Peptides as Switchable Molecular Scaffolds and Asymmetric Catalysts

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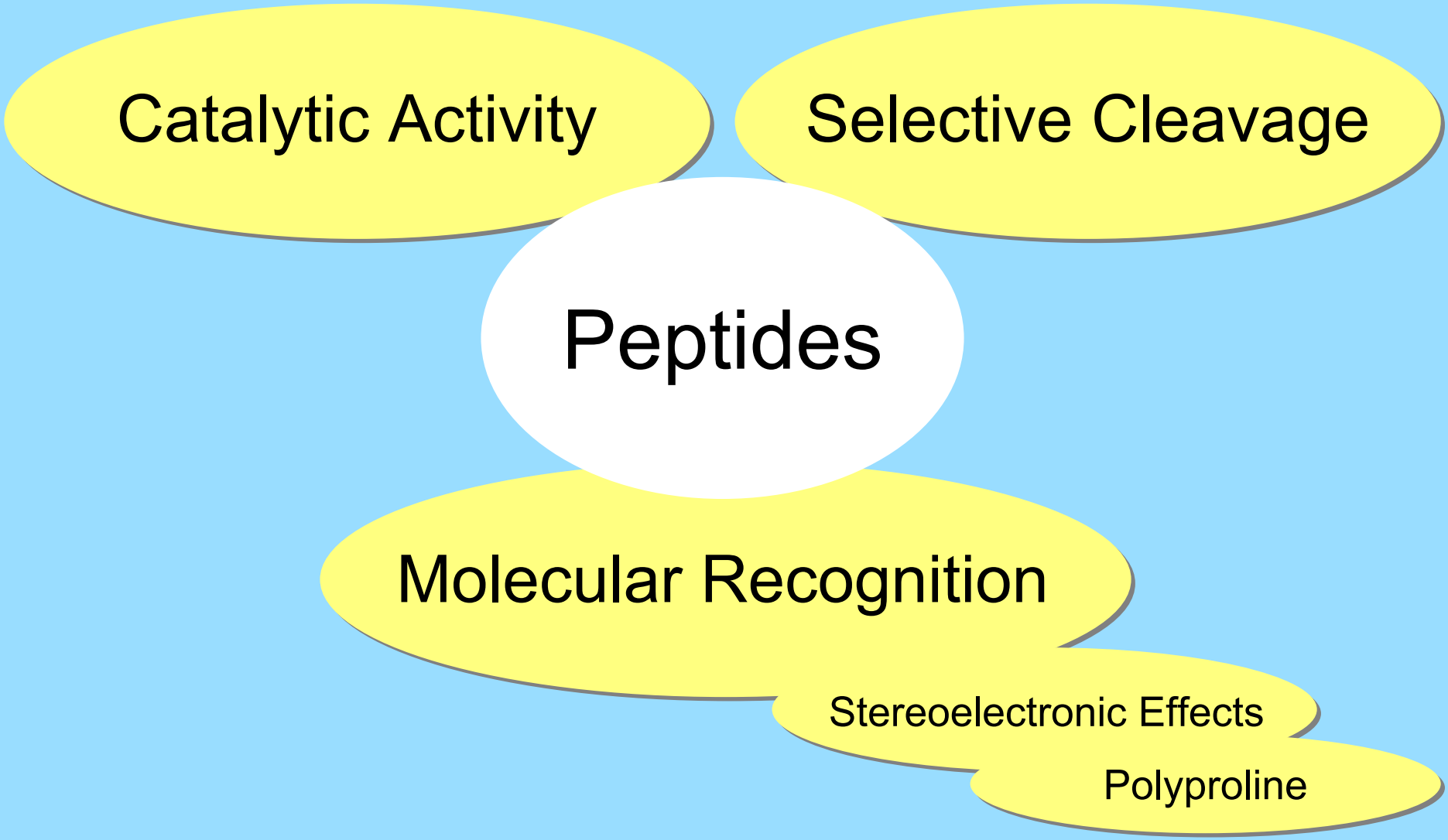
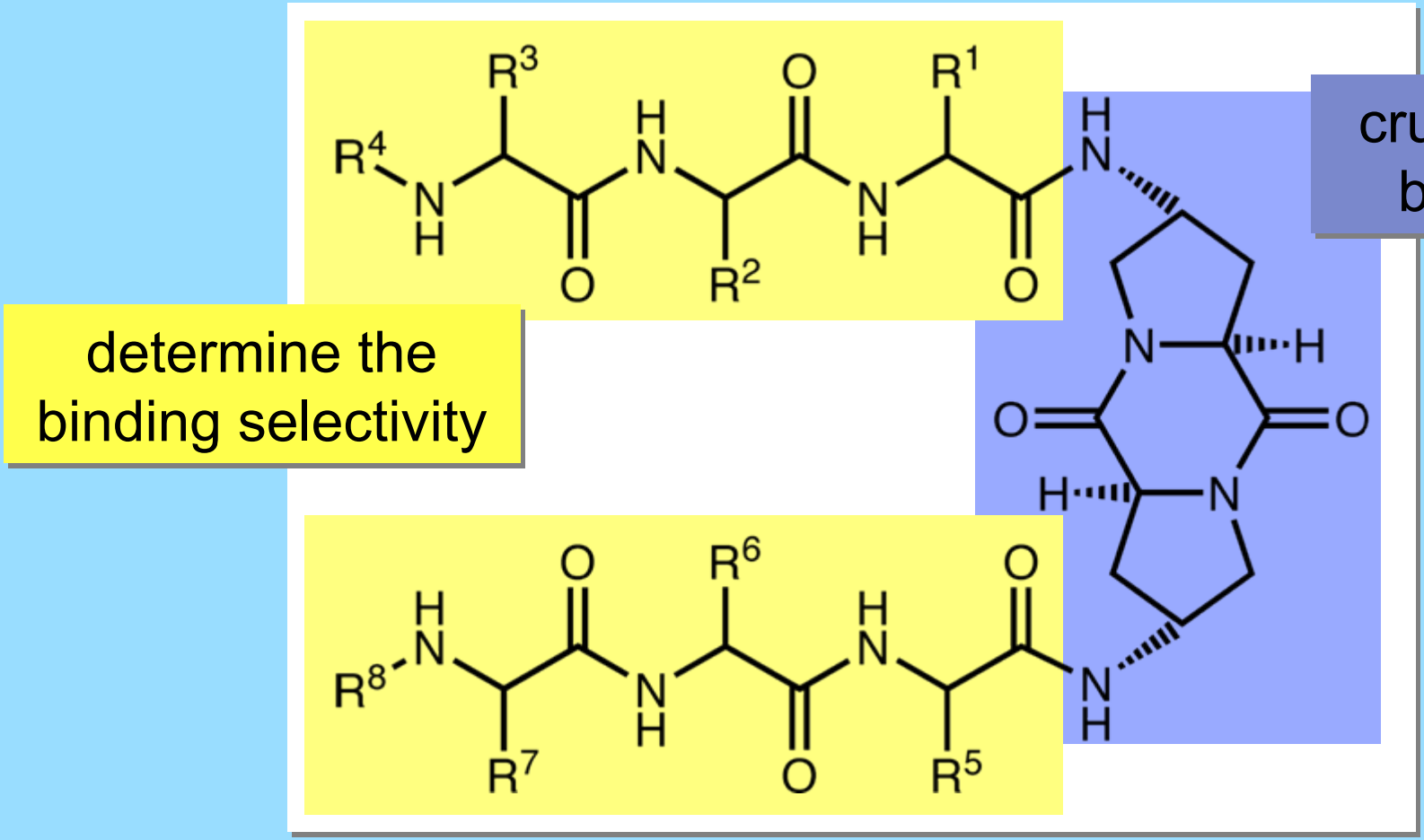

Catalytic Activity
Selective Cleavage
Peptides
Molecular Recognition
Stereoelectronic Effects
Polyproline

Diketopiperazine Receptors - Sequence Selective Binding of Short Peptides



H. Wennemers, M. Conza, M. Nold, P. Krattiger, *Chem. Eur. J.* **2001**, *7*, 3342. M. Conza, H. Wennemers, *J. Org. Chem.* **2002**, *67*, 2696.
H. Wennemers, M.C. Nold, M.M. Conza, K.J. Kulicke, M. Neuburger, *Chem. Eur. J.* **2003**, *9*, 442.
M. Conza, H. Wennemers, *Chem. Commun.* **2003**, 866. P. Krattiger, H. Wennemers, *Synlett* **2005**, 706.

Applications of Diketopiperazine Receptors

Sensors

Prof. J. Bargon, University Bonn

Material Sciences

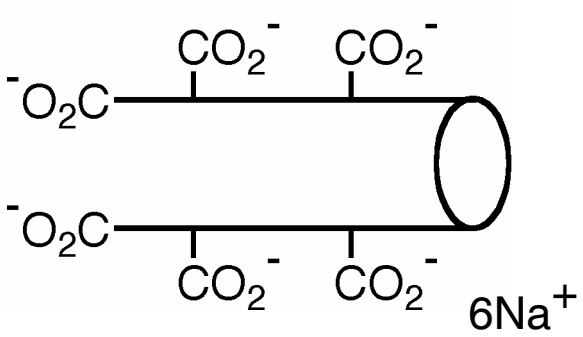
Prof. C. Faul, Bristol University

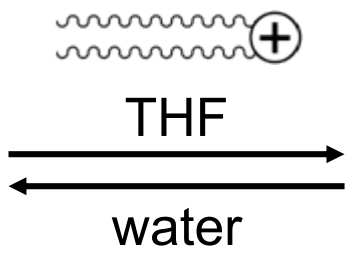
Diagnostics / Synthetic Antibodies

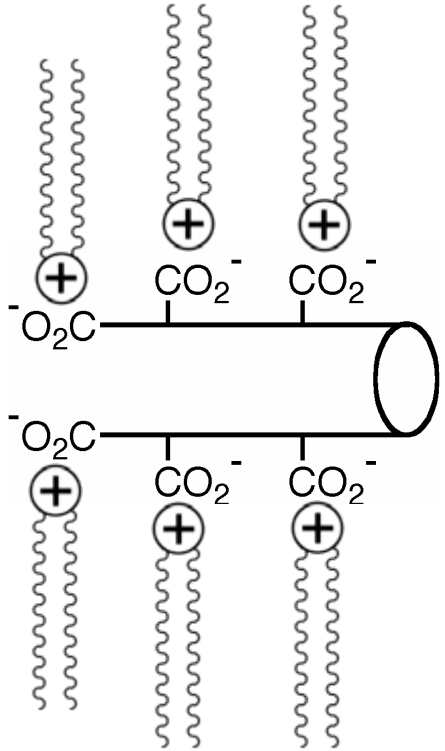
Prof. M. Przybylski, University of Konstanz
Prof. U. Jenal, Biocentre Basel

J.W. Lörgen, P. Krattiger, C. Kreutz, H. Wennemers, J. Bargon, *Sensors and Actuators B: Chemical* **2005**, *107*, 366.
P. Krattiger, C. JF Faul, H. Wennemers, *manuscript in preparation*.

Diketopiperazine Receptor-Surfactant Complexes



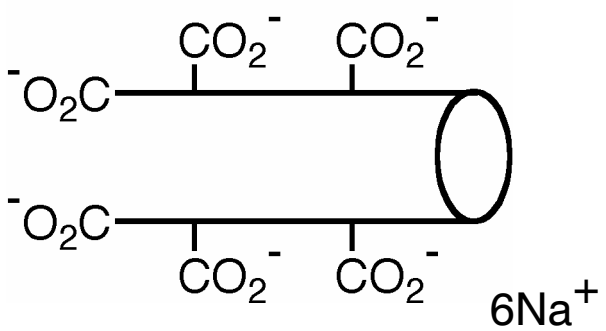


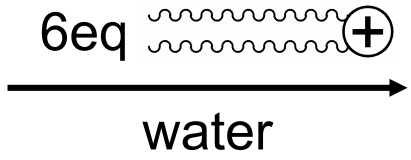


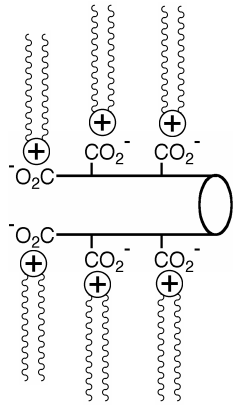
binds to
D/L-Arg-D/L-Arg-D/L-X
(X = Arg, Ser, Thr, or Cys)
in aqueous solution at pH 7 or 10
ΔG = 5-6 kcalmol^{-1}

no binding
to any of 29791 peptides

Material Properties of Diketopiperazine Receptor-Surfactant Complexes

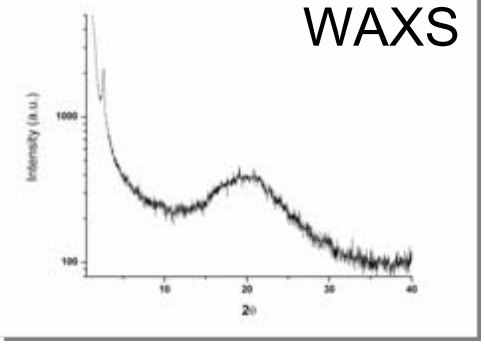




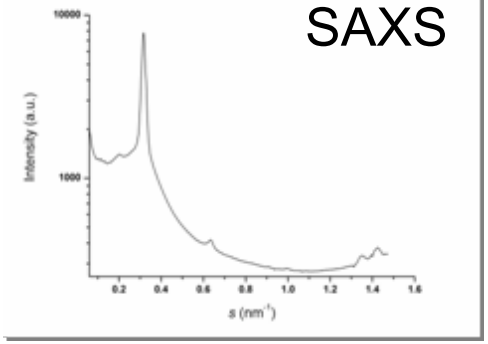


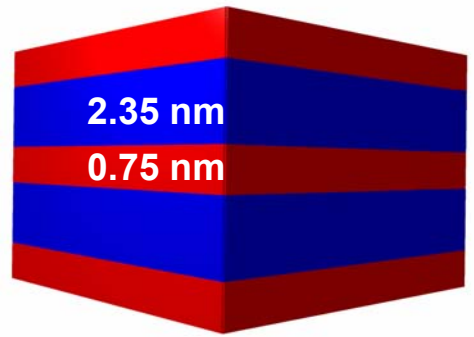
precipitate

WAXS



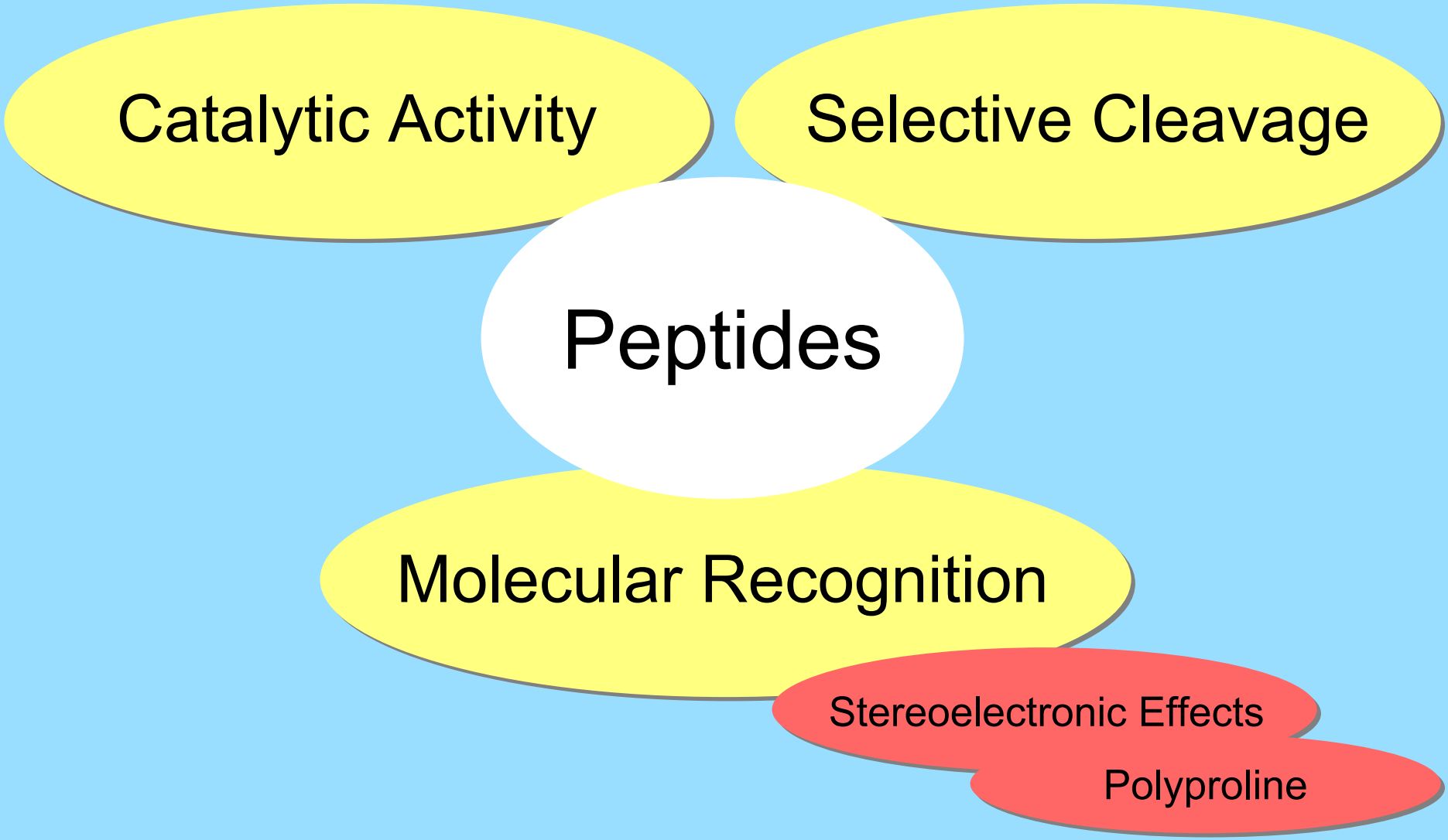
SAXS



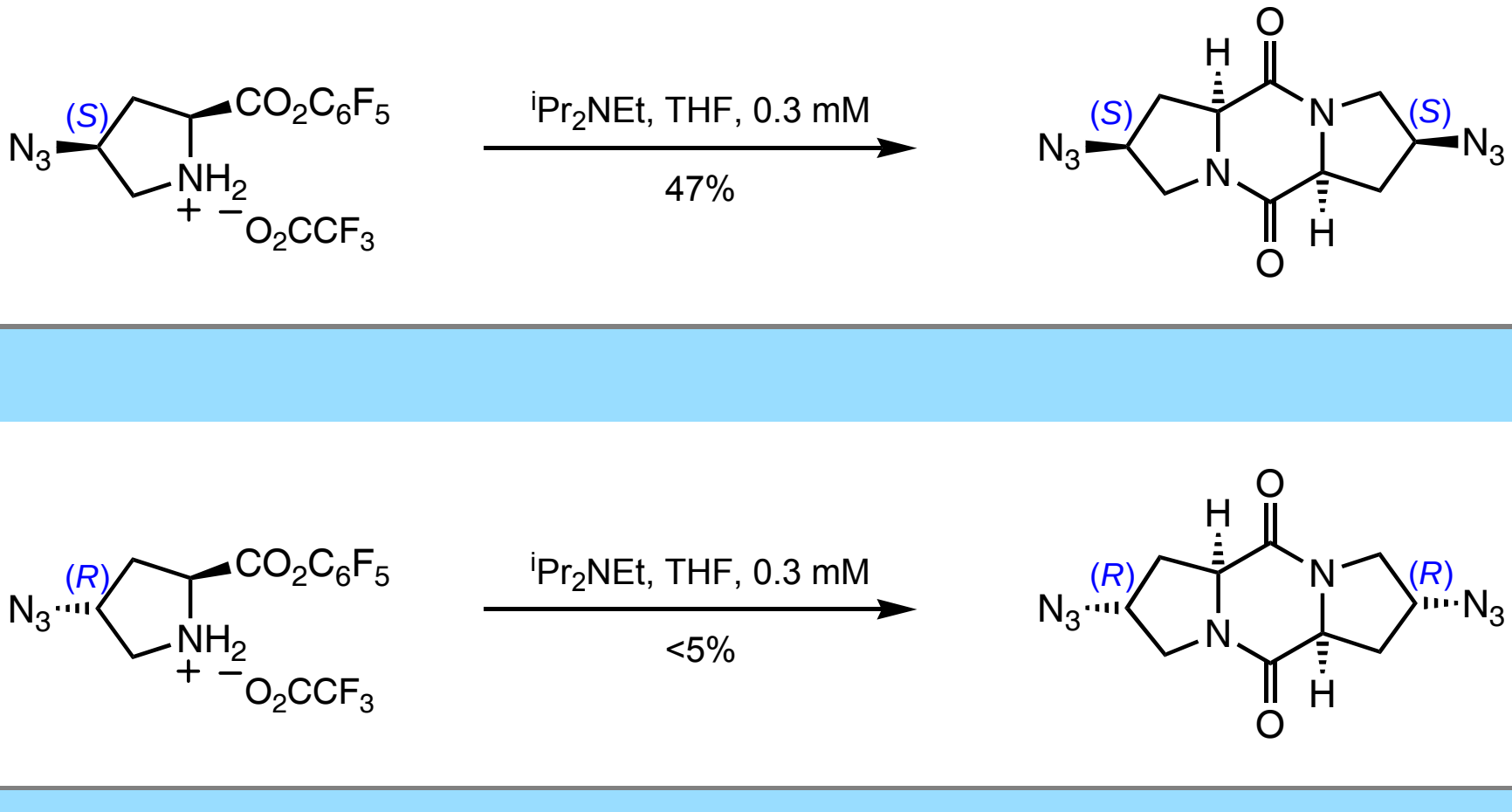


→ liquid crystalline properties

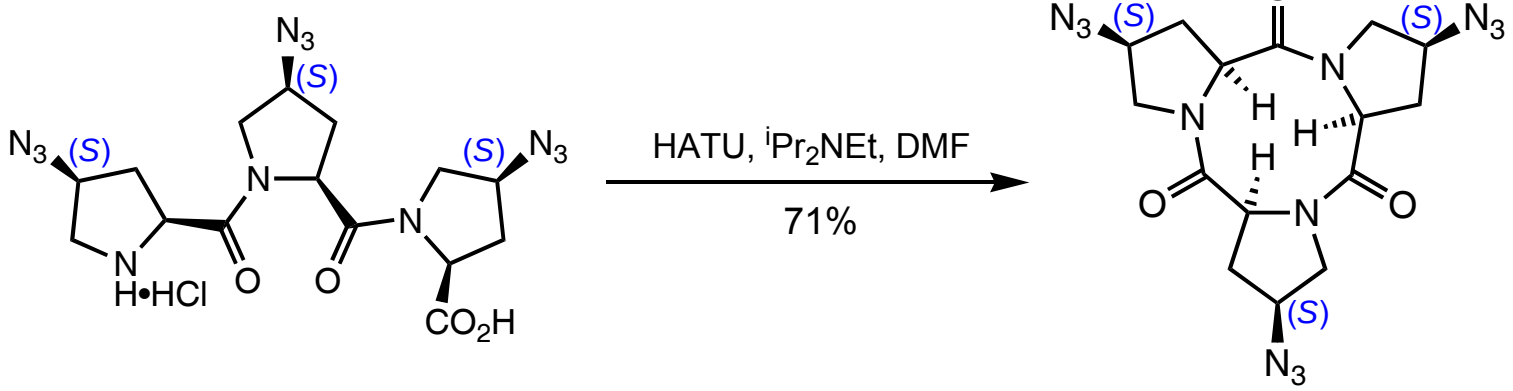
collaboration with Prof. Charl FJ Faul, Bristol University

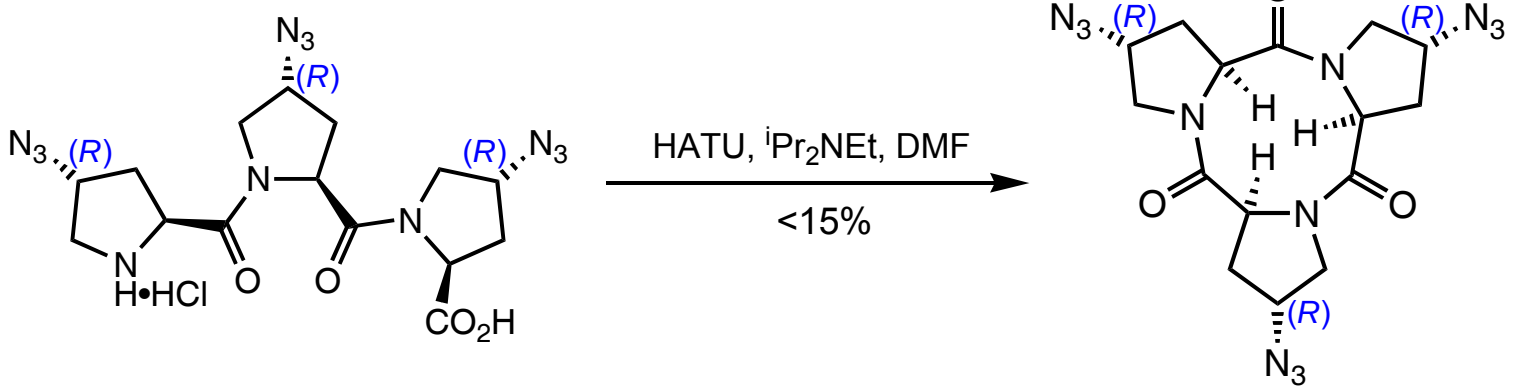

Catalytic Activity
Selective Cleavage
Peptides
Molecular Recognition
Stereoelectronic Effects
Polyproline

Syntheses of (4*S*,4´*S*)- and (4*R*,4´*R*)-Azp-Diketopiperazines



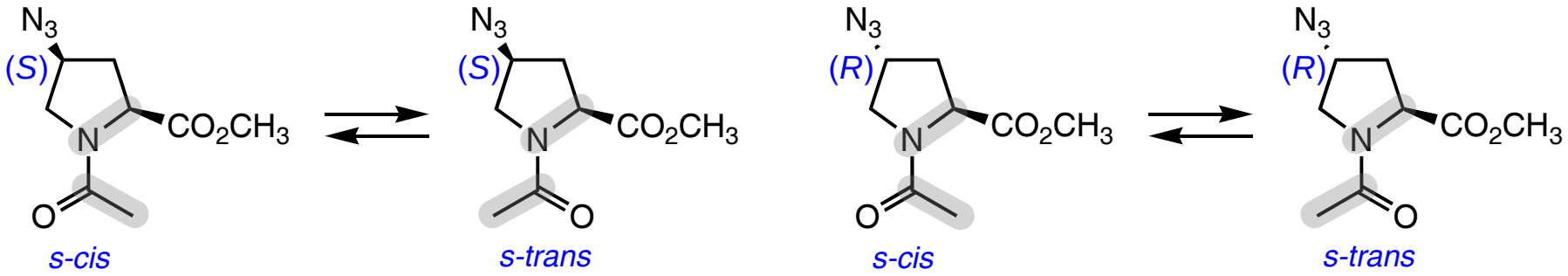
Syntheses of Cyclotri[(4*S*)- and (4*R*)-azidoproline]





The absolute configuration at C(4) influences the cyclisation tendency!

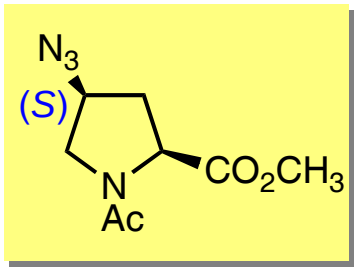
Influence of the configuration at C(4) on the *s-cis* : *s-trans* ratio

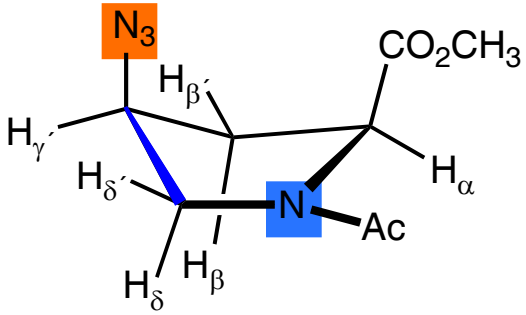


solvent	N-Ac-(4*S*)-N_3-Pro-OCH_3 *s-cis* : *s-trans*	N-Ac-(4*R*)-N_3-Pro-OCH_3 *s-cis* : *s-trans*
D_2O	1 : 2.6	1 : 6.1
d_7-DMF	1 : 1.6	1 : 3.8
d_5-pyridine	1 : 2.0	1 : 4.5
CD_3OD	1 : 1.7	1 : 4.0
d_6-acetone	1 : 1.6	1 : 3.7
$CDCl_3$	1 : 1.9	1 : 3.9
d_8-dioxane	1 : 2.0	1 : 4.2

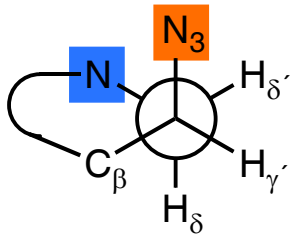
Conformational Analysis of the Pyrrolidine Rings

C(4)-endo-conformation



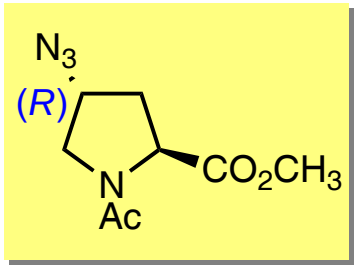


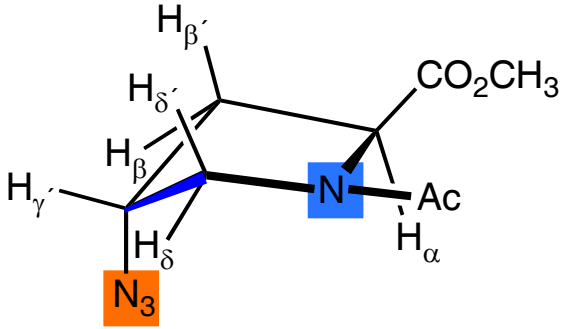
pseudo-axial position



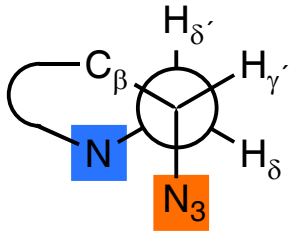
gauche conformation

C(4)-exo-conformation



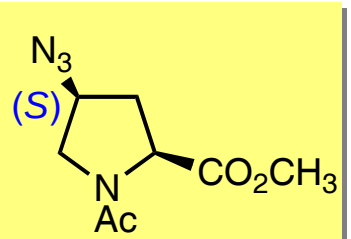


pseudo-axial position

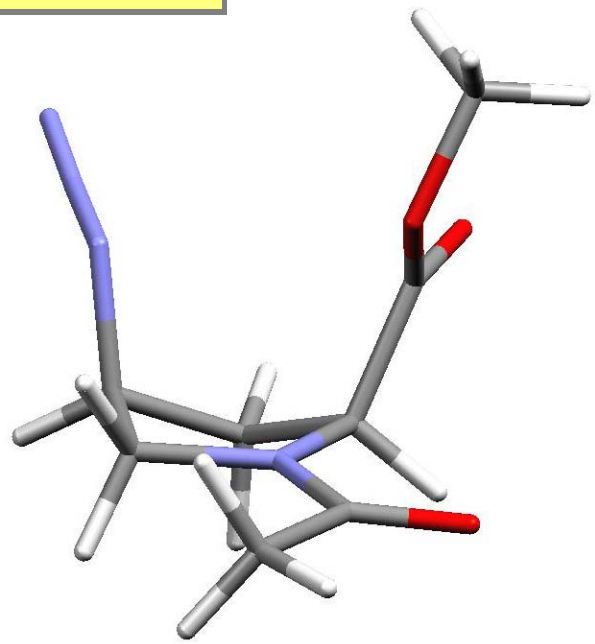


gauche conformation

An “azido gauche effect“ determines the conformation of the pyrrolidine rings



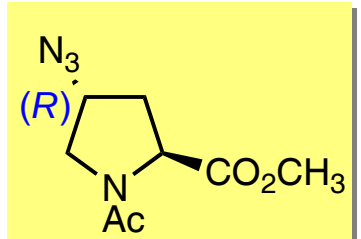
endo-conformation

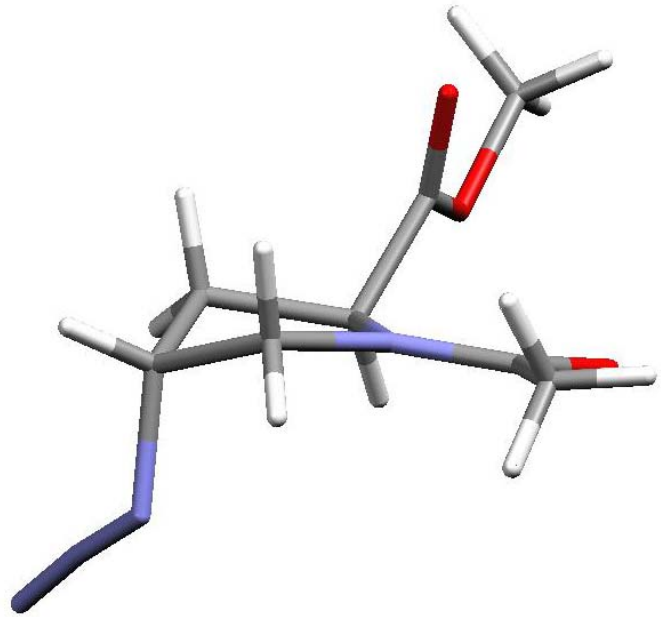


ab-initio calculations

RI-MP2/TZVP level, B3LYP/6-31G** structures

exo-conformation





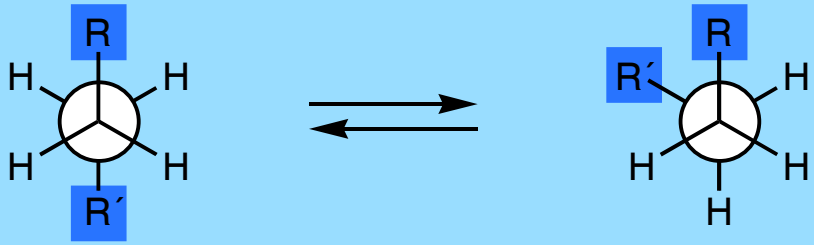
crystal structure analysis

(also ab-initio calculation)

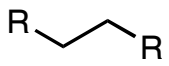
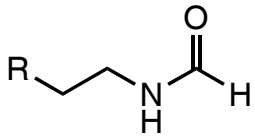
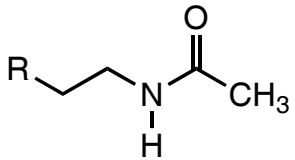
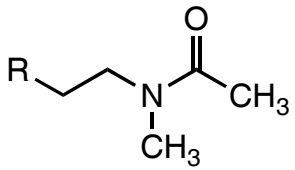
collaboration with Prof. Christian Ochsenfeld, University of Tübingen

The “Azido Gauche Effect“

anti



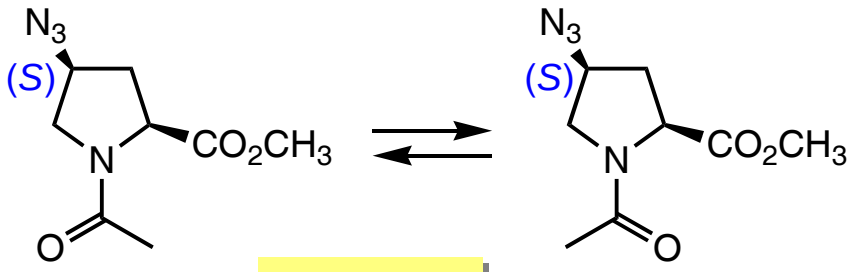
gauche

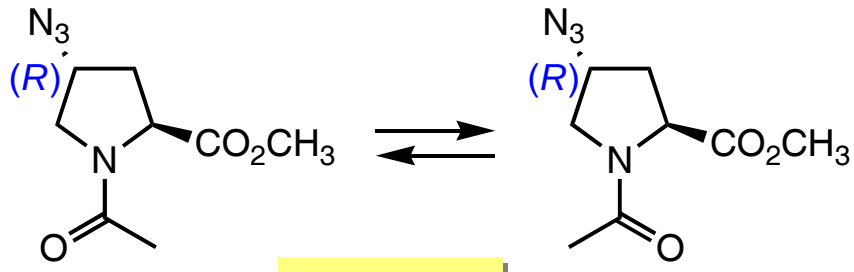
	$\Delta E = E_{anti} - E_{gauche}$ (kcal mol^{-1}) R = F	R = N_3
	0.9	1.3
	1.4	1.3
	1.4	1.6
	1.7	3.3

ab initio calculations using B3LYP/6-31G** for geometry optimization and RI_MP2/TZVP for energy optimisation

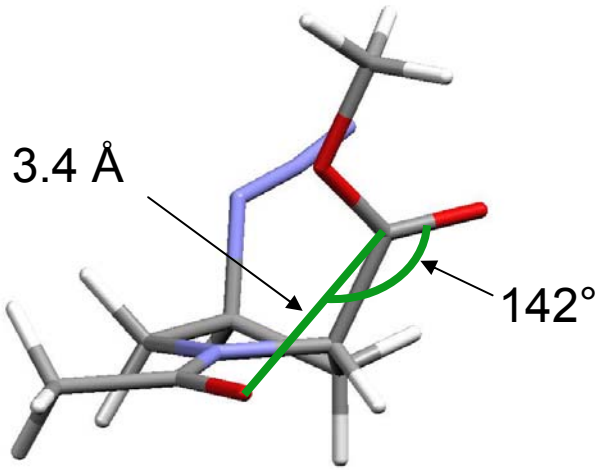
collaboration with Prof. Christian Ochsenfeld, University of Tübingen

What is the reason for the higher *s-trans* preference of (4*R*)-azidoproline?

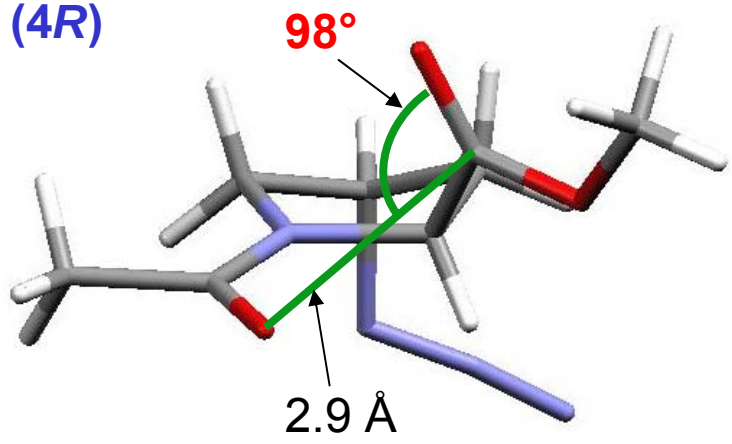




(4*S*)



(4*R*)

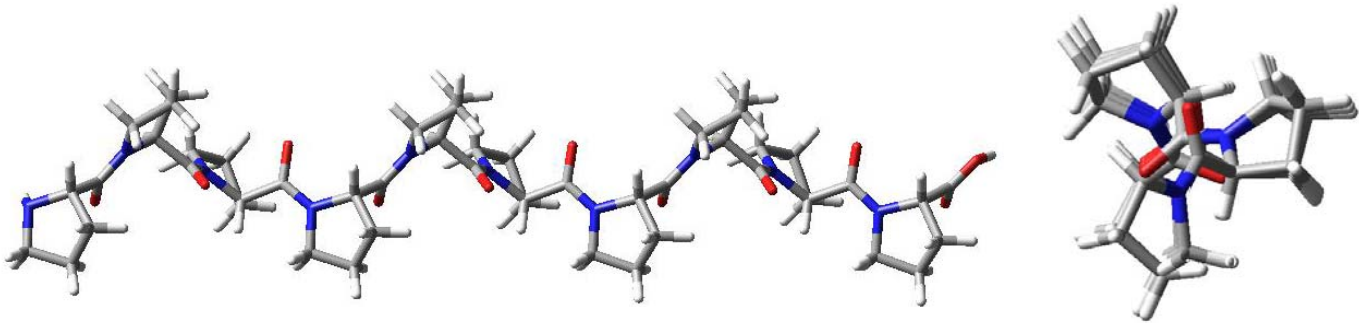


stabilizing n->π*-interaction

L.-S. Sonntag, S. Schweizer, C. Ochsenfeld, H. Wennemers, *J. Am. Chem. Soc. In press.*

Do azidoprolines effect the conformational stability of polyproline?

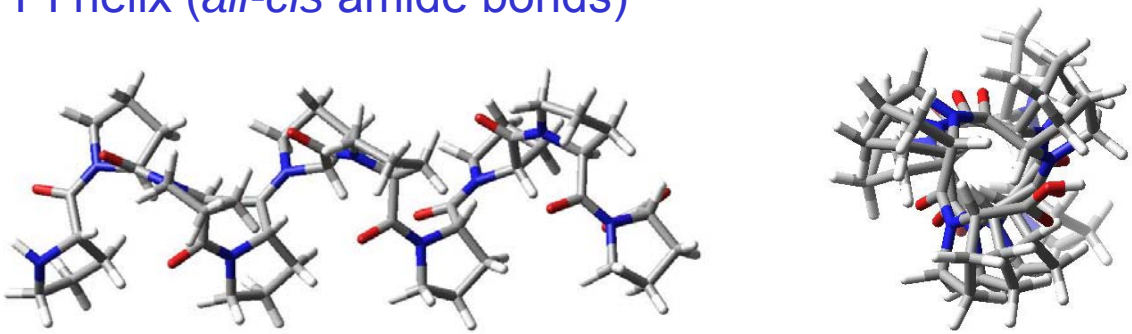
PPII helix (*all-trans* amide bonds)



in H_2O

should be stabilized by incorporation of (4*R*)-azidoproline

PPI helix (*all-cis* amide bonds)

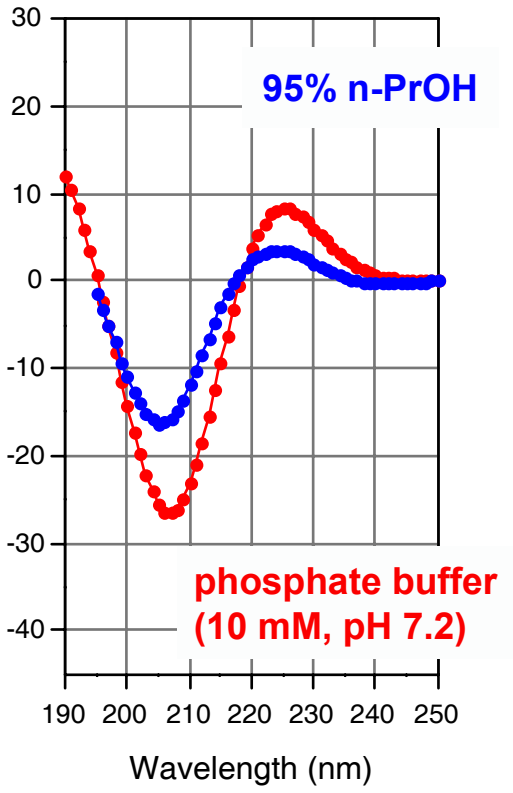


in n-PrOH

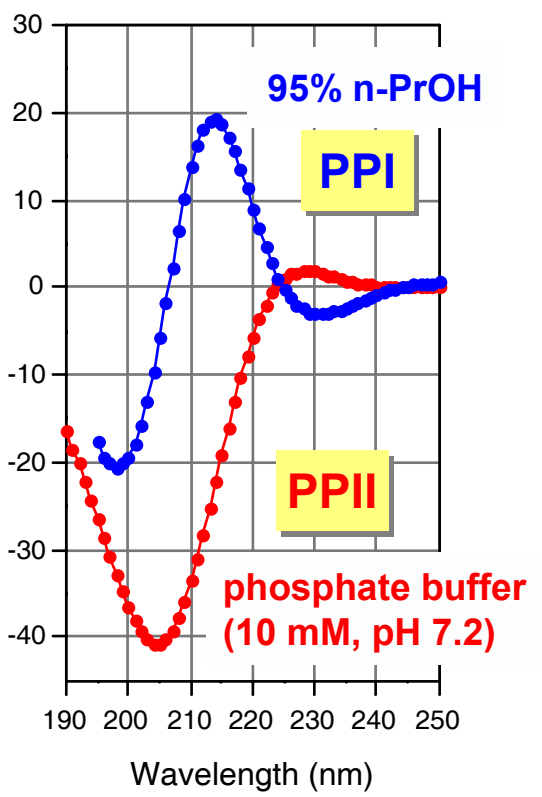
should be stabilized by incorporation of (4*S*)-azidoproline

Polyprolines containing (4*S*)- and (4*R*)-azidoprolines

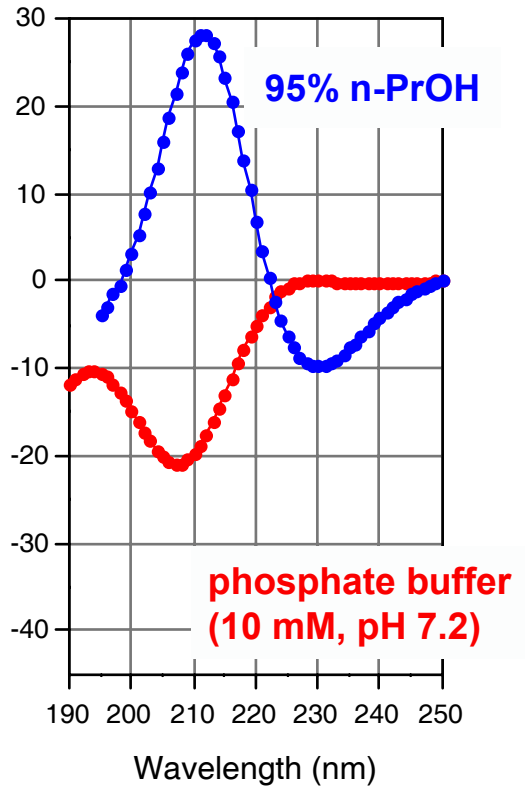
Ac-[(4*R*)Azp]$_9$-OH



Ac-[Pro]$_9$-OH



Ac-[(4*S*)Azp]$_9$-OH

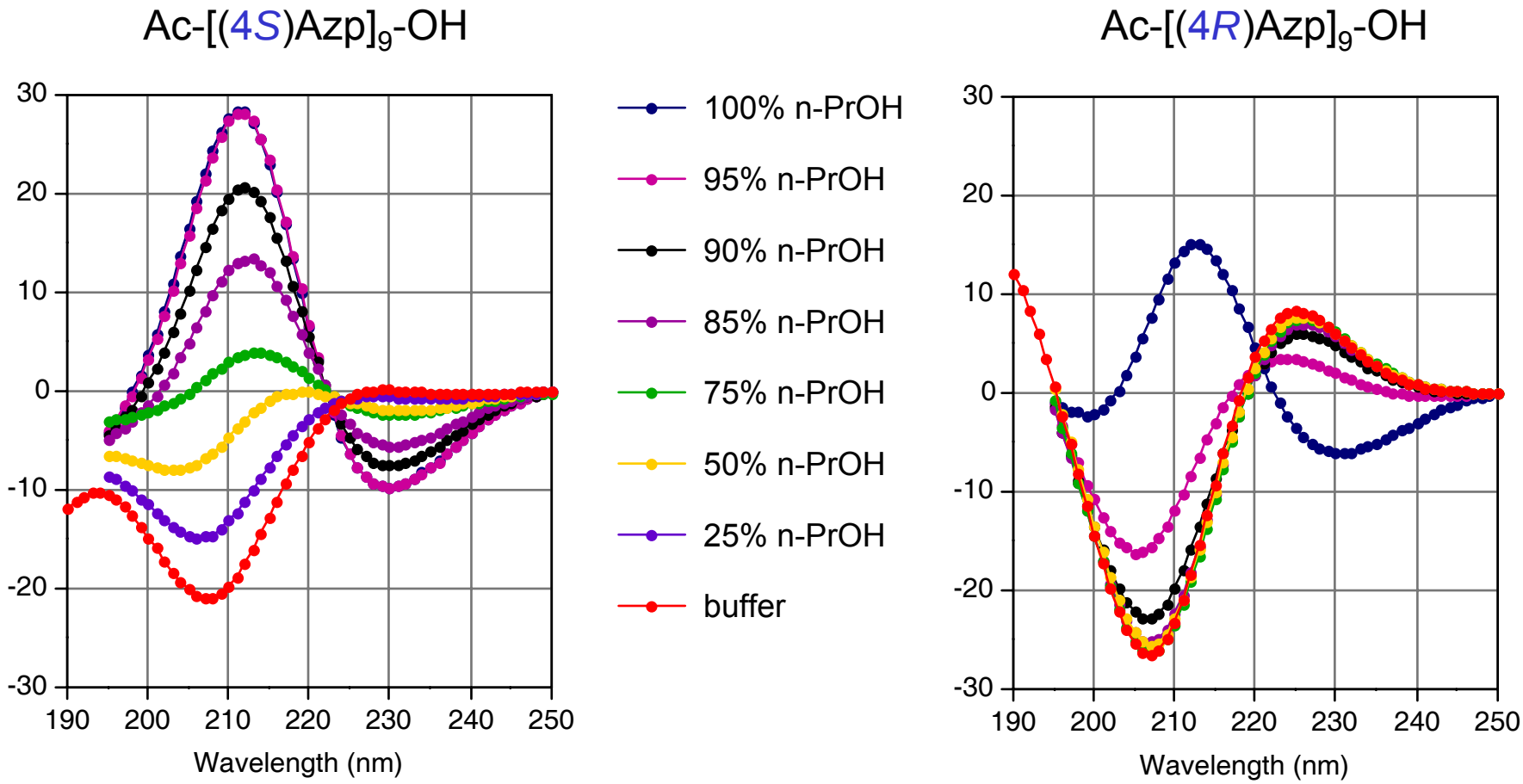


(4*R*)Azp stabilizes PPII

(4*S*)Azp stabilizes PPI

(4*R*)Azp stabilizes PPII, (4*S*)Azp stabilizes PPI - further support

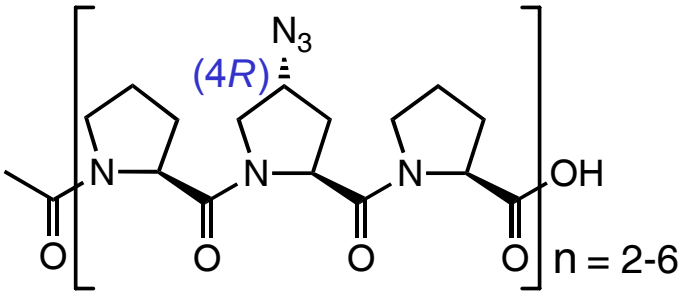
CD-spectra in different mixtures of n-PrOH in phosphate buffer (10 mM, pH 7.2).

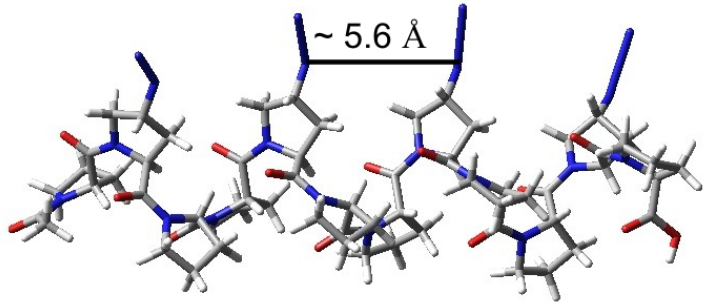


(4*R*)Azp and (4*S*)Azp are tools to tune the *s-cis*:*s-trans* ratio of Xxx-Pro bonds.

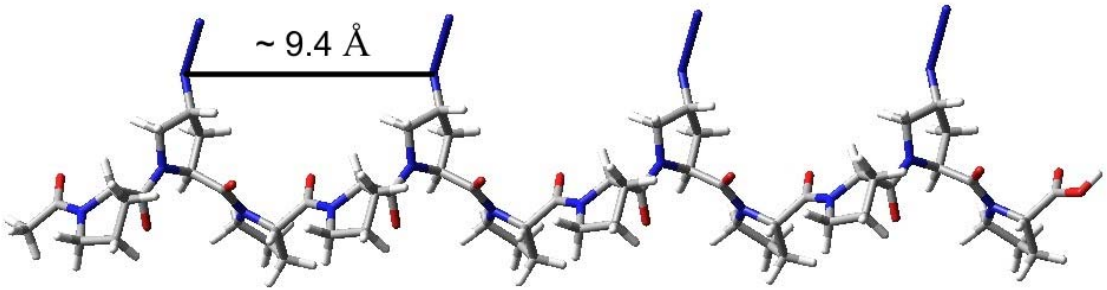
Azp containing Polyprolines as Functionalizable Scaffolds?

Models: Oligoprolines with (4*R*)Azp in every third position





PPI



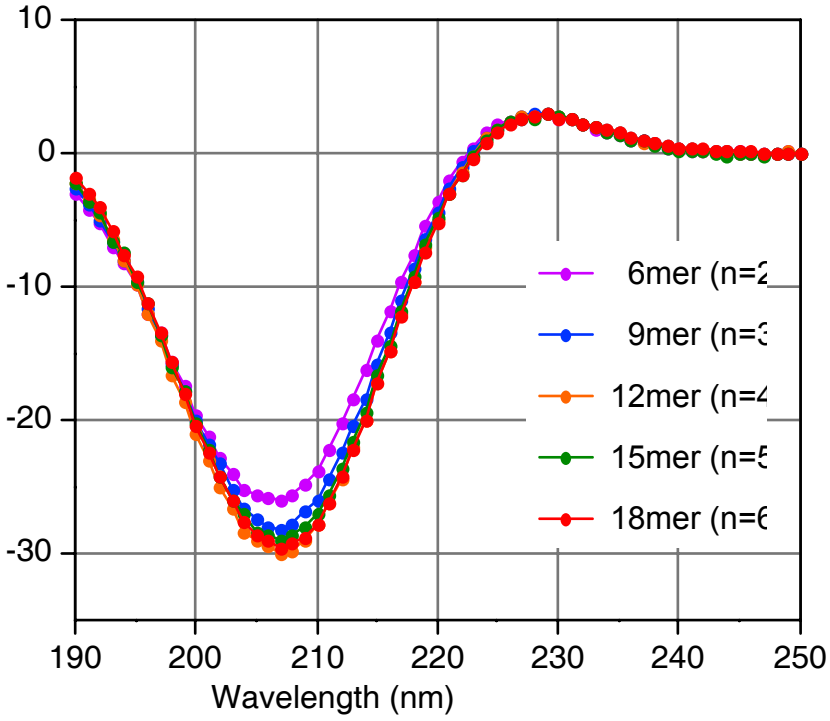
PPII

- What is the minimal chain length for conformationally well-defined PPI and PPII helices?
- Is functionalization possible?

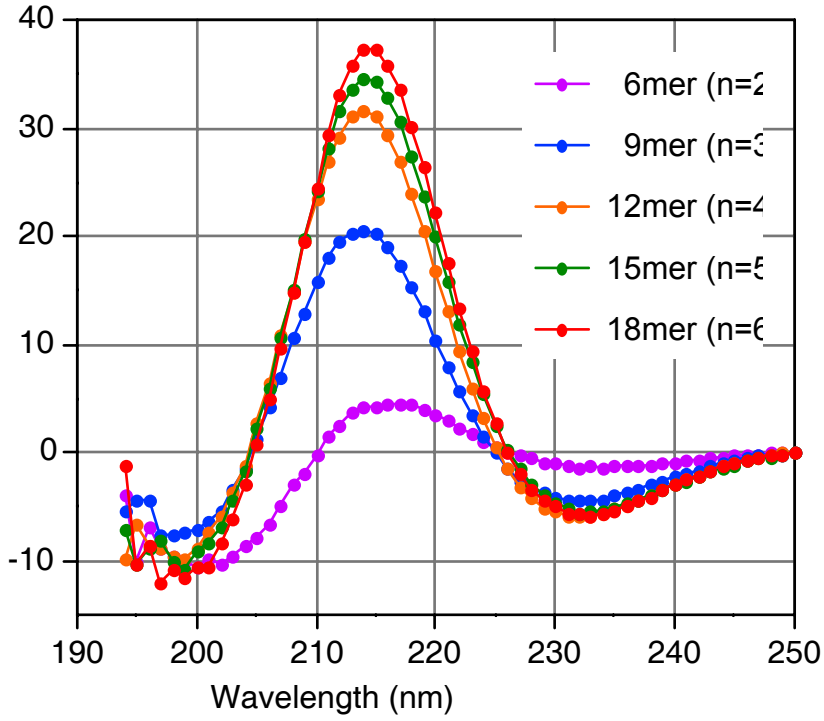
What is the minimal chain length for stable PPII and PPI helices?

Ac-[Pro-(4*R*)Azp-Pro]$_n$-OH (n=2-6), c = 630 mM/residue, T = 25°C

phosphate buffer (10 mM, pH 7.2)

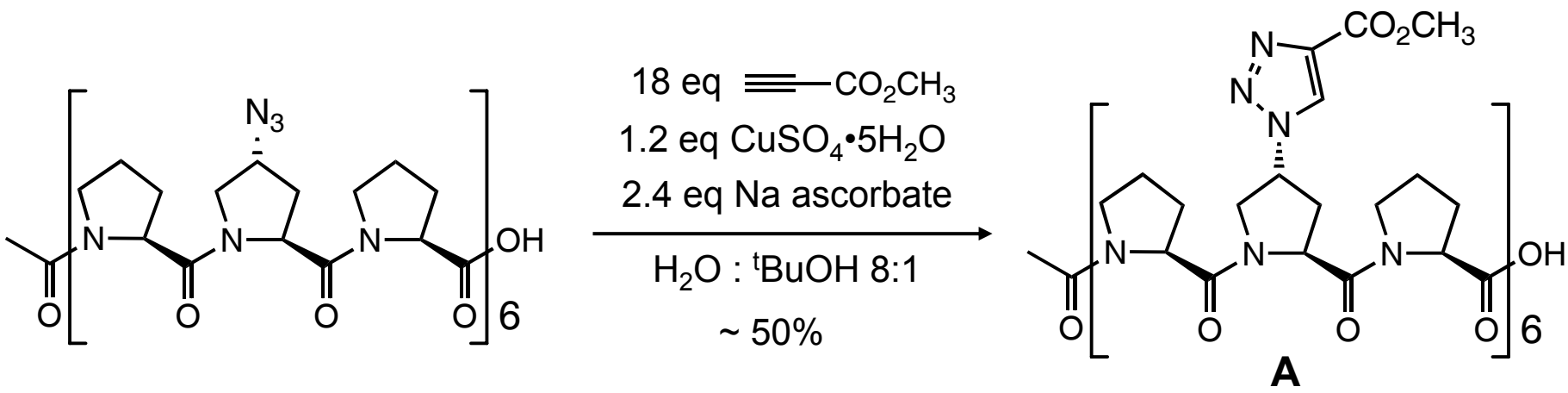


n-PrOH



Azp containing oligoprolines adopt already at short chain lengths conformationally well-defined PPI and PPII helices

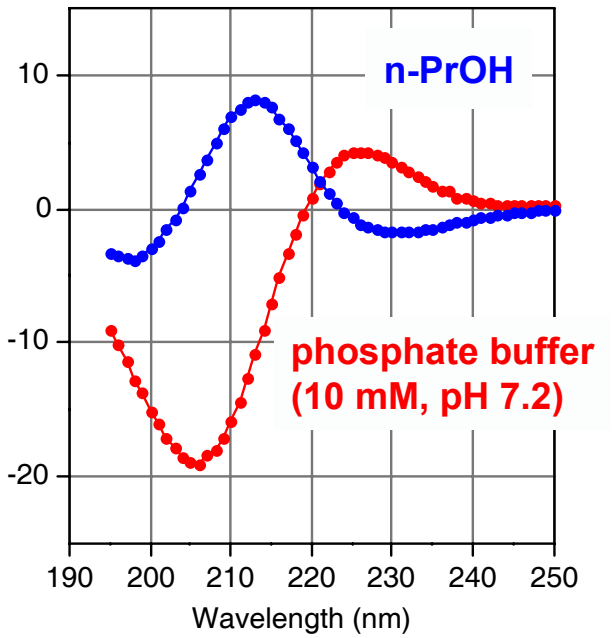
Functionalization via “Click Chemistry“



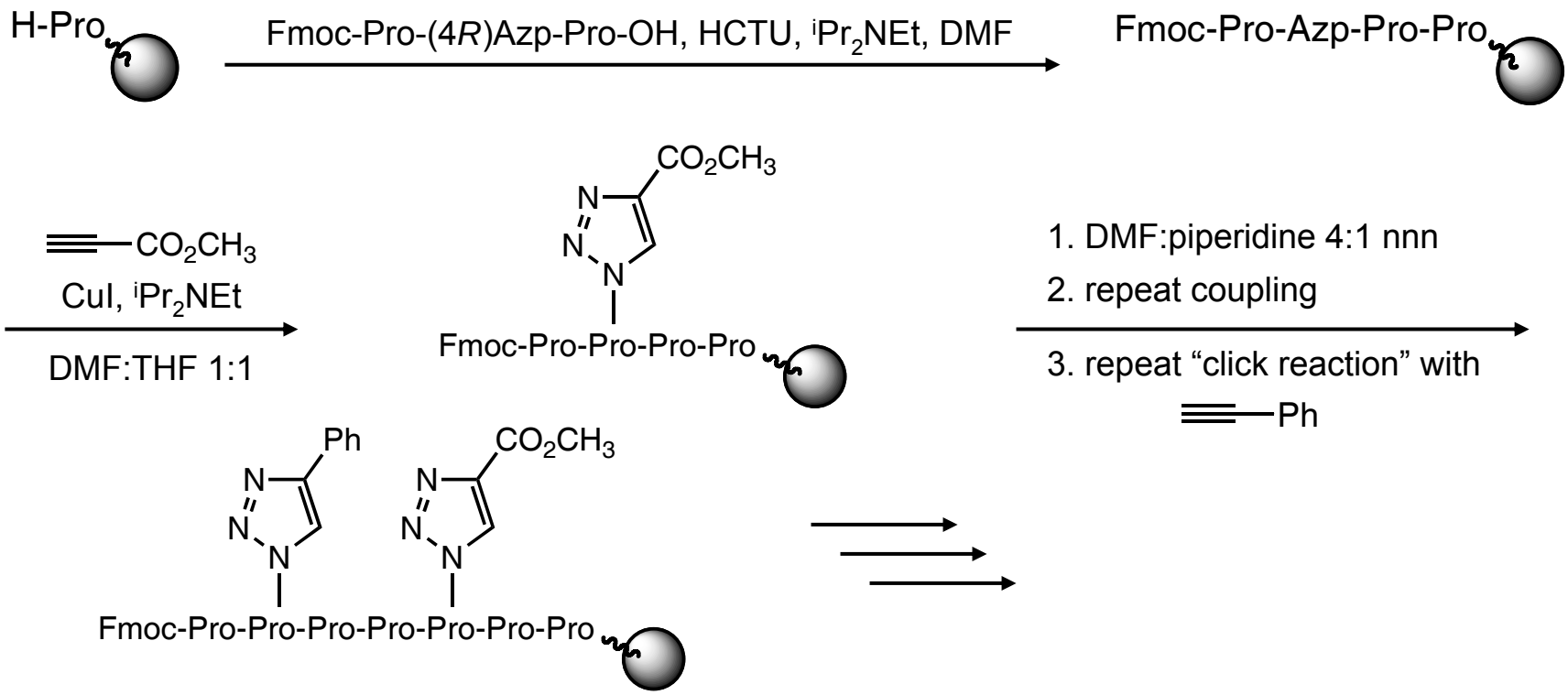
CD spectra of **A**

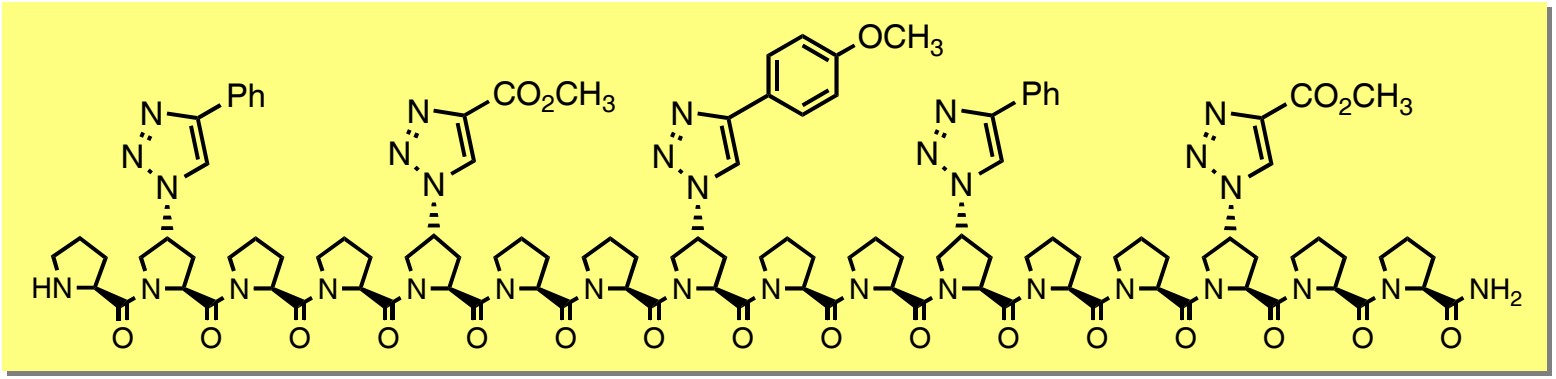
in n-PrOH: **PPI**

in phophate buffer: **PPII**



Differential Functionalization via “Click Chemistry“

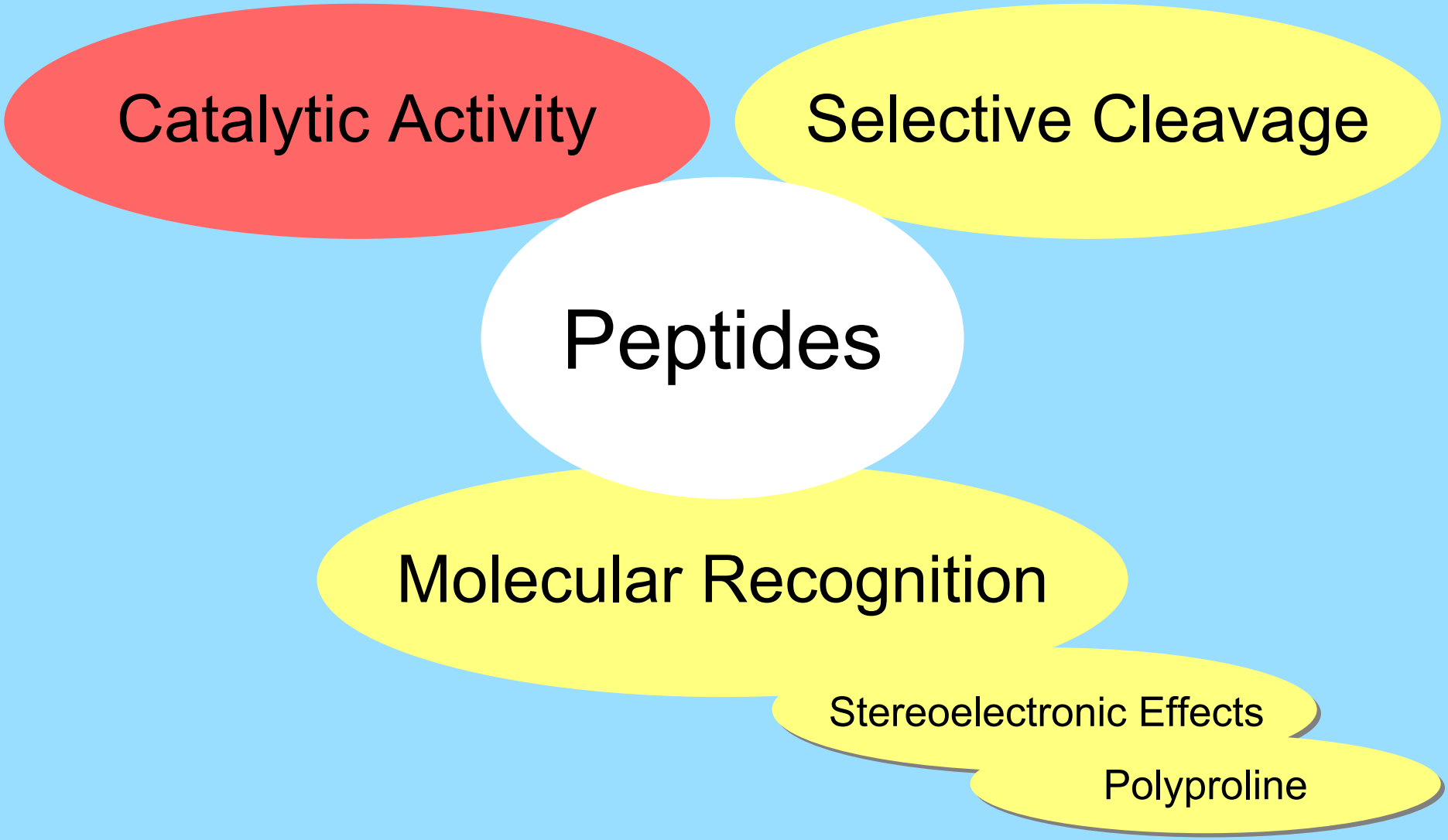




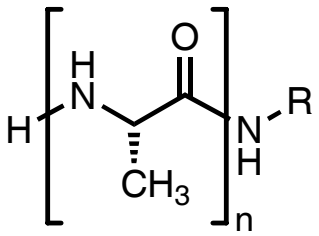
Summary and Outlook

- (4*R*)Azp stabilizes, (4*S*)Azp destabilizes the PPII helix
- an “azido gauche effect” and a n->π* interaction determines the conformation of (4*R*)Azp
- Azp containing oligoprolines are conformationally well-defined and switchable scaffolds that allow for further functionalization

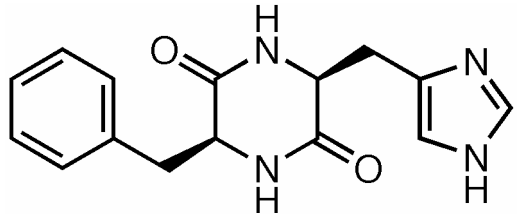
- Use of Azp as tools to tune the conformation of the PPII helix
- development of liquid crystalline materials, antibiotics, ……


Catalytic Activity
Selective Cleavage
Peptides
Molecular Recognition
Stereoelectronic Effects
Polyproline

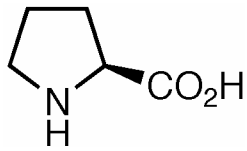
Peptidic Catalysts



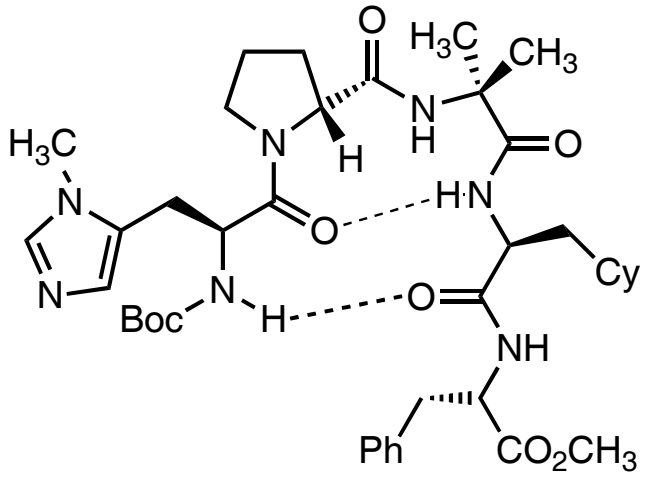
S. Juliá,1983
A. Berkessel, 2001



S. Inoue, 1992

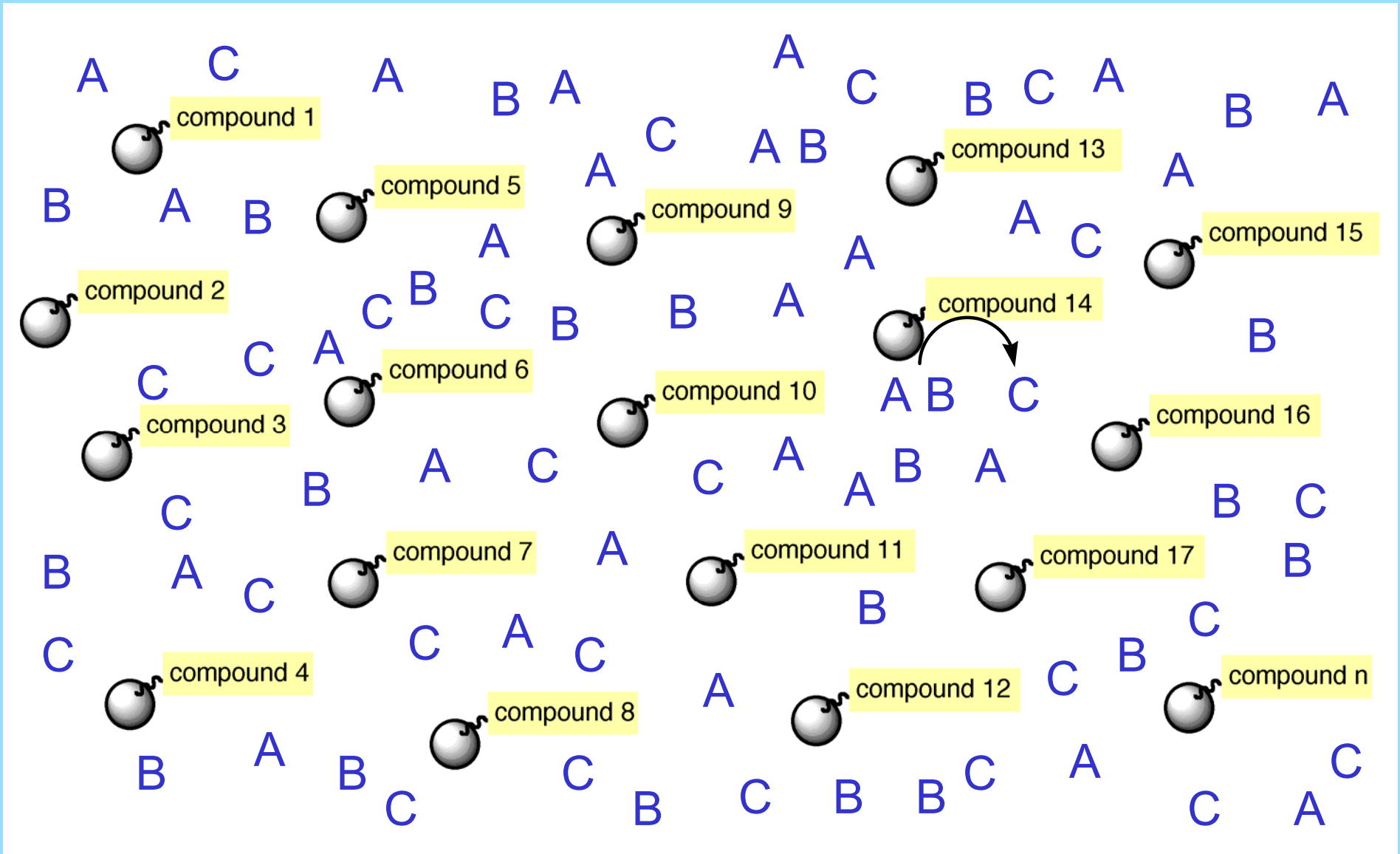


Hajos/Parrish/Wiechert, 1971
B. List, C.F. Barbas 2000, ...

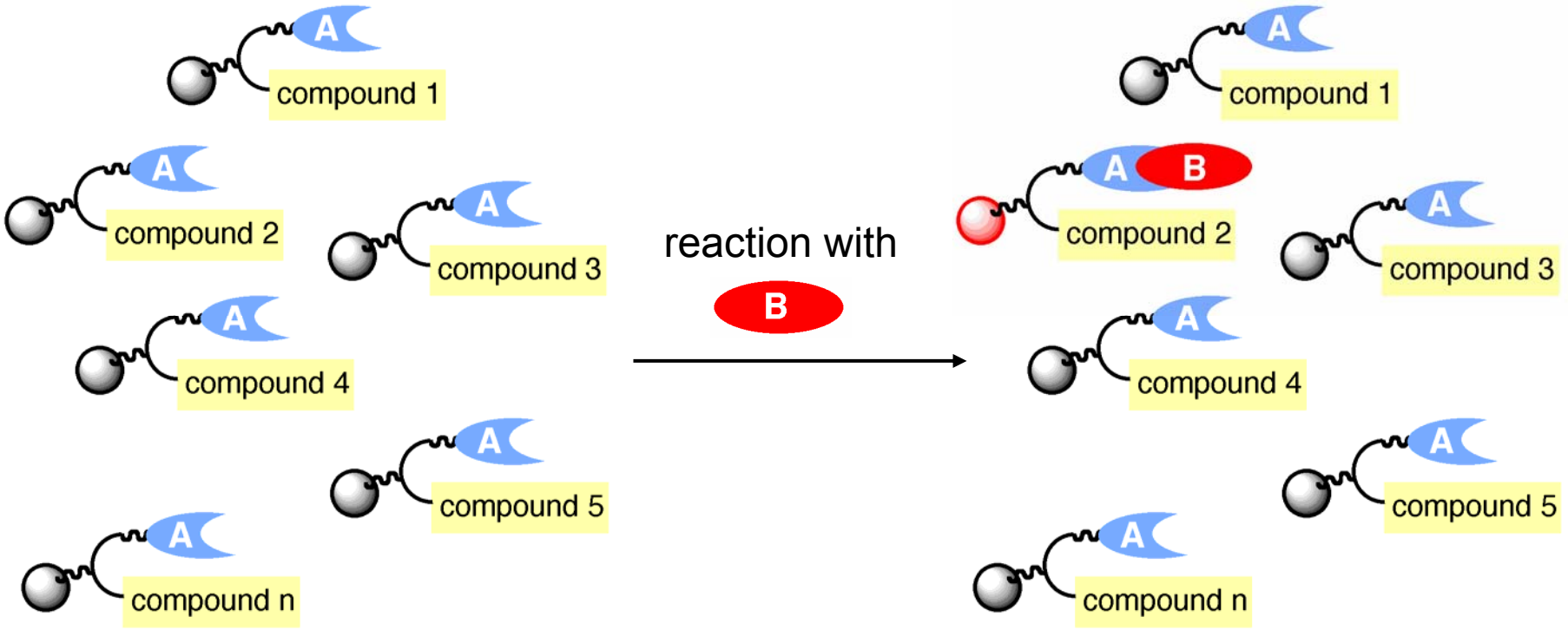


S.J. Miller, 1998

How to find the catalyst?



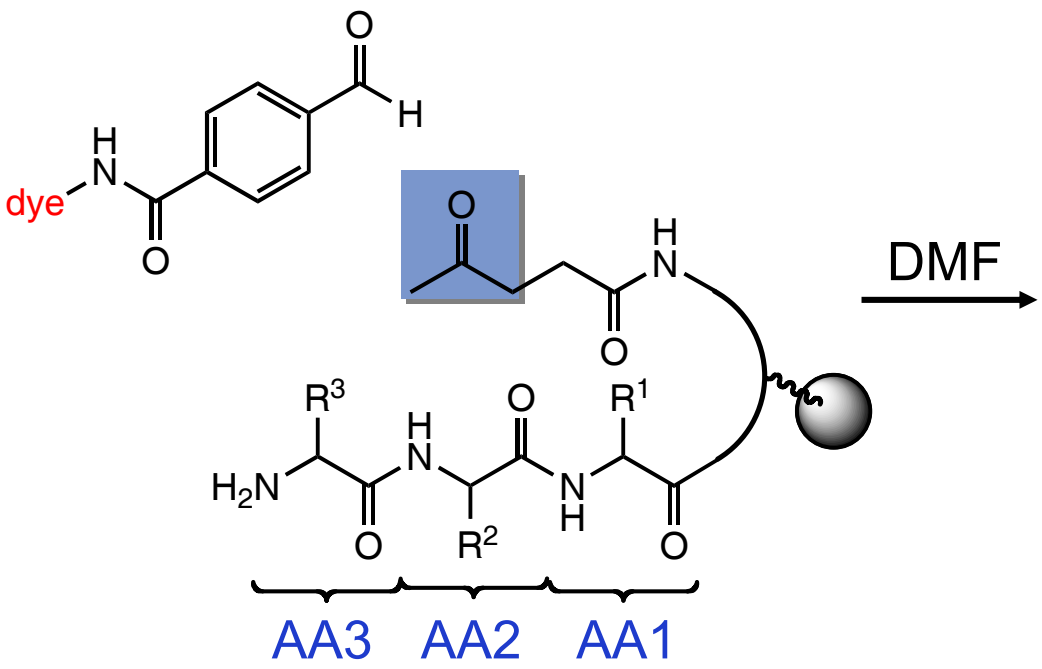
Catalyst-Substrate Co-immobilisation

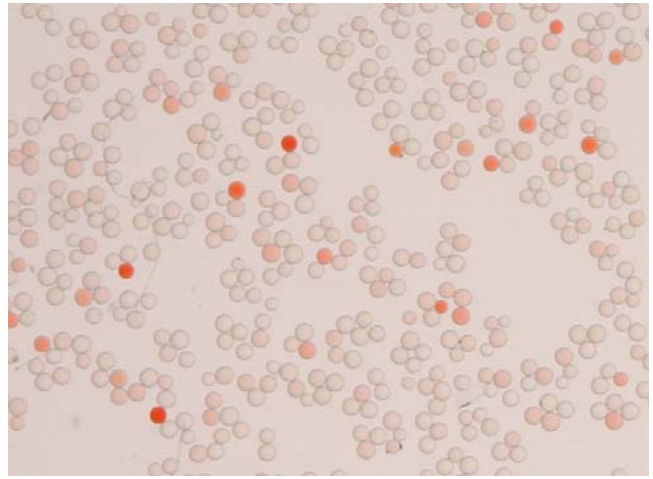


Only *compound 2* is an active catalyst!

P. Krattiger, C. McCarthy, A. Pfaltz, H. Wennemers, *Angew. Chem. Int. Ed.* **2003**, *42*, 1722.

Screening Peptides for Catalysts of Aldol Reactions



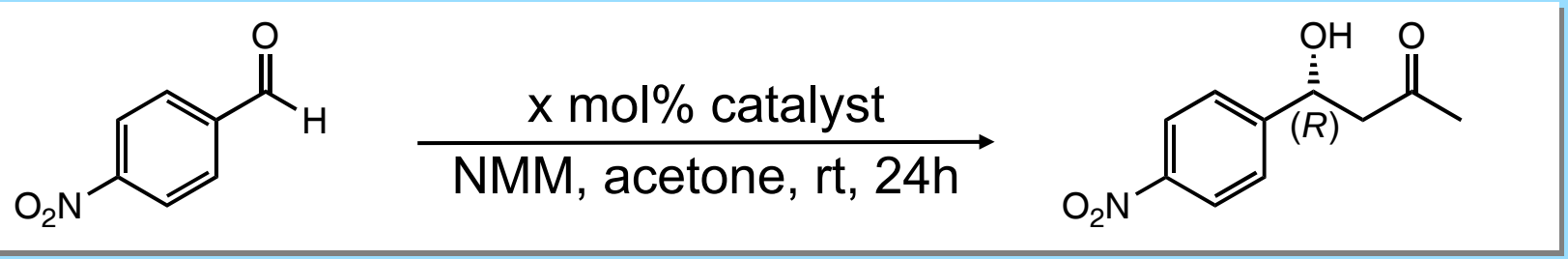


AAn = 15 different D- and L-amino acids ⟶ 15^3 = 3375 tripeptides

consensus sequences: H-Pro-Pro-Asp-NHR

H-Pro-D-Ala-D-Asp-NHR

Solution Phase Experiments

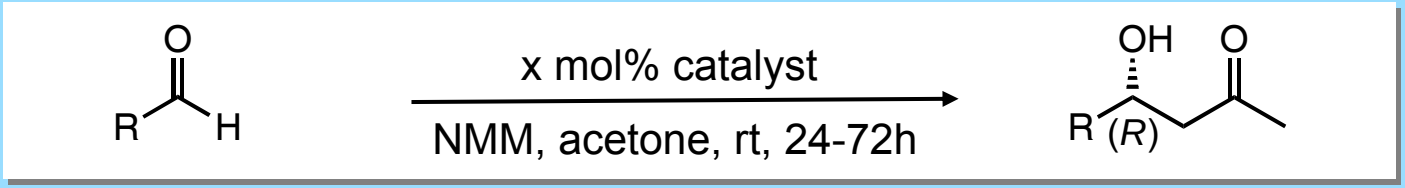


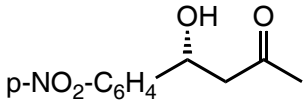
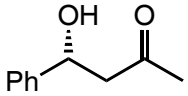
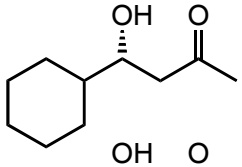
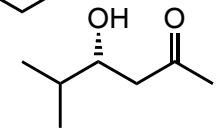
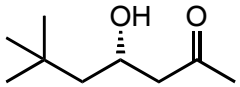
catalyst		mol%	yield (%)	ee (%)	abs. conf.
H-Pro-D-Ala-D-Asp-NH_2		10	73	70	*R*
H-Pro-D-Ala-D-Asp-NH_2	(-20°C)	10	53	81	*R*
H-Pro-Pro-Asp-NH_2		1	99	80	*S*
H-Pro-Pro-Asp-NH_2	(-20°C)	5	98	90	*S*
H-Pro-OH[a]		30	68	76	*R*
H-Pro-OH	(-20°C)	30	nd	71	*R*

[a] B. List, *J. Am. Chem. Soc.* **2000**, *122*, 2395.

P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers, *Org. Lett.* **2005**, *7*, 1101.

....other substrates

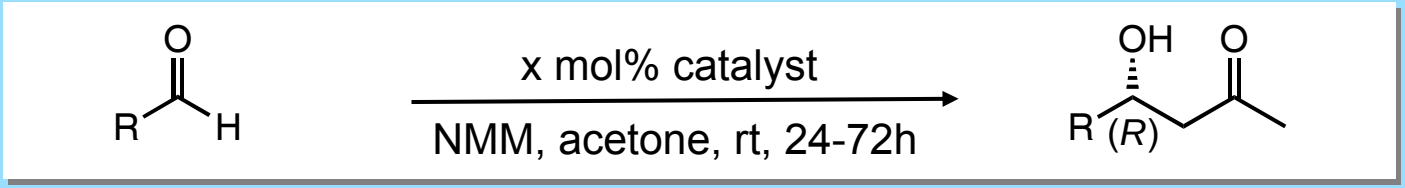


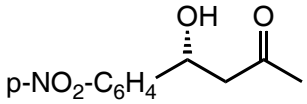
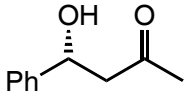
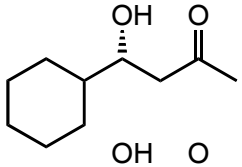
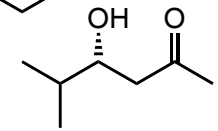
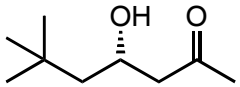
Product	H-Pro-OH[a] yield (%) / ee (%)	H-Pro-D-Ala-D-Asp-NH_2 yield (%) / ee (%)	H-Pro-Pro-Asp-NH_2 yield (%) / ee (%)
	68 /	73 /	99 /
	62 /	58 /	69 /
	63 /	56 /	66 /
	97 /	75 /	79 /
	22 /	24 /	28 /
	30 mol%	10 mol%	1 mol%

[a] B. List, *J. Am. Chem. Soc.* **2000**, *122*, 2395.

P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers, *Org. Lett.* **2005**, *7*, 1101.

....other substrates

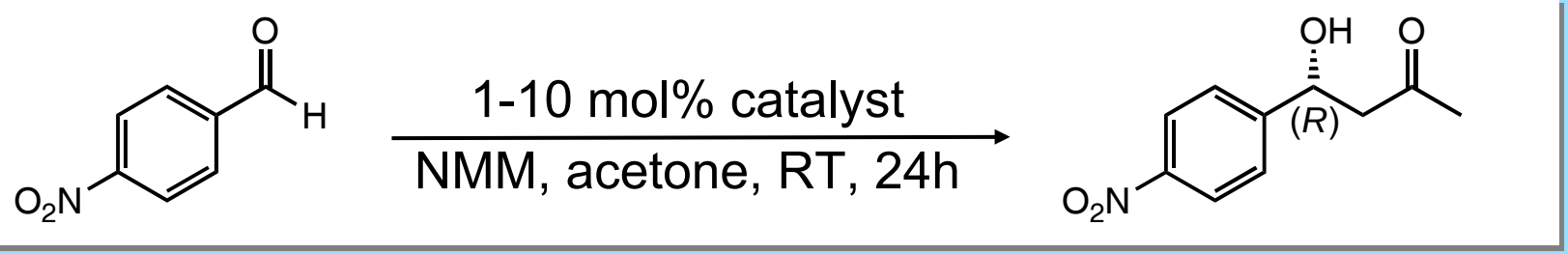


Product	H-Pro-OH[a] yield (%) / ee (%)	H-Pro-D-Ala-D-Asp-NH_2 yield (%) / ee (%)	H-Pro-Pro-Asp-NH_2 yield (%) / ee (%)
	76 (*R*)	70 (*R*)	80 (*S*)
	60 (*R*)	66 (*R*)	78 (*S*)
	84 (*R*)	83 (*R*)	82 (*S*)
	96 (*R*)	91 (*R*)	79 (*S*)
	36 (*S*)	70 (*S*)	73 (*R*)
	30 mol%	10 mol%	1 mol%

[a] B. List, *J. Am. Chem. Soc.* **2000**, *122*, 2395.

P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers, *Org. Lett.* **2005**, *7*, 1101.

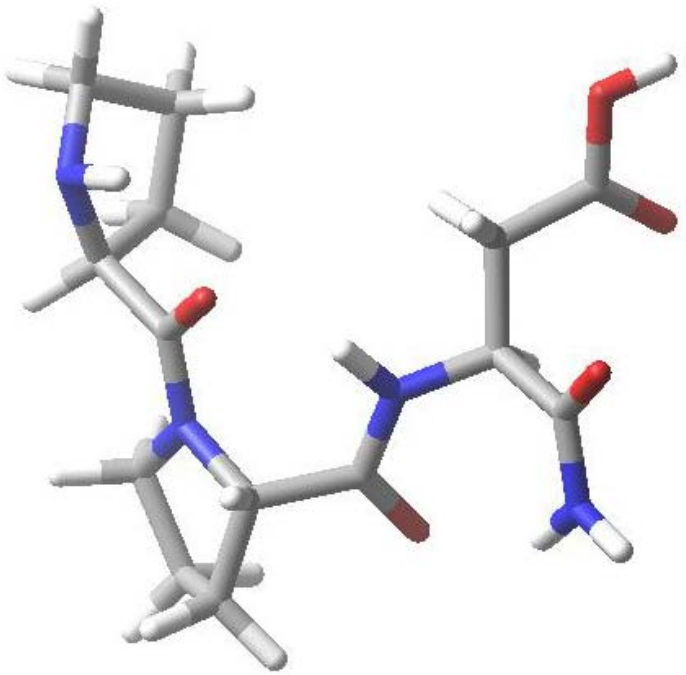
....other peptides



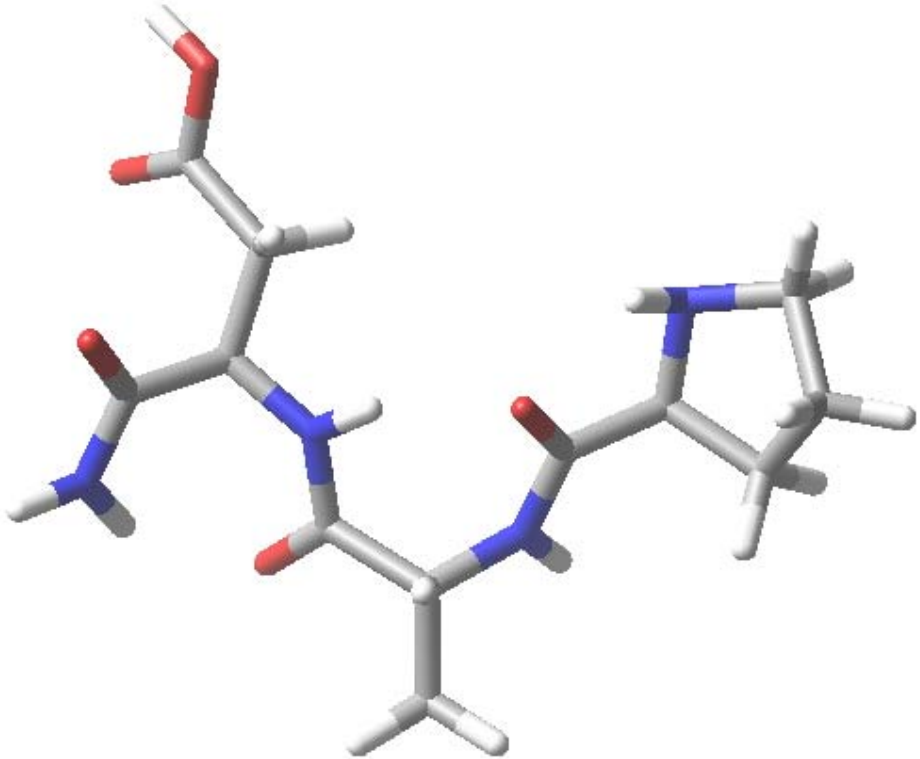
catalyst		yield (%)	ee (%)	abs. conf.
H-Pro-Pro-Asp-NH_2		99	80	*S*
H-Pro-Pro-Asp-NH_2	(-20°C)	98	90	*S*
H-Pro-Pro-Asn-NH_2		23	50	*S*
H-Pro-Pro-Glu-NH_2		60	62	*S*
H-D-Pro-D-Pro-Asp-NH_2		27	11	*R*
H-Pro-Pro-OH		26	7	*S*
H-Pro-D-Ala-D-Asp-NH_2		73	70	*R*
H-Pro-D-Ala-D-Asp-NH_2	(-20°C)	53	81	*R*
H-Pro-Aib-D-Asp-NH_2		73	62	*R*
H-Pro-D-Ala-OH		38	27	*R*

Conformational Analysis

H-Pro-Pro-Asp-NH_2



H-Pro-D-Ala-D-Asp-NH_2



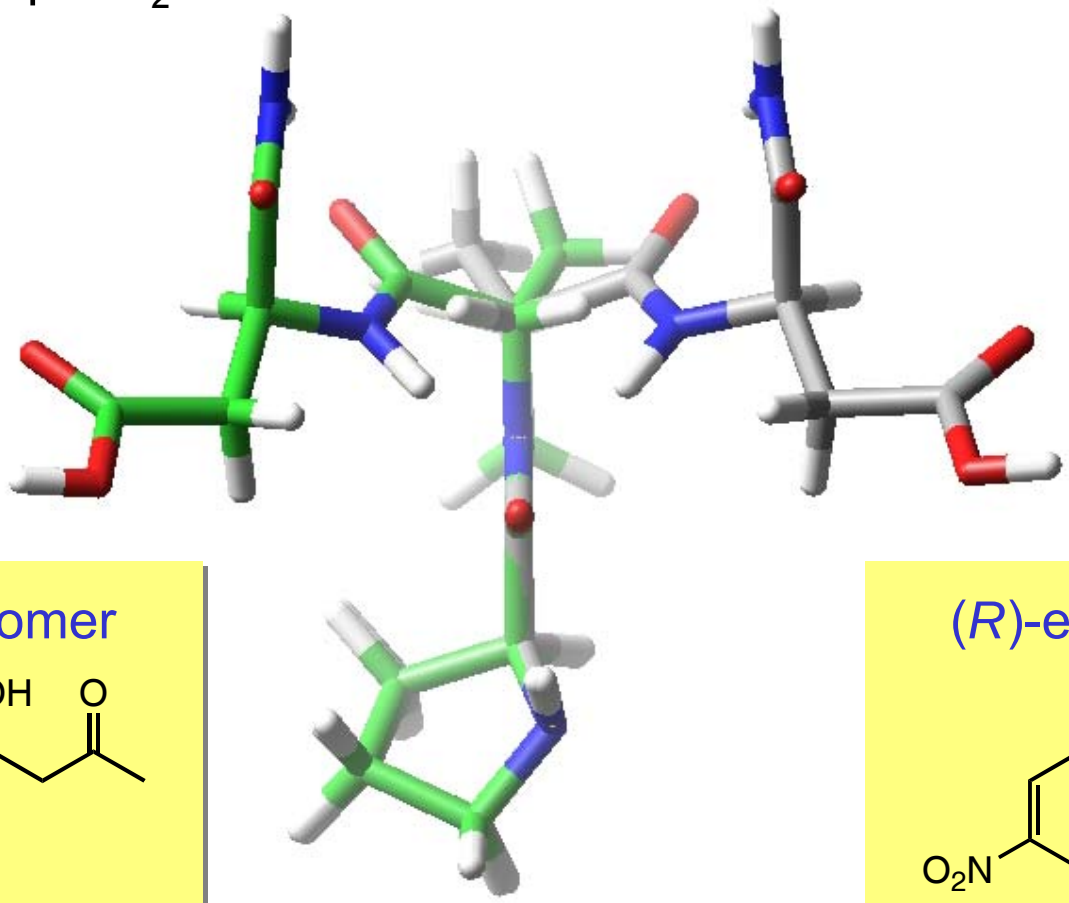
turn-conformations!

MacroModel 8.0, OPLS force field

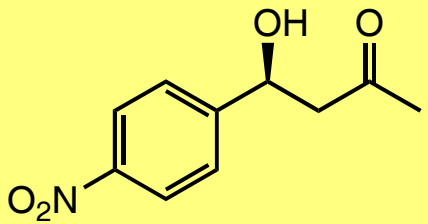
Conformational Analysis

H-Pro-Pro-Asp-NH_2

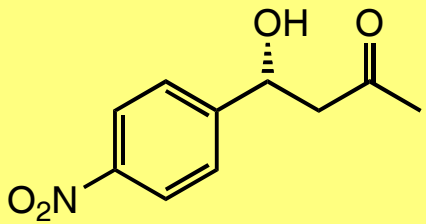
H-Pro-D-Ala-D-Asp-NH_2



(*S*)-enantiomer

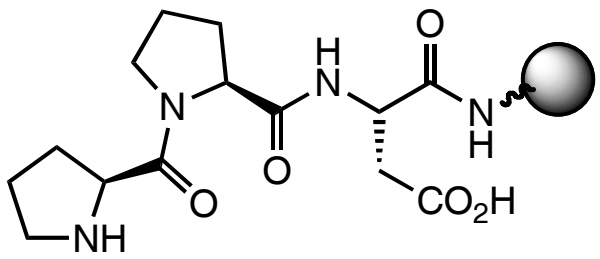


(*R*)-enantiomer

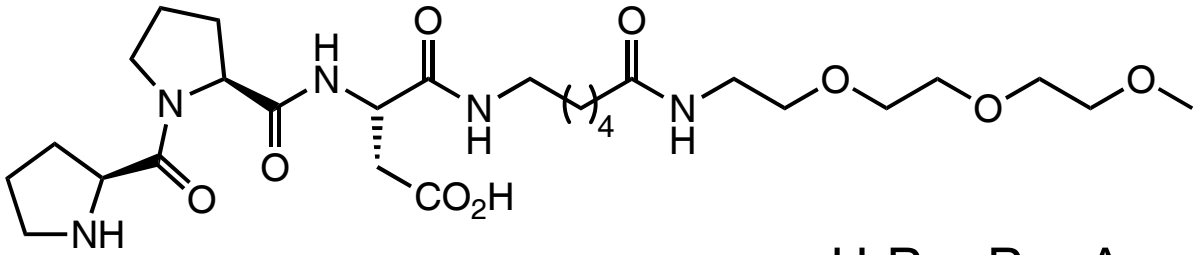


P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan, H. Wennemers, *Org. Lett.* **2005**, *7*, 1101.

Solid-supported and Pegylated H-Pro-Pro-Asp-NHR



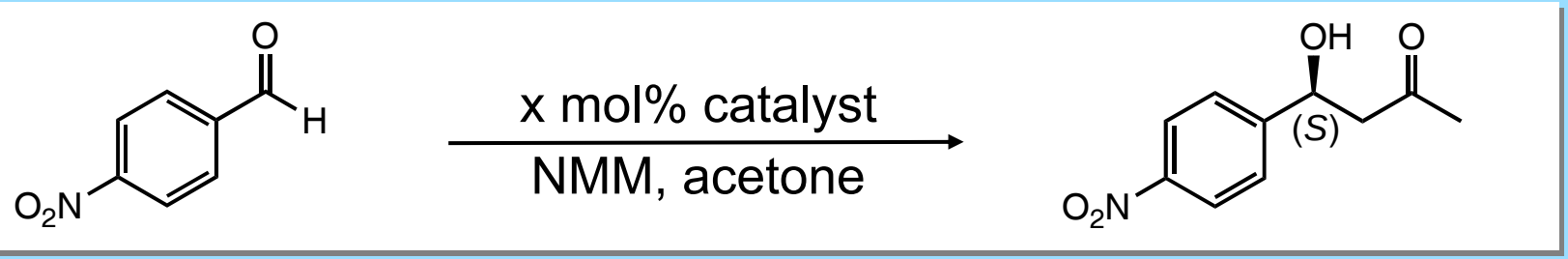
= H-Pro-Pro-Asp-resin (PPD-resin)



= H-Pro-Pro-Asp-Ahx-PEG

J. D. Revell, D. Gantenbein, P. Krattiger, H. Wennemers, *Biopolymers (Pept. Sci.)* **2006**, *84*, 105.

Pegylated H-Pro-Pro-Asp-NHR

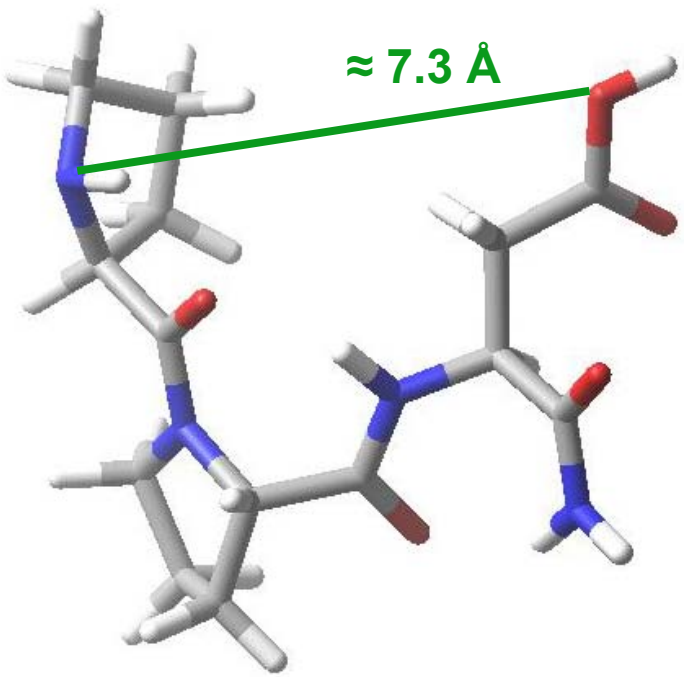


catalyst		mol%	yield (%)	ee (%)	time
H-Pro-Pro-Asp-NH_2		1	99	80	4
H-Pro-Pro-Asp-NH_2	(-20°C)	5	98	90	24
H-Pro-Pro-Asp-Ahx-PEG		1	94	80	1
H-Pro-Pro-Asp-Ahx-PEG	(-20°C)	1	96	91	4
H-Pro-Pro-Asp-Ahx-PEG		0.5	93	80	4
H-Pro-Pro-Asp-Ahx-PEG	(-20°C)	0.5	95	91	8

J. D. Revell, D. Gantenbein, P. Krattiger, H. Wennemers, *Biopolymers (Pept. Sci.)* **2006**, *84*, 105.

Conformational Analysis

H-Pro-Pro-Asp-NH_2



Distance between the secondary amine and the carboxylate is $\geq$ 3 Å longer compared to proline.

↓

H-Pro-Pro-Asp-NH_2 a catalyst for 1,4-additions?

Outlook

- substrate/reaction scope, mechanism

- What is the optimal size of a catalytically active peptide?

- Peptides as catalysts for other reactions, e.g. Cycloadditions?

  ....non-covalent catalysts

Acknowledgements

Current Coworkers

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Kirsten Koch
Michael Kümin
Jana Lubkoll
Dr. Jefferson D. Revell
Lucy Rush
Dr. Nitya Srinivas
Markus Wiesner

Former Coworkers

Dr. Matteo Conza
Dr. Matthias Nold
Dr. Philipp Krattiger
Catrina Ragettli
Dr. Sebastian Sonntag

Collaborators

Prof. Charl Faul (Bristol University)
Prof. Wolfgang Meier (Basel University)
Prof. Joachim Bargon (University of Bonn)
Prof. Christian Ochsenfeld (University of Tübingen)

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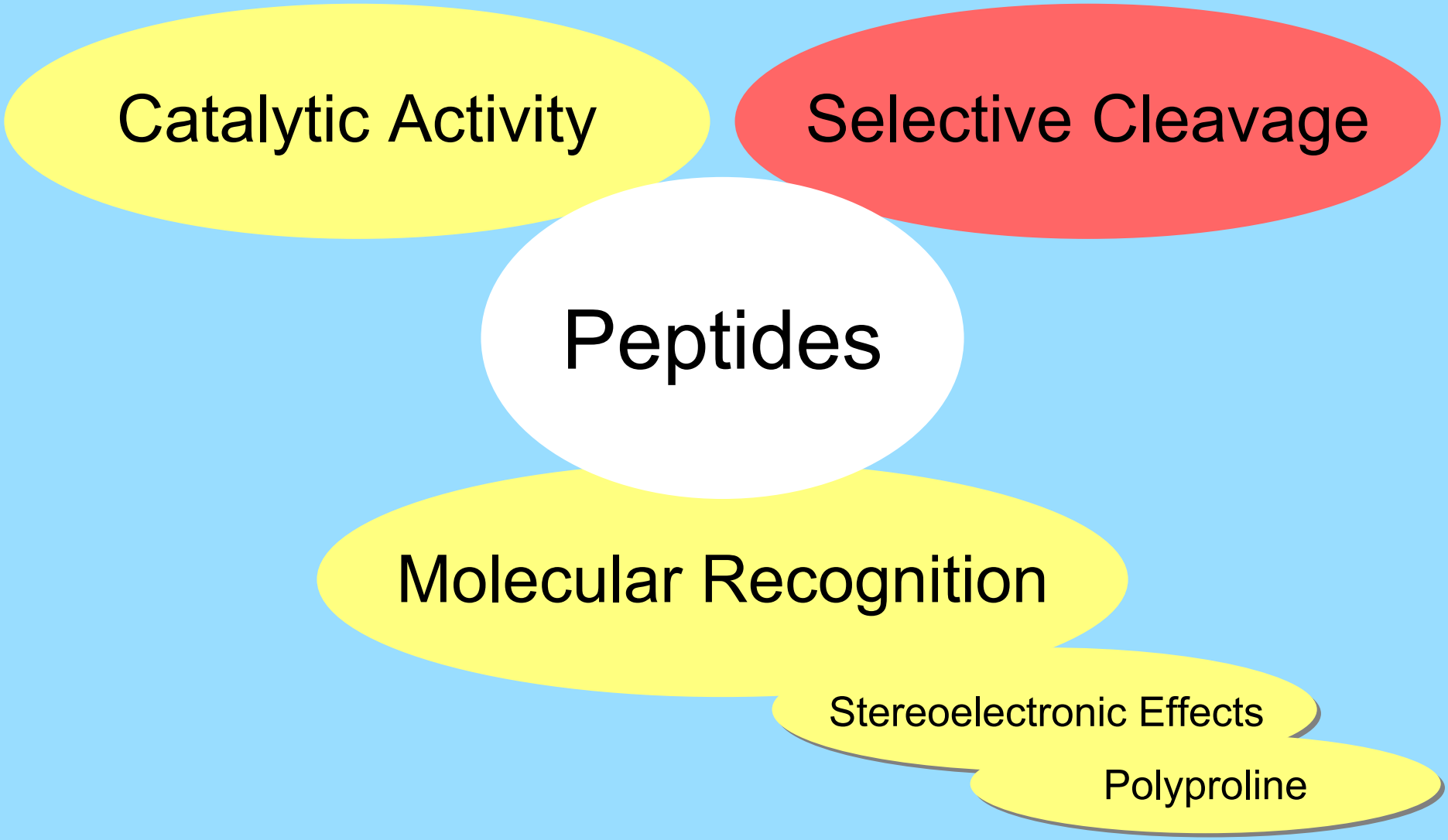
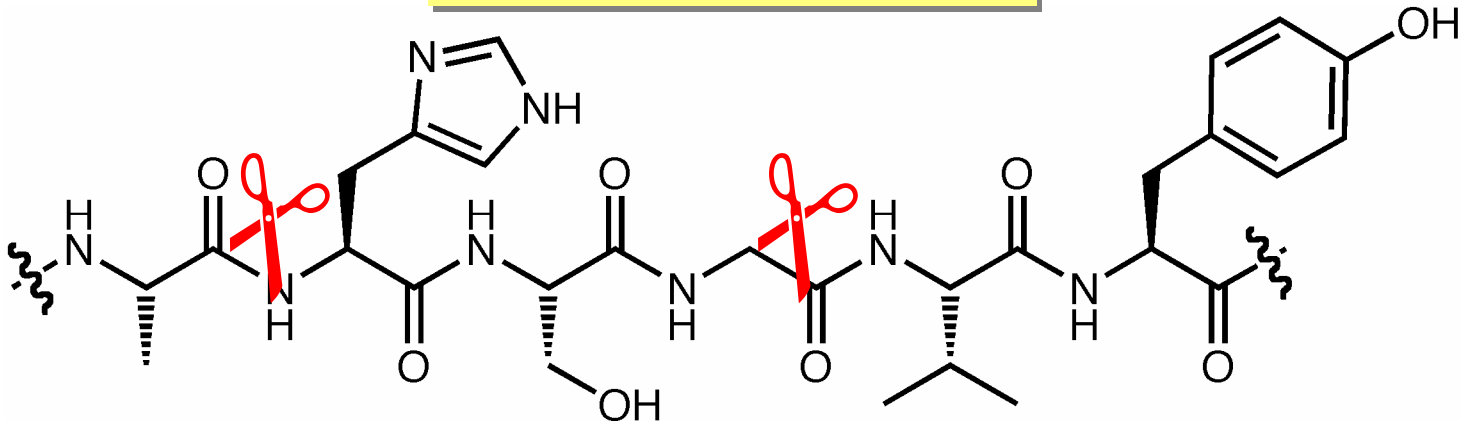
Roche Foundation
BACHEM AG


Catalytic Activity
Selective Cleavage
Peptides
Molecular Recognition
Stereoelectronic Effects
Polyproline

Protein Damage

for example, Fenton reaction: Fe^{3+}, reducing agent, H_2O_2

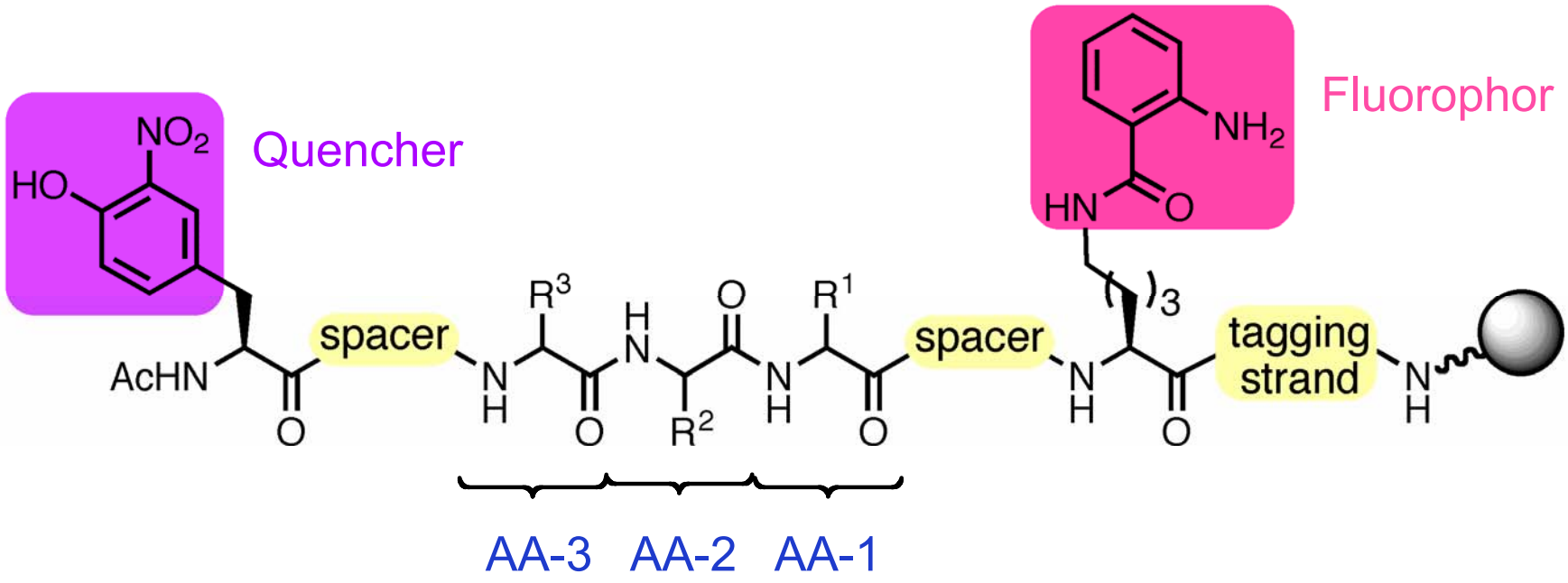
backbone damage



side-chain damage

Is the cleavage sequence dependent?

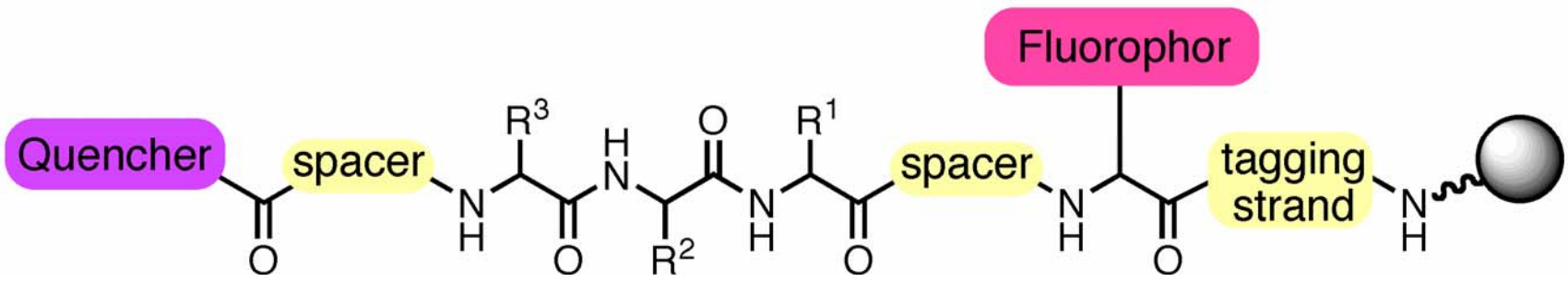
Fluorophor-Quencher-Peptide Library



AA-n = 31 different D- and L-amino acids

→ 31^3 = 29791 peptides

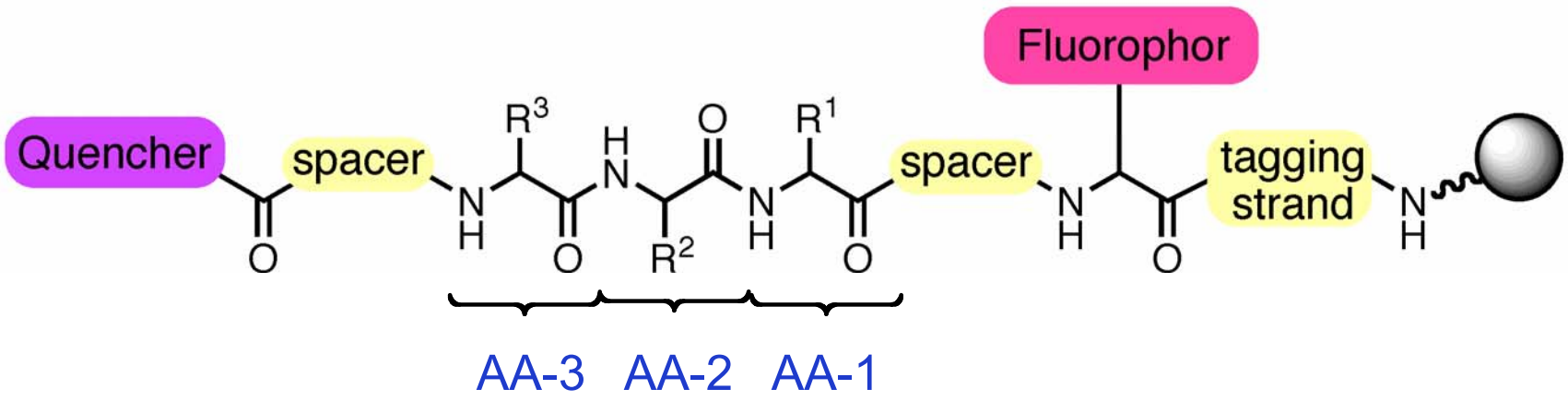
Cleavage under “Fenton Conditions“



HEPES buffer pH 5

1. $FeCl_3$
2. H_2O_2, NaAscorbate

Cleavage Selectivities



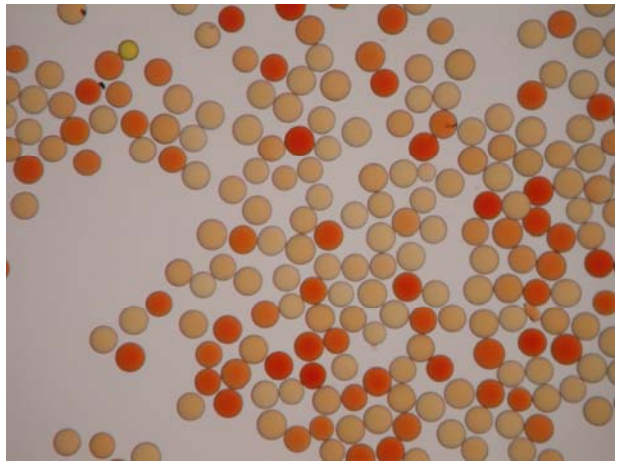
AA-3	AA-2	AA-1	frequency found
L/D-Asp/Glu	L/D-Asp/Glu	X	34%
X	L/D-Asp/Glu	L/D-Asp/Glu	46%
L/D-Asp/Glu	X	L/D-Asp/Glu	13%

Fe-Peptide Complexes

studies on solid support

tripeptide library complexed with $FeCl_3$
followed by staining with KSCN

red beads: carboxylic acid-rich peptides



studies in solution

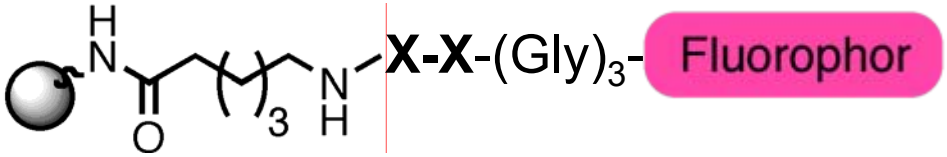
Ac-Glu-Glu-$NHCH_2CH_2CH_3$

binding to $FeCl_2$: $K \approx 10^3$-$10^4\ M^{-1}$

Ac-Gly-Gly-$NHCH_2CH_2CH_3$

no binding to $FeCl_2$ observed

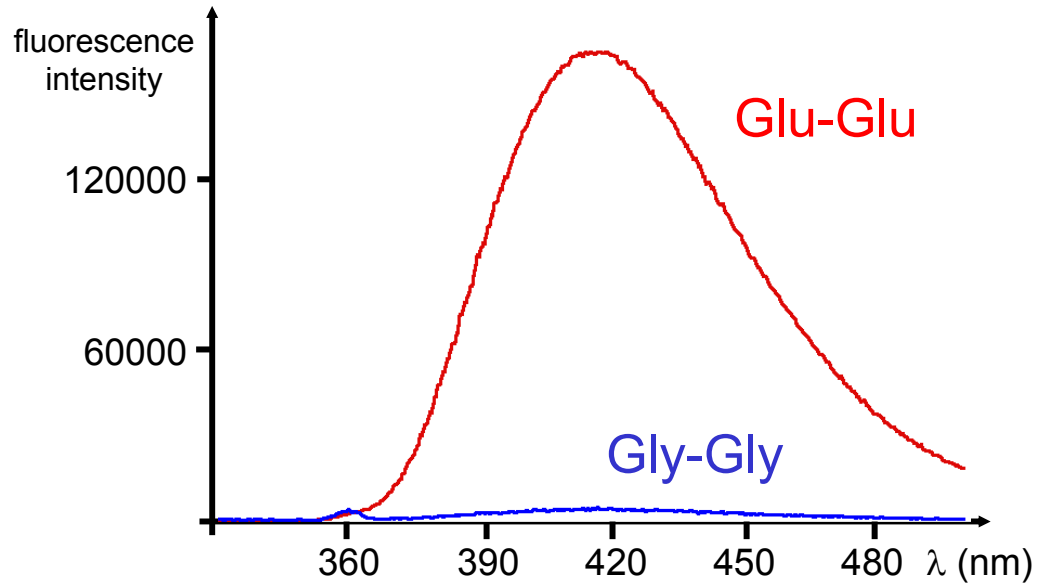
Cleavage Studies with Solid-supported Peptides



X-X = Glu-Glu
X-X = Gly-Gly

1. $FeCl_3$
2. H_2O_2, NaAsc
3. MnO_2 quench

filter and measure fluorescence of solution



M. Nold, H. Wennemers, *Chem. Commun.* **2004,** 1800; M. Nold, K. Koch, H. Wennemers *Synthesis* **2005**, 1455.