

Supporting Information

Lipid Membrane Interaction of Peptide/DNA Complexes Designed for Gene Delivery

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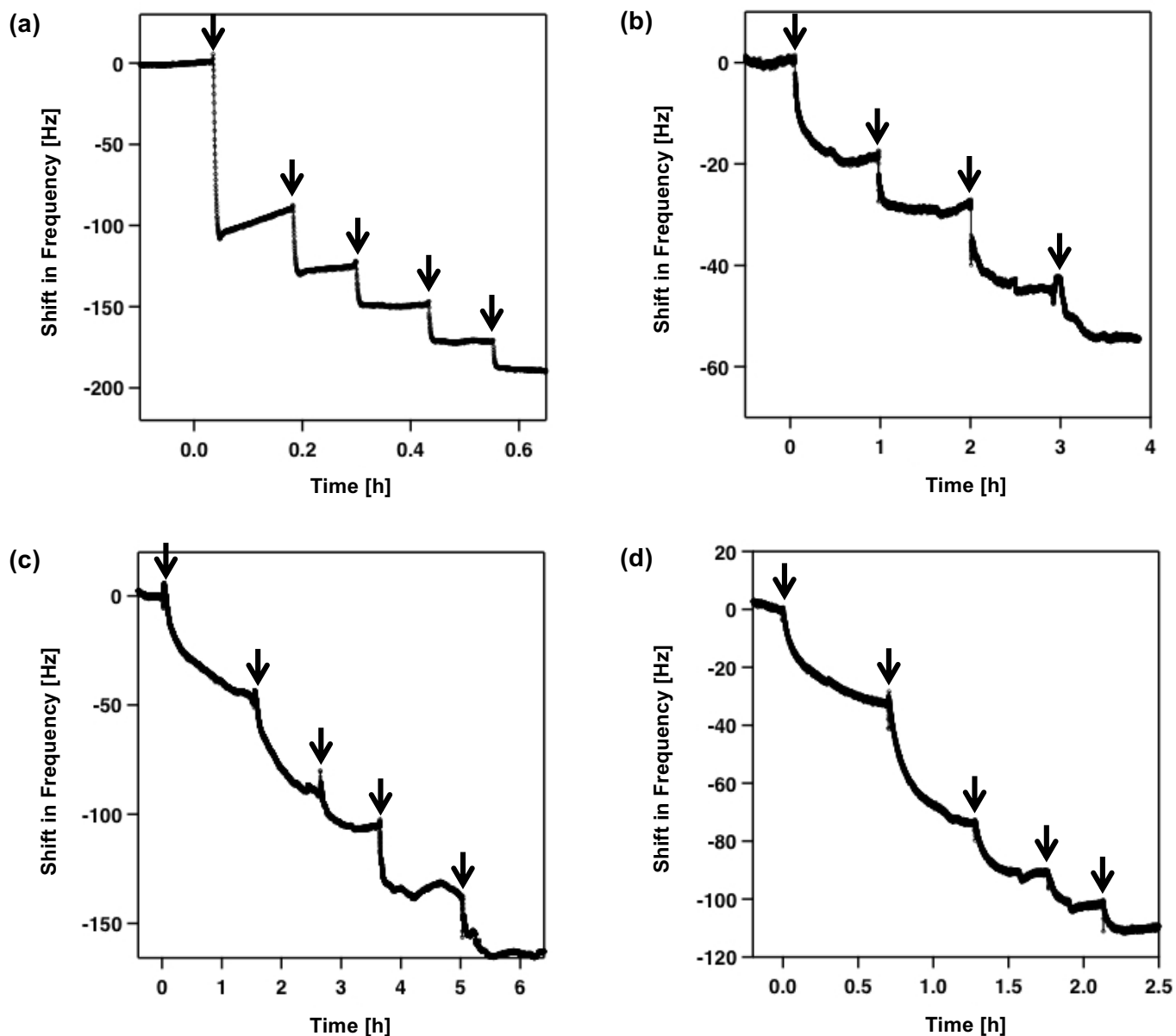


Figure S1. Time-course plots showing the binding of Cytcox **(a)**, BP100 **(b)**, Cytcox-KH₉ **(c)**, and BP100-KH₉ **(d)** to Plasma-MM. The arrows indicate the injection of the peptide. The concentrations of the injected amounts of Cytcox, BP100, Cytcox-KH₉, and BP100-KH₉ were 41, 2.2, 1.3, and 0.7 μ M, respectively.

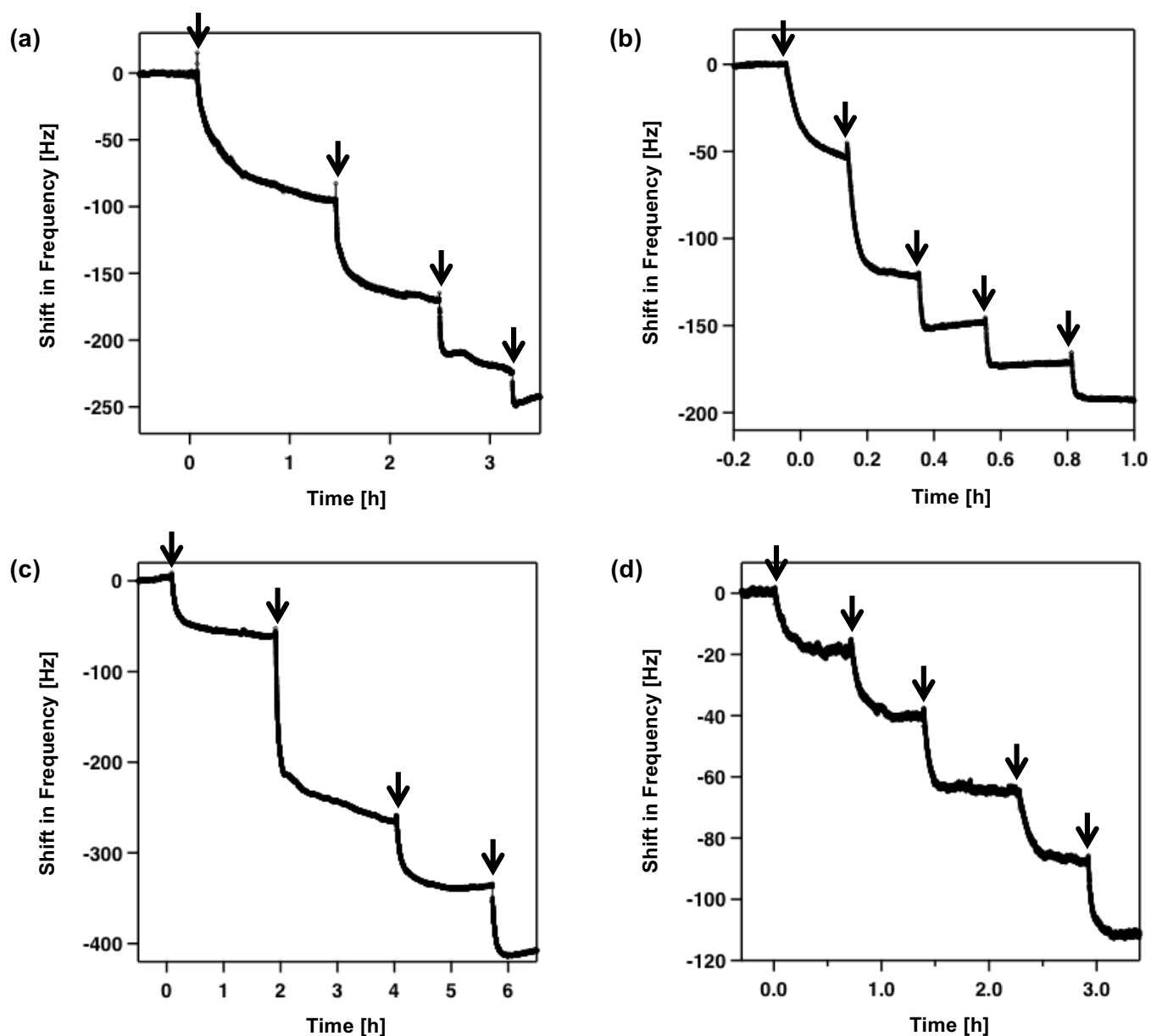


Figure S2. Time-course plots showing the binding of Cytcox **(a)**, BP100 **(b)**, Cytcox-KH₉ **(c)**, and BP100-KH₉ **(d)** to Mito-MM. The arrows indicate the injection of the peptide. The concentrations of the injected amounts of Cytcox, BP100, Cytcox-KH₉, and BP100-KH₉ were 8.3, 9.1, 1.3, and 0.7 μ M, respectively.

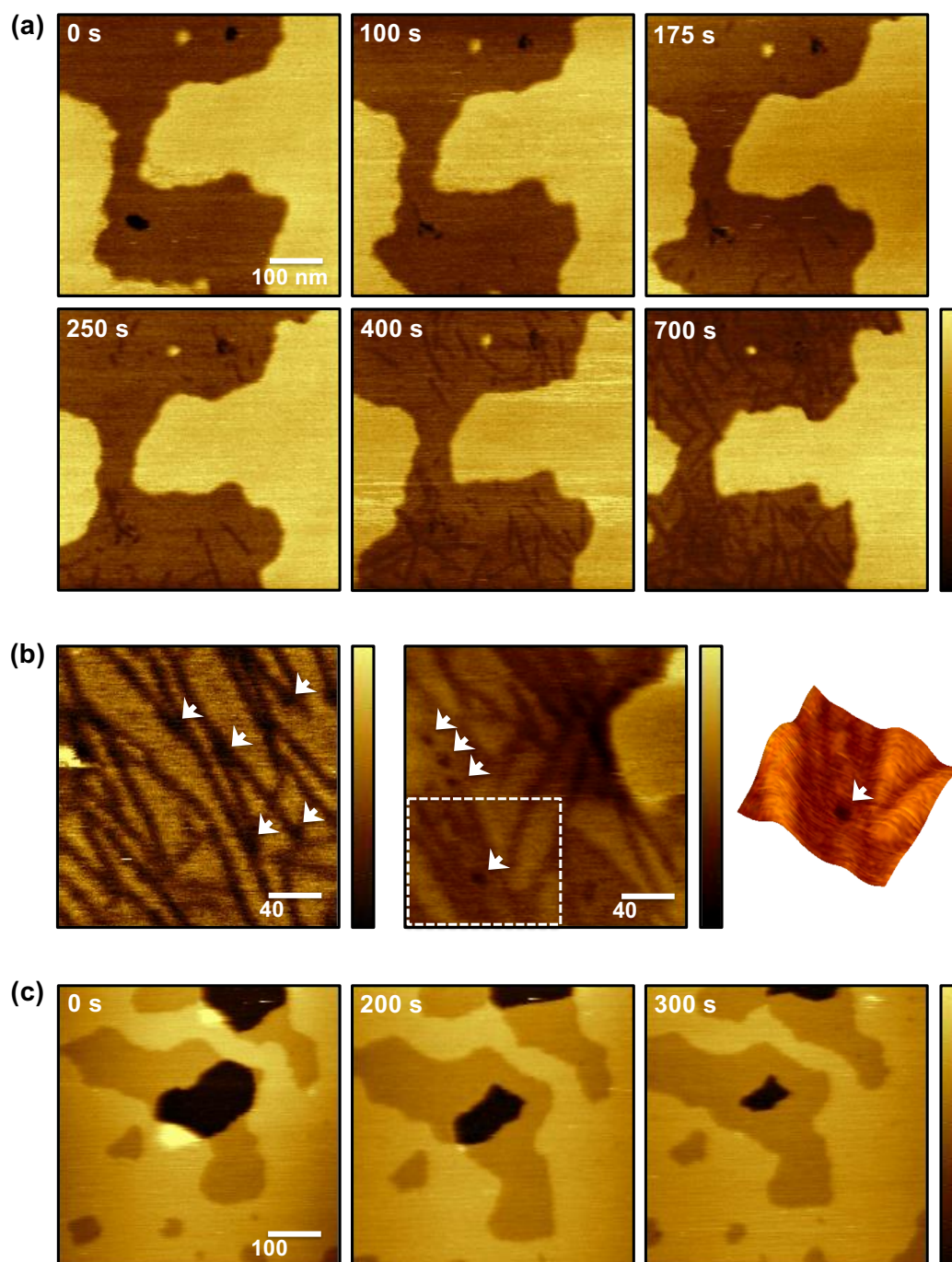


Figure S3. HS-AFM height images showing the changes in Plasma-MM in the presence of 8 μM Cytcox (a, b) and 9 μM BP100 (c). “0 s” in A and C correspond to approximately 5 and 9 min after addition of Cytcox and BP100, respectively. The arrows in B point to the pore-like defects. The 3D image is the region in the dashed line box. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); $Z_{\max}(\text{a}) = 9 \text{ nm}$, $Z_{\max}(\text{b}) = 2.5 \text{ nm}$ (left), 6 nm (right), and $Z_{\max}(\text{c}) = 10 \text{ nm}$.

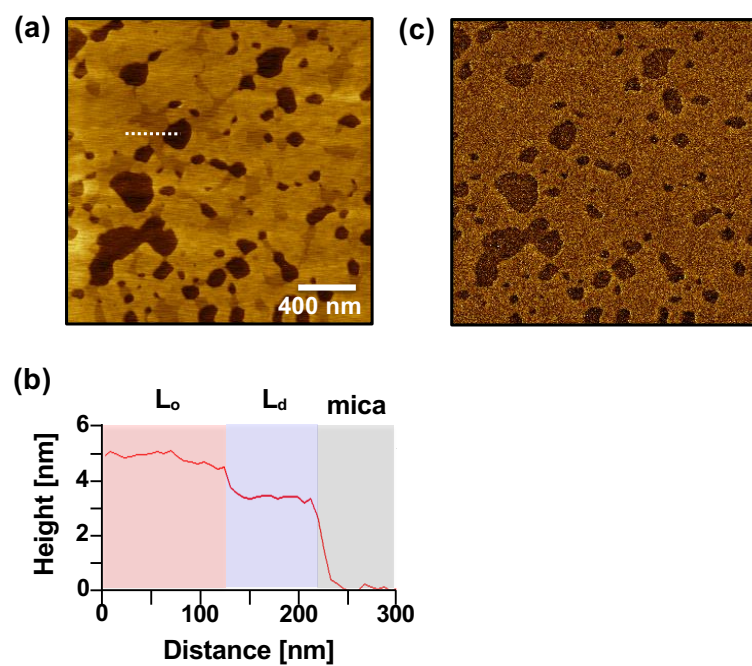


Figure S4. HS-AFM height (a) and phase (c) images showing the height and phase variation in plasma-MM. (b) Height profile along the dashed line in (a).

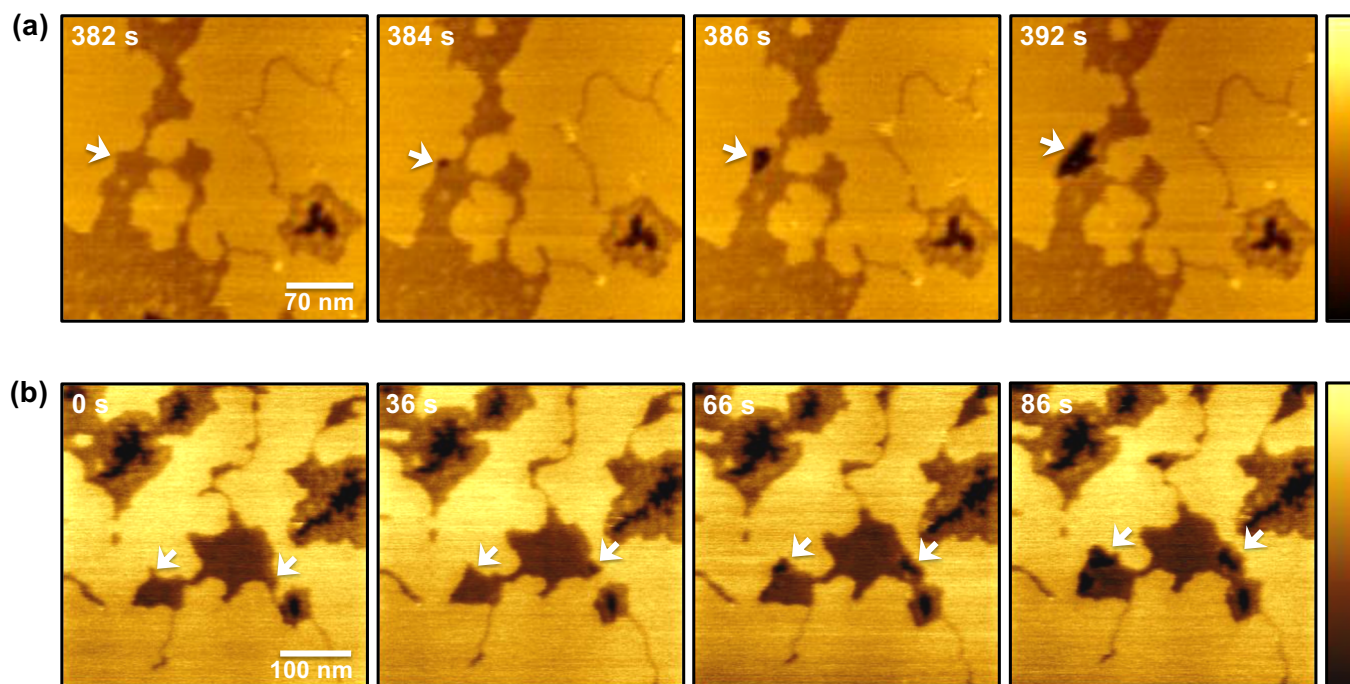


Figure S5. HS-AFM height images showing the pore formation in Plasma-MM in the presence of 3 μM Cytcox-KH₉ (a) and 0.5 μM BP100-KH₉ (b). “0 s” in (b) corresponds to 28 min after addition of BP100-KH₉ and 26 min after the start of observation in Figure 4b. The arrows point to the pores. The color bar indicates the Z-range between 0 (darkest) to Z_{max} (brightest); Z_{max} (a, b) = 5 nm.

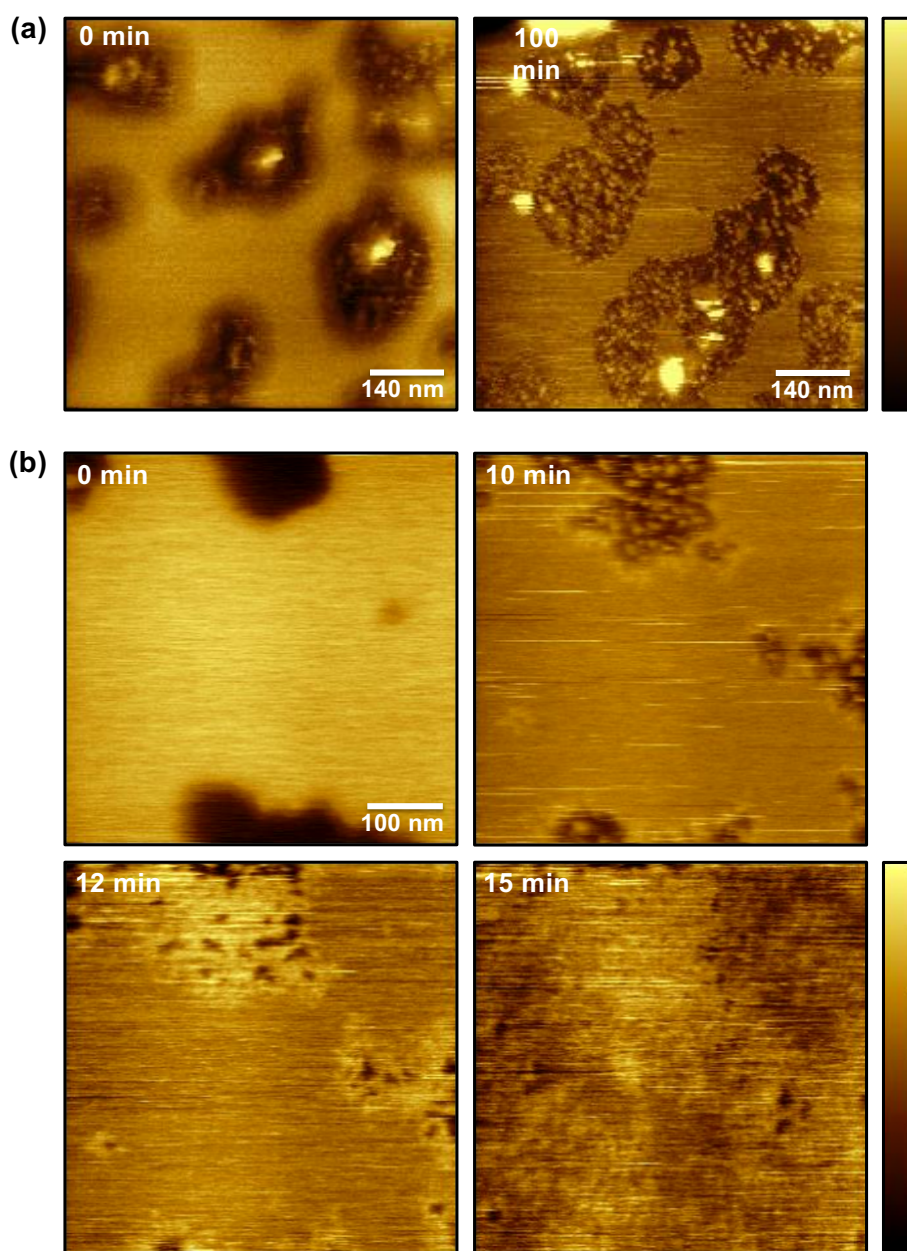


Figure S6. HS-AFM height images showing the changes in Mito-MM in the presence of 12 μM Cytcox (a) and 1.3 μM Cytcox-KH₉ (b). “0 min” corresponds to the time just after addition of peptide. The color bar indicates the Z-range between 0 (darkest) to Z_{max} (brightest); Z_{max} (a) = 7 nm and Z_{max} (b) = 6 nm (0 min, 10 min), 4 nm (12 min), 3 nm (15 min).

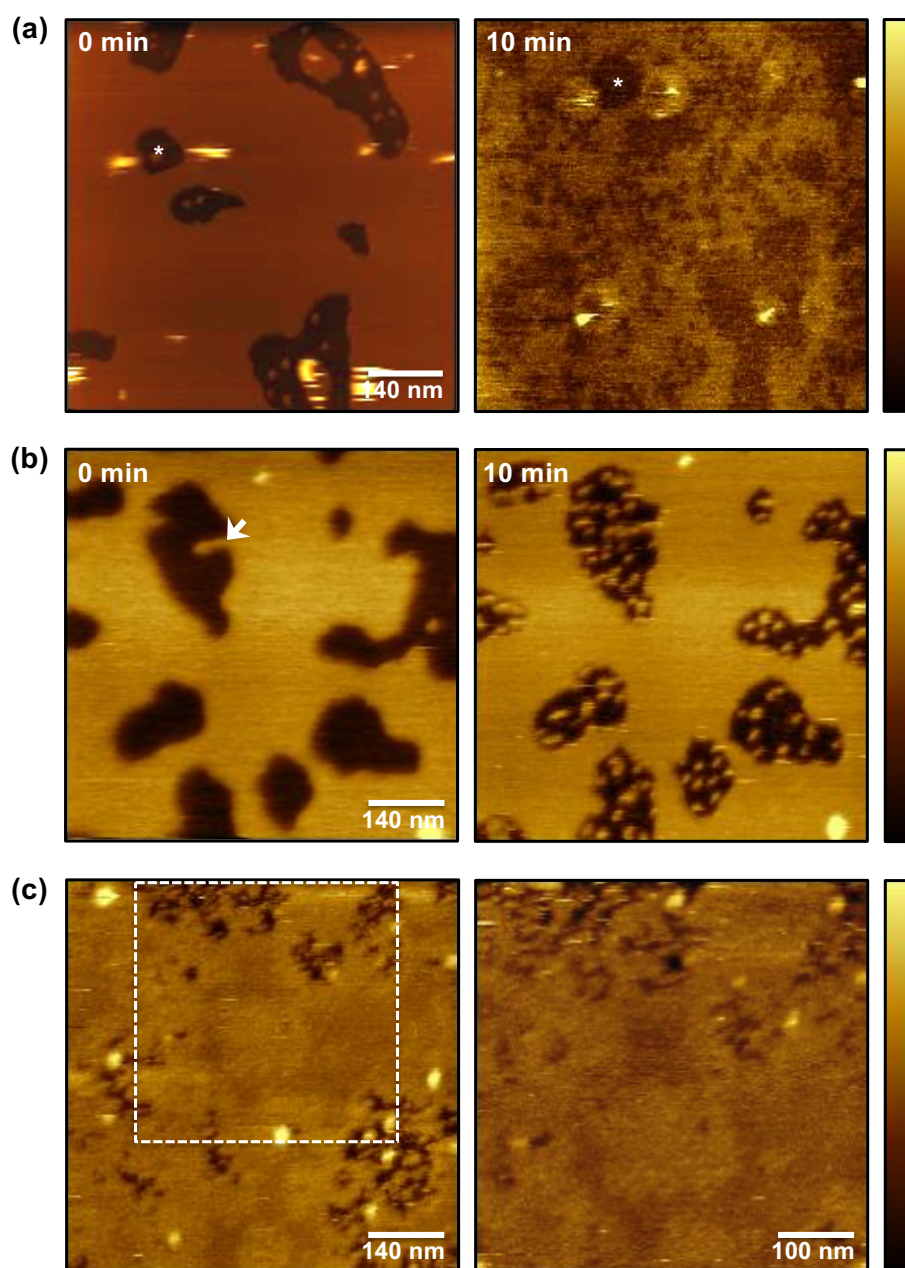


Figure S7. HS-AFM height images showing the changes in Mito-MM in the presence of 9 μM BP100 (a) and 0.4 μM BP100-KH₉ (b, c). “0 min” corresponds to the time just after addition of peptide. The arrow in (b) points to the tubule-like structure protruding from the membrane in the presence of BP100-KH₉. The images in (c) show the thin layer formed on Mito-MM 45 min after addition of BP100-KH₉. The image to the right is the higher-resolution image of the region in the dashed line box. The color bar indicates the Z-range between 0 (darkest) to Z_{max} (brightest); Z_{max}(a) = 40 nm (0 min), 5 nm (10 min), Z_{max}(b) = 12 nm, and Z_{max}(c) = 10 nm.

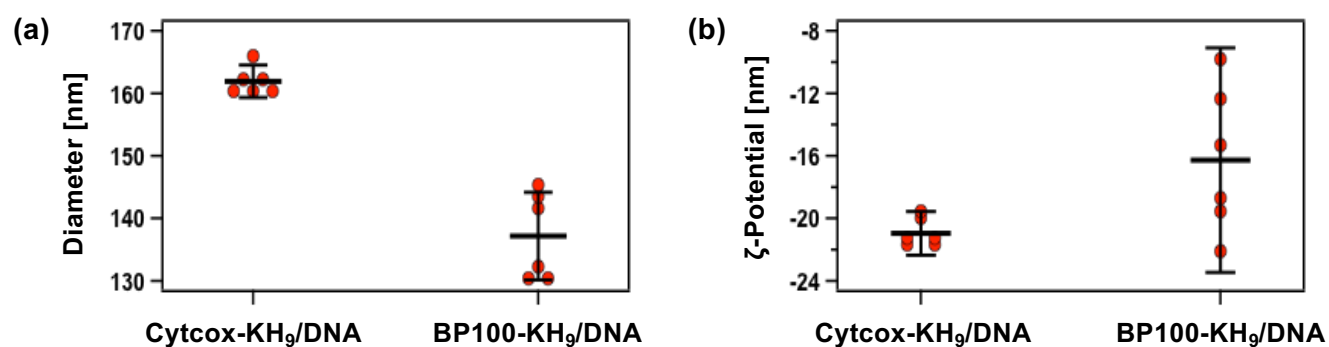


Figure S8. Hydrodynamic size **(a)** and ζ -potential **(b)** of Cytcox-KH₉/DNA and BP100-KH₉/DNA complexes measured by DLS. The number of data points was 6 for each measurement. The highest and lowest lines indicate the standard deviation from the mean value (centered line).

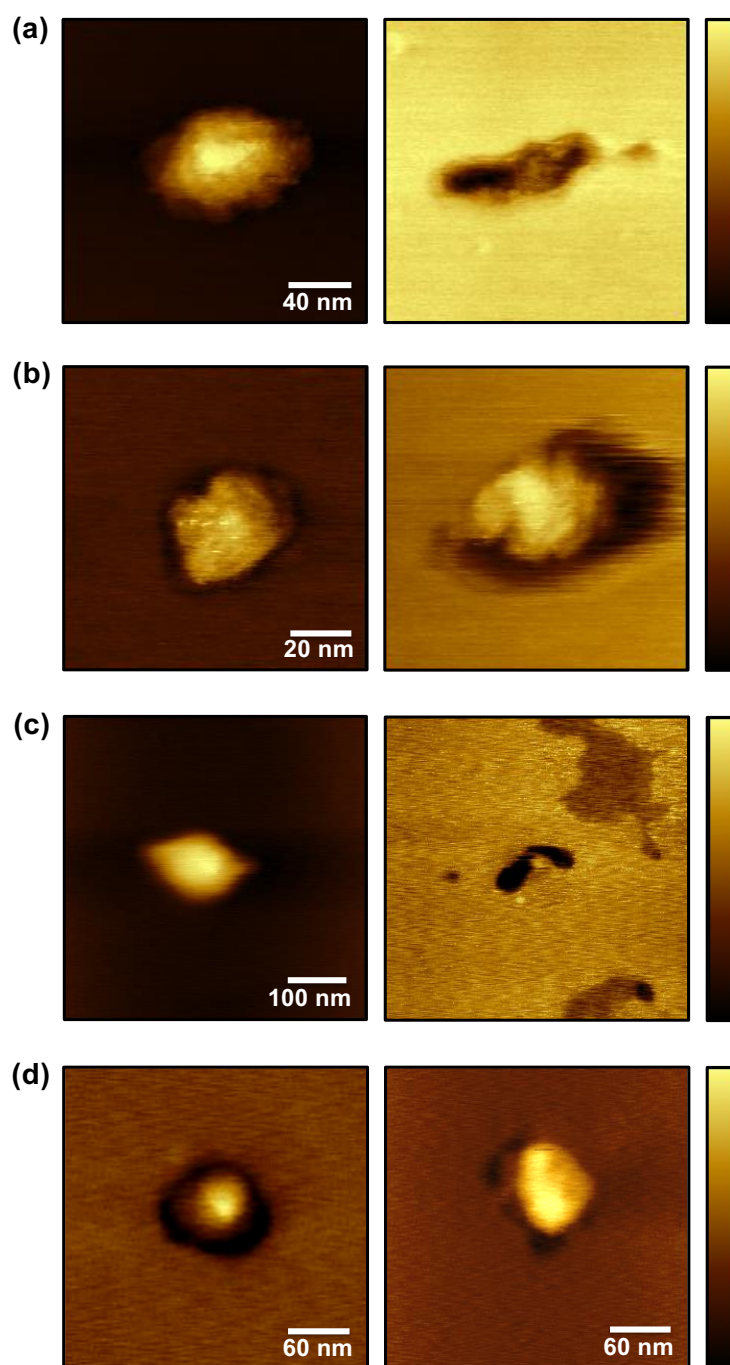


Figure S9. HS-AFM height images showing the complexes of Cytcox-KH₉/DNA **(a-c)** and BP100-KH₉/DNA **(d)** on Plasma-MM. The images to the right in (a)-(c) were recorded at a higher imaging force to reveal the disrupted region by Cytcox-KH₉/DNA complex. The images in (d) show the disrupted regions of the membrane around BP100-KH₉/DNA complex. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); $Z_{\max}(\text{a}) = 22 \text{ nm}$ (left), 6 nm (right), $Z_{\max}(\text{b}) = 6 \text{ nm}$ (left), 8 nm (right), $Z_{\max}(\text{c}) = 40 \text{ nm}$ (left), 6 nm (right), and $Z_{\max}(\text{d}) = 13 \text{ nm}$.

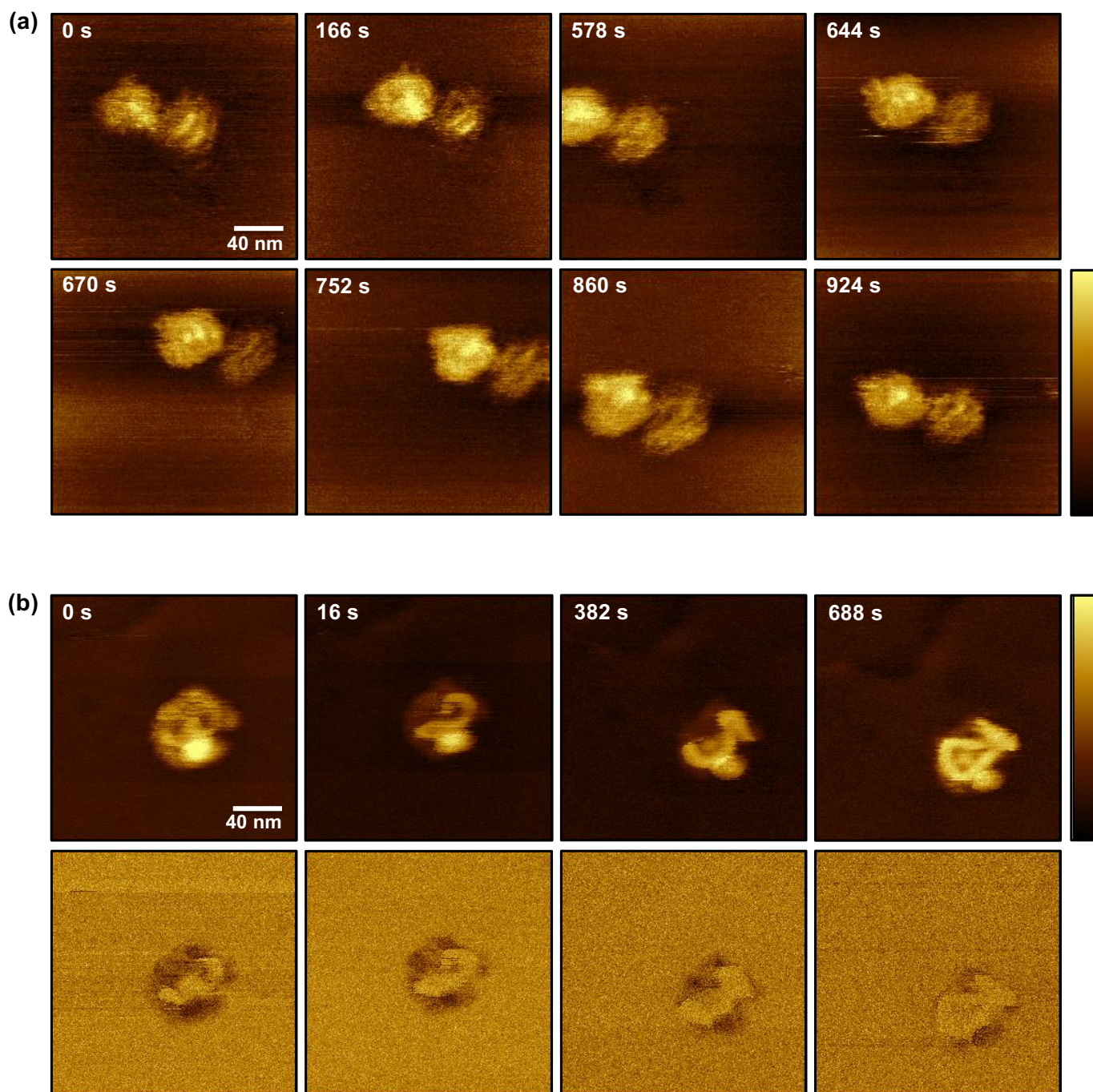


Figure S10. HS-AFM height images of Cytcox-KH₉/DNA complex on Plasma-MM (**a**, **b**) at pH 5.8 in 20 mM MES containing 150 mM NaCl. The phase images in the lower row in (**b**) show the DNA-rich region of the complex on the peptide-rich darker region. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); $Z_{\max}(\text{a}) = 10$ nm and $Z_{\max}(\text{b}) = 8$ nm.

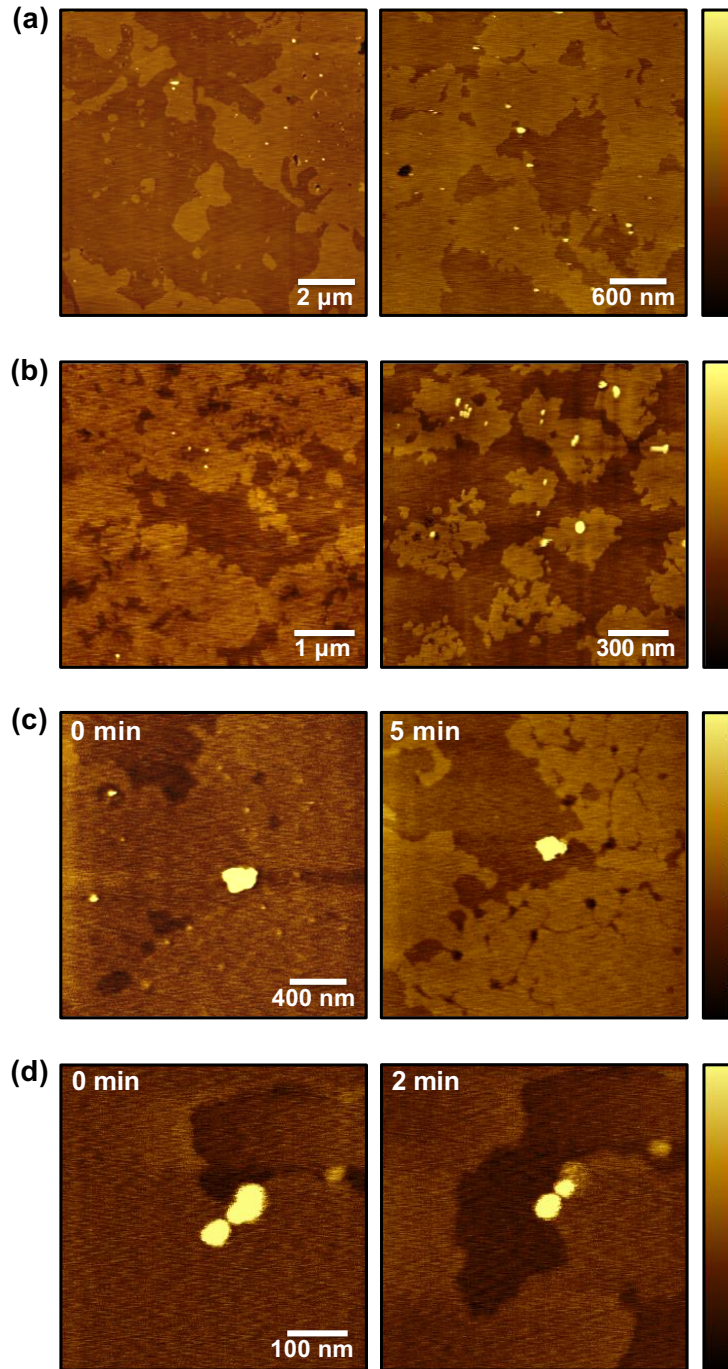


Figure S11. HS-AFM height images showing the localization of Cytcox-KH₉/DNA complex **(a)** and BP100-KH₉/DNA complex **(b)** on L_o domains of Plasma-MM and fluidization of L_o domains by Cytcox-KH₉/DNA complex **(c)** and BP100-KH₉/DNA complex **(d)**. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); Z_{\max} (a) = 15 nm, Z_{\max} (b) = 10 nm, Z_{\max} (c) = 7.5 nm (0 min), 10 nm (5 min), and Z_{\max} (d) = 7 nm.

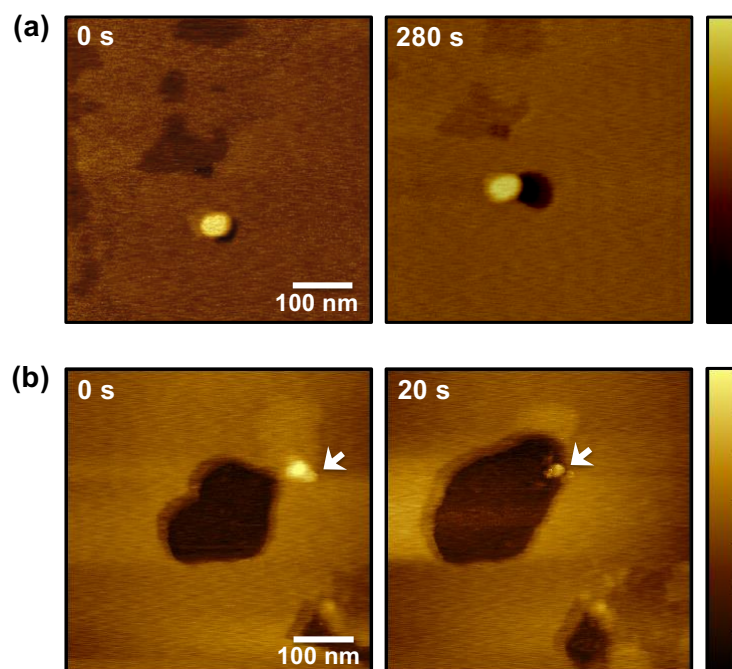


Figure S12. HS-AFM height images showing the pore formation by Cytcox-KH₉/DNA complex in Plasma-MM **(a, b)**. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); $Z_{\max}(\text{a}) = 10 \text{ nm}$ (0 s), 14 nm (280 s) and $Z_{\max}(\text{b}) = 14 \text{ nm}$.

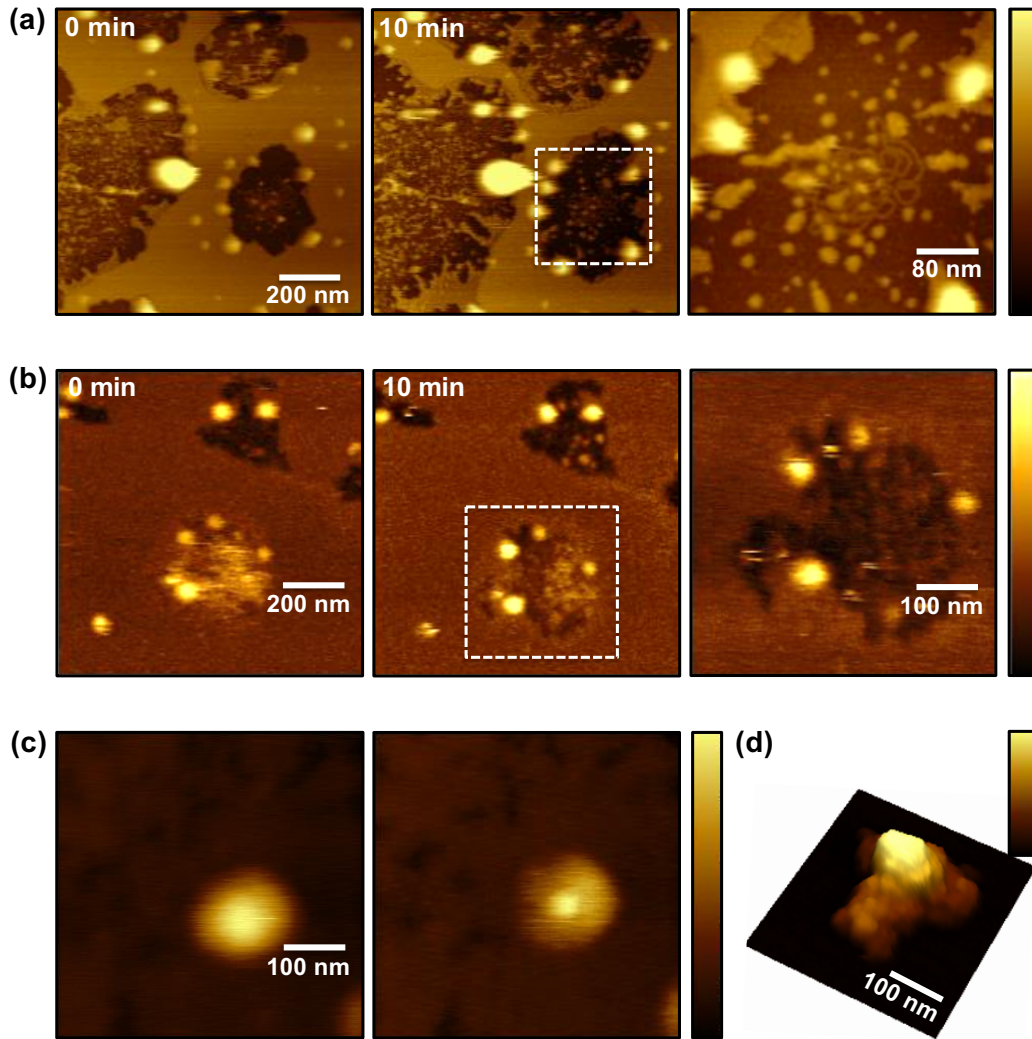


Figure S13. HS-AFM height images showing the disruption in Mito-MM caused by Cytcox-KH₉/DNA complex **(a)** and BP100-KH₉/DNA complex **(b)**. “0 min” corresponds to 4 h in a and b after addition of 40 μ L of complex into the imaging medium. The third images in A and B are the higher-resolution images of the regions within the dashed line boxes in the second images. The images in **(c)** and **(d)** show the intact complex and the partially-dissociated complex on the membrane. The image to the right in (c) was recorded at a higher imaging force. The color bar indicates the Z-range between 0 (darkest) to Z_{\max} (brightest); $Z_{\max}(\text{a}) = 20$ nm, $Z_{\max}(\text{b}) = 25$ nm, $Z_{\max}(\text{c}) = 32$ nm (left), 25 nm (right), and $Z_{\max}(\text{d}) = 100$ nm.

Movies

Movie 1. Cytcox-induced changes in Plasma-MM. Scan rate was 0.5 frame/s.

Movie 2. BP100-induced changes in Plasma-MM. Scan rate was 0.5 frame/s.

Movie 3. Cytcox-KH₉-induced changes in Plasma-MM. Scan rate was 0.5 frame/s.

Movie 4. BP100-KH₉-induced changes in Plasma-MM. Scan rate was 0.5 frame/s.

Movie 5. BP100-KH₉-induced changes in Plasma-MM. Scan rate was 0.5 frame/s.
(continuation of Movie 2)

Movie 6. Cytcox-KH₉-induced changes in Mito-MM. Scan rate was 0.5 frame/s.

Movie 7. BP100-KH₉-induced changes in Mito-MM. Scan rate was 0.5 frame/s.