RAPID AND EXTENSIVE EPITOPE FINGERPRINTING OF MONO- AND POLYCLONAL ANTIBODIES

Statistical Analyses based on NGS and Novel Library Technologies

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Fraunhofer IZI

Main Contents

Feel free to click back and forth within the presentation using hyperlinks. Best to start with your field of interest, save the technology part for the end or better ask someone from us to explain it in detail.

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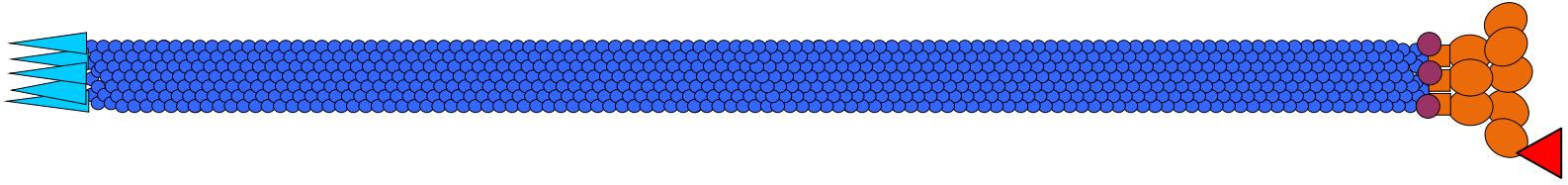

modifying the rules of the game

STATISTICAL PEPTIDE PHAGE DISPLAY

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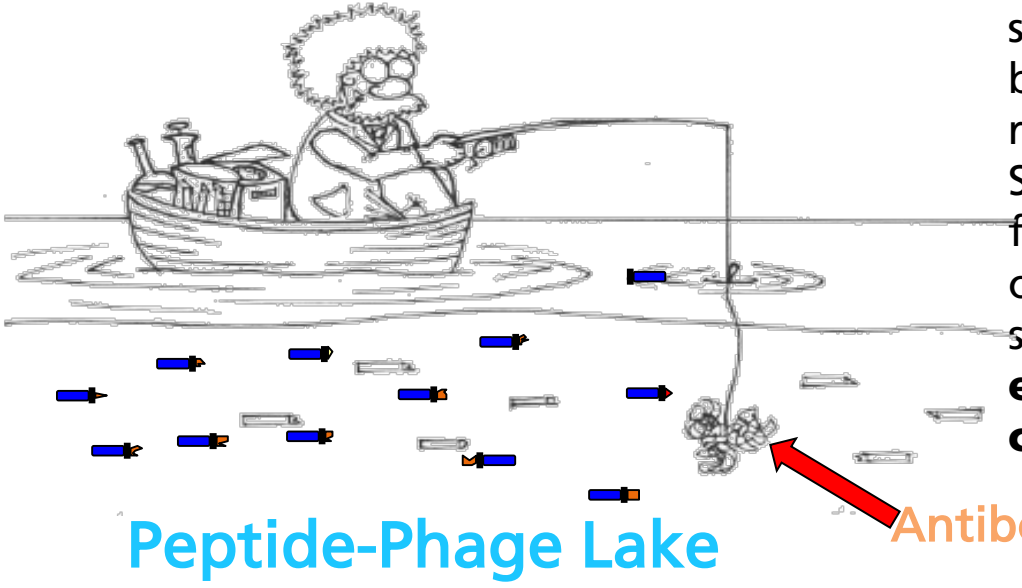
Fingerprinting Antibody Epitopes



Standard peptide phage display is, as most people will agree to, a kind of lottery with respect to the sequences finally identified as binders. After many years without real progress Next Generation Sequencing (NGS) is now more frequently being used. But this is only partially improving the results, since it is still **relying on the enrichment of individual clones.**

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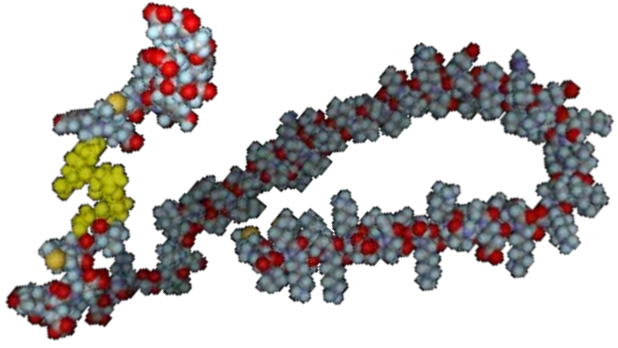
Epitope Mapping of Monoclonal Antibodies
the old fashioned way

Peptide library CPL3 on anti-alpha-Synuclein-mAB 10D2 (Roboscreen)

```
                          106             120         130         140
SYUA_HUMAN (106)          GAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA
Sc24-GATC-PL1-F-408607    --GELGWRDQSMNDPANSAYIYSSDPG--------
Sc20-GATC-PL1-F-408607    ----GELGWRDGYDPWNSLYALFGSSDPG------
```



pdb Structure 1XQ8
"Human Micelle-Bound Alpha-Synuclein"
Ulmer TS, Bax A, Cole NB, Nussbaum RL, Structure and dynamics of micelle-bound human alpha-synuclein
J. Biol. Chem. 280, p.9595-9603

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Epitope Mapping of Monoclonal Antibodies

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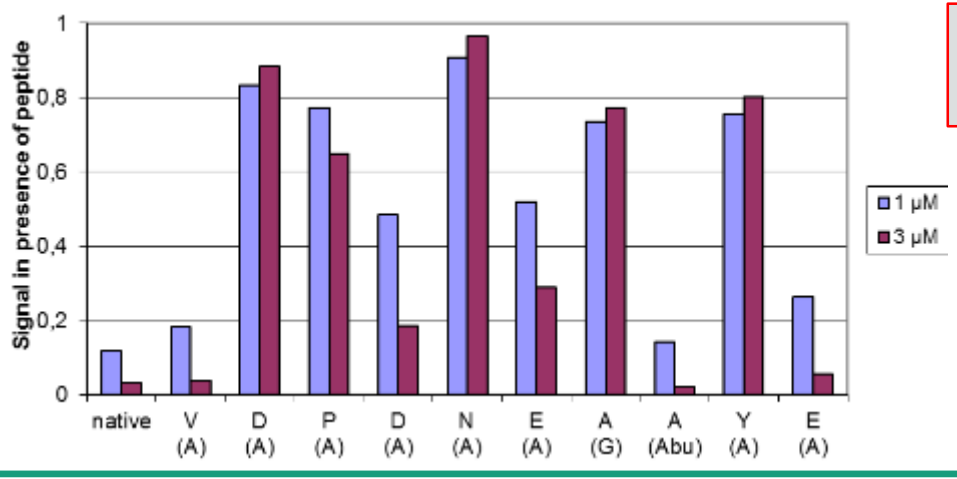
Sc20-GATC-PL1-F-408607  ----GELGWRDGYDPWNSLYALFGSSDPG------
Peptide Scan (Ala-Scan) ----------PVDPDNEAYEMP------------
```



In total > 4 months at least one person, costs > €20,000

Results from Ala-Scan competition of mAb binding to the target: Modified peptides with values above 0.5 can be regarded as loss of binding activity, i.e. this amino acid position is critical for binding.

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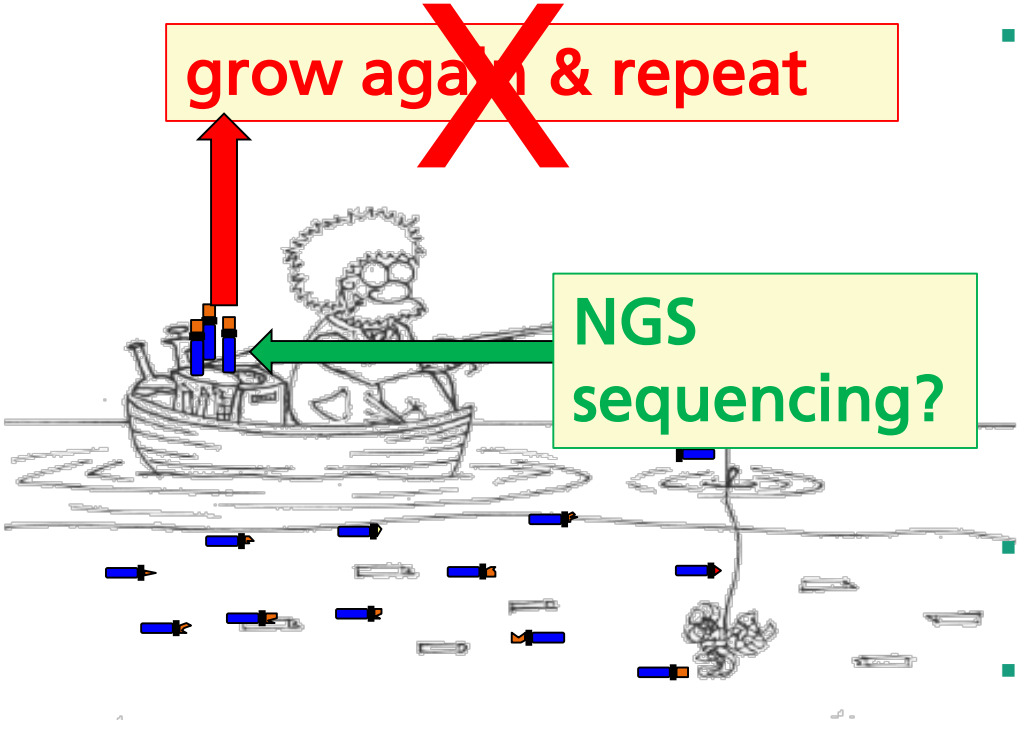
Fingerprinting Antibody Epitopes



- Applying a new combination of
  - Statistically reliable random peptide phage library
  - Optimized NGS protocols
  - Stringent sequence data filters
  - Specially designed software for calculating statistics of short motifs
- …this allows to include not only enriched sequences…
- …and gives access to hundreds of sequences in the analyses, which would otherwise be discarded.

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Waste of time and information in peptide phage display

10^9 clones?	primary standard library	10^9 clones = different peptides?
10^5 clones?	1st selected sub-library	Contains possibly all potential binders, but we cannot identify them against 99% background!
10^3 clones?	further selected sub-libraries	loosing information and „toxic“ sequences, enrichment of „garbage“
10^2 clones?	phage clone ELISA, sequencing, peptide synthesis	Limitation to 20-200 clones

at least 4 weeks

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Re-Inventing the Usage of Peptide Phage Display

A project was started and initially publicly funded from 2012-2015 that combined know how from Fraunhofer IZI (phage display, immunology) and PolyQuant GmbH (precision libraries, programming/software)

- Redesign of a phagemid vector for minimal expression levels and alternative cloning procedures (Patent granted)
- New design of a randomized peptide gene based on trinucleotide synthesis with limited amino acid usage to maximize library complexity (Patent granted)
- Optimized NGS procedures for Illumina sequencers
- New software and algorithms to handle and analyze the enormous amount of sequence data from NGS

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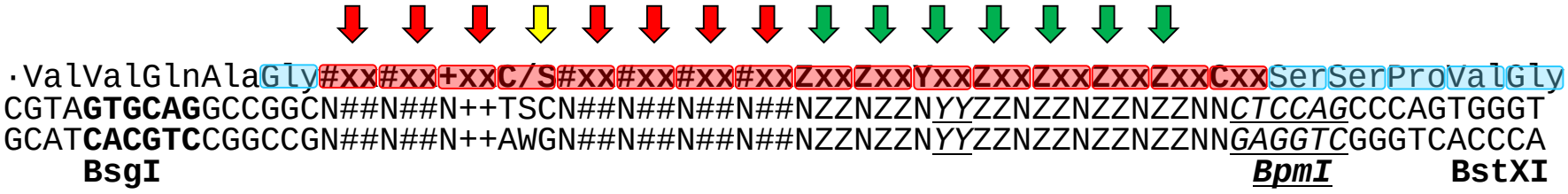
Library Design ENTE-1

Extremely diverse library



NYY: any codon ending on certain non palindromic NN
NZZ: any codon (no Trp no Met)
N##: any codon (no Cys no Met)
N++: any codon MUST end with a K, NO Cys
NNC: any codon ending on C
(or NNK instead of N++)

- NO Cys
- Cys/Ser
- reduced codon set with Cys

Trinucleotide based synthesis (by PolyQuant GmbH)

- Max 18 codons per position
- Reduced probability of too close Cys
- Reduction of Met and Trp codons
- Boost of primary library and selected sub libraries through recombination by type IIs restriction cleavage

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Extremely stable library

Statistics: Library diversity

	ENTE-1 before expansion	ENTE-1 final library	Ph.D.™-12* (commercial library)	ENTE-1 after mAB 10D2 1st selection	ENTE-1 after mAB 10D2 2nd selection
Total number	**1,241,361**	**2,800,721**	**17,609,210**	**294,193**	**411,931**
Sequence found 1X	1,186,637 (96%)	2,018,083 (72%)	736,953 (4.2%)	76,972 (26.2%)	16,574 (4%)
Sequence found 2X	22,853 (3.7%)	351,921 (25.1%)	114,791 (1.3%)	37,533 (25.5%)	4,401 (2.1%)
Sequence found 3X	2,002 (0.5%)	24,957 (2.7%)	47,187 (0.8%)	16,079 (16.4%)	1,492 (1.1%)
Sequence found 4X	21 (0.1%)	838 (0.1%)	26,184 (0.6%)	7,074 (9.6%)	759 (0.7%)
Sequence found 5X	131 (0.1%)	71	17,098 (0.5%)	3,338 (5.7%)	478 (0.6%)
Sequence found 6X	48	14	11,727 (0.4%)	1,672 (3.4%)	437 (0.6%)
Sequence found 7X	21	6	8,801 (0.3%)	817 (1.9%)	309 (0.5%)
Sequence found 8X	5	5	7,057 (0.3%)	504 (1.4%)	268 (0.5%)
Sequence found 9X	4	1	5,531 (0.3%)	297 (0.9%)	231 (0.5%)
Sequence found 10X	6	2	4,678 (0.3%)	191 (0.6%)	228 (0.5%)
Sequence found 11X	2	1	3,972 (0.2%)	142 (0.5%)	187 (0.5%)
Sequence found 12X	2	1	3,326 (0.2%)	100 (0.4%)	155 (0.5%)
Sequence found 13X	1		2,939 (0.2%)	70 (0.3%)	156 (0.5%)
Sequence found 14X	2		2,542 (0.2%)	56 (0.3%)	119 (0.4%)
Sequence found 15X	1		2,253 (0.2%)	46 (0.2%)	110 (0.4%)
Sequence found 16X			2,074 (0.2%)	45 (0.2%)	95 (0.4%)
Sequence found 17X			1,825 (0.2%)	36 (0.2%)	98 (0.4%)
Sequence found 18X			1,713 (0.2%)	32 (0.2%)	110 (0.5%)
Sequence found 19X			1,495 (0.2%)	22 (0.1%)	90 (0.3%)
Sequence found 20X			1,366 (0.2%)	31 (0.2%)	85 (0.4%)
Sequence found **> 20X**			65,305 (89.1%)	308 (5.6%)	2,224 (84.5%)
Sequence found **> 100X**			20,241 (82.1%)	26 (1.6%)	631 (67.1%)
Sequence found **> 1000X**			2,844 **(56.6%)**	0	45 (29%)

*Matochko et al. Methods 58 (2012) 47–55

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Statistics of the new library ENTE-1

Statistically even library

	Gly	#xx	#xx	+xx	C/S	#xx	#xx	#xx	#xx	Zxx	Zxx	Yxx	Zxx	Zxx	Zxx	Zxx	Cxx	Ser	Ser
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	0	39398	90956	186896	522	106968	111974	119732	125638	145486	143878	330	115070	141808	150810	148728	210284	0	0
C	0	416	442	972	1568218	1508	384	382	514	166848	166862	326870	148224	181926	178966	186930	550978	0	0
D	0	121218	147304	448	54	132024	128594	132772	135180	133122	133244	444	125948	119076	122484	89678	373274	0	0
E	0	265552	214760	200	52	181604	168106	166774	173500	171608	183564	361690	164792	154616	157474	156350	120	0	0
F	0	79882	93234	132368	782	121944	133130	124916	123726	135898	135512	388	135932	148972	141866	140308	419896	0	0
G	2580938	118170	102104	123210	288	103164	98142	103158	103184	105932	118254	210228	103892	100738	99158	103104	302	0	0
H	0	183728	210022	276224	218	193046	199854	198690	201420	198948	197830	712	215206	199326	197608	200372	662	0	0
I	0	261530	148836	670	60	152052	131096	125206	131572	121100	125694	166342	125310	121888	120926	117576	307086	0	0
K	0	123962	113660	270	44	96312	88342	82500	84762	85532	90728	141044	88136	78988	75258	70150	92	0	0
L	0	76996	101696	135064	180	107394	121148	123968	121546	126398	114968	454	142988	135062	129778	143870	600	0	0
M	0	136	132	190552	146	90	90	82	118	92	68	44	126	106	72	80	8	0	0
N	0	242394	173660	222964	164	160878	146182	139142	137072	100932	98022	396	99174	94478	96654	91812	225742	0	0
P	0	133074	151342	179242	464	155758	144530	145170	148064	144820	140742	260560	161584	139658	139098	141702	150	0	0
Q	0	167382	203670	276748	198	184028	211236	213484	203750	205056	187362	398642	221298	207610	212524	222028	32	0	0
R	0	59562	111702	155384	522	104284	117846	123140	121788	122634	121240	189740	125332	125692	121258	132628	368	0	0
S	0	97316	107976	145310	1007238	129726	126148	125332	121664	129168	127548	664	130660	138544	134466	131084	868	2580938	2580938
T	0	155464	132292	164596	254	128358	126256	123696	122298	122874	122012	436	125248	121086	124680	125946	320	0	0
V	0	215518	194452	214970	384	197740	191790	190844	194094	197602	214250	324	190598	199846	205314	204654	722	0	0
W	0	83942	127436	174576	336	159568	171438	179928	164760	160	82	280202	562	62	54	26	56	0	0
Y	0	155298	155262	274									171456	172490	173912	489378	0	0	

No such codon in the oligonucleotide, error rate of Illumina MiSeq

Naive ENTE-1 library

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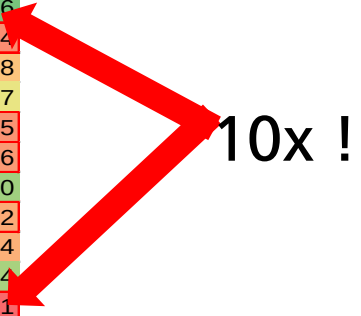
Statistics: Amino Acid Distribution in „Normal“ Libraries

Almost 10x difference in amino acid statistics: C7C library , NNK synthesis (312,352 sequences)

data from: Dias-Neto et al:Next-generation phage display: integrating and comparing available molecular tools to enable cost-effective high-throughput analysis. PLoS One. 2009 Dec 17;4(12)

	A1	A2	A3-	A4	A5	A6	A7
A	3398	3223	3422	3475	3592	3697	4161
R	4564	5649	5669	5316	4691	4484	5326
N	826	701	755	888	750	822	834
D	1531	1385	1636	1101	1472	1654	1288
C	2387	2171	2188	2215	2635	2402	2227
Q	902	1131	1159	656	750	816	885
E	1253	1318	1368	1009	1265	1362	936
G	5308	5196	4750	4652	5350	5273	4220
H	1062	1089	1273	715	901	880	1082
I	1143	1045	1064	1616	1223	1167	1124
L	4233	4402	4484	3440	3934	3941	3994
K	614	585	564	766	568	682	531
M	928	858	669	1080	773	876	658
F	1906	1648	1613	1441	1783	1597	1190
P	1723	1882	2059	1751	1769	1704	3384
S	3909	3881	3768	5875	4564	4382	5047
T	1334	1310	1298	2595	1555	1591	2143
W	1701	1824	1460	1823	1802	1847	1309
Y	1267	1170	1163	918	1124	1121	1003
V	4629	4149	4254	3283	4117	4320	3276



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New way of data evaluation

NGS Data Preparation

NGS data used for sequence analysis from the original data set is filtered

- Not only highest quality sequences must be used! Cloning and PCR artefacts are removed.
- Remaining data is indexed and stored in a data base
- In standard approaches all 3-mer and 4-mer peptide motifs are indexed, frequency and probability are calculated, compared and related sequences can be retrieved and analyzed..

Id	Motif	Count	Freq	Expect	Enrichment (log!)
66091	DPEN	2445	3,17412	5,12765	1,95354
65785	DPPN	2365	3,18856	5,12765	1,93909
4168	NEVY	2340	3,19318	5,12765	1,93447
66128	DPDN	1459	3,39834	5,21223	1,81389
4740	NEAY	1727	3,3251	5,12765	1,80255
65998	DPHN	1272	3,45791	5,21223	1,75433
100344	PPNE	1387	3,42032	5,16191	1,74159
33118	EWIW	125	4,46548	6,13503	1,66955
5080	NDEY	1252	3,46479	5,12765	1,66286
65750	DPON	1204	3,48177	5,12765	1,64588

First selection round on mAB 10D2!

Count = Total number in data set
Freq = -log(count/total sequences)
Expect = -log(theoret./total sequences)
Enrichment = (Expect-Freq)

Mapping of mAB 10D2, epitope:
DMPV**DPDNEAY**EMPS

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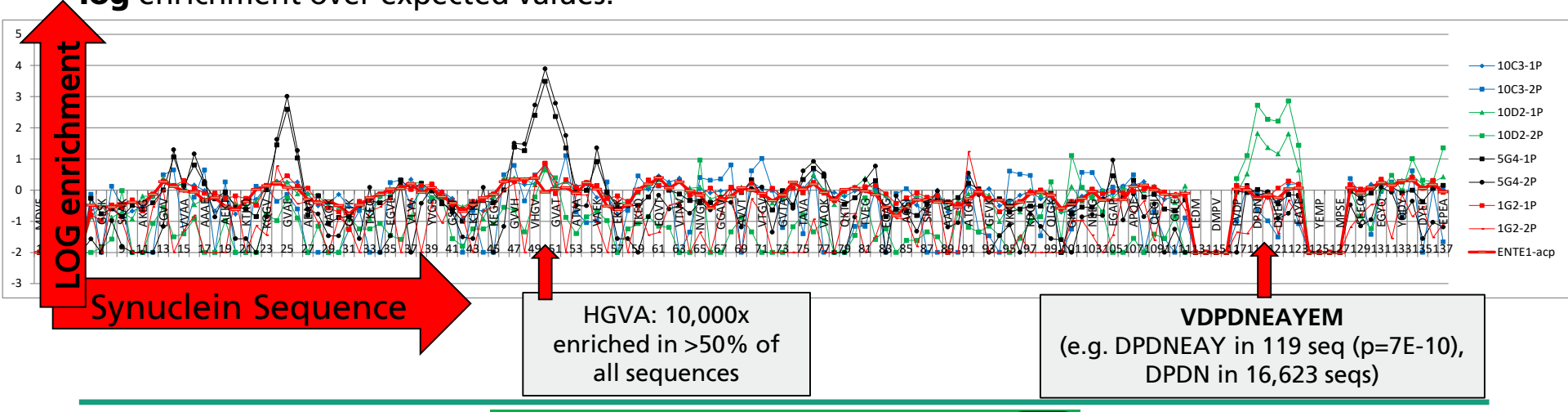
Motif Search in NGS Data –Example Synuclein mABs

- Instead of searching for sequences, we are looking for enriched motifs, provided the data sets are large enough (>200,000 peptides => >1 Mio 4-mers)
- The motif enrichment (NOT THE FREQUENCY) in data sets from selection experiments can be plotted against the entire alpha synuclein protein's 4-mer sequences. This curve reveals potential epitopes. (Antibodies from AJ Roboscreen GmbH)

Explanation: blue/green/black different monoclonal antibodies; red non specific data sets; Y-axis is **log** enrichment over expected values.



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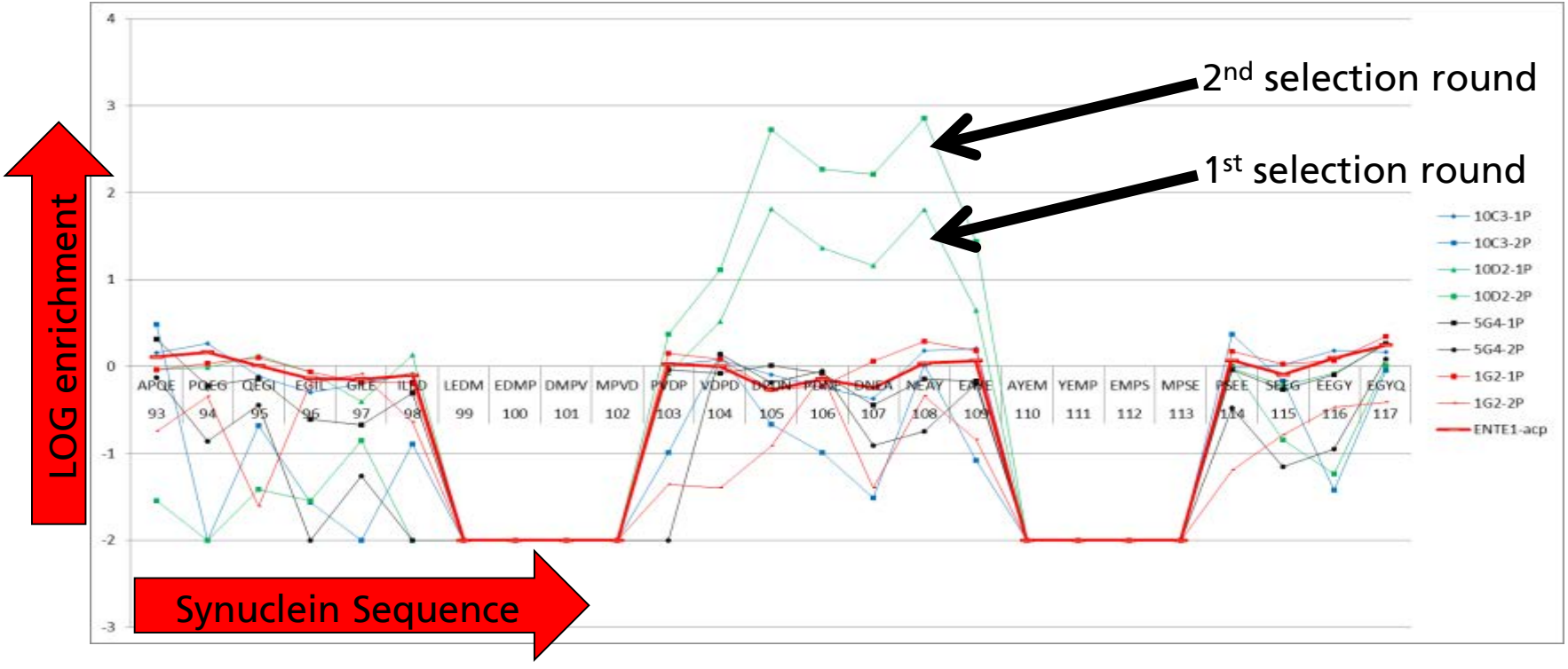

93-APQEGILEDMPV**DPDNEAY**EMPSEEGYQ-117

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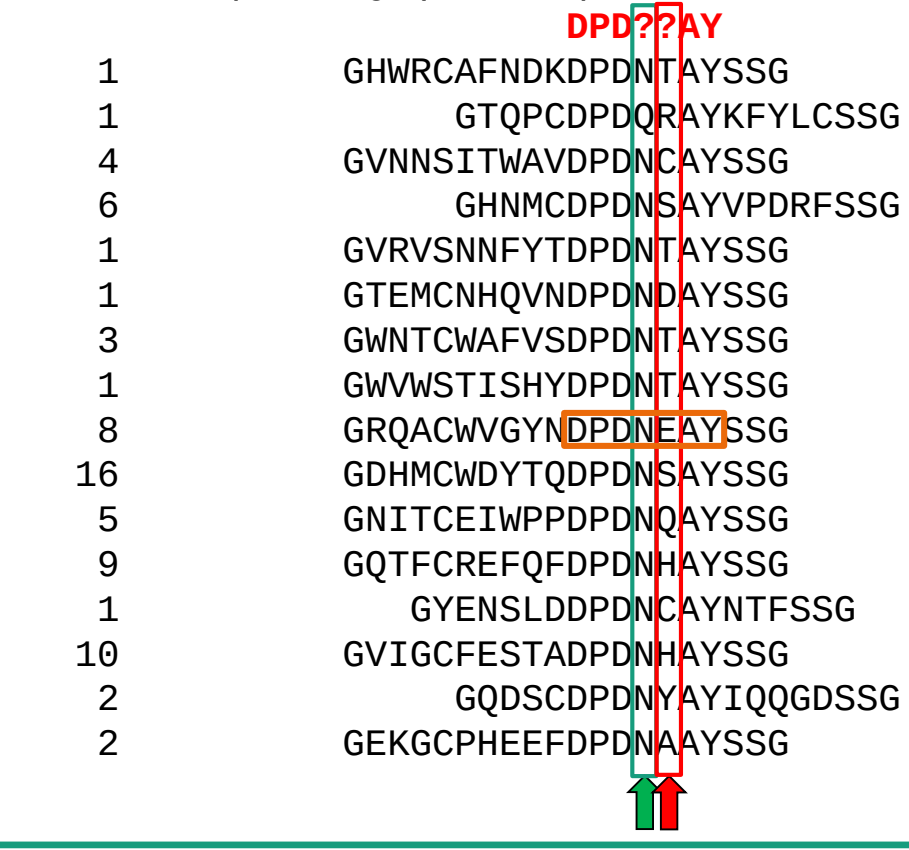
Epitope Fingerprinting – „Manual Analyses“

1st round panning (all sequences with DPDxxAY)



DMPV**DPDNEAY**EMPS

DPDNxAY

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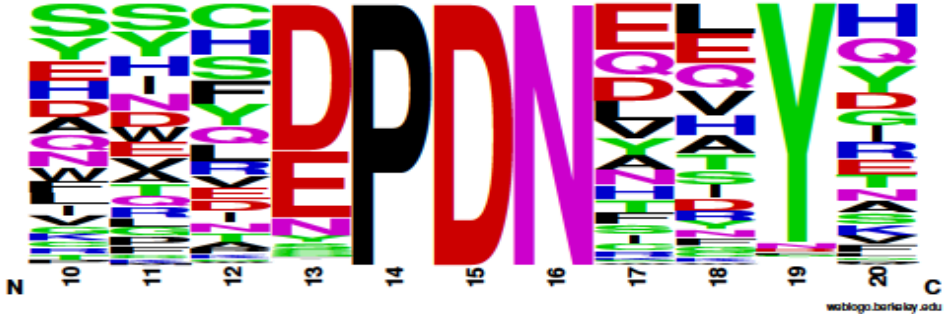
Epitope Fingerprinting Statistics

Multiple special software tools for analyses

	A4	A3	A2	A1	M	B1	B2	B3
C	0	27	1	225	DPDN	33	14	1
P	28	15	46	16	DPDN	0	0	0
G	102	32	77	5	DPDN	5	65	0
A	92	90	78	20	DPDN	45	73	4
V	38	59	190	59	DPDN	144	79	0
I	111	43	130	64	DPDN	34	33	0
L	106	54	57	113	DPDN	117	145	0
M	8	0	30	0	DPDN	0	0	0
F	9	65	39	107	DPDN	70	24	0
Y	35	128	117	111	DPDN	78	68	1442
W	119	79	67	10	DPDN	9	17	2
T	181	18	86	16	DPDN	111	71	0
S	17	160	134	128	DPDN	30	80	0
N	122	143	43	23	DPDN	230	149	2
Q	104	60	34	87	DPDN	172	286	1
R	13	30	62	146	DPDN	12	69	0
K	51	52	21	8	DPDN	9	9	0
H	139	94	92	229	DPDN	112	127	0
E	51	80	87	37	DPDN	110	46	4
D	133	230	68	55	DPDN	136	102	1
count	1459	1459	1459	1459		1457	1457	1457

An alternative display of the previous slide's data is this table of N- and C-terminal amino acid statistics. Less useful for the untrained eye, but more informative with respect to total numbers. Naive sequence's amino acid frequencies with thick frame.

The amino acids surrrounding the motif are sorted by similarity and not by alphabetic order to facilitate reading and understanding the output.



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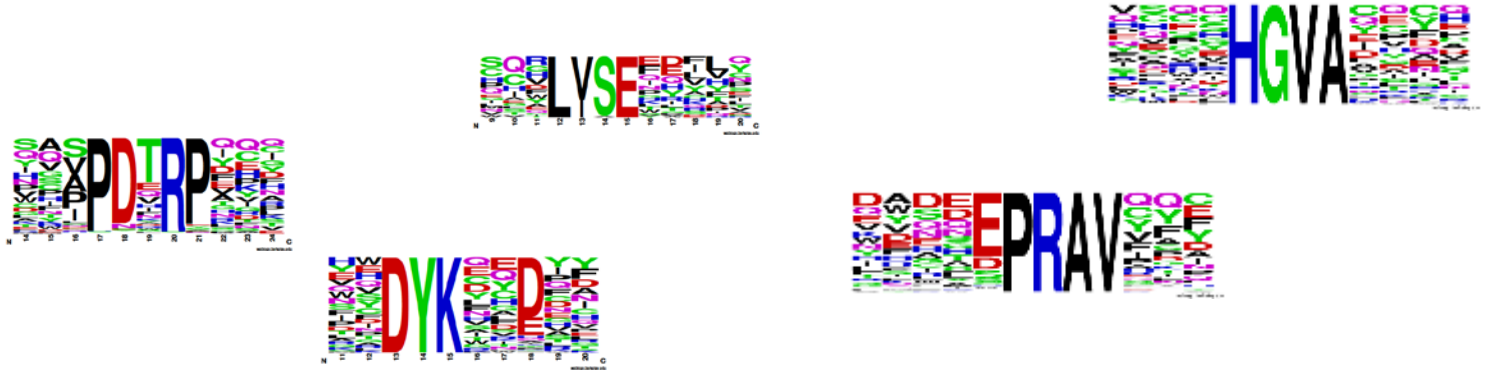

Epitopes beyond the resolution of single amino acids

FINGERPRINTING EPITOPES OF MONOCLONAL ANTIBODIES

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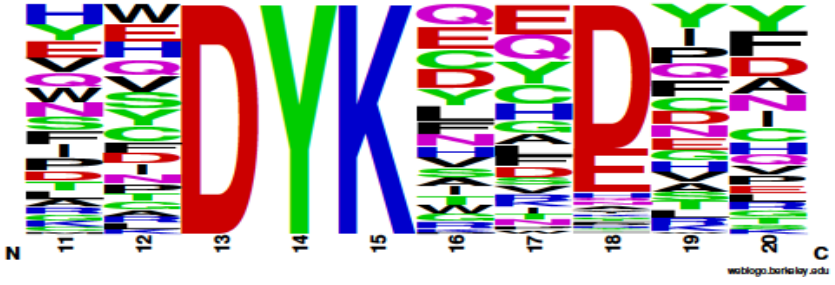
Comparing Different Antibodies - Specificity

- Fingerprinting renders in depth understanding of even minor differences in the epitope of seemingly identical antibodies
- Example: The epitope for the two well-known FLAG™ antibodies is regarded to be the peptide DYKDDDDK
- Displayed as here in „web-logo“ style fingerprint explains immediately the higher specificity of FLAG M2 generated from several hundred sequences sharing the binding motif. FLAGM2 is considered to be more specific.

FLAG-M1

FLAG-M2



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Comparing Different Antibodies – Checking Identities

Two antibodies binding to mucin-1, both binding the repetitive motif, databases searched for PDTRP motif variations, single selection round!

BD anti-CD227, data based on 769 individual resp. 271 different sequences after first selection, 2.8x enriched; Dataset: 377,990 seq.

Non commercial mAB, data based on 1013 individual resp. 406 different sequences after first selection, 2.5x enriched; Dataset: 451,834 seq.

Compare naive library, data based on only 262 individual resp. 255 different sequences, 1.027x (=not) enriched; Dataset: 949,676 seq.

You get what you ask for!

-> important is enrichment

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Surprise?

The c-myc tag and the mAB 9E10 are famous tools in molecular biology:

- Antigen: EEQKLISEEDLLRKRREQLKHKLEQLRNSCA (Synthetic peptide of human c-Myc 408-438)

- Mapping against full lenght c-Myc reveals an additional, much stronger epitope AAAK**LVSE**KLAS vs. expected EEQK**LISE**EDLL
- Fingerprinting confirms EEQK**LISEEDL**L, despite dominant valine

Guess the structure of the antigen!

- Potential explanation for Val/Ile indifference: Helical structure of target

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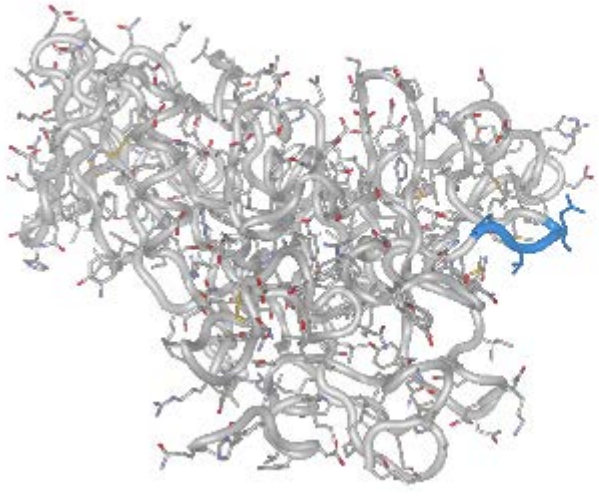
Structural Epitopes

1F9 is a monoclonal antibody raised against AMA-1 a major malaria antigen.



AMA-1

Structure of the Malaria Antigen AMA1 in Complex with a Growth-Inhibitory Antibody. Coley, A.M., Gupta, A., Murphy, V.J., Bai, T., Kim, H., Foley, M., Anders, R.F., Batchelor, A.H. (2007) Plos Pathog. 3: e138

Results:

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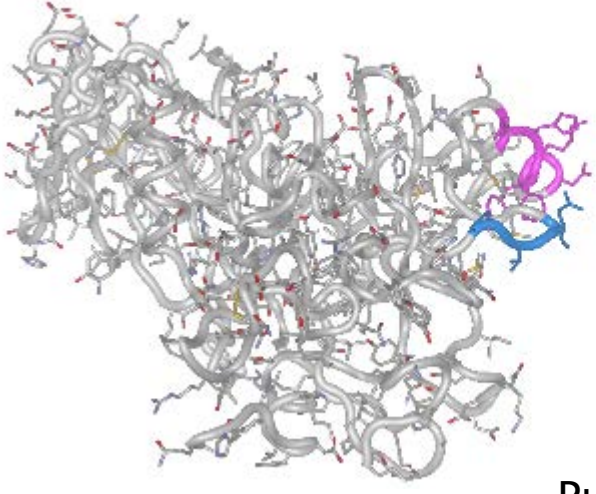
Fraunhofer IZI

Structural Epitopes

1F9 is a monoclonal antibody raised against AMA-1 a major malaria antigen.

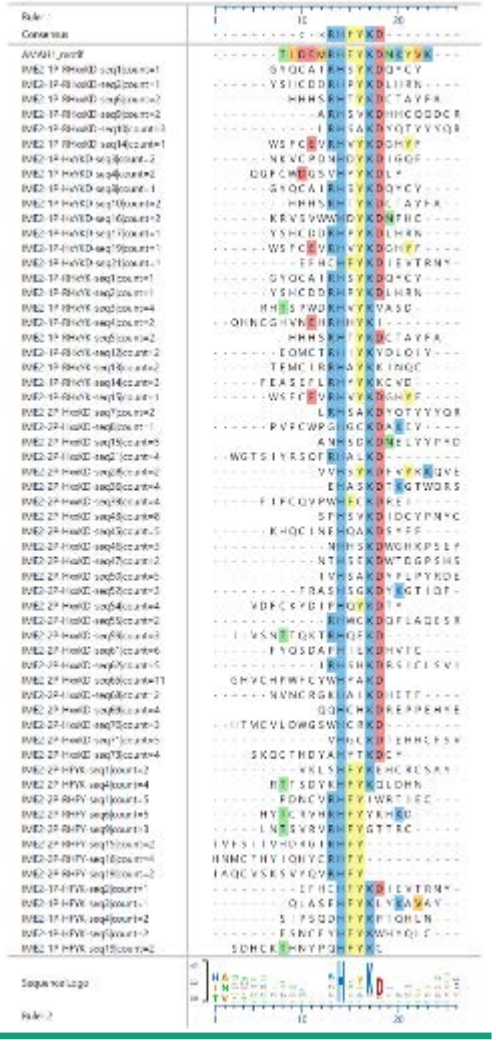


AMA-1

Published from structure: HFYK

„Pepscan“:

194-TLDEMRHFYKDNK-206



Fraunhofer IZI

Structural Epitopes

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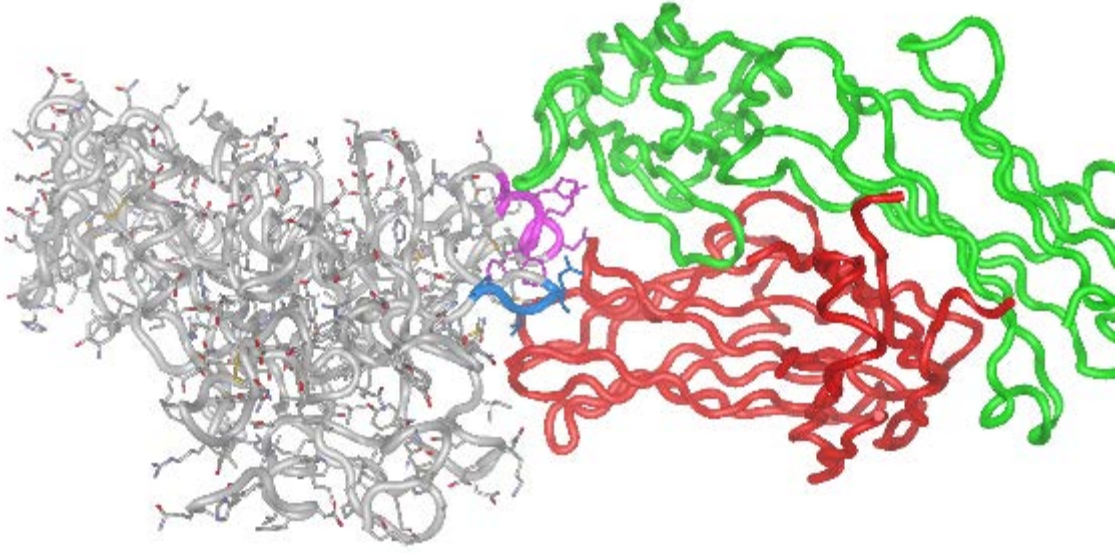

AMA-1 and monoclonal antibody 1F9

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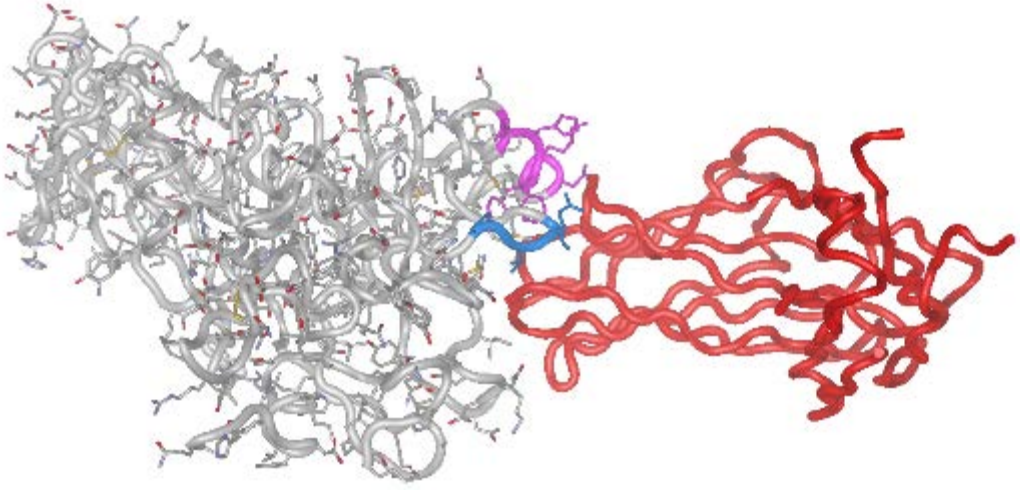
Structural Epitopes

1F9 is a monoclonal antibody raised against AMA-1 a major malaria antigen.



AMA-1 and 1F9 heavy chain

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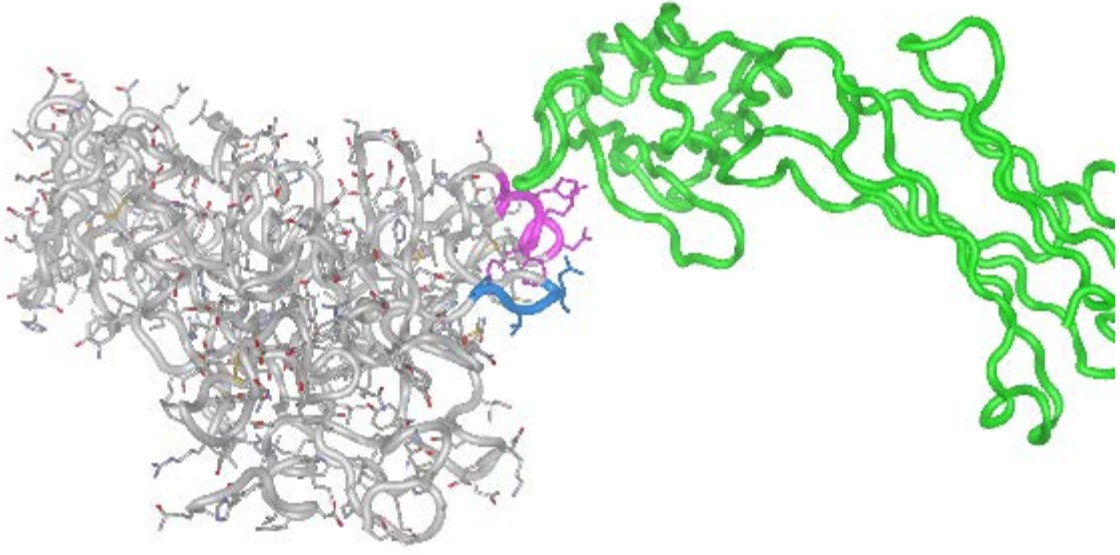
Structural Epitopes

1F9 is a monoclonal antibody raised against AMA-1 a major malaria antigen.



AMA-1 and 1F9 light chain

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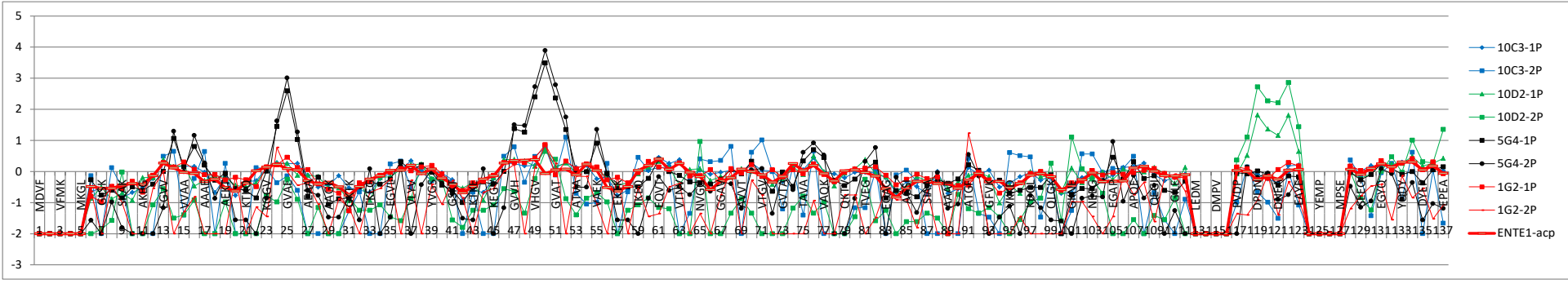
Summary of Technology

The combination of a stable peptide library with a novel NGS data evaluation works very reliable in the identification of continuous and dis-continuous epitopes. Results of selections experiments are reproducible on the level of motif statistics!

The main limits observed so far:

- Biased data sets by strong enrichment of individual clones or unspecific hydrophobic junk
- Data from selections on less than ca 100 antibody molecules become less reliable



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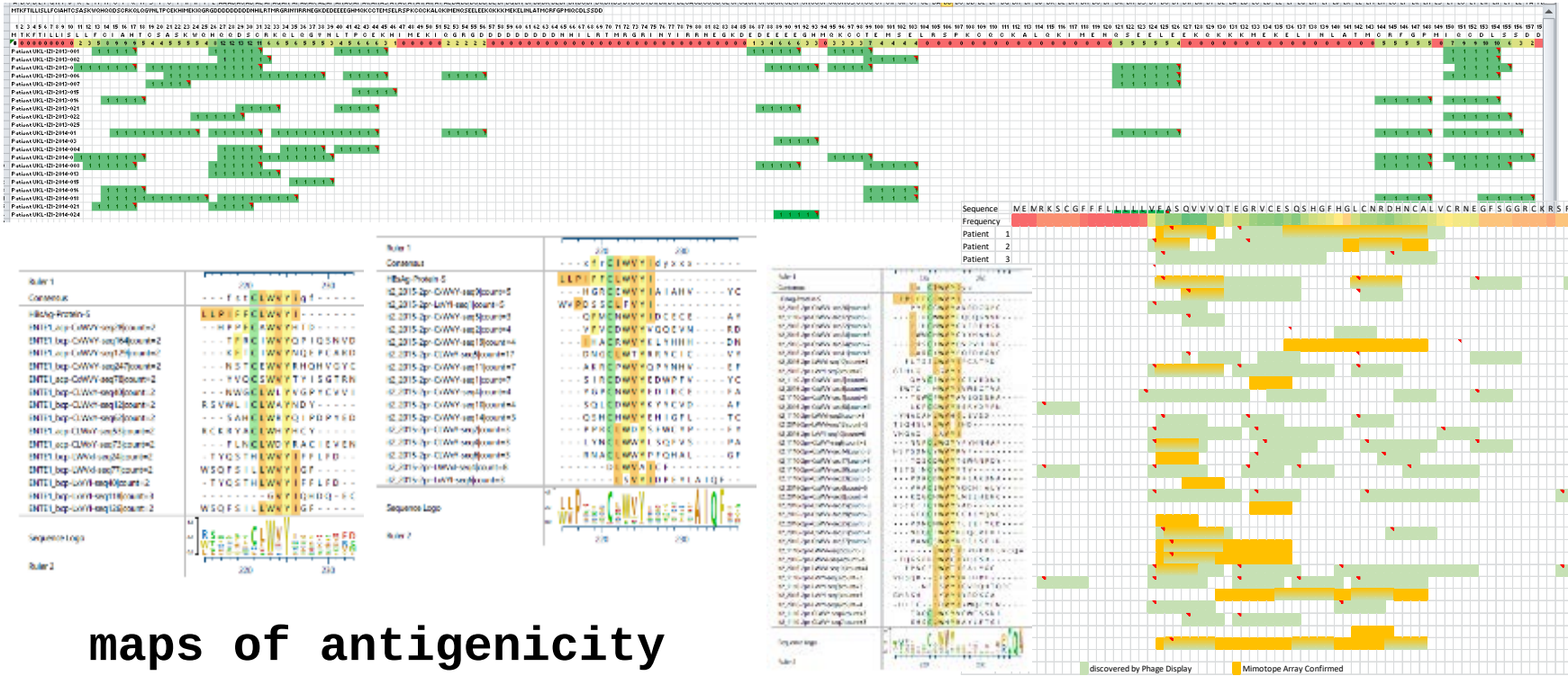
Fraunhofer IZI



maps of antigenicity

FINGERPRINTING ANTIBODY EPITOPES IN SERUM

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Fraunhofer IZI

Fingerprinting Antibody Epitopes in Serum

- Serum samples collected from one patient over several years have been used for this immunome study. The results have been compared for vaccine antigens received in this time period.
- Hepatitis Antigen epitope signal strength varies before and after vaccination, epitopes shift with the time
- Epitopes from influenza virus immunisation can be also mapped. In addition an infection can be seen with a different H3N2 virus.

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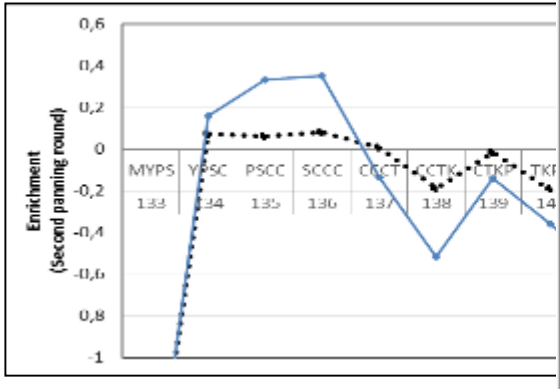
Example HBsAg

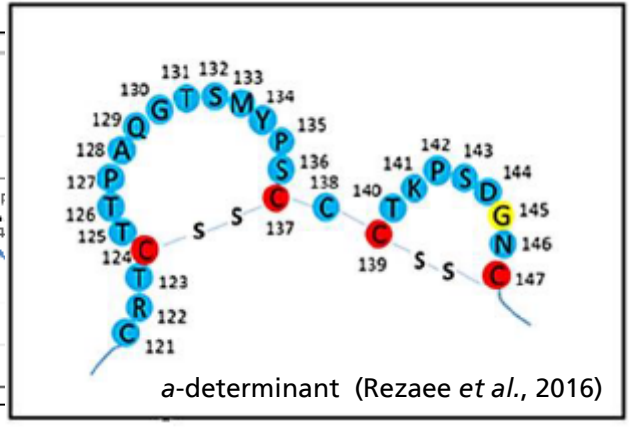
Several motif related to the Hepatitis B epitope have been identified. The significant but very unusual epitope below showed an interesting change with respect to motif frequencies.

Antigen	Motif	First panning round		Second panning round			
		Enrichment	Unique seq	Enrichment	Unique seq	Found motifs	Count
	PSCC	0,08287	17	0,25451	24	VVTSYGIFSQCPSCCC	1**
GTSMYPSCCCTKPSDGNC	SCCC	0,17992	19	0,33903	20	WVNCNIYR**SCCCT**RKD	4
	CCCT	0,23766	14	0,29074	13		

**more single sequences with this motif found

Sample ID	Date
Engerix-B 03.2010	
S-10	25.11.2010
S-12	18.12.2012
S-0214	02.2014
S-1014	22.10.2014
S-1114	26.11.2014
S-15	07.12.2015
Engerix-B 12.2015	
S-16	17.01.2016





Fraunhofer IZI

Example HBsAg

Antigen	Motif	First panning round		Second panning round			
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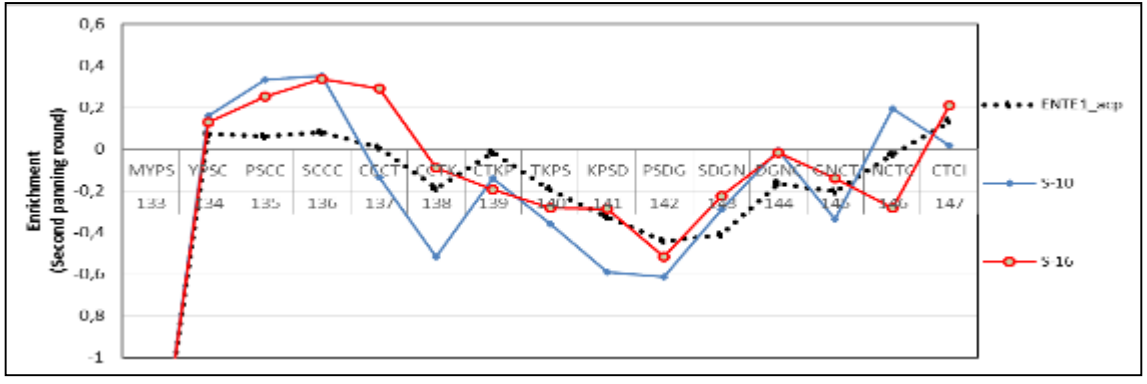
Engerix-B 12.2015	
S-16	17.01.2016



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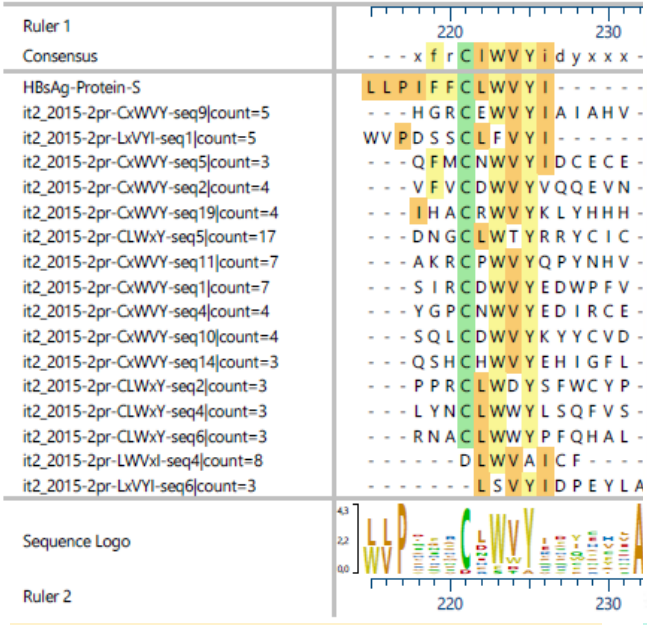
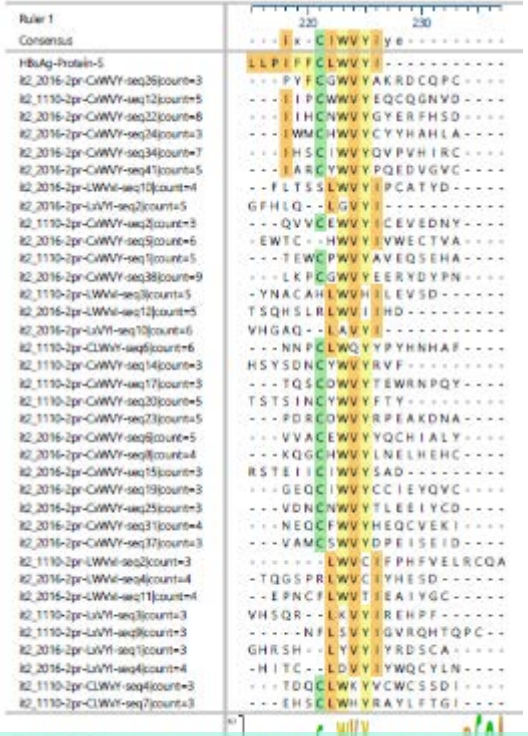
Fraunhofer IZI

Motif Enrichment – HbsAg C-Terminal Epito

Not just numbers – sequence similarities count

Comparing naive library vs. pre-boost vs. post boost sera: Only sequences found with at least 4 aa identity to the antigen's C-terminal epitope are





Naïve library
15 sequences >1x; 1 >3x / data set 2,191,037

Pre boost serum
27 sequences >1x ; 16 >2x / data set 253,288

Post boost serum
65 sequences >1; 37 >2 / data set 476,099

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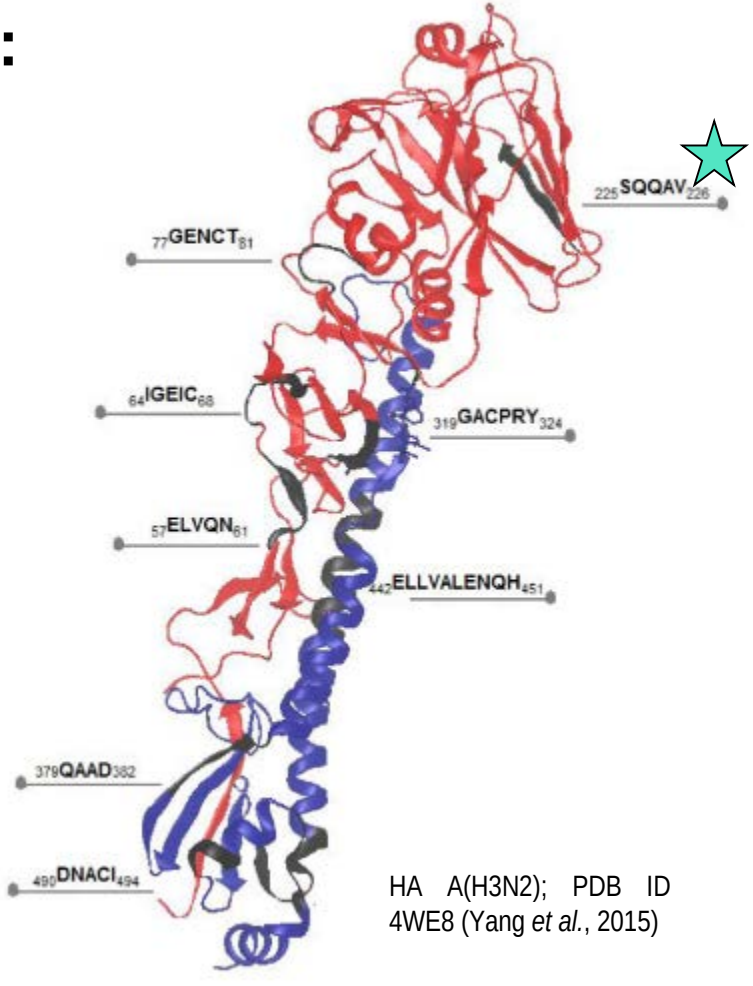
Fraunhofer IZI

Identified Epitopes from Influenza: HA - A(H3N2) Texas/50/2012 virus

- Nine potential epitopes identified
- Four epitopes described in the literature
- One epitope in the receptor binding site

(residues 219-228) [Yang et al., 2015; 10.1016/j.virol.2014.12.024]



HA A(H3N2); PDB ID 4WE8 (Yang *et al.*, 2015)

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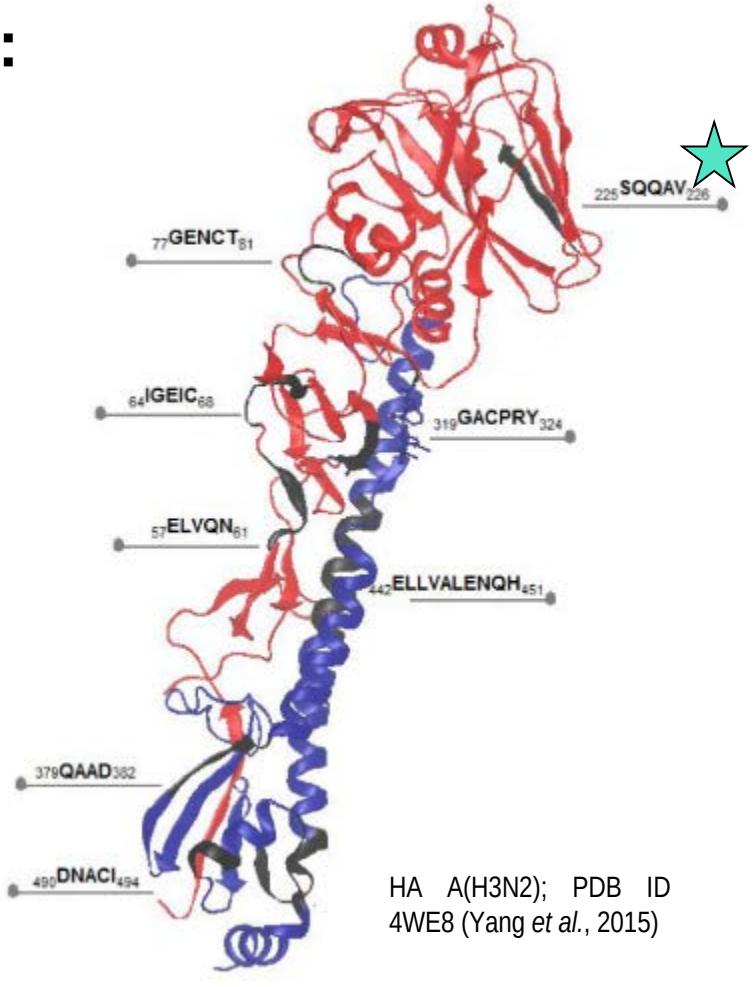
Fraunhofer IZI

Identified Epitopes from Influenza: HA - A(H3N2) Texas/50/2012 virus

Ruler 1	220	240
Consensus	- - - - - - q i v - q S Q Q A V x - - - - - - - -
HA A-Texas-50-2012 H3N2	S G R I T V S T K - R S Q Q A V I P N I G f R P R I R
it2_1114-2pr - QQAV - seq28 - count: 9	- - - H H N S Y D A Q A Q Q A V R F Y - - - - - - - -
it2_1114-2pr - QQAV - seq18 - count: 8	- - - - - - S T P C V T Q Q A V I E V P D F - - - - -
it2_1014-2pr - QQAV - seq8 - count: 11	- Q W H C K Q E - - N H Q Q A V I V C - - - - - - -
it2_1114-2pr - SQQA - seq59 - count: 10	- - K H Q C Y T L - Q S Q Q A H I A Y - - - - - - -
it2_1114-2pr - SQQA - seq4 - count: 5	- - - - - - - - I D - A S Q Q A P H E Q Y H F F D - -
it2_2015-2pr - QQAV - seq12 - count: 4	- - - - - - Y D H S T G Q Q A V E P C D L Y - - - - -
it2_2015-2pr - QQAV - seq11 - count: 5	- - - - - - W I W S Y L Q Q A V K G Y I I - - - - -
it2_1114-2pr - QQAV - seq4 - count: 4	- - - - - - - E S - R S Q Q A V A R G A L P E A - - -
it2_1114-2pr - SQQA - seq87 - count: 5	- R G S S V G I Q - S S Q Q A N Y N - - - - - - - -
it2_1114-2pr - QQAV - seq32 - count: 5	- K F R C Y Q Q - - D Y Q Q A V C Q A - - - - - - -
it2_2015-2pr - QQAV - seq26 - count: 5	- I L F C I E H V - P C Q Q A V G C - - - - - - - -
it2_2015-2pr - QQAV - seq29 - count: 7	- - P S H S A G E S T L Q Q A V Q Y - - - - - - - -
it2_2015-2pr - QQAV - seq33 - count: 4	T A N C - E V L Y - Q L Q Q A V R N - - - - - - - -
it2_1114-2pr - SQQA - seq3 - count: 7	- - - - - - - H H - T S Q Q A H D L W Y H Q D C - - -
it2_1114-2pr - SQQA - seq5 - count: 4	- - - - - - - S E - W S Q Q A Y C A G F K C P C - - -
it2_1114-2pr - SQQA - seq45 - count: 4	- - - - - - E F V - S S Q Q A L V E D L - N Y A - - -
it2_1114-2pr - SQQA - seq52 - count: 5	A Q C Y S Q Q A A - W S A Q C F - - - - - - - - - -
it2_1114-2pr - SQQA - seq88 - count: 5	- E V V S S F P T - V S Q Q A Q V C - - - - - - - -
it2_1114-2pr - SQQA - seq60 - count: 14	- - V G M C I N W - E S Q Q A Q L Q F - - - - - - -
it2_2015-2pr - QQAV - seq25 - count: 14	- F L Q C N V Q S - D T Q Q A V C D - - - - - - - -
it2_1014-2pr - SQQA - seq4 - count: 4	- - - - - - - - V P - A S Q Q A W T H P E Y S L F - - -
it2_1014-2pr - SQQA - seq28 - count: 4	- - - - - - Q H T - C S Q Q A A V Y S P Y P F - - - -
it2_1014-2pr - SQQA - seq30 - count: 4	- - - - - - D S V - C S Q Q A H C W F T - L A Y - - -
it2_1014-2pr - SQQA - seq54 - count: 8	W S Q W S T I I Q - P S Q Q A - - - - - - - - - - -
it2_1014-2pr - QQAV - seq10 - count: 4	- D N F C Y Q A - - P V Q Q A V F V C - - - - - - -
it2_1014-2pr - QQAV - seq27 - count: 5	- R T A S W Q F V - G P Q Q A V N N - - - - - - - -
it2_1014-2pr - QQAV - seq28 - count: 9	- T Q W S Y R F Q - Q G Q Q A V E D - - - - - - - -

72 sequences with the active site motif, only those >3x are shown (vs 17 2x in larger naive data set)



HA A(H3N2); PDB ID 4WE8 (Yang *et al.*, 2015)

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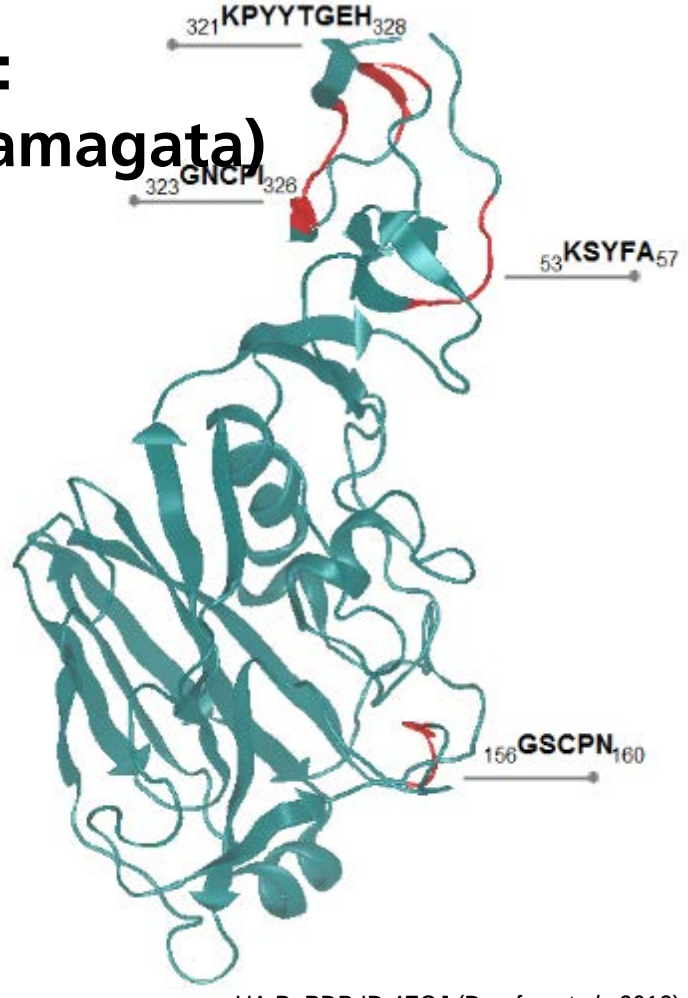
Fraunhofer IZI

Identified Epitopes from Influenza: HA - B Massachussetts/02/2012 (Yamagata)

- Five potential epitopes identified
- Four epitopes confirmed in the literature
- Cross-reactive neutralizing epitopes



HA B; PDB ID 4FQJ (Dreyfus *et al.*, 2013)

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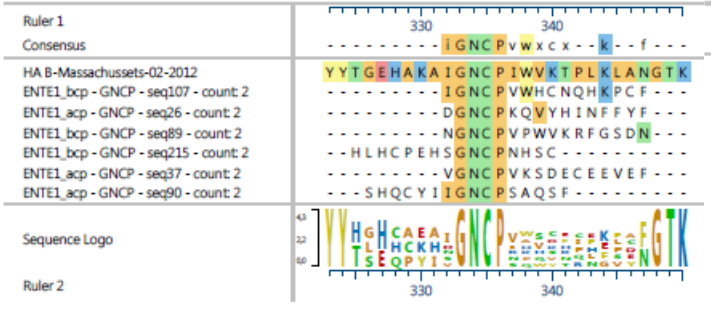
Fraunhofer IZI

Identified Epitopes from Influenza: HA - B Massachussetts/02/2012 (Yamagata)

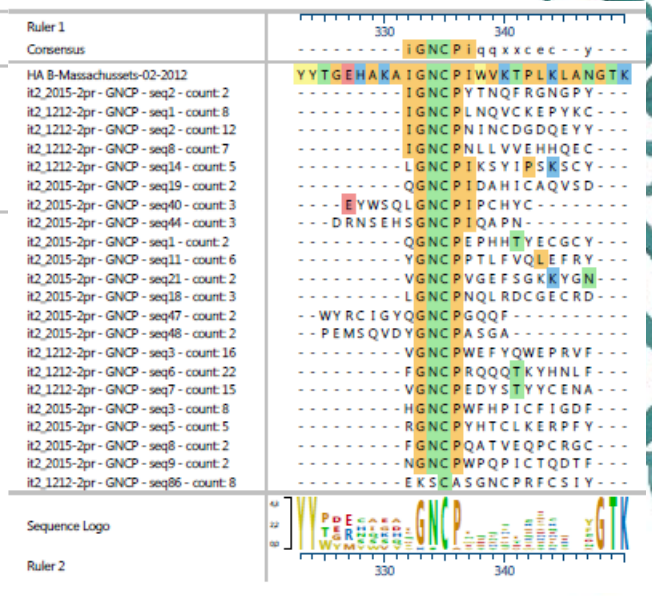
- Comparing naive and selected library

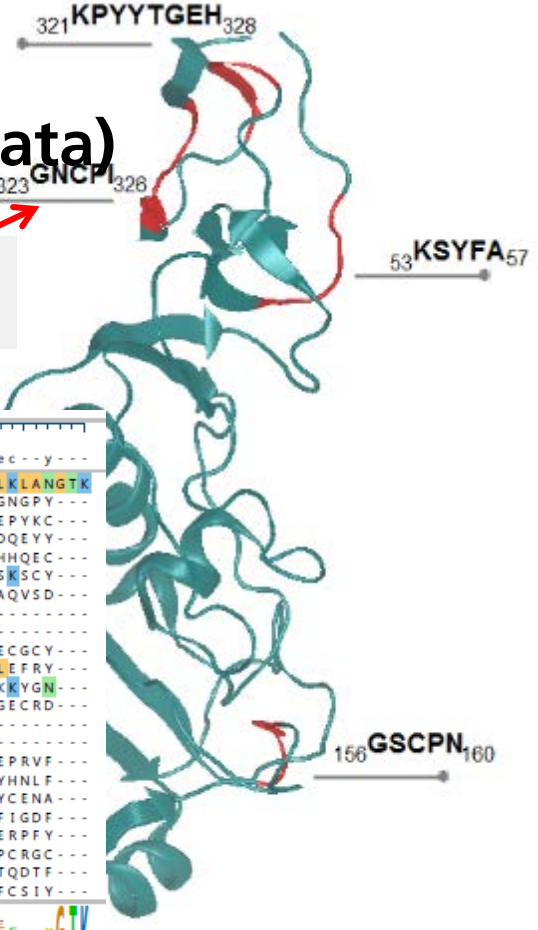
$_{332}$IGNCPIWVKT$_{341}$
Yasugi *et al.*, 2013

Naïve Dataset: 2,191,037 sequences



Selection Dataset: 511,986 sequences





HA B; PDB ID 4FQJ
(Dreyfus *et al.*, 2013)

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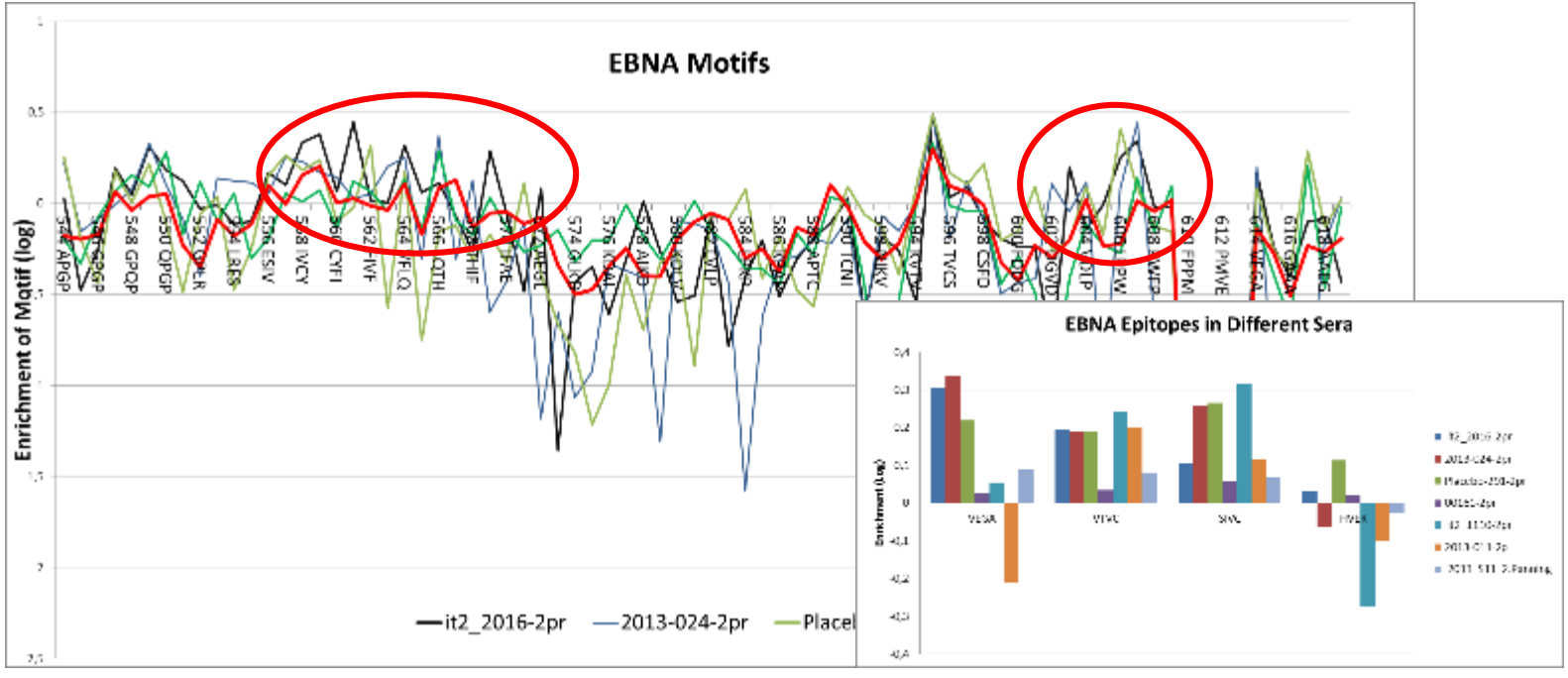
Fraunhofer IZI

Epstein-Barr-Virus Signatures

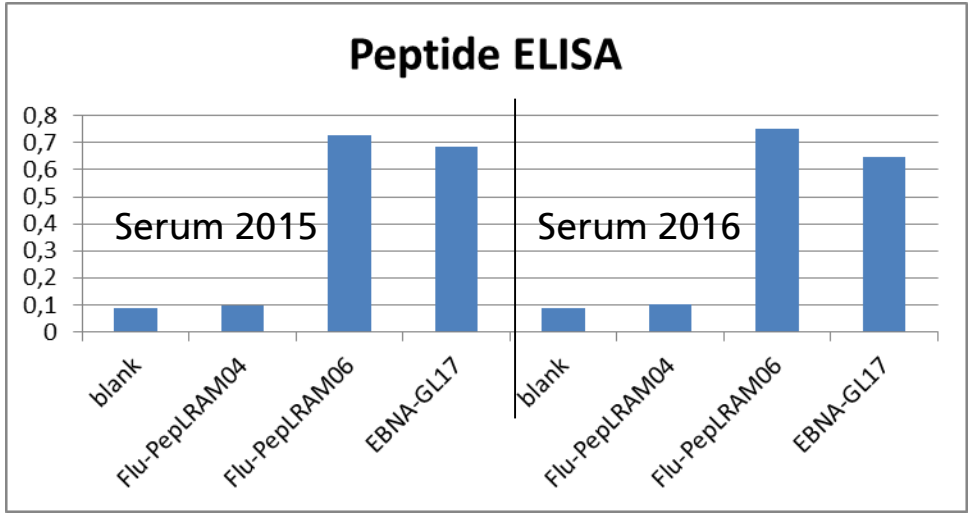
- Being present in most of the population, EBNA1 C-terminal signatures have been found in almost every serum and are useful as internal markers



Fraunhofer IZI

Verification in ELISA

We have established a new method to easily attach peptides to ELISA plates, with these or peptide arrays usually 50-80% of all peptides from in silico data show binding to antibodies.



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Fraunhofer IZI

a drop of blood

FINGERPRINTING SERUM ANTIBODY EPITOPES FOR DIAGNOSTICS

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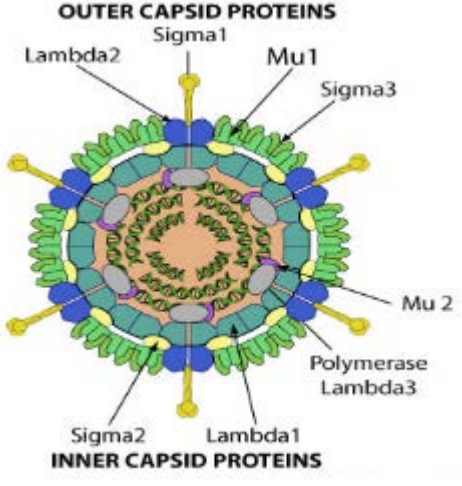
Fingerprinting Serum Antibody Epitopes for Diagnostics

Because of the broad information obtained from epitope fingerprints, it is a straight forward procedure to use epitope/mimotope peptides for immune diagnostics.

This study was carried out with sera from different mice strains infected with the Orthoreo virus, a common problem in animal facilities.



Pictures: ©ViralZone 2013, Swiss Institute of Bioinformatics; http://viralzone.expasy.org/all_by_species/105.html

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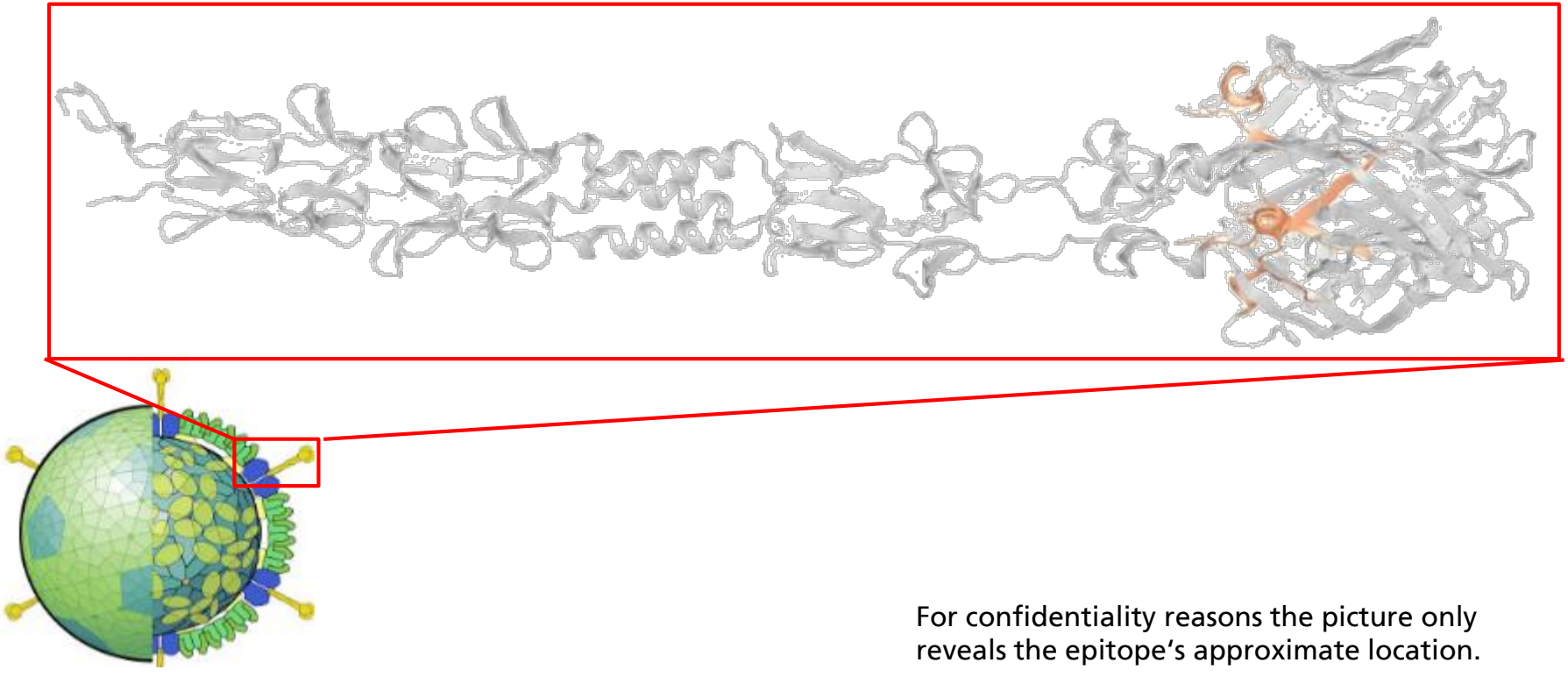
Orthoreovirus

Two main epitopes have been discovered on Sigma 1



For confidentiality reasons the picture only reveals the epitope's approximate location.

Virus Picture: ©ViralZone 2013, Swiss Institute of Bioinformatics; http://viralzone.expasy.org/all_by_species/105.html

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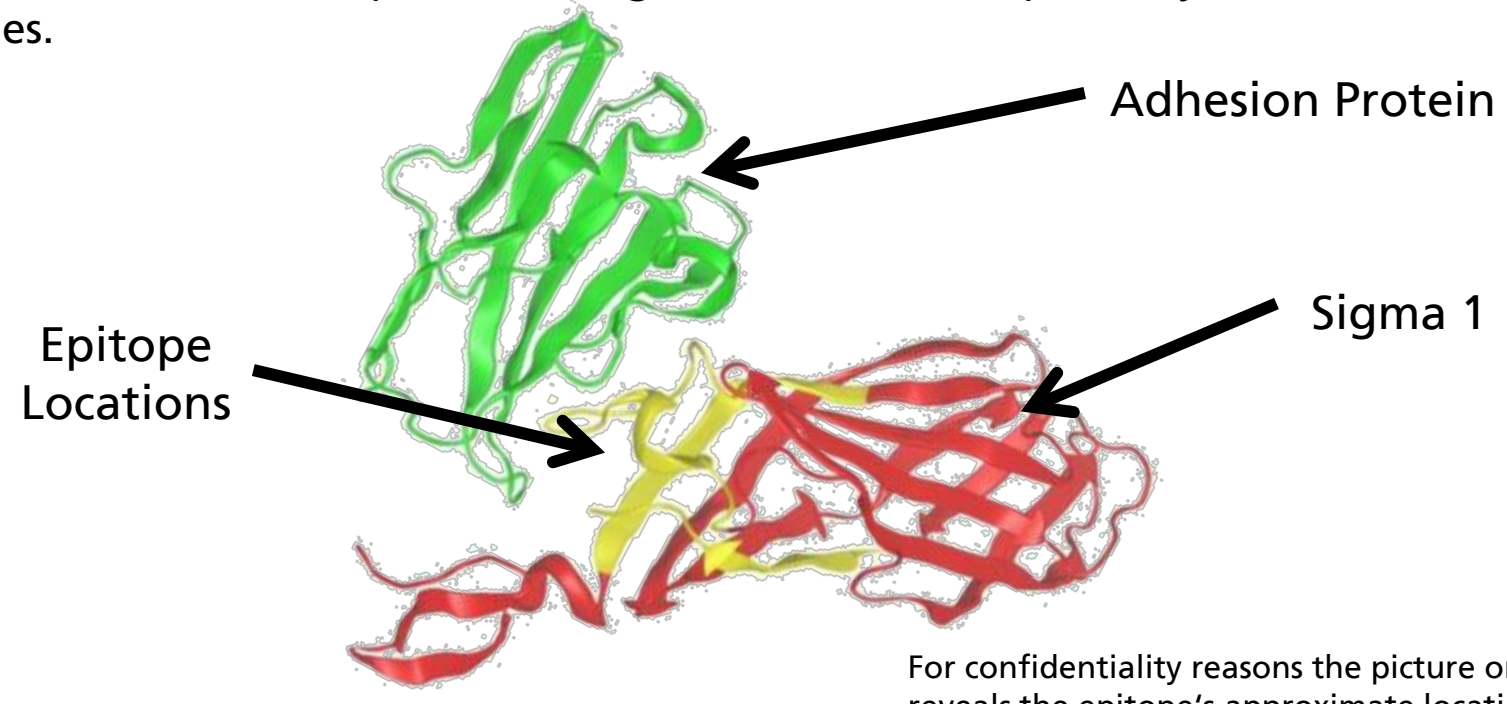
Orthoreovirus

The epitopes are located at the same site where the interaction with the cellular adhesion molecule takes place. Binding antibodies would probably have neutralizing activities.



For confidentiality reasons the picture only reveals the epitope's approximate location.

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Fraunhofer IZI

Results from Peptide Arrays

Peptides EM6 and EM8

Sera

	EM6		EM8	
	V=1:100, P=27ng, X=1,9	V=1:100, P=81ng, X=2	V=1:100, P=27ng, X=1,5	V=1:100, P=81ng, 1,8
$VL1_N$	0,92	0,80	0,66	0,70
$VL10_N$	1,24	0,99	0,69	0,72
$VL11_N$	1,05	1,10	0,89	0,73
$VL3_N$	1,78	1,91	1,22	1,20
$VL5_N$	-0,01	0,05	0,01	0,00
$VL6_N$	1,89	1,76	1,07	1,99
$VL7_N$	0,53	0,67	0,57	0,68
$VL9_N$	0,59	0,70	0,45	0,52
$VL13_P$	5,08	4,30	2,79	2,30
$VL15_P$	3,55	4,50	2,23	2,48
$VL16_P$	2,87	2,54	1,99	1,99
$VL17_P$	4,00	3,28	2,19	2,45
$VL19_P$	4,17	4,13	2,34	2,82
$VL21_P$	5,01	6,71	3,15	4,44
$VL23_P$	3,15	3,34	1,51	1,94
$VL24_P$	3,80	2,62	2,01	2,29
$VL31_P$	1,98	2,17	1,66	1,81
$VL33_P$	2,60	3,07	2,53	2,87
$VL41_P$	3,83	4,00	2,02	2,73
$VL43_P$	3,69	4,32	1,97	2,90

- 2 peptide densities on the array
- serum dilution 1:100

Signal over average of all spots

Healthy

Orthoreo Virus Infected

All peptides were synthesized without optimisation as found in the results of the epitope fingerprinting!

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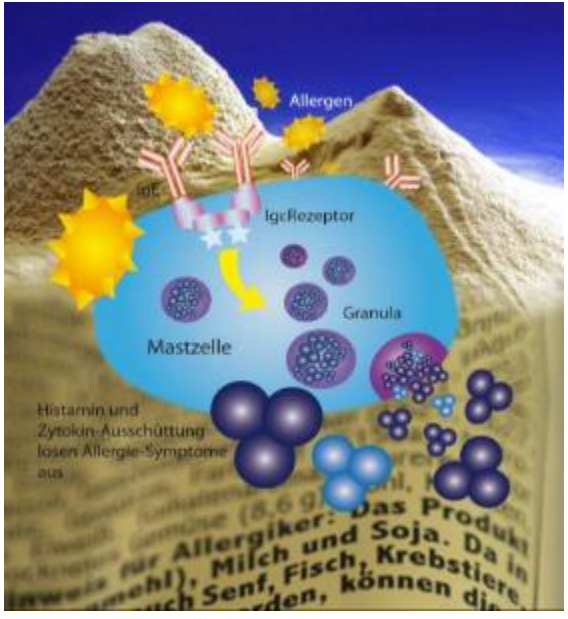
Fraunhofer IZI



„dangerous food in hostile environment“

FINGERPRINTING ANTIBODY EPITOPES IN ALLERGY

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Fraunhofer IZI

Fingerprinting Antibody Epitopes in Allergy

Identification of allergenic epitopes in soybean proteins and their application for allergy diagnostics, food analysis and generation of controlled hypo-allergenic food ingredients had been the goal of a large Fraunhofer consortium. The basis is the analysis of sera from a biobank collecting now >500 well characterized patient sera.

Despite the low abundance of IgE in serum: From 50 patient sera more than 300 epitopes identified for soy alone (partially patented) and potentially relevant of these and other food allergies were tested in peptide micro arrays.

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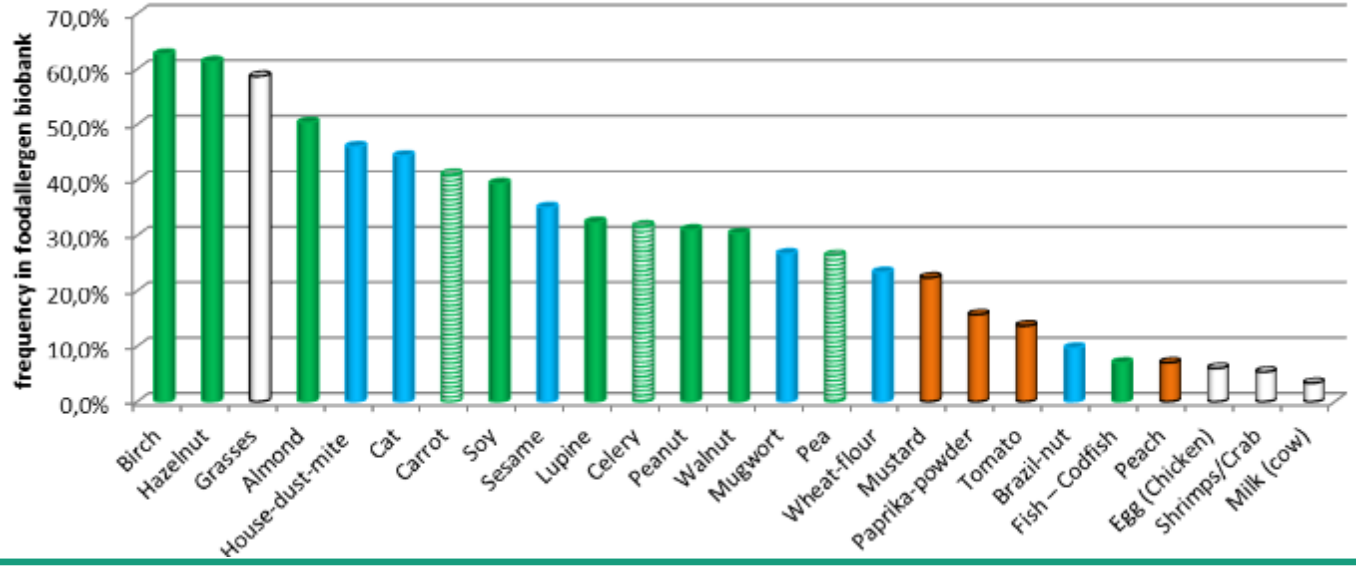
Fraunhofer IZI

Additional Product Options

- The composition of the biobank allowing the identification of diagnostic epitopes for foodallergens
- Those in green are available.
- Epitopes for additional allergens can be extracted from existing data (blue) or found by analyzing existing patient material (orange)



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Visualisation of Epitope Walking

Comparison of data from different sera allows to define the epitope spreading or epitope walking as a results of antibody maturation in different patients.

This example shows only a few mimotopes with similarity to an epitope in the allergen Gly m 5 from 12 patient sera.

RAELSEQDIFVIPAGYPVVVNA	**Gly m 5.01**
GVQRSEQDIFTEPEAHD	>S06|count=2
GEPQSEQDIFQKCEKQF	>S08|count=1
GEAHCKHSEQDICAIAN	>S06|count=4
GNLACWYNEQDIFVAHD	>S03|count=1
GAGFCYQQYLEQDIFVY	>S03|count=2
GEEFSQLHAFEQDIFSC	>S06|count=46
GTKRCFNVYPCQDIFVI	>S18|count=1
GYYPSVGHQTCQDIFVA	>S03|count=8
GIARSNQDIFVYDAHKC	>S03|count=9
GLNRCDIFVIPECELHN	>S09|count=2
GSENCDIFVIQEIQSCF	>S09|count=3
GTFHSIQQQRDIFVIVI	>S08|count=1
GSWPSLEIFVIPIFVQI	>S07|count=112
GYQWSTEHIFVIPCQRA	>S08|count=4
GLNRCDIFVIPECELHN	>S09|count=2
GIRACWQAPFVIPAGIC	>S16|count=1
GSAVCSYGFVIPAGCII	>S12|count=5
GLVSSQLTQFVIPAHCD	>S02|count=2
GGTQCLQFKVIPAGHFC	>S03|count=40
GEKHSEDPFLVIPAGGD	>S21|count=8
GTSQSEAVIPAGHHEHY	>S21|count=15
GTVVCFIWWPAGYPVDC	>S11|count=6
GLKVCHGPAGYPVCPCD	>S18|count=3
GIWGSPAGYPEECVVRD	>S12|count=3
GIQRSAGYPVFKDVDID	>S01|count=4

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Gly m 2S Albumin Map

aa sequence of Gly m 2S Albumin

Epitopes

Patient ID

Maps of all major soy allergens could be generated!

possible cross-reactivity with other proteins because of similar sequences

Epitopes of Soybean potein	Epitopes of peanut	Epitopes of Ricinus
LFCIAHTCS		LLFIIAN
SASKWQH		
QQDSCR		
SCRKQL		
KQLQGVN		
NLTPCEK	NLKPCE	
QGRGD		
EDEEEEG		
QKCCT	QRCCD	
TEMSEL		
CKALQK		
NQSEELEEK		
MCRFGP		
IQCDLS	RCDLD	

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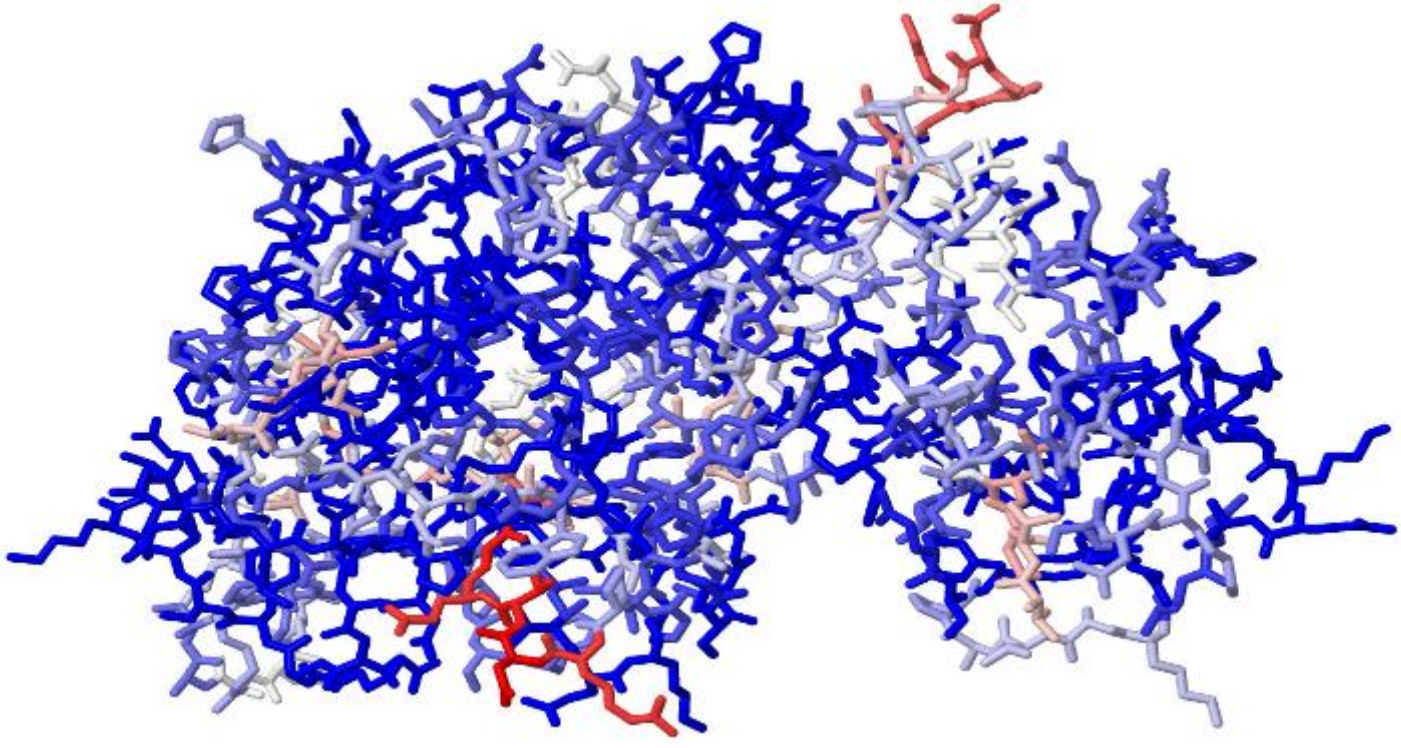
Epitope Frequency 3D-Map



Epitope frequency 3D-map of basic 7S protein from soy
(red = most frequent; blue = not found, motifs in 50 patient sera)

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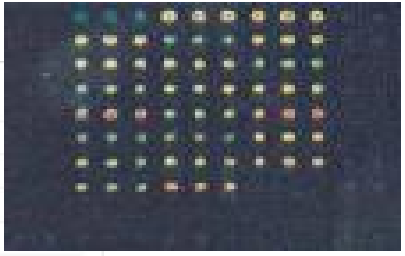
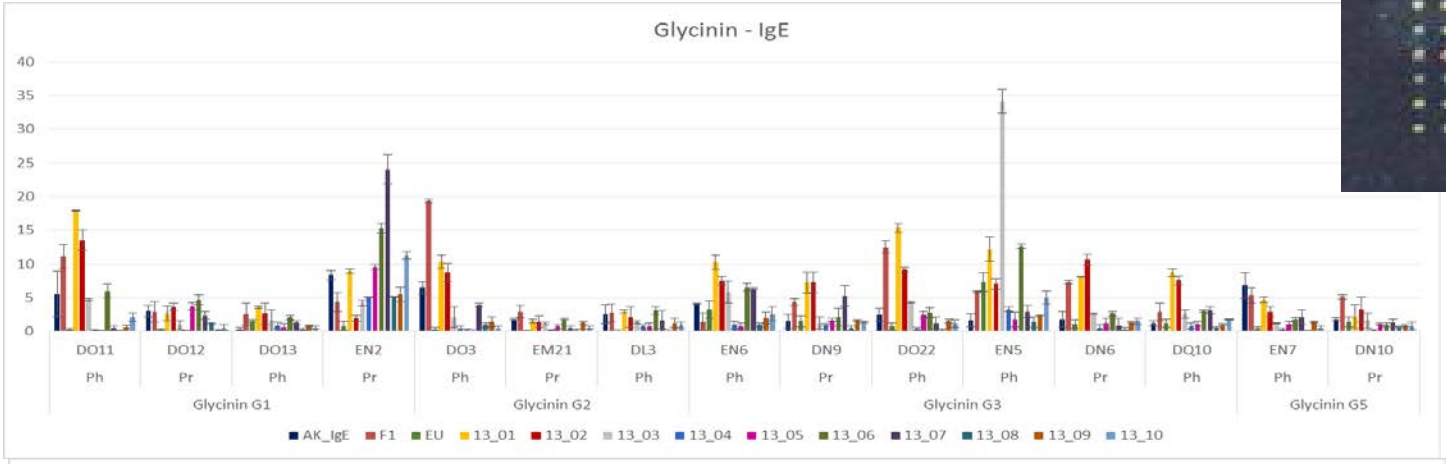
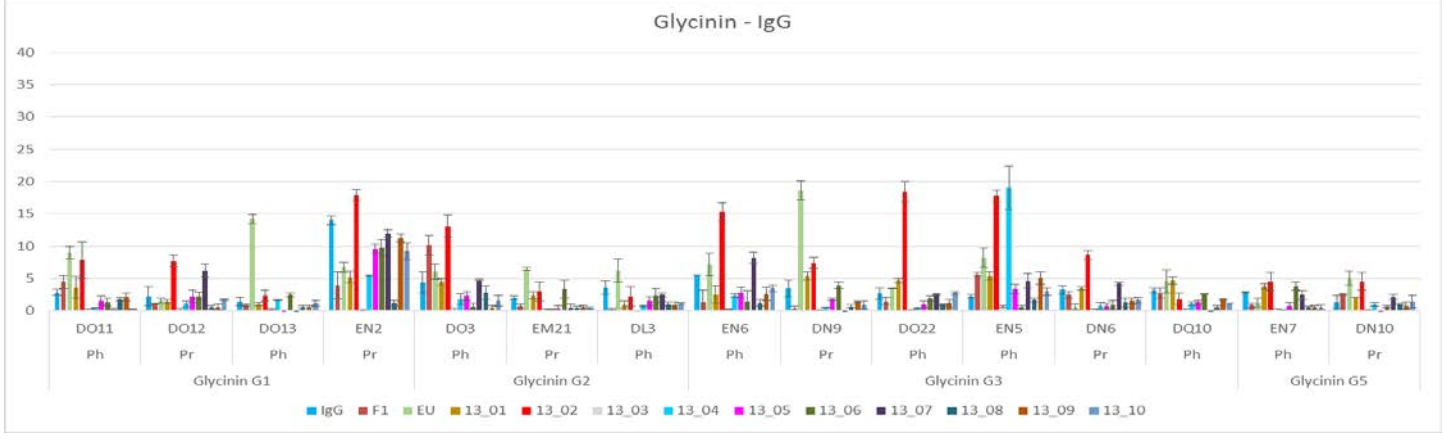
Validation in Peptide Arrays: IgE vs IgG







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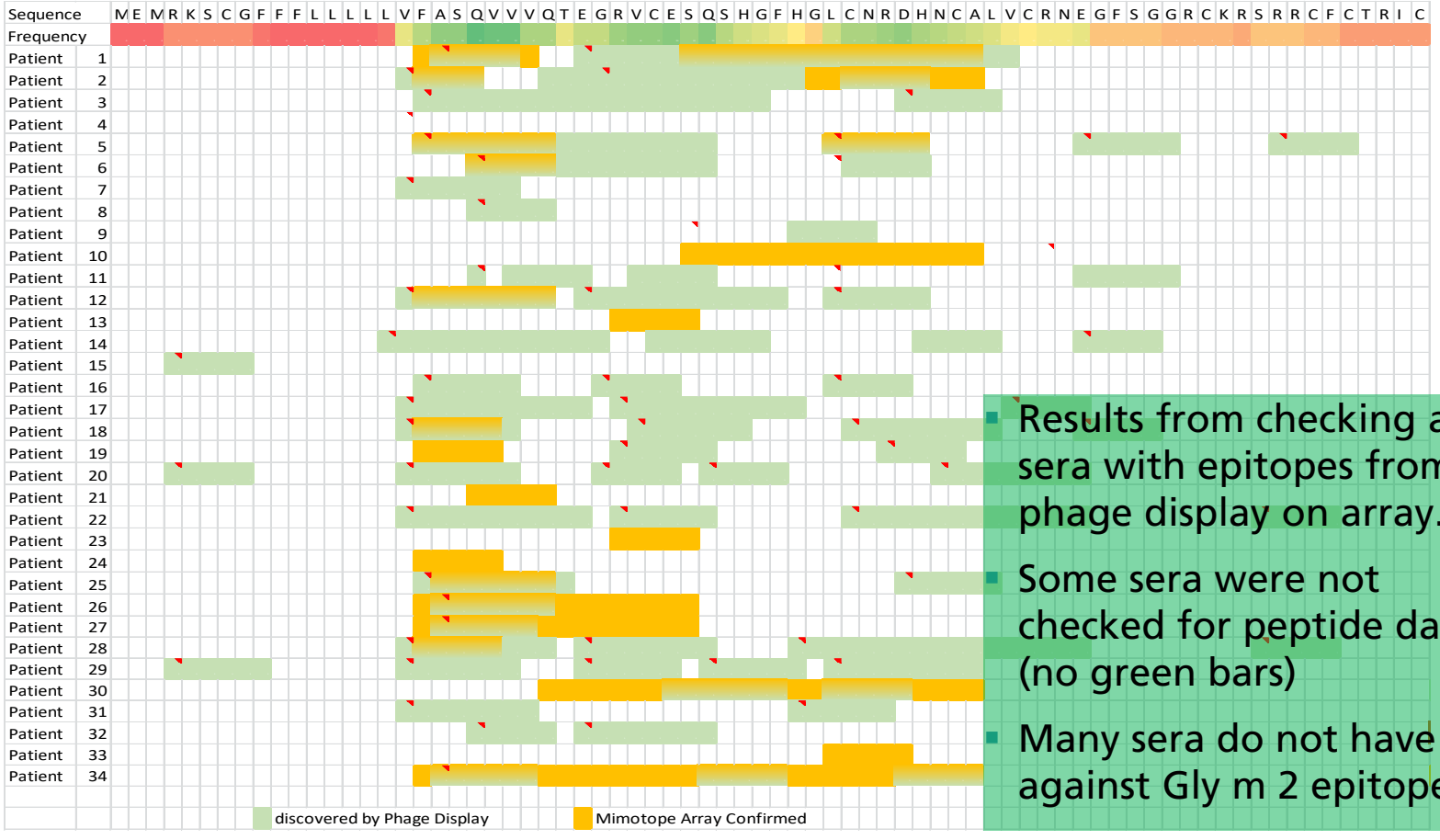
Fraunhofer IZI

IgE Epitopes of Gly m 2 – Defensin II



- Results from checking all sera with epitopes from phage display on array.
- Some sera were not checked for peptide data (no green bars)
- Many sera do not have IgE against Gly m 2 epitopes

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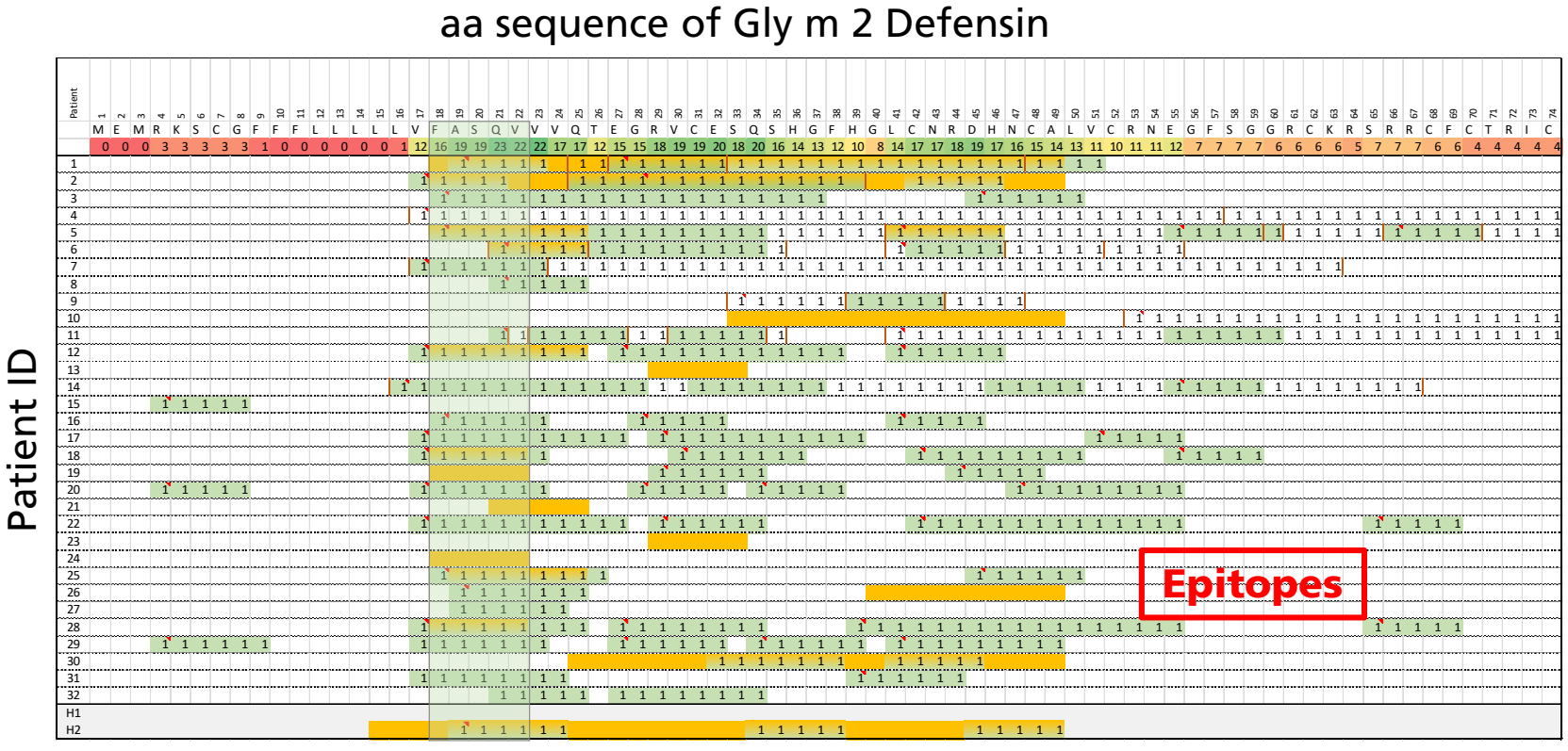
Fraunhofer IZI

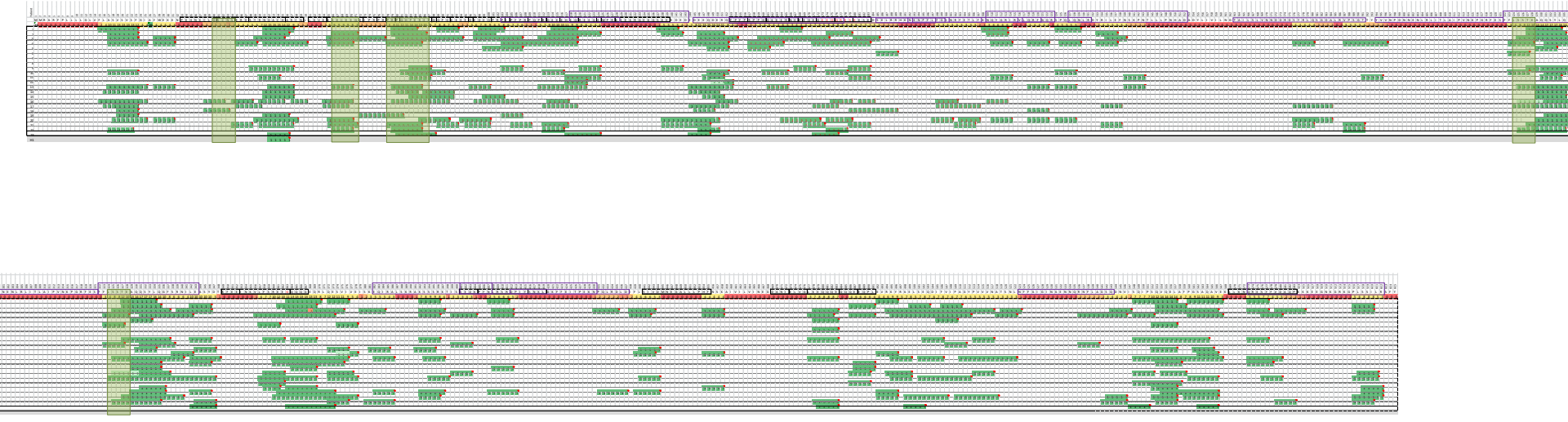
Antigenicity Maps



Fraunhofer IZI

Antigenicity Maps

aa sequence of beta-conglycinine



Peptide sequences in boxes are potentially specific and recognized by IgE!

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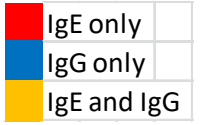
Fraunhofer IZI

Soy Allergy

Preliminary results of peptide arrays



Protein	Peptide origin	Peptide code	Sequenz	IgE-Binder	IgG-Binder	total	Purity
Gly m BD 28K	Ph	**DO4**	GYNPCR**QEEDEE**LHHKC	9	7	16	>75
	Ph	DO24	QD**QEDEE**D	5	2	7	>90
Gly m KTI	Ph	**DO5**	GTHFSKAVLG**KKNHG**DEF	10	4	14	>90
	Pr	EM22	SLA**KKNHG**LSR	3	2	5	>85
	Pr	EN1	IRFIAEGHPLSL	4	3	7	>90
Gly m 6.01	Ph	**DO11**	SD**KYQE**EFQPR	10	1	12	>85
	Pr	**EN2**	EFL**KYQQ**EQG	7	1	8	>80
Gly m 6.02	Ph	DO3	GVYNSQVDDEEEQNQRD	7	1	8	>90
Gly m 6.03	Ph	**EN6**	HVVEQEFLD	26	11	38	>90
	Pr	DN9	RRFYLAGNQEQEFL	3	2	6	>90
	Ph	**EN5**	ISQQDRHRI	28	13	42	>80
Gly m BD 30K	Ph	**DO17**	GEYSCDITYT	34	16	50	>60
	Pr	EL13	GYGSADGVDYW	5	3	8	>85
	Pr	**DO15**	YHNHEEEAKR	23	7	30	>75
	Ph	**DO7**	GEFS**KKYL**QAP	17	2	19	>90
	Ph	**DO6**	GPVQ**KKYL**FDS	11	5	16	>90
	Pr	**EN3**	VYHNHEEEAK	13	17	30	>90
Gly m 8	Pr	DO20	KCQCKALQKI	4	0	4	>85
Gly m 5.01	Pr	EM18	PRPRPQHPERE	23	5	28	>90
	Ph	**DO19**	SHHDQPRQHA	12	6	18	>70
	Ph	DN5	NIELVGLKEQQQRQQQ	5	3	8	>90
	Ph	EM20	AIN**QSCNS**HPI	9	3	12	>90
	Pr	DN7	**QSCNS**ERDSYRNQ	3	0	3	>85
	Ph	**EM15**	HITF**SREEG**SI	12	2	14	>85
	Pr	**EM14**	KVLF**SREEG**QQQ	21	9	31	>90
	Ph	DO21	FFLSSTEAQ	9	2	11	>90
Gly m 5.02	Pr	**EN17**	VEEEEECEEGQI	32	17	50	>90
Gly m 2	Ph	EN15	SANQVVVQTHV	3	4	7	>90
	Ph	EN16	EVSFASQVLIY	6	3	9	
Gly m agglutii	Pr	DN11	HAAANVVGNKESQREAR	8	1	9	>75
PR5	Ph	DK3	GVINCETFDQYQPIEIGC	8	2	10	>90
Beta Amylase	Pr	EN9	PGIGDFQSYD	4	0	4	>85

Fraunhofer IZI

Soy Allergy

Preliminary results of peptide arrays with native sequences (Pr) and mimotopes from phage display (Ph)

Protein	Peptide origin	Peptide code	Sequenz	IgE-Binder	IgG-Binder	total	Purity	13_03	13_07	13_11	13_12	13_13	13_16	13_23	13_24
Gly m BD 28K	Ph	**DO4**	GYNPCR**QEEDEE**LHHKC	9	7	16	>75								
	Ph	DO24	QD**QEEDEE**D	5	2	7	>90								
Gly m KTI	Ph	**DO5**	GTHFSKAVLG**KKNHG**DEF	10	4	14	>90								
	Pr	EM22	SLA**KKNHG**LSR	3	2	5	>85								
	Pr	EN1	IRFIAEGHPLSL	4	3	7	>90								
Gly m 6.01	Ph	**DO11**	SD**KYQE**EFQPR	10	1	12	>85								
	Pr	**EN2**	EFL**KYQQ**EQG	7	1	8	>80								
Gly m 6.02	Ph	DO3	GVYNSQVDDEEEQNQRD	7	1	8	>90								

IgE only
IgG only
IgE and IgG

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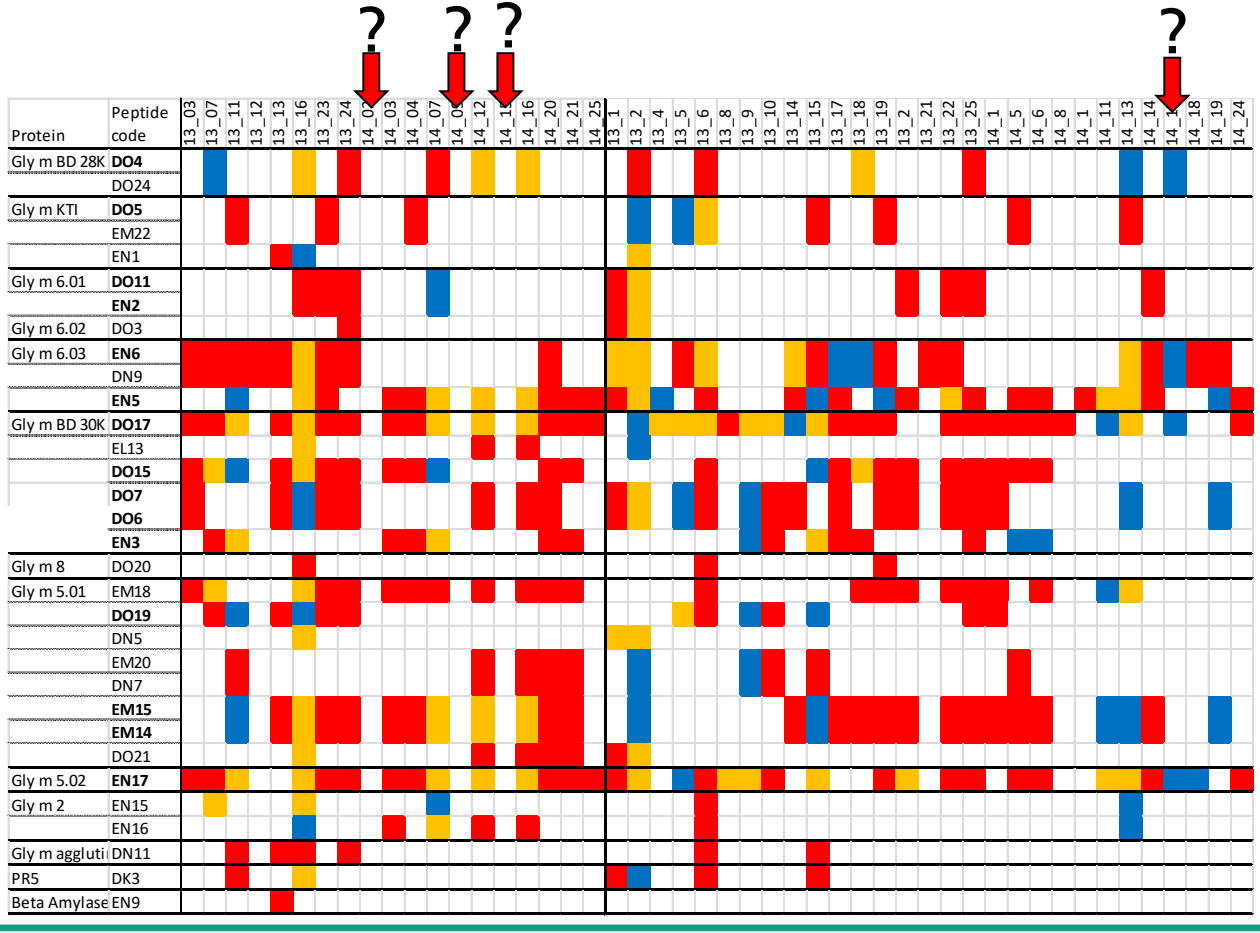
Fraunhofer IZI

Soy Allergy – strange patients



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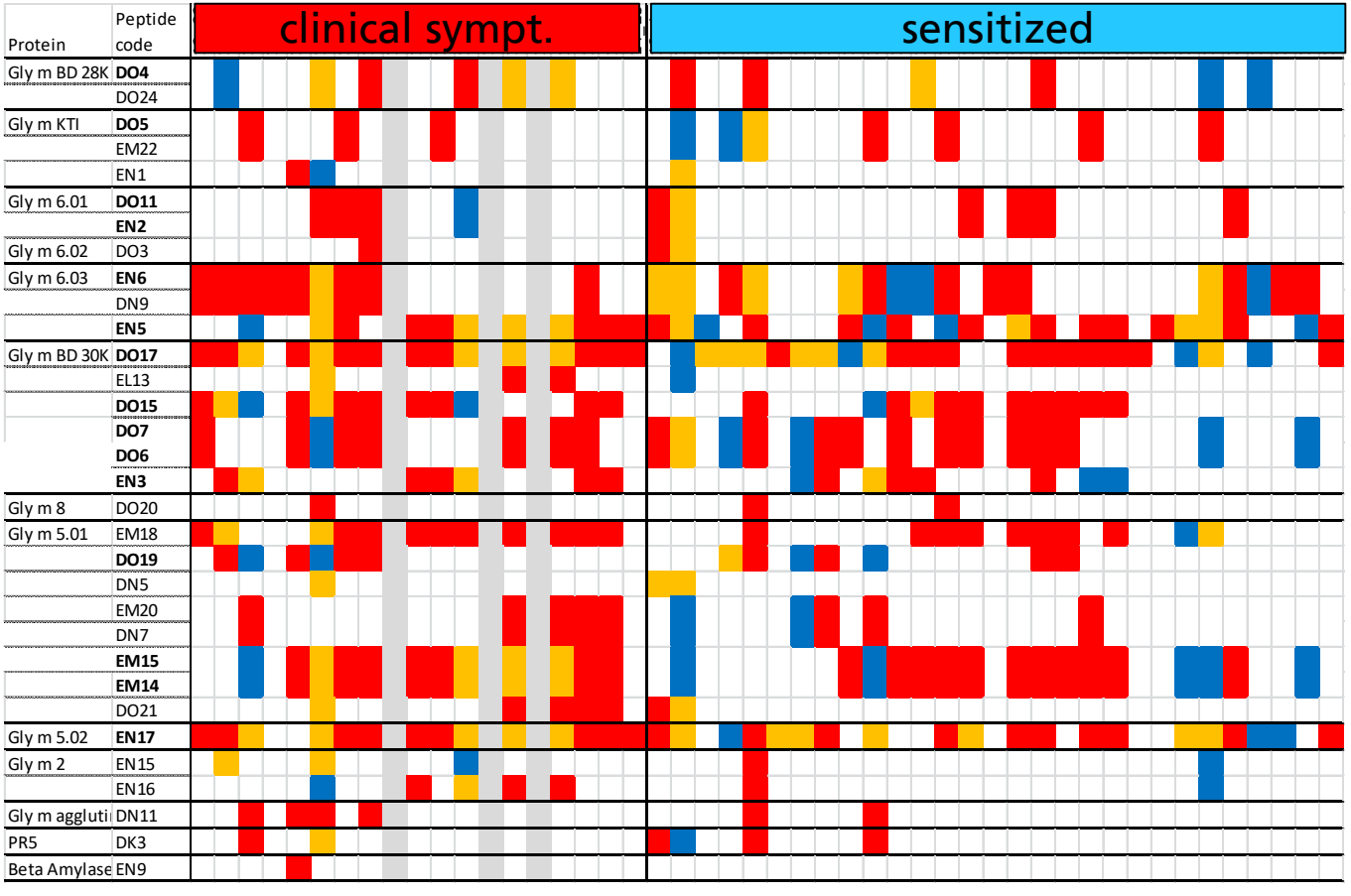
Fraunhofer IZI

Soy Allergy – by patient's symptoms



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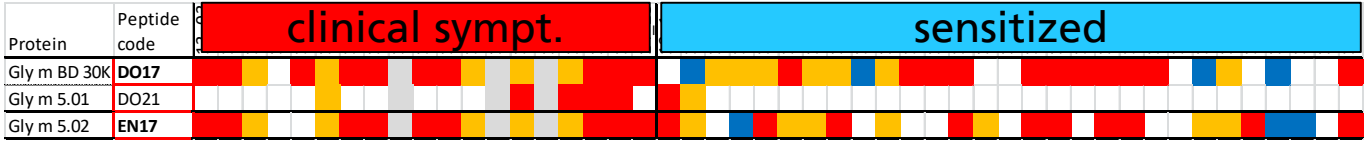
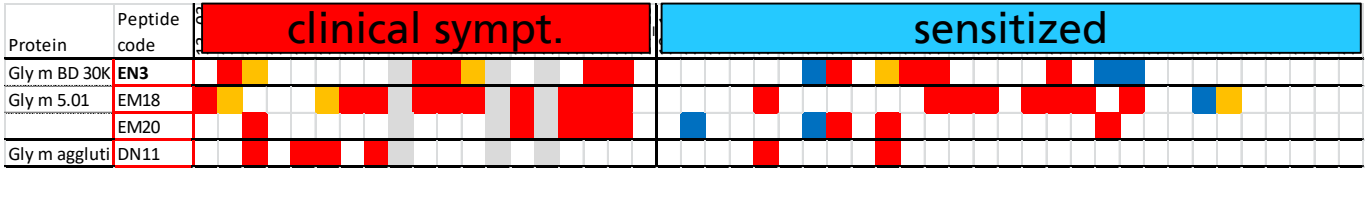
Fraunhofer IZI

Soy Allergy

Could this replace a prick test?



Could this replace a food challenge?



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Fraunhofer IZI

Nut Allergies

At present our biobank contains more than 300 sera. The frequency of sensitization to food allergens allows interesting insights into potential cross reactivities.

- Sensitivities against a single allergen from the family of legumes (soy, pea, lupine) are rare. Most persons recognized at least two!
- Tree nuts are long known for cross reactivities. Only 22 out of 105 sera with nut sensitisation tested so far with arrays reacted to hazelnut alone

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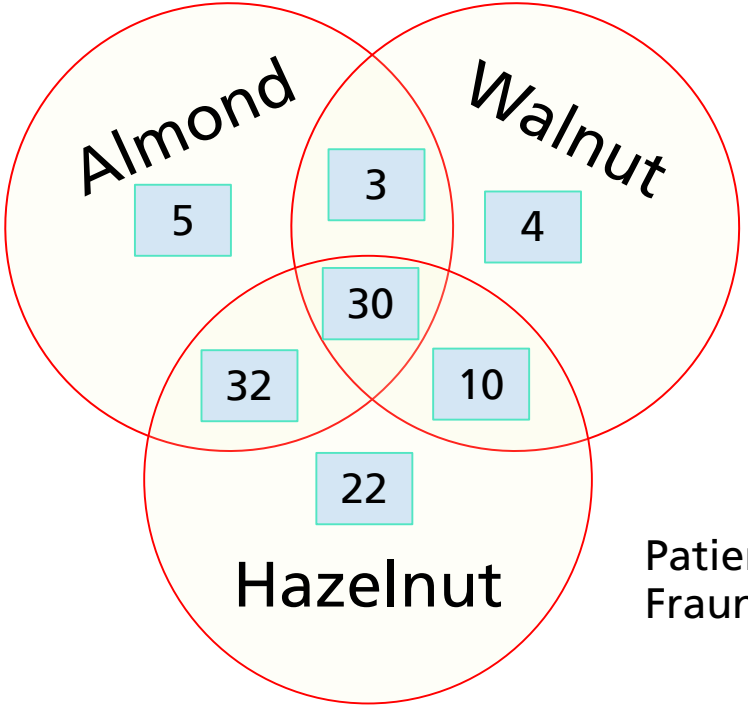
Is there a hazelnut allergy?



Patient sensibilisation to tree nuts in Fraunhofer IZI Biobank (April 2019)

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Fraunhofer IZI

Peptide Epitopes Tracking Nut Allergies

Tests with patient sera revealed several peptide epitopes with higher specificity to certain combinations of tree nut allergies, **„hazelnut“ ≠ „hazelnut“ !**

Number of sera	30	10	22	32	3	5	4	28
Allergen:	+ hazelnut + almond	+ hazelnut - almond	+ hazelnut - almond	+ hazelnut + almond	- hazelnut + almond	- hazelnut + almond	- hazelnut - almond	- hazelnut - almond
Peptide	+ walnut	+ walnut	- walnut	- walnut	+ walnut	- walnut	+ walnut	- walnut
FX18	44%	40%	41%	29%	33%	0%	25%	18%
HL17	17%	20%	27%	19%	67%	20%	0%	14%
GL1	20%	20%	36%	25%	67%	20%	25%	11%
GL3	10%	30%	9%	19%	67%	40%	0%	4%
DO22	10%	0%	14%	3%	67%	20%	75%	7%
DO2	14%	20%	14%	3%	67%	0%	25%	11%
EN10	10%	20%	9%	0%	67%	20%	25%	4%
FH22	3%	10%	5%	0%	67%	20%	25%	0%
EN6	3%	0%	14%	0%	33%	20%	25%	0%
EO12	7%	0%	5%	0%	67%	20%	0%	7%
ID17	14%	10%	18%	6%	0%	0%	0%	4%

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Fraunhofer IZI

Peptide Epitopes Tracking Nut Allergies

Selections of peptide pairs with low background and relatively high specific IgE binding show: The „only-hazelnut“ and e.g. the „hazelnut-almond“ sensitized serum are different.

Number of sera		30	10	22	32	3	5	4	28
Allergen:		+ hazelnut + almond + walnut	+ hazelnut - almond + walnut	+ hazelnut - almond - walnut	+ hazelnut + almond - walnut	- hazelnut + almond + walnut	- hazelnut + almond - walnut	- hazelnut - almond + walnut	- hazelnut - almond - walnut
Peptidcombin.									
FX18	DO22	51%	40%	45%	32%	67%	20%	100%	18%
FX18	DO2	54%	60%	45%	32%	67%	0%	50%	18%
HL17	GL3	27%	40%	27%	32%	67%	60%	0%	14%
GL3	EN10	20%	50%	18%	19%	100%	60%	25%	7%
GL3	FH22	14%	40%	14%	19%	100%	60%	25%	4%
GL3	EN6	14%	30%	23%	19%	67%	60%	25%	4%
GL3	EO12	17%	30%	14%	19%	100%	60%	0%	11%
GL1	ID17	34%	30%	55%	32%	67%	20%	25%	14%
GL1	GL3	24%	30%	41%	29%	67%	60%	25%	11%

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Conclusions

Peptide phage display in this novel set up can indeed be used to reliably identify epitopes of serum antibodies.

The observed peptides can be used to rapidly generate immuno diagnostic arrays for screening sera and to characterize individual immune reactions.

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TUMOR AND TISSUE BINDING PEPTIDES

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Peptide Fingerprints of Tissues

- Starting in late 2012 we have collected tumor and healthy tissue binding peptide phage.
- By now we have collected binding phage from more than 85 sample pairs of different tumors in collaboration with the CNUHH, a very large cancer hospital in South Korea, and partners in Heidelberg and Leipzig:
    - Colon / Colorectal
    - Stomach
    - Brain
    - Liver
    - Lung
    - Myeloma
    - Head and neck cancer
- These have been sequenced with NGS and yielded millions of sequences
- Analyses of the data has led with a very high success rate to the identification of several tumor and tissue binding peptide motifs.

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Peptide Fingerprints of Tissues – Example RGD Motif

- RGD peptides are known to bind integrins, in particular on tumor tissues and other rapidly growing cells
- The RGD amino acids alone can bind integrin expressing cells, but natural integrin ligands have additional amino acids influencing selectivity and affinity
- Can statistical analysis of a large number of sequences help in identifying optimal candidates?

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Peptide Fingerprints of Tissues

Motif analysis: Amino acids adjacent to RGD motifs are different in tumor and healthy tissue, the naive library's statistics are as expected from the original set up.

Lung

	A5	A4	A3	A2	A1	M	B1	B2	B3	B4	B5
C	12	53	52	75	75	RGD	98	39	62	54	10
P	53	31	74	55	37	RGD	37	18	16	43	3
G	15	37	36	34	20	RGD	24	5	28	18	4
A	32	44	27	26	24	RGD	18	42	22	44	5
V	77	78	31	79	94	RGD	46	30	16	14	22
I	40	35	33	38	0	RGD	47	22	34	36	28
L	38	27	12	35	60	RGD	12	12	25	9	27
M	15	14	3	0	0	RGD	0	0	0	0	0
F	28	46	46	51	35	RGD	29	72	34	17	7
Y	46	48	40	37	18	RGD	66	50	70	44	34
W	26	68	65	31	29	RGD	5	1	7	0	0
T	22	34	62	49	50	RGD	31	26	16	1	10
S	37	46	61	62	38	RGD	26	21	24	8	21
N	57	54	46	60	15	RGD	23	31	19	16	1
Q	82	45	118	56	60	RGD	41	62	29	29	25
R	31	20	40	25	29	RGD	10	26	6	7	9
K	47	62	24	28	41	RGD	29	3	17	5	9
H	51	69	32	50	31	RGD	40	33	34	14	17
E	33	36	40	30	57	RGD	31	27	44	18	19
D	60	11	16	26	45	RGD	23	10	24	37	4

Lung Cancer

	A5	A4	A3	A2	A1	M	B1	B2	B3	B4	B5
C	11	87	104	92	106	RGD	69	75	76	53	24
P	68	85	43	52	42	RGD	48	33	33	58	29
G	35	71	66	79	75	RGD	38	29	20	16	27
A	41	61	24	26	44	RGD	24	24	20	22	9
V	112	123	83	140	60	RGD	71	63	21	24	13
I	50	47	90	44	0	RGD	31	39	39	35	12
L	60	49	21	50	57	RGD	26	13	10	5	21
M	12	9	0	8	0	RGD	0	0	0	0	0
F	47	66	29	49	86	RGD	52	72	32	32	31
Y	118	34	75	94	93	RGD	43	55	94	51	19
W	45	57	97	26	44	RGD	11	9	27	21	0
T	36	39	51	114	37	RGD	30	23	19	7	13
S	31	70	94	66	67	RGD	31	33	15	9	10
N	101	48	41	60	51	RGD	35	40	32	32	3
Q	74	110	159	97	116	RGD	44	59	51	37	47
R	71	28	81	45	41	RGD	65	30	21	16	26
K	40	69	35	32	51	RGD	73	7	41	0	16
H	66	68	60	85	74	RGD	70	52	32	54	47
E	55	76	59	33	73	RGD	52	43	34	26	16
D	78	35	20	50	56	RGD	12	45	32	24	13

Starting Library

	A5	A4	A3	A2	A1	M	B1	B2	B3	B4	B5
C	12	53	62	49	44	RGD	42	44	47	39	20
P	35	30	32	32	22	RGD	17	26	29	15	15
G	25	18	39	21	26	RGD	14	26	13	9	7
A	24	18	14	31	27	RGD	5	26	18	9	17
V	41	42	40	52	43	RGD	46	35	25	21	21
I	34	34	27	18	29	RGD	26	28	32	30	15
L	27	16	20	17	30	RGD	29	15	13	6	9
M	6	5	4	5	0	RGD	0	0	0	0	0
F	33	22	20	31	28	RGD	22	23	39	27	13
Y	45	24	33	37	36	RGD	41	38	52	46	21
W	22	33	34	13	20	RGD	15	7	6	7	0
T	34	35	20	29	31	RGD	10	19	9	16	16
S	18	58	47	58	55	RGD	27	28	15	12	12
N	37	38	33	36	25	RGD	14	13	20	21	6
Q	43	47	55	35	41	RGD	62	39	40	31	23
R	25	31	20	33	16	RGD	24	21	20	15	12
K	18	21	21	26	25	RGD	18	26	21	19	10
H	39	49	33	42	55	RGD	41	32	24	18	29
E	35	41	49	43	50	RGD	39	32	18	21	20
D	24	18	30	25	30	RGD	10	13	24	30	11

similiar

dissimiliar

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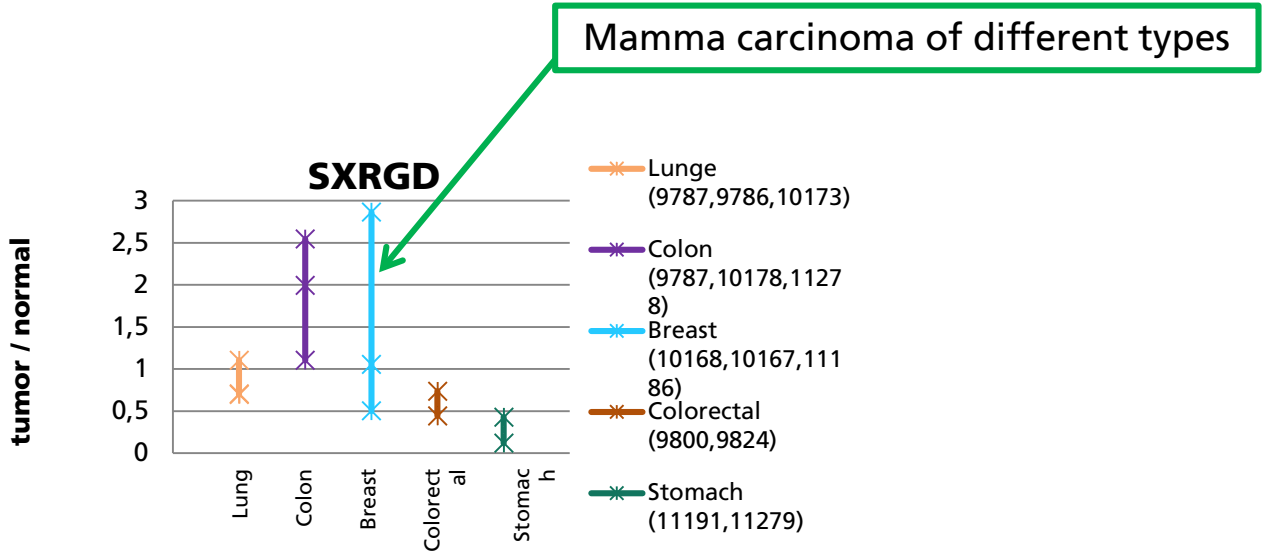
Next Step: sorting out tissue specific motifs



Relative frequency of a motif in a individual patient:

We ask whether this motif is more frequently occuring than other RGD sequences in the tumor tissue than in the surrounding tissue

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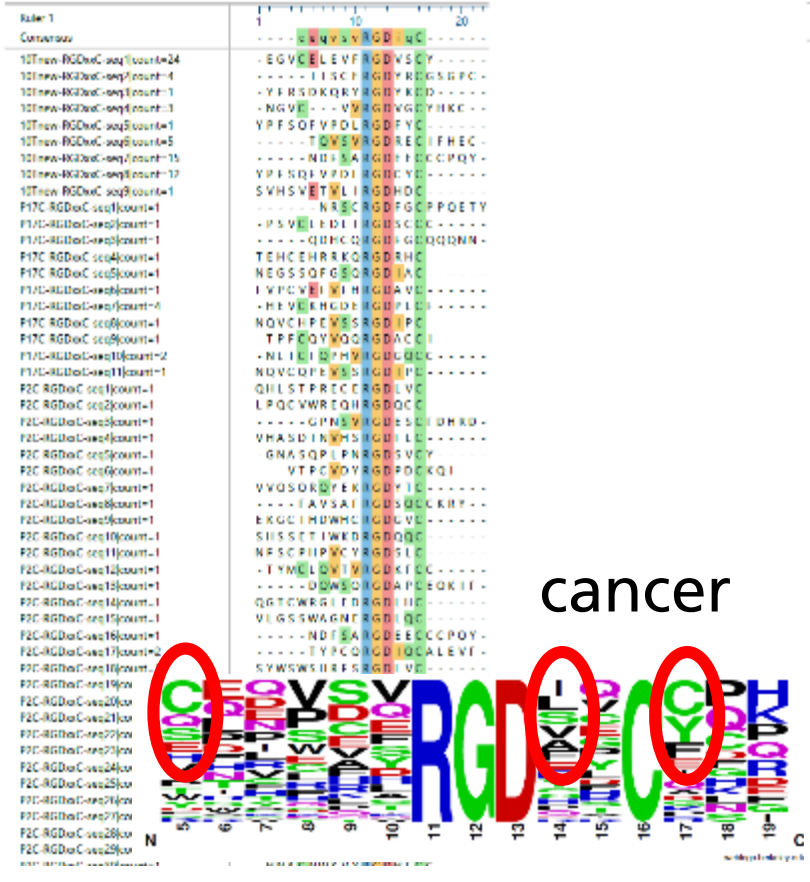
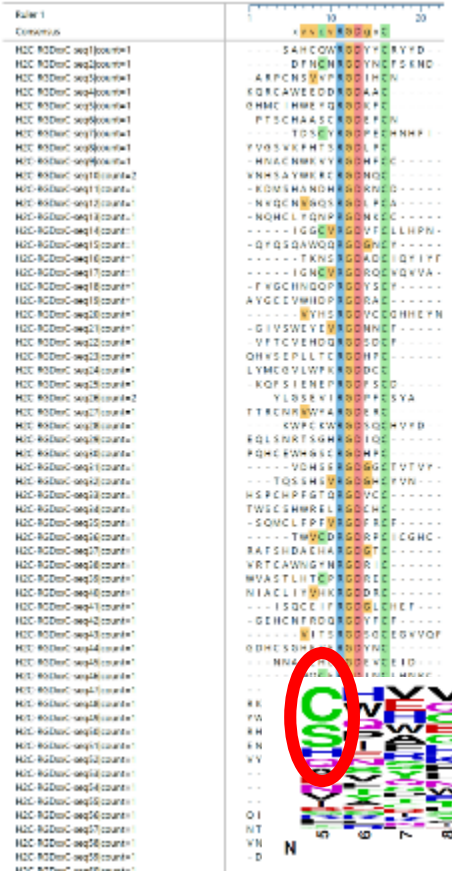
Fraunhofer IZI

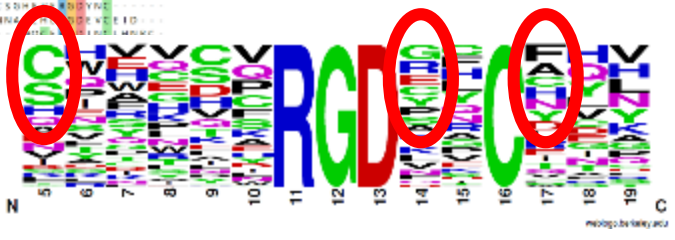
Alignment: Example Colon Cancer / RGD**C



cancer



healthy



Alignment of sequences binding colon cancer containing RGDxxC motif

(Only a fraction of all sequences shown)

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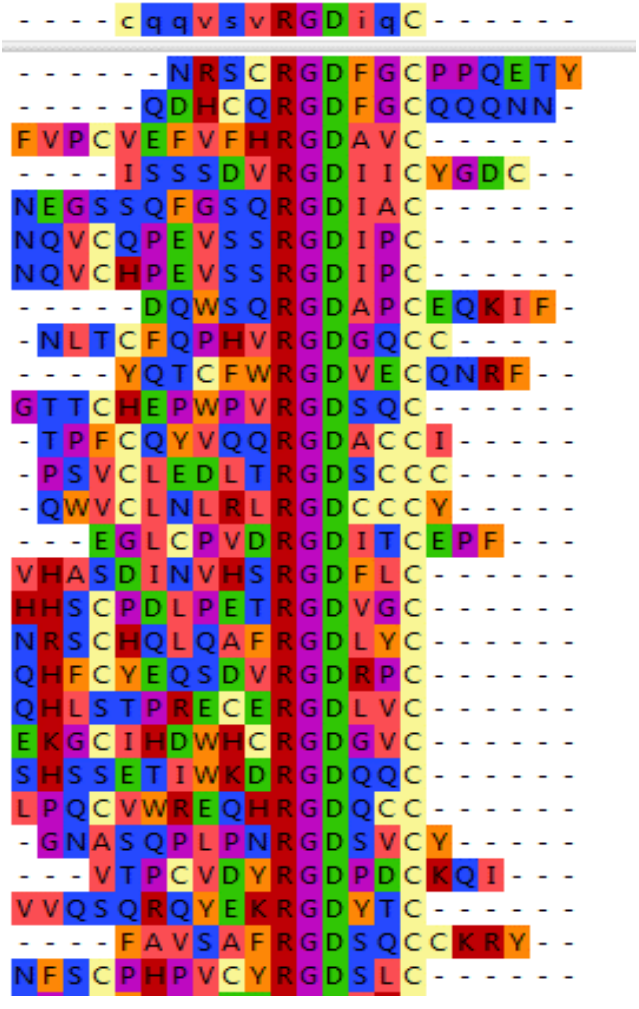
Fraunhofer IZI

Next Step: Aligment

Retrieve all sequences with the motif from datasets with similar tissues. Presented are only a few sequences!



Alignment of sequences binding colon cancer containing RGDxxC motif

(Only a fraction of all sequences shown)

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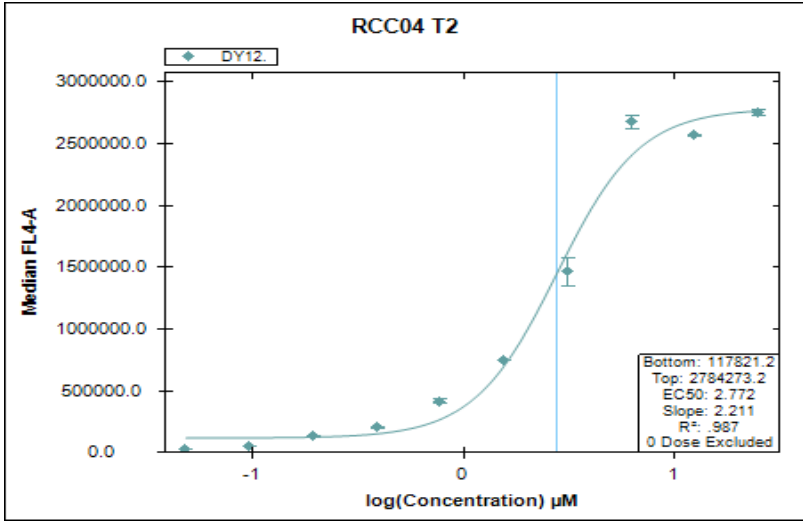
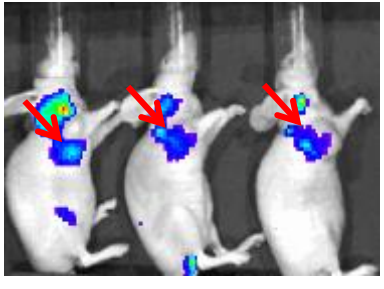
Application of Tumor Binding Peptides

- FACS analysis of fluorescent dye labelled tumor cell binding peptides



- These peptides work even *in vivo* in mice
  - HT29 xenograft



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Fraunhofer IZI

SYSTEM FOR TARGETED DELIVERY AND CONTROLLED DRUG RELEASE

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Fraunhofer IZI

System for Targeted Delivery and Controlled Drug Release

A novel targeted drug delivery and release system has been developed and successfully tested in a xenograft tumor model.

The system consists of **nanoparticles** equipped with **tumor targeting peptides**. These particles are designed to carry drugs that can be released by **local application of an external trigger**. A first proof-of-concept *in vivo* study for a tumor targeting nanosystem was successfully finished. A patient derived xenograft tumor model in the mouse was used. Initial results show no side effects but a significant inhibition of tumor growth. Furthermore the delivery and release system is more efficient than the standard therapy with the non-encapsulated drug.

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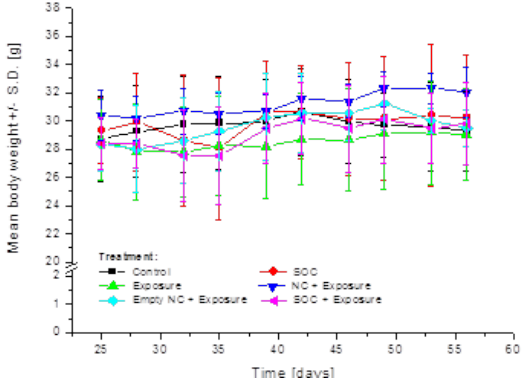
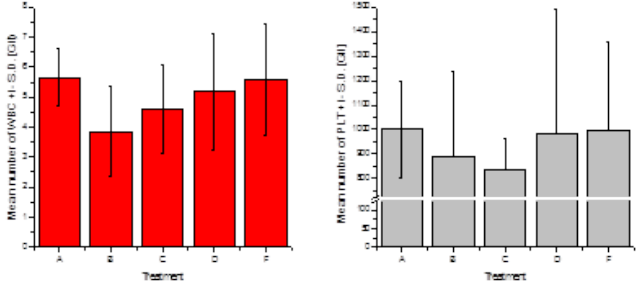
No side effects due to targeted drug delivery and release



Impact of a combined treatment on body weight. REN11619 bearing mice were treated at day 0, 2 and 4 after stratification and body weight was measured at indicated days.



Impact of a combined treatment on blood composition. Mice were treated at day 0, 2 and 4 after stratification and blood samples were taken at day 36 and analyzed for composition. Treatments: (A) Saline, (B) SOC, (C) Exposure, (D) NC+Exposure, (F) SOC + Exposure

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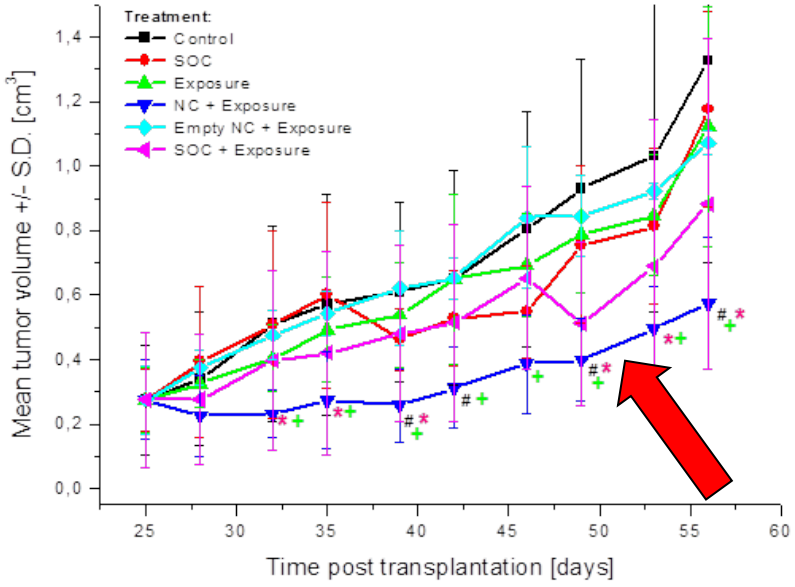
Proof of concept: Inhibition of tumor growth

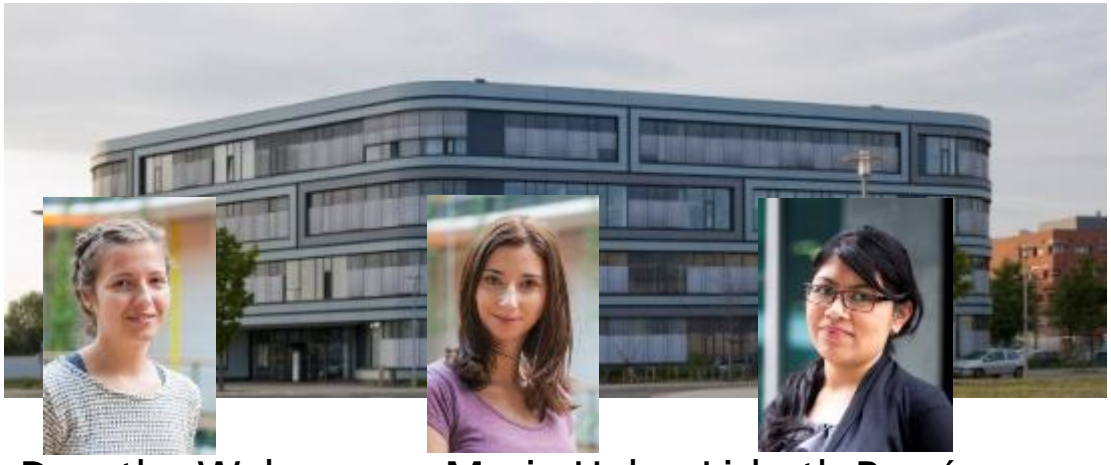


Xenograft tumor model treated with nanocarrier for targeted drug delivery and triggered drug release.

Renal PDX REN11619 bearing mice were treated at day 0, 2 and 4 after stratification with the nanocarrier, followed by trigger exposure 2 h post treatment.
#: significantly different to control; *: significantly different to SOC, +: significantly different to Exposure. Mann-Whitney nonparametric U-test, $p<0.05$.

Thanks to….





Nicolas Delaroque, Karolin Kern, Dorothe Wehrmann, Maria Helm, Lisbeth Ramírez Caballero, Aastha Jain, Markus Puder and many more…
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