Supplementary Materials for:

Programmed Self-Assembly of DNA Nanosheets with Discrete Single-Molecule Thickness and Interfacial Mechanics: Design, Simulation, and Characterization

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Text S1

The DNA sequences for F-unit:

A: GTGCTGTATC CCATGTTACCTTCCAC CGACTGACTCTGACTGCGATG CCATGTTACCTTCCAC CTGATCTCTG
B: GATACAGCAC CCATGTTACCTTCCAC CATCGCAGTCAGAGTCAGTCG CCATGTTACCTTCCAC CAGAGATCAG
C_{chol}: GTGGAAGGTAACATGG AAAAA - TEG - cholesterol

The DNA sequences for F-unit with short arms:

A_{short}: GTGCTGTATC TACCTTCCAC CGACTGACTCTGACTGCGATG TACCTTCCAC CTGATCTCTG
B_{short}: GATACAGCAC TACCTTCCAC CATCGCAGTCAGAGTCAGTCG TACCTTCCAC CAGAGATCAG
C_{chol}: GTGGAAGGTAACATGG AAAAA - TEG - cholesterol

Table S1: The DNA sequences for F-unit

			Melting temperature (T_m)	
	Part	**Sequence**	**DPBS***	**$MgCl_2$****
F-unit	Sticky ends	SE1: GTGCTGTATC SE1*: GATACAGCAC	31.2 ºC (0.1 µM) 37 ºC (1 µM)	35.7 ºC (0.1 µM) 41.7 ºC (1 µM)
		SE2: CTGATCTCTG SE2*: CAGAGATCAG	29 ºC (0.1 µM) 34.9 ºC (1 µM)	33.4 ºC (0.1 µM) 39.6 ºC (1 µM)
	Arm	CCATGTTACCTTCCAC	52.2 ºC (0.1 µM) 56.3 ºC (1 µM)	54.9 ºC (0.1 µM) 59.1 ºC (1 µM)
	Core	CGACTGACTCTGACTGCGATG	63.7 ºC (0.1 µM) 66.9 ºC (1 µM)	65.2 ºC (0.1 µM) 68.3 ºC (1 µM)
F-unit (short arm)	Arm	TACCTTCCAC	31.4 ºC (0.1 µM) 37.6 ºC (1 µM)	35.9 ºC (0.1 µM) 42.3 ºC (1 µM)

Oligoanalizer simulation was performed with the following conditions.

* 1x DPBS buffer: 150 mM Na^+
** 1x TAE/12.5 mM $MgCl_2$ buffer: 50 mM Na^+ and 12.5 mM Mg^{+2}

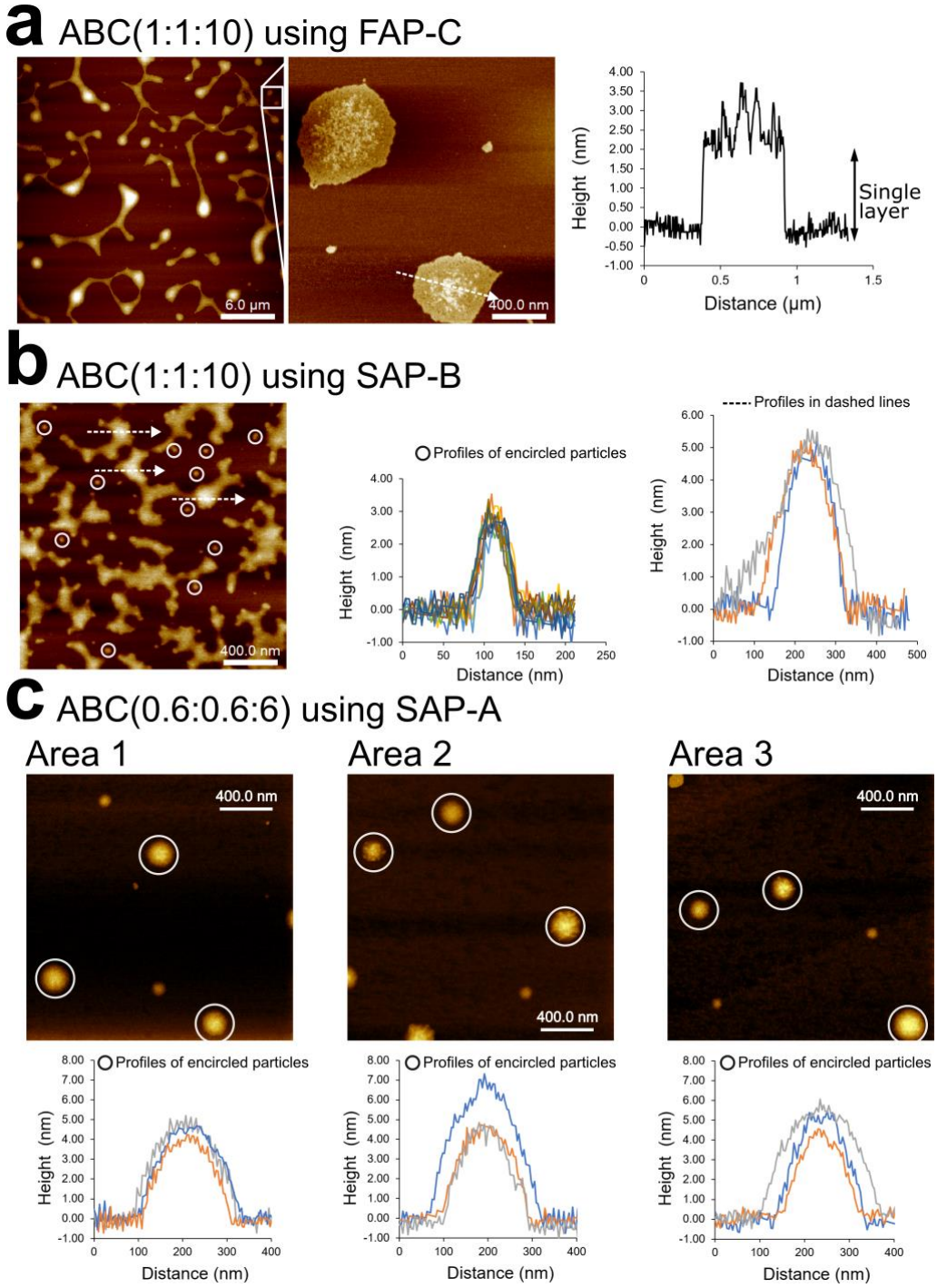

Figure S1. AFM characterization of F-unit superstructures assembled in liquid phase. (**a**) ABC(1:1:10) using FAP-C. A height profile is shown. [A] = 50 nM. (**b**) ABC(1:1:10) using SAP-B. The height profile of encircled particles and asymmetrical structures are shown. [A] = 50 nM. (**c**) ABC(0.6:0.6:6) using SAP-A. Three scanning areas (top), and the height profile of particles (bottom) are shown. [A] = 30 nM.

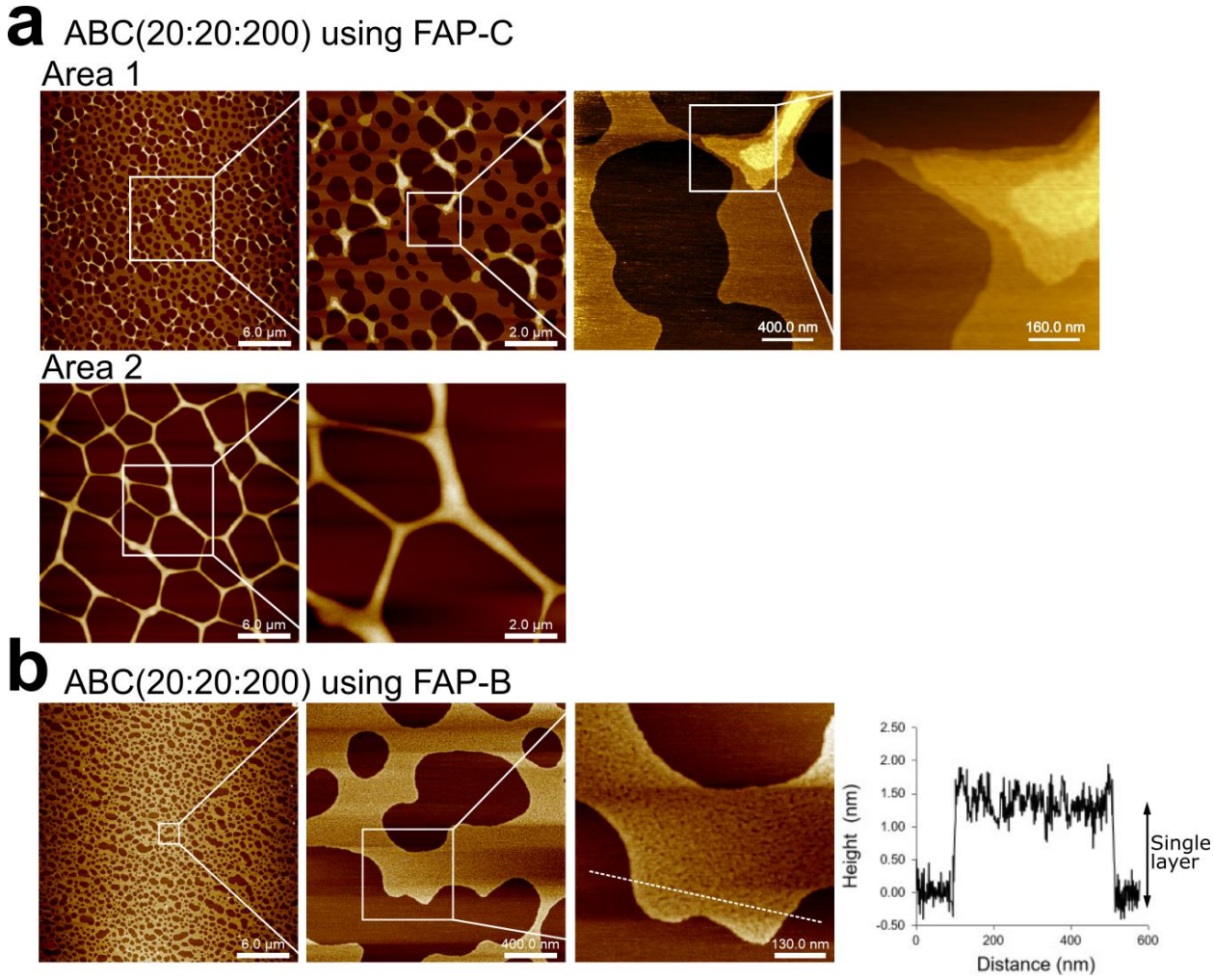

Figure S2. AFM characterization of F-unit superstructures assembled in liquid phase (**a**) ABC(20:20:200) using FAP-C. Two scanning areas are shown. Area 1 shows a multilayer nanosheet. Area 2 shows a network of connected nanotubes. [A] = 1 µM. (**b**) ABC(20:20:200) using FAP-B. A height profile is shown. [A] = 1 µM.

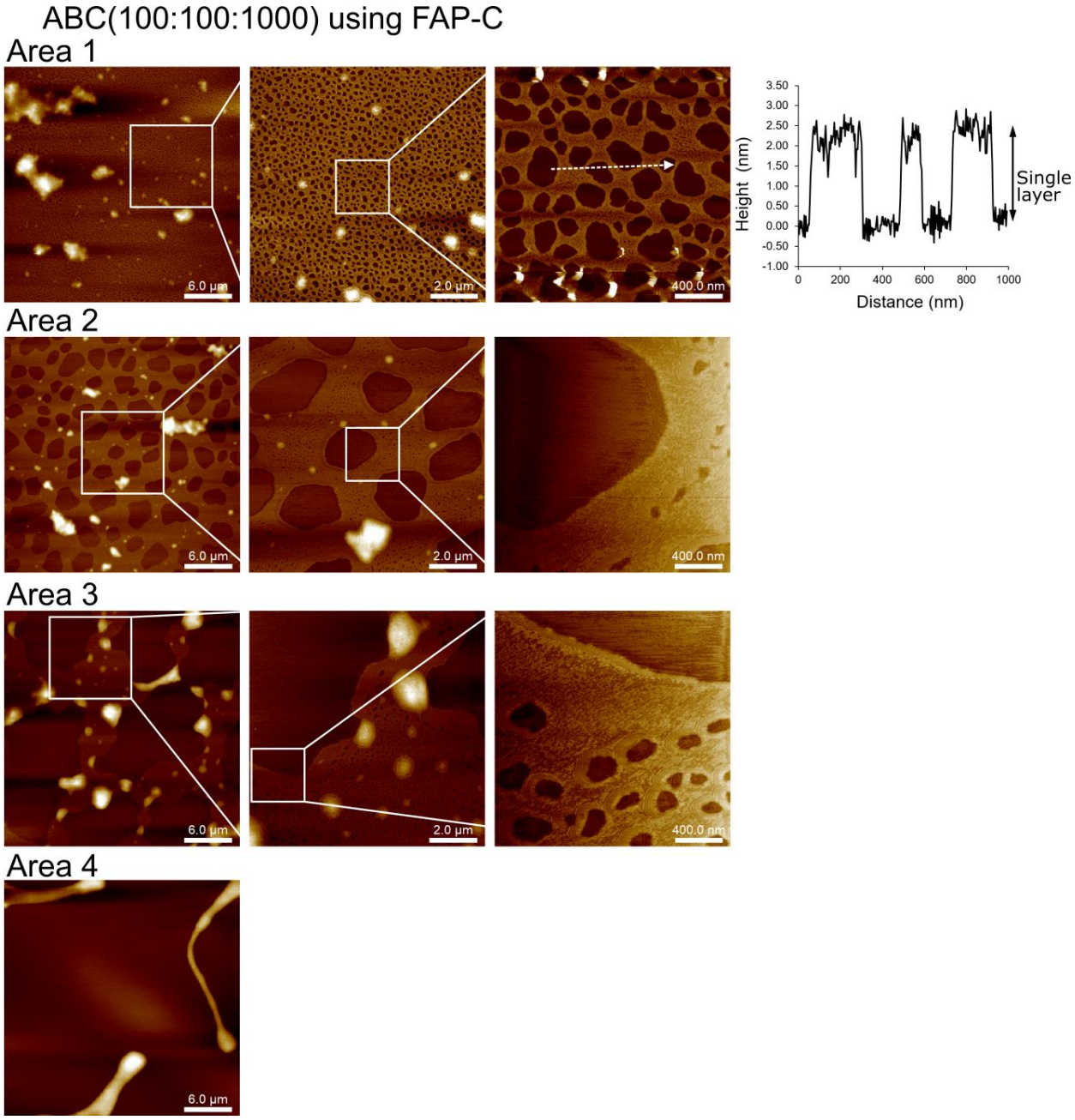

Figure S3. AFM characterization of F-unit superstructures assembled in liquid phase. ABC(100:100:1000) using FAP-C. Four scanning areas are shown. Area 1 shows a large coverage of a single-layer nanosheet with particles on top. [A] = 5 µM.

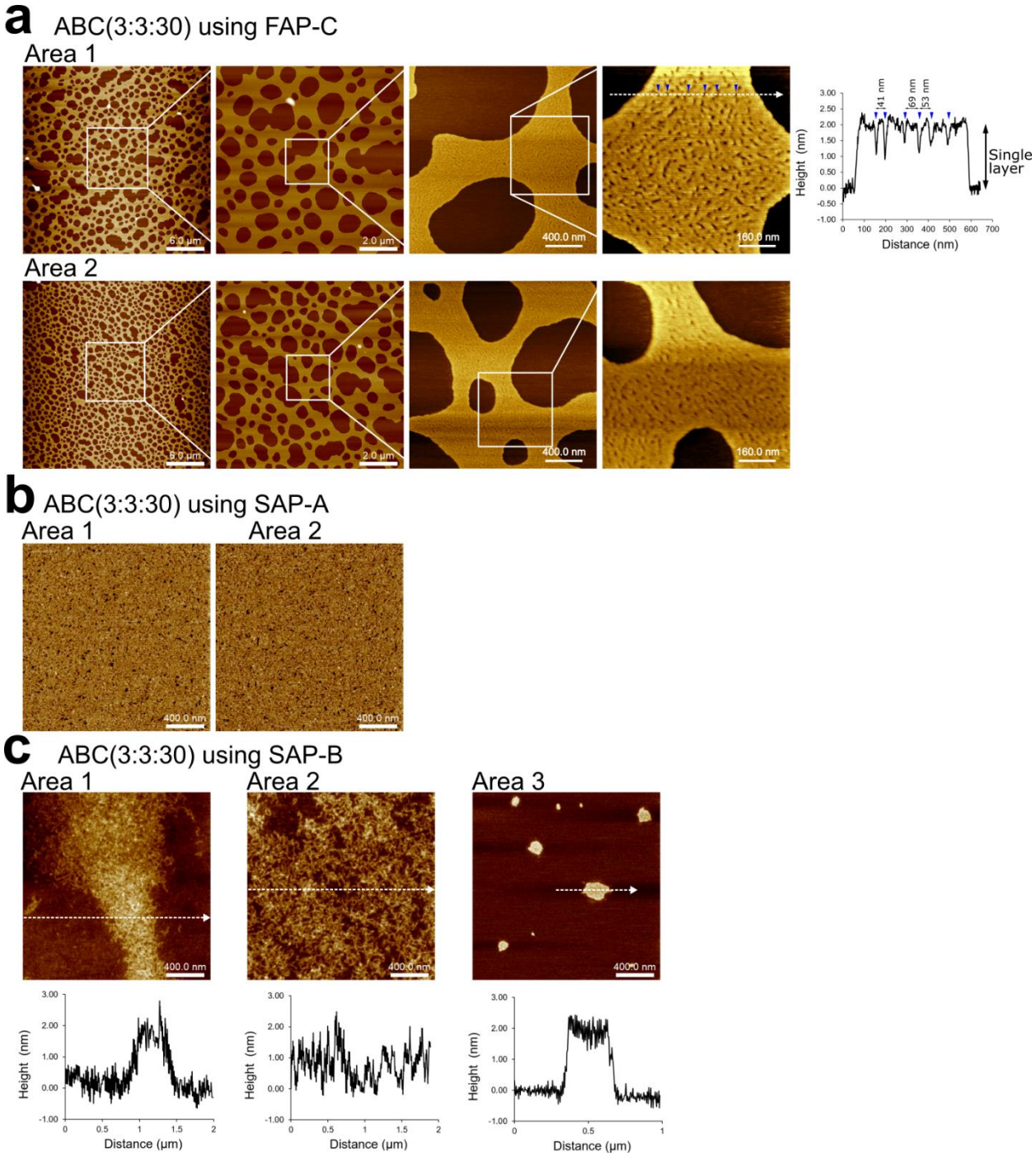

Figure S4. AFM characterization of F-unit superstructures assembled in liquid phase. (**a**) ABC(3:3:30) using FAP-C. Two scanning areas, and a representative height profile are shown. [A] = 150 nM. (**b**) ABC(3,3,30) using SAP-A. Two scanning areas are shown. [A] = 150 nM. (**c**) ABC(3,3,30) using SAP-B. Three scanning areas (top) with representative height profiles (bottom) are shown. [A] = 150 nM.

Text S2

By assuming that the surface density of C_{chol} (21 bases plus the hydrophilic flexible TEG linker) at the oil/aqueous interface is limited by the radius of gyration of DNA and the electrostatic repulsion between C_{chol} strands, the space occupied by each C_{chol} can be approximated as the sum of the radius of gyration of the DNA strand and the length of the flexible TEG linker, which are ~2 nm (interpolating from [1]) and ~2.25 nm, [2] respectively. As a result, the cholesterol of C_{chol} could be separated ~6 nm, which could be longer due to the electrostatic repulsion between the DNA's backbones. This separation is in the same order of magnitude as the distance between cholesterol modifications in the F-unit design in Section 3.1 of the Main Text, i.e., a minimum distance of ~3 nm and a maximum distance of ~14 nm.

Supplementary Information references

1. Sim, A.Y.L.; Lipfert, J.; Herschlag, D.; Doniach, S. Salt Dependence of the Radius of Gyration and Flexibility of Single-Stranded DNA in Solution Probed by Small-Angle X-ray Scattering. *Phys Rev E* **2012**, *86*, 21901, doi:10.1103/PhysRevE.86.021901.

2. Palma, E.; Klapper, D.G.; Cho, M.J. Antibodies as Drug Carriers III: Design of Oligonucleotides with Enhanced Binding Affinity for Immunoglobulin G. *Pharm Res* **2005**, *22*, 122–127, doi:10.1007/s11095-004-9017-z.