Real time partitioning of octadecyl rhodamine B into bead supported lipid bilayer membranes reveals quantitative differences in saturable binding sites in DOPC and 1:1:1 DOPC/SM/Cholesterol membranes

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Supplementary Material

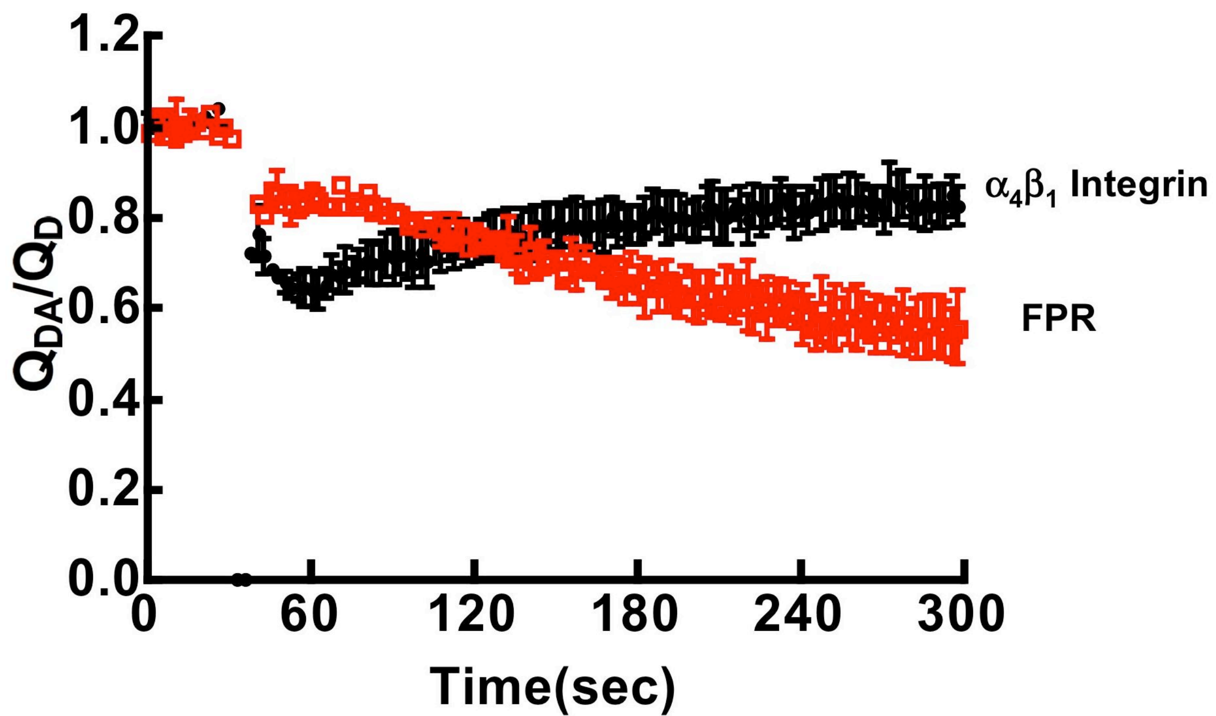

Figure S1. Exposure of cells to an isotropic environment of R18 (see Fig. 1) reveals differential FRET quenching at sites of integrin and FPR expression. Parallel real-time measurements of FRET quenching of LDV-FITC and 4pepFITC show a rapid (*2 sec* ≤ t) 40% drop in LDVFITC fluorescence that is followed by a gradual recovery of fluorescence of the donor at integrin sites. This unusual characteristic of the integrin-FRET time course is the result of two factors. First, the early kinetics of distribution of R18 among water, serum albumin and membranes as described in Figure 6 exposes cells to a transiently high dose of R18 (i.e. $[R18]_{0,\ t=0}$=1.76μM and $[R18]_{0,\ t=>10ms}$ = 1.0nM) that is available for transfer to sites on heterogeneous cell membranes. Second, the leveling off, or recovery of donor quenching for the remainder of the time course is the result of probe internalization, as shown in the confocal micrographs in

Figure 1. Probe internalization depletes acceptor density from the surface, where $[R18]_{eq.} \approx 1nM$ of monomeric R18 in the aqueous phase is insufficient to counterbalance the internalization process. In contrast, local FRET at FPR sites shows a 15% initial drop in donor intensity, which is followed by a slow monotonic decrease in donor intensity because internalization at FPR sites is minimal. When cells are exposed to an isotropic bath of R18, the real time topographical distribution of R18 on the cell surface is governed by the frequency of local density fluctuations, which present free area holes for probe insertion at each receptor site. Cumulative site density of acceptors and the distance of closest approach between the donor and the surrounding acceptors govern the relative efficiency of FRET at the locus of each receptor. The distance of closest approach (r_c) in the cell-based study reflects the size of the receptors, or conformation (the integrin), the presence of boundary lipid (membrane raft), which excludes or hinders the partitioning of R18 near the FPR (see Figure 1). These issues will be published elsewhere.