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Integrins as Drug Targets: Rational and Combinatorial Development of Selective Ligands for Integrins

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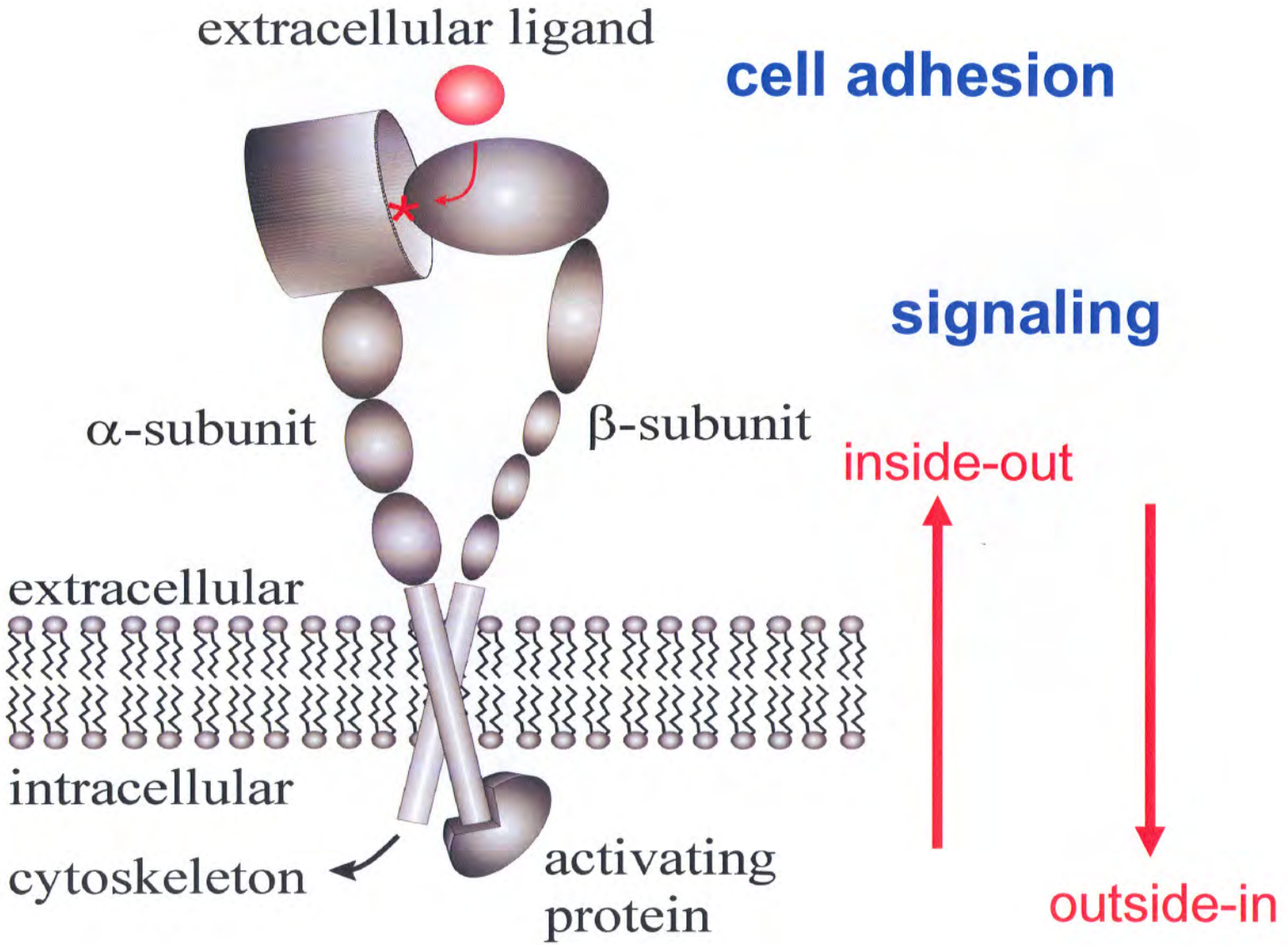
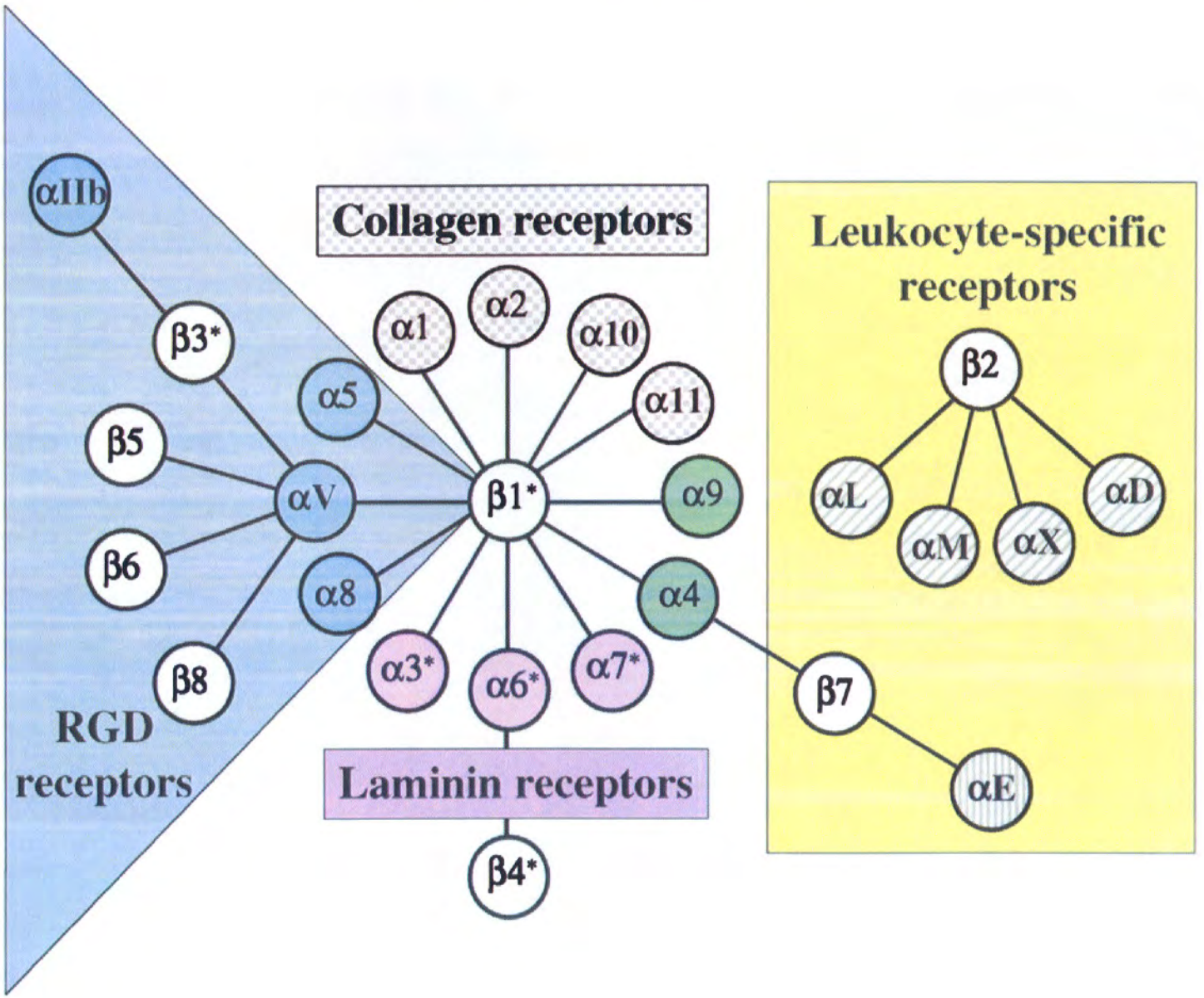
Outline

1. **Introduction**
2. RGD Peptides
3. Applications
   - 3.1. Therapy
   - 3.2. Diagnosis
   - 3.3. Biomaterials
4. From Peptides to Non-Peptides
5. Mechanism of Signal Transduction
6. Non-RGD Peptidomimetics

Biology of Integrins



The Integrin Receptor Family



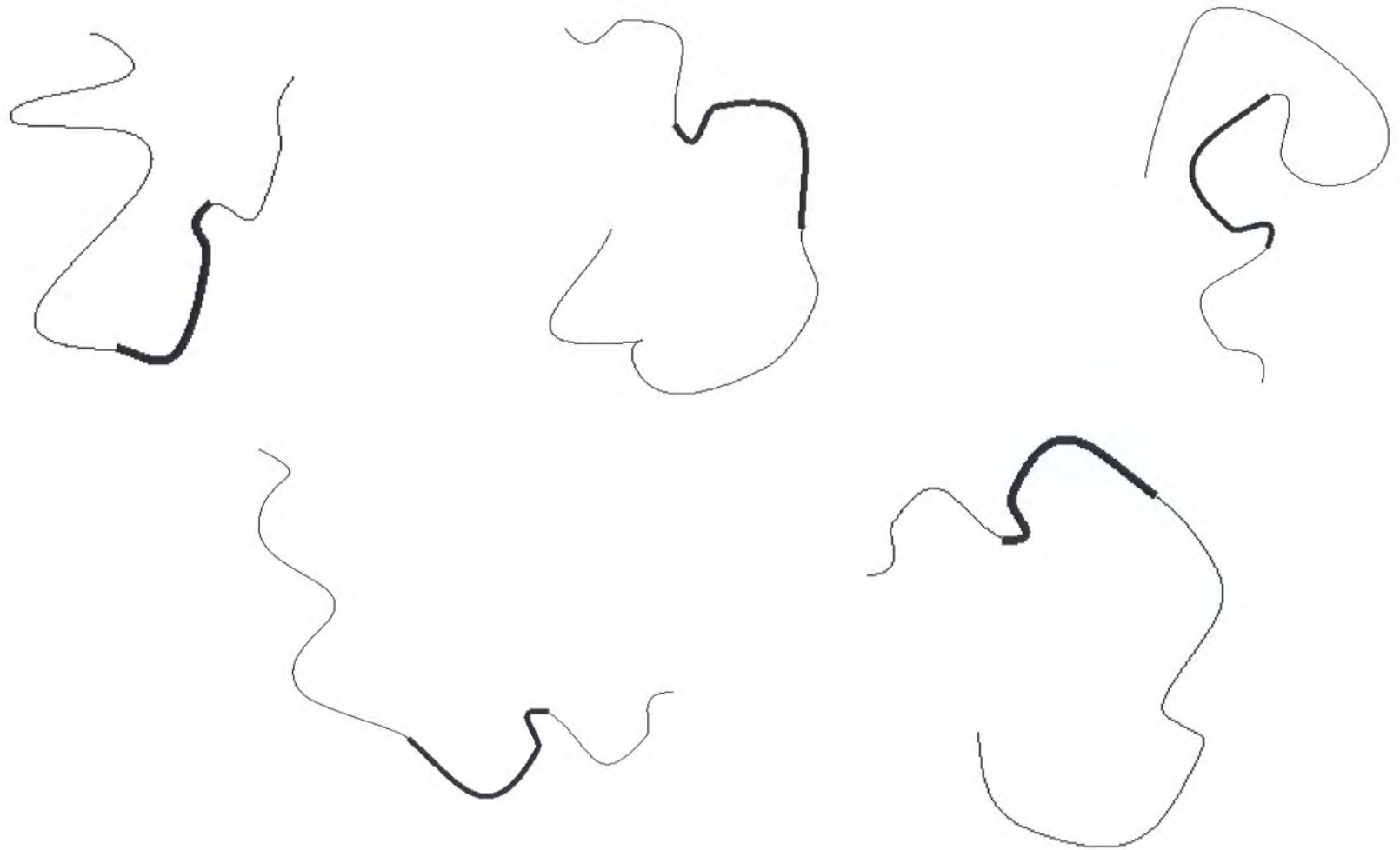
Integrines and Their Ligands (blue: RGD binding)

	α1	α2	α3	α4	α5	α6	α7	α8	α9	α10	αE	αL	αM	αX	αD	αIIb	αv
β1	Col Ln	Col Ln Fn	Col Ln5 Fn	Fn In VCAM-1	Fn	Ln	Ln	Fn Vn Tn	Col Ln Tn	Col							Vn Fn Opn
β2												ICAM-1 ICAM-2	iC3b Fb Factor X ICAM-1 ICAM-2	iC3b Fb	ICAM-3 VCAM-1		
β3																Fb Fn Vn vW F Tsp	Vn Ln Fn vWF Tsp Tn Opn Fib Fbu
β4						Ln Ep											
β5																	Vn, Fn Opn, Fb vWF
β6																	Fn, Fb Tn
β7				MadCAM -1, Fn VCAM-1							E-Cad						
β8																	Vn

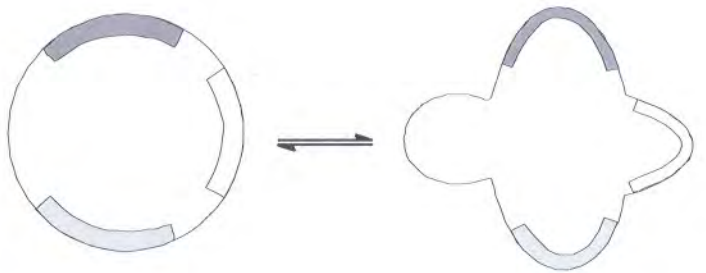
Where comes the specifity ?

- additional binding epitopes ?
- different bioactive conformations ?
- both ?

A Linear Peptide Can Adopt Multible Conformations

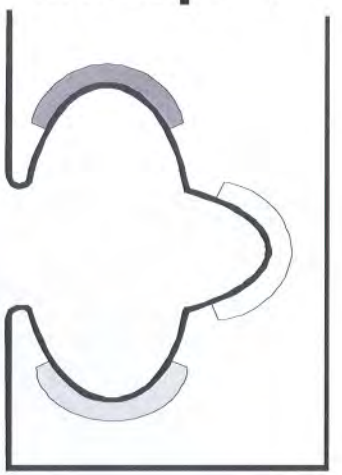


Flexible Molecules in Solution



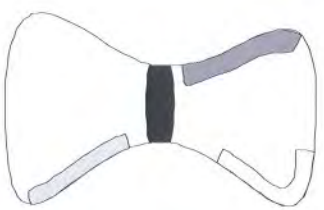
not active active

Receptor



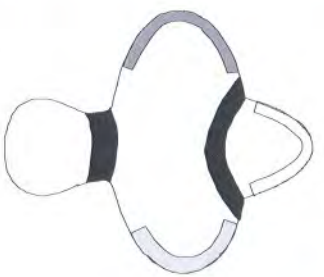
The more the structure of the free ligand in solution resembles the structure of the ligand in the complex, the stronger the binding, i.e. the more active is the ligand

Fixation of the conformation via cyclization



inactive

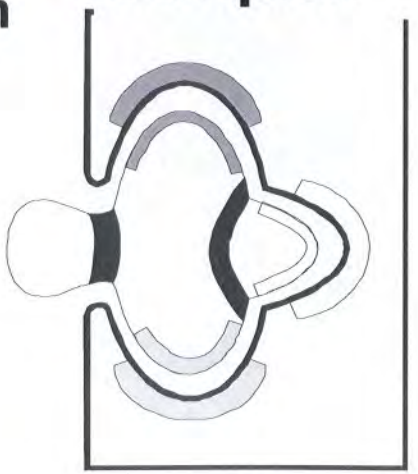
mismatched case



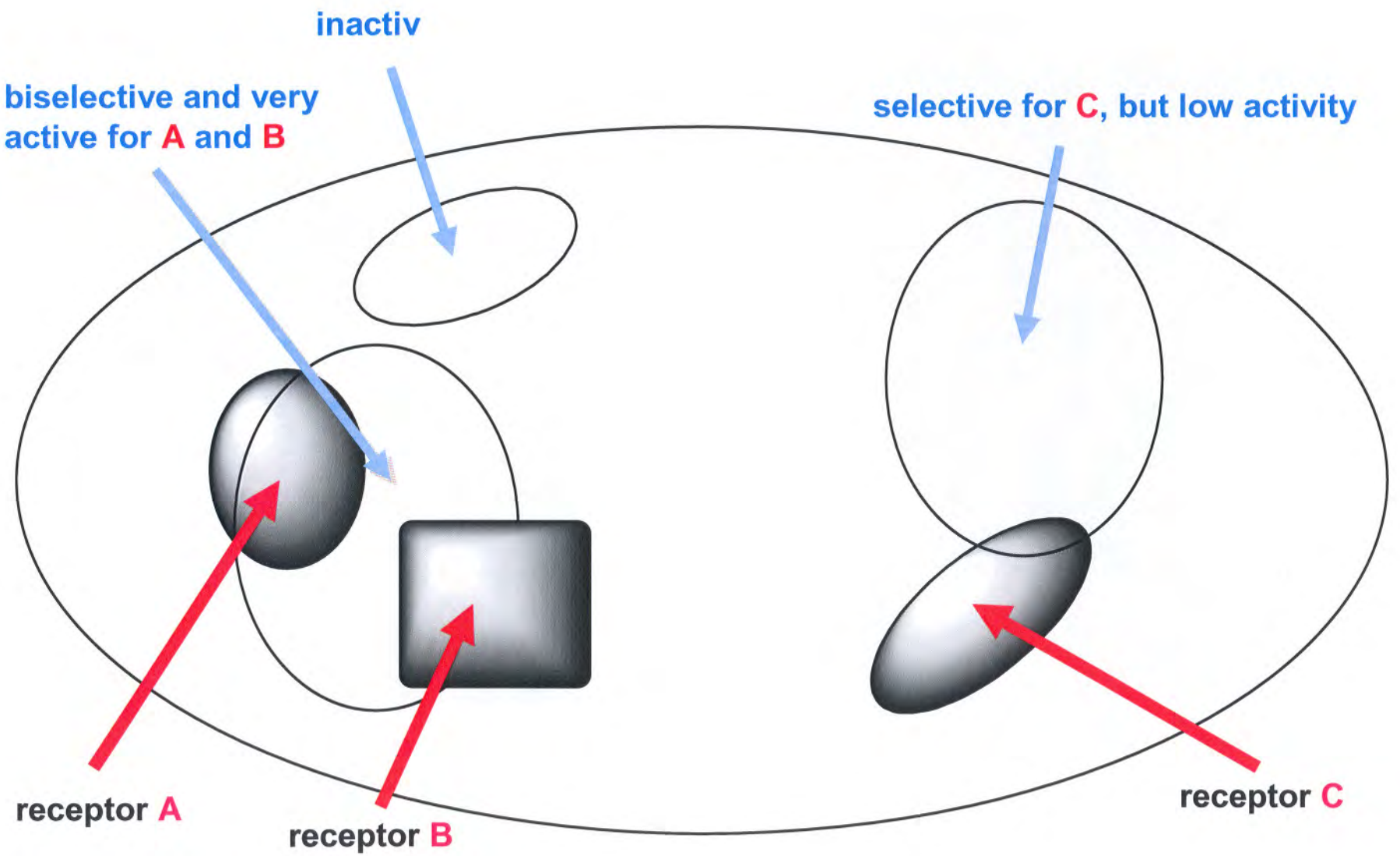
super-active

matched case

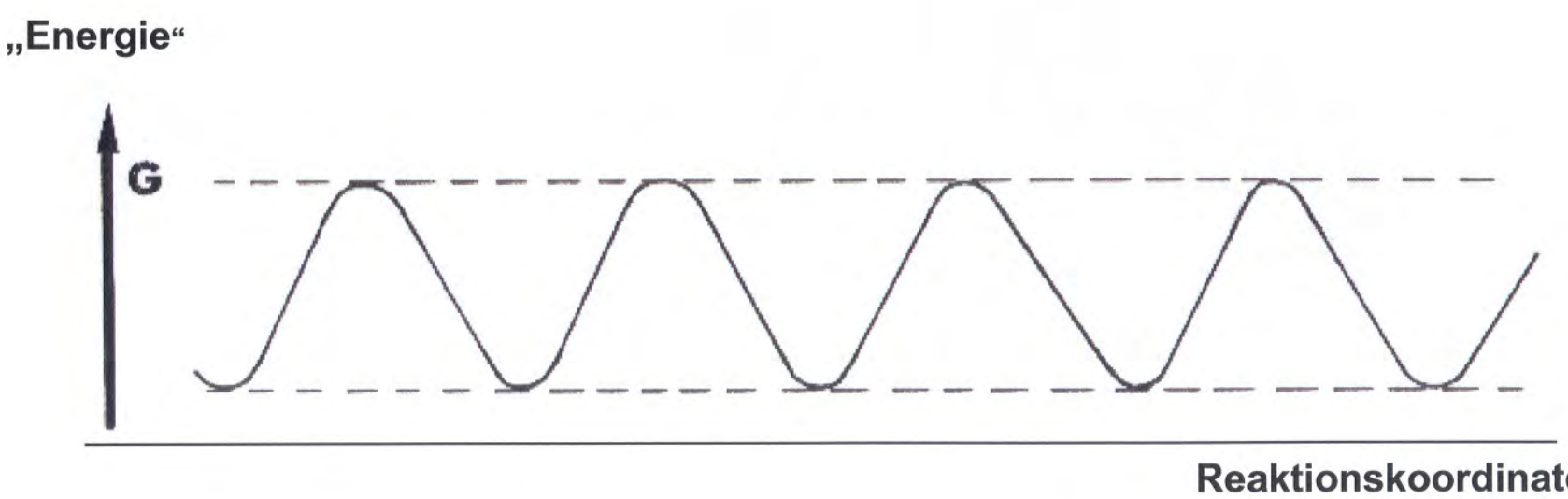
complex



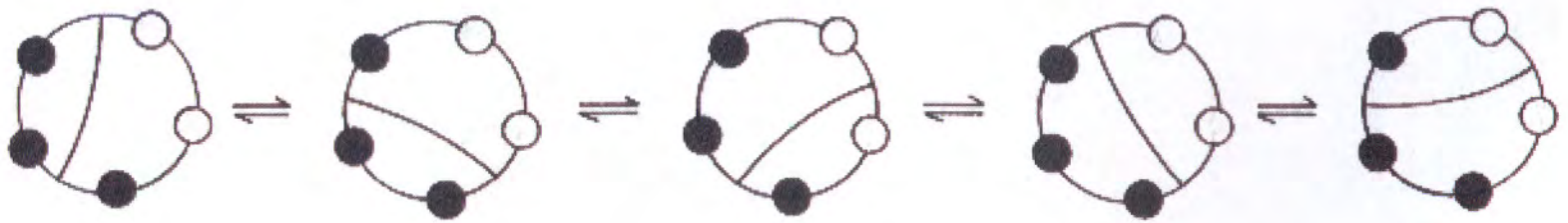
The Conformational Space for Flexible and "Rigid" Ligands

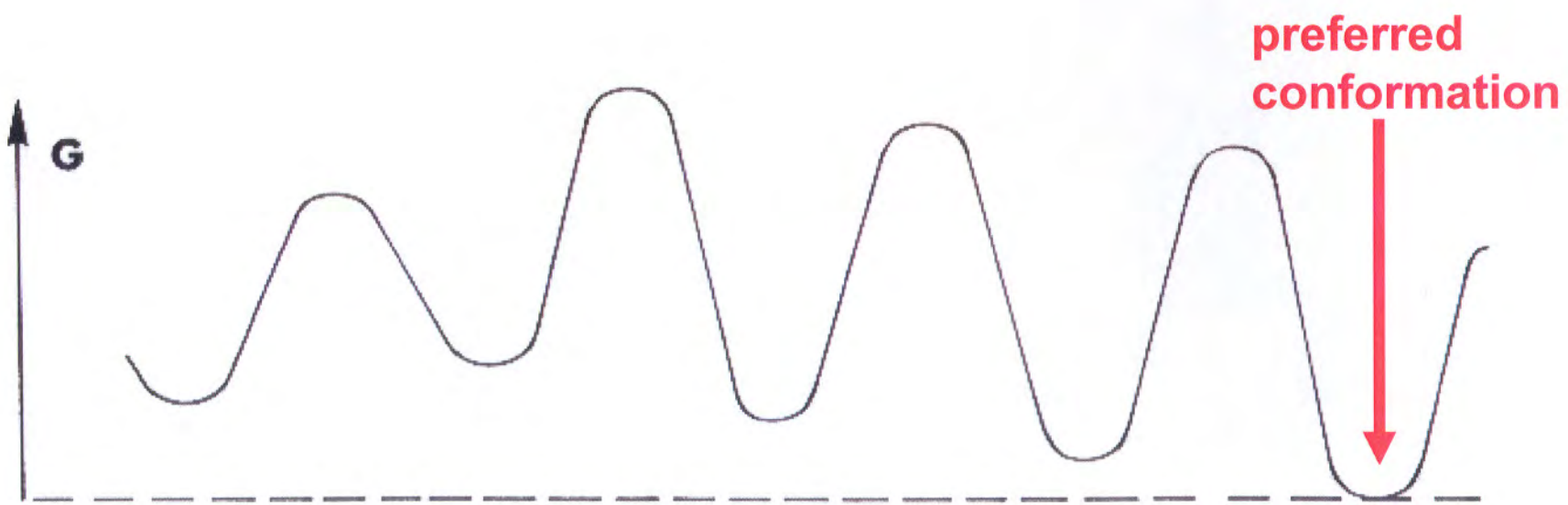


A Cyclic Peptide of Five Identical Amino Acids Exhibits a Fast Equilibrium of Five Degenerated Identical Structures (Effektive C_5 Symmetry)



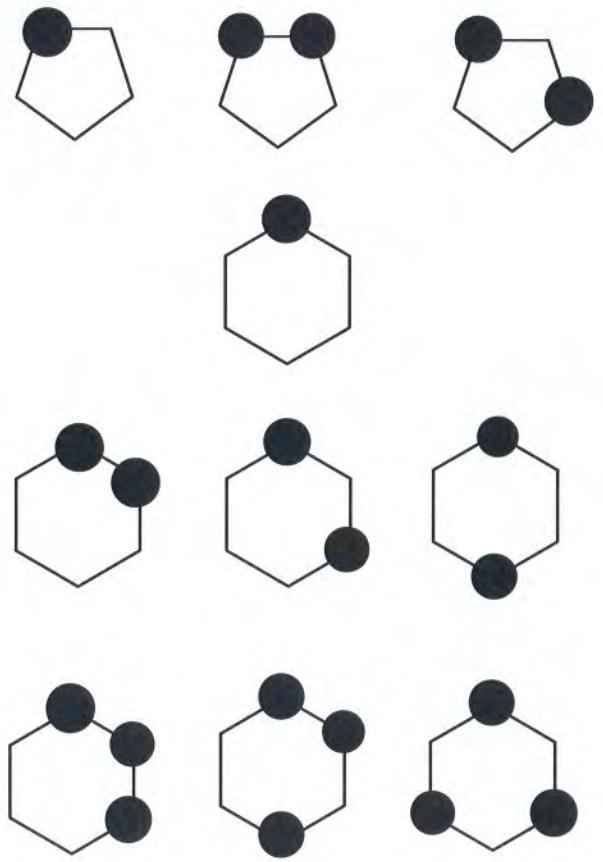
Distortion of Symmetry





Search for Preferred Conformations

(often incorrectly called "rigid" conformation)



the chiralty of the amino acids controls the backbone conformation

penta- and hexapeptides

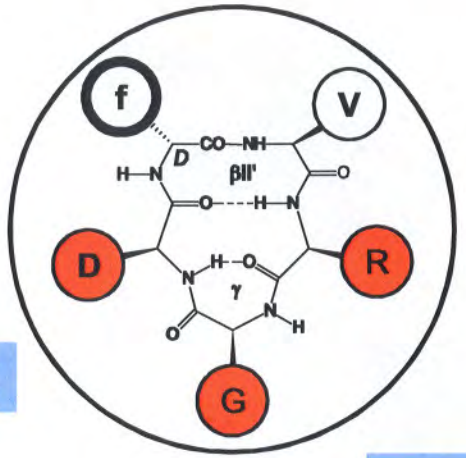
● **= D-Ala, Gly, D-Pro, L-Pro**

all corners = L-Ala

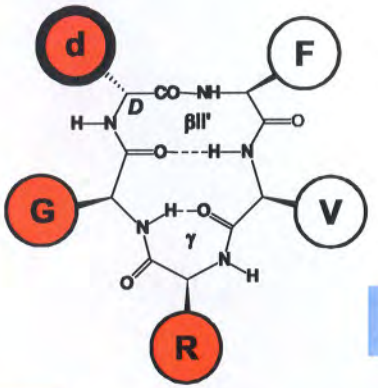
H. Kessler, R. Gratias, G. Hessler, M. Gurrath, G. Müller; Conformation of cyclic peptides. Principle concepts and the design of selectivity and superactivity in bioactive sequences by 'spatial screening'; *Pure & Appl. Chem.* **1996**, *68*, 1201-1205

D-Amino Acid Scan of cyclo(RGDFV)

linear reference GRGDSPK: 1.2 µmol

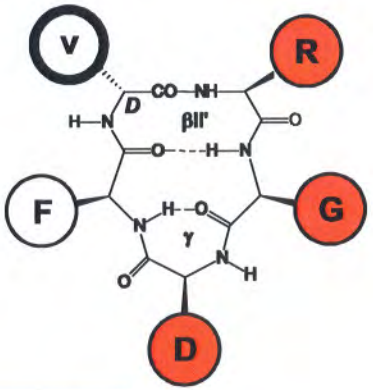


0.002 µmol

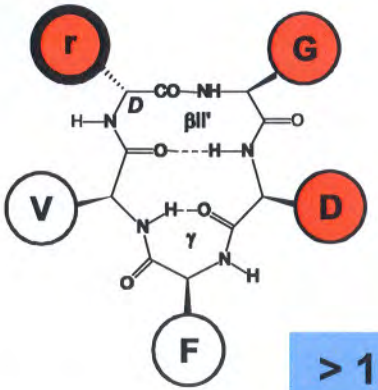


> 10 µmol

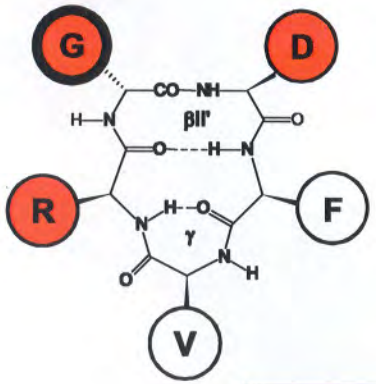
IC_{50} ($\alpha v\beta 3$) against vitronectin



0.011 µmol



> 10 µmol

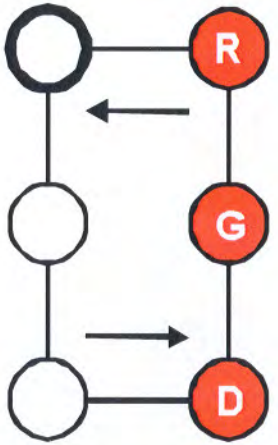


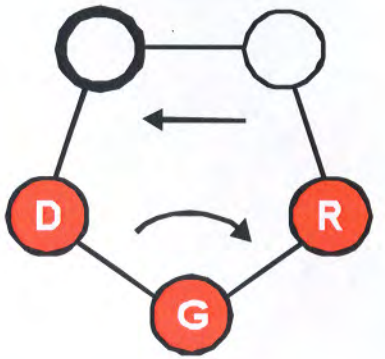
0.15 µmol

M. Aumailley, M. Gurrath, G. Müller, J. Calvete, R. Timpl, H. Kessler; *FEBS Lett*. **1991**, *291*, 50-54.

Selectivity of Peptide Integrin Antagonists

linear: flexible = nonselective





streched: αIIbβ3-selective **kinked: αvβ3-selective**

cyclic: rigid = selective

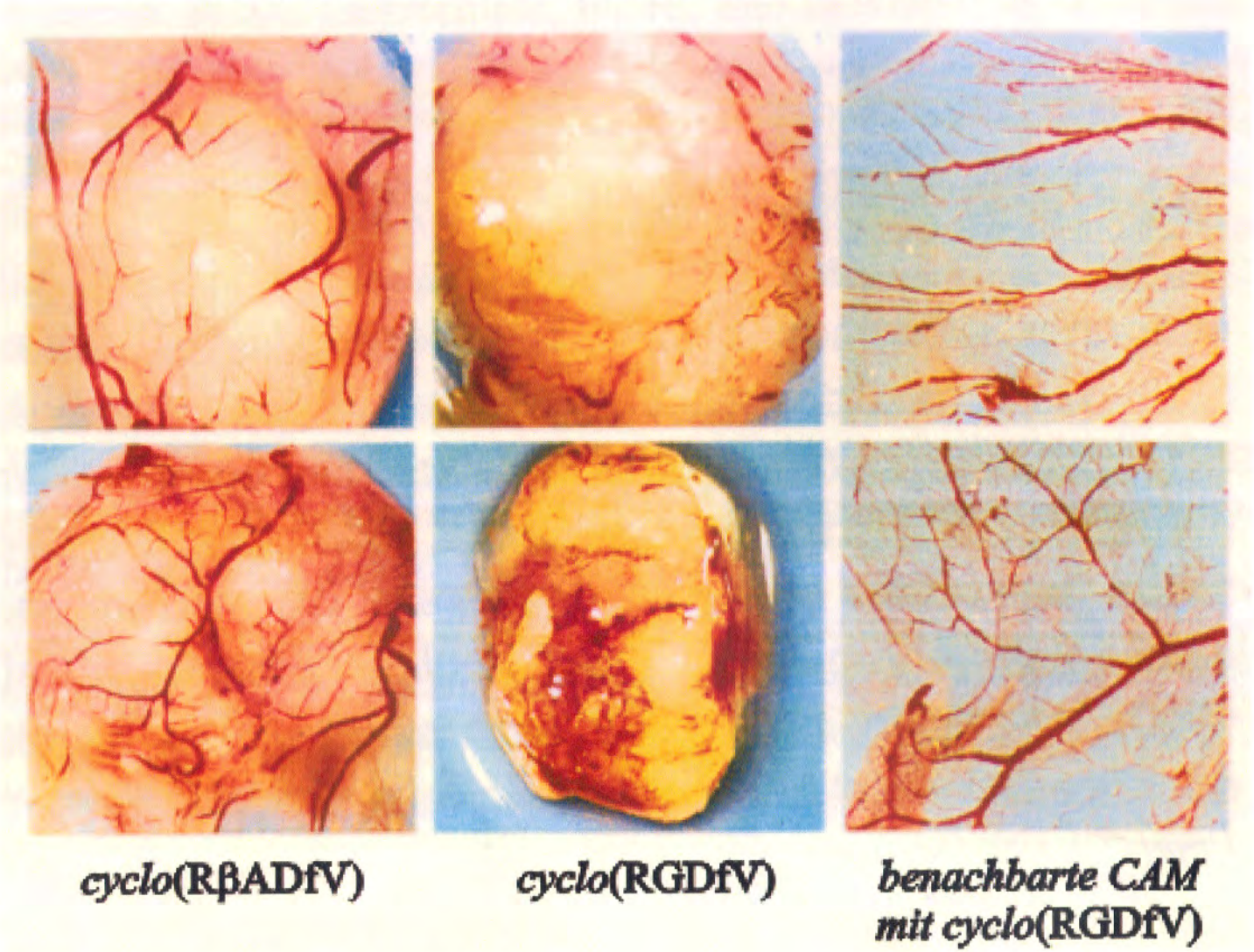
M. Gurrath, G. Müller, H. Kessler, M. Aumailley, R. Timpl; *Eur. J. Biochem.* **1992**, *210*, 911-921.

Conformation can differentiate between different α integrin subunits

M. Pfaff, K. Tangemann, B. Müller, M. Gurrath, G. Müller, H. Kessler, R. Timpl, J. Engel; *J. Biol. Chem*. **1994**, *269*, 20233-20238

G. Müller, M. Gurrath, H. Kessler; *J. Comp-Aided Mol. Design* **1994**, *8*, 709-730.

Inhibition of Neoangiogenesis of Human Tumours on Chicken Chorioalantioc Membranes



Brooks et al. Cell *79*, 1157 – 1164 (1994)

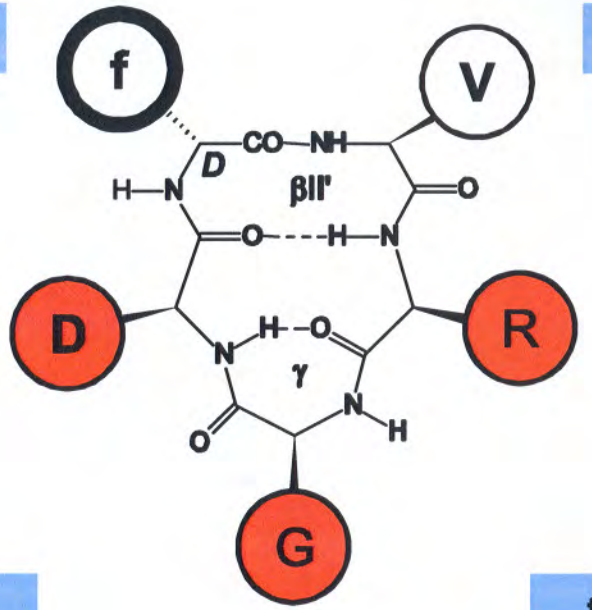
Derivatization of the RGD-Cyclopentapeptide

stereoisomers and inverso peptides

lipid anchor

N-methylation

β-turn mimetics



sugar amino acids

peptide bond isosters

peptoids

thiopeptides

reduced amide bonds

R. Haubner, D. Finsinger, H. Kessler; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374-1389

Effect of N-Methylation of the Peptide Bonds in cyclo(RGDfV)

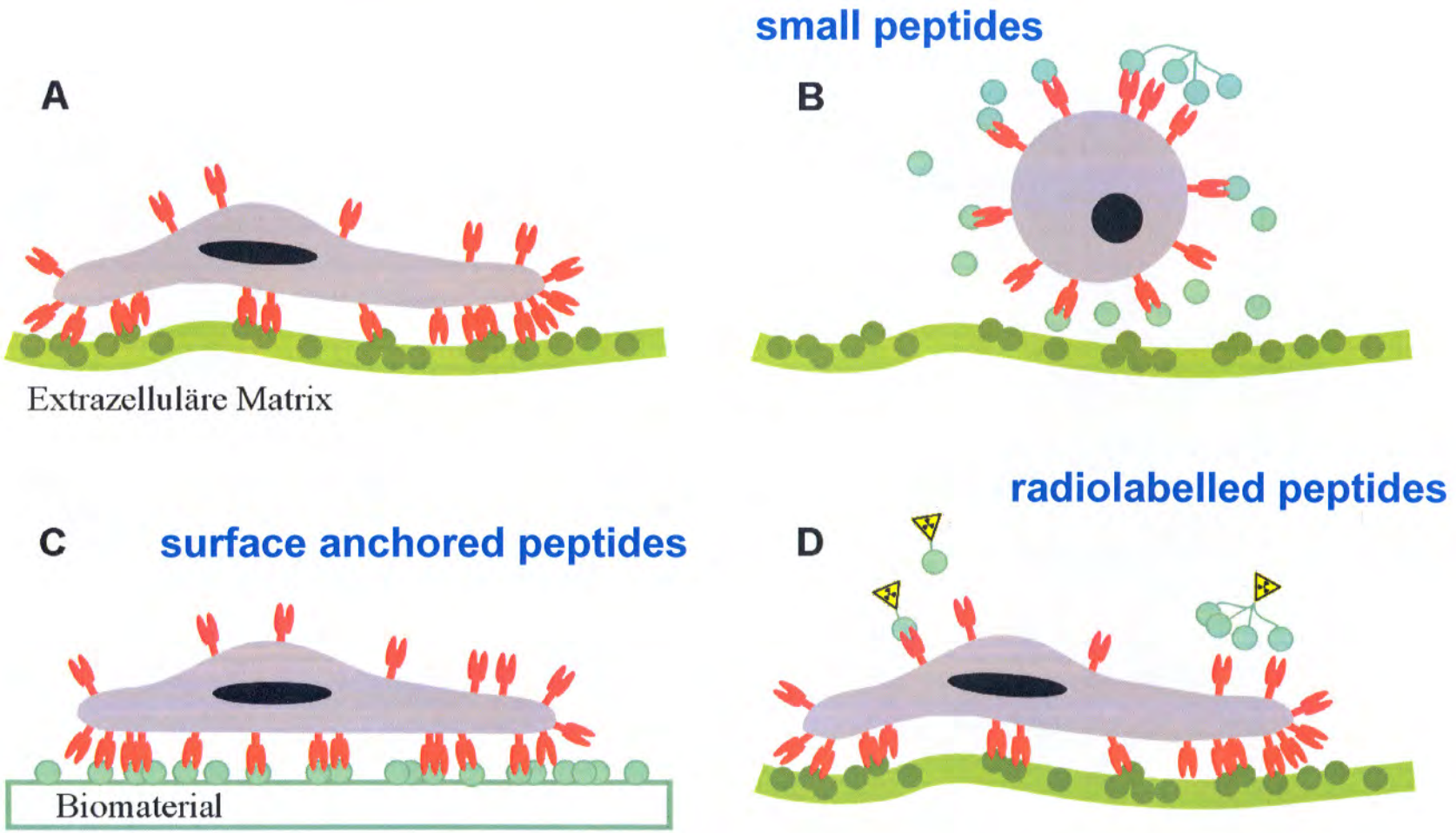
	IC_{50} [nM] ($\alpha v\beta 3$)
cyclo(-[NMe]R-GDfV)	5.5
cyclo(R-[NMe]G-DfV)	45
cyclo(RG-[NMe]D-fV)	560
cyclo(RGD-[NMe]f-V)	1400
cyclo(RGDf-[NMe]V-)	0.58

(Cilengitide, EMD 121974)

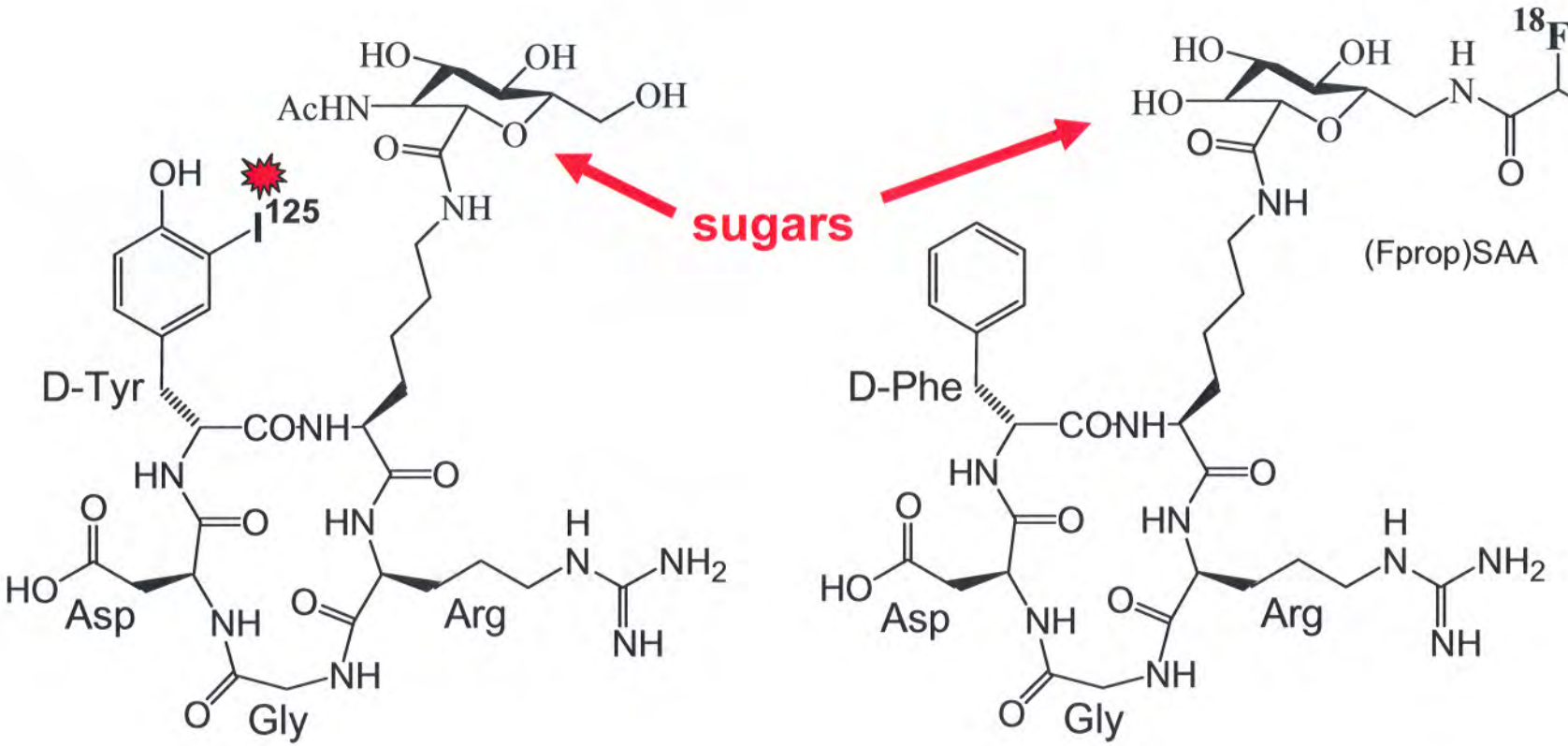
clinical phase II for treatment of different cancers
in 2004: *orphan drug* status for treatment of glioma

Dechantsreiter E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler et al., *J. Med. Chem.* **1999**, *42*, 3033-3040

Applications of Integrin Ligands



Glycosylated RGD-Containing Peptides: Tracer for Tumor Targeting and Angiogenesis Imaging with Improved Biokinetics



Cancer Res. **2001**, *61*, 1781

J. Nucl. Med. **2001**, *42*, 326

RGD peptide without a sugar residues exhibited low contrast because of rapid clearance also from the tumor tissue

[^{18}F]Galacto-RGD
Axillary Lymph Note Metastasis / Melanoma

CT

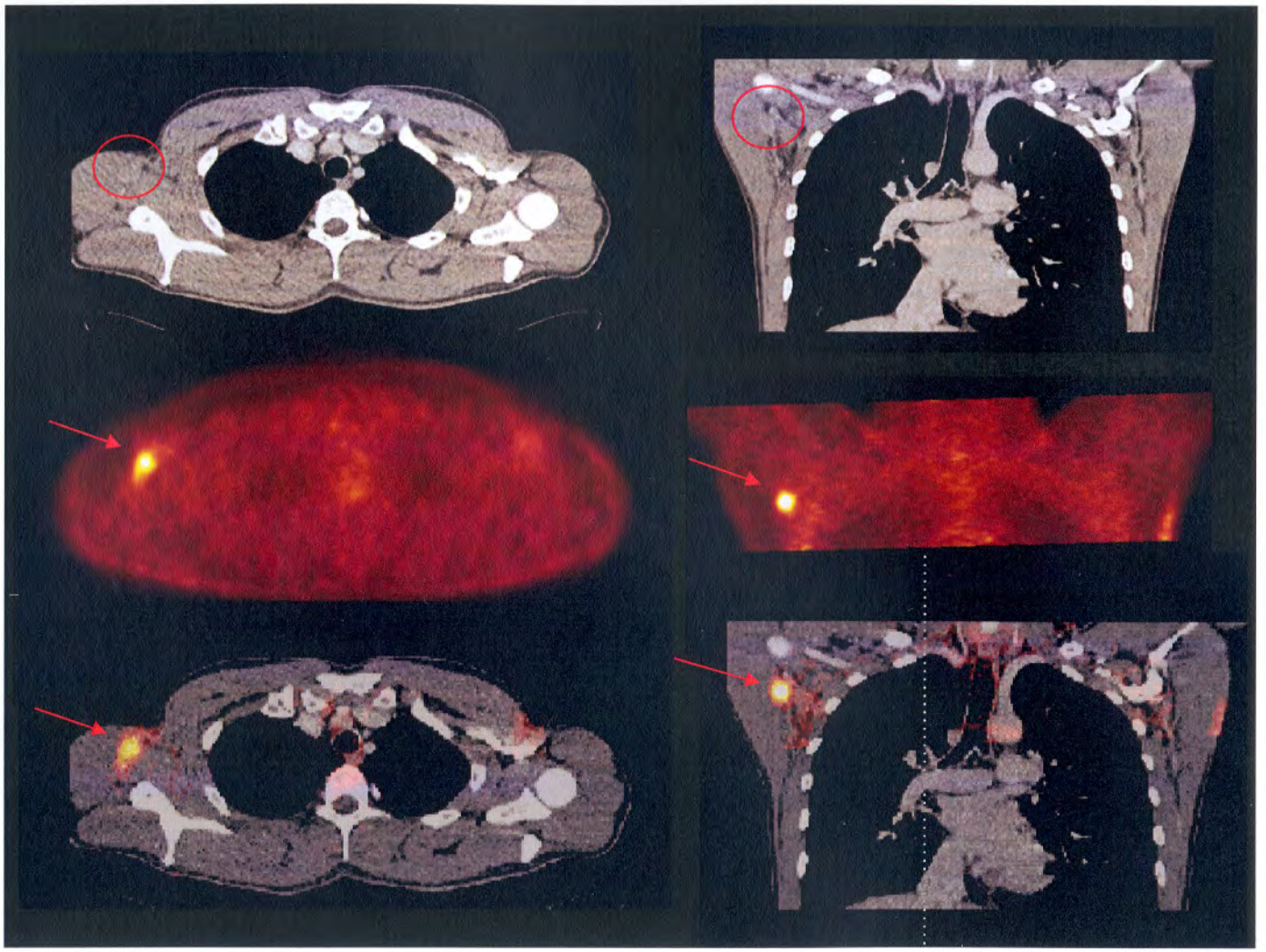
PET

Galacto-RGD

220 MBq; 2h p.i.

PET/CT-Fusion

Galacto-RGD



Beer, Haubner et al. (unpublished)

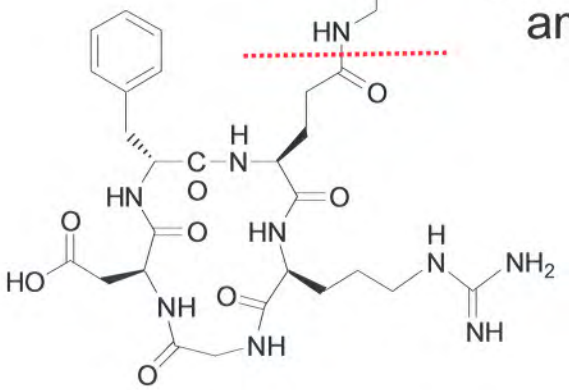
Monomeric and Dimeric ^{18}F-c(RGDfE)-Peptides

AOE: Aminooxy-essigsäure

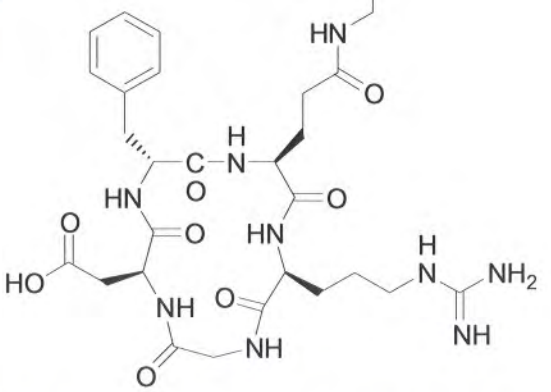
[^{18}F]FBA:
4-[^{18}F]Fluorbenzaldehyd

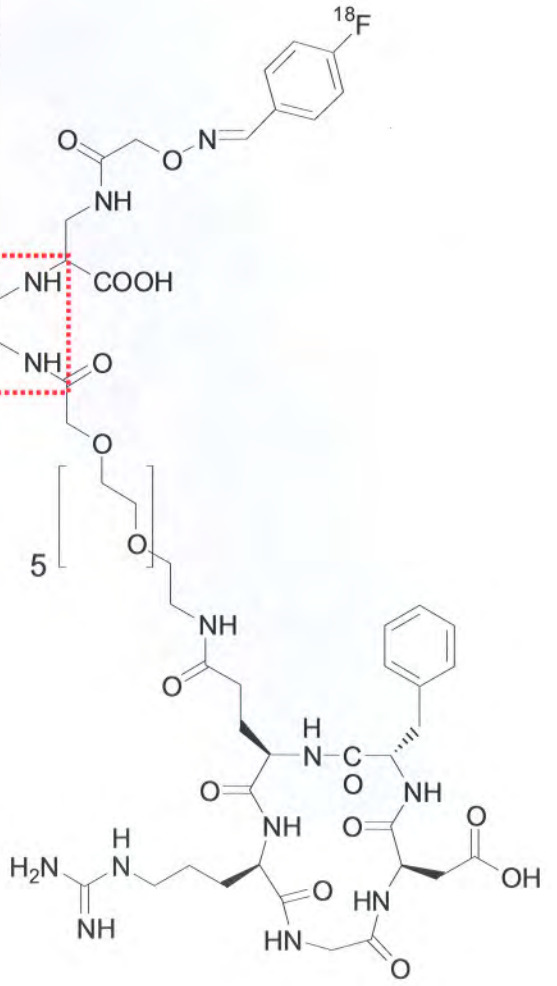
Dpr: Diaminopropionsäure

Hegas:
Heptaethylenglykol-
aminocarbonsäure



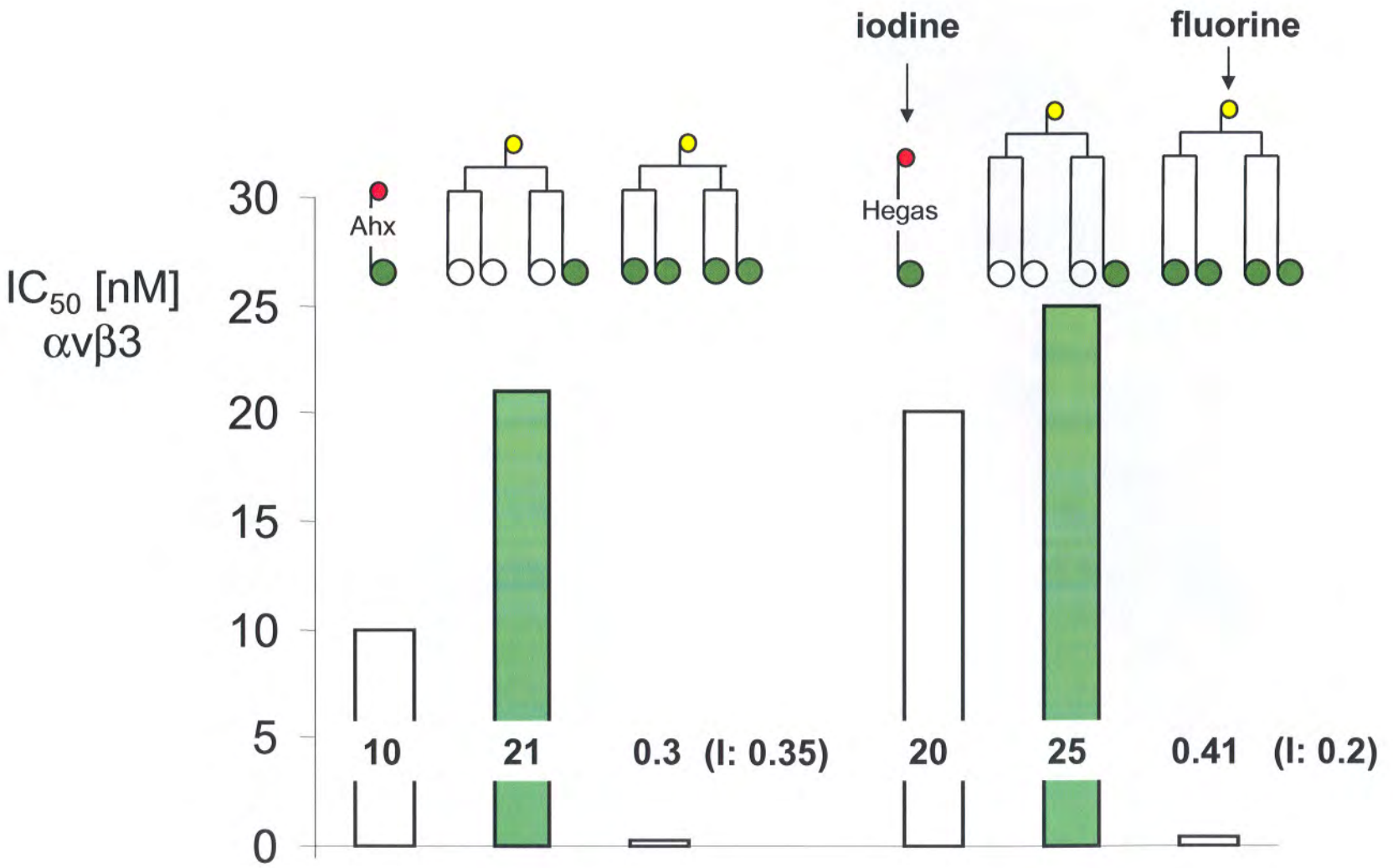
c(RGDfE)





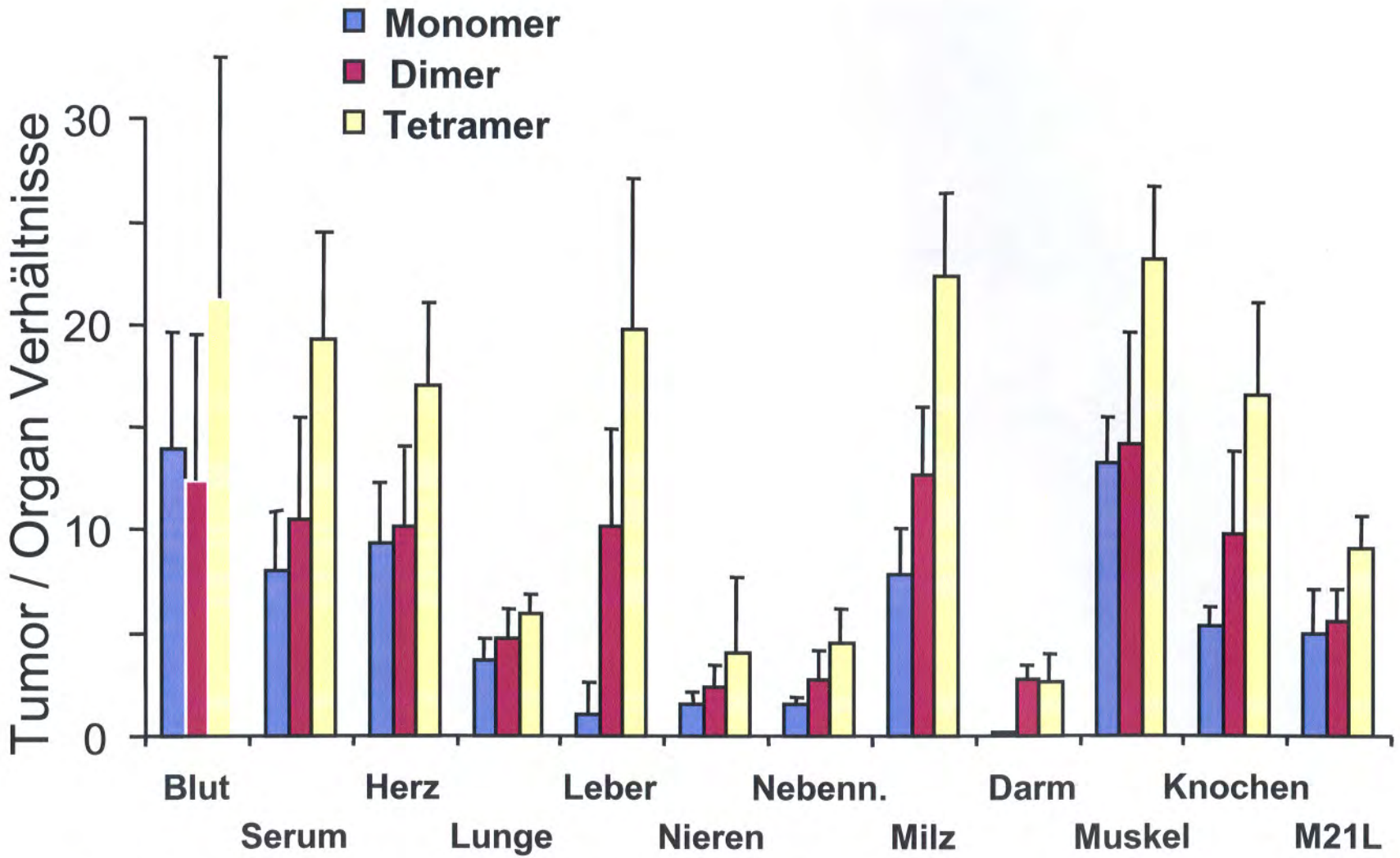
Thumshirn G et al. *Chemistry* **2003**, *9*, 2717-25

Multimeric RGD Peptides



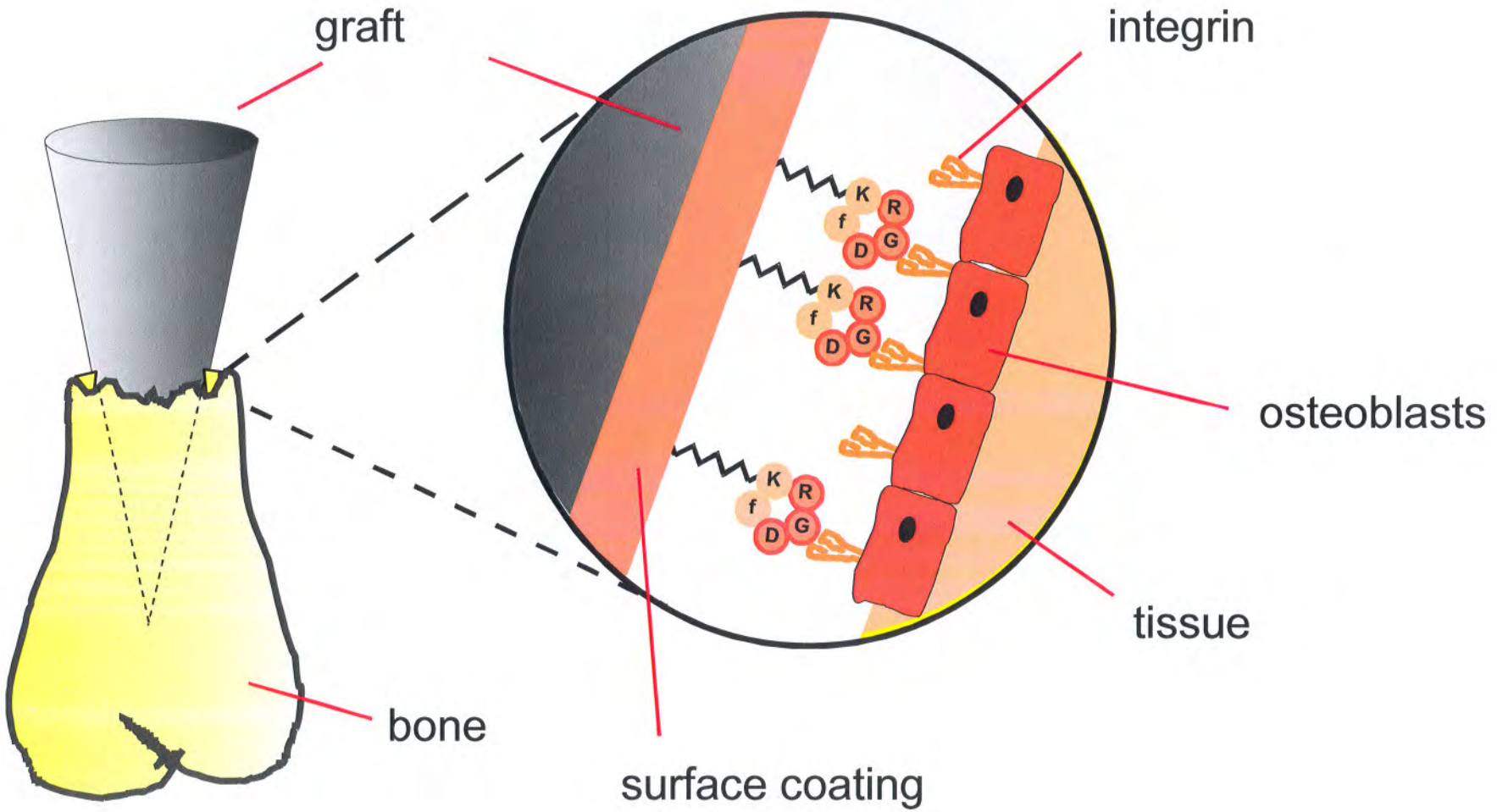
Tumor to Organ Rations

nude mice, M21-(αvβ3+)-melanoma, 2 h p.i. (n=3-5)

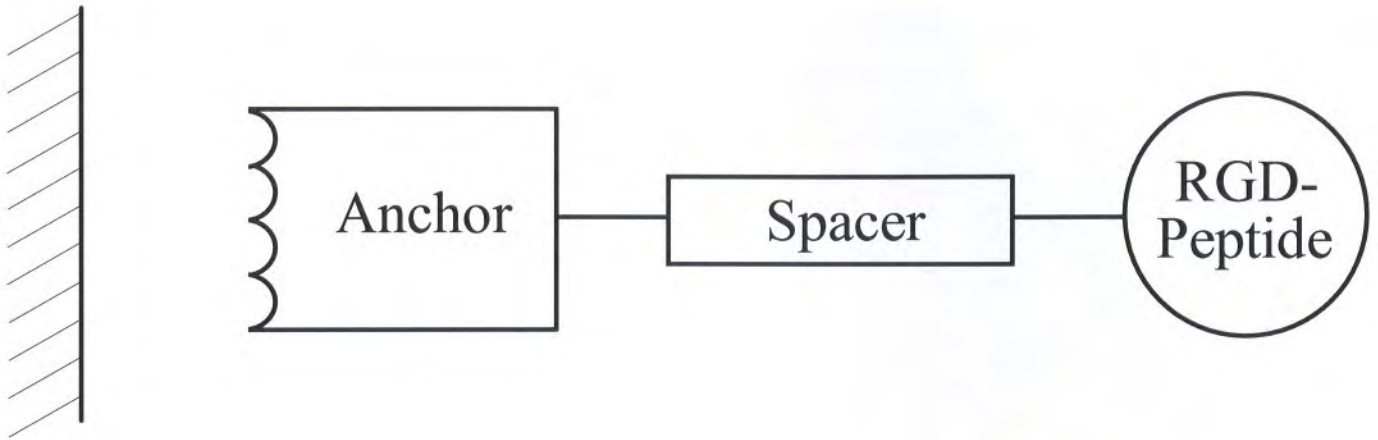


Poethko T et al. J Nucl Med. **2004**, *45*, 892-902.

Surface Coating of Biomaterials



Design of Coating Molecules

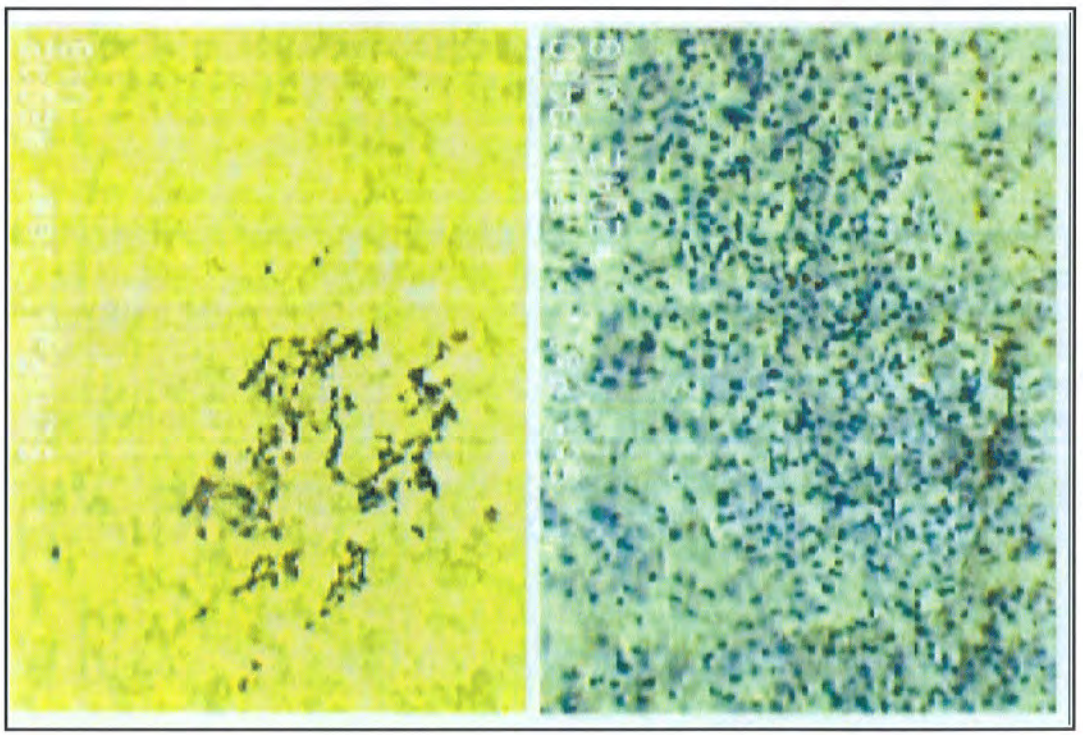


PMMA	acrylate	aminohexanoic acid $(Ahx)_n$	(RGDfK)-NH_2
$Ca_3(PO_4)_2$	thiolate	HEGAS	(RADfK)-NH_2
Ti / TiO_2	phosphonate		(RGDfE)-COOH
gold	isonipecotic acid		(RADfE)-COOH
SiH			
-NH_2 (BSA)			

Applications of αv Selective Inhibitors

Biomaterials

- surface anchoring (biocompatibility)

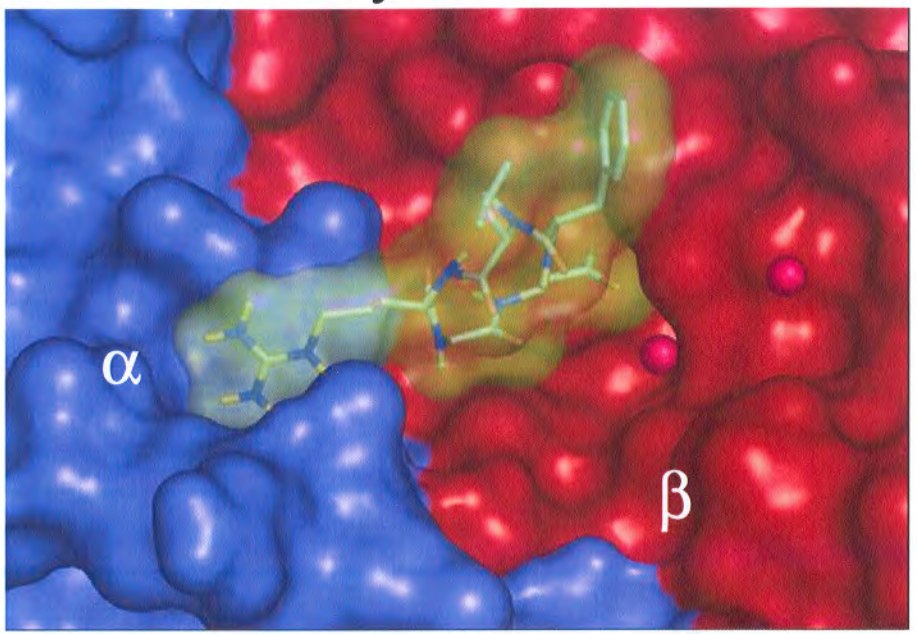


not coated **RGD coated**

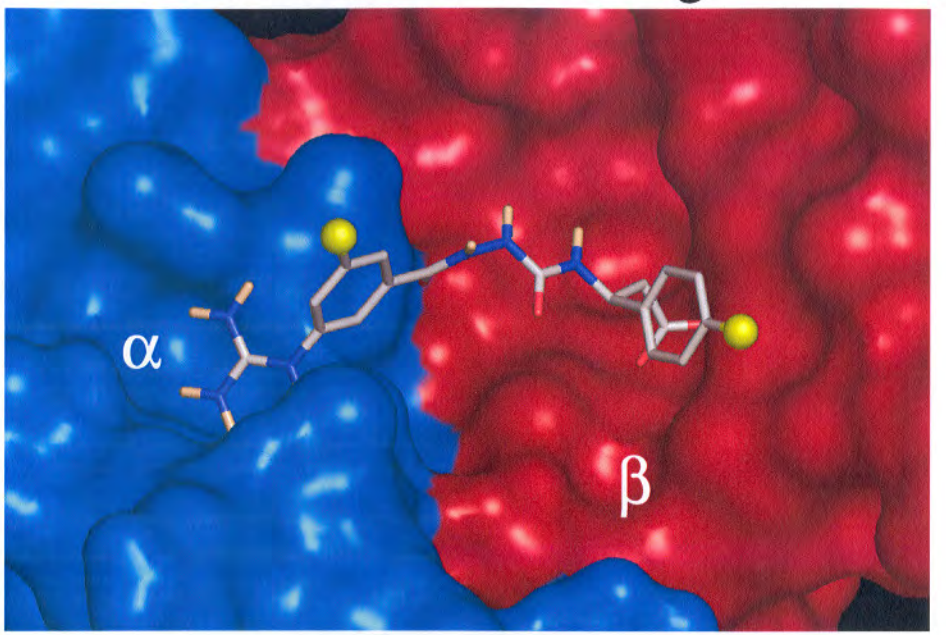
M. Kantlehner et al., *Angew. Chem. Int. Ed.* **1999**, *38*, 4, 560-562.

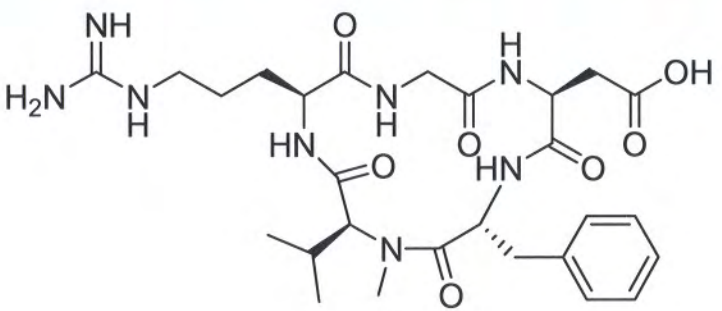
Modeling of Non-Peptidic Ligands for Coating of Surfaces

X-ray structure

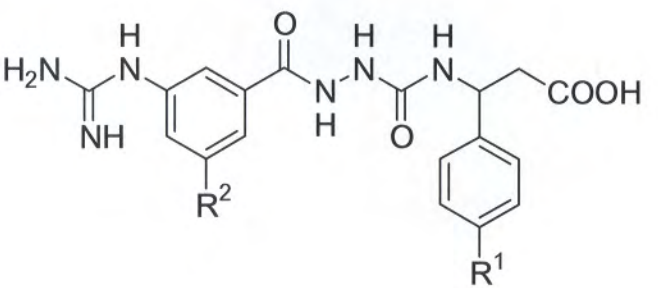


automated docking



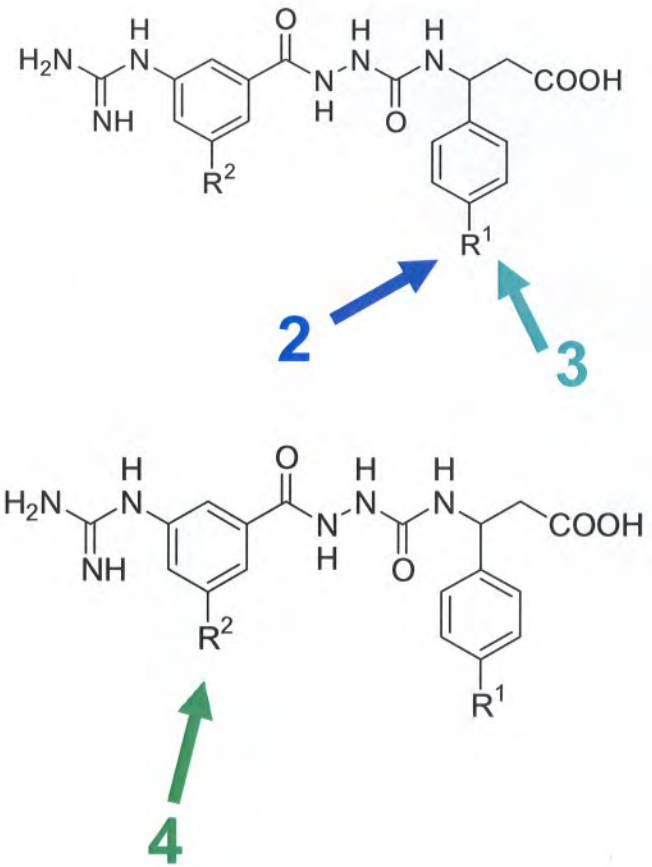


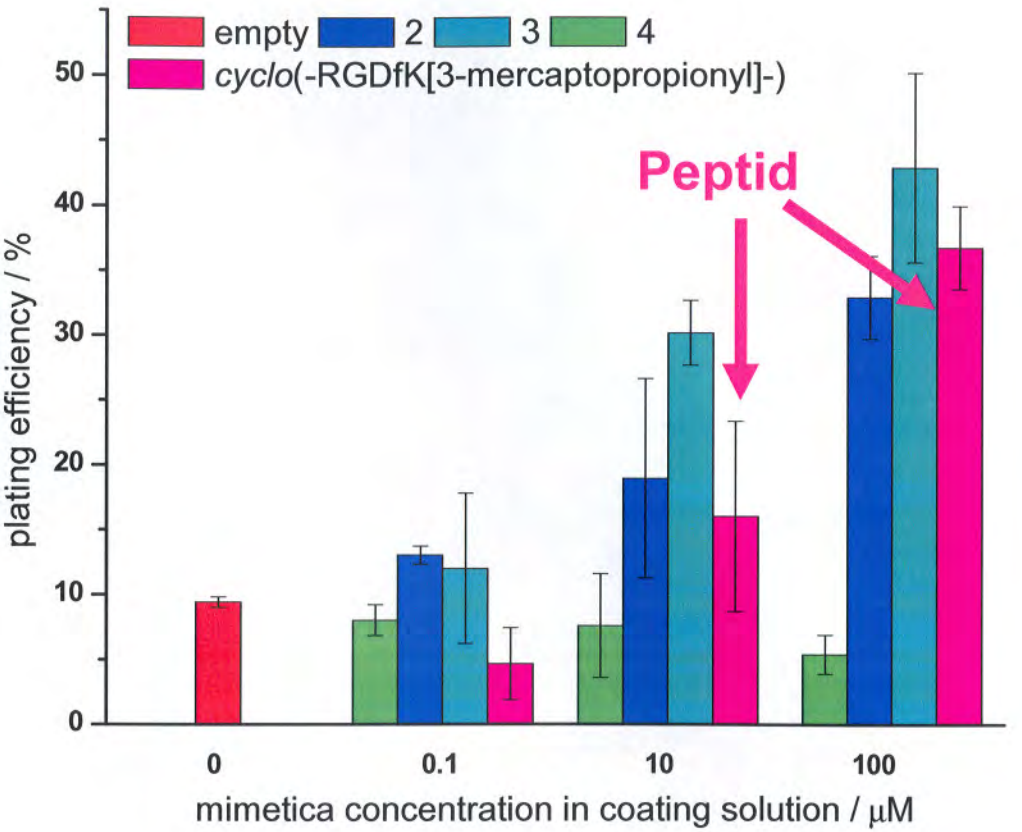
Cilengitide (**I**)



L.Marinelli, A. Lavecchia, K.E. Gottschalk, E. Novellino, H. Kessler, *J. Med. Chem.* **2003**, *46*, 4393-4404.

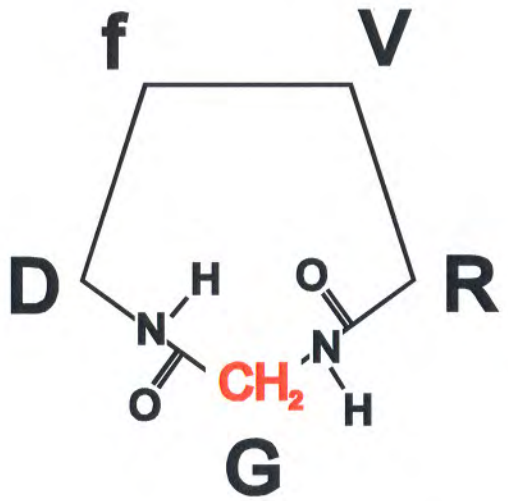
Stimulated Cell Adhesion on Titanium

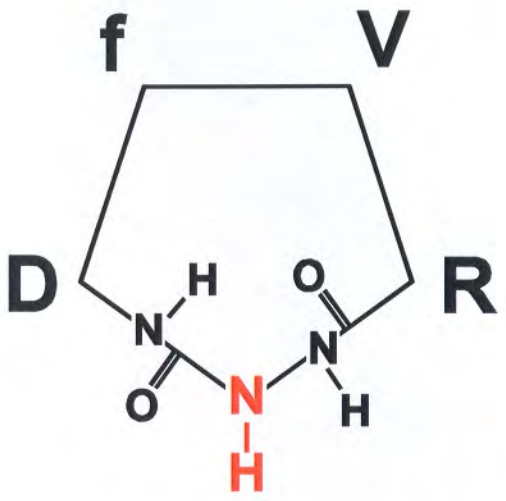




C. Dahmen, J. Auernheimer, A. Meyer, A. Enderle, S.L. Goodman, H. Kessler, Angew.Chem. **2004**, in press

Aza Peptide Mimic

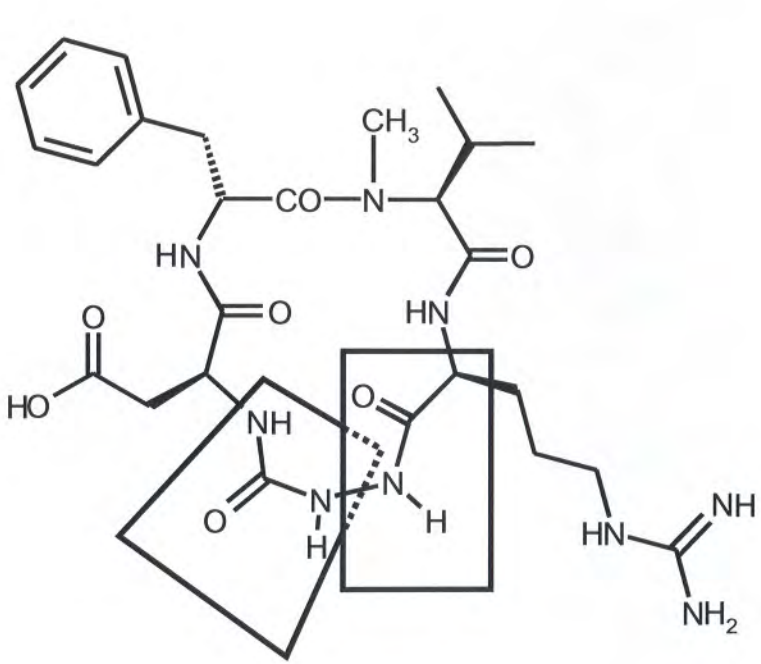


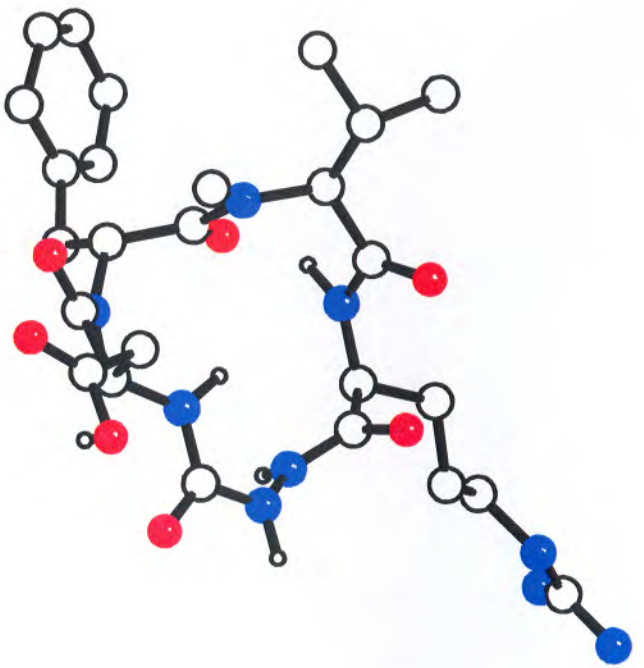


lead	
IC_{50} =	2.5 nM αvβ3
	1700 nM αIIbβ3

Aza-Gly-derivative	
IC_{50} =	3.5 nM αvβ3
	5800 nM αIIbβ3

Structure of cyclo(R*aza*GDf[NMe]V)

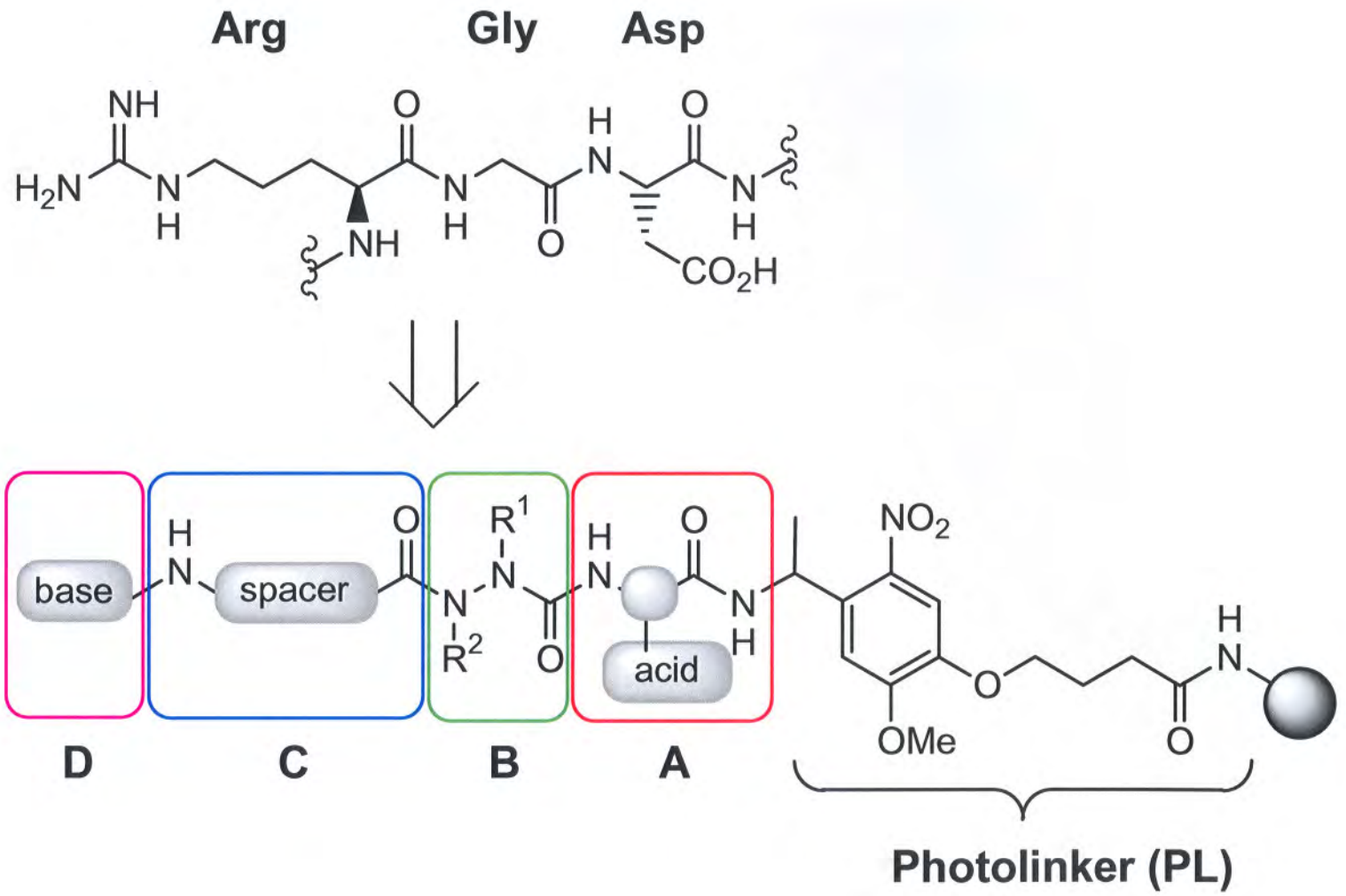




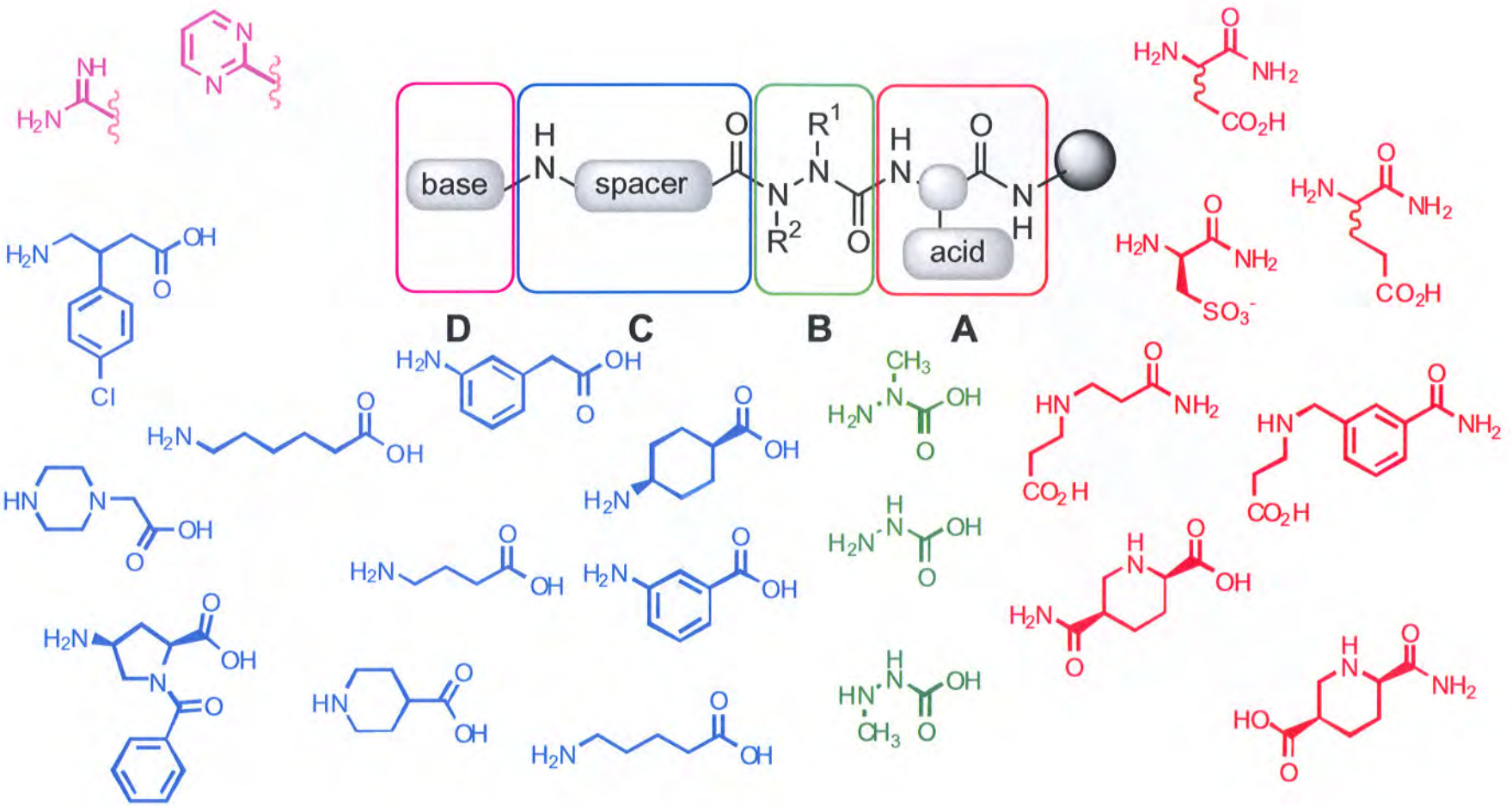
cyclo(-Arg-*aza*Gly-Asp-D-Phe-[NMe]-Val-)

IC_{50} 6 nM ($\alpha v\beta 3$)

Design of Modularly Assembled RGD Mimetics



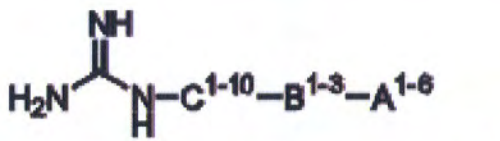
Design of an RGD Mimetic Library: Building Blocks A-D



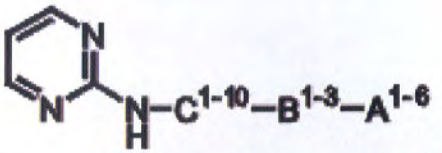
C.Gibson G. A. G. Sulyok, D. Hahn, S. L. Goodman, G. Hölzemann, H. Kessler . *Angew. Chem. Int. Ed. Engl.* **2001**, *40*,165-169.

On Bead Screening of Libraries with 330 Compounds

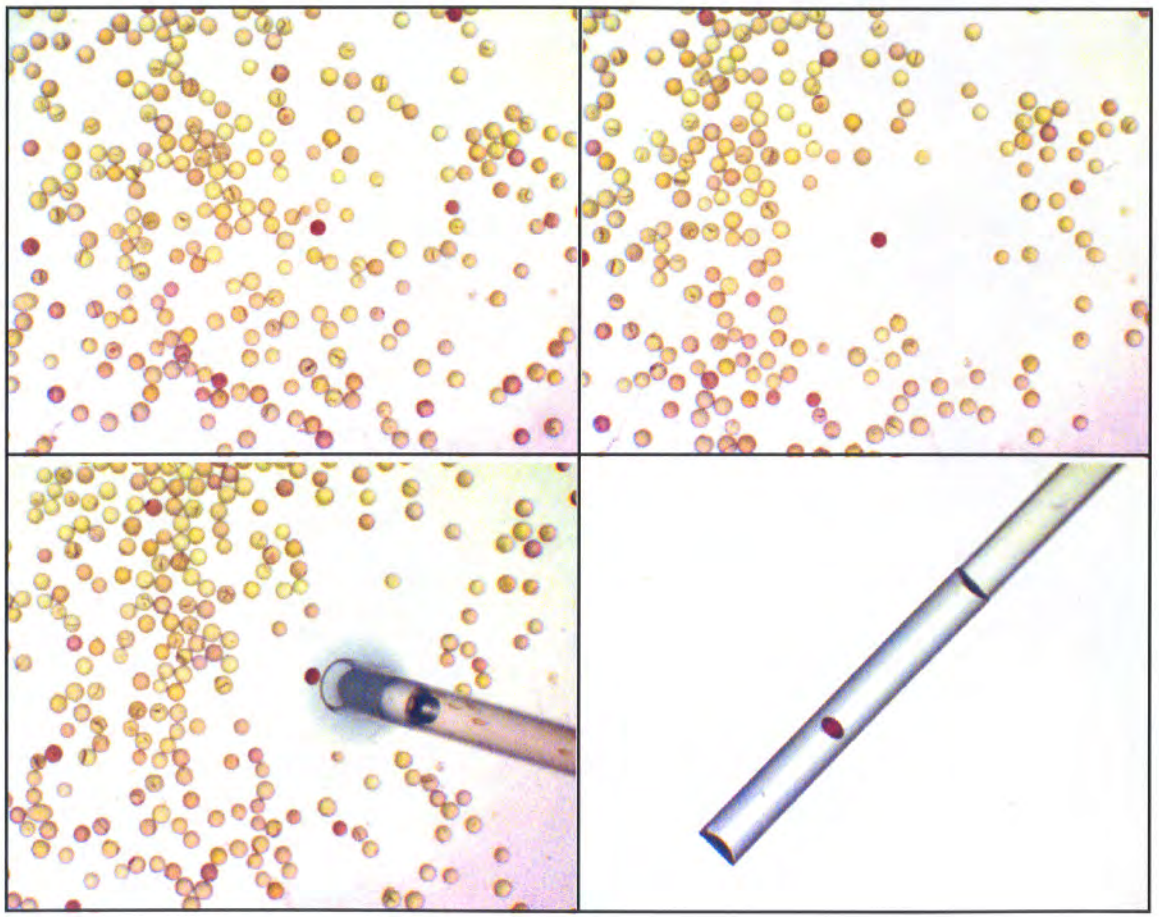
guanidine library



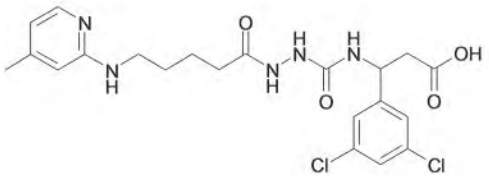
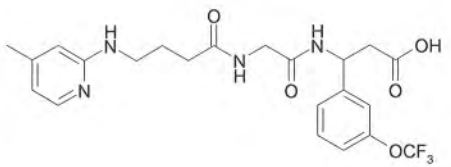
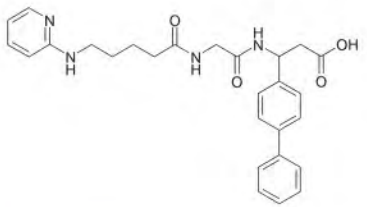
pyrimidine library



Selection of an Active Compound



Some Selected αvβx Inhibitors

Compound	IC_{50} (nM) on integrin			
	αvβ3	αvβ5	αvβ6	αIIbβ3
cyclo(RGDf(NMe)V)	4	70	550	600
cyclo(R*aza*GDfV)	4	500	6000	6000
	64	2500	2	> 10000
	45	7	0.2	> 10000
	0.45	2670	0.6	4050

S. L. Goodman, G. Hölzemann, G. A. G. Sulyok, H. Kessler,*J. Med. Chem.* **2002**, *45*, 1045-1051

Evolution of a Lead Structure:The RGD Sequence

GRGDSPK
Identification of binding sequence

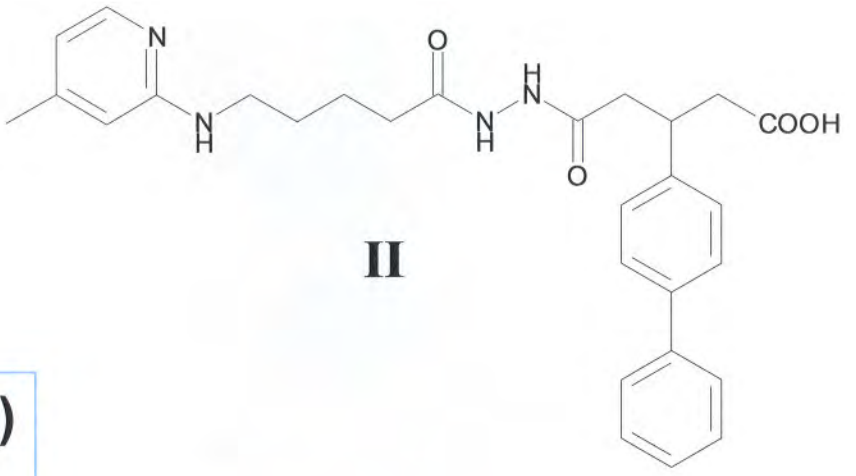
c(RGDfV)
Cyclisation and ´spatial screening´

c(RGDf[NMe]V)
Optimisation

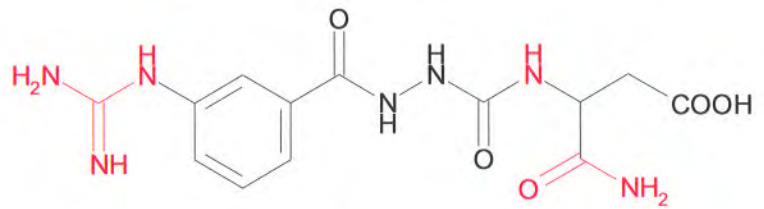
c(R*aza*GDf[NMe]V)
Peptidomimetics

I
RGD mimetics (combinatorial library)

II
Optimisation (Pfizer´s ´Rule of 5´)



II



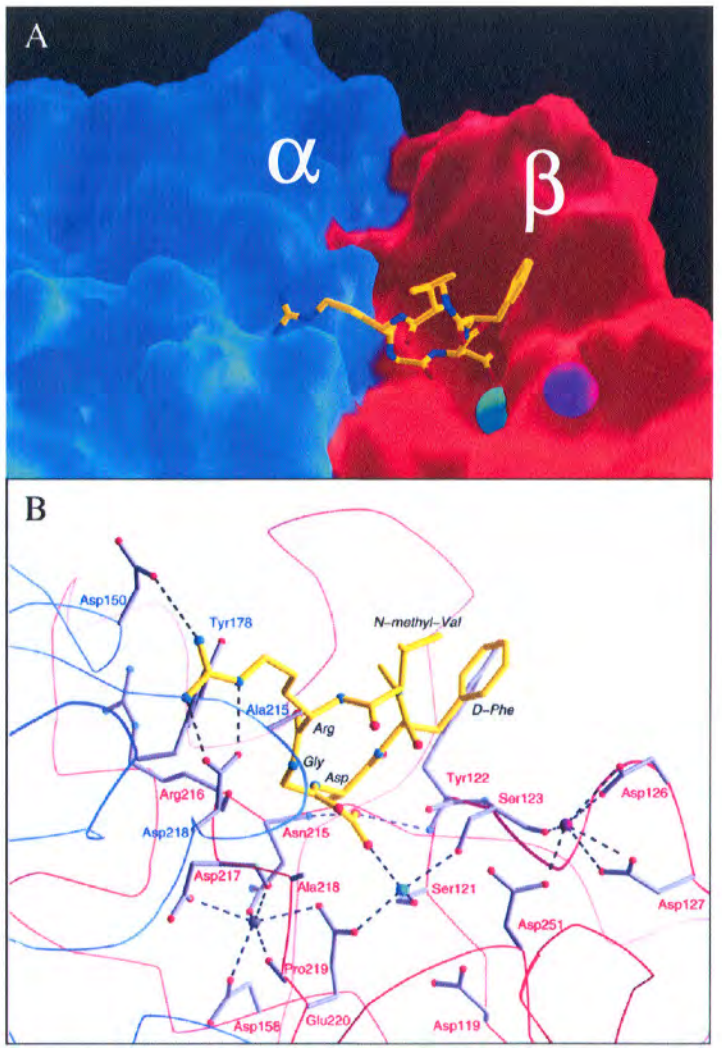
I

Small molecules can distinguish between different β subunits

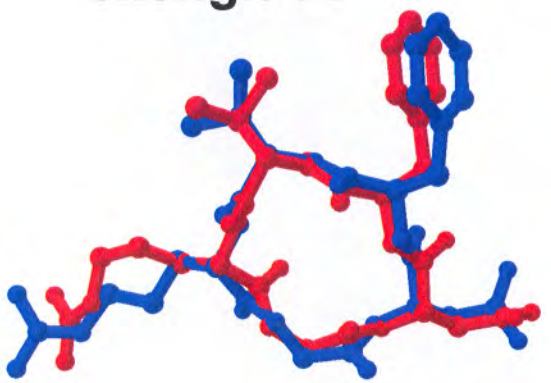
and between αIIb and αv

→ **small ligands bind in the interphase between α and β**

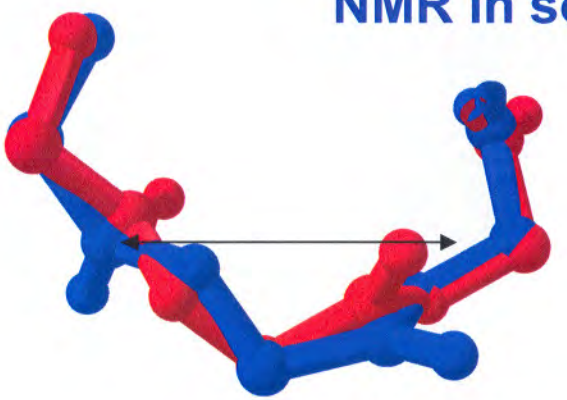
Integrin Structure – the Complex (2002)



cilengitide



X-ray in complex
NMR in solution



Xiong et al. *Science* **2002**, *296*, 151 - 155

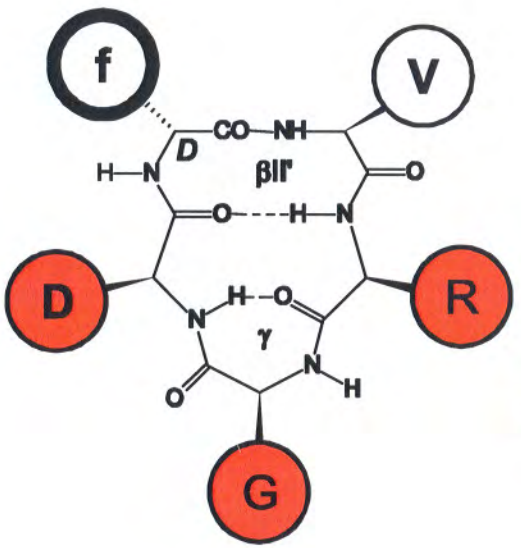
Structural Basis of SAR

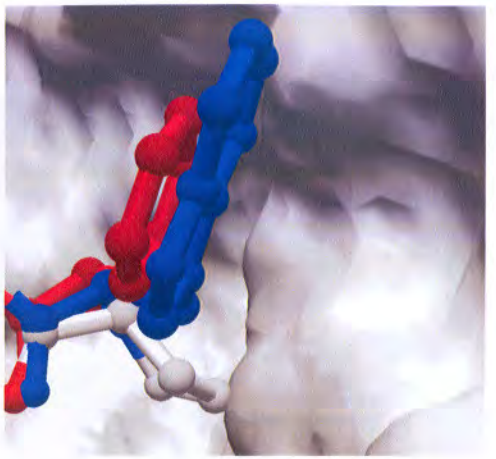
Experimental activities

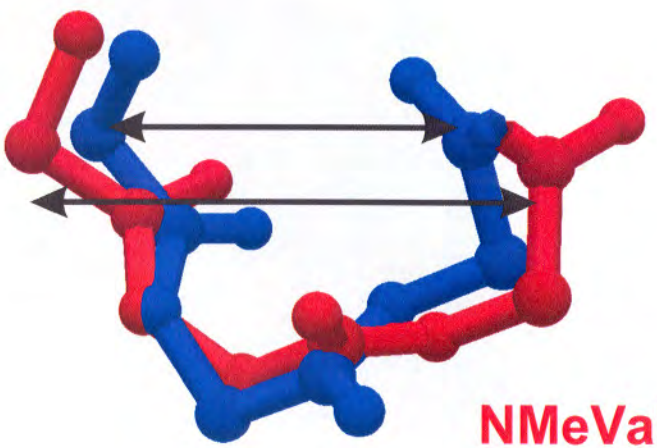
D-Phe 2.5 nM

D-Trp 0.2 nM

D-Pro 580 nM







Val 2.5 nM

NMeVal 0.6 nM

higher selectivity

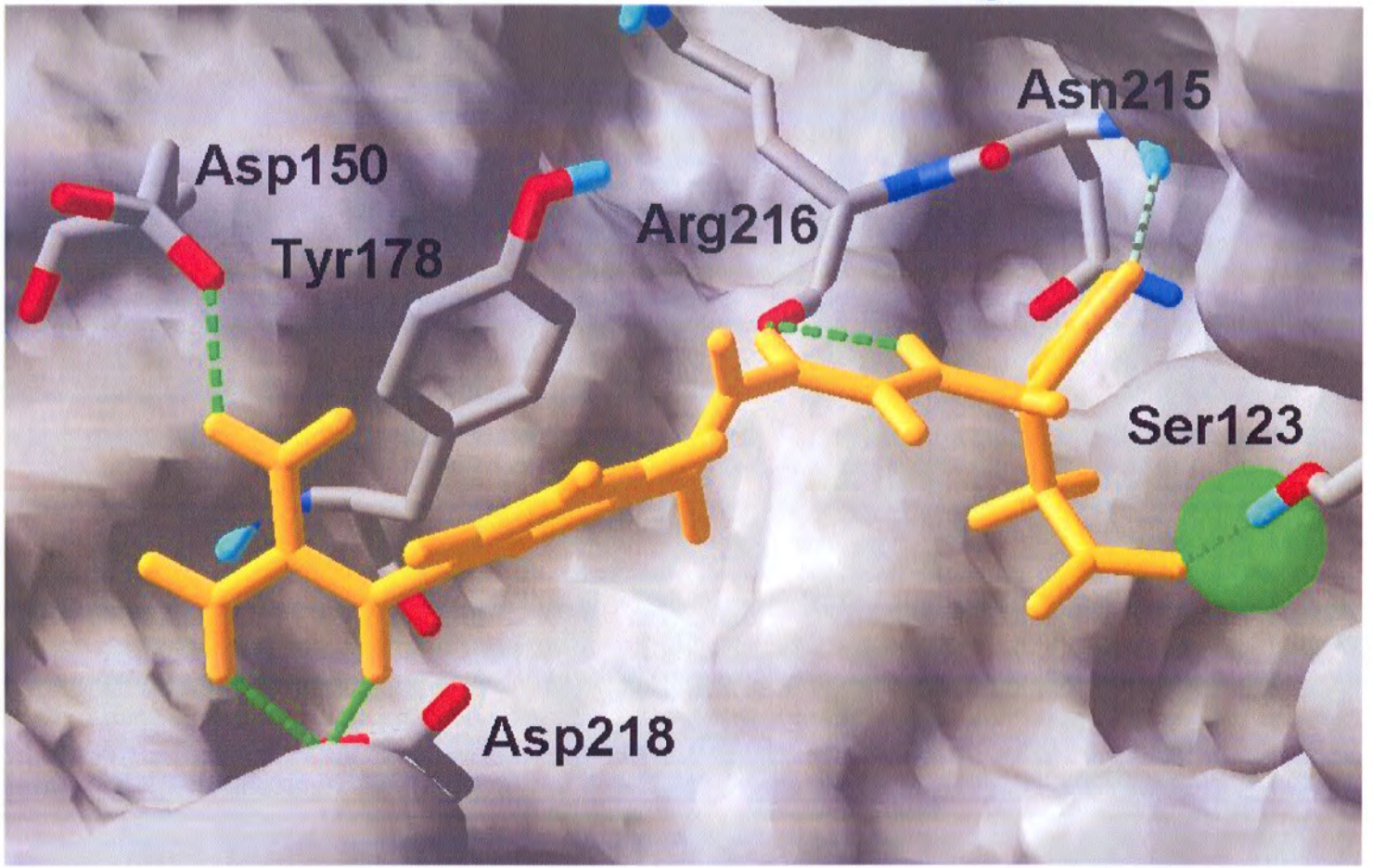
Val

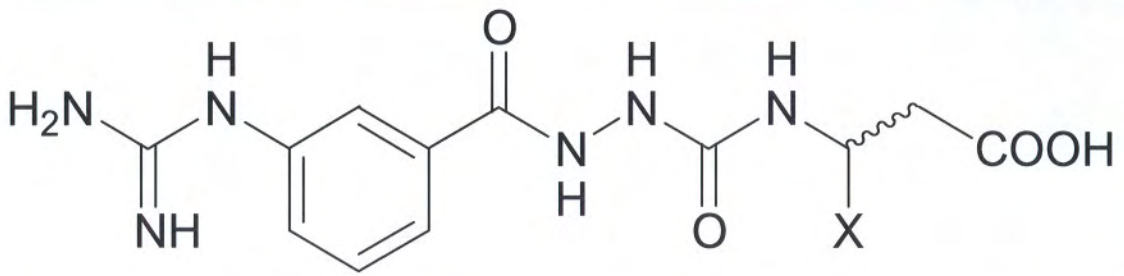
NMeVal

V

a

Docking of Non-Peptidic Ligands

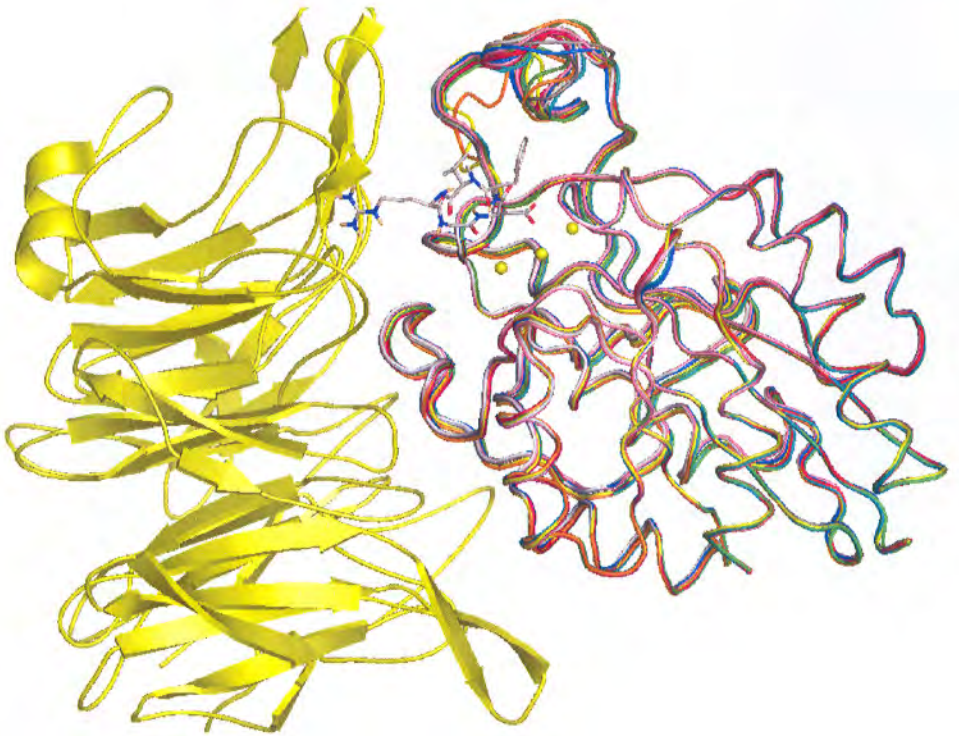




L.Marinelli, A. Lavecchia, K.E. Gottschalk, E. Novellino, H. Kessler,
J. Med. Chem. **2003**, *46*, 4393-4404.

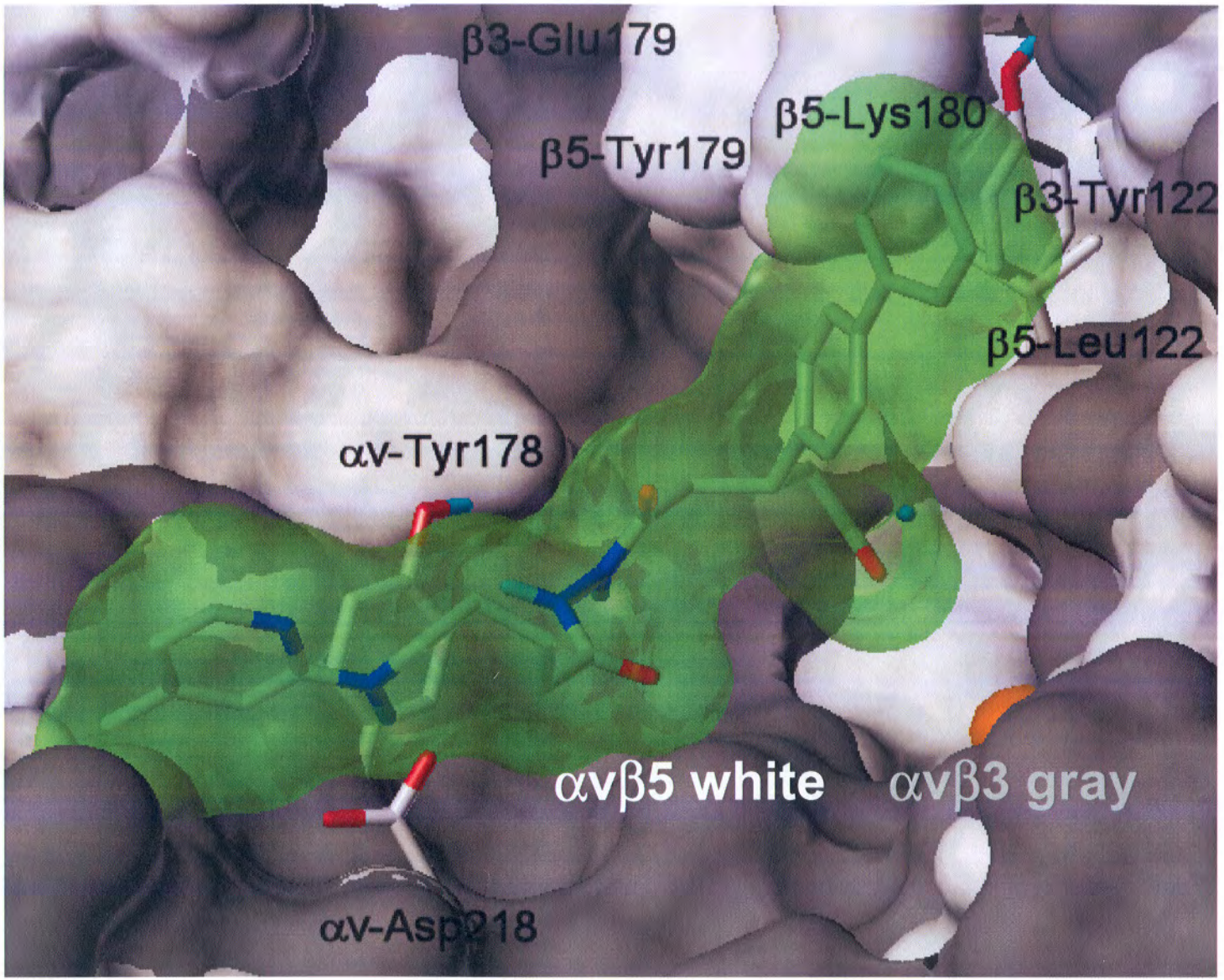
Homology Models of β_5

- Secundary structure of $\alpha_v\beta_3$ (yellow) with RDG-ligand (grey)
- Homology models of β_5 subunit (different colors) show great similarity except SDL (Lys159 – Phe189)



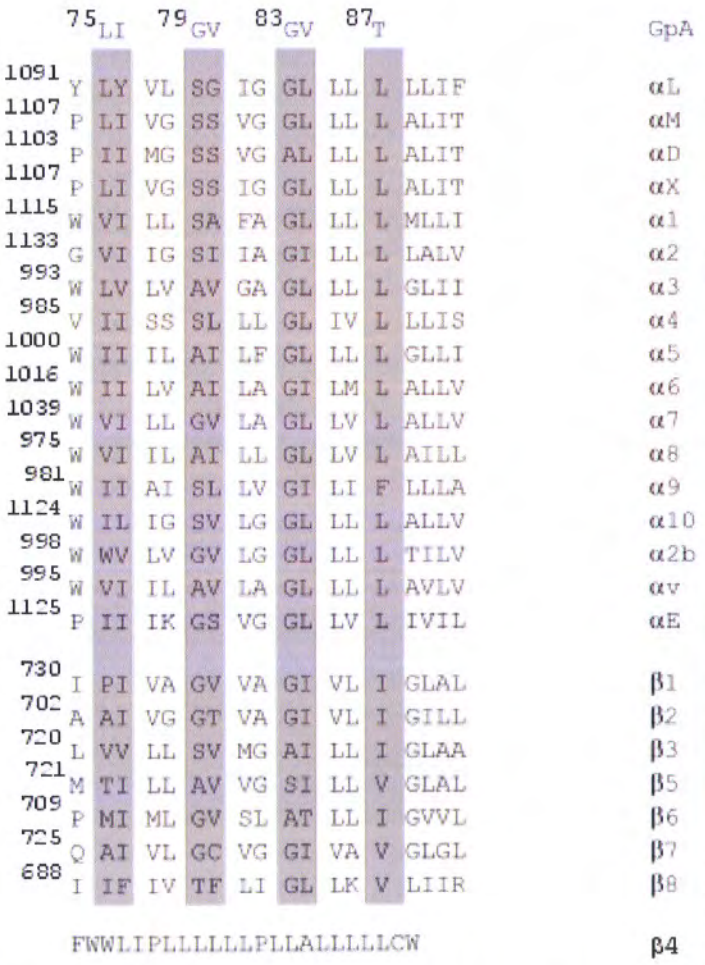
L.Marinelli, K.E. Gottschalk, A. Meier, E. Novellino, H. Kessler, *J. Med. Chem.* **2004**, *47*, 4166-4177.

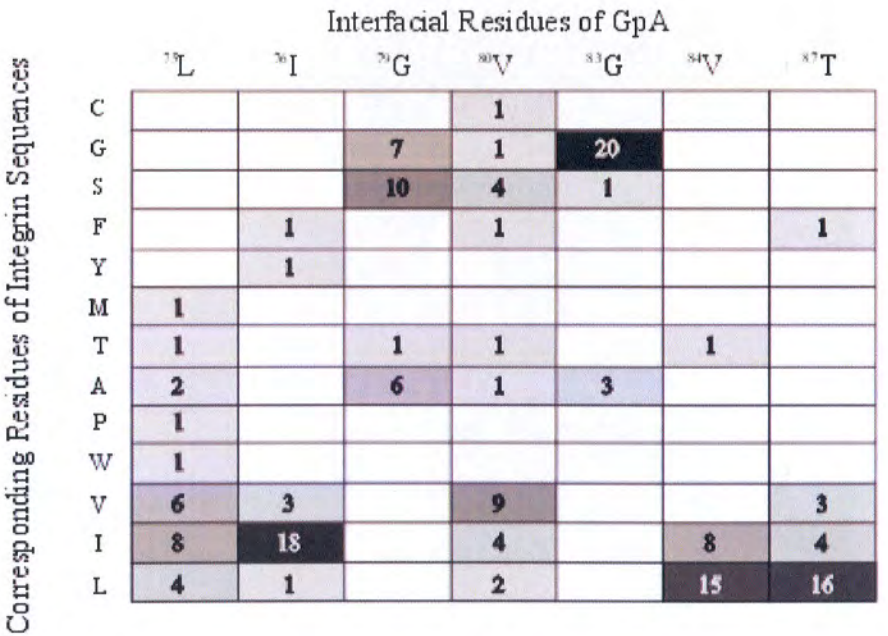
Selectivity between αvβ5 and αvβ3



Glycophorin A and Integrins Have a Common Dimerization Motif

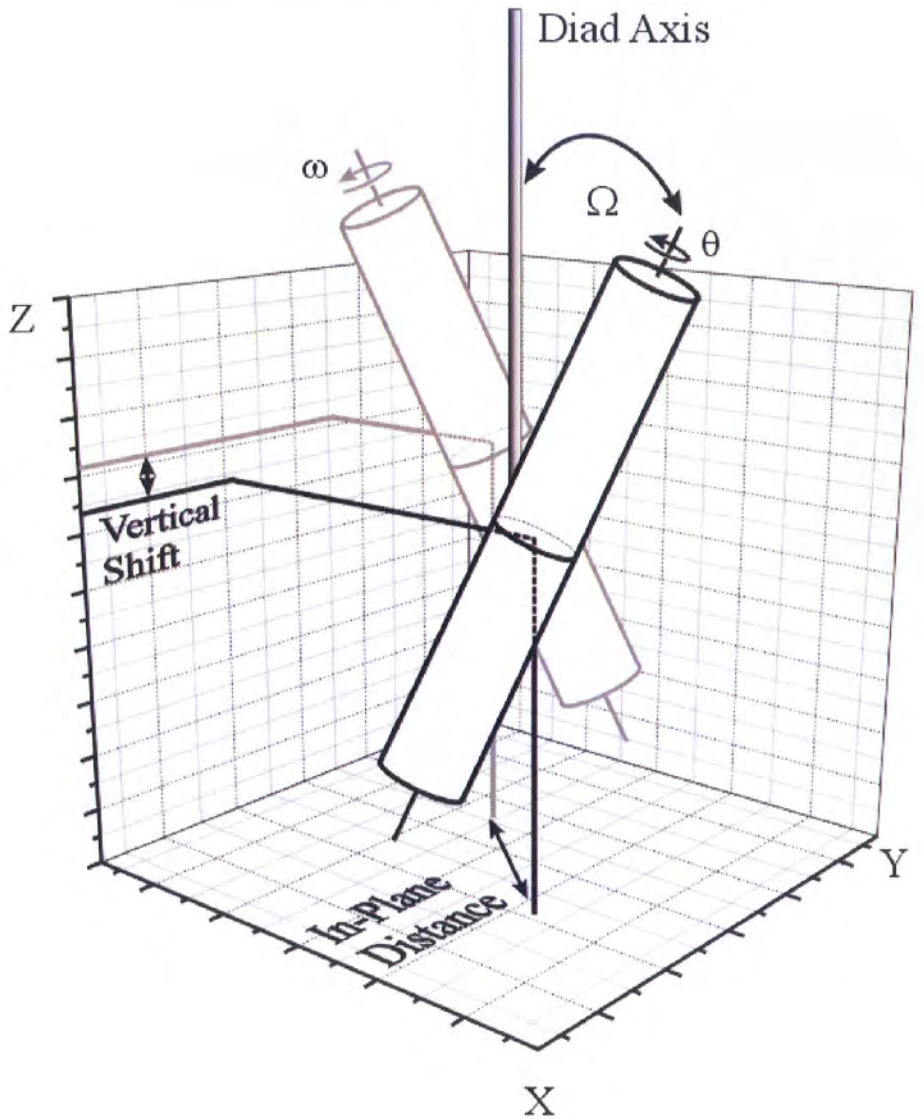
GXXXG motif





K. Gottschalk, P.D. Adams, A.T. Brunger, H. Kessler, Protein Science **2002**, *11*, 1800 - 1812

Global Search of Helix-Helix Interactions: Degrees of Freedom

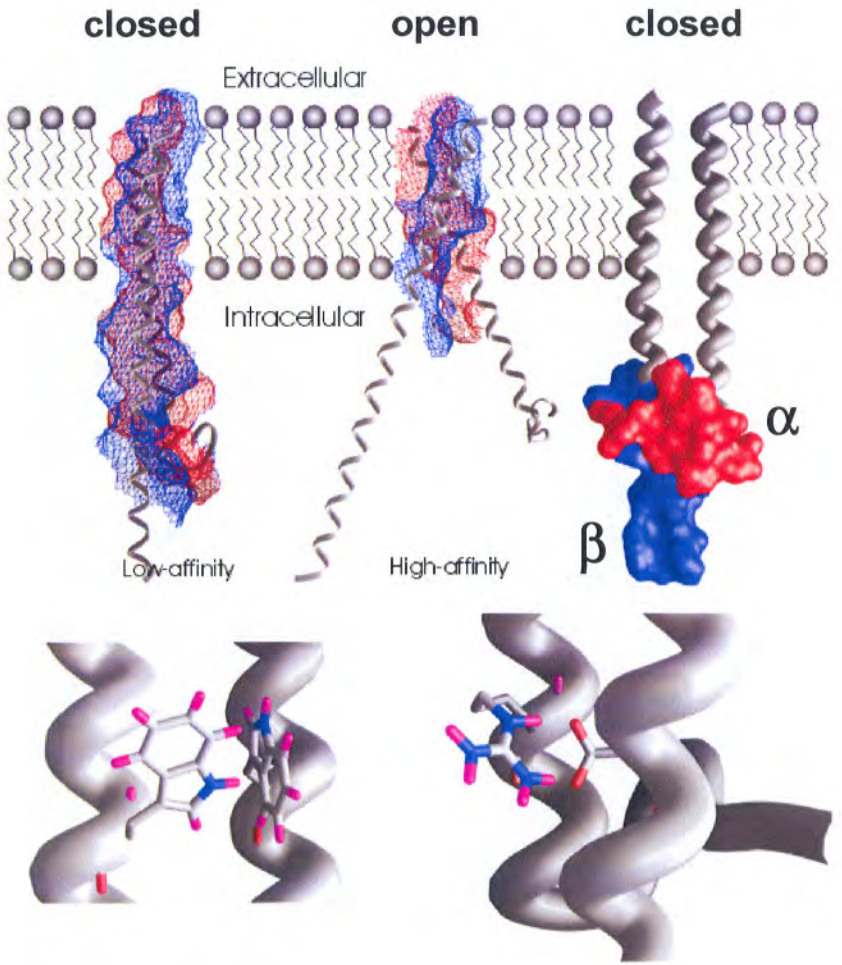


Integrin Subtypes Globally Searched

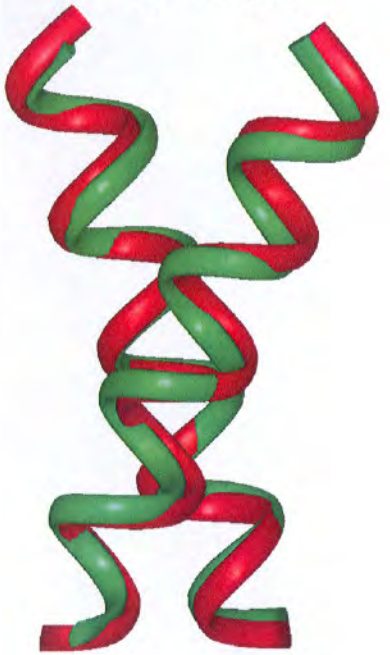
	β1	β2	β3	β5	β6	β7	β8
α1	X						
α2	X						
α3	X						
α4						X	
α5	X						
α6	X						
α7	X						
αD		X					
αL		X					
αM		X					
αV	X		X	X	X		X
αIIb			X				

16 different integrins

Open and Closed Conformation
Comparison with Experimental Results



Similarity of the open form and GpA



GpA: red

Integrin: green

Haas et al. *J.Biol.Chem.***1996**, *271*, 6017-26

Vallar et al.*J.Biol.Chem.***1999**, *274*,17257-66

Hughes et al. *J.Biol.Chem.* **1996**, *271*, 6571-4

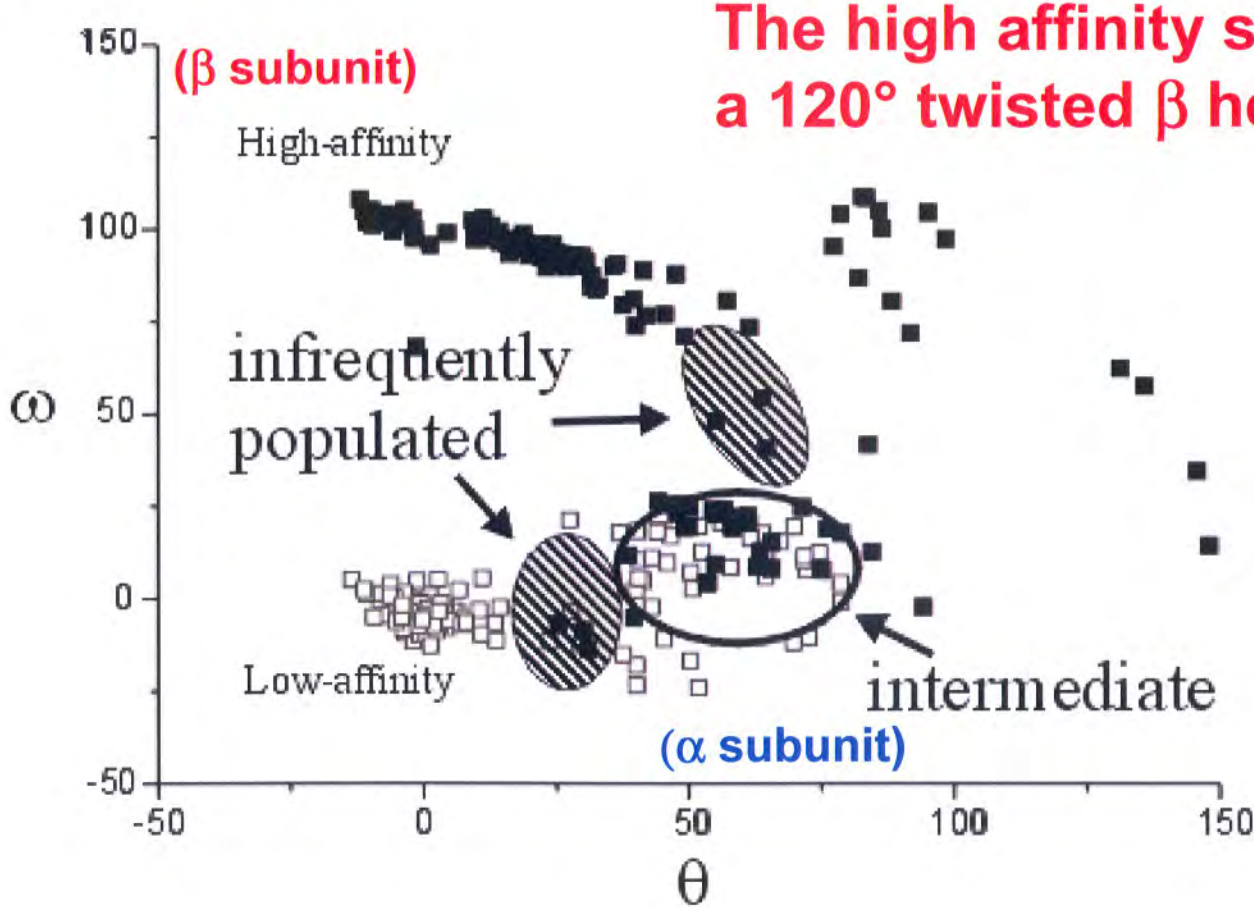
Lu et al *J.Biol.Chem.***2001**, *276*, 14642-8

MacKenzie et al. *Science* **1997**, *276*, 131-133

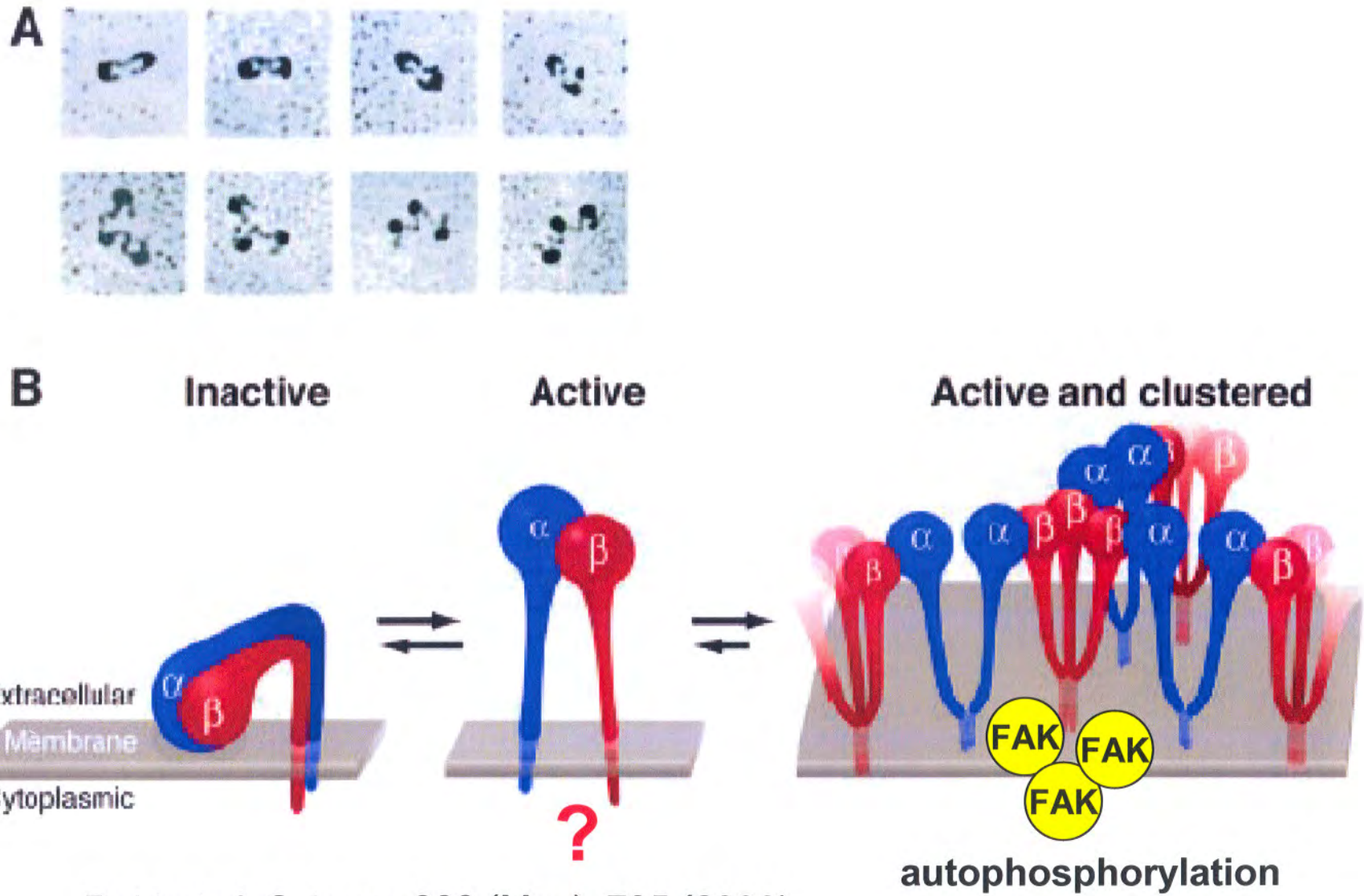
Transition Between High-Affinity and Low-Affinity State: Three States?

High temperature molecular dynamics simulations starting from open and closed conformations (150 calculations per starting structure)

The high affinity state has a 120° twisted β helix

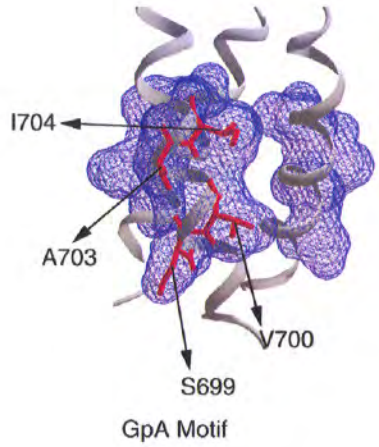


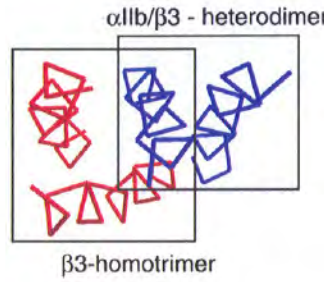
Oligomerization of Integrin Subunits from αIIbβ3

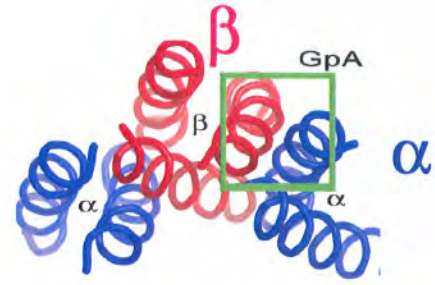


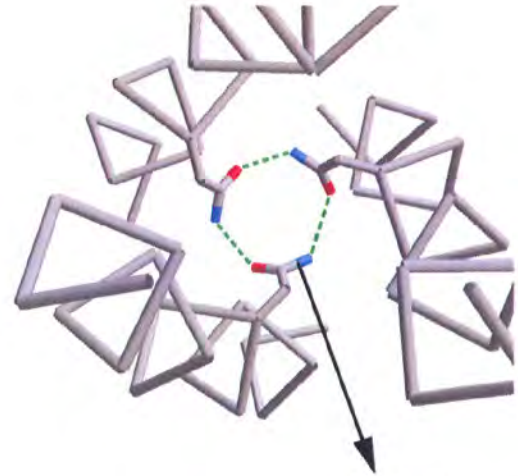
R. Li et al. Science 300 (May), 795 **(2003)**

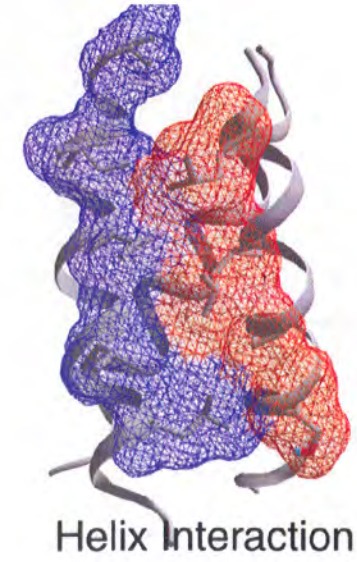
The Trimer of the β Subunite allows Dimers of α to Associate





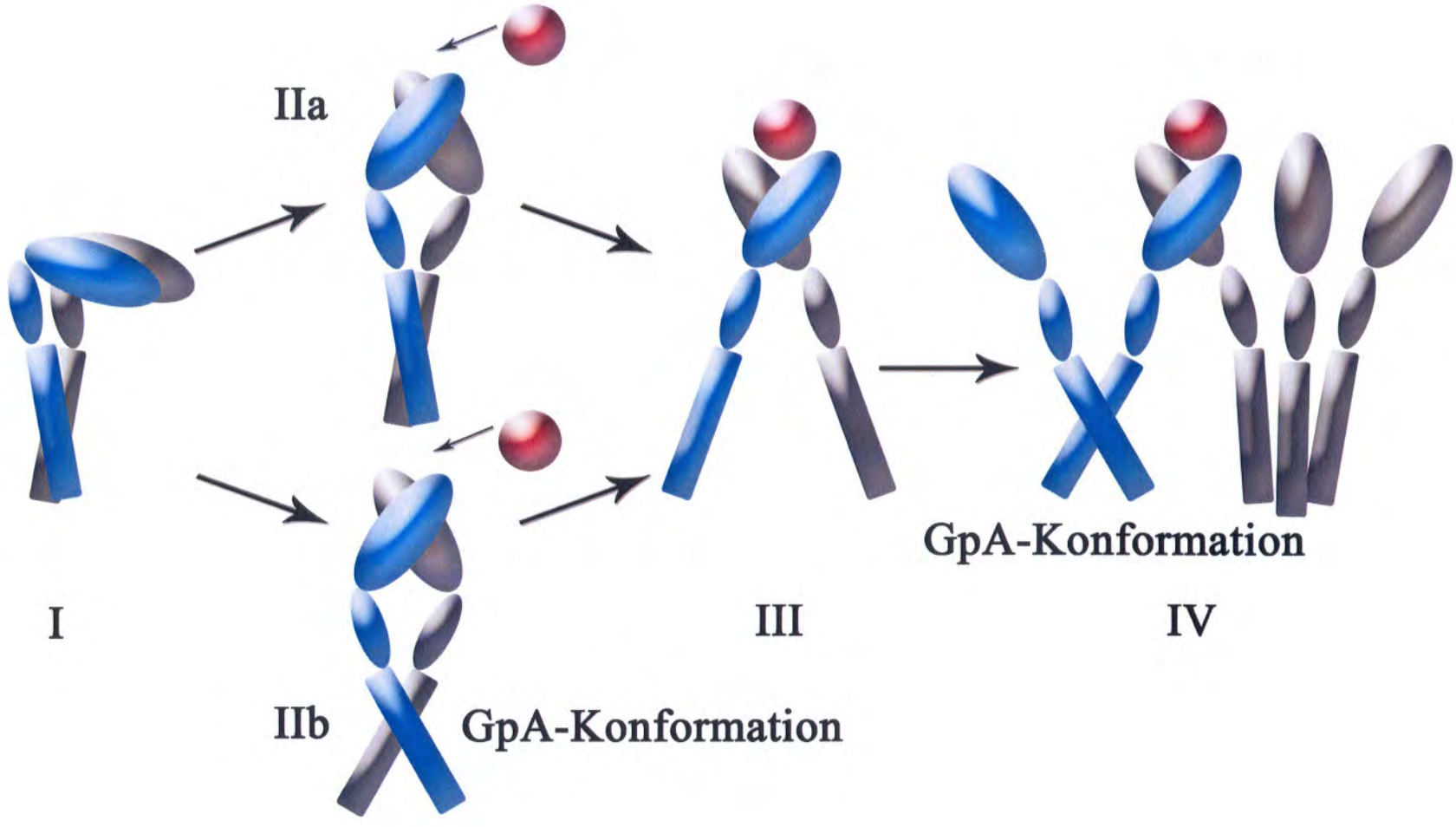






K. Gottschalk, H. Kessler, *Structure* **2004,** *12*, 1109-1116

Activation of Integrins and Formation of Focal Adhesions



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cyclic peptides	Marion Gurrath	R. Timpl	(MPI Martinsried)
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	Michael Dechantsreiter	S. Goodman	(Merck KGaA)
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	Harald Schmidt	G. Hölzemann	(alle Merck KGaA)
		M. Wiesner	
non-peptide	Christoph Gibson		
	Gabor Sulyok		
radio-labeling	Armin Modlinger	M. Schwaiger	alle
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