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Supporting Information

Intracellular Delivery of Antisense Peptide Nucleic Acid by Fluorescent Mesoporous Silica Nanoparticles

Xing Ma,^{†,‡,§} Gitali Devi,^{†,§} Qiuyu Qu,[†] Desiree-Faye Kaixin Toh,[†] Gang Chen,^{†} Yanli Zhao^{†,‡*}*

[†]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, 637371, Singapore.

E-mail: zhaoyanli@ntu.edu.sg, rnachen@ntu.edu.sg

[‡]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore.

[§]These authors contributed equally to this work.

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Figure S1. (a) XRD patterns and (b) N₂ adsorption/desorption isotherms of MSNP(FITC)-SH, MSNP(FITC)-NH₂ and solid SiO₂-SH.

Figure S2. SEM image of MSNP(FITC)-SS-NH₂.

Figure S3. FT-IR spectra of various NSMPs functionalized without the disulfide linkage. (1) MSNP(FITC)-NH₂, (2) MSNP(FITC)-COOH, and (3) MSNP(FITC)-PNA(Cy5).

Figure S4. Comparison of TEM images between (a) MSNP(FITC)-SS-NH₂ and (b) solid SiO₂-SS-NH₂.

Figure S5. (a) Fluorescence intensities of MeOH solutions containing various amount of (A) MSNP(FITC)-SS-NH₂ and (B) solid SiO₂-SS-NH₂ treated with 20 μg fluorescamine for 30min; (b) Surface functional groups quantified by the fluorescence intensities at 475nm according to the standard curve in Figure S6. The surface functional groups of both MSNP(FITC)-SS-NH₂ and solid SiO₂-SS-NH₂ were quantified by using fluorescamine. Methanol solutions with known APTES concentrations were utilized as standard to quantify the primary amine concentrations (Figure S6), and thus the density of -SS-NH₂ group was obtained.

Figure S6. Standard curve of the primary amine concentrations quantified by fluorescamine. Methanol solutions containing APTES with known concentrations were used for the standard curve measurement.

Figure S7. HPLC profiles for PNAs synthesized. In panels (a), (c) and (d), the data were taken for the HPLC-purified PNAs. In panel (b), the HPLC profile was for the crude conjugation products of PNA and Cy5 dye. A clear peak separation was achieved, and the PNA(Cy5) peak was collected and used in this study.

Figure S8. MALDI-TOF MS for PNAs synthesized.

Figure S9. CLSM images of HeLa cells treated with MSNP(FITC)-PNA(Cy5) ($50 \mu\text{g mL}^{-1}$) for 24 h. (a) FITC channel, (b) Cy5 channel, (c) overlay of FITC, Cy5 and DAPI, and (d) enlarged area of the dashed box in (c). After 24h incubation, the red fluorescence still largely overlapped with green fluorescence from MSNP(FITC), indicating no PNA(Cy5) release from MSNP(FITC)-PNA(Cy5).

Figure S10. CLSM images of HeLa cells treated with free PNA(Cy5) (1 μ M) for 24h. (a) DAPI channel, (b) Cy5 channel, and (c) overlay of DAPI and Cy5. Red fluorescence of Cy5 was barely observed inside HeLa cells, suggesting that PNA(Cy5) cannot be taken up by cells without the help of the nanoparticle carrier.

Figure S11. Relative Bcl-2 protein expression levels (top) and corresponding Western blot data (bottom) for HeLa cells treated with (a) MSNP(FITC)-SS-PNA(-), (b) MSNP(FITC)-PNA(Bcl-2), (c) free PNA(Bcl-2), and (d) MSNP(FITC)-SS-PNA(Bcl-2-mm) at various concentrations. HeLa cells without any treatment were used as the control.

Figure S12. MTT cytotoxicity assay indicating low cytotoxicity of the nanoparticles under low to moderate concentrations. No obvious cytotoxicity was found for free PNA, consistent with the poor uptake of free PNA (Figure S10). MSNP(FITC)-SS-COOH and MSNP(FITC)-SS-PNA(Bcl-2) exhibited similar low cytotoxicity even at high concentrations, which would not influence the protein expression assay by Western blot. The concentrations of free PNA used here (ranging from 1.7 nM to 2 μM) were equivalent to the PNA amounts on MSNP(FITC)-SS-PNA(Bcl-2).

Table S1. BET surface areas and pore volumes of MSNP(FITC)-SH, MSNP(FITC)-NH₂ and solid SiO₂-SH.

Samples	BET Surface Area (m ² g ⁻¹)	Pore Volume (cm ³ g ⁻¹)
MSNP(FITC)-SH	790.06	0.585
MSNP(FITC)-NH ₂	797.38	0.541
Solid SiO ₂ -SH	34.58	0.28

Table S2. Zeta potential measurements of various MSNPs ($20 \mu\text{g mL}^{-1}$) in distilled H_2O .

Samples	ζ -Potential (mV)
MSNP(FITC)-SH	-33.6 ± 4.74
MSNP(FITC)-SS-NH ₂	32.5 ± 4.07
MSNP(FITC)-SS-COOH	-42.2 ± 5.36
MSNP(FITC)-SS-PNA(Cy5)	-9.10 ± 3.68
MSNP(FITC)-NH ₂	31.6 ± 5.12
MSNP(FITC)-COOH	-39.6 ± 3.79
MSNP(FITC)-PNA(Cy5)	-10.2 ± 3.22
Solid SiO ₂ -SH	-27.5 ± 5.74
Solid SiO ₂ -SS-NH ₂	20.3 ± 5.21