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Electronic Supplementary Information (ESI)

Reversibility and two state behavior in the thermal unfolding of oligomeric TIM barrel proteins

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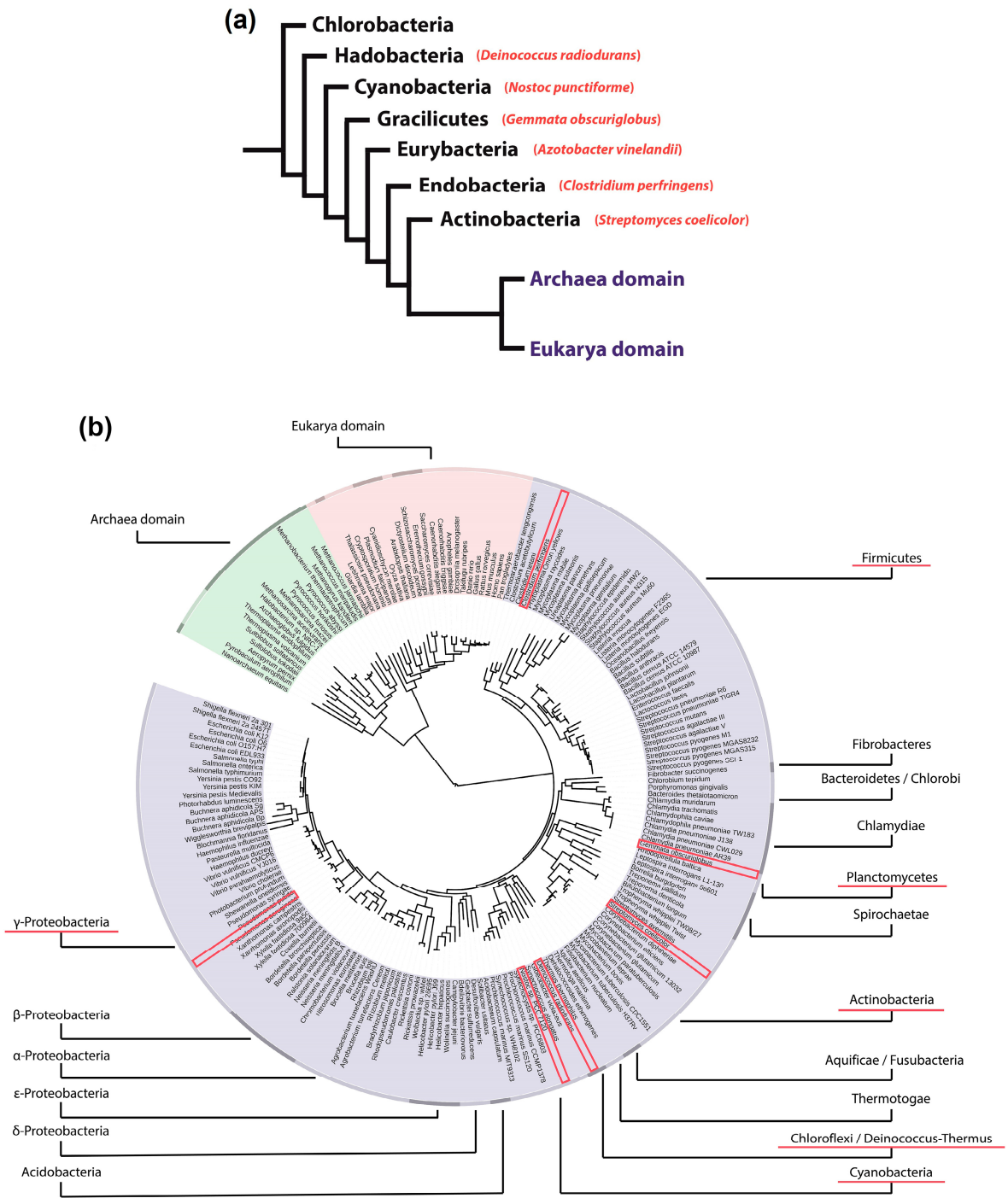

Fig. S1. Phylogenetic representations of BacTIMs. **(a)** Cladogram of bacterial supertaxa (according to ref. 1) showing the species from which the indicated TIM sequence was studied. **(b)** Phylogenetic tree showing the TIMs studied in this work (modified from ref. 2). For *Av*TIM phylum (γ-Proteobacteria) only an approximate location in the phylogenetic tree is shown.

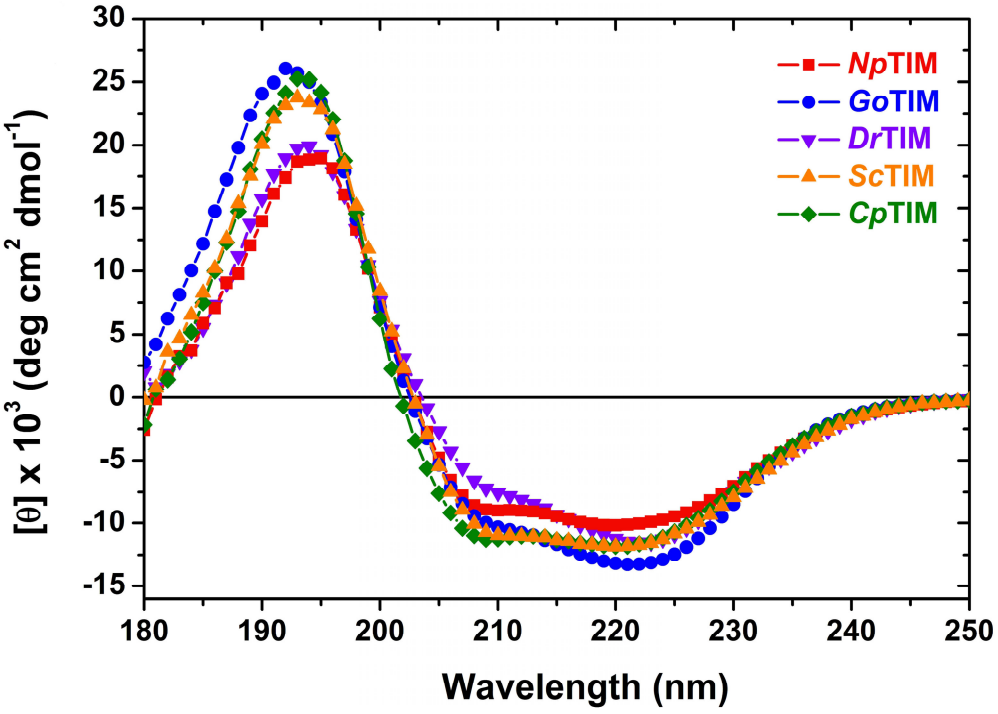

Fig. S2. Far-UV CD spectra of BacTIMs at 25 °C. Data shown are the average of five scans recorded using a protein concentration of 15 μM in buffer C (10 mM NaH_2PO_4 pH 8.0).

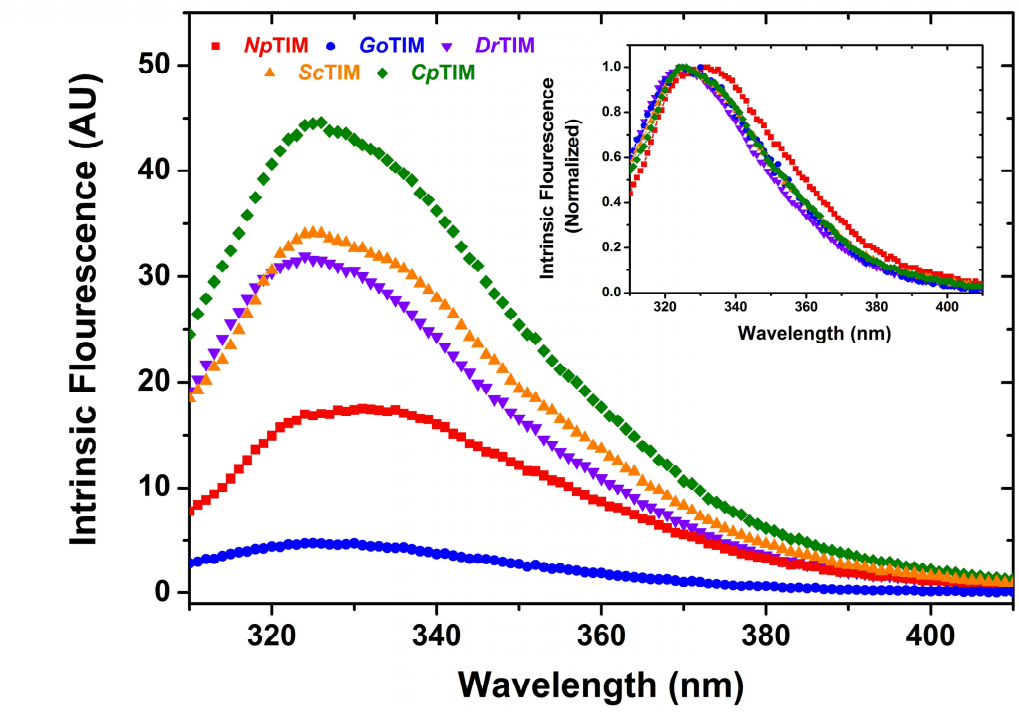

Fig. S3. Intrinsic tryptophan fluorescence spectra of BacTIMs. The spectra shown are the average of five scans recorded using a protein concentration of 15 µM at 25 ºC in buffer C (10 mM NaH_2PO_4 pH 8.0). The excitation wavelength was 295 nm. The inset shows normalized data.

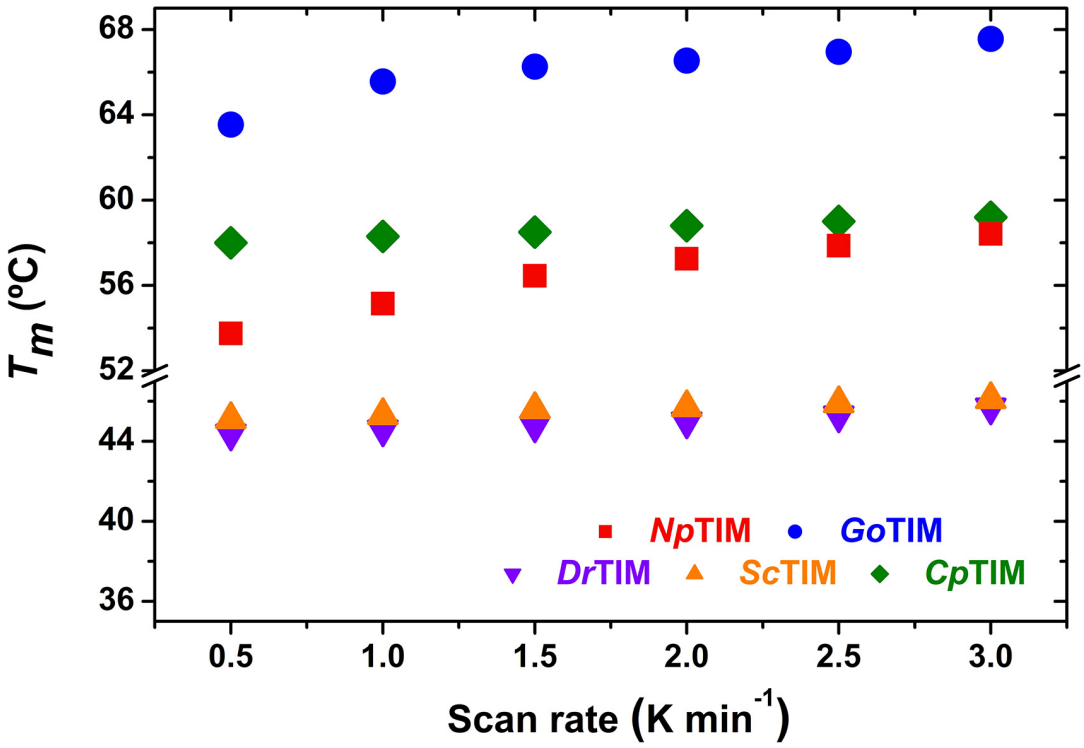

Fig. S4. Dependence of the T_m with respect to scan rate for BacTIMs. In all experiments, protein concentration was 15 μM.

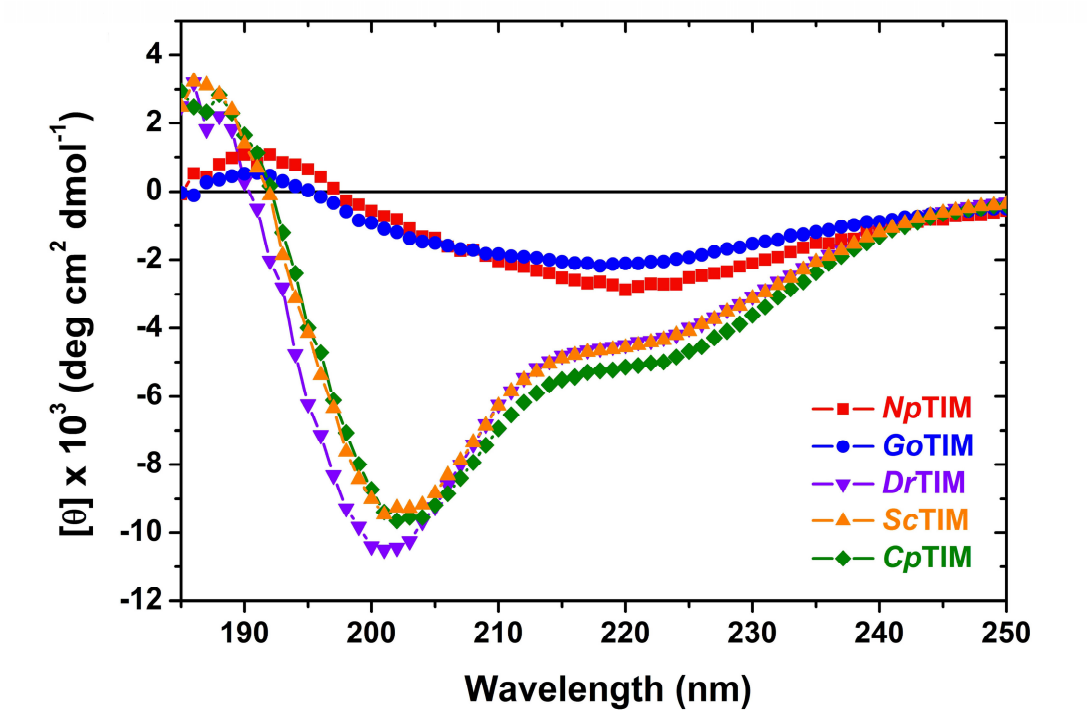

Fig. S5. Far-UV CD spectra of BacTIMs at 80 °C. Data shown are the average of five scans recorded using a protein concentration of 15 μM in buffer C (10 mM NaH_2PO_4 pH 8.0).

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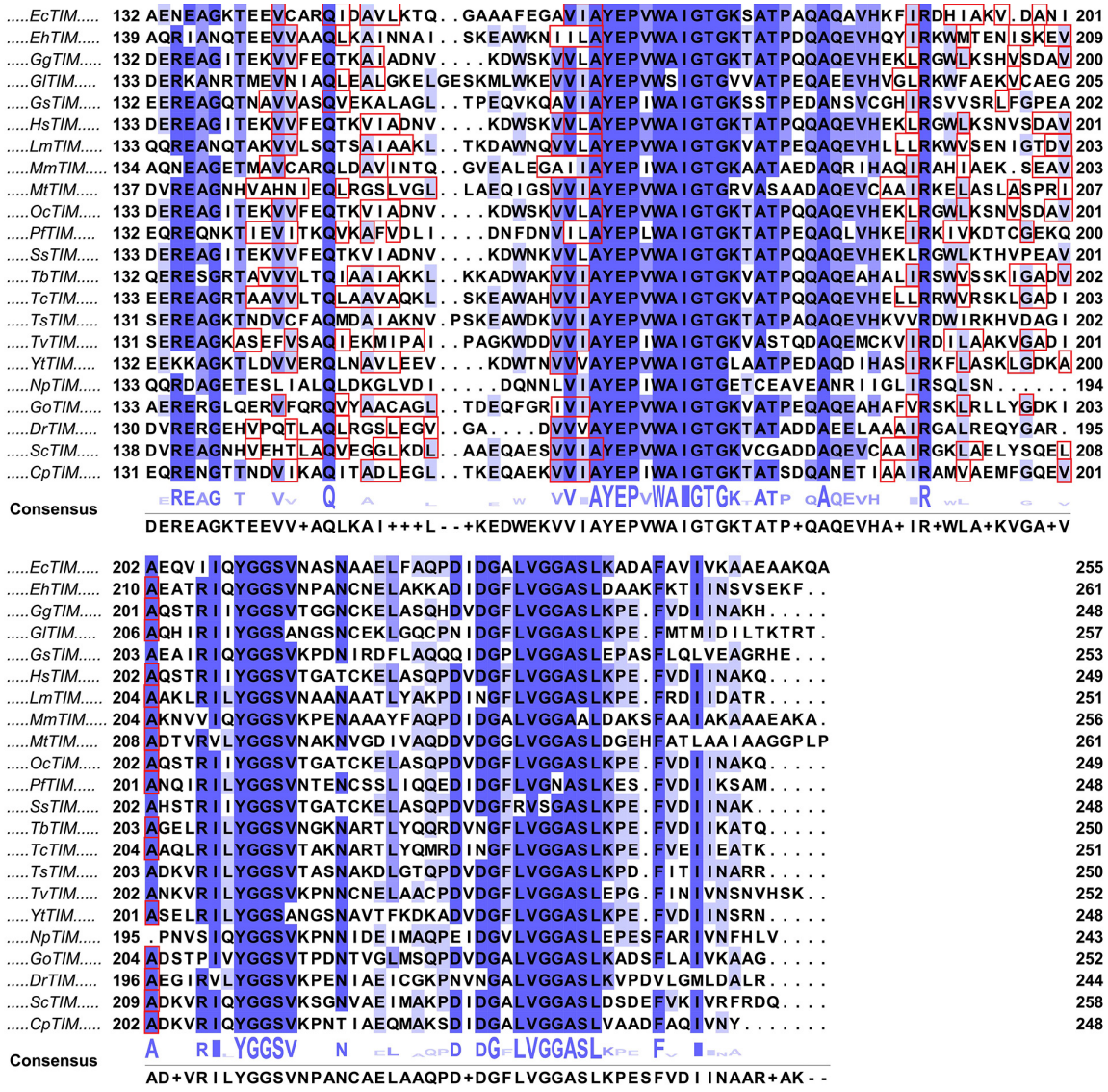

Fig. S6. Sequence alignment of diverse TIMs used for comparison. The conserved residues in these TIMs are shown on the *consensus line* and represented from blue light to dark in accordance to increase conservation. In red squares are shown the residues (both internal and boundary) involved in the formation of the biggest cavity in these structures, except for *Np*TIM, *Ss*TIM and *Ts*TIM for which there is not structural data reported.

Table S1. Thermodynamic parameters for the irreversible thermal unfolding of *Np*TIM and *Go*TIM.

Thermodynamic parameter	Bacterial TIMs		Eukaryotic TIMs				
	***Np*TIM**	***Go*TIM**	***Gl*TIM[a]**	***Hs*TIM[a]**	***Lm*TIM[b]**	***Tb*TIM[b]**	***Tc*TIM[b]**
Activation free energy (kJ mol^{-1}) from average of individual fits to DSC thermograms (Eqs. (3) and (4))	368 ± 46	716 ± 63	NR	NR	312 ± 5	397 ± 5	809 ± 7
Activation free energy (kJ mol^{-1}) from Arrhenius plot (Eq. (2))	368 ± 17	716 ± 25	367 ± 7	361 ± 4	315 ± 3	398 ± 5	793 ± 18
Activation free energy (kJ mol^{-1}) from consistency test using several scanning rates (Eq. (5))	343 ± 21	715 ± 88	NR	NR	347 ± 39	420 ± 55	715 ± 137
Activation free energy average (kJ mol^{-1})	360 ± 25	715 ± 46	NR	NR	325 ± 21	406 ± 29	774 ± 75
$m^{\ddagger}$(kJ mol^{-1} M^{-1})	1.67	8.12	3.94	2.77	1.47	1.69	8.32
m_{eq} (kJ mol^{-1} M^{-1})	27.95[c]	27.77	30.02	30.09	30.80	30.35	30.67
$m^{\ddagger}$ / m_{eq}	0.06	0.29	0.13	0.09	0.05	0.06	0.27
Number of residues unfolded in the transition state[d]	30	148	67	45	24	28	136

[a] From Ref. 18. [b] From Ref. 19. [c] Because the structure of *Np*TIM is not known, the ΔASA value used for the calculation of m_{eq} is the average of ΔASA from all three-dimensional structures employed for structural comparison (57388 ± 1365 A^2; Table 4). [d] Calculed from: $N_r(m^{\ddagger}/m_{eq})$, where N_r is the total number of residues in the dimeric protein, and $m^{\ddagger}/m_{eq}$ is the degree of unfolding as estimated from the urea *m* values. NR: value not reported.

Table S2. Data collection and refinement statistics of *Go*TIM, *Dr*TIM, *Sc*TIM and *Cp*TIM.

Data collection[a]				
	***Go*TIM**	***Dr*TIM**	***Sc*TIM**	***Cp*TIM**
PDB ID	4Y96	4Y90	4Y9A	4Y8F
Resolution range (Å)	36.36-1.58 (1.64-1.58)	36.13-2.09 (2.17-2.09)	43.03-2.29 (2.37-2.29)	37.59-1.54 (1.59-1.54)
Space group	P 6_5 2 2	R 3 2	P 4_3	C 1 2 1
Unit cell dimensions				
a, b, c, (Å)	124.8, 127.8, 134.2	169.6, 169.6, 202.3	86.1, 86.1, 134.0	75.3, 49.6, 71.5
α, β, γ, (º)	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 90.0	90.0, 120.0, 90.0
Total reflections	779530 (107888)	261090 (36321)	128420 (19245)	142402 (17348)
Unique reflections	81335 (8192)	64962 (6342)	43475 (4241)	33930 (3130)
Multiplicity	9.6 (9.0)	4.0 (3.9)	3.0 (3.1)	4.2 (3.7)
Completeness (%)	99.3 (97.1)	99.7 (98.6)	99.7 (98.1)	99.2 (94.4)
Mean I / sigma (I)	29.0 (4.8)	11.0 (2.9)	10.0 (3.0)	14.6 (3.1)
R-merge	0.040 (0.487)	0.097 (0.464)	0.071 (0.371)	0.062 (0.448)
Refinenment statistics				
	***Go*TIM**	***Dr*TIM**	***Sc*TIM**	***Cp*TIM**
R_{work} / R_{free} (%)	17.8 / 20.1	13.8 / 18.6	23.1 / 28.2	14.8 / 18.1
Average B-value ($Å^2$)	24.9	25.9	35.8	16.6
Protein	23.1	24.8	35.9	14.1
Ligand	32.8	49.3	---	31.5
Solvent	35.2	33.2	32.7	29.5
Number of atoms	4446	8069	7943	2362
Protein	3777	7142	7690	1977
Ligand	14	87	---	11
Water	639	832	250	372
Protein residues	500	976	1018	251
RMS (bonds) (Å)	0.007	0.011	0.009	0.010
RMS (angles) (º)	1.11	1.30	1.33	1.24
Ramachandran favored (%)	98.43	97.74	96.15	98.47
Ramachandran allowed (%)	1.18	1.85	3.56	1.15
Ramachandran outliers (%)	0.39	0.41	0.30	0.38
Clashcore	1.83	1.25	5.41	1.76

[a] Statistics for the highest-resolution shell are shown in parentheses.

Table S3. Matrix with identity sequence percentage (below) and average RMSD values (top) for IrrevTIMs and RevTIMs.[a]

Identity sequence percentage / RMSD values		IrrevTIMs																RevTIMs			
		***Ec*TIM**	***Eh*TIM**	***Gg*TIM**	***Gl*TIM**	***Gs*TIM**	***Hs*TIM**	***Lm*TIM**	***Mm*TIM**	***Mf*TIM**	***Oc*TIM**	***Pf*TIM**	***Ss*TIM**	***Tb*TIM**	***Tc*TIM**	***Ts*TIM**	***Tv*TIM**	***Yf*TIM**	***Np*TIM**	***Go*TIM**	***Dr*TIM**	***Sc*TIM**	***Cp*TIM**
IrrevTIMs	***Ec*TIM**	-----	1.51	1.30	1.53	1.48	1.39	1.13	1.24	1.10	1.50	1.27		1.41	1.48		1.24	1.08		1.49	1.37	1.36	1.46
	***Eh*TIM**	41	-----	0.83	1.50	1.16	0.97	1.09	1.65	1.15	0.93	1.04		0.82	1.07		0.90	1.21		1.51	1.20	0.87	1.07
	***Gg*TIM**	45	50	-----	1.01	0.92	0.40	0.65	1.20	1.05	0.34	0.85		0.61	0.73		0.62	0.68		1.36	1.00	0.87	1.22
	***Gl*TIM**	40	43	44	-----	1.21	1.01	1.05	1.09	1.37	0.98	1.30		1.10	0.88		1.04	1.12		0.86	1.49	1.45	1.52
	***Gs*TIM**	39	41	39	38	-----	0.99	1.49	1.51	1.20	0.94	1.30		1.13	1.30		1.11	1.24		1.33	1.07	0.88	0.78
	***Hs*TIM**	44	49	90	45	38	-----	0.93	1.26	1.06	0.22	0.96	ND	0.69	0.79	ND	0.64	0.87	ND	1.13	1.29	0.91	1.17
	***Lm*TIM**	42	51	50	46	43	50	-----	1.16	1.13	0.91	0.84		0.46	0.51		1.03	0.92		1.46	1.05	1.23	1.39
	***Mm*TIM**	65	41	40	35	41	40	38	-----	1.42	1.33	1.36		1.40	1.17		1.17	0.99		1.31	1.39	1.44	1.26
	***Mf*TIM**	41	36	37	38	43	37	38	41	-----	1.11	0.93		1.11	1.25		1.15	1.10		1.49	0.61	0.70	1.40
	***Oc*TIM**	44	49	89	45	38	98	50	40	37	-----	1.10		0.76	0.87		0.63	0.86		1.14	1.13	0.92	1.11
	***Pf*TIM**	39	44	43	40	37	42	45	38	35	42	-----		0.87	0.93		1.07	1.01		1.52	1.10	1.01	1.42
	***Ss*TIM**	43	48	87	44	37	93	49	39	35	93	42	-----						ND				
	***Tb*TIM**	42	43	51	44	40	52	69	38	38	51	43	39	-----	0.47		0.89	0.98		1.48	1.07	0.96	1.35
	***Tc*TIM**	42	45	50	46	41	52	68	39	39	51	43	50	74	-----	ND	0.91	0.88	ND	1.38	1.49	1.17	1.48
	***Ts*TIM**	49	48	61	47	39	59	46	39	38	59	41	58	47	50	-----				ND			
	***Tv*TIM**	40	44	48	41	43	47	43	40	39	47	38	46	42	42	48	-----	1.02		1.15	1.01	0.89	0.95
	***Yf*TIM**	44	43	53	48	38	52	46	42	39	52	42	51	48	46	49	46	-----	ND	1.49	1.27	1.15	1.47
	***Np*TIM**	39	38	37	33	47	36	36	43	40	36	33	35	34	32	34	38	35	-----			ND	
RevTIMs	***Go*TIM**	46	41	46	39	45	45	39	43	44	45	40	43	41	40	45	39	40	38	-----	1.25	1.43	1.44
	***Dr*TIM**	38	34	38	36	41	38	37	39	48	37	33	37	37	39	38	39	37	40	40	-----	0.76	1.35
	***Sc*TIM**	42	40	38	38	48	37	40	40	65	37	34	36	37	38	40	43	37	46	45	49	-----	0.94
	***Cp*TIM**	45	42	41	38	55	40	42	44	41	40	40	40	40	40	41	43	40	48	44	42	52	-----

[a] ND: average RMSD value not determined because there is no structural data for *Np*TIM, *Ss*TIM and *Ts*TIM.

Table S4. Amino acidic composition used in the sequence comparison of IrrevTIMs and RevTIMs.

Structural property	*Dr*TIM	*Sc*TIM	*Cp*TIM	Average RevTIMs	Average IrrevTIMs
Amino acid composition (%)					
Alanine (A)	17.0	13.6	12.4	14.3 ± 2.0	12.0 ± 3.1
Cysteine (C)	1.2	1.6	2.4	1.7 ± 0.5	1.5 ± 0.4
Aspartic acid (D)	4.9	7.4	4.8	5.7 ± 1.2	4.4 ± 1.4
Glutamic acid (E)	8.1	7.4	9.6	8.3 ± 0.9	7.2 ± 1.3
Phenylalanine (F)	0.4	1.6	2.8	1.6 ± 1.0	3.2 ± 0.9
Glycine (G)	10.9	8.9	7.2	9.0 ± 1.5	8.8 ± 1.4
Histidine (H)	2.0	3.1	2.0	2.4 ± 0.5	2.3 ± 0.6
Isoleucine (I)	3.2	5.0	8.0	5.4 ± 1.9	6.9 ± 1.2
Lysine (K)	3.6	7.0	7.6	6.1 ± 1.7	6.9 ± 1.8
Leucine (L)	10.1	8.5	4.8	7.8 ± 2.2	7.1 ± 1.3
Methionine (M)	1.6	1.9	3.2	2.2 ± 0.7	1.6 ± 0.7
Asparagine (N)	3.2	2.3	4.8	3.4 ± 1.0	4.0 ± 1.2
Proline (P)	4.0	2.3	2.8	3.1 ± 0.7	3.5 ± 0.9
Glutamine (Q)	2.8	3.9	3.6	3.4 ± 0.4	4.0 ± 1.1
Arginine (R)	4.9	3.5	2.8	3.7 ± 0.9	3.6 ± 0.8
Serine (S)	4.9	3.9	2.4	3.7 ± 1.0	5.1 ± 1.1
Threonine (T)	4.5	4.3	7.2	5.3 ± 1.3	5.1 ± 0.9
Valine (V)	8.9	9.7	8.8	9.1 ± 0.4	9.2 ± 1.1
Tryptophan (W)	1.2	0.8	0.8	0.9 ± 0.2	1.5 ± 0.5
Tyrosine (Y)	2.4	3.5	2.4	2.8 ± 0.5	2.0 ± 0.6
Physicochemical amino acid properties (%)					
Charged (DEHKR)	23.5	28.3	26.7	26.2 ± 2.0	24.4 ± 1.9
Positively charged (HKR)	10.5	13.6	12.4	12.1 ± 1.2	12.7 ± 1.3
Negatively charged (DE)	13.0	14.7	14.3	14.0 ± 0.8	11.6 ± 1.4
Aliphatic (AGILPV)	54.3	48.1	43.8	48.7 ± 4.3	47.5 ± 3.3
Aromatic (FHWY)	6.1	8.9	8.0	7.7 ± 1.2	9.0 ± 0.8
Polar (DEKNQR)	27.5	31.4	33.1	30.7 ± 2.3	30.1 ± 2.4
Neutral, polar (CNQSTY)	19.0	19.4	22.7	20.4 ± 1.7	21.8 ± 2.7
Neutral, non-polar (AFGILMPVW)	57.5	52.3	50.6	53.5 ± 2.9	53.8 ± 2.9
Hydrophobic (CFILMVW)	26.7	29.1	30.7	28.8 ± 1.6	31.0 ± 1.4
Small size (ACGSTV)	47.4	41.9	40.2	43.2 ± 3.1	41.8 ± 3.0
Medium size (DEHILMNPQ)	40.1	41.9	43.4	41.8 ± 1.4	41.0 ± 2.9
Large size (FKRWY)	12.6	16.3	16.3	15.1 ± 1.8	17.2 ± 2.1

Table S5. Secondary structure elements and stabilizing interactions for IrrevTIMs and RevTIMs.

Structural property	*Dr*TIM	*Sc*TIM	*Cp*TIM	Average RevTIMs	Average IrrevTIMs
Secondary structure elements (%)					
α-helix	44	48	43	45.0 ± 2.2	44.9 ± 2.1
β-strand	21	20	21	20.7 ± 0.5	21.8 ± 1.0
Random coil	35	32	36	34.3 ± 1.7	33.3 ± 1.8
Stabilizing interactions					
Total H-bonds of the oligomer	490	503	510	501 ± 8	521 ± 24
Interface H-bonds	27	19	24	23 ± 3	28 ± 5
Total salt bridges of the oligomer	37	50	44	44 ± 5	34 ± 7
Interface salt bridges	2	2	12	5 ± 5	6 ± 3

Table S6. Dimeric TIM PDB files used in the structural comparison of IrrevTIMs and RevTIMs.

Organism	Code	PDB ID	Resolution (Å)	Spacial group	Asymmetric unit[a]	Conformation of active site loops		Reference
						Subunit A	Subunit B	
Clostridium perfringens	*Cp*TIM	4Y8F	1.54	C 1 2 1	Monomer	Open	Open	This work
Deinococcus radiodurans	*Dr*TIM	4Y90	2.10	R 3 2	Tetramer	Open	Open	This work
Entamoeba histolytica	*Eh*TIM	1M6J	1.50	P 2_1 2_1 2	Dimer	Open	Open	3
Escherichia coli	*Ec*TIM	4K6A	1.80	P 2_1 2_1 2_1	Dimer	Open	Open	4
Gallus gallus	*Gg*TIM	1TPH	1.80	P 2_1 2_1 2_1	Dimer	Closed	Closed	5
Gemmata obscuriglobus	*Go*TIM	4Y96	1.58	P 6_5 2 2	Dimer	Closed	Open	This work
Geobacillus stearotermophilus	GsTIM	1BTM	2.80	P 2_1 2_1 2	Dimer	Closed	Closed	6
Giardia lamblia	*Gl*TIM	2DP3	2.10	I 2 2 2	Monomer	Closed	Open	7
Homo sapiens	*Hs*TIM	2JK2	1.70	P 2_1 2_1 2_1	Dimer	Open	Open	8
Leishmania mexicana	*Lm*TIM	1AMK	1.83	C 1 2 1	Monomer	Closed	Closed	9
Moritella marina	*Mm*TIM	1AW2	2.65	P 1 2_1 1	Dimer	Open	Open	10
Mycobacterium tuberculosis	*Mt*TIM	3TA6	1.41	C 1 2 1	Dimer	Open	Open	11
Oryctolagus cuniculus	*Oc*TIM	1R2R	1.50	P 2_1 2_1 2_1	Dimer	Open	Open	12
Plasmodium falciparum	*Pf*TIM	1YDV	2.20	C 1 2 1	Dimer	Open	Open	13
Saccharomyces cerevisiae	*Yt*TIM	1NF0	1.60	P 2_1 2_1 2_1	Dimer	Open	Closed	14
Streptomyces coelicolor	*Sc*TIM	4Y9A	2.30	P 4_3	Dimer	Open	Open	This work
Trichomonas vaginalis[b]	*Tv*TIM	3QSR	2.05	P 2 2_1 2_1	Monomer	Open	Open	15
Trypanosoma brucei	*Tb*TIM	5TIM	1.83	P 2_1 2_1 2_1	Dimer	Open	Closed	16
Trypanosoma cruzi	*Tc*TIM	1TCD	1.83	P 2_1 2_1 2_1	Dimer	Closed	Open	17

[a] The number of molecules in asymmetric unit was determined by the Matthews coefficient analysis by solvent content percentage in the crystal.

[b] Structural comparison of *Tv*TIM was realized with the three-dimensional structure of its dimeric Ile45 variant.

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