Supplementary Material

Single Molecule Microscope:

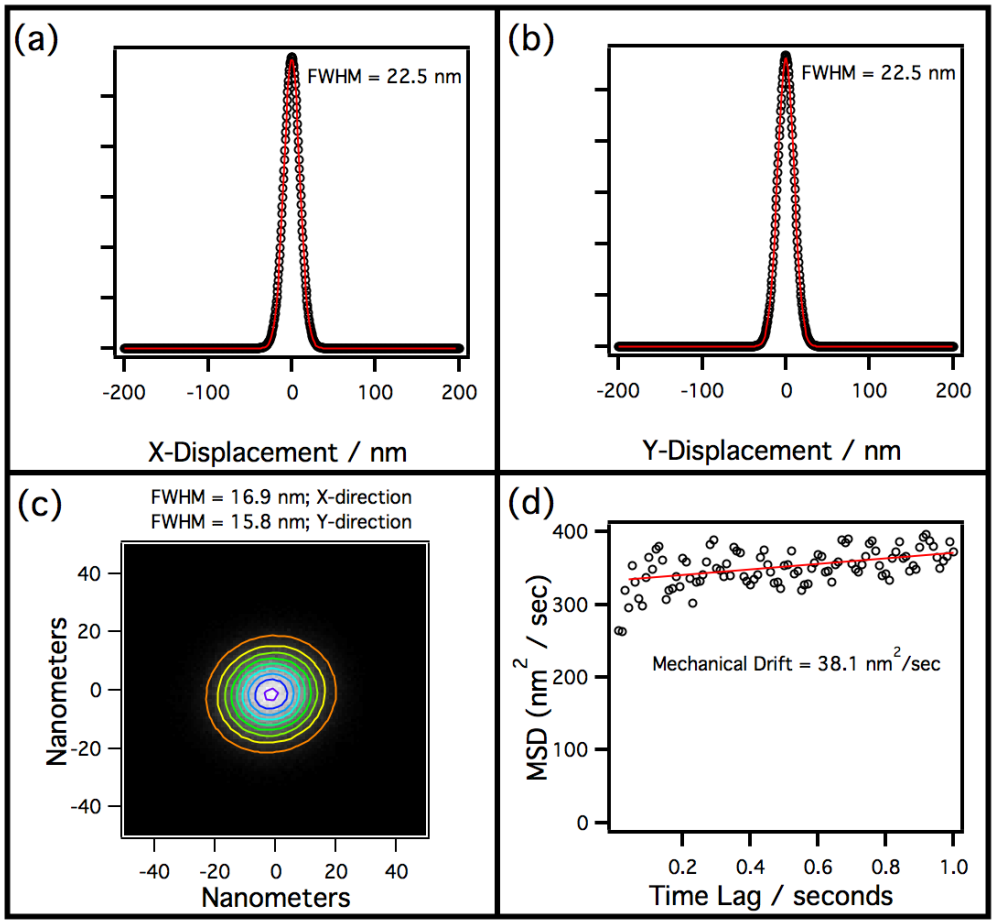

Figure S1. The microscope stability was characterized with a back illuminated zero-mode-waveguide (ZMW). The illumination intensity was adjusted to match the fluorescence intensity of a single Alexa Fluor probe molecule excited with a 532 nm cw-Nd:YAG laser attenuated to 1 mW. The results presented here matched well with a previous stability study that utilized Quantum Dots covalently bonded to glass coverslips and illuminated with a 532 nm laser. [1] Since both methods gave identical results, the ZMW method was chosen as the standard technique to characterize mechanical stability in the laboratory because of its convenience. All data presented above was collected with 10 ms time resolution and under identical conditions of the experiments described in this work. The data analysis was identical to that described in the experimental section of this article. The peak position of each diffraction-limited spot was determined from its centroid. (a-b) Frame-to-frame displacement in the x and y directions were identical (isotropic) with a FWHM of 22.5 nm which was taken as the spatial resolution (mechanical stability) of the instrument, (c) diffraction limited spots tracked over 500 frames (5 seconds) gave a total dispersion of ~16.5 nm with a eccentricity of 0.3247, (d) a mean squared displacement (MSD) analysis showed that the mechanical drift was 38.1 nm^2 / sec.

Histograms of Diffusion Coefficient for Single Molecule Tracking Studies:

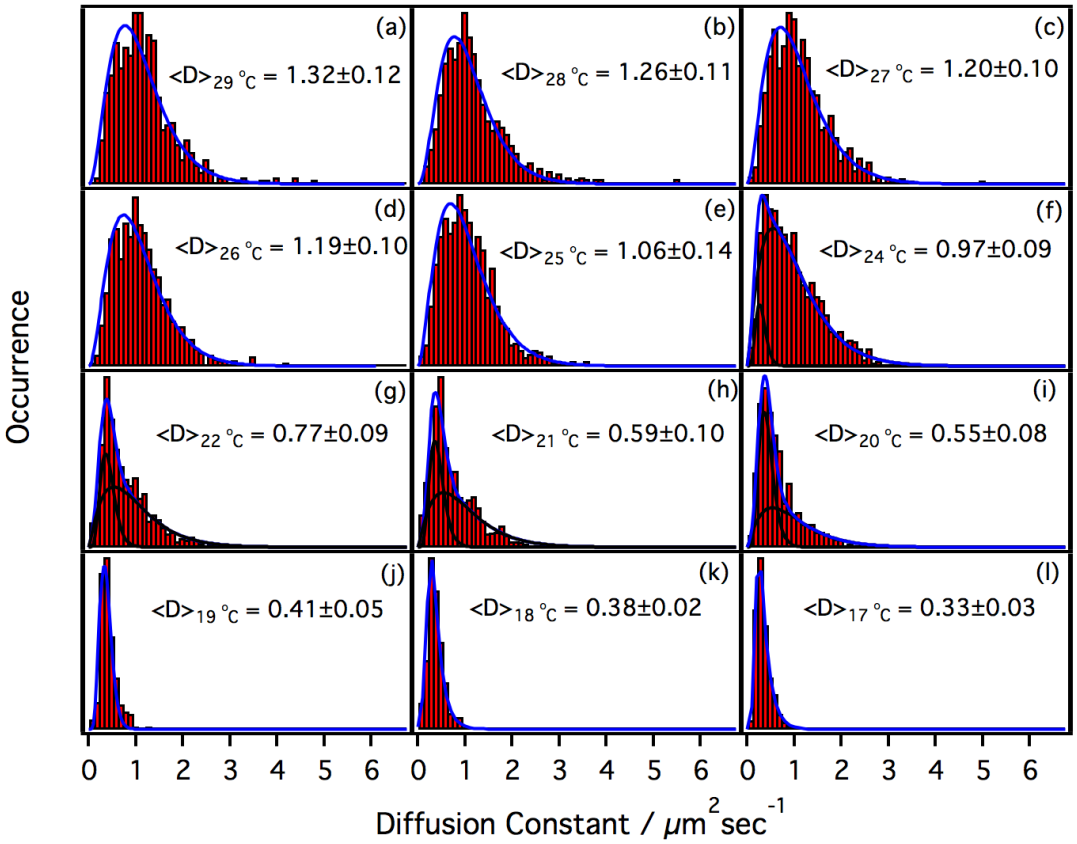

Figure S2. Histograms of diffusion coefficients of individual receptors in DMPC / PEG-PE / Brain-PS cushioned assemblies. The solid blue lines are the total fits of the data and the solid black lines are the constituents of those fits. (a) 29 ºC, (b) 28 ºC, (c) 27 ºC, (d) 26 ºC, (e) 25 ºC, (f) 24 ºC, (g) 22 ºC, (h) 21 ºC, (i) 20 ºC, (j) 19 ºC, (k) 18 ºC, and (l) 17 ºC. The data in (a-e) and (j-l) could be fit with a single Gaussian and the data in (f-i) had to be fit with at least two Gaussians. The 30 ºC, 23 ºC, and 16 ºC data is presented in the body of the manuscript.

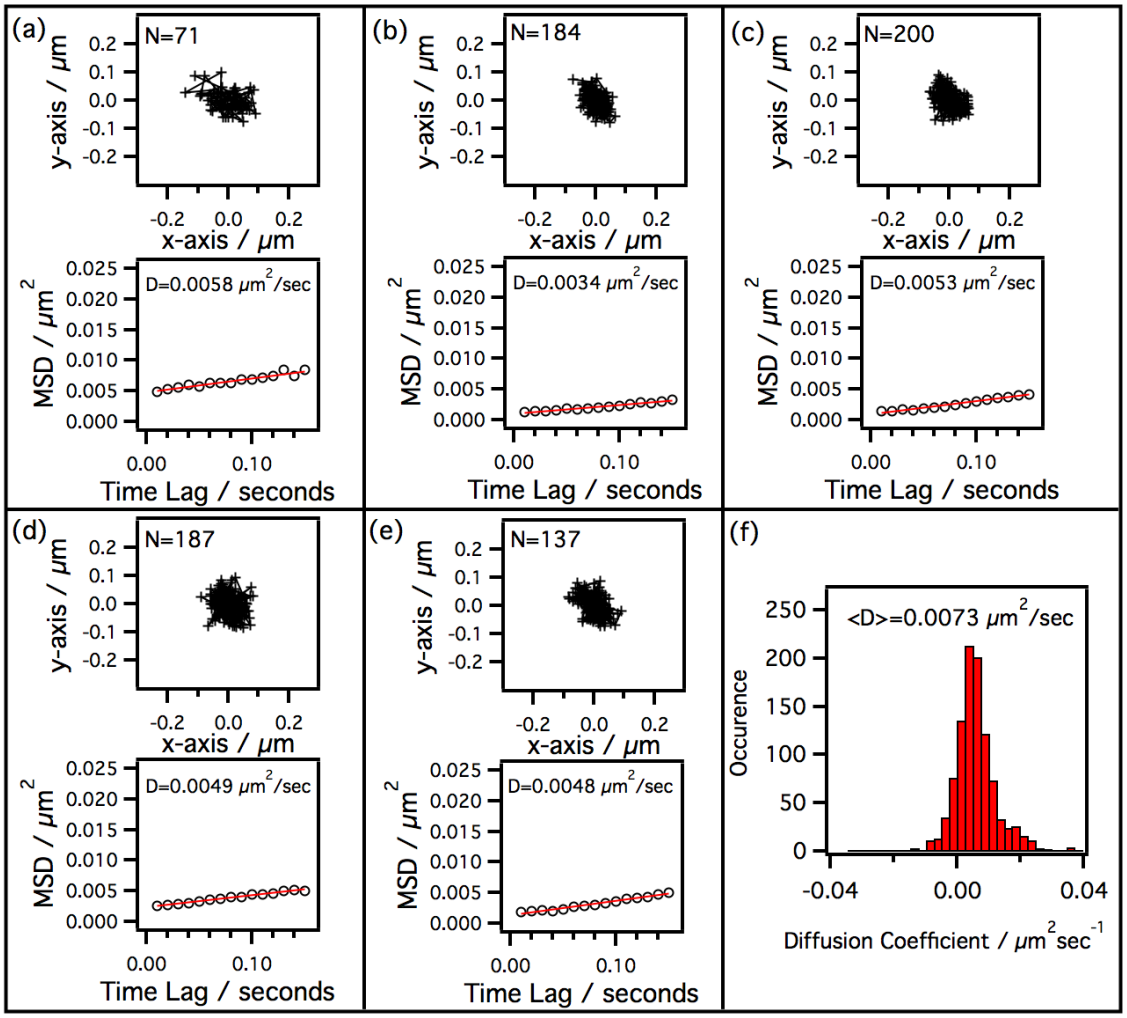

Figure S3. At 15 °C nearly all Annexin V TM proteins in the DMPC / PEG-PE / Brain-PS membrane traveled less than 150 nm during the course of its track, which was the minimum requirement to be considered as a mobile particle. Therefore, all discrimination was lifted when analyzing data collected at 15 °C and all labeled Annexin V was analyzed regardless of how far it traveled during its particular track. (a-e) Example tracks of Annexin V at 15 °C, the upper graph depicts the track of the particle and the lower curve is the MSD vs. $n\Delta t$. N is the number of frames the TM protein was tracked and D is the diffusion coefficient determined from equation 4. All MSDs were plotted on the same scale as data collected at 16 °C (figure 5(a and b)) and all MSD curves gave linear relationships. (f) Histogram of Ds determined for all Annexin V TM proteins regardless of total motion during individual tracks, <D> is the mean value of the diffusion coefficients determined from the MSD analysis. While we have considered these particles to be 'immobile' because of their very small movement in the x or y direction, a case could be made that Annexin V still diffuses in this temperature range but has an **extremely** slow rate of the diffusion.

Table S1. Results of FRAP experiments on Rhodamine-DMPE in the DMPC / PEG-PS / Brain-PS assemblies.

	DMPC / PEG-PE / BRAIN-PS	
Temperature (^{o}C)	**FRAP Recovery**	**D (μm^2/sec)**
17.9	92.5%	1.11 ± 0.10
18.4	90.6%	1.18 ± 0.11
19.2	92.7%	1.22 ± 0.11
20.4	92.3%	1.41 ± 0.14
21.8	93.3%	1.80 ± 0.12
23.3	93.2%	2.11 ± 0.16
24.4	92.4%	2.35 ± 0.16
25.5	92.5%	2.41 ± 0.10
27.1	92.2%	2.45 ± 0.10
28.5	93.2%	2.53 ± 0.11
29.7	93.9%	2.57 ± 0.10
31.1	93.0%	2.63 ± 0.11
32.2	93.5%	2.67 ± 0.08

Reference:

(1) Poudel, K. R.; Keller, D. J.; Brozik, J. A. *Langmuir* **2011**, *27*, 320-7.