

Figure S1: (A) Domain-based description of 1 dye labelling complex. (B) Domain-based description of 3 dye labelling complex.. (C) Domain-based description of a branched DNA complex.



*Figure S2:* (A) Correlations between average DNA-probe and GFP intensities. Each plot shows the average intensities of the nuclear regions of at least cells. The lines through the data indicate least square fits to the data ( $R^2$  ranges from 0.84-0.98). (B) Comparisons of theoretical and measured amplification ratios for the reporting complexes analyzed in C. Theoretical amplification ratios indicate the maximum number dyes that can be integrated into a complex. Experimental ratios are determined by dividing the slopes from the fits in C by the slope obtained using a linear complex containing a single dye: the P(1,1,1) complex. Error bars indicate standard deviation from fitted regression for each point



Figure S3: Summary of P(3,9,18) data. (A) Representative GFP and Cy3 image of P(2,9,18) similar to Figure 2A. (B) Linear correlation plot for P(2,9,18) (purple) plotted over previously shown data (C) Representative image of tetrahedral structure made using NanoEngineer1 software



Figure S4: Representative images from stathmin labeling control experiments. Images are from both DNA based branched complex probes and from dye conjugated secondary antibodies as well as from both epifluorescent and confocal microscopes. Images show similar extents of label in cytoplasmic regions as well as similar degrees of textured punctate staining indicating labeling of stathmin-1.



*Figure S5:* Purification of branching complexes. (A) Representative FPLC chromatagram. Fully annealed complexes are present in highest concentrations in Fractions 9,10,11. These fractions are used in downstream purification. (B) Verification native PAGE gel. Lanes 8-12 Show fractions (8-12) for P(2,9,9) complex 1 followed by the 25bp ladder (Promega), Gels show sufficient purifiations of fully formed complexes.

 Table S1. List of oligonucleotide sequences used in design of DNA probe complexes. /5Hexynyl/, /5AmMC6/, /5Cy3/, /3IABkFQ/ indicate a 5' hexynyl, 5' amine, Cy3, 3' lowa Black FQ quencher modification, respectively. T signifies Target Strand. BC signifies Branched Complex.

Target Strands	Amine Strands
P(2,3,3) T & P(2,3,9) T	/5AmMC6/ TTTTTTTTTGG CCA CCG AGA CAA TAC GCA GGA CCC
P(1,1,1) T & P(1,1,3) T	/5AmMC6/ TTTTTTTTTGT GTA CCG GAA ACA TCG GCG AAT TAG
P(3,3,9) T & P(3,3,18) T	/5AmMC6/ TTTTTTTTTTTT ACG CGG TAC ACC TGT GCG GAT ATA
	Hexynyl Strands
P(2,3,3) T & P(2,3,9) T	/5Hexynyl/ TTTTTTTTGG CCA CCG AGA CAA TAC GCA GGA CCC
P(1,1,1) T & P(1,1,3) T	/5Hexynyl/ TTTTTTTTGT GTA CCG GAA ACA TCG GCG AAT TAG
P(3,3,9) T & P(3,3,18) T	/5Hexynyl/ TTTTTTTTTTT ACG CGG TAC ACC TGT GCG GAT ATA
P(2,3,3) & P(2,3,9) BC1	Long Strands
BC1-1	ACT TAA TGA ACG CTA GCA TCA CGC CGG TAC TTA CAA GGT ATA GTA TAC CAG GAG GGC TTG
	TTG TAA GTA CCG GCG TGA GAG ACC GTT AAG CTA CCT GCG AGT GTA CCG GAA ACA TCG GCG
BC1-2	AAT TAG AGG TAG CTT AAC GGT CTC ATA GGC ACA GAT CGA ACC GCT CGT GTA CCG GAA ACA TCG GCG
BC1-3	AAT TAG
BC1-4	AAT TAG
	Covering Strands
BC1-5	AAG GTA TAG TAT ACC AGG AGG GCT TGT C
BC1-6	AAA AAA AAT TCG CCG ATG TTT CCG AC
P(3,9,9) & P(3,9,18) BC2	Long Strands
BC2-1	CAC TGT TCA CTT CAC GCT TTG GTC GCT TCC ACG AAC GAG CAT CCA TGT GAG GGC GCT TAT
	GTT CGT GGA AGC GAC CAA TAC TCG TTT CAC CGT CAA CCT GCG GTC TGG CGA ATG TAG CGT
BC2-2	TTG ACG GTG AAA CGA GTA ATT GAC GTA TGC TGG TTC CGA GAG GTC TGG CGA ATG TAG CGT
BC2-3	
BC2-4	ATG TGC TC
	Covering Strands
BC2-5	CAA ATA CAA TAA GCG CCC TCG TGC AA
BC2-6	GTA TGA GCA CAT ACG CTA CAT TCG TT
P(3,9,9) & P(3,9,18) BC3	Long Strands
BC3-1	CAT TCC GAG AAG GTC GAA TCG CAC GAC ATT CCG GTG ATT TGG ACG CAG GCA CAT ACG CTA
BC3-2	CAC CGG AAT GTC GTG CGA GCG AGA AAT CAC AGC ATA CGC GTT TTA CGC GGT ACA CCT GTG
	TAT GCT GTG ATT TCT CGC TGA CAC CAA GGC AAC CAT GAT CTA TTA CGC GGT ACA CCT GTG
BC3-3	CGG ATA TA ATG GTT GCC TTG GTG TCA TTC GAC CTT CTC GGA TTC GTT TAG TTA CGC GGT ACA CCT GTG
BC3-4	CGG ATA TA
	Covering Strands
BC3-5	AAC GAA TGT AGC GTA TGT GCT CAT AC
BC3-6	AAA AAA AAA TAT CCG CAC AGG TGT ACC AT
Dye Complex Strands	
P(1,1,1) & P(2,3,3) Q	CGT AAT AGC GCT AGT CTC/3IABkFQ/
P(1,1,1) & P(2,3,3) D	/5Cy3/TTTCTT GTC AAT TCG CCG ATG TTT CCG GTA CAC
P(1,1,1) & P(2,3,3) L	GT CGG AAA CAT CGG CGA ATT TTT TTT GAG ACT AGC GCT ATT ACG
P(1,1,3) & P(2,3,9) Q	/5Cy3/TTTCTT GTC AAT TCG CCG ATG TTT CCG GTA CAC/3Cy3Sp/
P(1,1,3) & P(2,3,9) L	/5Cy3/GT CGG AAA CAT CGG CGA ATT TTT TTT GAG ACT AGC GCT ATT ACG
P(3,9,9) D	/5Cy3/TTT CAA ACG TAT CCG CAC AGG TGT ACC GCG TAA
P(3,9,9) L	AT GGT ACA CCT GTG CGG ATA TTT TTT TTT GAG ACT AGC GCT ATT ACG
P(3,9,18) D	/5Cy3/TTT CAA ACG TAT CCG CAC AGG TGT ACC GCG TAA /3Cy3Sp/
P(3,9,18) L	AT GGT ACA CCT GTG CGG ATA TTT TTT TTT TTT GAG ACT AGC GCT ATT ACG

[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21] [22] [23] [24] [25] [26] [27]

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