## STRUCTURAL BASIS OF MOLECULAR RECOGNITION BY LEISHMANIA SMALL HYDROPHILIC ENDOPLASMIC RETICULUM-ASSOCIATED PROTEIN, SHERP, AT MEMBRANE SURFACES

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From the <sup>‡</sup>Division of Cell and Molecular Biology, Centre for Molecular Microbiology and Infection and the <sup>§</sup>Division of Molecular Biosciences, Department of Life Sciences, Imperial College London, Exhibition Road, London SW7 2AZ, and the <sup>¶</sup>Department of Crystallography, Institute of Structural and Molecular Biology, Birkbeck College, University of London, London WC1E 7HX, and the <sup>∥</sup>Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York YO10 5YW, and the \*\*Membrane Protein Laboratory, Diamond Light Source Limited, Harwell Science and Innovation Campus, Chilton, Didcot, Oxfordshire OX11 0DE, United Kingdom, and the <sup>‡‡</sup>Institute for Cellular and Molecular Biology, The University of Texas at Austin, 1 University Station A4800, Austin, Texas 78712, USA <sup>1</sup>To whom correspondence should be addressed: Division of Cell and Molecular Biology, CMMI, Flowers Building, Imperial College London, Exhibition Road, London SW7 2AZ, United Kingdom. Tel.: 44-20-7594-5298; Fax: 44-20-7594-5297; E-mail: k.brown@imperial.ac.uk FIGURE S1 2D <sup>1</sup>H-<sup>15</sup>N HSQC NMR spectrum of *L. major* SHERP at 4 °C in the absence of SDS or phospholipids. The poor amide <sup>1</sup>H dispersion is indicative of a lack of tertiary structure.



FIGURE S2 <sup>1</sup>H-<sup>15</sup>N heteronuclear NOE spectrum of *L. major* SHERP at 4 °C in the absence of SDS or phospholipids. The spectrum shown is a summation of the reference and NOE spectrum corresponding to (0.8 times the reference – NOE spectrum). Residual signals thus correspond to those with a significant <sup>1</sup>H-<sup>15</sup>N NOE contribution arising from extensive internal motion on the ps timescale, which for the free SHERP is observed in essentially all residues (for comparison with Figure S2).



FIGURE S3 SDS-PAGE avidin-column fractions analyzed to identify SHERP-SBED complexes containing a biotinylated label. Lanes labeled correspond to the flow-through (FT), wash (W) and elution (E) fractions. Bands labeled with numbers correspond to sample numbers shown in Table S1 below.



## FIGURE S4 Amino acid sequence alignment between the L. major and T. thermophilus

**V-ATPase subunit B.** A ClustalW2 alignment is shown. Identical residues (blue), conserved substitutions (green) and semi-conserved substitutions (yellow) are colored according to ClustalW2 conventions (see http://www.ebi.ac.uk/Tools/clustalw2/index.html and Larkin M.A., et al. (2007) *Bioinformatics* **23**, 2947-2948).

		*	20	*	40	*	60		
L maj subB	:	MGRDEEHVRVLSKQ	ELLATHIKEL	NESYSVKPHL	EYTTIRA <mark>VN</mark> G	PLVILEDVRK	P <mark>TFA</mark> E <mark>I</mark>	:	60
T the subB	:	M	DLLKK		EYTGITY <mark>IS</mark> G	PLLFVENAKD	LAYGAI	:	32
	-			•				-	
		*	80	*	100	*	120		
I. mai subB									120
$\underline{\mathbf{m}}$ + ho gubB	:							:	0.2
1_clie_subb	•	VDIRDGIGKVKGGÇ	VILV <mark>O</mark> BEIAV	TÕAL T <mark>RIIG</mark> TI		EDVARDOVOR.		•	92
		+	140	+	160	+	100		
T med subD									100
L_maj_subB	:	FNGSGIPIDNGPP		I PINPRARV II	PEEMIQTGIS	SIDVMTSISR	GQKIPL	:	180
T_the_subB	:	ENGIGKLIDGTLL	TPEKRLPTTG.	LPLNPVARRKI	2E <mark>0F</mark> I0IGIS	TTDVMNTLVR	GQK <mark>L</mark> PL	:	152
		*	200	*	220	*	240		
L_maj_subB	:	FSG <mark>A</mark> GLPHNEIAAQ	21 <mark>VRQA</mark> G <mark>L</mark>	VKREG-KTEDI	F <mark>C</mark> VVFAAMG <mark>V</mark>	NQETARFFRT.	EFE <mark>QN</mark> G	:	236
T_the_subB	:	FSG <mark>S</mark> GLP <mark>ANEIAAÇ</mark>	<mark>IARQA</mark> T <mark>V</mark> RPD	<mark>LS</mark> G <mark>EG</mark> EKEEPI	F <mark>AVVFAAMG</mark> I	T <mark>QR</mark> ELS <mark>Y</mark> FIQ	EFE <mark>RT</mark> G	:	212
		*	260	*	280	*	300		
L_maj_subB	:	SMEKTVLFLNLAN	PTIERI <mark>V</mark> TPR	L <mark>ALT<mark>T</mark>AEYLA</mark> Y	<mark>ZD</mark> C <mark>G</mark> KHVLVI	LTDM <mark>SS</mark> YADA	LRE <mark>VS</mark> A	:	296
T_the_subB	:	ALSRSVLFLNKADI	PTIERI <mark>L</mark> TPR	M <mark>ALT<mark>V</mark>AEYLA<mark>I</mark></mark>	FEH <mark>D</mark> YHVLVI	LTDM <mark>TN</mark> YCEA	LRE <mark>IG</mark> A	:	272
		*	320	*	340	*	360		
L maj subB	:	AREE <mark>V</mark> PGRRG <mark>F</mark> PGY	MYT <mark>NLACIYE</mark>	RAGRVL <mark>GR</mark> AGS	S <mark>I</mark> TOIPILSM	P <mark>N</mark> DDITHPIP	DLTGYI	:	356
T the subB	:	AREE <mark>I</mark> PGRRG <mark>Y</mark> PGY	MYT <mark>DLATIYE</mark>	RAGVVEG <mark>K</mark> KGS	S <mark>V</mark> TOIPILSM	PDDDRTHPIP	DLTGYI	:	332
					~				
		*	380	*	400	*	420		
I. mai subB	•	TEGOT YVDROTHNE		ST.SRT.MKNAT		GUSNOMYAAY		•	416
T the subB		TEGOTOLSRELHRK		ST.SRLM <mark>NNGV</mark>					392
1_ene_bubb	•	110616 <mark>10</mark> 101010				2 VOD 2 L OILL		•	572
		*	110	*	160	*	180		
I mai cubB			ים ד <mark>ים דע</mark> ד ד <mark>רוםי</mark>	VEE <mark>UVETCOC</mark> I					176
L_maj_subb	•			AFE <mark>RAFICOG</mark> I				•	4/0
subB	•	KK <mark>UVALIGEDALT</mark>	ADARA LQI AD	AT B <mark>R</mark> T T INQG	5- <mark>511K91</mark> FR	T <mark>ATA</mark> WATTO		•	491
		JL	EOO						
				405					
L_maj_subB	:	NKIDMKTRDEFYDF	HPGRK	: 495					
T_the_subB	:	<mark>KRIS</mark> KDHI <mark>GKYY</mark> GÇ	<mark>ek</mark> le <mark>e</mark> iwgapQ	ALD : 478					

FIGURE S5 Recombinant *L. major* SHERP binding to *T. thermophilus* V-ATPase demonstrated by surface plasmon resonance. *T. thermophilus* V-ATPase at 0.2 mg/ml in 10 mM sodium acetate buffer, pH 5.0 was immobilized to a CM5 chip by amine coupling. Injection of *L. major* SHERP at a flow rate of 5 ul/min is shown for concentrations ranging from 0.5  $\mu$ M (red) to 15  $\mu$ M (dark blue) are shown along with the associated fits (dashed lines) to the raw data based upon a 1:1 Langmuir binding model. Binding was measured as response units over time. See main article for more details on the experimental data and analysis.



## TABLE S1

Summary of mass spectrometric analyses of *Leishmania major* proteins isolated from cross-linking studies with recombinant *L. major* SHERP

Sample	Molecular	MOWSE	GeneDB	Description		
No.	Mass (kDa)	Score <sup>a</sup>	Identifier			
1	55	301	LmjF28.2430	vacuolar ATP synthase		
				subunit B, putative		
2	25	121	LmjF23.0040	peroxidoxin		
				tryparedoxin peroxidase		
3	27	381	LmjF36.5010	40S ribosomal protein SA,		
				putative		
4	68	111	LmjF29.1760	paraflagellar rod protein 1D,		
				putative		
5	71	574	LmjF28.2780	heat-shock protein hsp70,		
				putative		

<sup>a</sup> Pappin, D. J., Hojrup, P., and Bleasby, A. J. (1993) Curr. Bio. 3, 327-332