JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Computational Design of a β-Peptide That Targets Transmembrane Helices

Scott J. Shandler,[†] Ivan V. Korendovych,[†] David T. Moore,[†,‡] Kathryn B. Smith-Dupont,[†] Craig N. Streu,[‡] Rustem I. Litvinov,[‡] Paul C. Billings,[†] Feng Gai,[§] Joel S. Bennett,[‡] and William F. DeGrado*[,†,§]

[†]Department of Biochemistry and Biophysics, [‡]Department of Hematology, and [§]Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

Supporting Information

ABSTRACT: The design of β-peptide foldamers targeting the transmembrane (TM) domains of complex natural membrane proteins has been a formidable challenge. A series of β-peptides was designed to stably insert in TM orientations in phospholipid bilayers. Their secondary structures and orientation in the phospholipid bilayer was characterized using biophysical methods. Computational methods were then devised to design a β-peptide that targeted a TM helix of the integrin $\alpha_{IIb}\beta_3$. The designed peptide (***β*-CHAMP**) interacts with the isolated target TM domain of the protein and activates the intact integrin in vitro.

The discovery of the β-peptide class of foldamers[1−6] has resulted in the development of β- and mixed α,β-peptides capable of binding specific biological targets.[2,7,8] Considerable progress has been made in the design of β-peptides that compete for water-soluble protein−protein interactions or bind to the surface of membranes.[2,7−11] However, the design of β-peptide foldamers that target the transmembrane (TM) domains of complex natural membrane proteins has been a formidable challenge, which is addressed in the present paper. Our first objective was to design β-peptides that stably insert in TM orientations in bilayers and to adapt biophysical methods to demonstrate their orientations. Next, computational methods were devised to design a β-peptide that targeted the α_{IIb} TM helix of the integrin $\alpha_{IIb}\beta_3$. The designed peptide indeed interacts with the isolated target TM domain of the protein and activates the intact integrin in vitro.

We chose the well-characterized platelet integrin $\alpha_{IIb}\beta_3$ as a model system for our design.[12] The α_{IIb} and β_3 TM domains associate in the resting state of the integrin, but this interaction is disrupted when the integrin is activated to bind to its extracellular ligand, fibrinogen. Thus, disruption of the $\alpha_{IIb}-\beta_3$ TM helical interaction, either by introducing disruptive mutations[13−16] or by addition of α-peptides that compete for this interaction,[17−20] leads to integrin activation. Our goal here was to design a β-peptide that binds specifically to the α_{IIb} TM domain. This endeavor not only tests and extends our fundamental understanding of the mechanism of TM helix−helix interactions but also takes an important step toward the goal of designing non-natural molecules that specifically target TM regions of proteins in a sequence-specific manner.

We previously developed the computed helical antimembrane protein (CHAMP) method to enable the computational design of α-peptides that target TM helices of natural proteins.[18] The original CHAMP method uses the crystallographic database of membrane proteins to define interacting TM helical pairs whose backbones are compatible with the target sequence and that serve as starting points for a side-chain repacking algorithm. As there are no experimental structures of TM β-peptides, let alone TM β-peptides interacting with α-peptides, it was necessary to discover an optimal pose of the β-peptide backbone onto the α-peptide and design a sequence of the β-peptide that stably interacts with the α-peptide in this pose. We began with the previous model of a complex of the α_{IIb} with a successfully designed CHAMP peptide to guide the selection of the ***β*-CHAMP** sequence. In our previous work,[18] the CHAMP peptide targeted a GXXXG motif in the α_{IIb} sequence. The exposed backbone atoms of the Gly residues in this motif are recognized by a similar motif in the CHAMP peptide, resulting in interhelical backbone−backbone interactions that help drive helix association in membranes. Therefore, we positioned a β-peptide poly-(homo-Gly) [poly(hGly)] helix against the GXXXG of the α_{IIb} helix, using a grid search and the CHARM force field to determine the optimal position of the β-helix against the α-helix.

There are two common helix types for β-amino acids: while the 14-helix is the most stable one for β^3-substituted amino acids in polar solvents, preliminary studies showed the coexistence of a 12-helix conformation.[21] Thus, both were considered in the design of the CHAMP peptide. In each case, multiple poses of the poly(hGly) backbone were exhaustively sampled to discover the optimal backbone orientation. The sequence of the β-peptide was then outfitted with side chains using a packing algorithm that evaluates various side chains in low-energy rotamers to obtain combinations that optimize the geometric complementarity between the β-peptide and the α-helical target.[22] The β-peptide positions that directly contacted the main chain could not sterically accommodate a side chain and hence remained hGly. The design was completed by inclusion of Trp and Lys residues near the termini to orient the peptide in the bilayer properly. The initial design focused on the 14-helix, and the backbone docking restraints induced an $(hGly\text{-}X\text{-}X)_3$ motif with the hGly residues lined along one face of the helix (Figure 1). Somewhat surprisingly, this same motif shows a good fit for the 12-helix; the major difference is that the hGly residues line up along the helix in the threefold screw of the 14-helix but spiral around the helix in the 12-helix. This difference leads to different predicted packing angles in the complex.

Received: May 7, 2011
Published: July 22, 2011

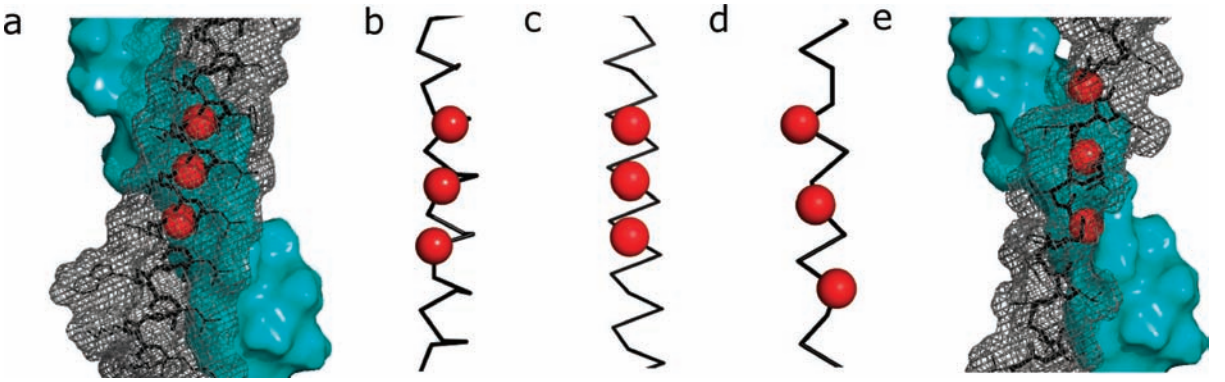

Figure 1. Design of ***β*-CHAMP**. (a) Docking of a CHAMP peptide on the α_{IIb} TM helix (cyan). The α-carbons of Gly residues are shown as red spheres.[18] (b–d) Positions of the interacting motif on (b) an α-helix (GX_3GX_3G), (c) a 14-helix (GX_2GX_2G), and (d) a 12-helix (GX_2GX_2G). (e) Final sequence of ***β*-CHAMP** modeled as a 12-helix. C_3 atoms of the GX_2GX_2G motif are shown as red spheres.

Table 1. Sequences of the Designed *β*-CHAMP Peptides[a]

β-CHAMP	KKKVLWVLVGLLGLIGFIVVLVVKKK
β-CHAMP G14I	KKKVLWVLVGLL*I*LIGFIVVLVVKKK
β-CHAMPscr	KKKVVVIVGIVLVFLGLVLWLGLKKK

[a] One-letter codes refer to the corresponding β^3-amino acids.

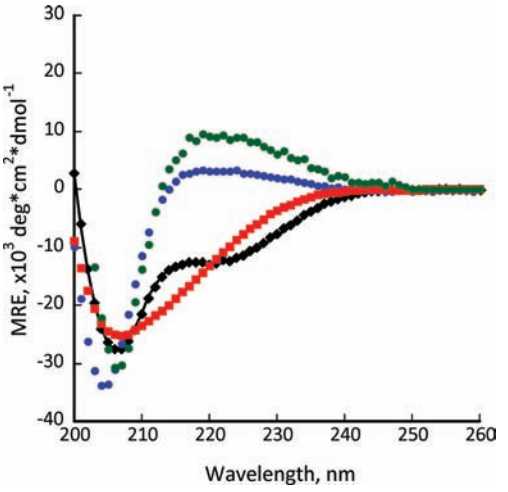

Figure 2. CD spectra of ***β*-CHAMP** in DPC micelles (blue ●; 40 μM peptide, 10 mM DPC, 10 mM phosphate buffer, pH 7.4), 7:3 POPC/POPG vesicles (green ●; 167 μM total lipid, 1:100 peptide:lipid ratio), and TFE (red ■; 40 μM peptide) and in the presence of 1 equiv of α_{IIb} TM peptide (black ◆; DPC micelles, 40 μM total peptide). The black solid curve represents a linear combination of the CD spectra of the α_{IIb} TM peptide and the 12-helix form of ***β*-CHAMP** in equal proportions.

To test the designed binding mode, we prepared two control peptides, ***β*-CHAMP G14I** and ***β*-CHAMPscr**, by disrupting the GXXGXXG motif via mutation of the central β^3-homoglycine (hG14) to β^3-homoisoleucine and by scrambling the ***β*-CHAMP** sequence, respectively. All of the β-peptides were synthesized using optimized microwave-assisted solid-phase peptide synthesis techniques as outlined in the Supporting Information (SI); the TM α-peptide was synthesized using a previously established

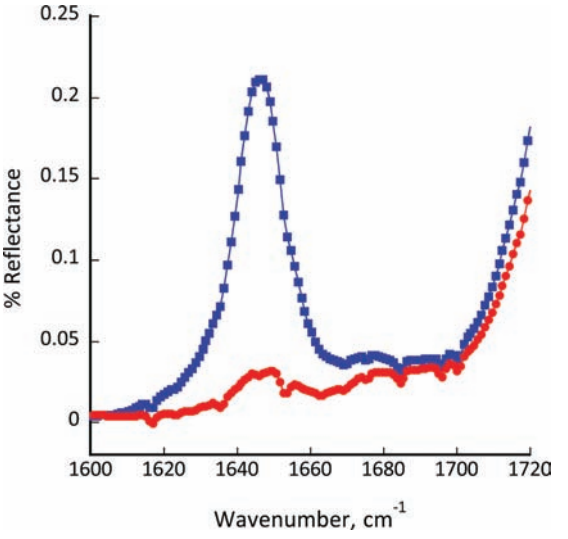

Figure 3. FT-IR/ATR spectrum of ***β*-CHAMP** in 7:3 POPC/POPG lipid membranes (1:80 peptide:lipid ratio). The blue and red traces represent light polarized parallel and perpendicular, respectively, to the plane of incidence.

protocol.[23] The final sequences are shown in Table 1. Our protocols allowed us to consistently obtain high-purity products in high yield.

The circular dichroism (CD) spectrum of ***β*-CHAMP** was measured in trifluoroethanol (TFE), dodecylphosphatidylcholine (DPC) micelles, and phospholipid vesicles composed of a 7:3 mixture of palmitoyl oleoyl phosphatidylcholine (POPC) and palmitoyl oleoyl phosphatidylglycerol (POPG) (Figure 2). In the DPC micelles and phospholipid vesicles, there were minima at 204 and 206 nm, respectively, and a maximum at ~220 nm, indicative of the 12-helix structure. The CD spectrum of ***β*-CHAMP** in TFE suggested an equilibrium between the 12- and 14-helix structures, consistent with increasing destabilization of the 12-helix with increasing solvent polarity.[21,24] The spectra of the control ***β*-CHAMP** peptides followed the same pattern, showing higher 14-helix content in TFE.[21] Also, when ***β*-CHAMP** was mixed with the α_{IIb} TM peptide under conditions wherein they formed a 1:1 complex (Figure 2), the spectrum was well-described as a mixture of those for the TM α-helix and the

dx.doi.org/10.1021/ja204215f |*J. Am. Chem. Soc.* 2011, 133, 12378–12381

Table 2. Association of DNP-Labeled β-Peptides with the α_{IIb} TM Peptide in DPC Micelles As Measured by AUC

peptide	monomer MW (Da)	MW_{obs} (Da) without α_{IIb} TM peptide	MW_{obs} (Da) with α_{IIb} TM peptide
β-CHAMP	3420.56	4380 ± 61	6737 ± 71
β-CHAMP G14I	3476.67	3542 ± 172	3905 ± 106
β-CHAMPscr	3445.81	3446 ± 147	3542 ± 172

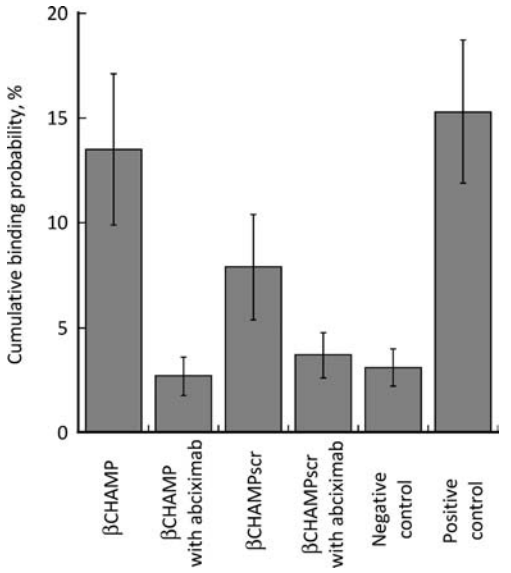

Figure 4. Rupture force spectroscopy data on the interaction of isolated surface-bound $\alpha_{IIb}\beta_3$ and fibrinogen in the presence of the **β-CHAMP** peptides (5 μM). The negative control represents TFE and the positive control 2 mM Mn^{2+}. The difference between the β-**CHAMP** data (column 1) and the β-**CHAMPscr** data (column 3) is statistically significant ($p = 0.008$).

12-helix form of the β-peptide, strongly suggesting that **β-CHAMP** binds its target in a 12-helix conformation.

To determine the orientation of the **β-CHAMP** peptides in phospholipid bilayers, they were examined in hydrated multilayers by attenuated total internal reflectance (ATR) IR spectroscopy (Figure 3 and Figure S2 in the SI). The order parameter of the amide I′ band (1646 cm^{-1}) of **β-CHAMP** was 0.83, indicative of the helix being aligned close to the membrane normal.[25−28]

The binding of the **β-CHAMP** peptides to their target α_{IIb} TM protein was studied using analytical ultracentrifugation (AUC) in density-matched DPC micelles. In order to exclude the contribution of α_{IIb} TM peptide absorbance, we labeled the N-termini of the β-peptides with a 2,4-dinitrophenyl (DNP) group. Analysis of the sedimentation equilibrium profile at 356 nm allowed us to monitor the radial distribution of the β-peptide, which is sensitive to its association with the intended target. In the absence of the α_{IIb} TM peptide, the designed **β-CHAMP** and control peptides sedimented with an apparent molecular weight close to that expected for the corresponding monomers. A small but significant increase in the molecular weight was observed for the **β-CHAMP**, suggesting a small degree of homodimerization that was likely due to the GXXGXXG motif, as neither **β-CHAMP G14I** nor **β-CHAMPscr** showed

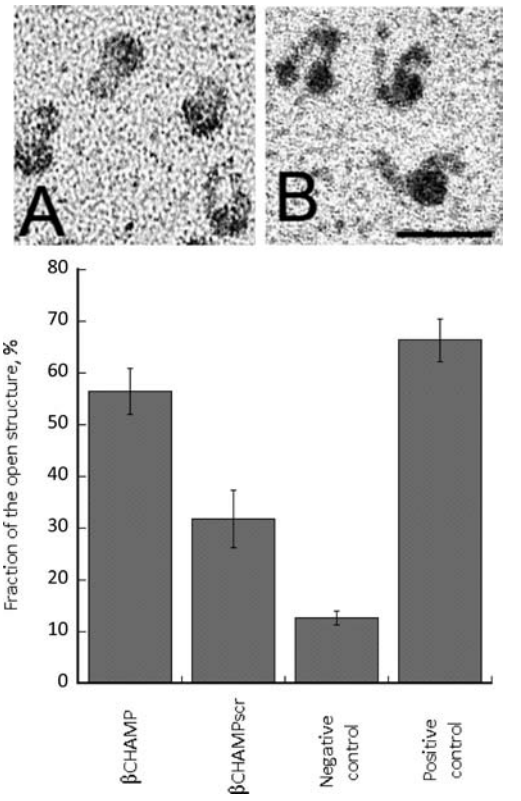

Figure 5. TEM images of purified $\alpha_{IIb}\beta_3$ in the (A) "closed" and (B) "open" forms, corresponding to inactive and active conformations, respectively. Scale bar = 30 nm. The bar graph shows the fraction of the open structure in the presence of the **β-CHAMP** peptides (5 μM). The negative control represents TFE and the positive control 2 mM Mn^{2+}.

any evidence of self-association. In the presence of the α_{IIb} TM peptide, **β-CHAMP** sedimented with a molecular weight consistent with a 1:1 complex with the target, while **β-CHAMP G14I** and **β-CHAMPscr** displayed no association with the α_{IIb} TM peptide (Table 2 and Figures S3−S8).

Having established the interaction of **β-CHAMP** with the α_{IIb} TM domain, we explored whether **β-CHAMP** can disrupt the interaction of the α_{IIb} and β_3 TM domains to enable the activated $\alpha_{IIb}\beta_3$ to bind fibrinogen. Activation of $\alpha_{IIb}\beta_3$ was monitored by optical-trap-based single-molecule rupture force spectroscopy. Full-length $\alpha_{IIb}\beta_3$ was isolated from human platelets as described in Supporting Information, and the rupture forces between the surface-bound $\alpha_{IIb}\beta_3$ and fibrinogen were measured after incubation of the integrin with **β-CHAMP** or **β-CHAMPscr**. The β-peptides were flanked with poly(ethylene glycol) groups on the N-terminus to enhance their solubility. The cumulative probabilities of $\alpha_{IIb}\beta_3$ binding to fibrinogen in the presence of β-peptides are shown in Figure 4. In order to assess nonspecific background binding, the experiments were also performed in the presence of the $\alpha_{IIb}\beta_3$-specific Fab antibody fragment abciximab, which prevents the interaction of fibrinogen and the integrin. **β-CHAMP** almost completely activated $\alpha_{IIb}\beta_3$ in the absence of the antibody fragment and had essentially no activity in the presence of abciximab. The integrin-activating effect of the control peptide **β-CHAMPscr** was substantially lower. Mn^{2+}, which was previously shown to activate $\alpha_{IIb}\beta_3$,[29] was used as a positive control, and solvent (TFE) was used as a negative control. The rupture force spectroscopy results were also confirmed by

transmission electron microscopy (TEM) data (Figure 5) that clearly showed the opening of the $\alpha_{IIb}\beta_3$ structure (a morphological equivalent of activation) as a result of the interaction of an α_{IIb} TM helix with ***β*-CHAMP**. These results showing strong and selective interaction of ***β*-CHAMP** with the α_{IIb} TM domain are in good agreement with the AUC data.

In conclusion, these studies indicate the feasibility of designing β-peptides to target TM helices of natural proteins. This requires the design of a β-peptide that spans the bilayer, which in the present case was accomplished by inclusion of a block of apolar residues sufficiently long to span the bilayer as well as Trp and Lys residues near the headgroup regions of the bilayer. The TM orientation was established using polarized IR spectroscopy. Specific interactions with the α_{IIb} TM peptide were computationally designed by (1) placing Gly residues at regular spacings along one face of the 12-helix, (2) optimizing the interaction of the 12-helix with the α_{IIb} TM peptide, and (3) optimizing the placement of other side chains in ***β*-CHAMP** to allow favorable van der Waals contacts with the α-helical target. The interaction was shown to be specific using variants of the ***β*-CHAMP** sequence. These studies provide a well-defined and automated approach to the design of β-peptides that recognize membrane targets.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Details of the computational design, preparation, and experimental (AUC, CD, FT-IR) characterization of the peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
wdegrado@mail.med.upenn.edu

■ ACKNOWLEDGMENT

The authors thank Chandrasekaran Nagaswami for technical assistance with TEM. This work was supported by the NIH (Grants GM54616, GM60610, and HL40387) and NSF MRSEC.

■ REFERENCES

(1) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.
(2) Kritzer, J. A.; Stephens, O. M.; Guarracino, D. A.; Reznik, S. K.; Schepartz, A. *Bioorg. Med. Chem.* **2005**, *13*, 11.
(3) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366.
(4) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252.
(5) Lee, E. F.; Sadowsky, J. D.; Smith, B. J.; Czabotar, P. E.; Peterson-Kaufman, K. J.; Colman, P. M.; Gellman, S. H.; Fairlie, W. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 4318.
(6) Di Blasio, B.; Pavone, V.; Lombardi, A.; Pedone, C.; Benedetti, E. *Biopolymers* **1993**, *33*, 1037.
(7) Sadowsky, J. D.; Fairlie, W. D.; Hadley, E. B.; Lee, H. S.; Umezawa, N.; Nikolovska-Coleska, Z.; Wang, S. M.; Huang, D. C. S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 139.
(8) Stephens, O. M.; Kim, S.; Welch, B. D.; Hodsdon, M. E.; Kay, M. S.; Schepartz, A. *J. Am. Chem. Soc.* **2005**, *127*, 13126.
(9) Sadowsky, J. D.; Schmitt, M. A.; Lee, H. S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966.
(10) Bautista, A. D.; Appelbaum, J. S.; Craig, C. J.; Michel, J.; Schepartz, A. *J. Am. Chem. Soc.* **2010**, *132*, 2904.
(11) Imamura, Y.; Watanabe, N.; Umezawa, N.; Iwatsubo, T.; Kato, N.; Tomita, T.; Higuchi, T. *J. Am. Chem. Soc.* **2009**, *131*, 7353.
(12) Bennett, J. S. *J. Clin. Invest.* **2005**, *115*, 3363.
(13) Zhu, H.; Metcalf, D. G.; Streu, C. N.; Billings, P. C.; DeGrado, W. F.; Bennett, J. S. *J. Mol. Biol.* **2010**, *401*, 882.
(14) Luo, B. H.; Takagi, J.; Springer, T. A. *J. Biol. Chem.* **2004**, *279*, 10215.
(15) Litvinov, R. I.; Vilaire, G.; Li, W.; DeGrado, W. F.; Weisel, J. W.; Bennett, J. S. *Biochemistry* **2006**, *45*, 4957.
(16) Partridge, A. W.; Liu, S.; Kim, S.; Bowie, J. U.; Ginsberg, M. H. *J. Biol. Chem.* **2005**, *280*, 7294.
(17) Yin, H.; Litvinov, R. I.; Vilaire, G.; Zhu, H.; Li, W.; Caputo, G. A.; Moore, D. T.; Lear, J. D.; Weisel, J. W.; DeGrado, W. F.; Bennett, J. S. *J. Biol. Chem.* **2006**, *281*, 36732.
(18) Yin, H.; Slusky, J. S.; Berger, B. W.; Walters, R. S.; Vilaire, G.; Litvinov, R. I.; Lear, J. D.; Caputo, G. A.; Bennett, J. S.; DeGrado, W. F. *Science* **2007**, *315*, 1817.
(19) Caputo, G. A.; Litvinov, R. I.; Li, W.; Bennett, J. S.; DeGrado, W. F.; Yin, H. *Biochemistry* **2008**, *47*, 8600.
(20) Tang, J.; Yin, H.; Qiu, J.; Tucker, M. J.; DeGrado, W. F.; Gai, F. *J. Am. Chem. Soc.* **2009**, *131*, 3816.
(21) Korendovych, I. V.; Shandler, S. J.; Montalvo, G. L.; DeGrado, W. F. *Org. Lett.* **2011**, *13*, 3474.
(22) Korendovych, I. V.; Senes, A.; Kim, Y. H.; Lear, J. D.; Fry, H. C.; Therien, M. J.; Blasie, J. K.; Walker, F. A.; DeGrado, W. F. *J. Am. Chem. Soc.* **2010**, *132*, 15516.
(23) Rathore, N.; Gellman, S. H.; De Pablo, J. J. *Biophys. J.* **2006**, *91*, 3425.
(24) Korendovych, I. V.; Kim, Y. H.; Ryan, A. H.; Lear, J. D.; DeGrado, W. F.; Shandler, S. J. *Org. Lett.* **2010**, *12*, 5142.
(25) Tucker, M. J.; Getahun, J.; Nanda, V.; DeGrado, W. F.; Gai, F. *J. Am. Chem. Soc.* **2004**, *126*, 5078.
(26) Goormaghtigh, E.; Raussens, V.; Ruysschaert, J. *Biochim. Biophys. Acta* **1999**, *1422*, 105.
(27) Axelsen, P. H.; Braddock, W. D.; Brockman, H. L.; Jones, C. M.; Dluhy, R. A.; Kaufman, B. K.; Puga, F. J. *Appl. Spectrosc.* **1995**, *49*, 526.
(28) Axelsen, P. H.; Citra, M. J. *Prog. Biophys. Mol. Biol.* **1996**, *66*, 227.
(29) Litvinov, R. I.; Nagaswami, C.; Vilaire, G.; Shuman, H.; Bennett, J. S.; Weisel, J. W. *Blood* **2004**, *104*, 3979.