



## Supporting Information

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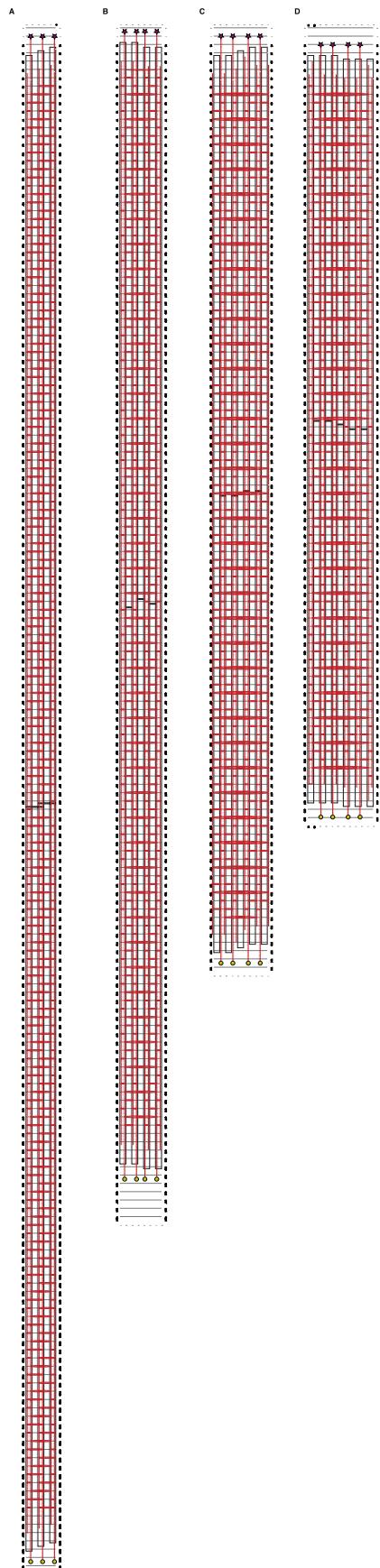
69451 Weinheim, Germany

## Rigid DNA Beams for High-Resolution Single-Molecule Mechanics\*\*

*Emanuel Pfitzner, Christian Wachauf, Fabian Kilchherr, Benjamin Pelz, William M. Shih,  
Matthias Rief, and Hendrik Dietz\**

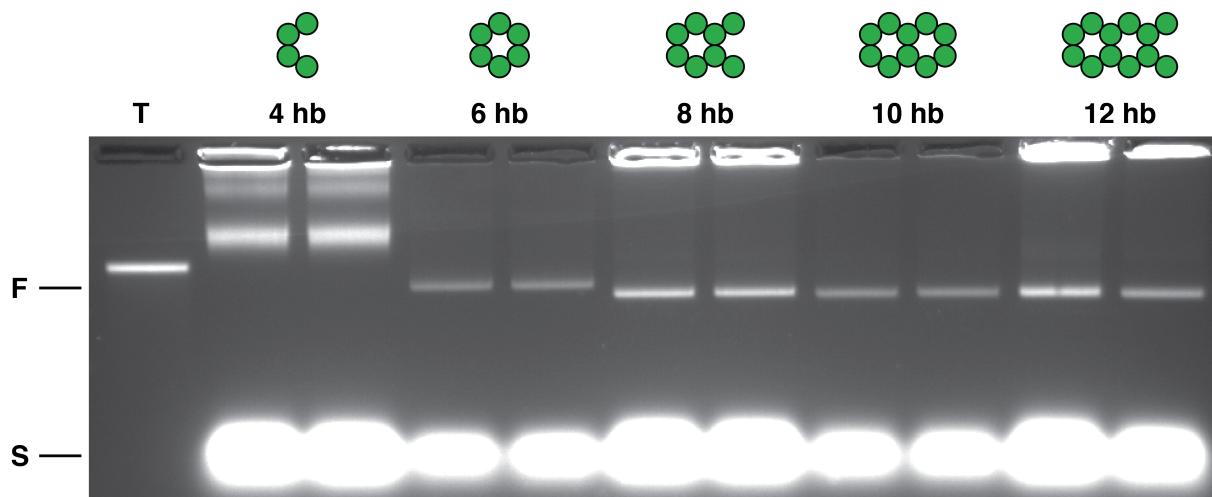
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Figure S1



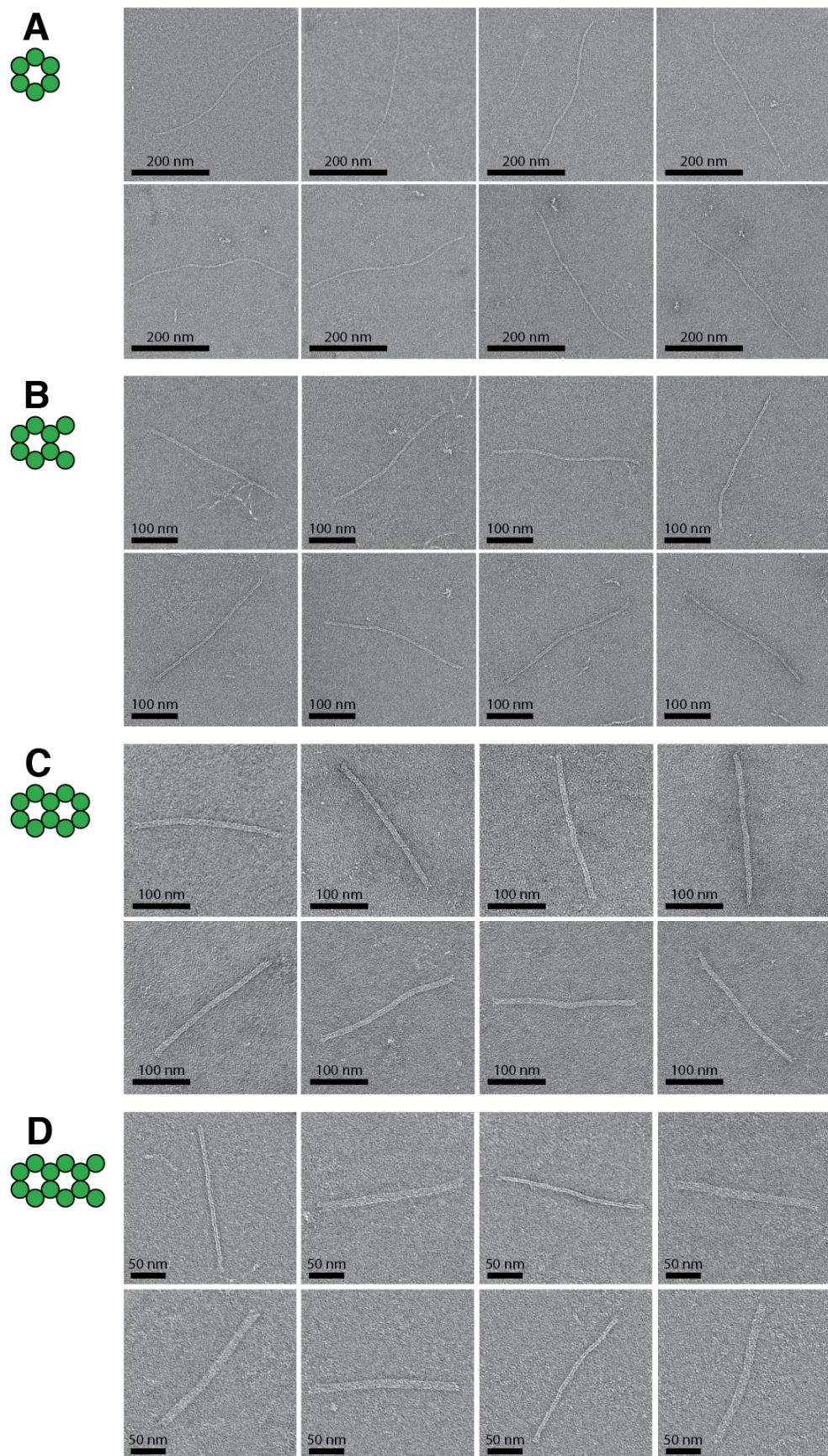
**Fig. S1:** caDNAno design diagrams. Scaffold strand path is depicted with black lines, and staple strand paths are shown as red lines.

Figure S2



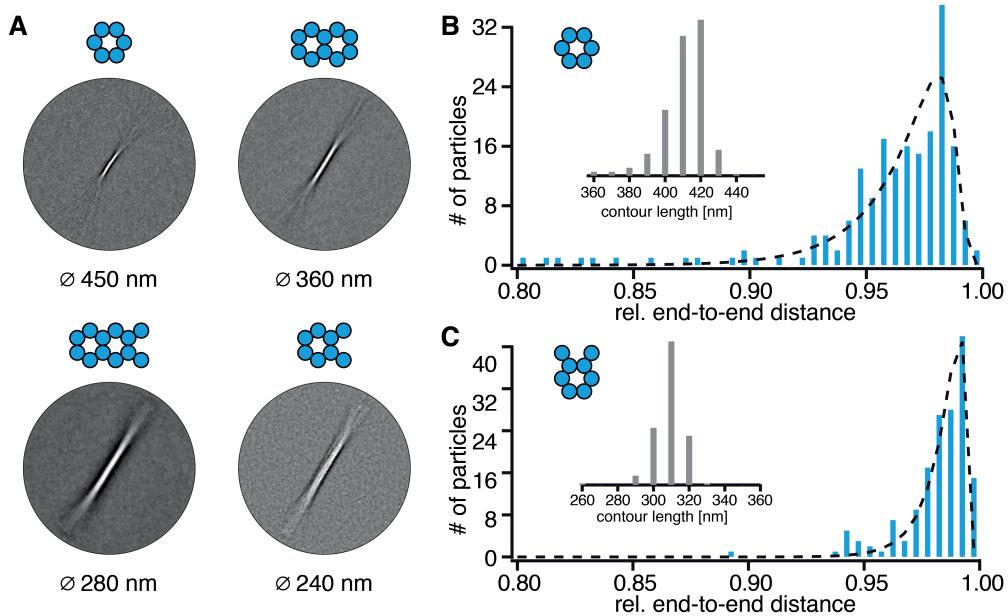
**Fig. S2:** Gel-electrophoretic bundle folding quality assessment. Photograph of a 2% ethidium-bromide stained 2% agarose gel on which a reaction products were electrophoresed. The gel also includes a four helix bundle not described in the main text. The low electrophoretic mobility of the folded species as compared to the other bundle types is due to the high flexibility (random-coil-like) of this bundle type. The four helix bundle was thus not considered for usage as a linker system. "T" marks the lane on which the template strand was electrophoresed. "F" marks the folded species, "S" excess staple strands.

Figure S3



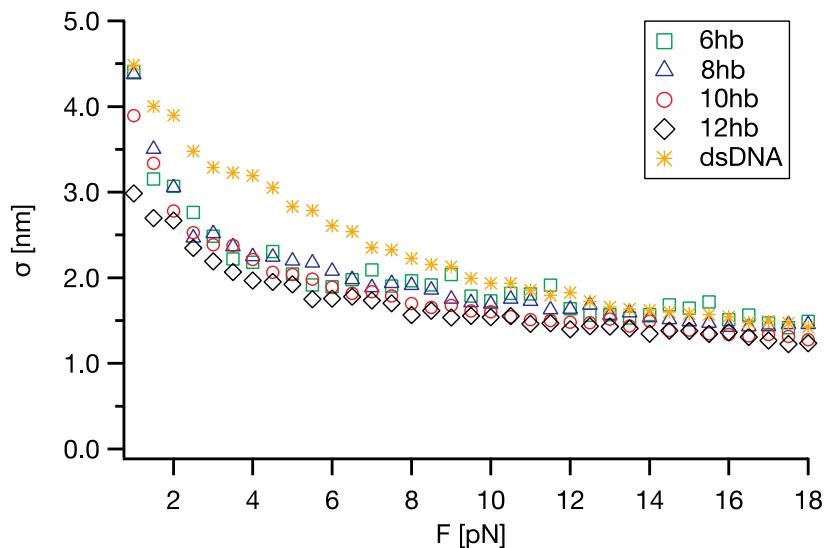
**Fig. S3:** Exemplary negative-stain transmission electron micrographs of purified DNA helix bundle particles.

Figure S4



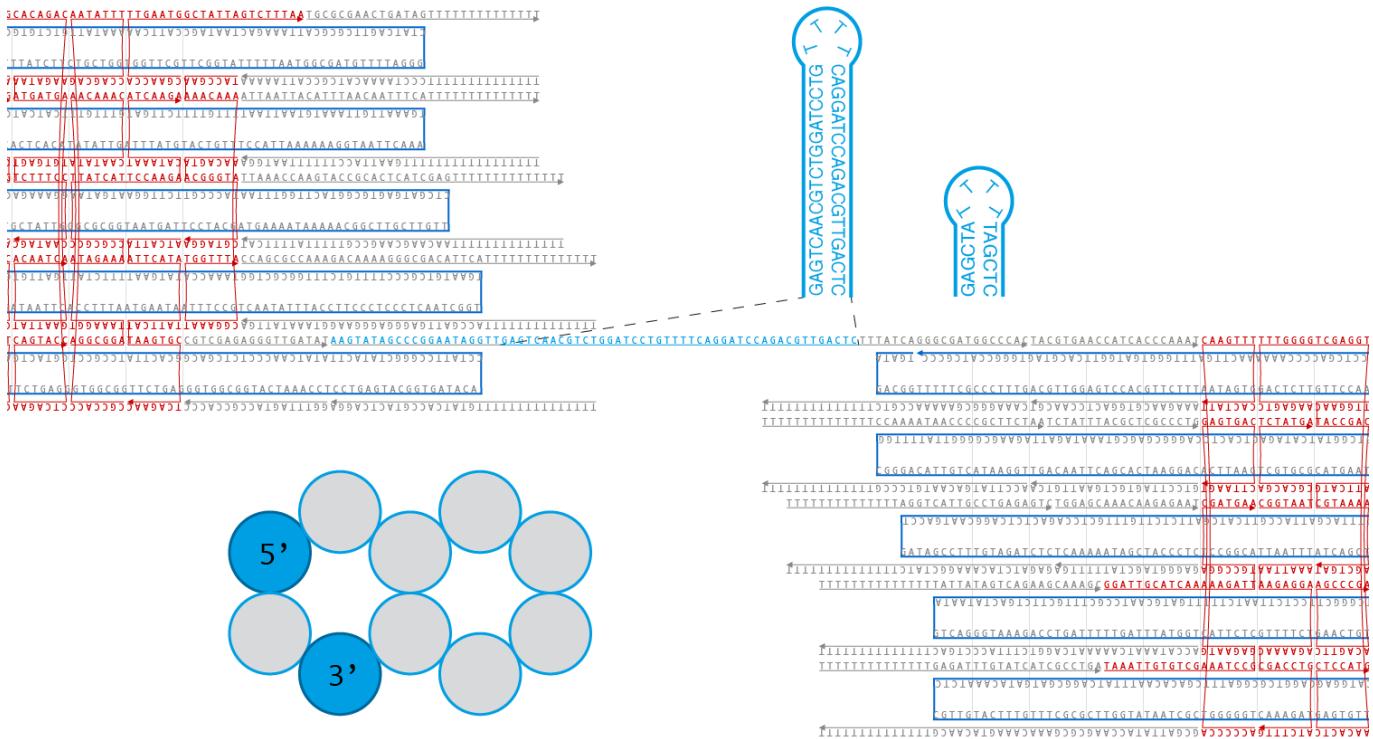
**Fig. S4:** (A) Average TEM-micrographs obtained by rotational alignment of single particle micrographs of each bundle type against a randomly chosen reference micrograph. (B,C) Blue Bars: Histograms of the relative end-to-end distance observed for individual six (B) and eight (C) helix bundle particles. The absolute contour lengths (inset, grey bars) were determined by manual backbone tracing, where the two endpoints of a backbone trace were used to determine the absolute end-to-end distance. Dashed lines: fit by a semi-flexible beam model. <sup>[10]</sup>

Figure S5



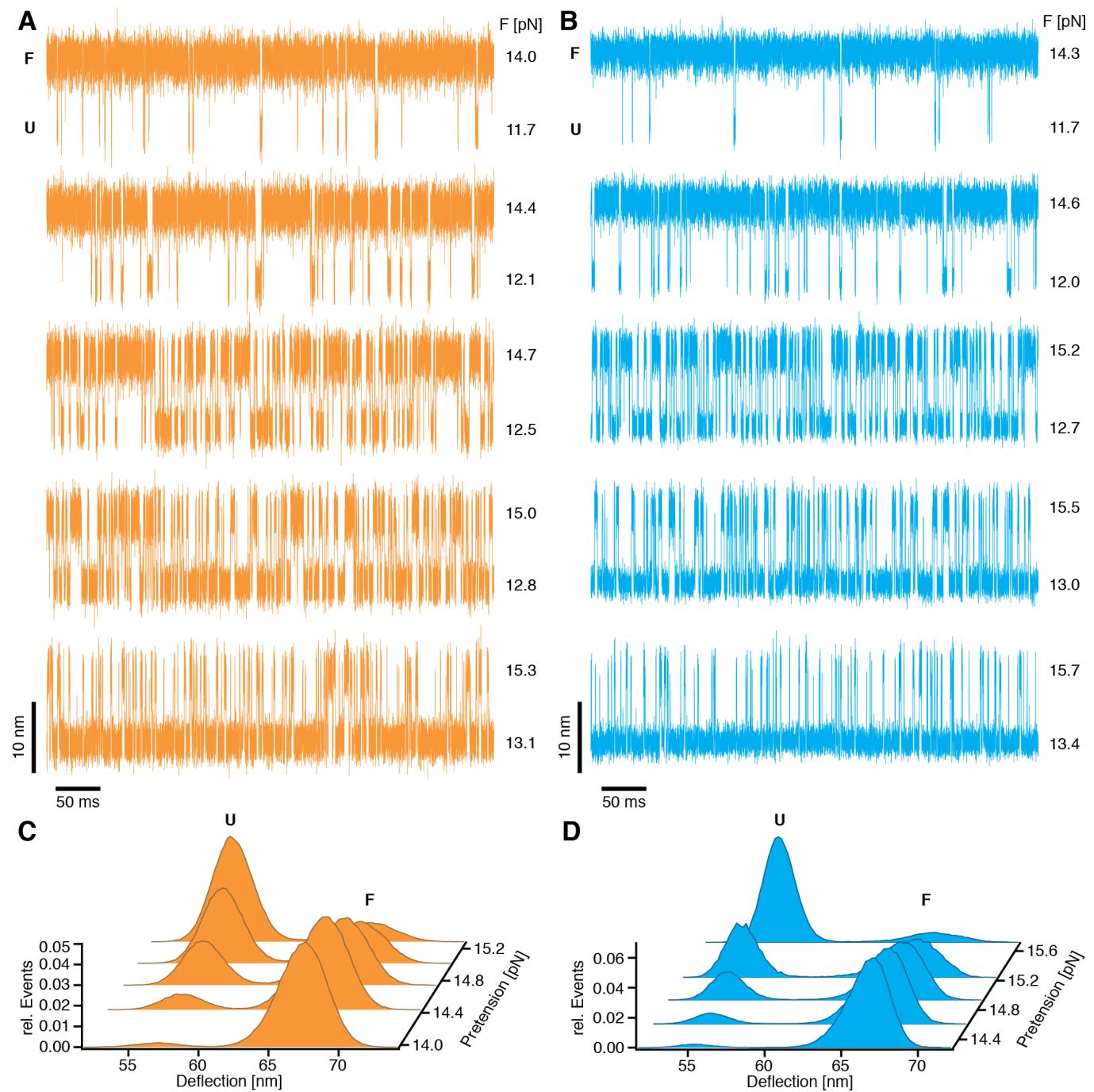
**Fig. S5:** Force-dependent noise-amplitudes for stiff DNA helix bundle linkers vs. conventional dsDNA linkers.

Figure S6



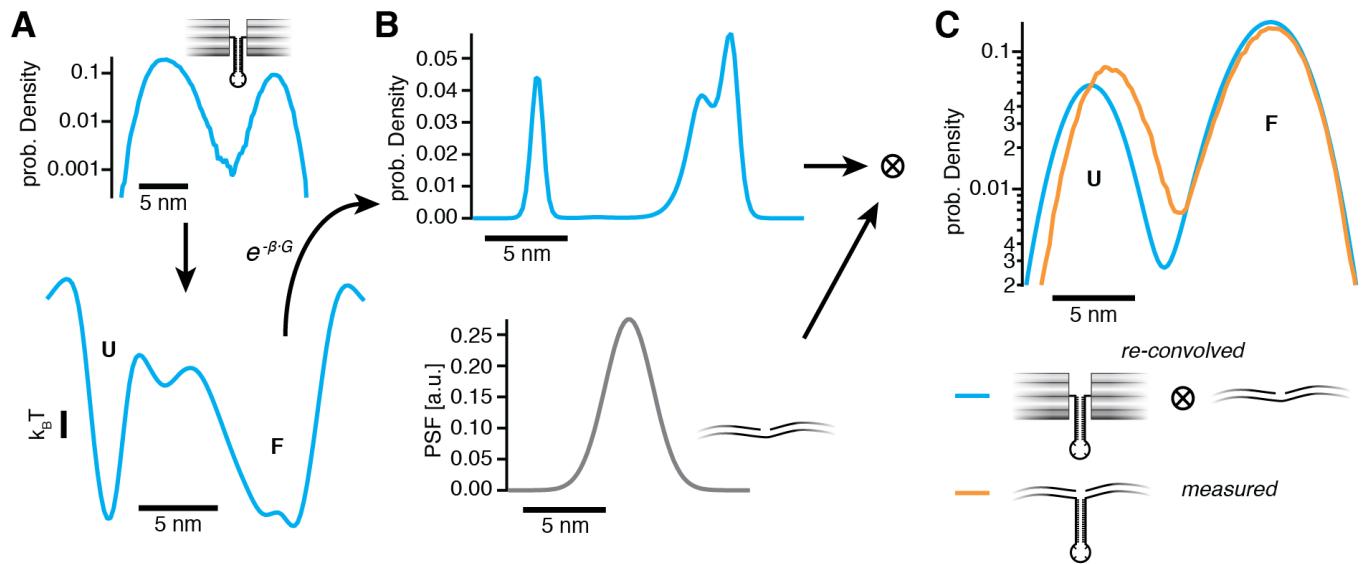
**Fig. S6:** Design diagram for hairpin-helix bundle constructs used for the experiments described in figures 3 and 4.

Figure S7



**Fig. S7:** Additional constant-distance data as in Fig. 3 obtained for different loads. Orange traces: data collected with conventional linker system. Cyan traces: data collected with stiff ten helix bundles.

Figure S8



**Fig. S8:** Schematic explanation of the workflow for deconvolution of a deflection histogram to extract the energy landscape for the 20-bp DNA hairpin from figure 3, followed by reconvolution using the point-spread-function of the conventional dsDNA handle system.

## Sequence Information

### 6 helix bundle

Start	End	Sequence	Length
0[370]	4[357]	CCCGCTTAAATTAAGTGTATCAAAAGGGTTAAGAGGCCAAC	42
0[412]	4[399]	TAATGAGTAAACGTCATAATTTAAAGGCTTCGGAAAG	42
0[454]	4[441]	AACATACGTGGAAGCCCTTATTTCAAGGATAATTGT	42
0[496]	4[483]	CTGTTCTAAAGTGTTGTACCAAATCATTTGGCGAA	42
0[538]	4[525]	GATCCCCCGGCCAACGAAATAAGCAAGCTGTAGACACTA	42
0[580]	4[567]	GGCTTAAAGGAGTGCAGTAACTCAACCAAGCTGCGGACACTAC	42
0[622]	4[609]	TCTCCAAGGAAAGCAACGGCGAGTAAAGGCAAGCTTCA	42
0[706]	4[693]	AATTTAAAGGCTTAAATTAATCAAAAGTAACTGAGGAA	42
0[748]	4[735]	ACTTTTGTTAGGICACATTCACCGATTGGCACCGTATTC	42
0[790]	4[777]	ATGCTGATGTAATAGAAAATCATATGTGACACTTGGCG	42
0[832]	4[819]	TTTAACTATAAAAGGCAAGAACACACCCCTTATGCTT	42
0[874]	4[861]	CAATAGFCGACATCTGAGAAATAGCATGAAACCGAGGTC	42
0[916]	4[903]	TGAAAGACTTAACTACGGCATGTTAAAGGCCCTCAGATGCG	42
0[958]	4[945]	TCTGTAACATCTGCGGACAGGAGGAAACCCAGACAGT	42
1[185]	3[398]	TTTTATACGGCTATCGGTTTAAATCCTGAACTGCGTC	42
1[427]	4[440]	TCACGCATTCGGCAACATTCACCGAACAGCAACAGGAA	42
1[469]	3[482]	ATAACACAAACCGCTTGTATAAGGATTATGATCTGG	42
1[511]	3[524]	TITCTGCTCATTTTGTGCTAAATAAAAGCCCTTATG	42
1[553]	3[566]	ACAGGAACCAACCTAACTAAAGTGGTTAACTATTC	42
1[595]	3[608]	ATCTGCTTCCCCTATTTCAAACTTGTGTCGACGGCTTC	42
1[679]	3[692]	GAACCCCTTCAGCAGACCATTTACAGGAAATGTTAGT	42
1[721]	3[734]	AATATTTAGGCTAACCTGATGAGCAAGGGGAGGCAACG	42
1[763]	3[776]	AGGCCAACACAGCTGGCTTACCTGGCTTAAACAC	42
1[805]	3[818]	GCAGGAACTTCTTCTCGCTCATAGCGGAATACAGTAA	42
1[847]	3[860]	CCIGAAACGCCCTAAATACAGGAAATACCCAAATTC	42
1[889]	3[902]	TCTAAAGTTTCTCTCTAGAGCCGCAACACTTCAGAACG	42
1[931]	3[944]	ATCAATAGGACGACACCACAGGCGCAGAAATAGAAAAT	42
1[384]	0[371]	AATGTTAGGAAACGACACCACAGGCGCAGAAATAGAAAAT	42
1[426]	0[413]	AATTTTGGAAACCCCTGATGAGCAAGGGGAGGTTGGGTC	42
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1[594]	0[581]	TAATTAACCTAACAGGGTAAACACATTTTGTAAAGG	42
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1[804]	0[791]	TITCTGCTTAACTGCAACAGCTGATGAGCAACAGCTGT	42
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# 10 helix bundle

Start	End	Sequence	Length
[1][31]	[8][38]	CCACTACGGTAAACCATACCCAAATTGGGATTATAACCGCCG	43
[1][385]	[8][399]	AGAAGTGGGGTTAGTCAGGACGCCAACAGAGGCCCTTA	42
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[0][90]	[9][97]	AGTGTGAGGACAIAATCGGAAGAGGACGAGGCG	35
[0][111]	[9][18]	GAATAGCAGGGAGCCCCGAAAGGCACAAAGGAA	35
[0][132]	[9][19]	AAAATCTTGACGGGAAAGCCAATACCTTGA	35
[0][153]	[9][160]	GATGTCGGTGAGAAAGGGGAAGTCGGTGT	35
[0][174]	[9][181]	CAGCAGGGAAAGGAAAGGAGGACTAAAGGCTGG	35
[0][195]	[9][202]	GGCGCTCTAGGCCGCTGGCAACAGCGGAGTA	35
[0][216]	[9][223]	GCCCTGAGGTCACGCTGGCGCACAGCATGATATT	35
[0][237]	[9][244]	CTATGTCACCCGGCGCGTACCGTAA	35
[0][258]	[9][265]	CAACAGTGGCCGAAAGGCGTAAAGGCGTAA	35
[0][265]	[9][265]	TCTCTTACGAGGTAACAGGAA	28
[0][279]	[9][286]	CGCCAGGTGGCTTACGGGAAAGGCAAAATCTTGA	35
[0][300]	[9][307]	AGAGCGGATAACGCTTACGGCATAATAATIG	35
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[0][342]	[9][349]	CTGICGTGGAGCCGATTAATGATCATGAA	35
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[0][426]	[9][433]	AACGTTAAAGAGTCAGGAAAGGAGCAGGCC	35
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[0][468]	[9][475]	CTTACATCTTGTAGTAAACAAATAAT	35
[0][489]	[9][496]	TGAGGATCACTGGCTGAGTAAAGGGAGA	35
[0][510]	[9][517]	CGCGCTACAACTATCGCCAAACAAATTC	35
[0][531]	[9][538]	ACTAACATAATTCGAAAGAACATTCATACAT	35
[0][552]	[9][559]	ATCTAACCGCCGCTTACGTCAGCAGGAGT	35
[0][573]	[9][580]	TTAGGAGGAAAGGCTGGCACAGACCTTAAAC	35
[0][594]	[9][601]	GTCAGITATTGTCAGCTAACAGCCGGAAAC	35
[0][615]	[9][622]	CAAAACCGGAAAGGATTAAACGGCCCTGAGTAA	35
[0][636]	[9][643]	ACTTCAGGAGATGCTGGACTTCGCGGAA	35
[0][657]	[9][664]	AAAATGAGCAGTAAATAAAGGGCAATGAA	35
[0][678]	[9][686]	GCAACCGGCCAACAGGATAGTACGAGGACTC	36
[0][699]	[9][706]	ATTAACATCTGACCTGAAAGGCCAACATAGGATT	35
[0][720]	[9][721]	AACAGAGTACGGCAGACAGCTAACAGACGACC	35
[0][741]	[9][756]	CGAACCAITGAATGCTTAACTTAA	29
[0][748]	[9][748]	TACCGGAAAGAACGAAAGGATATGGTTA	28
[3][31]	[9][37]	TCTCTAATCTTACGGCTGGACTCAAC	30
[2][55]	[9][34]	GTCTTAAAGGAGTGGGGTTTGTAGGAGA	36
[2][391]	[9][391]	ATTGACATCGGAGGAGTGGACTAATGGAGATTT	42
[2][748]	[9][742]	AACAGTAAACGGCTGGAG	21
[5][24]	[9][242]	ATTGGCGAGACTGGAGAACAGTGTGTA	32
[5][63]	[9][69]	CGGTAACTGGCAAGTATGTA	21
[5][252]	[9][531]	GCATTTGCAAACTATCA	21
[6][69]	[8][631]	AAATTAAAGGGAAAGGAAACCGGCTGATCTTG	35
[4][90]	[8][84]	TATICAACAAATATCIAATGGGAAAGAAT	35
[4][111]	[8][105]	GTCTTAAAGGAGTGGGGTTTGTAGGAGA	35
[4][132]	[8][126]	GGGGAGGGAGGAGCTTACGGGAGGCCGATA	35
[4][153]	[8][147]	TGTGTAGAGGATTTAAATGAGTGAATCCATTA	35
[4][174]	[8][161]	TTAAATGTTGCTCTTGGAGGCGCATAGTACCTT	35
[4][195]	[8][189]	TTTATGCACTTATAGGCTTACATCAATACAG	35
[4][216]	[8][210]	ATTCGAACGTTAACTGAAAGGAGGAAACG	35
[4][237]	[8][231]	ACTTTCGCTGACATACCCAACTACATCGACA	35
[4][258]	[8][252]	AAAACATATATGACATAGTATCATCAGCCTT	35
[4][279]	[8][273]	AAAGCTATCTGGAAACGCCAACCTGCGCTTGC	35
[4][300]	[8][294]	AGCAAAACAGTACCTGGGAGGCCGATA	35
[4][321]	[8][315]	GGCAACGGGAACTTGGAGATGATGTTAACACC	35
[4][342]	[8][336]	TCCAATACCATAAACGAACTATGAGGATTT	35
[4][363]	[8][357]	CTAATAGTGTCAACAACTGAGGTTTCTTCA	35
[4][384]	[8][378]	GAAGAAAGGTTATTTAAATCTACCATCAGCTT	35
[4][405]	[8][405]	GAATTAACTGAAAGCTTAATGAGAAGAATAAAACAA	42
[4][426]	[8][420]	AGGGAGTGGCCGAGGCCAACGGGAAAAAAA	35
[4][447]	[8][441]	CAAGAGGATACCCACCCGGCAGTA	35
[4][468]	[8][462]	AAAATGAAAGCCAAAACCGCCCAAAACAAAGGAA	35
[4][489]	[8][483]	CGATTTAACATCGGAAACAGCAGTGA	35
[4][510]	[8][504]	TATCCCAATCTTACTTCTTCTGACTTCAA	35
[4][531]	[8][525]	AAAATAAAGGAAAGGCCCTTAAAGGTTA	35
[4][552]	[8][546]	AGAGCTTAAACAAAGCTTGGCTGATTAACGTTAG	35
[4][573]	[8][567]	AAAGCTTACCGGAGGACGTAAGATAAGAAAGTTT	35
[4][594]	[8][588]	ATTITAGGAAATCACGAGTCGCTTCTATGT	35
[4][615]	[8][609]	TGCTTATGAGTACAGCACCAGTAAAGTGGAT	35
[4][636]	[8][630]	AGCGCTTGCGATTAACGCTTACGTCATGACT	35
[4][657]	[8][651]	CCCGACTAAATACCTAACGTTACGTCACCGTAAAC	35
[4][678]	[8][672]	GGCGGGCAACATTAACAGGAGCTGGCCAA	35
[4][699]	[8][693]	CTTATCAGGACACCCATTGGGAGAAGGATCTCCCT	35
[4][720]	[8][714]	AAGCGAAATTCTGCTACCGGCTTTCGCGCC	35
[4][741]	[8][735]	ATCATTAATCATATTTATTAATGAGTGGCTGAAAC	35
[4][769]	[8][759]	CGAACCGCTTATTTATTAACCAAGAT	32
[7][44]	[5][62]	GGATGAGTCATTAAGGATTTGGCGGAGGATGAA	33
[7][71]	[3][90]	AAAGACTTCGTTCTCTAGCACTTAACTGGCTTGC	42
[7][88]	[3][111]	TAATTCGACCACTCCCGGGTTGATATAAGGCCAC	42
[7][119]	[3][132]	CGACGAAAGGCTGGGAGCTGGGATGAA	42
[7][140]	[3][153]	ACAGCTGCAAAAGATAAAAGGAGGATCTGGCTT	42
[7][161]	[3][174]	CTTAAACAACTGCTTAAACGGTAAATTAACGGCTT	42
[7][182]	[3][195]	AAAGGGTAACTCTTAACTGGATTAATGCAAGGGTTAAT	42
[7][203]	[3][216]	GCTTACGGAGGAAAGATCAGCTTACGGCTGTAAGCTA	42
[7][224]	[3][237]	TATAATGGGGAGAATAGGAAACGGCTATGGGCTCTCGT	42
[7][245]	[3][258]	GTITTAATGACCACTTGGGAGGAGGAGGAGT	42
[7][266]	[3][279]	TAACGGTGTGGCTTACGGACTTACGGTCAATG	42
[7][287]	[3][300]	TCCATTAAGCCGCTTAAATGAGGCGTAAACCGTCTG	42
[7][308]	[3][321]	ATTCCTGAAATTGCGCTGGATTCTGGGACCCGCTCA	42
[7][329]	[3][342]	TAGTTGAAATCATAAAGGCTTAAAGGAGTGGCTGAAAC	42
[7][350]	[3][363]	TTGGCAATAGGGGGTAAAGCTGCTGAGCTGATGAA	42
[7][371]	[3][385]	GTIILAGCCTGGCATGGATGGCTGATGCGTAA	42
[7][392]	[3][392]	GCCCCCTGAAACGGCCTAACAGGCTTGGGGACCGGA	42
[7][413]	[3][426]	GGGTAACTGGCTTAAAGTGTATGAGGAAATGGGGAA	42
[7][434]	[3][447]	AGAGGAAAATACAAATCTTACAGCTGGTTGAGTAA	42
[7][455]	[3][468]	IGATGAAATACACACAGCTTACAGGAGGCTT	42
[7][476]	[3][489]	AGCAAGATGTTAAATGGAGAATGCGCAAAATTTGGAT	42
[7][497]	[3][510]	AACTGCTATCAACAAACGGCACAATGAGAACATGAA	42
[7][518]	[3][524]	CTTITTAACAGGAGGAG	21
[7][539]	[3][552]	ATAGCGGAATTGGGTTAAAGGAAATATACTAAAACAG	42
[7][560]	[3][573]	AAAGGAAACGGGCTGGCAAGGTAATCGGCTGAGT	42
[7][581]	[3][594]	TAATACTCTGAACTTGGCAGACGAGTGGAGAGTCA	42
[7][602]	[3][615]	AACCTGGCTTACACAAACATGTCAGCAATTGACTT	42
[7][623]	[3][636]	TTATACAAACGAAAGGCCAACGGCTTACGGTACATA	42
[7][644]	[3][657]	AACGTAGTGGGGGAAATGAGTCGCTGAGGACGAGC	42
[7][665]	[3][678]	AAAGGTGGGTTTAAAGGAAATACGCTTACGGTGT	42
[7][686]	[3][699]	AAAGCGAAGGTTACCTAGGAGTGGCTGCTGAT	42
[7][707]	[3][720]	AAGGTTATGAGTAACTAACTGGCTGAAATCAAAGAG	42
[7][728]	[3][741]	ATAGAAACCGGCCCTATCACTAACGACATAATCAAGA	42
[7][743]	[3][763]	GAAGGAAAATATGAAATTAATTA	23
[7][744]	[2][56]	TAATATGIGGAAATACGGAGAATGGAGTGGCTTAAGT	40
[7][77]	[2][70]	TCTACTGTTAAAGTCGCGGTTGGAATCACTGGTAAAGT	49
[9][98]	[4][91]	CAGACGGTCTACGACATGGGGTGAATGTTAATGAAATGA	49
[9][119]	[4][112]	ACCGAAACTGGGAAATATGAGAAGCTTCTGGAGCAGAAAAGGAGACA	49
[9][140]	[4][133]	AGAGGACTAGAGTCTGAGCTGTTAACAGCGGCAAGATGTTCAAA	49
[9][161]	[4][154]	CAGACGAGGAGGGGATTCTCGGGGTTCTGGCTGAGTAA	49
[9][182]	[4][175]	CTGACCTGGCAACTGGTGGATTAGTGGTTGTTGAAATATT	49
[9][203]	[4][196]	CTTGACAAACAAAGGGCTAAAGGGGATGTTGCTTTGTTA	49
[9][224]	[4][217]	ATTACCTCTGTTACCTGTTATCGGTTACGGCTTAAACAGCCCTT	49
[9][245]	[4][238]	AAGCTGCGAGGAGGAGGGCTGGCGATCGGTTGCGGAA	49

[9][266]	[4][259]	AGCGCTTGAAGGAAATTCGGAATTAGGCGGCAACTGCTTCCTGTTGACCA	49
[9][287]	[4][280]	ACCGAAAATCTGAGCTGCTGAAAGGGCTTACCTAACATGAGGAT	49
[9][308]	[4][301]	GGCTTGTAGGAAATGTTAGTCGCTTCTGGCTGGCTGAAACACAAAATTA	49
[9][329]	[4][322]	AACTTAAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][350]	[4][343]	CTTCTTAAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][371]	[4][364]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][392]	[4][427]	CAGCTGCTGAAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][413]	[4][448]	ATATTCATCCCTCATGGCAATGACCTGGCTTACAGGAA	49
[9][434]	[4][469]	CTCTTAAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][455]	[4][490]	AGCGCTTGAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][476]	[4][511]	CTTCTTAAAGGAAATACGACATGCACTTACGGCTTACAGGAA	49
[9][518]	[4][531]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][539]	[4][551]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][560]	[4][571]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][581]	[4][581]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][602]	[4][595]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][623]	[4][616]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][644]	[4][637]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][665]	[4][658]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][686]	[4][679]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][707]	[4][719]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][728]	[4][727]	GGCTCATGAGTAACTGGCTGAAAGGCTGTTGTTGACCTGAA	49
[9][749]	[4][748]	GGAGGTTTGTAGTGGCCGCCCCGTCGAG	29

**biotin-modified**  
 GTCAAGGGGAAACCGCTCCCTCG-Biotin  
 ATTGCAACCTTATGAACTATGTCGGGCAATCTC-Biotin  
 Biotin-ATTCTCATACCTATTAATATAGTCAGAAAGCAAAAGC  
 Biotin-ATATCTAATCTCTGAGATTTGCTACATCGCTGA

**digoxigenin-modified**  
 Dig-TATTCACCCCTACCTCTAAACATCGCCATTAAAGA  
 Dig-TACCCAACTTCTTGTGAACTTACCTTCTTAAATGGA  
 CCAGGGCAAAAGACAAAGGGGECATTCATCTCA-Dig  
 AGGGTTOATAAGTATAGCCCAGGAAATAGGACTCCCTT-Dig

