Electronic Supplementary Material (ESI) for Nanoscale.
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Supplementary Figures

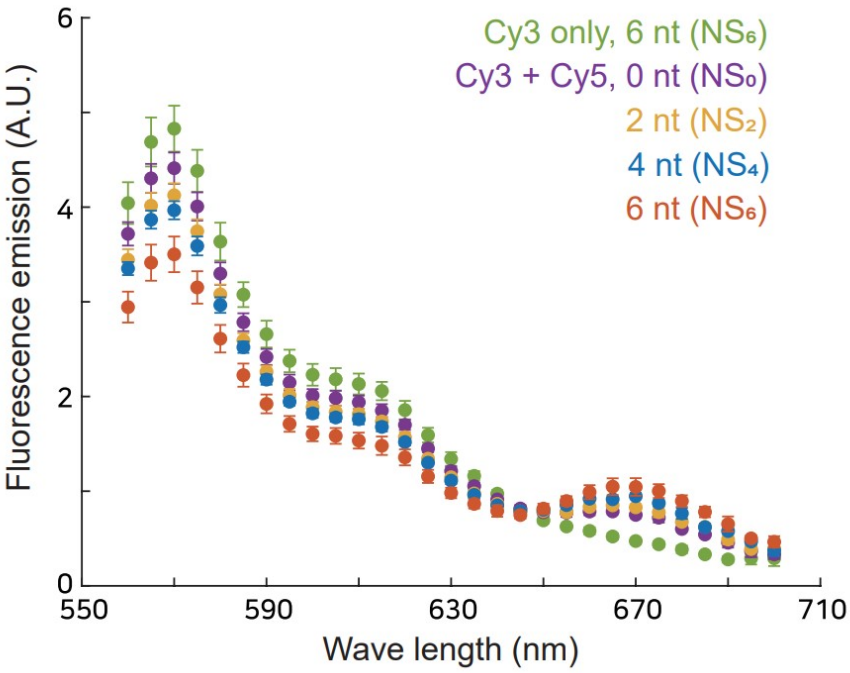

Fig. S1. Fluorescence emission spectra of DNA nanostars with different structural flexibilities. For each construct, fluorescence emission is measured under excitation at 520 nm wavelength of light. 200 nM DNA nanostars are used in 100 mM NaCl and 30 °C. Error bars are standard deviations.

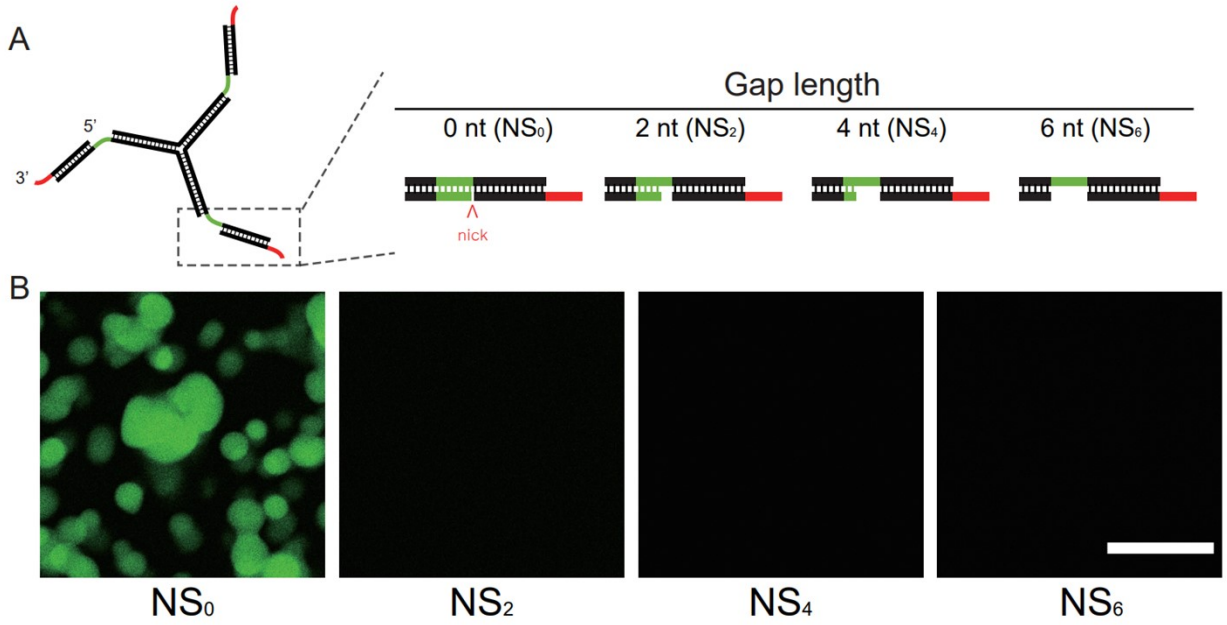

Fig. S2. The effect of the gap length on the phase separation of DNA nanostars. (A) Schematics of an alternative design of DNA nanostars with a varying degree of flexibility. In this design, the single stranded gaps are closed by extending the oligo composing the core of DNA nanostars. (B) Fluorescence images of solutions of DNA nanostars with different flexibilities. The nanostar design shown in (A) is used here. Total 5 μM of DNA nanostars are used in 350 mM NaCl buffer, and 10% of DNA nanostars contain a single FAM-labeled DNA strand. Scale bar, 50 μm.

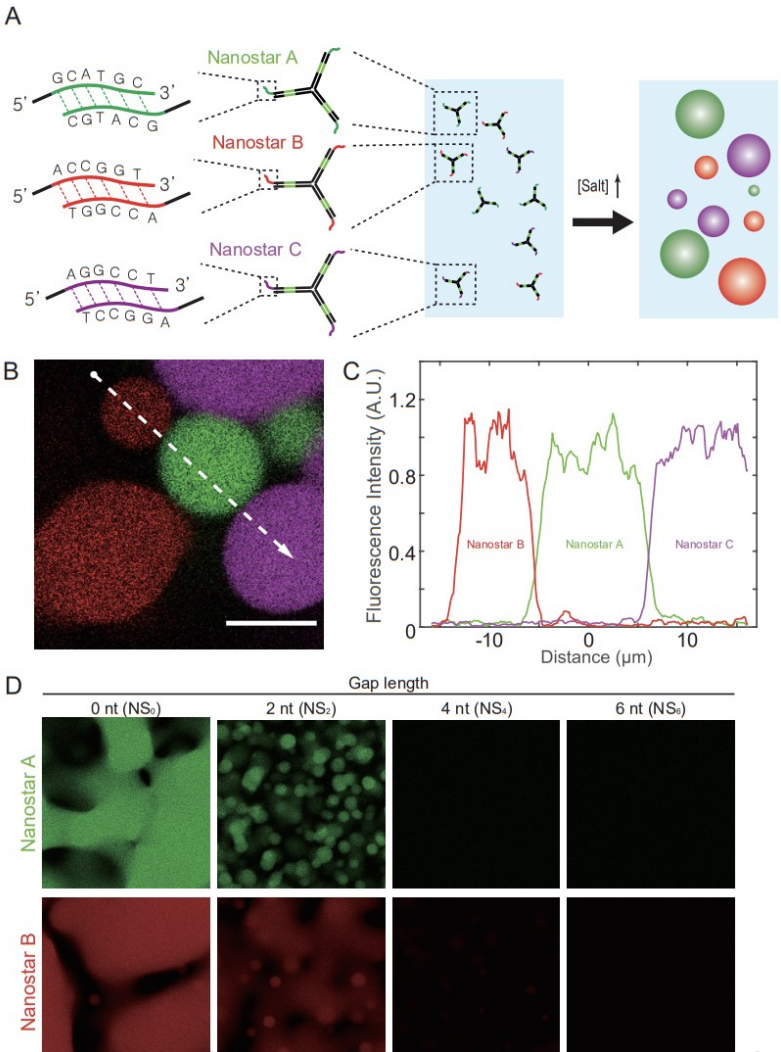

Fig. S3. DNA nanostars with different sticky ends form distinct immiscible condensed phases. (A) Schematic of an experiment performed in (B) and (C) to probe interactions between different sticky ends. DNA oligos for three types of DNA nanostars are annealed separately to assemble individual nanostars. A low salt condition (100 mM of NaCl) is used to keep the solution in the diffusive state even after these DNA nanostars were mixed. Increasing the salt concentration to 350 mM triggers condensation of DNA nanostars, leading to formation of three immiscible condensed phases. Here, nanostars without any gaps, NS_0, are used. (B) Fluorescence image of a mixture comprised of three different types of DNA nanostars. DNA nanostar A, B and C are labeled with FAM, Cy3, and Cy5, respectively. (C) Line profiles of DNA microgels in (B). A moving averaging of 9 pixels (1 μm) is performed. (D) Phase separation behaviors of DNA nanostars with different flexibilities. Each type of DNA nanostars has distinct sticky ends as shown in (A). Scale bar, 20 μm.

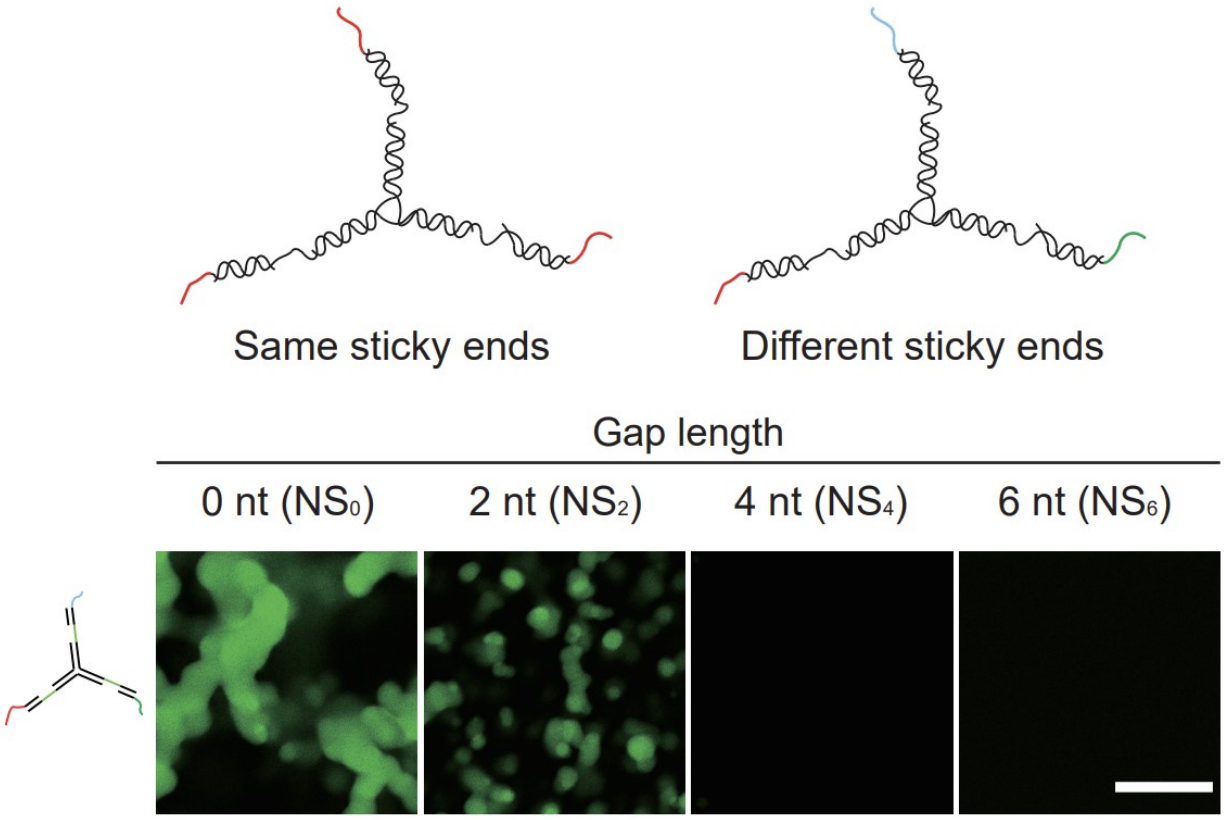

Fig. S4. Phase behavior of heterogeneous DNA nanostars with their sticky ends all different. (Top) Schematics of homogeneous and heterogeneous DNA nanostars used in this study. Homogeneous nanostars harbor identical sticky ends at the end of each arm, but heterogenous ones have different sticky ends. (Bottom) Fluorescence images of solutions of heterogeneous DNA nanostars with different flexibilities. For all samples in Fig S4, 5 μM DNA nanostars are used in 350 mM NaCl buffer and 30 °C. 10 % of the DNA nanostar contains a DNA strand labeled with a FAM dye. Scale bars, 20 μm

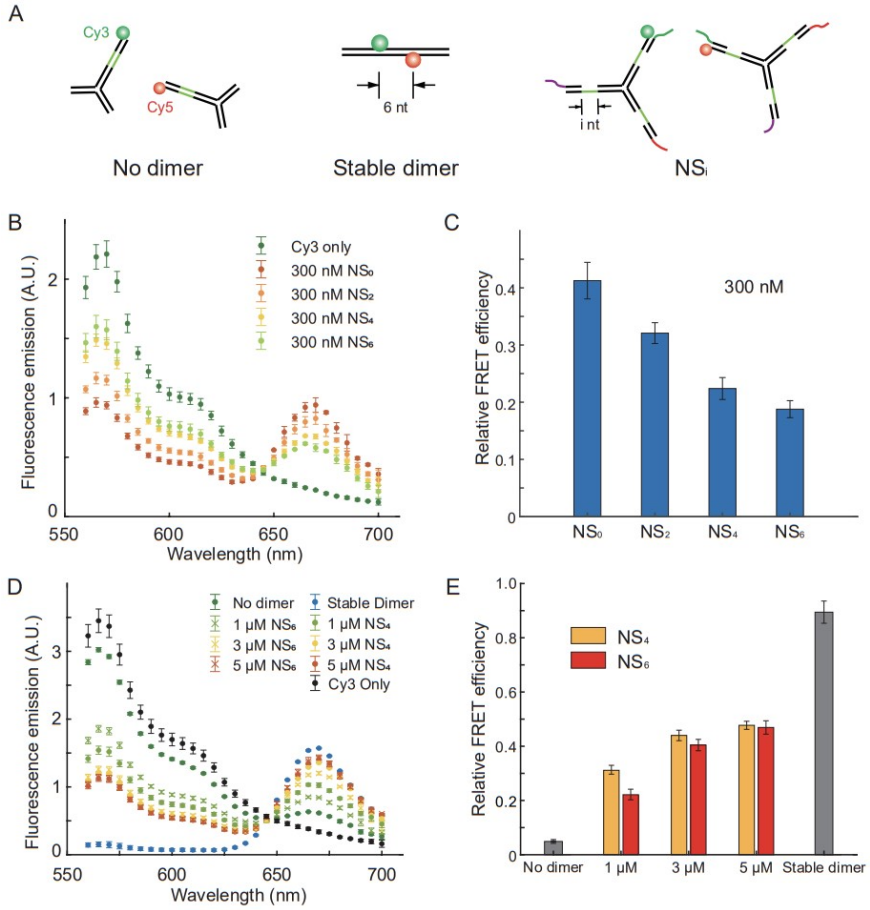

Fig. S5. FRET experiments to measure sticky-end bindings. (A) Schematics of FRET constructs used. The "no dimer" constructs are nanostars that do not display any sticky ends. The "stable dimer" construct is comprised of two oligos in the fully-hybridized form where Cy3-Cy5 FRET pairs are distanced 6 nts away, identical to the length of sticky ends. (B) Fluorescence emission spectra of nanostars with different gap lengths. Samples are excited with 520 nm wavelength of light, and fluorescence emission from 520 nm to 700 nm is measured. Equimolar (150 nM) amounts of Cy3- and Cy5 labeled nanostars are used in 350 mM NaCl buffer at 30 °C. Errorbars are standard deviations. (C) The relative FRET efficiency of the DNA nanostars with different gap lengths. The data in (B) are used to compute relative FRET efficiencies. Error bars are standard deviations. (D) Fluorescence emission spectra of nanostars at different concentrations. In the "no dimer" sample, equimolar amounts of Cy3- and Cy5 labeled nanostars (2.5 μM each) are mixed. The "Cy3 only" sample contains 2.5 μM of Cy3-labeled nanostars of the "no dimer" construct. The "stable dimer" sample contains 2.5 μM of dsDNA labeled with the FRET-pair. In the case of NS_i samples, equimolar amounts of Cy3- and Cy5 labeled nanostars are present at designated total nanostar concentrations. For samples at 1 and 3 μM, fluorescence emission spectra are rescaled using corresponding Cy3-only samples to facilitate their comparison with other data. (E) The relative FRET efficiencies of data in (D). Errorbars are standard deviations.

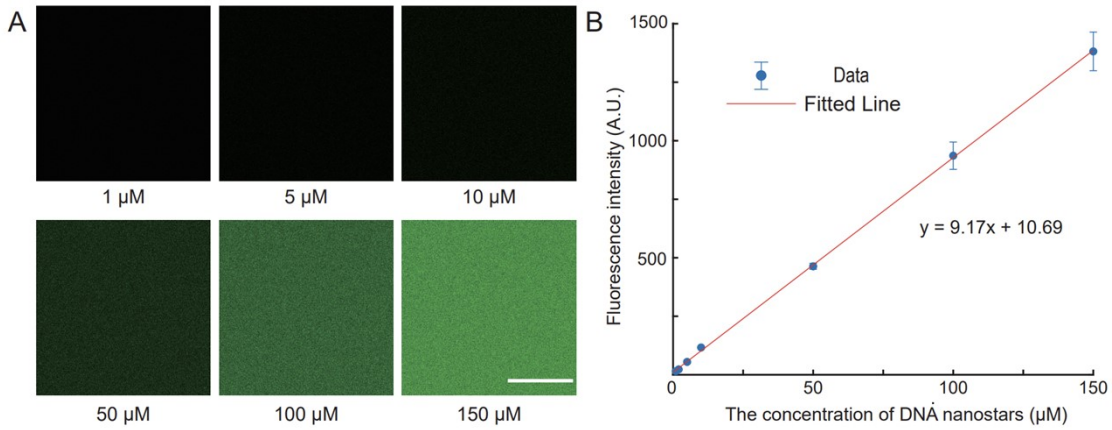

Fig. S6. The calibration curve between fluorescence intensity and nanostar concentration. (A) Fluorescence images of the DNA nanostars at different concentrations. DNA nanostars without sticky ends were used. Scale bar 50 μm. (B) The calibration curve between fluorescence intensity and DNA nanostar concentration is constructed.

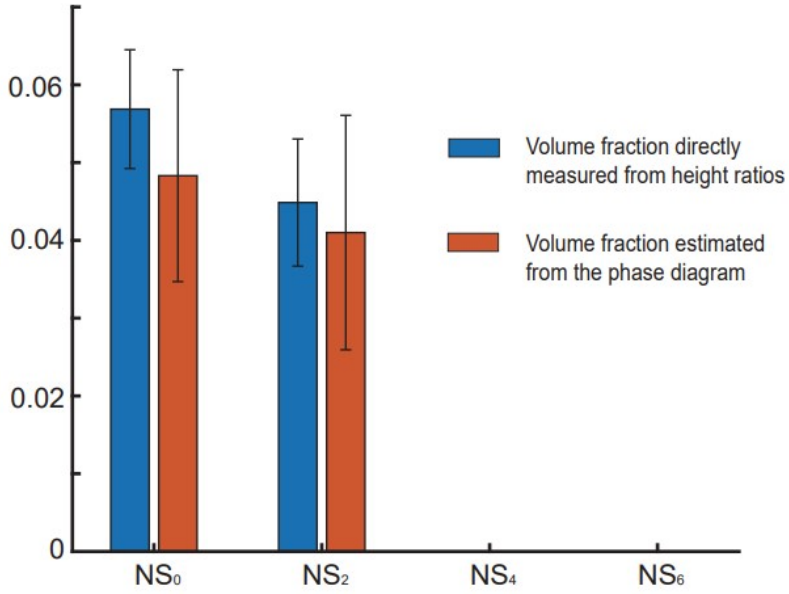

Fig. S7. The condensed phase volume fractions of DNA nanostars quantified using two orthogonal approaches. Using the concentrations of dilute (C_d) and condensed (C_c) phases in the phase diagram, the condensed phase volume fraction (ϕ_c) can be determined; when the total concentration of DNA nanostars is C_t, the solute conservation gives a relation, $C_t = C_d(1- \phi_c)+C_c \phi_c$. The condensed phase volume fractions are estimated using phase diagrams and the total nanostar concentration used in Fig. 2B (red), and then compared with values directly measured using three-dimensional confocal microscopy (blue). The latter data are replicas of those in Fig. 2B.

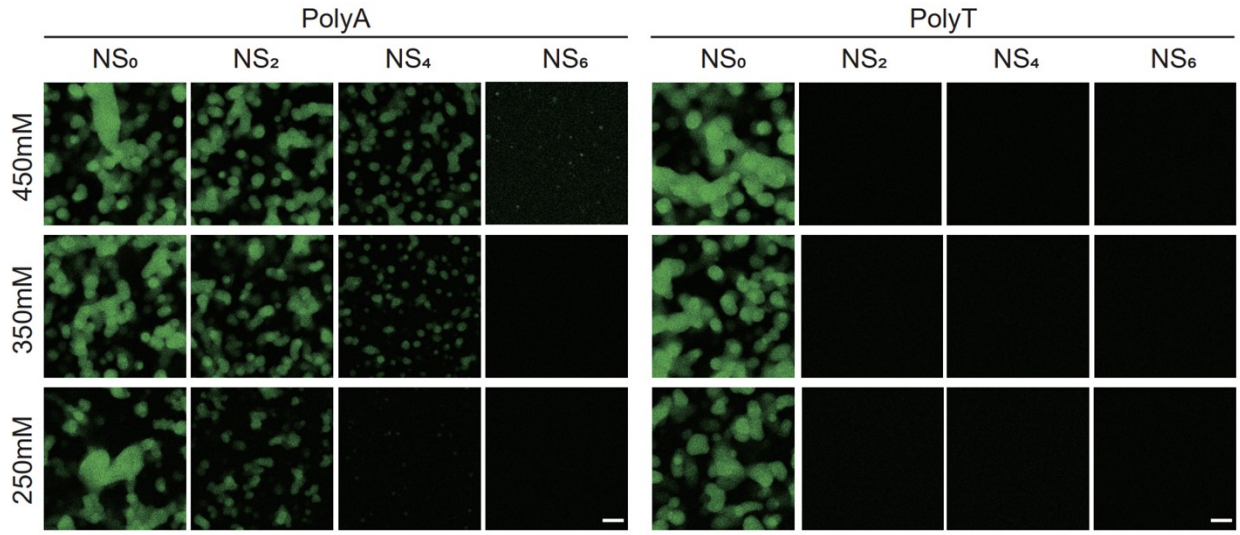

Fig. S8. The phase behavior of DNA nanostars with poly-dT or poly-dA gaps. Fluorescence images of solutions of DNA nanostars with different lengths of poly-dA (A) or poly-dT (B) gaps. Total 5 μM of DNA nanostars are used for each salt condition, and 10% of DNA nanostars contain a single FAM-labeled DNA strand. Scale bar, 20 μm.

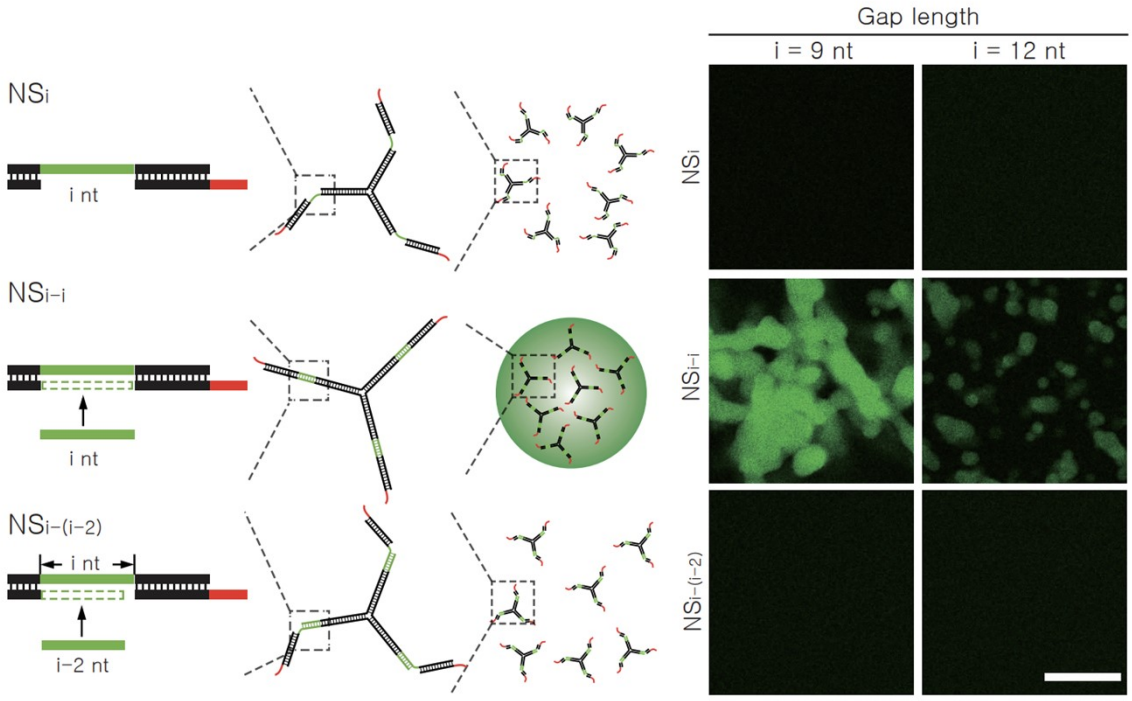

Fig. S9. Fluorescence images of DNA nanostars with longer gaps. Each sample solution contains 5 μM of DNA nanostars, and 15 μM of signal strands are added for NS_{i-i} and $NS_{i-(i-2)}$. All samples are in 500 mM NaCl. Scale bars, 50 μm

Supplemental Information

Oligonucleotide sequences

The sequences of the oligonucleotide strands were designed using the web-based software NUPACK. Sequences used in this study are shown in tables S1-S9. The set of {S1, S2, S3} or {AS1, AS2, AS3} forms the core part of DNA nanostars. "G#_*i* nt" oligos hybridize with the core part, where '#' represents the sticky-end type and '*i*' is the gap length. In the tables, bases written in italic are gaps, and the underlined bases correspond to sticky-ends. For oligos forming the core part of nanostars, the color-coding is such that for each table regions of identical colors are mutually complementary each other.

Table S1. The core of DNA nanostars (Fig. 1-5, S1, S3; used together with oligos in Table S2)

Name	5'-Sequence-3'	Puri.
S1	GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
FAM/Cy3 /Cy5_S1	(FAM/Cy3/Cy5)-GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
S2	GAG AGT AGA TTG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G	HPLC
Cy5_S2	(Cy5) - GAG AGT AGA TTG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G	HPLC
S3	GAG AGT AGA TTG *TGA GTA* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C	HPLC
Cy3_S3	GAG AGT AGA TTG *TGA GTA* CAT GGA TGT CGT CAA C T - (Cy3) - T GAA CAC GTT CAG GTA C	HPLC
T1	*TAC TCA*	HPLC

Table S2. The nanostar arms with sticky ends (Fig. 1-5, S1, S3; used together with oligos in Table S1)

Name	5'-Sequence-3'	Puri.
G1_6nt	CAA TCT ACT CTC GCA TGC	HPLC
G1_4nt	*CA*C AAT CTA CTC TCG CAT GC	HPLC
G1_2nt	*CTC A*CA ATC TAC TCT CGC ATG C	HPLC
G1_0nt	*TAC TCA* CAA TCT ACT CTC GCA TGC	HPLC

Table S3. The core of heterogeneous DNA nanostars (Fig. S4-5; used together with oligos in Table S4)

Name	5'-Sequence-3'	Puri.
AS1	GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
FAM/Cy3/	(FAM/Cy3/Cy5) - GAG AGT AGA TTG *TGA GTA* GTA CCT	HPLC

Cy5_AS1	GAA CGT GTT CTT CCT AAT GAT GCT CGT G	
AS2	GTT CCA GTT TAG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC TTT CCT G	HPLC
AS3	GAT TGA ATA CGG *TGA GTA* CAG GAA AGT CGT CAA CTT GAA CAC GTT CAG GTA C	HPLC

Table S4. Arms of heterogeneous DNA nanostars with sticky ends all different (Fig. S4-5; used together with oligos in Table S3)

Name	5'-Sequence-3'	Puri.
AG1_0nt	*TAC TCA* CAA TCT ACT CTC GCA TGC	HPLC
AG2_0nt	*TAC TCA* CTA AAC TGG AAC ACC GGT	HPLC
AG3_0nt	*TAC TCA* CCG TAT TCA ATC AGG CCT	HPLC
AG1_2nt	*C TCA* CAA TCT ACT CTC GCA TGC	HPLC
AG2_2nt	*C TCA* CTA AAC TGG AAC ACC GGT	HPLC
AG3_2nt	*C TCA* CCG TAT TCA ATC AGG CCT	HPLC
AG1_4nt	*CA* CAA TCT ACT CTC GCA TGC	HPLC
AG2_4nt	*CA* CTA AAC TGG AAC ACC GGT	HPLC
AG3_4nt	*CA* CCG TAT TCA ATC AGG CCT	HPLC
AG1_6nt	CAA TCT ACT CTC GCA TGC	HPLC
AG2_6nt	CTA AAC TGG AAC ACC GGT	HPLC
AG3_6nt	CCG TAT TCA ATC AGG CCT	HPLC

Table S5. Arms of homogeneous DNA nanostars with single type of sticky ends (Fig. S3; used together with oligos in Table S1)

Name	5'-Sequence-3'	Puri.
G2_6nt	CAA TCT ACT CTC ACC GGT	HPLC
G2_4nt	*CA*C AAT CTA CTC TCA CCG GT	HPLC
G2_2nt	*CTC A*CA ATC TAC TCT CAC CGG T	HPLC
G2_0nt	*TAC TCA* CAA TCT ACT CTC ACC GGT	HPLC
G3_6nt	CAA TCT ACT CTC AGG CCT	HPLC
G3_4nt	*CA*C AAT CTA CTC TCA GGC CT	HPLC
G3_2nt	*CTC A*CA ATC TAC TCT CAG GCC T	HPLC
G3_0nt	*TAC TCA* CAA TCT ACT CTC AGG CCT	HPLC

Table S6. DNA nanostars with gaps closing from the core oligo (Fig. S2)

Name	5'-Sequence-3'	Puri.
SS1_4nt	GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G*TA*	HPLC
SS2_4nt	GAG AGT AGA TTG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G*TA*	HPLC

SS3_4nt	GAG AGT AGA TTG *TGA GTA* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C*TA*	HPLC
SS1_2nt	GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G*TA CT*	HPLC
SS2_2nt	GAG AGT AGA TTG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G*TA CT*	HPLC
SS3_2nt	GAG AGT AGA TTG *TGA GTA* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C*TA CT*	HPLC
SS1_0nt	GAG AGT AGA TTG *TGA GTA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G*TA CTC A*	HPLC
SS2_0nt	GAG AGT AGA TTG *TGA GTA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G*TA CTC A*	HPLC
SS3_0nt	GAG AGT AGA TTG *TGA GTA* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C*TA CTC A*	HPLC
FAM_G1	(FAM)- CAA TCT ACT CTC GCA TGC	HPLC

Table S7. DNA nanostars with longer gaps (Fig. S9; used together with oligos in Table S2)

Name	5'-Sequence-3'	Puri.
S1_9nt	GAG AGT AGA TTG *TGA GTA AGA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
FAM-S1_9nt	(FAM) - GAG AGT AGA TTG *TGA GTA AGA* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
S2_9nt	GAG AGT AGA TTG *TGA GTA AGA* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G	HPLC
S3_9nt	GAG AGT AGA TTG *TGA GTA AGA* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C	HPLC
T1_9nt	*TCT TAC TCA*	HPLC
T1_7nt	*TCT TAC T*	HPLC
S1_12nt	GAG AGT AGA TTG *TGA GTA AGA AAG* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
FAM-S1_12nt	(FAM) - GAG AGT AGA TTG *TGA GTA AGA AAG* GTA CCT GAA CGT GTT CTT CCT AAT GAT GCT CGT G	HPLC
S2_12nt	GAG AGT AGA TTG *TGA GTA AGA AAG* CAC GAG CAT CAT TAG GTT GTT GAC GAC ATC CAT G	HPLC
S3_12nt	GAG AGT AGA TTG *TGA GTA AGA AAG* CAT GGA TGT CGT CAA CTT GAA CAC GTT CAG GTA C	HPLC
T1_12nt	*CTT TCT TAC TCA*	HPLC
T1_10nt	*CTT TCT TAC T*	HPLC

Table S8. DNA oligos for the "stable dimer" model and the "no dimer" model (Fig. S5)

Name	5'-Sequence-3'	Puri.
Nodimer A_S1	(Cy3) - CGG GTC GTG GTC TGA GTA GGA GAT GCA GAT GTC GTT GCG TCA CAG CTG ACT C	HPLC
Nodimer A_S2	GGT AAC GAC TCG GGG CTT CGA CAT CTG CAT CTC CTA CTC AGA CCA CGA CCC G	HPLC
Nodimer A_S3	GAG TCA GCT GTG ACG CTT GCC CCG AGT CGT TAC C	HPLC
Nodimer B_S1	(Cy5) - CGT GCT CGG TAG TGA GTA CCT TAC TGC TTT CGC GTT CGC GGG TTG AGC CCC G	HPLC
Nodimer B_S2	TAG TTC TAG ATC AGG CTT CGC GAA AGC AGT AAG GTA CTC ACT ACC GAG CAC G	HPLC
Nodimer B_S3	CGG GGC TCA ACC CGC GTT GCC TGA TCT AGA ACT A	HPLC
Stabledimer_S1	GGT TGC CG - (Cy3) - *GCA TGC* CGT GCT CG	HPLC
Stabledimer_S2	CGA GCA CG - (Cy5) - *GCA TGC* CGG CAA CC	HPLC

Table S9. Diffusion coefficients calculated from FRAP data

NaCl [mM]	i (NS_i)	D [$\times 10^{-3} \mu m^2 / sec$]
500	4	112.7 $\pm$ 4.5
500	2	62.2 $\pm$ 2.4
500	0	21.3 $\pm$ 0.3
350	0	27.6 $\pm$ 0.7
200	0	66.3 $\pm$ 2.6