

## Supporting information for

# Formation and structure of PEI/DNA complexes: quantitative analysis

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## Materials and Methods

### Preparation of PEI/DNA complexes at different ratio

PEI/DNA complexes at different ratios measurement were prepared as follows. We mixed 2  $\mu$ L of 500 nM ATTO550 labeled DNA solution and 1  $\mu$ L of PEI solution, the concentration of which was 12.5 ng/ $\mu$ L, 50 ng/ $\mu$ L, 125 ng/ $\mu$ L, 12.5  $\mu$ g/ $\mu$ L and 175  $\mu$ g/ $\mu$ L respectively, in 200  $\mu$ L of TE solution and incubated the mixture at 25 °C for 30 min. The concentration of labeled DNA fragments in all final mixture was 5 nM. The molar ratio between PEI to DNA in the mixture was 0.5, 2, 5, 500, and 3600 respectively. One PEI contains 581 N and one DNA contains 132 P. So the corresponding N:P ratio (the molar ratio between N in PEI and P in DNA) is 2.2, 8.8, 22, 2200 and 15840 respectively. This ratio indicates the ratio of positive to negative charges for single molecules.

### Fluorescence spectra measurement to confirm the quench of dye

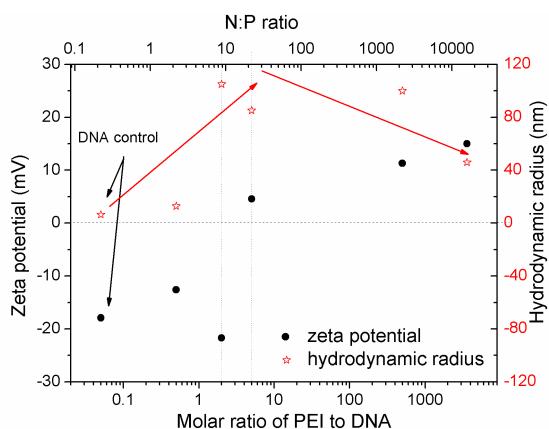
The fluorescence spectra were recorded on USB 2000+ spectrophotometer (Ocean-Optics, USA) in the spectral range 190–1000 nm in quartz micro cuvette (10 mm of path length). The fluorescence was excited using He-Ne laser (543 nm). The experiments were performed in Okolab incubator at 25 °C. The concentration of DNA in the final mixture was 50 nM. The PEI/DNA molar ratio was 5 (N:P ratio 22). The complexes were incubated at 25 °C for 30 min before measurement. Free DNA was used as a control.

## Results and discussion

### DLS and zeta potential measurement on the PEI/DNA complexes of different ratio

We measure the hydrodynamic radius and zeta potential of PEI/DNA complexes formed at different PEI/DNA ratios (Fig. S1). We find that the size of PEI/DNA complex increases and then decreases with the PEI/DNA ratio. Our result well agrees with previous study<sup>1</sup>. We also find that the zeta potential of PEI/DNA complexes changes from negative to positive with the increase of PEI/DNA ratio. The change of zeta potential (and consequently the charge) from negative to positive

occurs at a PEI/DNA molar ratio between 2 and 5 (N:P ratio between 8.8 and 22).



**Fig. S1** Influence of PEI/DNA ratio on the zeta potential and hydrodynamic radius of PEI/DNA complexes. The zeta potential of PEI/DNA complexes change from negative to positive with the addition of PEI. The conversion from negative to positive occurs at PEI/DNA molar ratio between 2 and 5 (N:P ratio between 8.8 and 22). DLS measurement shows that the particles size increases and then decreases with PEI/DNA ratio (the trend is marked as red arrow). Black arrow denotes free DNA as a control.

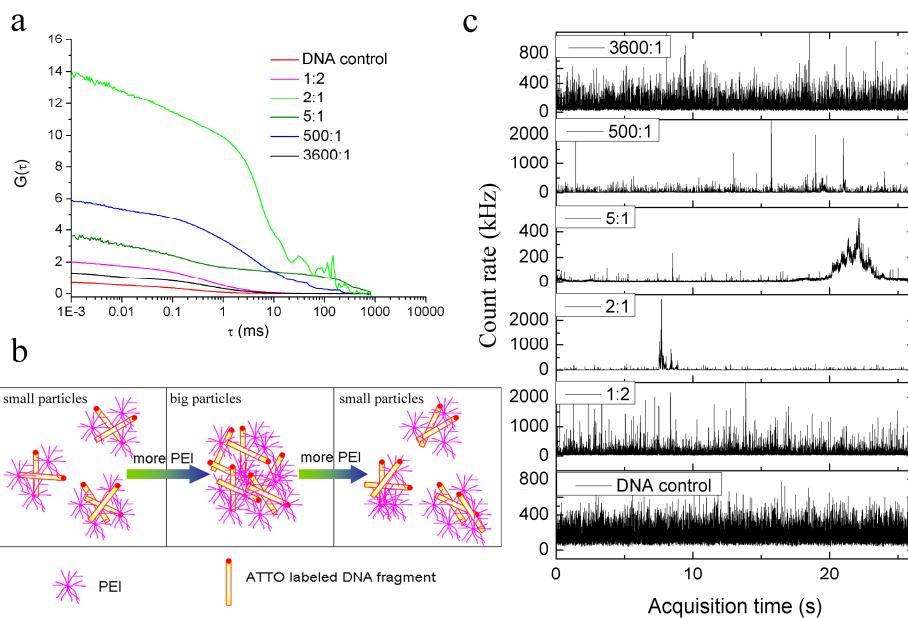
### FCS measurement on the PEI/DNA complexes of different ratio

We use FCS to monitor changes of hydrodynamic radius of PEI/DNA complexes (Fig. S2a). The residue time of PEI/DNA complexes, which is proportional to their size, increase and then decrease with the PEI/DNA ratio (Fig. S2b). We should notice that at PEI/DNA molar ratio 2, 5 and 500 (N:P ratio 8.8, 22 and 2200), the FCS curve cannot be fitted because of the formation of huge complexes. At these ratios, some prerequisites of fitting is not valid for huge PEI/DNA complexes. We assume that the tracers should be treated as points in comparison to the focal volume and the FCS signal comes solely from translational diffusion across the focal volume. However, when big PEI/DNA complexes form, they can no longer be treated as points and meanwhile some other factors may also contribute to the fluctuation of fluorescent signals, such as rotational diffusion.

In the following study, we confirm the change of the size of

PEI/DNA complexes with PEI/DNA ratio by the fluorescence fluctuation profiles (Fig. S2c). We have shown in Fig. S1 that the size of PEI/DNA complexes increases and then decreases with the PEI/DNA ratio, indicating the concentration of PEI/DNA complexes decreases and then increases with the PEI/DNA ratio. The frequency of the fluctuation is proportional to the concentration of the complexes. Therefore, we expect to observe a decrease and then an increase of fluctuation frequency with the PEI/DNA ratio. In our study, when the PEI/DNA molar ratios increases from 0.5 to 3600 (N:P ratio 2.2 and 15840), the number of fluctuations first

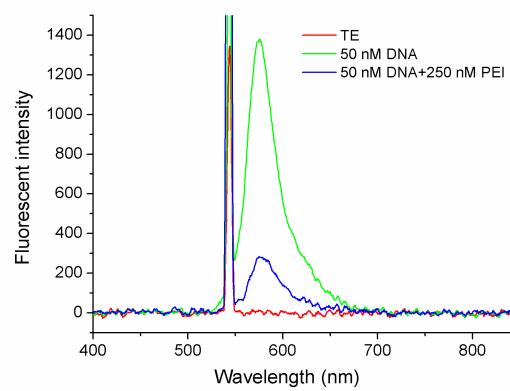
decreases and then increases again, which is just the same as we expected. The intensity of the fluctuation is proportional to the size of the complexes. So the intensity of fluctuations should increase and then decrease with the PEI/DNA ratio. But at PEI/DNA molar ratio of 5 (N:P ratio 22) the amplitude is so small that it cannot be simply reconciled with many DNA molecules in a complex. We assume that there is significant fluorescence quenching inside a complex. In order to prove this assumption we perform fluorescence intensity measurements in free DNA and also in PEI/DNA solutions (next section).



**Fig. S2** (a) FCS curve of PEI/DNA complexes formed at PEI/DNA molar ratio of 0.5, 2, 5, 500 and 3600. The corresponding N:P ratio is 2.2, 8.8, 22, 2200 and 15840, respectively. The residue time is inversely proportional to the diffusion coefficient of complexes and proportional to the size of complexes. We can roughly see an increase and then a decrease of the particles size with PEI/DNA ratio. (b) Schematically image showing the trend that the size of PEI/DNA complexes goes with PEI/DNA ratio. (c) Original FCS signal shows that the number of complexes in the focal volume decreases and then increases with PEI/DNA ratio (all is due to not only the change of size but also significant fluorescence quenching at some PEI:DNA ratio).

### Quenching of fluorescence from ATTO550 caused by DNA aggregation

The interaction between PEI and DNA packages many ATTO550 dyes in one PEI/DNA complex. Therefore, the brightness of the complexes should increase with their size. However, the fluorescent intensity decreases 3 to 4 times after the addition of PEI (Fig. S3). A simple explanation is that the surrounding solution of ATTO550 dye changes upon DNA condensation. The brightness of a dye depends on its surrounding solution. In PEI/DNA complexes, the water is expelled from the inside. So the surrounding solution of ATTO550 dyes in a complex is different from that of free ones. As a result the fluorescence of inner ATTO550 dyes decreases. The final brightness of the PEI/DNA complexes is the competition result of these two aspects.



**Fig. S3** Fluorescent spectra showing a quenching of ATTO550 dyes upon DNA condensation. High peak at 543 nm is the signal from the laser.

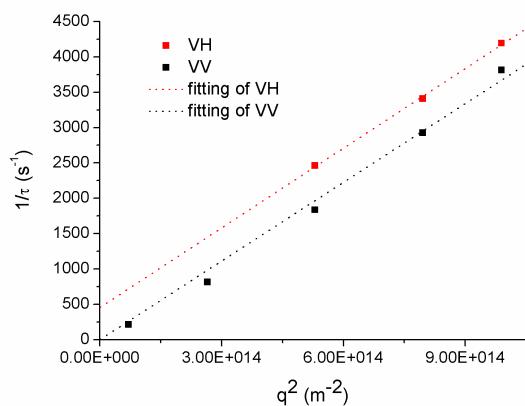
### Optical anisotropy of PEI/DNA complexes

Optical anisotropy of PEI/DNA complexes gives useful

information concerning the arrangement of DNA and PEI in the complexes. We used depolarized DLS (DDLS)<sup>2</sup> measurement to investigate the optical anisotropy of PEI/DNA complexes. In depolarized DLS measurement, the relation between decay efficiency  $1/\tau$  and wavevector  $q$  is

$$\frac{1}{\tau} = D_T q^2 + 6D_R, \quad (1)$$

where  $D_R$  is the rotational diffusion coefficient and  $D_T$  is the translational diffusion coefficient. In order to confirm the accuracy of DDLS measurement (VH component), we also performed a corresponding DLS measurement (VV component) as a comparison.



**Fig. S4** DDLS and DLS fitting lines of PEI/DNA complexes formed at PEI/DNA molar ratio of 0.5 (N:P ratio 2.2) using 75 nM of DNA, indicating a regular arrangement of chemical bonds in the PEI/DNA complexes. Please note the intercept of the DDLS line with the vertical axis indicating rotational diffusion of the whole complex.

In Fig. S4, the slopes of VV and VH fitting line are the same, indicating that the translational diffusion coefficients of PEI/DNA complexes measured by DLS and DDLS are the same (check on the accuracy of our measurements). We observe that the VH line does not intercept the vertical axis at zero indicating rotational diffusion of the complex. VH component of the scattered light in most cases comes from the anisotropy of the chemical bonds enhanced by their regular spatial arrangement. Such arrangements give a necessary contrast for observation of rotations in the DDLS measurement. The observation of the rotational signal clearly reveals a regular arrangement of chemical bonds in the PEI/DNA complexes, but we cannot state on this basis that the shape of the complex is elongated.

## References

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