Supplementary Material (ESI) for *Soft Matter*

†Electronic Supplementary Information (ESI):

Protein decorated membranes by specific molecular interactions

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1. Microscopy of objects formed by self-assembling of metal-functionalised copolymers

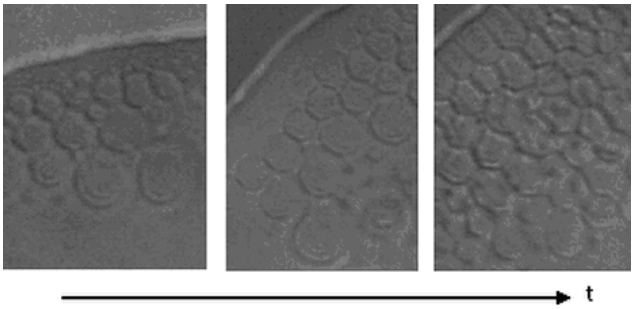

Figure S1. Images of a drop of PB_{39}-PEO_{36}-SA-OH/ PB_{39}-PEO_{36}-SA-TrisNTA.d-Ni^{2+} 10:1, in time. Solvent: Milli-Q water; c(polymer)/μM 5. Microscope: Transmission Microscope Leica JPK, magnification: 100x10 Pol. 1.

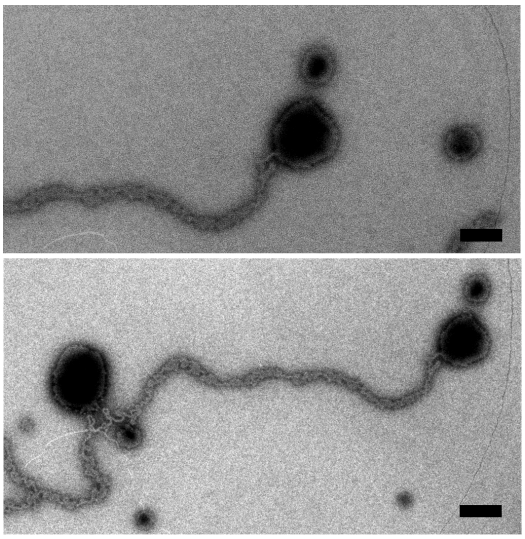

Figure S2: Cryo-TEM images of PB_{60}-PEO_{34}-SA-OH/PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+}solutions in PBS, extruded through a 400 nm pore PC membrane, showing the different populations present in the sample. Scale bar is 100 nm.

2. Dynamic Light Scattering, DLS

Table S1. Dynamic light scattering data[a]

Polymer type	Diameter of membrane pore/ nm	$d_{h/}$ nm	Vesicles population
PB_{60}-PEO_{34}-SA-OH/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} in PBS	80	244 ± 56 753 ± 236	20 (%) 80 (%)
PB_{60}-PEO_{34}-SA-OH/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} in PBS	400	782 ± 154	100(%)
PB_{60}-PEO_{34}-SA-OH in PBS	80	164 ± 35 466 ± 140	20 (%) 80 (%)
PB_{60}-PEO_{34}-SA-OH in PBS	400	750 ± 250	100 (%)
PB_{39}-PEO_{36}-SA-OH/0.1PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} in water	80	52 ± 16 244 ± 75	39 (%) 61 (%)
PB_{39}-PEO_{36}-SA-OH/0.1PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} in water	400	240 ± 62	100 (%)
PB_{39}-PEO_{36}-SA-OH in water	80	74 ± 26 318 ± 103	34 (%) 66 (%)
PB_{39}-PEO_{36}-SA-OH in water	400	109 ± 25 503 ± 160	8 (%) 92 (%)

The hydrodynamic diameter evolution as a function of the vesicle statistical composition and the pore diameter of the membrane used for extrusion are shown in **Table S1**. Samples sizes are always under one micrometer and are bimodal for objects formed after extrusion through 80 nm membrane, while the objects created from PB_{60}-PEO_{34}-SA-OH/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} and PB_{39}-PEO_{36}-SA/0.1PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} extruded through a 400 nm membrane, are unimodal. When extruded through 80 nm membranes, the probes presents two populations, of relative proportions are either 20 (%)/80 (%) and 40 (%)/60 (%) for objects dispersed in PBS and water respectively. Objects created with PB_{39}-PEO_{36}-SA-OH and PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} are systematically smaller than those created PB_{60}-PEO_{34}-SA-OH and PB_{60}-PEO_{34}-SA-NTA-Ni^{2+}, as qualitatively expected. However, direct comparison is biased by different nature of the dispersant. Objects created with PB_{60}-PEO_{34}-SA-OH are systematically larger than the membrane where they were extruded from, while an important proportion of objects (40 (%) in volume) created from PB_{39}-PEO_{36}-SA-OH is always below the membrane diameter. A significant part of the probes volume is composed of objects usually larger than 200 nm, and this irrespective of the dispersant used and extrusion membrane used to form them.

3. Further modeling of the SAXS data from PB_{60}-PEO_{34}-SA-OH/PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+}solutions

Figure S3 presents the fit of the PB_{60}-PEO_{34}-SA-OH/PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+}solutions in PBS, using a core-shell model (red line) and an infinite by long parallelepiped added to a core-shell model (green line), as a first approximation. The Table S2 shows the fit data obtained upon data analysis. Comparison between the fit and the different models shows that the data support, the presence of worms-like structures and vesicles in the proportion 1:5, that is the majority of the sample is composed of vesicles. This is supported by Cryo-TEM, Figure S2. The slight increase in membrane thickness is likely to be caused from a calculation artifact rather than an indeed existing difference with the simpler core-shell model, as the A parameter and vesicle thickness are close together.

Table S2. Fitting parameters of SAXS data from PB_{60}-PEO_{34}-SA-OH/PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+}solutions in PBS.

polymer type	scale factor ratio	A*/ nm	B*/ nm	C*/ nm	membrane thickness/ nm	vesicle diameter/ nm
core/shell model	0	-	-	-	10.2 ± 0.2	> 400
core/shell model and infinite length parallelepiped summed	0.2	10.7 ± 0.2	17.2± 0.2	> 500	10.7 ± 0.2	> 400

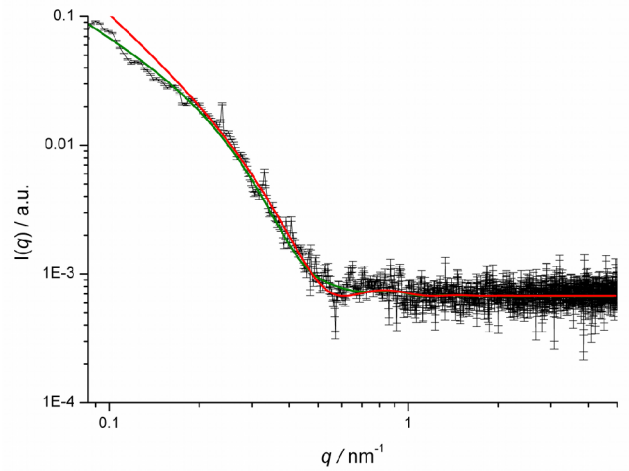

Figure S3. SAXS data from PB_{60}-PEO_{34}-SA-OH/PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+}solutions in PBS, extruded through a 400 nm pore PC membrane (black line), and the corresponding fitting using a simple core-shell model (red line) and a weighted sum model containing weighted amounts of a core-shell and a parallelepiped model (green line).

4. FCS of protein-bound vesicles in various environmental conditions

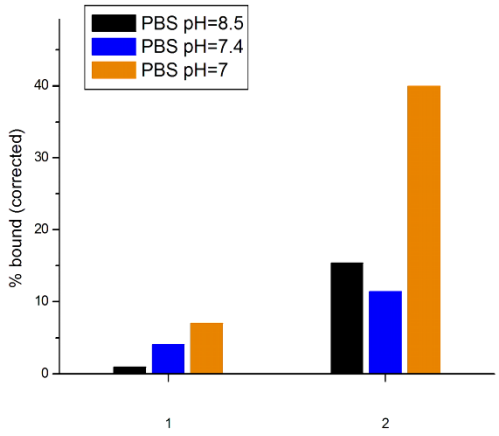

Figure S4. Protein-bound fraction of PB_{60}-PEO_{34}-SA-/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} vesicles: after 5 minutes incubation time (1), and after 4 hours incubation time (2), in PBS buffer, at different pH values (black - pH 8.5, blue – pH 7.4, yellow – pH 7.0).

The fraction of protein-bound vesicles has to be corrected for differences in the fluorescence quantum yield of bound and free protein using eq.S1**:**

$$c = \frac{F}{F + \alpha^2 - F\alpha^2}\ , \qquad \text{(S1)}$$

where c corresponds to the corrected fraction of vesicle-bound protein, F stands for the measured fraction of vesicle-bound protein, and α is the ratio of counts per molecules (cpm) in the free and bound state, respectively (in this experiment the calculated α is 0.64 for Ni(II) doped vesicles).

The fraction of the protein-bound vesicles was plotted against the metal content, and the data were fitted by a Langmuir isotherm, as previously used for Ni^{2+}-NTA functionalised liposomes.[46]

5. SAXS data for protein-bound vesicles

Table S3. Small-Angle X-Ray scattering fitting parameters after protein binding to the metal centers at vesicles surface[a)]

Polymer type	Diameter of membrane pore /nm	Thickness/ nm	Vesicle diameter/ nm
PB_{60}-PEO_{34}-SA-OH/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} in PBS	80	12.9 ± 0.2 [b)]	> 400 [b)]
PB_{60}-PEO_{34}-SA-OH/0.1PB_{60}-PEO_{34}-SA-NTA.d-Ni^{2+} in PBS	400	10.2 ± 0.2 [c)]	> 400 [c)]
PB_{39}-PEO_{36}-SA-OH/0.1PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} in bidistilled water[d)]	80	na	na
PB_{39}-PEO_{36}-SA-OH/0.1PB_{39}-PEO_{36}-SA-NTA-Ni^{2+} in bidistilled water[d)]	400	na	na

6. Langmuir isothermes

To obtain a homogeneous polymer film at the air/water interface with Ni(II)-NTA groups facing towards the water we measured surface pressure area isotherms on a Langmuir trough.

At low surface pressures and mean molecular areas >1500 $Å^2$ it is assumed that the polymer films are in an expanded state ("pancake" conformation), with the hydrophobic PB blocks lying flat at the air-water interface. The insoluble PB chains are anchored to the interface by water-soluble PEO blocks. The PEO segments are assumed to adopt a flattened conformation at the interface.[37] At low surface pressures and mean molecular areas >1500 $Å^2$ it is assumed that the polymer films are in an expanded state ("pancake" conformation), with the hydrophobic PB blocks lying flat at the air-water interface. The insoluble PB chains are anchored to the interface by water-soluble PEO blocks. Upon compression, an increase in surface pressure and compressibility modulus of both polymers is measured, indicating that the films undergo a transition from a gas-like to a more condensed, i.e.,

liquid-like phase. In this region, the PEO blocks extend into the subphase increasing intermolecular interactions by hydrogen bonding[37] while the water-insoluble PB blocks serve as an anchor to the interface.

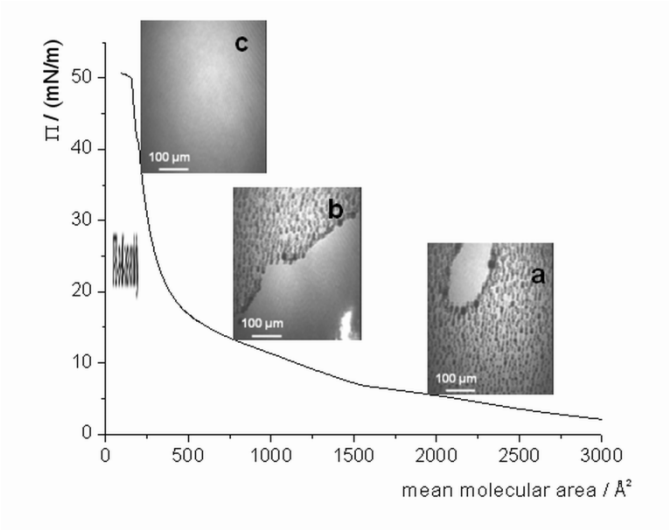

Figure S5: Langmuir compression isotherm of the diblock copolymer PB_{60}-PEO_{34}-OH in bidistilled water, with the corresponding BAM-images (a) – (c). Images correspond to a film at a surface pressure of: a) 7.0 mN/m, b) 15.0 mN/m, c), and 40 mN/m. The transfer experiments have been performed with a surface pressure corresponding to c).