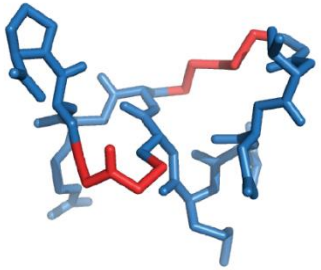
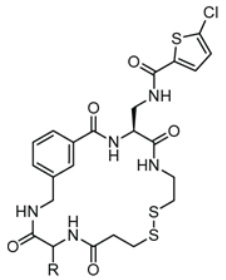
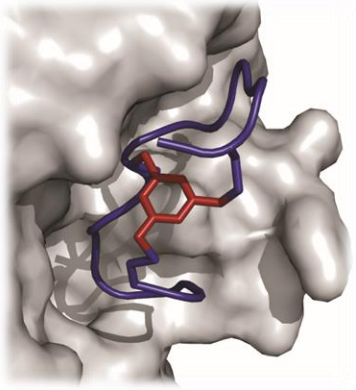
Cyclic peptides and high-throughput technologies for addressing unmet medial needs







Christian Heinis, EPFL

Laboratory of Therapeutic Proteins and Peptides

My background

drug development technologies

(bi)cyclic peptides

macrocycle drugs

phage display

ligand development

combinatorial chemistry

high-throughput screening

My background

drug development technologies

(bi)cyclic peptides

macrocycle drugs

phage display

ligand development

combinatorial chemistry

high-throughput screening

diseases & targets

protease targets

plasma kallikrein, FXII, FXI, thombin, ...

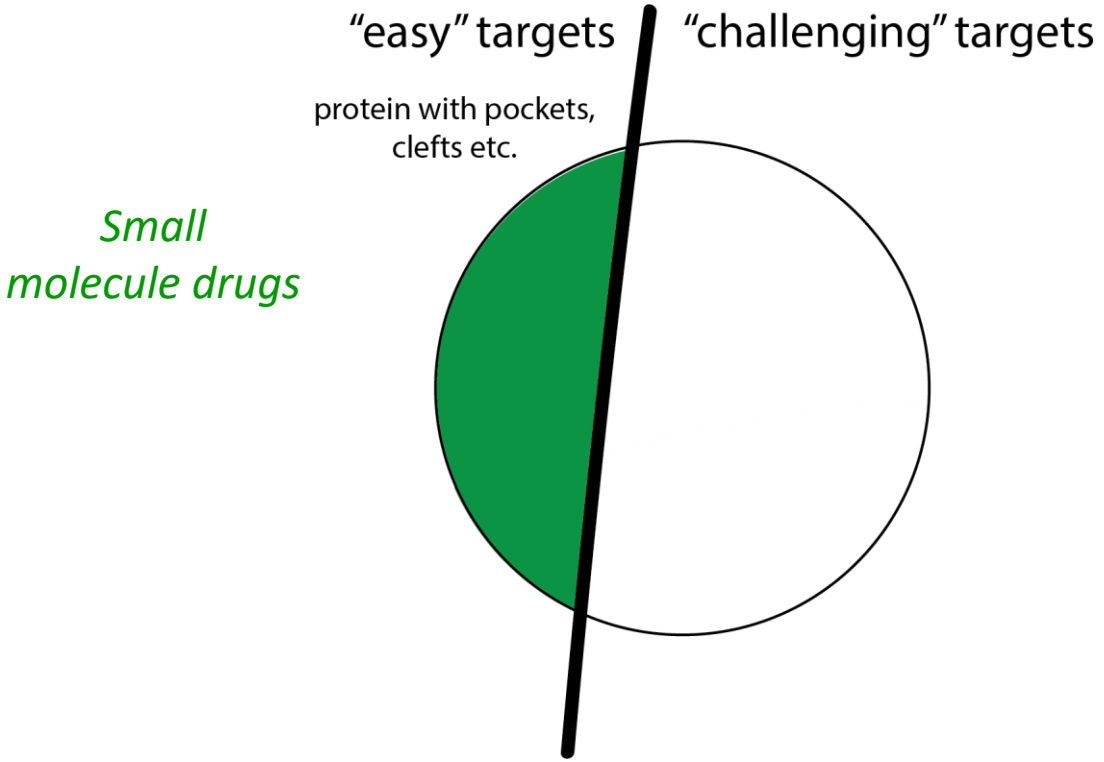
protein-protein interactions

IL-23R, KRAS, β-catenin, Keap1, ...

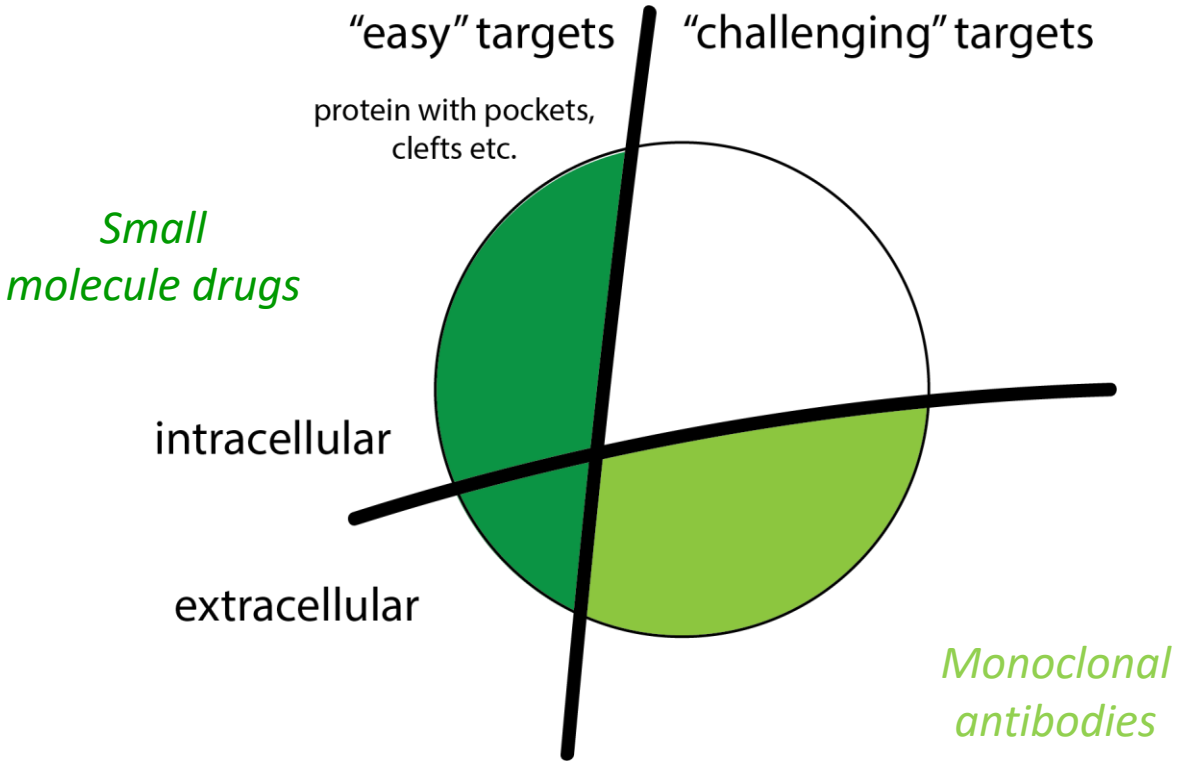
thrombotic diseases

cancer

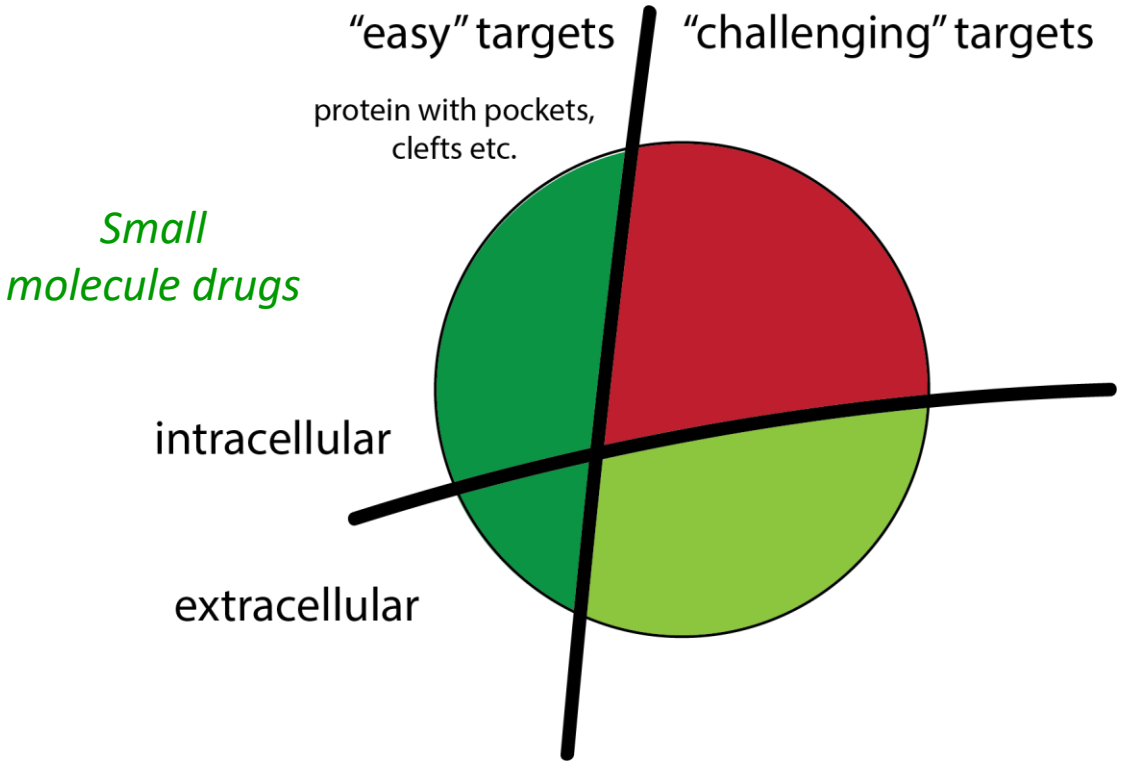
Why new drug modalities / technologies?



Why new drug modalities / technologies?



Why new drug modalities / technologies?

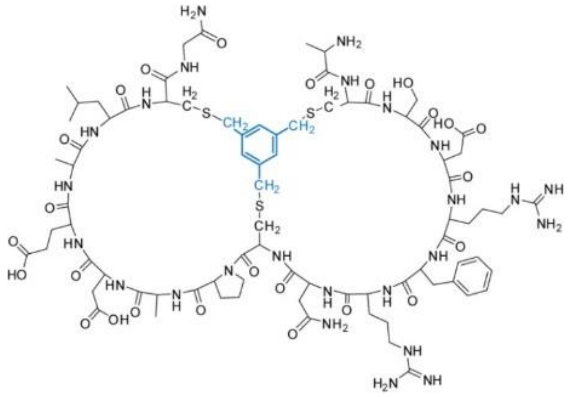


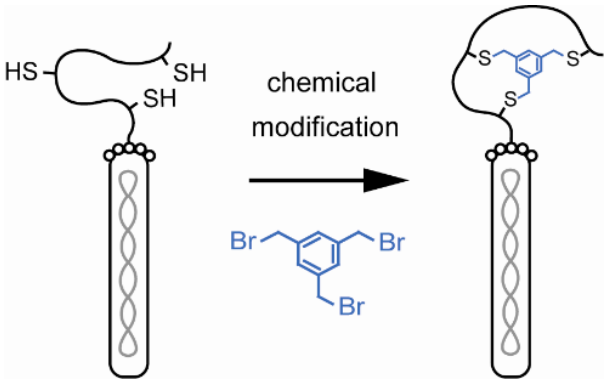
KRAS
MYC
β-catenin
NF-kB
KEAP1
....

TNFα
VEGF
IL-6
....

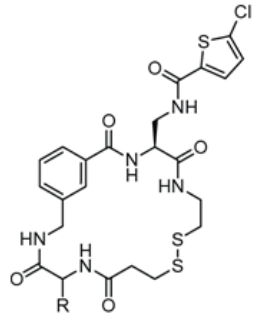
Talk overview

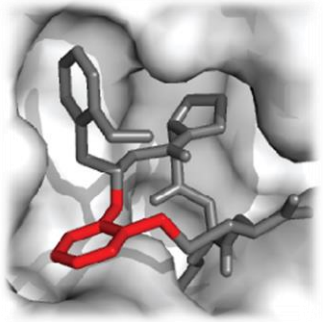
1. Bicyclic peptides developed by phage display





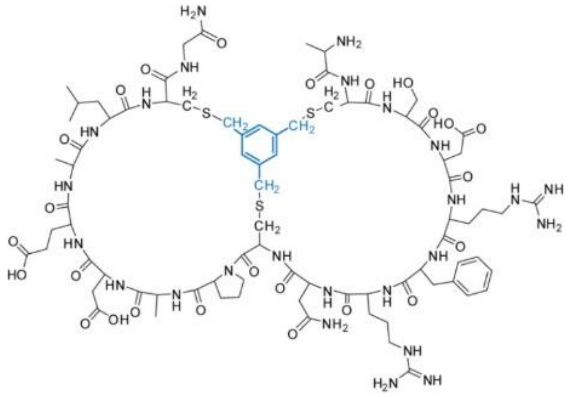
2. Peptide macrocycles developed by high-throughput synthesis at a nanomole scale

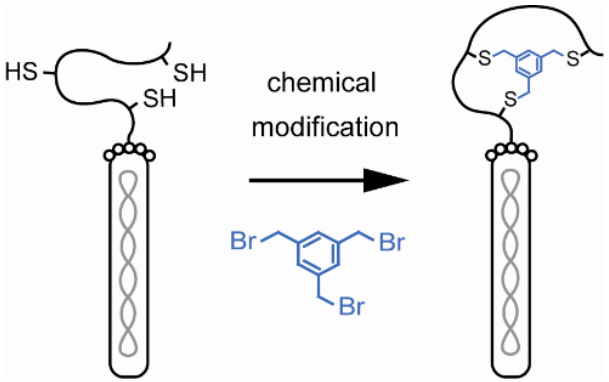




Talk overview

1. Bicyclic peptides developed by phage display





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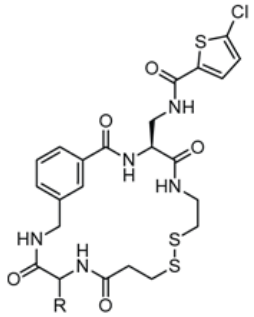
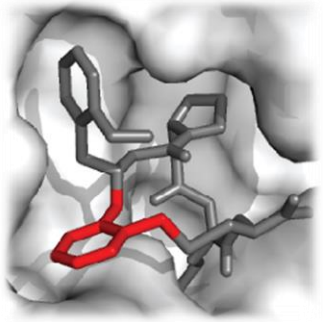
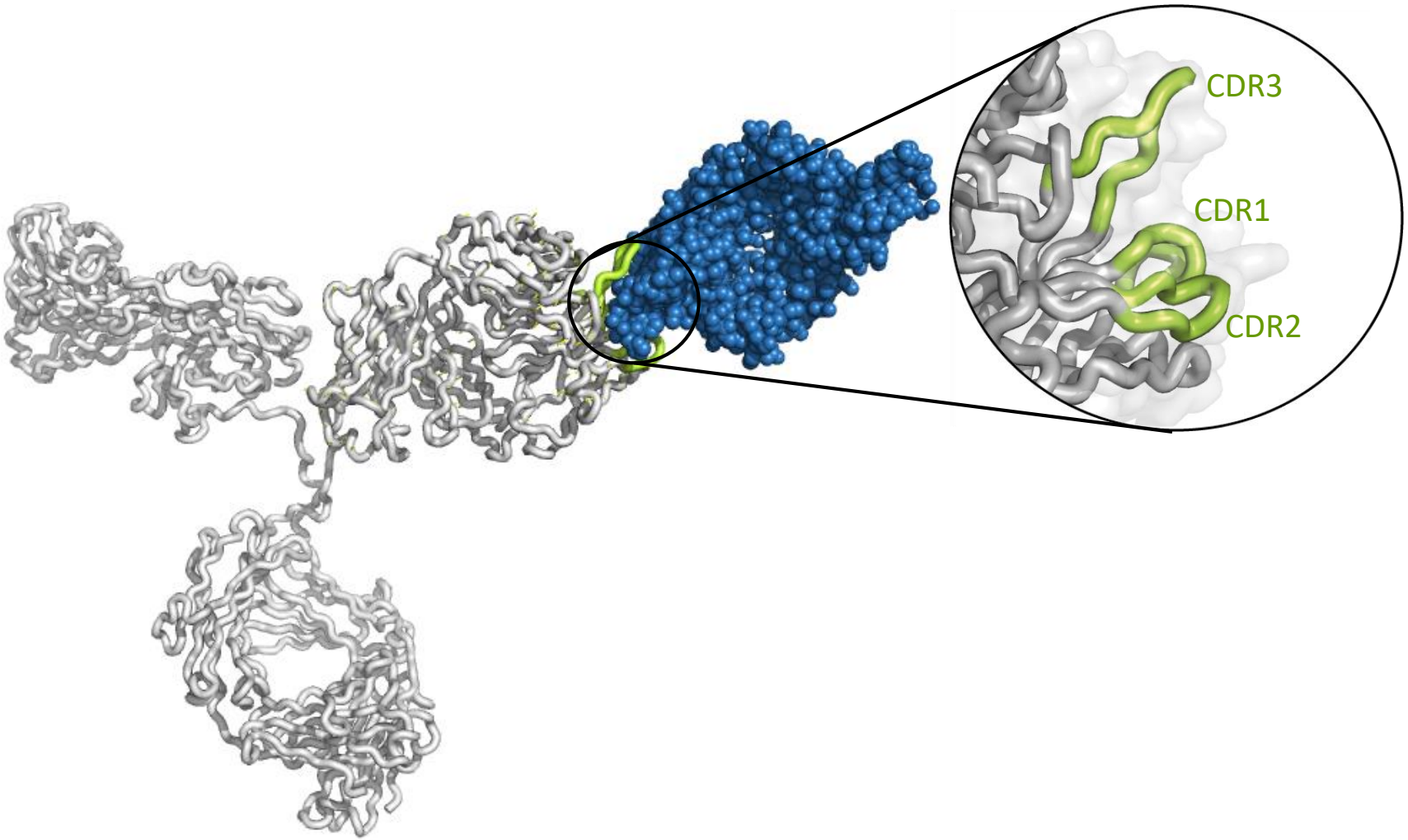
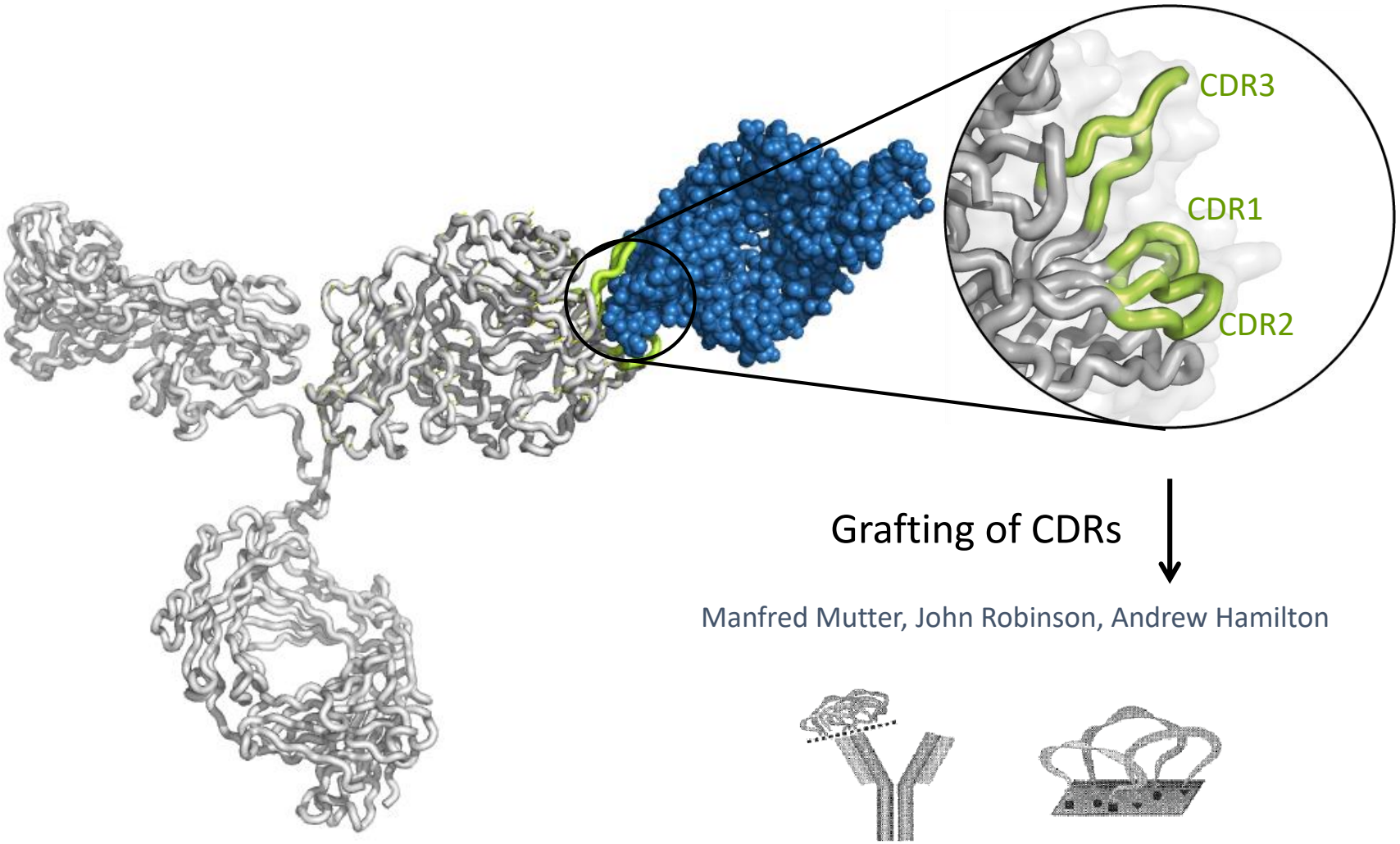




Miniaturization of antibodies



Miniaturization of antibodies

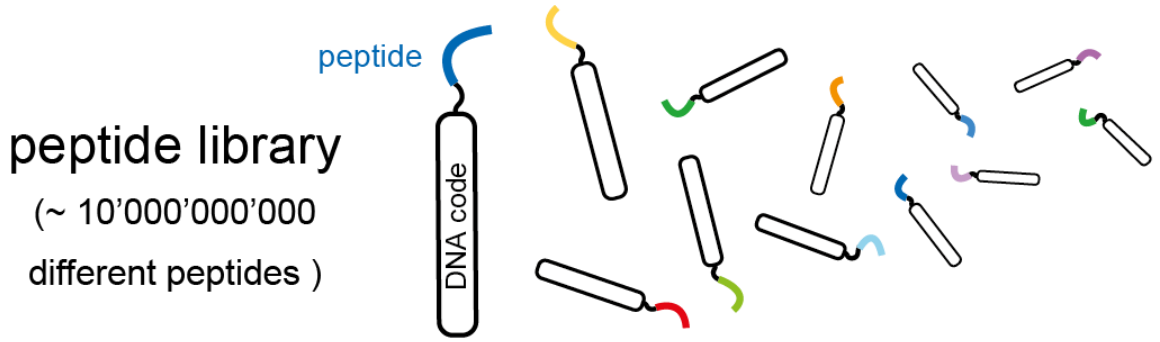


Grafting of CDRs

Manfred Mutter, John Robinson, Andrew Hamilton

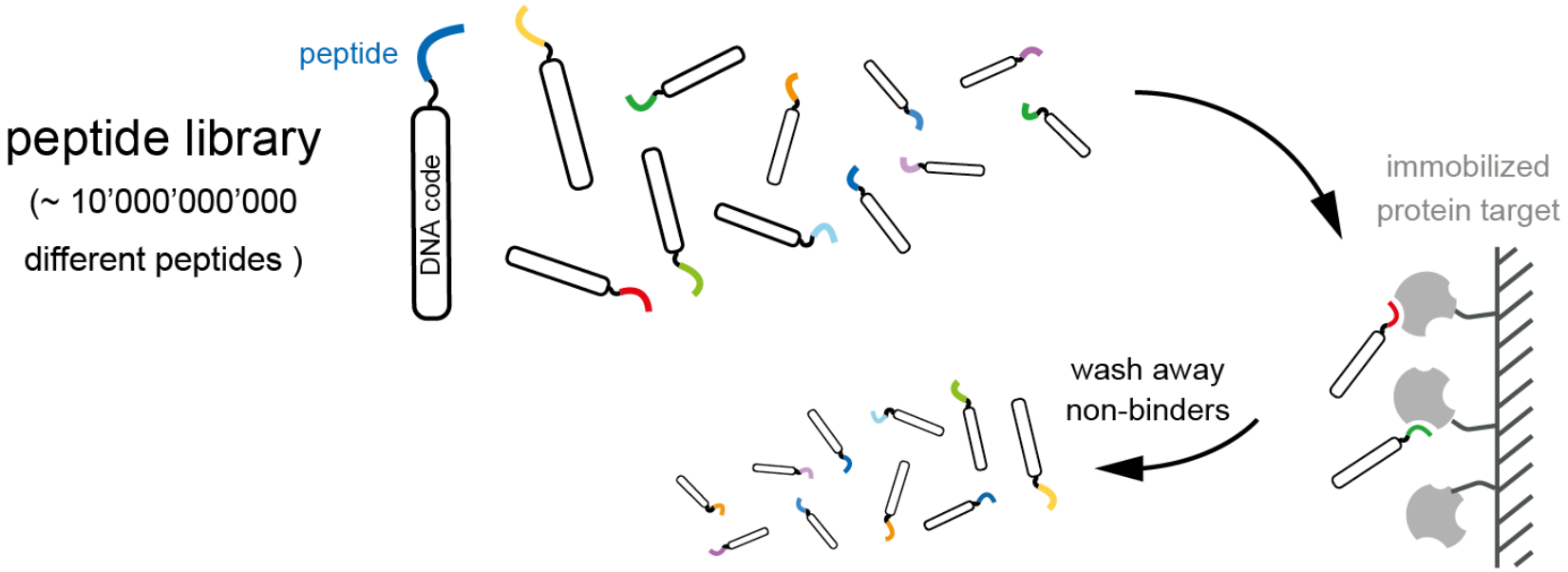
Figure from Mutter, *et al.*, *Angewandte*, 1996)

Phage display technology



Smith, G.P., *Science*, 1985

Phage display technology



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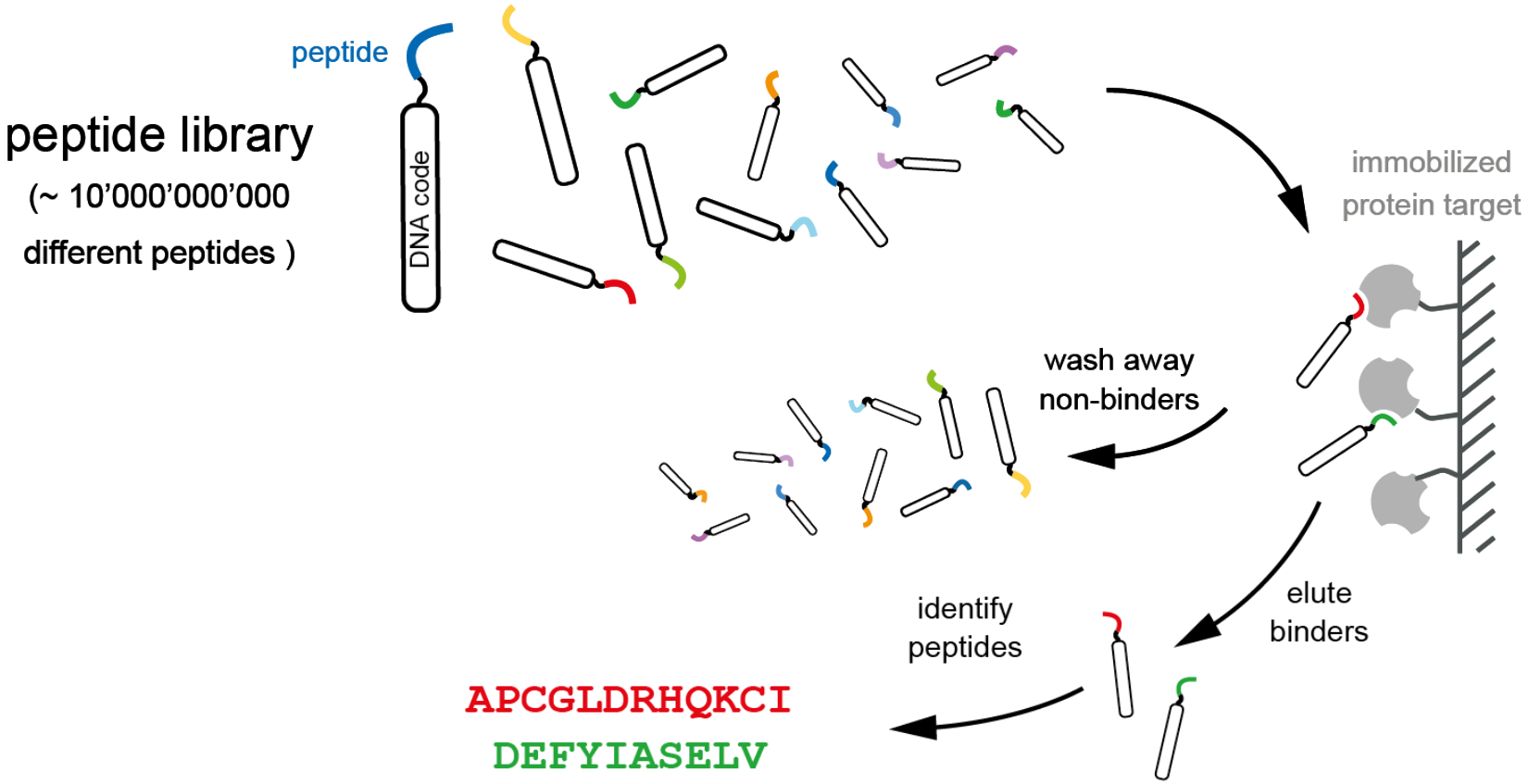
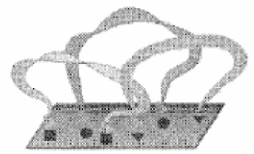
Phage display technology

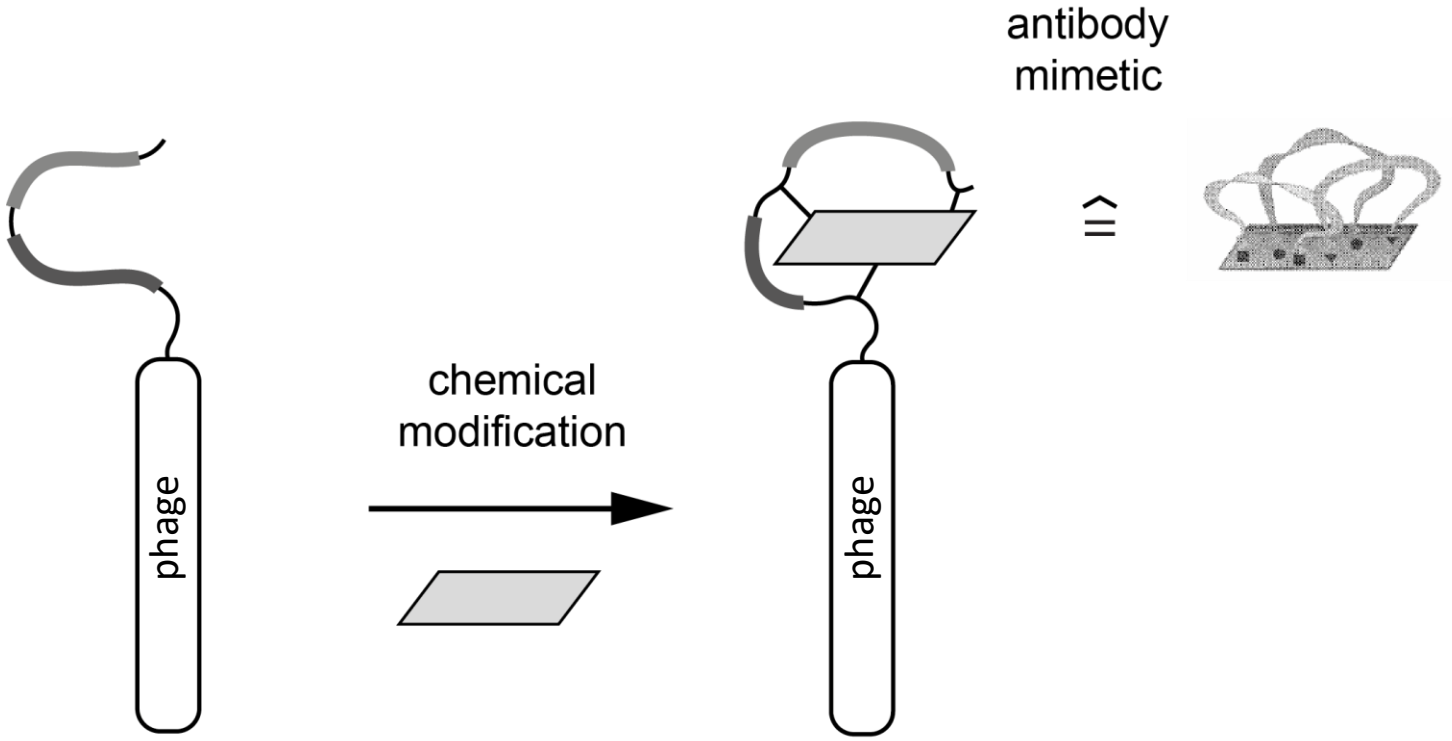


Smith, G.P., *Science*, 1985

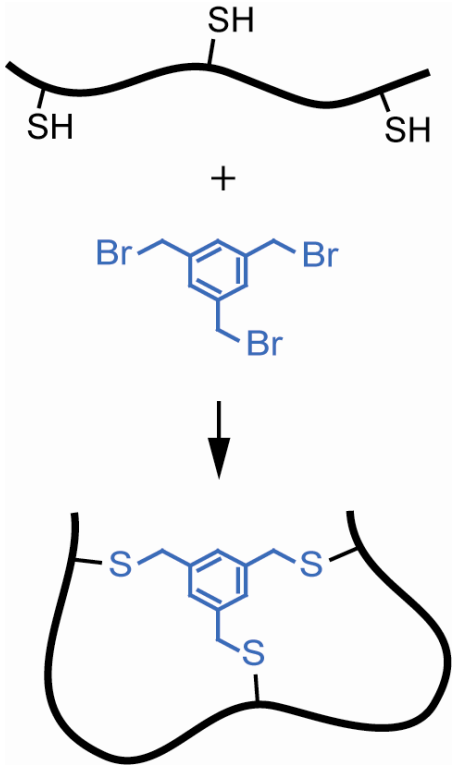
Phage-encoded antibody mimetics



Phage-encoded antibody mimetics



Bicyclic peptides



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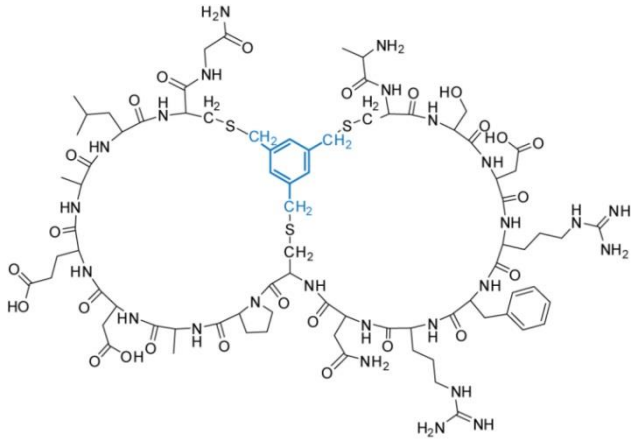
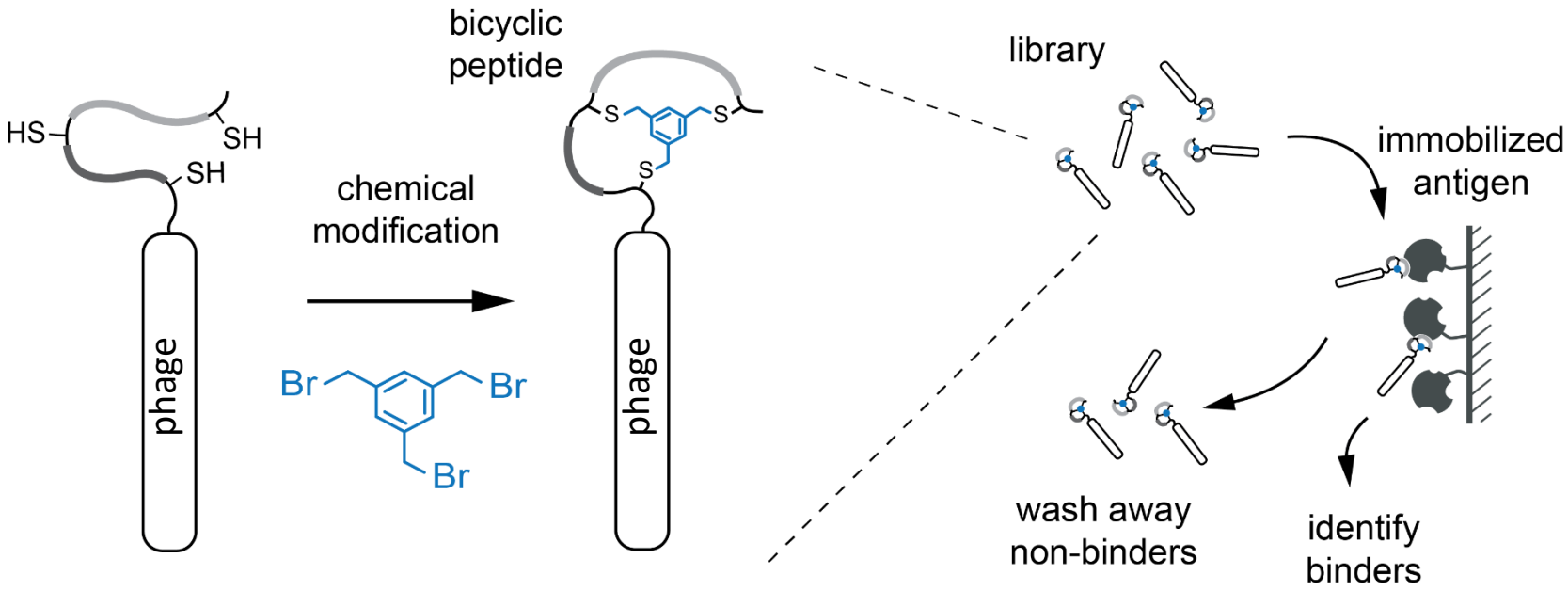
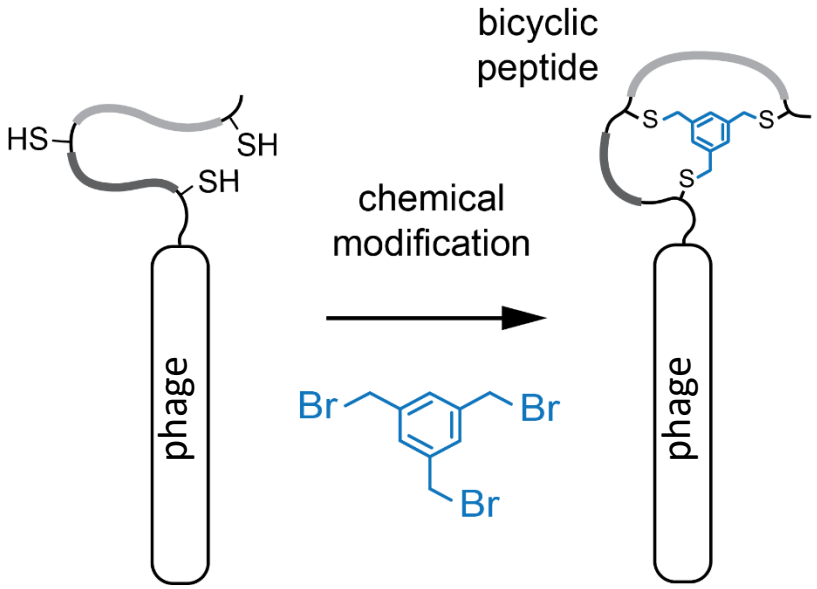


(Timmerman, P., Beld, J., Puijk, W.C. and Meloen, R.H., *ChemBioChem*, 2005)

Phage selection of bicyclic peptides



Phage selection of bicyclic peptides

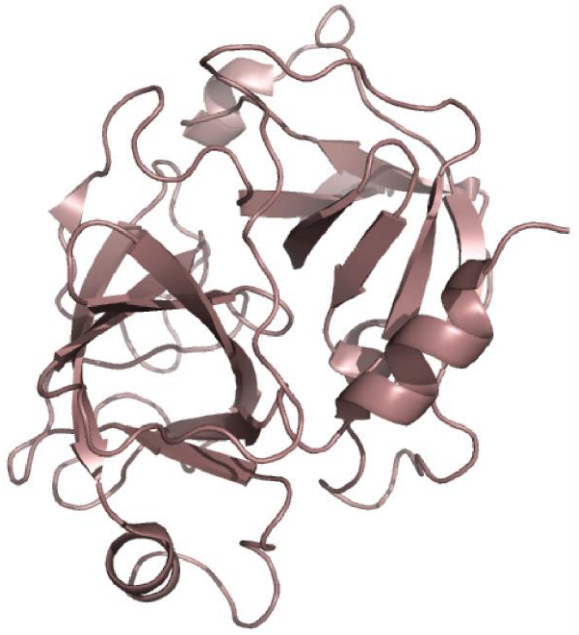


MRC Laboratory of Molecular Biology

Laboratory of Molecular Biology (LMB), Cambridge, UK

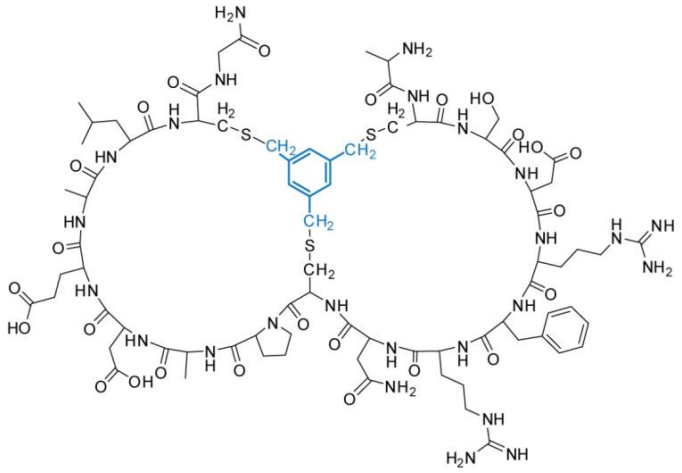
Sir Gregory Winter

First phage-selected bicyclic peptide



plasma kallikrein

K_i = 2.9 nM



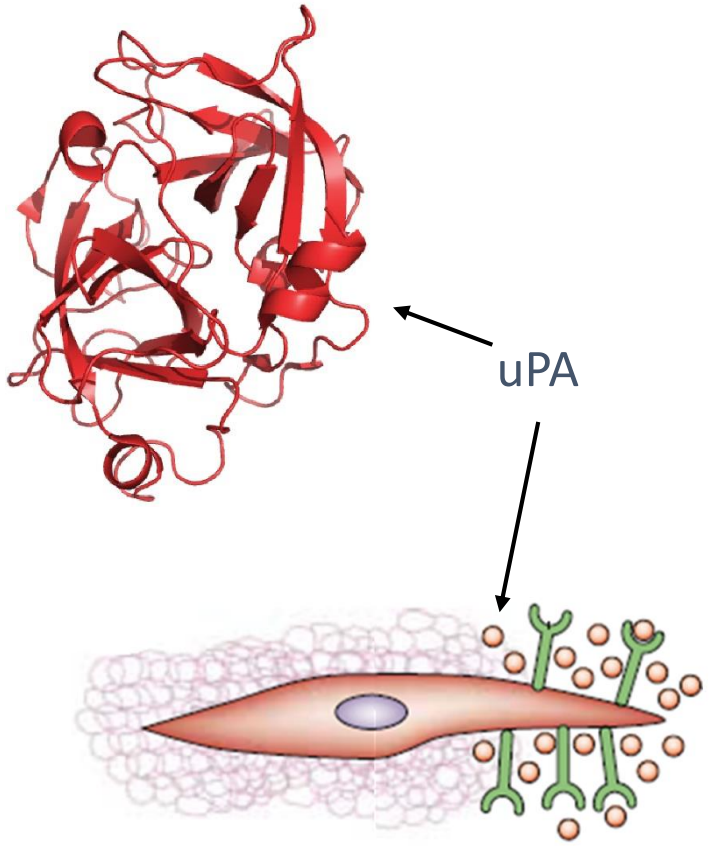
bicyclic peptide PK15

Heinis, C., Rutherford, T., Freund, S. and Winter, G., *Nat. Chem. Biol.,* 2009

Phage selection of bicyclic peptides

Example target: urokinase-type plasminogen activator (uPA)

- Trypsin-like serine protease
- Overexpressed in some tumor cells (secreted)
- Proteolytic activity was reported to promote tumor growth and metastasis formation

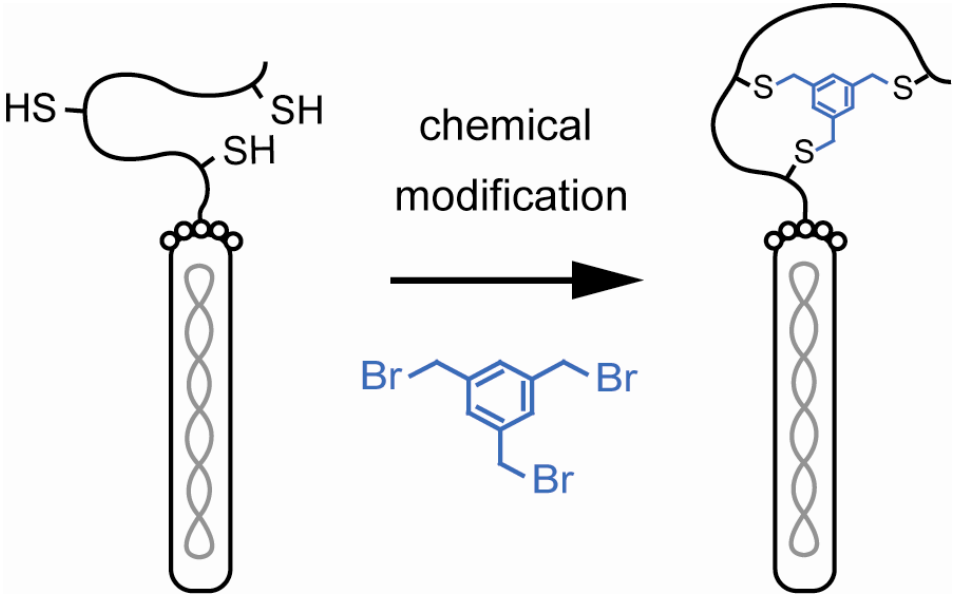


Phage library

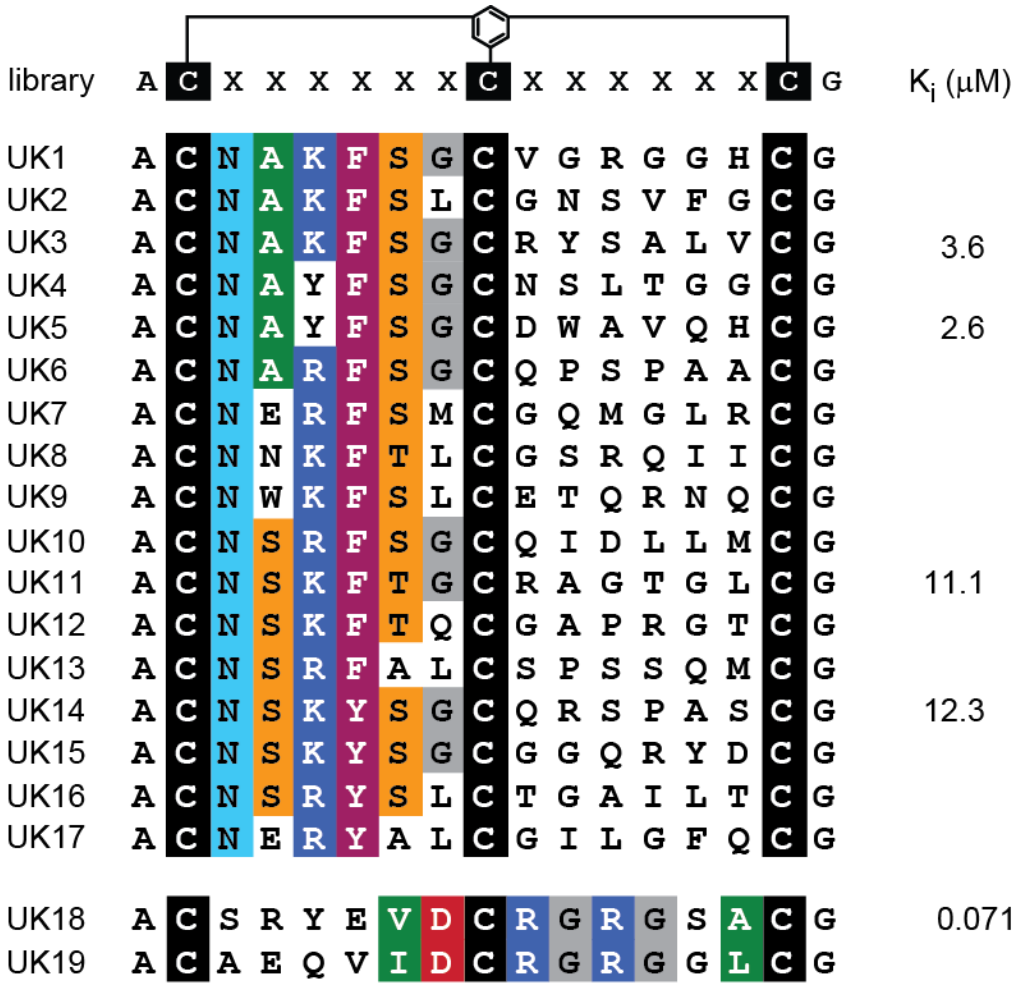
library size:

> $4x10^9$

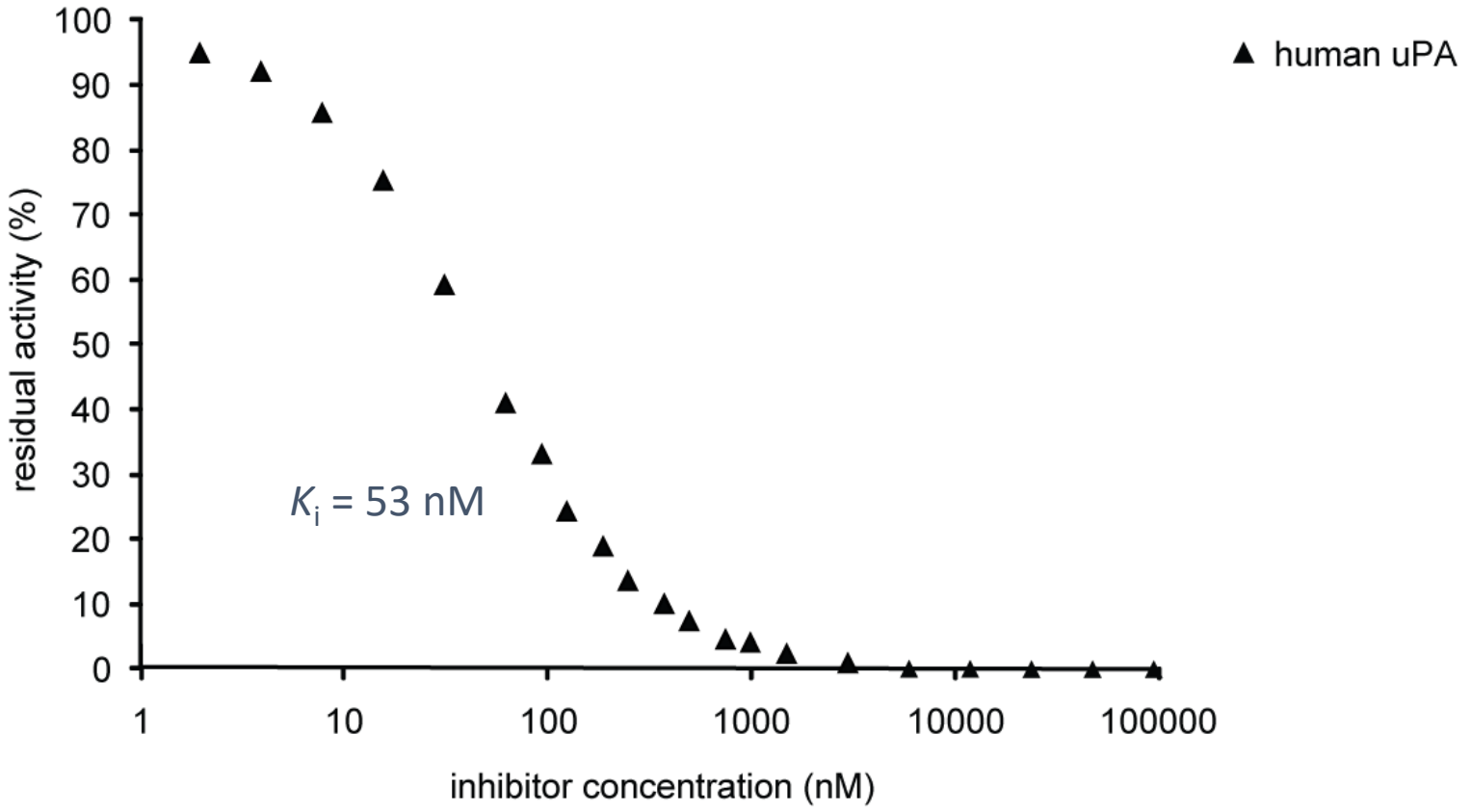
transformants



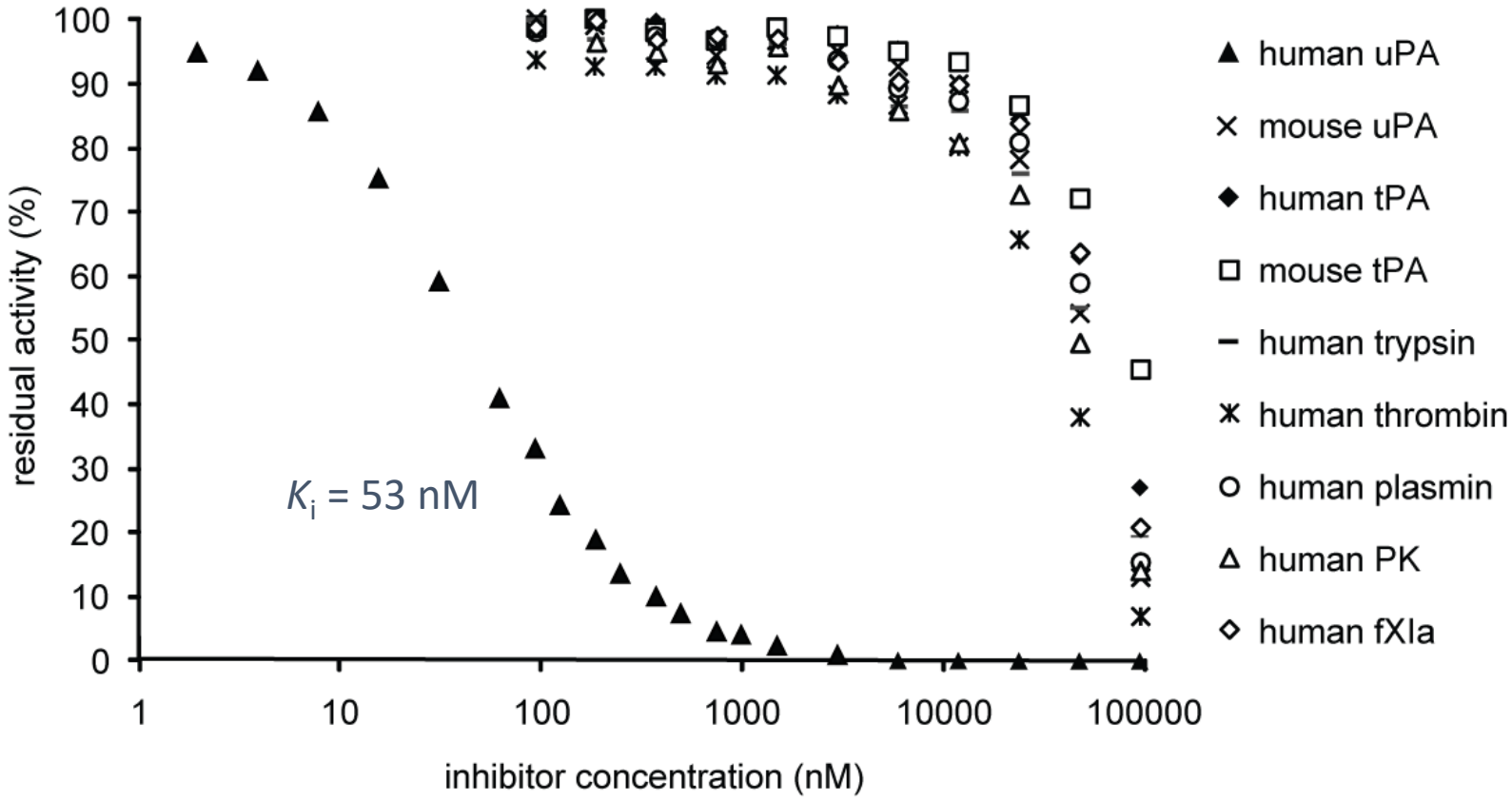
Isolated peptides



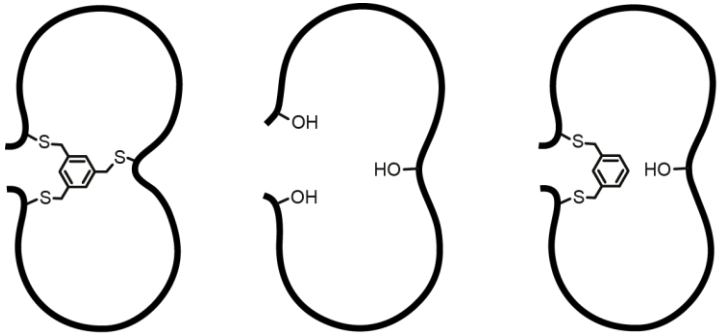
Inhibitory activity of UK18



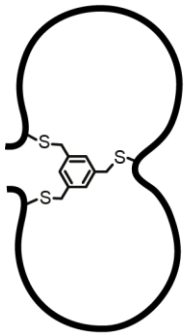
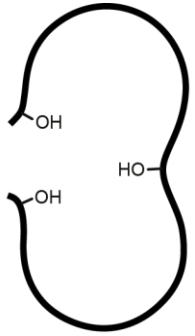
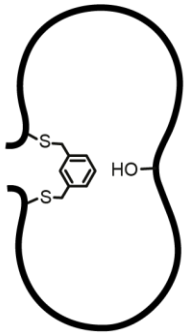
Inhibitory activity of UK18



Is bicyclic configuration important?

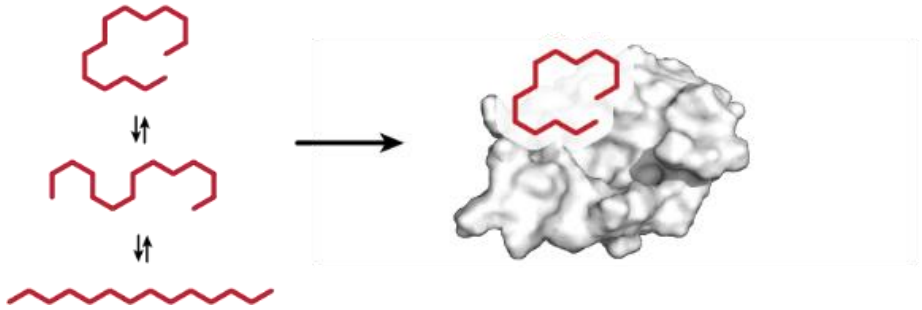


Is bicyclic configuration important?

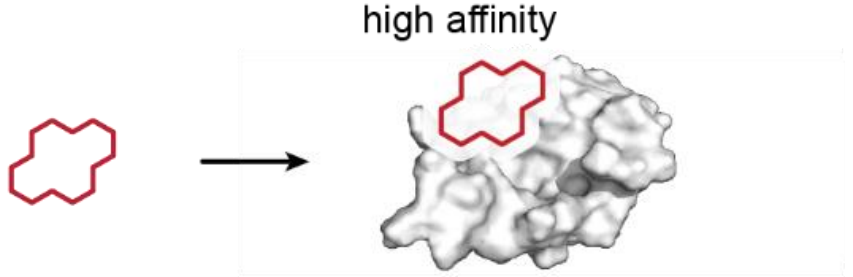
			
Inhibitory constant (K_i)	53 nM	17’500 nM	383 nM

Smaller entropic penalty → Higher affinity

Linear

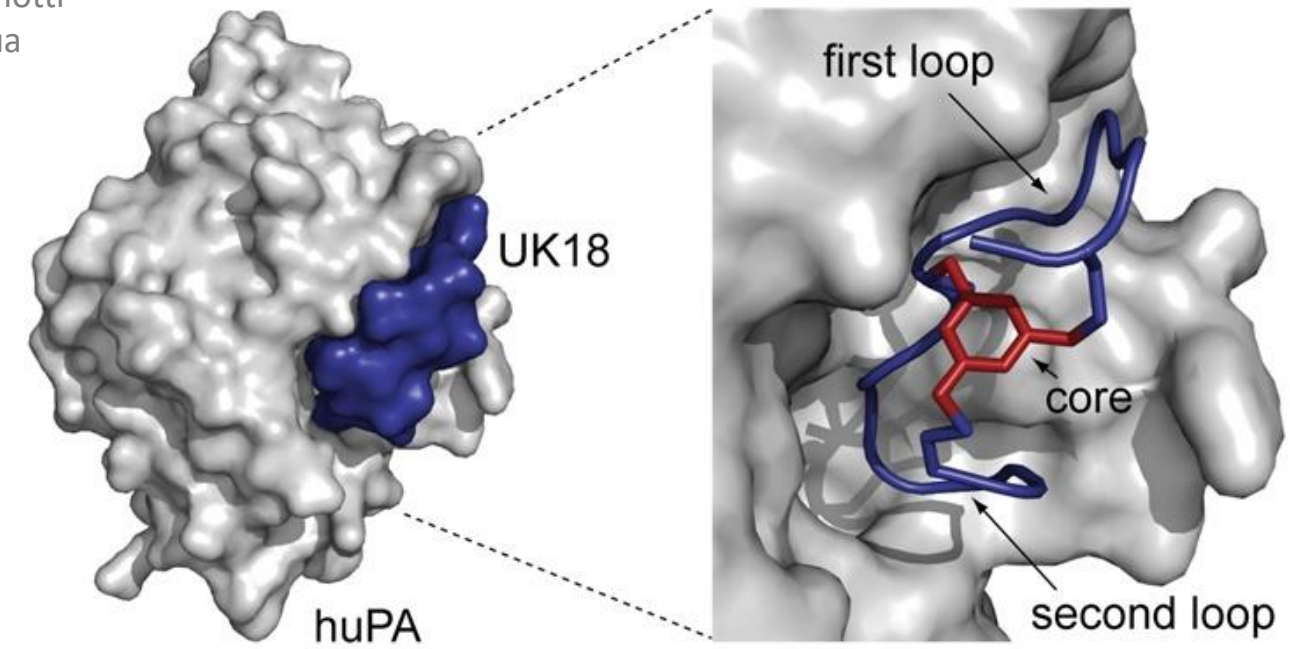


Cyclic



Structure of uPA-UK18 complex

Collaboration with
Prof. Giuseppe Zanotti
University of Padua



1.9 Å resolution

> 700 Å^2 binding interface

Angelini, A., Cendron, L., Chen, S., Touati, J., Winter, G., Zanotti, G. and Heinis, C., *ACS Chem. Biol.*, 2012

Bicyclic peptides to a broad range of targets

Plasma kallikrein	K_i = 300 pM
uPA	K_i = 28 nM
MMP-2	K_i = 1.5 nM
PSA	K_i = 47 nM
Her2	K_d = 270 nM
Notch1 receptor	K_d = 170 nM
β-catenin	K_d = 6 μM
CAIX	K_i = 50 nM
Sortase A	K_i = 1.2 μM
Factor XIIa	K_i = 370 pM
G-actin	K_d = 5 nM
TNFα	K_d = 300 nM
FKBP12	K_d = 0.5 μM
IL-17	K_d = 300 nM
Factor XIa	K_i = 10 nM
....	

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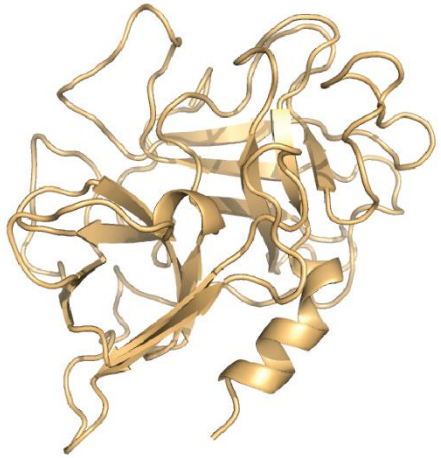
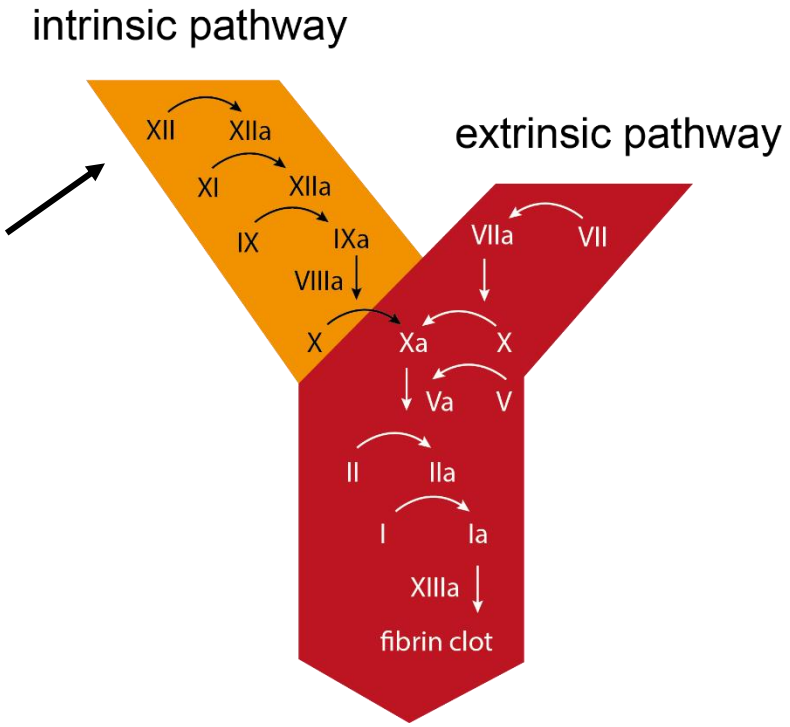
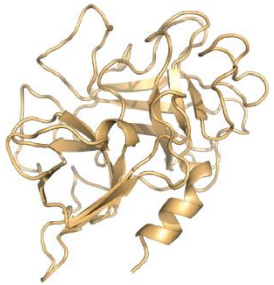


FXIIa

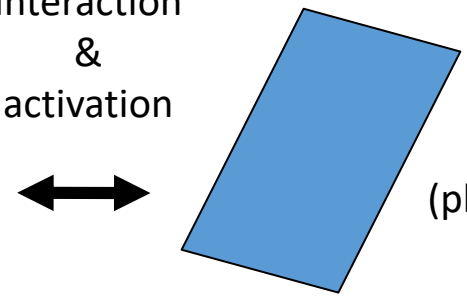


Contact activation

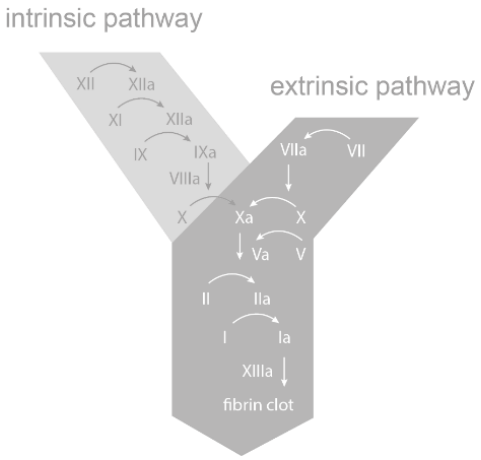
FXII
(= zymogen)



interaction
&
activation



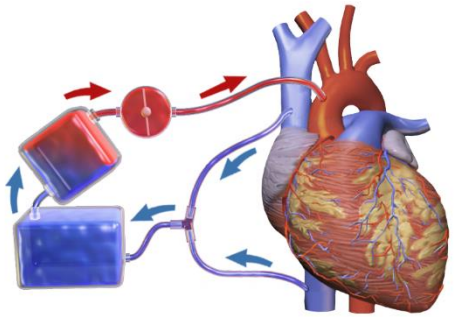
artificial surface
(plastic, membrane, etc.)



Contact activation in CPB and ECMO

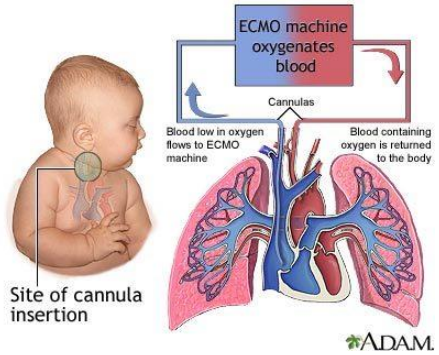
Cardiopulmonary bypass surgery

(CPB)



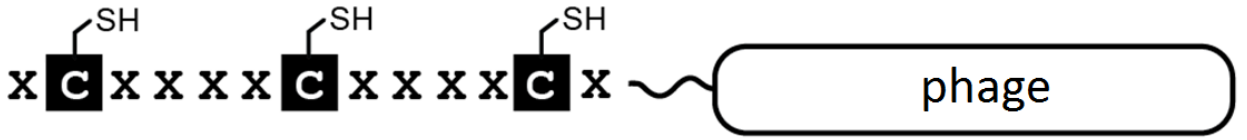
Extracorporeal membrane oxygenation

(ECMO)

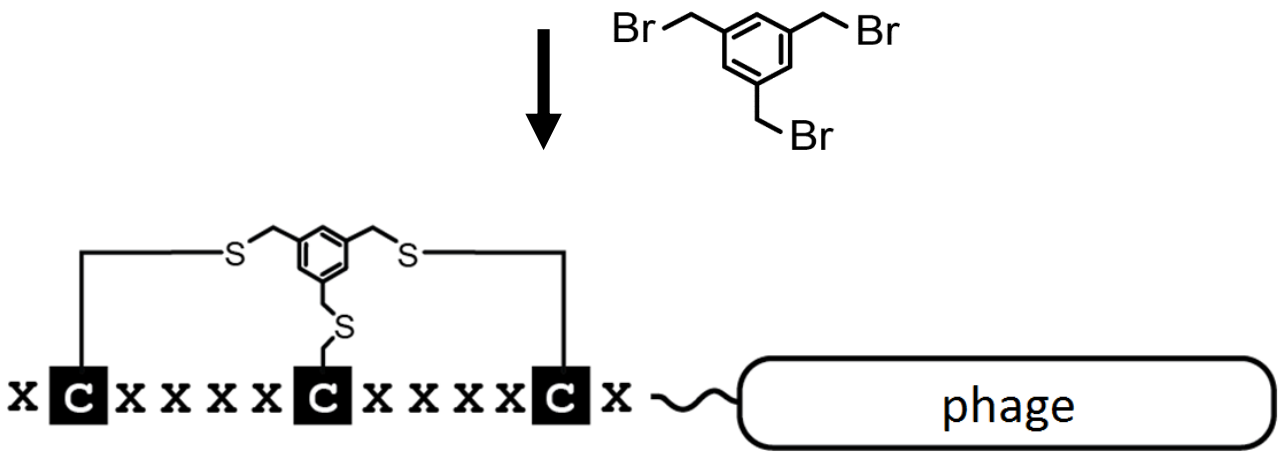


- Strong anticoagulation required (heparin) → bleeding risks
- Activation of kinnin-kininogen pathway → inflammation, organ damage

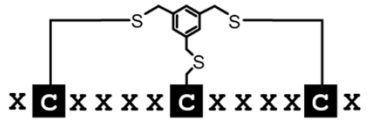
Phage selection of bicyclic peptide FXIIa inhibitor



X = random amino acid, > 3 x 10^8 different peptides

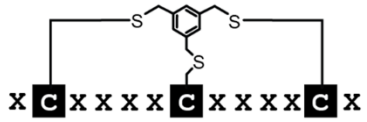


Isolated peptides



peptide:	sequence:	abundance:
FXII301	A C D A R P C P Q T Y C L	28
FXII302	Q C N A R P C P S S Y C R	2
FXII303	G C M G R P C P V S Y C F	2
FXII304	S C G G R P C P P A Y C K	22
FXII305	G C L G R P C P M A Y C S	13
FXII306	G C W A R P C P L A L C Q	1
FXII307	G C A A R P C P L T A C W	1
FXII308	G C H G R P C P L Q Y C K	1
FXII309	R C Y A N P C P I S Y C R	1
FXII310	S C S G R R C P P S Y C K	1
FXII311	V C V Q K F C W R G W C P	4
FXII312	A C Q Q Q F C W R G W C P	2
FXII313	L C E Y T L C W R G W C P	2
FXII314	H C R Y V F C W R G W C P	2
FXII315	N C V N R Y C W R G W C S	1
FXII316	W C V M Q K C L M Q Y C E	2
FXII317	Y C V K D K C L Q A M C S	2
FXII318	S C V V G K C L V Q Y C A	1
FXII319	H C V Q G K C L V Y M C G	1
FXII320	Q C V P F K C L L H L C A	1
FXII321	G C V L G K C L Q D F C Q	1
FXII322	Y C V P L K C L W D R C E	1
FXII323	R C S A G G C C E R V C E	1
FXII324	T C T T Q G C A F R L C S	1
FXII325	W C T H Q R C V K A R C C	1
FXII326	Y C Q G T K C V K E R C C	1
FXII327	L C Q P R K C V K A L C C	1
FXII328	S C A N Q R C V K S L C C	1
FXII329	T C L C K R C I K E L C C	1
FXII330	V C T Y R K C V K E L C C	1
FXII331	G C Q A R A C A K F L C C	1
FXII332	G C G R N I C V K N L C C	1

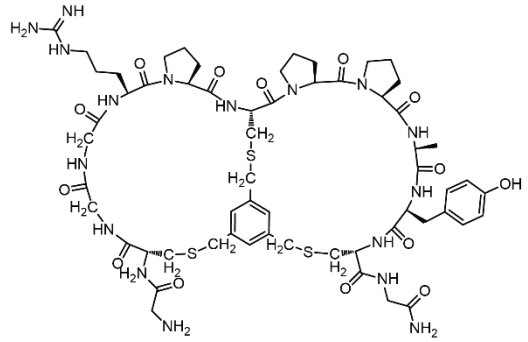
Isolated peptides



peptide:	sequence:	abundance:	K_i for FXIIa (μM):
FXII301	A C D A R P C P Q T Y C L	28	20.5 +/- 5.2
FXII302	Q C N A R P C P S S Y C R	2	4.7 +/- 1.5
FXII303	G C M G R P C P V S Y C F	2	5.0 +/- 1.3
FXII304	S C G G R P C P P A Y C K	22	3.1 +/- 0.5
FXII305	G C L G R P C P M A Y C S	13	5.0 +/- 1.5
FXII306	G C W A R P C P L A L C Q	1	10.2 +/- 4.6
FXII307	G C A A R P C P L T A C W	1	33.5 +/- 5.9
FXII308	G C H G R P C P L Q Y C K	1	11.2 +/- 4.4
FXII309	R C Y A N P C P I S Y C R	1	
FXII310	S C S G R R C P P S Y C K	1	7.8 +/- 3.2
FXII311	V C V Q K F C W R G W C P	4	> 75
FXII312	A C Q Q Q F C W R G W C P	2	
FXII313	L C E Y T L C W R G W C P	2	
FXII314	H C R Y V F C W R G W C P	2	
FXII315	N C V N R Y C W R G W C S	1	
FXII316	W C V M Q K C L M Q Y C E	2	
FXII317	Y C V K D K C L Q A M C S	2	
FXII318	S C V V G K C L V Q Y C A	1	
FXII319	H C V Q G K C L V Y M C G	1	
FXII320	Q C V P F K C L L H L C A	1	
FXII321	G C V L G K C L Q D F C Q	1	
FXII322	Y C V P L K C L W D R C E	1	
FXII323	R C S A G G C C E R V C E	1	
FXII324	T C T T Q G C A F R L C S	1	> 75
FXII325	W C T H Q R C V K A R C C	1	
FXII326	Y C Q G T K C V K E R C C	1	
FXII327	L C Q P R K C V K A L C C	1	
FXII328	S C A N Q R C V K S L C C	1	
FXII329	T C L C K R C I K E L C C	1	
FXII330	V C T Y R K C V K E L C C	1	
FXII331	G C Q A R A C A K F L C C	1	
FXII332	G C G R N I C V K N L C C	1	

Bicyclic peptide FXIIa inhibitor

1st generation



K_i = 1.2 μM

Baeriswyl, V., *Calzavarini, S., Gertschheimer, C., Diderich, P., Angelillo-Scherrer, A. and Heinis, C., J. Med. Chem.*, 2012

Screening larger structural diversities

3x3 library

X C X X X C X X X C X ~ phage

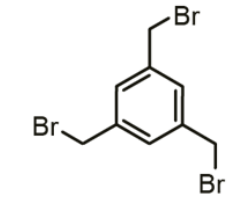
4x4 library

X C X X X X C X X X X C X ~ phage

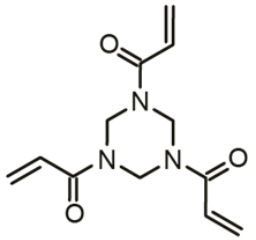
6x6 library

A C X X X X X X C X X X X X X C G ~ phage

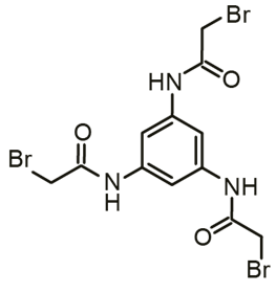
TBMB



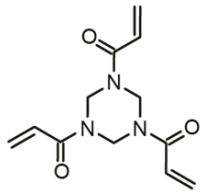
TATA



TBAB



Bicyclic peptide isolated with TATA



3x3 library

peptide:	sequence:	abundance:	K_i (μM):
FXII501	K C W G A C L N T C P	4	
FXII502	R C W G A C L N V C S	2	1.2
FXII503	K C W G A C L N A C P	1	
FXII504	V C W G A C L N V C P	1	1.9
FXII505	Q C W G A C L N V C N	4	
FXII506	A C W G A C Q N V C R	5	6.3
FXII507	F C N P Y C M M K C A	1	
FXII508	D C N P Y C Q M K C M	1	
FXII509	R C W Y G C V P Q C Y	6	1.0
FXII510	F C T K E C L Q S C Y	3	
FXII511	R C L Q K C F P P C L	1	
FXII512	G C C Y L R L S C R	2	0.16
FXII513	G C C Y L R Y L C S	1	0.35

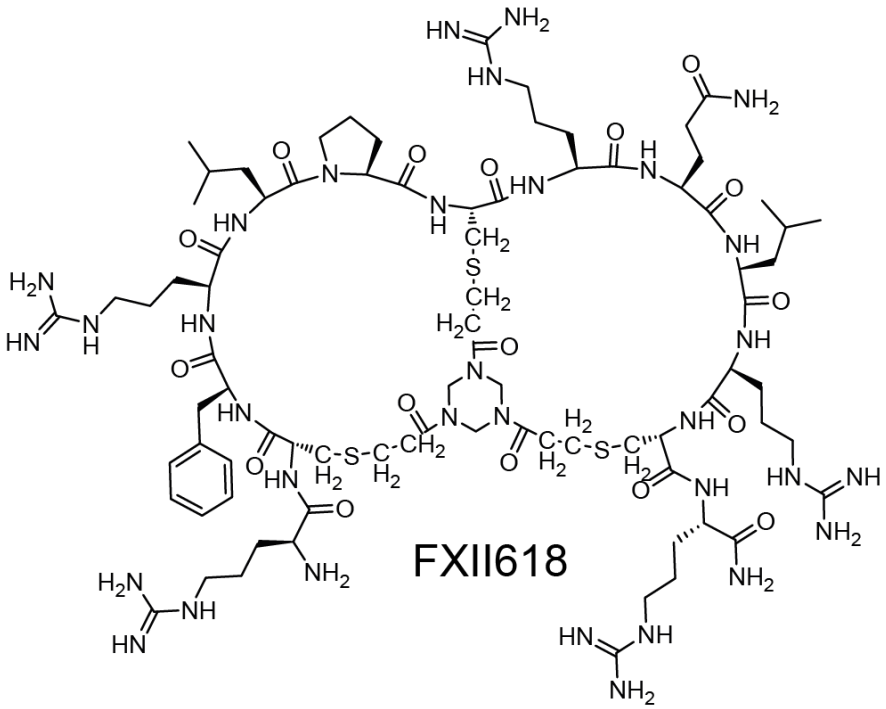
4x4 library

peptide:	sequence:	abundance:	K_i (μM):
FXII514	L C S R L P C E Q L P C N	11	10.2
FXII515	L C P R L S C H Q L G C S	4	2.5
FXII516	R C F R L P C A Q L S C E	1	0.16
FXII517	N C Q Y M R C P P M S C L	3	
FXII518	S C M A T Q C L N Q L Y G	4	

6x6 library

peptide:	sequence:	abundance:	K_i (μM):
FXII519	A C W G A A A G C V Q G A S N C G	1	
FXII520	A C W G A A A G C V S V H Q S C G	1	
FXII521	A C W G A A A K C S Q T W S G C G	1	
FXII522	A C W G A A A K C P Q T A A Q C G	1	
FXII523	A C W G A A A T C N Q Q Q L V C G	1	
FXII524	A C W G A A A H C Q L P K D R C G	1	
FXII525	A C W G A A V G C N S P R G P C G	1	
FXII526	A C W G A A V S C E T Q S S L C G	1	
FXII527	A C W G A A L S C N P Q G S R C G	1	8.5
FXII528	A C W G A A Q R C K V M Q G E C G	1	3.7
FXII529	A C W G A A Q K C K M N G Q N C G	1	
FXII530	A C W G A A Q L C G R P Q M P C G	1	
FXII531	A C W G A A Q A C I P Q M A A C G	1	
FXII532	A C W G A A Q H C Q A S N N S C G	1	
FXII533	A C W G A A S G C N Q T G M D C G	1	
FXII534	A C W G A A S A C Q Q G G A K C G	1	2.4
FXII535	A C W G A A S R C T V I S G R C G	1	
FXII536	A C W G A A R G C P D Q H G V C G	1	
FXII537	A C W G A A K L C G A A R D G C G	1	
FXII538	A C W G A A E R C K Q A D N S C G	1	

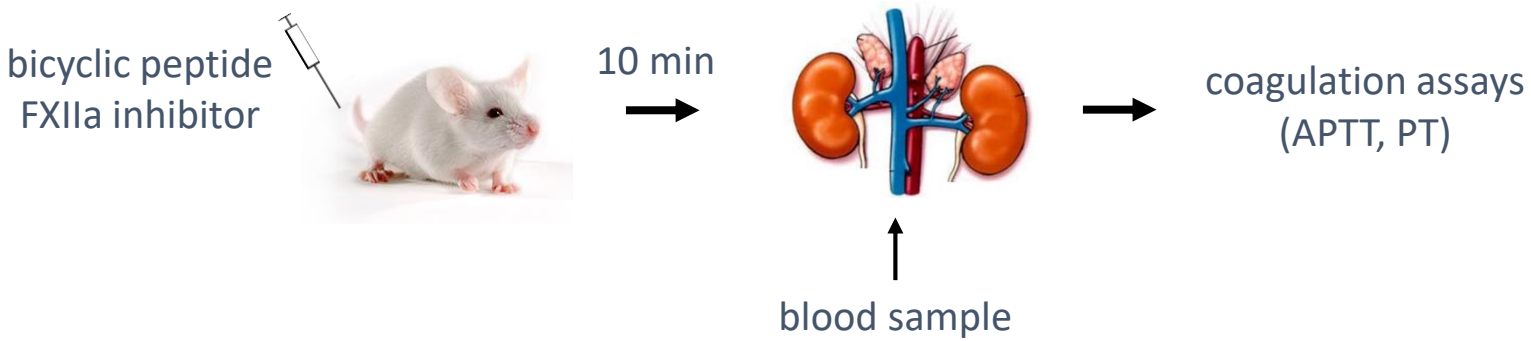
Affinity maturation

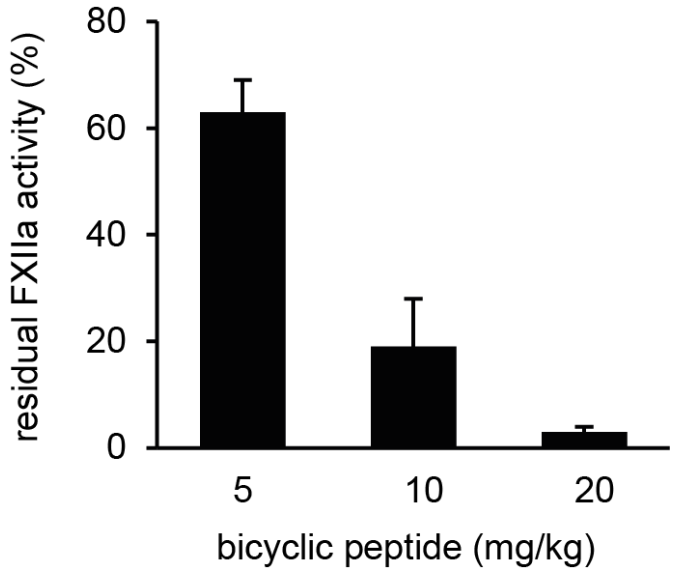


Protease	K_i (nM)
hFXIIa	8 ± 0.7
mFXIIa	93 ± 4
htPA	> 50,000
huPA	> 50,000
hFVIIa	> 50,000
hFXa	> 50,000
hFXIa	> 50,000
hPK	> 50,000
hthrombin	> 50,000
hplasmin	> 50,000

Baeriswyl, V., Calzavarini, S., Chen. S., Zorzi, A., Bologna, L., Angelillo-Scherrer, A. and Heinis, C., *ACS Chem. Biol.*, 2015

Inhibition of intrinsic coagulation pathway (in vivo)





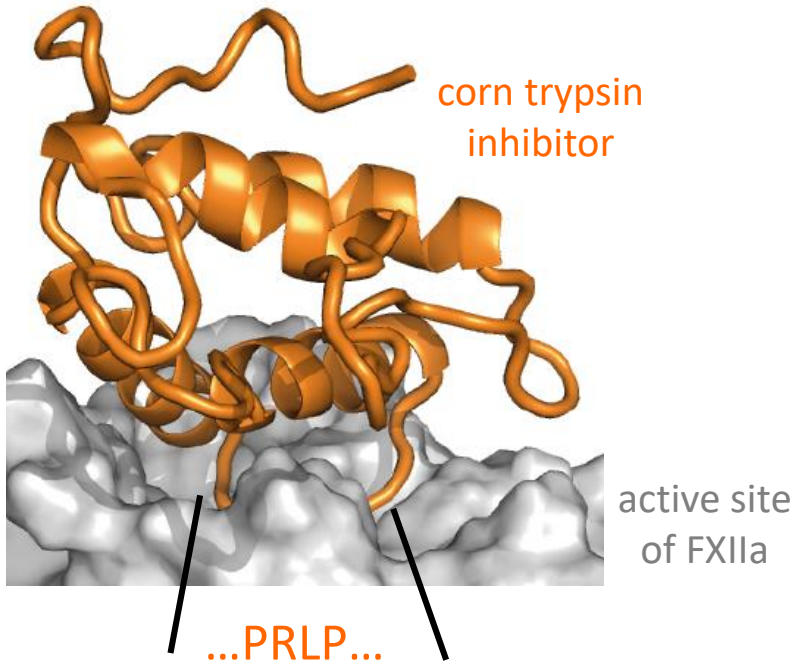
Collaboration with group
Prof. Anne Angelillo-Scherrer
(Inselspital Bern)

Sequence similarity to natural FXIIa inhibitor

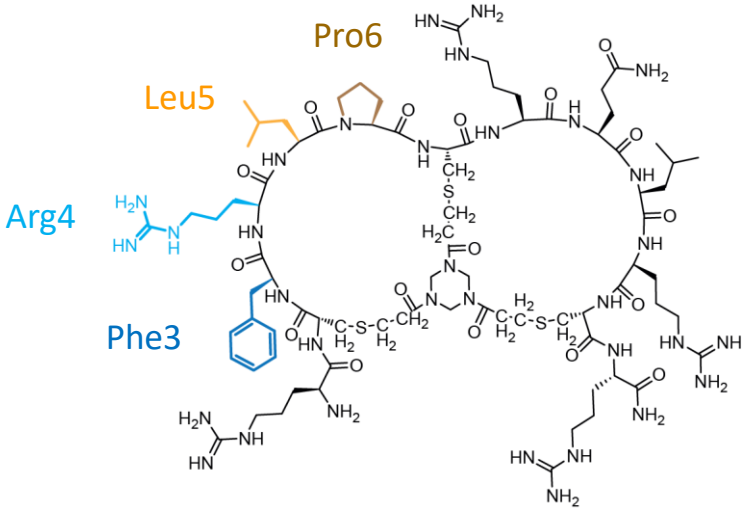
peptides selected against FXIIa:

FXII572	R	C	P	R	L	P	C	A	Q	L	Q	C	P
FXII573	I	C	P	R	L	P	C	A	Q	L	R	C	V
FXII574	V	C	P	R	L	P	C	G	Q	L	R	C	G
FXII575	I	C	P	R	L	P	C	R	Q	L	S	C	M
FXII576	V	C	P	R	L	P	C	H	Q	L	R	C	G
FXII577	R	C	P	R	L	P	C	E	Q	L	R	C	A
FXII578	I	C	P	R	L	P	C	A	Q	L	V	C	T
FXII579	L	C	P	R	L	P	C	G	Q	L	R	C	T
FXII580	I	C	P	R	L	P	C	G	Q	L	S	C	Q
FXII581	R	C	P	R	L	P	C	E	Q	L	A	C	G
FXII582	G	C	P	R	L	P	C	A	Q	L	R	C	S
FXII583	I	C	P	R	L	P	C	S	Q	L	R	C	A
FXII584	K	C	P	R	L	P	C	G	Q	L	R	C	A
FXII585	A	C	P	R	L	P	C	A	Q	L	P	C	R
FXII586	R	C	P	R	L	P	C	S	Q	L	V	C	G
FXII587	R	C	P	R	L	P	C	A	Q	L	E	C	Q
FXII588	I	C	P	R	L	P	C	A	Q	L	K	C	R
FXII589	L	C	P	R	L	P	C	A	Q	L	R	C	P
FXII590	A	C	P	R	L	P	C	S	Q	L	K	C	G
FXII591	F	C	P	R	L	P	C	A	Q	L	K	C	S
FXII592	V	C	P	R	L	A	C	G	Q	L	R	C	D
FXII593	R	C	P	R	L	M	C	A	Q	L	H	C	P
FXII594	G	C	P	R	L	M	C	A	Q	L	Q	C	W
FXII595	R	C	P	R	L	A	C	E	Q	L	H	C	V
FXII596	P	C	P	R	L	A	C	G	Q	L	R	C	F
FXII597	V	C	P	R	L	Q	C	H	Q	L	R	C	T
FXII598	I	C	P	R	L	A	C	G	Q	L	R	C	E
FXII599	V	C	A	R	L	P	C	A	Q	L	K	C	Q
FXII600	A	C	F	R	L	P	C	L	Q	L	A	C	H
FXII601	R	C	F	R	L	P	C	S	Q	L	T	C	V
FXII602	R	C	A	R	L	P	C	H	Q	L	H	C	L
FXII603	R	C	A	R	L	P	C	H	Q	L	K	C	S
FXII604	K	C	L	R	L	F	C	D	Q	L	P	C	R
FXII605	G	C	F	R	L	L	C	S	Q	L	K	C	A
FXII606	W	C	L	R	L	Q	C	E	Q	L	M	C	G

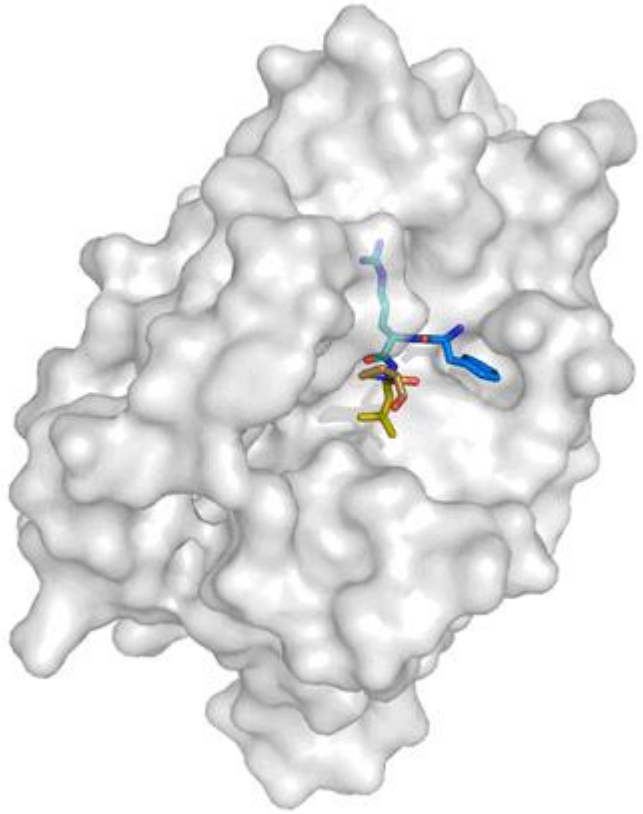
Corn trypsin inhibitor (CTI):



Structure model

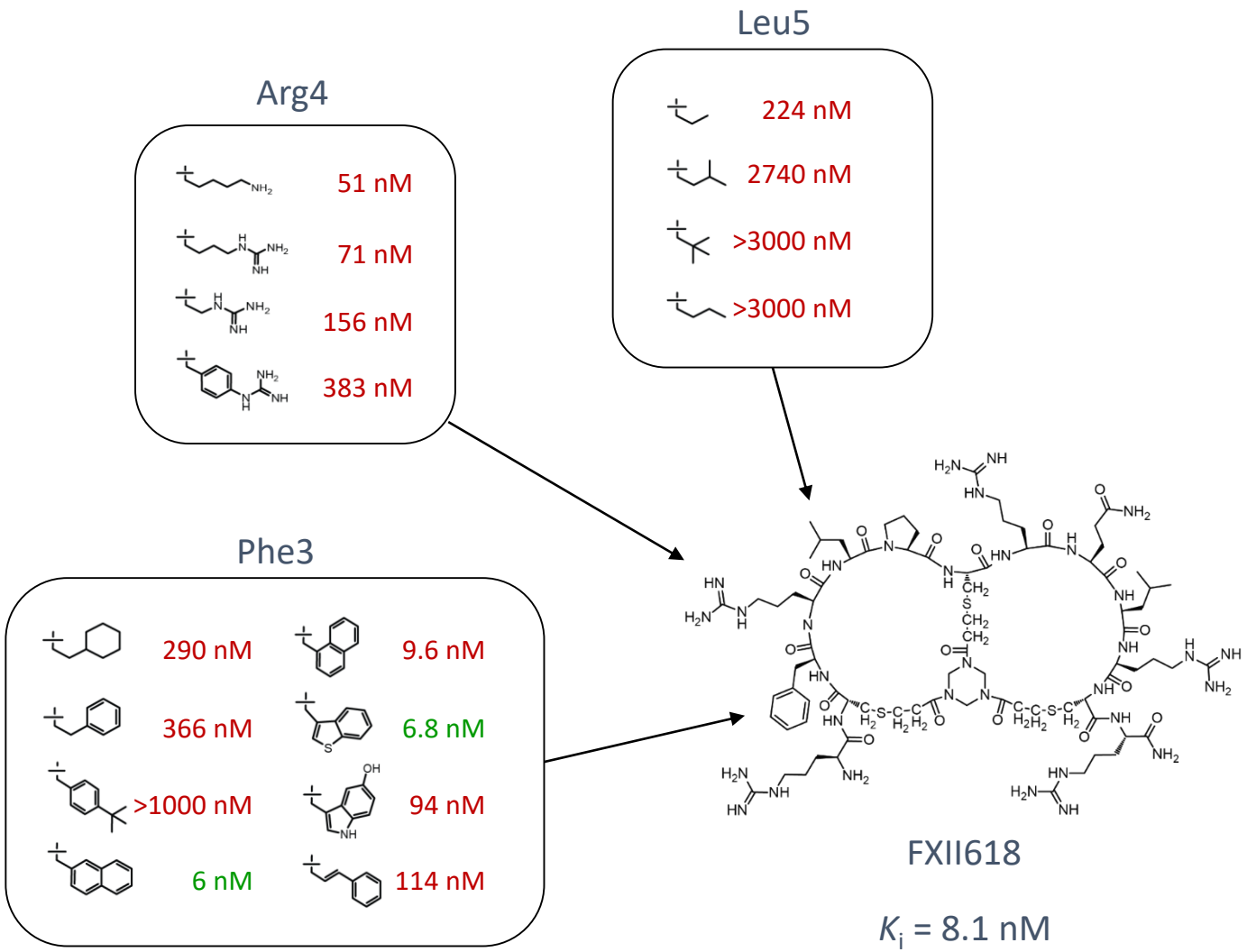


FXII618



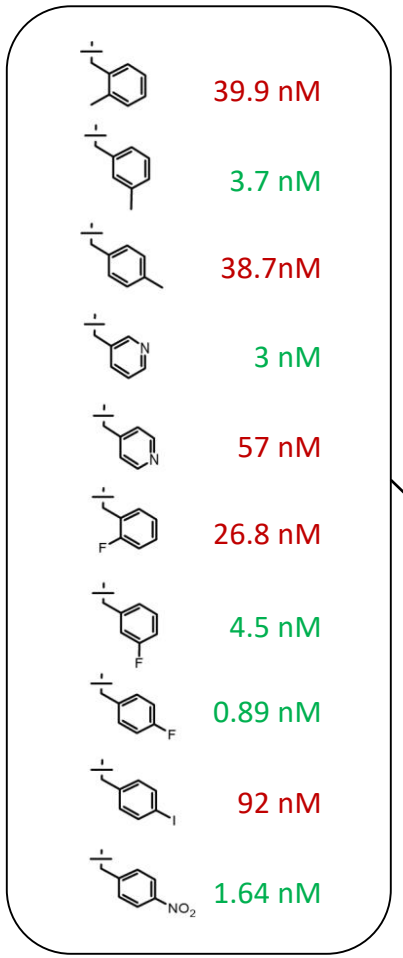
Project of Dr. Simon Middendorp

Affinity maturation with non-natural amino acids

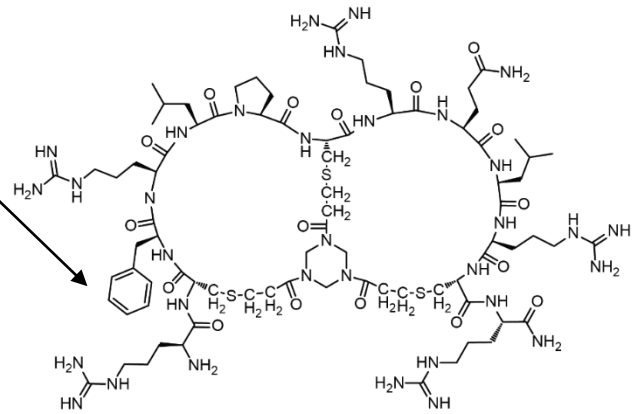


Affinity maturation with non-natural amino acids

Phe3



9-fold improved



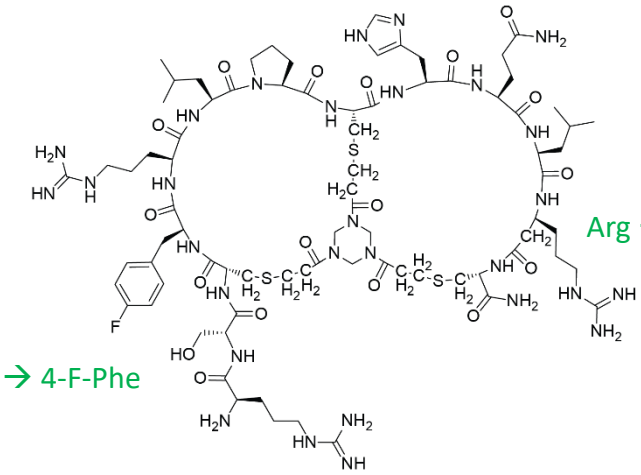
FXII618

K_i = 8.1 nM

Middendorp, S.J., Wilbs, J., Quarroz, C., Calzavarini, S., Angelillo-Scherrer, A. and Heinis, C., *J. Med. Chem.*, 2016

Combination of all mutations

FXII900

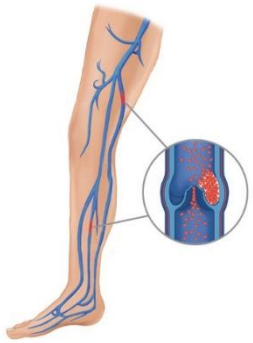


Affinity:	K_i = 370 pM
Selectivity: (over homologous plasma proteases)	> 50’000-fold
Stability: (human plasma)	$t_{1/2}$ > 4 days
Activity in plasma: (APTT, human, ex vivo)	EC_{2x} = 1.3 μM
Pharmacokinetics: elimination half-life $t_{1/2}$ (β)	20 min (mouse) 15 min (rabbit) 40 min (pig)

Collaboration with
Prof. Robert Rieben, University of Bern

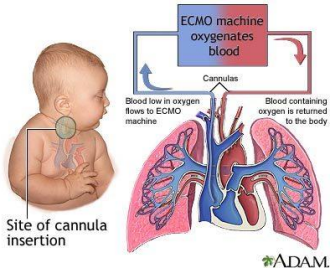
Evaluation of inhibitor in vivo

1. Thrombosis



Ferric chloride-induced thrombosis mouse model

2. Contact activation



Extracorporeal membrane oxygenation (ECMO) in rabbits

Prevention of thrombosis

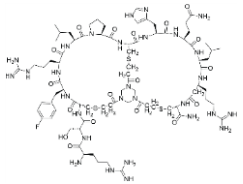
Ferric chloride-induced thrombosis mouse model

rhodamine 6G i.v.

(→ fluorescent cells/platelets)

t = -20 min

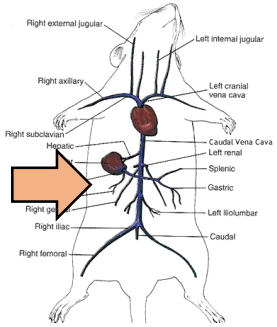
FXII900



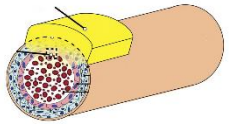
5 mg/kg s.c.

t = -15 min

expose mesenteric arteries



7.5% $FeCl_3$ (soaked in filter paper)



t = 0 min

intravitreal microscopy

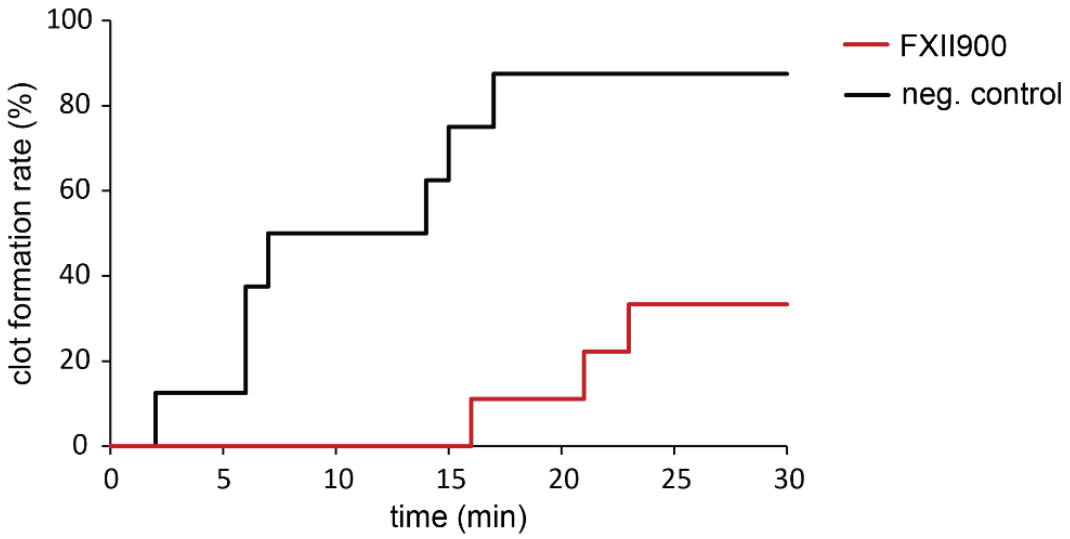
t = 0 - 30 min

Study performed in collaboration with
Prof. Anne Angelillo-Scherrer and Ms Raja Price (University of Bern)

Prevention of thrombosis

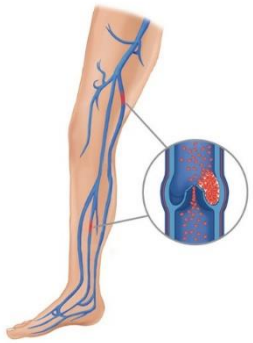
Ferric chloride-induced thrombosis mouse model

Time to clot formation:



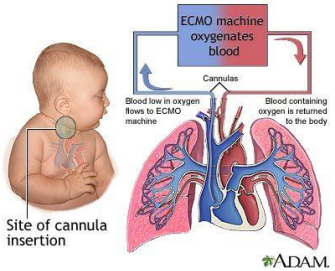
Evaluation of inhibitor in vivo

1. Thrombosis



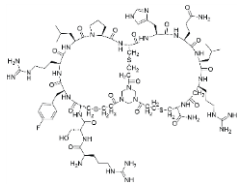
Ferric chloride-induced thrombosis mouse model

2. Contact activation



Extracorporeal membrane oxygenation (ECMO) in rabbits

Inhibition of coagulation during ECMO in rabbit model



FXII900

bolus 2 mg/kg i.v.

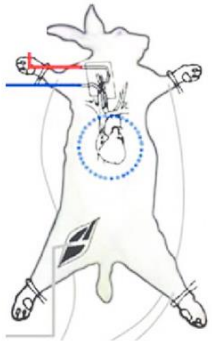
t = -20 min

mount to ECMO device

→

infusion 0.075 mg/kg min i.v.

t = 0 min



t = 0 – 4 hr

Monitor parameters:

- ACT
- aPTT
- Resistance
- Bleeding times

- Blood pressure
- HCT
- Platelet count
- WBC

measure clot volume in ECMO device

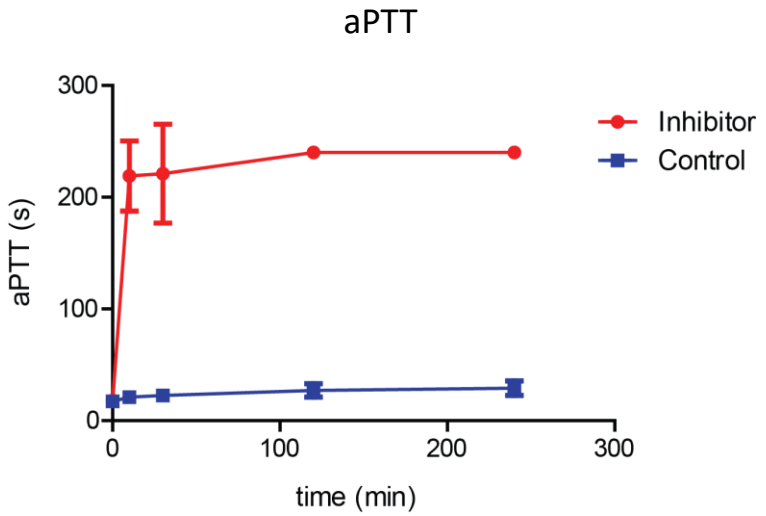
→

t = 4 hr

Study performed in collaboration with
Prof. Keith Cook and Ms Alida Cooke (Carnegie Mellon University)

Inhibition of coagulation during ECMO in rabbit model

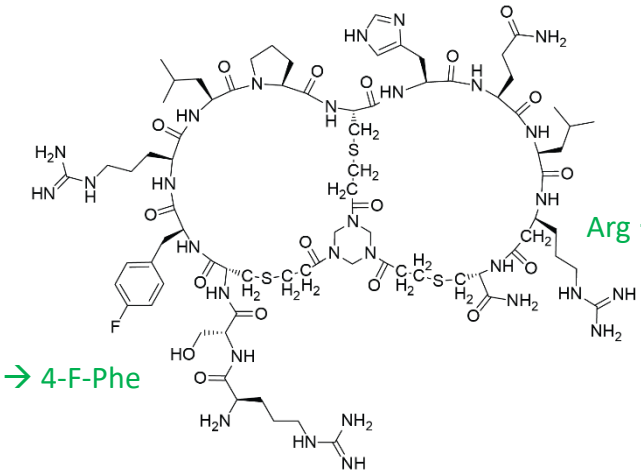
- Maximal prolongation of aPTT
- Clot volume in ECMO oxygenator was reduced > 3-fold
- Normal bleeding time



Study performed in collaboration with
Prof. Keith Cook and Ms Alida Cooke (Carnegie Mellon University)

Combination of all mutations

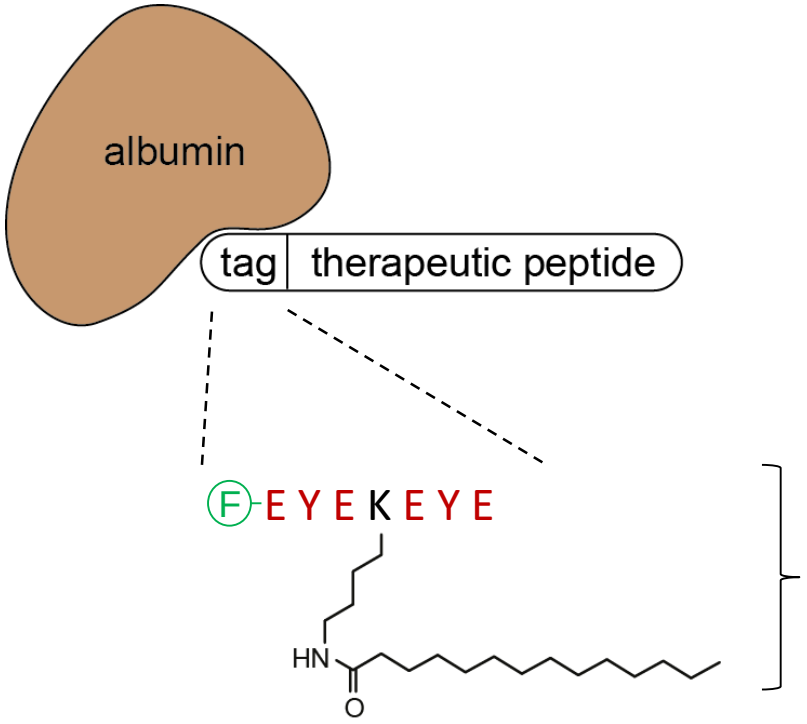
FXII900



Affinity:	K_i = 370 pM
Selectivity: (over homologous plasma proteases)	> 50'000-fold
Stability: (human plasma)	$t_{1/2}$ > 4 days
Activity in plasma: (APTT, human, ex vivo)	EC_{2x} = 1.3 μM
Pharmacokinetics: elimination half-life $t_{1/2}$ (β)	20 min (mouse) 15 min (rabbit) 40 min (pig)

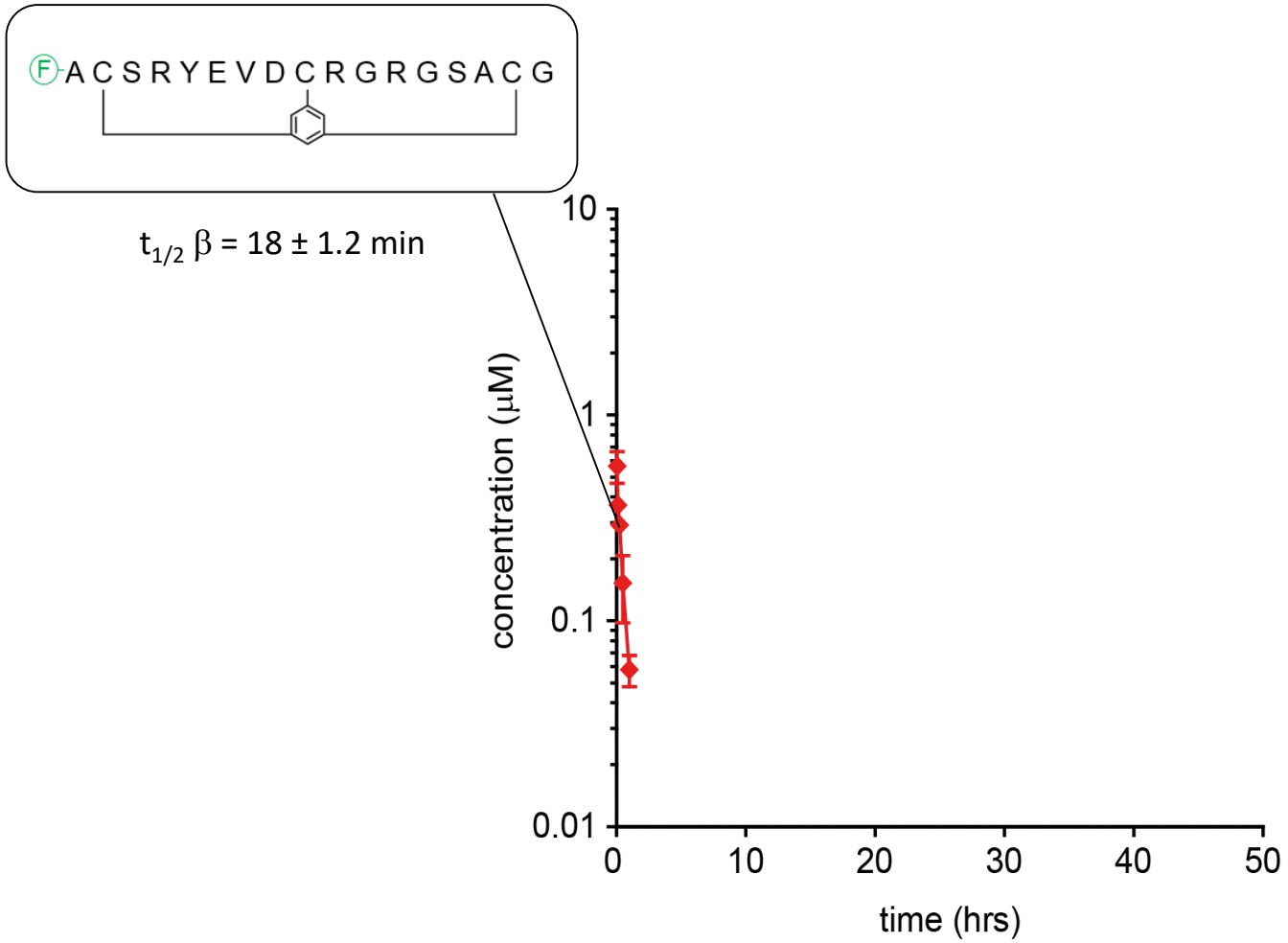
Collaboration with
Prof. Robert Rieben, University of Bern

Short peptidic albumin-binding tag

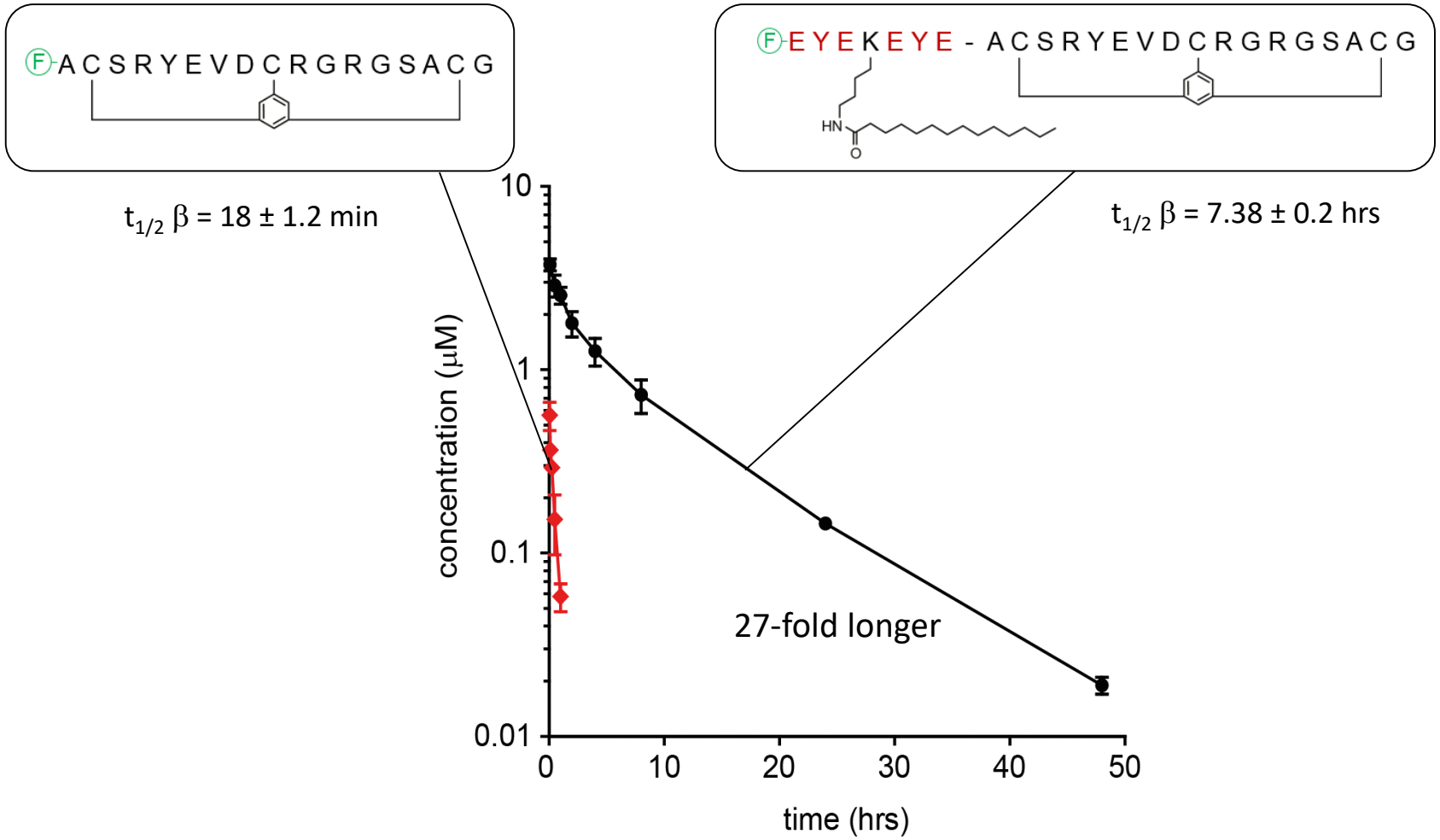


K_d human albumin = 39 ± 3 nM

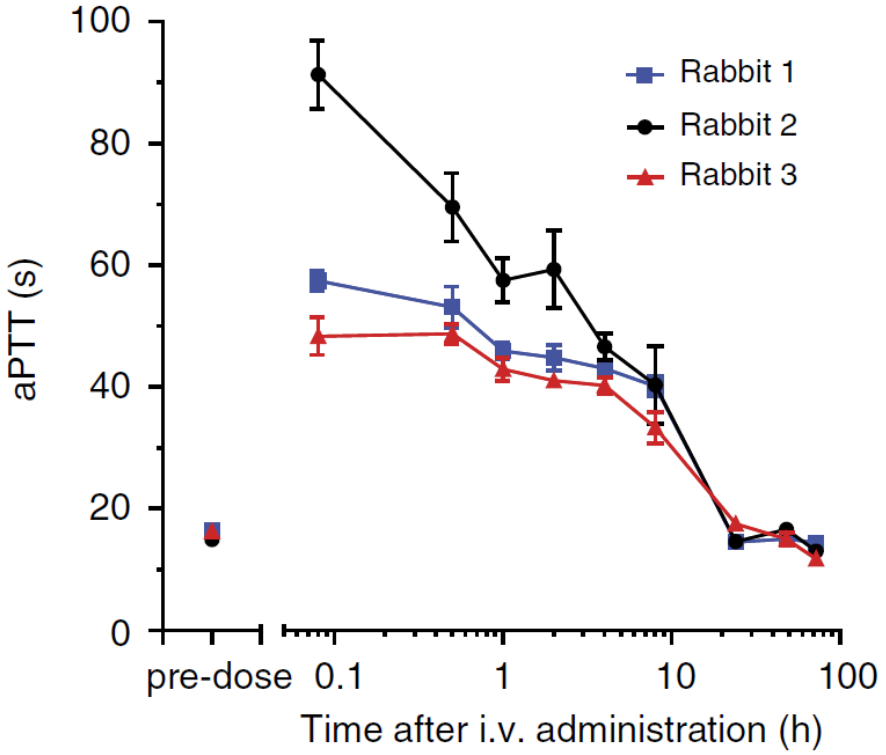
Pharmacokinetics in rats



Pharmacokinetics in rats



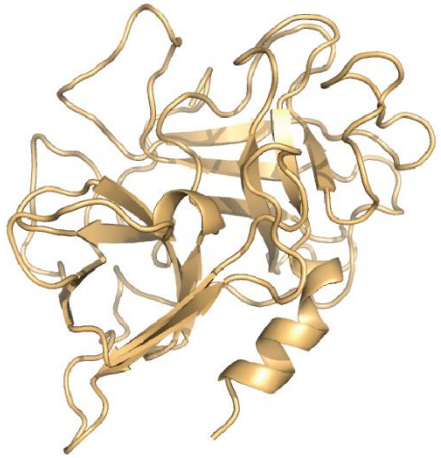
Application to FXII inhibitor



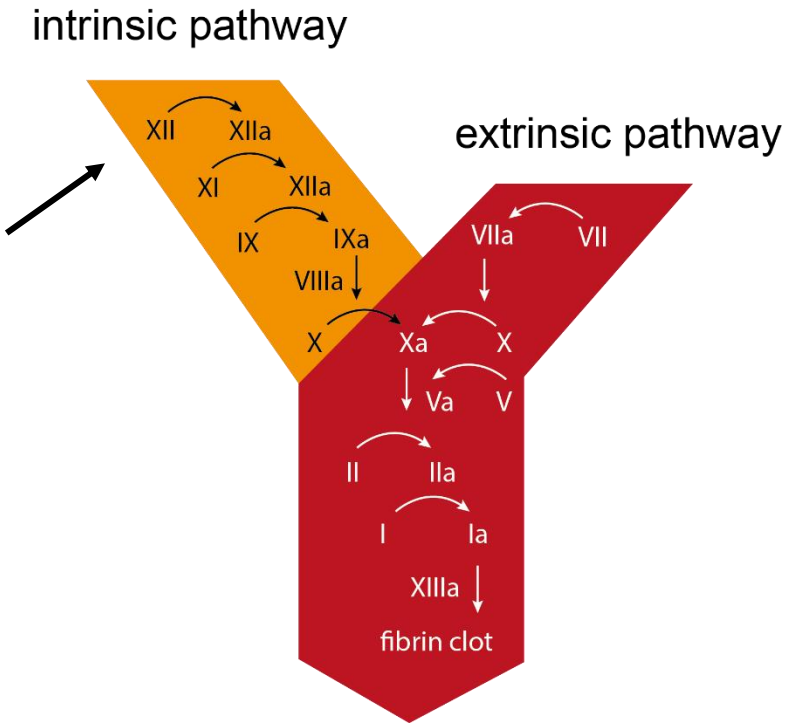
Zorzi, A., Wilbs, J., Middendorp, S., Deyle, K. and Heinis, C., *Nature Communications*, 2017

Bicyclic peptides to a broad range of targets

Plasma kallikrein	K_i = 300 pM
uPA	K_i = 28 nM
MMP-2	K_i = 1.5 nM
PSA	K_i = 47 nM
Her2	K_d = 270 nM
Notch1 receptor	K_d = 170 nM
β-catenin	K_d = 6 μM
CAIX	K_i = 50 nM
Sortase A	K_i = 1.2 μM
Factor XIIa	K_i = 370 pM
G-actin	K_d = 5 nM
TNFα	K_d = 300 nM
FKBP12	K_d = 0.5 μM
IL-17	K_d = 300 nM
Factor XIa	K_i = 10 nM
....	



FXIIa



Spin-off company Bicycle Therapeutics

Spin-off

- Founded in 2009, based in Cambridge, UK
- > 120 employees
- Listed at Nasdaq (BCYC)

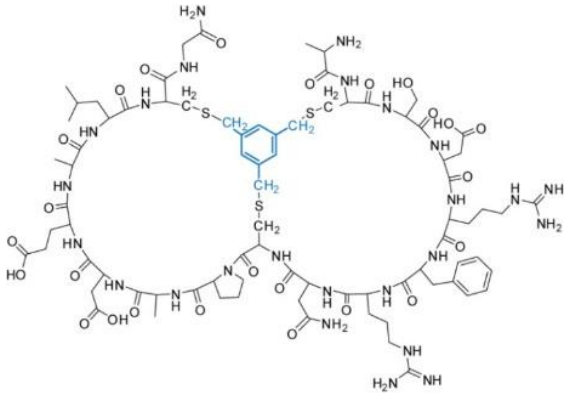
Bicycle

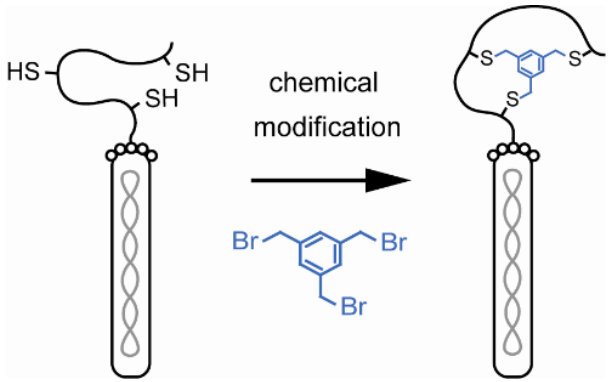
Three bicyclic peptides in clinical trials

- BT5528 (anti-EphA2 peptide drug conjugate, Phase I/II
- BT8009 (anti-Nectin-4 peptide drug conjugate, Phase I/II
- BT5528 (anti-MT1-MMP peptide drug conjugate, Phase I/IIa

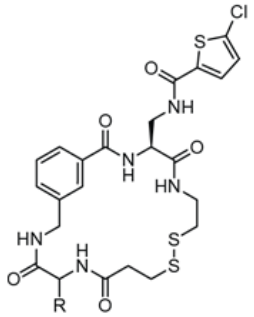
Talk overview

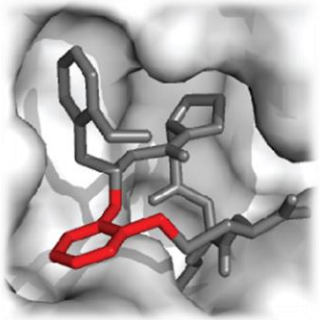
1. Bicyclic peptides developed by phage display



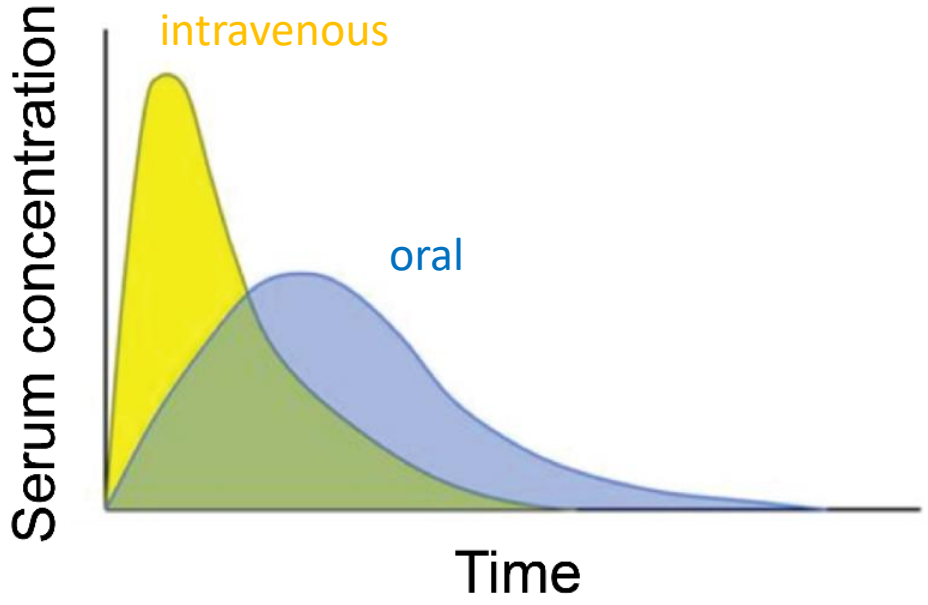


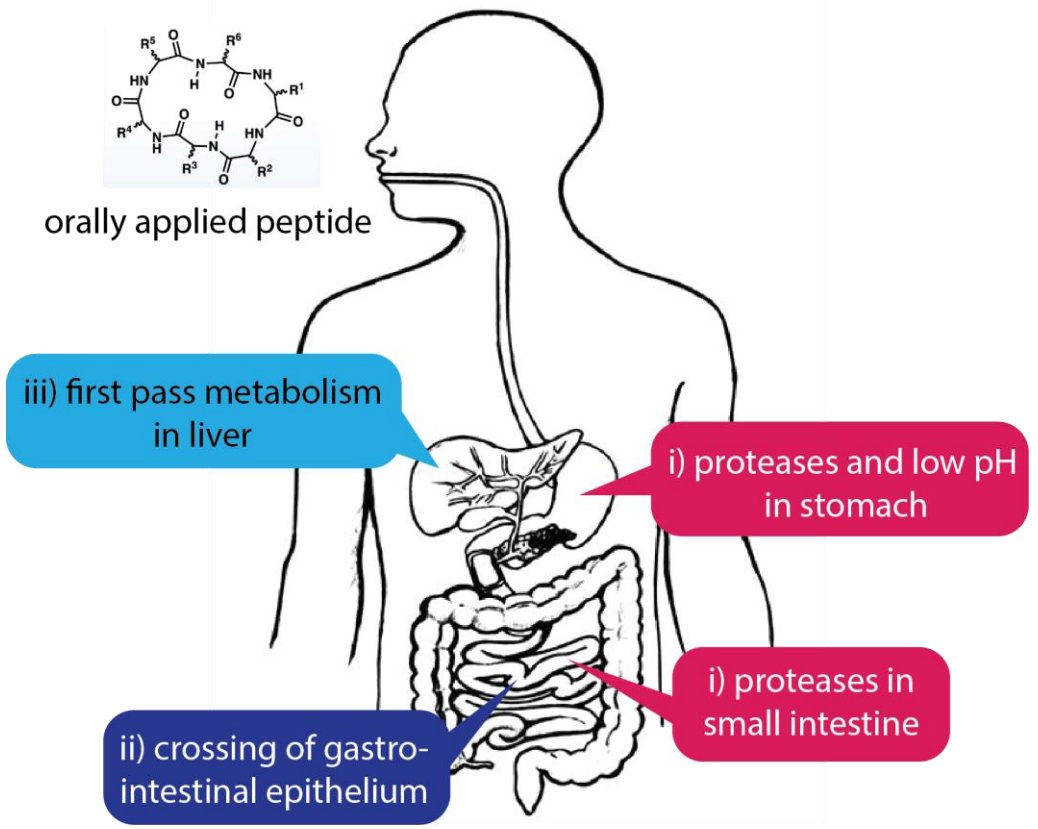
2. Peptide macrocycles developed by high-throughput synthesis at a nanomole scale





Can peptides be applied orally?





Which cyclic peptides can cross membranes?

Oral vs. not oral macrocyclic drugs

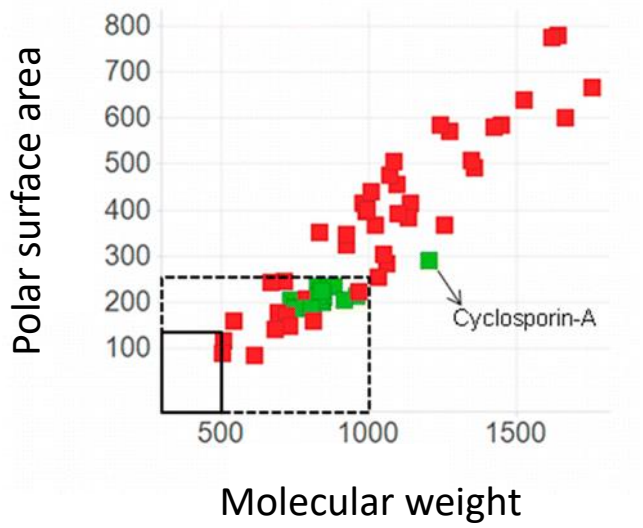


Figure from Giordanetto and Kihlberg, J. Med. Chem., 2014

Journal of Medicinal Chemistry

How Big Is Too Big for Cell Permeability?

Pär Matsson[†] and Jan Kihlberg[*,‡]

Which cyclic peptides can cross membranes?

Oral vs. not oral macrocyclic drugs

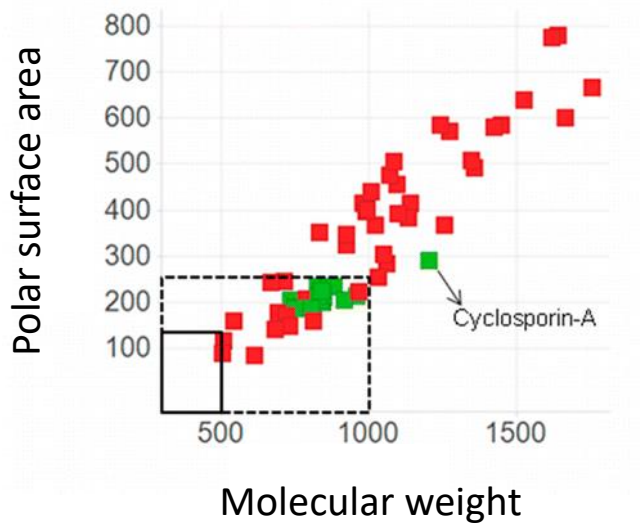


Figure from Giordanetto and Kihlberg, J. Med. Chem., 2014

Journal of Medicinal Chemistry

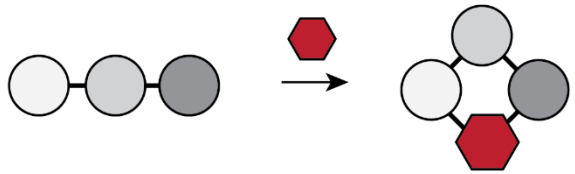
How Big Is Too Big for Cell Permeability?

Pär Matsson[†] and Jan Kihlberg[*,‡]

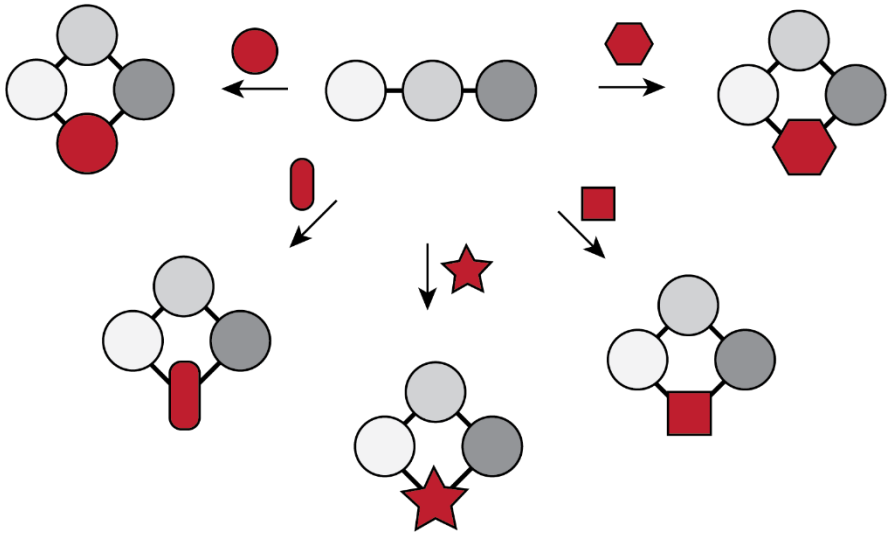
Key factors:

- Molecular weight: < 750 Da
- Polar surface are: < 250 Å^2
- Number of H-bond donors: < 6

Combinatorial synthesis of peptide-based macrocycles



Combinatorial synthesis of peptide-based macrocycles



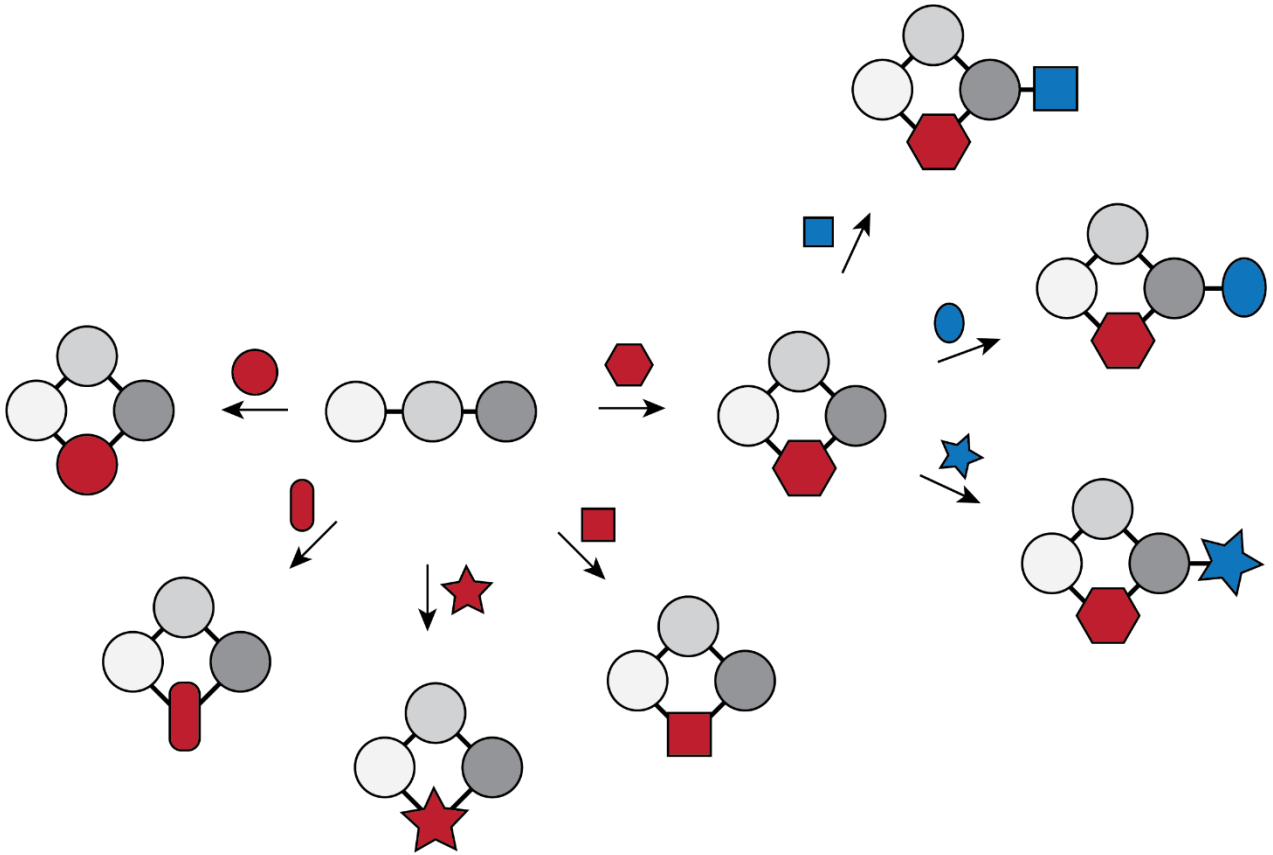
Number example:

1,000 peptides

x

10 linkers

Combinatorial synthesis of peptide-based macrocycles



Number example:

1,000 peptides

x

10 linkers

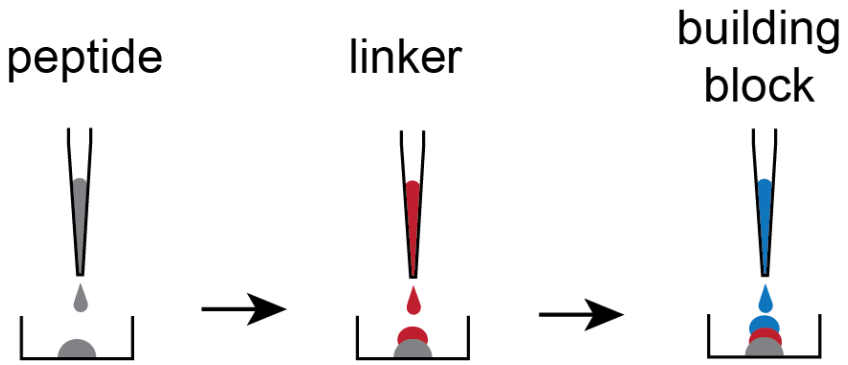
x

10 building blocks

= 100,000 macrocycles

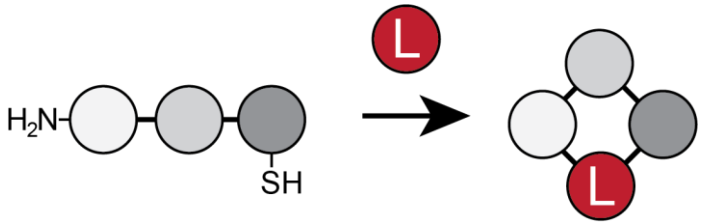
Combinatorial synthesis of peptide-based macrocycles

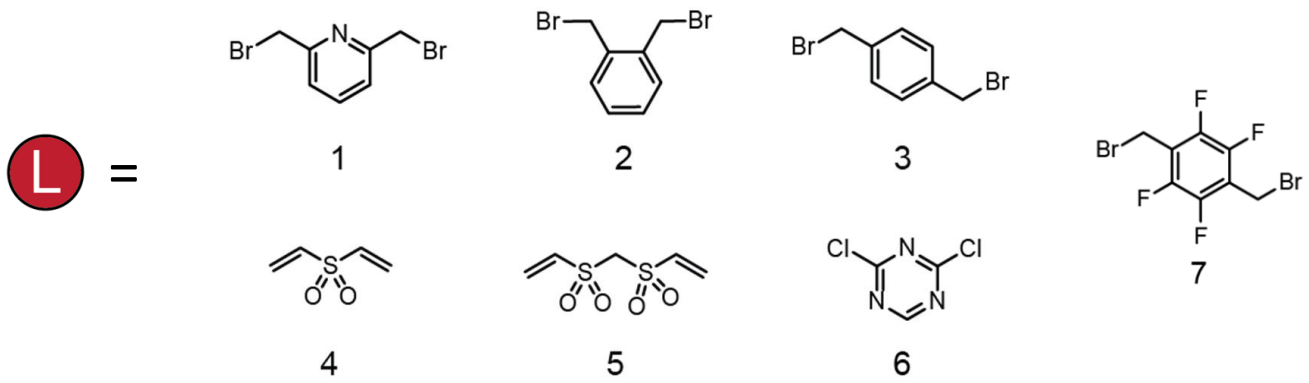
Combine reagents, react & screen



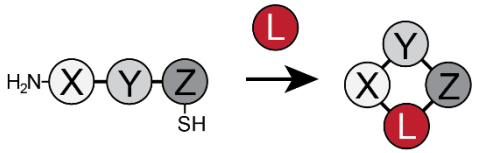
No purification → large libraries

Efficient cyclization reaction: Thiol-to-amine ligation

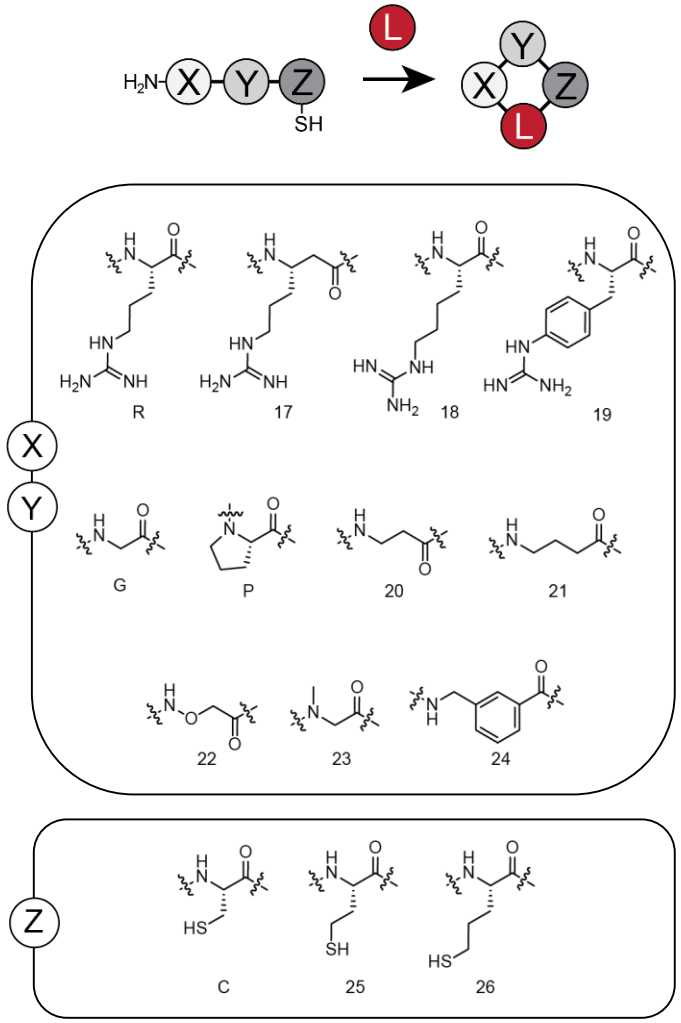




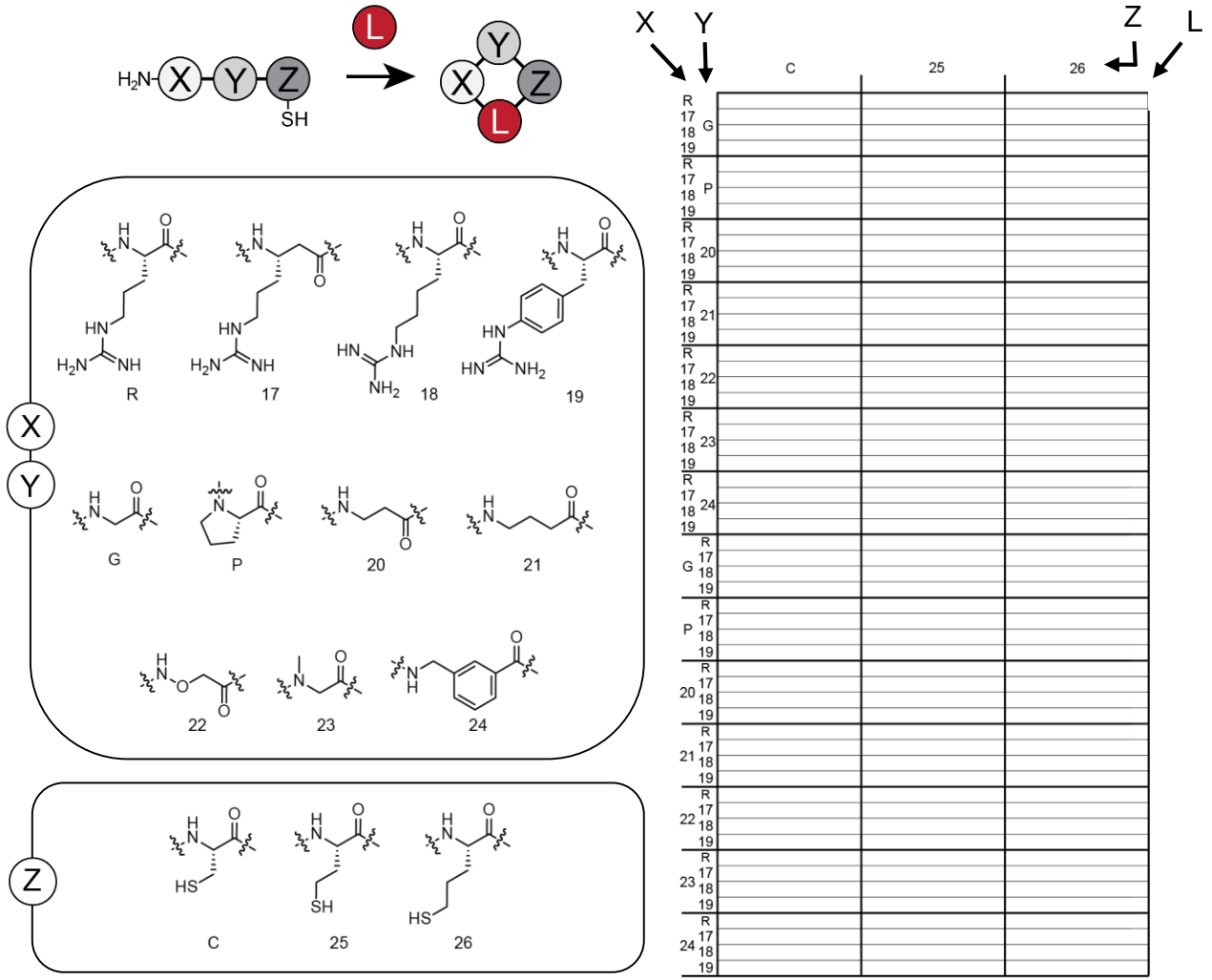
Pilot-scale library



Pilot-scale library

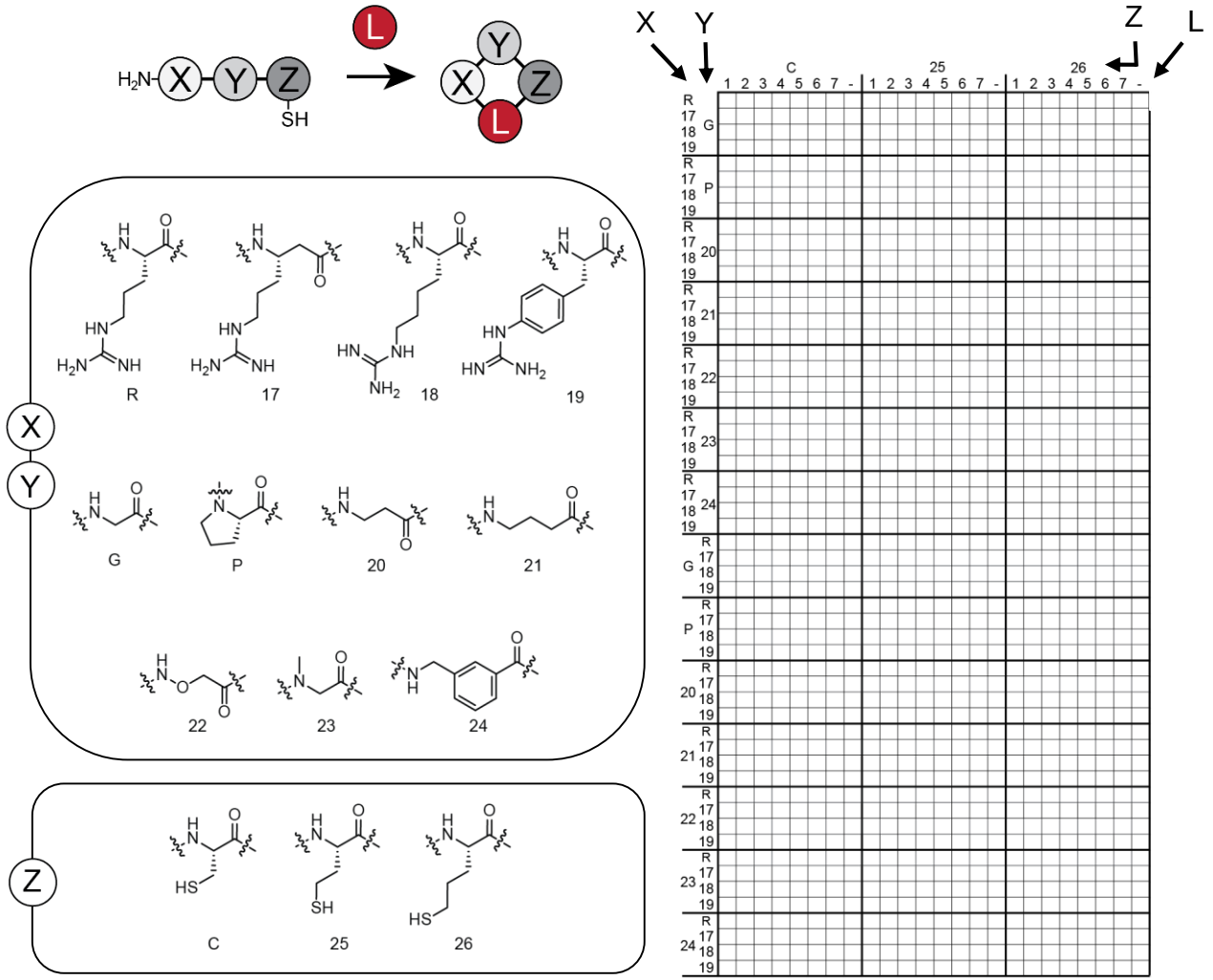


Pilot-scale library



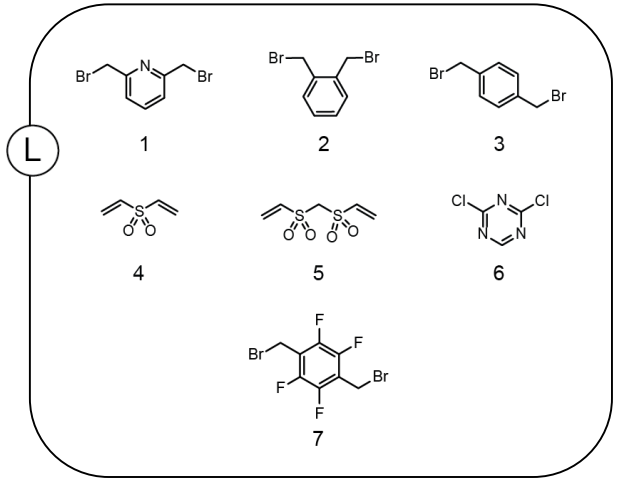
Solid-phase peptide synthesis of 168 peptides

Pilot-scale library

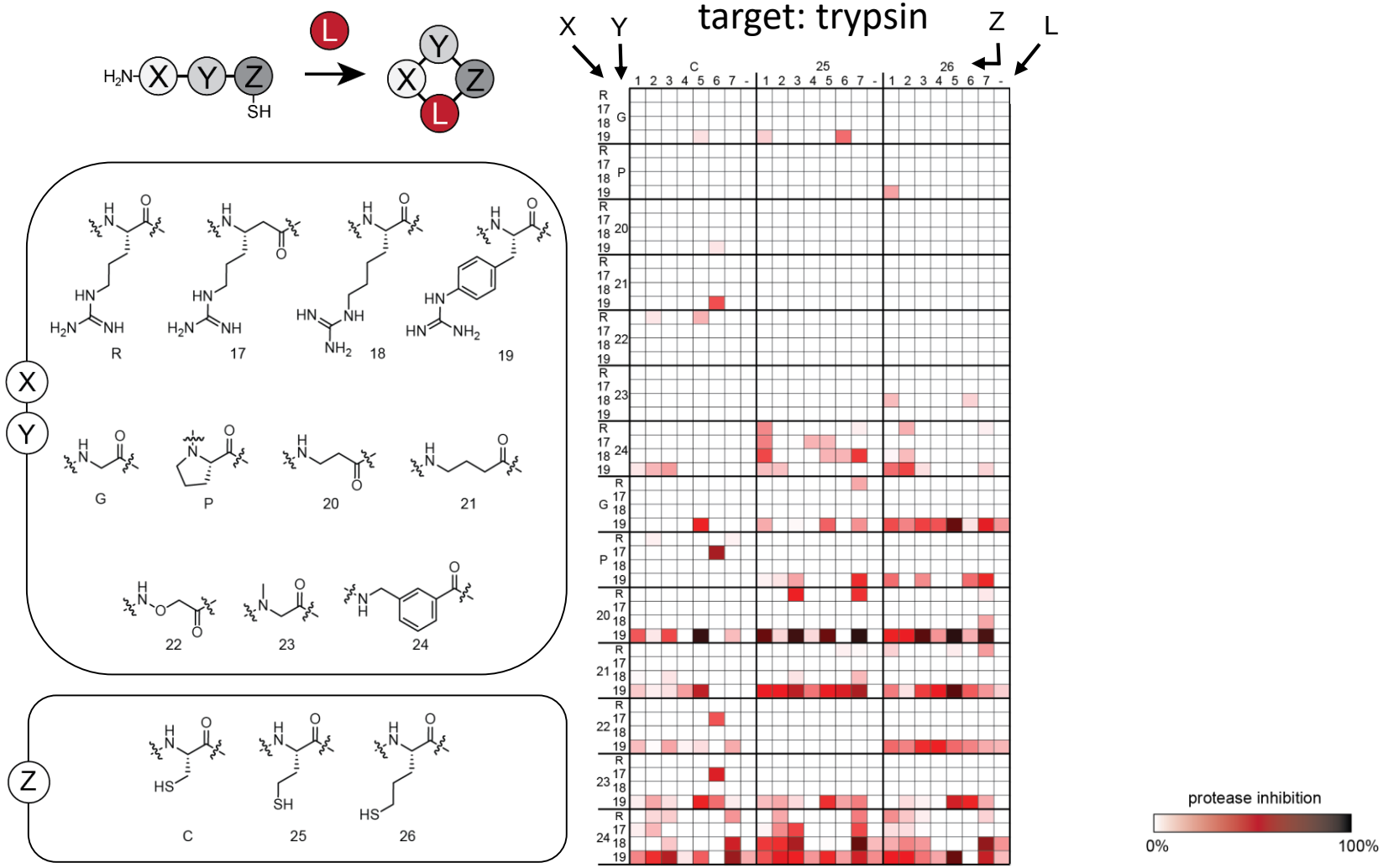


168 peptides × 7 linkers

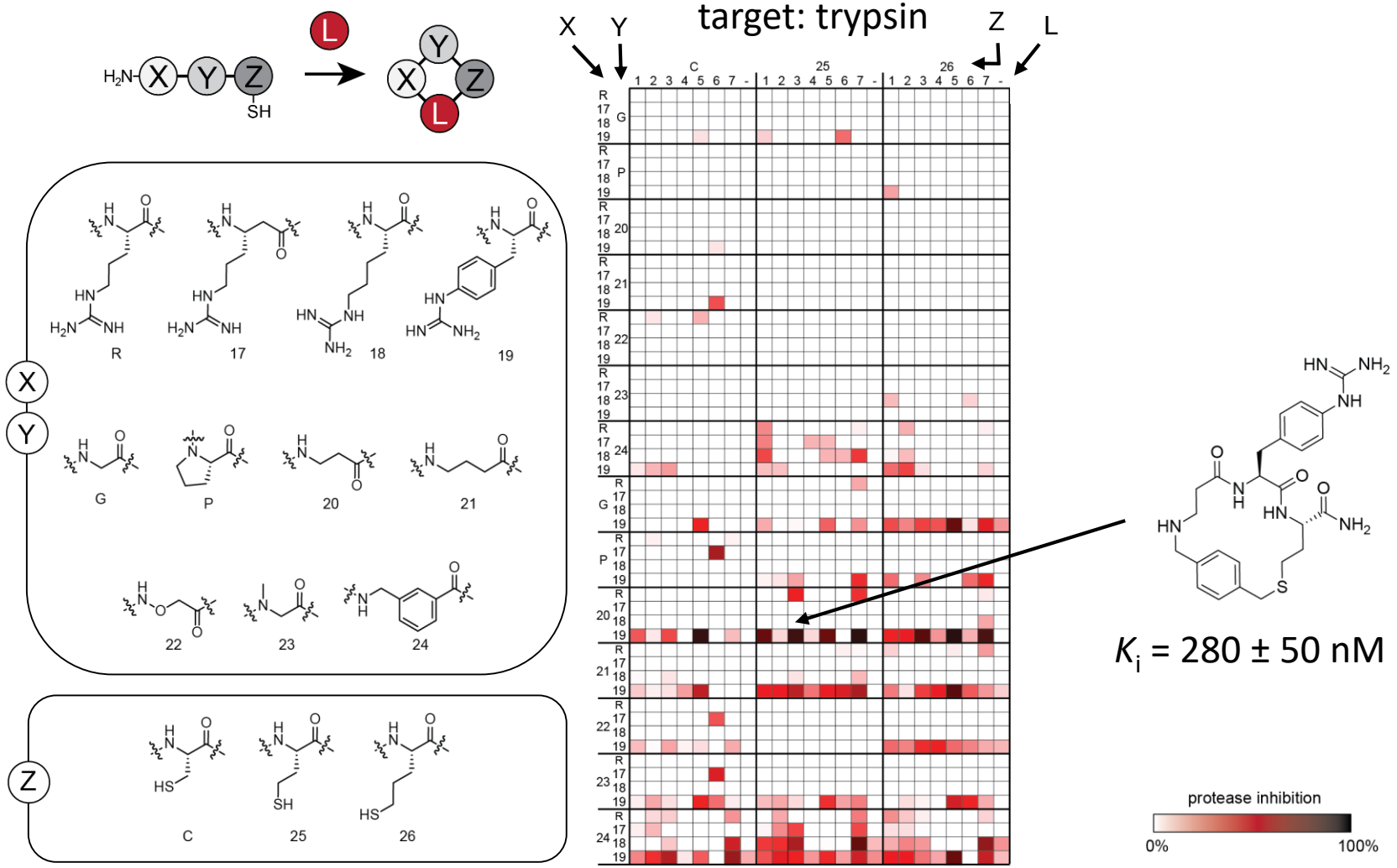
= 1176 macrocycles



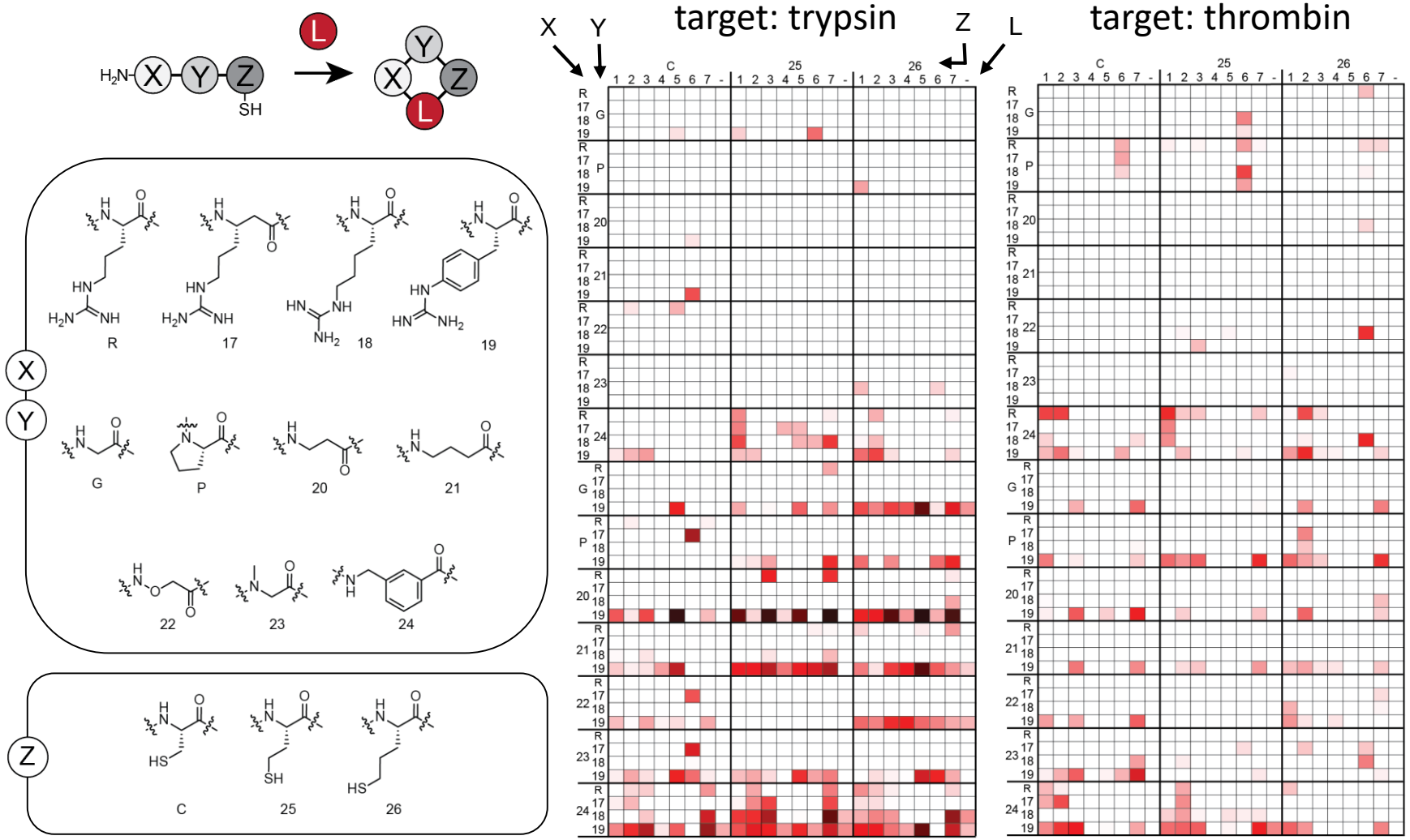
Pilot-scale library



Pilot-scale library

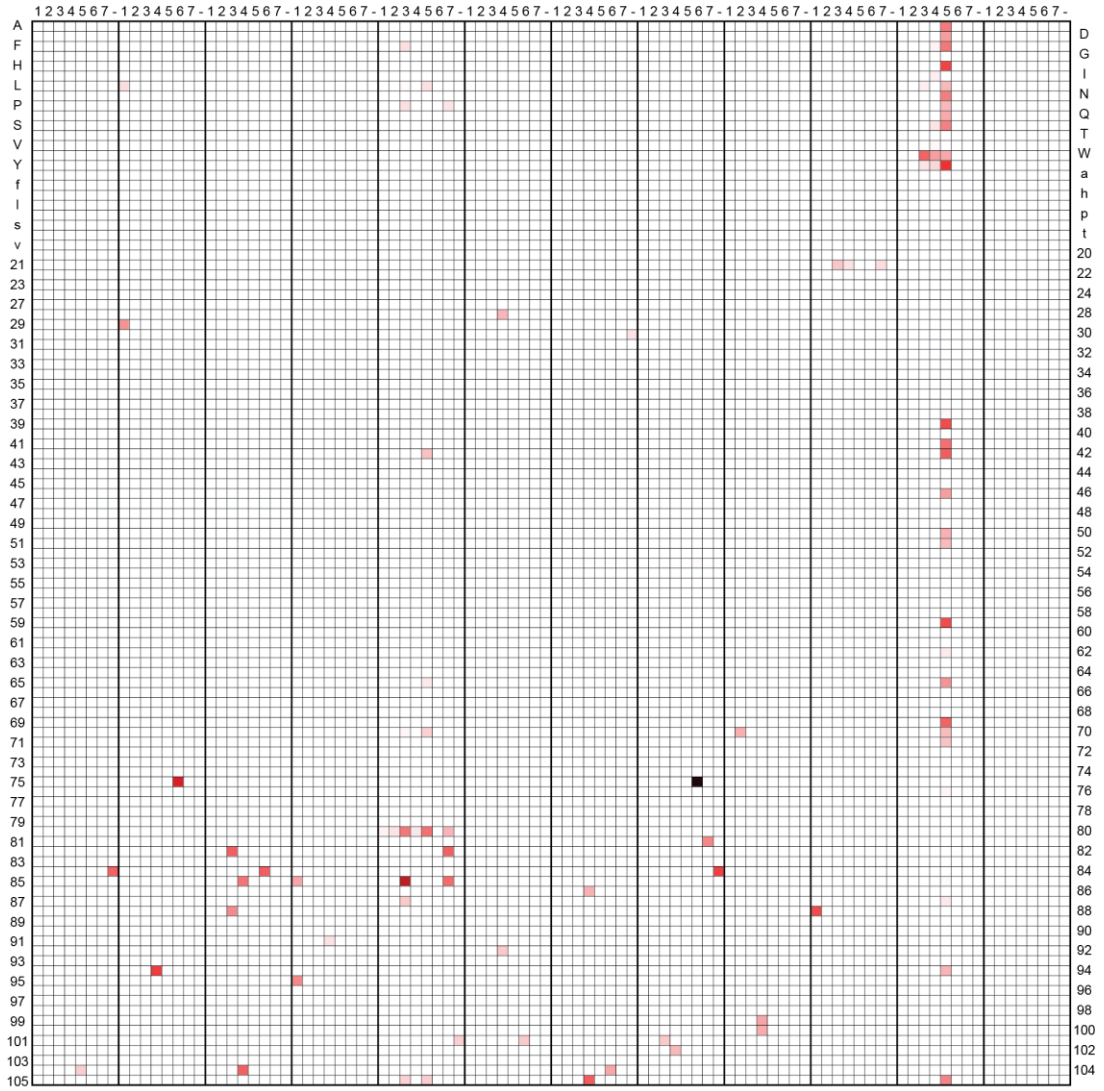


Pilot-scale library



8,988 macrocycle library

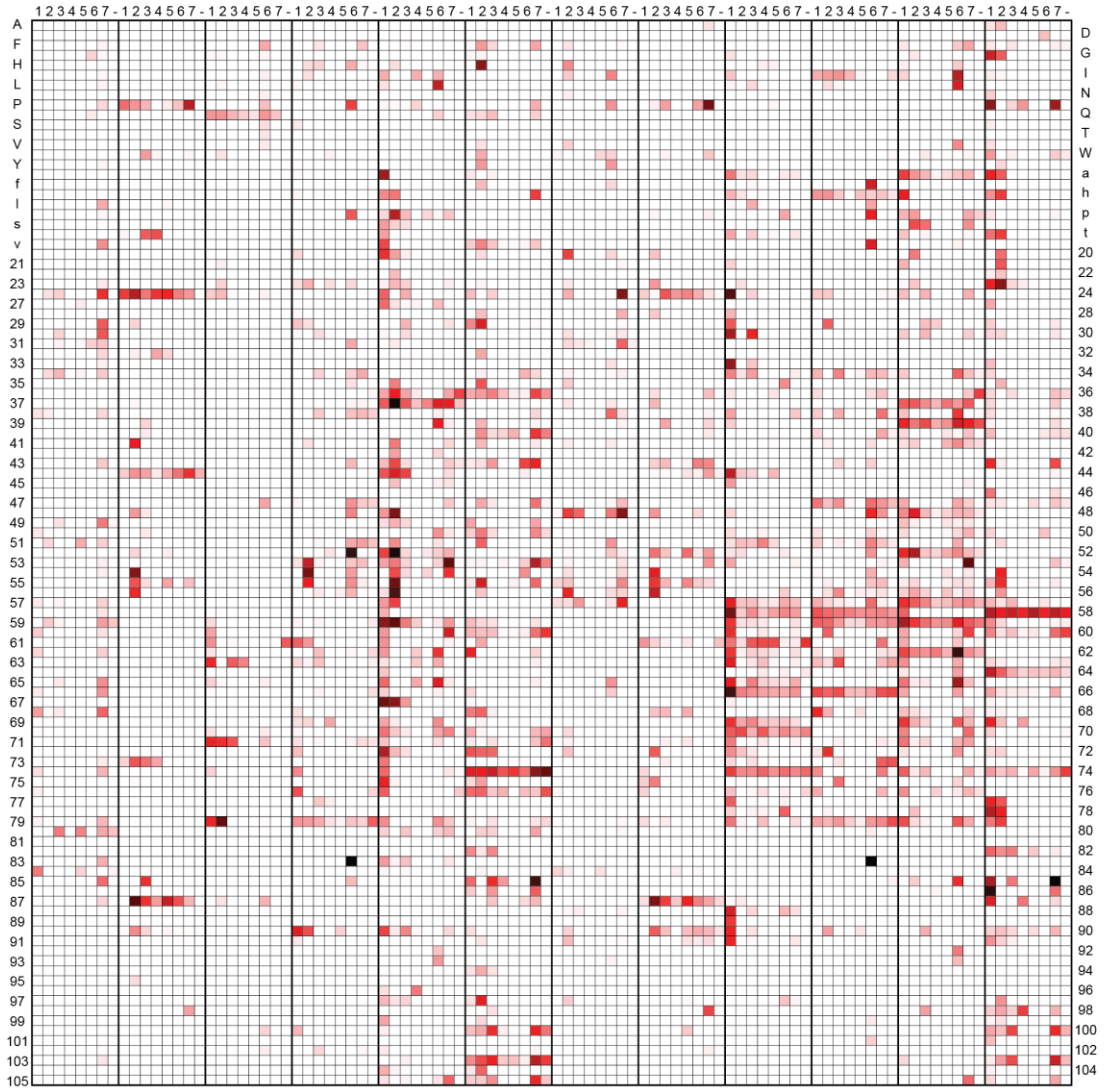
tissue kallikrein 5
inhibition



1,284 peptides × 7 linkers

8,988 macrocycle library

thrombin inhibition

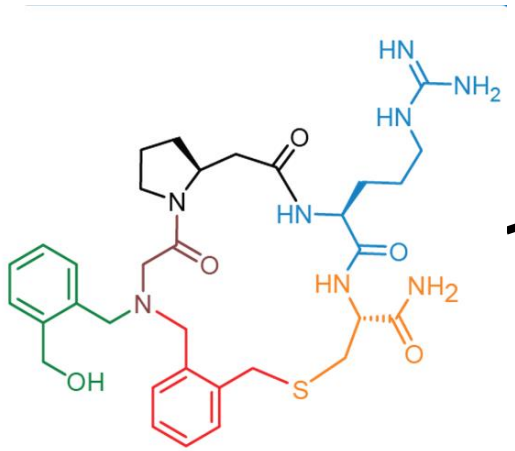


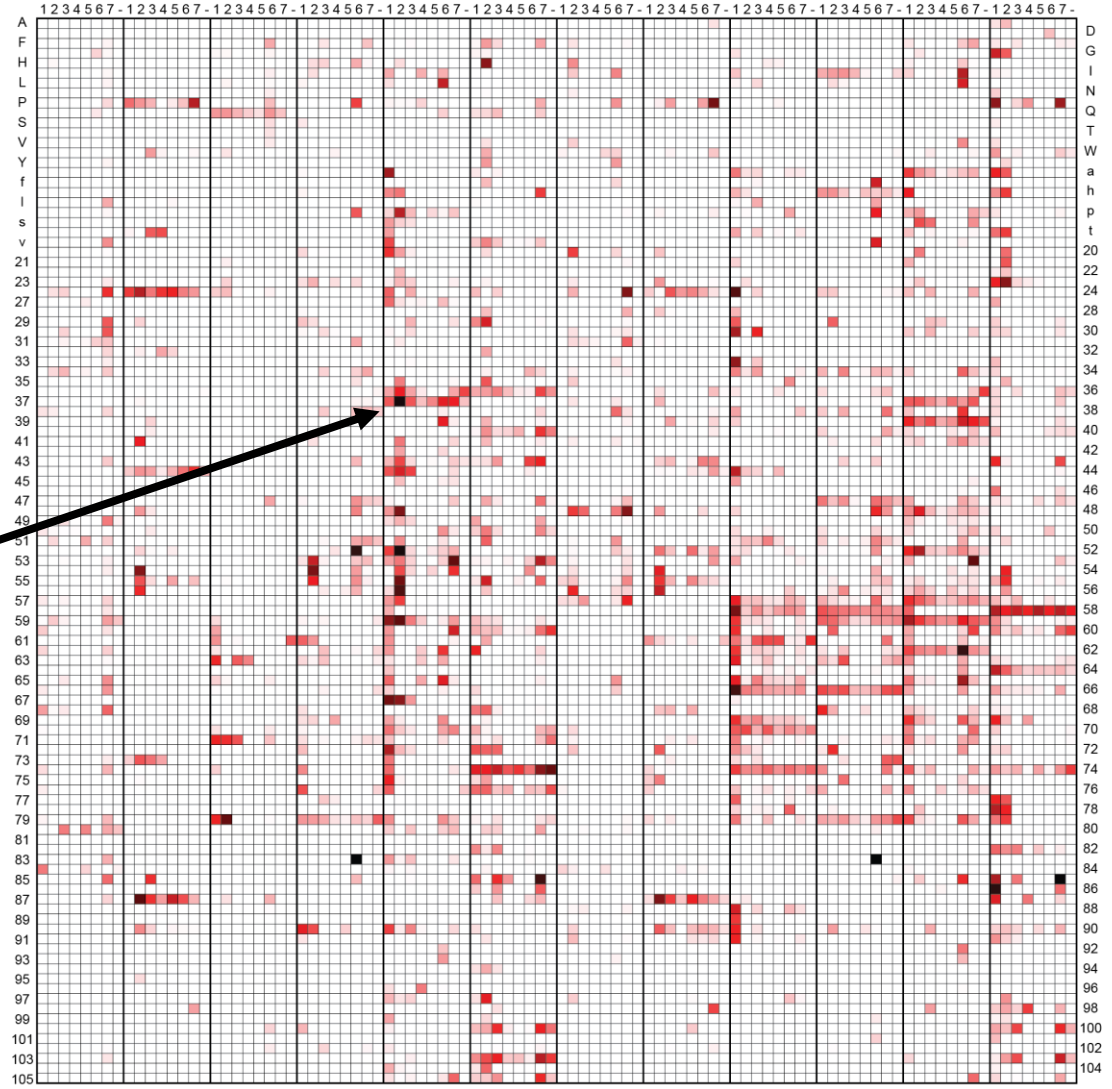
1,284 peptides × 7 linkers

8,988 macrocycle library

thrombin inhibition

0% 100%

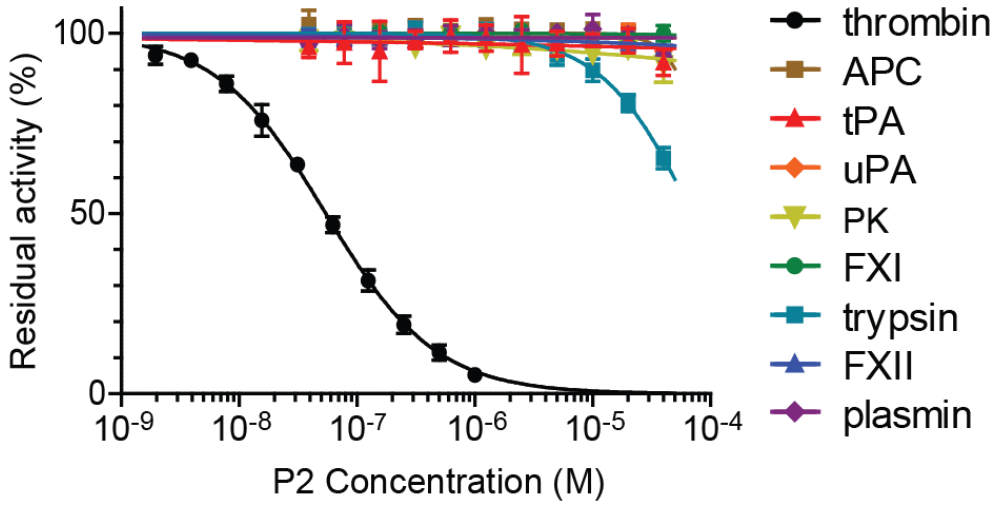




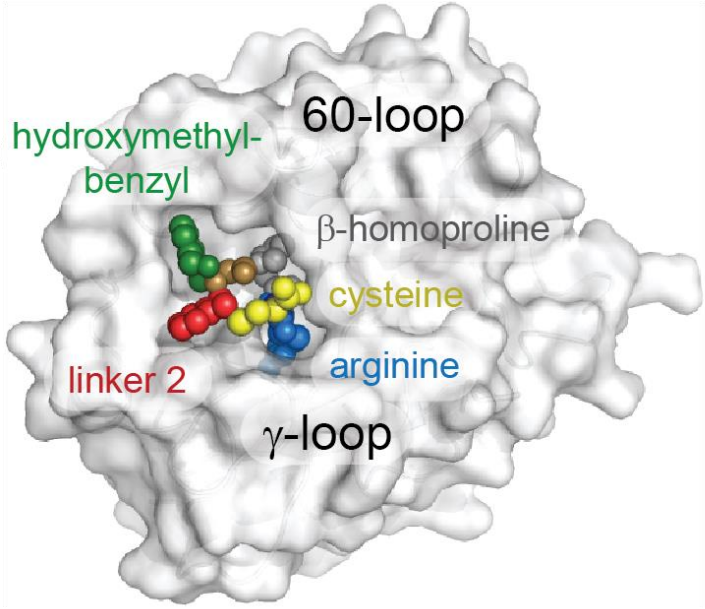
1,284 peptides × 7 linkers

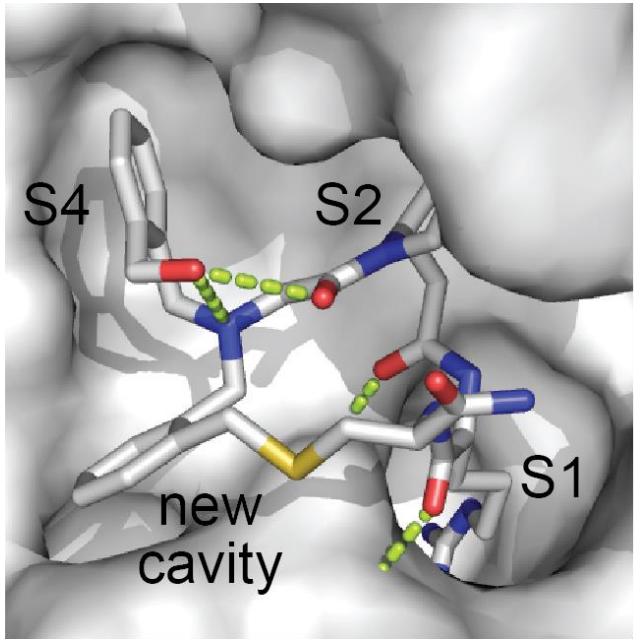
Thrombin inhibitor: specificity

K_i = 42 nM



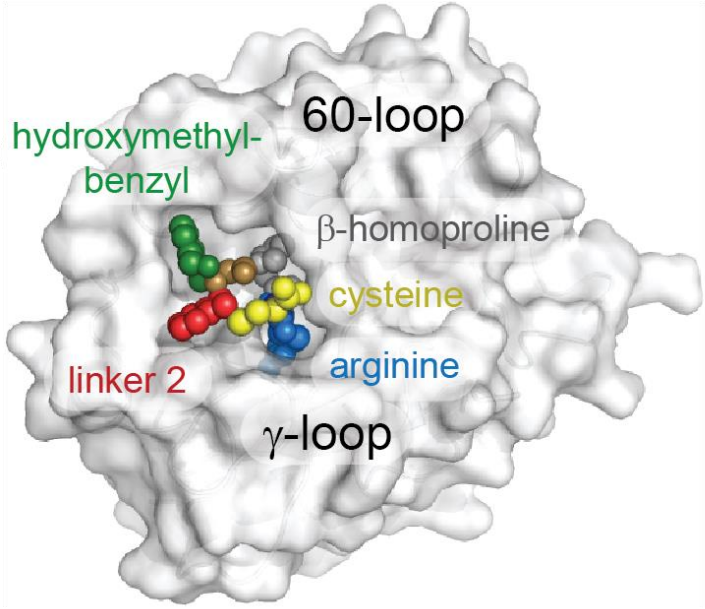
Thrombin inhibitor: structure

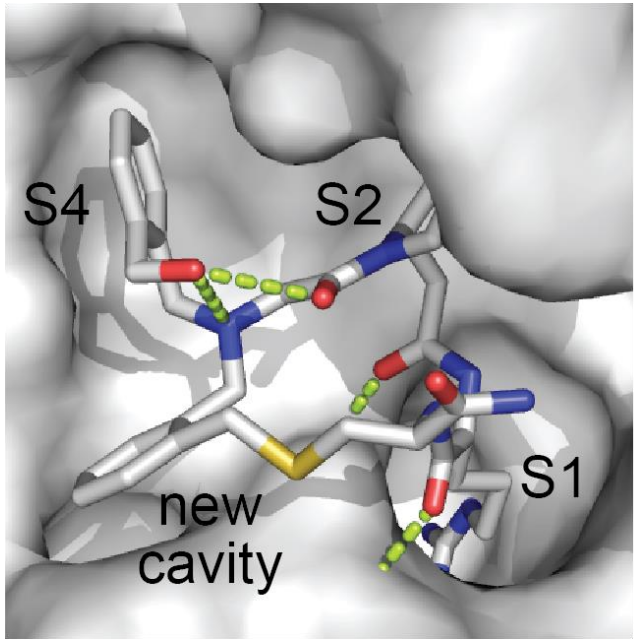


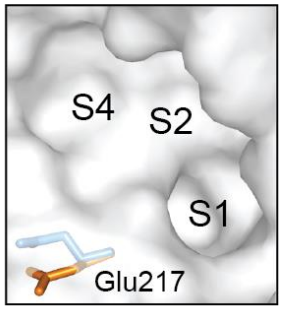


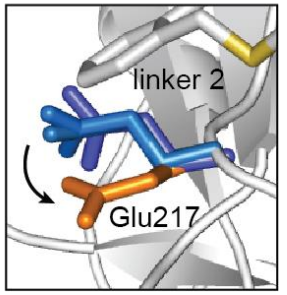
Structure solved by
Prof. Alessandro Angelini (Ca' Foscari University of Venice) and Prof. Laura Cendron (University of Padova)

Thrombin inhibitor: structure



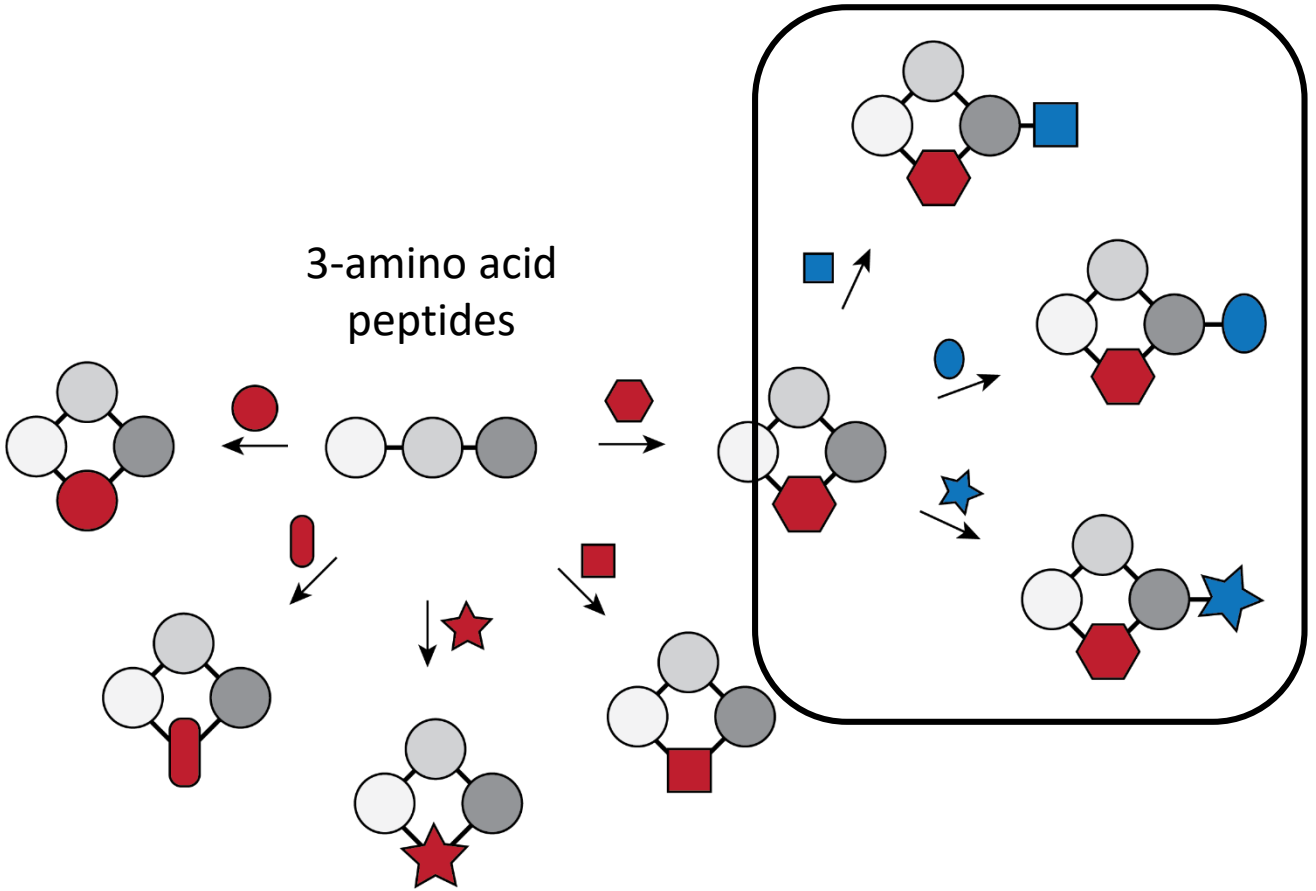






Kale, S.S., Bergeron-Brlek, M., Wu, Y., Kumar, M.G., Pham, M.V., Bortoli, J., Vesin, J., Kong, X.-D., Franco Machado, J., Deyle, K., Gonschorek, P., Turcatti, G., Cendron, L., Angelini, A. and Heinis, C., *Science Advances*, 2019

Combinatorial synthesis of peptide-based macrocycles



Number example:

1,000 peptides

x

10 linkers

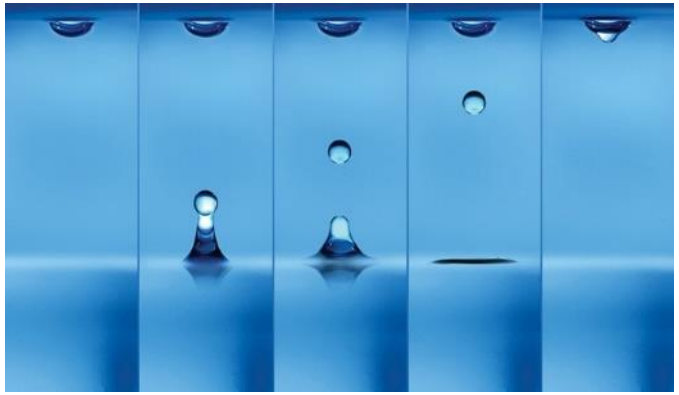
x

10 building blocks

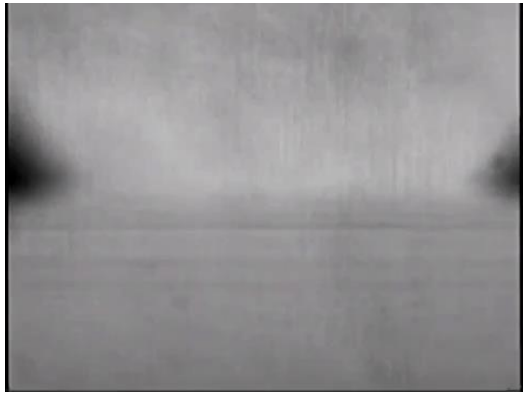
= 100,000 macrocycles

Macrocycles at a nanomole scale

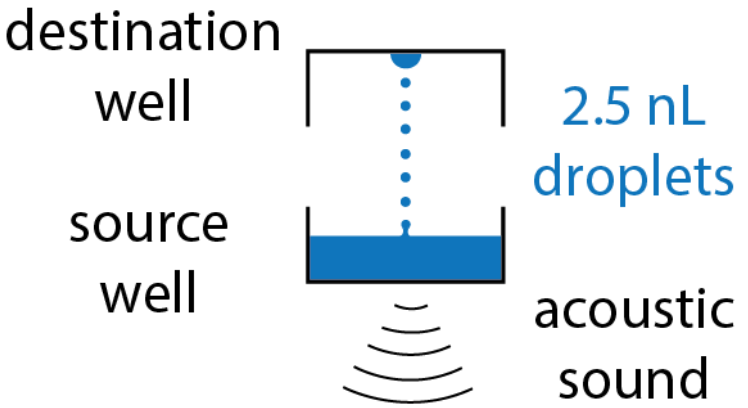
Acoustic droplet ejection (ADE)



Acoustic dispensing
(image from C&EN)



Acoustic dispensing
(film from Labcyte)



Macrocycles at a nanomole scale

Acoustic droplet ejection (ADE)

Our acoustic dispensing platform

1536 microwell plates

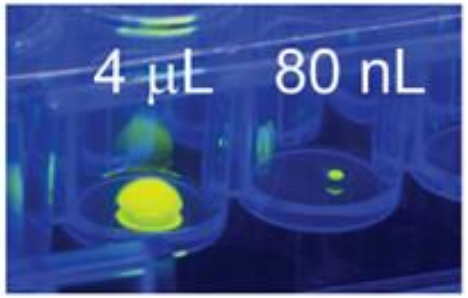


Photo of droplets (fluorescein)

Macrocycles at a nanomole scale

Acoustic droplet ejection (ADE)

Our acoustic dispensing platform

1536 microwell plates

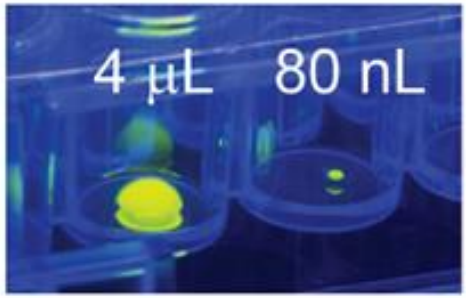
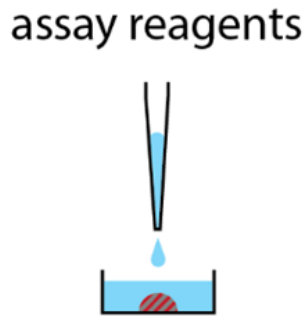
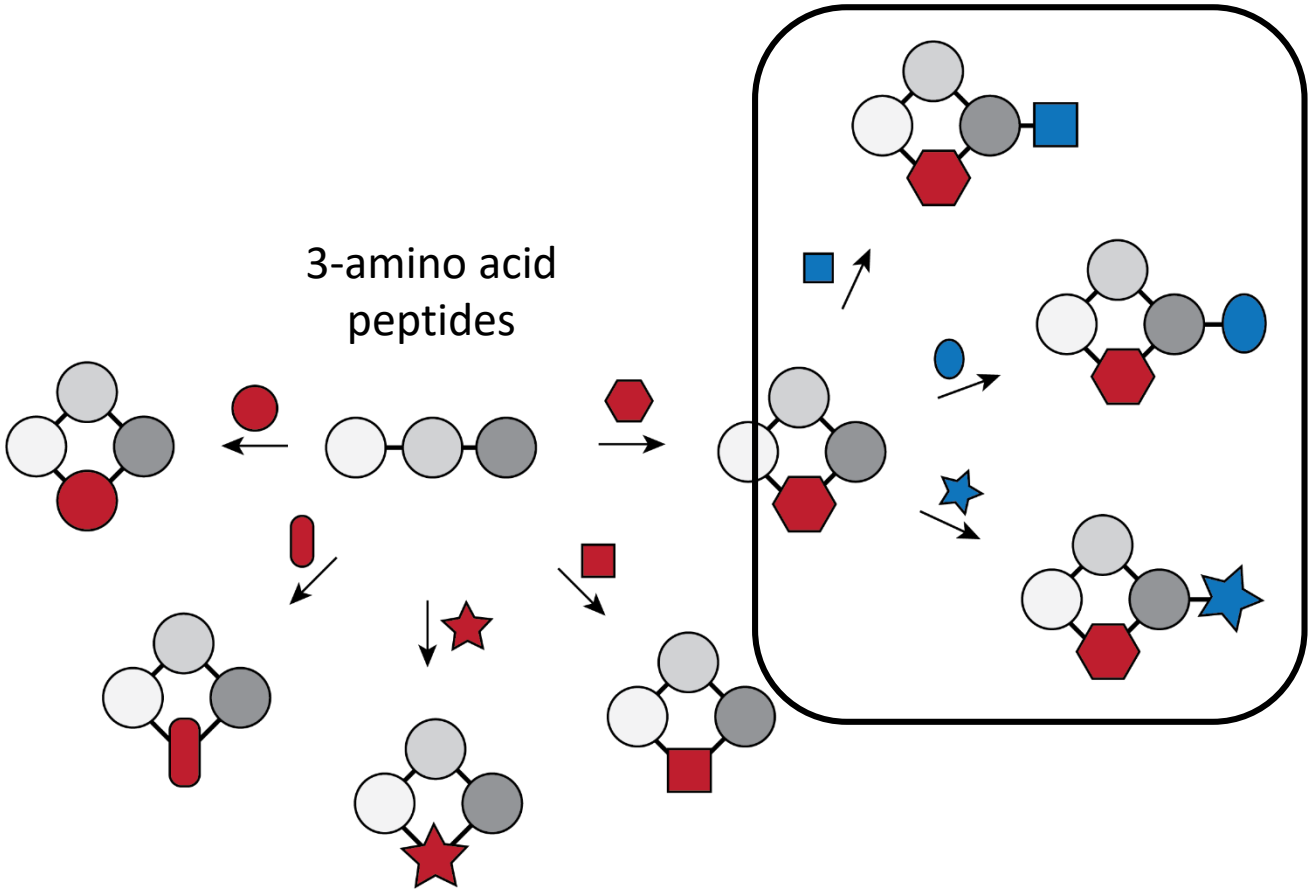


Photo of droplets (fluorescein)

synthesis and assay
in the same microwell plate



Combinatorial synthesis of peptide-based macrocycles



Number example:

1,000 peptides

x

10 linkers

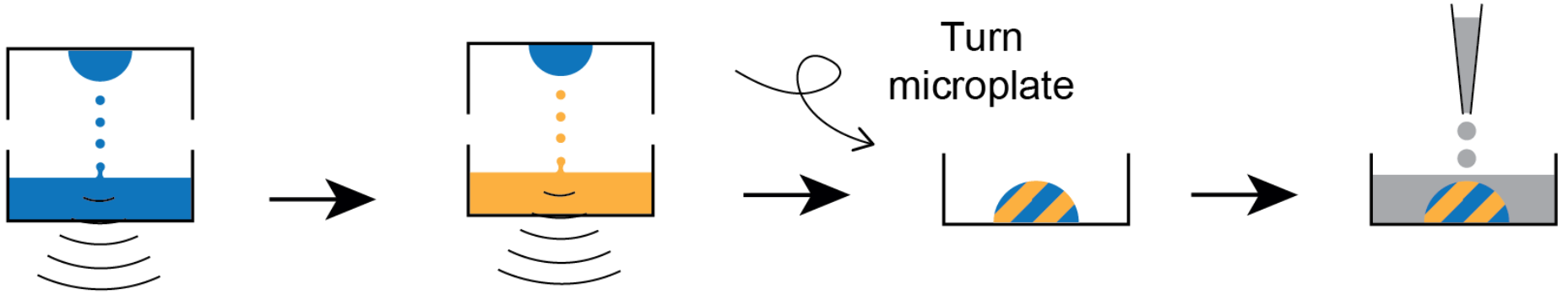
x

10 building blocks

= 100,000 macrocycles

Acylation of amino groups

Synthesis of macrocycles at a nanomole scale



20 nL
Scaffold (8 mM)
DABCO (150 mM)
in DMSO

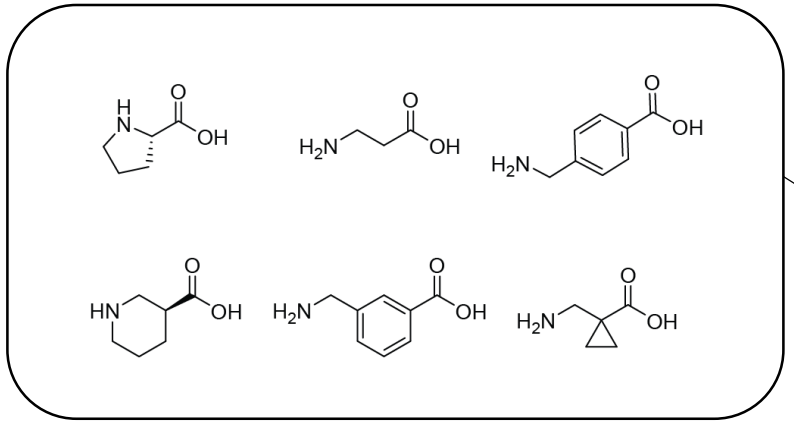
20 nL
Carboxylic acid (80 mM)
HBTU (80 mM)
DABCO (80 mM)
in DMSO

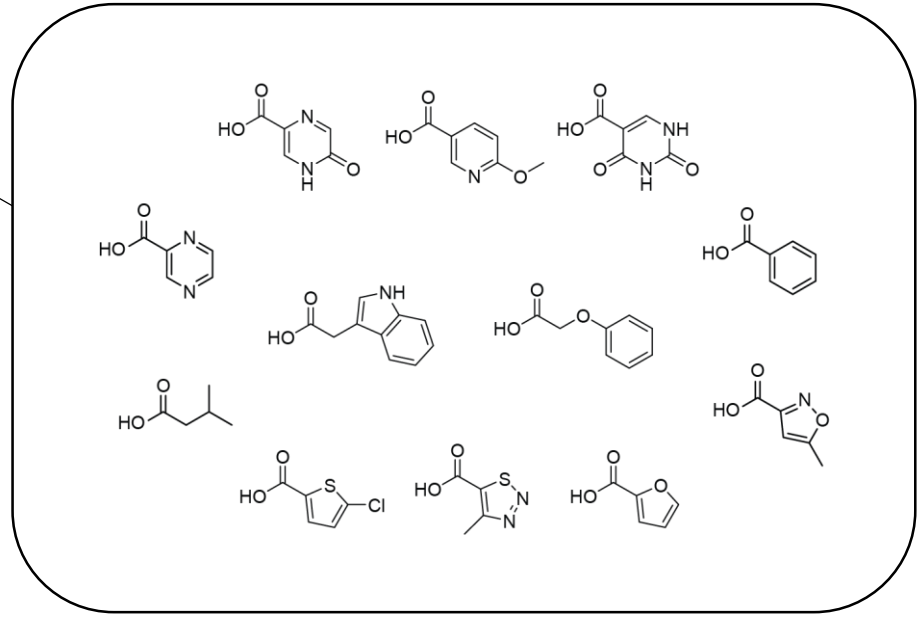
1. Acylation reaction
at RT, 5 hrs
2. Quenching
with Tris

Addition of
thrombin (target)
and thrombin
substrate

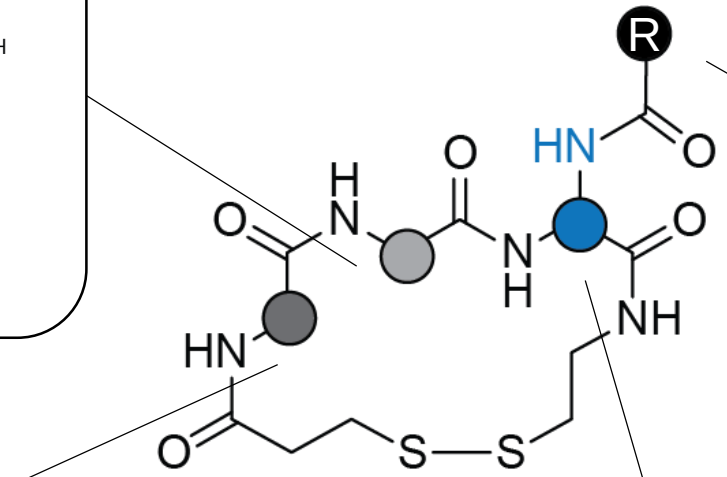
Synthesis of 3,840 macrocycles

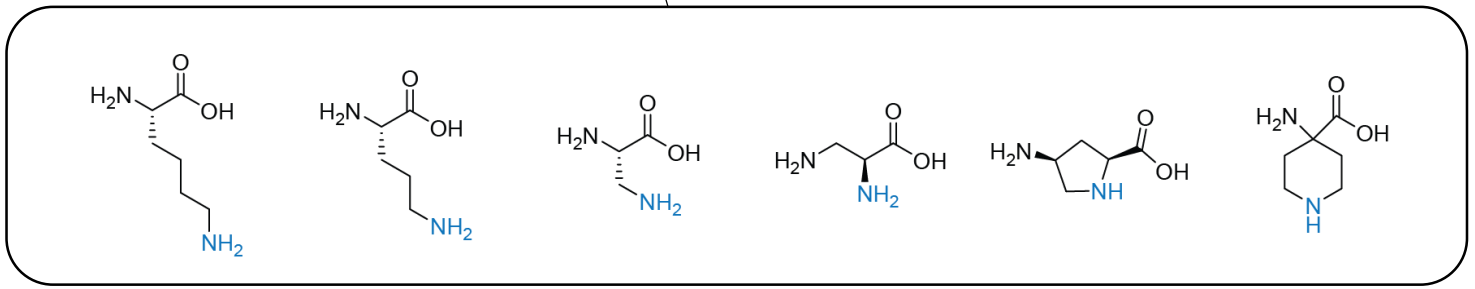
384 scaffolds × 12 carboxylic acids



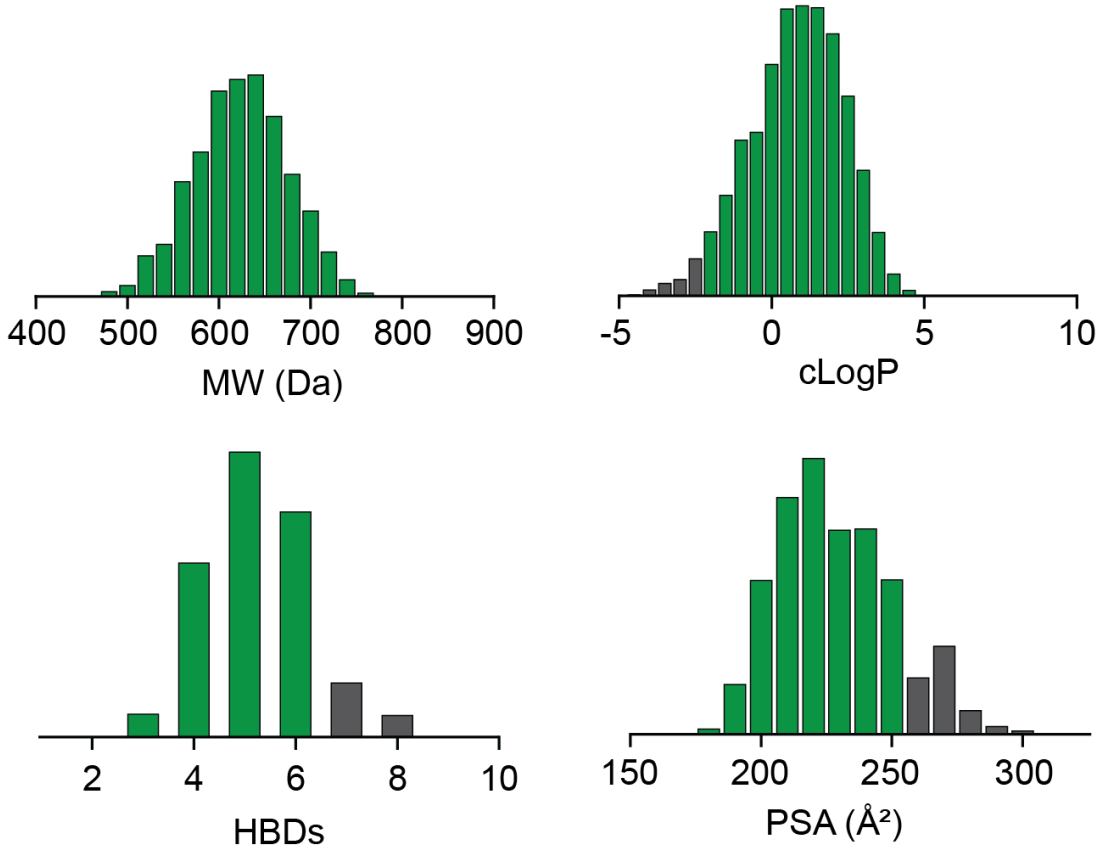


F, H, I, L, Q,
S, V, W, Y,
a, f, s, v, w, y

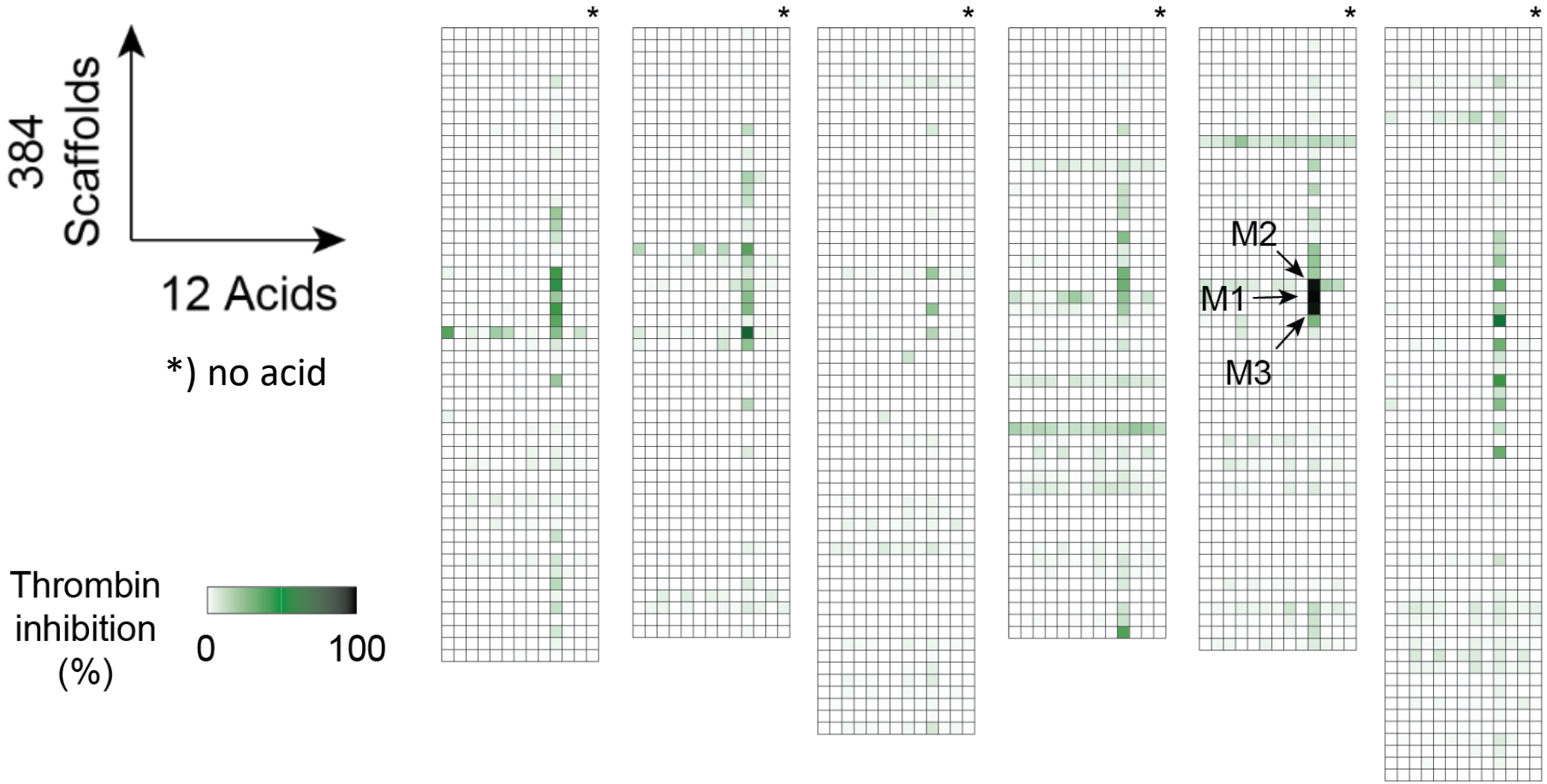




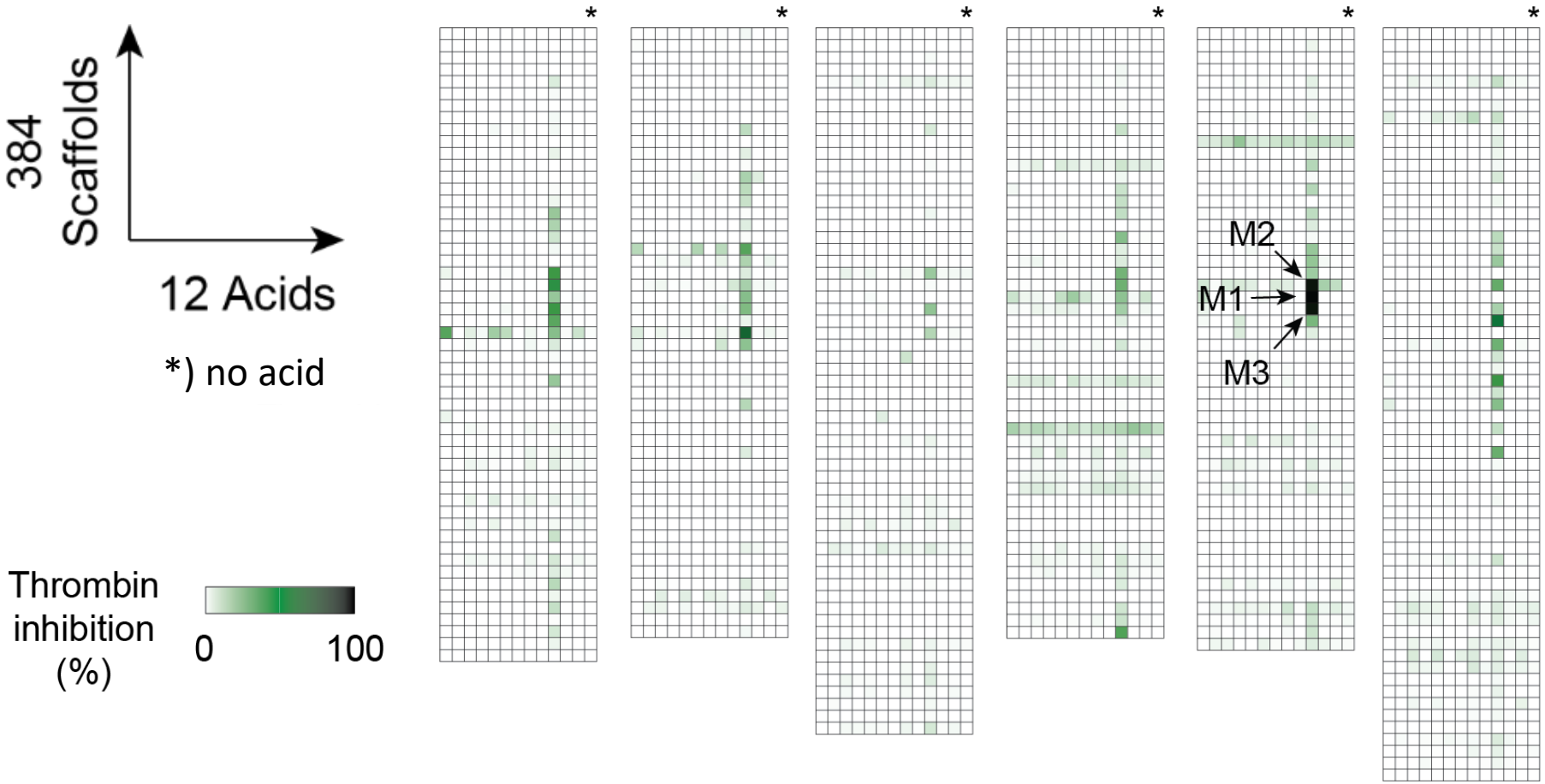
Synthesis of 3,840 macrocycles tailored for thrombin

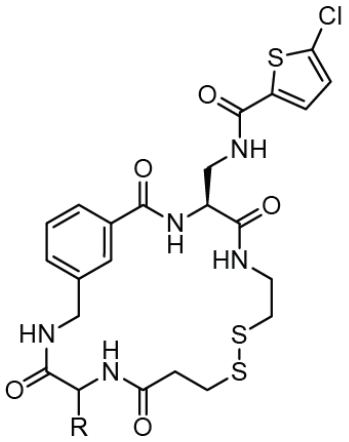


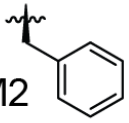
Thrombin inhibition screen



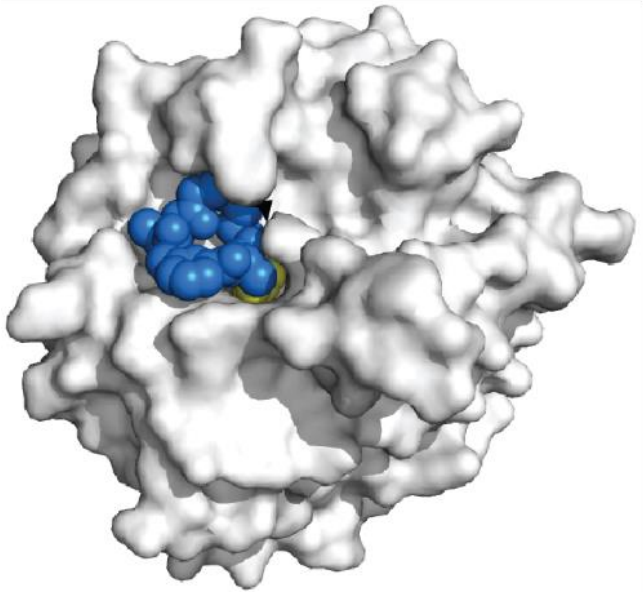
Thrombin inhibition screen

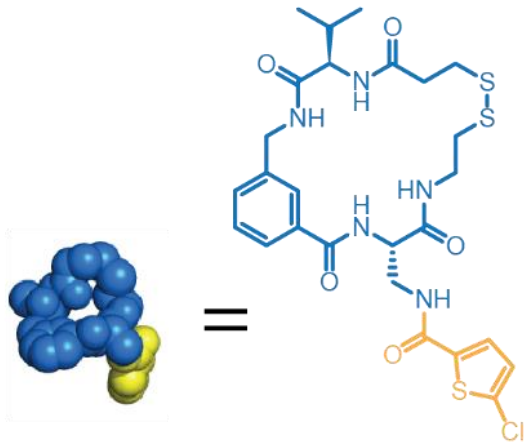


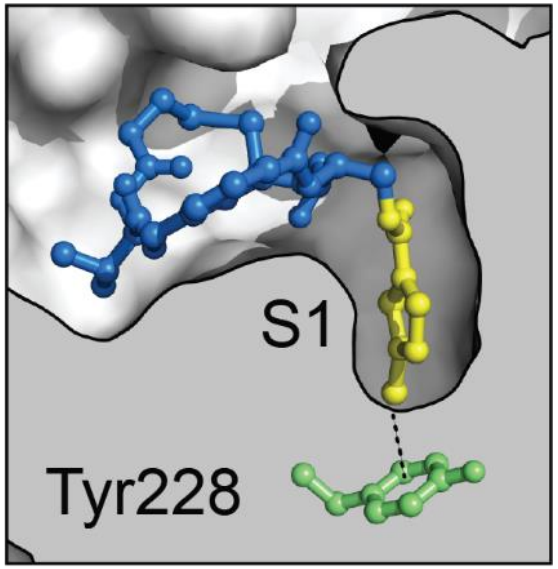


R =	K_i (nM)
M1 	44 ± 1
M2 	165 ± 38
M3 	125 ± 8

X-ray structure

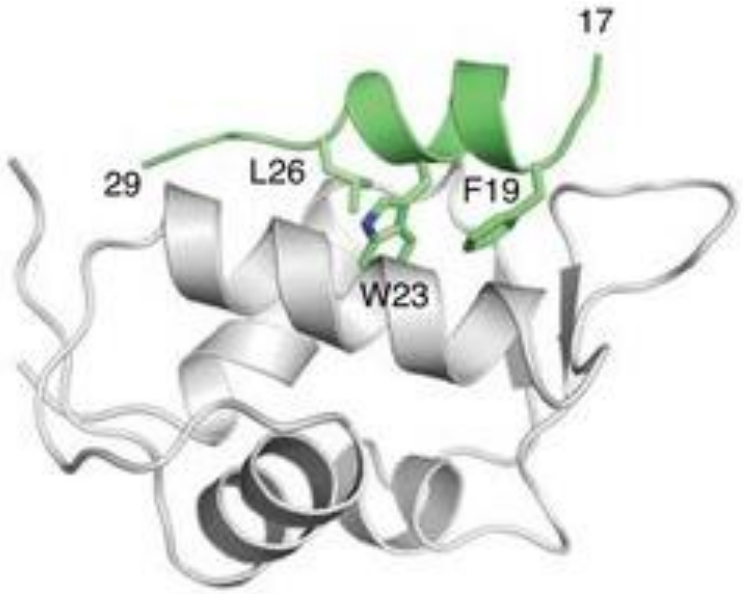






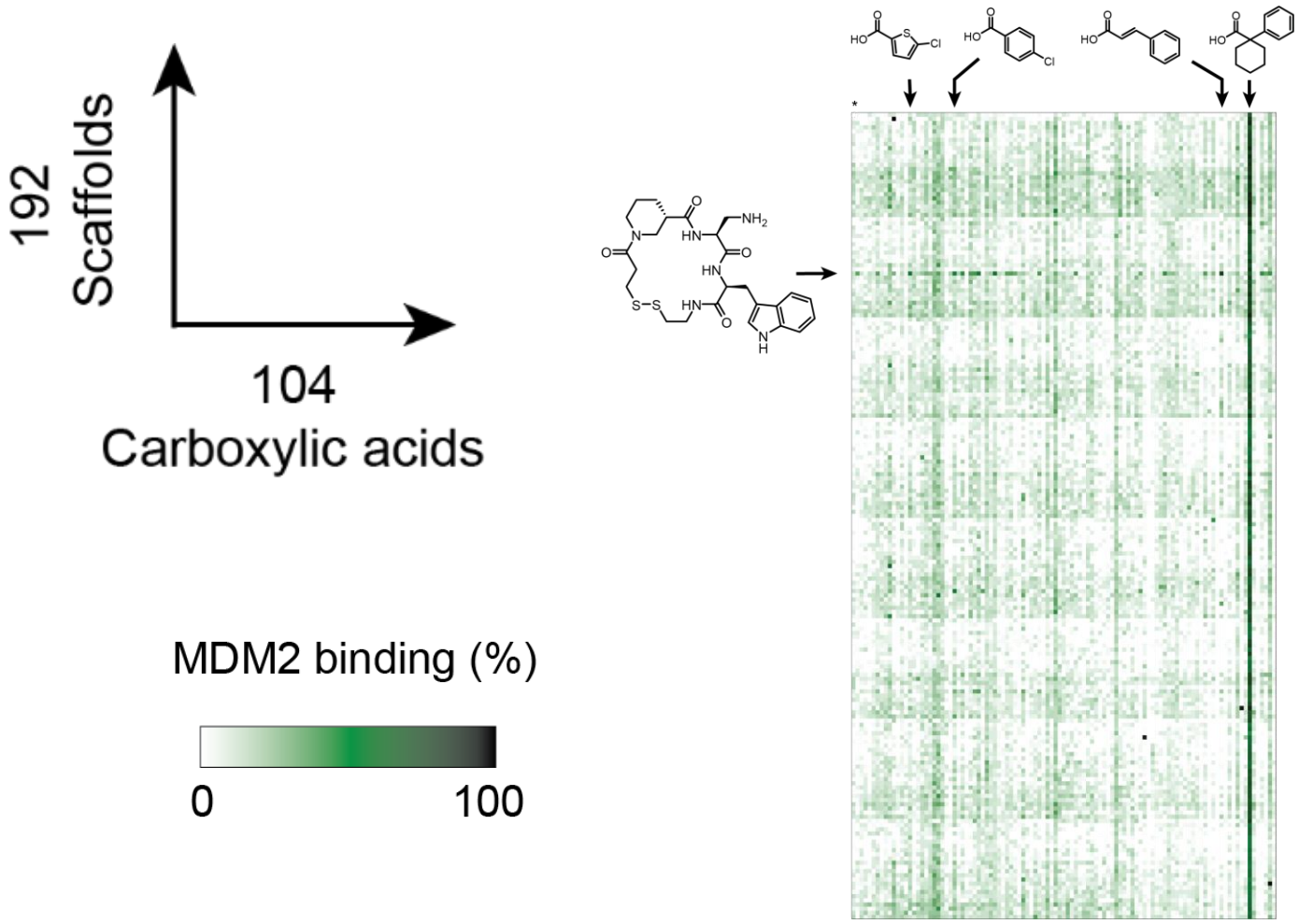
Structure solved by
Prof. Alessandro Angelini (Ca' Foscari University of Venice) and Prof. Laura Cendron (University of Padova)

Inhibition of protein-protein interactions (PPIs)

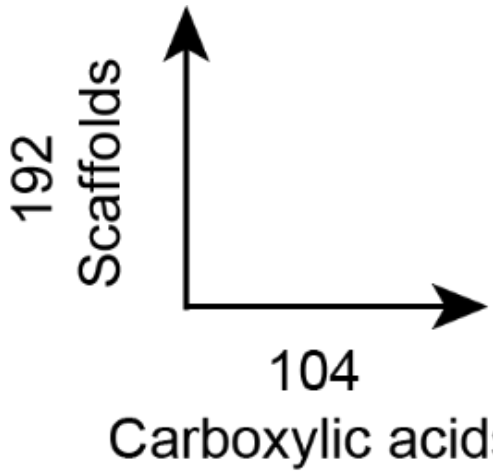


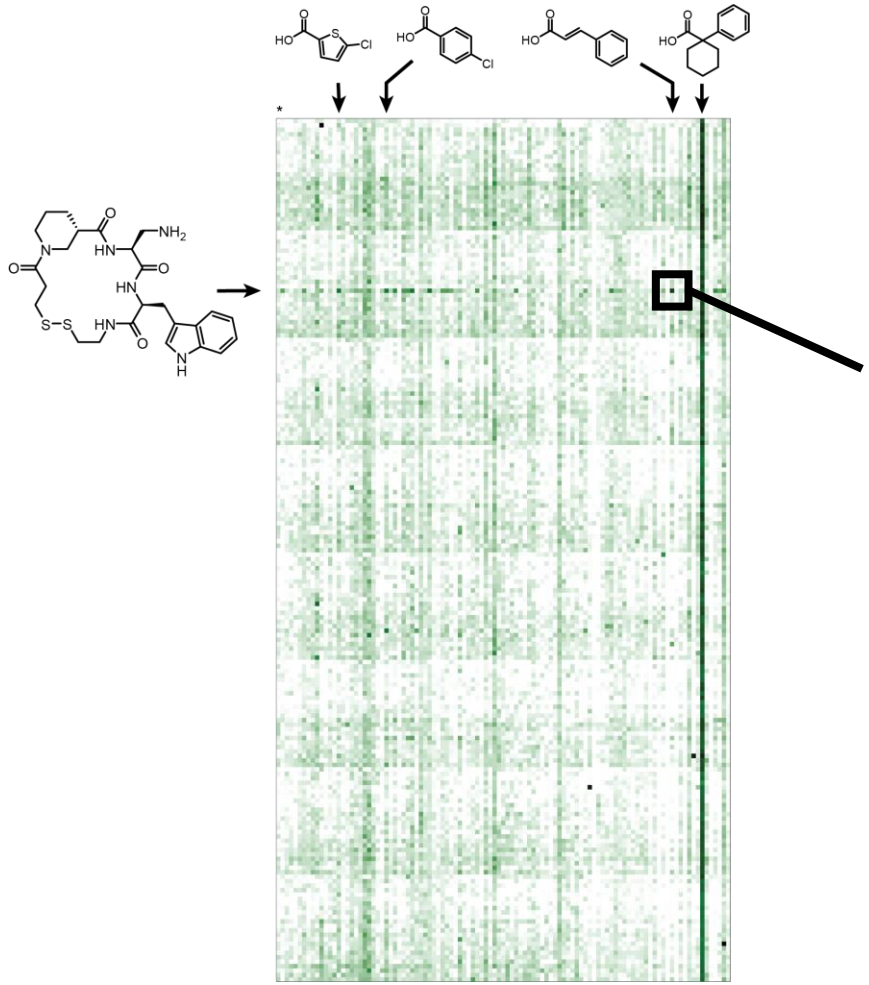
p53-MDM2

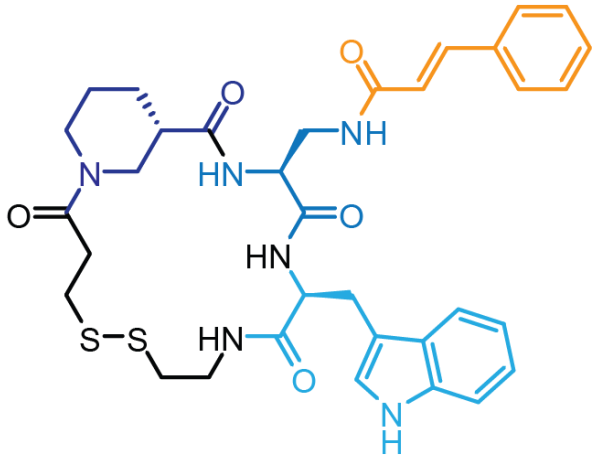
Synthesis of a 19,968 macrocycles tailored for MDM2/p53



Synthesis of a 19,968 macrocycles tailored for MDM2/p53



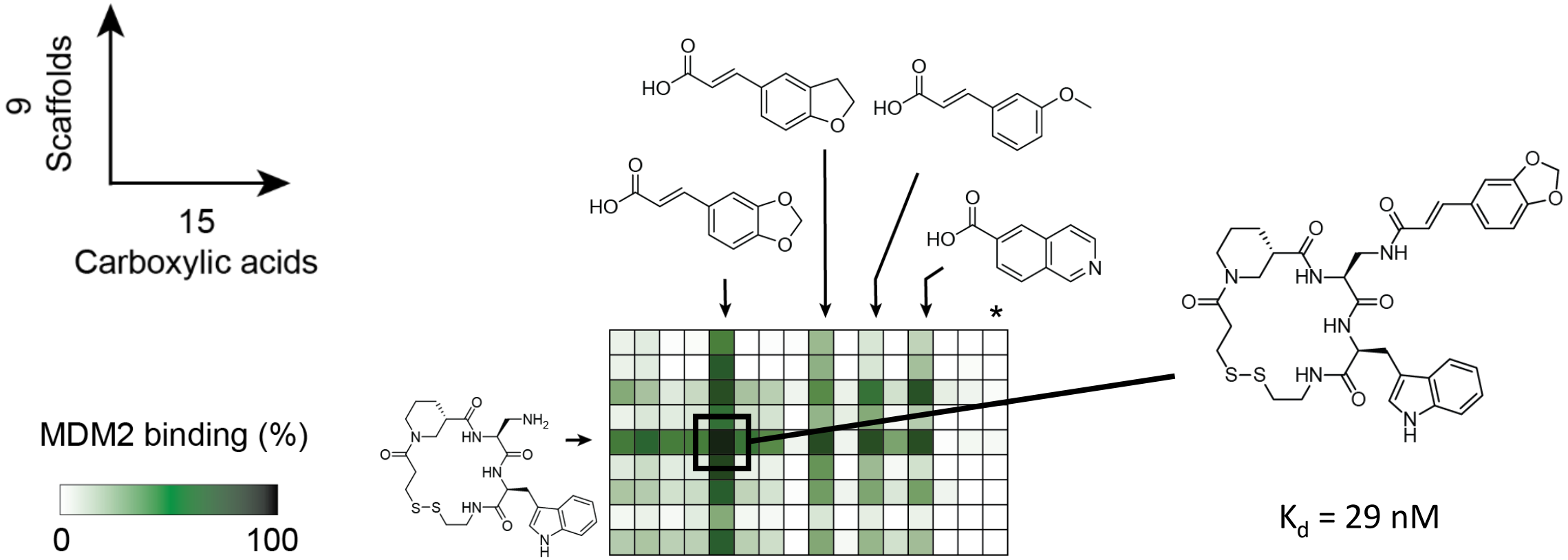




K_d = 370 nM

MDM2 binding (%)

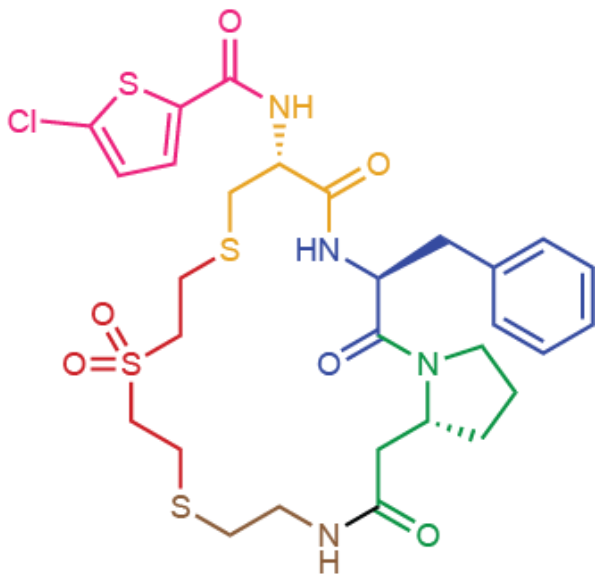
Rapid improvement of binding affinity



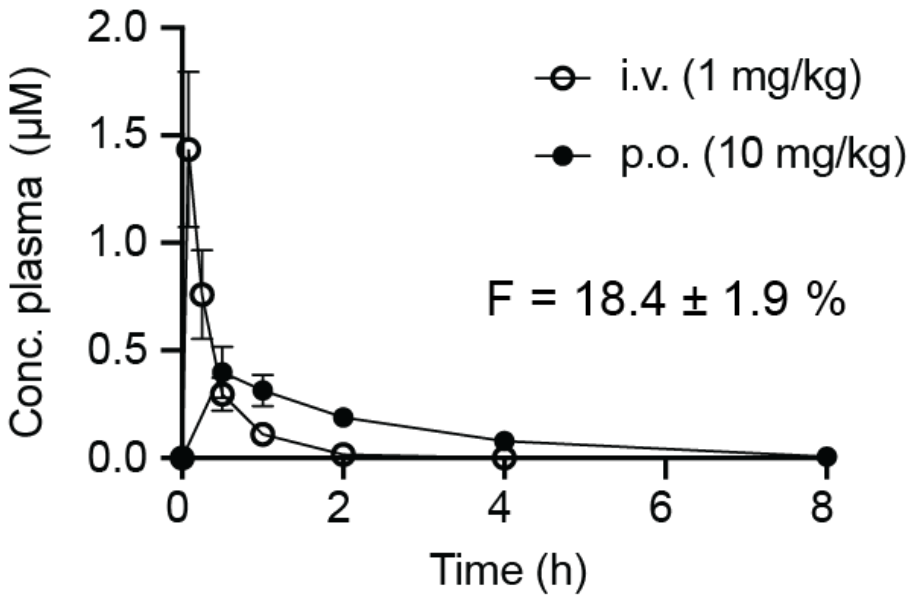
Habeshian, S., Merz, M.L., Mothukuri, G.K., Schüttel, M., Bognar, Z., Diaz-Perlas, C., Vesin, J., Bortoli Chapalay, J., Turcatti, G., Cendron, L., Angelini, A. and Heinis, C., *Nature Communications*, 2022

Are they membrane permeable / orally available?

Stable linkers

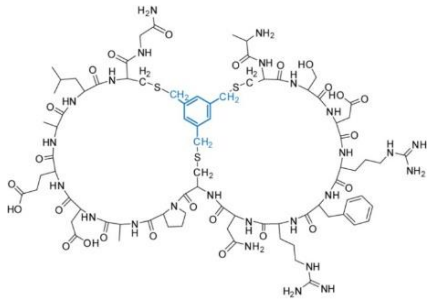


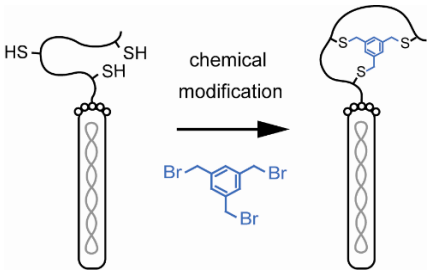
Oral availability (rat)



Summary

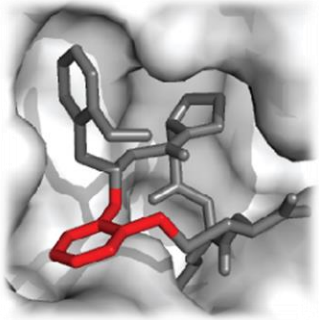
Bicyclic peptides & phage display





- High affinity, specificity
- Fast development
- Works with many targets
- Good for extracellular targets
- Limits: needs injection, short half-life

Macrocycles & screening



- High affinity, specificity
- Also intracellular targets
- Oral application
- Limits: harder to develop for challenging targets