Electronic Supplementary Material (ESI) for Nanoscale.

SUPPORTING INFORMATION

(Poly)Cation-Induced Protection of Conventional and Wireframe DNA Origami Nanostructures

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Supporting Notes

Note S1: Formulas for calculating the N/P charge ratio of a polyplex

Formula 1. $\frac{N}{P}$ Ratio

$$\frac{\text{nmoles of polycation} \times \text{number of amine groups per polycation}}{\text{nmoles of origami} \times \text{number of phosphate groups per origami}}$$

Formula 1a. Calculating nmoles of polycation

$$\frac{\text{concentration of polycation } \left(\frac{\text{mg}}{\text{ml}}\right) \times \mu\text{l of polycation} \times 10^3}{\text{molecular weight of polycation } \left(\frac{\text{g}}{\text{mol}}\right)}$$

Formula 1b. Calculating the number of amine groups per polycation

$$\frac{\text{Molecular weight of polycation} \times \text{number of amine per monomer}}{\text{molecular weight of one monomer}}$$

Formula 1c. Calculating nmoles of the origami

$$\frac{\text{concentration of origami } \left(\frac{\text{ng}}{\mu\text{l}}\right) \times \mu\text{l of origami}}{\text{molecular weight of origami } \left(\frac{\text{g}}{\text{mol}}\right)}$$

Formula 1d. Calculating the molecular weight of a typical origami

$$\text{number of base pairs} \times \text{ average molecular weight of one base pair } \left(660 \ \frac{\text{g}}{\text{mol}}\right)$$

or

$$\text{number of nucleotides} \times \text{ average molecular weight of one nucleotide } \left(330 \ \frac{\text{g}}{\text{mol}}\right)$$

Formula 1e. Number of phosphate groups in one origami

$$\text{number of base pairs per origami}$$

Note S2. Calculating the number of phosphate groups and molecular weight of origamis

The NR and the NB were self-assembled using p8064 and p7249 single stranded scaffold, respectively. While designing, up to 99% of the scaffolds have been used to avoid non-paired loop. Hence, the number of phosphate groups in NR and NB can be calculated simply by doubling the number of scaffold base pairs. The WN was designed by Daedalus software and has a non-paired loop of 200 base pairs.

DNA origami	Number of phosphate groups	Molecular weight (g/mol)
NR	= (8064*2) = 16128	5322240 (5322 kDa)
NB	= (7249*2) = 14498	4784340 (4784 kDa)
WN	= (7249*2) -200= 14298	4718340 (4718 kDa)

Note S3. Calculating the number of amine groups per polycation

The average molecular weight of chitosan oligosaccharide lactate (M_n ~ 4000-6000 Da) is considered to be 5000 Da. As mentioned by the supplier, this product is deacetylated from chitin by 90%. So, we modified **formula 1b** for calculating the number of amine per chitosan as follows:

$$\text{Number of amine per chitosan} = \frac{\mathbf{0.9}\times\text{molecular weight of chitosan}\times\text{number of amine per monomer}}{\text{molecular weight of one monomer}} = \frac{0{,}9\times5000\times1}{161} = 28$$

Number of amine groups per LPEI-5 kDa, LPEI-10 kDa, and LPEI-25 kDa are 116, 232 and 581, respectively. However, all LPEIs regardless of their different degree of polymerization (DP) have the similar charge density.

Cationic polymers	Chitosan	LPEI-5 kDa	LPEI-10 kDa	LPEI-25 kDa
Molecular weight of the polymer (g/mol)	5000	5000	10000	25000
Molecular weight of monomer	161	43	43	43
Number of amine per monomer	1	1	1	1
Number of amine per polymer	28	116	232	581

Note S4. **Preparation of a polyplexes at a defined N/P charge ratio**

The volume of the polycation stock solution needed to prepare a polyplex at a defined N/P charge ratio can be calculated as follows:

$$\text{Volume (µl) of polycation} = \frac{\frac{N}{P} \times nmol\ of\ all\ phosphates \times molecular\ weight\ of\ polymer\ (\frac{g}{mol}) \times 0.001}{concentration\ of\ polycation\ stock\ solution\ \left(\frac{mg}{ml}\right) \times number\ of\ amine\ groups\ per\ polymer}$$

$$nmol\ of\ all\ phosphates = nmoles\ of\ origami \times number\ of\ phosphate\ groups\ per\ DNO$$ (**Note S1**)

As an illustration, to prepare the polyplex of NR (10 µl containing 1 nmol phosphate) with chitosan at N/P 2, 3.3 µl of chitosan (stock concentration of 0.1 mg/ml) is needed:

$$\text{Volume (µl) of chitosan (0.1 mg/ml)} = \frac{2 \times 1\ nmol \times 5000\ (\frac{g}{mol}) \times 0.001}{0.1\ \left(\frac{mg}{ml}\right) \times 28} = 3.6\ \mu l$$

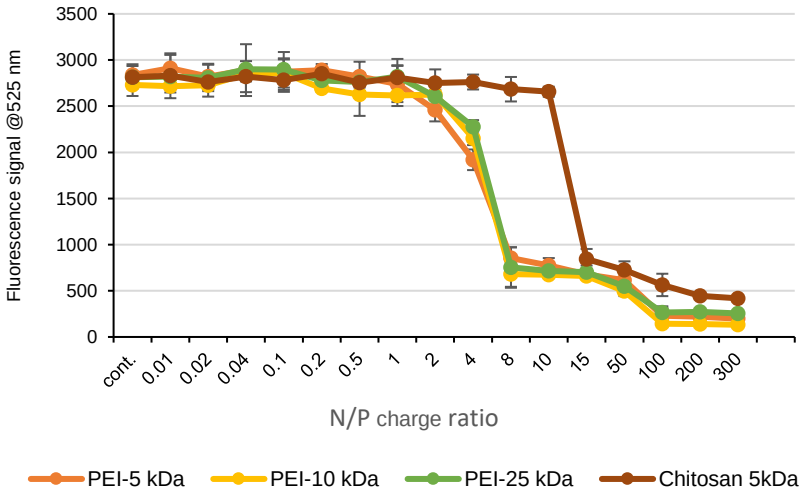
Then, 6.4 µl of TRIS buffer is added to 3.6 µl of chitosan (0.1 mg/ml) to bring up the total volume to 10 µl, followed by adding 10 µl of NR. The process of condensation is entropically driven, and polyplexes are formed spontaneously upon mixing of cationic polymers with the origami. In a similar pattern, the volume of chitosan needed to prepare polyplexes at other N/P ratios can be calculated:

Pipetting scheme for preparing the polyplex between the NR (10 µl of 1 nmol phosphate) and chitosan at different N/P ratios				
N/P ratio	**1**	**2**	**10**	**20**
Chitosan stock solution (mg/ml)	0.05	0.1	0.5	1
µl of stock solution to take	3.6	3.6	3.6	3.6
µl of TRIS buffer to take	6.4	6.4	6.4	6.4

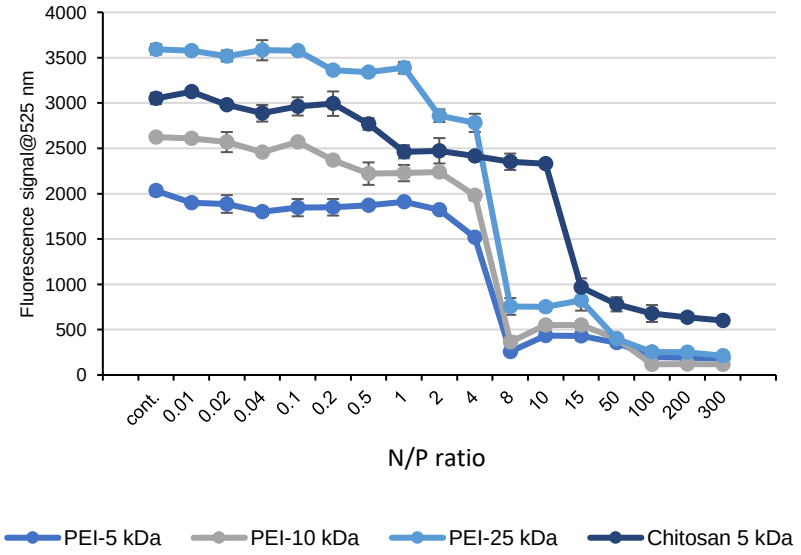
Note S5. The PicoGreen displacement assay

The charts illustrate the results of PicoGreen displacement assay for the polyplex formation between **a)** WN and **b)** NB with LPEI of different DP (5, 10 and 25 kDa) and chitosan (5 kDa). Y and X axis represent fluorescence signal at 525 nm and N/P charge ratio, respectively. Similar to the LPEI-NR polyplex, the LPEI-NB and LPEI-WN polyplexes showed the fluorescence signal reduction at N/P 8. LPEI of higher DP showed sharper signal decrement. Chitosan-origami polyplexes exhibited signal reduction at N/P ratio of 15. The data were normalized in graph **a.** Scale bars represent the standard deviations.

a) WN-polycation complex



b) NB-polycation complex



Note S6. Decomplexation of origami-LPEI/chitosan polyplexes

Polyanions such as dextran sulfate [1] and chondroitin sulfate [2] have been used for decomplexation of polyplexes. We tested dextran sulfate with two different DP (4 kDa and 40 kDa). The concentration of dextran sulfate, which is used for decomplexation is described by the A/P charge ratio. Here, A/P is the ratio between the sulfate groups of the polyanion (A) to the phosphate group of the origami (P). The volume of dextran sulfate needed to decomplex a polyplex at a defined A/P ratio can be calculated as follows:

$$\text{Volume (µl) of dextran sulfate} = \frac{\frac{A}{P} \times nmol\ of\ all\ phosphates \times molecualr\ weight\ of\ dextran\ sulfate\ (\frac{g}{mol}) \times 0.001}{concentration\ of\ dextran\ sulfate\ \left(\frac{mg}{ml}\right) \times number\ of\ sulfate\ per\ dextarn\ sulfate}$$

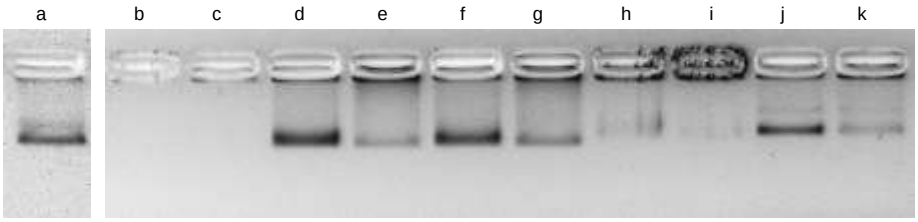
Molecular weight of dextran sulfate (g/mol)	**4000**	**40000**
Molecular weight of one monomer	366	366
Number of sulfate per monomer	2	2
Number of sulfate per polymer	22	220

As an illustration, to decomplex a polyplex (N/P 4, including 0.15 nmol phosphate) with dextran sulfate (M_w~4 kDa) at A/P 1000, 5.4 µl of dextran sulfate (5 mg/ml) is needed.

$$\text{Volume (µl) of dextran sulfate-4 kDa (5 mg/ml)} = \frac{1000 \times 0.15\ nmol \times 4000\ (\frac{g}{mol}) \times 0.001}{5\ \left(\frac{mg}{ml}\right) \times 22} = 5.4\ \mu l$$

Note S7. AGE analysis of polyplexes after decomplexation with dextran sulfate

Decapsulation of polyplexes require excess amount of dextran sulfate (DS). DS of higher molecular weight (M_W ~ 40 kDa) was more efficient for decomplexation (lanes d, f and j) compared to DS with M_W ~ 4 kDa (lanes e, g and k). Chitosan-origami polyplexes were more resilient towards the decapsulation treatment (lanes h and i) compared to LPEI-origami polyplexes. Decapsulation of chitosan-origami polyplexes could be efficiently performed by making the media slightly basic by adding NaOH (lanes j and k).

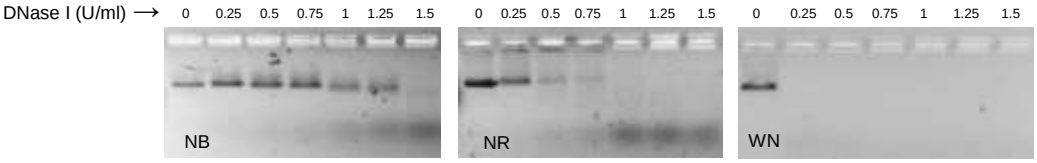


Lanes: a) reference NR; **b)** LPEI-NR polyplex; **c)** chitosan-NR polyplex; **d)** LPEI-NR polyplex+ DS (40 kDa); **e)** LPEI-NR polyplex +DS (4 kDa); **f) L**PEI-NR polyplex+ DS (40 kDa) + 30 min heating at 37 ˚C; **g)** LPEI-NR polyplex + DS (4 kDa), 30 min heating at 37 ˚C; **h)** chitosan-NR polyplex+ DS (40 kDa), 30 min heating at 37 ˚C; **i)** chitosan-NR polyplex+ DS (4 kDa), 30 min heating at 37 ˚C; **j)** chitosan-NR polyplex + DS (40 kDa) +NaOH; **k)** chitosan-NR polyplex+ DS (4 kDa)+ NaOH. In this experiment, LPEI-25 kDa has been used for preparing all LPEI-NR polyplexes. The N/P ratio of all polyplexes was set to 4. Dextran sulfate was added in A/P of 1000.

Note S8. DNase1 titration assay of naked origamis

Origamis were incubated with 0.25-1.5 U/ml DNase I at 37 ˚C for 1 and 2 h. At the end of the reaction, the samples were immersed in ice bath and the DNase I was deactivated with DTT and EGTA. Based on the AGE images, the nucleolytic degradation is design dependent. The NBs having closely packed structure showed to be the most stable origami. The NB could tolerate up to 1.25 U/ml of DNase I in 1 h, while NR hardly withstand 0.5 U/ml of DNase I. The WN showed to be the most vulnerable towards degradation by nucleases.

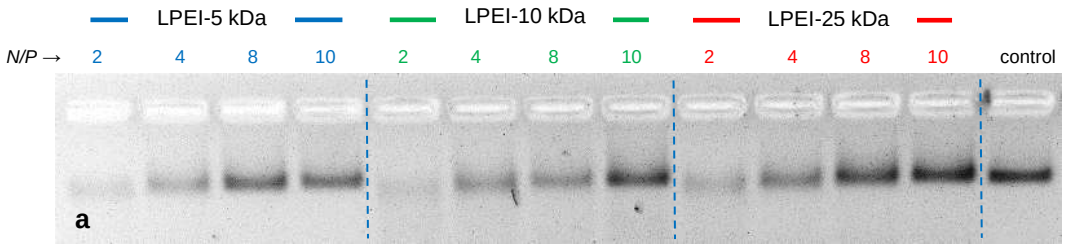
a) 1h of incubation

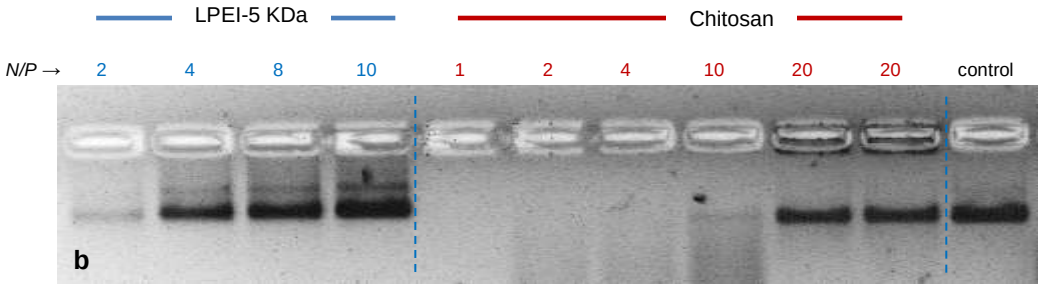


b) 2h of incubation

Note S9. Stability of polyplexes towards DNase1

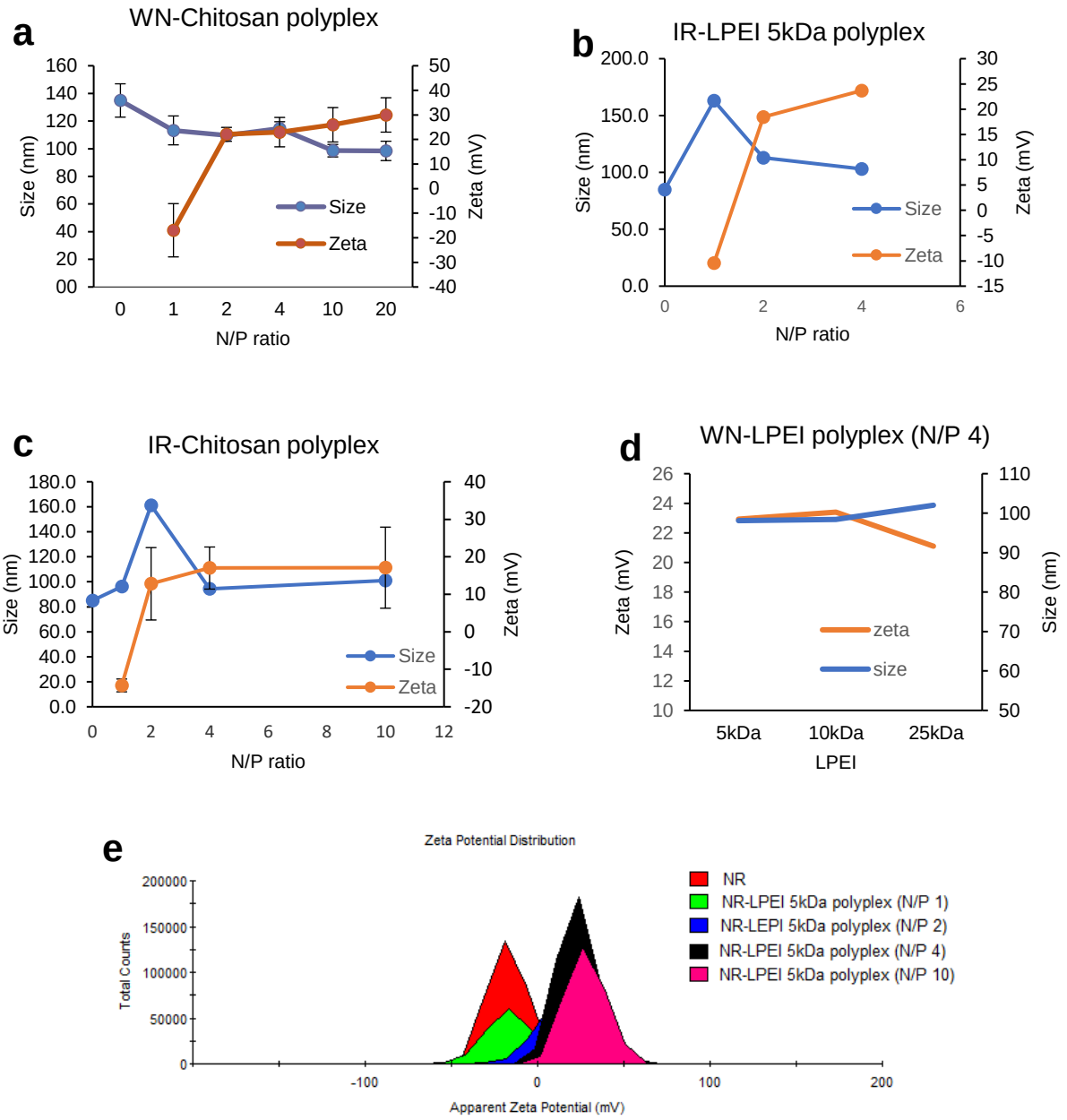
The images illustrate AGE analysis of **a)** of LPEI-NB polyplexes and **b)** LPEI-5 kDa and chitosan coated WN after one day of incubation with 10 U/ml DNase1 at 37 ˚C. At the end of each reaction, the nucleolytic activity was stopped by adding DTT and EGTA, as previously described. The polyplexes were decapsulated with dextran sulfate (M_W ~ 40 kDa) prior to loading into the gels. Based on this set of experiments, preparing polyplexes at higher N/P ratio leads to the higher protection towards DNase I. Similar trend was observed for oligolysine coated DNA nanostructures by Ponnuswamy et al. [2]. As can be seen in image b, LPEI protects DNA nanostructures more efficiently compared to chitosan. While LPEI-5 kDa could sufficiently shield encapsulated WN towards DNase I at N/P 8, comparable protection was achieved only at N/P 20 with chitosan.





Note S10. DLS measurements

The Z-average hydrodynamic diameter and the surface charge of DNA origamis and the polyplexes were determined by dynamic light scattering (DLS) using Zeta-sizer Nano ZS (Malvern Instruments Ltd., UK) at 25°C. All samples were measured in triplicate. All measurements performed in Mill-Q water using freshly-made sample. For naked origami, the measurements were also performed in Milli-Q water containing 5 mM $MgCl_2$, which showed the same DLS results as when using Milli-Q water without salt supplementation.



Graph a-e) the intensity-based hydrodynamic size (nm) and zeta potential (mV) of WN-chitosan polyplex (**graph a**), IR-LPEI 5kDa polyplex (**graph b**), and IR-LPEI 5kDa polyplex (**graph c**) at different N/P ratios; **graph d)** shows the size and zeta potential data of WN coated with LPEI of different DP at N/P 4.; **graph e)** illustrates the increase in zeta potential of NR upon coating with LPEI 5kDa.

Note S11. Addressability of aptamer-functionalized origami after coating with polycations at variant N/P ratio.

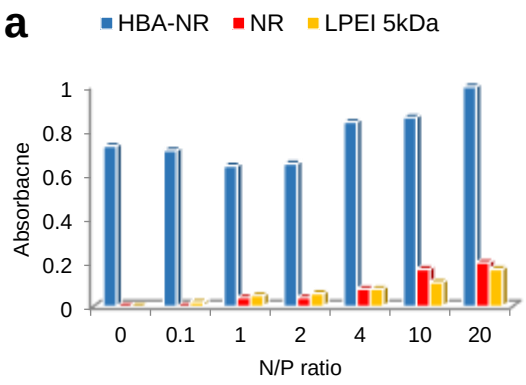
Hemin-binding aptamer (HBA) is a G-rich single stranded DNA sequence typically consists of four triplets of G separated by short A, T or C spacer. Many G-rich sequences have been already developed through SELEX technology (Systematic Evolution of Ligands by Exponential Enrichment), with low to very high catalytic activity towards Hemin. We selected PS2.M (5'-GTGGGTAGGGCGGGTGG-3'), which has a high catalytic activity (K_d: 439 nM). [3]

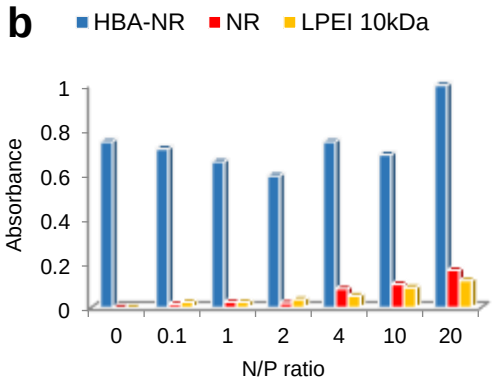
Up to 24 staple strands have been protruded from the NR surface with a 5-nm length linker (5'-AAAAGAAAAGAAAAA-3') followed by the PS2.M sequence. The self-assembly of HBA-functionalized nanorod (HBA-NR) has been performed similarly as discussed in "materials and methods" section in the main paper, with the slight difference that PS2.M extended staple strands added in 20:1 ratio compared to the scaffold. As HBA-NR has a high tendency for aggregation, the self-assembly was performed in folding buffer including 15 mM $MgCl_2$. Additionally, after PEG purification, the precipitated sample was resuspended and kept in buffer supplemented with 6 mM $MgCl_2$.

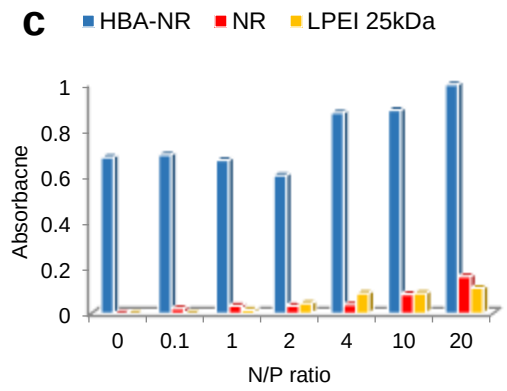
For aptamer activity measurements, 10 µl DNA nanostructures (40 nM) were mixed with 10 µl polycations of varying concentration to get polyplex of defined N/P ratio. The resulted polyplex (20 µl) was then mixed with 60 µl HEPES buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, 1% DMSO, pH 7.4). Afterwards, 5 µl hemin (0,04 mM) followed by 10 µl of ATBS (15 mM) was added. The reaction was initiated by adding 5 µl H_2O_2 (12 mM). Absorption at 421 nm was measured as a function of time (up to 1 h) with the EnSpire multimode plate reader (Perkin Elmer). As control, the absorbance of NR (and its polyplexes) and the polycations was also examined.

The result analysis showed that coating HBA-NR by polycations has no blocking effect on the aptamer activity. Interestingly, the activity of HBA-NR has been increased slightly after coating with polycations and upon increasing N/P ratio.

Note S11. Continued…







Graph a-c) absorbance of HBA-NR and NR before and after coating with LPEI 5kDa (**graph a**), LPEI 10 kDa (**graph b**) and LPEI 25 kDa (**graph c**) at different N/P ratios. The absorbance of polycations at different concentrations (corresponding to the defined N/P ratio used for polyplex formation) was also examined as control. X and Y axis represent N/P ratio and normalized absorbance (6 min after initiation of reaction), respectively.

Note S12. Addressability of Enzyme-functionalized origami after coating with polycations at variant N/P ratios

Three biotinylated staple strands were protruded from NR surface to be then loaded with HRP-conjugated streptavidin. The biotinylated NRs (biotin-NRs) were self-assembled as discussed in "materials and methods" section in the main paper, with the slight difference that biotinylated staple strands were added in 20:1 ratio compared to the scaffold. The purified Biotin-NR was then incubated with excess amount of HRP-conjugated streptavidin (15 molecules of enzyme per origami). For this aim, purified Biotin-NR (200 µl, 36 nM) was incubated with HRP-conjugated-streptavidin (200 µl, 540 nM) at r.t for 12 h. The folding buffer (Tris 5 mM, EDTA, 5 mM NaCl) containing 14 mM $MgCl_2$ was used as the reaction media. Finally, the sample was purified using PEG purification method to remove the unbound HRP enzymes. The precipitated HRP-loaded origami (HRP-NR) was then resuspended in folding buffer including 16 mM $MgCl_2$. As enzyme might bind non-specifically to the origami, the non-biotinylated NR was also treated with enzyme, PEG purified and further included as the control in the measurements. The concentration of HRP-NR or NR was assessed by Nanodrop at 260 nm.

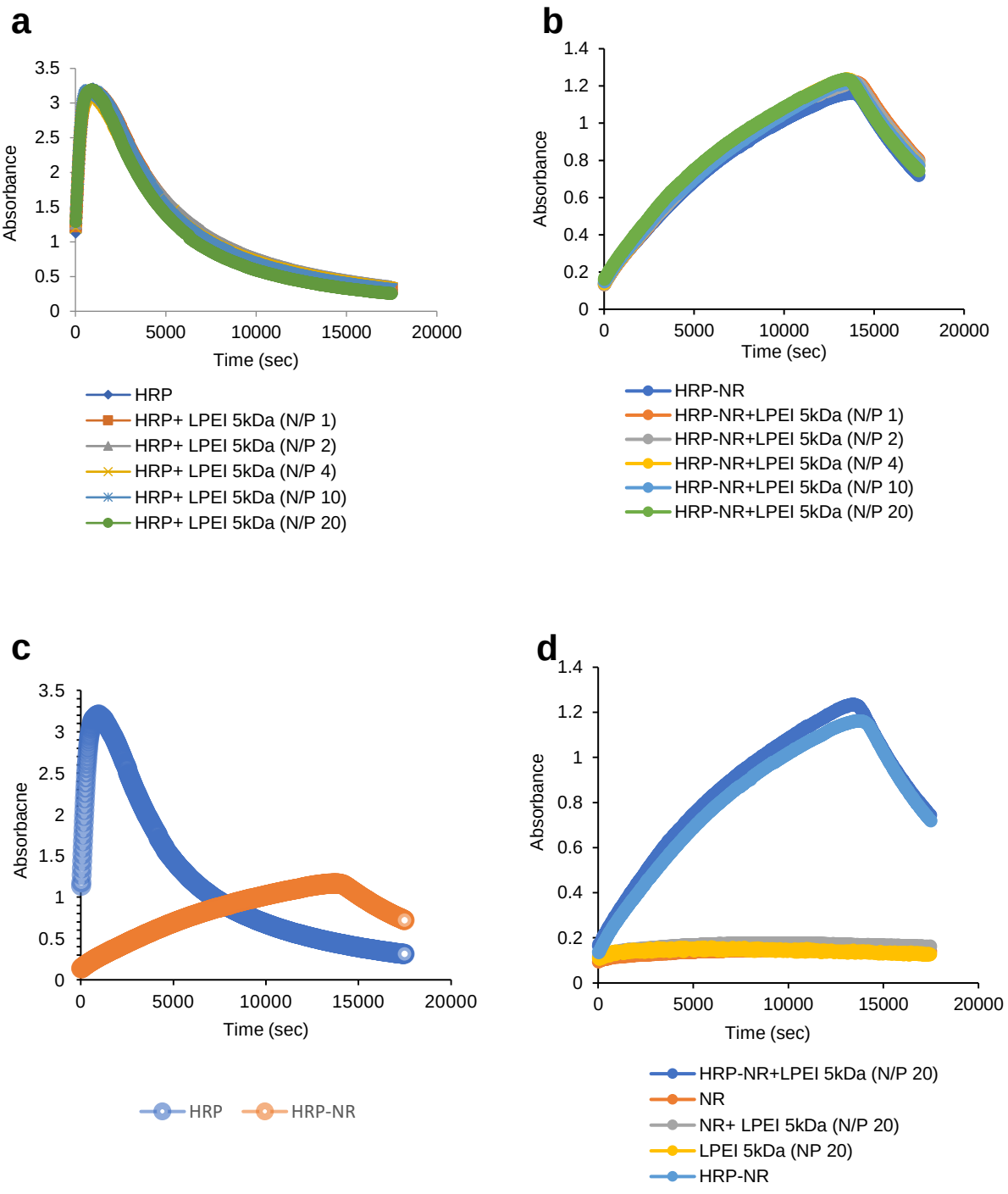
For enzymatic activity measurements, 6.5 µl DNA nanostructures (36 nM) were mixed with 6.5 µl of polycations to reach defined N/P ratio of 1, 2, 4, 10 and 20. The final 12.5 µl of polyplex was then mixed with 72,5 µl of HEPES buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, 1% DMSO, pH 7.4). Afterwards, 10 µl of ATBS (15 mM) was added. The reaction was initiated by adding 5 µl of H_2O_2 (12 mM). Absorption at 421 nm was measured as a function of time (up to 5 h) with the EnSpire multimode plate reader (Perkin Elmer). As control, polycations at different concentration (corresponding to the defined N/P ratio used for polyplex formation) were also tested. Additionally, the enzymatic activity of the HRP enzyme in the absence and presence of polycations was examined.

Our results showed that polycations have no remarkable hindrance or reduction effect on the catalytic activity of HRP (**graph a**) or HRP-functionalized origami (**graph b**). However, the kinetic of HRP enzyme has been dramatically changed after binding to the DNA origami (**graph a-c**). As an illustration, free HRP enzyme reached its peak absorbance value (3.3), 15.5 min after initiating the catalytic reaction. However, HRP-NR reach its absorbance peak (1,2) only 3.7 h after initiating the catalytic reaction. Changing the kinetics of enzyme upon attachment to the nanoscale structures has been already reported elsewhere.[4]

Our experiments additionally prove that the non-specific binding between enzymes and origami structures is insignificant, since the non-biotinylated NR showed negligible catalytic activity (**graph d**). Hence, PEG purification is a straightforward and efficient method for purification of enzyme-functionalized origami and removing non-bound enzymes. [5]

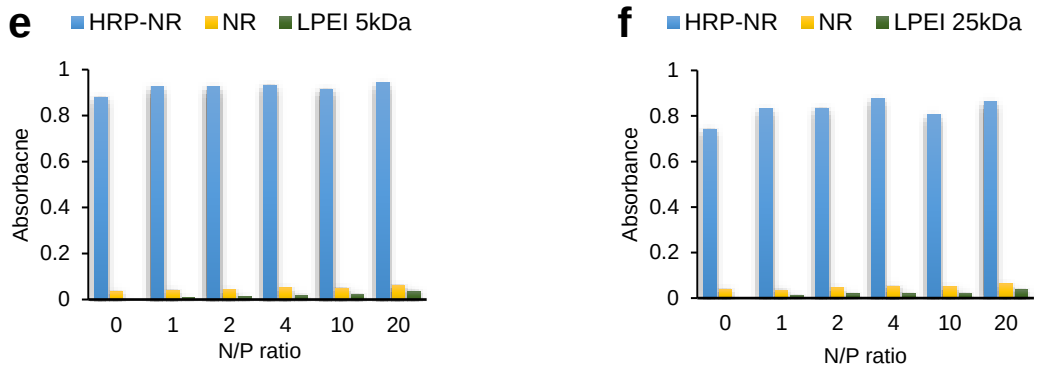
In the current represented data, LPEI-5kDa was used. Similar trend was observed for LPEI-25 kDa and chitosan.

Note S12. Continued…



Graph a) the colorimetric assay of HRP in the absence or presence of LPEI 5kDa (at different polycation concentration corresponding to N/P ratio of 1,2,4,10 and 20) as a function of time; **b)** the colorimetric assay of HRP-functionalized origami (HRP-NR) in the absence or presence of LPEI 5kDa (at different N/P ratios) as a function of time; **c)** the colorimetric assay of HRP and HRP-NR was monitored over time; **d)** the colorimetric assay of HRP-NR and NR (non-biotinylated NR which treated with HRP) in the absence or presence of LPEI-5kDa (at N/P 20) monitored over time. Y and X axis represent absorbance at 421 nm and times (sec), respectively.

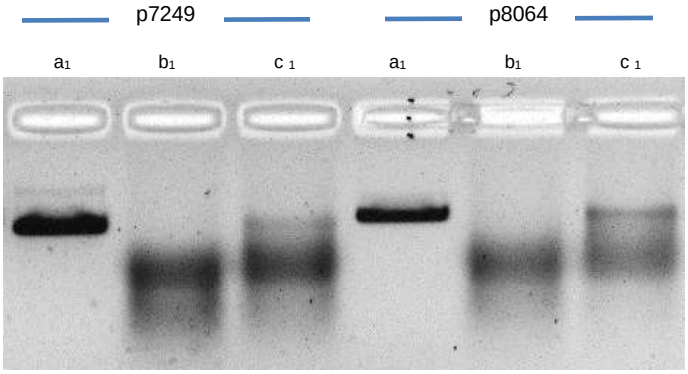
Note S12. Continued…



Graph e and f) the absorbance (@421 nm) of HRP-NR and NR (non-biotinylated NR treated with HRP) in the absence or presence of LPEI 5kDa (**graph e**) or LPEI 25kDa (**graph f**) at different N/P ratios, 3.7 h after initiation of the reaction by H_2O_2. As control, polycations at different concentration (corresponding to the defined N/P ratio used for polyplex formation) were also tested.

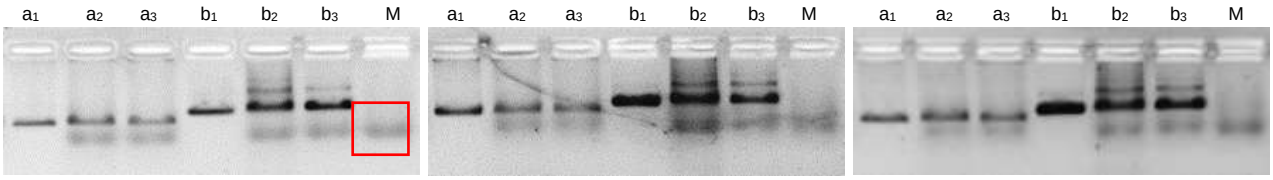
Note S13. Stability of p7249 and p8064 single stranded scaffolds in cell culture media

The image illustrates the AGE analysis of p7249 and p8064 scaffolds after 1 h of incubation in DMEM+10% FBS at 37 ˚C. a_1) the scaffold in 16 mM $MgCl_2$ supplemented TRIS buffer (control); b_1) the scaffold in DMEM+10%FBS; c_1) the scaffold in DMEM+10%FBS supplemented with 16 mM $MgCl_2$.

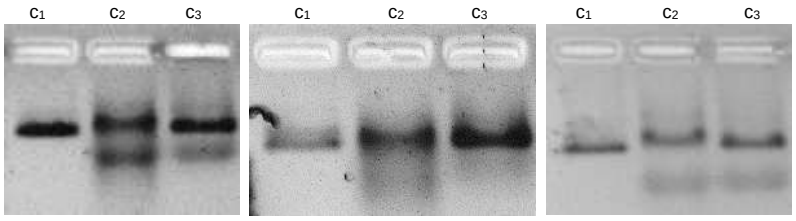


Note S14. Satbility of of naked origamis in cell culture media

The images illustrate the AGE analysis of naked origamis after incubation in cell culture media at 37 ˚C. The stability of origamis were monitored over time. a , b and c represent NR, WN and NB, respectively. Lanes a_1, b_1, c_1) the control origami; a_2, b_2, c_2) the ORIGAMI in DMEM+10%FBS; a_3, b_3, c_3) the ORIGAMI in DMEM+10%FBS supplemented with 16 mM $MgCl_2$. The lane M is the media alone. The band circled in red represnts FBS proteins.



1-day 3-days 1-week



1-day 3-days 1-week

Note S15. Stability of naked origamis in RPMI+10%FBS and TB+10%FBS.

The images show the AGE analysis of naked origamis after incubation in RPMI or TRIS buffer (TB) both supplemented with 10%FBS. The origamis were incubated for 12 h (image a) or 24 h (image b) at 37 ˚C. RPMI+ or TB+ samples were supplemented with 16 mM $MgCl_2$. As can be seen, origamis are degraded in TB+10%FBS but not in RPMI+10%FBS. Supplementing the TB with 16 mM $MgCl_2$ did not delay the degradation by FBS. The WN is degraded faster than the NB and the NR. The most stable DNA origami is the NB, which has a closely packed structure.

Image a) origamis after 12 h of incubation in FBS (10%) supplemented RPMI/RPMI+ or TB/TB+

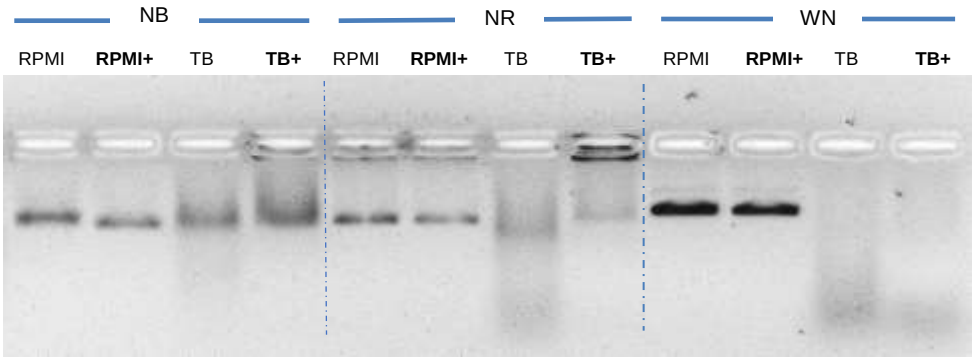
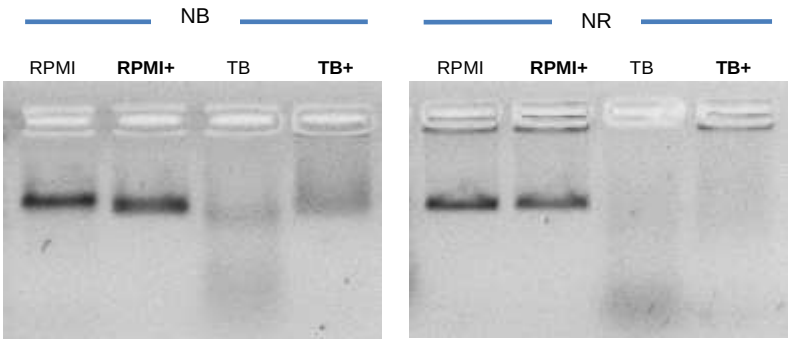


Image b) origamis after 24 h of incubation in FBS (10%) supplemented RPMI/RPMI+ or TB/TB+



Note S16. Stability of naked origamis in DMEM+10%FBS and TB+10%FBS.

The images illustrate the AGE analysis of naked origamis after incubation in DMEM or TB both supplemented with 10%FBS. The origamis were incubated for 12 h (image a) or 24 h (image b) at 37 ˚C. Origamis are degraded in TB+10%FBS but not in DMEM+10%FBS, confirming the positive effect of basal media on the protection of DNA nanostructures. Here again, corresponding to experiments in Note S8 and S12, the design dependency of the nucleolytic degradation was observed. As nsTEM analysis of cell culture treated origamis showed no recognizable structural difference compared to their control origami, the slower electrophoretic mobility of origamis after incubation in cell culture media can be attributed to the attachment of FBS proteins (Figure S10).

Image a) origamis after 12 h of incubation in FBS (10%) supplemented DMEM or TB

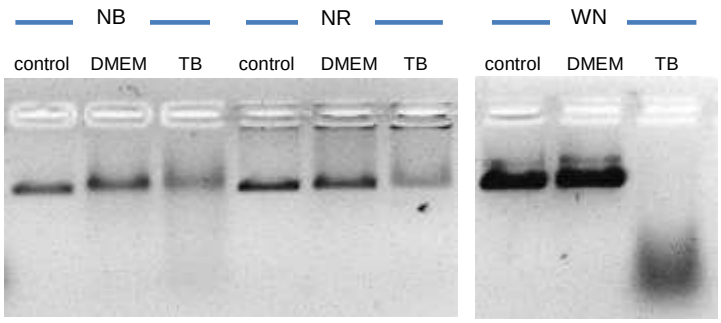
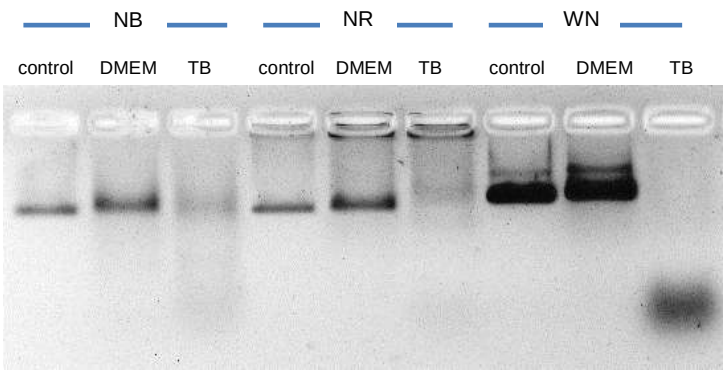
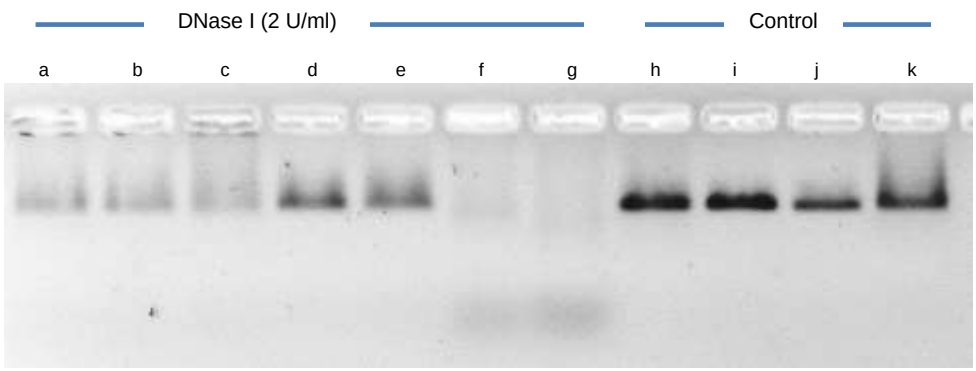


Image b) origamis after 24 h of incubation in FBS (10%) supplemented DMEM or TB



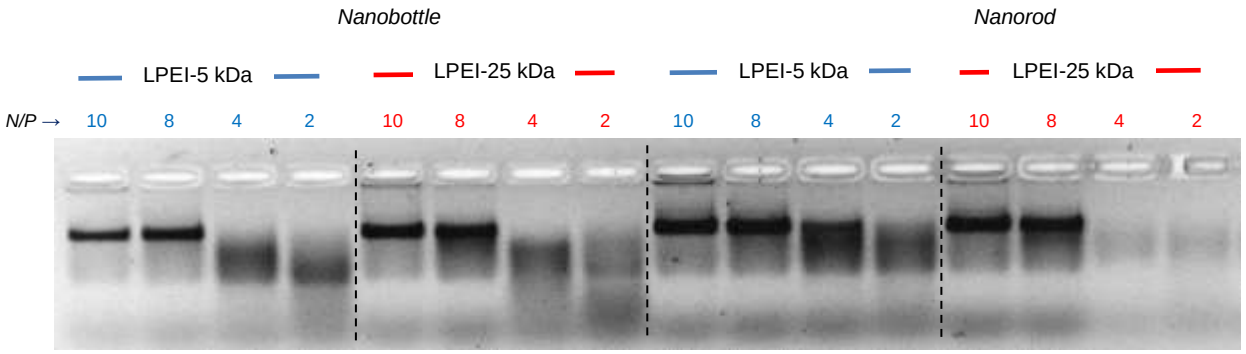
Note S17. Nucleolytic degradation of the NB by 2 U/ml DNase1 in the presence of different media

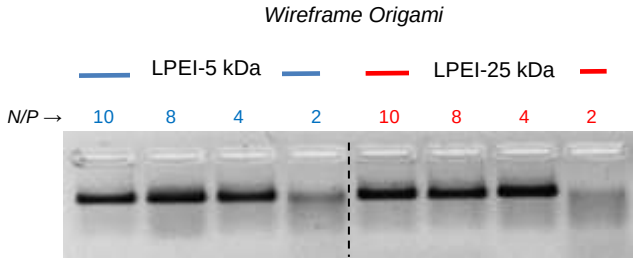
The image shows the AGE analysis of nucleolytic degradation of the NB by 2 U/ml DNase I in the presence of different media after 2 h of incubation at 37 ˚C. All reactions were supplemented with 16 mM $MgCl_2$. Lanes **a)** DMEM+10%FBS; **b)** RPMI+10%FBS; **c)** EBM-2+10%FBS; **d)** DMEM; **e)** EBM-2; **f)** TB; **g)** TB+10%FBS. No DNase I was added to **h)** DMEM+10%FBS; **i)** DMEM; **j)** TB and **k)** TB+10%FBS. The samples were loaded into the gel pockets with no prior treatment. AGE analysis clearly shows that growth media such as DMEM, RPMI and EBM-2 protect the NB against extra nucleases. Lanes d and e have higher intensity due to the lack of FBS and extra serum nucleases in media.



Note S18. Stability of LPEI polyplexes towards 10% FBS.

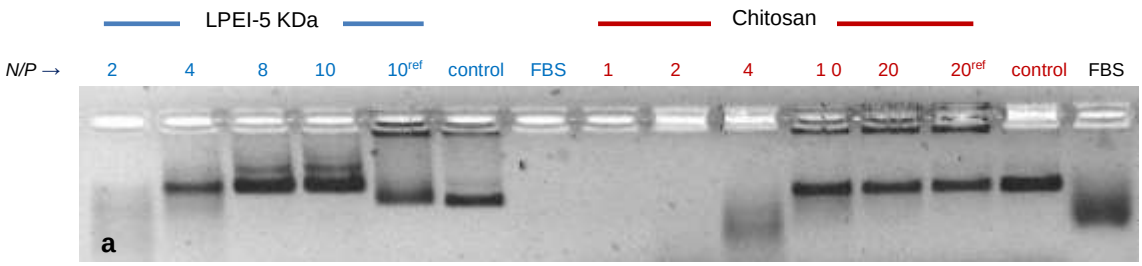
The images illustrate the AGE analysis of LPEI-origami polyplex after incubation in TB+10%FBS for one day at 37 ˚C (no Mg added). The polyplexes were decapsulated with dextran sulfate (M_W ~ 40 kDa) prior to loading into the gels.



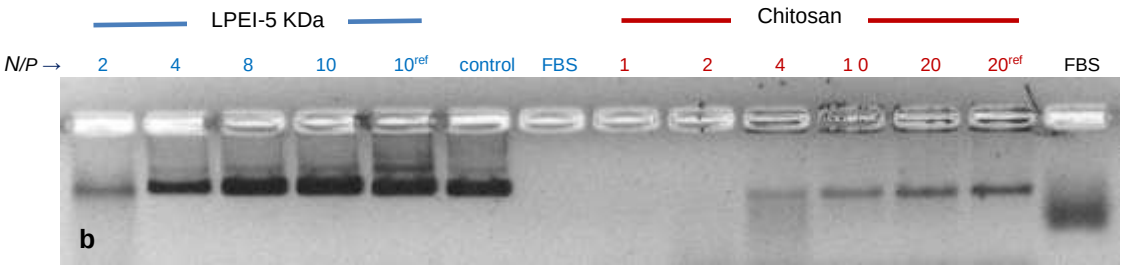


Note S19. Stability of LPEI-5 kDa and chitosan polyplexes towards 10%FBS

The images illustrate the AGE analysis of LPEI-5 kDa or chitosan polyplexes after incubation in TB+10%FBS for one day at 37 ˚C ($MgCl_2$ free). The samples were treated with proteinase K followed by decomplexation by dextran sulfate.



Polyplex of NR



Polyplex of WN

The last lane in images a and b represent reference FBS, which has not been treated with proteinase K. Lanes 5 (denoted as 10^{ref}) and 13 (denoted as 20^{ref}) are control polyplexes with no FBS exposure. Lanes 6 (denoted as control) represent the control NR (image a) and WN (image b), which kept at TB supplemented with 16 mM $MgCl_2$.

Supporting Figures

Figure S1. AGE analysis of self-assembled DNA nanostructures before and after PEG purification. "U" stands for unpurified self-assembly reaction mixtures and "P" stands for PEG purified samples.

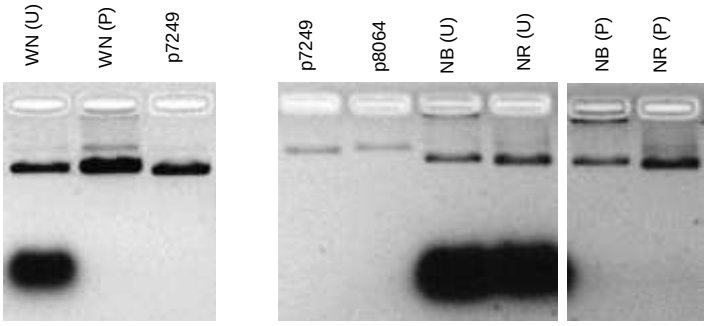
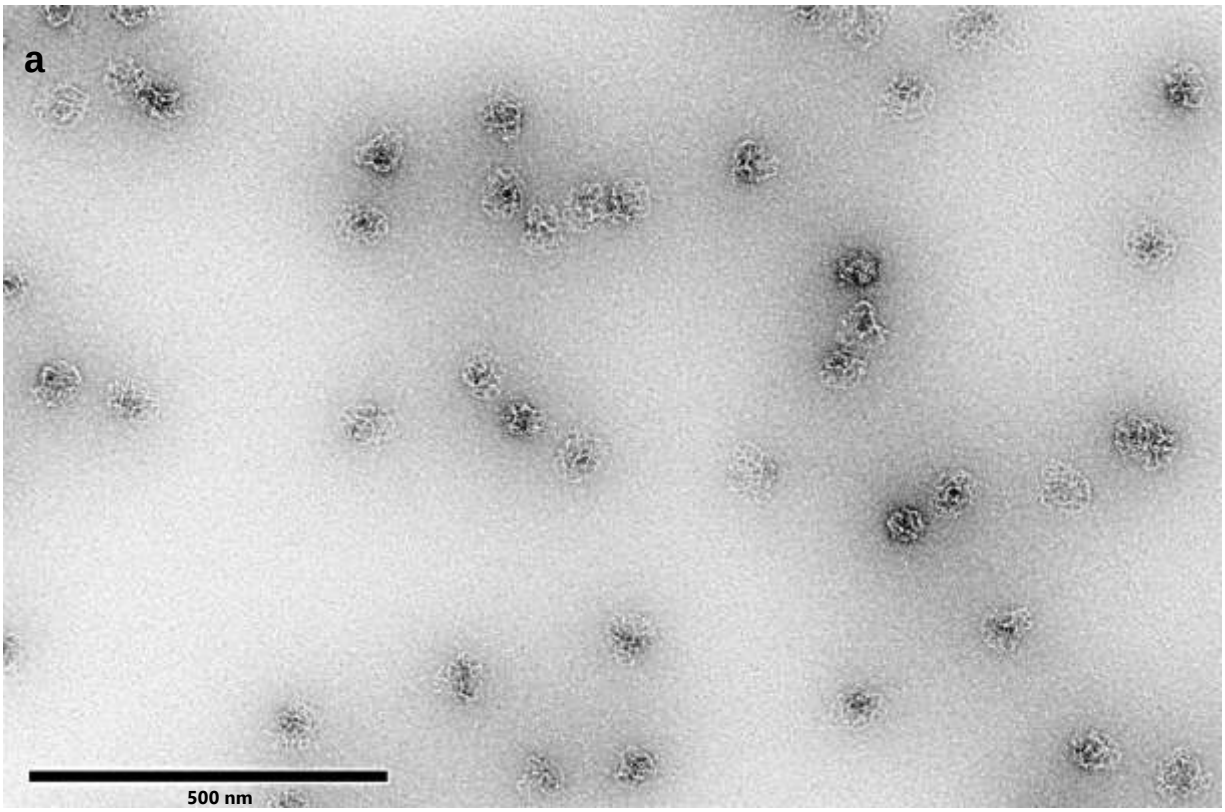
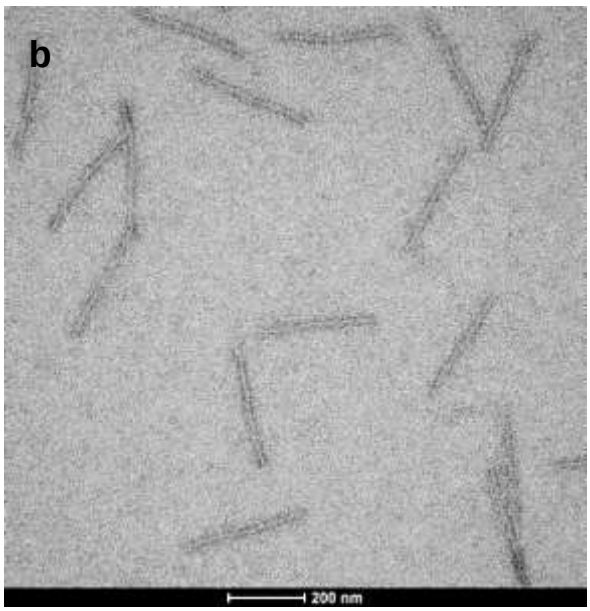


Figure S2. Negative stain TEM micrographs of purified nanostructures; **a)** WNs, **b)** NRs and **c)** NBs.





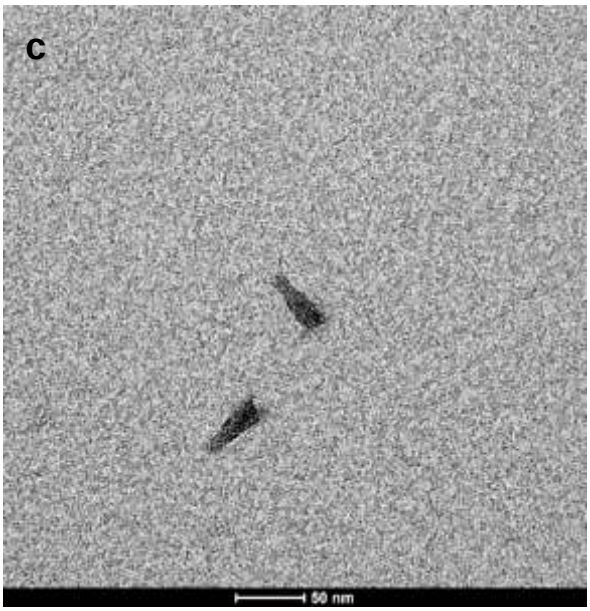


Figure S3. Gel retardation assay for the NR complexed with LPEI-10 kDa at N/P ratios of 0.01-20. Each lane contains 1 nmol NR. The first lane is the reference NR.

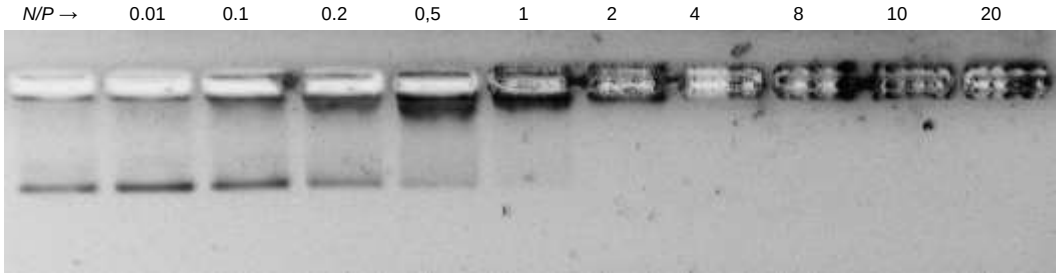


Figure S4. Negative stain TEM micrographs of LPEI.5 kDa-WN polyplex (N/P 4) after decomplexation with dextran sulfate. In addition to the core DNA origami (image a), spherical structures were also observed (image b), which can be attributed to the incomplete decapsulation process. The WN has a very open configuration, so the polycation can be trapped within the structure, being hardly accessible to polyanions. The scale bars in the images a and b are 200 nm and 500 nm, respectively.

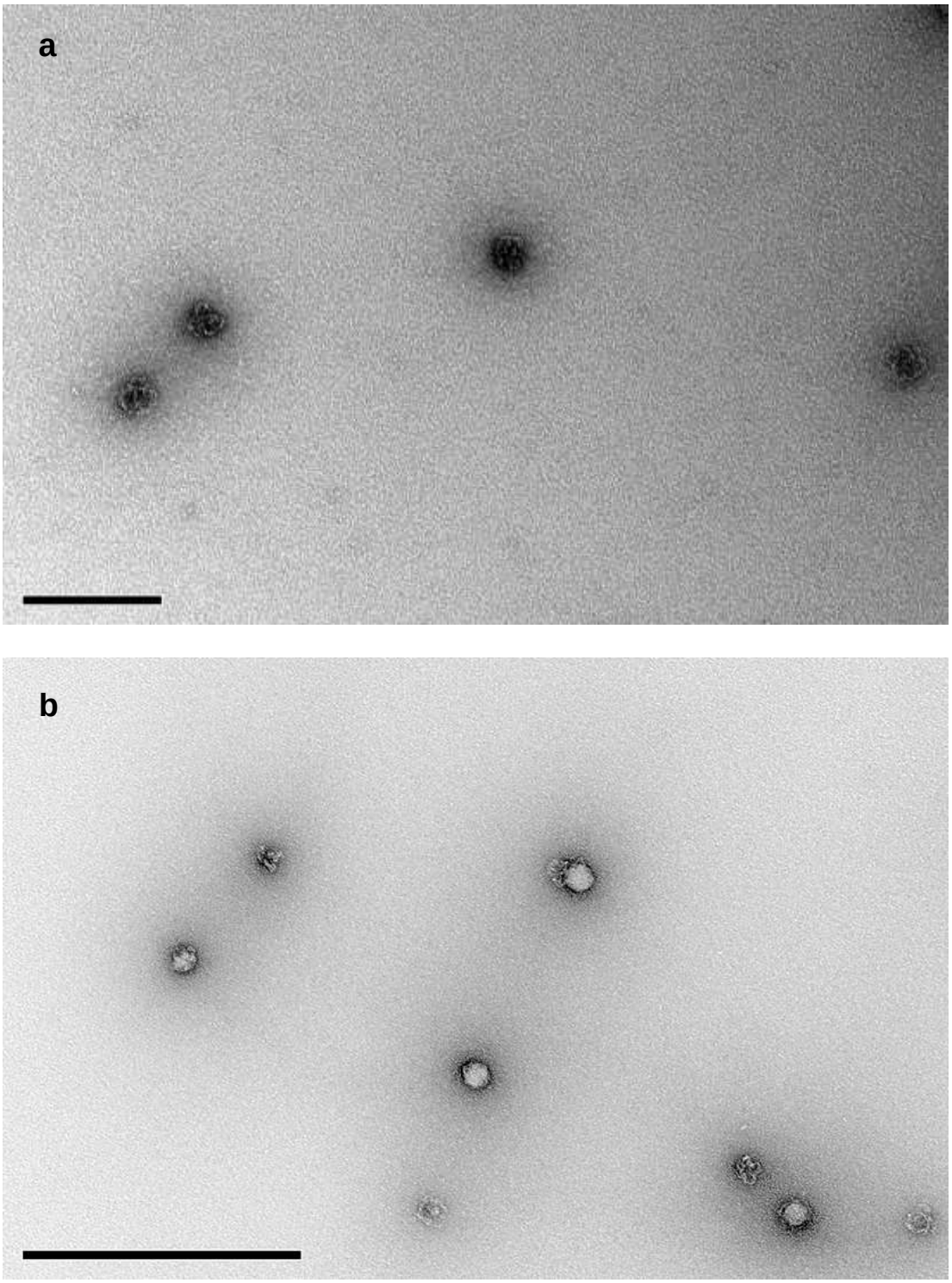


Figure S5. Negative stain TEM micrographs of **a)** NBs and **b)** NRs coated with LPEI-25 kDa at N/P 8 after decapsulation with dextran sulfate. Scale bars are 500 nm.

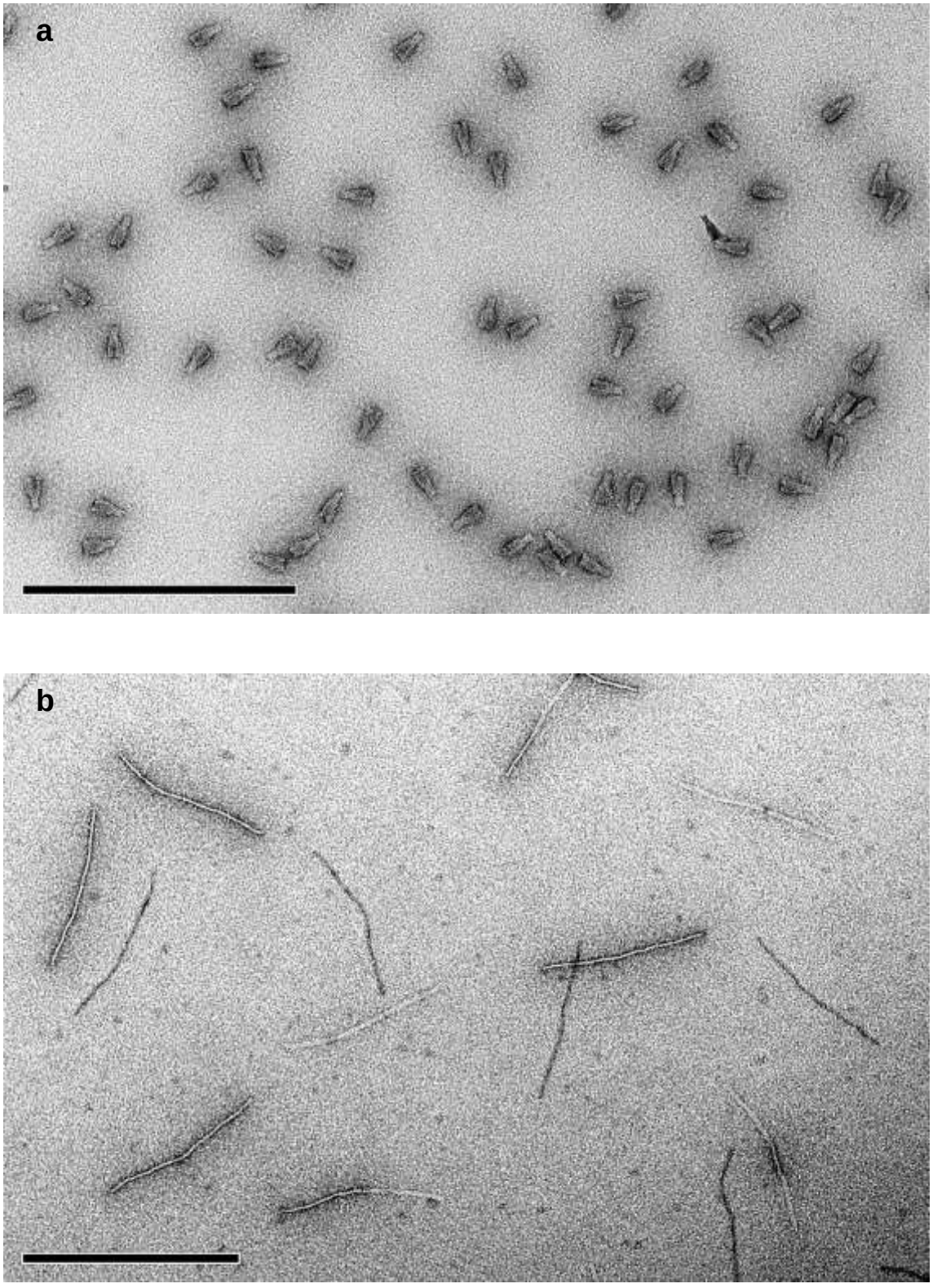


Figure S6. Negative stain TEM micrographs of NRs coated with chitosan at N/P ratio of 10. In addition to single NRs (image a), high degree of aggregation and NR-ribbon formation were also observed (image b). Scale bars are 500 nm.

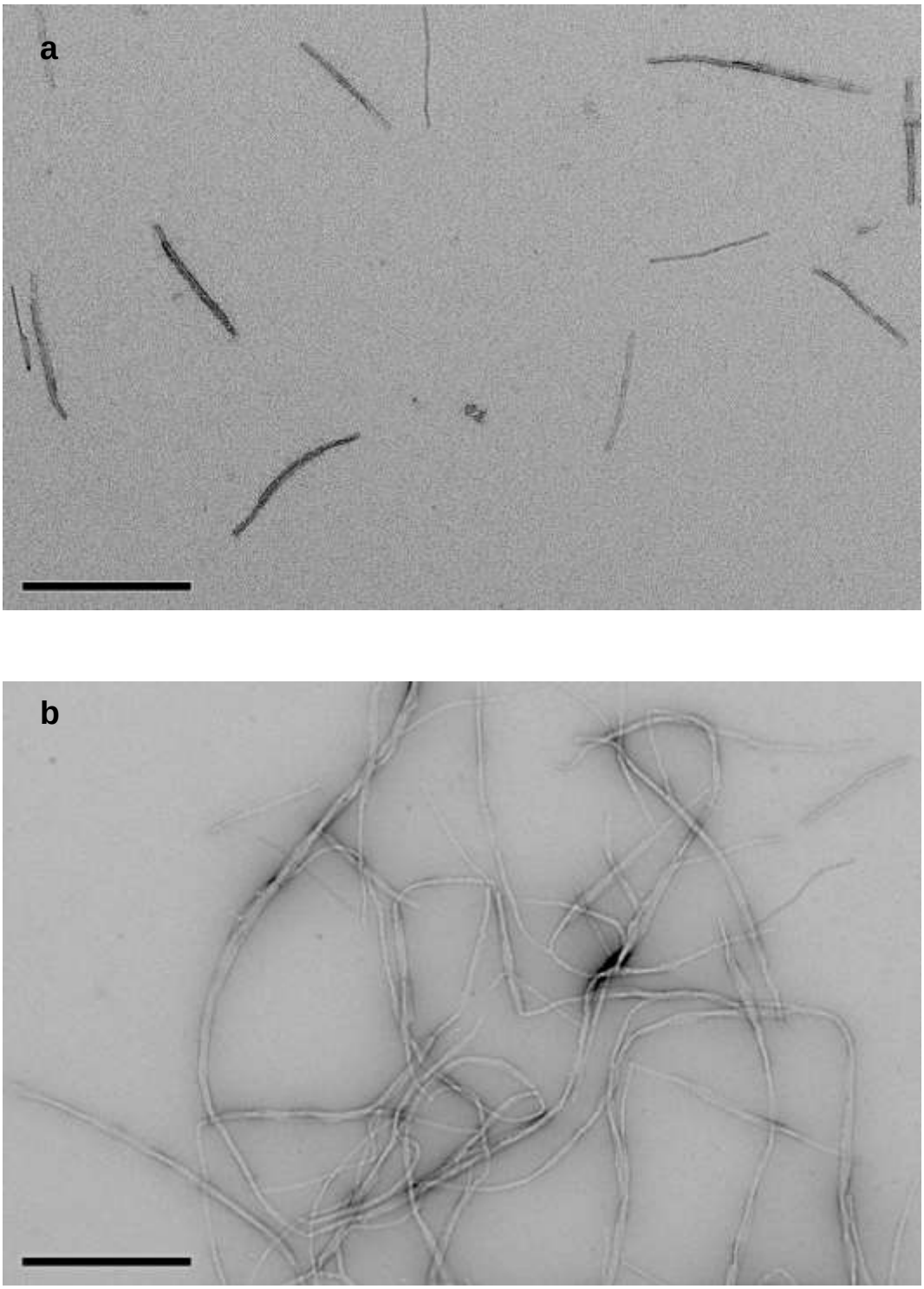


Figure S7. Negative stain TEM image of NBs coated with chitosan at N/P ratio of 10. Scale bar is 500 nm.

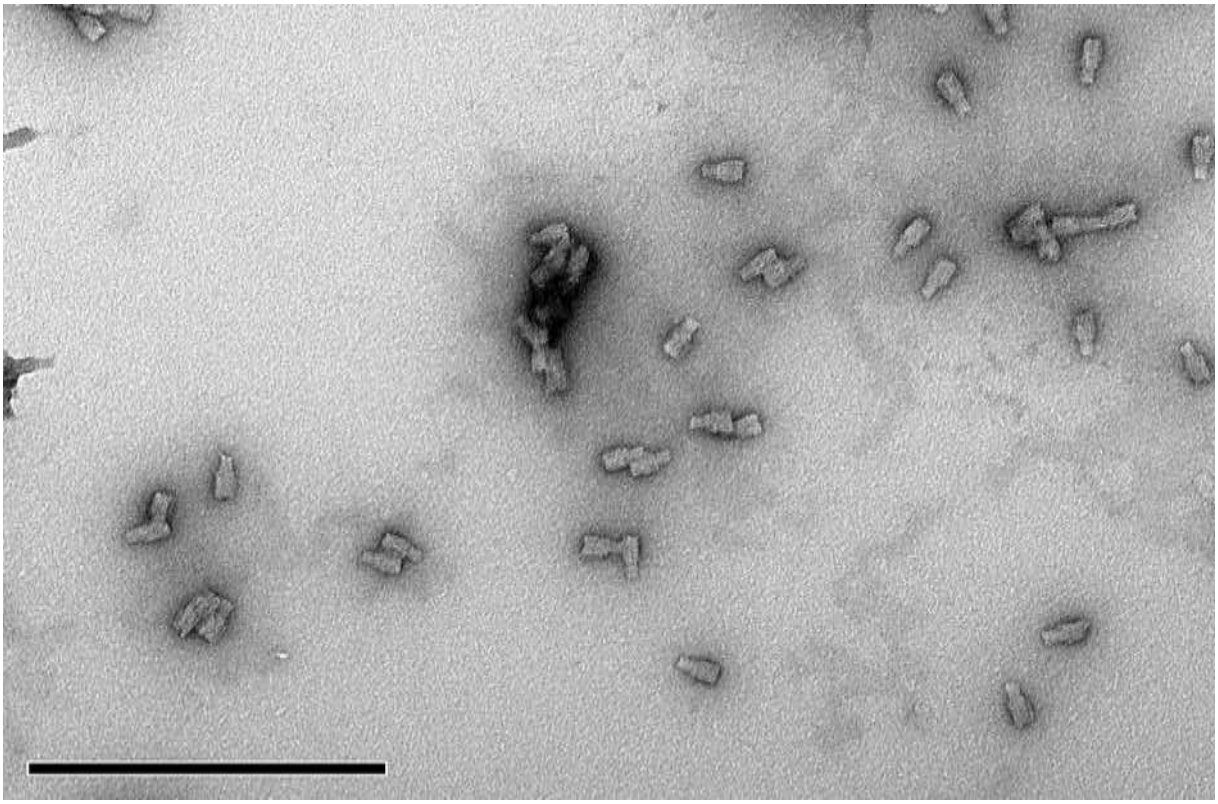


Figure S8. Negative stain TEM micrographs of the WN coated with chitosan at N/P ratio of 10 (images **a-f**). Images **g-i** are the same batch of the polyplex after decapsulation by iterative treatment with dextran sulfate and ultra-centrifugation by Amicon ultrafiltration column (MWCO~ 100 kDa). Scale bars are 50 nm.

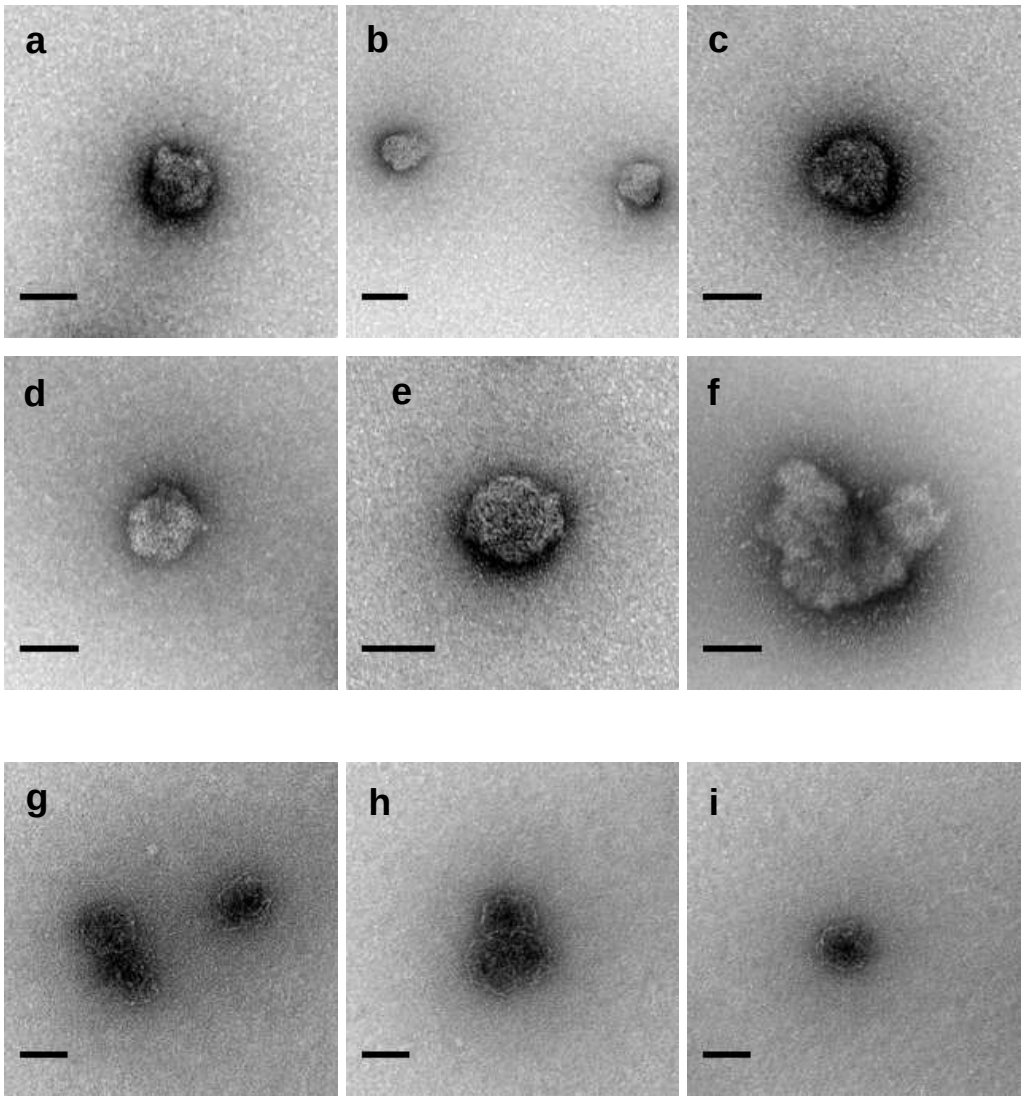


Figure S9. Negative stain TEM image of NBs (gel extracted sample) incubated for one day at 37 ˚C in DMEM+10%FBS. Scale bar is 500 nm.

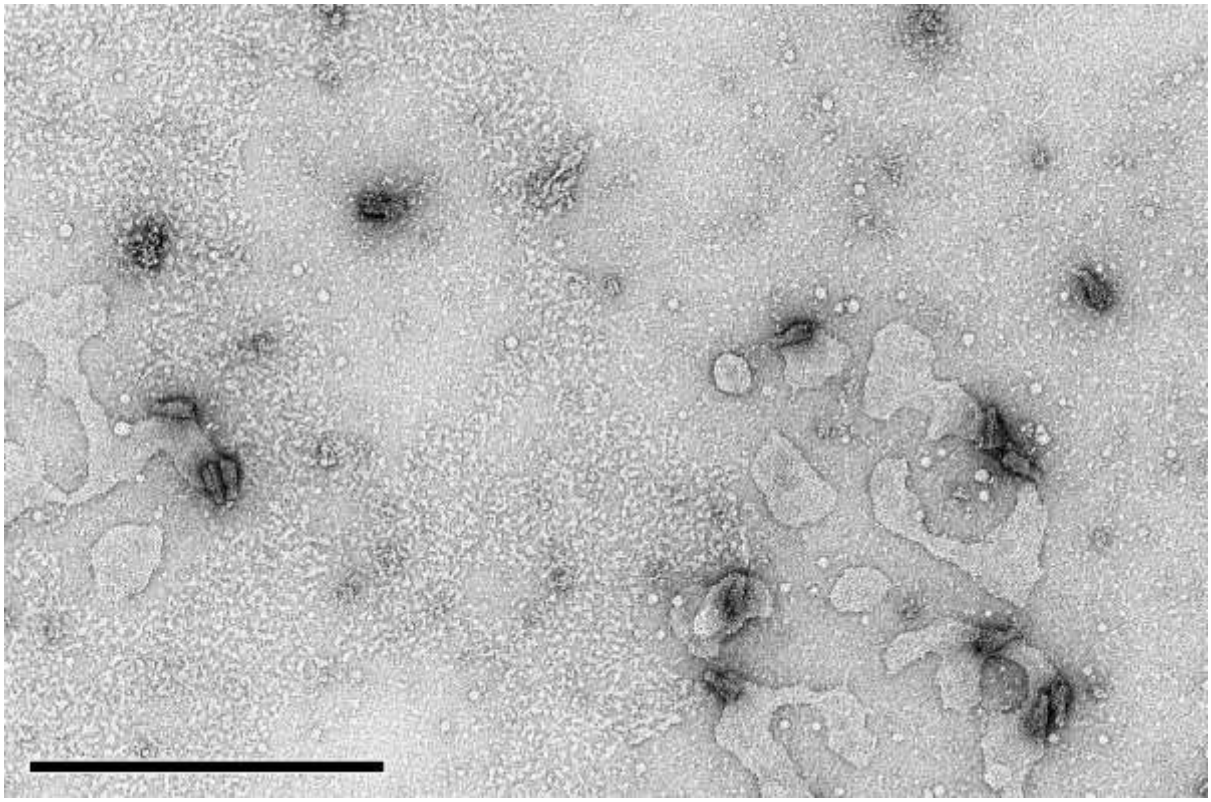


Figure S10. Negative stain TEM micrograph of NRs (gel extracted sample) after one day of incubation in DMEM+10%FBS at 37 ˚C. The NRs together with serum proteins can be seen. Scale bar is 500 nm.

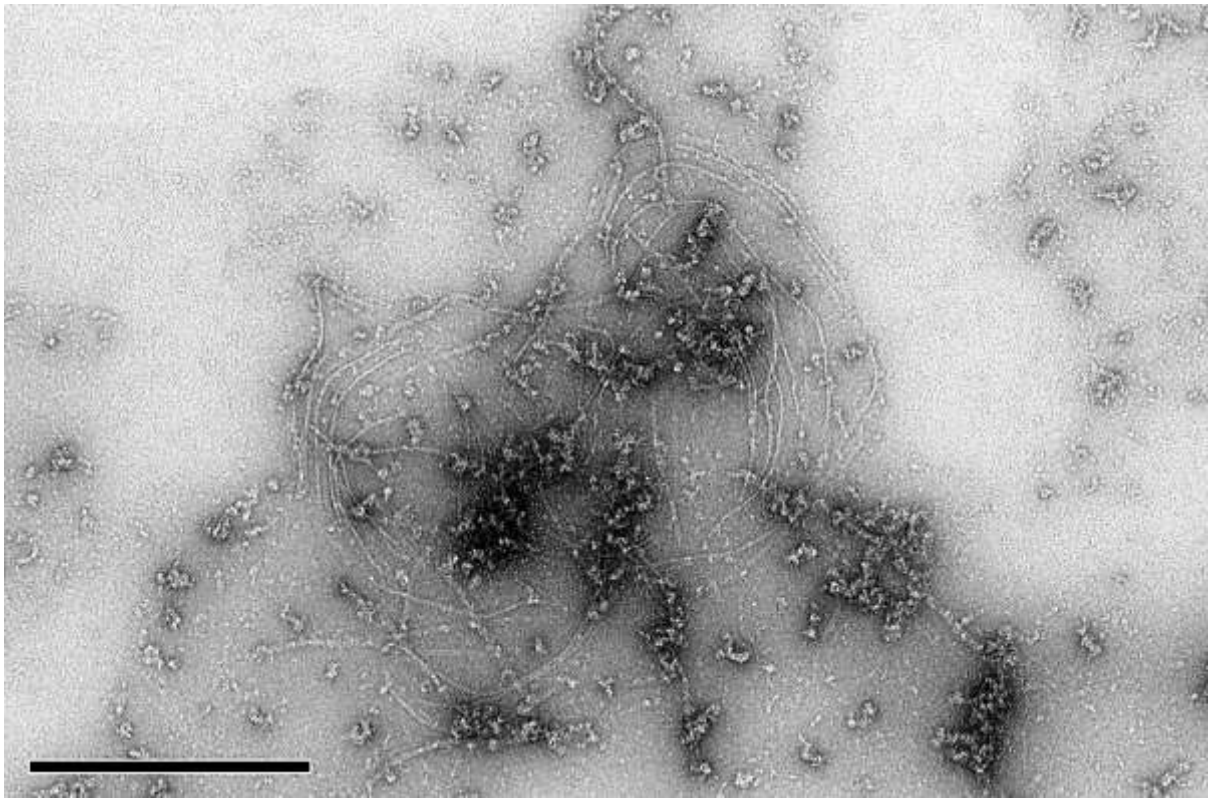


Figure S11. Negative stain TEM micrograph of WNs (gel extracted sample) after one day incubation in DMEM+10% FBS at 37 ˚C. Scale bar is 500 nm.

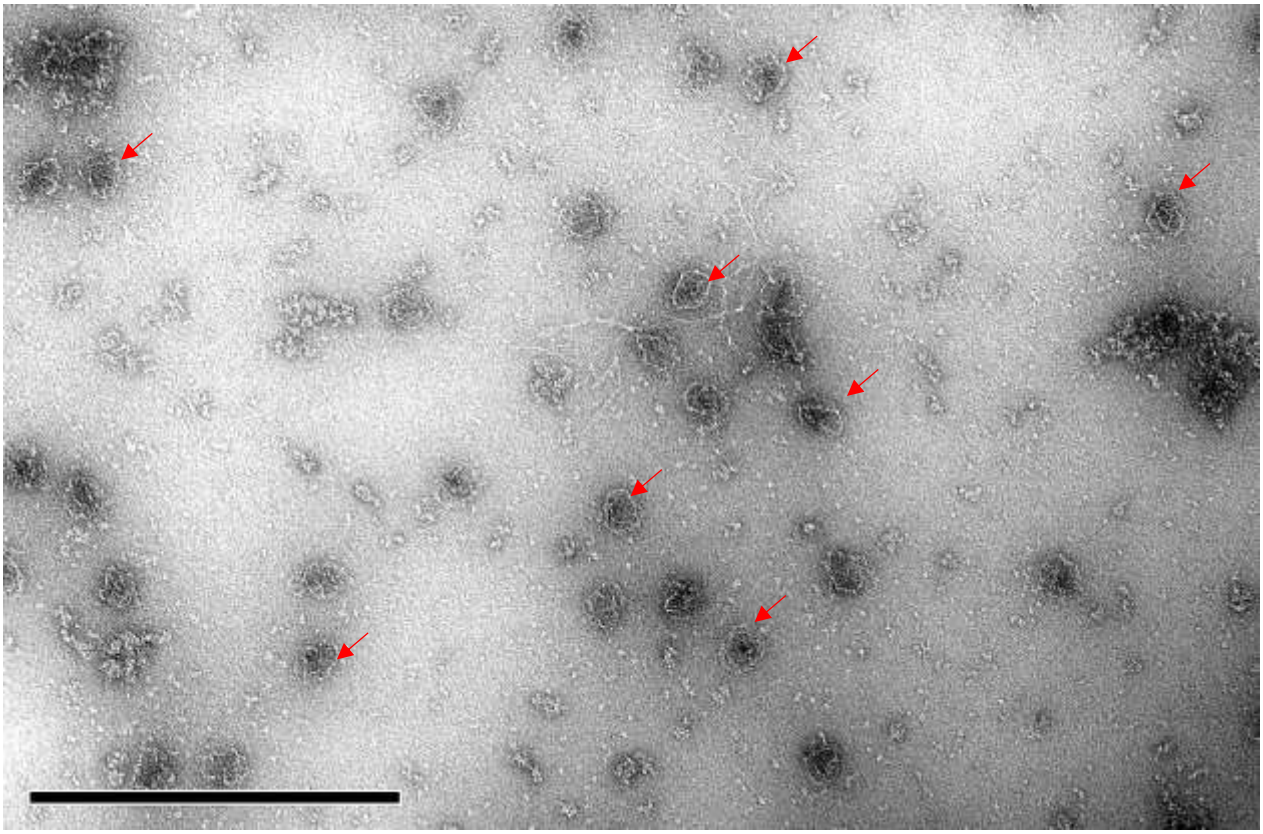
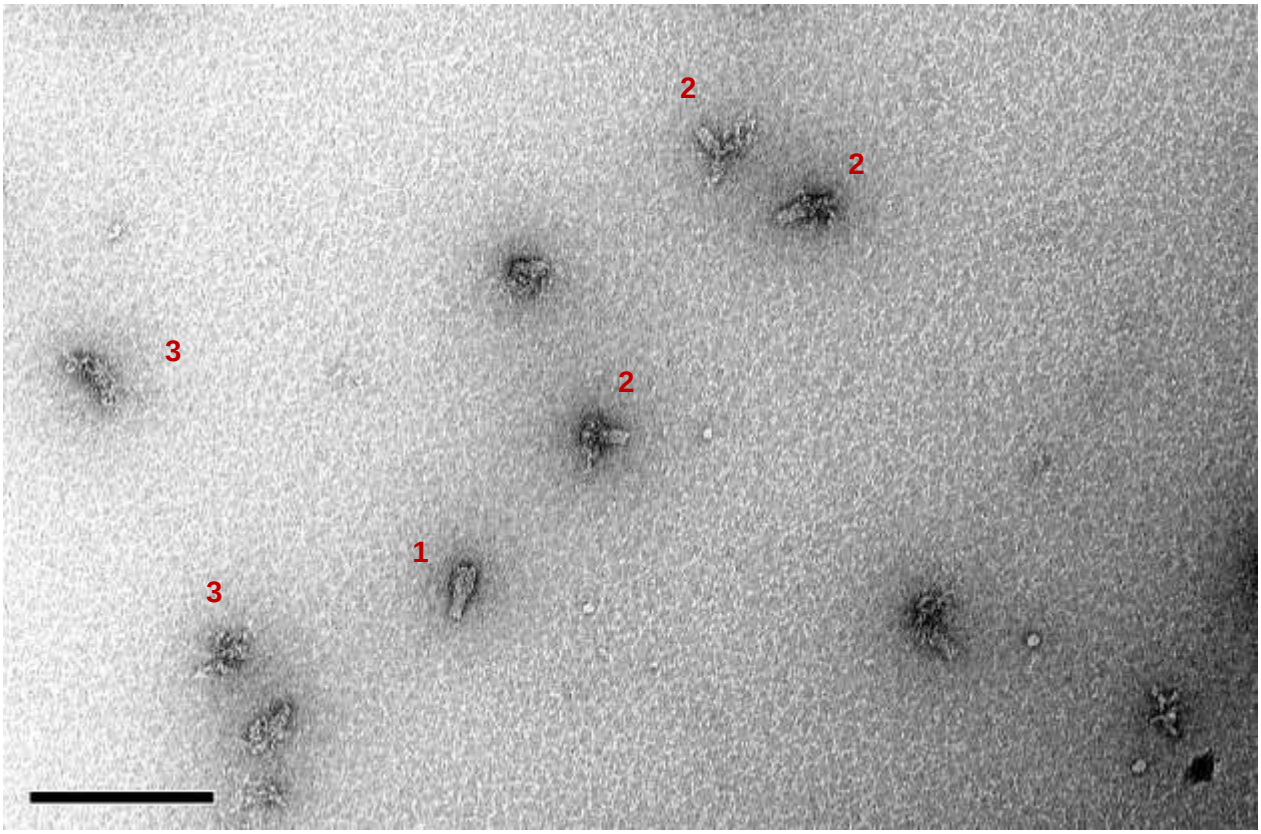


Figure S12. Negative stain TEM micrograph of NBs in DMEM incubated for one day at 37 ˚C. Nanostructures denoted with "**1**" are the intact NBs, "**2**" are the partially degraded NBs and "**3**" are fully degraded nanostructures. The nsTEM images reveal that denaturation initiated exclusively from the bottom of nanostructures. Scale bar is 200 nm.



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