Electronic Supplementary Material (ESI) for RSC Advances

This journal is © The Royal Society of Chemistry 2013

*Supplementary Information*

# Design, synthesis, characterisation and *in vitro* studies of hydrophilic, colloidally-stable, $^{64}$Cu(II)-labelled, ultra-small iron oxide nanoparticles in a range of human cell lines

**Karina Pombo-García,[a] Kristof Zarschler,[a] Jose A. Barreto,[b] Julia Hesse,[a] Leone Spiccia,[b,*] Bim Graham,[c,*] Holger Stephan[a,*]**

[a] Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstrasse 400, 01328 Dresden, Germany. Fax: +49 351 260 3232. Tel: +49 351 260 3091. E-mail: h.stephan@hzdr.de

[b] School of Chemistry, Monash University Clayton, VIC, 3800, Australia. Fax: +61 (0)3 9905 4597. Tel: +61 (0)3 9905 4526. E-mail: leone.spiccia@monash.edu

[c] Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, 3052, Australia. Fax: +61 (0)3 9903 9582. Tel: +61 (0)3 9903 9706. E-mail: bim.graham@monash.edu

# 1. Characterization of OPA-USPIONs

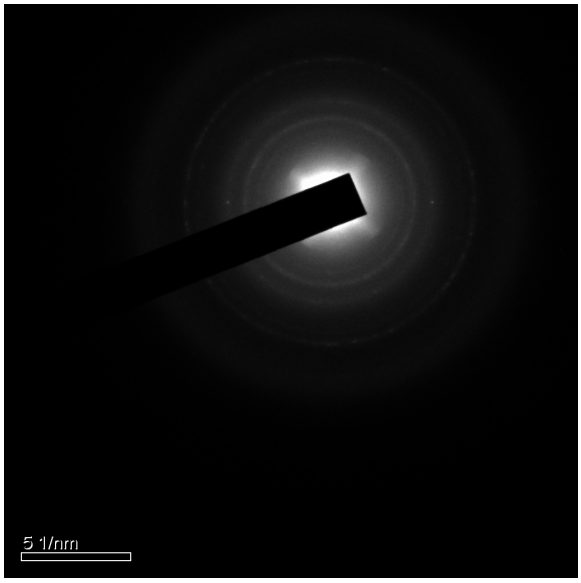


**Figure S1**. Selected area electron diffraction (SAED) pattern of the USPIONs, revealing the high crystallinity of the magnetite core ($Fe_3O_4$).

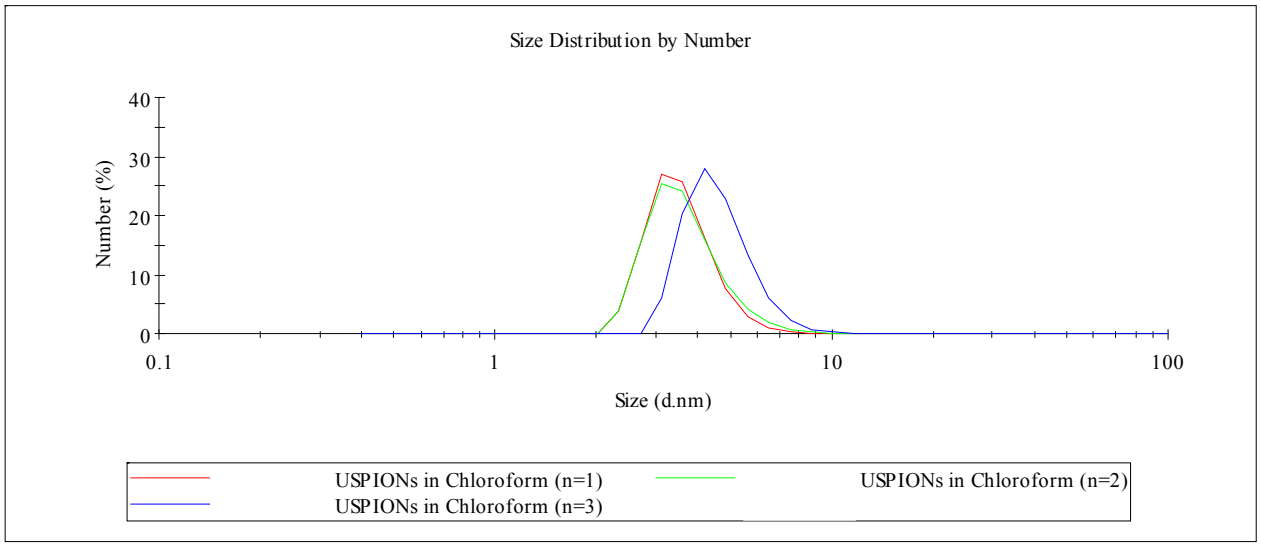


**Figure S2**. Size distribution of USPIONs in chloroform measured in triplicate by DLS confirming the stability of the nanoparticles in organic solvent.

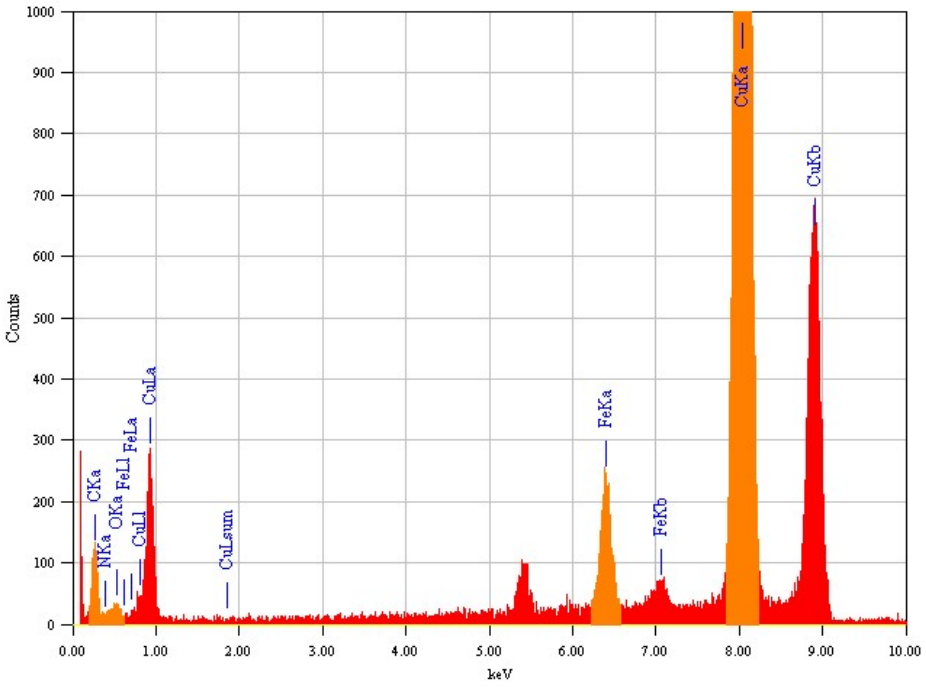


**Figure S3**. Energy-dispersive X-ray spectroscopy (EDS) analysis of OPA-USPIONs, confirming the presence of iron in the core of the USPIONs and nitrogen from the OPA coating. High levels of copper belong to the EDS column itself.

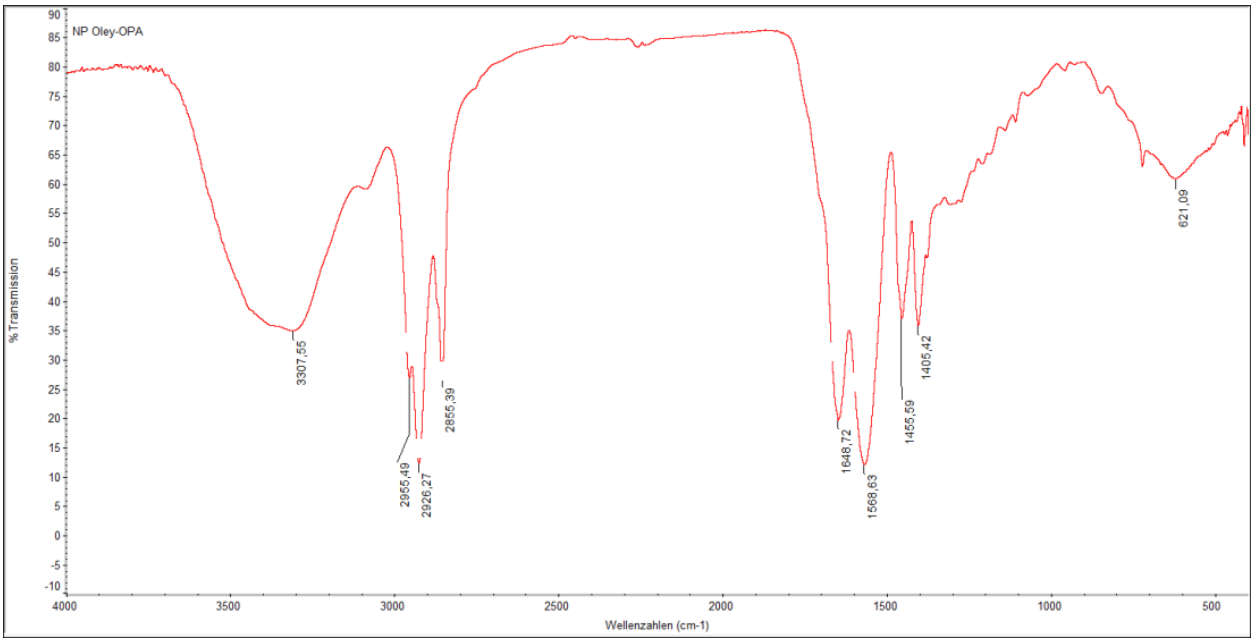


**Figure S4.** FTIR spectrum of OPA-USPIONs.

## 2. Colloidal stability of OPA-USPIONS

The colloidal stability of the OPA-USPIONs was further examined by incubating 50 μg $mL^{-1}$ solutions of the nanoparticles for 1 h in the following media: (i) PBS, (ii) Dulbecco's PBS, (iii) Dulbecco's modified Eagle's medium (DMEM), (iv) Dulbecco's modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS) (Table S1; Figures S5–S7), and (v) salt solutions of differing concentrations (0–154 mM NaCl) (Fig. S8). The results indicate little or no aggregation under the conditions trialled, which reflects the high degree of stabilisation afforded by the negatively-charged OPA polymer coating.[S1,S2] The hydrodynamic size measurements for the nanoparticles incubated in DMEM + 10% FBS should be treated with caution as adsorbed protein may have influenced the light scattering properties of the core particles.[S3] OPA-USPIONs were also incubated in total rat plasma and complete serum, however the polydispersity of the prepared samples (arising from the large amount of protein present) precluded the collection of reliable DLS data.

**Table S1.** Average hydrodynamic diameters ($D_h$) of OPA-USPIONs after 1 h of incubation in different media.

| Medium | PBS | Dulbecco's PBS | DMEM | DMEM |
|---|---|---|---|---|
| Additives | - | $CaCl_2$, $MgCl_2$ | - | 10% FBS |
| $D_h$ | 9.6 ± 1.3 | 14.7 ± 5.7 | 18.9 ± 6.4 | 43.2 ± 5 |

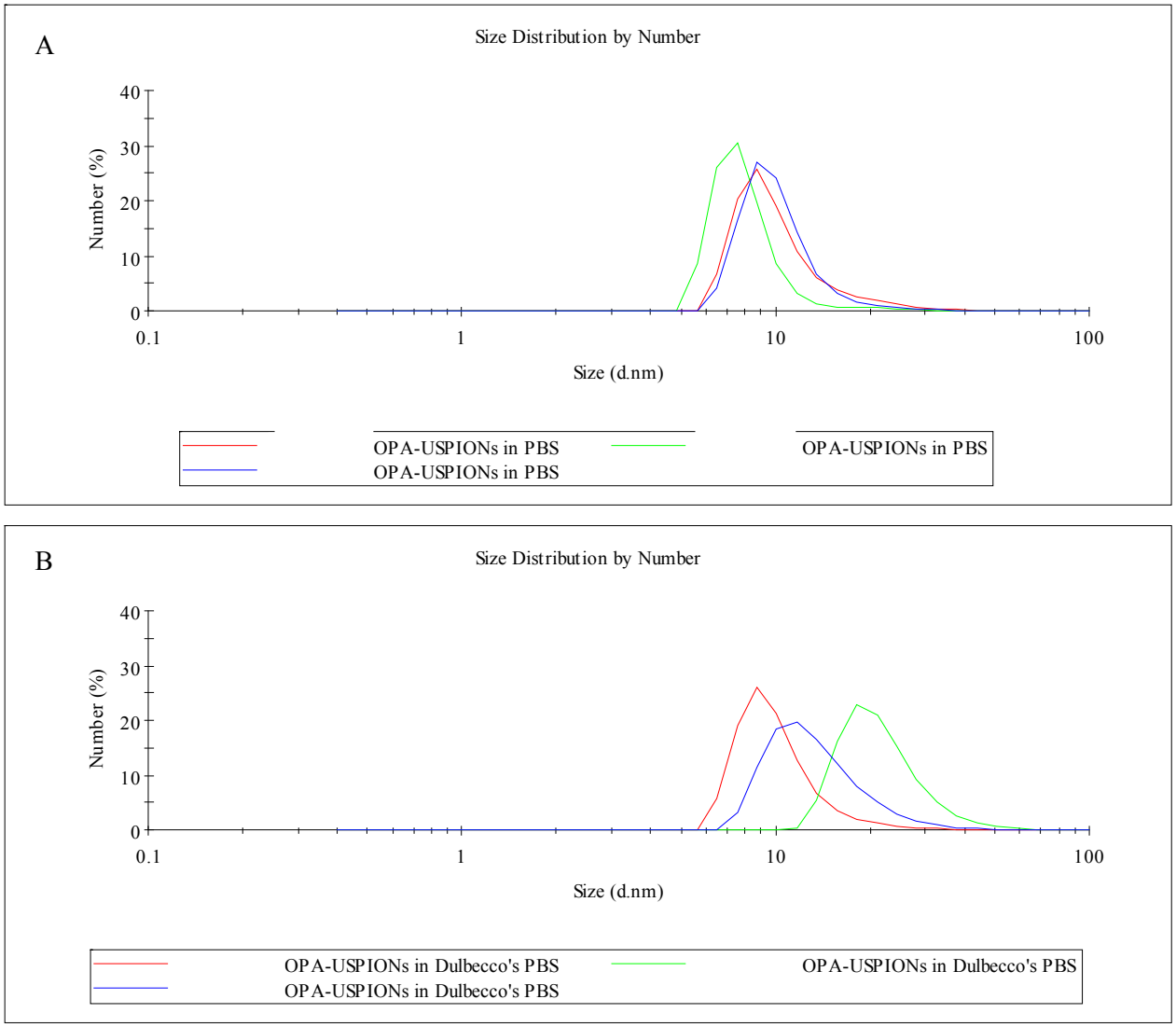


**Figure S5.** Number size distributions measured by DLS of OPA-USPIONs in PBS (A) and Dulbecco's PBS (B). Measurements were performed within one hour of solution preparation. Three replicates are shown.

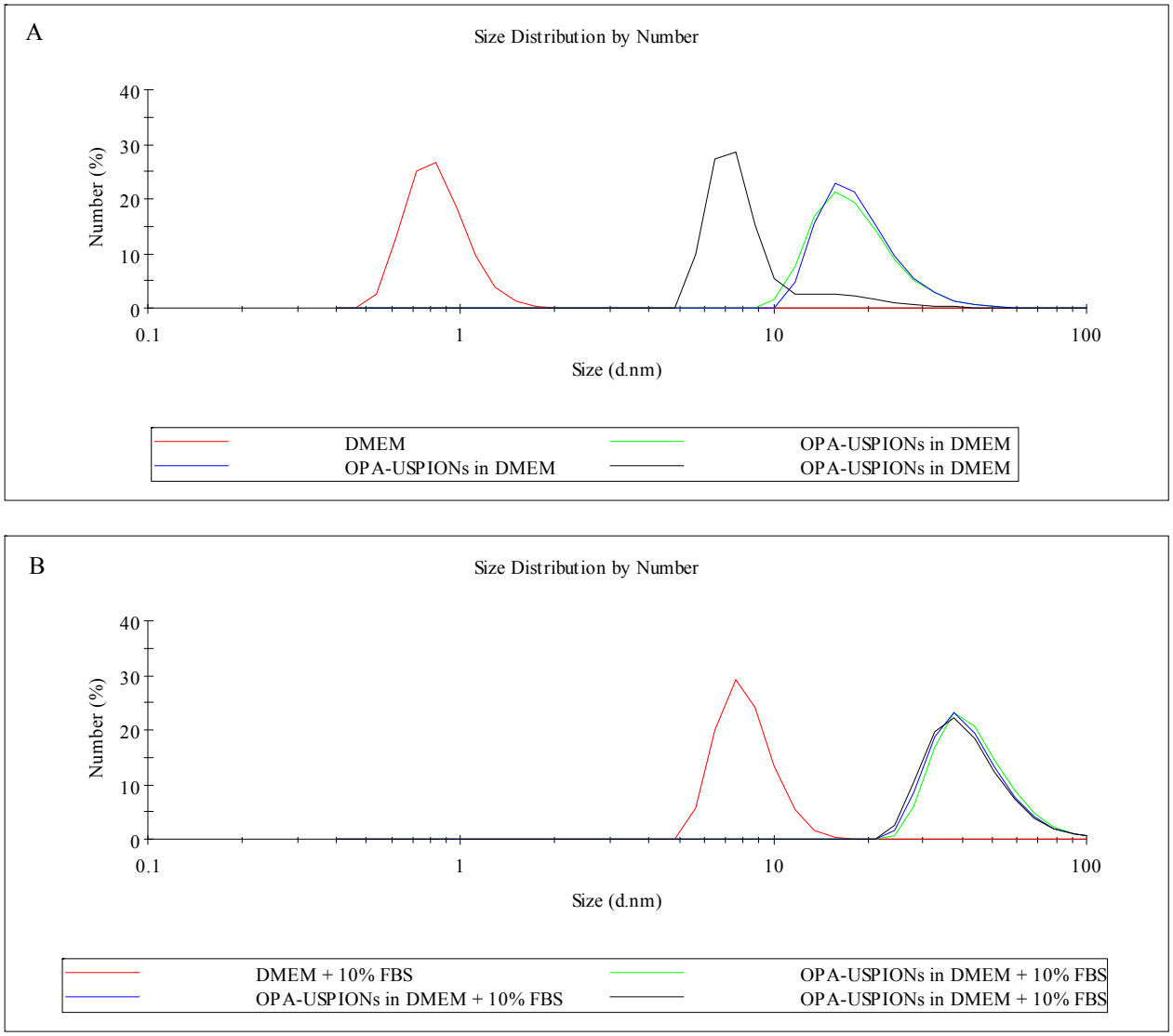


**Figure S6.** Number size distributions measured by DLS of OPA-USPIONs in DMEM (A) and DMEM + 10% FBS (B). Measurements were performed within one hour of solution preparation. The peak centred at *ca.* 1 nm in panel A is due to DMEM itself, whilst the peaks at *ca.* 8 nm in panel B are due to the proteins present in FBS.

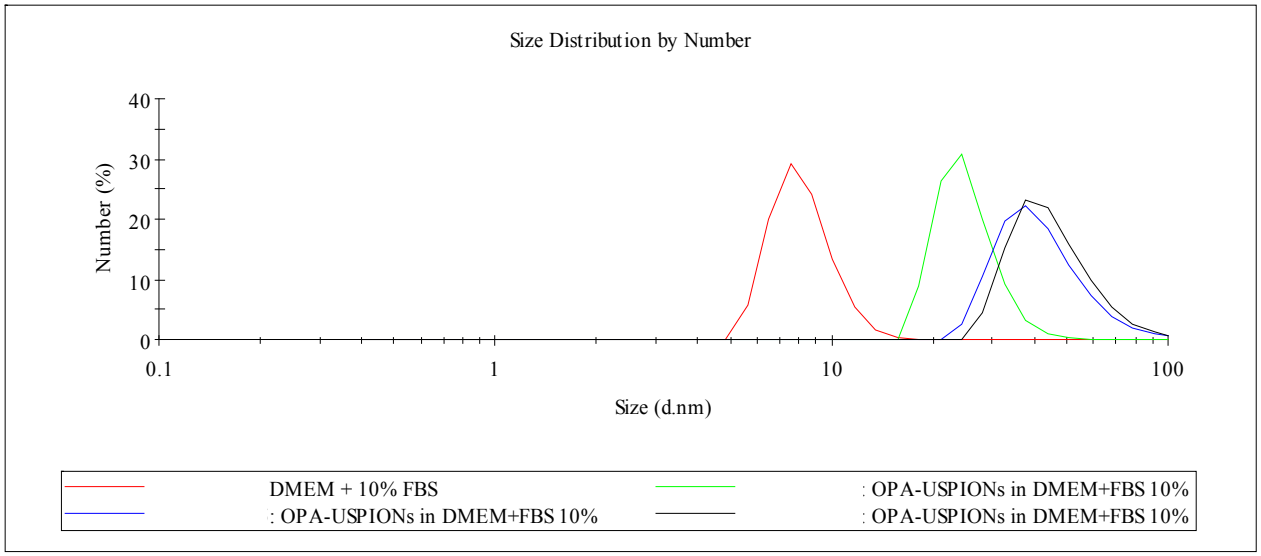


**Figure S7.** Number size distributions measured by DLS of OPA-USPIONs in DMEM + 10% FBS after three hours of incubation. The signal at *ca.* 8 nm is due to the proteins present in FBS.

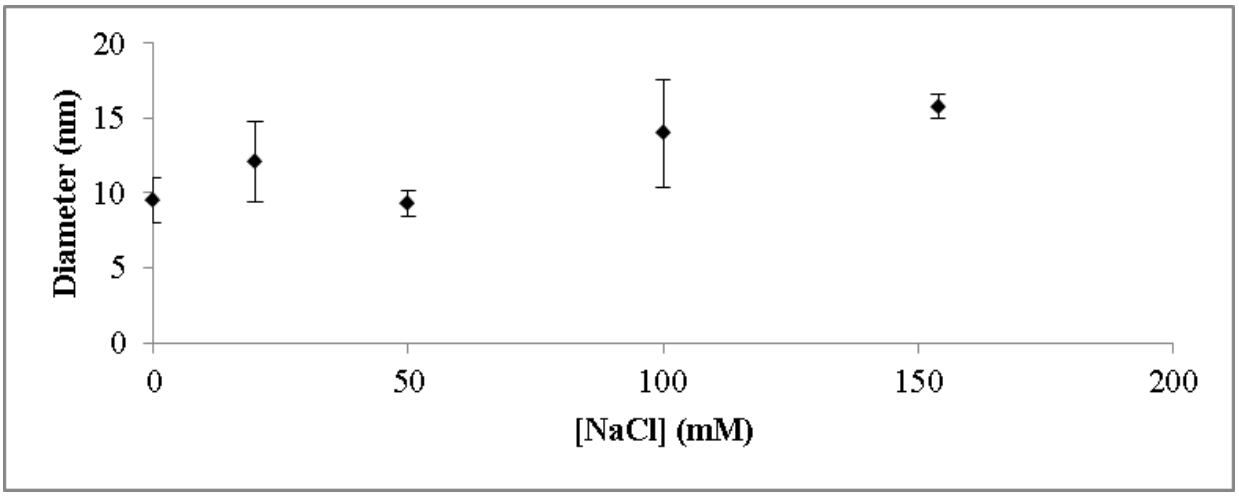


**Figure. S8**. Measured hydrodynamic diameter of OPA-USPIONs in aqueous salt (NaCl) solutions of differing concentration at 25°C.

## References

S1. M. Pavlin and V. B. Bregar, *Dig. J. Nanomater. Bios.*, 2012, **7**, 1389-1400.

S2. D. Hühn, K. Kantner, C. Geidel, S. Brandholt, I. De Cock, S. J. H. Soenen, P. Rivera-Gil, J.-M. Montenegro, K. Braeckmans, K. Müllen, G. U. Nienhaus, M. Klapper and W. J. Parak, *ACS Nano*, 2013, **7**, 3253-3263.

S3. Y. Park, R. D. Whitaker, R. J. Nap, J. L. Paulsen, V. Mathiyazhagan, L. H. Doerrer, Y.-Q. Song, M. D. Hürlimann, I. Szleifer and J. Y. Wong, *Langmuir*, 2012, **28**, 6246-6255.