Computational design of a β–peptide that targets transmembrane helices

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SUPPORTING INFORMATION

Computational Design. The NAPOLI computational foldamer design algorithm[S1] was used to design the sequence by optimizing the interactions between an idealized β-peptide helix (both 14- and 12-helical structures) and a model transmembrane region of the α_{IIb} integrin (α_{IIb}-TM, `AIPIWWVLVG VLGGLLLLTI LVLAMWK`). The β-peptide (β^3-homoglycine) was allowed six degrees of freedom while fixing the backbone of α_{IIb}-TM but allowing its rotamers to vary. NAPOLI filtered out β-peptide poses with backbone (BB) clashes and optimized the β-peptide sequence using a Monte Carlo and exhaustive enumeration algorithms (**Figure S1**). Each algorithm operated in the context of a unique search space; rigid body transformation (RBT) space was enumerated, while sequence space was optimized using a Monte Carlo algorithm.

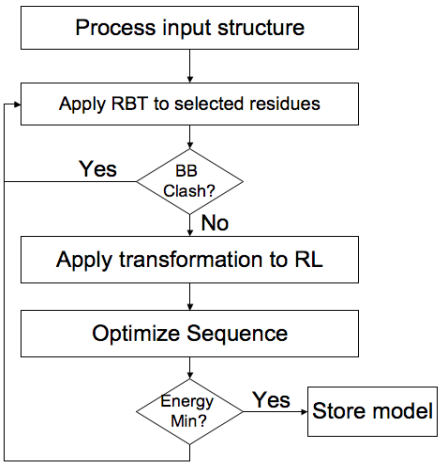

Figure S1. Process flow diagram for NAPOLI's flexible docking algorithm. RL stands for rotamer library.[S2]

The evaluation step for the rigid-body translation/rotation step was a simple clash filter based on inter-atomic distance and atomic radii. NAPOLI's implementation of the CHARMM27[S3] molecular mechanics energy function was used for sequence optimization. Since this design project made use of fixed bond lengths and angles, these terms were disabled in NAPOLI to improve computational efficiency. Atomic radii were scaled to 95% of the force field value to account for these constant parameters. A distance-dependent dielectric of 10 was used.

Reference Energies. Reference energies for the 20 β^3 -amino acids were included in the calculations to normalize the contributions made by each amino acid in the overall energy calculation. The reference energies were calculated using the central residue in an idealized 14-helix. The average energy for the top 10% energetically favorable rotamers for each residue was used as the reference energy for a given identity.

Table S1. Self energies for β^3-amino acids. Energies are added on a per residue basis during the sequence optimization process. Proline is not an allowable mutation for this simulation, no reference energy was calculated.

Amino acid	Reference energy	Amino acid	Reference energy
Ala	1.06053	Met	2.54102
Cys	1.57805	Asn	3.33143
Asp	3.14559	Gln	3.34744
Glu	2.00603	Arg	8.90945
Phe	-1.68754	Ser	0.61257
Gly	-0.06740	Thr	1.53606
His/Hid/Hie	1.28849	Val	-0.08374
Ile	1.07692	Trp	0.52935
Lys	2.14835	Tyr	-1.09898
Leu	1.81771	Pro	N/A

Peptide synthesis. β-peptides were synthesized using a published procedure.[S4] α-peptides were synthesized using a Symphony automated synthesizer at room temperature using a protocol optimized for TM peptides.[S5] The full-length peptide was cleaved from solid support using TFA:trisisopropylsilane (TIS):ethanedithiol (EDT):H_2O (94:2.5:2.5:1 v/v) for 2.5 hours, precipitated in cold ethyl ether and dried in vacuo. The peptide was dissolved in trifluoroethanol (TFE) and diluted with water and acetonitrile to the final solvent mixture composition of 4:3:2 (TFE:CH_3CN:H_2O), and purified on a Vydac C4 column.

The identity and purity of the polypeptides was evaluated on a Bruker MicroFlex MALDI mass spectrometer and Agilent Series 1100 analytical HPLC.

FT-IR/ATR Data analysis. Sample preparation, data collection, and analysis was performed using the methods described by Tucker *et al.*[S6] and Goormaghtigh *et al.*[S7] A solution containing palmitoyl oleoyl phosphatidylcholine (POPC) and palmitoyl oleoyl

phosphatidylglycerol (POPG) (POPC/POPG = 7:3, 15 mM in chloroform) was combined with the appropriate amount of peptide from a 138 μM stock solution (in TFE) of peptide to a final volume of 120 μL of 1:75 mol peptide:lipid ratio. The sample was added dropwise to a Ge crystal (50 mm x 10 mm x 2 mm SPT45) and allowed to dry in air. The crystal was then placed in a hydration chamber and allowed to rehydrate for at least 24 hours. During data acquisition, a mixture of nitrogen and D_2O gas was passed over the crystal. Spectra were obtained with a Magna-IR 860 spectrometer with a Harrick's Horizon multiple-reflection attachment for ATR measurements. 128 scans were averaged for each spectrum. The dichroic ratio was determined for the amide I band via Gaussian fitting at the major peak (1646.938 cm^{-1}). Initial analysis was conducted using previously published standards[S8] and resulted in an S' order parameter of 0.82 with regards to the amide I IR stretch. An order parameter of 1.0 corresponds to alignment parallel to the Z-axis, while an order parameter of -0.5 corresponds to alignment perpendicular to the Z-axis.

Analytical Ultracentrifugation. Equilibrium sedimentation was used primarily to determine the association state of the peptides, and also to provide an estimate of the association constants. The experiments were performed in a Beckman XL-I analytical ultracentrifuge (Beckman Coulter) using six-channel carbon-epoxy composite centerpieces at 25 °C. Peptides were co-dissolved in TFE (Sigma) and dodecyl phosphatidylcholine (DPC, Avanti Polar Lipids). The organic solvent was removed under reduced pressure to generate a thin film of peptide/detergent mixture, which was then dissolved in buffer previously determined to match the density of the detergent component (10 mM phosphate buffer (pH= 7.4) buffer containing 2.1 mM TCEP). The final concentration of DPC is 15 mM in all of the samples. Data at different measurement speeds (30 - 45 krpm) were analyzed by global curve-fitting of radial concentration gradients (measured using optical absorption) to the sedimentation equilibrium equation for monomer-tetramer equilibria among the peptides included in the solution. Peptide partial specific volumes were calculated using previously described methods[S9] and residue molecular weights corrected for the 52% D_2O exchange expected for the density-matched buffer. The solvent density (1.053 g/ml) was measured using a Paar

densitometer. Aqueous solution molar extinction coefficients at 280 nm were calculated using the program Sednterp.[S10] These coefficients were multiplied by the molar detergratio concentration units.

In order to study heteromeric association of α_{IIb}-TM with β-peptides we installed a chromophore (2,4-dinitrophenol, DNP) on the β-peptide N-termini, and the sedimentation equilibrium was fitted to a single species model:

$$Abs = E + \varepsilon_a c_{0a} l \exp\left[\frac{\omega^2}{2RT} M_a (r^2 - r_0^2)\right] \quad \textbf{(S1)}$$

where E = baseline (zero concentration) absorbance, c_o is the peptide/detergent ratio at r_o, ε_a is the molar extinction coefficient of DNP (11,340 $M^{-1}cm^{-1}$ at 356 nm),[S11] l is the optical path length, ω =2π*RPM, R= 8.3144 $\times 10^7$ erg $K^{-1}mol^{-1}$, T is temperature in K, M_a is the buoyant molecular weight the heterodimer.

Molecular weight was obtained from the buoyant molecular weight using:

$$M_w = M(1 - \bar{\nu}\rho) \quad \textbf{(S2)}$$

where M is the buoyant molecular weight, $\bar{\nu}$ is the partial specific volume and ρ is the solution density.

Circular Dichroism Spectrometry. CD spectrometry experiments were carried out using a J-810 spectropolarimeter (JASCO). Peptide samples were prepared in TFE or detergent micelles (10-15 mM DPC) in 10 mM phosphate buffer (pH = 7.4). Measurements were conducted at 20°C in 1 nm step mode with a response time of 4 seconds in a 1 mm path length quartz cuvette. The samples in DPC micelles were allowed to equilibrate for several hours prior to measurements. Three independent measurements were collected and averaged. For CD measurements in lipid bilayers, POPC/POPG (7:3) vesicles containing peptide were sonicated to decrease scattering.

Integrin $\alpha_{IIb}\beta_3$ isolation from platelets. Platelets (Blood Bank, Hospital of the University of Pennsylvania) were pelleted by centrifugation (1000 g, 20 min, 20 °C) in 50 mL sterile Falcon tubes. The supernatant was aspirated and each pellet was resuspended in ~ 5 mL HEN buffer (10 mM HEPES, pH 6.5, 150 mM NaCl, 3 mM

EDTA, 1 μM PGE_1) and pelleted by centrifugation. The resulting pellets were resuspended in ~ 25 mL HEN buffer containing Halt™ protease inhibitor cocktail (Pierce), flash frozen in liquid nitrogen and stored at -80 °C. Prior to use, slowly thawed (room temperature) platelets were disrupted by nitrogen cavitation at 1300 psi for 10 minutes followed by extrusion into a centrifugation tube on ice. The resulting turbid suspension was centrifuged for 15 minutes at 4 ˚C and 7000 g. The supernatant was decanted and centrifuged for 60 min at 4 ˚C and 30,000 g. The supernatant was decanted and the pellet resuspended in the minimum volume of resuspension buffer (10 mM HEPES, pH 6.8, 150 mM NaCl, 1 mM $CaCO_3$, 1mM $MgCl_2$, 1% Trition 100x). After dissolution, the resuspended pellet was centrifuged in 1.5 mL tubes at 10,000 g and 4 ˚C for 10 min. The supernatant from this spin was decanted into a Falcon tube containing a prewashed (1x in 10 mL resuspension buffer) concanavalin A agarose resin (~100 μL/mL resuspension buffer; Sigma) and agitated lightly with a nautilating mixer for 2 hours at 4 ˚C. The resin was centrifuged for 2 min and the supernatant removed. The concanavalin A resin was then washed (2x 10 mL resuspension buffer) by lightly agitating for 10 minutes at 4 ˚C. Finally, the integrin was eluted by repetitive addition of 1 mL elution buffer (200 mM methyl-α-D-mannopyranose, 10 mM HEPES (pH 6.8), 150 mM NaCl, 1 mM $CaCO_3$, 1 mM $MgCl_2$, 80 mM n-octyl-β-D-glucoside), followed by agitation for 10 min at 4 ˚C and elution. Integrin fractions were tested for purity by gel electrophoresis on 4-12% bis/tris gels. The cleanest fractions were combined and concentrated to 1 mg/mL using Amicon® Ultra 30k Centrifugal filters. Protein concentration was confirmed using the BCA technique.

Optical Trap-Based Single-Molecule Rupture Force Spectroscopy. Rupture force spectroscopy was performed basically as described earlier.[S12] Briefly, to measure the interactions between fibrinogen and purified $\alpha_{IIb}\beta_3$, suspension of fibrinogen-coated 2-μm-latex beads was introduced into a chamber containing 5-μm-integrin-coated motionless pedestals and a single bead was trapped by the laser beam. An integrin-coated pedestal was brought to within 1-2 microns of the trapped bead. The position of the laser trap was then oscillated in a triangular waveform at 10 Hz with a pulling velocity of 36 μm/s (loading rate of 8,000 pN/s). The distance separating the pedestal and the bead was

reduced in 10-nm steps using the piezostage until repeated contacts are observed. When fibrinogen is bound to $\alpha_{IIb}\beta_3$, the bead stops moving and a linear increase in force signal is registered. The results of many experiments under similar conditions were averaged so that each rupture force histogram represented from 10^3 to 10^4 repeated contacts of more than 10 different bead-pedestal pairs. The number of events was plotted against the average force after normalizing for the total number of interaction cycles. The percentage of events in a particular force range represents the probability of rupture events at that tension. Optical artifacts observed with or without trapped latex beads produce signals that appeared as forces below 10 pN. Accordingly, rupture forces in this range were not considered when the data were analyzed.

To reveal effects of the β-peptides on the ligand-binding integrin activity, $\alpha_{IIb}\beta_3$ was incubated at 37 °C for 30 min with each peptide in 20 mM HEPES buffer, pH 7.4, containing 150 mM NaCl and 3 mM $CaCl_2$. The peptides were added from solution in TFE to a final concentration of 5 μM at the peptide/$\alpha_{IIb}\beta_3$ molar ratio 2:1. $\alpha_{IIb}\beta_3$ was immobilized in the buffer containing the same peptide concentration and the measurement were also made in the presence of the peptide. Negative controls without peptides contained a corresponding amount of TFE in the buffer. When Mn^{2+} was used to activate $\alpha_{IIb}\beta_3$, the integrin was pretreated with 2 mM Mn^{2+} for 30 min at 37 °C, and then it was immobilized in the buffer containing the same Mn^{2+} concentration. $\alpha_{IIb}\beta_3$ antagonist, abciximab, was added at 100 μg/mL final concentration to the suspension of fibrinogen-coated beads before they were introduced into the chamber.

Transmission electron microscopy (TEM) of purified integrin $\alpha_{IIb}\beta_3$ in the absence and presence of the β-peptides was performed as previously described.[S13] $\alpha_{IIb}\beta_3$ was incubated at 37°C for 30 min either with 2 mM $MnCl_2$ (positive control) or a peptide in 20 mM HEPES buffer, pH 7.4, containing 150 mM NaCl and 3 mM $CaCl_2$ at a final peptide concentration of 5 μM at the peptide/$\alpha_{IIb}\beta_3$ molar ratio 2:1. We prepared rotary shadowed samples by spraying a dilute solution of molecules in a volatile buffer (0.05 M ammonium formate) and glycerol (30-50%) onto freshly cleaved mica and shadowing it with tungsten in a vacuum evaporator (Denton Vacuum Co., Cherry Hill, NJ). All specimens were examined in a FEI/Philips 400 electron microscope (Philips Electronic

Instruments Co., Mahwah, NJ), operating at 80 kV and at a magnification of 60000×. Integrin molecules were found either in the inactive state or in the active state with their transmembrane stalks closed or open, respectively. The majority of inactive $\alpha_{IIb}\beta_3$ molecules had a closed configuration with their stalks touching at their tips. By contrast, when β-CHAMP was present, most of the $\alpha_{IIb}\beta_3$ molecules had an open configuration with separated stalks. By contrast, **β-CHAMPscr** induced only moderate activation at the same concentrations.

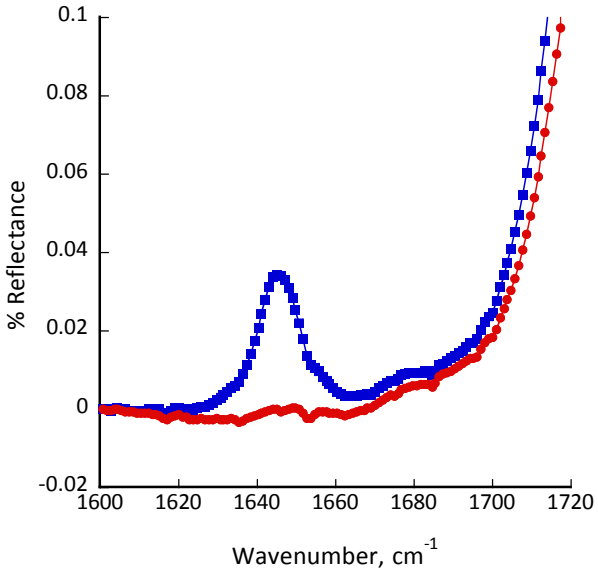

Figure S2. FT-IR/ATR spectrum of β-CHAMPscr in POPC/POPG (7:3) lipid membranes (1:75 peptide:lipid ratio). The blue and red traces represent light polarized parallel and perpendicular, respectively, to the plane of incidence.

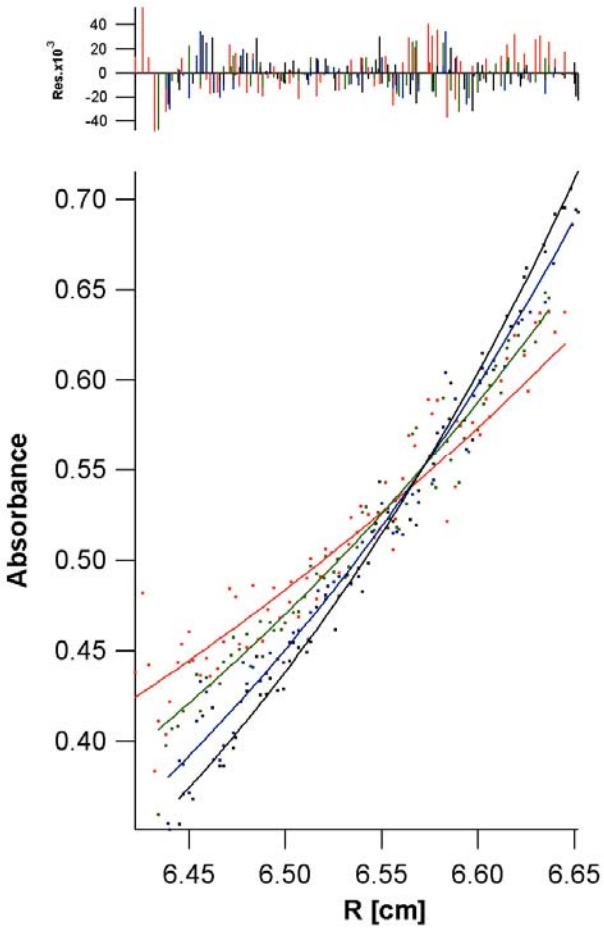

Figure S3. Sedimentation equilibrium profile at 356 nm of β-CHAMP-DNP peptide (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM). The partial specific volume and the solution density were fixed at 0.85 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 4360±81 Da.

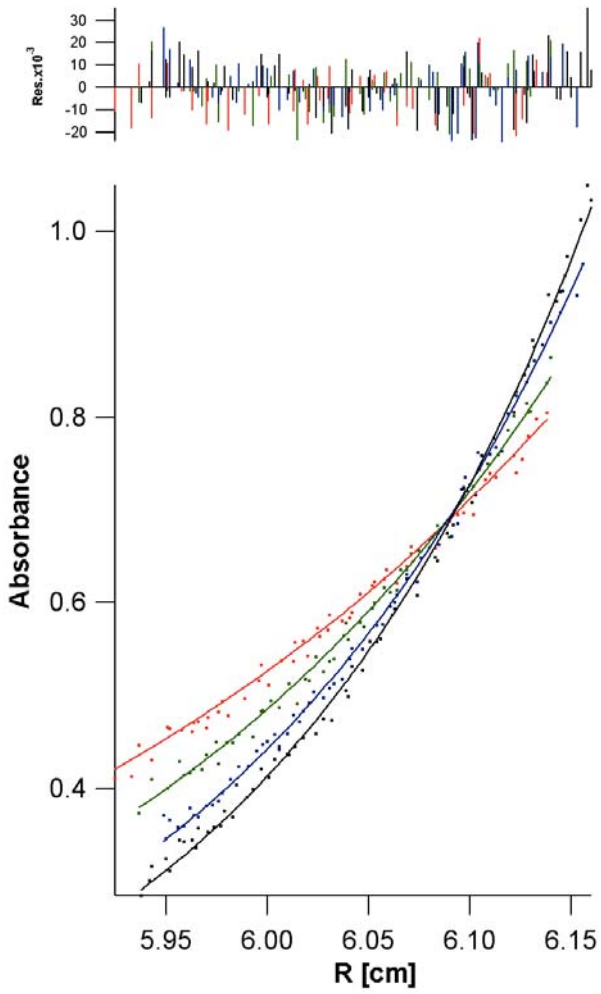

Figure S4. Sedimentation equilibrium profile at 356 nm of β-CHAMP-DNP peptide (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM) in the presence of 1 equiv of α_{IIB}-TM peptide. The partial specific volume and the solution density were fixed at 0.85 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 6737±71 Da.

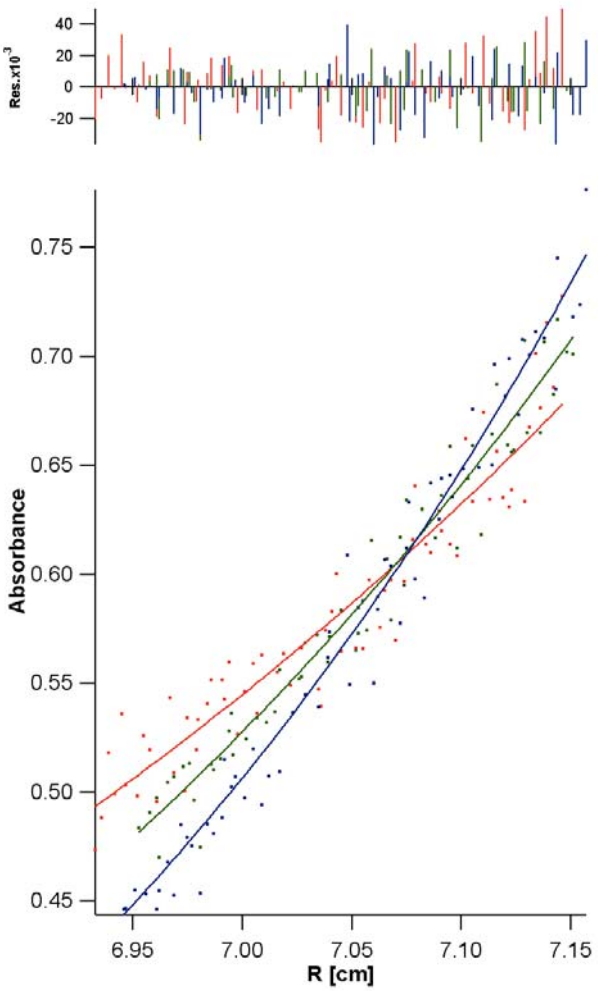

Figure S5. Sedimentation equilibrium profile at 356 nm of β-CHAMPscr-DNP peptide (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM). The partial specific volume and the solution density were fixed at 0.845 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 3446±147 Da.

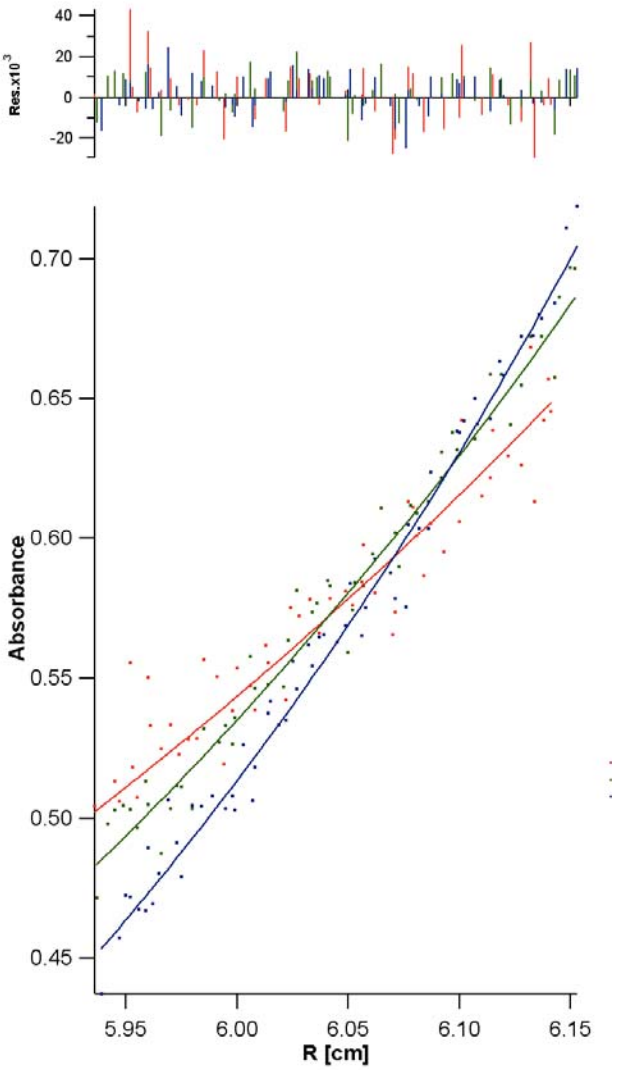

Figure S6. Sedimentation equilibrium profile at 356 nm of β-CHAMPscr-DNP (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM) in the presence of 1 equiv of α_{IIb}-TM peptide. The partial specific volume and the solution density were fixed at 0.845 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 3542±172 Da.

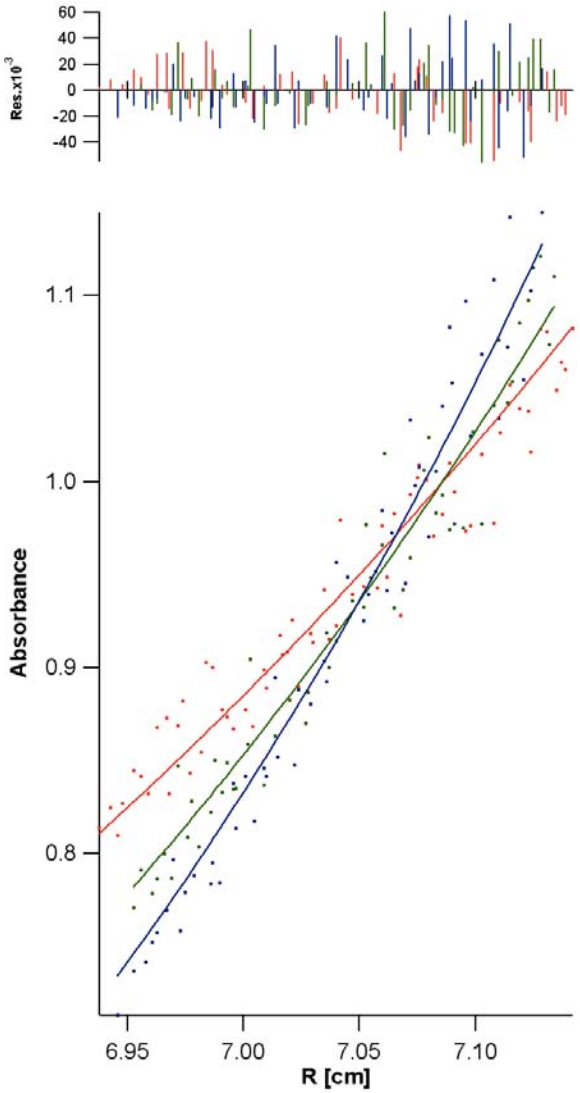

Figure S7. Sedimentation equilibrium profile at 356 nm of β-CHAMP G14I-DNP peptide (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM). The partial specific volume and the solution density were fixed at 0.85 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 3542±172 Da.

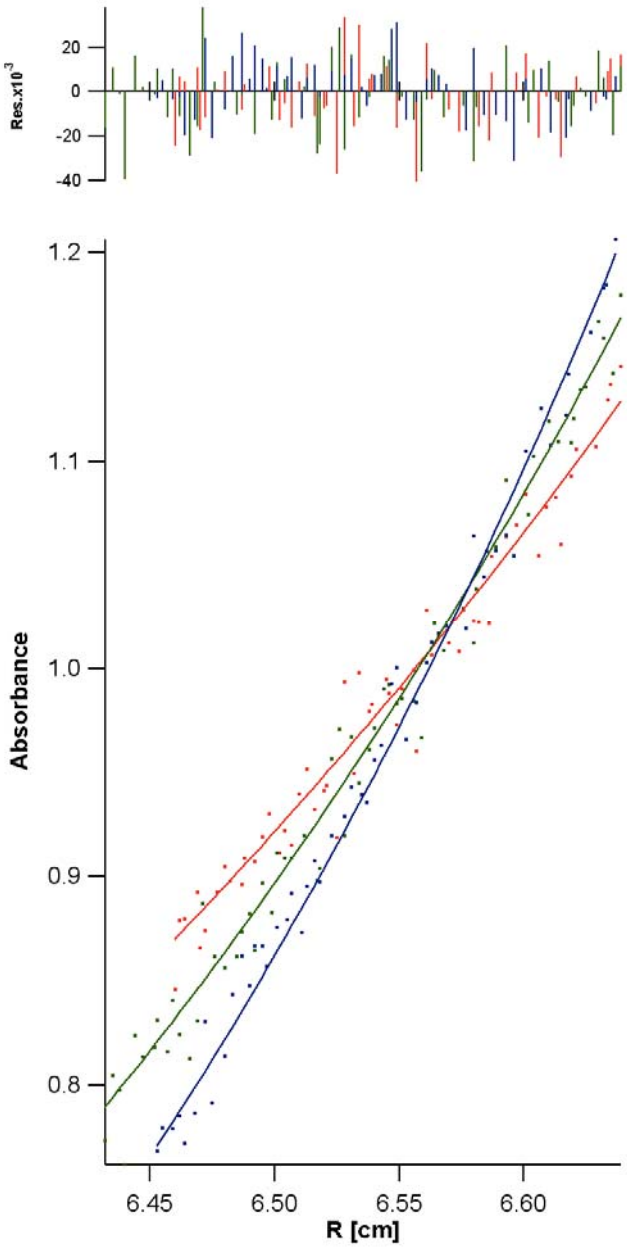

Figure S8. Sedimentation equilibrium profile at 356 nm of β-CHAMP G14I-DNP (50 μM) in density-matched DPC micelles (15 mM) in phosphate buffer (10 mM, pH 7.4) containing TCEP (2.1 mM) in the presence of 1 equiv of α_{IIb}-TM peptide. The partial specific volume and the solution density were fixed at 0.85 mL/g and 1.053 g/mL, respectively. The data were analyzed using a global fitting routine. The average molecular weight obtained from the fit is 3905±106 Da.

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