Structural basis of myelin-associated glycoprotein adhesion and signalling

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Myelin-associated glycoprotein (MAG) is a myelin-expressed cell-adhesion and bi-directional signalling molecule. MAG maintains the myelin-axon spacing by interacting with specific neuronal glycolipids (gangliosides), inhibits axon regeneration and controls myelin formation. The mechanisms underlying MAG adhesion and signalling are unresolved. We present crystal structures of the MAG full ectodomain, which reveal an extended conformation of five Ig domains and a homodimeric arrangement involving membrane-proximal domains Ig4 and Ig5. MAG-oligosaccharide complex structures and biophysical assays show how MAG engages axonal gangliosides at domain Ig1. Two post-translational modifications were identified—N-linked glycosylation at the dimerization interface and tryptophan C-mannosylation proximal to the ganglioside binding site—that appear to have regulatory functions. Structure-guided mutations and neurite outgrowth assays demonstrate MAG dimerization and carbohydrate recognition are essential for its regeneration-inhibiting properties. The combination of trans ganglioside binding and cis homodimerization explains how MAG maintains the myelin-axon spacing and provides a mechanism for MAG-mediated bi-directional signalling.

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Myelination of axons enables enhanced conduction velocity in both the central and peripheral nervous system (CNS and PNS) of vertebrates. It also provides electrical insulation and a decrease of the capacitance, as well as physical protection and metabolic support of long axons. Myelin-associated glycoprotein (MAG) adhesion and signalling at the myelin–axon interface regulates the formation and maintenance of myelinated axons, thus playing an important role in the development of the nervous system. Aberrant MAG function, for example from mutations that likely cause misfolding, or anti-MAG autoimmunity, has been associated with demyelination and neurodegenerative disorders, such as corticospinal motor neuron disease also known as hereditary spastic paraplegias, Pelizaeus–Merzbacher disease-like disorder, demyelinating anti-MAG peripheral neuropathy, and multiple sclerosis.

MAG is a type 1 single-pass transmembrane protein expressed on myelinating oligodendrocytes in the CNS and Schwann cells in the PNS. MAG is the fifth highest expressed protein in myelin of the CNS. It is highly enriched at the innermost (adaxonal) myelin membrane along the internode, where it contacts the axon. MAG is also found on other myelin structures, such as the mesaxon, Schmidt-Lanterman incisures and paranodal loops. MAG adhesion maintains the myelin–axon spacing (periaxonal space), the lack of flexible linker residues and a previously reported C2-type Ig fold (Supplementary Fig. 2). The three crystal structures of the full extracellular segment of mouse MAG in two different crystal forms that diffracted to a maximum resolution of 3.8 and 4.3 Å. These crystals were obtained by enzymatic deglycosylation of MAG1–5 or reductive lysine methylation of glycosylated MAG1–5 (see ‘Methods’ section). In addition, crystals of a shorter construct, consisting of the three N-terminal domains (MAG1–3), diffracted to a maximum resolution of 2.1 Å. The structures were solved by molecular replacement with individual Ig domains from homologous proteins. The exceptionally high-solvent content of the two MAG1–5 crystal forms (91 and 85%, Supplementary Fig. 1) aided in obtaining phases of sufficient quality for initial model building (see also Table 1 and ‘Methods’ section for details).

In all three crystal forms MAG has an extended collinear conformation (Fig. 1). Only consecutive Ig domains interact with each other via hydrophobic interfaces (buried-surface area ranging from 243 to 690 Å²) and short inter-domain linkers of up to two residues (Fig. 1a). Domains Ig1 and Ig2 form the largest interface in which the Ig2 loops at the N-terminal ‘head’ side interact with the A2-B (Ig domain β-strand numbering) side of Ig1 (Fig. 1a). The three other inter-domain interfaces are exclusively formed in a head-to-tail manner involving loops at the N-terminal ‘head’ and C-terminal ‘tail’ side of the Ig domains (Fig. 1a). As predicted from the primary sequence the N-terminal Ig1 domain of MAG has a V-type Ig fold like other Siglec family members and domains Ig3 and Ig4 are of the C2 type. Domains Ig2 and Ig5, however, have a C1-type Ig fold, contrary to the predicted C2-fold (Supplementary Fig. 2). The three crystal structures of MAG are similar to each other with only small differences within the domains (r.m.s.d. ranging from 0.93 to 2.13 Å) and inter-domain angle rotation differences ranging from 3.4 to 17.4°, the largest difference is in the domain Ig2–Ig3 angle (Fig. 1b). The combination of hydrophobic inter-domain interfaces, the lack of flexible linker residues and a previously predicted inter-domain disulfide between Ig1 and Ig2 (C37–C165) explains the limited inter-domain flexibility observed between the three different crystal forms.

Results

MAG has an extended conformation. We determined crystal structures of the full extracellular segment of mouse MAG (MAG1–5) in two different crystal forms that diffracted to a maximum resolution of 3.8 and 4.3 Å. These crystals were obtained by enzymatic deglycosylation of MAG1–5 or reductive lysine methylation of glycosylated MAG1–5 (see ‘Methods’ section). In addition, crystals of a shorter construct, consisting of the three N-terminal domains (MAG1–3), diffracted to a maximum resolution of 2.1 Å. The structures were solved by molecular replacement with individual Ig domains from homologous proteins. The exceptionally high-solvent content of the two MAG1–5 crystal forms (91 and 85%, Supplementary Fig. 1) aided in obtaining phases of sufficient quality for initial model building (see also Table 1 and ‘Methods’ section for details).

MAG signalling is bidirectional, engaging in both axon-to-myelin as well as myelin-to-axon signalling. MAG has been extensively studied as one of three classic myelin-associated inhibitors of central nervous system regeneration, the other ligands being Nogo66 and oligodendrocyte myelin glycoprotein. MAG inhibits neurite outgrowth and collapses axonal growth cones in a sialic acid binding-dependent manner. It does so as S-MAG binds to zinc and microtubules and this is postulated to have a structural function in mature myelin.

From earlier rotary-shadowed electron microscopy (EM) and sedimentation velocity analytical ultracentrifugation (AUC) studies it was hypothesized that the extracellular segment of MAG has a back-folded Ig-horseshoe type structure, but the estimated maximum dimensions of 8.8 and 18.5 nm determined by AUC and EM, respectively, deviate substantially. In the absence of any high-resolution structural data on MAG or its interaction with ganglioside ligands, the conformation of the five Ig domains, the extracellular specificity-determining parameters and the mechanisms underlying MAG adhesion and bi-directional signalling are unresolved. Using a combination of structural, biophysical and cellular techniques, we provide the structural basis of MAG-mediated adhesion and identify a dimerization-dependent mechanism that explains how MAG regulates axon-to-myelin and myelin-to-axon signalling, and controls myelin–axon spacing.

MAG is post-translationally modified. The structures reveal MAG is post-translationally modified at several sites. MAG contains seven disulfides, five of which are canonical for Ig domains. Cysteines 37 and 165 form an inter-domain disulfide between Ig1 and Ig2, and cysteines 421 and 430 form an...
proteins35. The W22-attached a rare post-translational modification present on several secreted WxxW motif (W22 is the first tryptophan) for C-mannosylation, determined in human MAG by mass spectrometry analysis34. We glycans and previously eight N-linked glycosylation sites were (Supplementary Fig. 3). In addition, MAG carries N-linked additional intra-domain disulfide in Ig5, as shown previously33 sequence reveals that this tryptophan is part of the canonical (Supplementary Fig. 6). Indeed, analysis of the MAG primary side chain of W22 suggests this residue is C-mannosylated W22. In all three crystal forms, electron density proximal to the N406, N450 and N454).

Table 1 | Data collection and refinement statistics.

| Data collection | MAG1–3 unliganded | MAG1–3 ligand bound | MAG1–5 deglycosylated | MAG1–5 lysine-methylated |
|----------------|--------------------|---------------------|----------------------|------------------------|
| Space group | P1 | P1 | P32 | P62 |
| Cell dimensions | 43.06, 60.4, 79.22 | 43.61, 60.12, 79.47 | 278.9, 278.9, 62.52 | 101.2, 101.2, 687.5 |
| a, b, c (Å) | 72.70, 86.71, 83.01 | 71.86, 86.51, 82.95 | 90, 90, 120 | 90, 90, 120 |
| Resolution (Å) | 42.73–2.12 (0.118) | 56.79–2.30 (0.38–2.30) | 69.72–3.80 (4.03–3.80) | 114.62–4.30 (4.81–4.30) |
| Rmerge | 0.064 | 0.157 | 0.234 | 0.115 |
| Mean I/σ | 1.8 (0.9) | 5.9 (1.6) | 9.2 (1.6) | 15.6 (1.3) |
| Rwork/Rfree | 0.985 (0.565) | 0.997 (0.565) | 0.998 (0.242) | 0.998 (0.242) |
| Completeness (%) | 96.2 (95.7) | 97.5 (95.7) | 100.0 (100.0) | 100.0 (100.0) |
| Redundancy (%) | 3.6 (3.5) | 4.5 (4.3) | 9.6 (9.7) | 35.7 (36.9) |

Refinement

| Resolution (Å) | 43–2.1 | 57–2.3 | 70–3.8 | 115–4.3 |
| No. reflections | 42,931 | 42,931 | 35,257 | 15,430 |
| Rwork/Rfree | 0.224/0.262 | 0.224/0.254 | 0.203/0.230 | 0.266/0.282 |
| No. atoms | 5,054 | 4,977 | 3,841 | 3,942 |
| Protein | 54 | 84 | 46 | |
| Water | 54 | 34 | | |
| Average B-factors | 71.1 | 70.9 | 144.2 | 414.5 |
| Protein | 106.8 | 102.5 | 129.4 | |
| Ligand/ion | 60.8 | 59.7 | | |
| r.m.s. deviations | 0.003 | 0.002 | 0.006 | 0.008 |
| Bond lengths (Å) | 0.002 | 0.583 | 1.218 | 1.384 |
| Bond angles (°) | 1.69 | 1.49 | 2.38 | 2.36 |
| Molprobity percentile | 94th | 99th | 99th | 99th |

Each data set was collected from a single crystal.

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additional intra-domain disulfide in Ig5, as shown previously33 (Supplementary Fig. 3). In addition, MAG carries N-linked glycans and previously eight N-linked glycosylation sites were determined in human MAG by mass spectrometry analysis34. We observe glycan electron density for five of those equivalent sites in the mouse MAG structures (on asparagine 99, 223, 246, 315 and 406; Supplementary Fig. 4). The differences in these observations arise from one N-linked glycosylation site that is not conserved (N106 in human MAG is a threonine in mouse) and from poorly resolved electron density for the other two sites (on N450 and N454) that are situated in a flexible loop. One additional N-linked glycan is revealed by clear electron density on N332, in contrast to the previous study that did not find this residue in a glycopeptide analysis34. Electron density at the N332-linked D-mannopyranosyl group has explained by the preference of the bulky tryptophan, covalently attached to the C1 of the mannose, to be in the equatorial position. We confirmed the C-mannosylation of W22 by in-gel trypsin digestion of MAG1–5 followed by liquid chromatography-mass spectroscopy (LC-MS/MS) analysis of the (glyco)peptide fragments (Supplementary Fig. 7). In addition, native mass spectrometry of wild type and mutated MAG1–5, in which the second tryptophan of the C-mannosylation motif is substituted for glutamine (W25Q, resulting in WxxQ), showed a mass shift of —221 Da in accordance with a loss of C-mannosylation and confirming the importance of the WxxW motif (Supplementary Fig. 6).

MAG1–5 crystal structures reveal a dimeric arrangement. MAG1–5 forms a symmetry-related dimer at a crystallographic two-fold rotation axis in both MAG1–5 crystal forms. They share the same interface (Fig. 2a) on domains Ig4 and Ig5, which buries a surface area of 2,037 Å2. The CC’FG face of Ig4 binds to the ABDE face of Ig5 of the symmetry-related molecule and vice versa, thus forming two equivalent hemi-interfaces. The interface is mostly hydrophobic apart from the negatively charged E395 in Ig4, with hydrophilic residues lining the edges of the interface (Fig. 2b).

To validate the interface we generated two interface mutations based on the structures, that we predicted to either disrupt dimerization (I473 to E) or enhance it (N406 to Q). The hydrophobic I473 in the middle of the hydrophobic interface of Ig5 was mutated to a negatively charged glutamate, to ensure disturbance of the hydrophobic effect as well as introducing (regular mannose is 4C1, Supplementary Fig. 6). This ring-flipped conformation is in agreement with previous nuclear magnetic resonance studies on mannosyl-tryptophan36 and can be explained by the preference of the bulky tryptophan, covalently attached to the C1 of the mannose, to be in the equatorial position. We confirmed the C-mannosylation of W22 by in-gel trypsin digestion of MAG1–5 followed by liquid chromatography-mass spectroscopy (LC-MS/MS) analysis of the (glyco)peptide fragments (Supplementary Fig. 7). In addition, native mass spectrometry of wild type and mutated MAG1–5, in which the second tryptophan of the C-mannosylation motif is substituted for glutamine (W25Q, resulting in WxxQ), showed a mass shift of —221 Da in accordance with a loss of C-mannosylation and confirming the importance of the WxxW motif (Supplementary Fig. 6).
electrostatic repulsion with the opposing E395 in Ig4 (Fig. 2c). N406 carries an N-linked glycan and in the glycosylated lysine-methylated crystals of MAG1–5, the density for this glycan suggests that it sterically clashes with its symmetry partner in the dimer (Fig. 2d). We hypothesized that a dimer with increased affinity would form, were this glycan absent. Remarkably, the N406Q mutant that lacks this glycan was the only MAG construct that showed a distinct dimer peak in size exclusion chromatography (SEC; Supplementary Fig. 8). The other glycans are not expected to interfere with dimerization, also not when considering myelin-specific N-linked glycans. We confirmed that MAG dimerizes in solution via the Ig4–Ig5 interface by analysing glycosylated and deglycosylated forms of MAG 1–5, MAG1–3 (that lacks the dimerization domains) and the aforementioned mutants in small angle X-ray scattering (SAXS) and AUC experiments.

SAXS confirms dimerization interface. As predicted, SAXS analysis indicated disruption of dimerization for MAG1–3 and MAG1–5 I473E, whereas dimerization is enhanced for MAG1–5 N406Q and deglycosylated MAG1–5 compared with wt MAG1–5. The molecular mass (M_m) based on the extrapolated intensity at zero scattering angle (I_0, scaled for concentration to bovine serum albumin (BSA)), the radius of gyration (R_g), the maximum interatomic distance (D_max) and the Porod volume all show these trends (Fig. 2, Supplementary Fig. 9 and Table 2). The data show that at similar concentrations, MAG1–5 I473E appears smaller than wt MAG1–5 and MAG1–5 N406Q appears larger than wt MAG1–5. This can be attributed to a shift in the monomer–dimer equilibrium; MAG1–5 I473E has a lesser and MAG1–5 N406Q a greater propensity to dimerize compared with wt MAG1–5.

Furthermore, the MAG1–5 I473E pair distance distribution function P(r) and derived D_max, the ab initio models as well as the Kratky plots confirm that MAG has an extended conformation and behaves as a semi-rigid rod in solution (Fig. 2f,g,i). Whereas the SAXS data for glycosylated MAG1–5 and MAG1–5 I473E fit best to scattering curves calculated from single chains of the crystal structures of MAG1–3 and MAG1–5, respectively (χ = 2.87 and 2.95, Supplementary Fig. 10), the glycosylated MAG1–5 N406Q SAXS data fits best to scattering curves calculated from the dimer structure (χ = 3.45, Supplementary Fig. 10). Both glycosylated and deglycosylated MAG1–5 wt SAXS data fit best to scattering curves calculated from a combination of monomeric and dimeric crystal structures (χ = 4.05 for glycosylated and 3.72 for deglycosylated MAG1–5, Fig. 2) and Supplementary Fig. 11). Furthermore, ab initio models based on the SAXS data of deglycosylated MAG1–5 I473E and MAG1–5 N406Q agree remarkably well with the crystal structures of the monomer and dimer of MAG1–5, respectively (Fig. 2g, Supplementary Fig. 12, χ^2 of the model-to-data fit are 1.05 and 1.33 for MAG1–5 I473E and MAG1–5 N406Q, respectively). These data confirm MAG dimerizes via domains Ig4 and Ig5 and that the MAG1–5 chains have an extended and relatively rigid conformation.

The MAG1–5 dimer is weak in solution with a K_d of 3.8 × 10^2 μM. To quantify the affinity of MAG dimerization in solution, we performed sedimentation equilibrium AUC (SE-AUC) experiments for wt MAG1–5, deglycosylated wt MAG1–5, MAG1–3, MAG1–5 I473E and MAG1–5 N406Q constructs. For each sample a global analysis was performed for different concentrations, centrifugal speeds and wavelengths together (Supplementary Figs 13–16), except for MAG1–5 N406Q, which appeared to suffer...
**Figure 2 | MAG forms dimers via domains Ig4 and Ig5.** (a) Superposition of the crystallographic dimers observed in the crystals of MAG1-5 deglycosylated (blue) and MAG1-5 lysine-methylated (orange). The two-fold axis is indicated by a black line with an ellipse on top, N- and C-termini indicated with N and C for one monomer. (b) The dimer is formed by two equivalent symmetry-related hemi-interfaces, which are mostly hydrophobic (yellow) with hydrophilic (grey), positively charged (blue) and negatively charged (red) residues lining the edges. (c) Zoom of the interface in deglycosylated MAG1-5, indicating isoleucine 473, which was mutated to glutamate to disturb the interface. (d) 2Fo – Fc electron density at a contour level of 1.0 σ of the N-linked glycan on N406 in the lysine-methylated MAG1-5 crystal structure, showing the steric hindrance with its symmetry mate. Glycosylation at this site was prevented by mutating N406 to glutamine to obtain a MAG variant with enhanced dimerization properties. (e) SAXS Log I versus Q plots for glycosylated MAG variants: MAG1-5 wt (blue), MAG1-5 I473E (red), MAG1-5 N406Q (green) and MAG1-3 (purple), same colouring used in (e–j). (f) Paired distance distribution functions of the same MAG variants as in (e). (g) DAMMIF *ab initio* modelling for deglycosylated MAG1-5 I473E (red) and MAG1-5 N406Q (green), showing remarkable similarity to the crystal structures of monomeric MAG (left, grey) and the crystallographic dimer (right, grey), respectively (see Supplementary Fig. 13 for fits). (h) Guinier plots for the different glycosylated MAG variants confirm that MAG behaves as a semi-rigid rod in solution and not as flexible beads-on-a-string. (i) OLIGOMER fit of the MAG monomer and crystallographic dimer to the glycosylated MAG1-5 wt data, using the glycosylated and lysine-methylated crystal structures. OLIGOMER gives a 0.72:0.28 (monomer:dimer) ratio at a MAG concentration of 52 μM.
from aggregation during the experiment. Similar to the SAXS analysis, the MAG1–5 I473E and MAG1–3 SE-AUC data fit best to a single species that agrees with the $M_m$ of a monomer (Table 3). For wt MAG1–5 and deglycosylated MAG1–5, we could fit the data to a monomer–dimer equilibrium, with $K_{d}$ of 3.8 × 10^2 and 1.7 × 10^3 μM, respectively (Table 3). On the basis of a monomer–dimer equilibrium with these $K_{d}$, dimer fractions of 18% for glycosylated wt MAG1–5 and 24% for deglycosylated wt MAG1–5 are expected to be present in the SAXS experiments (calculated at 52.2 and 37.5 μM for glycosylated and deglycosylated MAG, respectively). Indeed, the presence of a mix of monomers and dimers is observed in the SAXS data of both glycosylated and deglycosylated MAG1–5 (Fig. 2j, Supplementary Fig. 11). The lack of dimers in the AUC experiments for MAG1–5 I473E and MAG1–3 and the higher affinity for deglycosylated MAG1–5 compared with glycosylated MAG1–5 further confirm that MAG forms dimers via domains Ig4 and Ig5 (Table 3).

**Structural basis of ligand recognition by Ig1.** MAG binds to sialic acids of gangliosides with its N-terminal V-type Ig domain and has a preference for a Neu5Ac-sialic acids of gangliosides with its N-terminal V-type Ig domain moiety. We observed unmodelled electron density in the $2F_o – F_c$ and $F_o – F_c$ maps of the deglycosylated MAG1–5 structure close to R118 in the putative ligand-binding site (Fig. 3a). Native mass spectrometry of purified MAG1–5 revealed a mixture of free and two ligand-bound MAG1–5 forms with mass differences of 835 ± 2 Da compared with free MAG1–5 (Fig. 3b). The 854 Da ligand possibly corresponds to a tetrasaccharide comprising the aforementioned Neu5Ac–α2,3-Gal–β1,3-GlcNAc trisaccharide plus another hexose. The 854 Da ligand might correspond to a similar tetrasaccharide where Neu5Ac is replaced by Neu5Gc, a mammalian sialic acid variant not produced by humans. These ligands are likely co-purified in their interaction with GT1b ganglioside incorporated into liposomes. The set-up we used, MAG1–5 coupled at the C-terminus to a streptavidin-coated surface plasmon resonance (SPR) chip and GT1b-containing liposomes in the mobile phase, enables avidity-enhanced interactions that also occur in trans between cells (Fig. 3f, see ‘Methods’ section). Indeed we observed specific binding of GT1b liposomes to wt MAG1–5, no interactions with the ligand-binding mutants MAG1–5 R118A, T128A and Y127A and reduced interaction with MAG1–5 Y65A (Fig. 3f). In addition, the MAG1–5 W25Q mutant that lacks the tryptophan mannosylation on W22, still interacted with GT1b liposomes in this assay. Remarkably, this W25Q mutant appeared to have higher affinity for the GT1b liposomes compared with wt MAG. This suggests that rather than contributing to the interaction strength, this tryptophan mannosylation on W22, still interacted with GT1b liposomes in this assay. In summary, we have shown that MAG interacts with membrane-bound gangliosides via the side chain of R118, the CC′-loop and the F and G β-strands of the N-terminal V-type Ig1 domain and that the W22 mannosylation does not enhance ganglioside binding.

Ligand interaction of MAG is similar to the sialic acid recognition of other Siglec family members (Fig. 3g). In an experiment, MAG1–5 and MAG1–3 SE-AUC data fit best to a single species that agrees with the $M_m$ of a monomer (Table 3). For wt MAG1–5 and deglycosylated MAG1–5, we could fit the data to a monomer–dimer equilibrium, with $K_{d}$ of 3.8 × 10^2 and 1.7 × 10^3 μM, respectively (Table 3). On the basis of a monomer–dimer equilibrium with these $K_{d}$, dimer fractions of 18% for glycosylated wt MAG1–5 and 24% for deglycosylated wt MAG1–5 are expected to be present in the SAXS experiments (calculated at 52.2 and 37.5 μM for glycosylated and deglycosylated MAG, respectively). Indeed, the presence of a mix of monomers and dimers is observed in the SAXS data of both glycosylated and deglycosylated MAG1–5 (Fig. 2j, Supplementary Fig. 11). The lack of dimers in the AUC experiments for MAG1–5 I473E and MAG1–3 and the higher affinity for deglycosylated MAG1–5 compared with glycosylated MAG1–5 further confirm that MAG forms dimers via domains Ig4 and Ig5 (Table 3).

**Table 2 | SAXS data collection and parameters.**

| Sample               | $M_m$ (kDa) | Concentration (μM) | Temperature (K) | $R_g$ (nm) | $M_m$ based on $I_0$ (kDa) | $D_{max}$ (nm) | Porod volume (nm^3) | SASBDB accession code |
|----------------------|-------------|--------------------|----------------|------------|-----------------------------|--------------|---------------------|-----------------------|
| MAG1–5 wt glycosylated | 64.9        | 52.2               | 293            | 6.8        | 76.8                        | 23.8         | 153.8               | SASDB55               |
| MAG1–5 I473E glycosylated | 64.9        | 46.2               | 293            | 6.0        | 64.4                        | 20.6         | 117.1               | SASDB26               |
| MAG1–5 N406Q glycosylated | 63.7        | 33.3               | 293            | 7.3        | 82.0                        | 25.6         | 193.2               | SASDB36               |
| MAG1–3 glycosylated   | 39.8        | 43.7               | 293            | 3.9        | 43.0                        | 13.0         | 59.3                | SASDB46               |
| MAG1–5 wt deglycosylated | 56.8        | 37.5               | 293            | 7.3        | 75.4                        | 25.5         | 166.2               | SASDBF6               |
| MAG1–5 I473E deglycosylated | 56.8        | 45.1               | 293            | 6.0        | 61.1                        | 21.2         | 99.6                | SASDB56               |
| MAG1–5 N406Q deglycosylated | 56.8        | 33.6               | 293            | 7.8        | 93.9                        | 29.0         | 216.4               | SASDB66               |
| MAG1–3 deglycosylated | 36.6        | 38.2               | 293            | 3.9        | 39.9                        | 12.6         | 49.4                | SASDB76               |
S Iglec5 (ref. 32), the MAG Ig1 CC'-loop (residues 64–70) seems to undergo conformational selection on ligand binding. This loop adopts a single conformation when ligand is bound, whereas it can have several conformations (including the ligand-bound conformation) or is unstructured in the different unliganded MAG crystal forms (Fig. 3e). Furthermore, this loop adopts different conformations in the Siglec-1, -5 and -7 structures (both unliganded and ligand-bound forms, Fig. 3g)30–32. The combination of our structural and biophysical data on MAG–ganglioside interaction, with that of others on MAG’s specificity for Neu5Acα2,3-Galβ1,3-GalNac (ref. 18) establishes the structural basis of ganglioside recognition by MAG.

Neurite outgrowth inhibition depends on MAG dimerization

We tested different MAG variants in neurite outgrowth assays to determine the role of MAG dimerization for neuronal plasticity inhibition (Fig. 4). In agreement with previous data20,38, MAG1–5 wt on coverslips inhibited neurite outgrowth of hippocampal neurons compared with poly-L-lysine (PLL)-covered slips (Fig. 4a,b). Other dimeric variants (MAG1–5 N406Q and MAG1–5-Fc) inhibited neurite outgrowth to a similar level (Fig. 4d,f). The monomeric MAG1–5 I473E and MAG1–3 wt on the other hand showed no significant neurite outgrowth inhibition (Fig. 4c,e). Interestingly, MAG1–5-Fc R118A, which is dimeric but lacks sialic acid binding properties, showed neurite outgrowth stimulation instead of inhibition, compared with PLL (Fig. 4g). These data indicate that dimerization through domains Ig4–Ig5 and the ability to bind sialic acid moieties on the neuronal surface are required for neurite outgrowth inhibition signalling by MAG for hippocampal neurons.

Discussion

MAG controls adhesion and signalling between myelinating cells and axons. In contrast to earlier studies28,29, we find that MAG does not fold back onto itself like an Ig-horseshoe as in the L1CAM and axonin neuronal adhesion molecules. Instead, our data show that the extracellular region of MAG has an extended shape with limited inter-domain flexibility, similar to several other cell adhesion molecules such as SYG, Cadherin and Nectin family members39–41.

The structure of MAG is the first of a full extracellular portion of a Siglec family member. Besides the common N-terminal V-type Ig domain for recognizing sialic acid moieties, Siglecs vary in the number of additional Ig domains; from 1 up to 16. Comparison of the structures of MAG and Siglec5 reveals a different inter-domain orientation between domains Ig1 and Ig2, likely due to differences in amino acids at the interface (Supplementary Fig. 17 and Supplementary notes).

By binding to axonal gangliosides, MAG maintains a defined spacing between the innermost myelin surface and the axon surface6–12.42. This myelin–axon spacing has been reported to be 12–14 nm based on electron micrographs of chemically fixed myelin tissue10–12. However, analysis of more recent electron micrographs of high-pressure frozen myelin that does not suffer from fixation induced artefacts43 reveals an axon–myelin spacing of 9–12 nm. This periaxonal diameter matches well with a straightforward model that follows from our structural data of the MAG dimer and the MAG–ganglioside interaction; two opposing membrane surfaces are spaced 10 nm apart when the membrane-proximal C-termini of the MAG dimer are positioned on one membrane (the structures lack only two residues to the transmembrane helix) and the MAG dimer-bound gangliosides are positioned on the other membrane (Fig. 5). Although the two crystal forms of the full extracellular segment of MAG have different inter-domain angles (3.4°–17.4°), the overall arrangement and resulting structure-based axon–myelin spacing is similar (see Fig. 2a). The agreement of intermembrane distance determined from high-pressure frozen EM on myelin tissue43 and here by structural and biophysical data on the extracellular segment of MAG indicates that in the periaxonal space, MAG is dimerized in cis via domains Ig4–Ig5 when bound to axonal gangliosides in trans.

Intriguingly, in this model, the unusual tryptophan C-mannosylation on W22 is positioned at the interface of MAG and the extracellular leaflet of the axonal membrane (Fig. 5, Supplementary Fig. 18). The WxxW motif is conserved among MAG orthologues in vertebrates from fish to human, but is absent in all other Siglec paralogs (Supplementary Fig. 6). This suggests that tryptophan mannosylation is specific for the function of MAG. We showed that the mannosyl group does not enhance the binding of MAG to GT1b ganglioside liposomes but may weaken it. Possibly, tryptophan mannosylation of MAG provides specificity to sialylated ligand interactions. Alternatively, the close proximity of the mannosyl group to the axonal membrane during MAG–ganglioside interaction may indicate a regulatory role in axonal membrane engagement.

The buried-surface area of the MAG dimerization interface formed by Ig4 and Ig5 is large (2,037 Å²) and hydrophobic. We find, however, that the affinity of MAG dimerization via this interface in solution is low (Kd of 3.8 x 10^2 μM). The weak interaction is probably important in the native context, where MAG is expressed on the cell surface, as the cis dimer can be trans stabilized by interaction with gangliosides on the opposing axonal membrane. For the N-Cadherin family of cell adhesion molecules it has been shown that affinities as weak as 10 mM in solution are functionally important in the context of a trans-stabilized cis-dimer39.

Dimerization of MAG may serve two purposes. It provides a mechanism to restrain the intermembrane distance, since a cis–trans stabilized MAG dimer would restrict angular freedom with respect to the membrane more than a trans only stabilized MAG monomer. In this sense, the MAG dimer could function as a ‘molecular leaf spring’ that maintains the well-defined spacing between the axonal membrane and the adaxonal myelin membrane along the internode. In addition, dimerization of MAG could enable compaction of the periaxonal space. The weak cis-interaction of MAG, if not stabilized in trans, may ensure enough monomer is available to bridge a wider periaxonal spacing (of up to 16 nm, based on the length of a MAG monomer) that may exist during myelin formation. Even greater distances could be bridged if MAG binds to sialylated N- or O-linked glycans of axonal surface glycoproteins before reaching

**Table 3 | SE-AUC parameters.**

| Sample          | Model:                 | M_m floated/fixed | M_a (kDa) | logK_d (K_d) | K_d (μM) |
|-----------------|------------------------|-------------------|-----------|--------------|-----------|
| MAG1–5 wt glycosylated | Monomer–dimer equilibrium | Fixed          | 62.6      | 3.42         | 382       | 1.17     |
| MAG1–5 I473E glycosylated | Single species          | Fixed          | 62.3      | -            | -         | 1.18     |
| MAG1–3 glycosylated      | Single species          | Fixed          | 40.8      | -            | -         | 1.31     |
| MAG1–5 wt deglycosylated | Monomer–dimer equilibrium | Fixed          | 56.9      | 3.78         | 167       | 0.94     |
**Figure 3 | Structural characterization of ligand recognition by the N-terminal domain Ig1 of MAG.** (a) $2F_o - F_c$ electron density map displayed at a contour level of 1.3 $\sigma$ before placing any ligand for refinement in the MAG$_{1-5}$ deglycosylated crystal structure (blue), showing a density that fits well with the Neu5Ac-$\alpha$Gal-$\beta$GalNAc trisaccharide (orange). (b) Native mass spectrometry reveals two species that have a mass difference of $854 \pm 1.4$ Da, presumably because of oligosaccharide ligand binding. The deconvolved mass versus intensity spectrum (blue) is shown together with the 20 $\times$ amplified version of this spectrum for masses above 57.2 kDa (orange) to highlight the similar pattern of trimmings between the unliganded and ligand-bound form. (c) $F_o - F_c$ (soaked-unsoaked) electron density at a contour level of 3.0 $\sigma$ of MAG$_{1-3}$ at the ganglioside binding site of chain B, showing the unbiased electron density changes that resulted from binding of the 3'-Sialyl-N-acetyllactosamine (Neu5Ac-$\alpha$2,3-Gal-$\beta$1,4-GlcNAc) ligand and concomitant small conformational rearrangements. Residues involved in ligand engagement (sticks), as well as the C-mannosylation (yellow) on W22 are shown. The first two sugars of the 3'-Sialyl-N-acetyllactosamine (orange) fit the density well. (d) Protein-ligand interactions with hydrogen bonds indicated by dashes and Van der Waals' contacts by curved blue lines. (e) Comparison of the four unliganded (grey) and the two ligand-bound structures of MAG; MAG$_{1-5}$ deglycosylated (blue) and soaked MAG$_{1-3}$ (purple). The CC' loop adopts different conformations in the unliganded structures yet appears to undergo conformational selection by interactions of Y65 in this loop with the ligand. (f) GT1b ganglioside liposome SPR confirms the contribution of contact residues from the crystal structures. Surprisingly, the W25Q mutant that lacks the tryptophan mannosylation on W22 shows enhanced ligand binding. (g) Similar ligand recognition by four different Siglec family members; MAG (purple, MAG$_{1-3}$ structures), Siglec1 (red), Siglec5 (green) and Siglec7 (blue). Shown are unliganded forms (lighter colours), ligand-bound forms (darker colours) with sialylated ligands and the conserved arginine (stick representation, R118 in MAG) that forms a salt bridge with the carboxylic acid group of the sialic acid. The structurally heterogeneous CC' loop is also indicated.
Surprisingly, a dimeric variant that does not bind sialic acids (MAG1–5-Fc monomeric MAG constructs) inhibit neurite outgrowth compared with PLL. 

**Figure 4 | MAG dimerization and sialic acid-binding are required for neurite outgrowth inhibition of hippocampal neurons.** Dissociated neuron cultures were prepared from P1 hippocampus and grown for 2 days _in vitro_ (DIV2) on (a) PLL (20 µg ml⁻¹) as a control or on coverslips coated with PLL and MAG proteins (60 µg ml⁻¹); (b) MAG1–5 wt, (c) MAG1–5 I473E, (d) MAG1–5 N406Q, (e) MAG1–3 wt, (f) MAG1–5-Fc wt or (g) MAG1–5-Fc R118A. Cultures are immunostained with antibodies against III-tubulin and DAPI. Quantification of the length of the longest neurite is shown in (h). n = 6 mice, One-way ANOVA with Tukey _post hoc_ test was used, *P < 0.05, **P < 0.01. Error bars represent s.e.m. Only dimeric and not monomeric MAG constructs inhibit neurite outgrowth compared with PLL. Surprisingly, a dimeric variant that does not bind sialic acids (MAG1–5-Fc R118A) appears to stimulate neurite outgrowth.

the gangliosides. When _trans_ interactions with gangliosides have been established and possibly local concentrations are elevated due to the abundance of ganglioside ligands, formation of MAG _cis_-dimers is triggered concomitant with compaction of the periaxonal diameter to its final spacing (Fig. 5).

Dimerization of MAG can be regulated by modulating glycosylation on N406. Large and charged glycans on N406 can obstruct dimerization because of steric clashes and coulombic repulsion. Trimming all MAG N-linked glycans down to single N-Acetylglucosamines by deglycosylation with Endo-H or preventing glycosylation on N406 by mutating it to glutamine enhances dimerization (Fig. 2, Supplementary Figs 8–12 and 16, Tables 2 and 3). This glycosylation site might play a regulatory role during myelin development and myelination-related pathologies. MAG glycosylation changes during development and abnormal glycosylation of MAG correlates with myelination deficiencies. Possibly, modulation of N406 glycosylation, either at the biosynthesis level or by extracellular trimming, affects MAG dimerization and thereby impacts on the myelin–axon interaction (see Supplementary notes for details).

Myelin-to-neuron signalling with MAG as a ligand can inhibit neurite outgrowth. Studies based on MAG truncations, chimeras and mutant versions suggested that the inhibitory properties reside in domains Ig4 and Ig5, and in the sialic acid binding site. For example, a chimeric protein consisting of Siglec1 Ig domains 1–3 plus MAG Ig4-5, but not Siglec1 domains 1–3 alone, has neurite outgrowth inhibition properties similar to MAG. We confirmed that the sialic acid-binding properties of MAG are required for neurite outgrowth inhibition in hippocampal neurons as the MAG1–5-Fc R118A mutant that lacks ganglioside binding properties does not inhibit, but surprisingly, stimulates neurite outgrowth (Fig. 4g). This has not been reported before and may be an interesting new avenue for therapeutic intervention to enhance central nervous system regeneration. In addition we show that domains Ig4 and Ig5 are essential for MAG dimerization and that neurite outgrowth inhibition is abrogated by the I473E point mutation that monomerizes MAG (Fig. 4e,c). Thus, it is the dimerization of MAG that is required for neurite outgrowth inhibition, rather than direct interactions of domains Ig4 and Ig5 with neuronal receptors, as previously suggested. Although other protein receptors have been identified that mediate the neurite outgrowth-inhibiting signalling by MAG, it has been shown that direct clustering of gangliosides by antibodies also leads to inhibition of neurite outgrowth of hippocampal neurons. The combination of our data and that of others indicates that MAG dimerization at domains Ig4 and Ig5 and sialic acid binding at domain Ig1 induces neurite outgrowth inhibition for hippocampal neurons, by clustering of gangliosides.

Axon-to-myelin signalling with MAG as a receptor controls myelin formation. Antibody-mediated extracellular clustering of the L-MAG isoform activates Fyn kinase and Fyn activation is essential for the initiation of myelination. Our structures show that the C-termini of MAG1–5 are separated by 5.4 nm in the dimer, bringing the cytosolic regions into close proximity (Fig. 5). Probably, L-MAG dimerization as a result of _trans_ interaction...
with gangliosides on the axon brings the cytosolic regions of MAG into close proximity to trigger activation of Fyn, similar to Fyn activation by signalling lymphocytic activation molecule clustering in immune cells. Whether MAG forms higher-order clusters that are triggered by dimerization needs to be established, but preference of both MAG and Fyn for lipid rafts suggests that both proteins can be locally enriched in the membrane to assist clustering.

Methods

Generation of mutants and mutagenesis. MAG constructs were generated by polymerase chain reaction (PCR) using mouse c-MAG (IMAGE 4009200) as a template and primers to start at (UNIPROT) residue number 20 (after the signal peptide) and end at residue 325 for MAG-1 and residue 508 for MAG-3. Point mutants were also created by PCR, either by a two-step PCR with overlapping primers (W22A, W25Q, Y65A, R118A, I128A, T128A, I473E,) or by a single-step PCR using non-overlapping phosphorylated primers (N406Q). All constructs were subcloned using BamH1/NotI sites in pUPE107.03 (cystatin secretion signal peptide, C-terminal His-tag), unless indicated otherwise.

Large-scale expression and purification. Constructs were transiently expressed in N-acetylglucosaminyltransferase I-deficient (GnTI−) Epstein–Barr virus nuclear antigen I (EBNA1)-expressing HEK293 cells in suspension (U-protein express). Medium was collected 6 days after transfection and cells were spun down by 10 min of centrifugation at 1,000 r.p.m. Supernatant was concentrated fivefold and dialyzed against 500 mM NaCl, 25 mM HEPES pH 7.8 (IMAC A) using a Quixstand benchtop system (GE Healthcare) with a 10 kDa molecular weight cut-off (MWCO) membrane. Cellular debris was spun down for 10 min at 9,300 g and the concentrate was filtered with a glass fibre prefiltre (Minisart, Sartorius). Protein for purification by Nickel–nitrioltriacetic acid (Ni–NTA) affinity chromatography followed by SEC on a Superdex200 HiLoad 16/60 column (GE Healthcare) equilibrated in SEC buffer (150 mM NaCl, 20 mM HEPES pH 7.5). Protein was concentrated to 7–14 mg ml−1 using a 30 kDa MWCO concentrator before plunge freezing in liquid nitrogen and storage at −80 °C.

Crystalization and data collection. Since initial crystallization attempts did not yield 0.99998 Å)

Small angle X-ray scattering. SAXS was performed at the ESRF BM29 BioSAXS beamline equipped with a 2D Pilatus 1 M detector (DECTRIS, Switzerland), operating at an energy of 12.5 kV. MAG constructs were dialyzed against SEC buffer using a 10 kDa MWCO membrane. The concentrations were determined by ultraviolet–visible spectroscopy on a nanodrop ND-1,000 spectrophotometer. Similar concentrations were selected for all samples to allow comparison (see Table 2). SAXS data were collected at 20 °C in a nitrogen beam and exposure time and the scattering of the solvent-blank (SEC buffer) was subtracted, following standard procedures. The curve was scaled using a BSA reference so that the I0 represents the M0 of the sample. Radiation damage was monitored by comparing curves collected from the same sample, only curves without radiation damage were merged. A single concentration was used for all measurements, no extrapolation to zero concentration was performed. Data were analysed using PRIMUS90, GNOM91, DAMMIF92, CRYSOL93 and OILIGOMER94 of the ATSAS95 suite.

Analytical ultracentrifugation. Sedimentation equilibrium experiments were carried out in a Beckman Coulter ProteomeLab XL-I and a Beckman Optima XL-A analytical ultracentrifuge. Either 12 or 3 mm centerpieces with quartz windows were used, 12 mm for the lowest concentrations and 3 mm for the others. An-60 and An-50 Ti rotors (Beckman) were used to carry out the measurements. MAG constructs were diluted with and dialyzed against SEC buffer using a 10 kDa MWCO membrane. Input concentrations of 3.8, 35.3 and 89.0 μM (MAG1−3, wt glycosylated), 16.8, 27.2, 35.5 and 144 μM (MAG1−5, wt glycosylated), 3.6, 8.3, 20.5, 35.6 and 70.5 μM (MAG1−5, lysine-methylated), and 1.9, 3.9, 7.8 and 15.6 μM (MAG1−5, lysine-methylated, wt glycosylated) were used. Sedimentation equilibrium runs were performed at 20 °C and at 7,500, 14,000 and 20,000 r.p.m. Absorbance was determined at 250, 280 and 300 nm with SEC buffer as reference. Buffer density and viscosity were determined by SEDNTERP as 0.99823 g ml−1 and 0.001002 mPas, respectively.

In-gel digestion and LC-MS/MS. MAG1−3 was separated by SDS-PAGE, and cut from gel for digestion with trypsin (Promega). The gel band was cut to small pieces, washed in Milli-Q water and treated with acetonitrile to shrink the gel pieces. The sample was then incubated in 1 g/l 1.4-dithiothreitol for 60 min at 60 °C, treated with acetonitrile, alkylated with 10 g/l iodoacetamide for 30 min at room temperature in the dark and subsequently washed with ammonium bicarbonate and treated with acetonitrile, twice. The gel pieces were then incubated on ice for 90 min with 30 mg ml−1 trypsin. Excess trypsin was removed, the gel pieces were covered in ammonium bicarbonate, and the samples were subsequently incubated overnight at 37 °C. The digested samples were collected and remaining sample was extracted from the gel pieces by treatment with acetonitrile. The solution with the peptides was subsequently dried in a speedvac and the peptides resuspended in 10 mM sodium, 5% dimethylformamide in water.

Peptides were separated by reversed phase LC coupled on-line to an Orbitrap Elite for MS/MS analysis. The nano-LC consists of an Agilent 1200 series LC system equipped with a 20 mm ReproSil-Pur C18-AQ (Dr Maisch GmbH) trapping column (packed in-house, i.d., 100 μm; resin, 5 μm) and a 400 mm ReproSil-Pur C18-AQ (Dr Maisch GmbH) analytical column (packed in-house, i.d., 50 μm; resin, 3 μm) arranged in a vented-column configuration. The flow was passively split to 100 nl min−1. We used a standard 45 min gradient from 7–30% acetonitrile in aqueous 0.1% formic acid. All precursors were fragmented by both EThCAd and HCD. Data were searched against a custom database of recombinant protein sequences, including the MAG constructs used here, with trypsin as protease, allowing up to two missed cleavages. We used a 50 p.p.m. precursor mass window and 0.02 Da fragment mass window. The C-mannosylated peptide, with
Native mass spectrometry. Purified protein samples were deglycosylated with Endo-H as for crystallization, followed by buffer exchange to 150 mM ammonium acetate pH 7.5, using Vivaspin®500 10 kDa MWCO centrifugal filter units. Samples were loaded onto gold-coated borosilicate capillaries prepared in-house for nanoelectrospray ionization. Samples were analysed on a modified Orbitrap extended mass range (thermo fisher) for high mass ions.

Surface plasmon resonance. MAG1–5 wt and mutants cloned in-frame with an N-terminal cystatin secretion signal and a C-terminal biotin acceptor peptide and His6-tag (sequence AAAGGGLNDIFEAQKIEWHEGRTKHHHHHH), were biotinylated in HEK293 cells by co-transfection with E. coli BirA biotin ligase with a sub-optimal secretion signal (in a pUPE5.02 vector), using a DNA ratio of 9:1 (MAG DNA: pCUBA DNA, m per m). Additional sterile biotin (10 mg ml⁻¹ Tris-buffered biotin per 4 ml HEK293 culture) was supplemented to the medium a few hours after transfection. MAG mutants were purified from the medium by Ni-NTA affinity purification. Purity was evaluated by SDS-PAGE and coomassie staining and biotinylation by a streptavidin gel-shift assay followed by 96 His, Western blot (Supplementary Fig. 19). C-terminally biotinylated MAG proteins were spotted on a G-STRIP SensEye chip (Siens) with a Continuous Flow Microspotter (Wasatch Microfluidics) using a 8 × 6 format. The C-terminal coupling of MAG to the surface mimics the native, membrane attached topology. SEC buffer with 0.05% tween was used as a spotting buffer and the coupling was quantified using 1 mM biotin in SEC buffer solution.

GT1b ganglioside liposomes were prepared as described previously. In the lipids, the dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylglycerol, cholesterol and GT1b gangliosides were mixed in a molar ratio of 40.3:34.2:40.9:1.3 in a chloroform/methanol mixture (6:1, v per v). The lipid mixture was dried under vacuum and then resuspended in a thin film of lipids. Liposomes were formed by addition of 1 ml of SEC buffer per 21.7 μmol of lipid mixture and 11 freeze-thaw cycles. As a negative control, liposomes with the lipid composition but lacking GT1b were prepared using the same protocol. Liposomes were extruded through a 100 nm membrane with a mini-extruder (Avanti Polar Lipids) at 70°C.

SPR experiments, with the liposomes in the mobile phase and the MAG constructs attached to the surface, were performed on an MX96 SPRi instrument (IBIS Technologies) equipped with a CX flow cell, using the CX vesicle run protocol (IBIS Technologies) equipped with a CX flowcell, using the CX vesicle run protocol. Liposomes were injected into the flowcell and binding was examined with a running buffer and an association time of 60 min at a temperature of 25°C. As a running buffer, SEC buffer without any detergent was used. Preliminary removal of co-purified lipids before the runs and regeneration after runs was performed by washes with 0.5% SDS in phosphate-buffered saline ( PBS) followed by 5 mM NaCl. Data was zeroed and referenced using Sprintr 1.11 (IBIS Technologies).

Neurite outgrowth assays. MAG1–5-Fc constructs were generated by subcloning into pUPE7.12 vector using BamHI/NotI restriction sites (Fc is C-terminal of MAG, expressed in HEK293 cells and purified by protein-A affinity purification using standard protocols. Coverslips were all coated overnight at 4°C for 3–4 h to allow immobilization of the different MAG variants. Coverslips were then washed with phosphate-buffered saline (PBS) and blocked with 1% bovine serum albumin (BSA) in PBS for 1 h. Coverslips were then incubated with primary antibody (donkey anti-mouse Alexa Fluor 488, 1:750) for 2 h at room temperature. After three PBS washing steps, coverslips were incubated with 4′,6-diamidino-2-phenylindole (DAPI—Sigma) for 10 min. After several PBS washes, coverslips were mounted with Fluoroshield. Coverslips were visualized using a Zeiss Axioplan A1 using a 20× objective. Images were analysed using Fiji (version 2.0.0.7) by tracing the longest neurite of a hippocampal neuron (positive for tubulin and DAPI). Significance was determined using a one-way ANOVA (Fisher’s LSD posthoc test for multiple comparison) (GraphPad Prism version 6.07). Every construct was compared with control PLL or MAG wt. All quantitative assessments in this manuscript were performed while being unaware of the condition to avoid observer bias. During analysis, raw data were named in a descriptive way, without revealing experimental group information. Fluorescent microscopic imaging was always done with the same settings within each experiment, and analysing techniques were standardized. A single person analysed all data obtained within the experiments. Significant levels for the following proteins: MAG1–5-Fc wt (**, 348 neurons counted), MAG1–5 N406Q (*, 385 neurons counted), MAG1–3 Fc wt (**, 364 neurons counted) and MAG1–3 Fc R118A (****, 369 neurons counted) compared with PLL (395 neurons counted). Constructs MAG1–3 I473E (*, 342 neurons counted), MAG1–3 wt (**, 353 neurons counted) and MAG1–3 Fc R118A (****, 348 neurons counted) were significant compared with MAG1–3 wt. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Data availability. Coordinates and structure factors for MAG1–5 deglycosylated (spacegroup P3212), MAG1–3 methylated (spacegroup P622), MAG1–3 (spacegroup P21) and MAG1–3 ligand bound (spacegroup P21) have been deposited in the Protein Data Bank with accession numbers 5EFS, 5ELU, 5ERF and 5EFV, respectively. All SAXS data is made available at the small angle scattering databank (SASDB) with the accession codes SASDB55 (glycosylated MAG1–5 wt), SASDB26 (glycosylated MAG1–3 I473E), SASDB36 (glycosylated MAG1–3 N406Q), SASDB46 (glycosylated MAG1–3 wt), SASDB46 (deglycosylated MAG1–3 wt), SASDB56 (deglycosylated MAG1–3 I473E), SASDB66 (deglycosylated MAG1–3 N406Q) and SASDB76 (deglycosylated MAG1–3 wt).

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Author Contributions
M.F.P. and B.J.C.J. designed the experiments. M.F.P. generated constructs, purified proteins and did all the structural biology (SAXS and X-ray diffraction). M.F.P. and D.M.E.T.-W. performed SE-AUC experiments and analysed the data. J.S. performed mass spectrometry experiments and analysed the data together with A.J.R.H.; S.L. performed neurite outgrowth experiments and analysed the data together with R.J.P.; B.J.C.J. supervised the project. M.F.P. and B.J.C.J. wrote the manuscript with input from all authors.

Additional information
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