The Dual Nature of Biomimetic Melanin

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Supporting Information

1. Materials and Methods

1.1 Materials

All reagents, solvents and chemicals were purchased from Sigma-Aldrich and used without modification, unless otherwise stated.

1.2 UV-Vis Spectroscopy

The experiments were carried out in air-equilibrated solutions at 25 °C. UV–Vis absorption spectra were recorded with a Perkin-Elmer Lambda 45 spectrophotometer using quartz cells with different path lengths that were 1.0 cm, 2mm and 50 mm.

Dynamic Light Scattering (DLS)

DLS measurements were performed with Zetasizer Nano ZS Malvern Panalytical using PMMA semi-micro cuvettes (BRAND).

1.3 Transmission Electron Microscopy (TEM)

TEM images were received using a Philips TEM CM 100 electron microscope at an accelerating voltage of 80 kV. For the acquisition, the samples were deposited on Formvar on 400 mesh Cu grids supplied by TED PELLA INC.

2. Synthesis and Characterization of PDA

2.1 Synthesis of Polydopamine (PDA)

Polydopamine (PDA) was prepared by the spontaneous oxidation of dopamine hydrochloride in aerobic and alkaline conditions.[1] Initially, 40 mL of ethanol and 90 ml of deionized water were mixed in a 250 mL round bottom flask. In the flask were added 2 mL of 28-30% NH4OH and the mixture was stirred vigorously for 10 min. In the next step, a dopamine hydrochloride solution was prepared by solubilizing 400 mg of dopamine hydrochloride in 10 mL of deionized water. The dopamine solution was injected fast into the flask under vigorous stirring. An immediate colour change was observed which evolved in time from light yellow to dark brown. The reaction was carried out for 24hours under vigorous stirring. After 24 hours, the nanoparticles (NPs) were retrieved by centrifugation at 14000 rpm for 20minutes. The first supernatant was

kept as isolated polymer. Then, NPs were washed with deionized water three times at 10.000rpm and finally redispersed in water.

3. Field-flow fractionation (FFF)

Field-flow fractionation (FFF) is a soft, size-based separation technique performed in the absence of stationary phase thanks to the presence of a transversal field; when the field is a perpendicular flow of solvent, the technique is named flow-FFF or F4. This technique can separate and characterize populations such as proteins, colloids, polymers and particulate materials up to about 100 µm in size and preserving the native state. The micro-volume variant of FFF, hollow-fiber flow-field flow fractionation (HF5) offers high performance, short analysis time and low dilution at the same time for particles analysis of different nature, also allowing for applications where a disposable device is needed to avoid cross-contamination.

In HF5 the separation is achieved without a stationary phase and by an external flow-field, perpendicular to the parabolic flow in an empty, porous capillary channel ("fiber"). The system used was a commercial Wyatt Dualtec (WTC) set up with an 1200 Agilent HPLC tower (Agilent Technologies) and UV detector. Light Scattering was performed with a Wyatt Heleos (WTE). Particle retention is inversely proportional to the hydrodynamic diffusion coefficient of the analyte and, consequently, to its *MW* or hydrodynamic size. Bigger particles have a lower diffusion coefficient and therefore a higher retention time. Selectivity is particularly high in the high-*MW* and nanometer-size range reaching the micrometer range. Downstream coupling of the fiber with detectors such as MALS and DAD allows for size (R_G),mass (Mw) and shape characterization. The latter is obtained through a conformation plot, i.e. a double logarithmic logMW – logRg plot, where the shape value v is the slope of the regression line. It is theoretically defined as v = 0.33 for solid spheres, v = 0.5–0.6 for random-coil, v = 0.7-0.8 for branched polymers, and v ~1 for rod-like structures.

An FFF method requires two different steps (Figure S1). (1) A Focus-injection step in which the analytes are focused in a narrow band at the beginning of the fiber/channel (S1a). During this phase molecules smaller than the fiber cut off (10 kDa) are filtered trough (2) An Elution step in which the analytes are separated based on their diffusivity (S1b). The separation profile (fractogram) produced (S1c) is a plot of the signal against elution time.

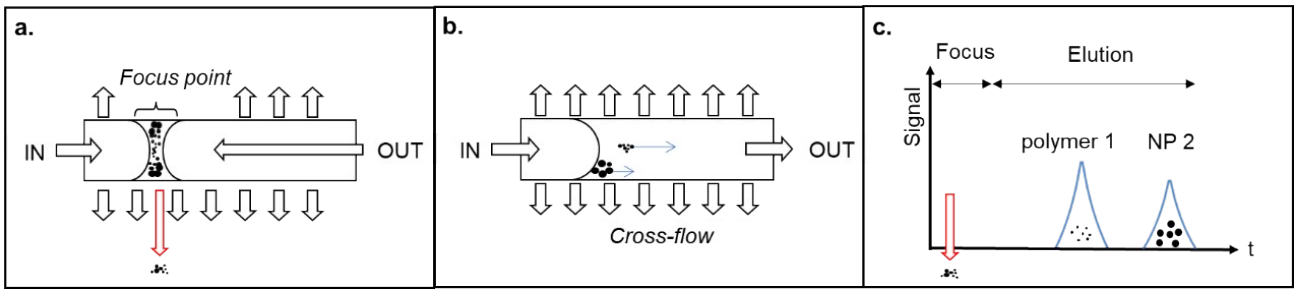

Figure S1. method and output schematics. a, b) flow representation of the focus (a) and elution (b) steps of an HF5 experiment; fiber is represented as longitudinal cross-section. C) schematisation of an output signal (fractogram) of an FFF separative experiment.

Particle recovery (expressed as area percentage ratio between a zero-crossflow analysis and separation method) was >99% indicating no sample loss and no surface interactions with the fiber in the separation conditions.

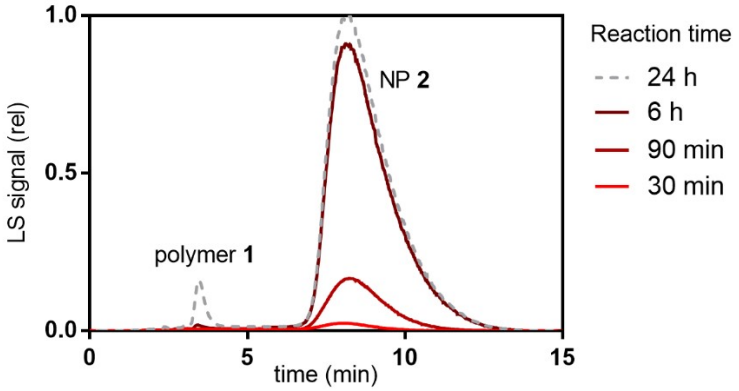

Figure S2. Light scattering signal (LS) evolution of the DA reaction batch at different reaction times (0.5, 1.5 and 6 hours, red traces) against 24h (grey dotted line).

Light Scattering (LS) signal gives information on both the amount and the size of the eluted particles, its intensity being linked to both parameters (and scattering angle) as opposed to concentration detectors such as UV. This allows to understand the relative smaller size of polymer 1 even though the software computes a high r_G value (corresponding to an entangled supramolecular object).

In the first six hours of DA oxidation (Figure S2) it is possible to observe the rising second band (NP **2**) which is associated to the formation of **2.** Though the reaction time is very low, the LS signal is already visible indicating that the size of the particles under formation is high from the beginning. After the six hours mark the generation process of **2** is nearly stopped while **1** starts to form. The low LS intensity associated to **1** is caused by its low size/mass which is confirmed even after 24h. indeed at this time mark population 1 is quantitatively predominant but its LS signal is still very low.

Further information on morphology is obtained from the v value calculated from isolated **1** and **2** after 24h reaction time. (Figure S3)

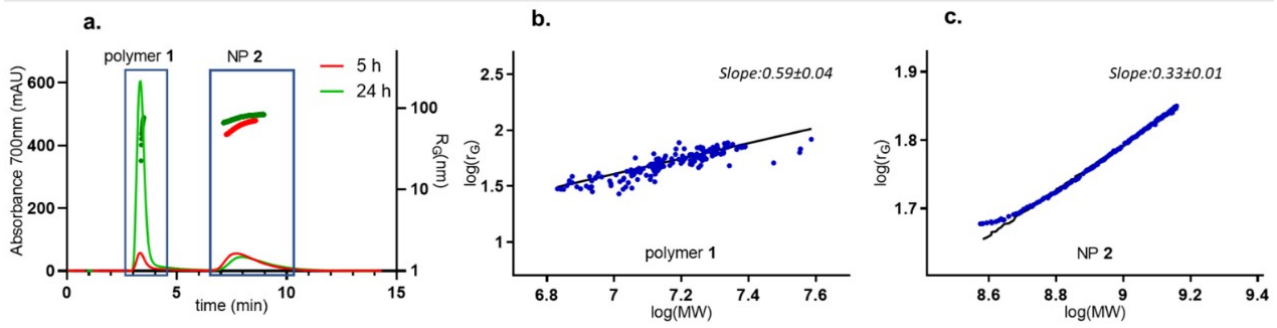

Figure S3. a) absorbance fractograms of the PDA raw reaction batch after a reaction time of 5 hours (red) and 24 h (green). Dotted traces: r_G values calculated for **1** and **2**. b) Conformation plot of **1** as supernatant of the centrifugation step. The slope of the conformation plot is coherent with a random coil conformation. c) Conformation plot of **2** isolated through centrifugation and resuspended in water/ethanol. The slope of the conformation plot is coherent with a solid sphere conformation.

The etching of NP **2** into polymer **1** was observed by dispersing **2** in the reaction media and monitoring its evolution over time through FFF analysis (Figure S4): signal corresponding to **2** decreases while polymer **1** starts forming until complete conversion after 3 weeks.

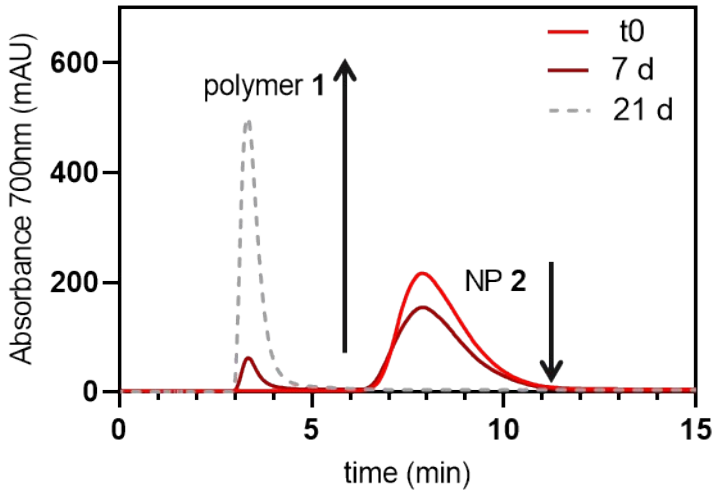

Figure S4. Absorption signal evolution of **2** after isolation trough centrifugation and resuspension in alkaline environment, FFF analysis. Red line: t0 and 1 week. Dotted line 21 days from isolation, where it is possible to observe the complete disappearance of **2** and rising of **1** suggesting an «etching» process converting **2** in **1**.

4. TEM of PDA NPs

TEM images of PDA NPs were retrieved after their purification and redispersion in water. TEM (Figure S5) images revealed the presence of spherical particles with diameter around 160nm.

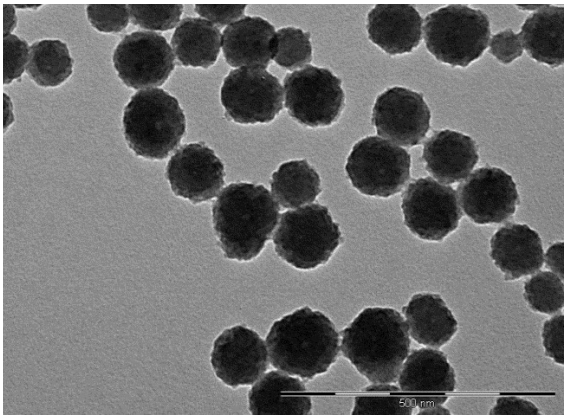

Figure S5. TEM image of PDA NPs.

5. Kinetic analysis.

A simple first order kinetic model was used to fit the increase of the absorbance as a function of time. In particular the reaction was modelled as:

$$n\,DA \rightarrow PDA$$

And the formation of PDA was considered to follow a first order kinetics:

$$c_{PDA}(t) = c_{PDA}(\infty)(1 - e^{-kt})$$

Or, since absorbance is proportional to the concentration:

$$A(t) = A(\infty)(1 - e^{-kt})$$

As shown in figure 1b this model allowed to fit the experimental data giving a rate constant 0.084 h-1.

6. DLS Measurements

Dynamic light scattering measurements were performed at 1 hour time interval during the reaction.

As shown in figure S6 the measured hydrodynamic radius was about 50-70 nm during the first six hours of reaction. Moreover the scattering intensity stopped increasing after 4 hours of reaction,

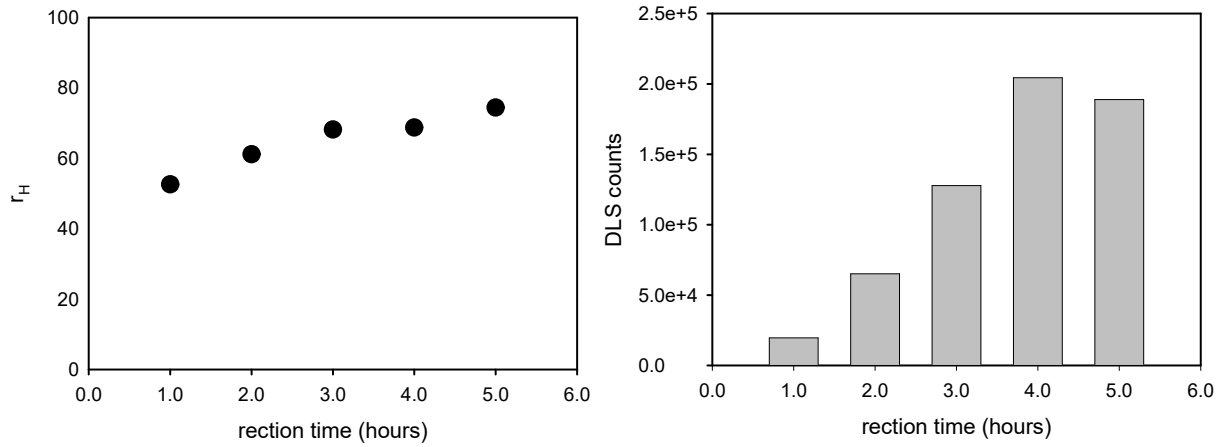

Figure S6. Left. Hydrodynamic radius of the NP measured as a function of the reaction time by DLS. Right. Light scattering intensity measured as a function of the reaction time by DLS

7. Zeta-Potential

Zeta-potential measurements were performed with Zetasizer Nano ZS Malvern Panalytical using folded capillary Zeta cell (Malvern).

8.Electrophoresis

Electrophoresis Native agarose gel electrophoresis was performed on an Owl Easycast B-Series Horizontal Gel Systems Model B2. Electrophoresis was performed in a 1 cm thick 5% agarose gel. The run was performed applying a potential of 30 V

7. Fluorescence Spectroscopy

Fluorescence spectra were recorded with a Horiba Jobin Yvon Fluoromax-4, a Perkin-Elmer LS-55 or an Edinburgh FLS920 equipped with a photomultiplier Hamamatsu R928 phototube using quartz cuvettes 1.0 cm path length.

Excitation-emission fluorescence maps of each sample was determined using a FluoroMax-4 system (Horiba Scientific; Kyoto, Japan), in quartz cuvettes (1 cm).

8 DPPH Assay for Antioxidant Activity.

DPPH radical scavenging activity was measured according to the literature. Briefly, 0.2 mM of DPPH solution in 95% ethanol was prepared before use, and then either **P** or **NP** were dispersed in water and mixed with 1.8 mL of the DPPH solution. The scavenging activity was evaluated by monitoring the absorbance decrease at 516 nm after it remained in the dark for 20 min. DPPH radical scavenging activity was calculated as $\%_{deg}=[1-(A_i-A_j)/A_c]\times 100\%$, where A_c is the absorbance of DPPH solution without neither **P** or **NP**, A_i is the absorbance of the samples of **P** or **NP** mixed with DPPH solution, and A_j is the absorbance of the samples of **P** or **NP** themselves without DPPH solution.