

Supplementary Figure S1:
Gel electrophoresis data

2% Agarose gels, stained with EtBr, run at 70 V.

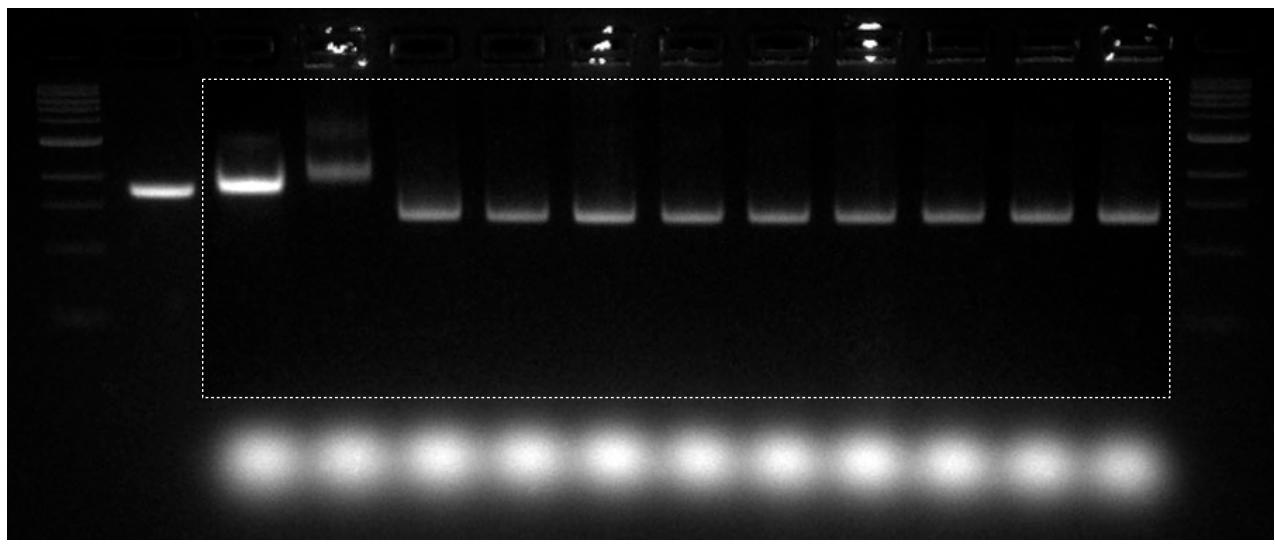
Photographed with Alpha Imager HP

ref = scaffold DNA used in self-assembly reactions

M = 1 kb DNA Ladder from New England BioLabs

6 helix bundle; 2 day annealing in MgCl₂ [mM]

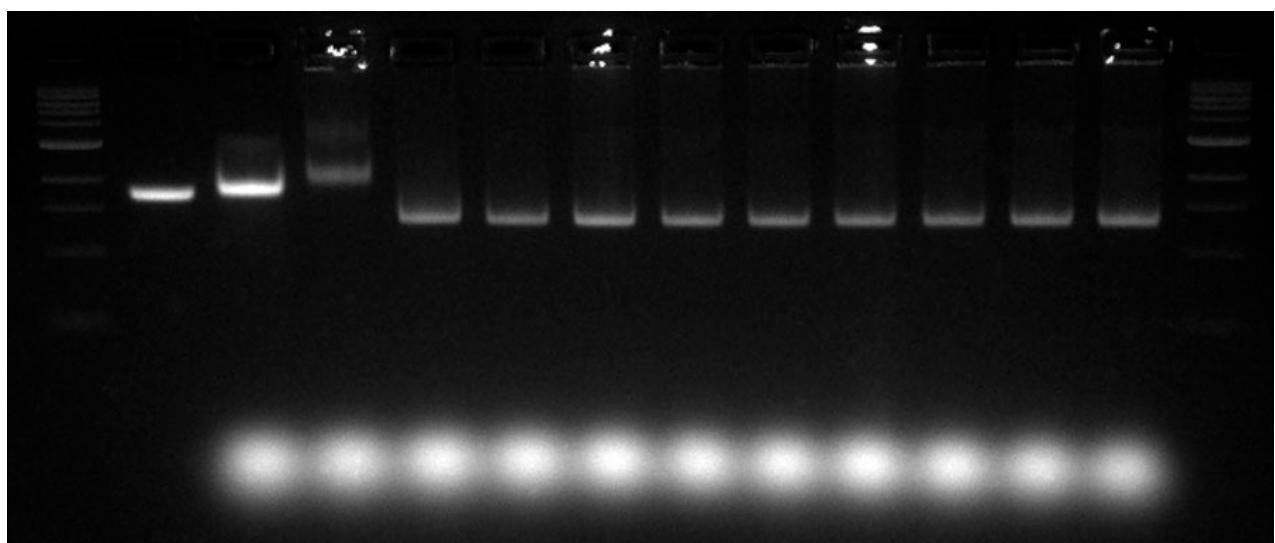
M ref 0 2 4 6 8 10 12 14 16 18 20 M



Region of interest auto-leveled

6 helix bundle; 2 day annealing in MgCl₂ [mM]

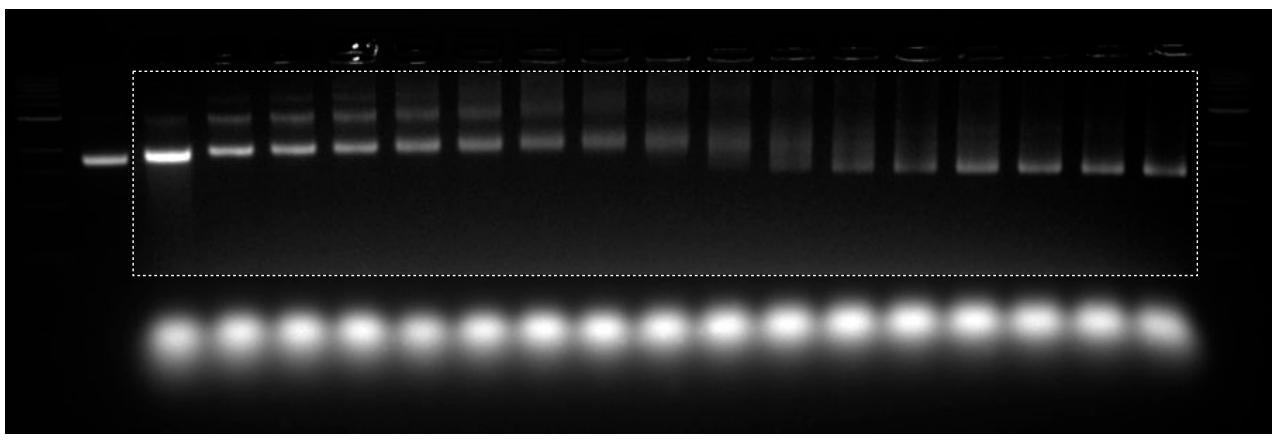
M ref 0 2 4 6 8 10 12 14 16 18 20 M



Globally auto-leveled

6 helix bundle; 2 day annealing in NaCl [mM]

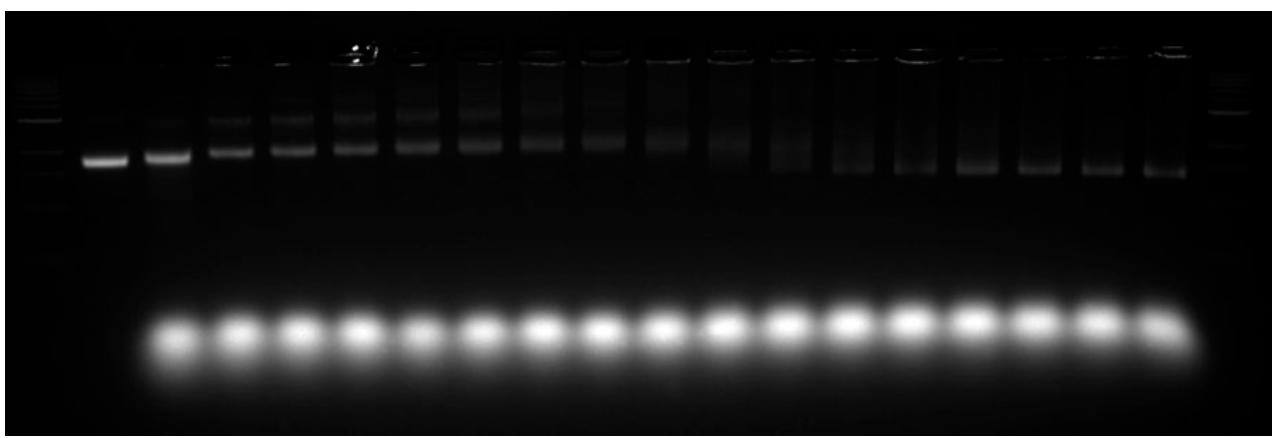
M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M



Region of interest auto-leveled

6 helix bundle; 2 day annealing in NaCl [mM]

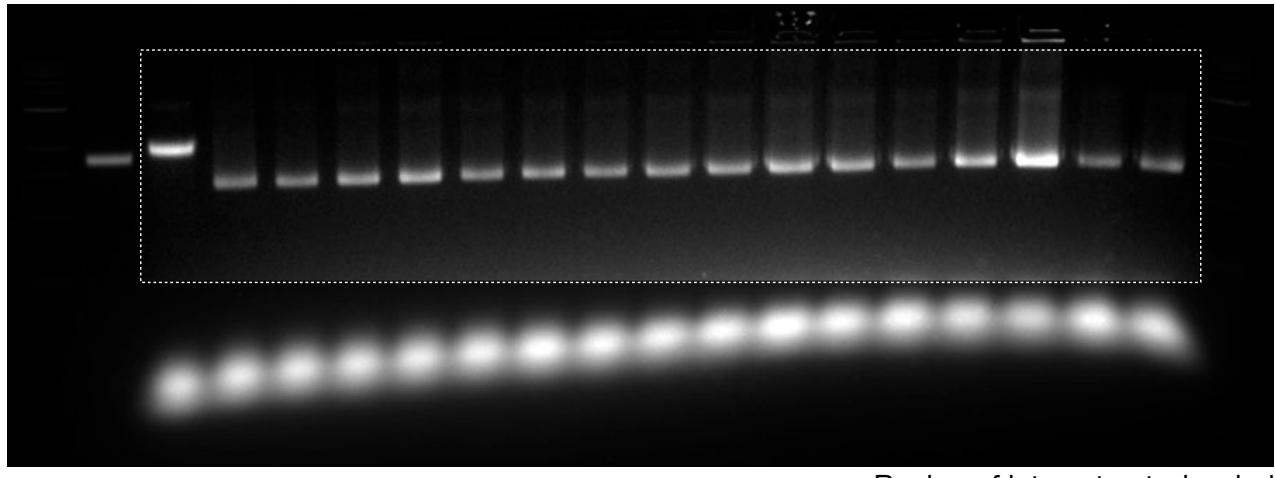
M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M



Globally auto-leveled

6 helix bundle; 2 day annealing in NaCl [M]

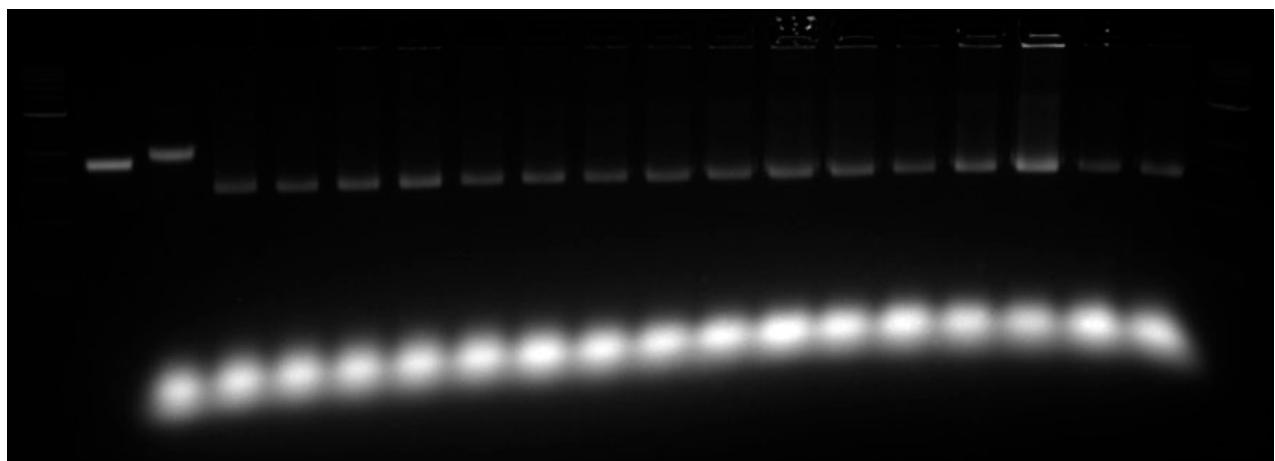
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

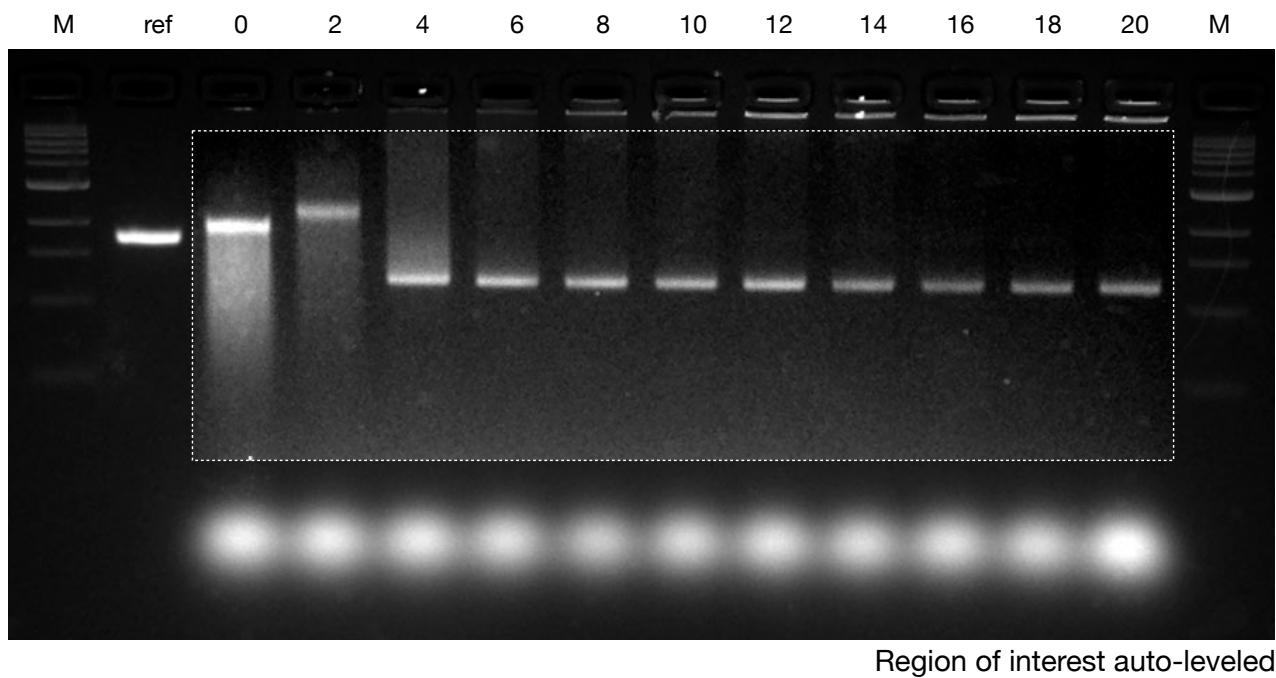
6 helix bundle; 2 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M

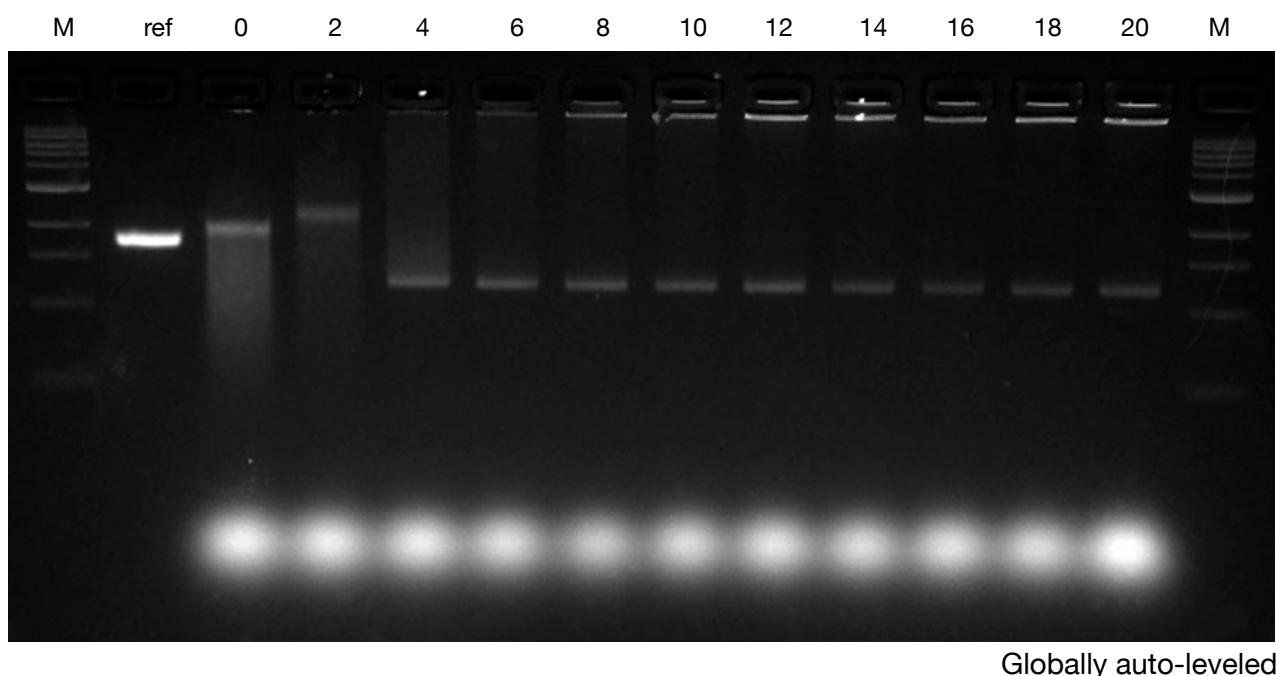


Globally auto-leveled

8 helix bundle; 12 day annealing in MgCl₂ [mM]

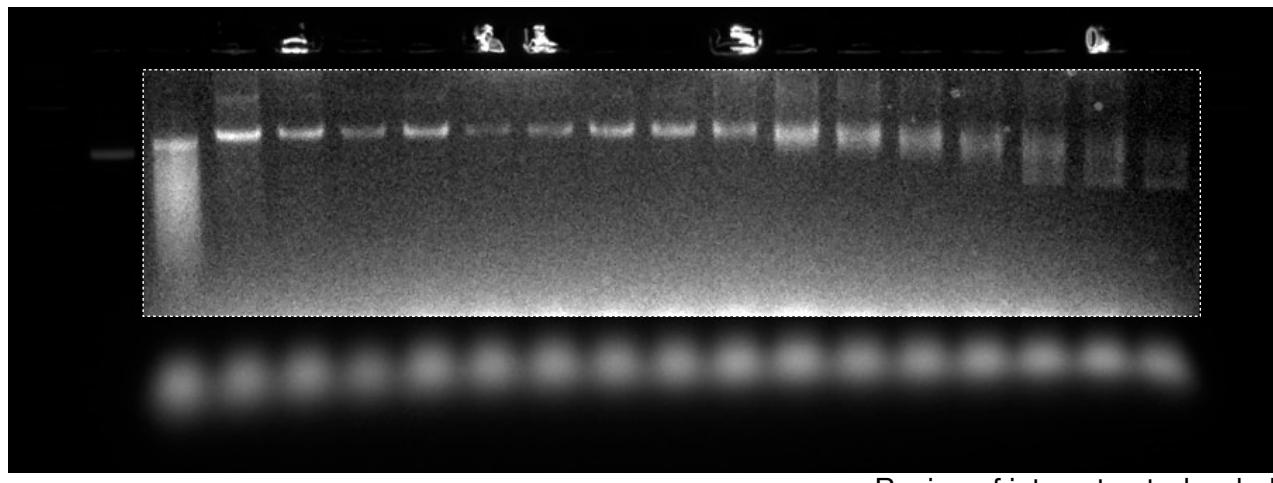


8 helix bundle; 12 day annealing in MgCl₂ [mM]



8 helix bundle; 12 day annealing in NaCl [mM]

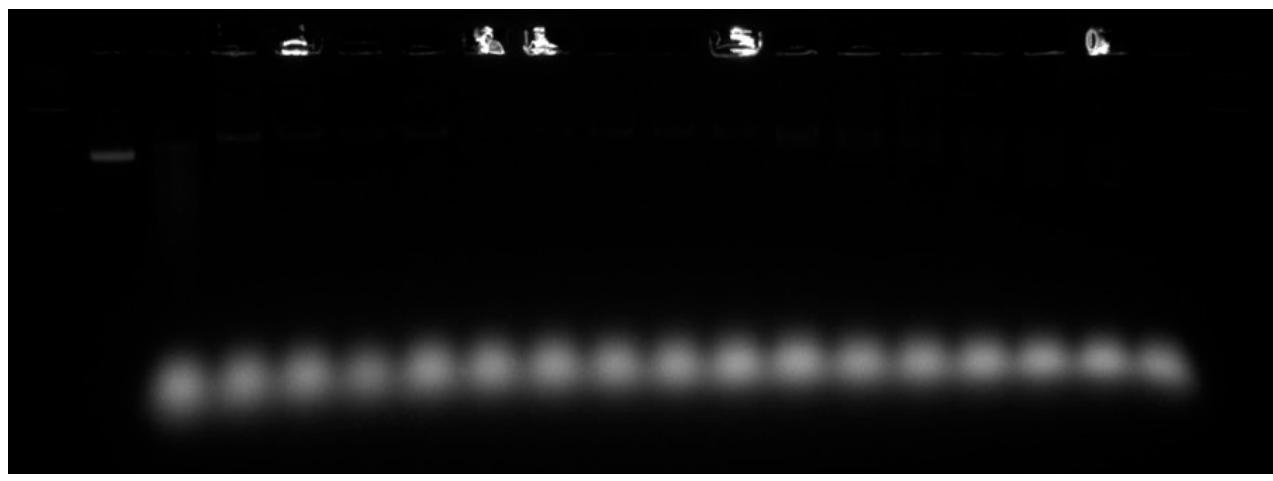
M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M



Region of interest auto-leveled

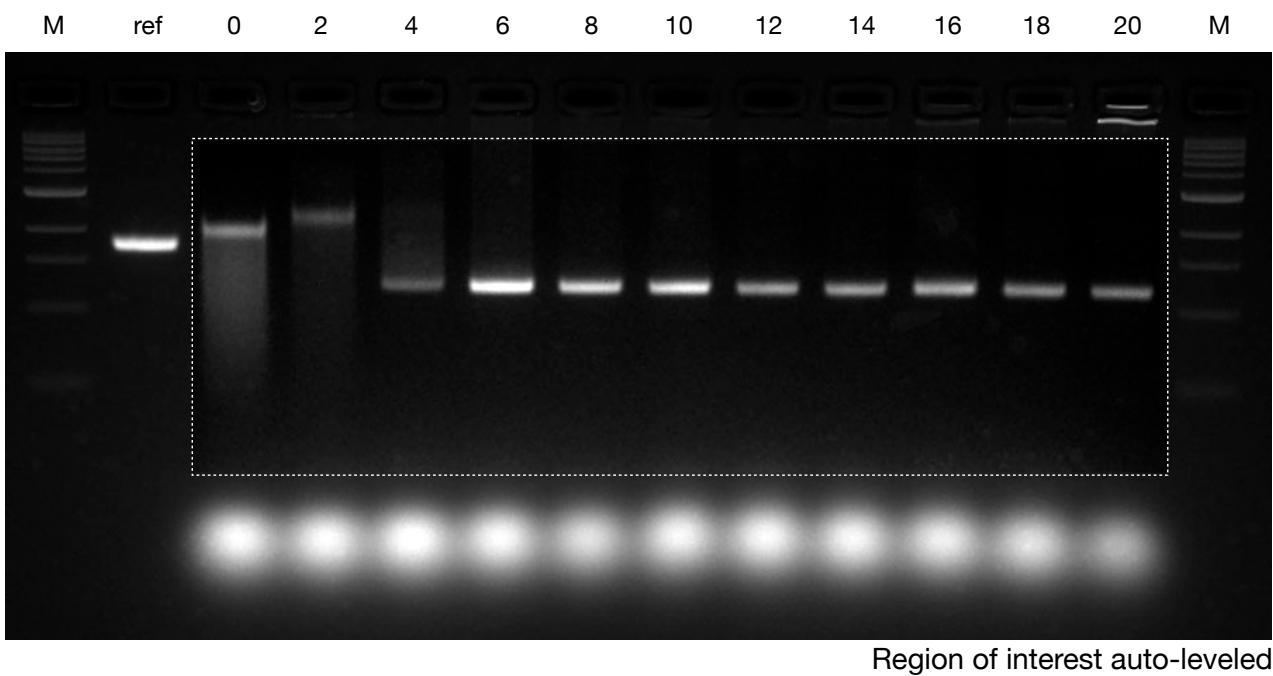
8 helix bundle; 12 day annealing in NaCl [mM]

M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M

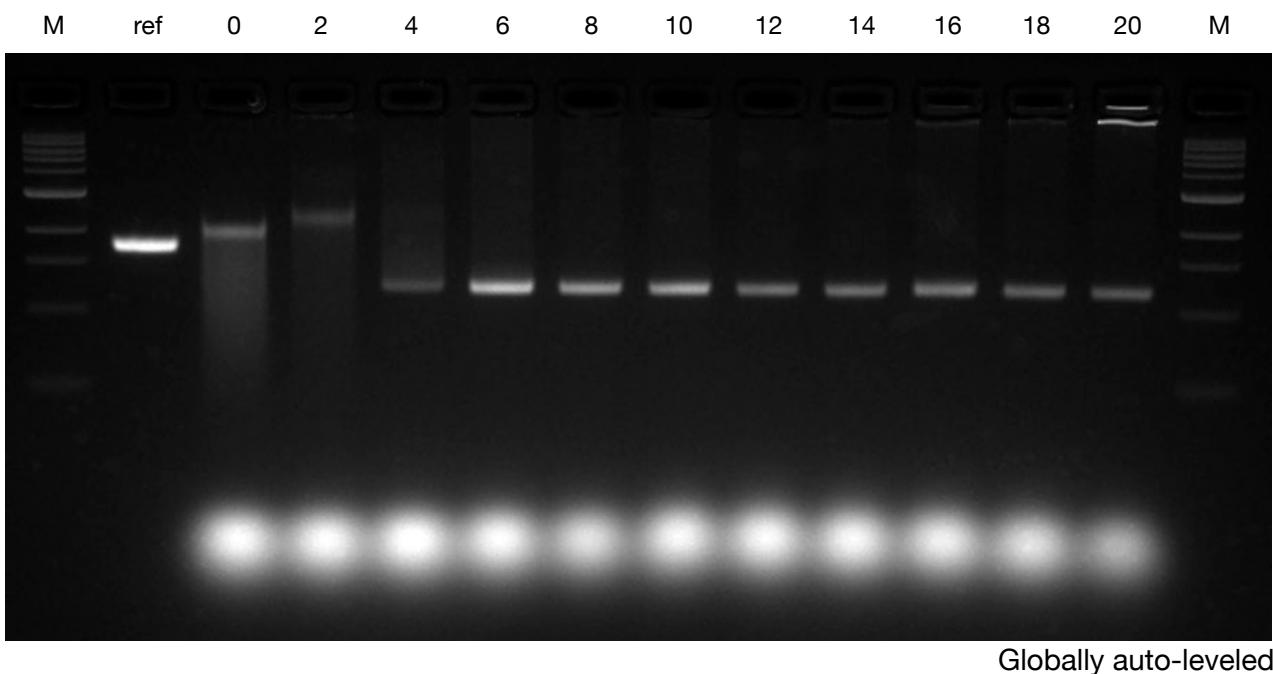


Globally auto-leveled

10 helix bundle; 12 day annealing in MgCl₂ [mM]

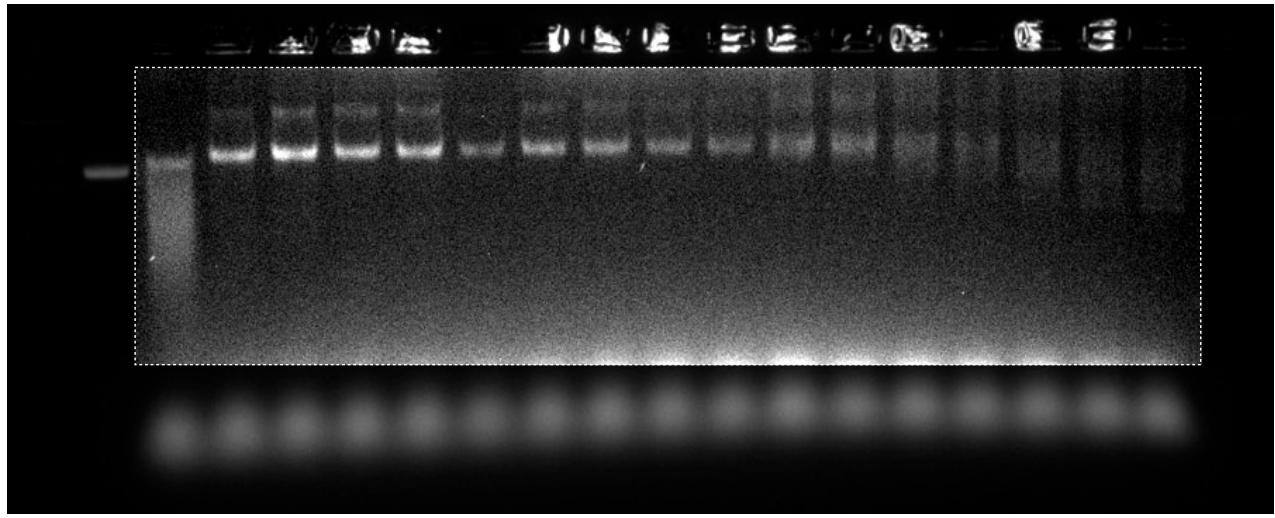


10 helix bundle; 12 day annealing in MgCl₂ [mM]



10 helix bundle; 12 day annealing in NaCl [mM]

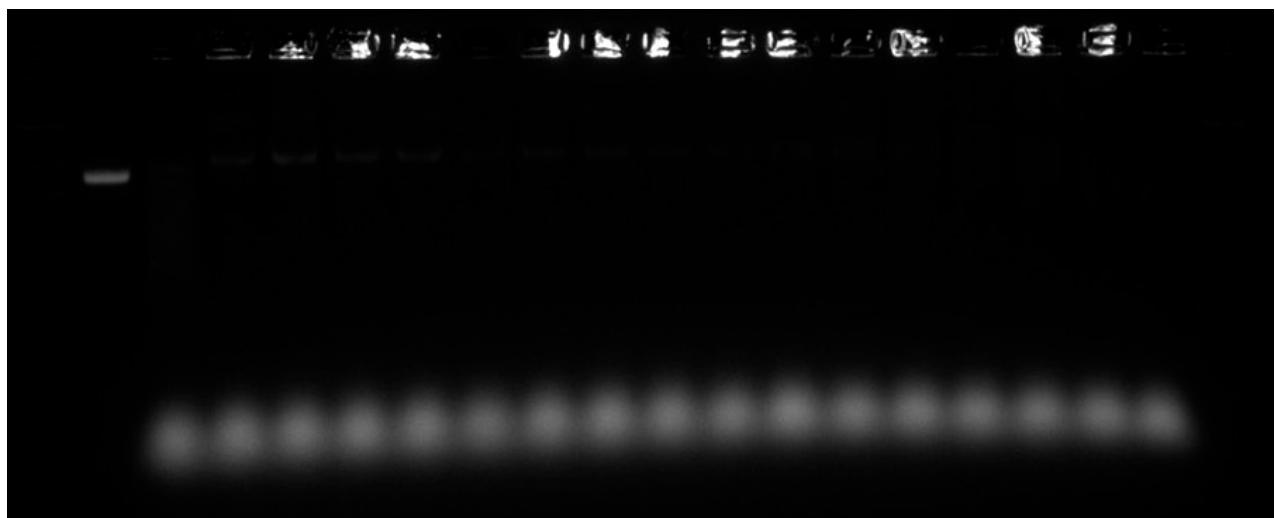
M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M



Region of interest auto-leveled

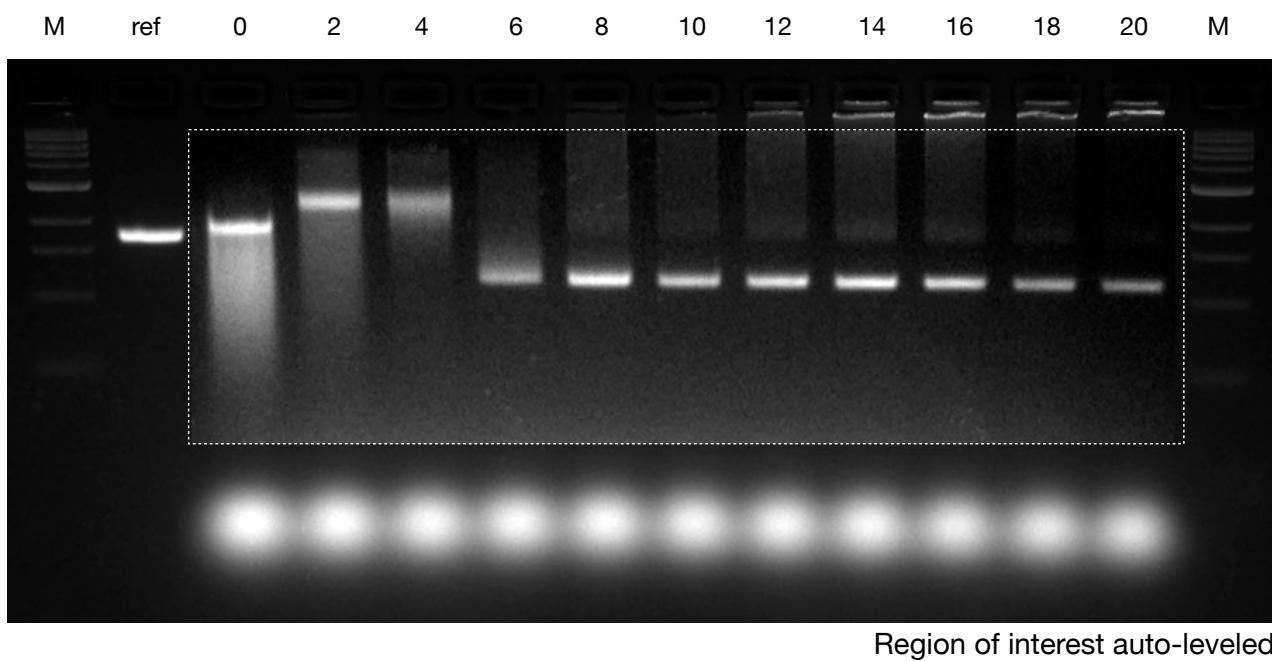
10 helix bundle; 12 day annealing in NaCl [mM]

M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M

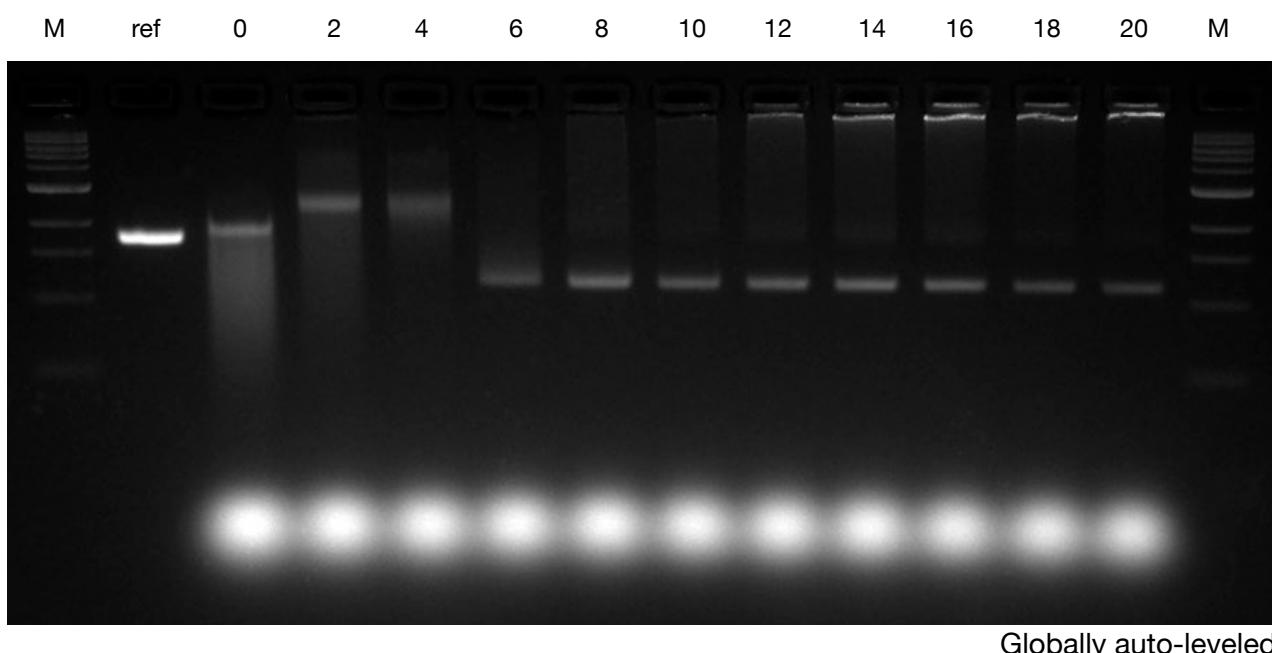


Globally auto-leveled

12 helix bundle; 12 day annealing in MgCl₂ [mM]

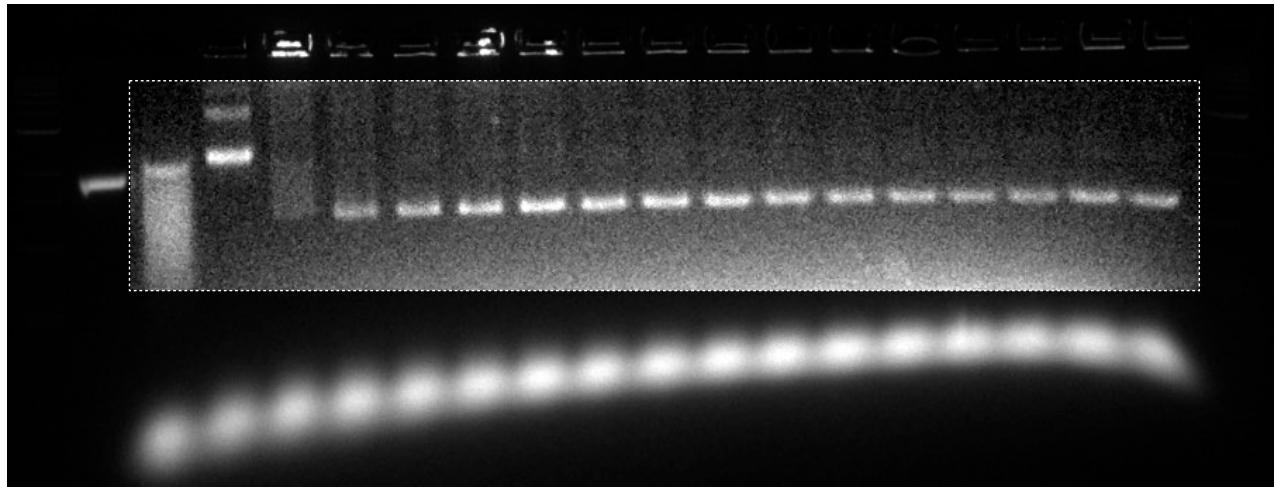


12 helix bundle; 12 day annealing in MgCl₂ [mM]



12 helix bundle; 12 day annealing in NaCl [M]

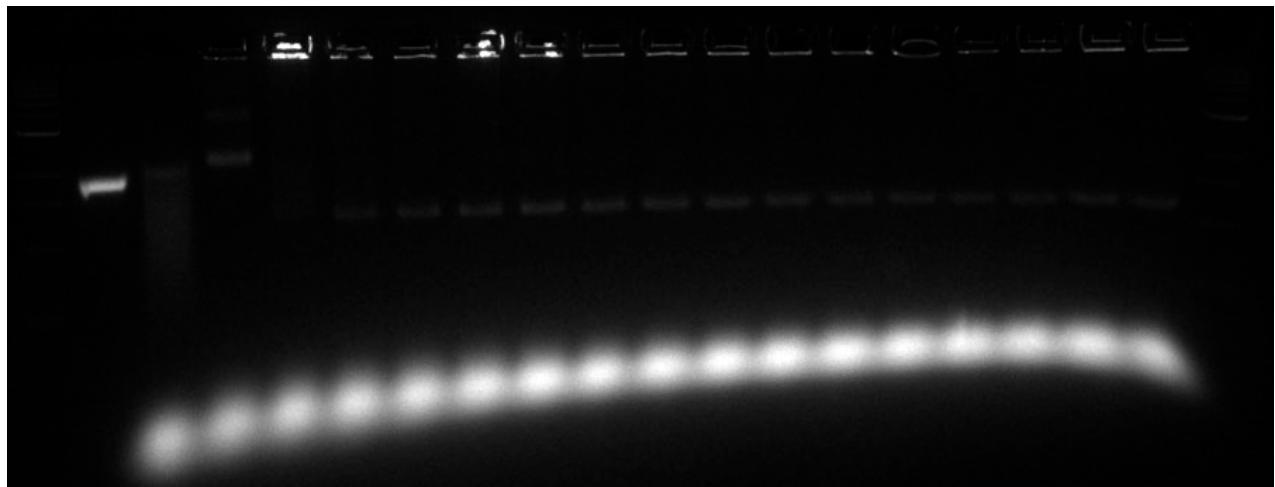
M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M



Region of interest auto-leveled

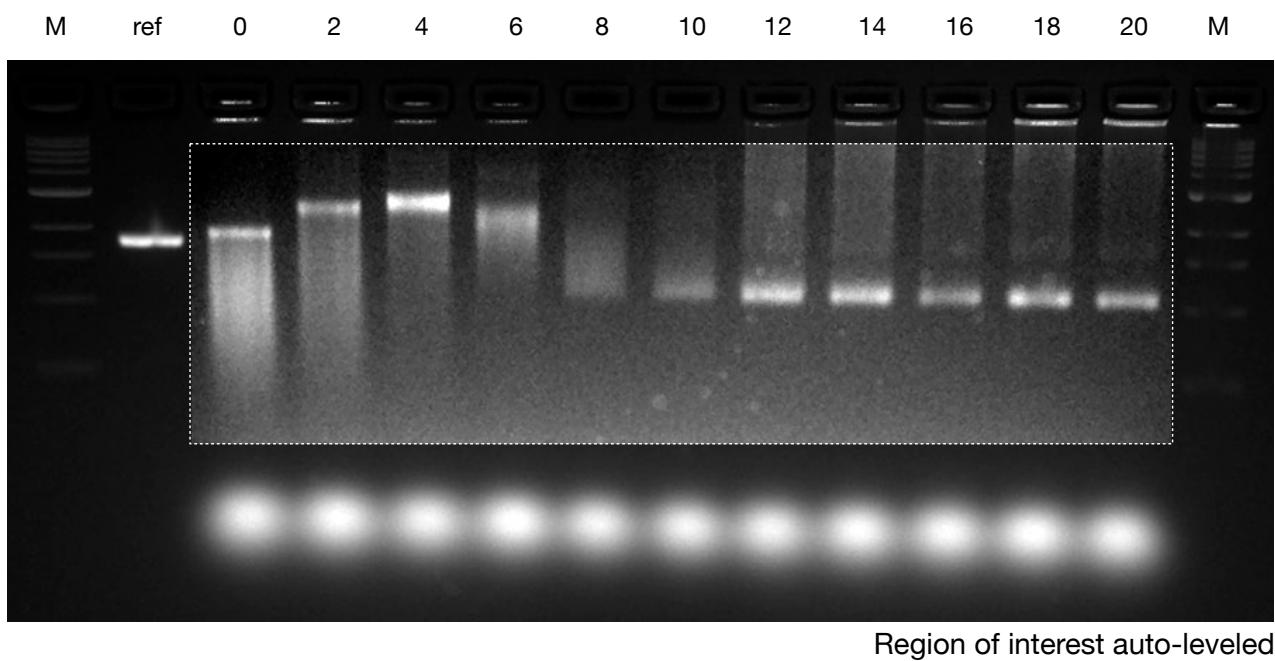
12 helix bundle; 12 day annealing in NaCl [M]

M ref 0 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 M

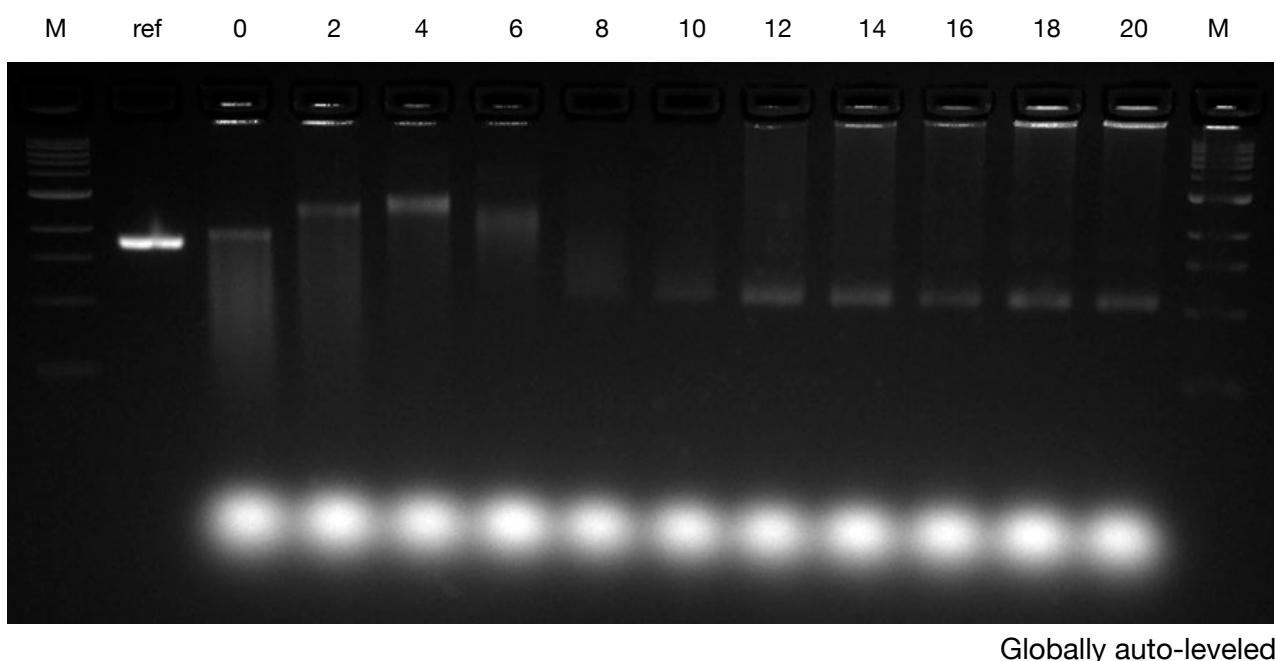


Globally auto-leveled

18 helix bundle; 12 day annealing in MgCl₂ [mM]

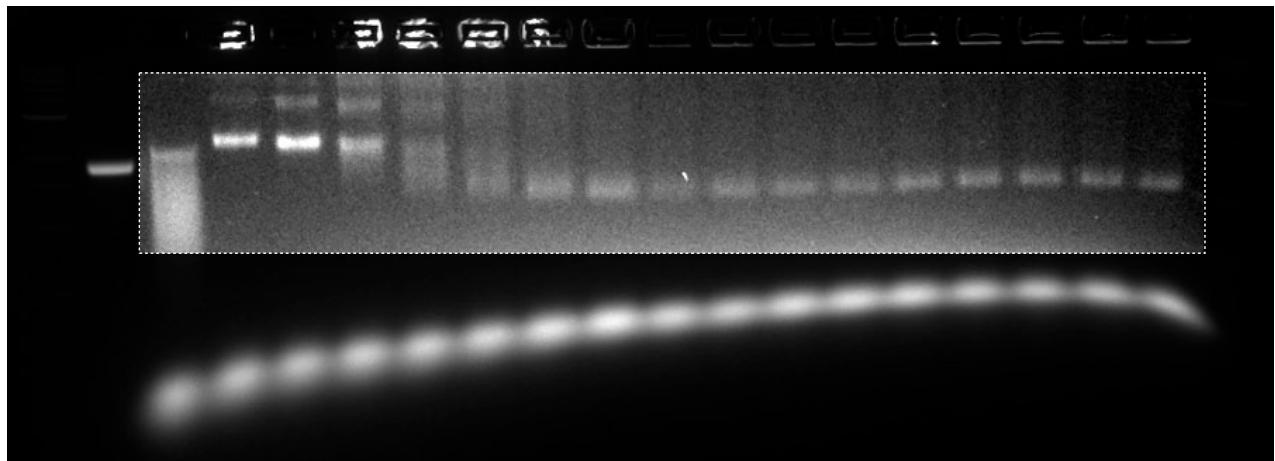


18 helix bundle; 12 day annealing in MgCl₂ [mM]



18 helix bundle; 12 day annealing in NaCl [M]

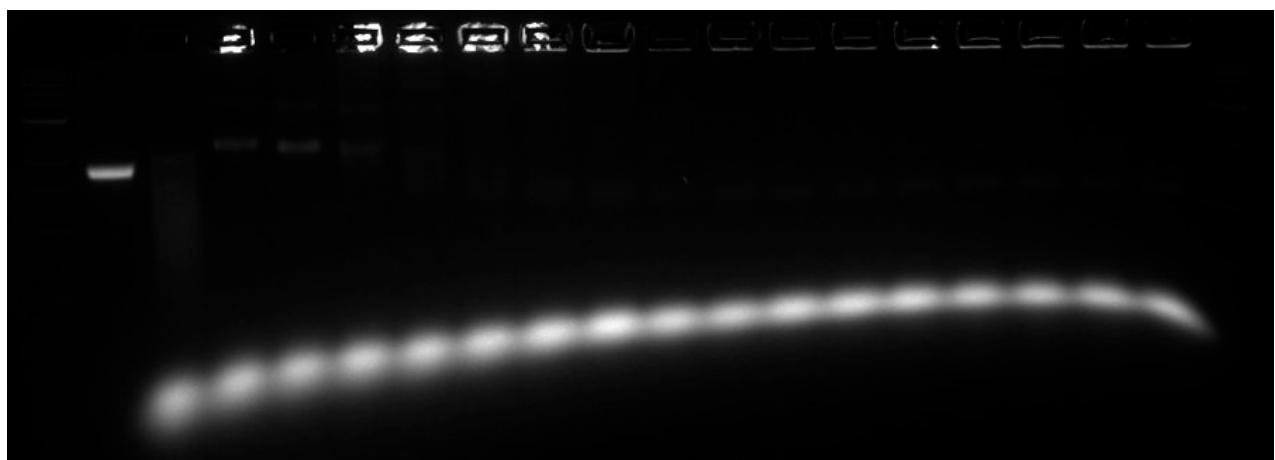
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

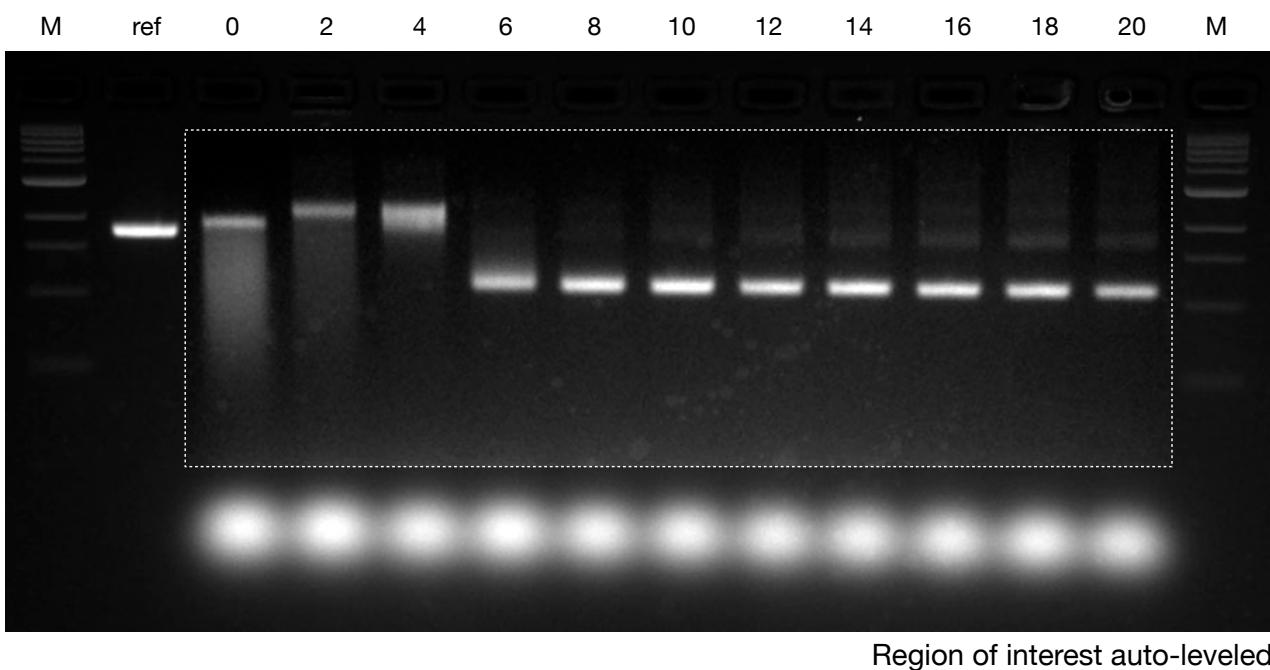
18 helix bundle; 12 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



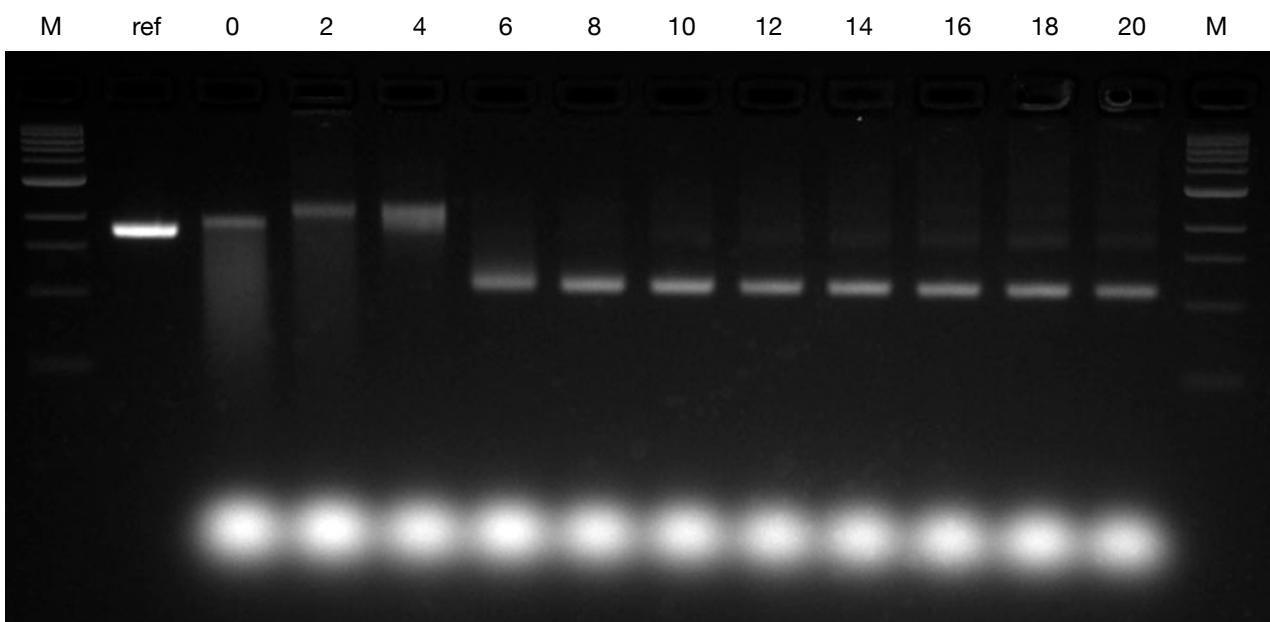
Globally auto-leveled

24 helix bundle; 12 day annealing in MgCl₂ [mM]



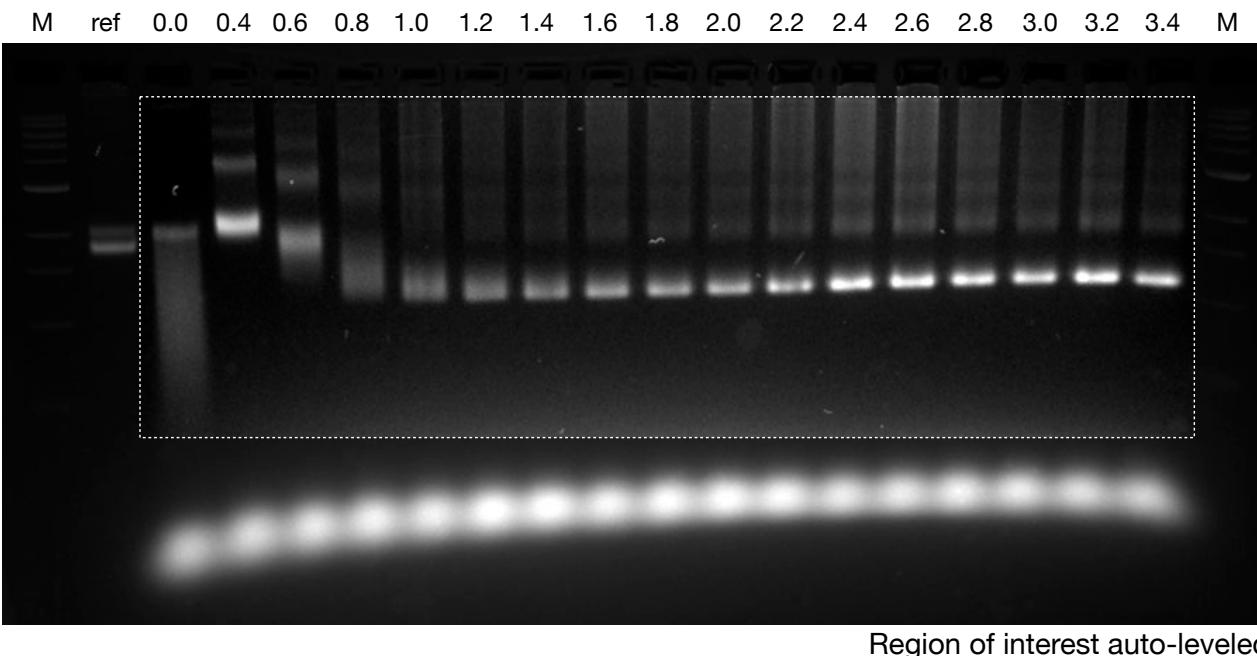
Region of interest auto-leveled

24 helix bundle; 12 day annealing in MgCl₂ [mM]

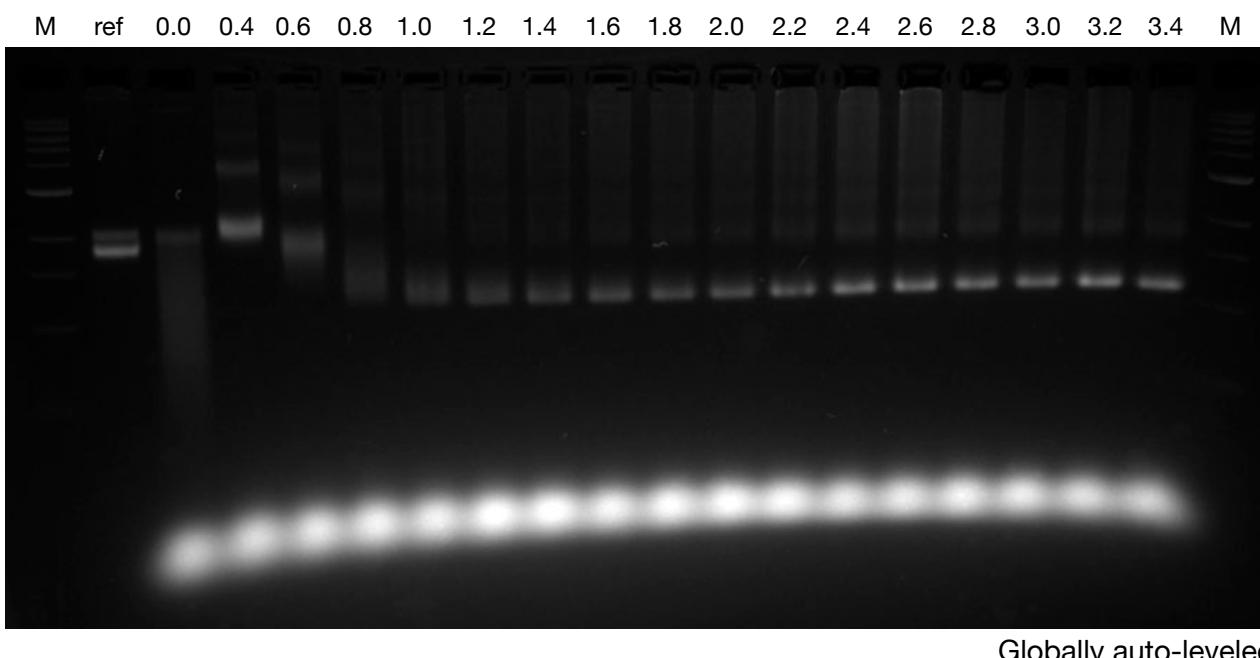


Globally auto-leveled

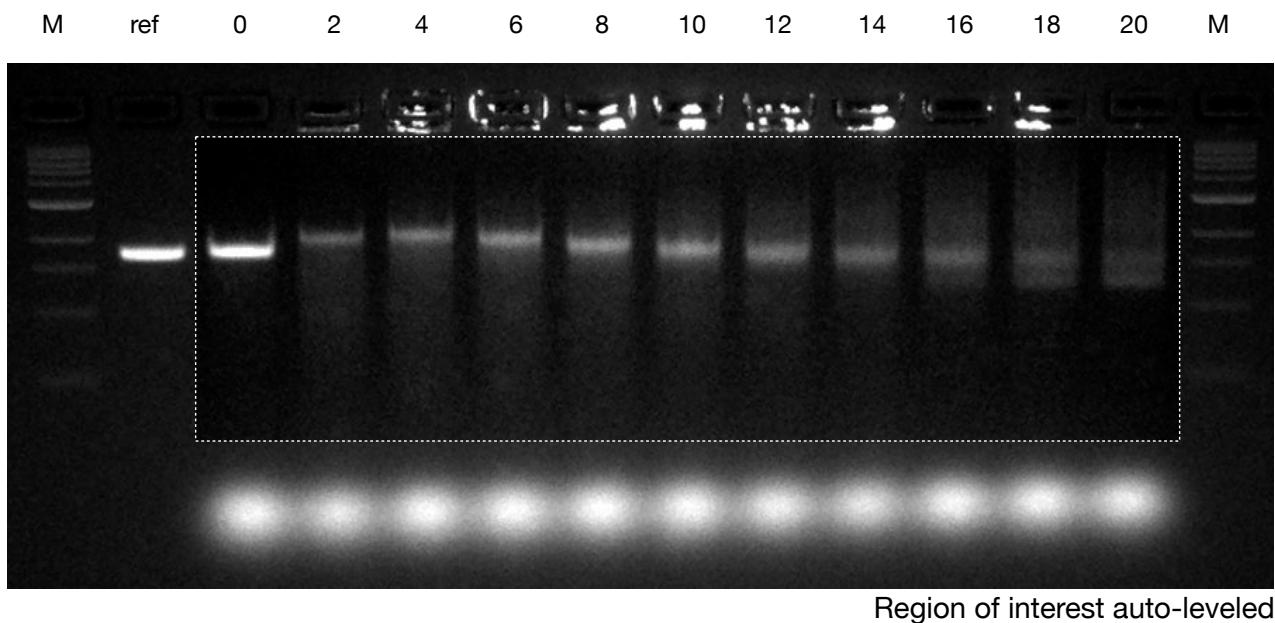
24 helix bundle; 12 day annealing in NaCl [M]



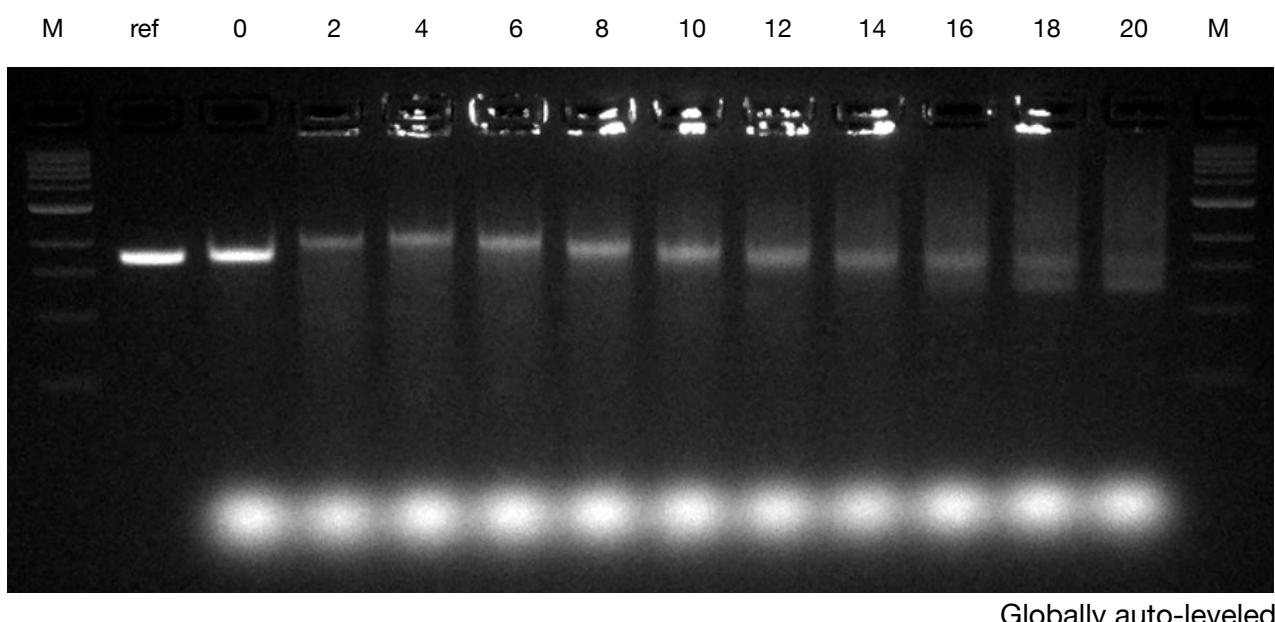
24 helix bundle; 12 day annealing in NaCl [M]



42 helix bundle, backbone nick rule 1; 3 day annealing in MgCl₂ [mM]

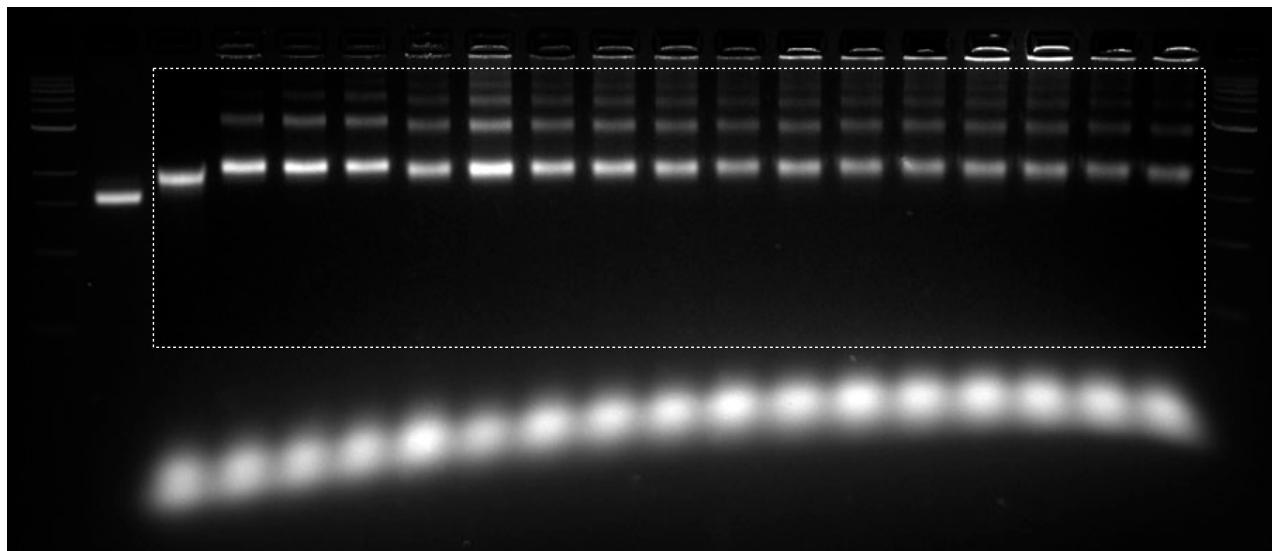


42 helix bundle, backbone nick rule 1; 3 day annealing in MgCl₂ [mM]



42 helix bundle, backbone nick rule 1; 3 day annealing in NaCl [M]

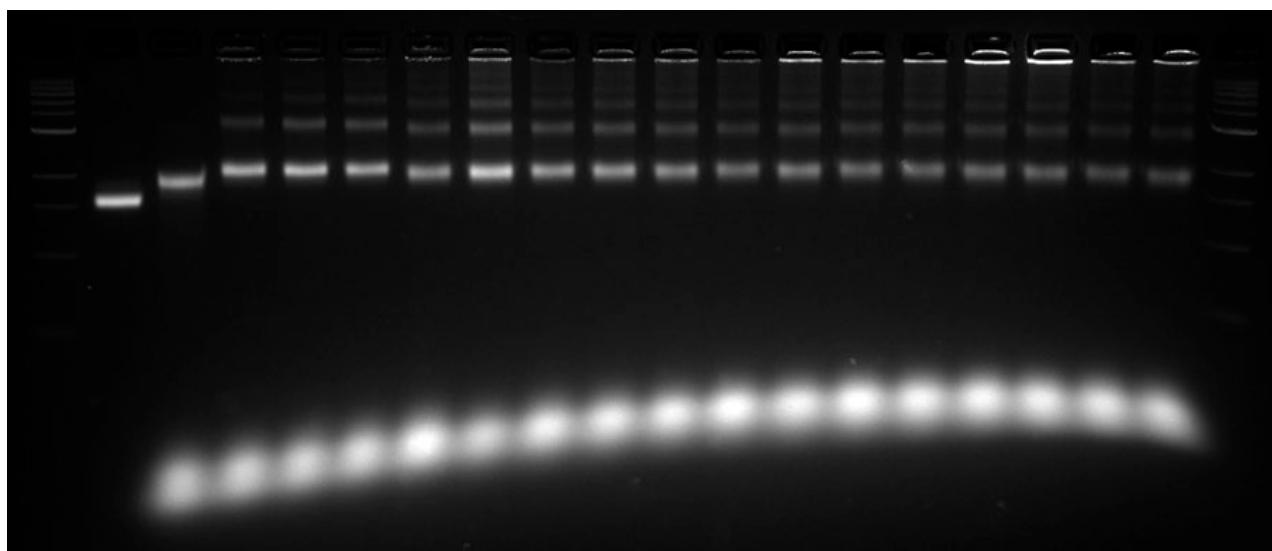
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

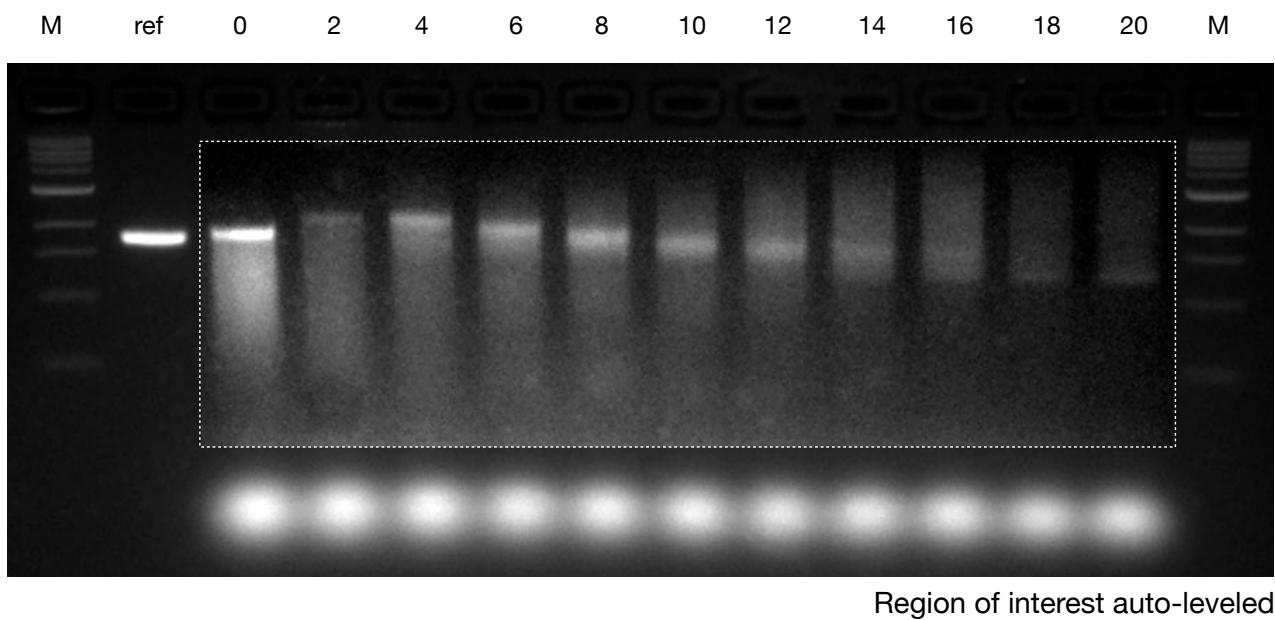
42 helix bundle, backbone nick rule 1; 3 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



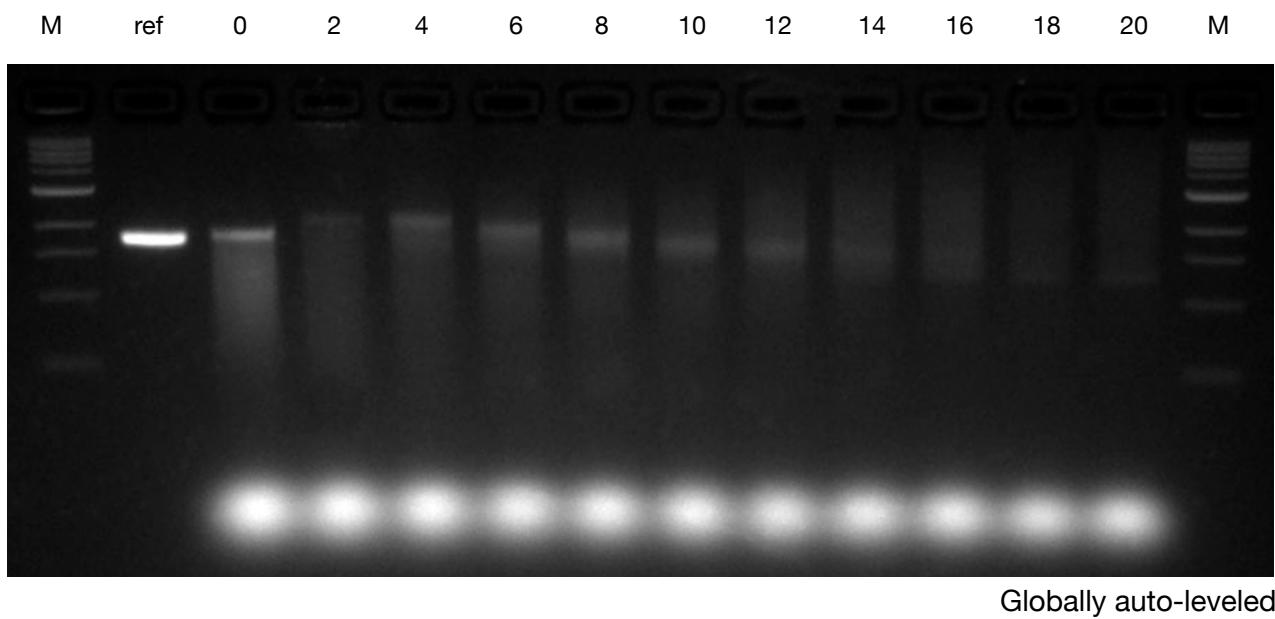
Globally auto-leveled

42 helix bundle, backbone nick rule 1; 12 day annealing in MgCl₂ [mM]



Region of interest auto-leveled

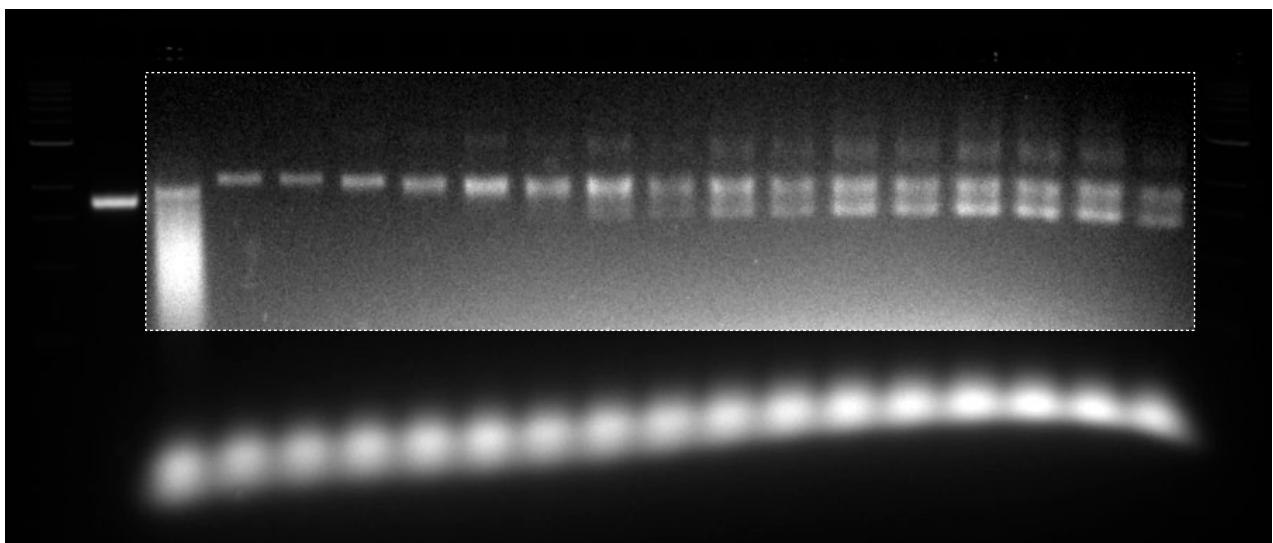
42 helix bundle, backbone nick rule 1; 12 day annealing in MgCl₂ [mM]



Globally auto-leveled

42 helix bundle, backbone nick rule 1; 12 day annealing in NaCl [M]

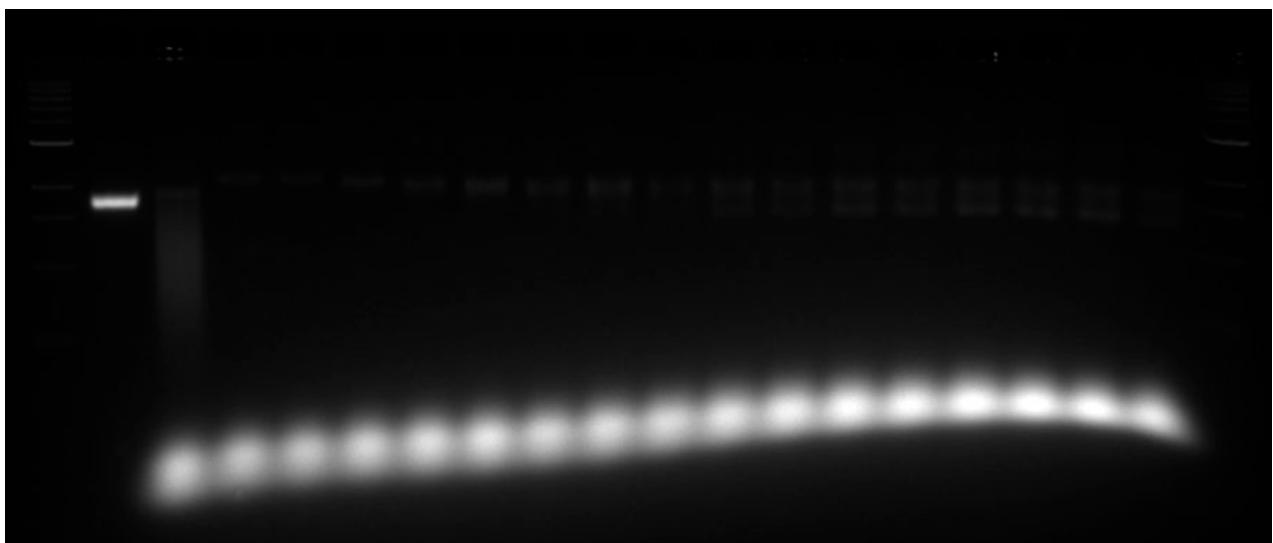
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

42 helix bundle, backbone nick rule 1; 12 day annealing in NaCl [M]

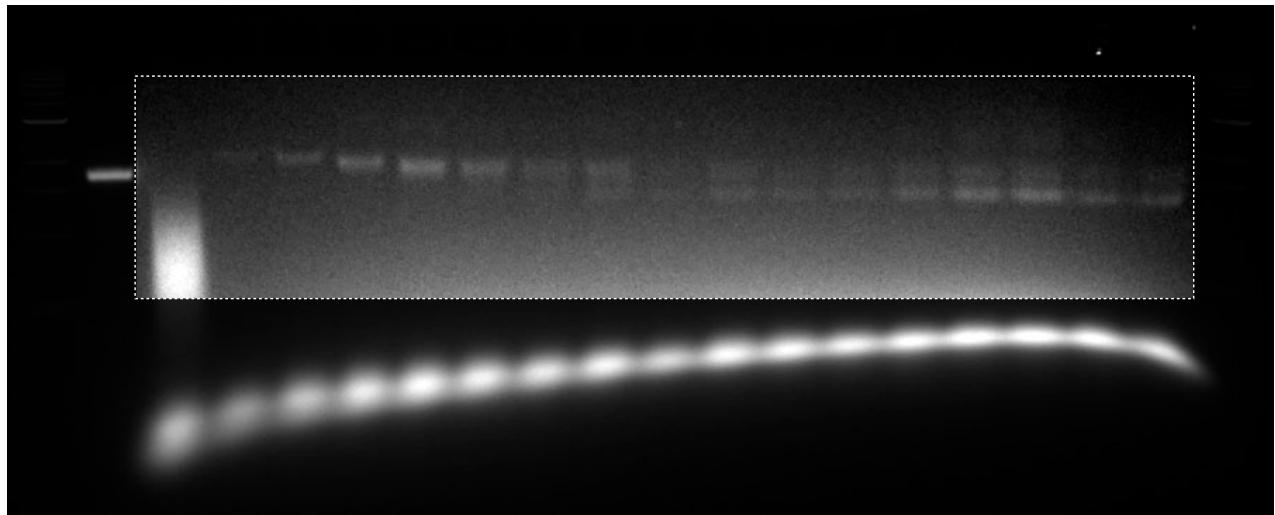
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Globally auto-leveled

42 helix bundle, backbone nick rule 1; 23 day annealing in NaCl [M]

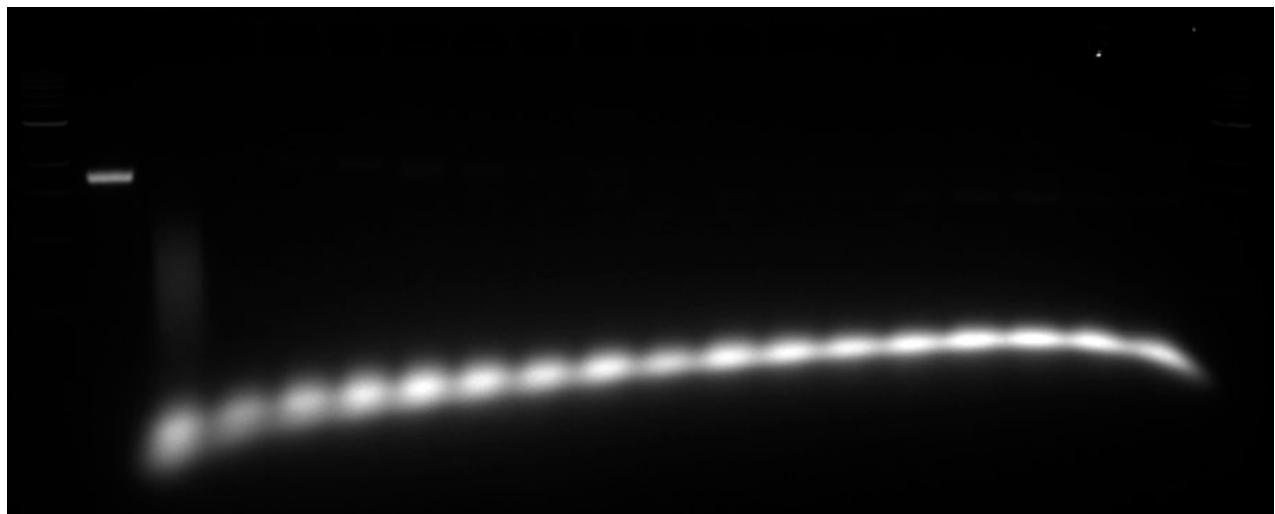
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

42 helix bundle, backbone nick rule 1; 23 day annealing in NaCl [M]

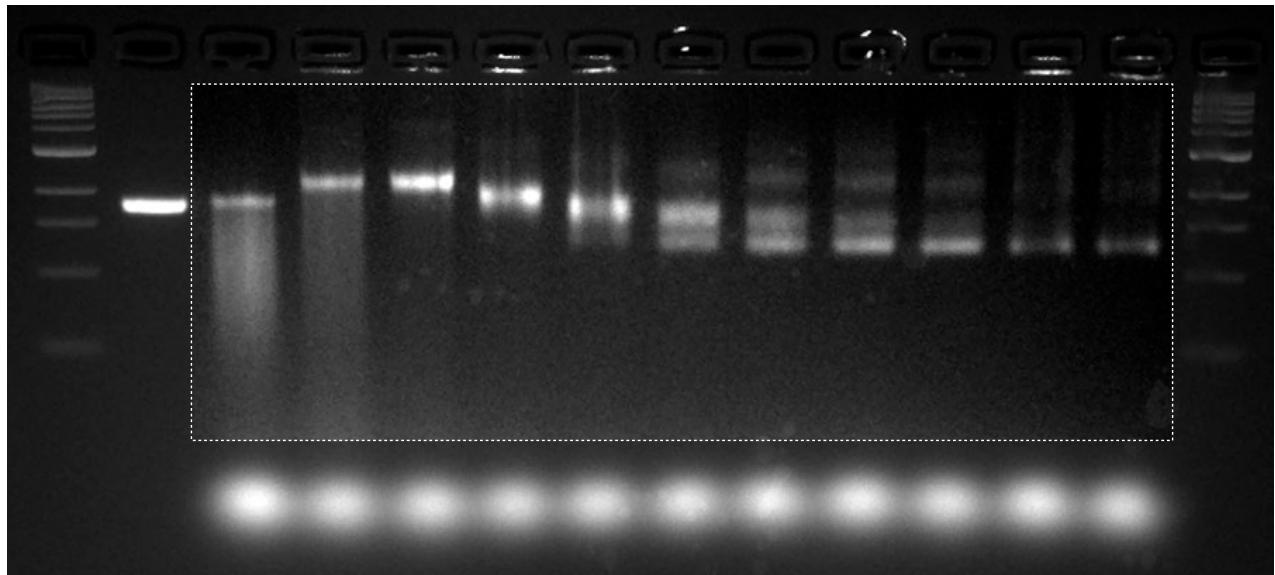
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Globally auto-leveled

42 helix bundle, backbone nick rule 2; 12 day annealing in MgCl₂ [mM]

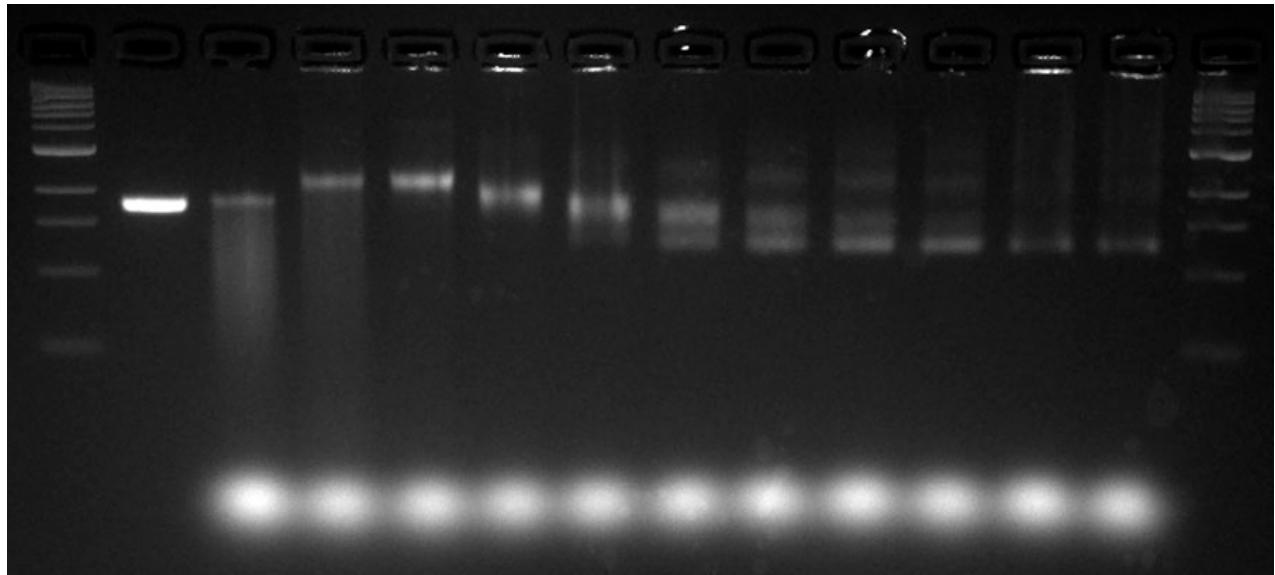
M ref 0 2 4 6 8 10 12 14 16 18 20 M



Region of interest auto-leveled

42 helix bundle, backbone nick rule 2; 12 day annealing in MgCl₂ [mM]

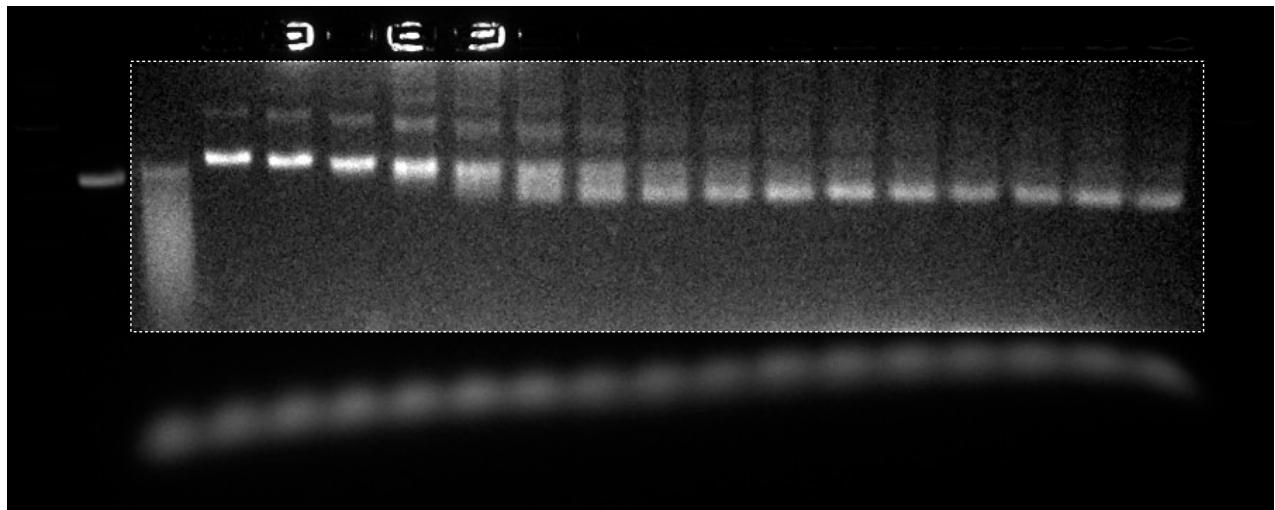
M ref 0 2 4 6 8 10 12 14 16 18 20 M



Globally auto-leveled

42 helix bundle, backbone nick rule 2; 12 day annealing in NaCl [M]

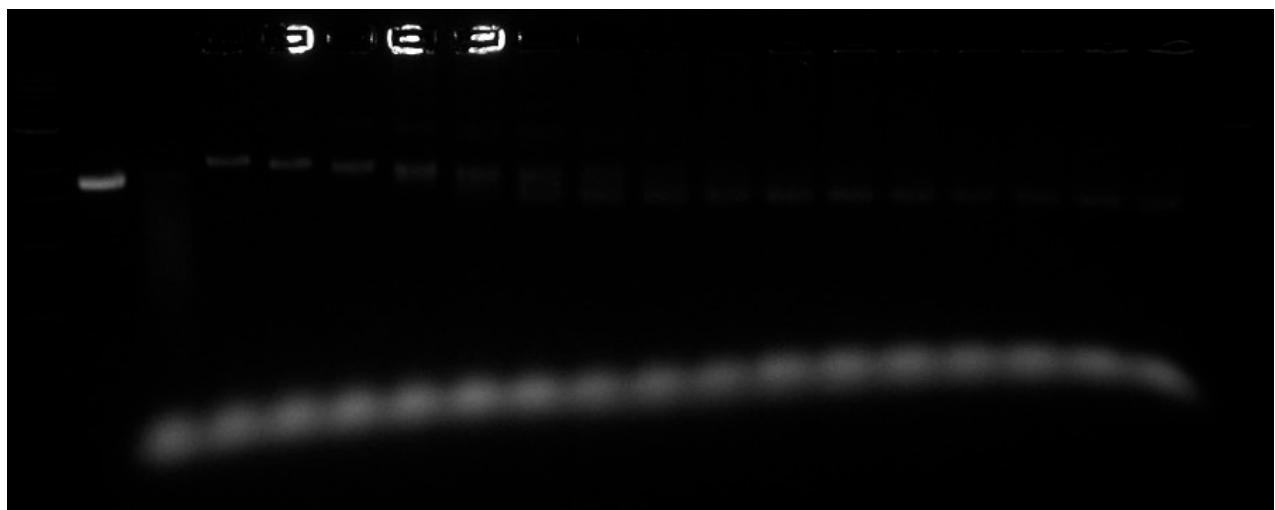
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

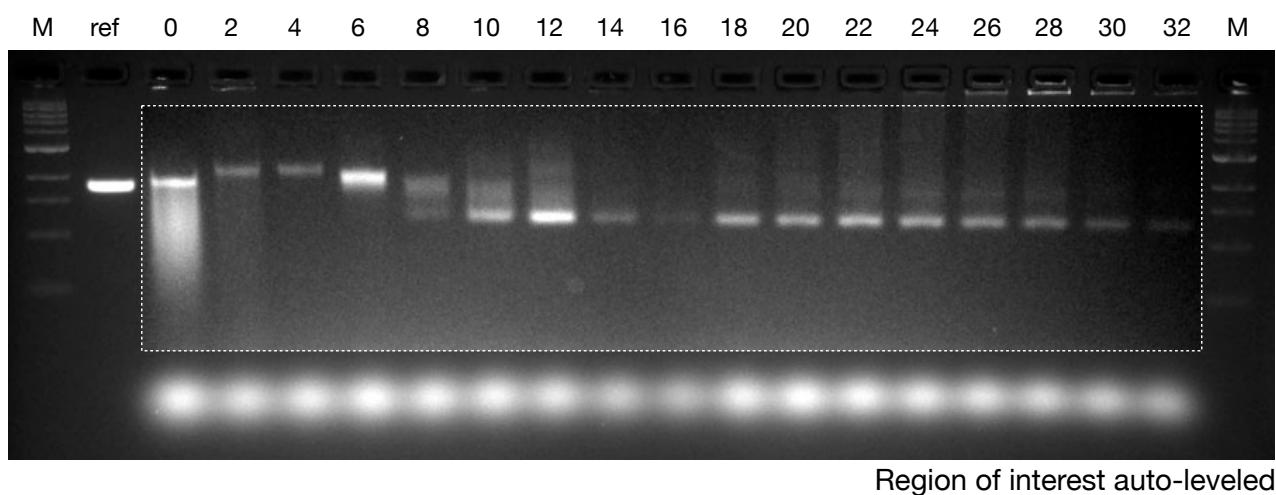
42 helix bundle, backbone nick rule 2; 12 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M

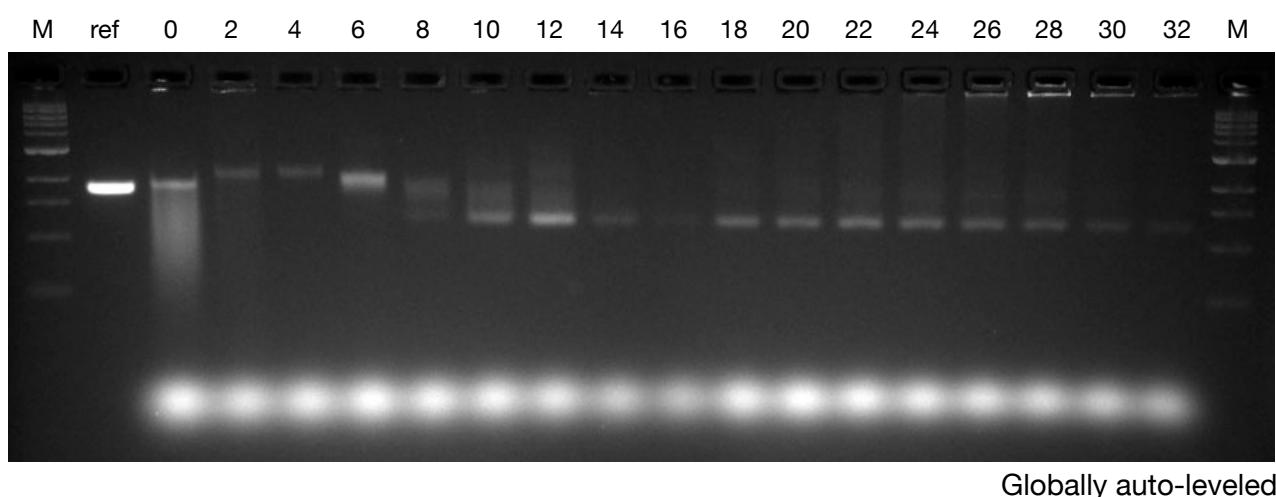


Globally auto-leveled

42 helix bundle, backbone nick rule 3; 12 day annealing in MgCl₂ [mM]

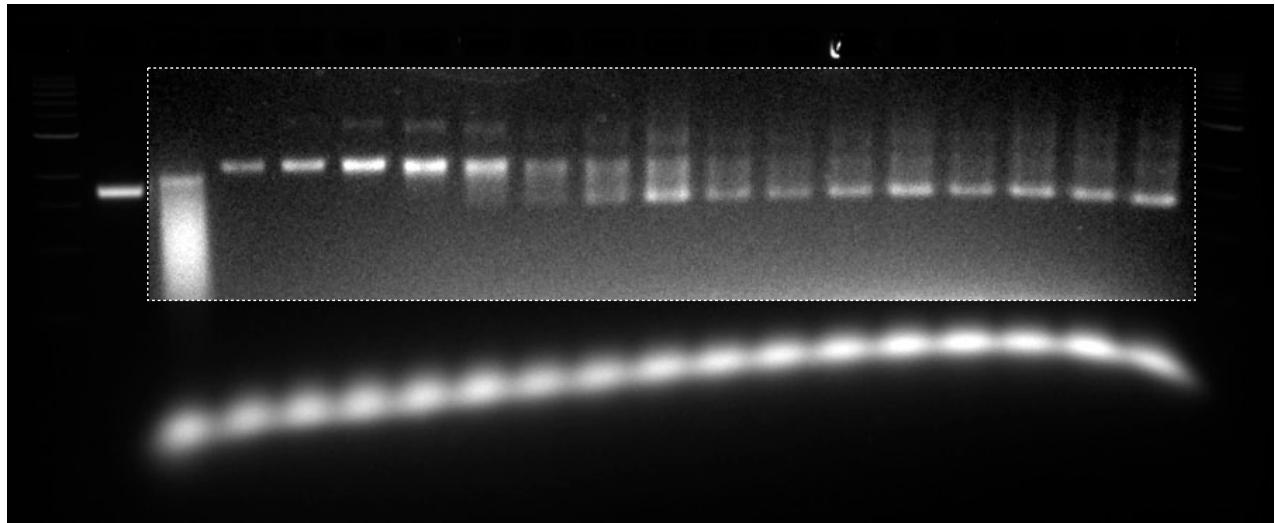


42 helix bundle, backbone nick rule 3; 12 day annealing in MgCl₂ [mM]



42 helix bundle, backbone nick rule 3; 12 day annealing in NaCl [M]

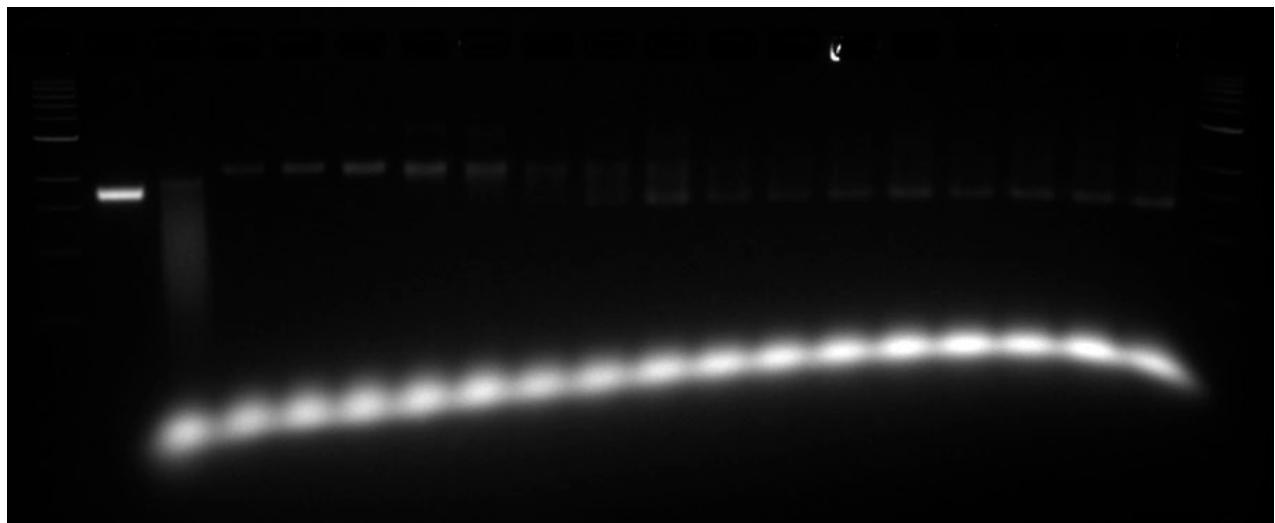
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

42 helix bundle, backbone nick rule 3; 12 day annealing in NaCl [M]

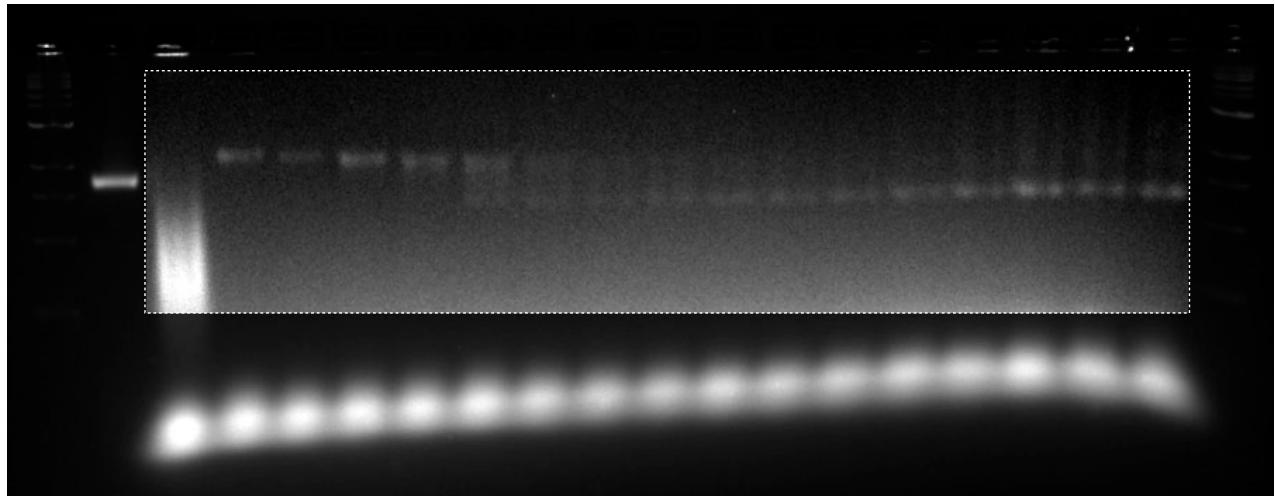
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Globally auto-leveled

42 helix bundle, backbone nick rule 3; 23 day annealing in NaCl [M]

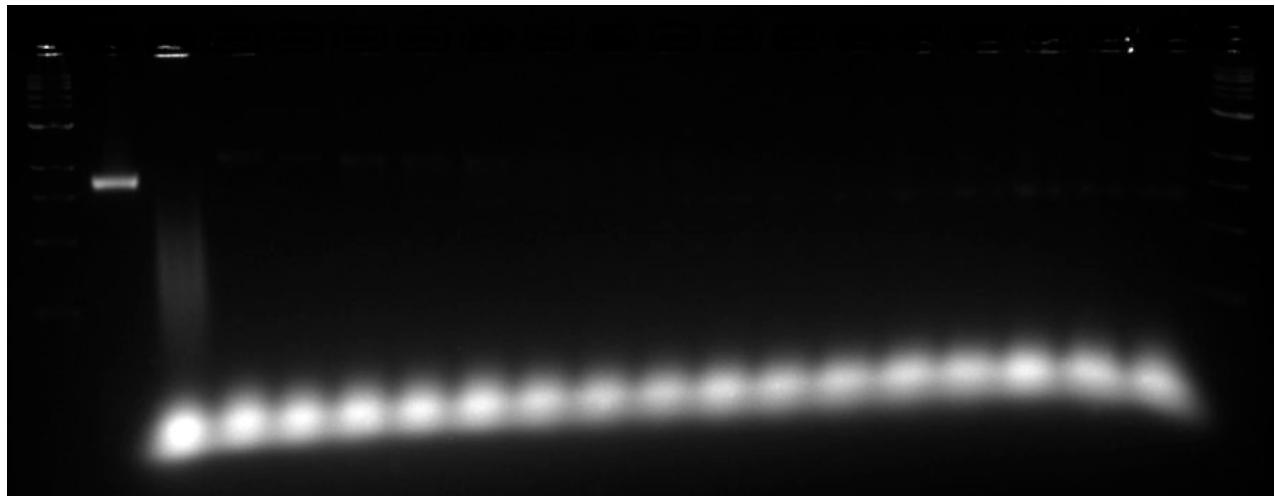
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

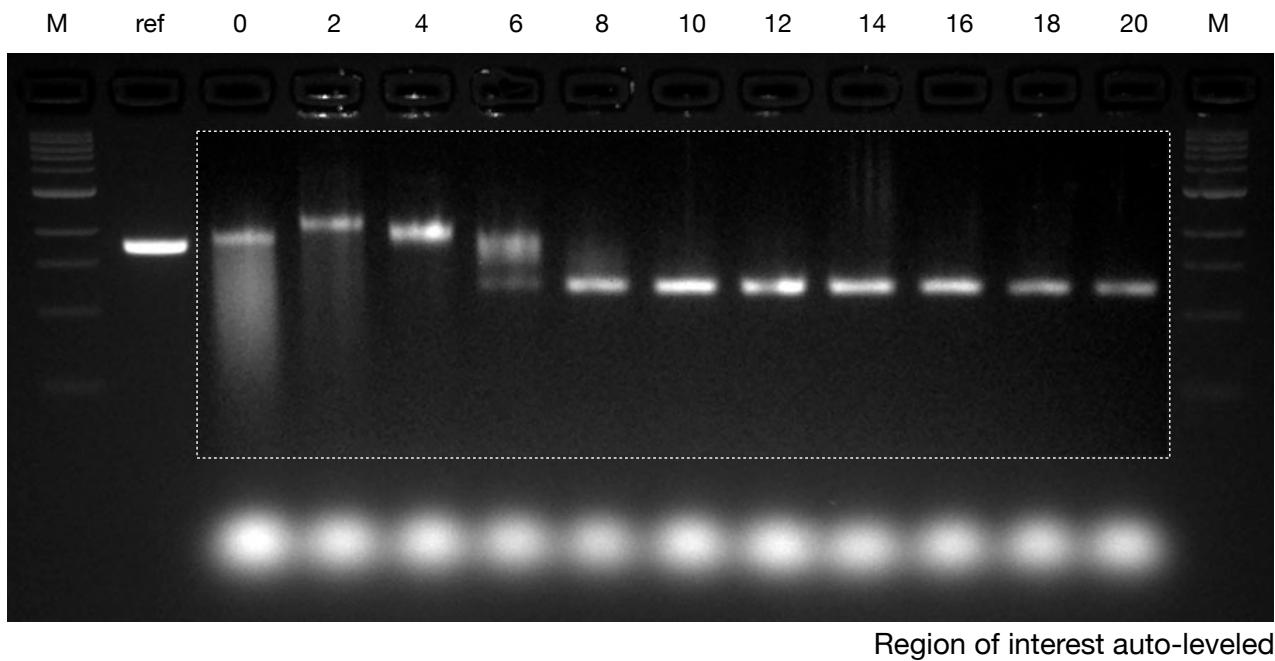
42 helix bundle, backbone nick rule 3; 23 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M

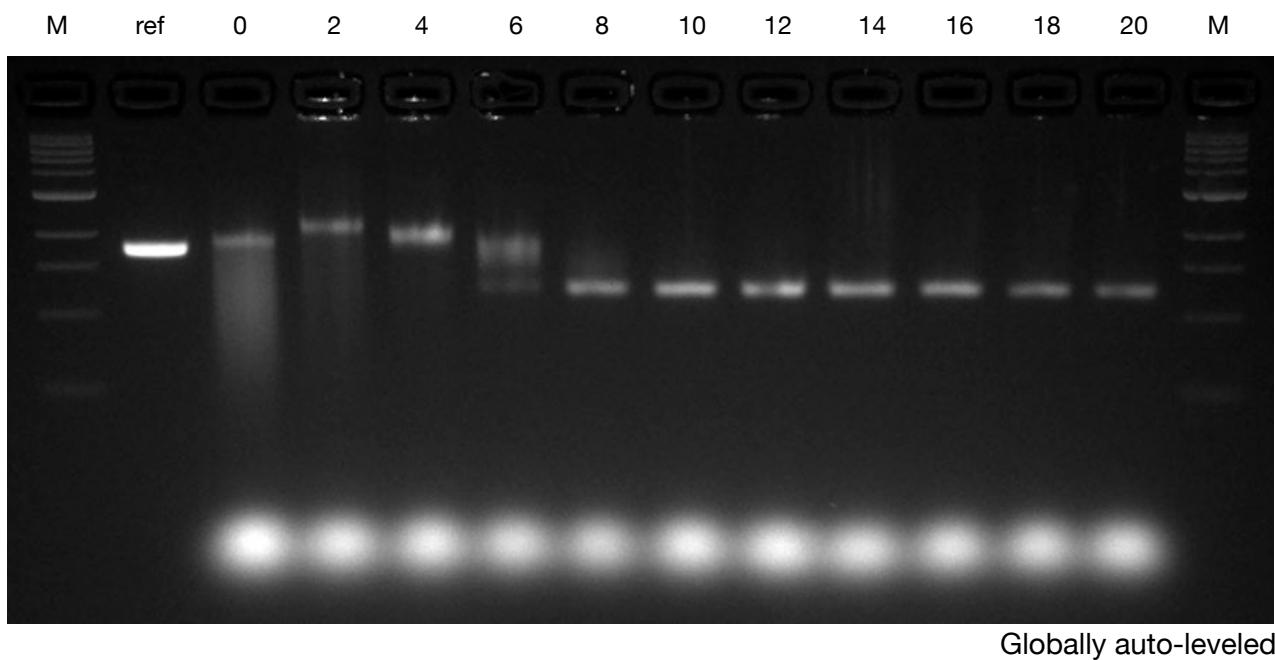


Globally auto-leveled

42 helix bundle, backbone nick rule 4; 12 day annealing in MgCl₂ [mM]

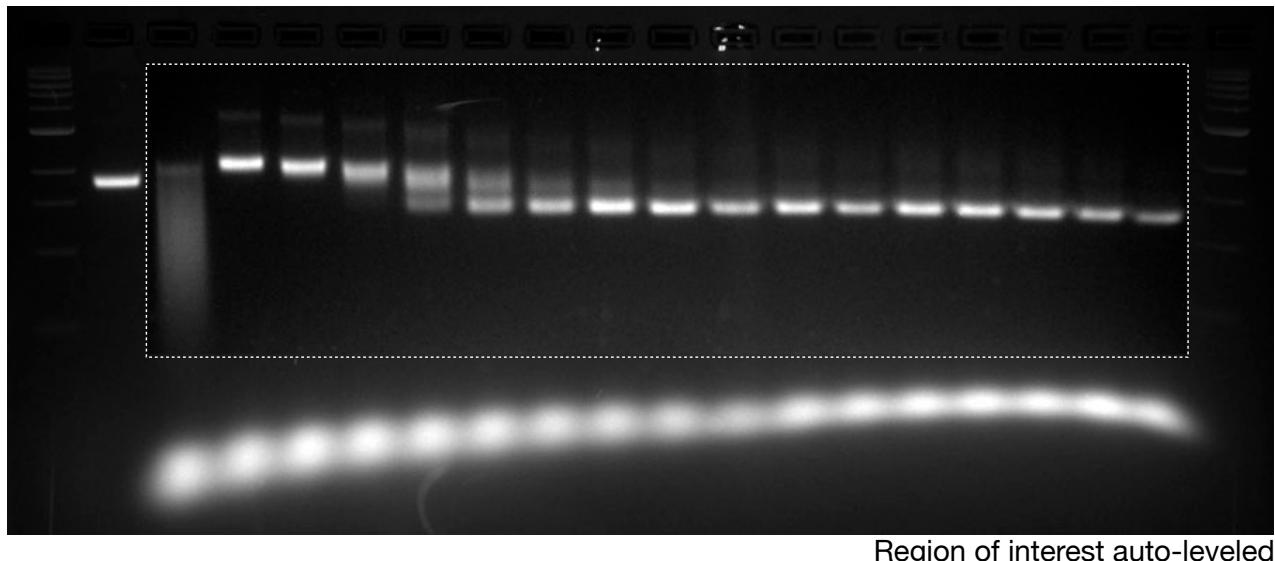


42 helix bundle, backbone nick rule 4; 12 day annealing in MgCl₂ [mM]



42 helix bundle, backbone nick rule 4; 12 day annealing in NaCl [M]

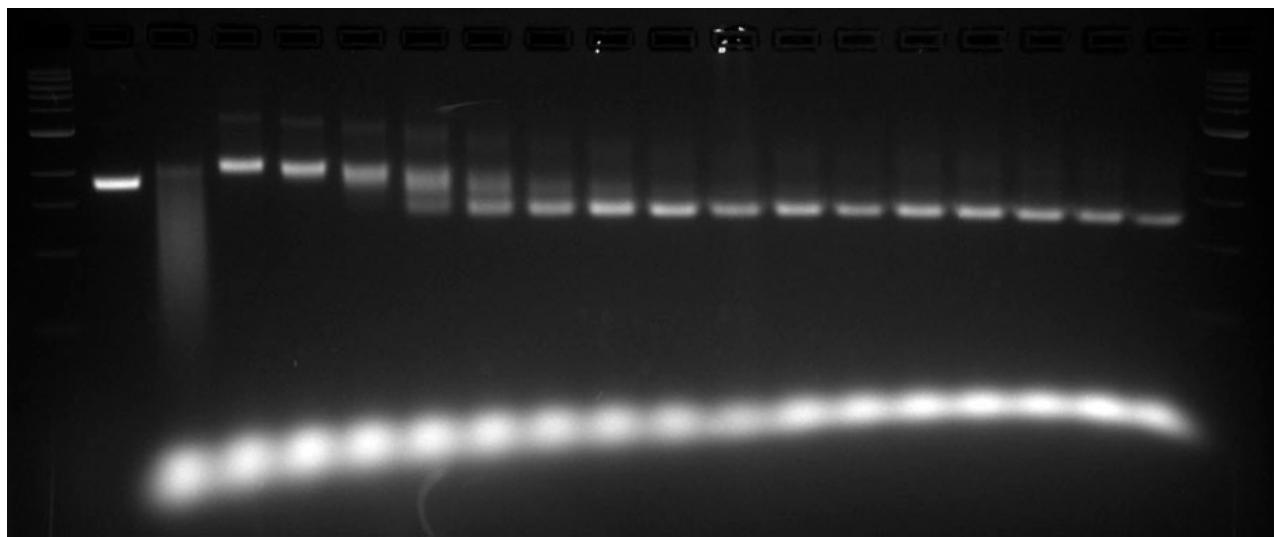
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

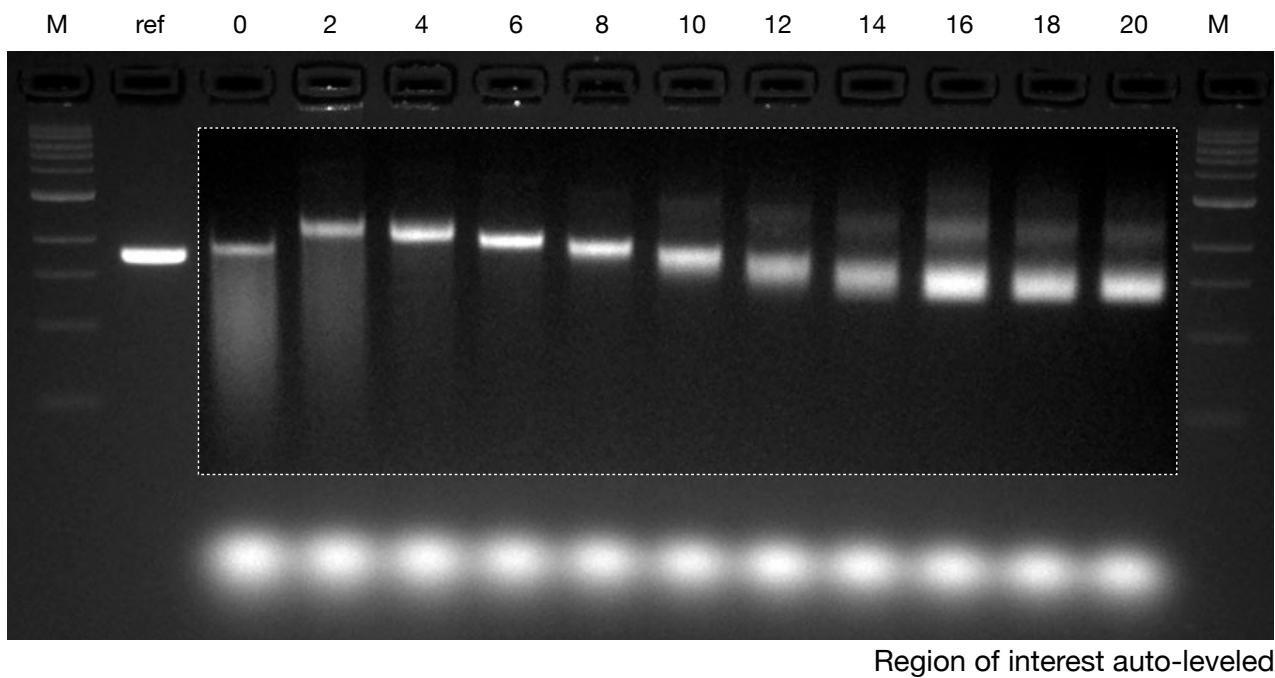
42 helix bundle, backbone nick rule 4; 12 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



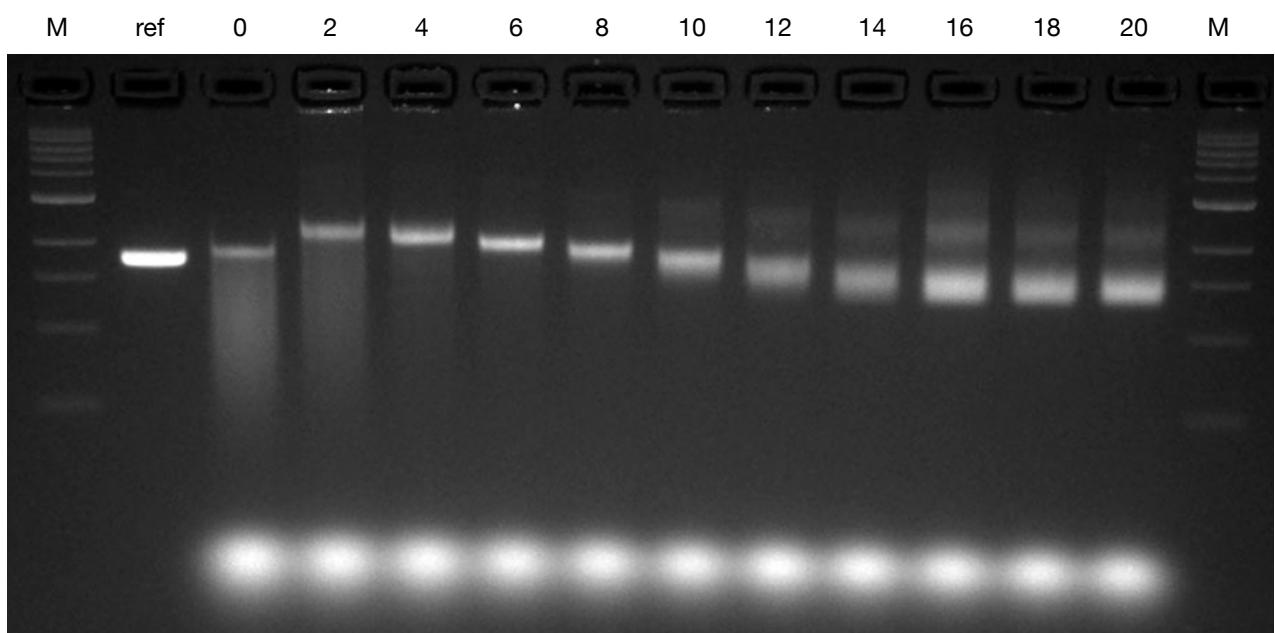
Globally auto-leveled

100 helix bundle; 12 day annealing in MgCl₂ [mM]



Region of interest auto-leveled

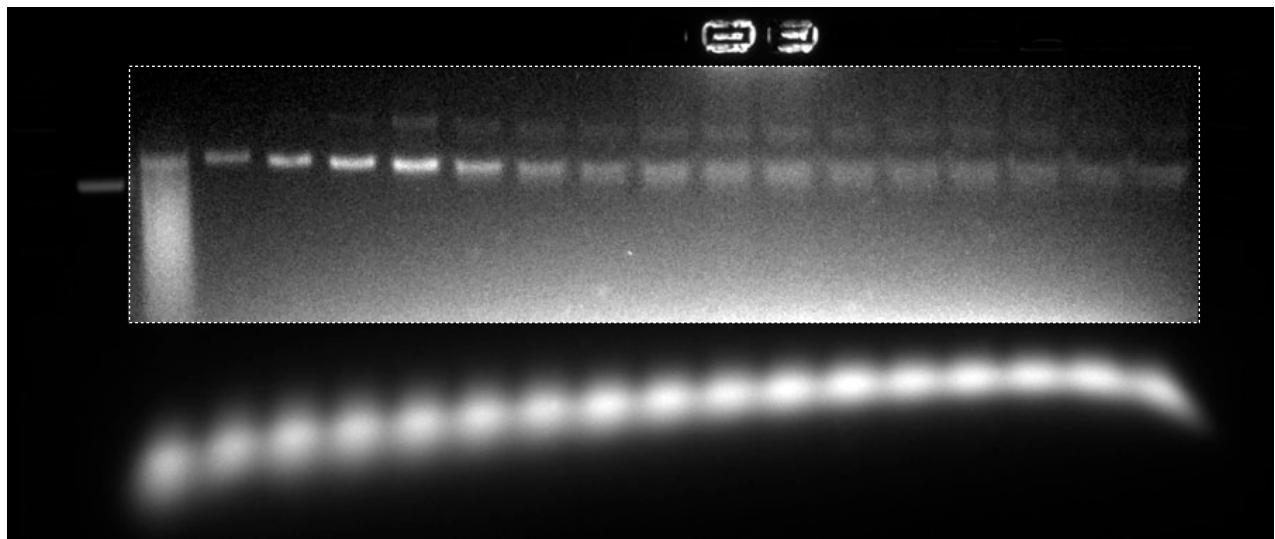
100 helix bundle; 12 day annealing in MgCl₂ [mM]



Globally auto-leveled

100 helix bundle; 12 day annealing in NaCl [M]

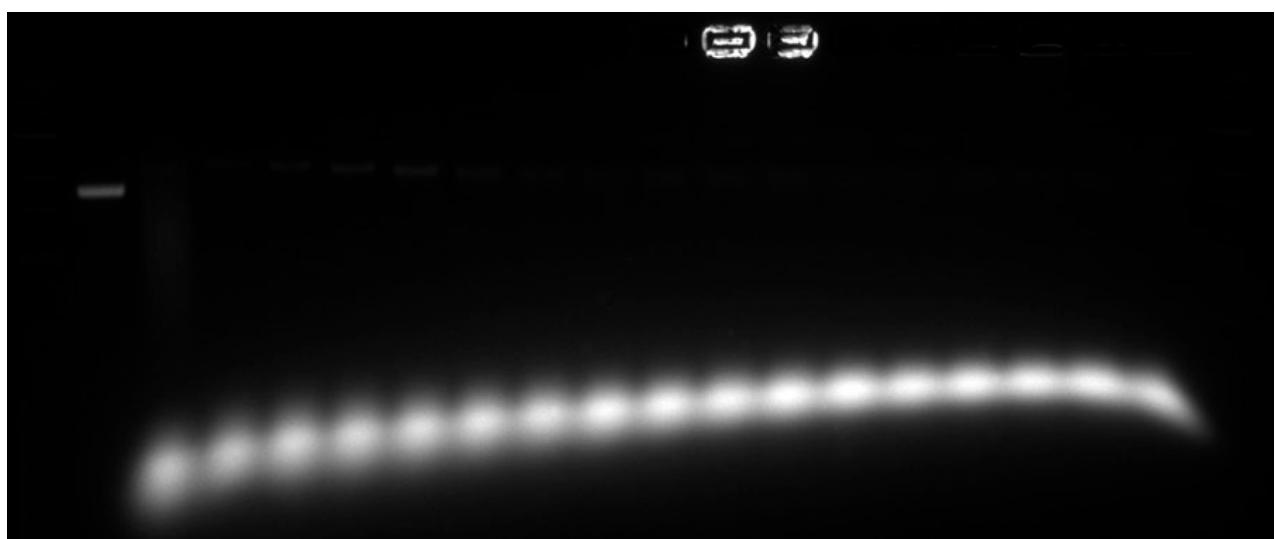
M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M



Region of interest auto-leveled

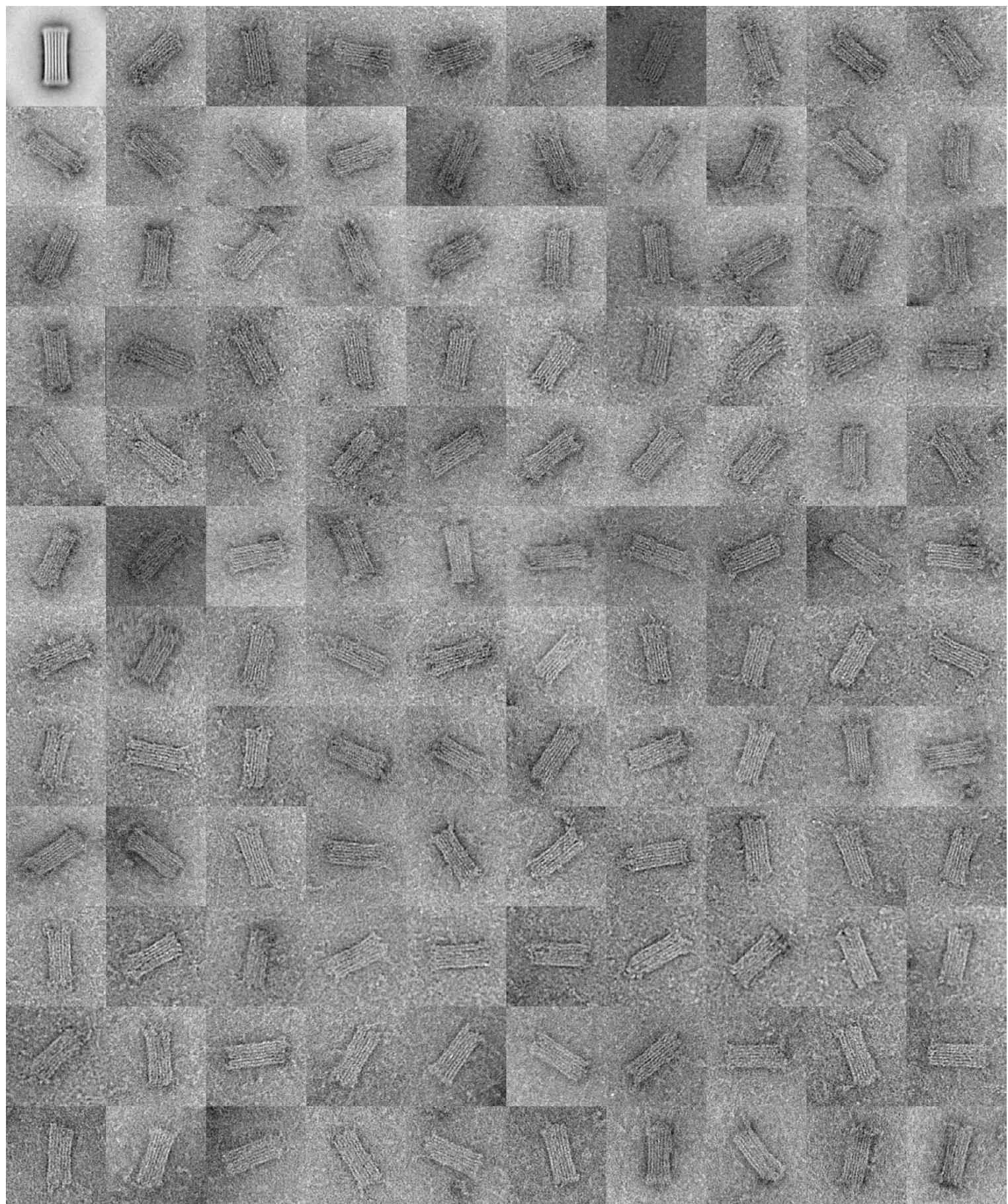
100 helix bundle; 12 day annealing in NaCl [M]

M ref 0.0 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 M

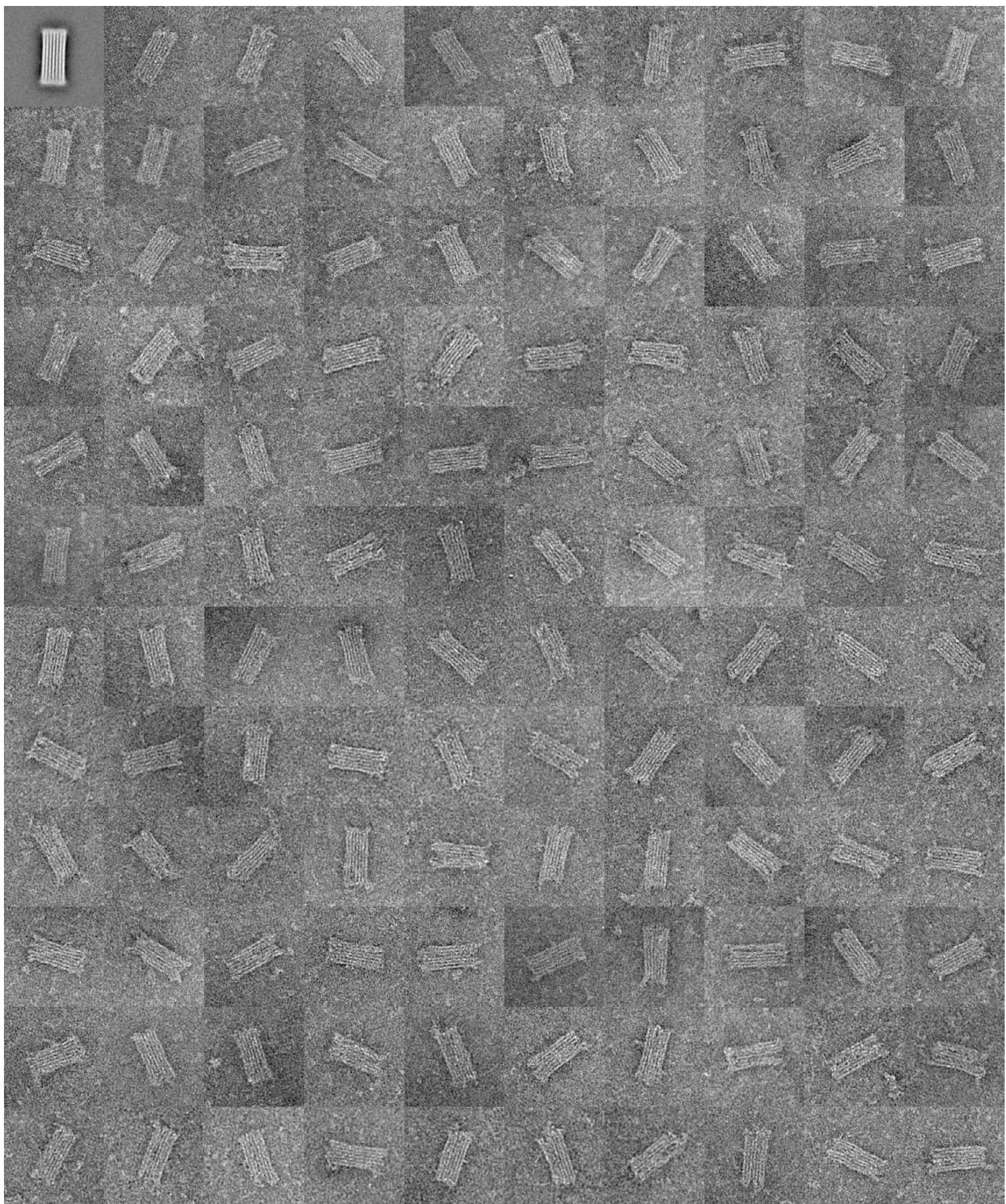


Globally auto-leveled

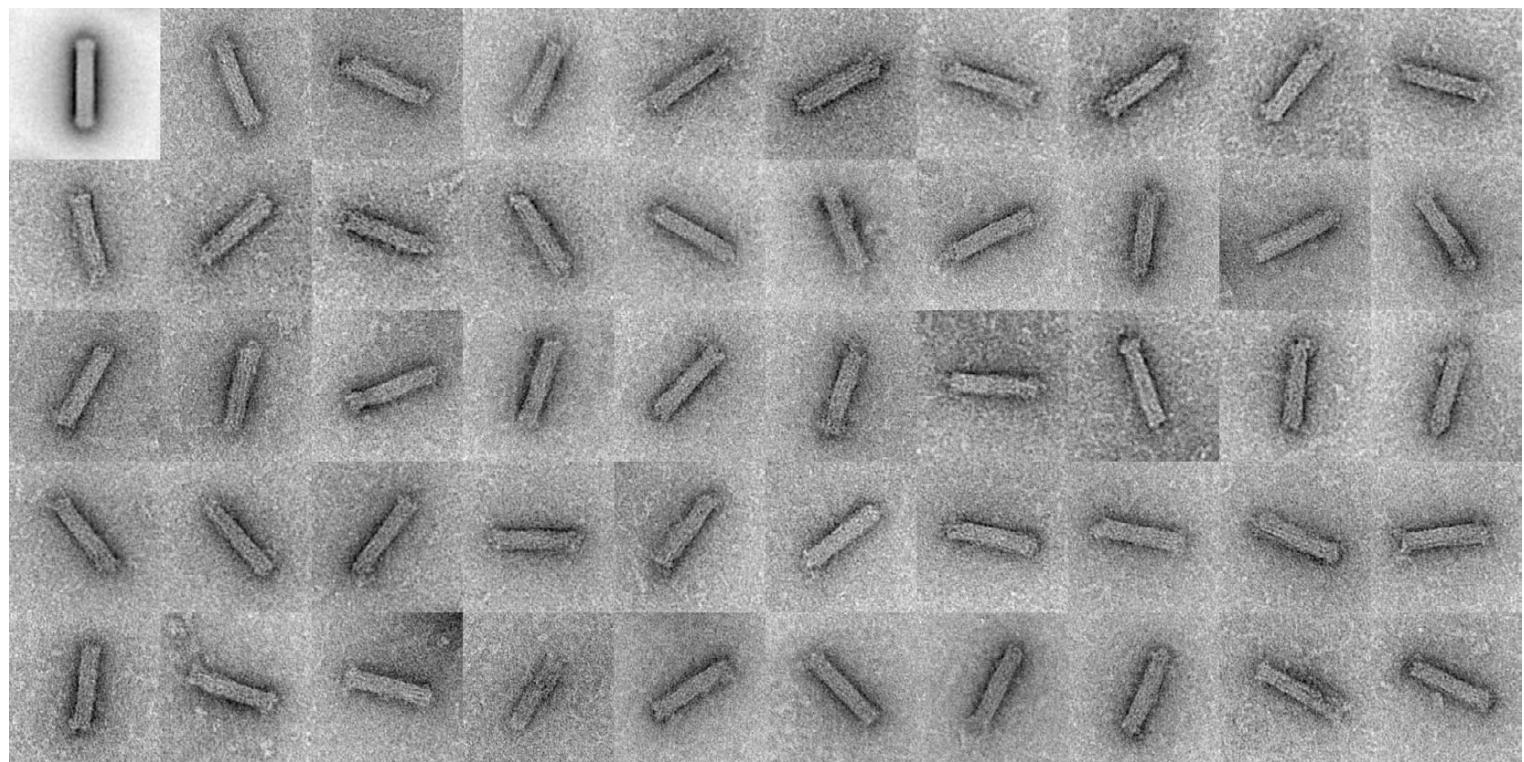
**Supplementary Figure S2:
42 helix bundle particle micrographs,
backbone nick rule 3**



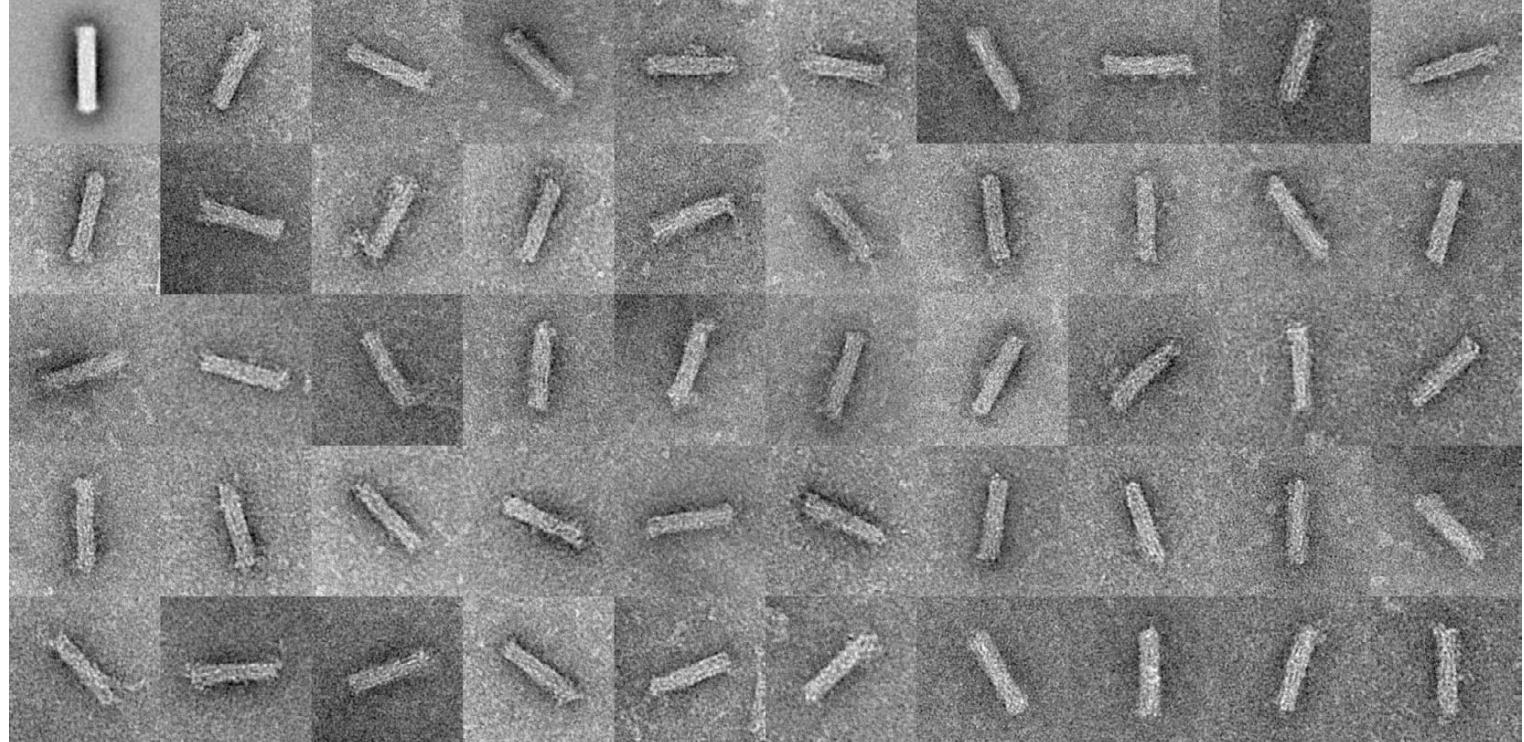
Negative-stain TEM micrographs of 42 helix bundle, backbone nick rule 3. 6 day annealing in 20 mM MgCl₂ unpurified, **flat views**. Each micrograph: 100nm x 100nm. Top row, image #1 = average micrograph;



Negative-stain TEM micrographs of 42 helix bundle, backbone nick rule 3. 6 day annealing in 2.4 M NaCl unpurified, **flat views**. Each micrograph: 100nm x 100nm. Top row, image #1 = average micrograph;



Negative-stain TEM micrographs of 42 helix bundle, backbone nick rule 3. 6 day annealing in 20 mM MgCl₂ unpurified, **side views**. Each micrograph: 100nm x 100nm. Top row, image #1 = average micrograph;



Negative-stain TEM micrographs of 42 helix bundle, backbone nick rule 3. 6 day annealing in NaCl unpurified, **side views**. Each micrograph: 100nm x 100nm. Top row, image #1 = average micrograph;

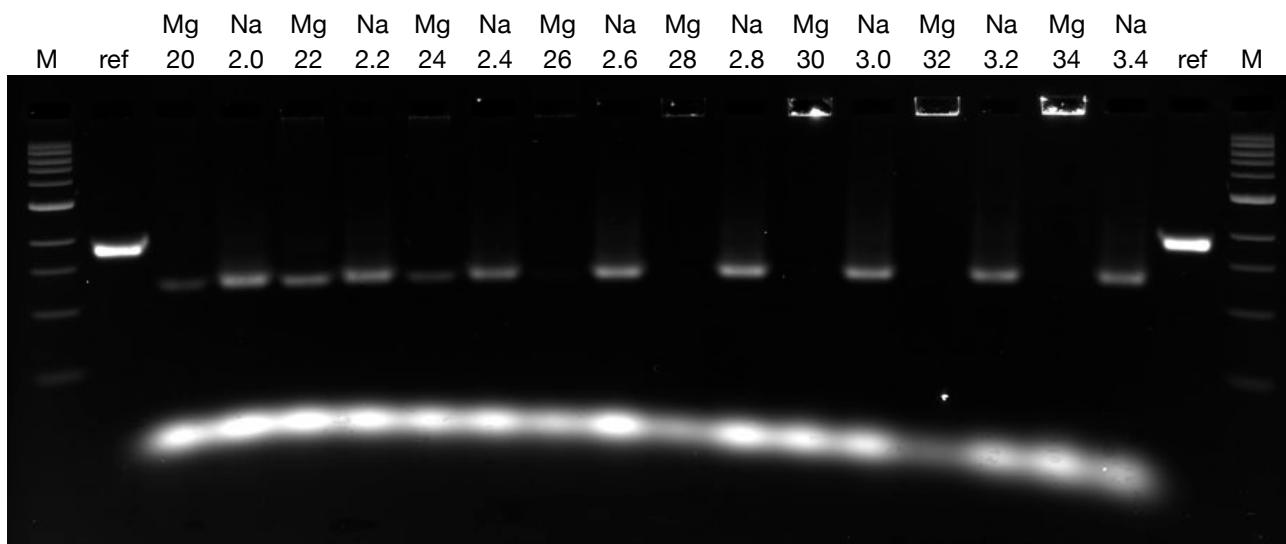
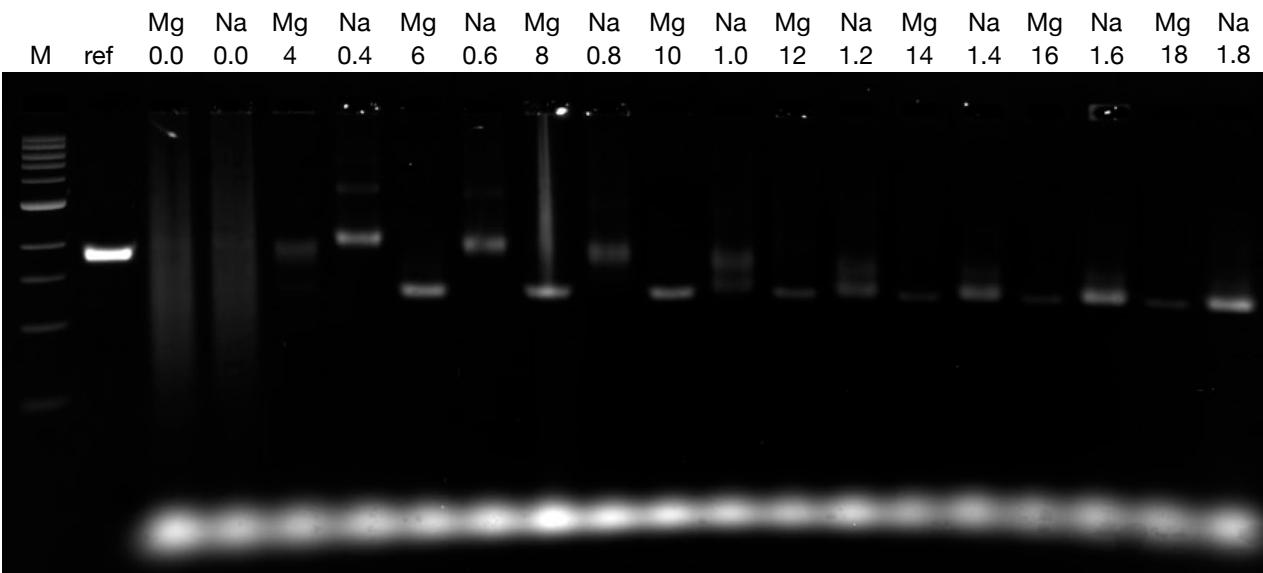
Supplementary Figure S3:
Electrophoretic mobility comparison

42 helix bundle rule 4 and 24 helix bundles annealed for 12 days in either MgCl₂ or NaCl. All samples have been adjusted to 18mM MgCl₂ plus 1.8M NaCl 12 hours before the gel was run. Original annealing concentrations are shown above the gel.

ref = scaffold DNA used in self-assembly reactions

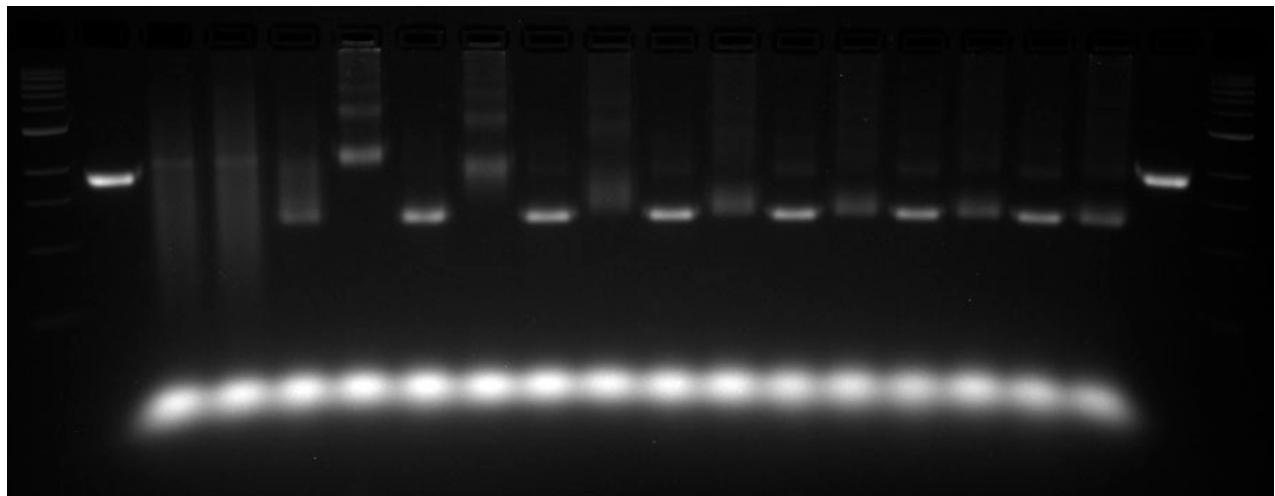
M = 1 kb DNA Ladder from New England BioLabs

42 helix bundle rule 4; 12 day annealing in either MgCl₂ [mM] or NaCl [M]
- all samples adjusted to 18mM MgCl₂ plus 1.8M NaCl

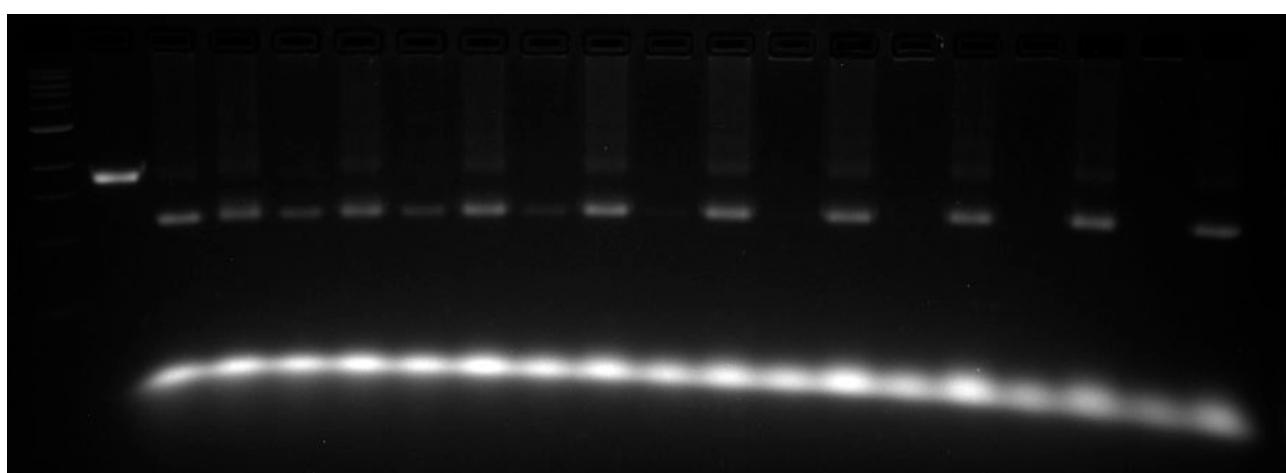


24 helix bundle; 12 day annealing in either MgCl₂ [mM] or NaCl [M]
- all samples adjusted to 18mM MgCl₂ plus 1.8M NaCl

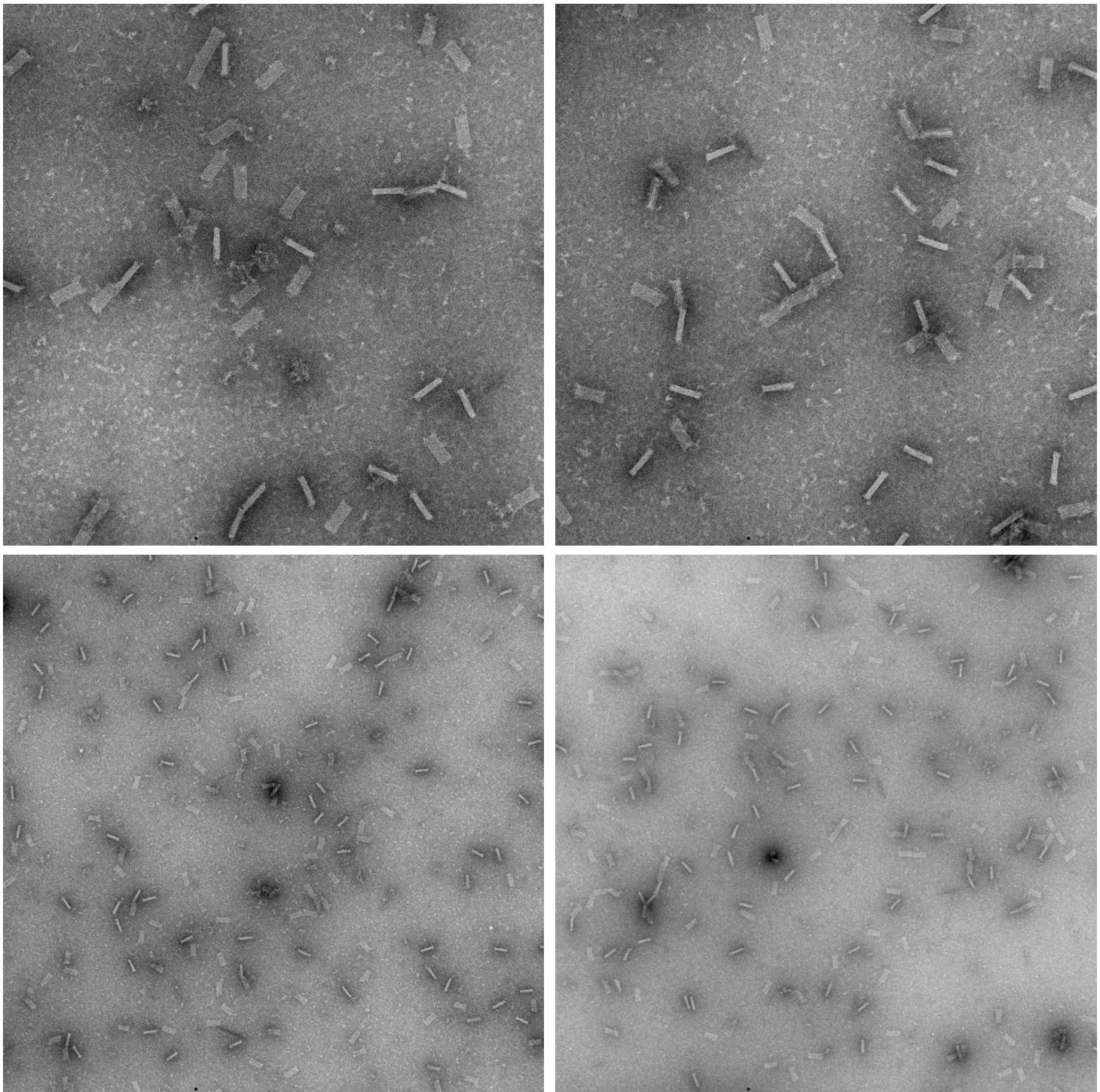
M	ref	Mg	Na	Mg	Na	M												
0.0	0.0	4	0.4	6	0.6	8	0.8	10	1.0	12	1.2	14	1.4	16	1.6	ref	M	



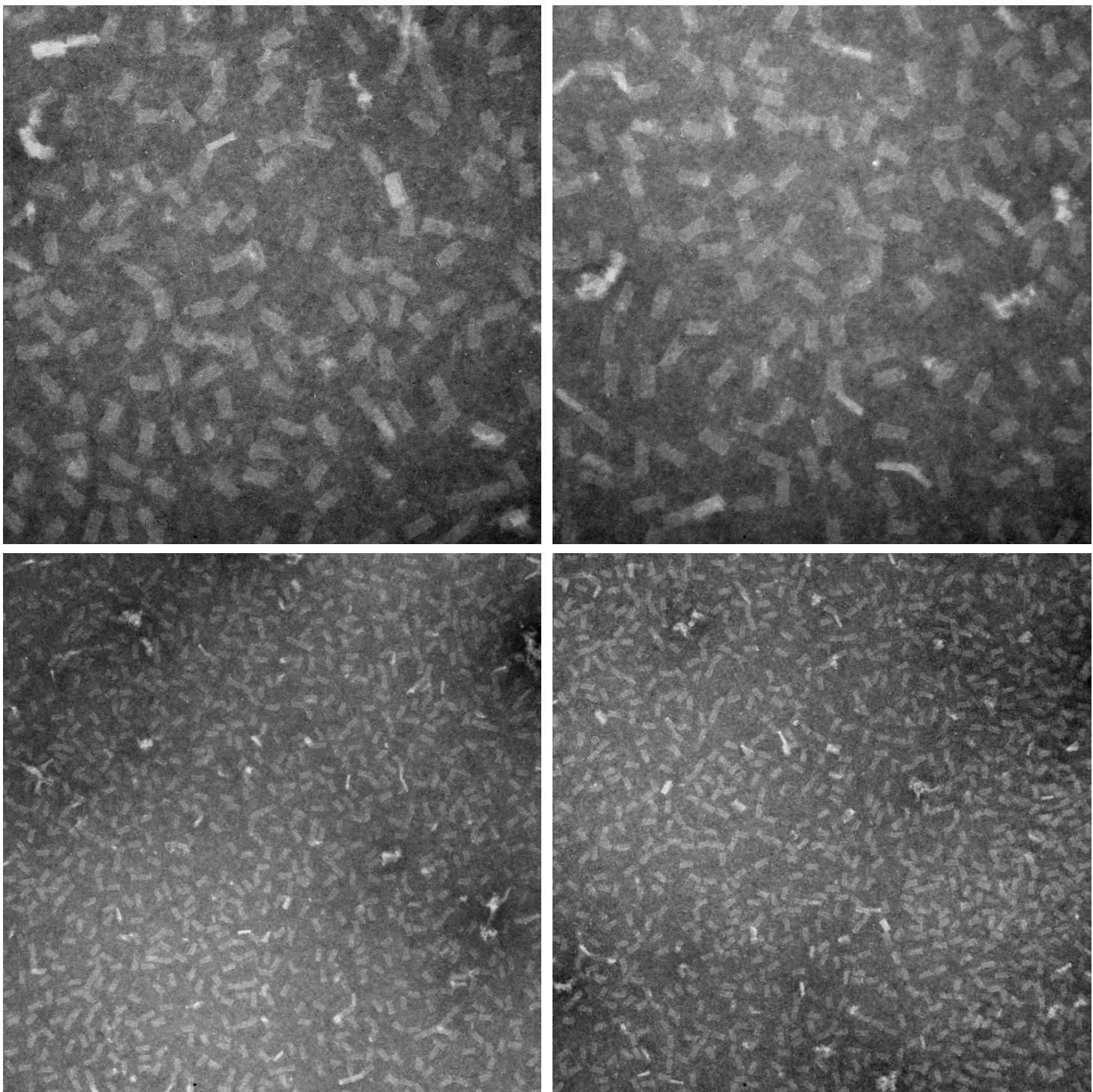
M	ref	Mg	Na	M														
18	1.8	20	2.0	22	2.2	24	2.4	26	2.6	28	2.8	30	3.0	32	3.2	34	3.4	



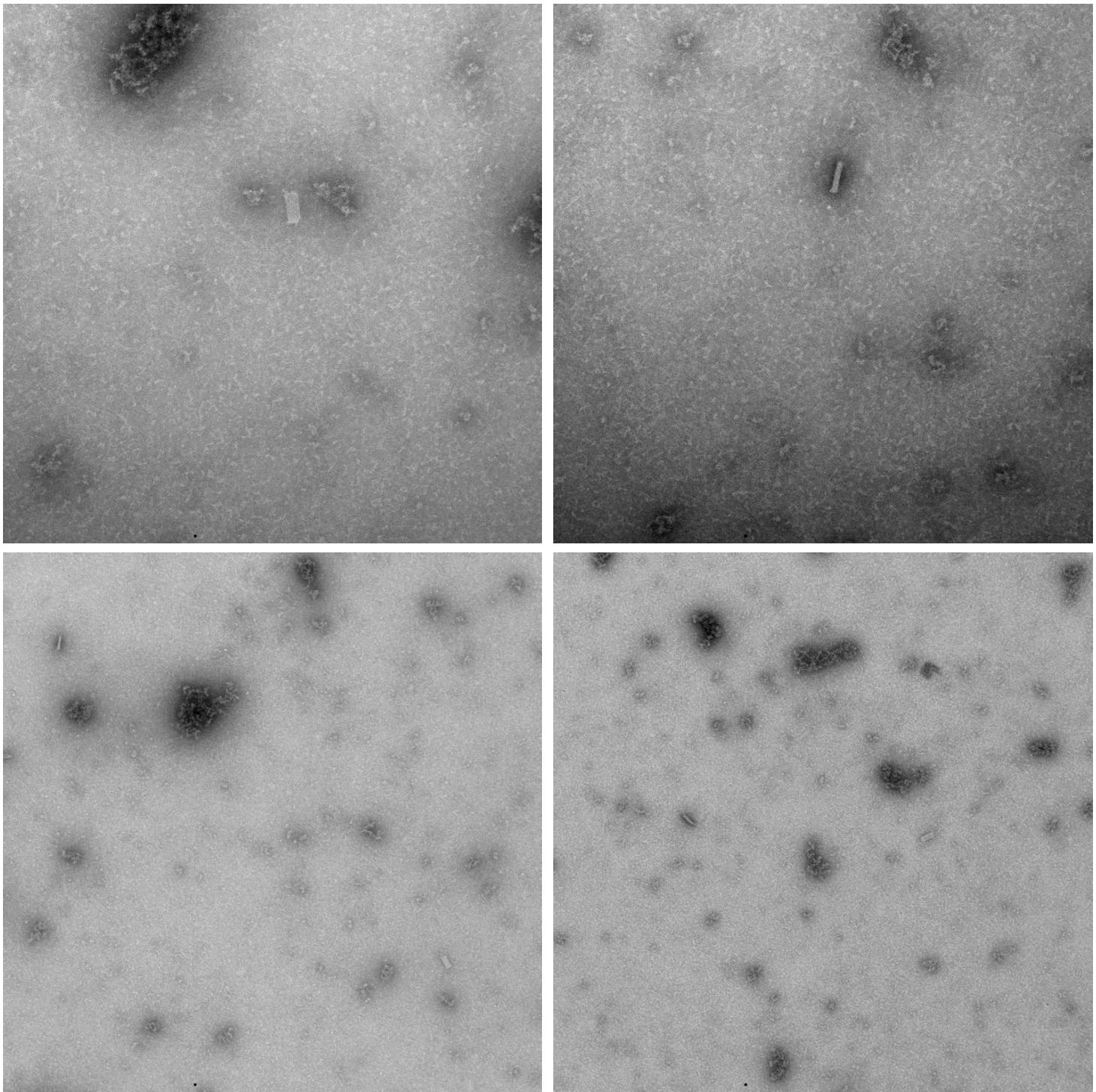
Supplementary Figure S4:
TEM images of all tested structures



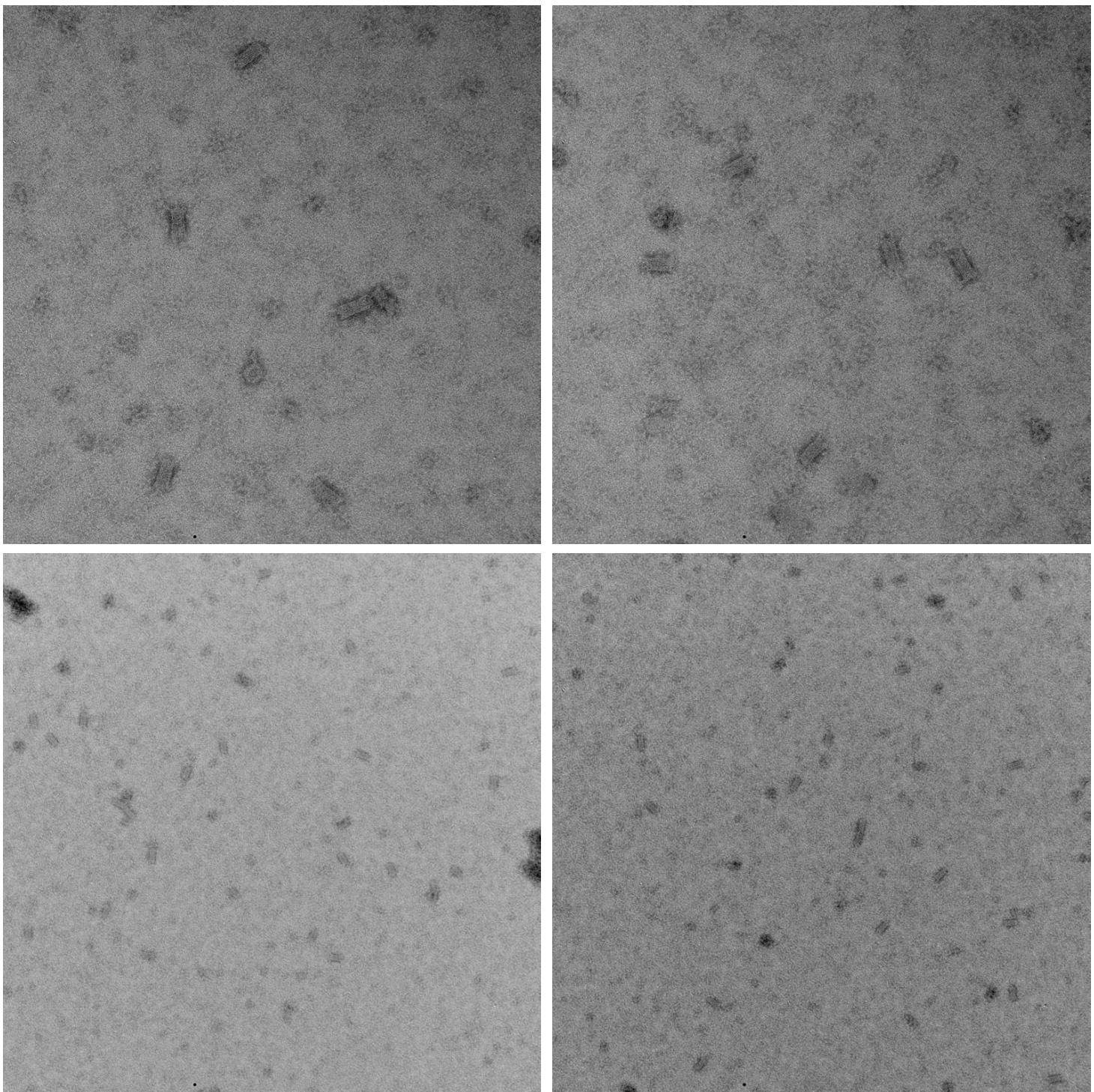
42 helix bundle rule 4, 12 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



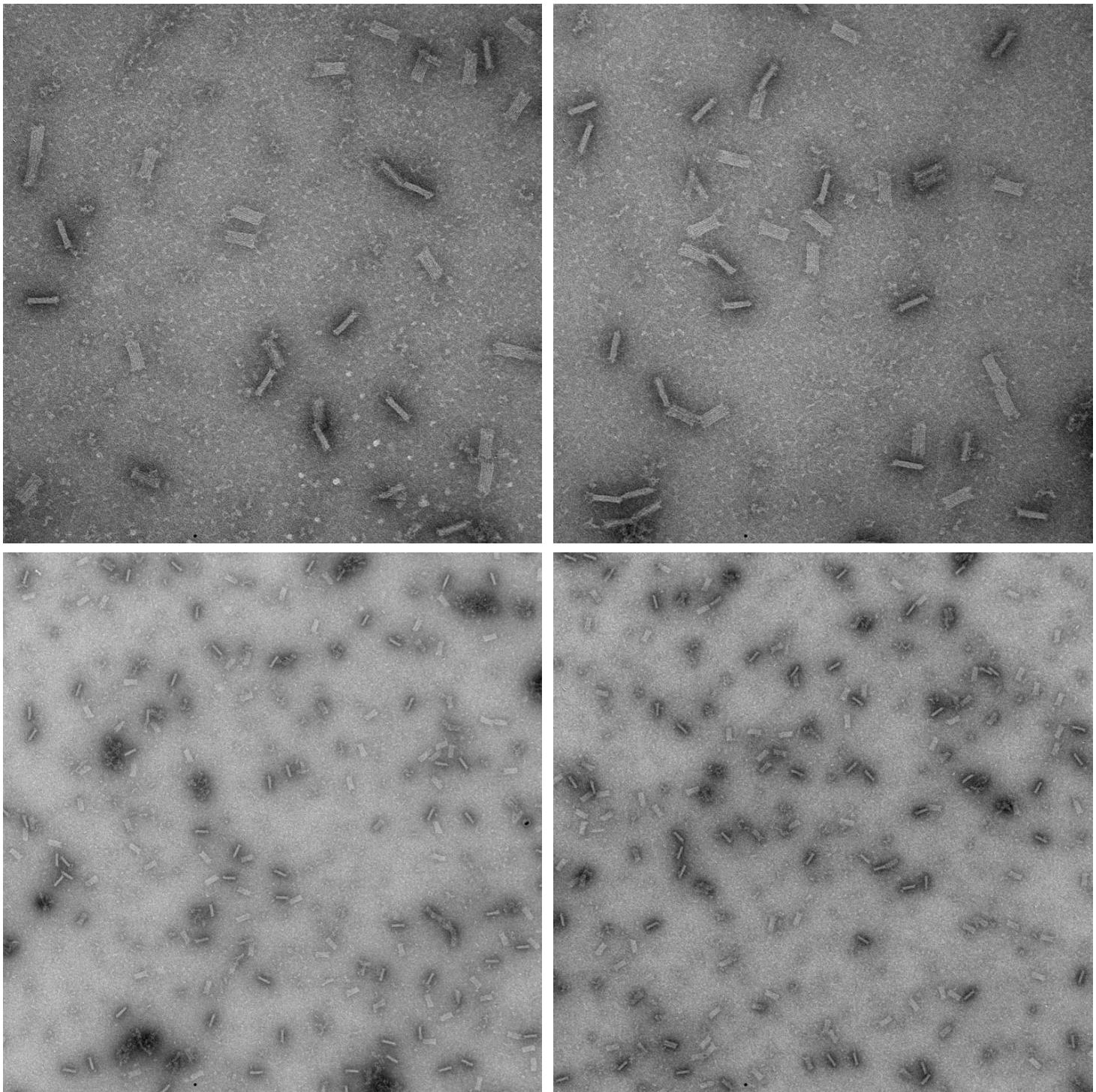
42 helix bundle rule 4, 12 day annealing in 2.4 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



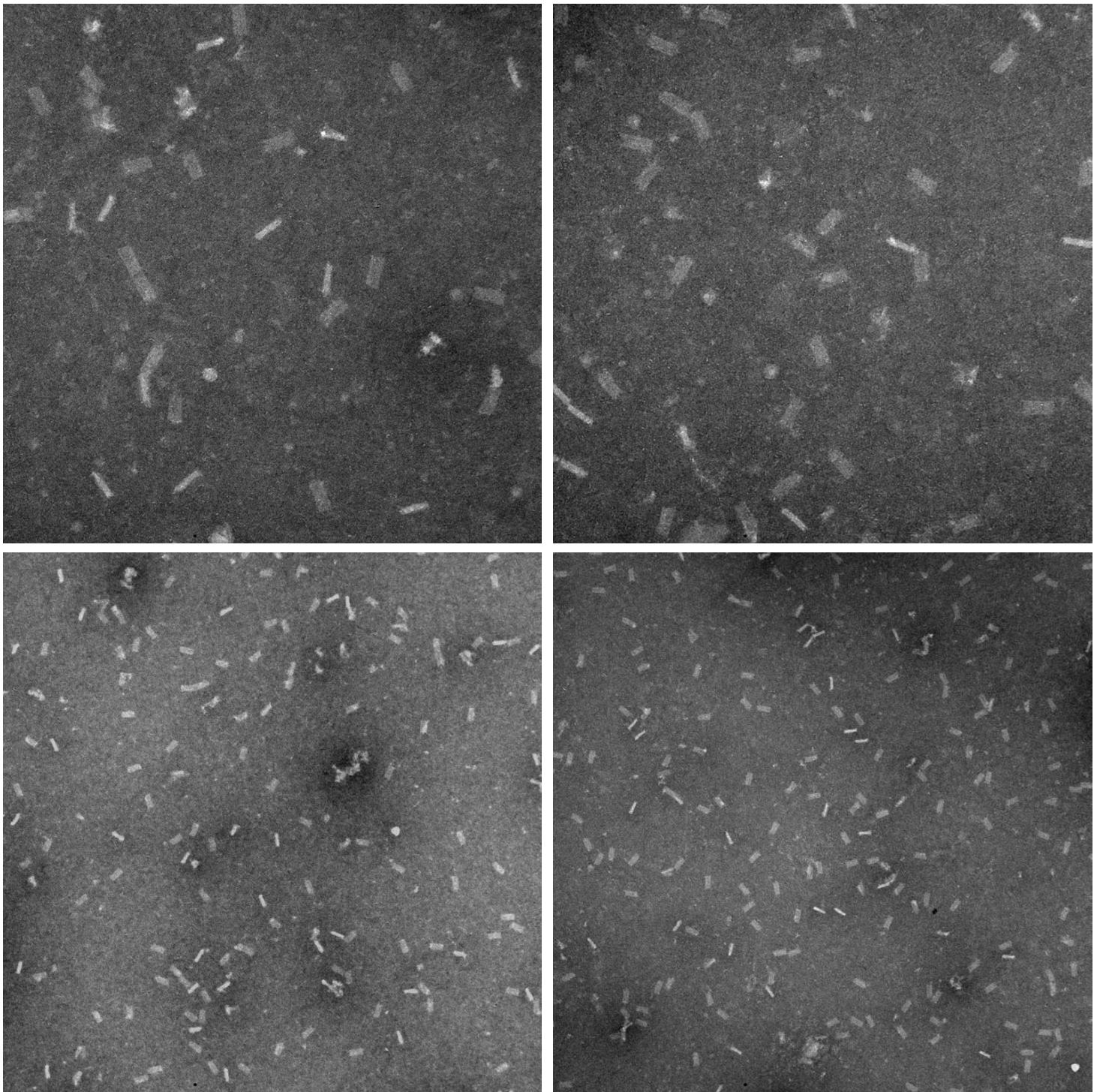
42 helix bundle rule 3, 12 day annealing in 24 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



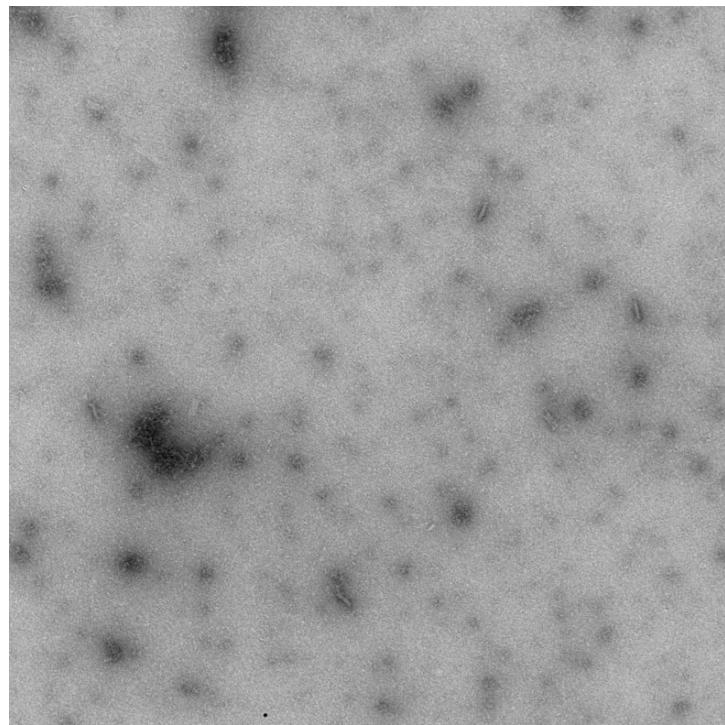
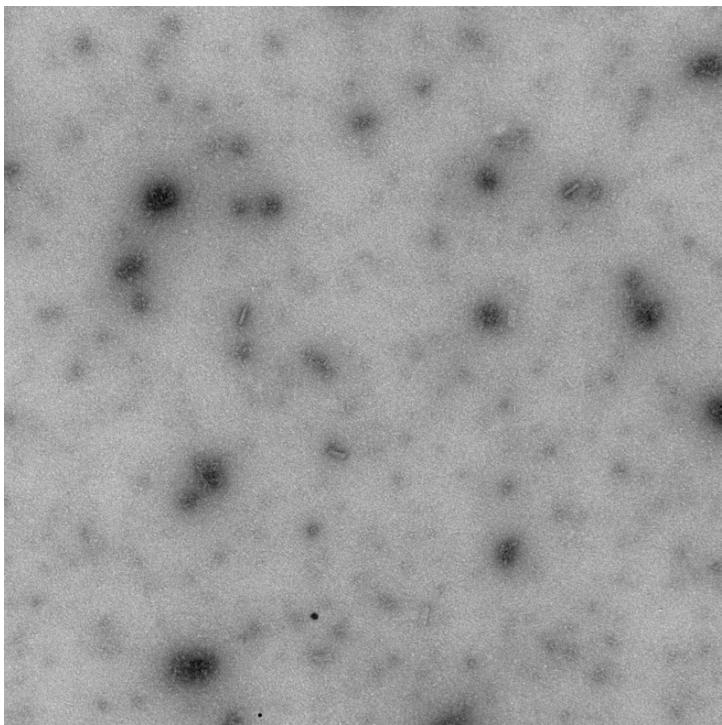
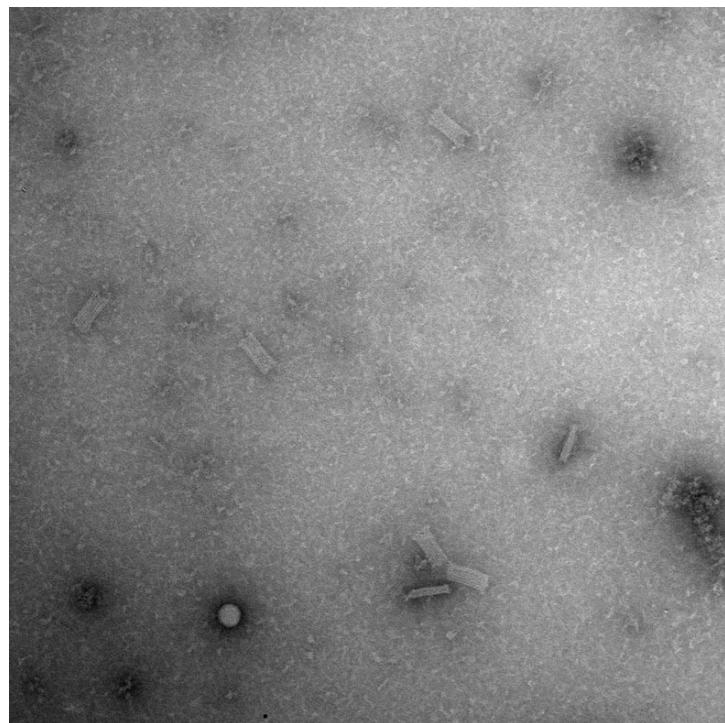
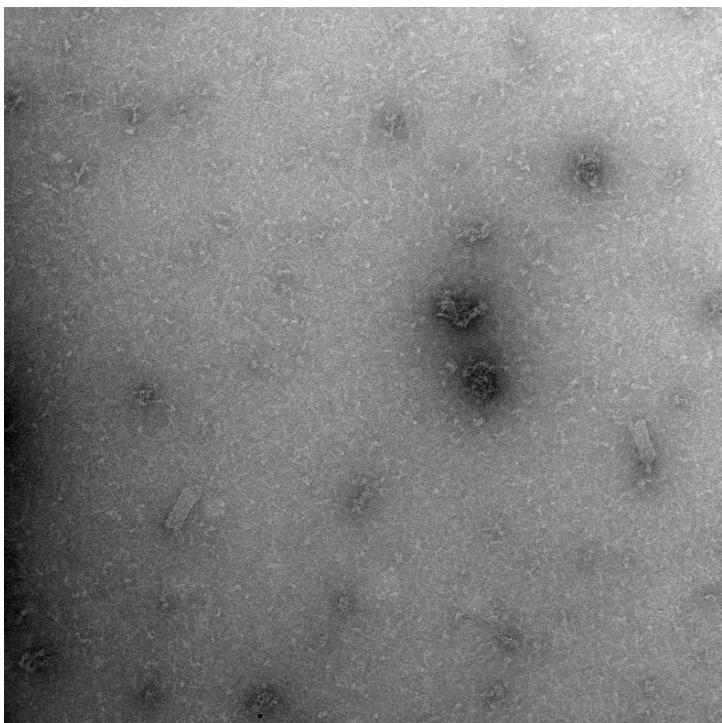
42 helix bundle rule 3, 12 day annealing in 2.4 mM NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



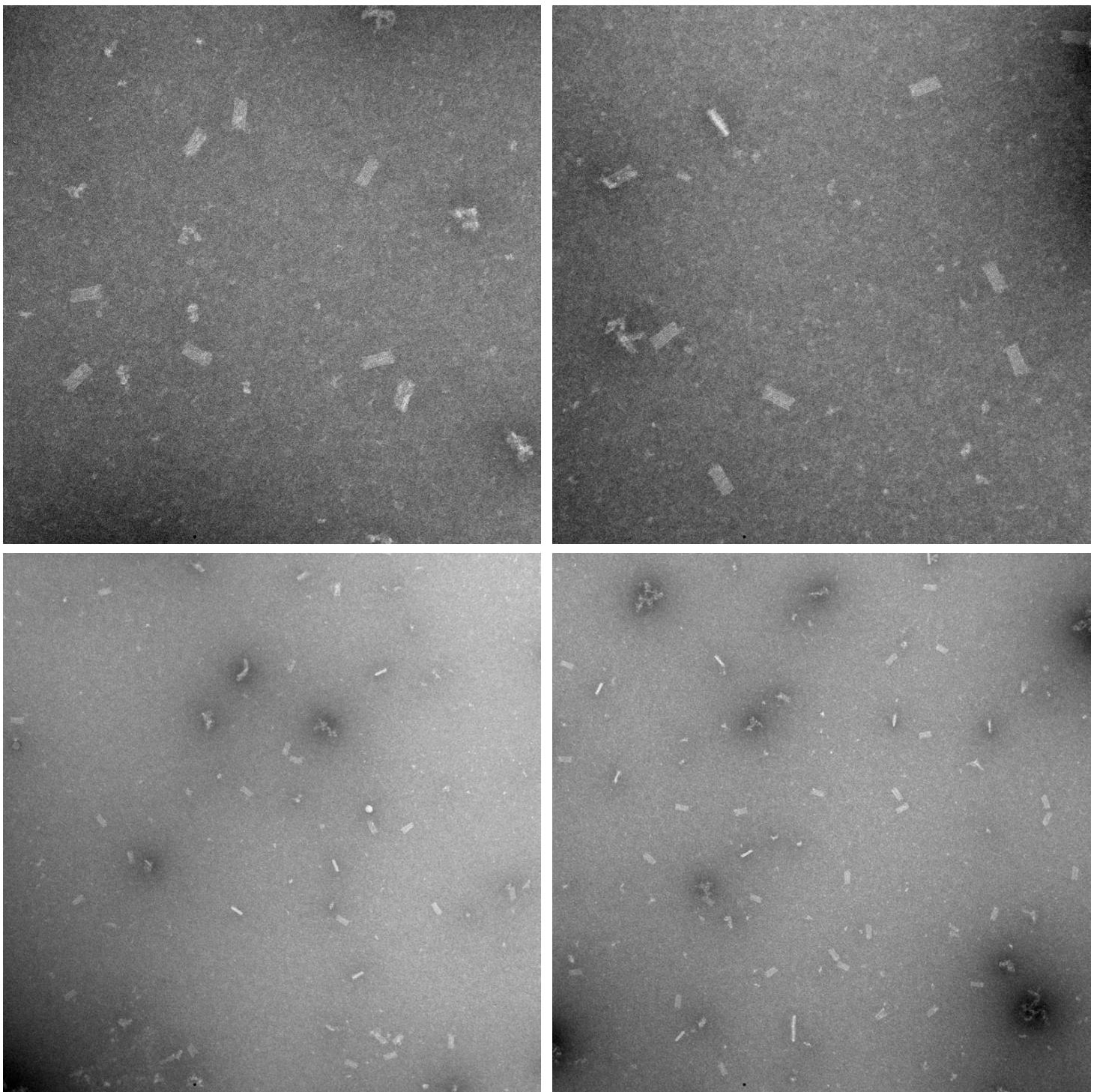
42 helix bundle rule 2, 12 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



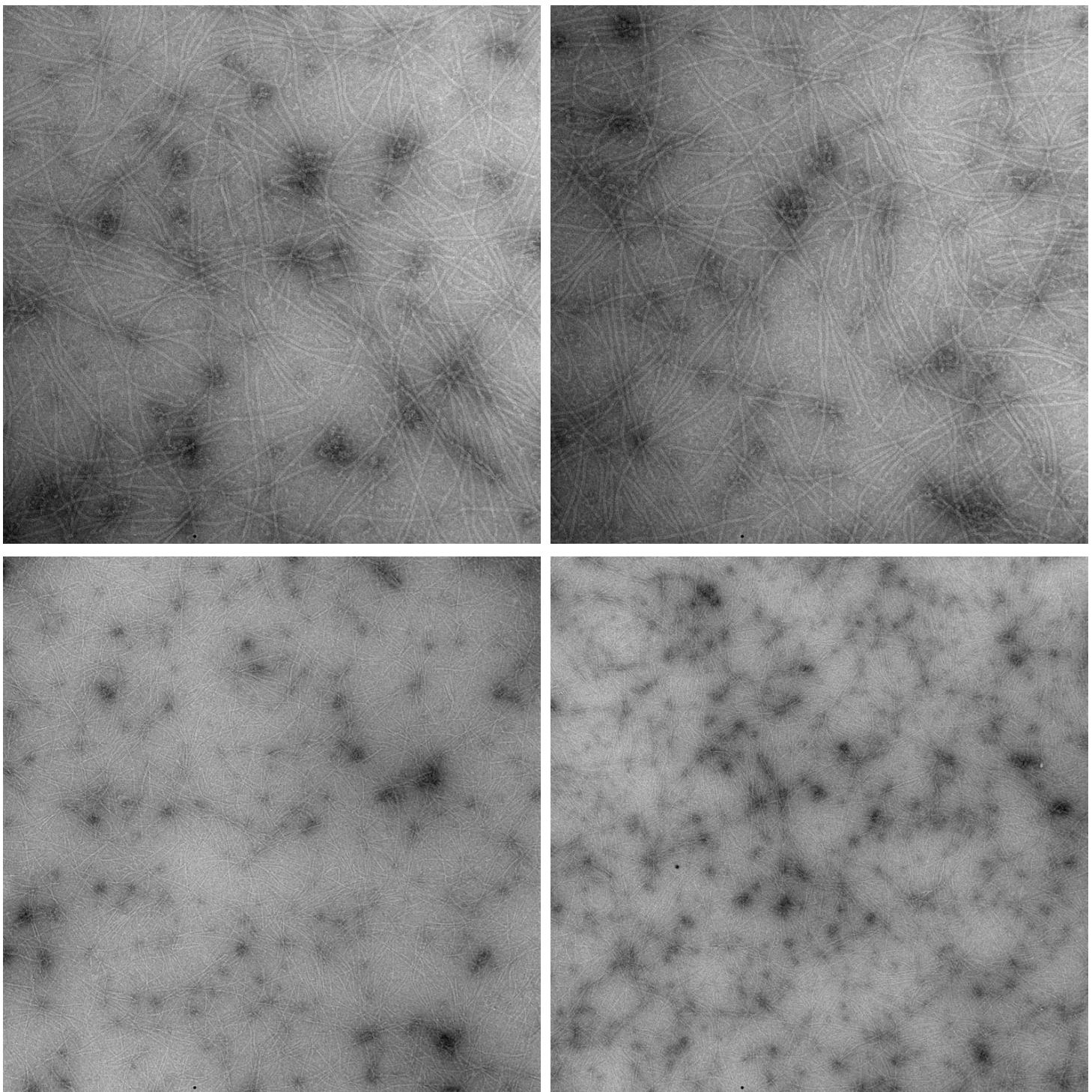
42 helix bundle rule 2, 12 day annealing in 2.4 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



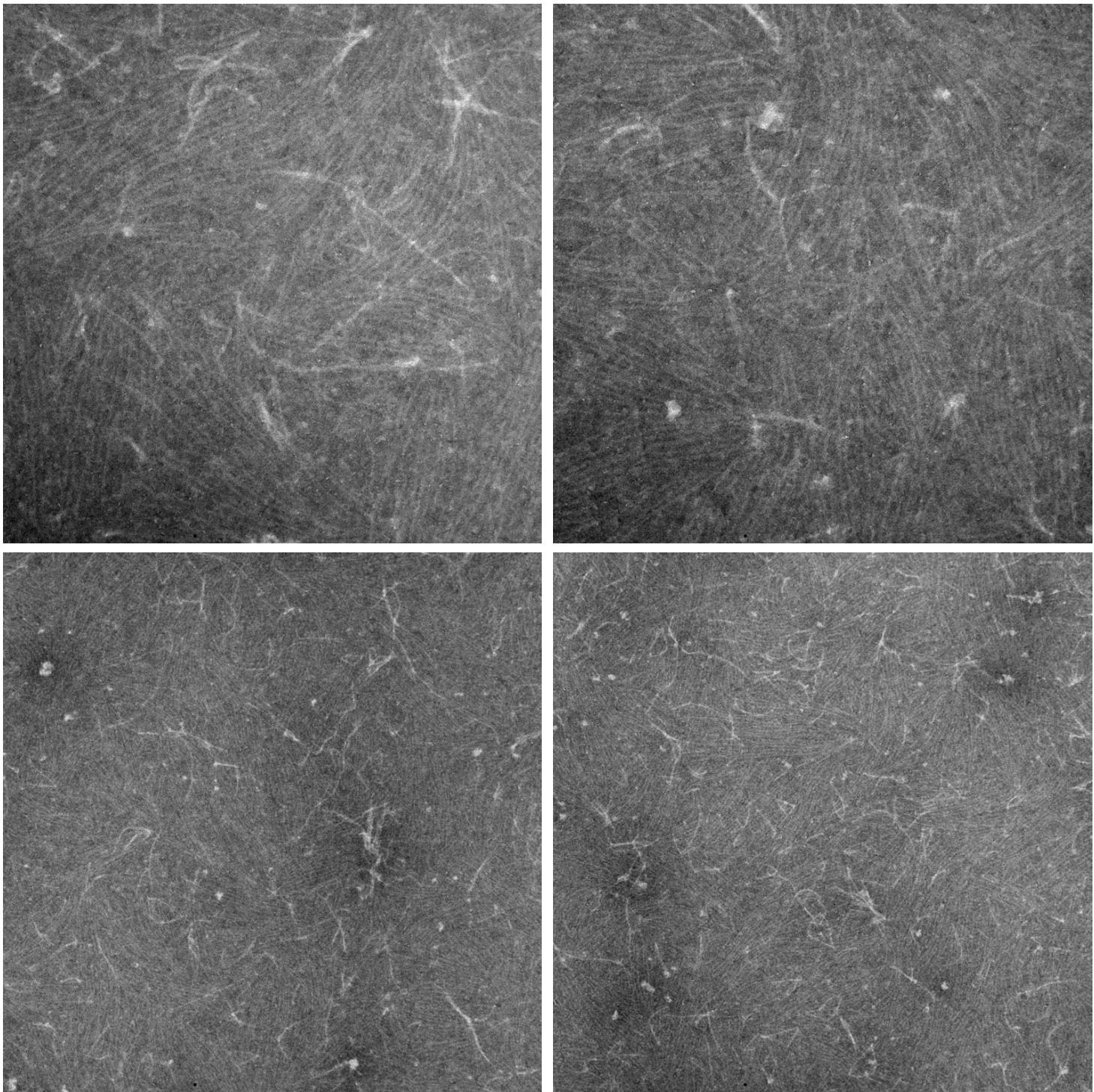
42 helix bundle rule 1, 12 day annealing in 24 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



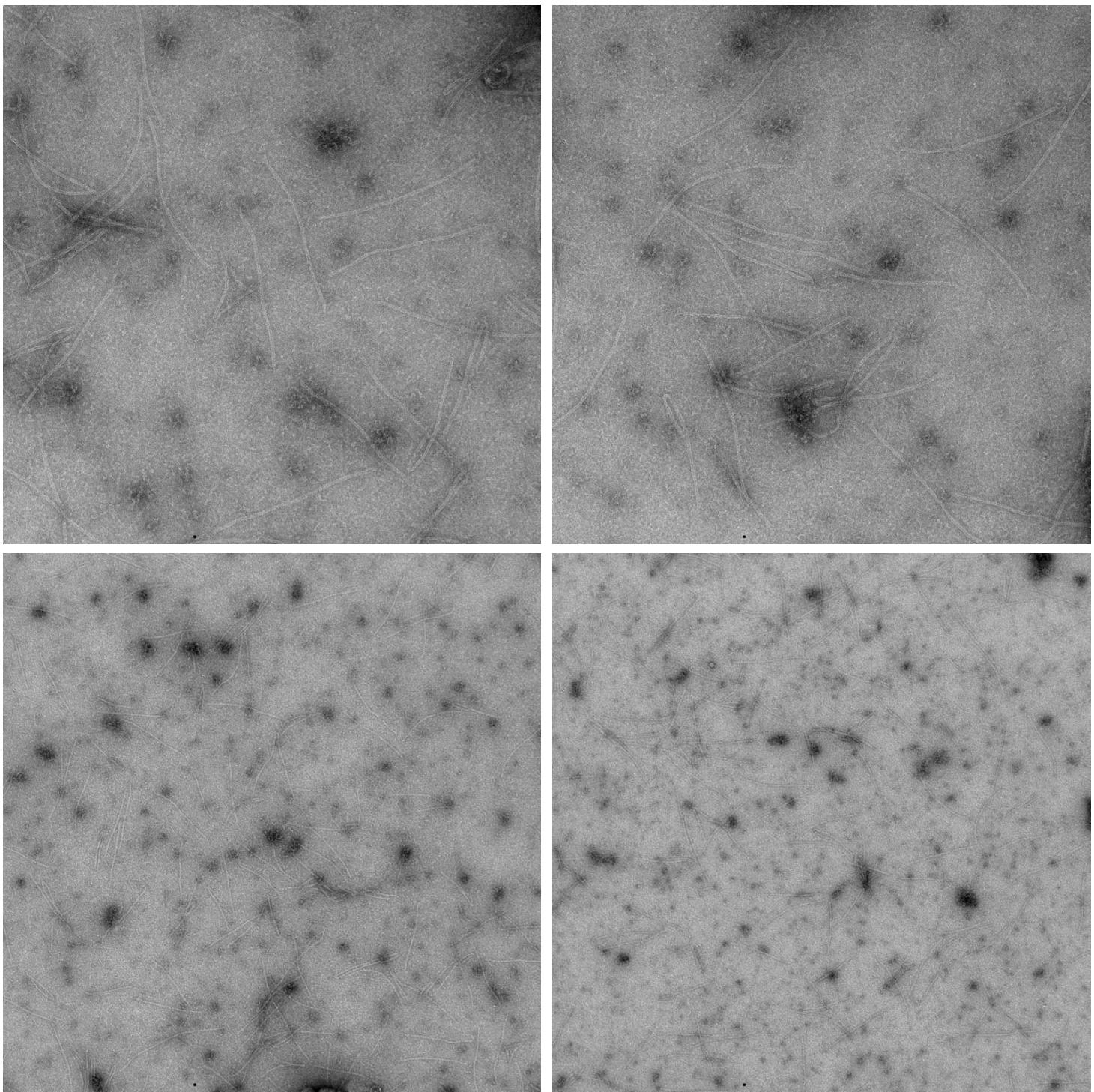
42 helix bundle rule 1, 12 day annealing in 2.4 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



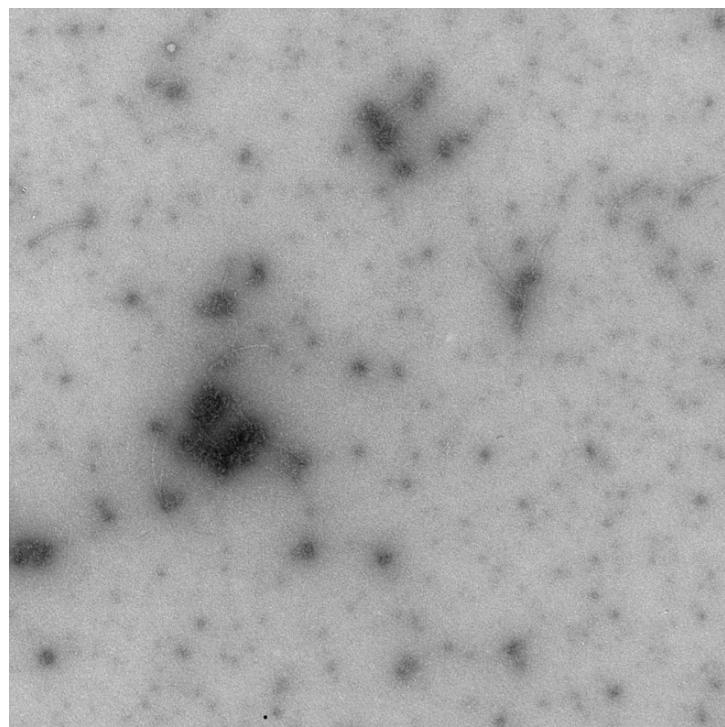
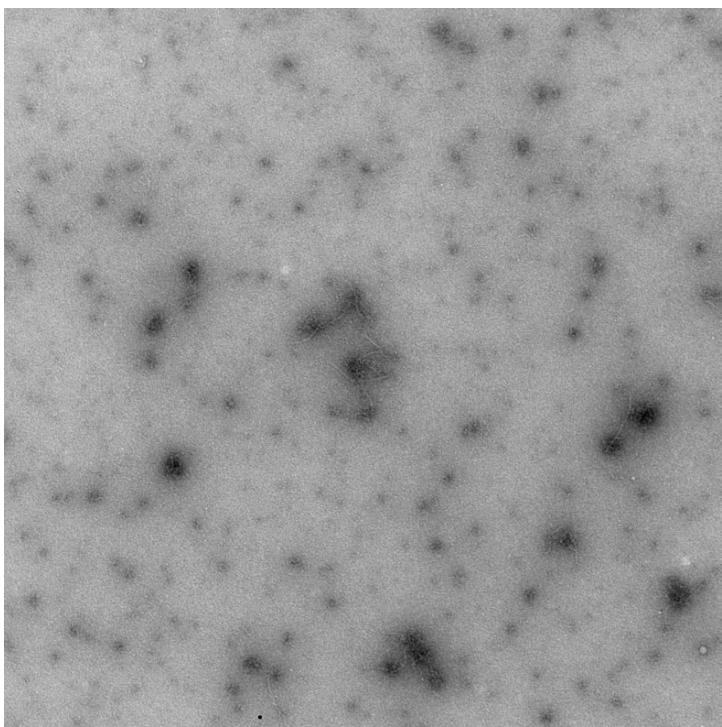
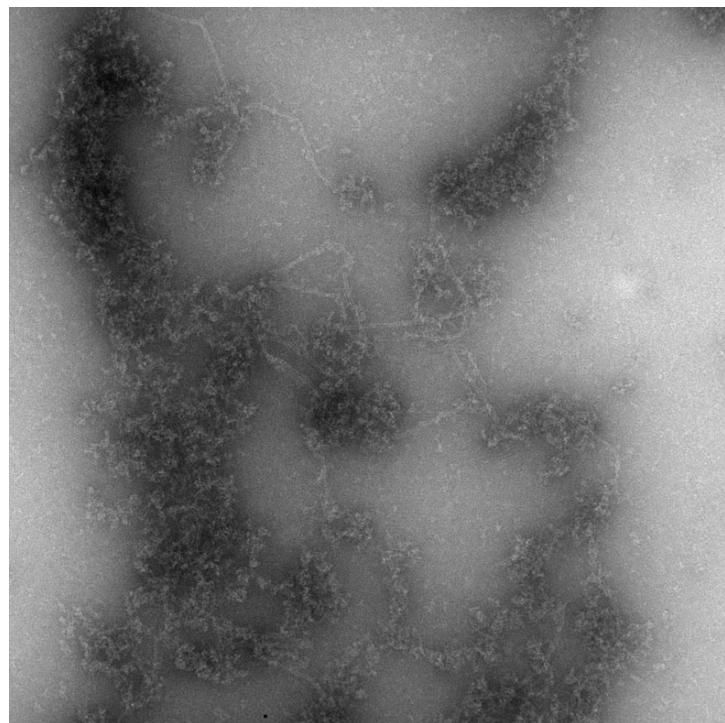
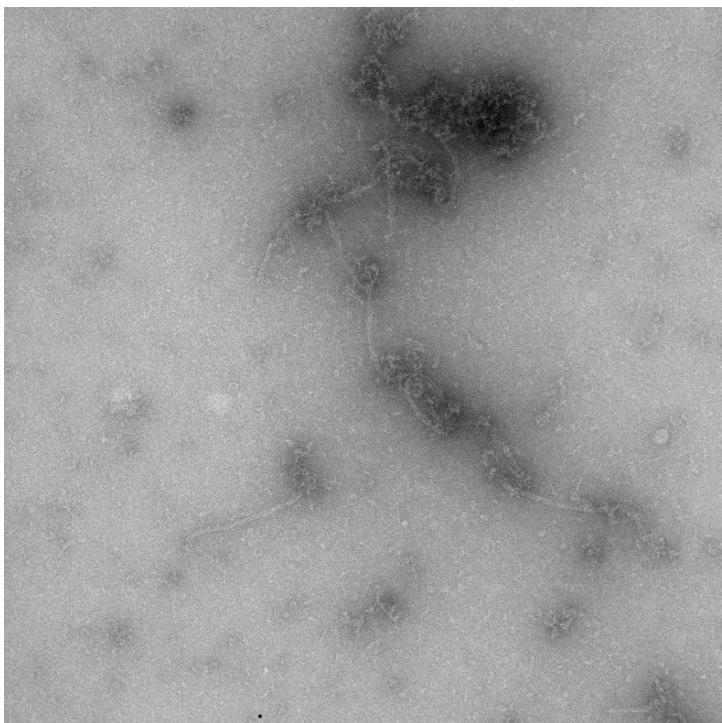
6 helix bundle, 2 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



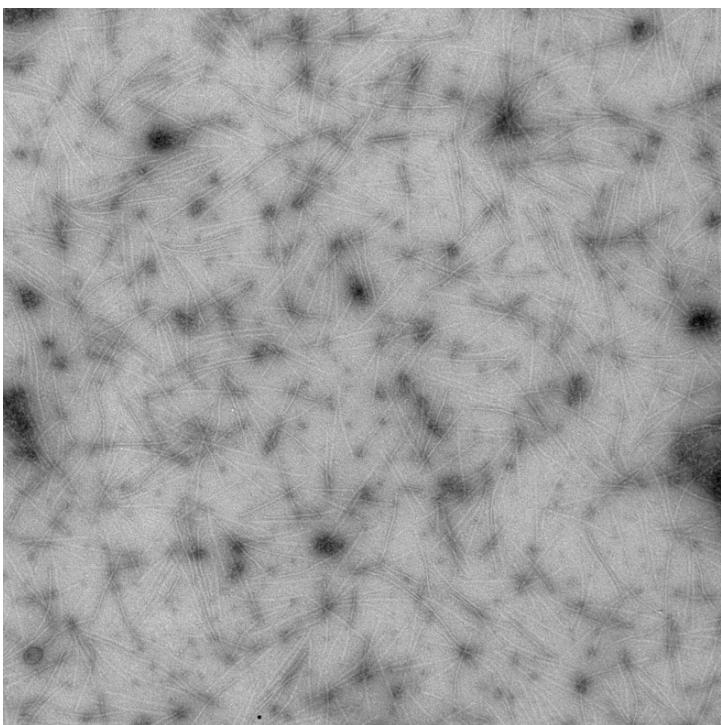
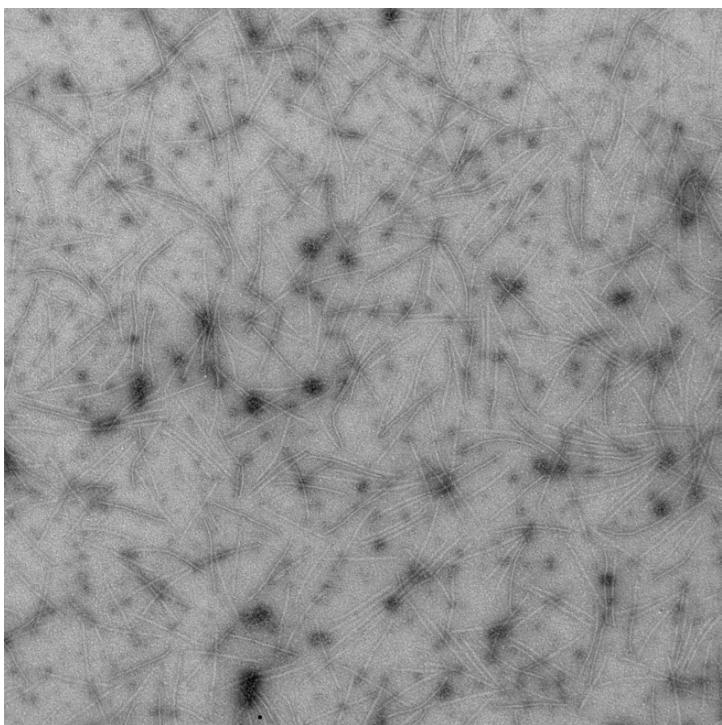
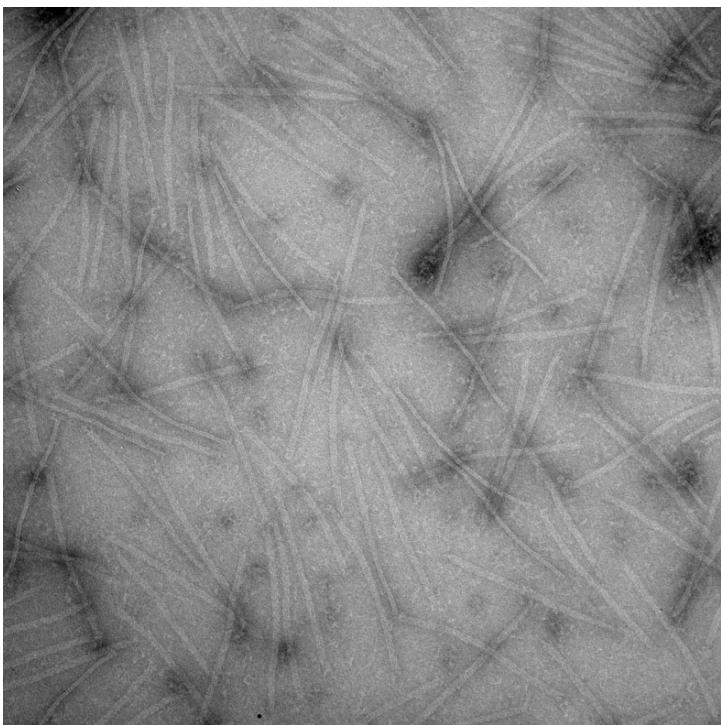
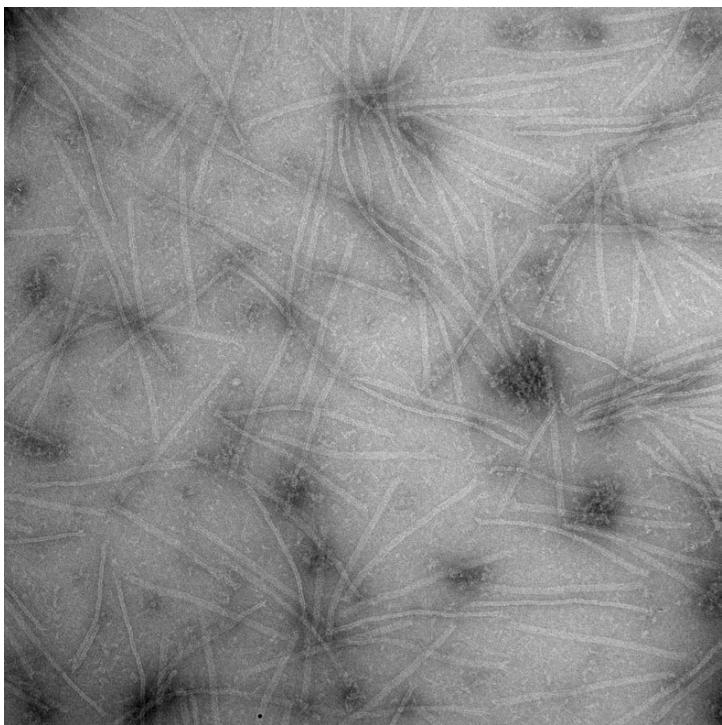
6 helix bundle, 2 day annealing in 2.4 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



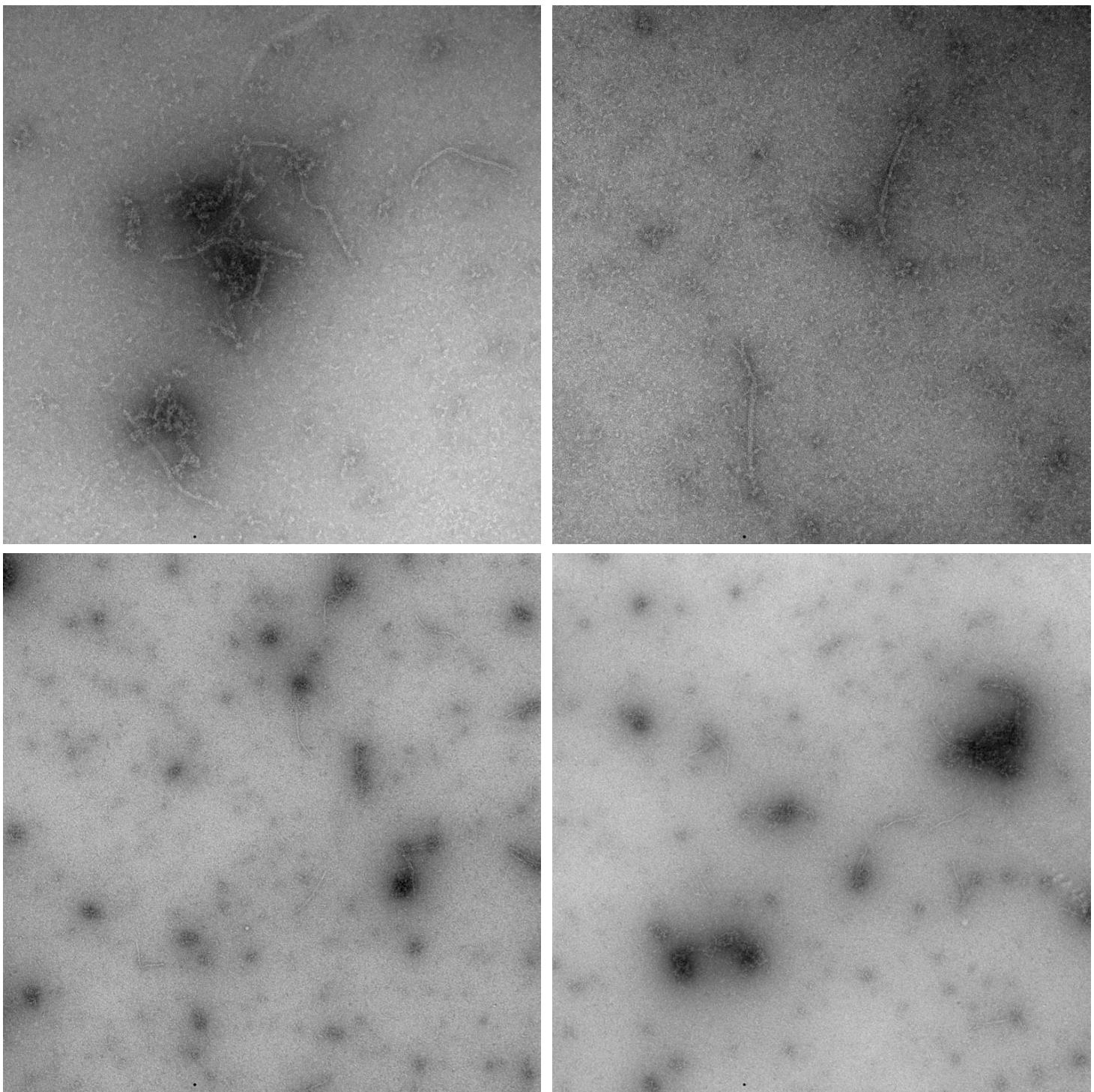
8 helix bundle, 12 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$. Bottom images taken at 11500x magnification, field of view 2.5 $\mu\text{m} \times 2.5 \mu\text{m}$.



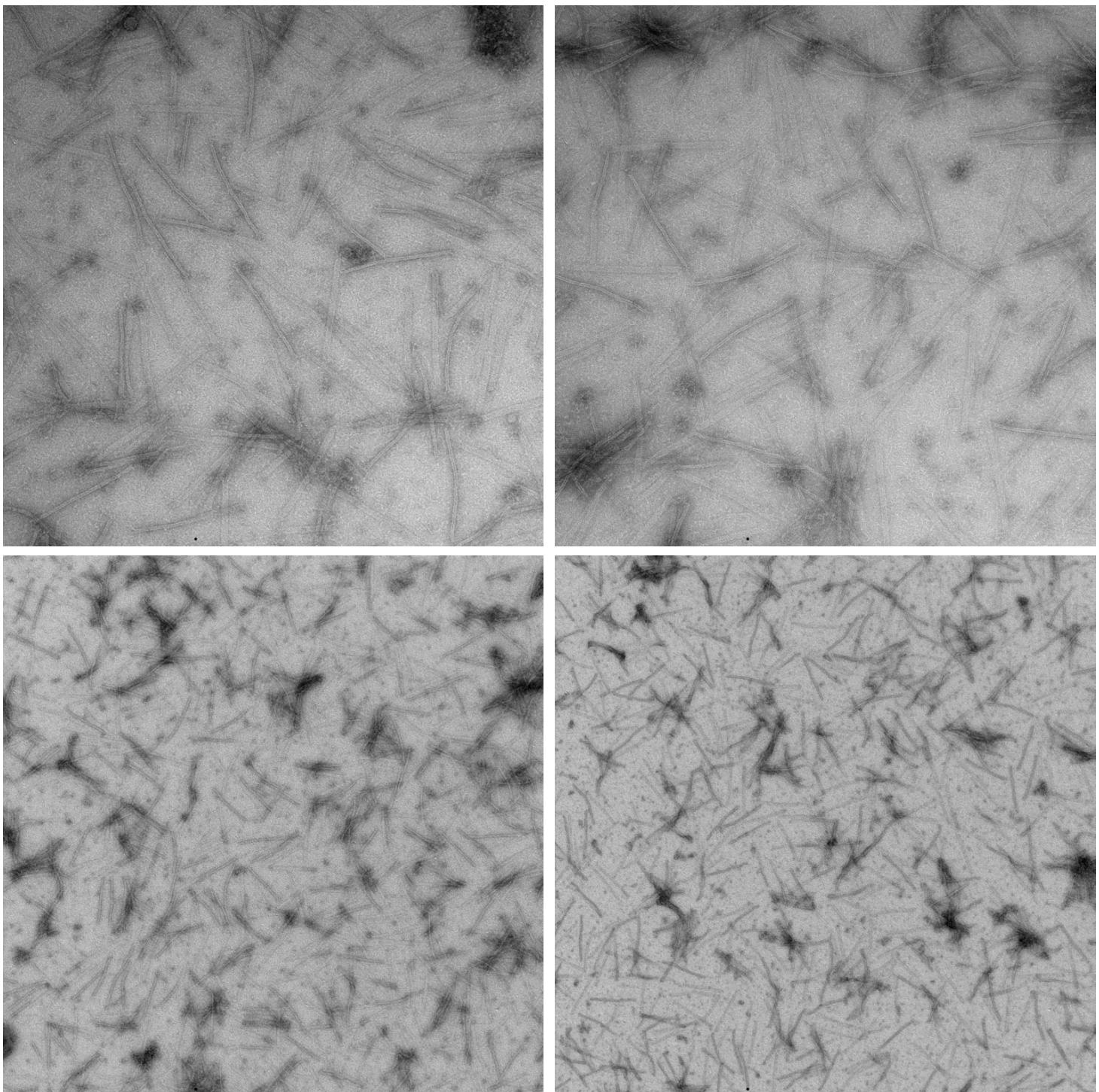
8 helix bundle, 12 day annealing in 340 mM NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$. Bottom images taken at 11500x magnification, field of view 2.5 $\mu\text{m} \times 2.5 \mu\text{m}$.



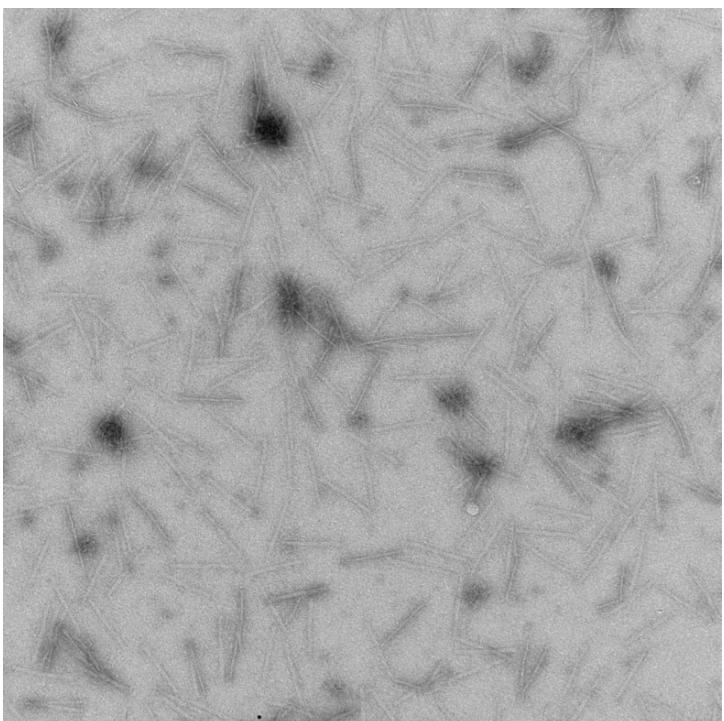
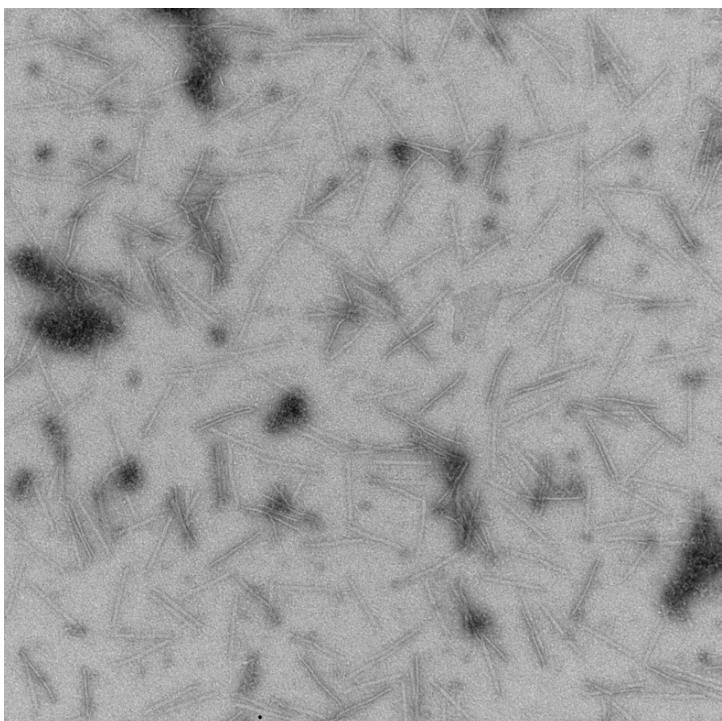
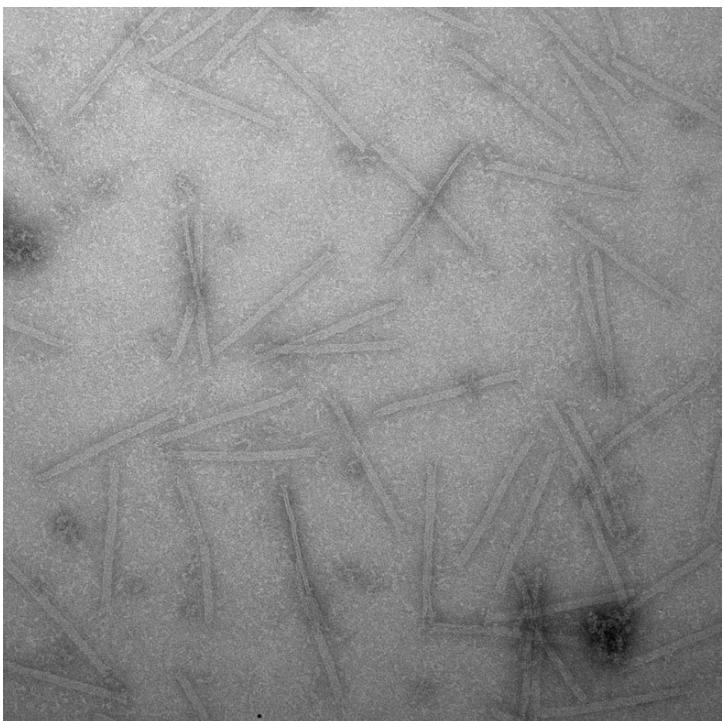
10 helix bundle, 12 day annealing in 12 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



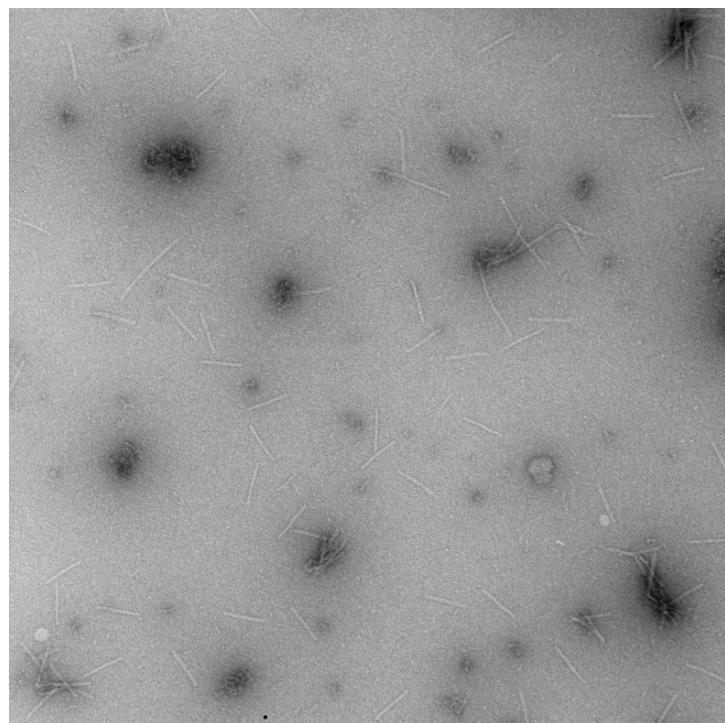
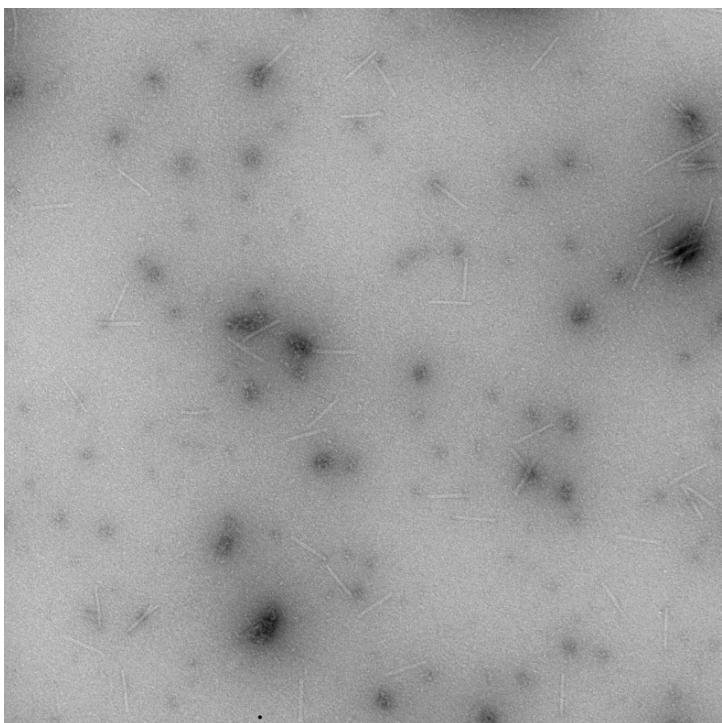
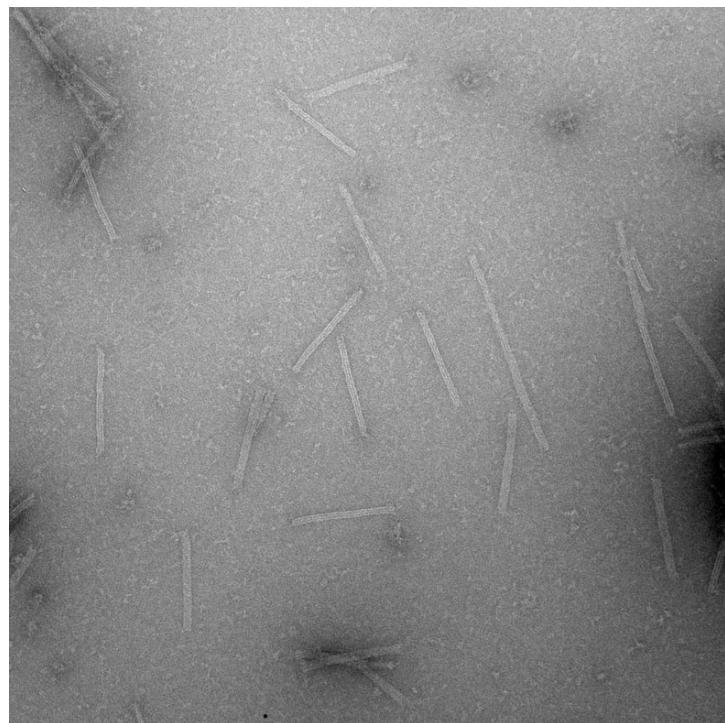
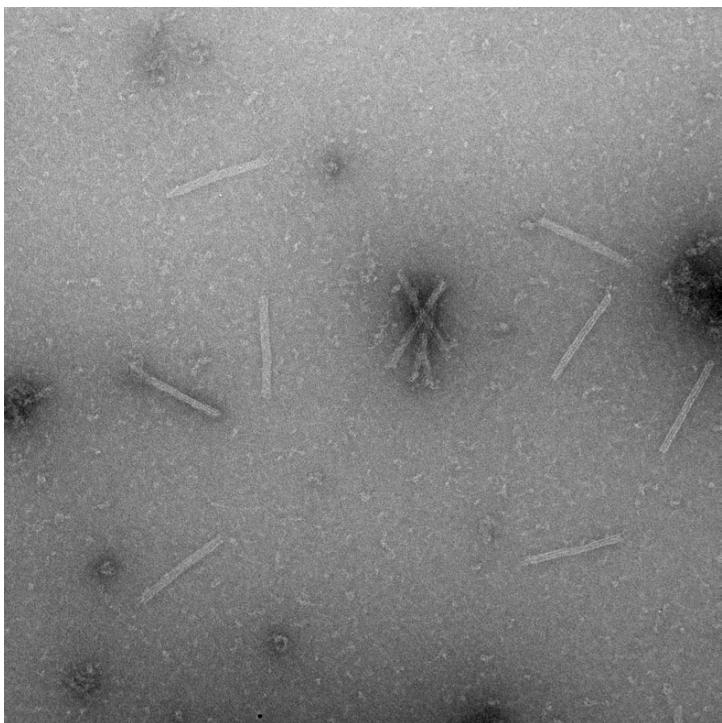
10 helix bundle, 12 day annealing in 340 mM NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



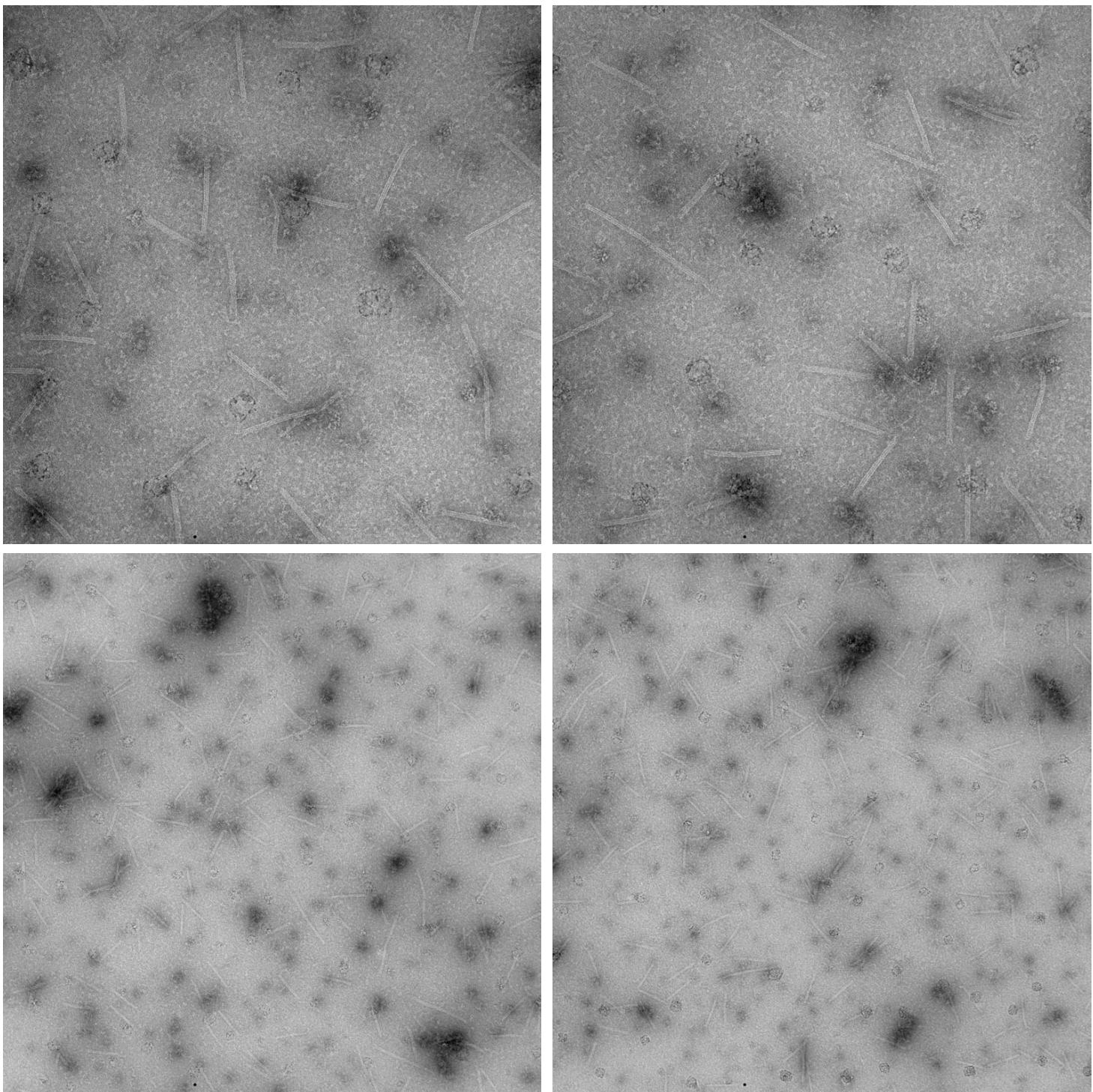
12 helix bundle, 12 day annealing in 12 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



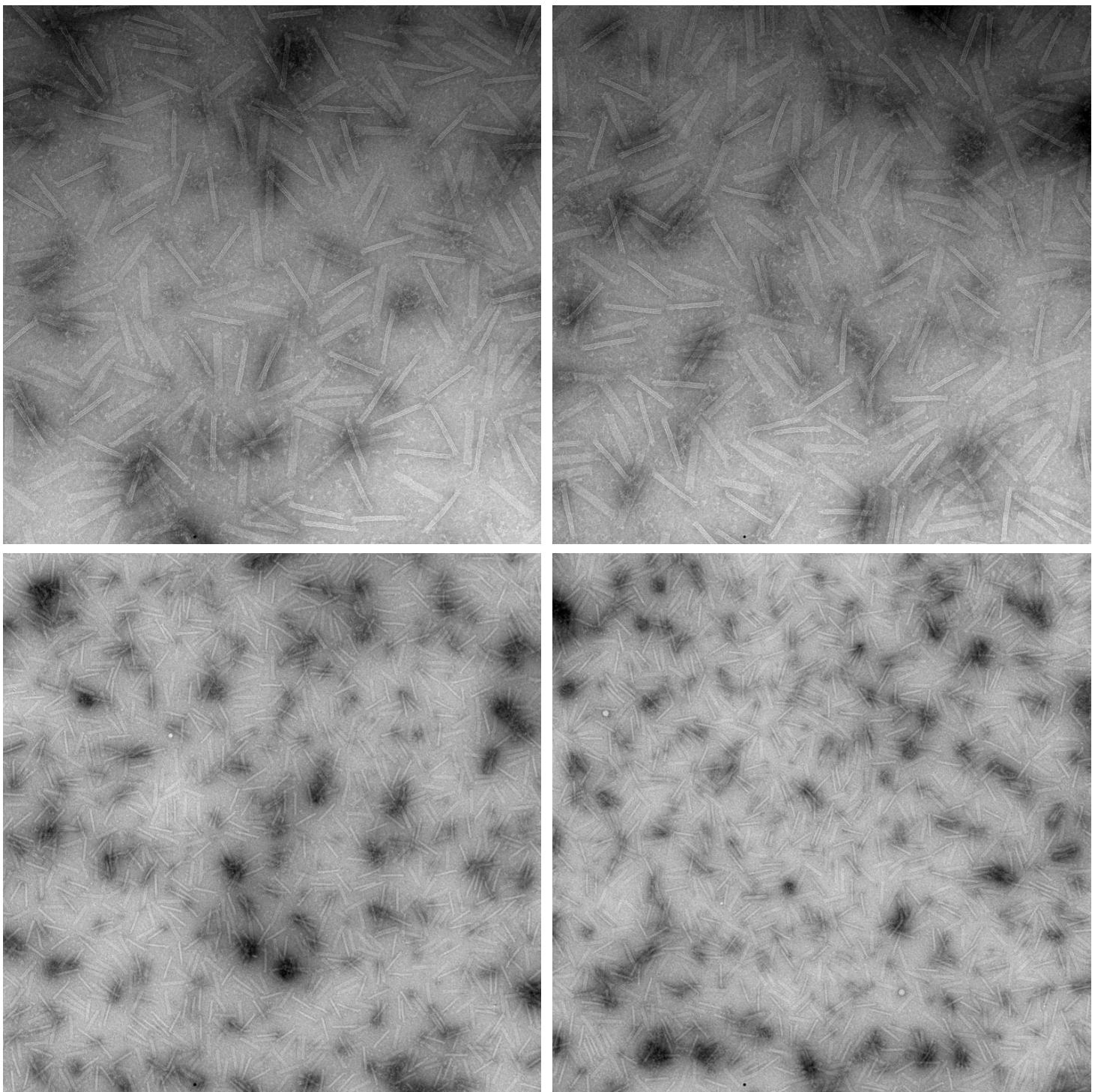
12 helix bundle, 12 day annealing in 1.2 mM NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$. Bottom images taken at 11500x magnification, field of view 2.5 $\mu\text{m} \times 2.5 \mu\text{m}$.



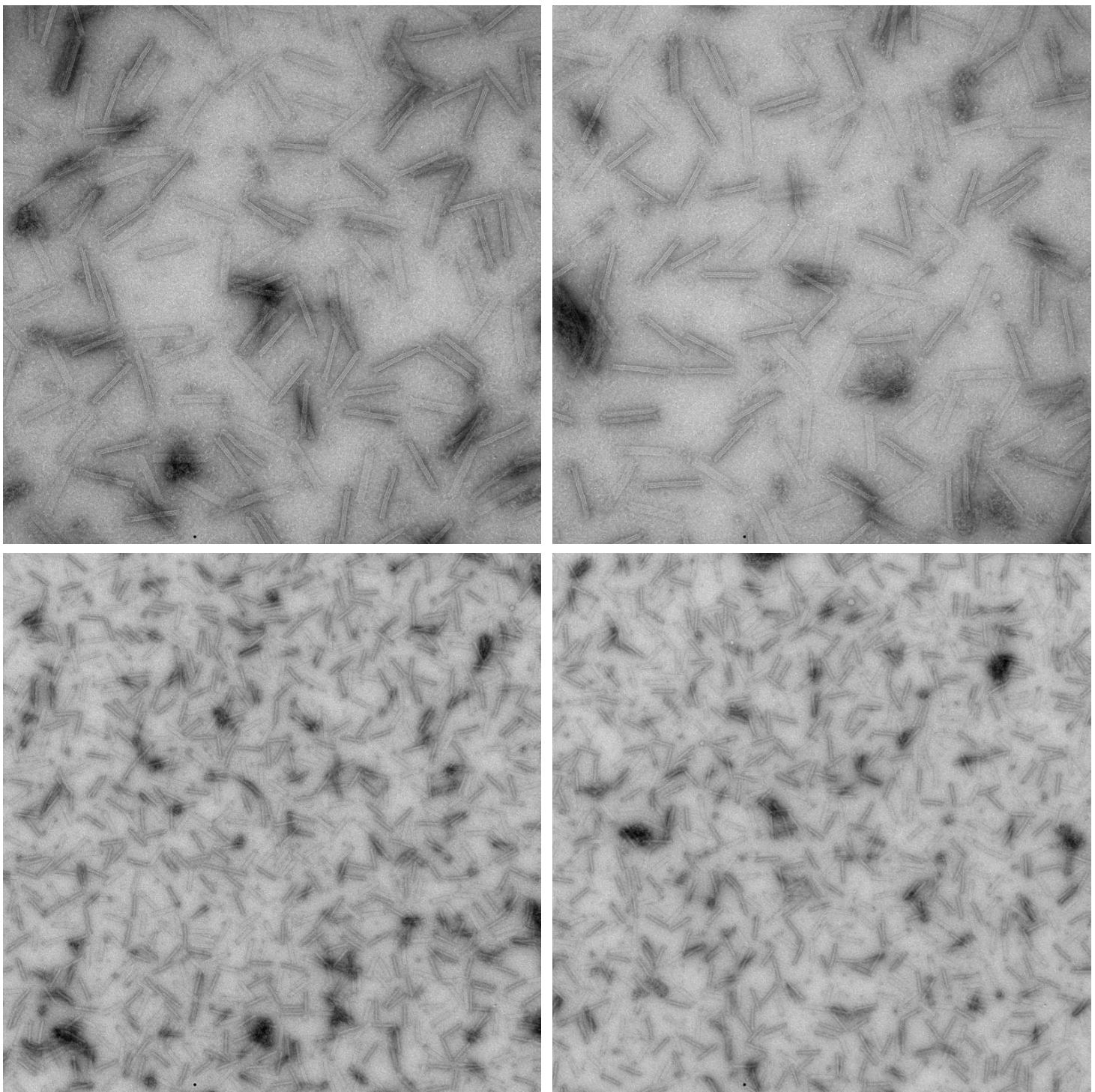
18 helix bundle, 12 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.



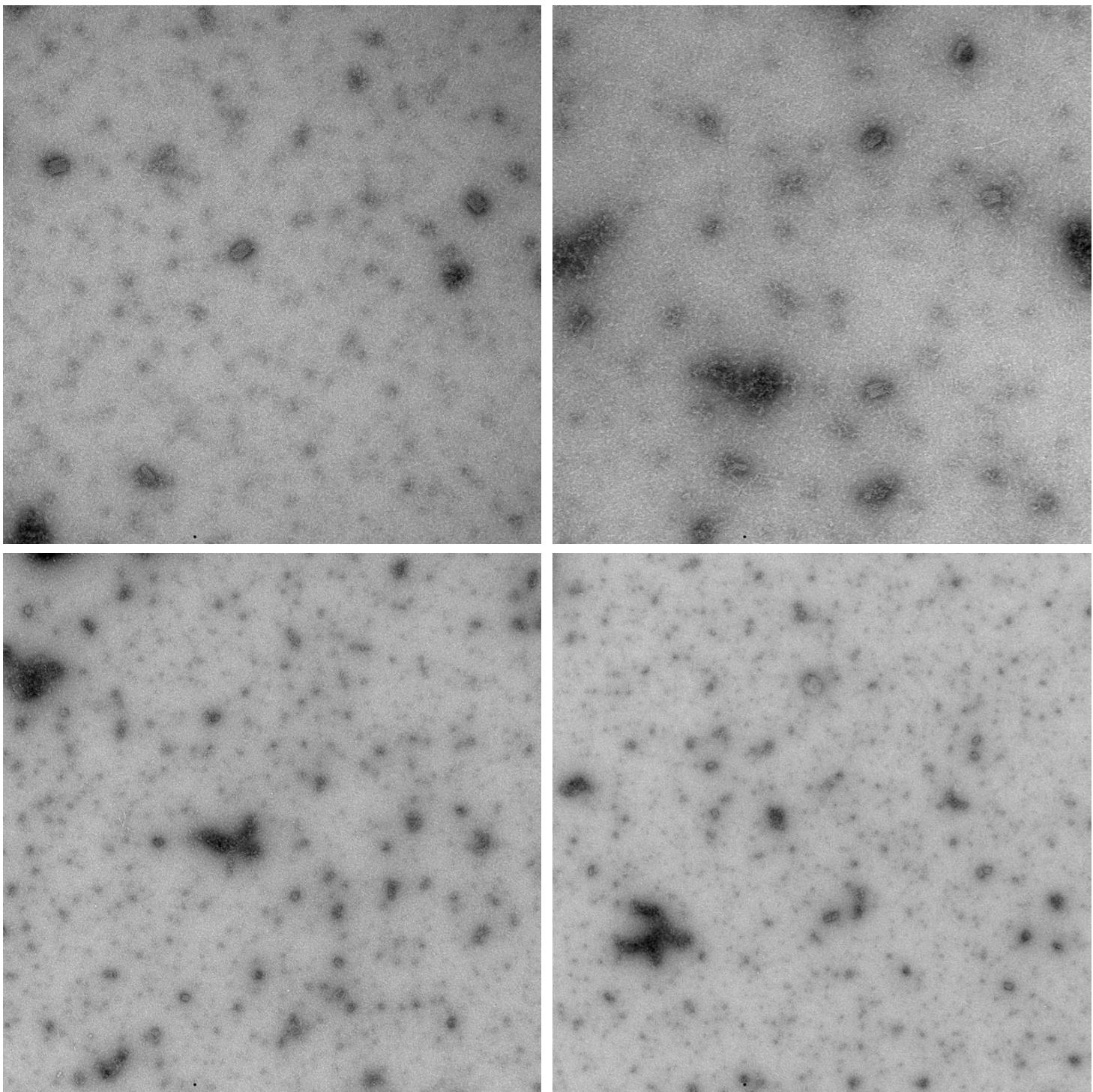
18 helix bundle, 12 day annealing in 2.0 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$. Bottom images taken at 11500x magnification, field of view 2.5 $\mu\text{m} \times 2.5 \mu\text{m}$.



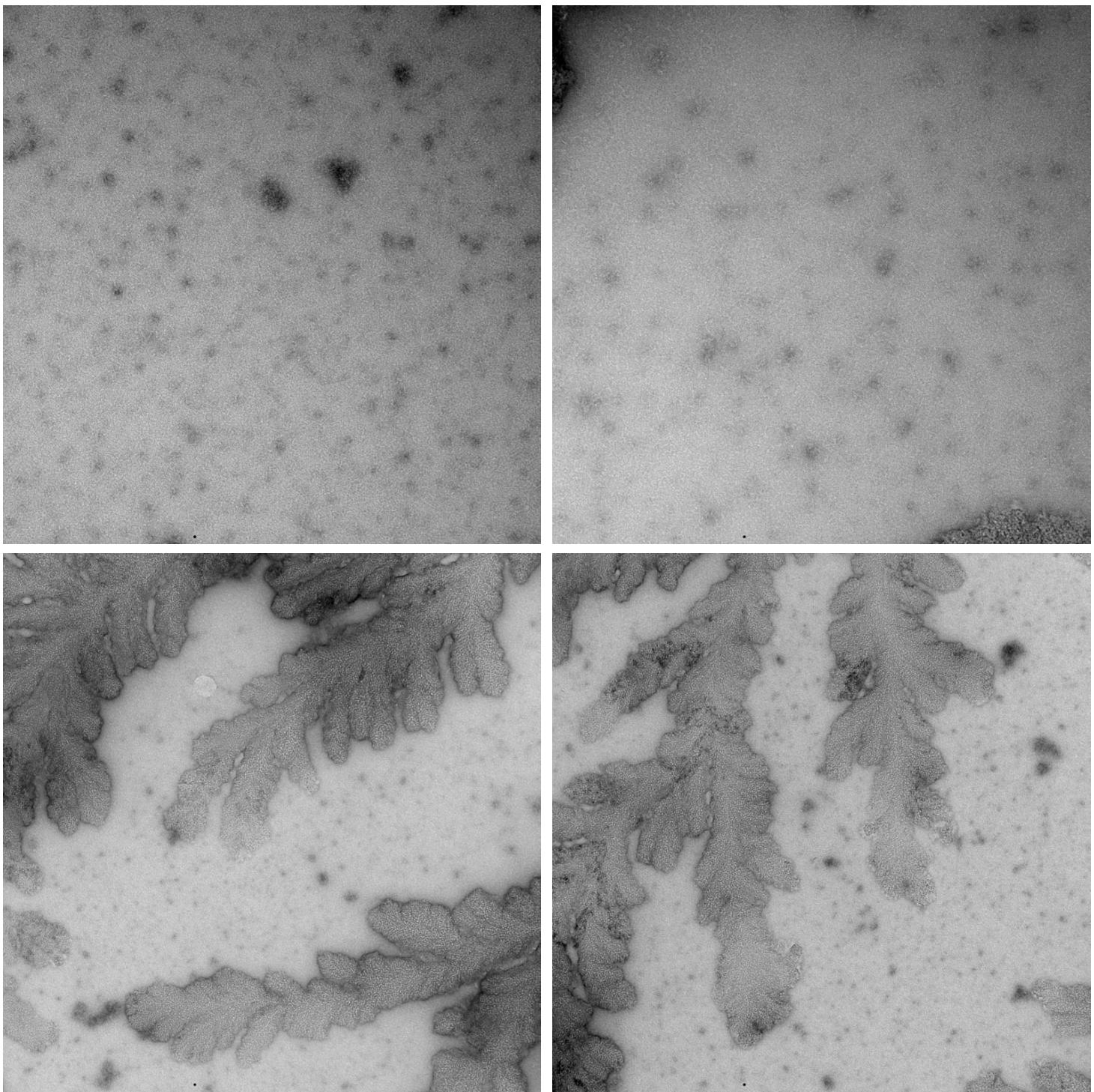
24 helix bundle, 12 day annealing in 16 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



24 helix bundle, 12 day annealing in 1.6 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm . Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm .



100 helix bundle, 12 day annealing in 20 mM MgCl₂, unpurified. Top images taken at 28500x magnification, field of view 1.0 μm x 1.0 μm. Bottom images taken at 11500x magnification, field of view 2.5 μm x 2.5 μm.

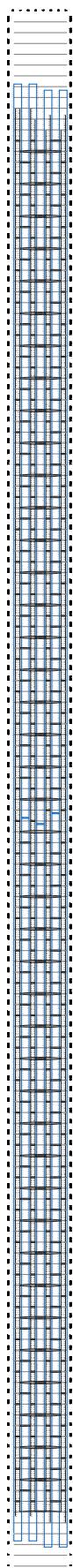


100 helix bundle, 12 day annealing in 3.4 M NaCl, unpurified. Top images taken at 28500x magnification, field of view 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$. Bottom images taken at 11500x magnification, field of view 2.5 $\mu\text{m} \times 2.5 \mu\text{m}$.

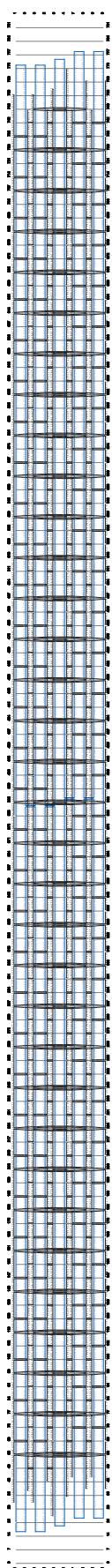
Supplementary Figure S5
caDNAno design diagrams



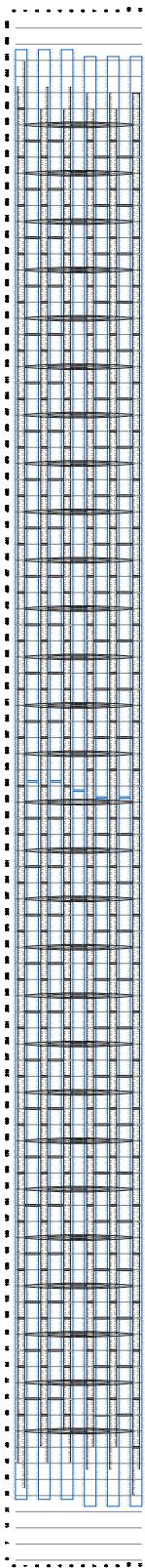
Scaffold / staple layout diagram
for 6 helix bundle



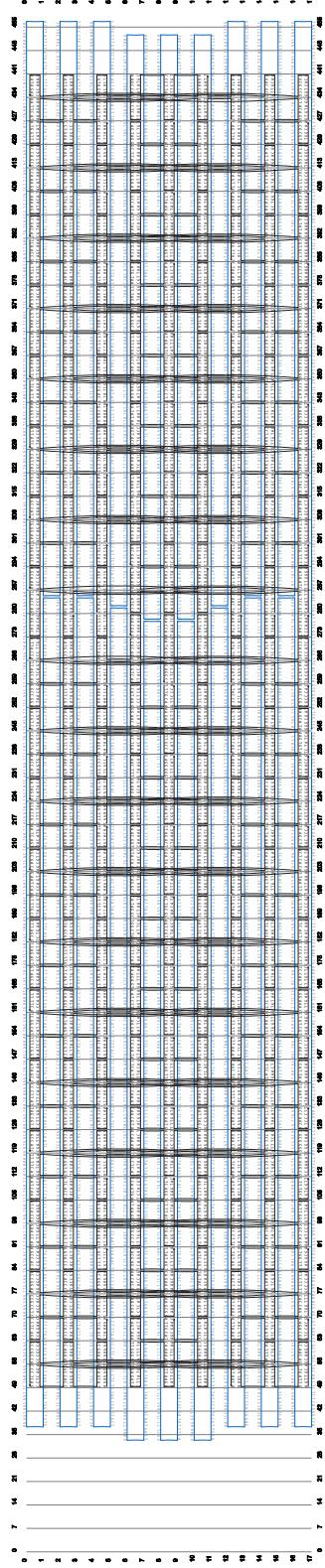
Scaffold / staple layout diagram
for 8 helix bundle



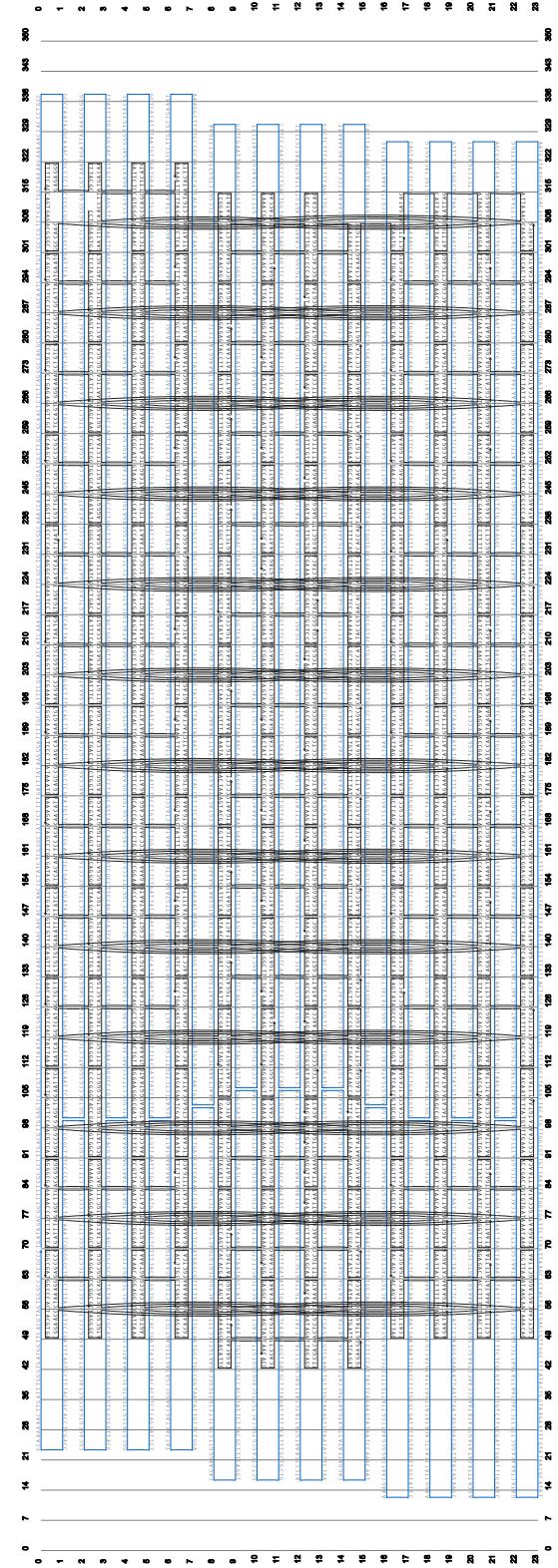
Scaffold / staple layout diagram
for 10 helix bundle



Scaffold / staple
layout diagram for 12
helix bundle

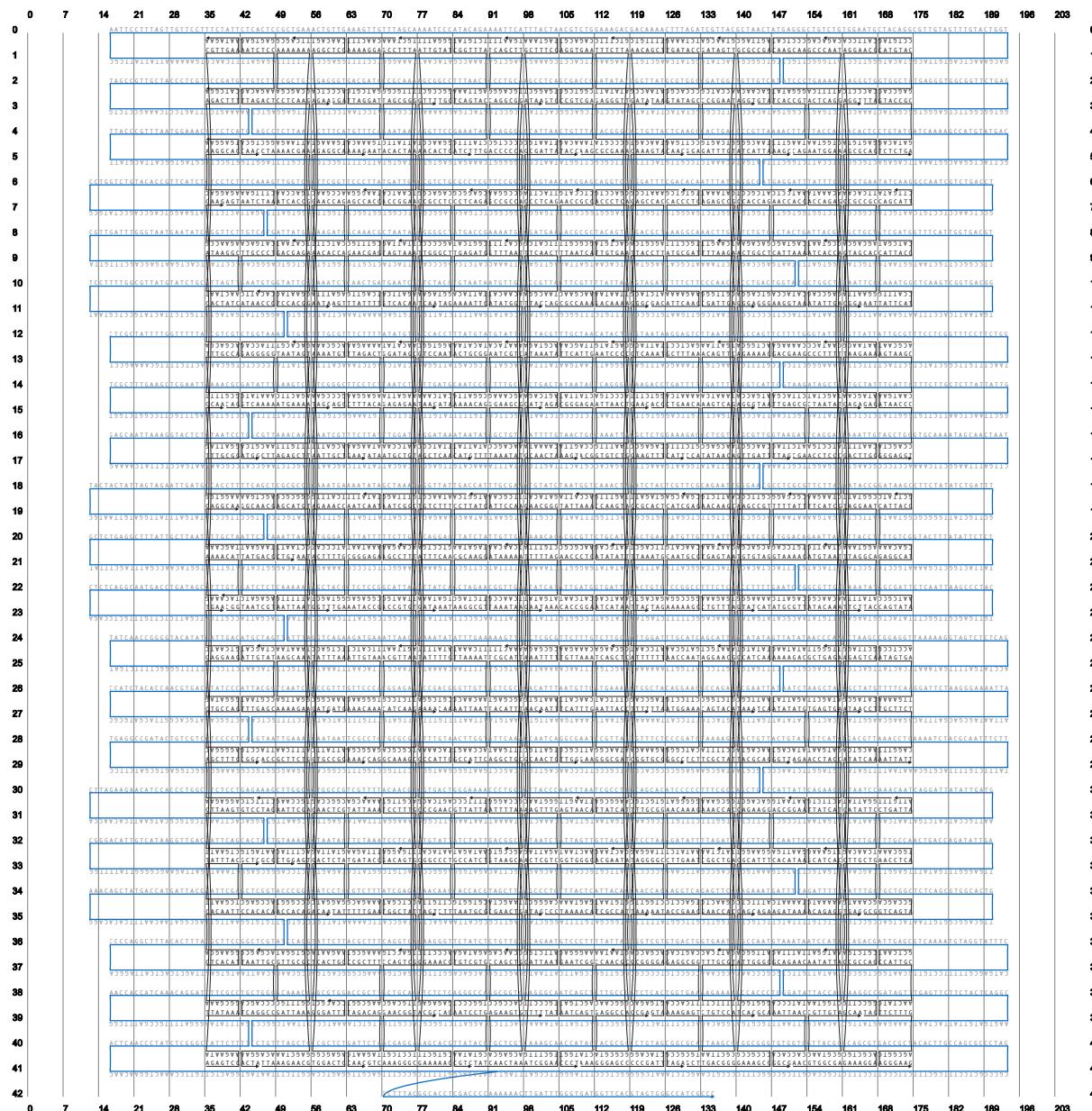


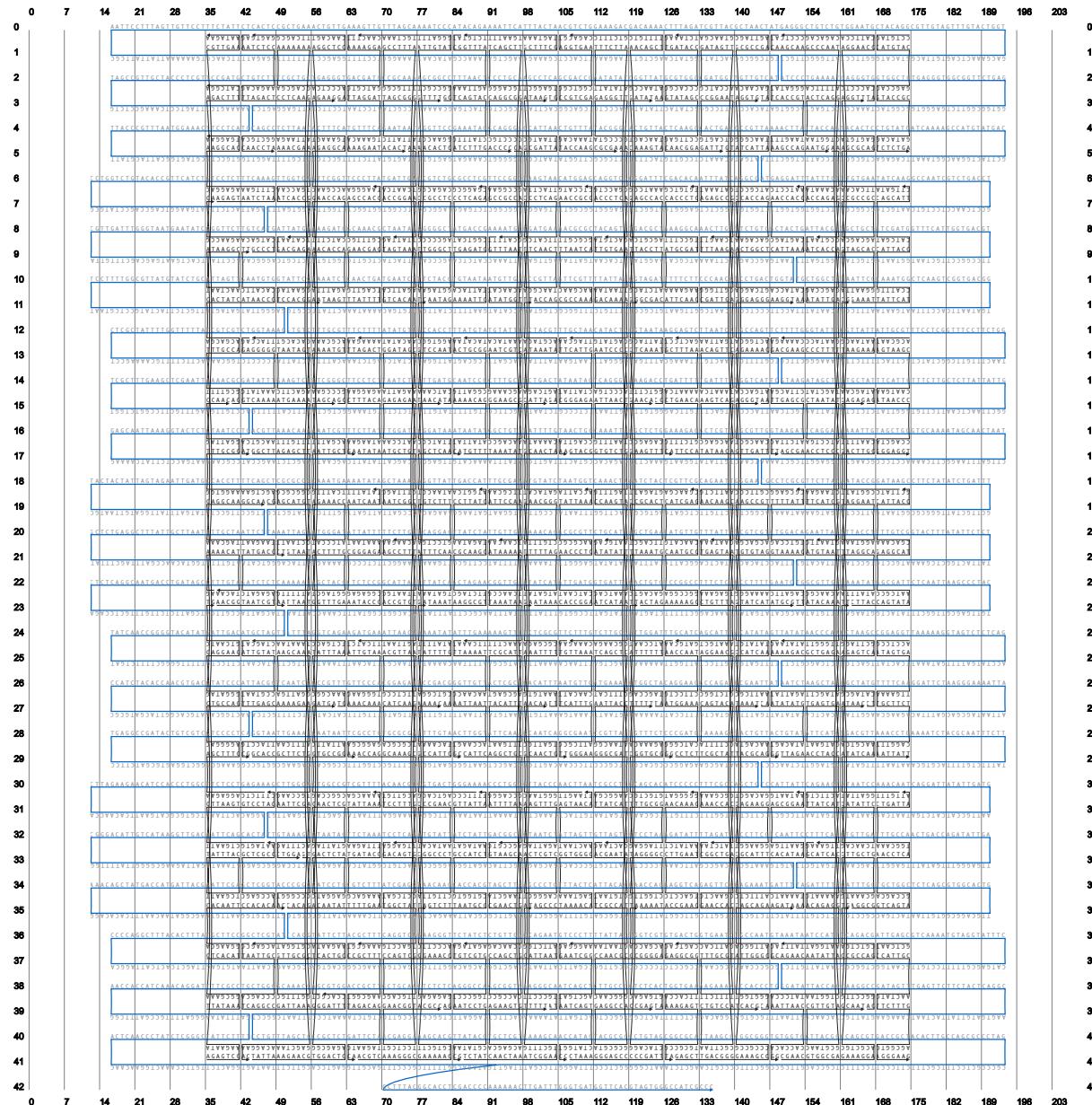
Scaffold / staple
layout diagram for 18
helix bundle



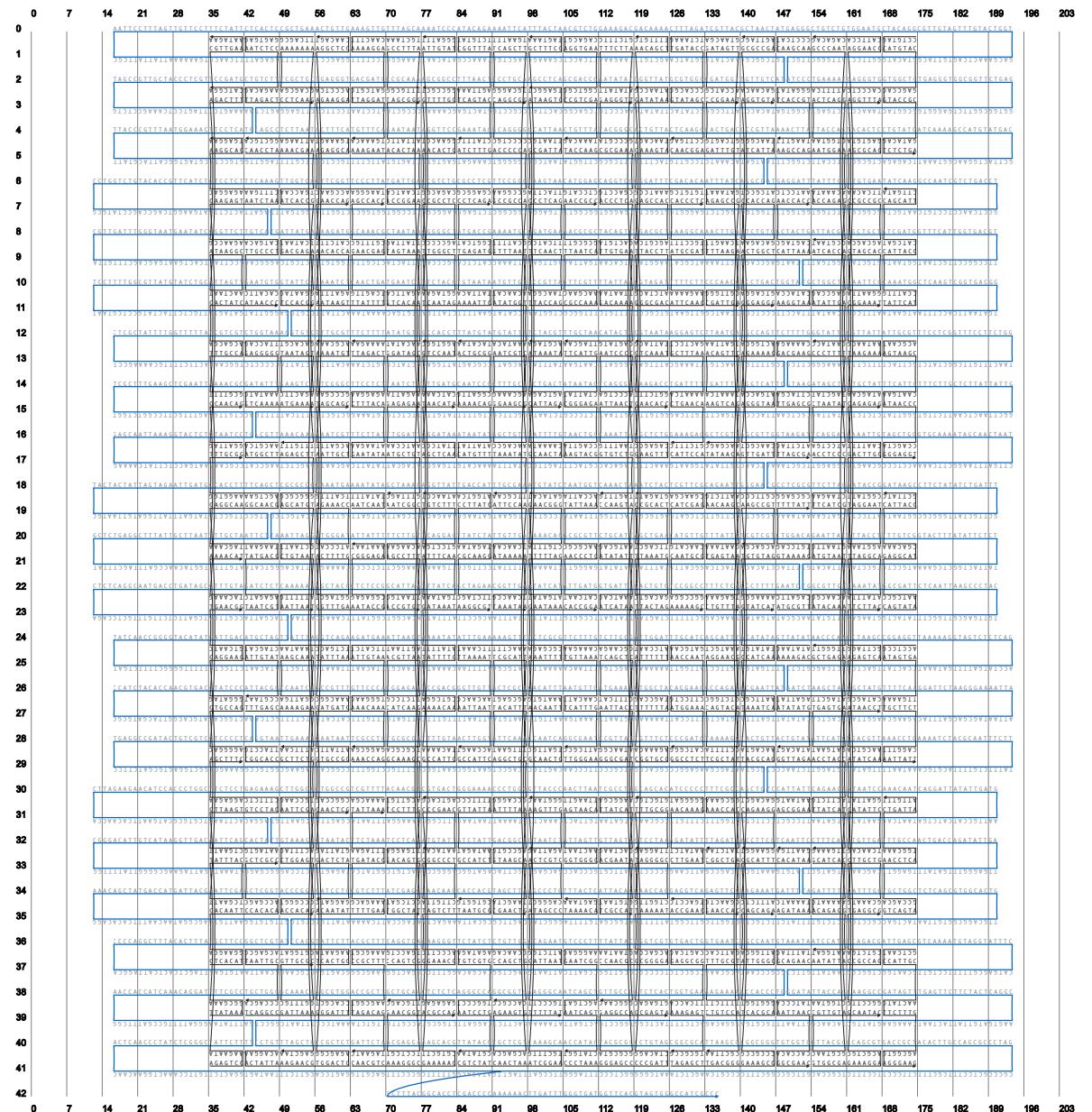
Scaffold / staple layout diagram
for 24 helix bundle

Scaffold / staple layout diagram for 42 helix bundle, backbone nick rule 1

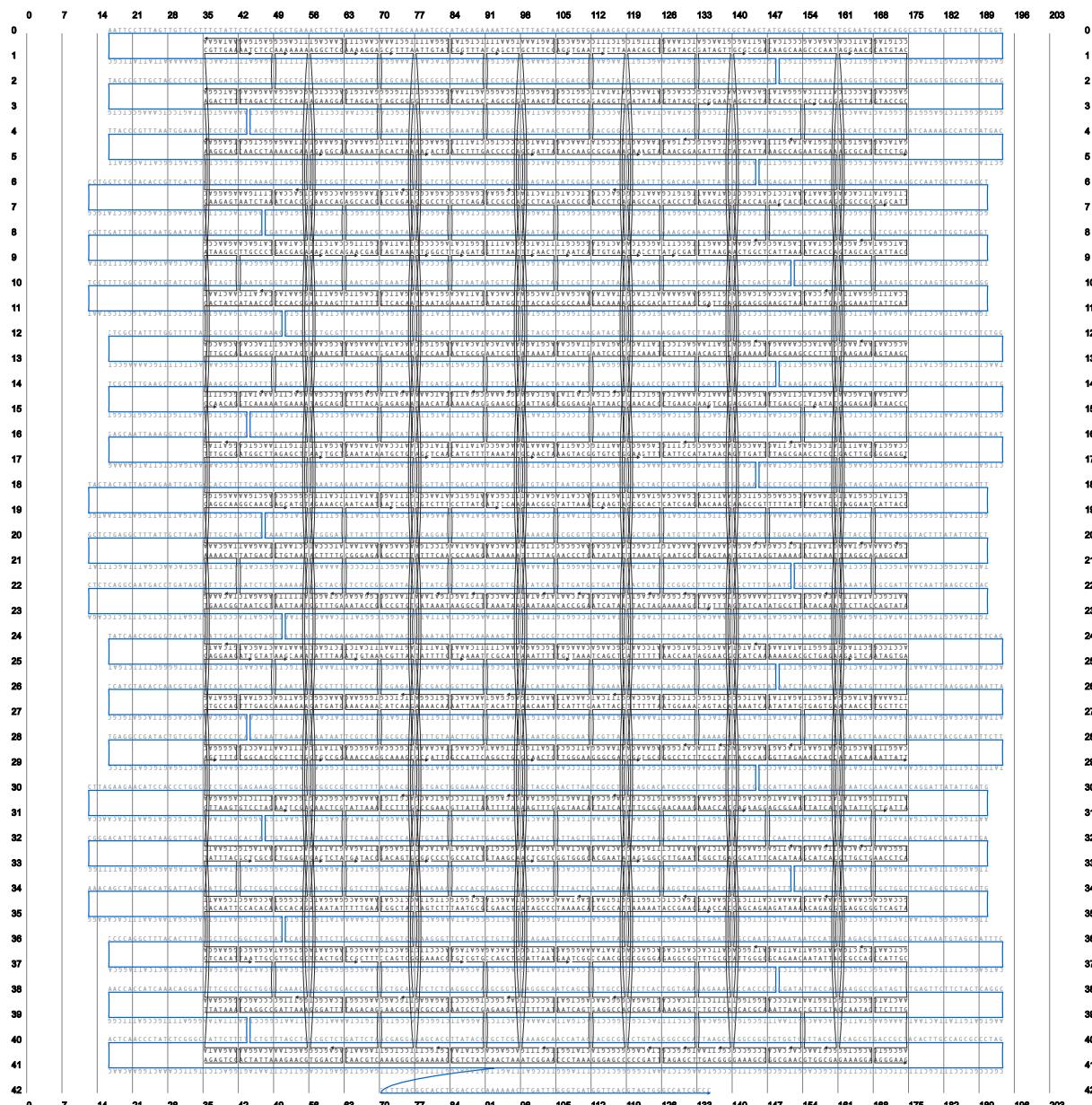




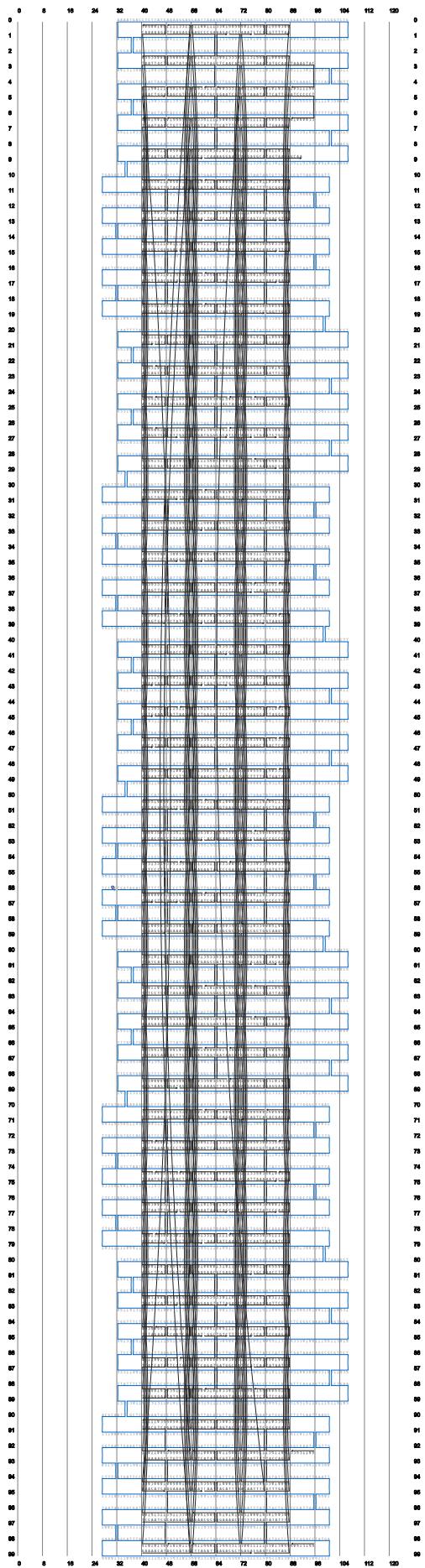
Scaffold / staple layout diagram for 42 helix bundle, backbone nick rule 2



Scaffold / staple layout diagram for 42 helix bundle, backbone nick rule 3



Scaffold / staple layout diagram for 42 helix bundle, backbone nick rule 4



Scaffold / staple layout diagram for 100 helix bundle

Supplementary Tables S1-S6:
Thermal annealing ramps

Table S1: 1.5 day ramp

°C	min/°
65	5
64 - 57	15
56 - 25	60
4	forever
total	1d 9h 5min

Table S2: 2 day ramp

°C	min/°
65	5
64 - 57	15
56 - 25	90
4	forever
total	2d 35min

Table S3: 3 day ramp

°C	min/°
65 - 57	1
56	2
55	60
54	120
53 - 51	360
50	500
49 - 46	360
45	240
44	120
43 - 41	60
40	30
39 - 36	15
35 - 25	2
10	forever
total	2d 16h 23min

Table S4: 6 day ramp

°C	min/°
65	5
64 - 57	15
56 - 25	270
4	forever
total	5d 21h 35min

Table S6: 23 day ramp

°C	min/°
65	5
64 - 57	15
56 - 25	1080
4	forever
total	23d 8h 5min

Table S5: 12 day ramp

°C	min/°
65	5
64 - 57	15
56 - 25	540
4	forever
total	11d 17h 5min