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# MARveling at parasite invasion

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## Abstract

Micronemal proteins (MICs) are key mediators of cytoadherence and invasion for *Toxoplasma gondii*. Emerging evidence indicates that carbohydrate binding facilitates *Toxoplasma* entry into host cells. TgMIC1s recently solved structure reveals the presence of novel specialized domains able to discriminate between glycan residues. Comparison with *Plasmodium* erythrocyte-binding antigen 175 reveals that terminal sialic acid residues may represent a shared but tailored invasion pathway among apicomplexan parasites.

### Keywords

Apicomplexa; *Toxoplasma*; microneme; structure; invasion

## The secrets of a successful pathogen

*Toxoplasma gondii* is an obligate intracellular parasite with a broad host range [1]. A member of the phylum Apicomplexa that includes the medically virulent pathogens *Plasmodium falciparum* (human malaria) and *Eimeria tenella* (poultry coccidiosis), *T. gondii* is capable of causing severe opportunistic disease in neonates and immunocompromised individuals [2]. Compared to viral and bacterial infections that rely on the host for entry, invasion is a highly active process for *Toxoplasma* and other apicomplexans.

The success of *T. gondii* as a widely distributed pathogen centers on its ability to invade virtually any nucleated cell using secreted parasite proteins. Among this arsenal of proteins are the micronemal proteins (MICs) [3]. MICs function in cellular adhesion and link the parasite actin/myosin system to the host surface, leveraging parasite entry into the host cell [4]. TgMIC1, one of the first micronemal proteins to be characterized, contributes to both cell invasion and parasite virulence [5]. Although the importance of receptor-ligand interactions during the first step of invasion of Apicomplexa has been established [4, 6–9]., identification of host-cell receptors and the structure of parasite ligands have been largely elusive. In an elegant study recently published in *EMBO J*. [10], Blumenschein et al., reveal by X-ray crystallography and carbohydrate microarrays that TgMIC1 binds sialylated oligosaccharides using a micronemal adhesive repeat (MAR) [10]. MAR bears no resemblance to other sialic acid lectins, including a key plasmodial ligand involved in host recognition: *P. falciparum* erythrocyte-binding

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antigen 175 (PfEBA175). This study represents a major advance in our understanding of invasion by delineating the structure of an important parasite ligand and the identification of its host receptor.

## Mysteries of *Toxoplasma* adhesion

TgMIC1 is tightly associated with two other MICs called TgMIC4 and TgMIC6. As the 'meat' of the complex, TgMIC1 is sandwiched between its partners, it promotes folding of TgMIC6, and it efficiently binds host receptors using its two MAR domains even in the absence of TgMIC4 and TgMIC6 [11]. In this multifunctional capacity, TgMIC1 contributes to microneme targeting [12], attachment to the host cell surface [6], and parasite virulence [5]. Although many pieces seemed to be falling into place regarding its function, several unknowns remained. For example, lactose was implicated as a receptor for TgMIC1 based on affinity chromatography, yet little evidence exists regarding the specificity of this interaction or its significance for *Toxoplasma* attachment to host cells [13]. Also, structural analysis of TgMIC1's C-terminal domain revealed a galectin-like fold with the potential to bind carbohydrates; however, key sugar-binding residues were absent and in their place stood hydrophobic amino acids that form the protein binding interface with TgMIC6 [14]. Additionally, based on primary sequence the TgMIC1 tandem MAR domains loosely resemble thrombospondin type 1 (TSP1) repeats that possess carbohydrate binding activity in thrombospondin and other proteins, but TSP1 repeats typically recognizes sulfated glycosaminoglycans (GAGs) such as heparin and have not been associated with binding lactose or other sugars. Finally, although negatively charged carbohydrates such as GAGs and sialic acid have been implicated as receptors for *Toxoplasma* attachment, the parasite ligands responsible for binding such carbohydrates remain largely obscure.

## Atomic resolutions

Peering into the molecular basis of *Toxoplasma* adhesion, Blumenschein et al. [10] used X-ray crystallography to solve the structure of the TgMIC1 tandem MAR domains, which together comprise the TgMIC1 N-terminus (TgMIC1-NT). Surprisingly, these domains adopt a novel fold unrelated to any other including TSP1. Each MAR domain consists of a small, distorted barrel of five β-strands flanked on one side by an antiparallel helical bundle. Although MAR1 and MAR2 share only 27% sequence identity, their structures are highly alike except for a short extension on MAR2 that forms a β-finger possibly involved in binding TgMIC4. Native TgMIC1 and recombinant TgMIC1-NT were shown to bind host cells but, unexpectedly, these interactions were not disrupted by lactose, galactose, or heparin. The study used a carbohydrate microarray encompassing more than 200 diverse oligosaccharides to interrogate the sugar binding specificity of TgMIC1, the first application of such technology for a protozoan. Strikingly, TgMIC1-NT bound exclusively to oligosaccharides containing terminal sialic acids. This specificity was elegantly confirmed by showing that: (i) Sialic acids dose-dependently obstruct both binding of TgMIC1 to host cells and parasite invasion; (ii) Sialidase treatment of host cells impairs TgMIC1 binding and parasite invasion; and (iii) Each MAR domain is capable of binding sialic acids according to nuclear magnetic resonance (NMR) measurements.

TgMIC1-NT crystals soaked with α-2,3-sialyl-*N*-acetyllactosamine or α-2,6-sialyl-*N*-acetyllactosamine revealed binding in a shallow pocket that comprised six contiguous residues (amino acids 216–221) of MAR2. Although neither sugar occupied the MAR1 binding pocket, this is likely because crystal contacts in this region provided insufficient space for binding. MAR1 may also have a lower binding affinity than MAR2. Also, the affinity of individual MAR domains is low and binding may be cooperative, requiring use of both sites. This notion is further supported by the strong propensity of TgMIC1-NT to recognize branched,

multisialylated glycans. Binding was particularly robust with sialyl residues separated by five to eight carbohydrate units, presumably because this spacing allows occupation of both binding pockets.

## A tail of two (or more) sugars

Whereas *Toxoplasma* tachyzoites invade and replicate in virtually any animal cell except erythrocytes, *P. falciparum* merozoites only infect erythrocytes. This specificity is dictated largely by apical secretory proteins including PfEBA175, a micronemal protein that recognizes the major erythrocyte surface protein glycophorin A [15]. Like the MAR domains of TgMIC1, PfEBA175 binds sialic acid residues through tandem N-terminal adhesive domains called Duffy Binding Ligands (DBLs) [16]. While TgMIC1 and PfEBA175 share these characteristics, they are fundamentally different on the structural level (Figure 1, Table 1). TgMIC1-NT is monomeric, whereas PfEBA175 is a dimer. The features they recognize on sialic acid are distinct and the adhesive domains differ in sequence, fold, and spacing (Figure 1, Table 1) [10, 16]. Most notably, each TgMIC1 MAR domain contains a central sialic acid binding pocket [10] whereas PfEBA175 displays three sialic acid binding pockets at each of two dimer interfaces [16]. Thus, while these pathogens employ MICs that recognize a similar receptor for invasion the basic mechanisms involved differ dramatically. Perhaps given the broad distribution of sialic acids among animal tissues it is not surprising that they make attractive receptors. More remarkable is how the differences in sialic acids may be selectively exploited during parasite recognition of its host [17]. For example, the *Plasmodium* species that infects chimpanzees, *P. reichenowei*, preferentially binds to N-glycolylneuraminic acid (Neu5Gc), the predominant sugar on chimp erythrocytes [15, 18, 19]. By contrast, the human pathogen, *P. falciparum*, displays a marked predilection for the metabolic precursor of Neu5Gc, Neu5Ac [19]. This sugar is found on human erythrocytes. Intriguingly, humans are the only primates unable to synthesize Neu5Gc [17]. The authors of the recent structural study of TgMIC1 speculate that the high binding affinity of TgMIC1 for polyvalent carbohydrates possessing two or more sialic acid resides such as gangliosides (enriched in neuronal tissue), may be a reflection of the asexual life cycle of *Toxoplasma* where cysts are formed within the brain [10, 20].

Correct spacing and configuration of sialic acid binding sites on the individual parasite ligands might dictate another level of host cell specificity. The sialic acid binding sites for TgMIC1 are closer together than those for PfEBA175 (Table 1). These ligands may have evolved to be highly complementary to their respective receptors. This may be especially true for *Eimeria* EtMIC3, a protein that harbors seven sequential MAR domains. Molecular modeling of EtMIC3 predicts a regular arrangement of binding pockets laterally positioned along its length [10]. Perhaps this multivalent configuration is specifically tuned for complementarity with heavily sialylated glycoproteins in the intestinal mucosa and epithelium where *Eimeria* thrives.

## Concluding remarks and future perspectives

Like most major advances, the important insight provided by Blumenschein *et al.* inspires several new questions. Does *Toxoplasma* rely on sialic acid binding for invasion of certain cell types more than others? This is hinted at by the finding that tachyzoite invasion is inhibited by only 30% in sialic acid deficient Chinese hamster ovary cells [21] whereas a >85% invasion block was seen in human fibroblasts exhaustively treated with sialidase or preincubated with excess sialic acid [10]. Such differences might reflect the parasite's ability to opt for the most appropriate invasion pathway for each cell type, depending on receptor availability. Accordingly, genetic evidence suggests that other micronemal proteins also contribute significantly to tachyzoite adhesion [9, 22], although in these cases the cognate receptors are less well defined. Do MAR domains contribute to *T. gondii*'s broad host range? Other MAR domain-expressing parasites such as *Eimeria* and *Neospora* have a much more limited host

range. Therefore any contributions MAR domains make in this respect must be through recognition of an appropriately narrow or wide array of sialic acid structures. Do MAR domains recognize sialic acids in the context of particular glycoproteins? Perhaps MAR domains discriminate receptors by recognizing features of the polypeptide in addition sialylated moieties, thereby providing yet another layer of specificity. With these and other radiating lines of investigation the new molecular picture of MAR domain structure and function will likely ripple through the field for some time to come.

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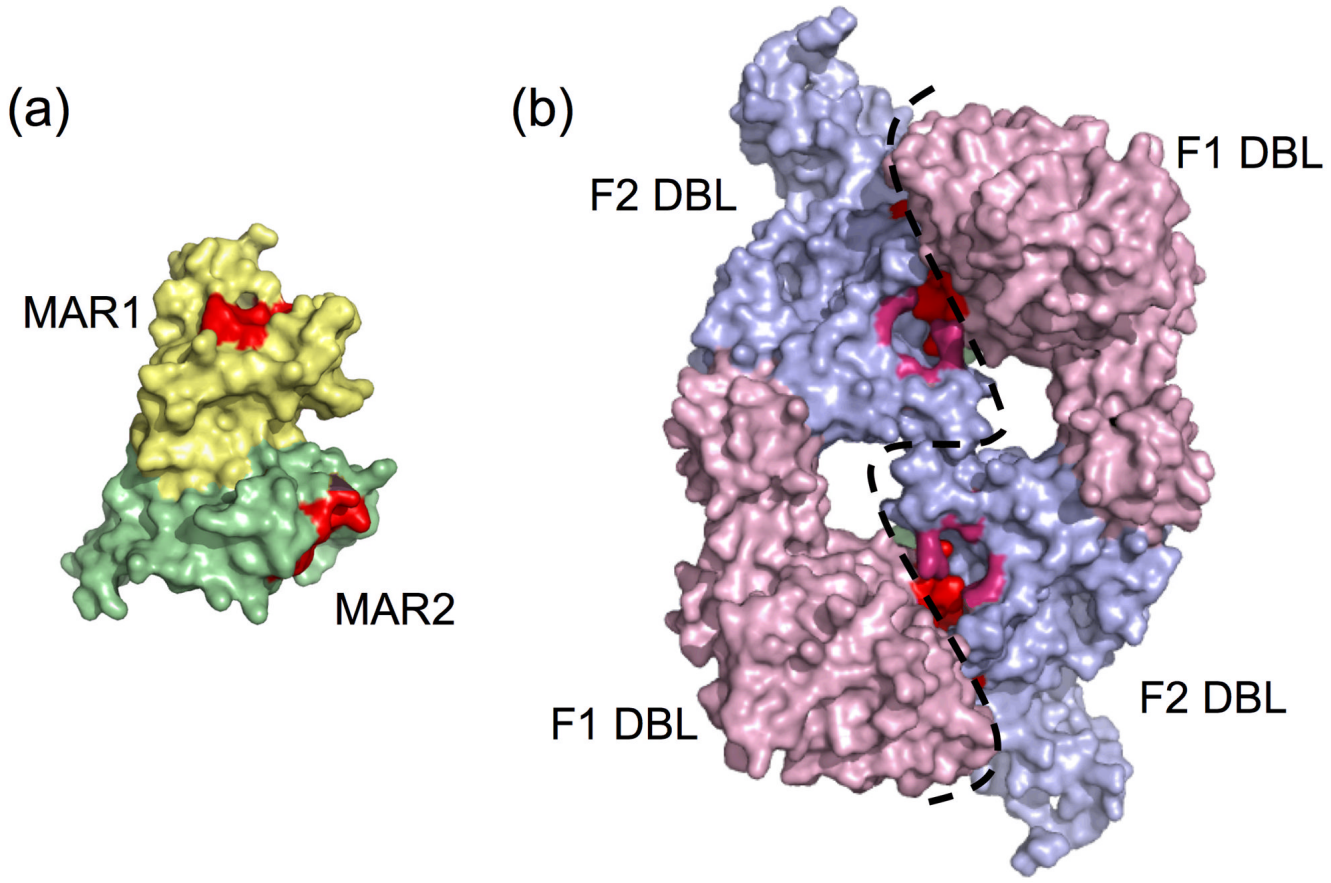


**Figure 1.**
Structural comparison of sialic acid binding domains of TgMIC1 (a) and PfEBA175 (b). The TgMIC1-NT (PDB 2JH1) is monomeric and comprised of two domains, MAR1 [23] and MAR2 (green) each with one shallow SA binding pocket (red). The N-terminal region of PfEBA175 (PDB 1ZRL) consists of two domains, F1 DBL (light pink) and F2 DBL (blue) that form a homodimer with approximate two-fold symmetry. SA binding sites are positioned at the dimer interface (dashed line) and are colored pink (sites 1 and 2), red (sites 3 and 4) and green (sites 5 and 6, only partially visible from this angle).

**Table 1**

Comparison of Parasite Ligands[a]

| Properties | TgMIC1 | PfEBA175[b] |
|---|---|---|
| Target cell | Nucleated Cell[c] | Erythrocyte |
| Target receptor | Branched carbohydrates with two or more terminal sialic acids[d] | N-acetylneuraminic acid (Neu5Ac)(α2, 3)-Gal on glycophorin A (GpA) |
| No. of ligand subunits | Monomer | Dimer |
| Adhesive domain nomenclature | MAR | DBL |
| Secondary structure composition | β-sheet enriched | α-helices enriched |
| No. of disulfide bonds formed within parasite protein | 6–8 | 13 |
| Receptor-ligand biochemistry | Novel hydrogen bonding | Salt-bridge bonding |
| Contact residues involved in glycan binding[e, f] | YY219[g], R217[g], **T126**[g], K216[g], H218[g], **T220**[g]. | N417[h], **R422**[h], N429 [h], **K439**[h], D422[h], K28 [h]; **N33**[i], N550[i], N551[i], **Y552i**[i], K553[i], M554[i]; T340[j], **K341**[j], D342[j] V343[j], Y415[j], Q542[j], Y546[j], K28[j], N29[j], R31[j], S32[j] |
| No. of glycan binding sites | 2 | 6 |
| Location of glycan binding sites | Occurs centrally with each MAR domain | Occurs at dimer interface |
| Spacing between glycan binding sites | 32.3 Å[k] | 40.4 to 44.8 A depending on the pair |

[a] Parasite protein is referred to as the ligand in Blumschein et al. [10]

[b] EBA175 structural components were obtained from Tolia *et al.* [16]

[c] Tissue cyst tropisms are observed in the central nervous system, eye, and muscle tissue [20, 24, 25].

[d] Optimal binding for the MAR domains occurs when five to eight carbohydrates units separate the sialic acid termini.

[e] Bold residues: Mutagenesis of these residues greatly decreased glycan binding.

[f] Residues between K216 and E221 form a shallow binding pocket in the MAR2 domain and most of these residues make specific, direct contacts with the sialy moiety[10].

[g] Key binding residues for TgMIC1. Note: double mutant T126/T220 completely abrogates glycan binding.

[h] Contact sites for binding of glycans 1 and 2.

[i] Contact sites for binding of glycans 3 and 4

[j] Contact sites for binding of glycans 5 and 6

[k] Å=Angstrom