Diagnosis of occupational asthma and rhinitis: usefulness of recombinant allergens (component resolved diagnosis), metabolomics and other new aspects
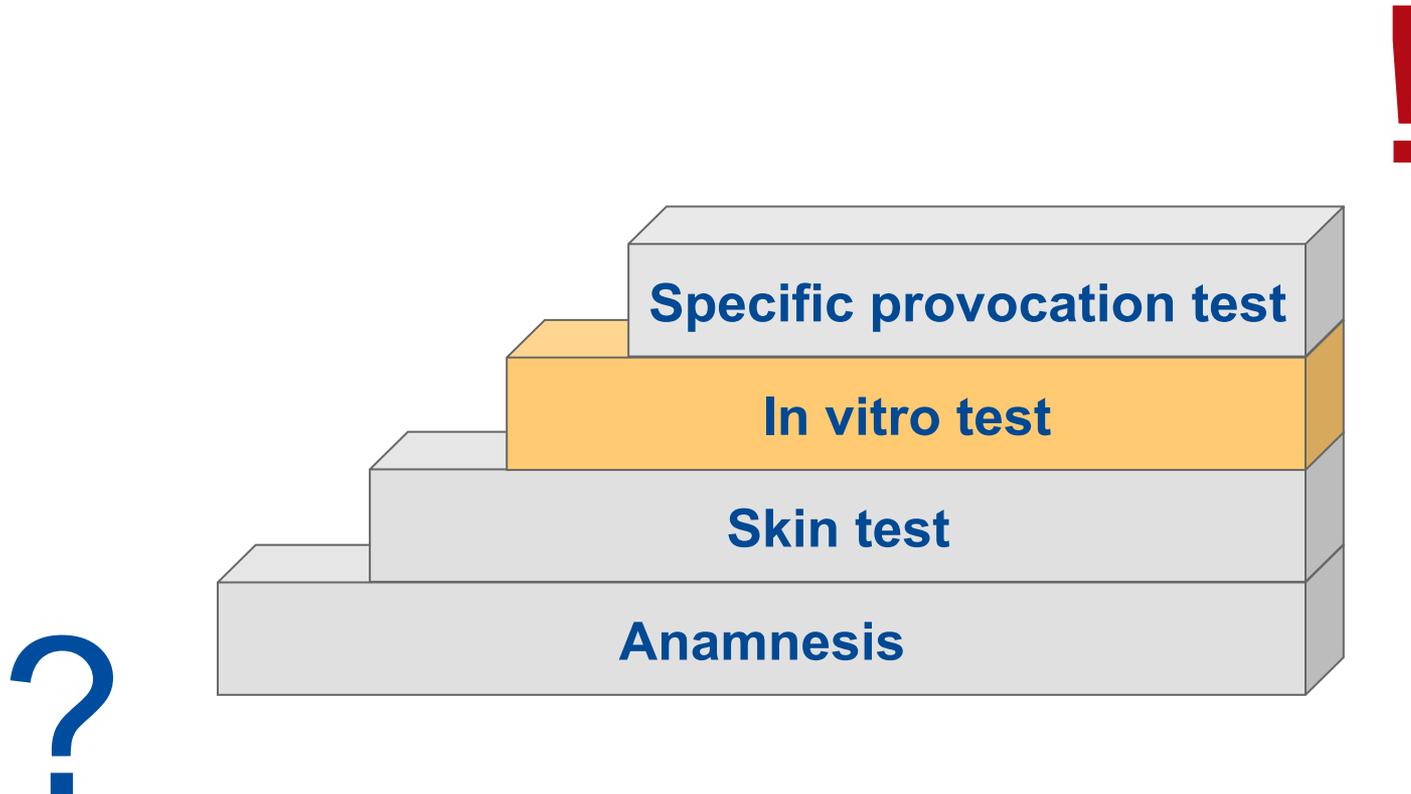
Monika Raulf
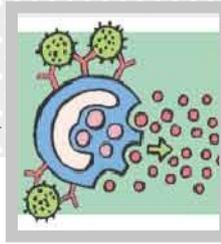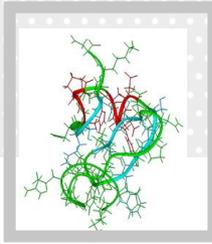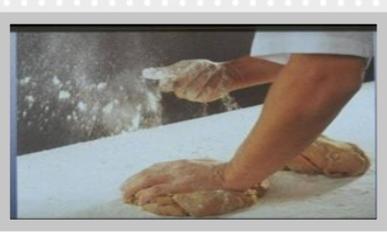
6th Jack Pepys Workshop, Toronto
May 13th, 2016

Allergy diagnostic

Specific provocation test

In vitro test

Skin test

Anamnesis

In vitro-test systems

2. Anti-IgE

IgE

Allergen-(Mix)

Solid phase
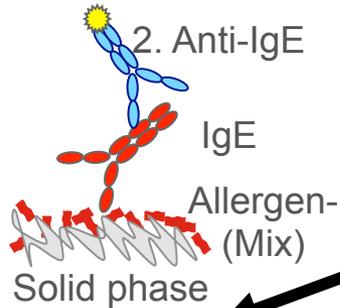
Specific IgE detection

Cellular tests

soluble mediators

Allergen extracts

Single allergens
- recombinant
- nativ

Basophils
- CAST
- FlowCAST
- histamine release

PBMC*2
- LTT

from eosinophils
- ECP

from mast cells/ basophils
- tryptase

Current standard

CRD*1

Complementary tests

*1component- resolved diagnosis   *2peripheral blood mononuclear cells

Occupational allergen sources

Flour dust

Isocyanates

Wood dust

Cow hair

Mold

Laboratory animals
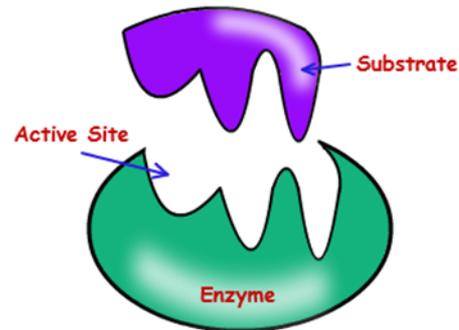
Enzymes

Natural rubber latex

(Storage-)mites

„Allergen" - is an ambiguous term

Allergen source: raw material from which allergenic extracts are obtained

Allergen extract: mixture of allergenic and non-allergenic molecules solubilized from a defined (usually) source

Allergen molecule: Proteins or glycoproteins identified with serum IgE of sensitized or allergic patients
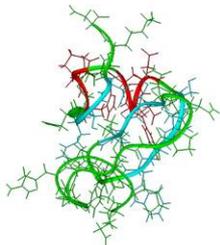
What about recombinant allergens?
From the allergen source to the allergen molecule

Allergen source   **Allergen extract**   **Specific allergen component**   **Cross reacting allergen component**
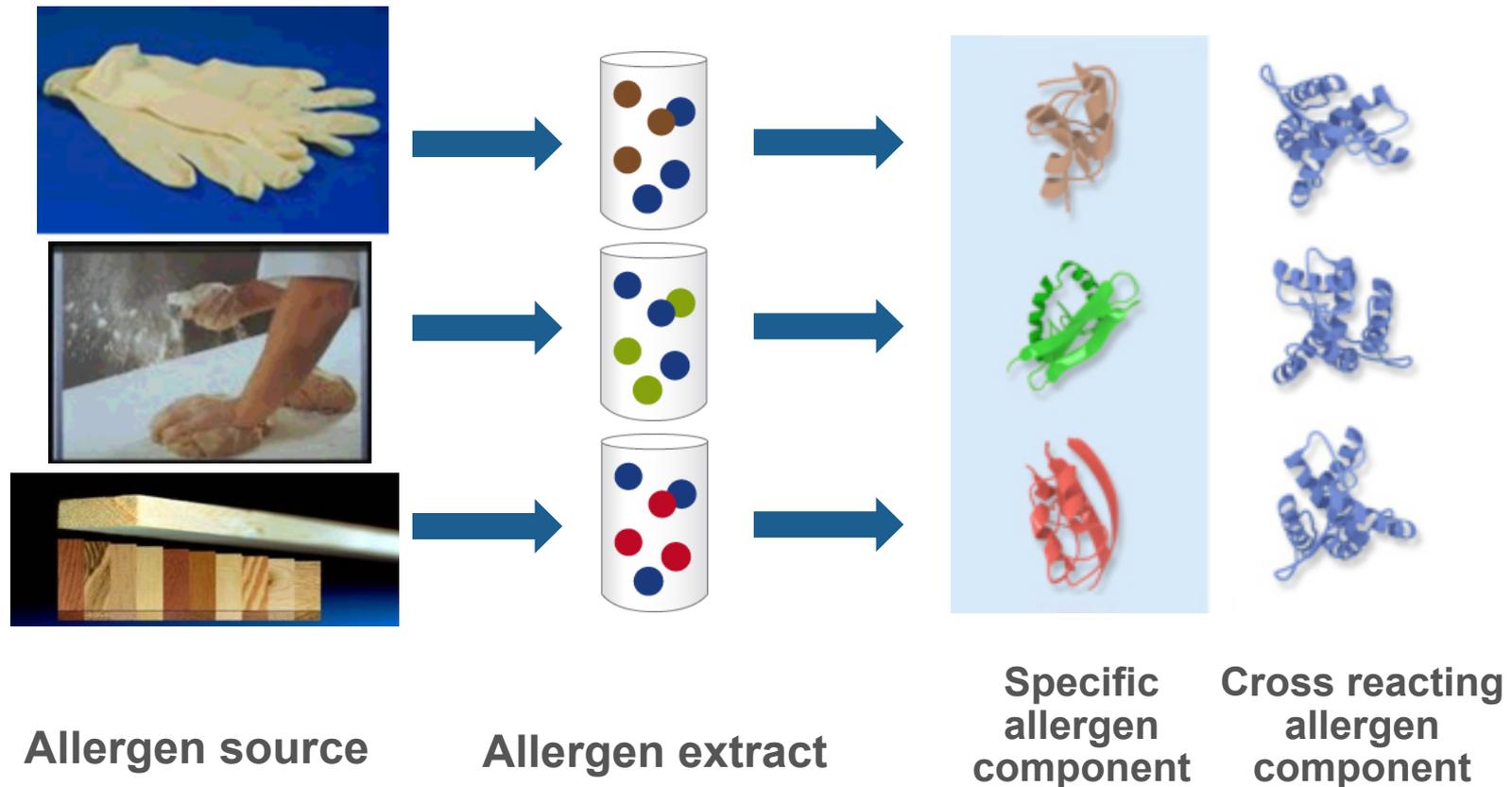
Referring to Huss-Marp/Thermo Fisher Scientific

Purified and recombinant allergens can be used

- alone (Singleplex method)

- in combination with CRD
  e.g. in the Microarray (Multiplex method)

- spiked in extracts

- combined as extract surrogate
  (so far not in use)

Methodical reasons for a molecular allergy diagnosis

Allergen source/ extract (A, B, C)

A — a1, a2, a3

B — b1, b2, b3, c1   **C** — c2

Reasons for the use of allergen molecules

Missing or barely available	Defined clinical role (e.g. risk)	IgE cross-reactivity	Genuine (primary) sensitization

Allergen molecules

a2 | a3 | b3 c1 | c2

Effects for the test:

• **Limit of quantitation (LoQ)** ↑ • Increasing of test sensitivity	• **Analytical specificity** ↑ • Limit of quantitation (LoQ)	Marker allergen of **cross-reactivity**	Marker allergen of **species-specific sensitization**

Variations:

1	2	3	4
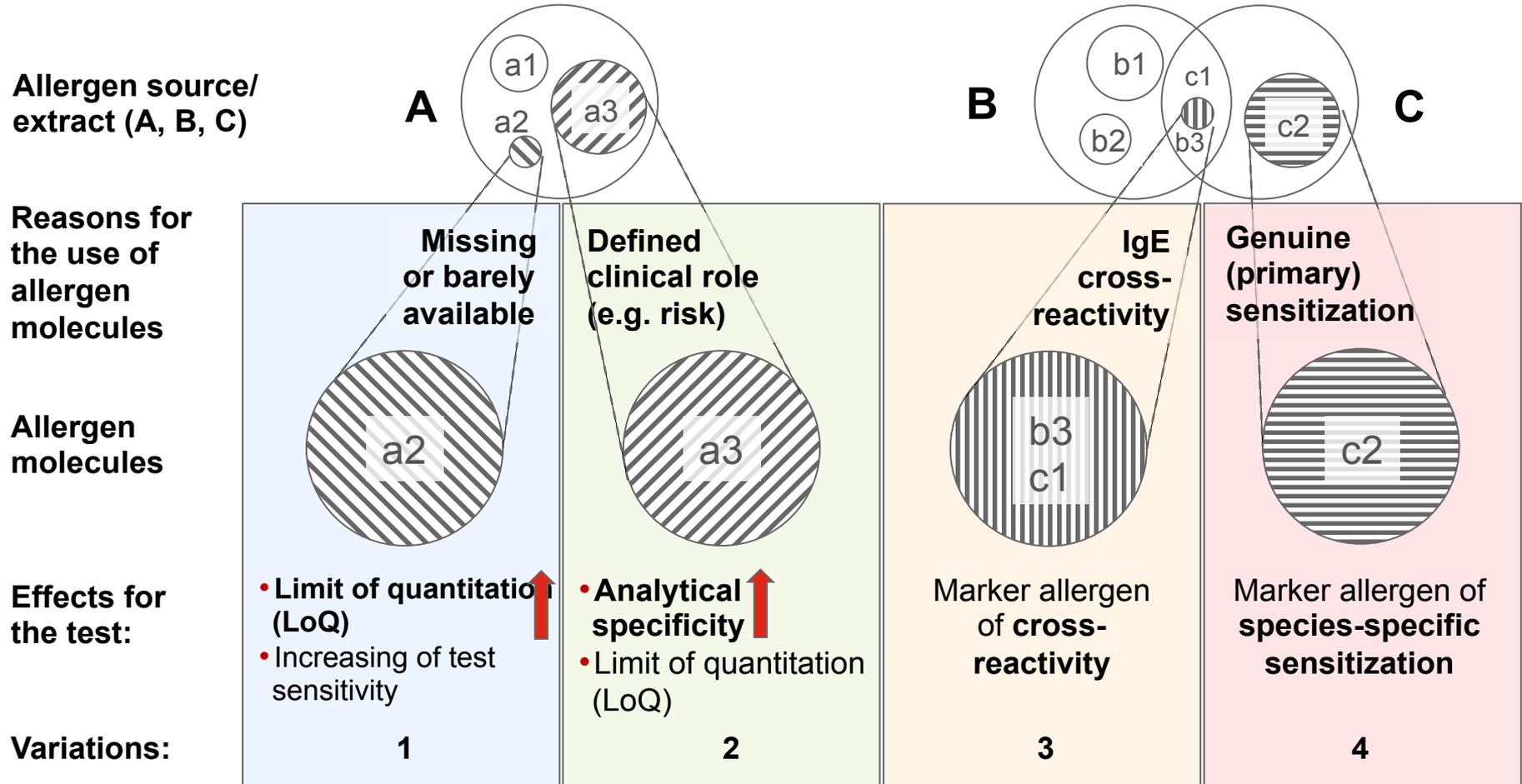
Allergens associated with a higher or lower risk

Higher risk

Profilin
(Cor a 2, Pru p 4, Mal d 4, Cuc m 2, Dau c 4)

PR-10
(Ara h 8, Cor a 1, Mal d 1, Cuc m 1, Dau c 1)

Lower risk

nsLTPs
(Pru p 3, Cor a 8, Ara h 9, Tri a 14)

Storage proteins
(Ara h 1, Ara h 2, Cor a 9, Gly m 5, Gly m 6)

PR-10
(Gly m 4)

Wheat ω5-gliadin
(Tri a 19)
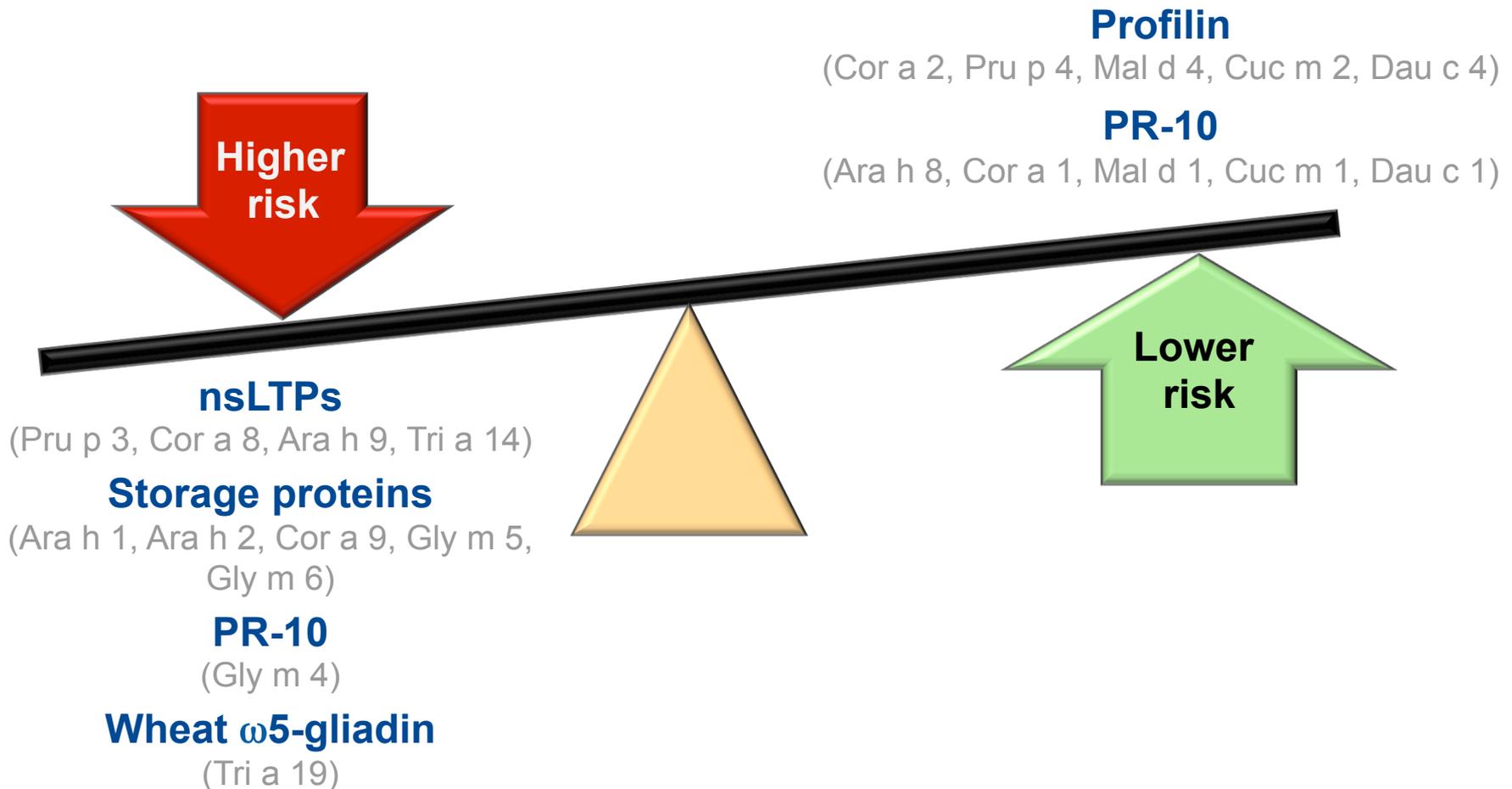
What can we learn from the "Latex Story "?

Latex allergy diagnostic "State of the art"

☺ Allergens are characterized, Hev b 1 - 15

☺ **r**Hev b 1, 3, 5, 6.01, 6.02, 7, 8, 11 available

☺ Sensitized patients clearly recognized **major allergens** (HCW versus SB)

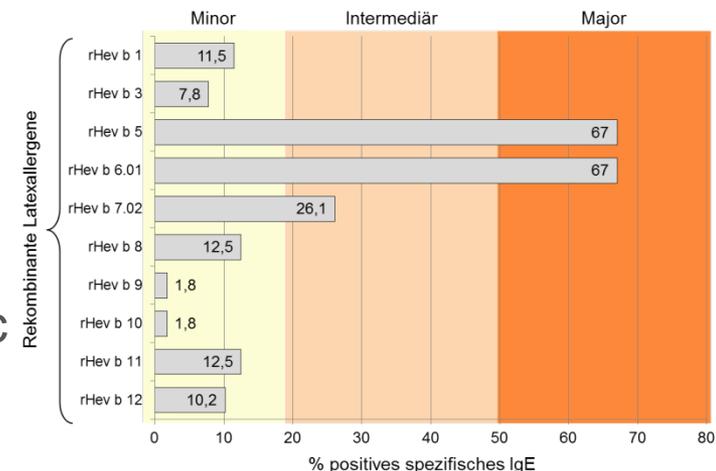☺ Spiking of the latex extract with the stable **rHev b 5** improved the in-vitro diagnostic

☹ Latex SPTs are no longer available

but

☺ Component-resolved in-vitro diagnostic is possible (diagnostic workflow)

Rekombinante Latexallergene	Minor	Intermediär	Major
rHev b 1	11,5		
rHev b 3	7,8		
rHev b 5			67
rHev b 6.01			67
rHev b 7.02	26,1		
rHev b 8	12,5		
rHev b 9	1,8		
rHev b 10	1,8		
rHev b 11	12,5		
rHev b 12	10,2		

% positives spezifisches IgE

Marker allergens for latex allergy

"True" latex allergens Hev b 1, 3, 5, 6

If positive, clinical relevant latex allergy; latex allergen prevention necessary

Latex profilin Hev b 8, CCD

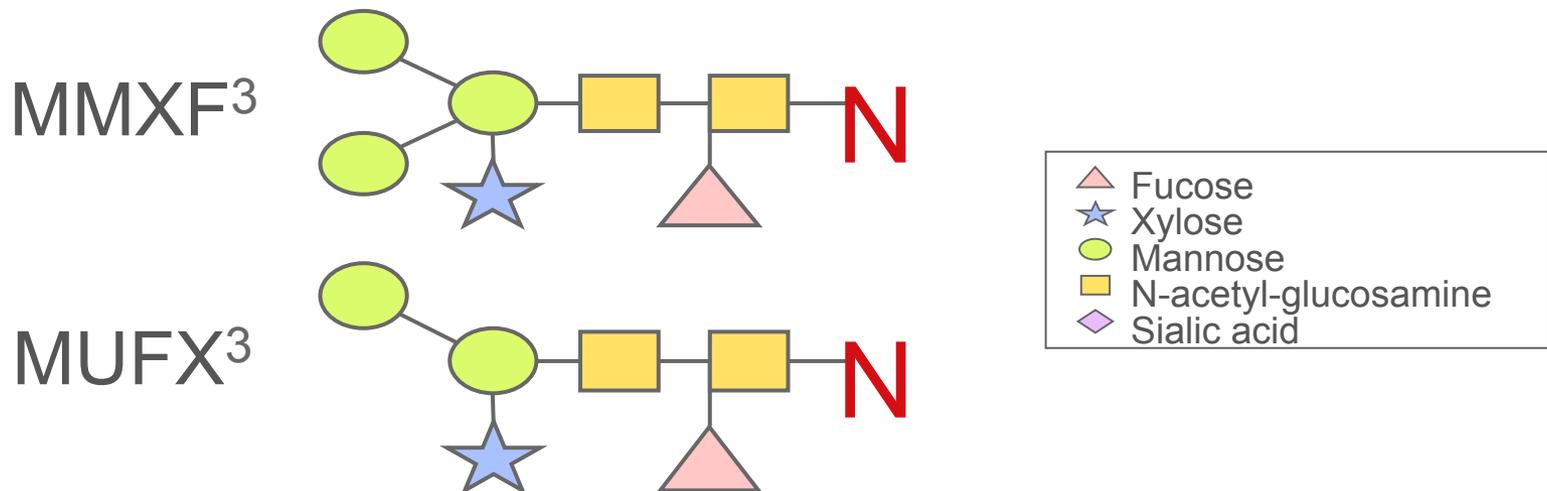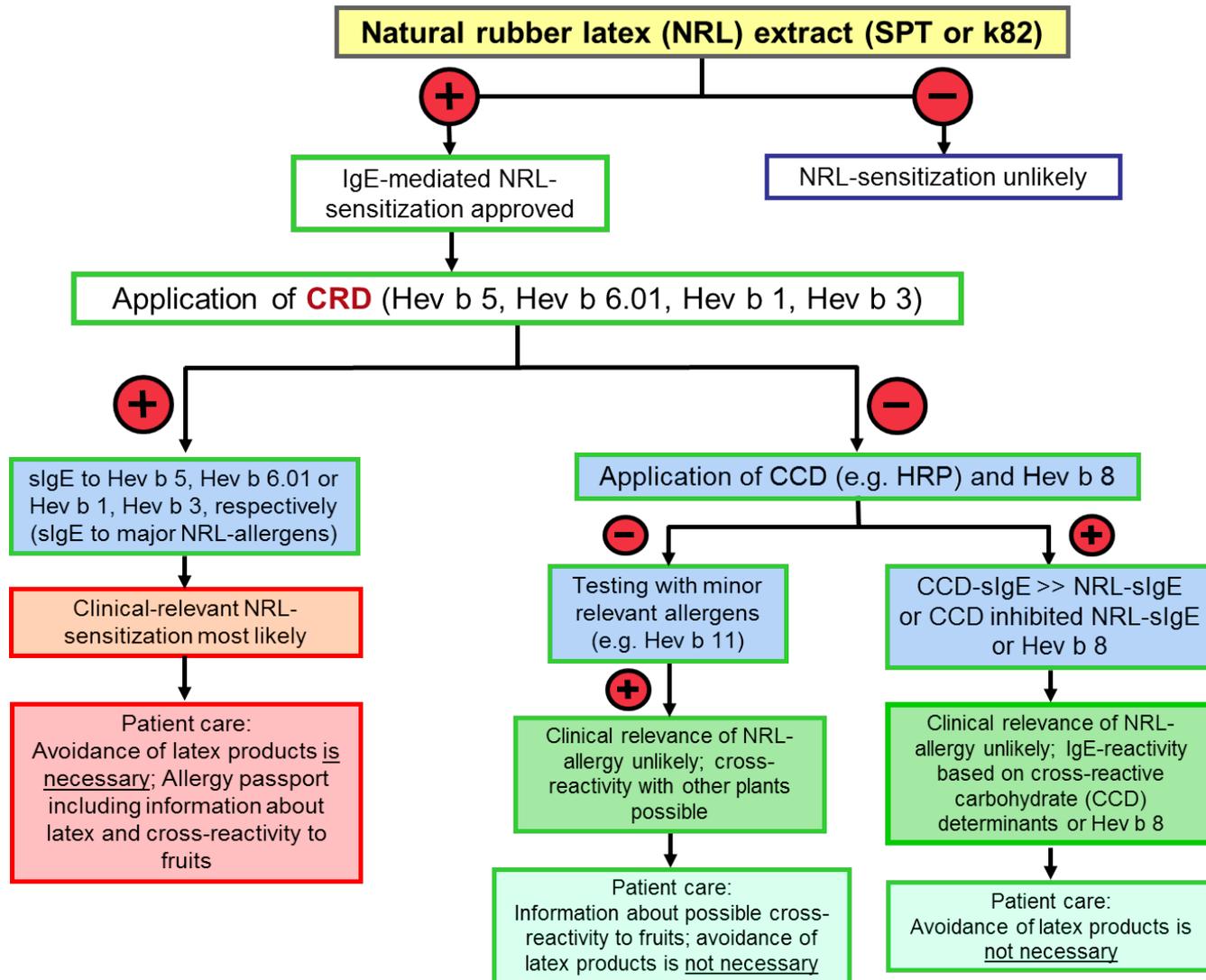**In asymptomatic patients, if Hev b 8 $\oplus$ and 'true' latex allergens $\ominus$, avoidance of latex contact is not necessary; no clinical relevant latex allergy**
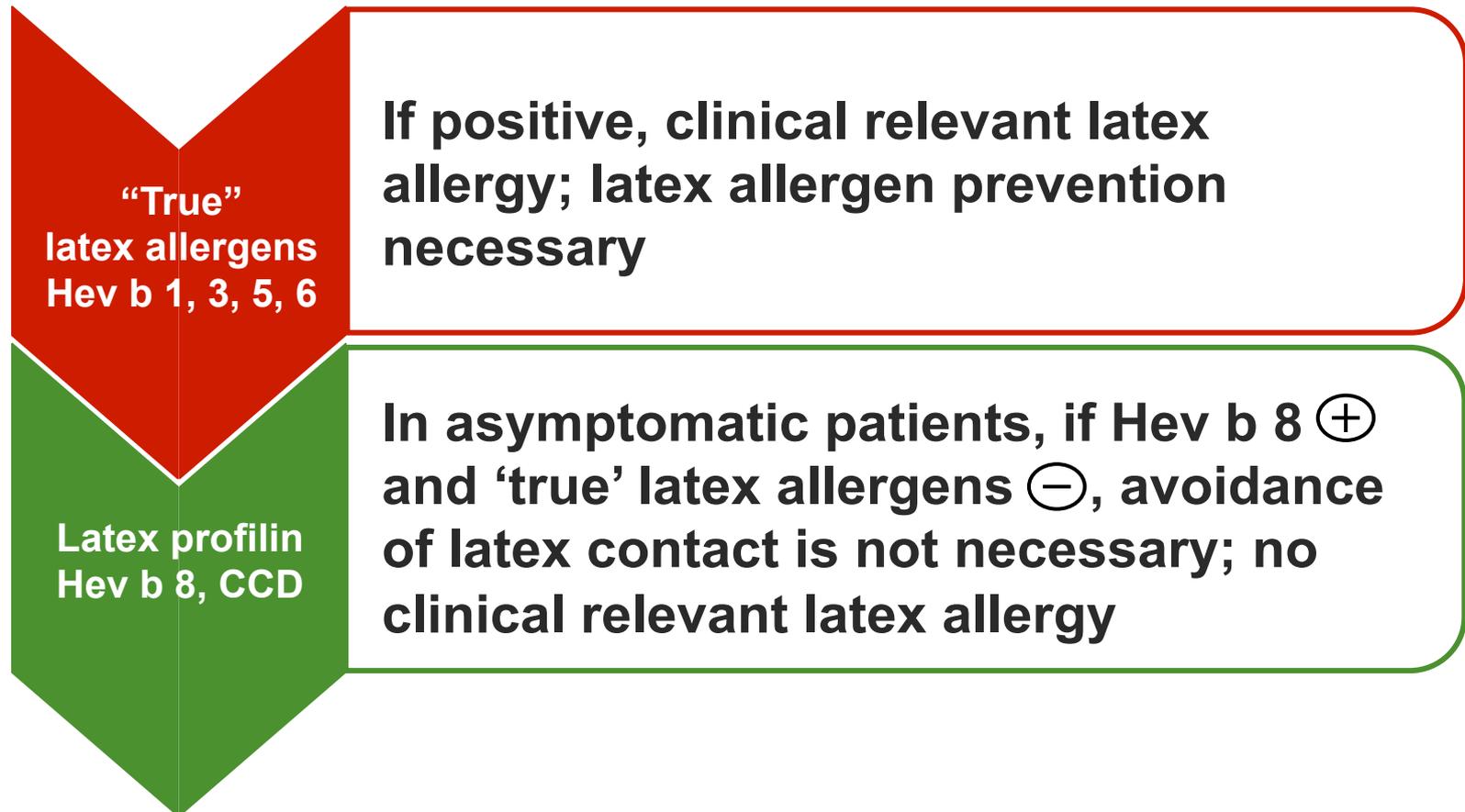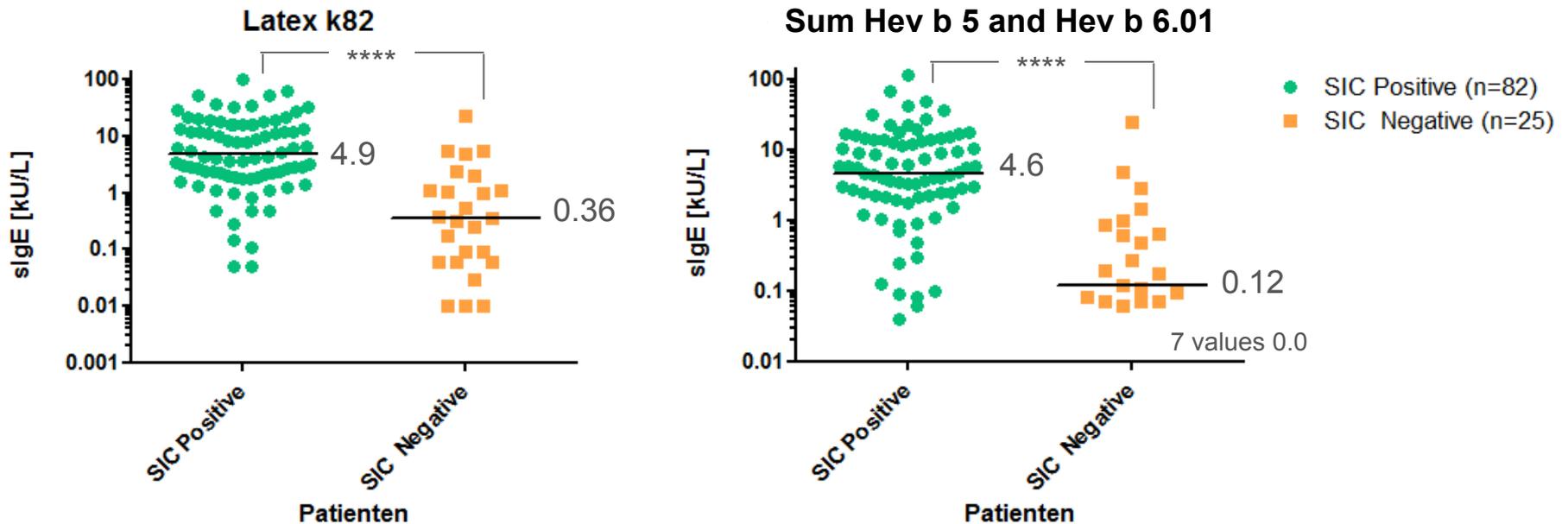
Cross-reactive Carbohydrate Determinants (CCD)

- Most allergens, particularly of plant origin, reveal a glycan-associated IgE reactivity.

- Glycan epitopes may share significant structural elements with allergens of other, non-related protein families. This feature predestinates them to be an important cause of a large variety of cross-reactions.

MMXF[3]

MUFX[3]

N

△	Fucose
☆	Xylose
●	Mannose
■	N-acetyl-glucosamine
◆	Sialic acid

Latex-sIgE vs. provocation Hev b 5 + Hev b 6.01-sIgE vs. provocation

Latex k82

Sum Hev b 5 and Hev b 6.01

- SIC Positive (n=82)
- SIC Negative (n=25)

	sIgE-Cut-Off [kU/L]	Sensitivity [%]	Specificity [%]	PPV	NPV	Youden-Index	Likeli-hood ratio	AUC
Latex k82	≥ 1.12	85 (76-92)	76 (55-91)	92 (84-97)	61 (42-78)	0.61 (0.42-0.81)	3.6	0.84 (0.75-0.94)
Sum Hev b 5 and Hev b 6.01	**≥ 1.497**	**79 (69-87)**	**88 (69-97)**	**96 (88-99)**	**56 (40-72)**	**0.67 (0.5-0.84)**	**6.61**	**0.86 (0.77-0.94)**

Vandenplast, …, Raulf et al. Allergy 2016

Baker's asthma

- One of the oldest recognized occupational diseases (First described by Bernardino Ramazzini (1633-1714) in "De Morbis artificum diatriba")
- One of the most common forms of occupational asthma

Examples:

in France: Incidence of baker's asthma among young bakers ranges from 0.3 to 2.4 cases per 1000 person-years [Remen et al. 2010]

in Norway: Incidence of occupational asthma among male bakers 2.4 and female 1 case per 1000 person-years [Leira et al. 2005]

in Germany: Incidence of occupational asthma among bakers ~2 cases per 1000 person-years [BGN, personal communication]

~ 10 % of all bakers develop asthma during their working life period

Potential allergens in bakeries

- **Wheat flour**
- Rye flour
- Further cereal flours (e.g. barley)
- Enzymes (α-amylase, cellulase etc.)
- Soy, Lupine flour
- Storage mites
- Flour pests (including flour worm, flour moth)
- Moulds
- Egg yolk and white, sesame seed, nuts, poppy etc.

The most relevant allergenic wheat fractions for baker´s asthma are the water-/salt-soluble albumins and globulins.

Which allergens are important?
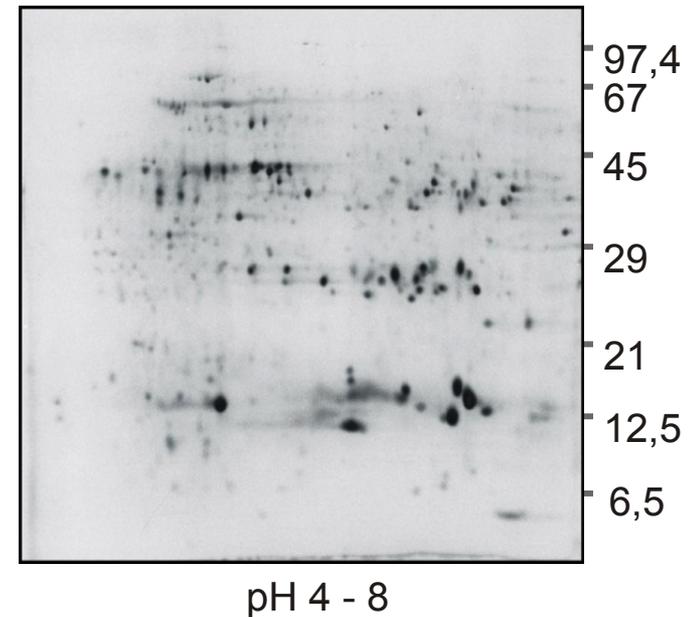
Molecular allergy diagnosis for baker's asthma

Proteomic approach

- 2D-electrophoresis and
- 2D-immunoblotting

e.g. 1995 Posch et al.
1997 Weiss et al.
2001 Sander et al.

➡ High number of proteins/peptides with IgE-binding capacity were identified

➡ High interindividual variation of 2D IgE-binding profiles in patients with baker´s asthma

97,4
67
45
29
21
12,5
6,5

pH 4 - 8

Identified wheat flour allergens I

- **α-amylase inhibitors MW 14-14 KDa**

  Tri a 15   Monomer (Wheat) alpha-amylase inhibitor 0.28 (WMAI-0.28)

  Tri a 28   Dimeric alpha-amylase inhibitor (WDAI-0.19)

  Tri a 29.01   Tetrametric alpha-amylase inhibitor (WTAI-CM1)
  Tri a 29.02   Tetrametric alpha-amylase inhibitor (WTAI-CM2)

  Tri a 30   Tetrametric alpha-amylase inhibitor (WTAI-CM3)

- **Thiol reductase homologue 27 KDa**
  Tri a 27

- **Serine protease inhibitors**
  Tri a 39 SPILA – Serine protease inhibitor-like protein   9.9 KDa
  Tri a 33 – Serpin   40-43 KDa

Identified wheat flour allergens II

Tri a 12	Profilin	14 KDa
Tri a 14*	Wheat nonspecific lipid transfer protein 1	9 KDa
Tri a 18	Agglutinin isolectin 1	
Tri a 19	Omega-5 gliadin, seed storage	65 KDa
Tri a 21	Alpha/beta gliadin	32.7 KDa
Tri a 25	Thioredoxin H	13.4 KDa
Tri a 26	High molecular weight glutenin	88 KDa
Tri a 31	Triosephosphate-isomerase (TPIS)	27 KDa
Tri a 32	1-cys-peroxiredoxin	23.9 KDa
Tri a 34	Glyceraldehyde-3 phosphate-dehydrogenase	36.5 KDa
Tri a 35	Dehydrin	12.4 KDa
Tri a 36	Low molecular weight glutenin GluB 3-23	40 KDa
Tri a 37	Alpha purothionin	12 KDa

*isoforms: Tris a 14.01.101 (nsLTP 9.1); Tri a 14.0201 (nsLTP 9.7)

Limitation of the studies to evaluate the importance of single allergens in baker's asthma

- Different patients (from different countries, different exposure)
- Different methods (SPT, ELISA, Dot-blot, (1D/2D-)Western-blot, microarray, etc.)
- Tested with single or only few purified proteins in natural or recombinant forms

Using a (complete) panel of identified wheat flour allergens

in recombinant form for determination of the IgE-binding profile

Characteristics of the study group

101 Bakers   (40 German, 37 Dutch, 24 Spanish)

    f4:   7.47 kU/L; (0.55 - 83.3 kU/L)

    gx1:   1.79 kU/L; (0.01 - >100 kU/L)

29 Controls   (10 German, 10 Dutch, 9 Spanish; 59% asthma, 72% rhinitis, grass pollen positive and wheat positive)

    f4:   1.14 kU/L; (0.36 – 9.1 kU/L)

    gx1:   81.3 kU/L; (3.63 - 705 kU/L)

Sander I, Rihs HP, Doekes G, Quirce S, Krop E, Rozynek P, van Kampen V, Merget R, Meurer U, Brüning T, Raulf M: J Allergy Clin Immunol 2015; 135: 1529-1537

19 Recombinant wheat flour allergens and 2 CCDs

- **Tri a 15** (WMAI-0.28)
- **Tri a 28** (WDAI-0.19)
- **Tri a 29.01** (WTAI-CM1)
- **Tri a 29.02** (WTAI-CM2)
- **Tri a 30** (WTAI-CM3)
- **Tri a 12.0102** (Profilin)
- **Tri a 14.02** (nsLTP)
- **Tri a 34** (GAPDH)
- **Tri a 33** (Serpin)
- **Tri a 31** (TPIS)
- **Tri a 21** ($\alpha\beta$-Gliadin)
- **Tri a 25** (Thioredoxin H)
- **Tri a 32** (1-cys-Peroxiredoxin)
- **Tri a 27** (Thiol reductase)
- **Peroxidase 1**
- **Tri a 35** (Dehydrin)
- **Tri a 39** (SPILA)

- **HRP- Horse Radish Peroxidase (MMXF)**
- **MUXF (Glucan Bromelain)**

- **Tri a 19** (ω-5-Gliadin)*
- **Tri a 14.01** (nsLTP)* (*ThermoFisher)
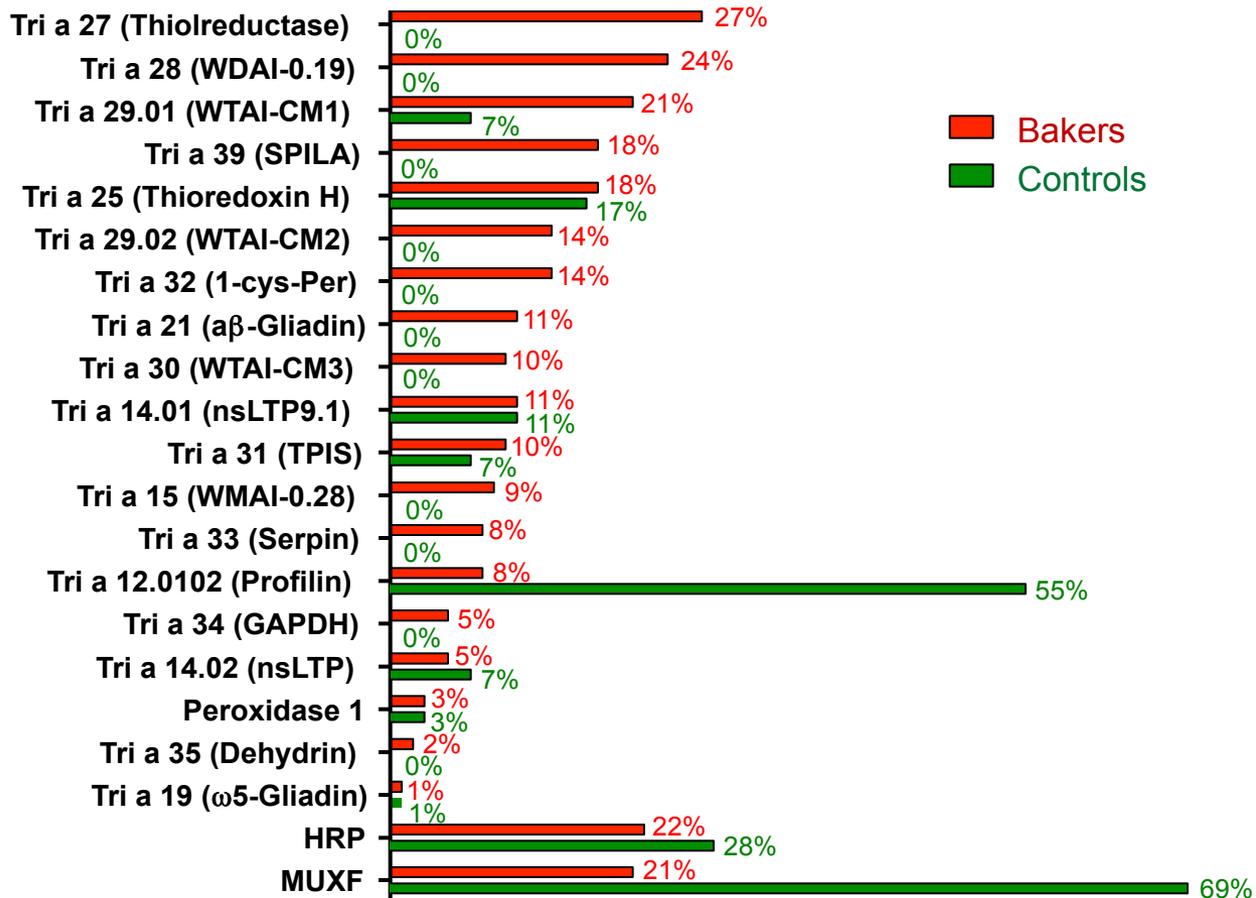
Binding to Streptavidin ImmunoCAPs
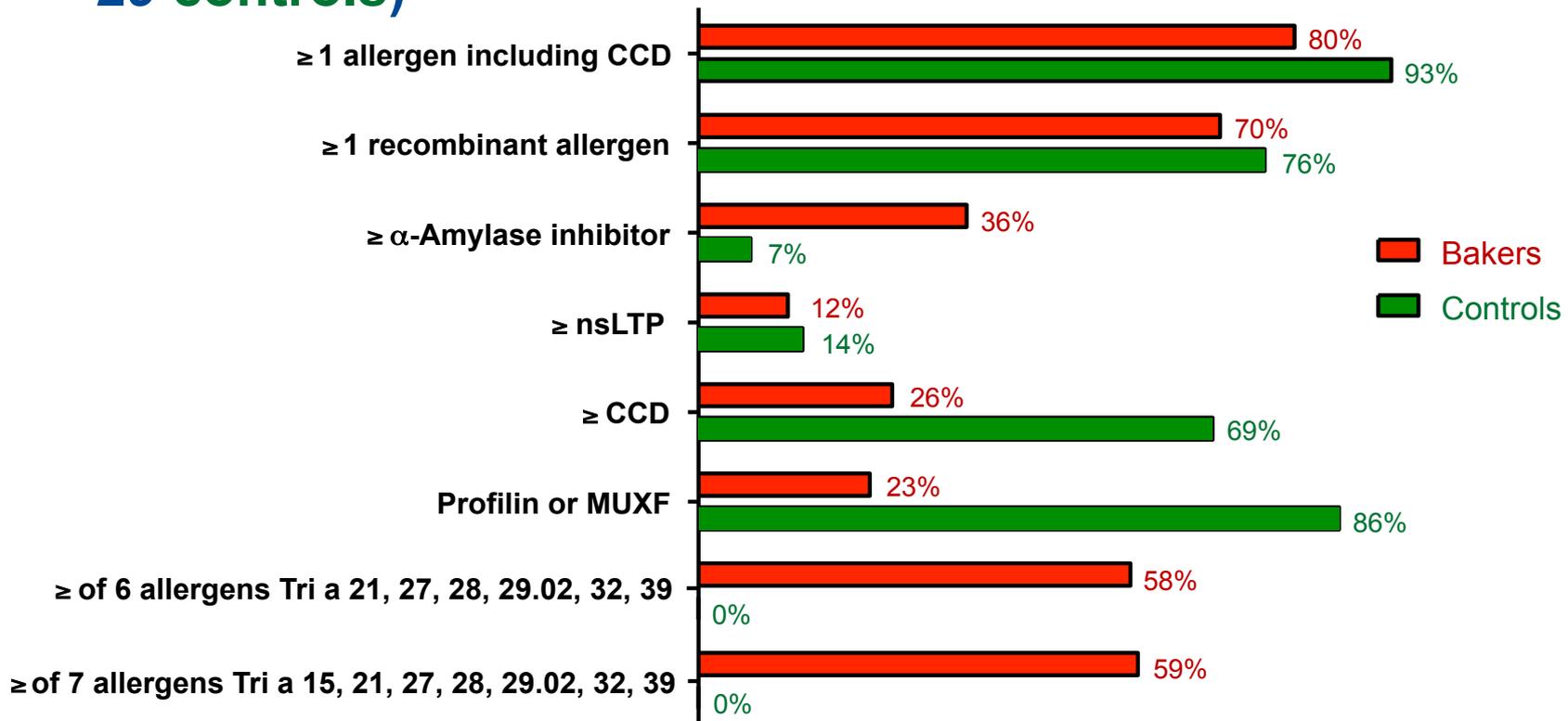
MBP or TRX-His-S were also tested

according to
Sander I, Rozynek P, Rihs HP, van Kampen V, Chew FT, Lee WS, Kotschy-Lang N, Merget R, Brüning T, Raulf-Heimsoth M: Allergy 2011; 66: 1208-1215
Sander I, Rihs HP, Doekes G, Quirce S, Krop E, Rozynek P, van Kampen V, Merget R, Meurer U, Brüning T, Raulf M: J Allergy Clin Immunol 2015; 135: 1529-1537

Sensitization profile of 101 symptomatic bakers and 29 controls

Bakers
Controls

Allergen	Bakers	Controls
Tri a 27 (Thiolreductase)	27%	0%
Tri a 28 (WDAI-0.19)	24%	0%
Tri a 29.01 (WTAI-CM1)	21%	7%
Tri a 39 (SPILA)	18%	0%
Tri a 25 (Thioredoxin H)	18%	17%
Tri a 29.02 (WTAI-CM2)	14%	0%
Tri a 32 (1-cys-Per)	14%	0%
Tri a 21 (aβ-Gliadin)	11%	0%
Tri a 30 (WTAI-CM3)	10%	0%
Tri a 14.01 (nsLTP9.1)	11%	11%
Tri a 31 (TPIS)	10%	7%
Tri a 15 (WMAI-0.28)	9%	0%
Tri a 33 (Serpin)	8%	0%
Tri a 12.0102 (Profilin)	8%	55%
Tri a 34 (GAPDH)	5%	0%
Tri a 14.02 (nsLTP)	5%	7%
Peroxidase 1	3%	3%
Tri a 35 (Dehydrin)	2%	0%
Tri a 19 (ω5-Gliadin)	1%	1%
HRP	22%	28%
MUXF	21%	69%

✓ No major allergen detectable

Summary: Sensitization to grouped allergens (101 bakers and 29 controls)

Chart — Sensitization percentages:

| Allergen group	Bakers	Controls
≥ 1 allergen including CCD	80%	93%
≥ 1 recombinant allergen	70%	76%
≥ α-Amylase inhibitor	36%	7%
≥ nsLTP	12%	14%
≥ CCD	26%	69%
Profilin or MUXF	23%	86%
≥ of 6 allergens Tri a 21, 27, 28, 29.02, 32, 39	58%	0%
≥ of 7 allergens Tri a 15, 21, 27, 28, 29.02, 32, 39	59%	0%

For bakers, in most cases the wheat flour CAP values were higher than the sum of IgE to single allergens, whereas for control subjects the sum was higher

Baker's asthma and CRD

- All soluble fractions contain allergens; the most relevant for baker's asthma are the **albumins** and **globulins**.

- In 2D-immunoblots of the salt-/water-soluble fraction more than **100 different allergen spots** were detected.

- The **allergen spectrum differs** individually.

- So far, no **common major wheat allergen** could be identified, but **Tri a 27** and **Tri a 28** are most frequent.

- **Tri a 19** (**ω-5-Gliadin**; marker allergen for WDEIA) **is not** a relevant allergen for baker's asthma.

- So far, for routine diagnosis allergen **specific IgE tests with whole wheat flour** extracts remain mandatory because of superior diagnostic sensitivity.

- **Component-resolved diagnosis** might improve the diagnosis of baker's asthma and help to **differ between grass pollen, respiratory wheat flour** and **wheat-induced food allergy** (differentiation of occupational sensitization and sensitization caused by cross-reactivity is possible).

Laboratory animal allergens

Mus m 1

Rat n 1

Ory c 1

Ory c 3

Ory c 4

Cav p 1

Cav p 2

Cav p 3

Cav p 4

Cav p 6

Pho s 21

Mes a 1

As common for the most mammalian inhalant allergens, the major allergens from mouse, rat, guinea pig, hamster and rabbit are lipocalins
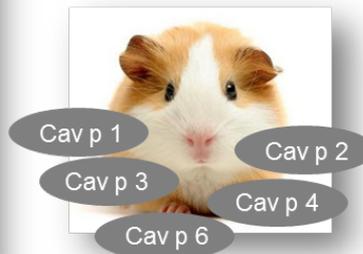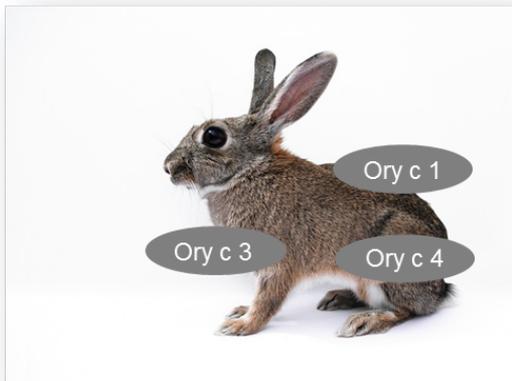
Laboratory animal allergy (LAA)

- Allergen-specific IgE to the suspected **animal allergen extracts** is the common and recommended step. Specific IgE determination in the case of LAA based on extracts prepared from epithelia, serum-/urine protein as mixture or alone.

- Dual sensitization to rat and mouse urinary allergens reflects **cross-reactive molecules** rather than atopy and therefore the determination of the **primary sensitizer is difficult**.

- There is not enough evidence to advice the use of single molecules for *in-vitro* diagnosis. **Single animal allergens**, relevant for LAA, **are not commercially available** so far for routine testing.

- Impact of **individual molecules on severity of symptoms** is still unknown.
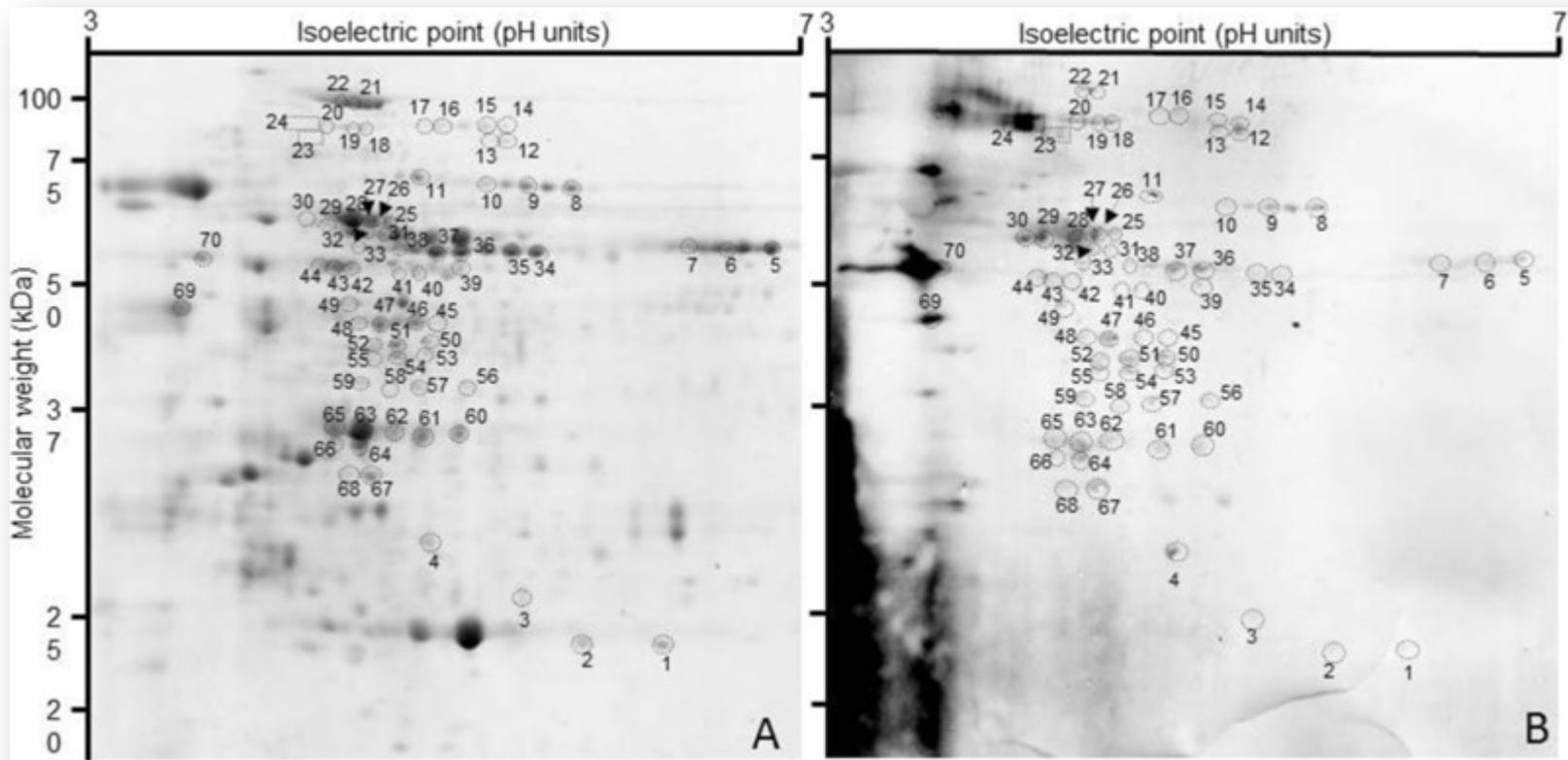
Molecular approach for Hypersensitivity pneumonitis (HP)

Example:

Farmer´s lung disease

Proteomic – 2D-Electophoresis – detection of immune reactive *S. rectivirgula* proteins

Barrera et al, 2014; Proteomics Clin

- Identification of **25 farmer´s lung-specific proteins** via MS (e.g. proteases SR2, SR4, SR14, SR21; glycosidase SR9)

- Production of **17 recombinant proteins with immune reactivity**

- **ELISA-testing**
  with sera from 41 farmer´s lung-patients
  and from
  43 healthy, exposed controls
  (from France and Switzerland)

- Evaluation if the proteins can contribute to a **differentiation between diseases and exposed patients**

ELISA-testing with recombinant proteins
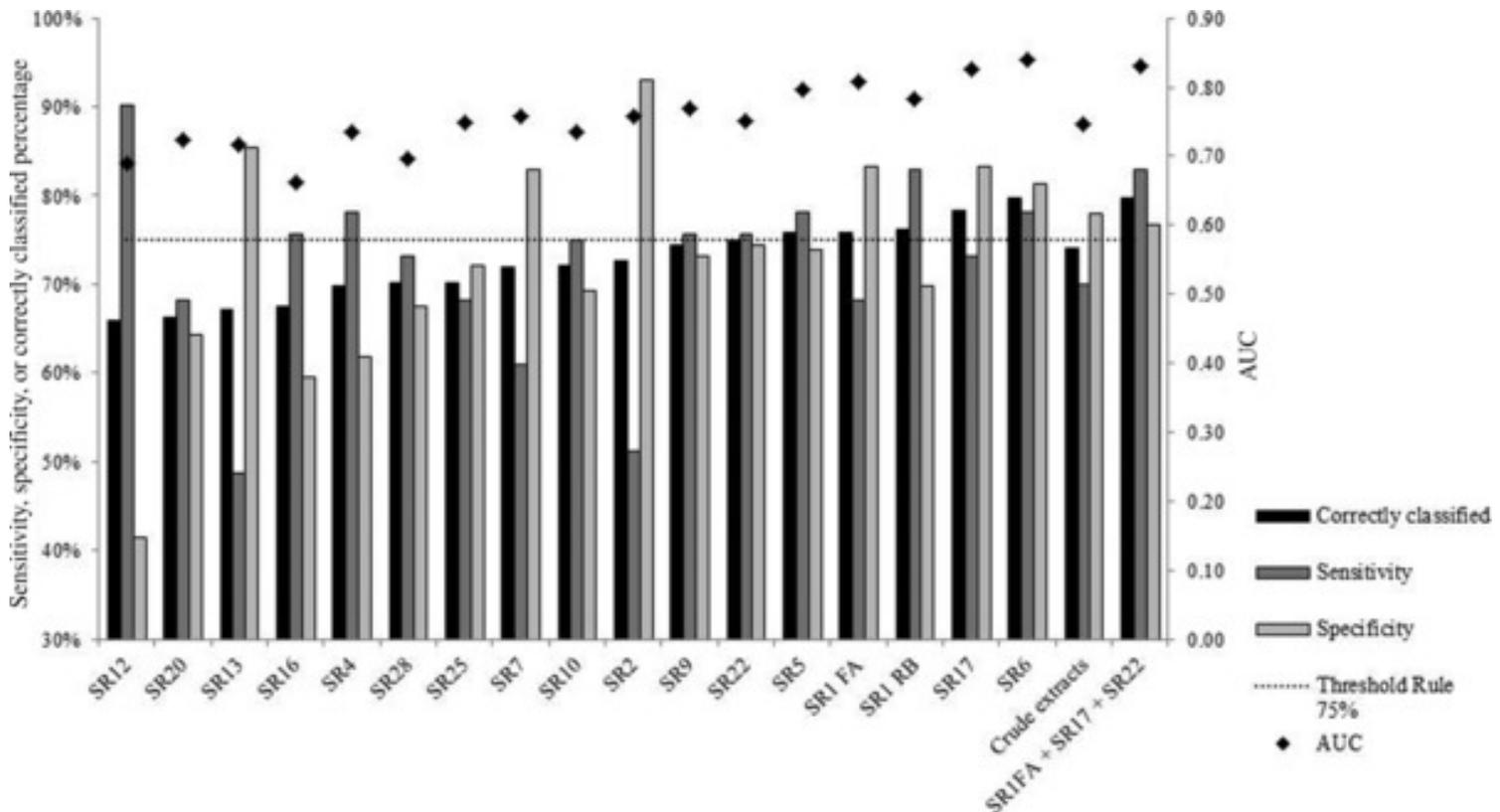
Combination of SR1FA + SR17 + SR22 in ELISA
83% sensitivity and 77% specificity

Perspectives

- Immune precipitation with
  S. rectivirgula-extract   40% sensitivity

- ELISA with
  S. rectivirgula-extract   70% sensitivity

- ELISA with
  SR1FA, SR17, SR22   83% sensitivity
   + 77% specificity

  ➢   but not all responded to actinomycetes

  ➢   in the future: production of a panel of recombinant proteins of the four species *S. rectivirgula*, *W. sebi*, *Lichtheimia corymbifera*, *Eurotium amstelodami* (*A. vitis*) for the diagnosis of farmer´s lung disease

Summary

- Wide range of occupational allergens, often **individual case reports** ⇒ only few allergen sources are characterized on the molecular level; but an essential prerequisite for the production of suitable allergen extracts is knowledge about relevant allergens.

- **Molecular allergy diagnostic** only useful for latex. For baker's asthma and for LAA no relevant single allergens are commercially available so far.

- **Component-resolved diagnosis** might help to differ between occupational sensitization and sensitization caused by cross-reactivity between environmental allergens.

- **CCD-reagents** can be used as *in vitro* **screening-tools** to discriminate between 'true' allergy and clinical not relevant cross-reactivity.

- **Complementary tests** (e.g. BAT (FlowCAST, CAST), inhibition test, serum or urine biomarkers etc.) may be helpful, but they need further validation.

- There is a broad range of unmet needs in the case of OA to improve diagnosis and therapy.
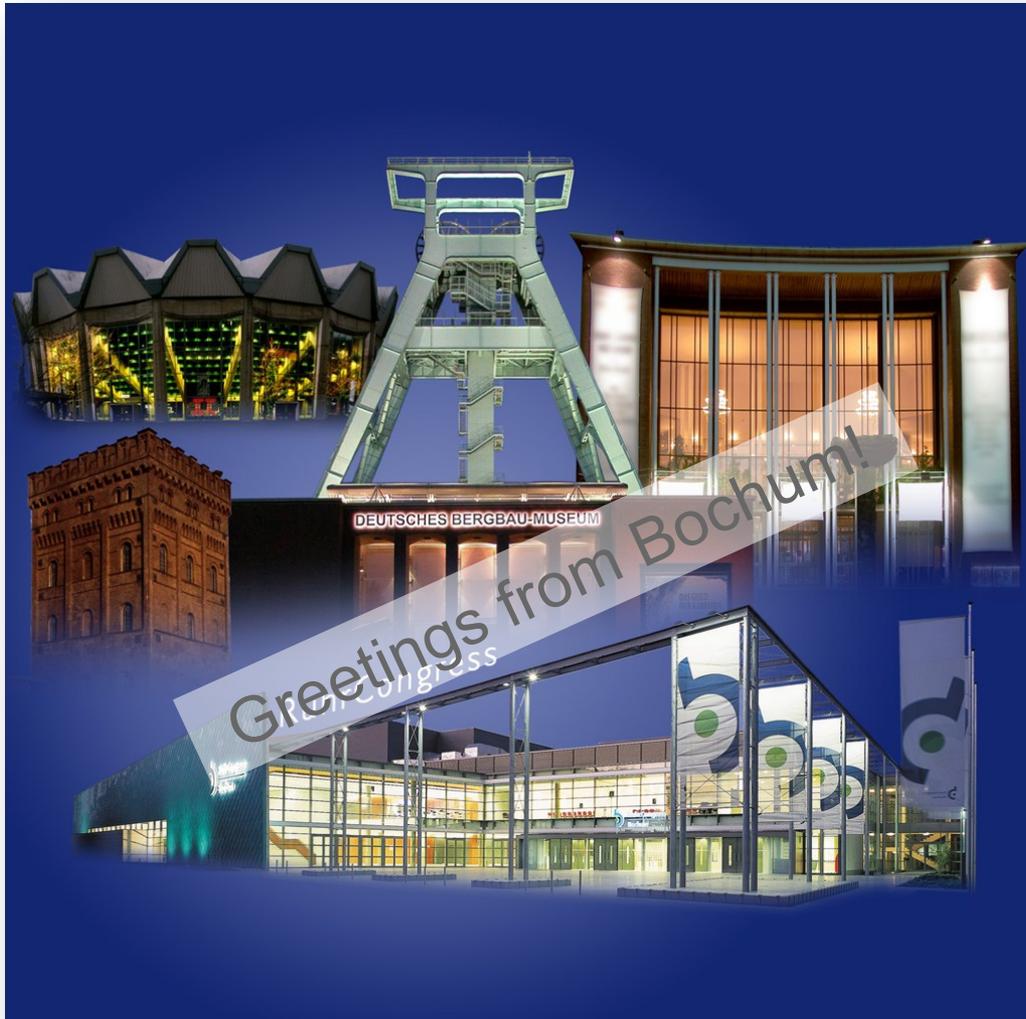
Bochum, Germany

Thank you for your attention!