Cations as switches of amyloid mediated membrane disruption mechanisms: Calcium and IAPP

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Supporting methods

ThT fluorescence assays: The kinetics of peptides amyloid formation were measured using the increase of fluorescence emission upon binding of the commonly used amyloid specific dye thioflavin T. Samples were prepared by adding 1 μL of the peptide stock solution to 100 μL of 10 mM HEPES solution, 100 mM NaCl, pH 7.4, containing 10 μM ThT (final peptide concentration was 2.0 μM) in the presence of LUVs at the concentration of 200 μM. Where present, calcium ion concentrations were 50, 100, 200 or 400 μM. Experiments were carried out in Corning 96 well non binding surface plates. Time traces were recorded using Biotek Synergy 2 plate reader using a 440 nm excitation filter and a 485 emission filter at room temperature, shaking samples for 10 seconds before each read. All measures were done in triplicate.

Supporting figures

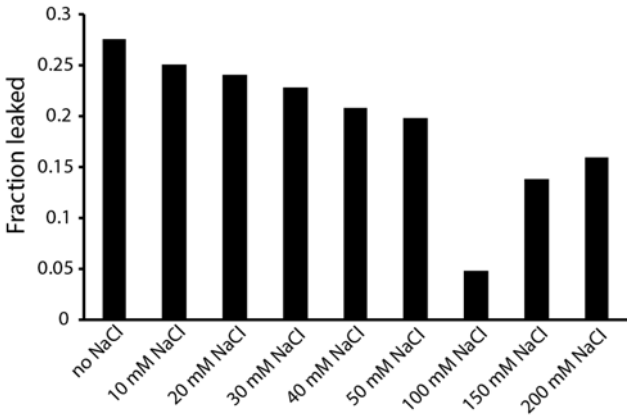

Figure S1. Dye release from model membranes at different NaCl concentrations. Dye release from 200 μM LUVs of POPC/POPS (molar ratio 7/3) after 24 hours in the presence of the indicated concentrations of NaCl in the outside buffer. All experiments were made in 10 mM HEPES buffer, pH 7.4 at 25^{o}C. The concentration of 6-carboxyfluorescein inside the LUVs is 70 mM and no NaCl is present in the inside buffer. Results are the average of three experiments.

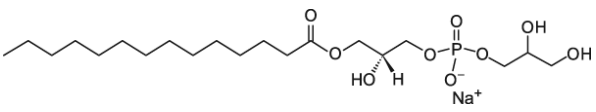

Figure S2. Molecular structure of LysoPG.

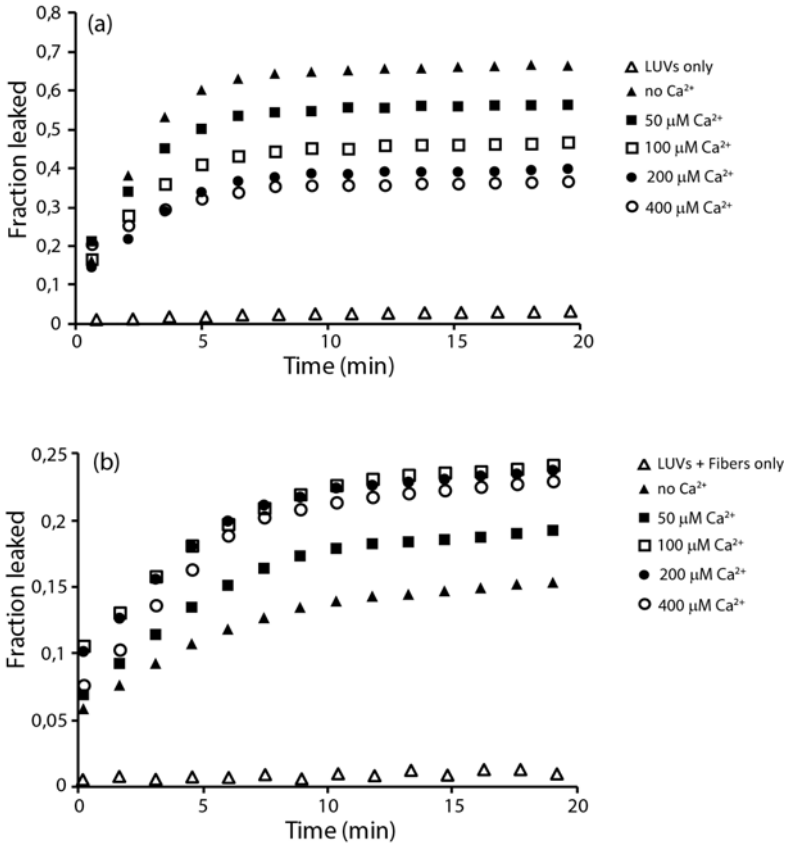

Figure S3. Dye release from model membranes induced by hIAPP in the presence and absence of calcium ions. Kinetics of dye release from 200 µM LUVs of POPC/POPS (molar ratio 7/3) in the absence (a) or presence (b) of 1 µM preformed hIAPP fibers, induced by 2 µM of monomeric hIAPP at the Ca^{2+} concentrations indicated. All experiments were made in 10 mM HEPES buffer, 100 mM NaCl, pH 7.4 at 25°C. Results are the average of three experiments.

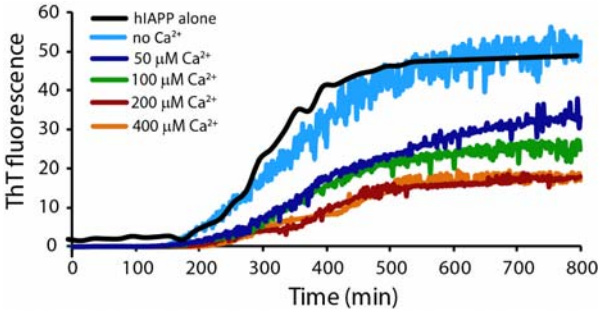

Figure S4. Kinetics of hIAPP fiber formation measured by the ThT fluorescence assay. ThT fluorescence of 2 µM hIAPP in the presence of 200 µM of LUVs POPC/POPS (molar ratio 7/3) in the presence of the indicated concentrations of Ca^{2+}. All experiments were made in 10 mM HEPES buffer, 100 mM NaCl, pH 7.4 at 25°C. Results are the average of three experiments.

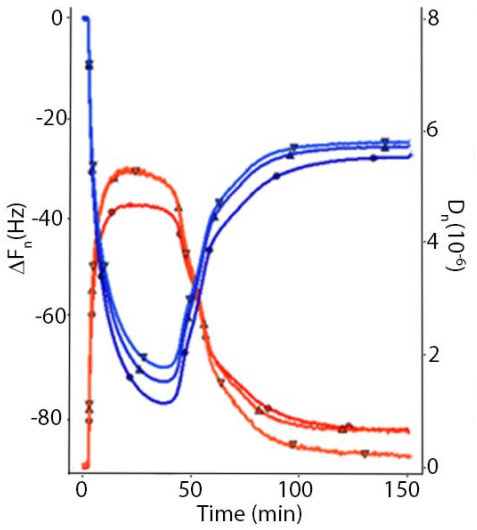

Figure S5. QCM-D analysis of the formation of supported lipid bilayer. QCM-D curves of frequency (blue curves) and dissipation (red curves) shifts for POPC/POPS (molar ratio7/3) after vesicle adsorption (from 0 to ~40 minutes), rupture (~40 to ~90 minutes) and SLB formation (after ~90 minutes).

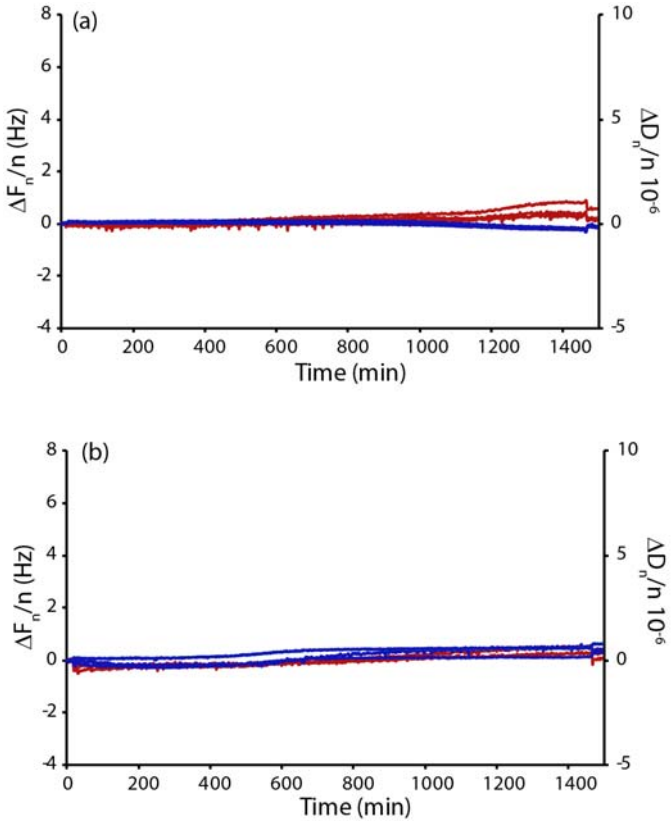

Figure S6. QCM-D analysis of the interaction of rat IAPP (rIAPP) and supported lipid bilayer. QCM-D traces of the interaction of POPC/POPS (molar ratio 7/3) SLB with rIAPP in the absence (a) or in presence (b) of 100 µM of Ca^{2+} ions. (Blue lines: ΔF3, ΔF5 and ΔF7; red lines: D3, D5 and D7). All experiments were made in 10 mM HEPES buffer, 100 mM NaCl, pH 7.4.

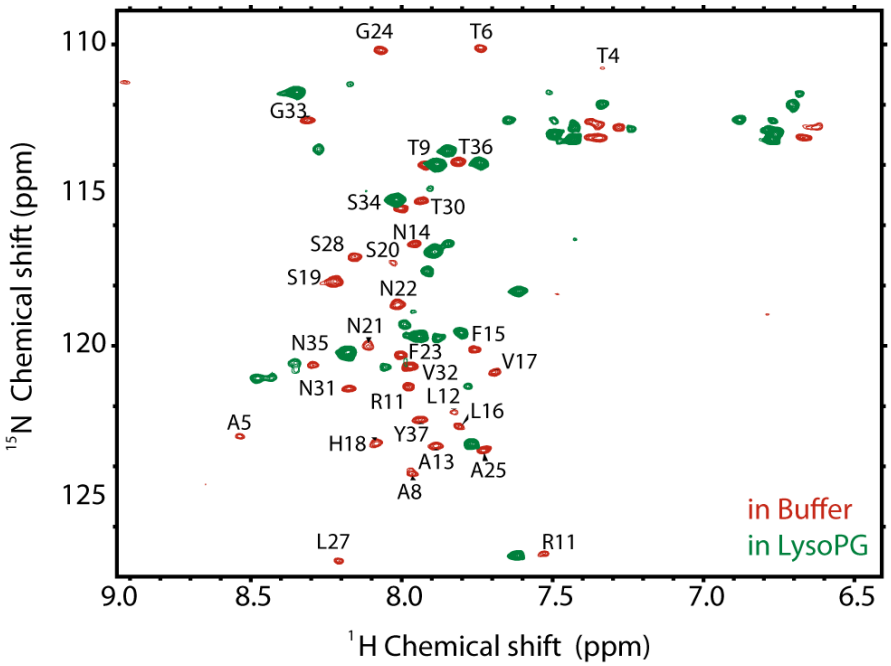

Figure S7. 2D ^{15}N-^{1}H SOFAST-HMQC NMR spectra of hIAPP with and without LysoPG. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP in the presence (green) and absence (red) of 25 mM LysoPG. Resonance assignment corresponds to hIAPP in the absence of LysoPG.

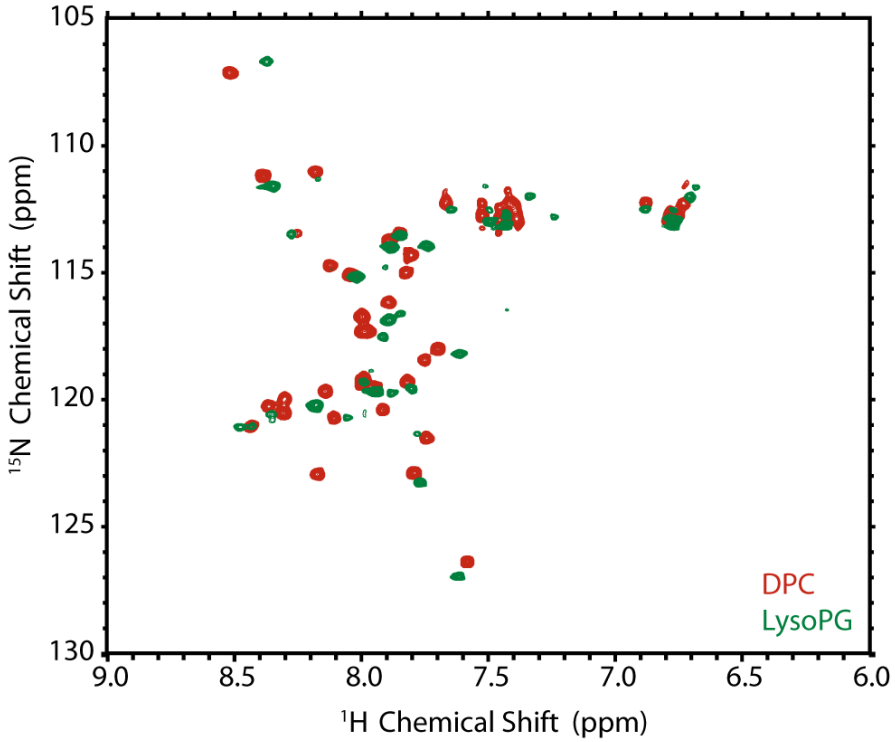

Figure S8. 2D ^{15}N-^{1}H SOFAST-HMQC NMR spectra of hIAPP bound to DPC and LysoPG. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP bound to LysoPG (green) and DPC (red) micelles.

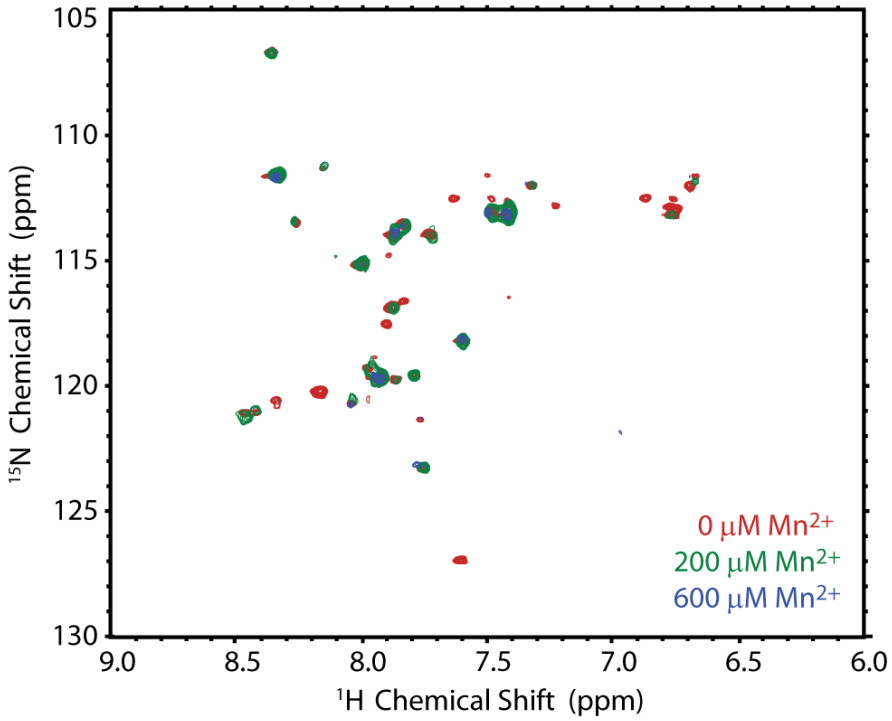

Figure S9. Paramagnetic quenching of hIAPP bound to LysoPG in the absence of Ca^{2+}. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP bound to 25 mM LysoPG with the indicated concentration of $MnCl_2$.

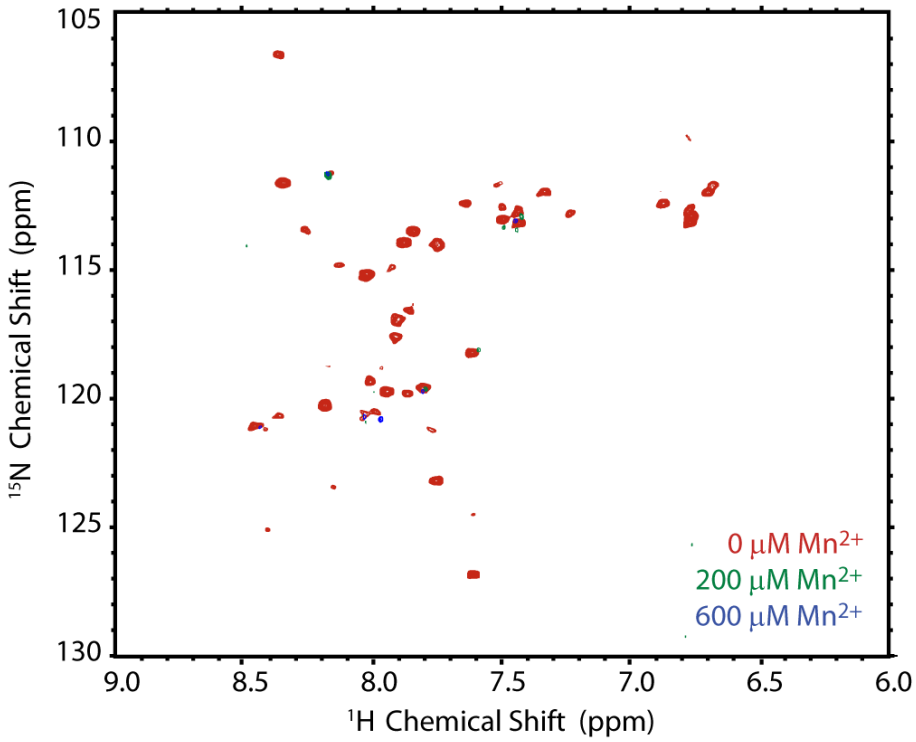

Figure S10. Paramagnetic quenching of hIAPP bound to LysoPG in the presence of Ca^{2+}. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP bound to 25 mM LysoPG in the presence of 12.5 mM $CaCl_2$ with the indicated concentrations of $MnCl_2$.

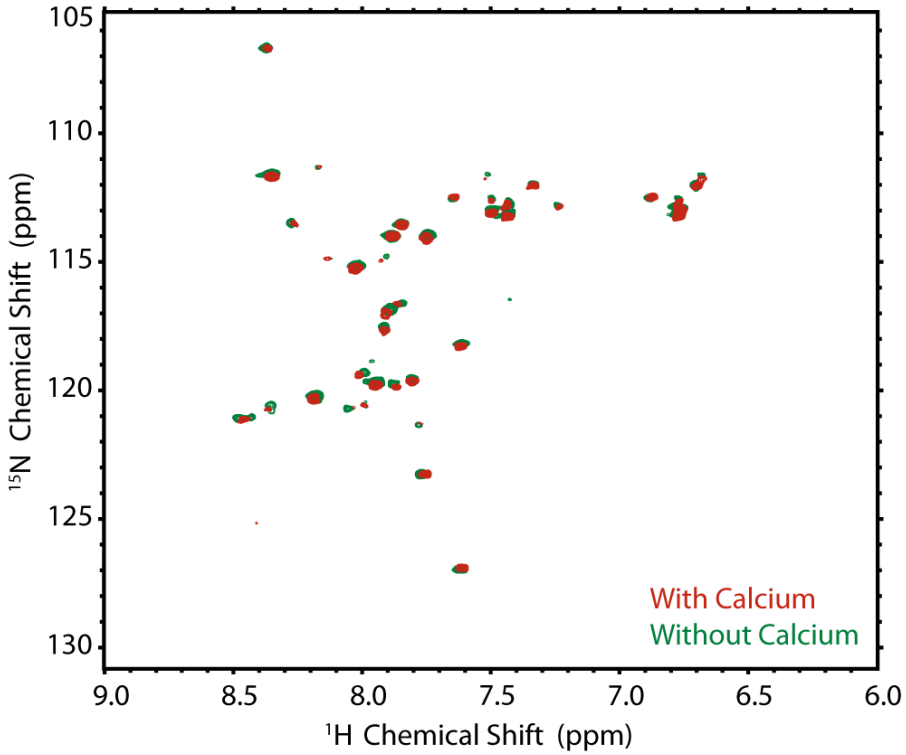

Figure S11. 2D ^{15}N-^{1}H SOFAST-HMQC NMR spectra of hIAPP bound to LysoPG with and without Ca^{2+}. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP bound to 25 mM LysoPG with (red) and without (green) 12.5 mM $CaCl_2$.

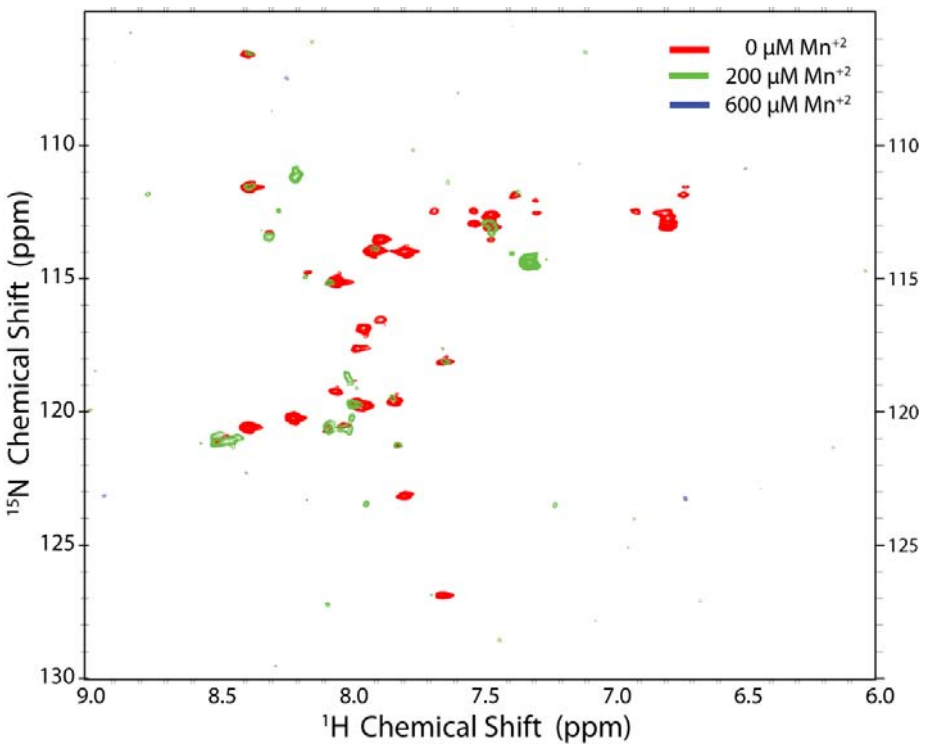

Figure S12. Paramagnetic quenching of hIAPP bound to LysoPG in the presence of Mg^{2+}. 2D ^{15}N-^{1}H SOFAST-HMQC spectra of hIAPP bound to 25 mM LysoPG in the presence of 12.5 mM $MgCl_2$ with the indicated concentrations of $MnCl_2$.

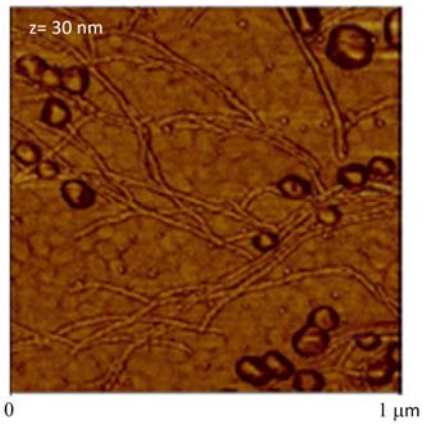

Figure S13. AFM images of hIAPP on Mica after 24h.

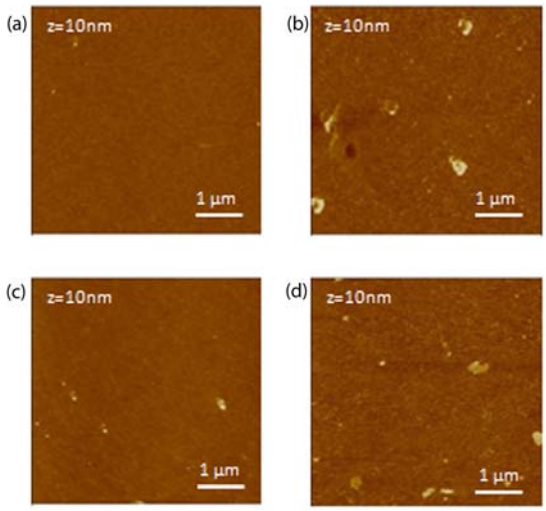

Figure S14. AFM images of rIAPP on SLB with and without calcium ions. (a) POPC/POPS (molar ratio 7/3) SLB after exposure to 2 µM rIAPP for 2 hours; (b) POPC/POPS (molar ratio 7/3) SLB exposed to 2 µM rIAPP for 24 hours; (c) POPC/POPS (molar ratio 7/3) SLB after exposure to 2 µM rIAPP and 100 µM Ca^{2+} ions for 2 hours; (d) POPC/POPS (molar ratio 7/3) SLB, after exposure to 2 µM rIAPP and 100 µM Ca^{2+} ions for 24 hours.