

## Supporting Information-

### Investigation of Binary Lipid Mixtures of a Triple-Chained Cationic Lipid and Phospholipids Suitable for Lipofection

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## **Content**

1. TEM images of different lipid mixtures	p. 3
2. Cell Culture Screening	p. 18
3. Correlation Functions of DLS Measurements of Lipoplexes	p. 21

1. TEM images of different lipid mixtures:

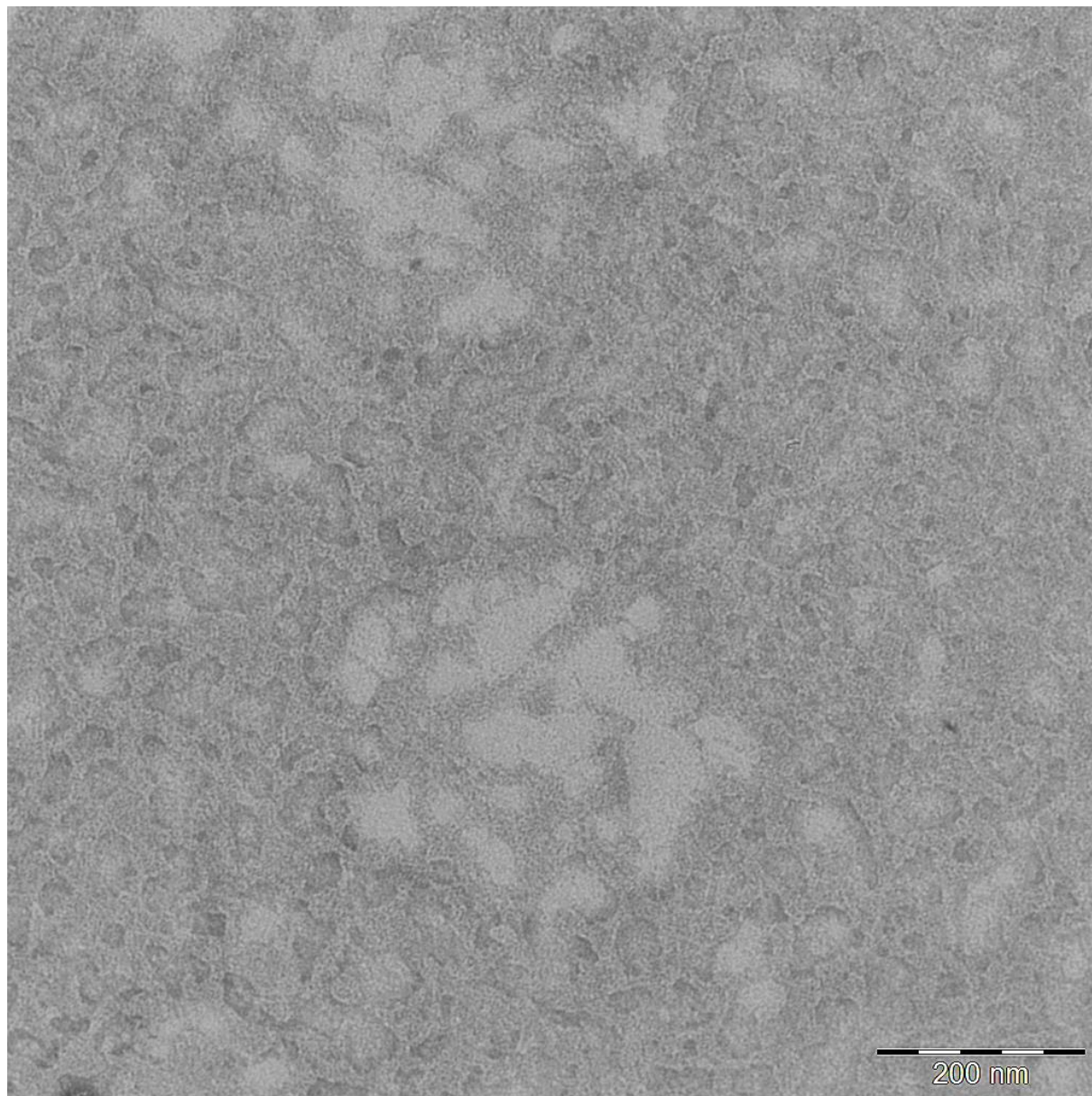


Figure S1. **DiTT4**/DOPE mixture  $X_{\text{DiTT4}} = 0.2$ .

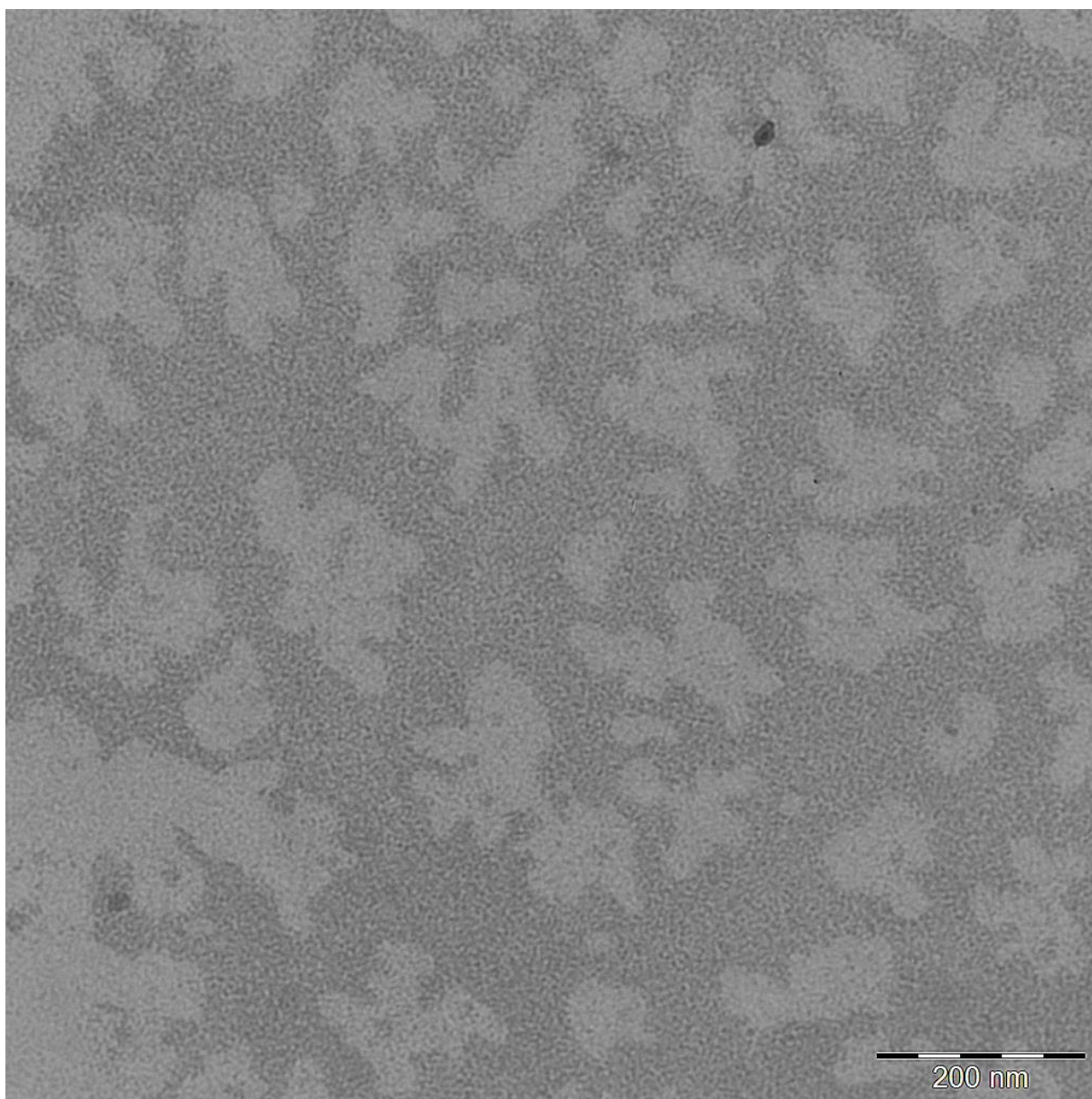


Figure S2. **DiTT4**/DOPE mixture  $X_{\text{DiTT4}} = 0.4$ .

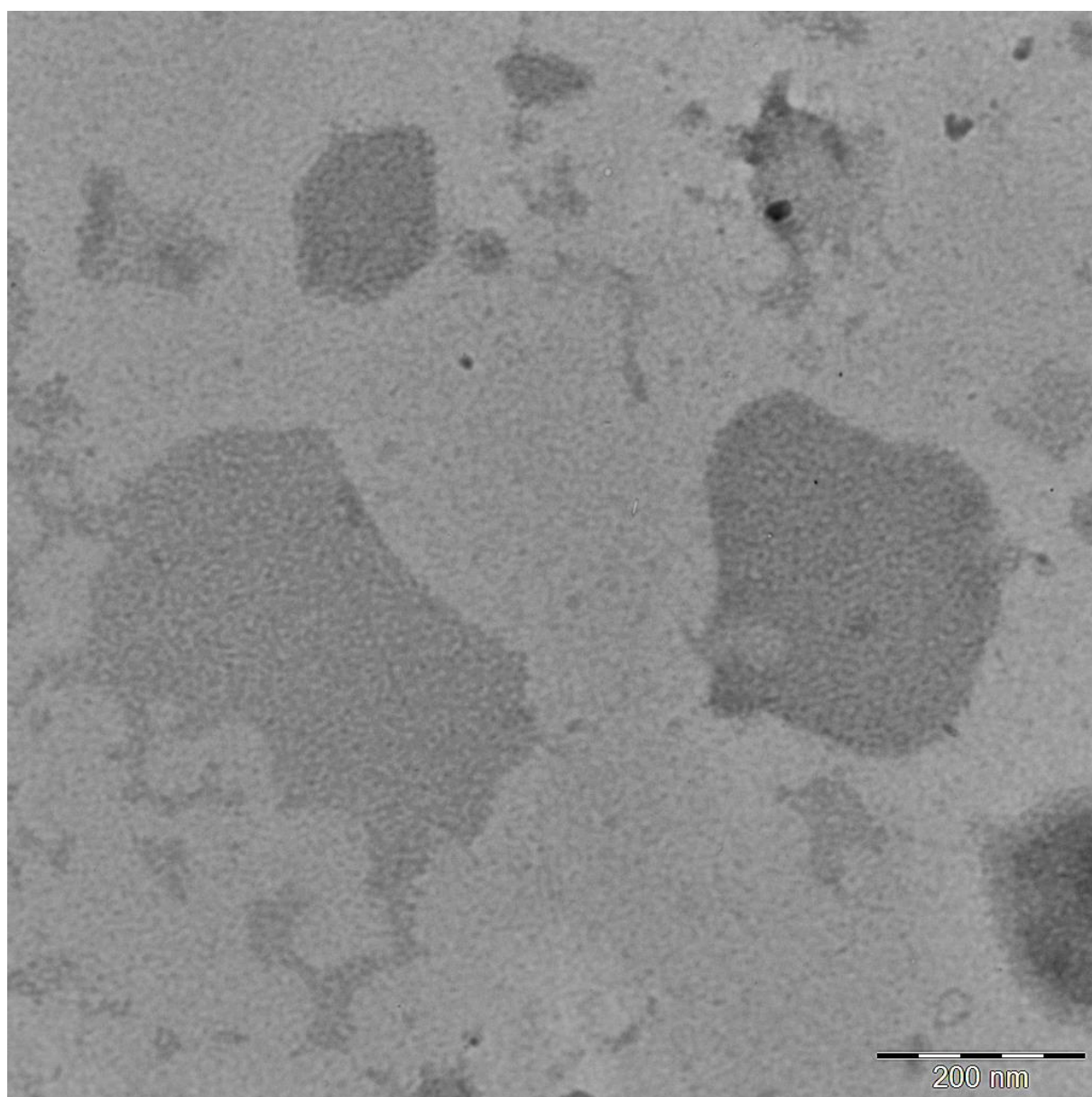


Figure S3. **DiTT4**/DOPE mixture  $X_{\text{DiTT4}} = 0.5$ .

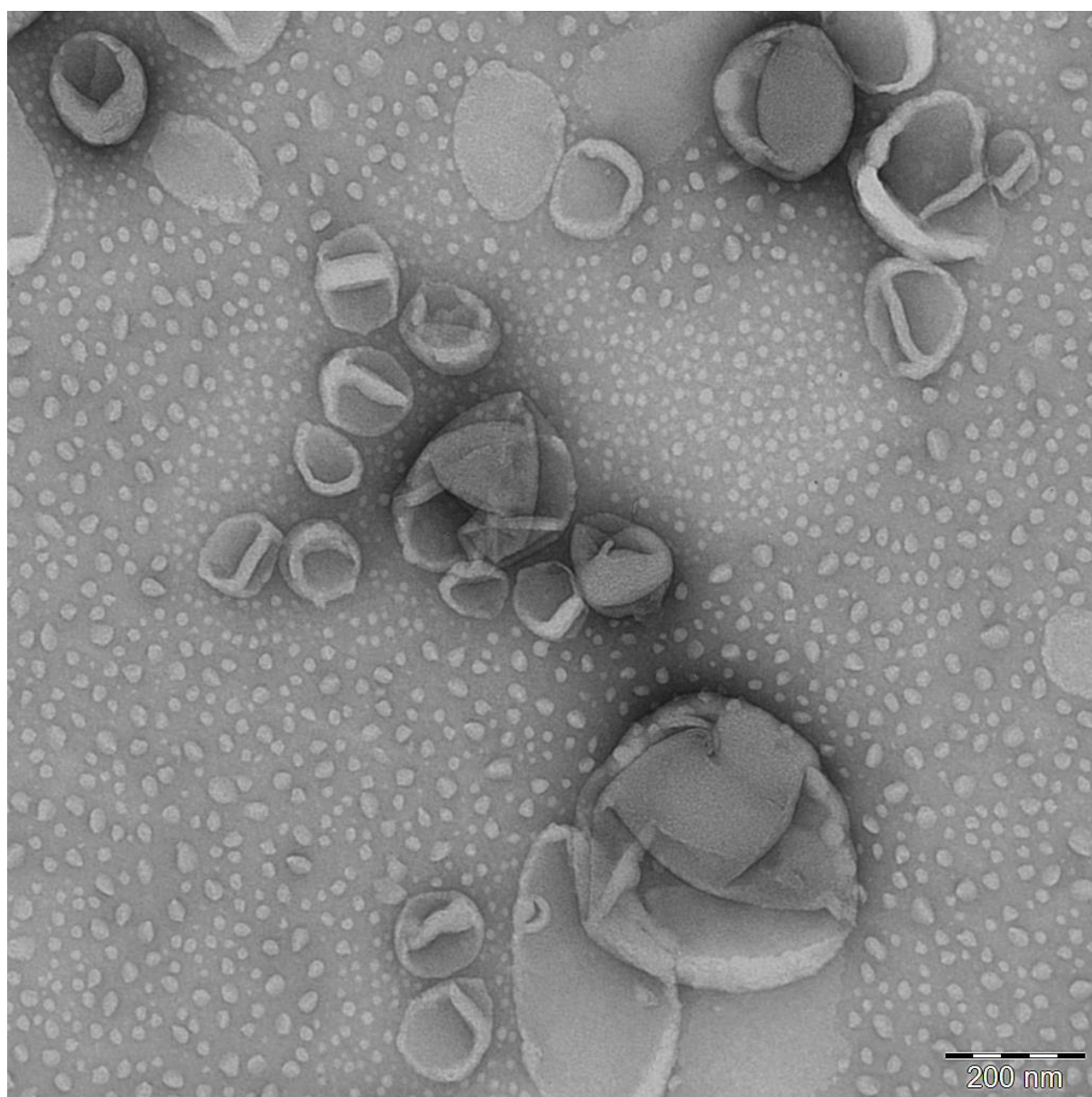


Figure S4. **DiTT4**/DOPE mixture  $X_{\text{DiTT4}} = 0.6$ .

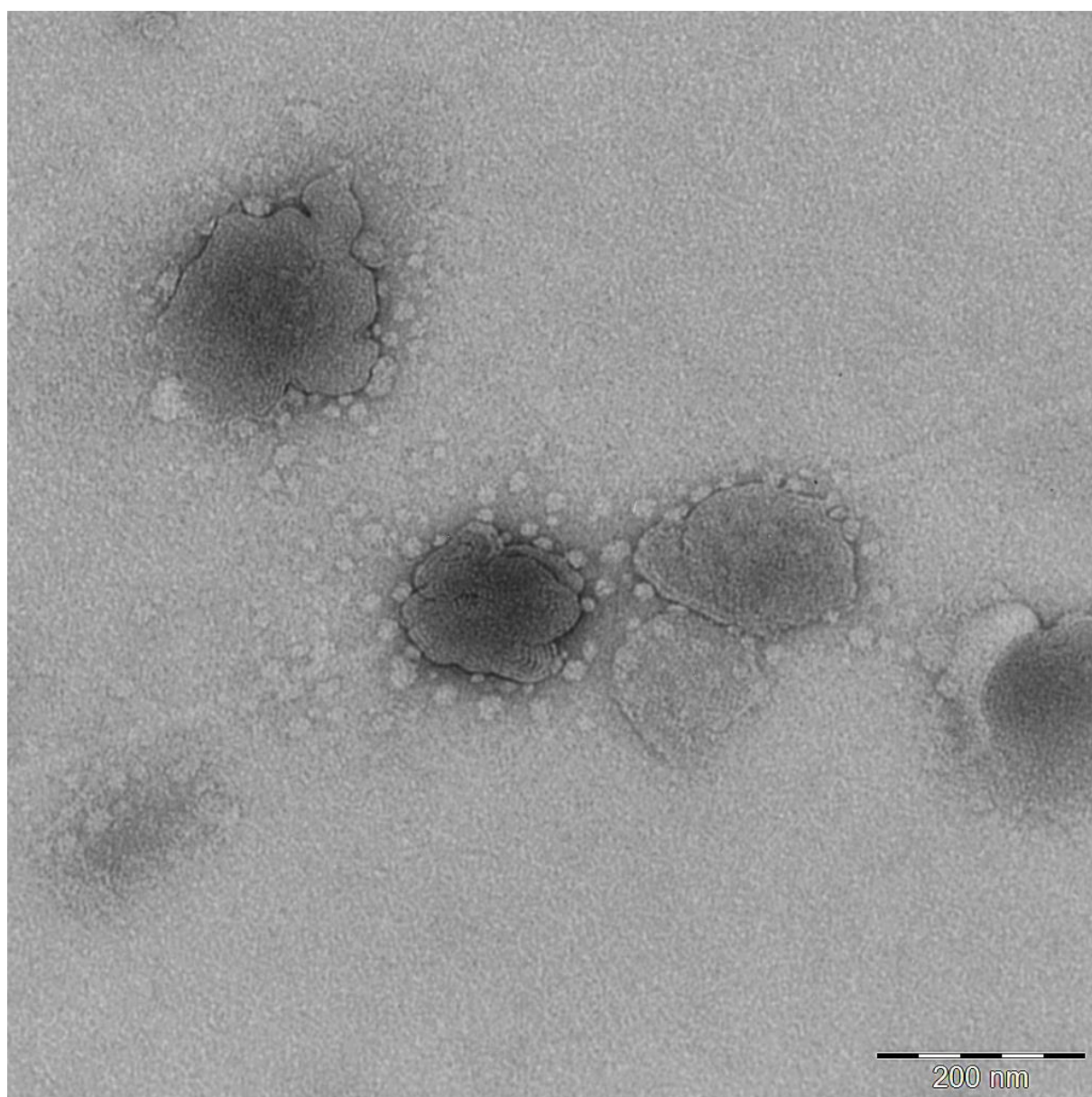


Figure S5. **DiTT4**/DOPE mixture  $X_{\text{DiTT4}} = 0.8$ .

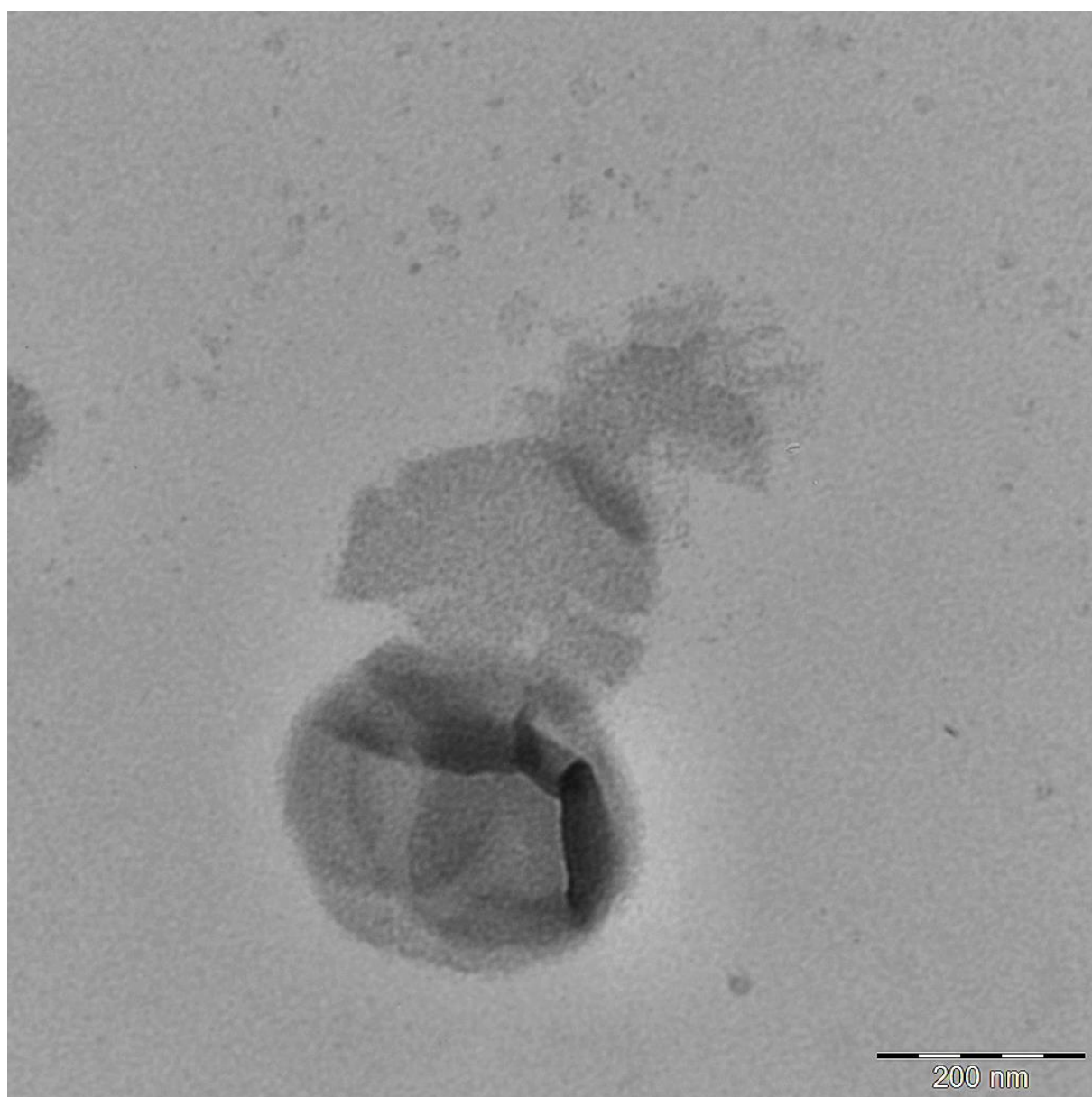


Figure S6. **DiTT4**/DMPE mixture  $X_{\text{DiTT4}} = 0.2$ .



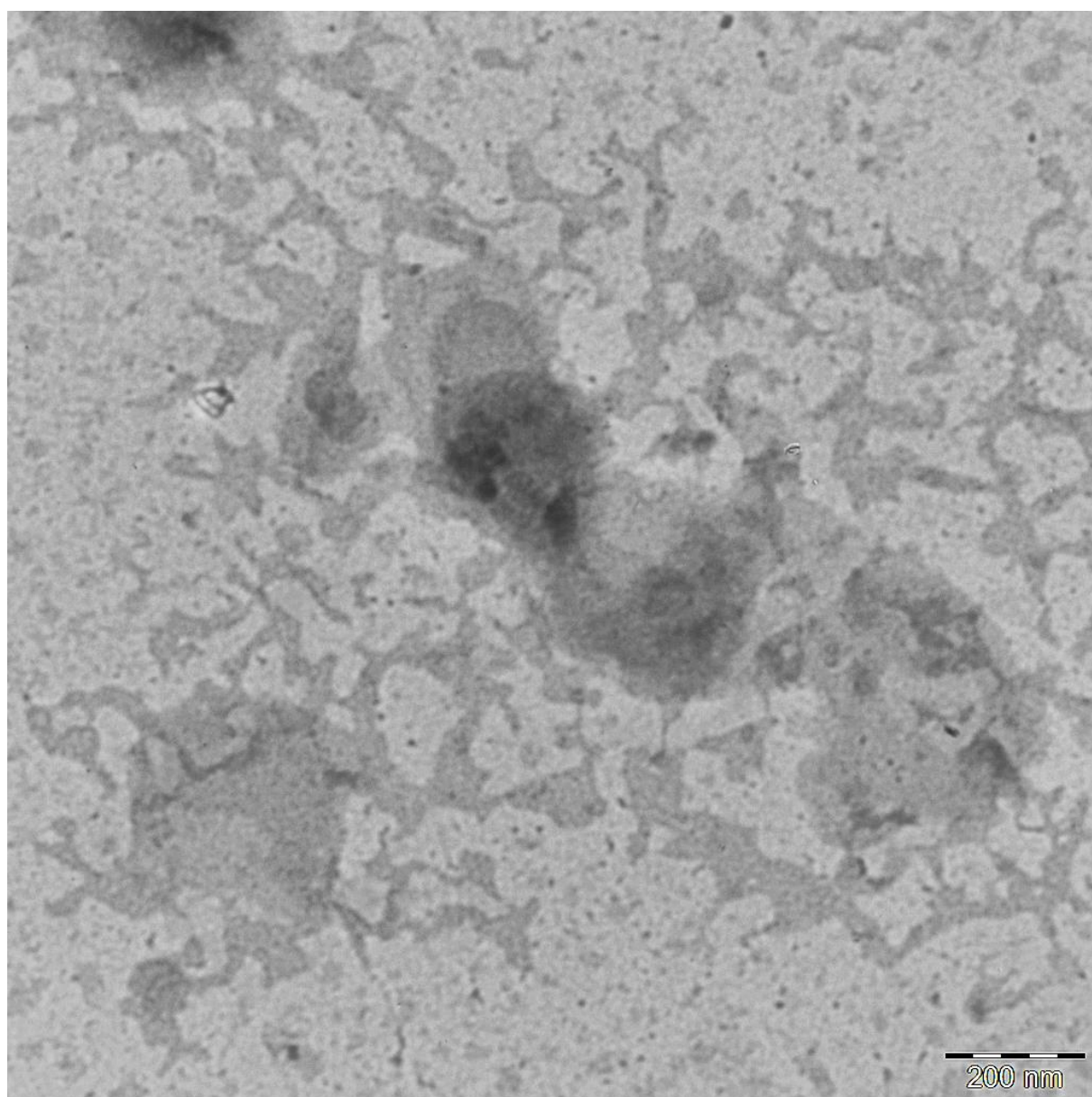


Figure S7. **DiTT4**/DMPE mixture  $X_{\text{DiTT4}} = 0.4$ .

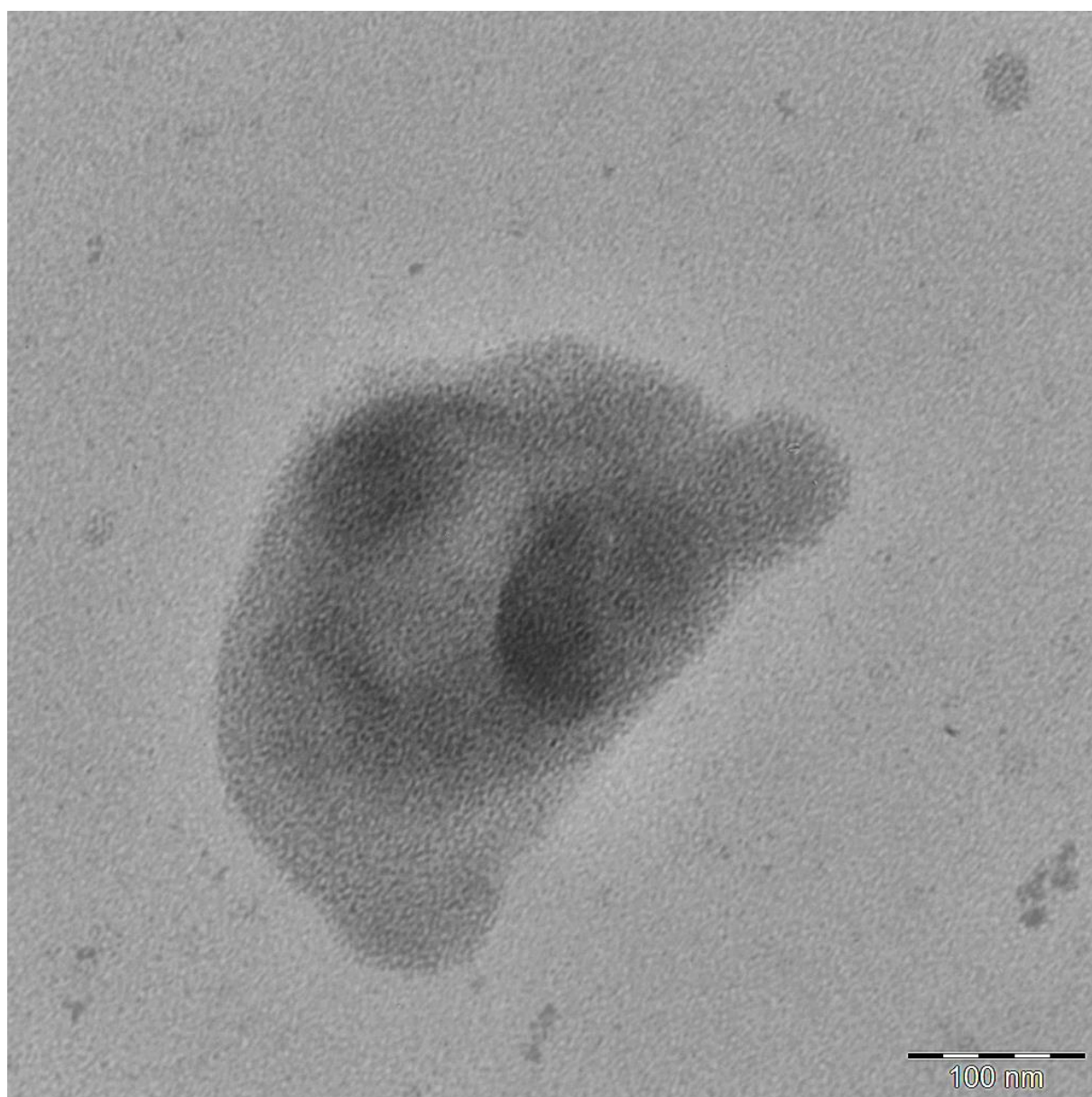


Figure S8. **DiTT4**/DMPE mixture  $X_{\text{DiTT4}} = 0.5$ .

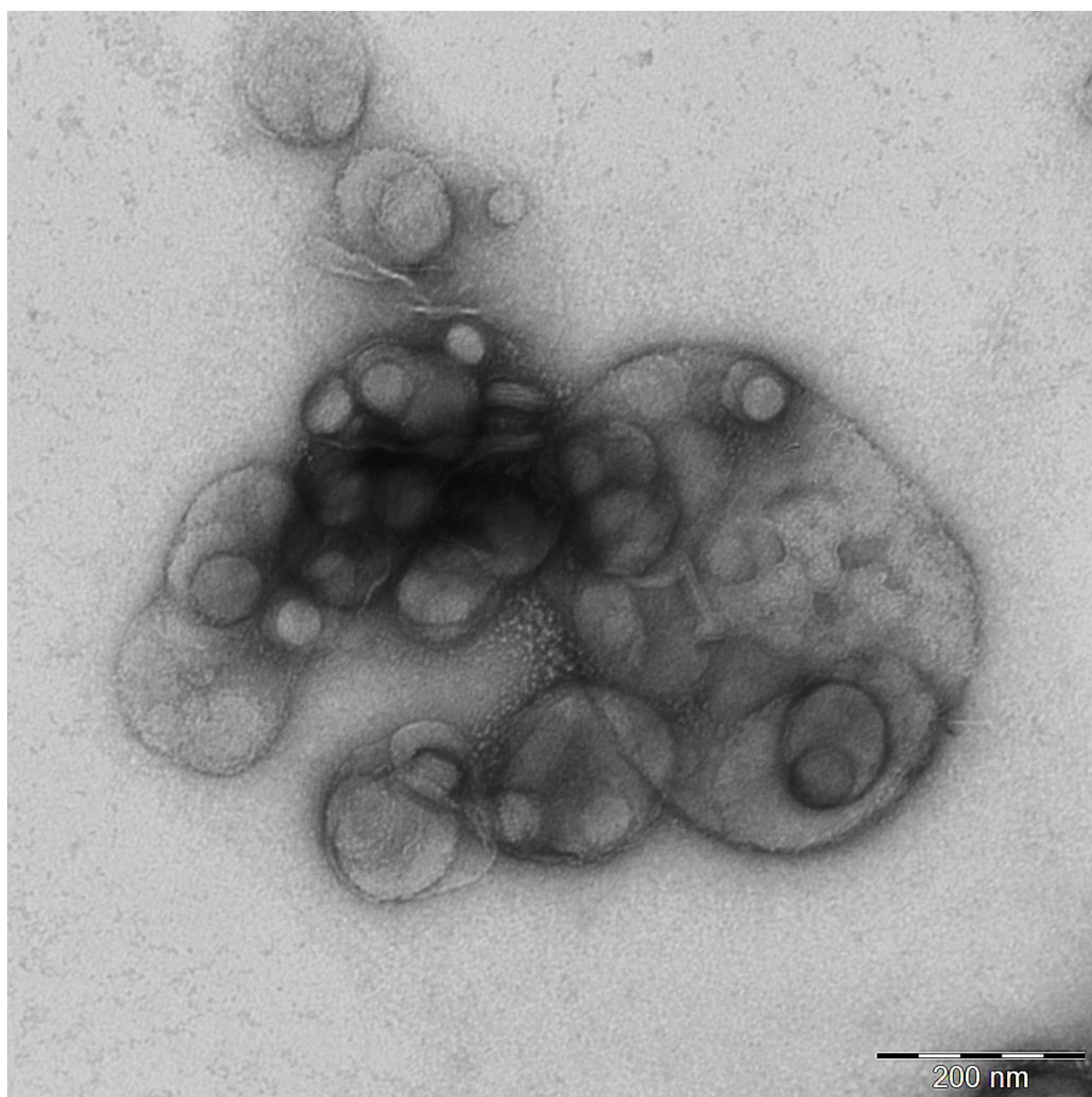


Figure S9. **DiTT4**/DMPE mixture  $X_{\text{DiTT4}} = 0.6$ .

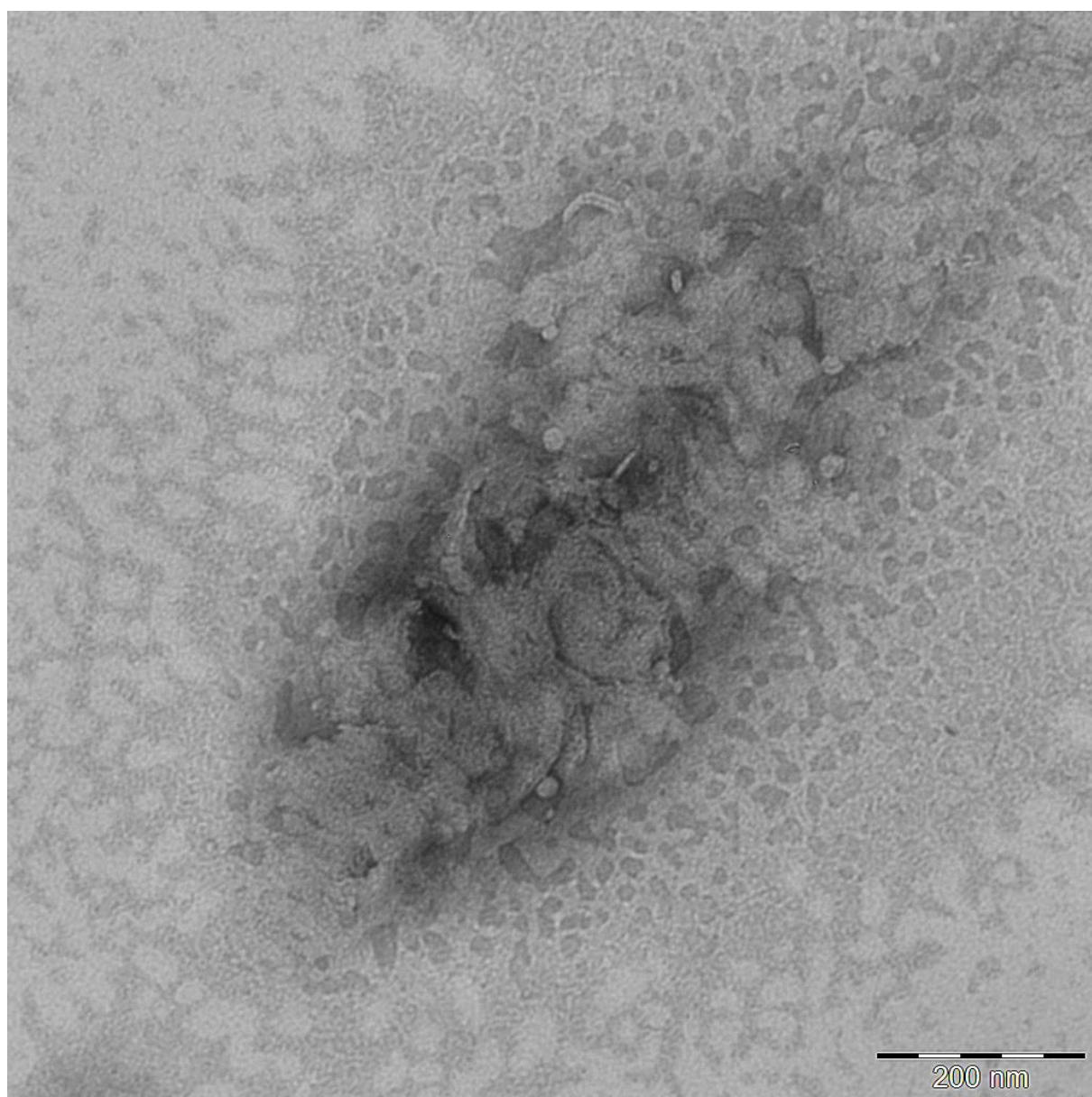


Figure S10. **DiTT4**/DMPE mixture  $X_{\text{DiTT4}} = 0.8$ .

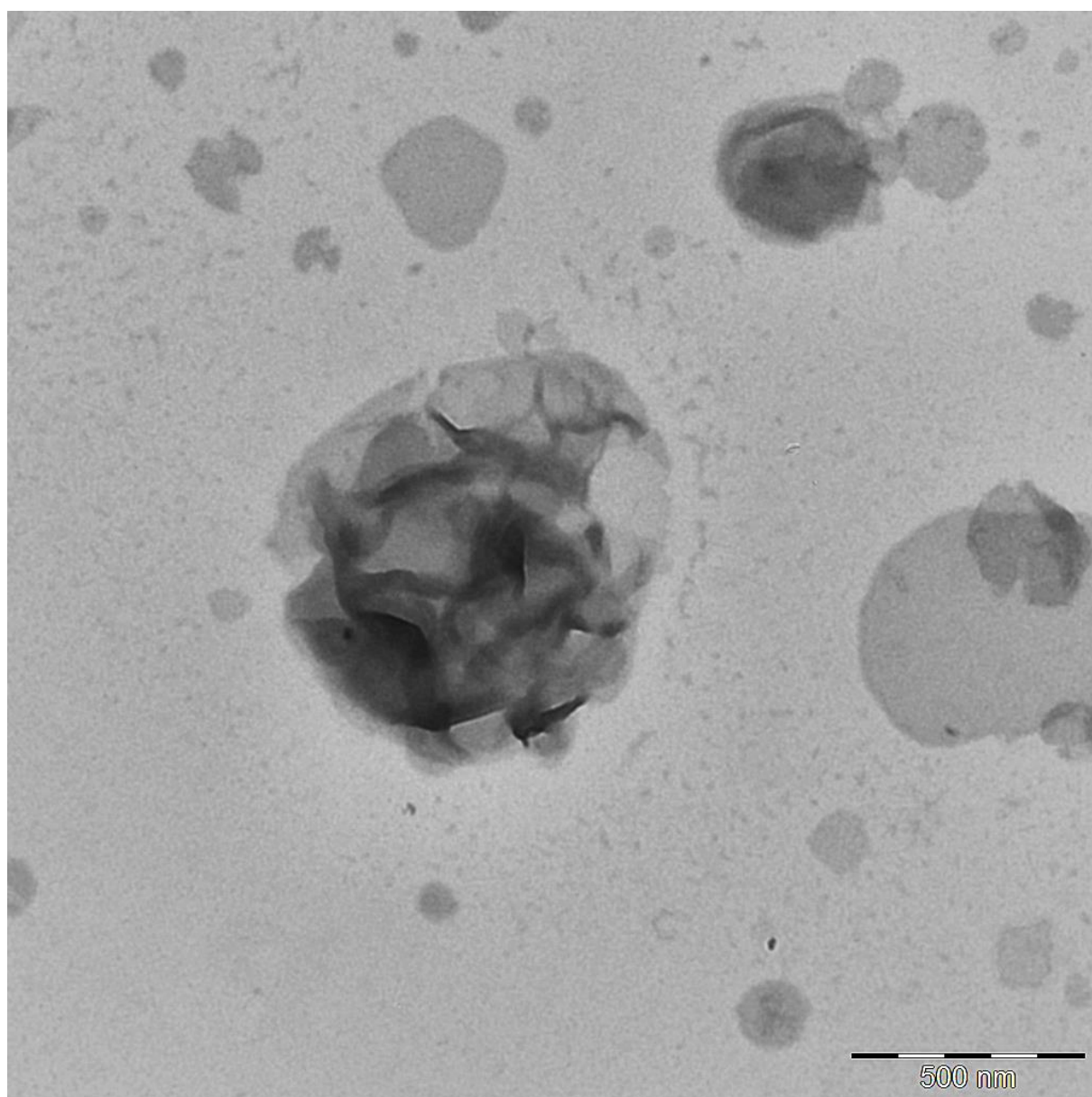


Figure S11. **DiTT4**/DMPC mixture  $X_{\text{DiTT4}} = 0.2$ .

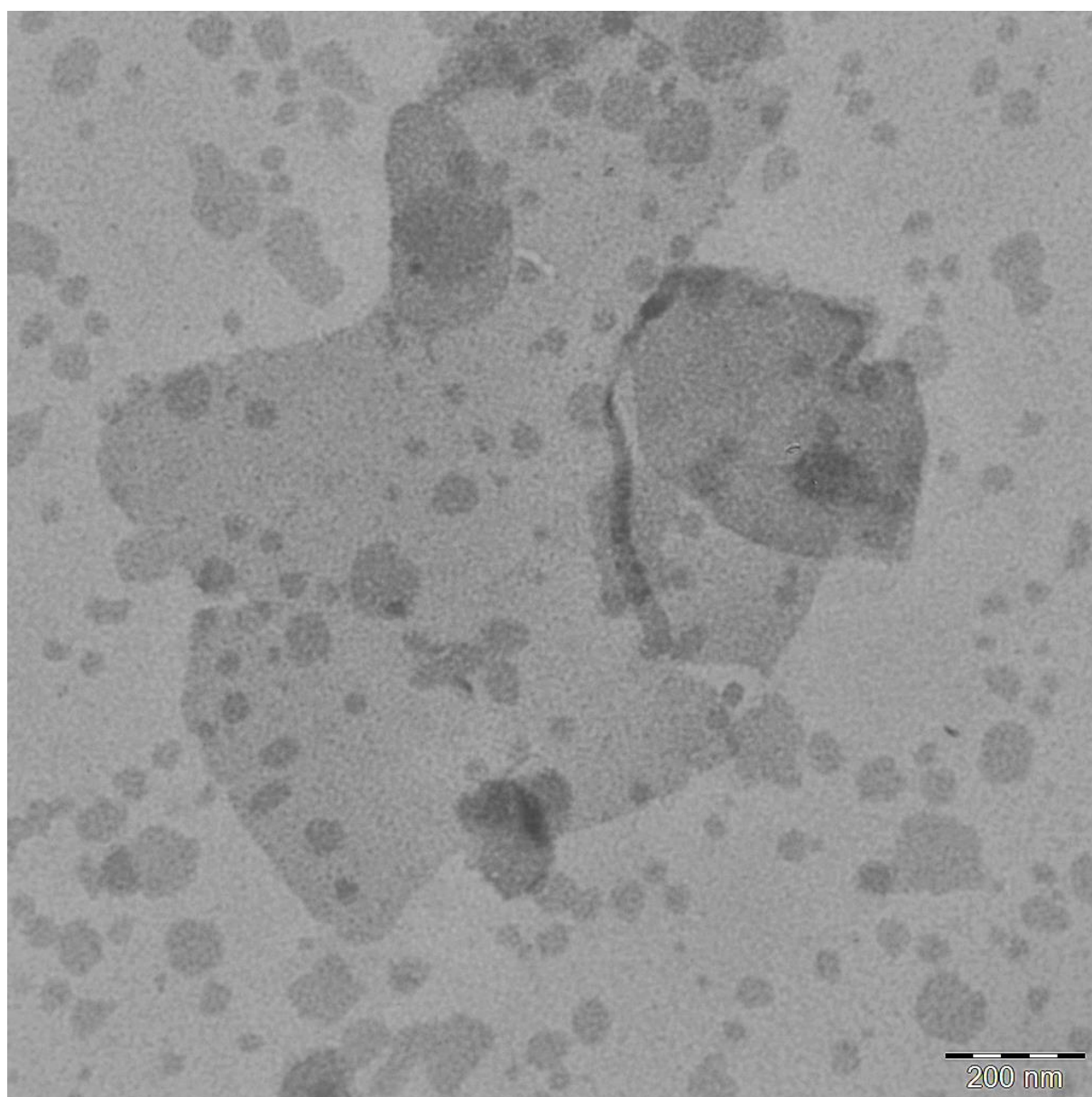


Figure S12. **DiTT4**/DMPC mixture  $X_{\text{DiTT4}} = 0.4$ .

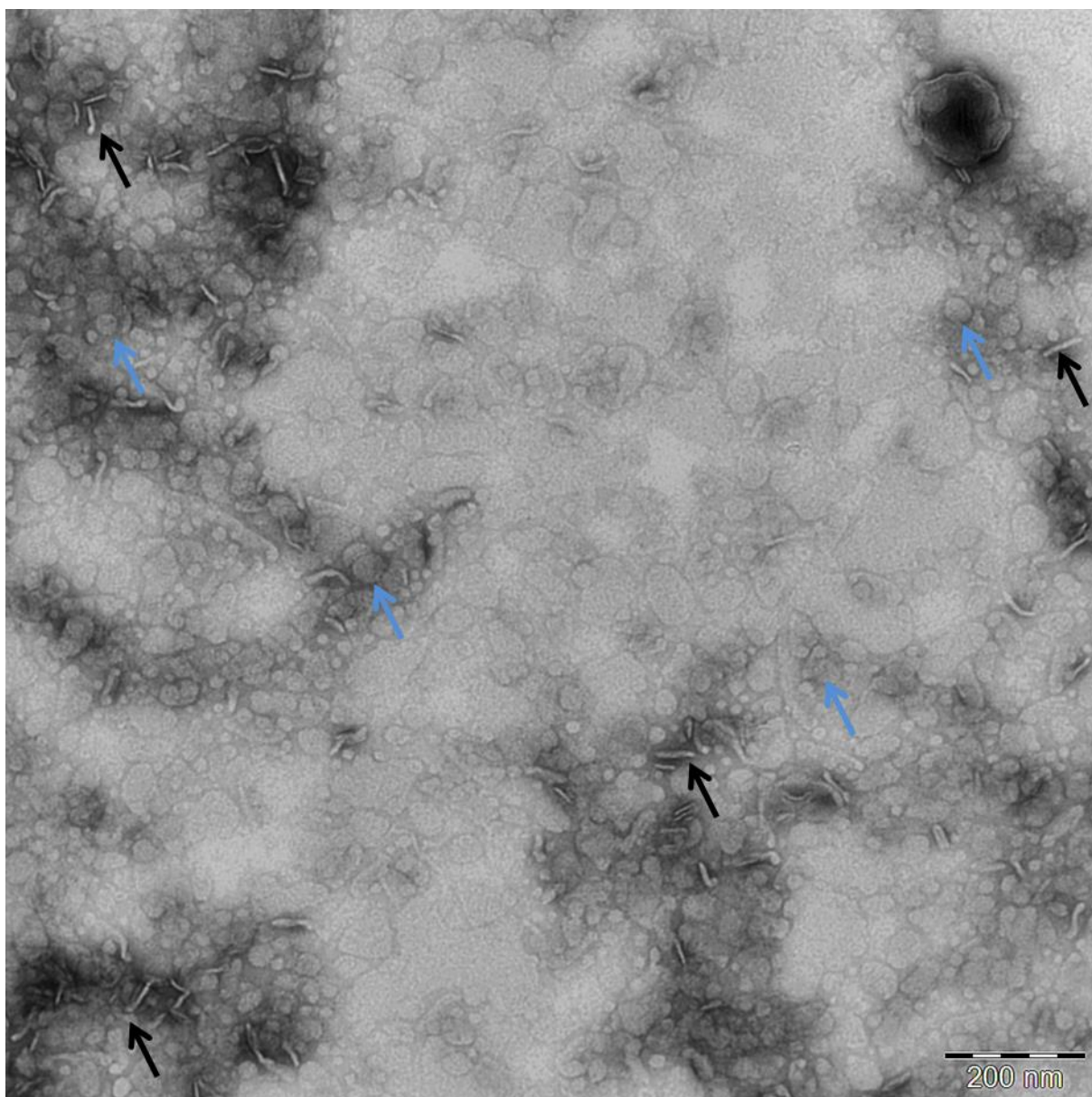


Figure S13. **DiTT4**/DMPC mixture  $X_{\text{DiTT4}} = 0.5$ . The arrows indicate disks observed edge-on (black arrows, disc oriented perpendicular to the surface of the grid) and face-on (blue arrows, disc oriented parallel to the surface of the grid), respectively, an observation indicating the presence of disc-like structures.

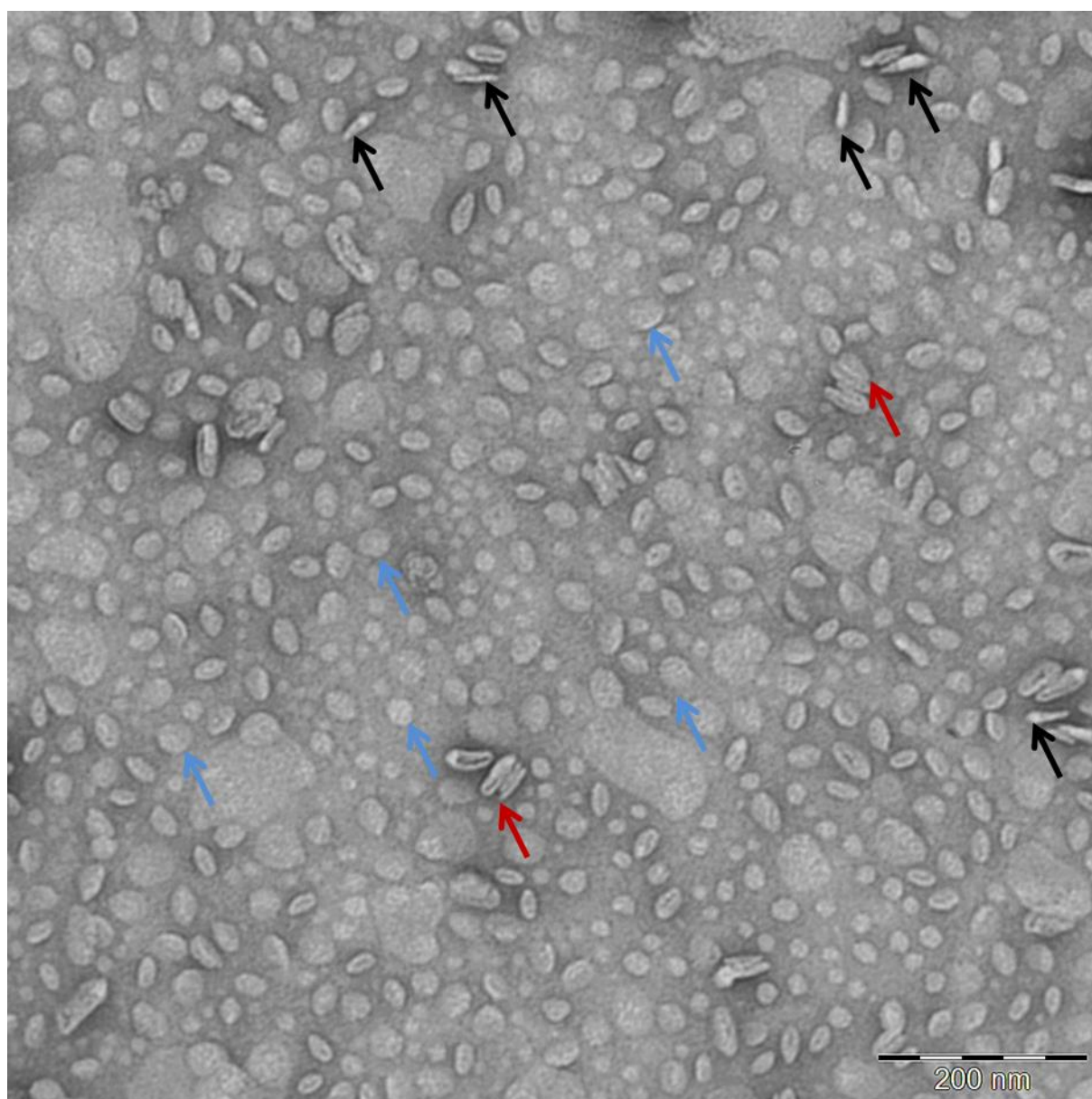


Figure S14. **DiTT4**/DMPC mixture  $X_{\text{DiTT4}} = 0.6$ . The arrows indicate disks observed edge-on (black arrows, disc oriented perpendicular to the surface of the grid) and face-on (blue arrows, disc oriented parallel to the surface of the grid), respectively, an observation indicating the presence of disc-like structures. The red arrows indicate stacks of discs in the edge-on view. The arrangement to stacks is also typical for discs in TEM.



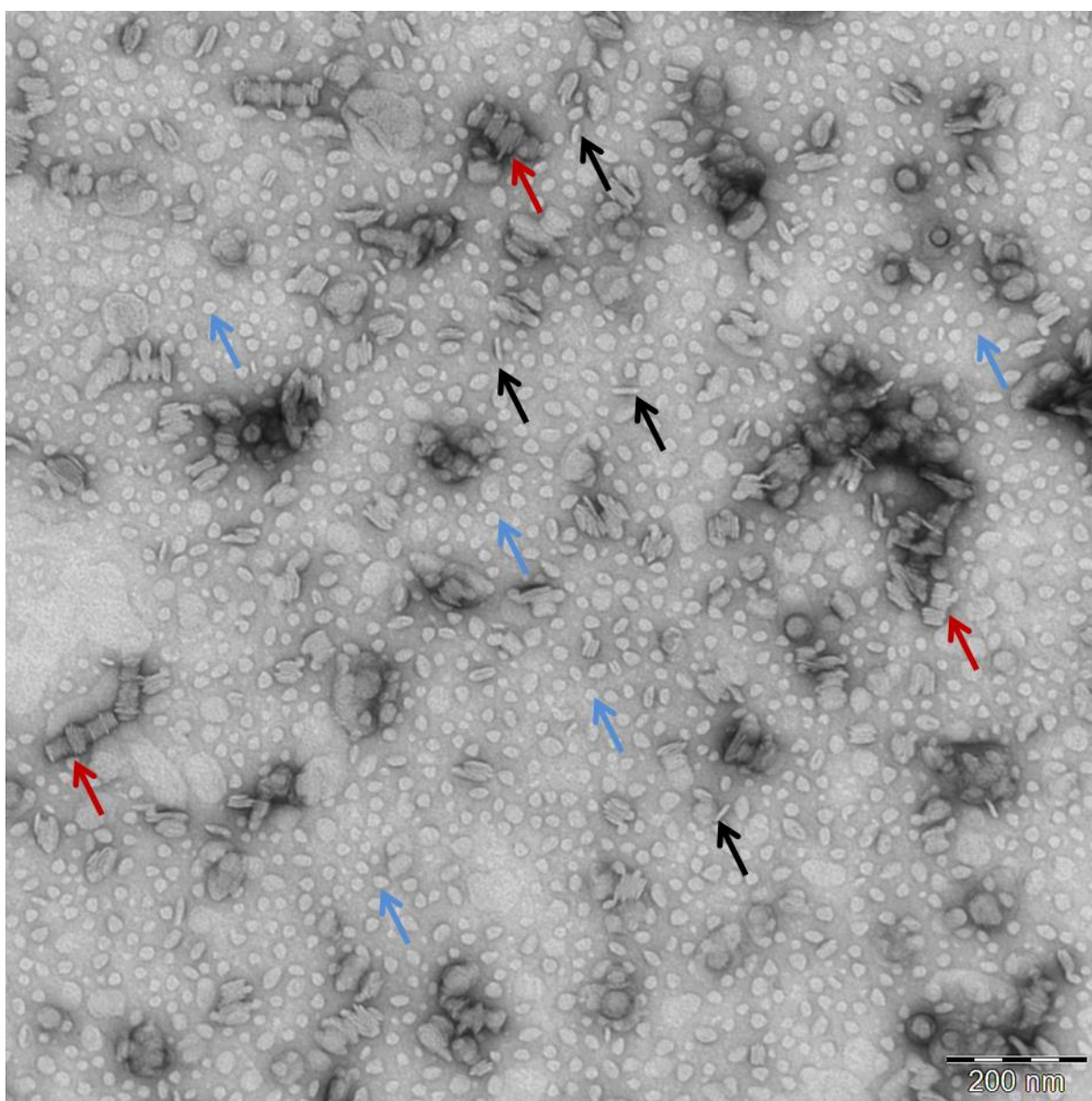


Figure S15. **DiTT4**/DMPC mixture  $X_{\text{DiTT4}} = 0.8$ . The arrows indicate disks observed edge-on (black arrows, disc oriented perpendicular to the surface of the grid) and face-on (blue arrows, disc oriented parallel to the surface of the grid), respectively, an observation indicating the presence of disc-like structures. The red arrows indicate stacks of discs in the edge-on view. The arrangement to stacks is also typical for discs in TEM.

## 2. Cell Culture Screening:

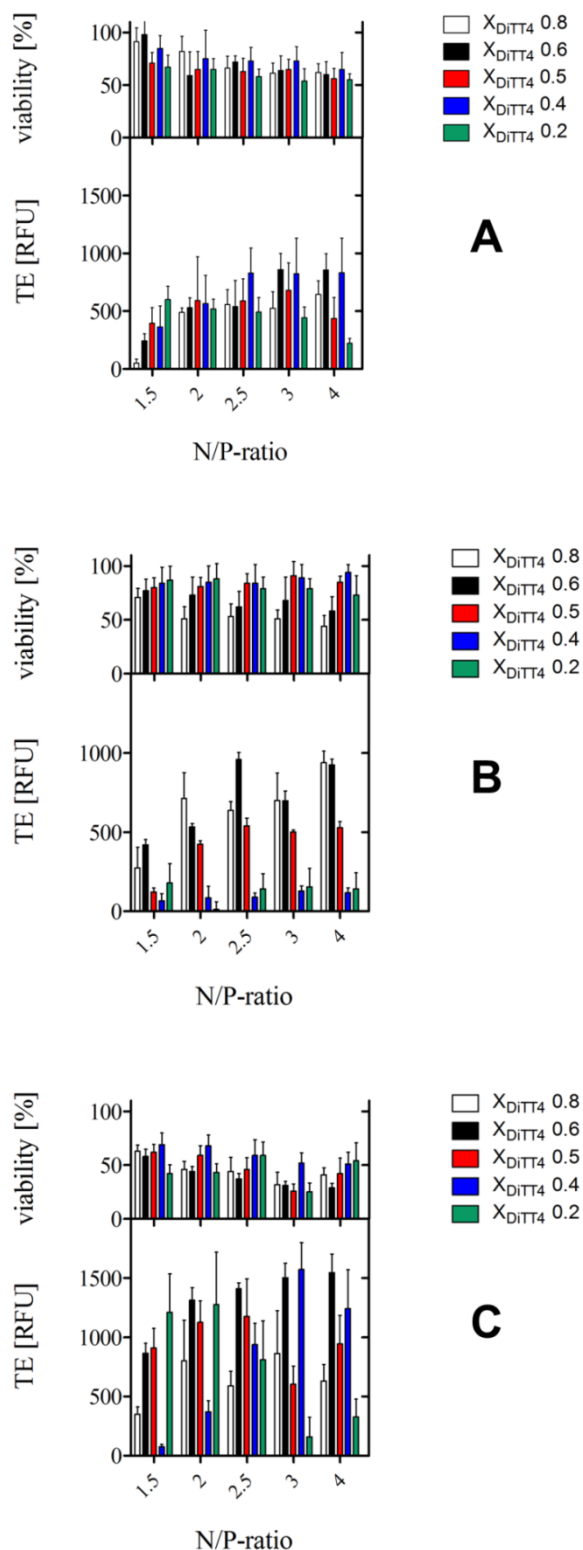
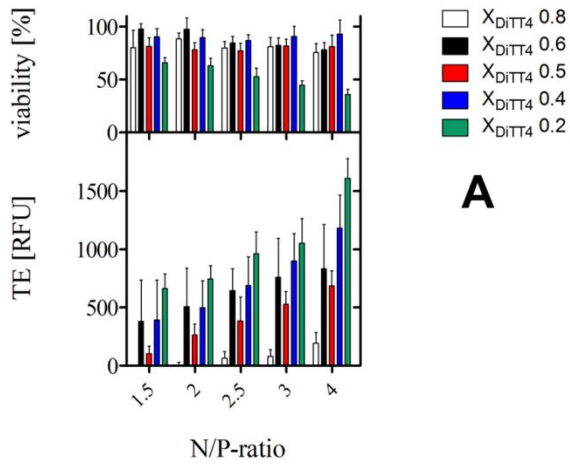
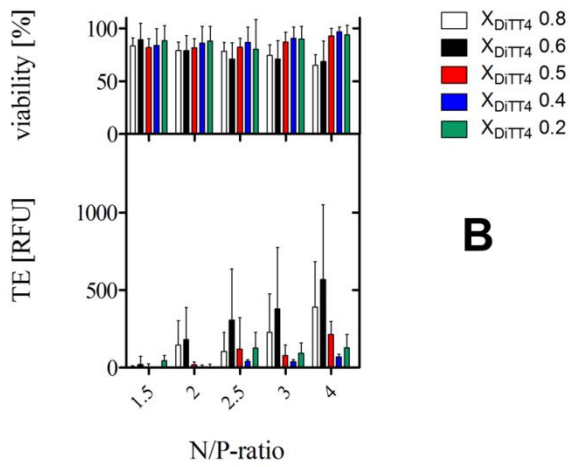


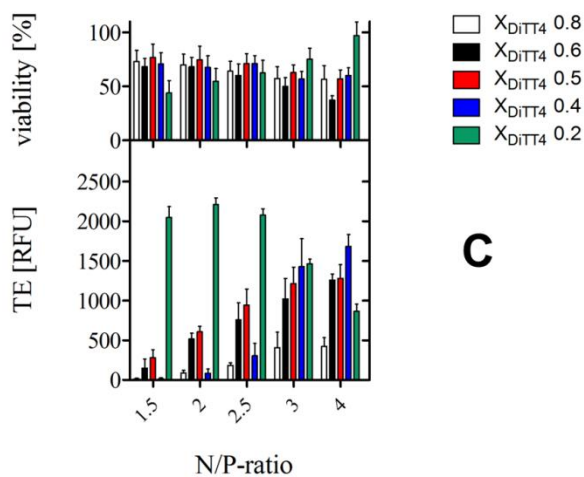
Figure S16. Transfection efficiency (TE) of lipoplexes at different N/P-ratios and the corresponding cell viability 24 h after the transfection of A549 cells in absence of serum during the incubation time of the lipoplexes. Following lipid mixtures were used: **DiTT4/DOPE** (A), **DiTT4/DMPE** (B) and **DiTT4/DMPC** (C) with different molar fractions of **DiTT4**.



**A**



**B**



**C**

Figure S17. Transfection efficiency (TE) of lipoplexes at different N/P-ratios and the corresponding cell viability 24 h after the transfection of A549 cells in presence of 10% serum during the incubation time of the lipoplexes. Following lipid mixtures were used: **DiTT4/DOPE** (A), **DiTT4/DMPE** (B) and **DiTT4/DMPC** (C) with different molar fractions of **DiTT4**.

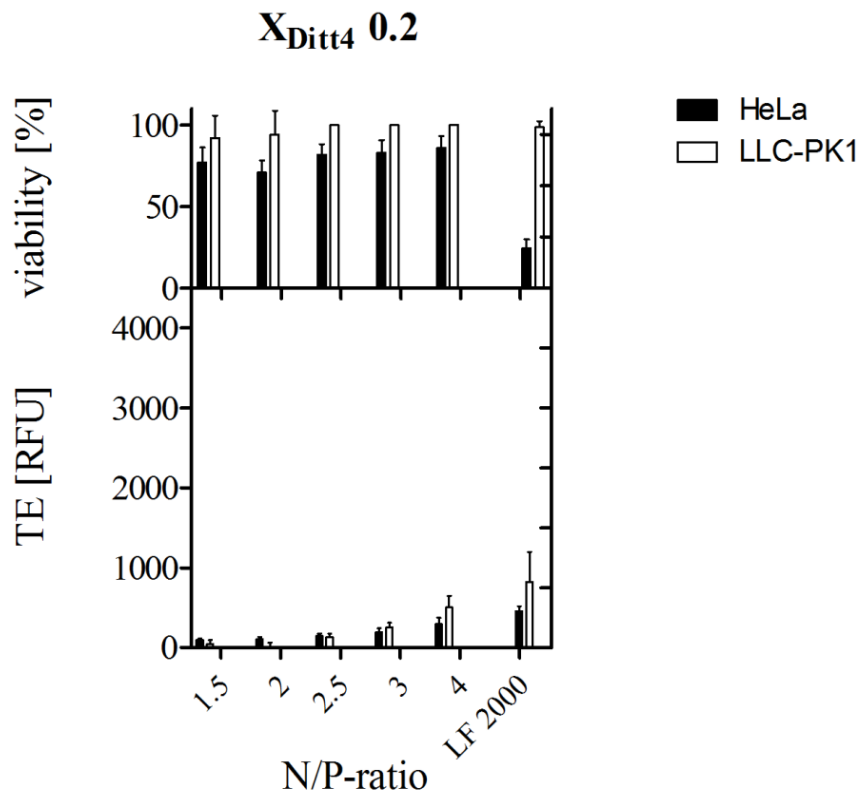


Figure S18. Transfection efficiency and the corresponding cell viability of **DiTT4**/DMPC ( $X_{\text{DiTT4}} = 0.2$ ) lipoplexes as a function of the N/P-ratio 24 h after the transfection of HeLa and LLC-PK1 cells in presence of 10% serum during the incubation time of the lipoplexes. LF 2000 is the standard Lipofectamine 2000<sup>®</sup> in the most effective DNA/Lipofectamine 2000<sup>®</sup> ratio.

### 3. Correlation Functions of DLS Measurements of Lipoplexes:

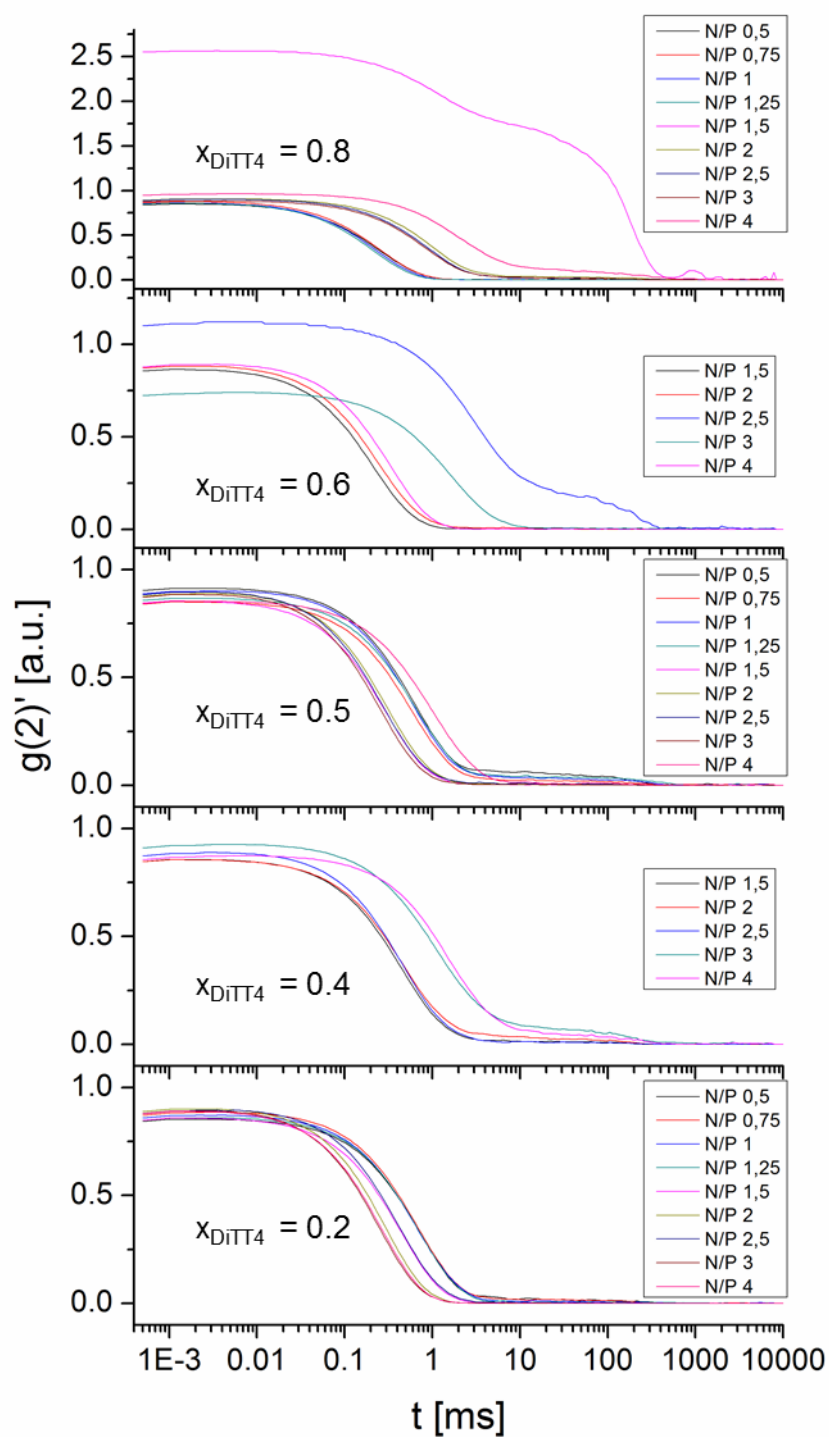


Figure S19. Correlation functions of lipoplex dispersions in HEPES buffer pH 7.3 determined by DLS. The used lipid composition was **DiTT4**/DOPE with different  $x_{\text{DiTT4}}$  values which were complexed with pDNA at different N/P-ratios (see legend).

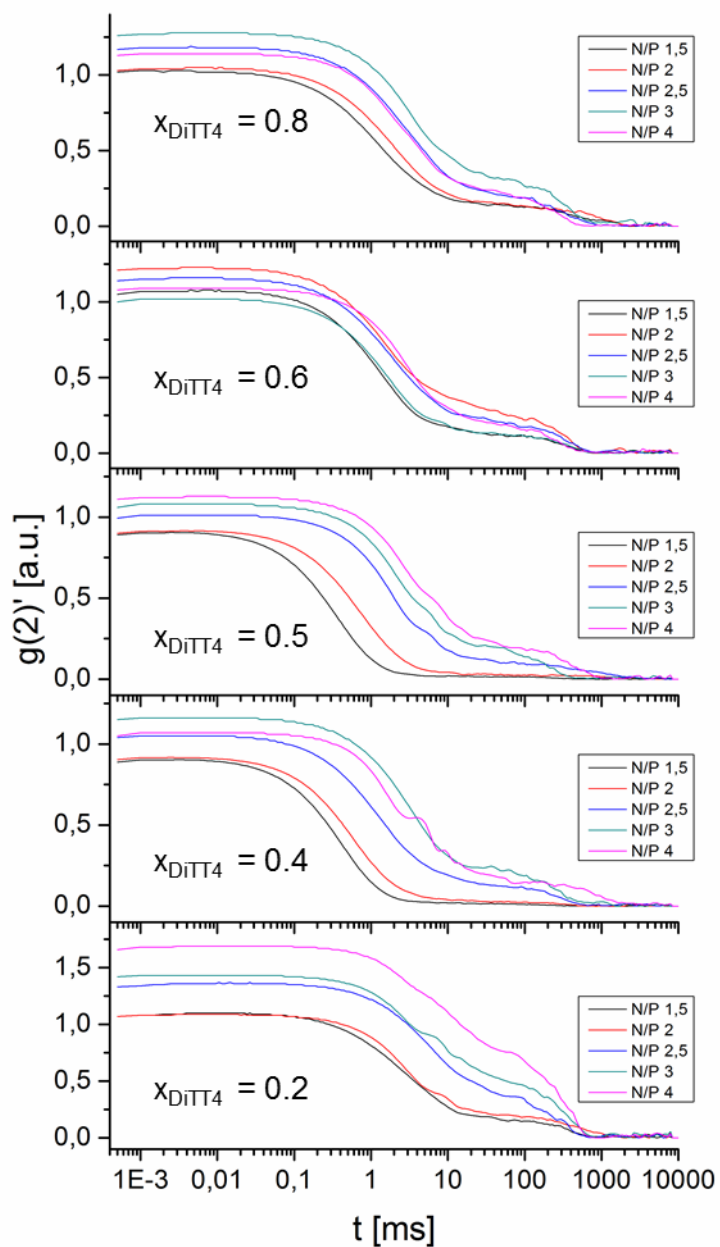


Figure S20. Correlation functions of lipoplex dispersions in HEPES buffer pH 7.3 determined by DLS. The used lipid composition was **DiTT4**/DMPE with different  $x_{\text{DiTT4}}$  values which were complexed with pDNA at different N/P-ratios (see legend).

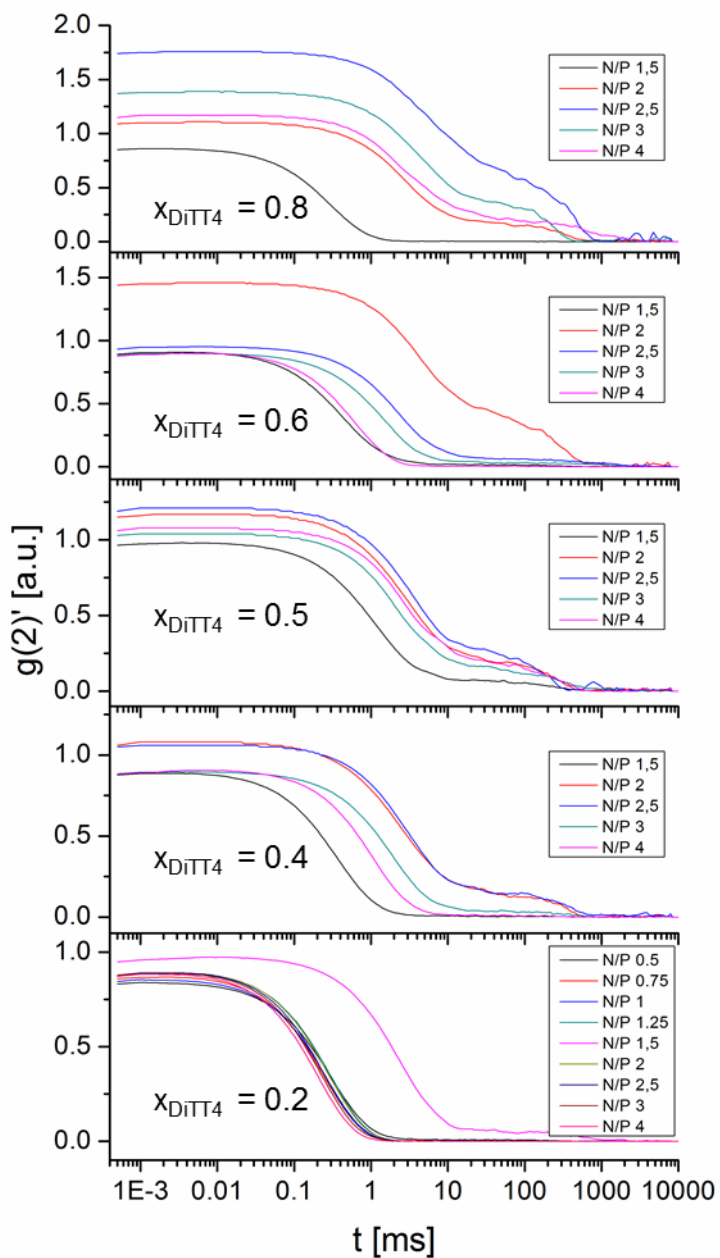


Figure S21. Correlation functions of lipoplex dispersions in HEPES buffer pH 7.3 determined by DLS. The used lipid composition was **DiTT4**/DMPC with different  $x_{\text{DiTT4}}$  values which were complexed with pDNA at different N/P-ratios (see legend).