SUPPORTING MATERIAL

Modeling the Assembly of the Multiple Domains of α-actinin-4 and its Role in Actin Crosslinking

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Running title: On the core structure of multi-domain ACTN4

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A5D7D1 ACTN4 Bos taurus	0	K A	E	T	AA	N	R	i	СК	d v	/ L	A	v	NC	E	N	EH	L	м	EI		E	R	LA	s	D	LI	E	w	I E	R	T
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O3B7N2 ACTN1 Bos tourus		K A	E	т	AA	N	R	i.	СК	d v	/ L	A	v	NC	E	N	EC		м	EI	D Y	E	ĸ	LA	s	D		E	w	L E	R	T
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Q5RCS6 ACTN4 Pongo abelii	т	р т	D	т.	AC	Q	v	I.	AS	F	ĸ	v	L	A G	D	к	NF	1	т	A	EE	L	R	RE	L	Р	PC	Q	А	EN	r c	I.
P57780 ACTN4 Mus musculus	т	р т	D	т.	AC	Q	v	I.	AS	F	ĸ	v	L	AG	D	к	NF	1	т	A	EE	L	R	RE	L	Р	PC	ο	А	EN	r c	1
090X00 ACTN4 Rattus norveaicus	т	р т	D	т	AC	Q	v	I.	AS	F	к	v	L	A G	D	к	NF	1	т	A	EE	L	R	RE	L	Р	PC		A	EN	r c	1
A5D7D1 ACTN4 Bos taurus	т	р т	D	т	AC	Q	v	I.	AS	F	к	v	L	AG	D	к	NF	1	т	A	EE	L	R	RE	L	Р	PC		А	E	r c	1
090734 ACTN4 Gallus gallus	т	р т	D	т	AC	0	v	Ì.	AS	F	ĸ	v	L	AG	D	к	NY	1	т	A	EE	L	R	RE	E L	Р	PE	0	A	EN	10	1
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02PFV7 ACTN1 Macaca fascicularis		рт	D	т	A	0	v	M	AS	F	K	1	L	AG	D	K	NY	1	T	VI	DF	L	R	RF	1	P	P	0	A	E	10	1
07TPR4 ACTN1 Mus musculus		рт	D	т	A	0	v	M	AS	F	K	i.	L	AG	D	K	NY	1	T	E		1	R	RF	1	P	P	0	A	E	(0	i
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03B7N2 ACTN1 Bos taurus		рт	D	T	A		v	M	A	F	K	i	1	AG	D	K	NY	1	Т	V		1	R	RF	- 1	P	P		A	E	10	1
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P18091 ACTN Drosophila melanoaaster	T	рт	D	T	AF	0	v	1	DS	F	R	i.	L	AA	D	ĸ	PY	i	L	PI		i.	R	RF	1	P	P	0	A	E	(0	i
Q7PKQ5 ACTN Anopheles gambiae		DT	D	T	AE	Q	v	1	DS	F	R	I	L	AS	D	R	PY	1	L	PI	DE	L	R	RE	L	P	PC	0	A	E	rc	1

Figure S1. Residues that are predicted to stabilize the ABD-neck-CaM2 ternary complex are highly conserved among non-muscle ACTNs. Multiple sequence alignment (MSA) of non-muscle ACTNs is shown for only the regions around the contact residues (marked above each alignment, with numbering based on human ACTN4 sequence) validated in Figs.6–8. ACTN sequences are taken from the manually annotated (reviewed) section of UniProt (1); accession IDs are shown on the far left. Residues in the alignment are colored as follows: blue (positively-charged), pink (negatively, charged), green (polar), and gray (non-polar). MSA was generated using the implementation (with default parameters) of Clustal Omega (2) at the UniProt website.

А		\downarrow \downarrow
	WT N-ter	1-MVDYHAANQSYQYGPSSAGNGAGGGGSMGDYMAQEDDWDRDLLLD-45
	DSC	сссссссссссссссссссссссссссснннннннннссс
	HNNC	ссссссссссссссссссссссссссссссссссссссс
	MLRC	CEEEEcccccEEccccccccccccCCCHHHHHHcccccccHHHHcc
	PHD	сссЕссссссссссссссссссснннннсЕссссссссс
	Predator	000000000000000000000000000000000000000
	Sec.Cons.	ccccccccccccccccccccccccccccccccccccccc
В		↓ ↓
	MUT N-ter	1-MVDEHAANQSYQYGPSSAGNGAGGGGSMGDEMAQEDDWDRDLLLD-45
	DSC	сссннннннсссссссссссссснннннннннннссс
	HNNC	CCHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	MLRC	сссссссссЕЕссссссссссссссссннннннсснннннсс
	PHD	ссссссссссссссссссссссссссснннннссссссс
	Predator	ссснннннссссссссссссссссссснннннссссссс
	Sec.Cons.	ссснососососососососососснининисснонинсос

Figure S2. Consensus secondary structure predictions show a disorder-to-order transition around **Y31 for the Y4E/Y31E double phosphomimic mutant.** Predictions of secondary structure were performed for (*A*) wild-type (WT) and (*B*) Y4E/Y31E mutant (MUT) sequences of the 45-residue ACTN4 N-terminal using the Network Protein Sequence Analysis (NPS@) web server (3). Consensus predictions are shown in bold for both sequences. The consensus prediction for the double mutant shows more consecutive residues adopting helical conformations around residue 31, consistent with the helicity plot shown in Fig. 10 *B*. Arrows point to the two mutation sites.

SUPPORTING REFERENCES

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