensitized to mealworm based on SPT, BAT, Immunoblot and CAP mealworm
- 87% of the Shrimp allergic patients had a positive DBPCFC to mealworm
- Shrimp allergic patients are at risk when eating mealworm


Universitair Medisch Centrum Utrecht

TNO innovation for life

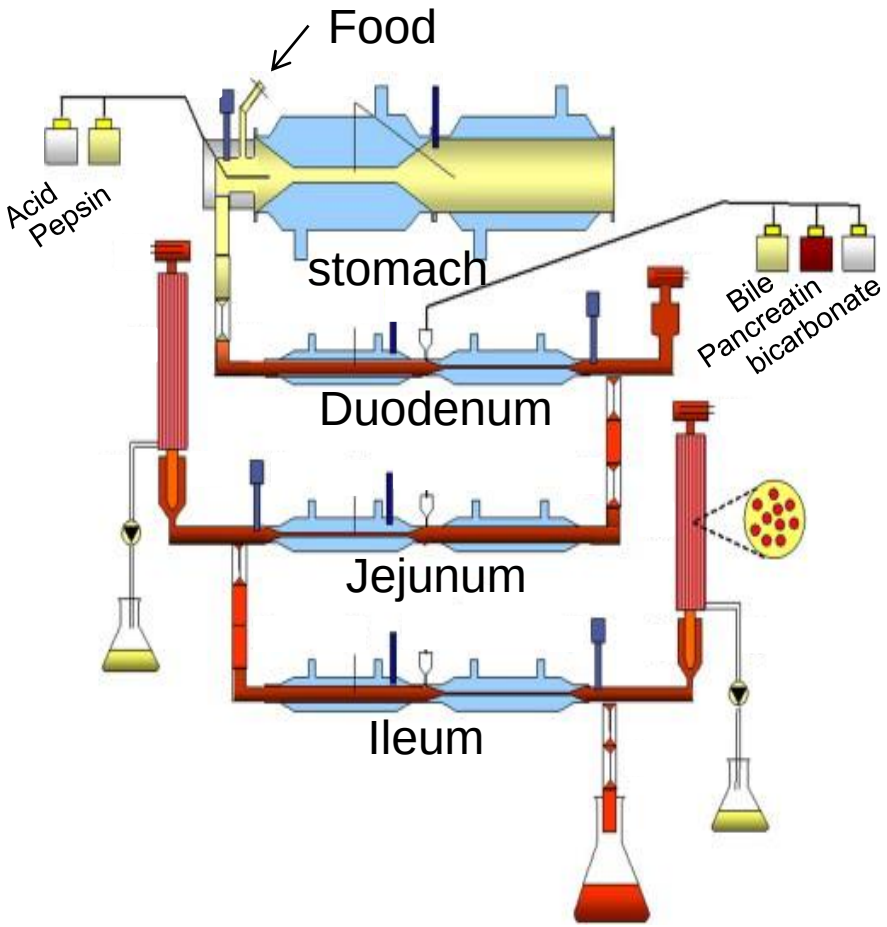
Conclusions De novo sensitisation

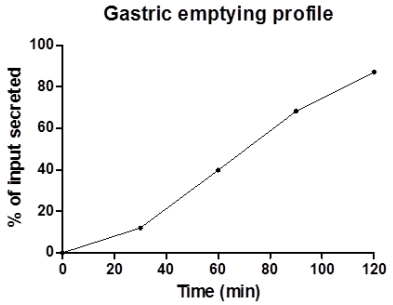
- All subjects (n=4) were atopic and sensitized to mealworm according to SPT, BAT, Immunoblot and CAP mealworm.
- Two subjects had a positive DBPCFC to mealworm, but were not allergic to shrimp.
- Test population was to small to draw conclusions.
- New strategies are needed to assess De novo sensitisation.

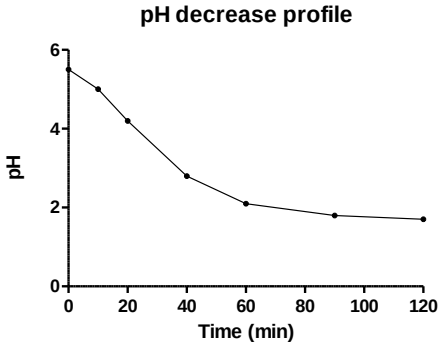
Food for thought

- Static vs Dynamic digestion
- Matrix
- Bioactivity after digestion
- Bioactivity after transport

TNOs Intestinal model (TIM)





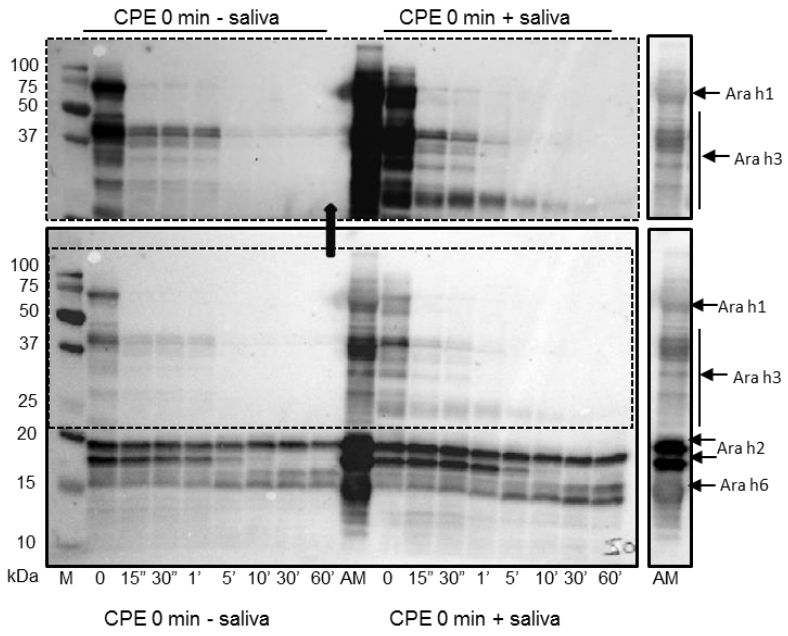


You Tube: hJImi6iK3gQ

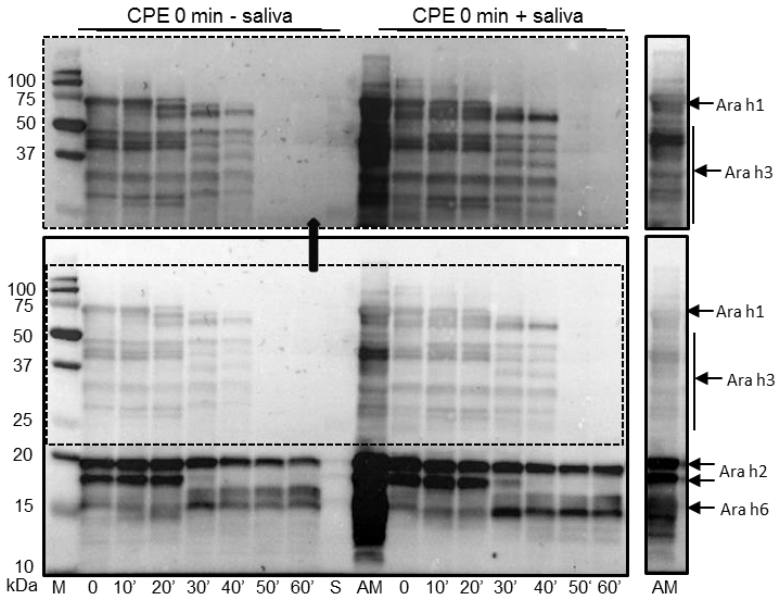
Bio-accessibility after digestion: Static vs Dynamic digestion (TIM)

Immunoblot

Static digestion CPE

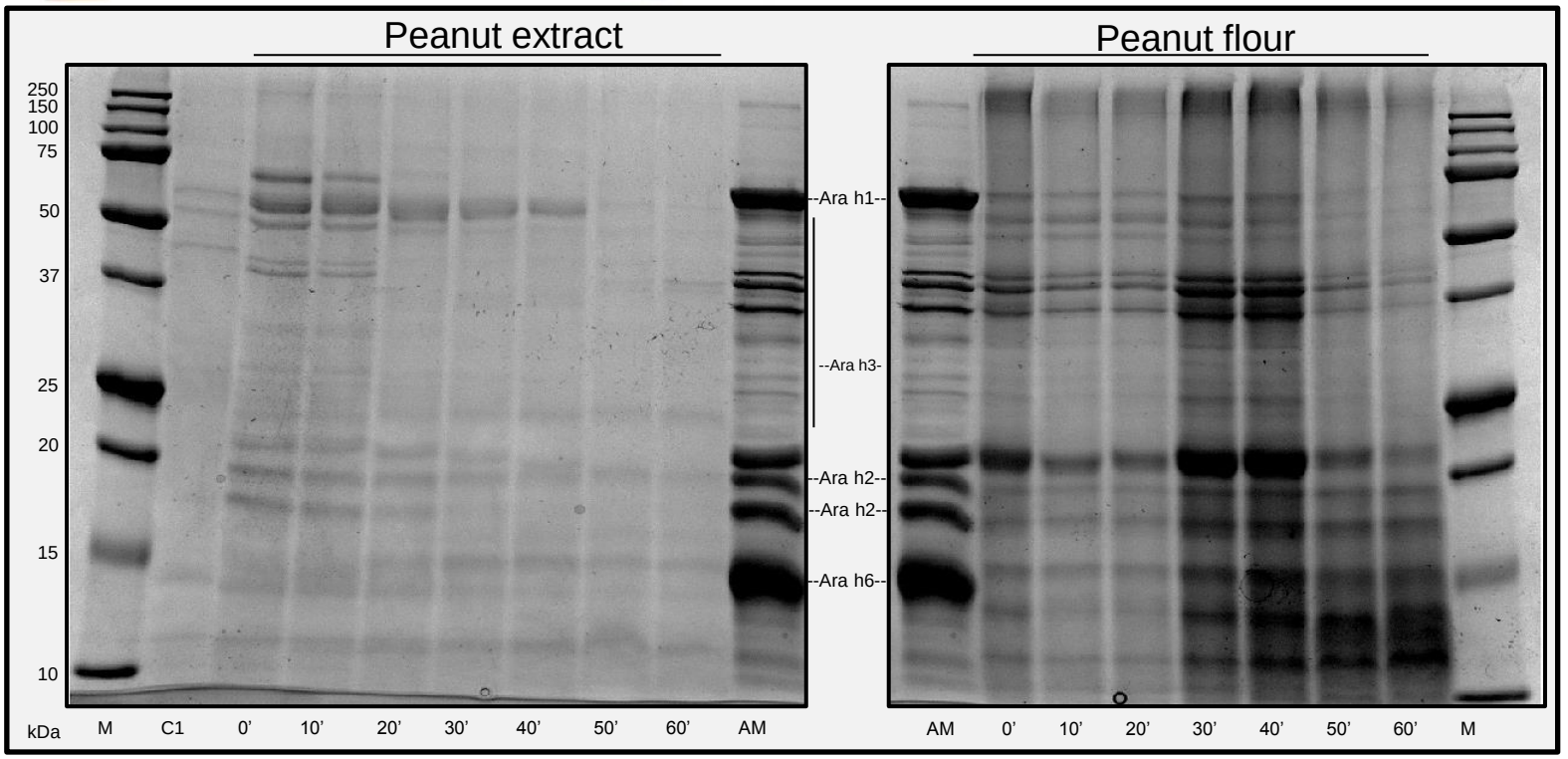


Dynamic digestion CPE



Intact protein will reach the Intestine due to gastric emptying

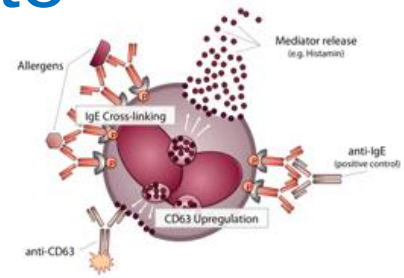
Digestion kinetics of peanut flower is different from peanut extracts in TIM

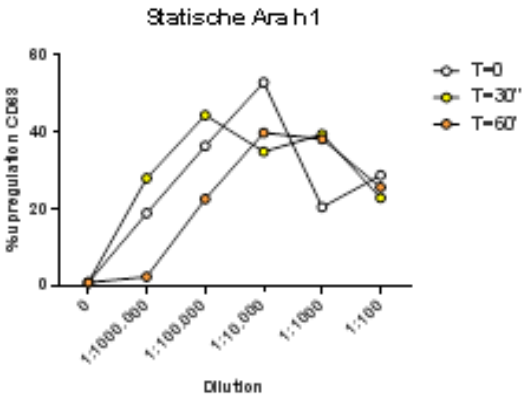


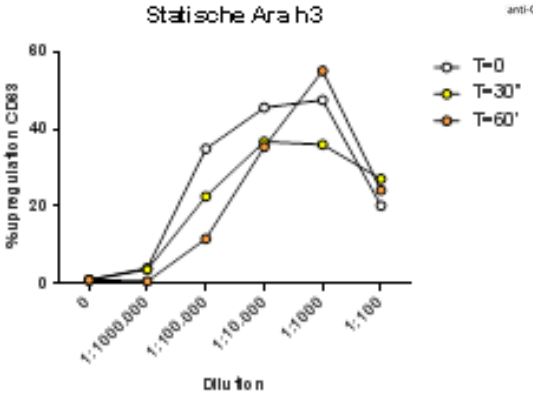
Verhoeckx et a

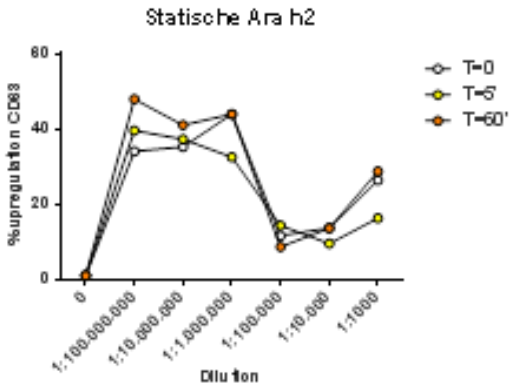
Digested Ara h 1 and 3 still able to activate basophils

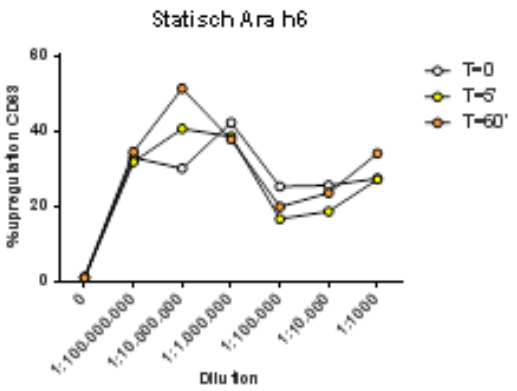
Basophil activation



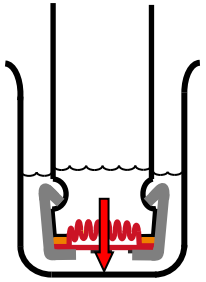




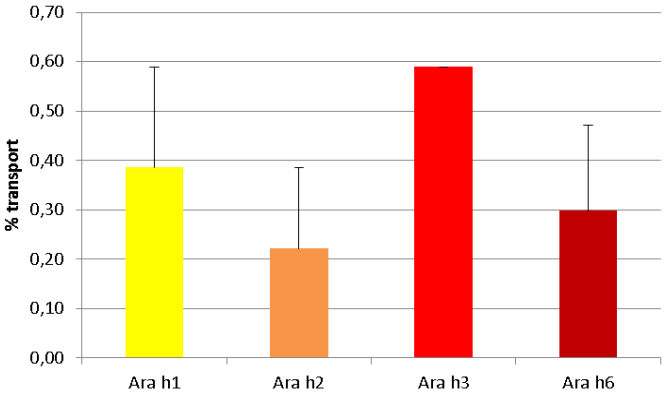




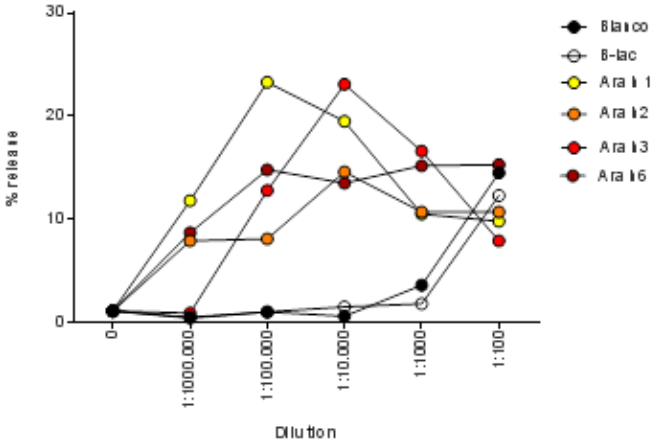
Ara h 1 and 3 lose reactivity after transport



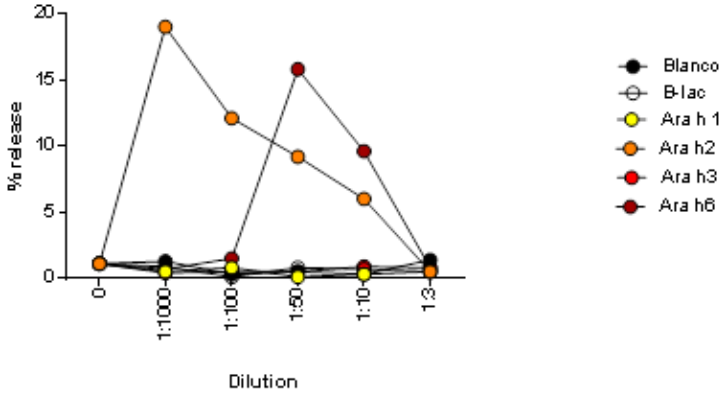
% transport



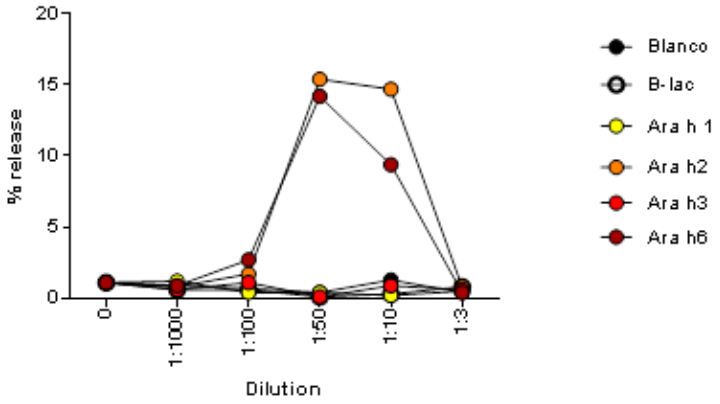
Apical

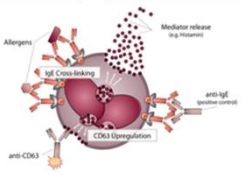


Basolateral Pig 1

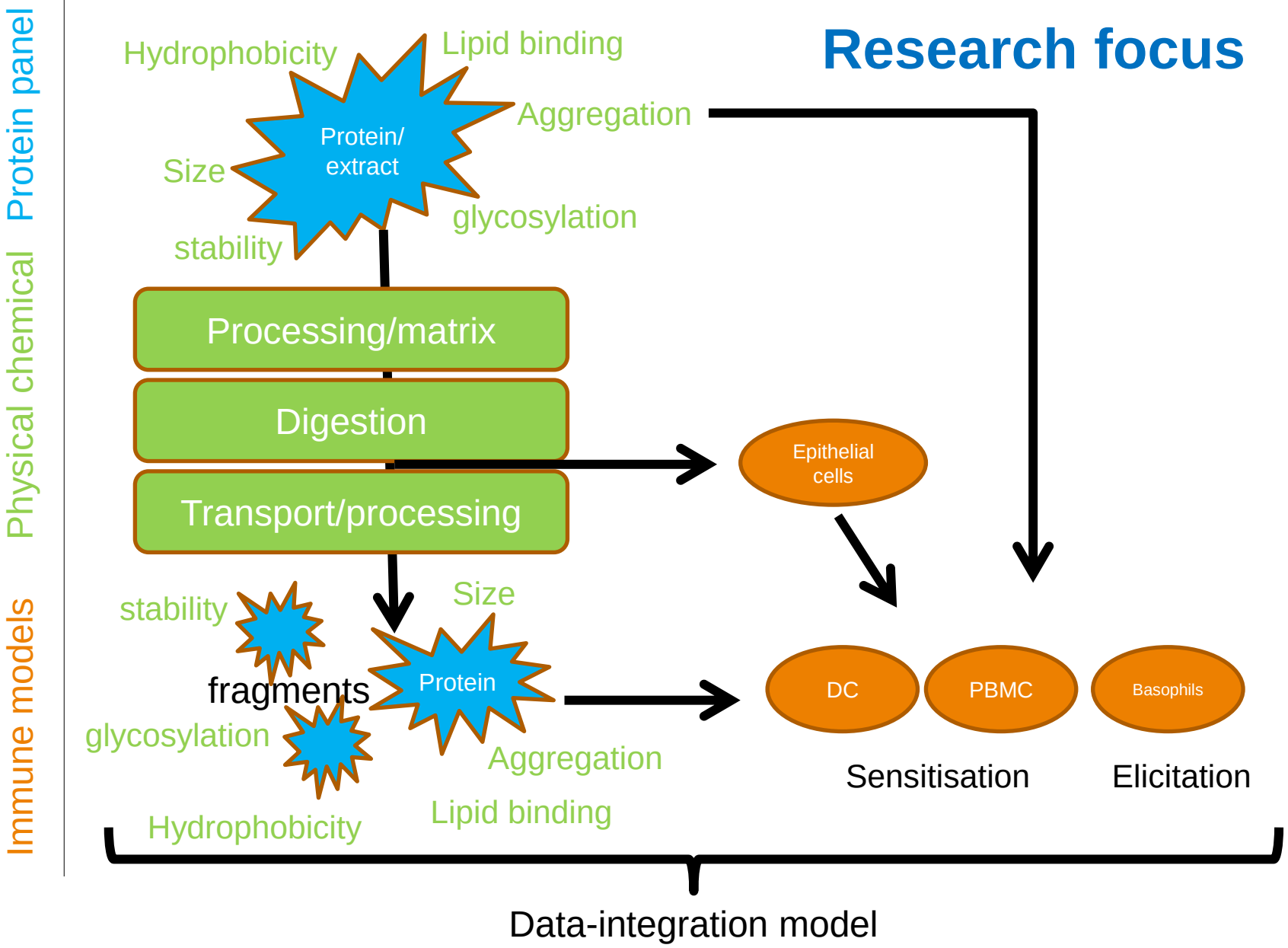


Basolateral Pig 2





Research focus



Improvement Allergy Risk assessment initiatives

- TNO Shared Research Program Food Allergy
- COST Action ImpARAS

Shared Research Program Food Allergy

Towards a Food Allergy Free World

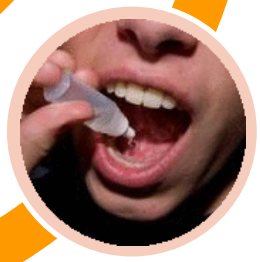
Goals

Protect the allergic consumer

4-6 years

Prevent introduction strong allergenic foods

6-8 years



Preventive & curative strategies

10-15 years

Program Lines

1: Allergen & allergy management

2: Allergenicity assessment of (novel) food proteins

3: Effect assessment and markers to improve diagnostics, prognostics and monitoring of immune health interventions

ImpARAS

Improving Allergy Risk Assessment Strategy for new food proteins

Aim:

To build an interdisciplinary European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity risk assessment strategy

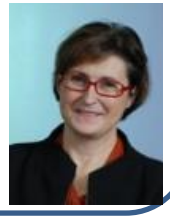
ImpARAS

Chair: Kitty Verhoeckx

Vice Chair: René Crevel

Working group 1
Physical chemical properties and Analysis

Karin Hoffmann-Sommergruber



Working group 2
In vitro methods

Erwin Roggen

Working group 3
In vivo methods

Liam O'Mahony

Working group 4
Risks assessment and clinical perspectives

Anne Constable

Website: www.imparas.eu

ImpARAS

1st International Conference

Improving Allergy Risk Assessment Strategy for new food proteins

November 24-26, 2015
University of Belgrade
Belgrade – Serbia

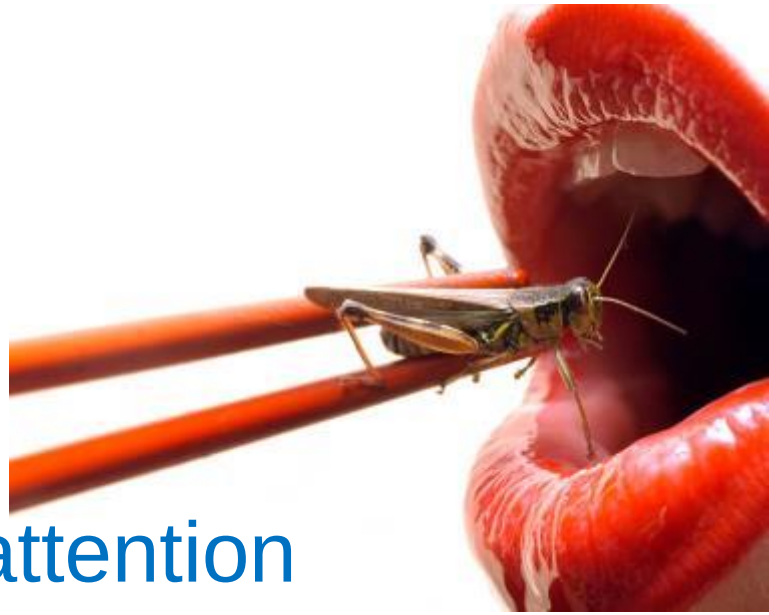
For more information please visit:
http://imparas.eu/meetings/

Contact: Iván López (ivan.lopez@csic.es)

TNO innovation for life

University of Belgrade

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY



Thank you for your attention

Kitty Verhoeckx
Scientist, TNO
T: +31 (0)88 8665136
@: Kitty.verhoeckx@tno.nl