SUPPLEMENTARY FIGURE LEGENDS

Fig S1. Structural details of the ternary complex and comparison with the LIM3^{TES}-EVH1^{Mena} binary complex.

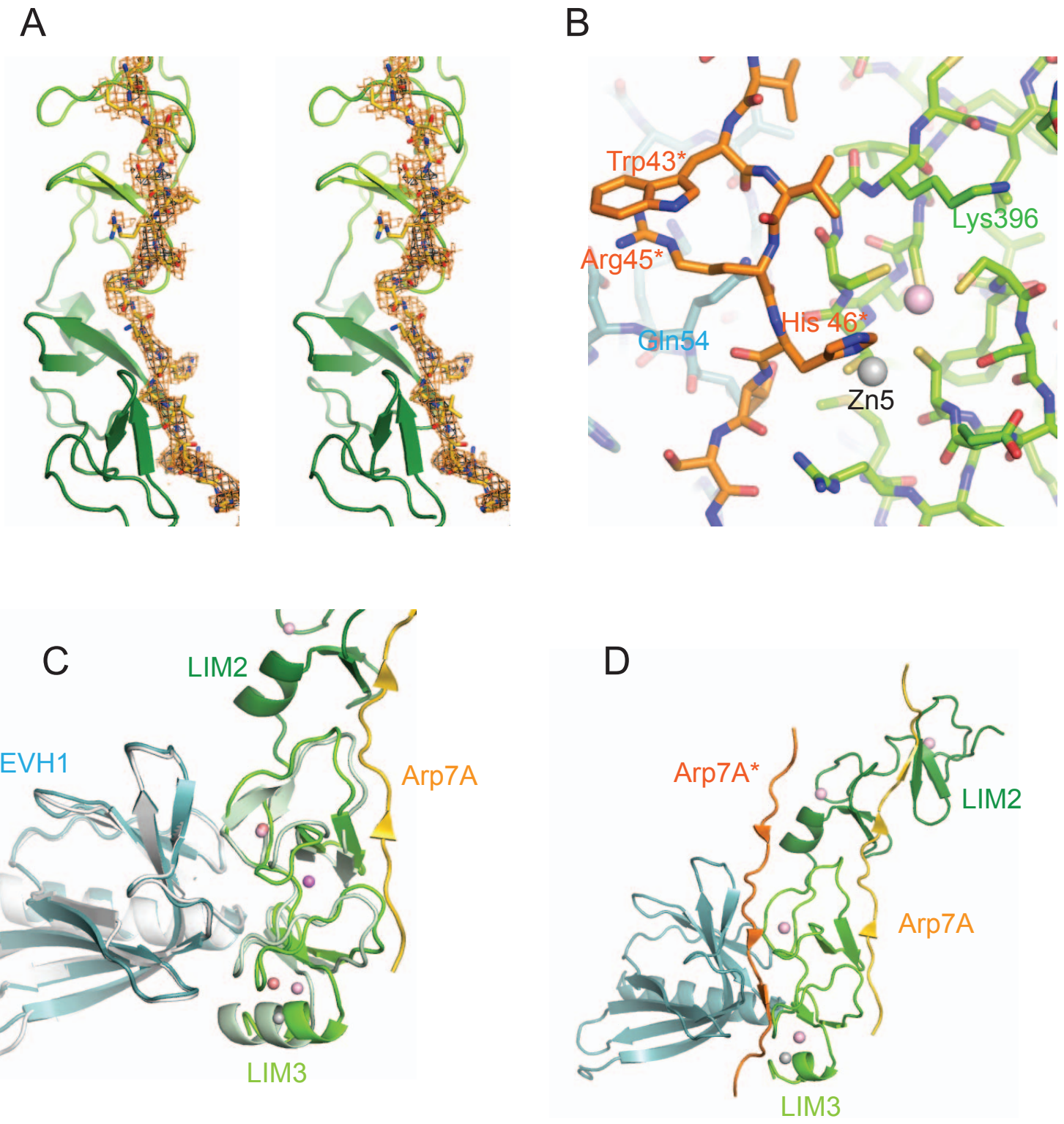
(A) Electron density (orange) of 28-49^{Arp7A} in the ternary complex before inclusion in the crystallographic refinement. (B) Close-up of the contact region centred on the symmetry-related Arp7A His46 with the non-canonical fifth zinc ion associated with the LIM3 domain. (C) Superposition of the EVH1 domains from the ternary 1-65^{Arp7A}:LIM2-3Tes:EVH1^{Mena} (PBD code 2XQN) and binary LIM3^{Tes}-EVH1^{Mena} (PBD code 2IYB) complexes. Protein chains in the ternary complex are coloured as in Fig. 5(A); the binary complex is in white. (D) In the ternary complex, a symmetry related peptide Arp7A* (orange) lies in a shallow groove between EVH1 and LIM3 in the ternary structure, pushing them apart.

Fig S2. Comparison of tandem LIM domain orientations.

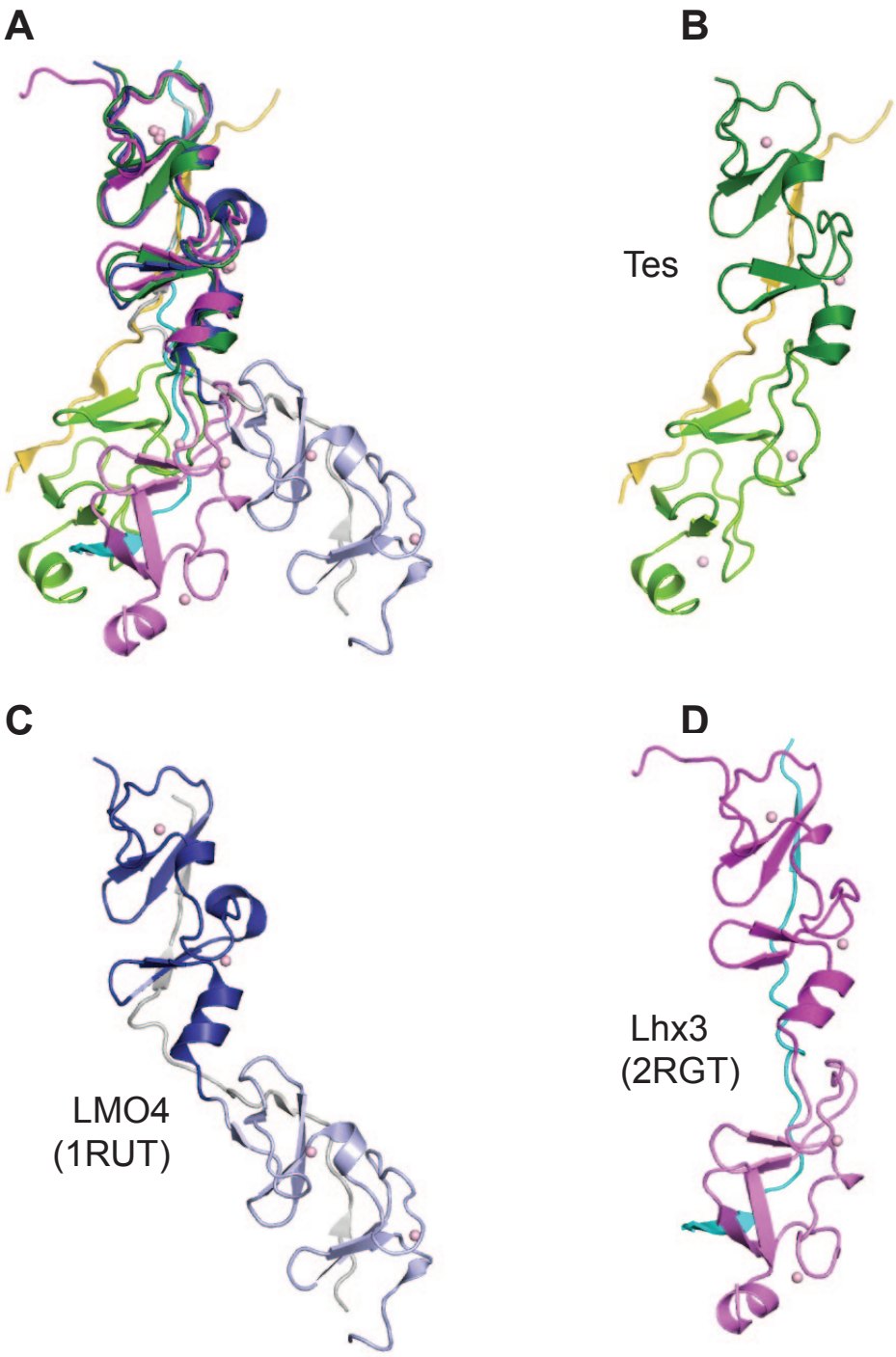
(A) All three tandem LIM domain crystal structures with bound ligand (gold) superimposed using the Cα atoms of the first LIM domain (LIM2 in Tes-Arp7A, LIM1 in 1RUT and 2RGT). The three separate structures are shown for clarity in B, C, and D. (E) Structure-based sequence alignment for the tandem LIM domains. Zinc-binding residues are indicated in pink; pocket-forming residues in green, with those close to A38 of Arp7A in italics; EVH1Mena binding residues in orange; other conserved residues in bold black.

Table S1.

Shows the analysis of protein contacts within the 1-65^{Arp7A}:LIM2-3Tes:EVH1^{Mena} ternary complex using the PISA server (39).



Boëda et al, Fig.S1.



E

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Tes 297 -EKPRCAGCDELIFSNEYTQAENQNWHLKHFCCFDCDSILAGE--IYVMVNDKPVCKPCYVKNHAVV
Lhx3 30 PEIPMCAGCDQHILDRFILKALDRHWHSKCLKCSDCHVPLAER---CFSRGESVYCKDDFFKRFGTK
LMO4 19  SWKRCAGCGGKIADRFLLYAMDSYWHSRCLKCSSCQAQLGDIGTSSYTKSGMILCRNDYIRLFGNS

Tes   --CQGCHNAIDPEVQRVTYNNFSWHASTECFLCSCCSKCLIG-QKFMPV-EGMVFCSVECKKRMS 421
Lhx3  --CAACQLGIPPTQVVRRAQDFVYH--LHCFACVVCKRQLATGDEFYLMEDSRLVCK-ADYETAK 152
LMO4  GACSACGQSIPASELVMRAQGNVYH--LKCFTCSTCRNRLVPGDRFHYI-NGSLFCE-HDRPTAL 145
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Boëda et al, Fig.S2.

Interface Summary

	Tes		Arp7A	
Selection range **Class** **Symmetry operation** **Symmetry ID**	T Protein x,y,z 1_555		A Protein x,y,z 1_555	
Number of atoms interface surface total	152 648 973	(15.6%) (66.6%) (100.0%)	117 165 169	(69.2%) (97.6%) (100.0%)
Number of residues interface surface total	41 125 125	(32.8%) (100.0%) (100.0%)	19 22 22	(86.4%) (100.0%) (100.0%)
Solvent-accessible area, Å² interface total	1104.2 8384.9	(13.2%) (100.0%)	1327.3 3181.7	(41.7%) (100.0%)
Solvation energy, kcal/mol isolated structure gain at complexation average gain P-value	-112.7 -4.1 -5.4 0.632	(100.0%) (3.6%) (4.8%)	-6.9 -10.0 -8.0 0.347	(100.0%) (144.1%) (115.1%)

Hydrogen bonds

##	Tes	Dist. [Å]	Arp7A
1	T:PHE 403[N]	2.85	A:GLN 29[O]
2	T:ARG 374[N]	3.01	A:ARG 34[O]
3	T:GLN 373[NE2]	2.81	A:ASP 35[OD1]

Salt bridges

##	Tes	Dist. [Å]	Arp7A
1	T:GLU 317[OE2]	3.80	A:LYS 39[NZ]

4	T:TYR 340[N]	3.09	A:LYS 39[O]
5	T:GLU 338[N]	3.77	A:ALA 41[O]
6	T:GLY 337[N]	2.79	A:ALA 41[O]
7	T:GLN 315[N]	2.97	A:VAL 42[O]
8	T:TYR 313[N]	3.12	A:VAL 44[O]
9	T:GLN 401[O]	3.33	A:ALA 31[N]
10	T:ARG 374[O]	2.84	A:ARG 34[N]
11	T:THR 376[OG1]	3.41	A:ARG 34[NE]
12	T:PRO 370[O]	3.02	A:ALA 38[N]
13	T:TYR 340[O]	3.08	A:LYS 39[N]
14	T:GLU 317[OE2]	3.80	A:LYS 39[NZ]
15	T:GLY 337[O]	2.82	A:ARG 40[NH1]
16	T:GLU 338[O]	3.02	A:ALA 41[N]
17	T:GLN 315[O]	2.89	A:VAL 42[N]
18	T:GLU 312[OE1]	3.51	A:TRP 43[NE1]
19	T:TYR 313[O]	2.94	A:VAL 44[N]

Disulfide bonds

##	Structure 1	Dist. [Å]	Structure 2
No disulfide bonds found			

Covalent bonds

##	Structure 1	Dist. [Å]	Structure 2
No covalent bonds found			

Interfacing residues (not a contact table)

Display level: Residues

Inaccessible residues
Solvent-accessible residues
HSDC Residues making **H**ydrogen/**D**isulphide bond, **S**alt bridge or **C**ovalent link
Interfacing residues
ASA Accessible Surface Area, Å² **BSA** Buried Surface Area, Å² **Δ^{i}G** Solvation energy effect, kcal/mol |||| Buried area percentage, one bar per 10%

##	Tes	HSDC	ASA	BSA	Δ^{i}G
1	T:GLU 297		156.02	0.00	-0.00
2	T:LYS 298		109.31	0.00	0.00
3	T:PRO 299		42.09	0.00	0.00
4	T:ARG 300		126.98	0.00	-0.00
5	T:CYS 301		0.49	0.00	0.00
6	T:ALA 302		46.29	0.00	-0.00
7	T:GLY 303		44.84	0.00	0.00
8	T:CYS 304		47.26	0.00	-0.00
9	T:ASP 305		87.54	0.00	-0.00
10	T:GLU 306		105.91	0.00	0.00

##	Arp7A	HSDC	ASA	BSA	Δ^{i}G
1	A:LEU 28		226.45	116.63 ||||||	1.53
2	A:GLN 29	H	143.01	88.05 ||||||	0.84
3	A:THR 30		138.77	50.41 ||||	0.17
4	A:ALA 31	H	93.45	88.98 |||||||||	0.86
5	A:SER 32		97.12	40.86 |||||	0.23
6	A:LEU 33		185.61	103.55 ||||||	1.58
7	A:ARG 34	H	214.39	76.36 ||||	-0.18
8	A:ASP 35	H	125.77	31.12 |||	-0.26
9	A:GLY 36		59.32	44.11 |||||||	0.39

11	T:LEU 307		54.99	0.00	0.00
12	T:ILE 308		1.15	0.00	0.00
13	T:PHE 309		146.22	0.00	0.00
14	T:SER 310		64.74	0.00	-0.00
15	T:ASN 311		145.08	37.28 |||	-0.06
16	T:GLU 312	H	106.77	50.42 |||||	0.12
17	T:TYR 313	H	83.57	48.84 ||||||	-0.09
18	T:THR 314		9.20	9.20 |||||||||	0.15
19	T:GLN 315	H	138.91	61.64 |||||	-0.37
20	T:ALA 316		11.70	5.36 |||||	0.09
21	T:GLU 317	HS	76.23	22.86 |||	-0.19
22	T:ASN 318		152.76	0.00	0.00
23	T:GLN 319		75.84	0.00	-0.00
24	T:ASN 320		32.44	0.00	0.00
25	T:TRP 321		32.58	0.00	0.00
26	T:HIS 322		45.50	0.00	-0.00
27	T:LEU 323		58.47	18.06 ||||	0.29
28	T:LYS 324		154.64	0.00	0.00
29	T:HIS 325		82.46	0.00	-0.00
30	T:PHE 326		9.17	2.19 |||	0.03
31	T:CYS 327		34.33	0.00	-0.00
32	T:CYS 328		1.11	0.00	0.00
33	T:PHE 329		135.49	0.00	0.00
34	T:ASP 330		58.55	0.00	-0.00
35	T:CYS 331		64.72	0.00	0.00
36	T:ASP 332		105.83	0.00	0.00
37	T:SER 333		53.87	0.00	-0.00
38	T:ILE 334		75.67	4.85 |	0.08
39	T:LEU 335		5.78	4.10 |||||||	0.02
40	T:ALA 336		31.01	26.58 ||||||||	0.19
41	T:GLY 337	H	86.89	69.41 |||||||	0.07
42	T:GLU 338	H	112.73	15.29 ||	-0.09
43	T:ILE 339		92.60	61.85 ||||||	0.99
44	T:TYR 340	H	54.66	53.80 ||||||||	0.26

10	A:PRO 37		132.42	76.35 |||||	1.01
11	A:ALA 38	H	94.97	72.12 |||||||	1.12
12	A:LYS 39	HS	189.18	81.38 |||||	-0.32
13	A:ARG 40	H	209.49	74.66 ||||	-0.76
14	A:ALA 41	H	98.12	98.12 |||||||||	1.01
15	A:VAL 42	H	120.73	45.85 ||||	-0.03
16	A:TRP 43	H	180.65	94.71 |||||	0.92
17	A:VAL 44	H	129.39	84.69 ||||||	0.78
18	A:ARG 45		148.84	29.21 ||	0.38
19	A:HIS 46		181.09	30.18 ||	0.75
20	A:THR 47		131.26	0.00	0.00
21	A:SER 48		115.11	0.00	-0.00
22	A:SER 49		166.60	0.00	0.00

45	T:VAL 341		33.71	32.20 ||||||||	0.52
46	T:MET 342		74.26	9.13 ||	-0.01
47	T:VAL 343		24.64	0.00	0.00
48	T:ASN 344		135.27	0.00	-0.00
49	T:ASP 345		127.57	0.00	-0.00
50	T:LYS 346		45.28	0.00	-0.00
51	T:PRO 347		20.25	0.00	0.00
52	T:VAL 348		2.18	0.00	0.00
53	T:CYS 349		12.34	0.00	0.00
54	T:LYS 350		48.91	0.00	-0.00
55	T:PRO 351		90.01	0.00	-0.00
56	T:CYS 352		18.37	0.00	0.00
57	T:TYR 353		16.95	7.90 |||||	-0.08
58	T:VAL 354		78.66	0.00	-0.00
59	T:LYS 355		100.87	0.00	0.00
60	T:ASN 356		90.12	0.00	0.00
61	T:HIS 357		59.15	0.00	0.00
62	T:ALA 358		56.82	0.00	0.00
63	T:VAL 359		54.58	0.17 |	0.00
64	T:VAL 360		67.94	0.00	0.00
65	T:CYS 361		0.12	0.00	0.00
66	T:GLN 362		76.61	0.00	0.00
67	T:GLY 363		44.92	0.00	0.00
68	T:CYS 364		48.87	0.00	0.00
69	T:HIS 365		150.36	0.00	-0.00
70	T:ASN 366		88.08	0.00	-0.00
71	T:ALA 367		52.75	0.00	0.00
72	T:ILE 368		1.01	0.00	0.00
73	T:ASP 369		55.78	0.00	-0.00
74	T:PRO 370	H	32.70	32.41 ||||||||	-0.15
75	T:GLU 371		102.75	12.83 ||	0.03
76	T:VAL 372		75.12	4.17 |	-0.02
77	T:GLN 373	H	157.35	80.14 ||||||	-0.21
78	T:ARG 374	H	68.56	60.53 ||||||||	-0.23

79	T:VAL 375		32.46	31.29 |||||||||	0.50
80	T:THR 376	H	83.85	52.41 |||||||	-0.26
81	T:TYR 377		92.25	26.06 |||	-0.04
82	T:ASN 378		129.18	0.00	-0.00
83	T:ASN 379		152.32	0.00	0.00
84	T:PHE 380		66.47	0.00	-0.00
85	T:SER 381		13.58	0.00	-0.00
86	T:TRP 382		7.82	0.00	0.00
87	T:HIS 383		38.87	0.00	0.00
88	T:ALA 384		52.27	8.86 ||	0.14
89	T:SER 385		36.19	0.00	-0.00
90	T:THR 386		94.89	0.00	0.00
91	T:GLU 387		142.31	0.00	-0.00
92	T:CYS 388		11.37	0.00	0.00
93	T:PHE 389		4.06	3.75 |||||||||	0.06
94	T:LEU 390		47.47	0.00	-0.00
95	T:CYS 391		3.68	0.00	0.00
96	T:SER 392		46.93	0.00	0.00
97	T:CYS 393		42.71	0.00	-0.00
98	T:CYS 394		63.49	0.00	0.00
99	T:SER 395		74.31	0.00	-0.00
100	T:LYS 396		96.33	0.00	-0.00
101	T:CYS 397		44.18	0.00	-0.00
102	T:LEU 398		0.98	0.98 ||||||||||	-0.01
103	T:ILE 399		61.50	21.90 ||||	0.35
104	T:GLY 400		81.34	35.82 |||||	0.12
105	T:GLN 401	H	87.82	13.98 ||	-0.07
106	T:LYS 402		146.47	57.07 ||||	0.88
107	T:PHE 403	H	64.87	63.45 |||||||||	0.32
108	T:MET 404		52.27	22.76 |||||	0.36
109	T:PRO 405		45.01	9.71 |||	0.16
110	T:VAL 406		69.63	0.00	0.00
111	T:GLU 407		150.38	0.00	-0.00
112	T:GLY 408		13.88	0.00	-0.00

113	T:MET 409		95.27	0.00	-0.00
114	T:VAL 410		5.52	0.00	-0.00
115	T:PHE 411		5.47	0.00	-0.00
116	T:CYS 412		26.53	5.34 |||	-0.05
117	T:SER 413		29.60	7.17 |||	0.11
118	T:VAL 414		85.60	12.43 ||	0.17
119	T:GLU 415		117.82	0.00	0.00
120	T:CYS 416		14.38	0.00	-0.00
121	T:LYS 417		74.90	0.00	-0.00
122	T:LYS 418		110.27	0.00	0.00
123	T:ARG 419		160.89	0.00	0.00
124	T:MET 420		93.91	0.00	-0.00
125	T:SER 421		138.37	0.00	-0.00