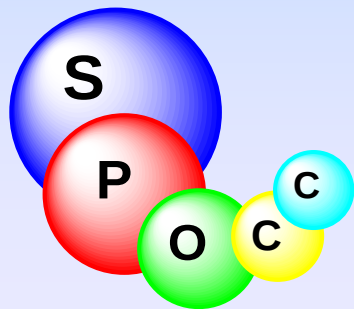




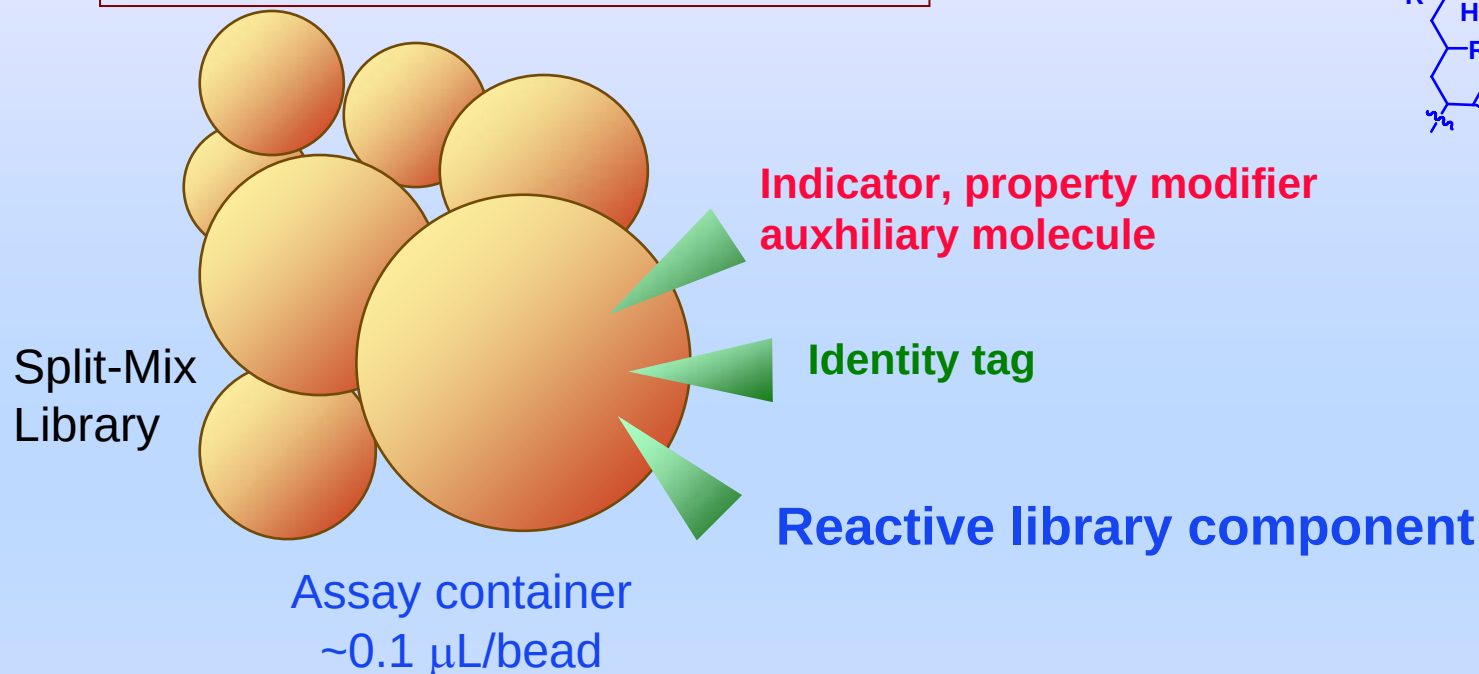
Methods of Structure Assessment in OBSC Libraries

M. Meldal, F. Diness, Grith Hagel, R. Michael, S.F. Christensen

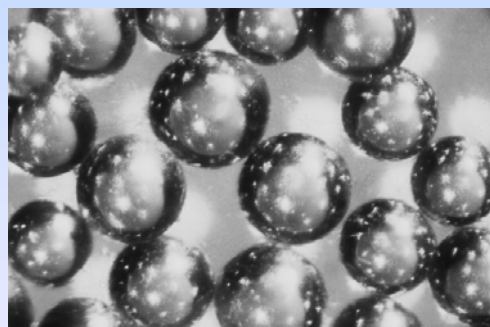


Centre for **S**olid **P**hase **O**rganic **C**ombinatorial **C**hemistry
Carlsberg Laboratory, Valby, Denmark, mpm@crc.dk

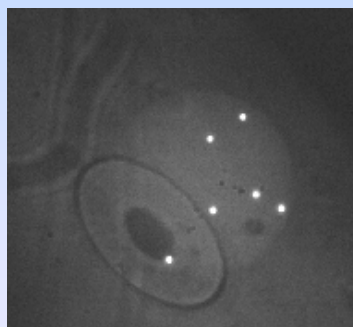
"One Bead Some Compounds"
A very general assay format



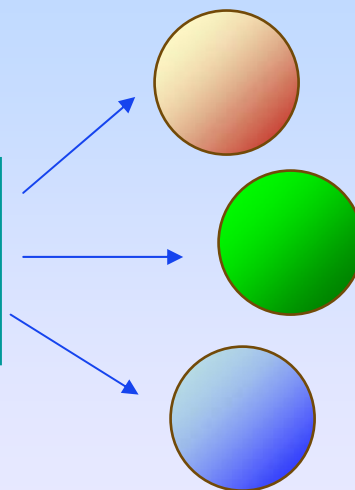
Cells on Beads



PEG-Based resin

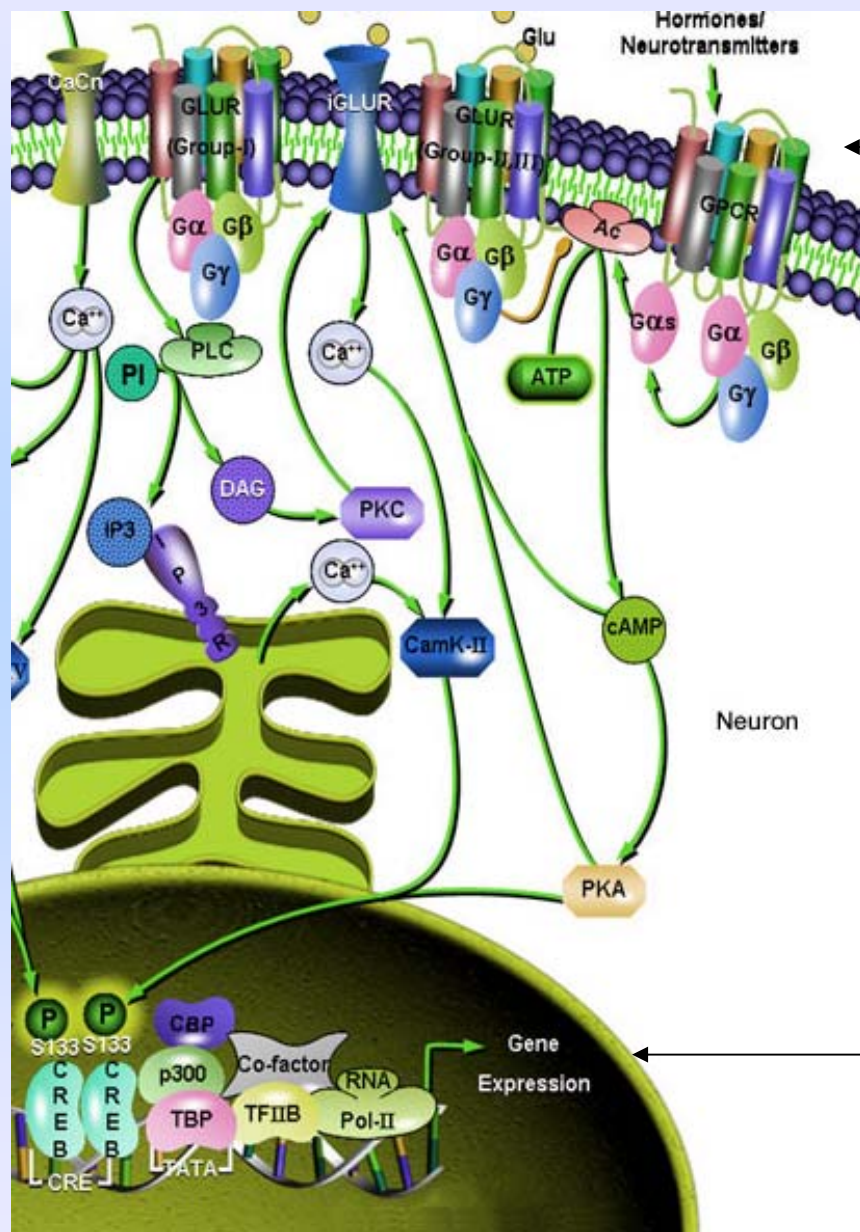


Enzyme reaction
Chemical reaction
Cellular interaction?



Casette for Expression of GPCR + Reporter

Morten Meldal et al.
Carlsberg Laboratory

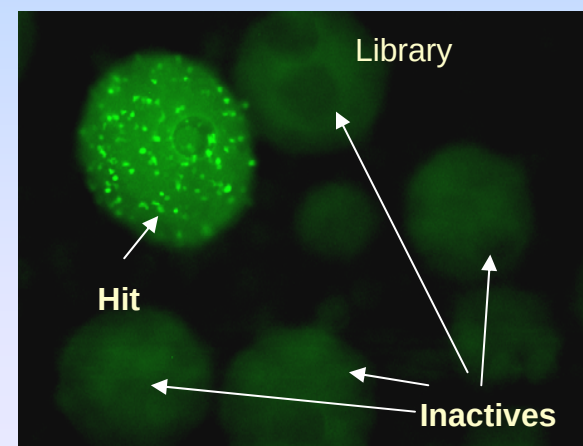
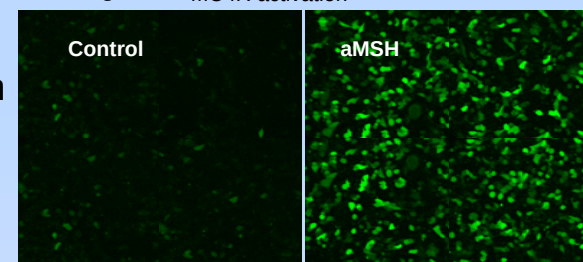
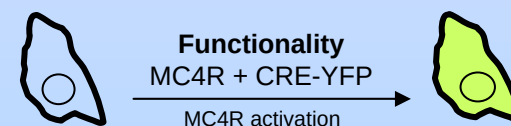


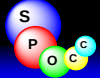
Receptor

Single vector:
Stable Expression
HEK-293 Celline

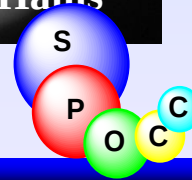
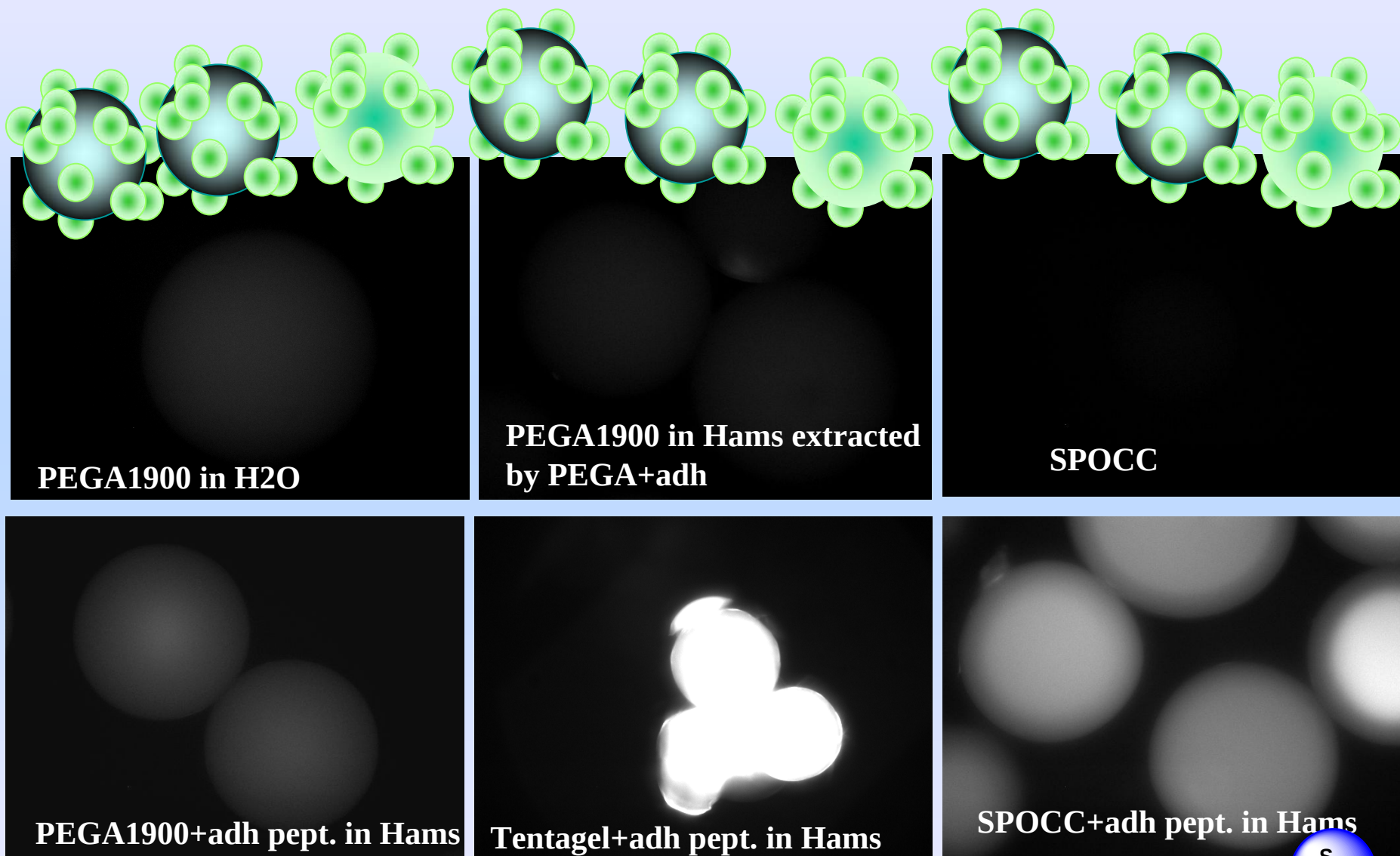
Primary screen

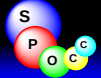
YFP
or
DS-Red





Cells on beads: Background



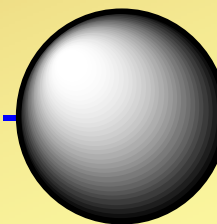
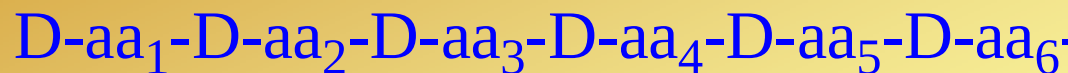


Cell adhesion peptides derived by CombiChem

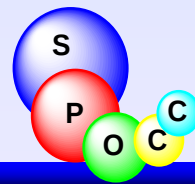
Samuel Denmeade et al. :	Prostate	QMARIPKRLARH-
Kit Lam et al.:	Prostate	DNRIRLQAXXX-
Kit Lam et al.:	Lung A549	c-NGRGEQ-c-
Kit Lam et al.:	Lymphoma	XXXpLDX-
Kit Lam et al.:	Lung Bronc.	Nle-DXXXX-
Horst Kessler et al.:	Jurkat (lym)	Iqui-LD-
Horst Kessler et al.:	Integrins	RGD-peptides
Nomizu Motoyoshi et al.:	Tissue repair	Laminin Peptides

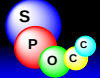
New concept: weak multivalent interaction

- General adhesion towards cell-lines used in screening
- Metabolic stability
- Diversity

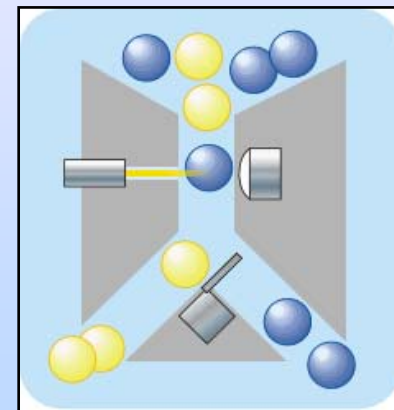
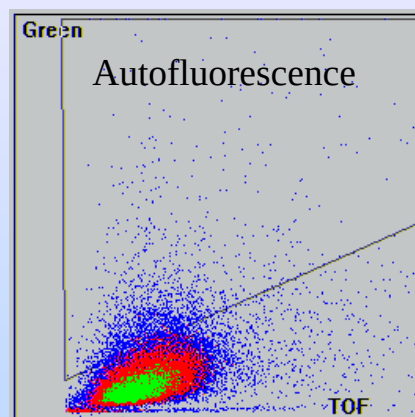


PEGA₁₉₀₀





Cell adhesion on D-amino acid library



Auto fluorescence removed

U2OS/GFP + beads + beadsorter: 75.000 beads

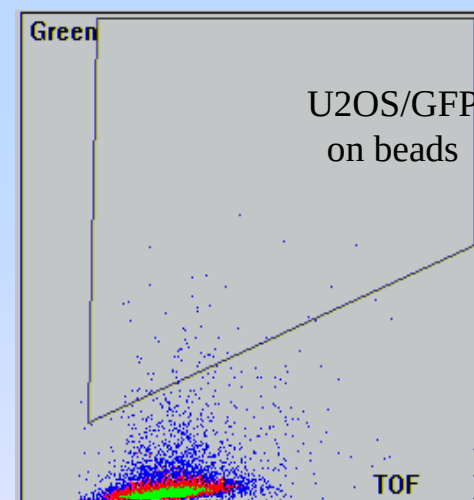
Recovered from sorting: 536 beads

Hek293 adhesion :~40 beads isolated

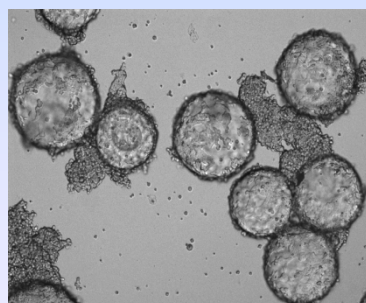
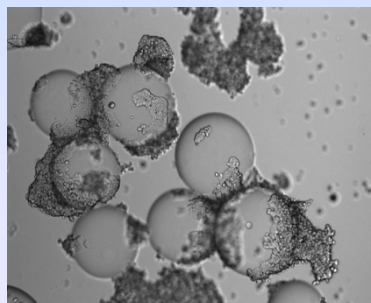
Sequencing

Re-synthesis

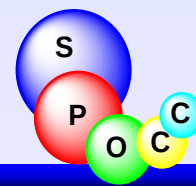
Adhesion study: 50-100 beads/sequence



Weak adhesion



Strong adhesion



Motif

Best

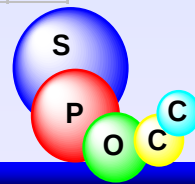
LEU	ARG	LEU	LYS	PRO	LYS			
LEU	ARG	HIS	ARG	LEU	LYS			
ILE	ARG	TYR	ARG	LEU	ARG			
	ALA	GLN	ARG	PRO	ARG	TRP		
		TYR	ARG	TRP	ARG	ILE	ALA	
			ARG	MET	LYS	LEU	HIS	LYS
			LYS	MET	ARG	TYR	CYS	GLN
	THR	LYS	ARG	LEU	LYS	THR		
THR	LYS	GLY	LYS	ALA	LYS			
ALA	LYS	THR	ARG	HIS	ARG			
ASN	ARG	PRO	ARG	VAL	ARG			
			ARG	CYS	LYS	LYS	LEU	ILE
		ARG	LYS	LEU	ARG	VAL	ASN	
			LYS	PRO	LYS	MET	MET	CYS
		LYS	LYS	MET	ARG	PHE	TRP	
		ARG	ARG	ALA	ARG	GLN	ASP	
			ARG	GLU	LYS	GLY	ALA	ARG
ALA	ARG	LYS	ARG	ILE	ARG	GLN		
ALA	LYS	ILE	ARG	GLU	LYS	ARG		
	ARG	ILE	ARG	GLN	ARG			
	ARG	GLN	ARG	ILE	ARG			

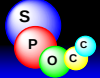
Fair

	LYS	PHE	GLY	GLN	LYS	CYS
	ARG	HIS	TRP	GLY	ARG	ILE
	LYS	TRP	PRO	HIS	HIS	ARG
	LYS	VAL	TYR	MET	HIS	LYS
	ARG	SER	ALA	LYS	ARG	(CYS)
LYS	ARG	GLU	SER	LYS	ARG	
VAL	ARG	THR	VAL	ARG	VAL	
	ARG	ALA	PHE	LYS	TYR	TYR
	LYS	MET	PRO	LYS	LYS	ASN

Intermediate

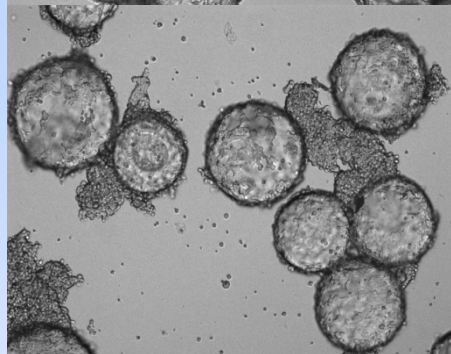
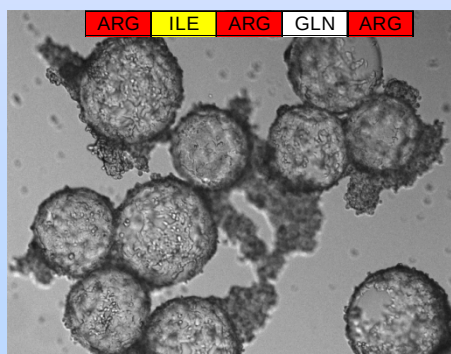
TRP	TYR	ALA	LYS	ARG	ARG			
	PRO	PHE	ARG	LYS	LYS	(CYS)		
			LYS	LYS	LYS	PHE	TYR	TYR
		GLU	ARG	LYS	ARG	CYS	THR	
	VAL	PRO	ARG	LYS	VAL	GLN		
			ARG	ARG	LEU	LEU	PRO	ILE
ARG	SER	ALA	LYS	ARG	CYS			
			LYS	LYS	GLN	PHE	TRP	PHE
		ILE	ARG	LYS	HIS	LEU	ILE	
		PRO	ARG	ARG	VAL	VAL	ILE	
			LYS	ARG	GLU	SER	LYS	ARG
	ILE	THR	ARG	ARG	THR	GLN		
LYS	MET	PRO	LYS	LYS	ASN			



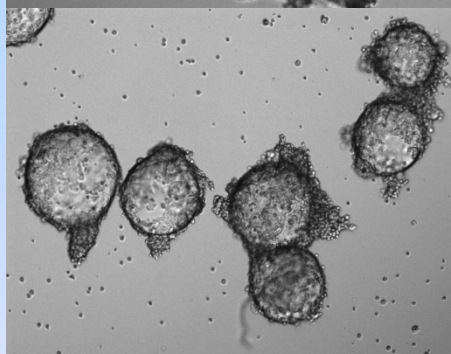
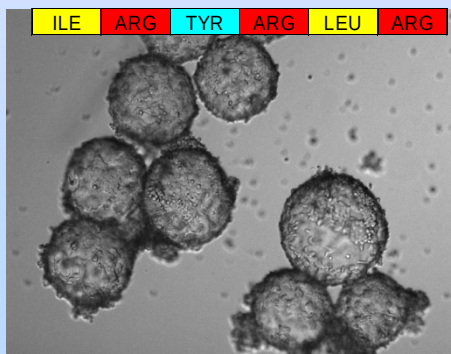


PEGA-Cell adhesion peptides from library

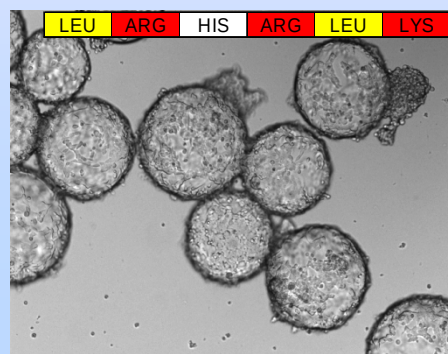
From screening of cellular adhesion on 75.000 PEGA library beads



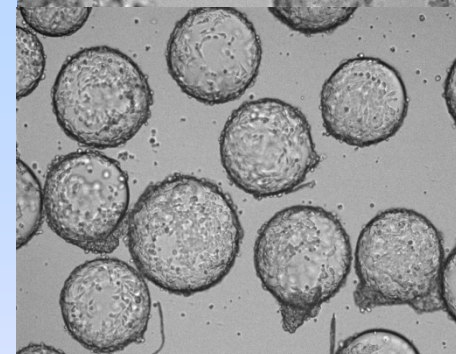
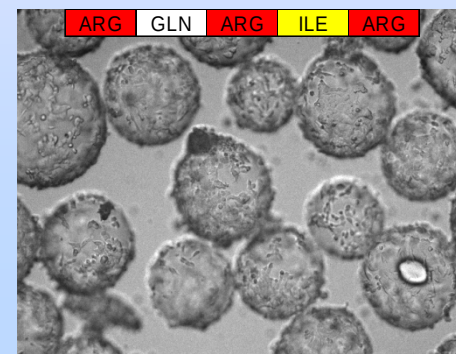
Some cell clusters
70-100% coverage



Few big cell clusters
50-70% coverage

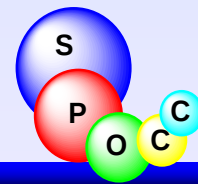


Few big cell clusters
60-80% coverage

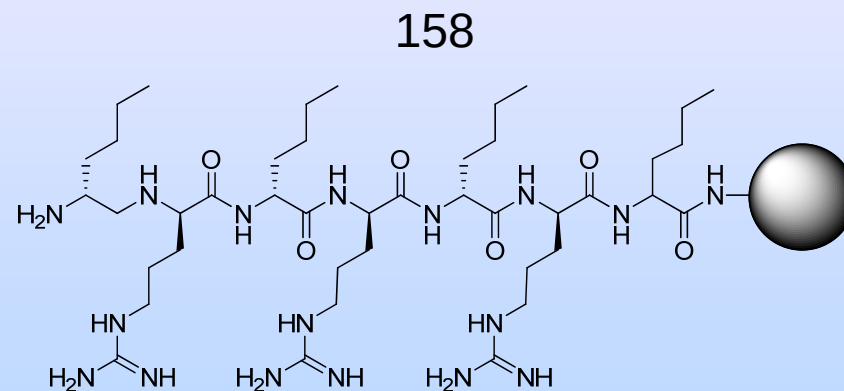
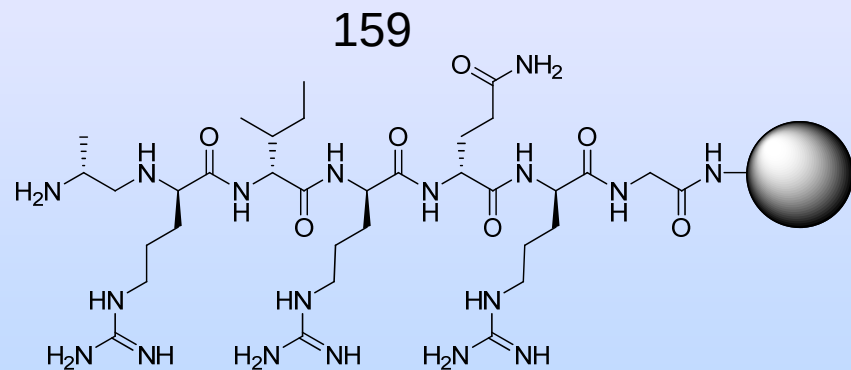


Few non-attached cells
50-75% coverage
Many beads

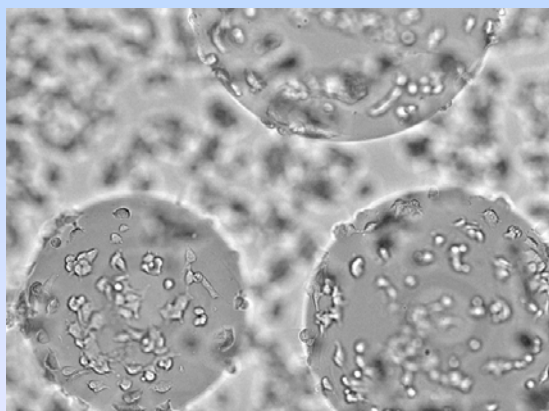
All D-amino acids



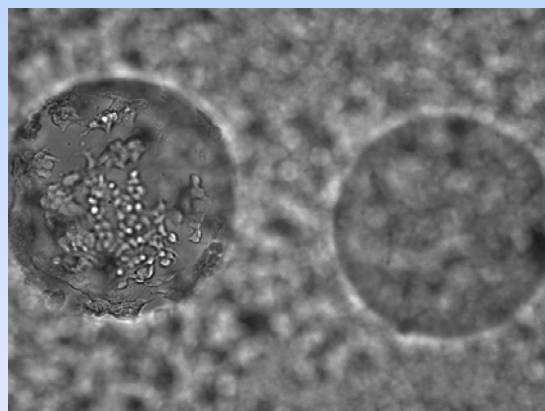
PEGA-Cell adhesion peptide function



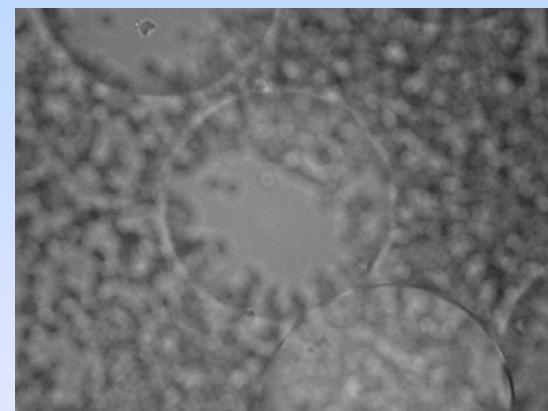
159



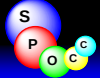
159



158



158



Interaction with phosphatidylcholine bilayer

Mainly superficial Phosphodiester – arg interaction

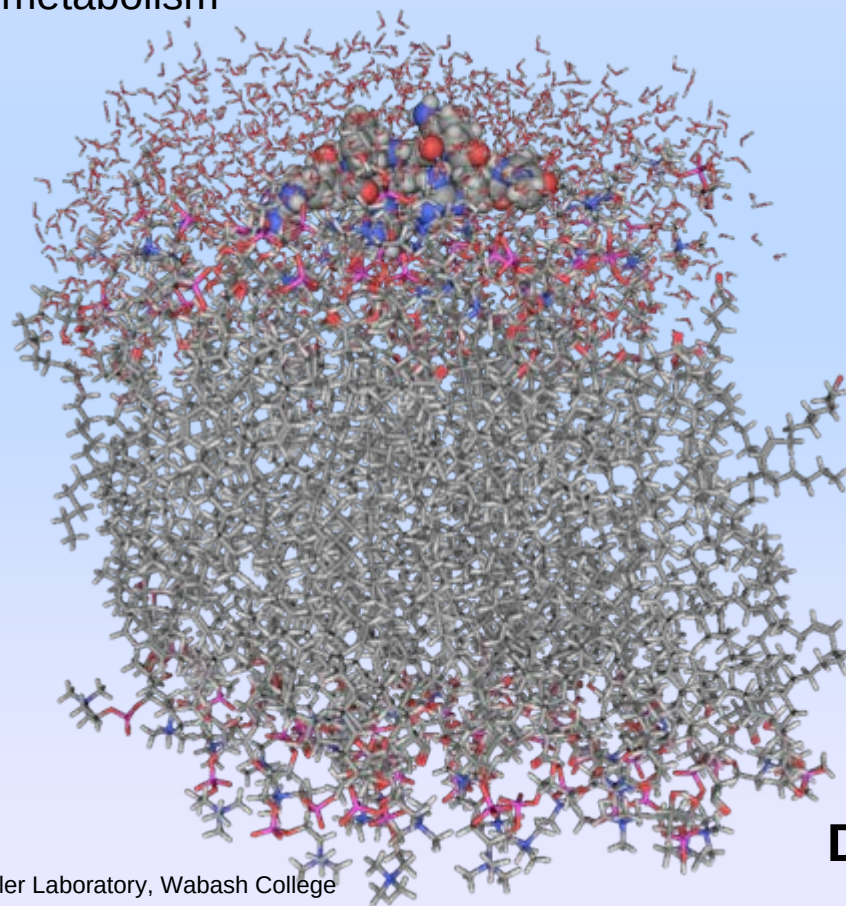
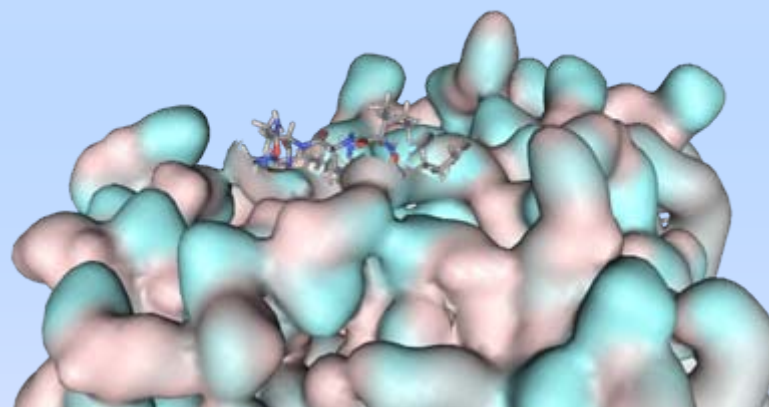
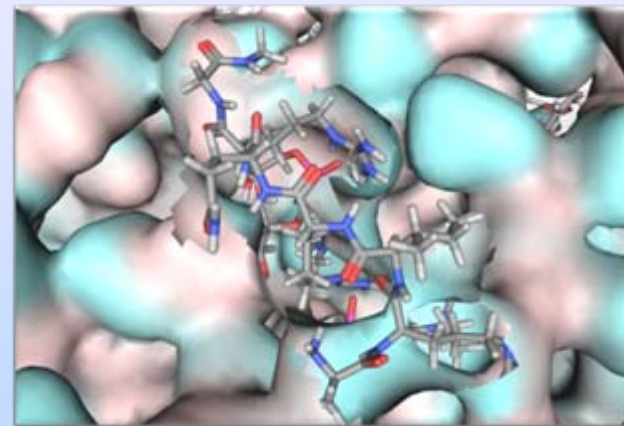
Probably:

No cell penetration

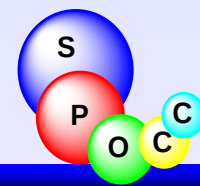
No membrane thinning

No pore formation

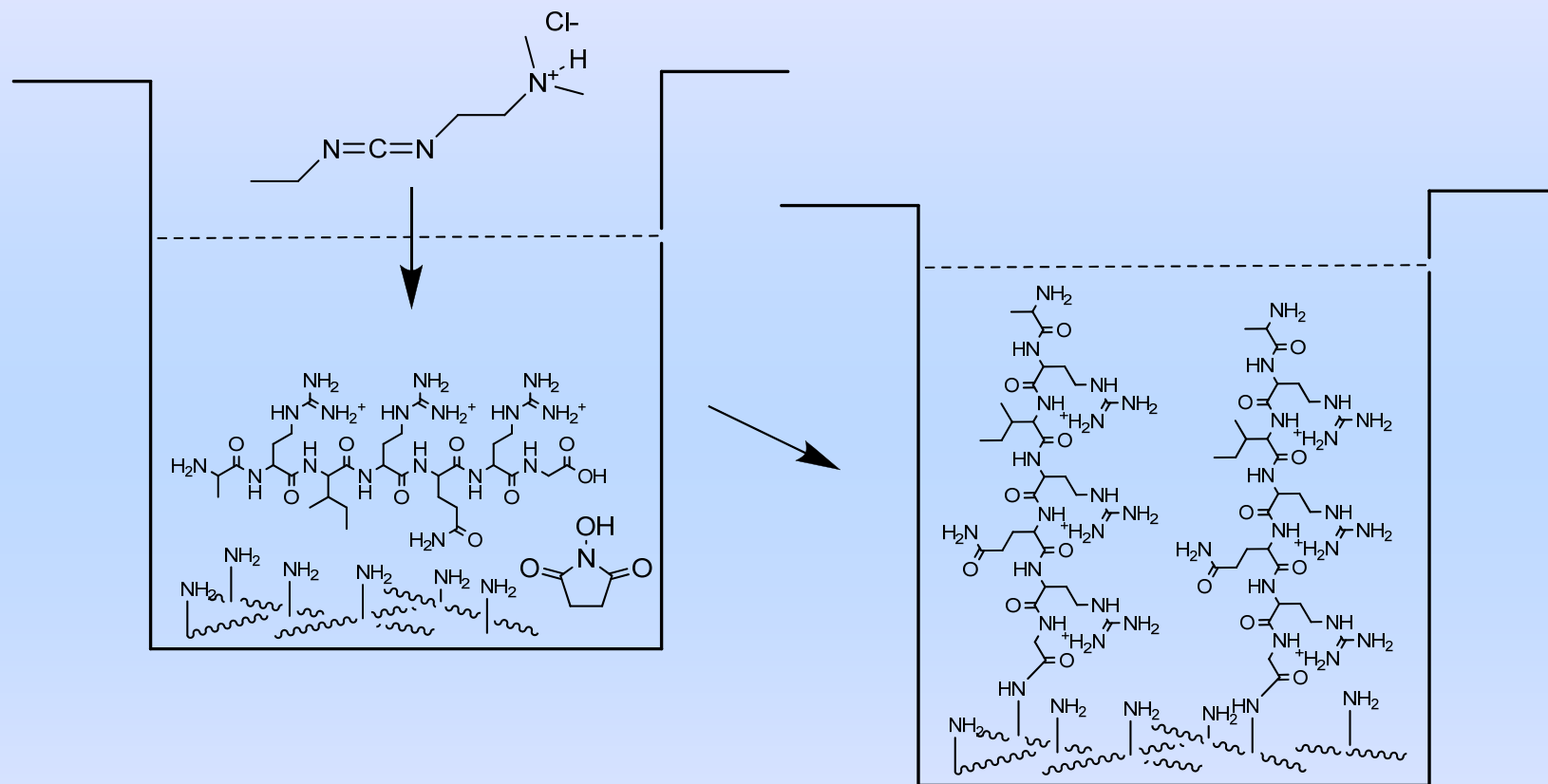
No metabolism

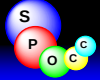


DOPC



Peptide conjugation to plates





Even cell distribution on adhesion peptide

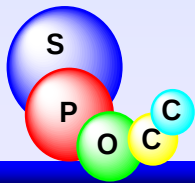
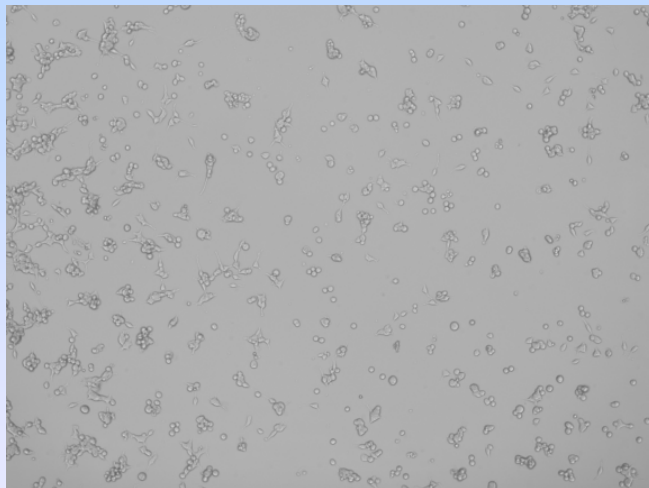
Cells growing on a Cell culture glass plate (Nunc) for 24 hours

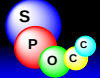
Cells growing on H-arirqrg-peptide-D-Lys-Plate for 24 hours

Edge



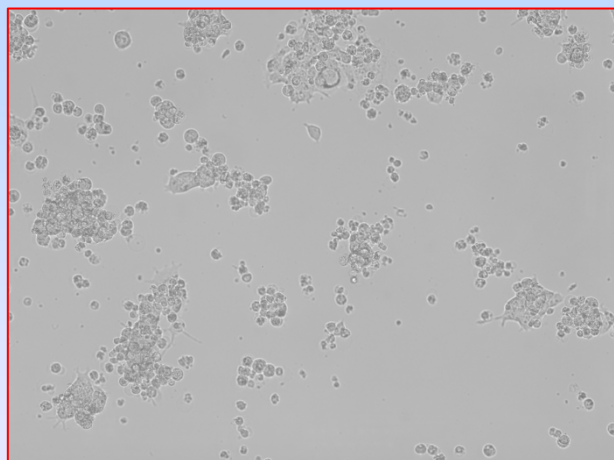
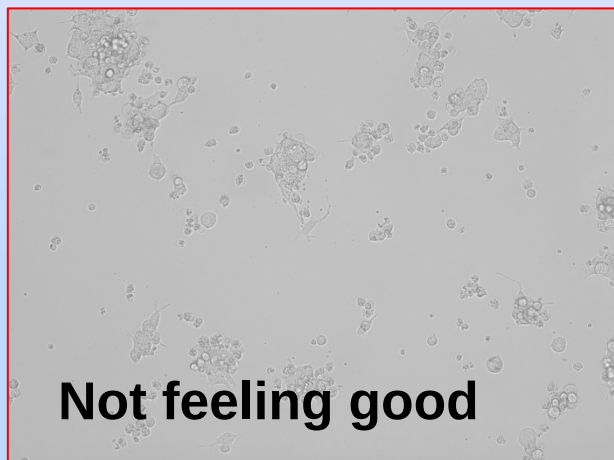
Center





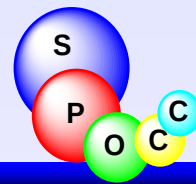
Extended adhesion time – HEK₂₉₃ cell culture

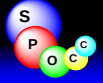
Cells growing on the two plates for **6 days**



Cell culture plates

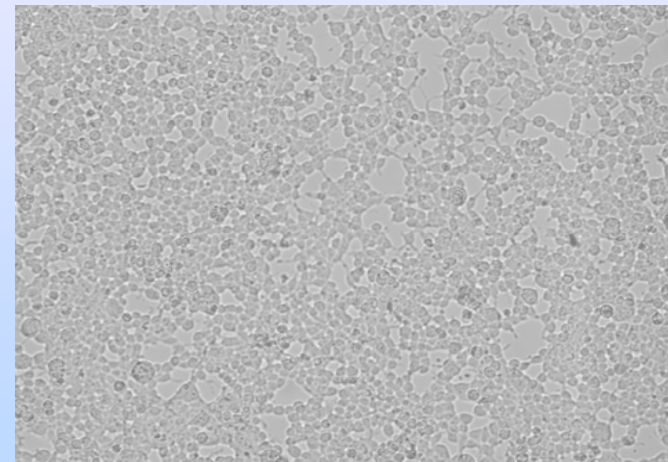
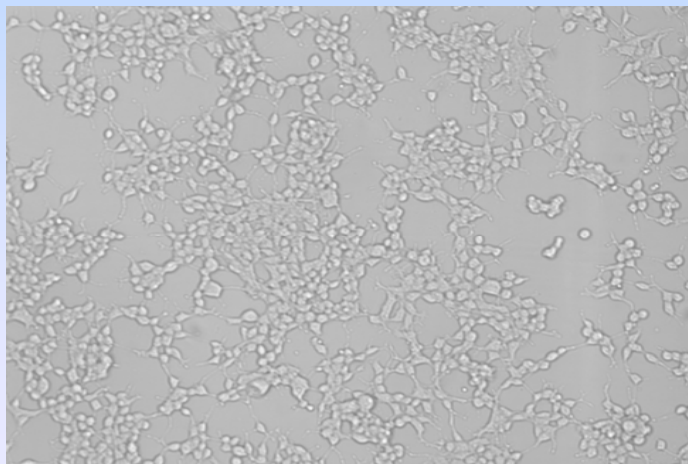
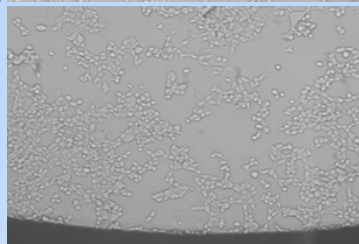
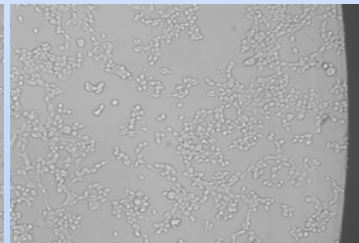
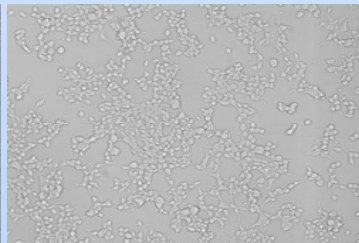
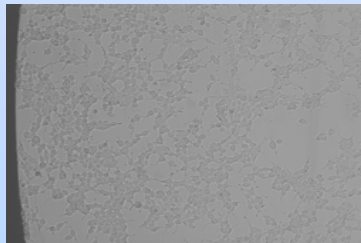
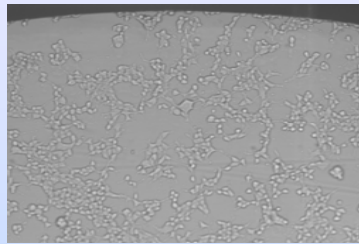
Adhesive peptide plates



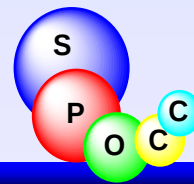
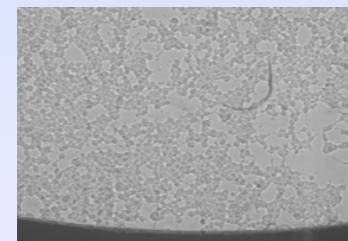
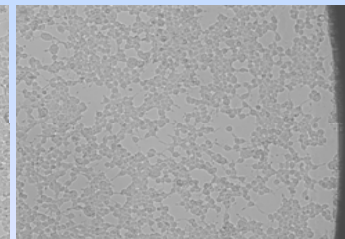
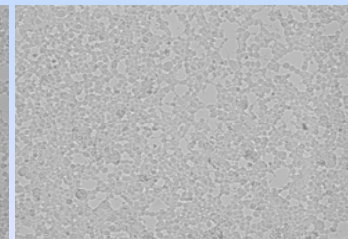
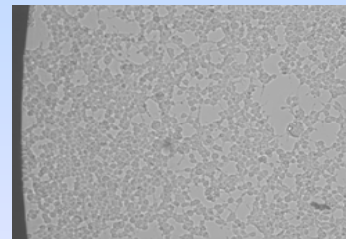
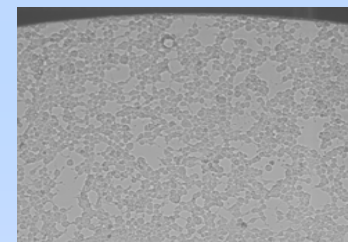


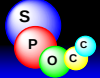
Improved cell culture – HEK₂₉₃ cell (10000) culture (3 days)

Poly-D-Lys

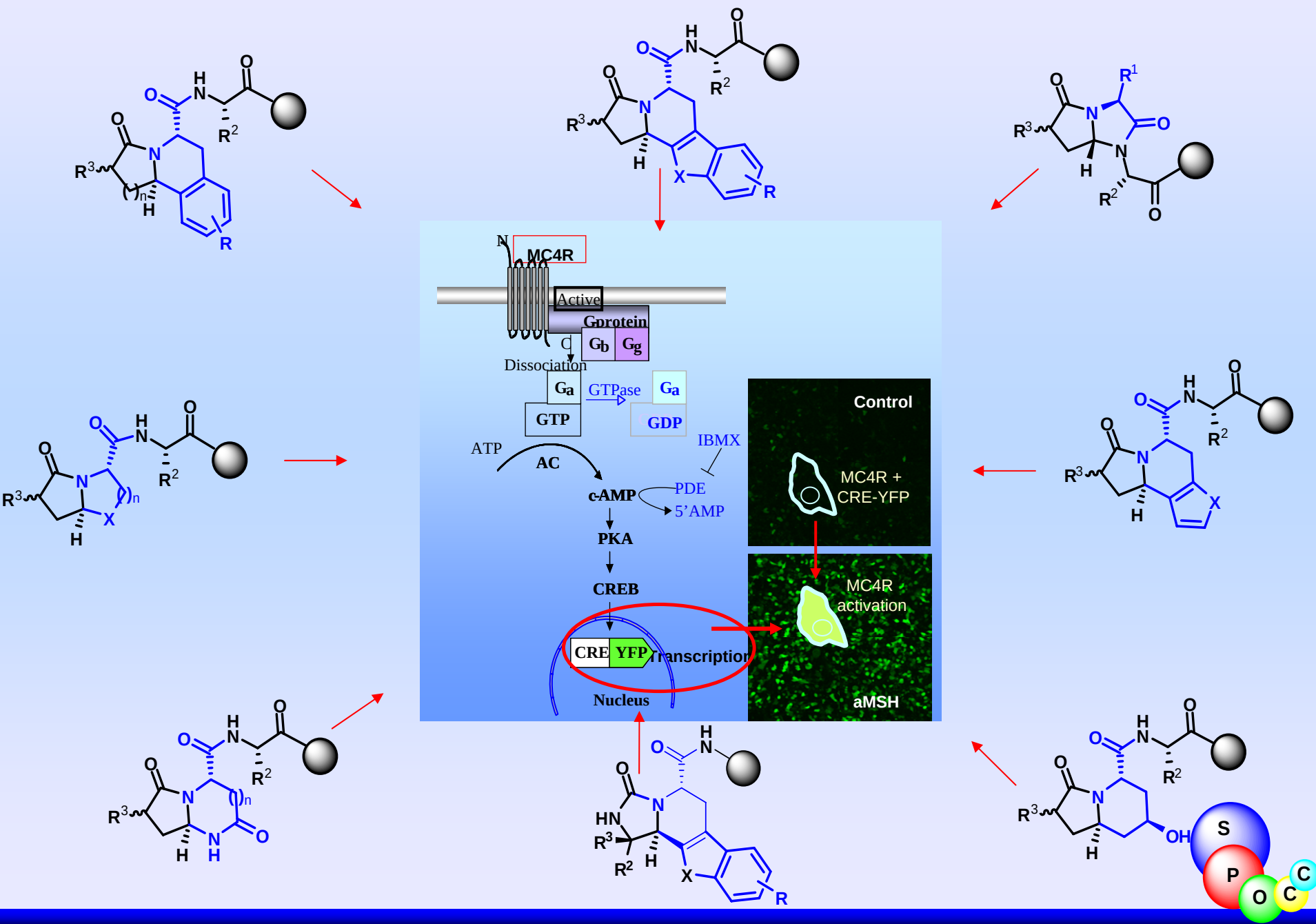


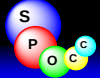
Adhesive peptide



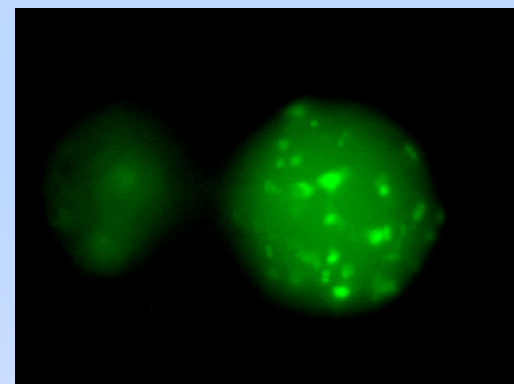
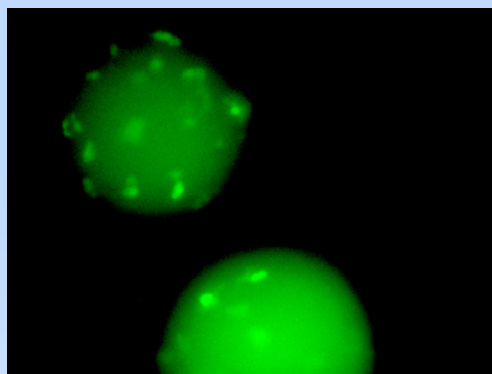
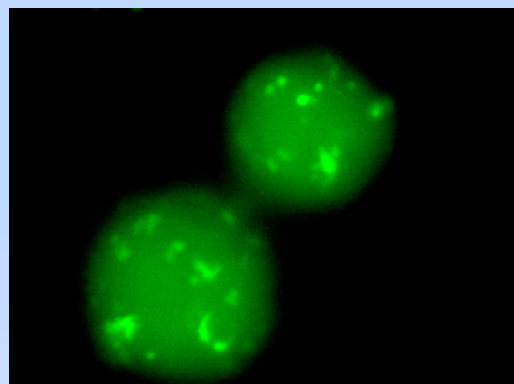
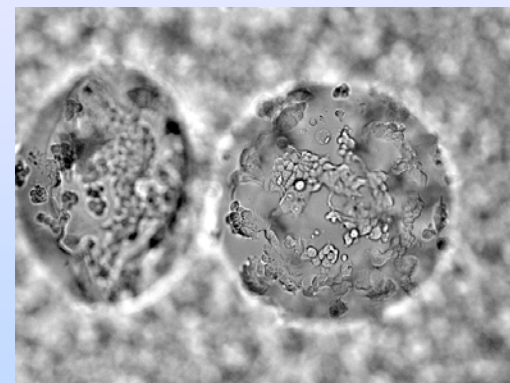
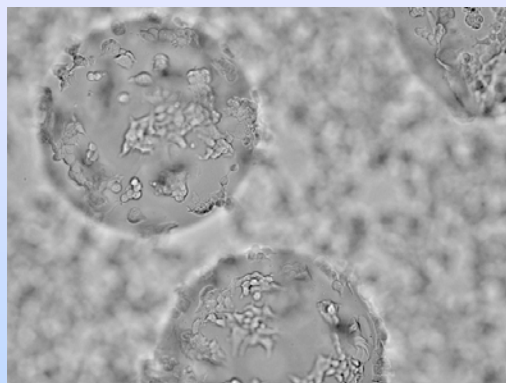
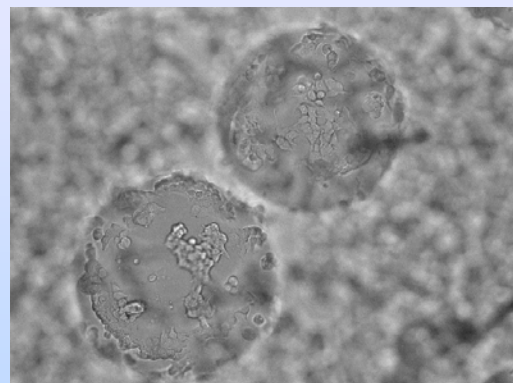


Scaffolds by N-acyliminium Cascade Chemistry



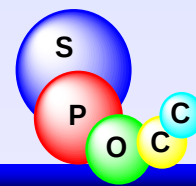
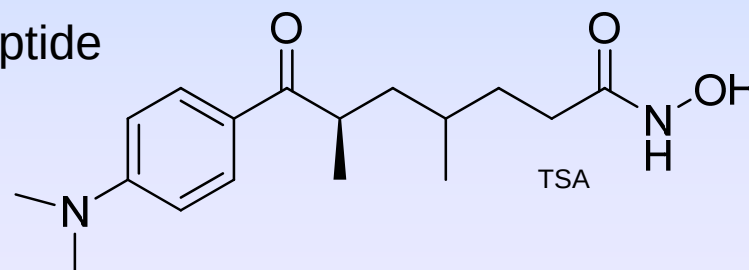


The cell's-on-bead assay with single vector MCR4/YFP



Beads Adh + resin bound activation peptide

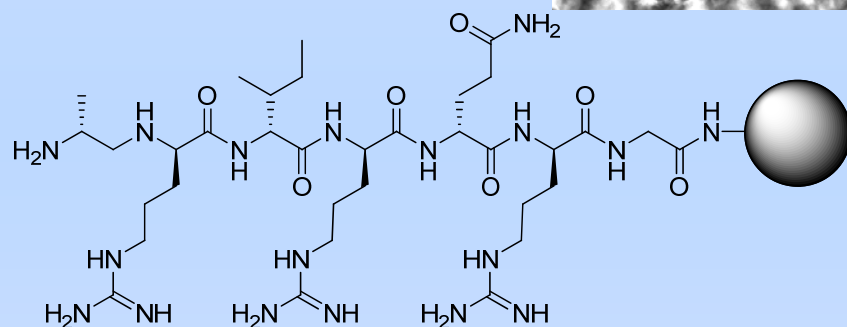
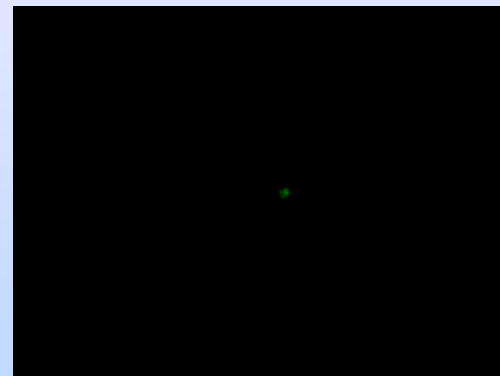
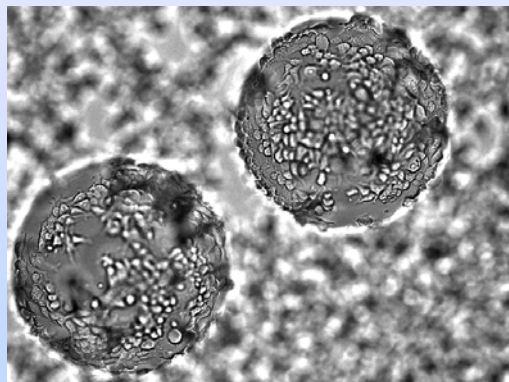
NO TSA required



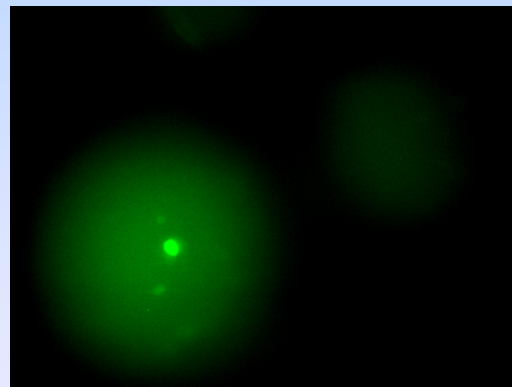
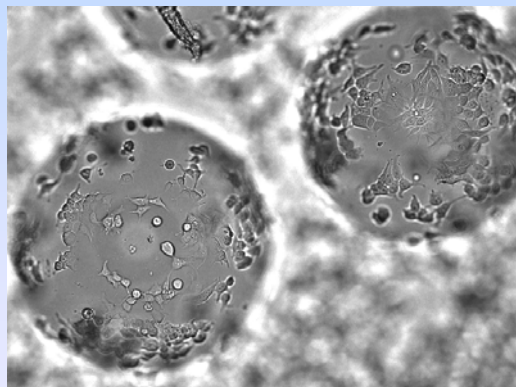
PEGA-Cell adhesion peptide: negative control

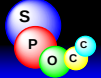
No ligand

Adhesion peptide only



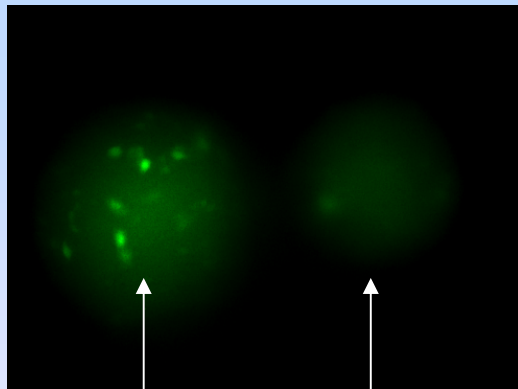
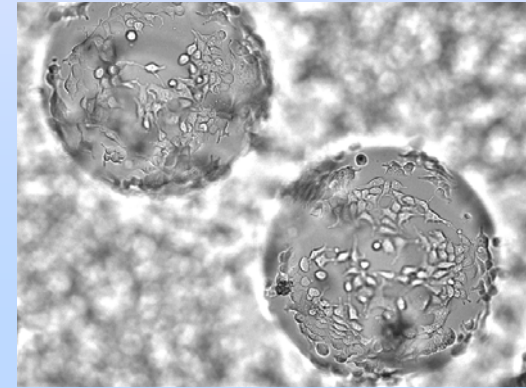
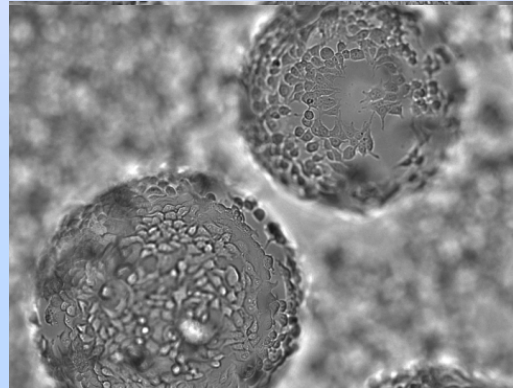
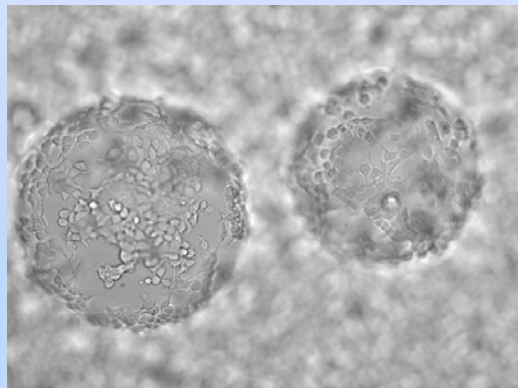
No Fluorescence





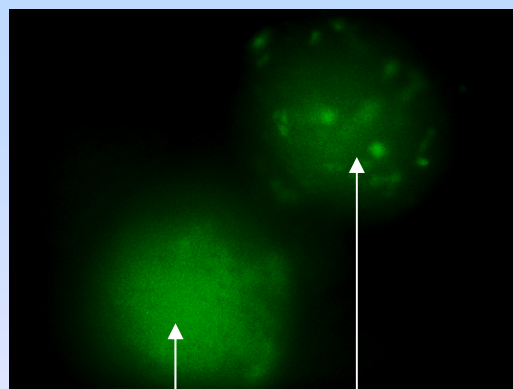
PEGA-Cell adhesion peptide H-arirqrg-

Mixed beads of control and 100 μ M (on resin) ligand
NO TSA



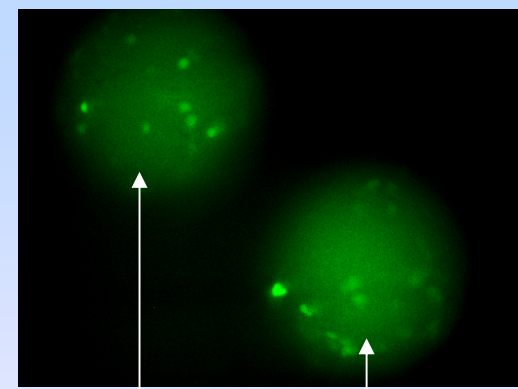
Active

Control



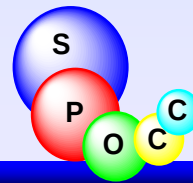
Control

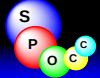
Active



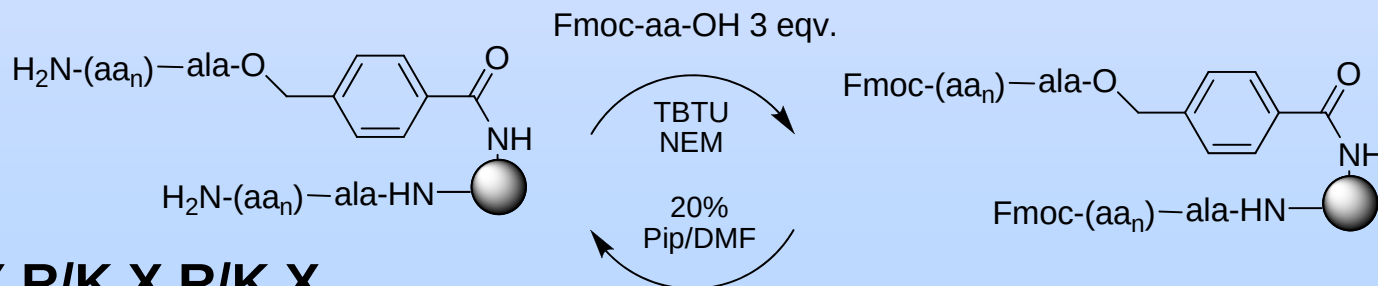
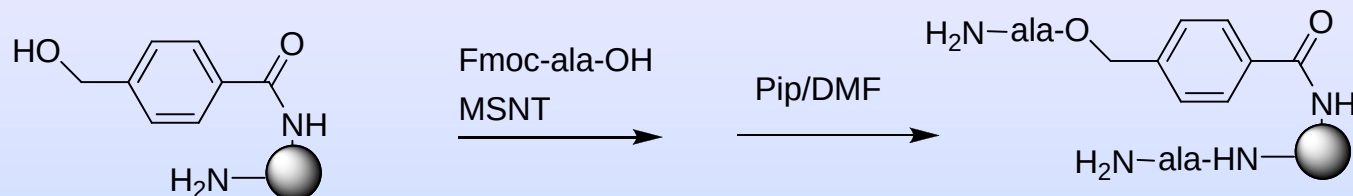
Active

Active





Second adhesion screen of 55.000 D-aa - peptides

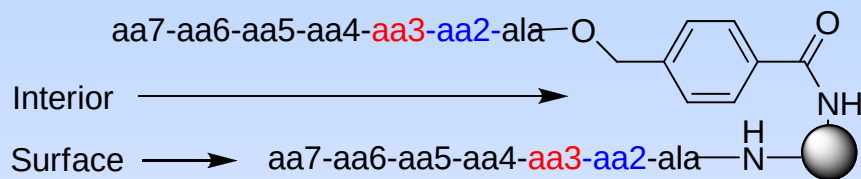


BIAS **X R/K X R/K X R/K X**

Columns / aa in 20 column synthesizer

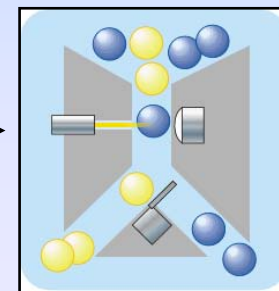
aa7	aa6	aa5	aa4	aa3	aa2
l 4	r 9	l 2	k 8	p 2	k 5
i 4	k 9	h 2	r 8	l 2	r 8
y 4	l 2	y 2	l 2	i 2	h 2
k 2		p 2	t 2	y 2	m 1
r 2		m 2		t 2	t 1
a 4		t 2		k 2	i 1
		k 2		h 2	n 2
		i 2		v 1	
		d 2		m 1	
		e 2		q 2	
				d 2	

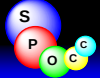
All D-amino acid library of adhesion peptides



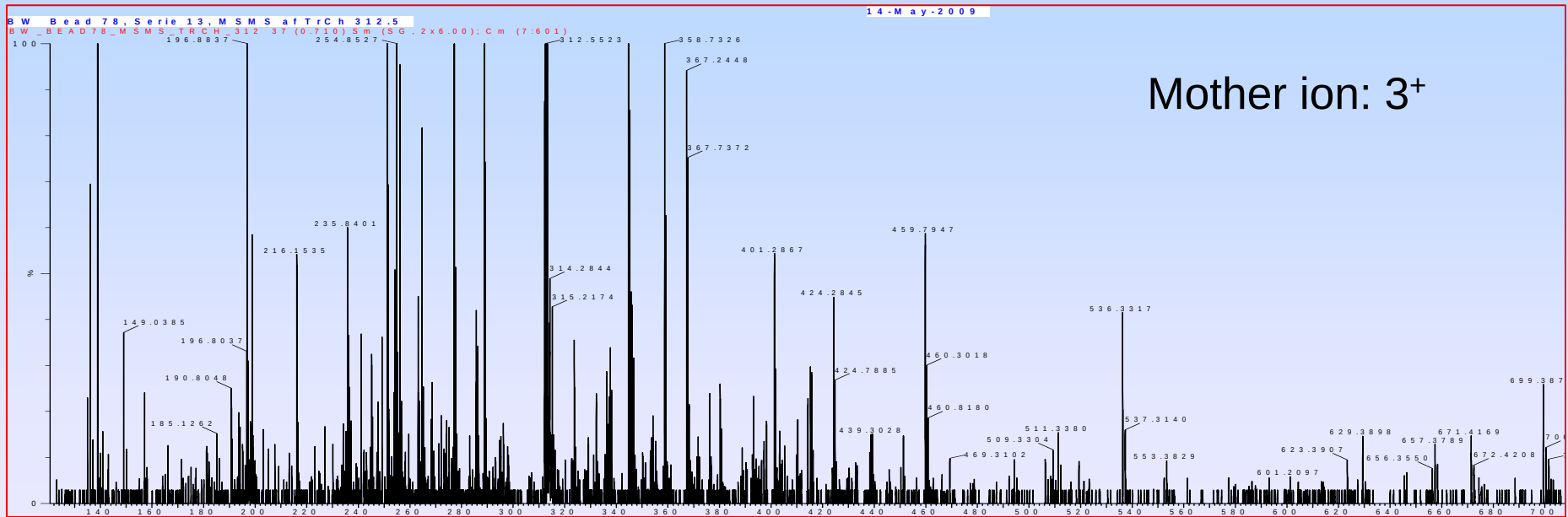
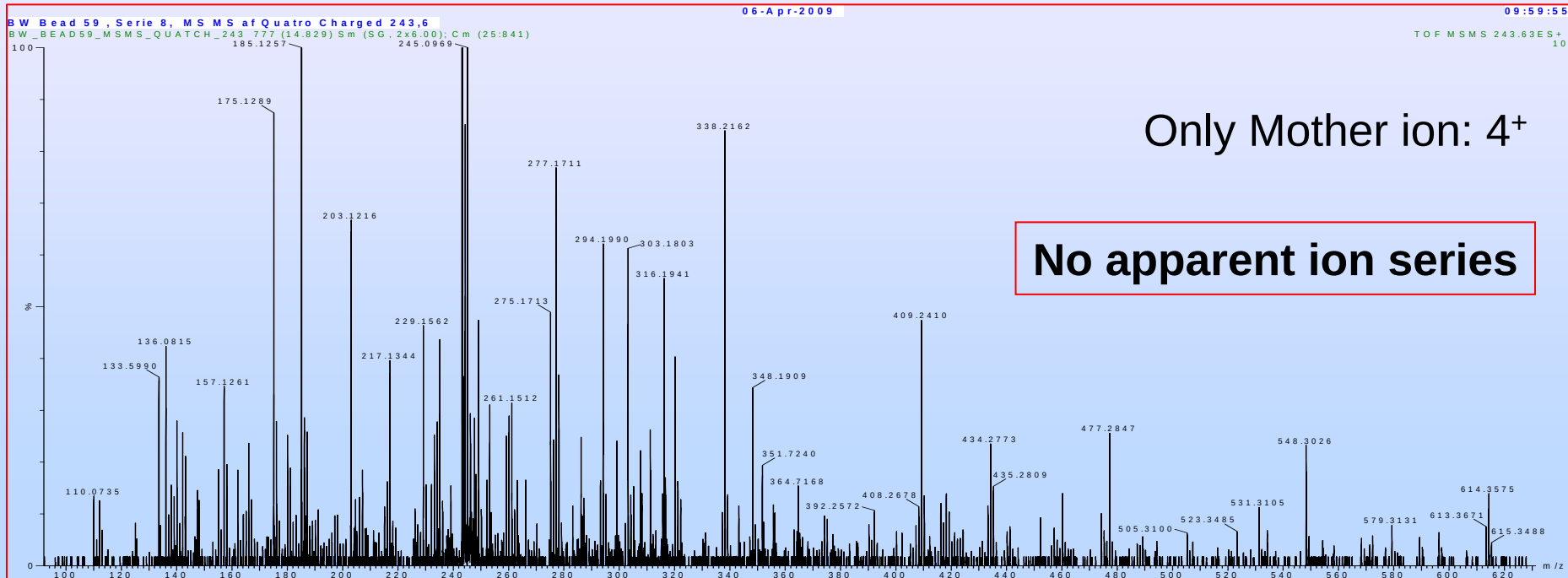
Incubation with HEK293

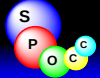
Stirring then sorting





Second adhesion screen of 55.000 D-aa - peptides





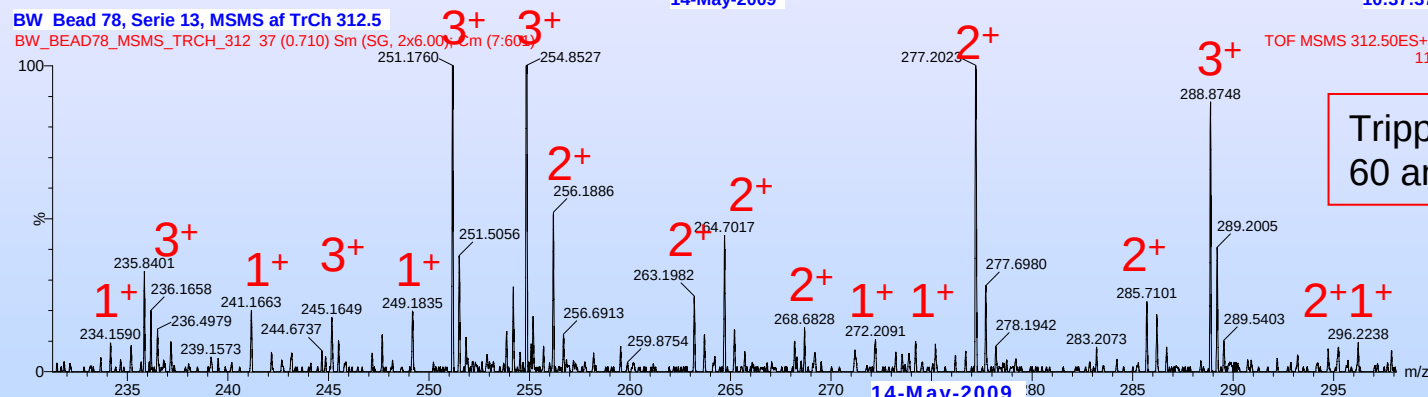
Second adhesion screen of 55.000 D-aa - peptides

BW Bead 78, Serie 13, MSMS af TrCh 312.5

BW_BEAD78_MSMS_TRCH_312 37 (0.710) Sm (SG, 2x6.00); Cm (7.601)

14-May-2009

10:37:37



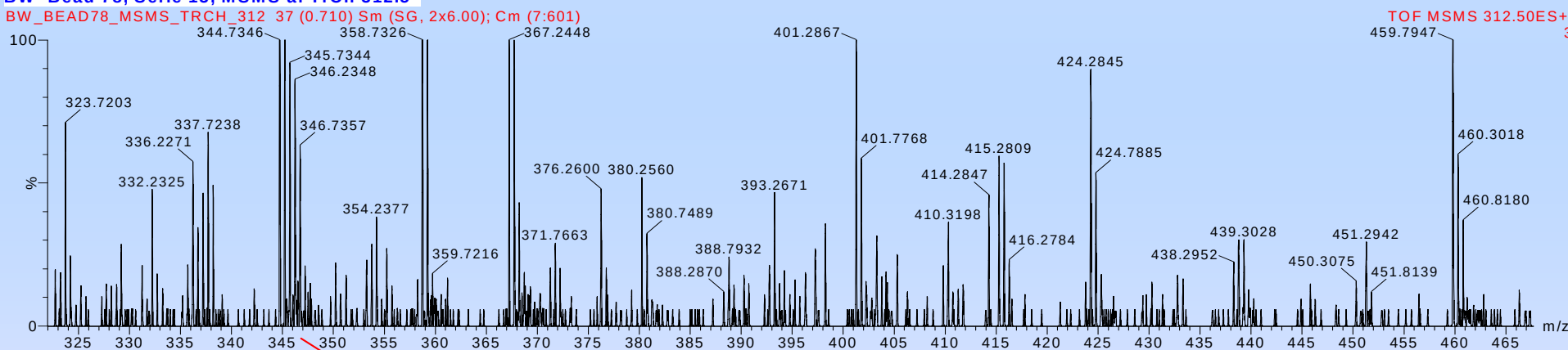
Tripple charged mother ion
60 amu: 1+, 2+ and 3+ ions

BW Bead 78, Serie 13, MSMS af TrCh 312.5

BW_BEAD78_MSMS_TRCH_312 37 (0.710) Sm (SG, 2x6.00); Cm (7.601)

14-May-2009

10:37:37

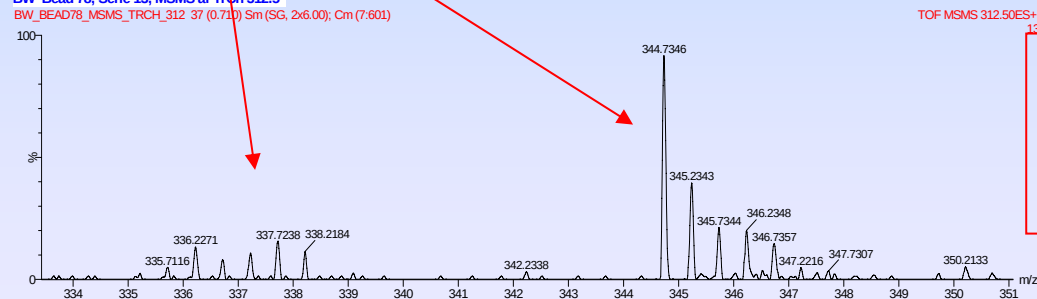


BW Bead 78, Serie 13, MSMS af TrCh 312.5

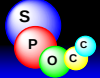
BW_BEAD78_MSMS_TRCH_312 37 (0.710) Sm (SG, 2x6.00); Cm (7.601)

14-May-2009

10:37:37



- 1) Determine accurate mass within 50 ppm
- 2) Compose virtual candidates
- 3) Assign/Edit peak-listing
- 4) Score ~ Y0, Y1, B1, B2 & B3 series



Second adhesion screen of 55.000 D-aa - peptides

Virtual mass determination for all library members
and selection by accurate mass determination

Mass-Sequence Calculator:

File Edit Tools Help Project title Project

	BB-1	BB-2	BB-3	BB-4	BB-5	BB-6	BB-7	BB-8	BB-9
▶	V	L	R	L	K	P	K	A	-OH
		I	K	H	R	L	R		
		Y	L	Y	L	I	H		
		K		P	T	Y	M		
		R		M		T	T		
		A		T		K	I		
				K		H	N		
				I		V			
				D		M			
				E		Q			
*						D			

~55.000 Compounds

Residues (max 15)

Max BB's/residue

Mass

Treshold

BB select

Edit

H- 1.007825037
-OH 17.002739677
-NH2 16.018724082
A 71.037113833
C 103.009185633
D 115.026943113
E 129.042593187
F 147.068413981
G 57.021463759
H 137.058911923
I 113.084064055
K 128.0949631
L 113.084064055
M 131.040485781
N 114.042927518
O 114.079313026
P 97.052763907
Q 128.058577592
R 156.101111116
S 87.032028473
T 101.047678547
V 99.068413981
W 186.079313026
Y 163.063328621
Abz 119.037113833
K(Abz) 229.121512219
YNO2 208.048406872
Fmoc 222.06807965
Boc 100.052429576
Cbz 134.036779502
Aloc 84.021129428
For 27.99491464
Ac 42.010564714
Bz 104.026214788
Piv 84.057514936
tBu 56.062600296
Bzl 91.054775259
All 40.031300148
Me 14.015650074
Et 28.031300148

Y-series

H- 1.007825037
H-R 157.108936153
H-RL 270.193000208
H-RLI 383.277064263
H-RLIR 539.378175379
H-RLIRY 702.441504
H-RLIRYK 830.5364671
H-RLIRYKA 901.573580933
H-RLIRYKA-OH 919.58414564

Y1-series

L 114.091889092
LI 227.175953147
LIR 383.277064263
LIRY 546.340392884
LIRYK 674.435355984
LIRYKA 745.472469817
LIRYKA-OH 763.483034531

B1-series

-OH 19.018989751
-OHA 90.056103584
-OHAK 218.151066684
-OHAKY 381.214395305
-OHAKYR 537.315506421
-OHAKYRI 650.399570476
-OHAKYRIL 763.483634531
-OHAKYRILR 919.584745647

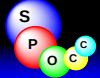
B2-series

A 72.04493887
AK 200.13990197
AKY 363.203230591
AKYR 519.304341707
AKYRI 632.388405762
AKYRIL 745.472469817
AKYRILR 901.573580933

B3-series

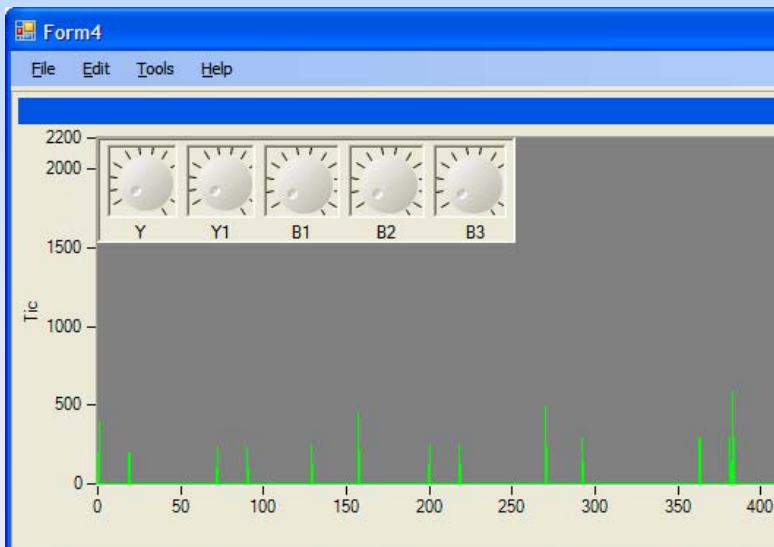
K 129.102788137
KY 292.166116758
KYR 448.267227874
KYRI 561.351291929

C 12.000000 P 30.973763400 Mg 23.985045000
H 1.007825037 S 31.972071800 Ti 204.97441000
B 11.009305300 Si 27.976928400 Tl 47.947946700
Br 78.918336100 Ag 106.90509500 Se 79.916520500
Cl 34.968852729 Al 26.981541300 Sn 119.902199000
F 18.998403250 Ca 39.962590700 Zn 63.929145400
Hg 201.97632000 Cr 51.940509700 e 0.00054857990943
I 126.904477000 Cu 62.929599200
K 38.96371 Fe 55.934939300
N 14.003074008 Pb 207.97664100
Na 22.989769700 Pd 105.90347500
O 15.994914640 Li 7.016004500



Second adhesion screen of 55.000 D-aa - peptides

Scoring structures selected
by accurate mass determination
by virtual fragment matching of
MSMS data



Form2

File Edit Tools Help

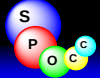
Score

M / z	Intensity	z	Peptide	Score
253.1965	24.8	1	H-YKIRIRA-OH	919.58414561
256.7135	8.4	2	H-YLLRKRA-OH	919.58414561
262.5336	4.2	3	H-YLLRQRA-OH	919.547760102
263.7214	3.5	2	H-YLKRLRA-OH	919.58414561
267.2169	4.6	2	H-YLKRIIRA-OH	919.58414561
268.2115	72.9	3	H-YLIRKRA-OH	919.58414561
269.2141	11.2	2	H-YLIRQRA-OH	919.547760102
270.2242	11.2	1	H-KRLRYIA-OH	919.58414561
275.1989	2.8	1	H-KRLLYRA-OH	919.58414561
276.8876	3.4	3	H-KRYRLIA-OH	919.58414561
277.7295	28.5	2	H-KRYRIIA-OH	919.58414561
280.2097	3.2	1	H-KRYLLRA-OH	919.58414561
285.2313	7.5	1	H-KRYLIRA-OH	919.58414561
286.7321	4.7	2	H-KRIRYIA-OH	919.58414561
287.2500	6.0	1	H-KRILYRA-OH	919.58414561
288.7230	3.2	2	H-KLLRYRA-OH	919.58414561
289.2267	4.2	2	H-KLYRLRA-OH	919.58414561
290.7471	5.3	2	H-KLYRIRA-OH	919.58414561
291.8862	8.9	3	H-KLIRYRA-OH	919.58414561
293.1880	3	1	H-RLKYIA-OH	919.58414561
294.1880	3	2	H-RLLYKA-OH	919.58414561
295.2205	3.0	1	H-RRYKLI-OH	919.58414561
302.7202	2.9	2	H-RRYKIIA-OH	919.58414561
302.7202	2.9	1	H-RRYLLKA-OH	919.58414561
302.7202	2.9	1	H-RRYLIKA-OH	919.58414561
302.7202	2.9	1	H-RRYLKIA-OH	919.58414561
302.7202	2.9	1	H-RRYLQIA-OH	919.547760102
302.7202	2.9	1	H-RRKLYIA-OH	919.58414561
302.7202	2.9	1	H-RRIKYIA-OH	919.58414561
302.7202	2.9	1	H-RRILYKA-OH	919.58414561
302.7202	2.9	1	H-RKLYIA-OH	919.58414561
302.7202	2.9	1	H-RKLLYRA-OH	919.58414561
302.7202	2.9	1	H-RKYRLIA-OH	919.58414561
302.7202	2.9	1	H-RKYRIIA-OH	919.58414561
302.7202	2.9	1	H-RKYLLRA-OH	919.58414561
302.7202	2.9	1	H-RKYLIRA-OH	919.58414561
302.7202	2.9	1	H-RKIRYIA-OH	919.58414561
302.7202	2.9	1	H-RKILYRA-OH	919.58414561
302.7202	2.9	1	H-RLKYRA-OH	919.58414561
302.7202	2.9	1	H-RLLYKA-OH	919.58414561
302.7202	2.9	1	H-RLYKLRA-OH	919.58414561
302.7202	2.9	1	H-RLYKIRA-OH	919.58414561
302.7202	2.9	1	H-RLYRLKA-OH	919.58414561
302.7202	2.9	1	H-RLYRIKA-OH	919.58414561
302.7202	2.9	1	H-RLYRKIA-OH	919.58414561
302.7202	2.9	1	H-RLYRQIA-OH	919.547760102
302.7202	2.9	1	H-RLYLKRA-OH	919.58414561
302.7202	2.9	1	H-RLYLQRA-OH	919.547760102
302.7202	2.9	1	H-RLKRYIA-OH	919.58414561

Intensity Threshold 1 Mass Difference 0.0475

RefM 500 Corr 0.000

Cyclopeptide Sodium H-RLKRYIA-OH 919.58414561 279



Second adhesion screen of 55.000 D-aa - peptides

HEK293-cells stick and hits
may be sorted automatically

Bias of aa's in 20 columns

aa7	aa6	aa5	aa4	aa3	aa2
l 4	r 9	l 2	k 8	p 2	k 5
i 4	k 9	h 2	r 8	l 2	r 8
y 4	l 2	y 2	l 2	i 2	h 2
k 2		p 2	t 2	y 2	m 1
r 2		m 2		t 2	t 1
a 4		t 2		k 2	i 1
		k 2		h 2	n 2
		i 2		v 1	
		d 2		m 1	
		e 2		q 2	
				d 2	

All D-Amino acids

H- **K** **L** **H** **R** **I** **R** **A** -OH

H-KLYKYRA-OH
H-KLYKHRA-OH
H-KLYKI/LRA-OH
H-KLYRPRA-OH
H-RLYRVRA-OH
H-KLPRVKA-OH
H-KLERVKA-OH
H-KLKRI/LKA-OH
H-KLHRQKA-OH
H-KLHR/I/LKA-OH
H-KLHR/I/LKA-OH
H-KLHR/I/LKA-OH
H-RLHR/I/LRA-OH
H-RLHRYRA-OH
H-RLTRTRA-OH

H- **K** **R** **X** **R** **Y** **R** **A** -OH

H-KRYRYKA-OH
H-KREKYKA-OH
H-KKMKTKA-OH
H-KKERPRA-OH
H-RRHRYRA-OH
H-RRHRVRA-OH
H-RRLIRQRA-OH
H-KKMRMRA-OH
H-RKPKVKA-OH
H-RRMRDHA-OH

H- **K** **R** **Y** **R** **V** **N** **A** -OH

H-KRPRI/LIA-OH
H-KKYKVNA-OH
H-KRHRVNA-OH
H-RRILKTIA-OH
H-RRTRYIA-OH
H-RRYRKIA-OH

H-RLI/LTPRA-OH
H-KLI/LLI/LKA-OH
H-RLKRYIA-OH
H-RLTKYIA-OH
H-RLDRQHA-OH
H-KKYL I/LRA-OH
H-ARPRDRA-OH
H-ARDKDRA-OH
H-YRI/LKHRA-OH
H-YRI/LKKRA-OH
H-I/LRKL I/LRA-OH
H-I/LKKLPKA-OH
H-ARHLPKA-OH
H-ALKRVHA-OH