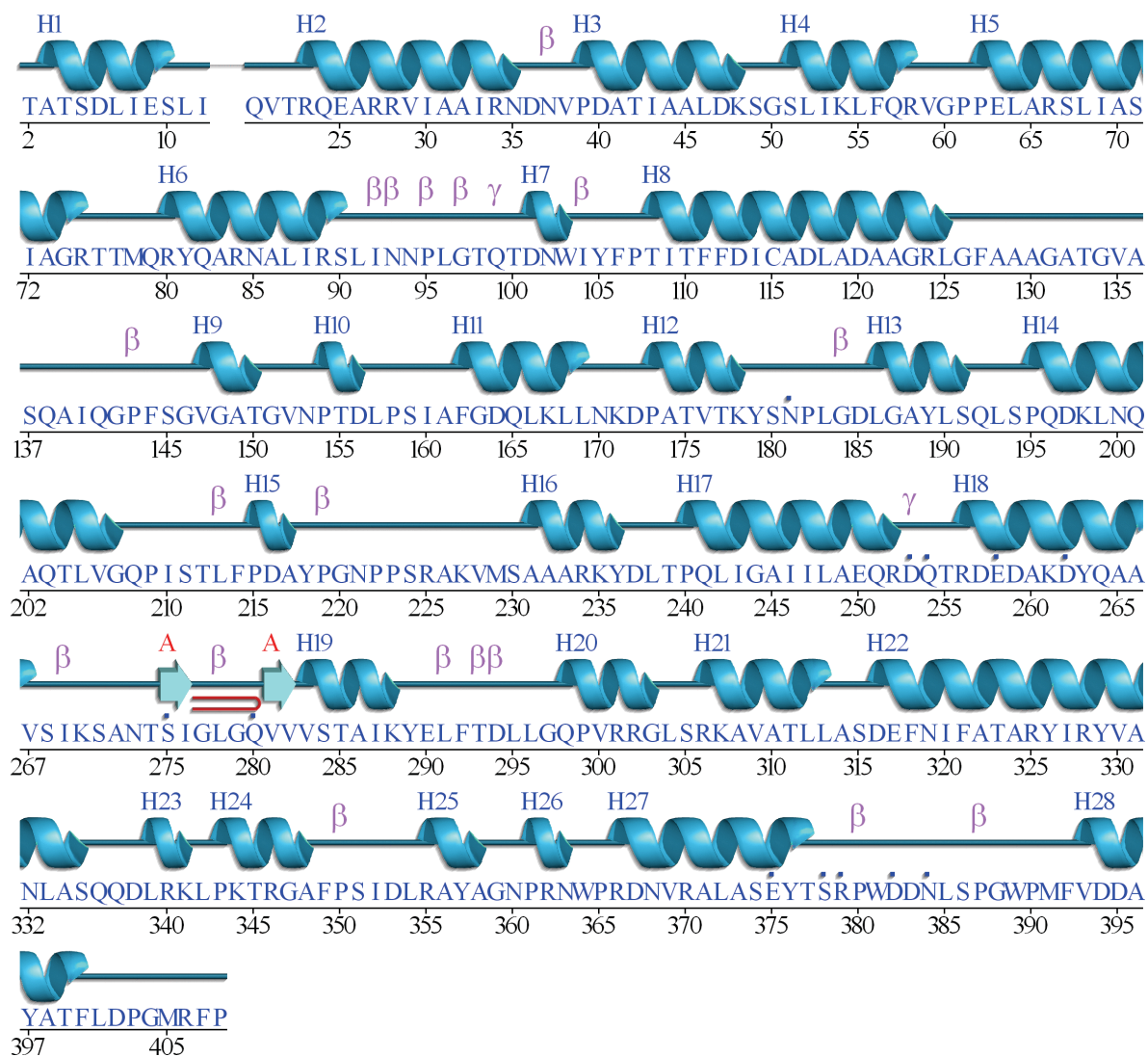
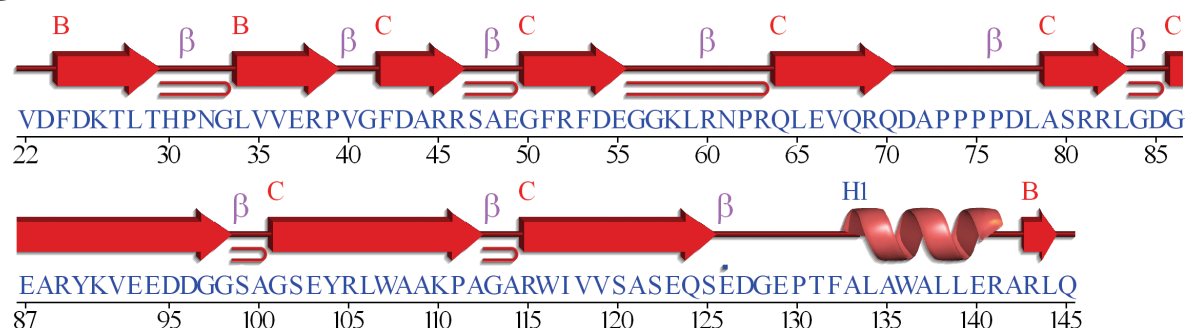


Supplementary Table S1. Bond angles of the Ca coordination spheres

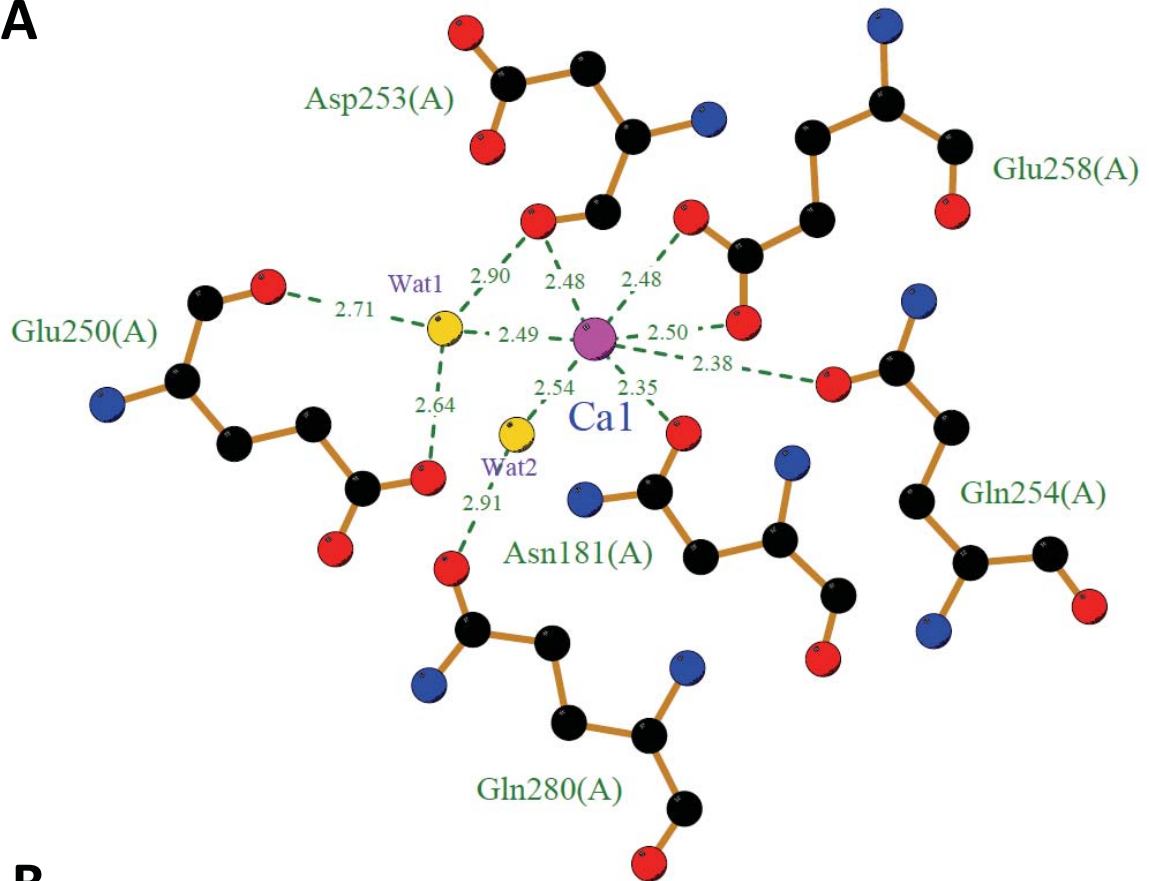
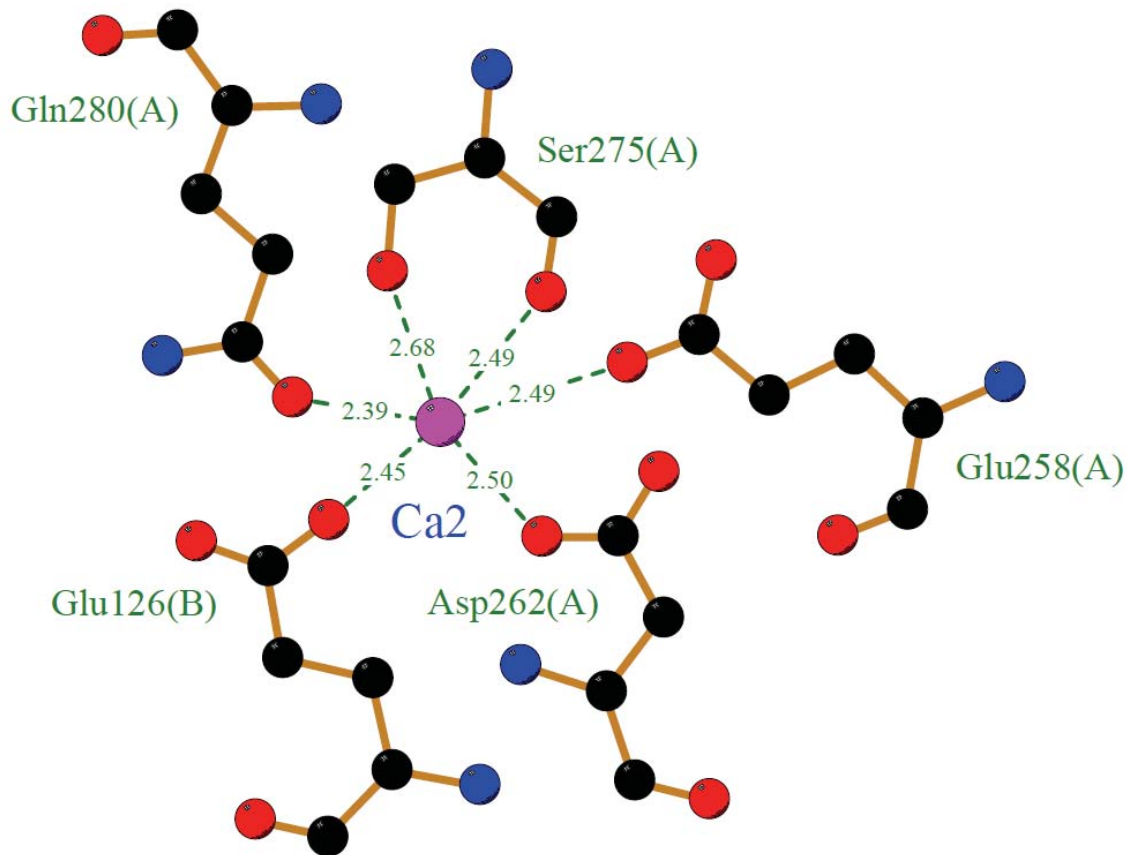
Ligand-Ca²⁺-ligand	Bond angles (°)
Ca1	
Tse3 Asn181 O δ -Ca1-Tse3 Gln254 O ϵ	160.44
Tse3 Glu258 O ϵ 1-Ca1-Wat1	152.38
Tse3 Glu258 O ϵ 2-Ca1-Wat1	146.27
Tse3 Glu253 O-Ca1-Tse3 Gln258 O ϵ 2	139.87
Tse3 Glu253 O-Ca1-Wat2	133.59
Tse3 Gln254 O ϵ -Ca1-Wat2	121.36
Tse3 Asn181 O δ -Ca1-Tse3 Glu253 O	82.70
Tse3 Glu253 O-Ca1-Tse3 Gln254 O ϵ	88.13
Ca2	
Tsi3 Glu126 O ϵ 2-Ca2-Tse3 Asp262 O δ 1	102.12
Tsi3 Glu126 O ϵ 2-Ca2-Tse3 Asp262 O δ 2	148.00
Tse3 Glu258 O ϵ 2-Ca2-Tse3 Ser275 O γ	125.58
Tse3 Glu258 O ϵ 2-Ca2-Tse3 Ser275 O γ	150.19
Tsi3 Glu126 O ϵ 1-Ca2-Tse3 Gln280 O ϵ	145.84
Tse3 Asp262 O δ 1-Ca2-Tse3 Glu258 O ϵ 2	96.01
Tsi3 Glu126 O ϵ 2-Ca2-Tse3 Glu258 O ϵ 2	80.52
Tse3 Asp262 O δ 2-Ca2-Tse3 Gln280 O ϵ	88.68
Ca3	
Tse3 Ser378 O γ -Ca3-Tse3 Asp382 O δ 1	157.37
Tse3 Arg379 O-Ca3-Tse3 Asn384 O δ	165.45
Tse3 Glu375 O ϵ 1-Ca3-Wat3	148.61
Tse3 Glu375 O ϵ 2-Ca3-Wat3	160.50
Tse3 Ser378 O γ -Ca3-Tse3 Arg379 O	91.40
Tse3 Asp382 O δ 1-Ca3-Tse3 Asn384 O δ	81.99
Tse3 Glu375 O ϵ 1-Ca3- Tse3 Ser378 O γ	71.10
Tse3 Glu375 O ϵ 2-Ca3- Tse3 Asp382 O δ 1	84.20

Supplementary Table S2. Details of Tse3-Tsi3 interactions

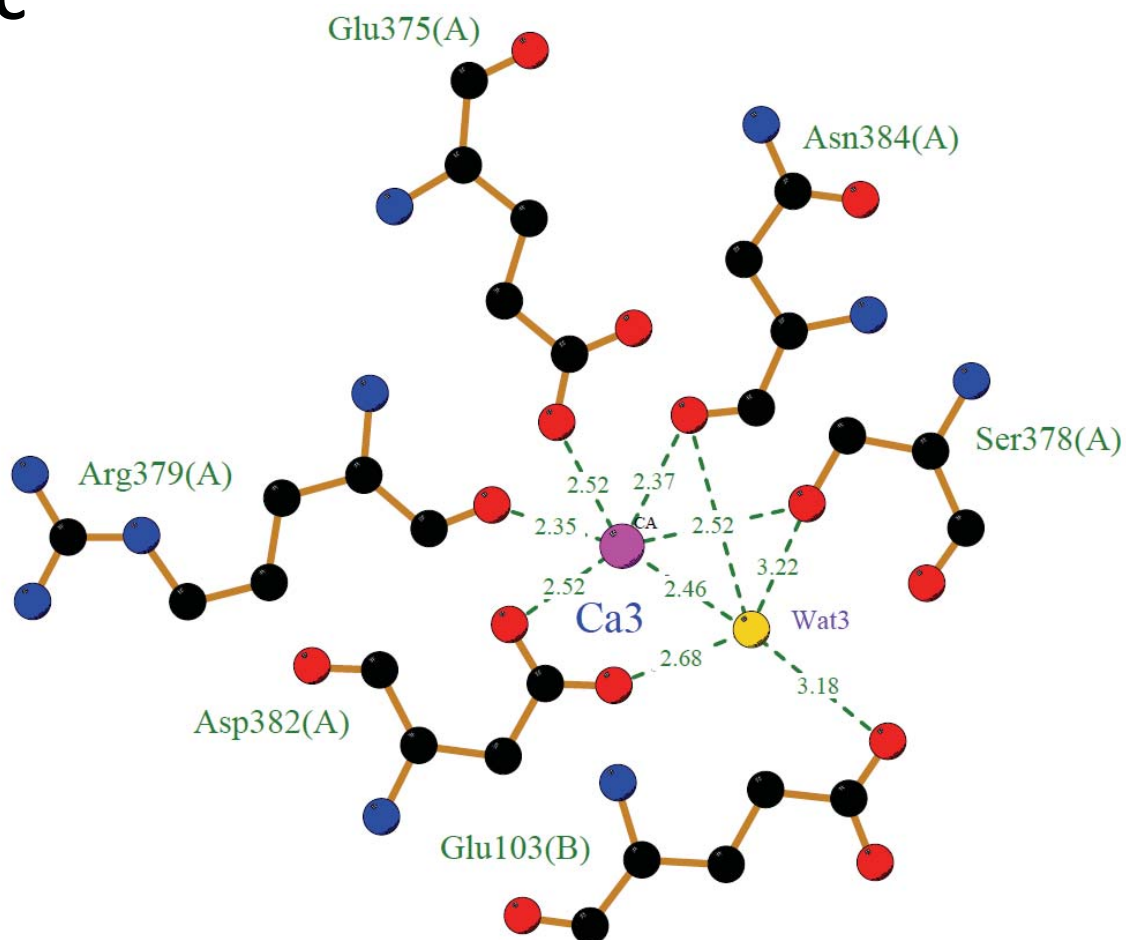
Hydrogen bonds (≤ 3.3 Å)		
Hydrogen donor	Hydrogen acceptor	Distance (Å)
Tse3: Lys171 N ζ	Tsi3: Glu129 O	2.69
Tse3: Glu258 O ϵ 2	Tsi3: Glu126 O ϵ 2	3.19
Tse3: Lys261 N ζ	Tsi3: Glu126 O ϵ 1	3.12
Tse3: Lys261 N ζ	Tsi3: Asp127 O δ 1	2.78
Tse3: Ser275 O γ	Tsi3: Ser99 O	2.87
Tse3: Ser275 N	Tsi3: Glu126 O ϵ 1	2.91
Tse3: Ser275 O γ	Tsi3: Glu126 O ϵ 1	3.11
Tse3: Ser275 O γ	Tsi3: Glu126 O ϵ 2	3.02
Tse3: Lys288 N ζ	Tsi3: Asp96 O δ 2	3.22
Tse3: Arg379 N	Tsi3: Glu103 O ϵ 1	2.82
Tse3: Ser386 O γ	Tsi3: Arg60 O	2.76
Tse3: Gly388 N	Tsi3: Leu59 O	2.97
Tsi: Arg60 N η 2	Tse3: Glu250 O ϵ 1	2.83
Tsi: Arg60 N η 1	Tse3: Asp253 O δ 2	2.85
Tsi: Ser99 O γ	Tse3: Glu250 O ϵ 2	2.64
Tsi3: Ser99 N	Tse3: Tyr376 O	2.97
Tsi3: Ala100 N	Tse3: Thr377 O	3.22
Tsi3: Gln124 N ϵ 2	Tse3: Thr377 O	2.85
Tsi3: Glu126 O ϵ 2	Tse3: Glu258 O ϵ 2	3.19
Tsi3: Glu126 O ϵ 1	Tse3: Asp262 O δ 1	2.97
Ionic interactions (≤ 6.0 Å)		
Tse3: Lys171	Tsi3: Glu129	
Tse3: Glu250	Tsi3: Arg60	
Tse3: Asp253	Tsi3: Arg60	
Tse3: Lys261	Tsi3: Glu126	
Tse3: Lys261	Tsi3: Asp127	
Tse3: Lys379	Tsi3: Asp96	
Tse3: Arg379	Tsi3: Glu103	
Tse3: Arg379	Tsi3: Asp95	

A**B**

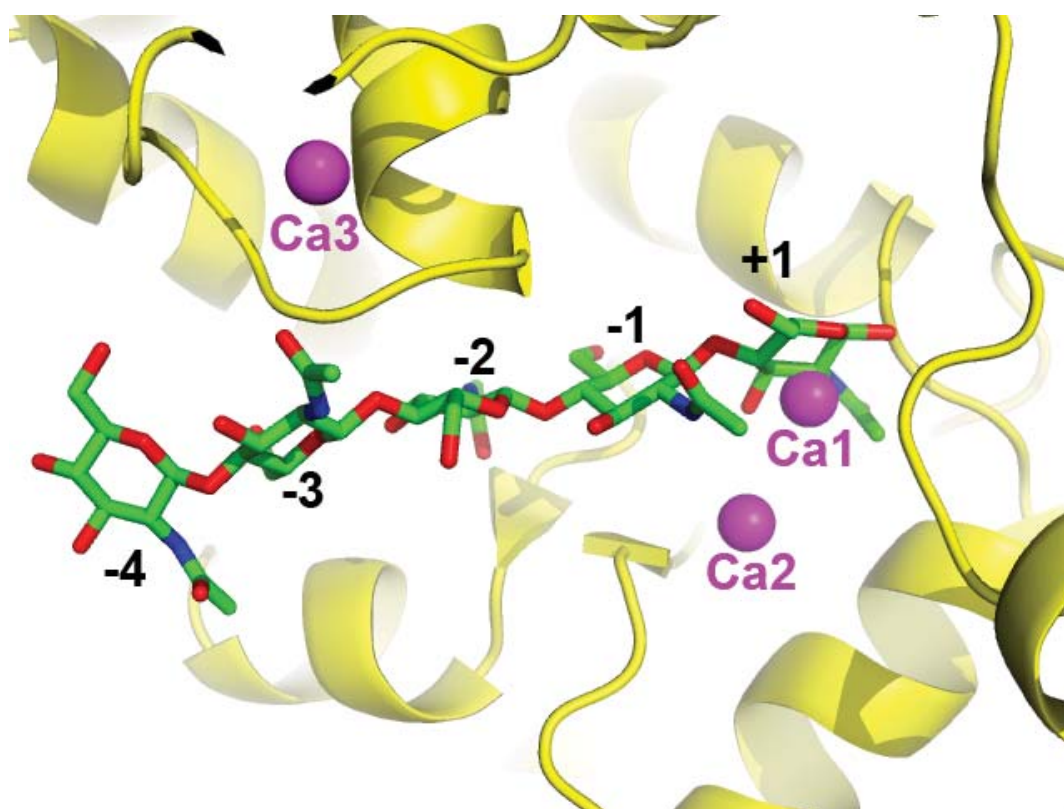
Supplementary Figure S1. Secondary structure distribution of Tse3 (A) and Tsi3 (B). α -helices and β -strands are represented by helical ribbons and schematic arrows. β -sheets are numbered as A, B and C. β -turns and γ -turns are labeled with Greek letters “ β ” and “ γ ” respectively. Residues contacting calcium ions are labeled with small blue dots.

A**B**

C



Supplementary Figure S2. Schematic diagrams showing the 3 Ca²⁺ binding sites of Ca1 (A), Ca2 (B) and Ca3 (C). The pictures were generated using LigPlot⁺ (Laskowski & Swindells, 2011).



Supplementary Figure S3. Modelling of bound substrate in Tse3 by replacing *E. coli* soluble MltE with overlaid Tse3 catalytic domain in the structure with a PDB code 4HJZ (Fibriansah *et al.*, 2012). The substrate-binding subsites from -4 to +1 are labeled with reference to the position and orientation of the chitopentase present in structure 4HJZ.