SFB F018 „Molecular and Immunological Strategies for Prevention, Diagnosis and Treatment of Type I Allergies“

The Structure of Environmental Allergens and Structure-based Epitope Mapping

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Outline

Immunological Background

Allergen Structures

Virtual Epitope Mapping

Outlook

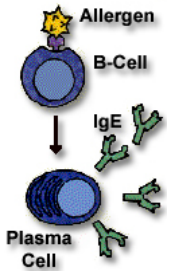
Allergy: Definition and Concept

"Allergy" (Clemens von Pirquet 1906) ≡ Type I Hypersensitivity

Allergens ≡ Antigens (Exogenous: Pollen, Plant Food, Mite, Animals)

Antibodies: Immunoglobulin E molelcules (IgE)

- 3 hypervariable (HV) regions ≡ CDRs



first allergen contact
antibody production

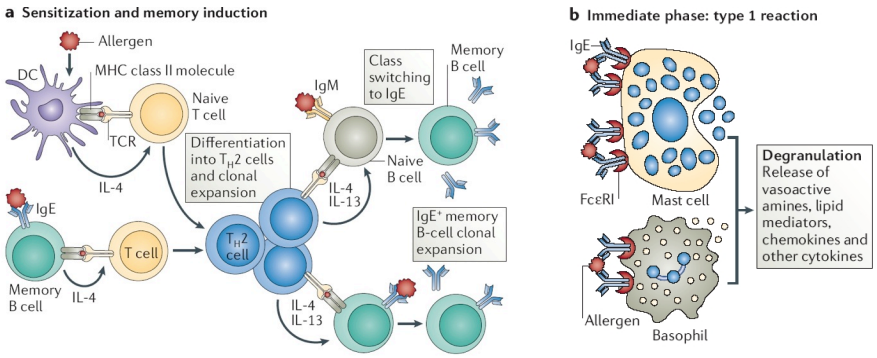
(Hyper)sensitization
IgE bound to Granulocytes

repeated exposition to allergen
allergic reaction

Allergy: Definition and Concept

Sensitization

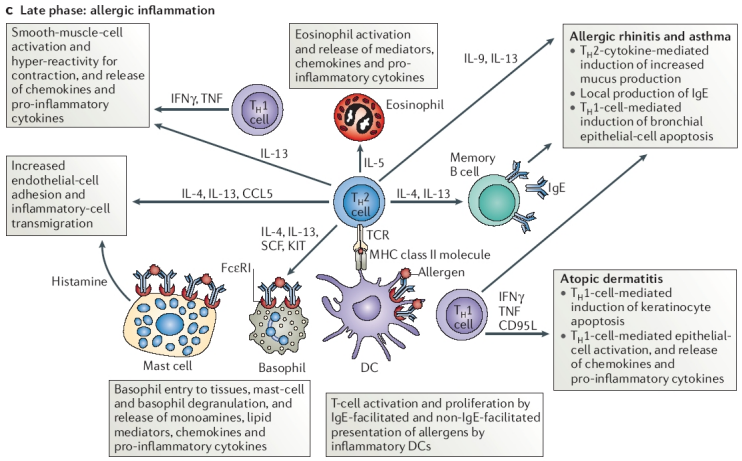
Immediate Type I reaction



Larrche, Akdis and Valenta, NAT REV IMMUNOL, 2006

Allergy: Definition and Concept

Late phase reactions

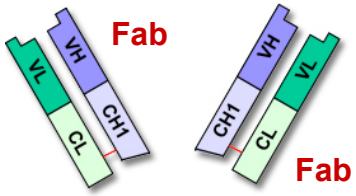


Larrche, Akdis and Valenta, NAT REV IMMUNOL, 2006

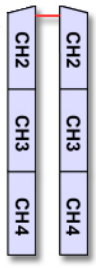
The IgE Antibody

Two heavy ε + two light polypeptide chains

- 2 x (4 + 1) constant domains
- 2 x (1 + 1) variable domains
- ε chain: ~ 550 amino acid residues
- light chains (λ and κ): ~ 211-217 aa



Digestion with proteases leads to one Fc and two Fab fragments



- **Fc** (Fragment, crystallizable) Ig-type specific
- binds to various cell receptors, e.g. FcεR I, II

- **Fab** (Fragment, antibody-binding) is variable
- complementary to a specific antibody

Immunotherapy

- **Protective Immunanswer**
- generation of blocking IgG antibodies

- **Desensitization** (enhance T_H1 over T_H2 pathway)

IT **traditionally done with extracts (pollen, house dust, animal dender) --> risk of anaphylactic reactions**

Cloning of allergens and recombinant production allows:

- **CRD** (component resolved diagnosis)
- **CRIT** (component resolved IT)
- concept of **Hypoallergenic Derivatives**

Epitope Mapping

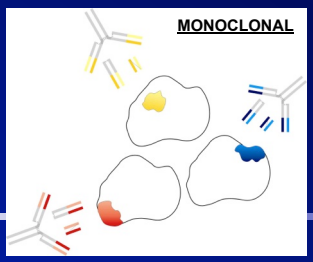
Conformational Epitopes

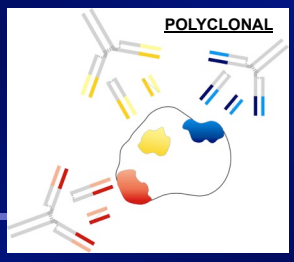
Linear Epitopes
Peptide scan
Fragments
√

Complex structures
X-ray crystallography
Allergen – Ab / F_{ab} / scF_v

NMR - methods
H/D exchange
relaxation enhancement

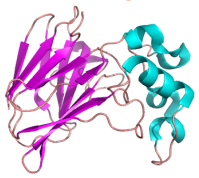
Virtual epitope mapping
Prediction of conformational epitopes on the basis of structure and cross-reactivity of allergens
Development and evaluation of "similarity" indices





Allergen families / Test systems

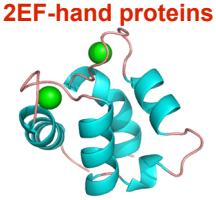
TLPs (PR-5 group)



Pru av 2
Mal d 2
Act d 2
Zeamatin

Thaumatin
Osmotin
PR5d

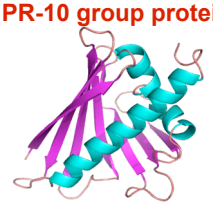
2EF-hand proteins



Phl p 7
Che a 3
Bet v 4

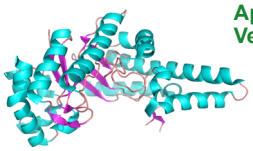
Calbindin
Parvalbumin
Psoriasin
Calmodulin

PR-10 group proteins



Bet v 1
Api g 1
LIPR-10(3)
Pru av 1

Hyaluronidases

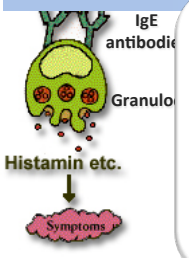


Api m 2
Ves v 2

The structural basis for allergenicity

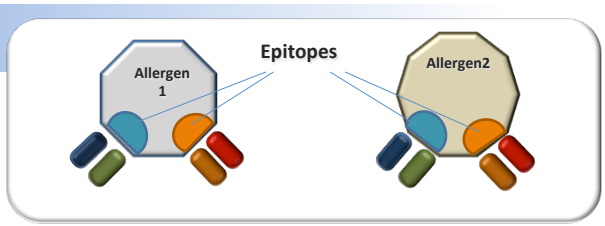
CROSS-REACTIVITY

Hypoallergenic vaccines



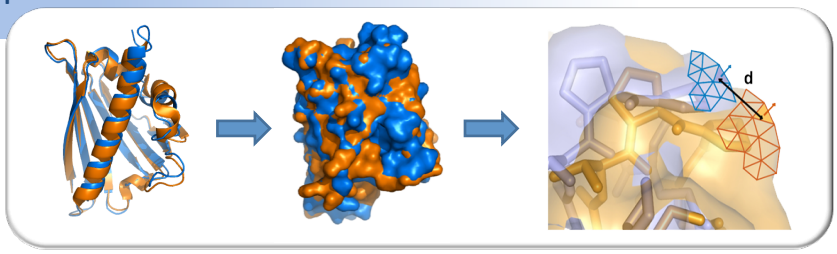
- recognized by antibodys, but not able to cross-link IgE
- induce T-cell response → allergic reaction will be suppressed later

Cross-reactivity

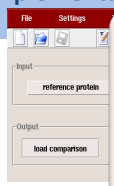


Surface comparison

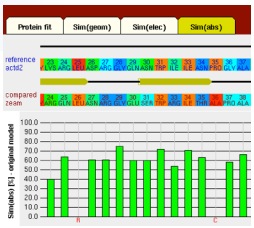
Principle



Implementation



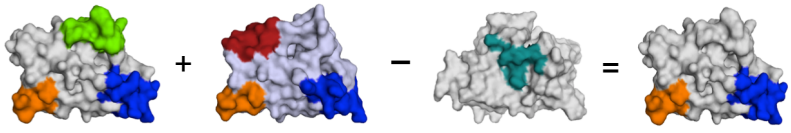
- Sequence alignment (T-Coffee)[1]
- (Global) model superposition
- Side chain normalization
- Surface calculation (MSMS)[2]
- Electrostatic potential calculation (APBS)[3]
- Surface feature mapping + comparison



[1] Notredame et al. (2000). **J. Mol. Biol. 302 (1),** 205-17
[2] Sanner et al. (1996). **Biopolymers 38 (3)**, 305-320
[3] Baker et al. (2001). **Proc. Natl. Acad. Sci. USA 98**, 10037-10041

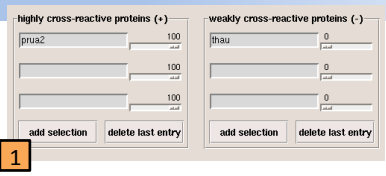
Localization of conformational epitopes

Principle

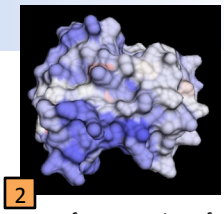


Reference allergen | Cross-reactive protein | Non-Cross-reactive protein | **Prediction**

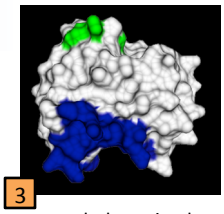
Implementation



1 assignment of related proteins cross-reactivity correlation



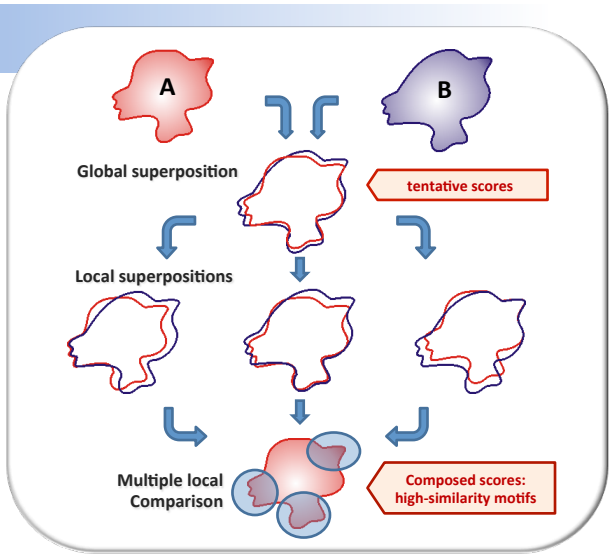
2 surface-mapping of similarity differences



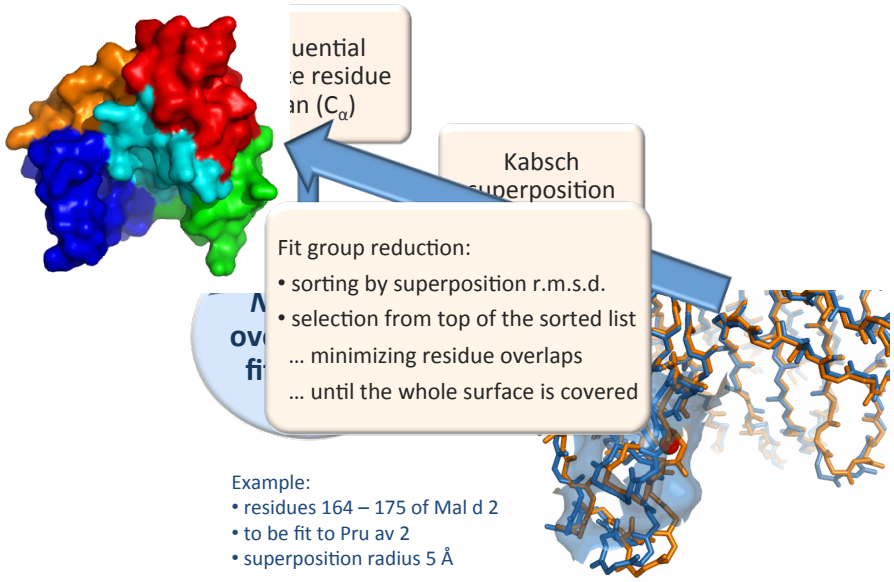
3 patch detection by filtering/cluster recognition

‘Adaptive ‘ surface fitting

Principle

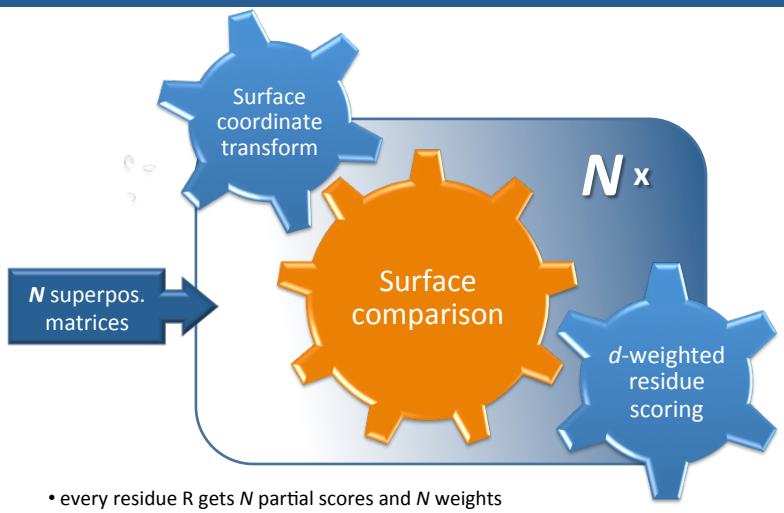


Pre-identification of local surface motifs



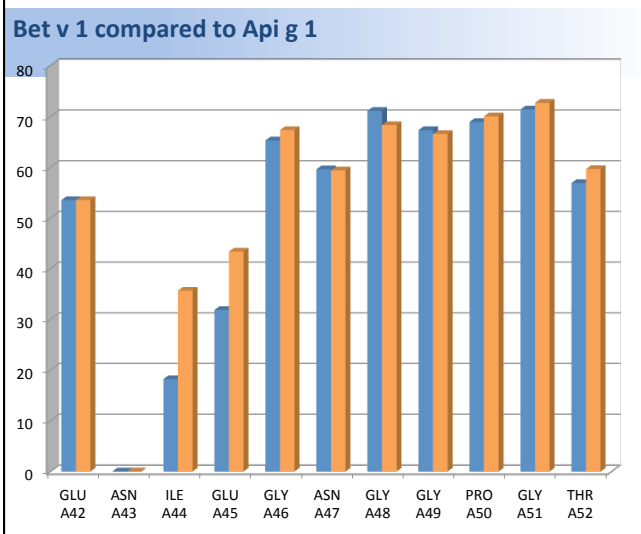
Example:
- residues 164 – 175 of Mal d 2
- to be fit to Pru av 2
- superposition radius 5 Å

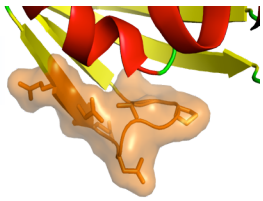
Multiple surface alignment & comparison



- every residue R gets *N* partial scores and *N* weights
- highest score for the R defining the fitting group center:
  w = 1.0, if *d* = 0.0 Å; w = 0.0 if d = 10.0 Å (radius)
- after the *N* comparisons, composed scores are obtained by weighted averaging

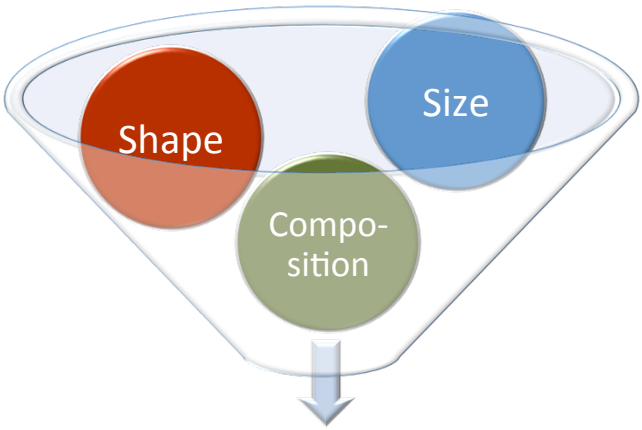
Effect on similarity scores





residue	global	adaptive
A42	53,5	53,5
A43	0	0
A44	18,2	35,6
A45	31,8	43,4
A46	65,3	67,3
A47	59,6	59,4
A48	71,1	68,3
A49	67,3	66,5
A50	68,9	70,0
A51	71,4	72,7
A52	56,9	59,6

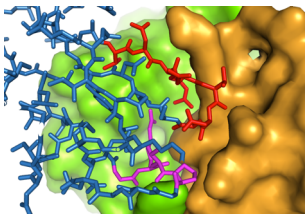
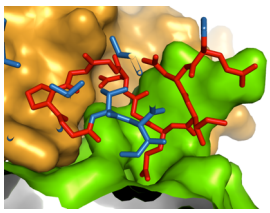
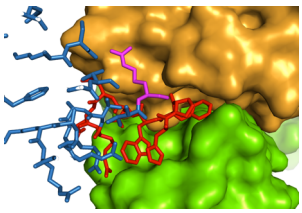
Epitope/patch feature analysis



Description of conformational epitopes

Pattern analysis → Classification (?)

Class prototypes in literature

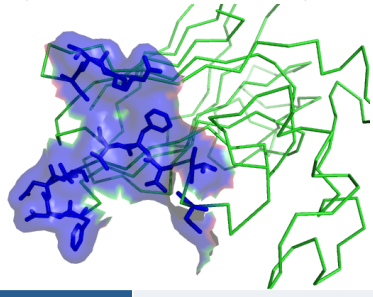
Discontinuous	Transitional	Continuous
Lysozyme – F_{ab} (HyHEL-5)[1]	*Bet v 1 – F_{ab} (BV16)*[2]	*Api m 2 – F_{ab} (21E11)*[3]
		
Size: 1337 Å² (14 residues)	Size: 1165 Å² (15 residues)	Size: 1274 Å² (9 residues)
Accessibility: 62.7 %	Accessibility: 54.8 % (core loop 67.4 %)	Accessibility: 69.9 %
Motif(s): strand-turn-strand + turn	Motif(s): strand-turn-strand + strand	Motif(s): helix-turn-helix
Composition: 1 + **7** + 1 + **4** + 1 residues	Composition: **10** + 1 + 1 + 2 + 1 residues	Composition: **8** + **1** residues
Polar : Apolar: 10 : 4	Polar : Apolar: 8 : 7 (core loop 4 : 6)	Polar : Apolar: 6 : 3
Interactions: salt bridges (2), hydrogen bonds, vdW contacts	Interactions: hydrogen bonds (8), vdW contacts	Interactions: salt bridges (2), hydrogen bonds (9), vdW contacts (11)

[1] Cohen et al. (2005). **Acta Cryst. D61**, 628. [2] Mirza et al. (2000). **J. Immun. 165**, 331. [3] Padavattan et al. (2007). **J. Mol. Biol. 368**, 742.

Study cases

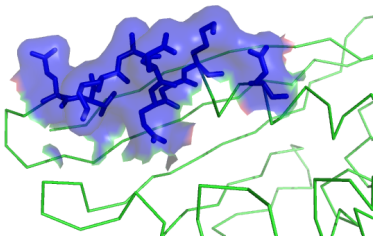
Discontinuous

Act d 2 (Zeamatin : Mal d 2, Pru av 2)



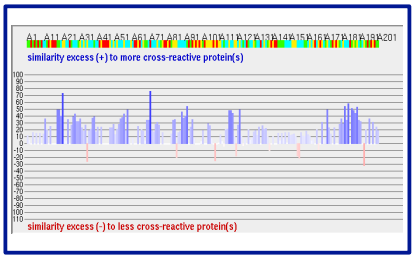
Continuous

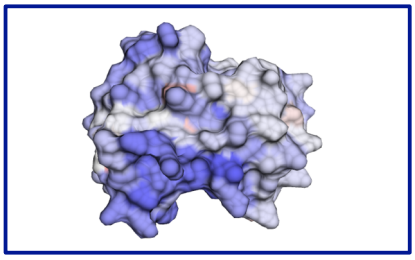
Bet v 1 (Api g 1, Pru av 1 : PR 10.1, PR 10.2)

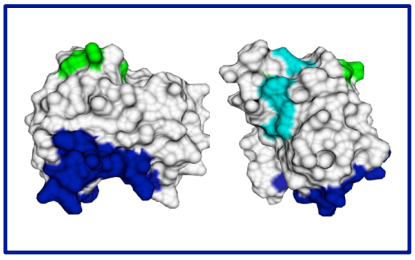


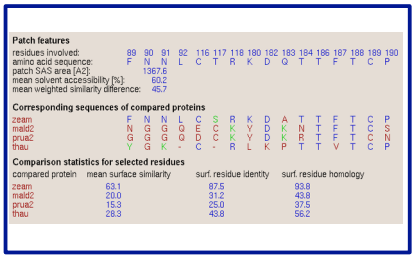
Size	1003 Å² (12 residues)	674 Å² (7 residues)
Accessibility	61.6 %	61.5 %
Motifs	turn, loop, loop-sheet	loop-helix
Composition	4 + 3 + 5 residues	6 + 1 residues
Polar : Apolar	8 : 4	6 : 1

TLPs : reference model Act d 2

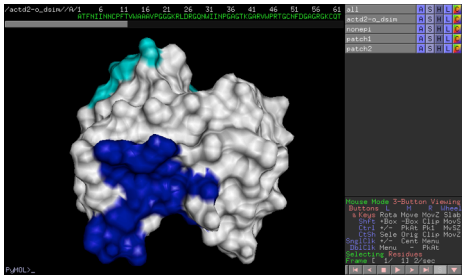


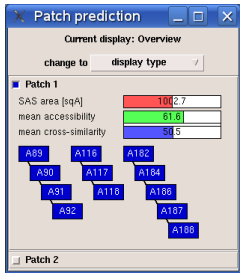


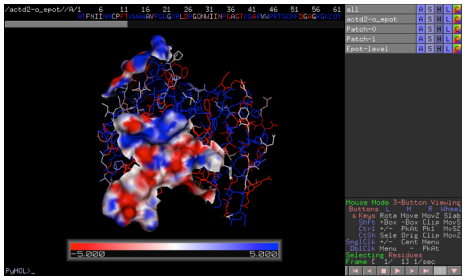


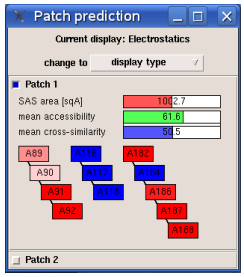


Software-assisted feature analysis









Future work

1. Validation experiments for predicted epitopes:
   - Mutation of key residues, binding assays
   - Characterization of Fab complexes (NMR, X-ray)
2. Extension of the study: more allergen families
3. Software optimization, documentation, distribution

Biochemie und Molekulare Biomedizin

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