Supporting Information

Nanomechanical Properties of Protein-DNA Layers with Different Oligonucleotide

Tethers

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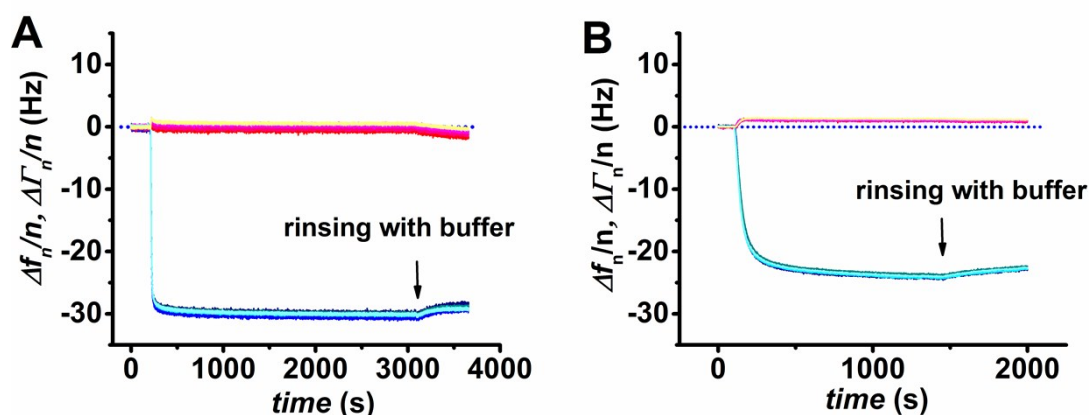
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## Table of contents   page

Frequency f_n/n and bandwidth Γ_n/n shifts belonging to the QCM-D measurements presented in Figures 1-4, and Figure 6 in the main manuscript.

Instead of the shift of dissipation factors ΔD_n the shifts of the bandwidth $\Delta\Gamma_n/n$ is shown in the figures below.

Figure S1. Frequency (blue) and bandwith (red) shifts (overtones 5-13) observed in situ by QCM-D for adsorption/desorption of apododecin DtE on monolayers of flavin-terminated dsDNA with 20 bp (**A**) and ssDNA with 20 bases (**B**) as shown in the cartoon in **Figure 1** of the main manuscript. The color code of the overtones in this as well as in the other figures is for the frequency overtones 5, 7, 9, 11, and 13 navy, blue, dark cyan, cyan, and light cyan and for the bandwith overtones 5, 7, 9, 11, and 13 red, pink, magenta, light magenta, and light yellow, respectively.
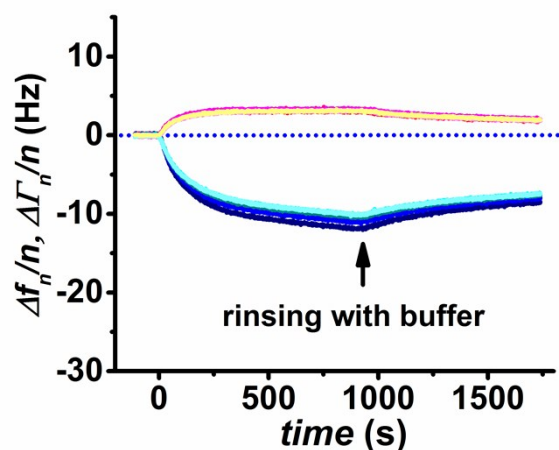
Figure S2. Frequency (blue) and bandwith (red) shifts (overtones 5-13) observed in situ by QCM for adsorption/desorption of apododecin DtE on monolayers of flavin-terminated dsDNA with 100 bp as shown in the cartoon in **Figure 2** of the main manuscript.
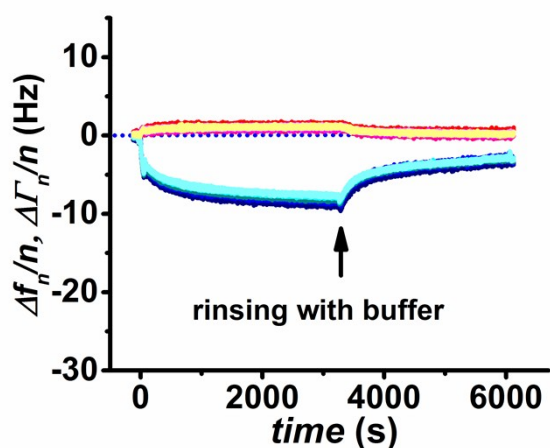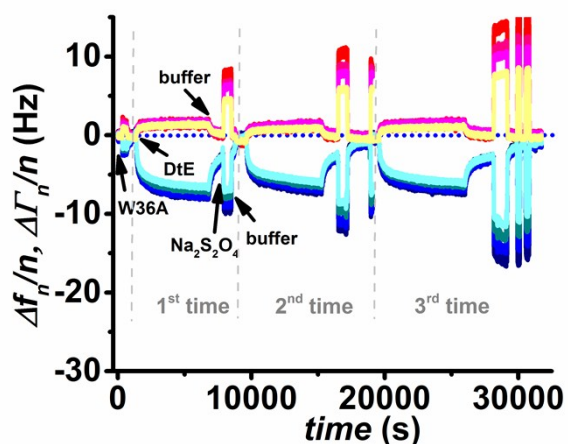
Figure S3. Frequency (blue) and bandwith (red) shifts (overtones 5-13) observed in situ by QCM for adsorption/desorption of apododecin DtE on a monolayer of flavin-terminated dsLNA with 7 bp as shown in the cartoon in **Figure 3** of the main manuscript.

Figure S4. Frequency (blue) and bandwith (red) shifts (overtones 5-13) observed in situ by QCM-D measurements. Three times the adsorption of apododecin DtE on monolayers of flavin-terminated dsLNA with 7 base pairs, followed by rinsing with buffer solution, and subsequently by rinsing with an oxygen-free buffered sodium dithionite solution for flavin reduction in order to release remaining DtE molecules captured by multi-ligand binding was measured. For the second and third chemical reduction step the period for rinsing with sodium dithionite had to be subsequently expanded. Prior to the first incubation of DtE a non-binding apododecin variant, W36A, was incubated as negative control at a concentration of 5 µM and subsequently rinsed with buffer to ensure that there was no unspecific binding.[1,2]
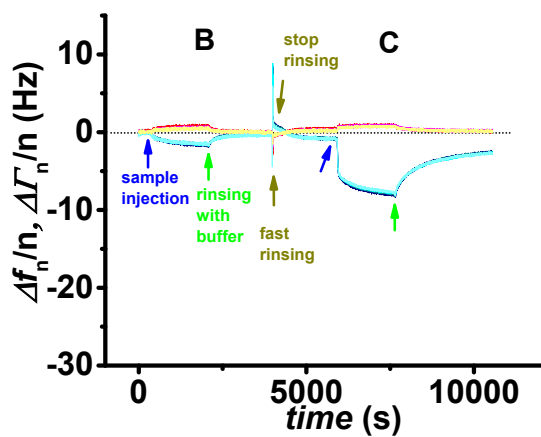
Figure S5. Frequency (blue) and bandwith (red) shifts (overtones 5-13) corresponding to the ligand addition and subsequent rinsing with buffer (start of rinsing is indicated by arrows) for a Cy5-labeled flavin-dsDNA ligand (**B**), and a Cy5-labeled flavin-ssDNA ligand (**C**) (corresponding to **Figure 6** in the main manuscript).

Analysis of the binding and unbinding kinetics of a Cy5-modified flavin-dsDNA and a Cy5-modified flavin-ssDNA ligand measured by SPFS

The unbinding kinetics of a Cy5-modified flavin-dsDNA and a Cy5-modified flavin-ssDNA ligand were analyzed from the SPFS kinetic curves shown in **Figure 5** in the main manuscript. The determination of the corresponding k_{off} values is shown in **Figure S6**.
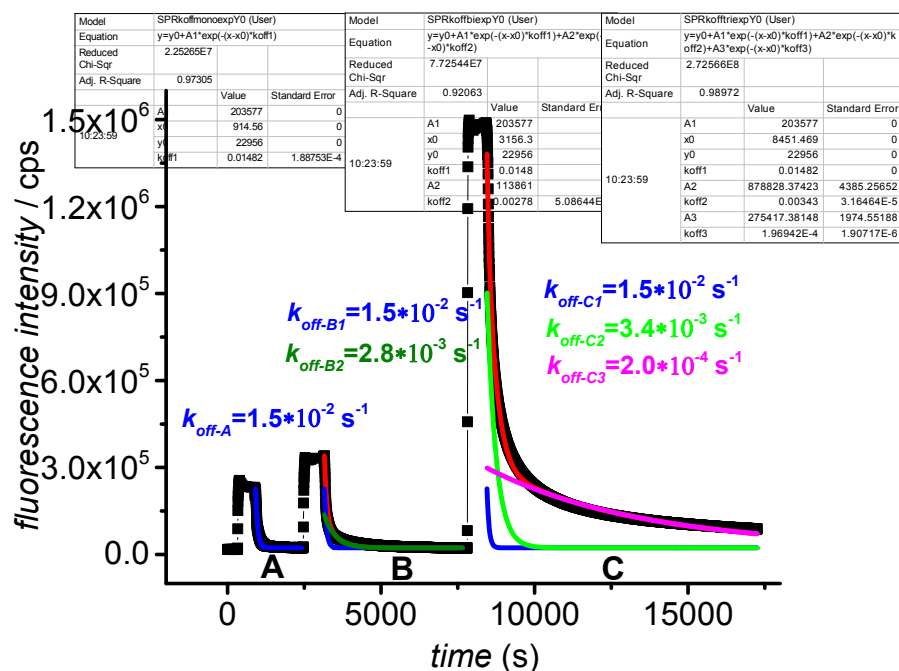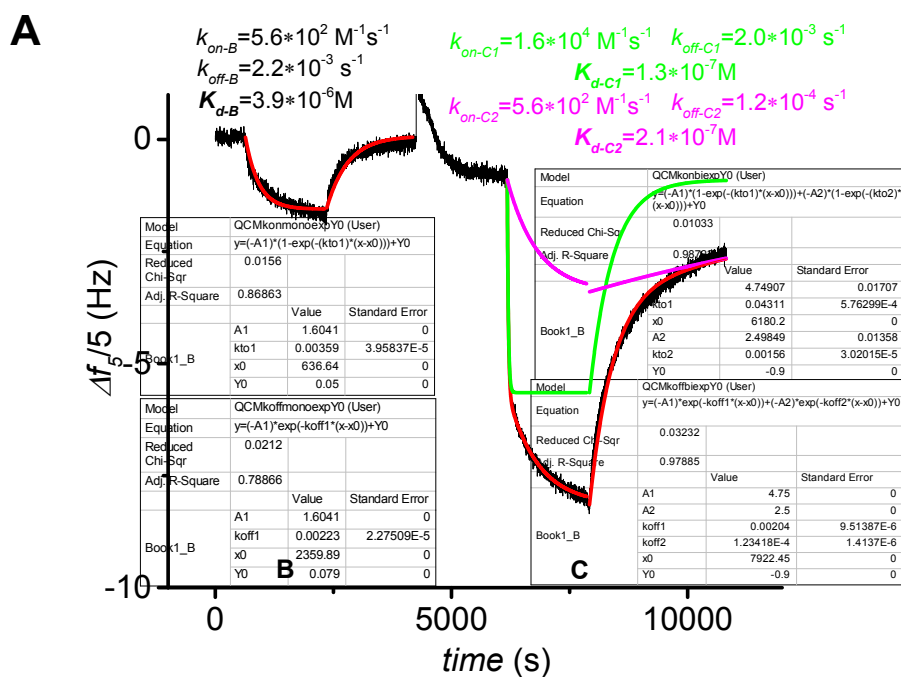

Figure S6. Analysis of the desorption kinetics including the determination of the k_{off} values from the SPFS kinetic scan curve measured during binding and subsequent rinsing with buffer for a Cy5-labeled flavin-dsDNA ligand (**B**), and a Cy5-labeled flavin-ssDNA ligand (**C**). Prior to binding and desorption of these ligands non-binding Cy5-labeled ssDNA (**A**) was incubated as negative control.

As shown by the negative control, *i.e.* the Cy5-labeled ssDNA without flavin anchor (**A**), the fluorescence instantaneously increased when the dye-DNA containing solution entered the flow cell. Therefore, only the decay in fluorescence upon rinsing with buffer could be used for data analysis (*i.e.* only the determination of k_{off} was possible). For data analysis the decay of the negative control was fitted mono-exponentially (blue curve) and it was assumed that there is nearly the same contribution of non-bound dye molecules to the overall fluorescence signal in the desorption kinetics of the Cy5-modified flavin-dsDNA and the Cy5-modified flavin-ssDNA ligand. Making this assumption a mono-exponential function with a value of $k_{offB2} = 2.8 \times 10^{-3}$ s^{-1} fits the decay curve of the Cy5-modified flavin-dsDNA ligand quite well. In contrast two exponential functions with values of $k_{off} = 3.4 \times 10^{-3}$ s^{-1} and $k_{off} = 2.0 \times 10^{-4}$ s^{-1} were necessary to fit the decay curve of the Cy5-modified flavin-ssDNA ligand. Apparently there is a difference in the kinetics whether a ligand from a singly occupied or a doubly occupied binding pocket gets desorbed. Assuming that not all binding pockets are doubly occupied, the green decay curve with a larger amplitude and a value of $k_{off} = 3.4 \times 10^{-3}$ s^{-1} corresponds to ssDNA ligands leaving a singly occupied pocket, whereas the pink curve with smaller amplitude and a value of $k_{off} = 2.0 \times 10^{-4}$ s^{-1} is attributed to ssDNA ligands being released from a doubly occupied pocket.

Analysis of the binding and unbinding kinetics of a Cy5-modified flavin-dsDNA and a Cy5-modified flavin-ssDNA ligand measured by QCM

In order to do a more accurate fitting of the k_{off} values and to determine also values for k_{on} also the kinetic QCM curve shown in **Figure 6** in the main manuscript were analyzed in **Figure S7A**. To further enhance the accuracy of the results, the experiment was repeated twice (**Figure S7B** and **S7C**) using a second independently modified chip. Average values are given (from three measurements performed on two different chips). As shown in **Figure S7**, for the flavin-dsDNA ligand mean values of $k_{on} = 1.3 \times 10^3$ M^{-1} s^{-1} and $k_{off} = 3.5 \times 10^{-3}$ s^{-1} were determined. From these values a dissociation constant of $K_d = 2.7 \times 10^{-6}$ M ($K_d = k_{off}/k_{on}$) can be calculated. This K_d value obtained by adding the flavin-dsDNA ligand to a stable dodecin monolayer is about five times larger than the K_d value obtained previously by adding apododecin to a dsDNA monolayer with a relative flavin surface coverage of 10% with respect to the total amount of double-stranded DNA (dsDNA).[3] This difference is tolerable if it is taken into account that, while in the previous approach (by adding apododecin to a partially flavin terminated DNA layer) the probability for multi-ligand binding could not be excluded completely, in the current study (by adding a monodentate flavin ligand to a dodecin layer) multi-ligand binding is not possible at all, and therefore the current value can be regarded as being more accurate.

B

$k_{on-B}=1.0*10^3$ M⁻¹s⁻¹ $\quad k_{on-C1}=2.0*10^4$ M⁻¹s⁻¹ $k_{off-C1}=2.1*10^{-3}$ s⁻¹
$k_{off-B}=3.0*10^{-3}$ s⁻¹ $\qquad K_{d-C1}=1.1*10^{-7}$ M
$K_{d-B}=3.0*10^{-6}$ M $\quad k_{on-C2}=7.6*10^2$ M⁻¹s⁻¹ $k_{off-C2}=1.7*10^{-4}$ s⁻¹
$\qquad\qquad K_{d-C2}=2.2*10^{-7}$ M

Model	QCMkonmonoexpY0 (User)	
Equation	y=(-A1)*(1-exp(-(kto1)*(x-x0)))+Y0	
Reduced Chi-Sqr	0.01124	
Adj. R-Square	0.88957	
	Value	Standard Err
A1	1.4	0
kto1	0.0055	7.42189E-5
x0	305.9	0
Y0	-0.2	0

Model	QCMkoffmonoexpY0 (User)	
Equation	y=(-A1)*exp(-koff1*(x-x0))+Y0	
Reduced Chi-Sqr	0.03048	
Adj. R-Square	0.51898	
	Value	Standard Error
A1	1.4	0
koff1	0.00302	4.9452E-5
x0	1160.5	0
Y0	-0.37	0

Model	QCMkonbiexpY0 (User)	
Equation	y=(-A1)*(1-exp(-(kto1)*(x-x0)))+(-A2)*(1-exp(-(kto2)*(x-x0)))+Y0	
Reduced Chi-Sqr	0.0099	
Adj. R-Square	0.98567	
	Value	Standard Error
A1	4.72416	0.01809
kto1	0.05122	7.36707E-4
x0	2871.7	0
A2	2.26858	0.01468
kto2	0.00205	3.61013E-5
Y0	-0.416	0

Model	QCMkoffbiexpY0 (User)	
Equation	y=(-A1)*exp(-koff1*(x-x0))+(-A2)*exp(-koff2*(x-x0))+Y0	
Reduced Chi-Sqr	0.02206	
Adj. R-Square	0.9835	
	Value	Standard Error
A1	4.72	0
A2	2.27	0
koff1	0.00209	7.42408E-6
koff2	1.68085E-4	6.46462E-7
x0	4518.8	0
Y0	-0.4	0

(Plot: $\Delta f_5/5$ (Hz) vs time (s), y-axis 0 to -10, x-axis 0 to 10000)

C

$k_{on-B}=2.4*10^3$ M⁻¹s⁻¹ $\quad k_{on-C1}=1.2*10^4$ M⁻¹s⁻¹ $\quad k_{off-C1}=2.5*10^{-3}$ s⁻¹
$k_{off-B}=5.4*10^{-3}$ s⁻¹ $\qquad K_{d-C1}=2.1*10^{-7}$ M
$K_{d-B}=2.3*10^{-6}$ M $\quad k_{on-C2}=7.7*10^2$ M⁻¹s⁻¹ $\quad k_{off-C2}=2.8*10^{-4}$ s⁻¹
$\qquad\qquad K_{d-C2}=3.6*10^{-7}$ M

Model	QCMkonmonoexpY0 (User)	
Equation	y=(-A1)*(1-exp(-(kto1)*(x-x0)))+Y	
Reduced Chi-Sqr	0.0123	
Adj. R-Squar	0.8526	
	Value	Standard Err
A1	1.3	0
kto1	0.0112	2.4363E-4
x0	268.9	0
Y0	0.2	0

Model	QCMkoffmonoexpY0 (User)	
Equation	y=(-A1)*exp(-koff1*(x-x0))+Y0	
Reduced Chi-Sqr	0.0079 1	
Adj. R-Squar	0.9312	
	Value	Standard Err
A1	1.3	0
koff1	0.0053	6.4724E-5
x0	1521	0
Y0	0.3	0

Model	QCMkonbiexpY0 (User)	
Equation	y=(-A1)*(1-exp(-(kto1)*(x-x0)))+(-A2)*(1-exp(-(kto2)*(x-x0)))+Y0	
Reduced Chi-Sqr	0.01919	
Adj. R-Square	0.97728	
	Value	Standard Error
A1	4.5315	0.03604
kto1	0.0318	6.32308E-4
x0	2742.4	0
A2	2.02267	0.02741
kto2	0.0022	8.02378E-5
Y0	0.4	0

Model	QCMkoffbiexpY0 (User)	
Equation	y=(-A1)*exp(-koff1*(x-x0))+(-A2)*exp(-koff2*(x-x0))+Y0	
Reduced Chi-Sqr	0.02145	
Adj. R-Square	0.98453	
	Value	Standard Error
A1	4.53	0
A2	2.02	0
koff1	0.00254	1.05174E-5
koff2	2.7695E-4	1.38652E-6
x0	4170	0
Y0	0.4	0

(Plot: $\Delta f_5/5$ (Hz) vs time (s), y-axis 0 to -10, x-axis 0 to 5000)

Figure S7. Fits of the kinetic QCM curves (overtone 5) measured three times for absorption and desorption of a Cy5-labeled flavin-dsDNA ligand (**B**), and a Cy5-labeled flavin-ssDNA (**C**) in order to determine k_{on}, k_{off}, and K_d.

Appropriate fitting of the binding and unbinding kinetics of the flavin-ssDNA ligand was only possible by the sum of two exponential functions. Since in total more flavin-ssDNA ligands will enter an empty and leave a singly occupied binding pocket than enter a singly and leave a doubly occupied binding pocket, the binding and unbinding curve with the higher amplitude/prefactor shown in green is attributed to ssDNA binding to an empty and being released from a singly occupied binding pocket (the same assignment has been done previously for the fits of the SPFS curve shown in **Figure S6**). Here mean values of $k_{on} = 1.6 \times 10^4$ M⁻¹ s⁻¹, $k_{off} = 2.2 \times 10^{-3}$ s⁻¹, and $K_d = 1.4 \times 10^{-7}$ M were obtained. Apparently

for binding to an empty binding pocket the flavin-ssDNA ligand binds faster with a k_{on} value that is about one order of magnitude higher than for the flavin-dsDNA ligand, whereas both ligands follow nearly the same unbinding kinetics. As a consequence the dissociation constant of the apododecin complex with the flavin-ssDNA ligand is about one order of magnitude lower than with the flavin-dsDNA ligand. While after binding a single flavin-dsDNA ligand in each binding pocket the entrance to the pocket is blocked by the bulky dsDNA, after binding of a first flavin-ssDNA ligand per pocket a second flavin-ssDNA ligand may enter following slower kinetics. For the second binding event a mean value of $k_{on} = 7.0 \times 10^2$ M^{-1}s^{-1} has been determined. Interestingly the second ligand is captured quite efficient, which is reflected by a rather low average value of $k_{off} = 1.9 \times 10^{-4}$ s^{-1} (see pink curves in **Figure S7**). The binding of the second ligand seems to be stabilized by aromatic tetrade formation inside the binding pocket *i.e.* by π-stacking interaction between the two isoalloxazine moieties of the flavins and the two tryptophans W36 belonging to the apoprotein. Since for the second binding event not only k_{on} but also k_{off} is smaller than for the first, the value of K_d for the second binding/unbinding event ($K_d = 2.7 \times 10^{-7}$ M) is similar to the first ($K_d = 1.4 \times 10^{-7}$ M). It is further worth to note that the individual values for k_{off} determined from the kinetic SPFS and QCM curves are in good agreement.

References

1   M. Grininger, G. Nöll, S. Trawöger, E.-K. Sinner and D. Oesterhelt, *Biointerphases*, 2008, **3**, 51.
2   Y. Yu, B. Heidel, L. Parapugna Tamara, S. Wenderhold-Reeb, B. Song, H. Schönherr, M. Grininger and G. Nöll, *Angew. Chem., Int. Ed.*, 2013, **52**, 4950.
3   C. Gutiérrez Sánchez, Q. Su, H. Schönherr, M. Grininger and G. Nöll, *ACS nano*, 2015, **9**, 3491.