Methods of Structure Assessment in OBSC Libraries

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The “One Bead SOME Compounds” Solid Phase Assay

Morten Meldal et al.
Carlsberg Laboratory

The “One Bead Some Compounds” Solid Phase Assay
A very general assay format

Split-Mix Library

Identity tag

Assay container
~0.1 μL/bead

Indicator, property modifier auxiliary molecule

Reactive library component

PEG-Based resin

Enzyme reaction
Chemical reaction
Cellular interaction?

Cells on Beads
Casette for Expression of GPCR + Reporter

Morten Meldal et al.
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Functionality
MC4R + CRE-YFP
Inactives
Hit
Library

Primary screen
YFP or DS-Red

Receptor

Single vector:
Stable Expression
HEK-293 Cellline

Fluorescence microscopy
Control
aMSH

The ACS - Ralph F. Hirschmann Award Lecture 2009
**Cells on beads: Background**

- PEGA1900 in H2O
- PEGA1900 in Hams extracted by PEGA+adh
- SPOCC
- PEGA1900+adh pept. in Hams
- Tentagel+adh pept. in Hams
- SPOCC+adh pept. in Hams
### Cell adhesion peptides derived by CombiChem

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<th>Researcher</th>
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<td>Tissue repair</td>
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**New concept: weak multivalent interaction**
- General adhesion towards cell-lines used in screening
- Metabolic stability
- Diversity

\[
D-\text{aa}_1-D-\text{aa}_2-D-\text{aa}_3-D-\text{aa}_4-D-\text{aa}_5-D-\text{aa}_6-\text{PEGA}_{1900}
\]
Cell adhesion on D-amino acid library

Auto fluorescence removed
U2OS/GFP + beads + beadsorfer: 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
## Adhesion molecules from D-amino acid library

### Motif

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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

158 158
Interaction with phosphatidylcholine bilayer

Mainly superfacial Phosphodiester – arg interaction

Probably:
No cell penetration
No membrane thinning
No pore formation
No metabolism
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
Cells growing on the two plates for 6 days

Not feeling good

Excellent condition

Cell culture plates

Adhesive peptide plates
Improved cell culture – HEK_{293} cell (10000) culture (3 days)

Poly-D-Lys

Adhesive peptide
Scaffolds by N-acyliminium Cascade Chemistry

MC4R + CRE-YFP

Control

GTPase

GTPase

Ga

GTP

Atp

AC

c-AMP

PKA

CREB

Nucleus

Transcription

Dissociation

GTP

Ga

Gb

G-g

G-protein

PDE

5'AMP

Prot

CRE

YFP

Active

aMSH

IBMX

Prot

N

GDP

Ga

GDP

5'AMP

Prot

CRE

YFP

Nucleus

Transcription

Dissociation

GTP

Ga

Gb

G-g

G-protein

PDE

5'AMP

Prot

CRE

YFP

Nucleus

Transcription

Dissociation

GTP

Ga

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G-protein

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5'AMP

Prot

CRE

YFP

Nucleus

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

Beads Adh + resin bound activation peptide

NO TSA required

DT1058 51 14-05-2008
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 μM (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

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Second adhesion screen of 55,000 D-aa - peptides

Only Mother ion: 4+

No apparent ion series

Mother ion: 3+
Second adhesion screen of 55,000 D-aa - peptides

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

Tripple charged mother ion
60 amu: 1+, 2+ and 3+ ions
Second adhesion screen of 55,000 D-aa- peptides

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

~55,000 Compounds
Second adhesion screen of 55,000 D-aa - peptides

Scoring structures selected by accurate mass determination by virtual fragment matching of MSMS data
Second adhesion screen of 55,000 D-aa - peptides

HEK293-cells stick and hits may be sorted automatically

All D-Amino acids

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

H- K L Y K Y R A -OH
H- K L Y K H R A -OH
H- K L Y K I / L R A -OH
H- K L Y R P R A -OH
H- R L Y R V R A -OH
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Bias of aa’s in 20 columns

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