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Supporting Information

Systemic Gene Silencing in Plant Triggered by Fluorescent Nanoparticle Delivered Double-Stranded RNA

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Experimental

Plant materials and growth: Wild type *Arabidopsis* ecotype *Landsberg erecta* (*Ler*) seeds were sterilized with 75% (v/v) ethanol plus 0.03% x-triton for 2 min, followed by three rinses in 70% (v/v) ethanol and three rinses in sterile water. Seeds surface liquid was drained with filter paper and sown on MS (Murashige and Skoog Stock) plates with 1% agar. Plates were placed in the dark for 3 days at 4 °C, and then transferred to a plant growth chamber (22 °C, 16 h light/8 h dark cycle) under 120 μmol $m^{-2}s^{-1}$ white light.

Double-stranded RNA synthesis: Total RNA was isolated from inflorescence of wild-type *Arabidopsis* using RNA extraction kit (Huayueyang, China). cDNA synthesis was performed with TUREscript H^- Reverse Transcriptase (Aidlab, China), and PCR were amplified as follow: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 15 s, and 72 °C for 75 s; then 72 °C for 5 min. After purification, PCR product was cloned into the pMD19-T vector (TaKaRa), and double-stranded RNA (dsRNA) was synthesized using T7 RiboMAX™ express RNAi System according to the manufacture's instruction (Promega). The gene specific primers for cloning and dsRNA synthesis are listed in Supplemental Table S1.

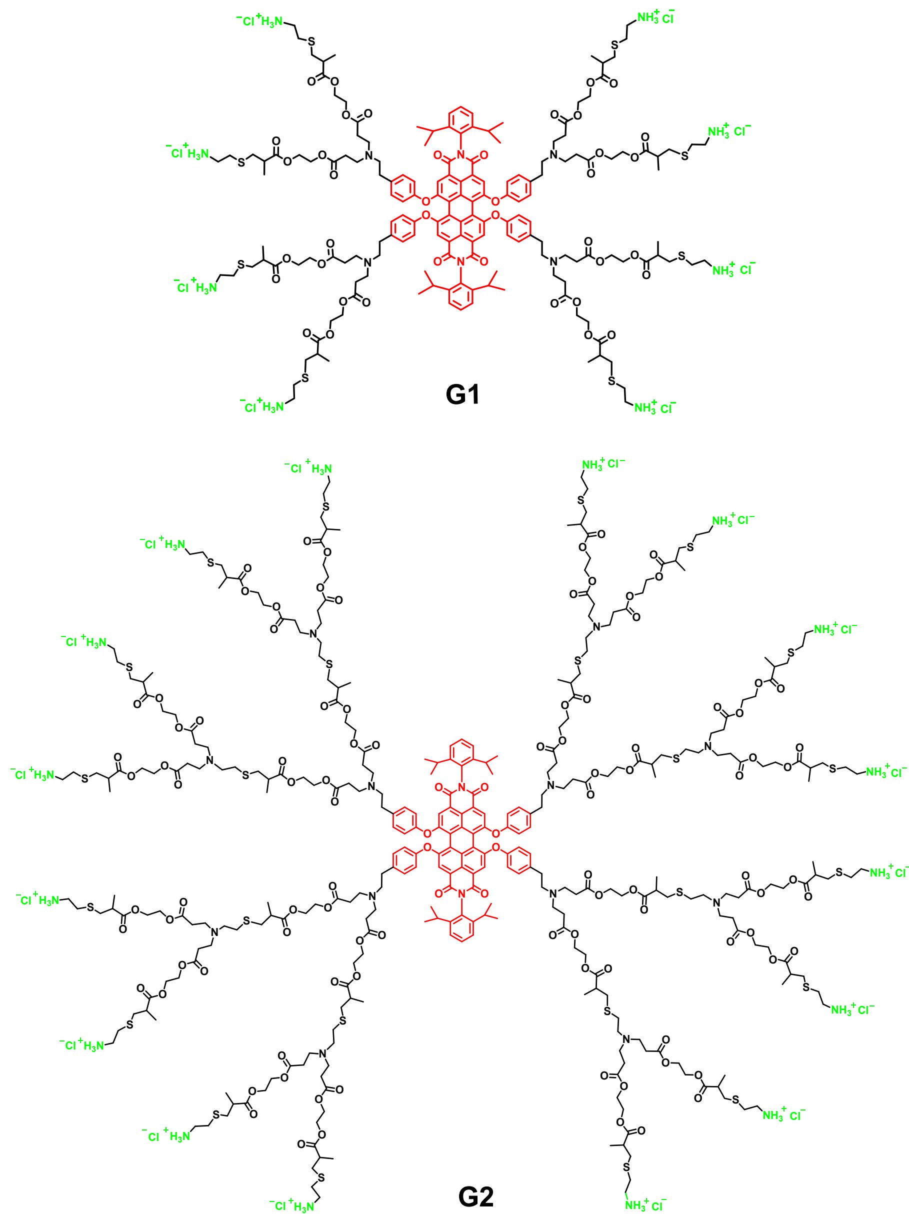
G2 delivers DNA in Arabidopsis: 30 μM CXR Reference Dye (Promega, Catalog C5411) was used to label the single-stranded DNA (20 bp, 100 μM) or double-stranded RNA. The gene carrier G2 and DNA or dsRNA were pre-incubated in culture medium at N/P ratio of 2:1 and then applied to *Arabidopsis* root. Fluorescence microscopy was used to take the fluorescent images.

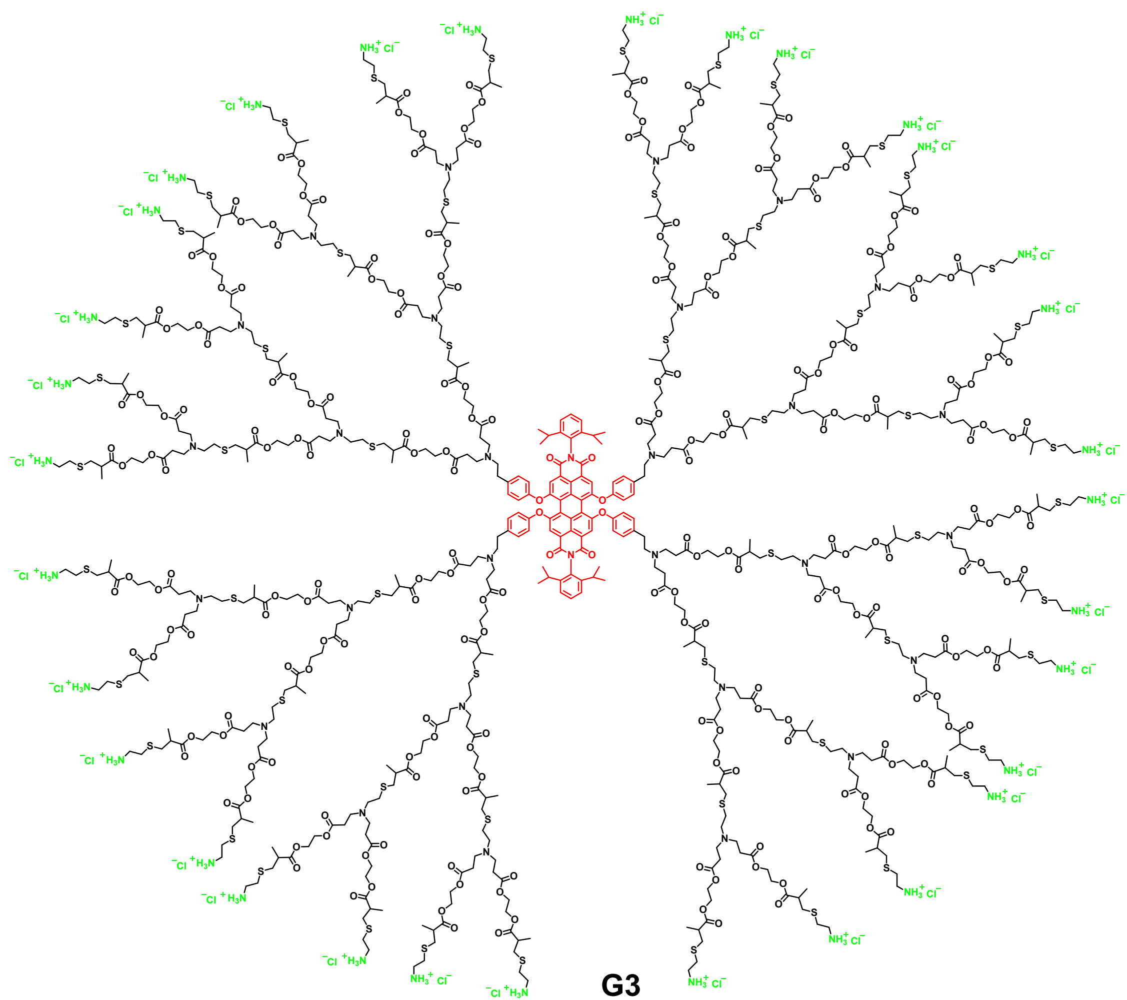
G2 delivered RNAi in Arabidopsis: The root of 10-day-old *Arabidopsis* was treated with 1 μg of dsRNA mixing with 3 μg of gene carrier G2 once every 24 h by pipette. Water plus 1 μg dsRNA were used as control. At least three biological replicates were performed for each treatment. Fluorescent images were taken 24 h after dsRNA application. The whole seedlings were sampled for RT-PCR analyses after 3 days continuous treatment, and phenotypic comparison were observed 5-7 days after dsRNA application.

RT-PCR: Total RNA was extracted using TRIzol reagent (Aidlab, China), and cDNA was synthesized from 2 μg of total RNA using FastQuant RT Kit (TIANGEN, China). Quantitative real time RT-PCR (qPCR) was performed on an ABI PRISM 7500 Real-Time PCR System (Applied Biosystems, USA). Each qPCR were performed with two biological replicates. Gene expression was evaluated by the $2^{-\Delta\Delta CT}$ method and normalized by *Arabidopsis ACTIN2*. Semi-quantitative PCR was carried out following the manufacturer's instructions using the same templates and primers as in the qPCR. The gene specific primers are listed in Supplemental Table 1.

Histologic section: The seedlings after 5-day continuous dsRNA treatment were used for histologic observation. Sample fixation, embedding and sectioning were performed as described [1]. The 10μm-thick sections were dewaxed twice in histoclear for 10 minutes each, followed by dehydration in two changes of isopropanol for 5 minutes each and air dried, and then mounted in neutral resins and observed under light microscope.

Gene accession numbers: Sequence information of this study can be found in the Arabidopsis Genome Initiative under the following accession numbers: *STM* (At1g62360), *WER* (At5g14750).





Scheme S1. Chemical structures of three cationic fluorescent nanoparticle.

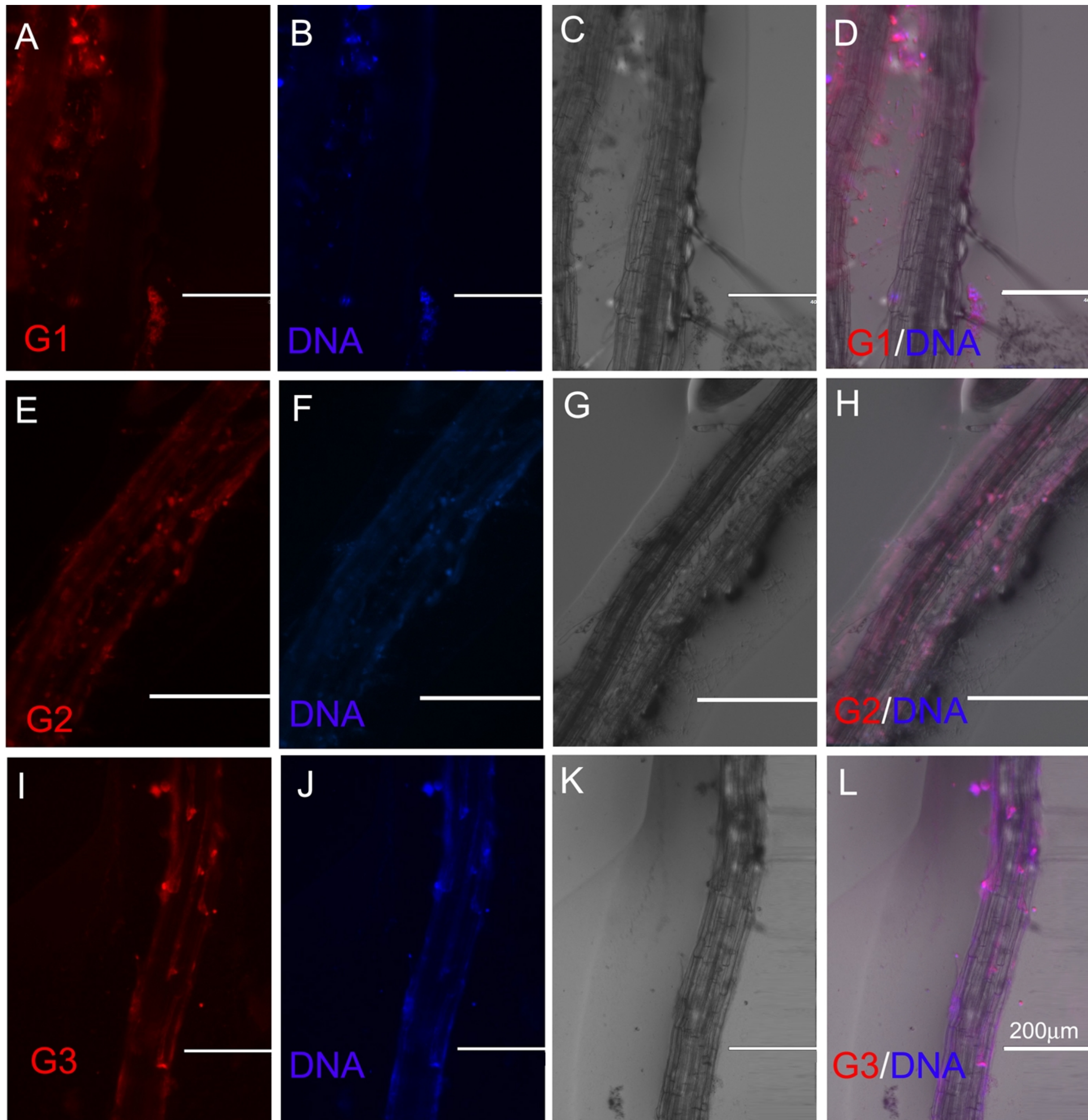


Fig. S1 Fluorescence images of G1, G2 and G3 with DNA in *Arabidopsis* root. (A-L) Fluorescence images of G1/DNA complex (A-D), G2/DNA complex (E-H) and G3/DNA complex (I-L) after 24 h treatment in *Arabidopsis* root. G1, G2, G3 are shown in red (A, E, I), DNA is shown in blue (B, F, J), (C, G, K) represent the bright field, and (D, H, L) show the corresponding merged field. Bars=200 μm.

Table S1. Primer information used in this study

Primers for cloning	
STM-F	5'-ATGGAGAGTGGTTCCAACAGC-3'
STM-R	5'-TCAAAGCATGGTGGAGGAGAT-3'
WER-F	5'-ATGAGAAAGAAAGTAAGTAGTAGTGG-3'
WER-R	5'-TCAAAAACAGTGTCCATCTATAAA-3'
Primers for double-stranded RNA synthesis	
dsSTM-F	5'-tgTAATACGACTCACTATAGGGGGGATAATAGTGATGGTCCGATG-3'
dsSTM-R	5'-tgTAATACGACTCACTATAGGGAGACATCCTGTTGGTCCCATAGA-3'
dsWER-F	5'-tgTAATACGACTCACTATAGGGGTAGTAGTGGTGACGAAGGAAAC-3'
dsWER-R	5'-tgTAATACGACTCACTATAGGGAAGTCCATCATGTTGGTGAGTG-3'
Primers for qRT-PCR/Semi-quantitative PCR	
qSTM-F	5'-CCTTGCTCCTCTTCCTCTTCT-3'
STM-R	5'-TAGCCTCGCCACAACCTC-3'
qWER-F	5'-CCGAATCCTACCGAAACATCAG-3'
qWER-R	5'-CCATCATGTTGGTGAGTGTGCT-3'
qACTIN2-F	5'-CCTTCGTCTTGATCTTGCGG-3'
qACTIN2-R	5'-AGCGATGGCTGGAACAGAAC-3'

1 X. Zhang, Y. Zhou, L. Ding, Z. Wu, R. Liu, E. M. Meyerowitz, *Plant Cell.*, 2013, **25**, 83.