Effects of Molecular Size and Surface Chemistry on Oligonucleotide Interfacial Dynamics

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Supporting Information

Single-molecule total internal reflection fluorescence microscopy was used to observe the dynamic behavior of ssDNA (1-50 nucleotides long)at the interface between aqueous solution and solid surfaces with varying hydrophobicity – oligoethylene oxide-modified fused silica (OEG0, and octadecyltriethoxysilane-modified fused silica (OTES). This approach permitted the extraction of residence time distributions and cumulative step size distributions over a range of temperatures. Due to the quantity of results obtained in these experiments, some details were omitted from the main text of the manuscript to focus on important conclusions. Therefore, additional raw data and the numerical values of all parameters are presented here, including data used to determine the photobleaching rate (Section I Figure S1), the detailed characteristic residence times of all populations (Section II Table S1 and S2), diffusion coefficients of multiple modes (Section III Table S3 and S4) and their population fractions. Example raw cumulative squared-displacement distributions are given here in section III (Figure S2).

I. Photobleaching Rate Determination Data

Time-lapse imaging of the surface was performed under continuous TIRF illumination. The time-lapse interval for image acquisition was 4 s and imaging was performed for 600 s. Single molecules were identified on a frame by frame basis, counted, and tabulated versus time. If a molecule disappeared for one or more frames but then reappeared in the same position, that molecule was considered to have undergone a blinking event as opposed to actual photobleaching. The results were then plotted and fit using an exponential decay expected from first order kinetics model. An example figure for fluorescein photobleaching experiments is shown in Figure S1.

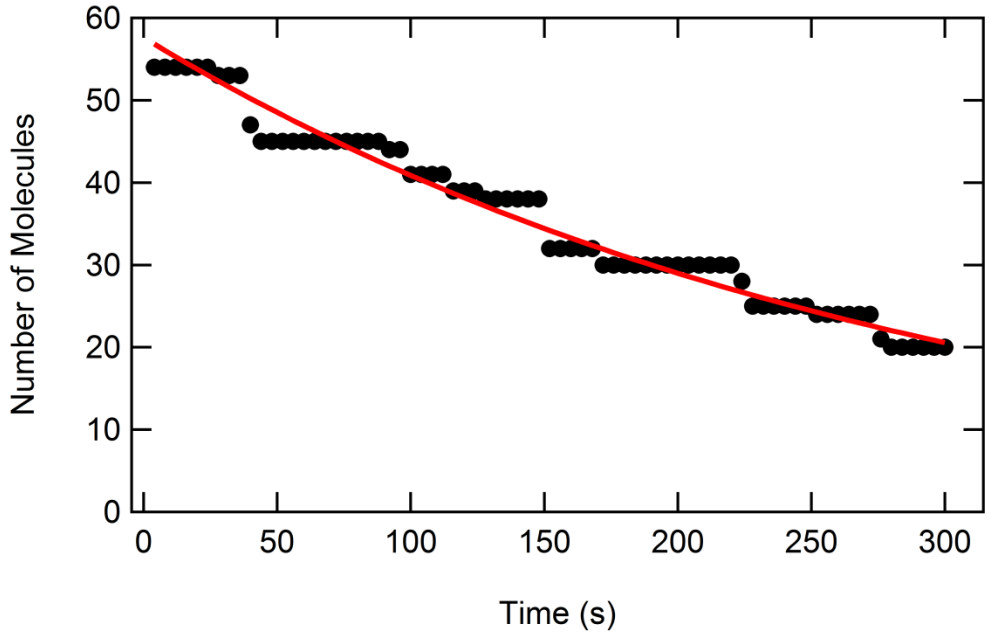

Figure S1. Number of molecules versus exposure time with best fit line based on a first order kinetic model.

II. Residence Time Distribution Parameters

Residence time distributions were fit to a tri- exponential function to characterize each observed population with a characteristic residence time and population fraction. The three populations were designated the short, moderate and long residence time modes based on the characteristic residence times. The parameters of the exponential fits for OTES and OEG are given in Table S1 and S2, respectively.

Table S1. Characteristic residence times for the short, moderate and long residence time modes and their respective population fractions for the OTES surface.

OTES							
		Short Mode		Moderate Mode		Long Mode	
Molecule	Temperature (K)	Characteristic Residence Time	Population Fraction	Characteristic Residence Time	Population Fraction	Characteristic Residence Time	Population Fraction
	283	0.276(7)	0.927(3)	1.28(4)	0.069(2)	7.7(4)	0.0040(3)
	293	0.41(1)	0.875(5)	1.88(7)	0.113(4)	9.1(4)	0.0115(8)
	303	0.40(1)	0.855(5)	1.86(6)	0.134(5)	13.3(5)	0.0112(6)
	313	0.35(1)	0.857(3)	1.45(4)	0.139(3)	10.3(5)	0.0040(2)
dCTP	323	0.31(1)	0.859(6)	1.20(5)	0.132(6)	5.5(2)	0.0090(8)
	283	0.39(1)	0.848(4)	1.81(5)	0.140(4)	10.4(4)	0.0116(6)
	293	0.41(1)	0.851(5)	1.87(6)	0.132(5)	10.1(3)	0.0161(8)
	303	0.44(1)	0.848(4)	1.87(6)	0.134(4)	9.8(2)	0.0180(8)
	313	0.41(1)	0.860(4)	1.82(5)	0.128(4)	10.9(2)	0.0131(5)
C5	323	0.396(9)	0.854(4)	1.74(4)	0.133(4)	9.0(2)	0.0130(5)
	283	0.36(1)	0.833(6)	1.62(5)	0.153(5)	8.4(3)	0.0143(9)
	293	0.36(1)	0.887(5)	1.63(6)	0.104(5)	9.8(4)	0.0090(6)
	303	0.28(1)	0.887(7)	1.17(7)	0.102(6)	6.6(4)	0.011(1)
	313	0.295(8)	0.897(4)	1.32(4)	0.095(3)	7.6(3)	0.0083(5)
C10	323	0.329(8)	0.920(3)	1.52(5)	0.075(3)	10.5(4)	0.0043(3)
	283	0.38(1)	0.861(6)	1.51(6)	0.126(6)	8.5(3)	0.0126(6)
	293	0.36(1)	0.883(5)	1.65(6)	0.108(4)	11.7(5)	0.0087(5)
	303	0.323(8)	0.896(3)	1.52(4)	0.097(3)	9.3(3)	0.0071(4)
	313	0.33(1)	0.901(4)	1.50(6)	0.089(4)	7.9(3)	0.0097(7)
C25	323	0.33(1)	0.905(4)	1.56(7)	0.087(4)	9.2(5	0.0077(7)
	283	0.26(1)	0.894(6)	1.54(4)	0.091(5)	8.81(1)	0.014(1)
	293	0.27(1)	0.899(6)	1.45(8)	0.089(5)	7.50(1)	0.011(1)
	303	0.234(9)	0.945(3)	1.28(8)	0.050(3)	8.89(1)	0.0047(5)
	313	0.269(3)	0.980(2)	1.4(1)	0.018(1)	6.850(3)	0.0023(3)
C50	323	0.269(2)	0.9898(5)	1.282(8)	0.009(1)	9.997(1)	0.0009(1)

Table S2. Characteristic residence times for the short, moderate and long residence time modes and their respective population fractions for the OEG surface.

OEG							
		Short Mode		Moderate Mode		Long Mode	
Molecule	Temperature (K)	Characteristic Residence Time	Population Fraction	Characteristic Residence Time	Population Fraction	Characteristic Residence Time	Population Fraction
	283	0.196(9)	0.888(4)	0.67(2)	0.100(4)	1.80(4)	0.012(1)
	293	0.237(8)	0.848(6)	0.79(2)	0.145(5)	2.5(1)	0.0078(9)
	303	0.158(6)	0.966(3)	0.83(2)	0.030(3)	5.2(2)	0.0039(5)
	313	0.160(6)	0.955(3)	0.74(3)	0.040(3)	4.8(1)	0.0048(4)
dCTP	323	0.167(4)	0.958(5)	0.90(5)	0.040(5)	5.1(4)	0.0036(6)
	283	0.195(2)	0.970(2)	0.91(5)	0.027(1)	3.6(2)	0.0035(3)
	293	0.207(5)	0.970(1)	1.03(5)	0.027(1)	3.7(5)	0.0026(2)
	303	0.187(6)	0.976(2)	1.08(6)	0.022(1)	7.0(6)	0.0018(2)
	313	0.196(4)	0.977(1)	1.00(2)	0.022(1)	6.1(5)	0.0014(1)
C5	323	0.190(1)	0.996(1)	1.04(9)	0.0038(1)	6.4(7)	0.0006(1)
	283	0.25(1)	0.896(7)	1.14(6)	0.096(6)	5.3(4)	0.0078(1)
	293	0.27(1)	0.846(9)	1.08(5)	0.140(8)	4.3(2)	0.0132(2)
	303	0.21(1)	0.955(4)	1.18(5)	0.041(3)	7.2(5)	0.0042(5)
	313	0.24(1)	0.946(2)	1.14(5)	0.049(2)	6.7(3)	0.0053(4)
C10	323	0.20(1)	0.940(5)	1.09(4)	0.055(4)	7.0(2)	0.0054(6)
	283	0.39(1)	0.850(5)	1.83(6)	0.131(4)	10.5(2)	0.0191(8)
	293	0.37(1)	0.896(5)	1.69(7)	0.094(5)	9.6(4)	0.0106(7)
	303	0.39(2)	0.861(8)	2.0(1)	0.132(7)	11.5(9)	0.0076(1)
	313	0.32(1)	0.902(5)	1.95(9)	0.084(4)	12.1(6)	0.0137(1)
C25	323	0.21(1)	0.924(6)	1.94(4)	0.070(6)	9.9(5)	0.0063(6)
	283	0.40(1)	0.847(5)	1.78(5)	0.136(5)	12.7(2)	0.0168(6)
	293	0.39(1)	0.881(5)	2.02(8)	0.108(5)	15.1(7)	0.0109(7)
	303	0.41(1)	0.873(4)	2.04(5)	0.115(4)	14.0(4)	0.0117(5)
	313	0.39(1)	0.877(4)	1.95(6)	0.111(4)	11.5(4)	0.0116(6)
C50	323	0.39(1)	0.887(1)	2.06(6)	0.103(3)	13.4(5)	0.0102(6)

III. Cumulative Squared Displacement Distributions

Experimental cumulative squared-displacement distributions were created by sorting the squared-displacement data and ranking each data point. The distributions demonstrating the effect of temperature on squared displacement are shown in Figure S2 for the C5 probe on both OTES and OEG surfaces, and demonstrate that squared displacement initially increases on the OTES surface up to 30C and then decreases with a continued increase in temperature. A multiple-Gaussian function was fitted to each cumulative distribution to extract population fractions and their respective diffusion coefficients. The best-fit parameters are given in Tables S3 and S4.

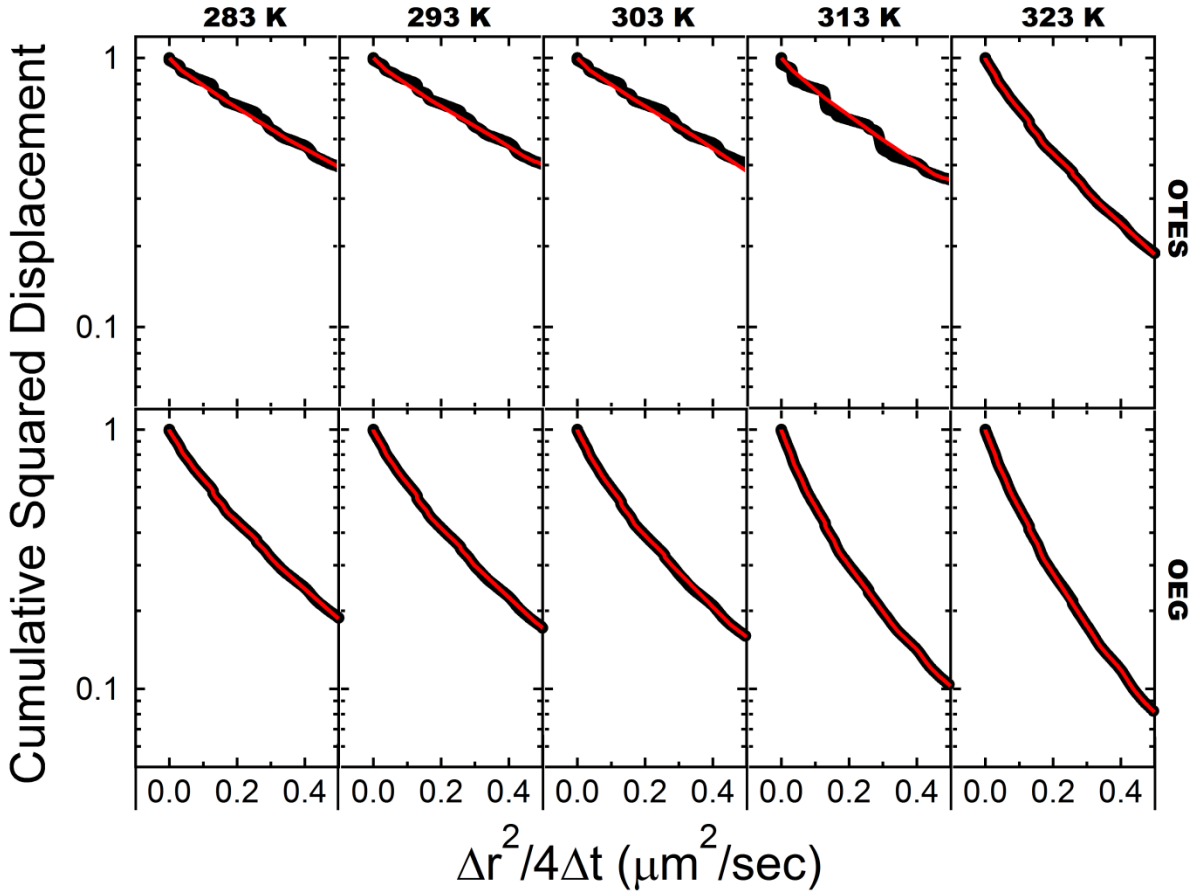

Figure S2. Semi-log plots of the cumulative squared displacement distributions of C5, on OTES modified fused silica (top row), and OEG-modified fused silica (bottom row). Error bars are present in the figure but are generally smaller than the symbols.

Table S3. Diffusion coefficients and respective population fractions for the flying mode of diffusion for each surface/probe combination.

Flying Mode					
Surface		OTES		OEG	
Molecule	Temperature (K)	Diffusion Coefficient $\mu m^2/s$	Population Fraction	Diffusion Coefficient $\mu m^2/s$	Population Fraction
	283	0.1763(1)	0.2411(3)	0.2808(1)	0.8074(2)
	293	0.2284(1)	0.4739(2)	0.2943(1)	0.8044(3)
	303	0.2982(1)	0.8959(3)	0.2972(3)	0.8383(9)
	313	0.2729(1)	0.6738(2)	0.2974(3)	0.828(1)
dCTP	323	0.2368(1)	0.5309(3)	0.2964(3)	0.816(1)
	283	0.3107(1)	0.8519(2)	0.3669(1)	0.9486(3)
	293	0.4062(1)	0.9900(3)	0.3864(1)	0.9410(3)
	303	0.4466(1)	0.9975(3)	0.3659(1)	0.9334(3)
	313	0.3321(2)	0.8027(3)	0.4228(2)	0.9554(3)
C5	323	0.2430(1)	0.5912(2)	0.3618(3)	0.8709(6)
	283	0.2117(1)	0.4521(2)	0.2650(1)	0.5413(3)
	293	0.2768(1)	0.7400(2)	0.2640(1)	0.6504(3)
	303	0.3043(1)	0.8454(1)	0.2868(4)	0.726(1)
	313	0.2789(1)	0.7289(3)	0.3003(3)	0.8054(9)
C10	323	0.2658(1)	0.6884(4)	0.2904(3)	0.8298(9)
	283	0.2545(1)	0.6402(4)	0.2338(5)	0.5281(2)
	293	0.2529(1)	0.5986(2)	0.2471(4)	0.5797(1)
	303	0.2487(1)	0.6340(2)	0.2416(5)	0.5922(2)
	313	0.2481(1)	0.5865(2)	0.2685(1)	0.7551(4)
C25	323	0.2536(1)	0.6387(2)	0.2850(1)	0.8079(6)
	283	0.3370(1)	0.8329(9)	0.2597(5)	0.4837(1)
	293	0.3412(1)	0.839(1)	0.2766(4)	0.5550(1)
	303	0.3233(1)	0.796(2)	0.2579(8)	0.4882(2)
	313	0.3386(1)	0.835(1)	0.2657(7)	0.4871(2)
C50	323	0.3574(1)	0.8657(7)	0.2853(5)	0.5810(2)

Table S4. Diffusion coefficients and respective population fractions for the crawling mode of diffusion for each surface/probe combination.

Crawling Mode					
Surface		OTES		OEG	
Molecule	Temperature (K)	Diffusion Coefficient $\mu m^2/s$	Population Fraction	Diffusion Coefficient $\mu m^2/s$	Population Fraction
	283	0.0419(1)	0.7589(3)	0.0603(1)	0.1925(2)
	293	0.0471(1)	0.5260(2)	0.0495(2)	0.1956(3)
	303	0.0486(2)	0.1041(3)	0.0326(4)	0.1617(9)
	313	0.0646(1)	0.3262(2)	0.0325(5)	0.172(1)
dCTP	323	0.0713(1)	0.4691(3)	0.0341(4)	0.184(1)
	283	0.0489(1)	0.1481(2)	0.01038(3)	0.0514(3)
	293	0.0551(2)	0.0100(2)	0.01257(3)	0.0590(3)
	303	0.0569(3)	0.0025(3)	0.01476(3)	0.0666(3)
	313	0.0695(2)	0.1973(3)	0.01557(3)	0.0446(3)
C5	323	0.0700(3)	0.4088(2)	0.01638(3)	0.1291(6)
	283	0.0534(1)	0.5479(2)	0.06088(1)	0.4587(3)
	293	0.0572(1)	0.2600(2)	0.06462(1)	0.3496(3)
	303	0.0456(2)	0.1546(1)	0.03858(5)	0.2742(9)
	313	0.0775(1)	0.2711(3)	0.03227(4)	0.1946(9)
C10	323	0.0692(1)	0.3116(4)	0.03852(4)	0.1701(9)
	283	0.0507(2)	0.3598(4)	0.06057(2)	0.4720(2)
	293	0.0576(3)	0.4014(2)	0.06152(2)	0.4203(1)
	303	0.0649(1)	0.3660(2)	0.05618(2)	0.4078(2)
	313	0.0613(1)	0.4135(2)	0.05105(1)	0.2449(4)
C25	323	0.0613(1)	0.3613(2)	0.03862(1)	0.1921(6)
	283	0.0263(3)	0.1671(9)	0.05587(2)	0.5163(1)
	293	0.053(2)	0.161(1)	0.05565(2)	0.4450(1)
	303	0.0309(3)	0.204(2)	0.05992(3)	0.5118(2)
	313	0.0231(2)	0.165(1)	0.05892(3)	0.5129(2)
C50	323	0.0201(3)	0.134(1)	0.05893(3)	0.4190(2)