Control of Frictional Coupling of Transmembrane Cell Receptors in Model Cell Membranes with Linear Polymer Spacers

Oliver Purrucker,[1] Anton Förtig,[2] Rainer Jordan,[2,*] Erich Sackmann,[1] and Motomu Tanaka[1,3,†]

[1]*Lehrstuhl für Biophysik E22, Technische Universität München, D85748 Garching, Germany*

[2]*Lehrstuhl für Makromolekulare Stoffe, Technische Universität München, D85748 Garching, Germany*

[3]*Physikalisch-Chemisches Institut, Universität Heidelberg, D69120 Heidelberg, Germany*

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The planar plasma membrane model with linear polymer spacers with defined lengths enables the control of the frictional coupling between incorporated transmembrane proteins (human platelet integrin) and the solid substrate. This mimics the viscous environment provided by the extracellular matrix of cells. The friction coefficient can be calculated quantitatively from the diffusion coefficient of integrin, measured by fluorescence recovery after photobleaching. The obtained results demonstrate a clear influence of the length and lateral density of polymer chains on the mobility of transmembrane proteins.

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The natural plasma membrane separates the intracellular space from the surrounding environment under preservation of selective material exchange across it. In the *fluid mosaic model* [1], the cell membrane is described as a ''two-dimensional oriented solution of integral proteins in the viscous phospholipid bilayer solvent.'' The model has been improved over the years by allowing for lateral phase separation within multicomponent membranes, binding of proteins to cytoskeleton or extracellular matrices, confinement of proteins to domains by the cytoskeleton and tight junctions, and formation of large protein-lipid aggregates as functional complexes [2,3].

Among several artificial cell membranes, a lipid bilayer deposited onto a planar substrate (supported membrane) possesses an excellent mechanical stability to coat macroscopically large surfaces. In spite of remarkable success as plasma membrane model, the close proximity of the membrane to the substrate does not provide a sufficiently thick lubricant layer to avoid denaturing of protein subunits facing to the solid. Particularly, the direct protein-substrate contact causes strong suppression of the lateral diffusion of transmembrane proteins. For instance, this can be overcome by deposition of ultrathin polymer supports (typical thickness ≤ 100 nm) that separate the membrane from the underlying solid substrate [4]. Previously, we reported that a thin cellulose (≈ 10 nm) film significantly improves the lateral, homogeneous distribution, the mobility, and the functionality of transmembrane cell receptors [5]. More recently, we demonstrated the fabrication of another class of supported membranes, which uses hydrophilic poly(2-methyl-2-oxazoline) spacers to increase the membrane-substrate distance [6]. This so-called polymer-tethered membrane can serve as suitable platform for incorporation of transmembrane proteins, where we observed a clear dependence of the homogeneity of protein distribution on the length of the polymer spacers. In fact, as reported by some *in vitro* studies, the lateral mobility of cell adhesion molecules is known to significantly influence the strength of cell adhesion due to the cooperative accumulation of ligand-receptor pairs [7].

Here, we demonstrate that the lateral mobility of the transmembrane receptors in this model membrane can be controlled by the lateral density and length of polymer spacers, which mimics the natural role of the extracellular matrix. As a receptor molecule, we chose human platelet Integrin aIIbb3 receptors. They are cell adhesion molecules expressed on human blood platelets, and play a critical role in thrombosis and hemostasis [8]. The theory of Evans and Sackmann [9] was consulted to evaluate the influence of the frictional coupling of reconstituted membrane proteins to the solid substrate on their diffusion.

As a basic theory of diffusion, the continuum hydrodynamic model proposed by Saffman and Delbrück was chosen [10,11]. Here, a lipid membrane can be considered as a two-dimensional continuum, while the concreteness of the lipid molecular structure is ignored. The motion of proteins is driven by random, fluctuating forces provided by unbalanced collisions with the solvent molecules (= lipids) and is resisted by the frictional forces inherent in a viscous solvent (=lipid bilayer). The diffusing molecule (=protein) is represented as a cylinder of height h and radius R. Its diffusion coefficient D is given by the Einstein relation, $D = k_B T/f$, where k_B is the Boltzmann constant, T the temperature, and f is the drag coefficient.

However, in contrast to the classical theory that assumes a ''free'' lipid membrane with a symmetric boundary condition the interfacial drag σ of the membrane on the substrate has to be considered, when the membrane is associated with a solid substrate by a thin lubricant film (Fig. 1).

In the work of Evans and Sackmann [9], σ was assumed to be proportional to the local velocity v of the membrane relative to the substrate $\sigma = b_s v$, where b_s is the intrinsic friction coefficient between the membrane and the substrate. b_s is therefore dependent on the viscosity η_l of the lubricant film within the membrane-substrate cleft and its thickness d, $b_s = \eta_l/d$. The drag coefficient f can be

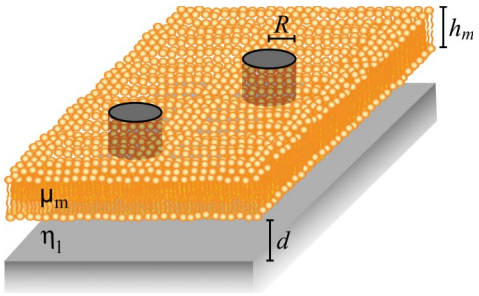

FIG. 1 (color). Hydrodynamic model of the membrane (viscosity μ_m) in close proximity of a substrate, separated by a thin lubricant layer of thickness d and viscosity η_l. Transmembrane proteins are treated as cylinders with height h and radius R.

expressed as a function of the dimensionless particle radius ε of the diffusant,

$$f = 4\pi\mu_m\left(\frac{1}{4}\varepsilon^2 + \frac{\varepsilon K_1(\varepsilon)}{K_0(\varepsilon)}\right), \quad (1)$$

where μ_m is the two-dimensional membrane viscosity, K_0 and K_1 are modified zero and first orders Bessel functions of the second kind. ε is defined as $\varepsilon = R\sqrt{b_s/\mu_m}$. Since b_s is dependent on the liquid viscosity η_l and the distance d between membrane and substrate, the dimensionless particle radius ε is a measure for the environment of the particle (= protein) with radius R. Dependent on the magnitude of ε, one can discuss two limiting cases. When the membrane is very weakly coupled to the substrate ($\varepsilon \ll 1$), the Saffman-Delbrück law still holds,

$$f = 4\pi\mu_m\left(\ln\frac{1}{\varepsilon}\right)^{-1}. \quad (2)$$

On the other hand, when the membrane is strongly coupled to the substrate surface ($\varepsilon \gg 1$), one obtains

$$f = \frac{\pi\eta_l R^2}{d}. \quad (3)$$

Diffusion of transmembrane cell receptors.—The preparation of polymer-tethered membranes and incorporation of fluorescently labeled integrin $\alpha_{\mathrm{IIb}}\beta_3$ were reported in our previous account [6]. Since the homogeneity of the integrin distribution depends on the length of the tether, only lipopolymers with larger degrees of polymerizations n were used in this study: DS-PMOx$_{33}$-Si with $n = 33$, and DS-PMOx$_{104}$-Si with $n = 104$. All the other abbreviations are explained in the experimental section.

After reconstitution, the diffusivity and mobile fraction of integrin receptors were measured by fluorescence recovery after photobleaching (FRAP) technique (the diameter of the bleaching spot: 9.3 μm). Figure 2 shows a typical fluorescence intensity recovery curve, which yielded the diffusion coefficient of $D = (0.10 \pm 0.01)\ \mu\mathrm{m}^2\,\mathrm{s}^{-1}$ and the mobile fraction of $R = (26 \pm 1)\%$.

In Table I, the results of FRAP experiments are summarized. The diffusivity and mobile fraction of incorporated proteins exhibited a systematic dependence on lipopolymer length and density. Comparing the results on membranes with 5 mol% ($X_p = 0.05$) and 0.5 mol% ($X_p = 0.005$) DS-PMOx$_{33}$-Si in the underlayer, an increase in the diffusion coefficient D (from 0.08 to 0.11 $\mu\mathrm{m}^2\,\mathrm{s}^{-1}$) and the mobile fraction R of integrin (from 16% to 21%) could be observed. The increase in the polymer chain length from $n = 33$ to $n = 104$ led to higher values of D and R: $D = 0.13\ \mu\mathrm{m}^2\,\mathrm{s}^{-1}$ and $R = 24\%$.

To confirm that the obtained R values coincide with the fraction of mobile proteins but not with the drift in the optical pathway, a second bleaching experiment was carried out subsequently at the same spot. Here, much higher recovery rates ($R \approx 90\%$) could be gained, while the corresponding diffusion coefficients D were almost identical. This result verifies that the relatively low recovery rates (15%–25%) correspond to the fraction of integrins that protrude their large extracellular domains to the bulk solution [5]. Another possible scenario is the clustering of integrin receptors that can also lead to a lower mobile fraction R [12].

On the other hand, the increase in diffusivity and mobile fraction with decreasing density of lipopolymer tethers can be explained by a lower viscosity of the interlayer between membrane and substrate, since $D \propto \eta_l^{-1}$ [see Eq. (3)]. Assuming an average area of $A_l \approx 0.7\ \mathrm{nm}^2$ per phospholipid in the fluid state [13], the average distance between lipopolymers can be approximated to be $d_p = \sqrt{A_l/X_p} \approx 3.8$ nm ($X_p = 0.05$) and $d_p \approx 11.8$ nm ($X_p = 0.005$). Therefore, the increase in the molar fraction of lipopolymers, i.e., the larger volume fraction of polymer chains within the water reservoir, causes an increase in the entire viscosity of the interlayer.

The increase in diffusivity and mobile fraction with increasing length of lipopolymer spacers can be explained by the enlargement of the interlayer thickness between membrane and substrate, since $D \propto d$ [see Eq. (3)].

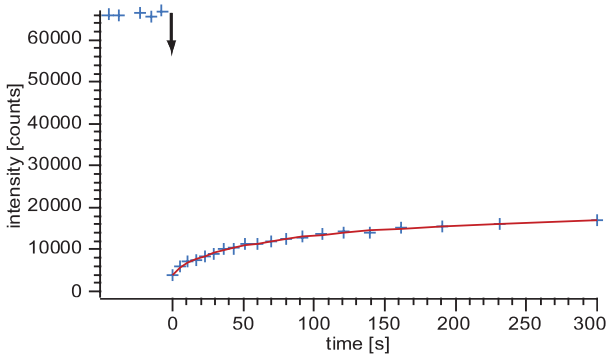

FIG. 2 (color). Fluorescence intensity recovery curve, the line illustrates the fit of the data according to the theory of Soumpasis [25]. The arrow denotes the moment of the laser bleaching pulse at $t = 0$ s. Proteoliposomes were spread onto a dry Langmuir-Blodgett monolayer composed of 0.5 mol% DS-PMOx$_{104}$-Si and 99.5 mol% SOPC. The evaluation of the experimental data yielded $D = (0.10 \pm 0.01)\ \mu\mathrm{m}^2\,\mathrm{s}^{-1}$ and $R = (26 \pm 1)\%$.

TABLE I. Diffusion coefficients D and mobile fractions R of integrin $\alpha_{IIb}\beta_3$ incorporated into polymer-tethered membranes with various concentrations of DS-PMOx$_n$-Si lipopolymers and SOPC matrix lipids in the underlayer.

Lipopolymer fraction		D (μm^2 s^{-1})	R (%)
5 mol%	DS-PMOx$_{33}$-Si	0.08 ± 0.05	16 ± 6
0.5 mol%	DS-PMOx$_{33}$-Si	0.11 ± 0.04	21 ± 10
0.5 mol%	DS-PMOx$_{104}$-Si	0.13 ± 0.06	24 ± 8

The estimated mobile fraction R of integrins in membranes with 0.5 mol% DS-PMOx$_{104}$-Si of $R = (24 \pm 8)\%$ agrees well with the value reported by Gönnenwein *et al.* [5] for the integrin doped membrane deposited onto a cellulose film with a dry film thickness of 5 nm.

The only reports on diffusion measurements of proteins reconstituted into polymer-tethered membranes are from Tamm and co-workers using poly(ethylene glycol) spacers [14,15]. The diffusion coefficients of cytochrome b_5, annexin V [14], and target SNARE [15] were in the range of 0.3–1.3 μm^2 s^{-1}, but a quantitative discussion on the physical origin of lateral diffusion was missing. Furthermore, a systematic control of the length and the density of polymer spacers has not been performed. Thus, this is the first quantitative study that demonstrates the control of frictional coupling between proteins and solid substrates using tunable polymer spacers.

Determination of frictional coupling.—Figure 3 represents a plot of the dimensionless mobility $m = 4\pi\mu_m/f$ versus the dimensionless particle radius ε, calculated from Eq. (1). Thus, the friction coefficient $f = k_BT/D$ can be derived using the measured diffusion coefficients D. For the two-dimensional membrane viscosity, a value of $\mu_m = 1.6 \times 10^{-10}$ N s m^{-1} was chosen [16].

From the dimensionless particle mobilities m, the corresponding dimensionless particle radii can be obtained, $\varepsilon = 8.3$ (5 mol% DS-PMOx$_{33}$-Si), $\varepsilon = 6.8$ (0.5 mol% DS-PMOx$_{33}$-Si), and $\varepsilon = 6.1$ (0.5 mol% DS-PMOx$_{104}$-Si). As depicted in the figure, the classical Saffman-Delbrück theory [Eq. (2)] does not hold, since the values of ε are all $\varepsilon > 1$. The approximation for $\varepsilon \gg 1$ [Eq. (3)] is also not applicable to this system because the deviation from the exact theory is still large when $\varepsilon < 10$.

By assuming the radius of the transmembrane part of integrin $\alpha_{IIb}\beta_3$ to be $R = 0.64$ nm, the friction coefficient b_s can be evaluated [17,18].

Table II summarizes calculated values of b_s for integrin, which are in the range of 1.5 to 2.7×10^8 N s m^{-3}. Decreasing the fraction of lipopolymers from 5 to 0.5 mol%, which coincides with an increase in lateral distance from 3.8 to 11.8 nm while keeping the length of the polymer constant ($n = 33$), the friction coefficient was reduced to 1.8×10^8 N s m^{-3}. A reduction of b_s to 1.5×10^8 N s m^{-3} and hence a further decoupling of the membrane from the substrate was achieved by introduction of longer polymer spacers ($n = 104$), which led to an increase in distance from $d = 2.3$ nm (DS-PMOx$_{33}$-Si) to 4.8 nm (DS-PMOx$_{104}$-Si), measured by fluorescence interference contrast microscopy [19–21]. In literature, there are only a few reports on frictional coupling of diffusants in bilayer membranes. Kühner *et al.* [16] investigated the diffusion of phospholipids in bilayer membranes on polyacrylamide polymer supports. There, the values of b_s were about 10 times smaller than that of integrin in our experiments. But a comparison is difficult, since their system was quite undefined, for instance the distance between membrane and hydrated polymer support (with a thickness of several μm) was unknown. In contrast, in our experimental system, parameters such as membrane-substrate distance and lateral tether density can be controlled much more precisely.

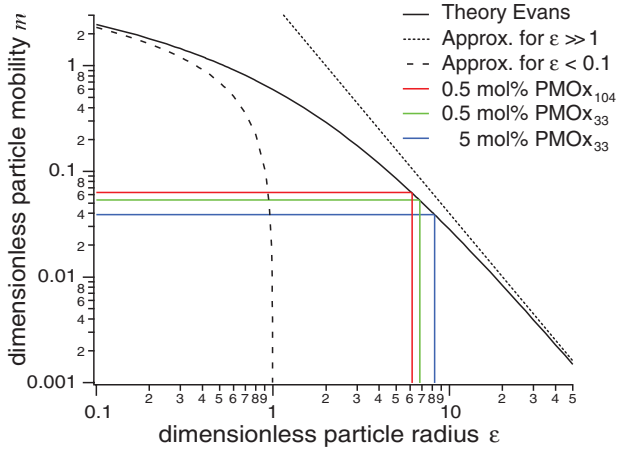

FIG. 3 (color). Dimensionless particle mobility $m = (4\pi\mu_m D)/(k_BT)$ vs dimensionless particle radius ε according to Eq. (1). The dashed line reflects the Saffman-Delbrück theory for weak coupling [$\varepsilon < 0.1$, Eq. (2)], whereas the dotted line visualizes the approximation for strong coupling [$\varepsilon \gg 1$, Eq. (3)].

The three-dimensional viscosity η_l of the lubricant layer is determined by its thickness and the friction coefficient of the diffusant ($\eta_l = b_s d$). Since d is known from interferometric measurements [21], η_l can be calculated for the presented polymer-tethered membrane model. Using DS-PMOx$_{33}$-Si lipopolymers, the viscosity decreases from 0.63 to 0.42 N s m^{-2} by enlargement of the average tether distance from 3.8 to 11.8 nm. Hence, the lower volume fraction of polymer in the lubricant layer led to a reduction of η_l. Furthermore, when keeping the tether

TABLE II. Friction coefficients b_s of integrin $\alpha_{IIb}\beta_3$ and liquid viscosity η_l of the water reservoir of membranes tethered with DS-PMOx$_n$-Si lipopolymers.

Lipopolymer fraction		b_s (N s m^{-3})
5 mol%	DS-PMOx$_{33}$-Si	2.7×10^8
0.5 mol%	DS-PMOx$_{33}$-Si	1.8×10^8
0.5 mol%	DS-PMOx$_{104}$-Si	1.5×10^8

density constant (0.5 mol%) but extending the length of lipopolymers from $n = 33$ to $n = 104$ (DS-PMOx$_{104}$-Si), which coincides with a raise in membrane-substrate distance from $d = 2.3$ to 4.8 nm, the average viscosity η_l increases to 0.71 N s m^{-2}. This agrees with the former finding of an influence of the volume fraction of polymer in the membrane-substrate interlayer. It is noteworthy that the obtained three-dimensional viscosities η_l were about 500 times larger than the viscosity of bulk water (0.001 N s m^{-2}). On the other hand, for the experimental system used in this study, the undulation of the membrane does not seem to play an important role. First, the average distance between the membrane and the substrate (<5 nm) seems much smaller than the distance between a freely undulating 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) membrane and a solid substrate, which is beyond 30 nm [22]. Second, the average distance between homogeneously distributed lipopolymer tethers (3.8–11.8 nm) (see Ref. [23]) is much smaller than the lateral correlation distance of the thermal undulation of a freely undulating SOPC membrane, which is in the order of 700–800 nm [22].

This report demonstrates that the plasma membrane model with tunable lipopolymer tethers enables us to precisely adjust the frictional coupling between transmembrane proteins and underlying solid substrate. From the lateral diffusion coefficients measured by FRAP experiments, the effect of (i) the lateral lipopolymer density and (ii) the degree of polymerization n of polymer spacers could be quantitatively discussed within a framework of the theoretical model proposed by Evans and Sackmann. Such a well-defined model of cell membranes is a powerful tool to mimic the viscous environment provided by the extracellular matrix.

Experimental section.—The synthesis of the used poly(2-methyl-2-oxazoline) lipopolymers has been reported previously [6,24]. They are composed of a distearoyl lipid moiety, a trimethoxysilane surface anchoring group, and a hydrophilic poly(2-methyl-2-oxazoline) polymer spacer with the degrees of polymerization $n = 33$ (DS-PMOx$_{33}$-Si) and $n = 104$ (DS-PMOx$_{104}$-Si). As matrix lipid, SOPC, was used (Avanti Polar Lipids, Alabaster, USA).

The polymer-tethered lipid membrane with reconstituted transmembrane cell receptors integrin $\alpha_{\mathrm{IIb}}\beta_3$ was prepared in two steps according to our previous reports [6,24]. The proximal leaflet of the lipid membrane was established by Langmuir-Blodgett transfer (at $\Pi = 30$ mN m^{-1}, $T = 20\,^{\circ}$C) of a lipid-lipopolymer monolayer of appropriate mixture onto a glass slide. The distal leaflet was prepared by fusion of proteoliposomes containing integrin $\alpha_{\mathrm{IIb}}\beta_3$.

Fluorescence recovery after FRAP measurements were performed analog to our previous accounts at the same experimental setup at room temperature ($T \approx 25\,^{\circ}$C) [6,24]. The theory of Soumpasis [25] was chosen to fit the evaluate the diffusion coefficients and mobile fractions of integrin.

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*Also at Department of Chemistry, Chemical Engineering and Material Science, Polytechnic University Six Metrotech Center, Brooklyn, NY 11201, USA.
†Electronic address: tanaka@uni-heidelberg.de

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