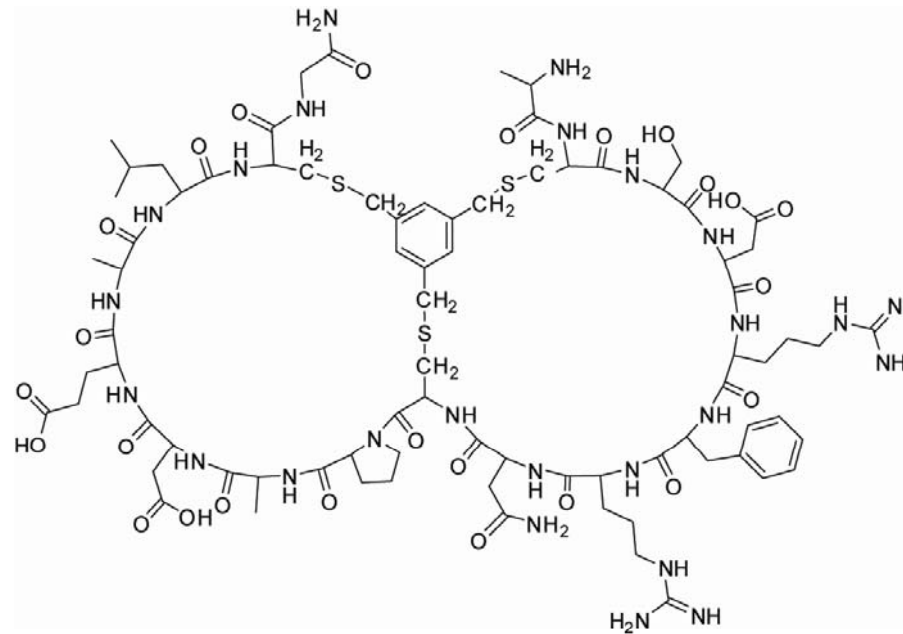
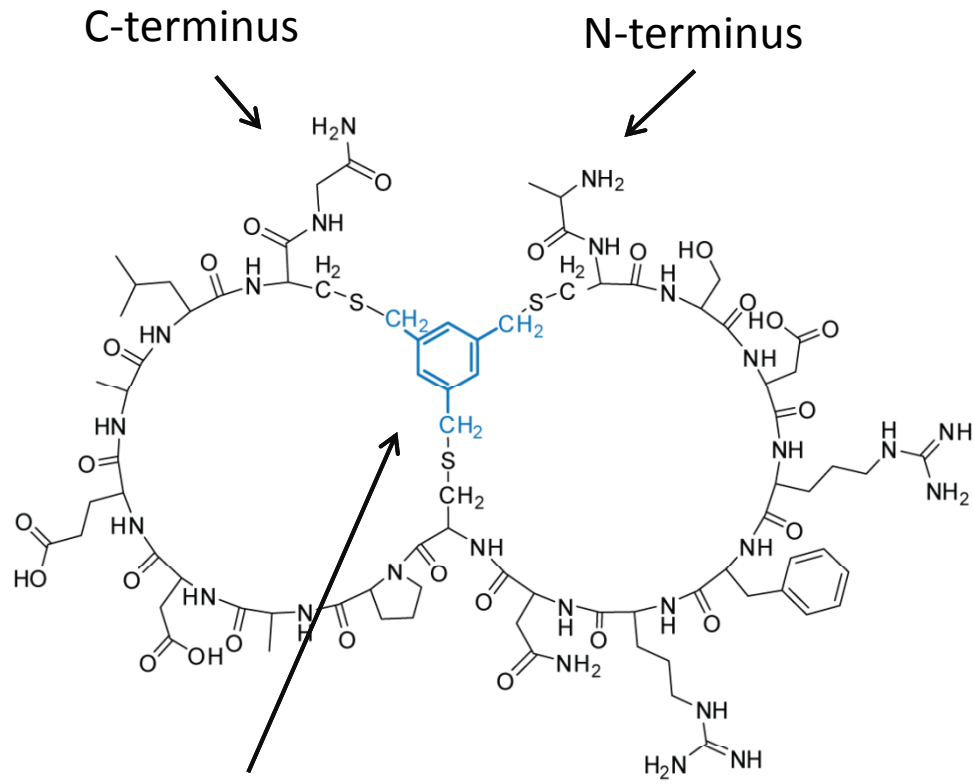


Phage-encoded combinatorial libraries of multi-cyclic peptides

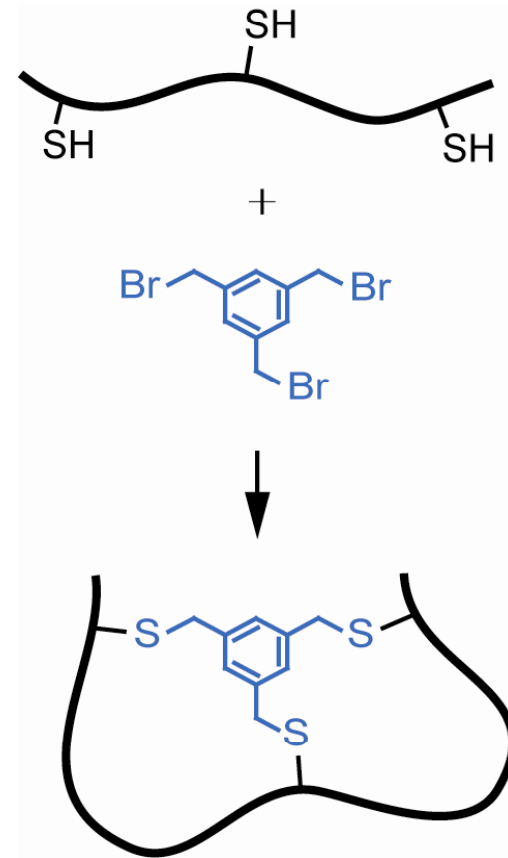
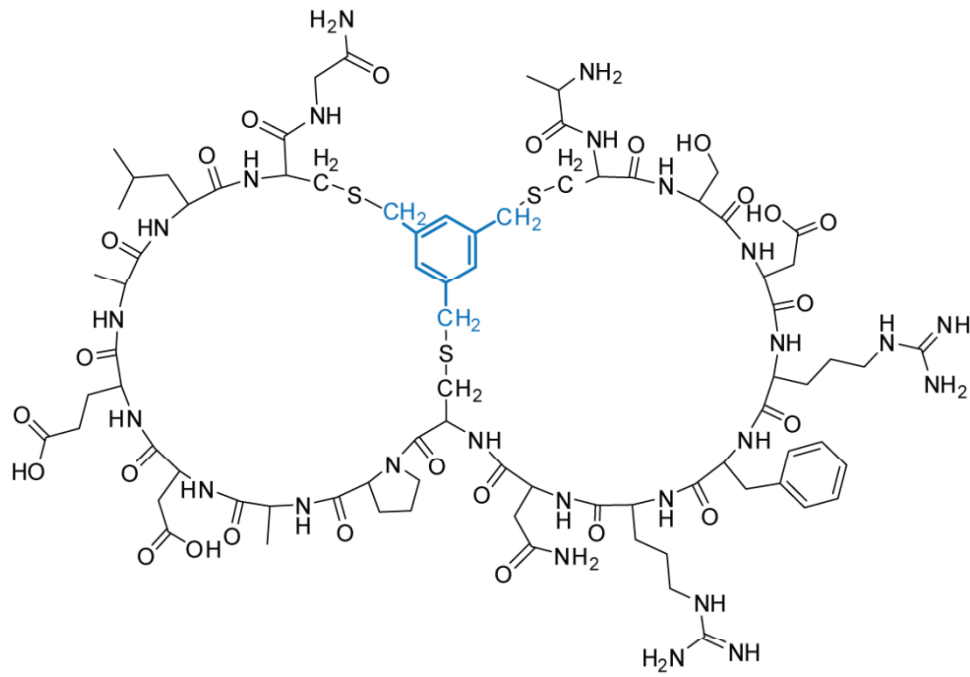


Bicyclic peptides



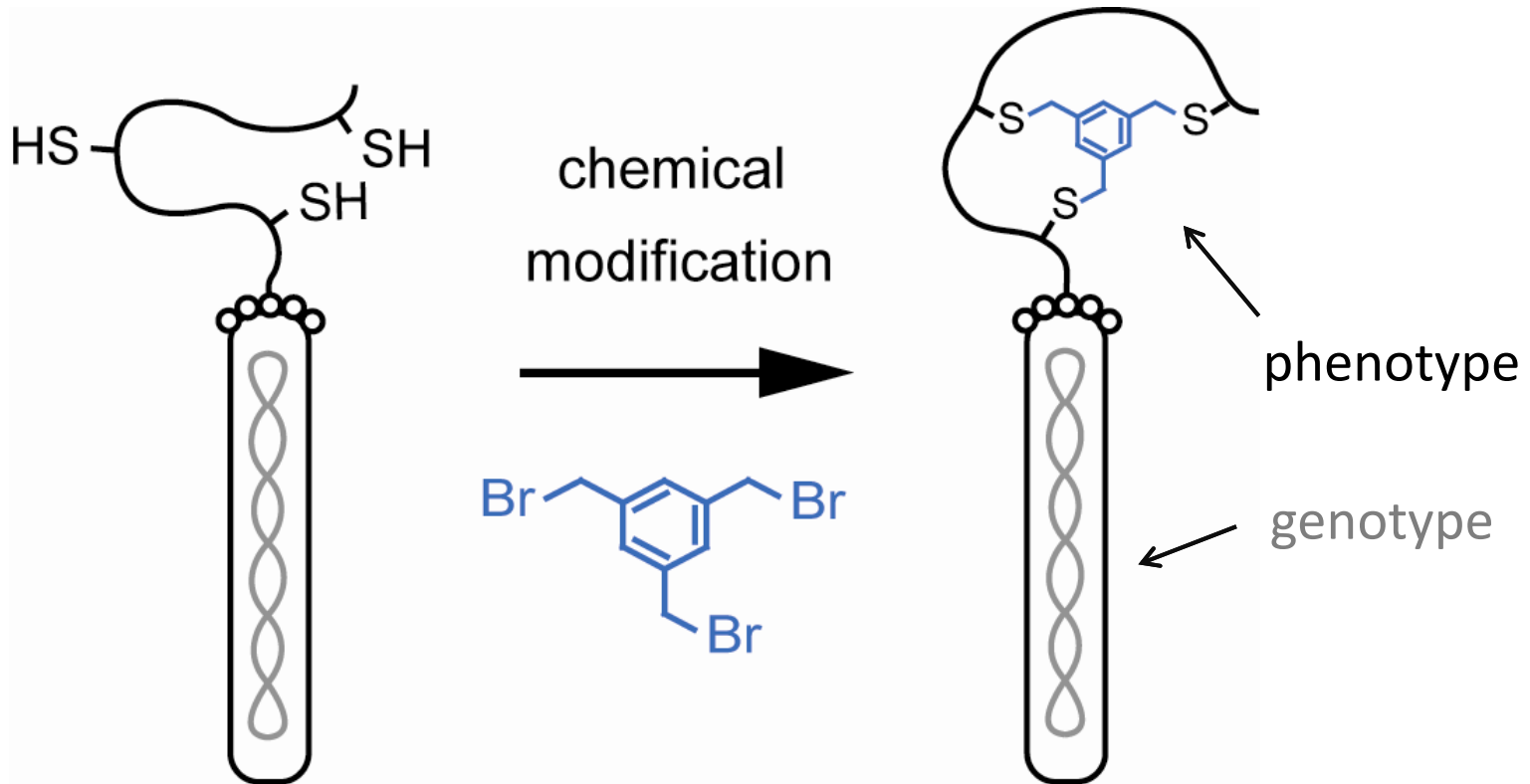
Small molecule core

Bicyclic peptides



(Timmerman, P. *et al.*,
ChemBioChem, 2005)

Phage-encoded bicyclic peptides



Talk outline

Part I: Development of bicyclic peptide phage display method



Laboratory of Molecular Biology (LMB),
Cambridge, UK



Sir Gregory
Winter

Talk outline

Part I: Development of bicyclic peptide phage display method



Laboratory of Molecular Biology (LMB),
Cambridge, UK



Sir Gregory
Winter

Part II: Bicyclic inhibitors of the tumour-associated protease uPA

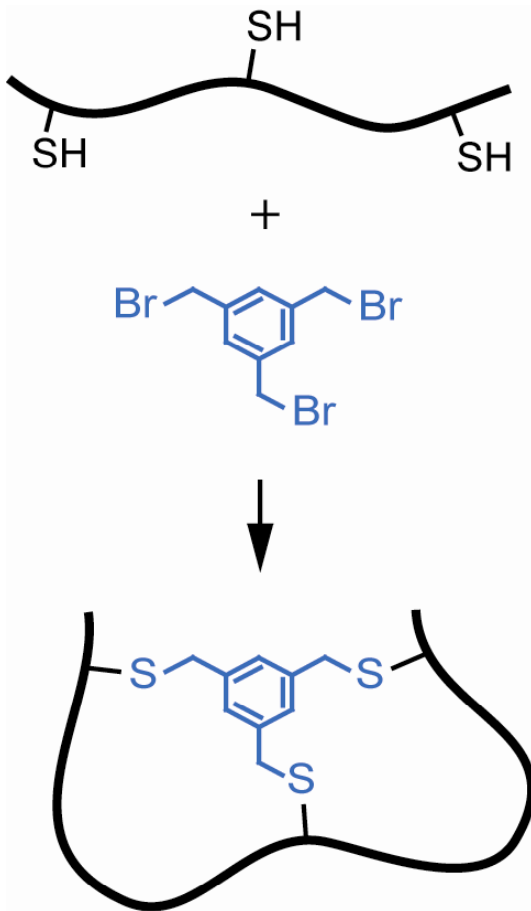


Ecole Polytechnique Fédérale
de Lausanne (EPFL)



Dr Alessandro
Angelini

Chemical reaction

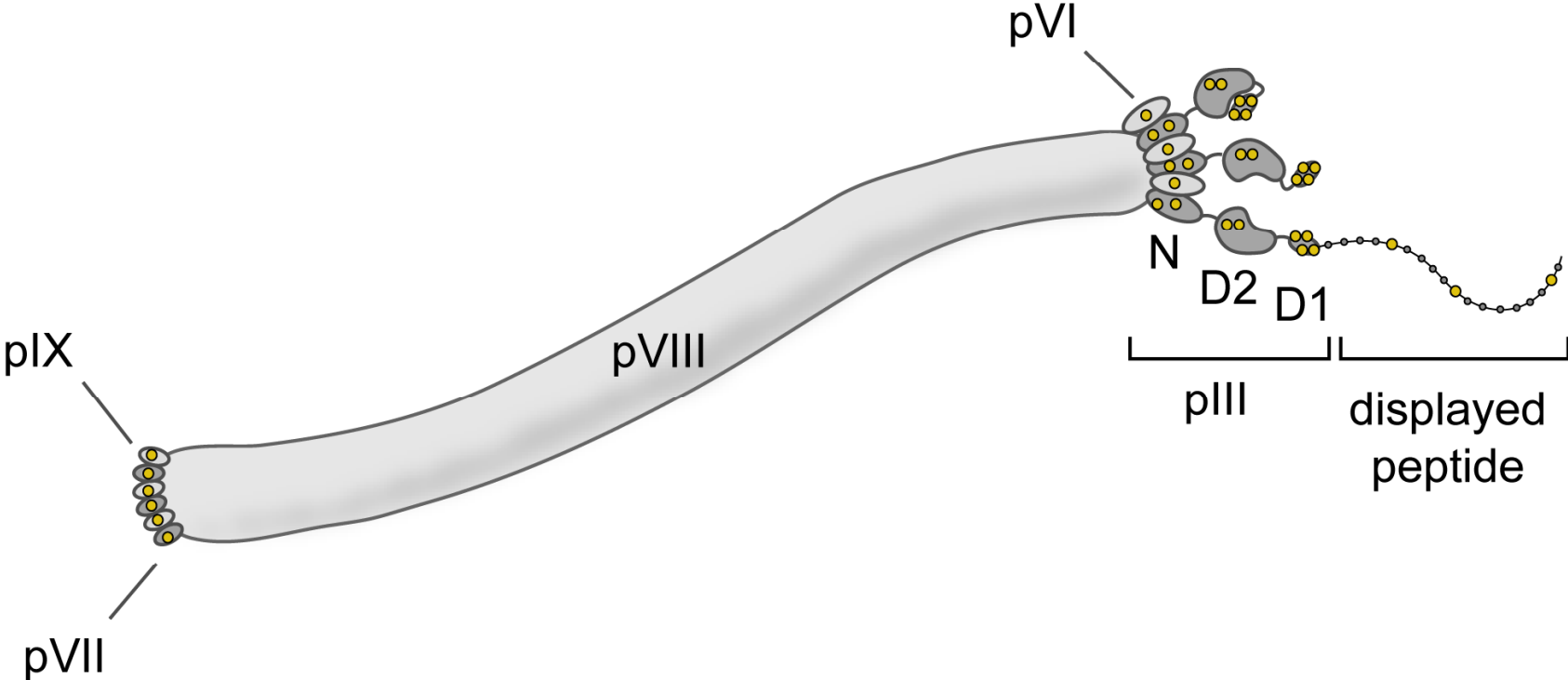


Reaction conditions:

- aqueous buffer
- 20% acetonitrile
- pH 7.8
- RT
- 1hr

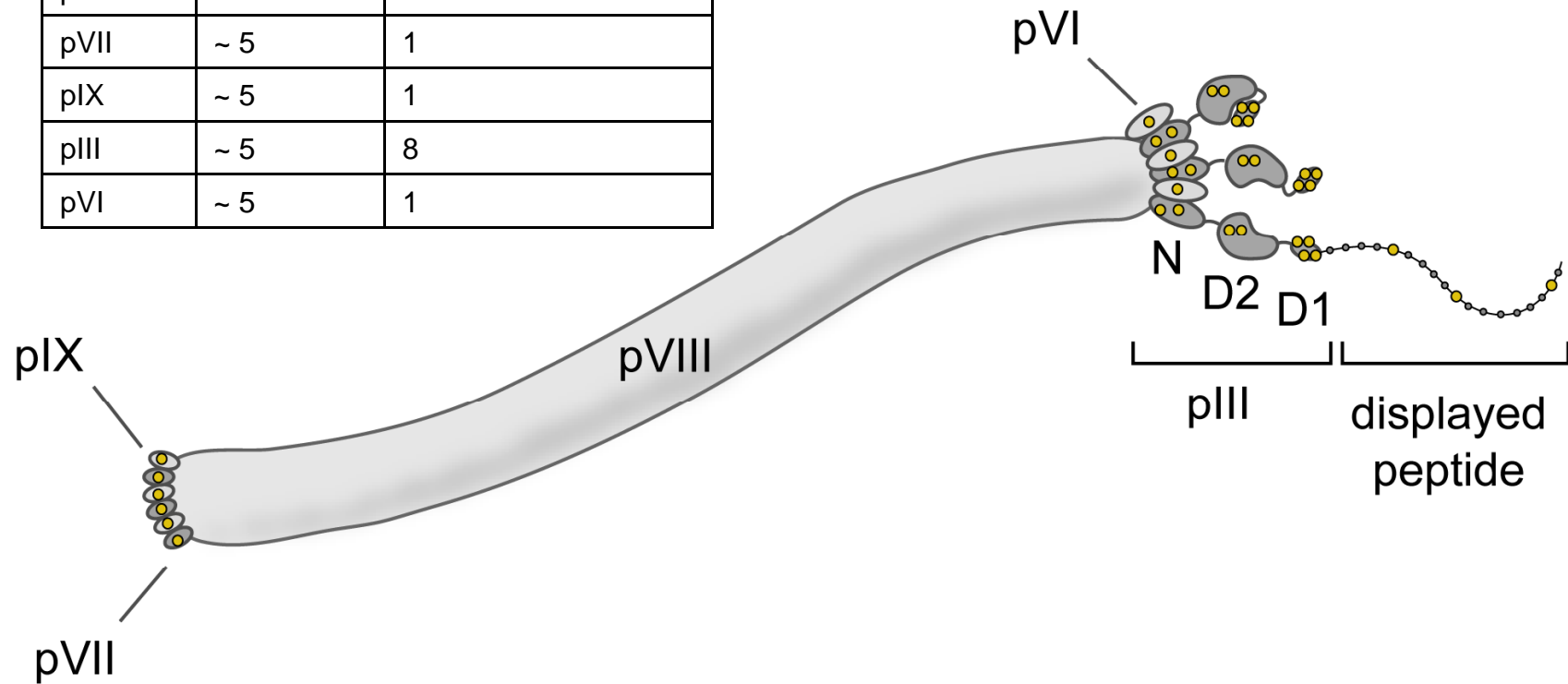
(Timmerman, P. *et al.*, *ChemBioChem*, 2005)

Cysteine residues in the M13 phage



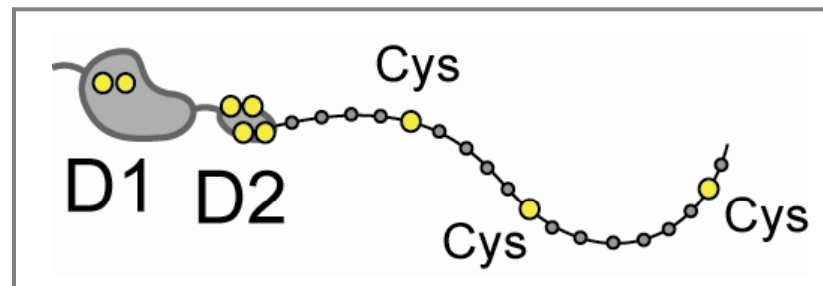
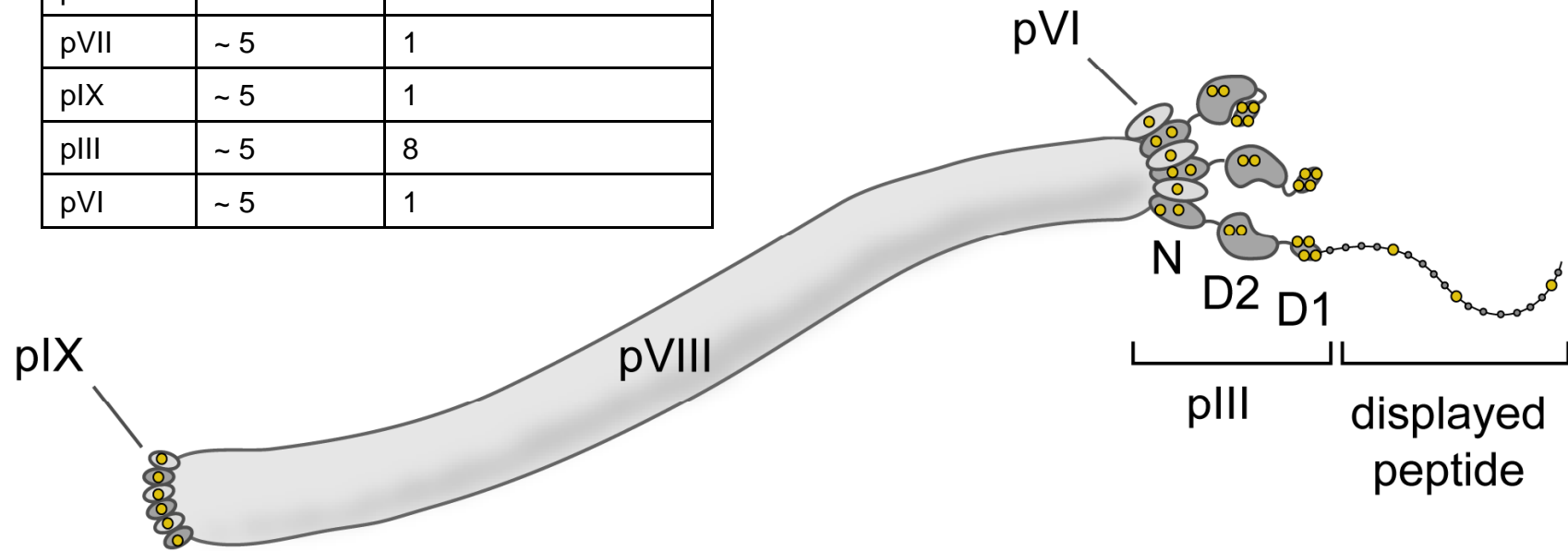
Cysteine residues in the M13 phage

phage protein:	copies per phage:	number of cysteines per coat protein:
pVIII	2700	0
pVII	~ 5	1
pIX	~ 5	1
pIII	~ 5	8
pVI	~ 5	1



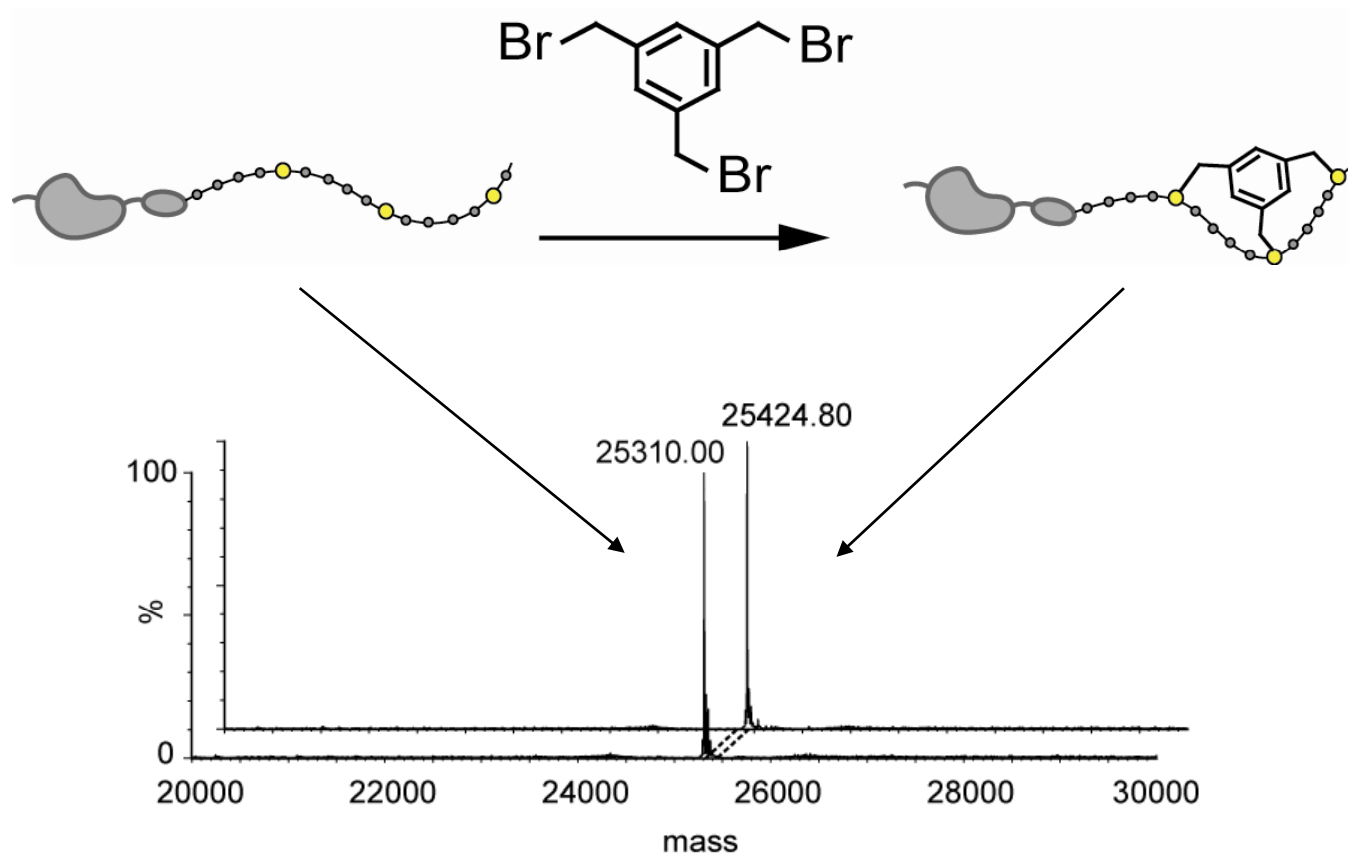
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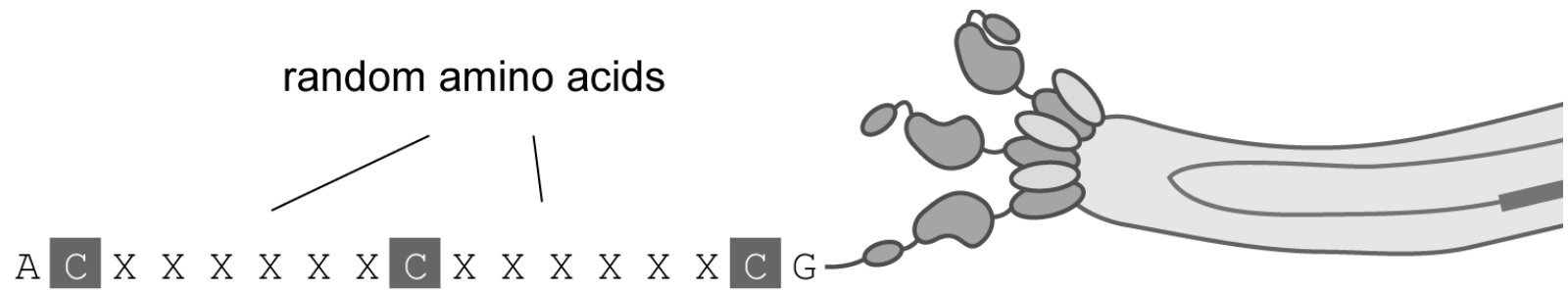


Phage with disulfide-free gene-3-protein

(Kather, I. *et al.*, *JMB*, 2005)

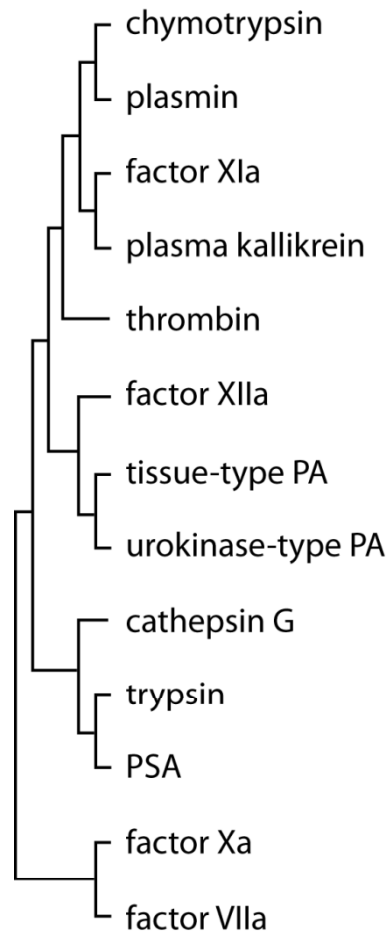


Phage peptide library

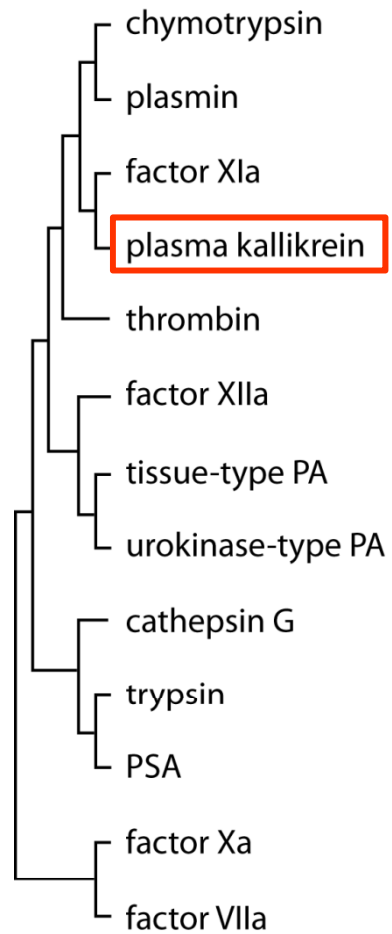


library size: $> 4 \times 10^9$ transformants

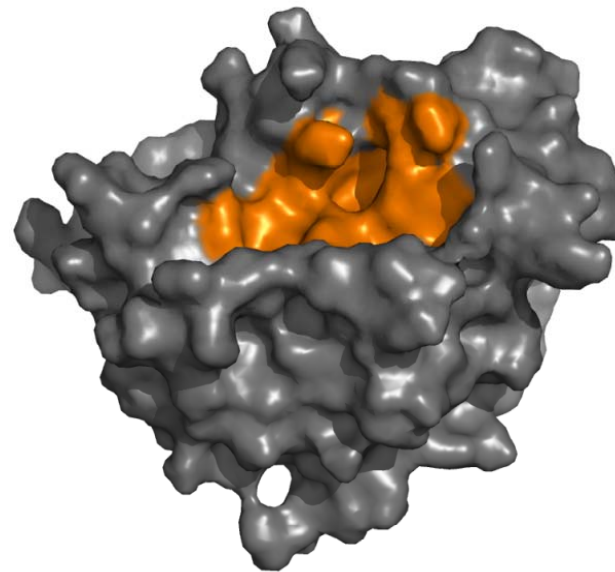
Targets: serine proteases (class S1A)



Targets: serine proteases (class S1A)



Plasma kallikrein



Plasma kallikrein with **substrate-binding pocket**

Sequences of selected peptides

Mutant:	Amino acid sequence:	IC ₅₀ (nM):	
		TBMB reacted	linear peptide
PK1	A C D S R F R N C P W S M S G C G	28	>10000
PK2	A C S D R F R N C P L W S G T C G		
PK3	A C R D I R F R C N Y D V A V C G		
PK4	A C S T E R R Y C P I E I F P C G	33	7181
PK5	A C A P W R T M C L N I D G P C G	21	5707
PK6	A C A P W R T A C Y E D L M W C G		
PK7	A C P V W R T L C M E S E G V C G		
PK8	A C F P L W R T C V H E P T M C G		
PK9	A C W Q V Q V N C R V N F G K C G	39	>10000
PK10	A C G G N S D R C R V N N I S C G		
PK11	A C G R G D Q T C R V N W H P C G		
PK12	A C - - V H N Y C R V N W V T C G		
PK13	A C G T G E G R C R V N W T P C G		

Sequences of affinity matured peptides

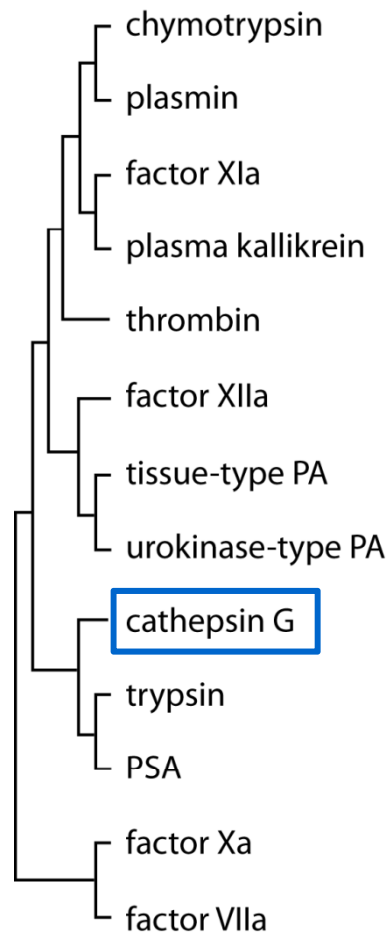
Mutant:	Amino acid sequence:	IC ₅₀ (nM):
PK14	A C S D R F R N C P A D E R E C G	1.7
PK15	A C S D R F R N C P A D E A L C G	
PK16	A C S D R F R N C P V D E A L C G	
PK17	A C S D R F R N C P V D E W L C G	
PK18	A C S D R F R N C P G D E S L C G	
PK19	A C S D R F R N C P Y T L H D C G	
PK20	A C S D R F R N C P Y V S S D C G	
PK21	A C S D R F R N C P Y S E G D C G	
PK22	A C S D R F R N C P V W D S S C G	
PK23	A C S D R F R N C P V S E S A C G	

Inhibitor specificity

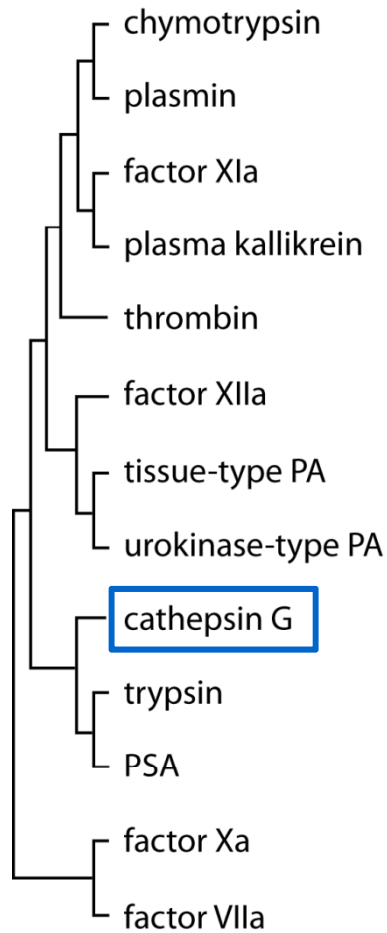
Protease	Sequence identity*	IC₅₀ (nM)
Human plasma kallikrein		1.7
Mouse plasma kallikrein	79%	> 10'000
Factor Xla	63%	> 10'000
Thrombin	36%	> 10'000

*) compared to human plasma kallikrein

Cathepsin G selections



Cathepsin G selections



Mutant:	Amino acid sequence:
CG1	A C E Y G D L W C G W D P P V C G
CG2	A C E Y D V G F C W D G F G Q C G
CG3	A C L F D A G F C Q Q H S T E C G
CG4	A C I F D L G F C H N D W W N C G
CG5	A C L F D L G F C G G G E G P C G
CG6	A C P R I E G F C L P I F S D C G
CG7	A C L R A Q E D C V Y D R G F C G
CG8	A C T R G S G D C T Y D F G F C G

Heinis, C. *et al.*, Nat. Chem. Biol., 2009

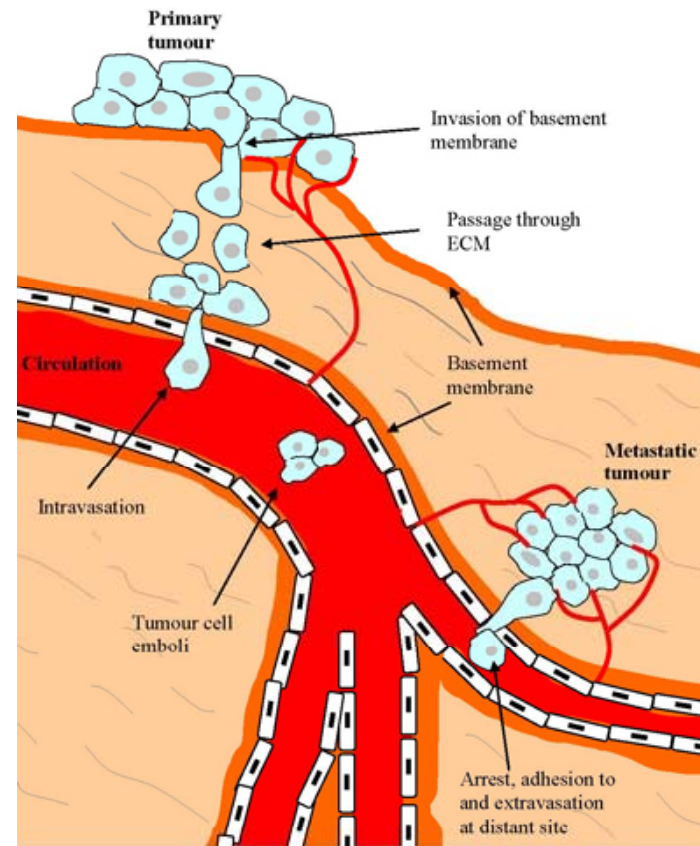
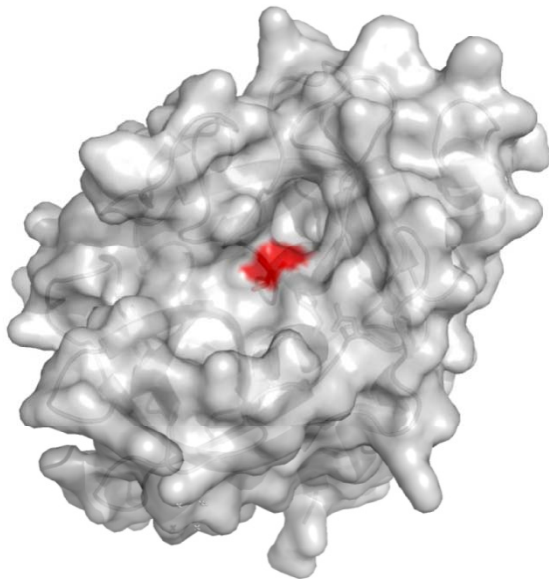
Part II: Therapeutic potential of bicyclic peptides?



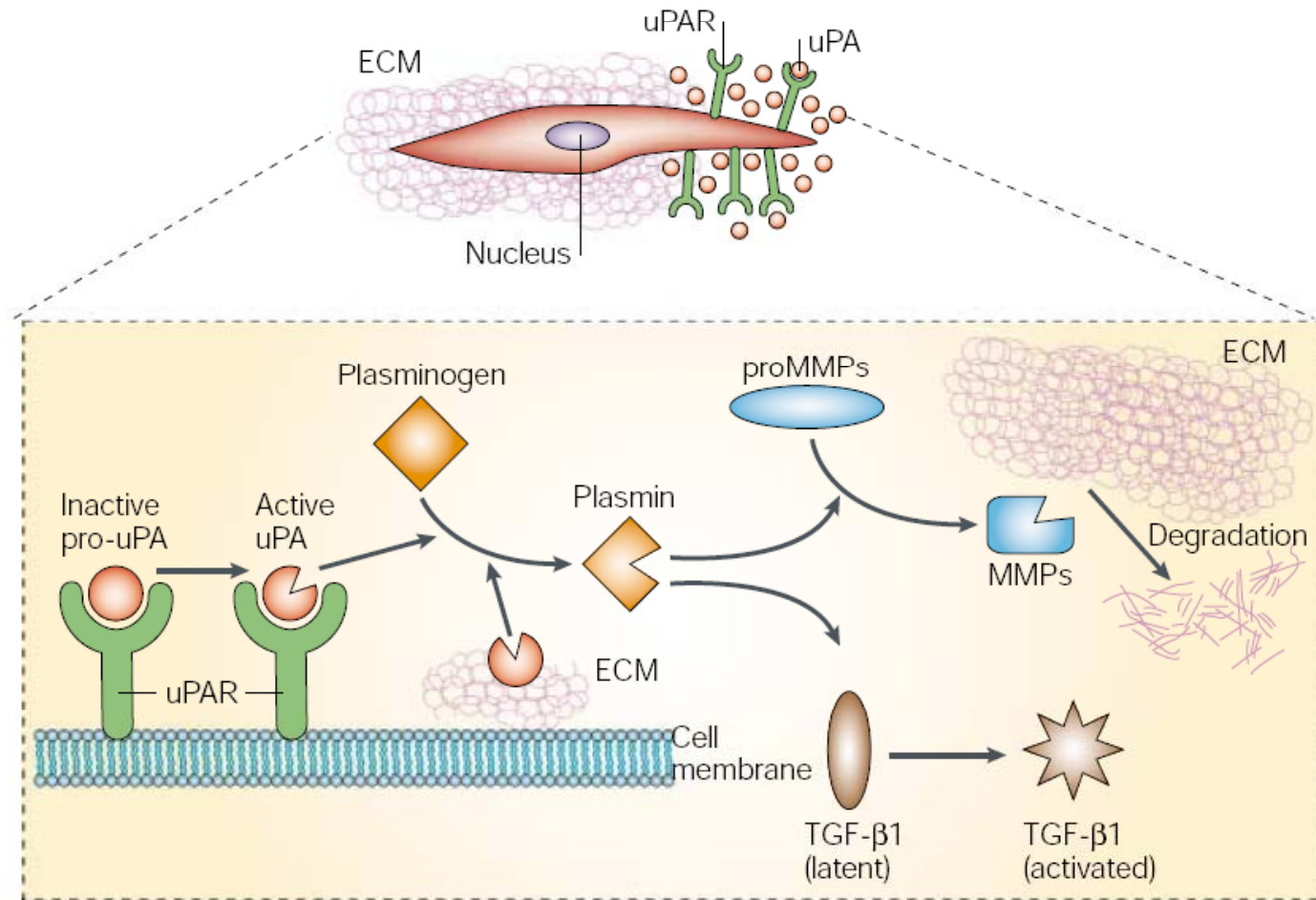
EPFL, Lausanne, Switzerland

Urokinase-type plasminogen activator (uPA)

Structure of uPA
(25 kDa)

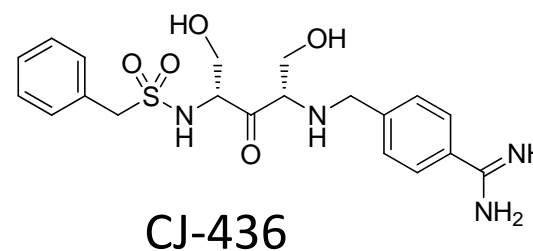
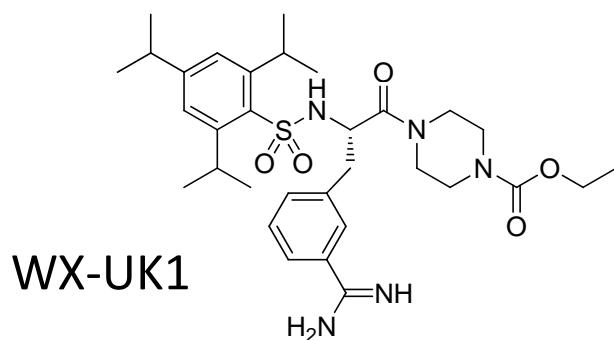


Urokinase-type plasminogen activator (uPA)



Available uPA inhibitors

Inhibitor	Activity
WX-UK1 (small molecule in clinical trials)	$K_i = 410 \text{ nM}$
CJ-436 (most selective/potent small molecule)	$K_i = 20 \text{ nM}$
Upain-1 (best monocyclic peptide)	$K_i = 6700 \text{ nM}$
DS2 (monoclonal antibody against murine uPA)	$K_D = 6.4 \text{ nM}$



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Dr. Agnes Jaulent



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Shiyu Chen

Lisa Pollaro

Dr. Carly Huitema

Dr. Julia Morales Sanfrutos

Philippe Diderich

Inmaculada Rentero

Vijay Tejwani



Bicycle Therapeutics:

Dr. John Tite (CEO)

www.bicycletherapeutics.com

