

Supplementary Materials

Protein-Assisted Room-Temperature Assembly of Rigid, Immobile Holliday Junctions and Hierarchical DNA Nanostructures

Saminathan Ramakrishnan, Sivaraman Subramaniam, Charlotte Kielar, Guido Grundmeier, A. Francis Stewart, and Adrian Keller

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1. Red β -mediated annealing and purification of 88mer HJ assemblies.

12.5 μ l of each 88mer ssDNA (1 μ M) were pipetted into four individual sterile 1.5 ml Eppendorf tubes. 370 μ l of 87 μ M Red β (pre-incubated at 37°C for 20 minutes) was pipetted into each tube and the final volumes were adjusted to 1250 μ l by addition of 870 μ l of sterile B1 buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, and 1 mM MgCl₂). The reaction contents were mixed well by gentle pipetting and incubated at 25°C for 20 minutes. Afterwards, the contents of all four tubes were mixed in a 15 ml Falcon tube (Fisher scientific) and incubated overnight at 25°C. Following overnight incubation, the reaction mixture was loaded using a 5 ml sample loop onto the preparative superdex 200 column (16/600) (GE lifesciences) connected to Äkta purifier (GE healthcare). The column was pre-equilibrated with B2 buffer (100 mM Tris-HCl pH 8.0, 150 mM NaCl and 1 mM MgCl₂). Preparative size exclusion chromatography was carried out at 1 ml/minute flowrate. The eluting fractions were collected and based on the profile of the peaks, only the summit fractions from the peaks were analyzed and pooled. Purified Red β -assembled HJs were analyzed by AFM imaging as well as on 8% native PAGE or 4-16% gradient BlueNative PAGE gels followed by colloidal coomassie staining to visualize protein. 2% sodium borate agarose gel electrophoresis was carried out in order to visualize DNA.

2. Gel analysis of the purified and enriched 88mer HJ structures and corresponding AFM images.

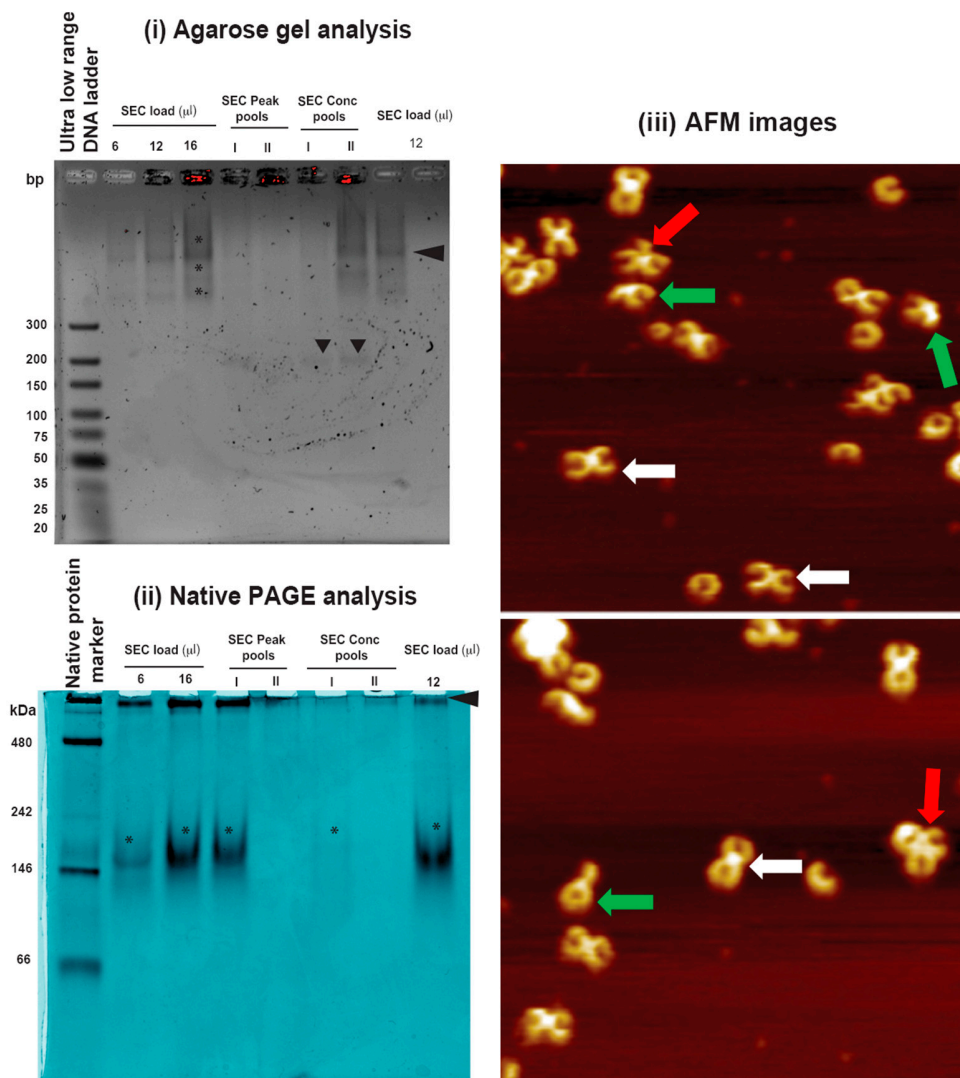
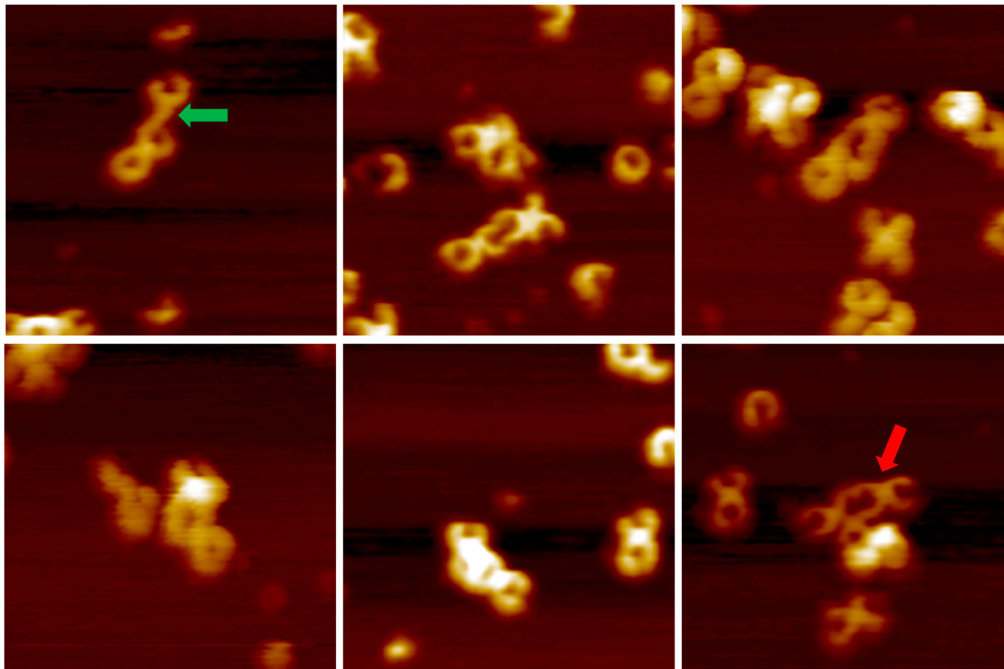


Figure S1. Purification and enrichment of single HJ species using SEC. (i) 2% sodium borate agarose analysis shows the concentrated HJ structures after purification using SEC. The asterisks (*) indicate the HJ structures. The lower arrowheads (faint bands) indicate the free DNA. The black sideways arrowhead depicts the HJ structures imaged by AFM. (ii) 8% native PAGE analysis shows the SEC purified HJ structures. The asterisks (*) and the black sideways arrowhead indicate different HJ structures. (iii) The AFM analysis of purified 88 mer HJ species shows completely formed structures (white arrows) as well as structures missing single arms (green arrows) and structures with additional arms (red arrows). The C-shaped structures represent DNA-protein filaments that did not participate in the annealing of complementary sequences and HJ formation. These individual C-shaped structures often interfere with other structures that are getting annealed and create HJ structures with multiple arms. The AFM images are 300 x 300 nm² in size and 4 nm in height.

3. Additional AFM images of hierarchical structures after SEC purification.

(i) Three-ring structures



(ii) Other hierarchical structures

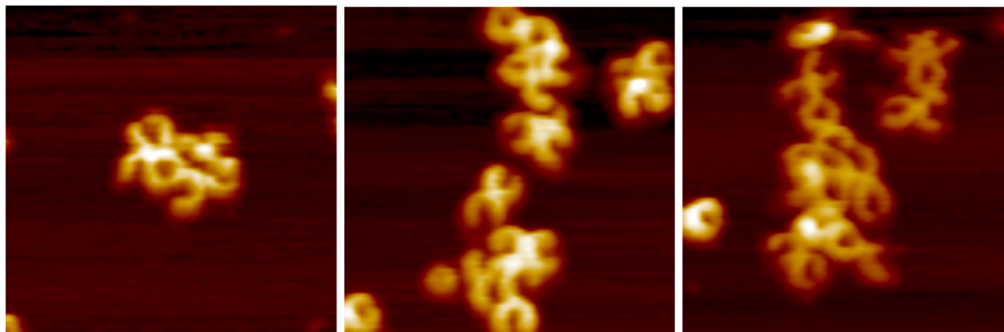


Figure S2. AFM images of concentrated and purified hierarchical structures after SEC. The AFM images show mainly two different structures, *i.e.*, (i) three-ring structures and (ii) irregular hierarchical structures. The three-ring structures are often observed with an arm missing (green arrow) or additional arms present (red arrow) due to non-specific annealing. The irregular hierarchical structures are the result of non-specific annealing of free C-shaped DNA-protein filament in the SEC pool. The examples are given to show the annealing efficiency of Red β at room temperature. The AFM images are 200 x 200 nm² in size and 4 nm in height.

4. AFM images of individual 88mer HJ structures and hierarchical structures without purification.

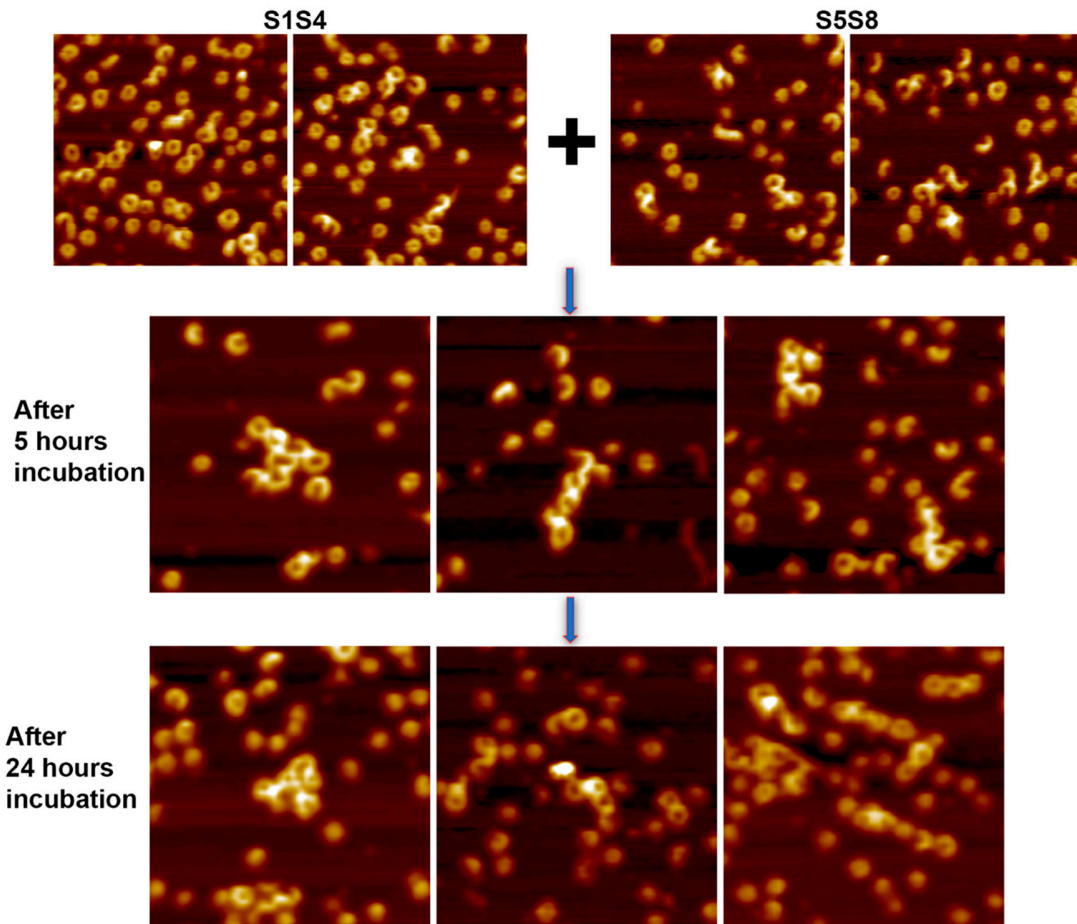


Figure S3. The AFM images of S1S4 and S5S8 show individual HJ structures with sticky ends, assembled in the one-pot annealing reaction without purification. Many completely formed HJ structures are observed but accompanied by additional Red β rings and C-shaped DNA-protein filaments. The AFM images of hierarchical structures assembled after 5 hours and 24 hours reveal many irregular structures, which are the result of non-specific annealing of free C-shaped DNA-protein filaments in the pool. Purification and enrichment of individual HJ species are therefore necessary to remove the excess of Red β rings and C-shaped filaments. The AFM images are 300 x 300 nm² in size and 4 nm in height.

5. Oligonucleotide sequences.

Table 1. ssDNA sequences used for 44mer HJ structure

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	CGATGCGAAATCTGTACGCGCACCACGTGAAGGTGCTAGTCCCC	59	44	S1
2	GAATGTGTTGATGGTTAAGTTCTGCGCGTACAGATTTTCGCATCG	46	44	S2
3	GTGTCTATCATTAGCGGCTCAAGAACTTAACCATCAACACATTC	41	44	S3
4	GGGGACTAGCACCTTCACGTGGTTGAGCCGCTAATGATAGACAC	55	44	S4

Table 2. ssDNA sequences used for 66mer HJ structure

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	ATGCCGCATACAACAAGTGGGGGGATGAATAGGATGTACTGCCT TTTTTATCGACTGTCCAATATG	44	66	S1
2	CATATTGGACAGTCGATAAAAAAGGCAGTACATACTTGGTCCACT CTGGTACAAGATGAGGTGGAT	42.43	66	S2
3	ATCCACCTCATCTTGTACCAGAGTGGACCAAGTAGTGCGCGATT GACGTTTCGAGGCGGACTCTCCT	55	66	S3
4	AGGAGAGTCCGCCTCGAACGTCAATCGCGCACTCCTATTCATCC CCCCACTTGTTGTATGCGGCAT	56	66	S4

Table 3. ssDNA sequences used for 88mer HJ structure

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	CAGTTCCTTAAGACATCGACGTAGGACCTTGATCAACGCAGCTAT GACTTCAGAGATTCTCTTATCTGGAAGCGTCCACGAACCGGCC	52	88	S1
2	GGCCGGTTTCGTGGACGCTTCCAGATAAGAGAATCTCTGAAGTCAT TTGTACACTTTTCTATTACGAGCAAAGCCTGGTATACTAGAA	45	88	S2
3	TTCTAGTATACCAGGCTTTGCTCGTGAATAGAAAAGTGACAAACC CACATTGCCATCGGGCGAAATGGGGTGTGCAGCGCATGCTCG	50	88	S3
4	CGAGCATGCGCTGCACACCCCATTTGCCCCGATGGCAATGTGGGT	56.8	88	S4

	AGCTGCGTTGATCAAGGTCCTACGTCGATGTCTTAGGGAAGT			
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Table 4. ssDNA sequences used for hierarchical structure assembly (S1S4)

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	GATTCCTCCGGGCGAATTTACCCAGTGAACGCTGGTCCCCGC GGTCATGCTGAAGGCGATAGTA	58	66	S1
2	GCGGGGACCAGCGTTCCTGTTATTGGGTATGACGAGCTTAG ATTTGGTATATGGTCTCGACTA	50	66	S2
3	TAGTCGAGACCATATACCAAATCTAAGCTCGTCATACCAATAAG ATGTACGTTGGCGGACAGTTACATATTGTGCGCTGCCGGTGGC	48	88	S3
4	GCCACCGGCAGCGCACAATATGTAAGTGTCCGCCAACGTACATC GTAAATTCGCCCCGAAGGAATCCCGTAACGAGGCCACATTTTG	53	88	S4

Table 5. ssDNA sequences used for hierarchical structure assembly (S5S8)

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	ATGTTGATCGGGAGAGGTCGAACCCTCTTTGATATTTGTGACTTT CGGGGCAACTATTTCTCGGCGACCACGTTCCGGTGACGGACCT	52	88	S5
2	AGGTCCGTCACCGAACGTGGTCGCCGAGGAAATAGTTGCCCCGA CAGGAAGCTAACTAACATGAGACAAAAATGTGGCCTCGTTACGG	53	88	S6
3	TTCATGTTAGTTAGCTTCCTGACGCCTGGCCGTATCTTCGGCTT ACTATCGCCTTCAGCATGACC	52	66	S7
4	AGCCGAAGATACGGCCAGGCGTAAGTCACAAATATCAAAGAGGG TTCGACCTCTCCCGATCAACAT	50	66	S8

Table 6. ssDNA sequences used for hierarchical structure assembly (S9S12)

No.	Sequence (5'-3')	GC (%)	Length	Identifier
1	CCAATACGTGCTCGCCGTCCAGCGACTCTTAATTGCGTTGGTGT TTTTGAGTGAGTCTCTAACCCG	51	66	S9
2	CGCCAGTCGTCTATGGCGCTGACGGGTTAGAGACTCACTCAAAA AGGCGATGGTGAGCATGTCTGG	56	66	S10
3	CTGGACGGCGAGCACGTATTGGCCAGACATGCTCACCATCGCCT ATCATAAGCGCACCCGCTGCCC	62	66	S11
4	TCAGCGCCATAGACGACTGGCGGGGCAGCGGGTGCGCTTATGAT ACACCAACGCAATTAAGAGTCG	58	66	S12