**Materials and methods**

**Preparation of nanopatterned surfaces**

The production of gold nanopatterned glass surfaces was based on self-assembly of diblock copolymer micelles strategy \(^1,^2\). Patterned glasses were prepared by spin coating of 100 μl solution, then W10 gas (90% Ar/10% H\(_2\)) plasma-treatment for 45 min (0.4 mbar; 350 W), leaving solid gold nanoparticles of about 8 nm on the glass, with varied spacing, in the range of 30-120 nm.

**Passivation of nanopatterned glass surfaces**

Nanopatterned substrates were passivated by first activating the glasses with oxygen plasma for 10 min (0.4 mbar; 150 W) and coating the underlying glass with a layer of PEG-ethoxysilane (Mw=2000 gr/mol). Nanostructured glasses were immersed overnight, under an inert N\(_2\) atmosphere at 80°C in anhydrous toluene (99.8%; Sigma-Aldrich, Israel) containing 5 mg/ml PEG-ethoxysilane, 1% (v/v) triethylamine 99.7% (ACROS organics, USA/Belgium) and 0.01% (v/v) water \(^3,^4\). Thereafter, the substrates were rinsed 10 min with ethyl acetate then methanol 99.8% (Bio-Lab, Israel) twice each, sonicated for 2 min in fresh methanol and dried under nitrogen.

**Synthesis of SN528, a peptidomimetic ligand of αIIb/β3 integrin**
General Procedures and

**Solvents and Reagents.** Solvents were purchased from *Aldrich, Fluka, Merck and Prolabo.* Reactions sensitive to oxygen or water were performed in flame-dried reaction vessels under an argon atmosphere (99.996%). Protected Fmoc-amino acids and coupling reagents were purchased from *Novabiochem* (Schwalbach, Germany), *Iris Biotech GmbH* (Marktredwitz, Germany) and *Medalchemy* (Alicante, Spain). All other chemicals and organic solvents were purchased from commercial suppliers at the highest purity available and used without further purification.

**Analysis.** Semi-preparative HPLC was carried out on a *Beckmann* instrument (system gold, solvent delivery module 126, UV detector 166) using an YMC ODS-A column (20 × 250 mm, 5 μm), at a flow rate of 8 mL/min. Linear gradients using H₂O (0.1 % (v/v) TFA) and MeCN (0.1 % (v/v) TFA) were run over varying periods of time. Analytical HESI HPLC-MS (*heated electrospray ionization mass spectrometry*) was performed on a LCQ Fleet (*Thermo Scientific*) with a connected UltiMate 3000 UHPLC focused (*Dionex*) on C18-columns: Accucore C18, 80 Å, 2.6 μm, 50 x 2.1 mm (for 5 minute measurements) (*Thermo Scientific*). Linear gradients (5% 95% acetonitrile content) with H₂O (0.1% v/v formic acid) and acetonitrile (0.1% v/v formic acid) as eluents were used.

**GP1 Loading of tritylchloride (TCP) resin.** Solid phase synthesis was carried out using TCP resin (0.94 mmol/g) following standard Fmoc strategy. Fmoc-Aax-OH (1.2 eq.) was attached to the TCP resin with *N,N*-diisopropylethylamine (DIEA, 2.5 eq.) in anhydrous DCM (10 mL/g resin) at room temperature for 1 h. The remaining trityl chloride groups were capped by addition of a solution of MeOH/DIEA (4/1, v/v, 1 mL/g resin) for 15 min. The resin was filtered and washed thoroughly with DCM (2x), *N*-methyl-2-pyrrolidone (NMP, 3x) and MeOH (5x). The loading capacity was determined by weight after drying the resin under vacuum.

**GP2 Solid phase N-Fmoc deprotection.** The resin-bound Fmoc peptide was treated with 20 % piperidine in NMP (v/v) for 5 min and a second time for 10 min. The resin was washed with NMP (5x).

**GP3 Solid phase coupling with HATU/HOAt.** A solution of the acid (2 eq.), HATU (2 eq.), HOAt (2 eq.), DIEA (5 eq.) in NMP was added to the resin-bound free amine peptide and shaken for 1.5 h at room temperature. The resin was washed with NMP (5x).
**GP4 Solid phase N-Alloc deprotection.** The resin was washed with DCM (3x) and then treated with a solution of *tetrakis*-triphenylphosphinepalladium (0.25 eq.) and phenylsilane (10 eq.) in DCM at ambient temperature. Care had to be taken due to gas evolution and the pressure had to be released from the reaction vessel from time to time. After 30 min of shaking, the mixture was filtered and the resin washed twice with a 0.5% solution of DDTC (sodium *N*,*N*-diethylldithiocarbamate) in DMF and a 0.5% solution of DIEA in DMF. The washing procedure was repeated (3x) and the resin washed with NMP (5x).

**GP5 Cleavage from TCP resin.** The resin-bound compound was treated three times with a 20% solution of HFIP (hexafluoroisopropanol) in DCM (v/v) for 10 min. The collected solutions were concentrated *in vacuo*.

**GP6 Cbz and trityl deprotection in solution.** Deprotection was achieved by treatment with TFA/TIS/H₂O (95/2.5/2.5, v/v/v) in solution for 4 h. The mixture was coevaporated with toluene (3x).

**4-(3-(6-(3-(tritylthio)propanamido)hexanamido)propoxy)benzoic acid (1)**

Compound 1 was synthesized according to literature (see SI). ⁵

**4-(2-(1-((benzyloxy)carbonyl)piperidin-4-yl)ethoxy)benzoic acid (2)**

Compound 2 was synthesized according to literature. ⁶

**(S)-2-(4-(3-(6-(3-mercaptopropanamido)hexanamido)propoxy)benzamido)-3-(4-(2-(piperidin-4-yl)ethoxy)benzamido)propanoic acid (SN528)**

Prepared from *N*<sub>a</sub>-Fmoc-*N*<sub>b</sub>-Alloc-l-2,3-diaminopropionic acid according the following reaction sequence: Loading of 100 mg resin (GP1), Fmoc deprotection (GP2), coupling of 4-(3-(6-(3-(tritylthio)propanamido)hexanamido)propoxy)benzoic acid (1) (GP3), Alloc deprotection (GP4), coupling of 4-(2-(1-((benzyloxy)carbonyl)piperidin-4-yl)ethoxy)benzamido (2) (GP2), cleavage from the resin (GP5), Trityl and Cbz deprotection (GP6). After semi-preparative HPLC purification compound SN528 was obtained as colorless solid (10.28 mg, 14.4 µmol). RP-HPLC (5-95 %, 5 min) Rt = 2.10 min. MS (HESI): m/z = 357.94 [m+2H]<sup>2+</sup>, 714.33 [m+H]<sup>+</sup>. The IC50 value was determined via an ELISA-like competitive integrin binding assay ⁷ and showed an activity for αIIbβ3 of 3.58 nM.
Functionalization of nanopatterned glass surfaces

Functionalization of the nano-patterned glass substrates was carried out after the substrates passivation process using SN528, (Mw = 713.88 gr/mol). Nanopatterned surfaces that were passivated but not functionalized, served as negative controls. Surfaces were incubated for 1.5 hr at RT with SN528 (25 μg/ml in sterile MiliQ-water), forming SH-gold bonds. Specimens were then rinsed x5, for 10 min each, in sterile MiliQ-water followed by blocking with 1 mg/mlsolution Bovine Serum Albumin (BSA, Sigma-Aldrich, Israel) in sterile PBS, for 20 min. Final washing was with PBS and with Tyrode’s buffer, pH=7.4, containing Dextrose 5 mM, KCl 2.9 mM, NaH₂PO₄ 0.36 mM, HEPES 10 mM, and NaCl 140 mM.

Preparation of Fibrinogen-coated surfaces

Glass coverslips (20x20x0.15 mm) were cleaned with piranha solution, rinsed and sonicated in MiliQ-water. After drying, under nitrogen flow, glasses were sterilized under ultraviolet light for 20 min. The cleaned coverslips were then incubated with 50 μg/ml human fibrinogen (Sigma, Israel) in sterile PBS, for 1 hr, then rinsed (2x5 min) in PBS. Finally, blocking with BSA solution in sterile PBS (1mg/ml, 20 min incubation) was conducted, to reduce non-specific platelet attachment, followed by washing with PBS and with Tyrode’s buffer.

Seeding of platelets on nanopatterned surfaces and immunofluorescence labeling

Human platelets in Platelet-Rich Plasma were isolated from freshly drawn blood of different healthy donors (6), donors were both male and female of ages 25-72 (under Weizmann Institute IRB approval). The donor-to-donor variability was very low, based on the results obtained over multiple experiments. Each experiment was repeated between 3-5 times. The blood was collected into PT Vacutainer tubes (containing sodium citrate). After 10 min of incubation at RT, the blood was centrifuged at 800 rpm for 10 min, and the supernatant, containing platelets and plasma, was collected. Fresh platelets were seeded (10-15x10⁶ per 20x20 mm nanopatterned coverslips) and incubated for 1 hour at 37°C in humidified atmosphere of 5% CO₂ and 95% air). Surfaces were then gently washed with PBS (containing Ca++ and Mg++),
fixed and permeabilized for 2.5 min in PBS containing 0.1% Triton X-100 (Sigma-Aldrich, Israel and 3% paraformaldehyde, then post-fixed with 3% paraformaldehyde in PBS for 25 min. Thereafter, specimens were washed x3 with PBS and triple-labeled with mouse anti-human vinculin monoclonal antibodies (Sigma-Aldrich, Israel), rabbit anti zyxin antibodies and phalloidin-TRITC (1:500). Following 1 hr at RT, the platelets were washed with PBS, and further incubated for 45 min with the secondary antibodies (FITC alexa 488-conjugated anti mouse IgG, and Cy5 647 anti rabbit IgG). After washing with PBS, coverslips were mounted in Elvanol (Mowiol 4-88, Serafon, Ashdod, Israel). Examination of the fixed specimens was carried out with a DeltaVision Elite/ GE Healthcare) using X20/0.7 or X100/1.4 oil objectives. Images were acquired using a CoolSNAP HQ2 CCD camera (Roper Scientific, USA)

**Live-cell imaging and Interference Reflection Microscopy (IRM)**

Platelet attachment and spreading on the nanopatterned surfaces was monitored in real time using the DeltaVision Elite® system, using the WoRx6.0 software. Coverslips were glued, using Picodent twinsil® (gold particles facing up) to the punched (17 mm) bottom of 35 mm petri dishes. Platelets, suspended in Tyrode’s solution, were seeded directly onto the nano-patterned surfaces. Phase-contrast and interference reflection microscopy (IRM) time-lapse imaging of several fields per specimen were carried out using X40/0.75 air objective and X100/1.3 oil objective respectively, at 5 or 10 sec. between frames.

**Cell counting and spreading**

In order to determine the number of platelets attached to the nano-patterned surfaces, images of 180 fields, on average, were acquired, using X20 air objective and analyzed by semi-automated ImageJ macro, using particle analysis method. In every chosen field, image processing included subtracting background, setting of Otsu threshold and converting to mask fitting ellipse for each platelet edges. Following segmentation, automatic counting was performed, calculating also basic morphometric parameters (e.g. mean area, perimeter and axial ratio). Cell spreading and time spent in phases of spreading was determined qualitatively by criteria for defining each phase, they were as follows: The first time filopodia are visible and attached is regarded as the filopodial phase. The lamellipodial phase starts at the primary view of lamellipodial progression.
Routinely, two individuals (RZ and MBH) independently calculated the time spent in each phase.

**Morphometry and Fluorescence Ratio Imaging (FRI)**

Image processing for morphometric analysis was carried out on X100/1.3 oil objective, images, using locally-developed software compatible with the Priism Image Visualization Environment\(^8\)\(^9\). In brief, TRITC, FITC and Cy5 images were subjected to high pass filtration while setting permanent thresholds for all three colors. Cell segmentation was then performed manually. The relative intensities of vinculin and zyxin labeling were calculated using Fluorescence Ratio Imaging (using a locally-developed software, within the Priism environment). Ratio values were displayed in spectral log scale, ranging from 0.33 (blue) to 3.0 (red). All the ratios were normalized linearly by a constant that shifts their average toward a ratio of 1.0.

**High Resolution Scanning Electron Microscopy imaging**

Platelets plated on the nano-patterned surfaces were fixed with 2% PFA + 3% Glutaraldehyde in 0.1M Cacodylate buffer, pH=7.4. The cover-glasses were then dehydrated in ethanol series in MiliQ-water (30%, 50%, 70%, 96%, and 100%) and critical-point dried using a BalTec CPD030 system. The dry samples were coated with a thin layer of carbon in Edwards’s carbon coater. Visualization of the specimens was conducted either in InLens mode using secondary electron (SE) detector and back-scattered electron (BSE) detector in a high-resolution Ultra 55 SEM (Zeiss), using 30 nm aperture size, working distance of 5.0 mm and 3.0 kV voltage.

**Statistics**

Statistical calculations were performed using STATISTICA® software package, using ANOVA, T-test and Kolmogorov-Smirnov programs, as indicated. Standard error or standard deviation are displayed as indicated in the specific figure legends.

**Treatment of spreading platelets with Mn\(^{2+}\)**

Mn\(^{2+}\) (250 μM, final concentration) was added directly to the dish containing fibrinogen in Tyrode's-HEPES buffer pH 7.4 containing 5 mM dextrose, the platelets which were seeded (10-15\(\times\)10\(^6\)) in the microscope at 37°C in humidified atmosphere of 5% CO\(_2\) and 95% air.
Figure [S1]: Platelets seeded on fibrinogen with and without Manganese (Mn++) (A) Platelets displaying filopodial spreading or full spreading to fibrinogen (visualized by IRM). Black arrows point to platelets with filopodia whose spreading was arrested at the filopodial stage; (B)
Platelets were treated by Mn$^{++}$. Platelets displaying filopodial spreading or full spreading to fibrinogen after Mn$^{++}$ treatment. Black arrows indicate platelets that will start to progress into a lamellipodial form after the addition of manganese; (C) The platelets that were arrested in the filopodial stage show lamellipodial spreading (black arrows) (D) Fully spread platelets on fibrinogen at 60 min. The addition of Mn$^{++}$ was at time point 30 min. Black arrows indicate the progression of platelets spreading from filopodial spread to fully spread.

**Movie [S1]:** IRM movie showing the adhesion and spreading of platelets on fibrinogen-coated substrate (corresponding to Figure 3). 100x lens. 5 sec. frame rate. Total movie length 1 h.

**Movie [S2]:** IRM movie showing the adhesion and spreading of platelets on fibrinogen-coated substrate, with Mn$^{++}$ stimulation at 30 min time point (corresponding to Figure S1). 100x lens. 5 sec. frame rate. Total movie length 1 h.

**Movie [S3]:** Phase contrast movies showing the attachment and spreading of platelets on the nano-patterned surfaces with nano-gold spacing of: 30nm and 60nm (top, left to right); 80nm and 100nm (middle, left to right); 120nm (bottom). (Corresponding to Figure 4). 10 sec. frame rate. Total movie length 1 h.
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