

# High-resolution mapping of linear antibody epitopes using ultrahigh-density peptide microarrays

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# High-resolution Mapping of Linear Antibody Epitopes Using Ultrahigh-density Peptide Microarrays\*<sup>§</sup>

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# Antibody utility

Antibodies empowers numerous applications

- Scientific
- Clinical
- Diagnostic
- Industrial

Based on an extremely diverse repertoire of B cells each producing a unique antibody with a characteristic specificity

# Antibody specificity

Specificity is the essential characteristics

- What does it recognize?
- What does it not recognize?
- Any unintended cross-reactions?
- Any unwanted auto-reactions?

How do you identify, characterize and validate antibody epitopes?

# Proteins are important targets

Protein epitopes are traditionally divided into

- Conformational / discontinuous epitopes
- Linear / continuous epitopes

# Traditional B cell epitope discovery

Low throughput approaches aimed at identifying the

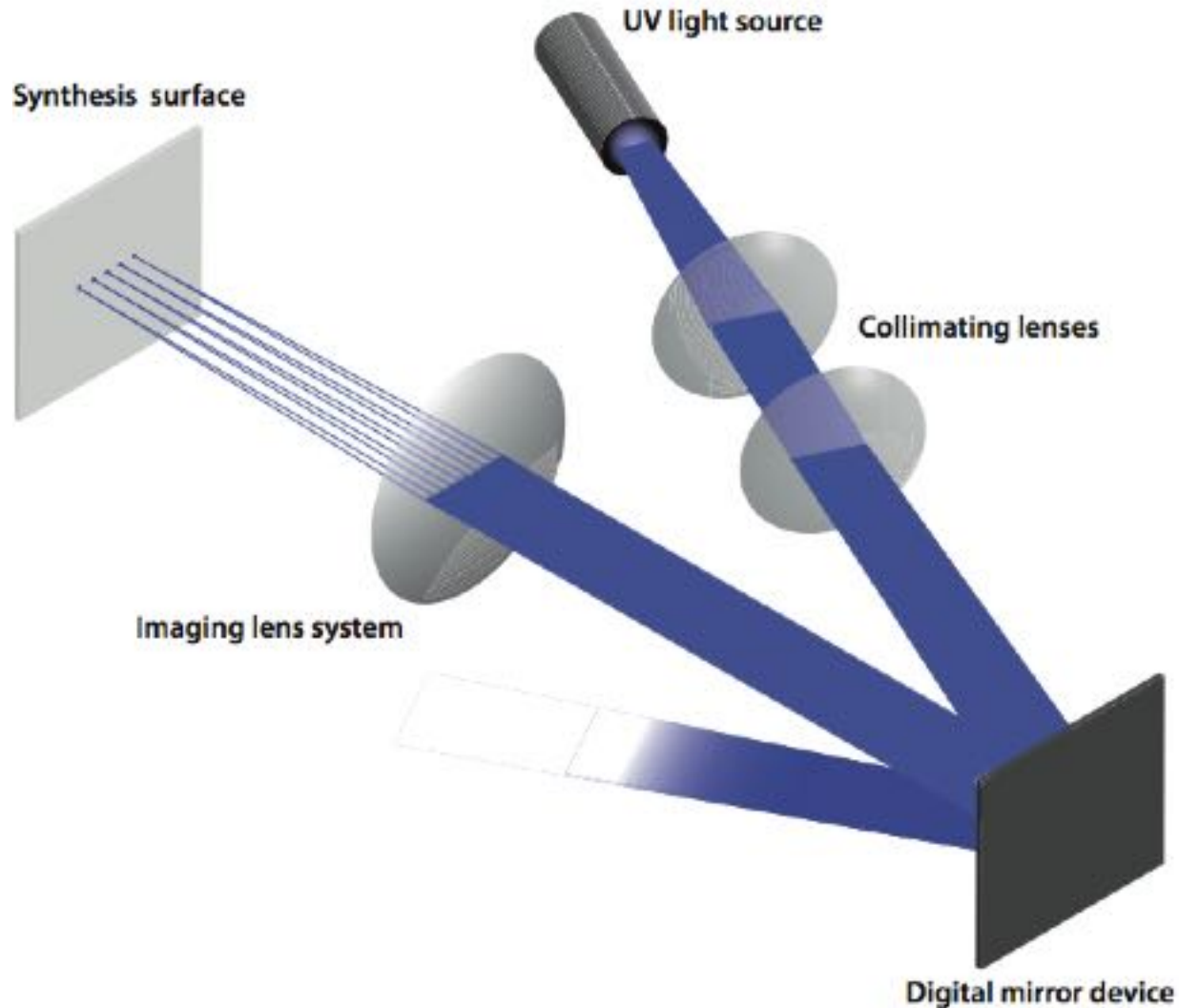
- nature and identity of the antigen (e.g. protein)
- detect interactions by RIA, ELISA, Western, immunohistochemistry, flow cytometry, SPR etc
- define molecular structure (mutational analysis, “fingerprint”, X-ray)

# Proteomics-based B cell epitope discovery

High-throughput approaches aimed at identifying epitopes through

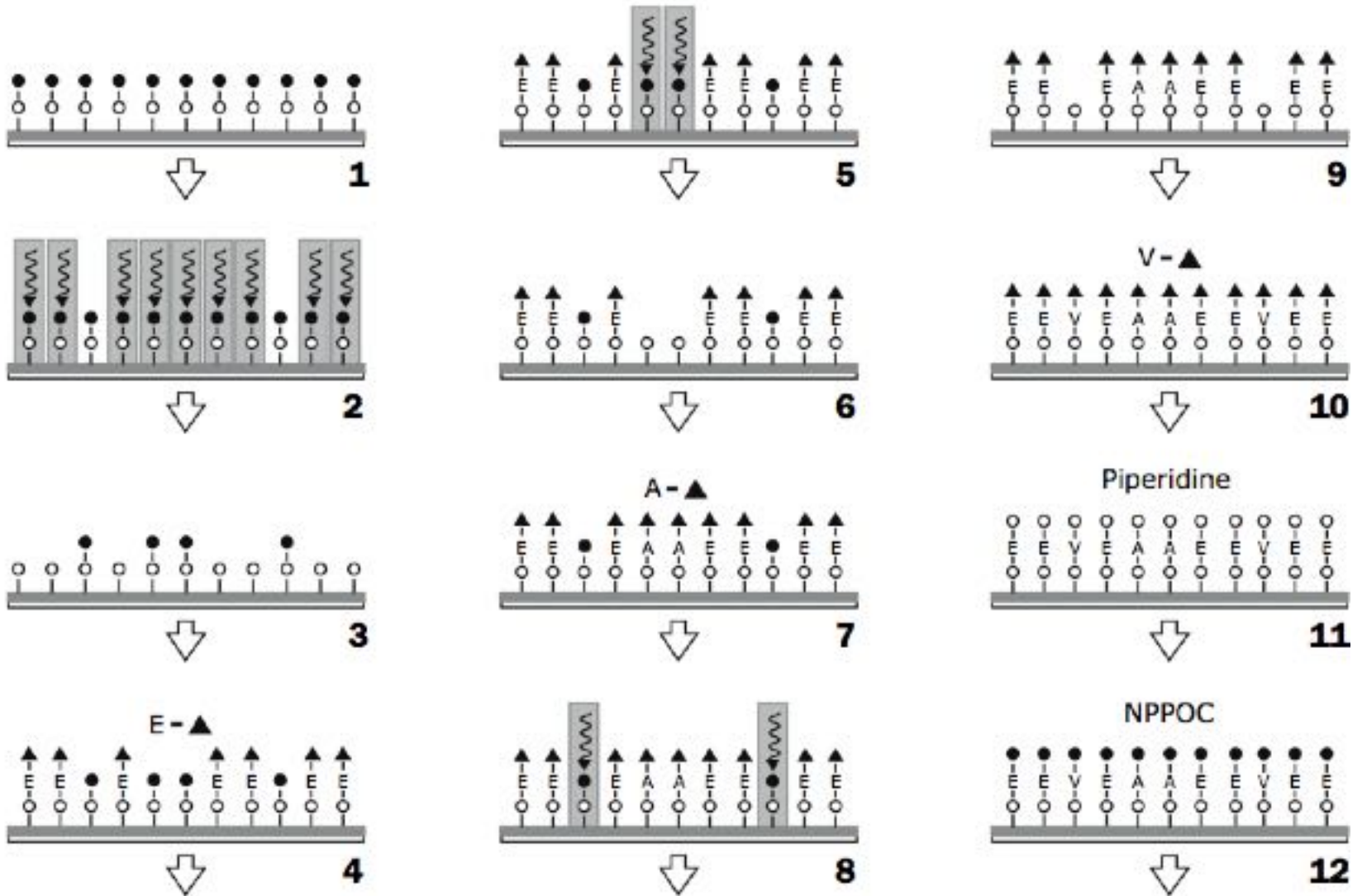
- phage-display
- in-vitro translation microarrays
- peptide microarray
- .....

# High-density peptide microarrays

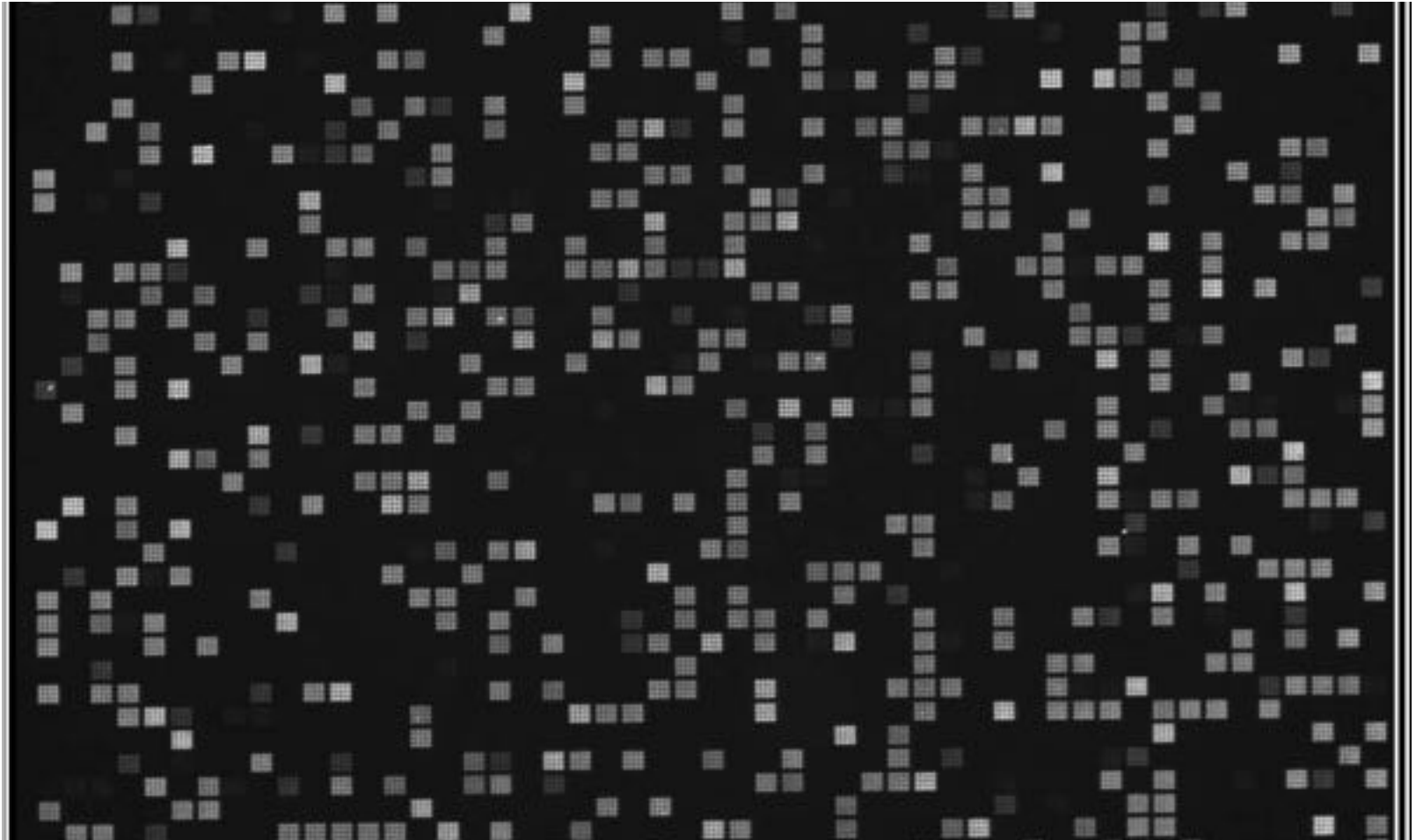




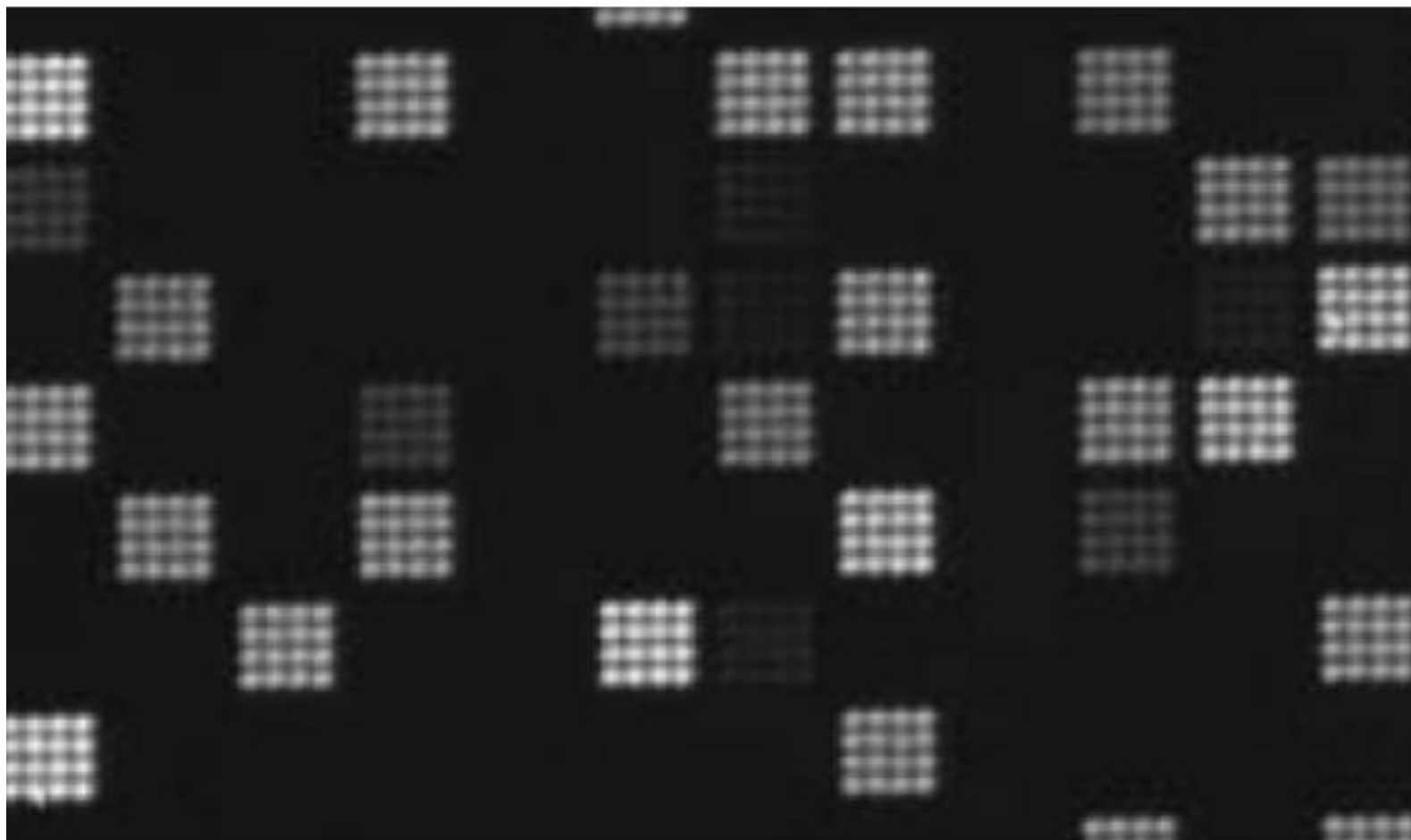
# High-density peptide microarrays



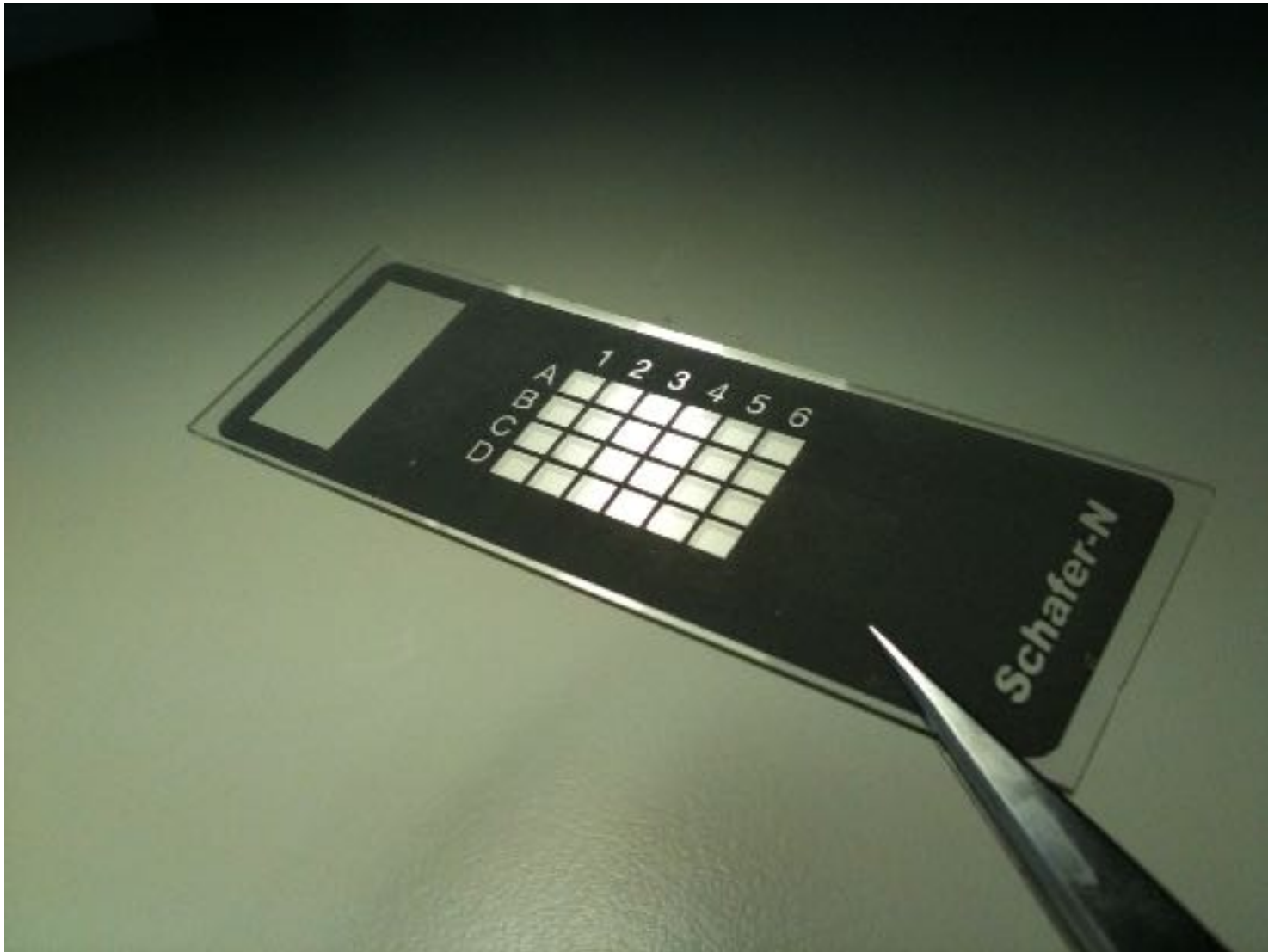
# High-density Peptide MicroArray



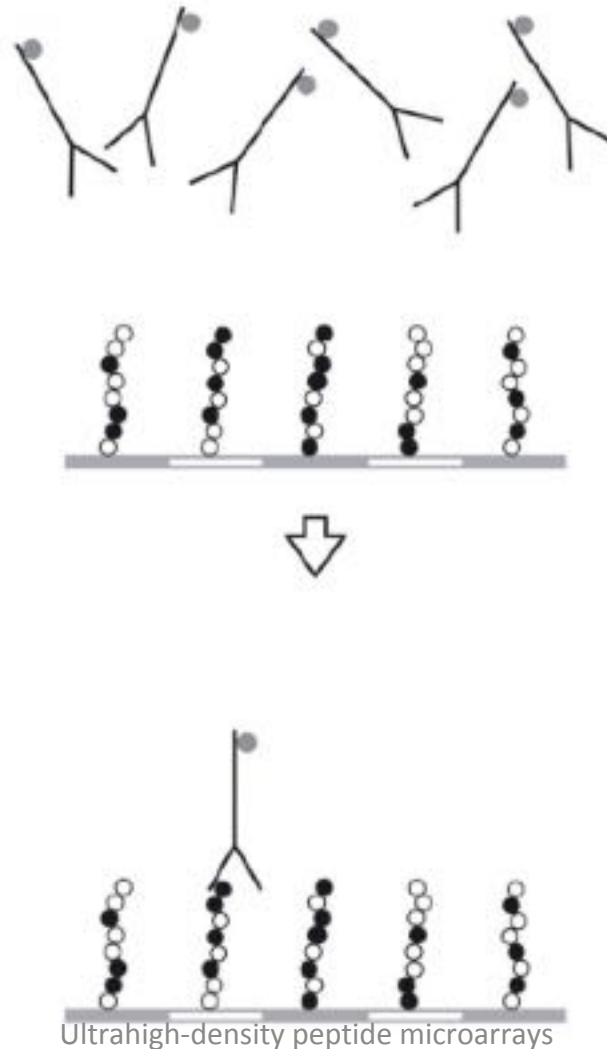
# Single mirror resolution



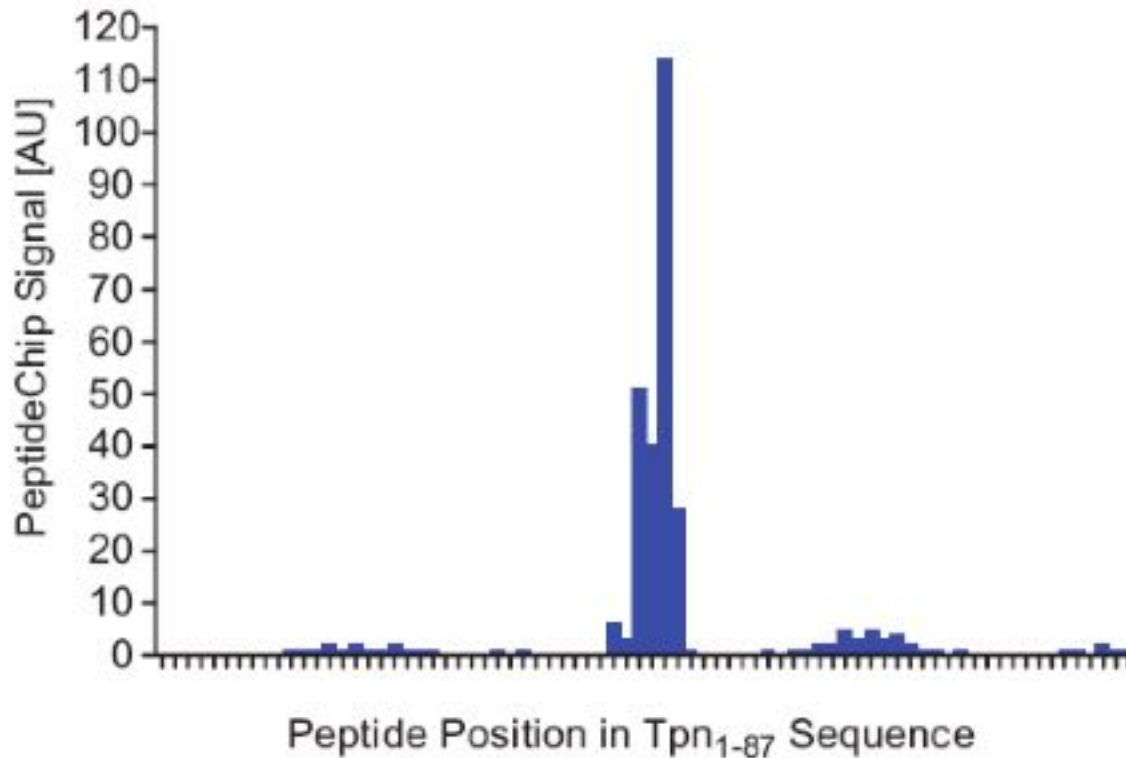
# Multiscreen (24 sectors)



# Antibody peptidomics



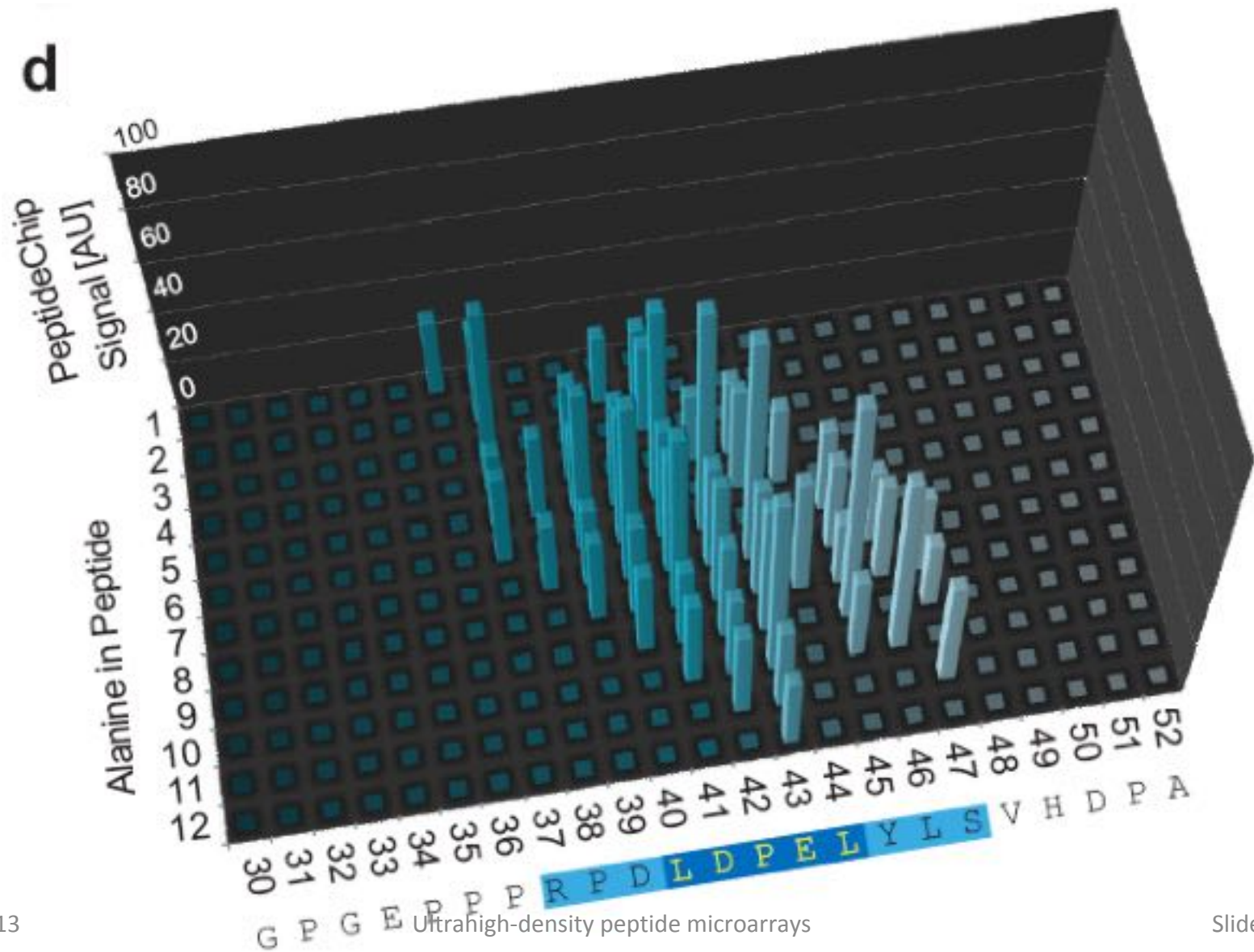
# Identifying linear epitopes for monoclonal Ab



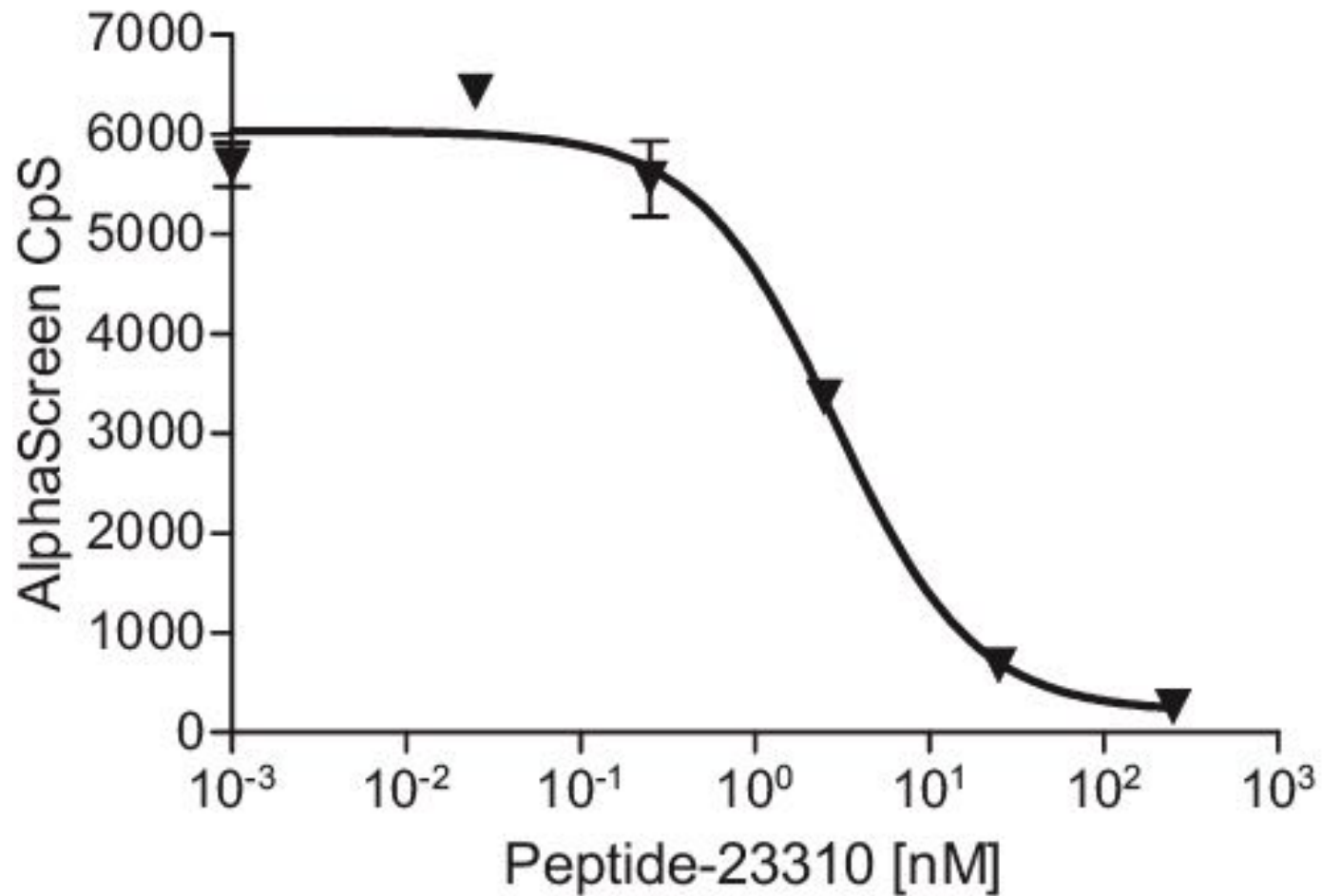
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# Antibody peptidomics

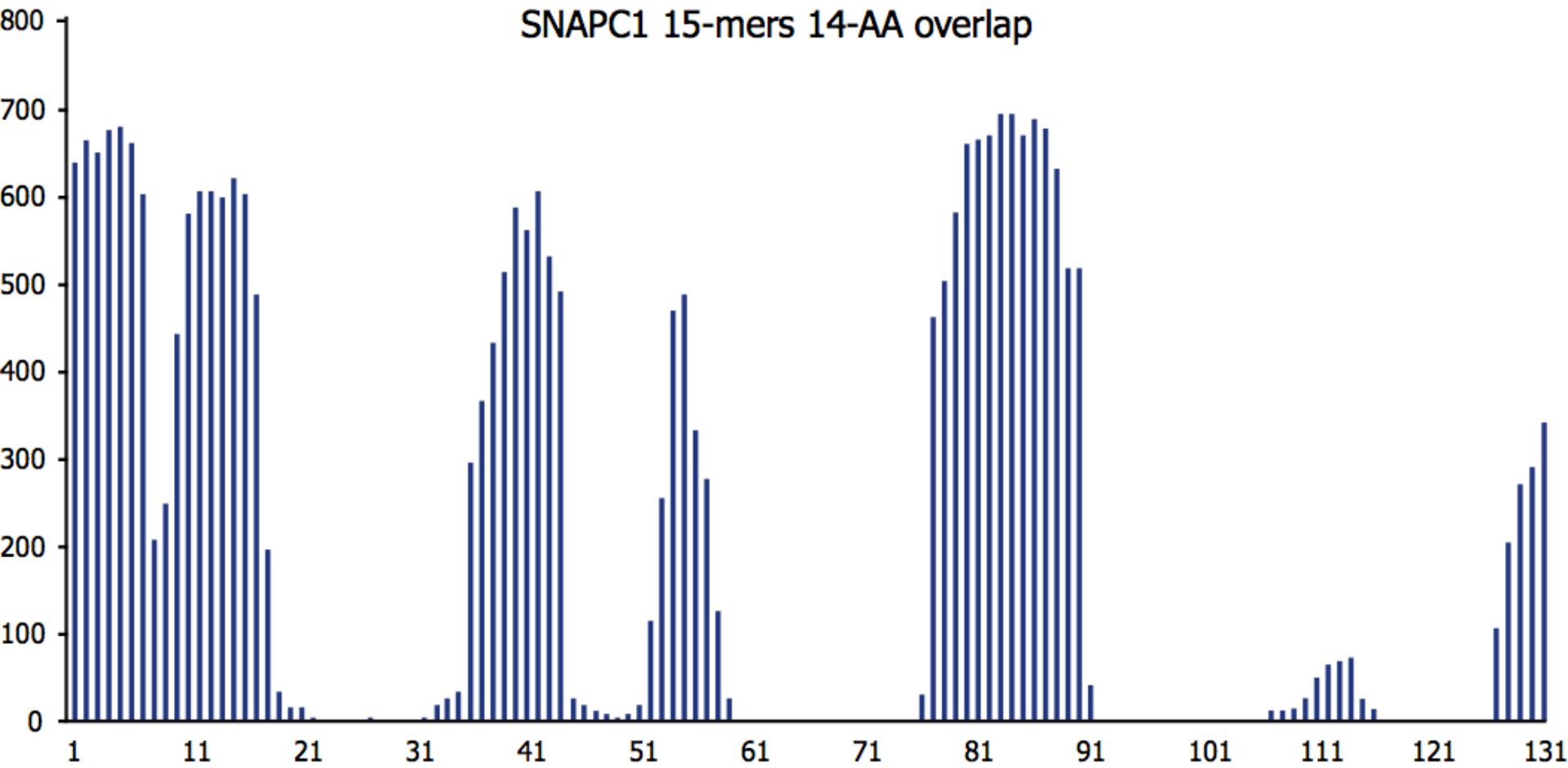


# High affinity peptide interactions





# Overlapping peptides



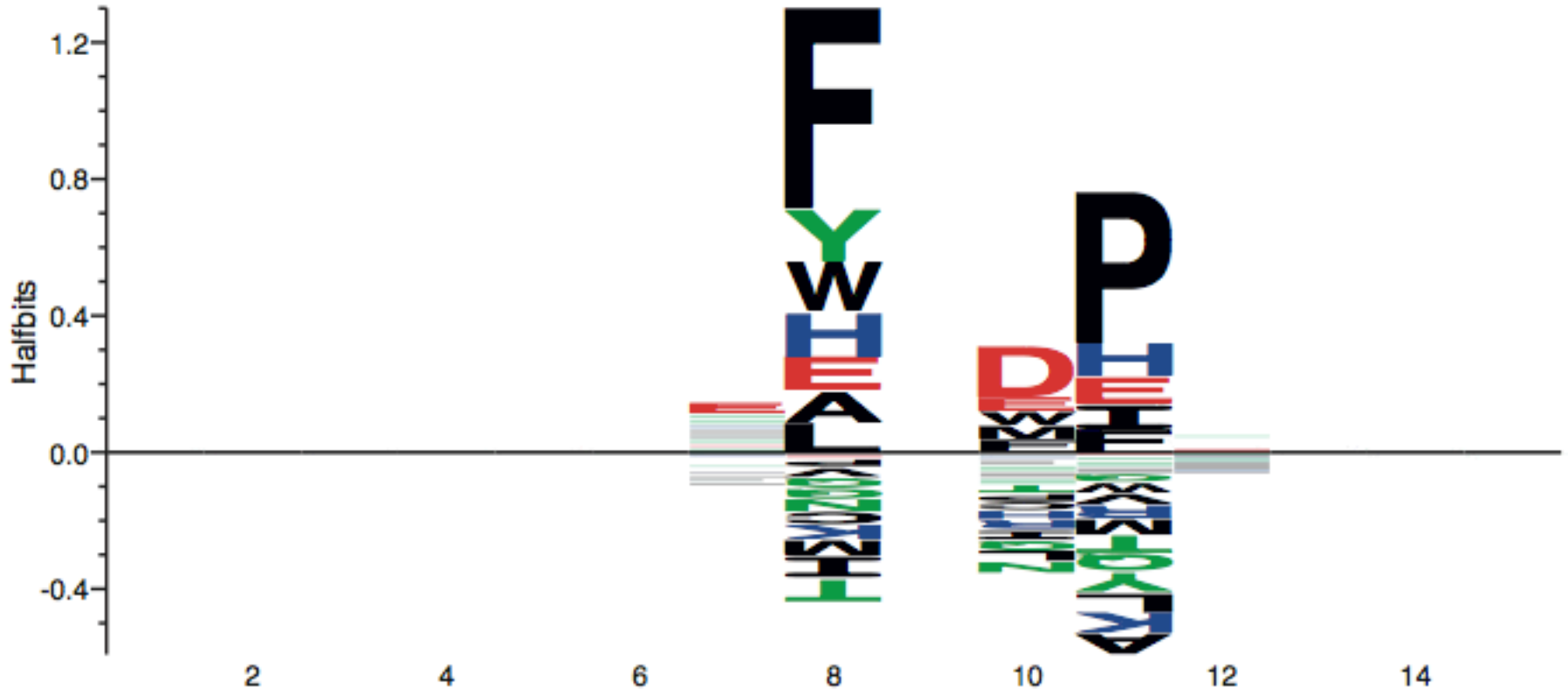
# Overlap / Length scan

```
20 RAEVTEEFKDPSPDRVMKLITSDVLEEMLNVDHYQNMKHVISVDKSKPKALKSLIKDDFFDNIKNIVLEHQQWHKDRKNPSLKSKTNDGEEKMIEGNSQETERCERAEESLAKIKSKAFSVVIQASKSRRHRCQVKLDSSDSDSASGQ
19 RAEVTEEFKDPSPDRVMKLITSDVLEEMLNVDHYQNMKHVISVDKSKPKALKSLIKDDFFDNIKNIVLEHQQWHKDRKNPSLKSKTNDGEEKMIEGNSQETERCERAEESLAKIKSKAFSVVIQASKSRRHRCQVKLDSSDSDSASGQ
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15 RAEVTEEFKDPSPDRVMKLITSDVLEEMLNVDHYQNMKHVISVDKSKPKALKSLIKDDFFDNIKNIVLEHQQWHKDRKNPSLKSKTNDGEEKMIEGNSQETERCERAEESLAKIKSKAFSVVIQASKSRRHRCQVKLDSSDSDSASGQ
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06 RAEVTEEFKDPSPDRVMKLITSDVLEEMLNVDHYQNMKHVISVDKSKPKALKSLIKDDFFDNIKNIVLEHQQWHKDRKNPSLKSKTNDGEEKMIEGNSQETERCERAEESLAKIKSKAFSVVIQASKSRRHRCQVKLDSSDSDSASGQ
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```

# Single substitution analysis

		Position														
Native		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		R	A	W	V	T	E	E	F	K	D	P	S	D	R	V
A		0.9	<u>1.0</u>	0.9	1.0	1.1	0.9	0.3	0.2	0.9	0.1	0.1	0.8	1.0	0.9	0.8
C		1.0	0.9	1.0	1.0	1.0	0.8	0.3	0.1	0.6	0.2	0.0	0.4	0.7	0.7	0.9
D		1.0	1.0	1.0	1.1	1.2	1.1	0.4	0.0	0.6	<u>1.0</u>	0.0	0.6	<u>1.0</u>	0.9	1.0
E		0.7	1.1	<u>1.0</u>	0.9	1.1	<u>1.0</u>	<u>1.0</u>	0.2	0.8	0.3	0.2	0.6	1.0	0.9	0.8
F		0.8	1.1	1.0	1.0	1.1	0.9	0.4	<u>1.0</u>	0.8	0.3	0.2	0.5	0.9	0.8	1.0
G		0.9	1.1	1.0	1.0	1.1	0.7	0.4	0.1	0.7	0.0	0.1	0.8	0.8	0.9	0.8
H		0.9	0.9	1.0	1.1	1.0	0.8	0.4	0.2	0.6	0.2	0.2	0.4	0.7	0.5	1.0
I		0.9	0.8	1.0	1.0	1.0	0.9	0.3	0.1	0.7	0.2	0.2	0.7	0.9	1.2	0.9
K		0.8	1.0	1.0	0.7	1.0	0.9	0.2	0.1	<u>1.0</u>	0.1	0.1	0.5	0.7	1.2	1.0
L		1.0	1.1	1.0	1.0	1.1	0.8	0.3	0.2	1.0	0.2	0.1	0.5	0.7	0.9	1.1
M		1.1	1.1	0.9	0.9	1.2	0.8	0.4	0.1	0.9	0.3	0.1	0.5	0.8	0.9	0.9
N		1.0	1.0	1.1	1.0	1.1	0.9	0.4	0.1	0.7	0.2	0.0	0.4	0.8	0.9	1.0
P		0.8	0.9	0.9	0.9	1.0	0.8	0.3	0.0	1.0	0.2	<u>1.0</u>	0.3	0.6	0.9	1.1
Q		1.1	0.9	0.9	1.1	1.1	0.9	0.3	0.1	1.0	0.2	0.1	0.5	0.9	0.9	1.1
R		<u>1.0</u>	0.8	1.1	1.0	0.9	0.8	0.2	-0.1	1.0	0.2	0.1	0.5	0.8	<u>1.0</u>	1.1
S		1.0	0.8	1.1	0.9	1.0	0.8	0.2	0.0	1.0	0.1	0.0	<u>1.0</u>	0.9	0.9	1.0
T		1.0	1.0	1.1	1.1	<u>1.0</u>	0.7	0.5	0.1	0.8	0.2	0.1	0.5	0.8	1.0	0.9
V		0.9	0.9	1.0	<u>1.0</u>	1.0	0.8	0.2	0.1	0.8	0.1	0.1	0.5	0.8	0.9	<u>1.0</u>
W		1.1	1.1	1.0	1.1	1.3	0.9	0.4	0.2	0.8	0.3	0.1	0.6	0.9	1.0	0.8
Y		0.9	1.2	1.1	1.1	1.1	0.6	0.5	0.3	0.7	0.1	0.1	0.7	0.9	1.0	1.1
Average Epitope		0.9	1.0	1.0	1.0	1.1	0.8	0.4	0.1	0.8	0.2	0.2	0.6	0.8	0.9	1.0
								E	F		D	P	S			

# Single substitution analysis

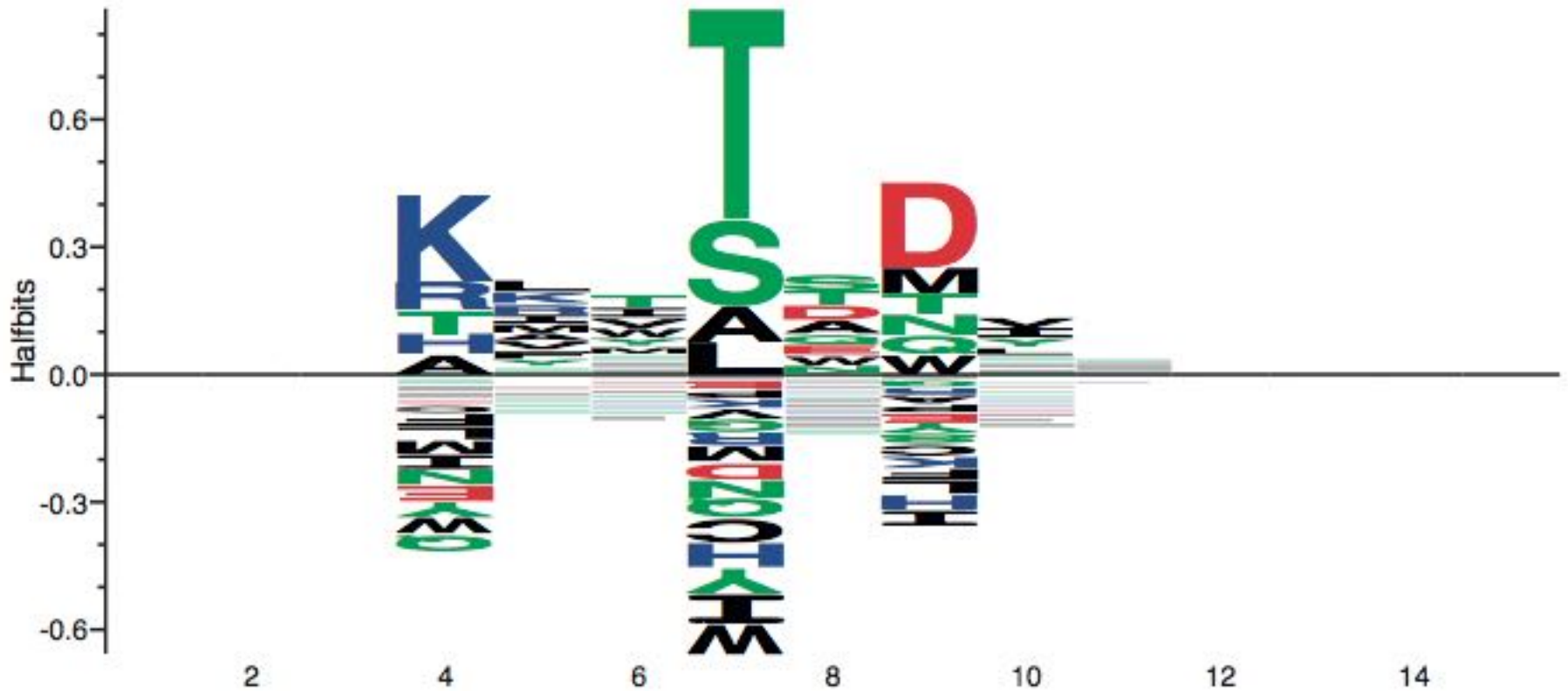




# Single substitution analysis

		Position														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Native		E	V	M	K	L	I	T	S	D	V	L	E	E	M	L
A		0.9	1.1	1.0	0.2	0.7	0.6	0.2	0.9	0.1	0.5	0.9	0.7	0.9	0.9	1.1
C		0.7	0.8	0.7	0.1	0.3	0.4	0.1	0.2	0.1	0.5	1.0	0.8	0.7	1.0	1.0
D		0.9	0.9	0.8	0.1	0.2	0.2	0.1	0.9	1.0	0.4	0.8	1.1	1.1	1.1	0.9
E		0.7	1.0	1.0	0.2	0.3	0.4	0.0	0.6	0.1	0.5	1.0	1.0	1.0	1.0	1.0
F		0.9	1.0	0.8	0.2	0.7	0.6	0.0	0.4	0.1	0.5	0.9	0.8	0.8	1.0	1.1
G		1.1	1.2	1.0	0.1	0.2	0.1	0.1	0.3	0.1	0.2	0.7	0.9	0.9	0.9	1.0
H		0.7	1.0	1.0	0.2	0.2	0.3	0.1	0.2	0.2	0.3	0.7	0.6	0.8	1.0	1.1
I		1.0	1.0	0.8	0.2	0.8	1.0	0.1	0.2	0.2	0.9	0.8	0.8	0.7	1.0	1.0
K		0.9	1.1	1.2	1.0	1.0	0.5	0.0	0.4	0.1	0.4	0.7	0.8	0.8	1.0	1.1
L		0.8	1.1	1.1	0.2	1.0	0.6	0.2	0.4	0.2	0.7	1.0	0.7	0.7	1.0	1.0
M		0.9	1.2	1.0	0.2	0.8	0.7	0.1	0.3	0.3	0.6	1.0	0.9	0.8	1.0	1.0
N		1.0	1.2	1.3	0.2	0.5	0.5	0.1	0.5	0.2	0.4	0.9	1.0	0.8	1.1	1.0
P		0.8	1.1	1.1	0.1	0.2	0.5	0.0	0.4	0.1	0.2	0.5	1.0	0.9	1.0	1.2
Q		0.9	1.1	1.1	0.2	0.6	0.7	0.1	0.6	0.2	0.6	1.1	0.9	0.8	1.1	1.0
R		1.0	1.2	1.1	0.3	0.8	0.5	0.1	0.2	0.1	0.5	0.9	0.9	0.7	1.0	1.1
S		0.9	1.2	1.1	0.0	0.5	0.5	0.4	1.0	0.1	0.4	0.6	0.9	0.9	0.9	0.9
T		0.9	0.8	1.1	0.3	0.5	1.2	1.0	0.9	0.3	0.4	0.6	0.8	0.9	0.9	1.1
V		0.8	1.0	0.8	0.1	0.7	0.9	0.1	0.2	0.1	1.0	1.0	0.7	0.7	1.1	1.1
W		0.8	1.1	0.9	0.2	0.4	0.9	0.1	0.6	0.2	0.5	1.1	0.8	1.0	1.0	1.1
Y		0.8	1.0	1.1	0.2	0.6	0.8	0.1	0.4	0.1	0.9	1.1	0.9	0.9	1.0	1.0
Average		0.9	1.1	1.0	0.2	0.5	0.6	0.2	0.5	0.2	0.5	0.9	0.9	0.8	1.0	1.0
Epitope					K	L	I	T	S	D	V					

# Single substitution analysis



# Scanning 22 PrESTs

Source PrEST	Epitope		Signal			ANOVA		Post-hoc LSD (1%)	Average in position indicated														
	Epitope	Length	AVE	SD	CV	F	P		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AIFM3	KSLD--P	7	202	42	21%	25.6	p < 0.000001	0.55	0.89	0.91	0.91	1.11	0.78	0.73	0.73	0.98	0.20	0.09	0.11	0.16	0.82	0.72	0.14
ANLN	G-GIKPF-E	9	123	11	9%	48.4	p < 0.000001	0.30	1.01	0.99	1.00	0.58	0.82	0.15	0.38	0.07	0.38	0.13	1.19	0.67	1.06	1.13	1.07
ANLN	Q-QSKDKS	8	293	42	14%	59.3	p < 0.000001	0.41	0.80	0.51	0.81	0.04	0.06	0.24	0.10	0.04	0.16	0.96	0.91	0.94	1.03	0.97	0.97
ANLN	T-NT--I--RLF	12	606	20	3%	54.9	p < 0.000001	0.20	0.97	0.95	1.00	0.51	0.94	0.55	0.51	0.88	0.86	0.23	0.86	0.89	0.67	0.19	0.24
AUTS2	D-LGRDFLL	9	648	18	3%	62.7	p < 0.000001	0.22	1.01	0.30	0.74	0.19	0.52	0.09	0.60	0.17	0.14	0.32	0.80	0.88	0.99	0.85	0.97
AUTS2	DPLR-PYR-LDI	12	134	27	20%	16.6	p < 0.000001	0.22	1.01	0.85	0.75	0.70	0.76	0.59	0.79	0.70	0.61	0.57	0.80	0.53	0.47	0.51	1.05
C22orf29	D-DLPLPDDY	10	439	41	9%	48.9	p < 0.000001	0.18	1.04	0.93	0.69	0.96	0.35	0.39	0.42	0.52	0.49	0.52	0.71	0.79	0.87	0.94	0.96
C22orf29	QDP-SF-EY	9	165	34	21%	38.1	p < 0.000001	0.38	0.99	0.14	0.23	0.47	0.94	0.21	0.32	0.92	0.23	0.22	1.69	0.98	0.96	0.92	0.91
CDNP1	LG-DPT	6	676	46	7%	111.8	p < 0.000001	0.27	0.93	0.99	0.93	0.90	0.93	0.85	0.11	0.08	0.93	0.13	0.17	0.19	0.84	0.90	0.99
CDNP1	PRF--E	6	271	49	18%	22.4	p < 0.000001	0.55	0.84	0.96	0.94	0.98	0.71	0.57	0.07	0.36	0.11	0.96	0.63	0.13	0.57	0.65	1.10
CRABP2	KVGEEFE	7	494	31	6%	94.1	p < 0.000001	0.18	0.96	0.97	0.96	0.98	0.82	0.20	0.22	0.13	0.51	0.21	0.40	0.43	0.88	0.95	0.98
EGFL6	LSSL	5	186	53	29%	15.3	p < 0.000001	0.46	1.11	0.72	0.66	0.25	0.17	0.25	0.41	0.29	0.66	0.91	1.06	1.03	0.90	0.87	1.17
EGFL6	DA--SIIFE-E	11	345	37	11%	43.3	p < 0.000001	0.26	1.01	0.97	0.70	0.71	0.76	0.82	0.08	0.13	0.14	0.28	0.59	0.76	0.57	1.03	1.02
EGFL6	EIAVDGVLL	9	88	6	7%	15.6	p < 0.000001	0.44	0.99	0.88	0.17	0.66	0.62	0.65	0.30	0.15	0.17	0.08	0.23	0.91	0.79	0.80	1.00
EGFL6	IGRLKLLP	9	377	38	10%	38.8	p < 0.000001	0.21	1.05	0.82	1.11	0.75	0.64	0.65	0.43	0.67	0.53	0.41	0.33	0.43	0.89	0.96	1.00
EGFL6	KLRVF	5	398	42	11%	12.2	p < 0.000003	0.27	0.95	0.91	0.86	0.43	0.58	0.55	0.61	0.58	0.78	0.75	0.92	0.80	0.88	0.94	1.05
EGFL6	NPADRD	6	559	40	7%	110.4	p < 0.000001	0.19	1.00	0.98	0.15	0.15	0.12	0.39	0.57	0.15	0.94	1.01	1.00	0.99	0.95	1.00	1.02
EGFL6	QD-EDDFD	8	358	18	5%	75.1	p < 0.000001	0.19	1.00	0.98	1.02	0.19	0.69	1.01	0.47	0.17	0.41	0.33	0.69	0.90	0.98	0.94	1.00
EGFR	D-IDDTF	7	665	18	3%	66.5	p < 0.000001	0.29	0.97	0.94	0.65	0.77	0.46	0.52	0.30	0.13	0.21	0.98	0.87	0.98	0.99	0.93	0.97
EGFR	NQPLN	5	406	34	8%	95.5	p < 0.000001	0.36	0.94	1.40	0.05	0.42	0.02	0.06	0.06	0.68	0.76	0.74	0.91	1.21	0.79	0.87	1.31
EGFR	R--LLS-L	8	659	27	4%	41.2	p < 0.000001	0.37	0.17	0.83	0.77	0.34	0.23	0.42	0.71	0.37	0.94	0.98	1.00	1.01	0.99	0.98	0.99
EGFR	R-AGSV-NP	9	188	19	10%	53.2	p < 0.000001	0.43	0.94	0.85	0.71	0.07	0.74	0.49	0.06	0.51	0.09	0.63	0.09	0.24	0.85	1.03	1.42
EGFR	VNSTFDS	7	200	17	9%	93.2	p < 0.000001	0.43	0.88	0.93	1.70	0.12	0.05	0.56	0.11	0.05	0.15	0.52	0.71	0.86	1.67	1.06	0.98
EPHRIN B3	PGKE-LP	7	643	25	4%	116.0	p < 0.000001	0.17	1.01	0.96	0.90	0.44	0.28	0.68	0.23	1.01	0.19	0.15	0.85	0.98	0.98	0.99	0.98
FOXO28	PSAALT--L	10	417	51	12%	49.1	p < 0.000001	0.42	0.86	0.12	0.02	0.11	0.09	0.19	0.14	0.09	0.78	0.78	0.31	0.98	0.90	0.78	0.98
FOXO28	RRE-SELRTK	10	178	24	13%	36.6	p < 0.000001	0.46	0.96	1.04	0.07	0.05	0.49	0.76	0.16	0.19	0.63	0.07	0.18	0.44	0.91	0.84	0.87
HER2-D2	DTFESMP	7	180	44	25%	35.7	p < 0.000001	0.34	1.01	1.13	0.97	1.12	1.13	0.33	0.38	0.50	0.15	0.19	0.15	0.32	1.07	0.95	0.71
HER2-D2	EDGTQ	5	114	21	19%	57.5	p < 0.000001	0.33	0.97	0.99	1.11	1.07	1.12	0.87	1.21	1.17	1.04	0.29	0.17	0.10	0.08	0.05	1.33
HER2-D3	APLQPEQL	8	661	28	4%	74.6	p < 0.000001	0.33	0.96	0.97	0.98	0.95	0.99	0.50	0.07	0.61	0.06	0.17	0.06	0.43	0.60	0.93	0.97
HER2-D3	RPED	4	706	10	1%	97.3	p < 0.000001	0.22	0.99	0.99	0.71	0.42	0.19	0.30	0.79	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99
HER2-D4	FGPEAD	6	69	17	25%	31.3	p < 0.000001	0.69	0.80	1.17	0.10	0.04	0.05	0.04	0.15	0.16	0.75	1.38	0.81	0.96	1.03	0.74	1.40
HER2-D4	SQFLRG-E	8	657	19	3%	84.7	p < 0.000001	0.33	1.02	0.08	0.04	0.05	0.59	0.60	0.12	0.83	0.34	1.02	0.95	0.92	0.91	0.99	1.01
HER2-D5	P-QPE	5	695	11	2%	47.6	p < 0.000001	0.20	0.94	0.98	0.98	0.99	0.96	1.00	0.85	0.56	0.89	0.59	0.68	0.44	0.84	0.90	0.99
HMCGR	IEIG-VG	7	163	27	17%	25.7	p < 0.000001	0.59	1.00	0.40	0.12	0.10	0.02	0.46	0.19	0.10	0.50	0.85	0.86	1.02	0.83	1.04	0.92
HMCGR	LSLMA-LA	8	179	17	9%	72.3	p < 0.000001	0.52	0.83	0.87	0.44	0.06	0.08	0.10	0.08	0.88	0.11	0.08	0.76	0.49	2.20	0.96	0.91
HMCGR	NEDLYIS-TM	10	519	36	7%	40.4	p < 0.000001	0.31	0.90	0.95	0.66	0.46	0.52	0.44	0.59	0.39	0.39	1.14	0.43	0.44	0.84	0.91	0.97
IL17RA	DLLPEDV-E	9	597	32	5%	64.4	p < 0.000001	0.33	0.95	0.90	0.67	0.52	0.53	0.35	0.18	0.05	0.16	0.88	0.24	1.06	0.95	0.77	0.96
RBM3	GGYDRYS	7	226	11	5%	81.8	p < 0.000001	0.20	0.99	0.92	0.93	0.96	0.80	0.61	0.14	0.15	0.16	0.09	0.52	0.88	0.94	0.83	0.92
RMB3	QA-ED-F	7	59	14	24%	28.0	p < 0.000001	0.48	1.00	0.66	0.18	0.45	0.92	0.20	0.18	1.92	0.19	0.81	0.89	1.07	1.21	1.08	1.10
SATB2	PRTASQSSL-N	11	38	4	10%	24.3	p < 0.000001	0.45	1.00	1.15	1.04	0.96	0.11	0.08	0.11	0.01	0.18	0.65	0.18	0.22	0.42	1.04	0.31
SATB2	RD-IYQDE-E	10	228	13	6%	75.9	p < 0.000001	0.20	1.00	1.03	0.93	0.31	0.44	1.12	0.25	0.18	0.50	0.22	0.22	0.81	0.71	0.98	0.84

EIP 2019

Ultra-high density peptide microarray



# High Sensitivity

Source PrEST	Epitope		Signal			ANOVA		Post-hoc LSD (%)	Average in position indicated														
	Epitope	Length	AVE	SD	CV	F	P		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AIFM3	KSLD--P	7	202	42	21%	25.6	p < 0.000001	0.55	0.89	0.91	0.91	1.11	0.78	0.73	0.73	0.98	0.20	0.09	0.11	0.16	0.82	0.72	0.14
ANLN	G-GIKPF-E	9	123	11	9%	48.4	p < 0.000001	0.30	1.01	0.99	1.00	0.58	0.82	0.15	0.38	0.07	0.38	0.13	1.19	0.67	1.06	1.13	1.07
ANLN	Q-QSKDKS	8	293	42	14%	59.3	p < 0.000001	0.41	0.80	0.51	0.81	0.04	0.06	0.24	0.10	0.04	0.16	0.96	0.91	0.94	1.03	0.97	0.97
ANLN	T-NT--I--RLF	12	606	20	3%	54.9	p < 0.000001	0.20	0.97	0.95	1.00	0.51	0.94	0.55	0.51	0.88	0.86	0.23	0.86	0.89	0.67	0.19	0.24
AUTS2	D-LGRDFLL	9	648	18	3%	62.7	p < 0.000001	0.22	1.01	0.30	0.74	0.19	0.52	0.09	0.60	0.17	0.14	0.32	0.80	0.88	0.99	0.85	0.97
AUTS2	DPLR-PYR-LDI	12	134	27	20%	16.6	p < 0.000001	0.22	1.01	0.85	0.75	0.70	0.76	0.59	0.79	0.70	0.61	0.57	0.80	0.53	0.47	0.51	1.05
C22orf29	D-DLPLPDDY	10	439	41	9%	48.9	p < 0.000001	0.18	1.04	0.93	0.69	0.96	0.35	0.39	0.42	0.52	0.49	0.52	0.71	0.79	0.87	0.94	0.96
C22orf29	QDP-SF-EY	9	165	34	21%	38.1	p < 0.000001	0.38	0.99	0.14	0.23	0.47	0.94	0.21	0.32	0.92	0.23	0.22	1.69	0.98	0.96	0.92	0.91
CDNP1	LG-DPT	6	676	46	7%	111.8	p < 0.000001	0.27	0.93	0.99	0.93	0.90	0.93	0.85	0.11	0.08	0.93	0.13	0.17	0.19	0.84	0.90	0.99
CDNP1	PRF--E	6	271	49	18%	22.4	p < 0.000001	0.55	0.84	0.96	0.94	0.98	0.71	0.57	0.07	0.36	0.11	0.96	0.63	0.13	0.57	0.65	1.10
CRABP2	KVGEFE	7	494	31	6%	94.1	p < 0.000001	0.18	0.96	0.97	0.96	0.98	0.82	0.20	0.22	0.13	0.51	0.21	0.40	0.43	0.88	0.95	0.98
EGFL6	LLSSL	5	186	53	29%	15.3	p < 0.000001	0.46	1.11	0.72	0.66	0.25	0.17	0.25	0.41	0.29	0.66	0.91	1.06	1.03	0.90	0.87	1.17
EGFL6	DA--SIIFE-E	11	345	37	11%	43.3	p < 0.000001	0.26	1.01	0.97	0.70	0.71	0.76	0.82	0.08	0.13	0.14	0.28	0.59	0.76	0.57	1.03	1.02
EGFL6	EIAVDGVLL	9	88	6	7%	15.6	p < 0.000001	0.44	0.99	0.88	0.17	0.66	0.62	0.65	0.30	0.15	0.17	0.08	0.23	0.91	0.79	0.80	1.00
EGFL6	IGRLKLLLP	9	377	38	10%	38.8	p < 0.000001	0.21	1.05	0.82	1.11	0.75	0.64	0.65	0.43	0.67	0.53	0.41	0.33	0.43	0.89	0.96	1.00
EGFL6	KLRVF	5	398	42	11%	12.2	p < 0.000003	0.27	0.95	0.91	0.86	0.43	0.58	0.55	0.61	0.58	0.78	0.75	0.92	0.80	0.88	0.94	1.05
EGFL6	NPADRD	6	559	40	7%	110.4	p < 0.000001	0.19	1.00	0.98	0.15	0.15	0.12	0.39	0.57	0.15	0.94	1.01	1.00	0.99	0.95	1.00	1.02
EGFL6	QD-EDDFD	8	358	18	5%	75.1	p < 0.000001	0.19	1.00	0.98	1.02	0.19	0.69	1.01	0.47	0.17	0.41	0.33	0.69	0.90	0.98	0.94	1.00
EGFR	D-IDDTF	7	665	18	3%	66.5	p < 0.000001	0.29	0.97	0.94	0.65	0.77	0.46	0.52	0.30	0.13	0.21	0.98	0.87	0.98	0.99	0.93	0.97
EGFR	NQPLN	5	406	34	8%	95.5	p < 0.000001	0.36	0.94	1.40	0.05	0.42	0.02	0.06	0.06	0.68	0.76	0.74	0.91	1.21	0.79	0.87	1.31
EGFR	R--LLS-L	8	659	27	4%	41.2	p < 0.000001	0.37	0.17	0.83	0.77	0.34	0.23	0.42	0.71	0.37	0.94	0.98	1.00	1.01	0.99	0.98	0.99
EGFR	R-AGSV-NP	9	188	19	10%	53.2	p < 0.000001	0.43	0.94	0.85	0.71	0.07	0.74	0.49	0.06	0.51	0.09	0.63	0.09	0.24	0.85	1.03	1.42
EGFR	VNSTFDS	7	200	17	9%	93.2	p < 0.000001	0.43	0.88	0.93	1.70	0.12	0.05	0.56	0.11	0.05	0.15	0.52	0.71	0.86	1.67	1.06	0.98
EPHRIN B3	PGKE-LP	7	643	25	4%	116.0	p < 0.000001	0.17	1.01	0.96	0.90	0.44	0.28	0.68	0.23	1.01	0.19	0.15	0.85	0.98	0.98	0.99	0.98
FOXB028	PSAALT--L	10	417	51	12%	49.1	p < 0.000001	0.42	0.86	0.12	0.02	0.11	0.09	0.19	0.14	0.09	0.78	0.78	0.31	0.98	0.90	0.78	0.98
FOXB028	RRE-SELRTK	10	178	24	13%	36.6	p < 0.000001	0.46	0.96	1.04	0.07	0.05	0.49	0.76	0.16	0.19	0.63	0.07	0.18	0.44	0.91	0.84	0.87
HER2-D2	DTFESMP	7	180	44	25%	35.7	p < 0.000001	0.34	1.01	1.13	0.97	1.12	1.13	0.33	0.38	0.50	0.15	0.19	0.15	0.32	1.07	0.95	0.71
HER2-D2	EDGTQ	5	114	21	19%	57.5	p < 0.000001	0.33	0.97	0.99	1.11	1.07	1.12	0.87	1.21	1.17	1.04	0.29	0.17	0.10	0.08	0.05	1.33
HER2-D3	APLQPEQL	8	661	28	4%	74.6	p < 0.000001	0.33	0.96	0.97	0.98	0.95	0.99	0.50	0.07	0.61	0.06	0.17	0.06	0.43	0.60	0.93	0.97
HER2-D3	RPED	4	706	10	1%	97.3	p < 0.000001	0.22	0.99	0.99	0.71	0.42	0.19	0.30	0.79	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99
HER2-D4	FGPEAD	6	69	17	25%	31.3	p < 0.000001	0.69	0.80	1.17	0.10	0.04	0.05	0.04	0.15	0.16	0.75	1.38	0.81	0.96	1.03	0.74	1.40
HER2-D4	SQFLRG-E	8	657	19	3%	84.7	p < 0.000001	0.33	1.02	0.08	0.04	0.05	0.59	0.60	0.12	0.83	0.34	1.02	0.95	0.92	0.91	0.99	1.01
HER2-D5	P-QPE	5	695	11	2%	47.6	p < 0.000001	0.20	0.94	0.98	0.98	0.99	0.96	1.00	0.85	0.56	0.89	0.59	0.68	0.44	0.84	0.90	0.99
HMCGR	IEIG-VG	7	163	27	17%	25.7	p < 0.000001	0.59	1.00	0.40	0.12	0.10	0.02	0.46	0.19	0.10	0.50	0.85	0.86	1.02	0.83	1.04	0.92
HMCGR	LSLMA-LA	8	179	17	9%	72.3	p < 0.000001	0.52	0.83	0.87	0.44	0.06	0.08	0.10	0.08	0.88	0.11	0.08	0.76	0.49	2.20	0.96	0.91
HMCGR	NEDLYIS-TM	10	519	36	7%	40.4	p < 0.000001	0.31	0.90	0.95	0.66	0.46	0.52	0.44	0.59	0.39	0.39	1.14	0.43	0.44	0.84	0.91	0.97
IL17RA	DLLPEDV-E	9	597	32	5%	64.4	p < 0.000001	0.33	0.95	0.90	0.67	0.52	0.53	0.35	0.18	0.05	0.16	0.88	0.24	1.06	0.95	0.77	0.96
RBM3	GGYDRYS	7	226	11	5%	81.8	p < 0.000001	0.20	0.99	0.92	0.93	0.96	0.80	0.61	0.14	0.15	0.16	0.09	0.52	0.88	0.94	0.83	0.92
RMB3	QA-ED-F	7	59	14	24%	28.0	p < 0.000001	0.48	1.00	0.66	0.18	0.45	0.92	0.20	0.18	1.92	0.19	0.81	0.89	1.07	1.21	1.08	1.10
SATB2	PRTASQSSLN	11	38	4	10%	24.3	p < 0.000001	0.45	1.00	1.15	1.04	0.96	0.11	0.08	0.11	0.01	0.18	0.65	0.18	0.22	0.42	1.04	0.31
SATB2	PD-IYQDE-F	10	228	13	6%	75.9	p < 0.000001	0.20	1.00	1.03	0.93	0.31	0.44	1.12	0.25	0.18	0.50	0.22	0.22	0.81	0.71	0.98	0.87



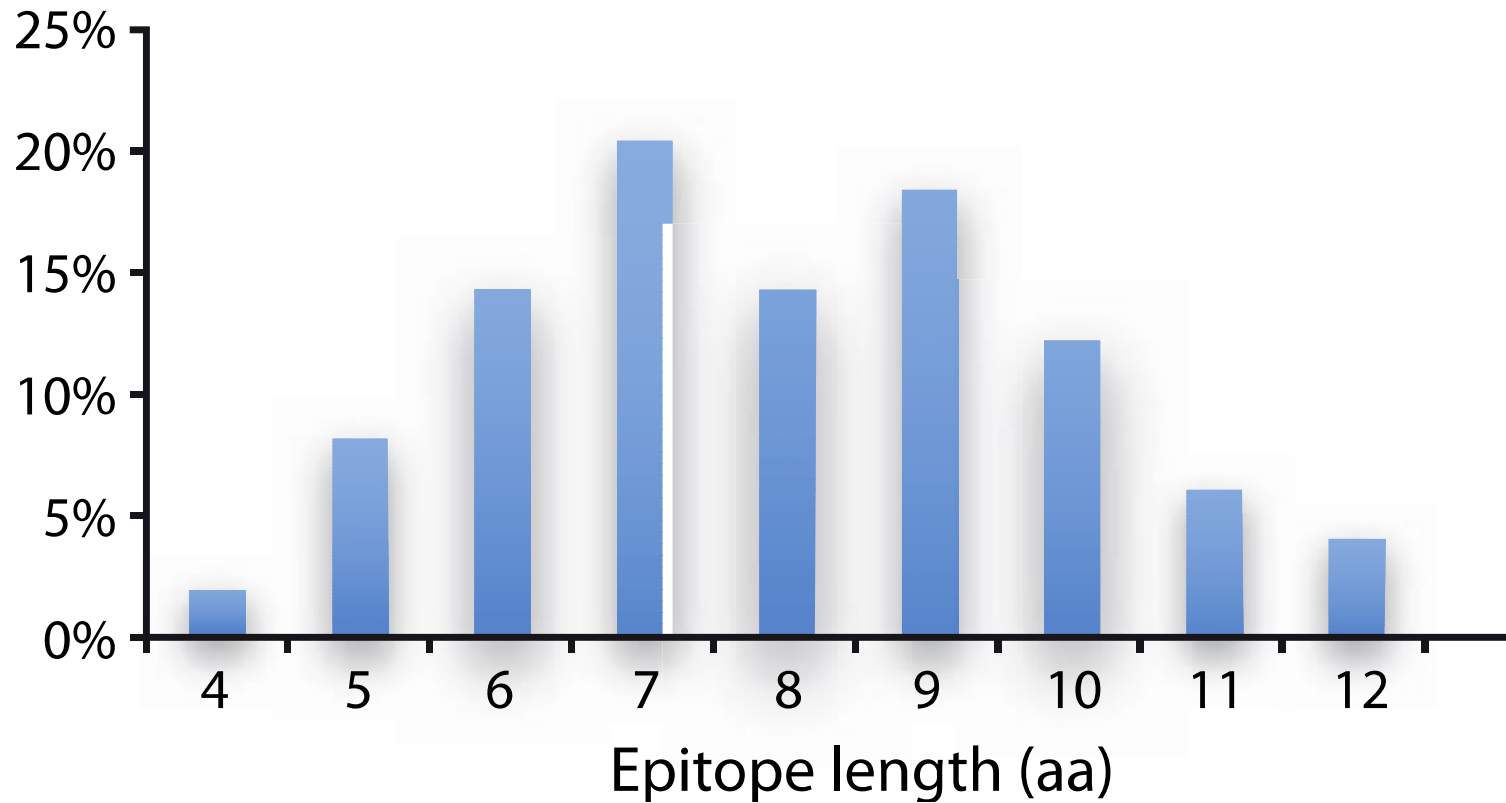
# Identify paired antibody specificities

Source PrEST	Epitope		Signal			ANOVA		Post-hoc LSD (1%)	Average in position indicated														
	Epitope	Length	AVE	SD	CV	F	P		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AIFM3	KSLD--P	7	202	42	21%	25.6	p < 0.000001	0.55	0.89	0.91	0.91	1.11	0.78	0.73	0.73	0.98	0.20	0.09	0.11	0.16	0.82	0.72	0.14
ANLN	G-GIKPF-E	9	123	11	9%	48.4	p < 0.000001	0.30	1.01	0.99	1.00	0.58	0.82	0.15	0.38	0.07	0.38	0.13	1.19	0.67	1.06	1.13	1.07
ANLN	Q-QSKDKS	8	293	42	14%	59.3	p < 0.000001	0.41	0.80	0.51	0.81	0.04	0.06	0.24	0.10	0.04	0.16	0.96	0.91	0.94	1.03	0.97	0.97
ANLN	T-NT--I--RLF	12	606	20	3%	54.9	p < 0.000001	0.20	0.97	0.95	1.00	0.51	0.94	0.55	0.51	0.88	0.86	0.23	0.86	0.89	0.67	0.19	0.24
AUTS2	D-LGRDFLL	9	648	18	3%	62.7	p < 0.000001	0.22	1.01	0.30	0.74	0.19	0.52	0.09	0.60	0.17	0.14	0.32	0.80	0.88	0.99	0.85	0.97
AUTS2	DPLR-PYR-LDI	12	134	27	20%	16.6	p < 0.000001	0.22	1.01	0.85	0.75	0.70	0.76	0.59	0.79	0.70	0.61	0.57	0.80	0.53	0.47	0.51	1.05
C22orf29	D-DLPLPDDY	10	439	41	9%	48.9	p < 0.000001	0.18	1.04	0.93	0.69	0.96	0.35	0.39	0.42	0.52	0.49	0.52	0.71	0.79	0.87	0.94	0.96
C22orf29	QDP-SF-EY	9	165	34	21%	38.1	p < 0.000001	0.38	0.99	0.14	0.23	0.47	0.94	0.21	0.32	0.92	0.23	0.22	1.69	0.98	0.96	0.92	0.91
CDNP1	LG-DPT	6	676	46	7%	111.8	p < 0.000001	0.27	0.93	0.99	0.93	0.90	0.93	0.85	0.11	0.08	0.93	0.13	0.17	0.19	0.84	0.90	0.99
CDNP1	PRF--E	6	271	49	18%	22.4	p < 0.000001	0.55	0.84	0.96	0.94	0.98	0.71	0.57	0.07	0.36	0.11	0.96	0.63	0.13	0.57	0.65	1.10
CRABP2	KVGEFFF	7	494	31	6%	94.1	p < 0.000001	0.18	0.96	0.97	0.96	0.98	0.82	0.20	0.22	0.13	0.51	0.21	0.40	0.43	0.88	0.95	0.98
EGFL6	LLSSL	5	186	53	29%	15.3	p < 0.000001	0.46	1.11	0.72	0.66	0.25	0.17	0.25	0.41	0.29	0.66	0.91	1.06	1.03	0.90	0.87	1.17
EGFL6	DA--SIIFE-E	11	345	37	11%	43.3	p < 0.000001	0.26	1.01	0.97	0.70	0.71	0.76	0.82	0.08	0.13	0.14	0.28	0.59	0.76	0.57	1.03	1.02
EGFL6	EIAVDGVLL	9	88	6	7%	15.6	p < 0.000001	0.44	0.99	0.88	0.17	0.66	0.62	0.65	0.30	0.15	0.17	0.08	0.23	0.91	0.79	0.80	1.00
EGFL6	IGRLKLLLP	9	377	38	10%	38.8	p < 0.000001	0.21	1.05	0.82	1.11	0.75	0.64	0.65	0.43	0.67	0.53	0.41	0.33	0.43	0.89	0.96	1.00
EGFL6	KLRVF	5	398	42	11%	12.2	p < 0.000003	0.27	0.95	0.91	0.86	0.43	0.58	0.55	0.61	0.58	0.78	0.75	0.92	0.80	0.88	0.94	1.05
EGFL6	NPARDR	6	559	40	7%	110.4	p < 0.000001	0.19	1.00	0.98	0.15	0.15	0.12	0.39	0.57	0.15	0.94	1.01	1.00	0.99	0.95	1.00	1.02
EGFL6	QD-EDDFD	8	358	18	5%	75.1	p < 0.000001	0.19	1.00	0.98	1.02	0.19	0.69	1.01	0.47	0.17	0.41	0.33	0.69	0.90	0.98	0.94	1.00
EGFR	D-IDDTF	7	665	18	3%	66.5	p < 0.000001	0.29	0.97	0.94	0.65	0.77	0.46	0.52	0.30	0.13	0.21	0.98	0.87	0.98	0.99	0.93	0.97
EGFR	NQPLN	5	406	34	8%	95.5	p < 0.000001	0.36	0.94	1.40	0.05	0.42	0.02	0.06	0.06	0.68	0.76	0.74	0.91	1.21	0.79	0.87	1.31
EGFR	R--LLS-L	8	659	27	4%	41.2	p < 0.000001	0.37	0.17	0.83	0.77	0.34	0.23	0.42	0.71	0.37	0.94	0.98	1.00	1.01	0.99	0.98	0.99
EGFR	R-AGSV-NP	9	188	19	10%	53.2	p < 0.000001	0.43	0.94	0.85	0.71	0.07	0.74	0.49	0.06	0.51	0.09	0.63	0.09	0.24	0.85	1.03	1.42
EGFR	VNSTFDS	7	200	17	9%	93.2	p < 0.000001	0.43	0.88	0.93	1.70	0.12	0.05	0.56	0.11	0.05	0.15	0.52	0.71	0.86	1.67	1.06	0.98
EPHRIN B3	PGKE-LP	7	643	25	4%	116.0	p < 0.000001	0.17	1.01	0.96	0.90	0.44	0.28	0.68	0.23	1.01	0.19	0.15	0.85	0.98	0.98	0.99	0.98
FOXB028	PSAALT--L	10	417	51	12%	49.1	p < 0.000001	0.42	0.86	0.12	0.02	0.11	0.09	0.19	0.14	0.09	0.78	0.78	0.31	0.98	0.90	0.78	0.98
FOXB028	RRE-SELRTK	10	178	24	13%	36.6	p < 0.000001	0.46	0.96	1.04	0.07	0.05	0.49	0.76	0.16	0.19	0.63	0.07	0.18	0.44	0.91	0.84	0.87
HER2-D2	DTFESMP	7	180	44	25%	35.7	p < 0.000001	0.34	1.01	1.13	0.97	1.12	1.13	0.33	0.38	0.50	0.15	0.19	0.15	0.32	1.07	0.95	0.71
HER2-D2	EDGTQ	5	114	21	19%	57.5	p < 0.000001	0.33	0.97	0.99	1.11	1.07	1.12	0.87	1.21	1.17	1.04	0.29	0.17	0.10	0.08	0.05	1.33
HER2-D3	APLQPEQL	8	661	28	4%	74.6	p < 0.000001	0.33	0.96	0.97	0.98	0.95	0.99	0.50	0.07	0.61	0.06	0.17	0.06	0.43	0.60	0.93	0.97
HER2-D3	RPED	4	706	10	1%	97.3	p < 0.000001	0.22	0.99	0.99	0.71	0.42	0.19	0.30	0.79	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99
HER2-D4	FGPEAD	6	69	17	25%	31.3	p < 0.000001	0.69	0.80	1.17	0.10	0.04	0.05	0.04	0.15	0.16	0.75	1.38	0.81	0.96	1.03	0.74	1.40
HER2-D4	SQFLRG-E	8	657	19	3%	84.7	p < 0.000001	0.33	1.02	0.08	0.04	0.05	0.59	0.60	0.12	0.83	0.34	1.02	0.95	0.92	0.91	0.99	1.01
HER2-D5	P-QPE	5	695	11	2%	47.6	p < 0.000001	0.20	0.94	0.98	0.98	0.99	0.96	1.00	0.85	0.56	0.89	0.59	0.68	0.44	0.84	0.90	0.99
HMCGR	IEIG-VG	7	163	27	17%	25.7	p < 0.000001	0.59	1.00	0.40	0.12	0.10	0.02	0.46	0.19	0.10	0.50	0.85	0.86	1.02	0.83	1.04	0.92
HMCGR	LSLMA-LA	8	179	17	9%	72.3	p < 0.000001	0.52	0.83	0.87	0.44	0.06	0.08	0.10	0.08	0.88	0.11	0.08	0.76	0.49	2.20	0.96	0.91
HMCGR	NEDLYIS-TM	10	519	36	7%	40.4	p < 0.000001	0.31	0.90	0.95	0.66	0.46	0.52	0.44	0.59	0.39	0.39	1.14	0.43	0.44	0.84	0.91	0.97
IL17RA	DLLPEDV-E	9	597	32	5%	64.4	p < 0.000001	0.33	0.95	0.90	0.67	0.52	0.53	0.35	0.18	0.05	0.16	0.88	0.24	1.06	0.95	0.77	0.96
RBM3	GGYDRYS	7	226	11	5%	81.8	p < 0.000001	0.20	0.99	0.92	0.93	0.96	0.80	0.61	0.14	0.15	0.16	0.09	0.52	0.88	0.94	0.83	0.92
RMB3	QA-ED-F	7	59	14	24%	28.0	p < 0.000001	0.48	1.00	0.66	0.18	0.45	0.92	0.20	0.18	1.92	0.19	0.81	0.89	1.07	1.21	1.08	1.10
SATB2	PRTASQSSL-N	11	38	4	10%	24.3	p < 0.000001	0.45	1.00	1.15	1.04	0.96	0.11	0.08	0.11	0.01	0.18	0.65	0.18	0.22	0.42	1.04	0.31
SATB2	RD-IYQDE-E	10	228	13	6%	75.9	p < 0.000001	0.20	1.00	1.03	0.93	0.31	0.44	1.12	0.25	0.18	0.50	0.22	0.22	0.81	0.71	0.98	0.84

EIP 2019

Ultra-high density peptide microarray

# B cell epitope length



# Validation

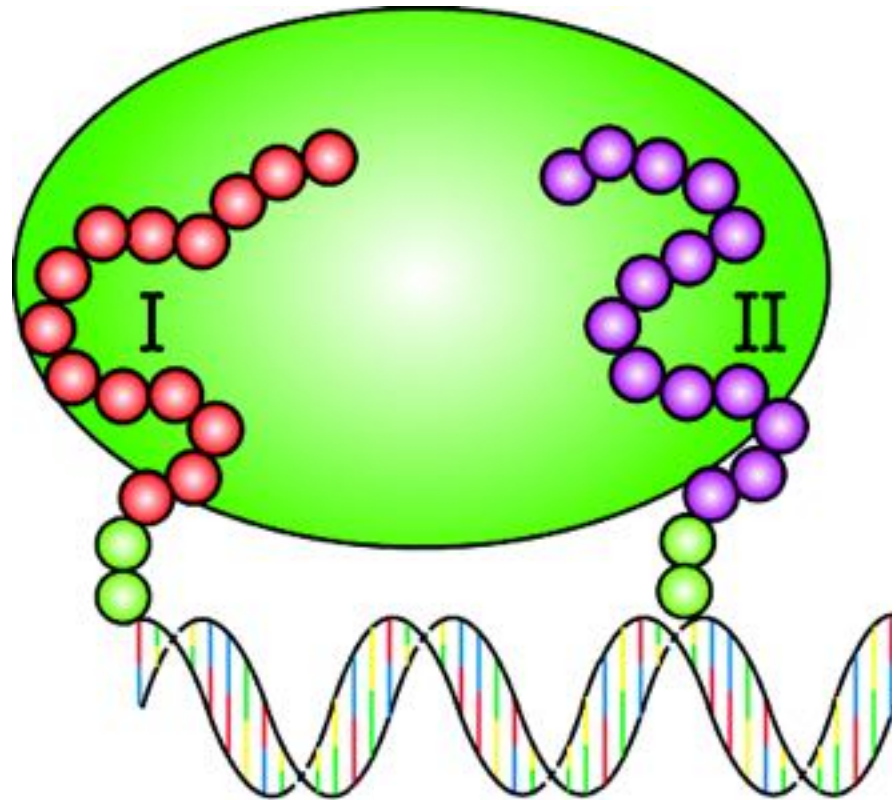
RAEVTEEFKDPSD DHYQNMKHV  
1 RAEVTEEFKDPSDRVMKLITSDVLEEMLNVDHDHYQNMKHV 40  
EF-DPS KLITSDV

LSLIKDDFFDNIKNIVLEHQQWHKDRKN  
41 ISVDKSKPKALKSLIKDDFFDNIKNIVLEHQQWHKDRKNP 80  
DKSKPK DFF-I-NIV

SKTNDGEEKMEG KAFSVV  
81 SLKSKTNDGEEKMEGNSQETERCERAESLAKIKSKAFSVV 120  
KTNDGEEKM

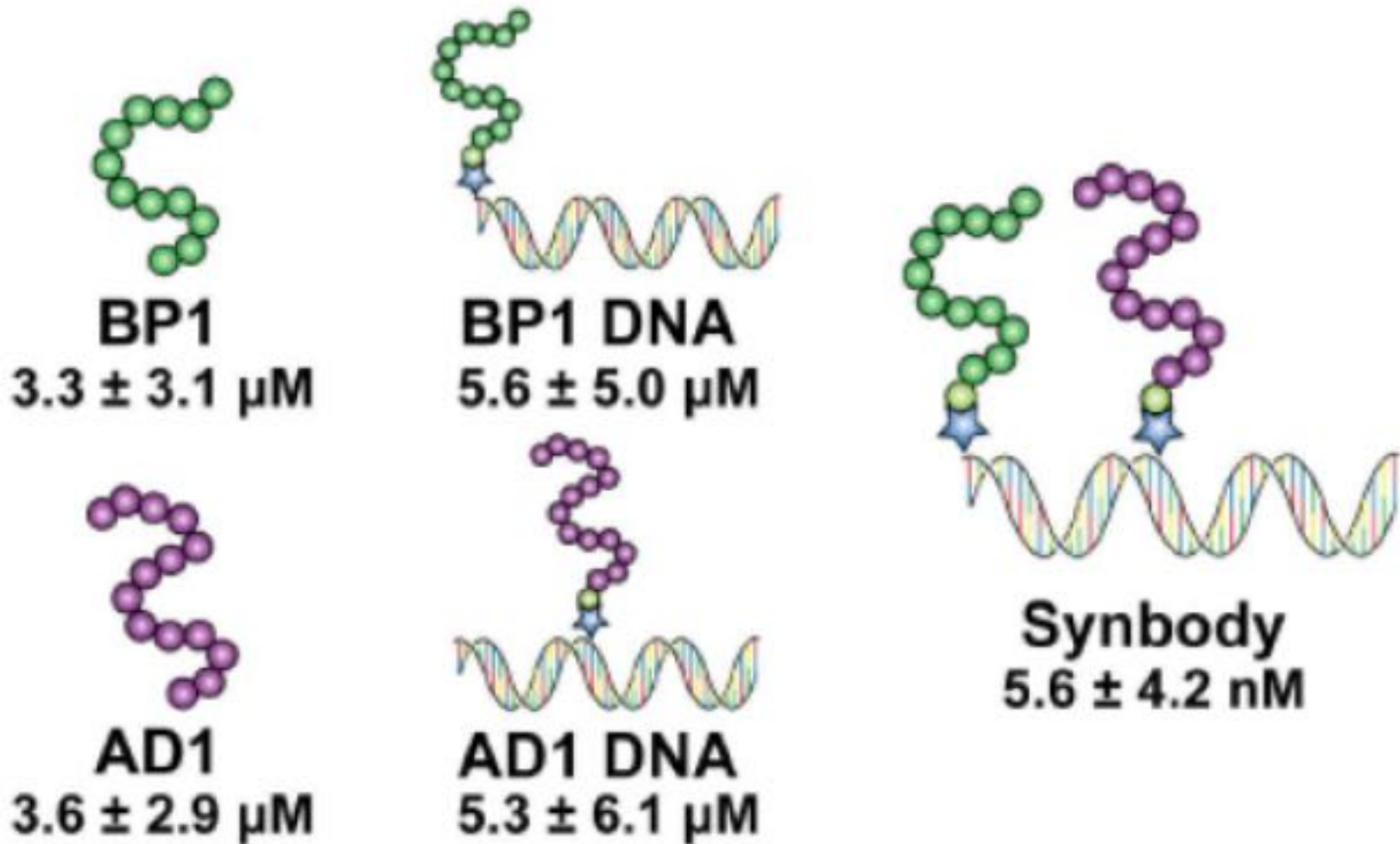
IQASKSRRHRQVKLDSSSDSDSASGQ  
121 IQASKSRHRQVKLDSSSDSDSASGQ  
L-S-D--S

# Can we address discontinuous epitopes?

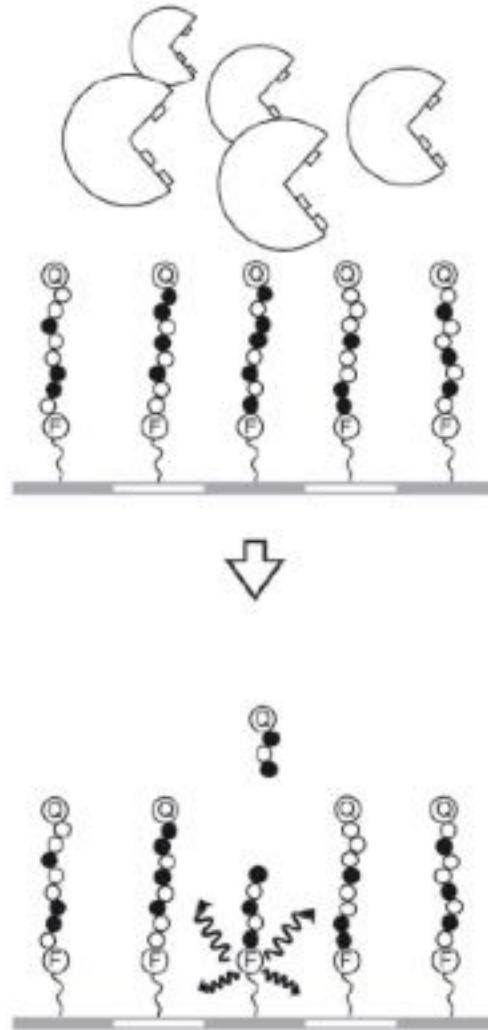


Williams et al, *J. Am. Chem. Soc.*, **2009**, *131* (47), pp 17233–17241

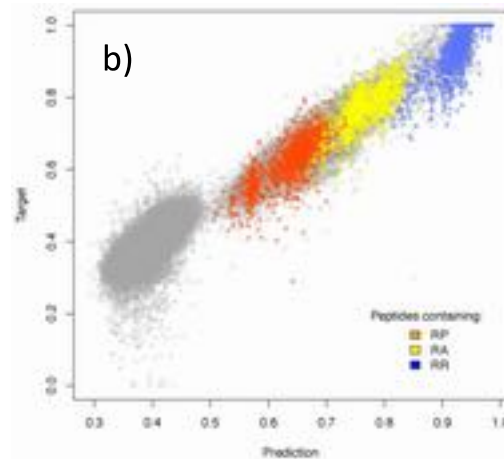
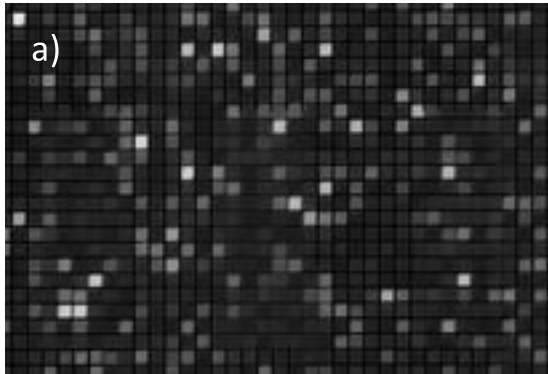
# Reconstructing a discontinuous epitope



# Protease peptidomics

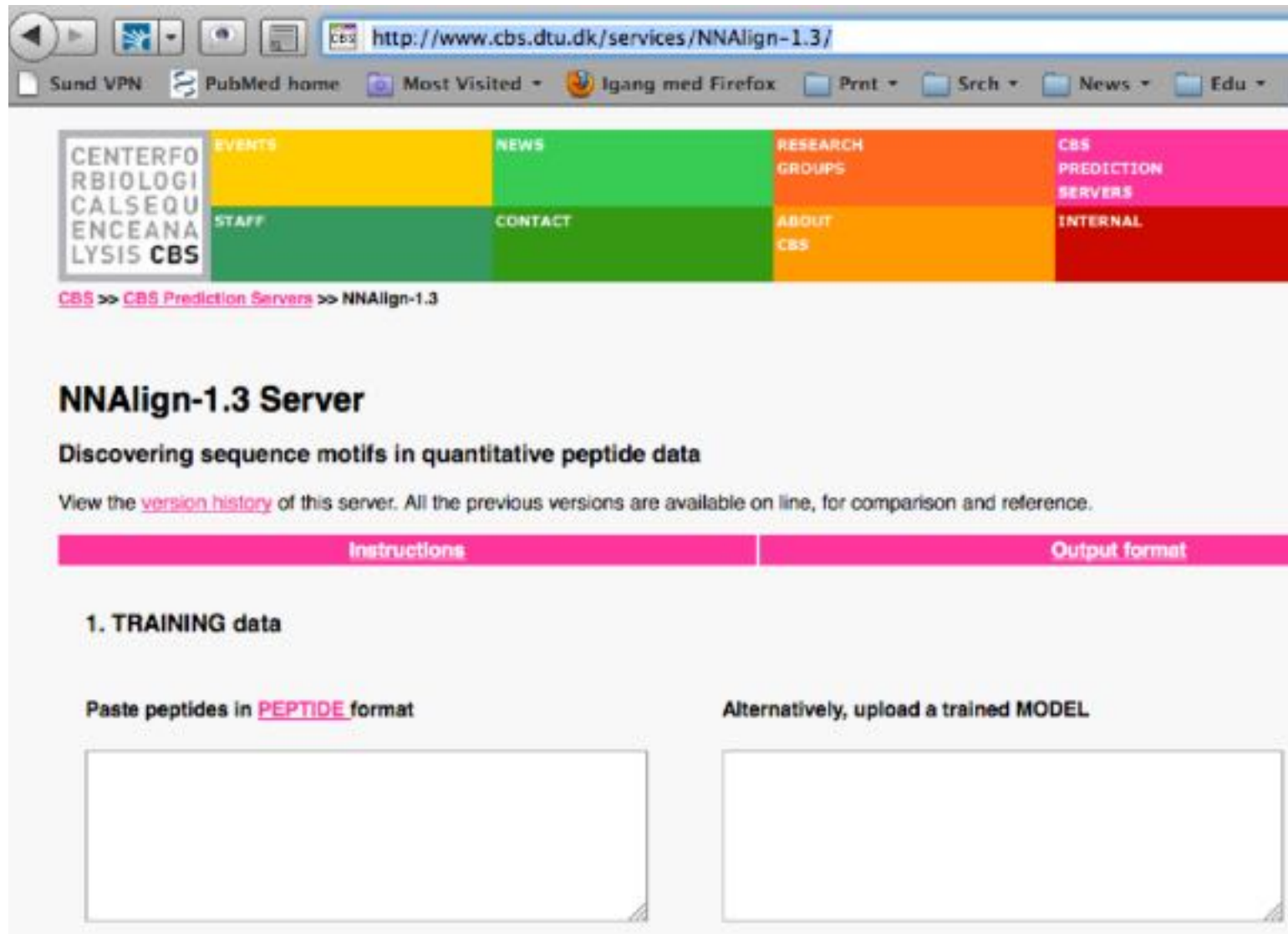


# Protease peptidomics



c)

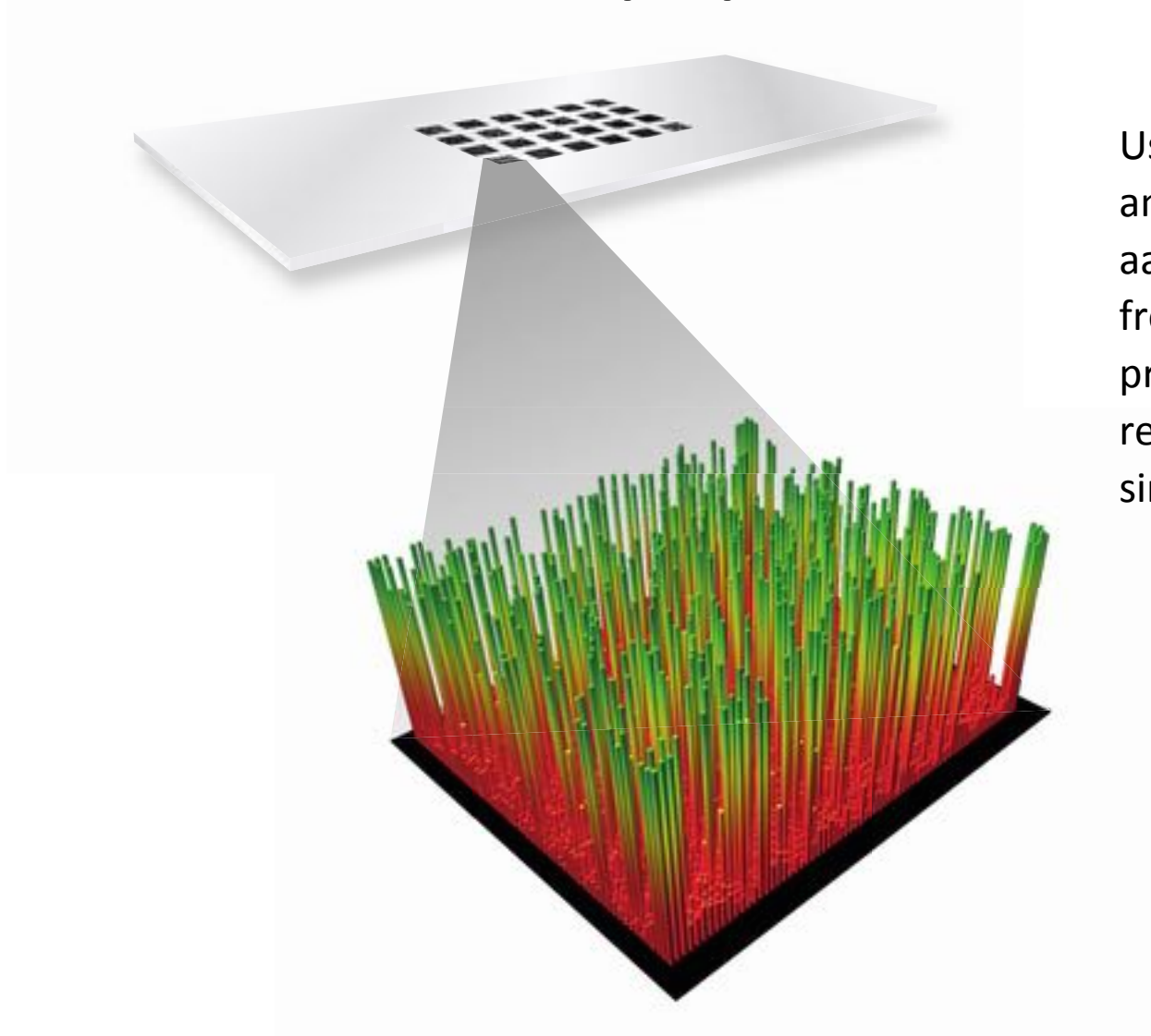
# End-user predictor: [NNAlign](http://www.cbs.dtu.dk/services/NNAlign-1.3/)



The screenshot shows a web browser window with the address bar displaying <http://www.cbs.dtu.dk/services/NNAlign-1.3/>. The browser's address bar also shows several bookmarks: Sund VPN, PubMed home, Most Visited, Igang med Firefox, Prnt, Srch, News, and Edu. The website's navigation menu is displayed in a grid of colored boxes: EVENTS (yellow), NEWS (green), RESEARCH GROUPS (orange), CBS PREDICTION SERVERS (pink), STAFF (dark green), CONTACT (dark green), ABOUT CBS (orange), and INTERNAL (red). The main content area features the CBS logo on the left and a breadcrumb trail: [CBS](#) >> [CBS Prediction Servers](#) >> [NNAlign-1.3](#). Below the breadcrumb trail is the heading **NNAlign-1.3 Server** and the subtitle **Discovering sequence motifs in quantitative peptide data**. A paragraph of text reads: "View the [version history](#) of this server. All the previous versions are available on line, for comparison and reference." Below this text is a horizontal navigation bar with two tabs: **Instructions** (selected) and **Output format**. The main content area is titled **1. TRAINING data** and contains two sections: "Paste peptides in **PEPTIDE** format" and "Alternatively, upload a trained MODEL". Each section has a large, empty text input box for user input.



# Proteome-wide peptide microarrays



Using 18-mers with an overlap of 12 aa, every 13-mer from the human proteome can be represented on a single slide

# PepChipOmics



**Schafer-N**

***Schafer-N***

**GenOptics / Horiba**



**JRC / EC**



**CBS / DTU**

CENTER FOR  
RIBIOMOLOGICAL  
SEQUENCE ANALYSIS  
CBS

**KTH**



**University of Mainz**



**University of Copenhagen**



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UNIVERSITÄT MAINZ

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# PepChipOmics

- Schafer-N ([www.schafer-n.com](http://www.schafer-n.com))
  - Claus Schafer-Nielsen
- Genoptics / Horiba
  - Emmanuel Maillart, Sophie Bellon
- JRC, Ispra
  - Pascal Colpo, Gerardo Marchesini
- Center for Biological Sequence Analysis, Technical University of Denmark
  - Massimo Andreatta, Morten Nielsen, Claus Lundegaard, & Ole Lund
- Royal Institute of Technology
  - Johan Rockberg, Peter Nilsson, Mathias Uhlen
- University of Mainz
  - Hans-Jorg Schild, Stefan Tenzer
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  - Thomas Østerbye, Lajla Bruntse Hansen & Soren Buus
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