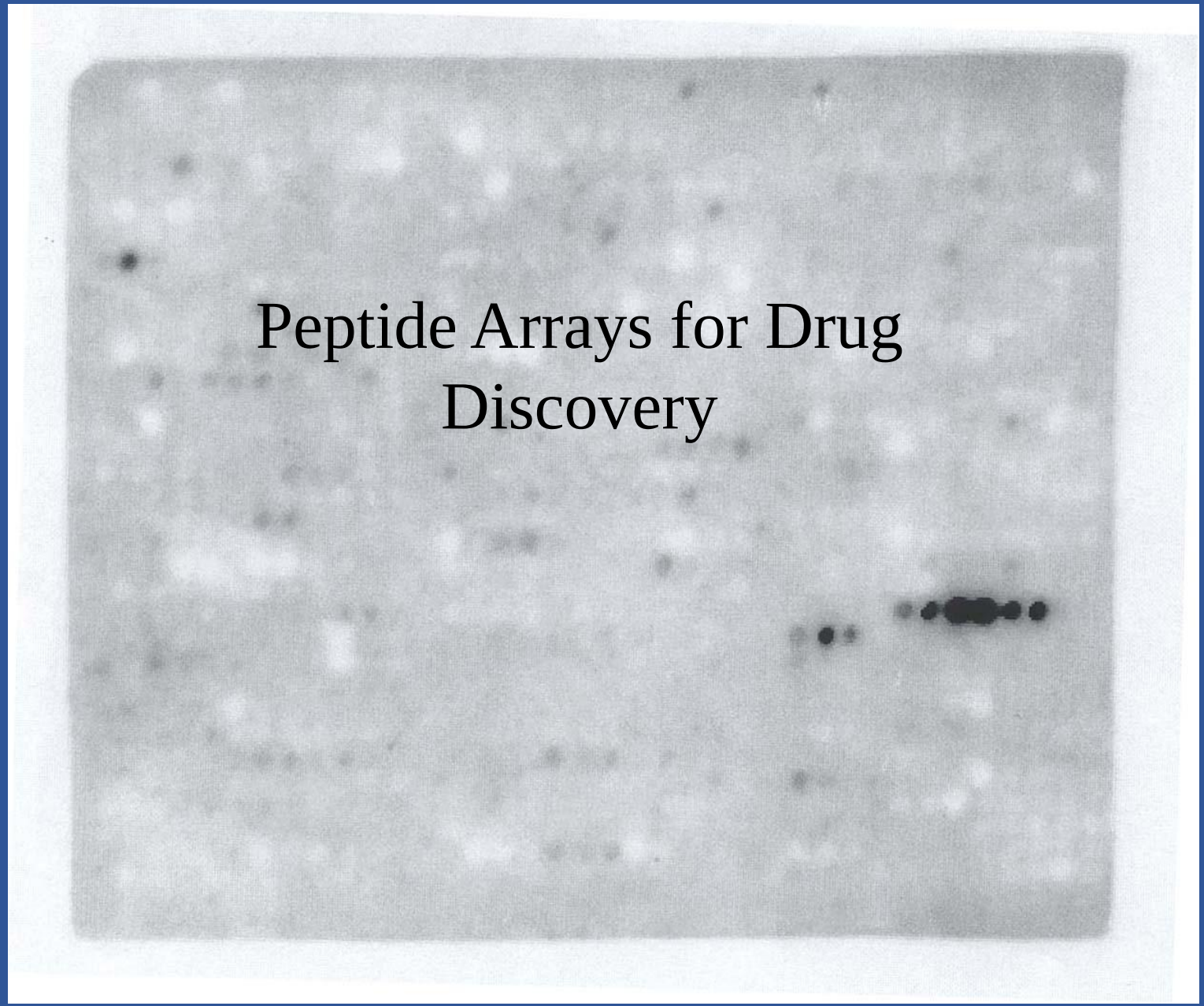
Peptide Arrays for Drug Discovery

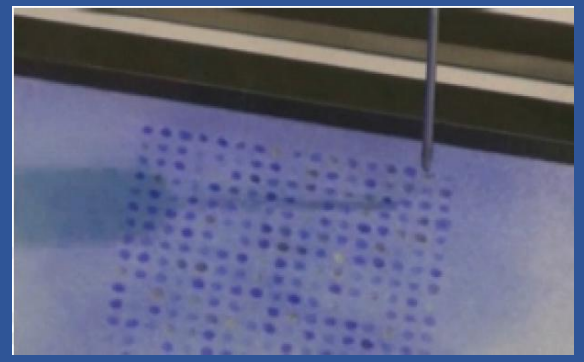


What you can do with a piece of Whatman filter paper

VERSA 110 Spotter

VERSA 110 Spotter

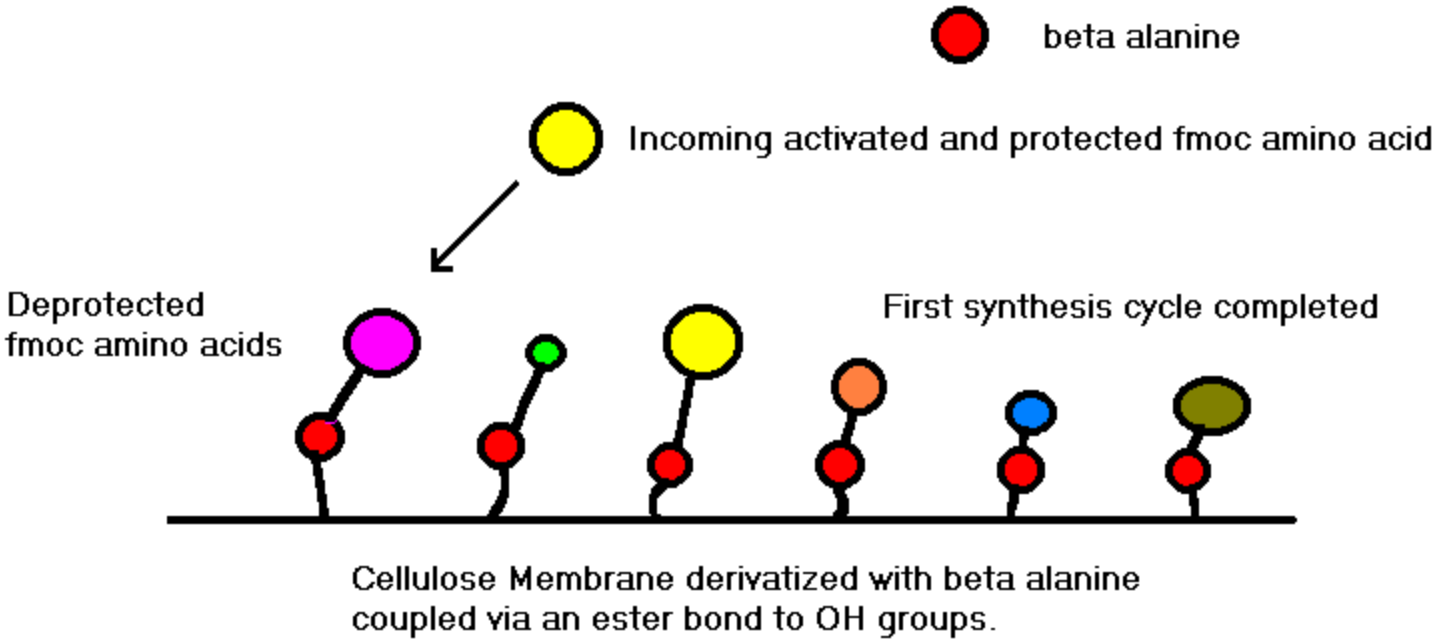
Deck layout



Results

Wash station

Synthesis of Peptides on a Cellulose Membrane



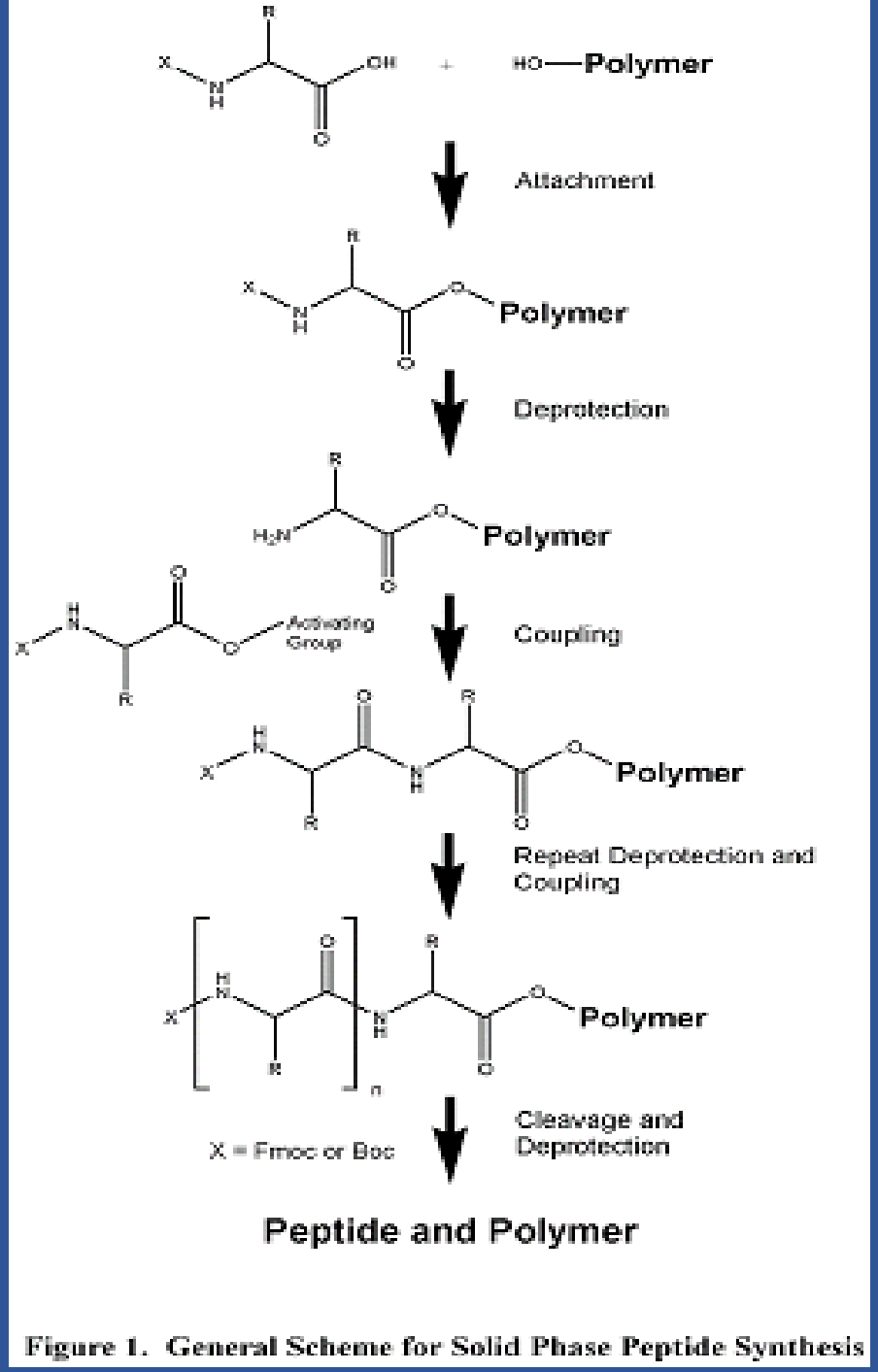


Figure 1. General Scheme for Solid Phase Peptide Synthesis

Cellulose membrane

Linked via ester bond to hydroxy group on cellulose membrane

Synthesis proceeds from the carboxy to amino terminal

Deprotection of primary amino group at each cycle followed by final deprotection of side chains. Peptides are not cleaved from the membrane after the final step as in usual solid phase synthesis.

Applications

- Peptide vaccines - identification of the antigen/antibody binding region, the epitope, with patient sera
- Ligand identification– identification of a binding partner by screening a peptide library using a peptide target region or protein
- Antimicrobial peptides – screening of a combinatorial library consisting of 48,000,000 peptides with the microbe of interest

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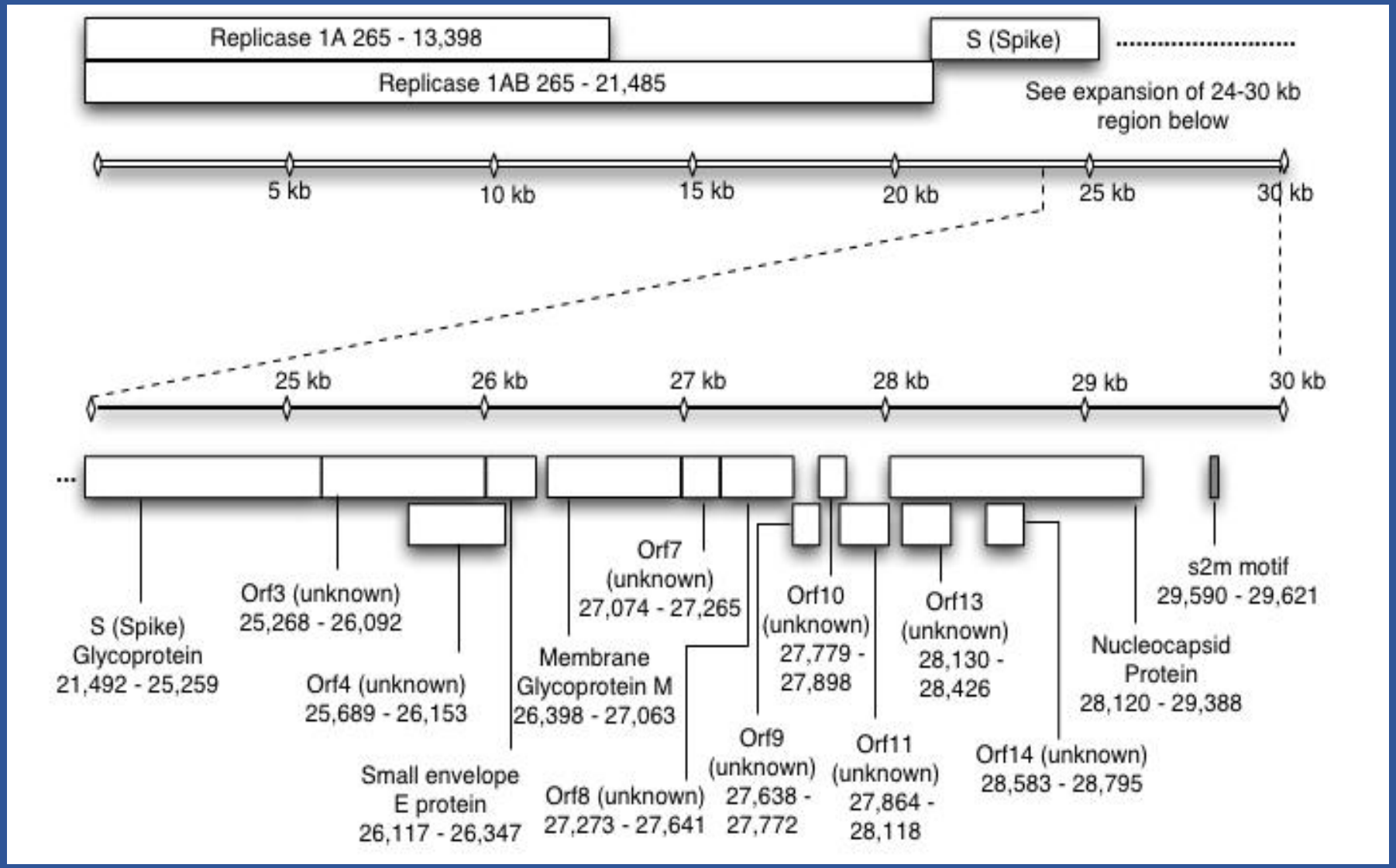
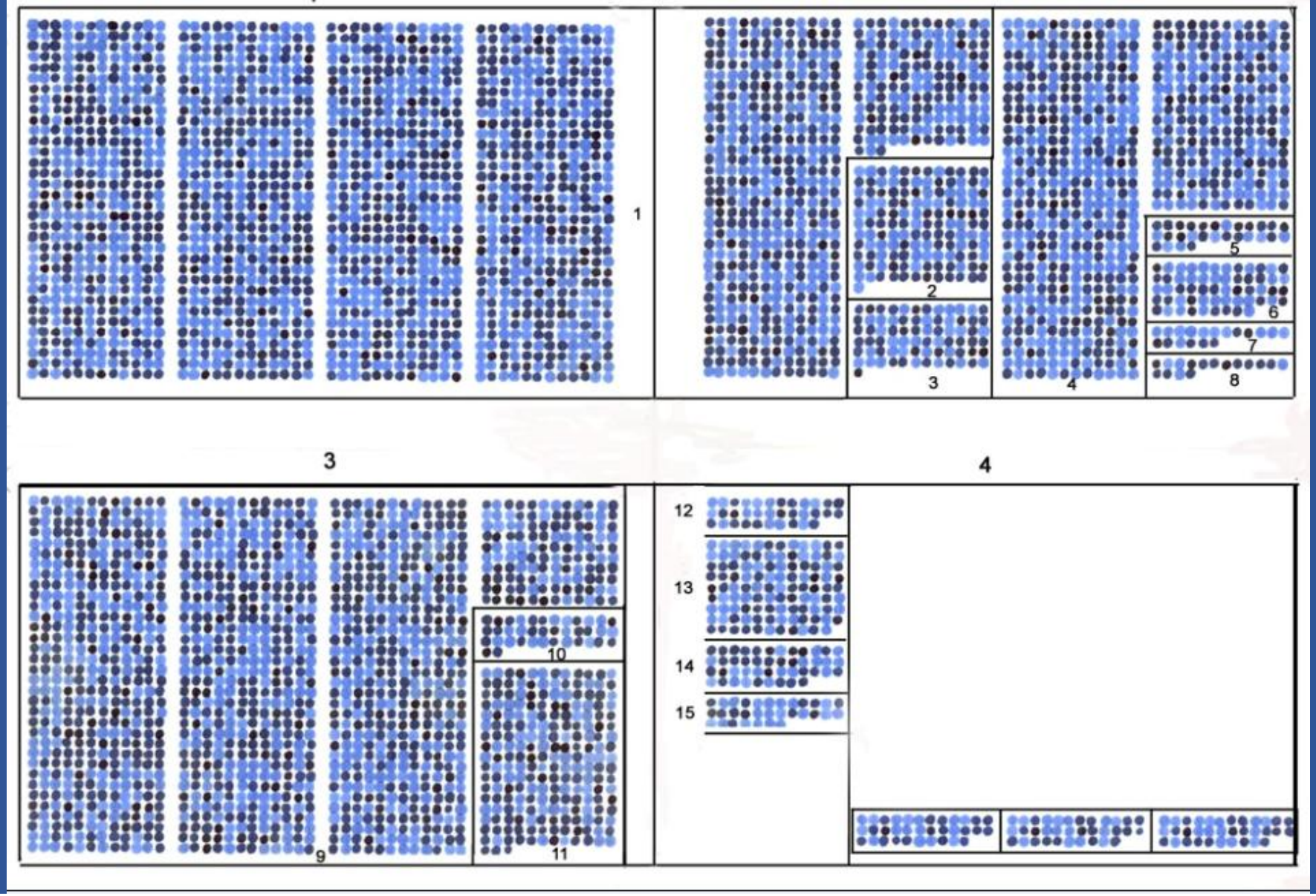
SARS OUTBREAK

- From November 2002 to July 2003 , 8442 probable cases of SARS were reported from 29 countries
- There were 916 deaths
- Toronto Canada reported 247 cases and 44 deaths


Replicase 1A 265 - 13,398
S (Spike)
Replicase 1AB 265 - 21,485
See expansion of 24-30 kb region below
5 kb
10 kb
15 kb
20 kb
25 kb
30 kb
25 kb
26 kb
27 kb
28 kb
29 kb
30 kb
S (Spike) Glycoprotein 21,492 - 25,259
Orf3 (unknown) 25,268 - 26,092
Orf4 (unknown) 25,689 - 26,153
Small envelope E protein 26,117 - 26,347
Membrane Glycoprotein M 26,398 - 27,063
Orf8 (unknown) 27,273 - 27,641
Orf7 (unknown) 27,074 - 27,265
Orf9 (unknown) 27,638 - 27,772
Orf10 (unknown) 27,779 - 27,898
Orf11 (unknown) 27,864 - 28,118
Orf13 (unknown) 28,130 - 28,426
Orf14 (unknown) 28,583 - 28,795
Nucleocapsid Protein 28,120 - 29,388
s2m motif 29,590 - 29,621



1.	ORF 1a	4. S protein	7. ORF 9	10. ORF 11	13. M protein
2.	ORF 3	5. ORF 7	8. ORF 10	11. N protein	14. ORF 13
3.	ORF 4	6. ORF 8	9. ORF 1b	12. E	15. ORF 14

Overlapping
Peptide sequences

1	R E P H R A A V T P	1133.6
2	E P H R A A V T P H	1114.6
3	P H R A A V T P H F	1132.7
4	H R A A V T P H F L	1148.7
5	R A A V T P H F L K	1139.7
6	A A V T P H F L K S	1071.4
7	A V T P H F L K S V	1099.5
8	V T P H F L K S V P	1125.6
9	T P H F L K S V P T	1127.5
10	P H F L K S V P T V	1125.6
11	H F L K S V P T V L	1141.6
12	F L K S V P T V L S	1092.3
13	L K S V P T V L S K	1073.3
14	K S V P T V L S K F	1107.3
15	S V P T V L S K F P	1076.3
16	V P T V L S K F P V	1087.6
17	P T V L S K F P V P	1085.6
18	T V L S K F P V P P	1085.6
19	V L S K F P V P P S	1072.4
20	L S K F P V P P S T	1074.3
21	S K F P V P P S T P	1058.3
22	K F P V P P S T P T	1071.5
23	F P V P P S T P T T	1044.4
24	P V P P S T P T T T	998.3
25	V P P S T P T T T L	1014.3
26	P P S T P T T T L L	1028.3
27	P S T P T T T L L V	1030.3
28	S T P T T T L L V C	1036.3
29	T P T T T L L V C P	1045.6
30	P T T T L L V C P T	1045.6
31	T T T L L V C P T P	1045.6
32	T T L L V C P T P S	1032.4

One amino acid
frameshift

11-EAPRRRSPSPTPTPGPSRRGPSLGASSHQHSRRRQGWLKEIRKLQKST-58

1EAPRRRSPSP

2PRRRSPSPTP

3RRSPSPTPTP

4SPSPTPTPGP

5SPTPTPGPSR

6TPTPGPSRRG

7TPGPSRRGPS

8GPSRRGPSLG

9SRRGPSLGAS

10RGPSLGASSH

11PSLGASSHQH

12LGASSHQHSR

13ASSHQHSRRR

14SHQHSRRRQG

15QHSRRRQGWL

16SRRRQGWLKE

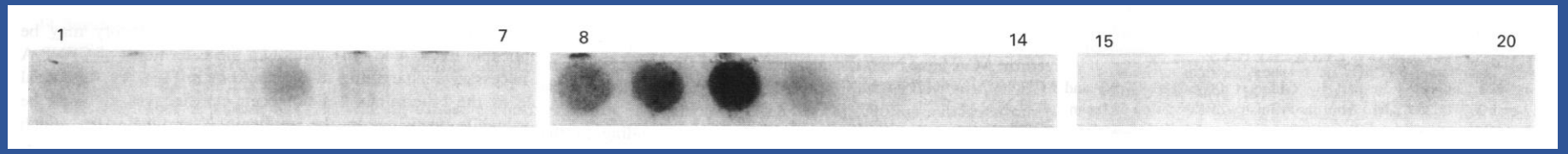
17RRQGWLKEIR

18QGWLKEIRKL

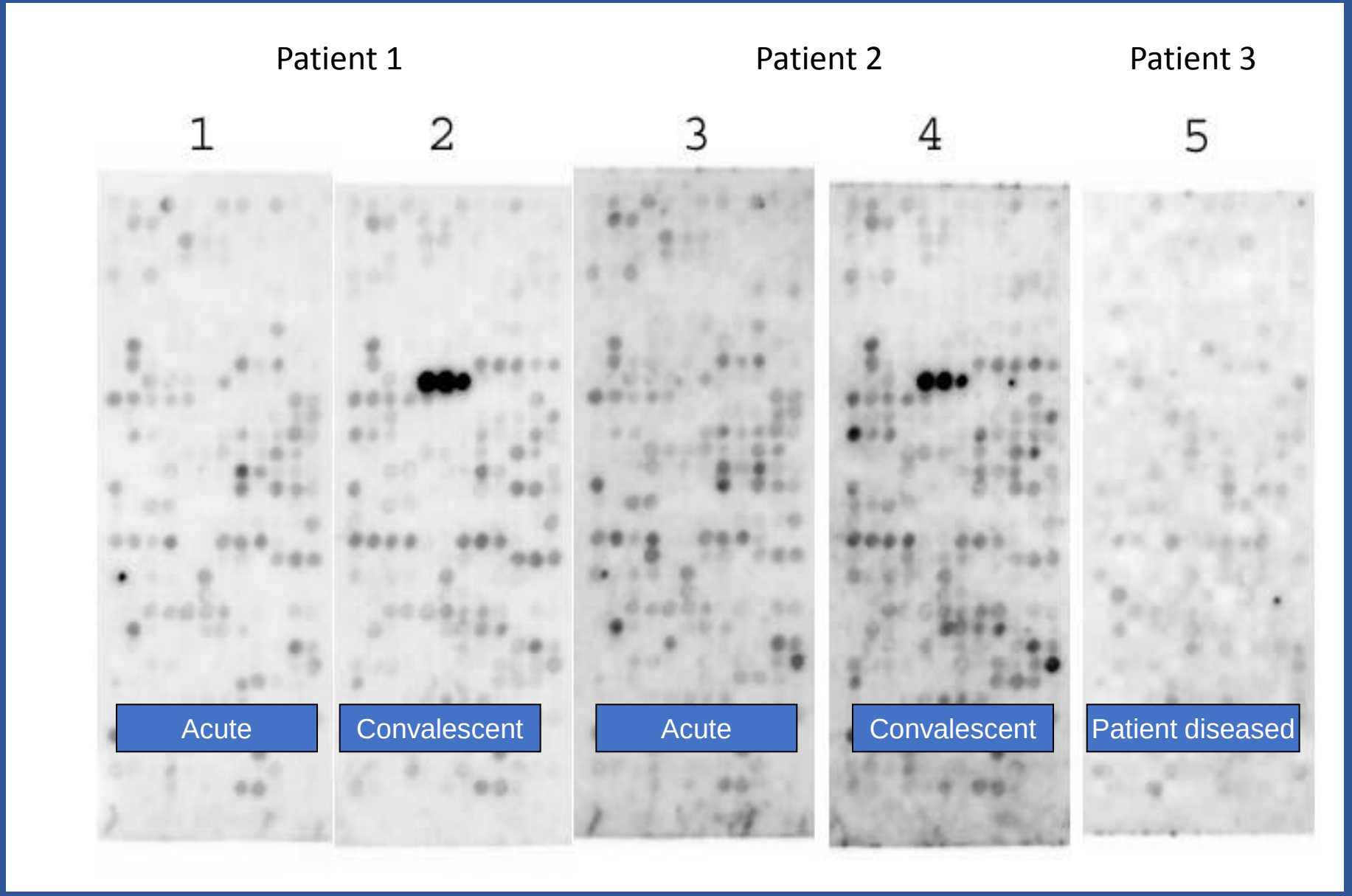
19WLKEIRKLQK

20KEIRKLQKST

1 8 9 10 20



The SARS peptide arrays were probed with patient and control sera and developed with anti IgA, IgG and IgM Secondary antibodies to identify epitopes specifically recognized by infected individuals.



SARS peptides recognized in convalescent sera (2 and 4) but not acute sera (1 and 3) or in a deceased case (5)


Vaccine

Applications

- Peptide vaccines - identification of the antigen/antibody binding region, the epitope, with patient sera
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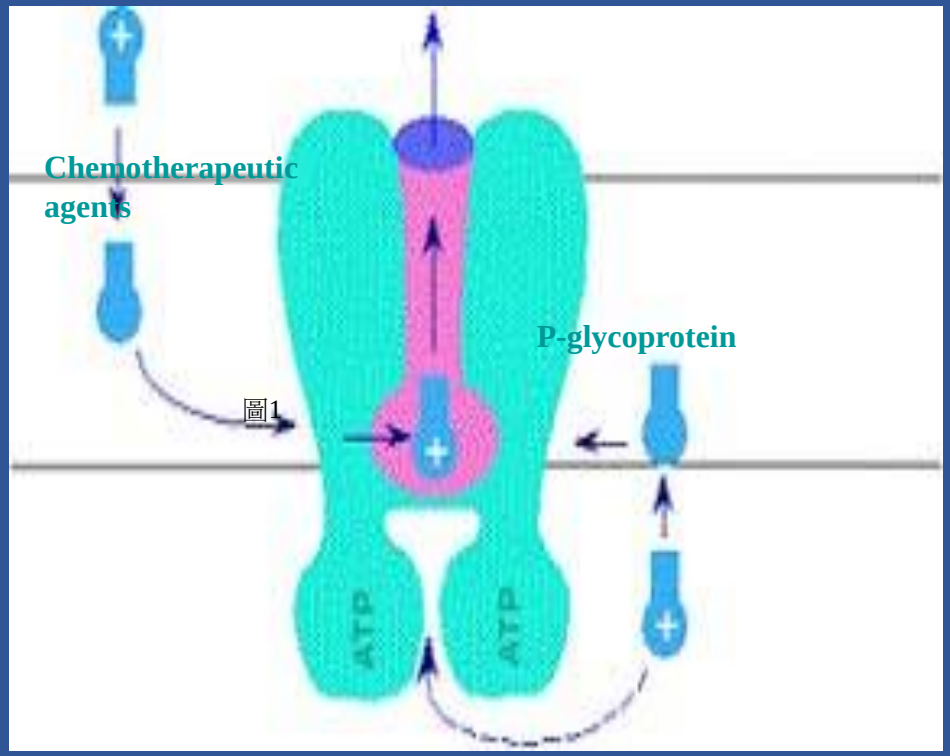
- Antimicrobial peptides – screening of a combinatorial library consisting of 48,000,000 peptides with the microbe of interest

Identification of Peptide Inhibitors of the Multidrug Transporter PgP

Identification of PGP Inhibitors

TM6 peptide was synthesized with a biotin tag



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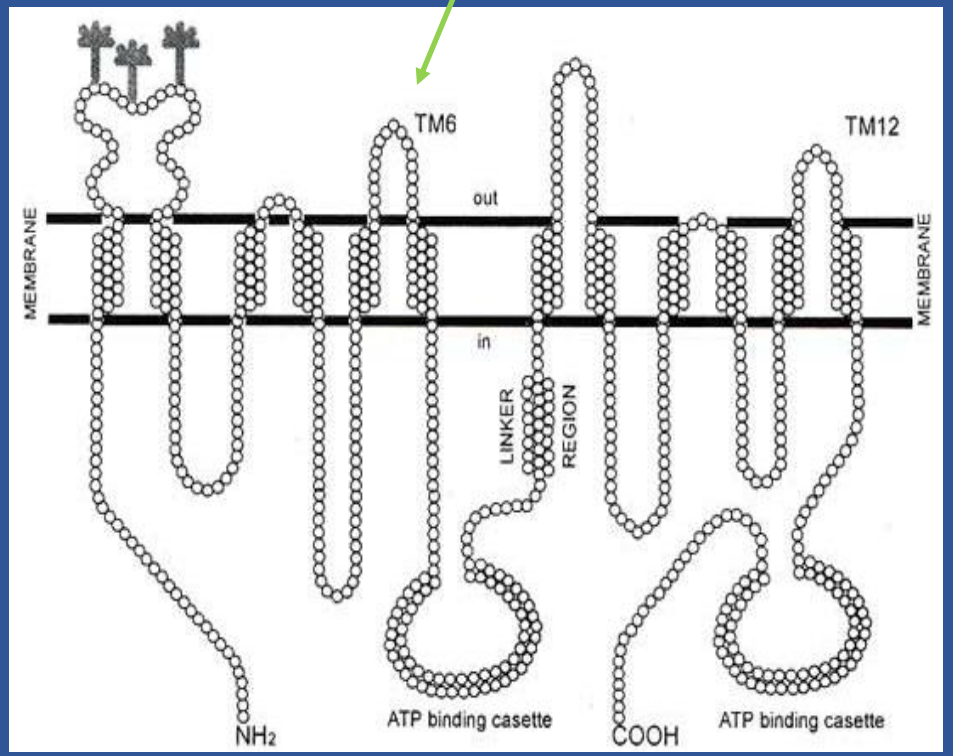
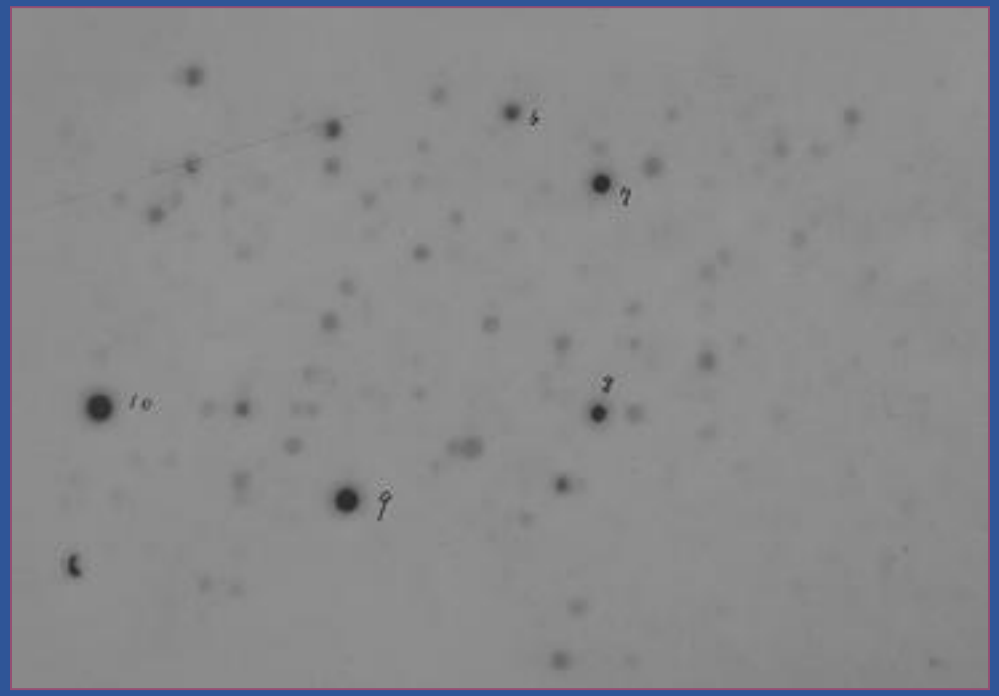


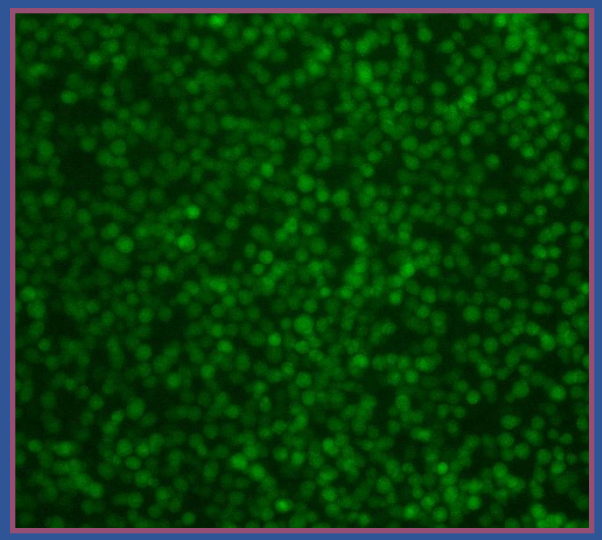
Diagram of PGP Molecule

Identification of PGP Inhibitors using proprietary array

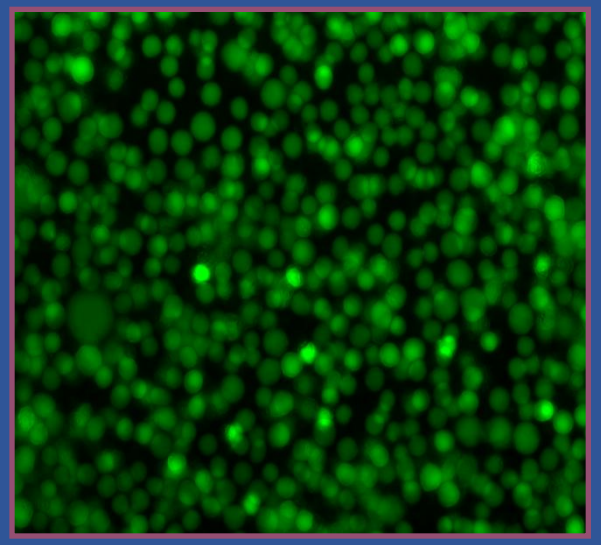
Using peptide TM6 to probe array; 5 strong hits were selected



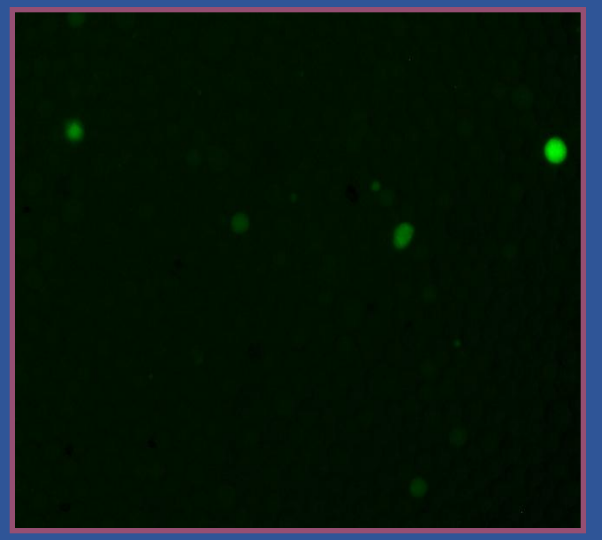
Inhibition of PGP using Calcein-AM as an indicator



0.25uM peptide P6+0.25uM Calcein AM, incubate for 15 minutes



Positive control: Verapmil + Calcein AM



Negative control: Calcein AM only

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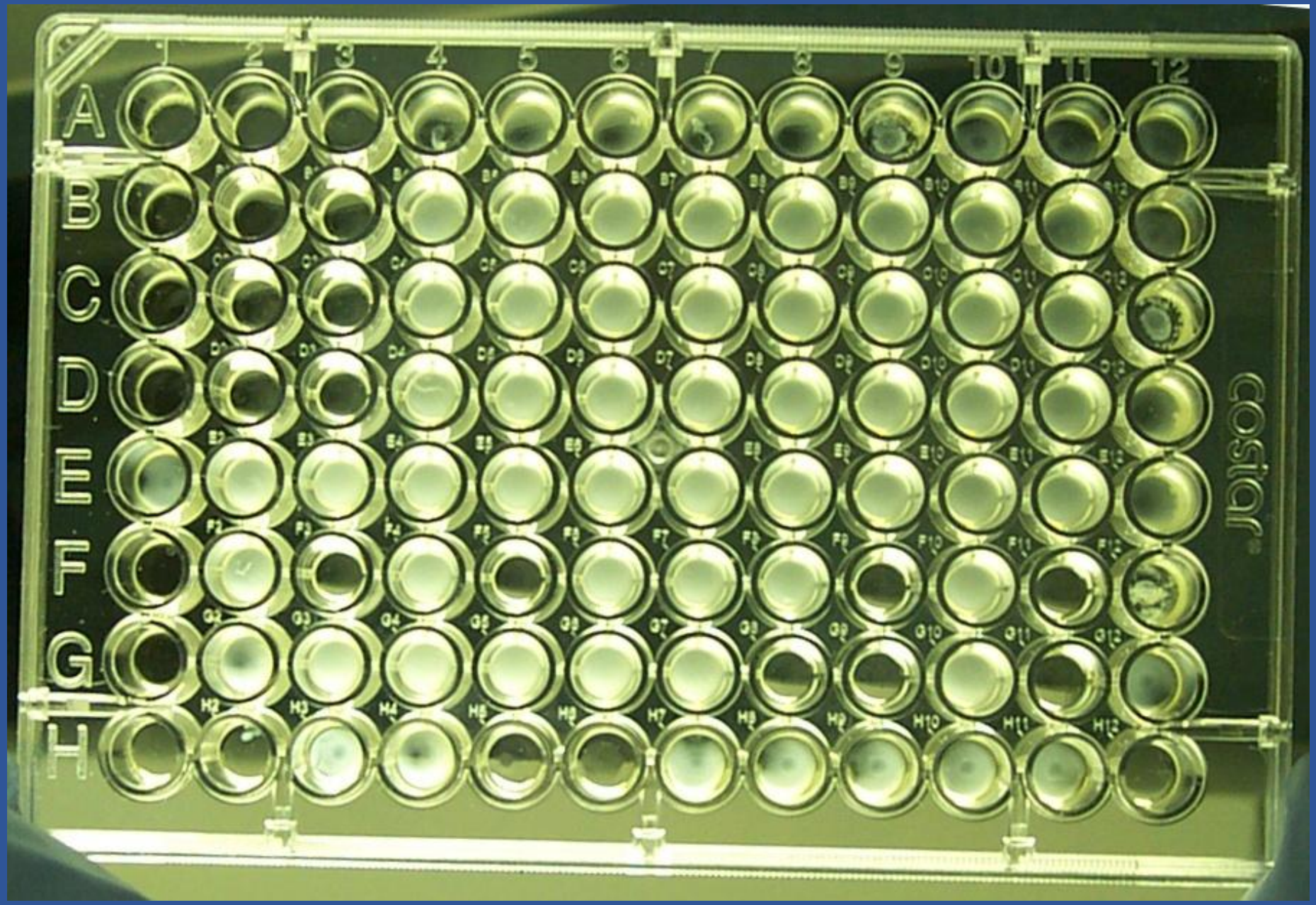
- Ligand identification– identification of a binding partner by screening a peptide library using a peptide target region or protein
- Antimicrobial agent – screening of a combinatorial library consisting of 48,000,000 peptides with the microbe of interest

Our peptide libraries contain up to 48,000,000 unique structures. Identification of the best candidate with highest affinity to any target is rapid and cost effective. Libraries can be Constructed with non natural amino acids ensuring stability to proteolytic degradation.

Combinatorial library Sequence motif: CKKPKKPK-X-X-B_1-B_2-X-X-C

B_1 \ B_2	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	AA	AD	AE	AF	AG	AH	AI	AK	AL	AM	AN	AP	AQ	AR	AS	AT	AV	AW	AY
D	DA	DD	DE	DF	DG	DH	DI	DK	DL	DM	DN	DP	DQ	DR	DS	DT	DV	DW	DY
E	EA	ED	EE	EF	EG	EH	EI	EK	EL	EM	EN	EP	EQ	ER	ES	ET	EV	EW	EY
F	FA	FD	FE	FF	FG	FH	FI	FK	FL	FM	FN	FP	FQ	FR	FS	FT	FV	FW	FY
G	GA	GD	GE	GF	GG	GH	GI	GK	GL	GM	GN	GP	GQ	GR	GS	GT	GV	GW	GY
H	HA	HD	HE	HF	HG	HH	HI	HK	HL	HM	HN	HP	HQ	HR	HS	HT	HV	HW	HY
I	IA	ID	IE	IF	IG	IH	II	IK	IL	IM	IN	IP	IQ	IR	IS	IT	IV	IW	IY
K	KA	KD	KE	KF	KG	KH	KI	KK	KL	KM	KN	KP	KQ	KR	KS	KT	KV	KW	KY
L	LA	LD	LE	LF	LG	LH	LI	LK	LL	LM	LN	LP	LQ	LR	LS	LT	LV	LW	LY
M	MA	MD	ME	MF	MG	MH	MI	MK	ML	MM	MN	MP	MQ	MR	MS	MT	MV	MW	MY
N	NA	ND	NE	NF	NG	NH	NI	NK	NL	NM	NN	NP	NQ	NR	NS	NT	NV	NW	NY
P	PA	PD	PE	PF	PG	PH	PI	PK	PL	PM	PN	PP	PQ	PR	PS	PT	PV	PW	PY
Q	QA	QD	QE	QF	QG	QH	QI	QK	QL	QM	QN	QP	QQ	QR	QS	QT	QV	QW	QY
R	RA	RD	RE	RF	RG	RH	RI	RK	RL	RM	RN	RP	RQ	RR	RS	RT	RV	RW	RY
S	SA	SD	SE	SF	SG	SH	SI	SK	SL	SM	SN	SP	SQ	SR	SS	ST	SV	SW	SY
T	TA	TD	TE	TF	TG	TH	TI	TK	TL	TM	TN	TP	TQ	TR	TS	TT	TV	TW	TY
V	VA	VD	VE	VF	VG	VH	VI	VK	VL	VM	VN	VP	VQ	VR	VS	VT	VV	VW	VY
W	WA	WD	WE	WF	WG	WH	WI	WK	WL	WM	WN	WP	WQ	WR	WS	WT	WV	WW	WY
Y	YA	YD	YE	YF	YG	YH	YI	YK	YL	YM	YN	YP	YQ	YR	YS	YT	YV	YW	YY


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
costar

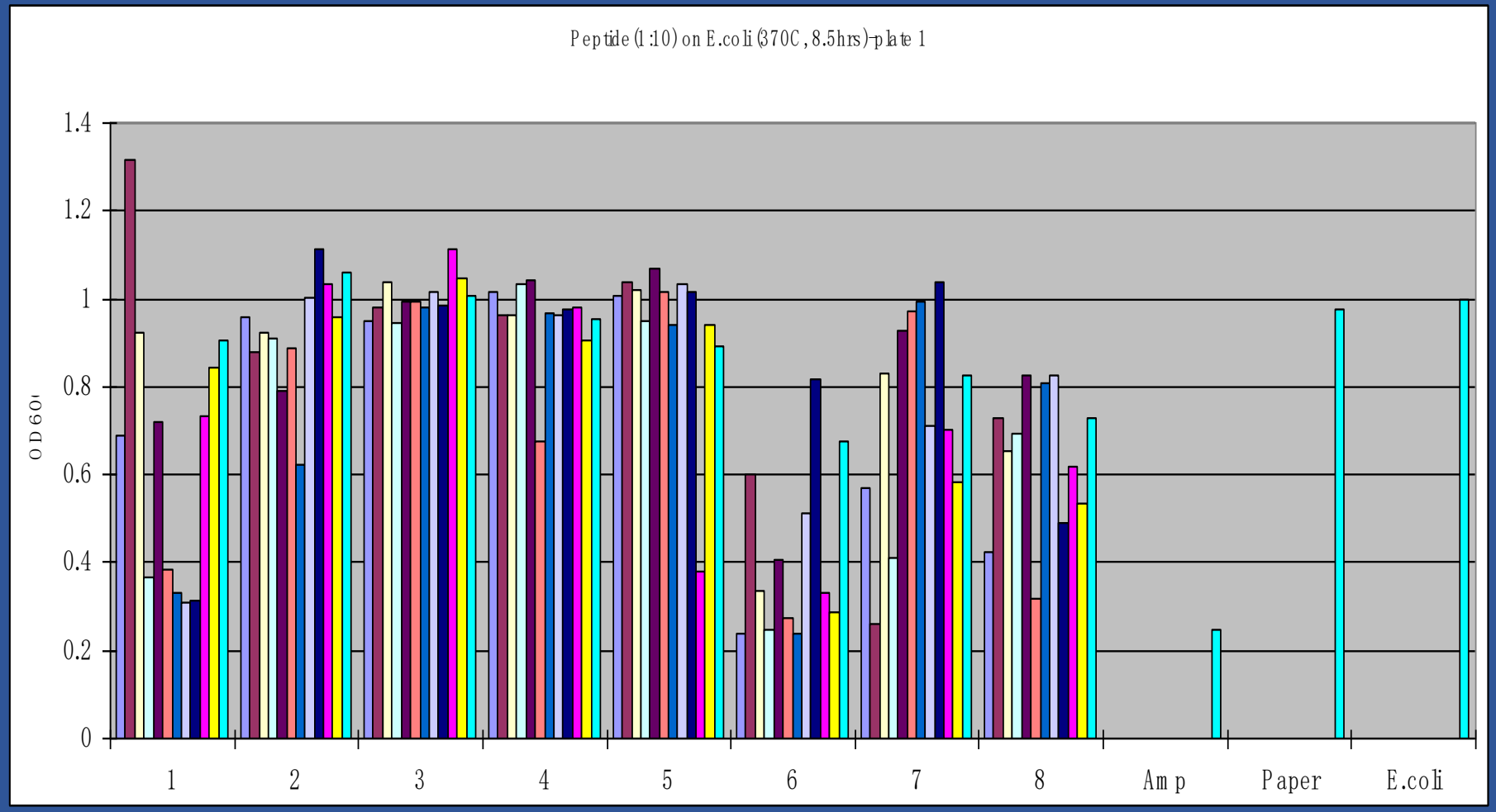


Plate 1 First cycle

Applications

- Peptide vaccines - identification of the antigen/antibody binding region, the epitope, with patient sera
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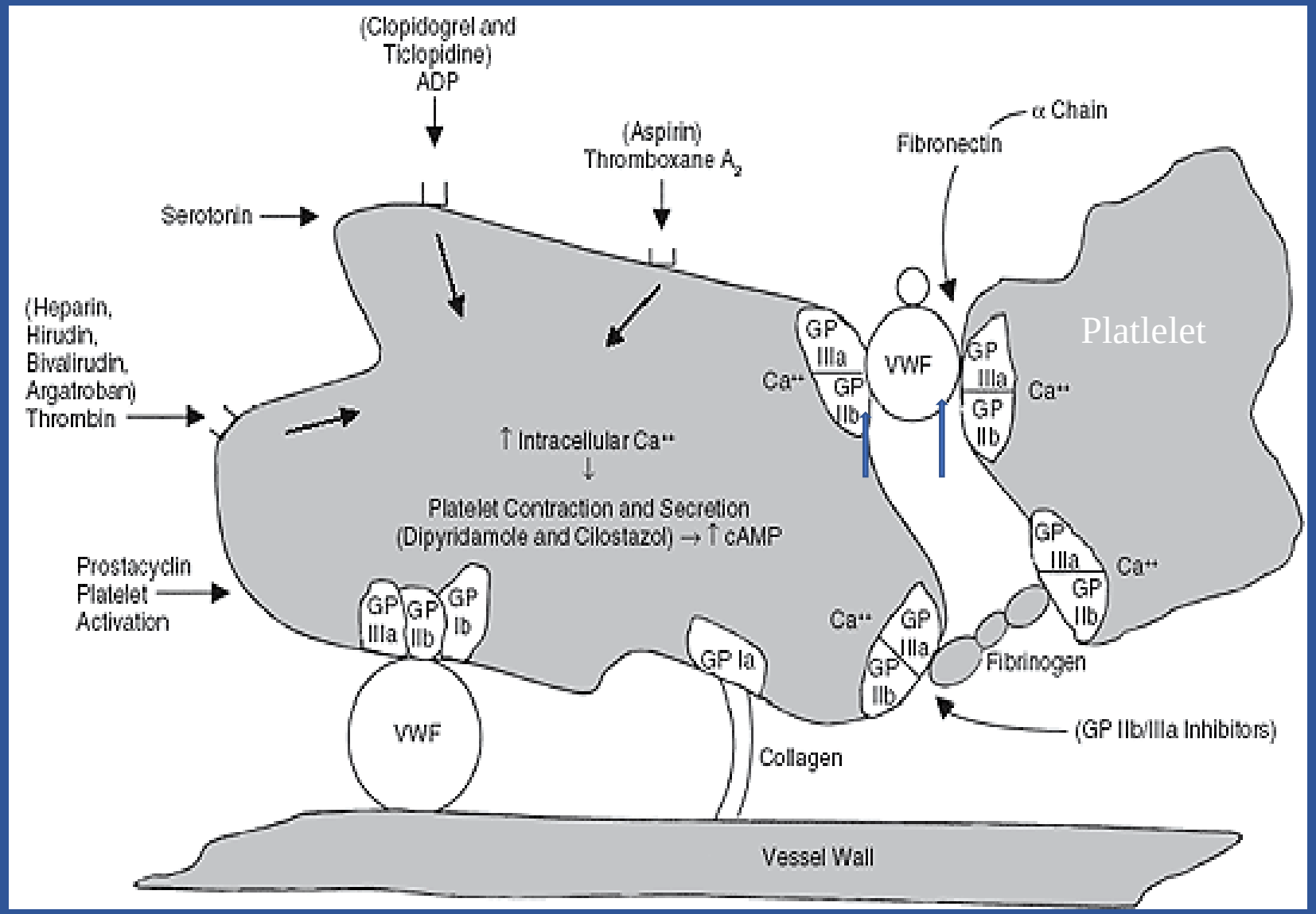
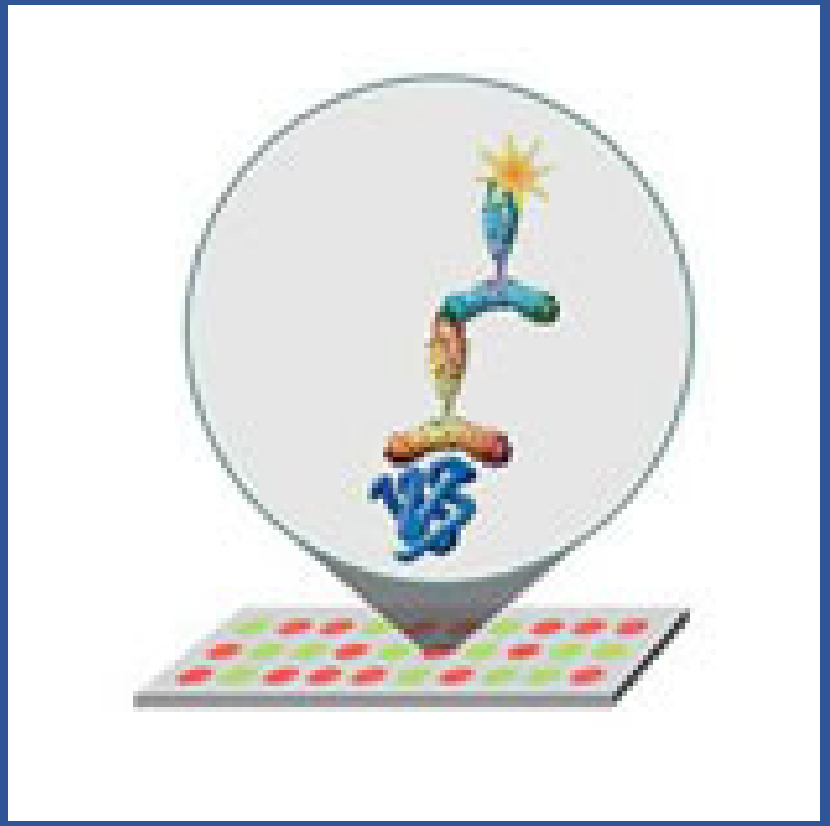
Antithrombotic Drug Design Using a Combination of Peptide Array Screening and Molecular Dynamics Simulation

At the molecular level, the critical events in thrombus formation, the adhesion and aggregation of platelets, are mediated by platelet integrins, GPIb, which upon conformational activation, binds to von Willebrand factor (vWf)

We wished to design a peptidomimetic that would exert GPIb-like receptor function and antithrombotic function by inhibiting the GPIb-vWf protein-protein interaction.

Peptides were chosen to block interaction of VWF and GP11b





Tagged Anti-antibody

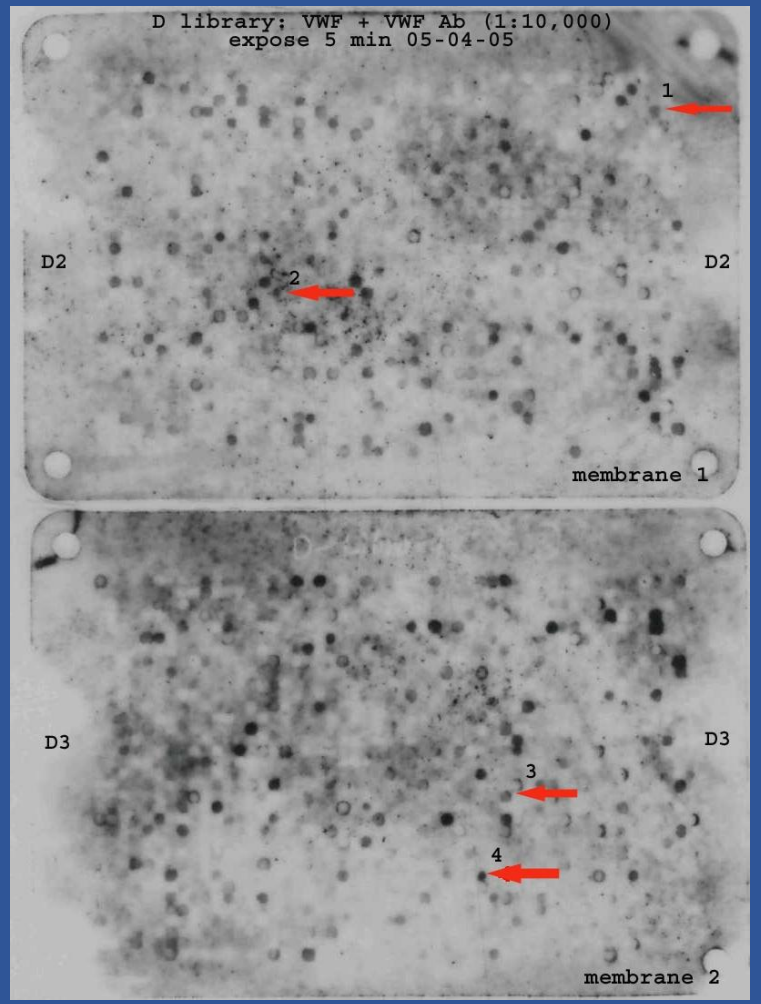
Antibody to VWF

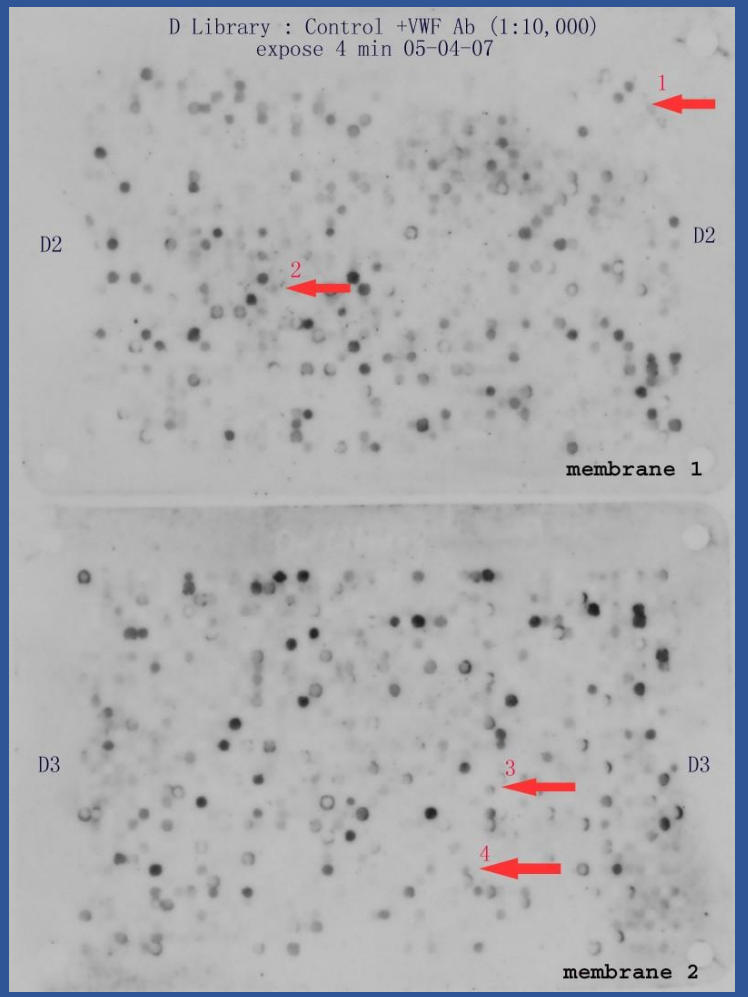
VWF

Peptide array

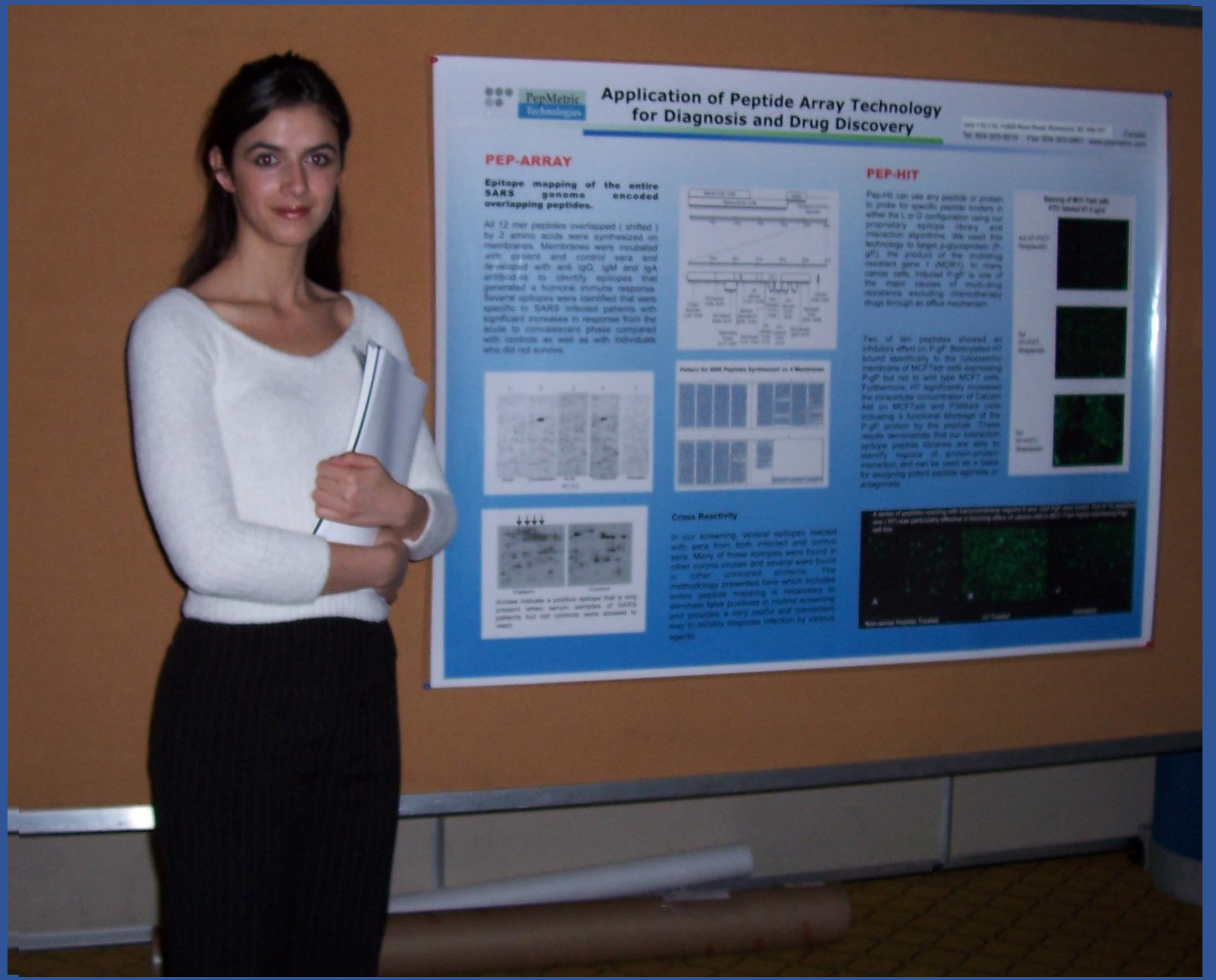
Screening for peptides binding to VWF

Four peptides were found that fit into the interaction site between Vwf and GP11b



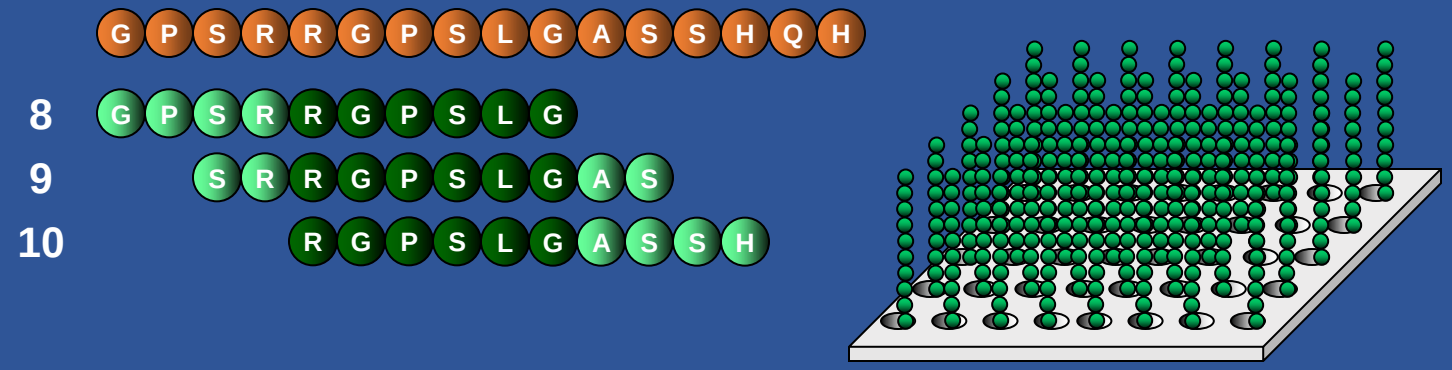


THANK YOU


PepMetric Technologies
Application of Peptide Array Technology for Diagnosis and Drug Discovery
PEP-ARRAY
Epitope mapping of the entire SARS genome encoded overlapping peptides.
All 12 mer peptides overlapped (shifted) by 2 amino acids were synthesized on membranes. Membranes were incubated with patient and control sera and developed with anti IgG, IgM and IgA antibodies to identify epitopes that generated a humoral immune response. Several epitopes were identified that were specific to SARS infected patients with significant increases in response from the acute to convalescent phase compared with controls as well as with individuals who did not survive.
Arrows indicate a positive epitope that is only present when serum samples of SARS patients but not controls were allowed to react.
Cross Reactivity
In our screening, several epitopes reacted with sera from both infected and control sera. Many of these epitopes were found in other corona viruses and several were found in other unrelated proteins. The methodology presented here which includes entire peptide mapping is necessary to eliminate false positives in routine screening and provides a very useful and convenient way to reliably diagnose infection by various agents.
PEP-HIT
Pep-Hit can use any peptide or protein to probe for specific peptide binders in either the L or D configuration using our proprietary epitope library and interaction algorithms. We used this technology to target p-glycoprotein (P-gP), the product of the multidrug resistant gene 1 (MDR1), in many cancer cells. Induced P-gP is one of the major causes of multi-drug resistance excluding chemotherapy drugs through an efflux mechanism.
Two of ten peptides showed an inhibitory effect on P-gP. Biotinylated H7 bound specifically to the cytoplasmic membrane of MCF7adr cells expressing P-gP but not to wild type MCF7 cells. Furthermore, H7 significantly increased the intracellular concentration of Calcein AM on MCF7adr and P388adr cells indicating a functional blockage of the P-gP protein by the peptide. These results demonstrate that our interaction epitope peptide libraries are able to identify regions of protein-protein interaction and can be used as a basis for designing potent peptide agonists or antagonists.

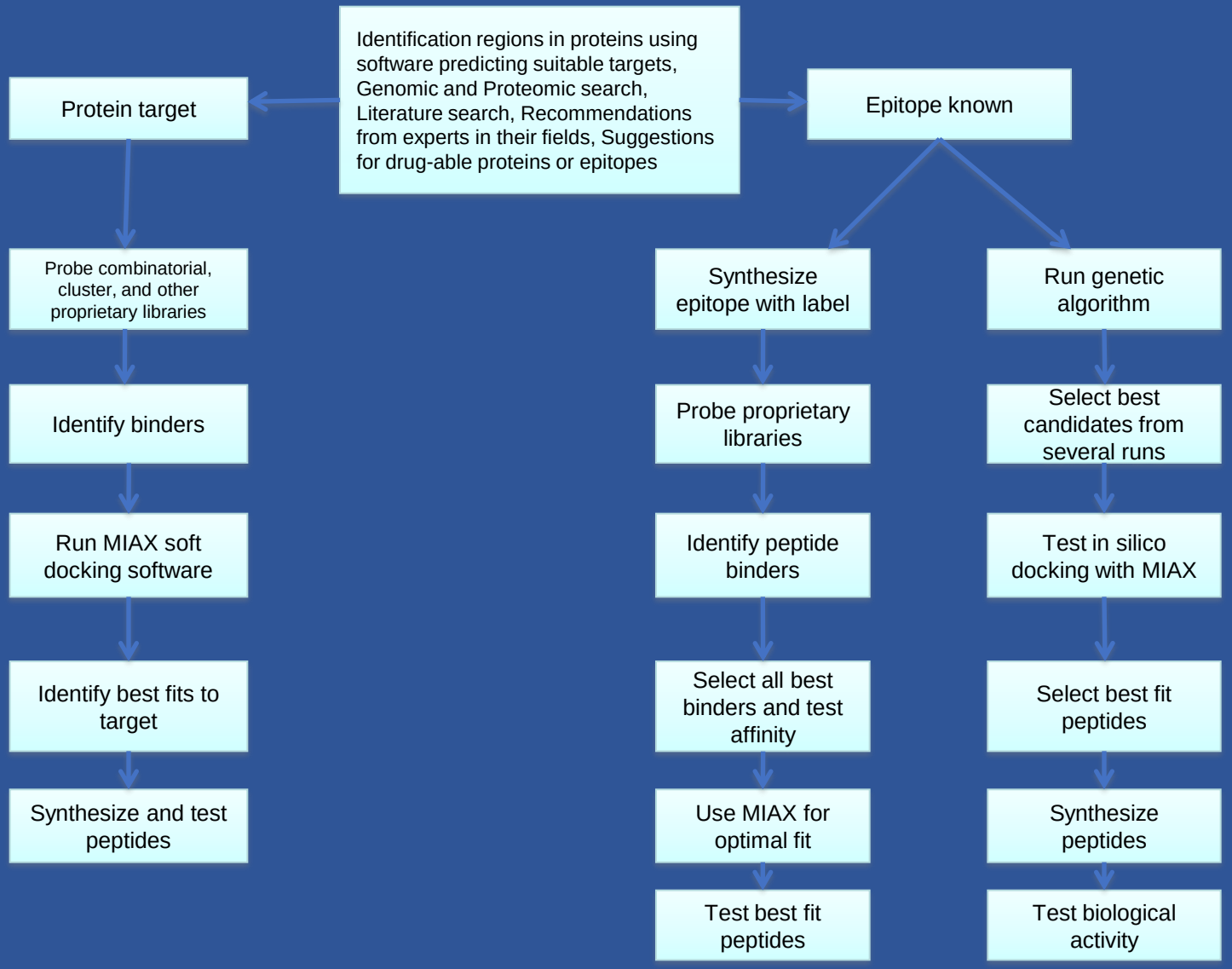
PEP-ARRAY Arrays of defined protein overlapping peptides

- Overlapping sequence design increases sensitivity
- Layer-by-layer direct synthesis
- “Stand-up” immobilization
- Wide choice of labeling, annotations and modifications
- Versatile reagent applications

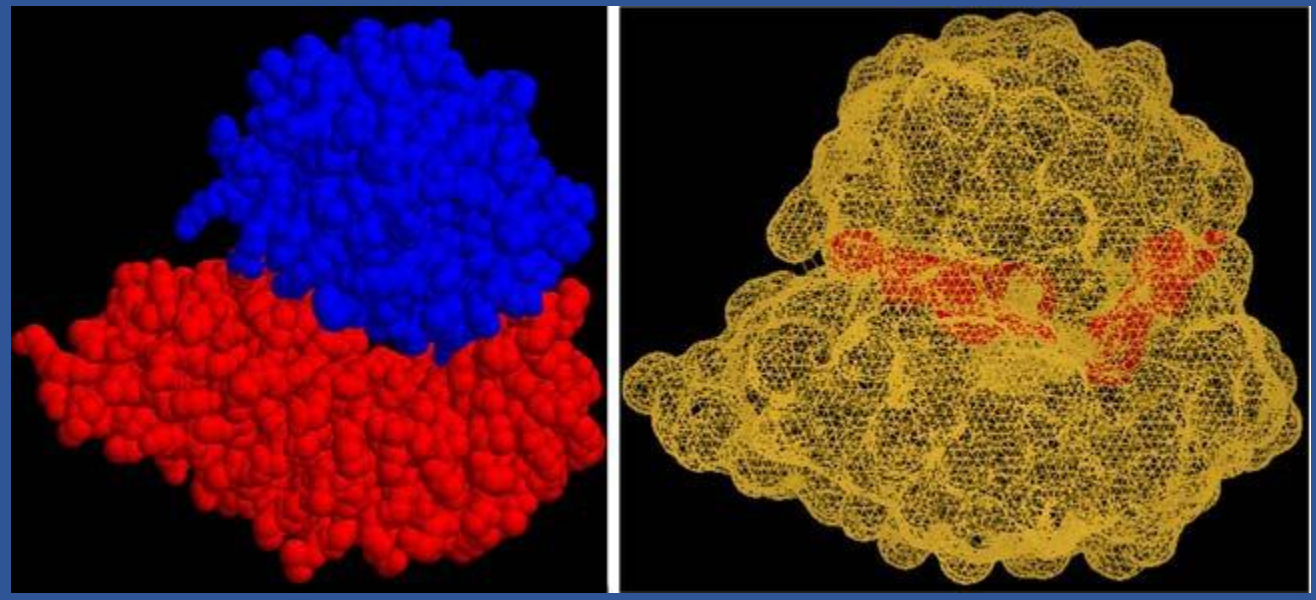


Targeted Discovery

Recognition peptide technology



The interaction interface of the GPIb-vWf complex



Spacefill model of the vWf-GPIb complex. (vWf=blue; GPIb= red). b) Computed interaction interface

Uses for custom designed peptide arrays on cellulose membranes

- Mapping and analysis of linear antibody epitopes with polyclonal and monoclonal antibodies or serum.
- Analysis of antibody paratopes (CDR derived peptides)
- Analysis of T-cell epitopes
- Mapping of linear binding sites in protein-protein/peptide interactions.
- Characterization of kinase substrates
- Peptide interactions with small ligands
- Peptide nucleic acid interactions
- Binding of metals
- Mapping and analysis of protease substrates
- Affinity enrichment and isolation of bound analyte
- Synthesis of mutated peptides for selection of better fit candidates
- Assembly of conformational epitopes by simultaneous synthesis of more
- than one peptide per spot on the arrays.
- Screening of combinatorial libraries to identify antibiotics, agonists or antagonists.

Confirmation of the applicability of this approach using peptide array screening and computer modeling was done by synthesis of D-pep3, which was chosen because of its solubility in physiological buffers. Its ability to prevent vWf-mediated platelet agglutination in a dose-dependent manner functionally validated the in silico process.