Electronic Supplementary Material (ESI) for Soft Matter.

Dynamic Exchange Controls the Assembly Structure of Nucleic-Acid-Peptide Chimeras

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Supplementary Information

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1. Supplementary Methods

s1. General

Chemical and reagents were purchased from Aldrich or Merck and unless otherwise specified used without further purification. Amino acids, resins and coupling reagents were purchased from Novabiochem and Alfa Aesar, and DMF was purchased from Biotech Grade. 5' hexynyl modified oligonucleotides were purchased from Bio- Synthesis.

s2. Synthesis and characterization

(a) Synthesis of the Azide-modified P9 peptide

Triflyl azide, used for modification of the n-terminus amine of the 9 amino acid peptide as follows. A suspension of sodium azide (3.3 mmol) in 4 mL of pyridine was cooled in an ice bath, triflic anhydride (2.78 mmol;Sigma-Aldrich) was added to the mixture by a syringe over 5 min of stirring, and the reaction was maintained in the ice bath for 2h. In parallel, the free amine peptide sequence was synthesized on a Rink amide MBHA resin using the standard Fmoc-based protocol. 100 mg of peptide-attached resin was allowed to swell in DMF for 20 min, followed by the addition of 0.02 mmol of $CuSO_4$ in MeOH (1 mL) and triethylamine (0.02 mol). The mixture was cooled in the ice bath for a few minutes, and the solution of triflyl azide in pyridine was added dropwise. The reaction mixture was allowed to warm to room temperature and reacted for 5h. The same procedure was repeated 3 times with fresh triflyl azide reagents. Upon completion, the reaction mixture was washed with DMF, DIPEA/DMF (99.5:0.5; 3 times; 2 min each), and finally with DMF, DCM and ether. The azido-peptide was obtained after cleavage and global deprotection with a mixture of TFA/TIS/water (95:2.5:2.5). The resin was removed by filtration under reduced pressure and the TFA was evaporated out of the peptide mixture. Cold ether, 8–10 times in volume, was added to precipitate the crude peptide. The peptide was purified by preparative HPLC using a Dionex Ultimate 3000 instrument with C18 reverse phase preparative column. The identity and purity of the peptide were analyzed on a Dionex 1100 HPLC using a reverse phase C18 column. ESI-MS mass spectrometry (LCMS Thermo Surveyor 355) analysis was performed to verify the molecular weight (measured Mw=1143).

(b) Synthesis of DNA-peptide conjugates via the 'click' chemistry

The azido-peptide (1 eq.) and 5' hexynyl DNA (1 eq.) were dissolved in water/tBuOH (1:1). $CuSO_4$ (1% mole eq.) and Tris((1-benzyl-4-triazolyl)methyl)amine (5% mole eq.) were added to the solution, followed by sodium ascorbate (0.5 eq.) and the mixture was shaken at 37°C for 3 hours. The reaction progress was monitored using the analytical Dionex 1100 HPLC with a reverse phase C18 column. Upon completion, the product was purified by HPLC, and analyzed by HPLC and MALDI-TOF mass spectrometry (measured MW = 4396 ssCon1, 4267 ssCon2). Only compounds with high purity (≥ 95% based on HPLC) were used for future studies.[1]

s3. Sample preparation for the spectroscopy and microscopy assembly experiments

Freshly prepared aqueous solutions were used for the assembly experiment by dissolving lyophilized powder into (10 mM) phosphate buffer pH 7. The concentrations of DNA and DNA-pep samples were determined by measuring the UV absorption of diluted stock solutions at 260 nm (the maximum absorption of DNA), using ε=99,000 L/(mole*cm) for DNA1 and ε=85,400 L/(mole*cm) for DNA2.

To samples containing dsDNA or dsCon have been added 10 mM $MgCl_2$ and were incubated at elevated temperature (>70 °C) for 10 min, cooled down to RT and then equilibrated for at least four more hours (unless written otherwise) before analyzed.

For the competition experiments, the competitive strand DNA1 was either added with ssCon1 and ssCon2, or separately, one hour after pre-incubation of ssCon1 and ssCon2 (and their assembly to the spherical structure). In the simultaneous co-assembly experiments, DNA1 was mixed with the two conjugates, the mixture heated to 70°C, and then cooled to room temperature. In contrast, for the strand-exchange experiments, the ssCon1 and ssCon2 was heated and cooled back to room temperature; DNA1 was added after 1h of equilibrating at the dsCon mixture at RT. In both cases, the time = 0 was considered right after heating the sample to 70°C.

(a) Characterization of the dsCon secondary structure domains by circular dichroism measurements

CD spectra were collected on a Jasco J-815 CD-spectrometer. All samples were prepared by dissolving powder of the peptide, DNA or conjugates in the phosphate buffer pH 7, and their final concentrations were re-assigned from the UV-absorption. CD spectra were recorded between 200 nm to 320 nm with data steps of 0.5 nm, scan speed of 10 nm*min^{-1}, response time 4s and bandwidth of 2 nm. The baseline buffer spectrum was measured and subtracted

from the sample spectra. All samples were measured in triplicate, and the results were averaged automatically. De-convolution of the CD data allowed to extract signals originating from the different assembly domains, containing peptide β-sheet, A-DNA, B-DNA or random coils. The deconvolution procedure was performed using the origin 2018 graphing & analysis software, by assigning the main peaks' central position based on the control experiments (e.g., with the P9 peptide alone) and/or available literature data.

(b) Structural characterization of the dsCon and related assemblies by atomic force microscopy imaging

A 3 µL aliquot of solution after equilibration as described above was deposited on a freshly cleaved mica surface. The sample was then slowly dried in air overnight. Topography images were acquired by AFM (SolverPro, NT-MDT, Ru) in tapping mode using noncontact tips BudgetSensors Multi75Al-G, 3 Nm@1, 75 kHz. Image processing, which included second order polynomial line fitting and cross-section analysis, was done using the NOVA AFM software.

(c) Structural characterization of the dsCon and related assemblies by cryogenic transmission electron microscopy imaging

Samples for direct imaging of the aqueous dispersions were prepared in the controlled environment box of a vitrification robot (Vitrobot, FEI), as follows: a 2 µL drop of the sample solution was deposited on a glow-discharged TEM grid, the excess liquid was automatically blotted with a filter paper, and the specimen was rapidly plunged into liquid ethane and transferred to liquid nitrogen, where it was kept until use. The samples were examined at minus 175°C using a FEI Tecnai 12 G2 TWIN TEM that operated at 120 kV in low-dose mode and with a few micrometers under focus to increase the phase contrast. The images were recorded with a Gatan charge-coupled device camera (model 794). Fiber lengths were analyzed by the Gatan microscopy suite software.

(d) Structural characterization of the dsCon and related assemblies by transmission electron microscopy imaging

A TEM copper grid with a 200-mesh carbon support (Electron Microscopy Sciences) was covered with 10 µL of a dsCon solution (50 µM) in 10 mM phosphate buffer pH 7, for 1 min before wicking the excess solution with filter paper. 10 µL of the staining solution, 2% uranyl

acetate, (Sigma–Aldrich), was added and incubated for 2 min; excess solution was wicked away, and the grids were placed in desiccators to dry under vacuum. A Hitachi H-7500 transmission electron microscope was used to image the samples at 75 kV. Fiber lengths were analyzed by the Gatan microscopy suite software.

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(e) Confocal fluorescence microscopy imaging of the dsCon assemblies

dsCon samples prepared as described above were incubated for 4h, then the respective fluorescence dyes (4′,6-diamidino-2-phenylindole, DAPI, and 2-[4-(Dimethylamino) phenyl]-3,6-dimethyl-1,3-benzothiazol-3-ium chloride, ThT) were added to the solution, which was then equilibrated for additional 30 min. For all compounds, 3 μL of the stained sample were applied on a microscope slide and covered by high performance cover glass D=0.17 mm. All samples were scanned with 405 nm laser on Zeiss LSM 880 with Airyscan and processed in Fiji ImageJ program.

(f) Dictating the double stranded conjugate melting temperatures

Samples prepared for the thermal melting analysis contained total concentration of 5μM DNA1-pep and DNA2-pep in 1:1 ratio, in a total volume of 200μL of 10 mM phosphate buffer pH 7 and the respective added salt. T_m was assigned using the change in absorption at 260 nm upon transition from duplex to single strand. The scans were performed on an Agilent Cary60 UV/Vis spectrometer with Temperature Controller TC125 using a cuvette of 1 cm pass length.

2. Supplementary tables

Table s1. Sequences of the molecules utilized in this study

Molecular name	Sequence
P9	N_3-βA-EFEFEFEA
DNA1	5'-hexynyl-GAATGGCCGG
DNA2	5'-hexynyl-CCGGCCATTC
ssCon1	3'-GGCCGGTAAG-triazole linker- βA-EFEFEFEA
ssCon2	3'-CTTACCGGCC-triazole linker- βA-EFEFEFEA

* (βA = β-alanine)

Table s2. Deconvolution of the CD ellipticity peak areas

Sample	Peak index	λ max (nm)	θ (mdeg)	Area integration	Area range (%)
dsCon	1	201.4	-0.98	5.8	8.7
	2	209.5	-0.61	3.7	7.9
	3	216.5	-0.18	0.8	1.7
	4	241.0	-0.33	9.8	20.8
	5	264.0	0.79	13.1	27.6
	6	277.3	0.65	15.8	33.3
dsDNA	1	210.7	-1.78	23.3	19.4
	2	265.5	1.90	43.8	37.3
	3	223.5	-1.67	52.5	43.3
P9	1	205.8	-1.44	18.4	50.9
	2	219.4	-1.03	8.1	25.9
	3	228.2	-0.51	6.3	20.2
	4	236.1	-0.19	1.0	3.0
dsDNA+P9	1	203.6	-1.03	4.0	4.4
	2	207.5	-1.68	11.5	12.6
	3	214.4	-1.88	24.7	27.0
	4	227.6	-0.87	13.0	14.2
	5	264.7	1.54	38.0	41.8

Table s3. Deconvolution of the CD ellipticity data

Sample	DNA conformation	λ max (nm)	θ (mdeg)	Area integration	Area range (%)	Sum area range (%)
dsCon+10 % DNA1	A	210.2	-0.99	9.7	**10.2**	**16.8**
		258.9	0.70	6.2	**6.6**	
	B	243.6	-1.87	43.2	**45.6**	**64.8**
		269.2	0.90	18.2	**19.2**	
dsCon+25 % DNA1	A	208.5	-3.06	64.8	29.5	**33.2**
		264.6	0.53	8.07	3.7	
	B	243.1	-1.94	40.5	18.4	**21.6**
		278.6	0.40	16.4	3.2	
dsCon + 50% DNA1	A	205.8	-0.44	8.7	7.8	**29.1**
		265.7	0.53	10.5	21.3	
	B	245.5	-1.12	20.9	42.6	**45.4**
		278.6	0.07	1.4	2.8	
dsCon+150 % DNA1	A	210.46	-0.95	8.5	12.6	**30.3**
		263.45	0.92	11.9	17.7	
	B	246.97	-1.03	7.9	11.7	**13.0**
		270.69	0.18	0.9	1.3	

Table s4. Average fiber length for assemblies formed under variable conditions

Entry #	ssCon1: ssCon2	Additives[a]		Fiber length (nm)[b]
		Seq (eq.)	Salt (mM)	
1	1:1	DNA1 (1.5)[a]	$MgCl_2$ (10)	±11040
2	1:1	-	-	800±300
3	1:1	-	$MgCl_2$ (5)	2000±1000
4	1:1	-	$CaCl_2$ (10)	110±50
5	1:1	-	NaCl (20)	1300±600
6	1:1	-	LiCl (20)	1900±900

[a]DNA1 was added after 1 h of incubation. [b]Fiber lengths were calculated based on ≥30 length values measured for the designated sample.

Supplementary Figures

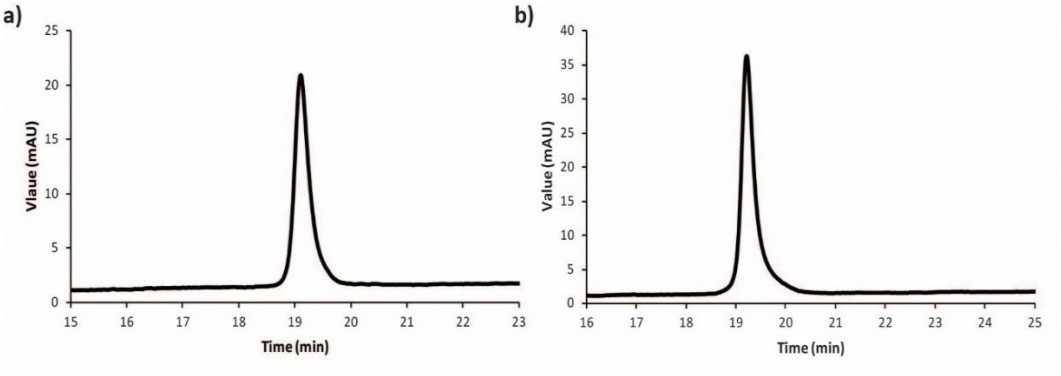

Figure s1. HPLC traces (detected at 260 nm) for a) pure ssCon1, and b) pure ssCon2.

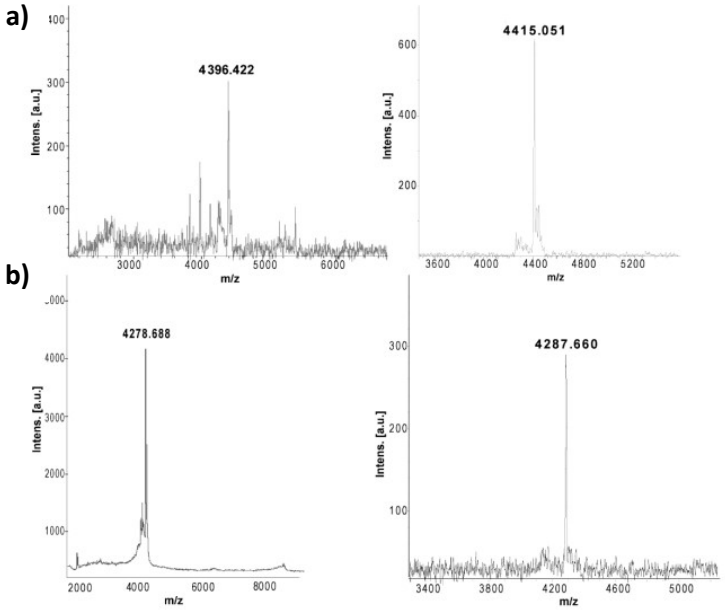

Figure s2. Characterization of the conjugates by MALDI-TOF MS. a) ssCon1 (calculated Mw = 4396; Mw = 4415 for Na^+ adduct), and b) ssCon2 (calculated Mw = 4267; Mw = 4288 for Na^+ adduct).

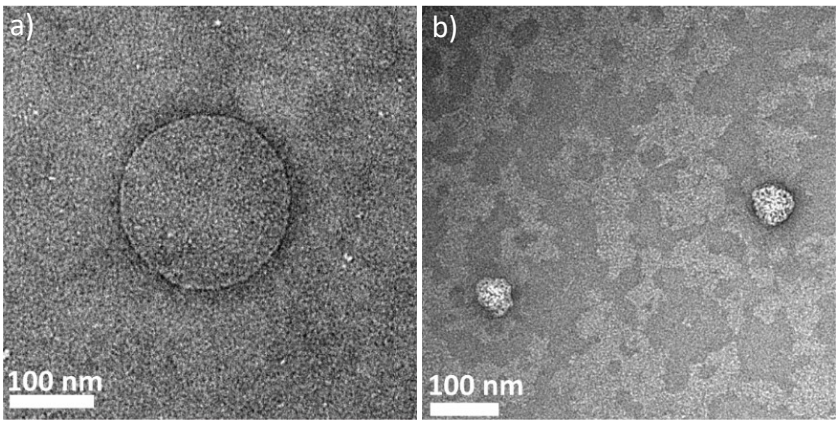

Figure s3. Images of assemblies observed in 50 μM dsCon solutions, featuring the formation of spherical particles. a,b) TEM images of self-assembled nanostructures obtained after negative staining with uranyl acetate. The sample contained 10 mM phosphate buffer and 10 mM $MgCl_2$, pH~7.

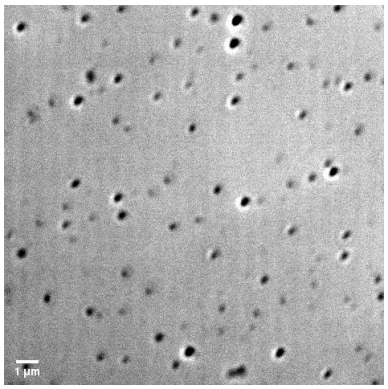

Figure s4. Bright field image of 50 μM dsCon solution, showing the formation of spherical particles in 10 mM phosphate buffer and 10 mM $MgCl_2$, pH~7.

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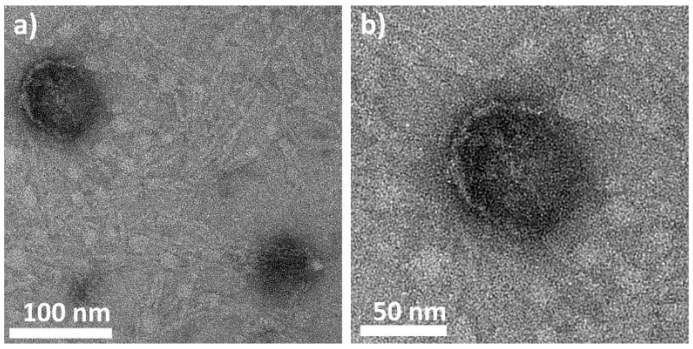

Figure s5. TEM images of 50 μM dsCon after incubation for 1h, showing the chimera's arrangement into spherical objects in 10 mM phosphate buffer and 10 mM $MgCl_2$, pH~7.

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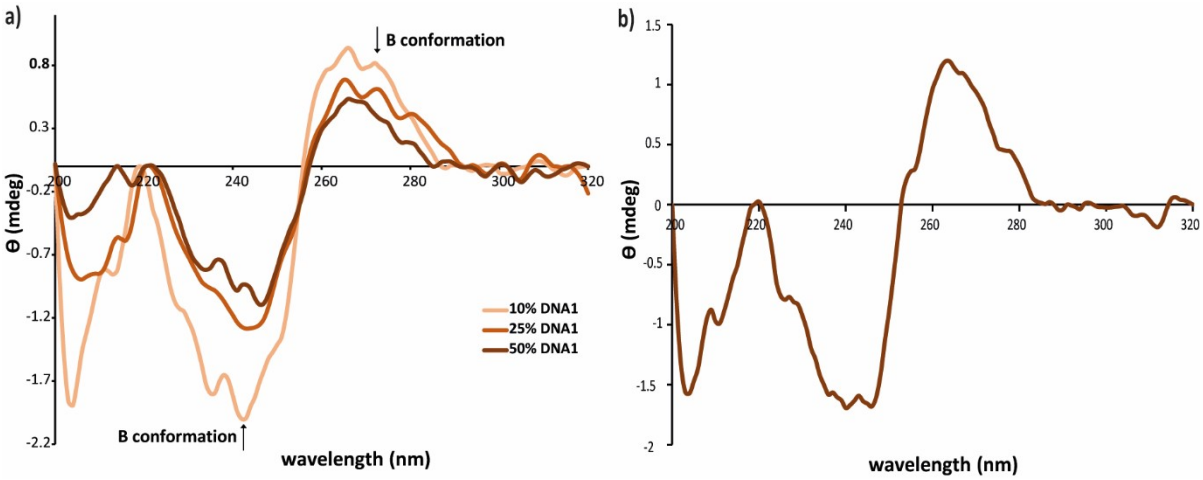

Figure s6. CD spectra of 10 µM dsCon and various concentrations of DNA1 as a competitor strand. a)10%, 25%, and 50% DNA1, and b) 150% DNA1. All samples contained 10 mM phosphate buffer and 10 mM $MgCl_2$, pH~7. Raw data was subjected to baseline correction and minimal smoothing (by the ORIGIN built-in protocol).

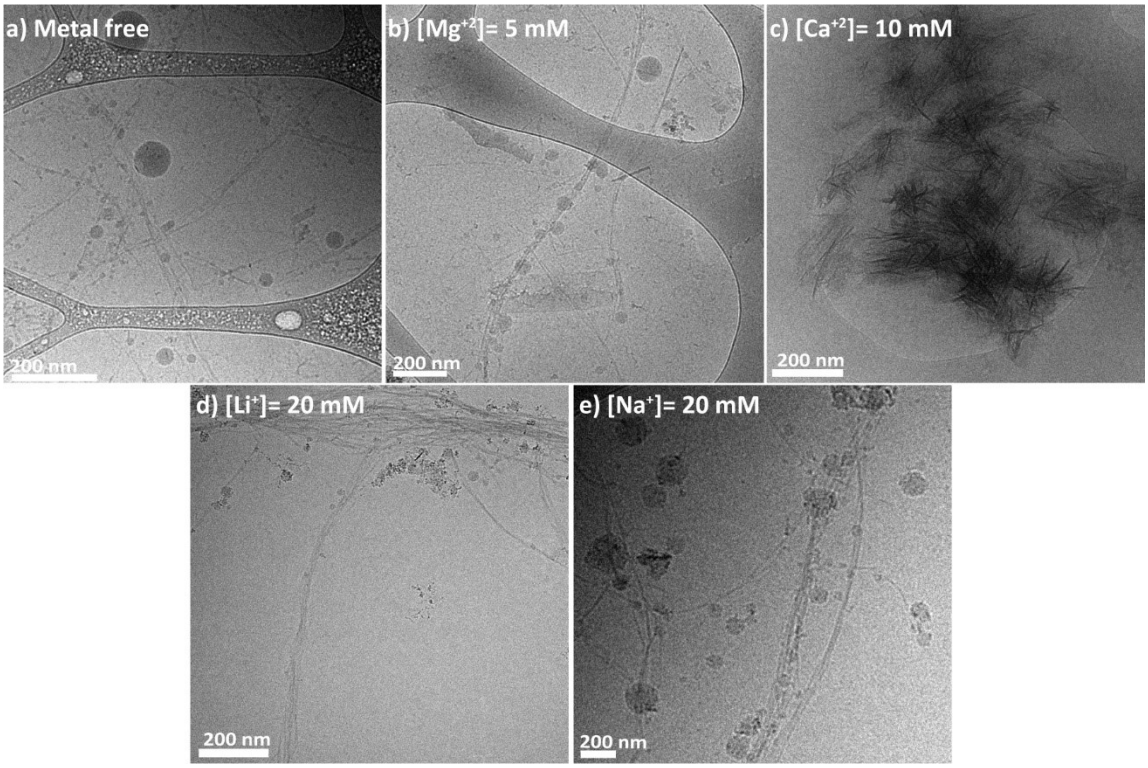

Figure s7. Cryo-TEM images of assemblies formed by 500 µM dsCon in the absence of metal cations (a) or in the presence of $[Mg^{+2}]$ = 5 mM (b), $[Ca^{+2}]$ = 10 mM (c), $[Li^{+}]$ = 20 mM (d), and $[Na^{+}]$ = 20 mM (e). All the samples contained 10 mM phosphate buffer, pH~7.

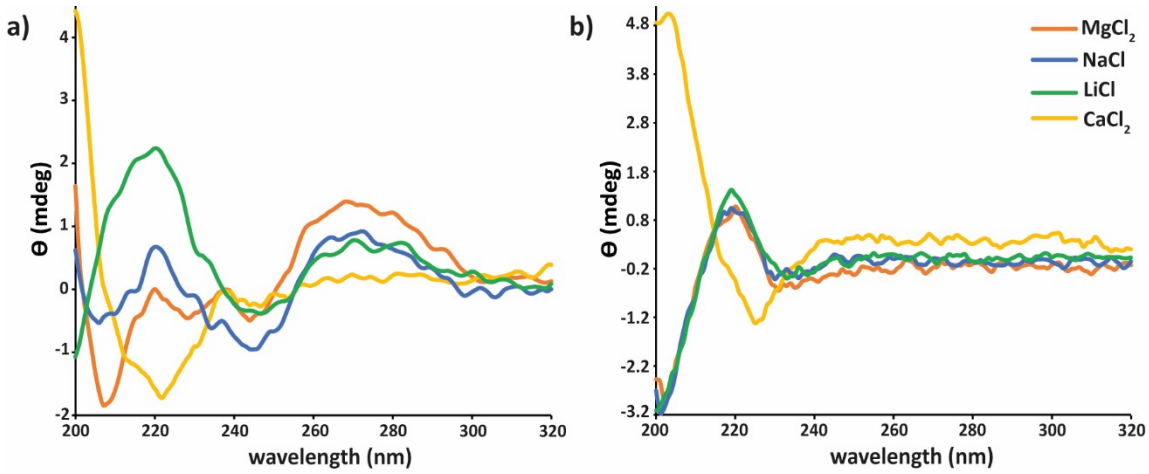

Figure s8. CD spectra of 10 µM dsCon (a), or 50 µM p9 (b) in the presence of different salts. All samples contained 10 mM phosphate buffer, pH~7. The positive cotton effect at ~220 nm indicates aromatic interactions by phenylalanine residues.

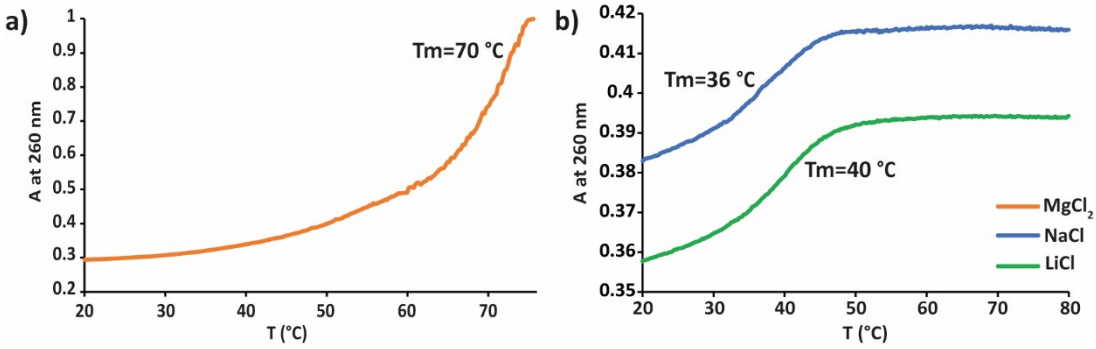

Figure s9. Temperature-dependent denaturation of 5 µM dsCon in the presence of 10 mM $MgCl_2$ (a) and 20 mM NaCl and LiCl (b). All samples contained 10 mM phosphate buffer, pH~7.

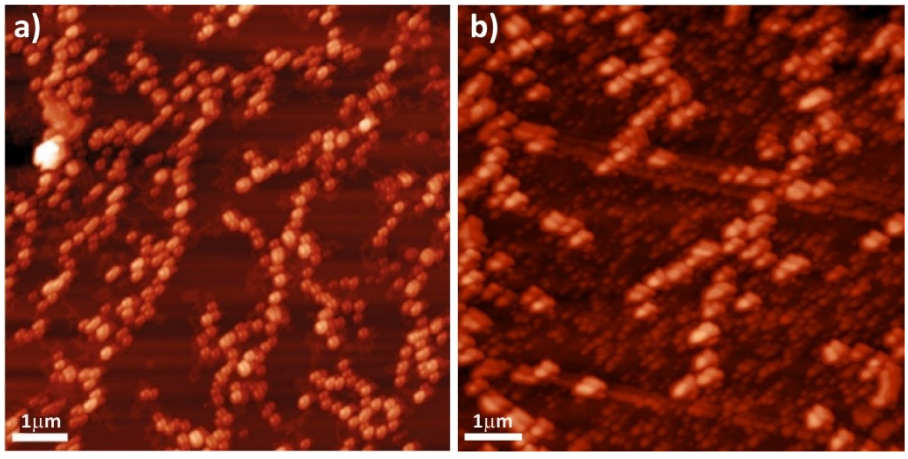

Figure s10. Additional AFM images of assemblies formed by 50 µM (a) and 25 µM (b) dsCon in the presence of 5 mM $MgCl_2$, displaying chain-like architectures. All samples contained 10 mM phosphate buffer, pH~7.

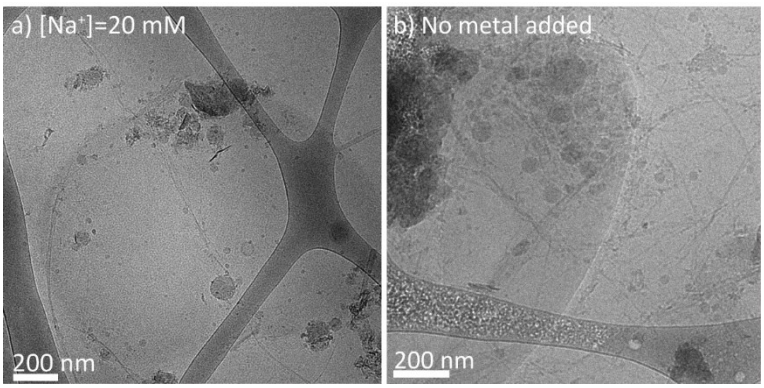

Figure s11. Representative membraneless large objects during the assembly of (500 μM) dsCon in the presence of 20 mM of sodium cation (a) or when no metal cations were added to the solution (b). All samples contained 10 mM phosphate buffer, pH~7.

References

1. A. Chotera, H. Sadihov, R. Cohen-Luria, P. A. Monnard and G. Ashkenasy, *Chem. Eur. J.* 2018, **24**, 10128.