A New Peptide Drug Modality; Helix-Loop-Helix Technology

Interprotein®

Interprotein Corporation

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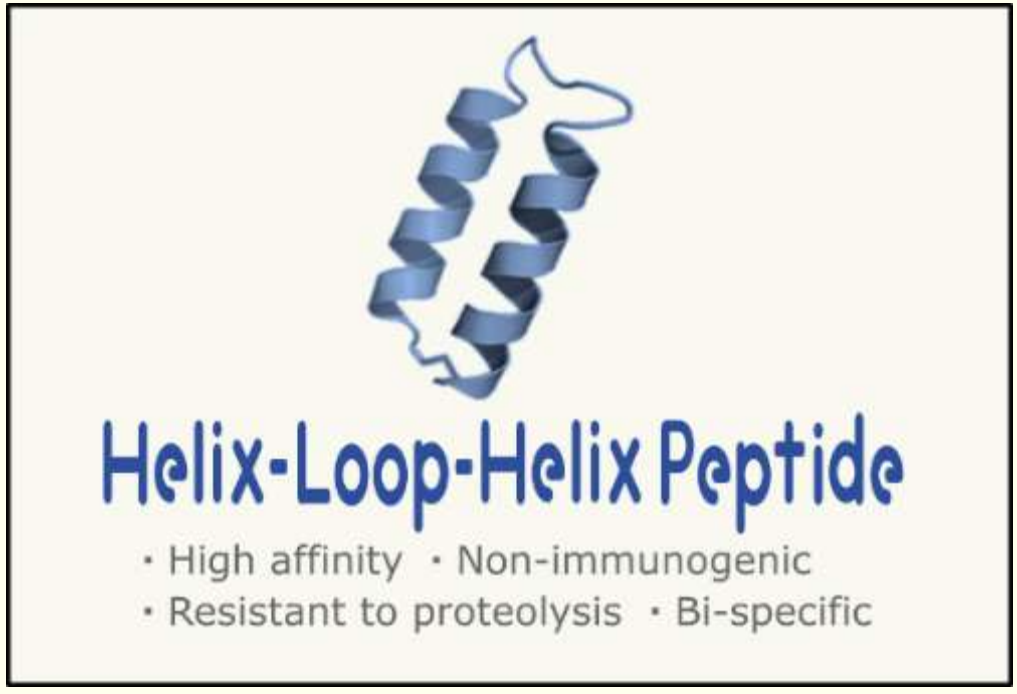
- Location: Osaka, Japan
- Year Established: 2001
- CEO & President: Masato Hosoda
- www.interprotein.com
- @interprotein

- Expertise: Development of PPI (protein-protein interaction) modulators with small molecules or peptide
- Business Model:
  - Strategic Alliance: Target discovery and Lead optimization
  - Licensing of a pipeline

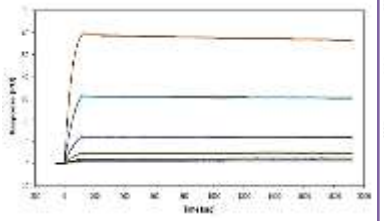
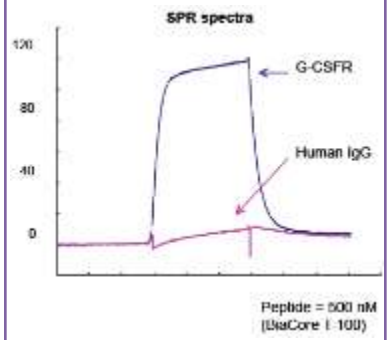
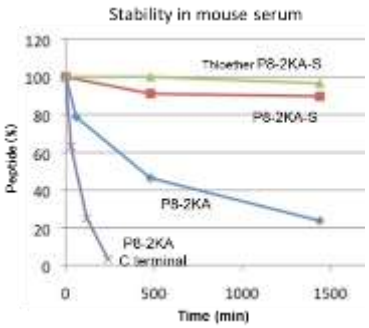
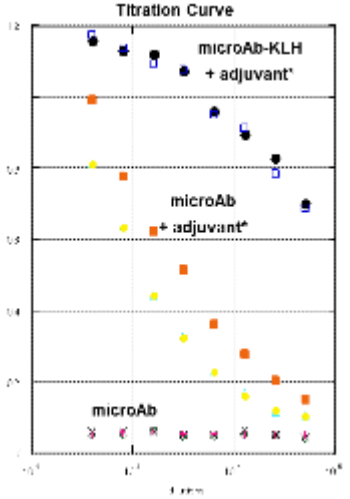
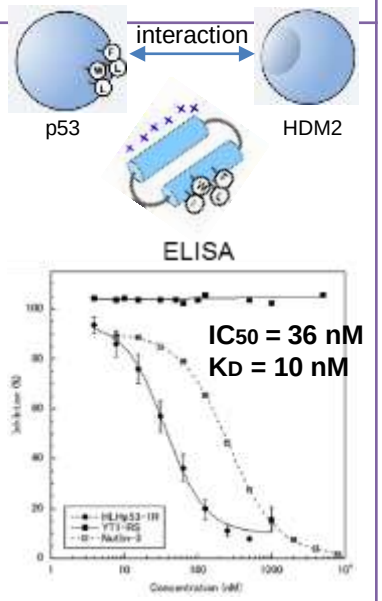
Platform Technology



- Conformationally-constrained Helix-Loop-Helix (HLH) peptide platform technology

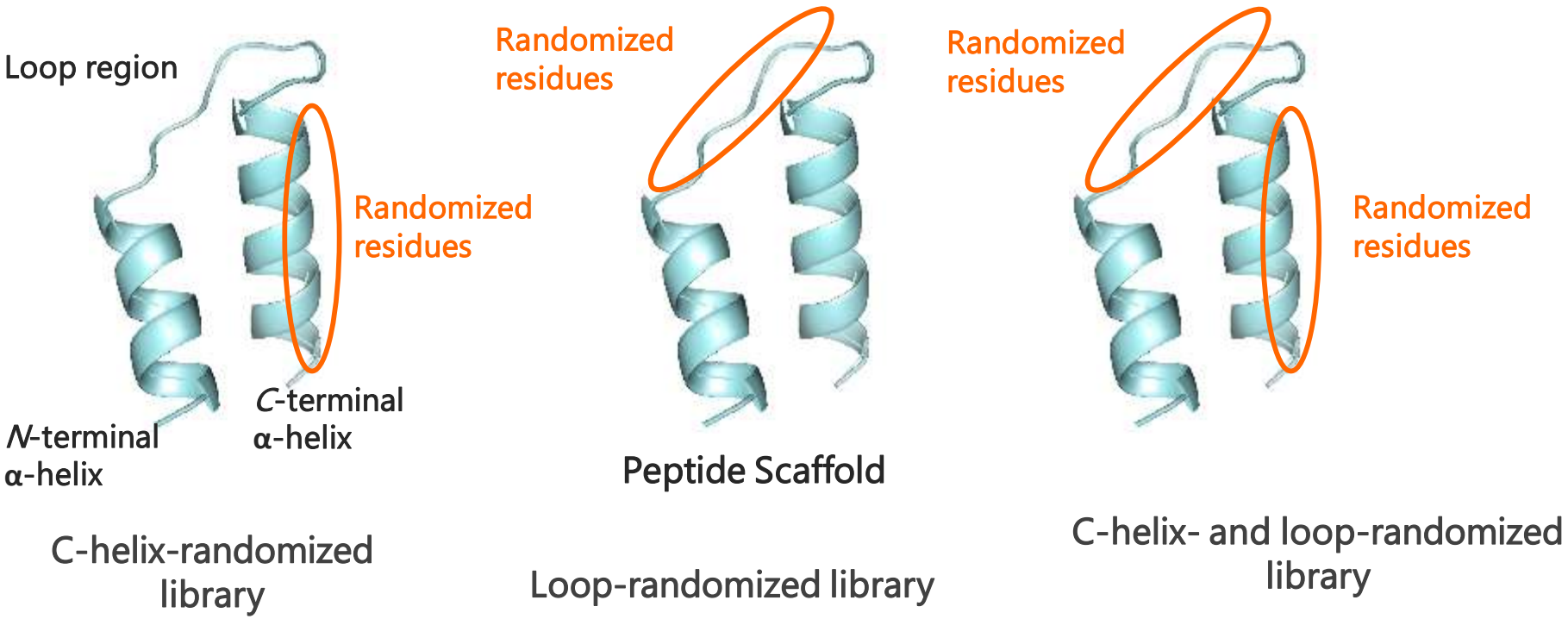
☐ High affinity
☐ Non-immunogenic
☐ Resistant to proteolysis
☐ Bi-specific

Advantageous Property of Helix-Loop-Helix Peptide in Drug Discovery

Affinity	Selectivity	Stability	Antigenicity	Applicability
High binding affinity to the target protein with K_D values of double-digit pM	**High selectivity for the target protein**	**High stability in serum (half life of cyclized peptide: more than 15 days)**	**Low risk for antigenicity (using only natural amino acids)**	**Broad applicability (α-helix-irrelevant, intracellular and extracellular PPIs)**
 **K_D = 34 pM**				

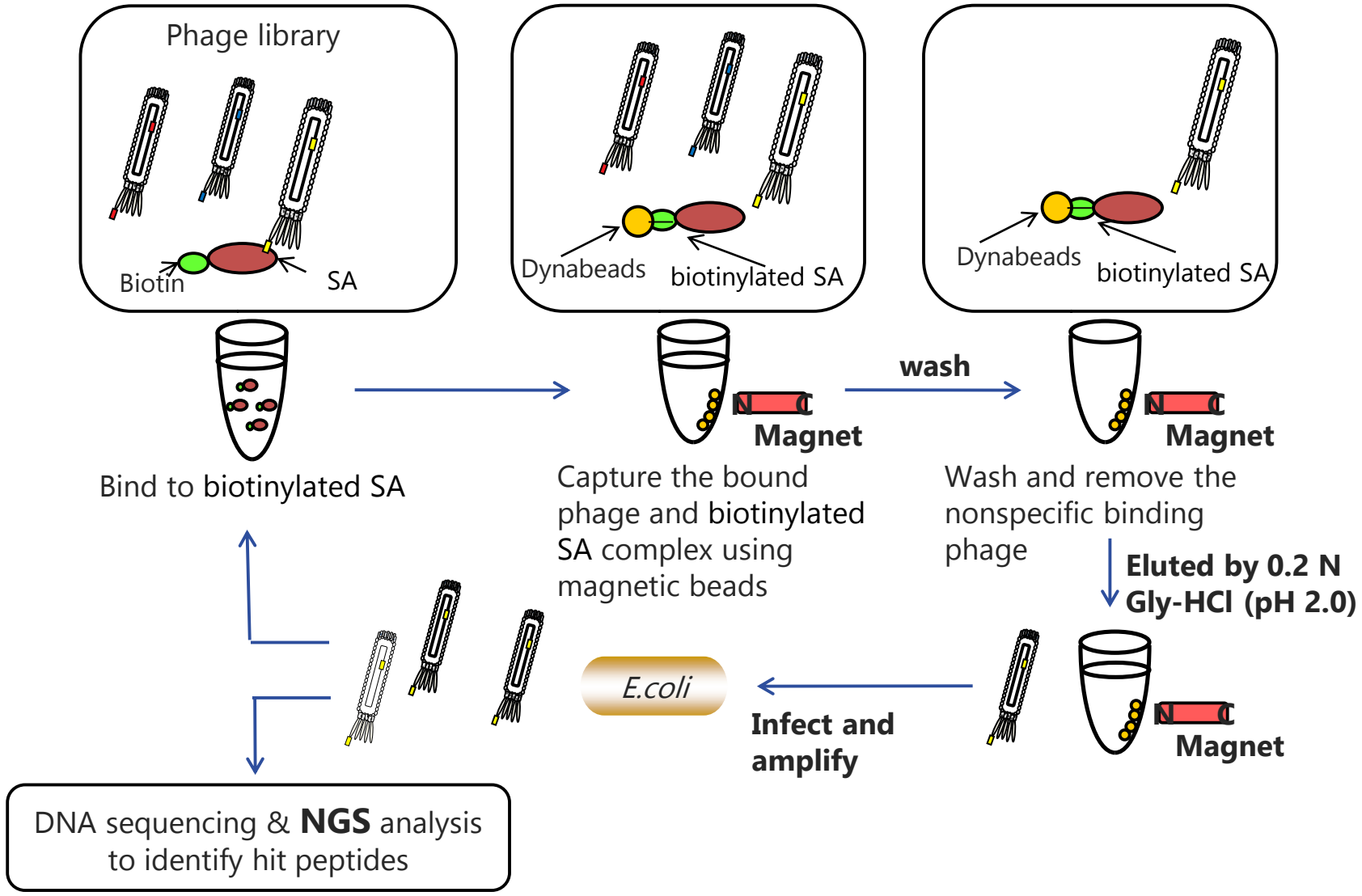
peptides	half life
P8-2KA	6.3 hour
P8-2KA-S	5.25 day
Thioether P8-2KA-S	15.25 day
P8-2KA C terminal	0.5hour

The largest-ever library size ensures production of high affinity binders against any targets



- We have constructed the ever largest size library of helix-loop-helix peptide (library size: 10^{10}) in our history
- This has enabled us to achieve 100% success rate in identifying high affinity binders ever since

Screening phage display libraries with biopanning



Enrichment of Streptavidin (SA)-binding phages by biopanning (model experiment)

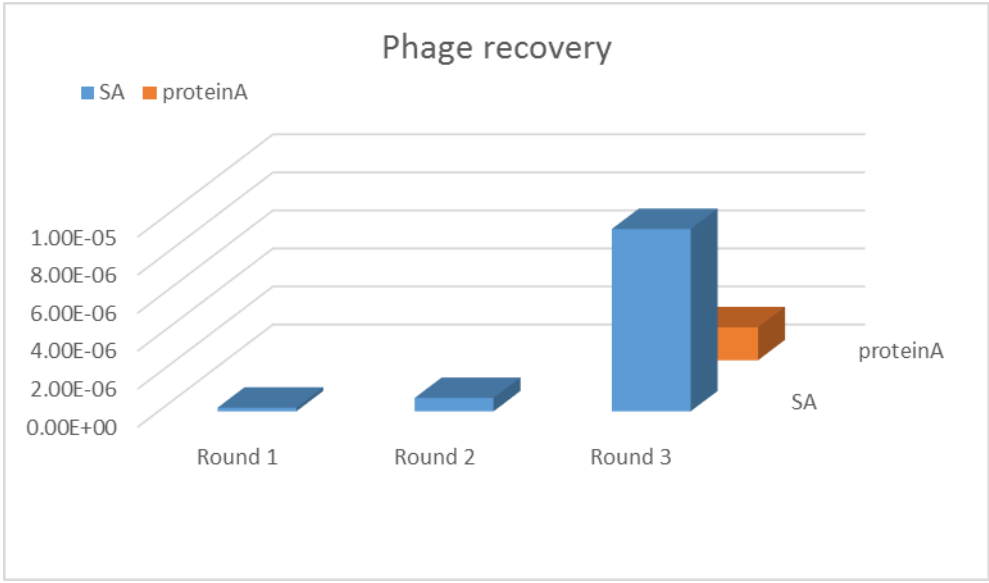
Ex. The results of 3-round biopanning against Streptavidin

				Dynabeads SA		Dynabeads Protein A	
Panning round	Concentration of biotin-streptavidin	Incubation time	Washing condition	Input phage	Output phage	Input phage	Output phage
1	1000 nM	Overnight at 4 °C	PBST 3 times	4.50E+11	8.60E+04	-	-
2	1000 nM	2 hr. on ice	PBST 3 times	7.80E+11	5.50E+05	-	-
3	100 nM	1 hr. on ice	PBST 10 times	4.90E+11	4.70E+06	4.90E+11	8.50E+05

Mixed libraries :

Mixture of CHR, LR, and CHLR was used for screening of Streptavidin (SA)-binding helix-loop-helix peptides. In Round 3, dynabeads Protein A was used as a negative control.

From Round 1 to 3, enrichments of output phage shows >50-fold. In Round 3, as compared with control dynabeads Protein A (which cannot capture the biotinylated SA), more than 5-fold of phages were captured from the target protein by using dynabeads SA.



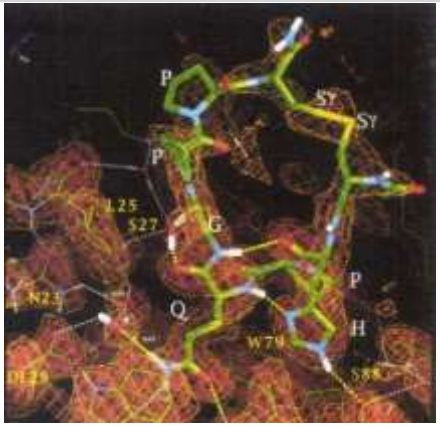
Identification of Streptavidin-binding Consensus Sequence

Streptavidin-binding peptide sequences isolated from mixed libraries

clone No.	amino acid sequence of peptides	frequency
kato-r4-01	AELAALEAELAALEGC**HPQ**GGKLCLLKAKLPTLKA	2
kato-r4-04	CAAELAALEAELAALEGR**HPQ**FPPPHGKLVGLKNKLCQLKKAC	2
kato-r4-07	CAAELAALEAELADHHYD**HPQ**DGKL*MLKWKLRELKVAC	1
kato-r4-10	CAAELAALEAELAALEGPH**HPQ**DPRSGKLHRLKKKLEDLKLAC	1
kato-r4-12	CAAELAALEAELAALEGY**HPQ**VPPPHGKLQMLKGKLRQLKEAC	1
kato-r4-02	CAAELAALEAELAALEGQ*WEAELRAGKLAALKAKLAALKAC	1
kato-r4-08	AELAALEAELAALEGNVTMYGKLPLLKHKLTTLKA	1
kato-r4-09	AELAALEAELAALEGNQGRGGKLQPLKQKLGTLKA	1

Library	Size
CHLR	5.1 X 10^8 transformants
LR	4.8 X 10^8 transformants
CHR	1.0 X 10^8 transformants
Total	**1.1 X 10^9 transformants**

Using Streptavidin as the target, after 4 rounds of enrichment/amplification, it was confirmed that the consensus sequence for Streptavidin-binding peptides with the HPQ motif was most strongly conserved.



The streptavidin-bound crystal structures of disulfide-bridge cyclic peptides cyclo-Ac-[C**HPQ**GPPC]-NH2

In house project (Helix-loop-helix peptides)

We are also developing a new modality, helix-loop-helix peptide for developing PPI inhibitors against following targets. http://www.interprotein.com/HLHP.html

- **Oncology, Hematology**

Project	Stage	Note
Tumor peptide	Lead optimization	We have identified a peptide that binds to tumor angiogenesis factor with pM found that the peptide is localized in a tumor, which releases a lot of the tumor factor, in a mouse model . We anticipate that this peptide can also be developed drug conjugates (PDC).
P53-MDM2, P53-FOXO4	Hit validation	We have identified a peptide that blocks p53-MDM2 interaction and thereby are now examining a correlation of the *in vitro* efficacy and peptide penetration We are also going to isolate p53-FOXO4 blockers for aging research.
TIM-3 inhibitor	Hit identification(≈lea generation)	Interprotein has identified candidates through phage-display and validated the to TIM-3 by SPR. The peptide without an epitope-tag has been synthesized and for its binding on Tim-3. We have recently isolated the peptide that can inhibit 9/TIM-3 interaction.
NKG2A/HLA-E/CD94	Discovery	We have designed peptides that are expected to bind NKG2A and inhibit NKG2A HLA-E/CD94.
KIR/HLA-C inhibitor	Discovery	We have designed peptides that are expected to bind KIR and inhibit KIR binding
IL-6	Discovery	We have isolates peptides that bind to TIM-3 by SPR through phage-display