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Supporting Information

Minimal Synthetic Cells to Study Integrin-Mediated Adhesion

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Chemicals
Purified lipids L-α-phosphatidylcholine (Egg, Chicken, egg PC) and L-α-phosphatidylglycerol, (Egg, Chicken, egg PG) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Biobeads SM-2 were supplied by Bio-Rad (Munich, Germany). All ingredients for Tris-buffered saline solution (TBS) were obtained from Roth, Germany and Triton X-100 (AppliChem, Germany). Fibrinogen from human plasma was purchased from Calbiochem (San Diego, CA). Collagen type I from calf skin was supplied by Sigma Aldrich (Munich, Germany). Phosphate buffered saline **(**PBS) tablets were purchased from Life Technologies GmbH (Darmstadt, Germany). GRGDSP peptide with a molecular weight of 587 Da (PSL, Heidelberg, Germany) and AB1967 anti-integrin αIIb antibodies were provided by Merck (Darmstadt, Germany). Selective ligands for integrin $\alpha_{IIb}\beta_3$ with a molecular weight of 498 Da (SN529) were a kind gift of Horst Kessler (Technische Universität München). Buffer A with pH 7.4 was prepared according to the protocol of Erb and Engel from 20 mM Tris-HCl (Carl Roth, Karlsruhe, Germany), 50 mM NaCl (Carl Roth) and 1mM $CaCl_2$ (Roth, Germany) [31]. Buffer B was prepared by adding 0.1% (w/v) of Triton X-100 to buffer A. For the activation of integrin 1mM $MnCl_2$ (Roth, Germany) and 1mM $MgCl_2$ (Roth, Germany) were added to buffer A during all QCM-D measurements. All solutions were prepared with nanopure water from a TKA GenPure system (TKA, Germany).

Protein Purification
Integrin $\alpha_{IIb}\beta_3$ was purified from outdated human blood platelets (Katharinenhospital Stuttgart, Germany) using TBS and Triton X-100. Affinity chromatography over Concanavalin A and Heparin columns was followed by gel filtration over Superdex 200 prep grade columns. All columns were purchased from GE Healthcare (Munich, Germany). Biological activity of the purified integrin was analyzed by an enzyme-linked immunosorbent assay (ELISA) using AB1967 anti-integrin α_{IIb} antibodies. Fibronectin was isolated from human plasma by gel filtration and affinity chromatography over a Sepharose CL-4B column (Sigma), followed by a gelatin Sepharose column (GE Healthcare). Fibronectin was eluted by 6 M urea (Sigma) in PBS and dialyzed against PBS before use.

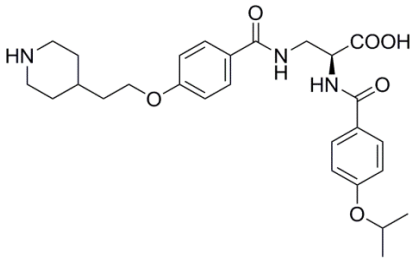
Integrin reconstitution into liposomes
We prepared liposomes by adapting the protocol of Erb and Engel and dried 50 mol% of egg PC and egg PG, respectively, in nitrogen and vacuum overnight.[31] When the dried lipids were dissolved in 1 ml of buffer B we added integrin at a 1:1000 integrin-lipid ratio and incubated the solution at 37°C for 2 hours. Triton X-100 was removed in two subsequent washing steps of 3.5 hours using 50mg/ml SM-2 Bio-beads. The size distribution of liposomes and integrin-liposomes was measured by dynamic light scattering (DLS) in a Malvern Zetasizer Nano ZS setup (United Kingdom).

Quartz crystal microbalance measurements with dissipation monitoring
Adsorption experiments with integrin-liposomes were performed with the QCM-D technique using an E4 System from Q-Sense (Västra Frölunda, Sweden). The measured signals from the piezoelectric quartz crystal sensor were the frequency shift ΔF and the energy dissipation change ΔD. SiO_2 coated sensor crystals were provided from Q-sense (QSX 303, Västra Frölunda, Sweden) and cleaned with a 2% Hellmanex III solution (Hellma, Germany) followed by exposure to oxygen plasma at 150 W with 0.4 mbar for 30 minutes (Gigabatch, PVA TePla AG, Germany). The system was operated in flow mode with a flow of 25 µl/min. Sample solution was continuously delivered to the crystal chamber by a peristaltic pump (Ismatec IPC, IDEX, Germany) and the working temperature was set to 21°C. Resonance frequency and dissipation were measured at 6 harmonics (15, 25, ..., 65 MHz) simultaneously. The operating frequency for the results reported here is the 7th overtone (35 MHz) and is normalized by the overtone. After equilibrating the system with buffer A containing 1mM $MgCl_2$ and 1mM $MnCl_2$ for 40 minutes (step I), the crystals were coated with either fibronectin, fibrinogen or collagen diluted in buffer A with $MgCl_2$ and $MnCl_2$ at a concentration of 50 µg/ml. During the adhesion of ECM proteins for 2.5 hours the frequency decreased and the dissipation increased accordingly (see Table 1, 3.-5. and Figure 2b to 2d, step II). Binding of fibrinogen (Figure 2b) for 2.5 hours and a consequent 30-min washing step (step III) with buffer A led to a reduction of the resonance frequency around $\Delta F_{Fg} = -98.8 \pm 2.2\ Hz$ and an increase in dissipation by roughly $\Delta D_{Fg} = 3.46 \pm 0.06 * 10^{-6}$. For fibronectin (Figure 2c) the frequency and dissipation were shifted by $\Delta F_{Fn} = -74.3 \pm 2.2\ Hz$ and $\Delta D_{Fn} = 3.04 \pm 0.07 * 10^{-6}$, respectively. Collagen binding (Figure 2d) led to a resonance frequency decrease of $\Delta F_{Col} = -151 \pm 4\ Hz$ and a dissipation increase of $\Delta D_{Col} = 34 \pm 1 * 10^{-6}$. After the washing step liposomes, integrin-liposomes or integrin were loaded into the flow system (step IV). Finally, in a fifth step the crystals were washed for 4 or more hours with buffer A containing $MgCl_2$ and $MnCl_2$.
We also calculated the thickness of the three different ECM protein layers on the quartz crystals according to the Voigt and the Sauerbrey model [26, 32] The Sauerbrey model, a model for thin rigid films, provides a lower limit of the layer thickness. The Voigt model also takes the viscoelasticity of the film into account, thus yielding more accurate results. As an estimate for the density of the protein coatings we used comparable values from literature in both models.[33] The fits were carried out with the software QTools from Q-Sense and are listed in Table S1. Our calculations show that all protein layers have a minimum thickness of 10 nm. This indicates that our protein coatings form a closed layer and can prevent any direct interaction between the crystals and the samples.

Table S1. Thickness calculations of the different ECM proteins on SiO_2 crystals according to the Voigt and Sauerbrey models. As an estimate for the protein density comparable values from [29] were used in both models.

	Fg		Fn		Collagen Type I	
Density [29]	Voigt (nm)	Sauerbrey (nm)	Voigt (nm)	Sauerbrey (nm)	Voigt (nm)	Sauerbrey (nm)
1.150 [g/cm^3]	16.1 ± 1.6	15.1 ± 0.3	17.4 ± 0.4	11.0 ± 0.6	56 ± 12	20.4 ± 0.5
1.300 [g/cm^3]	14.3 ± 1.5	13.3 ± 0.3	15.0 ± 0.4	9.7 ± 0.5	54 ± 8	18.0 ± 0.5

Synthesis of compound SN529



sn529

Chemical Formula: $C_{27}H_{35}N_3O_6$
Exact Mass: 497,25
Molecular Weight: 497,59

The compound was synthesized on solid support[1] according to Fmoc strategy[2] and bulid up analogous as reported by Heckmann et al.[3] After loading of 100 mg resin (real loading: ~74 µmol) with *N*-α-Fmoc-*N*-β- Alloc-L-diaminopropionic acid and Fmoc deprotection 4-isopropoxybenzoic acid was coupled. After Alloc deprotection and coupling of 4-[2-*N*-(benzyloxycarbonyl) piperidine-4-ylethyloxy]-benzoic acid[4] the compound was cleaved from the resin and Cbz was deprotected. After semipreparative HPLC purification SN529 was obtained as colorless solid (17.3 mg, 34.8 µmol, 47%). The IC_{50} value was determined via an ELISA-like competitive integrin binding assay[5] and showed an activity for αIIbβ3 of 30.8 nM. HPLC (10-90%, 5 min) R_t = 2.45 min. MS (ESI): m/z = 498.19 $[m+H]^+$, 995.12 $[2m+H]^+$. The compound was used as a Tirofiban[4,6] analogue with the intension for further functionalization.

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