The Molecular Basis for Cross-linking of actin filaments

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Cytoskeleton

- Cytosol of eukaryotic cells contains an array of fibrous proteins: ***cytoskeleton***

- Microfilaments:polimerisation of actin
- Microtubules built of tubulin
- Intermediate filaments built of one or more rod shaped subunits

Cytoskeleton

- Cytoskeletal fibres
  - Cell strength and rigidity
  - Control movements within the cell
  - Connect organnells or provide tracks for movements

Cytoskeleton

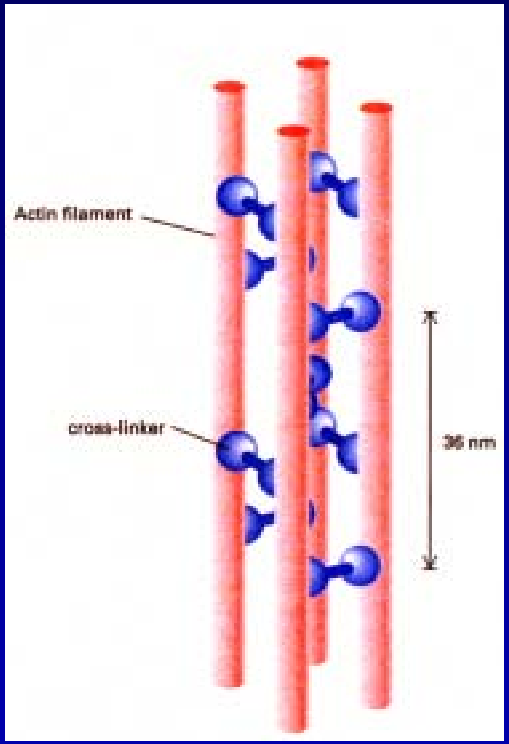
- Organisation principle:

- Large complex structures are built from small simpler components
- Crosslinking of cytoskeletal fibres into bundles, networks and gel-like lattices

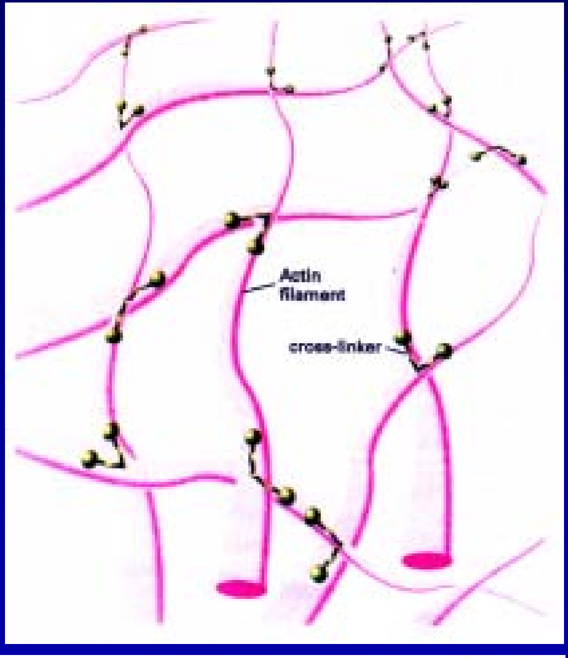
F-Actin-based cytoskeleton

- The most abundant cell protein
- Microfilaments involved in two basic mechamism of motility
    - Muscle contraction in association with myosin
    - Cellular movements, changes in the cell shape

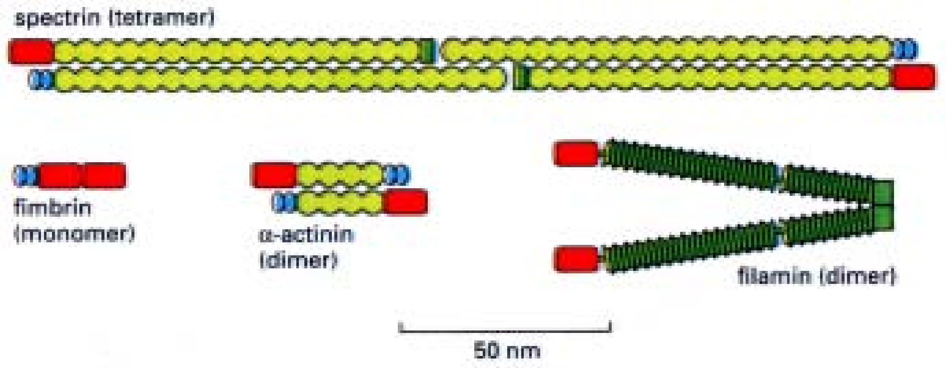
F-Actin-based cytoskeleton



Networks



Bundles



F-Actin cross-linking proteins

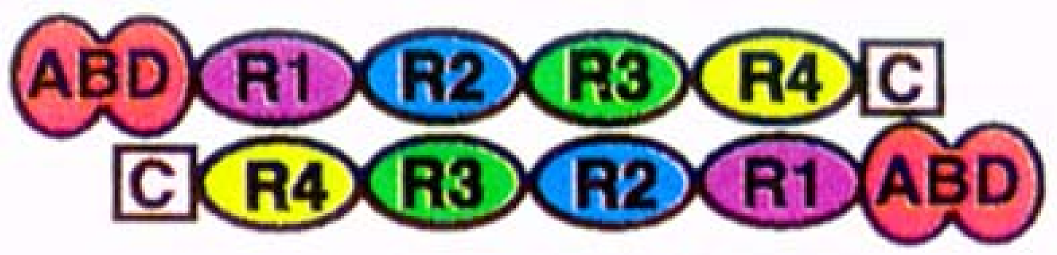
- Spectrin gene superfamily
- Share a commom actin-binding domain
- Length determined by α-helical or β-sheet motifs used as spacers
    - α-actinin (bundles)
    - Spectrin (networks)
    - Dystrophin (networks)
    - Filamin (networks)

Domain composition

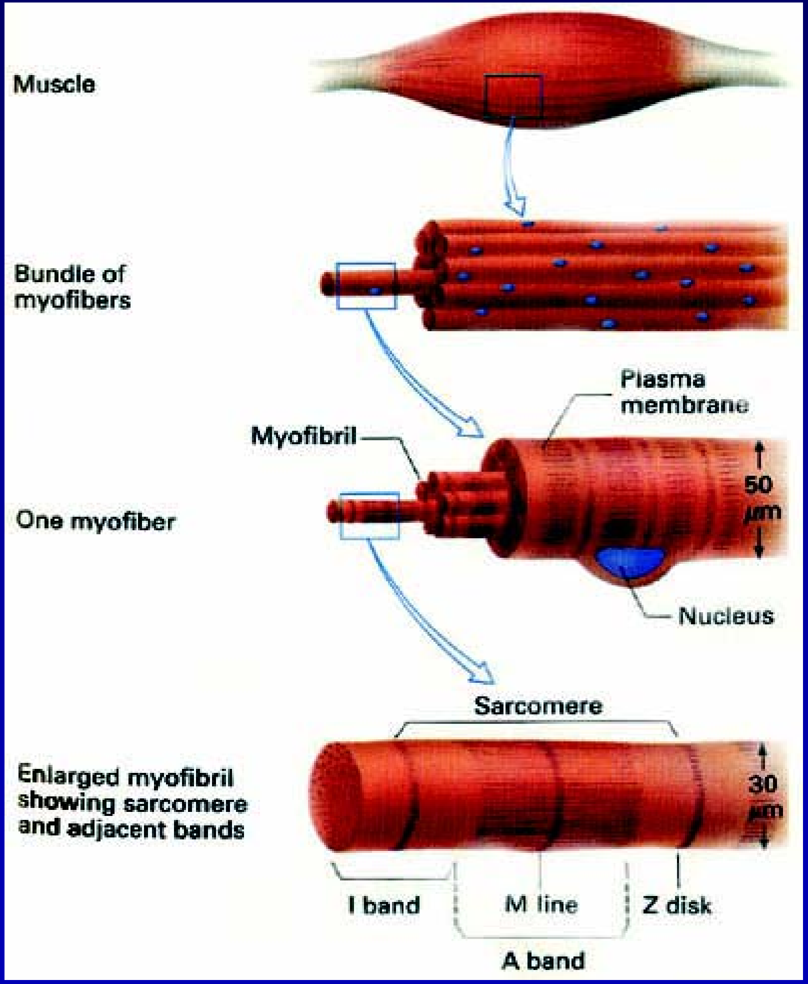
- Modular proteins
  - Central rod
    - 4 (α-actinin) - 24 (dystrophin) repeated units
  - Functional domains
    - Calmodulin hands
    - Actin binding domain
    - PH, SH3

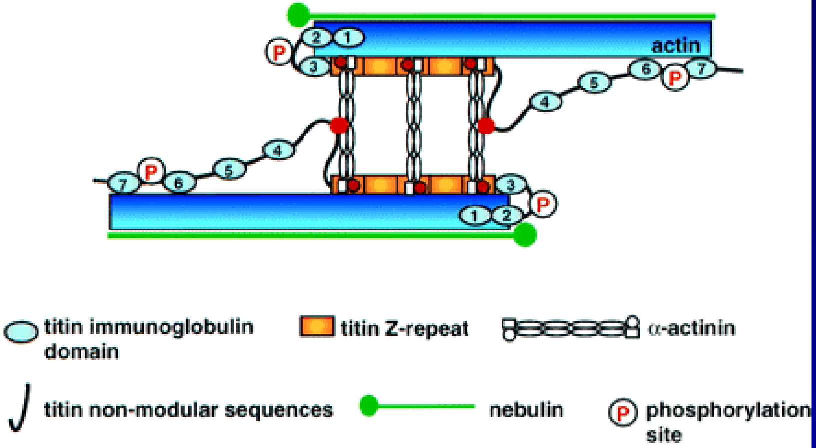
Alpha-actinin

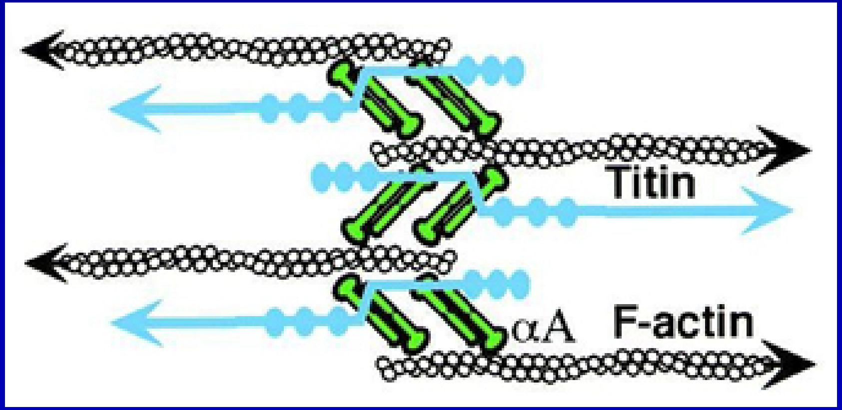
- Actin binding domain (ABS): 2 CH domains
- Rod domains: 4 spectrin-like repeats
- Calmodulin-like domain (CAM): 4 EF hands



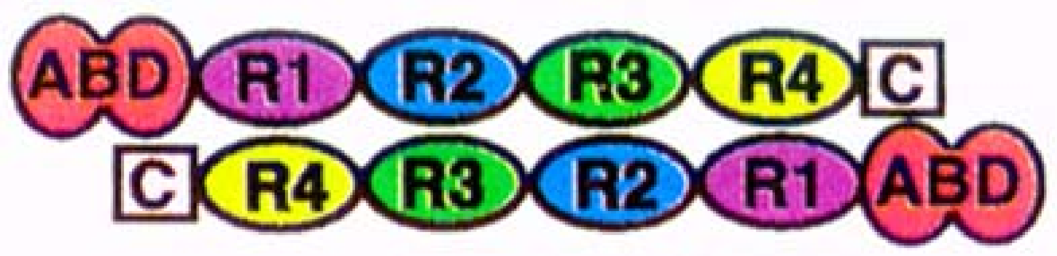
Muscle


Muscle
Bundle of myofibers
Plasma membrane
Myofibril
One myofiber
50 µm
Nucleus
Sarcomere
Enlarged myofibril showing sarcomere and adjacent bands
30 µm
I band
M line
Z disk
A band


actin
1
2
3
4
5
6
7
P
titin immunoglobulin domain
titin Z-repeat
α-actinin
titin non-modular sequences
nebulin
P phosphorylation site


Titin
αA
F-actin

Structural studies



- Actin binding domain of α-actinin

- Central two repeats of α-actinin

Purification and crystallization

- overexpressed in *E.coli* with His tag
- purified to homogeneity
- crystallized with PEG400
- at 4 C - 12 C
- protein conc. 170 mg/ml

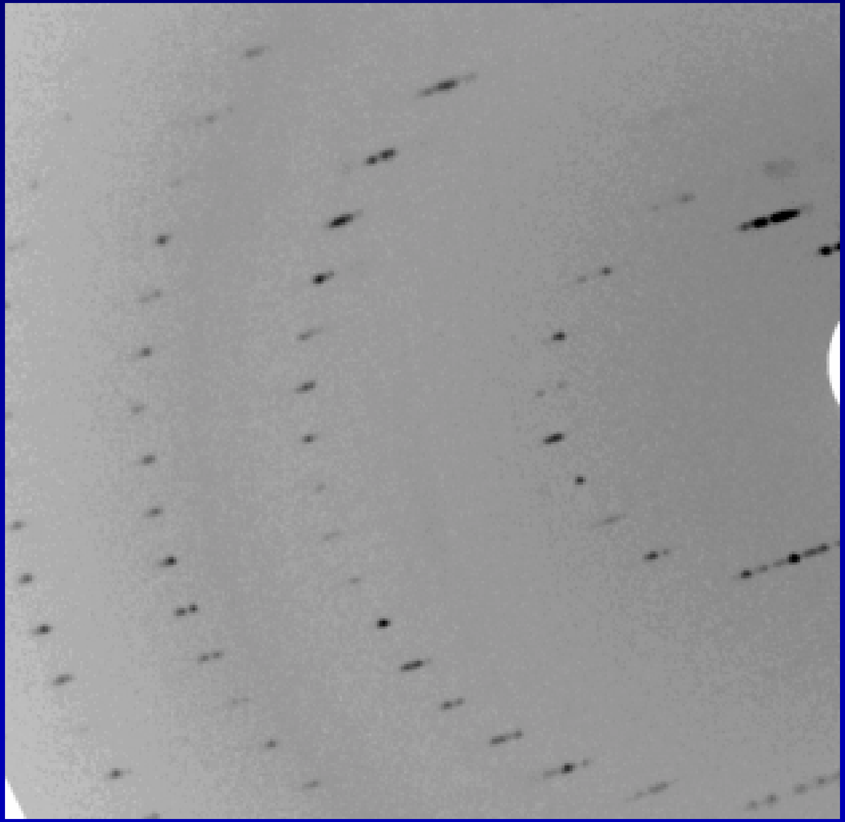
Oligomerisation state

- two-hybrid screen
- gel filtration
- analytical centrifugation (@1 mg/ml)

- stable dimer, monodisperse solution

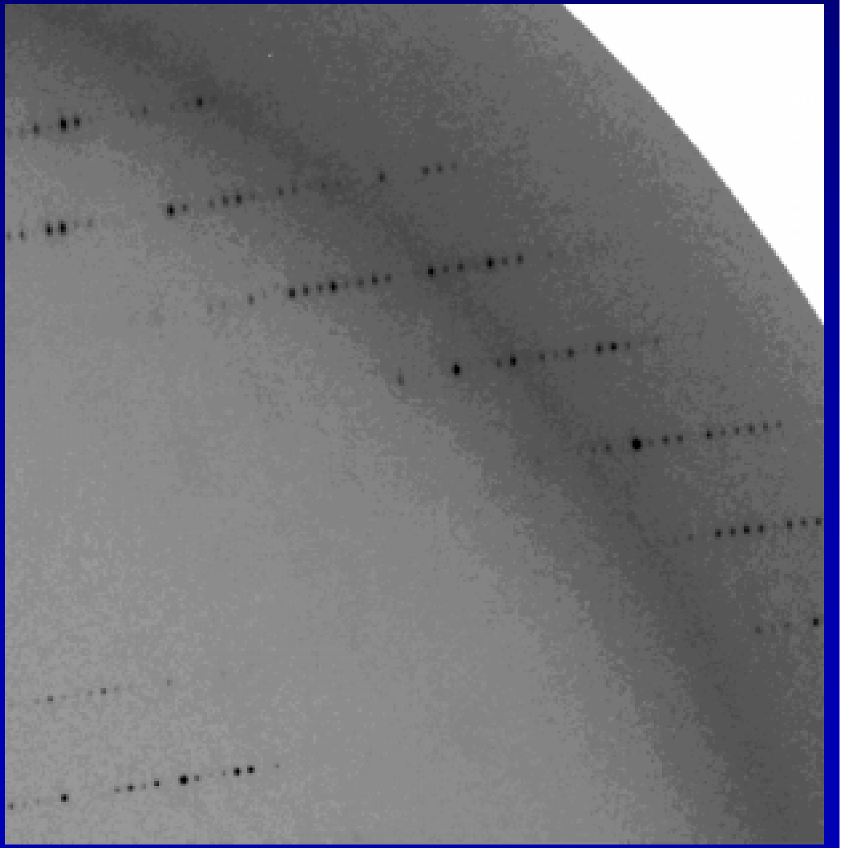
Diffraction Experiments

- Cell: a = b = 60 A, c = 390 A, $P6_522$
- Mar180 detector
- Rotating anode



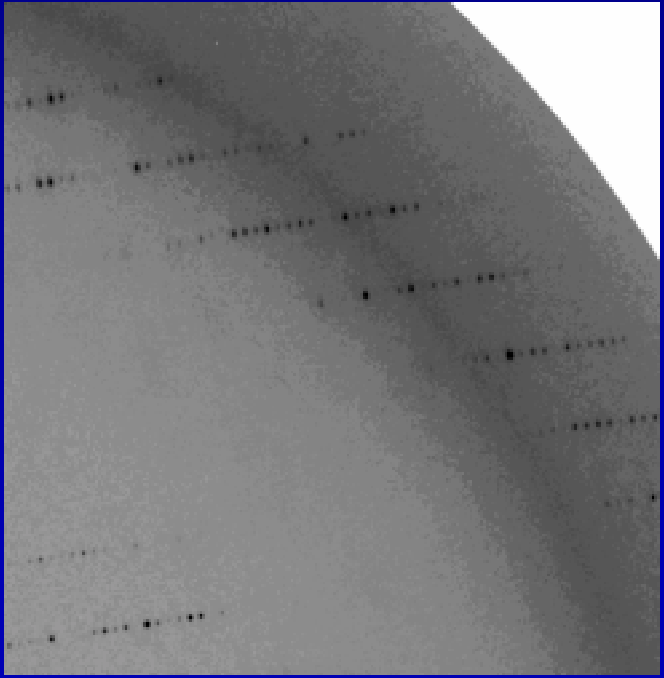
Diffraction Experiments

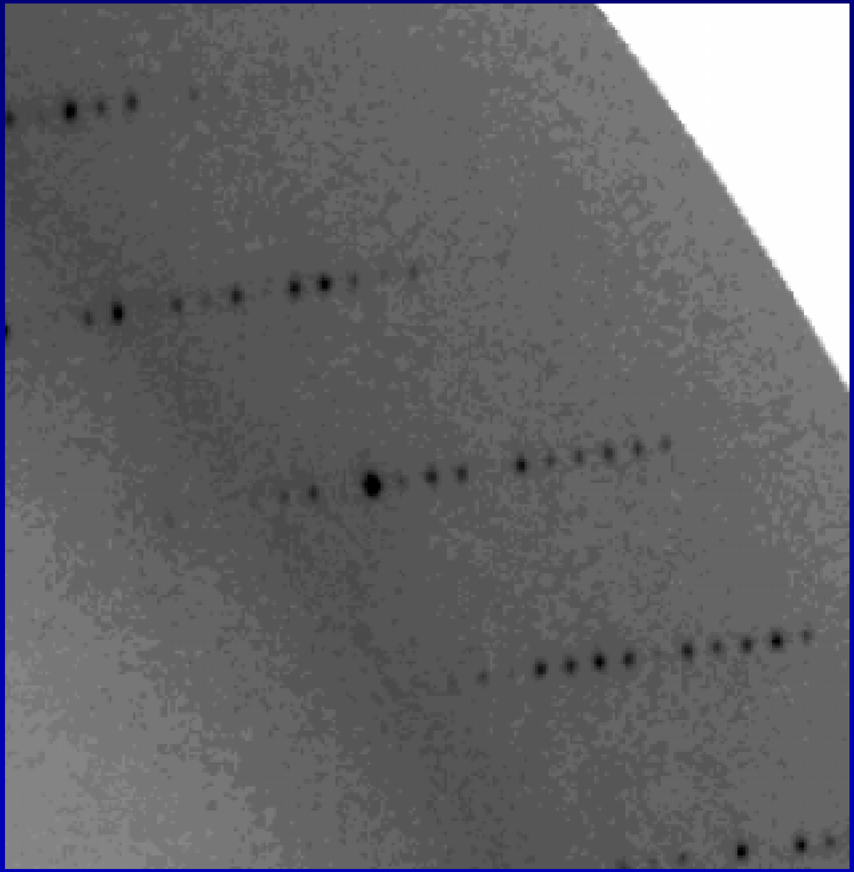
- Cell: a = b = 60 A, c = 390 A, $P6_522$
- Mar345 detector
- Rotating anode, mirrors



Diffraction Experiments

- Cell: a = b = 60 A, c = 390 A, $P6_522$
- MarCCD detector
- Synchrotron source
  - ID14-3 @ ESRF

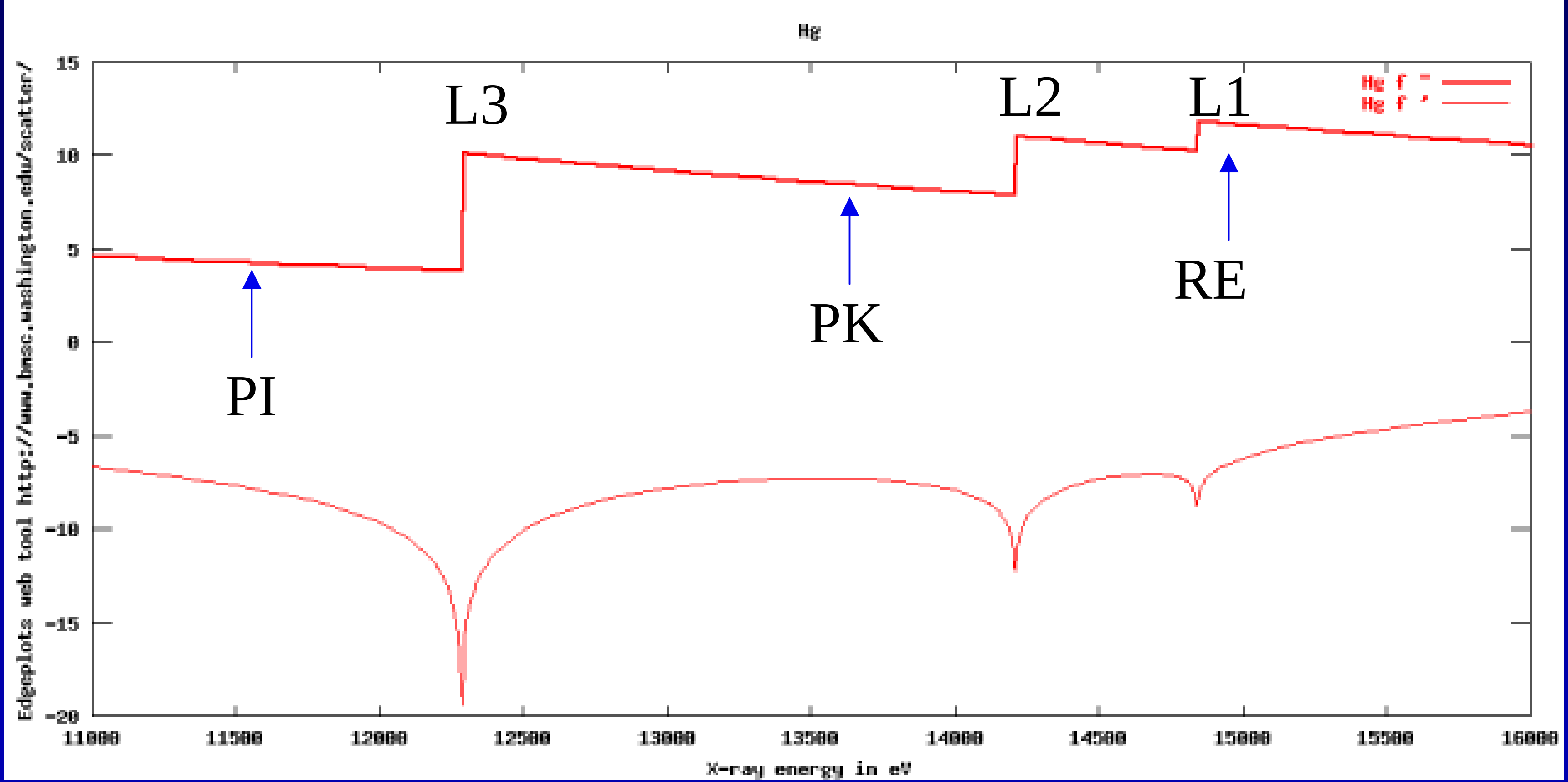




Heavy atom derivative search

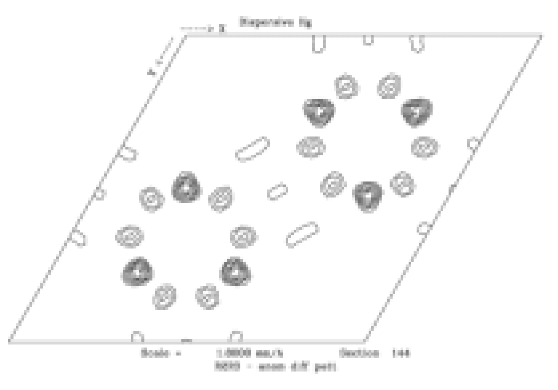
- One synchrotron trip dedicated to high-throughput search of heavy atom derivatives
  - Hg, Pt, Os, Au
    - Various compounds of each atom type
- Indentified Hg derivative

Anomalous dispersion experiment on Hg

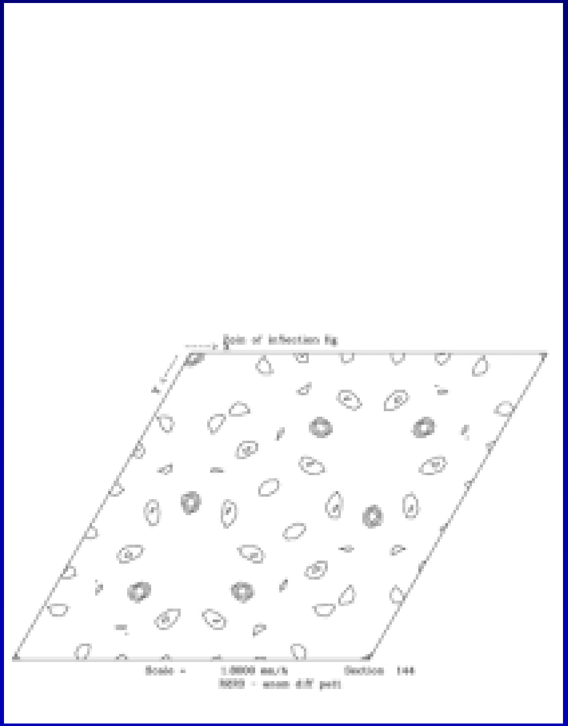


Pattersons

Dispersive



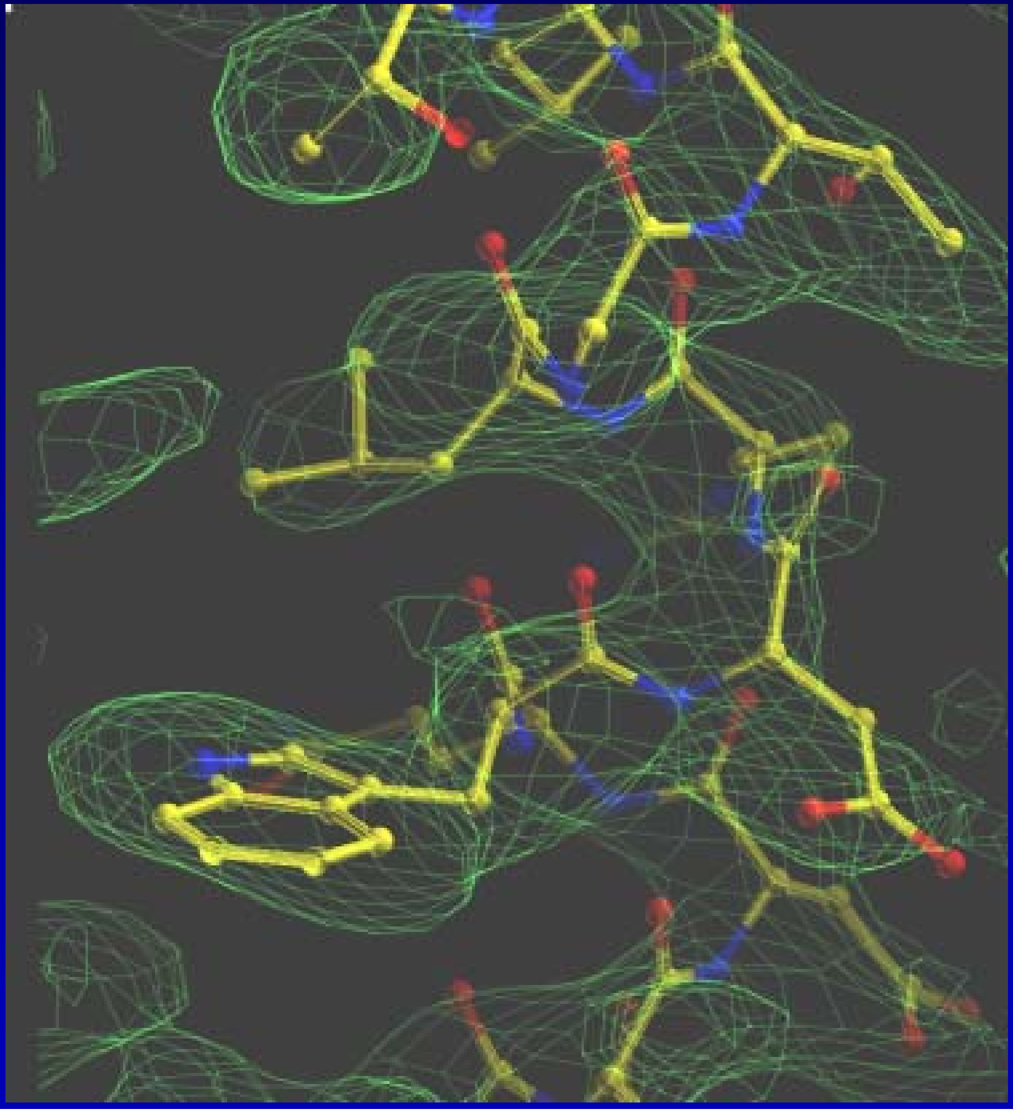
Anomalous



Data collection and phasing

	Remote	Inflection	PK	Native
Data collection				
Wavelength (Å)	0.8265	1.0078	0.9183	0.9470
Beamline@ESRF	BM14	BM14	BM14	ID14
Temperature (K)	100	100	100	100
Detector	MAR345	MAR345	MAR345	Off-line image plate
Space group		$P6_522$		
Cell (Å)		a=b=60.59 c=390.47		a=b=59.71 c=390.74
Unique reflections	10194	10194	10072	26307
Redundancy	6.4	7.4	6.4	6.1
Completeness %	97.8(96.7)	97.5(98.0)	97.4(99.4)	88.8(58.9)
Rmerge	0.069(0.136)	0.050(0.155)	0.052(0.135)	0.078(0.396)
Phasing				
Phasing power (iso/ano)	2.34/1.87	2.21/1.67	2.26/2.07	
RCullis		0.555		
Overall FOM		0.744		
Solvent flattenig				
Rfree				0.408

Experimental electron density map



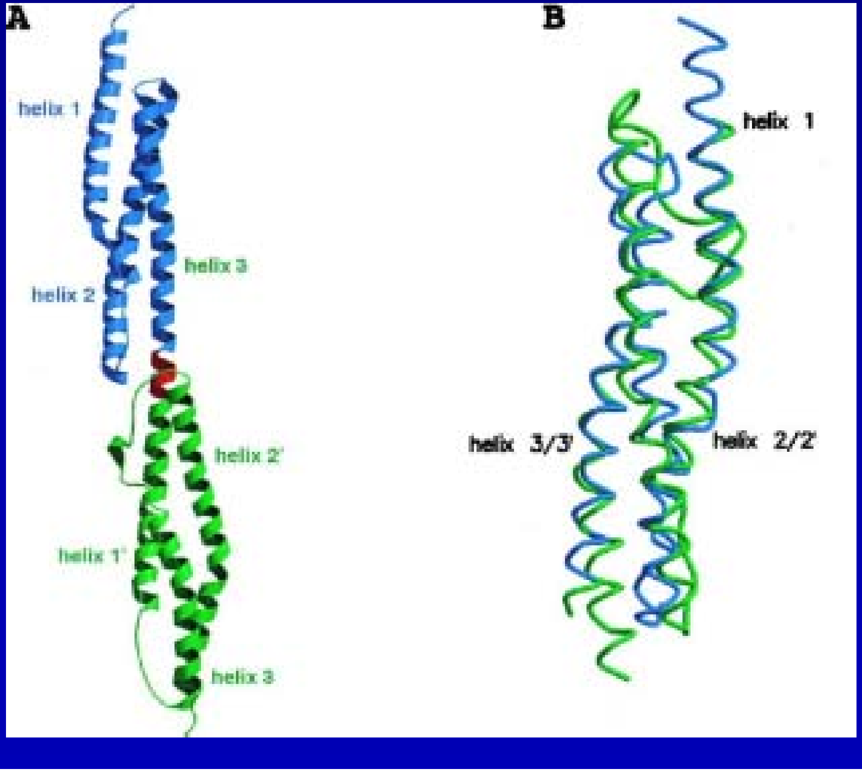
Refinement

Refinement statistics (CNS)

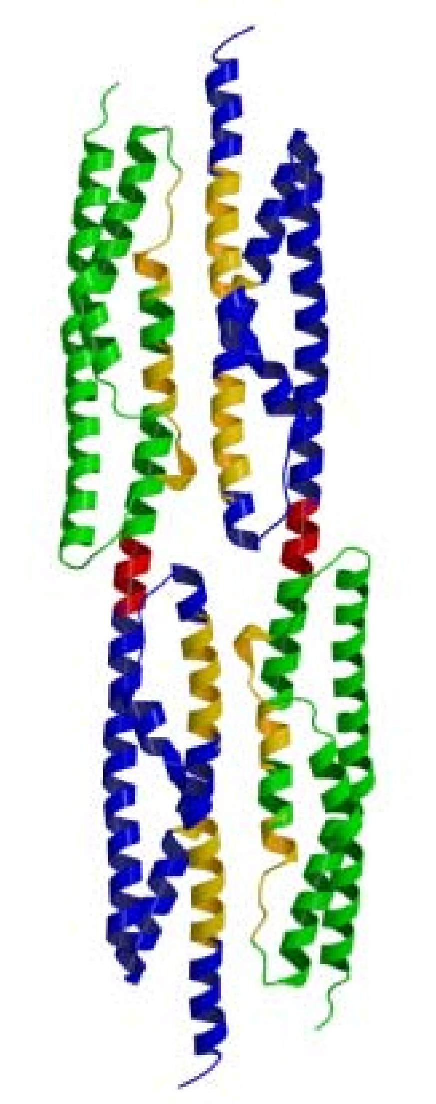
Resolution (A)	2.5
R-factor [1]	0.250
R- free	0.347
No. of protein atoms	2035
No. of water molecules	123
R.m.s deviation from ideal bond lenght (A)	0.015
R.m.s deviation from ideal bond angles (°)	1.76

[1] R-factor = $\sum|F_0-F_c| / \sum F_0$

Alpha-actinin central repeats

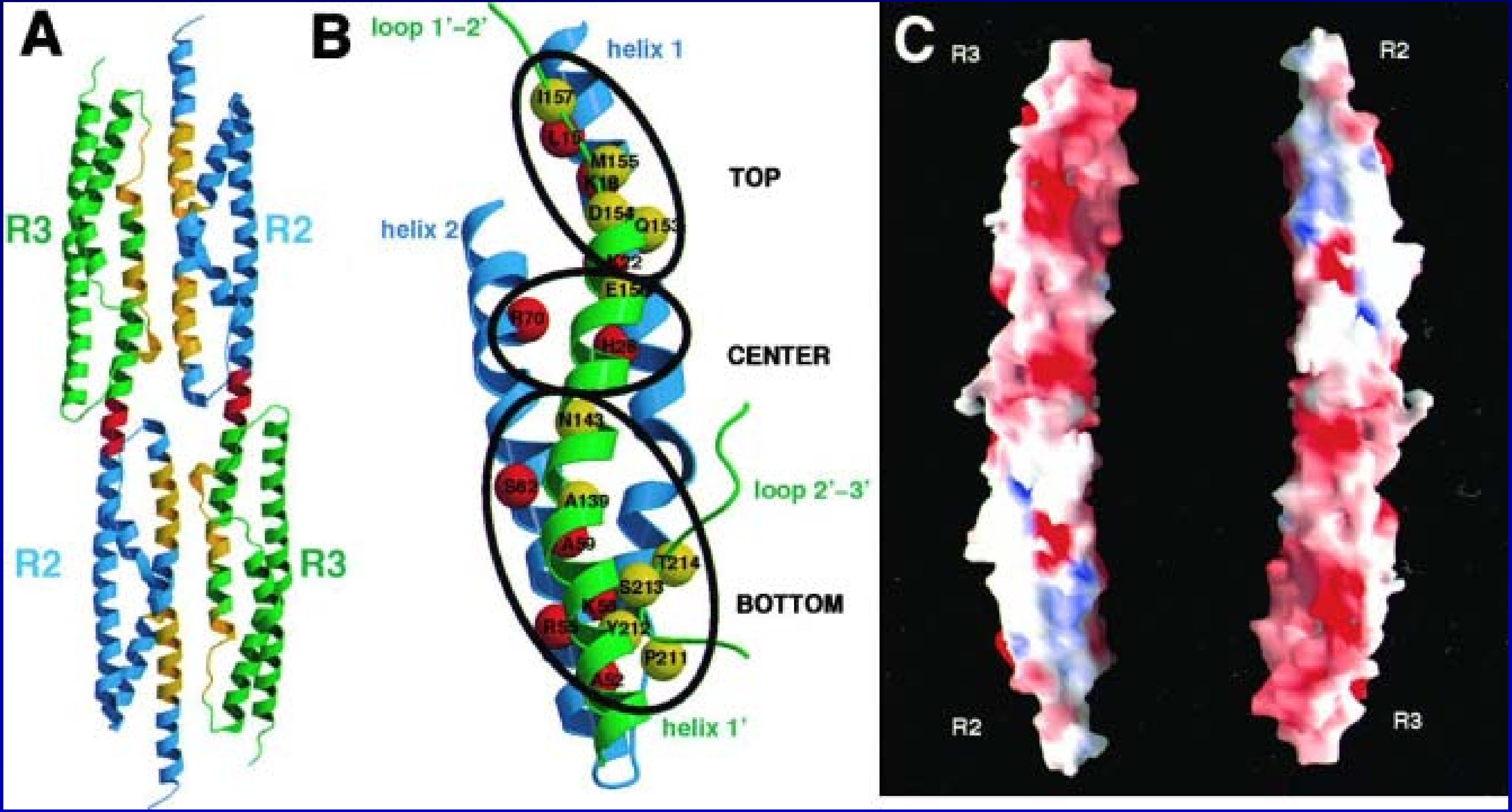


Alpha-actinin central repeats

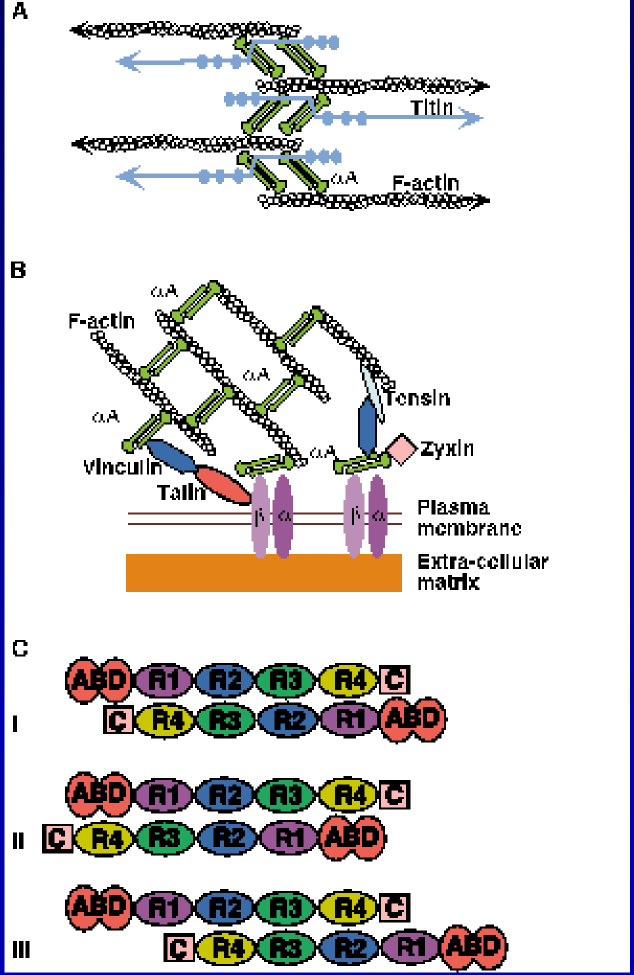


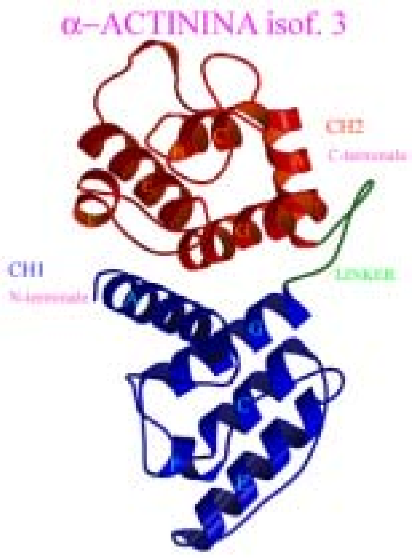
- Construct is dimer in crystal and in solution

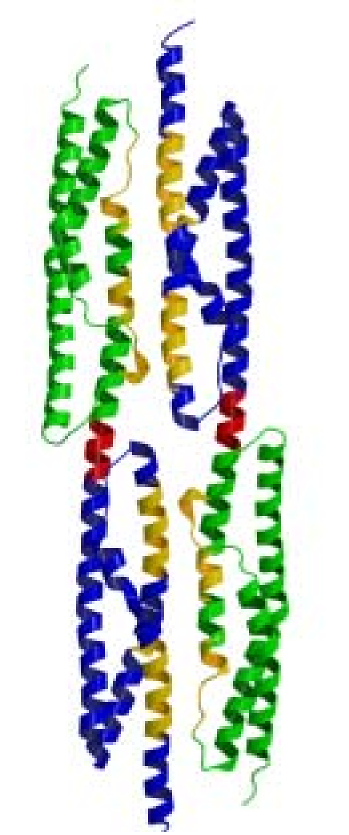
Alpha-actinin central repeats

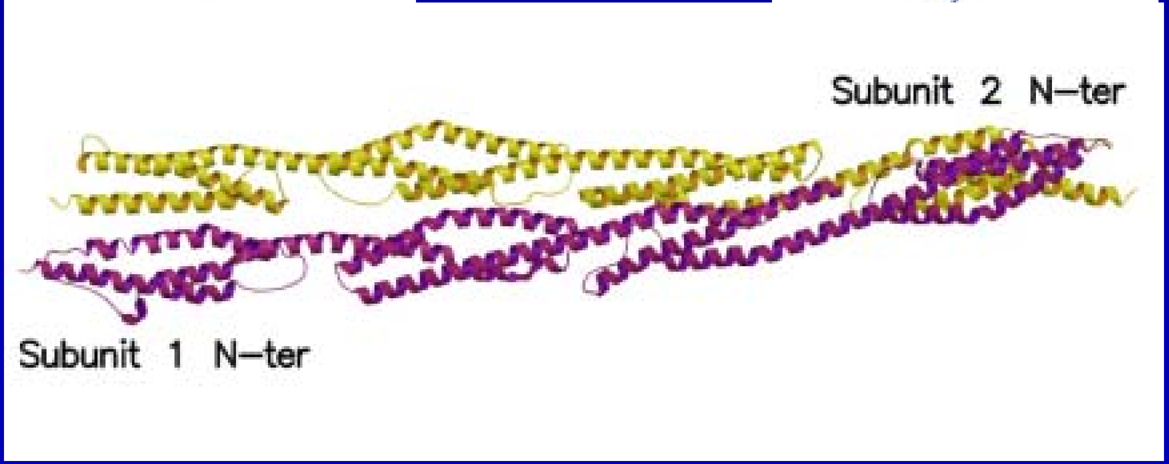


Alpha-actinin









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