

## Supporting Information

# Enhanced Biofilm Penetration for Microbial Control by Polyvalent Phages Conjugated with Magnetic Colloidal Nanoparticle Clusters (CNCs)

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**Synthesis and modification of magnetic CNCs.** All reagents used were analytical grade and available commercially. The DI water was obtained from a Milli-Q synthesis system (Millipore, Billerica, MA). The  $\text{Fe}_3\text{O}_4$  CNCs were synthesized by a solvothermal reaction.<sup>1</sup> Sodium acetate (4.32 g) and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (1.72 g) were dissolved in ethylene glycol (60 mL) under vigorous magnetic stirring. The obtained homogeneous yellow solution was transferred to a 100 mL Teflon-lined stainless-steel autoclave. After 8 h heating at 200°C, the products were washed with ethanol and DI water each for three times.

The  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  CNCs were prepared by a versatile solution sol-gel method.<sup>2</sup> An aqueous dispersion of the  $\text{Fe}_3\text{O}_4$  particles (17.5 mL, 0.02 g/mL) was added into a pure ethanol (70 mL) and concentrated ammonia solution (1.25 mL, 28 wt %), followed by ultrasonic dispersion for 15 min at room temperature. Afterward, 1.0 mL of tetraethyl orthosilicate (TEOS) was added dropwise, and the reaction proceeded under continuous mechanical stirring overnight. The resultant core-shell  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  CNCs were separated and washed with ethanol six times.

Amino groups were introduced onto the surface of  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  CNCs by conventional sol-gel reaction with APTES as a modifying agent.<sup>3</sup>  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  (20 mg) CNCs were dispersed in a mixed solvent of ethanol/DI water (25 mL, v/v=70/30).  $\text{NH}_4\text{OH}$  (50  $\mu\text{L}$ ) and (3-aminopropyl) triethoxysilane (APTES, 60  $\mu\text{L}$ ) were added under vigorous stirring. After 24 hours of reaction, the products ( $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$  core-shell CNCs) were washed with ethanol and DI water, respectively, three times.

Carboxyl groups were then functionalized by chemical reaction between the amino groups and succinic anhydride. The  $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$  core-shell CNCs obtained in the previous step were dispersed in N,N-dimethylformamide, followed by adding succinic anhydride (15 mg) to functionalize the CNC with adequate carboxyl groups. The reaction took place under vigorous

stirring overnight and the products ( $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$  core-shell CNCs) were washed.

Chitosan-coated  $\text{Fe}_3\text{O}_4$  (CS- $\text{Fe}_3\text{O}_4$ ) CNCs were synthesized via a versatile solvothermal reaction to obtain magnetic CNCs with porosity and high protonation of amino group.<sup>4</sup> Typically,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (1.50 g), NaOAc (3.6 g), chitosan (0.5 g), and PVP (1.0 g) were added into ethylene glycol (70 mL) to obtain a transparent solution under vigorous stirring. This mixture was then transferred to a 100 mL Teflon-lined stainless-steel autoclave for heating at 200°C for 8 h. The products were collected and washed exhaustively to remove the free chitosan.

**Sample calculations.** Phage immobilized onto CNCs (PFU/mg) = [Initial phage amount (PFU/mL) – Phage inactivated by EDC and NHS (PFU/mL) – Phage inactivated by CNCs (PFU/mL) – Total residual free phages amount (PFU/mL)] ÷ Total CNCs (mg/mL). The phages amounts were quantified in triplicate by double-layer plaque assay using mixed bacteria ( $\text{OD}_{600} = 0.5$ , equal ratio of *P. aeruginosa* and *E. coli*).

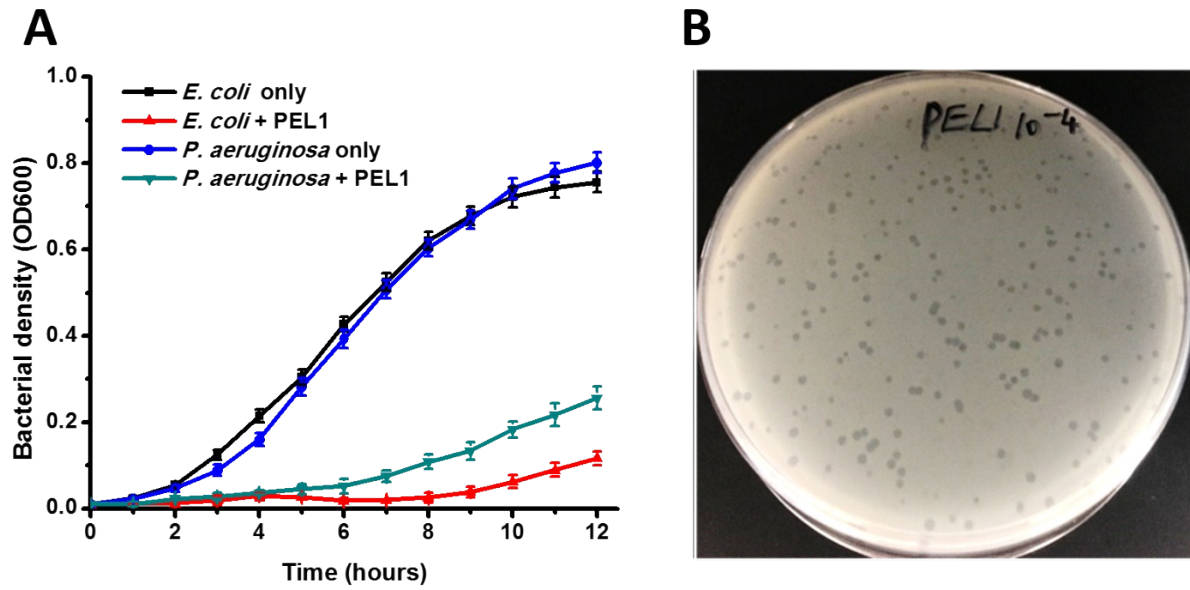


Fig. S1. **Polyvalent phage PEL1 infected both *E. coli* C3000 and *P. aeruginosa* PA01.** Phage PEL1 inhibited the growth of each bacterium during batch growth experiments at MOI of 10 (A), and formed clear plaques in the soft agar inoculated with both bacteria (B). Error bars indicate  $\pm$  one standard deviation from the mean of triplicate independent experiments.

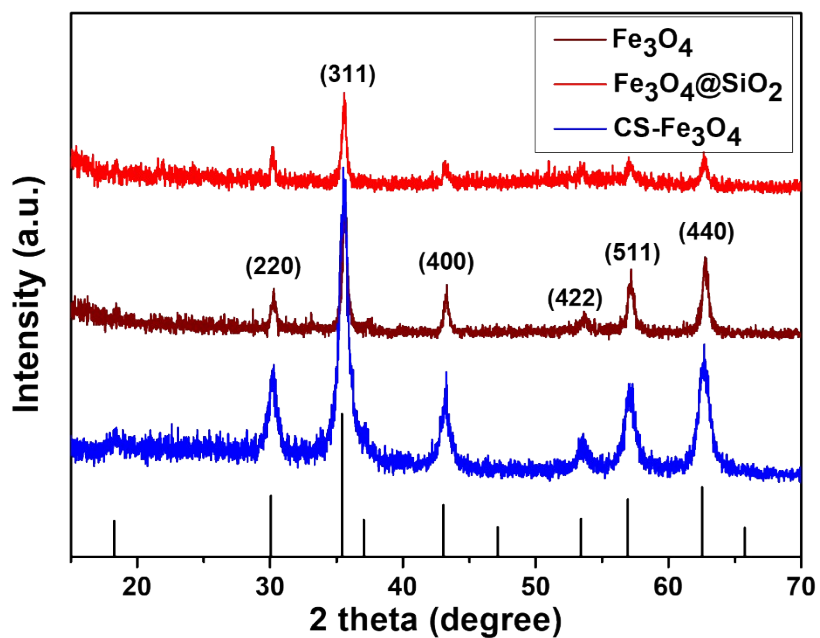


Fig. S2. **XRD patterns of synthesized magnetic materials.** JCPDS card NO. 65-3107 (black,  $\text{Fe}_3\text{O}_4$ , magnetite).

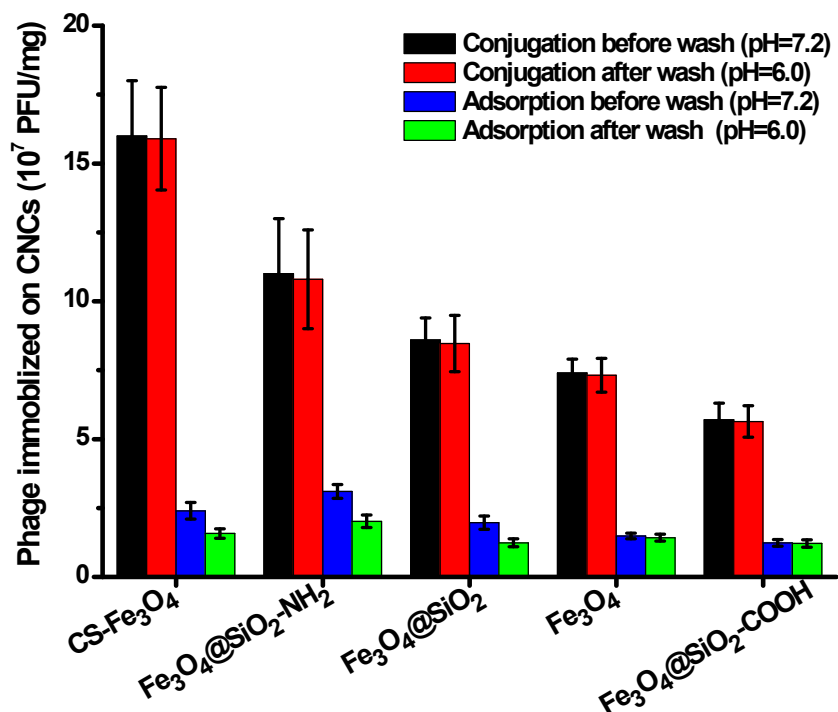
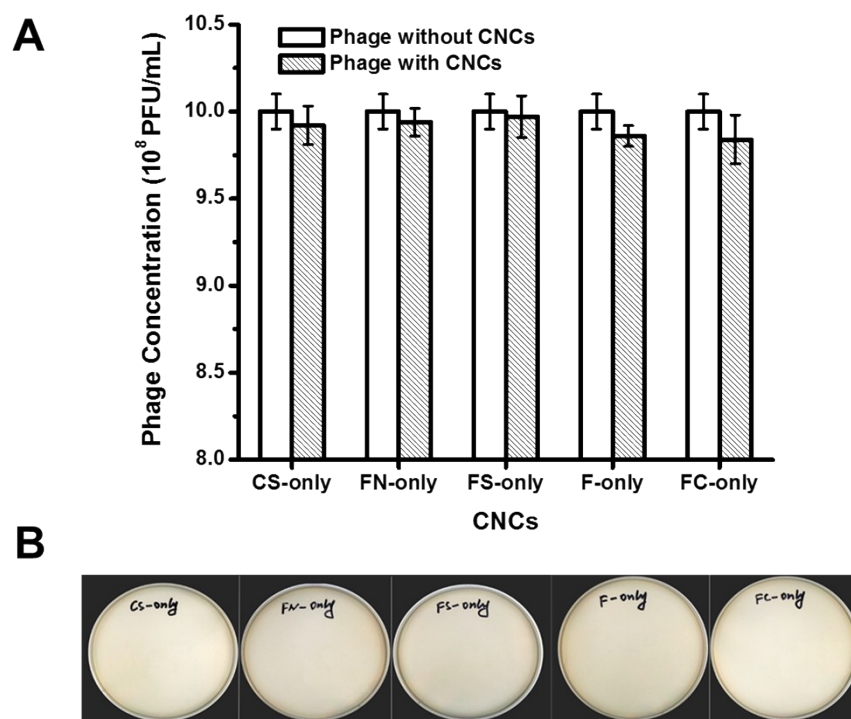


Fig. S3. **Phage PEL1 immobilized on CNCs due to conjugation and non-specific attachment.**

The number of phages immobilized onto CNCs by covalent bonds (i.e., conjugation) or adsorption was quantified by double-layer plaque assay as the difference between the initial phage amount and the total number of free phages remaining in the conjugation plus washing solutions. For estimating adsorption, EDC and NHS were omitted to avoid stimulating conjugation. During the washing step (repeated three times), PEL1-CNC complexes were vortexed at 240 rpm for 10 seconds and precipitated under magnetic field to detach loosely bound phages. Error bars indicate  $\pm$  one standard deviation from the mean of triplicate independent experiments.



**Fig. S4. Lack of phage inactivation (A) or bacterial inhibition (B) by different CNCs.** Panel A shows that these CNCs exerted little toxicity towards phage PEL1 since the sum of PFUs from free phages in bulk solution and attached phage on CNCs has no statistical difference with the PFUs in initial phage solution. Panel B shows no significant inhibitory effect on bacterial lawns (i.e., *E. coli* C3000 plus *P. aeruginosa* PA01) by CS-Fe<sub>3</sub>O<sub>4</sub> (CS-only), Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> core-shell CNCs (FS-only), Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub>-NH<sub>2</sub> CNCs (FN-only), Fe<sub>3</sub>O<sub>4</sub> CNCs (F-only) or Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub>-COOH CNCs (FC-only). Error bars indicate  $\pm$  one standard deviation from the mean of triplicate independent experiments.

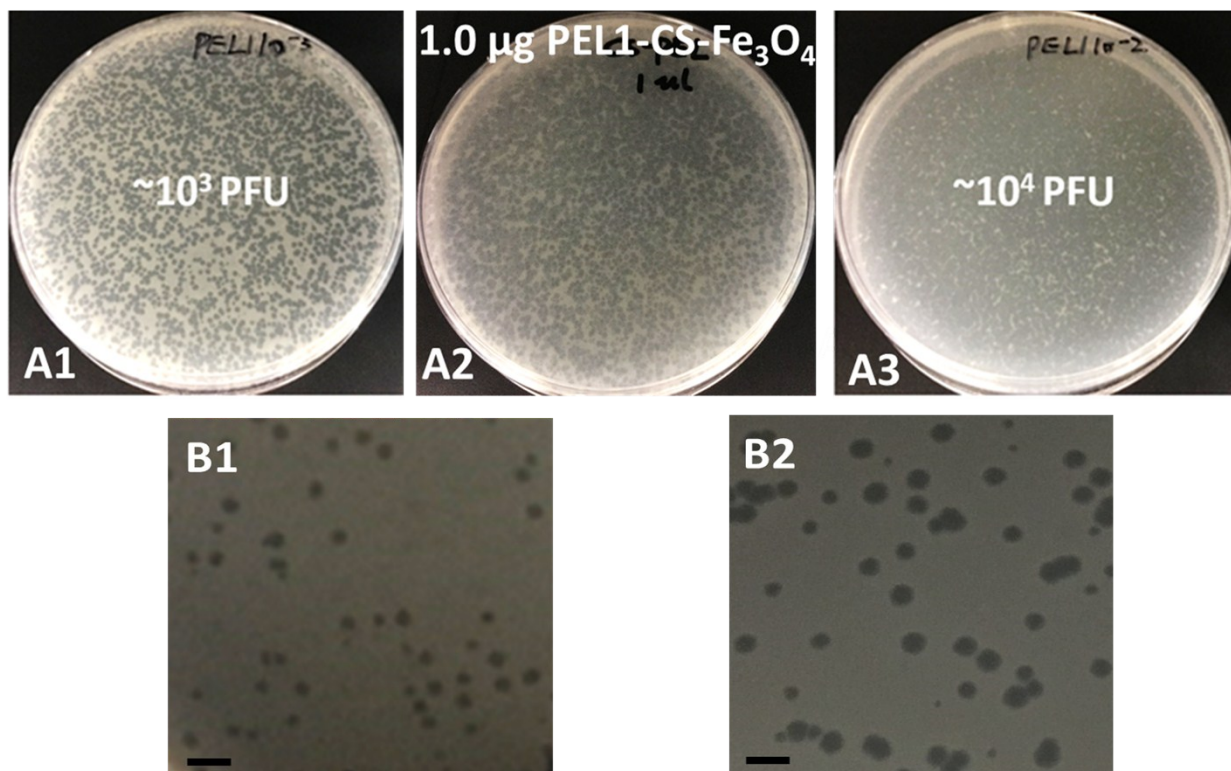


Fig. S5. **Plaque formation capability of PEL1-CS-Fe<sub>3</sub>O<sub>4</sub>.** Panel A1, A2 and A3 showed the plaque formation capability of 10<sup>3</sup> PFU of free phage, 1.0 μg PEL1-CS-Fe<sub>3</sub>O<sub>4</sub>, and 10<sup>4</sup> PFU of free phage, respectively. Panel B1 and B2 showed the plaque morphology from free phages and PEL1-CS-Fe<sub>3</sub>O<sub>4</sub> complexes, respectively. The plaque formation capability of 1.0 μg PEL1-CS-Fe<sub>3</sub>O<sub>4</sub> lay between 10<sup>3</sup>-10<sup>4</sup> PFU free phage. Further serial dilution and plating showed that 1.0 μg PEL1-CS-Fe<sub>3</sub>O<sub>4</sub> corresponded to approximately  $(5.2 \pm 0.7) \times 10^3$  centers of infection (COI) on the two-species bacterial lawn. Scale bar represents 10 mm.



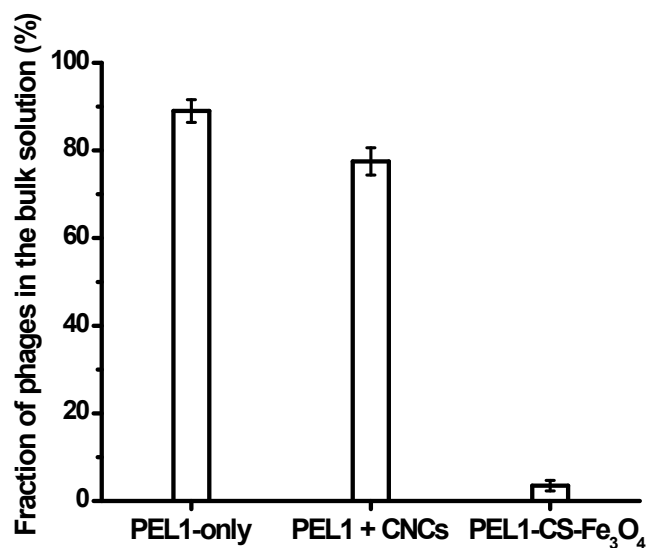


Fig. S6. **Fraction of phages remaining in the bulk solution 30 min after dosage.** Free phages (PEL1-only) are mainly dispersed in the bulk solution, while the conjugated phage (PEL1-CS-Fe<sub>3</sub>O<sub>4</sub>) effectively penetrate the biofilm under magnetic field. Combination of free phage and CNCs (PEL1 + CNCs) slightly enhanced the free phage dispersion. Error bars indicate  $\pm$  one standard deviation from the mean of triplicate independent experiments.

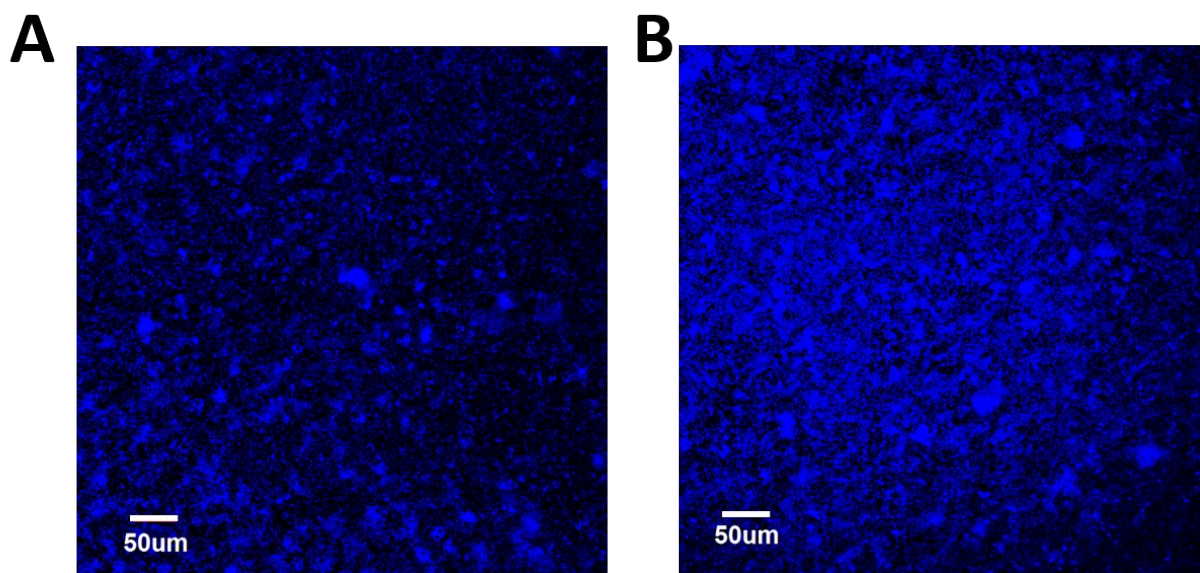


Fig. S7. **Migration of CNCs within biofilm disrupted the integrity of the biofilm.** Panel A shows disrupted biofilm due to horizontal migration of CNCs under magnetic field (660 gauss), and Panel B showed the intact biofilm without treatment by CNCs. Bacterial cells (live and dead) were stained with DAPI. Scale bar represents 50  $\mu\text{m}$ .

Table S1. **Growth parameters of phage PEL1<sup>a</sup>**

<b>Host</b>	<b>Adsorption rate constant (10<sup>-10</sup> mL/min)</b>	<b>Latent time (min)</b>	<b>Burst size (PFU/cell)</b>
<i>P. aeruginosa</i> PA01	10.9	35	75±6
<i>E. coli</i> C3000	7.38	40	98±6

<sup>a</sup> Data shown are the means of results from triplicate independent experiments in TSB medium supplemented with 10 mM Ca<sup>2+</sup>.

Table S2. Host range of phage PEL1

<b>Bacteria</b>	<b>ATCC #</b>	<b>Family</b>	<b>Infectivity <sup>a</sup></b>
<i>E. coli</i> C3000	15597	Enterobacteriaceae	++
<i>E. coli</i> K-12	10798	Enterobacteriaceae	++
<i>E. coli</i> NDM-1	BAA-2452	Enterobacteriaceae	++
<i>Enterobacter cloacae</i>	13047	Enterobacteriaceae	--
<i>Klebsiella oxytoca</i>	700324	Enterobacteriaceae	--
<i>Salmonella typhimurium</i>	700720	Enterobacteriaceae	--
<i>Serratia marcescens</i>	133880	Enterobacteriaceae	--
<i>P. aeruginosa</i> PA01	15692	Pseudomonadaceae	++
<i>P. aeruginosa</i> HER1018	BAA-47	Pseudomonadaceae	++
<i>P. aeruginosa</i> Migula	700829	Pseudomonadaceae	++
<i>P. putida</i>	700007	Pseudomonadaceae	--
<i>P. syringae</i>	19310	Pseudomonadaceae	--
<i>P. fluorescens</i>	13525	Pseudomonadaceae	--
<i>P. putida</i> F1	700007	Pseudomonadaceae	--
<i>Bacillus subtilis</i>	6051	Bacillaceae	--
<i>Bacillus cereus</i>	10987	Bacillaceae	--

<sup>a</sup> Spot tests and plaque assay showed infection (++) or no infection (--).

## Supplementary References

1. Y. Tang, S. Liang, S. Yu, N. Gao, J. Zhang, H. Guo and Y. Wang, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2012, **406**, 61-67.
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4. M. Shen, W. Jia, C. Lin, G. Fan, Y. Jin, X. Chen and G. Chen, *Nanoscale Research Letters*, 2014, **9**, 558-558.