Dr. Olga Vinogradova
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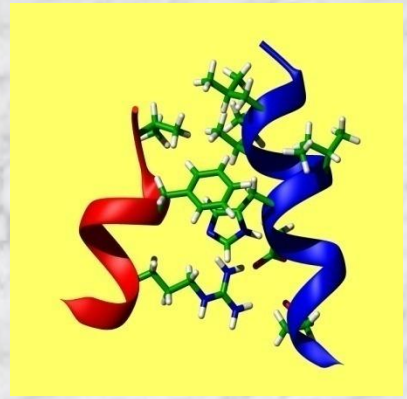
olga.vinogradova@uconn.edu

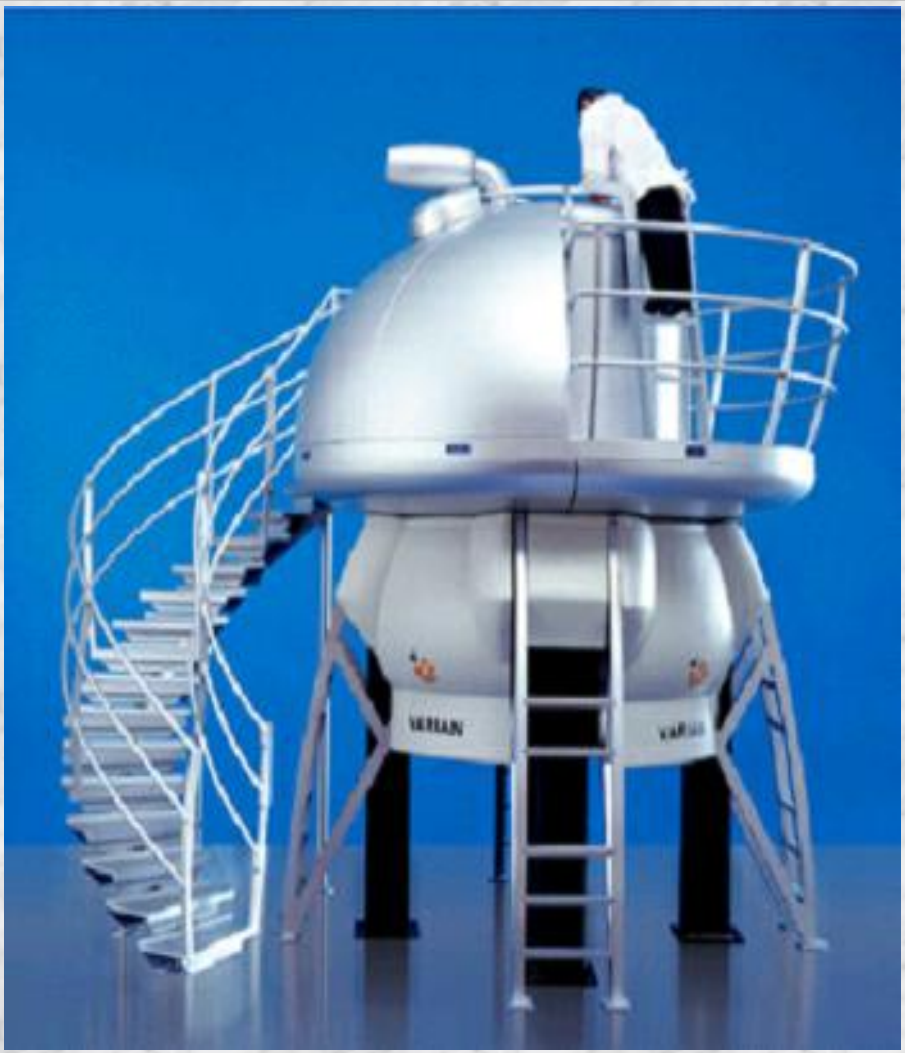


Structural Insights into Integrin Signaling

The 8th Joint National Medical and Scientific Conference

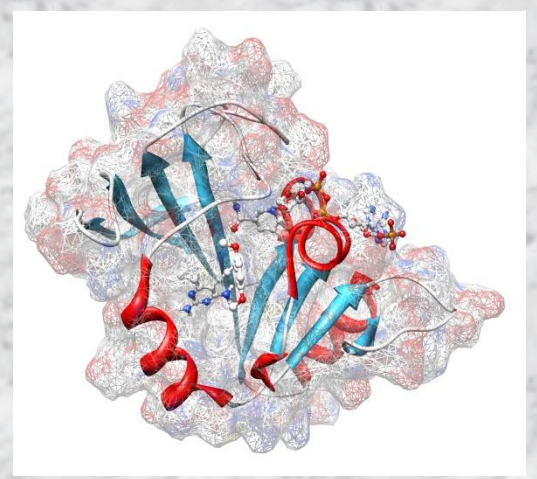
October 14, 2011

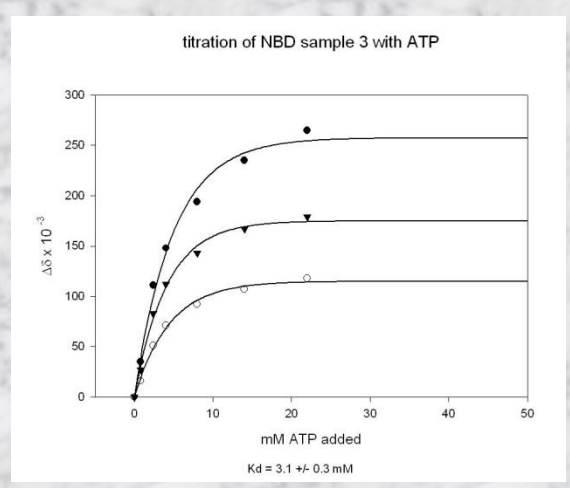
NMR

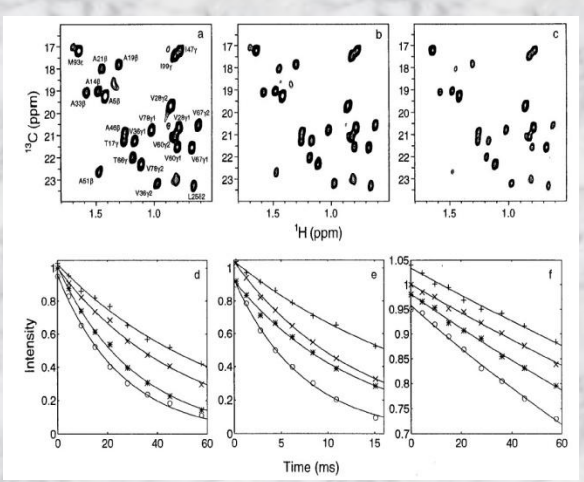


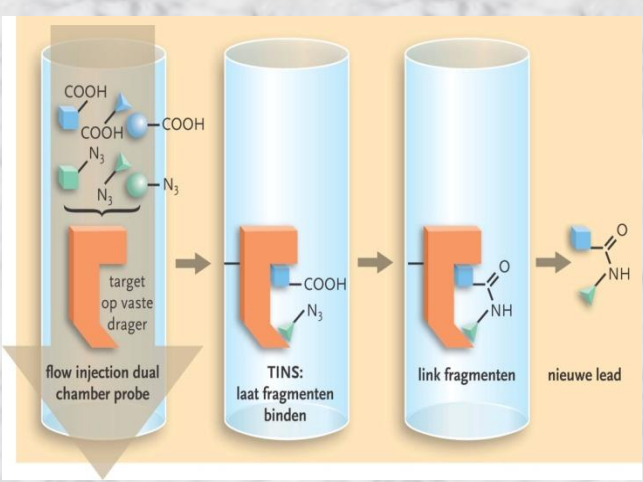
Biomolecular NMR:

3D structures
Detect/quantitate binding
Dynamics
Develop new pharmaceutical drugs









INTEGRINS

cell adhesion

cell migration

binding of soluble ligands

PHYSIOLOGY

platelet aggregation

cell proliferation and migration

angiogenesis

vasculogenesis

immune response

tissue integrity

apoptosis

PATHOLOGY

tumor growth and metastasis

atherosclerosis

thrombasthenia

DEVELOPMENT

bone development and remodeling

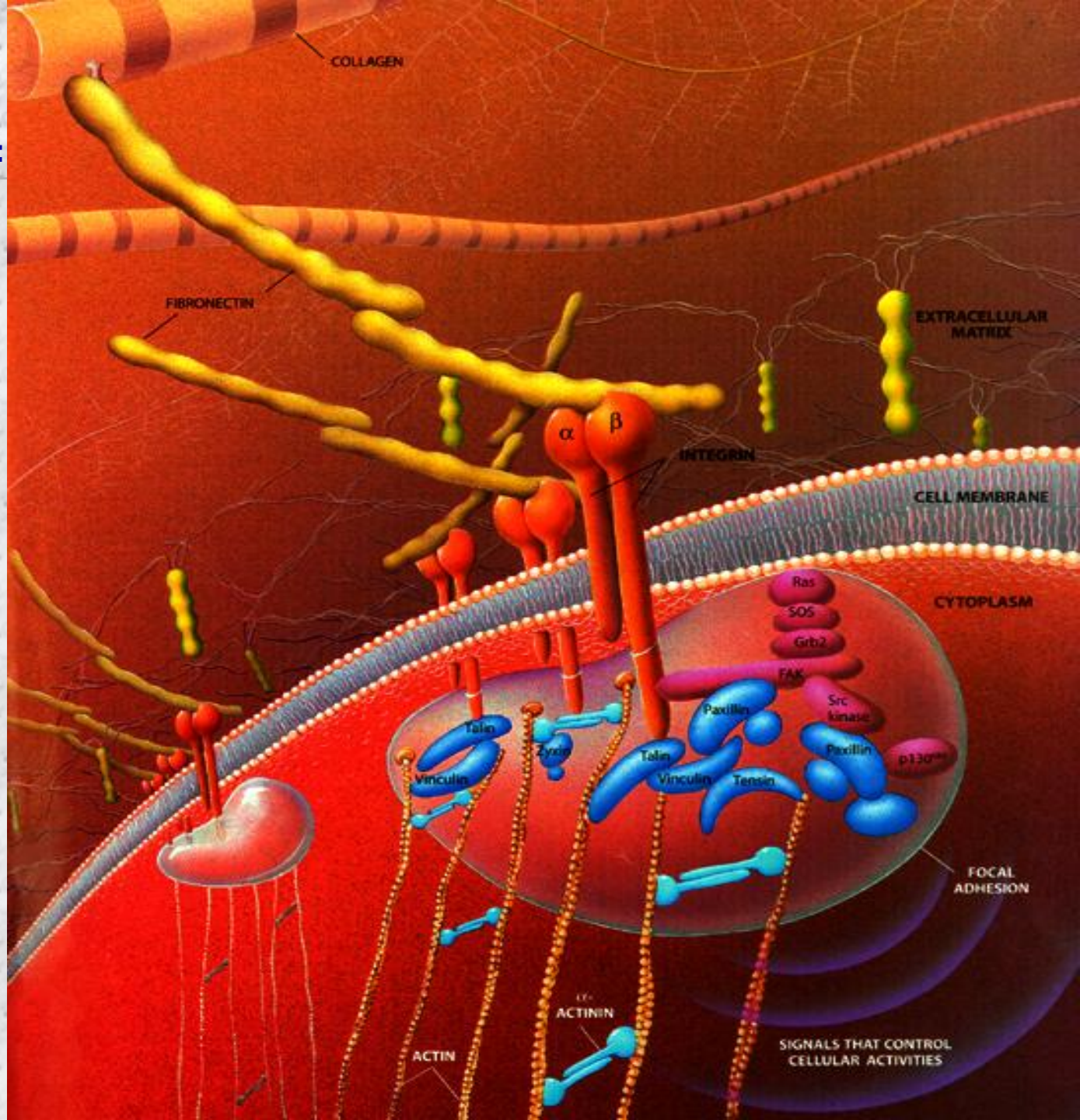
muscle development

extracellular matrix assembly

Integrins(24)

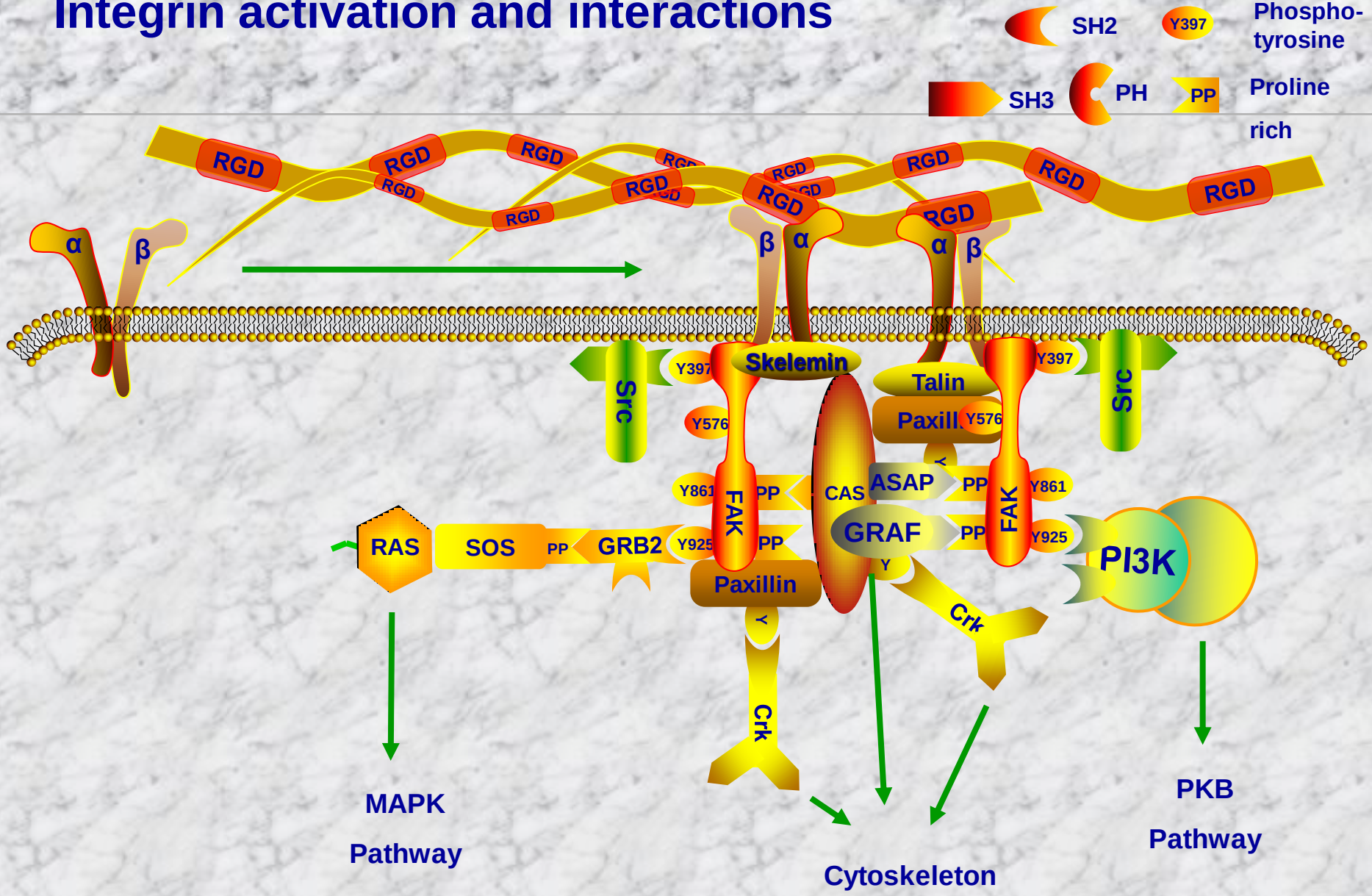
Cell Adhesion Receptors:

α (18)-β (8) heterodimers

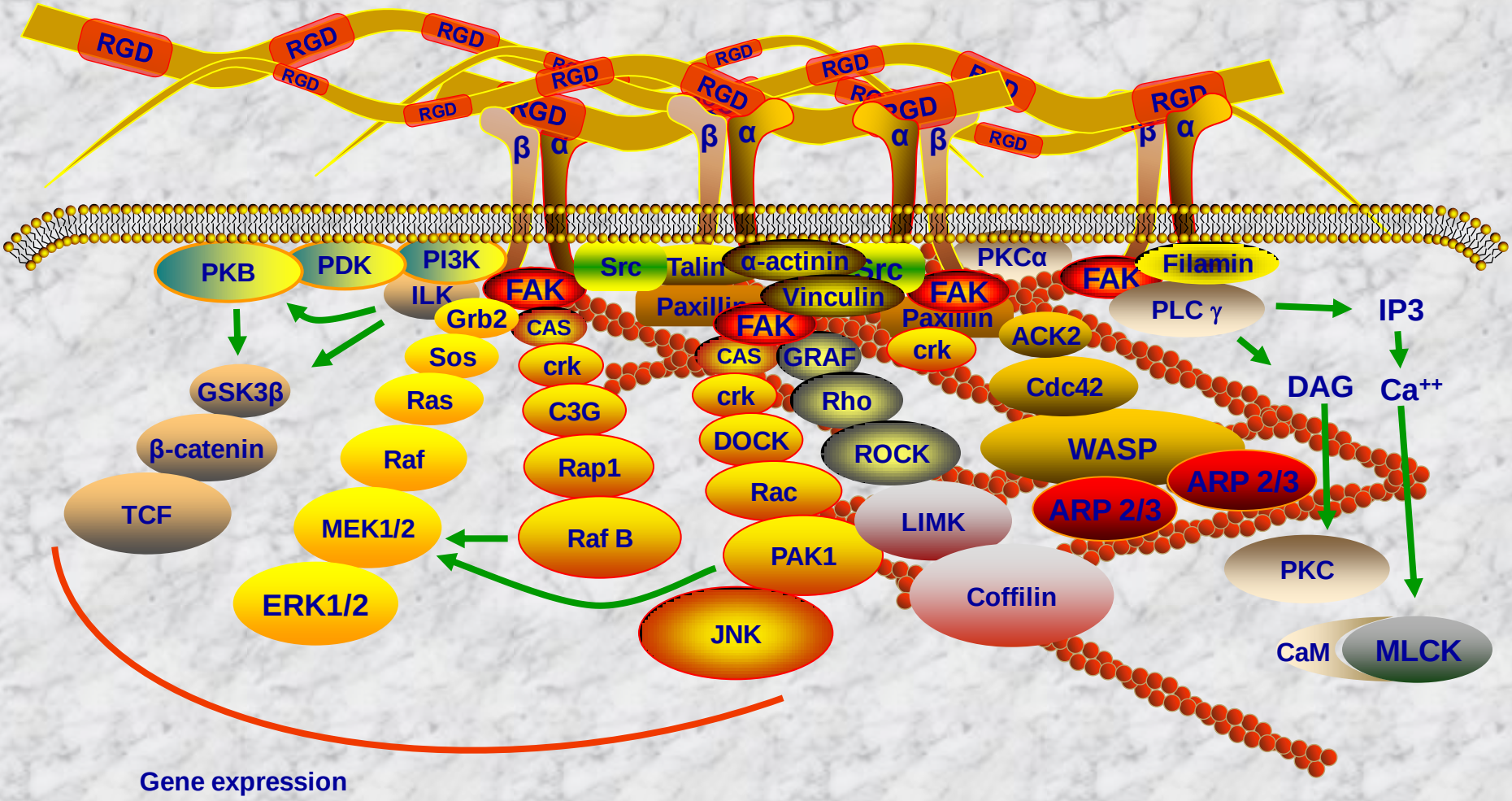


Slide adopted from Horwitz, Scientific American, May 1997

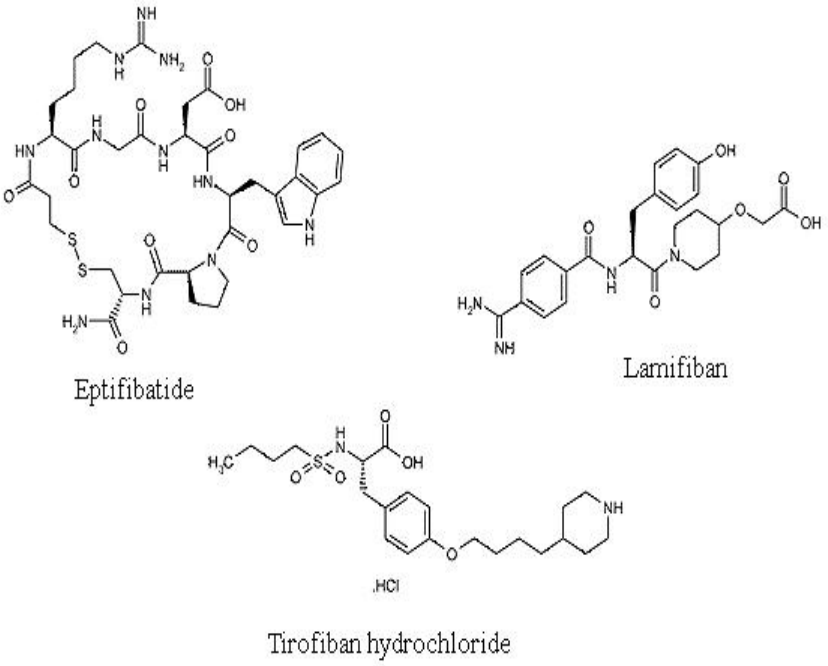
Integrin activation and interactions

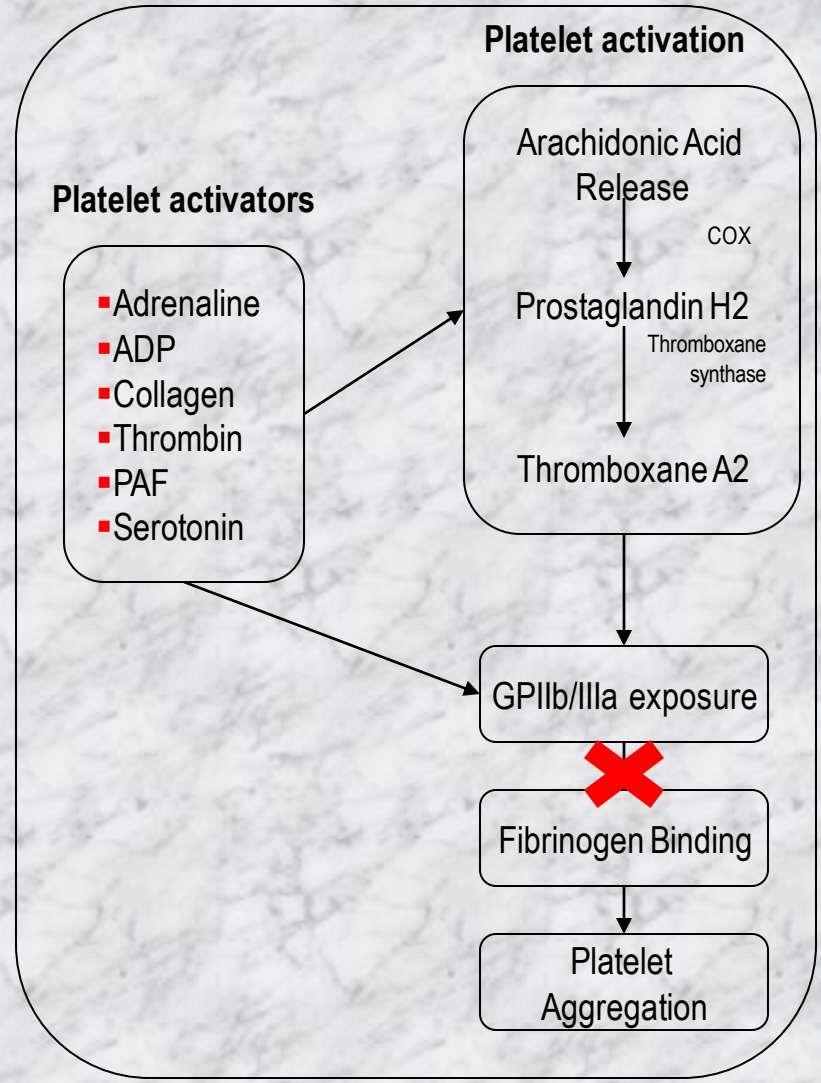


Integrin clustering and signal transduction



Currently marketed GPIIa/IIIb antagonists:





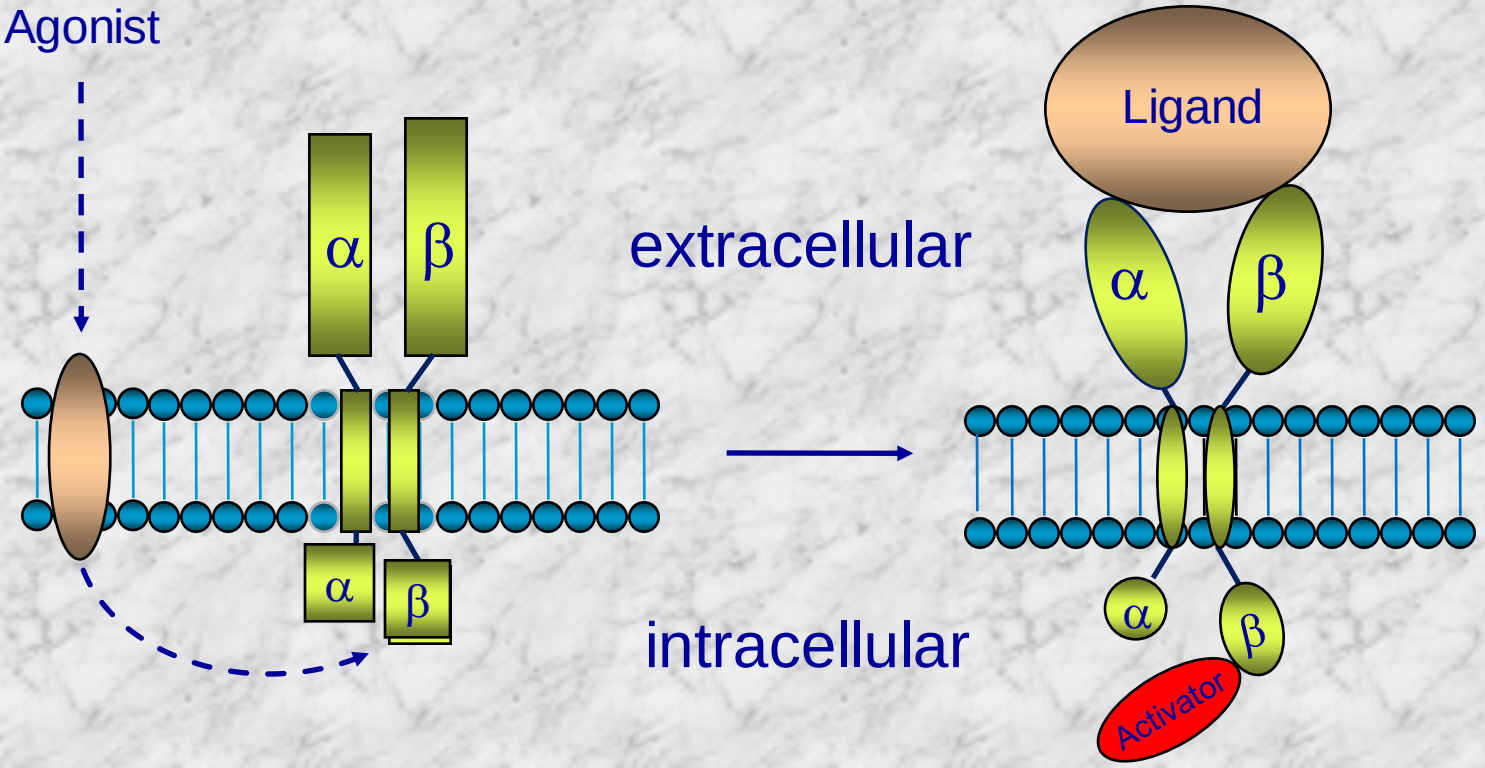
Most of integrins are expressed on cell surface in inactive or low affinity/avidity state

How is integrin activated at the molecular level?

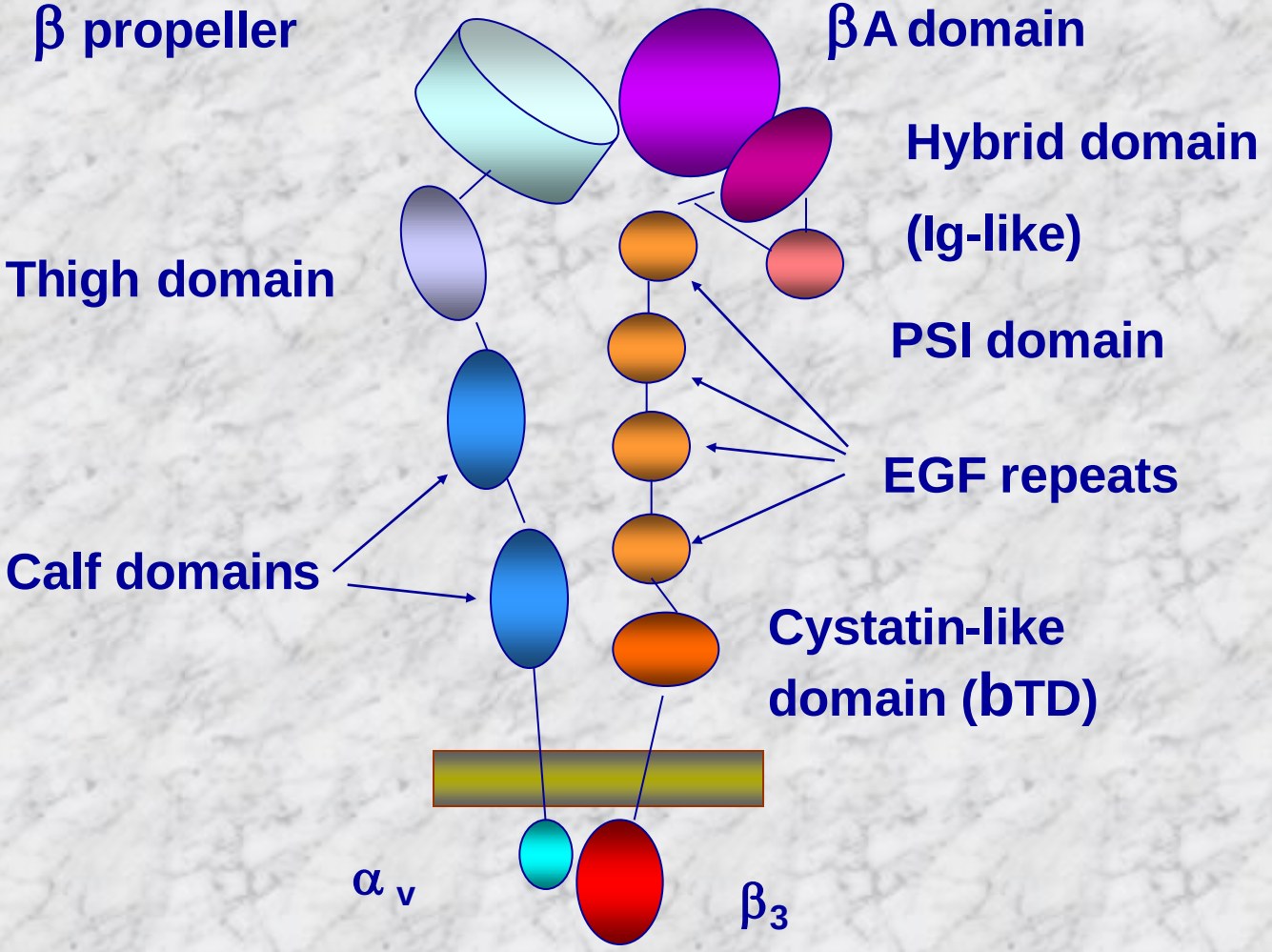
Platelet Integrin $\alpha_{IIb}\beta_3$

Hypothesis:

The α/β cytoplasmic tails of a latent integrin receptor form a "clasp" by interacting with each other in order to maintain the receptor in its inactive state. Binding of activators, such as cytoskeletal protein talin, to the cytoplasmic tails complex opens this "clasp", disrupt the interaction, thus initiating an inside-out conformational signaling for the receptor activation.

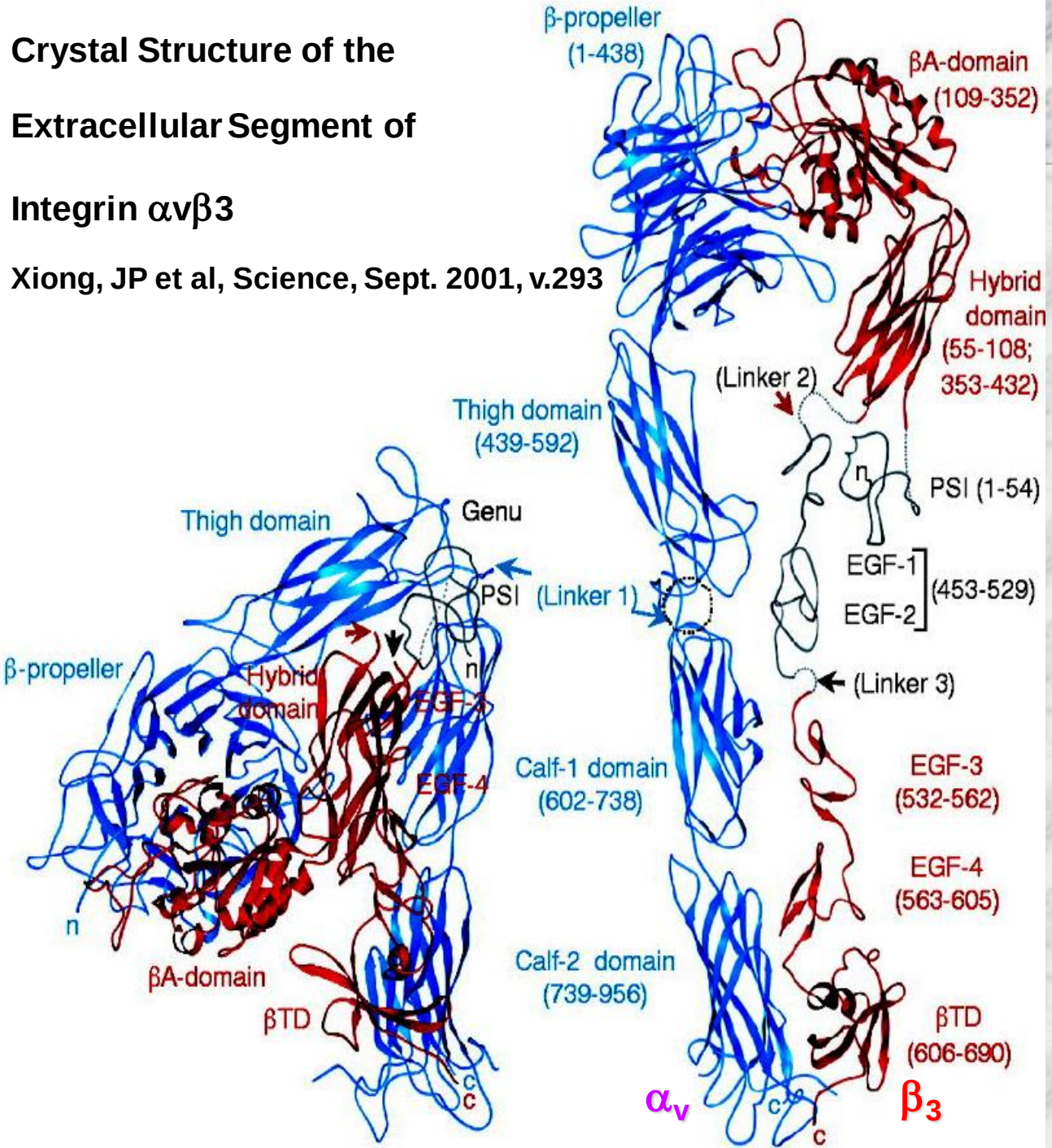


Modular Representation of Integrins

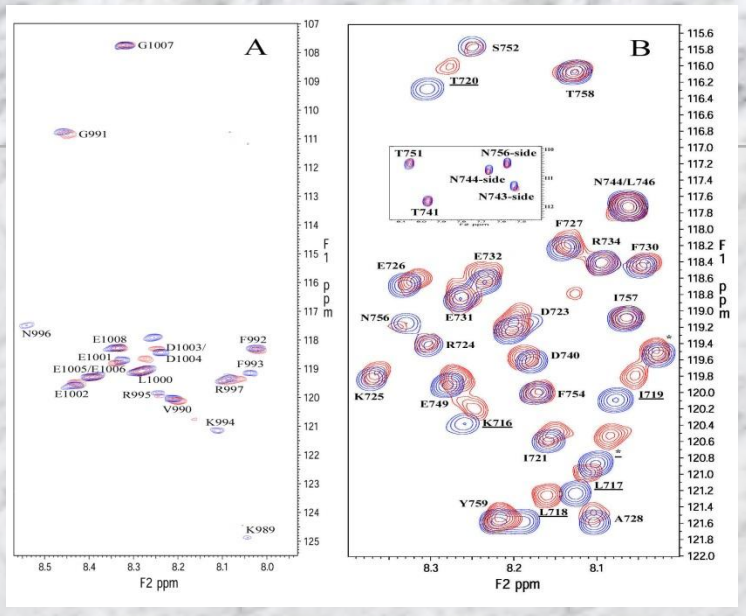


Crystal Structure of the Extracellular Segment of Integrin αvβ3

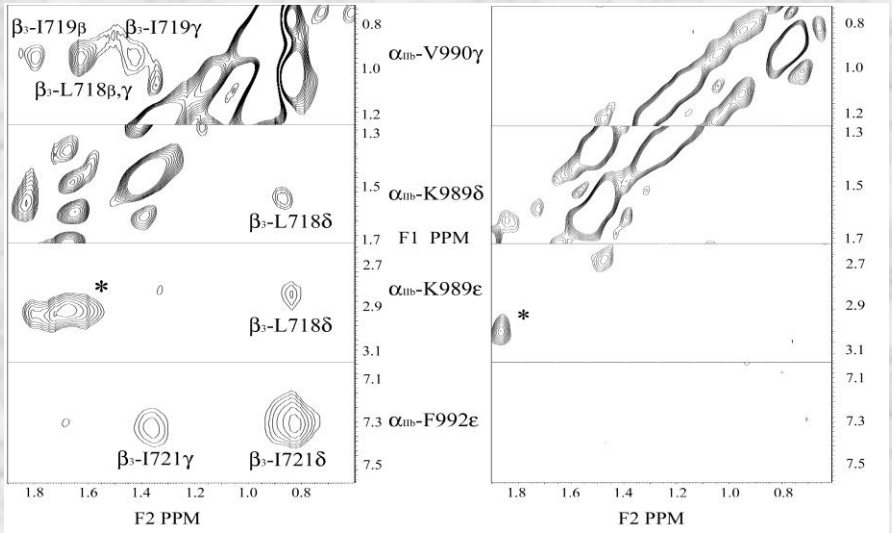
Xiong, JP et al, Science, Sept. 2001, v.293

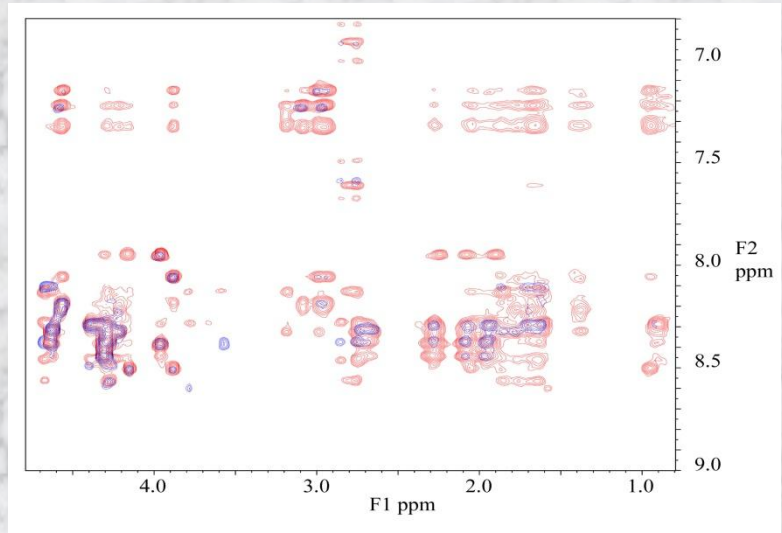


$\alpha_{IIb}\beta_3$ CT-complex

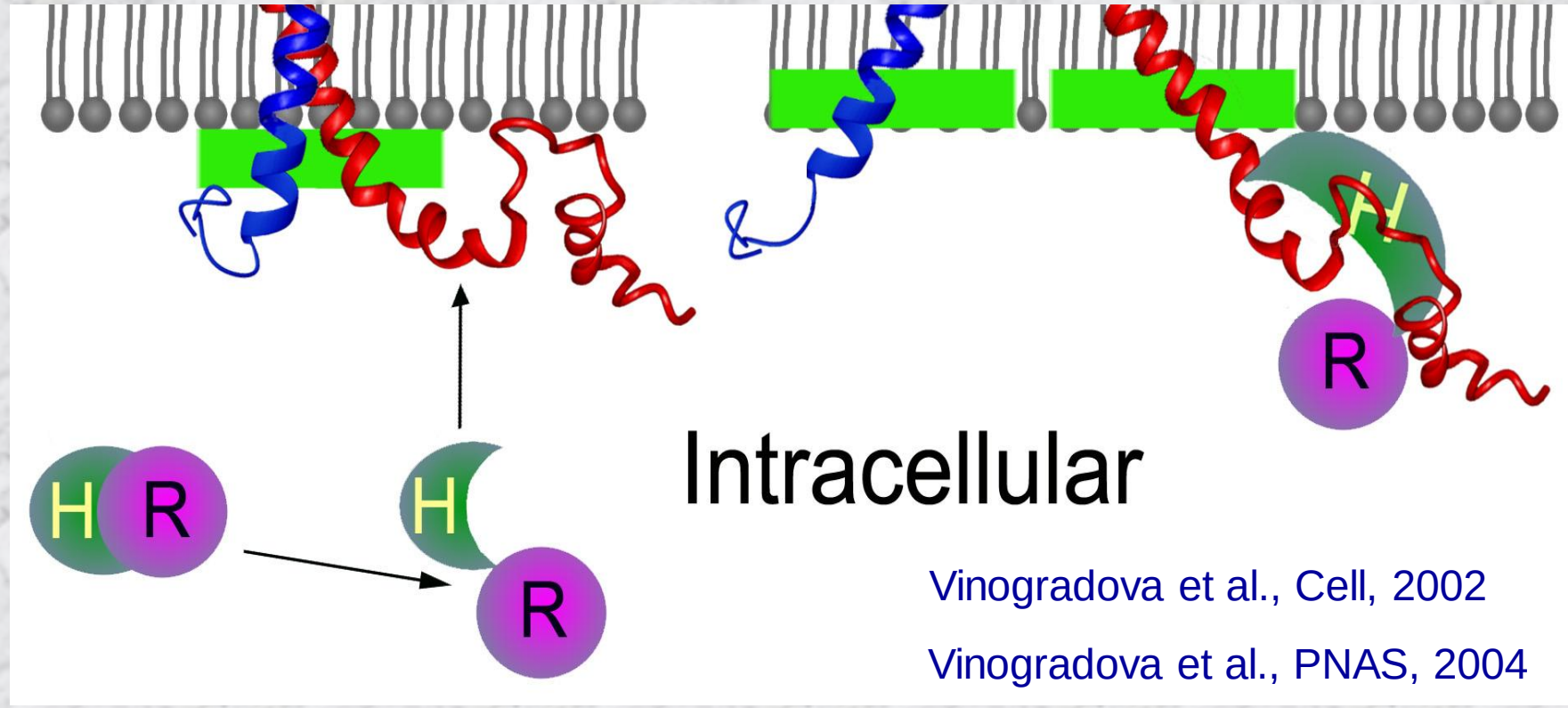


- HSQC Spectral Mapping of the Binding Sites
- Intermolecular Contacts (NOEs)
- Transferred NOEs
- Interactions with Membrane





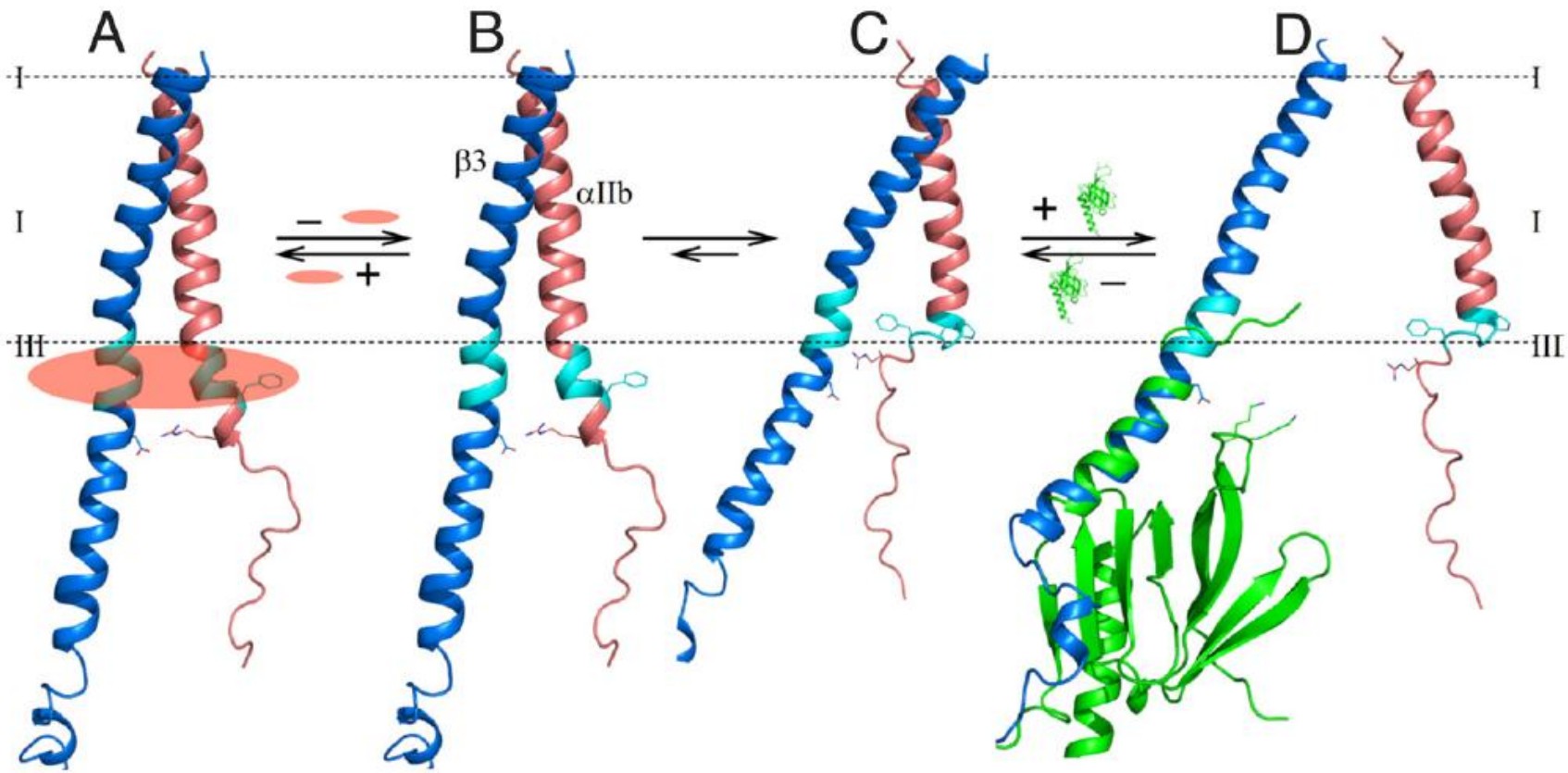
Membrane-mediated change of the cytoplasmic face during integrin activation



Vinogradova et al., Cell, 2002

Vinogradova et al., PNAS, 2004

A model for integrin inside-out Trans-Membrane signaling



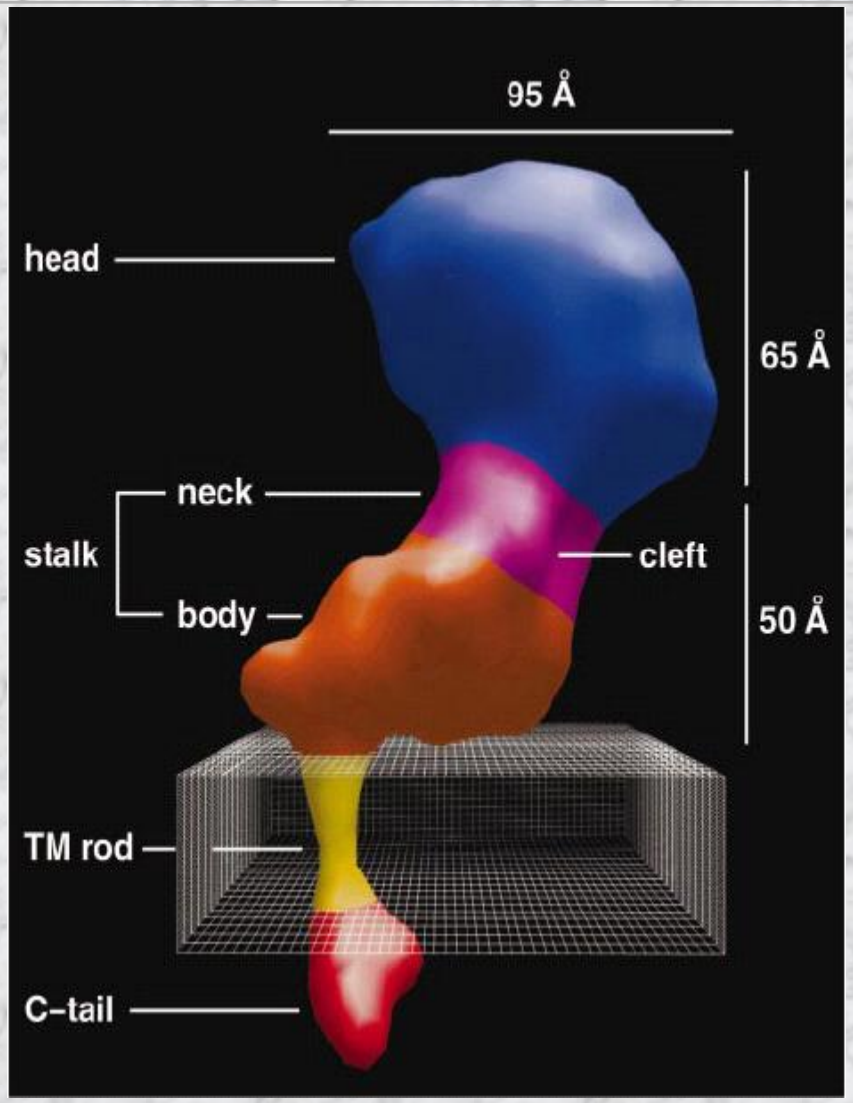
I. Extra-cellular Membrane
II. Hydrophobic Membrane Core
III. Intra-cellular Membrane
Inhibitory Ligand
Activation Ligand (e.g. Talin F3)

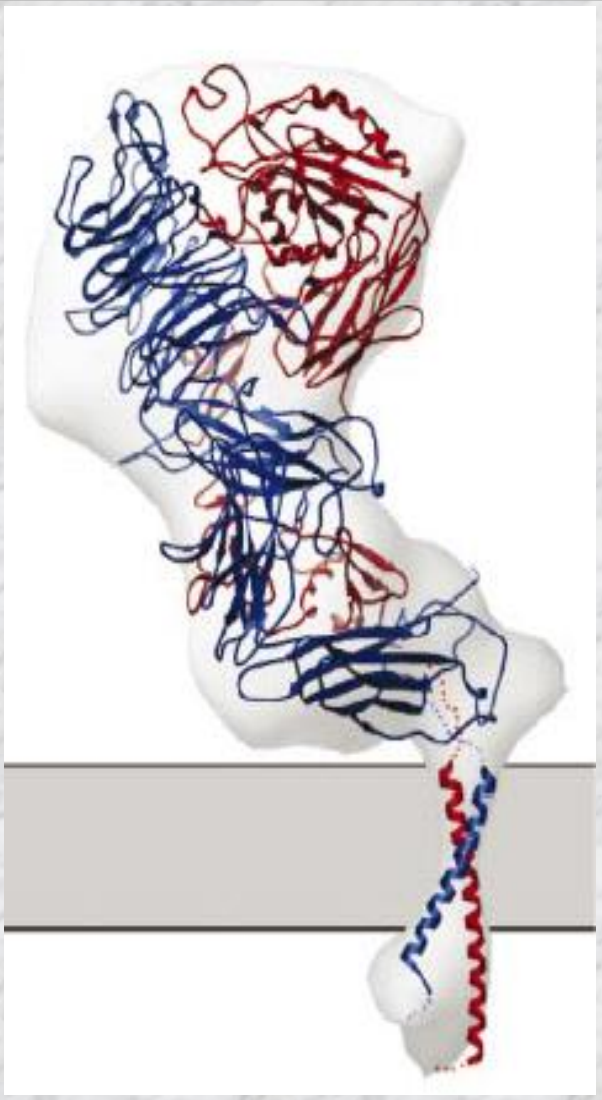
Lau et al., EMBO, 2009

Yang et al., PNAS, 2009

(Wegener et al., Cell, 2007)

CryoEM model of inactive integrin $\alpha_{IIb}\beta_3$

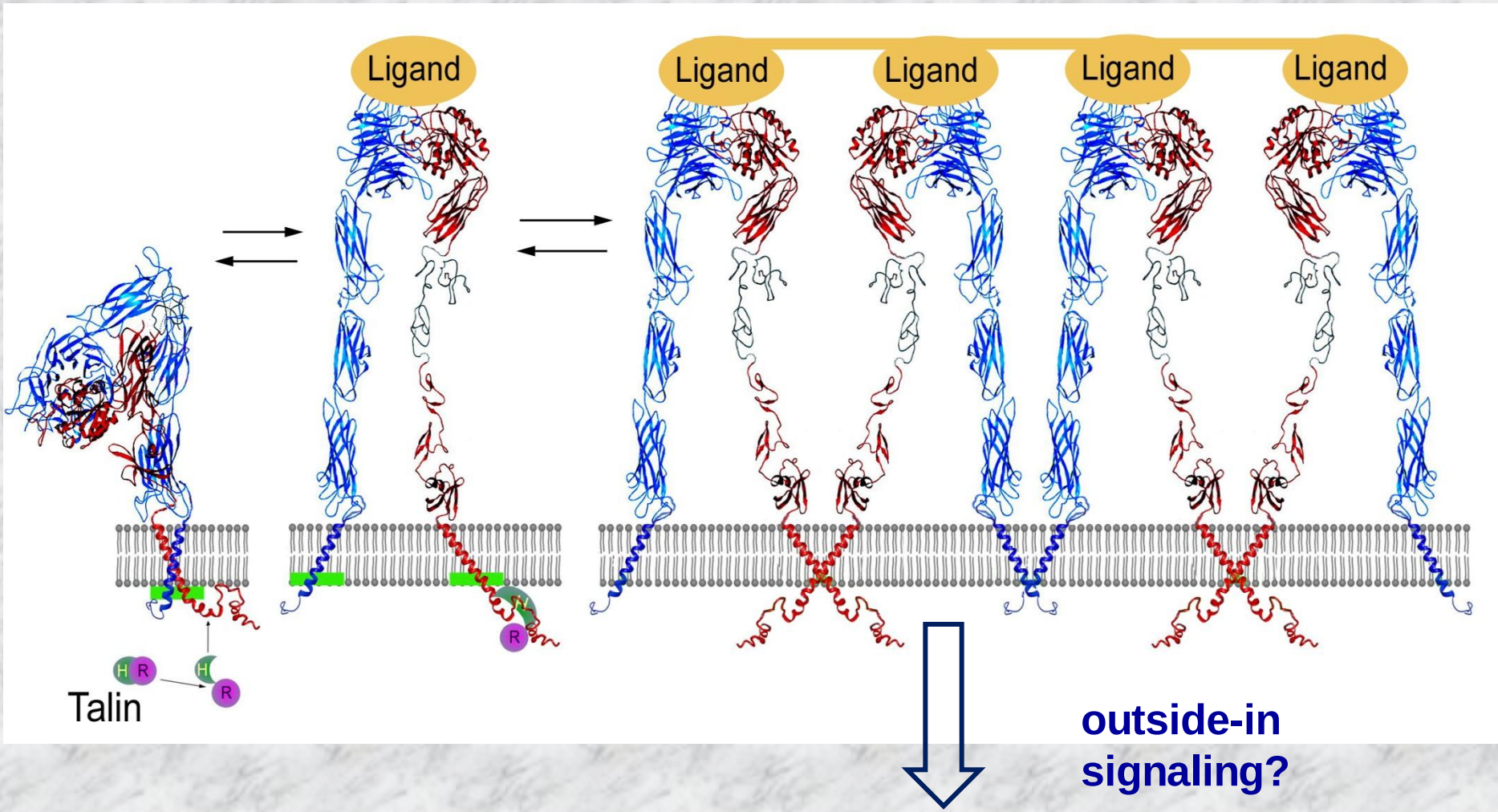




Adair and Yeager, 2002, PNAS

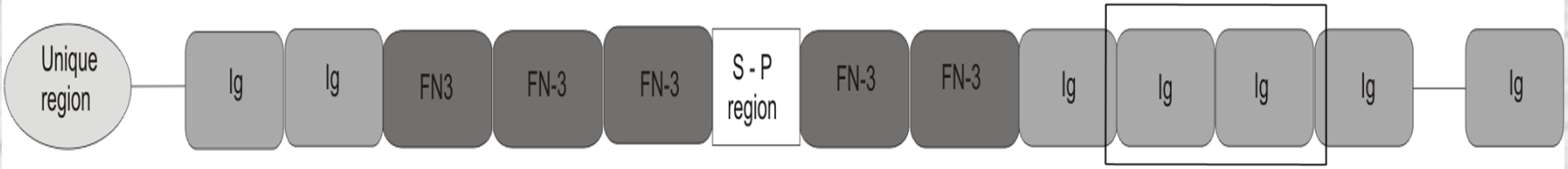
A model for integrin inside-out activation and clustering

Unclasping evidence and activation by Talin: NMR, EM, Mutational, RNA knockdown, FRET (*in vivo*)



Skelemin

A

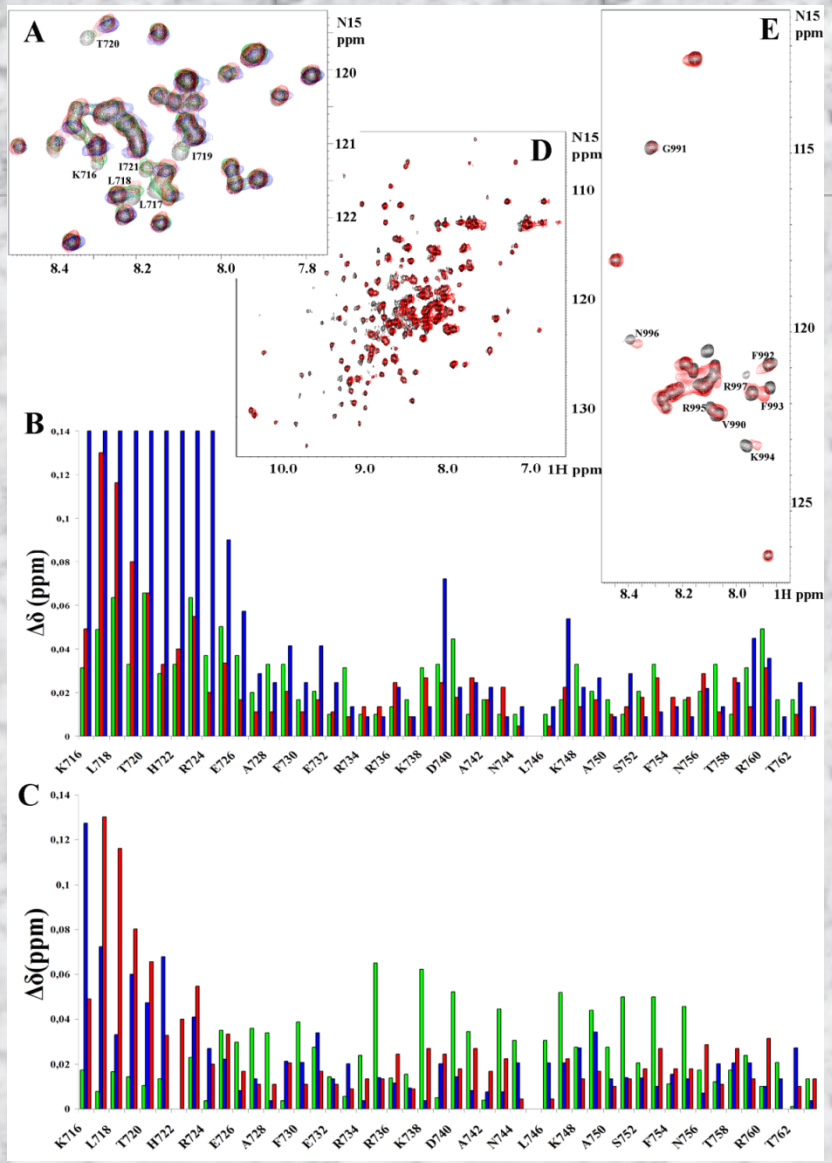


B

```
Sk-5  1329  ---QRQEWIRKQGPHFAEY-----LSWEVTGECNVLLKCKVANIKKETHIVWYKDEREIS  1380
Sk-4  1207  EEEMKRLLALSQEHKFPTVPTKSELAVEILEKGQVRFWMQAEKLSSNAKVSYIFNEKEIF  1266

Sk-5  1370   VDEKH----DFKDGICTLLITEFSKKDAGFYEVILKDDRGKDKSRLKLVDEAFQDLM---  1433
Sk-4  1257   EGPKYKMHIDRNTGIIEMFMEKLQDEDEGTYTFQIQDGKATGHSTLVLIGDVYKKLQKEA  1326

Sk-5    --
Sk-4  1326   EF                                                          1328
```


A
T720
I719
I721
K716
L718
L717
N15 ppm
120
8.4
8.2
8.0
7.8
D
N15 ppm
121
122
110
120
130
10.0
9.0
8.0
7.0 1H ppm
E
N15 ppm
G991
N996
F992
R997
F993
R995
V990
K994
115
120
125
8.4
8.2
8.0
1H ppm
B
Δδ (ppm)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
K716 L718 T720 H722 R724 E726 A728 F730 E732 R734 R736 K738 D740 A742 N744 L746 K748 A750 S752 F754 N756 T758 R760 T762
C
Δδ(ppm)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
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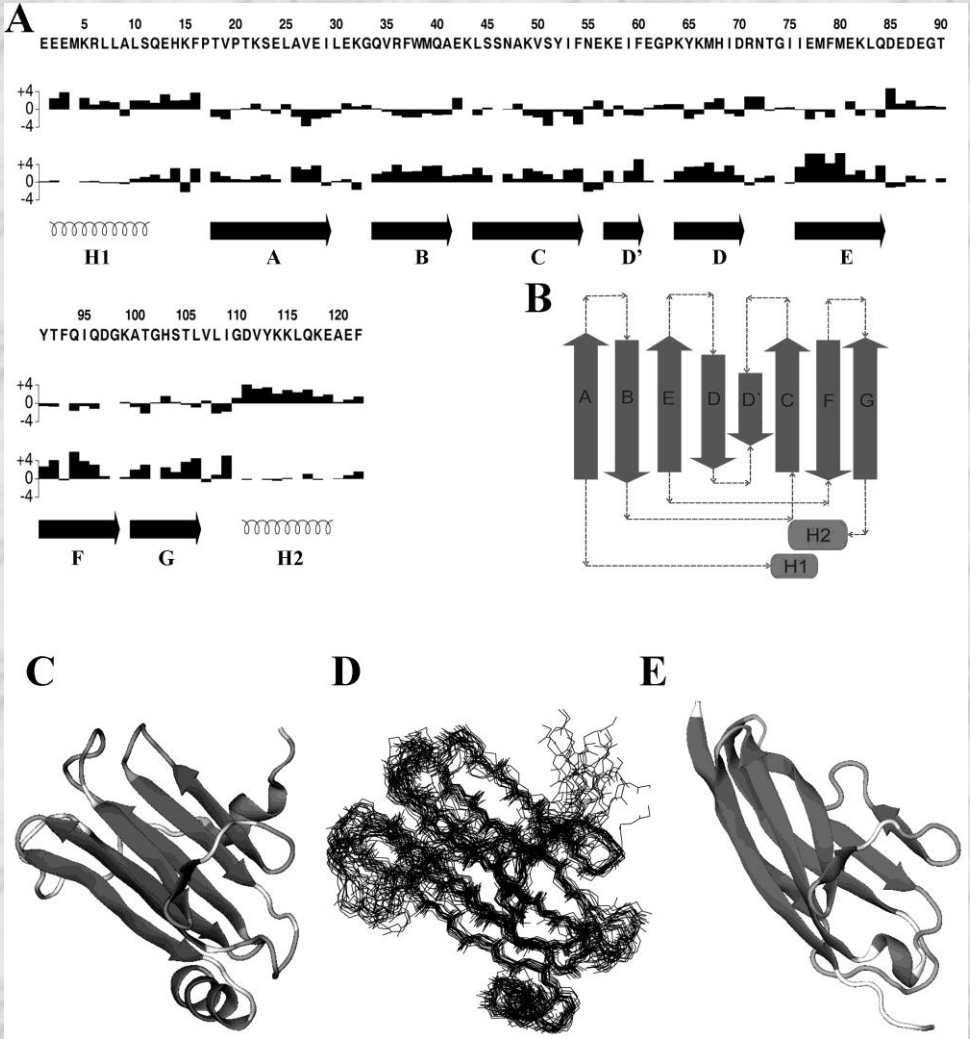
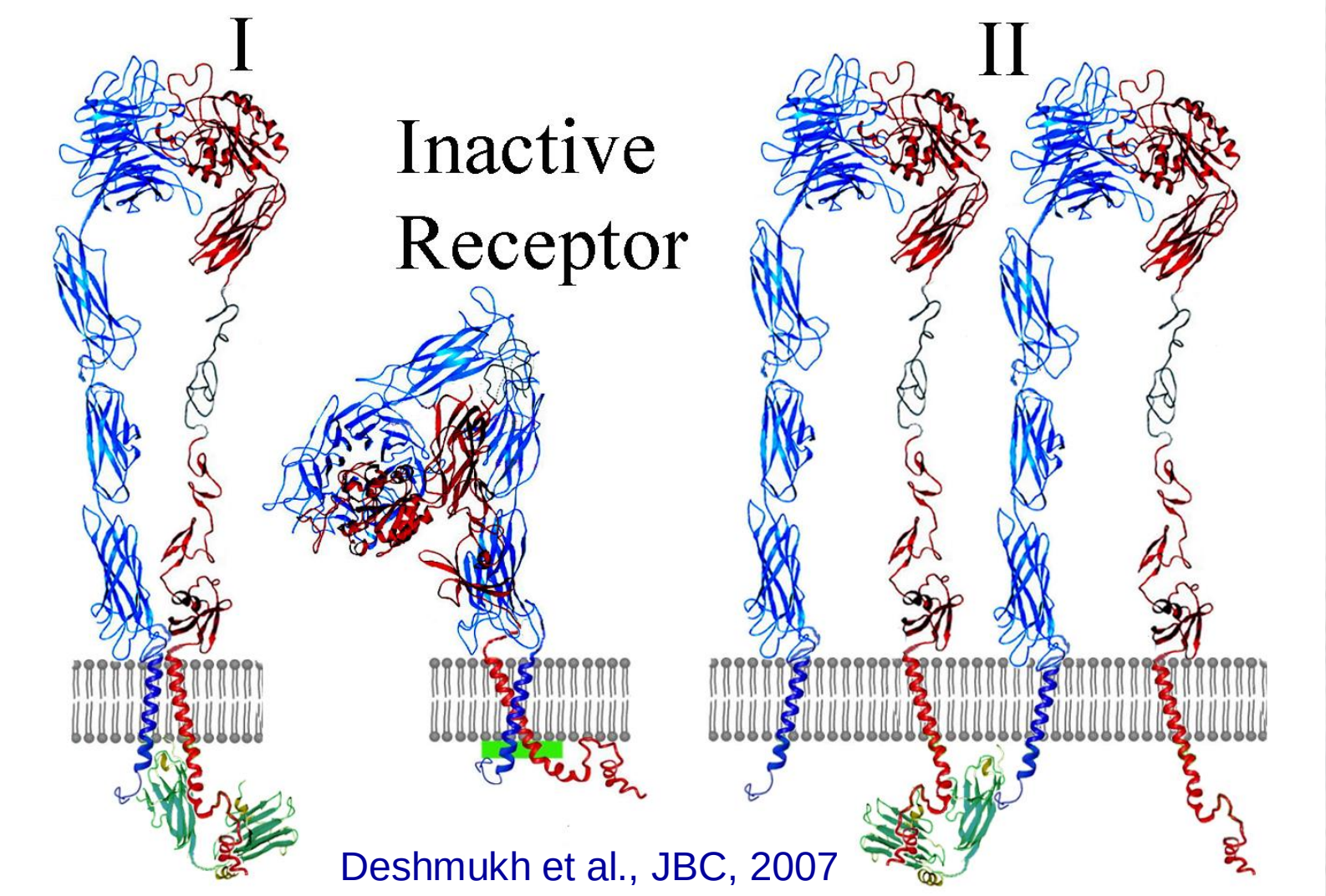
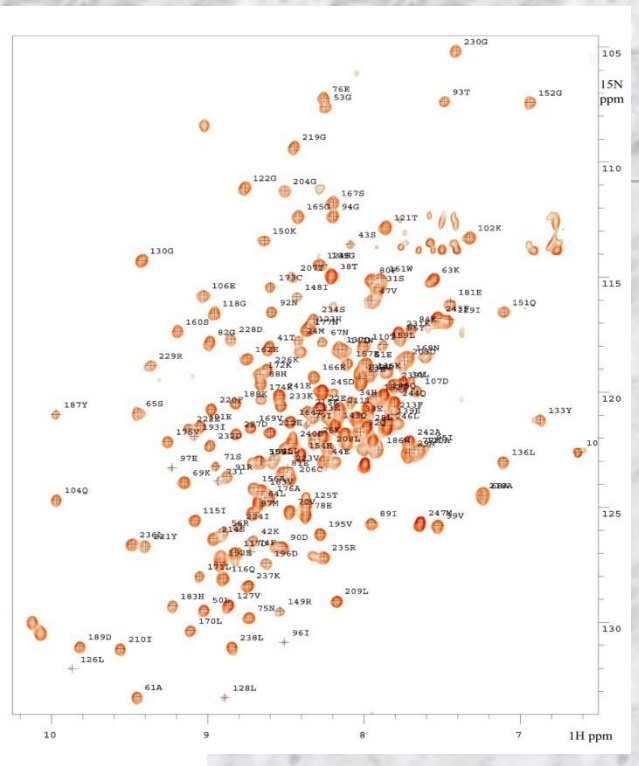

A
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
EEEMKRLLALSQEHKFPTVPTKSELAVEILEKGQVRFWMQAEKLSSNAKVSYIFNEKEIFEGPKYKMHIDRNTGIIEMFMEKLQDEDEGT
+4
0
-4
H1
A
B
C
D'
D
E
95 100 105 110 115 120
YTFQIQDGKATGHSTLVLIGDVYKKLQKEAEF
F
G
H2
B
A
B
E
D
D'
C
F
G
H2
H1
C
D
E

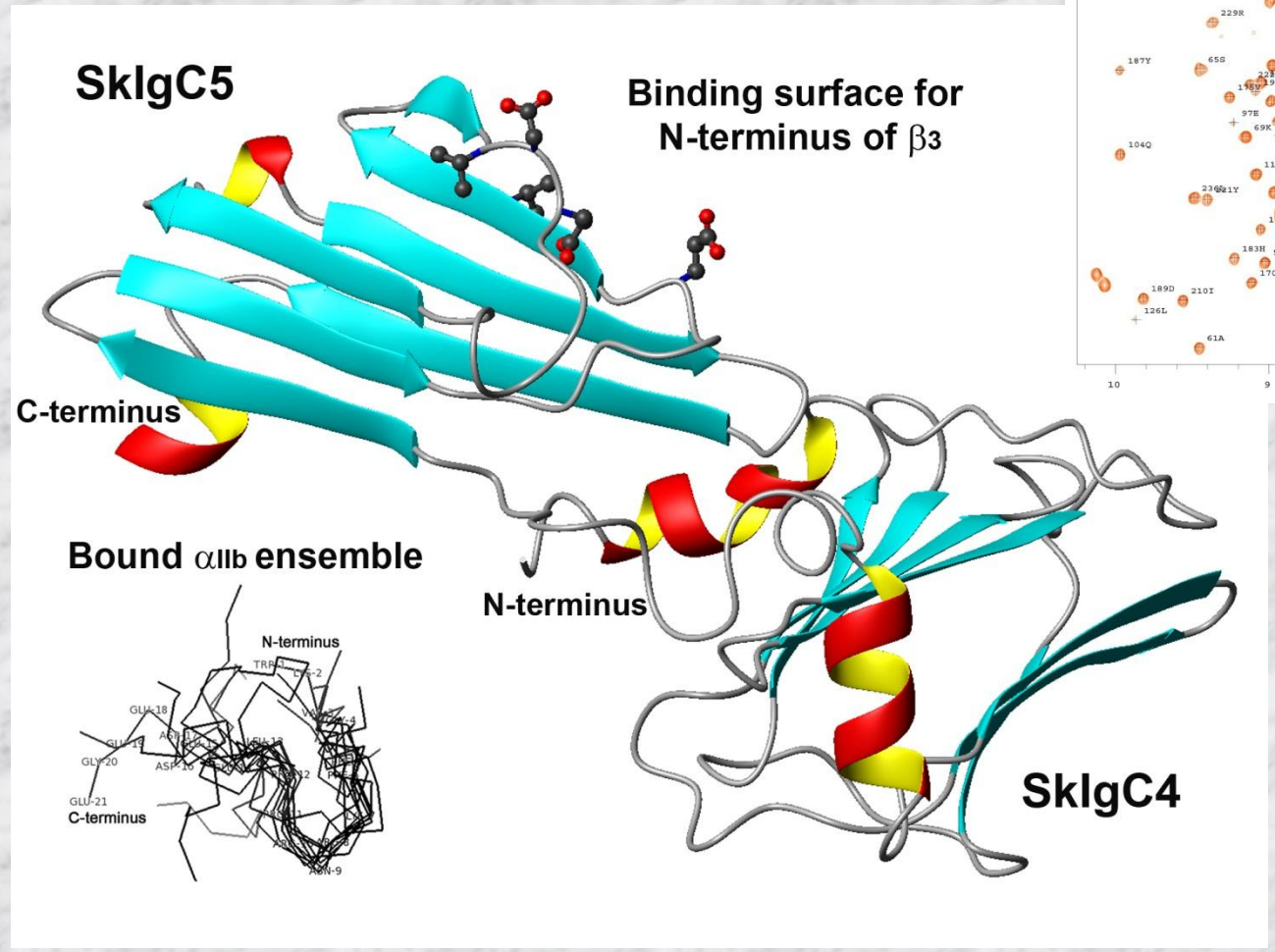
Suggested model for integrin-skelemin interaction



Deshmukh et al., JBC, 2007

Skelemin IgC45m structure



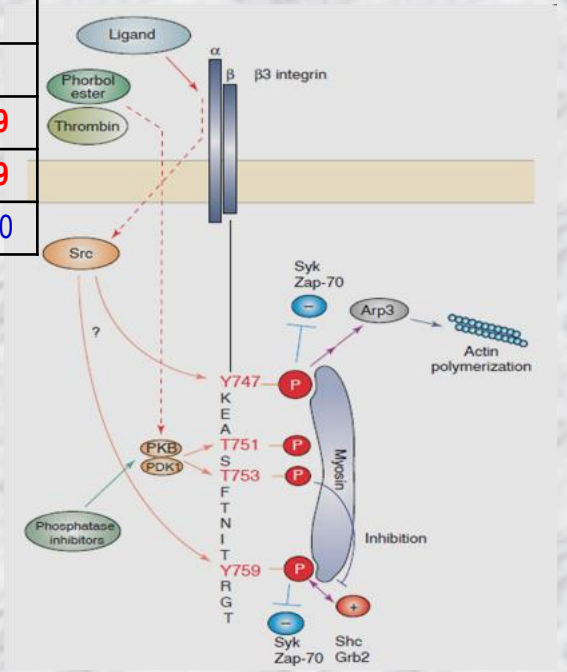


Exploring the phosphorylation of b_3 CT

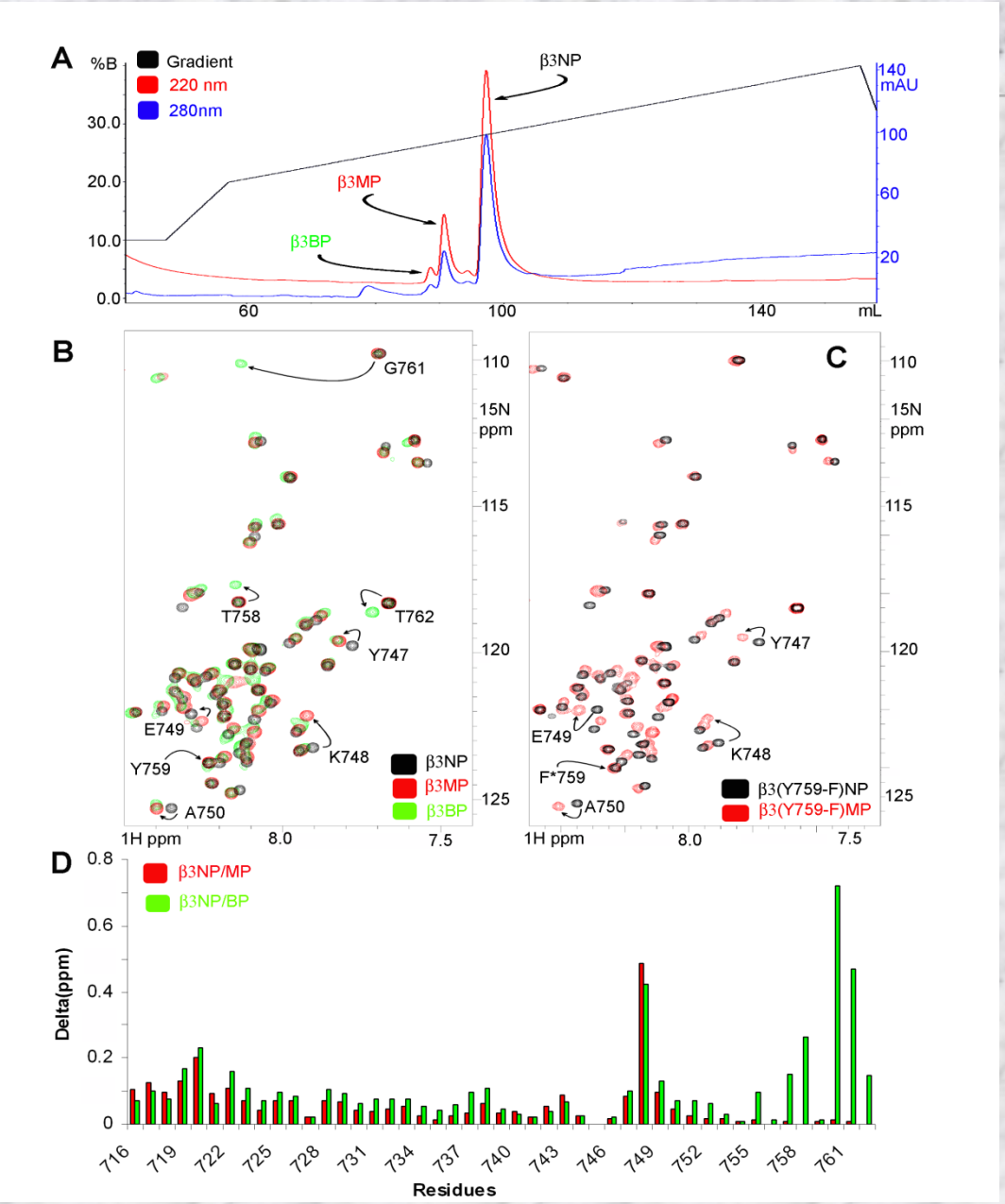
^{716}KLLITI**HDRKE**FAKFEEERARAKWDTAN**NPLY**^{747}KEA**T**^{751}S**T**^{753}FTNIT**Y**^{759}RGT^{762}

Phosphorylation site	Binding Protein	Effect on binding	Effect on Cells	Reference
Tyr747 and Tyr759	Myosin	+	Clot retraction	Jenkins, Nannizzi-Alaimo et al. 1998
Tyr747 and Tyr759	Syk	-	?	Woodside, Obergfell et al. 2002
Tyr747 and Tyr759	Zap-70	-	?	Woodside, Obergfell et al. 2002
Tyr759	Grb2	+	?	Law, Nannizzi-Alaimo et al. 1996
Tyr759	Shc-PTB	+	?	Cowan, Law et al. 2000
Tyr747	Talin	-	?	Anthis, Haling et al. 2009
Tyr747	Dok1	+	?	Anthis, Haling et al. 2009
Thr753	Shc	-	?	Kirk, Sanderson et al. 2000

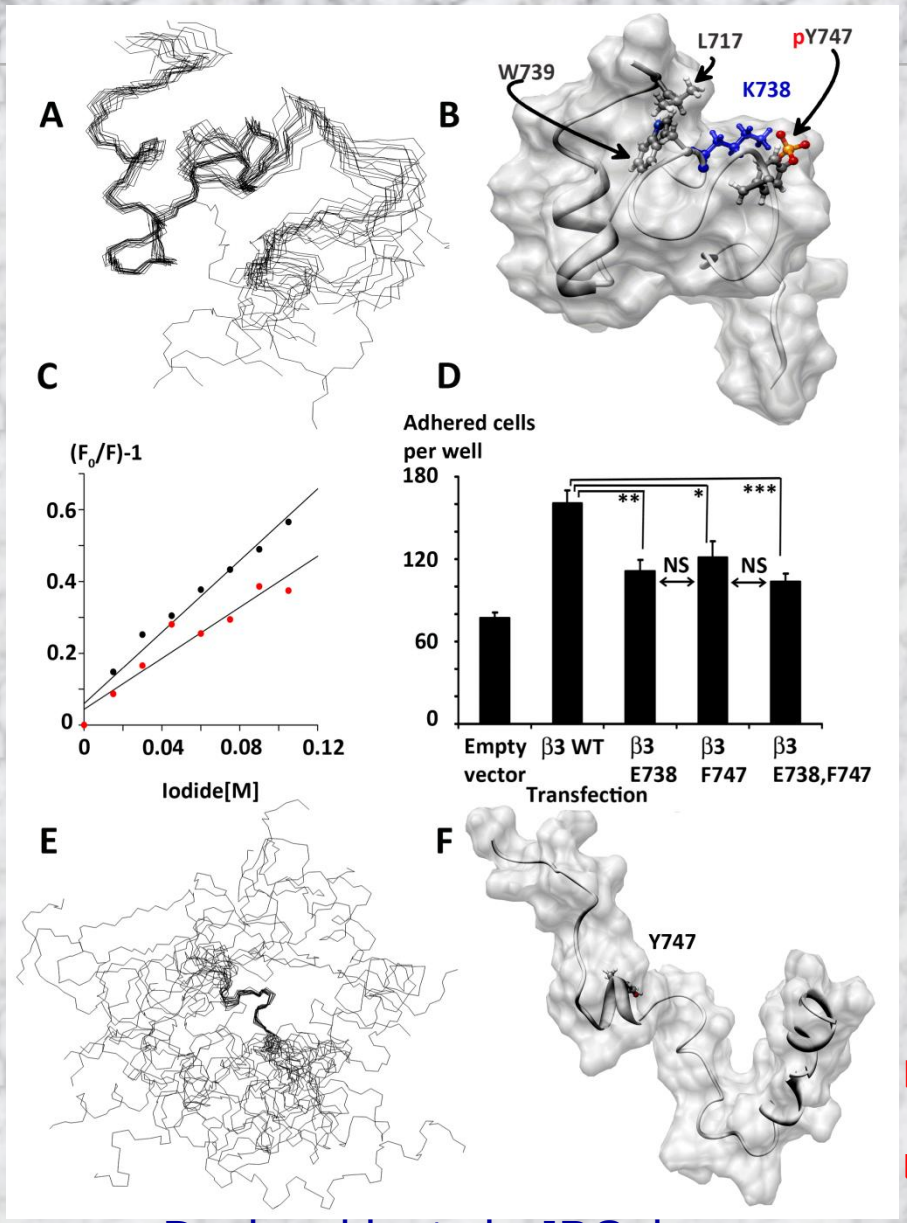
Fagerholm, Hilden et al. 2004

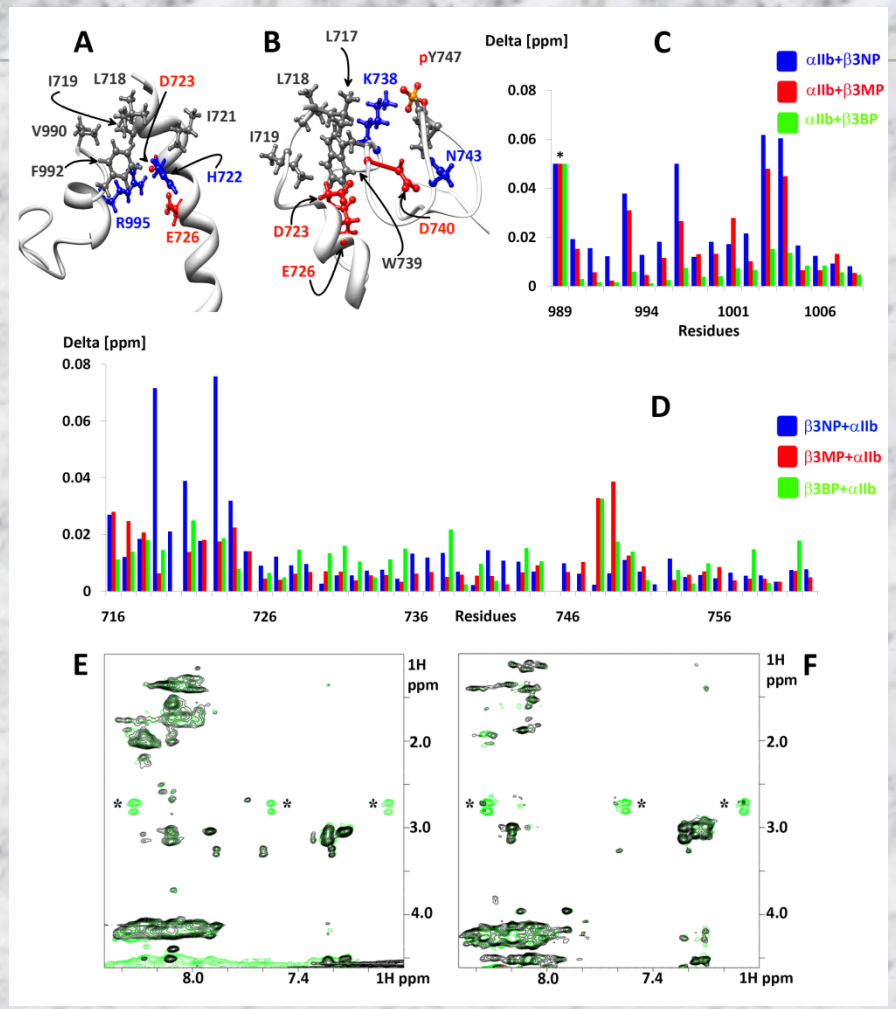


Role of tyrosine phosphorylation in Integrin outside-in



Structures of β_3NP and β_3MP

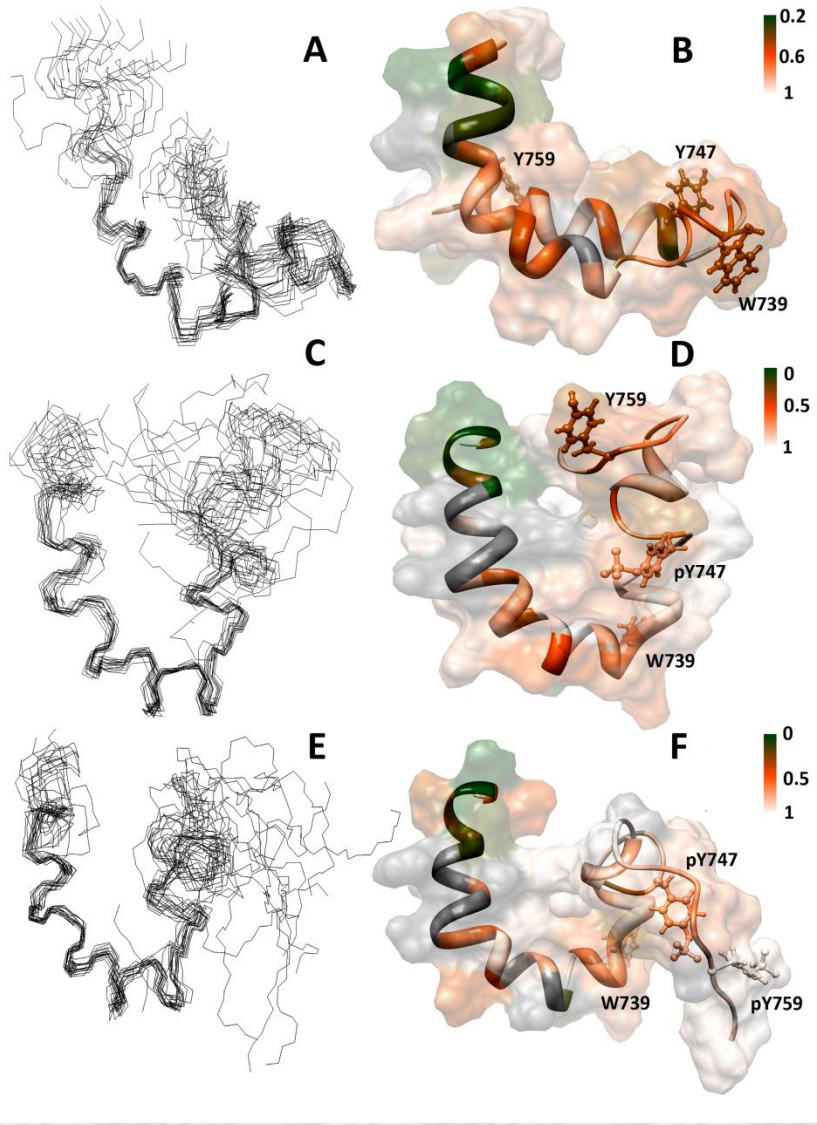


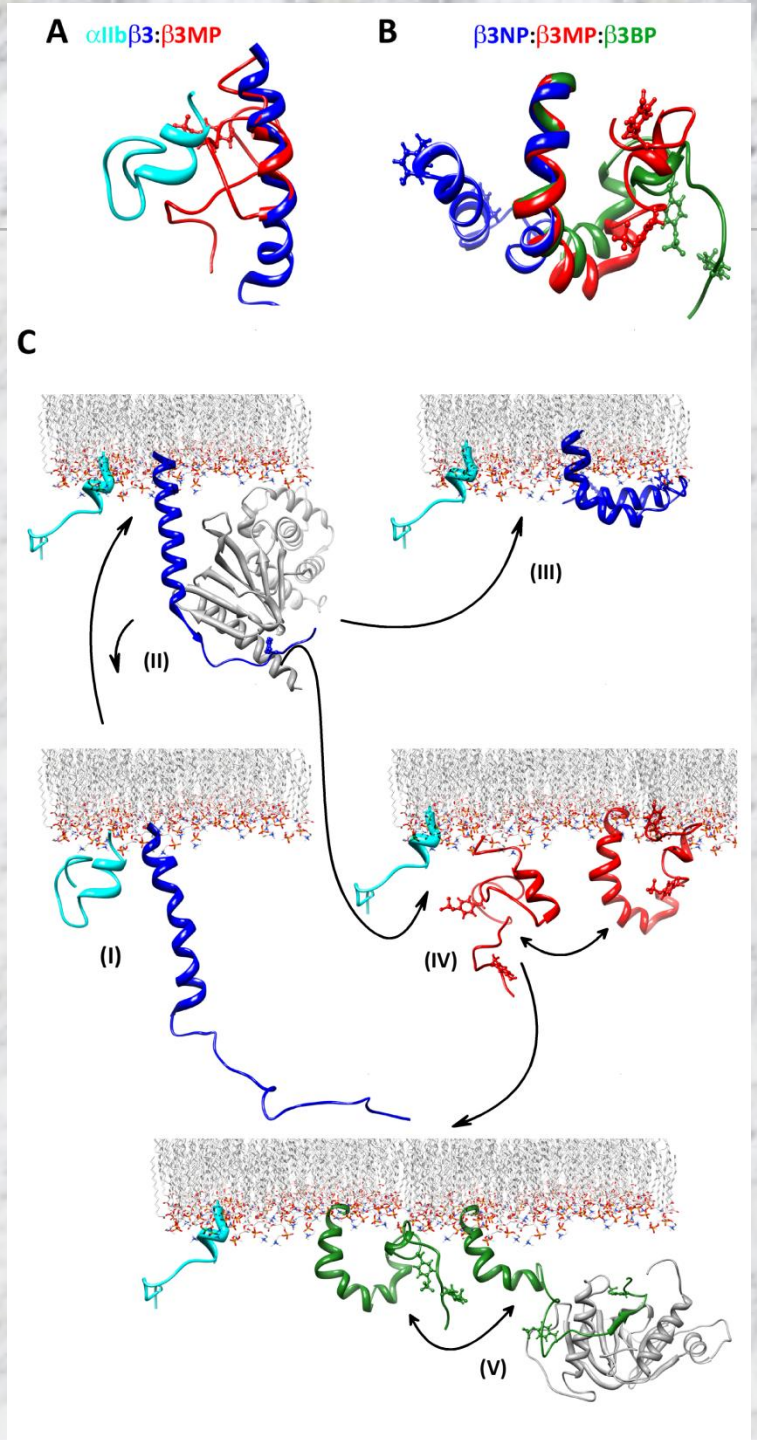


- Hydrophobic interactions:
  - α_{IIb} V^{990}-β_3 L^{718}, α_{IIb} V^{990} -β_3 I^{719}, α_{IIb} F^{992}-β_3 I^{721}
- Electrostatic interactions:
  - α_{IIb} R^{995}- β_3 D^{723}, α_{IIb} R^{995}- β_3 H^{722}, α_{IIb} R^{995}- β_3 E^{726}

Deshmukh et al., JBC, in press

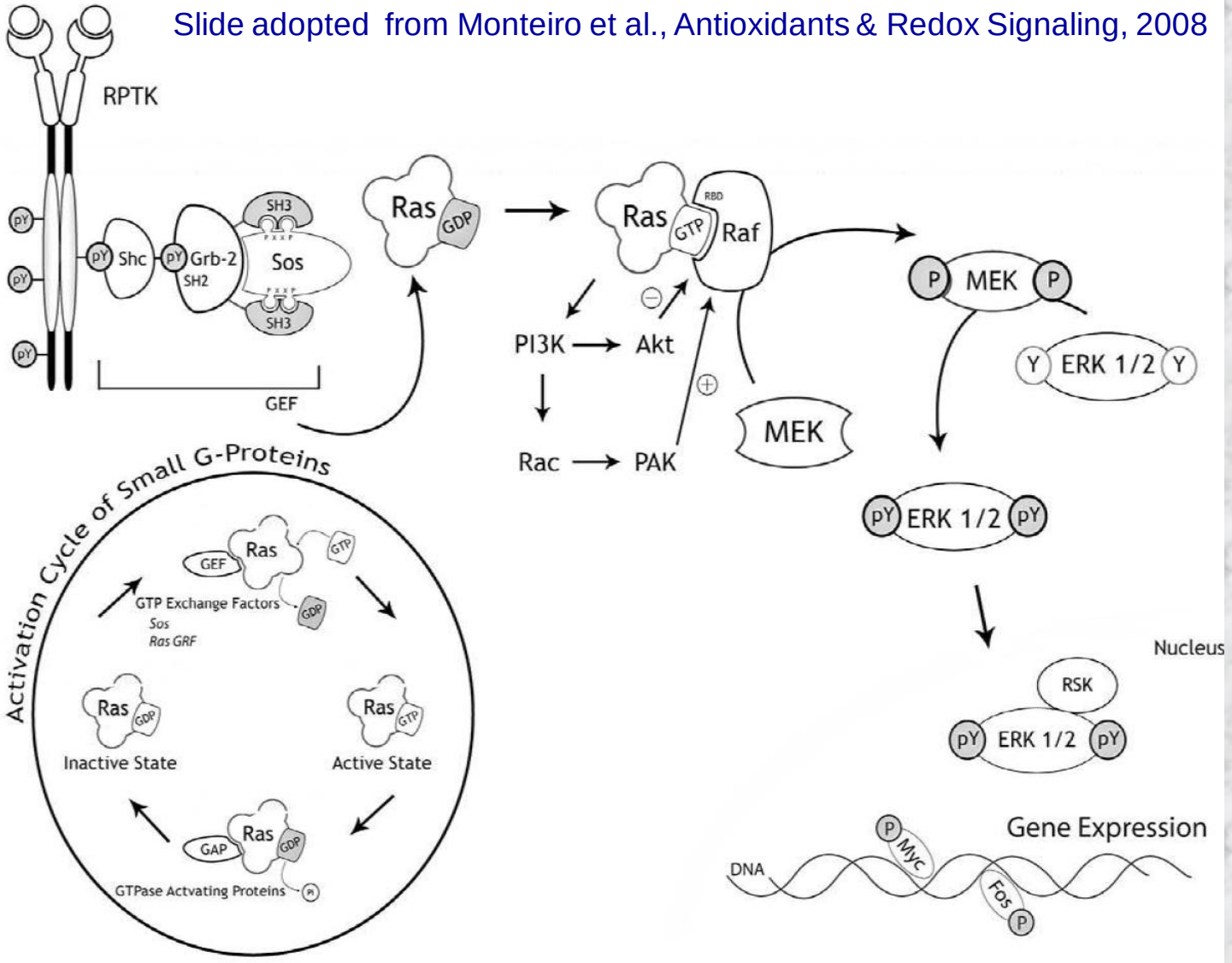
An equilibrium model of the integrin activation states



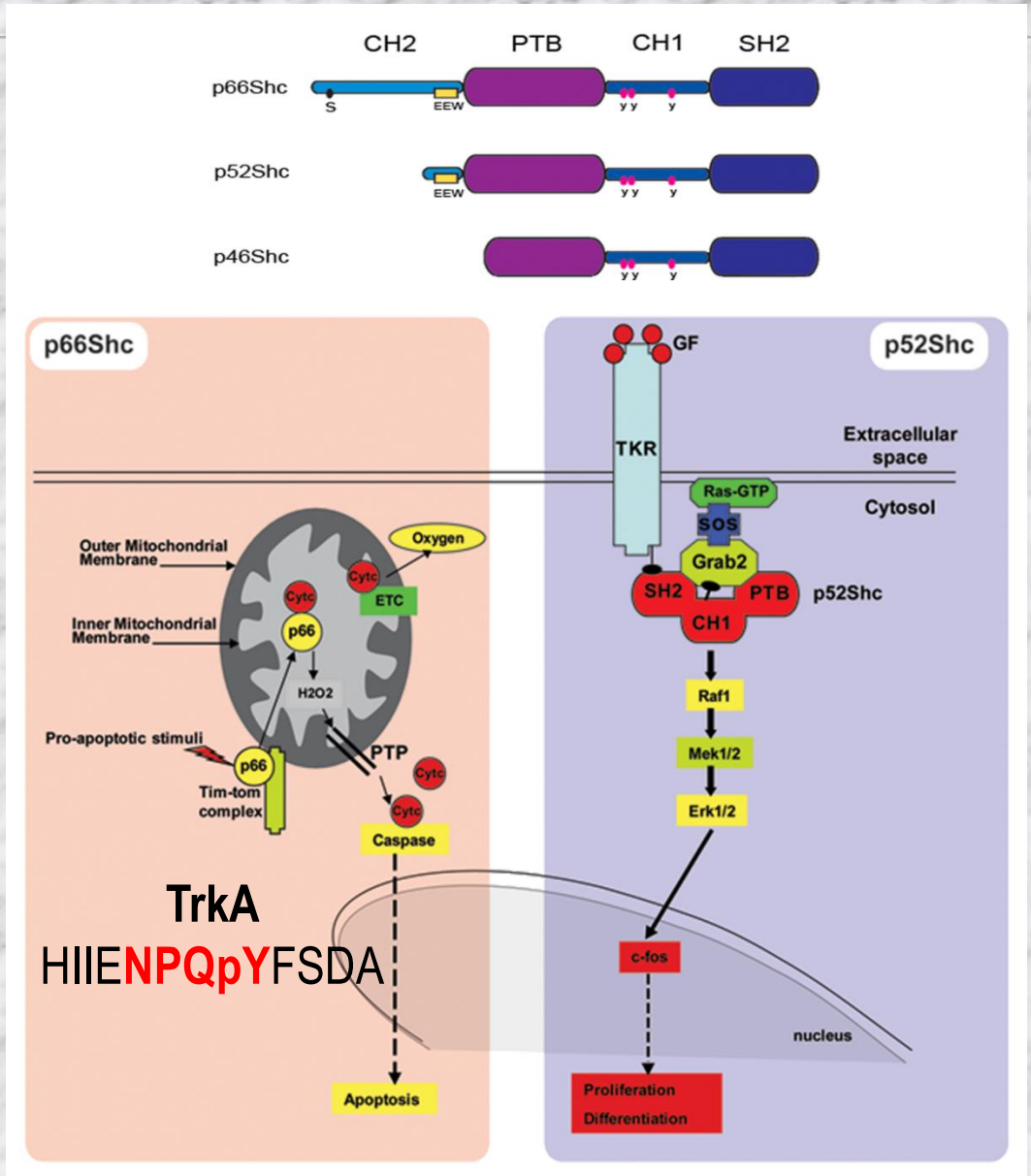


Tyrosine phosphorylation and SHC pTB binding in “outside-in” integrin signaling

Slide adopted from Monteiro et al., Antioxidants & Redox Signaling, 2008

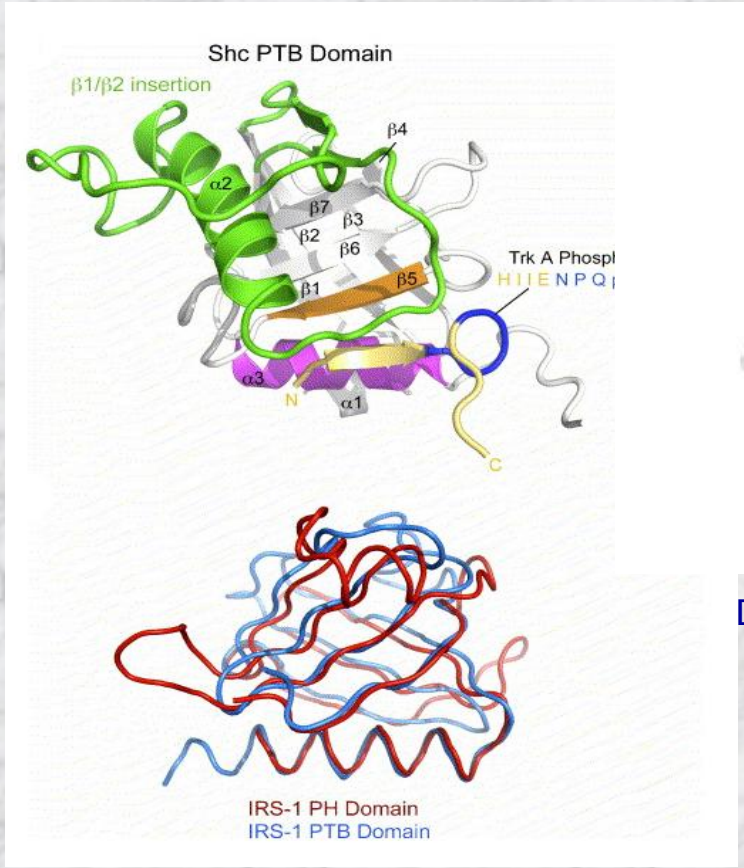


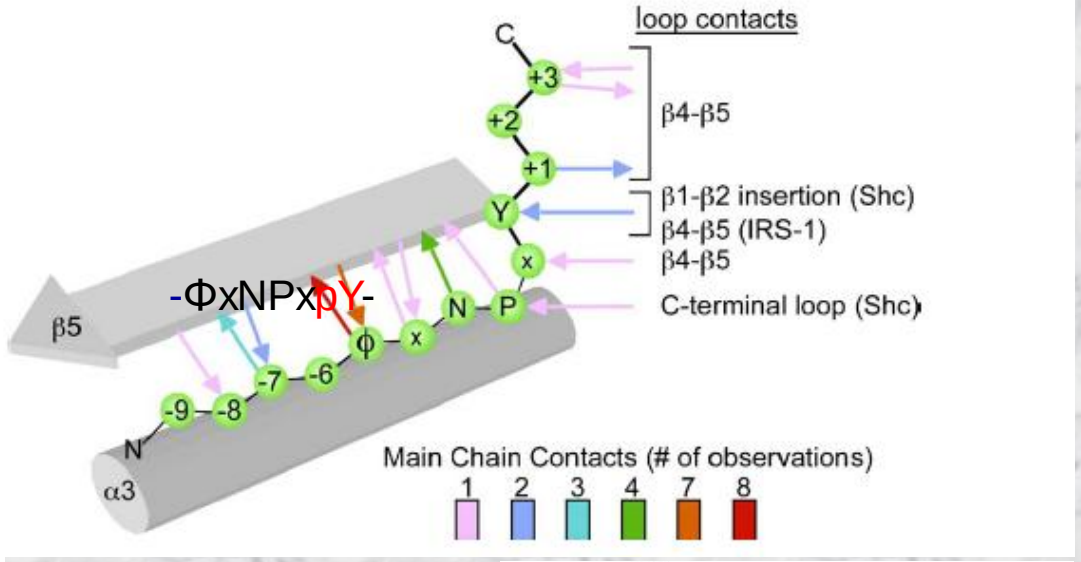
Shc isoforms and signaling



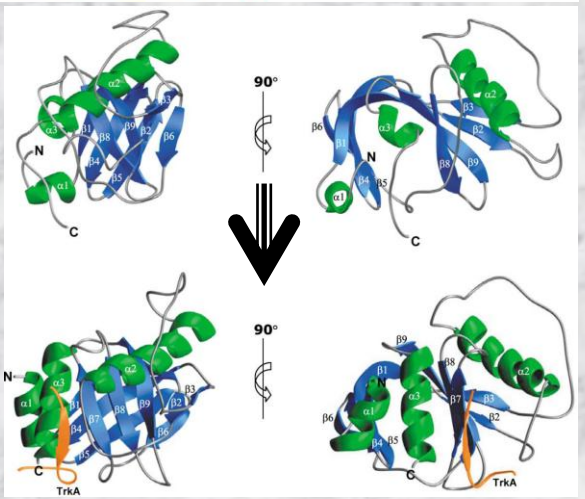
PhosphoTyrosine Binding (PTB) domain

Exhibit a conserved structural fold similar to pleckstrin homology (PH) domain





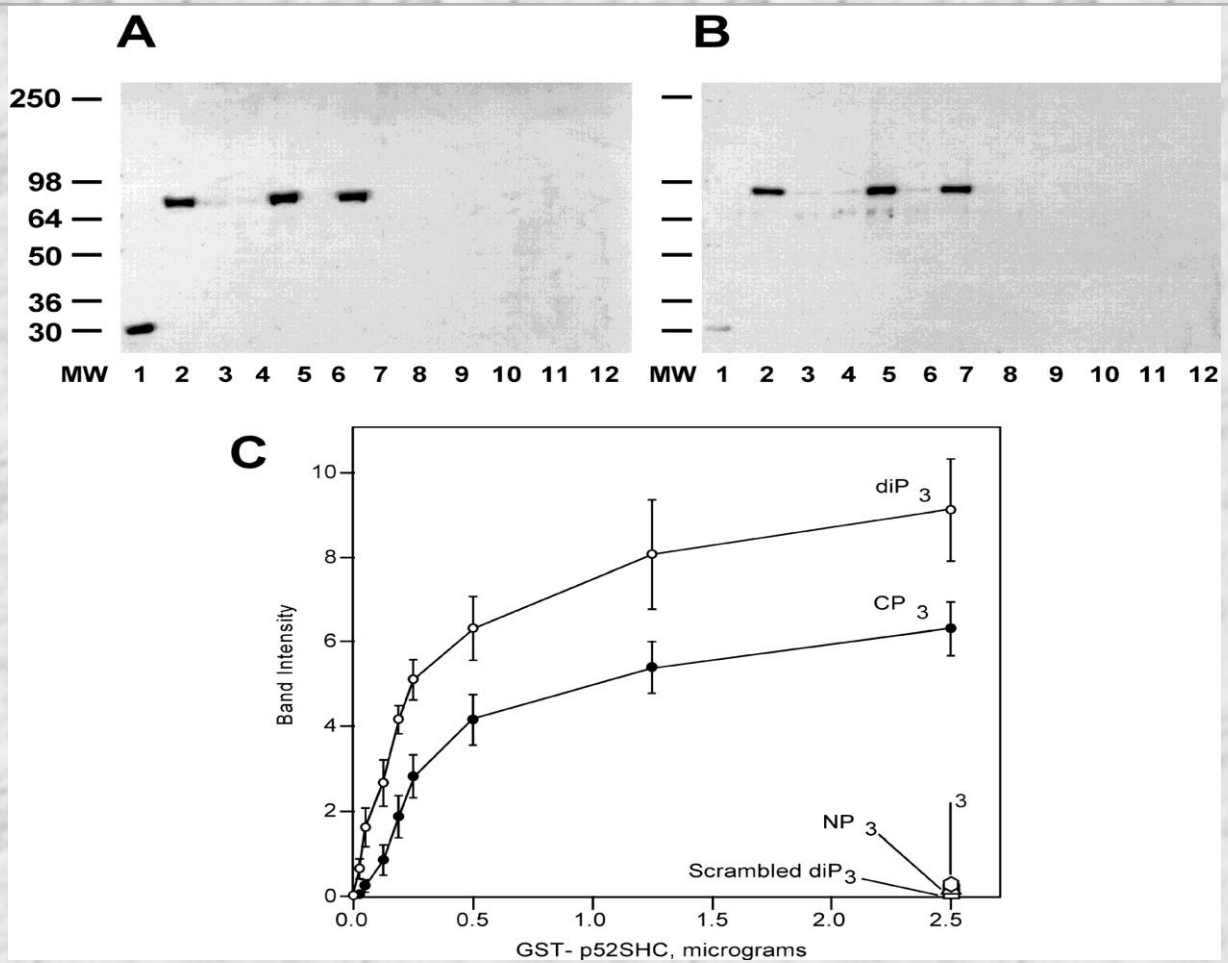
DiNitto and Lambright 2006



Farooq et al., Structure 2003

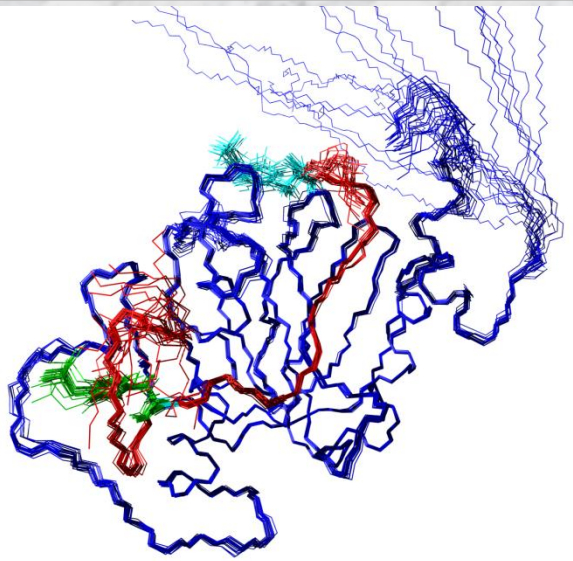
Protein	Peptide Ligand {sequence}	Kd(/μM)	Experimental method	PDB id	Reference:
Talin2 F2-F3	Integrin β1D-CT {GPKLLMIIHDRREFAKFEEKMNAKWDTQE**NPIY**KSPINN FKNPNYGRKAGL}	95	X-ray diffraction	3G9W	Anthis, Wegener et al. 2009
IRS1	IL4R {LVIAG**NPApY**RS}	2.0	Solution NMR	1IRS	Zhou, Huang et al. 1996
	IR {LYASS**NPEpY**LS}	90	Solution NMR	1IRS	
SNT	FGFR {HSQMAVHKLAKSIPLRRQVTVS}	10	Solution NMR	1XRO	Dhalluin, Yan et al. 2000
	TrkA {HIIE**NPQpY**FSDA}	5.0	Solution NMR	1XRO	
	TrkB {PVIE**NPQpY**FGIT}	5.0	Solution NMR	1XRO	
Dok1	RET {STWIE**NKLpY**GMSDGGK}	3.0	X-ray diffraction	1P5T	Shi, Ye et al. 2004
X11	βAPP {QNGEY**NPTY**KFFEQ}	0.5	X-ray diffraction	1AQC, 1X11	Zhang, Lee et al. 1997
Numb	NAK {GFS**NMSF**EDFP}	2.0	Solution NMR	1DDM	Zwahlen, Li et al. 2000
	LNX {LD**NPAY**}	2.0	Solution NMR	2NMB	Li, Zwahlen et al. 1998
	Mdm2 {YIGQYI}	-			
Dab1	ApoER2 {TKSMNFD**NPVY**RKT}	2.0	X-ray diffraction	1NTV, 1NU2	Stolt, Jeon et al. 2003
Shc	TrkA {HIIE**NPQpY**FSDA}	0.1	Solution NMR	1SHC	Zhou, Ravichandran et al. 1995
	ErbB3 {SAFD**NPDpY**WHSRLF}	0.3			Zhou, Harlan et al. 1995
	EGFR {SLD**NPDpY**QQDFF}	2.0			Farooq, Plotnikova et al. 1999
	IR {LYASS**NPEpY**LS}	4.0			Farooq, Plotnikova et al. 1999
	PTP-PEST {LLKAPLSFT**NPLH**SDDWHSDG}	-			Charest, Wagner et al., 1996

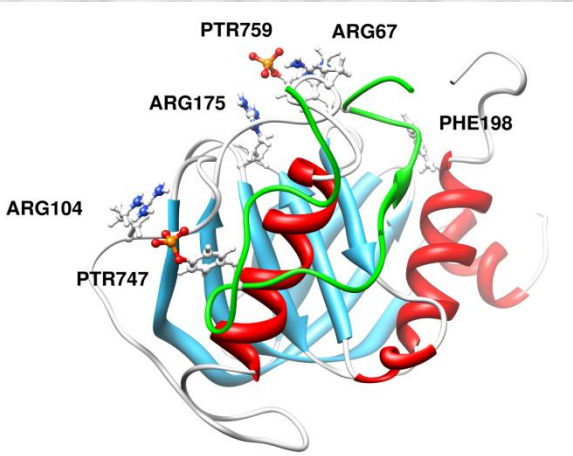
SHC pTB in “outside-in” integrin signaling



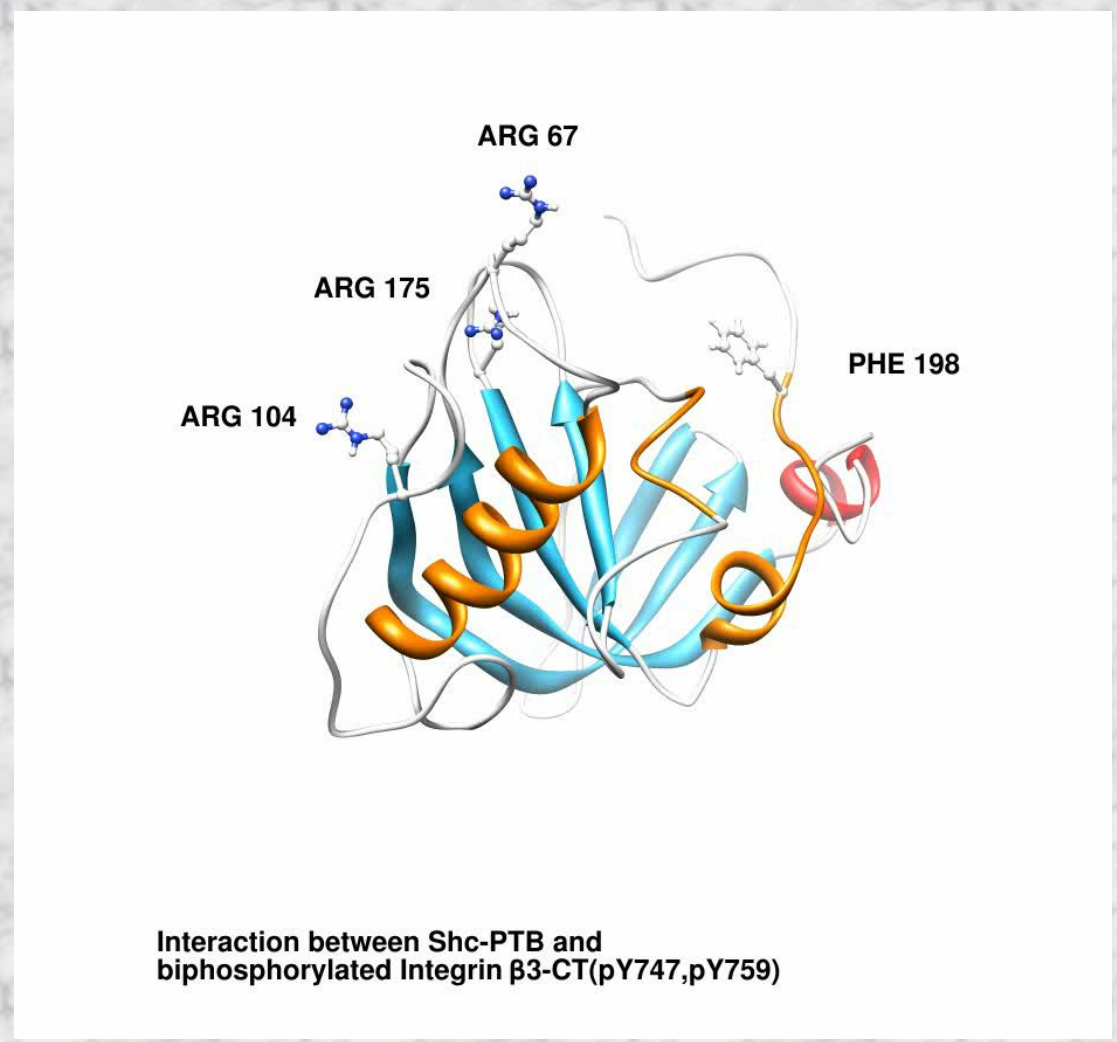
Cowan et al., 2000, JBC, 275, 36423–36429

Integrin β_3 Interaction with Shc-PTB



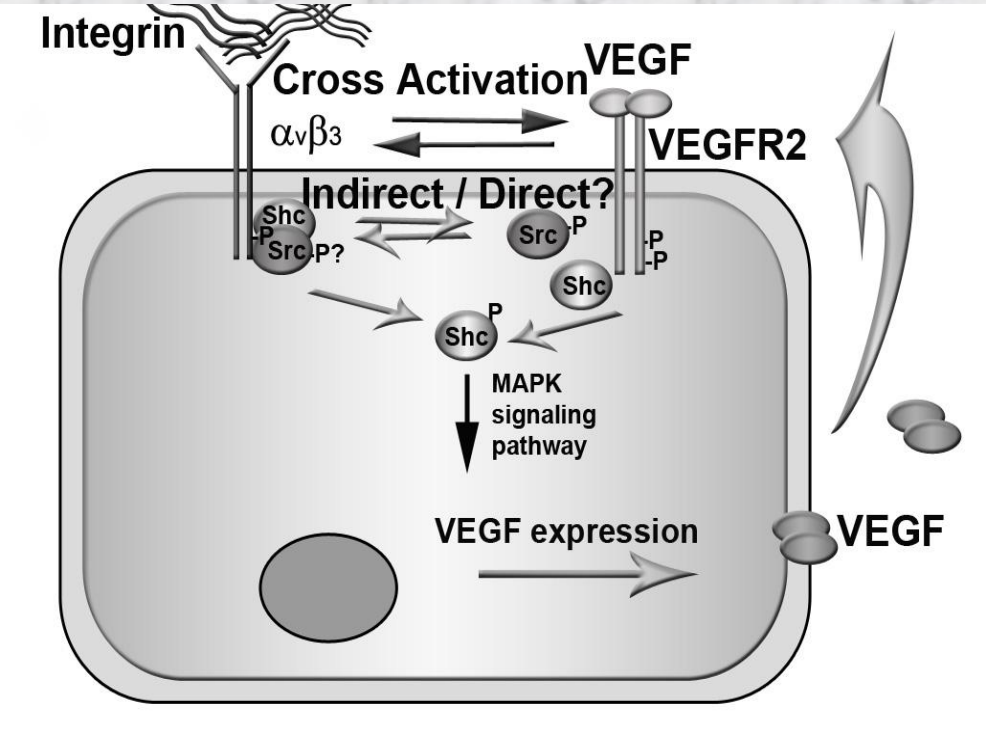


Interaction between Shc-PTB and
Biphosphorylated Integrin β3 (pY747, pY759)

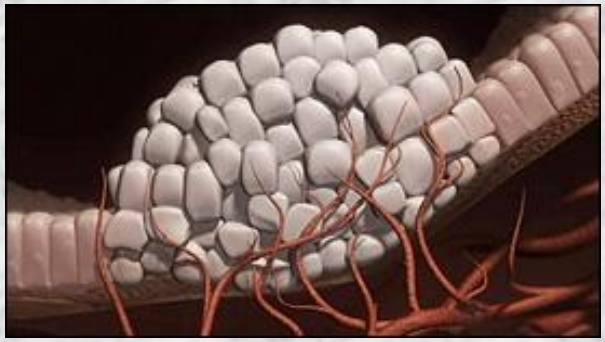


Interaction between Shc-PTB and
biphosphorylated Integrin β3-CT(pY747,pY759)

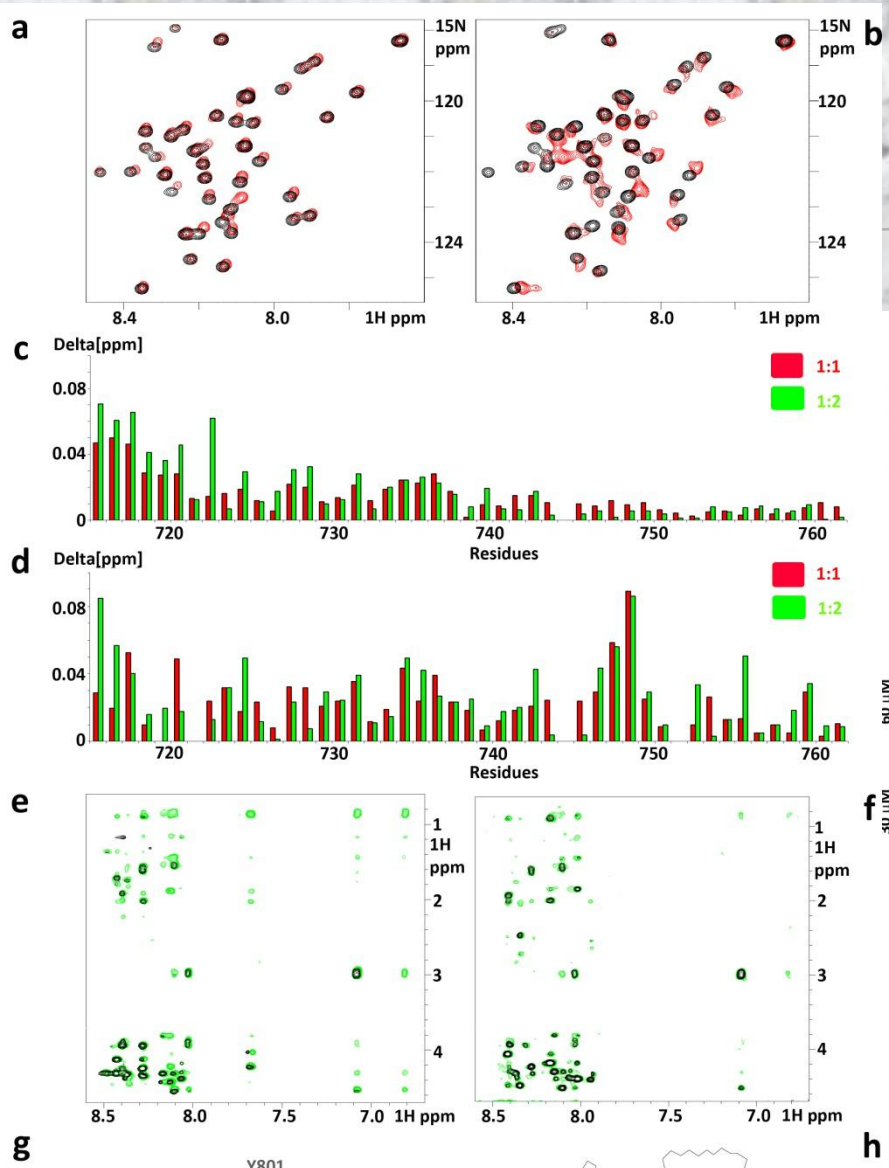
VEGFR/ Integrin interaction

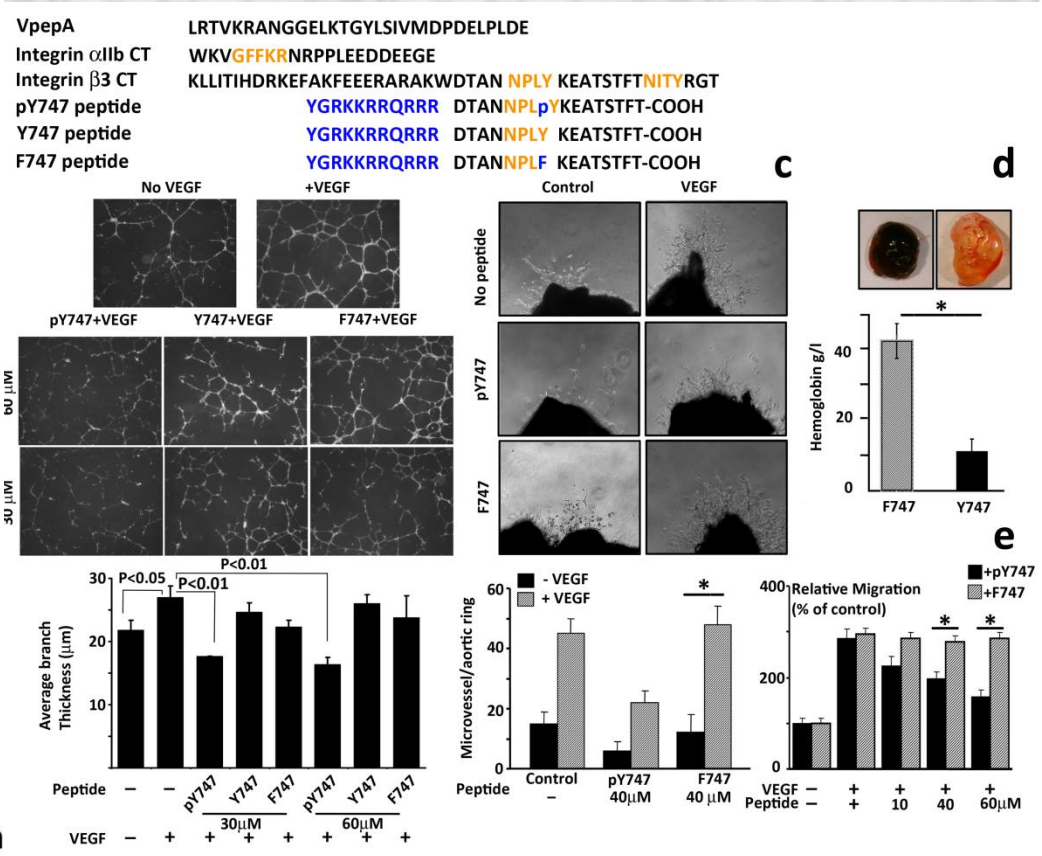


- Endothelial cells stimulation by VEGF induces high affinity state of $\alpha_V\beta_3$
- Activated $\alpha_V\beta_3$ interacts with VEGFR2
- VEGFR2 activation induces β_3 integrin tyrosine phosphorylation
- Integrin tyrosine phosphorylation is crucial for VEGF induced tyrosine phosphorylation of VEGFR2
- Thus, complex formation between VEGFR2 and $\alpha_V\beta_3$ is a crucial event in regulation of **angiogenesis**



β_3 interaction with VEGFR is phosphorylation dependent





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