EXTENDED DESCRIPTION OF THE METHODS OF ANALYSIS

6-Aminofluorescein assay for determination of best proteolipobead tethering conditions via flow cytometry. To determine the optimal proteolipobead tethering conditions, 4.7 μm microspheres after NHS-PEG_{3000}-NHS treatment are stained with 6-aminofluorescein using different incubation times and dye concentrations to determine optimal conditions for the activation of the microspheres with PEG_{3000}-NHS. Maximal labeling was attained within 1.5 h with 0.5 mM 5-aminofluorescein dye in pH 8 buffer with 10% DMSO (Figure S-1).

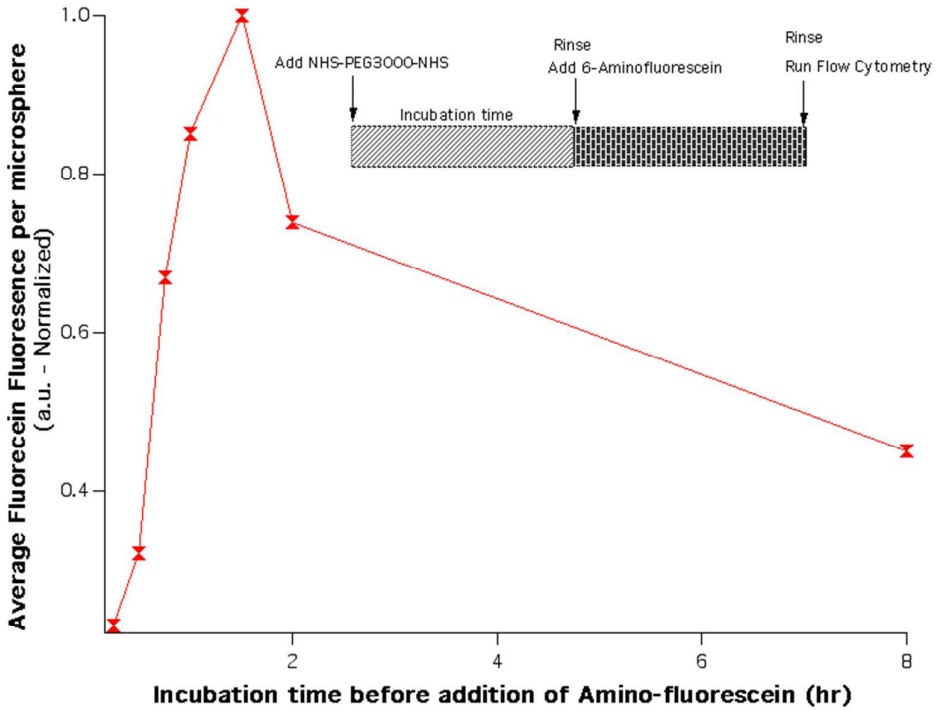

Figure S-1. The curve of relative mean channel fluorescence of fluorescein tagged to NHS-PEG_{3000}-NHS-activated 4.7 micron microspheres as the function of crosslinker incubation time before the addition of 6-aminofluorescein.

Confocal Microscopy Data Analysis. Confocal microscopy was used to image fluorescent alpha-helical peptide derivative, $K_3A_4L_2A_7L_2A_3K_2$-FITC within the biomembrane of the proteolipobead assemblies. Samples were imaged using a Leica TCS SP2 AOBS Confocal Microscope System equipped with argon ion and HeNe lasers. A 63X/1.4 NA oil immersion objective was used for all the images. FITC was excited using 488 nm line of a Ar/Kr laser and images were taken with the detection window set between 500-550 nm. The pinhole aperture was set at Airy value of 1.0, which was equivalent to >500 nm vertical slice of the bead in each Z section. Samples were compared under the same detector and laser settings in adjacent wells sharing the same coverglass by employing 8-well Lab-Tek® II #1.5 chambered coverglasses (Nunc, Thermo Fisher Scientific, Chicago, IL). The 3D reconstruction of XYZ-stacks was obtained using ImageJ 1.43. 1f with the 3D Viewer plugin (Author: Benjamin Schmid). In final image processing, all image adjustments were made identically to the

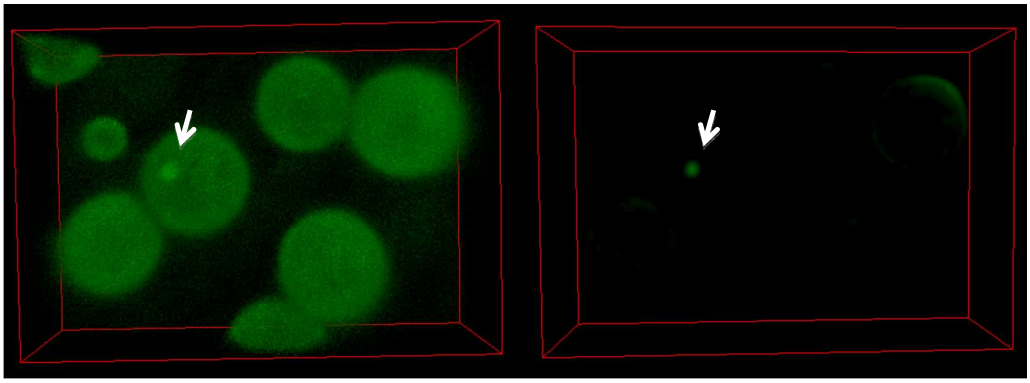

Figure S-2. Quantification of defects and inhomogeneities within the pPLB assemblies. The left 3D reconstruction was thresholded to reveal biomembrane defects (right image), shown by the white arrows. The size of the defect indicated was ~0.7 microns.

entire set of images that were under comparison. To examine defects and inhomogeneities within the pPLB assemblies, 3D reconstruction and thresholding

within ImageJ was used. Figure S-2 shows a representative analysis where the left 3D reconstruction was thresholded to reveal biomembrane defects (right image), shown by the white arrows. The size of the defect indicated was ~0.7 microns.

Fluorescence recovery after photobleaching studies were carried out using a built-in Leica method and a 512 x 32 pixel format was used (zoom value 16; scan speed 400 Hz, 488 nm AOTF 2%). And the image plane was set at the equator Z section of the proteolipobead. After 5 pre-bleach points a region of 1µm x 1µm on the bead was subjected to 50X laser intensity (AOTF 100%) for the duration of one scan. Recovery of fluorescence in the bleached area was subsequently monitored for 20 sec. Data was collected for the fluorescence intensity of the bleached region throughout the time course and corrected for overall photobleaching and analyzed using Igor Pro 6.0 (Wavemetrics, Eugene, OR). To estimate the diffusion coefficient, Mathematica 8.0 was used to estimate the value of time constant τ_D, using the formalism as [1,2]

$$F(t) = \alpha F_p \sum_{n=0}^{\infty} [(\frac{(-K)^n}{n!}) \frac{1}{(1 + n(1 + \frac{2t}{\tau_D}))}] + (1 - \alpha) F_o$$

Where:

F(t) = Fluorescence intensity of the bleached spot at time t after bleaching

F_p = Prebleach intensity

F_o = Intensity immediately after bleaching

$\alpha = \frac{F_\infty - F_o}{F_p - F_o}$ = Mobile fraction

K = Parameter related to the degree of bleaching

$\frac{F_o}{F_p} = \frac{(1 - e^{-K})}{K}$

The τ_D parameter is related to Diffusion coefficient as:

$D = \omega^2 / 4\tau_D$

Where ω is $1/e^2$ of the Gaussian radius of the bleaching laser.

The mobile fraction α and the degree of bleaching parameter K were calculated using the equations listed above. The above equation for F(t) was used to find a best single parameter fit in terms of τ_D (with fixed α and K), using the sum of the squares of residuals as the "goodness of fit" parameter. The best-fit value of τ_D was then used to estimate D in units of $\mu m^2/s$. Figure S3 displays a representative data set, from the tethered pPLBs with the central blue trace from the best fit yielding $\alpha = 0.97$ and $D = 0.010\ \mu m^2/s$. We used a value of 1 μm as an approximation of ω, the Gaussian radius of the bleaching laser beam. Values of α and D are reported as the average of N = 40 measurements with the error bars computed in terms of standard error.

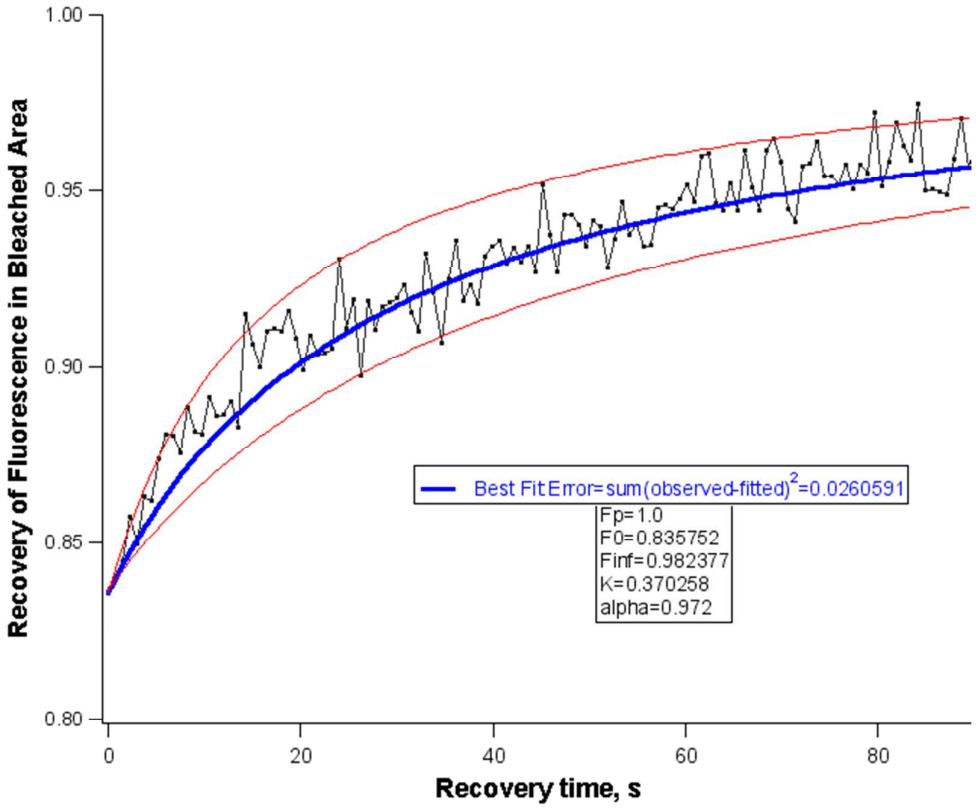

Figure S-3. Method for estimation of the mobile fraction, α and τ_D from FRAP data. The scatter points (black) are the FRAP data and the blue trace is from the "best fit" used to extract τ_D.

(1) Tsuji, A.; Ohnishi, S. *Biochemistry* **1986**, *25*, 6133.
(2) Klonis, N.; Rug, M.; Harper, I.; Wickham, M.; Cowman, A.; Tilley, L. *Eur Biophys J* **2002**, *31*, 36.