Supplementary Information

Antibiofilm surfaces based on the immobilization of a novel recombinant antimicrobial multidomain protein using self-assembly monolayers

Adriana R. Kyvik,[a] Ramon Roca-Pinilla,[d,f] Karla Mayolo-Deloisa,[a,e] Xavier Rodriguez Rodriguez,[a] Marc Martinez-Miguel,[a,b] Marta Martos,[a] M. Köber,[a,b] Nora Ventosa,[a,b] Jaume Veciana,[a,b] Judith Guasch,[a,b,c] Elena Garcia-Fruitós,[d] Anna Arís,[d] Imma Ratera[a,b]†

1. Fluorescence microscopy images of patterned mGFPH6

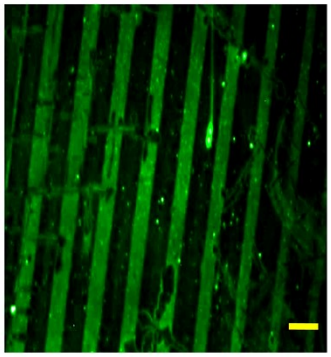


Figure S1. Fluorescence microscopy images of patterned mGFPH6 using the S-PEG-NTA-Ni SAMs strategy, obtaining **S-NTA-Ni-mGFPH6**. Micropatterns observed were created using the μCP technique with a PDMS stamp with a pattern of 20 μm. Scale yellow bars correspond to 30 μm.

2. Cyclic Voltammetry

A redox probe was used in order to study the electron transfer reactions between the redox probe and the gold surface under the functional mixed SAMs. As a redox probe it has been used hexaammineruthenium(III) chloride ($[Ru(NH_3)_6)]^{3+}$) because it is reported that can detect even the smallest defects in SAMs, which are invisible for other markers such as ferrocyanide [25]. The greater sensitivity of the $[Ru(NH_3)_6)]^{3+}$ probe can be explained by the location of its excess charge, located on the metal core in the ion center, whereas, in the case of hexacyano metal complexes, it is located on the terminal nitrogen atoms of cyano ligands [26]. Thus, as a consequence, [Ru(NH3)6)]3+ species can penetrate further into SAMs and diffuse along the SAM chains, so despite forming a very compact SAM, with the probable presence of some collapsed sites current signal can still appear in the recorded

voltammogram. Several studies have confirmed that the thicker the SAMs the higher the blocking properties [27].

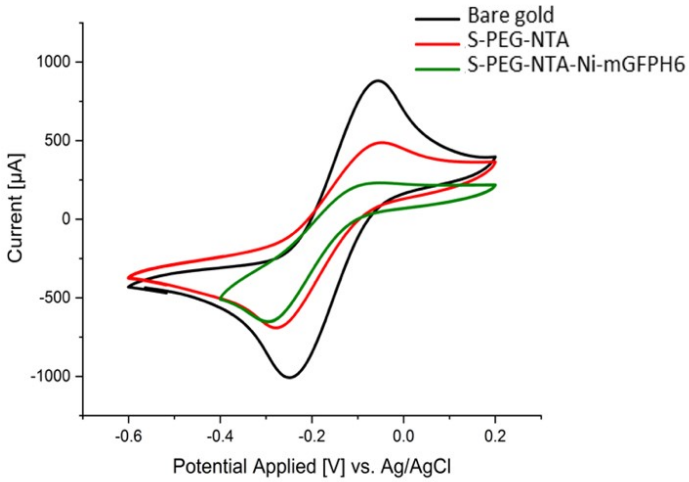


Figure S2. Cyclic voltammograms of bare gold, mixed SAMs and S-PEG-NTA-Ni-mGFPH6 SAMs. The electrolyte used is an aqueous KCl solution (50 mM) containing the redox probe $[Ru(NH_3)_6)]^{3+/2+}$ (5 mM). Scan rate used is 0.1 V/s.

3. Fluorescence profiles extracted from fluorescence images of immobilized JAMF1

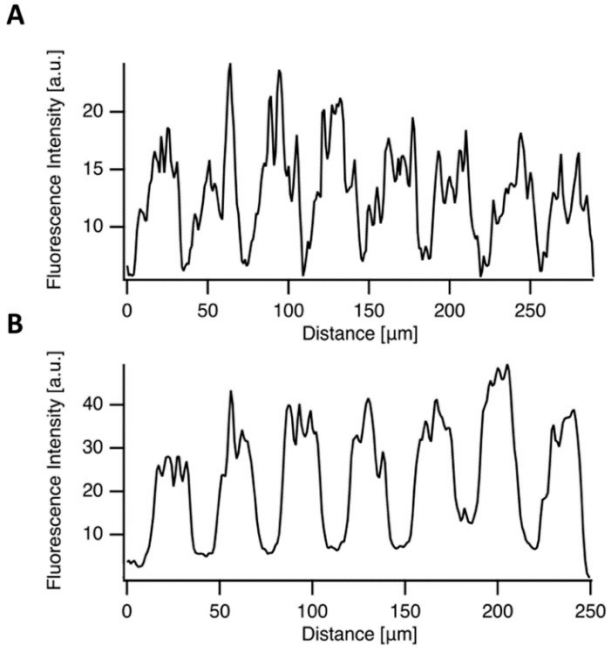


Figure S3. Fluorescence profiles extracted from fluorescence images of immobilized JAMF1: (A) soluble protein and (B) pNP after an immunostaining treatment. Specifically, these profiles correspond to the images portrayed in Figure 6 (A) and (B).

4. Cyclic voltammograms

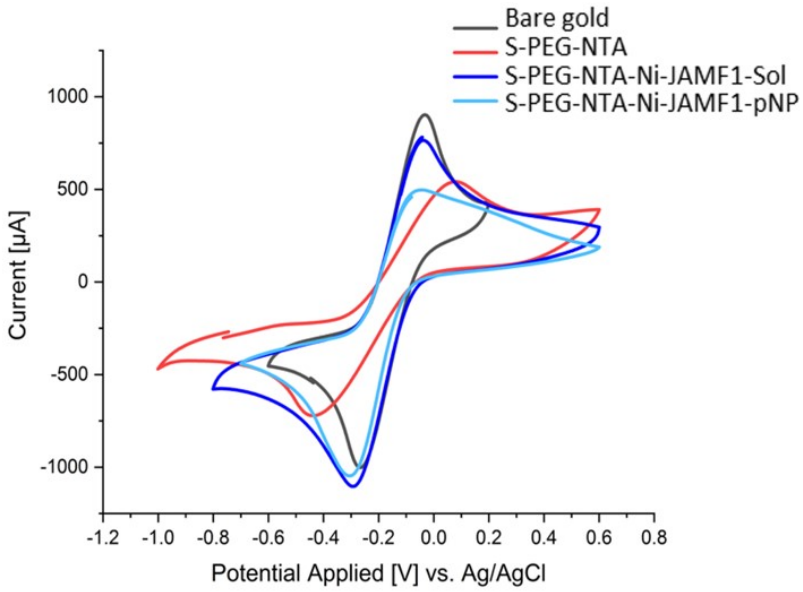


Figure S4. Cyclic voltammograms of bare gold, S-PEG-NTA, S-PEG-NTA-Ni-JAMF1-Sol and S-PEG-NTA-Ni-JAMF1-pNP. The electrolyte used was an aqueous KCl solution (50 mM) containing the redox probe $[Ru(NH_3)_6)]^{3+/2+}$ (5 mM). Scan rate used is 0.1 V/s.

5. Processing of AFM images (Figures 5 and 6)

For the AFM Image processing it has been used the software "Gwyddion". For the image processing of the topography the data has been leveled by mean plane subtraction and a flatten using 'Revolve Arc' function (30 pixel, 2,9 um, horizontal direction). No image processing for phase images. Only using the 'Revolve Arc' Flattening it has been possible to get a good image contrast to visualize the striped pattern. For the hight profiles, images have been treated with a mean Plane subtraction, a flatten base and offset subtraction.

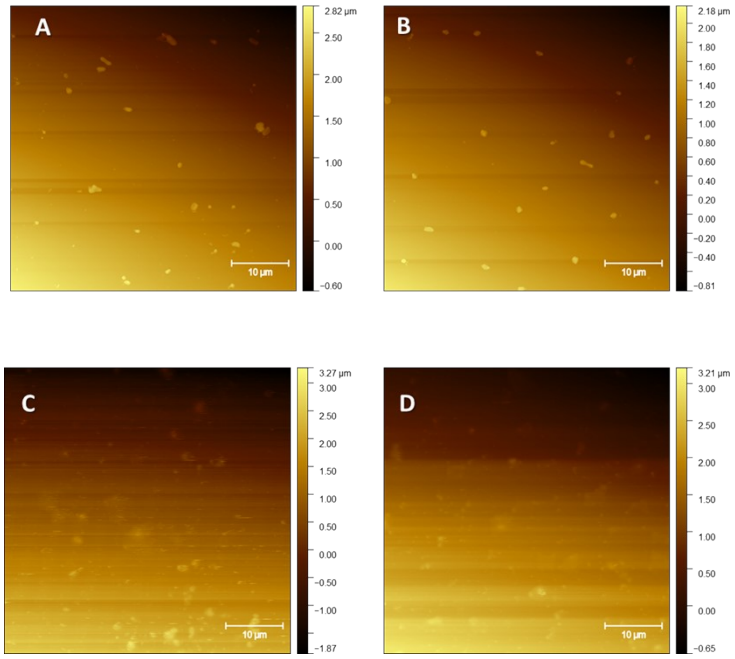


Figure S5. Unprocessed AFM images of A) 2 µm wide striped pattern of S-PEG-NTA-Ni-JAMF1-Sol; B) and their negative controls treated with EDTA (100 mM) S-PEG-NTA-Ni-JAMF1-Sol-Ctrl; C) 2 µm wide striped pattern of S-PEG-NTA-Ni-JAMF1-pNP and its negative control treated with EDTA (100 mM), S-PEG-NTA-Ni-JAMF1-pNP-Ctrl

6. XPS spectra

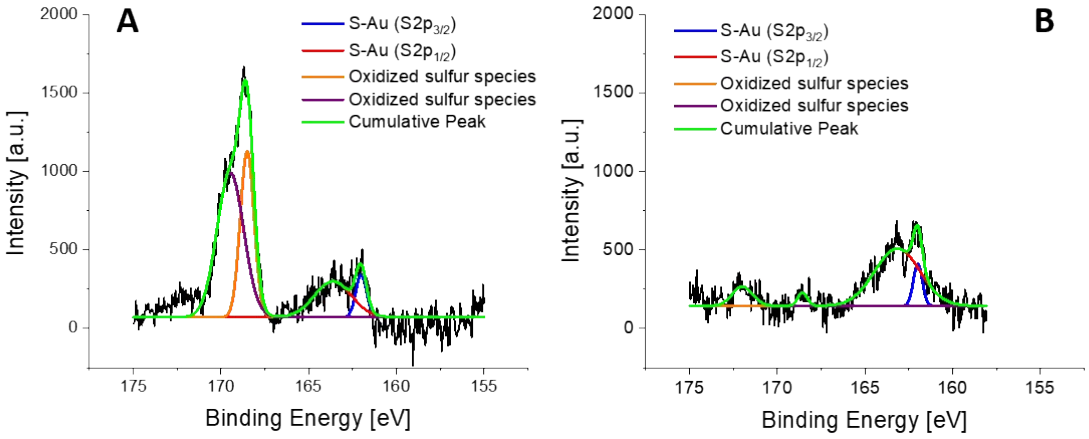


Figure S6. XPS deconvolutions of S 2p spectra for (A) S-PEG-NTA-Ni-JAMF1-Sol and (B) S-PEG-NTA-Ni-JAMF1-Sol-Ctrl (treatment with EDTA 10 mM).

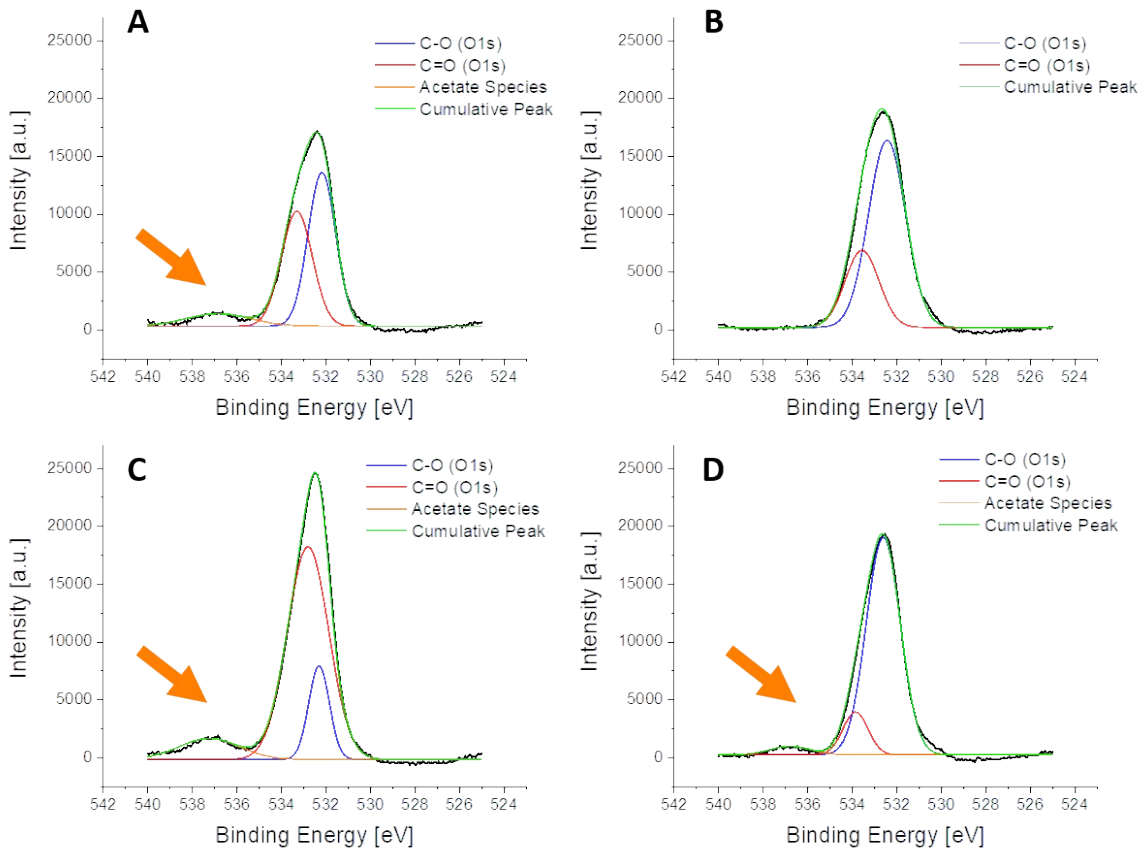


Figure S7. XPS deconvolutions of O 1s spectra for (A) S-PEG-NTA-Ni-JAMF1-Sol, (B) S-PEG-NTA-Ni-Sol- Ctrl (treatment with EDTA 10 mM), (C) S-PEG-NTA-Ni-pNP and (D) S-PEG-NTA-Ni-pNP-Ctrl (treatment with EDTA 100 mM). The yellow arrow indicates the contribution of acetate species.

7. Western Blots

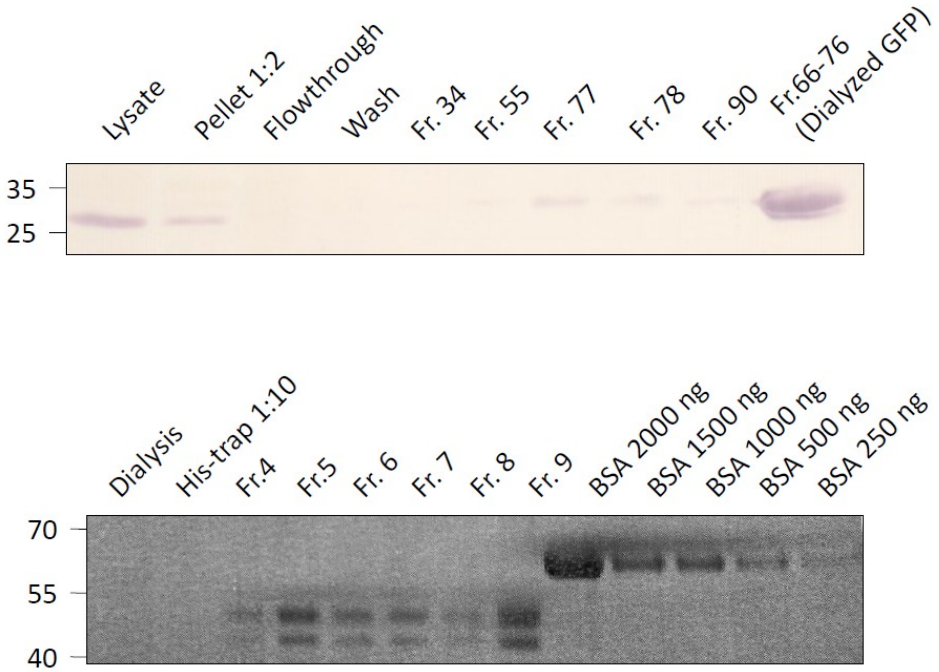
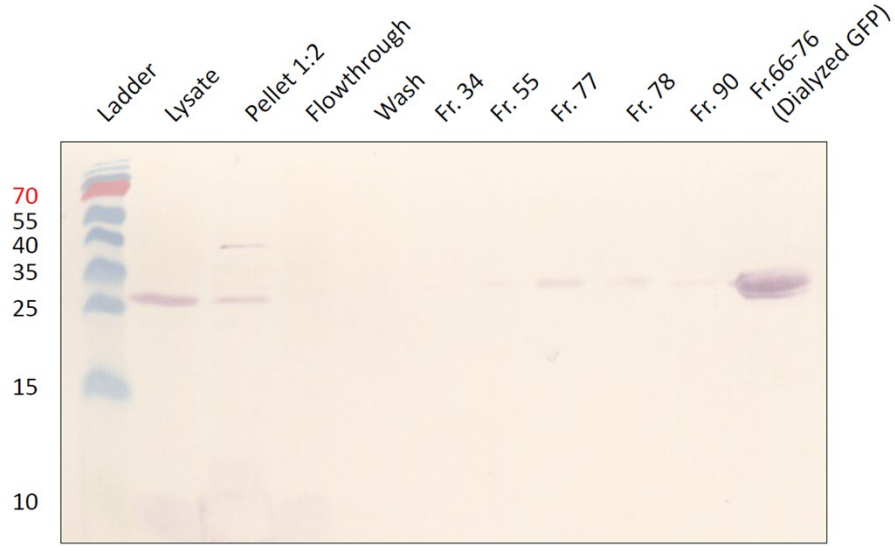


Figure S8. Western blot analysis to quantify JAMF1 (Bottom) and GFPH6 (Top).



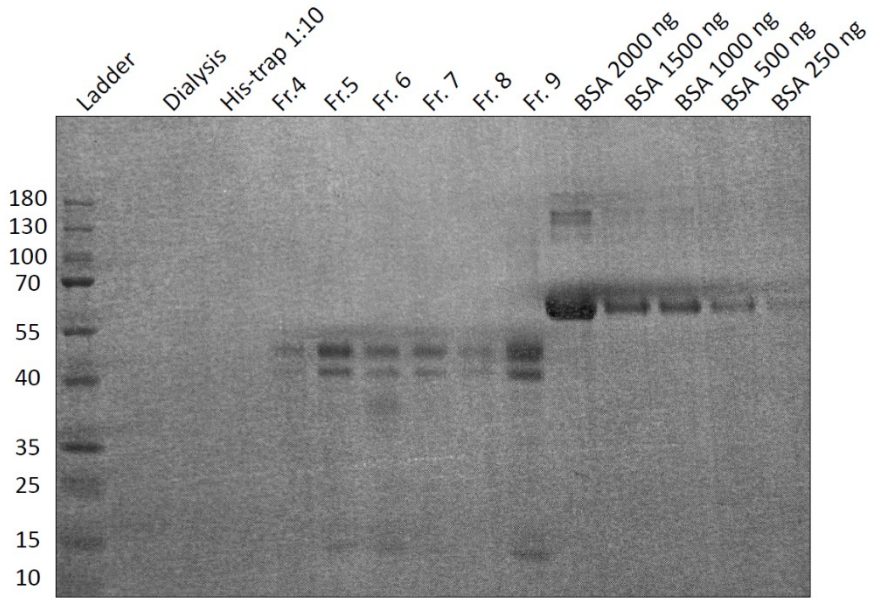


Figure S9. Uncropped images of the Western blot data with all samples and controls in the same blot with labelled lanes. JAMF1 (Bottom) and GFPH6 (Top).