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Computational design of a β–peptide that targets transmembrane helices

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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 that 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 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 have chosen the well characterized platelet integrin $\alpha_{IIb}\beta_3$ as a model system for our design.[12] The α_{IIb} and β_3 TMs 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 α_{IIb}–β_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 interaction, but takes an important step

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

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towards the goal of designing non-natural molecules that specifically target TM regions of proteins in a sequence-specific manner.

Previously we developed the CHAMP method (computed helical anti-membrane protein) to enable 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 sidechain 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, as well as to 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 inter-helical backbone-backbone interactions that help drive helix-association in membranes. Therefore, we positioned a β-peptide poly-homo-Gly (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 most stable for β^3-substituted amino acids in polar solvents, preliminary studies showed the co-existence of a 12-helical 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 sidechains using a packing algorithm that evaluates various sidechains 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 mainchain could not sterically accommodate a sidechain, and hence remained hGly. The design was completed by inclusion of Trp and Lys residues near the termini to properly orient the peptide in the bilayer. The initial design focused on the 14-helix and the backbone docking restraints induced an $(\text{hGly-X-X})_3$ motif with the hGly residues lined along one face of the helix. 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 3-fold 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.

To test the designed binding mode, two control peptides were prepared. We disrupted the GXXGXXG motif by mutating the central β^3-homo-glycine (hG14) to β^3-homo-isoleucine (**β-CHAMP G14I**) and scrambled the β-CHAMP sequence (**β-CHAMPscr**). All β-peptides were synthesized using optimized microwave-assisted solid phase peptide synthesis techniques outlined in the Supporting Information, TM α-peptide was synthesized using a previously established protocol.[23] The final sequences are shown in Table 1. Our protocols allow us to consistently obtain high purity products in high yield.

The CD spectrum of **β-CHAMP** was measured in trifluoroethanol (TFE), dodecyl phosphatidylcholine (DPC) micelles, and in phospholipid vesicles composed of a mixture of palmitoyl oleoyl phosphatidylcholine (POPC) and palmitoyl oleoyl phosphatidylglycerol (POPG) (7:3) (Figure 2). In DPC micelles and phospholipid vesicles there were minima at 204 nm and 206 nm, respectively, and a maximum at ca. 220 nm, indicative of the 12-helical structure. In TFE, the **β-CHAMP** CD spectrum suggested an equilibrium between 12- and 14-helical structures, consistent with increasing destabilization of the 12-helix with increasing solvent polarity.[21,24] Control β-CHAMP peptides followed the same pattern,

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showing higher 14-helical content in TFE.[21] Also, when the **β-CHAMP** mixed with the α_{IIb} TM peptide under conditions in which they formed a 1:1 complex (Figure 2), the spectrum was well described as a mixture of the TM α-helical and the 12-helical form of the β-peptide, strongly suggesting that the **β-CHAMP** binds its target in a 12-helical conformation.

To determine the orientation of the **β-CHAMP** peptides in phospholipid bilayers, they were examined in hydrated multilayers by attenuated total internal reflectance IR. (Figure 3, Figure S2, Supporting Information). 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 was studied using analytical ultracentrifugation (AUC) in density matched DPC micelles. In order to exclude the contribution of α_{IIb} TM 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 allows one 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 the 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 observed for the **β-CHAMP** suggests a small degree of homo-dimerization, which is likely due to the GXXGXXG motif, as both **β-CHAMP G14I** and **β-CHAMPscr** showed no evidence of self-association. In the presence of the α_{IIb} TM, **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 (Table 2, Figures S3–S8, Supporting Information).

Having established interaction of the **β-CHAMP** with the α_{IIb} TM domain, we explored whether **β-CHAMP** can disrupt interaction of the α_{IIb} and β_3 TM domains enabling 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 polyethylene-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 non-specific background binding, the experiments were also performed in the presence of the $\alpha_{IIb}\beta_3$-specific Fab antibody fragment, abciximab, that prevents interaction of fibrinogen and the integrin. **β-CHAMP** almost completely activates $\alpha_{IIb}\beta_3$ in the absence of the antibody fragment and has essentially no activity in the presence of abciximab. The integrin-activating effect of the control peptide **β-CHAMPscr** is substantially lower. Mn^{2+}, 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 data (Figure 5) clearly showing opening of the $\alpha_{IIb}\beta_3$ structure (a morphological equivalent of activation) as a result of an α_{IIb} TM helix interacting with **β-CHAMP**. These results that show strong and selective interaction of the **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 required the design of a β-peptide that spanned the bilayer, which was accomplished by inclusion of a block of apolar residues sufficiently long to span the bilayer, as well as inclusion of Trp and Lys residues near the headgroup region of the bilayer. The TM orientation was established using polarized IR spectroscopy. Specific

interaction with the α_{IIb} TM was 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 α_{IIb} TM; and 3) optimizing the placement of other sidechains in the **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 design β-peptides that recognize membrane targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Horne WS, Gellman SH. Acc Chem Res. 2008; 41:1399. [PubMed: 18590282]
2. Kritzer JA, Stephens OM, Guarracino DA, Reznik SK, Schepartz A. Bioorg Med Chem. 2005; 13:11. [PubMed: 15582447]
3. Seebach D, Gardiner J. Acc Chem Res. 2008; 41:1366. [PubMed: 18578513]
4. Goodman CM, Choi S, Shandler S, DeGrado WF. Nat Chem Biol. 2007; 3:252. [PubMed: 17438550]
5. Lee EF, Sadowsky JD, Smith BJ, Czabotar PE, Peterson-Kaufman KJ, Colman PM, Gellman SH, Fairlie WD. Angew Chem Int Ed Engl. 2009; 48:4318. [PubMed: 19229915]
6. Di Blasio B, Pavone V, Lombardi A, Pedone C, Benedetti E. Biopolymers. 1993; 33:1037. [PubMed: 8343584]
7. Sadowsky JD, Fairlie WD, Hadley EB, Lee HS, Umezawa N, Nikolovska-Coleska Z, Wang SM, Huang DCS, Tomita Y, Gellman SH. J Am Chem Soc. 2007; 129:139. [PubMed: 17199293]
8. Stephens OM, Kim S, Welch BD, Hodsdon ME, Kay MS, Schepartz A. J Am Chem Soc. 2005; 127:13126. [PubMed: 16173723]
9. Sadowsky JD, Schmitt MA, Lee HS, Umezawa N, Wang S, Tomita Y, Gellman SH. J Am Chem Soc. 2005; 127:11966. [PubMed: 16117535]
10. Bautista AD, Appelbaum JS, Craig CJ, Michel J, Schepartz A. J Am Chem Soc. 2010; 132:2904. [PubMed: 20158215]
11. Imamura Y, Watanabe N, Umezawa N, Iwatsubo T, Kato N, Tomita T, Higuchi T. J Am Chem Soc. 2009; 131:7353. [PubMed: 19432477]
12. Bennett JS. J Clin Invest. 2005; 115:3363. [PubMed: 16322781]
13. Zhu H, Metcalf DG, Streu CN, Billings PC, DeGrado WF, Bennett JS. J Mol Biol. 2010; 401:882. [PubMed: 20615419]
14. Luo BH, Takagi J, Springer TA. J Biol Chem. 2004; 279:10215. [PubMed: 14681220]
15. Litvinov RI, Vilaire G, Li W, DeGrado WF, Weisel JW, Bennett JS. Biochemistry. 2006; 45:4957. [PubMed: 16605263]
16. Partridge AW, Liu S, Kim S, Bowie JU, Ginsberg MH. J Biol Chem. 2004
17. Yin H, Litvinov RI, Vilaire G, Zhu H, Li W, Caputo GA, Moore DT, Lear JD, Weisel JW, DeGrado WF, Bennett JS. J Biol Chem. 2006; 281:36732. [PubMed: 17032655]
18. Yin H, Slusky JS, Berger BW, Walters RS, Vilaire G, Litvinov RI, Lear JD, Caputo GA, Bennett JS, DeGrado WF. Science. 2007; 315:1817. [PubMed: 17395823]
19. Caputo GA, Litvinov RI, Li W, Bennett JS, DeGrado WF, Yin H. Biochemistry. 2008; 47:8600. [PubMed: 18642886]
20. Tang J, Yin H, Qiu J, Tucker MJ, DeGrado WF, Gai F. J Am Chem Soc. 2009; 131:3816. [PubMed: 19256494]

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21. Korendovych IV, Shandler SJ, Montalvo GL, DeGrado WF. Org Lett. 2011; 13:3474. [PubMed: 21651308]
22. Korendovych IV, Senes A, Kim YH, Lear JD, Fry HC, Therien MJ, Blasie JK, Walker FA, DeGrado WF. J Am Chem Soc. 2010; 132:15516. [PubMed: 20945900]
23. Rathore N, Gellman SH, De Pablo JJ. Biophys J. 2006
24. Korendovych IV, Kim YH, Ryan AH, Lear JD, DeGrado WF, Shandler SJ. Org Lett. 2010; 12:5142. [PubMed: 20945888]
25. Tucker MJ, Getahun J, Nanda V, DeGrado WF, Gai F. J Am Chem Soc. 2004; 126:5078. [PubMed: 15099085]
26. Goormaghtigh E, Raussens V, Ruysschaert J. Biochim Biophys Acta. 1999; 1422:105. [PubMed: 10393271]
27. Axelsen PH, Braddock WD, Brockman HL, Jones CM, Dluhy RA, Kaufman BK, Puga FJ. Applied Spectroscopy. 1995; 49:526.
28. Axelsen PH, Citra MJ. Prog Biophys Mol Biol. 1996; 66:227. [PubMed: 9284452]
29. Litvinov RI, Nagaswami C, Vilaire G, Shuman H, Bennett JS, Weisel JW. Blood. 2004; 104:3979. [PubMed: 15319287]

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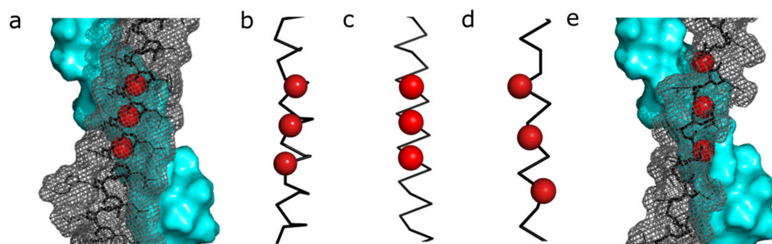

Figure 1.

Design of **β-CHAMP. a**) Docking of a CHAMP peptide on α_{IIb} TM helix (cyan), the α-carbons of Gly residues are shown as red spheres.[18] **b**) The position of the interacting motif on an α-helix (GX_3GX_3G); **c**) a 14 helix (GX_2GX_2G); **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 are shown as red spheres.

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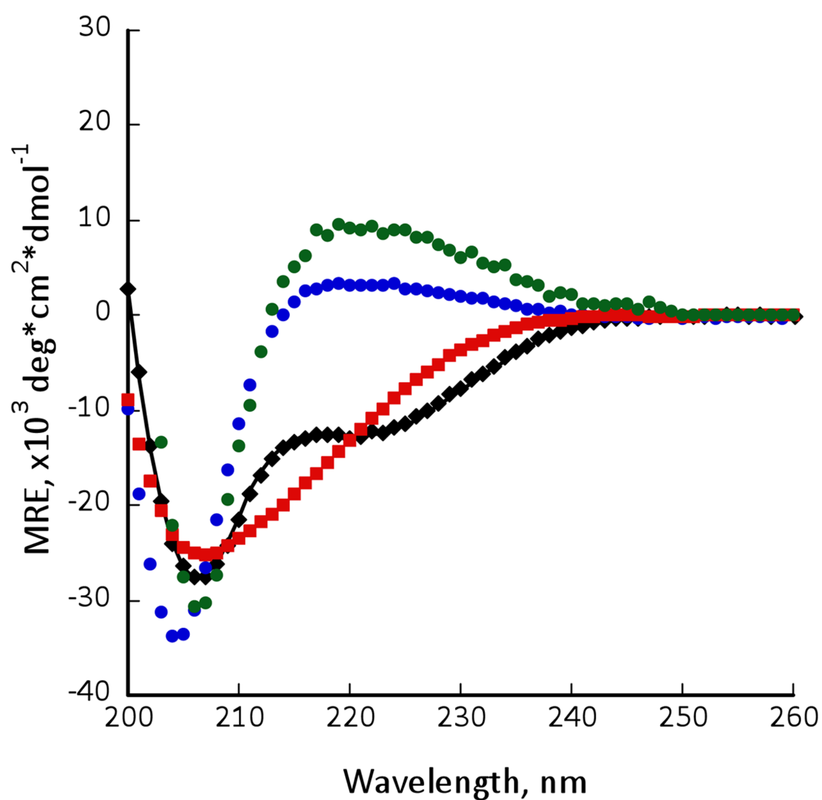

Figure 2.

CD spectra of **β-CHAMP** in DPC micelles (blue circles, 40 μM peptide, 10 mM DPC, 10 mM phosphate buffer, pH 7.4), POPC/POPG (7:3) vesicles (green circles, 167 μM total lipid, 1:100 peptide:lipid), and in TFE (red squares, 40 μM peptide). CD spectrum of **β-CHAMP** in the presence of 1 equiv. of α_{IIb}-TM (black diamonds, DPC micelles, 40 μM total peptide). The line represents linear combination of the 12-helical spectrum of **β-CHAMP** and the CD spectrum of α_{IIb}-TM in equal proportions.

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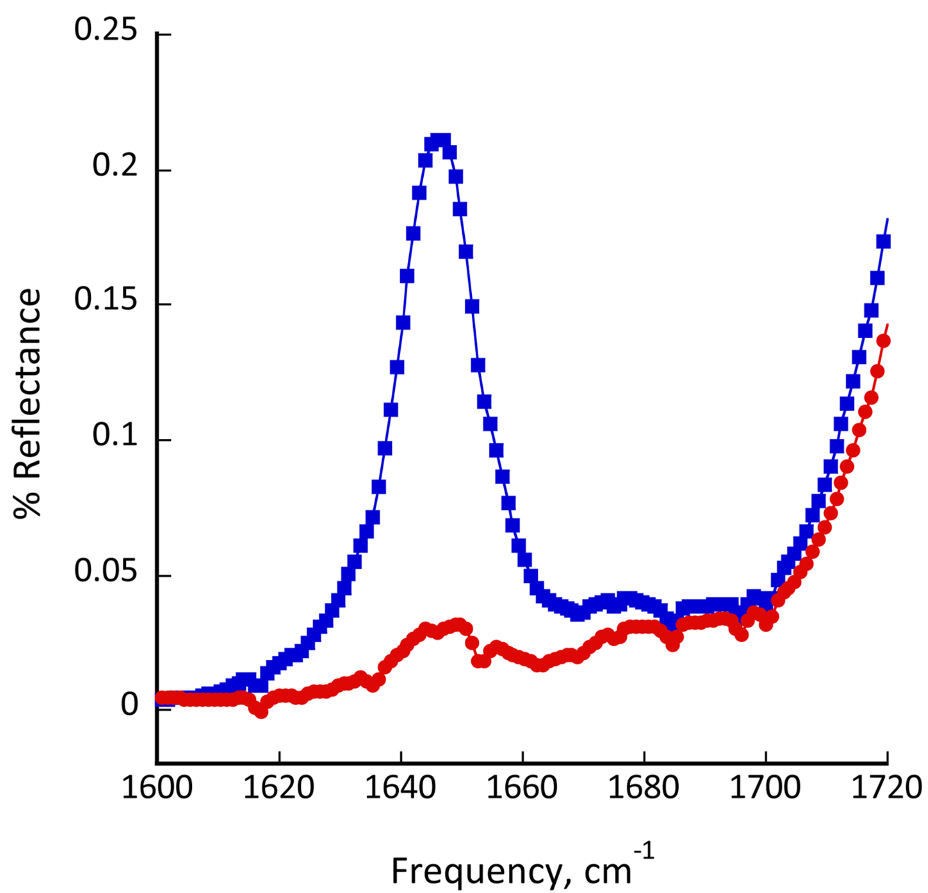

Figure 3.
FT-IR/ATR spectrum of **β-CHAMP** in POPC/POPG (7:3) lipid membranes (1:80 peptide:lipid ratio). The blue and red traces represent light polarized parallel and perpendicular, respectively, to the plane of incidence.

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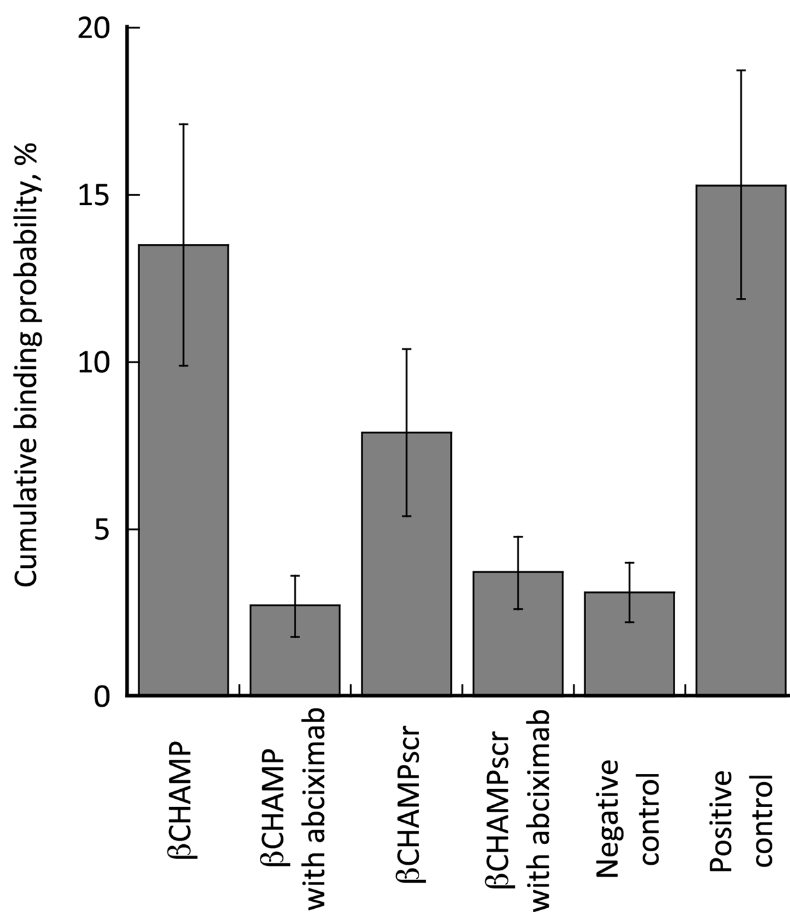

Figure 4.

Rupture force spectroscopy data on interaction of the isolated surface-bound $\alpha_{IIb}\beta_3$ and fibrinogen in the presence of the β-CHAMP peptides (5 μM). Negative control represents trifluoroethanol, 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 ($p=0.008$).

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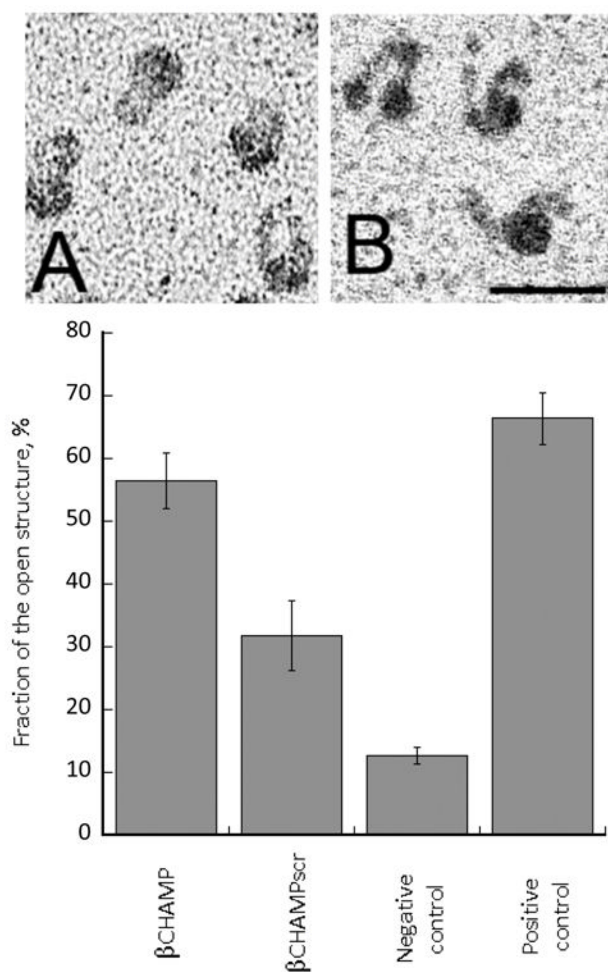

Figure 5.
Transmission electron microscopy of purified $\alpha_{IIb}\beta_3$ in "closed" (A) and "open" (B) forms, corresponding to inactive and active conformations, respectively. Magnification bar = 30 nm. The bar graph shows fraction of the open structure in the presence of the β-CHAMP peptides (5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

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Table 1

Sequences of the designed β-CHAMP peptides

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

*One letter code refers to the corresponding β^3-amino acid

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Table 2

Association of DNP-labeled β-peptides with α_{IIb}-TM in DPC micelles as measured by AUC

Peptide	Monomer MW, Da	MW_{obs} in absence of α_{IIb} TM peptide, Da	MW_{obs} in presence of α_{IIb} TM peptide, Da
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