

# Supporting Information

## Immobilization of Catalytic Virus-like Particles in a Flow Reactor

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### Materials and methods

All the reagents were purchased from Sigma Aldrich and used as received without a further purification. MilliQ water was used for buffer solutions and reactions (Millipore,  $R=18.2 \text{ M}\Omega \cdot \text{CM}^{-1}$ ). Citric acid stabilized gold nanoparticles were purchased from NanoComposix (7nm gold core, 0.05 mg/ml). Quartz glass cuvette were fabricated by Hellma-analytics, Quartz SUPRASIL (QS). Diced borofloat wafers (Schott) were used as flat substrates. Amicon Ultra centrifuge filters with 10k MWCO for particle preparation were purchased from Pall Corporation.

### Preparation of the flow reactor

CCMV-Au7B nanoparticles were immobilized in a microreactor chip using a PHD 22/2000 series syringe pump (Harvard Apparatus, United Kingdom) equipped with 250  $\mu\text{L}$  flat tip syringes (Hamilton). Syringes were connected to fused silica capillaries (100  $\mu\text{m}$  i.d., 362  $\mu\text{m}$  o.d., Polymicro Technologies) using Upchurch Nanoport<sup>TM</sup> assembly parts (i.e., Nano-Tight<sup>TM</sup> unions and fittings, Upchurch Scientific Inc.,

USA). A glass microreactor (Micronit Microfluidics) with a residual volume of 13  $\mu\text{L}$  (dimensions = 150  $\mu\text{m}$  width, depth = 150  $\mu\text{m}$ , length = 58 cm) was placed in a chip holder (Upchurch Nanoport<sup>TM</sup>).

## Synthesis and deposition of PFPS

*Undec-10-enoylchloride*. Under argon atmosphere, dimethylformamide (0.5 mL) was slowly added to a mixture of thionyl chloride (10 mL) and 10-undecylic acid (9.2 g, 50 mmol). The reaction mixture was refluxed at 80 °C. After 4 h, thionyl chloride was removed under reduced pressure and the residual materials was dried in high vacuum. The product undec-10-enoyl chloride was obtained as a brown liquid and used without a further purification. Yield: 100%. Analysis was carried out by converting the acid chloride to the corresponding ethoxide by stirring in ethanol for 2 h followed by heating under reduced pressure to remove the excess solvent. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 5.75 (ddt,  $J$  = 16.9, 10.2, 6.7 Hz, 1H), 4.90 (m, 2H), 4.07 (q,  $J$  = 7.1 Hz, 2H), 2.23 (t,  $J$  = 7.5 Hz, 2H), 1.98 (m, 2H), 1.57 (m, 2H), 1.32t (m, 2H), 1.25 (m, 8H), 1.20 (t,  $J$  = 7.1 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 173.79, 139.09, 114.16, 60.11, 34.37, 33.82, 29.31, 29.24, 29.15, 29.08, 28.92, 24.99, 14.27. m/z (ESI-ToF, pos. mode, DCM/MeOH): [M+Na]<sup>+</sup> 235.0208 calc. 235.1674.

*Perfluorophenylundec-10-enoate*. Undec-10-enoyl chloride (3.9 g, 17.7 mmol) was slowly added to a solution of pyridine (1.5 mL, 17.7 mmol) and pentafluorophenole (3.4 g, 18.3 mmol) in diethylether. The solution was refluxed for 1 h, filtrated (hot) and the remaining solvent was removed under reduced pressure. Purification was carried out using vacuum distillation (140 °C 0.07 mbar, steam temperature = 105 °C). Perfluorophenylundec-10-enoate (4.6 g, 13 mmol) was obtained as a colorless liquid. Yield: 73.5 %. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 5.81 (ddt,  $J$  = 16.9, 10.2, 6.7 Hz, 1H), 4.96 (m, 2H), 2.66 (t,  $J$  = 7.4 Hz, 2H), 2.04 (m, 2H), 1.77 (m, 2H), 1.35 (m, 10H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  = 169.74, 139.28, 114.33, 33.92, 33.49, 29.36, 29.22, 29.15, 29.01, 28.97, 24.90. <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>)  $\delta$  = 152.83 (m), 158.27, 162.48 m/z (ESI-ToF, pos. mode, DCM/MeOH): [M+Na]<sup>+</sup> 373.0884 calc. 373.1203.

*Perfluorophenyl-11-(triethoxysilyl)undecanoate (PFPS)*. Under argon atmosphere, perfluorophenylundec-10-enoate (2.0 g, 5.8 mmol) and triethoxysilane (1.3 g, 1.5 mL, 8.1 mmol) were stirred at r.t. Karstedt's catalyst (0.4 mL, 4 % in Xylole) was slowly added within 45 min. After 24 h, the product was distilled removing the remaining silane and xylole (170 °C, 0.35 mbar). The residue was purified using flash chromatography (silica, CHCl<sub>3</sub>) yielding a slightly brown liquid of perfluorophenyl 11-(triethoxysilyl)undecanoate (2.8 g, 5.4 mmol). Yield: 92.5 %. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 3.81 (q, *J* = 7.0 Hz, 6H), 2.65 (t, *J* = 7.4 Hz, 2H), 1.76 (m, 2H), 1.40 (m, 2H), 1.28 (m, 8H), 1.22 (t, *J* = 7.0 Hz, 9H), 0.63 (m, 2H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 169.74, 142.59, 140.77, 140.12, 139.21, 138.27, 136.71, 58.44, 33.49, 33.32, 29.57, 29.54, 29.35, 29.27, 29.00, 24.91, 22.90, 18.44, 10.52. <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>) δ = 152.88, 158.31, 162.52. m/z (ESI-ToF, pos. mode, DCM/MeOH): [M+Na]<sup>+</sup> 536.9995 calc. 537.2072.

*Deposition of PFPS*. The cleaned and activated substrates were immersed into a solution of PFPS (10mM, DCM) under argon atmosphere. After 24 hours, the substrates were rinsed with DCM for 30 seconds and immersed into DCM for 10 minutes followed by 2 min sonication in DCM. Modification with PFPS of the glass flow reactor was carried out following a similar methodology. A PFPS (10 mM, DCM) solution was injected into the channel with a flow rate of 0.5 μL/min a volume equal to 10 times the chip's volume was used to ensure optimal modification. The flow reactor was then washed with DCM to remove non-reacted PFPS, and dried with N<sub>2</sub> flow.

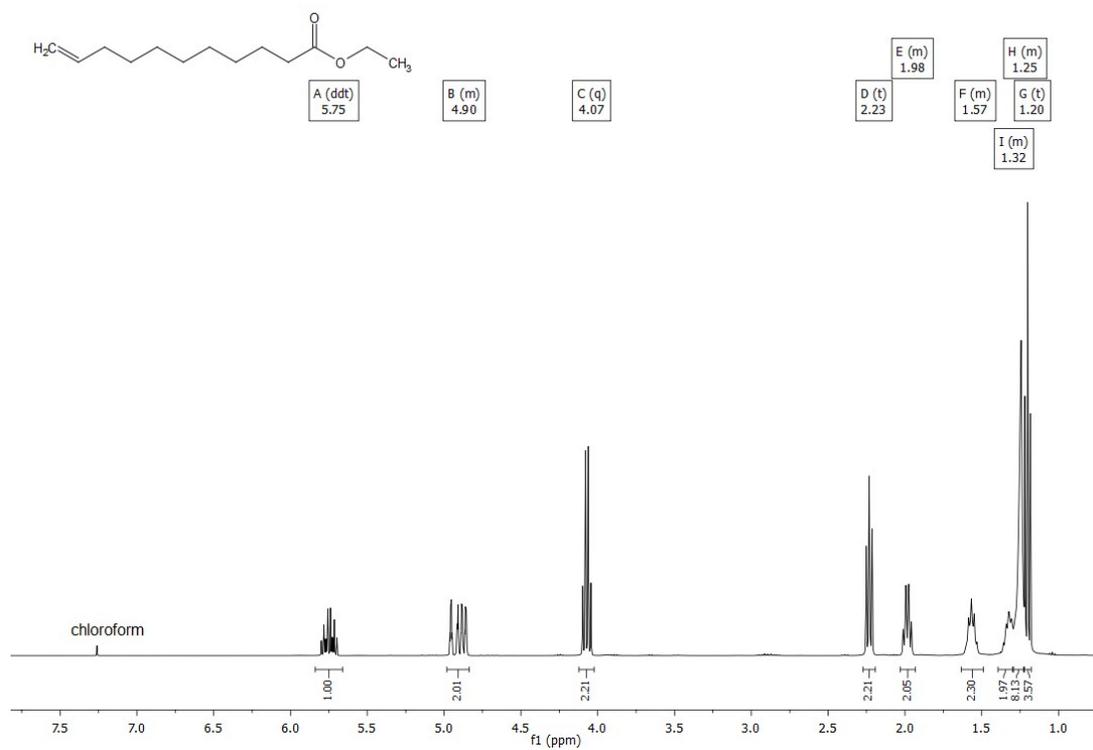


Figure S1.1. <sup>1</sup>H NMR spectrum of Undec-10-enylchloride.

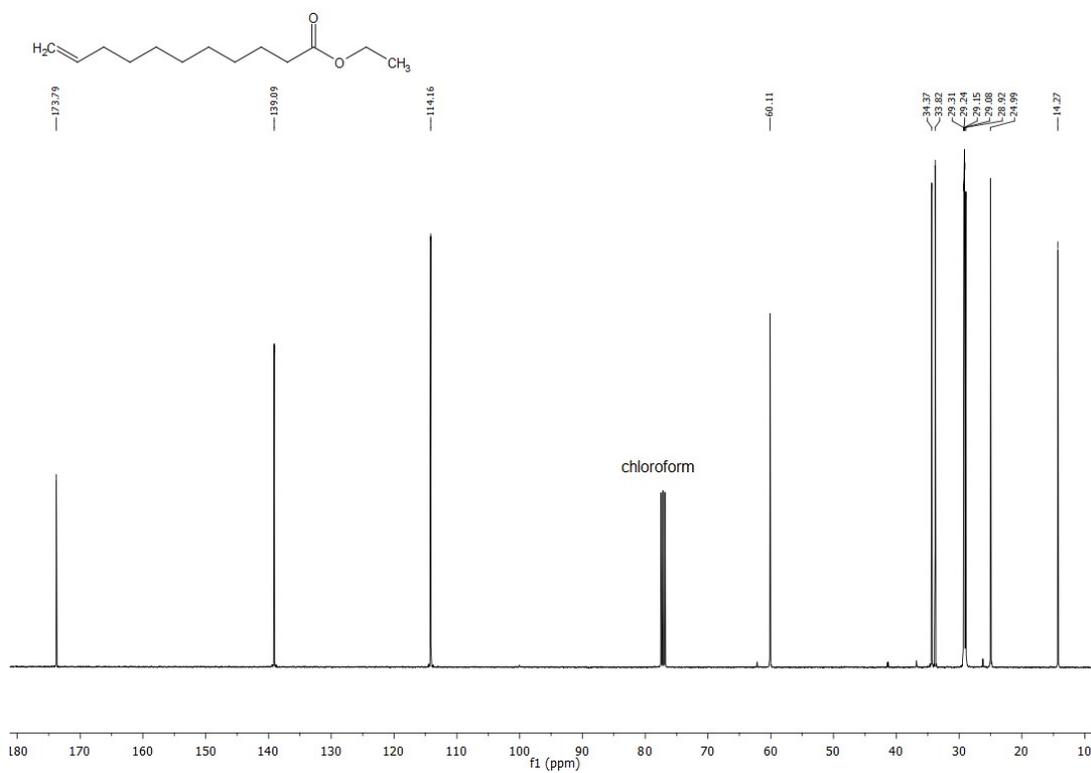


Figure S1.2. <sup>13</sup>C NMR spectrum of undec-10-enylchloride.

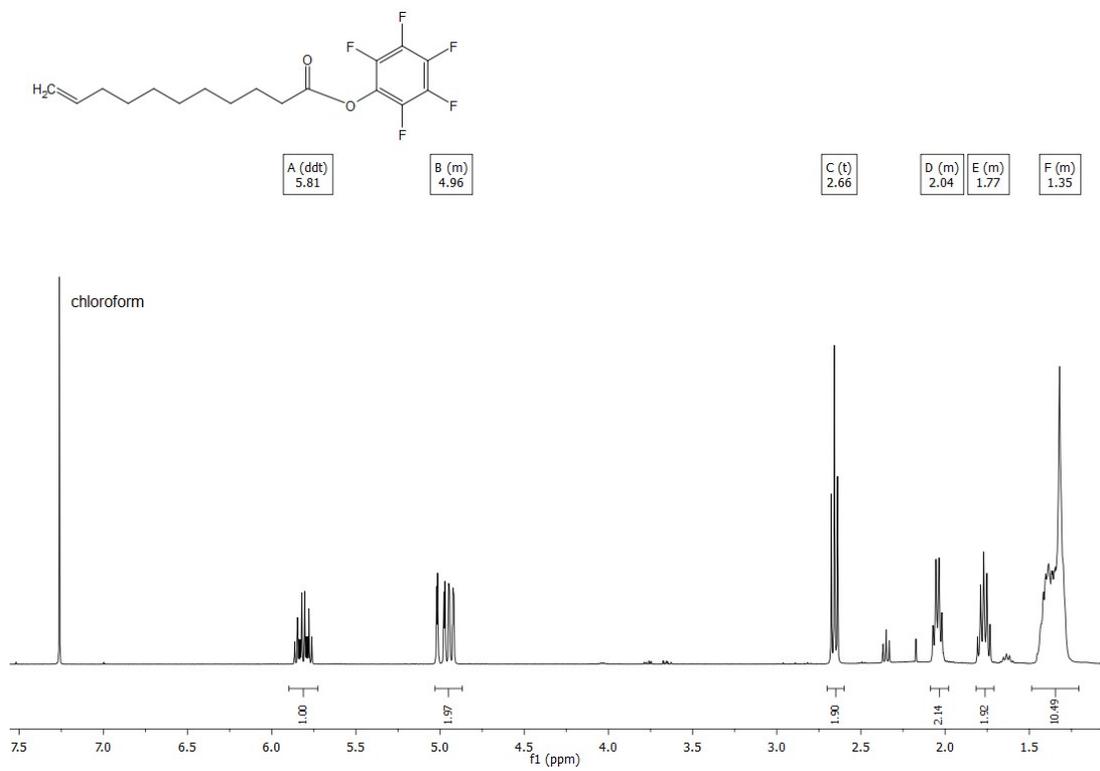


Figure S1.3. <sup>1</sup>H NMR spectrum of perfluorophenylundec-10-enoate.

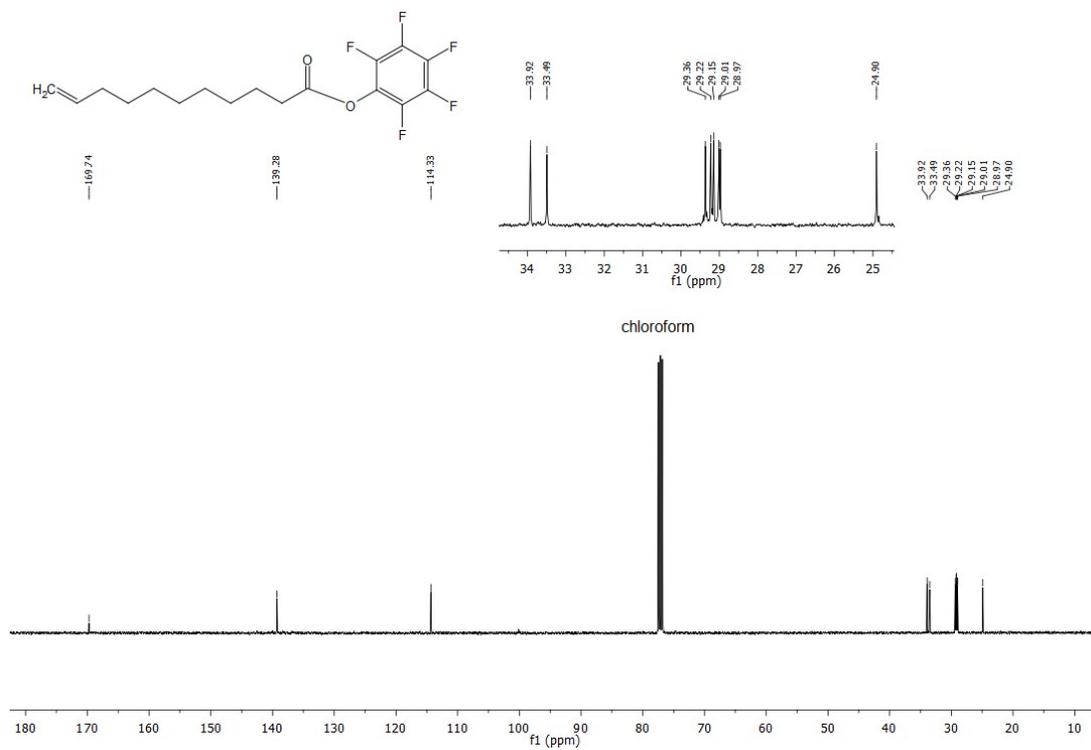


Figure S1.4.  $^{13}\text{C}$  NMR spectrum of perfluorophenylundec-10-enoate.

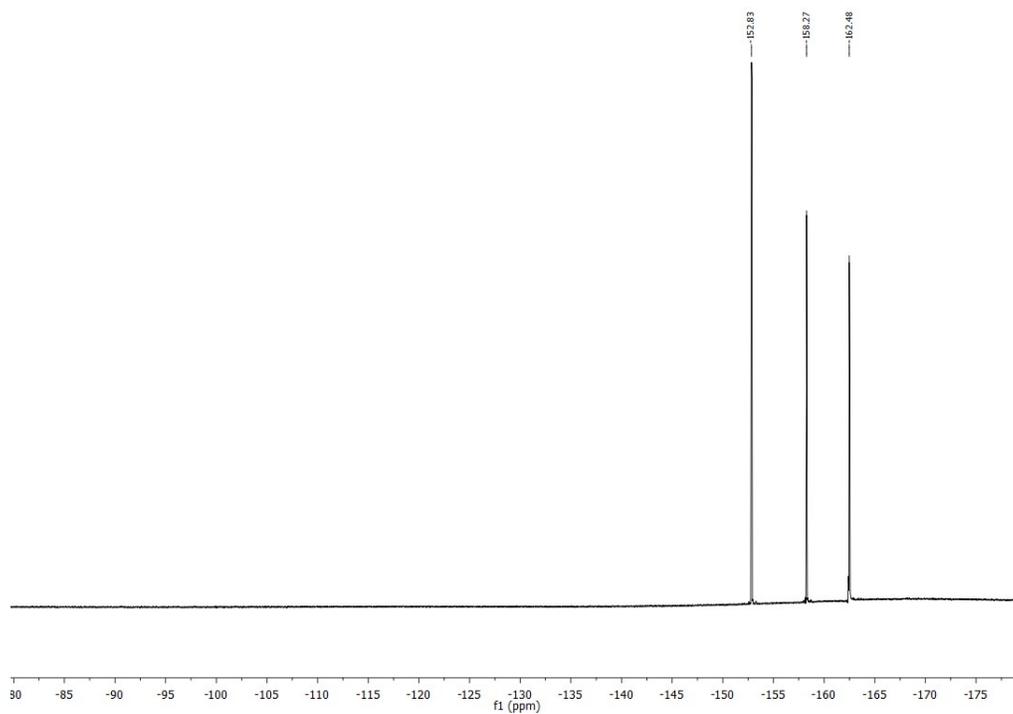


Figure S1.5.  $^{19}\text{F}$  NMR spectrum of perfluorophenylundec-10-enoate.

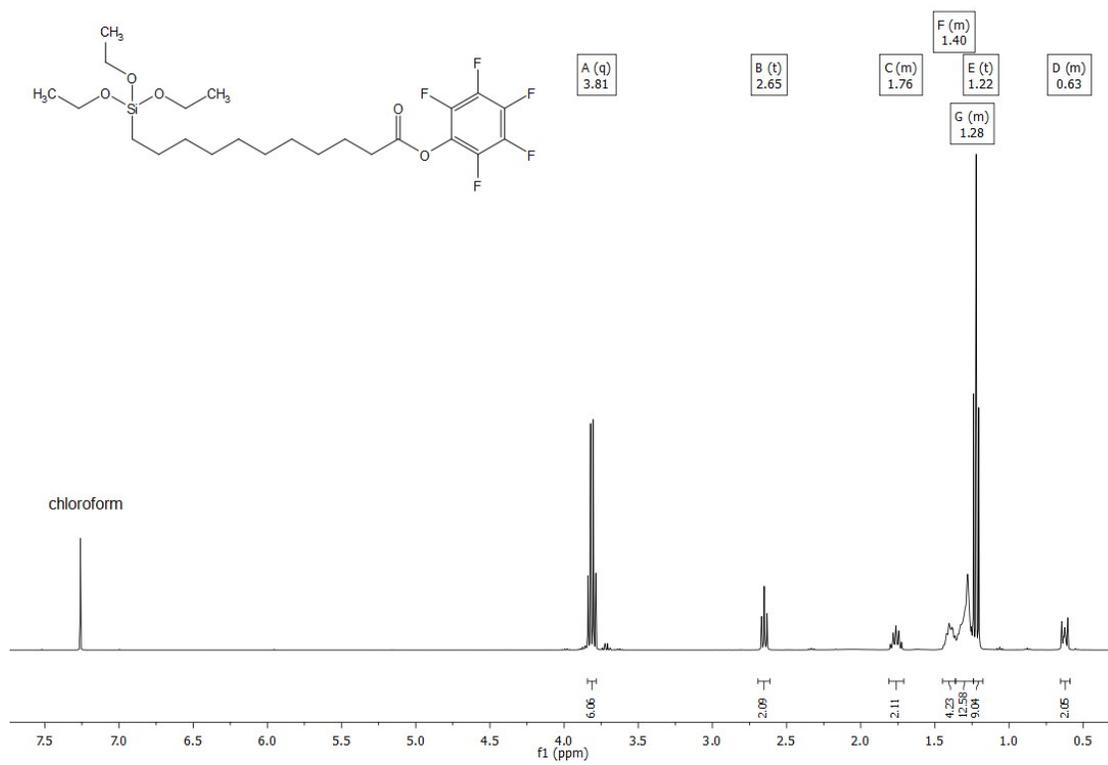


Figure S1.6.  $^1\text{H}$  NMR spectrum of perfluorophenyl 11-(triethoxysilyl)undecanoate.

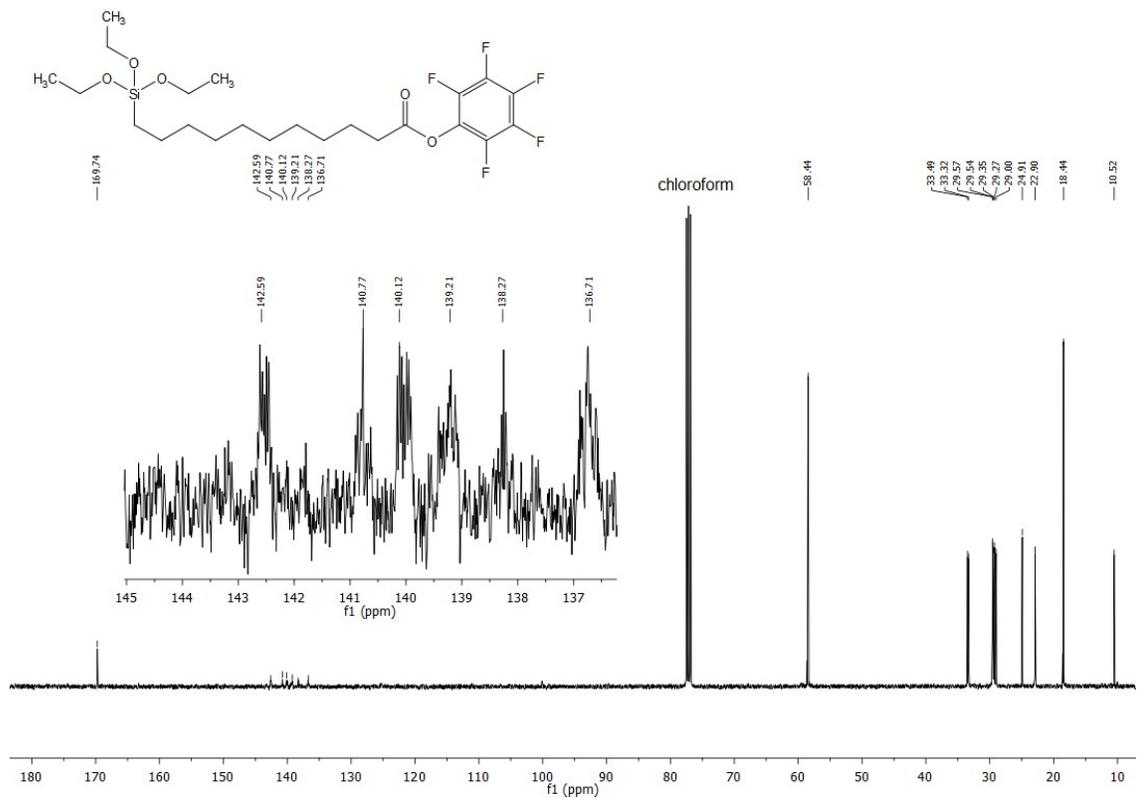


Figure S1.7.  $^{13}\text{C}$  NMR spectrum of perfluorophenyl 11-(triethoxysilyl)undecanoate.

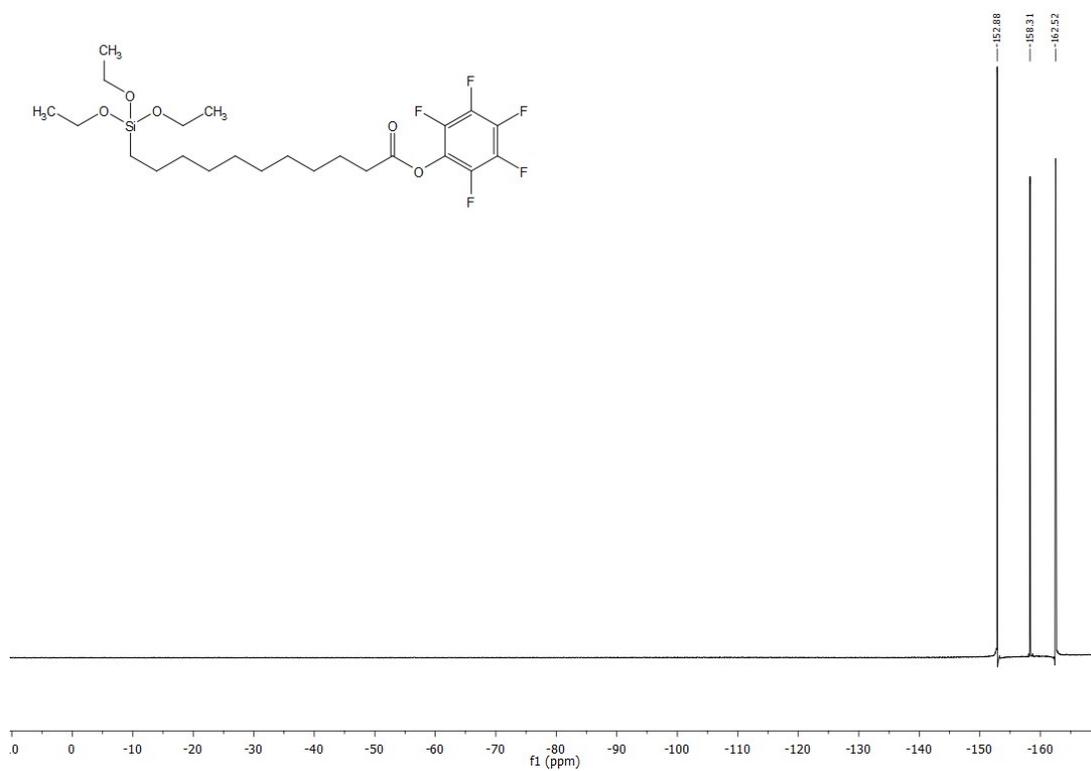


Figure S1.8. .  $^{19}\text{F}$  NMR spectrum of perfluorophenyl 11-(triethoxysilyl)undecanoate.

## 2. Immobilization of CCMV-Au7B

Substrates and microreactors were rinsed with water and activated by immersion in or cycling with piranha solution ( $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2=3:1$ ), and were subsequently rinsed with water and ethanol and dried with a stream of nitrogen. !Caution: Piranha solution reacts violently with organic material and has to be treated with utmost care! Substrates were immersed into a PFPS solution (DCM, 10 mM) for 16 hrs at r.t. under argon atmosphere. Microreactors were functionalized by cycling the solution for 16 hrs at a flow rate of 0.05  $\mu\text{L}/\text{min}$  at room temperature through the device. Both glass wafer and microreactor were rinsed with DCM to remove unreacted reagent and dried in a steam of  $\text{N}_2$ .

CCMV-Au7B hybrid nanoparticles were dispersed in a PB buffer (0.2 M phosphate, pH 7.2). The particles were drop-coated on a flat glass substrate overnight (12 hrs) in a closed vessel to avoid solvent evaporation. Microreactors were coated by cycling the solution for 12 hrs at a flow rate of 0.05  $\mu\text{L}/\text{min}$  at r.t. Both substrate and microreactor were rinsed with buffer solution to remove unreacted nanoparticles.

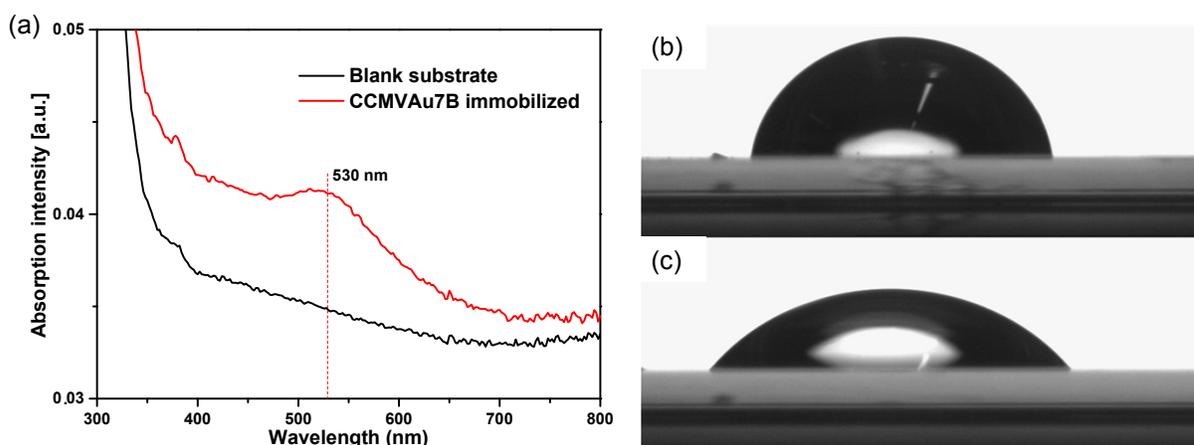


Figure S2.1. Analysis of flat substrates modified with PFPS and CCMVAu7B. (a) UV/Vis analysis (PFPS substrate as black line and CCMVAu7B as red line). Water contact angle analysis of substrates modified with PFPS (b) and CCMVAu7B (c).

## 2.2 Kinetic study of 4-NP reduction in flow reactor

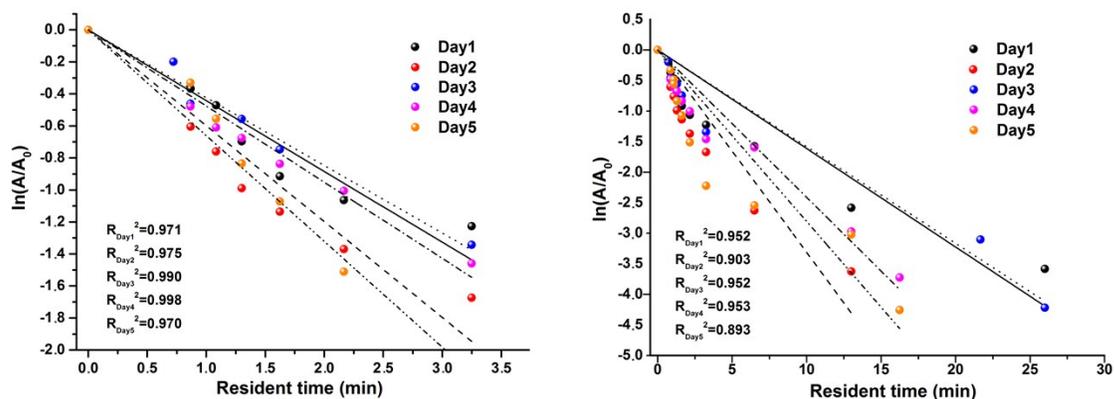


Figure S2.2. Plotted absorption maxima with a linear fit to determine the reaction rate constants of (a) the initial stage and (b) the overall reaction.

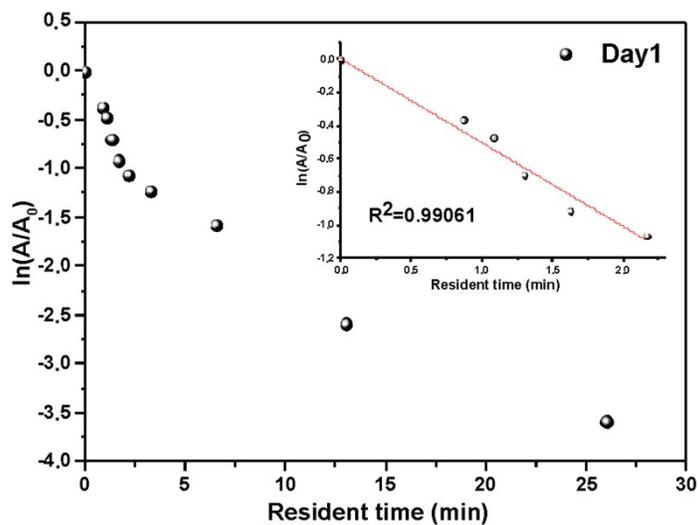


Figure S2.3. Plotted absorption maxima and linear fit to determine the reaction rate constants. Inset: regression analysis of the initial stage of the catalytic reaction.

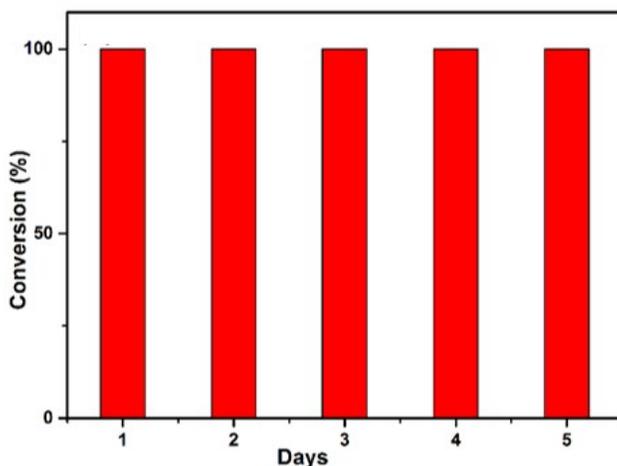


Figure S2.4. Conversion efficiency of the catalytic action of CCMV-Au7B in the microreactor converting the nitro-phenol to the corresponding amine. The reactor is run in continuous flow mode and the conversion is determined by measuring the residual phenol by in-situ UV/vis spectroscopy.

### 2.3 Catalytic activity study of free CCMV-Au7B NPs and immobilized CCMV-Au7B on the flat glass substrate

Estimation of the gold quantity during the different reaction conditions in order to compare to activity:

**a. Total number of Au atoms in free CCMV-Au7B solution**

**Reaction condition:** 150  $\mu$ L of CCMV-Au7B solution with  $A_{520} = 0.280$  was added into a mixed solution of 4NP and sodium borohydride, resulting in 0.5 mM of 4NP and 600  $\mu$ L total reaction volume. The reaction was monitored by UV-Vis spectrometer immediately after adding the catalyst.

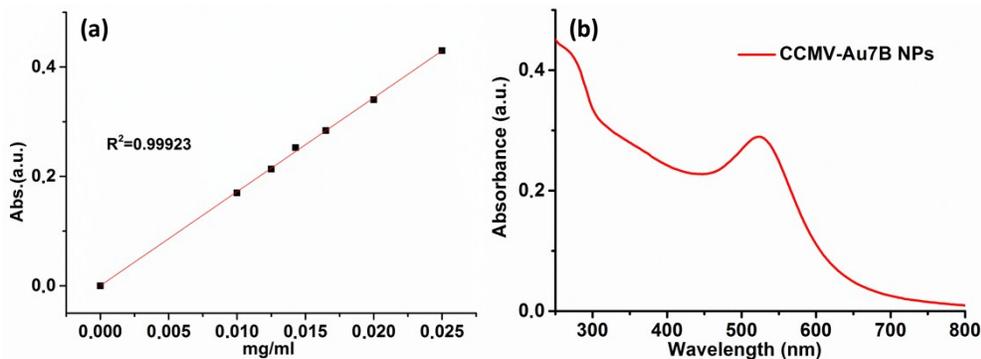


Figure S2.5. (a) Linear fit of UV-Vis absorption of Au7B at  $\lambda = 520$  nm with different concentrations; (b) UV-Vis spectrum of CCMVAu-7B in MQ for concentration calculation.

**Known:**

1. 0.025 mg/mL of Au7B with absorption of  $A_{520} = 0.43002$  contained  $0.85 \times 10^{13}$  particles/mL, which means 0.025 mg of Au7B contain  $0.85 \times 10^{13}$  Au7B particles ;
2. The concentration of Au NPs and the absorption at  $\lambda = 520$  nm are in linear relationship (Figure S2.5a).

**Therefore:** CCMV-Au7B in MQ for reaction with  $A_{520} = 0.280$  contained 0.0163 mg/mL of Au7B; with a volume  $V = 150 \mu\text{L}$  and  $M_w(\text{Au}) = 197 \text{ g/mol}$ , the total amount of Au atoms in reaction solution is  $= c \times V / M_w = (0.0163 \text{ mg/ml} \times 150 \mu\text{L}) / (197 \text{ g/mol}) = 12.4 \text{ nmol}$ .

**b. Total number of Au atoms on flat glass substrate**

**Reaction condition:** the CCMV-AuNPs immobilized glass substrate was added into 400  $\mu\text{L}$  of 0.5 mM 4NP and sodium borohydride mixed solution. Reaction was monitored by UV-Vis spectrometer immediately after adding glass substrate.

**Known:** Area of the CCMV-AuNPs immobilized glass surface:  $2.0 \text{ cm} \times 1.0 \text{ cm}$ , corresponding to  $2.0 \times 10^{14} \text{ nm}^2$ , while the coverage of CCMV-Au7B on glass substrate is 82% (see manuscript).

Total number of CCMV-Au7B particles =  $(S_{\text{immobilized glass surface}} / S_{\text{CCMV-Au7B cross-sectional area}}) \times 82\% = 2.0 \times 10^{14} \text{ nm}^2 / (\pi \cdot 9^2 \text{ nm}^2/\text{particle}) \times 82\%$

**Therefore:** the total number of CCMV-Au7B particles on glass surface =  $6.45 \times 10^{11}$ , while the mass of a single particles is:  $0.025 \text{ mg} / 0.85 \times 10^{13}$ . The mass of the Au7B particles on flat glass substrate =  $(6.45 \times 10^{11} / 0.85 \times 10^{13}) \times 0.025 \text{ mg} = 1.897 \times 10^{-6} \text{ g}$ . The total amount of Au atoms on glass substrate =  $1.897 \times 10^{-6} \text{ g} / (197 \text{ g/mol}) = 9.6 \text{ nmol}$ .

**c. Total number of Au atoms in flow reactor**

**Known:** The flow channel with a length of 58 cm, a diameter of 150  $\mu\text{m}$ , resulting in an inner surface area,  $A = 2.73 \times 10^{14} \text{ nm}^2$  with an assumed coverage of CCMV-Au7B on glass surface of 82% (see manuscript).

The total number of CCMV-Au7B particles =  $(S_{\text{inner surface area of flow channel}} / S_{\text{CCMV-Au7B cross-sectional area}}) \times 82\% = 2.73 \times 10^{14} \text{ nm}^2 / (\pi \cdot 9^2 \text{ nm}^2/\text{particle}) \times 82\%$

**Therefore:** the total number of CCMV-Au7B particles on inner surface =  $8.8 \times 10^{11}$ , while the mass of a single particles is:  $0.025 \text{ mg} / 0.85 \times 10^{13}$ ; the total amount of Au7B particles in flow reactor =  $(8.8 \times 10^{11} / 0.85 \times 10^{13}) \times 0.025 \text{ mg} = 2.588 \times 10^{-6} \text{ g}$ . The total amount of Au atoms in flow reactor =  $2.588 \times 10^{-6} \text{ g} / (197 \text{ g/mol}) = 13.1 \text{ nmol}$ .

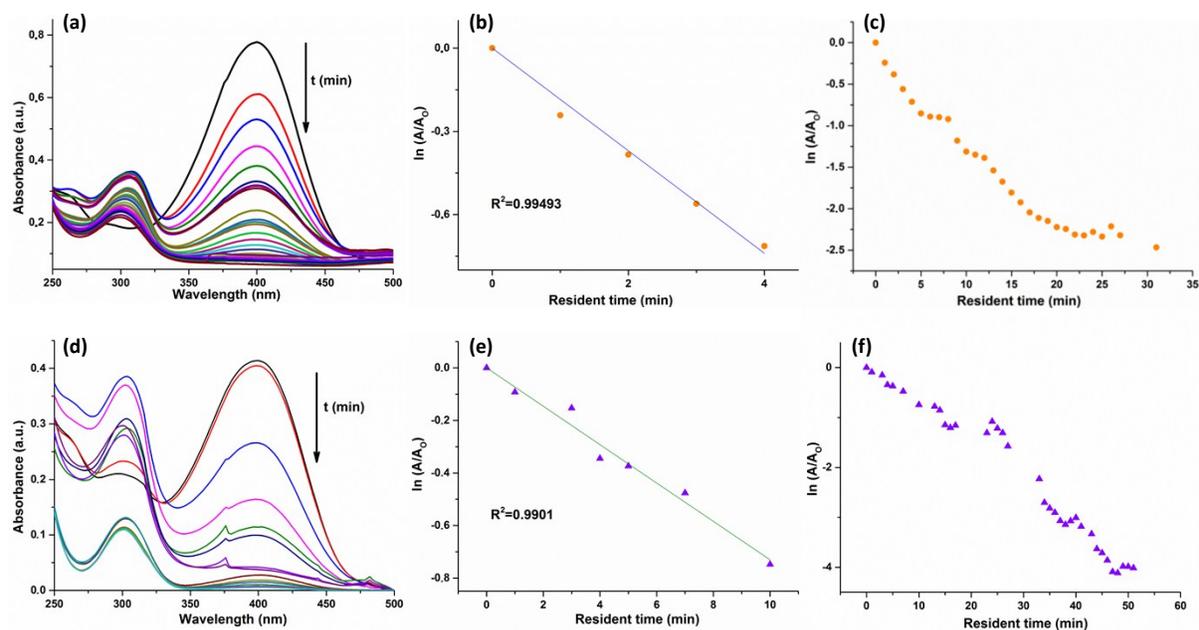


Figure S2.6. 4NP reduction catalyzed by CCMV-Au7B free particles: (a) UV-Vis spectra of 4NP reduction; (b) plotted absorption maxima to resident time at initial stage with a linear fit to determine the reaction rate constants and (c) plotted absorption maxima to resident time for the overall reaction. 4NP reduction catalyzed by CCMV-Au7B immobilized on flat glass substrate: (d) UV-Vis spectra of 4NP reduction; (e) plotted absorption maxima to resident time at initial stage with a linear fit to determine the reaction rate constants and (f) plotted absorption maxima to resident time for the overall reaction.

**Table S1. Comparison of the catalytic free NPs, immobilized NPs on flat glass substrate and in flow reactor**

Sample name	Rate constant (initial) ( $s^{-1}$ )	Total Au atoms (nmol)
CCMV-Au7B NPs	$3.08 \times 10^{-3} \pm 9.80 \times 10^{-5}$	12.4
CCMV-Au7B immobilized flat glass substrate	$1.21 \times 10^{-3} \pm 4.60 \times 10^{-5}$	9.6
CCMV-Au7B immobilized flow reactor	$1.10 \times 10^{-2} \pm 3.08 \times 10^{-5}$	13

**Note:** the reported numbers of gold atoms are intended for a qualitative comparison of the catalytic activity. The accuracy is limited by the size determination of the reactor surface and its coverage, but the observed trend is reliable.

Upon reaction and purification, the structure of the protein cage is converted to a net-like capping layer. UV-vis analysis reveals an intense absorption band at  $\lambda=530$  nm corresponding to the gold particles which are still present at the substrate (Figure S2.7b). The particles plasmon band shifts upon reduction reaction. This behavior is assigned to hydrogen adsorption.<sup>1</sup> Contact angle remains unchanged supporting our assumption (Figure S2.7c).

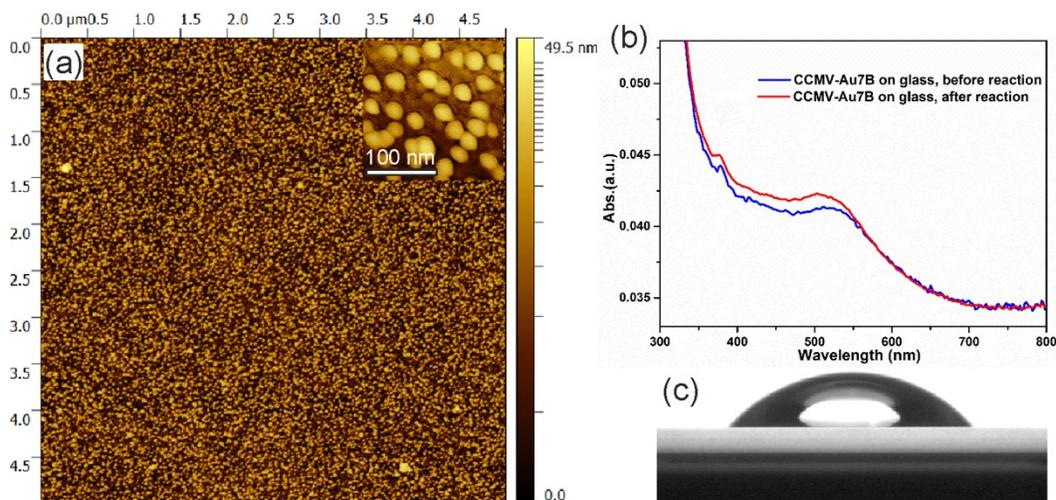


Figure S2.7. CCMV-Au7B on glass substrates after the catalytic reaction, a) AFM analysis; b) UV-vis analysis before and after the model catalytic reaction and c) contact angle analysis.

## Reference

1. M. M. Giangregorio, M. Losurdo, G. V. Bianco, A. Operamolla, E. Dilonardo, A. Sacchetti, P. Capezzuto, F. Babudri and G. Bruno, *J. Phys. Chem. C*, 2011, **115**, 19520-19528.