Supplemental Data

Ligand Capture of Human Blood Platelets on Surface Modified Planar Gold Surfaces.

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Supplemental Data

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ANALYTICAL DATA SHEET

Product Name:	**cyclo[Mpa-homoArg-Gly-Asp-Trp-Pro-Cys]-NH_2** Where Mpa = 3-mercaptopropionic acid		
Catalog No.	*PCS-30453-PI*	*Lot No.*	*922351*
Formula	$C_{35}H_{49}N_{11}O_8S_2$	*Molecular Weight*	*831.98*

Appearance *White powder*

ES-MS *MW calculated 831.32* *MW Found 831.39*

Amino acid analysis

Asp	*0.90 (1)*
Pro	*1.06 (1)*
Gly	*1.04 (1)*
Trp	*Not determined.*
Cys	*Present, not determined.*
homoArg	*not determined.*

Salt *Trifluoroacetate salt*

HPLC profile included (92.9%)

Prepared by
Original Date: December 28, 2010
CM
Revised Date: January 14, 2011
CM

Approved by

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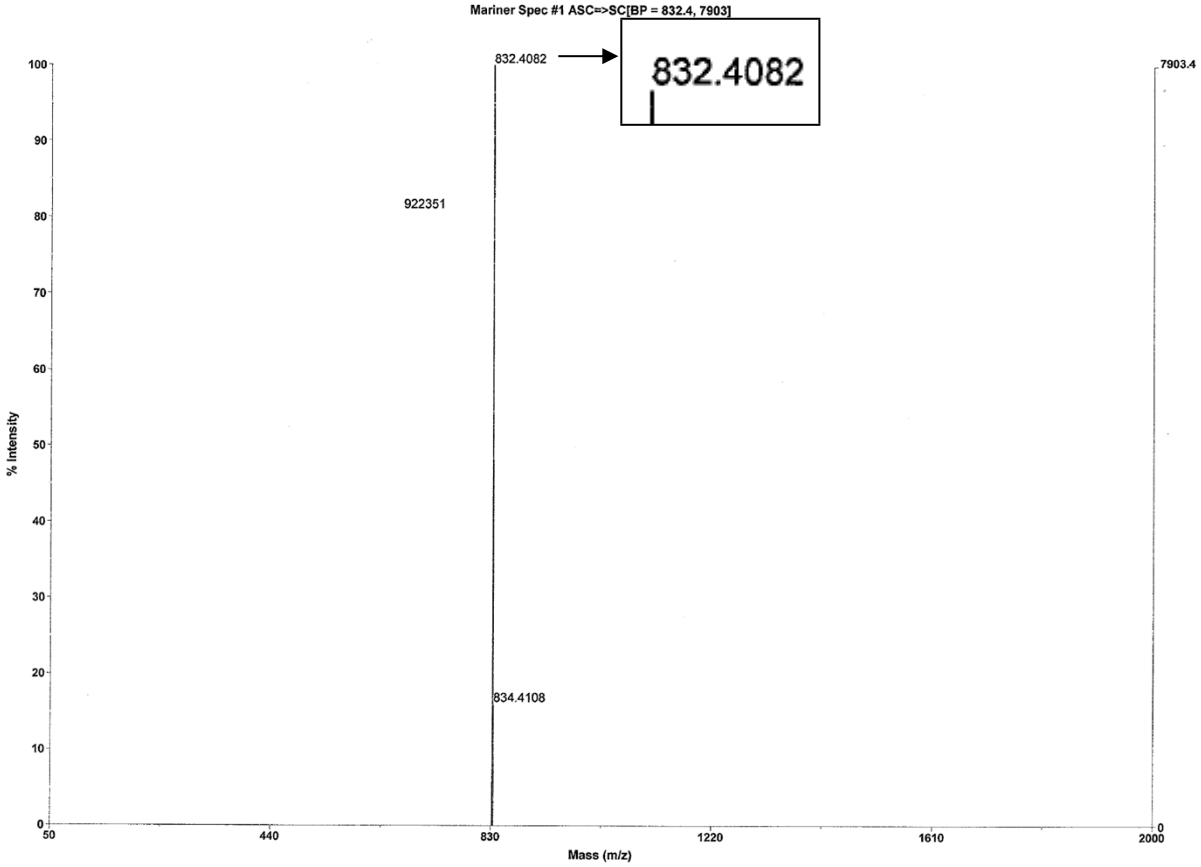
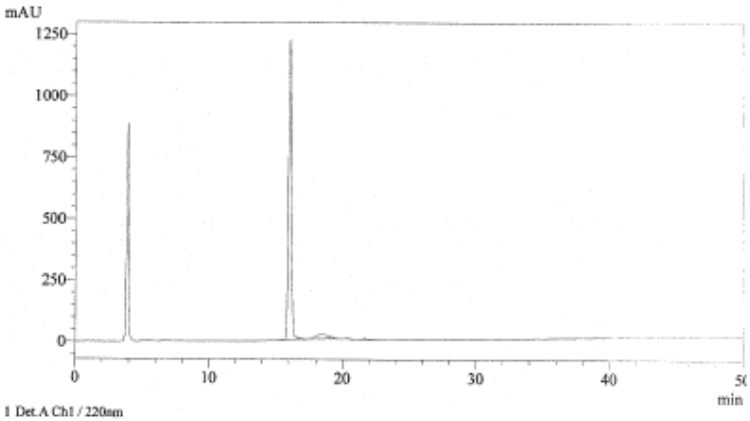


Gradient: 10-60% in 50 min.
Column: Jupiter 5u, C18, 300A, 547029-24, (4.6mm x 250 mm)
Buffer: A: 0.05% TFA in H2O B: 0.05% TFA in MeCN
Flow: 1mL/min



PeakTable

Detector A Ch1 220nm

Ret. Time	Area	Height	Area %
15.458	16722	1184	0.082
16.007	18955412	1220964	92.903
16.608	22021	1154	0.108
18.495	1179844	20264	5.783
19.172	119797	8494	0.587
20.339	15659	1175	0.077
21.711	94062	3152	0.461
			100.000

Fig. S1 Peptides International CoA including HPLC and MALDI-TOF-MS data confirming purity and identity of Eptifibatide cyclic peptide.

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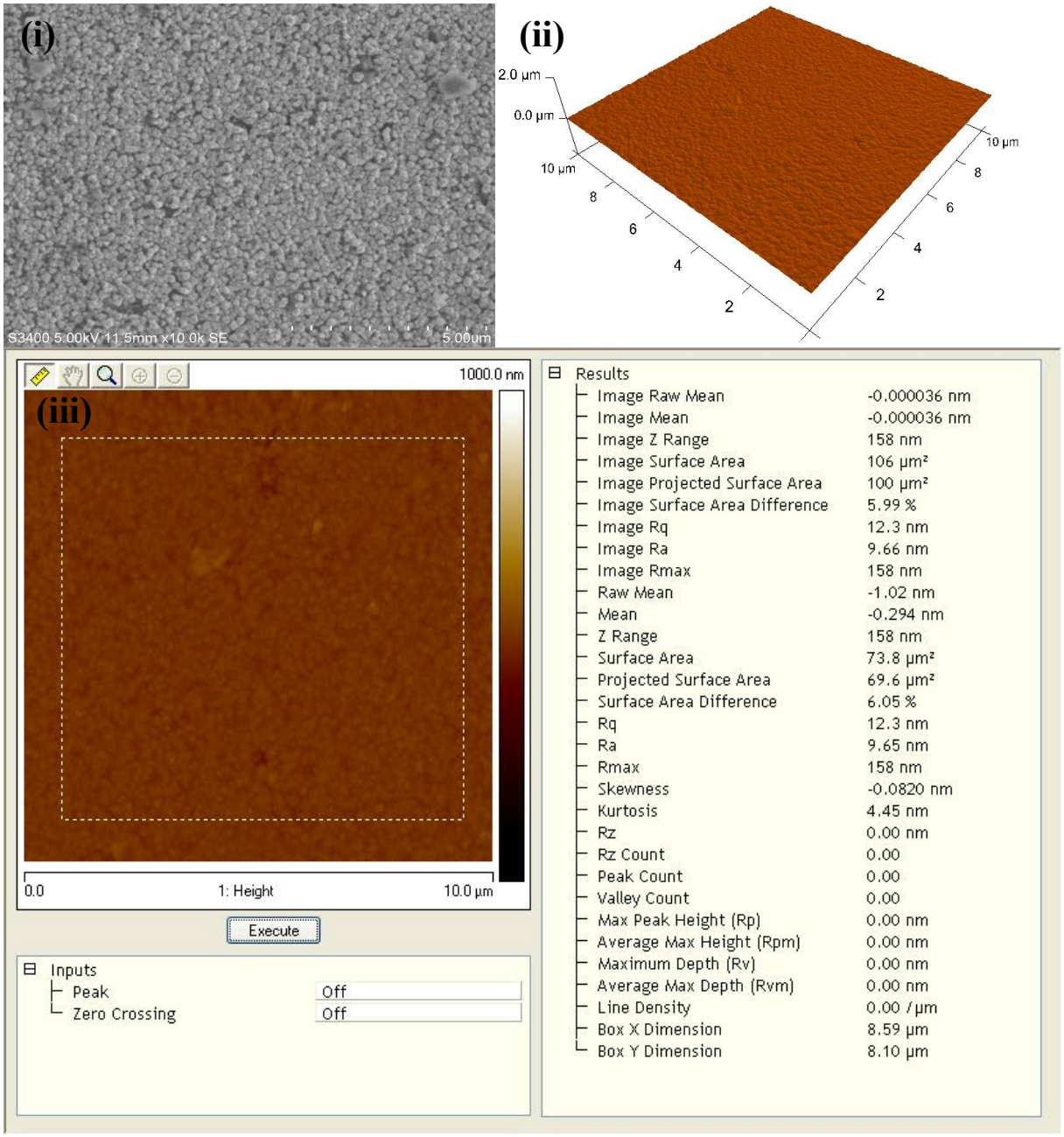

Fig. S2 (i) SEM image and (ii) AFM image of gold film of ~100 nm thickness electrochemically deposited onto silicon wafers coated with 1000 Å gold (Au) 525 µm thickness over a 50 Å titanium adhesion layer. (Bottom) Surface roughness measurement example suggesting the unmodified gold surface is rough with a root mean square (RMS) roughness of 12.3 nm (average RMS roughness was found to be 12.13±0.27).

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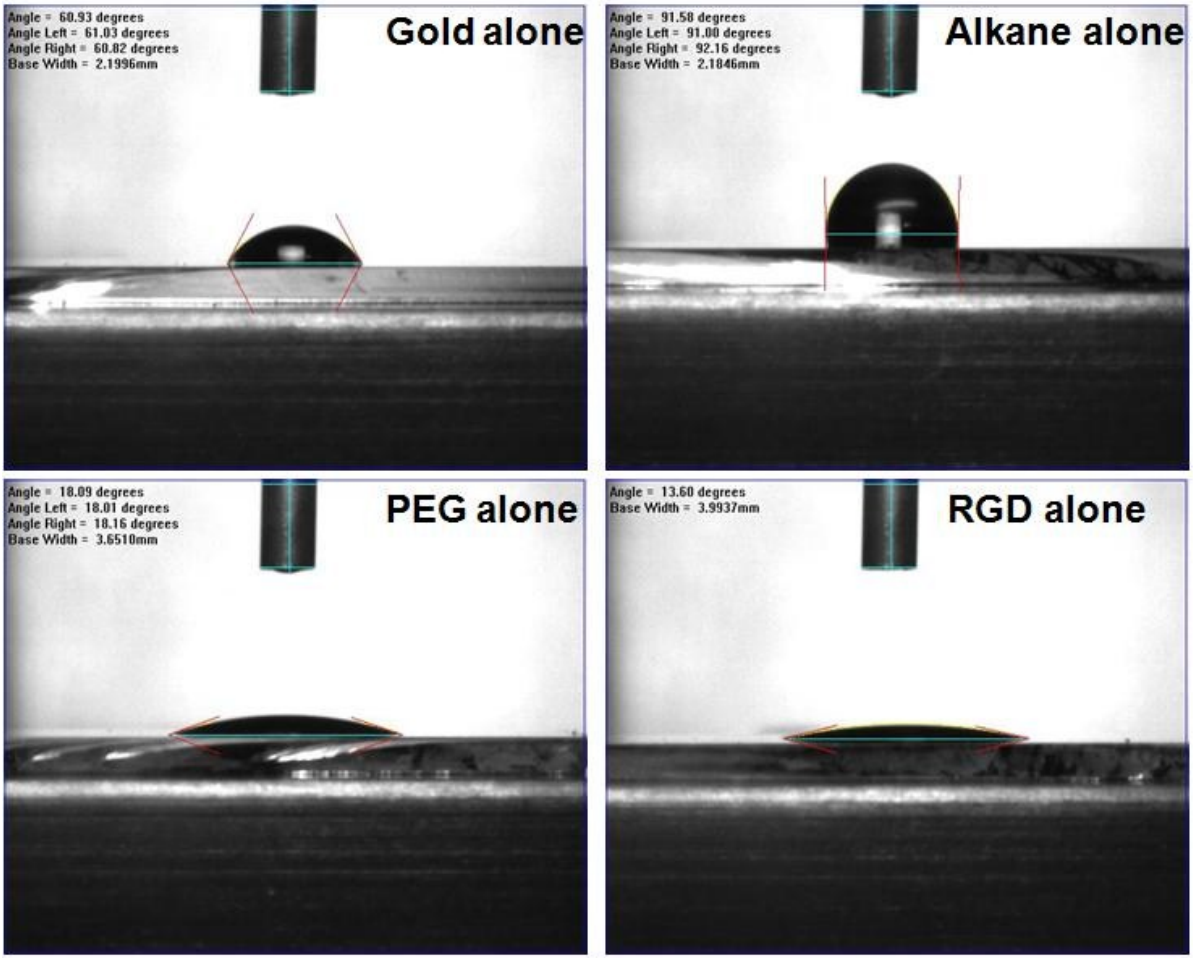
(A)



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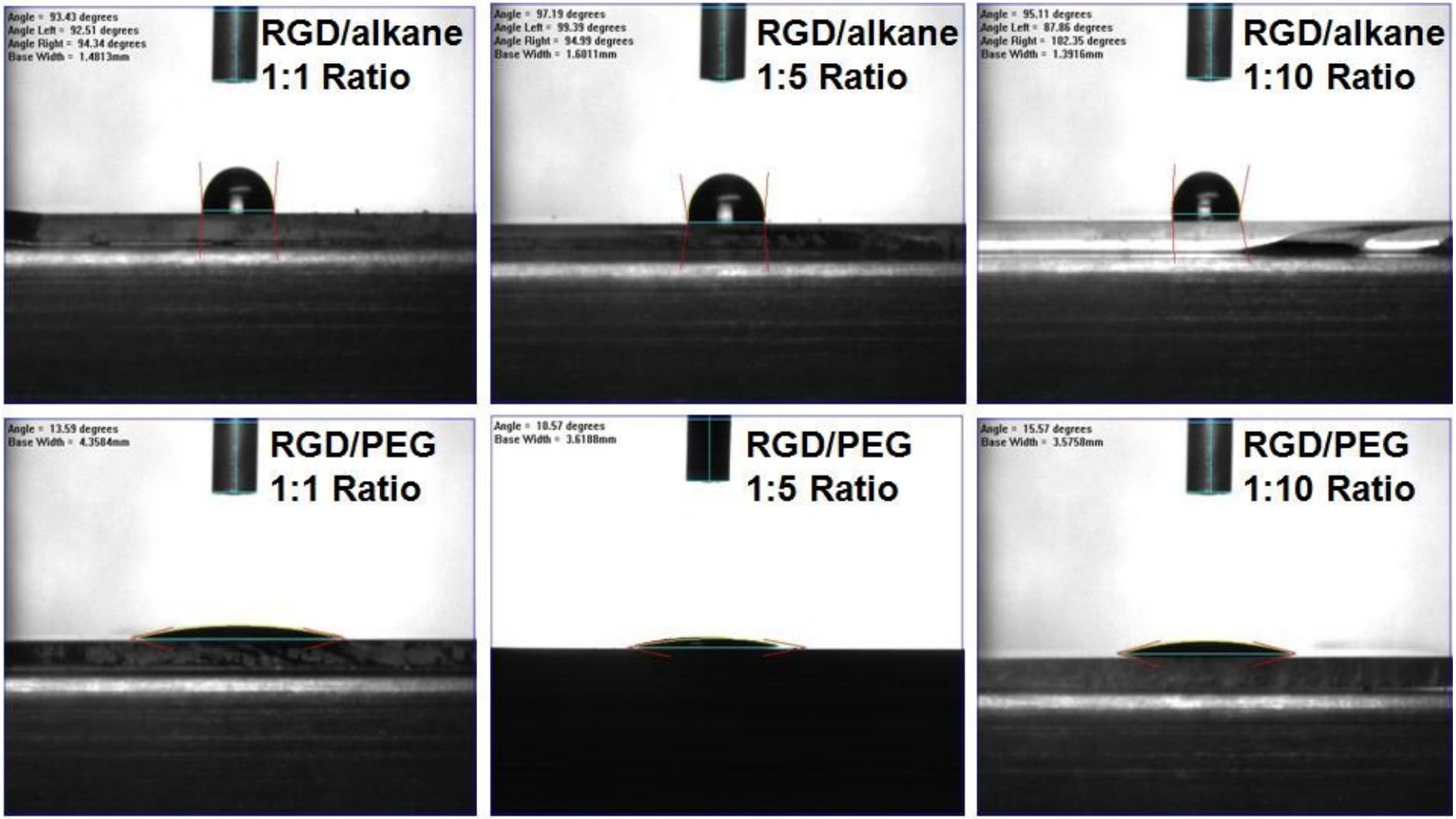
(B)



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(C)

Surface Modification	Water Contact Angle (°)
Unmodified gold	60.74 ± 1.33
Alkane alone	91.81 ± 2.30
RGD alone	13.53 ± 0.90
PEG alone	21.12 ± 2.96
RGD/Alkane 1:1 Ratio	85.14 ± 4.70
RGD/Alkane 1:5 Ratio	94.63 ± 1.77
RGD/Alkane 1:10 Ratio	98.54 ± 3.16
RGD/PEG 1:1 Ratio	11.64 ± 1.32
RGD/PEG 1:5 Ratio	11.69 ± 0.43
RGD/PEG 1:10 Ratio	14.10 ± 1.27

Fig. S3 Water contact angle measurements for (A) unmodified gold alone, alkane alone, RGD alone and PEG alone and (B) RGD/alkane and RGD/PEG 1:1, 1:5 and 1:10 ratios. (C) Summary table of water contact angle values for all surfaces used in this study. N=3 in all cases.

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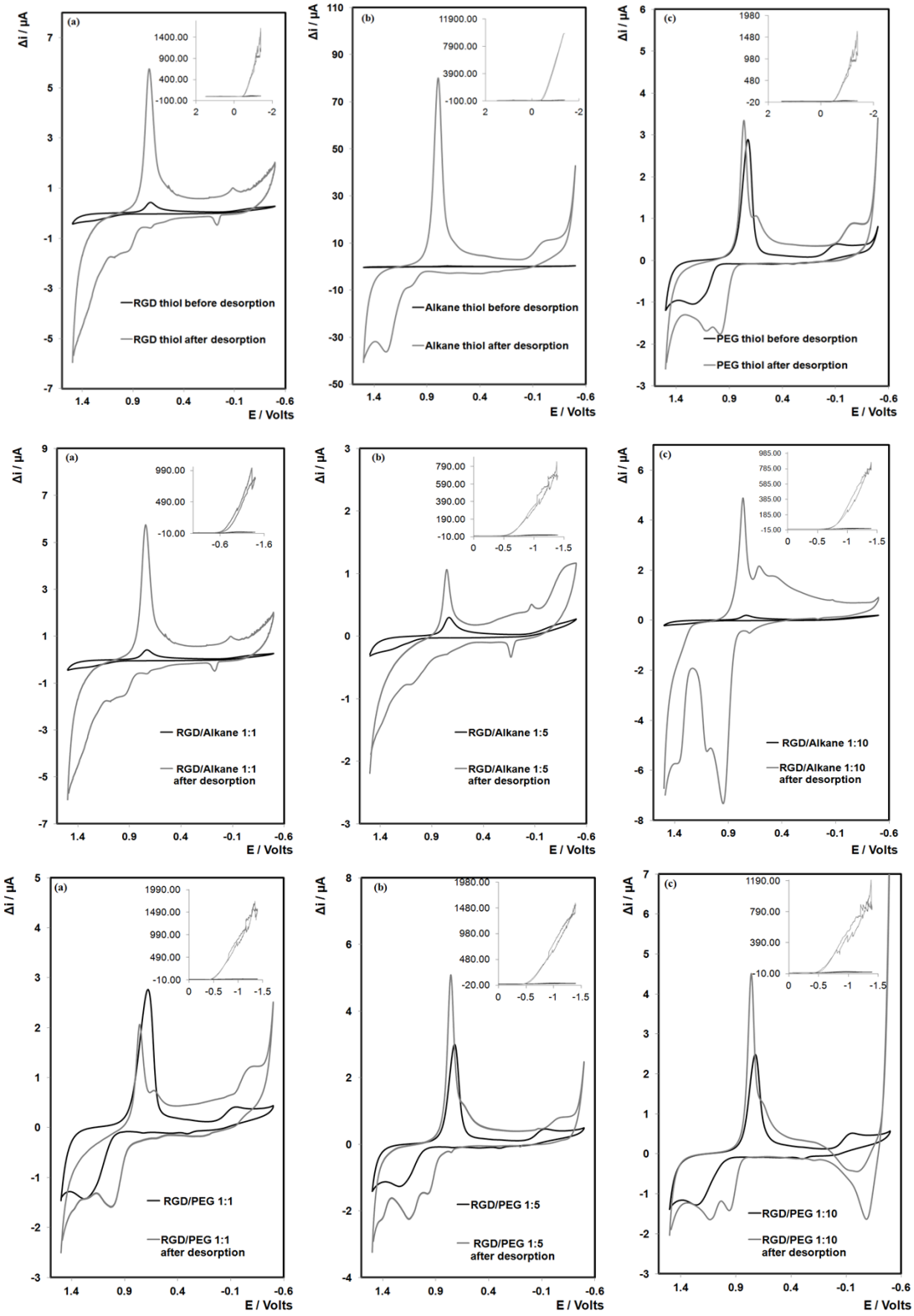

Fig. S4 (Top) Cyclic voltagrams presenting electrochemical desorption of (a) RGD alone, (b) alkane alone and (c) PEG alone. (Middle) Cyclic voltagrams presenting electrochemical desorption of (a) RGD/alkane 1:1 ratio, (b) RGD/alkane 1:5 ratio and (c) RGD/Alkane 1:10 ratio on gold planar electrode surfaces. Cyclic voltagrams presenting electrochemical desorption of (a) RGD/PEG 1:1 ratio, (b) RGD/PEG 1:5 ratio and (c) RGD/PEG 1:10 ratio on gold planar electrode surfaces. 0.4 V and 0.7V represents distinct gold oxidation reduction peaks. Inset: the peak at -1.1V is characteristic of thiol desorption from a gold surface. All CV spectra were recorded in in 0.1 M H_2SO_4 at 100 mVs^{-1}.

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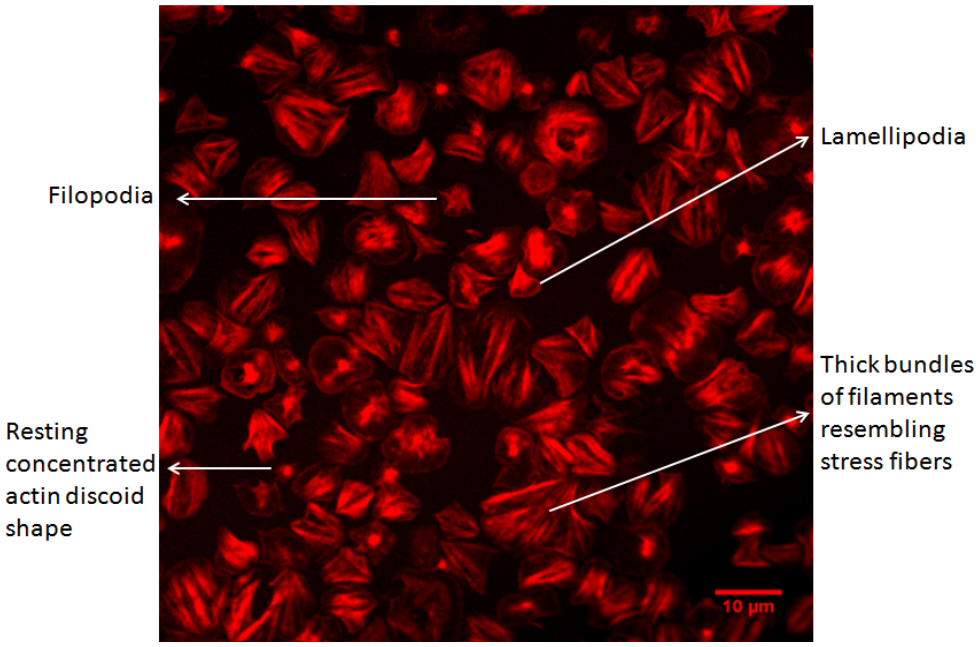

Fig. S5 Confocal luminescence image example of TRITC-phalloidin stained platelets illustrating different actin reorganizations including filopodia, lamellipodia, stress fibers and resting platelets presenting concentrated actin that is discoid in shape. 30 x 10^3± 2 x 10^3/µl washed platelets were incubated with a PEG modified planar surface for 45 minutes at 37°C, fixed with 3.8% PFA solution, stained with TRITC-phalloidin (1/100 dilution) for 30 minutes at room temperature and mounted with fluoroshield mounting media before imaging. Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 540 nm HeNe laser excitation.

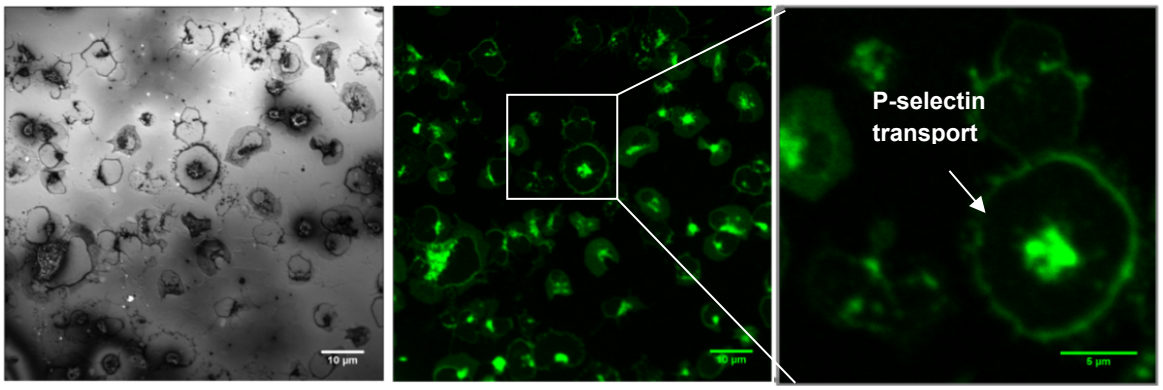

Fig. S6 Confocal luminescence images of glass activated platelets. Brightfield (grey) and CD62P (green). Far right: magnified section of image suggesting platelet activation via CD62P (green) transport towards platelet membrane. 30 x 10^3± 2 x 10^3/µl platelets were incubated with the glass surface for 45 minutes at 37°C. Bound platelets were incubated with CD62P (1/100 dilution) for 15-20 minutes at 37°C, fixed with 3.8% PFA solution for 10 minutes at room temperature and mounted using fluoroshield mounting media. Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) using a 488 nm Argon laser excitation.

Supplemental Data

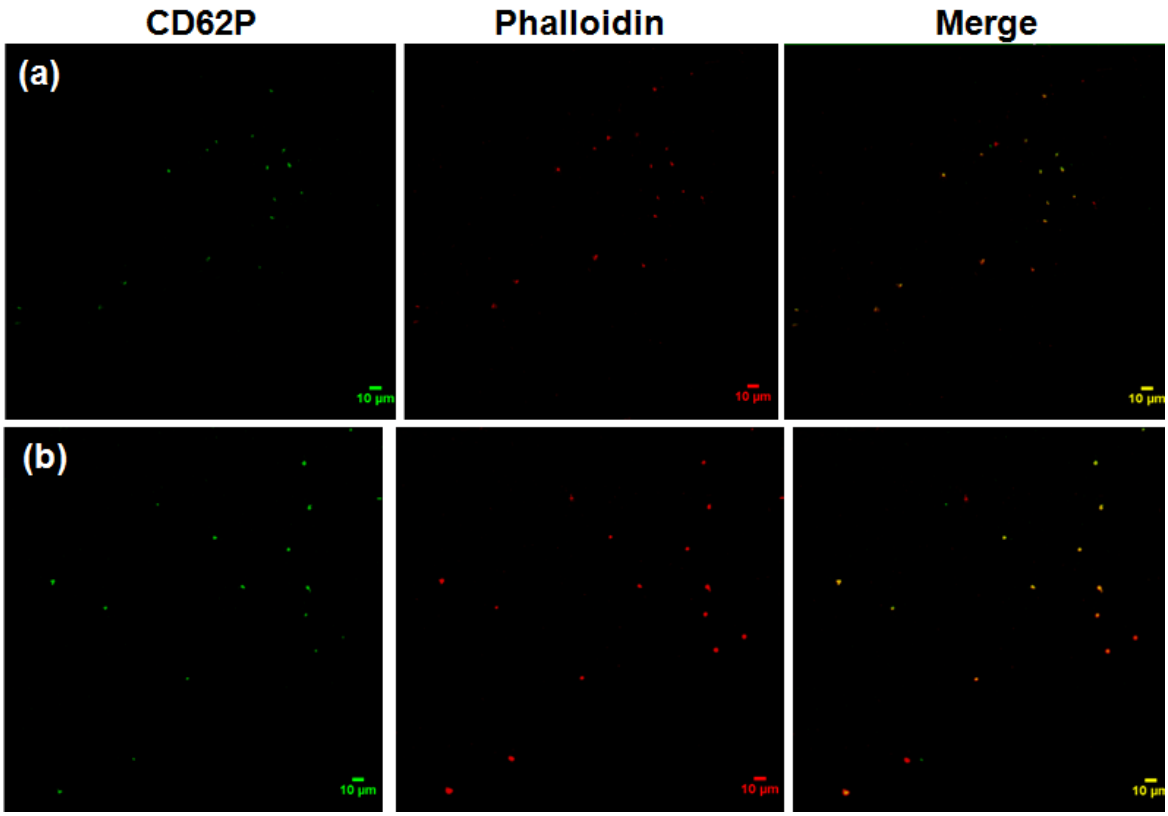

Fig. S7 Luminescent confocal images of platelets bound to (a) gold only and (b) alkane only coated planar gold surface and stained for both PE-CD62P (green) and TRITC-phalloidin (red). Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm argon and 540 HeNe laser excitation.

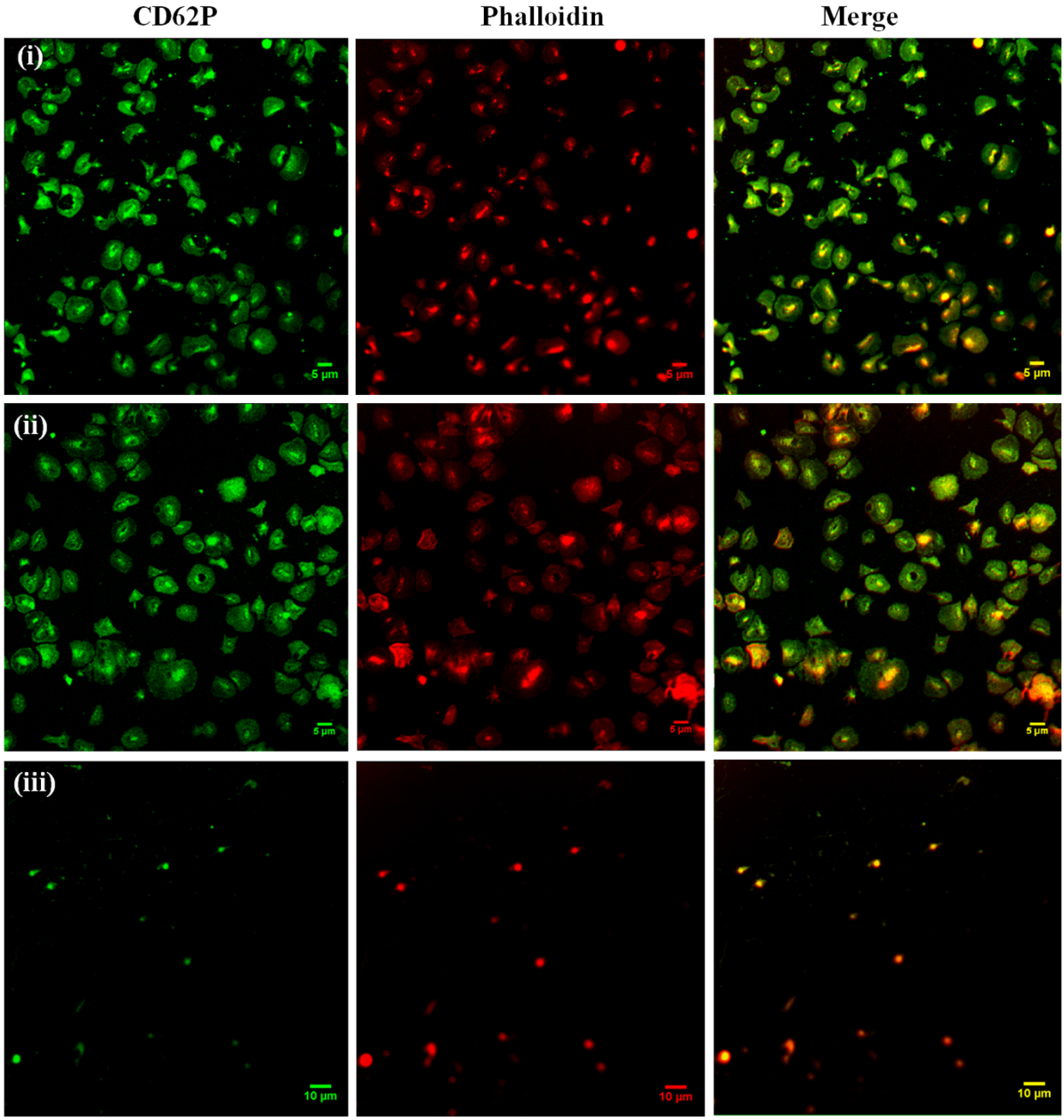

Fig. S8 Confocal luminescence images of platelets bound to planar gold surfaces modified with RGD:alkane (i) 1:1, (ii) 1:5, (iii) 1:10 ratio SAM surfaces stained for PE-CD62P (green) and TRITC-phalloidin (red). 30 x 10^3± 2 x 10^3/µl washed platelets were incubated with the modified surfaces for 45 minutes at 37°C. Bound platelets were incubated with CD62P (1/100 dilution) for 15-20 minutes at 37°C, fixed with 3.8% PFA solution for 10 minutes and stained for phalloidin (1/100 dilution, 30 minute incubation at room temperature). Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm Argon (CD62P) and 540 nm (phalloidin) HeNe laser excitation.

Supplemental Data

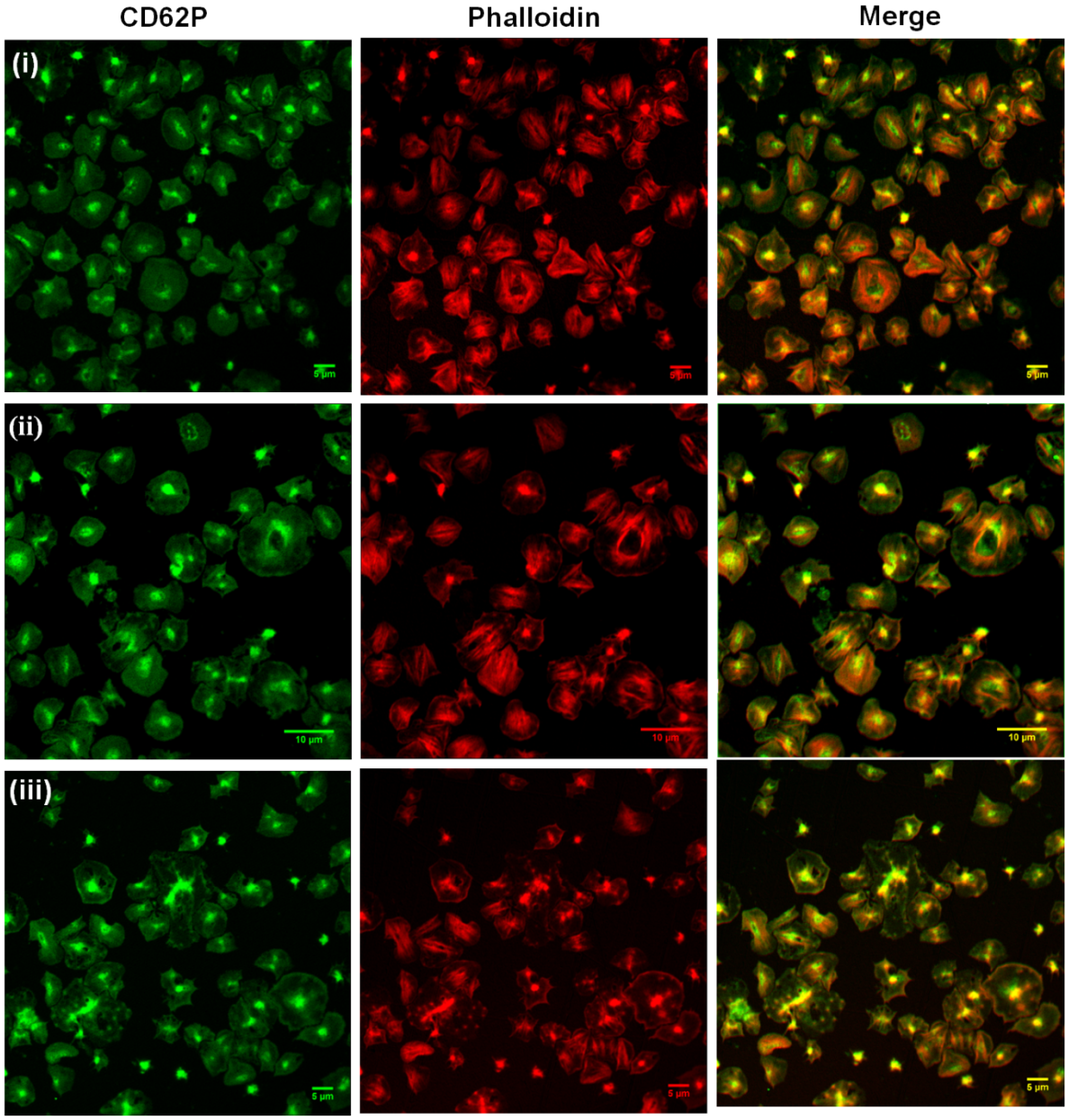

Fig S9 Confocal luminescence images of platelets bound to planar gold surfaces modified with RGD:PEG (i) 1:1, (ii) 1:5, (iii) 1:10 ratio SAM surfaces stained for PE-CD62P (green) and TRITC-phalloidin (red). 30 x 10^3± 2 x 10^3/µl washed platelets were incubated with the modified surfaces for 45 minutes at 37°C. Bound platelets were incubated with CD62P (1/100 dilution) for 15-20 minutes at 37°C, fixed with 3.8% PFA solution for 10 minutes and stained for phalloidin (1/100 dilution, 30 minute incubation at room temperature). Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm Argon (CD62P) and 540 nm (phalloidin) HeNe laser excitation.

Supplemental Data

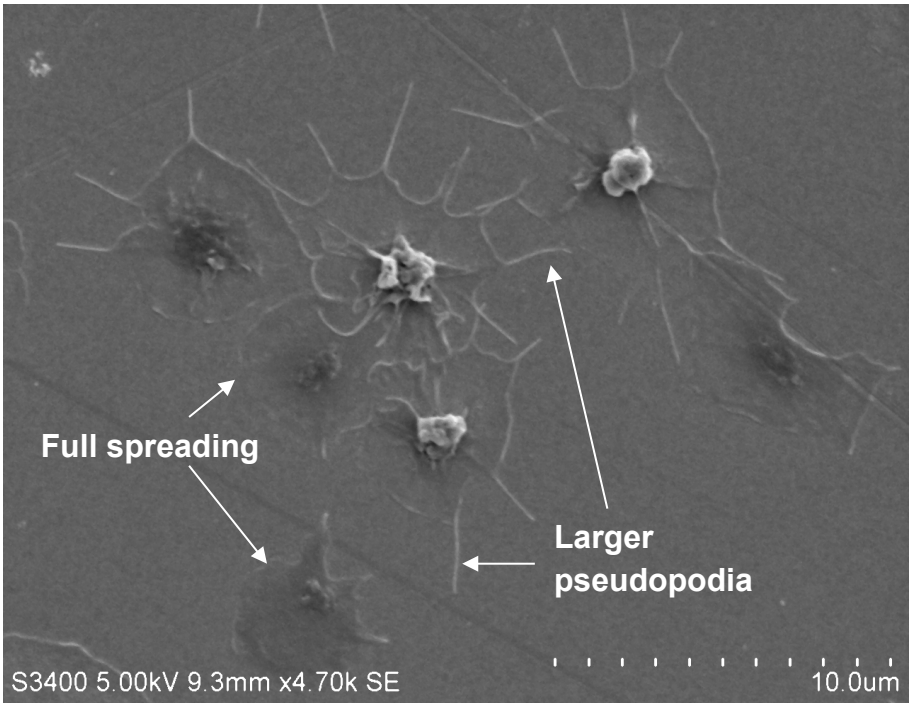

Fig. S10 SEM images of platelets treated with a thrombin (1 U/ml) for 10 minutes at room temperature prior to incubation for 45 minutes at 37°C with C-Ahx-GRGDS modified planar gold surfaces. White arrows highlight examples of larger pseudopodia formation and full platelet spreading. Images were recorded using 5.00 kV accelerating voltage. All images are reproducible, representative of N=3.

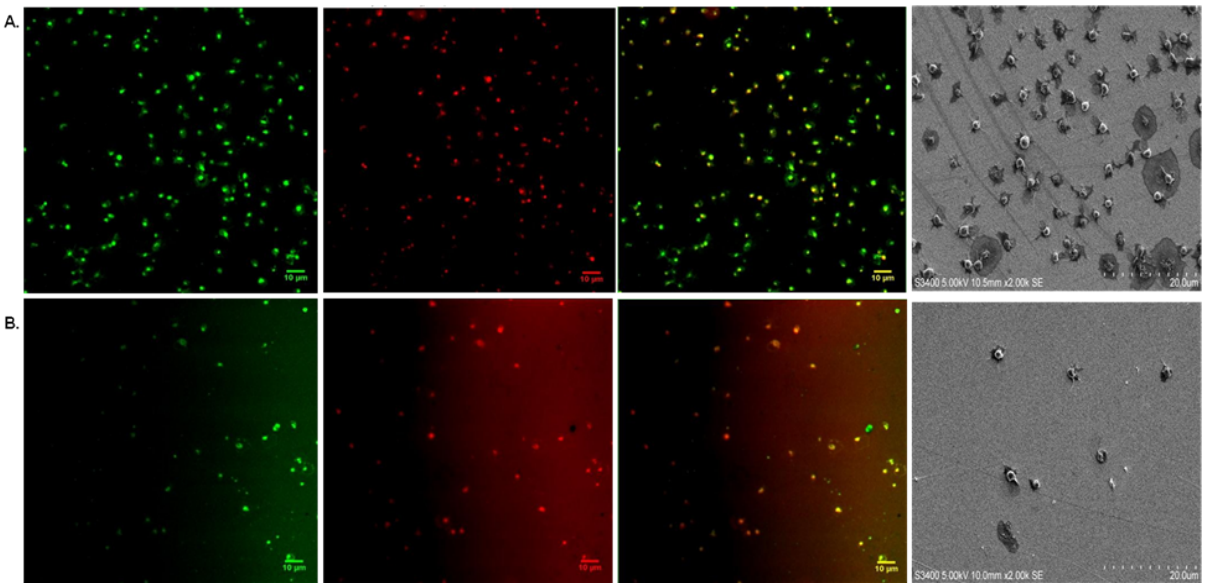

Fig. S11 Confocal luminescence and SEM images of platelets treated with (A) Mn^{2+} or (B) DTT (1 mM final concentration, 15 minutes incubation at room temperature) prior to incubation with planar gold surfaces modified with an RGD SAM. 4.5 x 10^6 platelets were incubated with the RGD SAM surfaces for 45 minutes at 37°C. Platelet fixation, staining, mounting, dehydration and sputter coating was carried out as described. Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm Argon (CD62P) and 540 nm (phalloidin) HeNe laser excitation. SEM images were recorded using 5.00 kV accelerating voltage. All images are reproducible, representative of N=3.

Supplemental Data

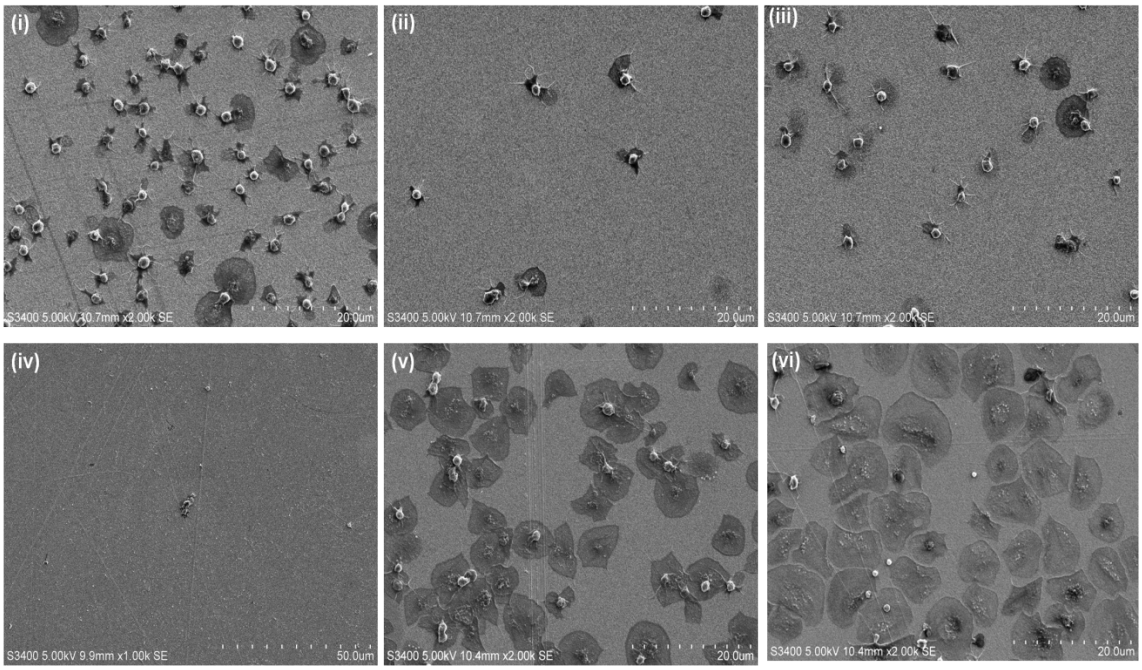

Fig. S12 SEM images of platelet adhesion at (top) C-Ahx-GRGDS and (bottom) PEG-COOH modified planar gold surfaces where platelets were treated post-bind with a 1 mM final concentration of (i, iv) PEG,-COOH (ii, v) C-Ahx-GRGDS or (iii, vi) cyclic RGD peptide drug, Eptifibatide bound to an RGD SAM surface. 30 x 10^3± 2 x 10^3/µl platelets were incubated with the C-Ahx-GRGDS or PEG-COOH SAM surfaces for 45 minutes at 37°C. Following adhesion, bound platelets were incubated with a 1 mM final concentration of PEG, RGD or Eptifibatide for 15 minutes at 37°C. Images were recorded using 5.00 kV accelerating voltage. All images are reproducible, representative of N=3.

Supplemental Data

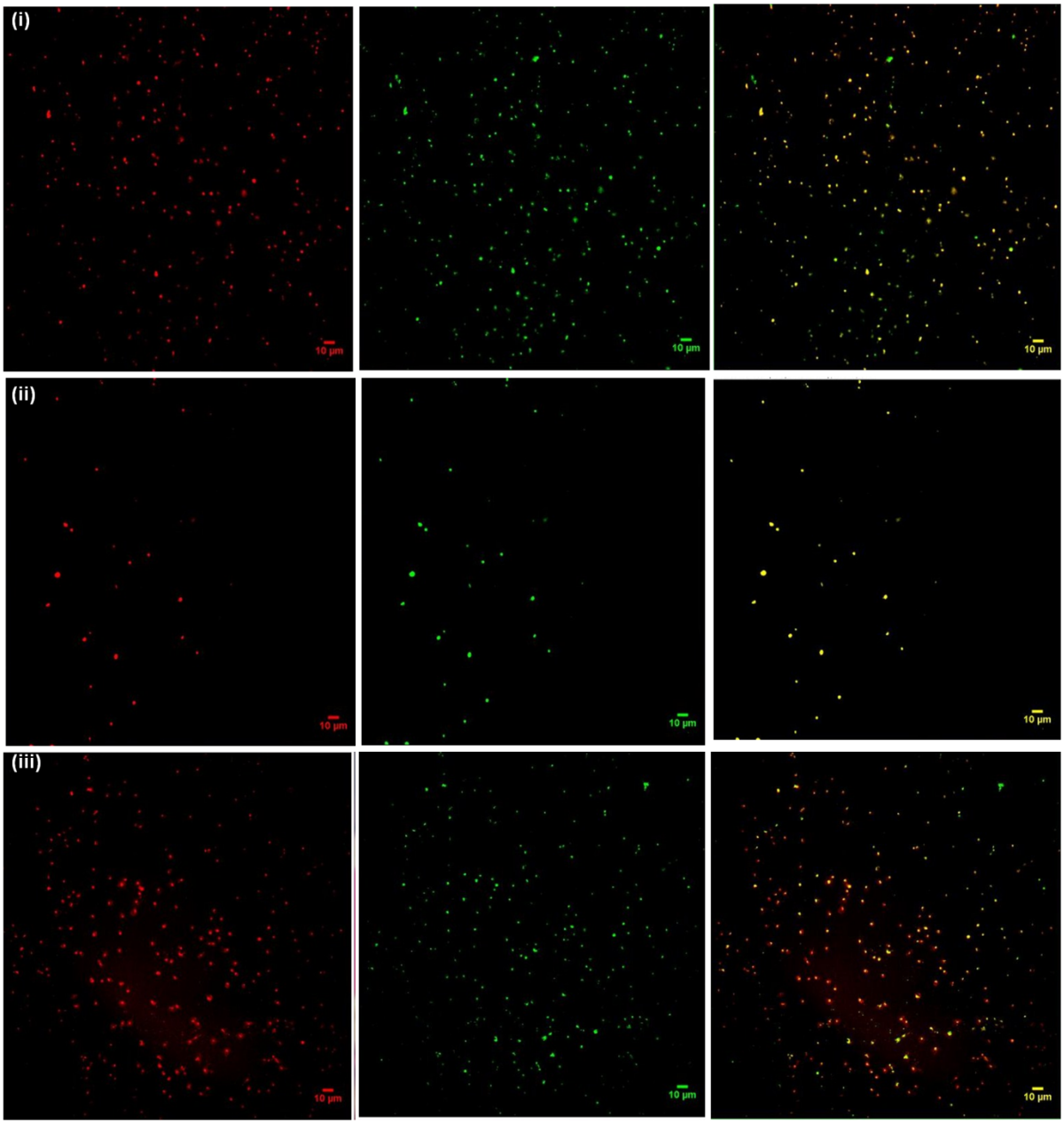

Fig. S13 Confocal luminescence images of platelet adhesion at C-Ahx-GRGDS modified planar gold surfaces where platelets were treated post-bind with a 1 mM final concentration of (i) PEG-COOH, (ii) C-Ahx-GRGDS or (iii) cyclic RGD peptide drug, Eptifibatide bound to an RGD SAM surface. 30 x 10^3± 2 x 10^3/µl platelets were incubated with the RGD SAM surfaces for 45 minutes at 37°C. Following adhesion, bound platelets were incubated with a 1 mM final concentration of PEG-COOH, C-Ahx-GRGDS or Eptifibatide for 15 minutes at 37°C Bound platelets were stained for CD62P (1/100 dilution) and phalloidin (1/100 dilution). Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm Argon (CD62P) and 540 nm (phalloidin) HeNe laser excitation. All images are reproducible, representative of N=3.

Supplemental Data

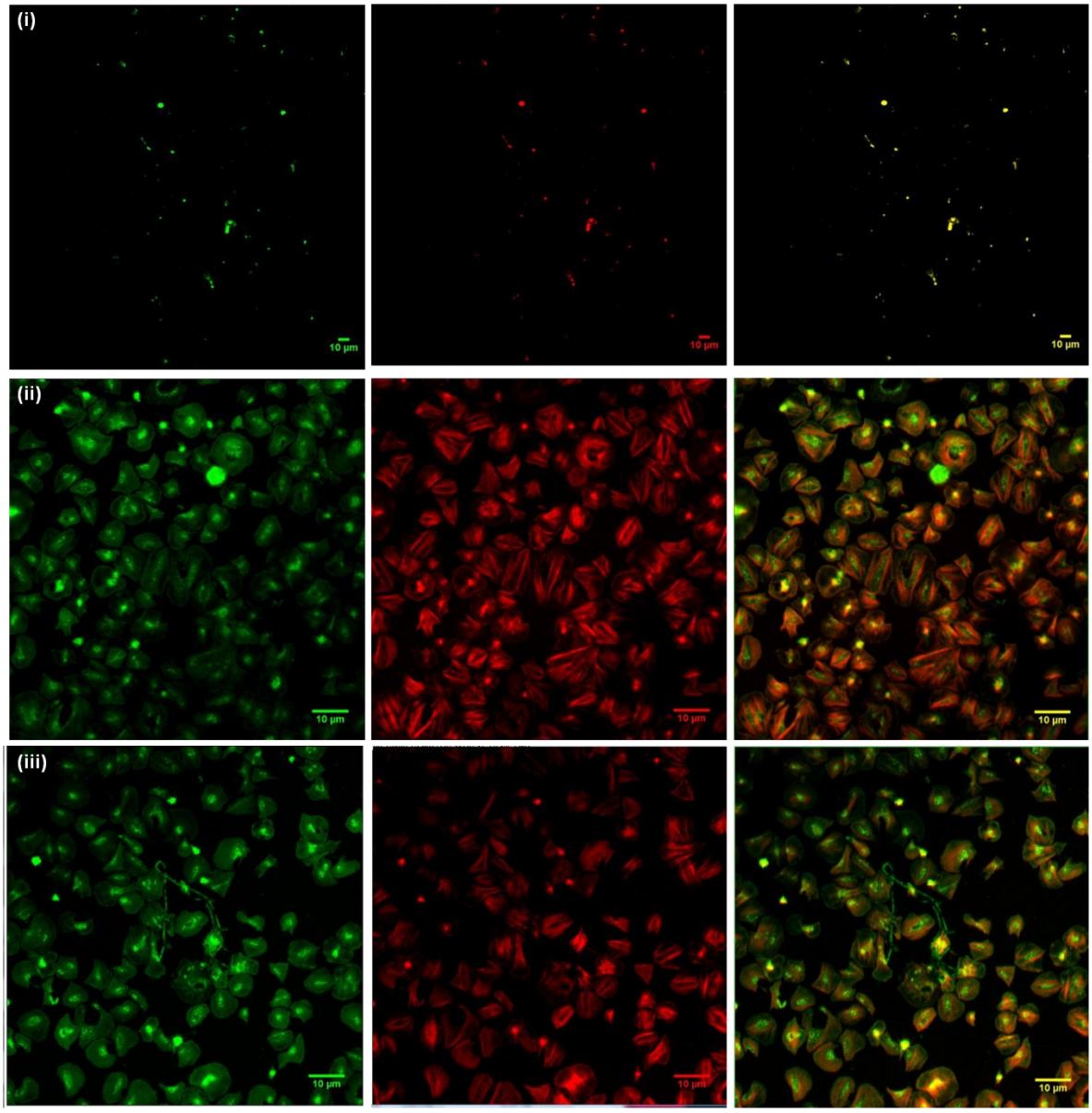

Fig. S14 Confocal luminescence images of platelet adhesion at PEG-COOH modified planar gold surfaces where platelets were treated post-bind with a 1 mM final concentration of (i) PEG-COOH, (ii) C-Ahx-GRGDS or (iii) cyclic RGD peptide drug, Eptifibatide. 30 x 10^3± 2 x 10^3/µl platelets were incubated with the PEG-COOH SAM surfaces for 45 minutes at 37°C. Following adhesion, bound platelets were incubated with a 1 mM final concentration of PEG-COOH, C-Ahx-GRGDS or Eptifibatide for 15 minutes at 37°C. Bound platelets were stained for CD62P (1/100 dilution) and phalloidin (1/100 dilution). Luminescence images were recorded using a 40x oil immersion objective lens (NA 1.4) with 488 nm Argon (CD62P) and 540 nm (phalloidin) HeNe laser excitation. All images are reproducible, representative of N=3.