Native ligands change integrin sequestering but not oligomerization in raft-mimicking lipid mixtures

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Supporting Material

SUPPORTING MATERIALS

The Photon Counting Histogram Algorithm

PCH algorithms were generated assuming a Gaussian point spread function (1, 2). To account for the non-Gaussian shape of the point spread function noticeable at low concentrations, the primary species was convoluted with a second species of low brightness and high number. The photon counting histograms were found by acquiring data from the ConfoCor graphs of frequency binned intensity data for 50 s runs. For fitting the data, variance and χ^2 were defined as follows:

$$\chi^2 = \frac{\sum_{k=k\,\min}^{k\,\max} \frac{(p(k)-PCH(k))^2}{p(k)(1-p(k))}}{k_{\max}-k_{\min}-d} \tag{3}$$

where d is the number of fitting parameters, $p(k)$ the experimentally determined probability of observing k photon counts and the PCH algorithm generates a probability of $PCH(k)$ of

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observing k photon counts. Because the values at different k may vary by several orders of magnitude, the differences are divided by a specialized variance, which is *p(k)(1-p(k))*. This can be fit by a least squares method. The residuals are as follows.

$$r(k) = (p(k) - PCH(k)) / \sqrt{p(k)(1 - p(k))} \tag{4}$$

The quality of the fit can be estimated from the χ^2 value and the residuals. In addition, multiple runs were collected for each data set. This enabled determination of error bars for both the PCH raw data and for the N_{avg}, ε, and X_{dimer} generated by the PCH algorithm.

Single Molecule Fluorescence Microscopy

Single molecule fluorescence microscopy was conducted using a procedure described recently (3, 4). The mean square displacement values were calculated as described previously (5) using cumulative distribution functions (CDFs) which also enabled determination of immobile fraction (IF). Based on the chosen number of time steps, 150, a deviation in $\langle r^2 \rangle$ of 1.5% can be obtained. Each CDF was analyzed for normal diffusion by determining χ^2 (using standard variance).

Determination of Quality of Lipid Bilayer Before and After Protein insertion and Detergent Extraction.

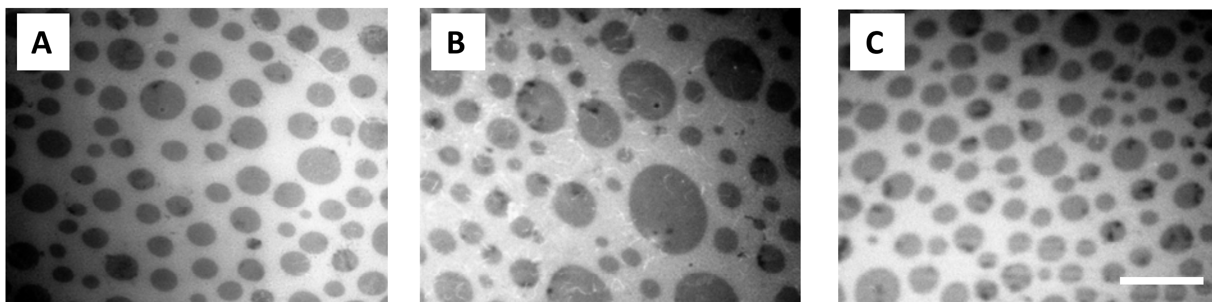

FIGURE S1 EPI micrographs of distribution of NBD-PE dyed lipids on bilayer before addition of proteins with detergents (A), after addition of proteins and detergents (B) and after rinsing off detergents with biobeads (C). Scale bar is 50 µm.

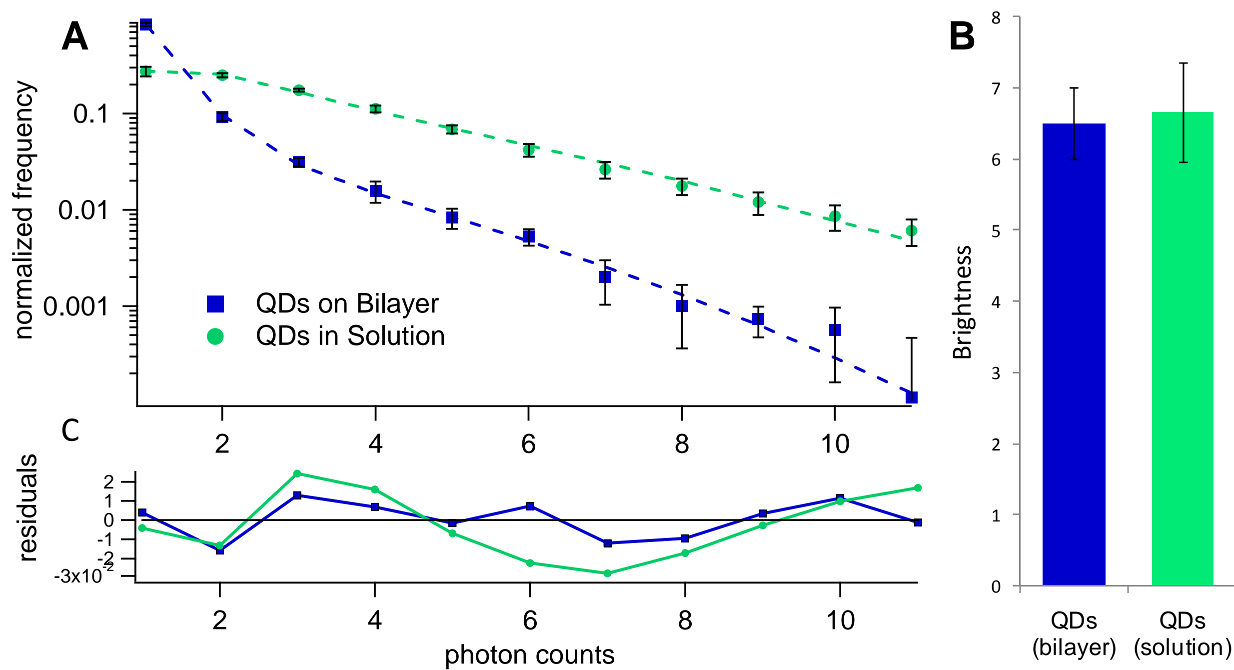

FIGURE S2 Graph showing PCH algorithm gives same count in solution as on a bilayer for quantum dots.

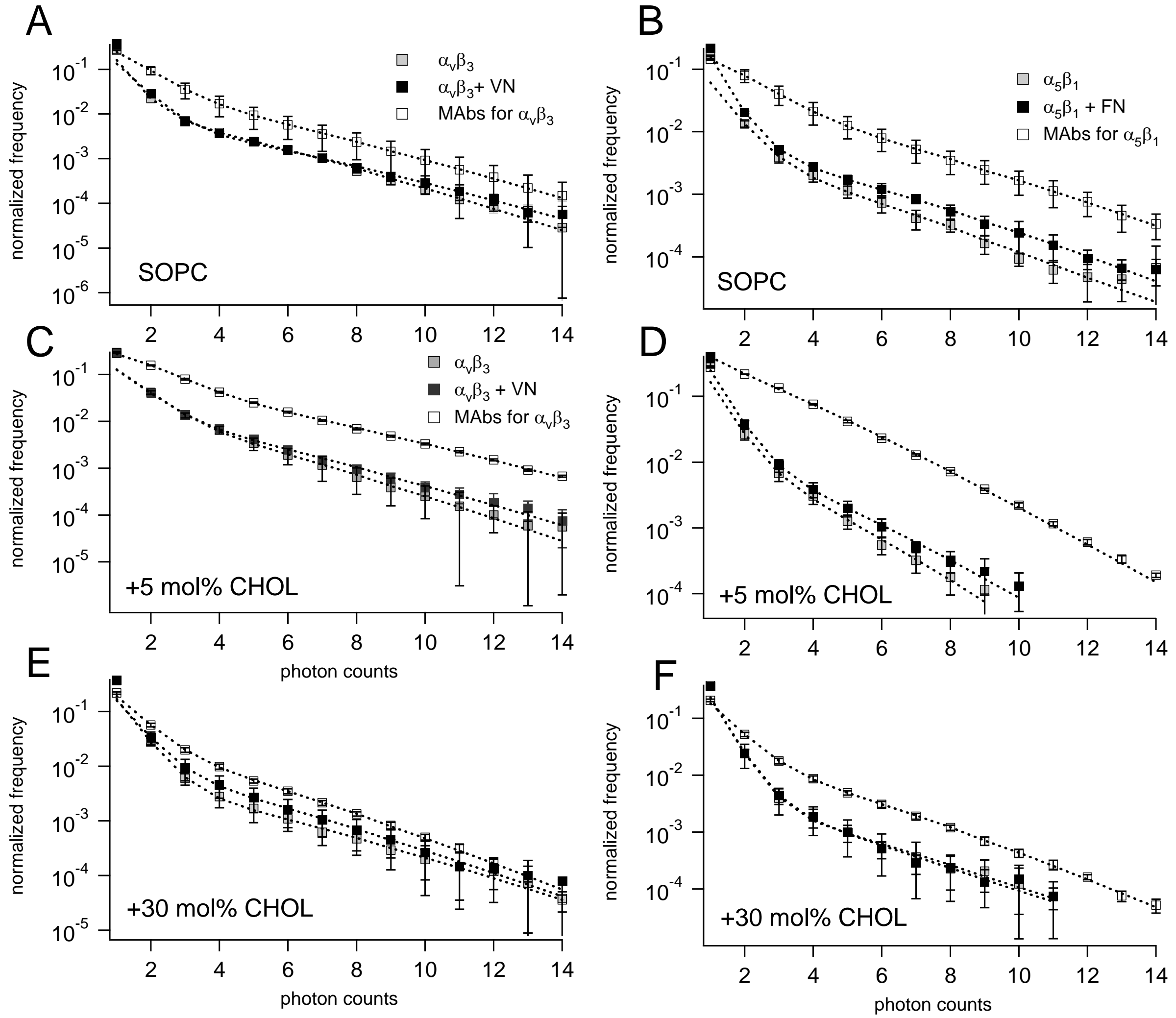

FIGURE S3 PCH curves for $\alpha_v\beta_3$ (*A, C, E*) and $\alpha_5\beta_1$ (*B, D, F*) before and after ligand binding in TYPE II bilayers with SOPC (*A,B*), SOPC + 5mol% CHOL (*C,D*), SOPC + 30 mol% CHOL (*E,F*) along with PCH curves for MAbs for integrins in solution (light markers – ECM ligand, dark markers + ECM ligand, open markers, MAbs). Dotted lines are best fit curves from PCH algorithm.

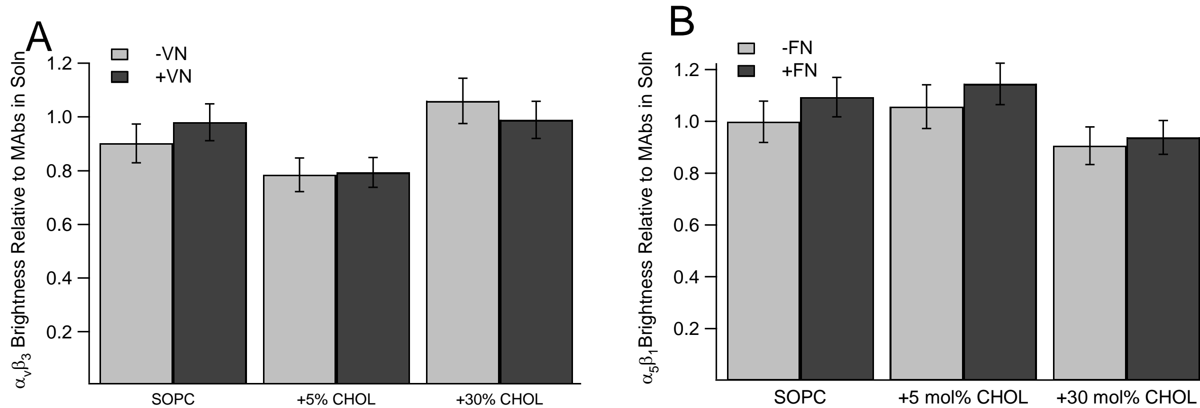

Figure S4 Brightness relative to MAbs in solution found through PCH analysis for $\alpha_v\beta_3$ (*A*) and $\alpha_5\beta_1$ (*B*) before (*light bars*) and after (*dark bars*) ligand binding.

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