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Supporting Information

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DNA-Directed Assembly of Capture Tools for Constitutional Studies of Large Protein Complexes

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1. Oligonucleotides

All oligonucleotides were HPLC purified and purchased by Sigma-Aldrich. The sequences of oligonucleotides used are summarized in Table 1. The structure of tiles X-A and X-B assembled from these oligonucleotides is schematically shown in Figure S1.

Table 1: Oligonucleotides used for assembly of the X-tiles:

name	**sequence (5'→3')**
Fsc-X1-T_{12}	[Flc] TTT TTT TTT TTT CGA CCG ATG AAT AGC GGT CAG ATC CGT ACC TAC TCG
Bio-X2-T_{12}	[Btn] TTT TTT TTT TTT CGA GTA GGT ACG GAT CTG CGT ATT GCG AAC GAC TCG
a-X3-T_{12}	[AmC12] TTT TTT TTT TTT CGA GTC GTT CGC AAT ACG GCT GTA CGT ATG GTC TCG
a-X4-T_{12}	[AmC12] TTT TTT TTT TTT CGA GAC CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG
BG-X3-T_{12}	[BG] TTT TTT TTT TTT CGA GTC GTT CGC AAT ACG GCT GTA CGT ATG GTC TCG
CH-X3-T_{12}	[CH] TTT TTT TTT TTT CGA GTC GTT CGC AAT ACG GCT GTA CGT ATG GTC TCG
BG-X4-T_{12}	[BG] TTT TTT TTT TTT CGA GAC CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG
CH-X4-T_{12}	[CH] TTT TTT TTT TTT CGA GAC CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG
X4-T_{12}	TTT TTT TTT TTT CGA GAC CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG

The acronym Flc at the beginning of the oligonucleotide sequence represents a Fluorescein modification at the 5' of the oligonucleotide. Btn stands for a Biotin modification, AmC12 for an amino group at a C12 linker, BG for a Benzylguanine modification and CH for a Chlorohexane modification.

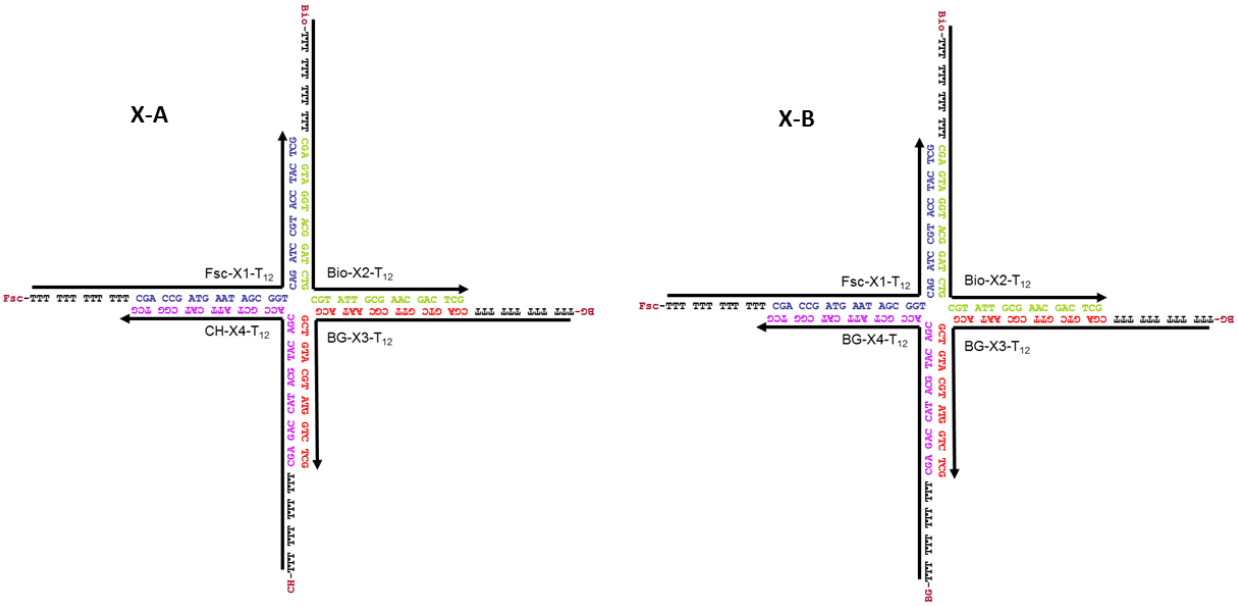

Figure S1: Schematic representation of tiles X-A and X-B.

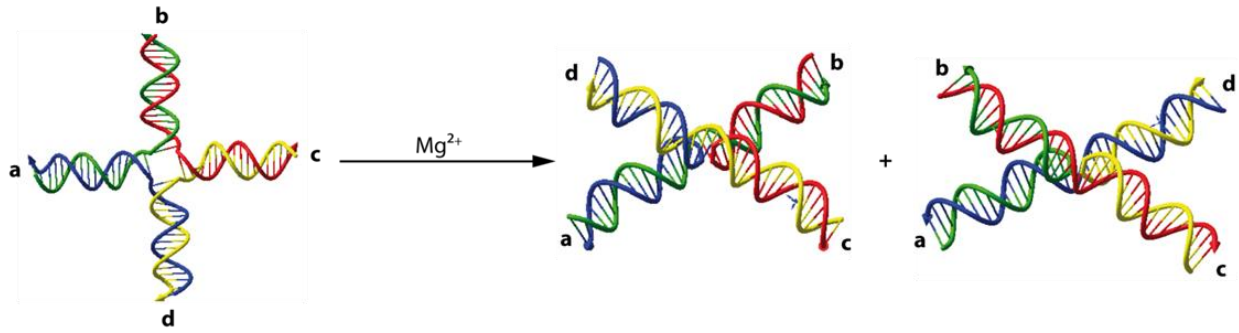

Figure S2: Illustration of the conformational freedom of the X-tiles used in this study. The schematic drawing illustrates two conformational isomers of an X-tile. The X-tiles represent a Holliday junction. In the presence of Mg^{2+} ions the extended conformation (left) can switch to form either one of two possible stereoisomers of the staggered conformation (right), which reveal a smaller angle of about 60° at the crossover.[1] Because Mg^{2+} ions were present in all buffers used in this study, X-tiles most likely adopted the staggered conformation. Which of the two staggered isomers is formed depends primarily on the local base composition at the junction site[1] as well as on the nature of the proteins binding to the end of the arms. The three conformations are in rapid equilibrium and the exact partition of the three species is unknown.

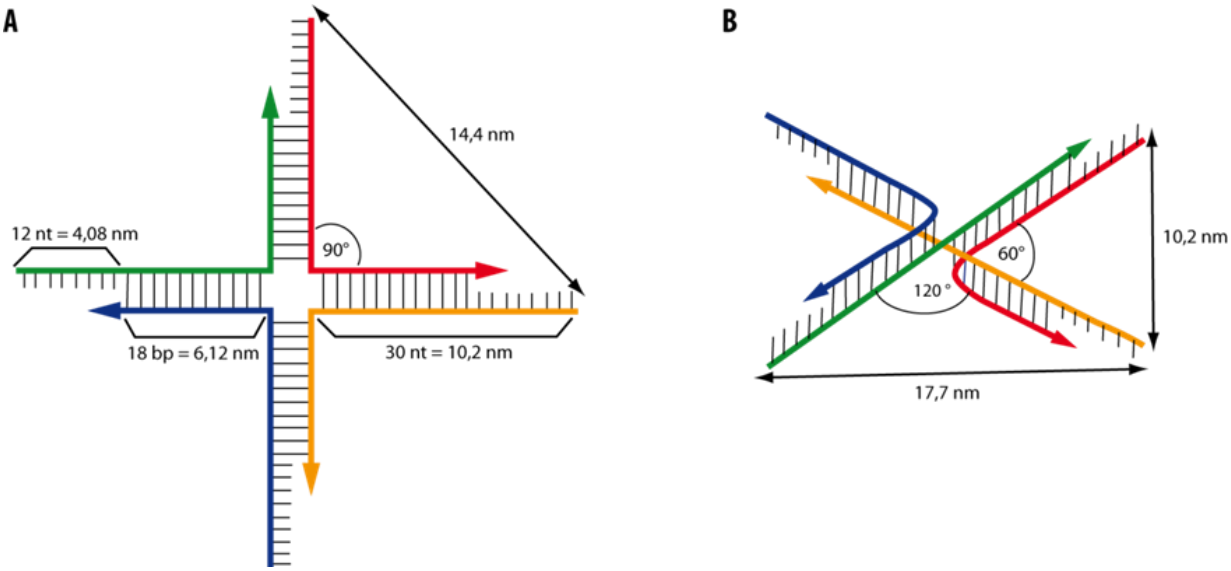

Figure S3: Schematic drawing and theoretical calculation of the distances inside the X-tile present in either the extended (**A**) or staggered conformation (**B**). The given distances were calculated under the assumption of stiff b-helical DNA conformation. Note that the two conformations are in rapid equilibrium (see Figure S2). Moreover, the single stranded regions at the end of the junction's arm are highly flexible. Therefore, a direct contact of tethered proteins should be possible.

2. Synthesis of BG- and CH- modified oligonucleotides

To derivatize oligonucleotides with the suicide tags O6-benzylguanine (BG) and 6-chlorohexane (CH), the amino-reactive N-Hydroxysuccinimide (NHS) compounds BG-NHS or CH-NHS were coupled to amino-modified oligonucleotides. To this end, the amino-modified oligonucleotides were initially desalted via NAP^{TM}-5 and NAP^{TM}-10 columns (GE Healthcare, cat. # 17-0853-02 and 17-0854-02) and dissolved in 0.5x PBS (3.5 mM NaH_2PO_4, 8 mM Na_2HPO_4, 150 mM NaCl, pH = 8.6). The oligonucleotides were then concentrated to about 450 µM by using a MWCO 5,000 Da Vivaspin column (Sartorius Biolab Products, cat. # VS0112). BG-NHS and CH-NHS were purchased from New England Biolabs (BG-GLA-NHS, cat. # S9151S) and Promega (HaloTag® Succinimidyl ester (O4) Ligand, cat. # P6751) respectively. The compounds were dissolved in anhydrous DMSO (Sigma-Aldrich, cat. # 41647) to a concentration of 10 mM and a 30-fold molar excess was added to the amino-modified oligonucleotides and incubated overnight with shaking at 1000 rpm at 25 °C.

Unreacted substrate was removed from the desired conjugate by buffer exchange (6x 200 µl) in 1x TEMg/NaCl (150 mM NaCl, 20 mM Tris, 2 mM EDTA, 12.5 mM $MgCl_2$, pH = 7.6) using a 5,000 MWCO Vivaspin column. Successful modification and purity was verified by gel electrophoresis (Figure S4).

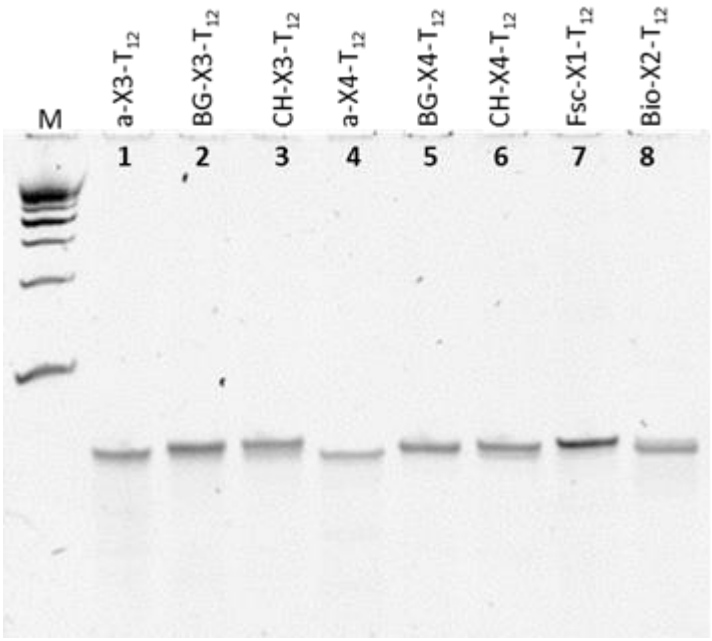

Figure S4: Gel analysis of the coupling reaction of BG-NHS or CH-NHS with amino-modified 48 nt oligonucleotides (a-X3-T_{12} and a-X4-T_{12}). Note the small shift between the bands of the amino-modified (lanes 1, 4) and the BG or CH modified oligonucleotides, which is indicative for the small increase in mass as a consequence of the coupling of the BG or CH ligand. A similar shift is also observed for the Fsc and biotin-modified oligomers (lanes 7, 8). Note also that the gel indicates the purity of all oligonucleotides used to assemble the X-tiles. Running conditions of this 20% denaturing polyacrylamide gel: TBE buffer, 220 V, RT, 60 min.

3. Assembly of X-Tiles and protein modified X-Tiles

Equimolar amounts of the four X-Tile oligonucleotides (X1-T_{12}, X2-T_{12}, X3-T_{12}, and X4-T_{12}, for sequences see Table 1) which were dependently from the experiment derivate, were mixed in 1x TEMg/NaCl buffer (150 mM NaCl, 20 mM Tris, 2 mM EDTA, 12.5 mM $MgCl_2$, pH = 7.6) with an endconcentration of 1 µM. The mixture was incubated for 1 h at 25 °C with agitation of 800 rpm. In a previous set of experiments, it was established that these conditions are well suited to quantitatively form the tiles (Figure S5).

For the gel electrophoretic analysis, about 1 pmol of oligonucleotides or oligonucleotide-protein conjugates were loaded on a 20% denaturing polyacrylamidgel. Protein-conjugated oligonucleotides were loaded on a non-denaturing 10% polyacrylamide gel and the protein-modified or unmodified X-tiles were loaded on a 6% or 8% non-denaturing polyacrylamide gel. For all gels 1x TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH = 8.0) was used as running buffer. Subsequent to the gel electrophoretic separation the gels were stained with SybrGold®. Detailed running conditions used in the various experiments are indicated in the respective figure legends.

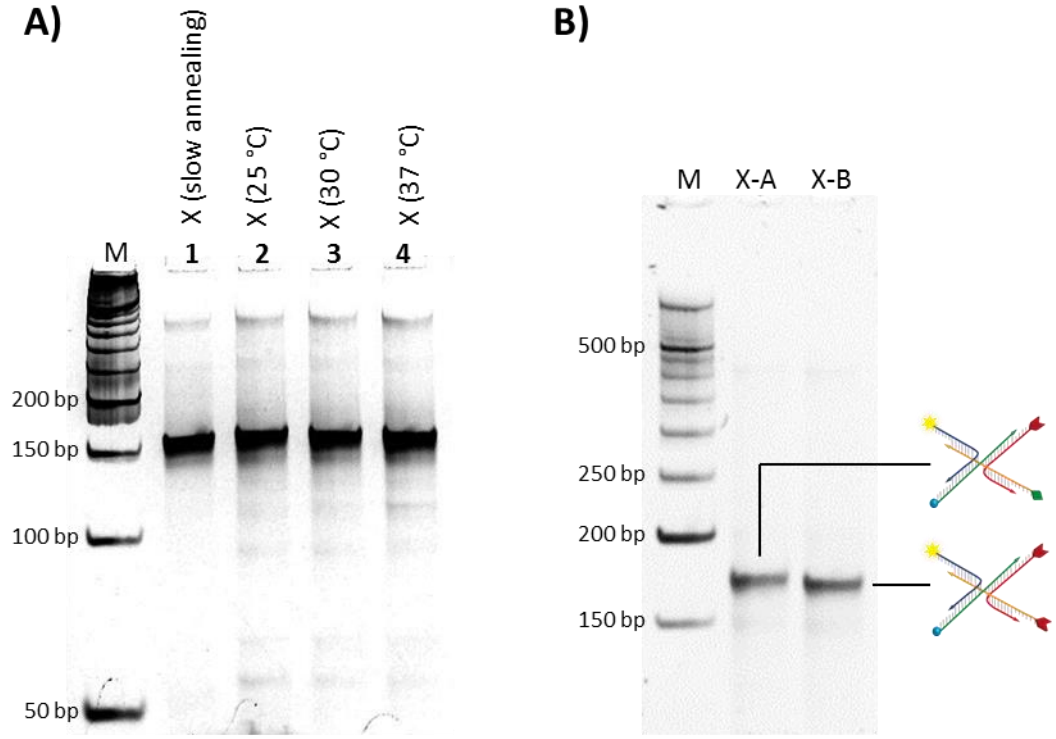

Figure S5: A) Gel analysis of variable conditions for the assembly of the X-tile. Conditions for slow annealing: temperature gradient which started at 2 min at 95 °C, 2 min cool-down to 65 °C, 2 min at 60 °C and then gradient cooling from 60 °C to 20 °C with -2 °C/min (lane 1). Else, the oligonucleotides were heated to 30 °C or 37 °C for 15 min and subsequently cooled down to room temperature (lanes 3, 4) or were only incubated at 25 °C for 30 min (lane 2). Note that all assembly protocols led to identical product formation, as indicated by electrophoretic analysis. Running conditions of this 8% non-denaturing polyacrylamide gel: TBE buffer, 150 V, 4 °C, 60 min. **B)** Assembly of tiles X-A and X-B using the four oligonucleotides Fsc-X1-T_{12}, Bio-X2-T_{12}, BG-X3-T_{12} and CH-C4-T_{12} (or BG-X4-T_{12}). The assembly was carried out at room temperature with 30 min incubation. Note that only a single species is generated. This is a 6% non-denaturing polyacrylamide gel. Running conditions: 60 min at 150 V and 4 °C using TBE as running buffer.

To assemble the protein-modified X-tiles, oligonucleotides were first conjugated with the desired Snap or Halo fusion protein and then assembled to the X-tile as described above. Conjugation of proteins with BG- or CH-modified oligonucleotides was achieved by mixing the BG- or CH-modified oligonucleotides (40 µM in TEMg/NaCl buffer) with a 5-fold molar excess of the CENP-C/CENP-T- or Snap-proteins and incubation for 1 h at 25 °C with agitation of 800 rpm. Successful protein conjugation was monitored by gel electrophoresis. Representative gel images are shown in Figure 2 of the main manuscript. Notably, it was necessary to increase the ion-strength of the buffer with 150 mM NaCl to prevent non-specific aggregation of the CENP-T-Halo protein which otherwise occurred in pure TEMg buffer (Figure S6). Subsequently, the additional oligonucleotides were added (6 µl / 80 µM) and the mixture was incubated for another 60 min at room temperature. Representative gel images are shown in Figure 3 of the main manuscript.

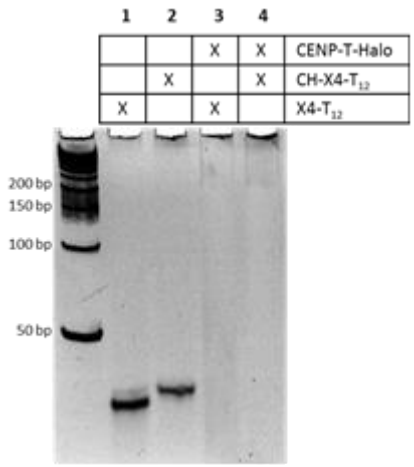

Figure S6: Non-specific aggregation of CENP-T-Halo protein in the presence of oligonucleotides. Note that the bands of both the CH-modified and the unmodified oligonucleotides X4-T_{12} and CH-X4-T_{12} (lanes 1, 2) disappear completely upon addition of 5 molar equivalents of the CENP-T-Halo protein (lanes 3, 4). Non-specific aggregation is indicated by increasing intensities of the bands within the gel pockets. This non-specific aggregation can be suppressed efficiently by addition of 150 mM NaCl to the TEMg buffer (see Figure 2, main manuscript). The running conditions of this 10% non-denaturing polyacrylamide gel were 220 V for 30 min at room temperature.

4. Cloning and recombinant expression of CENP-C and CENP-T proteins

CENP-C (residues 2 to 240) and CENP-T (residues 2 to 252) were PCR amplified using a forward primer carrying BglII and a reverse primer carrying BamH1-Stop-SalI and subsequently cloned in the first cassette of pGEX-6P-2rbs, a di- cistronic derivative of pGEX-6P vector generated in-house. SNAP or Halo tag were amplified with primers carrying BamH1 (forward) and SalI (reverse) and were cloned to respective pGEX – CENP-T or pGEX – CENP-C construct using the same sites resulting in C-terminal tagging. The veracity of constructs were checked by sequencing.

The constructs were expressed in BL21(DE) E.coli cells by IPTG induction and overnight growth at 18 degrees. Cells were lysed in Buffer A (25 mM Hepes pH 7.5, 300 mM NaCl, 1 mM DTT and 1 mM PMSF) by sonication. The proteins were purified using Glutathione Sepharose (GE Life Sciences) following manufacturer's protocols. After elution, the GST tag was removed by HRV 3C Prescision Protease (GE Healthcare Life Sciences) for a few hours on ice. CENP-C was subsequently purified using a RESOURCE 6ml column (GE Healthcare Life Sciences), while CENP-T was subjected to a 5ml Heparin column (GE Healthcare Life Sciences). Both sample were subsequently gel filtered over a S75 column (GE Healthcare Life Sciences). Samples were concentrated, flash frozen and stored at -80 degrees.

5. Pull-down assays

Before assembling into the DNA X-tiles or binding to the beads, CENP-T-Halo and –Snap fusion proteins were phosphorylated by CDK1-CyclinB (NEB) using manufacturer's protocols. This protocol leads to selective phosphorylation of CENP-T[2]. In practice, CENP-T-Halo was phosphorylated by incubation of a 67-fold molar excess of CENP-T-Halo (8.7 µM) over kinase CDK1 in the presence of 1 mM Mg/ATP for 90 min at 30 °C. Subsequently the protein was used without further purification for the conjugation with the CH-modified

oligonucleotides. Phosphorylation of CENP-T-Snap was carried out under the same conditions.

For pull-down assays, streptavidin (STV)-coated beads (Pierce Streptavidin UltraLink Resin, Thermo Scientific) were used to bind the four different protein-modified X-tiles **1** - **4**. Proteins without X-tiles served as control samples. The binding was carried out using either the biotin attached to the X-tiles or attached directly to CENP-T-Snap or CENP-C-Snap fusion proteins using a biotinylated benzylguanin ("Snap-biotin" reagent, New England Biolabs) following manufacturer's protocol. In a typical assay, 20 µL STV-beads per sample were used and washed two times with 300 µL bead buffer (300 mM NaCl, 12.5 mM $MgCl_2$, 10 mM Hepes, 1 mM EDTA, 1 mM TCEP, 0.05% Triton X-100). The beads were re-suspended in 50 µL solution containing the protein-modified X-tiles or control proteins (1 µM, 50 µL) and the mixture was incubated for 10 min on ice. To remove unbound materials from the beads, they were washed two times with 100 µL bead buffer.

Subsequently MIS12- and NDC80-complexes were added to the beads in a 1:1 ratio of prey:bait for samples containing only one bait-protein and in a 2:1 ratio for samples containing both bait-proteins and the negative controls, in a final volume of 50 µl for 30 min on ice. The beads were washed two times with 100 µL bead buffer and analysis of the bead-bound proteins was conducted by denaturing SDS gel electrophoresis using 4-12% gradient Tris-glycine gels (Life Technologies) and MOPS running buffer, according to manufacturer's conditions. Gel bands were quantified using ImageJ software (http://imagej.nih.gov/ij/index.html). Individual lanes were selected and baseline corrected. This allowed for the integration of the band intensities. Data are shown in Figure 4 of the main manuscript.

6. References

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[2] F. Malvezzi, G. Litos, A. Schleiffer, A. Heuck, K. Mechtler, T. Clausen, S. Westermann, *EMBO J* **2013**, *32*, 409-423.