Molecular recognition of Nucleic Acids by Nucleolipid-Dendrimer surface complexes

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Electronic Supplementary information

Materials and Sample Preparation

Deionized water, passed through a Milli-Q purification system (giving resistivity = 18.2 mΩ.cm, organic content ≤ 4ppb), and/or D_2O (Sigma-Aldrich) was used to prepare all solutions. Sodium chloride (NaCl, Suprapure 99.99 %) was obtained from Merck and Trizma Base (99.9% titration) was purchased from Sigma-Aldrich. The samples were prepared in 10 mM Tris-HCl buffer with a pH of 7.6 or 10 mM NaCl solution, adjusted to pH 7.2-7.4 by adding small volumes of concentrated hydrochloric acid (Merck, for analysis 37 %) or sodium hydroxide (Sigma-Aldrich). Poly(amidoamine) dendrimer (PAMAM) with ethylendiamine core, generation 4 (G4, 10% wt. in methanol) was purchased from Sigma-Aldrich. The dendrimer samples in methanol were dried in a vacuum oven for 1 day before dissolution in the appropriate solvent. Standard fully hydrogenous 1,2-dilauroyl-sn-glycero-3-phosphocoline (hDLPC) and DLPC with deuterated lauroyl chains (dDLPC) were purchased from Avanti Polar Lipids. Dilauroyl-phospatidyl-adenosine hydrogenated (hDLPA) and deuterated (dDLPA) were synthesized from the corresponding phosphatidylcholine as described previously[1] and obtained as an ammonium salt. Adenine, hydrochloric acid, chloroform, methanol and ammonia (33% aqueous solution) used in the synthesis were purchased from Fluka. Phospholipase D from *Streptomyces sp* AA586 used in the nucleolipid synthesis was a generous gift from Asahi Chemical Industry Co., Ltd (Tokyo, Japan). Oligonucleotides of 20 monomers based in adenosine (20dA) or thymidine (20dT) were custom synthesized by ATDbio Ltd. Luciferace T7 plasmid DNA of 4331 base pairs (Promega) was amplified and purified as described by Ainalem et al.[2] Double stranded DNA (dsDNA) was diluted to approximately 35 ppm in 10 mM Tris-Buffer and the purity and concentration were checked by UV-spectroscopy. The ratio between the absorbance at 260 nm and at 280 nm was higher than 1.8 indicating that the DNA sample has negligible protein contamination. Single stranded DNA(ssDNA) was prepared using the procedure described by Yang et al.[3] dsDNA was heated to 95 °C for 10-15 min and rapidly cooled in an ice bath for 30 min. The separation of the strands was confirmed by UV-spectroscopy where an increase of approximately 30% in the absorbance at 260 nm compared to the dsDNA sample is observed. To avoid that the ssDNA sample will renature, the solution was prepared immediately before injection.

Attenuated Total Reflection Fourier Transform Infrared Spectroscopy Measurements

Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectra were obtained in a Nicolet iS50 FT-IR Spectrometer (Thermo Scientific, United States). The instrument was equipped with a mutireflection ATR accessory (Specac Gateway, Kent, UK) containing a cell which allowed for the flow of liquid across the largest face of a trapezoidal polished silicon crystal. The IR beam was focused on the crystal and it penetrated a short distance (~ 1 μm) from the interface before it was totally reflected. As a result, this method allows obtaining information on the chemical bonds of the molecules close to the silica-water interface. The spectra were recorded with a wavenumber resolution of 4 cm^{-1} in a range between 4000 cm^{-1} to 1500 cm^{-1}. The signal at lower wavenumbers is dominated by the absorption of the Si bonds and therefore it is not accessible in the identification of the molecules at the interface. The flow of different solutions was controlled with a peristaltic pump. Before the addition of the samples, the cell was flushed with large amounts of D_2O until no further changes in the spectra of the bare silicon crystal were obtained which indicated that the system has been evacuated from a high percentage of the water vapor. All the measurements were done with 10 mM NaCl in D_2O. Spectra of the addition of each sample were taken continuously until the data showed a steady state. All spectra were corrected for complete removal of H_2O by subtraction with the scaled water vapor spectra as described by Clifton et al.[4] The peaks were determined with the instrument software (Omnic) and the detailed assignment is presented in SI table 1.

SI TABLE 1. Absorption bands obtained from ATR FT-IR spectroscopy of the different components of the system

Figure	Curve	Wavenumber (cm^{-1})	Assignment
1a	3 and 4	2962	CH_3 asymmetric stretch from the DLPA tail
1a	3 and 4	2926	CH_2 asymmetric stretch from the DLPA tail
1a	3 and 4	2872	CH_3 symmetric stretch from the DLPA tail
1a	3 and 4	2853	CH_2 symmetric stretch from the DLPA tail
1b and 1c	2	1645	C=O stretch from amides of PAMAM-G4
1b and 1c	3 and 4	1743	C=O stretch from the DLPA
1b and 1c	3 and 4	1632 to 1622	C=N and C=C ring vibration of the adenosine ring from the DLPA head
1b and 1c	3 and 4	1579 to 1576	In-plane ring vibration of the adenosine ring from the DLPA head
1c	4	1712	H-bonded to the C2=O stretch of thymines of the third strand for T*A-T base triplets
1c	4	1671 to 1655	C=O stretch of single stranded thymidine from 20dT

Neutron Reflectometry Measurements

NR measurements were performed on the time-of-flight reflectometers INTER at ISIS (Didcot, U.K.),[5] FIGARO at the ILL (Grenoble, France)[6] and the monochromatic angle-dispersive reflectometer NREX operated by the Max Planck Institut at Forschungs-Neutronenquelle Heinz Maier-Leibnitz (Garching, Germany). INTER was operated in a wavelength band between 1.5 to 17 Å, FIGARO with a wavelength range of 2 to 30 Å and NREX at a default wavelength of 4.3 Å. Data acquisitions were carried out at fixed incident angles of 0.7° and 2.3° at INTER and 0.62° and 3.8° at FIGARO. The reflectivity profiles shown are the ratio of the number of neutrons in the specular ridge of the reflected beam to those in the incident beam, corrected for background scattering, as a function of the momentum transfer, Q:

$$Q = \frac{4\pi \sin\theta}{\lambda} \tag{1}$$

where θ is the angle of incidence and λ is the wavelength. The setup for NR experiments at the solid-liquid interface have been described elsewhere.[7] Liquid flow cells with an internal volume of ~ 2 mL were employed for all the measurements. Silicon crystals (dimensions $l \times w \times h$ of 80 × 50 × 10 mm) with a SiO_2 layer were used as a substrate. The crystals were freshly polished (Siltronix, France) and cleaned using a dilute piranha solution composed of water, sulfuric acid (Merck, for analysis 95-97 %) and hydrogen peroxide (Merck, for analysis 30 %) in a 5:4:1 volume ratio for 20 min at 80 °C. Approximately 20 mL of solution were employed in every sample injection to ensure the efficient exchange of the bulk solution. The measurements were performed using two different nucleolipid isotopic contrasts, hDLPA and dDLPA, and three different solvent isotopic contrasts, D_2O, H_2O and a mixture of D_2O and H_2O in a 0.38 D_2O volume fraction to contrast match the scattering length density of silicon. The scattering length densities of each component were calculated based on their atomic composition, the scattering length of each atom and their molecular volume and they are displayed in SI table 2.

NR Data Evaluation

The neutron reflectivity profiles were analyzed using the software Motofit,[8] based on the interaction of neutrons with a stratified layers model and using the Abeles matrix method.[9] The parameters to fit for each layer were the thickness (t), the scattering length density (ρ) or the solvent volume fraction ($v_{solvent}$) and the roughness (δ). The layers were always 'sandwiched' between the two media, Si and solvent. The silica substrate was modeled as one layer in three regions (Si-SiO_2-solvent) and the adsorption of the polymer as two interfacial layers in four regions (Si-SiO_2-PAMAM-solvent). The adsorption of DLPA onto the preadsorbed PAMAM monolayers before rinsing with solvent was modeled as four interfacial layers in 6 regions: Si-SiO_2-PAMAM+$DLPA_{heads}$-$DLPA_{heads}$+$DLPA_{tails}$-$DLPA_{heads}$,-solvent and the reflectivity with the fits and the volume fraction profiles are shown in Figure S1. For the data obtained after rinsing with solvent the PAMAM/DLPA layers, the model employed was: Si-SiO_2-PAMAM-(DLPA)x5-solvent. We always employed the model that gave the best fits in multiple isotopic contrasts with the minimum number of layers in order to obtain the minimum number of fitting parameters. The adsorption of nucleic acid (20dT or ssDNA) was modeled as: Si-SiO2-PAMAM+nucleic acid-(nucleic acid-DLPA)x5-nucleic acid-solvent. If the layer contained more than two components (including solvent), the volume fractions were determined using the scattering length densities fitted for the layer of the equivalent solvent isotopic contrasts:

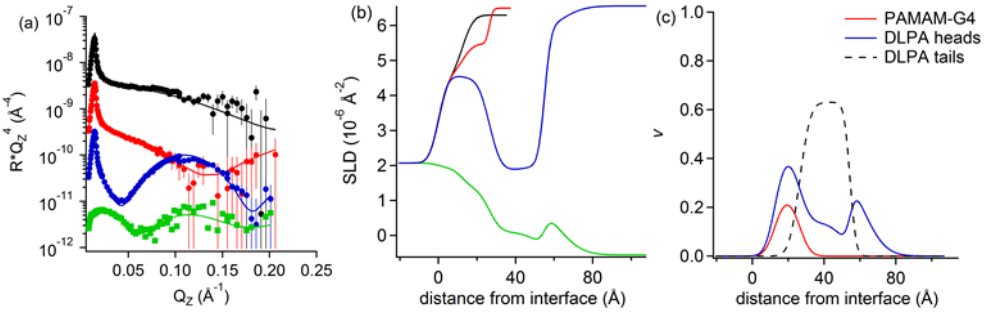
$$\rho_{layer} = \sum_i \rho_i v_i \tag{2}$$

where ρ_i and v_i are the scattering length density and the volume fraction of the component i respectively. The data with equivalent chemical layers in different isotopic contrasts were fitted with the MotoGlobal fit algorithm. The parameter errors are given by the variance-covariance matrix calculated using the Levenberg-Marquardt optimization.[10] The reflectivity data in multiple isotopic contrasts and the fitting parameters can be found in SI Figures 1 to 4 and SI Tables 3 to 13.

SI TABLE 2. Molecular volumes and Scattering Length Density (ρ) of the different components of the system

	Molecular Volume (Å^3)	ρ (10^{-6} Å^{-2})
PAMAM-G4 in H_2O/D_2O[a]	19290	1.2/2.2
Lauroyl chain[b] ($C_{22}H_{46}/C_{22}D_{46}$)	666	-0.39/6.8
PA head in H_2O/D_2O ($C_{15}H_{17}N_5O_{11}P$)[c]	448	3.4/4.3
20dT in H_2O/D_2O[d]	6204	2.8/3.1
20dA in H_2O/D_2O[d]	6310	3.4/4.1
DNA in H_2O/D_2O[e]	-	3.7/4.1
Solvent (H_2O/ D_2O/cmSi)	30	-0.56/6.36/2.07

[a] The scattering length density of the dendrimer in D_2O is affected by proton/deuterium exchange from the surface amine groups.[11] [b] The molecular volume was obtained based on the calculations of Armen et al.[12] [c] The molecular volume were obtained from Milani et al.[13] and the scattering length density in D_2O correspond to 4 exchangeable hydrogens of adenosine at pH 7. [d] The molecular volumes and scattering length densities were calculated using the biomolecular scattering length density provided by ISIS. 20dA and 20dT have 42 and 22 exchangeable hydrogens at pH 7 respectively. [e] The scattering length density of DNA was taken from Ainalem et al.[11]

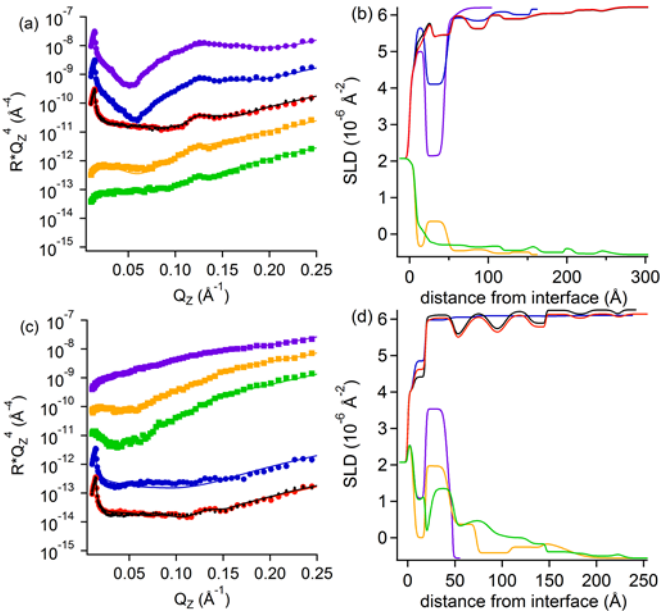


SI FIGURE 1. (a) Neutron reflectivity profiles, recorded using NREX, for a bare silicon crystal in D_2O (black circles), the adsorption of 100 ppm PAMAM-G4 in D_2O (red circes) and the addition of 0.1 mM hDLPA in D_2O (blue circles) and in H_2O (green squares) before rinsing with solvent and (b) the corresponding scattering length density profiles (SLD) as a function of the distance to the substrate (Si). The lines in (a) correspond to the calculated models and the data was offset in the y-axis for clarity. (c) Volume fraction (v) of PAMAM-G4, DLPA heads and DLPA tails as a function of the distance to the substrate (Si) before rinsing with solvent. The solvent was aqueous solution of 10 mM NaCl.

SI TABLE 3. Parameters obtained from the modeling of the NR profiles of the DLPA adsorption before rinsing with solvent (SI Figure 1)

Layer	t_i (Å)	δ (Å)	v_{SiO2}	$v_{PAMAM-G4}$	$v_{DLPA\ heads}$	$v_{DLPA\ tails}$	$V_{solvent}$
1	12.5 ± 0.5	4.5 ± 0.5	0.63 ± 0.03	0	0	0	0.37 ± 0.03
2	13.9 ± 0.5	4.7 ± 0.4	0	0.24 ± 0.01	0.41 ± 0.04	0	0.35 ± 0.04
3	28.6 ± 0.3	2.7 ± 0.7	0	0	0.14 ± 0.01	0.63 ± 0.01	0.23 ± 0.02
4	6.2 ± 0.4	10.4 ± 0.6	0	0	0.42 ± 0.02	0	0.58 ± 0.02

t_i represents the thickness of the layer i, δ the roughness between the layer i and the layer $i + 1$ (or the bulk) and v the volume fraction of the different components.



SI FIGURE 2. Neutron reflectivity profiles, recorded using INTER, for 0.1 mM hDLPA/D_2O (a, purple circles) and dDLPA/H_2O (c, purple squares) adsorbed onto a preadsorbed PAMAM-G4 monolayer on hydrophilic silica followed by rinses with H_2O (orange squares), D_2O (blue circles) and the addition of 200 ppm 20dT/D_2O before (black circles) and after rinsing with H2O (green squares) and D2O (red circles). (b) and (d) correspond to the scattering length density profiles (SLD) of (a) and (c) respectively as a function of the distance (d) to the substrate (Si). The lines in (a,c) correspond to the calculated models and the data was offset in the y-axis for clarity. The solvent was aqueous solution of 10 mM NaCl. The reflectivity for the addition of 20dT shows that multiple rinses with solvent have no effect on the reflectivity profiles.

SI TABLE 4. Parameters obtained from the modeling of the NR profiles of the hDLPA adsorption after rinsing with solvent before the addition of 20dT (SI Figure 2 a and b)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	7.2 ± 0.8	2	0.37
2	PAMAM-G4	12.6 ± 0.9	2	0.87
3	DLPA	27.9 ± 0.3	4	0.55
4	DLPA	28 ± 2	4	0.95
5	DLPA	26 ± 2	5	0.93
6	DLPA	27 ± 3	3	0.98
7	DLPA	25 ± 3	1	0.97

The parameters are described in the footnote of SI table 3.

SI TABLE 5. Parameters obtained from the modeling of the NR profiles for the addition of 200 ppm 20dt to the PAMAM/hDLPA layers. (SI Figure 2 a and b)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	7.2 ± 0.8	2	0.37
2*	PAMAM-G4+20dT	12.6 ± 0.9	8	0.69
3	20dT	8.4 ± 0.7	2	0.94
4	DLPA	25.3 ± 0.8	2	0.84
5	20dT	20.0 ± 0.9	4	0.92
6	DLPA	25 ± 1	3	0.87
7	20dT	19 ± 2	2	0.93
8	DLPA	33 ± 2	5	0.93
9	20dT	13 ± 3	4	0.93
10	DLPA	33 ± 4	1	0.96
11	20dT	12 ± 3	3	0.95
12	DLPA	31 ± 4	4	0.98
13	20dT	11 ± 4	12	0.95

The parameters are described in the footnote of SI table 3. * The volume fraction of PAMAM-G4 and 20dT in layer 2 were 0.13 and 0.18 respectively.

SI TABLE 6. Parameters obtained from the modeling of the NR profiles of the dDLPA adsorption after rinsing with solvent before the addition of 20dT (SI Figure 2 c and d)

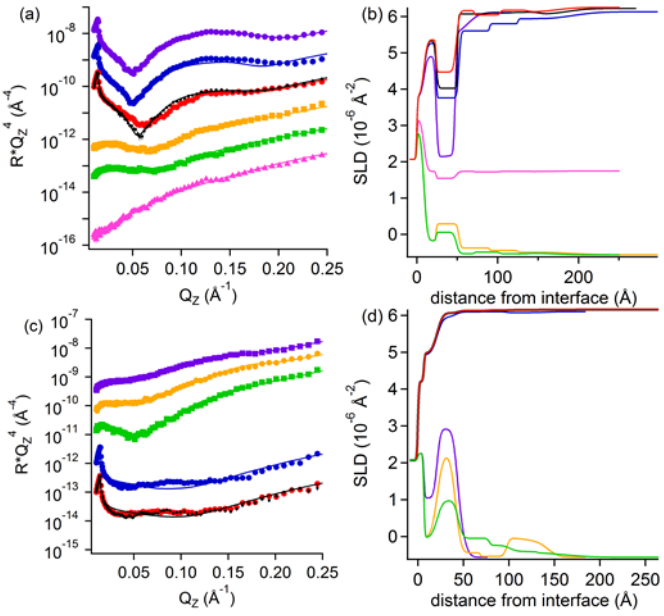
Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	6.2 ± 0.5	2	0.21
2	PAMAM-G4	12.4 ± 0.3	1	0.68
3	DLPA	25.0 ± 0.7	4	0.58
4	DLPA	29 ± 1	2	0.84
5	DLPA	36 ± 3	2	0.98
6	DLPA	34 ± 3	3	0.95
7	DLPA	27 ± 3	16	0.93

The parameters are described in the footnote of SI table 3.

SI TABLE 7. Parameters obtained from the modeling of the NR profiles for the addition of 200 ppm 20dt to the PAMAM/dDLPA layers (SI Figure 2 c and d)

Layer	Type	d (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	6.2 ± 0.5	2	0.21
2*	PAMAM-G4+20dT	12.4 ± 0.3	1	0.51
3	20dT	4.1 ± 0.3	4	0.93
4	DLPA	25.7 ± 0.4	3	0.68
5	20dT	12.7 ± 0.6	7	0.75
6	DLPA	25.5 ± 0.8	6	0.83
7	20dT	16.6 ± 0.9	6	0.81
8	DLPA	25 ± 1	4	0.91
9	20dT	18 ± 1	1	0.85
10	DLPA	28 ± 3	4	0.97
11	20dT	17 ± 4	4	0.97
12	DLPA	29 ± 4	4	0.99
13	20dT	5 ± 2	3	0.94

The parameters are described in the footnote of SI table 3. * The volume fraction of PAMAM-G4 and 20dT in layer 2 were 0.32 and 0.17 respectively.



SI FIGURE 3. Neutron reflectivity profiles, recorded using INTER, for 0.1 mM hDLPA/D_2O (a, purple circles) and dDLPA/H_2O (c, purple squares) adsorbed onto a preadsorbed PAMAM-G4 monolayer on hydrophilic silica followed by rinses with H_2O (orange squares), D_2O (blue circles) and the addition of 200 ppm 20dA/D_2O before (black circles) and after rinsing with H2O (green squares), D2O (red circles) and cmSi (pink triangles). (b) and (d) correspond to the scattering length density profiles (SLD) of (a) and (c) respectively as a function of the distance to the substrate (Si). The lines in (a,c) correspond to the calculated models and the data was offset in the y-axis for clarity. The solvent was aqueous solution of 10 mM NaCl. The slight difference in the reflectivity profiles after the addition of 20dA and the subsequent rinsing with solvent could not be modeled as 20dA adsorption but it might be attributed to changes in the conformation of the DLPA layer due to the change in the ionic strength of the bulk solution upon addition of the oligonucleotide.

SI TABLE 8. Parameters obtained from the modeling of the NR profiles of the hDLPA adsorption after rinsing with solvent before the addition of 20dA (SI Figure 3 a and b)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	8.8 ± 0.2	3	0.14
2	PAMAM-G4	15.0 ± 0.5	1	0.78
3	DLPA	28.2 ± 0.4	2	0.49
4	DLPA	38 ± 3	1	0.89
5	DLPA	35 ± 5	1	0.93
6	DLPA	34 ± 8	3	0.96
7	DLPA	34 ± 9	25	0.96

The parameters are described in the footnote of SI table 3.

SI TABLE 9. Parameters obtained from the modeling of the NR profiles for the addition of 200 ppm 20dA to the PAMAM/hDLPA layers (SI Figure 3 a and b)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	8.8	3	0.14
2	PAMAM-G4	15.0	1	0.78
3	DLPA	25.1 ± 0.3	3	0.63
4	DLPA	22 ± 3	1	0.98
5	DLPA	32 ± 5	2	0.96
6	DLPA	35 ± 8	4	0.98
7	DLPA	36 ± 7	22	0.97

The parameters are described in the footnote of SI table 3. The slight differences compared to SI table 8 are explained under SI Figure 3.

SI TABLE 10. Parameters obtained from the modeling of the NR profiles of the dDLPA adsorption after rinsing with solvent before the addition of 20dA (SI Figure 3 c and d)

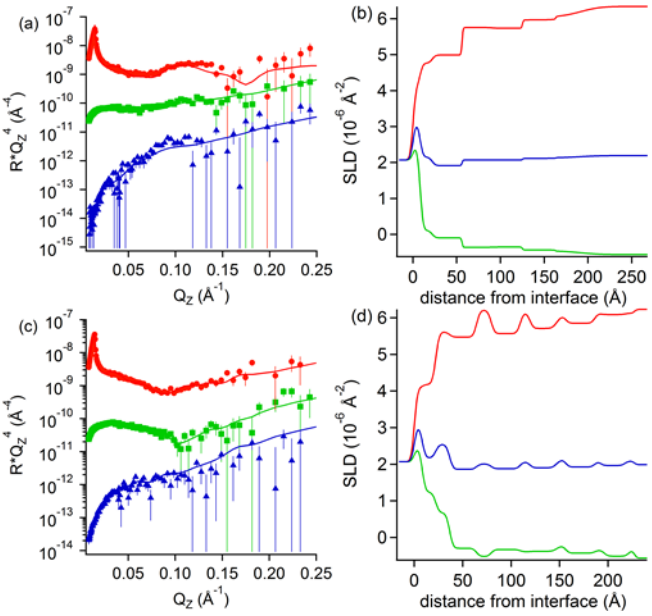
Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	6.5 ± 0.5	1	0.29
2	PAMAM-G4	14.5 ± 0.4	5	0.70
3	DLPA	20.4 ± 0.5	5	0.53
4	DLPA	27 ± 2	1	0.98
5	DLPA	29 ± 2	3	0.99
6	DLPA	23 ± 2	12	0.91
7	DLPA	23 ± 3	9	0.93

The parameters are described in the footnote of SI table 3.

SI TABLE 11. Parameters obtained from the modeling of the NR profiles for the addition of 200 ppm 20dA to the PAMAM/dDLPA layers (SI Figure 3 c and d)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	6.5 ± 0.5	1	0.29
2	PAMAM-G4	14.5 ± 0.4	5	0.70
3	DLPA	23.4 ± 0.7	4	0.74
4	DLPA	34 ± 4	2	0.91
5	DLPA	23 ± 5	6	0.95
6	DLPA	30 ± 11	1	0.97
7	DLPA	28 ± 11	25	0.98

The parameters are described in the footnote of SI table 3. The slight differences compared to SI table 10 are explained under SI Figure 3.



SI FIGURE 4. Neutron reflectivity profiles, recorded using FIGARO, for (a) 0.1 mM hDLPA adsorbed onto a preadsorbed PAMAM-G4 monolayer on hydrophilic silica after rinsing with solvent and (c) for the addition of 70 ppm ssDNA to the layers in (a). The isotopic contrasts were D_2O (red circles), H_2O (green squares) and cmSi (blue triangles). (b) and (d) correspond to the scattering length density profiles (SLD) of (a) and (c) respectively as a function of the distance to the substrate (Si). The lines in (a,c) correspond to the calculated models and the data was offset in the y-axis for clarity. The solvent was 10 mM Tris-HCl pH 7.6 buffer. No changes in the reflectivity profiles were observed after multiple rinses.

SI TABLE 12. Parameters obtained from the modeling of the NR profiles of the hDLPA adsorption after rinsing with solvent before the addition of ssDNA (SI Figure 4 a and b)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	8.2 ± 0.5	3	0.24
2	PAMAM-G4	13.9 ± 0.2	3	0.62
3	DLPA	34 ± 1	1	0.72
4	DLPA	35 ± 3	4	0.88
5	DLPA	34 ± 3	1	0.88
6	DLPA	38 ± 7	1	0.92
7	DLPA	28 ± 9	18	0.94

The parameters are described in the footnote of SI table 3.

SI TABLE 13. Parameters obtained from the modeling of the NR profiles for the addition of 70 ppm ssDNA to the PAMAM/hDLPA layers (SI Figure 4 c and d)

Layer	Type	t_i (Å)	δ (Å) ± 1	$v_{solvent}$ ± 0.01
1	SiO_2	8.2 ± 0.5	3	0.24
2*	PAMAM-G4+ssDNA	13.9 ± 0.2	3	0.33
3	ssDNA	14.3 ± 0.7	3	0.71
4	DLPA	27.9 ± 0.2	3	0.84
5	ssDNA	15 ± 1	3	0.99
6	DLPA	30 ± 2	3	0.86
7	ssDNA	10 ± 1	3	0.96
8	DLPA	28 ± 2	4	0.89
9	ssDNA	9 ± 2	3	0.92
10	DLPA	30 ± 4	3	0.92
11	ssDNA	6 ± 3	3	0.95
12	DLPA	25 ± 5	3	0.97
13	ssDNA	10 ± 5	2	0.95

The parameters are described in the footnote of SI table 3. * The volume fraction of PAMAM-G4 and ssDNA in layer 2 were 0.38 and 0.29 respectively.

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