Introduction

Eukaryotic pathogens of the genus *Trypanosoma* cause deadly disease in humans and livestock. Human African trypanosomiasis, or sleeping sickness, is caused by two subspecies, *Trypanosoma brucei rhodesiense*, causing an acute disease, or *Trypanosoma brucei gambiense*, causing a chronic infection [1]. Nagana, an economically debilitating wasting disease in African cattle is caused by *Trypanosoma brucei brucei* and other species including *Trypanosoma vivax* and *Trypanosoma congolense* [2]. *Trypanosoma vivax* infection in cattle has also been established in South America [3]. African trypanosomes exhibit several life stages including a bloodstream form (BSF) in the circulation of the mammalian host, a procyclic form (PCF) in the midgut of the tsetse fly vector and a metacyclic form [10]. These peptides do not bind, and thus do not kill, PCF *T. brucei*, which has a more rigid plasma membrane. Human cells, including erythrocytes, are refractory to SHP concentrations orders of magnitude higher than necessary to kill BSF *T. brucei* [10].

Here we report that trypanocidal SHP uniquely cause an increase in the rigidity of the interfacial region of the plasma membrane that is consistent with dramatic motility constriction and subsequent cell death. We present an explanation, based upon sequence analysis and the orientation and structure of lipid associated SHP, for these biophysical consequences.

Results

Susceptibility to SHP is Independent of VSG and Common to African Trypanosomes

An immediately apparent difference between the plasma membranes of BSF and PCF African trypanosomes is the lack of a dense coat of VSG in the insect stage cells. We tested metacyclic *T. b. brucei*, which do express a VSG coat, for susceptibility to SHP-
1 and found no killing activity (Fig. 1a). These data indicate that susceptibility is not due to specificity of SHP for VSG. Next we determined whether other African trypanosomes are sensitive to SHP. Bloodstream developmental forms of T. vivax and T. congolense are susceptible to killing by SHP-1 at concentrations similar to BSF T. brucei (Fig. 1b), indicating that SHP susceptibility is a characteristic of both human and veterinary pathogenic African trypanosomes.

Trypanocidal Activity Requires a C-terminal Positive Charge

Trypanocidal SHP are derived from apolipoproteins and exhibit the characteristics of secretory signal peptides, i.e. size (18–22 amino acids), a central hydrophobic region and a C-terminal putative signal peptidase cleavage site defined by specific amino acid patterns [10]. Although these peptides share physical features, the primary sequences are entirely different (Fig. 1c). We tested a third, distinct SHP (−3) for trypanocidal activity, also derived from an apolipoprotein [11] and possessing similar features as SHP-1 and -2 (Fig. 1c). Despite possessing the same general physical characteristics and binding to BSF T. brucei (Methods File S1, Figure S1), no trypanocidal activity was detected (Fig. 1d). Comparison of the three sequences revealed that an arginine at position -5 relative to the C-terminus is common to trypanocidal SHP-1 and -2, but is absent in SHP-3 (Fig. 1c). Substitution of an arginine for the leucine in this position of SHP-3 (SHP-3ΔR, Fig. 1c) confers trypanocidal activity (Fig. 1d). Replacement of the leucine with glutamate in SHP-3 (SHP-3ΔE, Fig. 1c) does not (Fig. 1d). Trypanocidal activity is conferred simply by a positive charge at the C-terminus, indicated by the trypanocidal activity of an SHP-3 variant in which the -5 leucine is replaced by lysine (SHP-3ΔK, Fig. 1d). Trypanocidal SHP-2 has a positive charge at both the N- and C-terminus, SHP-1 has a single positive charge at the C-terminus; thus we tested whether charge location is important by swapping the C-terminal arginine with the N-terminal aspartate of SHP-1 (SHP-1-swap, Fig. 1c). Rearranging these residues resulted in a loss of trypanocidal activity (Fig. 1d).

Trypanocidal SHP Rigidify the Interior and Interfacial Region of the Plasma Membrane

Trypanocidal SHP act at the plasma membrane but do not induce osmotic swelling or bursting [10]. Therefore we reasoned that any effect upon the BSF trypanosome must not result in a loss of...
of plasma membrane integrity. We investigated the rigidity of BSF *T. brucei* membranes, a property that can change without loss of membrane integrity, utilizing two anisotropic probes, diphenylhexatriene (DPH) that reports on the interior of the acyl chain region, and trimethylammonium-diphenylhexatriene (TMA-DPH) that is anchored at the membrane interface. Addition of either trypanocidal or non-trypanocidal SHP to BSF *T. brucei* results in increased rigidity of the interior of the plasma membrane (Fig. 2a). However only the trypanocidal SHP, SHP-1, -2, -3AR and -3AK increased the interfacial rigidity (Fig. 2b). These data indicate that rigidification of the interfacial region is likely involved in killing BSF trypanosomes.

**Trypanocidal SHP have Biophysical and Physiological Consequences**

Treatment of BSF African trypanosomes with SHP results in multiple physiological alterations. Addition of SHP-1 to BSF trypanosomes, which were subsequently immobilized in gelatin, decreases the fraction of laterally mobile surface exposed VSG (Fig. 2c). This effect may be directly due to increased membrane rigidity, or indirectly due to a decrease in the mobility of membrane spanning proteins and potential interactions with the VSG. Another physiological consequence, that may or may not be related to membrane rigidification, is SHP-induced changes in cell motility. Previously we reported that SHP-1 causes an initial hyperactivation followed by constricted motility and death [10]. Non-trypanocidal SHP-3 does cause some hyperactivation of BSF *T. b. brucei* (Movie S1 displays an untreated cell for comparison, Movie S2 presents a representative cell exhibiting SHP-3 induced hyperactivation), however subsequent constriction does not occur (Fig. 2d). Trypanocidal SHP-1 [10] and SHP-3AR (Movie S3, S4) induce both hyperactivation and subsequent constriction (Fig. 2d). Constricted motility may result in reduced hydrodynamic forces acting upon surface proteins.

**Figure 2. Membrane rigidity changes and physiological consequences of SHP.** The rigidity of the interior (a) or interfacial (b) region of the plasma membrane of BSF *T. b. brucei* treated with increasing concentrations of SHP-1 (blue ●), SHP-2 (grey ●), SHP-3 (red ■), SHP-3AR (green ▲), SHP-3DK (purple ▼), SHP-3AE (orange-open △), SHP-1swap (black-open ■) or solvent alone (DMSO, black ●) was determined by measuring the fluorescence depolarization of DPH or TMA-DPH respectively. (c) FRAP analysis of the mobile fraction of BSF *T. b. brucei* VSG in the presence (red ■) or absence (black ●) of 8 μM SHP-1. (d) Live BSF *T. b. brucei* treated with equimolue DMOSO (grey), 40 μM SHP-1 (blue), SHP-3 (red) or SHP-3AR (green) were visualized by DIC microscopy and scored for normal, hyperactivated and constricted motility as well as death at the indicated timepoints (see Movies S1, S2, S3 and S4 for examples of the normal, hyperactivated and constricted motilities respectively).

doi:10.1371/journal.pone.0044384.g002
Trypanocidal SHP Exhibit Shallow Penetration and Orient Perpendicular to the Plane of the Membrane

In order to understand why trypanocidal and non-trypanocidal SHP have different effects on the BSF plasma membrane, we determined the orientation of SHP-1 and -3 in lipid bilayers by parallax analysis [12]. Tryptophans were substituted at positions 1, 8, and 18 (N- to C-terminus, native tryptophan located at position 12) in SHP-1 and positions 1, 13, and 20 in SHP-3 (N- to C-terminus, native tryptophan located at position 5) (Table S1). These placements were chosen, and native tryptophan residues were replaced with glycine, in order to retain the hydrophobic profile of the original peptides. All of the substituted SHP-1 peptides show equivalent killing activity as well as membrane interaction (Methods File S1, Figure S2a, b). We determined the insertion depth of SHP tryptophans by ratiometric analysis of the quenching efficiency of liposomes containing 10 mol % brominated lipid at a shallow (6,7) and deep (9,10) position of the acyl chains. Trypanocidal SHP-1 penetrates shallowly into the hydrocarbon region and adopts a U-shaped conformation (Fig. 3a, Table S1). The two terminal tryptophans, positions 1 and 18, are located approximately 1.1 and 2.0 Å from the membrane interface respectively. The tryptophans at positions 8 and 12 are located approximately 7.8 and 5.1 Å from the interface respectively. Therefore, rather than aligning with the phospholipid acyl chains, SHP-1 inserts into the external leaflet parallel to the plane of the bilayer and proximal to the phospholipid headgroups. These data are consistent with an orientation that has been observed for the LamB signal peptide [13,14]. Non-trypanocidal SHP-3 and the tryptophan variants also exhibit membrane interaction (Figure S2c). Parallax analysis of SHP-3 indicates a tilted orientation with the C-terminus penetrating deeper into the bilayer, an orientation that has also been suggested for the LamB signal peptide (Fig. 3a, Table S1) [13]. The N-terminal tryptophan was inefficiently quenched, suggesting that it does not intercalate into the hydrocarbon region. The native tryptophan, position 5, inserts approximately 1.6 Å deep. The tryptophan at position 13 is located approximately 4.7 Å deep and the C-terminal tryptophan penetrates most deeply, to approximately 11.2 Å. This orientation precludes interaction of the C-terminus with the lipid headgroups.

Discussion

Changes in membrane rigidity as measured by fluorescence anisotropy have been shown to correlate with alterations in cellular physiology and biochemistry. Temperature induced rigidiﬁcation of platelet membranes results in a concomitant decrease in ﬂuid phase endocytosis [18]. Additionally in the type 1 diabetic disease state platelets exhibit an increase in the rigidity of the interior of the plasma membrane that results in a decrease in Na+/K+-ATPase activity [19]. In the case of BSF African trypanosomes, we have correlated increased membrane rigidity with changes in cell motility, the diffusion of rate of surface proteins and ultimately cell death. Therefore, membrane rigidiﬁcation may be trypanocidal by interfering with a variety of activities such as, but not limited to, lateral surface protein diffusion, ion channel function and/or the diffusion of small molecule nutrients into the BSF cell. It has been shown that increasing the rigidity of trypanosome membranes results in a redistribution of proteins normally localized to the flagellar membrane [20], posing the possibility that signaling pathways may be affected. Therefore trypanocidal SHP may have pleiotropic effects.

![Figure 3. Orientation and structure of SHP in lipid bilayers. (a) The depth of peptide penetration into the hydrocarbon region of model liposomes was determined via parallax analysis. Assuming a hydrocarbon bilayer thickness of 29 Å, the depths of tryptophans spanning SHP-1 (blue ●) and SHP-3 (red ■) are plotted against a background of the outer leaflet of a POPC bilayer. (b) Circular dichroism spectroscopy of SHP-1 in aqueous buffer (green ●) and in the presence of egg phosphatidylcholine liposomes (blue ●). (c) Molecular dynamic modeling of SHP-1 in a lipid environment. The backbone trace (top) illustrates a predominantly α-helical structure with disordered termini and an internal disordered region. Surface potential representations (N to C terminal, middle; C to N terminal, bottom) indicate positively charged patches (blue) formed by the N-terminal amino acid and the arginine at position 14. Non-polar and negatively charged regions are shown in white and red, respectively.](https://www.plosone.org/doi:10.1371/journal.pone.0044384)
In addition to directly killing BSF trypanosomes, SHP induce physiological changes that may attenuate the parasites’ ability to evade host immune effectors. The diffusion of VSG is altered in trypanosomes treated with SHP-1. The decrease in the number of mobile surface VSG may be due to increased membrane rigidity conferring greater drag upon the myristate anchors. Alternatively, or in addition to, increased membrane rigidity may hinder VSG diffusion through decreasing the mobility of membrane spanning proteins that subsequently interfere with the lateral surface flow of VSG. In either case hindering the flow of VSG to the flagellar pocket would likely delay the clearance of host defense molecules thereby promoting cell killing. Additionally, constricting the motility of BSF trypanosomes may effectively decrease hydrodynamic flow over the surface of the cell, a force necessary for maintaining directionality and movement of VSG to the anterior of the trypanosome [9], also delaying the clearance of VSG. Employing agents that increase the rigidity of the plasma membrane in pharmaceutical applications may therefore augment the host immune response to trypanosome infection.

The orientation of trypanocidal SHP in lipid bilayers differs from non-trypanocidal SHP in that both termini are proximal to the membrane interface. This orientation can be attributed to a positively charged residue at the -5 position relative to the C-terminus that is lacking in non-trypanocidal SHP. Swapping a negatively charged residue for the C-terminal arginine (SHP-1swap) will result in a similar hydrophobic profile and thus presumably induce the same orientation in lipid bilayers, however this change renders the SHP incapable of rigidifying the membrane interface and therefore non-trypanocidal. These data indicate a direct role for a C-terminal positively charged residue (arginine or lysine) in increasing interfacial rigidity and trypanocidal killing. Incorporating the requirement for a positive charge at the C-terminus and the hydrophobic bulge revealed by molecular dynamic modeling, we suggest a mechanism in which trypanocidal SHP are anchored in the membrane hydrocarbon region by the internal hydrophobic stretch and the positively charged patches at each termini coordinate negatively charged phosphates of the lipid headgroups. This model provides a plausible explanation for the increase in interfacial membrane rigidity by trypanocidal but not non-trypanocidal SHP.

The specificity of SHP for BSF African trypanosomes reveals a phenotype that may be taken advantage of for the development of pharmaceutical agents. Drugs that target the fluidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of resistance exhibited by these pathogens. Compensating for the rigidity of the plasma membrane may offer a means of circumventing the rapid onset of res...
dye. Labeled *T. b. brucei* cells were incubated with 8 µM SHP-1 for 10 min at 37°C. The fluorescently labeled cells were mixed 1:3 with 10% Type-A gelatin from porcine skin (Sigma-Aldrich, Steinheim) in PBS, pH 7.8, at 37°C. 4 µL of the cell gelatin mixture was applied into a cover slide sandwich and mounted into a temperature-controlled sample holder. The sample holder was cooled to 20°C until the cells were immobilized. Samples of SHP-1 treated and untreated MITat 1.6 wt/mL were prepared identically. Line FRAP measurements were performed at a constant temperature of 20°C. 10 pre-bleach and 100 post-bleach images were recorded at 2fps. VSG mobile fractions were determined according to Phair et al. (2004) [24] using double normalization. The mobile fraction refers to the percentage of mobile VSG molecules within the measured region. For example a mobile fraction of 50% means that half of the VSG molecules are mobile. The relative frequency indicates the proportion of cells that exhibit a given mobile fraction of VSG.

### Trypanosome Motility

All images and videos were acquired with an Axio Observer Z1 equipped with an AxioCam MRm controlled by AxioVision 4.6 software. Videos were acquired with live cells at a density of 1×10⁷ cells/mL in HMI 9 media with 10% fetal bovine serum, incubated with 40 µM SHP at 37°C. Trypanosomes were visualized at magnification 63× and videos were recorded with 100-ms acquisition times. The motility of BSF trypanosomes was scored directly or from video playback. Trypanosome motility was classified as normal, hyperactive or constricted as described previously [10]. An example of a trypanosome scored as normal is shown in Movie S1. Example hyperactive trypanosomes are shown in Movies S2 and S3, while those exhibiting constricted motility are visualized with the representative trypanosome presented in Movie S4. Data in figure 2D is shown as the average of at least duplicate trials with standard deviations.

### Parallax Analysis

The hydrocarbon penetration depth of tryptophans spaced throughout synthetic peptides corresponding to SHP-1 or SHP-3 (Table S1) was determined by parallax analysis with brominated phosphatidylcholine liposomes. Large unilamellar liposomes composed of egg phosphatidylcholine and 10 mol % 1-palmitoyl-2-(6,7-dibromo)stearoyl-sn-glycero-3-phosphocholine (shallow quencher) or 1-palmitoyl-2-(9,10-dibromo)stearoyl-sn-glycero-3-phosphoholine (deep quencher) were constructed by hydration through polycarbonate filters with 0.1 mm thickness, 29 Å [25], minus ZCF. The hydrocarbon penetration depth of tryptophans spaced 6 Å and 29 Å away from the center of the bilayer, respectively, was calculated from the equation [12]:

\[
Z_{\text{CF}} = L_{C1} + \left[ \ln \left( \frac{F_1}{F_2} \right) / r_C \right] / 2L_{C1}
\]

Where \(L_{C1}\) is the distance from the center of the bilayer to the shallow quencher, in this case 10.8 Å for 6,7-dibromo-PC [25], \(F_1\) is the intensity of tryptophan in the presence of the shallow quencher and \(F_2\) is the tryptophan intensity in the presence of the deep quencher, \(G\) is the mole fraction of quencher divided by the area of individual phospholipid (70 Å²), and \(L_{C1}\) is the difference in the depth of the two quenchers (2.7 Å) [25]. The hydrocarbon insertion depth of tryptophans is then given by one half the bilayer thickness, 29 Å [25], minus ZCF.

### Circular Dichroism

Spectra were recorded with a Jasco J-710 spectropolarimeter in a 1 mm quartz cuvette. Measurements were performed with a final concentration of 15 µM SHP-1 added from a stock of ethanol-solubilized peptide (final ethanol concentration 7.5 %) in 10 mM K₂PO₄, 50 mM Na₂PO₄, pH 7.5. Lipid associated peptide spectra were recorded with the addition of 0.1 µm unilamellar egg phosphatidylcholine liposomes at a peptide to lipid ration of 0.3. Spectra were averaged from four scans and the appropriate buffer scans were subtracted.

### Molecular Modeling

The tertiary structure of SHP-1 in a lipid environment was predicted using the web-based molecular dynamics simulation software PEPstr (http://www.imtech.res.in/raghava/pepstr/) [26]. The per-atom charge and radius were calculated by converting the PDB file obtained from PEPstr into a PQR file via the web-based PDB2PQR server (http://kryptonite.nbcr.net/pdb2pqr/) [27]. Values were calculated using the PARSE forcefield. The peptide was subsequently visualized with the PyMOL Molecular Graphics System, Version 1.4 (Schrodinger, LLC).

### Supporting Information

**Figure S1** SHP binding to BSF *T. b. brucei*. FITC-labeled SHP-1 (blue – ) and SHP-3 (green – ) were assayed for binding to BSF *T. b. brucei* via flow cytometry (no peptide, red – – – ). Trypanosomes were adjusted to 3×10⁶ cells/mL in HMI 9 media with 10% fetal bovine serum, 8 µM FITC-SHP-1 or FITC-SHP-3 was added and 30,000 cells were immediately counted. (DOC)

**Figure S2** Trypanosome killing and membrane interaction with SHP tryptophan variants. (a) Small hydrophobic peptide-1 trypanocidal variant (Table S1) SHP-1AW1 (orange ◆), SHP-1AW8 (green ■) and SHP-1AW18 (red ▲) were tested for trypanocidal activity. (b) The ability of SHP-1 trypanocidal variants, 1 µM SHP-1AW1 (orange – ), 1 µM SHP-1AW8 (green – ), 0.2 µM SHP-1AW1 (blue – ) and 0.2 µM SHP-1AW18 (red – ), and (c) SHP-3 trypanocidal variants, 1 µM SHP-3AW1 (orange – ), 1 µM SHP-3AW3 (blue – ), 1 µM SHP-3AW13 (red – – ) and 4 µM SHP-3AW20 (green – ), to interact with lipid bilayers was determined by monitoring the release of entrapped calcein from unilamellar egg phosphatidylcholine liposomes. (DOC)

**Table S1** Sequences and Quenching Data of SHP Tryptophan Variants. (DOC)

**Methods File S1** Contains methods for supplementary Figures S1 and S2. (DOC)

**Movie S1** Live, untreated BSF *T. b. brucei* exhibiting normal motility and visualized with DCI video microscopy. (MOV)
Movie S2  Live BSF T. b. brucei visualized via DIC video microscopy approximately 30 sec after addition of 40 μM SHP-3. Cells exhibit hyperactivated motility. (MOV)

Movie S3  Live BSF T. b. brucei visualized via DIC video microscopy approximately 30 sec after addition of 40 μM SHP-3AR. Cells exhibit hyperactivated motility. (MOV)

Movie S4  Live BSF T. b. brucei visualized via DIC video microscopy approximately 10 min after addition of 40 μM SHP-3AR. Cells exhibit constricted motility. (MOV)

Acknowledgments
We would like to thank all of the members of the Hajduk laboratory for constructive discussion, particularly Rujo Kief and David Seidman, as well as Zachary Wood. We thank Jeff and Ramona Urbauer for use of their CD spectropolarimeter.

Author Contributions
Conceived and designed the experiments: JMH AH NJG ME AM SH. Performed the experiments: JMH CS AH NJG PC. Analyzed the data: JMH AH NJG ME. Contributed reagents/materials/analysis tools: JMH ME AM SH. Wrote the paper: JMH SH.

References
1. Barrett MP, Burchmore RJS, Sitch A, Lazzari JO, Frasch AC, et al. (2003) The trypanosomiases. Lancet 362: 1469–1480.
2. Van den Bossche P, de La Rocque S, Hendrickx G, Bouyer JA (2010) Changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. Trends Parasitol 26: 236–243 (2010).
3. Jones TW, Davila AM (2001) Trypanosoma vivax – out of Africa. Trends Parasitol 17: 99–101.
4. Fenn K, Matthews KR (2007) The cell biology of Trypanosoma brucei differentiation. Curr Opin Microbiol 10: 539–546.
5. Chattopadhyay A, London E (1987) Parallax method for direct measurement of membrane-interactive properties of signal sequences of the phospholipids. Biochemistry 26: 39–45.
6. McIntosh TJ, Holloway PW (1987) Determination of the depth of bromine in bilayers formed from bromolipid probes. Biochemistry 26: 39–45.
7. Phair RD, Gorski SA, Misteli T (2004) Measurement of dynamic protein binding to chromatin in vivo, using photobleaching microscopy. Methods Enzymol 34: 11617–11624.
8. Shiflett AM, Faulkner SD, Cotlin LF, Widener J, Stephens N, et al. (2007) African trypanosomes: intracellular trafficking of host defense molecules. J Eukaryot Microbiol 54: 18–21.
9. Tyler KM, Fridberg A, Torrielli KM, Olson CL, Ciesiak JA, et al. (2009) Flagellar membrane localization via association with lipid rafts. J Cell Sci 122, 105–106.
10. Widener J, Nielsen MJ, Shiflett A, Mostrop SK, Hajduk S (2007) Hemoglobin is a co-factor of human trypanosome lytic factor. PLoS Pathog 3: 1250–1261.
11. Tyler KM, Fridberg A, Torrielli KM, Olson CL, Ciesiak JA, et al. (2009) Flagellar membrane localization via association with lipid rafts. J Cell Sci 122, 105–106.
12. Shiflett AM, Faulkner SD, Cotlin LF, Widener J, Stephens N, et al. (2007) African trypanosomes: intracellular trafficking of host defense molecules. J Eukaryot Microbiol 54: 18–21.
13. Tyler KM, Fridberg A, Torrielli KM, Olson CL, Ciesiak JA, et al. (2009) Flagellar membrane localization via association with lipid rafts. J Cell Sci 122, 105–106.
14. Widener J, Nielsen MJ, Shiflett A, Mostrop SK, Hajduk S (2007) Hemoglobin is a co-factor of human trypanosome lytic factor. PLoS Pathog 3: 1250–1261.
15. Greenfield NJ (2006) Using circular dichroism spectra to estimate protein secondary structure. Nat Protoc 1: 2076–2080.
16. Briggs MS, Cornell DG, Dlubh RA, Gierasch LM (1986) Conformations of signal peptides induced by lipids suggest initial steps in protein export. Science 233, 206–208.
17. Choquin V, Killian A, Breg J, d’Jongh HHJ, Boeles R, et al. (1995) PhoE signal peptide inserts into micelles as a dynamic helix-break-helix structure, which is modulated by the environment. A two-dimensional 1H NMR study. Biochemistry 34: 11617–11624.
18. Wolkers WF, Looper SA, Fontanilla RA, Tsvetkova NM, Tablin F, et al. (2003) Temperature dependence of fluid phase endocytosis coincides with membrane properties of pig platelets. Biochim Biophys Acta 1612: 154–63.
19. Vignini A, Moroni C, Nanetti L, Raffaelli F, Cester A, et al. (2011) Alterations of platelet biochemical and functional properties in newly diagnosed type 1 diabetes: a role in cardiovascular risk? Diabetes Metab Res Rev 27: 277–285.
20. Tyler KM, Fridberg A, Torrielli KM, Olson CL, Ciesiak JA, et al. (2009) Flagellar membrane localization via association with lipid rafts. J Cell Sci 122, 105–106.
21. Shiflett AM, Faulkner SD, Cotlin LF, Widener J, Stephens N, et al. (2007) African trypanosomes: intracellular trafficking of host defense molecules. J Eukaryot Microbiol 54: 18–21.
22. Shiflett AM, Faulkner SD, Cotlin LF, Widener J, Stephens N, et al. (2007) African trypanosomes: intracellular trafficking of host defense molecules. J Eukaryot Microbiol 54: 18–21.
23. Tyler KM, Fridberg A, Torrielli KM, Olson CL, Ciesiak JA, et al. (2009) Flagellar membrane localization via association with lipid rafts. J Cell Sci 122, 105–106.
24. Widener J, Nielsen MJ, Shiflett A, Mostrop SK, Hajduk S (2007) Hemoglobin is a co-factor of human trypanosome lytic factor. PLoS Pathog 3: 1250–1261.
25. McIntosh TJ, Holloway PW (1987) Determination of the depth of bromine atoms in bilayers formed from bromolipid probes. Biochemistry 26: 1783–1788.
26. Kaur H, Garg A, Raghava GP (2007) PEPstr: a de novo method for tertiary structure prediction of small bioactive peptides. Protein Pept Lett 14: 626–631.
27. Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA (2004) PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. Nucleic Acids Res 32: 665–673.