Supporting Information

Step-by-step build-up of covalent poly(ethylene oxide) nanogel films

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Build-up process type	Substrate	Anchoring layer	<F3> anchoring layer (Hz)	SH-component	Mal-component
A	Au	$Tetra_{112}$-SH	-56.6	$Tetra_{112}$-SH	$Tetra_{52}$-Mal
B	Au	Bis_5-SH	-4.6	$Tetra_{112}$-SH	$Tetra_{52}$-Mal
C	Au	Bis_5-SH	-4.6	Bis_5-SH	$Tetra_{52}$-Mal
C	Au	PEI	-25.0	Bis_5-SH	$Tetra_{52}$-Mal
C	SiO_2	PEI	-9.7	Bis_5-SH	$Tetra_{52}$-Mal
C	PDMS	PEI	-18.8	Bis_5-SH	$Tetra_{52}$-Mal
C	Au	SH-PEG-NH_2	-32.5	Bis_5-SH	$Tetra_{52}$-Mal
C	Au	Bis_{43}-SH	-52.0	Bis_5-SH	$Tetra_{52}$-Mal
C	Au	Bis_{43}-SH	-52.0	Bis_{43}-SH	$Tetra_{52}$-Mal

Table S1. Summary of the different build-up conditions studied.

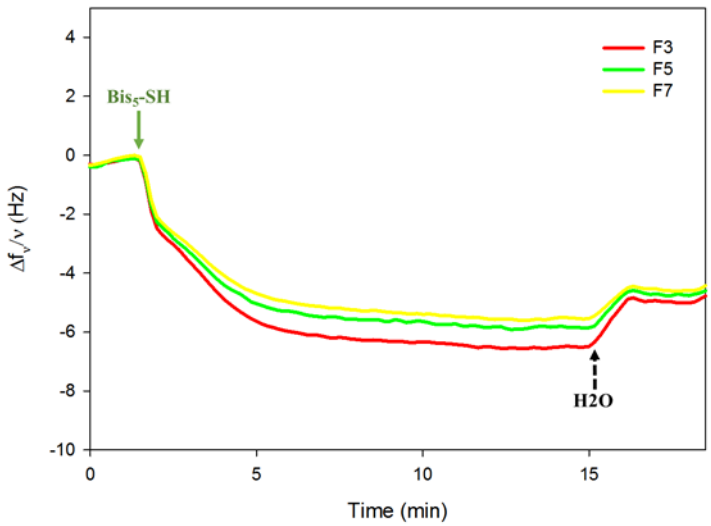

Figure S1. Evolution of the normalized frequency $\Delta f_v/v$ (for harmonics v = 3, 5 and 7) as a function of time with Bis_5-SH deposition. Bis_5-SH was used at a concentration of 10^{-2} M (3.25 mg.mL^{-1}).

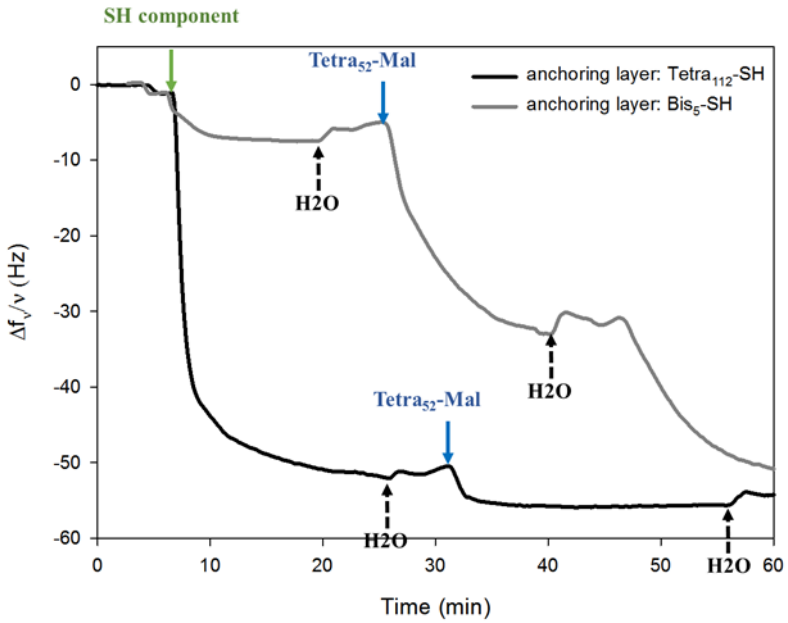

Figure S2. Evolution of the normalized frequency $\Delta f_v/v$ (for v = 3) as a function of time when either Bis_5-SH or $Tetra_{112}$-SH was deposited as a precursor layer and then a subsequent $Tetra_{52}$-Mal was injected. Bis_5-SH solution was used at a concentration of 3.25 $mg.mL^{-1}$, $Tetra_{112}$-SH and $Tetra_{52}$-Mal at a concentration of 5 $mg.mL^{-1}$.

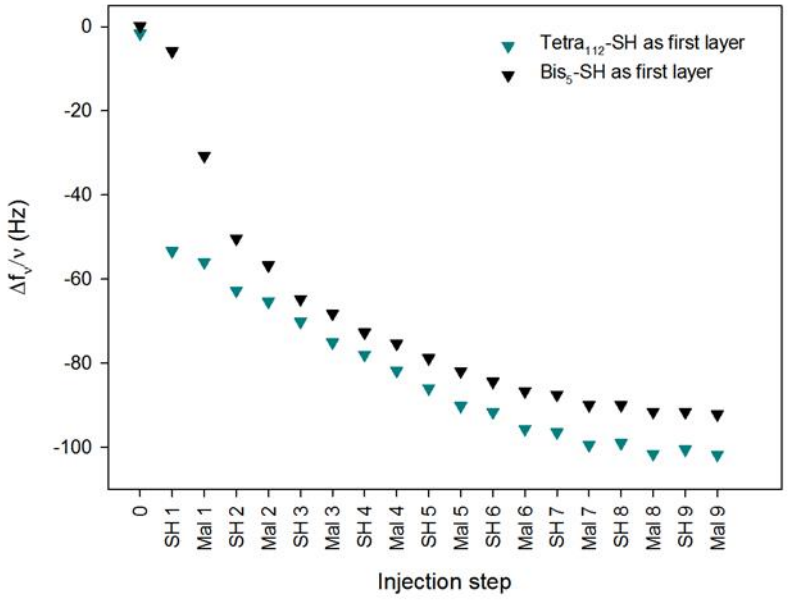

Figure S3. Evolution of the normalized frequency $\Delta f_v/v$ (for v = 3) as a function of injection step for a $Tetra_{112}$-SH/$Tetra_{52}$-Mal step-by-step build-up (with both solutions at 5 $mg.mL^{-1}$). The build-up processes on gold substrates start with a precursor layer of $Tetra_{112}$-SH or Bis_5-SH.

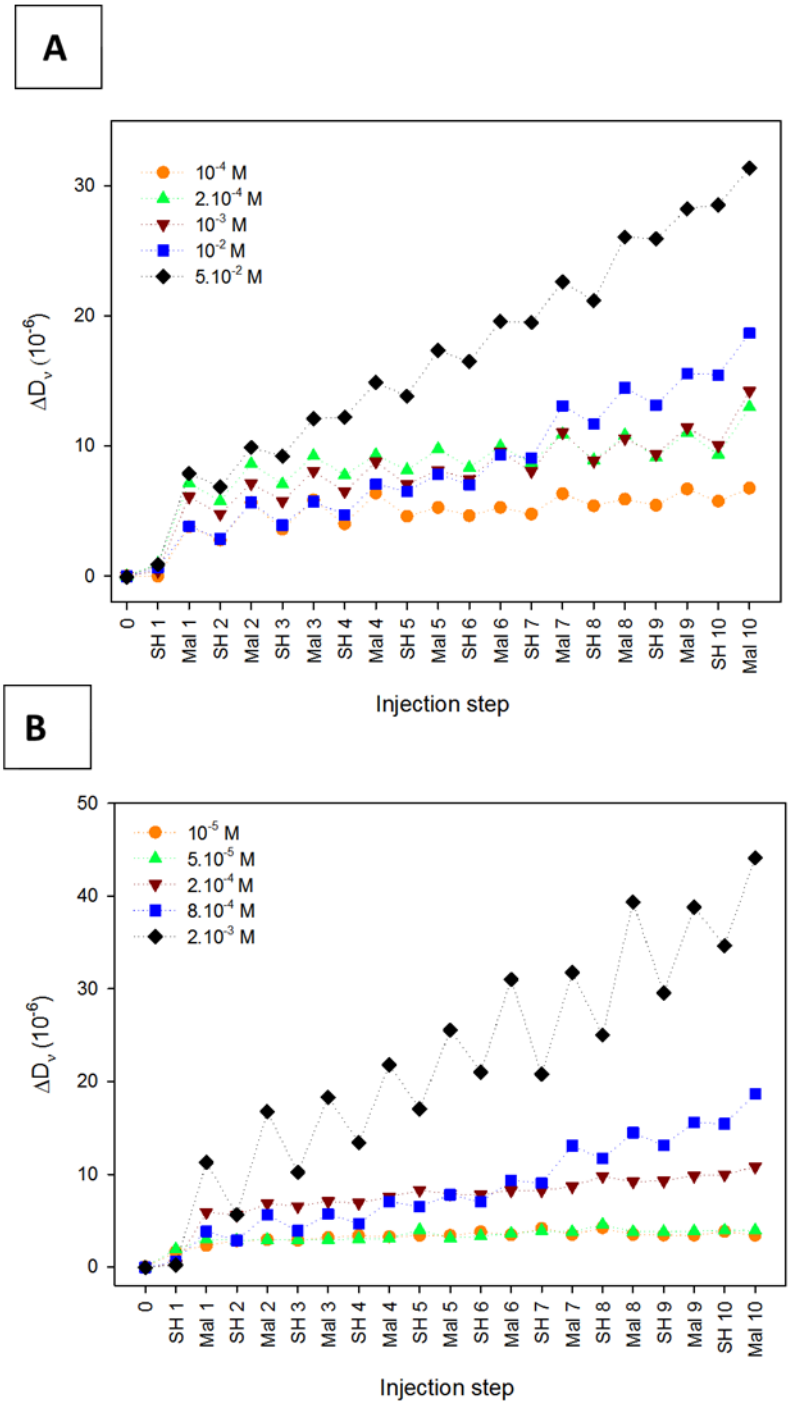

Figure S4. Evolution of the dissipation ΔD_v (for v = 3) as a function of injection step for a Bis_5-SH/$Tetra_{52}$-Mal step-by-step build-up. Concentrations of Bis_5-SH was varied from 10^{-4} M to 5.10^{-2} M (0.0325mg.mL^{-1} to 16.25 mg.mL^{-1} and concentration of $Tetra_{52}$-Mal was maintained constant at 8.10^{-4} M (8 mg.mL^{-1})(A). Concentration of Bis_5-SH was maintained constant at 10^{-2} M (3.25 mg.mL^{-1}) and concentration of $Tetra_{52}$-Mal was varied from 10^{-5} M to 2.10^{-3} M (0.1 to 20 mg.mL^{-1})(B).

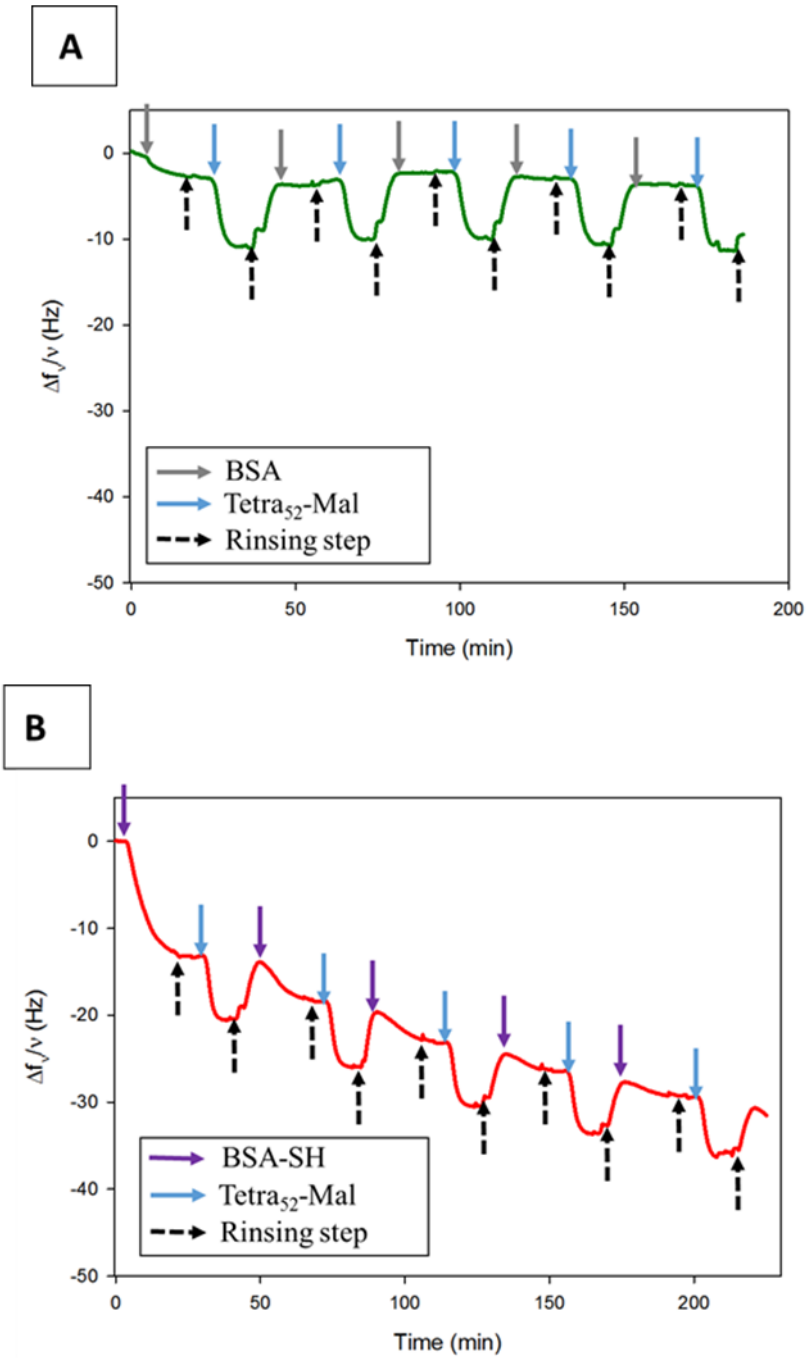

Figure S5. Evolution of the normalized frequency $\Delta f_v/v$ (for v = 3) as a function of time during BSA/Tetra$_{52}$-Mal (A) or BSA-SH/Tetra$_{52}$-Mal (B) step-by-step build-up processes. Tetra$_{52}$-Mal was introduced at 8 mg.mL^{-1} (8.10^{-4} M) and BSA or BSA-SH were used at a concentration of 0.3 mg.mL^{-1} (5.10^{-6} M).

Preparation of Biotin-EO_2-Mal

Biotin-EO_2-Mal has been prepared in three steps from according to the synthetic pathway given below. Experimental processes for each step have been reported elsewhere in literature.

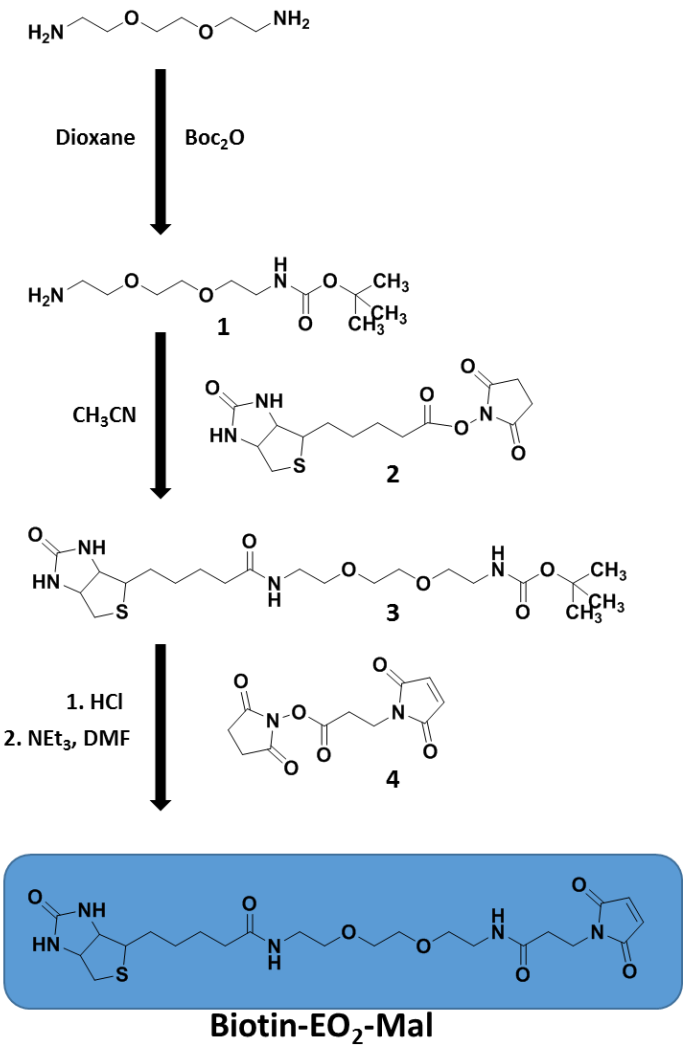

Figure S6. Synthesis of Biotin-EO_2-Mal.

Commercially available 2,2'-(ethylenedioxy)bis(ethylamine) (ref.: 385505 from Aldrich) is monoprotected with Boc_2O leading to compound **1** (S. Takayoshi et *al., Bioorganic & Medicinal Chemistry Letters*, **2007**, *17*(15), 4208-4212). Then this later is brought in contact with

activated biotin **2** (ref.: RL-1006 from Iris Biotech GmbH), yielding to **3** (J. Davila et *al*., *Langmuir*, **2013**, *29*(24), 7488-7498. Characterization of compound **3** was similar to the reported one: L. Myung-Ryul et *al.*, *Organic Letters*, **2005**, *7*(24), 5477-5480). *In situ* deprotection of the Boc group of **3** in acid condition, followed by the coupling with the activated maleimide derivative **4**, called Mal-OSuc (ref.: 63179 from Aldrich), provides **Biotin-EO_2-Mal**. The experimental preparation of **Biotin-EO_2-Mal** is described below.

Compound **3** (329.6 mg, 0.695 mmoles) is dissolved in 5mL of aqueous HCl solution (2.5M) leading to a slightly trouble solution. This mixture is stirred three hours at room temperature and freeze-dried, and thus used without further purification. This resulting white solid (261.1 mg, 0.695 mmoles) is diluted in a mixture DMF (11 mL) / NEt_3 (0.2 mL) and stirred 30 minutes at room temperature. Then, solid portions of Mal-OSuc **4** (1.2 equiv., 222.29 mg, 0.835 mmoles) are added into the reaction mixture and let stir 24 hours. Organic solvents are removed under reduced pressure leading to a yellow oil as residue. Flash chromatography on silica gel (150g, Silica gel 60-200µm from VWR Chemicals, ref.: 84893.290) with Acetonitrile/H_2O 8/2 including 1% of acetic acid as eluent allows to isolate 302,7 mg of a white solid corresponding to **Biotin-EO_2-Mal** (88% yield). Rf (SiO_2, Acetonitrile/H_2O 8/2 - 1%AcOH) = 0.79; ^{1}H NMR (D_2O, 400MHz): δ(ppm) 6.91 (*s*, 2H), 4.87 (*t*, ^3J=^3J =5Hz, 2H), 4.64 (*ddd*, $^3J_{cis}$=5.0Hz , ^3J=4.5Hz , ^3J=0.5Hz, 1H), 4.46 (*dd*, $^3J_{cis}$=5.0Hz , ^3J=4.5Hz, 1H), 3.84 (*t*, ^3J=^3J = 6Hz, 2H), 3.78 (*m*, 1H), 3.68 (broad *s*, 4H), 3.66 (*t*, ^3J=^3J =5.5Hz, 2H), 3.60 (*t*, ^3J=^3J =5.5Hz, 2H), 3.43 (*t*, ^3J=^3J =5.5Hz, 2H), 3.36 (*t*, ^3J=^3J =5.5Hz, 2H), 3.02 (*dd*, ^2J=13Hz, $^3J_{trans}$=5Hz, 1H), 2.82 (*d*, ^3J=13Hz, 1H), 2.56 (*t*, ^3J=^3J =6.5Hz, 2H), 2.31(*t*, ^3J=^3J =7.0Hz, 2H), 1.68 (*m*, 4H), 1.45 (*m*, 2H).