Electronic Supplementary Information

Self-Assembled Monolayer for AFM Measurements of Tobacco Mosaic Virus (TMV) at the Atomic Level

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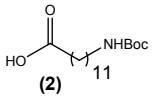
Synthesis of ureido silylated coupling agent (6):

All solvents were dried and distilled under inert atmosphere, according to literature procedures, immediately before use. ^{1}H and ^{13}C NMR spectra of compounds were recorded on Bruker DPX 300 spectrometer at room temperature in deuterated chloroform and using TMS as internal reference for the chemical shifts. Chemical shifts, δ, were represented in part per million (ppm) and coupling constants, *J*, in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintuplet and m = multiplet. High resolution mass spectra were obtained on hybrid Fisons-Instruments VG Micromass AutoSpec-EQ spectrometer using $LSIMS^{+}$ technique in 3-nitrobenzyl alcohol (NBA) as the matrix. Microanalyses of molecules were performed at the Service Central d'Analyse du CNRS, 69100 Villeurbanne.

Synthesis of 12-(tert-butoxycarbonylamino)dodecanoic acid **(2)**[1]



(2)

Aminododecanoic acid **(1)** (2 g, 9.3 mmol) was dissolved in a mixture of *tert*-butanol and water (3/1, 20 mL). The pH was adjusted to 13-14 with NaOH (1N). Di-*tert*-butyl dicarbonate (2.84 g, 13 mmol) was slowly added at 0°C. After 16 hours of stirring at 25°C, a solution of 10% HCl was slowly added at 0°C until pH 2. The aqueous phase was extracted 3 times with ethyl acetate. Organic phases were gathered, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford 2.52 g of product. (86%)

^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 4.57 (s, 1H, -NH-); 3.06 (m, 2H, -C$\underline{H_2}$-NH-); 2.31 (t, *J* = 7.5 Hz, 2H, -CH_2-CO-); 1.62 (quint, 2H, -C$\underline{H_2}$-CH_2-CO-); 1.42 (s, 9H, -C-$(CH_3)_3$); 1.25 (s, 16H, $(-CH_2-)_8$).

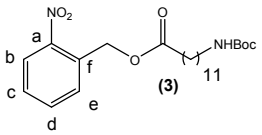
^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 179.02 ($\underline{C}O_{acide}$); 156.03 ($\underline{C}O_{Boc}$); 78.92 (-$\underline{C}_{Boc}$-); 40.53 (-$\underline{C}H_2$-NH-); 34.03 (-$\underline{C}H_2$-CO-); 29.92-28.33 (5 -CH_2-); 28,22 (CH_3); 26.61 (-$\underline{C}H_2$-CH_2-NH-); 24.64 (-$\underline{C}H_2$-CH_2-CO-).

FTIR (cm^{-1}): 1518 (δ_{NH}); 1686 ($\nu_{CO\ Boc}$); 1700 ($\nu_{CO\ acidic\ dimer}$); 1716 ($\nu_{CO\ acid}$); 2851 (ν_s CH_2); 2873 (ν_s CH_3); 2917 (ν_{as} CH_2); 2977 (ν_{as} CH_3); 3368 (ν_{NH}).

Anal. Calc. for $C_{17}H_{33}NO_4$ (%): C 64.73; H 10.54; O 20.29; N 4.44. Found (%): C 64.45; H 10.56; O 21.16; N 4.45.

SMHR-ESI Calculated for $C_{17}H_{33}NO_4Na$ [M+Na]+ 338.2301; found 338.2317.

Synthesis of 2-nitrobenzyl 12-(tert-butoxycarbonylamino)dodecanoate **(3)** [2, 3.]

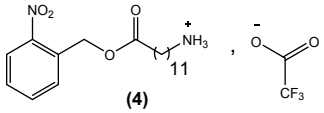


(3)

To a solution of compound **(2)** (2.33 g, 7.38 mmol) in anhydrous dichloromethane (20 mL) under inert atmosphere, were added 4-dimethylaminopyridin (0.9 g, 7.38 mmol), 2-nitrobenzylic alcohol (0.9 g, 5.9 mmol) and dicyclohexylcarbodiimide (1.52 g, 7.38 mmol). The reaction mixture was stirred for 20 hours at 25°C. DCU (dicyclohexylurea) was filtered. The solution was washed with water and the aqueous phase was extracted with CH_2Cl_2. All organic phases were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (CH_2Cl_2/Acetone : 98/2) to afford 2.12 g of compound **(3)** (80%).
^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.07 (d, J = 8.2Hz, 1H, H_b); 7.67 - 7.43 (m, 3H, H_e, H_c, H_d); 5.49 (s, 2H, -CH_2-Ar); 4.53 (s, 1H, -NH-); 3.08 (m, 2H, -CH_2-NH-); 2.39 (t, J = 7.5 Hz, 2H, -CH_2-CO-); 1.64 (quint., 2H, -CH_2-CH_2-CO-); 1.42 (s, 9H, -C-$(CH_3)_3$); 1.24 (s, 16H, $(-CH_2-)_8$).

^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.20 (CO_{ester}); 156.10 (CO_{Boc}); 147.78 (C_a); 133.73 (C_d); 132.39 (C_f); 129.19; 128.83 (C_c , C_e); 125.11 (C_b); 78,9 (-C_{Boc}-); 62.83 (-CH_2-Ar); 40.74 (-CH_2-NH-); 34.25 (-CH_2-CO-); 30.17 - 29.19 (7 -CH_2-); 28.22 (-CH_3); 26.88 (-CH_2-CH_2-NH); 24.98 (-CH_2-CH_2-CO-).
FTIR (cm^{-1}): 1337 ($\nu_{s\ NO2}$); 1521 (δ_{NH}, ν_{CN}); 1530 ($\nu_{as\ NO2}$); 1688 ($\nu_{CO\ Boc}$); 1748 ($\nu_{CO\ ester}$); 2852 ($\nu_{s\ CH2}$); 2878 ($\nu_{s\ CH3}$); 2919 ($\nu_{as\ CH2}$); 2975 ($\nu_{as\ CH3}$); 3372 (ν_{NH}).
Anal. Calc. for $C_{24}H_{38}N_2O_6$(%): C 63.98; H 8.50; O 21.31; N 6.22. Found (%): C 64.25; H 8.56; O 19.87; N 6.21.
SMHR-ESI Calculated for $C_{24}H_{38}N_2O_6Na$ [M+Na]+ 473.2622; found 473.2627.

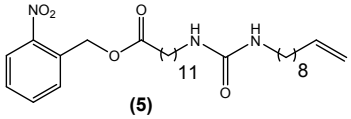
Deprotection of 2-nitrobenzyl 12-(tert-butoxycarbonylamino)dodecanoate **(4)**[4]



(4)

Trifluoroactetic acid (2.26 mL, 30.48 mmol) was added to a solution of **(3)** (1.14 g, 2.54 mmol) in dichloromethane (60 mL). The mixture was stirred at room temperature for 4 hours then evaporated to dryness. The excess of trifluoroacetic acid was eliminated by azeotropic distillation with toluene (30 mL).The product was then dried under reduced pressure to eliminate the residual toluene to give 1.14 g of pure compound **(4)** (97%).
^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.05 (d, J = 8.2 Hz, 1H,H_b); 7.89 (s, 3H, NH_3^+); 7.58 - 7.33 (m, 3H, H_e, H_c, H_d); 5.46 (s, 2H, -CH_2-Ar); 2.88 (s, 2H, -CH_2-NH_3^+); 2.37 (t, J = 7.5 Hz, 2H, -CH_2-CO-); 1.61 (quint., 4H, -CH_2-CH_2-NH_3^+ , -CH_2-CH_2-CO-); 1.24 (s, 14H, $(-CH_2-)_7$).
^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.26 (CO_{ester}); 147.5 (C_a); 133.78 (C_d); 132.26 (C_f); 129.07; 128.79 (C_c , C_e); 125.03 (C_b); 62.78 (-CH_2-Ar); 39.94 (-CH_2-NH_3^+-); 34.15 (-CH_2-CO-); 29.40 - 28.98 (6 CH_2); 27.46 (-CH_2-CH_2-NH_3^+), 26.33 (1 -CH_2-); 24.88 (-CH_2-CH_2-CO-).
FTIR (cm^{-1}): 1338 ($\nu_{s\ NO2}$); 1529 ($\nu_{as\ NO2}$); 1673 ($\nu_{as\ CO2-}$); 1744 ($\nu_{CO\ ester}$); 2853 ($\nu_{s\ CH2}$); 2922 ($\nu_{as\ CH2}$).
Anal. Calc. for $C_{21}H_{31}F_3N_2O_6$ (%): C 54.30; H 6.73; N 6.03. Found (%): C 53.29; H 6.72; N 5.88
SMHR-ESI Calculated for $C_{19}H_{31}N_2O_4$ [M-TFA] 351.2278; found 351.2286.

Synthesis of 2 nitrobenzyl 12-[(dec-9-enyl)ureido]dodecanoate **(5)**

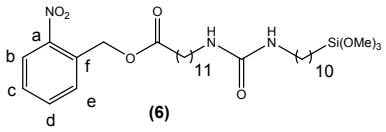


(5)

To a solution of ammonium trifluoroacetate salt **(4)** (0.34 g, 0.73 mmol) in dry dichloromethane (15 mL) under inert atmosphere, anhydrous triethylamine (0.12 mL, 0.80 mmol, and 10-isocyanatodecene[5] (0.17 g, 0.95 mmol), were added. The solution was stirred for 16 hours at room temperature then the solvent was evaporated. The solid obtained was then intensively washed with pentane and dissolved in dichloromethane, washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford 0.35g of the pure product **(5)** (92%).
^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.09 (d, J = 8.2 Hz, 1H, H_b); 7.68 - 7.44 (m, 3H, H_c, H_d, H_e); 5.87 - 5.71 (m, 1H, CH_2=CH-); 5.50 (s, 2H, -CH_2-Ar); 5.03 - 4.88 (m, 2H, CH_2=CH-); 4.38 (t, J = 5.4 Hz, 2H, 2 -NH-); 3.17 - 3.08 (m, 4H, 2 -CH_2-NH-CO-); 2.40 (t, J = 7.5 Hz, 2H, -CH_2-CO-); 2.06 - 1.97 (m, 2H, CH_2=CH-CH_2-); 1.65 (quint., 2H, -CH_2-CH_2-CO-); 1.54 - 1.40 (quint., 4H, 2 -CH_2-CH_2-NH-); 1.27 (s, 24H, 12 -CH_2-).
^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.32 (CO_{ester}); 158.44 ($CO_{urée}$); 147.76 (C_a); 139.29 (CH_2=CH-); 133.80 (C_d); 132.40 (C_f); 129.22; 128.89 (C_c ,C_e); 125.18 (C_b) ; 114.29 (CH_2=CH-); 62.91 (-CH_2-Ar); 40.77 (2 -CH_2-NH-); 34.29 (-CH_2-CO-); 33.91 (CH_2=CH-CH_2-); 30.37 - 27.02 (14 -CH_2-); 25.00 (-CH_2-CH_2-CO-).
FTIR (cm^{-1}): 1332 ($\nu_{s\ NO2}$); 1531 ($\nu_{as\ NO2}$); 1570 (δ_{NH}, ν_{CN}); 1615 ($\nu_{CO\ urée}$); 1645 ($\nu_{C=C}$); 1734 ($\nu_{CO\ ester}$); 2850 ($\nu_{s\ CH2}$); 2922 ($\nu_{as\ CH2}$); 3335 (ν_{NH}).

Anal. Calc. for $C_{30}H_{49}N_3O_5$ (%): C 67.76; H 9.29; O 15.04; N 7.90. Found (%): C 65.81; H 9.20; O 15.39; N 7.46.
SMHR-ESI Calculated for $C_{30}H_{49}N_3O_5Na$ [M+Na]+ 554.3564; found 554.3562.

Hydrosilylation of 2 nitrobenzyl 12-[(dec-9-enyl)ureido]dodecanoate **(6)** [6]



(6)

To a solution of compound **(5)** (26 mg, 0.05 mmol) in anhydrous toluene (0.5 mL), trimethoxysilane (32 µL, 0.25 mmol) and Karstedt's catalyst (28 µL, 1.25 µmol [Pt]) were added under inert atmosphere. The mixture was stirred for 3 hours at 65°C then cooled at room temperature. The solvent and the excess of trimethoxysilane were removed under reduced pressure, to afford compound **(6)**. Complete conversion of **(5)** was observed by ^{1}H and ^{13}C NMR.
^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.08 (d, *J* = 8.2 Hz, 1H, H_b); 7.67 - 7.44 (m, 3H, H_c, H_d, H_e); 5.50 (s, 2H, -C$\underline{H_2}$-Ar); 4.41 (s, 2H, 2 -N$\underline{H}$-); 3.56 (s, 9H, -Si(OCH_3)$_3$); 3.16 - 3.08 (m, 4H, 2 -C$\underline{H_2}$-NH-CO-); 2.40 (t, *J* = 7.5 Hz, 2H, -C$\underline{H_2}$-CO-); 1.64 (m, 2H, -C$\underline{H_2}$-CH_2-CO-); 1.52 – 1.41 (m, 4H, 2 -C$\underline{H_2}$-CH_2-NH-); 1.25 (s, 24H, 12 -CH_2-); 0.67 - 0.59 (m, 2H, -CH_2Si-).
^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.10 ($\underline{C}O_{ester}$); 158.52 ($\underline{C}O_{urée}$); 147.51 (C_a); 133.62 (C_d); 132.23 (C_f); 129.03, 128.72 (C_c, C_e); 125.04 (C_b); 62.71 (-$\underline{C}H_2$-Ar); 50.45 (-Si(OCH_3)$_3$); 40.51 (2 -CH_2-NH-); 34.12 (-$\underline{C}H_2$-CO-); 30.31 - 26.92 (15 -CH_2-); 24.83 (-$\underline{C}H_2$-CH_2-CO-); 22,53 (-$\underline{C}H_2$-CH_2-Si-); 9.12 (-CH_2Si-).
^{29}Si NMR (59 MHz, $CDCl_3$, δ ppm): -41.1.
FTIR (cm^{-1}): 1084-1194 (ν_{Si-O}); 1334 ($\nu_{s\ NO2}$); 1532 ($\nu_{as\ NO2}$); 1576 (δ_{NH}, ν_{CN}); 1617 ($\nu_{CO\ urée}$); 1736 ($\nu_{CO\ ester}$); 2850 ($\nu_{s\ CH2}$); 2925 ($\nu_{as\ CH2}$).

Chemical Surface Modification
Materials and Substrates[5]
* *Silicon wafers*
- The undoped Silicon-CZ wafers (1cm x 1cm, thickness 500 µm, one side polished) were supplied by BT Electronics.
- For PM-IRRAS experiments, the SiO_2/Au substrates were supplied by Optics Balzers AG: Goldflex-gold based metallic reflector (ref. 200785). Their absolute reflectance was higher than 98% in the 1.2-12 µm spectral range. The thickness of the SiO_2 layer, measured by ellipsometry, was 215 ± 7 Å, using a refractive index of 1.46 (I-elli 2000 NFT ellipsometer, λ = 532 nm.

* *Contact angles*
Contact angles were performed on a Krüss DSA 100 goniometer, at 20°C in static mode.
* *AFM Experiments*
AFM images of SAMs on silicon wafers were performed on Agilent 5500 AFM.
AFM images of TMVs:
- on SAMs were obtained with a multimode V microscope with a nanoscope 8 controller (Bruker, AXS) using OTESPA cantilevers (k = 42 N/m, F_0 = 303.41 kHz, Bruker AFM Probes).
- on mica were obtained with the same microscope using a silicon MPP-22100 cantilever (k= 0.9 N/m, Bruker AFM Probes).
* *PM-IRRAS Experiments*
PM-IRRAS spectra were recorded on a ThermoNicolet Nexus 670 FTIR spectrometer at a resolution of 4 cm^{-1}, by coadding several blocks of 1500 scans (30 minutes acquisition time). All spectra were collected in a dry-air atmosphere after 30 min of incubation in the chamber. Experiments were performed at an incidence angle of 75° using an external homemade goniometer reflection attachment, adding a ZnSe photoelastic modulator (PEM, Hinds Instruments, type III) after the polarizer.

Formation of Self-Assembled Monolayer **SAM-NO_2**:
The substrates were cleaned and activated just before grafting. They were intensively washed with milli – Q water (18 MΩ.cm). They were then sonicated in chloroform (15 min). After that the substrates were exposed to UV – ozone (homemade apparatus, λ = 185 – 254 nm) for 30 min and introduced into the silanization flask immediately and let under vacuum for 1h30.
Then, a solution of freshly synthesised compound **6** (32.7mg; 5.10^{-5} mol) in anhydrous toluene (50 mL) was prepared in a schlenk under inert atmosphere. A solution of trichloroacetic acid (TCA) (0.8 mg; 5.10^{-6} mol) in anhydrous toluene (50 mL) was prepared in another schlenk. Under inert atmosphere, anhydrous toluene (100mL) was introduced in the silanization

flask, and the silylated compound solution was added. After the addition of the trichloroacetic acid solution, the silanization flask was kept, under inert atmosphere, at 18°C for 12 hours.
The samples were washed in toluene, sonicated in toluene (5 min), chloroform (5 min) and milli – Q water (10 min), dried under vacuum before characterisation by PM–IRRAS and AFM.
Water contact angle: 71° ± 2

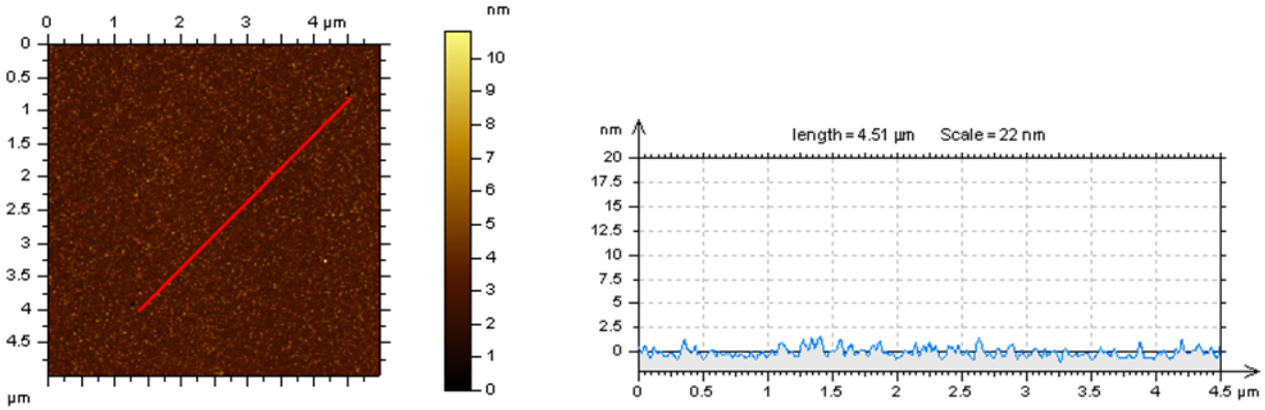


Figure S1: AFM height image (5 x 5 µm) of **SAM-NO_2** and corresponding surface profile.

o-Nitrobenzyl ester SAMs photoclivage[2, 7] **SAM-COOH** :
SAM-NO_2 were irradiated with a high-pressure mercury lamp (365 nm), coupled with a 280 nm cutoff filter (Pyrex plate) for preventing deterioration of the organic layer. The substrates were placed 2 cm far from the Pyrex plate and the Pyrex plate 3 cm far from the UV lamp then irradiated for 30 min, at room temperature. After deprotection, the substrates were cleaned using ultrasonic bath with ethanol and chloroform for 5 min then dried under vacuum before characterisation by PM-IRRAS.
Water contact angle: 55°± 2°

Carboxylic acid SAMs activation[5] **SAM-NHS** :
SAM-COOH were dipped in a solution of EDC (*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, 1 g), NHS (*N*-hydroxysuccinimide, 500 mg) and MES (2-(*N*-morpholino)ethanesulfonic acid hydrate, 1.2 g) in water (20 mL), for 3 hours. Then, activated SAMs were intensively washed with milli-Q water and sonicated (5 min) in chloroform before characterisation by PM–IRRAS.

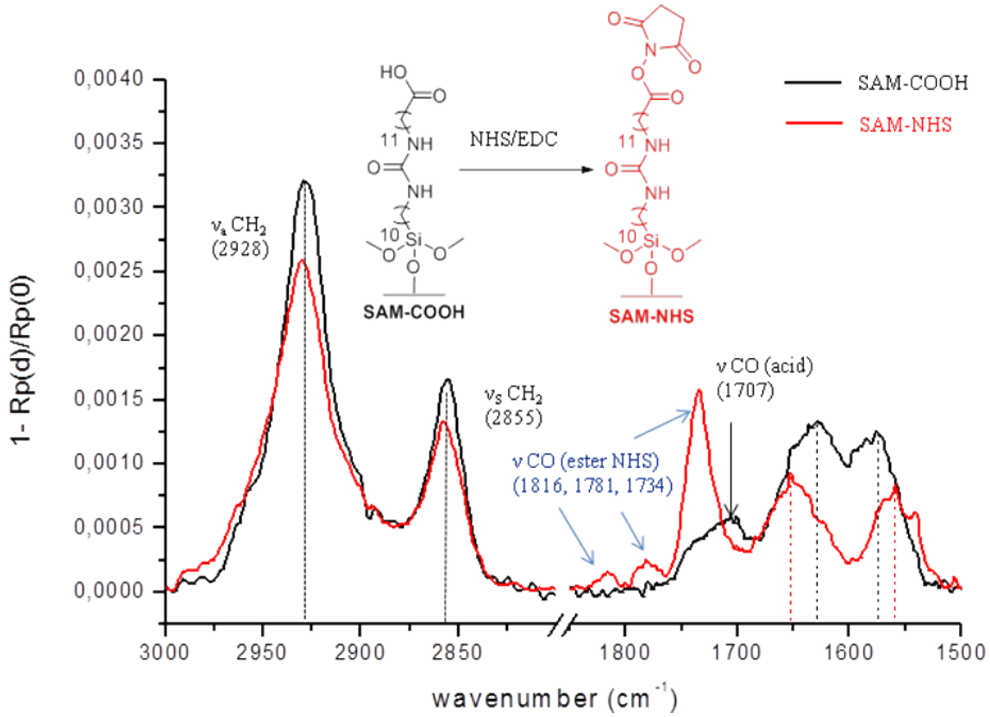


Figure S2: PM-IRRAS spectra of acid- and NHS-terminated monolayers, in the 3000-2800 cm^{-1} and 1850-1500 spectral ranges.

Immobilisation of Protein A gold nanoparticles **SAM-PA** :
Protein A gold nanoparticles (**GNPs-PA**, ø = 20.4 ± 0.6 nm, Protein A: 3.2 µg/mL) in a TRIS buffer solution (pH = 8.2) were purchased from tebu-bio SAS (78612 Le Perray-en-Yvelines, France). To a solution of **GNPs-PA** (0.25 mL), 10 mL of water was added. This solution was centrifuged 3 times for 30 min. The resulting nanoparticles were suspended in PBS (10 mL,), adjusted at pH = 8 with KOH (0.5 M). **SAM-NHS** was dipped in the PBS/**GNPs-PA** suspension, at 37 °C, for 2 hours. The modified surfaces were intensively washed with milli – Q water and sonicated in milli – Q water (5 min) to remove physisorbed particles before characterisation by PM–IRRAS and AFM.

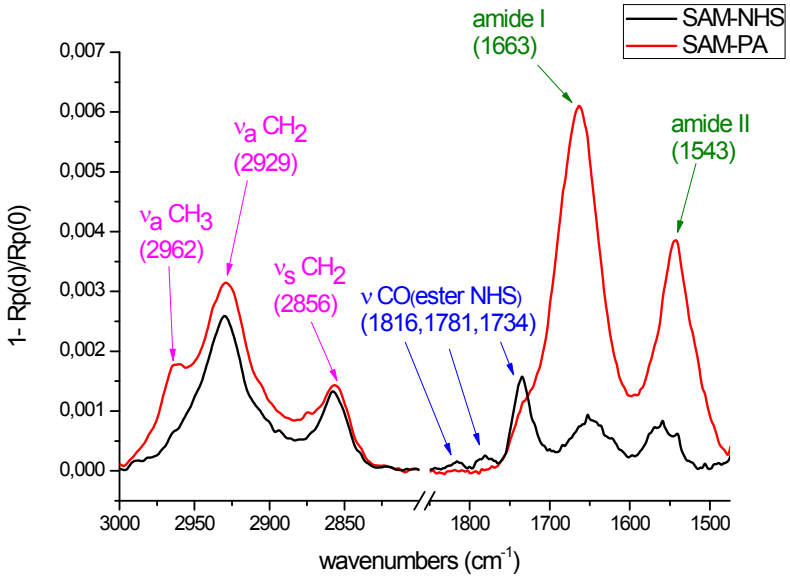


Figure S3: PM-IRRAS spectra of **SAM-PA** in the 3000-2800 cm^{-1} and 1850-1500 spectral ranges.

Immobilisation of Tobacco Mosaic Virus **SAM-TMV** :
TMV purification was performed essentially by differential ultracentrifugation. TMV particles were covalently attached to SAM using standard NHS/EDC chemistry.[8] TMV virus in 1 mM EDTA was prepared as described previously.[9] Carboxylated SAM was activated with 0.4 mg/mL NHS + 0.6 mg EDC in HQ water for 30 min. The surface was rinsed ten times with acetate buffer (10 mM, 100 mM NaCl, pH 4.5). A drop of 80 µL of acetate buffer was gently deposited on the activated SAM; then 20 µL of TMV virus (1.5 mg/mL) were inserted in the buffer drop. The substrate surface was rinsed gently with phosphate buffer (10 mM, 50 mM KCl, pH 7.5) and dried using a Laboport vacuum pump (KNF Neuberger, Trenton, NJ, USA).
On mica, TMV virus (1.5 mg/mL) diluted in HQ water was deposited 10 min; then the surface was rinsed gently with HQ water and finally dried as shown above.

Observation of denatured TMV particles with AFM:
Supernatant material, collected at the step of purification, contains denaturated TMVs (See AFM images on Figure 5).

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