Supplementary Material

Ff-nano, Functionalized 50 nm x 6 nm Nanorods Derived from Ff Filamentous Bacteriophage

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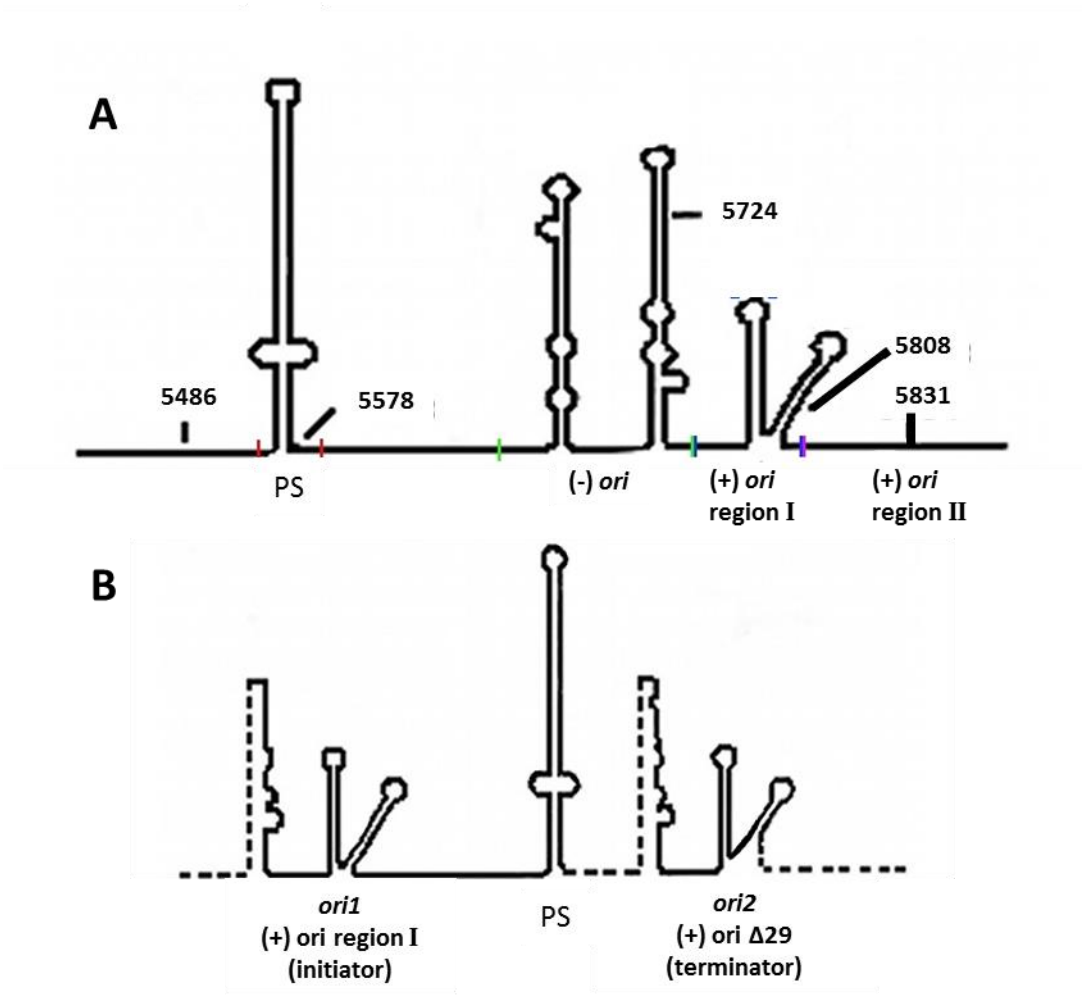
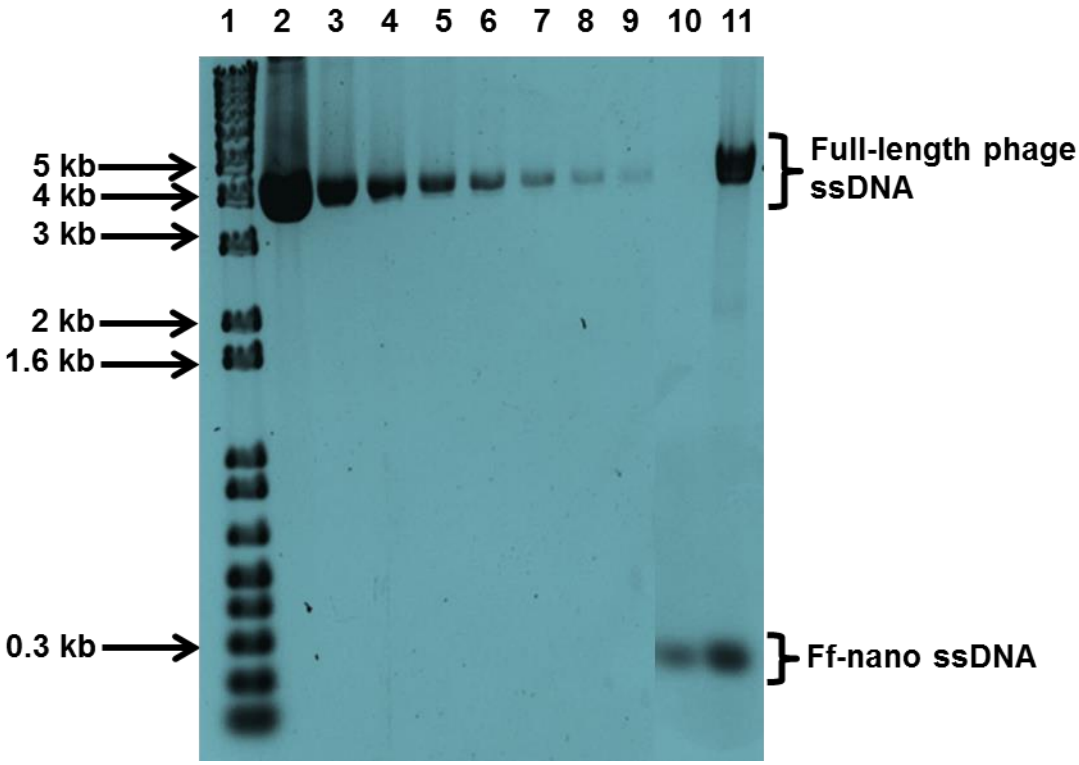
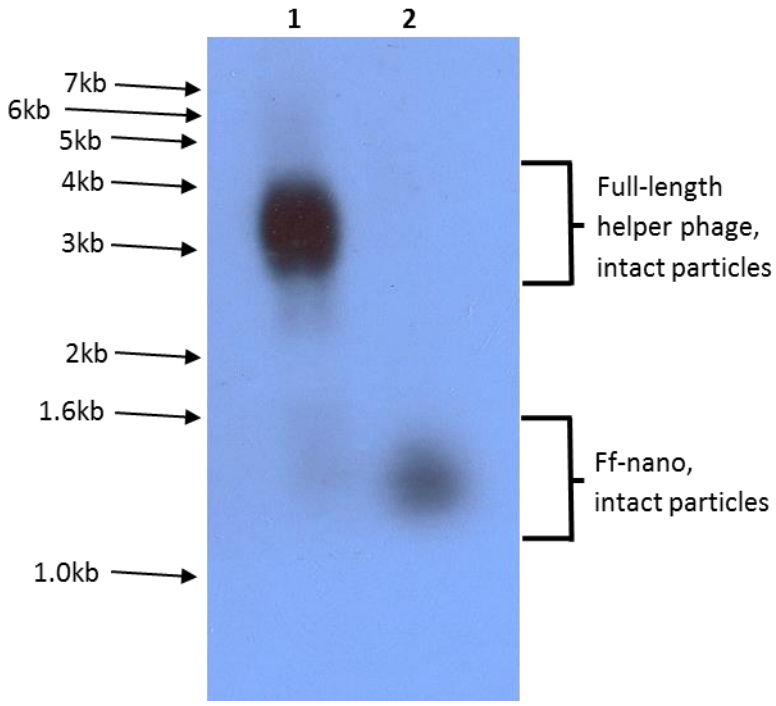
1. Supplementary Figures



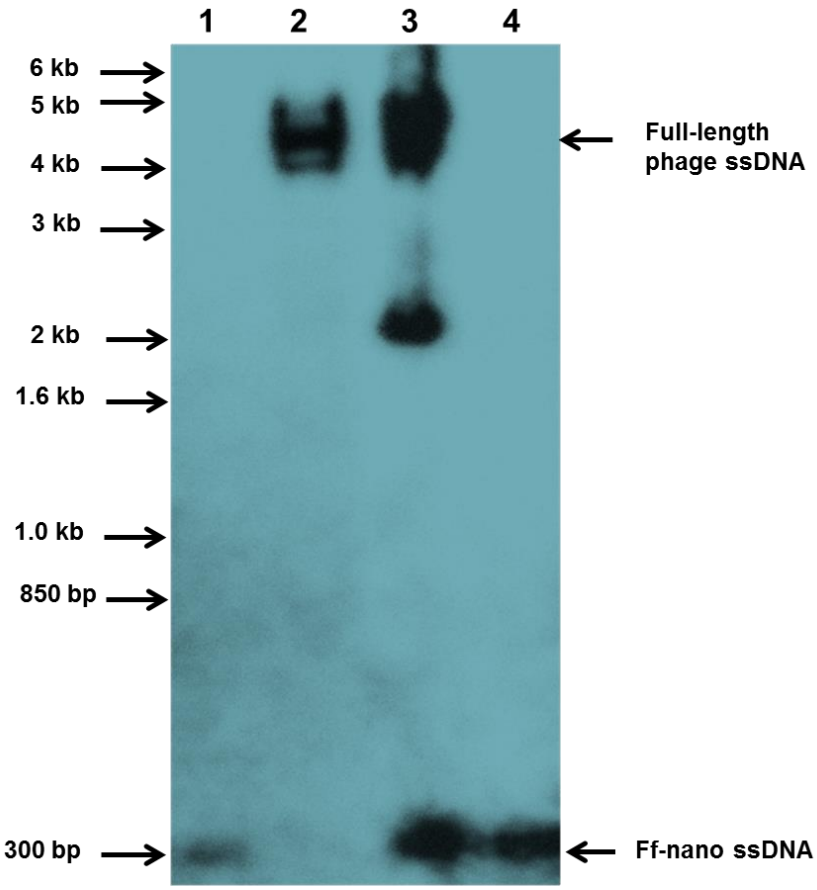
Supplementary figure 1. A. Ff wild-type intergenic sequence (IG) containing origin of replication. Packaging signal (PS); (-) *ori*, negative strand origin of replication; (+) *ori* region I, the region I of the positive strand origin of replication, (+) *ori* region II, the region II of the positive strand origin of replication. Numbers indicate nucleotide positions according to f1 sequence (Hill and Petersen, 1982). **B. The microphage (Ff-nano) origin of replication in pNJB7.** *ori1*, region I of the (+) origin of replication (initiator); PS, packaging signal; *ori2*, positive origin deletion mutant (serves as a terminator) (Specthrie et al., 1992).



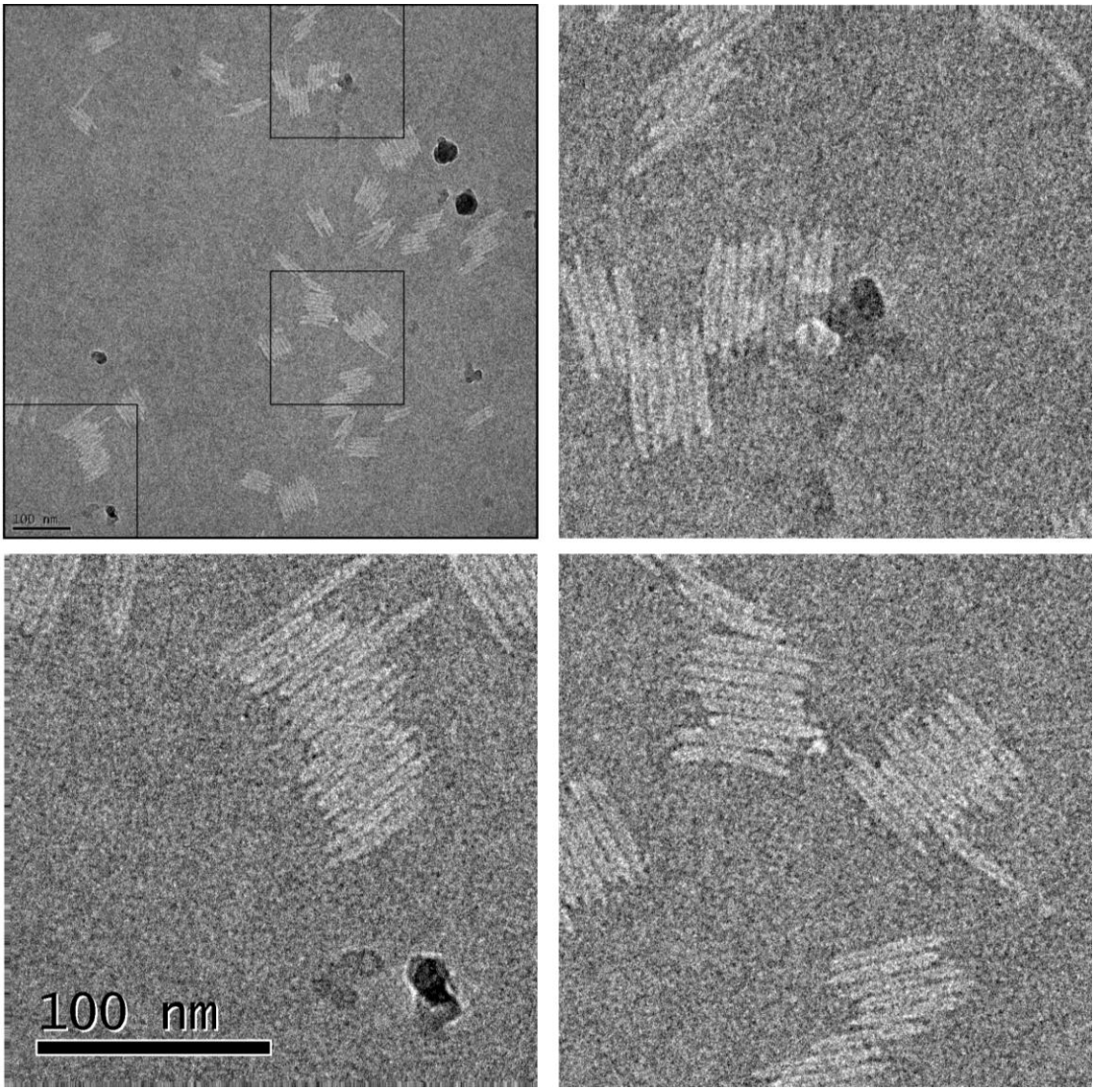
Supplementary figure 2. Quantification gel. Agarose gel electrophoresis of SDS-disassembled Ff-derived particles for quantification by densitometry. All samples were denatured by heating in SDS-containing buffer for 5 min at 100°C, to release ssDNA (please refer to Material and Methods section for details). The DNA was stained in ethidium bromide solution after electrophoresis. DNA bands were photographed using BioRad dark box and the band densities in the standard and test bands were determined using ImageQuant (Fuji). The amount of DNA and the copy number of the Ff-nano was calculated taking into account molecular weight difference relative to that of the full-length phage standard using Excel (Microsoft Office). Molecular weights were calculated based on the size and base composition as described (Rakonjac and Model, 1998). Lane 1, 1kb+ ladder (LifeTechnologies) that serves as a general migration marker; it is not suitable for size determination of phage circular ssDNA; Lanes 2-9, quantitation standard, two-fold serial dilutions of the f1 wild-type of known titre; 10, input into the preparative agarose electrophoresis Ff-nano-enriched lysate (5 µl out of 1 ml); 11, electropurified Ff-nano (5 µl out of 300 µl).



Supplementary figure 3. Separation of the intact full-length helper and Ff-nano by native agarose gel electrophoresis. Lanes: 1, Ff-derived particles precipitated by low PEG (2.5%); 2, Ff-derived particles precipitated by high PEG (15%). Arrows indicate the position of the dsDNA fragments of the 1kb^{+} ladder (Invitrogen). Samples were mixed with the native agarose gel electrophoresis loading buffer and loaded on the gel (please refer to the Material and Methods section for details)



Supplementary figure 4. Comparison of Ff-nano production by Rnano3 and R408-3 as helper phage. Preparations enriched for the Ff-nano (lanes 1 and 4) and the full-length helper phage (lanes 2 and 3) were tested to determine the amount of Ff-nano produced using Rnano3 vs. R408-3. Virions from the low-PEG (full-length phage enriched) and high-PEG (Ff-nano enriched) fractions were disassembled by heating (5 min at 100 °C in the presence of 1% SDS) and the released ssDNA was separated by electrophoresis on an agarose gel. DNA was blotted onto the appropriate DNA-binding membrane and visualized by Southern blotting using a labelled PCR-generated probe corresponding to the origin of replication and packaging signal (the intergenic (IG) sequence (using the ECL nucleic acids labelling and hybridization kit, GE Health, USA). Lanes: 1 and 4, Ff-nano enriched preparations produced using Rnano3 and R408-3 helper phage, respectively; 2 and 3, full-length phage enriched preparations produced using helper phage Rnano3 and R408-3, respectively. The arrows indicate 1 Kb Plus double-stranded linear DNA ladder bands (Life technologies). This standard is not suitable for direct comparison of ssDNA size in nucleotides. It has been used only to measure the progression of electrophoresis and position of bands due to lack of an appropriate ssDNA marker.



Supplementary figure 5: Cryo-negative electron micrograph image of purified R777-derived Ff-nano particles. This micrograph corresponds to Fig. 2A, except that it shows a wider vision field.

2. References

Hill, D.F., and Petersen, G.B. (1982). Nucleotide sequence of bacteriophage f1 DNA. *J Virol* 44, 32-46.

Rakonjac, J., and Model, P. (1998). Roles of pIII in filamentous phage assembly. *J Mol Biol* 282, 25-41. doi: 10.1006/jmbi.1998.2006.

Specthrie, L., Bullitt, E., Horiuchi, K., Model, P., Russel, M., and Makowski, L. (1992). Construction of a microphage variant of filamentous bacteriophage. *J Mol Biol* 228, 720-724.