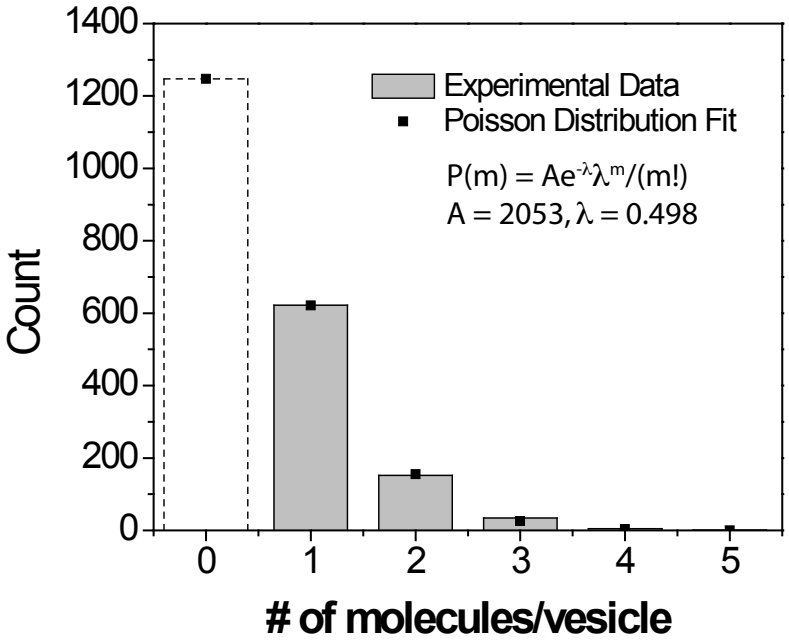
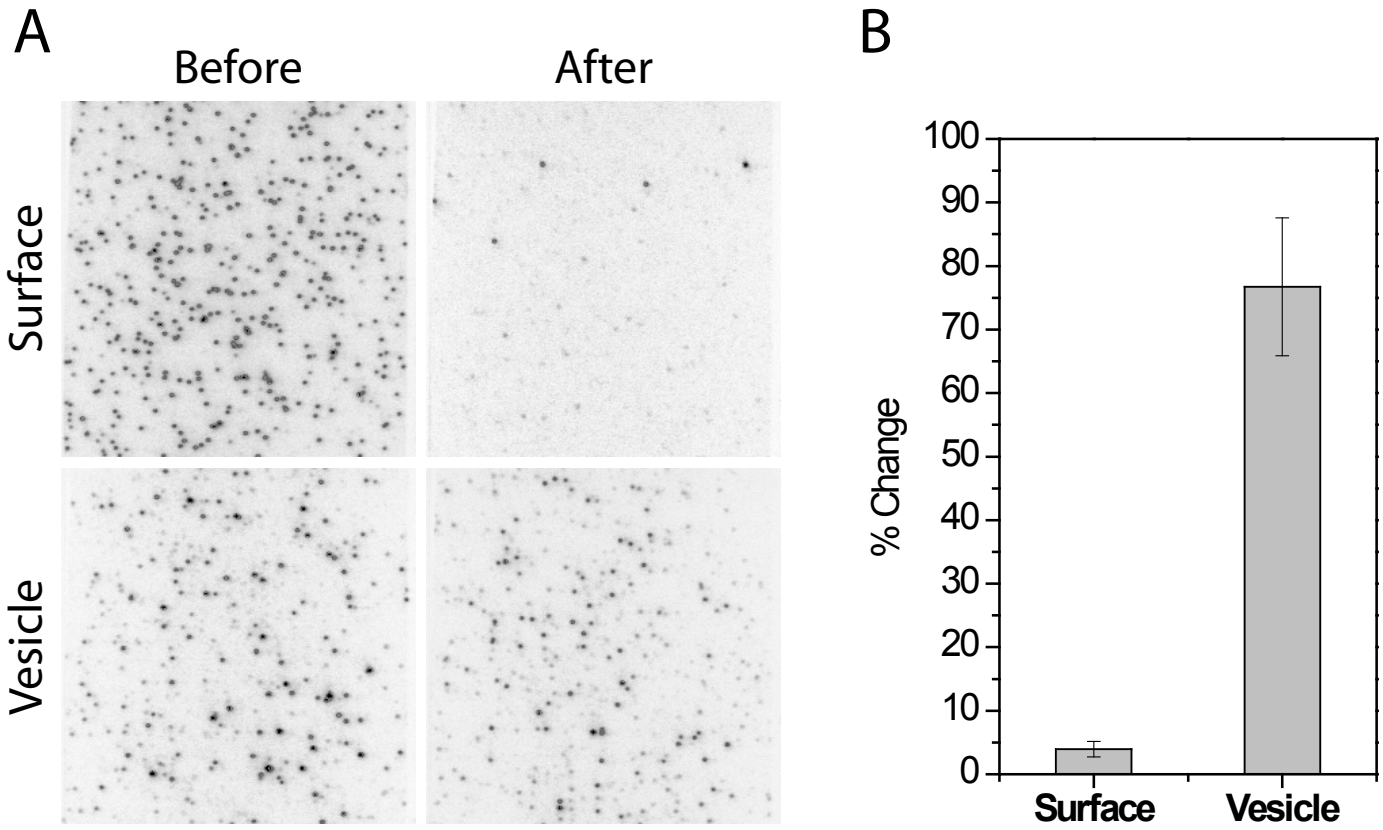
Supplemental Figure 1: Number of GQ-DNA per Vesicle



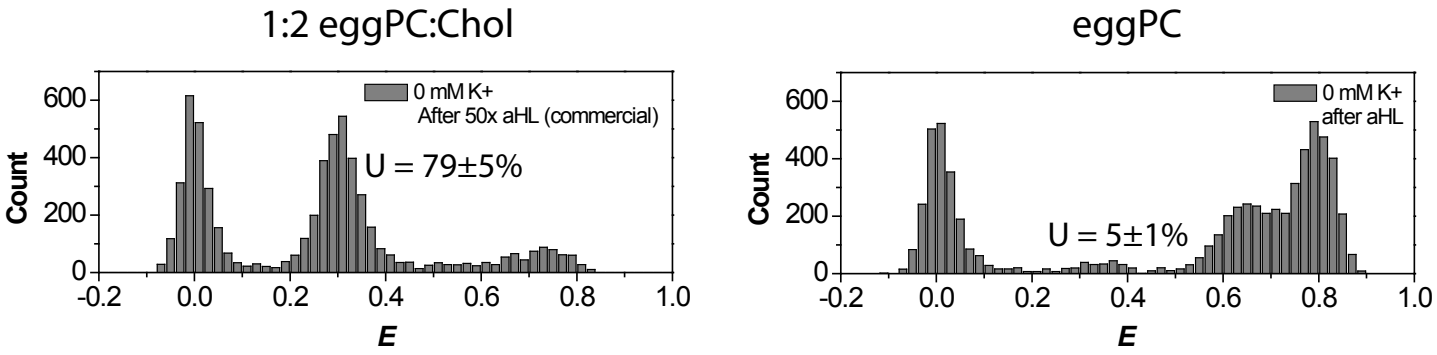
GQ-DNA encapsulated vesicle sample has been prepared as described in the Experimental Methods section. These vesicles were immobilized on the surface. The number of GQ-DNA molecules were quantified by counting the number of photobleaching steps of Cy5 dye (633 nm illumination). The number of molecules were plotted and fitted to Poisson distribution function. The overall average number of GQ-DNA per vesicle is below one (0.498).

Supplemental Figure 2: DNase Treatment



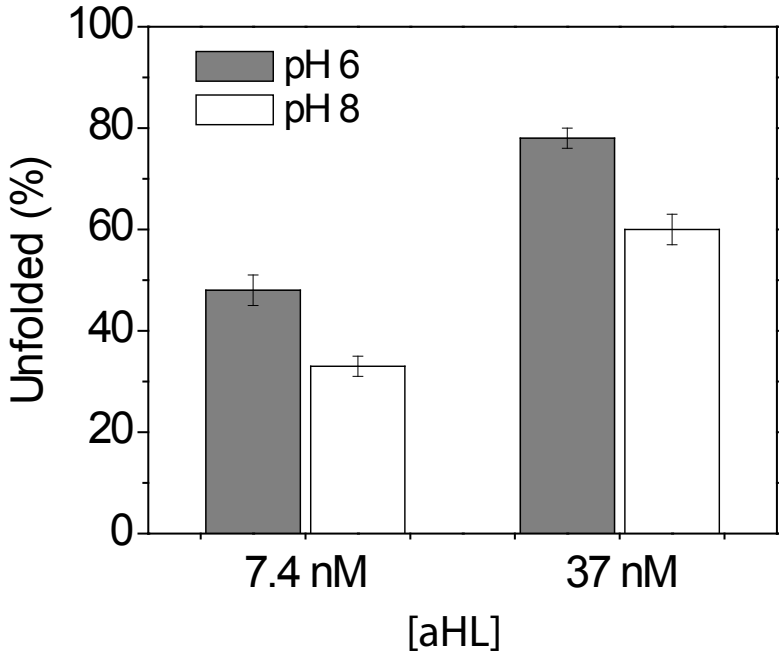
DNase Treatment. DNase I was added to flow chambers with surface immobilized GQ-DNA (surface) and with vesicle encapsulated GQ-DNA (vesicle). (A) Single molecule fluorescence images (TMR fluorescence) of before and after the DNase I treatment. Each dark spot represent a vesicle with GQ-DNA. These images show that GQ-DNA insde vesicles are protected from DNase I digestion. (B) Percent of molecules remained on the surface after the DNase I treatment normalized to counts before the treatment.

Supplemental Figure 3: Commercial aHL



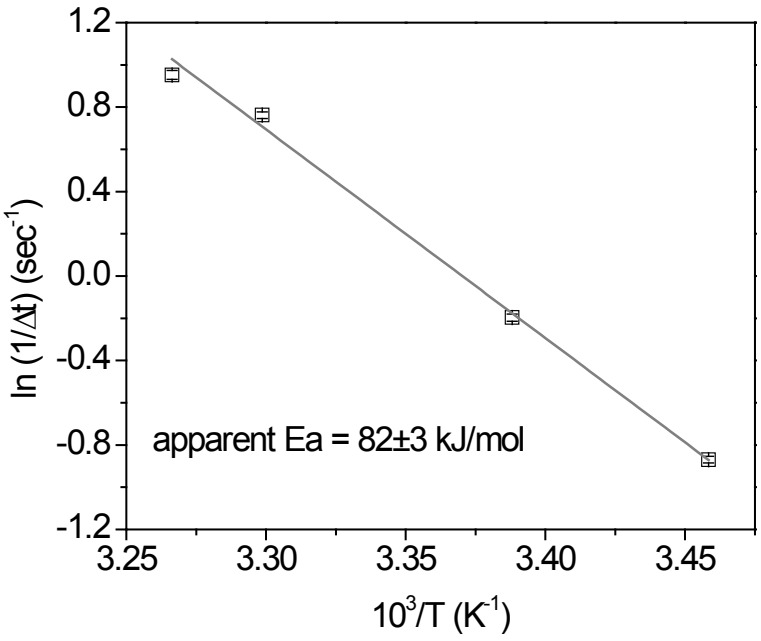
Commercial aHL. FRET histogram from GQ-DNA molecules inside 1:2 eggPC:Chol and eggPC vesicles are shown. Vesicles were incubated with 0.74 μM of commercial aHL (Calbiochem) solution for 30 min and washed with buffer containing no K^+. Comaprable level of 1:2 eggPC:Chol vesicles are permeated, but in contrast, only a small fraction of eggPC vesicles were permeated.

Supplemental Figure 4: pH Effect



pH effect. Surface immobilized 1:2 eggPC:Chol vesicles have been incubated with 7.4 nM and 37 nM aHL at pH 6.0 (10 mM MES, 50 mM NaCl) and 8.0 (10 mM Tris, 50 mM NaCl) for 60 min and washed with 10 mM Tris buffer (pH 8.0) contantaining no K^+. The relative unfolded populations (U) were quantified from respective histograms. The lower pH incubation consistently increased the efficiency of aHL membrane permeation.

Supplemental Figure 5: Rep translocation apparent activation energy



Rep translocation apparent activation energy. Arrhenius plot of Rep helicase translocation time (Δt) along partial duplex $(dT)_{80}$ and its fit are shown above. The data points have been fitted to a line and obtained the apparent activation energy of 82±3 kJ/mol.