SUPPORTING INFORMATION

FRET-Labeled siRNA Probes for Tracking Assembly and Disassembly of siRNA-Nanocomplexes

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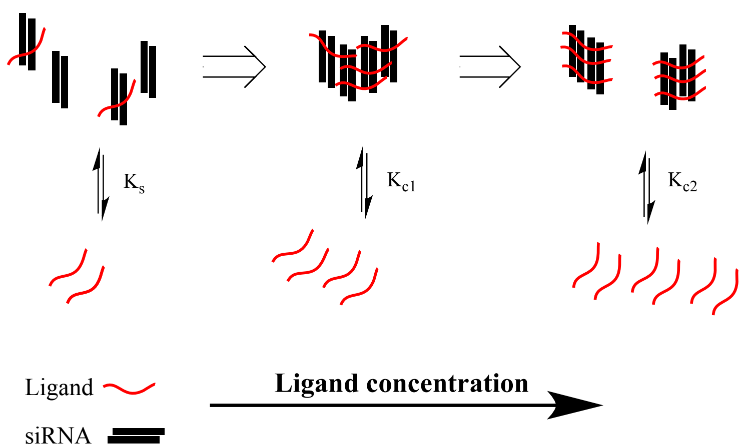

Figure S1. The two-state ligand-binding model as described by Lando and Teif. siRNA may exist in two states, the starting and complexed state, each represented with the equilibrium constants K_s and K_{c1} or K_{c2}. In the absence of ligand (delivery vehicle), nearly all the siRNAs exist in the starting state. Addition of ligand (delivery vehicle) shifts the equilibrium towards the complexed phase. Yet, at higher concentrations, redissolution of the complexed phase occurs (assuming more binding sites on siRNA exist) to give a second complexed phase, represented by K_{c2}.

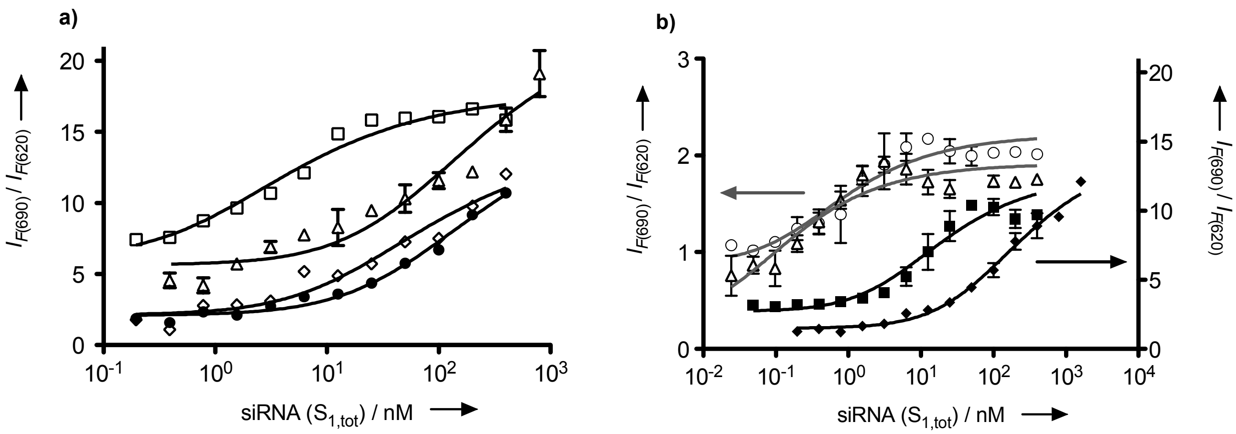

Figure S2. a) Ratiometric equilibrium binding FRET data of (□) C14-113, (△) ND98, (◇) C14-120, and (●) C16-96. b) Ratiometric equilibrium binding FRET data of (○) Ribojuice, (△) siPORT-Amine, (■) siPORT-NEOFX, and (◆) Lipofectamine. The lines are linear regression fits of the experimental FRET data to Eq. (2) and (3).

Derivation of Equilibrium binding model

$$S_1 + S_2 + DV \rightleftharpoons [(S_1 - S_2)_{DV}] + DV \tag{1}$$

$$K_D = \frac{[S_1][S_2]}{[(S_1 - S_2)_{DV}]} \tag{1a}$$

$$[S_1] = [S_{1,tot}] - [(S_1 - S_2)_{DV}] \tag{1b}$$

$$[S_2] = [S_{2,tot}] - [(S_1 - S_2)_{DV}] \tag{1c}$$

where $[S_1]$ = siAF594, $[S_2]$ = siAF647, $S_{1,tot}$ and $S_{2,tot}$ are the total amount of the respective siRNAs, DV = delivery vehicle (in excess), and $[(S_1 - S_2)_{DV}]$ represents nanocomplexes. For all the experiments conducted, $[S_{1,tot}] = [S_{2,tot}]$. By substituting eq. (1b) and (1c) into (1a), the following expression is obtained;

$$[(S_1 - S_2)_{DV}]^2 - (K_D + S_{1,tot} + S_{2,tot})\,[(S_1 - S_2)_{DV}] + S_{1,tot}\,S_{2,tot} = 0$$

with the quadratic solution;

$$[(S_1 - S_2)_{DV}] = \frac{K_D + S_{1,tot} + S_{2,tot} - \sqrt{(K_D + S_{1,tot} + S_{2,tot})^2 - 4S_{1,tot}S_{2,tot}}}{2} \tag{2}$$

The fraction of S_1 or S_2 in the complexed state can be represented with the FRET measurements via the following equation;

$$\frac{\text{FRET ratio}-\text{MIN}}{\text{MAX}-\text{MIN}} = \frac{[(S_1 - S_2)_{DV}]}{S_{1,tot}} \text{, where } S_{1,tot} \backsimeq S_{2,tot} \quad (3)$$