STRUCTURAL BIOLOGY COMMUNICATIONS

Volume 72 (2016)

Supporting information for article:

Crystal structures of two monomeric TIM variants identified via a directed evolution protocol selecting for l-arabinose isomerase activity

Mirja Krause, Tiila-Riikka Kiema, Peter Neubauer and Rik K. Wierenga

Supplementary Material

Table S1 Buffers used in the crystallization protocols

variants	crystallization buffers		cryo method
	protein buffer	well solution buffer	
A-TIM	20mM Tris pH 7.4, 10mg mL^{-1} protein	20 % PEG6000 100 mM citrate pH5.5	100 % paraffin oil (dried)
Ma18	10mM Tris pH 7.4 50 mM citrate, [a] 5mg mL^{-1} protein	1.75 M $(NH_4)_2HPO_4$ pH 8.2	100 % paraffin oil (dried)
Ma21	20mM Tris pH 7.4 100mM L-arabinose, 10mg mL^{-1} protein	21 % PEG6000 100 mM citrate pH 5.5 5% glycerol	cryo buffer: 21 % PEG6000 100 mM citrate pH 5.5 5% glycerol 15 % PEG400 10 mM L-arabinose

[a] Obtained by adding a 100mM citrate pH 5.5 solution to the protein storage buffer.

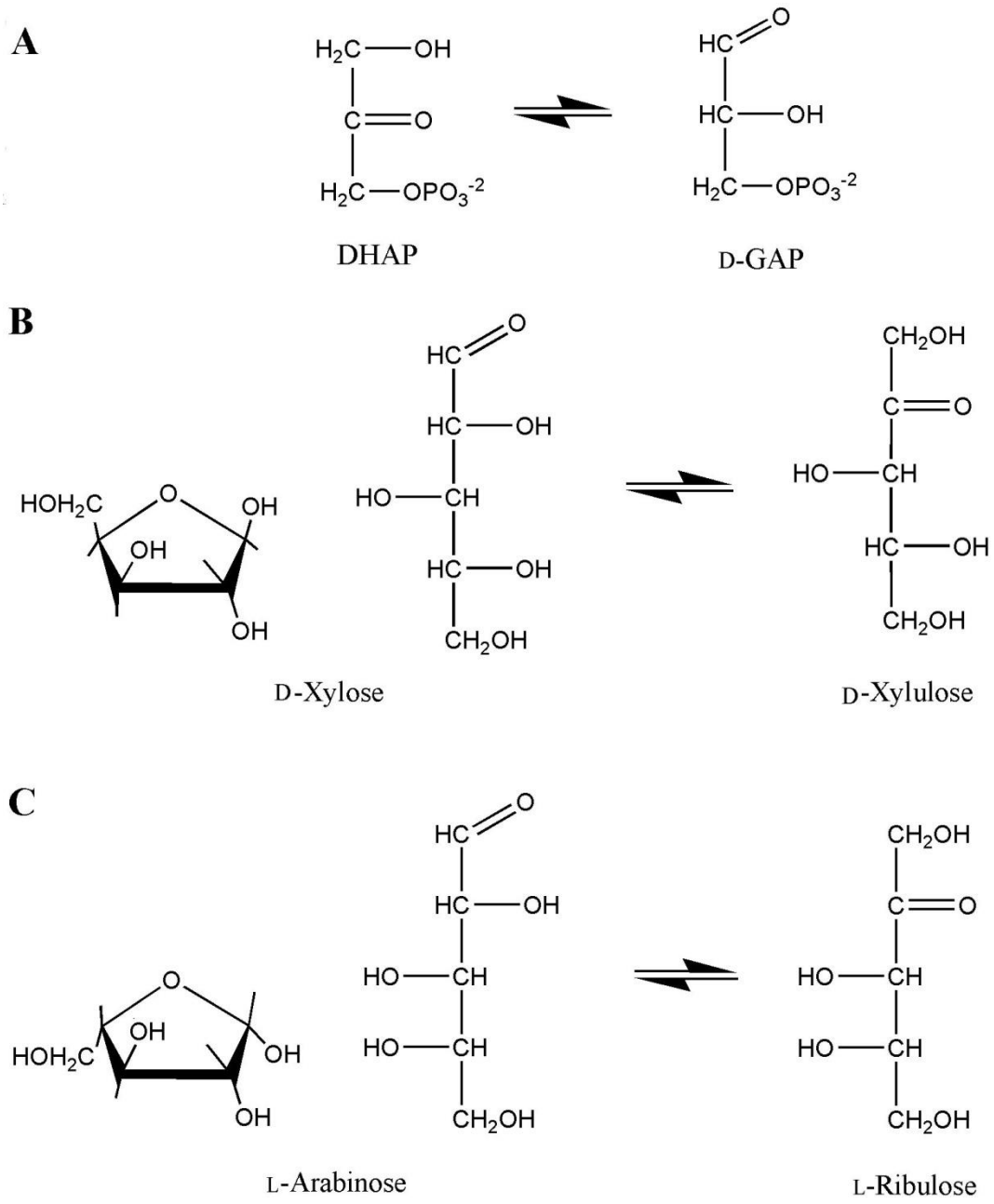

Figure S1 Overview of the reactions targeted in the directed evolution experiments, compared to the reaction catalyzed by TIM. The chemical structures of the substrate and the product of each reaction are shown in Fisher projections. If a ring structure of the substrate exists it is also displayed. A) The reaction catalyzed by wild type TIM, DHAP is dihydroxyacetone phosphate, D-GAP is D-glyceraldehye-3-phophate. B) The reaction catalyzed by D-xylose isomerase. C) The reaction catalyzed by L-arabinose isomerase.

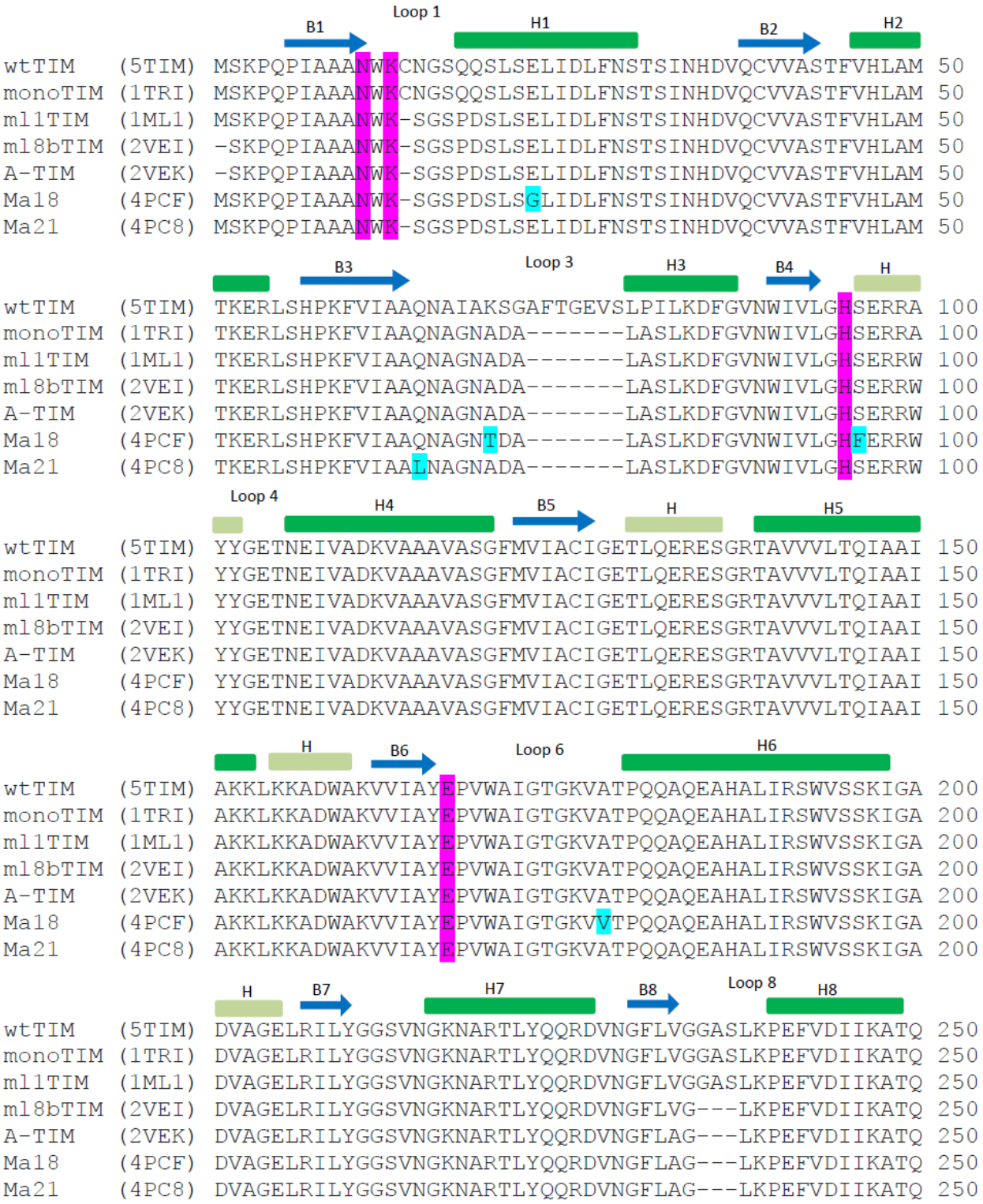

Figure S2 The structure based sequence alignment of wild type *Trypanosoma brucei brucei* TIM and its monomeric variants. The sequence numbering refers to the residue numbering of wild type TIM which was kept, even if residues were deleted. Secondary structure motifs of the wild type TIM of the framework helices and strands are marked with green bars (alpha helices) and blue arrows (beta strands) and the helices in the loop regions are colored by light-green bars. The four catalytic residues Asn11, Lys13, His95 and Glu167 are highlighted in magenta. Deletions are indicated by hyphens. The mutated positions of Ma18 and Ma21 with respect to A-TIM, are highlighted in blue.

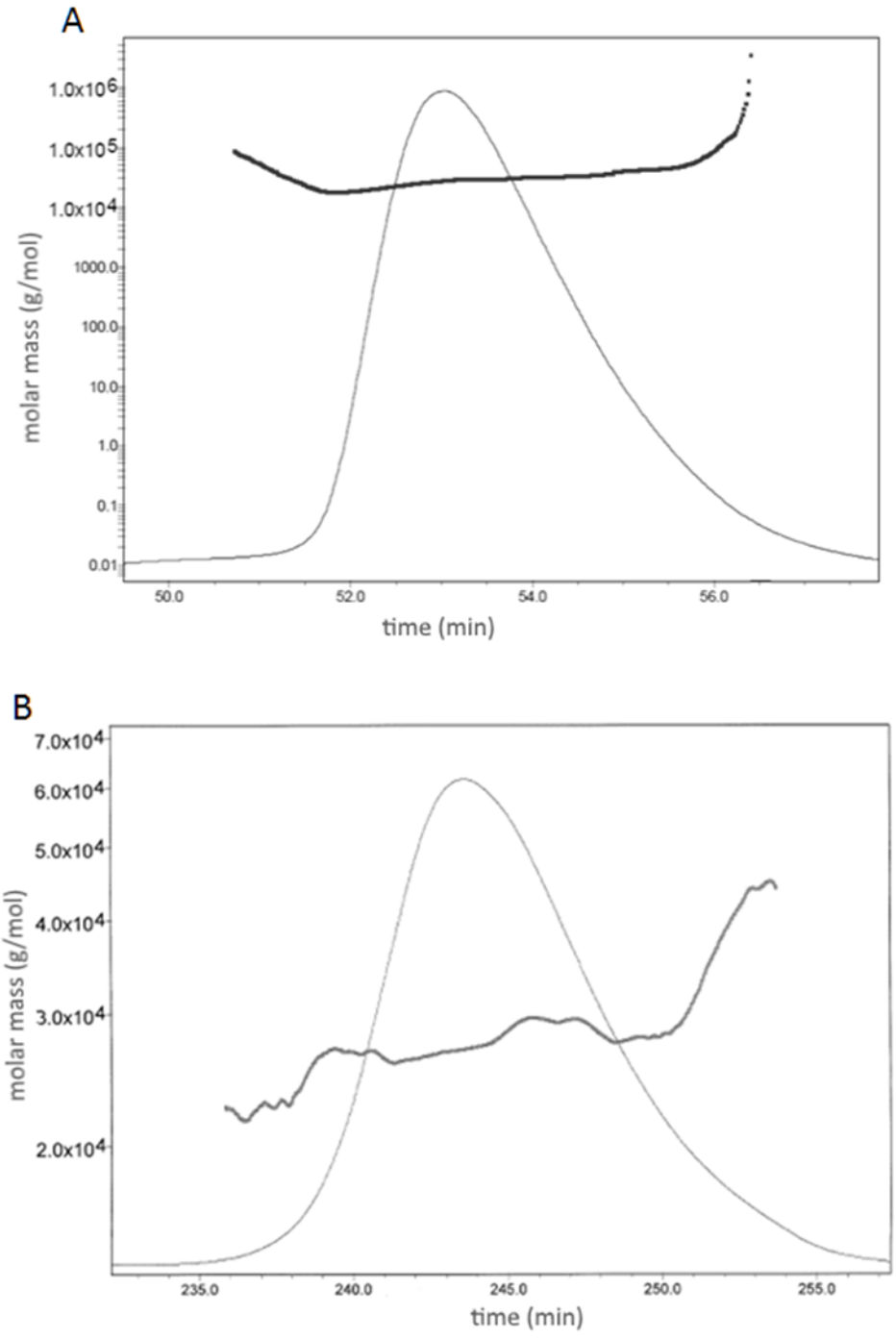

Figure S3 SLS graphs of A) Ma18 and B) Ma21. The horizontal axis refers to the elution time and the vertical axis shows the molecular weight calculated by the Astra software. The thin line is the UV-absorption trace and the thick line is the calculated molecular weight, being about 25kD for the peak fractions.

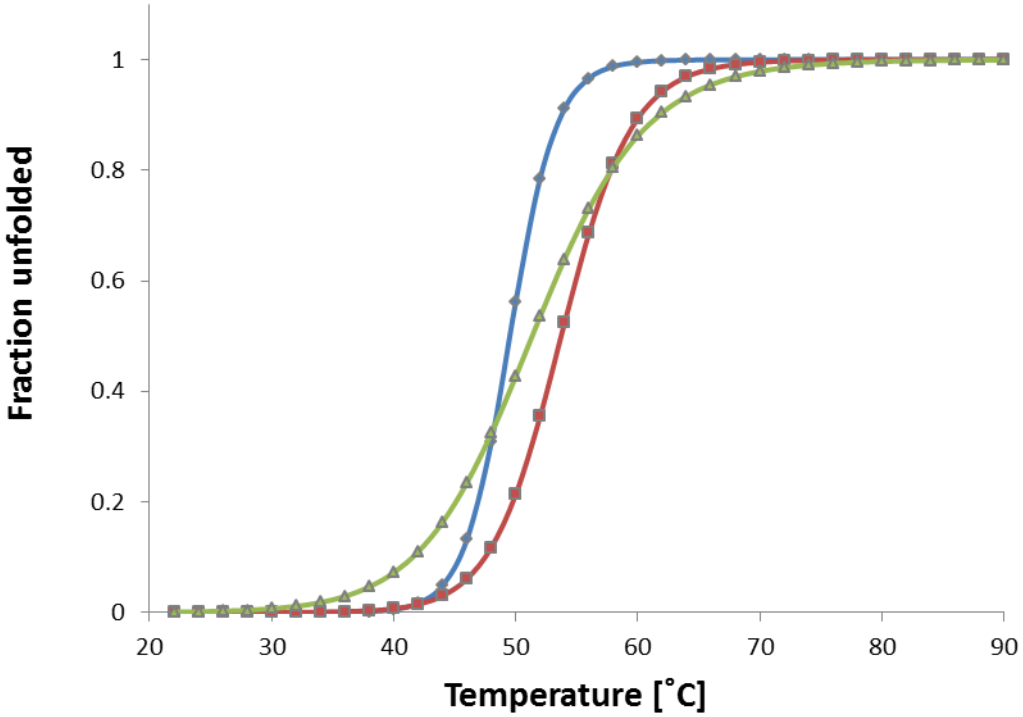

Figure S4 CD melting curves showing the percentages of unfolded protein as a function of the temperature. The calculated T_m value for A-TIM (blue, ♦) is 49.5 ± 0.1 °C, for Ma18 (green, Δ) it is 51.3 °C and for Ma21 (red,■) it is 53.7 ± 0.2°C.

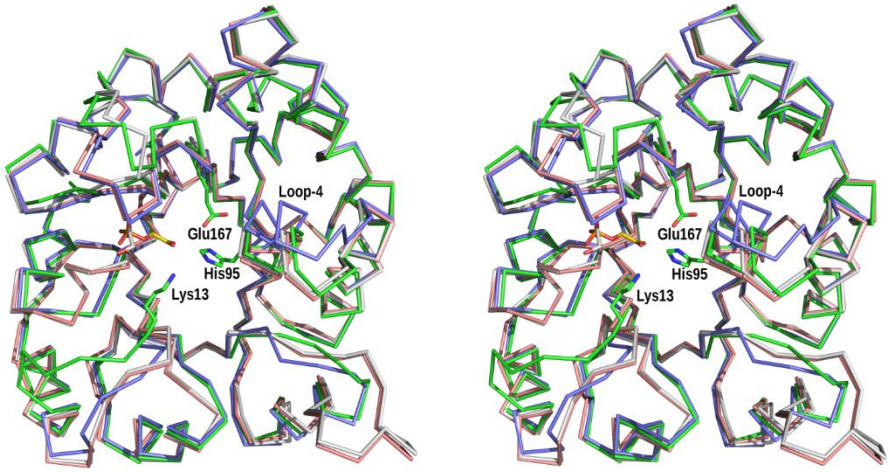

Figure S5 Comparison of the Cα-traces of Ma18 (green, PDB code: 4PCF, chain C, open), trypanosomal TIM (grey, PDB code: 5TIM, chain A, open), Ma21 (blue, PDB code: 4PC8, closed), and leishmanial TIM (pink, PDB code: 1N55, closed). The view is towards the "front" loops and into the active site. Also shown is the active site ligand PGA (yellow) as bound to loop-6 and loop-7 of wild type TIM (PDB code: 1N55) and glycolic acid (GOA) (grey) as bound to Ma21 (PDB code: 4PC8). It can be noted that in the Ma21 structure the helical segment of loop-4 has adopted an outward oriented conformation, as also observed in the crystal structure of monoTIM (PDB code: 1TRI) (Borchert *et al.*, 1993b).

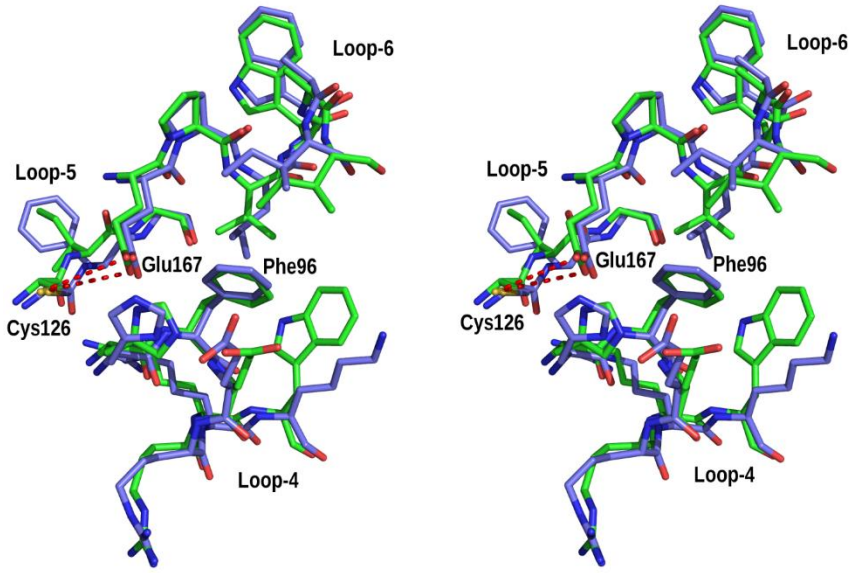

Figure S6 Comparison of the loop-4 region of Ma18 (green, PDB code: 4PCF) and *P. falciparum* TIM (blue, PDB code: 1YDV). In both structures the canonical Ser96 is replaced by a phenylalanine. Also shown is the highly conserved Cys126, near the Glu167 side chain, being hydrogen bonded to the side chain carboxylate oxygens of the catalytic glutamate, adopting its swung-out conformation.