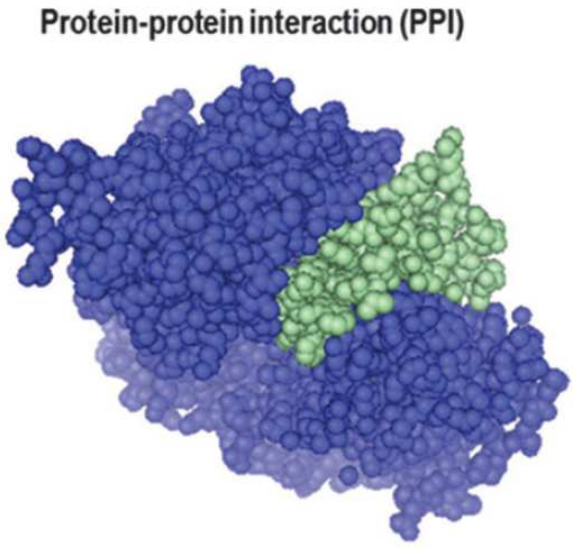
Library Strategy
for
Cyclic peptide

LS 2018/06/16 Koichi Kamiya

Protein-Protein Interaction

- **Protein-Protein Interaction (PPI) control many essential biological pathway.**
- **PPI could be potential drug targets.**
- **Contact surface area is typically very large at approximately 1500-3000 Å^2 (protein-small molecule interaction is 300-1000 Å^2.**
- **Binding pockets are often flat, featureless, and lack well defined grooves.**

The selectivity of small-molecular-weight compounds is often low.
While the selectivity of biologics is high, but they have low bioavailability.

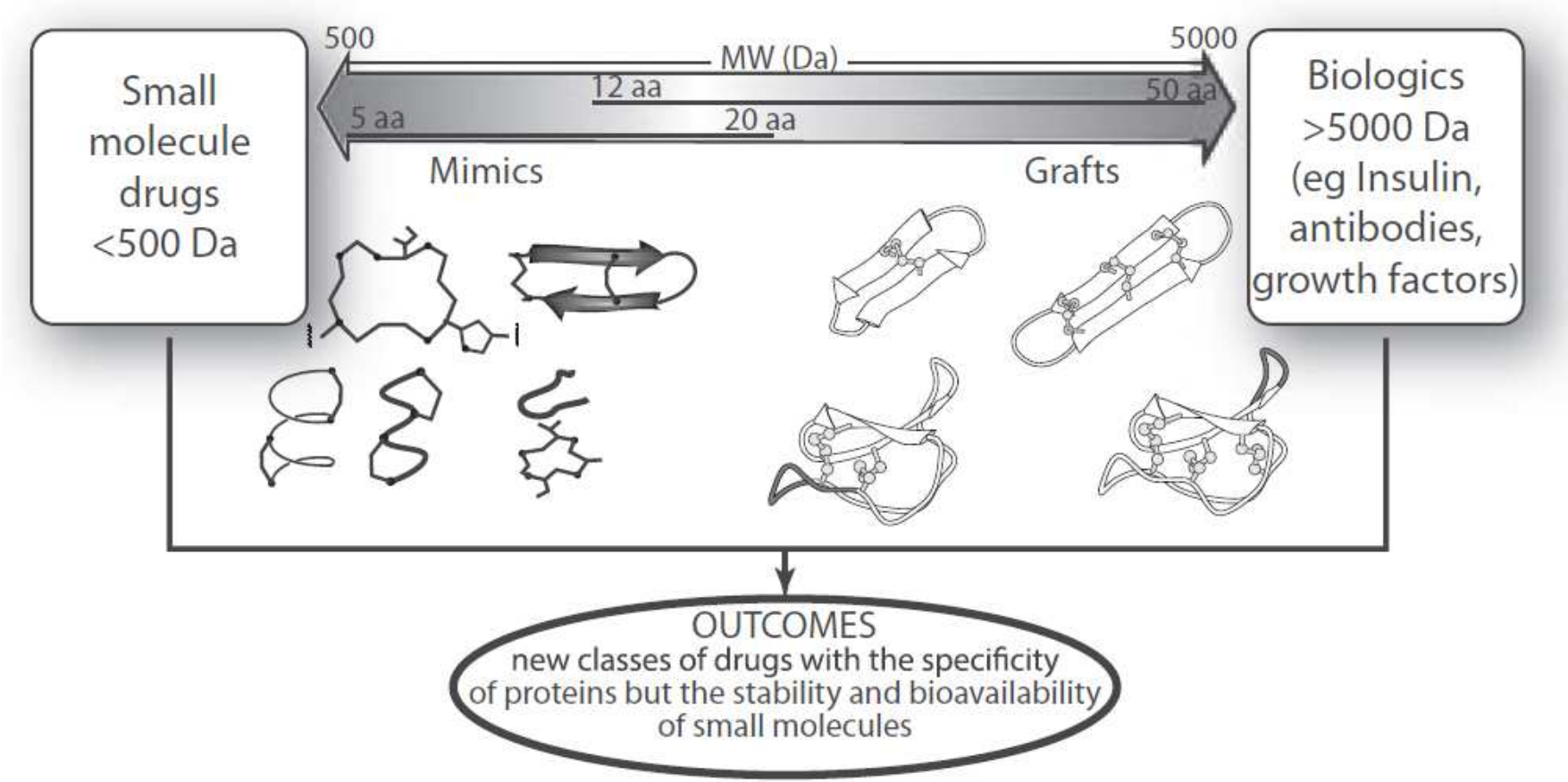


Nevola, L.; Giralt, E. *Chem. Commun*. **2015**, 51, *3302*.

Peptide as a Drug Candidate

- **orally bioavailability**
- **low target selectivity**

- **low bioavailability**
- **high target selectivity**
- **low cell permeability**

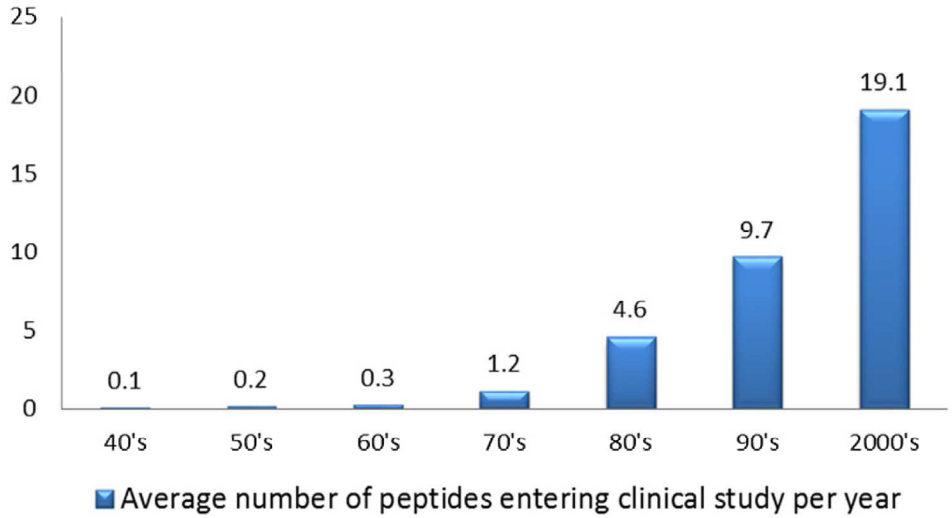


Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. *Chem. Biol. Drug. Dis*. **2013**, 81, *136*.

Peptide as a Drug Candidate

Advantages of Peptide

- **flexibility, which is translated into adaptability to large surfaces**
- **easy modularity, which increases structural diversity and consequently allows higher selectivity and potency**
- **size, which limits accumulation in tissue**
- **complete biocompatibility, which means low toxicity in humans**

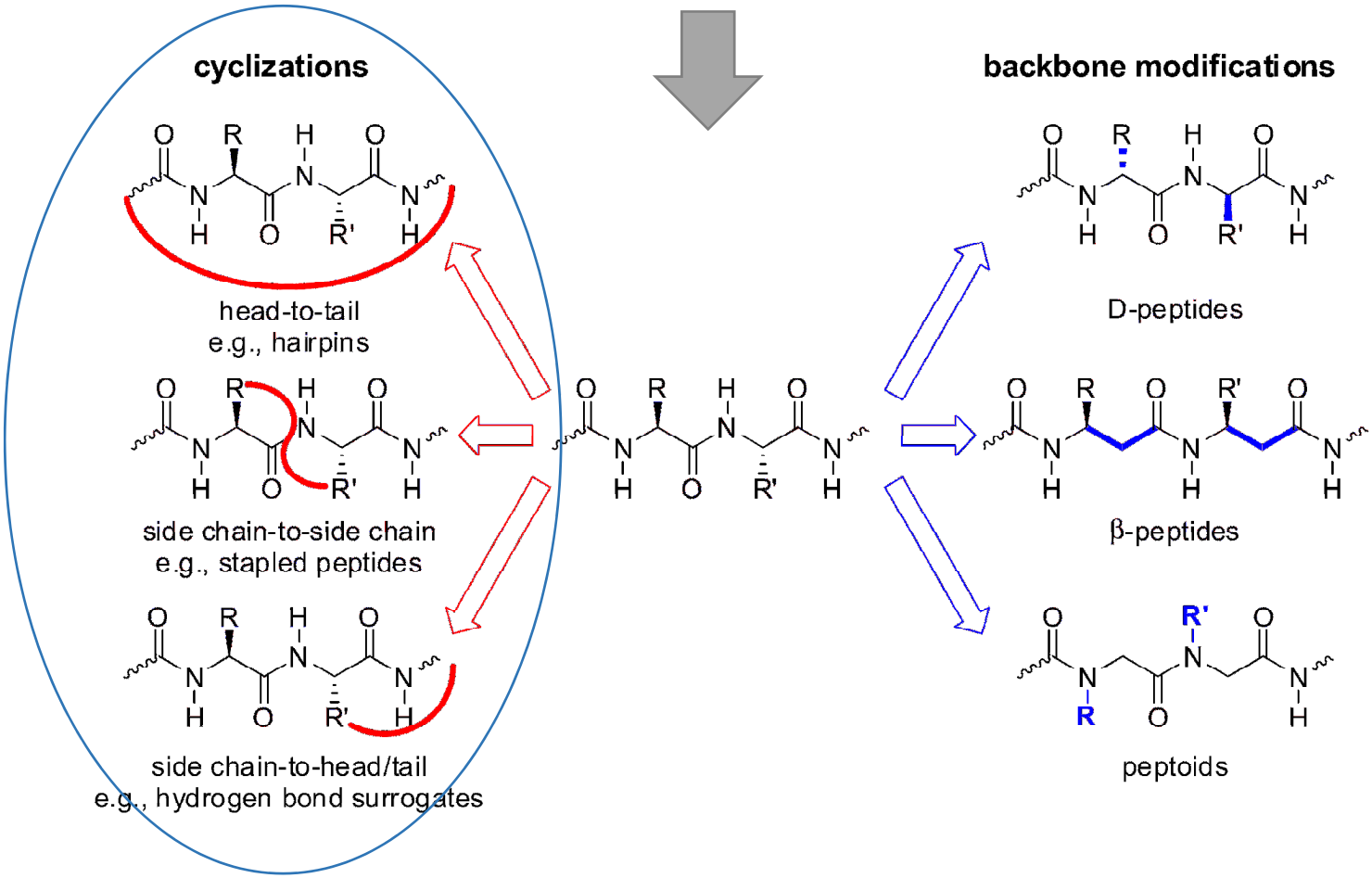


Peptides are good candidates for drug discovery.

Tsomaia, N. *Eur. J. Med. Chem*. **2015**, 94, *459*.

Main Challenge of Peptide Drugs

Increasing stability of the active conformation and decreasing susceptibility toward proteolysis are the most important goals.



Wójcik, P.; Berlicki, Ł. *Bioorg. Med. Chem. Lett*. **2016**, 26, *707*.

How to Find Peptide Drugs ?

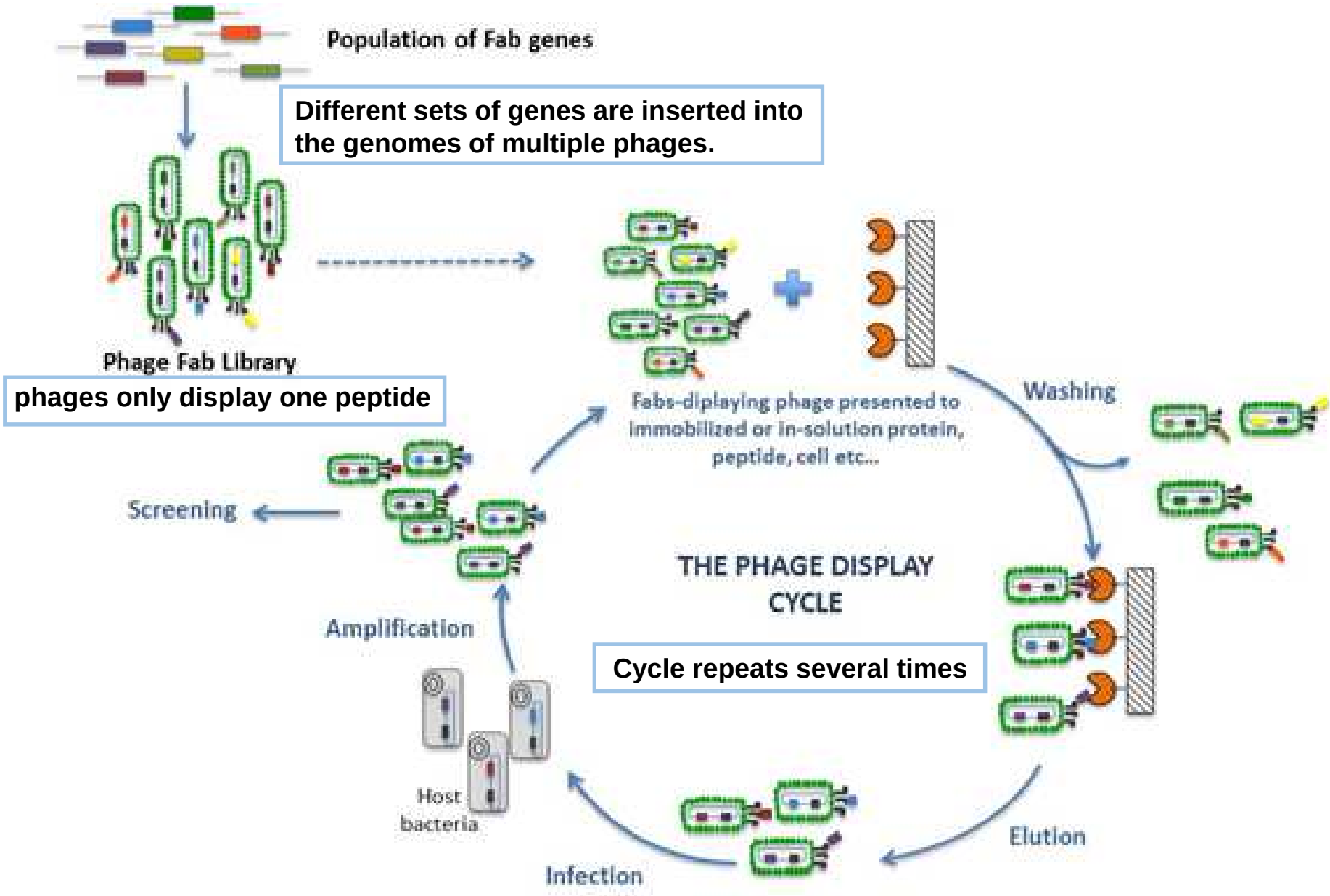
・Peptides can be easily synthesized chemically and biologically.

・Constructing combinatorial chemistry and high throughput screening are good method.

Method

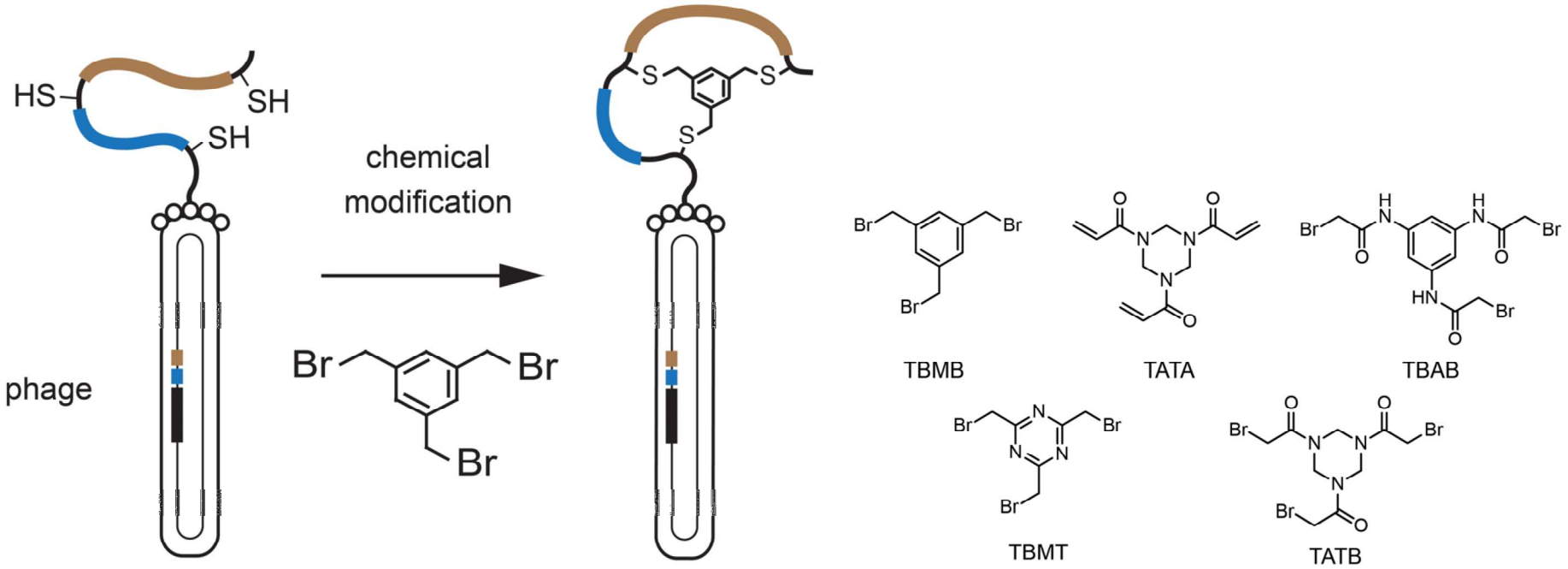
1. OBOC library
   - See 170322_LS_Hiroaki_ITOH
2. DNA-encoded library
   - See 171125_LS_Yuri_Takada
3. Phage display library
   - Today's focus

Phage Display Library



http://www.creative-biolabs.com/blog/wp-content/uploads/2016/12/phage-display.png

Previous Bicyclic Peptide from Phage Display



A major limitation

▪low scaffold diversity

Diversity is based only on variation of the amino-acid side chain, varying the number of amino acids or the chemical linkers

Deyle, K.; Kong, X.; Heinis, C. *Acc. Chem. Res.* **2017**, 50, *1866*.

Today's Main Paper

nature chemistry

ARTICLES

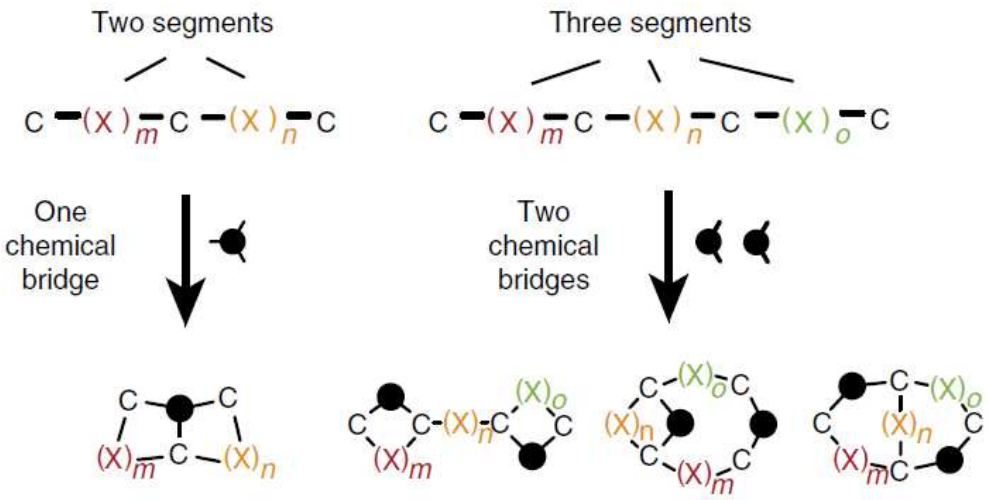
https://doi.org/10.1038/s41557-018-0042-7

Cyclization of peptides with two chemical bridges affords large scaffold diversities

Sangram S. Kale[1,2], Camille Villequey[1,2], Xu-Dong Kong[1,2], Alessandro Zorzi[1], Kaycie Deyle[1] and Christian Heinis[1*]

Successful screening campaigns depend on large and structurally diverse collections of compounds. In macrocycle screening, variation of the molecular scaffold is important for structural diversity, but so far it has been challenging to diversify this aspect in large combinatorial libraries. Here, we report the cyclization of peptides with two chemical bridges to provide rapid access to thousands of different macrocyclic scaffolds in libraries that are easy to synthesize, screen and decode. Application of this strategy to phage-encoded libraries allowed for the screening of an unprecedented structural diversity of macrocycles against plasma kallikrein, which is important in the swelling disorder hereditary angioedema. These libraries yielded inhibitors with remarkable binding properties (subnanomolar K_i, >1,000-fold selectivity) despite the small molecular mass (~1,200 Da). An interlaced bridge format characteristic of this strategy provided high proteolytic stability ($t_{1/2}$ in plasma of >3 days), making double-bridged peptides potentially amenable to topical or oral delivery.

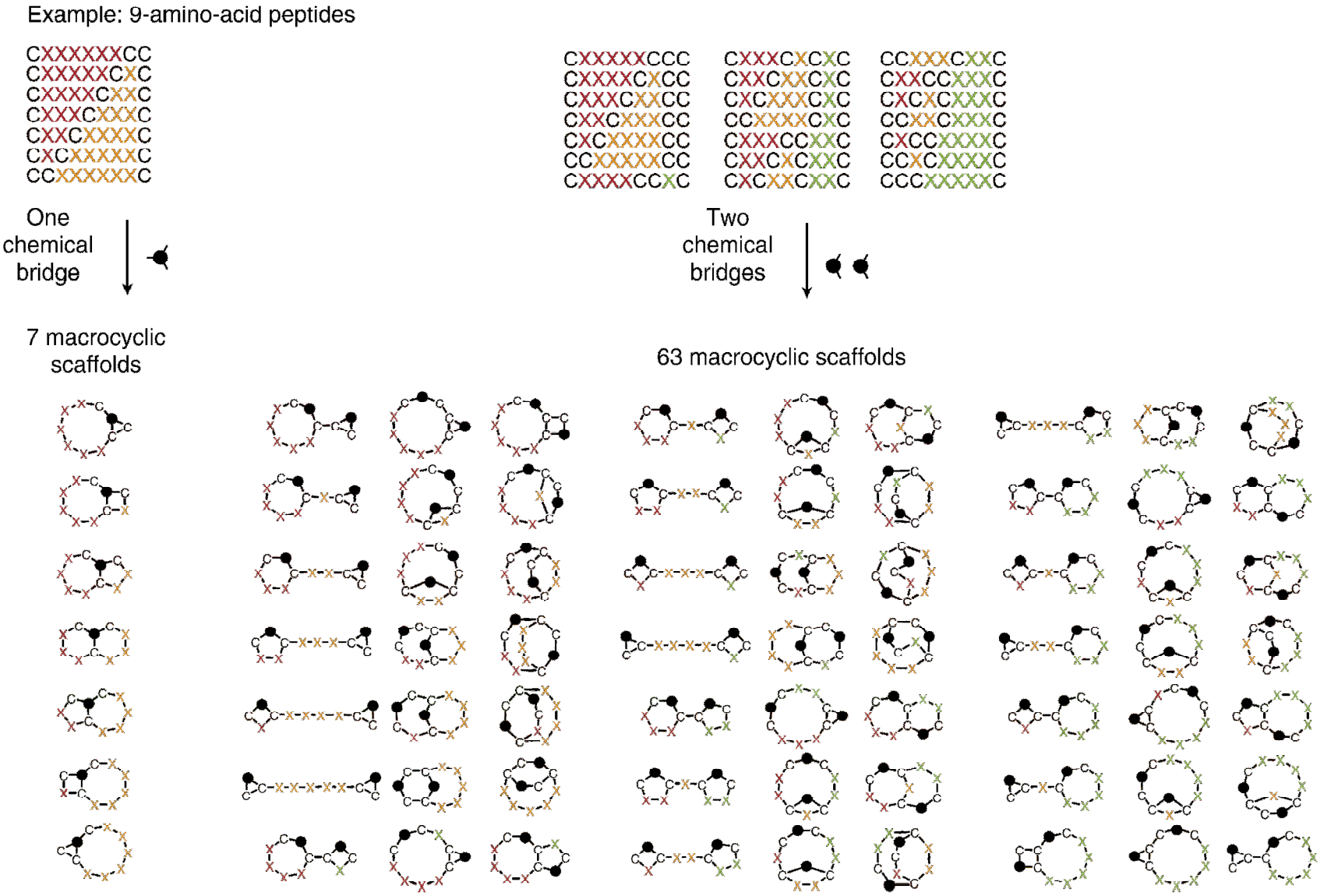
Bicyclic Peptide Scaffolds by Two Chemical Bridge



Peptide length	Number of possible macrocyclic scaffolds	
	One bridge	Two bridges
	length-2	$\frac{3 \times (\text{length-2})!}{2! \times (\text{length-4})!}$
8	6	45
9	7	63
10	8	84
11	9	108
12	10	135
13	11	165
14	12	198

Cyclization of peptides with two chemical bridges (connecting four cysteines) yields a much larger number of bicyclic peptide scaffolds than cyclization with one bridge.

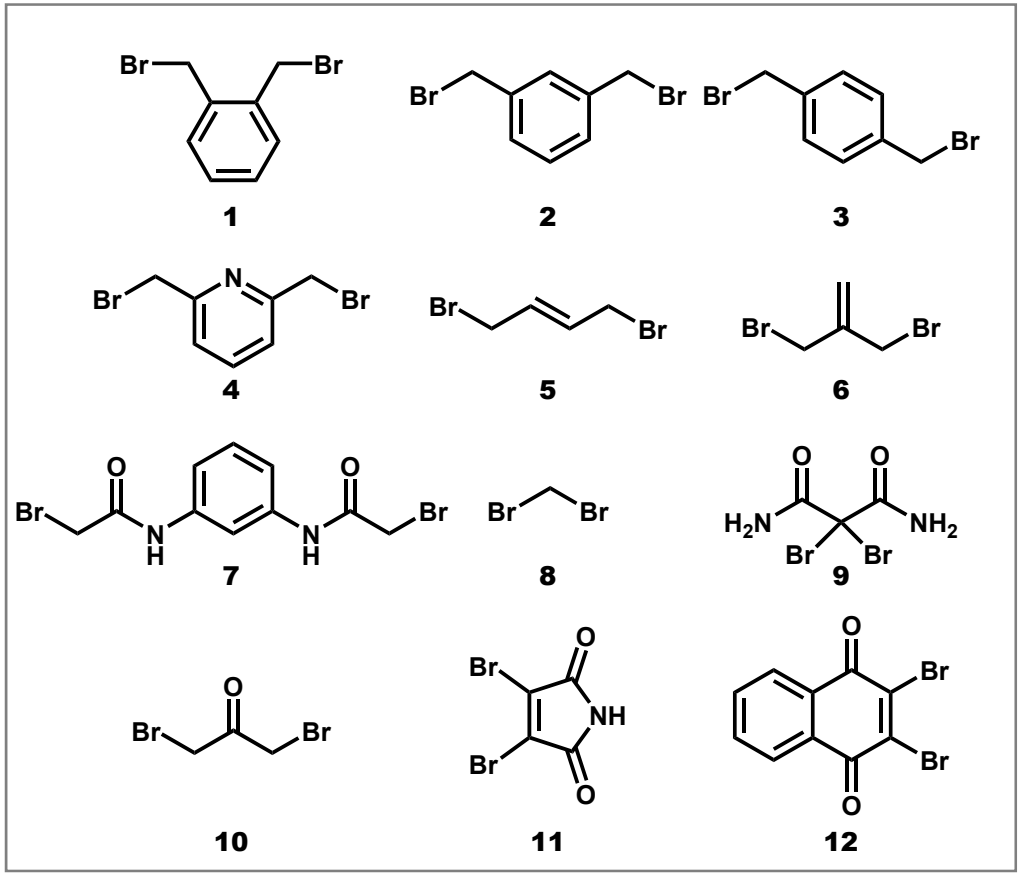
Example of Bicyclic Peptide Scaffolds (9 amino acid)



The number of scaffolds with two chemical bridges is 9 times larger than that with one chemical bridge.

Model Experiment of Chemical Linkers

- Model peptide (ACSRCVECGWCG-NH_2) was reacted with chemical linkers (**1-12**) to see if double-bridged peptide was produced.



Concentration (mM)

Bridge	0.1	0.2	0.4	0.8	1.6	3.2	6.4
1							
2	b,c	b,c	b,c	b,c	b,c	b,c	b,c
3	b,c	b,c	b,c	b,c	b,c	b,c	b,c
4	b,c	b,c	b,c	b,c	b,c	b,c	b,c
5	c	c	c	c	c	c	c
6	b	b	a				
7				d			
8				b	b	b	b
9			c	c			
10							
11	c	c	c	c	c	c	c
12	c	c	c	c	c	c	c

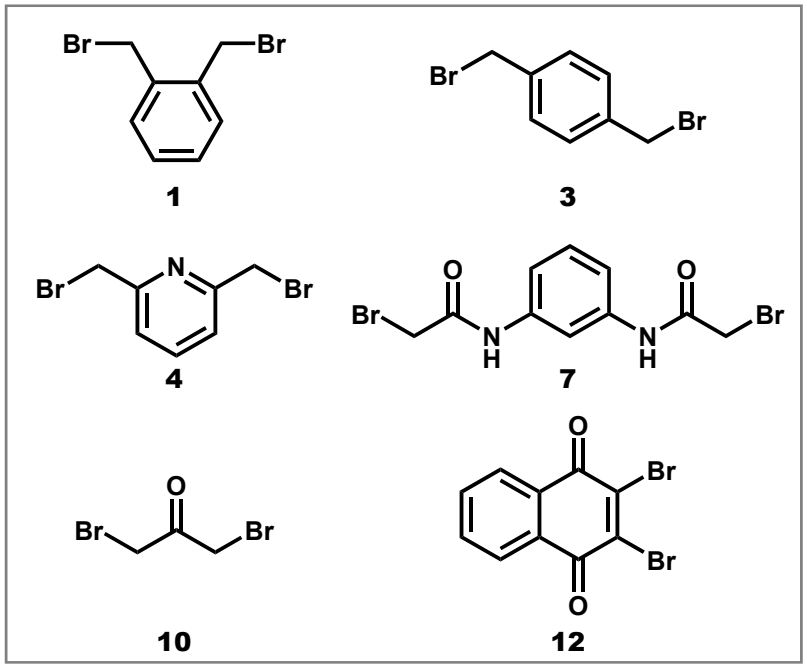
Major product:

- Unreacted peptide
- Double-bridged peptide
- Non-bridged modific.
- Not identified product

Eight of the 12 reagent (1-5, 10-12) produced the double-bridged peptide as the main product in a wide range of concentration.

The reactions are robust and would work efficiently.

Library Design



library 1

XCX_3CX_3CX-phage

X : any amino acids, C : cysteine

20% contain an additional cysteine

library 2

XCX_4CX_4CX-phage

X : any amino acids, C : cysteine

23% contain an additional cysteine

Each library was panned two rounds against immobilized plasma kallikrein.

Plasma Kallikrein and HAE

Plasma Kallikrein

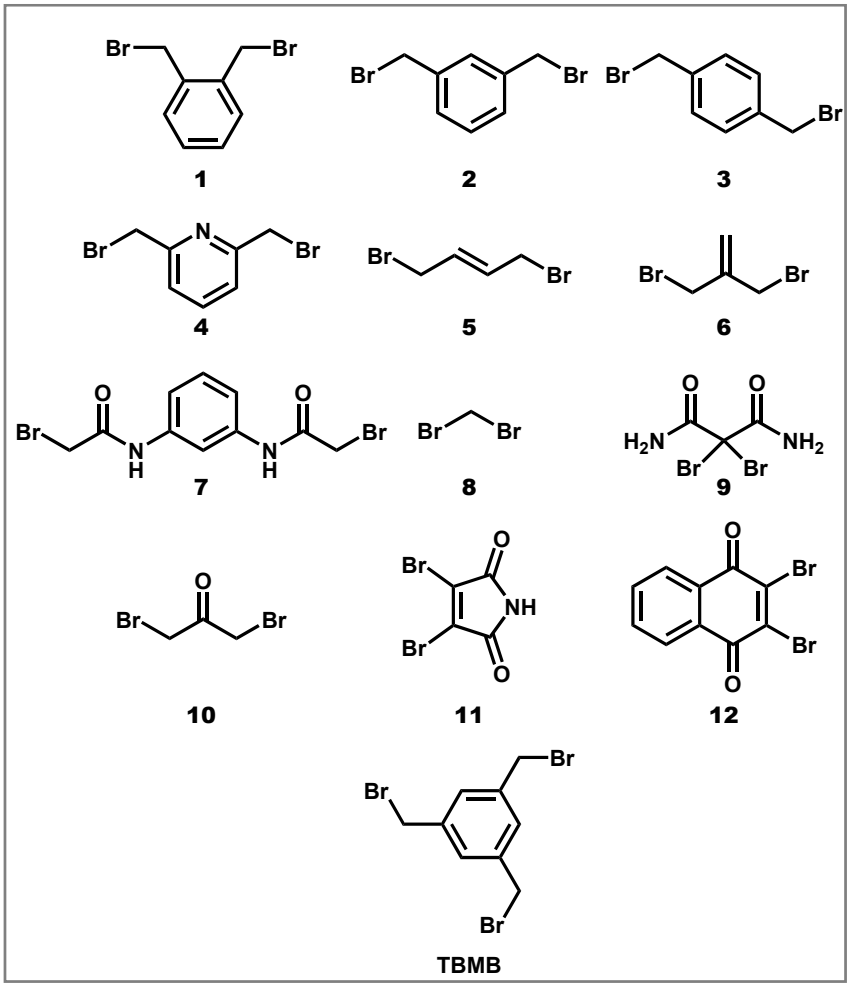
- **Selective cleavage of some Arg- and Lys- bonds, including Lys-Arg and Arg-Ser in (human) kininogen to release bradykinin**
- **This enzyme is formed from plasma prokallikrein (Fletcher factor) by factor XIIa**
- **An important target of the swelling disorder hereditary angioedema (HAE)**

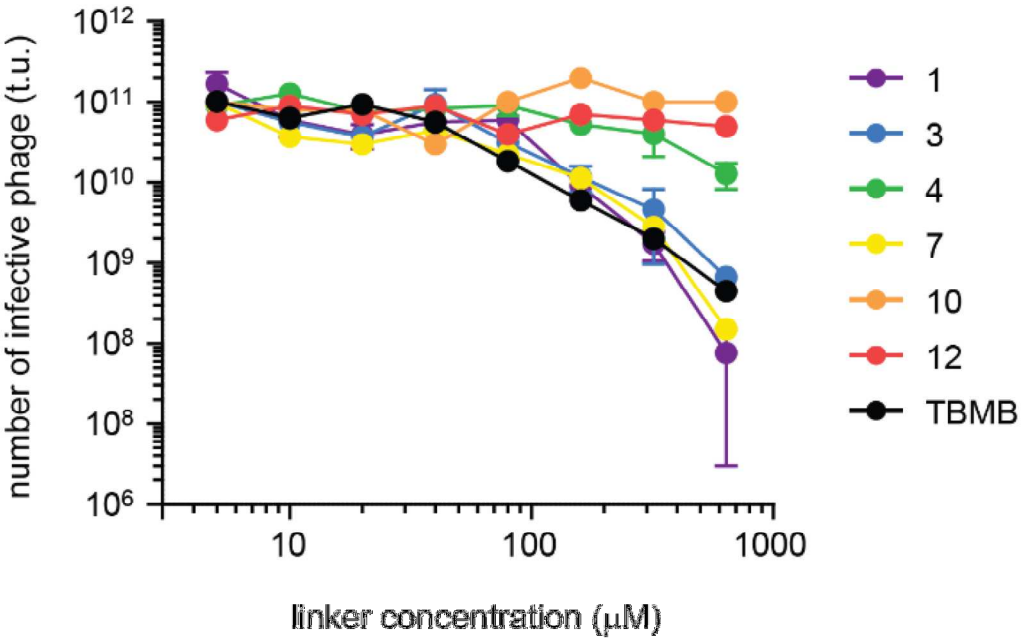
HAE

- **a disorder that results in recurrent attacks of severe swelling**
- **Attacks, without treatment, typically occur every couple of weeks and last for a few days**
- **There are three main types of HAE.**
  Type I and II are caused by a mutation in the SERPING1 gene that makes the C1 inhibitor protein while type III is often due to a mutation of the factor XII gene.
  This results in increased amounts of bradykinin which promotes swelling

Phage Infectivity after Reaction

Using library 1, whether the reaction affected phage infectivity was confirmed.





For most linkers, the reaction did not affect phage infectivity.

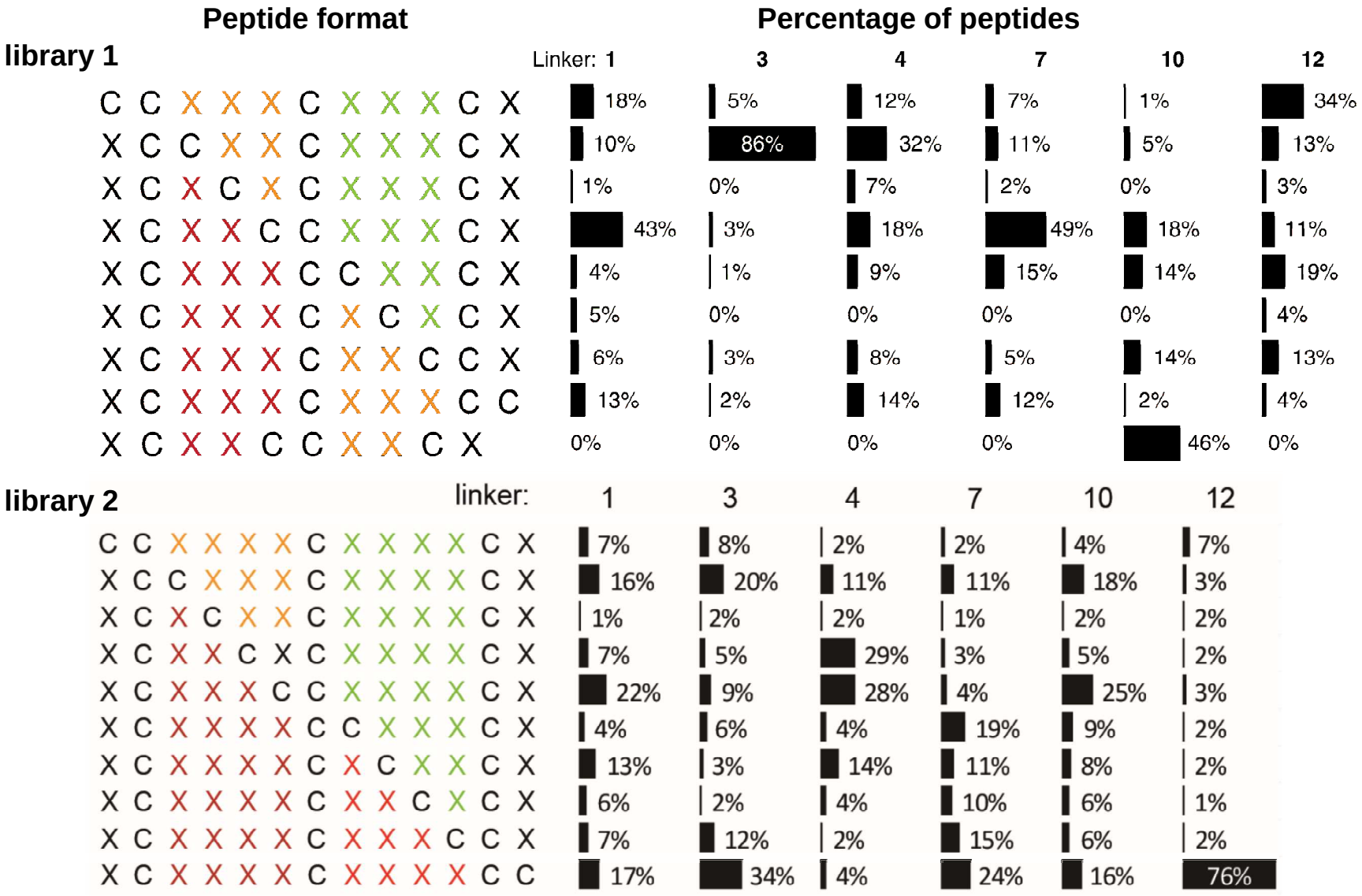
Number of Cysteine in Peptides

library 1 and library 2

selection	# cysteines	% peptides					
		linker 1	linker 3	linker 4	linker 7	linker 10	linker 12
round 2	0	1	1	0	1	1	1
	1	6	4	3	3	4	4
	2	24	15	13	11	20	8
	3	26	37	13	18	34	66
	4	43	44	70	66	41	22
round 3	0	3	2	1	2	3	1
	1	8	6	7	9	10	7
	2	10	17	14	17	26	13
	3	15	21	11	17	18	54
	4	63	53	66	53	42	25

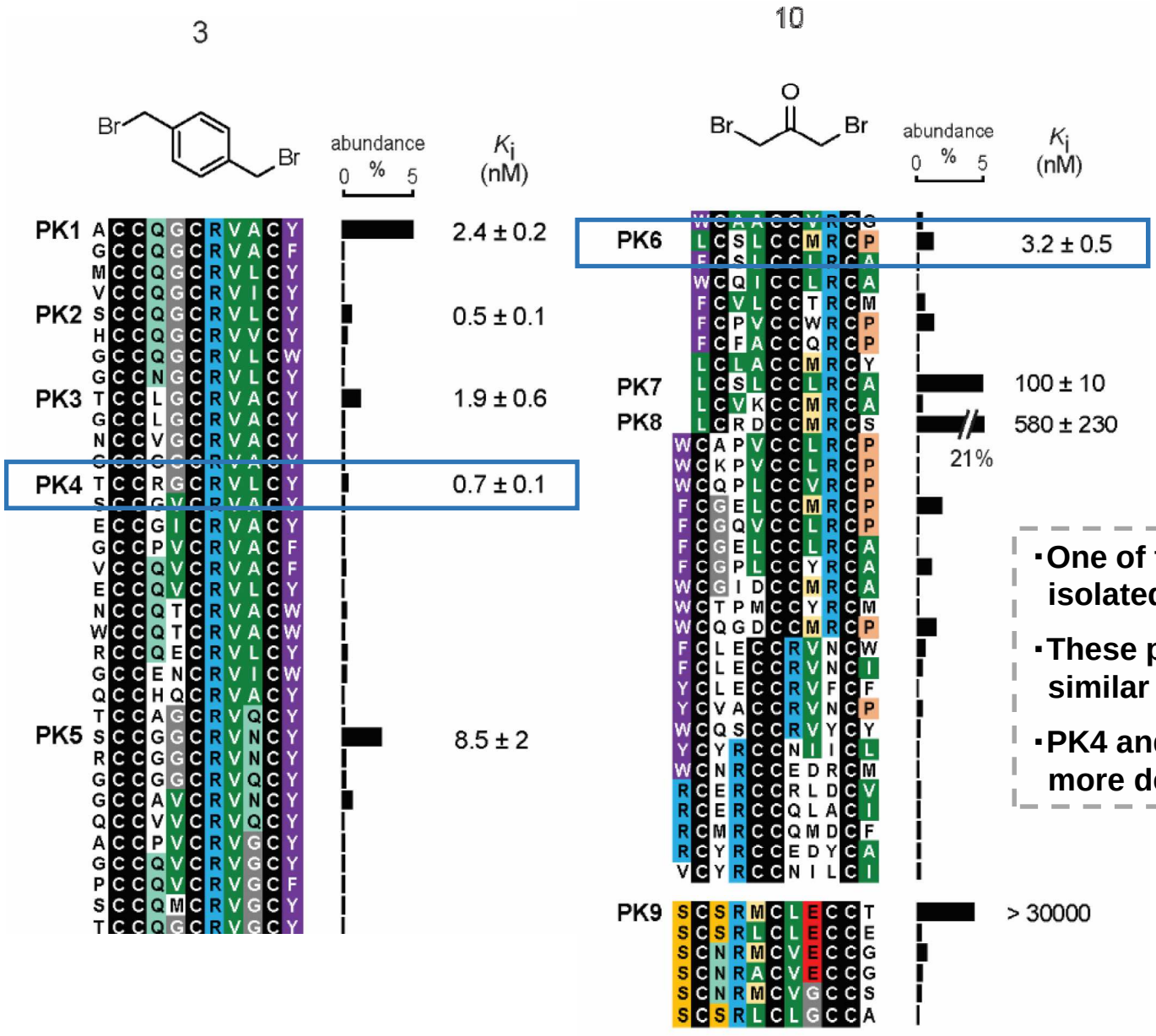
High-throughput sequencing of phage showed an enrichment for peptides containing four cysteines.

Phage Selection of Double-Bridged Peptides



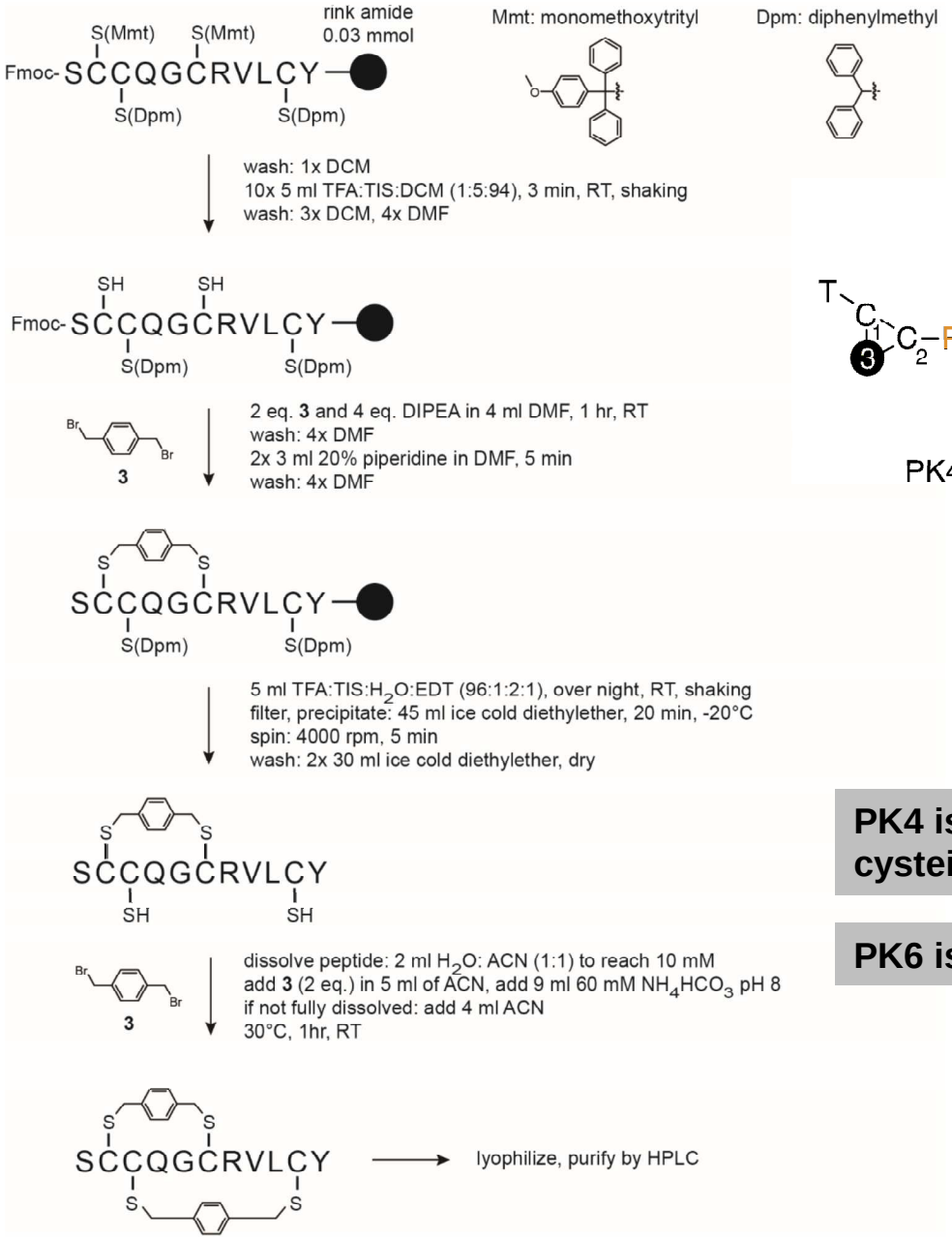
The strong enrichment of some peptide formats suggested that certain molecular scaffolds are particularly suited for target binding.

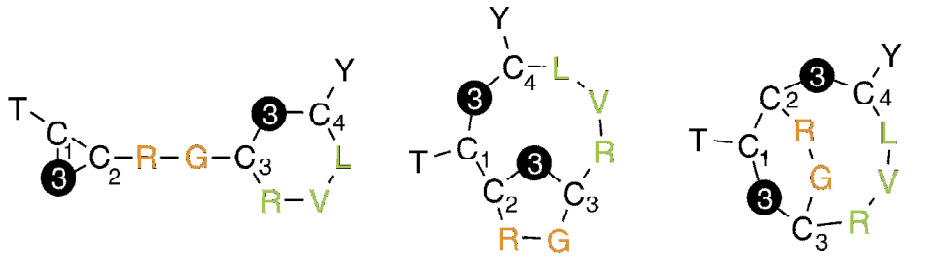
Consensus Sequences



- One of the peptide sequences isolated from library 1 and 2.
- These peptides showed similar sequences.
- PK4 and PK6 were picked for more detail analyzation.

Synthesis of PK4 isomers



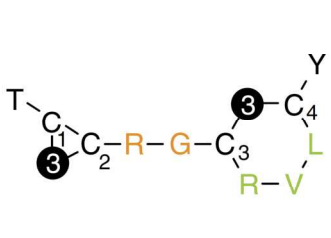


PK4 isomer 1 PK4 isomer 2 PK4 isomer 3

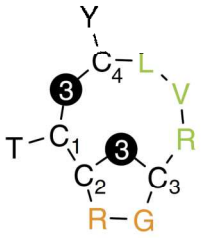
PK4 isomers were synthesized by using orthogonal cysteine protecting group Mmt and Dpm.

PK6 isomers were also synthesized in the same way.

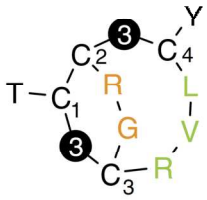
The Inhibition Constant Values of Each Isomers



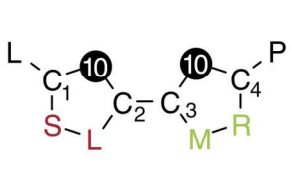
PK4 isomer 1



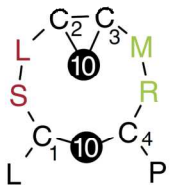
PK4 isomer 2



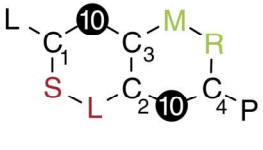
PK4 isomer 3



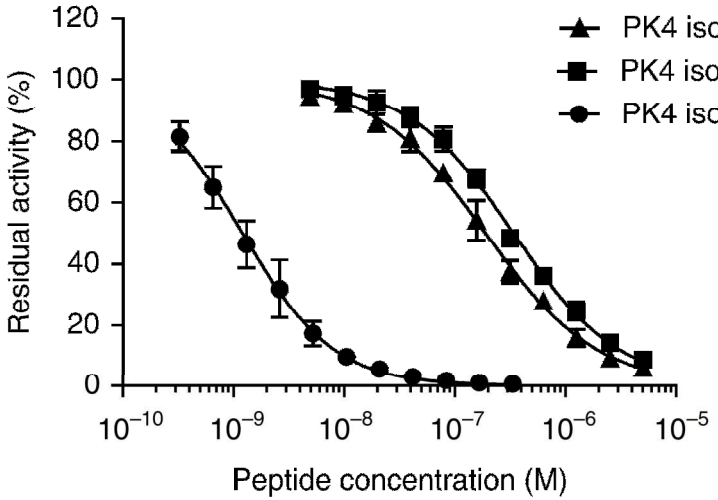
PK6 isomer 1



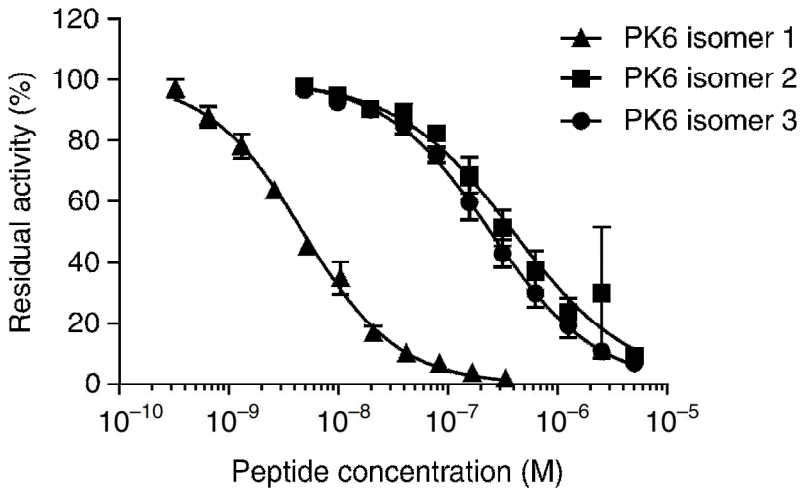
PK6 isomer 2



PK6 isomer 3



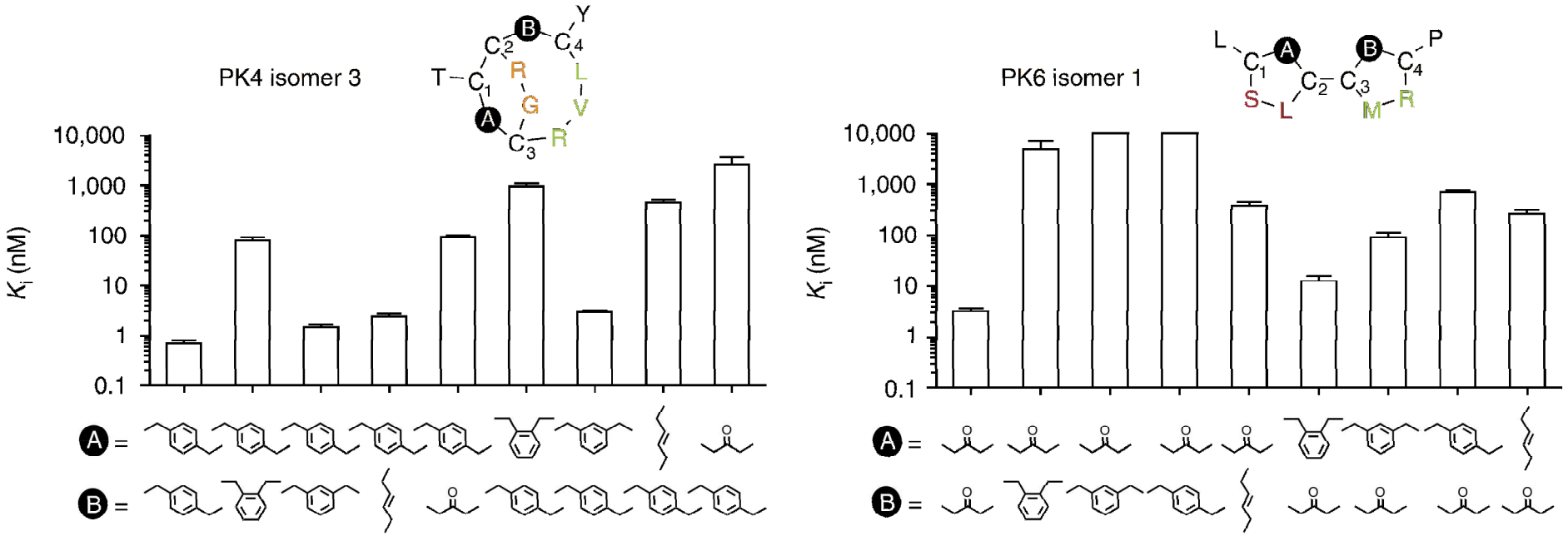
K_i (PK4 isomer 3) = 0.7 ± 0.1 nM



K_i (PK6 isomer 1) = 3.2 ± 0.5 nM

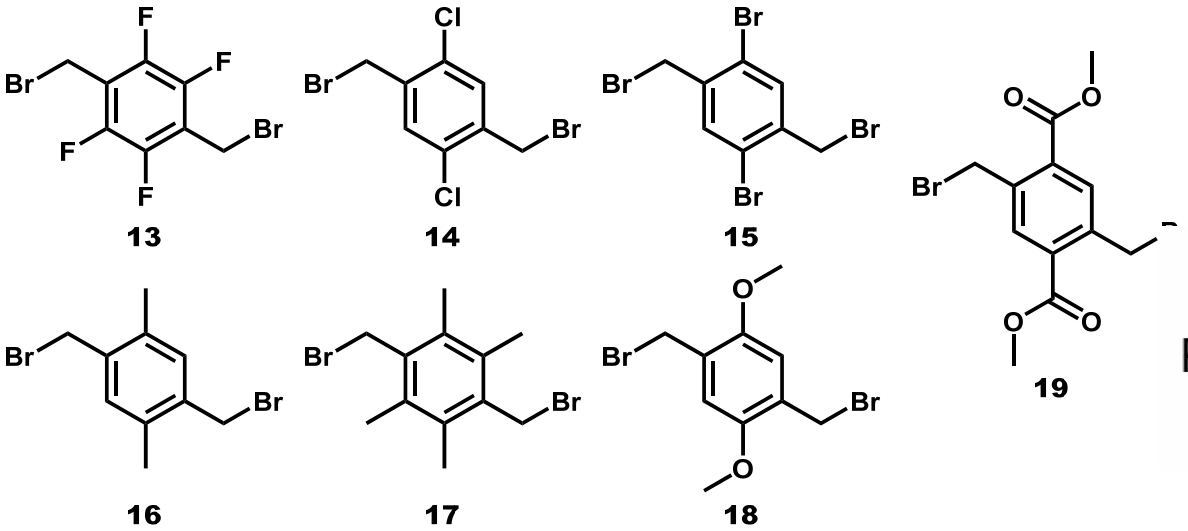
For each peptide, there was one isomer that was much more active than the other isomers. Certain scaffolds are important for binding.

Replacement of Chemical Bridges

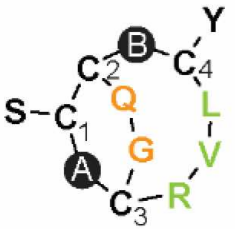


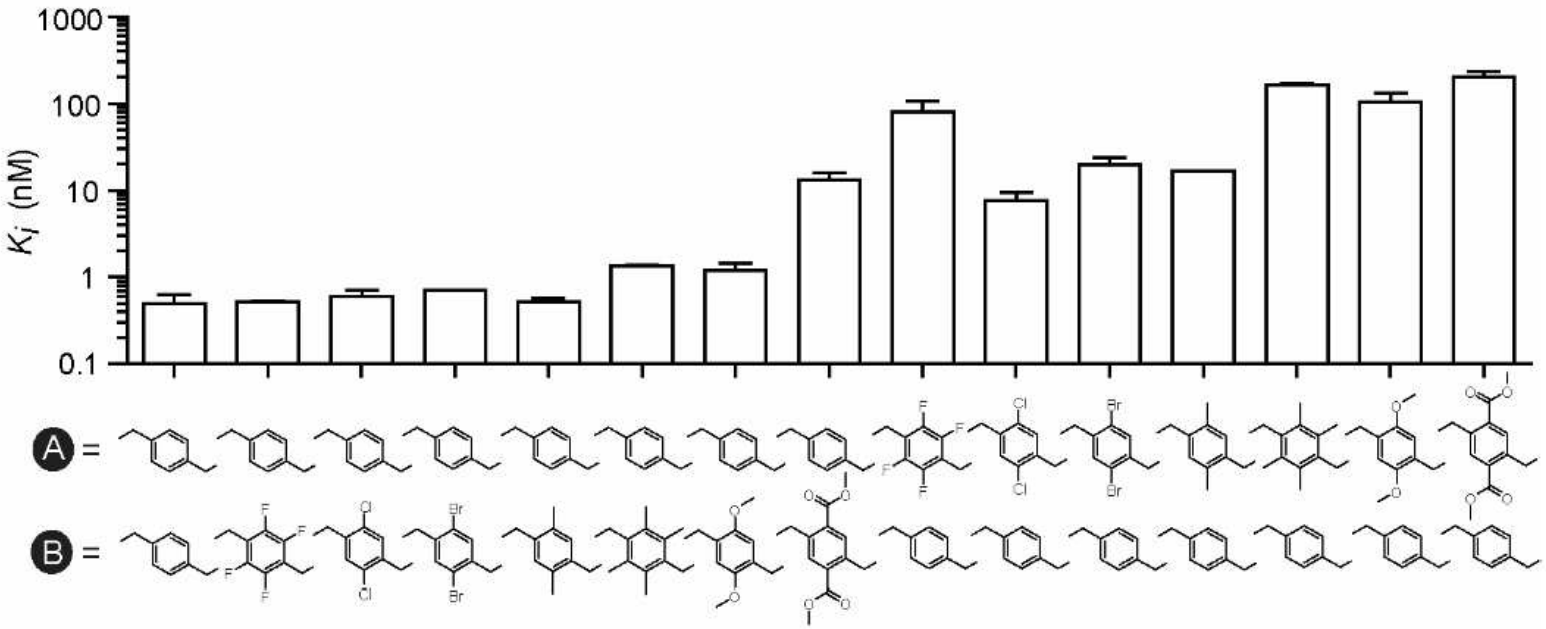
Even small structural changes in the bridges affected the binding affinity, indicating the important role of the bridges.

Changing Linker to Similar Structure



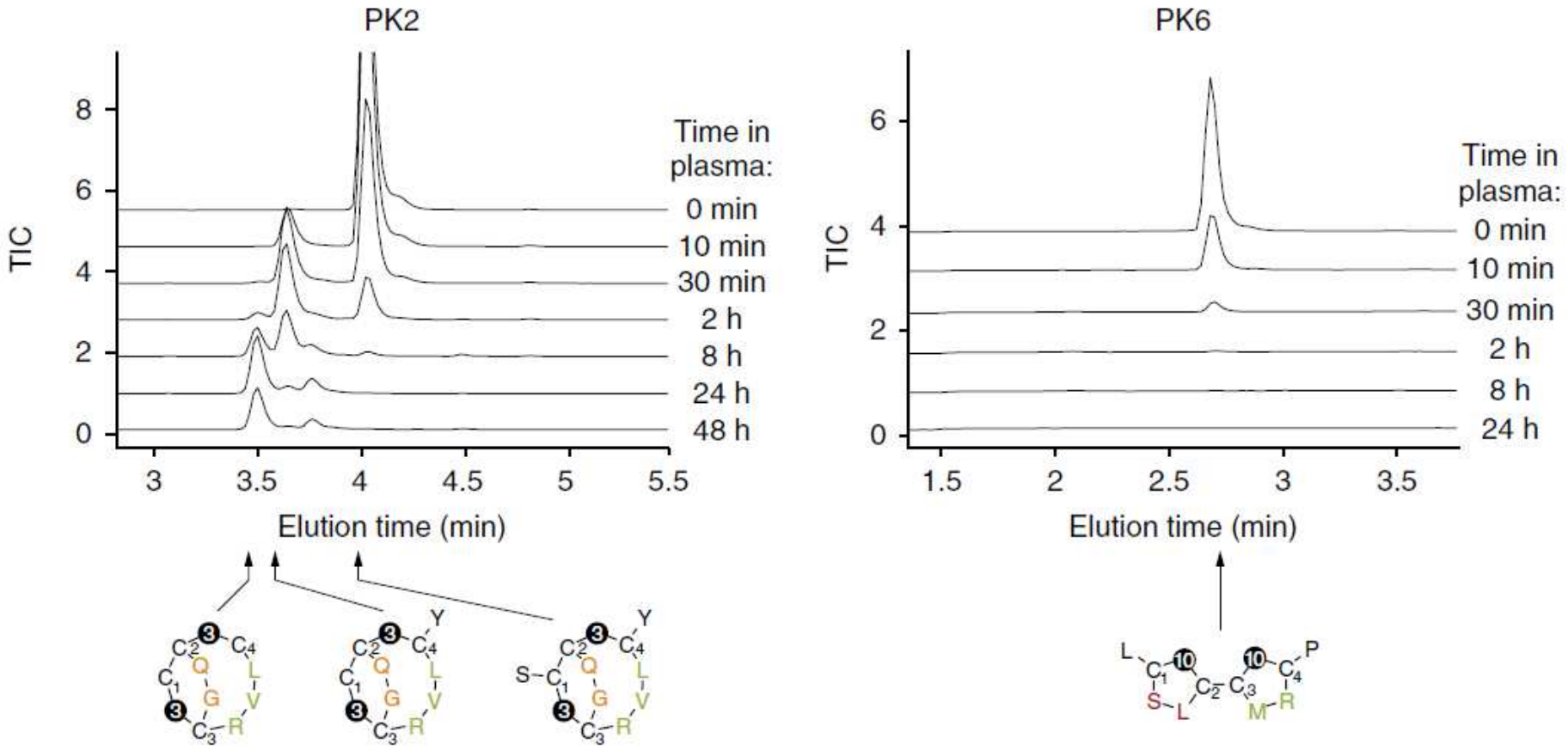
PK2 (isomer 3)





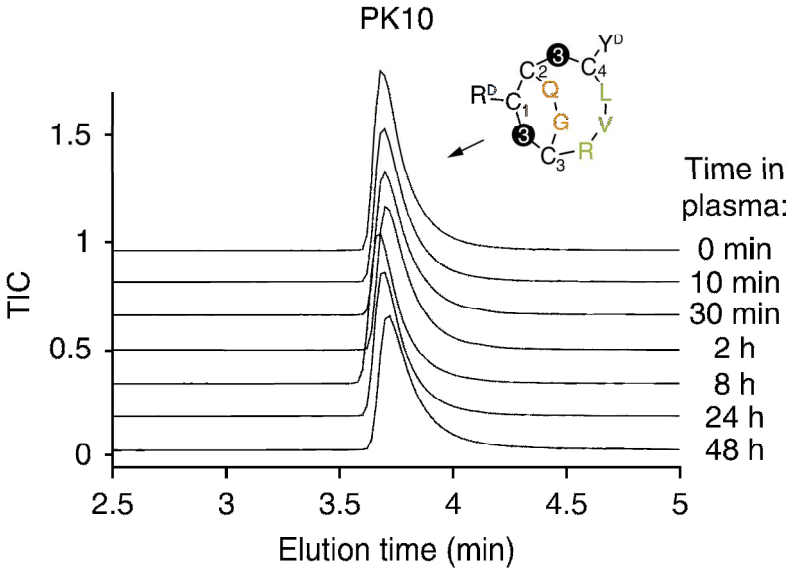
Linkers 13-19 could be applied in parallel to generate even larger diversities.

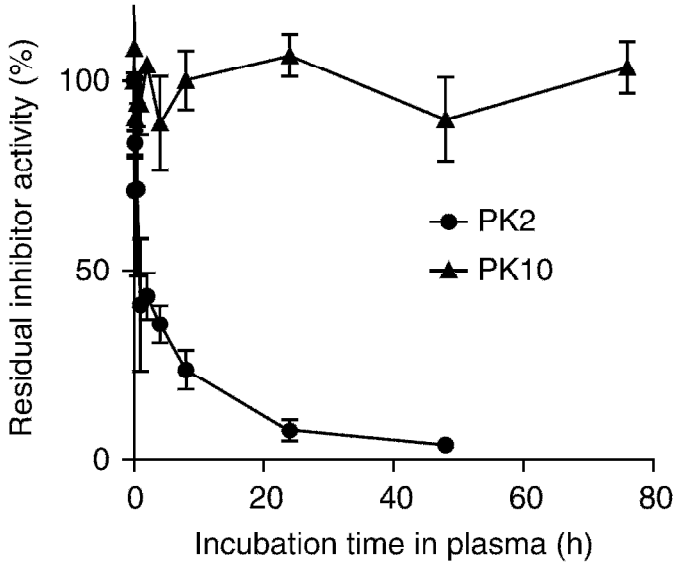
Proteolytic Stability in Human Plasma



Interlaced bridging that tightly connects the two macrocycles is important for proteolytic stability.

Changing N and C-terminal acids





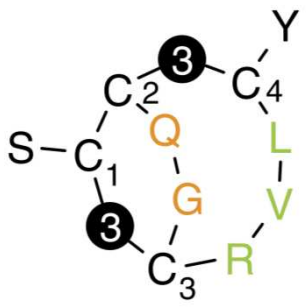
Position	Mutation	K_i (nM)
Ser1	Deletion	2.1 ± 0.5
	D-Ser	1.1 ± 0.3
	D-Thr	1.4 ± 0.0
	D-Arg	0.6 ± 0.1
	norArg	3.7 ± 0.4
	α-Methyl-Ser	1.5 ± 0.1
Tyr11	Deletion	54 ± 9.1
	D-Tyr	5.6 ± 0.9
	D-Phe	12.2 ± 4.1
	D-Trp	12.1 ± 4.6
	NMe-Tyr	3.0 ± 0.2

K_i (PK2) = 0.5 ± 0.1 nM

K_i (PK10) = 3.6 ± 0.5 nM

N-terminal acid and C-terminal acid change resulted in high proteolytic stability.

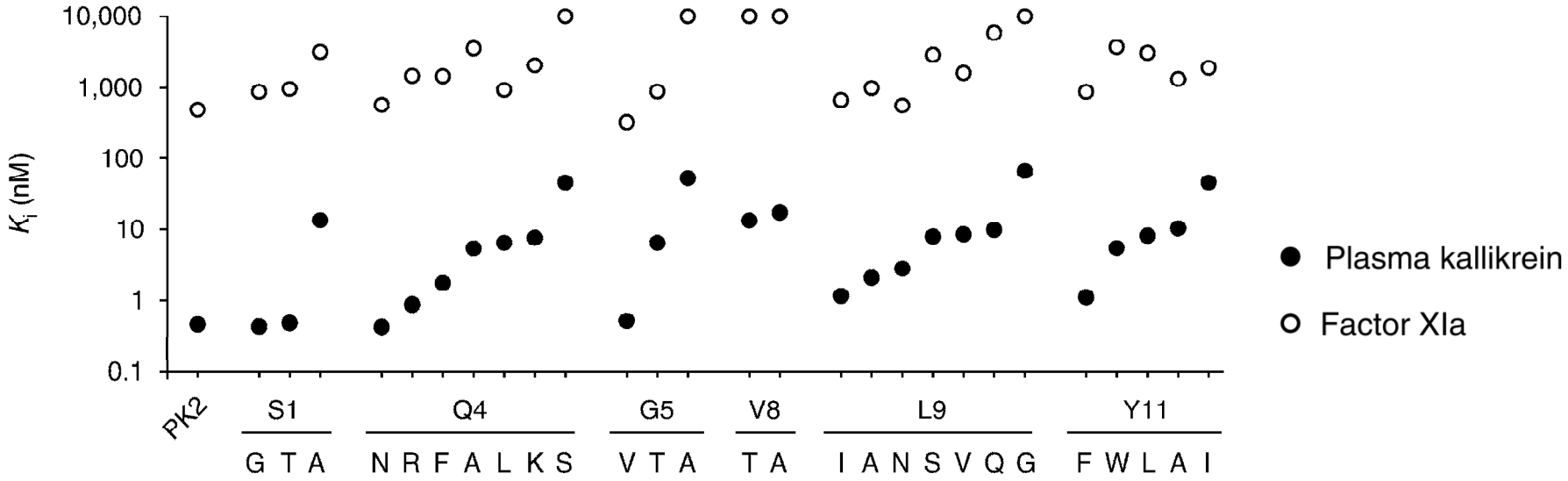
High Target Selectivity



PK2 isomer 3

	K_i (nM)			
	PK2	PK4	PK6	PK10
Plasma kallikrein	0.5 ± 0.1	0.7 ± 0.1	3.2 ± 0.5	3.6 ± 0.5
Factor XIa	580 ± 180	1300 ± 500	2700 ± 200	2500 ± 100
Factor XIIa	>30,000	>30,000	>30,000	>30,000
Thrombin	>30,000	>30,000	>30,000	>30,000
uPA	>30,000	>30,000	>30,000	>30,000
tPA	>30,000	>30,000	>30,000	>30,000
Plasmin	>30,000	>30,000	>30,000	>30,000
Factor Xa	>30,000	>30,000	>30,000	>30,000
Factor VIIa	>30,000	>30,000	>30,000	>30,000

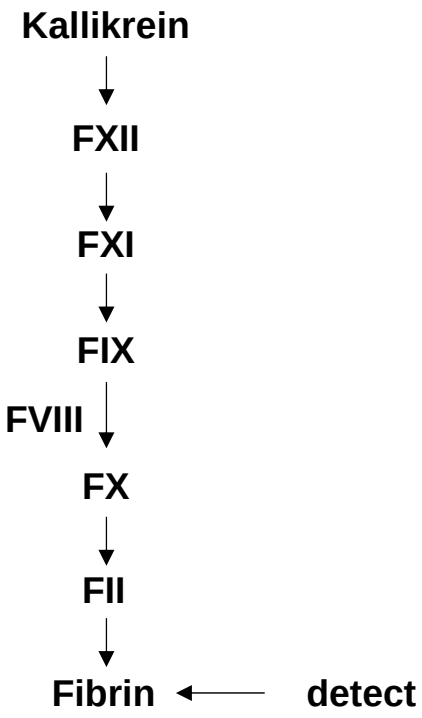
Inhibition of plasma kallikrein and a panel of structurally homologous or physiologically important paralogous proteases by isolated inhibitors. Average values and standard deviations of at least three measurements are shown.

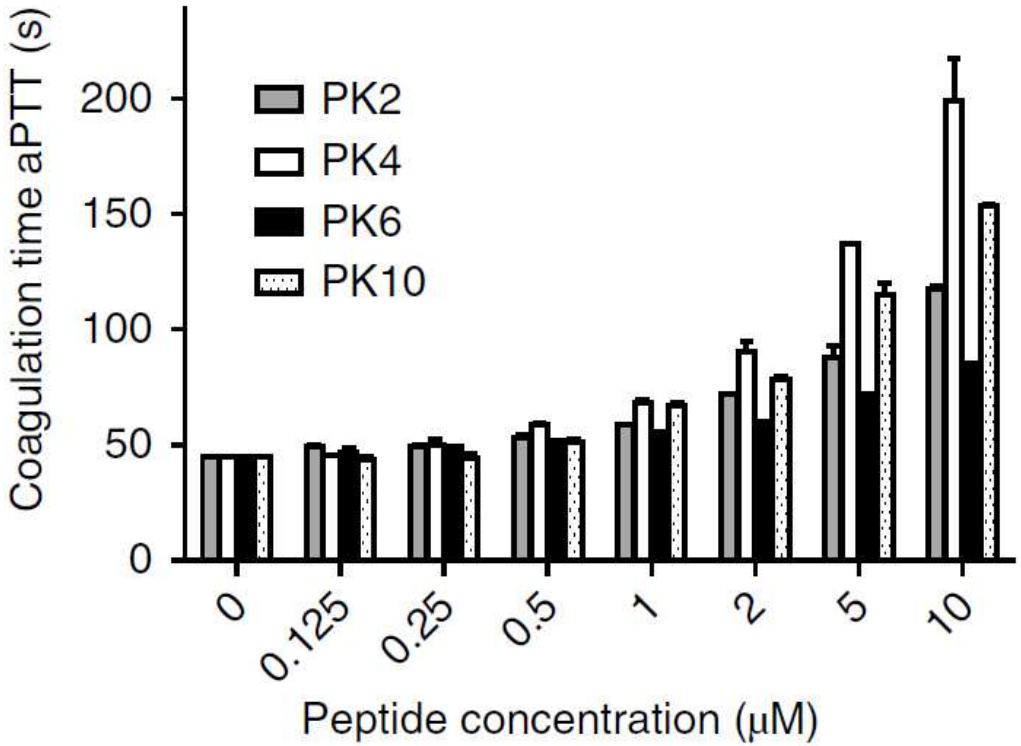


The backbone fits perfectly to the active site of plasma kallikrein and contributed to the target selectivity.

Ex Vivo Experiment

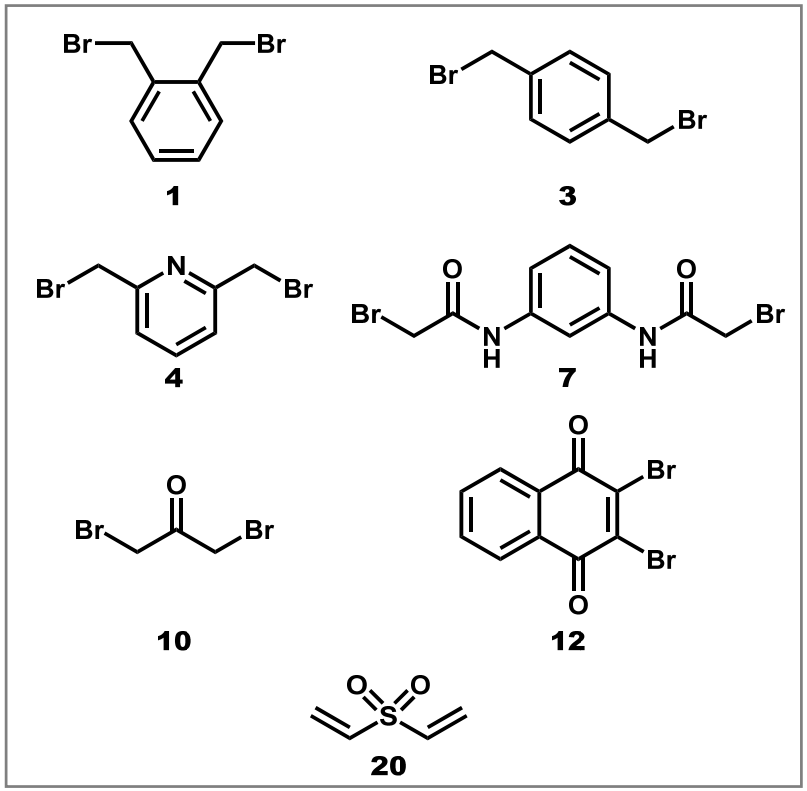
Intrinsic Pathway **(aPTT)**





All efficiently inhibited activation of the intrinsic coagulation pathway in human plasma.

Library for Another Target



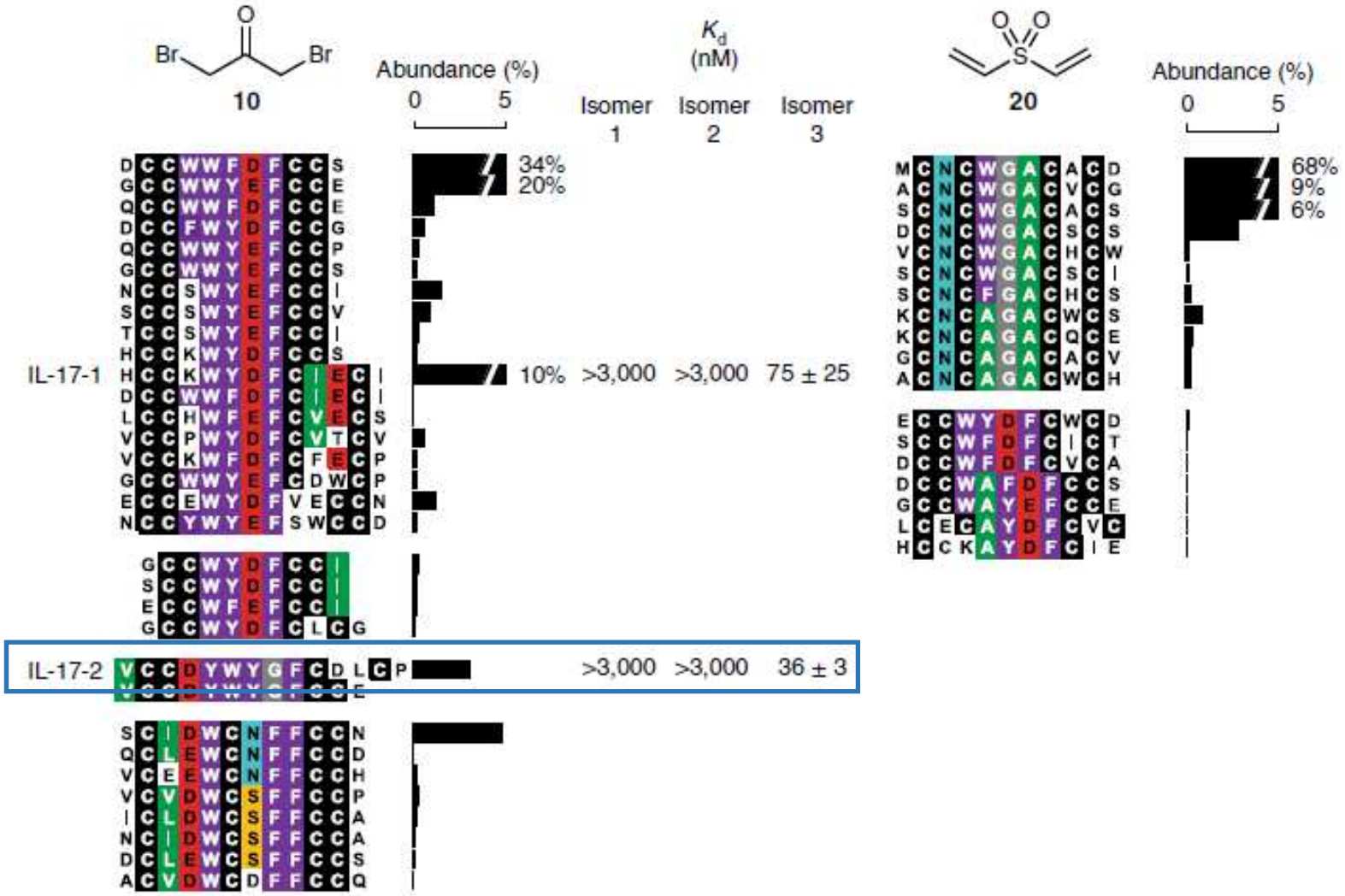
library

$XCX_mCX_nCX_oCX$-phage

X : any amino acids, C : cysteine

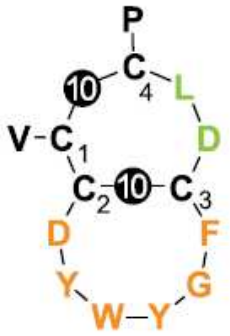
The library was panned three rounds against immobilized IL-17.

Consensus Sequences

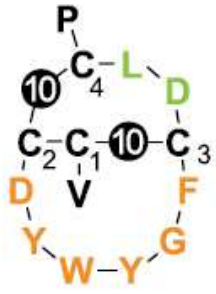


Activity of Each Isomers

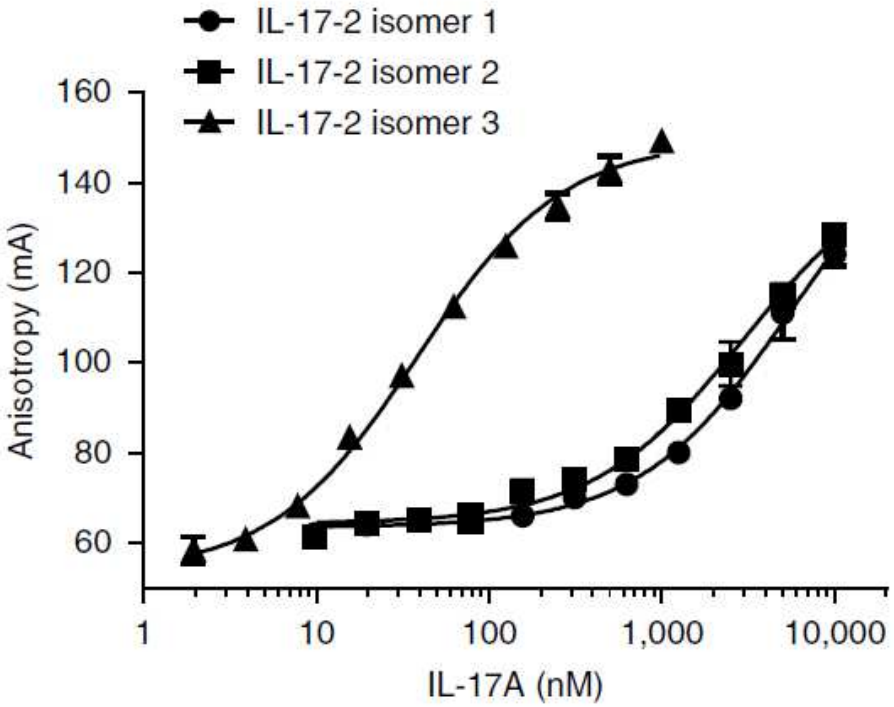
IL-17-2 isomer 1



IL-17-2 isomer 2



IL-17-2 isomer 3

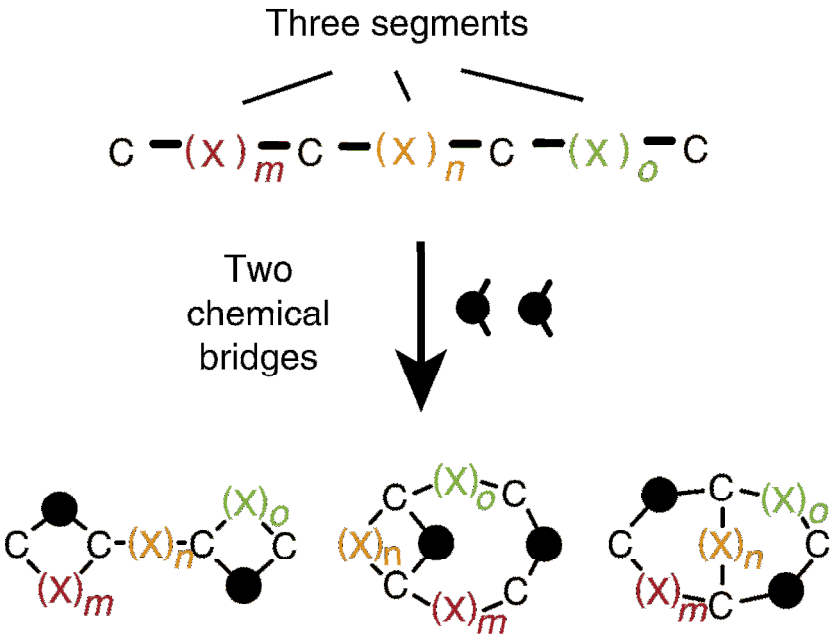


K_d (isomer 3) = 36 ± 3 nM

Isomer 3 was far more active than other two isomers

This library method could be applied to other targets

Summary



- **Macrocyclic peptide libraries with large structure diversities can be generated by cyclizing peptides with two chemical bridges.**
- **This approach yielded libraries comprising many more difficult macrocyclic scaffolds than previous libraries.**
- **Changing linkers could produce even lager library diversities.**