

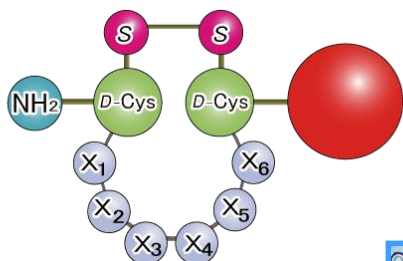
# One cyclic peptide immobilized on One Bead

Recently the molecular targeting therapy has been successfully applied for cancer chemotherapy, although in some cases side-effects are not negligible. Based on our bio-detection concept, that is, protein interaction can be mimicked by peptides, a novel cell-targeting concept designated peptide-vehicle has been proposed, which was conjugates consisting of the cancer cell recognition and cell penetrating peptides with anticancer drugs. The cancer cell capture elements was a cyclic peptide containing D-cysteines, which should discriminate normal and cancer cells. Several cell penetrating peptides has been prepared and conjugated. Anticancer drugs recycled from clinical use were purified and derivatized to load on the vehicle. For practical use patient cancer cells were obtained by surgery and primary culture has been succeeded. The efficacy of the present vehicle has been studied by using cancer cell lines and primary cultured human cancer cells.

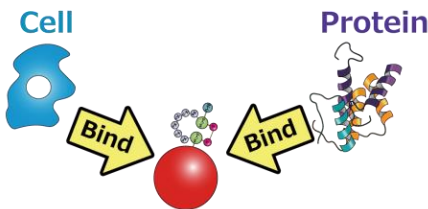
## Library of cyclic hexa-peptides with two D-cysteins immobilized on a gel-type resin (assays in aqueous media)

**X = 24 AA: 19 natural & 5 non-natural**  
**Diversity  $24^6 = \text{ca. } 200 \text{ millions}$**

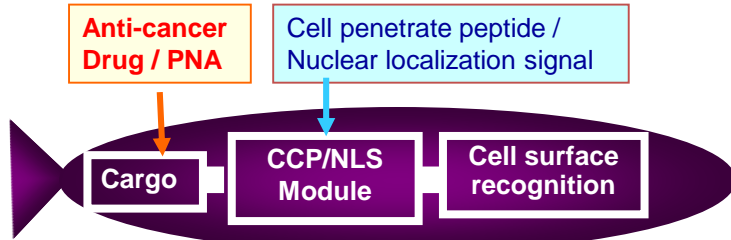
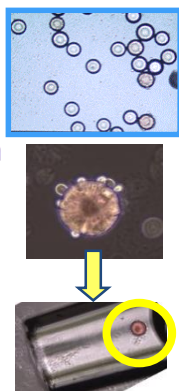
Characteristics (high quality): Prepared slit & combine; No mixture AA used; No inter-molecular SS; ca 100 mm particle; Peptide loading 100 pmole/ bead; 2.3 million beads/g; Selected single bead sequencing by Edman (Nokihara 1992)



Discovery → DDS



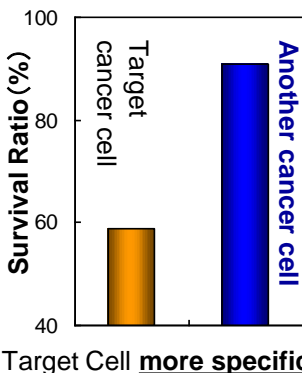
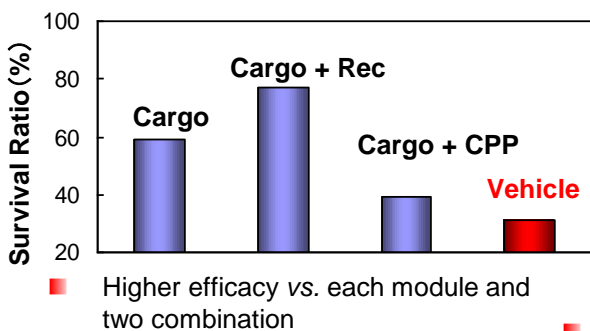
X = 24 natural & non-nat. AA  
 Diversity  $24^6 \text{ ca. } 200 \text{ millions}$



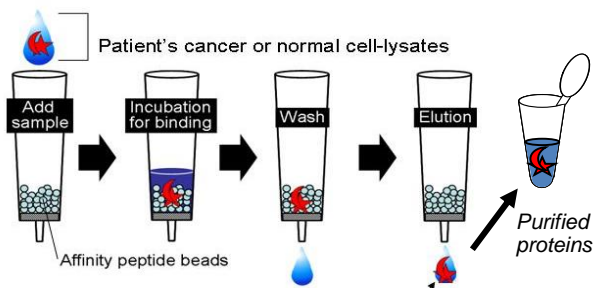
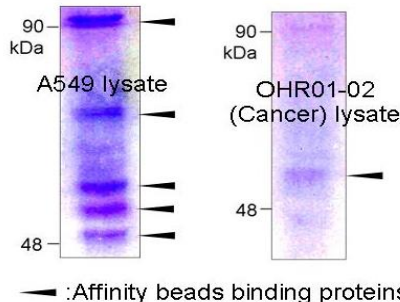
Peptide-Vehicle kills cancer cells more efficiently.

Nokihara, K., et.al, *Peptide Science* 2011: 73, 2012.  
 Hirata, A. & Nokihara, K., *Tetrahedron Lett.* 55, 4091, 2014.

### Efficacy for Peptide Vehicle



### Discovery of binding proteins purified by affinity columns



- Protein Separation ① HPLC
- ② SDS-PAGE + in-gel digestion
- ③ 2D Electrophoresis + in-gel digestion
- ④ Nano-LC MS/MS + OrbiTrap /MALDI-TOF/MS/MS

### Identification and Characterization



Peptides immobilized on to gel-type beads used for affinity columns [Nokihara, *Peptide Chemistry* 1993, 25, 1994] Elucidation for diseases specific proteins. → discovery of marker proteins