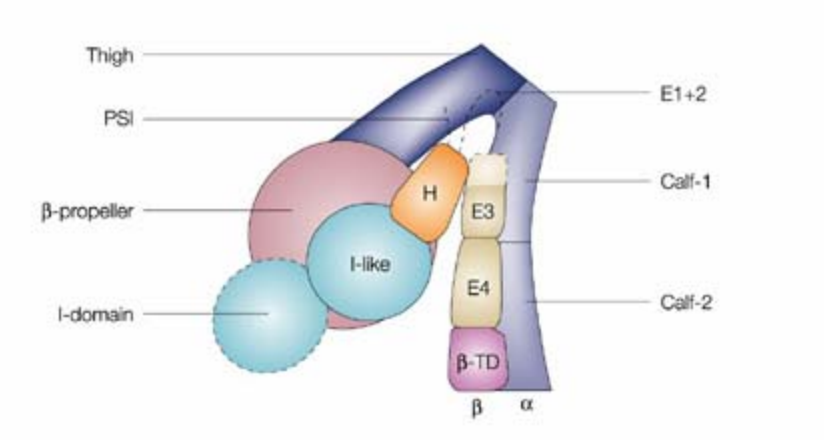
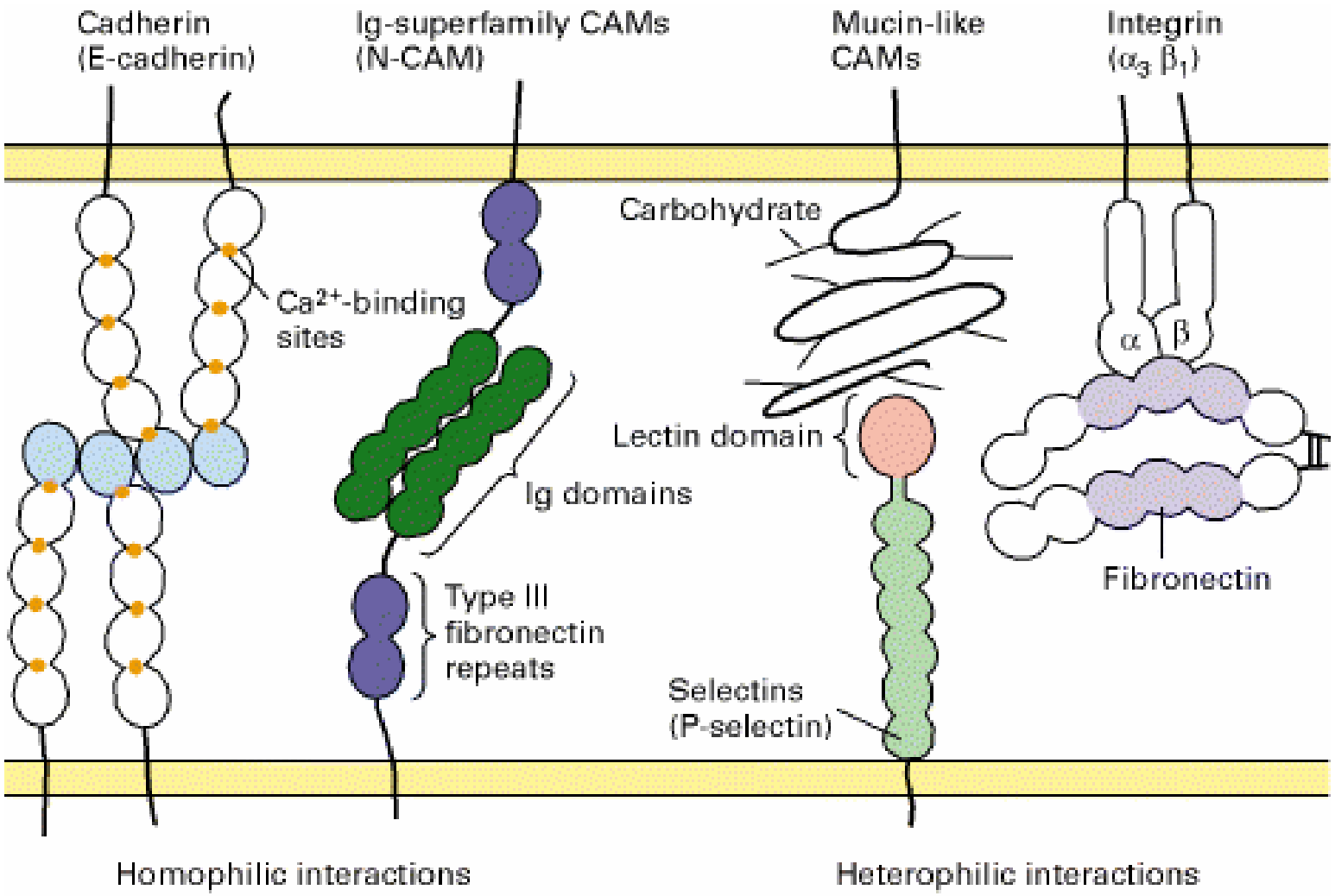
- Inhibiting protein-protein interactions with small molecules
- From Aspirin to Cox-2 inhibitors and beyond

Integrins

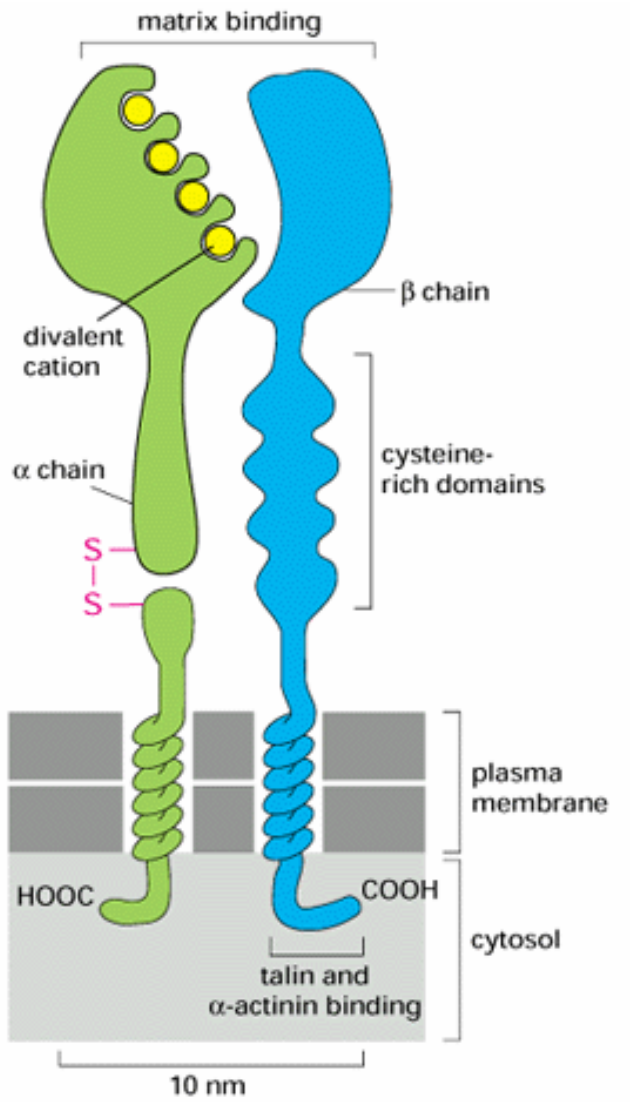
Inhibiting Protein-Protein Interactions



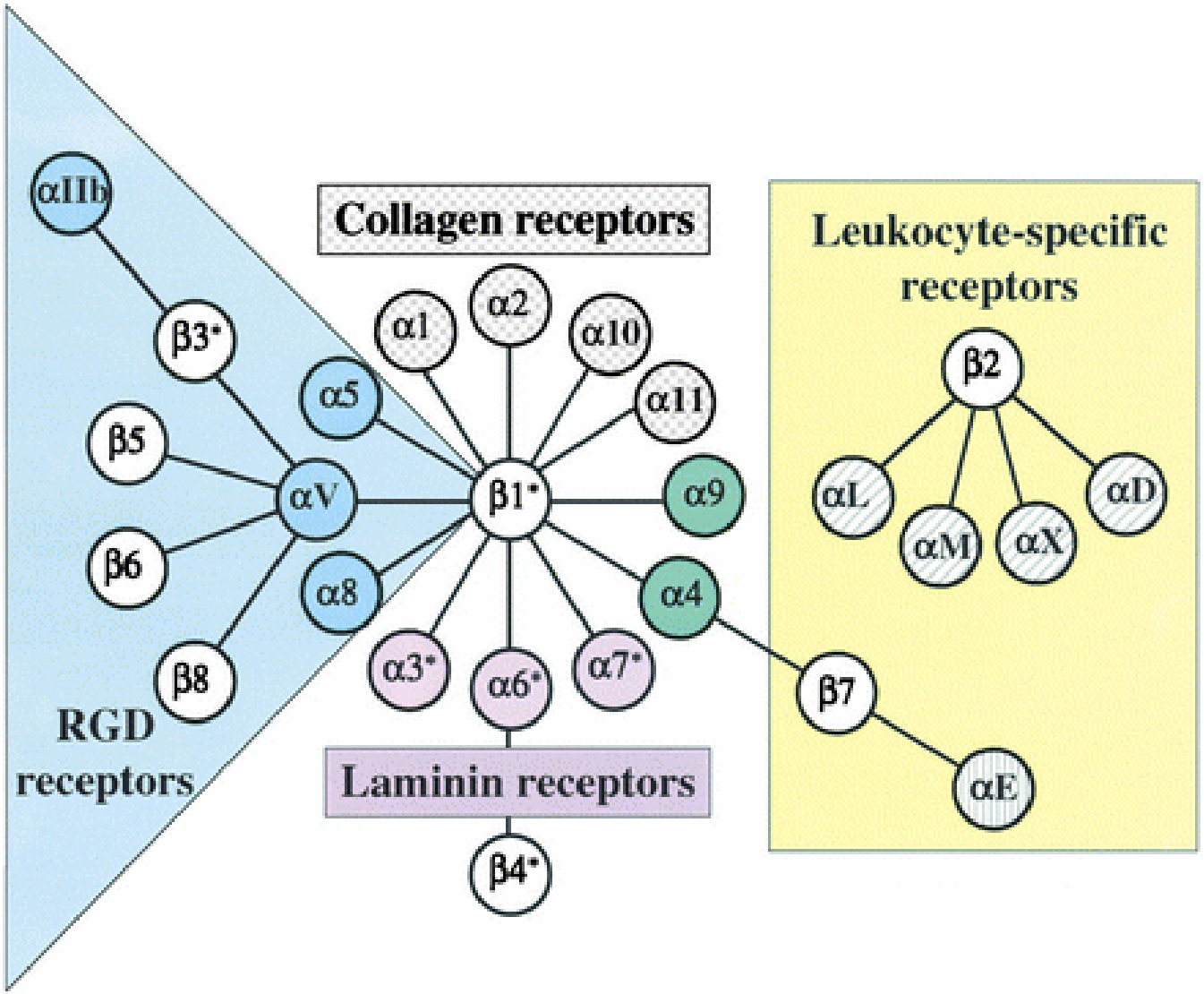
What Brings Cells Together?



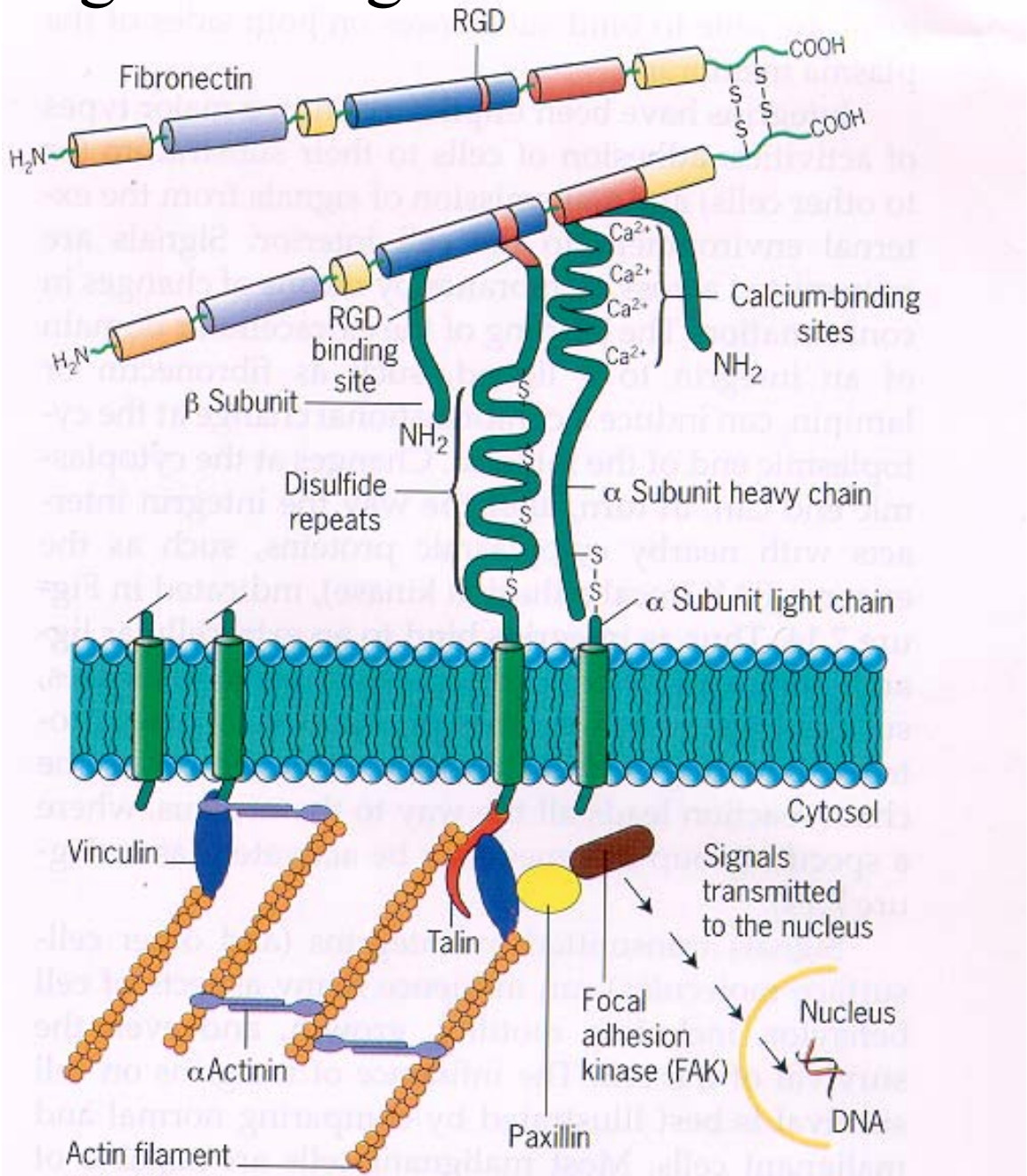
Integrins are Transmembrane Proteins



Integrins are Heterodimeric Proteins



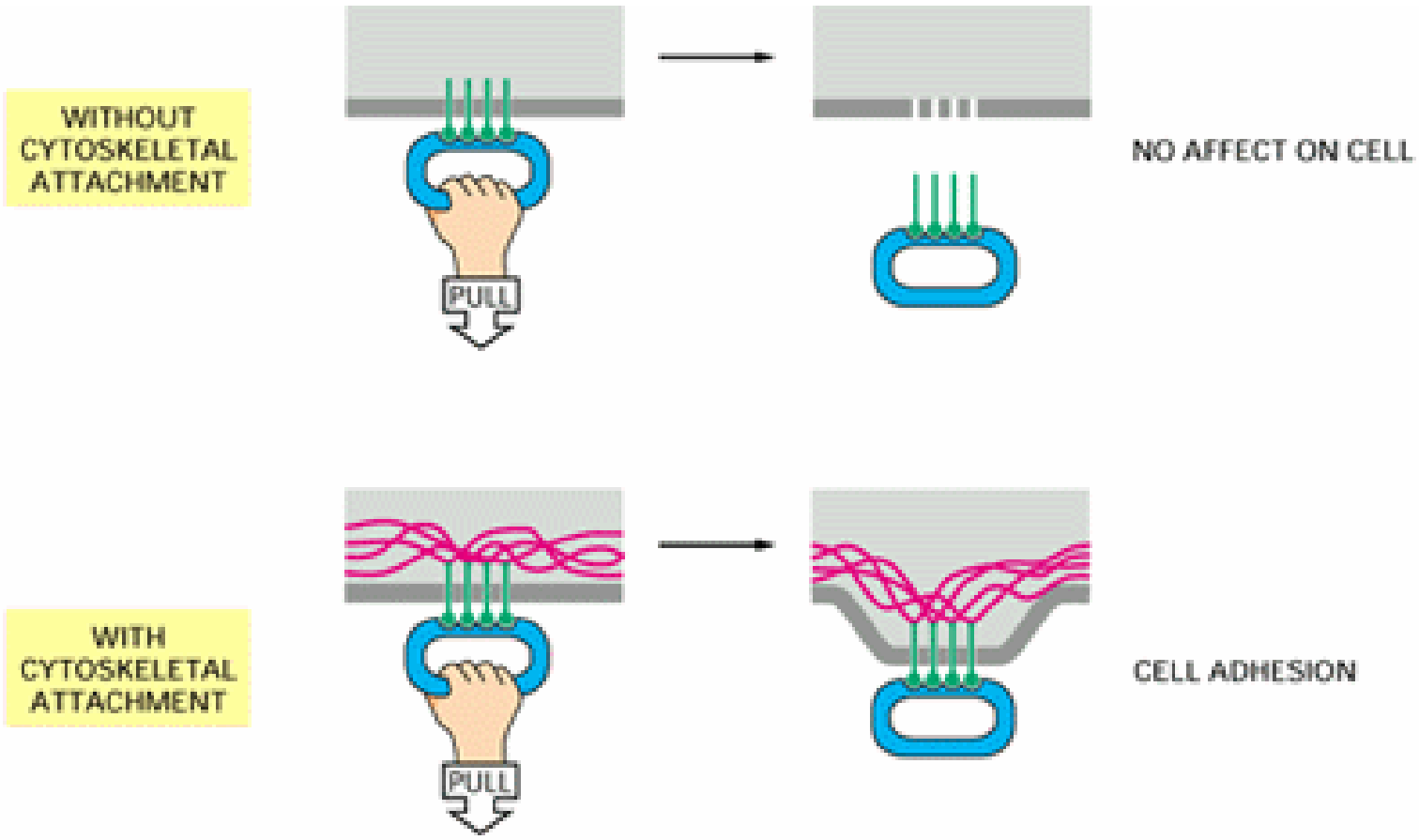
Ligand:Integrin Interactions - Fn



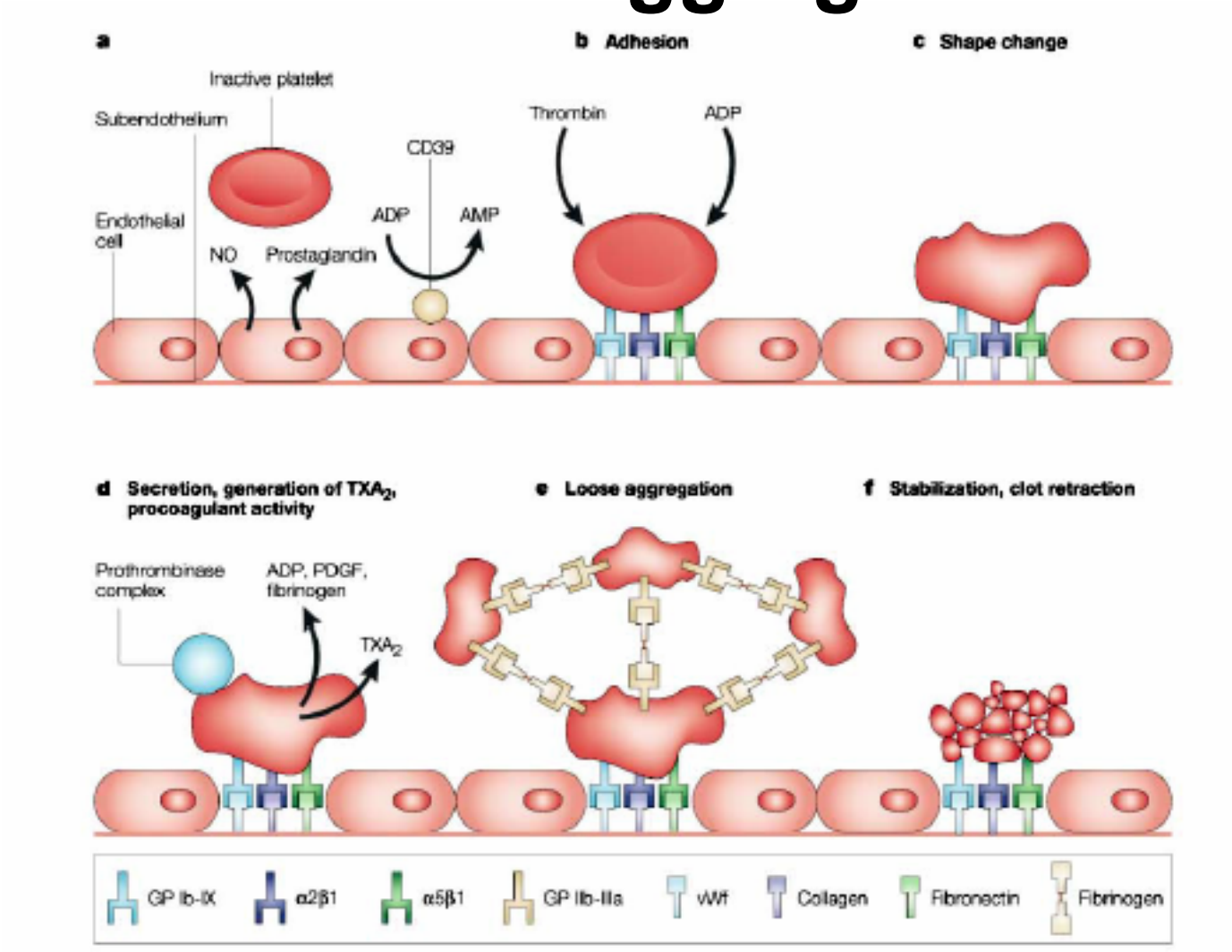
Cell and Molecular Biology by Karp 1999, Wiley and sons Publishing Inc. New York, NY

Hagman, MRL

Integrins are Holding Our Tissues Together

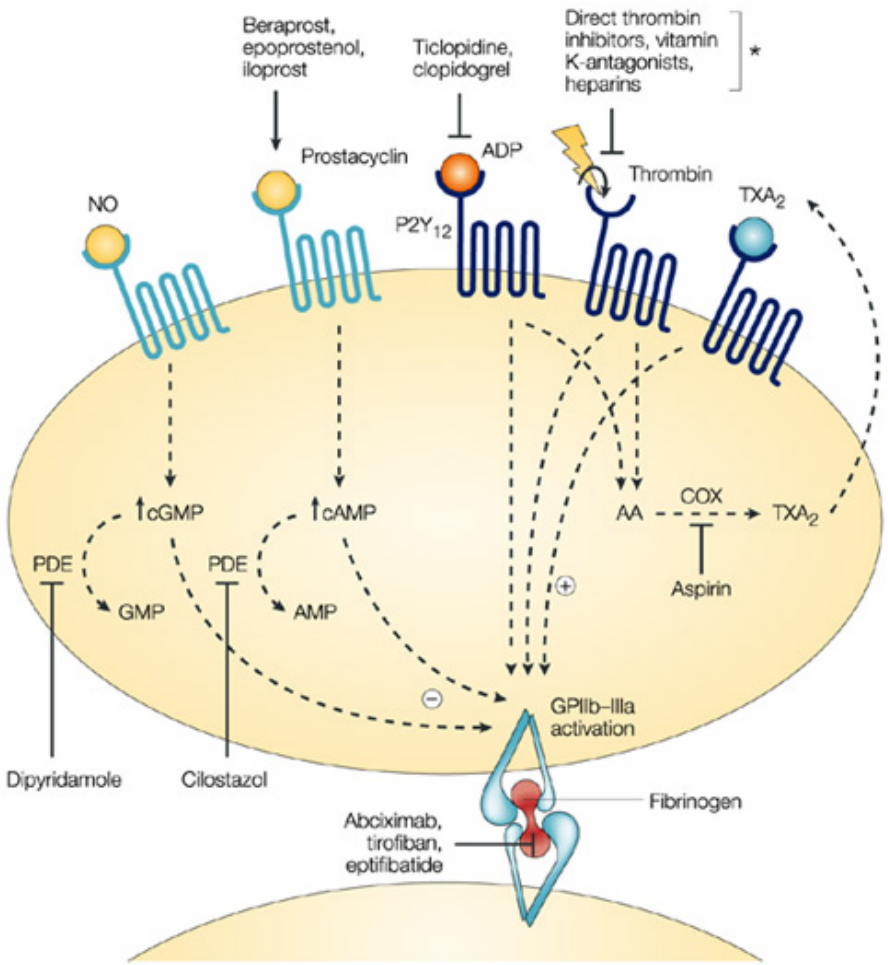


Integrins in Coagulation: Platelet Aggregation

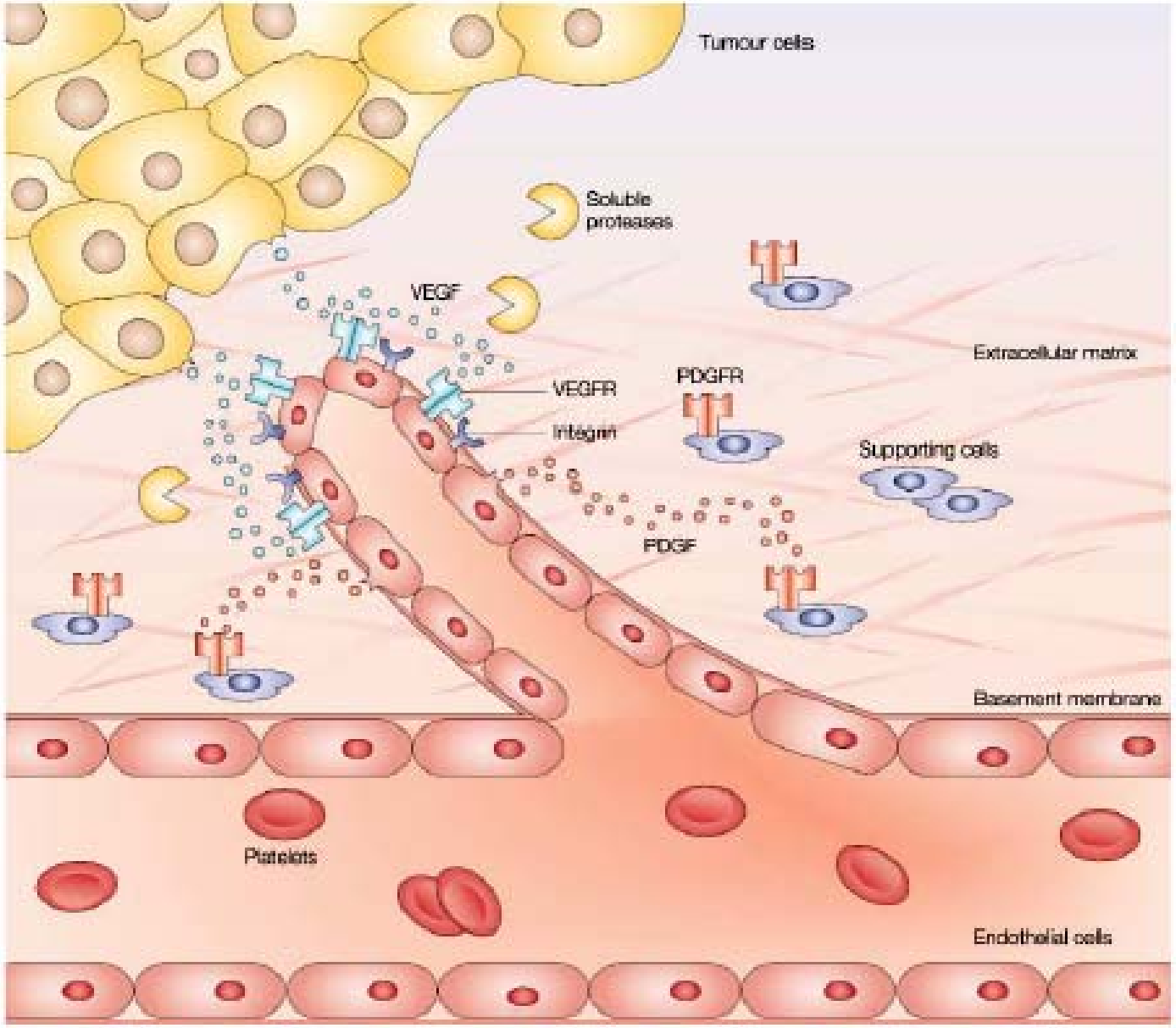


α2β1 αIIbβ3

Integrin Mediated “Final Common Pathway” for Platelet Aggregation

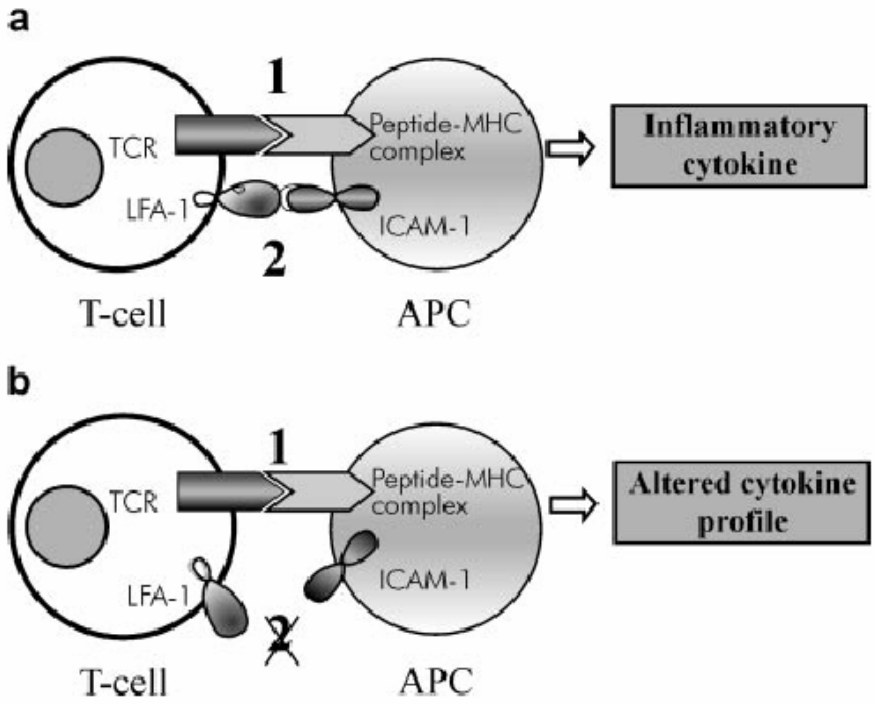


Integrins in Angiogenesis

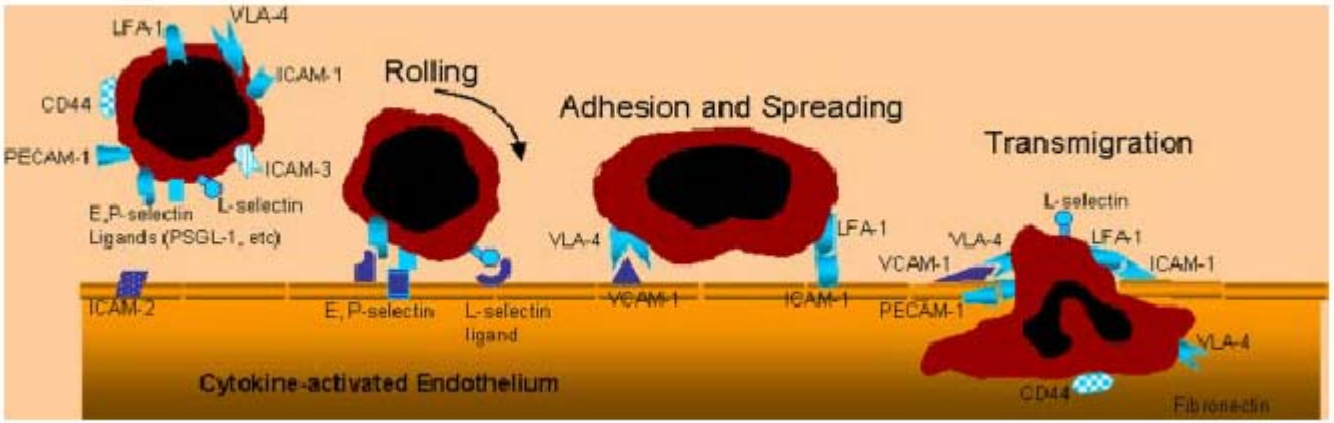


Integrins in Immune Response

Immune synapse:

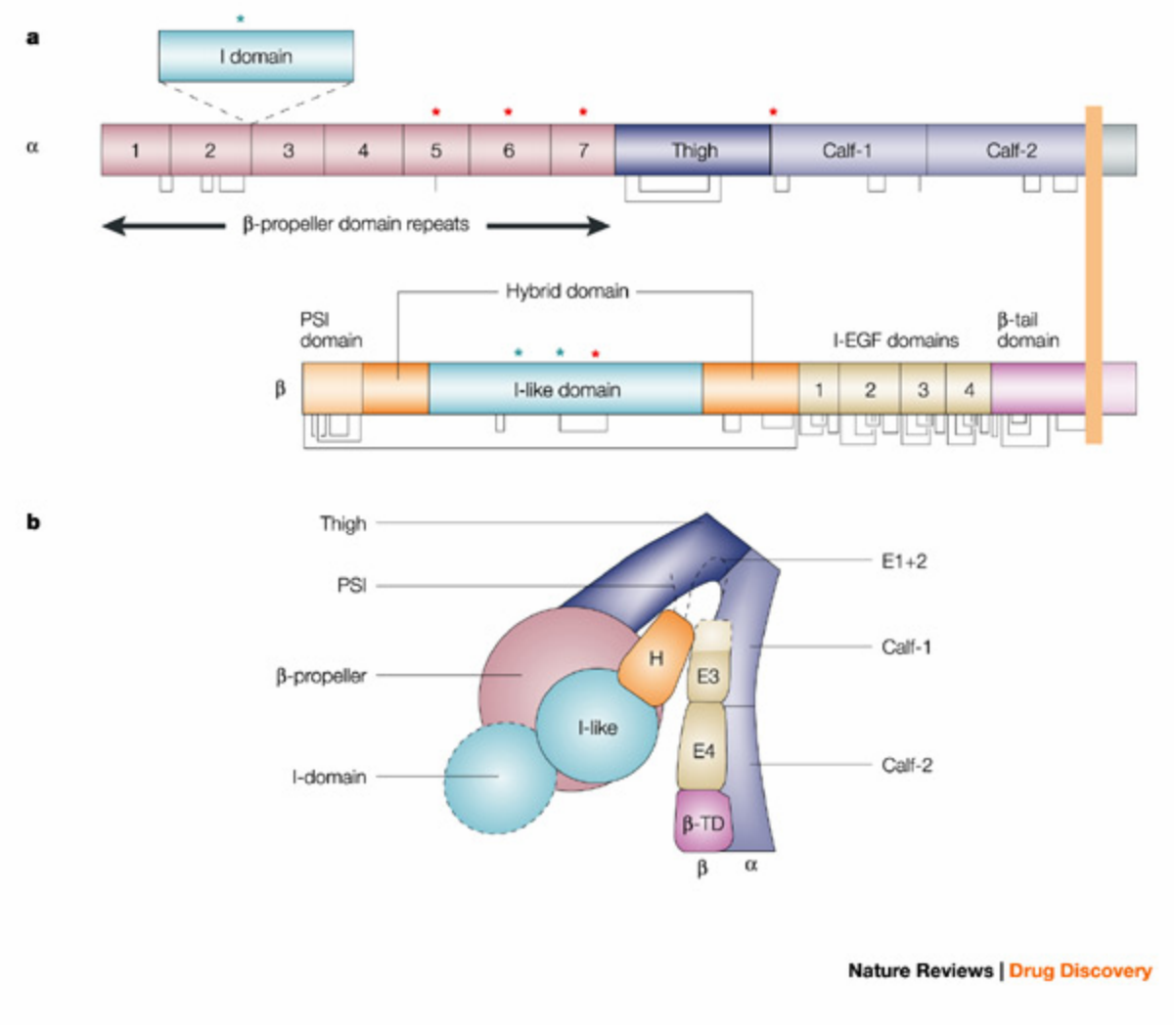


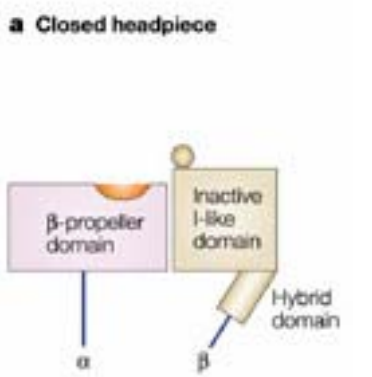
Activation and transmigration of leukocites:

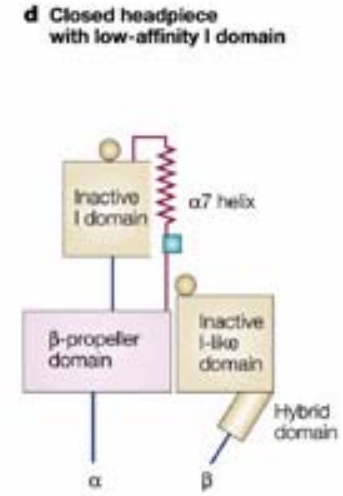


Leukocyte function-associated antigen (LFA-1, $\alpha_L\beta_2$, and CD11a/CD18) and very late antigen (VLA-4, $\alpha_4\beta_1$, and CD49d/CD29)

Structure of Integrin

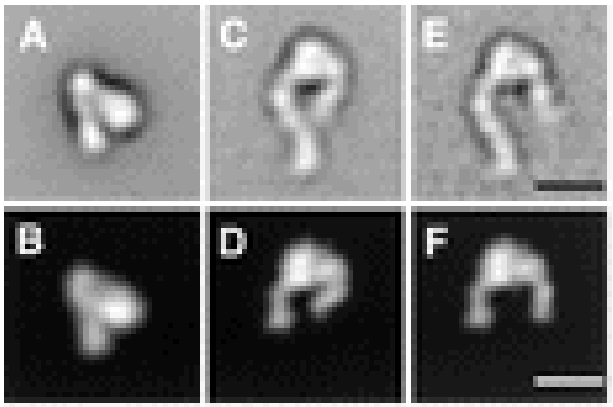


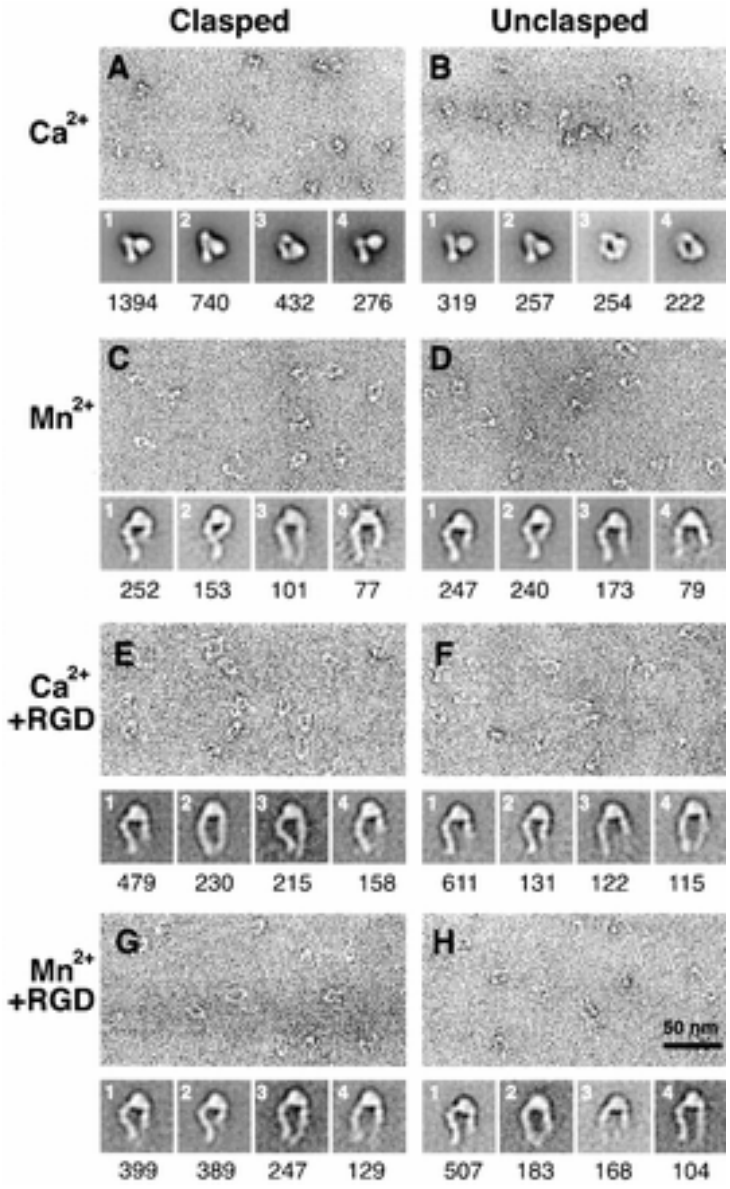




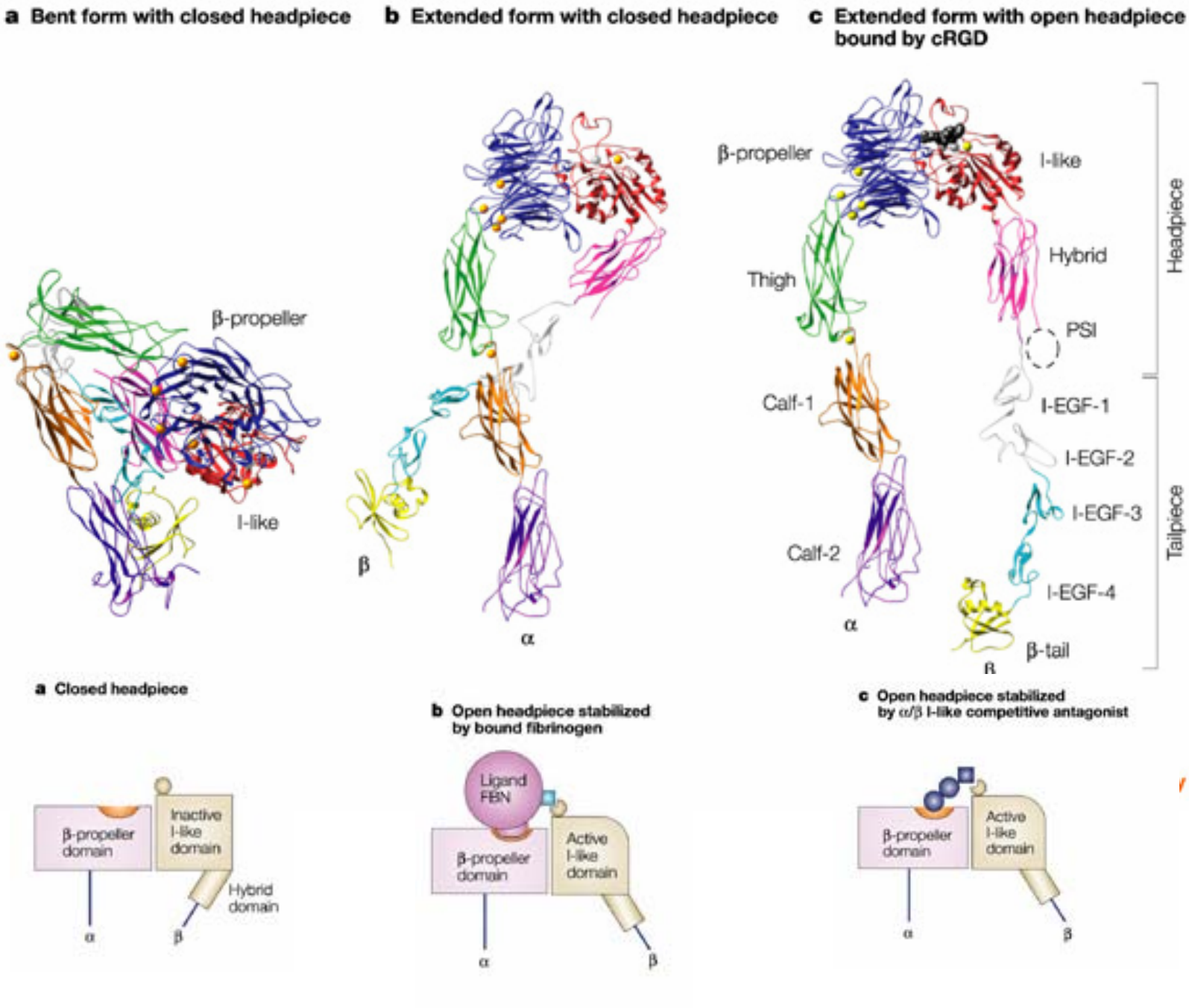
Annu. Rev. Biophys. Biomol. Struct. 2002. 31:485–516

Conformational Change – Caught on Camera!

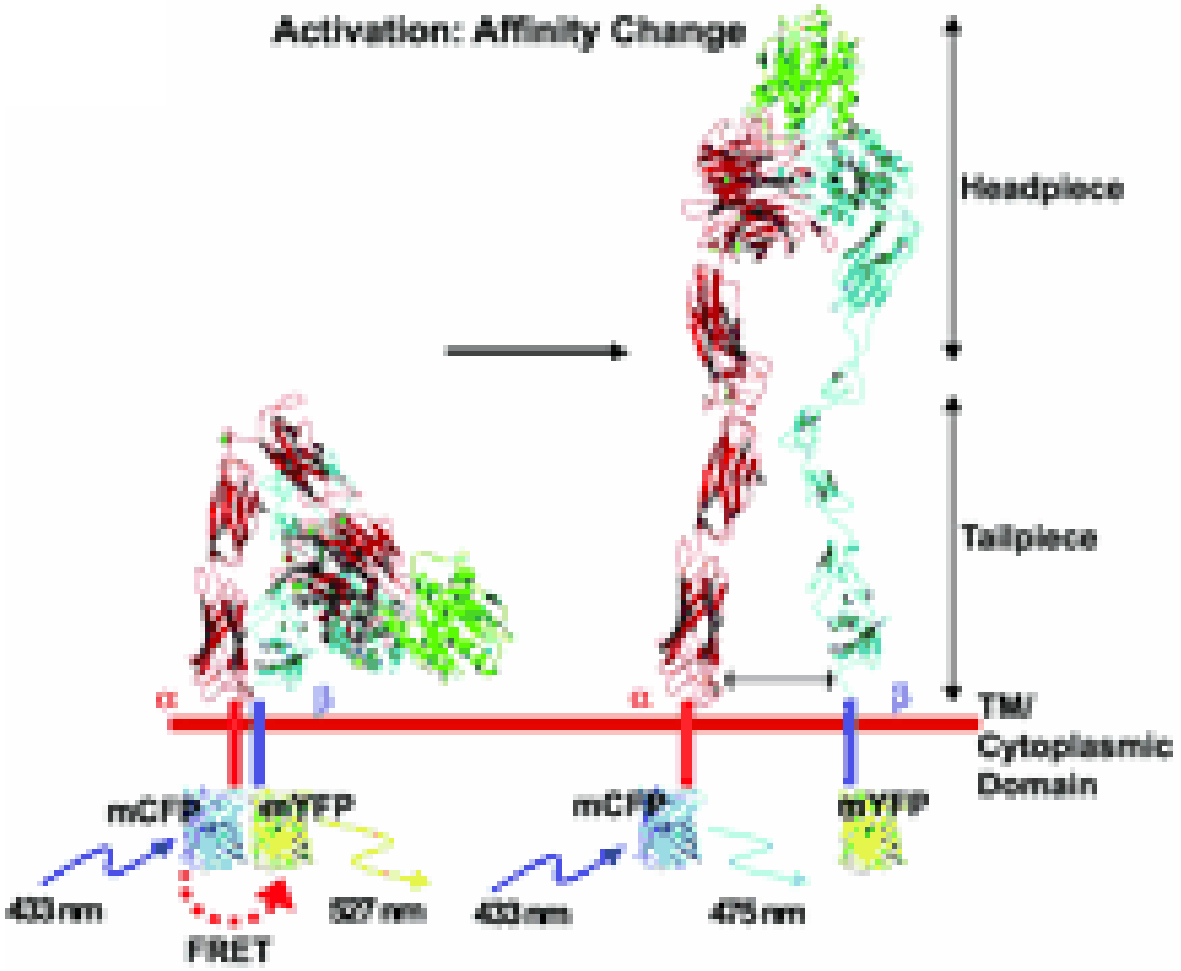




Timothy A. Springer Cell, Vol. 110, 599–611, September 6, 2002,


a Bent form with closed headpiece
b Extended form with closed headpiece
c Extended form with open headpiece bound by cRGD
β-propeller
I-like
β
α
β-propeller
I-like
Hybrid
Thigh
PSI
Calf-1
I-EGF-1
I-EGF-2
I-EGF-3
Calf-2
I-EGF-4
α
β-tail
β
Headpiece
Tailpiece
a Closed headpiece
b Open headpiece stabilized by bound fibrinogen
c Open headpiece stabilized by α/β I-like competitive antagonist
β-propeller domain
Inactive I-like domain
Hybrid domain
α
β
Ligand FBN
β-propeller domain
Active I-like domain
α
β
β-propeller domain
Active I-like domain
α
β

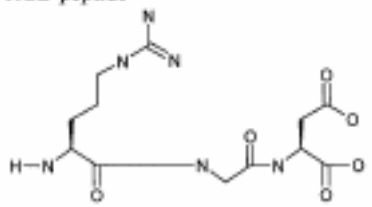
Conformational Change Occurs in Cells – as Well!



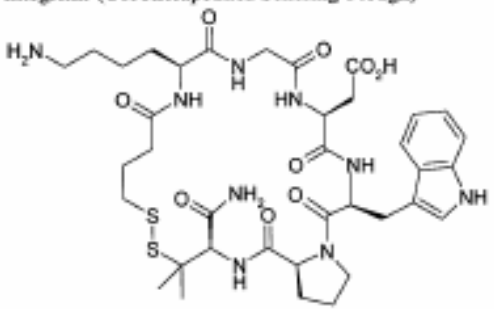
Timothy A. Springer 19 SEPTEMBER 2003 VOL 301 SCIENCE

αIIbβ3 α/β I-like Competitive Antagonists Platelet Aggregation Inhibitors

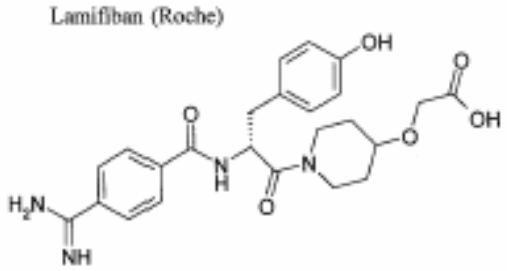
RGD peptide



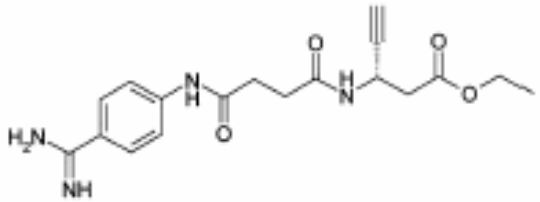
Integrilin (CorTherapeutics/Schering-Plough)



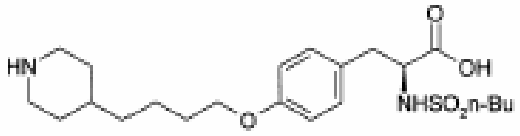
Lamifiban (Roche)



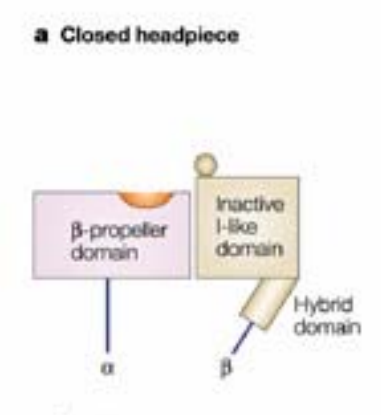
Xemilofiban (Searle)

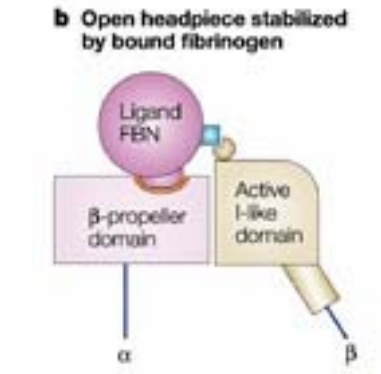


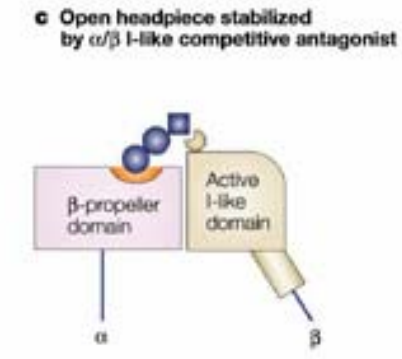
Tirofiban (Merck)



Disintegrins from snake venom!

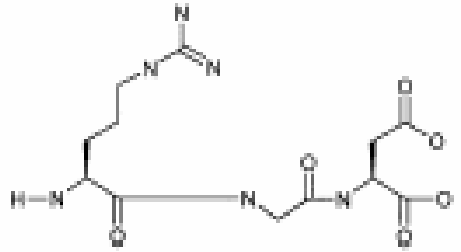




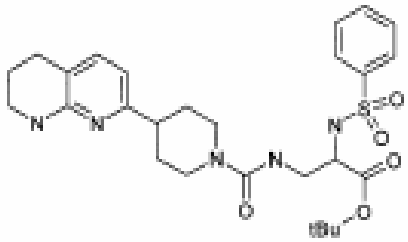


αvβ3 α/β I-like Competitive Antagonists Angiogenesis Inhibitors

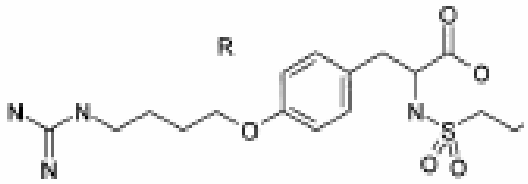
RGD peptide



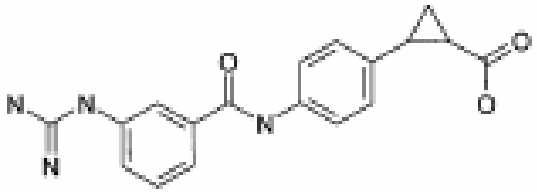
Merck&Co (WO9818461-A1)

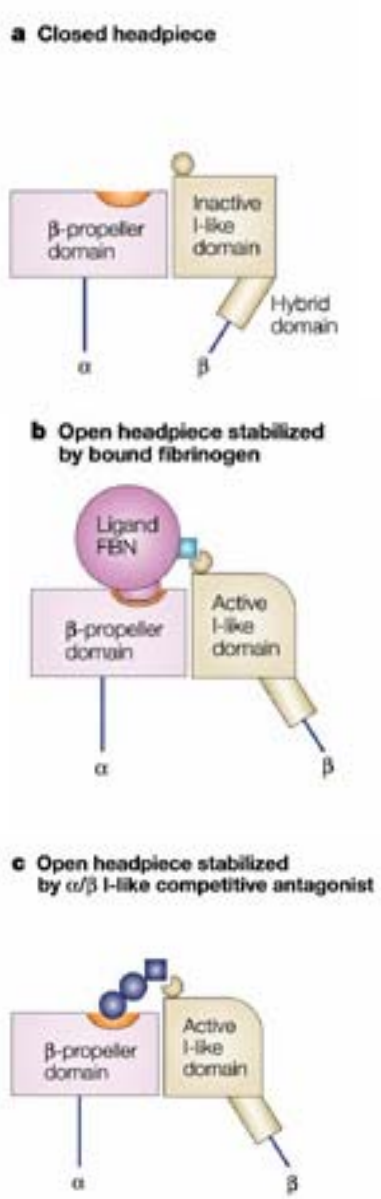


Merck KGaA (DE19548709-A)



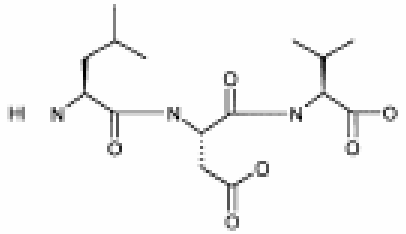
Searle (WO9736858-A1)



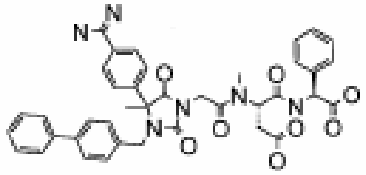


α4β1 α/β I-like Competitive Antagonists Autoimmune Diseases

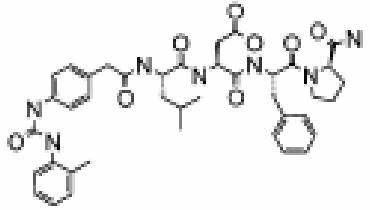
LDV peptide



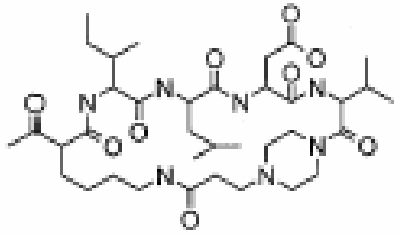
HMR (EP-842943-A2)



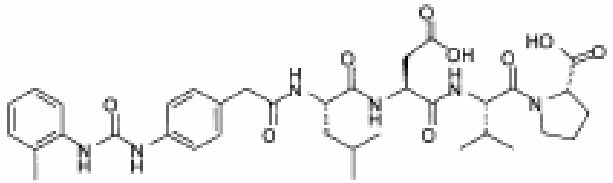
Cytel (WO9842656-A1)

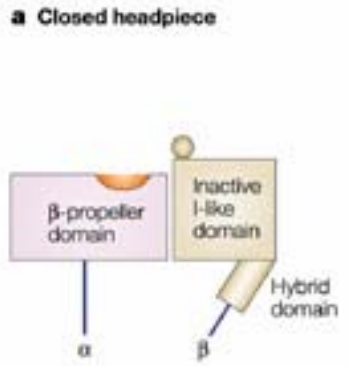


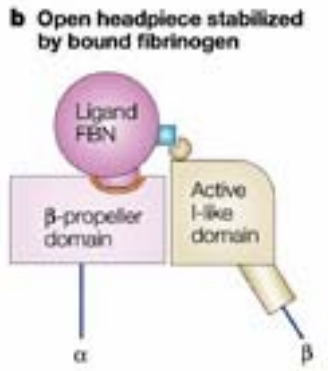
Zeneca (WO9620216-A1)

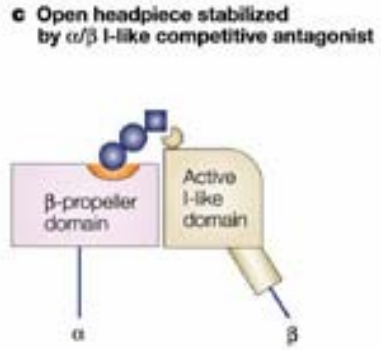


Biogen (BIO1211)









α_4-Antibodies and Antagonists are Active in Animal Models of Inflammation

- Airway inflammation.
  - Antigen sensitized / airways hyperresponsiveness (mice, rats, guinea pigs, and sheep)
- Other models of inflammatory disease.
  - EAE (rats, mice, and guinea pigs)
  - chronic colitis (cotton top tamarins)
  - adjuvant arthritis (rats)
  - atherosclerotic plaque formation (mice)
  - adoptive transfer model of diabetes (NOD mice)
  - restenosis (rabbits)
  - acute cardiac graft rejection (rats)

Merck Medicinal Chemistry Lead Generation

Sequence for VLA-4 Binding on VCAM-1 and Fibronectin

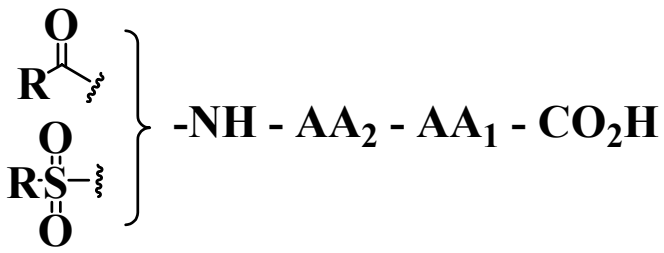
CD-loop of VCAM-1 Type III CS-1 domain of FN

-Q<u>IDS</u>P- -I<u>LDV</u>P-

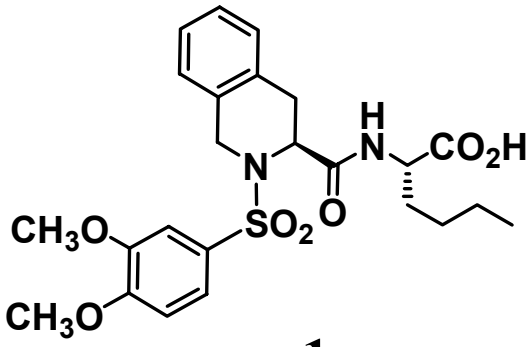
$R\text{-}CO_2H$

Initiated a focused screening effort on carboxylic acid containing compounds.

Lead Discovery from Combinatorial Library

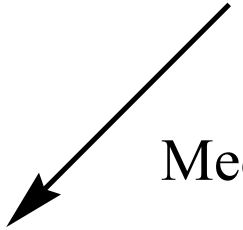


C-000,013
Capped dipeptide
combinatorial library



1

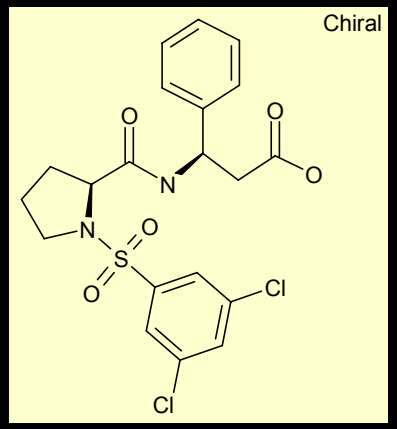
IC_{50} = 58 nM

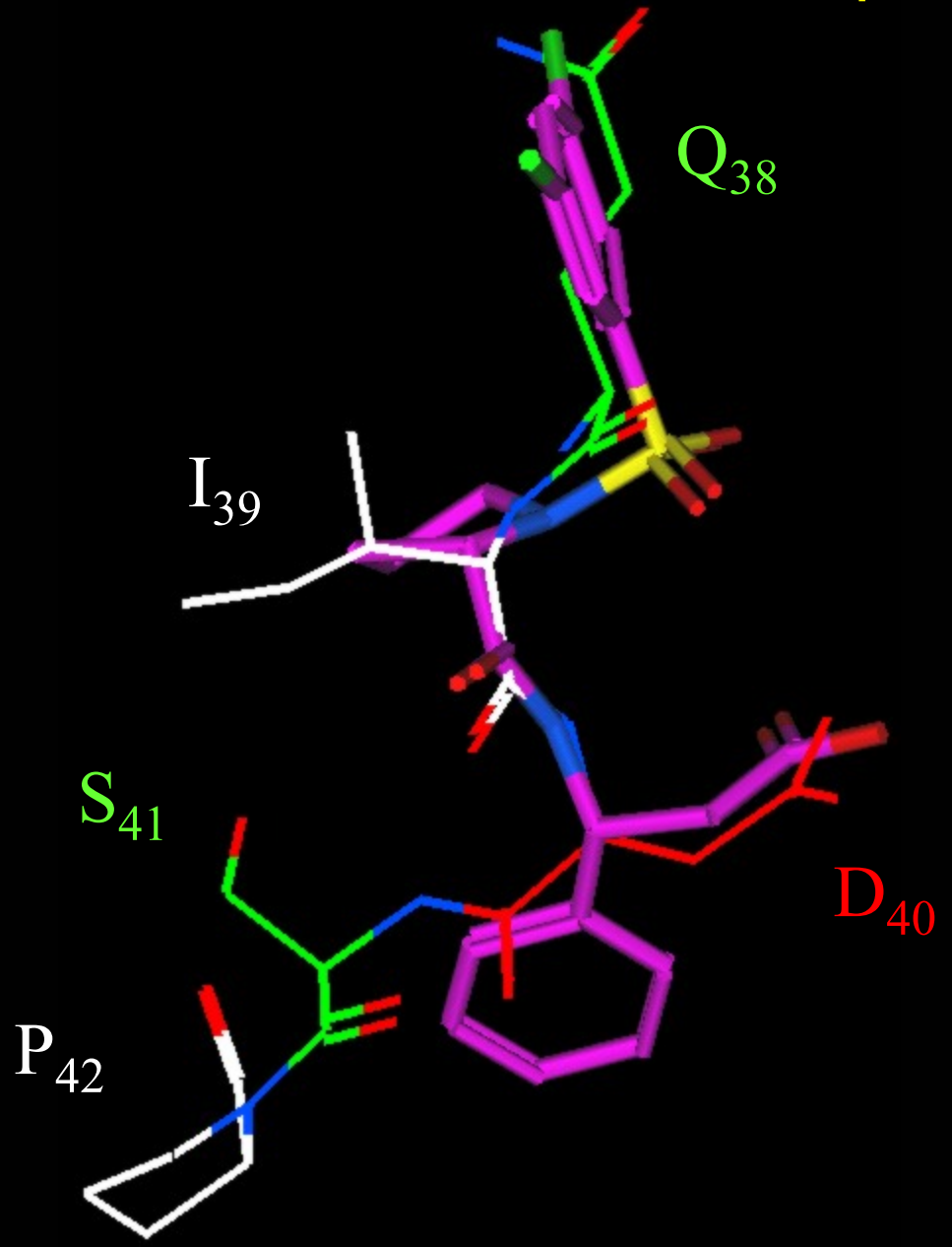


"Outsourced"
Medicinal Chemistry

- Solid phase synthesis
- Capped dipeptides
- Single compounds

Overlay of L-808116 with the CD loop of VCAM-1





Potential Therapeutic Targets for VLA-4 Antagonists

- Multiple Sclerosis
- Inflammatory bowel disease
  - Crohn's disease, ulcerative colitis
- Rheumatoid arthritis
- Chronic obstructive pulmonary disease (COPD)
- Asthma
- Atherosclerosis
- Diabetic retinopathy

<u>Orphan Diseases</u>

- Sickle cell anemia
- Mobilization of $CD34^{+}$ stem cells (cancer)
- Uveitis (ocular inflammation)

Potential Liabilities of VLA-4 Antagonists

Developmental Toxicity

- α_4 or VCAM-1 KO's are embryo-fetal lethal.
  - Failure of chorio-allantoic fusion and epi-myocardial fusion.
  - Heterozygous $\alpha_4^{(-/+)}$ embryos - normal development.
  - Conditional KO – normal development.

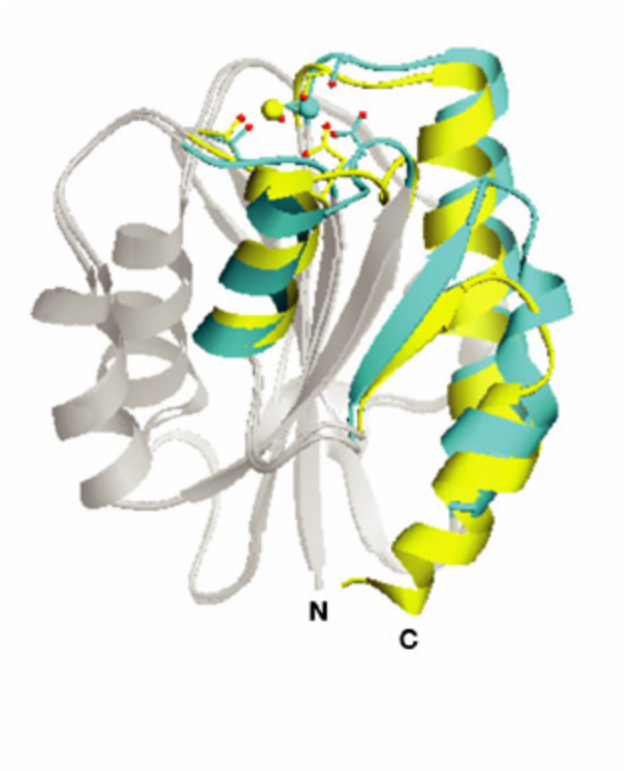
Hematology

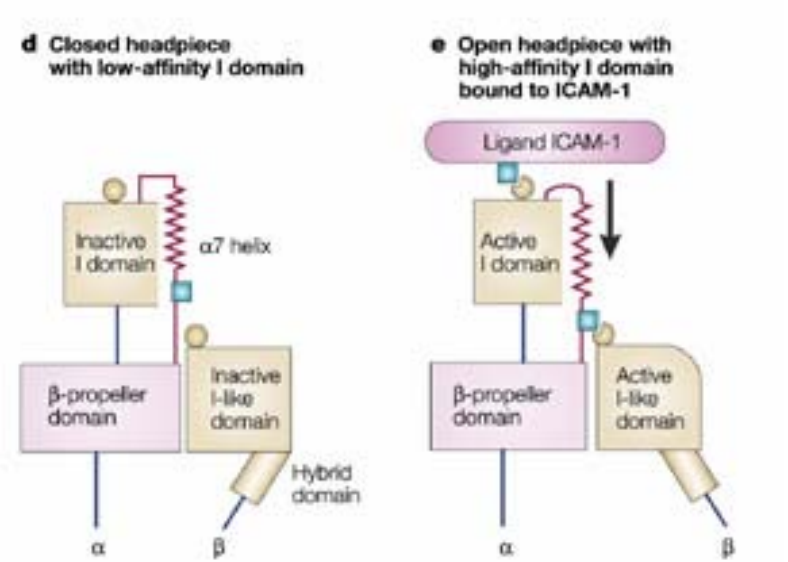
- Administration of anti-α_4 prenatally resulted in fetal anemia.
- Lymphocytosis observed in animals and humans.
- Neonatal lymphopoiesis.
- Hematopoietic progenitor cells.

Infection

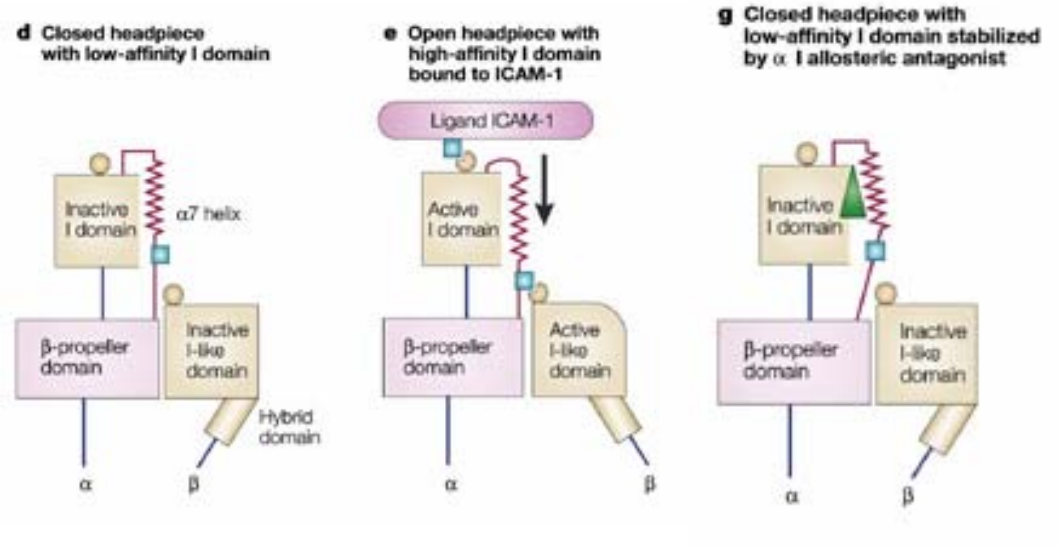
- Increased susceptability to respiratory infection.

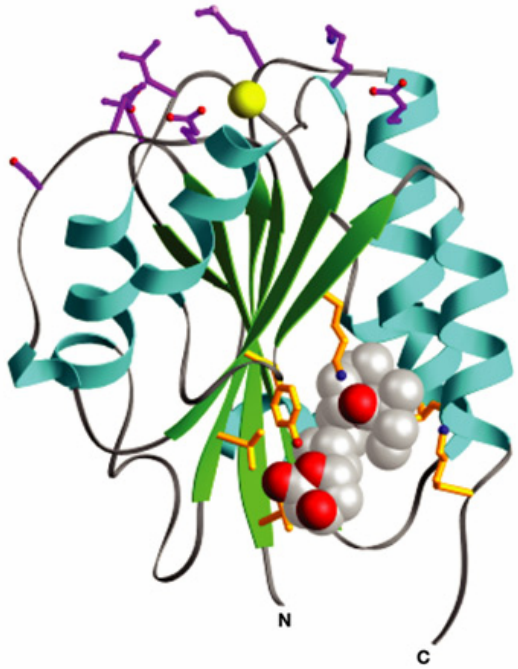
αLβ2 Integrin – Immune Response

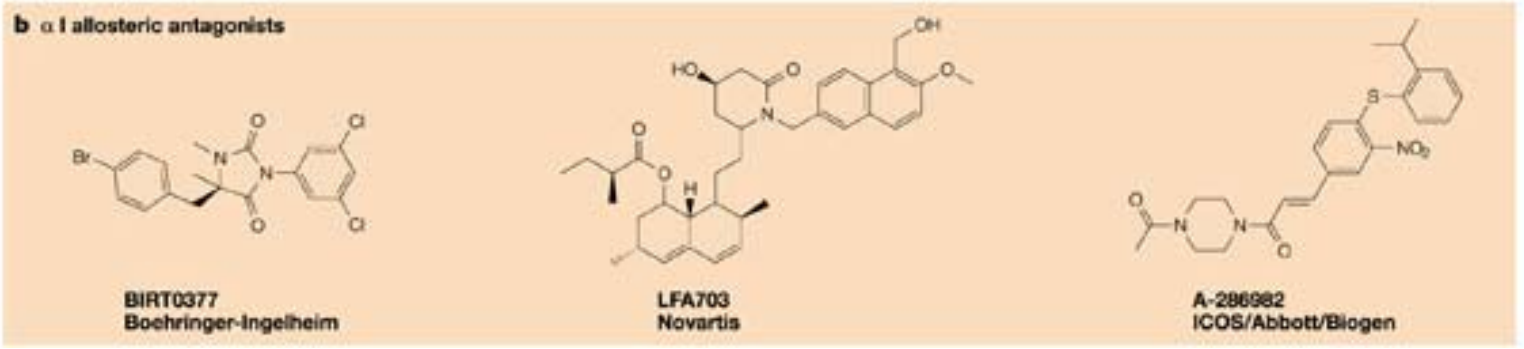




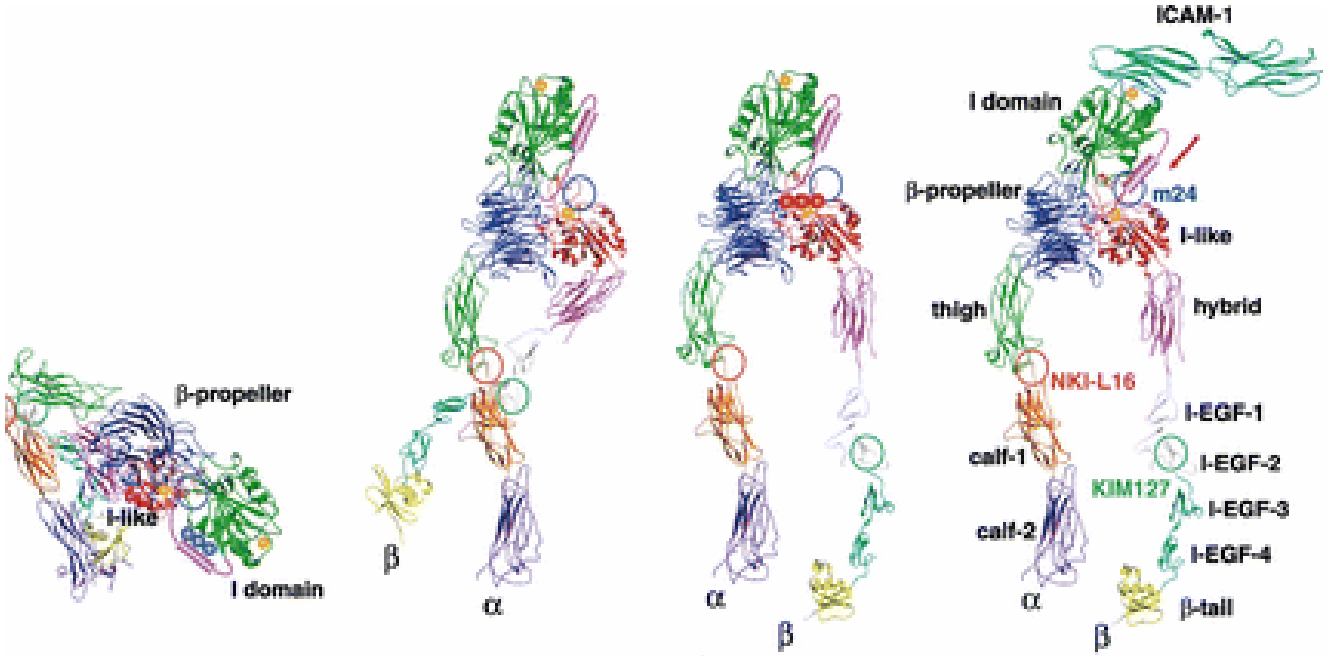
αLβ2 α I Allosteric Antagonist Autoimmune Diseases







αLβ2 α/β I-like Allosteric Antagonist

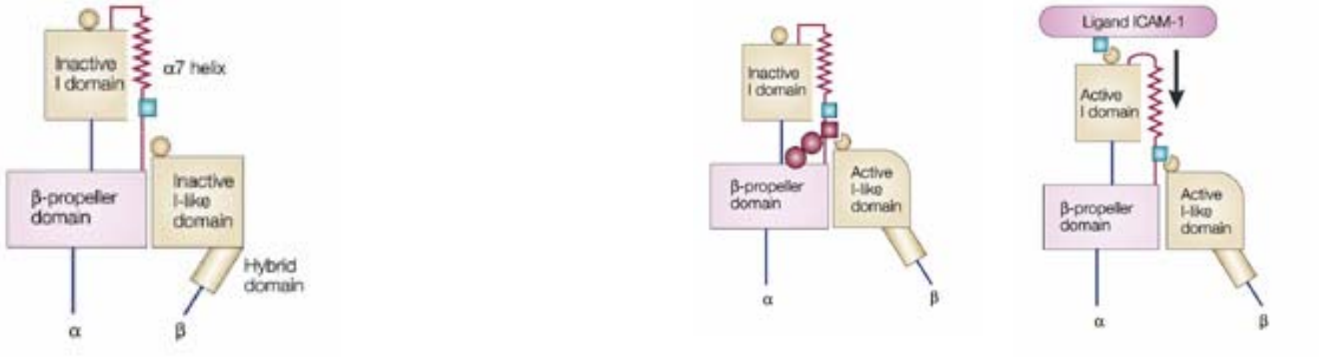


Bent form with closed headpiece
low-affinity I domain stabilized
α I allosteric antagonist

B. Extended form with closed headpiece and low-affinity I domain

C. Extended form with open headpiece and low-affinity I domain stabilized by α/β I-like allosteric antagonist

D. Extended form with open headpiece and high-affinity I domain stabilized by bound ICAM-1



Timothy A. Springer[1] Immunity, Vol. 19, 391–402, September, 2003

I-like Allosteric Antagonist vs. Competitive α4 Antagonist