Supporting Information

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In-situ Investigations on Gold Nanoparticles Stabilization Mechanisms in Biological Environments Containing HSA

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Figure S1. The experiment setup is located at EMBL at the PETRA III storage ring (DESY, Hamburg).
Figure S2. SAXS data for NPs in the stock solution. The data show that NPs were monodispersed without aggregation.
Figure S3. DLS results for citrate NPs. a) DLS autocorrelation function and size distribution by intensity for 40 nm citrate NPs in DI water and NaCl solution.
b) DLS autocorrelation function and size distribution by intensity for 5 nm citrate NPs in DI water and NaCl solution. For both sizes of NPs, the DLS correlation function in NaCl solution decays slower and the corresponding mean hydrodynamic diameter increases. c) Zeta potential in water and NaCl solution for citrate NPs, for both NPs zeta potential decreased after adding to NaCl solution.
Figure S4. DLS results for PEGylated NPs. a) DLS autocorrelation function and size distribution by intensity for 40 nm PEG NPs in DI water and NaCl solution. DLS autocorrelation function in NaCl solution and DI water decays in a same manner and the corresponding mean hydrodynamic diameter does not change. b) DLS autocorrelation function and size distribution by intensity for 5 nm PEG NPs in DI water and NaCl solution. There is small changes in the DLS autocorrelation function and hydrodynamic diameter in NaCl solution compared to DI water. c) Zeta potential in water and NaCl solution for PEGylated NPs, for both NPs zeta potential decreased after adding to NaCl solution.
**Figure S5.** Ordered structure factor in SASfit software for, a) FCC, b) BCC. As it is shown, the BCC peak positions and their relative intensities match the positions of the peaks in SAXS curves for 5 nm PEG NPs in NaCl solution.
Table S1. Fitting parameters for 5 nm PEG NPs in different environments.

| Contribution 1 | $5\ \text{nm\_PEG\_Pr\ 4\ mg/ml}$ | $5\ \text{nm\_PEG\_Pr\ 1000}$ | $5\ \text{nm\_PEG\_Pr\ 100}$ | $5\ \text{nm\_PEG\_NaCl}$ | $5\ \text{nm\_PEG\_Water}$ |
|----------------|-----------------------------------|---------------------------------|--------------------------------|----------------------------|-------------------------------|
| $P(q)$: (Sphere) | $\eta$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ |
| Polydispersity (Gaussian) | $N$ | $3.60\times10^{-20}$ | $3.10\times10^{-20}$ | $2.08\times10^{-20}$ | $3.08\times10^{-20}$ |
| $\sigma$ | $0.5$ | $0.7$ | $0.4$ | $0.5$ | $0.4$ |
| $X_0$ | $2.6$ | $3.6$ | $2.6$ | $2.6$ | $2.6\pm0.1$ |
| $S(q)$: (Sticky Hard Sphere) | $R_{HS}$ | $3$ | $3$ | $3$ | $3$ |
| $\tau$ | $0.1$ | $0.1$ | $0.1$ | $0.1$ | $0.2$ |
| $\phi_p$ | $0.1$ | $0.1$ | $0.1$ | $0.1$ | $0.1$ |

| Contribution 2 (Ordered NPs) | $R$ | $\eta$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ | $1.13154\times10^{12}$ |
|-----------------------------|-----|-------|--------------------------|--------------------------|--------------------------|--------------------------|
| $P(q)$: (Sphere) | Polydispersity (Gaussian) | $N$ | $3.3231\times10^{-25}$ | $3.6\times10^{-25}$ | $4.79\times10^{-25}$ | $4.58\times10^{-20}$ |
| $\sigma$ | $0.04$ | $0.04$ | $0.04$ | $0.04$ |
| $X_0$ | $2.6$ | $2.6$ | $2.6$ | $2.6$ |
| $S(q)$: (BCC-iso-Gaussian) | $a$ | $24.5\pm0.1$ | $22.1\pm0.1$ | $23.4\pm0.1$ | $22.9\pm0.1$ |
| $\sigma_{\text{a}}$ | $0.1$ | $0.1$ | $0.1$ | $0.1$ |
| $\delta$ | $0.06$ | $0.06$ | $0.06$ | $0.06$ |
| $cL$ | $5.9$ | $5.9$ | $5.9$ | $5.9$ |
| $\text{max(hkl)}$ | $3$ | $3$ | $3$ | $3$ |

| Contribution 3 (Protein) | $R$ | $\eta$ | $2.25\times10^{10}$ | $2.25\times10^{10}$ |
|--------------------------|-----|-------|----------------------|----------------------|
| $P(q)$: (Sphere) | Polydispersity (Gaussian) | $N$ | $1.80892\times10^{-17}$ | $7.06\times10^{-17}$ |
| $\sigma$ | $0.5$ | $0.5$ |
| $X_0$ | $2.7\pm0.1$ | $2.7\pm0.1$ |

| Background | $C_0$ | | | $C_4$ | | | $\alpha$ | | |
Table S2. Fitting parameters for 5 nm_citrate NPs in different environments.

| Contribution 1 | S nm_Citrate_Pr 4 mg/ml | S nm_Citrate_Pr 1000 | S nm_Citrate_Pr 100 | S nm_Citrate_NaCl | S nm_Citrate_Water |
|----------------|-------------------------|----------------------|---------------------|------------------|-------------------|
| P(q): (Sphere) | R                       | 1.13154E+12          | 1.13154E+12         | 1.13154E+12      | 1.13154E+12       |
|                | eta                     | 1.10E-24             | 1.40E-24            | 8.93E-25         | 8.38E-25          |
|                | s                       | 0.4                  | 0.4                 | 0.4              | 0.4               |
|                | XO                      | 2.6                  | 2.6                 | 2.6              | 2.6               |

| Contribution 2 (Ordered NPs) |
|-----------------------------|
| P(q): (Sphere) |
| R                           |                           |
| eta                         |                           |
| N                           |                           |
| Polydispersity (Gaussian)   |                           |
| s                           |                           |
| XO                          |                           |

| Contribution 3 (Protein) |
|--------------------------|
| P(q): (Sphere) |
| R                       |                           |
| eta                     |                           |
| Polydispersity (Gaussian)|                           |
| s                       |                           |
| XO                      |                           |

| Background |
|------------|
| C0         | 0           |
| C4         | 25.7        | 118.4        |
| alpha      | 2.5         | 2.6          |
Table S3. Fitting parameters for 40 nm PEG NPs in different environments.

| Contribution 1 | R | distr. | distr. | distr. | distr. | distr. |
|----------------|---|--------|--------|--------|--------|--------|
| P(q): (Sphere) | eta | 1.13154E+12 | 1.13154E+12 | 1.13154E+12 | 1.13154E+12 | 1.13154E+12 |
| Polydispersity (Gaussian) | N | 2.79E-27 | 2.96E-27 | 2.40E-27 | 1.86E-27 | 2.38E-27 |
| | s | 2.5 | 3.5 | 3.5 | 3.5 | 3.4 |
| | xo | 18.7 ± 0.1 | 18.7 ± 0.1 | 18.7 ± 0.1 | 18.7 ± 0.1 | 18.7 ± 0.1 |
| S(q): (Sticky Hard Sphere) | RHS | | | | | |
| | law | | | | | |
| | lp | | | | | |

| Contribution 2 (Ordered NPs) | R | distr. | distr. | distr. | distr. | distr. |
|-----------------------------|---|--------|--------|--------|--------|--------|
| P(q): (Sphere) | eta | | | | | |
| Polydispersity (Gaussian) | N | | | | | |
| | s | | | | | |
| | xo | | | | | |

| Contribution 3 (Protein) | R | distr. | distr. | distr. | distr. | distr. |
|--------------------------|---|--------|--------|--------|--------|--------|
| P(q): (Sphere) | eta | 2.25E+10 | | | | |
| Polydispersity (Gaussian) | N | 7.58E-22 | | | | |
| | s | 0.5 | | | | |
| | xo | 2.7 ± 0.1 | | | | |

| Background | C0 | | | | | |
| | C4 | | | | | |
| | alpha | | | | | |
**Table S4. Fitting parameters for 40 nm PEG NPs in different environments.**

| Contribution 1             | 40 nm_Citrate_Pr 4 mg/ml | 40 nm_Citrate_Pr 1000 | 40 nm_Citrate_Pr 100 | 40 nm_Citrate_NaCl | 40 nm_Citrate_Water |
|-----------------------------|--------------------------|-----------------------|----------------------|-------------------|---------------------|
| P(qi): (Sphere)            |                          |                       |                      |                   |                     |
| Polydispersity (Gaussian)  |                          |                       |                      |                   |                     |
| a                          | 1.13154E+12              | 1.13154E+12           | 1.13154E+12          | 1.13154E+12       | 1.13154E+12         |
| N                          | 2.79E-27                 | 5.63E-27              | 1.40E-27             | 1.43E-27          | 2.49E-27            |
| s                          | 2.4                      | 3.1                   | 1.4                  | 1.4               | 3.3                 |
| X0                         | 18.7                     | 18.7                  | 18.7                 | 18.7              | 18.7 ± 0.1          |
| S(qi): (Sticky Hard Sphere)|                          |                       |                      |                   |                     |
| RH5                        | 20.3                     | 21.3                  | 18.9                 |                   |                     |
| tau                        | 0.1055 ± 0.0002          | 0.1002 ± 0.0002       | 0.0973 ± 0.0002      |                   |                     |
| fp                         | 0.1                      | 0.1                   | 0.1                  |                   |                     |

| Contribution 2 (Ordered NPs) |
|------------------------------|
| P(qi): (Sphere)              |
| Polydispersity (Gaussian)    |
| a                            |
| sigma_a                      |
| delta                        |
| cl                           |
| clmax(hkl)                   |

| Contribution 3 (Protein)    |
|------------------------------|
| P(qi): (Sphere)              |
| Polydispersity (Gaussian)    |
| a                            |
| N                            |
| s                            |
| X0                           |

| Background                  |
|------------------------------|
| C0                           |
| C4                           |
| alpha                        |