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4 **The Actomyosin interface contains an evolutionary conserved**
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33 **Abstract**

34

35 *Plasmodium falciparum*, the causative agent of malaria, moves by an atypical process called
36 gliding motility. Actomyosin interactions are central to gliding motility. However, the details
37 of these interactions remained elusive until now. Here, we report an atomic structure of the
38 divergent *Plasmodium falciparum* actomyosin system determined by electron cryomicroscopy
39 at the end of the powerstroke (Rigor state). The structure provides insights into the detailed
40 interactions that are required for the parasite to produce the force and motion required for
41 infectivity. Remarkably, the footprint of the myosin motor on filamentous actin is conserved
42 with respect to higher eukaryotes, despite important variability in the *Plasmodium falciparum*
43 myosin and actin elements that make up the interface. Comparison with other actomyosin
44 complexes reveals a conserved core interface common to all actomyosin complexes, with an
45 ancillary interface involved in defining the spatial positioning of the motor on actin filaments.

46

47

48 Introduction

49

50 Malaria is the deadliest parasitic disease around the world, responsible for a half-million
51 deaths per year¹. *Plasmodium falciparum* (Pf), the causative agent of malaria, is able to
52 develop resistance to most of the treatments available today, creating the need for new
53 therapeutic targets². Like other Apicomplexan parasites, *P. falciparum* moves by an atypical
54 process called gliding motility³. The macromolecular complex necessary for force and motion
55 production is called the glideosome, comprised of an atypical myosin A (PfMyoA) and
56 filaments of the dynamic and divergent PfActin-1 (PfAct1)⁴. While the role of PfMyoA in the
57 movement of the parasite had been established⁵, it was only recently shown that PfMyoA is
58 essential for invasion of red blood cells by merozoites, the stage responsible for the symptoms
59 of malaria³. PfMyoA thus represents an efficient pharmaceutical target since blocking its
60 activity would impair both the motile and invasive stages of the parasite^{6,7}.

61 PfMyoA is a divergent myosin, sharing only 28.66% identity with non-muscle myosin
62 IIc (NM2c). Crystallographic structures of PfMyoA revealed an unforeseen N-term extension
63 of the heavy chain that extends over the N-terminal subdomain surface and that can be
64 phosphorylated on serine 19. Phosphorylation specifically tunes the properties of PfMyoA,
65 which can work in two regimes: phosphorylated PfMyoA moves actin at high speed but has
66 low ensemble force; unphosphorylated PfMyoA produces more force but at the expense of
67 speed⁶. These results led us to consider PfMyoA as a tunable molecular engine that could
68 function optimally either at high speed in highly mobile stages such as sporozoites that need
69 to move at more than 2 $\mu\text{m}\cdot\text{s}^{-1}$, or with high force in invasive stages such as merozoites that
70 produce forces of ~ 40 pN to invade the host cell⁸. Sequence comparisons indicate that an N-
71 terminal extension is also present in *Toxoplasma gondii* myosin A (TgMyoA), and
72 phosphomimetics in that region increased *in vitro* motility speeds, suggesting that the tunable
73 mechanism is likely conserved amongst the phylum of Apicomplexan parasites⁹.

74 Actin is one of the most conserved proteins amongst all eukaryotes. Despite being one
75 of the most divergent actins, PfAct1 shows 82% sequence identity with cytoplasmic γ -actin.
76 Given the dynamic nature of PfAct1 filaments, jasplakinolide (JAS), a cyclic peptide from the
77 sea sponge *Jaspis johnstoni* that is able to induce actin polymerization, has to be used to
78 stabilize PfAct1 filaments in structural investigations^{10,11}. Not surprisingly, monomeric and
79 JAS-stabilized filamentous PfAct1 exhibits features similar to mammalian monomeric G-actin
80 and filamentous F-actin^{10,11}. Sequence divergence is primarily located at the binding interface
81 of actin-binding proteins and the nucleotide-binding pocket¹⁰⁻¹². Furthermore, the contact sites
82 important for filament formation contain notable sequence deviations, particularly within the
83 DNase-1-binding loop (D-loop) which is essential for polymerization¹⁰. These sequence
84 differences result in a critical concentration for assembly (in the absence of JAS) that is an
85 order of magnitude higher than for skeletal muscle actin filaments or JAS-stabilized PfAct1¹³.
86 Moreover, PfAct1 filaments are highly unstable once ATP has been hydrolyzed and readily
87 depolymerize¹³, likely explaining why these filaments have been difficult to visualize *in vivo*.

88 The actomyosin complex is recalcitrant to crystallization. Before 2016, the description
89 of this interface by electron microscopy was limited to 7 Å resolution¹⁴⁻¹⁷. The recent
90 improvement in electron cryomicroscopy (cryo-EM) technology and software permitted five
91 mammalian actomyosin structures to be solved at a resolution of better than 5 Å: for human
92 non-muscle myosin 2c (NM2c) in the Rigor state at 3.9 Å resolution¹⁸; for porcine myosin VI
93 (Myo6) in the Rigor state (4.6 Å)¹⁹; and for rat myosin-Ib (myo1b) in the Rigor (3.9 Å) and in
94 two strong binding ADP (3.3 Å and 3.8 Å) states²⁰. All these structures correspond to the
95 states of the motor populated after force generation: the strong ADP state before ADP release,
96 and the nucleotide-free (Rigor) state that have the highest affinity for actin filaments (F-actin)
97 for most myosins. Because the myosin structural elements that bind F-actin are highly

98 divergent in sequence, the actomyosin interface was assumed to vary substantially among
99 myosin motors and is considered hard to predict¹⁹. This divergence explains why, despite the
100 fact that several structures are available at high-resolution, the structural features underlying
101 F-actin recognition by myosin are still highly debated. A better knowledge of the actomyosin
102 interface is crucial to understand which parts of the interface are general features, i.e. common
103 to all myosins, and which ones are specific to a particular myosin. In addition, which parts of
104 the interface are attributable to define a specific cellular function is currently unknown.

105 To understand the mechanism underlying force production by an atypical actomyosin
106 complex and how this interaction differs from previously described recognition of F-actin by
107 mammalian myosins²¹, we determined the cryo-EM structure of PfMyoA bound to
108 filamentous PfAct1 stabilized with JAS. Using likelihood-based reconstruction of helical
109 specimens and a real-space modeling approach aided by molecular dynamics for the
110 refinement of the model, we present a structure at a resolution of 3.8 Å. The PfActomyosin
111 interface highlights the conserved features that provide the structural basis for the ability of
112 PfMyoA to move both PfAct1 and mammalian actin filaments at similar speeds. Moreover,
113 comparison of this interface with the mammalian ones described previously allows us to
114 subdivide the actomyosin interface into two regions: (i) the conserved core interface that
115 governs filament recognition for all myosins, and (ii) the variable ancillary interface that
116 defines the orientation of the motor on the track and tunes specific motor functions.

117

118

119 **Results**

120

121 **High-resolution structure of the PfAct1/PfMyoA complex in the Rigor state.** Unlike
122 canonical skeletal muscle actin, PfAct1 filaments are dynamic and require a very high critical
123 concentration ($\sim 4 \mu\text{M}$) to polymerize¹³. Addition of JAS stabilizes PfAct1 filament dynamics
124 and decreases the critical concentration to the nanomolar range¹¹, which minimizes the
125 presence of actin monomers in the background of cryo-EM preparations^{10-12,22}. Indeed, a 3.8-
126 Å structure of JAS-stabilized PfAct1 filaments has been reported⁸, while a reconstruction of
127 unstabilized PfAct1 filaments has yet to be accomplished.

128 To identify experimental conditions for high-resolution structure determination of the
129 *Plasmodium falciparum* actomyosin complex, various PfMyoA constructs were used,
130 including the full-length protein and the motor domain. However, only sparse and non-
131 cooperative binding to both rabbit skeletal and JAS-stabilized PfAct1 filaments was observed
132 for all pairs. This lack of cooperativity contrasts with the highly cooperative nature of
133 essentially all other previously reported myosin motors binding to actin filaments, which
134 appear either fully decorated or as bare filament stretches in the same field of view in cryo-
135 EM preparations, significantly assisting in particle selection and image processing. Actin-
136 activated ATPase assays showed that the phosphomimetic mutation T417D located in the
137 hypertrophic cardiomyopathy (HCM) loop of the PfMyoA heavy chain (at the TEDS site)²³
138 resulted in a ~ 3 -fold enhancement in actin binding affinity while V_{max} is unchanged²⁴. The use
139 of a PfMyoA motor-domain construct with a T417D mutation increased occupancy of binding
140 to JAS-stabilized PfAct1 filaments in cryo-EM preparations (Figure 1A), although even with
141 the improved occupancy, PfMyoA binding remains sparse (Figure 1B).

142 Using these conditions, cryo-EM and Bayesian analysis of helical specimens²⁵ were
143 employed to determine a 3.8 Å cryo-EM reconstruction of the complex (Figure 1C,
144 Supplementary Figure 1). The reconstruction shows the converter in the post power-stroke
145 position, similar to the position obtained for actin bound myosin II in the Rigor state¹⁸. Using
146 the atomic models of the PfAct1¹¹ and PfMyoA in the Rigor-like state⁸ as starting points, we
147 were able to accurately place the protein chains throughout most of the density map and, in
148 regions with higher resolution, to confidently discriminate and place side chains (statistics in
149 Supplementary Table 1).

150 Regions of the motor further away from the PfAct1 filament such as the Converter and
151 a part of the N-terminal PfMyoA subdomain are less well defined (Figure 1D) in the
152 reconstruction but this is readily explained by increased atomic temperature factors in those
153 regions (Figure 1E). In fact, the model displays a characteristic repartition of the atomic
154 temperature factors: the lowest atomic temperature factors are located close to the
155 PfAct1/PfMyoA interface for the two motor subdomains (Upper-50 kDa and Lower-50 kDa)
156 involved in binding the PfAct1 filament. Higher atomic temperature factors are located in the
157 N-terminus (N-term extension and the SH3-like structural domain), the Transducer, the Relay
158 and the Converter. The regions that display the highest atomic temperature factors are located
159 in the Converter. This suggests a certain flexibility of the Rigor conformation of PfMyoA in
160 this region, as previously observed for other actomyosin reconstructions¹⁷. Actin and the
161 actomyosin interface are well defined in the electron density (Figure 1F-I). The tip of Loop2
162 (residues 632-635) is partially disordered (Figure 2H), but the interactions with actin are
163 visualized without ambiguity.

164

165 **Rigor complex formation occurs with only minor conformational changes to PfMyoA.**
166 Despite the availability of several high-resolution cryo-EM studies¹⁸⁻²⁰, one of the most
167 debated points has been the conformational changes induced by the formation of the
168 actomyosin assembly both in the actin filament and in the myosin motor. The PfMyoA motor

169 bound to the PfAct1 filament closely resembles the crystal structure of the Rigor-like
170 conformation⁸ with overall root-mean-square displacement (rmsd) of 0.84 Å. (Figure 2A,B),
171 There is no electron density at the active site of PfMyoA in this reconstruction that can be
172 attributed to a nucleotide (Figure 2C). This structure thus represents the Rigor state at the end
173 of the powerstroke in which no nucleotide is bound to the motor domain and, like Rigor
174 structures of other myosins, PfMyoA adopts an inner cleft closure in this state (Figure 2D).
175 The degree of cleft closure for the Rigor state of PfMyoA is similar to that of the Rigor states
176 of other myosins¹⁸⁻²⁰.

177 Not surprisingly, some actin-binding elements, namely the HCM-loop (407-430) and
178 Loop4 (363-387) differ in conformation between the PfAct1-bound Rigor and Rigor-like
179 crystal structures of PfMyoA. Note that these two actin-binding elements are quite flexible for
180 the unbound motor but become restrained when myosin binds actin (Figure 2B). As in the
181 Rigor-like crystal structure, the Rigor conformation of the PfMyoA motor is stabilized by a
182 number of atypical interactions involving several elements implicated in mechanical
183 communication during the power stroke as well as the PfMyoA specific N-terminal extension,
184 which is a key element of the unique tunable mechanism of force-production of PfMyoA via
185 its phosphorylation state⁸.

186
187 ***Complex formation does not shift the D-loop of PfAct1 but increases the helical twist.*** The
188 binding of PfMyoA to the PfAct1 filament also does not induce major changes in the
189 conformation of the PfAct1 filament. The structures of the bare¹¹ and decorated PfAct1
190 filaments superimpose with an rmsd of 0.67 Å (Figure 3A). Within the interface, only the D-
191 loop shows some modest changes (~1.4 Å maximum between C α positions) for residues 51-
192 55 that interact with PfMyoA. Notably, the re-orientation of the side chain of E51 is required
193 to avoid a steric clash with myosin (Figure 3B).

194 The helical parameters are changed slightly from the unbound PfAct1 filament with an
195 essentially identical subunit rise of 27.3 Å and a helical twist of -168.1°, a change in twist
196 from unbound PfAct1 filaments (-167.5°)¹¹ by 0.6°, a change similar to that observed for actin
197 binding of human myosin 2C and chicken myosin V^{18,26}. Despite this change in helical twist,
198 neither the intra- and inter-strand interactions nor the specific polar bonds established by the
199 plug are visibly altered in the decorated structure (Figure 3C). Thus, as in the unbound
200 structure¹¹, these interfaces are generally conserved with those in canonical actin and the key
201 differences explaining the intrinsically decreased stability of PfAct1 filaments¹¹, are still
202 present in the PfMyoA bound filament.

203 There is a substantial stabilization of PfAct1 when comparing the normalized relative
204 atomic temperature factor distributions of decorated and undecorated PfAct1 in the interface
205 region (Figure 3D). Surprisingly, the D-loop is the only part of the interface that does not
206 show relative stabilization. The D-loop is one of the few regions that differ in structure in the
207 PfAct1 filament compared with canonical actin. The conformations of the PfAct1 D-loop in
208 the bare or PfMyoA decorated structures are both shifted outward by about 1-2 Å in relation
209 to that of canonical F-actin¹¹. The JAS peptide (Figure 3E) and the ADP nucleotide
210 (Supplementary Figure 2) are seen without ambiguity in the electron density at the same
211 positions as in the undecorated filament.

212 It appears that the PfAct1 D-loop conformation is less sensitive to additional factors
213 binding to the filament than mammalian D-loops. For example, JAS stabilization of rabbit
214 skeletal actin filaments induces an open conformation of the D-loop^{27,28}, while JAS-stabilized
215 PfAct1 filaments adopt the closed conformation that most high-resolution structures of
216 canonical actin filament structures adopt^{11,27,29,30}. Similarly, binding of NM2c and Myo6
217 induces a shift in the D-loop position towards myosin in the corresponding actin subunit^{18,19}
218 while there is no perceivable shift in the PfAct1 D-loop upon PfMyoA binding. Sequence

219 differences between PfAct1 and canonical actin substantially alter the intra-strand interface
220 and the conformation of the D-loop. While in canonical F-actin, Q50 plays a critical role by
221 forming a N-H... π bond with Y170²⁹, the equivalent residue in PfAct1 is glutamate E49 that
222 adopts an opposite orientation.

223 This polymorphism could also explain why the open conformation is not observed in
224 JAS-stabilized PfAct1 filaments, although it would be compatible with the PfMyoA motor
225 domain positioning when bound to PfAct1. The open conformation results from a sharp bend
226 that occurs in the D-loop at residue Q43. Because the equivalent residue is proline P42 in
227 PfAct1, the conformation of the D-loop is restricted, and the open conformation cannot be
228 explored. The open conformation of the D-loop in other actins has been proposed to increase
229 the stability of the actin filament^{27,28,31}. The fact that this conformation cannot be adopted by
230 the D-loop of PfAct1 would thus contribute to the instability of PfAct1 filaments.

231
232

233 ***The actomyosin Rigor interface displays a conserved actin footprint.*** Among the Rigor
234 structures solved to date at high resolution, Myo1b and NM2c share a similar overall
235 orientation of the motor domain on the filament, while the Myo6 Rigor structure differs
236 significantly in the overall orientation²¹. Interestingly, comparison with these structures
237 indicates that PfMyoA adopts an overall orientation that is most similar to that of Myo6
238 (Figure 4A). Thus, the difference in overall orientation previously observed for Myo6
239 compared to other motors¹⁹ is not related to the motor directionality. This observation raises
240 the question of what dictates the differences in positioning of the motor and what are the exact
241 interactions formed at the actomyosin interface that govern the orientation. Both PfAct1 and
242 PfMyoA diverge significantly from the sequences of their mammalian counterparts, raising
243 the question of whether the Rigor interactions between PfMyoA and PfAct1 differ from those
244 previously described due to sequence adaptation co-evolution.

245 To study the actomyosin interface in the PfAct1/PfMyoA Rigor structure, the footprint
246 of the myosin on the PfAct1 filament was determined (Figure 4B, C). Similar to other
247 myosins²¹ PfMyoA interacts with the filament through five canonical structural elements.
248 These are the HCM-loop (407-430); Loop2 (625-643); Loop3 (566-580); Loop4 (363-387)
249 and the Helix-Turn-Helix (HTH, 540-565). The Activation Loop (A-loop) is also considered a
250 canonical actin binding element and has been reported to be a minor part of the Rigor
251 interface of NM2c and Myo1b^{18,20}. In PfAct1/PfMyoA, the activation Loop (524-539) is not
252 part of the Rigor interface, as is also the case for Myo6¹⁹. The mean actin/myosin interaction
253 area of all high-resolution Rigor actomyosin structures is 1607.5 Å² with a standard deviation
254 of 58.1 Å². Thus, the size of the interface is quite well preserved. Interestingly, almost all the
255 PfAct1 residues that interact with PfMyoA are conserved compared to canonical actins
256 (Supplementary Table 2; Supplementary Figure 4). The only region of divergence in this
257 footprint is the D-loop. Despite the divergence in sequence of the actomyosin system found in
258 the apicomplexan glideosome, the PfMyoA residues that are part of the interface define a
259 conserved actin footprint.

260

261 ***The footprint is defined by the recognition of a conserved central core interface with less***
262 ***extensive contributions from a more peripheral ancillary interface.*** The interactions
263 establishing contacts between actin and myosin in the PfAct1/PfMyoA structure were
264 compared to those of other Rigor structures to deduce how the divergent actin-binding loops
265 of distinct myosins accommodate binding to this conserved actin footprint. The conservation
266 of the residues involved in the actomyosin interface has been a subject of debate. Few
267 actomyosin structures are available at a sufficient resolution to confidently deduce the
268 position of residues and interactions made at the interface, and thus a structure fully
269 describing side chain interactions and solvent contribution is still unavailable. However,

270 sufficient differences in main chain loop interactions and some of the side chains defined in
271 cryo-EM maps provide enough information to describe the main features of the Rigor
272 interfaces (PfAct1/PfMyoA described here and in references¹⁸⁻²⁰). The study carried out with
273 Myo6 highlighted the divergence in the interface of this motor compared to previously
274 described actomyosin Rigor complexes¹⁹ whereas the study performed on Myo1b described
275 only minor differences with the NM2c interface²⁰. The PfAct1/PfMyoA interface was
276 compared to those of Rigor structures of NM2c, Myo1b and Myo6 after aligning the
277 structures using the F-actin region involved in myosin recognition. This analysis allowed us to
278 distinguish between a large core interface that is common among all four myosins, and a more
279 divergent and ancillary interface (Figure 4B,C). The core interface, which is essentially
280 completely conserved, is established by the HCM-Loop and most interactions of the HTH,
281 extended by conserved contributions of Loop2. The smaller ancillary interface which is more
282 divergent and established by the HS helix of the HTH, Loop3, Loop4 and, in some cases,
283 additional contributions from the Activation Loop and the strut (Supplementary Table 2). A
284 few additional interactions from Loop 2 and the HW helix may also complete the ancillary
285 interface for some Rigor structures. Interestingly, despite sequence divergence of PfAct1
286 compared to canonical actin, PfMyoA binds to the actin surface where the sequence is
287 conserved in both actins (Supplementary Table 2). Only two polymorphisms are found in
288 actin and they are located in the ancillary interface. They correspond to a residue that interacts
289 with Loop4 (^{Pf}V330, ^{Sk}I331) with no major impact on the interface and to a sequence
290 difference in the D-loop (^{Pf}E49, ^{Sk}G50).

291
292 ***The conserved core interface of Rigor actomyosin structures.*** The conserved core interface
293 of the Rigor state corresponds to the largest part of the interface, including interactions on
294 either side of the myosin inner cleft with the interface between the D-loop and subdomains 1
295 and 3 (SD1, SD3) of the filament (Figure 4B; Supplementary Table 2A). Most of this
296 interface corresponds to extensive apolar contacts with few specific interactions. Several
297 apolar interactions occur from residues of the HR helix or the turn of the HTH. While the
298 sequence of these residues varies amongst the four myosins, the regions of interaction on the
299 actin filament are conserved. The HTH structural element, the actin binding element most
300 conserved in sequence, comprises two invariably conserved residues that interact with actin in
301 a conserved fashion in all Rigor structures. The first conserved residue is an acidic amino-acid
302 from the HR-helix (D544 in PfMyoA) that establishes polar interactions with a serine of SD1
303 (S351 in PfAct1, S350 in canonical actin). The second conserved residue is a proline at the tip
304 region of the HTH (P549 in PfMyoA) that plugs into a hydrophobic cavity at the D-loop/SD1
305 interface between the two neighboring actin monomers. The main actin residues recognizing
306 this HTH turn region belong to SD1 and SD3, close to subdomain 2 (SD2) of the actin below.
307 While less preserved amongst the Rigor structures, residues on either side of this proline in
308 the HTH turn indeed provide additional hydrophobic interactions in the cavity in a lock-and-
309 key fashion (Supplementary Table 2A, Supplementary Figure 3).

310 The second part of the core interface involves the HCM-loop, which is a β -hairpin that
311 presents hydrophobic residues that establish contacts with actin via residues lining the cleft
312 between SD1/SD3 on the hydrophobic cleft (Supplementary Table 2A). The number of the
313 HCM-loop residues that interact with F-actin and their sequence have been reported to be
314 variable among myosins^{19,20}. While the PfMyoA HCM-loop residues that contact the PfAct1
315 filament do not correspond to either of the other myosin sequences, they anchor the HCM-
316 loop in a similar orientation on the actin surface. However, the variability in the nature of
317 these residues greatly influences the exact interface formed. For example, the same negative
318 actin surface residues (E335 in PfAct1, E336 in canonical actin) can either form polar
319 interactions with the first strand of the HCM-loop β -hairpin (PfMyoA, NM2c, Myo1b) or the

320 second strand (Myo2) (Supplementary Table 2A). Another example is the TEDS residue, a
321 consensus site for phosphorylation²³ found at the tip of the β -turn, which is either a negatively
322 charged residue (E,D) or one that can be phosphorylated (T,S). In the Rigor structures, this
323 residue only interacts with a positively charged residue on actin when the position is occupied
324 by a glutamate (i.e. Myo1b or the PfMyoA T417D mutant used in this study). Otherwise,
325 there is no interaction of this residue with actin at the Rigor interface.

326 Lastly, the C-terminal end of Loop2 complements the interaction in the core surface
327 by interacting with a negatively charged patch on SD1 in between the HCM-loop and HTH-
328 turn interfaces (Supplementary Table 2A). Loop2 is the most variable region of the actin
329 binding surface found on myosins – both in size and sequence. Despite this variability, Loop2
330 binds essentially to the same surface on the SD1 of F-actin and is usually stabilized when
331 binding to F-actin¹⁶. As it is the case for Myo1b, PfMyoA has a short Loop2. One common
332 feature among the Myo1b and PfMyoA Rigor complexes is that the interactions formed
333 involve positively charged residues of Loop2, extended to positively charged residues of the
334 strut (Supplementary Table 2A). Although these residues are not conserved in their position
335 (G633 and K634 in PfMyoA), they interact with the same residues of the actin surface (G24,
336 D25, D26 in PfAct1).

337
338 ***The ancillary interface of the Rigor structures.*** The ancillary interface (Figure 4C,
339 Supplementary Table 2B) comprises the most variable interactions that are also regions of the
340 interface distal from the main core. This actin-binding surface is composed of residues
341 interacting with the HS helix of the HTH, Loop3, Loop4 and, in some cases, the Activation
342 Loop. The N-terminus of the actin subunits is flexible and often disordered in the Rigor
343 structures, but it may participate in this ancillary interface. This is clearly indicated in the
344 cryo-EM map of the NM2c Rigor structure in which contacts with actin's N-terminal residue
345 E5 occur via residues of loop2 and the HW helix (Supplementary Table 2B; Supplementary
346 Figure 5A; Supplementary Figure 4). All four sites of the ancillary interface differ in the
347 nature of the contacts made among actomyosin structures (Supplementary Figure 3). (i)
348 Loop3 is highly variable in sequence and in length, but residues of the N-terminal part of its
349 sequence bind the same residues in SD1 although the exact contacts made for the different
350 Rigor myosin interfaces are different (Supplementary Figure 5B). (ii) Loop4 is also highly
351 variable in sequence and in conformation and has fewer and non-conserved contributions
352 compared to elements that form the core interface (Supplementary Figure 5C; Supplementary
353 Table 2B). The tip of the loop interacts with SD3 of actin. In the NM2c Rigor complex, there
354 are only minor contacts with SD3 and Loop4 and this region favors interactions with
355 tropomyosin¹⁸. It is tempting to speculate that the contribution of Loop4 to binding of the
356 filament is class-dependent and allows specificity in the recognition of the target actin
357 filament as well as tropomyosin interactions. Myo1b is indeed less efficient on actin-
358 tropomyosin filaments and this contributes to the selective localization of this myosin in
359 cells^{32,33}. (iii) The Activation Loop is not part of the Rigor interface of PfAct1/PfMyoA and
360 that of Myo6. Some contributions of this loop to the Rigor interface occur for NM2c and
361 Myo1b although these are relatively minor (Supplementary Table 2B). (iv) Lastly, the HS
362 helix of the HTH motif provides critical interactions with the D-loop and thus can extend the
363 interface close to the core (Supplementary Figure 5D; Supplementary Figure 3). In the PfAct1
364 and canonical actin structures, the D-loop is the structural element that explores the most
365 dynamic structural conformations; and these are influenced by the nucleotide-state of the
366 filament^{27,28}. PfAct1 is divergent from canonical actin in the D-loop conformation and this is
367 also where the main sequence differences of the footprint reside, i.e. the presence of a
368 glutamate (E49) instead of a glycine (G50) found in canonical actin. Interestingly, while most
369 myosins have a negatively charged residue on the HS helix facing G50, the sequence of

370 PfMyoA is a serine (S558) at this position, avoiding some charge repulsion between the
371 residues in contact. Myo6 has also a serine in this position and the conformation described for
372 the D-loop locally differs from that of Myo1b and NM2c, allowing it to optimize contacts
373 with the small serine side chain (Supplementary Table 2B). This D-loop conformation found
374 for a canonical F-actin when bound to Myo6, would not be compatible with the presence in
375 the myosin HS helix sequence of an aspartate or a glutamate. We conclude that the
376 interactions of the HS helix with the D-loop can cause local and possibly important induced
377 fits when the motor and the actin filament interact. This region of the ancillary interface close
378 to the core interface is likely to play an important role in defining how different myosins
379 would be more or less efficient depending on the age of the F-actin filament they are
380 interacting with³⁴.

381
382 ***The ancillary interface determines the position of myosin on F-actin in the absence of***
383 ***nucleotide.*** The motor positioning on the filament can vary greatly when the cryo-EM Rigor
384 structures are compared. The core interactions are at the center of the interface and are not
385 likely to influence this positioning significantly. In contrast, the ancillary interface probably
386 plays a more important role in positioning. The fact that the lower 50 kDa domain differs in
387 its positioning for PfMyoA compared to the other motors, is likely linked to the interactions
388 that occur between the HS helix and the D-loop, which themselves depend on the
389 conformation and sequence of the D-loop. In the case of Myo6, for which the difference in the
390 lower 50 kDa domain orientation is greater, optimization of interactions with Loop3 is likely
391 to be a key factor.

392 A closer look at the interface indicates how the core interactions can be conserved
393 despite a rather large difference in the position of the different motors on F-actin. Local
394 adaptation in the conformation of the actin residues near the C-terminus is also likely to
395 optimize the core interactions with the HR-helix. Another key element that may conserve the
396 core interactions involves flexibility in the HCM-loop, which optimizes the orientation of the
397 upper 50 kDa domain to accommodate ancillary interactions with Loop4. The flexibility at the
398 beginning and end of both the HCM-loop and Loop4 are restrained but could be essential for
399 allowing the repositioning of the upper 50 kDa domain resulting in conservation of the
400 footprint and the cleft closure in these different Rigor structures.

401
402 ***Motility assays show similar speeds with various actin and myosin pairs.*** The finding that
403 canonical actomyosin systems and the divergent *P. falciparum* actomyosin system share a
404 common interaction footprint was unexpected. To test whether this equivalence in footprints
405 translates to equivalence in functionality, motility assays were performed using PfMyoA and
406 smooth muscle myosin paired with either skeletal muscle actin, smooth muscle actin, or
407 PfAct1 filaments. To allow meaningful comparison, identical setups were used for evaluating
408 motility, i.e. the respective myosin motors were adhered to the coverslip and the speed of
409 unrestrained actin filaments being moved was evaluated. This setup should be insensitive to
410 the differences in twist between canonical actin and PfAct1 filaments.

411 Interestingly, no difference in speeds were observed when PfMyoA moved JAS-
412 stabilized PfAct1 filaments, skeletal muscle actin or smooth muscle actin. Although JAS has
413 profound effects on the polymerization properties of PfAct1¹³, the PfMyoA motor did not
414 discern a difference when it moved JAS-stabilized versus unstabilized PfAct1 filaments.
415 Likewise, when smooth muscle myosin was paired with JAS-stabilized PfAct1 filaments,
416 skeletal muscle actin, or smooth muscle actin, no difference in speeds were observed (Table
417 1). These assays demonstrate that PfMyoA moves all actin filaments with essentially identical
418 speed, and that the conventional motor smooth muscle myosin also moves PfAct1 filaments at

419 indistinguishable speeds to how it moves canonical actin filaments. Similarities in the
420 interaction footprint thus translate directly into similarities in motility speeds.

421

422 **Discussion**

423

424 The binding of PfMyoA to the PfAct1 filament does not induce major conformational changes
425 in the actin filament or in the motor. The cleft closure in the motor is similar to that found for
426 other myosin motors bound to actin¹⁸⁻²⁰ and the Rigor-like crystal structure of PfMyoA⁸. The
427 D-loop in the bound PfAct1 filament is quite similar to the conformation seen in other Rigor
428 structures, all of which have been reported to have a closed D-loop conformation, consistent
429 with the fact that the motor was bound to ADP F-actin filaments^{18-20,35,36}. The D-loop
430 conformation in PfAct1 filaments does not shift position upon PfMyoA binding, which differs
431 from at least two mammalian actomyosins, namely NM2c and Myo6 in the Rigor state^{18,19}.

432

433 The actomyosin interface is composed of a highly conserved actin core extended by
434 more variable ancillary regions. Comparison of currently available high-resolution cryo-EM
435 reconstructions of myosin strongly bound to actin filaments provides insights about the
436 conserved elements in the strong binding states of myosin motors: (i) a conserved motor
437 domain conformation with a specific cleft closure, (ii) a conserved surface on the bound actin
438 filament that participates in interaction with myosin and (iii) an absence of major
439 conformational changes of the filament when binding a motor, although a relative decrease in
440 the dynamics of some filament regions occurs.

440

441 Remarkably, the divergence in sequence of PfAct1 compared to canonical actin does
442 not impact residues of the footprint, with the notable exception of the D-loop, which is
443 variable in sequence. Thus, the myosin side of the interface remarkably adapts to recognize
444 the same residues of the footprint, by forming locally distinct interactions. This makes the
445 interface variable in detail, as previously observed^{19,20}, while retaining conserved general
446 principles. Local adaptations on myosin lead to recognition of the same residues on the actin
447 surface for the different myosins. Motility assays support these structural observations by
448 showing that a given myosin motor can move heterologous actins at the same speed as its
449 cognate actin.

449

450 The remarkable finding that there are conserved features among Rigor structures
451 spanning the phylogenetic tree from apicomplexans all the way to humans is important for
452 understanding how motors have evolved. Myosins are traditionally classified as members of a
453 superfamily based on phylogenetic analysis of their head domains³⁷. While these types of
454 phylogenetic relationships provide insights into how specific myosin functions are related
455 within and between families, the evolutionary history of myosin is not revealed by such
456 studies. This history can only be assessed by considering the evolution of entire organisms.
457 Except for the structure presented here, all other high-resolution actomyosin structures solved
458 up to now were mammalian.

458

459 Myosin is specific to Eukaryotes and does not occur in Bacteria or Archaea. The
460 phylogenetic relationship between *P. falciparum* and mammals is about as distant as it can be
461 within the eukaryotic branch of the phylogenetic tree of life. In the latest super-group model
462 of the tree³⁸, the branch that contains the TSAR (Telonemids, Stramenopiles, Alveolates, and
463 Rhizaria) super-group, which includes *P. falciparum* (Alveolata), and the one that contains
464 mammals (Amorphea), divide very near the root of the tree. While the exact position of the
465 root is still under debate, it is clear that the common ancestor of *P. falciparum* and mammals
466 is closely related to the Last Eukaryote Common Ancestor (LECA).

466

467 Extensive phylogenetic analysis indicates that the LECA had only two different
468 myosins present in its genome³⁹. The high-resolution structure of *P. falciparum* actomyosin,
469 in conjunction with the mammalian counterparts, thus gives us the opportunity to infer

469 features of the actomyosin complex and its interactions that were already present in the LECA
470 and have been preserved for over a billion years⁴⁰. The fact that the core actomyosin
471 interactions are essentially identical in all currently known structures, indicates that they have
472 been already present in this form in the LECA and did not change substantially during
473 evolution since then. It is likely that these core interactions are preserved among all
474 actomyosins. The plethora of myosin families and functions thus is independent of this core
475 interaction and is a consequence of secondary actomyosin interactions governed by the
476 divergent portions of the interface as well as other structural elements within specific myosins
477 that are far removed from the interface.

478 The common, evolutionary conserved interactions that occur for all Rigor structures,
479 provide an anchoring site of the motor on the actin filament that appears to be independent of
480 myosin type, species and function. Importantly, there is also an ancillary footprint on actin
481 with varying actomyosin interactions; the specificity of track recognition (e.g. selection
482 depending on the tropomyosin bound or depending on the age of the filament) resides in the
483 subtlety of the differences found in this interface. In addition, it is likely that these subtle
484 differences play a role in the evolution of cellular roles of specific myosins, since the time
485 spent in the actin-bound states is critical to define how long a motor stays bound to the
486 filament. Current evidence indicates that the internal rearrangements and the stabilization of
487 the Rigor state by interactions within the motor that involve the N-terminal extension can
488 greatly impact ADP release and the duty ratio for both Myo1b and PfMyoA^{8,41}. It remains to
489 be explored whether the specific variations among Rigor structures are also associated with
490 properties of the motor such as the duty ratio, which for several myosins depends mainly on
491 the ADP release rate. Defining whether possible local changes at the actin interface can tune
492 ADP release will require further investigation.

493
494

495 **Methods**

496

497 **Myosin expression and purification.** A truncated PfMyoA heavy chain (PlasmoDB ID
498 PF3D7_1342600/ GenBank accession number XM_001350111.1) coding for the motor
499 domain (amino acids 1-K768, PfMD), with a T417D mutation to enhance actin affinity,
500 obtained by PCR using gBlocks[®] gene fragments (Integrated DNA Technologies) with Sf9
501 cell preferred codons as template. Residue K768 was followed by a Gly-Ser linker and a C-
502 terminal FLAG tag. The PCR product was cloned into the baculovirus transfer vector
503 pAcSG2 (BD Biosciences) to make recombinant virus²⁴. The PfMD construct was co-
504 expressed with the PUNC chaperone in Sf9 cells. Cells were grown for 72 hrs, harvested and
505 lysed by sonication in 10 mM imidazole, pH 7.4, 0.2 M NaCl, 1 mM EGTA, 5 mM MgCl₂,
506 7% (w/v) sucrose, 2 mM DTT, 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 5 µg/ml
507 leupeptin, 2 mM MgATP. 3 mM MgATP was added prior to a clarifying spin at 200,000 x g
508 for 40 min. The supernatant was applied to a FLAG-affinity chromatography (Sigma), the
509 column washed with 10 mM imidazole pH 7.4, 0.2 M NaCl, and 1 mM EGTA and the myosin
510 eluted with the same buffer containing 0.1 mg/ml FLAG peptide. Peak myosin fractions were
511 pooled and concentrated using an Amicon centrifugal filter device (Millipore). PfMD was
512 dialyzed versus 10 mM imidazole pH 7.4, 10 mM NaCl, 1 mM MgCl₂, 1 mM EGTA, 1 mM
513 NaN₃, 1 mM DTT prior to use.

514

515 **Preparation of JAS-stabilized actin filaments.** PfAct1 (PlasmoDB
516 PF3D7_1246200/GenBank accession number XM_001350811.1) followed by a linker and
517 human thymosin-β4 (accession number AAI41977.1) was synthesized using gBlocks[®] gene
518 fragments with Sf9 cell preferred codons and cloned into pAcUW51 for expression in Sf9
519 cells. The initial Cys residue of actin was not included in the construct because it is not
520 present in the mature, processed protein. The thymosin-β4 gene was separated from the actin
521 gene by a 14-aa linker (ASSGGSGSGSGGA) to allow the thymosin-β4 enough flexibility to
522 occupy the barbed end binding site on the actin monomer, thus preventing the actin from
523 polymerizing with itself or native Sf9 cell actin. A HIS6 tag was added at the C terminus for
524 purification on a nickel affinity column. The final native residue of actin is Phe, which
525 provides a site for chymotrypsin cleavage to remove the linker, thymosin-β4, and the HIS6
526 tag completely. PfAct1 monomers were purified on a gel filtration column (Superdex 200
527 10/300 GL, GE Lifesciences 28990944) equilibrated in 5 mM Tris pH 8.0 at 4°C, 0.2 M
528 NH₄Ac, 0.2 mM CaCl₂, 0.2 mM NaATP, 0.5 mM DTT. The peak monomeric fractions were
529 converted to actin-MgATP by incubation with a 2-fold molar excess of MgCl₂ and 200 µM
530 EGTA for 10 min at 4°C. A 1.1-fold molar excess of jasplakinolide (Thermo Fisher J7473)
531 was added to the PfAct1-MgATP, followed by addition of one-tenth volume of a 10×
532 polymerization buffer (100 mM imidazole, pH 7.4, 500 mM KCl, 40 mM MgCl₂, 10 mM
533 EGTA, 10 mM DTT), and incubation at 37°C for 1 hour. Excess ATP was removed by
534 dialysis versus 10 mM imidazole, pH 7.4, 50 mM KCl, 4 mM MgCl₂, 1 mM EGTA, 1 mM
535 DTT.

536

537 **Optimized sample preparation and image acquisition for cryo-EM.** Various PfMyoA
538 constructs were screened in conjunction with Skeletal F-actin, *P. falciparum* actin 1 (PfAct1)
539 stabilized or not by JAS. These constructs included the full-length protein and the motor
540 domain. In our hands, the best samples were generated when myosin was incubated in
541 solution with the F-actin sample and vitrified within 15 minutes. Thus, four grids were
542 generated at a time. In general, we applied 5 µl of filamentous actin/myosin solution to
543 plasma cleaned holey carbon grid (C-flats 2/2, Protochips), incubated it for 1 min and

544 manually blotted with filter paper and achieved vitrification by plunging into LN₂ cooled,
545 liquified ethane using an in-house designed cryo-plunger.

546 Screening for the best sample mixture ratios and blotting conditions was performed on
547 a T12 Tecnai Spirit electron microscope (ThermoFisher Scientific) equipped with a 4Kx4K
548 Eagle camera (ThermoFisher Scientific), operated at a voltage of 120 kV. Data sets were
549 acquired on a Titan Krios electron microscope (ThermoFisher Scientific) equipped with an
550 extra-high brightness field emission gun (X-FEG) and operated at a voltage of 300 kV.
551 Although the sample preparation protocol was optimized, we had to screen for usable grids
552 and grid squares manually. Images were recorded with a back-thinned 4K×4K Falcon 2
553 (ThermoFisher Scientific) direct detection camera under minimal dose conditions using the
554 automatic data collection software EPU (ThermoFisher Scientific). Within each selected grid
555 hole, two positions were imaged, each with a total exposure time of 1s. Seven frames with a
556 total electron dose of ~60 electrons per Å² were collected. A total of 6073 image stacks were
557 collected in five separate, independent imaging sessions. The nominal magnification of
558 75,000 corresponds to a pixel size of 1.035 Å. The defocus range of the data sets was 0.7–2.8
559 μm.

560
561 ***Image processing and reconstruction.*** Dose weighting and motion correction were applied
562 using MotionCor2⁴² with anisotropic motion correction using 5 x 5 patches. The initial
563 defocus was estimated either using Gctf⁴³ or CTFFIND4⁴⁴, depending on the imaging session.
564 The data was then processed with the helical reconstruction routines in RELION3^{25,45}.
565 Briefly, the helices were divided into overlapping boxes that were essentially treated as
566 individual, independent particles (with modified Bayesian prior accounting for constraints
567 implied by helicity) to allow sorting of the segments into different conformations and
568 selecting the most well-defined of the conformations present in the sample, a prerequisite for
569 reaching high resolution. A total of 509,035 filament segments were extracted using a box
570 size of 336 × 336 pixels from 41,940 manually picked filaments. Two-dimensional reference-
571 free classification for the data set was carried out in RELION3 to eliminate bad segments
572 reducing the number of segments from 509,035 to 464,646. As an initial model, an in-house
573 rabbit skeletal actin reconstruction filtered to 40-Å resolution was used. An initial
574 reconstruction at 5.1 Å showing clear and detailed density for PfMyoA was obtained. After
575 several rounds of 3D classification and refinement followed by manual removal of bad
576 particles and careful enrichment of segments showing clear decoration using the initial
577 reconstruction as a reference, the estimated resolution of the reconstruction reached 3.8 Å
578 after processing using the 0.143 FSC cutoff gold-standard procedure implemented in
579 RELION3. The processing included RELION3-based CTF refinement, B-factor sharpening,
580 and application of a soft-edged mask generated in RELION3 corrected for helical edge effects
581 using pyCoAn, an extended python version of CoAn⁴⁶. Additional sharpening was applied
582 using the auto-sharpening routine in Phenix^{47,48}. The reconstruction was then symmetrized
583 within pyCoAn using the helical parameters refined by RELION3. Local resolution estimates
584 were calculated with ResMap⁴⁹.

585
586 ***Model building and refinement.*** The atomic models of the PfAct1 filament stabilized by JAS
587 (PDB code 5OGW; <https://www.rcsb.org/structure/5OGW>) and PfMyoA in the Rigor-like
588 state (PDB code 6I7D chain D; <https://www.rcsb.org/structure/6I7D>) as starting points. The
589 models were independently fitted into the map with the function “fit in map” in UCSF
590 Chimera⁵⁰. Building of Loop2 and manual refinement of the model was performed with
591 Coot⁵¹. A first step of real space refinement was performed with Phenix⁴⁸. In order to improve
592 the statistics at the interface and to compute realistic interactions, we performed an
593 independent run of molecular dynamics. The system was built with the CHARMM-GUI^{52,53},

594 using the Quick MD Simulator module. Only the actin binding elements of PfMyoA and
595 surfaces of the PfAct1 filament interacting with PfMyoA were relaxed in a box containing
596 explicit water (TIP3P) and salt (150 mM KCl) in the CHARMM36 force field, the rest of the
597 model being fixed during all the duration of the simulation. The duration of the simulation
598 was 10 ns in GROMACS (version 5.1.4)⁵⁴. The output of the simulation was then submitted
599 to another run of real space refinement. A final run of reciprocal space refinement was
600 performed with Refmac⁵⁵. To improve the overall consistency between the models used for
601 comparison, the published models of the Myo6 and Myo1b Rigor states were subjected to
602 reciprocal space refinement with Refmac. Quality indicators, including MolProbity⁵⁶ and
603 EMRinger⁵⁷ scores, were calculated with Phenix. The additional refinement step improved the
604 EMRinger score of Myo6 from 0.11 to 0.36 and the EMRinger score of Myo1b from 1.41 to
605 1.68. The Rigor state of NM2c was already refined with Refmac so the additional step was
606 not necessary for that model.

607
608 **Interface analysis.** All the actomyosin interfaces were analyzed automatically with the
609 software PDBsum⁵⁸ for protein-protein interfaces. The automatic analysis was complemented
610 by a manual analysis to check long-range interactions with a cut-off of 4 Å. Areas of
611 interaction were calculated with PISA⁵⁹.

612
613 **Motility assays.** For ensemble motility assays with PfMyoA and phosphorylated smooth
614 muscle myosin, MgATP insensitive heads were removed by a 20 min spin at 350,000 x g in
615 the presence of 1.5 mM MgATP and a three-fold molar excess of actin. Myosin
616 concentrations were determined using the Bio-Rad Protein assay. For PfMyoA motility
617 assays, the following solutions were added to a nitrocellulose-coated flow cell in 15 µl
618 volumes. 0.5 mg/ml biotinylated bovine serum albumin (BSA) in buffer A (100 mM KCl, 25
619 mM imidazole pH 7.5, 1 mM EGTA, 4 mM MgCl₂, and 10 mM DTT) was added and
620 incubated for 1 min. Three additions of 5.0 mg/ml BSA in buffer A were followed by a 2 min
621 wait. 25 µg/ml neutravidin (Thermo Fischer Scientific) in buffer A was added for 1 min and
622 then rinsed three times with buffer A. 120 µg/ml PfMyoA with a C-terminal biotin tag²⁴ was
623 flowed into the cell and incubated for 1 min followed by 3 rinses with buffer A. Skeletal,
624 smooth⁶⁰ and JAS stabilized PfAct1 filaments were incubated for 30 s followed by one rinse
625 with buffer A and one rinse with buffer B (buffer A plus chromobody, 0.5% (w/v)
626 methylcellulose, 25 µg/ml PfELC and 25 µg/ml PfMTIP and oxygen scavengers (50 µg/ml
627 catalase (Sigma), 125 µg/ml glucose oxidase (Sigma), and 3 mg/ml glucose). 50 nM
628 chromobody was used to visualize skeletal and smooth actins; 20 nM chromobody was used
629 for PfAct1 filaments. Motility was initiated by adding buffer B containing 2 mM MgATP
630 twice to the flow cell, followed by a 1 min wait for temperature to equilibrate to 30°C on the
631 microscope. Unstabilized PfAct1 filaments (freshly prepared for each experiment by
632 polymerizing at 8 µM for 10 min at 37°C), to which 20 nM actin chromobody and 2 mM
633 MgATP were added, was directly loaded into the flow cell with no subsequent washes.

634
635 Full-length phosphorylated smooth muscle myosin⁶⁰ was directly adhered to the
636 nitrocellulose-coated coverslip. 15 µl of 100 µg/ml myosin in 300 mM KCl buffer C (25 mM
637 imidazole pH 7.5, 1 mM EGTA, 4 mM MgCl₂, and 10 mM DTT) was flowed into the cell and
638 incubated for 1 min. Three 15 µl additions of 5.0 mg/ml BSA in 300 mM KCl buffer C were
639 followed by a 2 min wait, and then three 15 µl rinses with 25 mM KCl buffer C. Skeletal,
640 smooth and JAS stabilized PfAct1 filaments were incubated for 30 s followed by three 15 µl
641 rinses with 90 mM KCl buffer C. Next, one rinse of buffer D (90 mM buffer C plus
642 chromobody, 0.7% (w/v) methylcellulose, oxygen scavengers (50 µg/ml catalase (Sigma),
643 125 µg/ml glucose oxidase (Sigma), and 3 mg/ml glucose). 50 nM chromobody was used to

644 visualize skeletal and smooth actins; 20 nM chromobody was used for PfAct1 filaments.
645 Motility was initiated by adding buffer D containing 1 mM MgATP twice to the flow cell and
646 waiting 1 min for temperature to equilibrate to 30°C on the microscope. Unstabilized PfAct1
647 filaments (freshly prepared for each experiment by polymerizing at 8 μM for 10 min at 37°C),
648 to which 20 nM actin chromobody and 1 mM MgATP were added, was directly loaded into
649 the flow cell with no subsequent washes. Filaments were automatically tracked and analyzed
650 with the Fast Automated Spud Trekker analysis program (FAST, Spudich laboratory, Stanford
651 University, <http://spudlab.stanford.edu/fast-for-automatic-motility-measurements>). Reported
652 speeds are from a fit to a Gaussian curve. To assess the significance of differences in speed, a
653 two-tailed *z*-test was chosen because it is ideal for determining whether two population means
654 are different when the variances are similar, and the sample size is large. Samples were
655 assumed to be independent of one another and the populations from which the samples were
656 taken were assumed to be normally distributed. Actin filaments were visualized using a Nikon
657 ECLIPSE Ti microscope.

658

659 **Data availability**

660 Data supporting the findings of this manuscript are available from the corresponding authors
661 upon reasonable request. A reporting summary for this Article is available as a
662 Supplementary Information file.

663

664

665 The atomic models and cryo-EM maps are available in the PDB⁶¹ and EMDB databases⁶²,
666 under accession numbers 7ALN (<https://www.rcsb.org/structure/7ALN>) and EMD-11818
667 (<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-11818>) respectively.
668

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821

822 **Author contributions**

823 K.T., A.H., N.V. and D.H. designed research; J.P.R and H.L performed motility assays;
824 P.M.F. and E.B.K expressed and purified the *Plasmodium* proteins; X-P.X., M.F.S., N.V. and
825 D.H. generated the cryo-EM data and reconstructions; J.R-P. and D.A. built the model with
826 N.V., J.R. P. and A.H. analyzed the interface, and J.R.P. X-P.X., K.T., A.H., N.V., and D.H
827 analyzed data; J.R.P., K.T., A.H., N.V. and D.H. wrote the paper.

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829 **Competing Interests.**

830 The authors declare no competing interests.

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837 **Figure Legends:**

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839 **Figure 1:** *Structure of the PfMyoA motor domain/PfAct1 filament assembly* (a)
840 Representative micrograph (out of 6073) of JAS-stabilized PfAct1 filaments with bound
841 PfMyoA motor domain. The scale bar is 50 nm. (b) Representative classes (out of 1534) from
842 reference-free two-dimensional classification of segments showing lack of cooperative
843 binding (full decoration). The scale bar is 20 nm. (c) Reconstruction of JAS-stabilized PfAct1
844 filaments (pink) with bound PfMyoA motor domain (grey). The pointed (-) end of the
845 filament is up. (d) Local resolution mapped on the reconstruction. Values of color bar in Å.
846 (e) Atomic temperature factors mapped on the refined model. Values of color bar in Å² (f-i)
847 Density maps and atomic model for all the actin binding elements of PfMyoA at the
848 actomyosin interface. All the different actin binding elements are colored differently: The
849 hypertrophic cardiomyopathy loop (HCM) in pink; Loop2 in yellow; Loop3 in dark gray;
850 helix-turn-helix (HTH) in dark blue (see also Supplementary Fig. 1).

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853 **Figure 2:** *Structure of the PfAct1-bound PfMyoA motor domain* (a) Atomic model of actin-
854 bound PfMyoA motor domain with root mean square differences to the rigor-like unbound
855 structure mapped in thickness and color, from 0-5.5 Å. Only residues present in both
856 structures are shown. The hypertrophic cardiomyopathy loop (HCM), loop 4 (L4) and the
857 upper and lower 50kDa domains (U50, L50) are labeled. (b) Comparison of the normalized
858 atomic temperature factors between the bound (upper panel) and unbound (lower panel)
859 structures. The region shown corresponds to the box in A. (c) Density map and atomic model
860 in the active site of PfMyoA shows without ambiguity the absence of nucleotide. There is no
861 density in the cavity (black dashed lines) composed by the Switch-1 (green), Switch-2
862 (orange) and the P-loop (purple). (d) The density map and atomic model of the actin binding
863 cleft located between the upper 50 kDa (U50, in blue) and the lower 50 kDa (L50, pink)
864 domains.

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867 **Figure 3:** *Structure of the PfMyoA-bound PfAct1 filament* (a) Atomic model of filamentous
868 PfAct1 while bound to PfMyoA with root mean square differences to the bare PfAct1
869 filament structure mapped in thickness and color, from 0-2.5 Å. (b) The D-loop shows only
870 minor structural differences in the bound (cyan) compared to the bare (chocolate red) filament
871 (PDB code 5OGW; <https://www.rcsb.org/structure/5OGW>)¹¹ with the largest difference
872 occurring at residue E51. In the decorated filament, the conformation of the sidechain of E51
873 is reoriented in order to establish a polar contact of with S558 from the HTH of PfMyoA
874 (dark blue). (c) The plug conformation is identical in both structures and the specific polar
875 interactions are conserved. (d) Comparison of the normalized atomic temperature factors
876 between the bound (left) and unbound (right) PfAct1 structures. (e) Jasplakinolide (JAS) is
877 well defined in the density map of the PfMyoA bound PfAct1 structure.

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880 **Figure 4:** *Actomyosin interaction footprint analysis* (a) Overlay of the filament bound
881 structures of PfMyoA (teal) and Myosin 6 (Myo6; red) revealing very similar orientations in
882 relation to the filament. (b) Mapping of myosin footprints onto the actin filament surface.
883 Two longitudinal neighboring actin monomers are shown. The subdomains are labeled in the
884 upper monomer. The light blue region is contacted by all four myosins. The dark blue region
885 is contacted by all myosins except for PfMyoA. The orange region is contacted by Myo1b and
886 NM2c, the red region is contacted by NM2c only. (c) Mapping of the core (magenta) and
887 ancillary (Anci, teal) as defined by conservation of interactions (see supplementary table 2)

888 onto an open-book representation with the actin-filament surface on the left and the myosin
889 surface on the right. The inset in the lower right corner shows the primary myosin structural
890 elements partaking in interactions in the same orientation (HCM: hypertrophic
891 cardiomyopathy loop; HTH: helix-turn-helix motif; A-loop: activation loop).
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893 **Tables:**
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Table 1. *In vitro* motility speeds of different actin and myosin pairs.

Actin	PfMyoA ($\mu\text{m/s}$)	Smooth muscle myosin ($\mu\text{m/s}$)
PfAct1/JAS	3.55 \pm 0.53 (n=905)	0.53 \pm 0.19 (n=1441)
PfAct1 skeletal	3.98 \pm 0.67 (n=1545)	n.d.
smooth	3.85 \pm 0.59 (n=942)	0.62 \pm 0.29 (n=953)
	4.21 \pm 0.63 (n=1232)	0.51 \pm 0.22 (n=1019)

895
896 Values are mean \pm SD. Two separate experiments were performed for each condition. Each pairwise
897 combination of a given motor with different actins is statistically insignificant using a two-sided z-test.
898 All PfMyoA/actin pairs, $p \geq 0.416$; all smooth muscle myosin/actin pairs, $p \geq 0.774$
899 (PfMyoA/PfAct1/JAS vs. PfMyoA/PfAct1: $p = 0.604$; PfMyoA/PfAct1/JAS vs. PfMyoA/skeletal: $p =$
900 0.704; PfMyoA/PfAct1/JAS vs. PfMyoA/smooth: $p = 0.416$; PfMyoA/PfAct1 vs. PfMyoA/skeletal: $p =$
901 0.875; PfMyoA/PfAct1 vs. PfMyoA/smooth: $p = 0.803$; PfMyoA/skeletal vs. PfMyoA/smooth: $p =$
902 0.673; Smooth muscle myosin/PfAct1/JAS vs. Smooth muscle myosin/skeletal: $p = 0.799$; Smooth
903 muscle myosin/PfAct1/JAS vs. Smooth muscle myosin/smooth: $p = 0.954$; Smooth muscle
904 myosin/skeletal vs. Smooth muscle myosin/smooth; $p = 0.774$).
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