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Computational Design of Peptides That Target Transmembrane Helices

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A variety of methods exist for the design or selection of antibodies and other proteins that recognize the water-soluble regions of proteins; however, companion methods for targeting transmembrane (TM) regions are not available. Here, we describe a method for the computational design of peptides that target TM helices in a sequence-specific manner. To illustrate the method, peptides were designed that specifically recognize the TM helices of two closely related integrins (αβ₂ and αβ₃) in micelles, bacterial membranes, and mammalian cells. These data show that sequence-specific recognition of helices in TM proteins can be achieved through optimization of the geometric complementarity of the target-host complex.

Transmembrane (TM) helices play essential roles in biological processes, including signal transduction, ion transmission, and membrane-protein folding. Computational (1–4) and genetic methods (5, 6) are available to engineer antibody-like molecules that target the water-soluble regions of proteins; however, companion methods to target the TM regions are lacking. The design of TM helices that specifically recognize membrane proteins would advance our understanding of sequence-specific recognition in membranes and simultaneously provide new approaches to modulate protein-protein interactions in membranes. Here we describe a computational approach, designated computed helical anti-membrane protein (CHAMP), to design peptides that specifically recognize the TM helices of natural proteins (7).

Computational design. The design of a CHAMP peptide requires the selection of a backbone geometry for the CHAMP peptide-target complex, followed by computational selection of the CHAMP peptide’s amino acid sequence with a side-chain repacking algorithm. To simplify the selection of the backbone geometry, we used the growing database of membrane-protein structures, rather than relying on idealized helical dimers. The majority of the TM helix-helix pairs in TM proteins of known structure fall into a handful of well-defined structural motifs with recognizable sequence signatures (8). Thus, it is possible to predict a preferred mode of interaction between a target TM helix and other TM helices from the amino acid sequence alone. Once a preferred mode of interaction has been identified, examples of helix pairs from proteins of known structure can be used as backbone conformations for the design of a desired CHAMP peptide. The next steps are (i) to thread the sequence of the targeted TM helix onto one of the two helices of the selected pair and (ii) to select the amino acid sequence of the CHAMP helix with a side-chain repacking algorithm (1–4, 9).

As a stringent test of the CHAMP method, we focused on the recognition of the TM domains of two closely related and extensively studied platelet integrins, αβ₂ and αβ₃, as convenient biologically important targets (10–12). Both the αβ₂ and αβ₃ TM helices contain a small-X₃ small-motif, in which G (13) or other small residues (A and S) are spaced four residues apart (14) (Fig. 1, B and C). This sequence motif is associated with a high propensity to interact in a tightly packed “parallel GASG right motif” (8) with a right-handed helical crossing angle of ~40°. Indeed, site-directed mutagenesis (15–17) and modeling studies (15, 17, 18) suggest that the αβ₃ TM helix binds the β₃ TM helix with this geometry.

Five template backbones were tested in the design of a CHAMP peptide directed against αβ₃, and 15 were tested for αβ₂. The sequences of αβ₃ and αβ₂ were threaded onto either of the two helices in each template, yielding two different CHAMP peptides per template. A sequence for the opposing CHAMP helix was then selected with a Monte Carlo repacking algorithm that considers different combinations of side chains in low-energy rotamers (1–4, 9) [see Supporting Online Material (SOM)]. The rotameric states of the side chains in the target were allowed to vary, as were both the sequence and rotamers of the CHAMP side chains. A simple energy function that is based on a linearly dampened Lennard-Jones potential and a membrane depth-dependent knowledge-based potential (19) was then used to select the desired residues. This knowledge-based potential assured that residues with high propensities to occupy the interfacial and fatty acyl region of the bilayer were selected at appropriate positions. The membrane-exposed residues of the CHAMP helix were then randomly selected with a 60% probability of assigning L and a 10% probability of assigning A, I, F, or V.

The TM domains of α and αβ₃ are highly homologous (Fig. 1, B and C), and they both have small-X₃ small-motifs. Because a number of other integrin TM helices also contain a small-X₃ small-motif, a specific CHAMP peptide must recognize not only this global feature of its target, but also more fine-grained differences in surface topography. In the computed complexes with the lowest energy, the CHAMP sequences designed against α and αβ₃ both have G-X₃-G sequences that create a shallow concavity that is important for recognizing the small-X₃-small sequence on the integrin helices. However, the surrounding sequences differ in response to differences in the sequences of the target (Fig. 1, B and C), thereby providing specificity.

A CHAMP peptide with extensive geometric complementarity to its target (Fig. 1D and fig. S1) was selected based on its energy score, the uniformity of packing of the side chains at the CHAMP-target interface, and the ease of synthesis. [Peptides with multiple strings of β-branched amino acids and sequences with particularly high predictions for amyloid (20) were avoided.] The templates for the CHAMP peptide-target complexes were taken from polytopic proteins with no functional or structural relation to integrins, the CHAMP peptide

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against αv (anti-αv) was taken from the glycerol-3-phosphate transporter (1PW4, residues A64 to A86 and A121 to A141) (27), and anti-αIIb was taken from the photosystem I reaction center (1JB0, residues L43 to L65 and L114 to L140) (22). The designed peptides and their TM targets (αIIb-TM and αv-TM) (see SOM) were synthesized with solubility-enhancing groups appended to the C and N termini to facilitate membrane insertion (23, 24). Control peptides, in which the GX3G motifs were mutated (anti-αIIbmut and anti-αvmut) or scrambled (anti-αIIbscr), were also prepared to study the specificity of the designed sequences (Fig. 1E).

**CHAMP binding in micelles.** We used fluorescence resonance energy transfer (FRET) to evaluate the association of anti-αIIb with αIIb-TM in micelles. The titration of 7-hydroxycoumarin–tagged anti-αIIb as a FRET donor, with fluorescein–tagged αIIb-TM as a FRET acceptor, resulted in the quenching of the coumarin emission and the appearance of the fluorescein emission, indicating that the two peptides interacted (Fig. 2A). The apparent dissociation constant (Kd) for the αIIb-TM–anti-αIIb interaction was computed to be 0.32 ± 0.05 μM. The corresponding Kd expressed as a mole fraction (peptide versus detergent) is 3.2 ± 0.5 × 10^-4, which is relatively tight when compared with the range of 10^-2 (for weak associations) to 3 × 10^-3 for very strong associations (25), measured for TM-peptide associations in a similar micellar environment. Titrations with control peptides showed the specificity of the interaction; there was negligible binding between αIIb-TM and either anti-αIIbmut or anti-αv-TM (Fig. 2B). Furthermore, titrations with anti-αv showed that this peptide specifically recognized αv-TM (Kd = 3 ± 0.3 × 10^-3 as a mole fraction), but not αIIb-TM or anti-αv-TM (Fig. 2C).

Analytical ultracentrifugation of anti-αIIb and αIIb-TM, as well as anti-αv and αv-TM in micelles, indicated that these peptides formed homodimers, as well as heterodimerizing with their respective targets (fig. S2 and table S2). Both CHAMP peptides heterodimerized with

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**Fig. 1. (A)** Design of CHAMP peptides. A backbone geometry was selected for the CHAMP-target complex; for αIIb, the template was taken from two interacting helices in a much larger protein, the photosystem I reaction center (22). The original sequence was stripped off of the template, and the helices were extended to span the full length of a membrane. The sequence of αIIb-TM was threaded onto the right helix. The 14 positions selected for repacking are pink on the left helix. The final anti-αIIb CHAMP peptide sequence is shown on the left helix with the repacked positions in pink. (**B** and **C**) Human integrin TM targets and TM helices from homologous human integrins used in specificity assays. The sequences (B) are modeled in an idealized helical conformation (C). Common small (G, A, and S) residues are highlighted in red, and a common L on the binding interface is shown in purple. (**D**) Close-up of the predicted tightly packed interface between anti-αIIb and αIIb-TM. αIIb-TM is represented by a red surface with a blue hot spot. The anti-αIIb backbone is depicted in ribbon representation, with key positions designated for computational design shown in green. (**E**) Sequences of CHAMP designs and the control peptides. The residues repacked in the anti-αIIb and anti-αv peptides are shown in pink. (Lys)2 (23) or polyethylene glycol (24) was appended to the C and N termini as solubility-enhancing groups.

**Fig. 2.** Affinity and selectivity of CHAMP peptides for their target TM helices. (**A**) Fluorescence emission scans of coumarin–labeled anti-αIIb (64 nM) in the presence of different concentrations of fluorescein isothiocyanate (FITC)–tagged αIIb-TM in 10 mM 4-(2-hydroxyethyl)–1-piperazineethanesulfonic acid (HEPES), pH= 7.5, containing 1.0 mM C14-betaine at 25°C. (Inset) Concentrations as mole fractions. λex was set at 415 nm to selectively excite the coumarin. λem, excitation wavelength. (**B**) Corrected FRET signals of 64 nM coumarin–labeled anti-αIIb (red), anti-αv (green), or anti-αvmut (blue) in the presence of different concentrations of FITC–tagged αIIb-TM (10 mM HEPES, pH= 7.5, containing 1.0 mM C14-betaine at 25°C). Error bars represent SD of the mean. (C) Corrected FRET signals of 64 nM coumarin–labeled anti-αv (green), anti-αIIb (red), or anti-αvmut (orange) in the presence of different concentrations of FITC–tagged αv-TM (10 mM HEPES, pH= 7.5, containing 1.0 mM C14-betaine at 25°C). Error bars represent SD of the mean.
their targets at least as strongly as they homodimerized. This behavior is reminiscent of dominant-negative (DN) inhibitors of the helix-loop-helix family of transcription regulators, which engage in both homomorphic and heteromorphic interactions (26).

**Dominant-negative TOXCAT.** We next evaluated both the affinity and specificity of the CHAMP peptides for their targets when coexpressed in bacterial membranes with a DN-“TOXCAT” assay (Fig. 3). In TOXCAT, a TM sequence of interest is fused to a ToxR protein (TM-ToxR) that binds to the ctx promoter as a dimer, which induces expression of chloramphenicol acetyltransferase (CAT) (27, 28). In the DN assay, the TM1-ToxR fusion protein is coexpressed with a second fusion protein (TM2-ToxR*) containing a nonfunctional mutant of the ToxR domain. TM-driven dimerization leads to a ToxR-ToxR* dimer that is unable to bind the ctx promoter or to induce CAT synthesis (Fig. 3A). The resulting decrease in CAT activity can be used to monitor the formation of a heterodimeric TM complex. In principle, this assay could be accomplished by adding an exogenous synthetic peptide (29); however, it is difficult to assure that different peptides will be similarly inserted into the *Escherichia coli* inner membrane.

The anti-αIIb and anti-αv peptides both formed homodimers in bacterial membranes (Fig. 3B) with an affinity similar to that of the TM domain of glycoporphin A (GpA), which forms tight homodimers in this environment (27, 28). The CAT signal for the anti-αIIb-ToxR construct was attenuated by coexpression of anti-αIIb–ToxR* (Fig. 3B), validating the DN assay. When αIIb was used as the DN partner, the signal from anti-αIIb–ToxR was also strongly attenuated, indicative of heterodimer formation. The magnitude of the decrease in CAT signal due to heterodimerization of anti-αIIb with αIIb TM is particularly notable. The homodimerization of anti-αIIb and GpA (27, 28) are similar in affinity (Fig. 3B, first versus third bar). Thus, because the attenuation of the CAT signal in the DN-TOXCAT assay for an anti-αIIb-anti-αIIb homodimer is similar to that of the anti-αIIb–αIIb TM heterodimer (Fig. 3B, fourth versus fifth bar), the heterodimeric TM complex anti-αIIb–αIIb has similarly strong affinity to that of the anti-αIIb homodimer and therefore also to the GpA homodimer.

The TOXCAT assay also shows that anti-αIIb and anti-αv are highly specific for their targets versus other integrin TM domains. The TM domains of α2, α5, β3, or β1 failed to significantly interact with anti-αIIb, despite their high sequence and structural similarity to the αIIb TM (Fig. 1, B and C). Similarly, anti-αv selectively recognized the αv TM domain with much greater affinity than the α2, αIIb, β1, or β3 domains.

To probe whether anti-αIIb recognized its target in the intended manner, we measured the effect of mutating residues in anti-αIIb TM to decrease in CAT activity can be used to monitor the formation of a heterodimeric TM complex. In principle, this assay could be accomplished by adding an exogenous synthetic peptide (29); however, it is difficult to assure that different peptides will be similarly inserted into the *Escherichia coli* inner membrane.

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To probe whether anti-αIIb recognized its target in the intended manner, we measured the effect of mutating residues in anti-αIIb TM to
either V or A (Fig. 3C). Mutations to the residues predicted to occur at the helix-helix interface caused disruption of heterodimer formation. Very large effects were observed for buried residues within a 10-residue stretch (residues 11 to 20) spanning the primary interaction site, whereas only minor effects were observed for residues on the non-interacting side of the helix or the more distal sites (residues 23 and 24). Interestingly, the interaction face resembles a “Gly-zipper” (GXGXG) motif that has recently been shown to mediate intermolecular helix-helix associations in membranes (30).

**Platelet aggregation and adhesion.** To determine whether exogenously added CHAMP peptides recognize their targets in mammalian cells, we first determined whether they were capable of inserting into phospholipid bilayers without perturbing the integrity of the membrane. The addition of anti-\( \alpha_v \), or anti-\( \alpha_{lb} \) to phospholipid vesicles led to a large blue shift in the peptide’s W fluorescence spectrum, indicative of insertion into the hydrophobic region of bilayers (fig. S3). Furthermore, circular dichroism spectroscopy showed that the vesicle-bound peptides were helical (fig. S4), and attenuated total reflectance–infrared spectroscopy revealed that they adopted a transmembrane orientation, with their helix perpendicular to the bilayer surface (fig. S5). Moreover, anti-\( \alpha_{lb} \) and anti-\( \alpha_v \) did not lyse human erythrocyte membranes at the concentrations that were used in the following assays (fig. S6).

Pharmacological studies indicated that the CHAMP peptides interact with their target integrins in mammalian cells. The \( \alpha_{lb} \beta_3 \) and \( \alpha_v \beta_3 \) integrins are heterodimers whose \( \alpha \) and \( \beta \) subunits are composed of a large extracellular domain, a TM helix, and a short cytoplasmic domain. In the resting state, the TM helices of their \( \alpha \) and \( \beta \) subunits interact (15–18), whereas they separate when the integrins are activated by mutations or after treatment of cells with pharmacological agonists (15–18). Thus, the binding of a CHAMP peptide to the appropriate site on the TM helix of \( \alpha_{lb} \) or \( \alpha_v \) should disrupt dimerization with the \( \beta_3 \) TM helix, thereby shifting the conformational equilibrium of the integrin toward its activated state (Fig. 4A).

In platelets, \( \alpha_{lb} \beta_3 \) is in several-hundred-fold excess over \( \alpha_v \beta_3 \). Thus, a successful anti-\( \alpha_v \) peptide must recognize its target in the presence of a large excess of a closely related integrin, and the anti-\( \alpha_{lb} \) peptide must bind its target without affecting a minor population of \( \alpha_v \beta_3 \).

The physiological role of \( \alpha_{lb} \beta_3 \) is to induce platelet aggregation, which occurs through an interaction with its divalent ligand, the plasma protein fibrinogen. Anti-\( \alpha_{lb} \) rapidly induced platelet aggregation (Fig. 4B) in a dose-dependent manner when added at concentrations from 75 nM to 500 nM (fig. S7). Anti-\( \alpha_v \)–induced aggregation was only minimally affected by the platelet inhibitor prostaglandin E1 (PGE1) and by the adenosine 5′-diphosphate (ADP) scavenger apyrase, which indicated that it was independent of platelet signal transduction or secreted ADP. Furthermore, anti-\( \alpha_{lb} \)–induced aggregation was inhibited by agents that inhibit the function of \( \alpha_{lb} \beta_3 \)’s fibrinogen-binding site (Fig. 4B), including EDTA or the peptide RGDS (10).

In contrast, no platelet aggregation occurred when the platelets were exposed to 10 μM anti-\( \alpha_v \), which demonstrated that this peptide did not activate \( \alpha_{lb} \beta_3 \).

The integrin \( \alpha_v \beta_3 \) mediates the adhesion of platelets to the matrix protein osteopontin, potentially exposed to the circulating blood by rupture of an atherosclerotic plaque. Incubating platelets with anti-\( \alpha_v \) induced robust platelet adhesion to osteopontin (Fig. 4C), even in the absence of pharmacological platelet agonists. As expected from the FRET binding curves (Fig. 2C), the potency of anti-\( \alpha_v \) in inducing platelet adhesion to osteopontin was lower by a factor of 10 than that of anti-\( \alpha_{lb} \) in inducing platelet aggregation (fig. S9). Anti-\( \alpha_v \)–induced adhesion was prevented by agents that inhibit the interaction of \( \alpha_v \beta_3 \)’s extracellular ligand-binding site with osteopontin, including EDTA or the specific RGD-containing \( \alpha_v \beta_3 \) antagonist XJ735, which confirmed that anti-\( \alpha_v \)–induced platelet adhesion to osteopontin is mediated by \( \alpha_v \beta_3 \). Notably, there was no platelet adhesion to osteopontin when platelets were incubated with anti-\( \alpha_{lb} \) at concentrations that fully activate \( \alpha_{lb} \beta_3 \) (Fig. 4C), and anti-\( \alpha_{lb} \)mut and anti-\( \alpha_v \)mut had negligible effects in activating either integrin. Thus, these experiments indicate that anti-\( \alpha_v \) can specifically recognize and activate \( \alpha_v \beta_3 \) in the presence of a 400-fold excess of \( \alpha_{lb} \beta_3 \).

**Rupture force spectroscopy.** One potential ambiguity with the platelet experiments is that membrane peptides are intrinsically sticky, which could lead to nonspecific interactions that might cloud the interpretation of the results. Furthermore, the TM helices of integrins might engage in a number of homomeric interactions or heteromeric interactions with other membrane proteins (15–18), which may cause avidity effects relating to clustering and multivalent binding. Therefore, to measure the interaction at the single-molecule level, we used laser tweezers–based force spectroscopy to evaluate the activity and selectivity of anti-\( \alpha_{lb} \) and anti-\( \alpha_v \).
in platelets. The spectrum of rupture forces required to detach an osteopontin- or fibrinogen-coated bead from a platelet provides a measure of the ability of the CHAMP peptides to activate their target integrin and of the integrin to interact with its appropriate ligand. Histograms of rupture forces collected for a large number of contacts between platelets and ligand-coated beads are shown in Fig. 5. In the absence of CHAMP peptides, the binding probability decreased exponentially with increasing force, indicative of a nonspecific interaction (31). However, in the presence of 0.5 μM anti-αIIb (Fig. 5A), a peak is observed at a position similar to that observed when αIIbβ3 is stimulated by platelet agonists (31). The peak was eliminated by addition of the αIIbβ3-specific antibody, abciximab, which blocks the fibrinogen-binding site on the extracellular domain of αIIbβ3. Thus, the highly adhesive interaction between platelets and fibrinogen is mediated by activated αIIbβ3.

Exposing platelets to 10 μM anti-αv, produced a peak of rupture force between platelets and osteopontin-coated beads with a maximum at ~45 pN (Fig. 5B), characteristic of the interaction of αvβ3, and osteopontin-coated beads (32). This anti-αv-induced activation was eliminated by the αvβ3 antagonist XI735. There was no peak of specific rupture force between the platelets and the osteopontin-coated beads when the platelets were exposed to concentrations of anti-αv that fully activate αIIbβ3 (Fig. 5B, bottom panel). Demonstrating the specificity of the interaction, anti-αv failed to induce adhesion to fibrinogen-coated beads (Fig. 5A, bottom panel), and both anti-αIIbsc (Fig. 5A) and anti-αvmut (Fig. 5B) had negligible effects on the activation of either integrin. Thus, by activating specific integrin functions, these experiments confirm that anti-αIIb and anti-αv can specifically interact with αIIb and αv in situ in the plasma membrane of platelets.

Discussion. Lateral TM helix-helix associations play essential roles in membrane-protein folding, assembly, and signal transduction (33); what defines the specificity for their assembly (8)? For TM motifs similar to those studied here, there is only a 3 to 5 kcal/mol energetic difference (a factor of about 100 to 10,000) between a strongly associating TM helix dimer, such as GpA, and the nonspecific dimerization of randomly associating TM helices in micelles (25). For this class of helix-helix interaction motifs, nature appears to have modulated this energetic difference by (i) optimizing the geometric fit (34–36), electrostatic interactions, and weak C–H···O=C hydrogen bonds (37) between the two interacting helices and (ii) positioning the interacting sites on the two helices at precisely the same region of the bilayer. Similar specificity can now be engineered from first principles.

Given that there is only a small energetic difference between randomly associating TM helices and natural high-affinity dimers, it might appear that the design of a CHAMP peptide would require highly accurate computation of the enthalpy and entropy of peptide-peptide and peptide-phospholipid interactions. These calculations would require consideration of computationally challenging interactions, such as interhelical C–H···O=C hydrogen bonds (37) and local deviations from ideal helical geometry required to facilitate association of the helices. We largely circumvent the need for such accuracy by using a library of structurally defined helix pairs that are already in local minima with respect to interhelical backbone-backbone interactions and that position side chains for appropriate pairwise interactions. The problem then simplifies to selecting the best backbone from this library for the construction of a CHAMP peptide that maximizes the geometric complementarity between the CHAMP peptide sequence and its target. Although our scoring function for sequence selection is quite simple and we used only a subset of the available helix pairs, the procedure appears to be highly effective. The first two peptides designed with this protocol are described in this paper, whereas a third is described in the SOM. On the basis of this very limited sampling of three peptides, the method has been 100% successful.

The CHAMP design procedure is highly sensitive to the fine-grained topographic differences between the targets, which is consistent with the hypothesis that geometric complementarity is critical for tight and specific recognition. Although the sequences of the TM helices of αIIb and αv are similar, the sequences of anti-αIIb and anti-αv differ substantially, resulting in different selectivities. For example, anti-αIIb has a Gly-zipper motif (30), which is essential for the recognition of αIIb, whereas anti-αv has an M rather than the third G in this motif.

It should be possible to extend the CHAMP approach to other TM helix-association motifs, including ones involving polar side chains, which provide a strong driving force for helix-helix association (33). In this case, we would search for a helix pair library with structures in which one helix has the polar side chain(s) of interest and the second helix has appropriate side chains to bind it (38). Furthermore, the method could be modified to consider multispan bundles, rather than dimers, or to use “negative” design to select for sequences that avoid undesirable interactions; it should be possible to include a scoring function to avoid sequences that are particularly prone to amyloid formation (20), or one could computationally screen target CHAMP sequences against undesired targets to minimize off-target binding.

The activity of these CHAMP peptides is consistent with a push-pull mechanism of integrin activation, which postulates that separation of the α and β TM helices is a dominant signal for integrin activation (15, 17, 39). In this mechanism, integrins are activated by any perturbation that physically pushes the integrin TM helices apart (e.g., mutations that disrupt the α-β TM interface) or that pulls them apart via binding interactions that preferentially stabilize the separated state: CHAMP peptides activate by binding to a site in the α TM helix that physically blocks its interaction with the β TM helix, the binding of talin to the membrane-proximal cytoplasmic domain of the β subunit appears to impede its interactions with the α subunit (40), and the homo-oligomerization of the TM domains might activate by competing for heteromeric TM helix-helix associations (17).

More generally, CHAMP peptides should provide important reagents to probe the functional consequences of blocking protein-protein interactions in membranes, in a manner similar to the use of antibodies to study water-soluble regions of proteins. Previous investigators have shown that peptides from the TM regions of oligomeric proteins can disrupt the lateral assembly of the native complex (41–43). However, high concentrations of these TM peptides were required to elicit partial effects. Similarly, we have found that peptides from the TM regions of αIIb and β3 are substantially weaker activators of αIIbβ3 activation than anti-αIIb (44). It is likely that the affinity and specificity of the CHAMP peptides designed in this work could be further improved by genetic methods used previously for investigating membrane-peptide recognition (45), in a process similar to affinity maturation of antibodies. Given the growing appreciation of lateral TM helix associations in membrane-protein folding, assembly, and signal transduction (33), CHAMP peptides will provide much needed reagents for probing these processes.

References and Notes
7. Materials and methods are available as supporting material on Science Online.
13. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
Early Optical Polarization of a Gamma-Ray Burst Afterglow

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We report the optical polarization of a gamma-ray burst (GRB) afterglow, obtained 203 seconds after the initial burst of γ-rays from GRB 060418, using a ring polarimeter on the robotic Liverpool Telescope. Our robust (2σ) upper limit on the percentage of polarization, less than 8%, coincides with the fireball deceleration time at the onset of the afterglow. The combination of the rate of decay of the optical brightness and the low polarization at this critical time constrains standard models of GRB ejecta, ruling out the presence of a large-scale ordered magnetic field in the emitting region.

Gamma-ray bursts are the most instantaneous, powerful explosions in the universe and represent the most important new astrophysical phenomenon since the discovery of quasars and pulsars. Identified as the primary source of x-ray flashes on the sky, the most common type are the gamma-ray bursts (GRBs), which first became apparent in the late 1960s as x-ray flashes. In many cases, the detected x-ray flux implies an unphysically high explosion energy if assumed to be emitted isotropically by the source, the so-called energy catastrophe. Instead, focusing the energy into a narrow jet reduces the intrinsic energy output to a canonical value of ~1051 erg for most GRBs (3).

At the initial burst of γ-rays, the subsequent radiation produced at longer wavelengths (e.g., x-ray, optical, or radio), termed the “afterglow,” is generally accepted to be synchrotron radiation whose observed properties are consistent with a focused jet expanding at ultrarelativistic speeds into the interstellar medium. The production of synchrotron radiation requires the presence of a magnetic field, but the origin and role of the magnetic fields in GRB ejecta are a long-standing open issue. In turn, fundamental questions on the driving mechanism of the explosion, in particular, whether the relativistic outflow is dominated by kinetic (baryonic) or magnetic (Poynting flux) energy, remain unanswered (4, 5). The primary challenges in addressing these issues arise because GRBs are short-lived, compact, and lie at vast cosmological distances; our understanding of their physical nature is therefore inferred from the characteristics of their radiation, measured at the earliest possible time when the observed radiation is still sensitive to the properties of the original fireball.

The two main models of collimated relativistic outflows, or jets, that have been proposed are the hydrodynamical and the magnetized jet (5). Hydrodynamical jets have no dominant ordered magnetic field but instead produce synchrotron radiation from tangled magnetic fields, concentrated in the thin layer of the expanding shock front, that are generated locally by instabilities in the shock (6); the magnetic field does not influence the subsequent evolution of the jet. Models of these jets have been highly successful at reproducing a wide range of observed properties of GRBs (1, 2). A relativistic outflow from a central engine might have a weakly ordered or random magnetic field. As long as the magnetic field does not affect the dynamics of the jet, we classify it as a hydrodynamical jet. In contrast, magnetized jets are threaded with strong, globally ordered magnetic fields, which originate at the central source, are advected outward with the expanding flow, and may provide a powerful mechanism for collimating and accelerating the relativistic jet (7, 8). A magnetic driving mechanism is an attractive scenario to account for the prodigious energy outputs and vast accelerations required for GRB ejecta, as well as for overcoming energy-efficiency problems inherent in hydrodynamical models in which internal shocks must convert kinetic energy to radiative energy with sufficient efficiency to produce the observed γ-ray emission and prolonged central engine activity (9, 10).

Observationally, the fading rate of the afterglow emission alone is inadequate as a diagnostic for distinguishing between these theoretical jet models (11–13); in contrast, the polarization

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Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5820/1817/DC1

Materials and Methods

Figs. S1 to S9

Tables S1 and S2

References

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