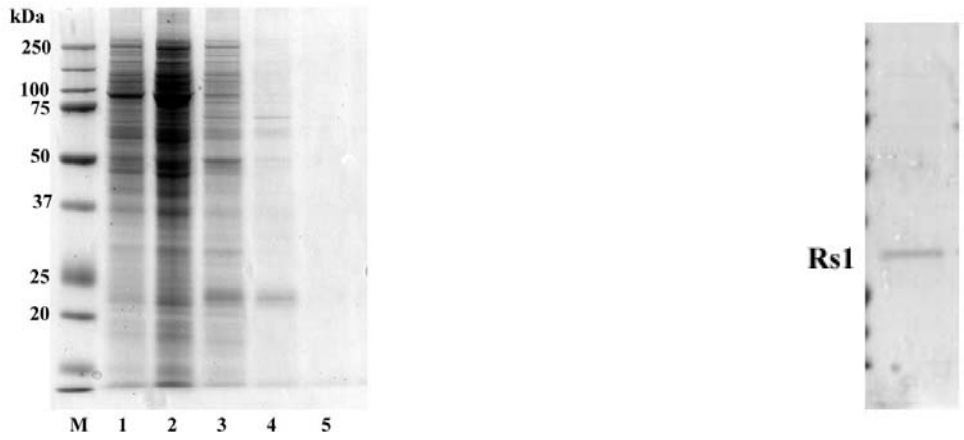
Supplementary Material

I. Protein purification

SDS-PAGE analysis of the Rs1 protein at different stages of purification. A. Recombinant Rs1 was purified fromSf9 cells as described in Materials and Methods. Proteins were visualized via SDS-PAGE and CBB staining. M-molecular weight size markers; 1- input; 2- flow through; 3-5- imidazole eluted fractions. B. To further remove the non-specific proteins, the imidazole fractions (3-5) were reloaded on a fresh pre-washed His Pur^{TM}-Cobalt resin and eluted with elute buffer (50 mM sodium phosphate, 250 mM NaCl and 250 mM imidazole, pH 7.4) in 1ml linear gradients (20-250 mM imidazole). (B) A fraction representing the purified His- Rs1.

A **B**



***LC–MS/MS* analysis:**

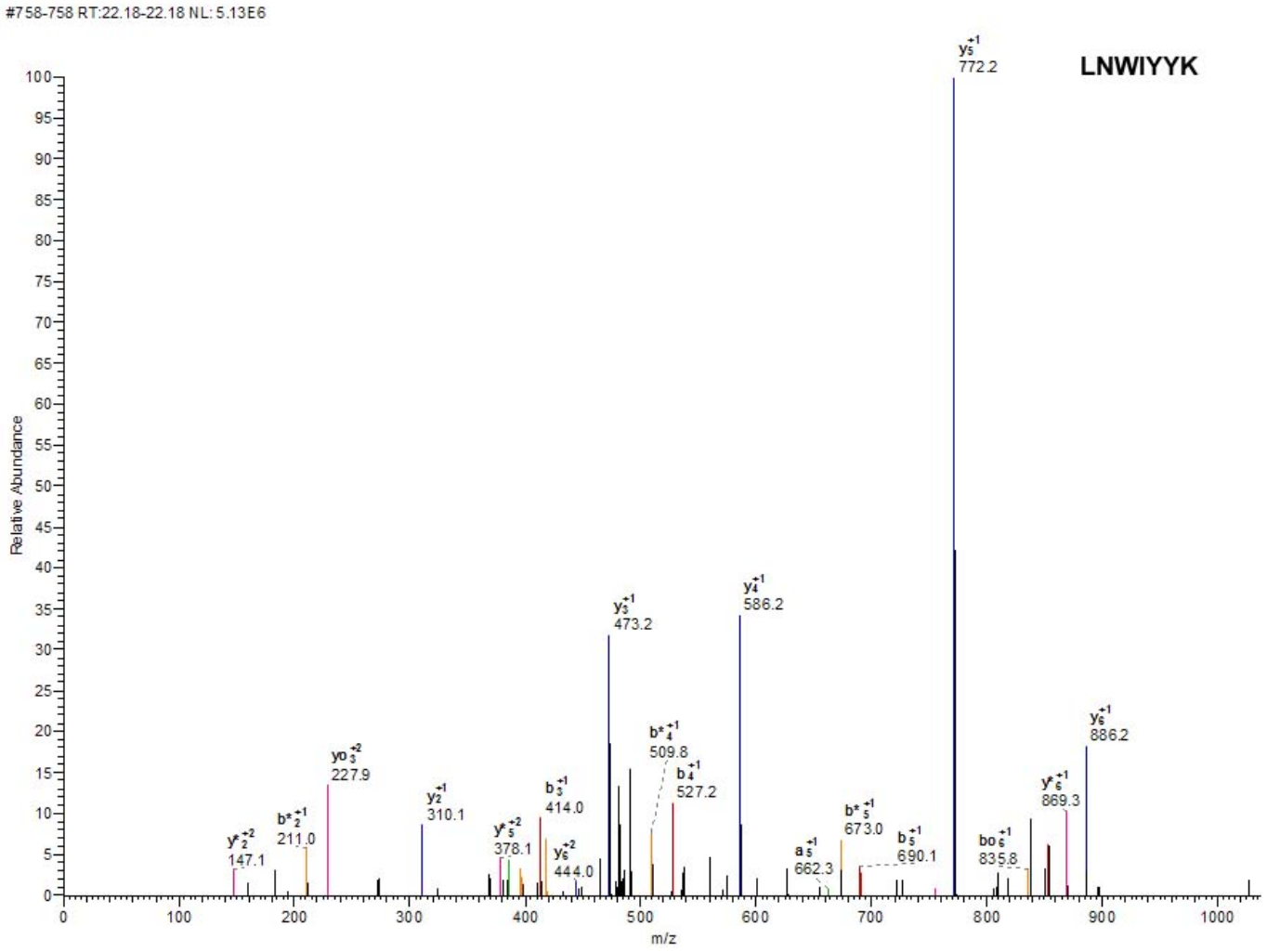
In-gel digestion: Following electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250. Coomassie-stained Rs1 band was excised from the gel and subjected to in-gel tryptic digestion in an Investigator ProPrep Robot (Digilab, Holliston, MA) essentially according to the manufacturer's protocol, but modified for digestion in 0.1% RapidGest (Waters, Milford, MA).

LC–MS/MS: Resulting in-gel digests were analyzed by LC–MS/MS on a LTQ XL mass spectrometer equipped with 2 Surveyor HPLC pumps (Thermo, San Jose, CA), and an Advance

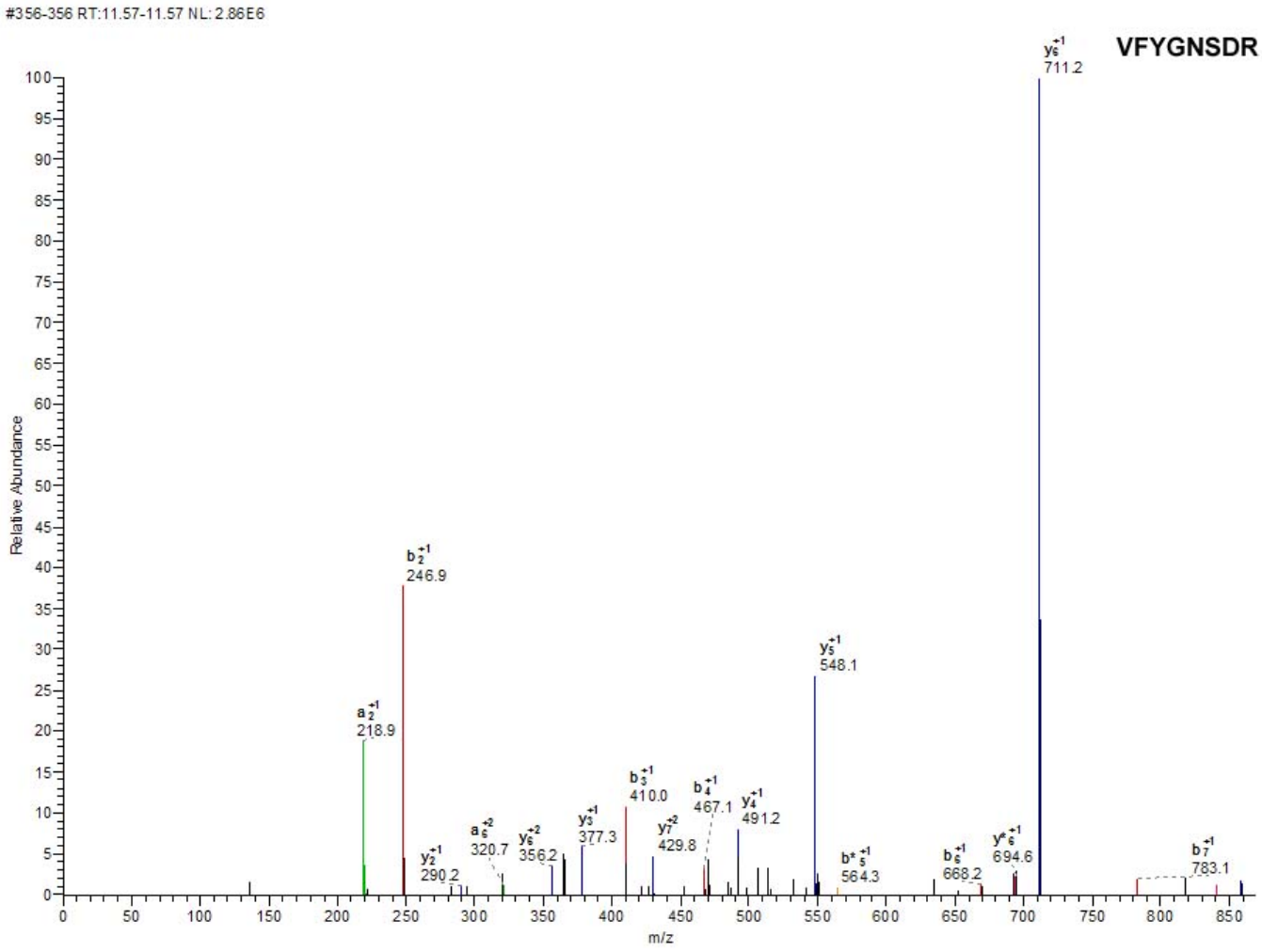
ESI source (Michrom Bioresources, Auburn, CA) and configured as previously described (REF: BBRC, 391, 78-84, 2010). Resulting MS/MS spectra were searched against the human database for peptide identification utilizing BioWorks 3.3.1 SP1 software (Thermo, San Jose, CA).

MS/MS Spectrum of the peptides corresponding to the sequence of RS1

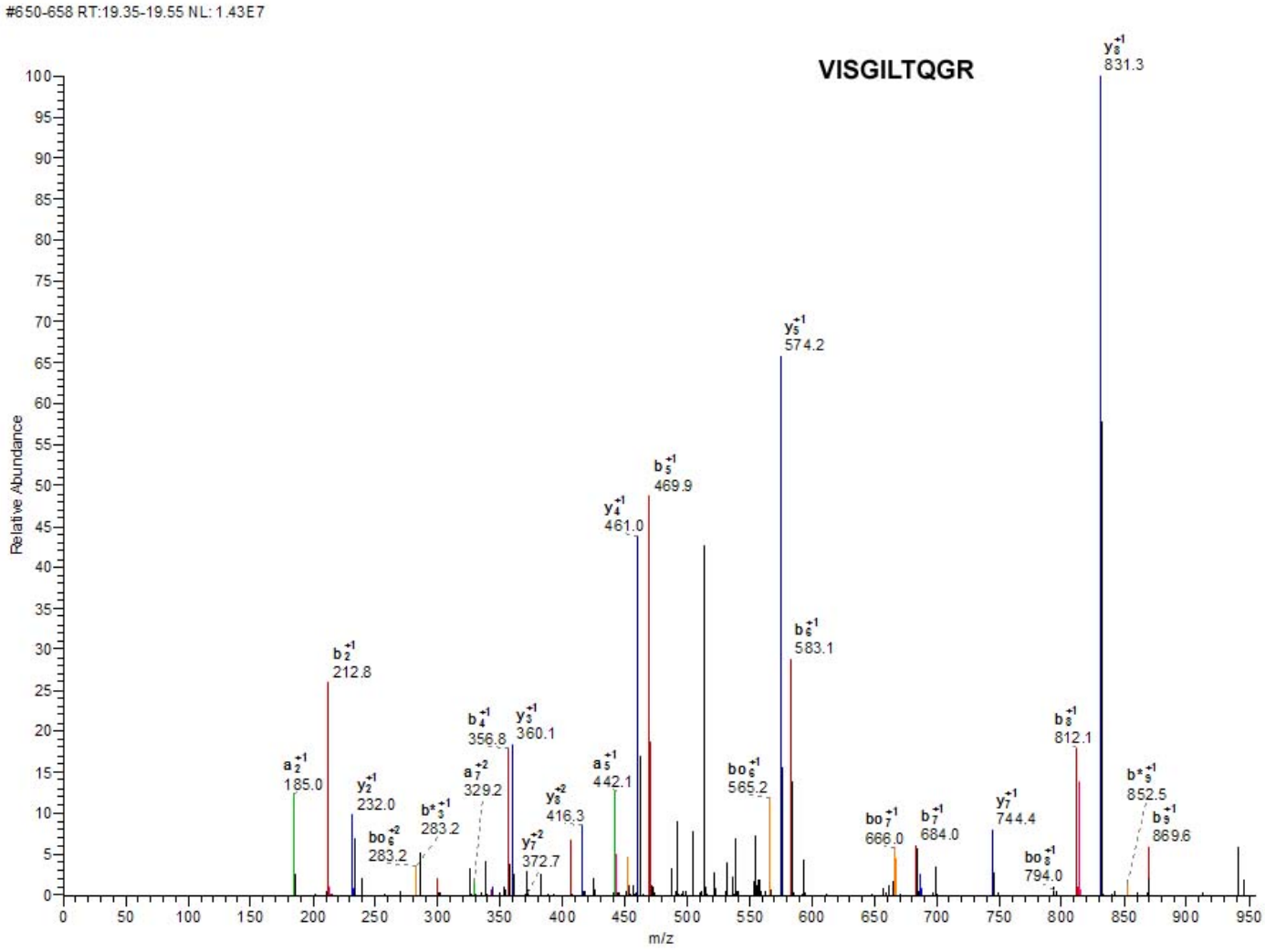
(A) LNWIYYK



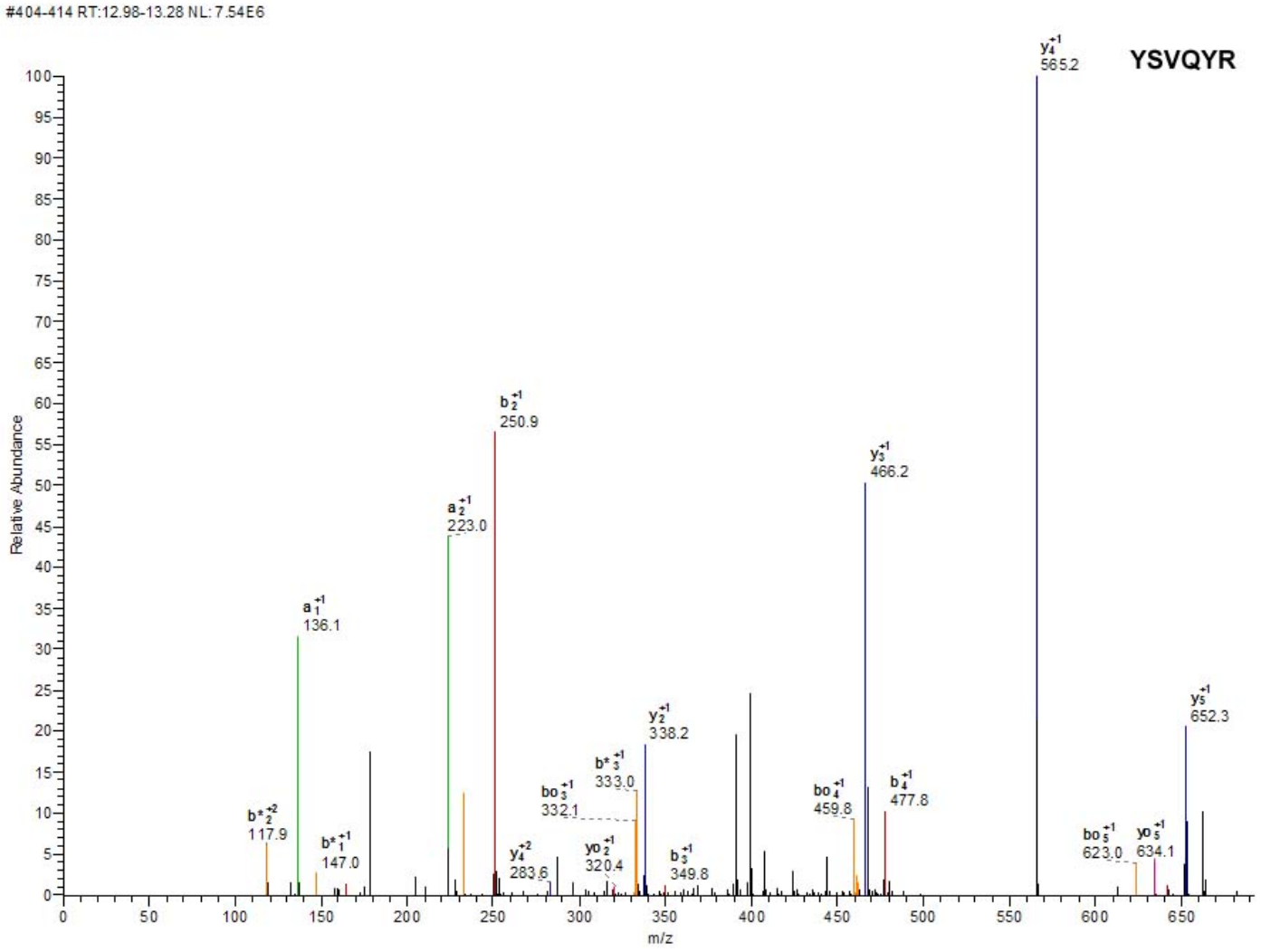
(B) VFYGNSDR


#356-356 RT:11.57-11.57 NL:2.86E6
VFYGNSDR
Relative Abundance
m/z
y6+1 711.2
b2+1 246.9
a2+1 218.9
y5+1 548.1
b3+1 410.0
b4+1 467.1
y4+1 491.2
y3+1 377.3
y7+2 429.8
a6+2 320.7
y6+2 356.2
y2+1 290.2
b*5+1 564.3
b6+1 668.2
y*6+1 694.6
b7+1 783.1

(C) VISGILTQGR



(D) YSVQYR


#404-414 RT:12.98-13.28 NL:7.54E6
YSVQYR
Relative Abundance
m/z
y1+1 565.2
b2+1 250.9
y3+1 466.2
a2+1 223.0
a1+1 136.1
y2+1 338.2
y5+1 652.3
b*3+1 333.0
bo3+1 332.1
bo4+1 459.8
b4+1 477.8
b*2+2 117.9
b*1+1 147.0
y4+2 283.6
yo2+1 320.4
b3+1 349.8
bo5+1 623.0
yo5+1 634.1

II. Time-lapse AFM of supported bilayers interacting with Rs1

Movie 1: Interaction of Rs1 with pure PS bilayer. The initial protein injection was made at the end of the first frame (see image disturbance toward the top of the first frame). The phase reorganization is evident during the four successive injections of protein. The shape of some of the calcium-rich, solid phase remains persistent during the whole experiment while the fluid phase appears to expand until almost full coverage is attained. Eventually, the system comes to a final equilibrium configuration.

Movie 2: Interaction of Rs1 with pure PS bilayer. The protein was injected immediately after the first frame. The height scale is 70nm, chosen to allow visualization of the stacks of multiple bilayer patches as they "dissolve" due to the presence of Rs1. Notice the shifting and reduction in area of overlying patches, while the bilayer attached to the substrate significantly expands. The reorganization of the phase structures during the protein adsorption is more difficult to see in this movie because of the height scale.

Movie 3: Interaction of Rs1 with a five-component lipid bilayer. The bilayer before protein introduction is continuous and has a rather complex, fractal-like, structure seen in the first frame of the movie. Upon injection of Rs1 the bilayer is severely disrupted with holes appearing in a pattern mirroring the phase structure of the bilayer itself. Eventually, the holes start "healing". Equilibrium is eventually established with a continuous bilayer and with the protein selectively adsorbed, in a pattern again mirroring the initial phase structure.

III. Control Experiments

Several experiments were performed for each lipid combination in the presence and absence of Ca^{2+} following the evolution of the bilayers before and after Rs1 injection. In all the experiments of this study imaging was performed continuously and over the same region from the moment the sample supported bilayer was introduced into the AFM for imaging until the final equilibrium was reached after Rs1 (or Rs1-antibody as is the case for Figure 6 in the manuscript) injections.

A. PS bilayer with Ca^{2+} moving toward equilibrium

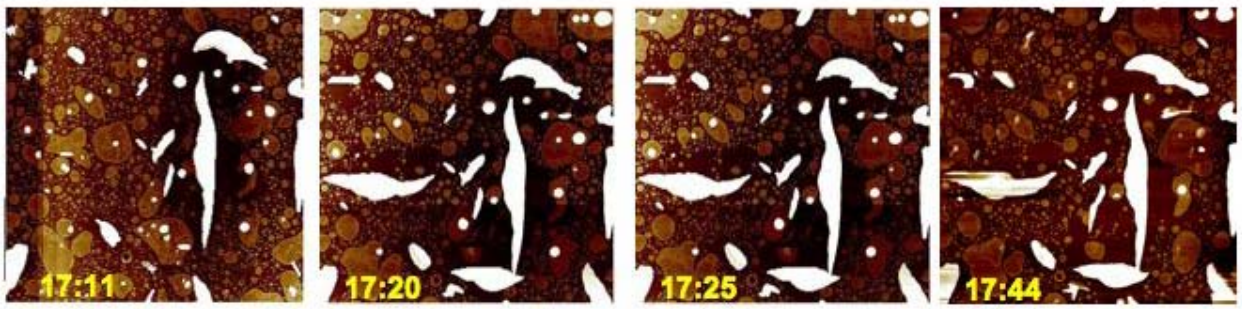


Figure SIII-*A*. Supported lipid bilayer was formed from PS vesicle solution containing Ca^{2+}. Imaging started shortly before 17:11pm. Some changes occurred during the first few minutes of imaging. By 17:44pm the bilayer reached equilibrium. No changes were observed for more than one hour after which Rs1 was injected.

B. Five-component lipid mix with Ca^{2+} moving toward equilibrium

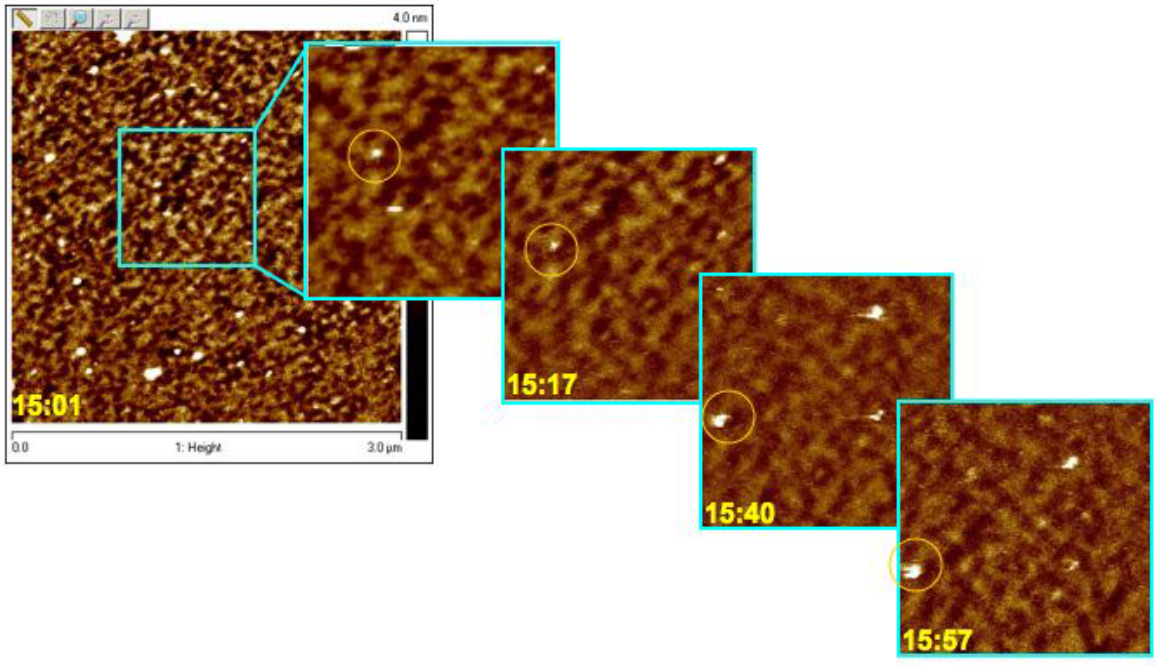


Figure SIII-*B.* Supported bilayer was formed form the five-lipid mix as described in Materials and Methods. The mix formed continuous bilayers with complex phase structure. Imaging was initiated shortly before 15:01pm. The changes observed were very small as can be seen by following a couple of small impurities (white spots). The bilayer was imaged for over an hour past 15:57 without noticeable changes upon which Rs1 was injected.

C. Rs1 adsorption to pure PS bilayer in the absence of Ca^{2+}

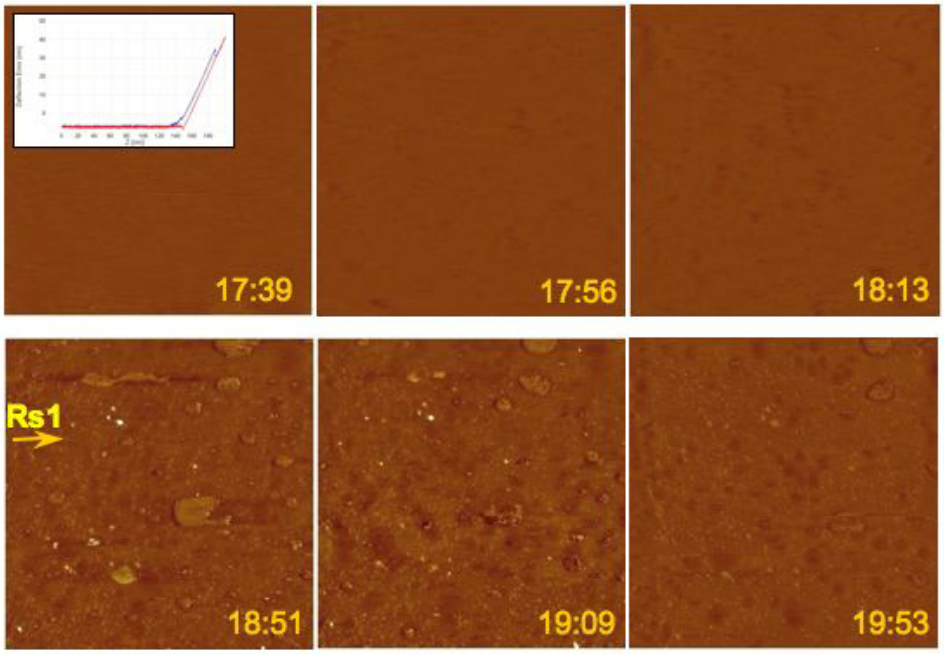


Figure SIII-*C.* Pure PS vesicle solution in the absence of Ca^{2+} resulted in uniform, continuous supported bilayers with small regions of a slightly lower phase. The presence of the bilayer was ascertained by the characteristic break-through discontinuity in the force-distance curve of indentation tests like the one shown in the inset of the first image (17:39). Rs1 was injected shortly before 18:51 and is seen randomly adsorbing, and even creating patches of protein on the PS bilayer. The interaction, however, was weak and probably non-specific since continued scanning removed the protein from the scanned surface. Moreover, no bilayer disruption or phase reorganization was observed.

D. **Rs1 adsorption to five-component lipid mix in the absence of Ca^{2+}**

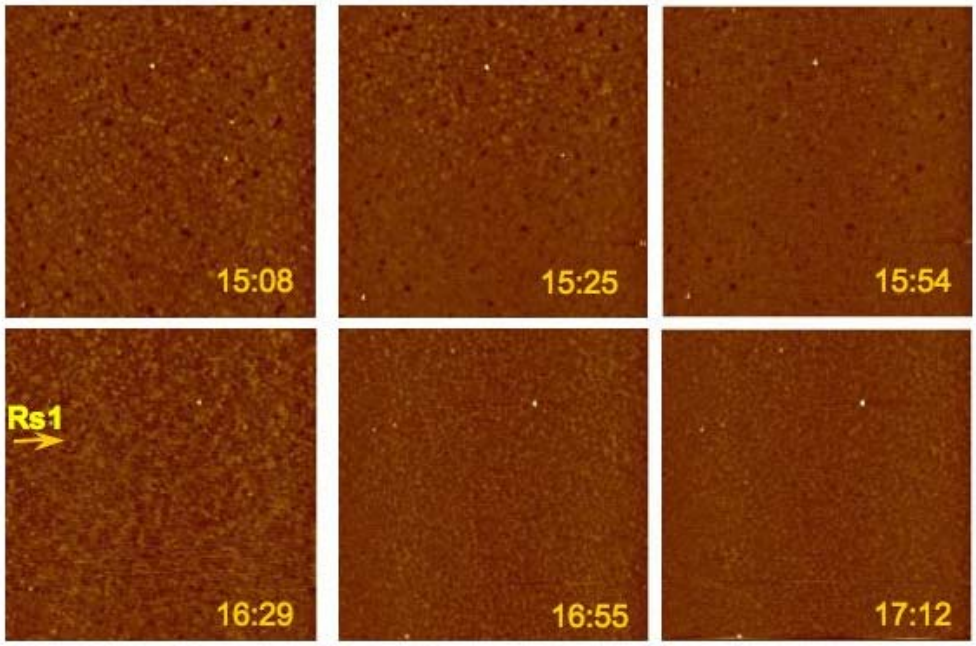


Figure SIII-*D*. A five-component lipid bilayer formed continuous supported bilayers with complex phase structure. Imaging was started shortly before 15:08pm and after about 1 hour of imaging no noticeable changes occurred. At that point Rs1 was injected. The protein did not adsorb and only subtle changes were seen in the bilayer which tended to vanish over time leaving the bilayer looking very much like before protein injection. Rs1 did not cause any disruption of the bilayer in the absence of Ca^{2+}.