Structural role of essential light chains in the apicomplexan glideosome

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Gliding, a type of motility based on an actin-myosin motor, is specific to apicomplexan parasites. Myosin A binds two light chains which further interact with glideosome associated proteins and assemble into the glideosome. The role of individual glideosome proteins is unclear due to the lack of structures of larger glideosome assemblies. Here, we investigate the role of essential light chains (ELCs) in *Toxoplasma gondii* and *Plasmodium falciparum* and present their crystal structures as part of trimeric sub-complexes. We show that although ELCs bind a conserved MyoA sequence, *P. falciparum* ELC adopts a distinct structure in the free and MyoA-bound state. We suggest that ELCs enhance MyoA performance by inducing secondary structure in MyoA and thus stiffen its lever arm. Structural and biophysical analysis reveals that calcium binding has no influence on the structure of ELCs. Our work represents a further step towards understanding the mechanism of gliding in *Apicomplexa*.
Apicomplexa are a phylum of intracellular, parasitic, single cell eukaryotes with high medical and agricultural relevance. For instance, Plasmodium species are the causative agents of malaria, that lead to 414,000 deaths per year. Another apicomplexan parasite, Toxoplasma gondii, infects more than 30% of the population worldwide with no clinical symptoms but can cause severe damage in immunocompromised patients and in pregnant women. Proliferation and transmission of these obligate endoparasites in their host organisms rely on efficient cell invasion. This active process is based on the motility of the membrane-embedded multi-protein complex referred to as the motor with actin filaments with surface transmembrane proteins such as GAP40, GAP45 and GAP50. Myosin A and its light chains interact with the C-terminus of the glideosome associated protein 45 (GAP45) to form a pre-complex in the earlier stages of intracellular parasite development, which subsequently assembles with the remaining glideosome members (GAP40 and GAP50). N-terminal palmitoylation modification at its N-terminus anchors MLC1 (MTIP) to the IMC, whereas N-terminal myristoylation and palmitoylation sites tie GAP45 to the plasma membrane. GAP45 is essential for the correct localization of MyoA with its light chains and GAP45 depletion leads to impairment of host cell invasion. Depletion of GAP40 or GAP50 changes the morphology of the parasites and the integrity of the IMC and thereby also alters the localization of MyoA and the light chains.

Structural information on individual members and sub-complexes of the glideosome are limited and the architecture of the entire glideosome is elusive. So far, only structures of P. falciparum PIGAP50 soluble domain, a T. gondii dimeric complex between the TgMyoA C-terminus and MLC1, a homodimeric complex in P. falciparum between PMy0A C-terminus and MTIP, and the motor domains of the T. gondii TgMyoA and P. falciparum PfMyoA are available.

Here, we present crystal structures of T. gondii and P. falciparum light chains bound to the respective MyoA C-termini in the presence of calcium, an additional calcium-free structure as well as the X-ray and NMR solution structures of the N-terminal domain of P. falciparum PfELC. We provide a thorough characterization of all identified interaction surfaces and discuss the differences between both species. We demonstrate that ELCs bind to a conserved binding site on MyoA to induce its a-helical secondary structure and stiffen the MyoA neck. Our work deepens the mechanistic understanding of the gliding motility in Apicomplexa.

Results

Structures of isolated ELCs. Crystal structures of T. gondii and P. falciparum MyoA and of their distal light chains MLC1 (MTIP) have already been determined. To shed light on the role of proximal essential light chains (ELCs), we studied their structure in isolation and in the context of their interaction partners. TgELC1 and TgELC2 share a high degree of sequence similarity (65.2%), whereas PfELC has only 40.6% similarity to TgELC1 (Supplementary Fig. 1a), pointing towards structural differences. Likewise, the disorder probability differs between T. gondii and P. falciparum ELCs (Supplementary Fig. 2a). We recombinantly expressed N-terminally His-tagged ELCs in E. coli (Supplementary Fig. 1b) and purified them to homogeneity. In spite of similar molecular weights, PfELC elutes earlier than TgELC2 when subjected to size exclusion chromatography (Supplementary Fig. 2b), indicative of a larger hydrodynamic radius for PfELC. Small angle X-ray scattering (SAXS) measurements further confirm that PfELC has a larger overall size in solution compared to TgELC2, with respective radii of gyration ($R_g$) of 2.71 ± 0.05 nm and 2.14 ± 0.05 nm (Supplementary Fig. 2d-e, Supplementary Table 2 and 3). The SAXS data also provide evidence that the increased $R_g$ of PfELC likely results from conformational flexibility (Supplementary Fig. 2f, Supplementary Table 3). This is also apparent from circular dichroism data which show that PfELC has lower $alpha$-helical and higher random coil content compared to TgELC2 (Supplementary Fig. 2c, Supplementary Table 2). To map the structured elements and disordered regions of PfELC, we performed triple-resonance NMR experiments that facilitated the near complete assignment of the amide backbone resonances (Supplementary Fig. 2j). Heteronuclear NOEs ($^{1}H$-$^{15}N$ NOE) and...
chemical shift analysis revealed that the protein consists of an \(\alpha\)-helical N-terminal domain, while the C-terminal part is disordered (Supplementary Fig. 2g). Based on this finding, we were able to determine the structure of the N-terminal PfELC fragment (amino acids 1–74, PfELC-N; see Supplementary Fig. 1b) by both X-ray crystallography to 1.5 Å resolution (Fig. 2a, Table 2) and by NMR spectroscopy (Fig. 2b, Supplementary Table 4). The lowest energy NMR conformers are very similar to the crystal structure, with an average backbone RMSD of 1.4 Å over residues 1–68. The N-terminal domain of PfELC has a typical calmodulin fold with two \(\alpha\)-helices (from N terminus red, orange, violet, cyan, loops and disordered regions in green).

Fig. 2 Crystal structure and NMR structures of PfELC N-terminal domain. a Crystal structure of the N-terminal domain of PfELC, residues 1–68. PfELC displays a typical calmodulin fold with two helix-loop-helix motifs. The degenerated EF hand loops do not bind any ion. In agreement with the NMR data of full length PfELC, the protein consists of four \(\alpha\)-helices (from N terminus red, orange, violet, cyan, loops and disordered regions in green). b Ten lowest-energy NMR structures of PfELC (residues 1–74, all atom RMSD of 1.23 Å) colored from lowest (blue) to highest (red) backbone RMSD compared to the crystal structure show that the loop of the PfELC first EF hand (residues 16–22) and the third helix (residues 40–47) display a certain degree of flexibility.

### Table 1 Overview of thermodynamic constants measured by ITC.

| Protein (cell) | MyoA peptide (syringe) | Molar ratio | \(K_d\) (nM) | \(\Delta H\) (kcal/mol) | \(-T\Delta S\) (kcal/mol) |
|----------------|------------------------|-------------|--------------|------------------------|------------------------|
| MTIP           | PfMyoA-\(\Delta\)ELC    | 0.74 ± 0.01 | 303 ± 43     | –14.4 ± 0.4            | 5.5                    |
| TgELC1         | TgMyoA-\(\Delta\)ELC    | 1.05 ± 0.01 | 36 ± 24      | –13.0 ± 0.2            | 3.2                    |
| TgELC1 (EDTA)  | TgMyoA-\(\Delta\)ELC    | 0.81 ± 0.01 | 57 ± 18      | –13.0 ± 0.6            | 3.4                    |
| TgELC2         | TgMyoA-\(\Delta\)ELC    | 0.85 ± 0.01 | 39 ± 12      | –15.0 ± 0.3            | 4.5                    |
| TgELC2 (EDTA)  | TgMyoA-\(\Delta\)ELC    | 0.77 ± 0.01 | 82 ± 7       | –18.0 ± 0.1            | 8.2                    |
| TgELC2\(\Delta\)10A | TgMyoA-\(\Delta\)ELC     | 0.79 ± 0.01 | 190 ± 25     | –17.0 ± 0.3            | 8.2                    |
| TgELC2\(\Delta\)79A | TgMyoA-\(\Delta\)ELC     | 0.84 ± 0.01 | 280 ± 34     | –18.0 ± 0.3            | 9.5                    |
| TgELC2\(\Delta\)510A | TgMyoA-\(\Delta\)ELC     | 0.88 ± 0.02 | 280 ± 85     | –18.0 ± 0.8            | 9.3                    |
| TgELC2\(\Delta\)102A | TgMyoA-\(\Delta\)ELC     | 0.79 ± 0.01 | 76 ± 26      | –16.0 ± 0.5            | 6.6                    |
| TgELC2\(\Delta\)102E | TgMyoA-\(\Delta\)ELC     | 0.77 ± 0.01 | 140 ± 26     | –18.0 ± 0.3            | 8.9                    |
| TgELC2\(\Delta\)102A+H110A | TgMyoA-\(\Delta\)ELC   | 0.75 ± 0.02 | 1100 ± 220   | –21.0 ± 0.9            | 12.0                   |

| Protein (cell) | Protein (syringe) | Molar ratio | \(K_d\) (nM) | \(\Delta H\) (kcal/mol) | \(-T\Delta S\) (kcal/mol) |
|----------------|-------------------|-------------|--------------|------------------------|------------------------|
| MTIP           | PfELC             | 0.86 ± 0.01 | 109 ± 6.2    | –13.4 ± 0.1            | 4.0                    |
| MTIP           | PfELC\(\Delta\)7 | 0.81 ± 0.01 | 260 ± 26     | –12.6 ± 0.2            | 4.0                    |
| TgELC1         | MLC1              | 0.92 ± 0.01 | 4.7 ± 2.5    | –39.1 ± 0.8            | 28.0                   |
| TgELC2         | MLC1              | 0.81 ± 0.01 | 0.6 ± 0.1    | –47.6 ± 0.1            | 35.0                   |
| TgELC2\(\Delta\)17A | MLC1              | 0.92 ± 0.01 | 4.6 ± 0.4    | –49.7 ± 0.2            | 38.0                   |
| TgELC2\(\Delta\)22A | MLC1              | 0.92 ± 0.01 | 5.2 ± 1.9    | –45.9 ± 0.7            | 35.0                   |
| TgELC2         | MLC1\(\Delta\)68A | 0.79 ± 0.01 | 1.2 ± 0.8    | –47.7 ± 0.2            | 36.0                   |
| TgELC2         | MLC1\(\Delta\)69A | 0.89 ± 0.01 | 2.3 ± 1.9    | –48.8 ± 0.5            | 37.0                   |
| TgELC2         | MLC1\(\Delta\)72A | 0.84 ± 0.01 | 4.3 ± 4.3    | –41.6 ± 0.9            | 30.0                   |

The thermodynamic parameters were fitted by a one site binding model with the MicroCal PEAQ-ITC Analysis Software.

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same structure as in the full-length context (Supplementary Fig. 2j). These results show that isolated PFECLC is monomeric in solution and adopts a calmodulin-like N-terminal fold and differs from TgELCs with a disordered C-terminal region.

Essential light chains bind conserved sequence of MyoA. Based on the available literature, T. gondii TgELCs and P. falciparum PFECLC bind to different sites of the MyoA C-terminus13,14,17. For PFECLC, two binding sites at the PfMyoA C-terminus (PfMyoA residues 775–799; see Fig. 3a and Supplementary Fig. 1c)15,16. These results show that isolated PFECLC is monomeric in solution and adopts a calmodulin-like N-terminal fold and differs from TgELCs with a disordered C-terminal region.

TgELCs form similar complexes with PfMyoA and MLC1. The successful formation of trimeric assemblies of MyoA with its light chain proteins allowed us to crystallize and determine the structures of the following complexes: (i) T. gondii MLC1/TgMyoA-C/TgELC1 complex at 2.4 Å resolution (hereafter named complex 1) and (ii) T. gondii MLC1/TgMyoA-C/TgELC2 complex at 2.3 Å resolution (hereafter named complex 2) (Fig. 4a, b, d, e, Table 2). Both complexes constitute a similar architecture. TgMyoA folds into an extended α-helix with a characteristic kink between residues 801–803 (angle of 139° in complex 1 and 137° in complex 2). Both TgELCs display a typical calmodulin fold with
Mutational analysis on TgELC2 (Table 1, Supplementary Fig. 3a) showed that disrupting one of the polar interactions or the conserved hydrophobic residues in the conserved MyoA region play a crucial role for complex formation. In agreement, the phosphomimetic mutation of residue S102, previously shown to be phosphorylated\(^2\), only had a moderate effect on the affinity of TgELC2 to the MyoA peptides, indicating that a single phosphorylation event is likely not sufficient to regulate complex formation (Table 1 and Supplementary Data). 

**PFELC binds PfMyoA in a structurally distinct manner.** To investigate whether the homologous complexes from T. gondii and P. falciparum are structurally similar, we determined the crystal structure of the \(P. falciparum\) trimeric complex (PfMyoA, MTIP, PFELC) at 2.5 Å resolution (Fig. 4g, Table 2). Overall, this structure resembles a similar fold and conformation compared to the \(T. gondii\) trimeric complexes, with the typical MyoA helix kink of 131° between the MTIP and PFELC binding sites. While the secondary structure elements are maintained, the position of the PFELC helices differ. The N-terminal lobe of PFELC aligns well to TgELCs structures (backbone RMSD of 2.5 Å to TgELC1), whereas two binding sites of PFELC at the very C-terminus of PfMyoA were proposed (black arrows)\(^3\), our data show that the actual binding site of PFELC encompasses the MyoA conserved region and is similar to the TgELC/ TgMyoA binding site (blue arrows). The blue boxed residues indicate residues involved in polar interactions with TgELC1 and TgELC2, while yellow boxed residues form polar interactions with MLC1 (see Fig. 4 and Supplementary Data). 

Due to the reduced number of interacting residues at the PFELC C-terminus (Supplementary Data), it seems plausible that C-terminal phosphorylation could play a regulatory role in binding of PFELC to PfMyoA. To test this hypothesis in vitro, we...
mutated residue S127, that has previously been shown to be phosphorylated in vivo\textsuperscript{29}, to a phosphomimetic aspartate residue and observed that the affinity for this variant to PfMyoA C-terminal peptide dropped twofold (Table 1 and Supplementary Fig. 3d). S127 does not directly interact with PfMyoA, but forms a polar interaction with PfELC residue N75, maintaining the tertiary structure of the C-terminal lobe. Based on available data, it is likely that phosphorylation of S127 has a direct impact on the interaction of PfELC with PfMyoA, however, in vivo experiments are necessary to study the impact of this phosphorylation on the glideosome assembly and function.

ELCs induce \( \alpha \)-helical structure in MyoA. Previous reports have shown that the presence of \textit{P. falciparum} and \textit{T. gondii} essential light chains increase the speed of the myosin A motor twofold\textsuperscript{14,16,17}. To understand the function of ELCs on a molecular level, we characterized TgELC2 in a free and bound state with TgMyoA-C\textsubscript{ELC} (see Supplementary Fig. 1c). On size exclusion chromatography, the dimeric complex of TgELC2 and TgMyoA-CELC elutes later than TgELC2 alone, indicating that the hydrodynamic radius of TgELC2 decreases upon binding of TgMyoA-CELC (Fig. 5a). To quantify the structural changes upon binding, we compared the parameters calculated from the SAXS data of TgELC2 alone and in complex with TgMyoA-CELC (Fig. 5b, c, Supplementary Fig. 4a, b, Supplementary Table 3). Changes in the dimensionless Kratky plot (Fig. 5b) as well as the drop of the radius of gyration (2.15 nm to 1.73 nm) and maximum particle size (6.7 nm to 5.5 nm, Fig. 5c) highlight that the dynamic TgELC2 protein undergoes compression upon interaction with the TgMyoA C-terminus. This rigid conformation...
allows the neck region to act as the lever arm of myosin and its stiffness directly correlates with the myosin step size and speed\cite{30,31,32}. Although our crystal structures show that TgMyoA-C forms a continuous \( \alpha \) helix, we noticed that both TgMyoA-C as well as PfMyoA-C are unfolded or partially unfolded in the absence of binding partners (Supplementary Fig. 4c). Indeed, the C-terminal amino acid residues of the recently published TgMyoA\cite{26} and PfMyoA\cite{27} motor domain structures could not be resolved, likely due to their intrinsically disordered nature. We hypothesized that the essential light chains can induce the formation of an \( \alpha \)-helical structure in MyoA upon binding. Therefore, we measured far-UV CD spectra of TgMyoA-C\textsuperscript{ELC} and TgELC2 in isolation and in complex (Fig. 5d). The data revealed that TgMyoA-C\textsuperscript{ELC} is predominantly unstructured while TgELC2 has an \( \alpha \)-helical fold. However, the CD spectrum of the dimeric complex displays a markedly higher \( \alpha \)-helical content than the sum of the spectra of the two individual components, suggesting that the content of the \( \alpha \)-helical secondary structure increased upon formation of the complex. We also observed a similar, albeit less pronounced effect for the TgELC1-TgMyoA-C\textsuperscript{ELC} and P. falciparum trimeric complex assembly (Supplementary Fig. 4d, e). We anticipate that the increase in \( \alpha \)-helical secondary structure content corresponds to the induction of the structure of the TgMyoA C-terminus, which in turn stiffens the TgMyoA lever arm. As a result, the myosins are capable of undergoing a larger step size and thus increase their speed, in agreement with the published functional measurements for both T. gondii and P. falciparum myosin A motors\cite{14,16,17}.

Calcium stabilizes but has no impact on complex assembly. The myosin light chains together with the myosin heavy chain neck region constitute a regulatory domain that in fluences the biochemical and mechanical properties of myosins either upon phosphorylation\cite{33-36} or by direct binding of calcium\cite{37,38}. Apicomplexan invasion is a tightly regulated process, which involves an increase in intracellular calcium concentration\cite{39}. To investigate the role of calcium bound in the first EF hand of both TgELCs, we determined an additional crystal structure of the calcium-free complex TgELC1/MLC1/MyoA-C at 2.0 Å resolution (complex 1f, Table 2). Complex 1f generally adopts...
the same conformation as complex 1. The MyoA-C helix is kinked at a similar angle (134°), and the binding interfaces between MLC1 and TgMyoA as well as between TgELC1 and

MyoA are identical to complex 1 (Supplementary Data). The first EF hand loop and the calcium binding residues remain in the same conformation as in complex 1 except for the side chain of aspartate 17 which is flipped by 120° and thereby enables the release of calcium from the binding pocket (Fig. 6b). In complex 1, calcium is coordinated in a tetragonal bipyramidal geometry by the carboxyl groups of side chains D15, D17, D19, the carbonyl group of E21 and two water molecules. In complex 2, calcium is similarly coordinated by the homologous side chain residues of D16, N18, D20, the carbonyl group of E22 and two water molecules. Additionally, in complex 2, these water molecules are further stabilized by interactions with the side chains of E27 and Q49. Contrary to T. gondii TgELCs, the homologous EF hand loop of PfELC (in isolation or in complex) is bent to the other side and does not possess the residues needed for coordination of calcium (Fig. 6b). In agreement with the presented crystal structures, calcium has no major influence on the secondary structure of individual TgELCs or PfELC (Supplementary Fig. 5a).

Powell et al. recently showed that the absence of calcium notably reduces the affinity of TgELC1 for the MyoA C-terminus15. To investigate this effect in both T. gondii essential light chains, we measured the affinity of TgELC1 and TgELC2 to the TgMyoA peptide with wild type proteins either in the presence of 5 mM calcium or 5 mM EDTA. Strikingly, the difference in affinity is only minor in both cases, with an observed twofold decrease in affinity in the presence of 5 mM EDTA compared to 5 mM calcium (Supplementary Fig. 5b). This is rather surprising, considering the fact that the regulatory role of calcium has been proposed for other myosin light chains35,40. Our binding data are supported by the available crystal structures, where a clear role for calcium regulation is not directly evident. While the presence of calcium affects the affinity of ELCs only to a minor extent, we observed a pronounced effect of calcium ions on the thermal stability of the trimeric complex in a concentration dependent manner (Fig. 6c, d, Supplementary Fig. 5c). This reveals that calcium ions bind TgELCs and mediate substantial stabilization of their sub-complexes, although they do not markedly change their structure or affinity. This is in agreement with previously published functional data, reporting that the absence of calcium does not alter the function of the myosin A motor in both P. falciparum17 and T. gondii13. It is likely that the presence of calcium could have a rather indirect effect, for example by modulating the activity of kinases which in return change the phosphorylation status of members of the glideosome41–43. In conclusion, calcium binding by the first EF hand of TgELCs does not structurally impact the formation of the complex but increases the stability of the complexes per se.

Light chain interactions do not trigger structural changes. Based on our structural work, we have shown that the formation of the TgMyoA-TgELCs dimeric complexes leads to large structural changes and folding of the MyoA C-terminus. In the trimeric complexes, interactions between the light chains have been proposed to mediate the transmission of regulatory signals from distal (MLC) to proximal light chain (ELC) light chains33. To assess the structural changes that could result from the interaction between the two light chains, we recorded SAXS data of the TgELC2-TgMyoA-CELC dimeric complex and compared them to the scattering profile calculated from complex 2 without MLC1 (Supplementary Fig. 6a). Based on a resulting $\chi^2$ of 1.16 Å, it is unlikely that TgELC2 undergoes structural changes upon trimeric complex formation. Similarly, MLC1 and MTIP adopt the same
conformation as in already described structures of their dimeric complexes with MyoA (PDB IDs 5vt9 and 4am0, respectively) and the key interactions remain unaltered in the presence of ELCs (Supplementary Fig. 6e–g, Supplementary Data).

However, the calcium-free crystal structure shows that these interactions are rather independent of the presence of calcium and conserved between complex 1 and complex 2 (Fig. 7). We additionally performed mutational analysis of the interacting residues at the interface of MLC1 and TgELC2. We observed only a minor decrease in affinity upon mutation, but the measured affinities reached the limitations of reliable high affinity ITC measurements (Table 1 and Supplementary Fig. 6d). This leaves open the possibility of cross-talk between the two light chains, however, we do not expect these to have a large impact on the overall structure and myosin motor function because the effect of the mutations at the light chain interface is only minor.

To complete our analysis, we examined whether the formation of the trimeric complexes impacts the structure of the MLC1 N-terminus. The disordered N-termini of MLC1 and MTIP are of particular interest because they are expected to anchor myosin A terminus. The disordered N-termini of MLC1 and MTIP are of the trimeric complexes impacts the structure of the MLC1 N-terminus upon binding to presumably GAP45, as previously reported structures of myosins in complex with their light chains suggest that the converter domains interact with the essential light chain to further stabilize the rigid lever arm and possibly transmit the structural changes from the myosin motor domain to the lever arm.44,45 Similarly, it has been proposed that TgELC1 might constitute a small binding interface with the TgMyoA converter domain.15 To investigate whether the crystal structures of T. gondii complexes are compatible with these observations and to ensure that they do not clash with the TgMyoA core, we built structural models of the TgMyoA motor and neck domain bound to MLC1 and TgELC1 or TgELC2 (Fig. 8).

In both cases, the energy-minimized models did not contain any clashes, indicating that our structures are compatible within the full-length context of TgMyoA (Fig. 8a, b). TgMyoA residues 762–818, which constitute the lever arm, maintained a continuous helix after energy minimization, with both TgELC1 and TgELC2 forming a small number of contacts with the TgMyoA converter domain. These contacts mainly involve the side chain of arginine 81 of TgELC1 or TgELC2 and residues 720–724 of TgMyoA, which is in agreement with the previously published HDX data.15 To further explore the dynamics of full-length TgMyoA with its light chains, we performed normal mode analysis in an all-atom representation on five energy-minimized models from complex 1 and complex 2, and subsequent deformation analysis which allowed us to identify potential hinge regions within these structures. In both cases, all five reconstructed models displayed nearly identical pattern of motions (see Supplementary Fig. 7a for complex 2): the structures undergo...
bending in the hinge region of TgMyoA residues 773–777 in two perpendicular directions (mode 7 and 8) as well as twisting in the same region (mode 9). In the remaining modes (modes 10 and higher), the movement further propagates throughout the lever arm helix up to TgMyoA residue 799. As a result, the deformation analysis of the 20 lowest energy modes predicts the hinge region of the TgMyoA lever arm between TgELCs and the converter domain, and an additional hinge between TgELCs and MLC1 (complex 2 in Fig. 8c and complex 1 in Supplementary Fig. 7b). Such dynamics of myosin light chains is similar to what has been previously described in conventional myosins43,46 and the flexibility in the first TgMyoA hinge could contribute to the efficient rebinding of the myosin motor domain to actin in the pre-power stroke state (Supplementary Fig. 7c)40. In conclusion, the structures of the trimeric complexes composed of the TgMyoA light chains and TgMyoA C-terminus are compatible with full-length TgMyoA and exhibit dynamics that are similar to the dynamics of conventional myosins.

Finally, ELCs generally interact with the myosin converter domain and likely stabilize the hinge region of the myosin neck between the ELC and the converter domain (TgMyoA residues 775–777)40,46. A small interaction interface between the converter domain and TgELC1 has also been suggested previously15. Our models now highlight that both TgELC1 and TgELC2 form polar interactions with the converter domain, however, these are not sufficient to maintain the rigid structure, and the TgMyoA hinge between ELC and the converter domain contributes to most of the movement of the myosin complex. Nevertheless, the normal mode analysis was performed in the absence of a bound nucleotide or actin and the interface between TgELCs and the converter domain might become more rigid once TgMyoA binds actin, as has been previously described for other myosins47.

**Discussion**

Although both gliding and invasion of apicomplexan parasites have been intensively studied in the past, the lack of structural data inhibits the broader understanding of these processes on a molecular level. Our work represents a further step towards grasping glideosome function and the mechanism of apicomplexan gliding and invasion. We have determined crystal structures of the glideosome trimeric sub-complexes of two main apicomplexan representatives, *P. falciparum* and *T. gondii*. Our structures together with binding data show that ELCs bind a conserved sequence of MyoAs. The C-terminus of PfELC is disordered in isolation compared to TgELCs and also adopts a distinct position when bound to PfMyoA, compared to *T. gondii* complexes. The structures also reveal potential regulatory phosphorylation sites on ELCs and our mutational analysis indicates that phosphorylation events can decrease the ELC binding affinity. We have further investigated the role of ELCs in glideosome assembly as well as the impact of calcium ions that we have observed to be bound in the first EF hands of TgELCs. An additional calcium-free structure of a *T. gondii* trimeric sub complex shows that no major structural changes occur upon calcium binding. Indeed, we observe that calcium ions have no impact on the assembly of the complexes but rather stabilizes the trimeric complexes per se. Finally, our biophysical analysis demonstrates that ELCs undergo compression upon binding to MyoA, which induces a helical structure and thereby stiffens the MyoA lever arms. Our functional observations explain previously published data showing that ELCs can double the speed of a myosin A motor whereas calcium has no effect. In conclusion, our study complements and rationalizes the role of glideosome components that have been previously observed while providing new structural and functional data that will be important in the future elucidation of glideosome structure and mechanism of apicomplexan gliding.

**Methods**

**Cloning.** Open reading frames encoding TgELC2 (*TGME49* _305050_) and TgMLC1 (*TGME49* _257680_) subcloned via NdeI/XhoI restriction enzymes into pET28a (+)–TEV vector were purchased from *GenScript*. The TgELC1 gene was cloned, by extending the *TGME49* _209442_ open reading frame (GenScript) into a pNIC28 _Bsa4_ vector via Bsal restriction sites. DNA sequences of PfELC (Pf3D7 _1017500_), PfELC-N (residues 1–74), PfMTIP (Pf3D7 _1246400_), PfMTIP-S (residues 60–204) and PfMTIP77–204 were amplified from *P. falciparum 3D7 cDNA* and cloned into a pNIC28 _Bsa4_ vector via Bsal restriction sites. These constructs have an N-terminal TEV-cleavable His-tag, TgMLC1-S (residues 66–146) was subcloned into a pNIC _C-terminal_ _TEV-cleavable His-tag_ and FLAG-tag. The sequence encoding TgMyoA-C was amplified by two complementary primers and cloned via

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**Fig. 8 Trimeric complexes modeled in the full-length MyoA context.** a Energy-minimized model of complex 1 as a part of TgMyoA. b Energy-minimized model of complex 2 as a part of TgMyoA. The models show that the crystal structures of the trimeric complexes are compatible with the structure of TgMyoA and maintain the α-helical structure of the TgMyoA lever arm. No clashes between TgMyoA and TgELCs were observed. c Deformation analysis of complex 2 identified two hinge regions in the lever arm of myosin A, which contribute to most of the observed dynamics of the protein complex within the 20 lowest-energy modes. The model is colored by deformation energy from lowest (violet) to highest (red). The hinges localize to the TgMyoA lever arm between the converter domain and the TgELC2 binding site (hinge 1, residues 773–777) as well as between the TgELC2 and MLC1 binding sites (hinge 2, residues 799–801). These deformations agree with the role of TgMyoA in the pre-power stroke state in the context of a power stroke cycle, where the myosin is probing the conformational space to bind to actin.
Nel-Nxl restriction enzymes into a pET-GB1 vector. This construct contains an N-terminal TEV-cleavable His-GB1 domain. Expression cassettes of His-TgELC1 and His-GB1-TgMyoA-C were then sub cloned via Nde/Nt/Xba restriction enzymes into a pYPC vector. The His-GB1-TgMyoA-C gene was then cut by Spel/XbaI restriction enzymes and inserted into Spel-cut pYCY-His-TgELC1 to construct the co-expression vector pYPC with TgELC1 and TgMyoA-C.

**Mutagenesis.** Site directed mutants were generated by blunt-end PCR. Briefly, the plasmid was ligated by primers which contain the alternative bases on their 5’ ends and anneal upstream and downstream of the target triplet. The PCR products were digested by DpnII (NEB) overnight at 37 °C and purified by a PCR purification kit (Qiagen). Subsequently, the 5’ ends of the PCR products were phosphorylated by T4 polynucleotide kinase (NEB), the products were purified and the free products of the plasmid re-ligated by T4 DNA ligase (NEB). The positive clones were subsequently selected and their sequence was verified by sequencing.

**Protein expression and purification.** The proteins were overexpressed in *E. coli* BL21(DE3) (MLC1, MTIP, MTIP-S, co-expressed TgELC1-TgMyoA-C + MLC1-S) or *E. coli* BL21-CodonPlus(DE3)-RIL (TgELC1, TgELC2, PIELC, PIELC-N, MLC1-S), in TB medium. The bacterial cultures were induced at OD600 nm of 0.6 with 0.25 mM IPTG and harvested after 4 h at 37 °C (TgELC1, TgELC2, PMDTP) or induced at OD600 of 0.6 by 0.2 mM IPTG and harvested after 16 h at 18 °C (PIELC, PIELC-N, MLC1). The expression of PIELC and PIELC-N for NMR measurements was performed in minimal expression medium as described elsewhere.

The cell pellets were resuspended in lysis buffer (20 mM NaP (pH 7.5), 300 mM NaCl, 0.5 mM TCEP) and buffer supplemented with 1 mM CaCl2. The complexes of TgELC1, TgELC2 or MTIP-S with the MyoA peptide (S777-V818) were mixed with 5-fold excess of 2-iodoacetamide. The samples were dialyzed against 1 L of gel filtration buffer supplemented with 1 mM CaCl2 or EDTA overnight at 4 °C and 2 μl of a 200 μM peptide solution was injected into 20 μl of protein to measure the interaction of the trimeric complex, first, the peptides were dissolved and the proteins dialyzed against gel filtration buffer supplemented with 1 mM CaCl2. The complexes of TgELC1 or MTIP-S with the MyoA peptide (S777-V818) in T. gondii, T. gondii and T. falciparum was first formed in 1:1:1 molar ratio, respectively, and incubated for 1 h at 4 °C. For measurement, 2 μl of 200 mM TgMLC-S or PIELC was injected 19 times into 20 μM of the pre-formed complex. The measurements were performed with a MicroCal PEAK-ITC (Malvern) at 25 °C. The data were processed using the MicroCal PEAK-ITC Analysis Software and fitted with a one-site binding model.

**Bioinformatics methods.** The homologous protein sequence were aligned with the program MAFFT. The protein disorder probability was calculated using the disEMBL server with loops and coils defined by dictionary of secondary structure of proteins. The secondary structure prediction of PIELC, TgELC2 and TgELC1 was calculated in JPred.

**Small angle X-ray scattering.** The SAXS data were collected at the P12 BioSAXS beamline at the PETRA III storage ring (DESY, Hamburg, Germany). The concentrated samples of TgELC2 and PIELC (10 mg/ml) were dialyzed against the buffer (20 mM NaP (pH 7.5), 150 mM NaCl, 0.5 mM TCEP for TgELC2; 20 mM Tris (pH 8.0), 150 mM NaCl, 0.5 mM TCEP for PIELC-N) overnight at 4 °C. Further, the samples were centrifuged (5 min, 15,000 g) and a dilution series of each sample (typically in a range of 0.5–10 mg/ml) and their corresponding solvent were measured at room temperature under continuous flow with a total exposure of 1 s (50 μs frames) at a pixel size of 1.8 Å, as well as the trimeric complexes using different constructs, were measured in 1:1 molar ratio, purified by SEC and concentrated to 10 mg/ml prior to measurement. The X-ray scattering data were measured in an on-line SEC-SAXS mode, using a MicroSAX-PEQ (GE Healthcare) at 0.25 μm with 10 frames recorded per second. The sample of PIELC was concentrated to 10 mg/ml and the X-ray scattering was measured in the on-line SEC-SAXS mode, using a SD200 5/150 column at 0.4 μl/min. The automatically processed data were further analyzed using the ATSas suite of programs CHROMIX and PRIMUS to determine the overall parameters and distance distribution, CRYSOL to compute the scattering from the crystal structures and ORTAL to compute the scattering from the crystal structures with dummy residues mimicking the missing flexible parts. The results of all SAXS measurements are summarized in Supplementary Table 3. All SAXS data and models have been deposited in the SASBDB (www.sasbdb.org) with accession codes: SASDH64, SASDH74, SASDH84, SASDH94, SASDHAA, SASDHC4, SASDH4 and SASDHE4.

**NMR.** All NMR experiments were conducted on a Bruker Avance III 800 NMR spectrometer equipped with a cryoprober at 288 K in 50 mM HEPES, 20 mM NaCl, 0.5 mM TCEP and 10% (v/v) D2O at pH 7.0, except for H(CCO)NH-TOCSY and H'(C(CO)NH-TOCSY. Circular dichroism. To estimate the secondary structure content of the proteins and peptides, we measured circular dichroism on a Chirascan CD spectrometer (Jasco). For spectrum measurements, the CD of each sample (typically in a range of 0.5–10 mg/ml) and buffer without EDTA overnight at 4 °C. The protein concentration was then adjusted to 100 μM (individually) in 50 mM NaP (pH 7.5), 300 mM NaCl, 5% glycerol, 15 mM imidazole, 0.5 mM TCEP) and subsequently 2× against 1 L of gel filtration buffer and varying concentrations of calcium chloride (0–500 μM). 10 μl of sample was loaded in the glass capillaries and heated from 20 °C to 95 °C with a heating rate of 1 °C/min. The fluorescence signals with an excitation wavelength of 280 nm and emission wavelengths of 330 and 350 nm were recorded and the melting temperatures of each sample was calculated as either the maximum of the derivative of fluorescence at 330 nm, and 350 nm, or as maximum of the derivative of the fluorescence recorded at 330 nm.
by (HB)CB(GCG)HD72 and aromatic HC(CHOH)TCOSY experiments and verified by the site-selective 13C labeling.

NOE distances determined from 3D-NOEY-HSQC experiments for 13N, 13C aliphatic nuclei and 13C aromatic nuclei (on 13C2C and 2-13C2C, glucose labeled samples). Phl-Psi dihedral angle constraints were derived using TALOS73. Structure calculations were performed using ARIA 2.5.474 and standard parameters. The lowest-energy models have been deposited in the PDB with accession numbers 6tj3, 6tj4, 6tj5, 6tj6, 6tj7, 6zn3. Secondary structure elements were determined from chemical shifts and the dynamics of the PdEPC backbone was probed using heteronuclear NOEs (1H–15N NOE). This 1H-based dynamics experiment allowed us to distinguish between rigid (1H–13N NOE > 0.7, secondary structure elements, helices), flexible (1H–13N NOE ~ 0.5–0.7, loops and turns) and extremely flexible (1H–13N NOE < 0.5, unfolded/ random coil) regions of the protein. Ramachandran analysis was performed by PROCHECK75.

(1H)13N NOE saturation was performed using a train of shaped 180° pulses in a symmetric fashion76–78 for 3 s and a total inter-scan relaxation period of 10 s. Data collection, processing and analysis details are summarized in Supplementary Table 4.

Crystallography. PdEPC-N was concentrated (5 kDa cut-off) to 26 mg/mL and 200 nL of the sample was mixed with 100 nL of reservoir solution (0.1 M Tris·HCl (pH 8.5), 0.2 M LiSO4, 30% PEG 4000). The crystals grew in sitting drop plates at 19 °C for 7 days.

The trimeric complex of MLC1-S, TgelC2 and TMyoA-C (S777–V818) was mixed in a molar ratio of 1:1:1:1, respectively. After 1 h of incubation, the trimeric complex was separated by gel filtration in 20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP using a Superdex 75 16/60 column (GE Healthcare). The fractions containing the peak of the trimeric complex were concentrated (5 kDa cut-off) to 10 mg/mL. The crystals grew for 7 days at 19 °C in sitting drop plates prepared by mixing 200 nL of the sample with 100 nL of reservoir solution (0.1 M imidazole/MES pH 7.5, 20% w/v ethylene glycol, 10% PEG 8000, 0.03 M of each di-ethylene glycol, tri-ethylene glycol, tetra-ethylene glycol and RMSDs. PdEPC-PISA86 was used to characterize the intermolecular interfaces. The atomic coordinates and the phase information were obtained by molecular replacement with Phaser82, with the structure of peptide-bound TgMLC1 (PDB ID 5vt9) as a search model in case of the trimeric complexes and the NMR structure as search model in case of the monomeric complexes.

Normal mode analysis. Normal mode analysis (NMA)86 was used to probe essential dynamics of the reconstituted trimeric models. The NMA was performed in an all-atom representation on the best five energy minimized models using the BIOSD software89. The deformation analysis was performed, using the first 20, 50 and 100 modes, and also on the first 10 modes separately. This allowed us to not only identify possible hinge points within the studied structures of trimeric complexes, but also to determine which hinges correspond to which modes.

Statistics and reproducibility. In all reported experiments, the protein samples were expressed and purified under identical experimental conditions. The figures represent the results from one experiment, unless stated otherwise. The CD experimental curves were recorded 10 times, averaged and buffer-subtracted. The SAXS data recorded in batch mode represent a buffer-subtracted average of 20 measurements of the same sample measured under continuous flow.

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on request. The data source underlying the charts in the main and supplementary figures is deposited in Figshare repository97. Coordinates and structure factors as well as NMR structures were deposited in the PDB at the Research Collaboratory for Structural Bioinformatics (RCSB) with the following identifying codes: 6tj3, 6tj4, 6tj5, 6tj6, 6tj7, 6zn3. The averaged and subtracted SAXS data were deposited in SASDB with the following identifying codes: SASDH64, SASDH74, SASHDH4, SASDHH4, SASDHB4, SASDHC4, SASDHD4 and SASDHE4. The structural models of full lengths MyoA-MLC1-ELCs have been uploaded to Zenodo98.

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Modeling. The modeling procedure was performed in Modeller version 9.187. We built 50 models for the TgMyoA residues 772–791. These 50 models were fused to the structure of TgMyoA (PDB ID 6dus; residues 33–771). All 50 models were tilted along the bond/dihedral angle between residue 771 and the first modeled residue, that is 772; at the same time, the residues 33–771 of the 6dus structure remained fixed. Thus, each of the produced models consisted of an intact crystal structure 6dus (till residue 771) and the novel modeled fragment of 772–791. Restraints in a form of i-i + 4 h-bonding pattern were imposed in order to ensure that all 50 models have an a-helical conformation along the whole length of the de modeled fragment, that is the junction between residues 771 and 772. The crystal structure of complex 1 (PDB ID 6js5) or complex 2 (PDB ID 6j7) were superposed on the 50 models using the TgMyoA residues 780–791. After superposition, the modeled conformation of this fragment was removed from the superimposed structures, which produced models consisting of an intact crystal structure of TgMyoA (PDB 6dus), the modelled helix of TgMyoA (residues 772–779) and the intact crystal structure of the complex 1 (50 models) or complex 2 (50 models), starting from the TgMyoA residue 780 of these structures. Next, all reconstituted complexes were screened against the existence of atomic clashes using the Chimera software88 and the best five models (both complex 1 and complex 2) were energy minimized by executing 1000 steps of conjugate gradient energy minimization in the NAMD program89. All energy minimizations were performed in a water box with ions.

Data collection and structure determination. The diffraction data of the trimeric complexes were collected at the P13 EMBl beamline of the PETRA III storage ring (c/o DESY, Hamburg, Germany) at 0.976 Å wavelength and 100 K temperature using a Pilatus 6 M detector (DECTRIS). The diffraction data of PdEPC-N were collected at the P14 EMBl beamline of the PETRA III storage ring (c/o DESY, Hamburg, Germany) at 1.033 Å and 100 K temperature using an Eiger 16 M detector (DECTRIS). The diffraction data were processed using XDS79, merged with Aimless80, or (STARANISO81 in case of the trimeric complexes, and phase information were obtained by molecular replacement with Phaser82, using the structure of peptide-bound TgMLC1 (PDB ID 5vt9) as a search model in case of the trimeric complexes and the NMR structure as search model in case of PdEPC-N. In all cases, the models were further built and refined in several cycles using PHENIX83, Refmac584, and COOT85. Data collection and refinement statistics are summarized in Table 2. In all structures, over 98% residues are in the favored region of the Ramachandran plot and each structure contains no more than one Ramachandran outlier. PyMOL was used to generate figures, measure the angle of the helical kink, inter-molecular angles, distances and RMSDs. PDBePISA86 was used to characterize the intermolecular interfaces. The atomic coordinates and the structure factors have been deposited in the PDB with accession numbers 6tj4, 6tj5, 6tj6, 6tj7 and 6zn3.
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