Selective removal of B cell epitopes from bacterial proteins

Exemplified by the generation of a novel C5a receptor antagonist for treatment of acute inflammation

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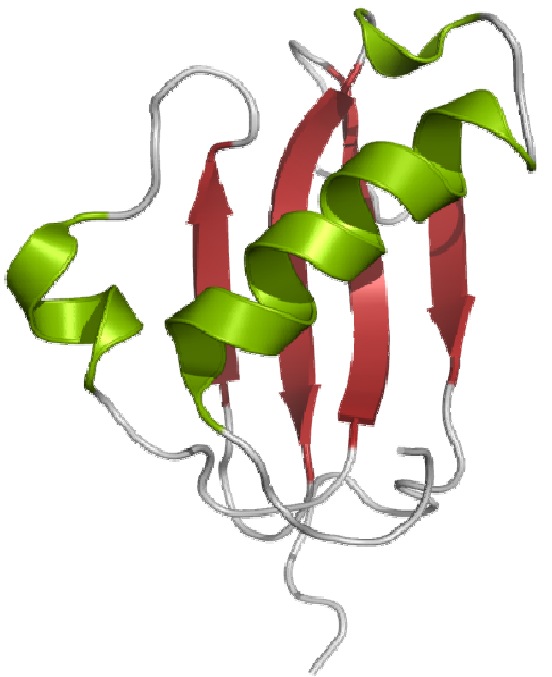
CHIPS

CHIPS – CHemotaxis Inhibitory Protein of *Staphylococcus aureus*

- 14.1 kDa protein, short half-life in the circulation
- Produced by > 60% of clinical *S. aureus* isolates
- Binds and blocks the human C5a receptor with nanomolar affinity
- Blocks the activation and migration of human neutrophils (anti-inflammatory)
- Anti-CHIPS antibodies are present in a majority of human sera



(Veldkamp, 2000; De Haas *et al*, 2004; Haas *et al*, 2004, 2005; Postma *et al*, 2004, 2005; Wright *et al*, 2007)

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Hypothesis and aim of the study

Hypothesis:
CHIPS is a potent C5aR antagonist, but would be a more effective and safe drug candidate if it was less reactive with human antibodies

Overall aim of the study:
To design a CHIPS variant with decreased interaction with pre-existing human IgG, yet retained biological function

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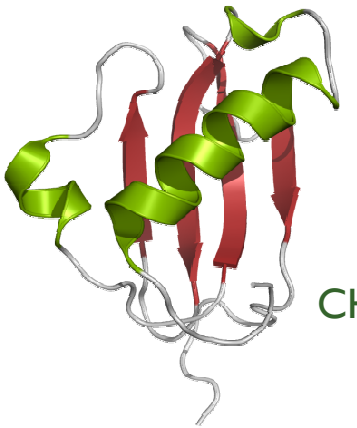
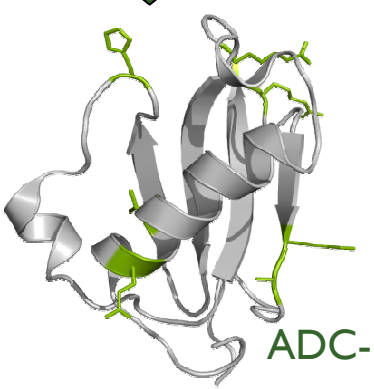
Two approaches to remove B cell epitopes

- Phage display of peptide libraries
  - mapping of B cell epitopes
  - SD mutagenesis to decrease CHIPS interaction with hIgG

- DNA recombination by FIND®
  - directed evolution of a new CHIPS variant with decreased interaction with hIgG





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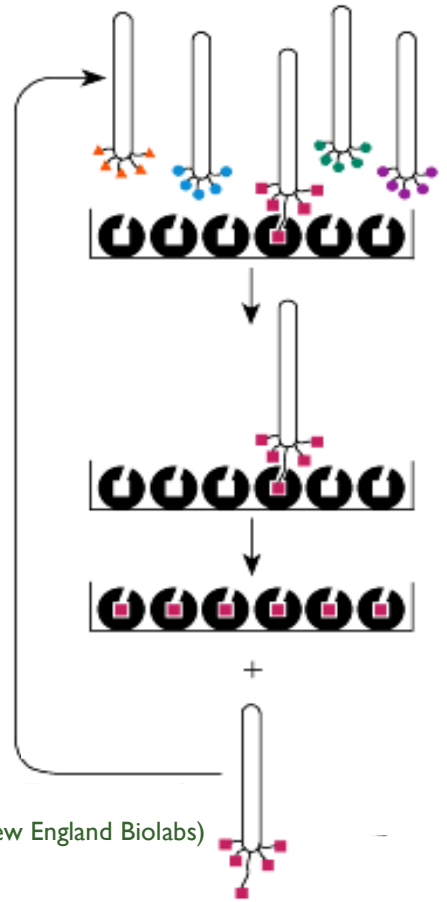
Mapping of B cell epitopes with phage display



Biopanning

(Adapted from New England Biolabs)

Group 1	M	N	K	T/S	x	x	x
	M	N	K	T	W	Y	P
	M	N	K	T	F	W	F
	M	N	K	T	F	F	S
	M	N	K	S	Y	H	L
	M	N	K	T	F	S	A
	M	N	K	T	F	V	D
	M	N	K	T	F	V	P
	M	N	K	V	Y	L	P
	M	N	K	S	Y	T	I
	M	N	K	Y	H	N	P
	F	N	K	S	Y	Y	G
	Y	N	K	S	F	F	M
	Y	N	K	S	F	F	P
	F	N	K	S	W	F	P
	L	N	K	T	F	Y	Y
	V	N	K	T	Y	W	K

Group 2	G	K	L	P	x	x	x
	G	K	L	P	I	A	M
	G	K	L	P	W	P	K
	G	K	L	P	I	P	Y
	G	K	L	P	P	P	I
	G	K	L	P	K	M	T
	G	K	L	P	K	E	S

(Gustafsson *et al* BMC Immunol, 2009)

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Mapping of B cell epitopes with phage display

Consensus group 1	M	N	K	T/S	x	x	x
Surface 1.1		N55	K100	T53	S107	Y108	
Surface 1.2		N55	K100	S107	Y108		
Surface 1.3		Q58	K100	S107	Y108		
Surface 1.4		N55	K54	T53	Y108		
Surface 1.5		N68	K69	G70	Y71	Y72	
Surface 1.6		N111	K95	Y94	Y97	Y71	
Consensus group 2	G	K	L	P	x	x	x
Surface 2.1		K69	L90	P35	K92	E67	

- Peptide phage display suggested six conformational and one linear epitope for anti-CHIPS IgG on CHIPS
- Mutational analysis identified amino acids important for the interaction with anti-CHIPS IgG
- CHIPS variants with reduced interaction with anti-CHIPS IgG were designed, but C5aR binding was affected

(Gustafsson *et al* BMC Immunol, 2009)

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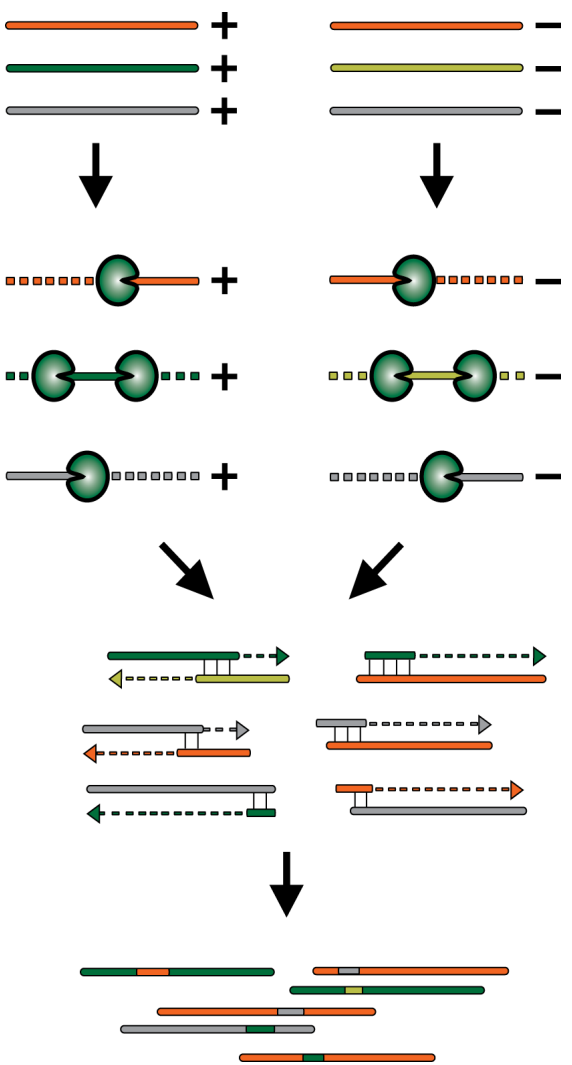
FIND® - Fragment INduced Diversity

Step 1:
Preparation of single-stranded DNA (ssDNA)

Step 2:
Exonuclease digestion to create ssDNA fragments

Step 3:
Recombination of ssDNA fragments through annealing and extension

Step 4:
Recombined full-length genes



- No prior information on protein structure, mechanism, active site, epitopes or binding sites needed
- Highly functional and diverse libraries are produced; 70-100% recombined genes with on average 1-2 recombinations per sequence
- Accumulation of benefical mutations and filtering out unwanted mutations

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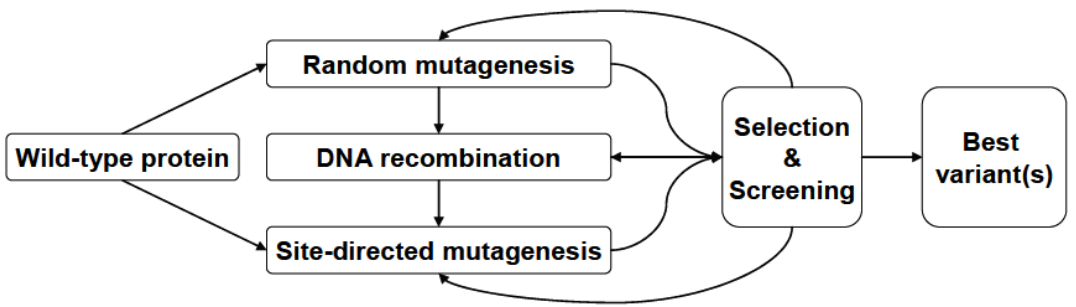
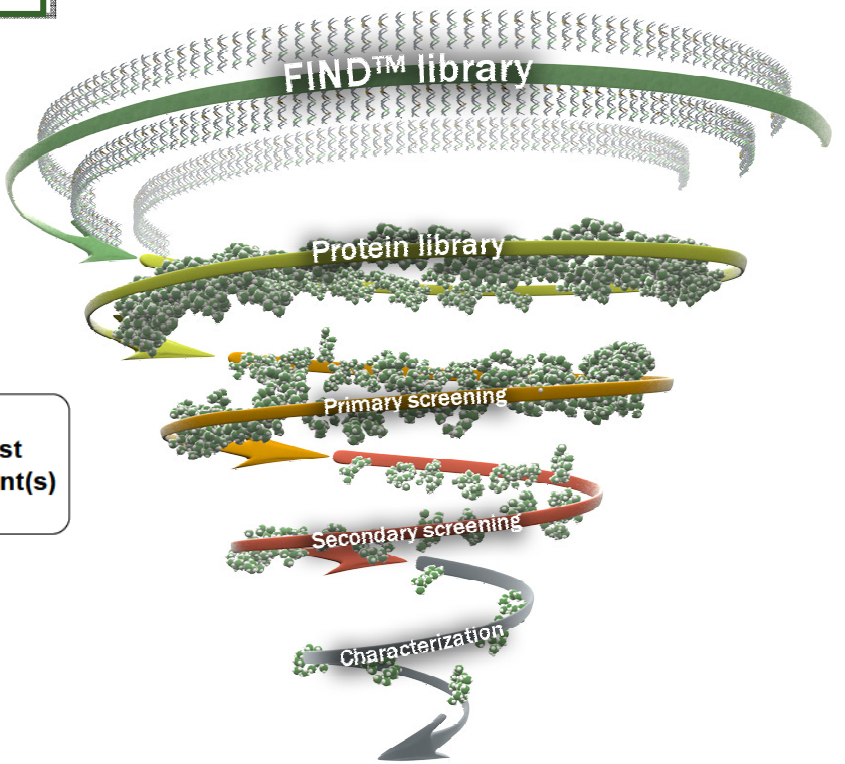
CHIPS optimization by FIND®

FIND® is performed in rounds of DNA recombinations and screening

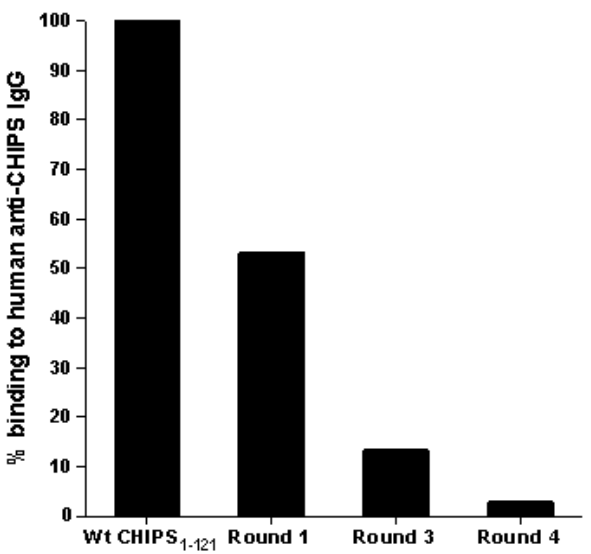




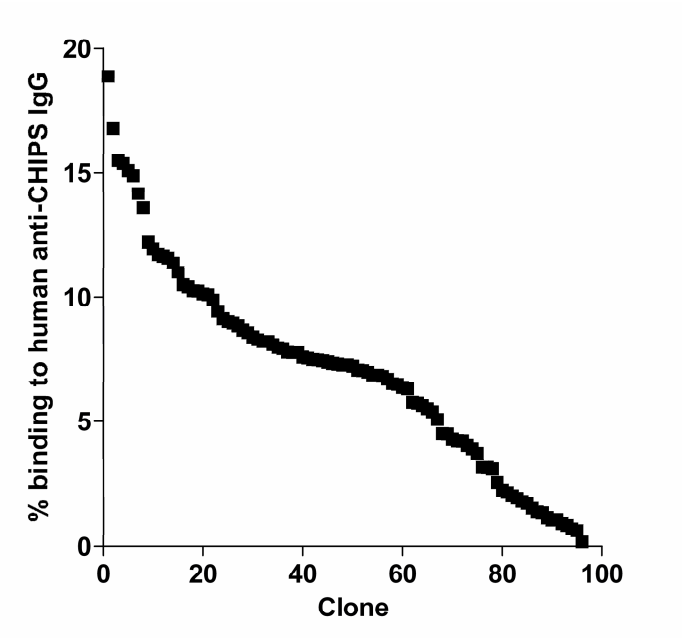
CHIPS optimization by FIND®

By the use of FIND®, CHIPS binding to hIgG was decreased by every round of evolution

Best clone/round



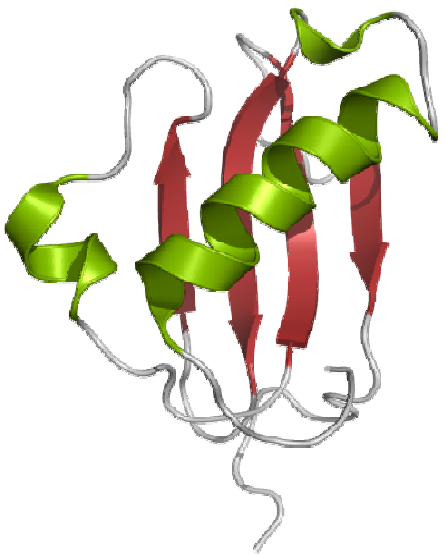
Distribution of clones in Round 4



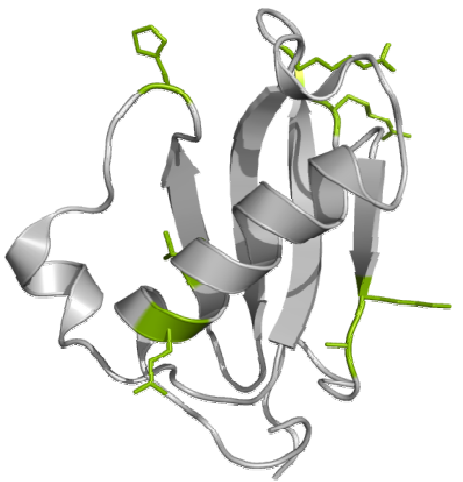
(Gustafsson *et al* accepted for publication in PEDS, 2009)

Characterization of CHIPS variants

CHIPS



ADC-1004



Thorough characterization of CHIPS variants:

- IgG binding
- Complement activation
- Biological function to block C5aR signaling and neutrophil migration
- Temperature stability
- T cell epitope content

(Gustafsson *et al* accepted for publication in PEDS, 2009)

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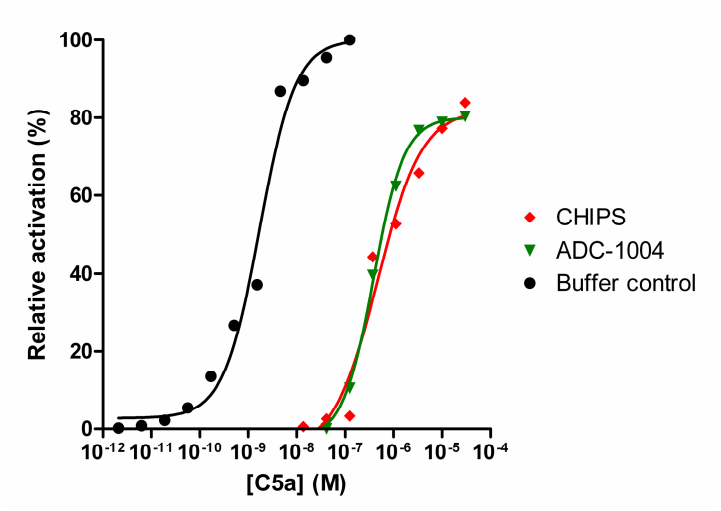
ADC-1004: Potency and Safety

Potency

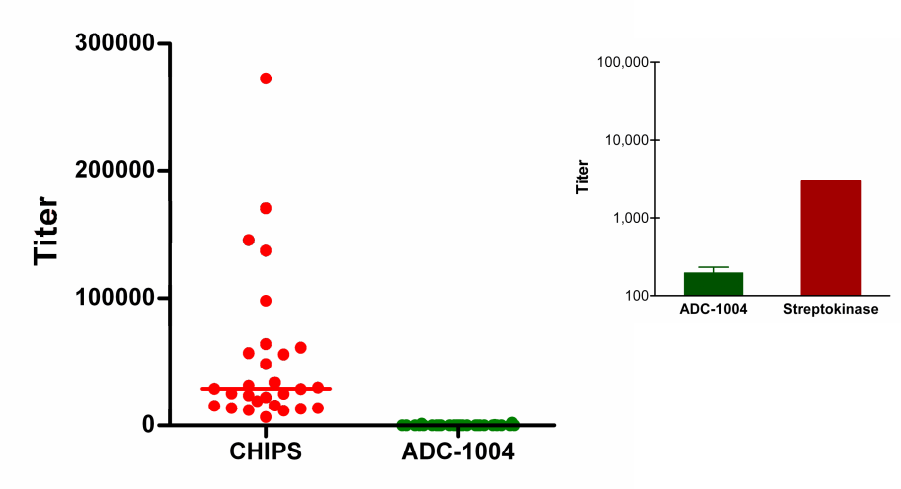
C5aR signaling inhibition



C5a induced intracellular Ca-flux, analyzed by FACS using Fluo-3AM labeled human neutrophils

Safety

Binding to individual human serum



Binding to hIgG in serum, analyzed by ELISA, n=28

- **ADC-1004 acts as an effective C5aR antagonist**

- **Reduction in binding to hIgG by 99%**

(Gustafsson *et al* accepted for publication in PEDS, 2009)

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T cell epitopes

In silico screening for T cell epitopes in CHIPS and ADC-1004 in collaboration with Algonomics.

	DRB1		DRB3/4/5	DQ	DP
	Strong	Medium	Strong	Strong	Strong
CHIPS	9	23	2	1	2
ADC-1004	7 (3)*	19 (7)	2 (1)	1 (1)	1 (1)

* The number of identical epitopes in ADC-1004 compared to CHIPS, are shown in brackets

Peptides binding to multiple HLAs of the same group (DRB1, DRB3/4/5, DP, DQ) are counted as one

- ADC-1004 is estimated to contain fewer T cell epitopes than CHIPS, but new epitopes have been introduced

- ADC-1004 is intended for acute conditions
  ➡ Not focus on removing T cell epitopes

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ADC-1004: Unmet medical need

Conditions in which complement is involved in the pathogenesis:

Chronic	Annual number of patients (USA)
Asthma	14 500 000
Psoriasis	5 500 000
Alzheimer disease	4 000 000
Rheumatoid arthritis	2 100 000
Age related macular degeneration	1 640 000
Systemic lupus erythematosus	500 000
Glomerulonephritis	300 000
Multiple sclerosis	250 000
Myasthenia gravis	36 000
Paroxysmal nocturnal hemoglobinuria	9 000

Acute	Annual number of patients (USA)
Burns	2 000 000
Myocardial infarction	1 500 000
Stroke	600 000
Sepsis	600 000
Angioplasty	440 000
Coronary artery bypass	360 000
ARDS	250 000
Open heart surgery	78 000
Transplantation	77 000

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ADC-1004: Conclusions from interim PoC study

The results from the interim study of 8 animals were very promising and indicates that:

- ADC-1004 substantially reduces the size of the myocardial infarction in relation to the area at risk

The second set of the study will be finalized during fall 2009

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Summary ADC-1004

- A novel antagonist of the C5a receptor
  Originally derived from *S. aureus*
- Small protein with short half-life (9.5 kDa)
  Suitable for treating acute inflammations
- High medical need
  No drug is available that effectively prevents and/or treats the consequences of acute inflammation
- Significant market potential
  Annual sales estimated to over a USD 1 billion.
- Early pre-clinical phase
  Proof-of-Concept in porcine AMI model ongoing
- IND planned 2010/2011

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