



## Data Article

# Supporting data on characterisation of linker switch mutants of *Plasmodium falciparum* heat shock protein 110 and canonical Hsp70

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## ABSTRACT

Here, we present data on characterisation of the linker of *Plasmodium falciparum* Hsp110 (PfHsp70-z) relative to the linker of canonical Hsp70s in support of a co-published article [1]. The linker of PfHsp70-z was switched with that of canonical Hsp70s, represented by PfHsp70-1 (cytosolic counterpart of PfHsp70-z) and *E. coli* Hsp70/DnaK. The datasets represent comparative analyses of PfHsp70-z, PfHsp70-1, and *E. coli* DnaK, relative to their linker switch mutants; PfHsp70-z<sub>1S</sub>, PfHsp70-1<sub>1S</sub>, DnaK<sub>1S</sub>, respectively. Intrinsic and extrinsic fluorescence spectroscopic analyses were employed to elucidate effects of the mutations on the structural features of the proteins. The structural conformations of the proteins were analysed in the absence as well as presence of nucleotides. In addition, stability of the proteins to stress (pH changes and urea) was also determined. Surface plasmon resonance (SPR) was employed to determine affinity of the proteins for ATP. The relative affinities of PfHsp70-z and PfHsp70-1 for the parasite cytosol localised, J domain co-chaperone, PfHsp40, was determined by SPR analysis. The effect of the linker of PfHsp70-z on the interaction of DnaK<sub>1S</sub> with DnaJ (a

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co-chaperone of DnaK), was similarly determined. These data could be used for future investigations involving protein-protein/ligand interactions as described in [1]. The raw data obtained using the various techniques here described are hosted in the Mendeley Data repository at [2].

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## Specifications Table

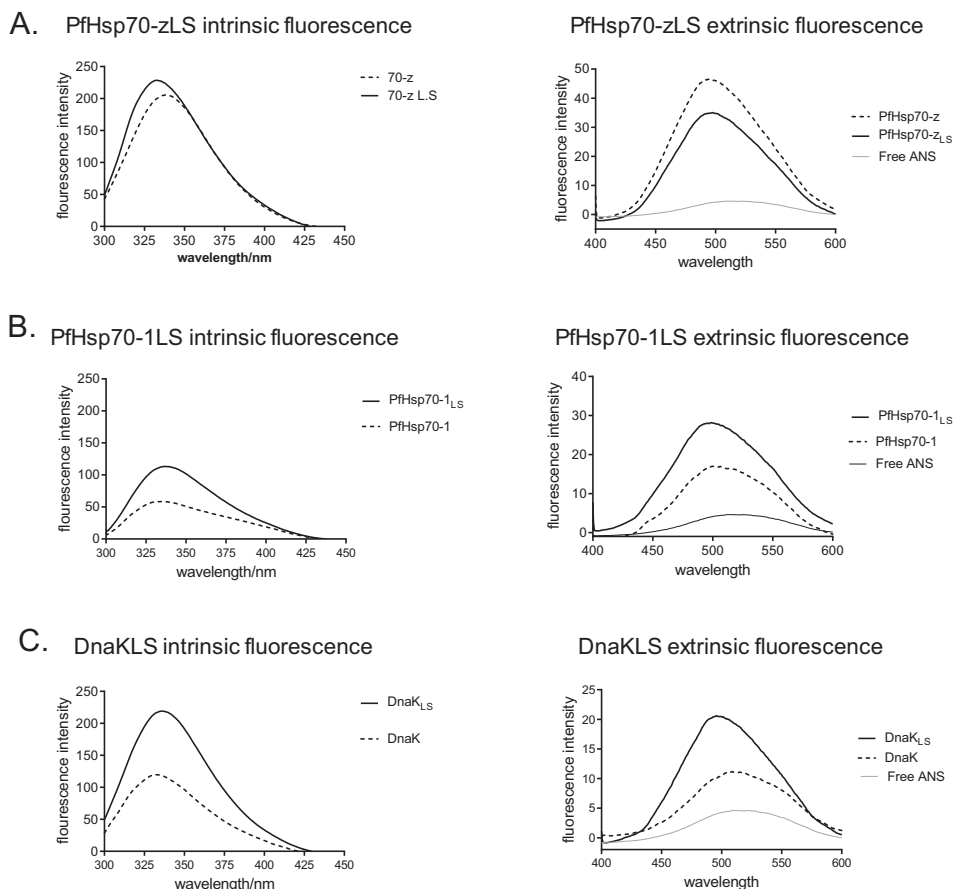
Subject	Biological Sciences
Specific subject area	Protein Biochemistry, Protein-Protein Interaction
Type of data	Table Graph Figure
How data were acquired	Instruments: JASCO FP-6300 spectrofluorometer (Jasco, Spain); BioNavis™ 420A ILVES MP SPR (BioNavis, Tampere, Finland) Software: TraceDrawer software version 1.8 (Ridgeview Instruments, Uppsala, Sweden)
Data format	Raw analysed
Parameters for data collection	Intrinsic fluorescence spectroscopic analysis: 300–450 nm Extrinsic fluorescence spectroscopic analysis; 8-anilino-1-naphthalene sulfonate (ANS) based: 400–600 nm Both intrinsic and extrinsic spectroscopic analyses were conducted on proteins subjected to pH range of 5–9 and urea at a concentration range of 0–8 M.
Description of data collection	Surface Plasmon Resonance: flow rate of 50 µl/min was used Extrinsic fluorescence analysis: Excitation at 390 nm and emission monitored at 400–600 nm Intrinsic fluorescence analysis: Excitation at 295 nM and emission monitored at 320–450 nM Surface plasmon resonance: Data were generated using TraceDrawer software.
Data source location	University of Venda, Thohoyandou, South Africa
Data accessibility	The data are provided in this article and the original raw data are provided [2].
Related research article	G. Chakafana, P.T. Mudau, T. Zininga, A. Shonhai, Characterisation of a unique linker segment of the <i>Plasmodium falciparum</i> cytosol localised Hsp110 chaperone. Int. J. Biol. Macromol. 180 (2021) 272–285.

## Value of the Data

- Data provides structure–function characterisation of the effect of swapping linker segments of canonical Hsp70s and Hsp110 of *P. falciparum*.
- The data can benefit structural biologists and biochemists working on protein structure–function assays and protein-protein/ligand interactions.
- The data could benefit protein biochemists investigating the role of protein motifs in regulating their stability to physiological stress and chemical denaturants.

## 1. Data Description

Chimeric linker switch (LS) variants of PfHsp70–1 (PlasmoDB accession number: PF3D7\_0818900), PfHsp70–z (PlasmoDB accession number: PF3D7\_088000) and DnaK (Uniprot accession number P0A6Y8); denoted as PfHsp70–1<sub>LS</sub>, PfHsp70–z<sub>LS</sub> and DnaK<sub>LS</sub>, were created

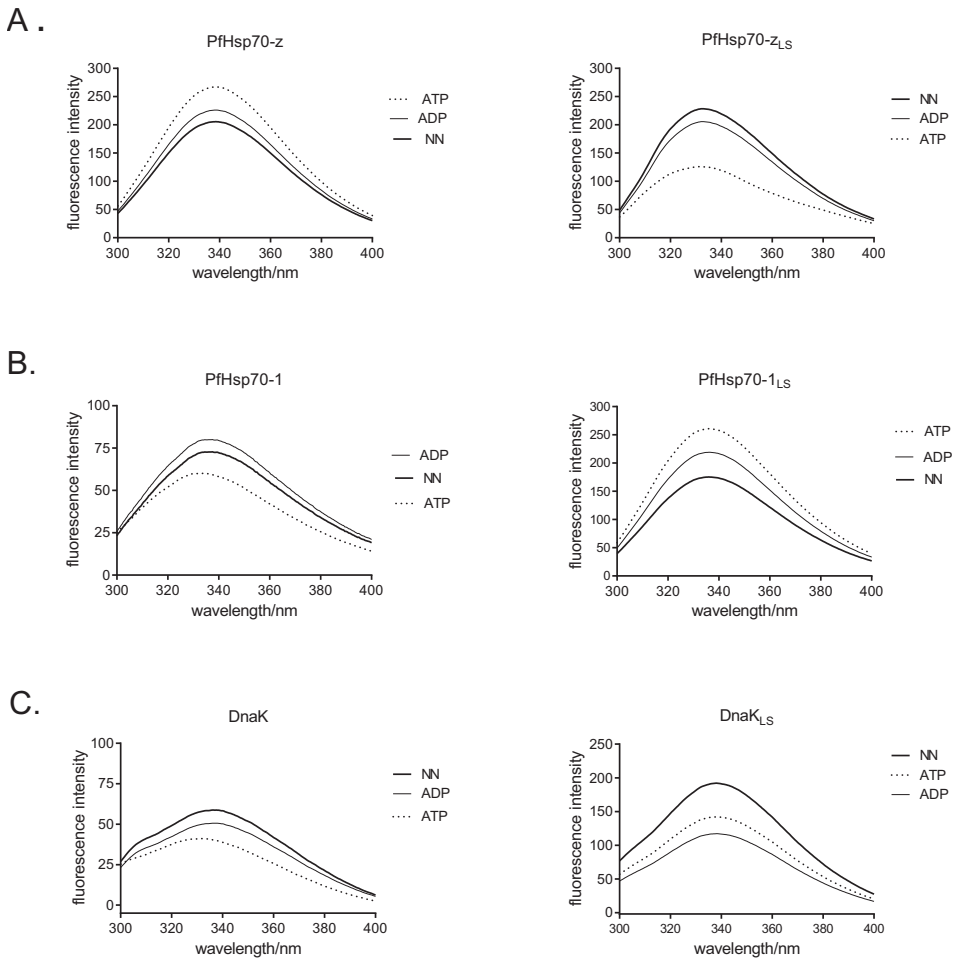


**Fig. 1.** Analyses of the linker mutants relative to wild type Hsp70s by intrinsic and extrinsic fluorescence.

by substitution mutations of the respective linkers. The effects of the linker mutations on the various features of the proteins such as stability, affinity for ATP, and Hsp40 co-chaperone binding were determined as outlined below.

### 1.1. Intrinsic and extrinsic fluorescence based analyses of PfHsp70-1, PfHsp70-z, and DnaK relative to their linker mutants

To determine the effects of the linker mutations on the tertiary structures of the Hsp70s, intrinsic and extrinsic fluorescence analyses were conducted on the recombinantly produced proteins using tryptophan and ANS fluorescence spectroscopy, respectively. DnaK harbors one tryptophan residue ( $W^{102}$ ) located in its nucleotide binding domain (NBD) while PfHsp70-1 possesses three tryptophan residues located in the NBD ( $W^{32}$  and  $W^{101}$ ) and the SBD ( $W^{593}$ ). PfHsp70-z harbors only two tryptophan residues located in its substrate binding domain (SBD) at positions  $W^{436}$  and  $W^{692}$ , respectively. The emission spectra for the proteins and their respective mutants are shown with the proteins exhibiting emission maxima within the 330–345 nm range (Fig. 1) as previously reported [3].



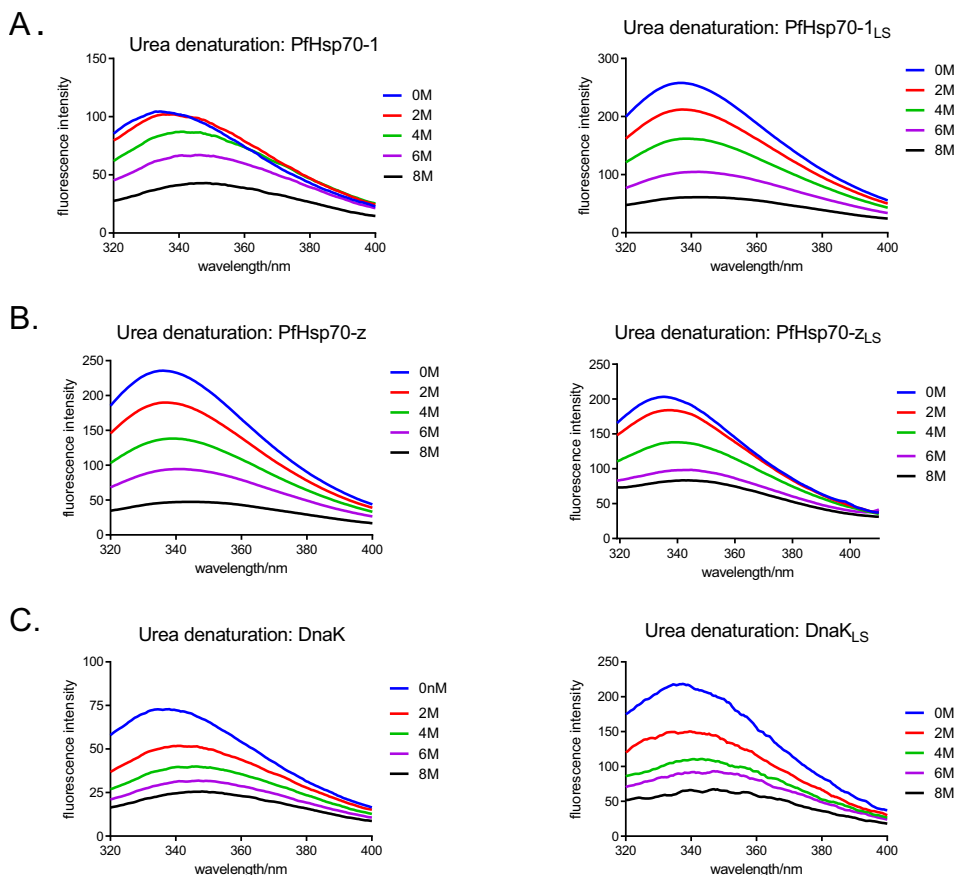
**Fig. 2.** Analysis of tertiary conformational changes induced by nucleotides on linker switch mutants versus wild type Hsp70s.

Tertiary structure conformations of recombinant Hsp70s were investigated using intrinsic (tryptophan) and extrinsic (ANS) fluorescence: (A) PfHsp70-z versus PfHsp70-z<sub>LS</sub> (B) PfHsp70-1 versus PfHsp70-1<sub>LS</sub> (C) DnaK versus DnaK<sub>LS</sub>.

*1.2. Data on the comparative effects of nucleotides on the conformations of the Hsp70s relative to their link switch mutants*

We further investigated the effect of nucleotides (ADP and ATP) on the tertiary structures of the three Hsp70s relative to their linker mutants using intrinsic (tryptophan) fluorescence analysis (Fig. 2).

Tertiary structural changes were monitored by intrinsic (tryptophan) fluorescence. Assays were conducted in the absence (NN) or presence of ADP/ATP: (A) PfHsp70-z versus PfHsp70-z<sub>LS</sub> (B) PfHsp70-1 versus PfHsp70-1<sub>LS</sub> (C) DnaK versus DnaK<sub>LS</sub>.



**Fig. 3.** Urea denaturation of recombinant Hsp70s tertiary structure.

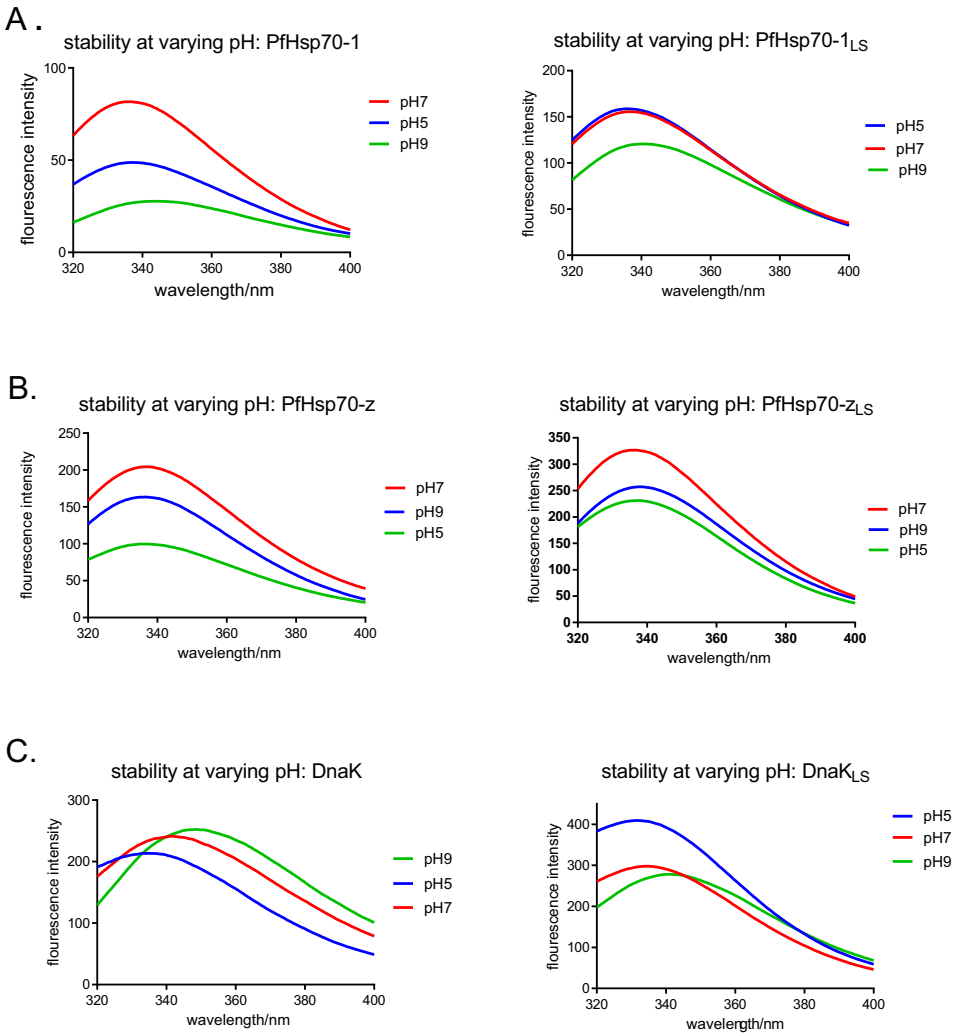
### 1.3. Comparative conformational stabilities of the Hsp70s and their mutants in response to urea treatment

The proteins were exposed to varying concentrations of urea (Fig. 3). As reported previously [2], the Hsp70s generally exhibited maxima between 330 and 340 nm at 0 M urea (Fig. 3). The spectral profiles of the wild type proteins relative to those of their linker switch mutants are shown (Fig. 3).

The recombinant Hsp70s were incubated in urea concentrations ranging from 0 to 8 M and tryptophan fluorescence readings were taken at each concentration. Fluorescence spectra for (A) PfHsp70-1/1<sub>LS</sub>; (B) PfHsp70-z/z<sub>LS</sub>; and (C) DnaK/K<sub>LS</sub> are presented.

### 1.4. Analysis of the effect of pH changes on the conformational stability of the Hsp70 chaperones and their linker derivatives

The proteins were subjected to varying pH levels (5.0, 7.0 and 9.0) and the resultant shifts in intrinsic fluorescence signals between wild type proteins and their linker derivatives were monitored (Fig. 4).



**Fig. 4.** Effect of pH on tertiary structure of linker mutants.

Effect of pH changes on the tertiary structure conformations of: PfHsp70-1/1<sub>LS</sub> (A); (B) PfHsp70-z/z<sub>LS</sub> (B); and DnaK/K<sub>LS</sub> (C), respectively.

1.5. Effect of linker switch mutations on the affinity of the Hsp70s for ATP

The relative affinities of the wild type proteins versus linker switch mutants for ATP were determined by SPR analysis and the resultant equilibrium binding affinity curve is shown (Fig. 5). The equilibrium binding constants ( $K_D$  values) were determined as shown (Table 1; Fig. 5).

Equilibrium binding affinity curve for wild type and linker mutants of PfHsp70-1, PfHsp70-z and DnaK at a concentration range of 0-5 nM.

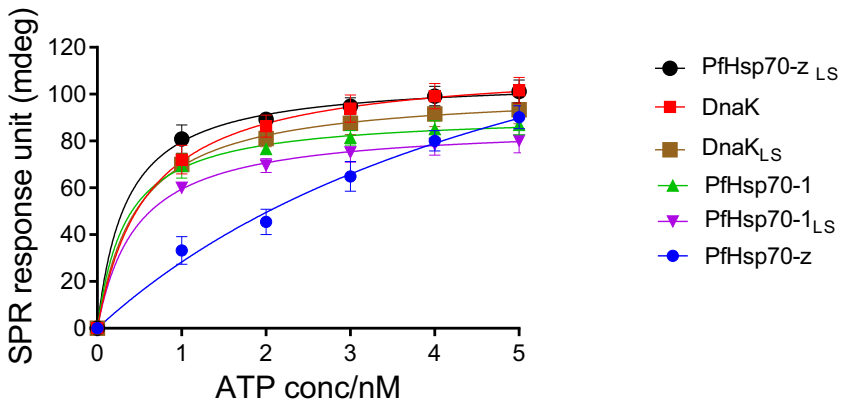


Fig. 5. Relative ATP binding profiles of wild type Hsp70s and their linker switch derivatives.

**Table 1**

Comparative binding affinities of Hsp70 and their linker mutants for ATP.

	$K_D$ ( $\mu\text{M}$ )	$\chi^2$
PfHsp70-1	0.174 ( $\pm 0.04$ )	1.73
PfHsp70-1 <sub>LS</sub>	0.537 ( $\pm 0.07$ )	2.71
PfHsp70-z	2.410 ( $\pm 0.10$ )	2.57
PfHsp70-z <sub>LS</sub>	0.442 ( $\pm 0.02$ )	1.12
DnaK	0.398 ( $\pm 0.08$ )	1.38
DnaK <sub>LS</sub>	0.314 ( $\pm 0.04$ )	1.06

The  $K_D$  values for the individual proteins were derived from three independent analyses. Standard errors generated are shown.

### 1.6. Interaction of Hsp70s with their respective Hsp40 co-chaperone

The interaction of PfHsp70-1 and PfHsp70-z with a *P. falciparum* cytosol localised co-chaperone, PfHsp40, was investigated by SPR analysis. Similarly, the interaction of DnaK with its Hsp40 co-chaperone, DnaJ was determined. The assay was conducted for wild type proteins and their linker derivatives. Analysis was conducted in the absence of nucleotide (NN) or presence of 5 mM ATP/ADP. Data representing the association ( $K_a$ ) and dissociation rate ( $K_d$ ) constants are summarised (Table 2). The associated equilibrium dissociation ( $K_D$ ) constants and the respective  $\chi^2$  data were previously reported [1]. Sensograms representing PfHsp40-PfHsp70-1/PfHsp70-1<sub>LS</sub> association in the absence of nucleotide and in the presence of ADP are shown (Fig. 6A-B). Further illustrated are sensograms representing interaction of PfHsp40 with PfHsp70-z/PfHsp70-z<sub>LS</sub> in the absence of nucleotide (Fig. 6C). The sensograms representing interaction of PfHsp40 with PfHsp70-1/PfHsp70-1<sub>LS</sub> in the presence of ATP and for PfHsp40-PfHsp70-z/PfHsp70-z<sub>LS</sub> association in the presence of either ATP or ADP were previously reported [1].

SPR kinetics data and sensograms for the association of DnaK/DnaK<sub>LS</sub> with the co-chaperone, DnaJ, investigated in the absence or presence of ATP/ADP are similarly illustrated (Table 2; Fig. 7).

**Raw data:** Direct URL to data: <http://dx.doi.org/10.17632/bsp89dh4nn.1>

Text files and graph generated data for intrinsic and extrinsic fluorescence analyses as well as surface plasmon resonance analysis.

**Table 2**

SPR kinetics for Hsp40-Hsp70 interaction.

Ligand	Analyte	Ka (1/Ms)	Kd (1/s)
DnaK	Dnaj (NN)	3.93 ( $\pm 0.03$ )e <sup>4</sup>	1.87 ( $\pm 0.07$ )e <sup>-2</sup>
	Dnaj (ADP)	1.19 ( $\pm 0.02$ )e <sup>4</sup>	2.71 ( $\pm 0.07$ )e <sup>-3</sup>
	Dnaj (ATP)	6.01 ( $\pm 0.01$ )e <sup>5</sup>	4.09 ( $\pm 0.09$ )e <sup>-3</sup>
DnaK <sub>LS</sub>	Dnaj (NN)	3.19 ( $\pm 0.09$ )e <sup>4</sup>	5.22 ( $\pm 0.02$ )e <sup>-2</sup>
	Dnaj (ADP)	2.11 ( $\pm 0.01$ )e <sup>5</sup>	6.76 ( $\pm 0.06$ )e <sup>-2</sup>
	Dnaj (ATP)	4.17 ( $\pm 0.07$ )e <sup>5</sup>	7.32 ( $\pm 0.06$ )e <sup>-2</sup>
PfhHsp70-1	PfhHsp40 (NN)	1.23 ( $\pm 0.03$ )e <sup>4</sup>	8.15 ( $\pm 0.05$ )e <sup>-2</sup>
	PfhHsp40 (ADP)	1.53 ( $\pm 0.03$ )e <sup>3</sup>	8.66 ( $\pm 0.06$ )e <sup>-2</sup>
	PfhHsp40 (ATP)	6.87 ( $\pm 0.07$ )e <sup>5</sup>	3.61 ( $\pm 0.01$ )e <sup>-2</sup>
PfhHsp70-1 <sub>LS</sub>	PfhHsp40 (NN)	6.04 ( $\pm 0.04$ )e <sup>3</sup>	9.55 ( $\pm 0.06$ )e <sup>-2</sup>
	PfhHsp40 (ADP)	2.20 ( $\pm 0.02$ )e <sup>3</sup>	1.18 ( $\pm 0.08$ )e <sup>-2</sup>
	PfhHsp40 (ATP)	6.14 ( $\pm 0.04$ )e <sup>3</sup>	3.87 ( $\pm 0.07$ )e <sup>-2</sup>
PfhHsp70-z	PfhHsp40 (NN)	3.37 ( $\pm 0.40$ )e <sup>4</sup>	7.13 ( $\pm 0.60$ )e <sup>-2</sup>
	PfhHsp40 (ADP)	6.58 ( $\pm 0.08$ )e <sup>3</sup>	3.96 ( $\pm 0.06$ )e <sup>-2</sup>
	PfhHsp40 (ATP)	7.52 ( $\pm 0.02$ )e <sup>3</sup>	3.69 ( $\pm 0.09$ )e <sup>-2</sup>
PfhHsp70-z <sub>LS</sub>	PfhHsp40 (NN)	9.02 ( $\pm 0.02$ )e <sup>3</sup>	1.26 ( $\pm 0.06$ )e <sup>-2</sup>
	PfhHsp40 (ADP)	6.17 ( $\pm 0.07$ )e <sup>3</sup>	4.29 ( $\pm 0.20$ )e <sup>-2</sup>
	PfhHsp40 (ATP)	7.25 ( $\pm 0.25$ )e <sup>3</sup>	3.79 ( $\pm 0.70$ )e <sup>-2</sup>

## 2. Experimental Design, Materials and Methods

### 2.1. Tertiary structure determination

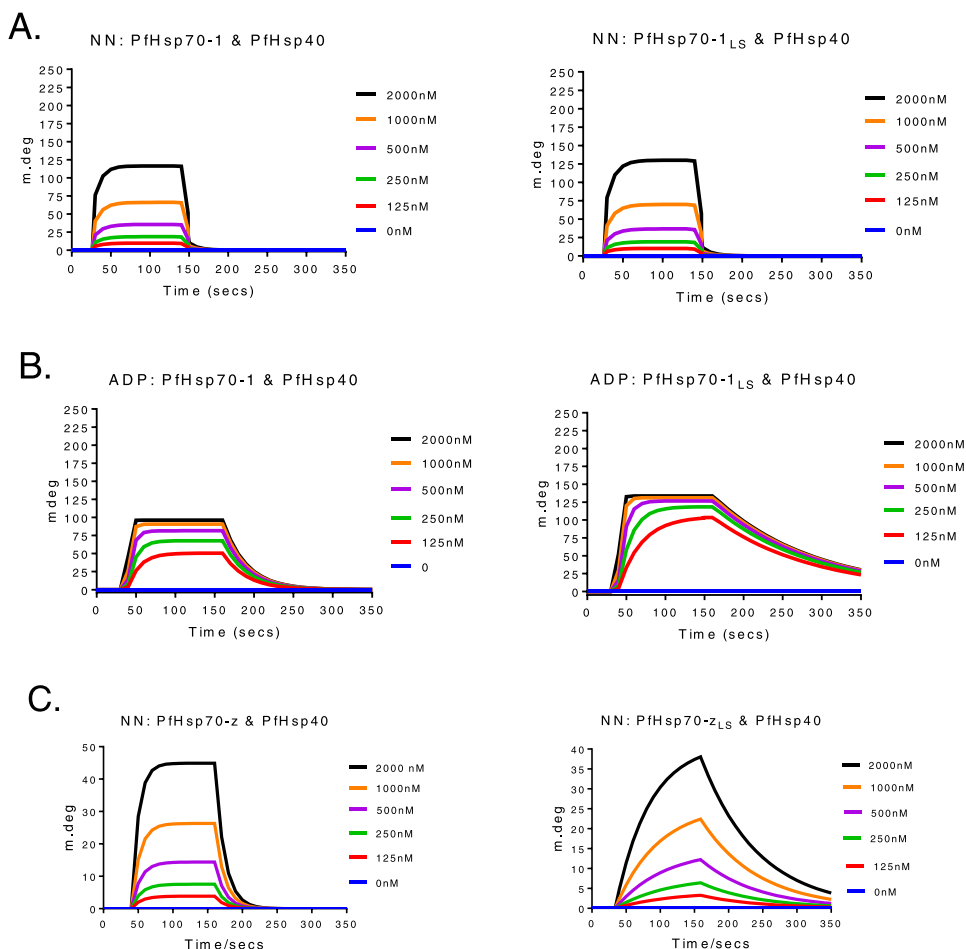
The tertiary structures of PfhHsp70-1<sub>LS</sub>, PfhHsp70-z<sub>LS</sub>, DnaK<sub>LS</sub> and their wild type forms were analysed by intrinsic (tryptophan) and extrinsic (1-Anilino-8-Naphthalene Sulfonate- ANS) fluorescence spectroscopic analyses. Tryptophan fluorescence spectroscopy assays were conducted as previously described [4]. The generated fluorescence spectra were analysed after initial excitation at 295 nm using a JASCO FP-6300 spectrofluorometer (JASCO Ltd, Spain). The emission spectra were monitored between 320 and 450 nm at a scan speed of 500 nm/min. The effect of nucleotides on the tertiary structure of each protein was determined by exposing the proteins to nucleotides (5 mM ADP/ATP) 15 mins ahead of the fluorescence readings as previously described [4]. The effect of urea and pH variation on the tertiary structures of the respective proteins was similarly investigated. Briefly, the recombinant proteins were left to sit for 30 mins in the presence of varying concentrations of urea (0 - 8 M) followed by spectral measurement. Spectra generated following subtraction of baseline accounted for by the buffer.

The tertiary structures of the recombinant Hsp70s were further validated by extrinsic fluorescence using an ANS assay as previously described [5]. ANS is a small amphipathic dye that is used as a probe to detect hydrophobic pockets on protein surfaces. Briefly, 200  $\mu$ M ANS was incubated together with 2  $\mu$ M of the respective Hsp70 for a total of 30 min at 25 °C in the dark. The binding of ANS to the respective Hsp70s was determined by monitoring the fluorescence spectra recorded between 400 and 500 nm after initial excitation at 390 nm. A total of 7 scans were collected and averaged for each sample. In addition, the fluorescence spectra of free ANS were also monitored as a negative control.

### 2.2. Surface plasmon resonance analysis

In order to investigate the effect of the linker mutations on the interaction of PfhHsp70-1, PfhHsp70-z and DnaK and their respective Hsp40 co-chaperone, SPR analysis was

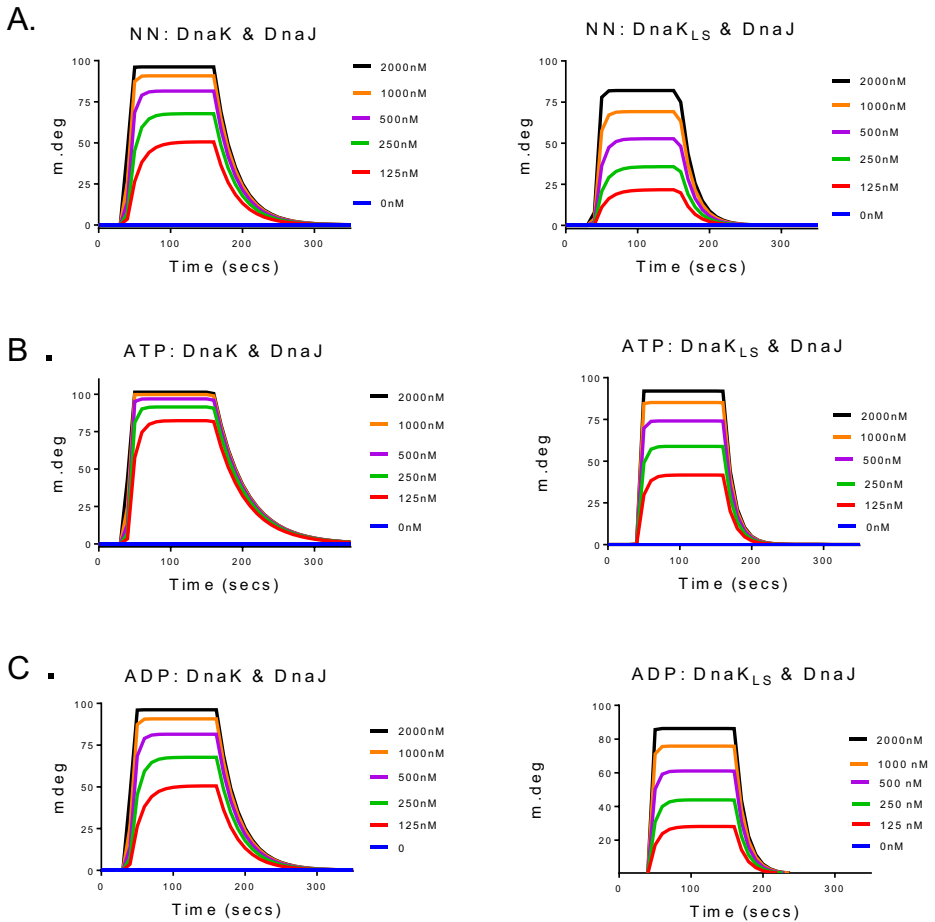




**Fig. 6.** Sensograms representing PfHsp40 interaction with PfHsp70-1/PfHsp70-z and their respective linker switch mutants.

SPR generated sensograms for the association of PfHsp40 with PfHsp701/PfHsp701LS were determined in the absence of nucleotide (NN; panel A) or presence of 5 mM ADP (panel B). (C) SPR generated sensograms for the association of PfHsp40 with PfHsp70-z/PfHsp70-zLS were determined in the absence of nucleotide (NN).

conducted using the BioNavis™ 420A ILVES MP SPR (BioNavis, Tampere, Finland) system. The assay was conducted at 25 °C. Recombinant forms of either PfHsp40 or DnaJ were injected as analytes at concentrations of 0, 125, 250, 500, 1000 and 2000 nM and a flow rate of 50 µl/min onto the immobilised Hsp70 ligands. Degassed PBS-Tween (4.3 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.4 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 3 mM KCl, 0.005% (v/v) Tween 20, and 20 mM EDTA; pH 7.4) was used as running buffer [6]. The following proteins were immobilized as ligands onto carboxyl methyl dextran (CMD) three-dimensional chips: PfHsp70-1, PfHsp70-1<sub>LS</sub>, PfHsp70-z, PfHsp70-z<sub>LS</sub>, DnaK and DnaK<sub>LS</sub>. Association between analyte and ligand was allowed for 3 min and dissociation was monitored for 5 min. The steady-state equilibrium constant data were processed and analysed using TraceDrawer software version 1.8 (Ridgeview Instruments, Sweden).



**Fig. 7.** Sensograms representing DnaK/DnaK<sub>LS</sub> interaction with DnaJ. SPR sensograms representing interaction of DnaJ with DnaK/DnaK<sub>LS</sub> as determined in the absence of nucleotide (NN) (panel A) or presence of 5 mM ATP (panel B) and in the presence of 5 mM ADP (panel C).

## Ethics Statement

No ethical clearance was required for this work.

## CRediT Author Statement

**Addmore Shonhai:** conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, resources, supervision, writing; **Graham Chakafana:** investigation, data curation, formal analysis, writing; **Pertunia Thendo Mudau:** investigation, formal analysis; **Tawanda Zininga:** data curation, formal analysis, methodology, supervision, writing.

All authors have read and agreed to the published version of the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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