Apical membrane antigen 1 (AMA1) is an essential component of the moving junction complex used by Apicomplexan parasites to invade host cells. We report the 2.0 Å resolution x-ray crystal structure of the full ectodomain (domains I, II, and III) of AMA1 from the pervasive protozoan parasite Toxoplasma gondii. The structure of T. gondii AMA1 (TgAMA1) is the most complete of any AMA1 structure to date, with more than 97.5% of the ectodomain unambiguously modeled. Comparative sequence analysis reveals discrete segments of divergence in TgAMA1 that map to areas of established functional importance in AMA1 from Plasmodium vivax (PvAMA1) and Plasmodium falciparum (PfAMA1). Inspection of the TgAMA1 structure reveals a network of apical surface loops, reorganized in both size and chemistry relative to PvAMA1/PfAMA1, that appear to serve as structural filters restricting access to a central hydrophobic groove. The terminal portion of this groove is formed by an extended loop from DII that is 14 residues shorter in TgAMA1. A pair of tryptophan residues (Trp353 and Trp354) anchor the DII loop in the hydrophobic groove and frame a conserved tyrosine (Tyr239), forming a contiguous surface that may be critical for moving junction assembly. The minimalist DIII structure folds into a cystine knot that probably stabilizes and orients the bulk of the ectodomain without providing excess surface area to which invasion-inhibitory antibodies can be generated. The detailed structural characterization of TgAMA1 provides valuable insight into the mechanism of host cell invasion by T. gondii.

Toxoplasma gondii, the etiological agent of toxoplasmosis, is a prevalent global pathogen capable of establishing acute and chronic infections in nearly all warm blooded animals (1, 2). Although largely asymptomatic in healthy individuals, T. gondii infections can be lethal to a developing fetus and immunocompromised cancer and AIDS patients (3–6). Toxoplasmosis can also result in severe ocular infections in both children and adults, and encysted forms of the parasite have recently been implicated in neuropsychiatric disorders, such as schizophrenia (7–9).

The success of T. gondii stems from its ability to persist in the environment, utilize several modes of transmission (10), and, importantly, to infect a broad range of host cells (1). A dominant feature that endows T. gondii and, in fact, all Apicomplexan parasites, including Plasmodium, Babesia, Cryptosporidium, and Neospora, with the ability to efficiently invade host cells is a multiprotein complex assembled at the moving junction (MJ)4 (2, 11). The MJ is an electron-dense, ringlike structure formed between the plasma membranes of the apical tip of the motile parasite and the target host cell (12). During invasion, T. gondii is rapidly engulfed within a parasitophorous vacuole (PV) as the MJ traverses in a posterior direction along the length of the parasite (13, 14). As it migrates, the MJ serves as a molecular sieve, selectively filtering host proteins from the PV (12, 15), thereby protecting the parasite from intracellular degradation (16).

Despite the critical role of the MJ in host cell invasion, only limited information exists describing the details of its assembly. This is due, in part, to the absence of structural information for the individual components. Importantly, however, studies with T. gondii have identified rhoptry proteins RON2, -4, -5, and -8 as forming part of the MJ complex targeted to the cytoplasmic face of the host cell membrane (17, 18). Despite an ambiguous orientation of TgRON2 in the membrane, recent studies have demonstrated a clear interaction between TgRON2 and the micronemal protein AMA1 (apical membrane antigen 1) (17–21), a core component of the MJ complex conserved across the phylum. A ligand-receptor model predicts that the parasite is able to provide its own ligand (TgAMA1) to the host cell-embedded RON complex (TgRON2/4/5/8) to promote invasion (18). This feature may explain the ability of Toxoplasma to invade its remarkably extensive cell range from a wide variety of warm blooded animals.

AMA1 was originally identified as an invariant surface antigen on Plasmodium knowlesi merozoites (22, 23), and monovalent Fab fragments of monoclonal antibodies against P. knowlesi AMA1 were sufficient to block in vitro invasion of erythrocytes (24). Subsequent genetic and immunological studies broadly established the importance of AMA1 as a core component of the invasion machinery (21, 25–27). Complete disruption of ama1 results in a lethal phenotype in Plasmodium

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4 The abbreviations used are: MJ, moving junction; TgAMA1, PfAMA1, T. gondii, P. vivax, and P. falciparum AMA1, respectively; PV, parasitophorous vacuole; RON, rhoptry neck protein.
(28) and T. gondii (26), whereas a conditional ama1 knock-out in T. gondii resulted in tachyzoites severely compromised for invasion (29). Immunological studies have shown that antibodies to both native and recombinant AMA1 recognize a conformational epitope and are protective in animal models of malaria infection (30–35). In addition, anti-AMA1 antibodies extracted from donor sera collected from areas endemic for malaria are both therapeutic and protective (36–38). The importance of AMA1 in both host cell invasion and immune regulation has prompted extensive study (19, 29, 39–43), including testing its potential as a malarial vaccine candidate.

Sequence analysis of AMA1 initially showed it to be a type I integral membrane protein, composed of a small intracellular C-terminal tail, a short trans-membrane region, and a large N-terminal ectodomain (26, 41). The three-domain architecture of the AMA1 ectodomain, originally proposed based on the disulfide bonding pattern (44), was definitively shown in the crystal structure of Plasmodium vivax AMA1 (PvAMA1). This seminal study established that DI and DII adopted a PAN (plasminogen, apple, nematode) motif (45), a module defining a carbohydrate receptors, while showing little structural homology for DIII. Subsequent structural characterization of a truncated ectodomain of P. falciparum (PfAMA1) incorporating DI and DII (39) allowed for delineation of surface loops disordered in the original PvAMA1 structure (43). Of particular interest was an extended non-polymorphic DII loop that, along with a network of surface loops on DI, formed part of an apical hydrophobic groove. Mutation of a tyrosine (Tyr251-PfAMA1) to an alanine located in the center of this groove was sufficient to abrogate binding to RONs (19), highlighting the importance of this structural feature in formation of the MJ complex (39). A molecular interaction role was also proposed for DIII based on the observations that, when expressed on Chinese hamster ovary cells, DIII was sufficient to bind to the Kx membrane protein on trypsin-treated erythrocytes (46). Although only the original PvAMA1 structure included DIII in the context of DI and DII (43), follow-up structural studies of PfAMA1 DIII alone and in complex with invasion inhibitory antibodies have provided further insight into potential functional roles for this domain (19, 40, 42, 47–49).

AMA1 from P. falciparum and P. vivax are highly homologous with respect to sequence and structure. Comparative sequence analysis, however, reveals significant levels of divergence with AMA1 from T. gondii and other Apicomplexan parasites. Intriguingly, several of these divergent stretches map to sites shown to participate in assembly of the MJ complex, immune regulation, and host cell adhesion in Plasmodium AMA1s. To accurately define the distinctive structural features of TgAMA1, we have solved and refined the crystal structure of the fully processed ectoplasmic region to 2.0 Å resolution. The highly ordered structure provides a nearly complete view of the inter- and intramolecular interactions of DI, DII, and DIII that comprise the ectodomain. The structure of TgAMA1 provides a critical step in defining its elusive role within the MJ and, more broadly, its contribution to the unique invasion characteristics of T. gondii.

**EXPERIMENTAL PROCEDURES**

**Bioinformatics**—Boundaries for DI, DII, and DIII were defined based on the paradigm established for PvAMA1 (43). Phylogenetic analysis was performed using MEGA 4 (50, 51), and multiple sequences were aligned using Kalign (52, 53). Accession numbers for aligned AMA1 sequences are as follows: T. gondii (ME49_055260), Neospora caninum (BAF45372), P. falciparum (XP_001348015.1), and Babesia bovis (AAS58045.1). The P. vivax (XP_001615447) sequence was modified to reflect the sequence crystallized by Pizarro et al. in 2005 (43).

**Cloning, Expression, and Purification**—A clone encoding the fully processed ectoplasmic domain of TgAMA1 was generated in a modified pAcGP67b vector (Pharmingen) incorporating a C-terminal hexahistidine tag and thrombin cleavage site. To generate TgAMA1 encoding virus for insect cell protein production, the TgAMA1 clone was transfected with linearized baculovirus DNA into Sf9 cells and amplified to a high titer. Hi-5 cells at 1.8 × 10^6 cells/ml were infected with amplified virus for 72 h, after which time the supernatant was harvested, concentrated, and applied to a HisTrapFF nickel affinity column. TgAMA1 was eluted with an increasing concentration of imidazole with fractions analyzed by SDS-PAGE and pooled based on purity. The hexahistidine tag was removed by thrombin cleavage, and TgAMA1 was further purified by size exclusion chromatography (Superdex 16/60 200) in HEPES-buffered saline (20 mM HEPE, pH 7.5, 150 mM NaCl). The final yield of purified TgAMA1 was ∼2 mg of purified protein/liter of insect cell culture.

**Crystallization and Data Collection**—Crystals of TgAMA1 were initially identified in the Index Screen (Hampton Research) and subsequently refined to a final condition of 20% polyethylene glycol 3350, 100 mM HEPES, pH 7.5, and 50 mM NaCl. Small crystals were observed after 2 days and grew to a final size of 0.5 × 0.1 × 0.1 mm within 6 days. The final drops consisted of 1.5 μl of protein (15 mg/ml) with 1.5 μl of reservoir solution and were equilibrated against 100 μl of reservoir solution. Cryoprotection of the TgAMA1 crystal was carried out in mother liquor supplemented with 5% glycerol and 5% ethylene glycol for 20 s and flash-cooled at 100 K directly in the cryostream. Diffraction data were collected on beamline 9-2 at SSRL (Stanford Synchrotron Radiation Laboratory) at a wavelength of 0.9794 Å. A total of 720 images were collected with a 1° oscillation and 2-s exposure.

**Data Processing, Structure Solution, and Refinement**—Diffraction data to 2.0 Å were processed using Imosfilm (54) and Scala (55) in the CCP4 suite of programs (56). Initial phases were obtained by molecular replacement using MOLREP (57) with the individual DI and DII domains of PfAMA1 (Protein Data Bank code 2Q8A) pruned with CHAINSAW (58) to better reflect the TgAMA1 sequence. No molecular replacement solution was obtained for DIII using a pruned or polyserine model. Tracing of the DIII chain was ultimately achieved using 4-fold non-crystallographic symmetry averaging. Solvent molecules were selected using COOT (59), and refinement was carried out using Refmac5 (57). The overall structure of TgAMA1 was refined to an R_max of 18.4% and an R_pvec of 24.8%. Stereochemical analysis performed with PROCHECK and SFCHECK.
T. gondii AMA1 Crystal Structure

### RESULTS AND DISCUSSION

**Domain Divergence; Comparative Sequence Analysis of DI, DII, and DIII**

Domain boundaries of the fully processed TgAMA1 ectodomain were defined based on the paradigm established for PvAMA1 and PfAMA1 (Fig. 1). TgAMA1 DI spans residues from Thr\(^{67}\) to Pro\(^{287}\) (residues numbered from initiation methionine in the signal sequence), DII spans from Asn\(^{288}\) to Asn\(^{415}\), and DIII spans from Phe\(^{416}\) to Ala\(^{487}\).

TgAMA1 is most closely related to AMA1 from *N. caninum*, with 75% sequence identity distributed over the entire ectodomain. Increased evolutionary divergence is observed with respect to AMA1s from *P. falciparum*, *P. vivax*, and *B. babesi*, with DIII, in particular, displaying less than 10% sequence identity.

Sequence analysis reveals a network of conserved cysteine residues in DI and DII, suggesting a conserved structural core. Several insertions and deletions in the primary sequence, however, map to functionally relevant sites in PvAMA1/PfAMA1 and may be responsible for the unique host cell invasion capabilities of *T. gondii*. Six invariant cysteines in TgAMA1 DI are supported by an overall moderate level of sequence identity with denoted species (on average 35%) (Fig. 1, top). Scale bars on the phylogenetic trees indicate evolutionary distances. Cysteine residues are shown in red and numbered with respect to disulfide bond partner based on the TgAMA1 crystal structure. Residues shown in blue are either invariant or highly conserved in at least four of the five sequences. Domain boundaries were defined based on the paradigm established for *Plasmodium* AMA1s (39). In CCP4 (56) showed excellent stereochemistry, with more than 95% of the residues in the favored conformations and no residues modeled in disallowed orientations of the Ramachandran plot. Overall, 5% of the reflections were set aside for calculation of \(R\)\(_{free}\). Data collection and refinement statistics are presented in Table 1.

### Table 1

Data collection and refinement statistics

| Parameters                              | Values             |
|-----------------------------------------|--------------------|
| Resolution range (Å)                    | 43.90–2.00 (2.05–2.00) |
| \(R\)\(_{cryst}\)                         | 0.184 (0.228)      |
| \(R\)\(_{free}\)                          | 0.248 (0.309)      |
| No. of reflections                      | 391,114 (56,271)   |
| Protein (chain A, B, D, E)              | 3225, 3202, 2932, 3149 |
| Solvent                                | 1177               |
| Glycerol                               | 6                  |
| \(R\)\(_{merge}\)                        | 0.074 (0.433)      |

**T. gondii AMA1 Crystal Structure**

![Phylogenetic tree and multiple sequence alignments of domains I, II, and III that comprise the ectodomain of AMA1 from *T. gondii* (TgAMA1), *N. caninum* (NcAMA1), *P. falciparum* (PfAMA1), *P. vivax* (PVAMA1), and *B. bovis* (BbAMA1). Scale bars on the phylogenetic trees indicate evolutionary distances. Cysteine residues are shown in red and numbered with respect to disulfide bond partner based on the TgAMA1 crystal structure. Residues shown in blue are either invariant or highly conserved in at least four of the five sequences. Domain boundaries were defined based on the paradigm established for *Plasmodium* AMA1s (39).
the insertions are highly polar or proline-rich and map to surface-exposed loops in PfAMA1 predicted to serve as structural filters in governing access to a central groove (39). A single eight-residue deletion at the N terminus of TgAMA1 DI may also carry functional implications as the additional residues in PvAMA1 (43) form an extended strand that connects DI and DIII. DII is approximately two-thirds the size of DI and encodes four invariant cysteines but is less well conserved, with an overall sequence identity of ~25% (Fig. 1, middle). The most unique feature of TgAMA1 DII is a deletion of 14 residues that maps to the non-polymorphic DII loop of PfAMA1 (39). Recent studies have shown that PfAMA1 DII loop presents an epitope recognized by an invasion-inhibitory monoclonal antibody and a T cell epitope implicated in the human response to Plasmodium infection (39, 43, 44). The greatest divergence among the AMA1 ectodomains, however, and surface loops removed (39). No molecular replacement solution was obtained for TgAMA1 DIII, and initial electron density maps were inadequate to trace or even manually position a DIII model. Phase improvement strategies incorporating 4-fold non-crystallographic symmetry averaging resulted in maps into which all but three amino acids of DIII were modeled. Each polypeptide chain in the unit cell is largely equivalent with respect to degree of modeled structure and organization as shown by root mean square deviations relative to chain A of 0.61 Å over 361 C Ï atoms (chain B), 0.53 Å over 353 C Ï atoms (chain D), and 0.44 Å over 386 C Ï atoms (chain E). Chain A is the most extensively modeled, yet a small section of loop in chain A (Gln338–Asp352) is reorganized with respect to the analogous regions in the three other NCS-related chains (supplemental Fig. 1). This alternate conformation appears to be a crystalization artifact arising from intermolecular packing.
Therefore, structural analysis of this region is based on the loop conformation observed in chains B, D, and E.

Overall Structure—The assembled TgAMA1 ectodomain extends 80 Å in height and, on average, 35 Å in width, as shown in Fig. 2B with respect to its predicted orientation to the membrane. Whereas the bulk of DI (Fig. 2B, purple) is positioned atop DII (Fig. 2B, green), a series of short N-terminal helices span the length of DII, resulting in the N terminus positioned within 21 Å of the C terminus of DIII (Fig. 2B, slate blue). DI and DII are intimately associated and form the bulk of the ectodomain, whereas the majority of the smaller DIII resides at the posterior, membrane-proximal region (Fig. 2B).

The DI domain is composed of small helical bundles, short twisted β-sheets, and an extensive network of random coils. Despite the low secondary structure content, DI is well ordered, due, in part, to the trio of stabilizing disulfide bonds (Fig. 2C). The core of DII is centrally located within the ectodomain with the exception of a 33-residue loop (termed the DII loop) that packs lengthwise against DI, forming an extended interface (Fig. 2B). The base of the DII loop is stabilized by a disulfide bond with a second disulfide bond stabilizing the DII core (Fig. 2C). A 25-residue tether connects the core of DIII to DI, making it possible for the DIII to be positioned at the posterior end of the ectodomain. Of the remaining 46 residues that comprise the DIII core, six are cysteines organized into three disulfide bonds that form a structurally ultrastable cystine knot with disulfide bond 8 (Cys452-Cys479) threading through a ring formed by bond 6 (Cys435-Cys459) and bond 7 (Cys477-Cys477) (Fig. 2C).

To probe the level of structural conservation, a DALI (60) search was individually performed with TgAMA1 DI, DII, and DIII. As expected, TgAMA1 DI shows a high level of structural homology to PfAMA1/PvAMA1 (39, 43), with Z scores ranging from 18 to 22. Intriguingly, however, no structural relationship was identified corresponding to the protein-protein or protein-carbohydrate interacting PAN superfamily (45), as was originally observed for PvAMA1 (43). It is likely that the insertions and deletions in TgAMA1 DI (Fig. 1) may limit its categorization as part of the PAN superfamily. Despite the lower sequence and structural (Z scores from 8 to 12.6) homology for DII resulting from the 14-residue deletion, a clear correlation (Z score of 7.5) is observed with PAN-containing proteins, such as hepatocyte growth factor. This structural feature suggests that DII may participate in ligand recognition. No statistical structural similarity was observed for DIII, consistent with less than 10% sequence identity observed.

Intimate Interfaces; Assembling the TgAMA1 Ectodomain—The highly ordered TgAMA1 structure provides an opportunity to thoroughly analyze the inter- and intramolecular interactions that stabilize the structural framework of the ectodomain. Each interdomain interface is formed from numerous non-covalent forces and substantial shape complementarity, as shown by a maximum complexation significance scores of 1.0 (61). To visualize the extensive nature of the interdomain interactions, an “open book” perspective is presented in Fig. 3 with residues contributing to the interfaces displayed in orange.

In total, more than 7350 Å² of surface area is buried upon assembly of the TgAMA1 ectodomain. The largest interface is formed between DI and DII, resulting in a buried surface area of ~4849 Å², with 2319 Å² contributed from DI and 2530 Å² from DII (Fig. 3A). The DI/DII interface is stabilized by 21 interdomain hydrogen bonds and three salt bridges (Asn288O-Glu330O; Asp102O-His357N; and Arg258Ne-Glu330Oe1). The role of polar interactions in defining the DI/DII interface is highlighted by an electrostatic surface representation that shows distinct complementary charged surfaces (Fig. 3A, middle). Additional polarity is provided by a small yet well ordered network of buried solvent that may also serve to increase shape complementarity. A major component of the DI/DII interface is contributed by the DII loop that extends from Gly333 to Arg369 and accounts for approximately half of the buried surface area between the two domains. Although the majority of the DII loop is structurally invariant across the four monomers, intramolecular packing results in a contorted segment (Gln338–Asp357) of the DII loop in chain A (Supplemental Fig. 1). As a result, defining the contributions of the DII loop to ectodomain stability is restricted to chains B, D, and E.
T. gondii AMA1 Crystal Structure

Structural Divergence in the Apical Region of TgAMA1; Functional Implications—Structural analysis reveals an extended groove at the anterior, or membrane distal tip, of the TgAMA1 ectodomain that extends 30 Å in length and averages ~10 Å in width (Fig. 4A, inset, horizontal black bar).

Surface loops (identified by yellow numbers in Fig. 4A, inset) span the length of the groove and probably serve as a selectivity filter in mediating access to the base of the groove as proposed for PfAMA1 (39). To assess potential functional implications of these loops, we present structural overlays with the analogous loops in PfAMA1 (Fig. 4A).

Loops 1 and 2 are centrally positioned on opposite sides of the groove, constricting the central segment to ~6 Å. Despite loop 1 being only three residues (Pro164, Ser165, and Gly166) longer than the analogous loop in PfAMA1, it is significantly reorganized (Fig. 4A, I) in structure yet well ordered as shown by low B-factors. In PfAMA1, this loop directly coordinates a series of invasion-inhibitory antibodies (40, 47), thereby playing a critical role in pathogenesis. The altered structure of loop 1 in TgAMA1 may, therefore, promote diversity in ligand recognition. A high degree of flexibility is observed in the apical region of loop 2, resulting in three unmod- eled residues, yet the overall size is similar between TgAMA1 and PfAMA1 (Fig. 4A, 2). A second set of loops, denoted as loops 3 and 4 in TgAMA1, extend the groove to incorporate the tip of the DII loop. The size of the β hairpin structure in loop 3 is largely conserved (Fig. 4A, 3), although the loop is shifted 1.5 Å toward the central groove in TgAMA1. This displacement is probably due to the reorganized DII loop that is much smaller in TgAMA1. A more striking structural reorganization coupled to the smaller DII loop is observed in loop 4 (Fig. 4A, 4), where the base of the loop provides a hydrophobic backstop with substantial shape complementarity to accommodate the DII loop. The tip of loop 4, however, is highly polar with Glu145, Lys146, Lys149, and Gln150 directed away from the base of the groove, where it may serve as the initial structural filter in defining appropriate ligands. Loops 5 and 6 are positioned at the periphery of the central groove and form a contiguous surface that appears to be critical in promoting correct orientation of the DII loop in the central groove (Fig. 4A, 5 and 6).

The core cystine knot of DIII displays a discontinuous epitope and contributes 637 Å² of buried surface area, with DII contributing 663 Å² for a total buried surface area of 1300 Å² (Fig. 3B). Fourteen hydrogen bonds complement a bifurcated salt bridge between the carboxylate side chain of Asp348 on DIII and the ε-amino group of Lys301 and the η-nitrogens of Arg303 on DII. The extended tether that connects DIII to the DI/DII core also contributes to the total buried surface area of 1400 Å² (740 Å² from DI and 660 Å² from DIII) (Fig. 3C). Despite the increased surface area relative to DII/DIII, the DI/DIII interface is stabilized by only six hydrogen bonds and one salt bridge (Arg303NH1-Glu303Oe1). Instead, DI/DIII stability relies on complementary hydrophobic surfaces where, for example, DI Phe31 and DIII Phe416 provide the most individual buried surface area of any interface residue. Overall, each domain is intimately associated with the remaining two domains to form a highly stable structure.

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Thus, the reorganization of TgAMA1 DIII may also contributed in mediating erythrocyte adhesion with PfAMA1 (46). This observation is especially interesting because DIII has been directly implicated in mediating the interaction between TgAMA1 and PvAMA1/PfAMA1. This observation underscores the importance of DIII in the context of the MJ complex formation.

Global Structural Rearrangement in TgAMA1 DIII—The structural reorganization of the TgAMA1 apical region (DI/ DIII) is likely to have a profound impact on assembly of the MJ complex, yet it is DIII that displays the most divergence between TgAMA1 and PvAMA1/PfAMA1. This observation is especially interesting because DIII has been directly implicated in mediating erythrocyte adhesion with PfAMA1 (46). Thus, the reorganization of TgAMA1 DIII may also contribute to the broad infectivity of T. gondii.

A comparative structural analysis revealed a rationale for why TgAMA1 DIII was substantially smaller than PvAMA1/ PfAMA1 DIII (Fig. 5). As described above, the N terminus of PvAMA1 DI is eight residues longer than in TgAMA1 with the additional residues adopting a single β strand that extends away from the DI core toward DIII. PvAMA1 DIII forms a saddle-like structure with a central groove to accommodate and stabilize this N-terminal extension. The shorter N-terminal region of TgAMA1 DI does not extend to DIII and therefore obviates the need for additional stabilizing features contributed by DIII. Interestingly, the minimalist structure of TgAMA1 DIII is sufficiently large to adopt the structurally ultrastable cystine knot that may serve as a foundation to properly orient the DI/DIII core with respect to the parasite cell membrane. In addition to a base structural role, the smaller TgAMA1 DIII provides little excess surface area to which growth inhibitory antibodies might be generated as recently suggested for PfAMA1 DIII (48). In this study, engineered peptidomimetics of PfAMA1 DIII were used to identify two immunodominant epitopes comprising the linear sequences KRIKN and DEGNKKII capable of generating a protective antibody response (48). With the exception of the two terminal isoleucine residues, the residues that comprise these epitopes are located in the divergent region of PfAMA1 DIII not represented in the smaller TgAMA1 DIII (Fig. 1).
CONCLUSIONS

*T. gondii* is one of the most successful parasites, yet a detailed molecular mechanism describing assembly and function of the MJ complex remains elusive. The highly ordered crystal structure of TgAMA1 presented herein reveals an intriguing level of divergence from its *Plasmodium* counterparts. While maintaining a conserved structural core in DI and DII, reorganized structural elements in TgAMA1 map to areas of established functional importance in PfAMA1, including a network of surface loops that frame a central hydrophobic groove. Because AMA1 is vital for parasitic invasion and has been previously shown to interact with a variety of other proteins during MJ formation, the implications of novel features leading to altered ligand binding sites are profoundly significant. More specifically, we predict that the hydrophobic groove (and in particular Tyr230) plays a key role in engaging RON2 during assembly of the MJ complex. Our structure of the complete TgAMA1 ectodomain will help catalyze a better understanding of the role AMA1 plays in host cell invasion by *T. gondii* and, indeed, all Apicomplexan parasites. The structural details provided here will also be useful to refine AMA1 vaccine development efforts for both *Plasmodium* and Toxoplasma.

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