Supporting Information

Purification of DNA nanoparticles using photocleavable biotin tethers

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QUANTIFICATION ESTIMATE OF aPCR SCAFFOLD RECOVERY. We performed 23 individual aPCR reactions of 50 µL each by using the OneTaq enzyme to produce ssDNA scaffold at a length of 1,644 nucleotides from the M13mp18 phage template as previously described.[1] We purified 20 of these tubes by running them on a 75 mL, 1.2% low-melt agarose gel at 100 volts for 30 minutes and then recovered the ssDNA via the Zymoclean Gel DNA Recovery Kit (#D4008). From the remaining 3 unpurified tubes, a portion was run alongside known amounts of purified sample in a 40 mL, 1.2% high-melt agarose gel at 100 volts for up to 30 minutes. Finally, we measured the individual ssDNA band brightness with the ImageJ Rectangle & Measurements Tools to create a standard curve from the known purified samples and estimate the ssDNA concentration from the unpurified sample.

DNA NANOSTRUCTURES DESIGN AND BIOTIN SITE SELECTION

48hb Design. The 48 helix bundle (48hb) was created using caDNAno2.[2] Structure file will be made available on nanobase.org and the cross-section helix layout (derived from caDNAno) is shown in **Figure S1**. The .JSON file was converted to a .PDB version using CanDo[3] and visualized in UCSF Chimera[4] to identify staple sites that would be PC-biotin modified. All sequences are available in **Table S2**.

6hb and PB Design. The other two structures were used using published works by Oktay et al.[1] Staples to be modified with PC-Biotin were identified in the same manner.

Choice of PC-Biotin staples were driven mainly by the accessibility of the staples in 3D atomic models. The staples chosen to tag with PC-Biotin were based on whether the 5' end of the staple strand was facing outward in the middle of a face or on the structure edge of 48hb. Five thymine residues were added to the beginning of the staple sequence to provide biotin/streptavidin binding flexibility.

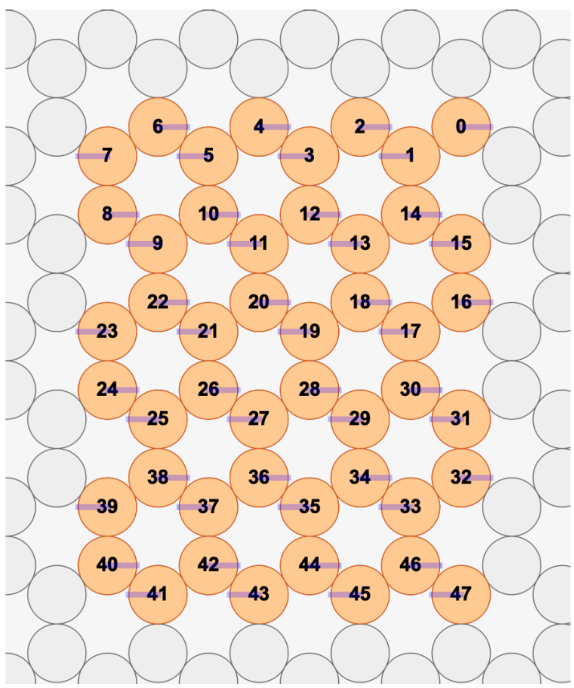

Figure S1: Cross sectional helix layout of the 48hb.

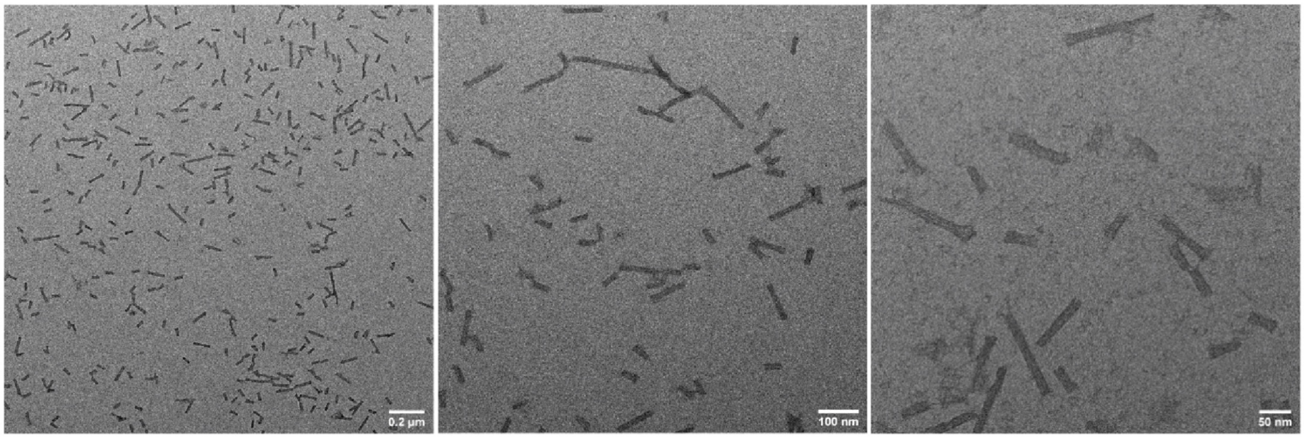

Figure S2: Representative TEM images of crude 48hb.

Photocleavable biotin modified staples for the 48hb.

Table S1: PC-Biotin staple sequences

Structure	Name	Sequence (5' -> 3')
48hb	48hb_128_PCBio (corner)	/5PCBio/TTTTTTTGCTCAGTACTGTATCAGCCCAATCCTGTAGTAAACAACGAA
	48hb_106_PCBio (face)	/5PCBio/TTTTTAATACCGATATACCTTTTCAAGCGCCATTCGAAA
6hb	6hb-1[2]-PCBio	/5PCBio/GTCAAAGGGCGAAAAACCGTCTA
	6hb-5[238]-PCBio	/5PCBio/TTTTTGTCAAAGGGCGAAAAACCGTCTA
PB	PB-17-PCBio	/5PCBio/GATTTTCAACCGCCTGCAACAGTGCCACGCT
	PB-24-PCBio	/5PCBio/AAAACAGAACATTTTGACGCTCAATCGTCTG
48hb-long linker	48hb_128_PCBioTEG (corner)	/5BiotinTEG/iSp18//iSpPC/TTTTTTTGCTCAGTACTGTATCAGCCCAATCCTGTAGTAAACAACGAA
	48hb_106_PCBioTEG (face)	/5BiotinTEG/iSp18//iSpPC/TTTTTAATACCGATATACCTTTTCAAGCGCCATTCGAAA

Table S2: 48hb staple sequences

Name	Start	Sequence	Length
48hb_2	45[102]	CACCCGCCACAGAACCGTTGAGATCCTTATGCGAT	35
48hb_3	5[108]	GGGTCACAATTCAGGTCGACTCTAGCCAGGGTTGGGAAGTAGTTTGTTTG	50
48hb_4	15[46]	ATTACGTTATATTTGAGCAGTTGAAAAGCATAGAAGATTAGTCTTGGG	48
48hb_6	0[93]	GGACTCCAACGCAGGCGAAAATCCTATTTAAGTCATTGTTG	41
48hb_9	3[129]	TCACGGGAAACCTAACTCTGTGTGACCGGGTACCG	35
48hb_12	17[101]	GTAAGCTATTTCCTGAGAGTCTGGAATTGTATTTTT	36
48hb_15	41[94]	GTTTATCAGCTCTCCAAAAAAGAGGATTGTGTATCGTCAGGTAATAATAA	50
48hb_19	17[59]	AGTATGAATAAGATGGCAATTCATCCGTATTAAGACTTTTCTGGTCTCAA	50
48hb_21	15[88]	CAGATTGTAAACGTTTCGAACGTGGTCAAAGGGCG	35
48hb_23	47[59]	CTGAAACATGACGTCATATCTCTGAAGAGCCGCGCCACCAGCG	43

48hb_24	8[45]	ACATTCTGGCCTACCTACCTTGCTGGTAATATGAGTAAAAGGT	43
48hb_28	2[80]	AGGAAGGGAAGAGCCGGCGACAACTAATATAATCCTGGGCTAT	43
48hb_32	45[60]	CAGCATGGCTAAATAGCCTAACGAGCTATTTTGCA	35
48hb_36	2[59]	GAGCGGGCGCTGATTTAGTGCCCGAATCAGATTGGAAGGGTTA	43
48hb_38	0[50]	GGCCCACTAAGCCCCCAGGGCGCACTATGGGGGATTTGTC	40
48hb_40	41[115]	GTGAATTTCTTTAATTTTAAAACACATTTGTAATCCCCCAAAAGAACAAA	50
48hb_43	43[52]	ACCCTCAGAACCCACCAGCAATGAAGGGAGAAAGCCGTTCAATAGCAAC	49
48hb_44	0[113]	AGTCCACTATGGTGGTAGCGGTCTGGGCGCGCATTAACTG	40
48hb_45	22[16]	GCAGGCACAGGTATTAATCAGTGAAATCCTGAGAAGT	37
48hb_47	31[46]	AAGTCAGATAATCGAGAATTTACGAATGCAGCGAG	35
48hb_54	32[23]	AAAATAAGAAGGGTAATAGAATTGAAACGCATGATTAAAAATTCACGA	48
48hb_57	40[24]	TAGCACCATTAAAAAGGGTATGGTTTTCTTACCCGACAAACAT	43
48hb_59	0[72]	AAAAACCGTCCGGGGAAAAAGCGAACGTATACTAAACATTG	41
48hb_60	31[88]	TTGATACCACATTCAGAAGAAACAAAATT	29
48hb_63	23[24]	CAAATATATTTATTACTAAAGCCAACAATAGAGAC	35
48hb_67	42[107]	AATTGCTTTCCTTGCAGGGAGTTAAGCGAAAAATGCCATATCGCGCGGA	49
48hb_69	5[45]	CATCTATCGGCATTTTGACGCTCAAAATAAAATAATGCGGTTATATGTCA	50
48hb_71	32[44]	GAGAACGTCAAACACCCATAAGAGGTTACCACAGT	35
48hb_74	46[44]	AGGAAGCCAGACCAGAGCAGAGCCTCGGTCAGTAATCAAAT	41
48hb_77	30[94]	AGGATGAATTAGGTTTAATTTCAACATGTAATCTTGACACCTCAGACCT	49
48hb_79	45[123]	CAACCGTACTAAATCAAAGAACGAGGCTCATTATACCAACATTATAGCA	49
48hb_80	23[45]	CATCTTCTGACAGAATAAGCTTAATATAAGTTTTAGCAAGGCATTTGCC	49
48hb_81	40[45]	TAGAGCCAGCATTGAGGGTCACAATCGCTCAAGTAA	36
48hb_83	2[92]	CCCCAGCGATTGGAGAGGCGGTTTCGGATTAAATTCGTAAATGC	44
48hb_84	31[109]	TTTTTCATCAGGAATTACAGAGGGTAAATATAGCAAAGTTTTAATGCG	48
48hb_85	46[23]	TTTCAAACAAGACAGGACACCACCGCGTTTGGAAACCACAG	41
48hb_89	40[87]	TTGCGGAATTATCACCGTCCTTTAGCGCCTTTAATTGTACTT	42
48hb_90	42[128]	TAAAAACAGCCGATATATTCGGTCGAGGGTACATTAAATCAAAGCCTAT	49
48hb_92	41[60]	ATCTTTCATCACGTAGAGATAGCCCTGTTTAAATATCCAATTTCAAATA	49
48hb_93	39[137]	AGAGCATAACTTGATACCGATAGTAACTAAACAGCGATTA	40
48hb_94	45[144]	TACTAGCCCGTCATTCAGT	19
48hb_95	6[80]	AACATCACTTGAATACTTCATTAAATCAAACCTAAATCATACCTTTTGAA	50
48hb_96	16[44]	GAAGATGAATAAATCGCTAATTACTGCTTCTGAGAAGAAACTATATTT	48
48hb_98	15[25]	GGATGAGTAAAGAGCCGGGTTATCCCAGCAGGCGG	35
48hb_99	8[87]	CGACGGGCAGATTCACCATAAAACATTAACCTATCATAGCAAC	43
48hb_100	6[59]	AGAACTCAAACACGCAAACACCAGCCACCTTGATAACCTATTT	43
48hb_101	0[30]	ACCCAAATCACTAAATCGTCACGCATGCGCCACGCCAGGGC	41
48hb_102	9[94]	TTCATTCGCAAATGGTACATGCTGTAGCTCAAGCTTAATCTT	42
48hb_103	24[129]	TGATAAGAGGTAAGTACGGTAGATTGGCGATCACTCCAGGAGTGAGCTG	49
48hb_106	23[74]	AATACCGATATACCTTTTCAAGCGCCATTCGAAA	34
48hb_112	2[38]	CAAGTGTAGCGGGAACCCAAAAGTTATTATCATATCAAAATTA	43
48hb_121	4[122]	TCGTGCCAGCTCAGGGTGATGTGAGTAAATCATTTTAGAAAAGGGTAGAA	50
48hb_125	6[101]	CACAACATACGAGCCGGTAGTGCCAAGCTTGCCGTTGTACCA	42

48hb_126	15[130]	GAAAAAAGCCTTTTAACTTCATCAGCGCATCAGAT	35
48hb_127	6[114]	GCTGCCTAATCCAGCTTGTTGGTGGGCAAGGGTTGTACGTT	41
48hb_128	47[122]	TTGCTCAGTACTGTATCAGCCCAATCCTGTAGTAAACAACGAA	43
48hb_132	31[67]	CCCCCGCGCCTTTATTTGAAAAATTCAACAATAAT	35
48hb_135	16[107]	GTAAGATTCAACCCTCATAAATCGCAAAGAAATTT	35
48hb_136	16[23]	TTTAGATTTTTTCATTTCAAACATTTAATTAGATAGCTCAAATCCTTT	48
48hb_138	45[82]	ACCGCCACCTCTAAAGTTTTGACCTTA	27
48hb_142	24[108]	GATGGCTTAGACATGTTTGATACATGGCTGCGCCGCTTCGTAAAGCTGA	49
48hb_150	5[66]	TAGCCCTGAGTAAATGGATTATTTACATTGCCAAT	35
48hb_151	16[94]	AGAGATCTACAATCTGAGTAATGTGTTT	28
48hb_156	5[24]	CACCCCAGAACAACGCTCATGGAAAAACAGAGTTTTGAAGCTGATGTAGA	50
48hb_158	47[81]	GGCTGAGACTACCGCCACCCTCTCGAATAACTTATCCTGAA	41
48hb_161	33[17]	CCAATAAACAGCCGACTTGCGGGAGGGTATTCATTAAACAATC	43
48hb_163	42[65]	CGTAAGTTTGCACCGACTTGAGCCTTGACGGCCACGGATGAGAATGGCA	49
48hb_164	4[101]	ATCGGCCAACGCGCGGAGTAGAAGCATAAAGTTGGTGCCGACC	43
48hb_166	42[86]	AAAAGGAGTCAGGTGGCCCTTTTTGTTAGCGTCCAATAAAGATT	44
48hb_168	45[31]	AATCCTCATTAAGTGTACTTTGTTTCCTAATTAGCCTTAAATC	43
48hb_170	32[73]	TCTTACCAACGAGCCTTTATTAGACATAGCAAGTAAGCAAAAT	43
48hb_171	43[102]	TGAAGTTAGCTACTTAGTGGCTGAGCCAAAAGTTGAGATAG	41
48hb_173	16[136]	TTCTAGCTGATGCCGGAGGCAAGGAATTATGACAATAAATGAA	43
48hb_176	2[122]	GAGTTGCAGCATCCGAAACAGGAAGGCAAACATGCCGGAGAGG	43
48hb_1	35[39]	ATATGAACAACGCACTCTAGAAGGAACGTCACCTACCATCATATTCCTG	49
48hb_5	10[128]	CGCGGTGCGGTAAGTTGGGTAACGAGGATCCAATTGTTATCC	42
48hb_7	6[148]	GGTCATAGCTGTTTCCACATTAACTCAGGAGTAA	34
48hb_8	8[155]	AGGGGGATGTGCTGCACGAATTCGT	25
48hb_10	26[44]	CCACAGTAGGACACCGGAATCATATAGTTAATGTAAATTGGCTATAAAA	49
48hb_11	26[37]	TAAAGACGCTGTAAATCAAAATCTAAGGAATGGCGCGTTGG	41
48hb_13	19[53]	AACCATCCTAACAAGCATTAACTGAAAATGATTTGATGACCTATTATT	48
48hb_14	18[90]	AATGCAAGAAAGCCTCAGAGCGTAAAATACATAACCCTTCATC	43
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48hb_18	35[109]	AACCTGATAACAAAAGAAATACGTGACAGCATCGGAACGCTGAGGGAG	48
48hb_20	3[8]	CCCGCCTCTTTAGACAACTACTGAGC	26
48hb_22	30[114]	AGAAAGAACTGTAGTAAATTGGGCGATATTCCATAGGCCCGG	42
48hb_25	10[58]	AACTTAACCGGGAGGCCGATTAAATTGCTTTAAATCAAGATT	42
48hb_26	19[8]	GAAGATTGTCTTTTTCCAAGTAATAT	26
48hb_27	35[8]	GAGATAGCAGGTCGGCCTTGATATTCAAACGGGGAAT	37
48hb_29	18[58]	CCAAAGTATTAATCCTTAGCTTGATATCAGGGCGAT	36
48hb_30	21[116]	GGGAGTCAGATCATTGATCATCGCGAGGCGCCAGACAGTTCAGGGATAG	49
48hb_31	38[148]	AGACTTTTTGCAAACTAAAATCACAATTCTAC	32
48hb_33	46[155]	ATATAAGTACGTAACACT	18
48hb_34	10[37]	CAGAGAGTCTTAGACAGGAACGGTGCTACAGTGAGGAATCAA	42
48hb_35	26[58]	GAGGTGAATTTGAGTGACTGAACCAGTTGGCGACGAGCAAG	41

48hb_37	21[18]	AGCATTTTCCCTGAGAGTAAAATAGCGCTTATGCGCGTAACCAC	44
48hb_39	18[37]	AGGATAATACTAATTTTTAAAGGGCGTGAACCATC	35
48hb_41	13[109]	TGTCGAGTAAAGGTCACTCCGGCACAAC	28
48hb_42	34[30]	AGAACGATTTTGGTAATATGCCCCCTGCCTATTTCGGAATAC	42
48hb_46	29[130]	ACACTTTTGCTCAAATGACCCTGAGAACCAGTTT	34
48hb_48	13[32]	TAGCGAATTACAGGTTTCTTATCCGTTTTGATGCCAGTTACA	42
48hb_49	21[130]	AAGCGAACGAGTGTCTGGAAGTTTTTGCTCCACCGGAACATGAGGTGAC	49
48hb_50	26[121]	TATCGCGAGCTCATACATAGATGGACATTAAGTTTTTCAGA	41
48hb_51	37[46]	ATGTATTTTGAGGGAAGGTAAATAATTTGGGGTAGCGACAGA	42
48hb_52	32[148]	GGCTTGCCCCGTTGGGAAGAAAAAT	25
48hb_53	11[67]	ATAAATACCGATAGCCCGTCACACGACCAGTTCGTCTGAGA	41
48hb_55	13[53]	TAGAGTTACAATACAGTAAGCAAAATTAGTTGCGTCTTTCCA	42
48hb_56	24[155]	TAGAGAGTACCTTTAACATTCCATA	25
48hb_58	13[74]	ACAGCCTGATCATCGGGATCATTAAGCTACAATTTTTAATCA	42
48hb_61	21[53]	ATAGCATTTTAACGCGCGAACAAACAAGAAAAACCACCAATGGAAAGCG	49
48hb_62	40[155]	AACAACCATCGCCCACGGCTTTGAG	25
48hb_64	18[135]	AACACAGTCACGGAACAGTCAGGATGACGAGAAACACCCGTAACAGAAC	49
48hb_65	34[148]	TTGAAAGAGAACCCTCGAACTAAAATCACCAT	32
48hb_66	23[7]	ACGCGAGAGTATCAATACAAATACCAG	27
48hb_68	42[155]	GAAAGGAACTGCGCCGAC	18
48hb_70	33[8]	TATCCCTCAGTGCCAGTGCCCGTATAAACAGTTAAAGT	38
48hb_72	4[148]	TGCCCGCTTTCCAGTCCAGTGAGGCCAGCTCAATAGGTTTC	41
48hb_73	18[16]	TACATAGATTCATTATCTAAAGCAAGTTTTTTGGGGT	37
48hb_75	11[95]	GTAAATTAAGCAATAAAAGCAATAACCTGTTCATCAAACTGC	42
48hb_76	26[100]	TTGTAGCTATTTAGCAAATGGGATCAACCCGTGCGTATCACGCTGGTTTG	50
48hb_78	37[67]	ACAAAAGACAAAATTATTCATTAAAGGTGGATATAAAAACAAGCC	45
48hb_82	26[65]	TTACGCCATATAAGGCGTTAAATACTAAATTAGGTTGGCGAACTGAACG	49
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48hb_97	34[122]	CAGATAGTAAGTACAGGTGAGAAAGAAATTAAAGAGAATCGAT	43
48hb_104	0[148]	AGATAGGGTTGAGTGTTGTTCCAAATCCCTGGCCCTGTTT	40
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48hb_107	47[94]	CAAGAGAAGGATTAGGATTTTAGTACCTCATTCCCTCATATTTTCTTGAA	50
48hb_108	34[128]	GGTCAATCATAACGGAGTCATCTTAAGTTTCGCAACGGCTAC	42
48hb_109	25[94]	CAAACTACGAAGGCACCATCGTCACCCTCAGCAAGGCCGTCG	42
48hb_110	21[109]	TCAACCATTATAAATATGCAACTACATTTTTTCGAGCTCGGGTAAATA	48
48hb_111	34[89]	AAGAAAAACCTGCTCCATGTGTAACGACTCAGAGCCAC	38
48hb_113	28[115]	TTGCCGAGGCACCAGGCGATTACCCCAGGAGGTAGCGGGGTT	42
48hb_114	36[16]	TAAACGACGAAATCGGCGATGAAACAAT	28
48hb_115	37[74]	TAAAGACTGTCTCAGAGCCTCGTCTTTCCAGCTCC	35
48hb_116	21[8]	TTGAAAAAGGTAATGTCCAGCGGAAT	26

48hb_117	25[84]	CGAAAGATGCTGAATACCGTGTGATAAATTTAACAGAAACGCTACA	46
48hb_118	7[8]	TTGCAAACCTGAAAATACGTAGACAAA	27
48hb_119	16[148]	GATATTCAACGTAAAACTAGCATGT	25
48hb_120	37[31]	TCCTCTTATTAGGAACCGCCTCCCTCCGCCGCCCCA	36
48hb_122	19[102]	AGCTATATTTCATTAAAAAGCAAATGTTTGATTAAAGAACGT	42
48hb_123	30[155]	TAATAAAACGTTTACCAG	18
48hb_124	37[115]	CACTTTCACGTGTATGGGATTTTGCCATTCCAAGACGGTGTA	42
48hb_129	16[78]	GTTTGGATTATACTTCCCTTTTATGCT	27
48hb_130	26[84]	AAGAGGGCGTCTGAGCAGTACACTAAACGGCGGATTGGAAAC	42
48hb_131	26[72]	ATGTAGATAAAAGAAAATAGCTATCACCCTCATTTACCGTAGA	43
48hb_133	28[155]	ATAAAAACCGAAAACGAG	18
48hb_134	10[84]	CAGGCAGCCTTTGATAATCAGAGCGGGAGACGTGCTATCAATAACAA	47
48hb_137	10[23]	TCAACAATATATAGAACCCTTCTGCAGGAAAAATATTACCGCCA	44
48hb_139	14[155]	TATGTACCCTCAAAAATA	18
48hb_140	12[155]	GTCTGGCCTGCCAGTTTG	18
48hb_141	39[8]	AAAGACCCATTAGGAAACGTCACCAATCCATCTTACTGGCAATAA	45
48hb_143	19[123]	AACTAAAAATGCTCATTCCAAAAATCGGCAAGTTTGGAACAAG	43
48hb_144	34[16]	CGCAACGGGTTAAGAACATTGCGTGCACGTACCAGAAGGAGC	42
48hb_145	26[155]	CATAAATCACCAACAGGT	18
48hb_146	37[137]	CCCGGAATTGCTTTCAACAGTTTCTACAAACCCGAACTGA	40
48hb_147	18[148]	AAGCCTTTAAACGCCACGGTTGACAAAAGAAT	32
48hb_148	28[23]	AATCAATAAATAAAGTACAGTATAGAAAAAGCCTGTTTAAAACTTAATC	49
48hb_149	39[24]	CATTCAACCGAAAATCACTCGATAGCAGCACCTAGCCCCTATT	43
48hb_152	2[148]	TTGCCCTTCACCGCCTTATAAATTAATCAGCGGTAATCCG	40
48hb_153	10[155]	CGACGACAGACGCCAGCT	18
48hb_154	17[8]	AAAGAAGCGAGGCGAACCTCCCATAT	26
48hb_155	20[148]	TAGTAGCATTGCATCTTCCTGTAACGGGCAAC	32
48hb_157	36[148]	GCGCGAAACCAGTTCAAAAATAGATACTTTTG	32
48hb_159	1[8]	GTGCCGATTTTGCGAAACCAAAACAG	26
48hb_160	7[136]	AGCTAGGCGATGCCTCTTAATTCTGGTGGCATGGTCTTTCTTT	43
48hb_162	18[65]	TTGTTTGAATATATATGTATCAAACCGGCTTTAATGGTTTGA	42
48hb_165	37[39]	ACGGAAGGAATTCAGCTAGCATGTACAAAATGCAG	35
48hb_167	44[155]	CGTCACCAGAGCGGAGTG	18
48hb_169	21[32]	TTAGAGAATACAACATGACCGAGGAGTTAAGCAGCATTATA	41
48hb_172	34[135]	GATAAGCTGCGAATAGGCAGGCGGATAAGTGCCGTCGAGA	40
48hb_174	11[137]	CCGTAACATCCCCTGTACGAGAGGCTATCATGACA	35
48hb_175	19[81]	ATGCGGATTCATAATATTTTGTTACTCCGTGGGAACCATTCCTCGGAA	48
48hb_177	37[8]	AAAAGATTCATAAACCGGAACCAGAGCGGTTGAGACCCACATGAG	45
48hb_178	28[72]	CAATCATCGTGAAGCGCACAGAGACAGTAAGAAGTATTAAGA	42

UV PHOTOCLEAVAGE SETUP USING STRING OF LIGHTS

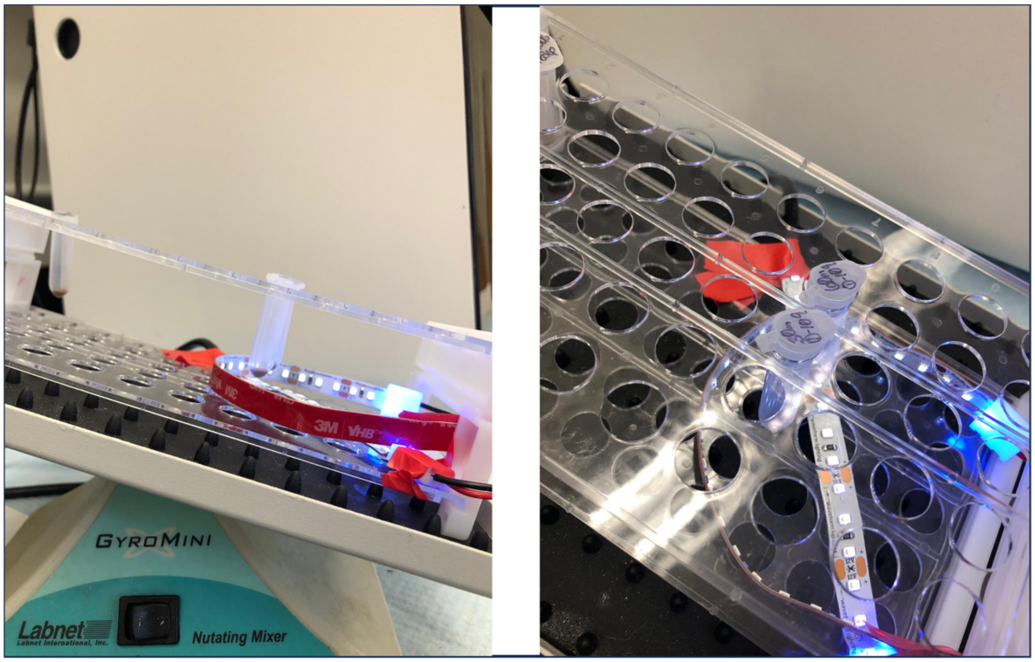

Figure S3: String of Lights for UV-photocleavage of the DNA NPs from the beads.

EFFECT OF DIFFERENT BEAD AMOUNTS ON 6HB AND PB PURIFICATION

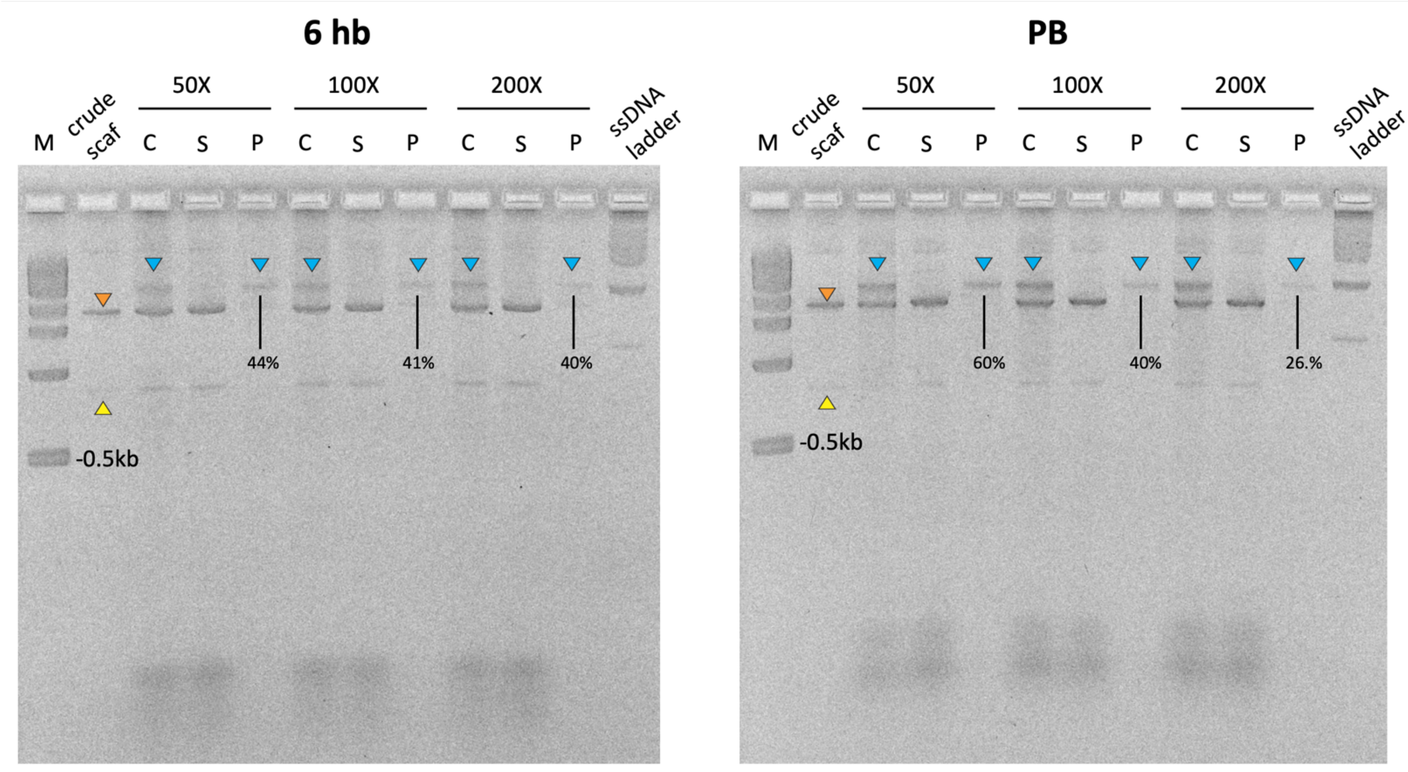

Figure S4: Agarose gel electropherogram testing different bead dosage on 6hb and PB purification. Color triangles represent: dsDNA byproduct (orange), ssDNA scaffold (yellow), and the formed PB NP (blue).

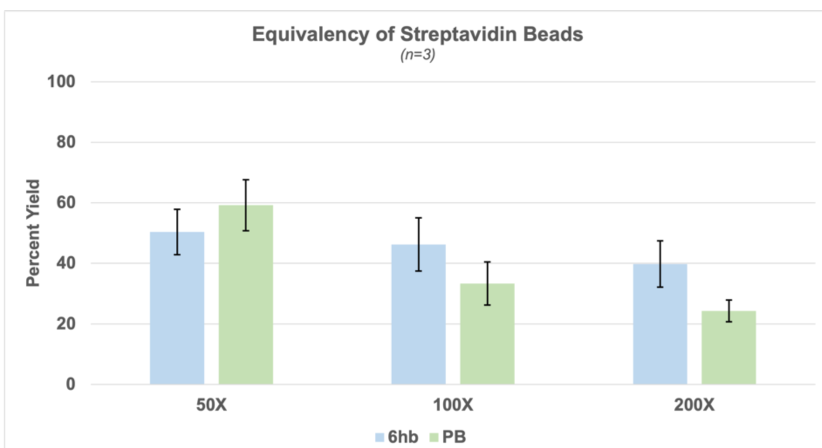

Figure S5: Yield of purification for 6hb and PB assembled using crude scaffold. Error bars represent standard deviation from the mean (n = 3).

YIELD OF aPCR-PURIFIED SCAFFOLD STRAND

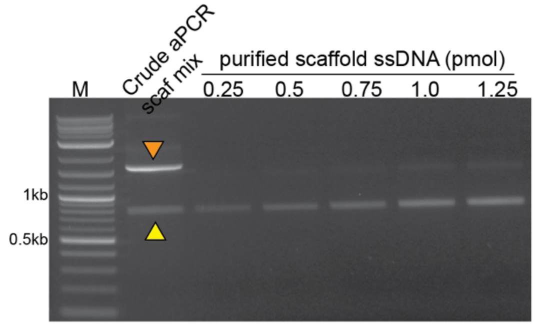

Figure S6: Estimation of scaffold concentration in crude aPCR mix using standardized pure scaffold series. Yellow triangle indicates scaffold and orange triangle indicates dsDNA by-product of the aPCR.

COMPARISON OF PC-BIOTIN PURIFICATION METHOD WITH EXISTING TECHNIQUES

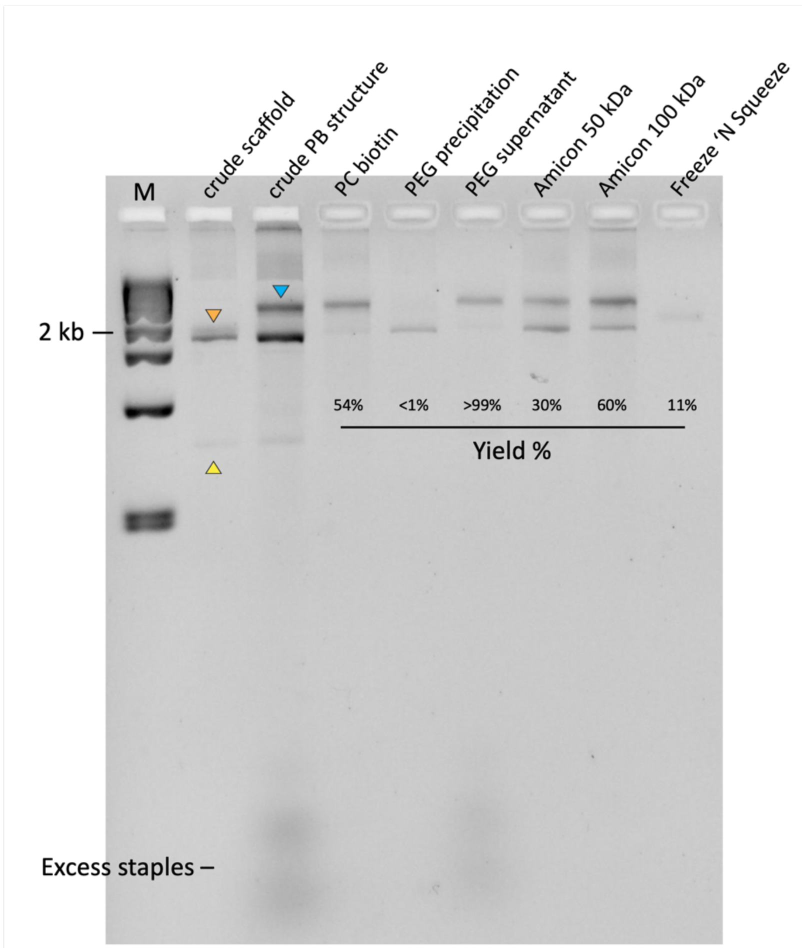

Figure S7: Yield and quality of PB purification via commonly used techniques. 2% agarose gel electropherogram showing PB purification using current method (PC biotin tag), PEG precipitation, two filter types of ultrafiltration, and gel extraction Freeze 'N Squeeze column. Color triangles represent: dsDNA byproduct (orange), ssDNA scaffold (yellow), and the formed PB NP (blue).

Table S3: Broad differences between the PC-biotin based purification technique and other leading techniques.

	Time	Quality	Requires concentration	Yield	Refs
PC-Biotin	4 hours	1 step custom scaffolded DNA NP purification	No	<90%*	**This work**
Precipitation	overnight	Cannot remove dsDNA aPCR byproduct; May be challenging to purify wireframe DNA NPs	No	>90%**	**5-6**
Ultrafiltration	2 hours	Cannot remove dsDNA aPCR byproduct	No	20-80%	**1, 7-9**
Gel-extraction	2-3 hours	Residual agarose particulates, variable yield	Yes	< 20%	**Figure S7**
Biotin-strand displacement	overnight	Theoretically 1 step, requires DSD staple extensions	Yes	> 90%	**9-10**

*Longer PC-biotin linker with 50-fold beads. **PEG precipitation on wireframe PB was 0% efficient but sufficient literature points to >90% efficacy of the technique to purify helix bundle DNA NPs. DSD = DNA strand displacement.

48HB PURIFICATION USING A LONGER PC-BIOTIN LINKER

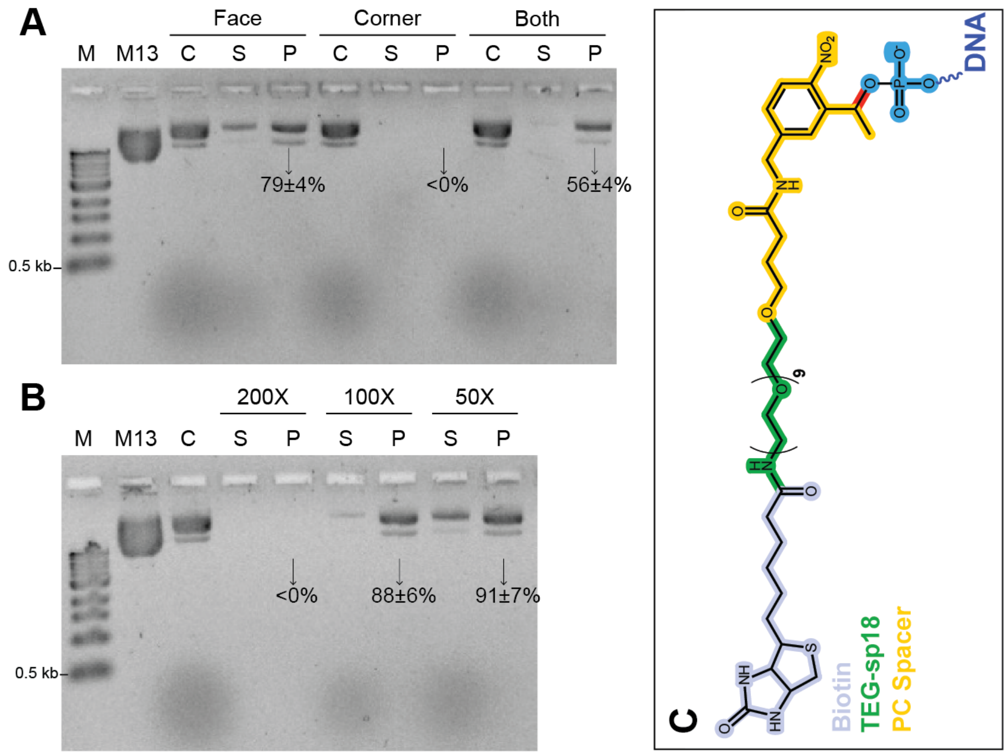

Figure S8: Purification of 48hb using BiotinTEG-sp18-PCspacer linker. 1% agarose gel electropherograms representing the yield of 48hb purification using a longer PC-biotin (referred to as PC-biotin-L) tether staples. (A) Gel separation and purification yield of 48hb when using either face or corner or both PC-biotin-L staples simultaneously and 200X BBC. Surprisingly, the face PC-biotin-L tether (staple 106 in **Table S1**) resulted in highest yield ~80%, even higher than when using both staples together. (B) Gel separation and purification of 48 hb (corner PC-biotin-L only) with 200-, 100-, 50-fold excess magnetic beads per PC-biotin-L staple. In this case, fewer beads lead to the highest yield of 91%. Sample abbreviations M13: scaffold only, C: Crude 48hb, S: supernatant, P: Photocleaved/purified 48hb. (C) Chemical formula of the PC-biotin-L tether. IDT formula: 5'-/BiotinTEG//sp18//PCspacer/. Standard deviation about the average yields were calculated from n = 3 samples.

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