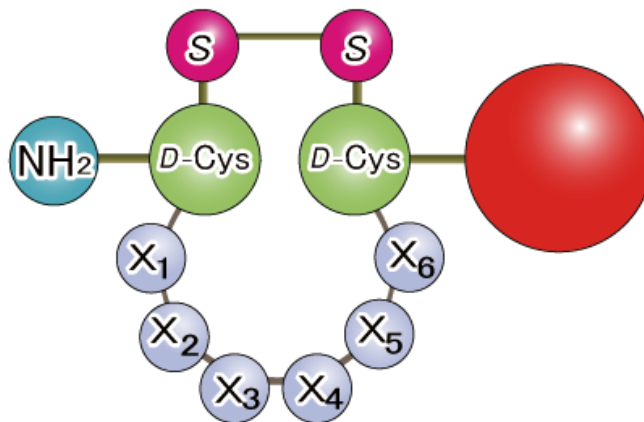


## High Quality One cyclic Peptide immobilized on One Bead (OPOB)



*Discovery for interacting Sequences*

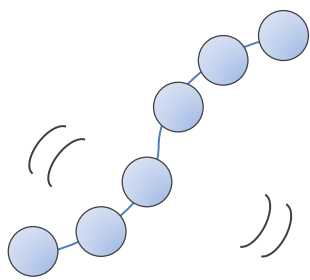
# Background

**Cyclic peptides library, immobilized on gel-type polymer support, is a reagent for discovery of the sequence that binds to the target molecule.**

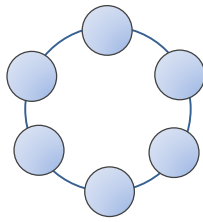
Current deconvolution method for the screened beads is Edman degradation, which is low throughput and free amino terminus is indispensable. Thus we have newly developed a higher throughput method by mass spectrometry in combination with the partial hydrolysis (protocols are in preparation).

## Basic architecture of the Peptide-Bead

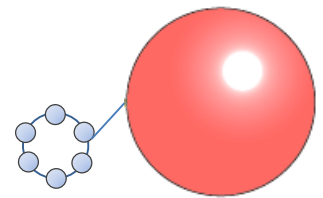
The peptide-beads consisting as the hexamer, constructed by 19 natural and 5 non-proteinogenic amino acids, with two *D*-cysteines to form an intra-molecular disulfide linkage (cyclized).



Linear peptide, very flexible structures



Rigid structures (expected higher affinities in recognition); Generally recognition requires 3-4 residues, although 3D structures are indispensable.

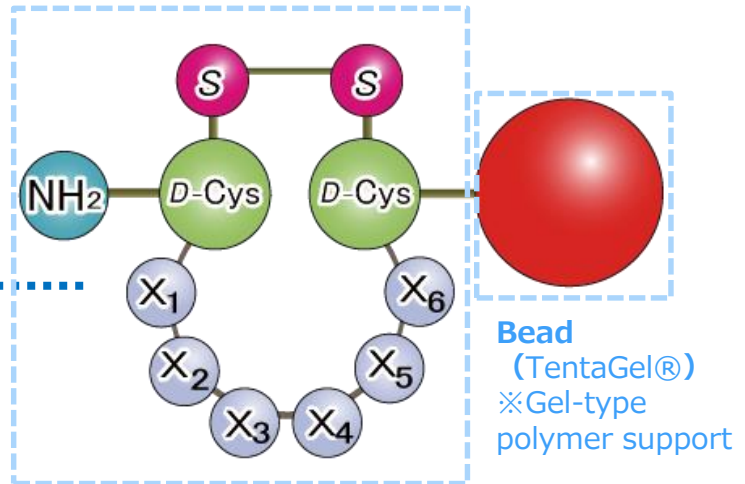


Peptides are immobilized on gel-type-polymer, thus allows assay in aqueous media, easier screening and picking up



24 kinds

19 natural amino acids  
5 non-proteinogenic amino acids, often used for drug development, large diversity!



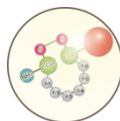
### Diversity of the sequence

$24^6 = 200 \text{ millions}$

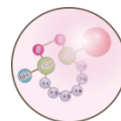
Proteins or cells (cell-surface proteins) are recognized, which can be picked up through screening (assays) and deconvoluted.

### Architecture of immobilized Peptides

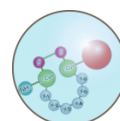
※Both termini of hexapeptides two *D*-cysteines are attached and cyclized, preventing enzymatic degradation during assays



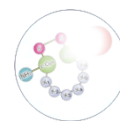
Sequence A



Sequence B



Sequence C

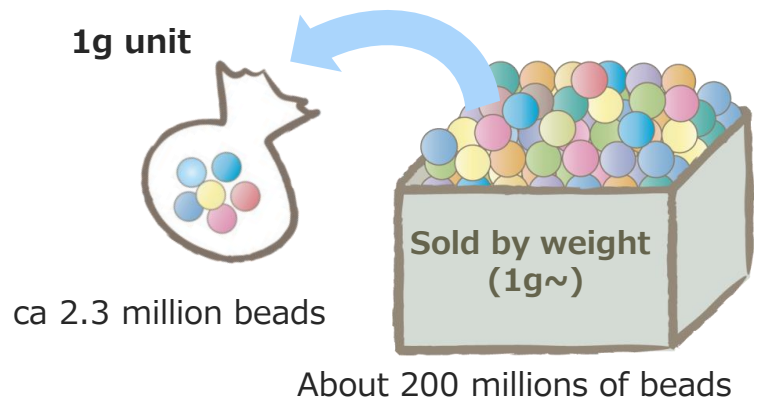


Sequence D

... } About 200 millions ways

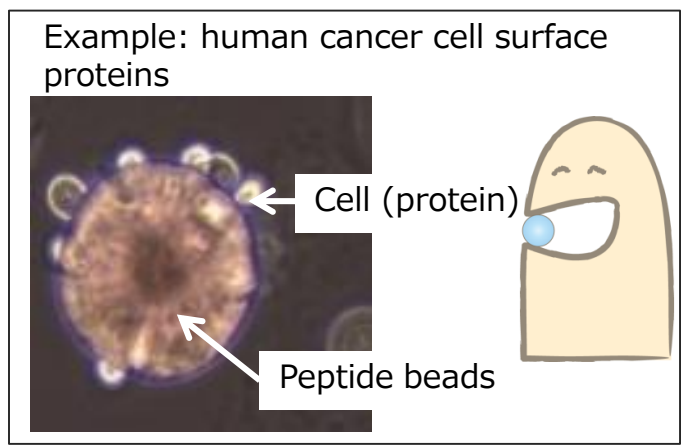
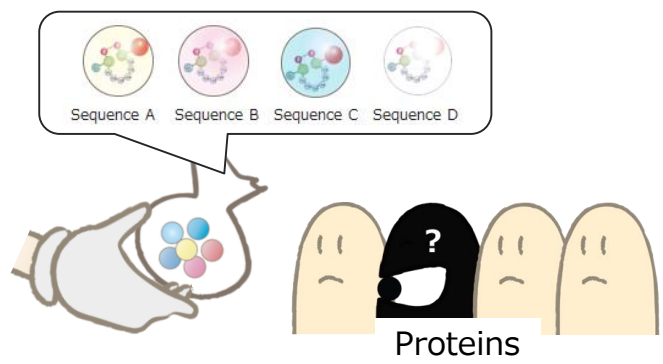
Offer

- Min. sales unit: 1 gram beads (equivalent to the dry weight) swelled in aqueous media containing ca. 2.3 million beads carrying 2.3 millions of high quality of cyclic octapeptides.



Screening and selection (Image)

Discovery of interacted of recognized beads → picking up → washing → deconvolution (sequence elucidation)



<p>Beads exposed to the target protein. Screening by the bead A</p> <p><b>No reaction</b></p> <p>①</p>	<p>Screening by the bead C</p> <p><b>Slightly reacted</b></p> <p>②</p>
<p>Screening by the bead B</p> <p><b>Too many beads screened</b></p> <p>③</p>	<p>Screening by the bead D</p> <p><b>Find bead recognized</b></p> <p>④</p> <p>Picking up the bead recognized by proteins. Washing and Edman degradation or novel method (in press), "partial hydrolysis with MS analyses" to elucidate the sequence(s)</p>

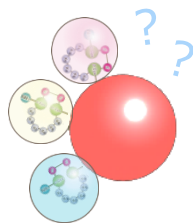
# Points

- **Resistant to enzymatic degradations during assays**
  - No inter-molecular cyclization (disulfide).
  - Two *D*-Cys for intra-molecule cyclization.
  - They are stable against endogenous enzymes.
- **High quality: thorough SPLIT & COMBINE procedure allow facile and higher-precision for deconvolution**
  - The polymer support used was gel-type TentaGel ®, swollen in aqueous media and lower non-specific absorption of proteins.
  - Contamination free Cyclic peptides immobilized on beads with the concept of One Peptide on One Bead (**OPOB**).

## Conventional mixture:

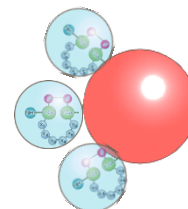
### Lower quality

Even finding the interacting subjects,  
Really recognized ?



## High quality OPOB

Immediately find  
recognition and  
sequence information



## Representative references

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Lebl M., Krchnak V., Sepetov N. F., Nikolaev V., Stierandova A., Safar P., Seligmana B., Thorpe D., Felder S., Lake D. F., Lam K. S., Salmon S. E. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Ed.; Mayflower Worldwide: Birmingham, 1994; pp 233–238.

Lau D. H., Guo L., Liu R., Song, A., Shao C., Lam K. S. Identifying peptide ligands for cell surface receptors using cell-growth-on-bead assay and one-bead one-compound combinatorial library. *Biotechnol. Lett.* 2002, 24, 497–500.

Ito H., Nokihara K., Soutome S., Ohyama T. Screening of peptides that inhibit bacterial binding to fibronectin using combinatorial peptide libraries. *Int. J. Pept. Res. Ther.* 2006, 12, 275–281.

Hirata A., Nokihara K. Construction of peptide-vehicles, bioconjugates having modules of cancer cell surface capture and cell-penetrating peptide with anticancer agents. *Tetrahedron Lett.* 2014, 55, 4091–4094.