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SMR/1499 - 2

INTERNATIONAL WORKSHOP ON PROTEOMICS: PROTEIN STRUCTURE, FUNCTION AND INTERACTIONS

(5 - 16 May 2003)

"Muscles, a complex network essential for life"

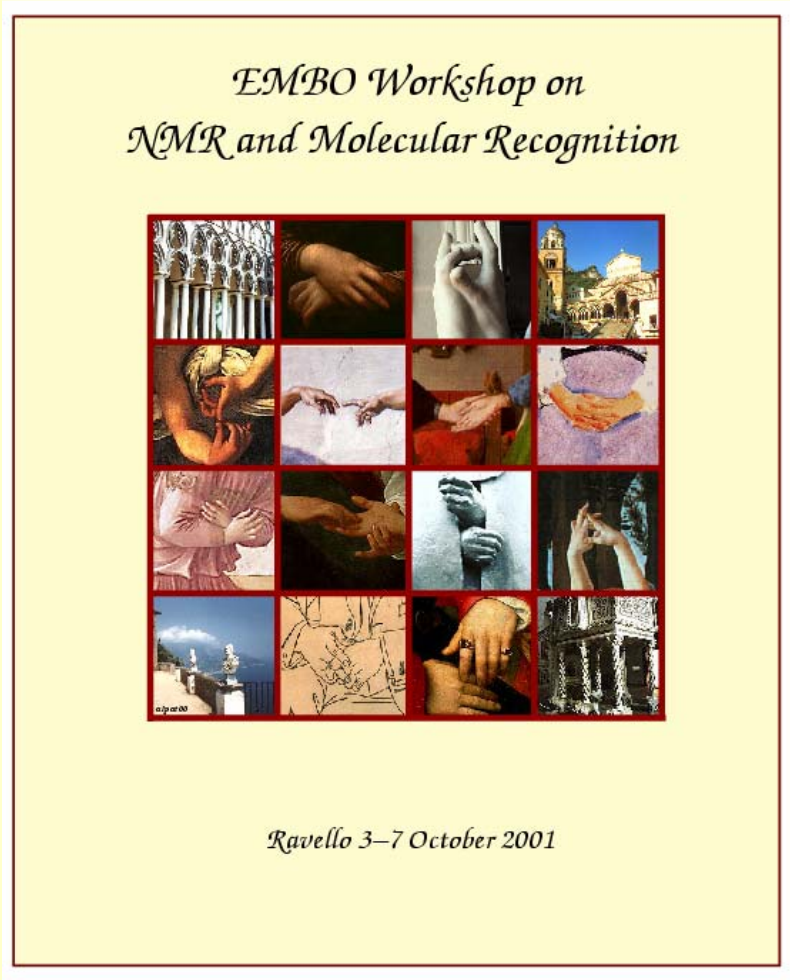
presented by:

A. Pastore
National Institute for Medical Research, London
United Kingdom

Muscles, a complex protein network essential for life

Molecular recognition and EF-hands

Trieste 2003



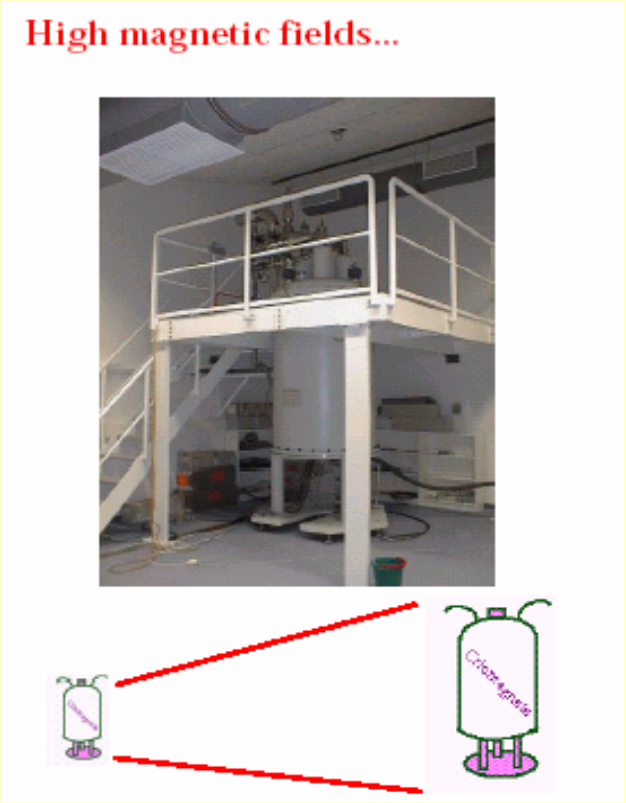
Nuclear Magnetic Resonance

Studies in solution

No need for crystals

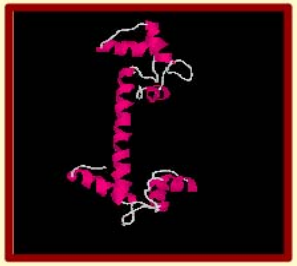
Dynamic behaviour

Excellent for weak complexes



From hands…

Interactions...

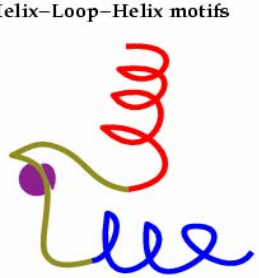


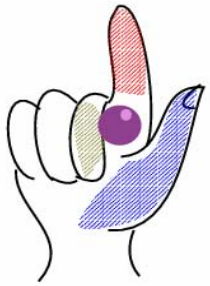
...EF–hands

A calcium-binding motif

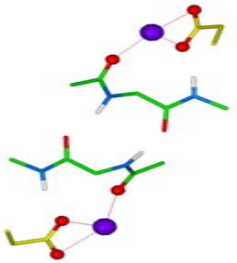
EF–hands

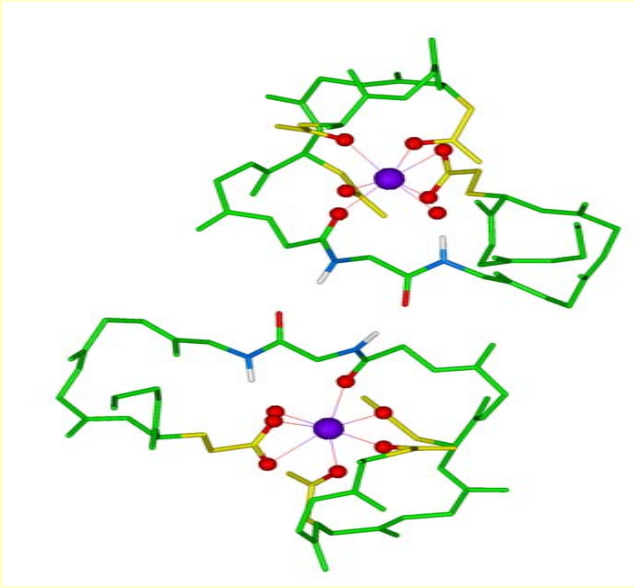
Helix–Loop–Helix motifs



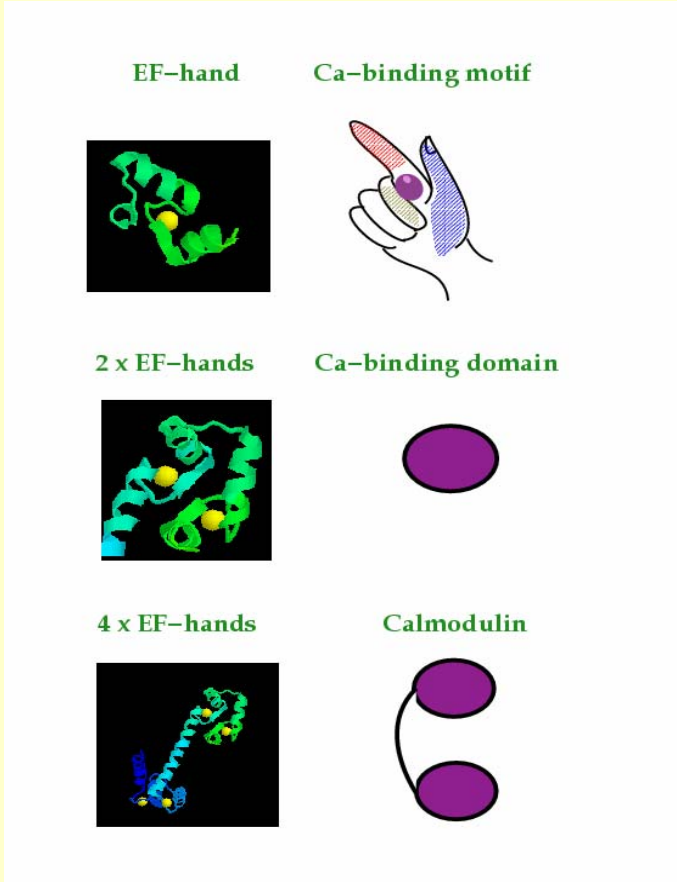


D-x-[DNS]-{#}-[DENSTG]-[DNQGHRK]-{GP}-[LIVMC]-
[DENQSTAGC]-x(2)-[DE]-[#]

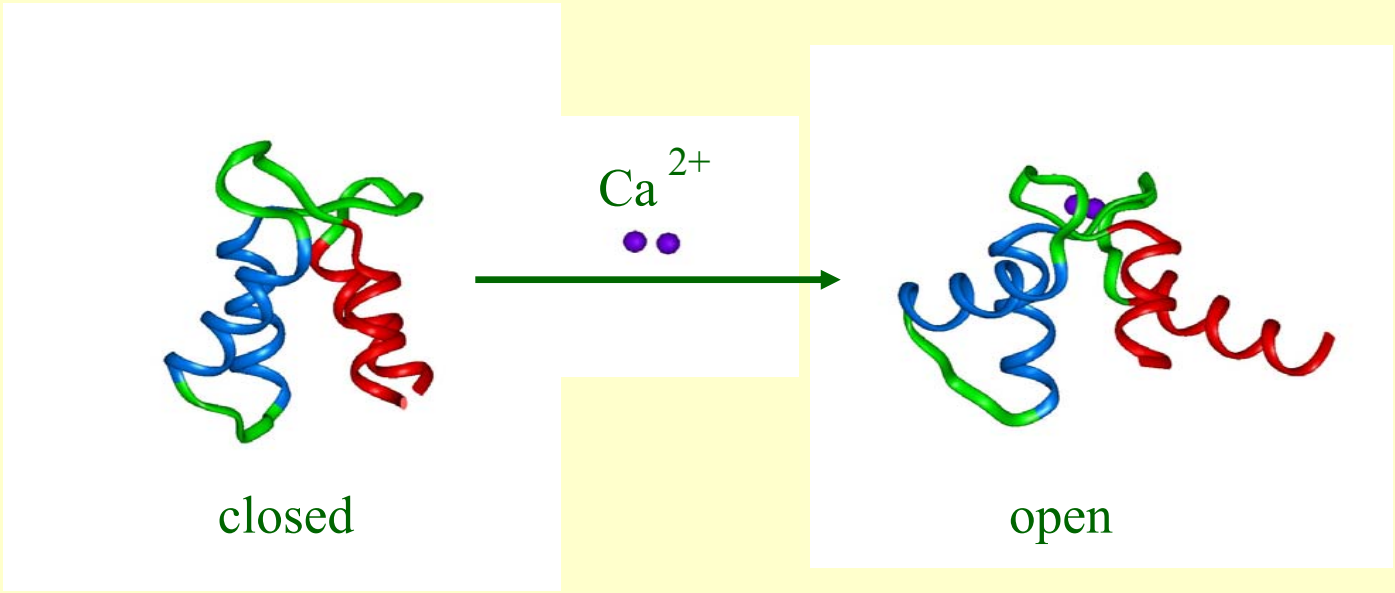




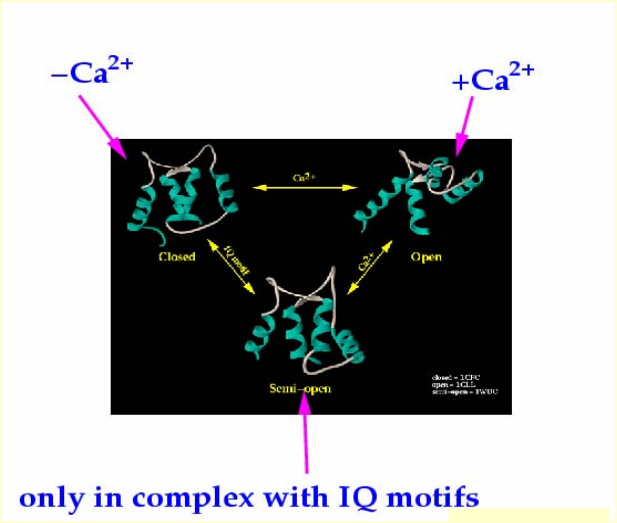
Hierarchical assembly



In the presence of Calcium…



Semi-open conformations

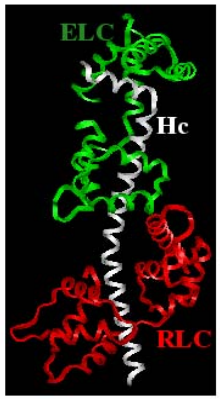


The semi−open conformation

has been observed

only in complex of EF−hands

with IQ motifs



IQxxIRGxxxRxxFY

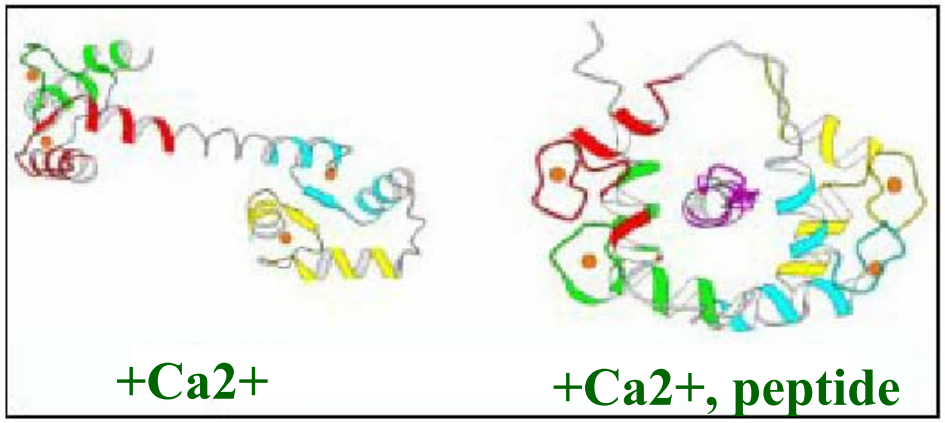
scallop myosin

ELC and RLC are
calcium insensitive

A three component system

In the presence of peptides calmodulin changes conformation, exposes its hydrophobic groups and wraps around the ligand

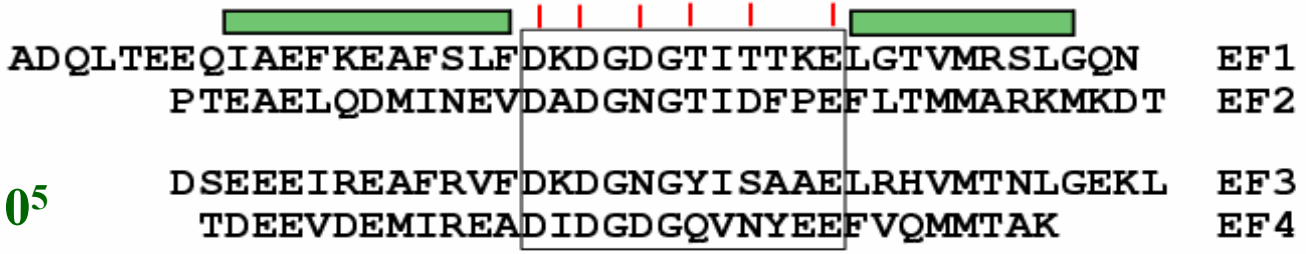
Free



Bound

Despite the sequence similarity…

N $6.8\ 10^4$



C $6.6\ 10^5$

Calcium affinities are different!

Questions 1

What determines a different calcium affinity?

Can we predict the affinities?

Protein plasticity

Amino-acid sequences of some CaM-binding domains and dissociation constants.

	Kd*
skMLCK ..... KRRWKKNFIAVSAANRFKKISSSGAL	1 nM
smMLCK ..... RRKWQKTGHAVRAIGRLSSM	1 nM
CaM-Kinase II .....LKKFNARRKLKGAILTTMLATRNFS	1 nM
Mellitin ..... GIGAVLKVLTTGLPALISWIKRKRQQ	3.5 nM
Titin P7 ..... IRTLKHRRYYHTLIKKDLNMVVSAARISCGGAIRSQKG	4.2 nM
Titin P10 ..... STKVIRTLKHRRYYHTLIKKD	11 nM
WFF Pep. ..... KRRWKKNFIAVSAANRFK	<0.2 nM
FFW Pep. ..... KRRFKKNFIAVSAANRWK	1.6 nM
Model Pep. ..... LKWKKLLKLLKKLLKKLLKLG	0.2 nM
PFkinase ..... FMNNWEVYKLLAHIRPPAPKSGSYTV	11.4 nM
Spectrin ..... KTASPWKSARLMVHTVATFNSIKE	<100 nM
Adenylate cyclase..... IDLLWKIAEAGARSAVG	580 nM
Neuromodulin ..... KAHKAAVKAVVASSRLGS	400 nM
Ca2+ pump .....LRRGQILWFRGLNRIQTQIKVVNFSSS	0.2 nM
Ca2+ pump-C28W.....LRRGQILWFRGLNRIQTQIKVVNFSSS	<1 nM
Ca2+ pump-C28A.....LRRGQILAFRGLNRIQTQIKVVNFSSS	18 nM
Ca2+ pump-C28Y.....LRRGQILYFRGLNRIQTQIKVVNFSSS	15 nM
Calcineurin ..... KEVIRNKIRAIGKMARVFSVLR	0.2 nM
CaM-Kinase ..... ARRKLKAAVKAVVASSRLG	3-10 nM
Phosphorylase b ..... GKGKVICLTVLASVRIYYQYRRVKP	6.5 nM
Phosphorylase b ..... LRRLIDAYAFRIYGHWVLGQQQNR	20 nM

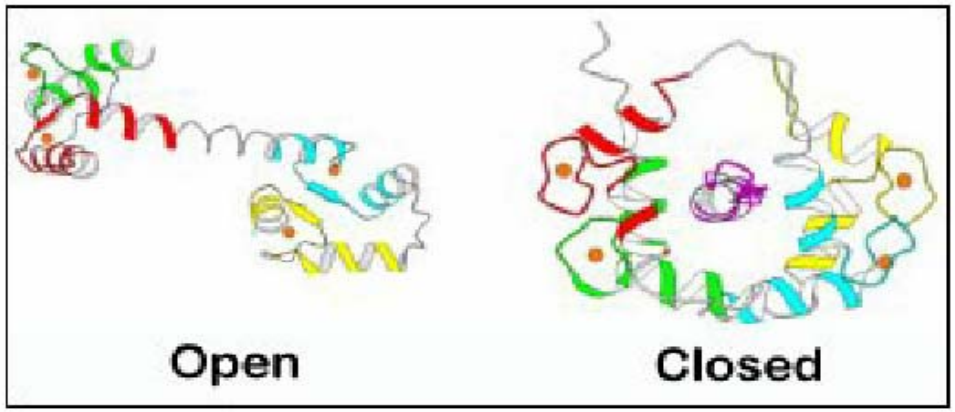
Which positions are important for the recognition?

```
Model Pep.      ...LKWKKLLKLLKKLLKKLLKLG    ....   0.2 nM
skMLCK    .....   KRRWKKNFIAVSAANRFKKISSSGAL    ...  1 nM
smMLCK    ......  RRKWQKTGHAVRAIGRLSSM    .........  1 nM
CaM-Kinase II  ..LKKFNARRKLKGAILTTMLATRNFS          1 nM
Mellitin      QQKRKIWSILAPLGTTLVKLVAGIG           3.5 nM
Mellitin       GIGAVLKVLTTGLPALISWIKRKRQQ         3.5 nM
```

Peptide recognition involves two anchoring points

In the presence of peptides calmodulin changes conformation, exposes its hydrophobic groups and wraps around the ligand

Free



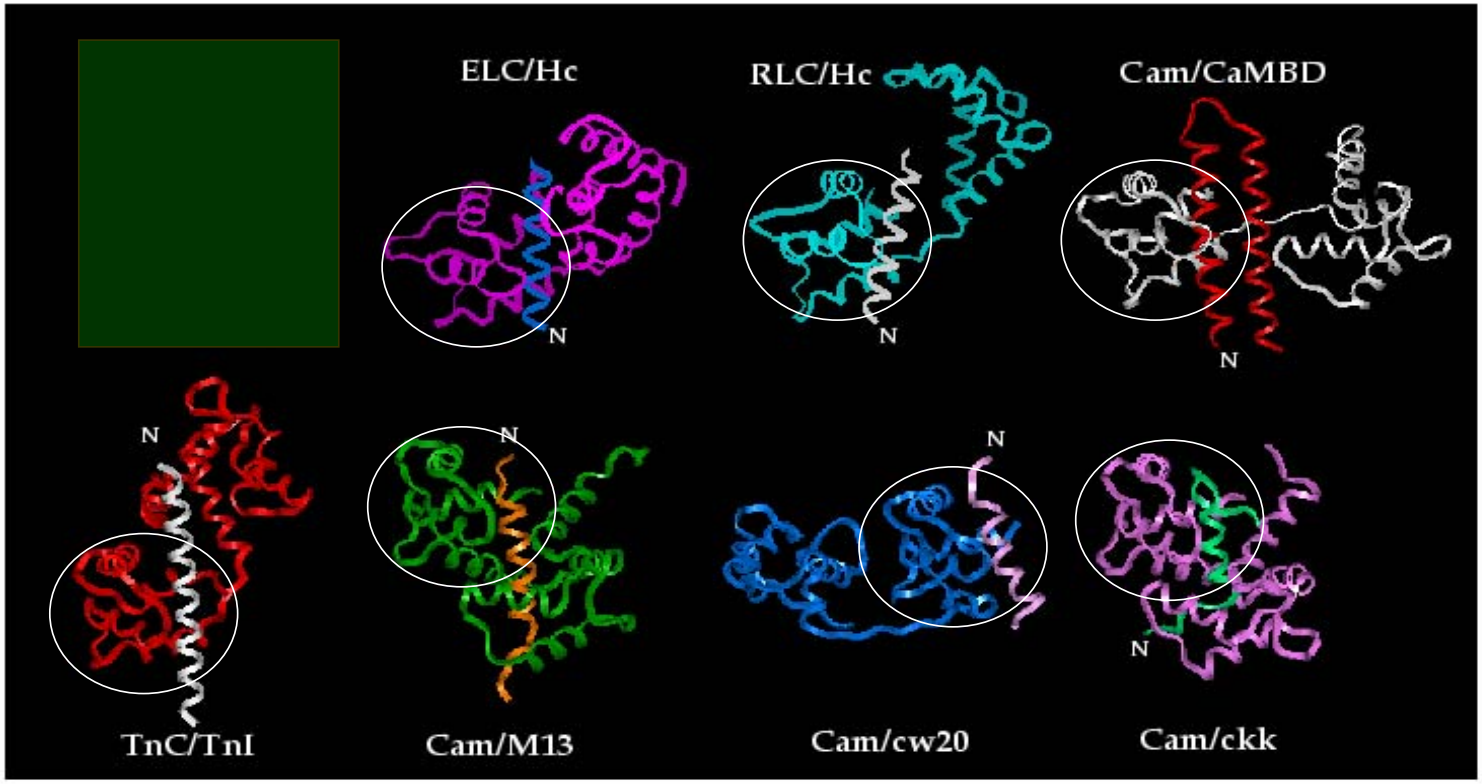
Bound

Questions 2

Can we predict whether a sequence will bind or not from first principles?

Can we predict the affinities?

Comparison of representative EF–hand complexes



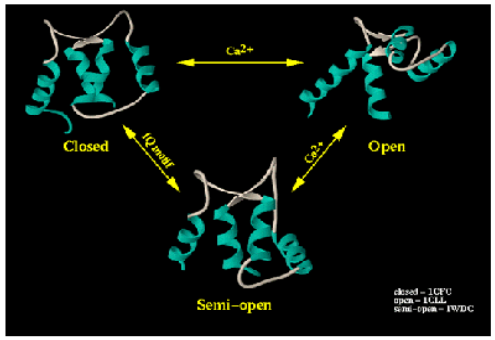
Questions 3

Why all complexes involve the C-terminal domain?

What determines the peptide direction?

Close, open and semi-open EF-hands

EF–hand domains can adopt a large repertoire of conformations (closed, open and semi–open).

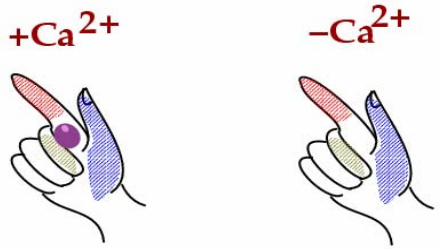


EF–hands are mostly involved in protein/protein interactions

Question 4

What determines an open, semi-open or close conformation?

Divergent EF-hands…



calcium insensitive EF–hands

How do they interact?

How are they regulated?

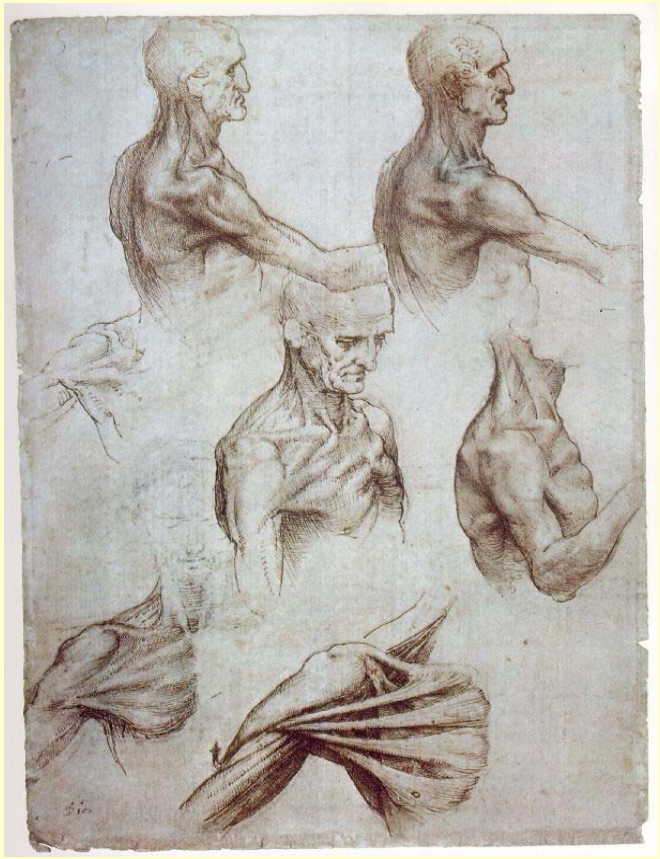
Some EF–hands have diverged through evolution

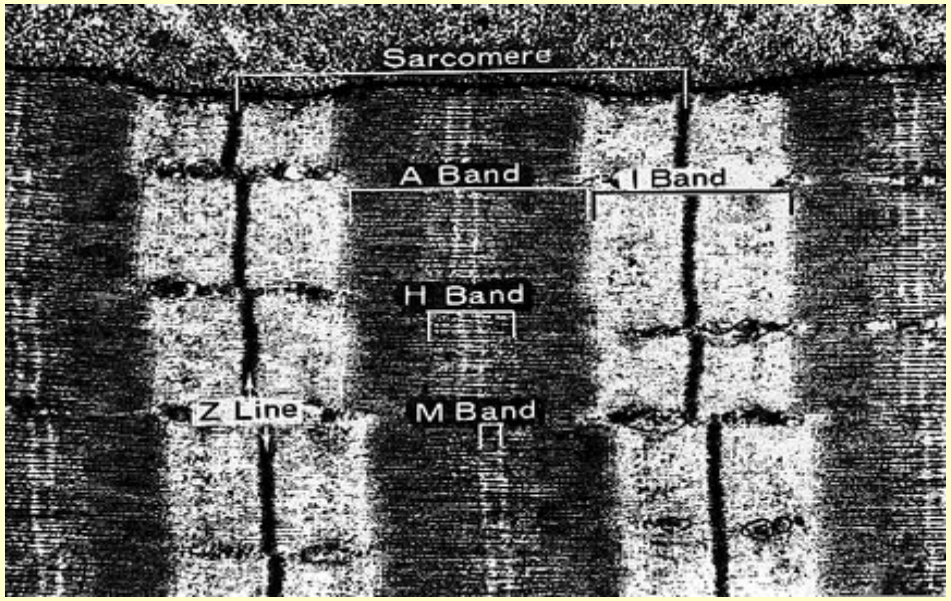
```
calmodulin   --ADQLTEEQIAEFKEAFSLFD--KDGDGTITTKELGTVMRSLG-------QNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKM
tnC          EARSYLSEEMIAEFKAAFDMFD--ADGGGDISVKELGTVMRMLG-------QTPTKEELDAIIEEVDEDGSGTIDFEEFLVMMVRQM
scalb-rlc    GVLTKLPQKQIQEMKEAFSMID--VDRDGFVSKEDIKAISEQLG-------RAPDDKELTAMLKEA-PGPLNFTMFLSIFSDKLSGT
scalc-elc    ---PKLSQDEIDDLKDVFELFDFWDGRDGAVDAFKLGDVCRCLG-------INPRNEDVFAVGGTH-KMGEKSLPFEEFLPAYEGLM
act-EF1234   RDAKGITQEQMNEFRASFNHFD--RRKNGLMDHEDFRACLISMG-------YDLGEAEFARIMTLVDPNGQGTVTFQSFIDFMTREP
spcn_chick   RNTTGVTEEALKEFSMMFKHFD--KDKSGRLNHQEFKSCLRSLGYDLPMVEEGEPDPEFESILDTVDPNRDGHVSLQEYMAFMISRE

calmodulin     ---KDTDSEEEIREAFRVFD--KDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGD--------GQVNYEEFVQMMTAK---
tnC            KEDAKGKSEEELAECFRIFD--RNADGYIDAEELAEIFRASGEHVTDEEIESLMKDGDKNND--------GRIDFDEFLKMMEGVQ--
scalb-rlc      D------SEETIRNAFAMFD--EQETKKLNIEYIKDLLENMGDNFNKDEMRMTFKEAPVEGG---------KFDYVKFTAMIKGSGEE
scalc-elc      D--CEQGTFADYMEAFKTFD--REGQGFISGAELRHVLTALGERLSDEDVDEIIKLTDLQED------LEGNVKYEDFVKKVMAGPYP
act-EF1234     ---ADTDTAEQVIASFRILA--SDKP-YILAEELRREL---------PPDQAQYCIKRMPAYSG-PGSVP-GALDYTSFSSALYGESDL
spcn_chic      T--ENVKSSEEIESAFRALS--SERKPYVTKEELYQNL---------TREQADYCISHMKPYMDGKGRELPSAYDYIEFTRSLFVN---
```

DKDGNGYISAAE
ASDKP–YILAEE

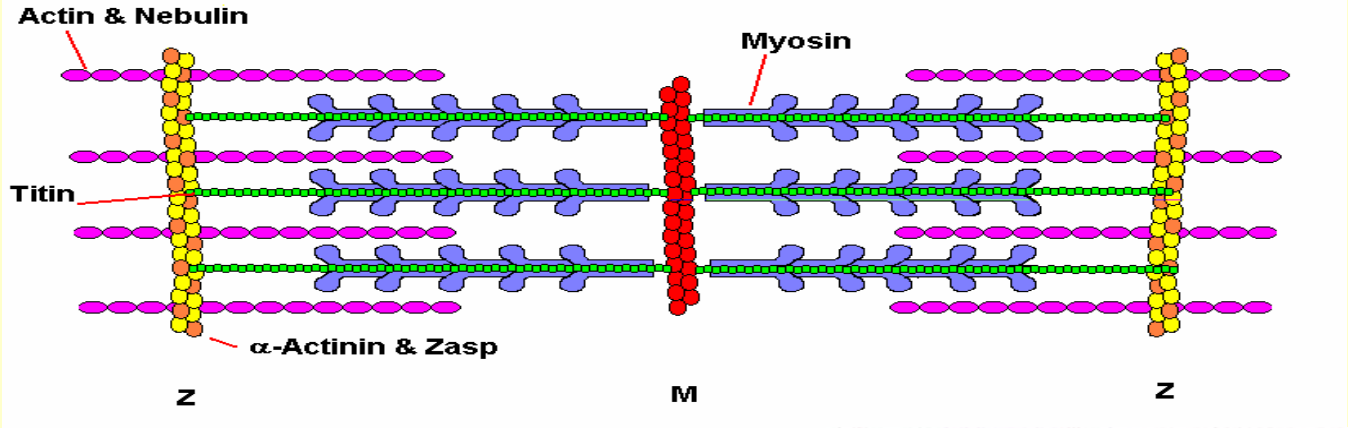
They have lost calcium–binding activity

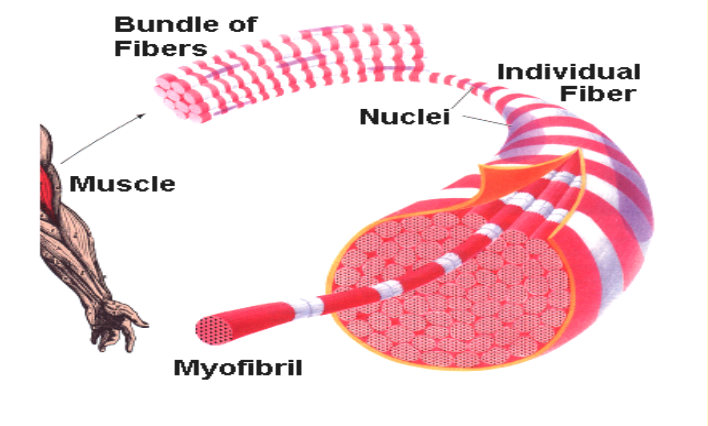
Muscles

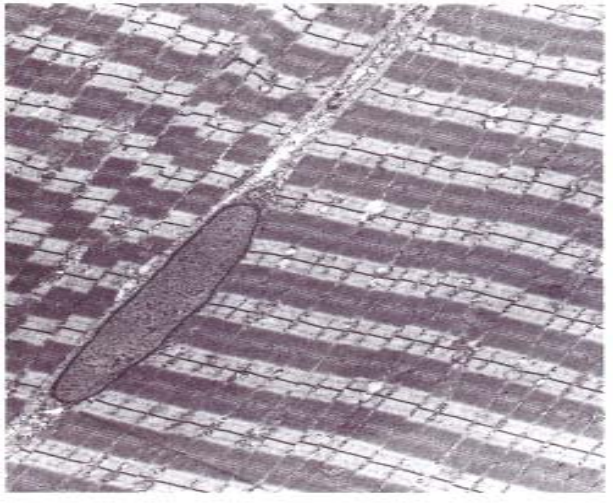




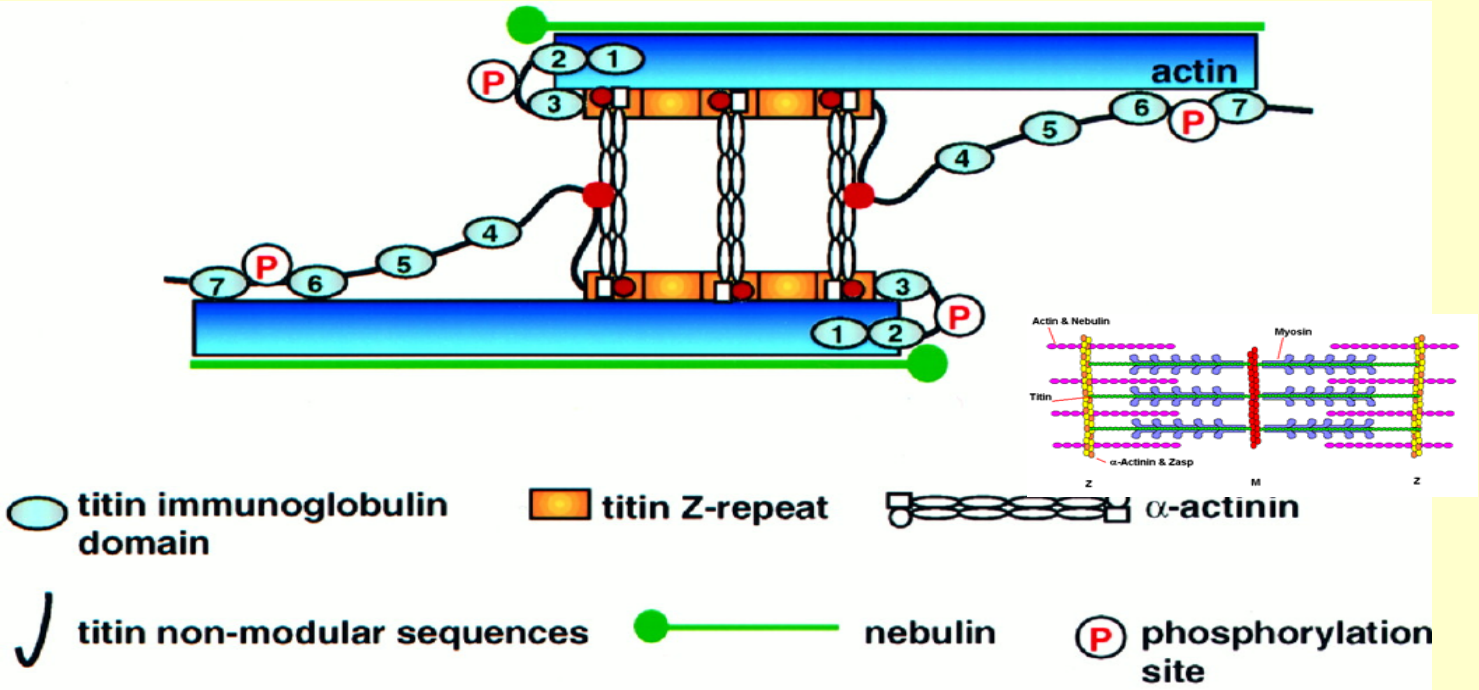
The Sarcomere







Interaction between Titin and α-actinin



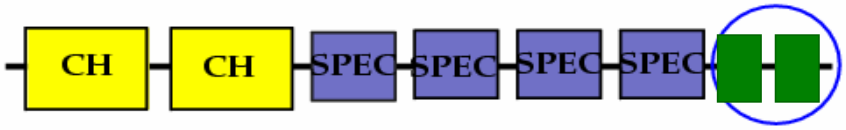
Young et al, EMBO (1998), 17, 1614-1624

Actin and myosin are the main actors in muscle contraction

Many other proteins are involved in muscle assembly

EF-hands in muscles…

α–Actinin

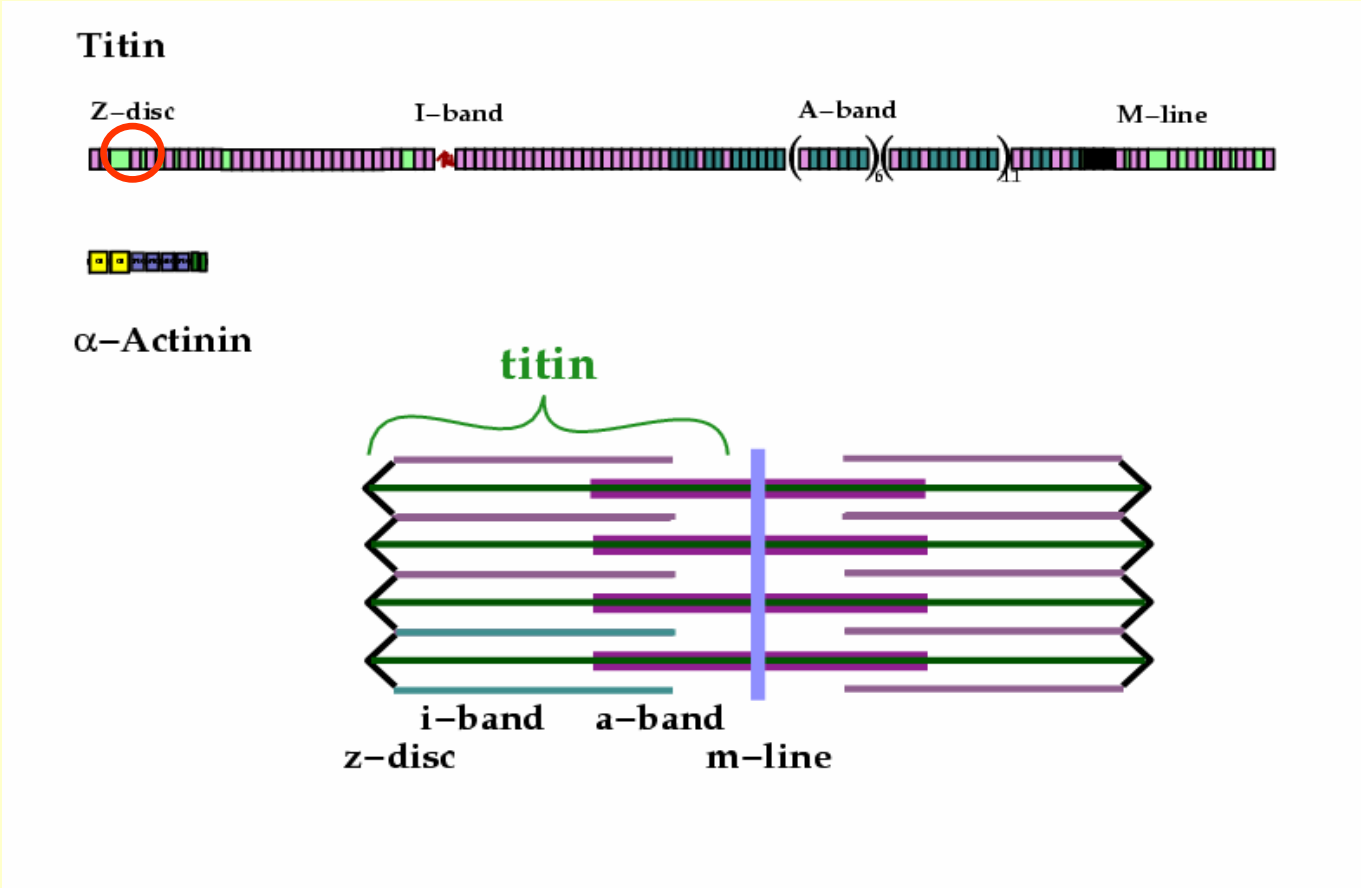


some degree of homology with calmodulin

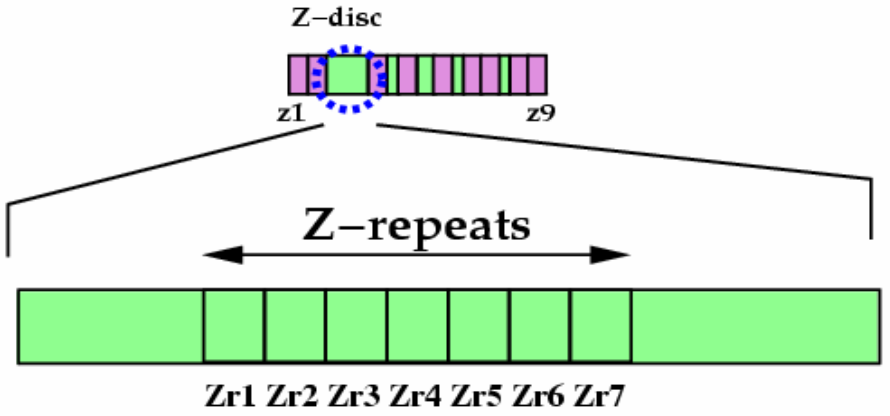
```
calmodulin     ADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKM
act-EF1234     RDAKGITQEQMNEFRASFNHFDRRKNGLMDHEDFRACLISMGYDLGEAEFARIMTLVDPNGQGTVTFQSFIDFMTREP

calmodulin     KDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREA-DIDGDGQ----VNYEEFVQMMTAK
act-EF1234     ADTDTAEQVIASFRILASD-KPYILAEELRRE-------LPPEQAQYCIKRMPQYTGPGSVPGALDYTSFSSALYGESDL
```

…but calcium insensitive

A titanic protein…

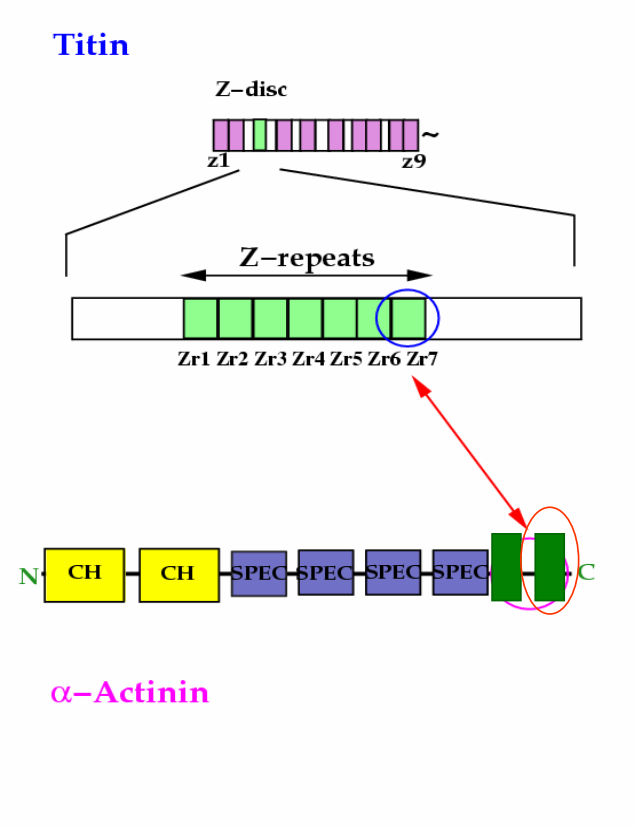


Z-repeats




```
          Zr1 -------KQDADKSA-AVATVVAAVDMARVREPVISA--VEQTAQRTTTTAVHIQP
          Zr2 -------QVRKEAEKTAVTKVVVAADKAKEQELKSRTK-EIITTKQEQMHVTHE--
          Zr3 -------QIRKETEKTPVPKVVISAAKAKEQETT--RIS-EEITKKQKQ--VTQEA-
          Zr4 --------IMKETRKTVVPKVIVATPKVKEQLLVSRGR-EGITTKREQVQITQEK-
          Zr5 --------IRQETEITAASMVVVATAKSTKLETVPGAQ-EETTTQQDQMHLSYEK-
          Zr6 --------MRKEAEKTALSTIAVATAKAKEQETILRTR-ETMATRQEQIQVTHG--
          Zr7 -------KVDVGKKAEAVATVVAAVDQARVREPREPGHLEESYAQQTTLEYGYKER
     TnI_1-47_ GLEEKRNRAITARRQHLKSVMLQIAATELEKEEGRREA-EKQNYLAEH--------
         ruler 1.......10........20........30........40........50......
```

The minimal units necessary for interaction



Z-repeats do not contain IQ motifs

…but they have a hydrophobic region

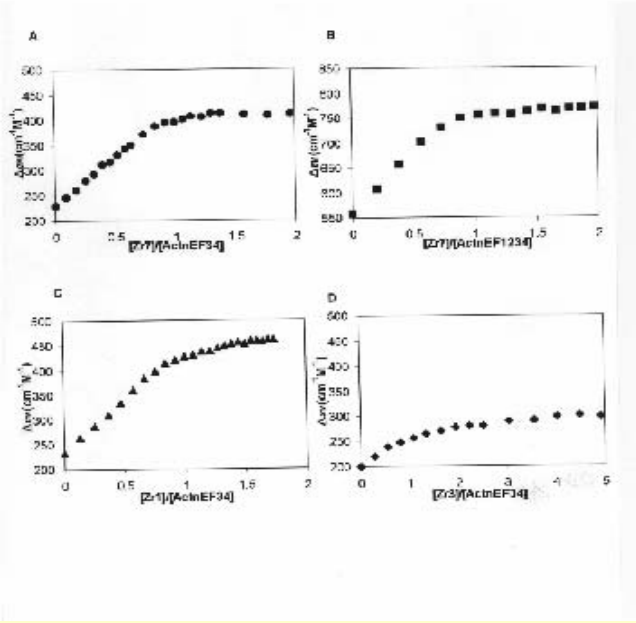
Questions 5

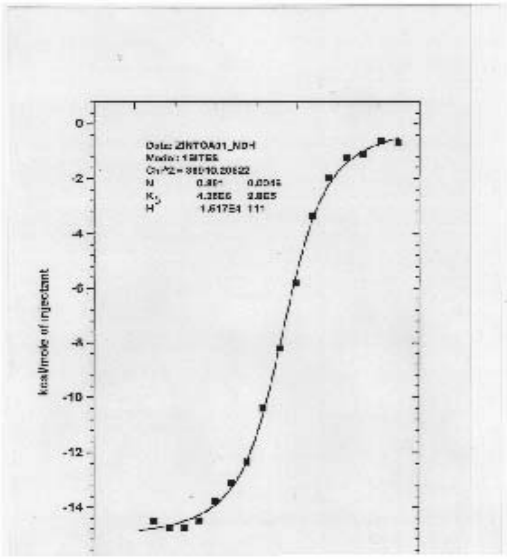
How does a calcium insensitive EF-hand interact with a non-IQ motif?

Which conformation adopt the EF-hand? And the peptide?

Affinities and stoichiometries were compared by CD and calorimetry studies

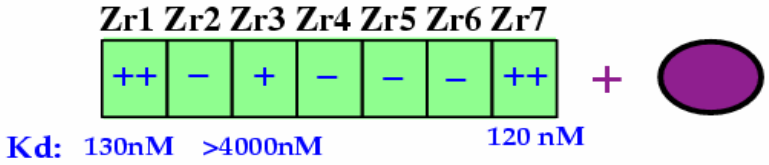
Zr1 Zr2 Zr3 Zr4 Zr5 Zr6 Zr7

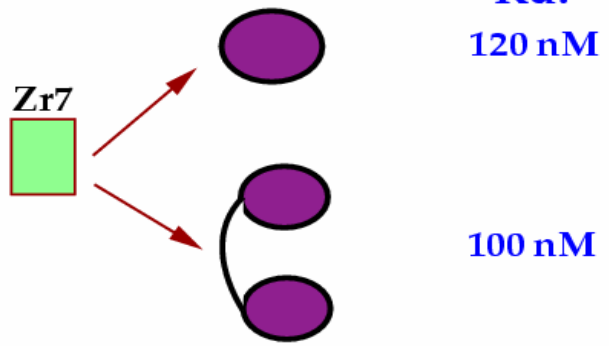




Summary of the binding affinity data:

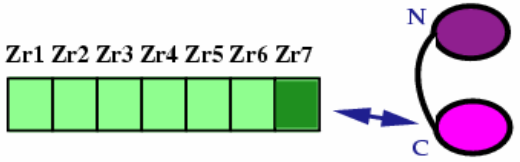
1:1 stoichiometries for all complexes





Act–EF34 seems to contain most of the high affinity sites

We solved the structure of Act–EF34/Zr7



Structure of Zr7 in the complex

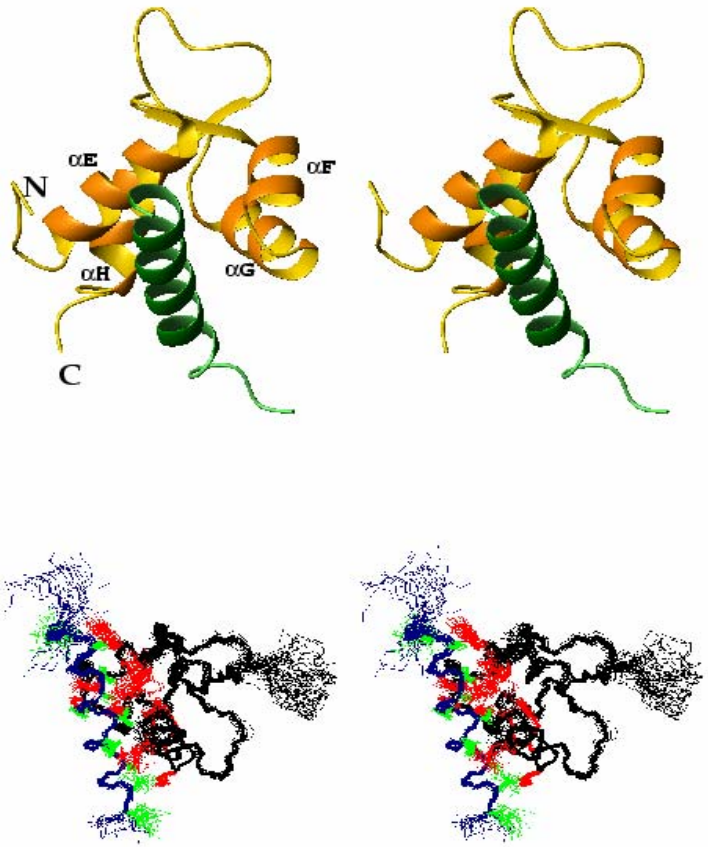
Zr7 is unstructured when isolated

~~~~~~~~~~~~~~~~~
KVGVGKKAEAVATVVAAVDQARVREPREPGHLEESYAQQTTLEYGYKER
~~~~~~~~~~~~~~~~~

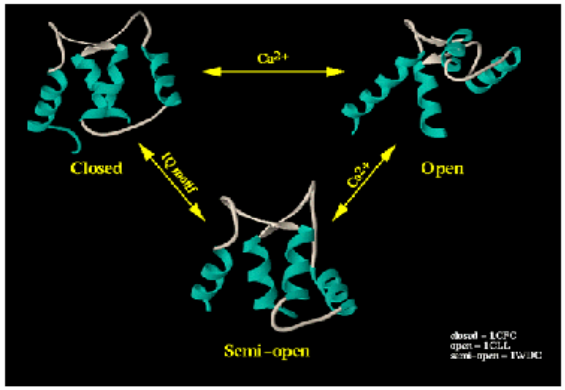
In the complex, Zr7 is helical from 8–25.

It remains unstructured from 26 to the end.

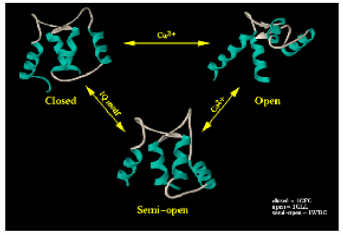
The structure of Act–EF34/Zr7

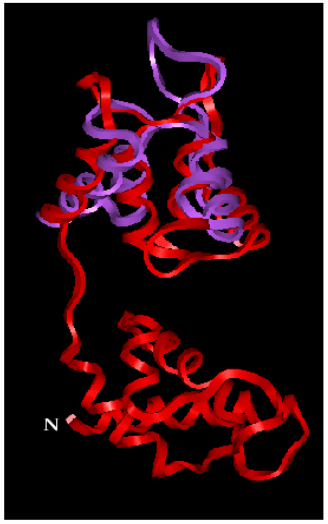


Close, semi–open or open conformation?



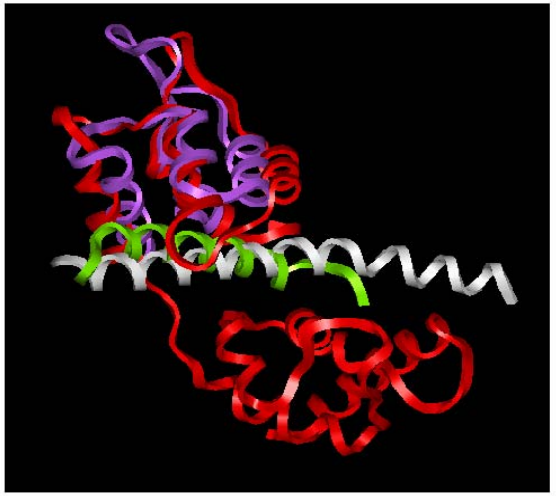
Semi-open



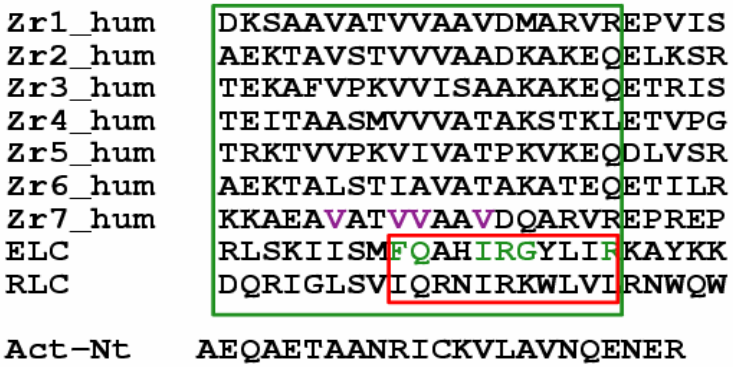


Comparison with scallop myosin RLC

scallop myosin and actinin

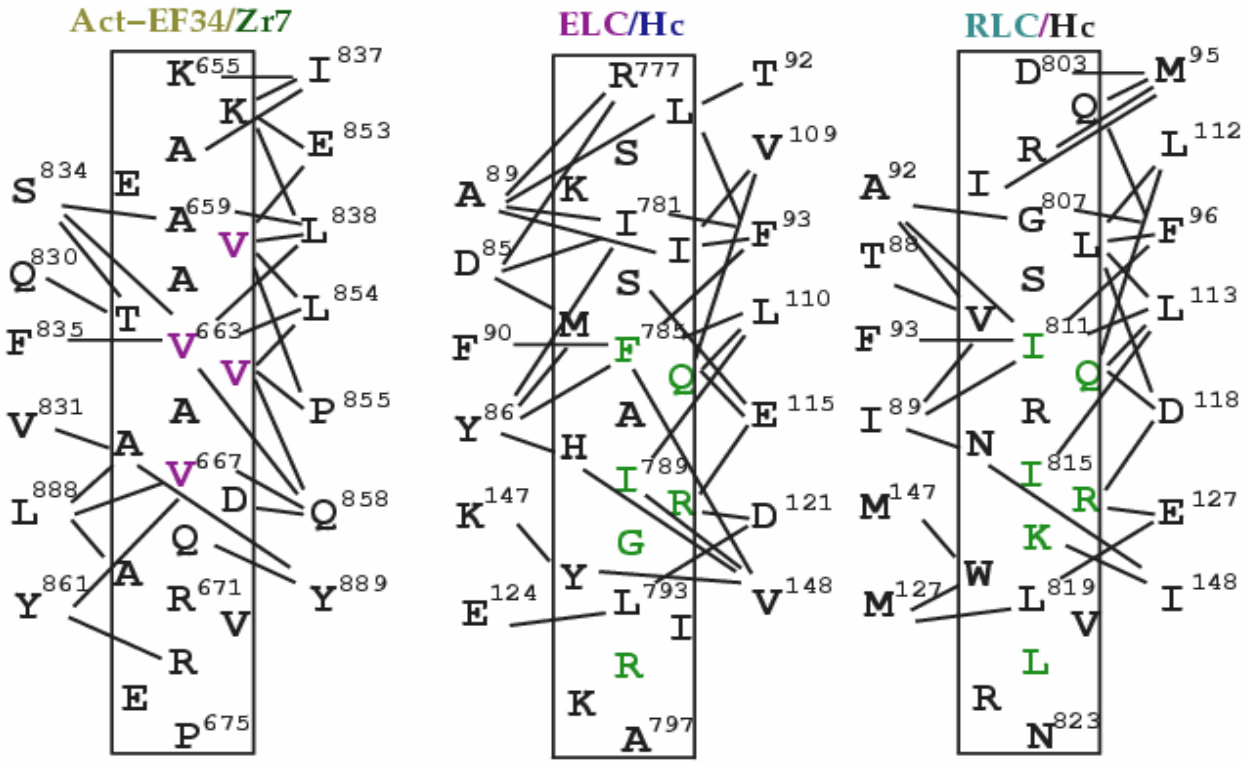


No IQ motif in Z–repeats

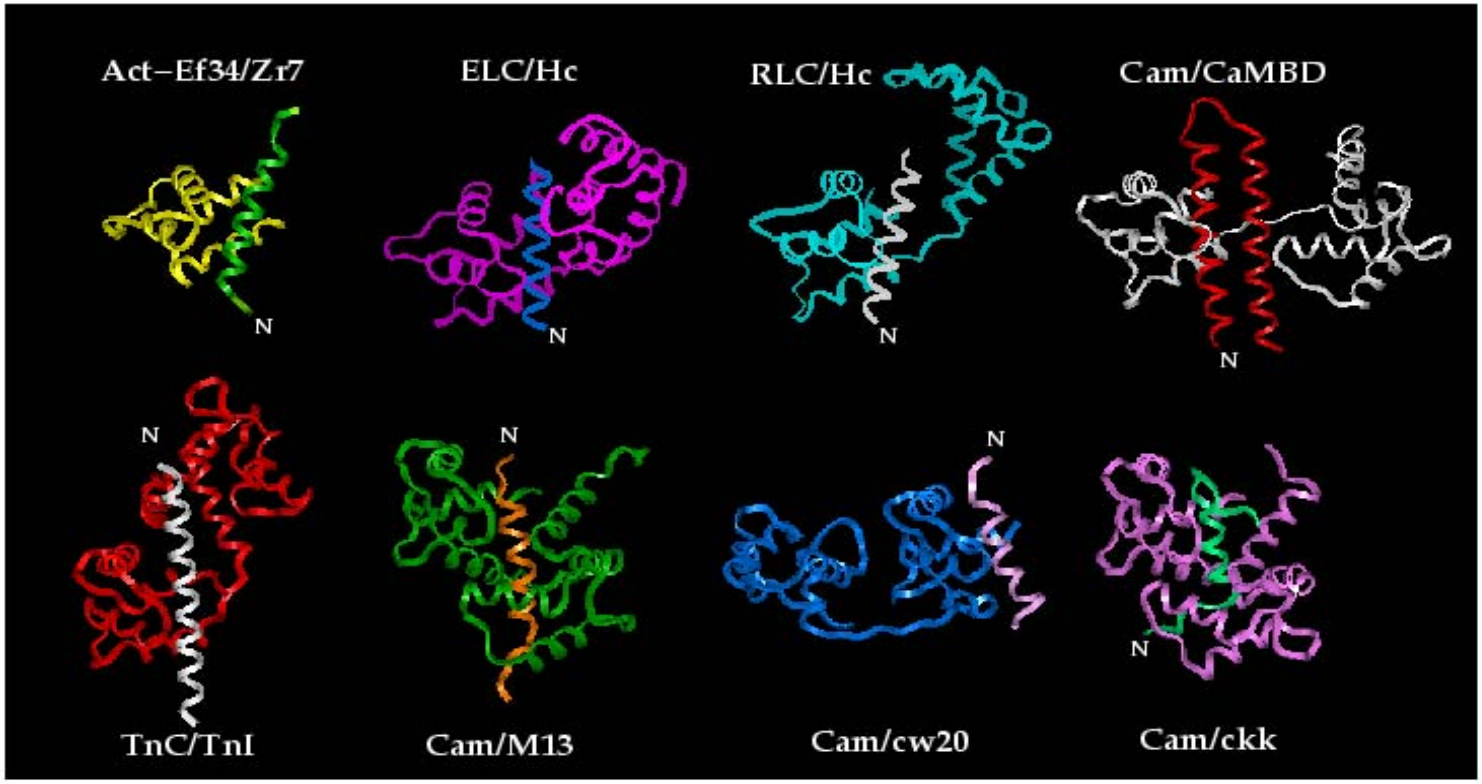


The IQ motif is replaced by 1–4–5–8 motif

Conservation of the contacts

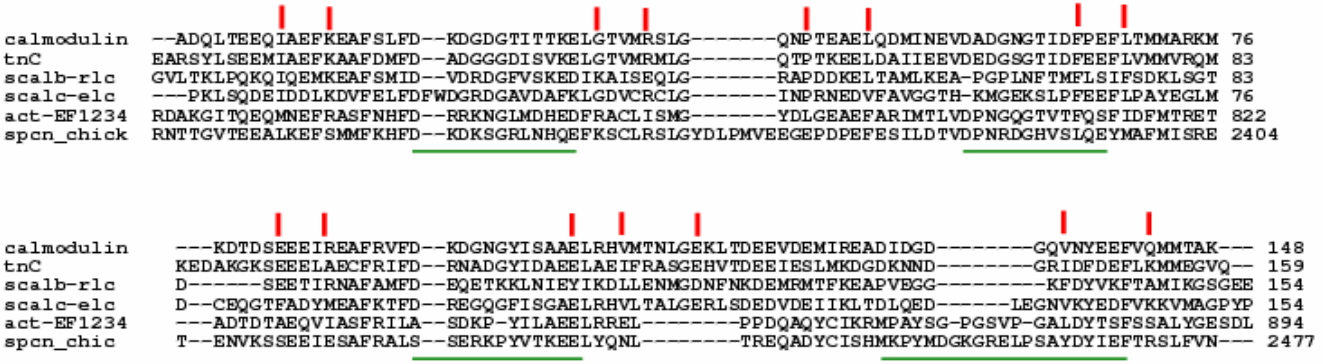


Comparison of representative EF–hand complexes



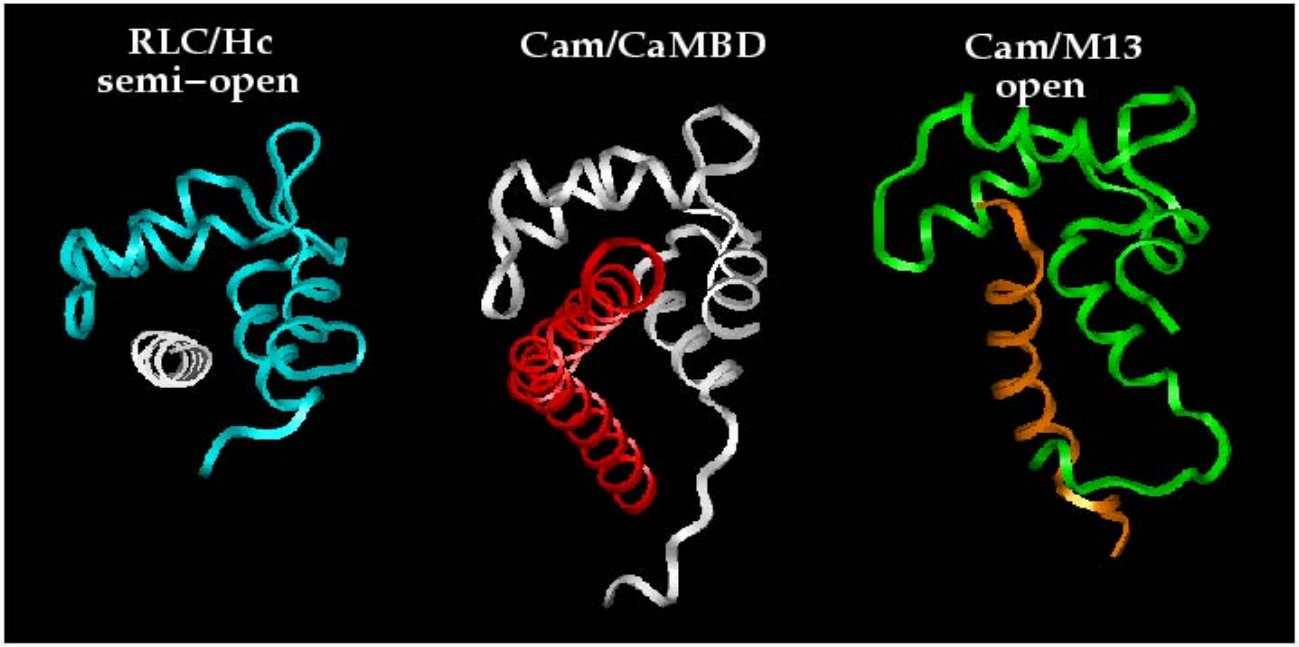
What is the secret of semi-open?

A semi–open conformation does not seem to be sequence related



A state induced by the target recognition?

Semi-open conformations in Ca2+-independent binding



Conclusions

– the C–terminal EF–hand domain of actinin forms stable complexes with some but not all titin Z–repeats

– the Act–EF34/Zr7 complex provides a paradigm for calcium independent target recognition by EF–hands

– calcium–independent binding induces a semi–open conformation

Despite their diversities, EF-hand recognition shares some basic features