Supporting Information

Transformation of ActoHMM Assembly Confined in Cell-Sized Liposome

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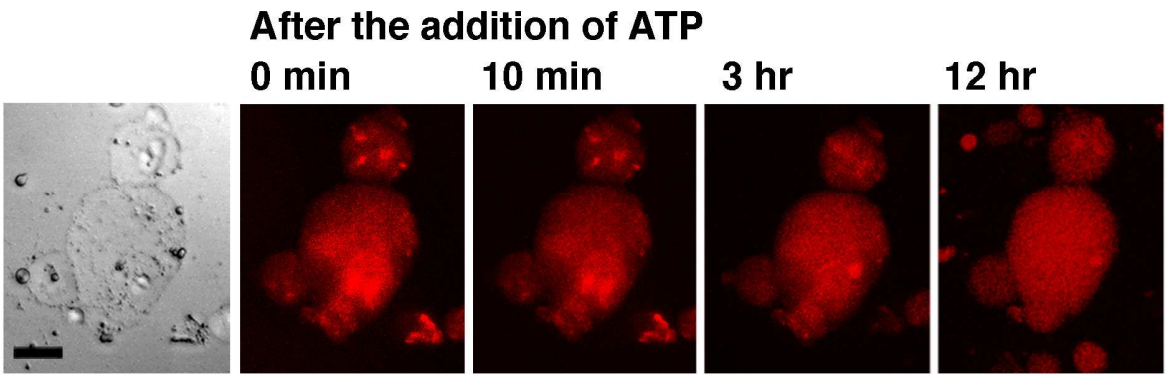

Figure S1. Fluorescence images indicating the disassembly process of F-actin bundles formed with the co-encapsulated HMM. Confocal microscopic observation of the time-dependent changes in the distribution of actoHMM after the addition of ATP to the liposome (left image: transmission, other images: fluorescence). ActoHMM-encapsulating giant liposomes with a DOPC bilayer membrane were made using the spontaneous transfer method. The concentrations of encapsulated F-actin and HMM are 50 and 5.0 μM, respectively. This Figure represents the larger neighboring area for the observation shown in Figure 2. F-actin is labeled with rhodamine-phalloidin. The time after the ATP supply through the added α-hemolysin is indicated at the top of each panel. The sample was observed for 3 hr at 25°C, and then left for 12 hr at 4°C. The picture on the right was taken after adjusting the temperature back to 25°C. Bars = 20 μm. Transmission images show the existence of small oil droplets around the liposome, which were squeezed out from the oil phase.[1]

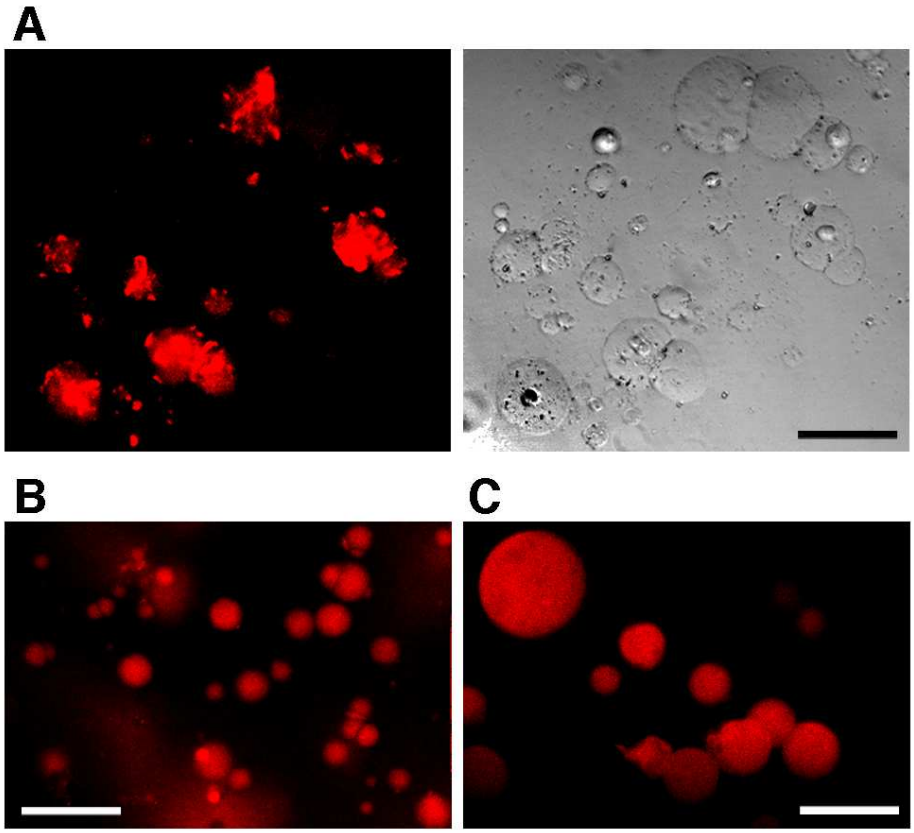

Figure S2. (A) Confocal microscopic images of actoHMM-encapsulating giant liposomes made from DOPC using the spontaneous transfer method (left: fluorescence, right: transmission).[2] The concentrations of encapsulated F-actin and HMM are 50 and 5.0 μM, respectively. (B and C) Confocal microscopic images of giant liposomes encapsulating only F-actin (B) or actoS-1 (C) obtained using the spontaneous transfer method (fluorescence images). The concentrations of encapsulated F-actin and S-1 are 50 and 60 μM, respectively. Fluorescence images show the distribution of rhodamine-phalloidin-labeled F-actin. Under these conditions, most liposomes are spherical and no protrusions develop. Bars = 100 μm.

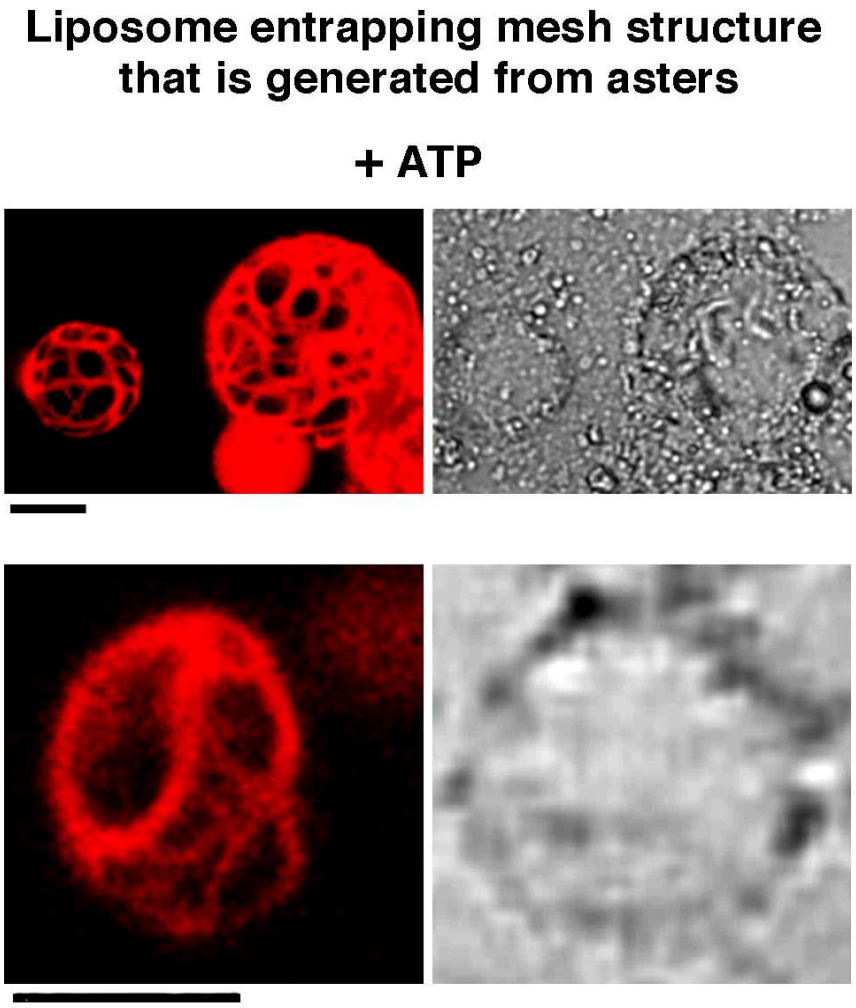

Figure S3. Giant liposomes that are entrapping an actoHMM mesh (left: fluorescence, right: transmission). The lipid composition was DOPC/DPPC/cholesterol. The concentrations of F-actin and HMM are 10 and 20 μM, respectively. Bars indicate 20 μm. Methylcellulose-formed actoHMM bundles are transformed to asters in the bulk solution by the addition of ATP. The asters are then encapsulated into cell-sized giant liposomes using the spontaneous transfer method after the pipetting procedure. By the suspension, the asters change in the mesh. ATP is supplied using treatment with α-hemolysin as described in Material and Methods.

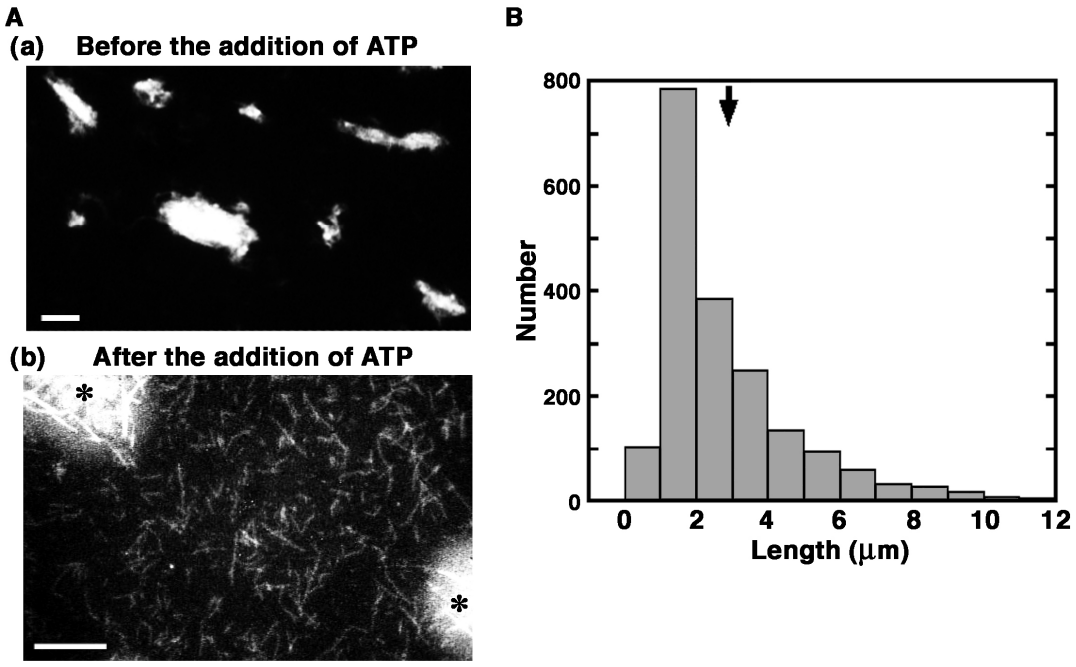

Figure S4. Length distribution of F-actin within bundles. (A) Actin bundles, which were prepared by the same procedure for the droplet formation in the oil, were attached to an HMM-coated glass surface (a). Subsequently, collapse of the bundles was observed by dark-field microscopy by the introduction of ATP. After the collapse of almost all of the bundles, debris of bundles was washed with the ATP-containing solution and then individual F-actin spread from bundles was observed by fluorescence microscopy and was measured length by use of ImageJ software (b). F-actin was labeled with rhodamine-phalloidin. Bars show 10 μm. Asterisks indicate the remaining debris of bundles. (a) and (b) show the representative images of bundles before the addition of ATP (dark-field) and F-actins after the addition of ATP (fluorescence) on a glass surface, respectively. The concentrations of F-actin and HMM used were 10 and 20 μM, respectively. (B) Histogram shows length distribution of F-actin within bundles (n=1,918). Arrow indicates the average length (2.9 μm).

As shown in Figure S1, after the addition of α-hemolysin and ATP, the crosslinked F-actins started to disassemble simultaneously inside many liposomes. Since the molecular weight of α-hemolysin is 33 kDa, the simply estimated molar ratio of the heptamer of the pore-forming protein to lipid is roughly 1:20000 in our conditions (the concentrations of α-hemolysin and lipid are 12.5 μg/ml and 1 mM, respectively). If the occupied area of the lipid molecule in the monolayer is a fraction of square nanometers, a liposome with a radius of 10 μm would be made from about 1×10^{10} lipids. Therefore, the number of pores that are opened in the liposomes by α-hemolysin may be sufficient to supply ATP to the actoHMM that is encapsulated in the liposomes.

Figure S2 shows giant liposomes encapsulating F-actin (50 μM) and HMM (5.0 μM) in the presence of $MgCl_2$. The small spherical objects situated around the liposomes or on their surfaces in the transmission images are attributed to oil droplets at the oil/water interface.[1] Inside of those liposomes, F-actin co-encapsulated with HMM assembles into large bundles or networks (Figure S2A).[2] We have confirmed the appearance of similar assemblies of F-actin in an aqueous solution in control experiments. On the other hand, in the case of liposomes encapsulating the same concentration of F-actin only, F-actins inside the liposomes exist in a homogeneous manner (Figure S2B).[2] Moreover, giant liposomes co-encapsulating the same concentration of F-actin and S-1, instead of HMM, showed a uniform distribution of F-actin, even under conditions where an excess molar ratio of S-1 was co-encapsulated (Figure S2C).[2]

Myosins are classified into two groups according to their head-structure, *i.e.* double- or single-headed myosins. Myosins belonging to the double-headed type, such as myosin-II, and a

double-headed derivative of myosin-II, such as HMM, have frequently been studied as a representative double-headed myosin.[3,4] On the other hand, S-1 has often been studied as a representative simple single-headed myosin. S-1 has only one actin-binding motor domain and is unable to crosslink F-actins. Thus, these results indicate that the crosslinking of F-actins by the double-headed HMM is the motive force for organizing the actin bundles and networks.[2]

References for Supporting Information

(1) Yamada, A.; Yamanaka, Y.; Hamada, T.; Hase, M.; Yoshikawa, K.; Baigl, D. *Langmuir* **2006,** *22,* 9824-9828.

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