Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2022

Supporting information

Coating Gold Nanorods with Self-Assembling Peptide Amphiphiles Promotes Stability and Facilitates in vivo Two-Photon Imaging

Elena A. Egorova,[a†] Gabriela Arias-Alpizar,[a,b‡] Redmar C. Vlieg,[c‡] Gert S. Gooris,[b] Joke A. Bouwstra,[b] John van Noort,[c] Alexander Kros,[a] and Aimee L. Boyle*[d]*

[a] Department of Supramolecular & Biomaterials Chemistry, Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands

[b] Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

[c] Leiden Institute of Physics, Leiden University, Leiden, The Netherlands

[d] Department of Macromolecular Biochemistry, Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands

† Current address: Elena A. Egorova, Sirius University, Sochi, Russia

‡ Gabriela Arias-Alpizar and Redmar C. Vlieg contributed equally.

Table of contents:

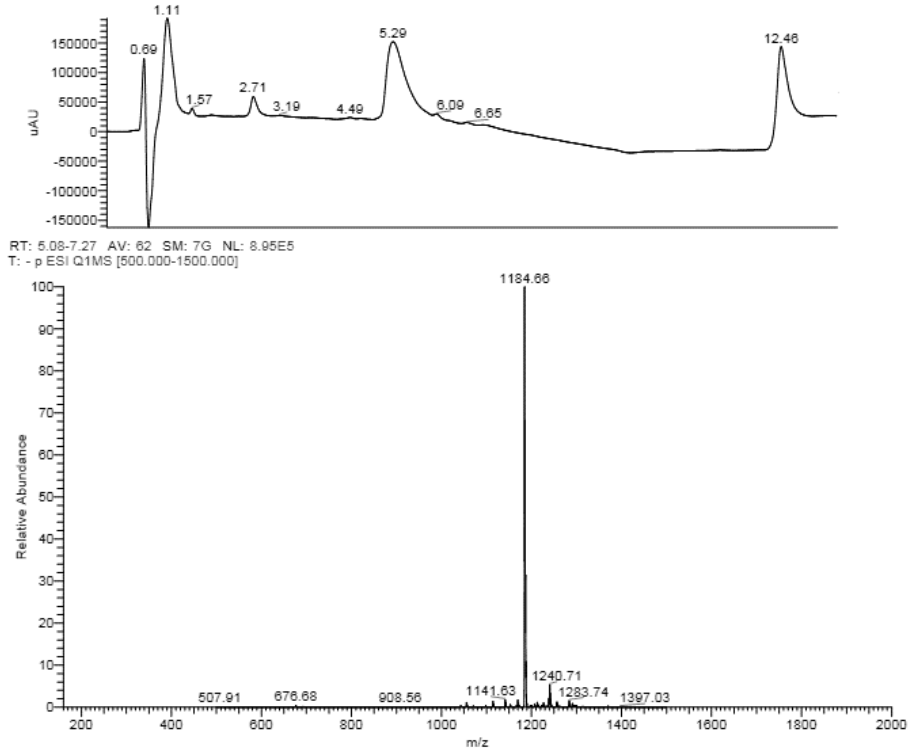


Figure S1. LC-MS spectrum for **1**. $[M\text{-}H]^{1-}_{theor}$ =1184.65.

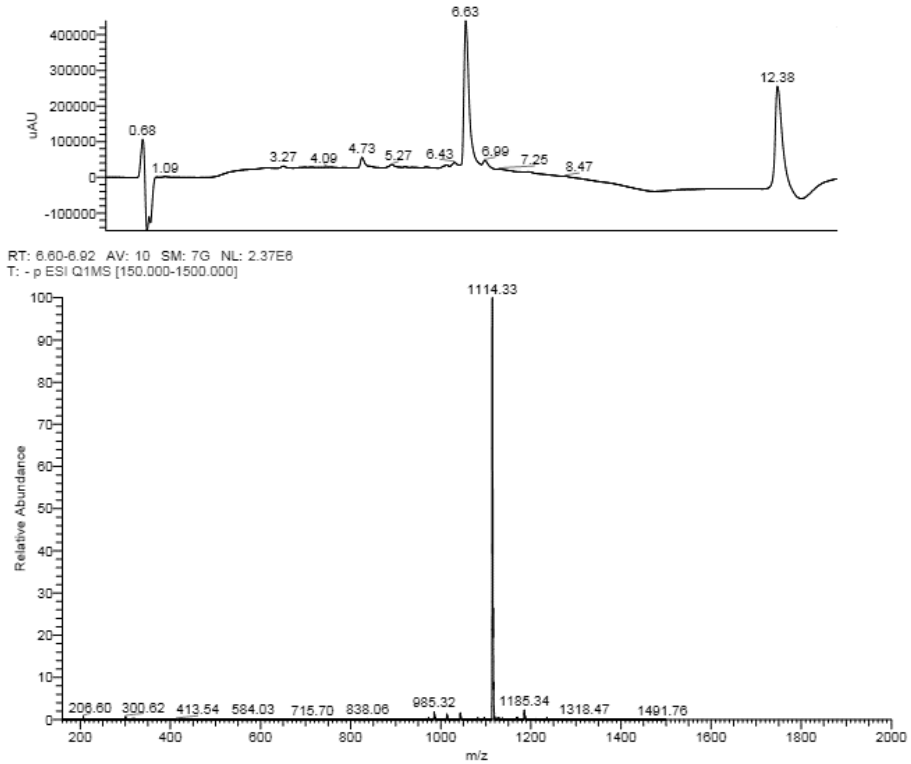


Figure S2. LC-MS spectrum for **2**. $[M\text{-}H]^{1-}_{theor}$ =1114.57.

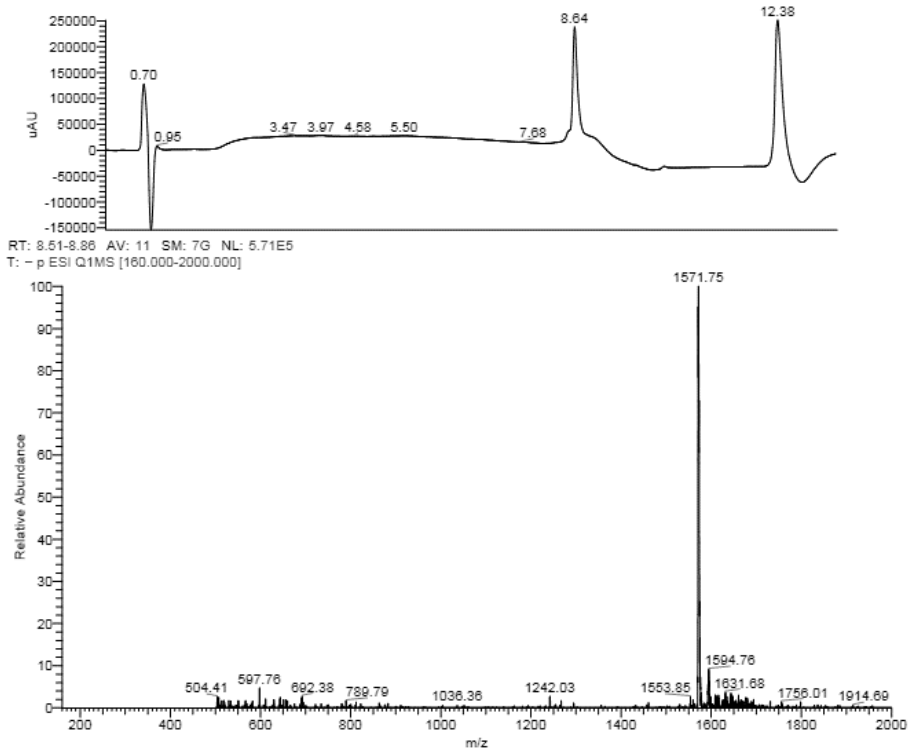


Figure S3. LC-MS spectrum for **3**. $[M\text{-}H]^{1-}_{theor}$ =1571.83.

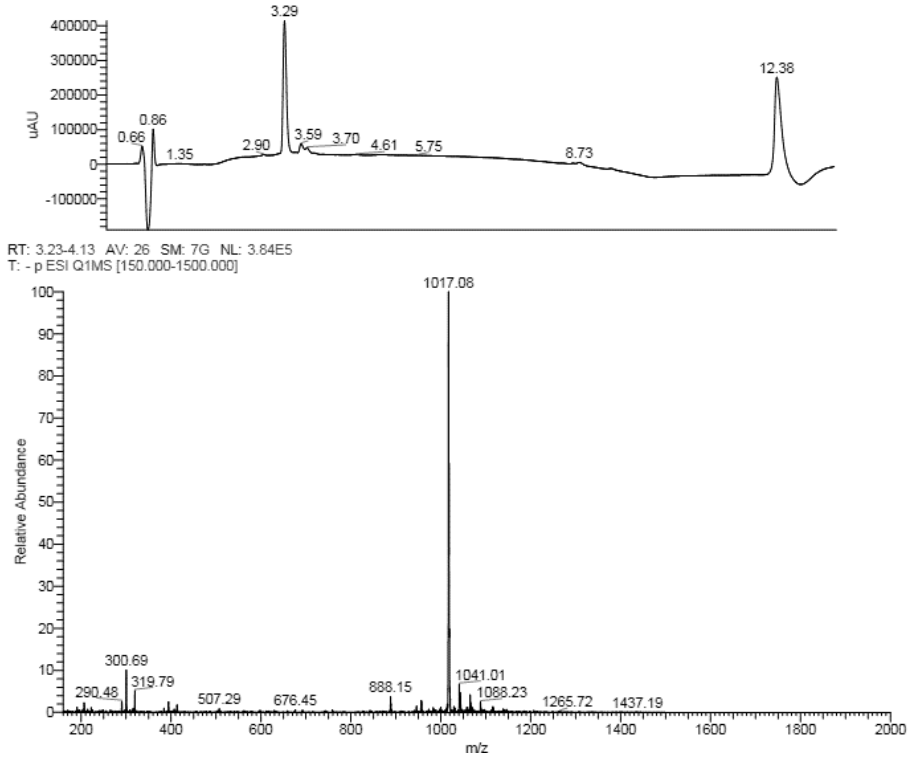


Figure S4. LC-MS spectrum for **4**. $[M\text{-}H]^{1-}_{theor}$ =1017.45.

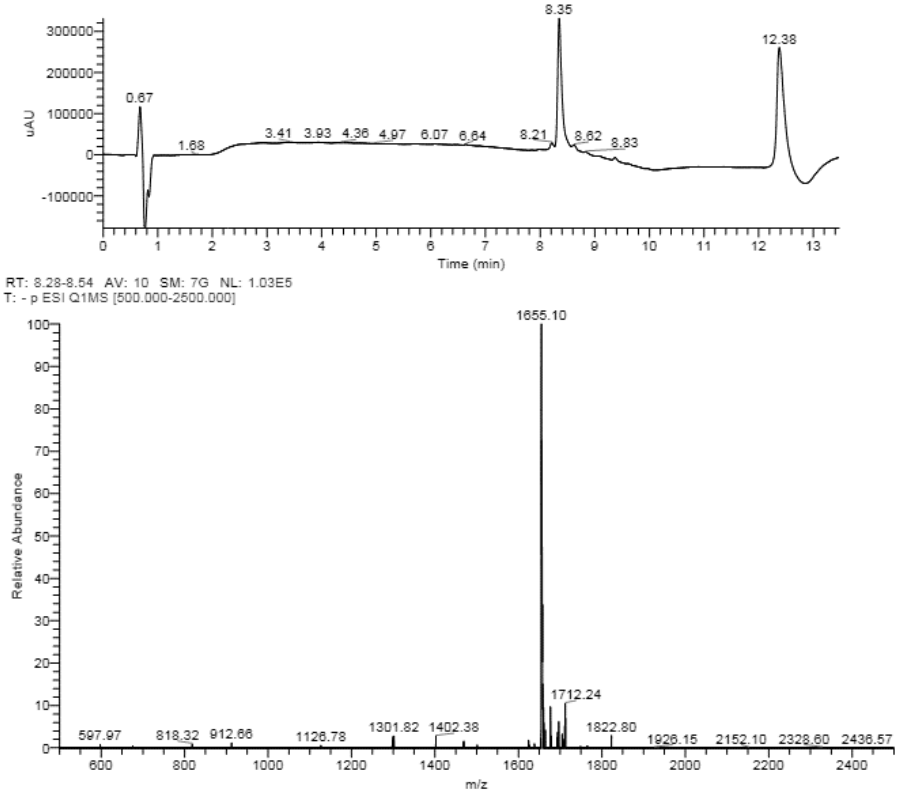


Figure S5. LC-MS spectrum for **1-W**. $[M-H]^{1-}_{theor}$ =1654.82.

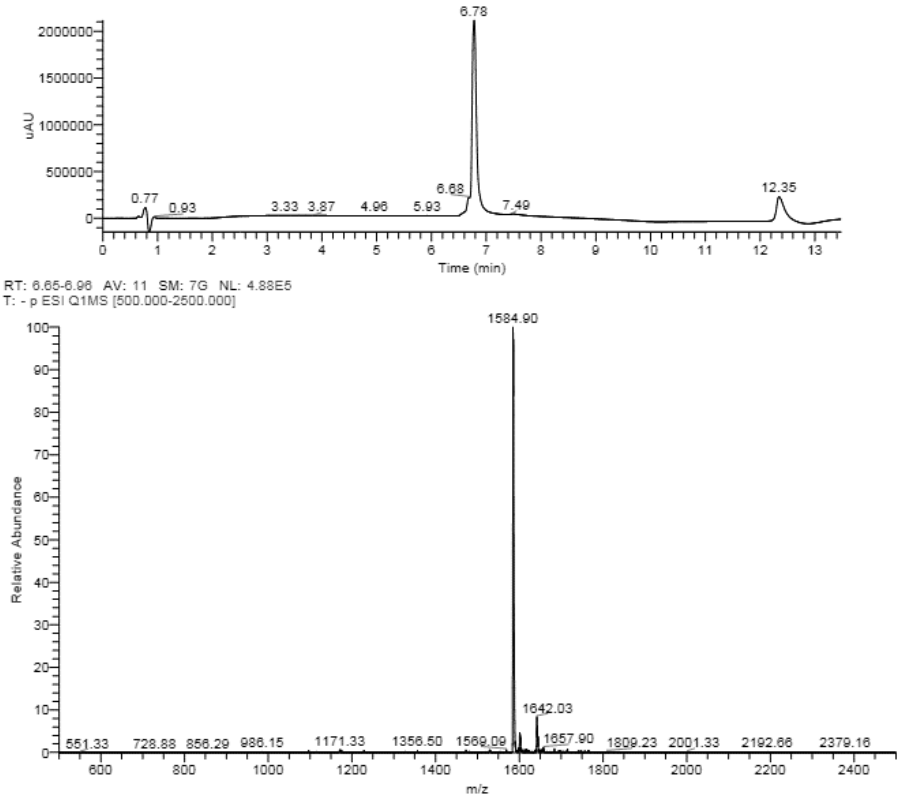


Figure S6. LC-MS spectrum for **2-W**. $[M-H]^{1-}_{theor}$ =1585.75.

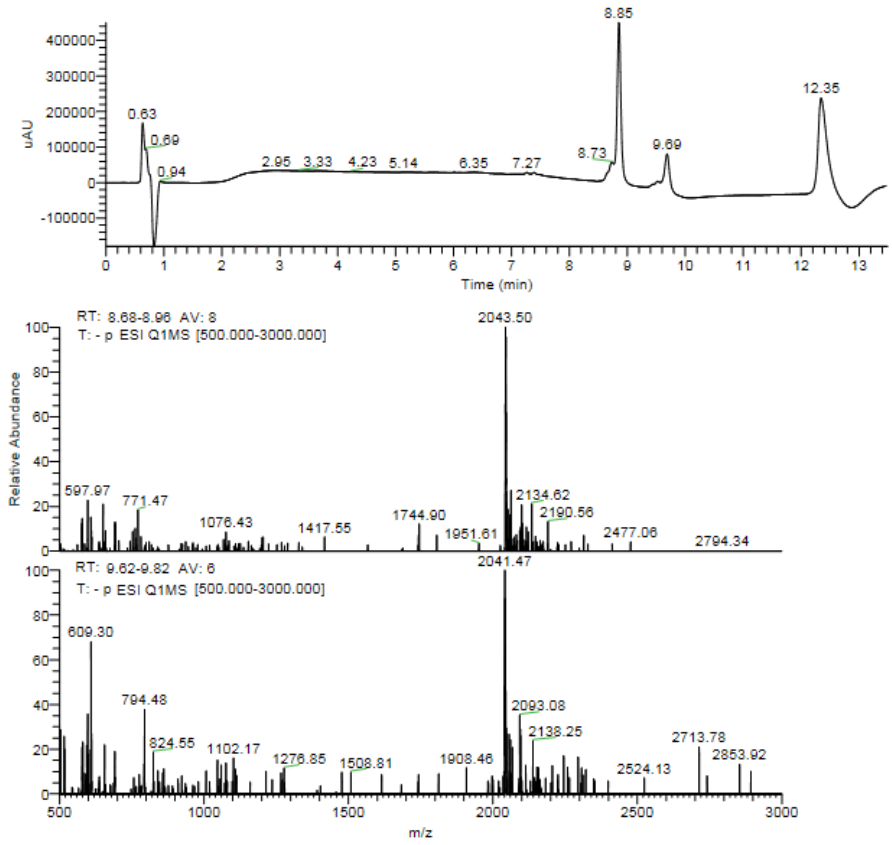


Figure S7. LC-MS spectrum for **3-W**. $[M-H]^{1-}_{theor}$ =2043.03; for the dimeric species $[M-2H]^{2-}_{theor}$ = 2041.50.

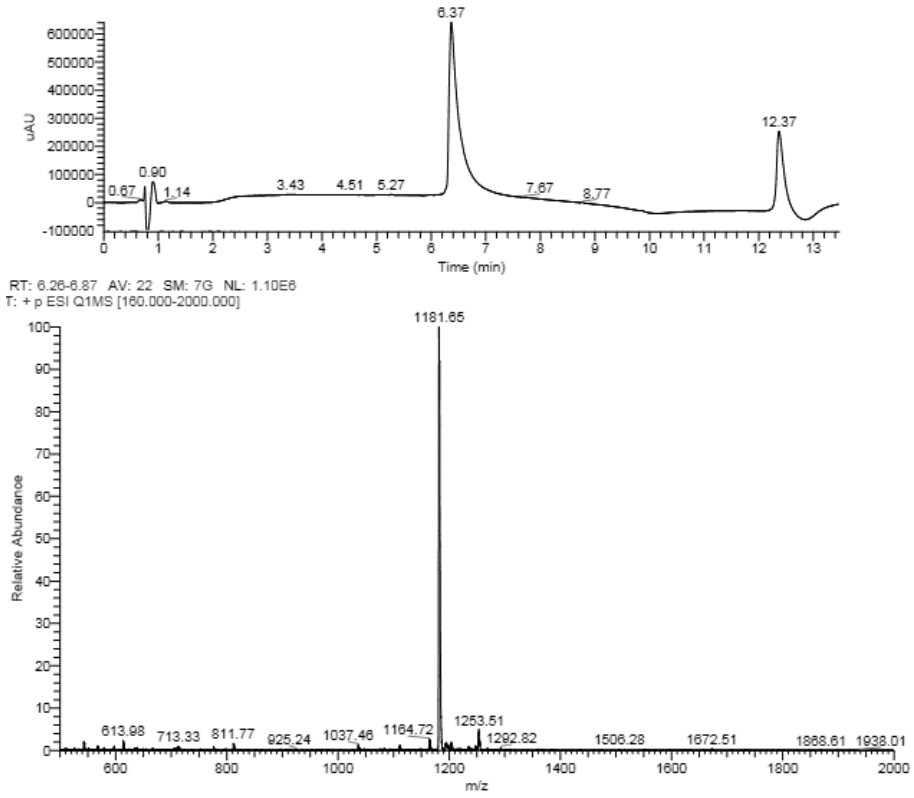


Figure S8. LC-MS spectrum for **1-K**. $[M+H]^{1+}_{theor}$ =1181.83.

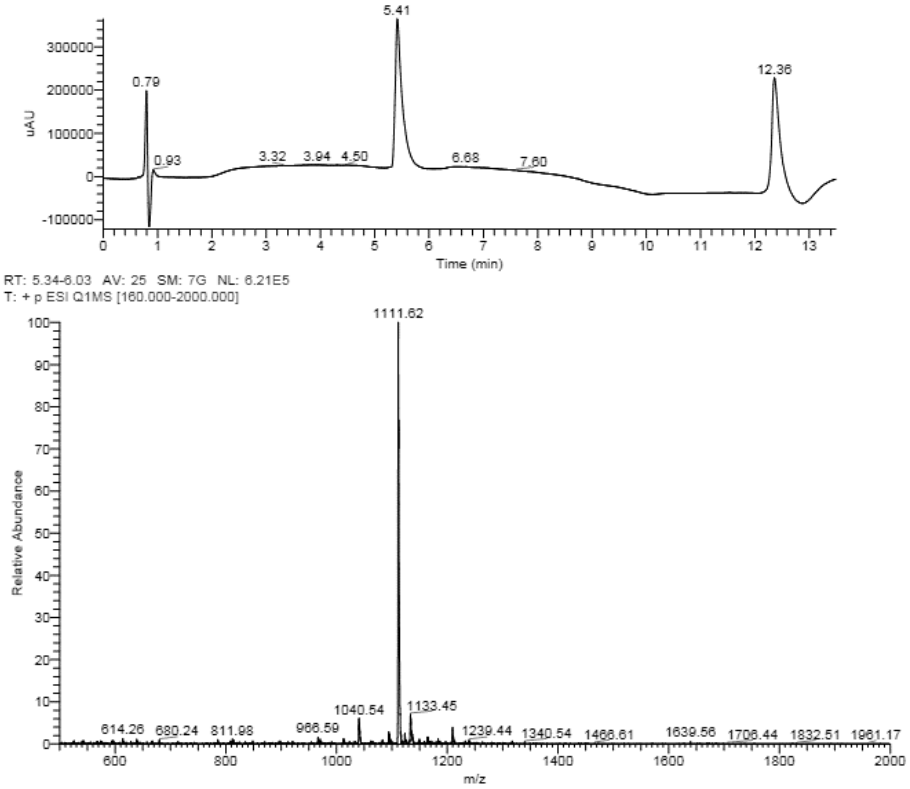


Figure S9. LC-MS spectrum for **2-K**. $[M+H]^{1+}_{theor}$ = 1111.75.

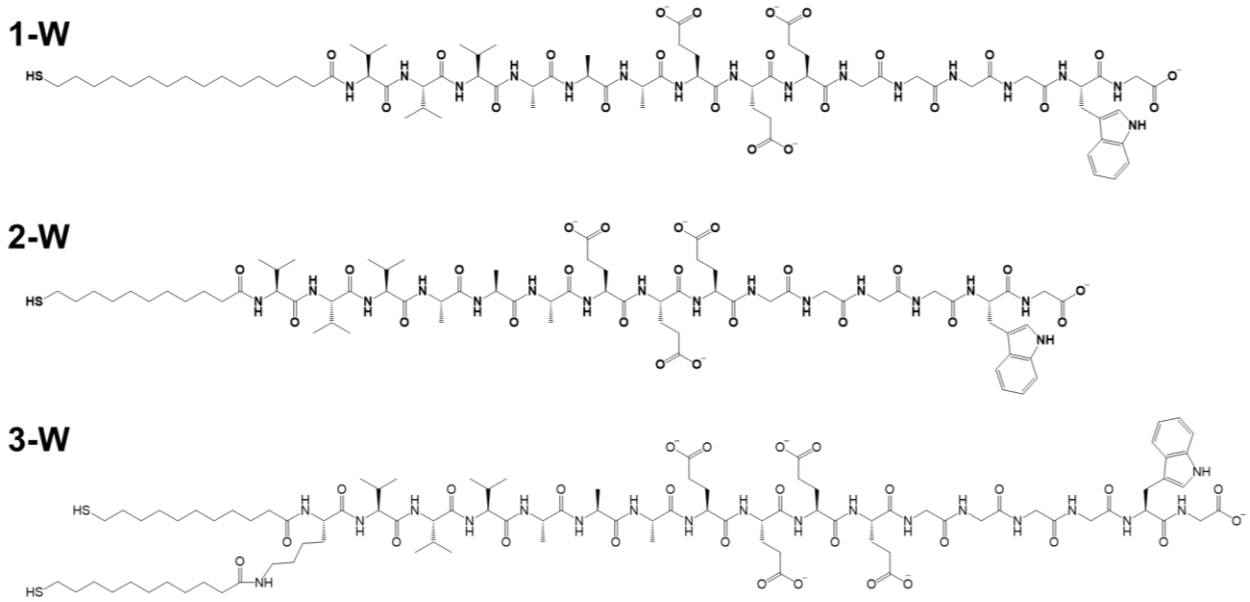


Figure S10. Chemical structures of thiolated PAs used to determine ligand coverage densities: **1-W** was used to determine the coverage density of **1**, **2-W** – of **2**, **3-W** – of **3**.

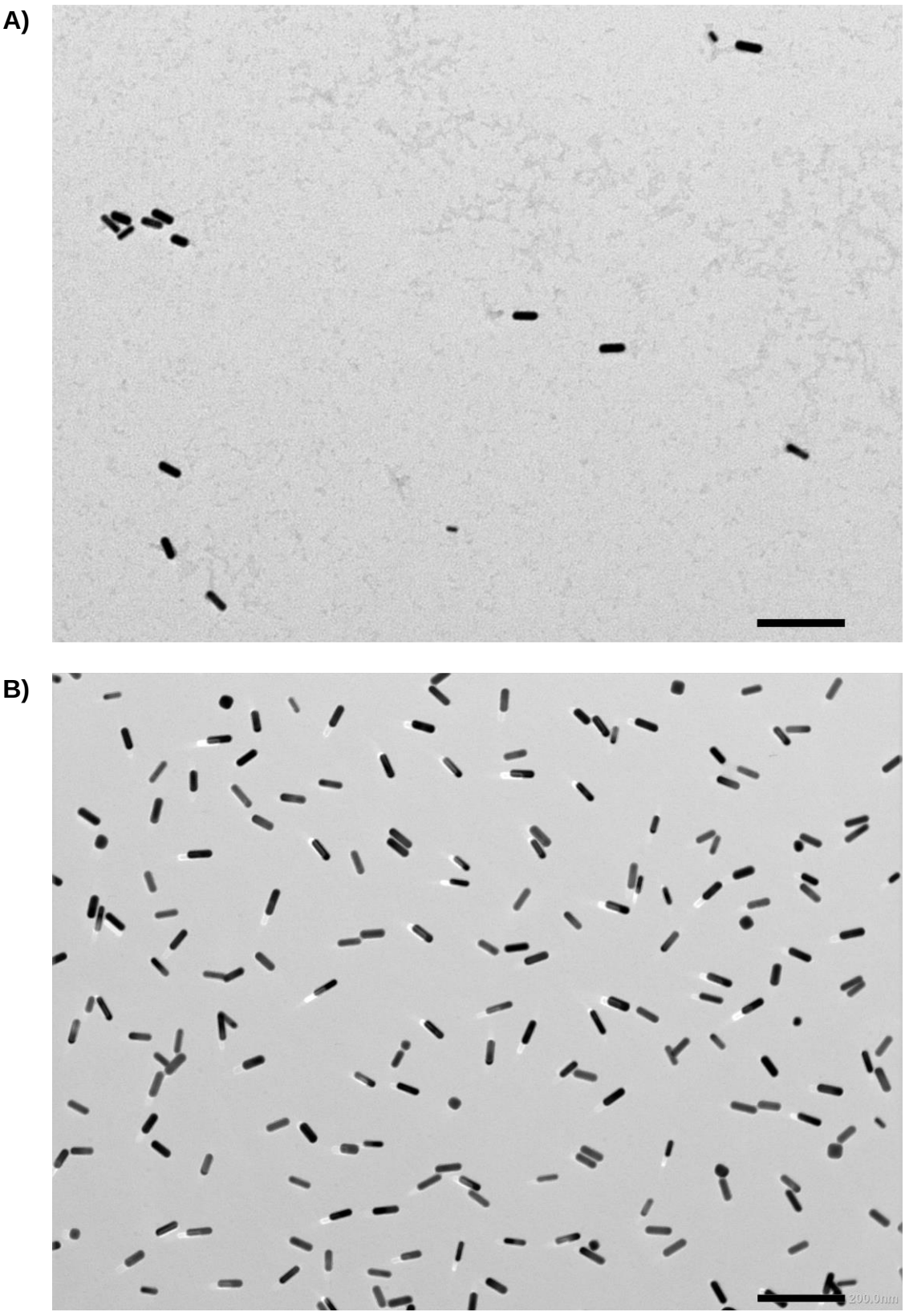


Figure S11. Low magnification images of (A) **GNR@1** in PBS and (B) **GNR@1-K** in MilliQ water. Scale bar = 200 nm. 0.5% uranyl acetate staining was used.

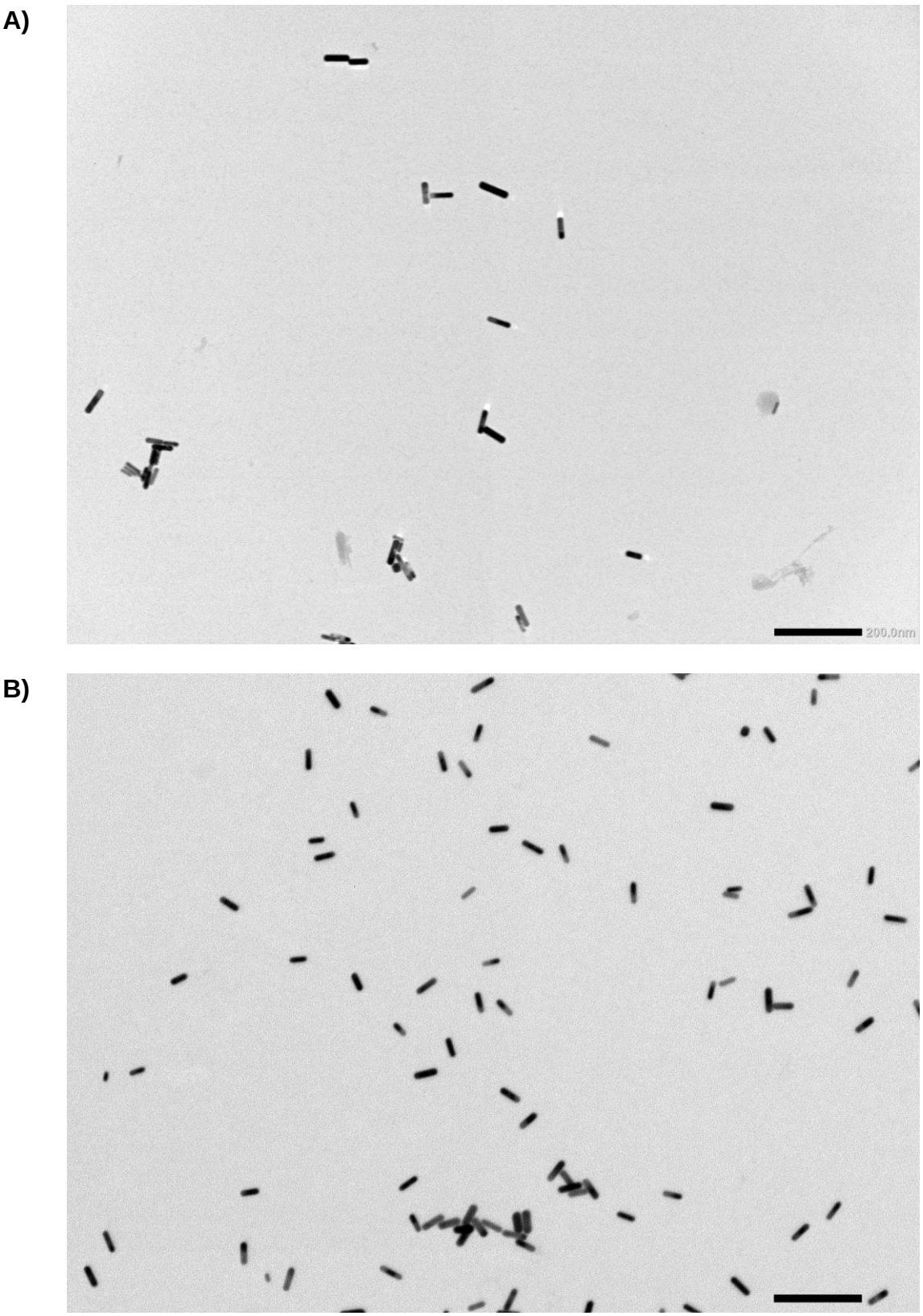


Figure S12. Low magnification images of (A) **GNR@2** in PBS and (B) **GNR@2-K** in MilliQ water. Scale bar = 200 nm. 0.5% uranyl acetate staining was used.

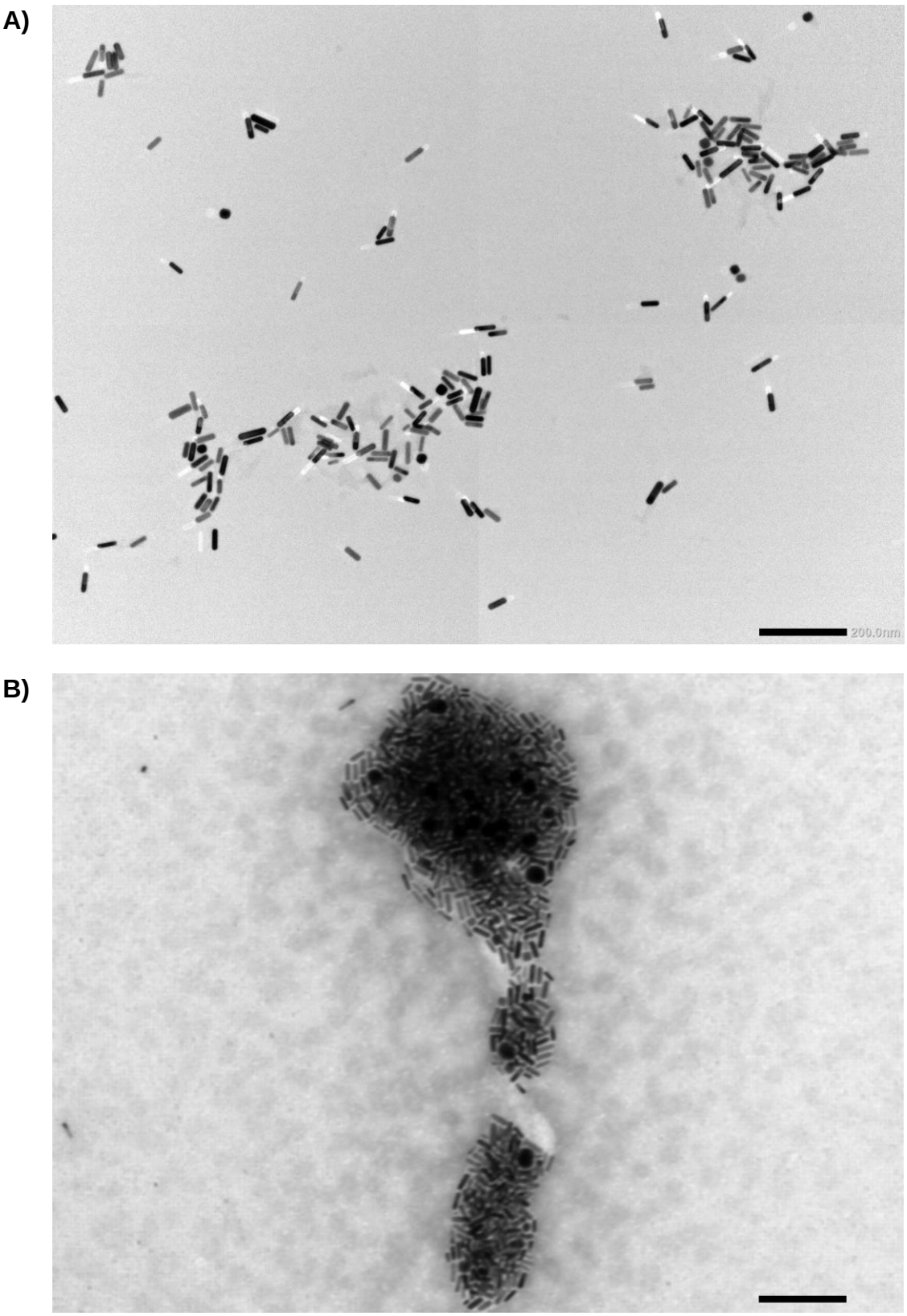


Figure S13. Low magnification images of (A) **GNR@3** in PBS and (B) **GNR@4** in PBS. Scale bar = 200 nm. 0.5% uranyl acetate staining was used.

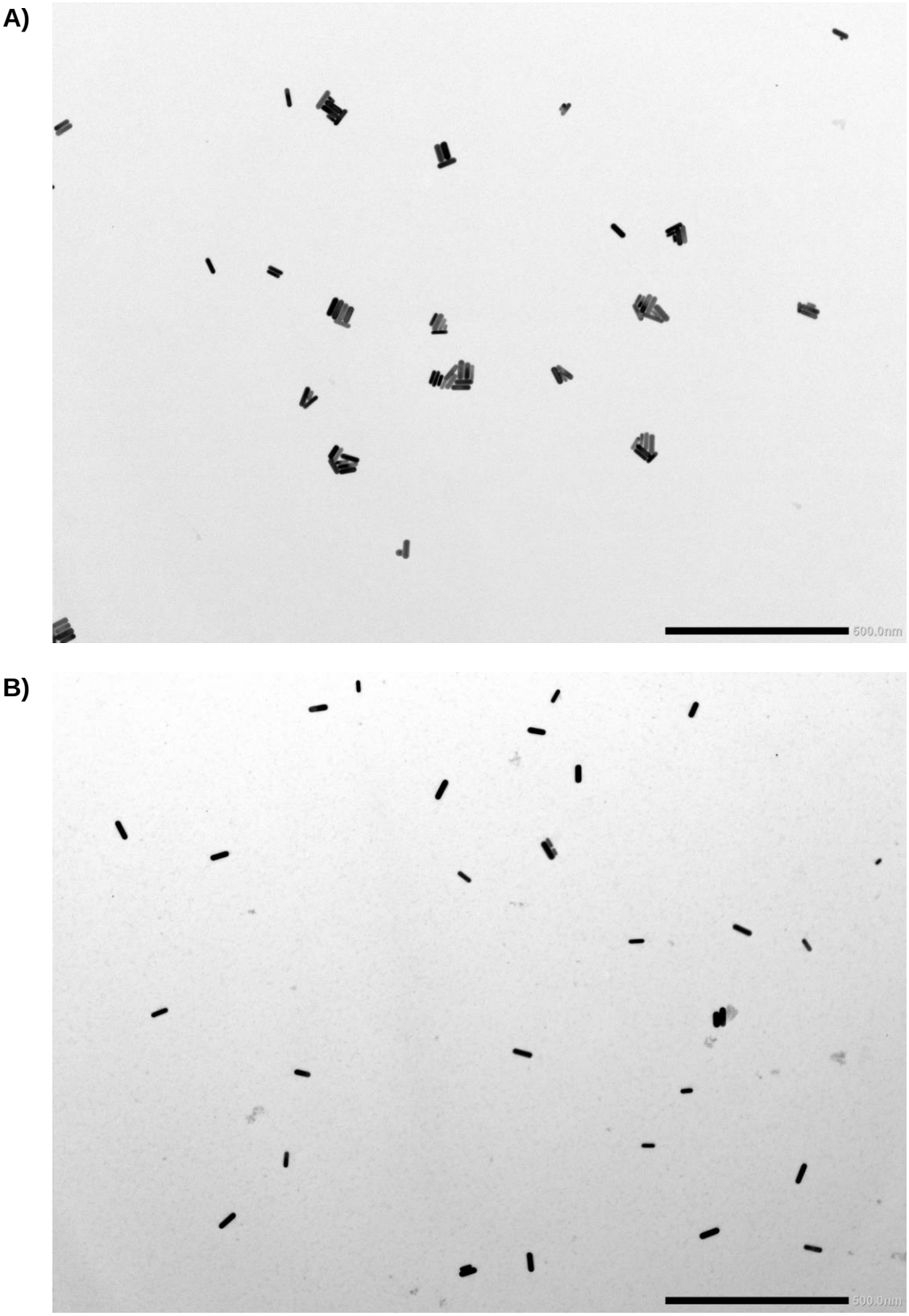


Figure S14. Low magnification images of (A) **GNR@PEG$_{750}$** in PBS and (B) **GNR@PEG$_{5000}$** in PBS. Scale bar = 500 nm. 0.5% uranyl acetate staining was used.

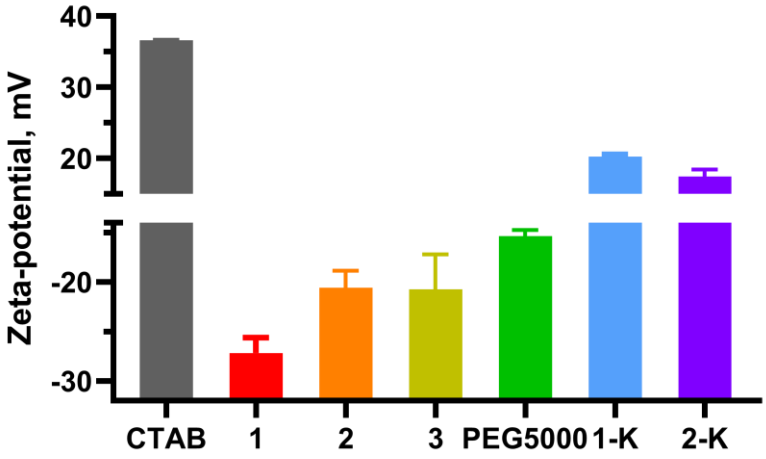


Figure S15. Zeta-potential values for GNRs with different protective coatings (**GNR@CTAB** – gray, **GNR@1** – red, **GNR@2** – orange, **GNR@3** – yellow, **GNR@PEG$_{5000}$** – green, **GNR@1-K** – blue, **GNR@2-K** – purple).

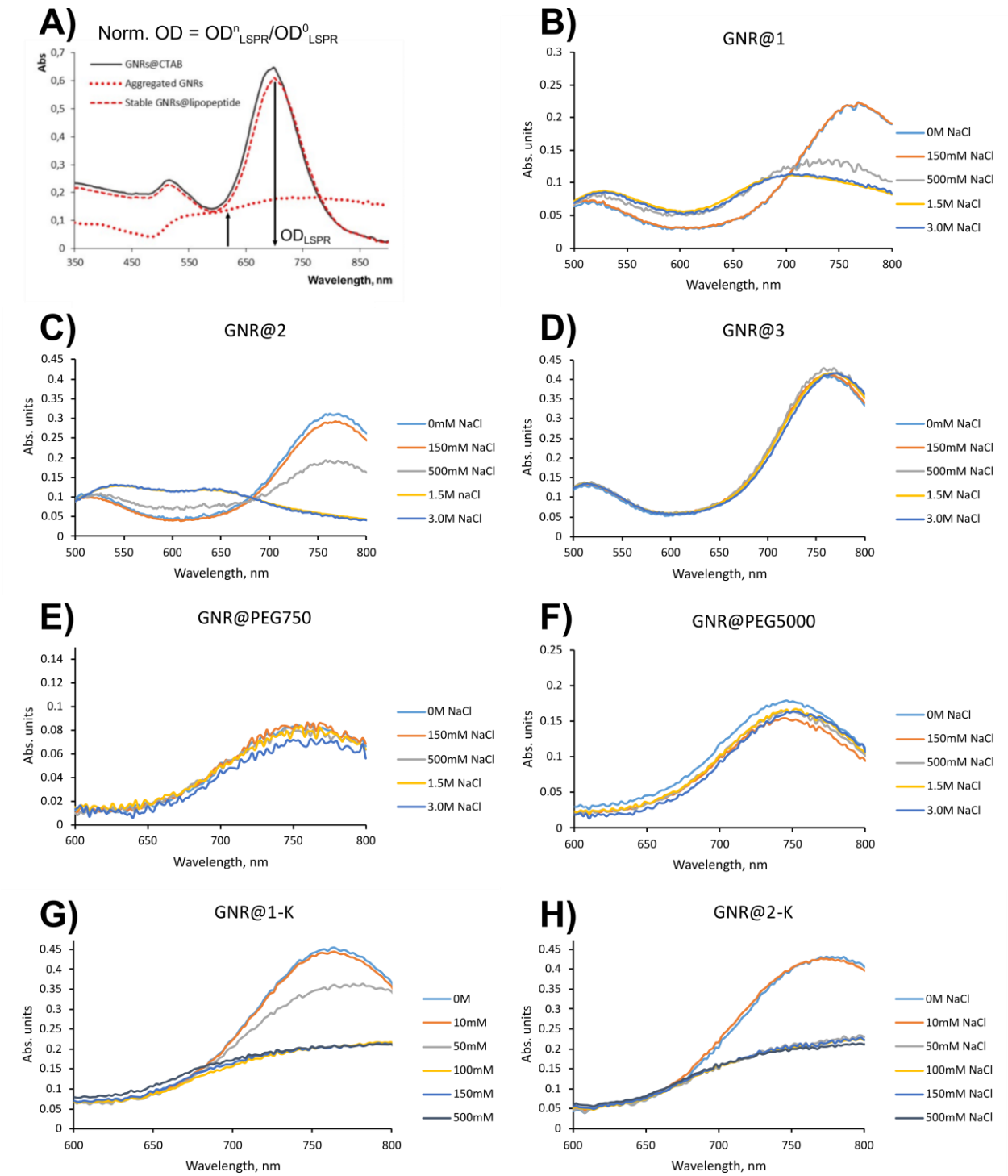
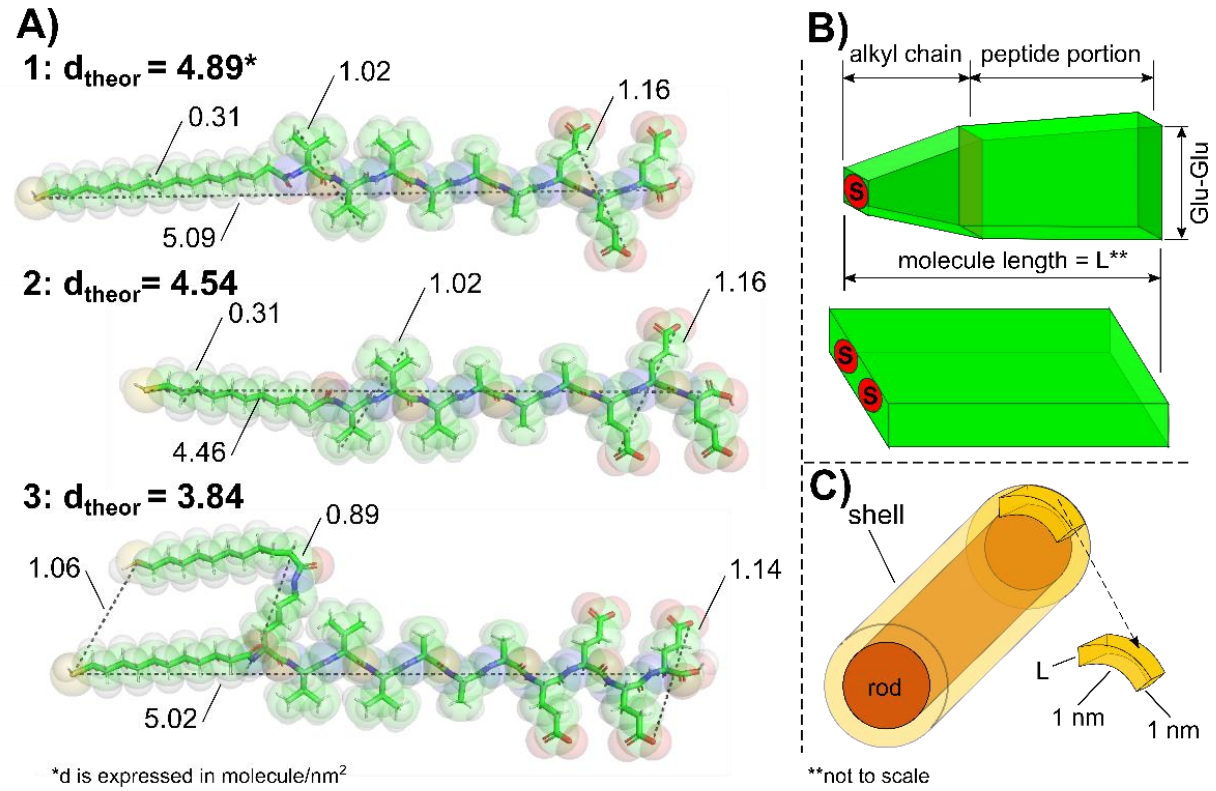


Figure S16. To obtain the normalized optical density (Norm. OD) as a measure of aggregation, the equation shown in (A) was used. UV-Vis spectra recorded during the NaCl assay for GNRs coated with (B) **1**; (C) **2**; (D) **3**; (E) **PEG$_{750}$**; (F) **PEG$_{5000}$**; (G) **1-K**; (H) **2-K**. Coated GNRs (originally dispersed in MilliQ water) were mixed with NaCl to yield varying final salt concentrations ranging from 150 mM to 3.0 M for negatively charged GNRs (B-F) and from 10 mM to 500 mM for positively charged GNRs (G,H). After mixing, samples were incubated for 15 min at room temperature. The sample with no salt added (0 M NaCl, light blue line) served as a reference to calculate Norm. OD (for results, see **Figure 3**). Measurements were carried out at room temperature.



Scheme S1. Simulation of theoretical coverage density of PAs on a cylindrical surface: (A) contour length and possible dimensions of PA molecules **1-3**; (B) schematics illustrating simplified geometries given to molecules **1,2** (top) and **3** (bottom); (C) a shell segment corresponding to 1 nm^2 of the gold surface occupied by 4.89 molecules of **1**, 4.54 molecules of **2**, or 3.84 molecules of **3**. The theoretical coverage density (d_{theor}) was expressed as the ratio between the shell volume and the volume of one molecule. Models were built using PyMol. Dimensions are given in nm.

The following equations (1-10) describe how the d_{theor} values in **Scheme S1** were calculated.

Molecules **1** and **2** were simplified into two adjacent right frustums: one describing the alkyl chain and the other the peptide segment (**Scheme S1**). The fully stretched molecules, as well as fully β-structured molecules, are flat and have a thickness corresponding to the alkyl chain (0.31 nm for all three molecules). Molecule volumes were calculated as follows:

$$V_{molecule} = V_I + V_{II}, \quad (1)$$

where $V_{molecule}$ is the volume of molecule **1** or **2** (dimensions shown in **Scheme S1**), V_I is the volume of the alkyl chain, and V_{II} is the volume of the peptide segment.

$$V_I = {}^1/_3 \cdot L_I \cdot \left(S_1 + S_2 + \sqrt{S_1 \cdot S_2}\right), \quad (2)$$

where L_I is the distance between the sulfur atom and the amide bond formed due to the coupling of the alkyl chain to the N-terminus of the peptide segment (L_I = 2.11 nm for molecule **1** and L_I = 1.49 nm for molecule **2**); where S_1 is the (square) area with the alkyl chain distance in the base (S_1 = (0.31 nm)^2 for both molecules); S_2 is the (rectangular) area with the Val-to-Val distance in the base (S_2 = (1.02 nm) × (0.31 nm) for both molecules),

$$V_{II} = {}^{1}/_{3} \cdot L_{II} \cdot \left(S_2 + S_3 + \sqrt{S_2 \cdot S_3}\right), \quad (3)$$

where L_{II} is the distance between the termini of the peptide segment (L_{II} = 2.98 nm for both molecules) and where S_3 is the (rectangular) area with the Glu-to-Glu distance in the base (S_3 = (1.16 nm) × (0.31 nm) for both molecules).

Molecule **3** was simplified in a different way: it was represented by a rectangular box with a base of $D_{alkyl\ chain}$ = 0.31 nm (the thickness of alkyl chain) by $D_{Glu-Glu}$ = 1.14 nm (Glu-to-Glu distance):

$$V_3 = L_{S-Cterm} \cdot D_{Glu-Glu} \cdot D_{alkyl\ chain}, \quad (4)$$

where $L_{S-Cterm}$ is the distance between the sulfur atom and the peptide C-terminus ($L_{S-Cterm}$ = 5.02 nm).

To calculate the shell volume V_{shell}, the volume of a gold rod segment V_{rod} and the volume of a coated rod segment $V_{rod+shell}$ was determined:

$$V_{rod} = \pi \cdot r_{rod}^2 \cdot h_{rod}, \quad (5)$$

where r_{rod} is the diameter of the rod (r_{rod} = 7.00 nm) and where h_{rod} is the rod length,

$$V_{rod+shell} = \pi \cdot (r_{rod} + L_{molecule})^2 \cdot h_{rod}, \quad (6)$$

where the length of the molecule $L_{molecule}$ is the distance between the sulfur atom and the C-terminus of the peptide segment (see **Scheme S1**). The shell volume is then:

$$V_{shell} = V_{rod+shell} - V_{rod}. \quad (7)$$

The lateral surface area of a rod is given by:

$$S_{lateral} = 2\pi \cdot r \cdot h_{rod}. \quad (8)$$

Next, we determined the number of molecules $N_{molecules}$ in a shell by dividing the shell volume by the volume of a single molecule:

$$N_{molecules} = {}^{V_{shell}}/_{V_{molecule}}. \quad (9)$$

Finally, the theoretical coverage density d_{theor} was calculated by dividing the number of molecules in a shell by the lateral surface area of a rod:

$$d_{theor} = {}^{N_{molecules}}/_{S_{lateral}} = {}^{L_{molecule}}/_{V_{molecule}} \cdot \left(1 + {}^{L_{molecule}}/_{2r_{rod}}\right). \quad (10)$$

The numerical values for the theoretical coverage density d_{theor} for the three molecules are presented in **Table S1**.

Table S1. Calculation of theoretical coverage densities for molecules **1-3** displayed on a cylindrical surface of 14 nm in diameter.

Molecule	$V_{molecule}$ (nm^3)	$L_{molecule}$ (nm)	d_{theor} (molecule/nm^2)
1	1.419	5.09	4.89
2	1.296	4.46	4.54
3	1.774	5.02	3.84

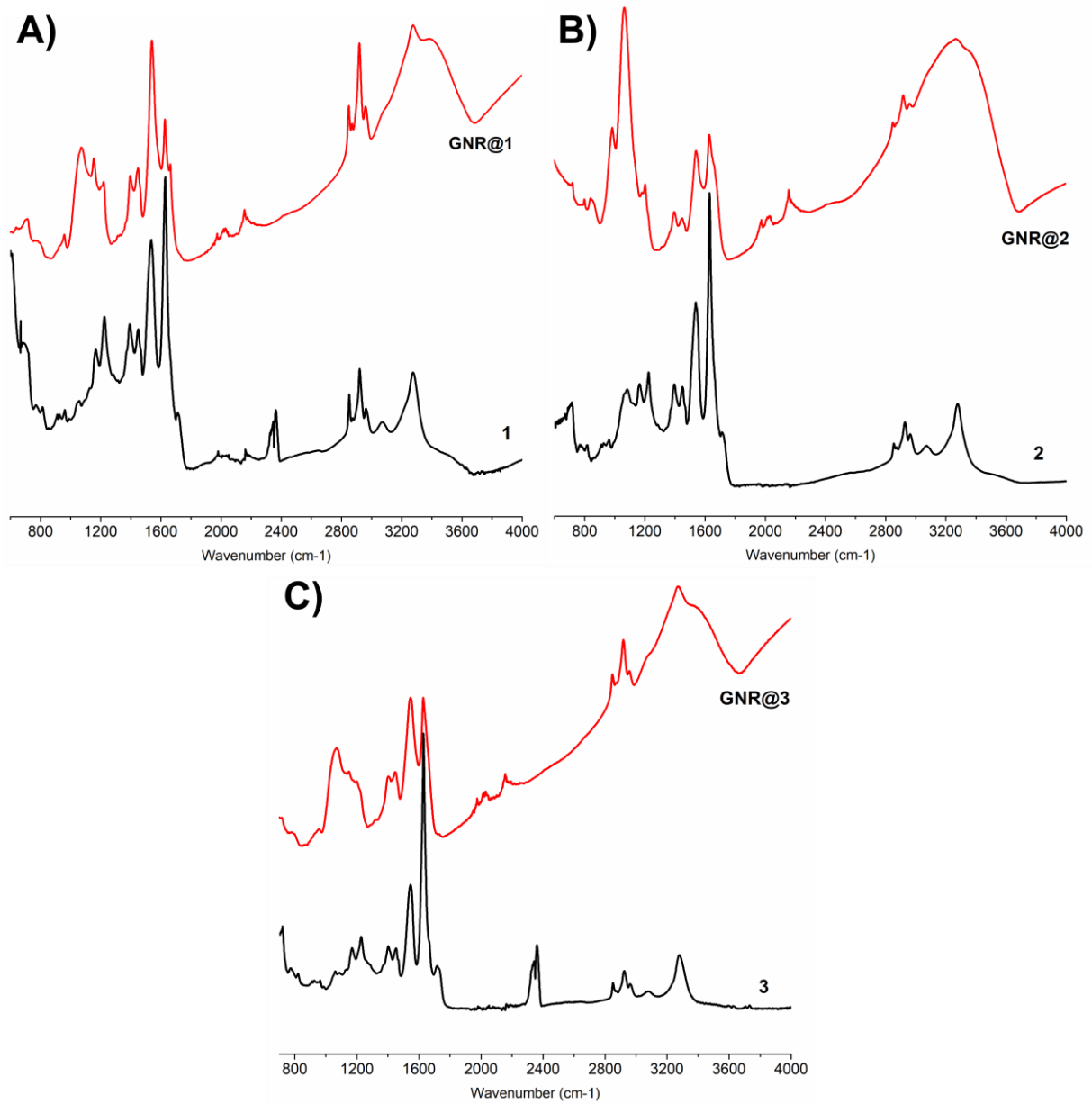


Figure S17. Full ATR-IR spectra of the coated GNRs and corresponding thiolated PAs: (A) **GNR@1**, (B) **GNR@2**, and (C) **GNR@3**. The PA traces were taken from our previous work.[1]

[1] Egorova, E. A.; van Rijt, M. M. J.; Sommerdijk, N.; Gooris, G. S.; Bouwstra, J. A.; Boyle, A. L.; Kros, A. *ACS Nano* **2020**, *14* (5), 5874–5886. https://doi.org/10.1021/acsnano.0c01021.

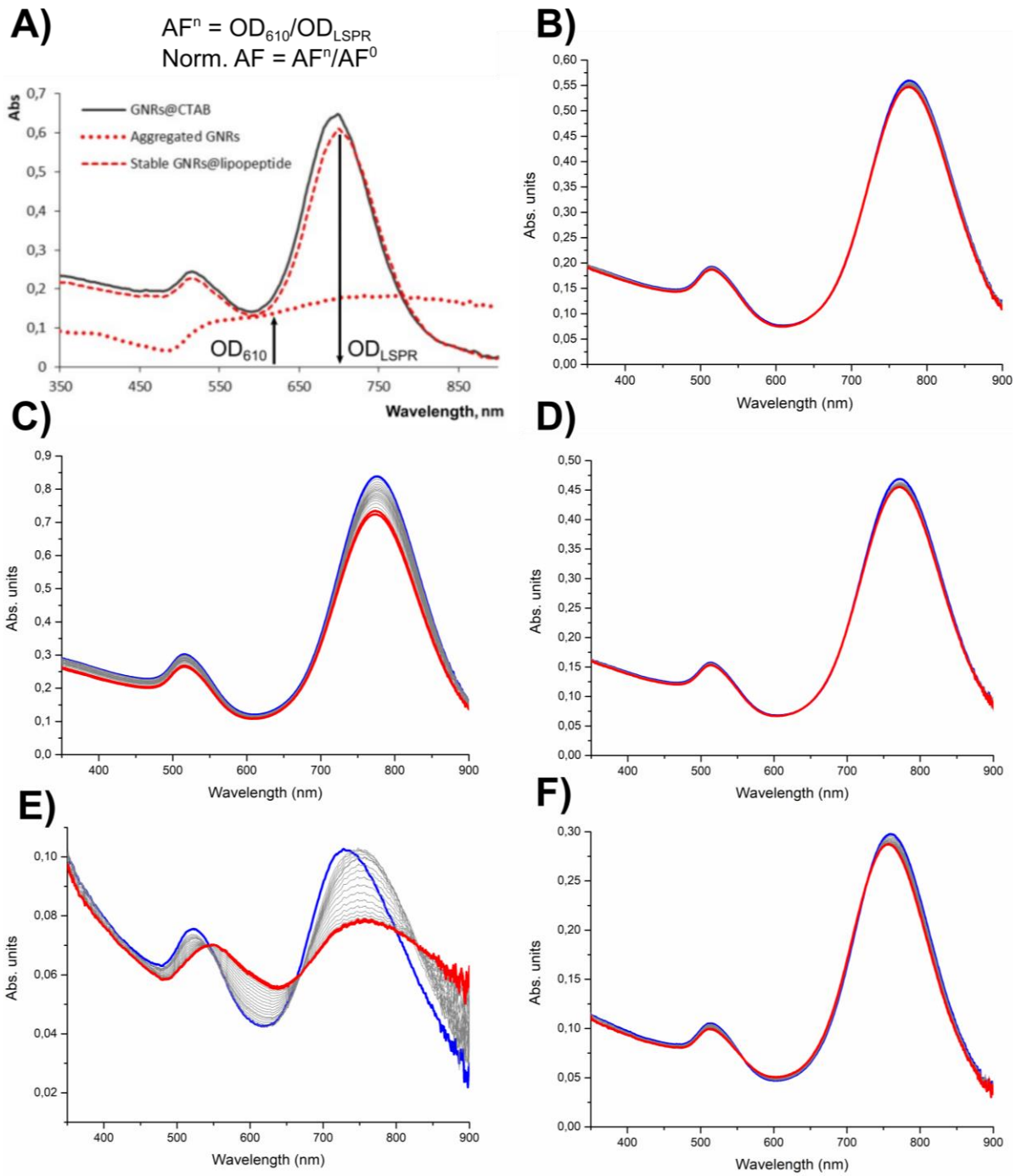


Figure S18. To obtain the normalized aggregation factor (Norm. AF) as a measure of aggregation, the equations shown in (A) were used. UV-Vis spectra recorded during the DTT competition assay to obtain the normalized aggregation factor (AF) for GNRs coated with: (B) **1**; (C) **2**; (D) **3**; (E) **PEG$_{750}$**; (F) **PEG$_{5000}$**. Coated GNRs (originally dispersed in MilliQ water) were mixed with DTT to yield a final DTT concentration of 1 M (blue line, t = 0 min), a UV-Vis spectrum was recorded every 5 mins (gray lines) over a course of 90 mins (red line, t = 90 mins). The sample at t = 0 min (blue line) served as a reference to calculate AF (for results, see **Figure 4D**). Measurements were carried out at room temperature.

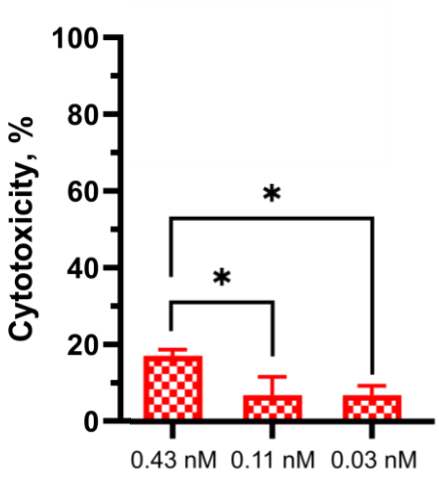


Figure S19. Cytotoxicity profile **GNR@3** in murine bone-marrow derived dendritic cells was evaluated with an LDH release assay according the manufacturer's manual. A negative control (PBS = 0% cytotoxicity) and positive control (assay lysis buffer = 100% cytotoxicity) were used to estimate the results. GraphPad Prism was used to perform statistical analysis. The error bars represent the mean and standard deviation. The sample was tested in three concentrations in triplicate.

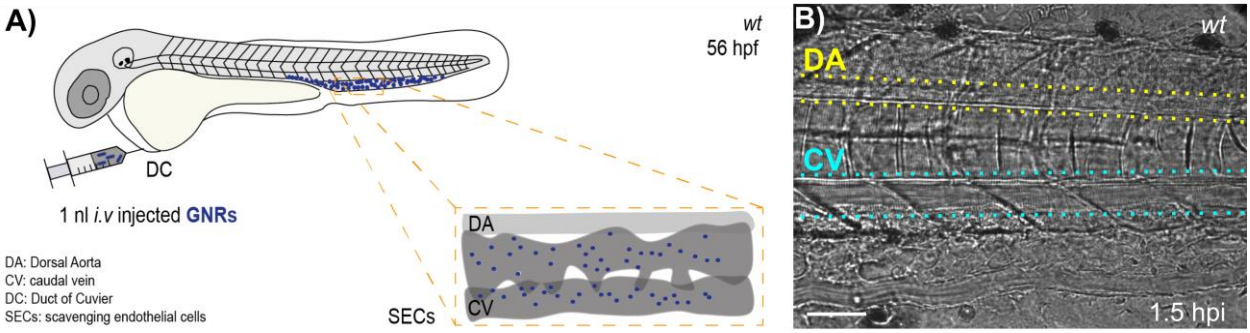


Figure S20. Transmission image of a zebrafish embryo injected with **GNR@3**. (A). Schematic showing the site of microinjection in a zebrafish embryo at 54-56 hours post fertilization (hpf) imaged with a two-photon microscope at 1.5 hours post injection (hpi). Volume of injection: 1 nL of coated GNRs (0.33 nM, PBS pH 7.2). In the zoomed-in box, the caudal region of the zebrafish embryo where the dorsal aorta (DA), the caudal vein (CV), and the scavenging endothelial cells (SECs) can be depicted. (B) A transmission image of a zebrafish embryo tail with the DA (in yellow) and CV (in cyan) indicated. Scale bar: 50 µm.