Mechanical Proteins



Stretching imunoglobulin and fibronectin domains of the muscle protein titin



NIH Resource for Macromolecular Modeling and Bioinformatics Theoretical Biophysics Group, Beckman Institute, UIUC Adhesion Proteins of the Immune System

Immunoglobulin Domains



AFM Studies of Titin And FN-III Modules



Reviewed in Fisher et al. Nature Struct. Biol. 7:719-724 (2001)

Contracting and Relaxing Muscle Muscle Alive Periosteum covering to the bone Tendon -Fascia -© 1994, 96 M.C. Skeletal Muscle-Epimysium Perimysium Fasciculus. Endomysium. Muscle Fiberthin filament thick filament (actin filament) (myosin filament) Z disc Z disc RELAXATION CONTRACTION Striations. Sarcolemma. Sarcoplasm. Nuclei **Needs ATP!** Filaments-Myofibrils. ~~~~~



Titin the Longest Protein in the Human Genome



Quantitative Comparison

Bridging the gap between SMD and AFM experiments

Steered Molecular Dynamics (SMD)



Force-pulling velocity relationship



Force-extension curve



Hui Lu, Barry Isralewitz

Stretching modular proteins – Detailed View



Schematic view and typical Extension vs. force plot



Extension occurs in two steps



Distribution of measured forces For step 1 and step 2

Sequence aligment of Ig modules from the proximal Ig region

	Α	A'				
		\rightarrow				
				*		
seqI1	-APKIFERI	QSQT <mark>VGQG</mark> SD <mark>AH</mark>	- FRVRVVGKPDPEC	E <mark>WYK</mark> N <mark>G</mark> VKIER <mark>SDR</mark>	IYWYWPEDN	56
seqI2	QLITFTQEL	Q <mark>DV</mark> VAKEKDTMA	TFECETSEPFVK-V	K <mark>WYK</mark> D <mark>G</mark> MEVHE <mark>GDK</mark>	- YRMHSDRK	56
seqI3	- VVEFVKEL	Q <mark>DIEVPE</mark> SYS <mark>G</mark> E	-LECIVSPENIE-G	K <mark>WY</mark> HND V <mark>EL</mark> KSN <mark>GK</mark>	-YTITSRR <mark>G</mark>	54
seqI4	- PIAILQGL	S <mark>D</mark> QK <mark>VCEG</mark> DI <mark>V</mark> Q	-LEVKVSLESVE-G	V <mark>WM</mark> KD <mark>G</mark> Q <mark>EV</mark> QP <mark>S</mark> DR	- <mark>V</mark> HIVI D KQ	54
seq15	DVITPL	K <mark>DVNVI<mark>EG</mark>TKAV</mark>	- LECKVSVPDVTSV	KWYLND EQIKPDDR	- <mark>VQ</mark> AIVK <mark>G</mark> T	53
seq16	K <mark>I</mark> IR <mark>GL</mark>	R <mark>DLTCTE</mark> TQNVV	-FEVELSHSGID-V	L <mark>W</mark> NFKDK <mark>EI</mark> K <mark>PS</mark> SK	- <mark>YK I</mark> EAH <mark>G</mark> K	52
seqI7	- GGAISKPL	T <mark>DQTVAE</mark> SQE <mark>AV</mark>	- FECEVANPDSK-G	EWLRDG KHLPLTNN	- IRSESDGH	54
seq18	KIKKTL	KNLTVTETQDAV	- FTVELTHPNVKGV	Q <mark>WIK</mark> N <mark>G</mark> VV <mark>L</mark> ESN <mark>EK</mark>	-YAISVKGT	53
seqI9	K <mark>I</mark> IKK <mark>P</mark> I	K <mark>DVTAL</mark> ENATVA	- FEVSVSHDTVP-V	K <mark>WF</mark> HKSV <mark>EI</mark> K <mark>PS</mark> DK	- <mark>HRL</mark> VS <mark>E</mark> RK	52
seqI10	HITKTM	KNIE <mark>VPE</mark> TKT <mark>A</mark> S	- FECEVSHFNVP - SI	MWLKNGVEIEMSEK	- <mark>FK I</mark> VVQ <mark>G</mark> K	52
seqI11	MITSML	K <mark>DINAEE</mark> KDTI <mark>T</mark>	- FEVTVNYEGIS-Y	K <mark>WLK</mark> NGVEIKS <mark>T</mark> DK	- <mark>CQ</mark> MRTKKL	52
seqI12	-HIEFRKHI	K <mark>DIKVLE</mark> KKRAM	-FECEVSEPDIT-V	QWMKDDQELQITDR	- <mark>IKI</mark> QK <mark>E</mark> KY	54
seqI13	-D <mark>V</mark> R <mark>I</mark> RSIKI	K <mark>EV</mark> QVI <mark>E</mark> KQR <mark>AV</mark>	-VEFEVNEDDVD-A	HWYKDGIEINFQVQER	- <mark>HKY</mark> VV <mark>E</mark> RR	56
seqI14	EPP Q <mark>V</mark> LQEL	Q <mark>PV</mark> TVQS <mark>G</mark> KPAR	- FCAMISGRPQPKI	S <mark>WYK</mark> EEQL <mark>L</mark> S <mark>TG</mark> FK	- <mark>CKF</mark> LH <mark>DG</mark> Q	56
seqI15	- PPAIITPL	Q <mark>DT</mark> V <mark>T</mark> SEGQPAR	- FQCRVSG-TDLKV	S <mark>WY</mark> SKDKK <mark>I</mark> K <mark>PS</mark> RF	- FRMTQFED	54
ruler	$1\ldots\ldots 1$	0		40	60	

	:	:	*			: :
seqI1	VCEL	VIRD	VTAED	SASIMVK	AINIAGETS	SHAFLLVQAK
seqI2	VHFI	SILT	ID <mark>T</mark> S <mark>D</mark>	AED <mark>YS</mark> CV	LVEDE-NVK	ITAKLIV <mark>EG</mark> A
seqI3	RQNI	TVKD	VTKED	Q <mark>G</mark> E <mark>YS</mark> FV	IDGKK	TTCKLKMKPR
seqI4	SHMI	LIED	MTKED.	A <mark>GNYSFT</mark>	IPALGLS	IS <mark>G</mark> RVSVYSV
seqI5	KQRI	'VI'NR	THASD	EGPYKLI	V <mark>G</mark> RVE	INCNLSVEKI
seqI6	IYKI	TV LN	MKDD	E <mark>G</mark> K YTFY .	A <mark>G</mark> – – – – ENM	<mark>FSG</mark> KLTVA
seqI7	KRRI	IIAA	TKLDD	I <mark>G</mark> E <mark>YTY</mark> K	VATSK	<mark>FS</mark> AKLKV <mark>E</mark> AV
seq18	IYSI	RIKN	CAIVD	ESV <mark>YG</mark> FR	L <mark>G</mark> RL <mark>G</mark> A	A <mark>S</mark> ARLHV <mark>E</mark> TV
seqI9	VHKI	MLQN	IS <mark>P</mark> SD	A <mark>G</mark> E <mark>YT</mark> AV	VGQLE	CKAKLFVETL
seqI10	L <mark>H</mark> QI	II MN	TSTED	SAE <mark>YT</mark> FV	CGNDQ	V<mark>S</mark>ATLTVTPI
seqI11	THSI	NIRN	VHFGD.	AAD <mark>YT</mark> FV.	A <mark>G</mark> KAT	STATLY <mark>VE</mark> AR
seqI12	VHRI	LIPS	TRMSD	A <mark>G</mark> K <mark>YT</mark> VV.	A <mark>G</mark> <mark>G</mark> NV	<mark>STA</mark> KLF <mark>VEG</mark> R
seqI13	IHRM	IFISE	TRQSD	A <mark>G</mark> E <mark>YT</mark> FV.	A <mark>G</mark> RNR	SSVTLYVNA <mark>P</mark>
seqI14	EYTI	LLIE	AFPED.	AAV <mark>YT</mark> CE.	AK <mark>NDYG</mark> VAT	ISASLSVEVP
seqI15	TYQI	EIAE	AY <mark>PED</mark>	E <mark>G</mark> T <mark>YT</mark> FV.	AN <mark>N</mark> A <mark>VG</mark> QVS	STANLSLEAP
ruler			70	80	90	0



87

Sequence aligment of Ig modules from the distal Ig region

	A		A'														
									*								
120	VKEIKDI	ILT-	ESEI	FVGS	AIFE	CLV	PST	AITT	WMK I	GSN	R	ESPK	HRF	IAD	GKDRK		57
121	PVRFV KT	LEEE	v <mark>r</mark> v	VKGQI	LYLS	CELI	N <mark>K</mark> -E	rd <mark>v</mark> v	WRKI	GKI	VE-	K <mark>P</mark> GR	IVF	GVI	GLMRA	. 5	58
122	RDWLVKP	IR <mark>D</mark> -	QHV1	PKG	AIFA		AKDT	PNIK	WFKG	YDE	PAE	PNDK	TEI	LRD	GNHLY	. 5	59
123	EVELLKP	IE <mark>D</mark> -	VTI	YEKE	ASFD	AEI	EAD	IPGQ	WKLK	GELI	R	P <mark>S</mark> PT	CEI	KAE	GG KRF	' 5	57
124	ELDFAVP	LK <mark>D</mark> -	VTV	PERR(<mark>ARF</mark> E	CVL	REA	NVI-	WSKG	PDI	K	SSDK	FDI	IAD	GKKHI	5	56
125	RLKFMSP	LE <mark>D</mark> -	Q <mark>T</mark> VI	KEGE	ATFV	CEL	HEK	MHVV	WFKN	IDAKI	н	TSRT	VLI	SSE	GKTHK	: 5	57
126	DPY FT VK	LHD-	K <mark>TA</mark> N	V <mark>E</mark> KD	SITL K	CEV	K-D	V <mark>P</mark> VK	WF <mark>K</mark> I	GEE	V	P <mark>S</mark> PK	YSI	KAD	GLRRI	5	56
127	LIEVEKP	LY <mark>G</mark> -	VEVI	FVGE	AHFE	IELS	EPD	V H <mark>G</mark> Q	WKLK	GQPI	T	A <mark>SP</mark> D	CEI	IED	GKKHI	5	57
128	PLIFITP	LSD-	νκνι	FEKDI	S <mark>AKF</mark> E	CEV	REP	KTFR	WLKG	TQE	T	GDDR	FEL	IKD	GTKHS	5	57
129	RLKFLTP	LK <mark>D</mark> -	V <mark>T</mark> AI	KEKE	AVFT	VELS	HDN	IRVK	WFKN	IDQR	H	TTRS	vsm	QDE	GKTHS	5	57
I30	DPY FTG K	LQ <mark>D</mark> -	Y <mark>T</mark> G	V <mark>E</mark> KDI	VIL Q	CEIS	KAD	A <mark>P</mark> VK	WF <mark>K</mark> I	GKEI	K	P <mark>S</mark> KN	IVA	КТ <mark>D</mark>	GKKRM	. 5	57
I31	EIKLVRP	LHS-	VEVI	METE	ARFE	TEIS	EDD	IHAN	WKLK	GEAI	ь	Q <mark>T</mark> PD	CEI	KEE	GKIHS	5	57
I32	VIGLLRP	LKD-	V <mark>T</mark> VI	ГА <mark>GE</mark>	ATFD	CELS	YED	I <mark>P</mark> VE	WYLK	GKKI	E	PSDK	VVF	RSE	GKVHT	5	57
I33	PVEFTKP	LED-	Q <mark>T</mark> VI	EEGA	AVLE	CEVS	REN.	akvk	WFKN	I <mark>GTE</mark> I	ь	K <mark>S</mark> KK	YEI	VAD	GRVRK	. 5	57
I34	HVEFLRP	LTD-	LQVI	REKEN	1 <mark>A</mark> RFE	CELS	REN.	<mark>a</mark> kvk	WFKI	GAE	K	K <mark>G</mark> KK	YDI	ISK	GAVRI	5	57
I35	EAVFTKN	LAN-	IEVS	SETD	IKLV	CEV	KPG.	AEVI	WYKG	DEE	I	E <mark>T</mark> GR	YEI	LTE	GRKRI	5	57
I36	AAEFISK	PQN-	LEII	L <mark>EGE</mark> I	(AEFV	CSI	KES	F <mark>P</mark> VQ	WKRI	DKT	E	SGDK	YDV	IAD	GKKRV	· 5	57
I37	-LRIVVP	LKD-	TRVI	K <mark>E</mark> QQI	S <mark>VVF</mark> N	(CEVI	NT <mark>EG</mark>	<mark>AKA</mark> K	WFRN	IEEA	F	DSSK	YII	LQK	DLVYT	5	56
I38	DLRIVEP	LK <mark>D</mark> -	IETI	MEKK	VTFW	CKVI	RLN	VTLK	WTKN	GEE	7 <mark>P</mark>	FDNR	VSY	RVD	КҮКНМ	. 5	57
I39	PTEFVEH	LE <mark>D</mark> -	Q <mark>T</mark> V	r <mark>e</mark> fdi	DAVFS	CQL	REK.	<mark>ANV</mark> K	WYRN	I <mark>GRE</mark>]	K	EGKK	YKF	EKD	GSIHR		57
I40	PVEIIRP	PQD-	ILE	A <mark>PG</mark> AI	D <mark>VV</mark> FL	AELI	N <mark>KD</mark> K	VEVQ	WLRN	NMV	v	Q <mark>G</mark> DK	HQM	MSE	GKIHR		57
ruler	1	.10.	• • •	2	20		30			40		5	0		60		

Key Residue

	: ::
120	LHIIDVQLSDAGEYTCVLRLGNKEK-TSTAKLVVEE-
121	LTINDADDTDAGTYTVTVENANNLECSSCVKVVEV
122	LKIKNAMPEDIAEYAVEIEGKRYPAKLTLGER
123	LTLHKVKLDQAGEVLYQALNAI T TAILTVKEI
124	LVINDSQFDDEGVYTAEVEGKKTSARLFVTGI
125	LEMKEVTLDDISQIKAQVKELS <mark>S</mark> TAQLKVLEA
126	LKIKKADLKDKGEYVCDCGTDK TKANVTVEAR
127	LILHNCQLGMTGEVSFQAANAK SAANLKVKEL
I28	MVIKSAAFEDEAKYMFEAEDKHTSGKLIIEGI
129	ITFKDLSIDDTSQIRVEAMGMS <mark>S</mark> EAKLTVLEG
I30	LILKKALKSDIGQYTCDCGTDK <mark>T</mark> SGKLDIEDR
I31	LVLHNCRLDQTGGVDFQAANVKSSAHLRVKPR
I32	LTLRDVKLEDAGEVQLTAKDFK <mark>T</mark> HANLFVKEP
I33	LVIHDCTPEDIKTYTCDAKDFK <mark>T</mark> SCNLNVVPP
I34	LVINKCLLDDEAEYSCEVRTAR TSGMLTVLEE
I35	LVIQNAHLEDAGNYNCRLPSSR <mark>T</mark> DGKVKVHEL
I36	LVVKDATLQDMGTYVVMVGAAR AAAHLTVIEK
I37	LRIR <mark>DAHLDD</mark> QAN <mark>Y</mark> NVSLTNH <mark>RGEN</mark> VK <mark>S</mark> AANLIVE <mark>E</mark> E
I38	LTIKDCGFPDEGEYIVTAGQDKSVAELLIIEA
I39	LIIKDCRLDDECEYACGVEDRKSRARLFVEEI
I40	LQICDIKPRDQGEYRFIAKDKEARAKLELAAA
ruler	





Water-Backbone Interactions Control Unfolding



time (ps)

Lu and Schulten, Biophys J.79: 51-65 (2000)



1 day/ns

Mu Gao



Fibronectins

Architecture and Function of Fibronectin Modules



• Highly extensible

Andre Krammer V. Vogel, U. Wash.

Fibronectin Matrix in Living Cell Culture



100 μ m



Extension (nm)

Atomic force microscopy observations

Ohashi et al. Proc. Natl. Acad. Sci USA 96:2153-2158 (1999)

Andre Krammer V. Vogel, U. Wash.

RGD Loop of FnIII₁₀

Krammer *et al. Proc. Natl. Acad. Sci USA* **96**:1351-1356 (1999)







Extension of 13 Å



fibronectir binding with RGD loop to integrin

integrin interacting with extracellular matrix

Andre Krammer V. Vogel, U. Wash.

http://www.ks.uiuc.edu

Probing Unfolding Intermediates in FN-III₁₀



FnIII₁₀ module solvated in a water box $55 \times 60 \times 367 \text{ Å}^3$ (**126,000 atoms**)

Steered Molecular Dynamics, periodic boundary conditions, NpT ensemble, Particle Mesh Edwald for full electrostatics

NAMD on Linux cluster of 32 Athlon 1.1GHz processors: **10 days/ns**



Mu Gao, U. Illinois

A. Krammer, D. Craig, V. Vogel, U. Wash.

Probing Unfolding Intermediates in FN-III₁₀



Specific stretching and unfolding pathway for constant force (500 pN) stretching: scenario with A rupturing first Complete stretching and unfolding pathway: after straightening and partial A-B separation, A, , or A+G or G rupture first

Modeling Fibronectin type III₁

NMR Structure + SMD Modeling

Mu Gao Collaboration with V. Vogel, David Craig, O. Lequin



Architecture of beta-sheets

This protein features a particularly strong (red) beta-sheet



Constant velocity pulling stretches and breaks first the green beta-sheet, exhibits then a stable intermediate, before the red beta-sheet is stretched and broken



Energetics of FN-III-1 during stretching



Adhesion Proteins of the Immune System

Strengthening the Immune System *M. Bayas and D. Leckband*

Infections are battled best by the human immune system when there exists a memory from a previous disease or vaccination. The first step in using this line of defense is recognition: Cells of the immune system capture antigens, e.g., microbes in the respiratory tract, then mature in the lymph system, and finally present on their surface pieces of the antigen to T-cells that may recognize the antigen and become activated. The recognition of the antigen by T-cells is dramatically enhanced through surface receptors, CD2 and CD58, on the T-cell and the antigen presenting cell. The receptors stick out from their cell, adhere to one another, and conjoin the T-cell and antigen presenting cell long enough to enable recognition and activation. The molecular basis of this adhesion has been probed in a recent <u>collaborative study</u> with UIUC chemical engineer <u>D</u>. Leckband. Starting from the available crystallographic structure of the CD2-CD58 complex the researchers carried out 90,000 and 100,000 atom simulations using <u>NAMD</u> and pulled the complex apart in steered molecular dynamics simulations. An analysis of the simulations with <u>VMD</u> revealed in atomic level detail how the human immune system is strengthened through elastic adhesion.



CD2-CD48 interaction enhances antigen recognition between t-lymphocyte cells and antigen presenting cells



CD2-CD-48: Cluster of differentiation (**CD**) antigen number for unique reactivity patterns with leukocytes.

TCR: t cell receptor.

MHC: major histocompatibility Complex.

Thousands of CD's with low adhesion affinity assist cells in antigen recognition

CD2-CD48 salt bridges contribute to specificity, but are they also important for the tensile strength of interaction? Yes!

How do Ig domains of CD2-CD48 contribute to adhesion elasticity: through unfolding and/or deformation? Through unfolding, even though Ig domains do not contain disulfide bonds!

Marcos Bayas, D. Leckband, K. Schulten, Biophys. J. 84:2223-2233, 2003



120 ps so far

• 1 - 0.05 Å / ps pulling velocity

Marcos Bayas, D. Leckband, K. Schulten, submitted

0.05 Å / ps **slow**



1 Å / ps



Marcos Bayas, D. Leckband, K. Schulten, Biophys. J. 84:2223-2233, 2003

Behavior of Non-Mechanical Proteins

Force-induced Unfolding of Other Domains

C2 domain of synaptotagmin I (all sheet protein)



- much less resistance to external forces than Ig and FnIII
- during unfolding hydrogen bonds not required to be broken in clusters

Lu and Schulten, Proteins, 35, 453-463 (1999)

NIH Resource for Macromolecular Modeling and Bioinformatics Theoretical Biophysics Group, Beckman Institute, UIUC

Classification of β Sandwich Domains



Lu and Schulten, Proteins, 35, 453-463 (1999)

NIH Resource for Macromolecular Modeling and Bioinformatics Theoretical Biophysics Group, Beckman Institute, UIUC

Force-induced unfolding of alpha-helical protein

Cytochrome C6 (all helix protein)



- no initial force peak
- much less resistance to external forces than Ig and FnIII
- during unfolding hydrogen bonds not required to be broken in clusters

Lu and Schulten, Proteins, 35, 453-463 (1999)

NIH Resource for Macromolecular Modeling and Bioinformatics Theoretical Biophysics Group, Beckman Institute, UIUC

Ubiquitin



Fatemeh Araghi, Timothy Isgro, Marcos Sotomayor

Monoubiquitylation versus multi-ubiquitylation



Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

Structure-Function Relationship



Proteasome Degradation





First peak when the first beta strand is stretched out

- SMD simulation, with constant velocity
- Box of water 70x240x70 A ~81K atoms
- smd velocity 0.4 A/ps
- smd spring constant 7 kcal/mol A^2

Ubiquitin Unfolding I



Ubiquitin Unfolding II



Pulling Dimer

- SMD (v=0.4 A/ps k=7 kcal/mol A^2) constant P
- Two monomers seperatre.



