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The closed MTIP-MyosinA-tail complex from the malaria parasite invasion machinery

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Abstract

The Myosin A-tail Interacting Protein (MTIP) of the malaria parasite links the actomyosin motor of the host cell invasion machinery to its inner membrane complex. We report here that at neutral pH *Plasmodium falciparum* MTIP in complex with Myosin A adopts a compact conformation, with its two domains completely surrounding the Myosin A-tail helix, dramatically different from previously observed extended MTIP structures. Crystallographic and mutagenesis studies show that H810 and K813 of Myosin A are key players in the formation of the compact MTIP:Myosin A complex. Only the unprotonated state of Myosin A-H810 is compatible with the compact complex. Most surprisingly, every side chain atom of Myosin A-K813 is engaged in contacts with MTIP. While this side chain was previously considered to prevent a compact conformation of MTIP with Myosin A, it actually appears to be essential for the formation of the compact complex. The hydrophobic pockets and adaptability seen in the available series of MTIP structures bodes well for the discovery of inhibitors of cell invasion by malaria parasites.

Keywords

Apicomplexa; IQ motif; gliding motility; myosin light chain; merozoite; sporozoite

Introduction

Malaria is one of the most devastating infectious diseases worldwide with 300 to 500 million cases and ~2 million deaths per year ^{1, 2}. Multiple *Plasmodium* species are responsible for infecting the human host, the most important ones being *P. falciparum* and *P. vivax*. Anopheline mosquitoes during a blood meal inject sporozoites into the skin which is followed by invasion of hepatocytes, leading a few days later to release of numerous merozoites, which

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Figure Preparation. All figures were prepared with Dino (www.dino3d.org) and rendered with POVRAY (www.povray.org).

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in their turn invade, grow and egress from erythrocytes. A multi-protein complex containing at least seven proteins located between the plasma membrane and the microtubule-supported inner membrane complex (IMC), empowers both substrate-dependent gliding motility and host cell invasion of *Plasmodium* and other Apicomplexan species^{3–5}. This invasion machinery includes (i) an adhesion protein linked via aldolase to actin filaments^{6, 7}; (ii) an actomyosin motor^{8, 9}; (iii) the Myosin A-tail Interacting Protein (MTIP) (Fig. 1) which bridges the unconventional *Plasmodium* Myosin A (MyoA) with the GAP45-GAP50 complex^{10–13}. This paper focuses on the Myosin A-MTIP interaction.

The current paper is an extension of earlier crystallographic studies on *P. knowlesi* MTIP (PkMTIP) which revealed not less than three structures of PkMTIP in a single asymmetric unit: two unliganded PkMTIP monomers and one PkMTIP in complex with the MyoA-tail¹⁴. Those studies showed that several hydrophobic MyoA-helix side chains are deeply buried in hydrophobic pockets of the flexible MTIP C-terminal domain. PkMTIP adopts an extended conformation in this complex with a long, central helix connecting the two PkMTIP domains. The present study of *P. falciparum* MTIP (PfMTIP) in complex with the MyoA-tail extends the previous study in important ways. While the numerous hydrophobic interactions between MTIP's C-terminal domain and the MyoA-tail helix are maintained, the long domain-connecting helix of the PkMTIP:MyoA complex is kinked thereby allowing also the N-terminal domain of MTIP, as well as the hinge region, to interact with the bound MyoA-helix. Most intriguingly, both our mutagenesis and structural studies show that MyoA-K813 plays a crucial role in forming the compact MTIP:MyoA complex. This MyoA-K813 occupies the 7th position of the so called "IQ motif"¹⁵ in myosin light chains and related proteins which form a PFAM family (PF00612) to which MyoA belongs (Fig 1B). Elegant studies by Terrak and coworkers¹⁶ on the so-called "IQ-motif" in myosin light chains from *S. cerevisiae* demonstrated that in that case a lysine at the 7th position prevented formation of the compact structure. Also in our own structural studies of the PkMTIP:MyoA complex¹⁴, we postulated that a clash of the lysine with the N-terminal domain of MTIP would prevent a closed conformation. Our current paper therefore not only provides a more complete picture of the interactions of MTIP with the MyoA-tail in the malaria parasite's invasion machinery, but also reveals a striking exception to what seemed to be a well obeyed rule in myosin complex formation with light chains¹⁷. The series of plasmodial MTIP structures now available provides insight into the major flexibility of the C-terminal domain and the hinge region of this key invasion protein of the world's most important protozoan pathogens. These studies on the plasmodial MTIP:MyoA-tail interactions, together with the recently published structure of *P. falciparum* aldolase interacting with the tail of the TRAP (thrombospondin related adhesion protein)¹⁸, are the initial steps towards an understanding of the malaria parasite's invasion machinery in atomic detail.

Results

The compact structure of MTIP in complex with MyoA

The 1.7 Å resolution structure of *P. falciparum* MTIP, comprising residues E60 to Q204, complexed with residues S803 to A817 of the *P. yoelii* MyoA-tail was solved at pH 7.5 by selenomethionine MAD phasing^{19, 20} (Table 1). Excellent electron density is observed for MTIP residues A63 to Q204 and for the MyoA-S803 to MyoA-A817 helix (Fig. 2). This complex shows unexpected and dramatic structural differences compared to our previous complex of *P. knowlesi* MTIP (PkMTIP), comprising residues K79 to L204, bound to the same *P. yoelii* MyoA-tail helix at pH 5.3 (Fig. 3·4)¹⁴. The compact structure is reminiscent of the complex of Myosin essential light chain and the heavy chain²¹ (see supplemental Fig. 1)

Comparison of the PfMTIP:MyoA and PkMTIP:MyoA complexes reveals that the N-terminal domain rotates by approximately 140° so that both domains of PfMTIP now surround the

MyoA-tail in a clamp-like manner burying 2107 Å² solvent-accessible surface area. This is significantly more than the 1389 Å² in the previous PkMTIP:MyoA complex¹⁴, where MTIP adopted an extended conformation (Fig. 3, 4). Residue S108 located in the N-terminal domain of MTIP forms a hydrogen bond with D173 of the C-terminal domain closing the “clamp” completely around the MyoA-tail in the current structure (Fig. 4B, 5A). Residues 135–140 of the PfMTIP:MyoA complex adopt a non-helical conformation, entirely different from the perfect α -helix seen in the PkMTIP:MyoA complex (Fig. 3). Residues in this hinge region connecting the N- and C-domains now contribute to complex formation by direct interactions with MyoA. A key hinge residue is the completely conserved D139 which forms salt bridges with MyoA-R806 and MyoA-H810 (Fig. 4A, 4B, 5A). The carboxylate of D139 moves 6.7 Å when the extended and compact complexes are compared. The new conformation of the hinge region is stabilized by a salt bridge between D137 of the hinge and K71 of the first helix of the N-terminal domain (Fig. 4A, 4B). In addition to this major change in the hinge residues, both domains of MTIP as well as the bound MyoA-helix have undergone a series of concomitant alterations, described below, to form the unexpected compact complex observed.

The interactions of MyoA with the PfMTIP N-terminal domain, which were entirely absent in the PkMTIP:MyoA complex, are involving a flat surface of the N-terminal domain with mixed hydrophobic and hydrophilic interactions (Fig. 3B). Nine residues of the N-terminal domain, mainly from the conserved loop L104 to I109, contact MyoA burying 716 Å² of solvent accessible surface. The core of the PfMTIP N-terminal domain itself hardly changes with an r.m.s. deviation of only 0.5 Å compared to that of PkMTIP. However, the new N-terminal helix α 1 of PfMTIP forms a second EF-hand with helix α 2, while the new helix α 0 makes stabilizing hydrophobic contacts with helix α 4 by bringing residues I65, L68, L128 and L131 close together.

The C-terminal domain of the PfMTIP:MyoA complex superimposes onto that of the PkMTIP:MyoA complex with an r.m.s. deviation of 1.1 Å for 64 residues, with the “framework”¹⁴ residues Y158 to D194 of α 6-loop- α 7 deviating by only 0.4 Å. Helices α 5 and α 8 move by 11 and 14 degrees, respectively, with respect to the framework to accommodate the MyoA-helix optimally. In both the compact and the extended complex the C-terminal domain of MTIP undergoes major conformational changes when compared to free MTIP, presenting a deep groove with similar hydrophobic pockets ready to accept hydrophobic side chains of the MyoA-helix (Fig. 3B).

The MyoA-helix main chain is shifted by a mere ~ 0.3 Å towards its C-terminus when superimposing the C-domains in the two complexes using the rigid framework residues Y158 to D194 (Fig. 2B). The conformations of the hydrophobic MyoA-tail residues L804, V807 and I811 interacting with the C-domain are very similar in the PfMTIP and PkMTIP complexes, but several residues now contacting the N-terminal domain, such as MyoA-R806, MyoA-K813 and MyoA-R814, adopt dramatically different conformations. MyoA-R806 moves its guanidinium moiety by ~6 Å to form a salt bridge with D139 of the MTIP hinge region. The side chain of MyoA-H810 is involved in important H-bonds to MyoA-K813 and PfMTIP-D139 (Fig. 5) whose critical importance will be discussed below. The guanidinium group of MyoA-R814 shifts by ~3.5 Å to interact with the C-terminal carboxylate of Q204 (Fig. 2B, 5A).

The Critical Lysine of Myosin A

MyoA-K813 is unexpectedly a key player in forming the compact PfMTIP:MyoA complex. Its side chain is entirely buried, completely different from it being fully exposed in the extended PkMTIP:MyoA complex¹⁴. The N ϵ of MyoA-K813 changes position by ~5 Å when the PfMTIP and PkMTIP complexes are compared (Fig. 2B). This amino group is engaged in extensive interactions with the N δ 1 of MyoA-H810, the backbone oxygen of I202 and the MyoA-K813 C-terminal carboxylate of Q204 (Fig. 5A). In addition to these extensive

hydrophilic contacts, the C β , C γ and C δ atoms of MyoA-K813 engage in hydrophobic interactions with the peptide units of MTIP-L104 and MTIP-G103 of the N-terminal domain. Previously, the side chain of MyoA-K813 was expected¹⁴ to bump into the N-terminal domain if the central helix would kink and therefore prevent adoption of a closed conformation by MTIP upon binding the MyoA-tail helix. This idea was supported by the fact that MyoA-K813 occupies the 7th position in the so-called “IQ motif” “IQxxxRGxxxR”¹⁵ which is, to the best of our knowledge, a glycine in all previously known closed structures of the family^{16, 17, 21–30}. Hence our PfMTIP-MyoA structure appears to be unique.

Analysis of yeast two hybrid variants and correlation with structural results

The importance of the contacts made by many of the MyoA and MTIP residues is completely confirmed in solution by mutagenesis and functional studies. These yeast two-hybrid studies were performed with *P. yoelii* MTIP which has no insertions or deletions compared to *P. falciparum* MTIP (Fig. 1). In particular, the loss of function due to the MyoA-K813A substitution is remarkable and underlines the crucial role of this myosin residue in the malaria parasite's invasion machinery.

The correspondence between the yeast-two hybrid studies (Fig. 6) and the structure (Figs. 3, 4, 5) is excellent in general since (using Py for *P. yoelii* and Pf for *P. falciparum*):

- i. PyMyoA-R806 makes contacts with A105 and D139 of PfMTIP (Fig. 5A) in good correspondence with the significant decrease in activity when this arginine is mutated into an alanine;
- ii. PyMyoA-H810 is involved in numerous interactions with MTIP in the PfMTIP:MyoA complex (Fig. 5A) in agreement with the significant decrease in activity when this residue is substituted by an alanine as well as by a glutamate;
- iii. PyMyoA-K813 is engaged in many contacts with MTIP in the PfMTIP:MyoA complex (Fig. 5B) explaining the significant decrease in activity when altered into an alanine;
- iv. The PyMyoA-M805L substitution was carried out as a control of the functional mutagenesis experiments. This residue makes hydrophobic interactions with the C-domain of MTIP (Fig. 5A). The change was expected to result in similar interactions in mutant and wt complex and this was indeed the case.
- v. PyMTIP-H97 corresponds to PfMTIP-Y97 (Fig. 1) and is not engaged in contacts with MyoA (not shown) explaining the lack of significant decrease in activity of the PyMTIP-H97A mutant;
- vi. PyMTIP-R100, is equivalent to PfMTIP-R100 (Fig. 1) which, is involved in extensive contacts of its main chain and its aliphatic side chain atoms with the main chain of MyoA-K813 and the aliphatic side chain carbons of MyoA-R812 (Fig. 5B) in agreement with the significant decrease in activity when PyMTIP-R100 is altered into an alanine. The decrease in activity when PfMTIP-R100 is altered into a glutamate is explained by the fact that the favorable hydrophobic contacts of the PfMTIP-R100 C δ with the MyoA-R812 aliphatic atoms are replaced by unfavorable contacts involving the newly introduced carboxylate of the glutamate side chain;
- vii. PyMTIP-A105 corresponds to PfMTIP-A105 (Fig. 1) which is engaged in contacts with MyoA-R806 (Fig. 5A) corresponding well with the significant decrease in activity when PyMTIP-A105 is altered into a phenylalanine;

- viii. PyMTIP-D139, equivalent to PfMTIP-D139 (Fig. 1), is a hinge region residue which makes extensive interactions with MyoA-R806 and MyoA-H810 (Fig. 5A) explaining the significant decrease in activity when PyMTIP-D139 is changed into an alanine.

Discussion

Factors Promoting the Closed Conformation

There are most likely two major factors responsible for the occurrence of the compact conformation in our current PfMTIP complex versus the extended conformation in the PkMTIP complex. First, MyoA-H810 needs to be in the correct protonation state. This residue is in the current structure engaged in contacts with the amino group of MyoA-K813 and with the carboxylate of MTIP D139 (Fig. 5A), which means that it must be unprotonated. At the pH of 7.5 of the compact PfMTIP:MyoA complex this is well possible, but at the pH of 5.3 of the PkMTIP:MyoA complex the imidazole is protonated and this would cause a very unfavorable interaction with MyoA-K813 were the structure compact.

Second, the slightly longer version of PfMTIP used for the structural studies, compared to that of PkMTIP, forms additional intra-MTIP interactions. Of the extra residues, K71 in PfMTIP is engaged in a salt bridge with D137 of the N-terminal domain thereby stabilizing the “kinked” conformation of what was the connecting helix in the PkMTIP complex (Fig. 4). This interaction was impossible to form in the PkMTIP complex since the residue equivalent to PfMTIP K71 was simply not present in the PkMTIP construct used¹⁴. The lysine at position 71 and an aspartate at position 137 are conserved in the *Plasmodium* MTIP's (Fig. 1). It seems safe to conclude therefore that the compact conformation (Figs. 3, 4, 5) in our new structure is of physiological relevance and also applies to the PkMTIP structure at physiological pH.

Interestingly, our previous structure of *P. knowlesi* MTIP with the MyoA helix suggested that MyoA K813 could be changed into almost any other residue (except possibly a Gly and a Pro) without affecting complex formation with only the C-terminal domain. Yet, the current yeast-two hybrid studies (Fig. 6) show that this is not the case. We like to point out, however, that the key aspect for detecting interactions via yeast two-hybrid studies is the affinity between two proteins, in this case between PfMTIP and PyMyoA. A weak affinity of the extended conformation of MTIP for the MyoA-helix is indicated by the presence of two *unliganded* PkMTIP:MyoA-tail subunits per asymmetric unit described in our previous work¹⁴. Moreover, a recent publication on affinities between calmodulin-binding proteins in the human proteome shows a wide range of binding affinities from 5 nM to 2 μ M³¹. These affinities probably reflect well the range for wild type MTIP-MyoA interactions given the overall similarities in complex formation¹⁷. Estojak et al.³² report that yeast two-hybrid studies detect affinities in the range of 20 nM up to 1 μ M. Therefore it is very likely that the MyoA K813A substitution leads to such low affinities of MTIP for the mutated MyoA that complex formation is undetectable by the yeast two-hybrid method.

Position 7 in the IQ motif

The IQ motif (IQxxxRGxxxR) family of light chains and related proteins has been studied extensively in Rhoads and Friedberg 1997¹⁵. Recently Houdusse et al. 2006³⁰ summarizes these results and describes in detail recognition features of a variety of IQ motifs leading to a modified IQ motif (ϕ Qvv ϕ Rn ϕ xn) where ϕ represents an apolar residue, v a variable residue and n residues interacting with the N-terminal domain of calmodulins³⁰. At the 7th position a glycine or other small residues like alanine or serine can be accommodated in a compact Myosin light chain complex and interact with the N-terminal domain. Replacing the residue at the 7th position by larger residues renders this favorable interaction of the N-terminal domain

with the IQ motif impossible, leading to an extended conformation of this class of proteins 17, 30.

The above results from previous studies would suggest an open extended conformation for the PfMTIP:MyoA complex, as also mentioned in our previous paper ¹⁴, but as demonstrated by mutagenesis and structural studies in the current work the lysine K813 of MyoA replacing the glycine at the 7th position of the IQ motif does not interfere with but instead *promotes* forming a compact conformation of the complex around neutral pH. The change in side chain conformation of MyoA-K813 avoids the anticipated clashes in the current PfMTIP:MyoA complex (Fig. 2B, 5 and Movie, Supplementary information). Most surprisingly therefore, instead of being an obstacle to adapt the compact conformation, the “forbidden” side chain of MyoA-K813 at the 7th position in the IQ-motif appears to promote the compact conformation observed in the PfMTIP:MyoA complex. Moreover every single atom of the lysine side chain is in contact with MTIP residues. Yeast two-hybrid studies carried out with a K813A substitution, fail to form a complex further supporting the importance of K813. Since this residue is invariable in plasmodial MyoA-tails we suggest a compact conformation in all these cases (see for further discussion Supplemental Figure 2 and 3). K813 is replaced by an arginine in *Toxoplasma* and *Cryptosporidium* (Fig 1B) but since the same hydrogen bonding network can be maintained when K813 is substituted by an arginine (data not shown) it is likely that a compact conformation also occurs in these two apicomplexan species.

The two previous unliganded structures of PkMTIP and the extended PkMTIP:MyoA complex ¹⁴ combined with our new compact PfMTIP:MyoA structure give a good feel for the dynamics (see Movie, Supplementary information) of this crucial protein-protein interaction in the invasion machinery of the malaria parasite. Not only the pockets of the C-domain ¹⁴, consisting of 18 residues in PfMTIP and the corresponding residues in PkMTIP, form interesting potential drug targets. Also the malleable C-domain, the connecting linker, and the first few helices of the N-terminal domain may be “frozen” by small molecules into a non-productive conformation (reminiscent of the functioning of e.g. non-nucleoside reverse transcriptase-inhibiting AIDS drugs ³³) thereby preventing the invasion machinery from functioning and prohibiting entry of malaria parasites into both hepatocytes and erythrocytes of the host.

Materials and Methods

Protein Expression and Purification

Cloning, expression and fermentation in *E. coli* of recombinant PfMTIP was performed according to a previously reported procedure used for PkMTIP ¹⁴. Cells were lysed by sonication in a buffer (buffer A) of 20 mM HEPES, 20 mM imidazole (pH 7.8), 200 mM sodium chloride and 10% glycerol, along with one “Complete-EDTA” tablet (Roche) and 1 mg/ml lysozyme. Protein was purified by using a NiNTA column (Qiagen). The protein was eluted with 200 mM Imidazole in buffer A. TEV protease (with a HisTag) was added to a concentration of about 1/50th of the total protein content and the solution was dialysed overnight against buffer A. The solution was again passed over the NiNTA column, this time collecting the flow through containing the protein from which the HisTag had been removed. The protein was further dialysed against a solution of 20 mM HEPES pH 7.8, 50 mM NaCl, 1 mM TCEP and then concentrated to 0.8 mM and flash frozen ³⁴ for long term storage at -80° C. Seleno-methionine substituted protein was made by first growing an overnight culture in 100 ml LB medium at 37°C under control of Kanamycin (50 mg/l) and Chloramphenicol (35 mg/l). The cells were spun down, then resuspended in minimal medium and divided among 4 1-liter batches of minimal medium with the same antibiotics, and grown at 37°C to an OD₆₀₀ of 0.5. The temperature was reduced to 18°C and after 30 minutes amino acids were added to suppress methionine production (to each 1 liter solution, 100 mg lysine, 100 mg threonine, 100 mg phenylalanine, 50 mg leucine, 50 mg isoleucine, 50 mg valine and 50 mg

selenomethionine were added). After a further 30 minutes IPTG was added to 1 mM and protein expression was allowed to continue overnight. The resulting cells were spun down, and subsequent purification followed the same protocol as for the native protein.

Initial Crystal Screening, Peptide co-crystallization and soaking

Initial crystallization conditions found during screening were optimized using the vapor diffusion sitting drop method to obtain the crystals used in the current study. 1 μ l of 0.77 mM PfMTIP in 20 mM HEPES pH 7.8, 50 mM NaCl, 1 mM TCEP and 2.26 mM MyoA-tail peptide Ac-SLMRVQAHIRKRMVA in 20 mM HEPES adjusted to pH 7.5 were mixed with 1 μ l of the reservoir solution in a 24 well Cryschem plate (Hampton Research) and incubated at 19° C. The reservoir solution for the native PfMTIP crystals contained 20% PEG3350 and 0.2 M Potassium Nitrate. For the selenomethionine substituted crystals the protein solution was at a concentration of 0.5 mM PfMTIP and 1.5 mM MyoA-tail peptide in a buffer of 20 mM HEPES pH 7.5, 100 mM NaCl and 1 mM DTT. The reservoir solution contained 30% PEG3350 and 0.17 M potassium thiocyanate. (Similar crystals were obtained in many of the PEG-Ion conditions (Hampton Research)). Crystals appeared over night with approximate dimensions of 400 $\times$ 50 $\times$ 50 μ m. Crystals were cryoprotected for data collection with mother liquor containing 20% glycerol and 2.5 mM *P. yoelii* MyoA-tail peptide prior to flash freezing in liquid nitrogen.

Data collection and structure determination

Diffraction patterns from native PfMTIP:MyoA crystals were collected at ALS 8.2.2 beamline with 1° rotation images and 5s exposure time, SeMet labeled crystals were collected at the SSRL 9-2 beamline at the Se K-absorption edge with 1° rotation images and 5s exposure time. Data reduction was carried out with Wedger ELVES³⁵ as a front end for Mosflm/Scala³⁶ from the CCP4 package³⁷. The crystals belonged to the orthorhombic space group P2₁2₁2₁, with one PfMTIP subunit and one 16mer *P. yoelii* MyoA-tail peptide per asymmetric unit yielding a solvent content of 41.4 %. Two Se sites were located with the program SHELXD³⁸ and further refined in autoSharp³⁹ resulting in a figure of merit of 0.44 and a phasing power of 1.44. An almost complete chain trace was obtained by ARP/wARP⁴⁰ from the selenomethionine data and placed into the native dataset for further phase extension to 1.7 Å. Manual rebuilding was carried out using the program Coot⁴¹. TLS refinement was performed with Refmac5⁴² using the TLS groups determined by the TLSMD webserver⁴³. The PfMTIP structure was refined to a crystallographic R_{work} of 16.7 % and R_{free} of 23.1 %⁴⁴. The r.m.s. deviations of the final model from ideal geometry are 0.006 Å for bond lengths and 0.934° for bond angles⁴⁵. The final models were analyzed with validation tools in Coot⁴¹ as well as MOLPROBITY⁴⁶, indicating 0.0 % outliers in a Ramachandran plot⁴⁷ (Table 1).

Mutagenesis and Yeast two-hybrid assays

A Gal4 DNA binding domain vector containing amino acids 803-817 of *P. yoelii* MyoA (pBD-MyoA) or a Gal4 activation domain vector containing full length *P. yoelii* MTIP (pAD-MTIP) was subjected to site directed mutagenesis to alter key residues using the QuikChange II SiteDirected Mutagenesis Kit (Stratagene). The DNA sequences of the resulting plasmids were confirmed and the plasmids were subsequently transformed into yeast strain PJ69-4a. Functional studies used a β -galactosidase activity essentially as described previously¹⁴. The values are represented as percentage wild type activity (pBD-MyoA + pAD-MTIP) and are the average values of duplicate aliquots from two independent yeast colonies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MTIP	MyosinA-tail Interacting Protein
MyoA	Myosin A
IMC	Inner Membrane Complex
Pf	Plasmodium falciparum
Pk	Plasmodium knowlesi
Py	Plasmodium yoelii

References

1. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005;434:214–217. [PubMed: 15759000]
2. Baird JK. Drug therapy: Effectiveness of antimalarial drugs. *New England Journal of Medicine* 2005;352:1565–1577. [PubMed: 15829537]
3. Kappe SHI, Buscaglia CA, Bergman LW, Coppens I, Nussenzweig V. Apicomplexan gliding motility and host cell invasion: overhauling the motor model. *Trends in Parasitology* 2004;20:13–16. [PubMed: 14700584]
4. Sibley LD. Intracellular parasite invasion strategies. *Science* 2004;304:248–253. [PubMed: 15073368]
5. Schmitz S, Grainger M, Howell S, Calder LJ, Gaeb M, Pinder JC, Holder AA, Veigel C. Malaria parasite actin filaments are very short. *J Mol Biol* 2005;349:113–25. [PubMed: 15876372]
6. Jewett TJ, Sibley LD. Aldolase forms a bridge between cell surface adhesins and the actin cytoskeleton in apicomplexan parasites. *Molecular Cell* 2003;11:885–894. [PubMed: 12718875]
7. Buscaglia CA, Coppens I, Hol WGJ, Nussenzweig V. Sites of interaction between aldolase and thrombospondin-related anonymous protein in *Plasmodium*. *Molecular Biology of the Cell* 2003;14:4947–4957. [PubMed: 14595113]
8. Meissner M, Schluter D, Soldati D. Role of *Toxoplasma gondii* myosin A in powering parasite gliding and host cell invasion. *Science* 2002;298:837–840. [PubMed: 12399593]
9. Sahoo N, Beatty W, Heuser J, Sept D, Sibley LD. Unusual kinetic and structural properties control rapid assembly and turnover of actin in the parasite *Toxoplasma gondii*. *Molecular Biology of the Cell* 2006;17:895–906. [PubMed: 16319175]
10. Herm-Gotz A, Weiss S, Stratmann R, Fujita-Becker S, Ruff C, Meyhofer E, Soldati T, Manstein DJ, Geeves MA, Soldati D. *Toxoplasma gondii* myosin A and its light chain: a fast, single-headed, plus-end-directed motor. *EMBO Journal* 2002;21:2149–2158. [PubMed: 11980712]
11. Bergman LW, Kaiser K, Fujioka H, Coppens I, Daly TM, Fox S, Matuschewski K, Nussenzweig V, Kappe SHI. Myosin A tail domain interacting protein (MTIP) localizes to the inner membrane complex of *Plasmodium* sporozoites. *Journal of Cell Science* 2003;116:39–49. [PubMed: 12456714]

12. Green JL, Martin SR, Fielden J, Ksagoni A, Grainger M, Lim B, Molloy JE, Holder AA. The MTIP-myosin A complex in blood stage malaria parasites. *Journal of Molecular Biology* 2006;355:933–941. [PubMed: 16337961]
13. Gaskins E, Gilk S, DeVore N, Mann T, Ward G, Beckers C. Identification of the membrane receptor of a class XIV myosin in *Toxoplasma gondii*. *Journal of Cell Biology* 2004;165:383–393. [PubMed: 15123738]
14. Bosch J, Turley S, Daly TM, Bogh SM, Villasmil ML, Roach C, Zhou N, Morrissey JM, Vaidya AB, Bergman LW, Hol WGJ. Structure of the MTIP-MyoA complex, a key component of the malaria parasite invasion motor. *Proc Natl Acad Sci U S A* 2006;103:4852–4857. [PubMed: 16547135]
15. Rhoads AR, Friedberg F. Sequence motifs for calmodulin recognition. *Faseb J* 1997;11:331–340. [PubMed: 9141499]
16. Terrak M, Wu G, Stafford WF, Lu RC, Dominguez R. Two distinct myosin light chain structures are induced by specific variations within the bound IQ motifs-functional implications. *EMBO J* 2003;22:362–71. [PubMed: 12554638]
17. Terrak M, Rebowski G, Lu RC, Grabarek Z, Dominguez R. Structure of the light chain-binding domain of myosin V. *Proc Natl Acad Sci U S A* 2005;102:12718–23. [PubMed: 16120677]
18. Bosch J, Buscaglia CA, Krumm B, Ingason BP, Lucas R, Roach C, Cardozo T, Nussenzweig V, Hol WGJ. Aldolase provides an unusual binding site for thrombospondin-related anonymous protein in the invasion machinery of the malaria parasite. *PNAS* 2007;104:7015–7020. [PubMed: 17426153]
19. Van Duyne GD, Standaert RF, Karplus PA, Schreiber SL, Clardy J. Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. *J Mol Biol* 1993;229:105–124. [PubMed: 7678431]
20. Hendrickson WA, Horton JR, LeMaster DM. Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. *EMBO J* 1990;9:1665–72. [PubMed: 2184035]
21. Houdusse A, Cohen C. Structure of the regulatory domain of scallop myosin at 2 Å resolution: implications for regulation. *Structure* 1996;4:21–32. [PubMed: 8805510]
22. Xie X, Harrison DH, Schlichting I, Sweet RM, Kalabokis VN, Szent-Gyorgyi AG, Cohen C. Structure of the regulatory domain of scallop myosin at 2.8 Å resolution. *Nature* 1994;368:306–12. [PubMed: 8127365]
23. Coureux PD, Sweeney HL, Houdusse A. Three myosin V structures delineate essential features of chemo-mechanical transduction. *Embo J* 2004;23:4527–37. [PubMed: 15510214]
24. Coureux PD, Wells AL, Menetrey J, Yengo CM, Morris CA, Sweeney HL, Houdusse A. A structural state of the myosin V motor without bound nucleotide. *Nature* 2003;425:419–23. [PubMed: 14508494]
25. Debreczeni JE, Farkas L, Harmat V, Hetenyi C, Hajdu I, Zavodszky P, Kohama K, Nyitrai L. Structural evidence for non-canonical binding of Ca²⁺ to a canonical EF-hand of a conventional myosin. *Journal of Biological Chemistry* 2005;280:41458–41464. [PubMed: 16227209]
26. Gourinath S, Himmel DM, Brown JH, Reshetnikova L, Szent-Gyorgyi AG, Cohen C. Crystal structure of scallop Myosin s1 in the pre-power stroke state to 2.6 Å resolution: flexibility and function in the head. *Structure* 2003;11:1621–7. [PubMed: 14656445]
27. Himmel DM, Gourinath S, Reshetnikova L, Shen Y, Szent-Gyorgyi AG, Cohen C. Crystallographic findings on the internally uncoupled and near-rigor states of myosin: further insights into the mechanics of the motor. *Proc Natl Acad Sci U S A* 2002;99:12645–50. [PubMed: 12297624]
28. Liu J, Taylor DW, Krementsova EB, Trybus KM, Taylor KA. Three-dimensional structure of the myosin V inhibited state by cryoelectron tomography. *Nature* 2006;442:208–11. [PubMed: 16625208]
29. Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA, Wesenberg G, Holden HM. Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 1993;261:50–8. [PubMed: 8316857]
30. Houdusse A, Gaucher JF, Krementsova E, Mui S, Trybus KM, Cohen C. Crystal structure of apo-calmodulin bound to the first two IQ motifs of myosin V reveals essential recognition features. *Proc Natl Acad Sci U S A* 2006;103:19326–31. [PubMed: 17151196]

31. Shen XC, Valencia CA, Szostak J, Dong B, Liu RH. Scanning the human proteome for calmodulin-binding proteins. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:5969–5974. [PubMed: 15840729]
32. Estojak J, Brent R, Golemis EA. Correlation of two-hybrid affinity data with in vitro measurements. *Molecular & Cellular Biology* 1995;15:5820–5829. [PubMed: 7565735]
33. Sarafianos SG, Das K, Hughes SH, Arnold E. Taking aim at a moving target: designing drugs to inhibit drug-resistant HIV-1 reverse transcriptases. *Curr Opin Struct Biol* 2004;14:716–30. [PubMed: 15582396]
34. Deng JP, Davies DR, Wisedchaisri G, Wu MT, Hol WGJ, Mehlin C. An improved protocol for rapid freezing of protein samples for long-term storage. *Acta Crystallogr D* 2004;60:203–204. [PubMed: 14684931]
35. Holton J, Alber T. Automated protein crystal structure determination using ELVES. *Proc Natl Acad Sci U S A* 2004;101:1537–1542. [PubMed: 14752198]
36. Leslie AGW. The integration of macromolecular diffraction data. *Acta Crystallogr D* 2006;62:48–57. [PubMed: 16369093]
37. Bailey S. THE CCP4 SUITE - PROGRAMS FOR PROTEIN CRYSTALLOGRAPHY. *Acta Crystallogr D* 1994;50:760–763. [PubMed: 15299374]
38. Schneider TR, Sheldrick GM. Substructure solution with SHELXD. *Acta Crystallogr D* 2002;58:1772–1779. [PubMed: 12351820]
39. Vonrhein, C.; Blanc, E.; Roversi, P.; Bricogne, G. Automated structure solution with autoSHARP. In: Doublie, S., editor. *Macromolecular Crystallography Protocols*. Humana Press; Totowa, NJ: 2005. p. 1-24.
40. Cohen SX, Morris RJ, Fernandez FJ, Ben Jelloul M, Kakaris M, Parthasarathy V, Lamzin VS, Kleywegt GJ, Perrakis A. Towards complete validated models in the next generation of ARP/wARP. *Acta Crystallogr D* 2004;60:2222–2229. [PubMed: 15572775]
41. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D* 2004;60:2126–2132. [PubMed: 15572765]
42. Murshudov GN, Vagin AA, Dodson EJ. REFINEMENT OF MACROMOLECULAR STRUCTURES BY THE MAXIMUM-LIKELIHOOD METHOD. *Acta Crystallogr D* 1997;53:240–255. [PubMed: 15299926]
43. Painter J, Merritt EA. A molecular viewer for the analysis of TLS rigid-body motion in macromolecules. *Acta Crystallogr D* 2005;61:465–471. [PubMed: 15809496]
44. Brünger AT. Free R value: a novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 1992;355:472–5. [PubMed: 18481394]
45. Engh RA, Huber R. Accurate bond and angle parameters for X-ray protein structure refinement. *Acta Crystallogr D* 1991;47:392–400.
46. Lovell SC, Davis IW, Adrendall WB, de Bakker PIW, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by C alpha geometry: phi, psi and C beta deviation. *Proteins-Structure Function and Genetics* 2003;50:437–450.
47. Ramakrishnan C, Ramachandran GN. Stereochemical criteria for polypeptide and protein chain conformations. II. Allowed conformations for a pair of peptide units. *Biophysical Journal* 1965;5:909–933. [PubMed: 5884016]

Fig. 1. Sequence alignments of *P. falciparum*, *P. vivax*, *P. knowlesi* and *P. yoelii* MTIP and MyoA
A) Secondary structure elements of the PfMTIP structure are depicted in the top rows of the alignment and the corresponding secondary structure elements of PkMTIP¹⁴ are below each row. The region “α4-hinge-α5 of PfMTIP” is depicted as a bar colored according to the domains involved as shown in Fig. 3A. This region corresponds to the continuous “central helix” of PkMTIP indicated with a bar in shades of blue. Conserved residues are in light red, completely conserved residues in dark red. The 29 crucial residues of PfMTIP for interaction with MyoA-tail are indicated with red asterisks above the PfMTIP sequence; the 17 previously identified residues of PkMTIP contacting MyoA-tail are show in blue asterisks below the sequence. The *P. yoelii* MTIP sequence is added to facilitate the description of the Yeast Two-hybrid studies.

B) MyoA-tail sequences in plasmodial pathogenic protozoa, *Toxoplasma gondii* and *Cryptosporidium hominis*. The canonical IQ motif ¹⁵ is shown above the alignment, with the critical 7th position highlighted in green. Residues involved in interactions with PfMTIP are indicated with black asterisks above the alignment.

Figure 2. Stereo views of the *P. yoelii* MyoA-tail

A) (2F_o-F_c) σA-weighted omit map after final refinement contoured at the 1σ level. All residues of the MyoA-tail used for co-crystallization are well defined.

B) Stereo diagram of the *P. yoelii* MyoA-tail helices in the PfMTIP:MyoA complex, in cyan, and in the PkMTIP:MyoA complex, in grey, after superposition of the “framework” Y158 to D194 of α6-loop-α7 in the C-domains. Only residues of the MyoA-tail bound to PfMTIP are labeled. Highlighted, with shifts in Å, are crucial conformational changes in the MyoA-tail side chains upon binding to PkMTIP at pH 5.3 or PfMTIP pH 7.5.

Fig. 3. Structural overview of the MyoA-tail bound to *Plasmodium* MTIP

A) The dramatic conformation difference of MTIP observed in the complexes with the MyoA-tail of *P. falciparum* MTIP and *P. knowlesi* MTIP. View along the MyoA-tail helix axis in red, after superposition of the “framework” Y158 to D194 of $\alpha 6$ -loop- $\alpha 7$ in the C-domains of PfMTIP and PkMTIP. PkMTIP is kept in blue shades, where the N-terminal domain is in lighter colors connected via the hinge region to the C-terminal domain in darker colors. The PfMTIP N-terminal domain is shown in yellow, the hinge region in sage and the C-terminal domain in orange. The center of mass of the N-terminal domain moves by ~ 24 Å, while rotating by $\sim 140^\circ$ when comparing the extended and compact complexes.

B) Stereo diagrams depicting the surface of the N-terminal domain (yellow; upper) and the C-terminal domain (orange; lower) of PfMTIP with the bound MyoA-tail helix as sticks. MTIP hinge region atoms are shown as sage. Regions of PfMTIP within 4 Å of the MyoA-helix are colored with hydrophobic atoms in green, oxygens in red and nitrogens in blue.

Fig. 4. Intra-MTIP contacts stabilizing the closure of *P. falciparum* MTIP in the compact PfMTIP:MyoA complex

A) View along the MyoA-tail helix (red) bound between the N-terminal (yellow) and C-terminal (orange) domain of PfMTIP. Some important contacting residues are highlighted as green sticks. The hinge region stretching from C134 to N140 is colored in sage.

B) Contacts between residues from the N-terminal and C-terminal domain of PfMTIP stabilizing the “clamp” of the N- and C-domains gripping the MyoA-tail.

C) Salt bridge between hinge region residue D137 and $\alpha 0$ residue K71, thereby stabilizing hydrophobic contacts between helix $\alpha 0$ from the N-terminal domain and the hinged region in the current PfMTIP:MyoA complex. Distances are shown in Å.

Fig. 5. Critical contacts of MyoA with *Plasmodium falciparum* MTIP

A) The hydrophilic network involving MyoA-His810. The MyoA-tail is shown as stick representation in grey, contacting residues of PfMTIP are colored according to their location, N-terminal residues are in yellow, hinge region residues in sage, and C-terminal residues in orange. MyoA-tail residues are labeled in red, PfMTIP residues in black, distances shown are in Å.

B) Each and every side chain atom of MyoA-K813 is interacting with PfMTIP residues in addition to a key interaction with unprotonated MyoA-H810. Stereodiagram depicting hydrophobic (magenta) and hydrophilic (black) interactions of the crucial residue MyoA-K813. Part of the MyoA-tail (grey) is shown with its interacting partners of the N-terminal domain (yellow) and C-terminal domain (orange). MyoA-tail residues are labeled in red and PfMTIP residues in black. For clarity distances were omitted.

Fig. 6. MyoA-MTIP interaction studies using the yeast two-hybrid system

Mutagenesis of key residues of the *P. yoelii* MyoA-tail or *P. yoelii* MTIP protein eliminate or reduce the interaction between these molecules, supporting the structural results. Yeast cells were transformed with either wildtype or mutant MyoA fused to the Gal4 DNA binding domain or either wildtype or mutant MTIP fused to the Gal4 activation domain. Values are represented as % wildtype activity. Experimental procedures are described in the materials and methods section.

Table 1
Data collection, phasing and refinement statistics

	Native	SeMet		
Data collection				
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁		
Cell dimensions <i>a</i> , <i>b</i> , <i>c</i> (Å)	37.2, 54.1, 76.3	37.4, 53.6, 76.7		
		<i>Peak</i>	<i>Inflection</i>	<i>Remote</i>
Wavelength	0.98	0.9789	0.9793	0.905
Resolution (Å)	20.0 - 1.7	28.5 -2.0	28.1 - 2.1	25.1 - 2.2
<i>R</i> _{sym}	0.055 (0.643)	0.089 (0.203)	0.098 (0.580)	0.081 (0.539)
<i>I</i> / <i>σI</i>	15.2 (1.8)	14.8 (10.6)	6.6 (1.6)	6.9 (2.2)
Completeness (%)	100 (100)	100 (100)	99.5 (100)	67.7 (70.9)
Redundancy	5.1 (3.8)	7.1 (7.2)	3.5 (3.5)	2.6 (2.4)
Refinement				
Resolution (Å)	20.0 - 1.7			
No. reflections	14110			
<i>R</i> _{work} / <i>R</i> _{free}	16.7/23.1			
No. atoms				
Protein	1204			
Ligand/ion	127			
Water	228			
<i>B</i> -factors (Å ²)				
Protein	30.2			
Ligand/ion	25.3			
Water	46.0			
R.m.s deviations				
Bond lengths (Å)	0.006			
Bond angles (°)	0.934			
Ramachandran plot				
favored	154/154 (100%)			
outliers	0/154 (0%)			
bad rotamers	1/141 (0.7%)			

* Highest resolution shell is shown in parentheses.