Supplemental Materials

Chaperone-like effect of the linker on the isolated C-terminal domain of rabbit muscle creatine kinase

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Table S1. Primer oligonucleotides used for the mutagenesis of RMCK.

Mutation	Primer
N100-R	5′-GGTGCTCGAGCTAGAAGCCCCCGTGGCGGTCCTG-3′
N112-R	5′-GGTGCTCGAGCTAGTGGTTGAGGTCGGTCTTGTG-3′
C101-F	5′-ATACATATGAAACCCACCGACAAGCACAAG-3′
C113-F	5′-ATACATATGGAGAACCTCAAAGGTGGGGAC-3′
C125-F	5′-ATACATATG TACGTGCTCAGCAGCCGCGTG-3′
F250S-F	5’- GAAGGAGGTCTCCCGCCGCTTCT -3’
F250S-R	5’- GCGGCGGGAGACCTCCTTCAT -3’
V255S-F	5’- GCTTCTGCTCGGGGCTGCAGAAGAT -3’
V255S-R	5’- CTGCAGCCCCGAGCAGAAGCG -3’
V347S-F	5’- GCTCGTCCGAGTCCGAGCAGGTG -3’
V347S-R	5’- GCACCTGCTCGGACTCGGACGAG -3’

Table S2. Biochemical and Biophysical properties of the WT and mutated RMCK.

	WT	C101	C113
CK activity	Yes	No	No
SEC elution volume (ml)	13.4	18.4	18.7
Oligomeric state	dimer	monomer	monomer
E_{max} – native (nm)	334	337	337
E_{max} – GdnHCl-denatured (nm)	351	351	351
E_{max} – heat-denatured (nm)	346	340	338.5
T_m-CD (°C)	31 ± 1, 63.4 ± 0.1	52.6 ± 0.2	47.7 ± 0.1
ΔH_m- CD (kJ/mol)	4 ± 1, 20 ± 1	9.7 ± 0.6	10.4 ± 0.4
T_m-I_{320}/I_{365} (°C)	55.3 ± 0.8	40 ± 4	42 ± 2
Aggregation after 24 h storage at 4°C	subtle	subtle	significant
k_{agg} at 46°C (s^{-1})	0.038 ± 0.002	0.041 ± 0.001	0.55 ± 0.02

Table S3. SAP scores for the three key residues at low and high temperatures

		C101	C113
Phe250	300K	0.011 ±0.008	0.08 ±0.02
	400K	0.011 ±0.009	0.10 ±0.03
	p-value	0.427	3.24e-6
Val255	300K	0.09 ±0.05	0.07 ±0.04
	400K	0.15 ±0.05	0.23 ±0.05
	p-value	1.76e-15	1.13e-46
Val347	300K	-0.04 ±0.04	0.09 ±0.03
	400K	-0.03 ±0.02	0.14 ±0.04
	p-value	0.028	6.75e-18
Leu115	300K	-0.002 ±0.01	0.09 ±0.06
	400K	-0.01 ±0.01	0.17 ±0.08
	p-value	5.15e-6	1.21e-15

The *p*-values are obtained by one-sided Student's *t*-test with a sampling size of 100.

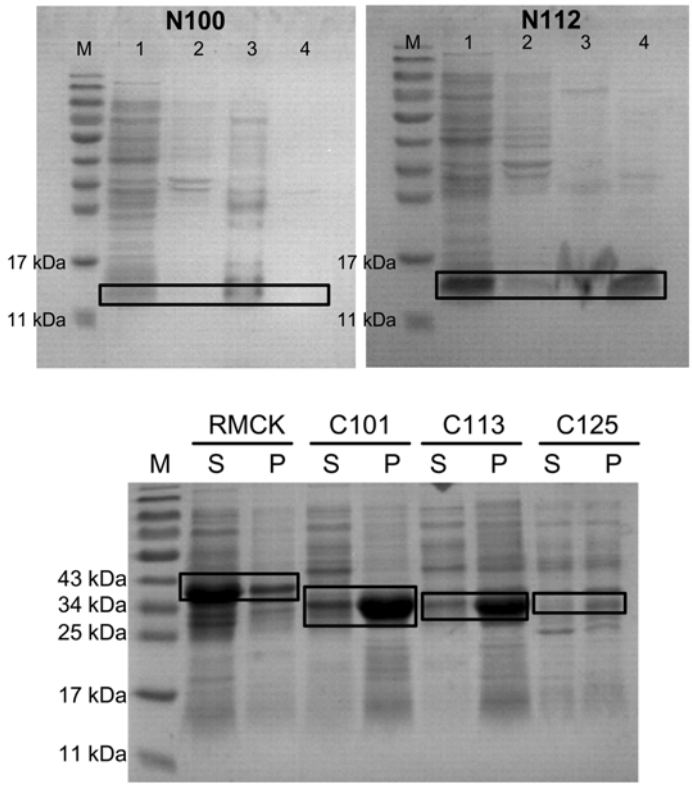

Fig. S1. Overexpression and purification of NTD (top) and CTDs (bottom) of RMCK. Lanes M and 1-4 represent marker, supernatant of the *E. coli* cells, precipitation of the *E. coli* cells, recombinant proteins purified by Ni^{2+}-column and final products purified by SEC, respectively. The positions of N100 and N112 are labeled. Most of the recombinant proteins existed in the supernatants of the *E. coli* cells. The expression yield of N100 was too low to get enough purified proteins, while that of N112 was about 0.2 mg/L. The purity of the final products of N112 was above 90% as evaluated by the SDS-PAGE analysis. The overexpression of the recombinant proteins were achieved by the addition of 0.1 mM IPTG, and the *E. coli* cells were cultured at 15°C for 24 h. The positions of the recombinant proteins on the SDS-PAGE are labeled by squares. S represents the supernatant of the lysates, while P is precipitant.

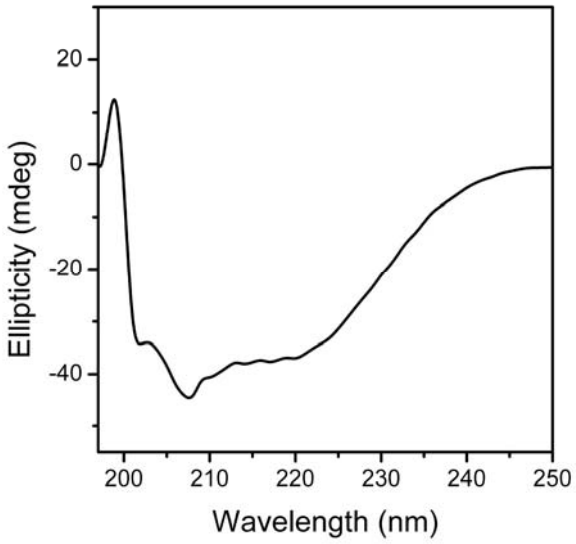

Fig. S2. CD spectrum of the NTD fragment N112. The final concentration of N112 was 6 μM. The CD spectra revealed that N112 was well-folded and dominated by α-helices as revealed by the two minimum peaks at 208 nm and 222 nm.

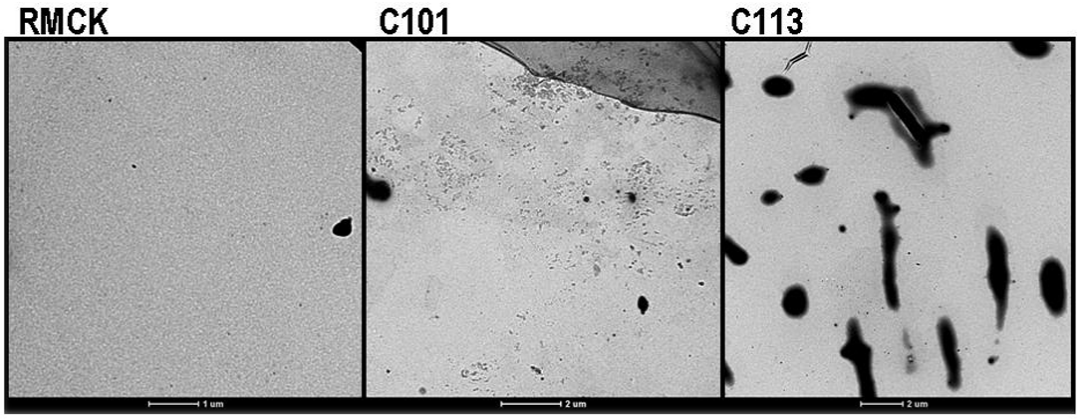

Fig. S3. The transmission EM images of RMCK, C101 and C113 incubated at 46°C for 10 min. The bar in the image of RMCK is 1 μm, while those of C101 and C113 are 2 μm. The EM samples were prepared by depositing about 3 μl protein samples onto a freshly glow-discharged carbon coated copper grid followed by negatively staining with 2% phosphotungstic acid for 2 min. The EM experiments were conducted on a FEI Tecnai Sprit transmission electron microscope at 120 kV. It is worth noting that the actual amount of aggregates of C113 was much higher than that appeared in the EM picture since parts of the amorphous aggregates were washed away from the grid.

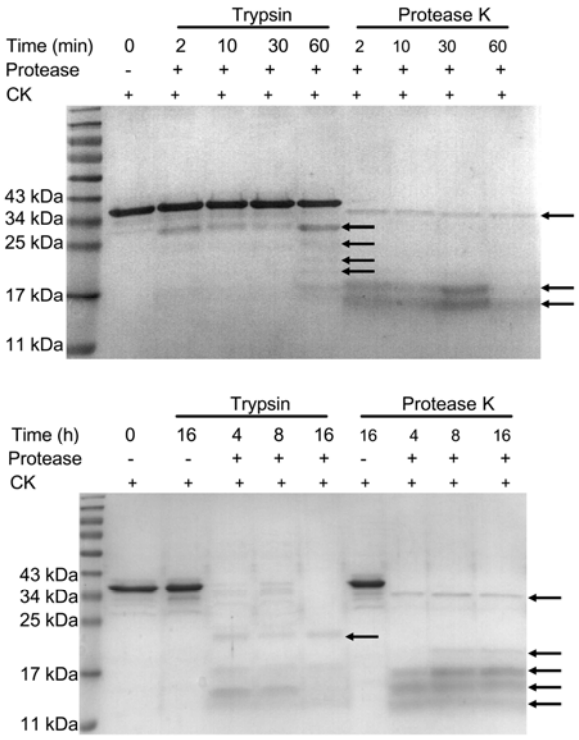

Fig. S4. Degradation of RMCK by site-specific protease trypsin and nonspecific protease K. The final concentrations of CK, trypsin, and protease K were 0.2, 0.02, and 0.02 mg/ml, respectively. Proteolysis by trypsin was conducted at 37°C, while that by protease K was at 25°C.

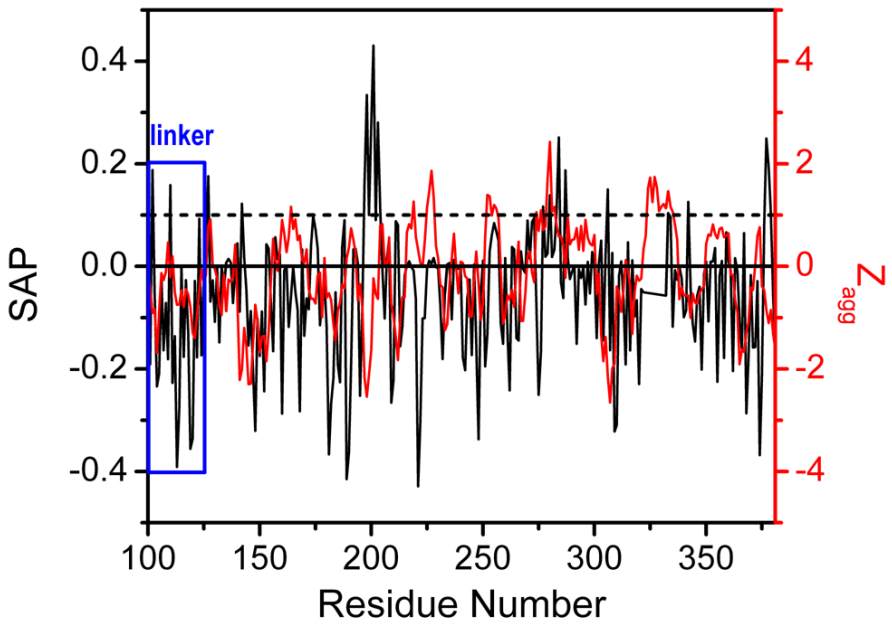

Fig. S5. Aggregation propensity profile of the CTD of RMCK. The intrinsic aggregation propensity Z_{agg} values were calculated according to the previously published Zyggregator method using the online computational algorithm at http://www-vendruscolo.ch.cam.ac.uk/zyggregator_test.php. The SAP values are also shown. The linker region is boxed. The SAP score evaluates the surface hydrophobicity of a residue based on three dimensional structures, while the Zyggregator method predicts the intrinsic aggregation propensity based on the primary sequences. The Zyggregator scores were 0.704, 0.709 and 0.717 for C101, C113 and C125, respectively. C125 exhibited the highest Zyggregator score, which was reduced gradually with the inclusion of the intact or truncated linker. Both the SAP and the Zyggregator scores indicated that the linker was aggregation-resistant but not aggregation-prone.

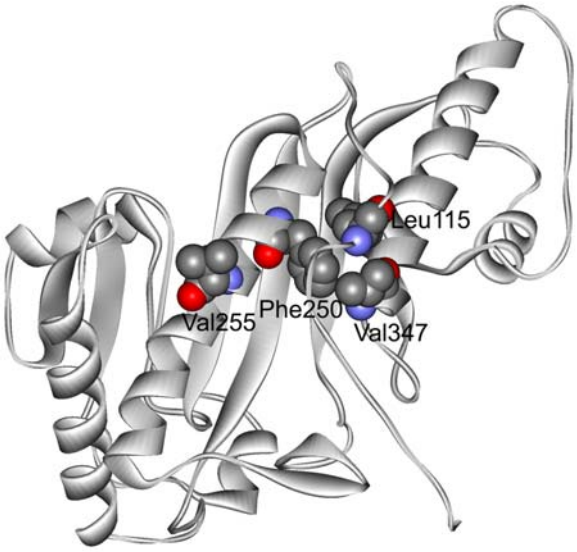

Fig. S6. Structure of the CTD of RMCK (PDB ID 2CRK). The positions of L115 in the linker and Phe250, Val255 and Val347 in CTD are highlighted by the space-filling model. The linker acts as a lid to protect the hydrophobic side chains of Phe250 and Val347, but not Val255 against exposure to solvent.