Independent vs. cooperative binding in polyethylenimine-DNA and poly(L-lysine)-DNA polyplexes

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Supporting Information

Hill plot model for multivalent ligand binding to multi-subunit substrate. Binding of a multivalent ligand such as cationic polymer to a multisubunit substrate such as DNA can be described by following binding equilibria:

Binding constant

DNA + P ⇌ DNA:P K_1

DNA:P + P ⇌ DNA:P$_2$ K_2

⋮

DNA:P$_{N-1}$ + P ⇌ DNA:P$_N$ K_N
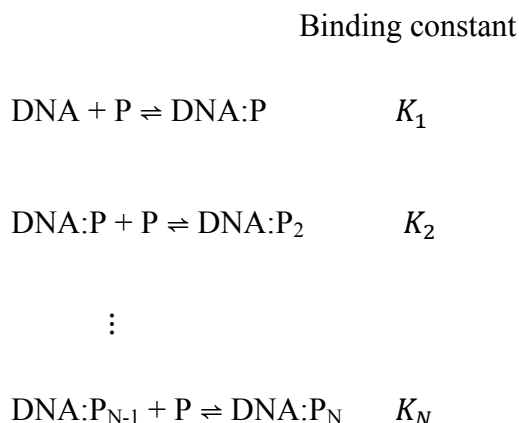
where P is one binding group of the ligand i.e. an active amine group of the polymer. The binding constant for the overall reaction DNA + NP ⇌ DNA:P$_N$ is

$$K_{tot} = K_1 \times K_2 \times ... \times K_N = \frac{[\text{DNA: P}_N]}{[DNA][P]^N}$$

The binding constants $K_1, K_2, ..., K_N$ will be unequal since the binding of an amine group to the DNA influences the binding off subsequent amine groups on the same DNA molecule, i.e. the binding is cooperative. If all phosphate groups of DNA are either unoccupied or all are occupied and no other situation is possible, the system is fully cooperative and N corresponds to the number of phosphate groups on the DNA. In practice the degree of cooperativity is less extreme and N is replaced with Hill coefficient α:

$$K_{tot} = \frac{[\text{DNA: P}_N]}{[DNA][P]^\alpha}$$

The proportion of DNA bound by the polymer, B, is

$$B = \frac{[\text{DNA:P}_\text{N}]}{[DNA] + [\text{DNA:P}_\text{N}]}$$

and the proportion of free DNA is

$$1 - B = \frac{[DNA]}{[DNA] + [\text{DNA:P}_\text{N}]}$$

Taking a ratio of these leads to

$$\frac{B}{1-B} = \frac{[\text{DNA:P}_\text{N}]}{[DNA]}$$

Combining this with the equation for K_{tot} :

$$\frac{B}{1-B} = K_{tot}[P]^\alpha = (K_{CO})^\alpha [P]^\alpha$$

where K_{CO} is the average of the individual binding constants $K_1, K_2, \ldots, K_N$. Taking the logarithm of both sides we obtain the Hill equation for multivalent ligand binding to multi-subunit substrate:

$$\ln\left(\frac{B}{1-B}\right) = \alpha\ln[P] + \ln(K_{CO})^\alpha$$

Since $\frac{B}{1-B} = \frac{A_{1,QY}}{A_2}$, we get

$$\ln\left(\frac{A_{1,QY}}{A_2}\right) = \alpha\ln[P] + \ln(K_{CO})^\alpha$$

which was used to determine the degree of cooperativity and binding constant for the present systems.

The values for the calculated curve of cooperative binding in **Figures 6** and **S4**, were obtained from

$$B = \frac{[P]^\alpha (K_{CO})^\alpha}{1 + [P]^\alpha (K_{CO})^\alpha}$$
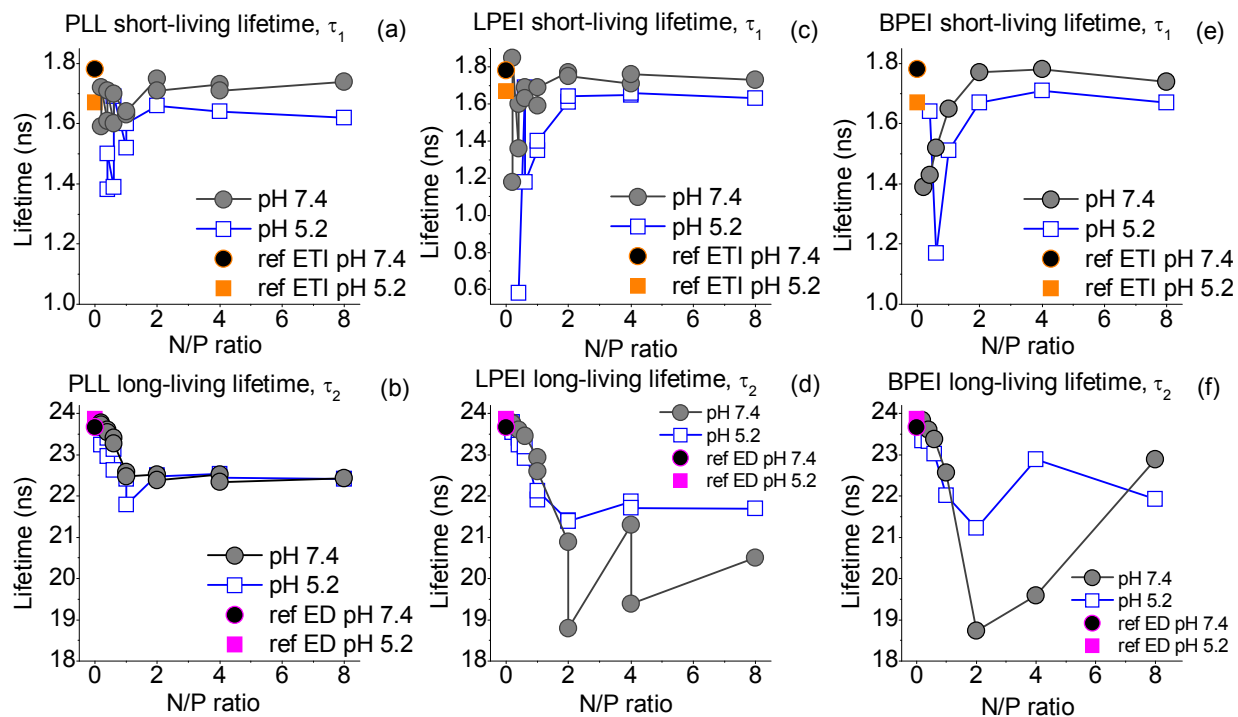
Figure S1. Fluorescence lifetimes of PLL (a, b), LPEI (c, d) and BPEI (e, f) at different pH-values. (ref ETI = free ETI in buffer at pH 7.4 (○) and at pH 5.2 (□), ref ED = ETI:DNA complex in buffer at pH 7.4 (○) and at pH 5.2 (□).) Short-living components (a, c, e) and long-living components (b, d, f).
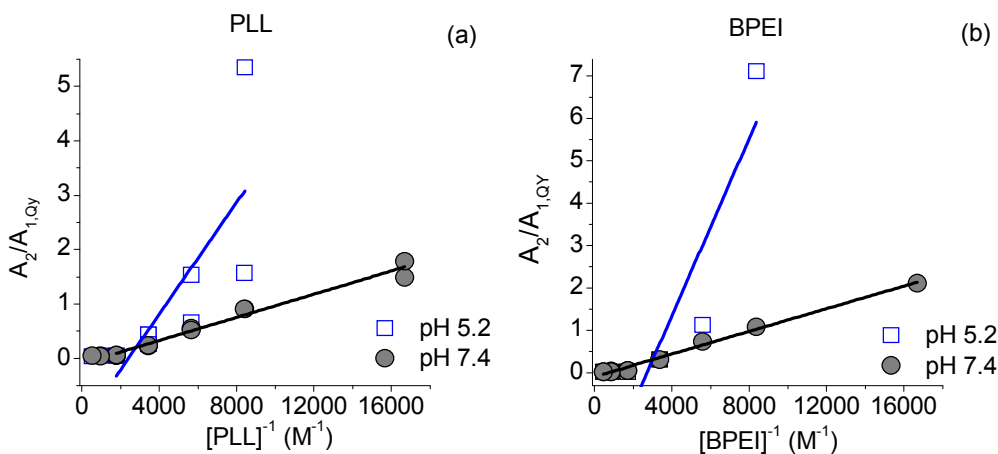

Figure S2. Ratio $A_2 / A_{1,QY}$ as a function of the inverse polymer concentration that the binding constant (as dm^{-3} mol) is equal to the inverse of the slope.
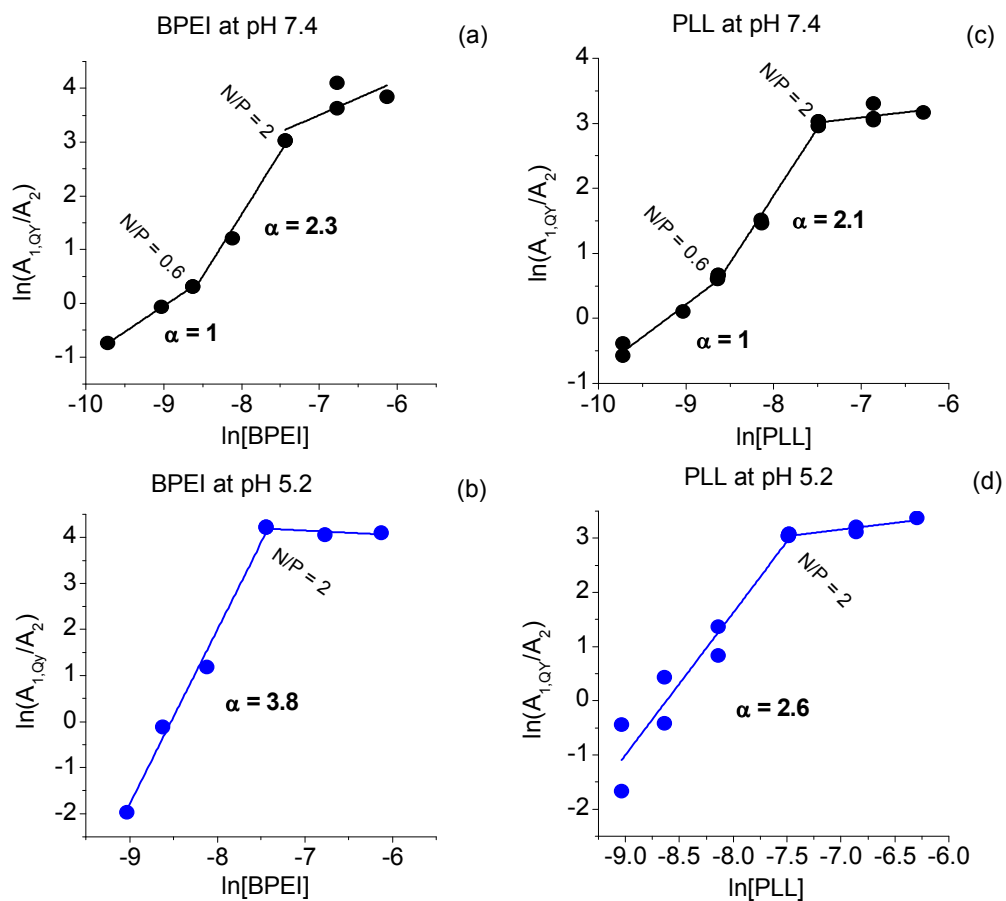
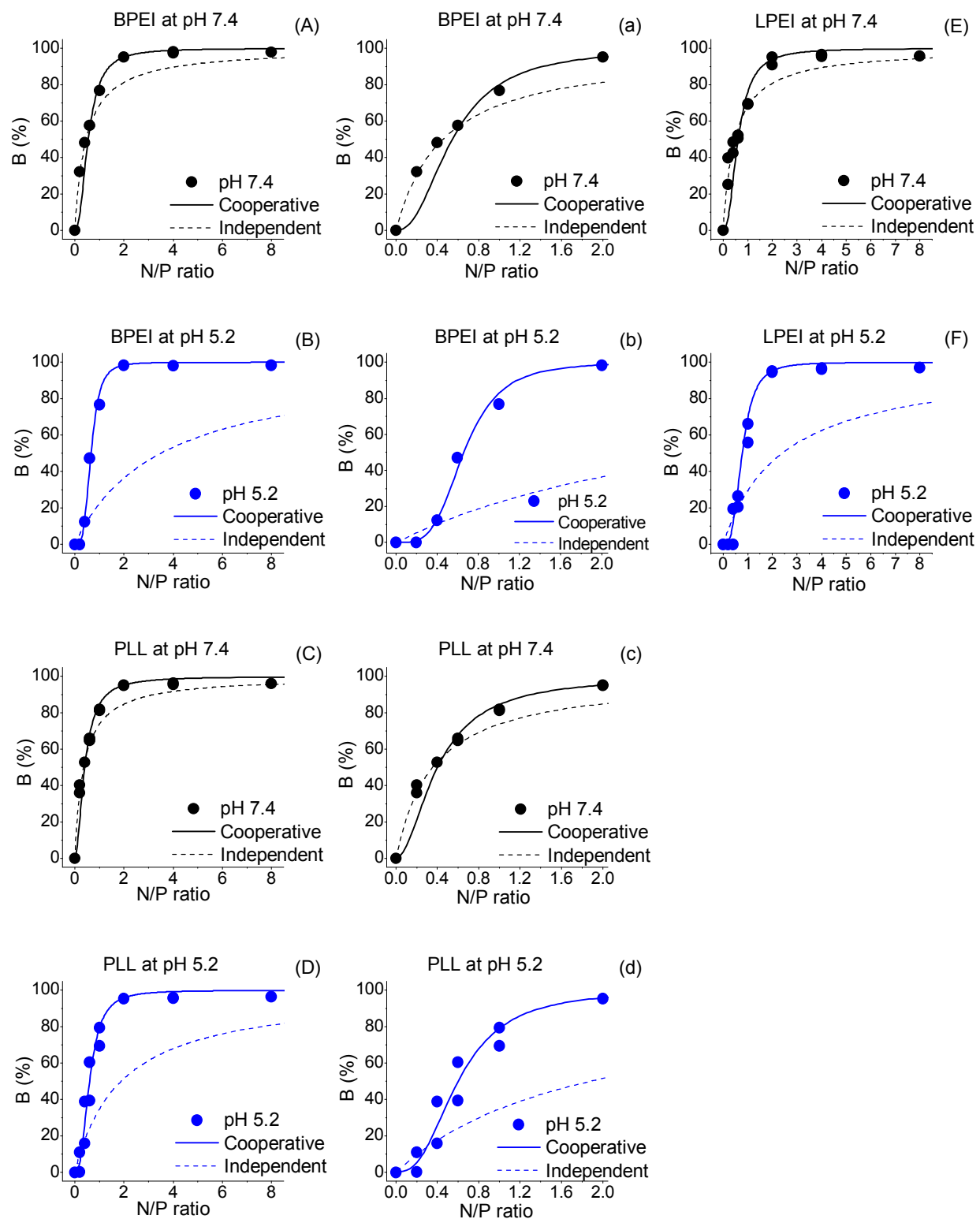**Figure S3.** Hill Plots of BPEI (a, b) and PLL (c, d) at pH 7.4 (a, c) and at pH 5.2 (b, d).

Figure S4. Independent vs. cooperative models for BPEI (A, a, B, b), PLL (C, c, D, d) and LPEI (E, F) at pH 7.4 (A, a, C, c, E) and at pH 5.2 (B, b, D, d, F).
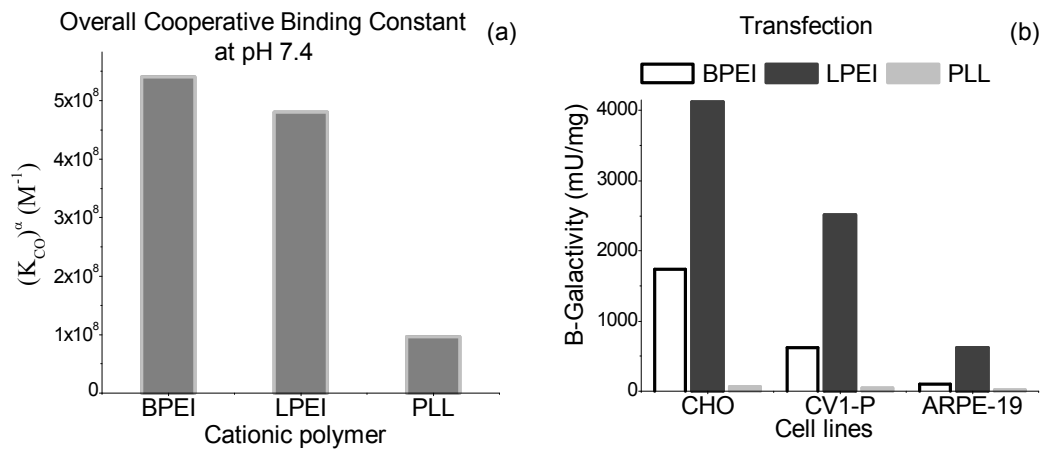
Figure S5. Overall cooperative binding constants at pH 7.4 (a) and transfection efficiencies in different cell lines at pH 7.2 (MES-HEPES-NaCl buffer) (b) for BPEI, LPEI and PLL.