

## Supporting Information for

### I-Motif-Stapled and Spacer-Dependent Multiple DNA Nanostructures

Jiangtao Ren<sup>‡a</sup>, Tianshu Wang<sup>‡a</sup>, Erkang Wang<sup>\*a</sup> and Jin Wang<sup>\*a, b</sup>

<sup>a</sup> State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China

<sup>b</sup> Department of Chemistry and Physics, State University of New York at Stony Brook, New York, 11794, USA

\* To whom correspondence should be addressed. Tel: (+86)431-85262003; Email: E-mail: [ekwang@ciac.ac.cn](mailto:ekwang@ciac.ac.cn); [jin.wang.1@stonybrook.edu](mailto:jin.wang.1@stonybrook.edu);

<sup>‡</sup>These authors contributed equally to this work.

**Materials.** Ultrapage-purified oligonucleotides (Table S1) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sangon Biotechnology Co., Ltd (Shanghai, China). 3-Aminopropyltriethoxysilane (APTES) and ethidium bromide (EB) were purchased from Sigma-Aldrich (USA). Acrylamide, N, N'-Methylenebisacrylamide and ammonium persulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) were obtained from Beijing Dingguochangsheng Biotechnology Co. Ltd (Beijing, China). Magnesium acetate (Mg(OAc)<sub>2</sub>) and Tetramethylethylenediamine (TEMED) were purchased from Beijing Chemical Works (Beijing, China) and AMRESCO Inc. (USA), respectively. 50 bp DNA ladder was purchased from New England Biolabs (Beijing), Ltd. The 6×loading buffer (pH=8.0 or 5.0) was prepared containing 25 mM Tris, 10 mM Mg(OAc)<sub>2</sub> and 36 % glycerol. TA buffer (pH=8.0 or 5.0) containing 25 mM Tris and 10 mM Mg(OAc)<sub>2</sub> was used for sample preparation and electrophoresis. Double distilled water was used throughout.

Table S1: The sequences of oligonucleotides used in this study.

<i>Oligos</i>	<i>Sequence (5' to 3')</i>
C1S0	ACTTAACTGCTAGCCGA CCCCCCTCCCCC
C2S0	TCGGCTAGCAGTGTAAGT CCCCCCTCCCCC
C1S1	ACTTAACTGCTAGCCGA T CCCCCCTCCCCC
C2S1	TCGGCTAGCAGTGTAAGT T CCCCCCTCCCCC
C1S2	ACTTAACTGCTAGCCGA TT CCCCCCTCCCCC
C2S2	TCGGCTAGCAGTGTAAG TTT CCCCCCTCCCCC
C1S3	ACTTAACTGCTAGCCGA TTT CCCCCCTCCCCC
C2S3	TCGGCTAGCAGTGTAAGT TTT CCCCCCTCCCCC
C1S5	ACTTAACTGCTAGCCGA TTTT CCCCCCTCCCCC
C2S5	TCGGCTAGCAGTGTAAGT TTTT CCCCCCTCCCCC
C1S10	ACTTAACTGCTAGCCGA TTTT TTTT CCCCCCTCCCCC
C2S10	TCGGCTAGCAGTGTAAGT TTTT TTTT CCCCCCTCCCCC

Fig. S1: Typical AFM images and their corresponding cross-sections of multimers produced by mixing of C1S0 and C2S0. Scale bars are 200 nm.

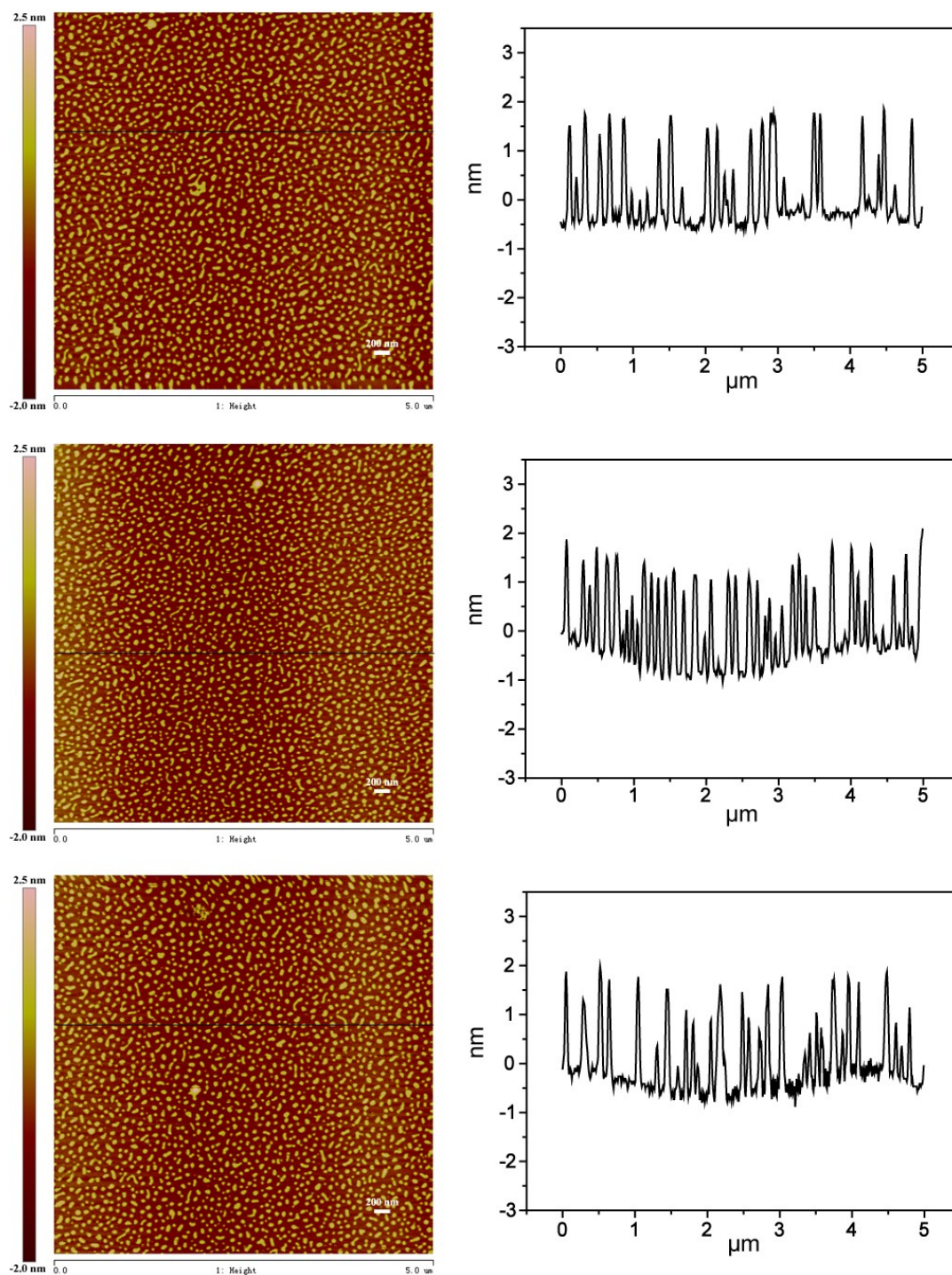


Fig. S2: Frequency distribution of length of multimer products produced from C1S0/C2S0.

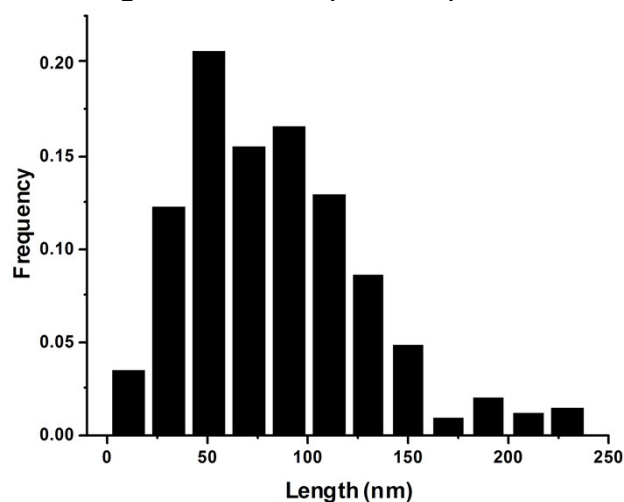


Fig. S3: (A) Native PAGE analysis of assembled products by mixing respectively annealed C1S0 and C2S0 at pH 5.0 (lane 3). Lanes 1 and 2 represent C1S0 and C2S0, respectively. (B) Native PAGE data of C1S10/C2S10 mixture (lane 6), for verifying formation of monomer structures. Lanes 4 and 5 represent C1S10 only and C2S10 only, respectively.

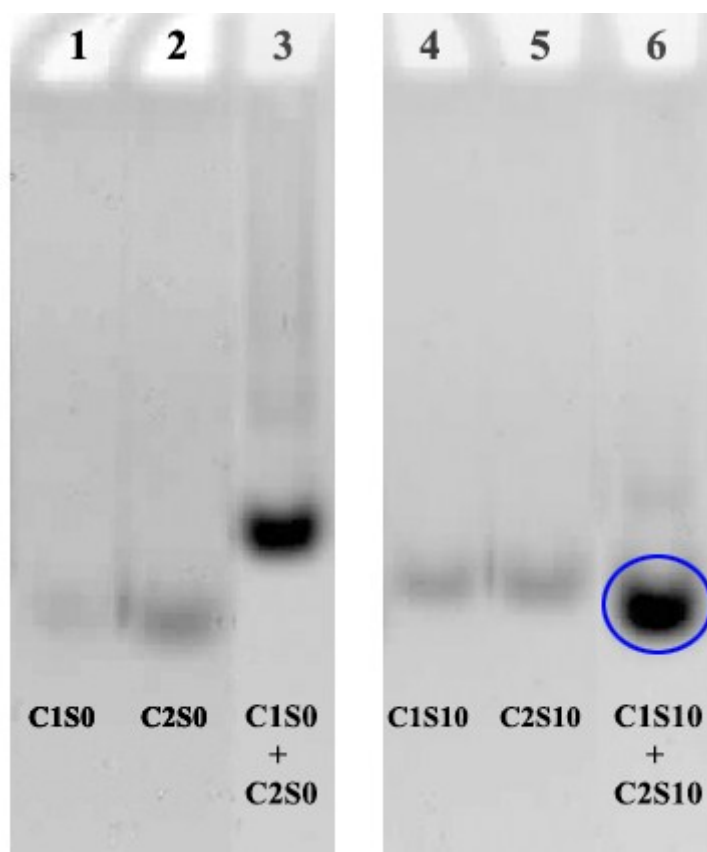


Fig. S4 The AFM images and their corresponding cross-sections of dimers produced from C1S2/C2S2 (A and B), and monomers produced from C1S10/C2S10 (C and D). Scale bars are 200 nm.

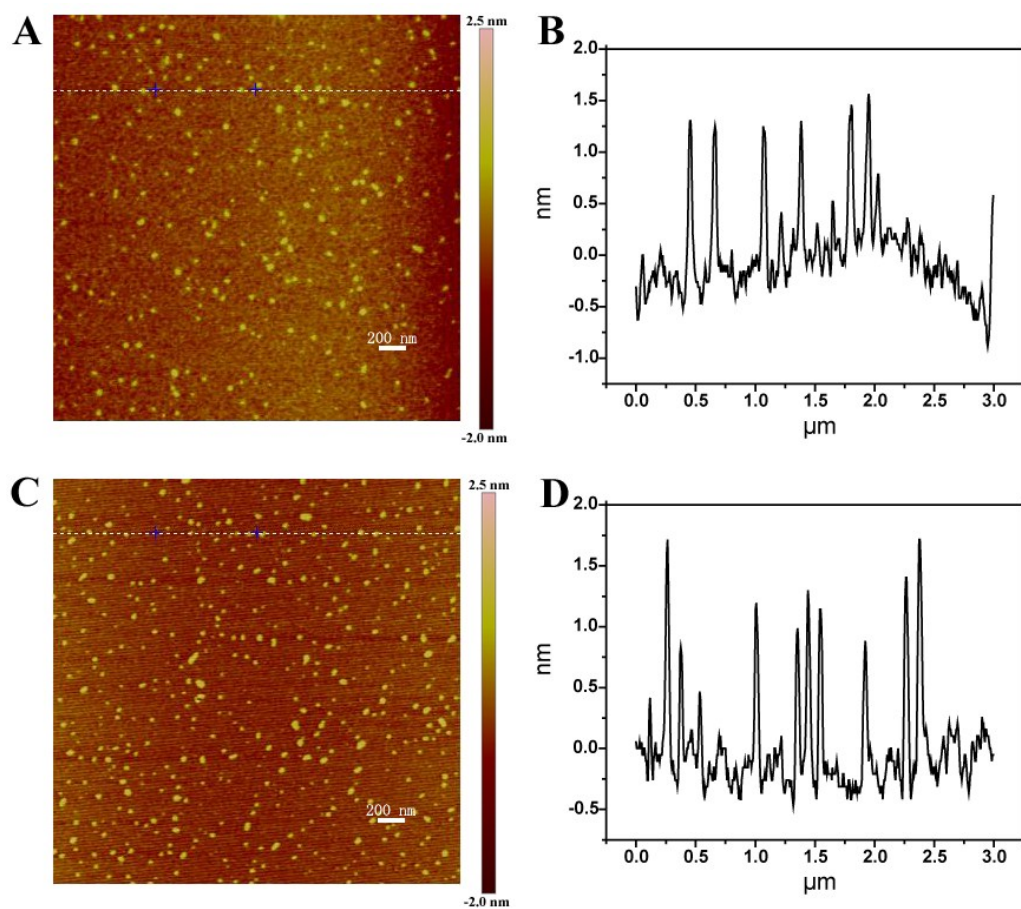


Fig S5: CD spectra of mixtures of C1S2/ C2S2 (A) and C1S5/ C2S5 (B) at pH 5 and pH 8, for demonstrating the formation of i-motif structures.

