Compositional and Structural Engineering of DNA Multilayer Films

ARTICLE *in* LANGMUIR · APRIL 2006

Impact Factor: 4.46 · DOI: 10.1021/la052581h · Source: PubMed

4 AUTHORS, INCLUDING:

Angus Johnston
Monash University (Australia)
84 PUBLICATIONS **3,805** CITATIONS

SEE PROFILE

Compositional and Structural Engineering of DNA Multilayer Films

Angus P. R. Johnston, Hideyuki Mitomo,† Elizabeth S. Read,‡ and Frank Caruso*

Centre for Nanoscience and Nanotechnology, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria 3010, Australia

We report the layer-by-layer (LbL) preparation of multilayered thin films that consist solely of DNA. The properties of the films were varied by assembling the layers from different oligonucleotide building blocks, which are composed of repeating homopolymeric units of nucleotides [adenosine (A), cytosine (C), guanine (G), and thymidine (T)] or "random" sequences. Films assembled from oligonucleotides with a single complementary unit did not show continual layer buildup. To form a repeating multilayer system, it was necessary for single-stranded DNA to be available for subsequent layers to hybridize. By using oligonucleotides with multiple nucleotide units, multilayer films were successfully assembled. We demonstrate that the thickness and swellability of the films can be controlled by the extent of hydrogen bonding (the G/C content of the oligonucleotide) and orientation of the oligomers. We have examined the stability and swellability of the films in solutions of varying salt concentration as well as in a denaturing urea solution. Stable, hollow DNA capsules were also formed by preparing the films on sacrificial colloidal templates, followed by removal of the core. The assembly of propagating structures through DNA hybridization paves the way for the engineering of DNA films with tailored composition, structure, and permeability, making them likely to find application in drug/gene delivery and biomolecular sensing.

Introduction

Thin films assembled from polymers have applications in drug/gene delivery,[1] sensing,[2] diagnostics,[3] and optoelectronic devices.[4] There are a number of approaches that can be used to construct thin polymer films, including spin coating,[5] in-situ polymerization,[6] and sol−gel deposition.[7] Recently, the layer-by-layer (LbL) technique[8] has emerged as a premier approach because of its simplicity and versatility. LbL polyelectrolyte films are predominantly assembled by the alternate deposition of positively and negatively charged polymers, where electrostatic forces facilitate layer build-up. Control over the film morphology can be achieved by altering the layer number and the assembly conditions and by incorporating different polyelectrolytes or other molecules at each layer.[8−11] Although there are many advantages of electrostatically depositing polymer layers, there are a limited number of systems that are stable, biocompatible, and responsive in the range of physiological conditions. The use of other forces, such as hydrogen-bonding[12,13] and hydrophobic interactions,[14] to induce the build-up of thin films is valuable because it allows additional control over the film properties in the physiological range. We recently reported a new method to form multilayer thin films, based on DNA hybridization.[15] We showed that these films could be assembled on macroscopic planar supports and on sacrificial colloidal particles that could subsequently be decomposed to yield hollow DNA capsules. Films synthesized solely from DNA are affected by a number of forces, including the hydrogen bonding of the nucleotides, the electrostatic repulsion of the phosphate backbone, and hydrophobic interactions of the aromatic rings in the nucleotide bases. Other systems have been studied where DNA is one component of the system;[9,16] however, their behavior is mostly governed by the electrostatic interactions which are used to assemble the films. DNA films prepared on alumina membranes were also recently reported, where the membrane can be dissolved to form DNA nanotubes.[17] These films, however, require R,ω-diorganophosphonate Zr(IV) to stabilize the film. Films assembled solely from DNA also permit control over the orientation of the oligonucleotides in the film and therefore the film properties. This is not possible with conventional polyelectrolyte multilayers assembled using electrostatic interactions.

In this paper, we examine the influence of the DNA composition on the film properties by comparing homopolymeric blocks of nucleotides with "random"[18] sequences of nucleotides. We also investigate the effect of using repeating blocks of homopolymeric nucleotides on the build-up and stability of the films. The stability of the films was monitored in solutions containing varying salt concentrations as well as in denaturing solutions (6 M urea).[19]

Five types of DNA films were investigated by using nine distinct DNA oligomers, as depicted in Scheme 1. The first four

* To whom correspondence should be addressed. Tel.: +61 3 8344 3461. Fax: +61 3 8344 4153. E-mail: fcaruso@unimelb.edu.au.

† Current address: Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259-S2-11, Nagatsuda, Midoriku, Yokohama 226-8503, Japan.

‡ Current address: University of Sheffield, Brook Hill, Sheffield S3 7HF, United Kingdom.

(1) Zhang, J.; Chua, L. S.; Lynn, D. M. *Langmuir* **2004**, *20*, 8015.
(2) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096.
(3) Schouten, S.; Stroeve, P.; Longo, M. L. *Langmuir* **1999**, *15*, 8133.
(4) Hartwich, G.; Caruana, D. J.; de Lumley-Woodyear, T.; Wu, Y. B.; Campbell, C. N.; Heller, A. *J. Am. Chem.* Soc. **1999**, *121*, 10803.
(5) Norrman, K.; Ghanbari-Siahkali, A.; Larsen, N. B. *Annu. Rep. Prog. Chem., Sect. C* **2005**, DOI: 10.1039/b408857n.
(6) Sano, M.; Lvov, Y.; Kunitake, T. *Annu. Rev. Mater. Sci.* **1996**, *26*, 153.
(7) Levy, D.; Esquivias, L. *Adv. Mater.* **1995**, *7*, 120.
(8) (a) Decher, G.; Hong, J. D. *Ber. Bunsen-Ges. Phys. Chem.* **1991**, *95*, 1430. (b) Decher, G. *Science* **1997**, *277*, 1232.
(9) Dubas, S. T.; Schlenoff, J. B. *Macromolecules* **1999**, *32*, 8153.
(10) Cho, J. H.; Quinn, J. F.; Caruso, F. *J. Am. Chem. Soc.* **2004**, *126*, 2270.
(11) Quinn, J. F.; Yeo J. C. C.; Caruso, F. *Macromolecules* **2004**, *37*, 6537.
(12) Stockton, W. B.; Rubner, M. F. *Macromolecules* **1997**, *30*, 2717.
(13) Wang, L.; Wang, Z. Q.; Zhang, X.; Shen, L. C.; Chi, L. F.; Fuchs, H. *Macromol. Rapid Commun.* **1997**, *18*, 509.
(14) Serizawa, T.; Kamimura, S.; Kawanishi, N.; Akashi, M. *Langmuir* **2002**, *18*, 8381.
(15) Johnston, A. P. R.; Read, E. S.; Caruso, F. *Nano Lett.* **2005**, *5*, 953.
(16) Caruso, F.; Rodda, E.; Furlong, D. N.; Niikura, K.; Okahata, Y. *Anal. Chem.* **1997**, *69*, 2043.
(17) Hou, S.; Wang, J.; Martin, C. R.; *J. Am. Chem. Soc.* **2005**, *127*, 8586. The DNA films prepared in this paper were assembled using five different DNA sequences and not as described here, where alternating layers of two DNA oligomers are deposited.
(18) The "random" sequence refers to the random ordering of bases, as opposed to the homopolymeric sequence. The "random" sequence and its complementary sequence were used to build-up the film.
(19) Rosenblum, B. B.; Oaks, F.; Menchen, S.; Johnson, B. *Nucleic Acids Res.* **1997**, *25*, 3925.

Scheme 1. Sequences of the Oligonucleotides Used in the Assembly of the DNA Multilayer Films[a]

single-block

5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 3′ $polyT_{30}$

5′ AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 3′ $polyA_{30}$

di-block

5′ TTTTTTTTTTTTTTTCCCCCCCCCCCCCCC 3′ $polyT_{15}C_{15}$

5′ AAAAAAAAAAAAAAAGGGGGGGGGGGGGGG 3′ $polyA_{15}G_{15}$

reverse-di-block

5′ CCCCCCCCCCCCCCCTTTTTTTTTTTTTTT 3′ $polyC_{15}T_{15}$

5′ AAAAAAAAAAAAAAAGGGGGGGGGGGGGGG 3′ $polyA_{15}G_{15}$

tetra-block

5′ TTTTTTTTTTTTTTTCCCCCCCCCCCCCCCTTTTTTTTTTTTTTTCCCCCCCCCCCCCCC 3′ $polyT_{15}C_{15}T_{15}C_{15}$

5′ AAAAAAAAAAAAAAAGGGGGGGGGGGGGGGAAAAAAAAAAAAAAAGGGGGGGGGGGGGGG 3′ $polyA_{15}G_{15}A_{15}G_{15}$

XY-di-block

5′ AGGGTCCTGAACTGA(X) TGTACCGTAAAGCCG(Y) 3′ XY

5′ TCAGTTCAGGACCCT(X′) CGGCTTTACGGTACA(Y′) 3′ X′Y′

[a] The scheme does not indicate the orientation or hybridization of the sequences.

films involved repeating nucleotide homopolymeric units. The first system (single-block) was assembled using oligomers of 30 repeating thymidine (T) residues ($polyT_{30}$) and 30 repeating adenosine (A) residues ($polyA_{30}$). The second system (di-block) was assembled from oligomers containing 15 repeating T residues followed by 15 repeating cytosine (C) residues ($polyT_{15}C_{15}$), and 15 repeating A residues followed by 15 repeating guanosine (G) residues ($polyA_{15}G_{15}$). G was paired in the same oligomer with A rather than T, because it is known that loose association between G and T can occur.[20] The third system investigated was a variation of the di-block system. DNA is not a symmetrical molecule, as the nucleotide units are linked by phosphate groups attached to the 5′ and the 3′ hydroxyl groups on the deoxyribose sugar. By convention, DNA sequences are written from 5′ to 3′. When DNA hybridizes to form a double helix, each oligomer is orientated in the opposite direction. That is, if one strand is orientated 5′ to 3′, the other is orientated 3′ to 5′. This means that the oligomer 5′ AG 3′ is different to 5′ GA 3′ and would only hybridize to 5′ CT 3′ and not to 5′ TC 3′. To investigate whether the orientation of oligomers within the film had an effect on the film properties, a di-block film was assembled using $polyA_{15}G_{15}$ and $polyC_{15}T_{15}$ (reverse-di-block). The fourth polymeric block system studied was a tetra-block system with alternating layers of $polyA_{15}G_{15}A_{15}G_{15}$ and $polyT_{15}C_{15}T_{15}C_{15}$. The fifth system investigated was a di-block system where each block contained a "random" sequence[18] with approximately the same percentage of A/T and G/C nucleotides (XY-di-block). The "random" blocks were designed to ensure there was no self-complementarity between either of the blocks or their complementary sequences. This could result in the formation of hairpin structures that would affect hybridization of each layer to the film.

We show that four of the five oligomer systems form stable films, each with different characteristics. The build-up and viscoelastic properties of the film were monitored using quartz crystal microgravimetry (QCM), and the stability of the films in different ionic strength solutions showed that the films were both stable and responsive at physiological conditions.

Experimental Section

Materials. Poly(ethyleneimine) (PEI, M_w = 25 000 g mol^{-1}), sodium chloride (NaCl), sodium citrate, hydrofluoric acid (HF), and 3-aminopropyltrimethoxysilane (APS) were obtained from Sigma-Aldrich. Unlabeled oligonucleotides (Scheme 1) were custom synthesized by Geneworks (Adelaide, South Australia). The fluorescently labeled oligonucleotide $polyT^*_{15}C_{15}$ (TTTTTTTTTTTTTTT-(T*)CCCCCCCCCCCCCCC) contained an internally modified T (T*) residue which was labeled with carboxytetramethylrhodamine (TAMRA) (TriLink Biotechnologies, San Diego). High purity water with a resistivity greater than 18 MΩ cm was obtained from an inline Millipore RiOs/Origin system (MilliQ water). QCM electrodes (Q-Sense AB, Västra, Frölunda, Sweden) and quartz microscope slides were cleaned with Piranha solution (70/30 v/v% sulfuric acid: hydrogen peroxide). *Caution! Piranha solution is highly corrosive. Extreme care should be taken when handling Piranha solution and only small quantities should be prepared.* A total of 500 mL of buffer containing 500 mM NaCl and 50 mM citrate (SSC buffer) was made with 4.8 g of citric acid and 14.6 g of NaCl. The pH of the buffer solution was measured with a Mettler-Toledo MP220 pH meter and adjusted to pH 6.5 using 1 M sodium hydroxide. Saline solutions containing 25, 50, 75, 100, 200, 300, and 500 mM NaCl were made using MilliQ water. A urea solution (6 M) containing 1 M NaCl and 100 mM citrate was used to denature the films. Silica particles, 3 μm in diameter, were purchased from MicroParticles GmbH (Germany).

Methods. *Quartz Crystal Microgravimetry.* QCM measurements were made using a Q-Sense D300 device with a flow cell (Q-Sense AB, Västra, Frölunda, Sweden). The temperature was kept constant at 23.4 °C during the experiments. A gold-coated 5 MHz AT-cut crystal was excited at its third overtone at ~15 MHz, and the change in the resonance frequency was recorded. The resulting frequencies were divided by 3 to be comparable to the results at the base frequency of 5 MHz. All frequency and dissipation values quoted are for the third overtone. The other overtones measured (fifth and seventh overtones) followed the same trend. Due to the large dissipation changes observed, the Sauerbrey equation[21] does not necessarily hold. Therefore, deposition of DNA is quoted as the corresponding frequency change, rather than as an absolute mass. After initially depositing a layer of PEI (1 mg mL^{-1} for 5 min), DNA (500 μL of 4 μM in SSC buffer) was adsorbed/hybridized to the film for 20 min.

(20) Evans, D. H.; Morgan, A. R. *Nucleic Acids Res.* **1986**, *14*, 4267.

(21) Sauerbrey, G. *Z. Phys.* **1959**, *155*, 206.

After each adsorption step, the film was washed with 2 mL of SSC buffer. For all systems investigated, a single block was deposited as the first DNA layer (polyT$_{30}$ for the repeating nucleotide systems, and X′ for the "random" block system).

Film Stability. To investigate the stability of the films to different salt concentrations, the assembled films were treated with decreasing concentration of saline solutions. After incubating the films in the saline solution for 5 min (or until no further change in the frequency and dissipation values was observed), the films were washed with SSC buffer to allow comparison of the frequency and dissipation values after the different treatments. The films treated with urea were incubated in the urea solution for 5 min. After the incubation, they were washed with SSC buffer, so a comparison with the untreated films could be made.

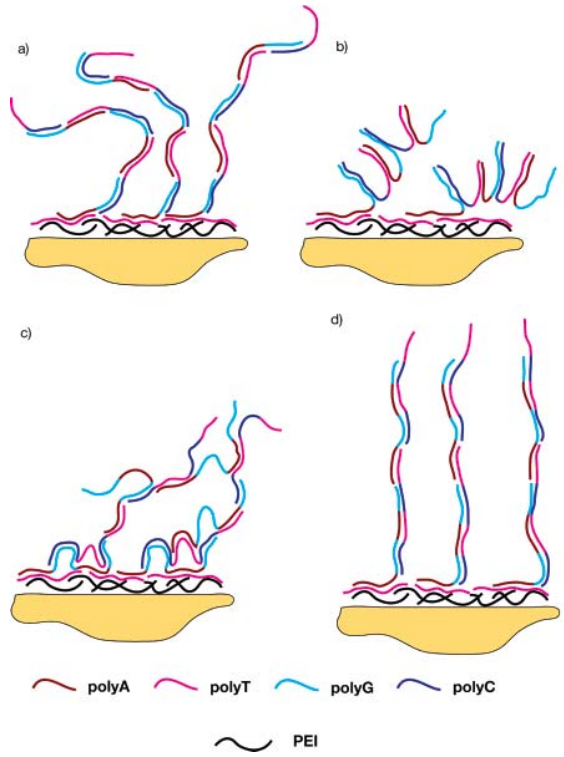
Hollow DNA Capsules. To impart a positive charge on the 3 μm silica particles, the spheres were coated with amine groups as follows. A total of 100 μL of a 1 wt % solution of particles was suspended in 1 mL of ethanol and 50 μL of 25 wt % ammonia solution. A total of 200 μL of APS was then added to the suspension, and the particles were allowed to react for 4 h. The particles were washed twice with ethanol and three times with MilliQ water. The DNA multilayers (polyT$_{30}$[polyA$_{15}$G$_{15}$/polyT*$_{15}$C$_{15}$]$_4$) were deposited on the particles by suspending 10 μL of the particles in 50 μL of a 10 μM DNA solution (in SSC buffer) and allowing the oligomers to hybridize to the surface for 20 min. After hybridization, the particles were washed three times in SSC buffer before the addition of the next layer. To form hollow capsules, the silica core was dissolved by mixing 50 μL of the particle suspension with 50 μL of 2 M HF. (*Caution! HF is highly toxic and great care must be taken when handling.*) After 2 min in the HF solution, the capsules were centrifuged at 4000 *g* for 5 min and washed 3 times with SSC buffer. The particles were imaged on a Leica TCS SP2 AOBS confocal microscope using an excitation wavelength of 543 nm. The images were processed and volume rendered using Imaris v4.2.

Results and Discussions

(a) Film Build-Up. As previously reported, alternately depositing single homopolymeric block oligonucleotides (polyT$_{30}$/polyA$_{30}$) resulted in limited film build-up.[15] The mass of DNA deposited in the first two layers of this system (corresponding to 8 and 7 Hz, respectively) was approximately equivalent to the mass expected for the deposition of a DNA monolayer on the surface. However, the addition of the third layer (the second polyT$_{30}$ layer) resulted in a lower amount of DNA deposited (corresponding to 3 Hz). This is because, after hybridization of the first two layers, additional layers can attach to the film only through competitive hybridization with the deposited layers. To form a repeating multilayer system, it is necessary for single-stranded DNA to be available for subsequent layers to hybridize. The DNA films formed are highly swollen (as seen by the high dissipation values), and as a result, the films include a significant amount of water. The swollen nature of the film also indicates that the Sauerbrey equation[21] does not necessarily hold, which means that the frequency may not correspond linearly to the film mass. For these reasons, the deposition of DNA onto the film is quoted as a frequency change, rather than as an absolute mass.

To promote the build-up of multilayer films, in all instances, the first layer adsorbed to the positively charged surface was polyT$_{30}$ (or in the case of the XY films, X′). This was to ensure that hybridization of the first di-block or tetra-block layer would result in a single-stranded tail of DNA being available for subsequent hybridization of the next layer (Scheme 2). In the case of the di-block system, if polyA$_{15}$G$_{15}$ was attached as the first layer, then both the T and C portions of polyT$_{15}$C$_{15}$ could hybridize to the surface. This would leave no single-stranded

Scheme 2. Schematic Illustration of the Possible Orientations of DNA Oligomers in the Film: (a) Di-Block System; (b) Reverse-Di-Block System, where the Film Build-Up Is Hindered by the Orientation of the Oligomers; (c) Tetra-Block System, where Both of the Complementary Units in the First Layer Hybridize to the Film; (d) Tetra-Block System, where an Excess of Oligomers in the Hybridizing Solution Induces the Maximum Number of Oligomers to Hybridize to the Film, Causing Only One of the Complementary Units to Hybridize to the Film[a]



[a] In all cases, a PEI layer was deposited to facilitate the electrostatic adsorption of a layer of polyT$_{30}$ to prepare the surface for assembly of each of the systems.

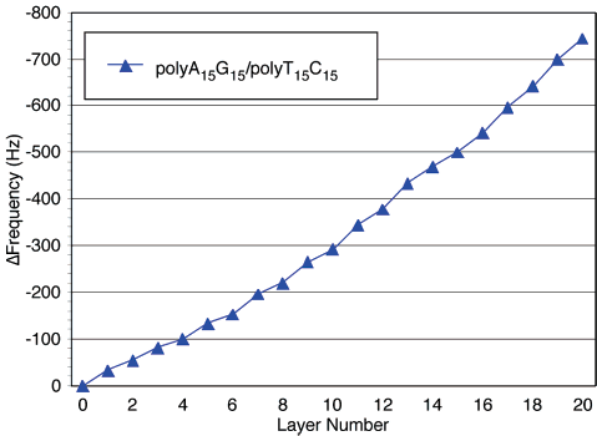


Figure 1. QCM data showing the assembly of a 20-layer di-block film (polyA$_{15}$G$_{15}$/polyT$_{15}$C$_{15}$). Layer 0 corresponds to the PEI/polyT$_{30}$ film. The odd layers correspond to the deposition of polyA$_{15}$G$_{15}$ and the even layers correspond to the deposition of polyT$_{15}$C$_{15}$.

portion for subsequent layers to hybridize. Build-up of the polyA$_{15}$G$_{15}$/polyT$_{15}$C$_{15}$ di-block multilayer system to 20-layers showed regular layer deposition (Figure 1). The build-up was shown to be base pair (nucleotide) specific by introducing noncomplementary oligonucleotides into the film. When the

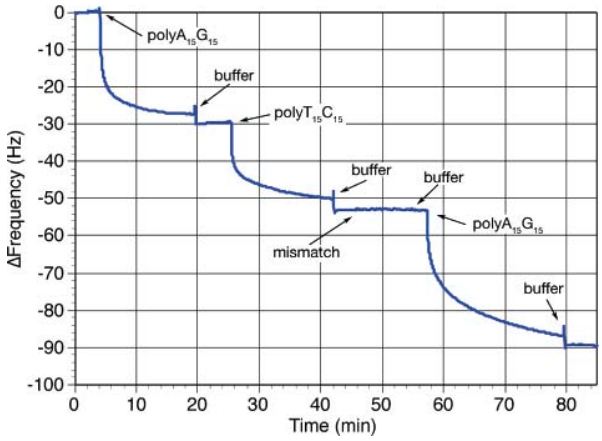


Figure 2. QCM frequency versus time for the polyA$_{15}$G$_{15}$/polyT$_{15}$C$_{15}$ di-block film. No change in the frequency was observed when a mismatched sequence (XY-di-block) was introduced to the film, however the film continued to grow following the introduction of the complementary sequence (polyA$_{15}$G$_{15}$).

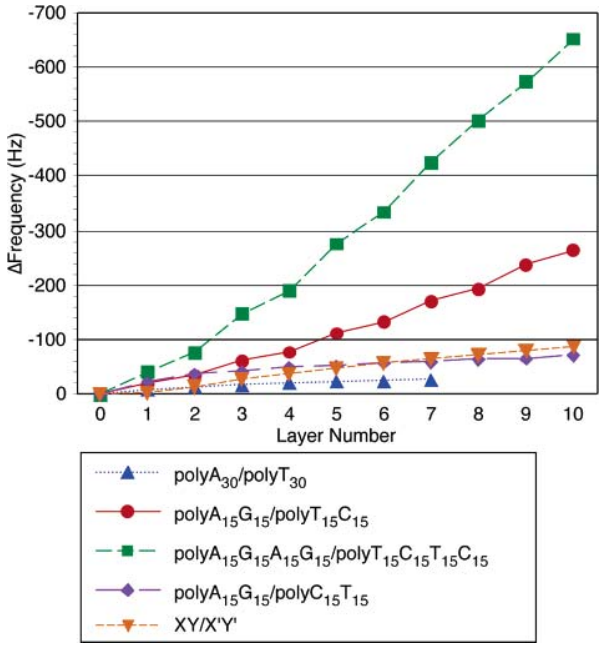


Figure 3. QCM data comparing the assembly of the five DNA films investigated. Layer 0 corresponds to the PEI/polyT$_{30}$ film (or PEI/X′ in the case of the XY/X′Y′ film). The odd layers correspond to hybridization of the polyA-containing layer (or XY layer) and the even layers correspond to hybridization of the polyT-containing layer (or X′Y′ layer).

noncomplementary DNA was introduced to the film, no deposition of the mismatched oligonucleotides occurred (Figure 2). This shows that the interaction between the multilayers is base pair specific and that the film is held together by hydrogen-bonding. We previously confirmed the presence of double-stranded DNA using PicoGreen, a dye that fluoresces green when bound to double-stranded DNA.[15]

A total film mass corresponding to 265 Hz was observed for the 10-layer di-block film (Figure 3). A comparison of the build-up of the di-block and the tetra-block films (Figure 3) shows that a much larger mass was deposited for each layer in the tetra-block film (total film mass corresponding to 652 Hz). There are two distinct ways the tetra-block system can hybridize to the surface. The first is where both the complementary blocks in the hybridizing oligonucleotides hybridize to the surface (Scheme 2c). The mass per layer for this system should be similar to the mass per layer for the di-block system, because although the mass of the oligonucleotides is double, as each oligonucleotide can hybridize twice to the surface, the surface area that each oligonucleotide can cover is also double. The alternative way the tetra-block system can hybridize is if there is a large excess of oligonucleotides in solution. Then the oligonucleotides may hybridize so that only one of the complementary blocks hybridizes with the film (Scheme 2d). This would result in double the mass of the di-block film, because the mass of the oligonucleotides is double, but the surface area that each oligonucleotide would cover would be the same. The high mass per layer shown in Figure 3 for the tetra-block system suggests that the orientation shown in Scheme 2d is most likely. Branching of the oligomer chains may also occur; however, as the film exhibits close to linear growth, the extent of branching is expected to be limited. Atomic force microscopy analysis of the films did not yield reliable images because in solution the swollen films deformed during imaging. Dry imaging of the film was hindered due to the presence of salt crystals. The films could not be rinsed in salt-free water to remove the crystals because the films disassembled (see section c, Film Stability).

To examine if there was a concentration dependence on the film structure and to determine if it was possible to induce the structure depicted in Scheme 2c, the concentration of DNA in the hybridizing solution was lowered to below the amount required to form a monolayer (0.5 μM). After the initial hybridization, two additional aliquots of the same diluted DNA solution (0.5 μM) were exposed to the film. The second addition resulted in a small increase in the mass of the film (corresponding to 9 Hz), whereas the third addition did not significantly change the film mass (data not shown). This indicates that the surface was saturated with complementary DNA. The mass deposited in the first bilayer (corresponding to 42 Hz) using this approach was approximately equivalent to the mass of the first bilayer in the di-block system (corresponding to 36 Hz) and much less than the mass deposited in the tetra-block system (corresponding to 77 Hz) using an excess of DNA. This suggests that the structure outlined in Figure 2c was formed.

The XY-di-block system showed a much smaller mass deposited per layer (corresponding to 7−12 Hz) compared with the di- and tetra-block systems (corresponding to 16−40 and 40−80 Hz, respectively) (Figure 3). This may be due to a number of reasons. In the di- and tetra-block systems, a homopolymeric block of T may hybridize anywhere along a block of homopolymeric A. Although the maximum amount of hydrogen-bonding would be achieved when all 15 units are hybridized, it also would be possible for two or more oligomers containing polyT to hybridize to the same polyA block. As there is only one correct alignment of the X and X′ sequence, only a 1:1 hybridization ratio would be expected. Additionally, as only one alignment of X and X′ would result in hybridization, the probability of this alignment is lower than the alignment of a region of polyA and polyT. This again could result in less XY and X′Y′ oligomers being deposited in each layer of the film.

The reverse-di-block system showed similar layer growth to the standard di-block system for the first two layers (Figure 3). However, after the addition of the first polyC$_{15}$T$_{15}$ layer, the mass of the subsequent layers was much less (corresponding to 2−6 Hz). This is most probably due to the strained nature of the reverse-di-block system (Scheme 2b). The orientation of oligomers hybridized in the di-block system lends itself to less hindered build-up of the layers, as the orientation of the growing

DNA layers could result in a continuous DNA strand (although it would be expected that crossing between the different strands would occur). However, with the reverse-di-block system, the direction of the growing DNA strand would have to reverse with the addition of each layer.

b. Film Viscoelasticity. When characterizing a film, apart from the mass of the film (which is related to the thickness), the viscoelasticity of the film reveals further important information about the structure of the films. A measure of the viscoelastic properties of the film can be obtained from the dissipation factor of the film.[22] The dissipation (D) is a measure of the damping of the crystal oscillation and is calculated from the energy lost by the crystal divided by the energy stored in the system. A plot of dissipation versus frequency (F) for the di-block system (Figure 4a) shows that for the addition of the poly$A_{15}G_{15}$ layer the frequency and the dissipation increase linearly, however, the addition of the poly$T_{15}C_{15}$ layer results in an increase in the frequency but no change in the dissipation.

A similar trend was observed for the dissipation versus frequency for the tetra-block system (Figure 4b), where the addition of the poly$T_{15}C_{15}T_{15}C_{15}$ layer resulted in only a small increase in the dissipation of the film. Interestingly, the change in frequency and dissipation for the addition of poly$A_{15}G_{15}A_{15}G_{15}$ did not follow a linear trend but rather an exponential type curve, where there was a greater increase in the dissipation for a corresponding frequency change as more DNA was deposited in the layer. Although the curve appears as if it would continue along the same trend if the incubation time was increased, both the F and D were allowed to equilibrate for 20 min so there was no further change in the frequency or the dissipation of the film. This suggests that the DNA that binds early on in the hybridization process is more rigidly bound to the film than the DNA that hybridizes toward the end. Also, as the number of layers increased, it became apparent that after the initial period where there was no increase in the dissipation of the film, towards the end of the hybridization of the poly$T_{15}C_{15}T_{15}C_{15}$ layer, there was a small increase in the dissipation. This suggests that, like for the poly$A_{15}G_{15}A_{15}G_{15}$, the DNA deposited toward the end of the hybridization is less rigidly bound than the DNA bound at the start. It is likely that this weakly bound material occupies single-stranded regions that remain after the initial hybridization of the DNA oligomers. It is also possible that the film undergoes a rearrangement, which changes the viscoelasticity of the film.

The hybridization of poly$T_{15}C_{15}$ and poly$T_{15}C_{15}T_{15}C_{15}$ to their complementary sequences corresponds to the pairing of C with G in the film. Hybridization of the nucleotides G and C results in three hydrogen bonds between the two DNA oligomers, whereas hybridization of A and T results in two hydrogen bonds. Correspondingly, the tightly bound G/C hybridization results in no increase in the viscoelasticity of the film, whereas in contrast, the weaker binding of A/T results in a significant increase. The plot of dissipation versus frequency for the XY-di-block system (Figure 4c) shows a linear increase in F and D for each layer. This confirms that the variation in the dissipation and frequency for the block systems is due to the different number of hydrogen bonds stabilizing the layers. In comparison, for a film assembled from poly(styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH), the change in frequency for the 5-bilayer film was similar to the change in frequency for a 5-bilayer di-block film; however, the dissipation changed by less than 1×10^{-6} (data not shown).

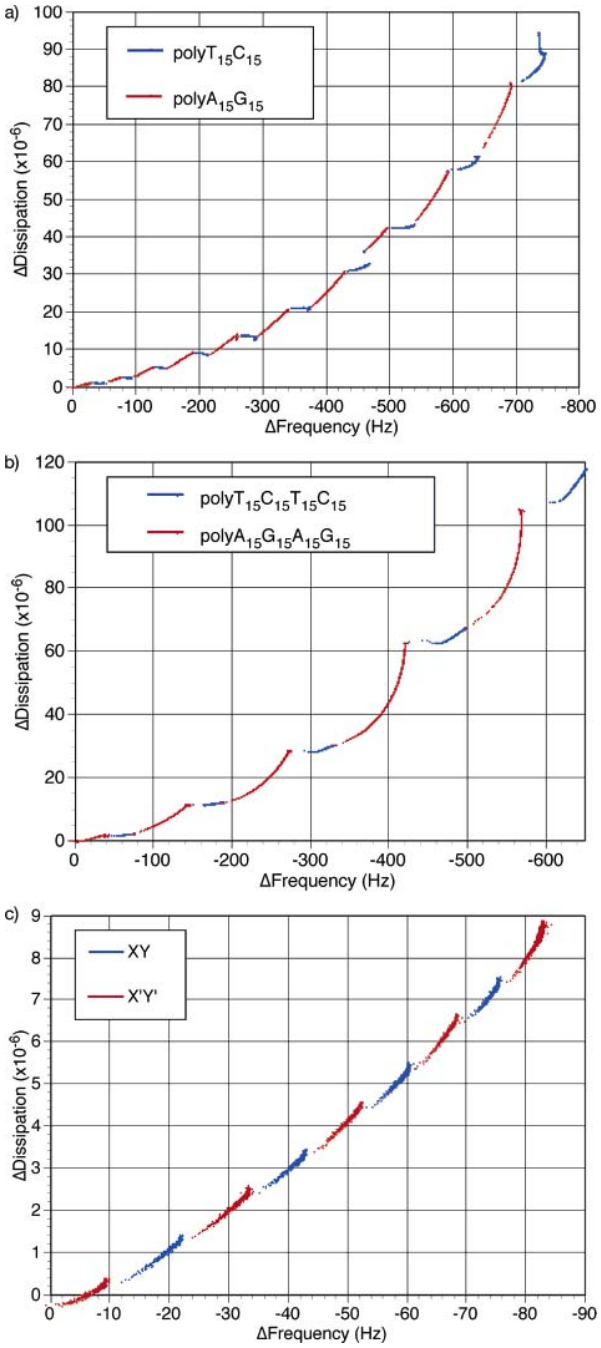


Figure 4. Dissipation versus frequency plot for the (a) 10 bilayers of di-block oligonucleotides, and five bilayers of (b) tetra-block and (c) XY-di-block oligonucleotides. The homopolymeric block systems showed a marked difference between the hybridization of the two layers, whereas the XY-di-block system showed similar build-up for each component.

c. Film Stability. The stability of each system to various conditions is an important property of the film. As the films consist entirely of negatively charged oligomers, the counterion in the solution plays an important role.[23] Positive ions (in this case sodium) shield the negatively charged layers, limiting repulsion. It is expected that lowering the salt concentration (the concentration of Na^+ ions) would increase the repulsion between the layers.

(22) Höök, F.; Rodahl, M.; Fredriksson, C.; Brzezinski, P,; Keller, C. A.; Voinova, M.; Krozer, A.; Kasemo, B. *Faraday Discuss.* **1997**, *107*, 229.

(23) Bai, Y.; Das, R.; Millet, I. S.; Herschlag, D.; Doniach, S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1035.

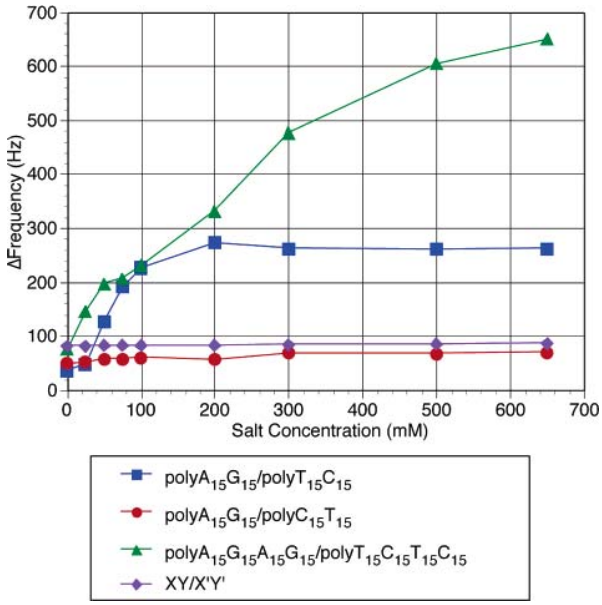


Figure 5. Effect of salt concentration on the stability of 5-bilayer DNA films.

For the tetra-block system (the system with the highest mass and highest *D:F* ratio), even a relatively small decrease in the salt concentration (100 mM) led to loss of mass from the film (Figure 5). This indicates that, although the mass of the film is large, a significant amount of the material in the film is only loosely bound, as more than 400 Hz (an apparent loss of 64% of the film mass[24]) was lost by lowering the salt concentration to 100 mM. At around 100 mM, the rate of mass lost from the film decreased, but below 50 mM a further 122 Hz was lost (a further 19%[24] of the original film mass). This indicates that there is both very loosely associated DNA which was lost as soon as the salt concentration was lowered, as well as more strongly bound DNA which was only lost when the salt concentration was lowered below 50 mM. In MilliQ water, approximately 12%[24] of the original film mass remained. This corresponds to the mass deposited in the first bilayer but is also equivalent to the mass of a 3-bilayer XY/X′Y′ film (the film with the lowest mass per layer). The di-block system was stable to salt concentrations down to 100 mM, but below this concentration, the film lost 85%[24] of the original film mass. The mass remaining corresponds to the mass deposited in the first bilayer and the mass of a 2-bilayer XY/X′Y′ film.

Both the reverse-di-block and XY-di-block systems appeared to be stable to all salt concentrations, with no significant mass loss observed for the XY-di-block system, whereas the reverse-di-block system retained 70%[24] of the film mass in MilliQ water (equivalent to the mass of 3 bilayers of the reverse-di-block system). The masses of these films were initially significantly lower than the masses of the di-block or tetra-block systems, and the fact that little mass was lost from the films indicates that DNA within the film is strongly bound. This also suggests that the mass loss seen in the reverse-di-block system may be due to loss of weakly hybridized material.

The dissipation values for the film also provide information on the swelling of the film at decreasing salt concentrations

(24) As the dissipation of the films was very high and treatment of the film with lower salt concentration further increased the dissipation of the film, the percent change in the frequency of the film may not correspond linearly to the absolute mass lost from the film. When the dissipation of the film is high, the mass of the film may be underestimated.

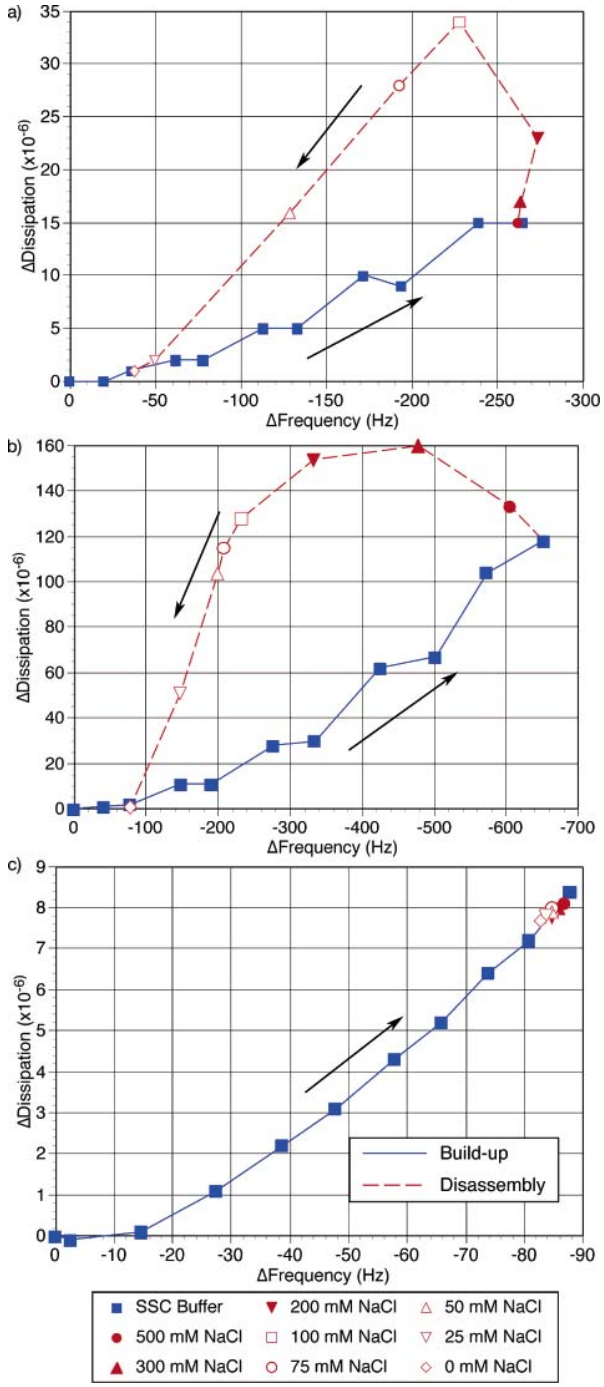


Figure 6. Effect of salt concentration (NaCl) on the viscoelasticity of 5-bilayer DNA films: (a) di-block, (b) tetra-block, and (c) XY-di-block films. The frequency and dissipation measurements after treatment with the salt solutions were taken in the same buffer in which the films were assembled.

(Figure 6). For the di-block system, although the mass of the film did not significantly change after treatment with salt solutions down to 100 mM, the dissipation of the film increased significantly. After treatment with 100 mM salt, the dissipation of the film more than doubled from 15 to 34. At lower salt concentrations, both the frequency and the dissipation dropped due to the loss of mass from the film. The changes observed indicate an irreversible morphological change in the film structure, as after the addition of the salt solution, the *F* and *D* were measured in the assembly buffer (see the Experimental Section). The *D* vs

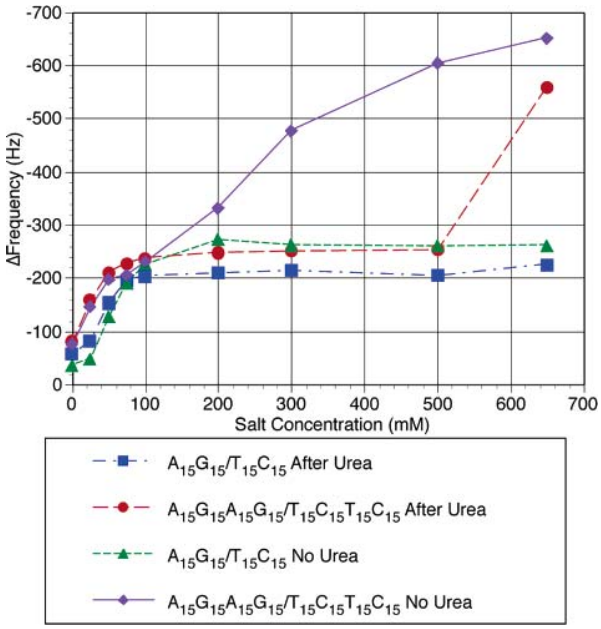


Figure 7. Effect of urea treatment on film stability. The value at 650 mM salt concentration refers to the mass of the film before urea treatment.

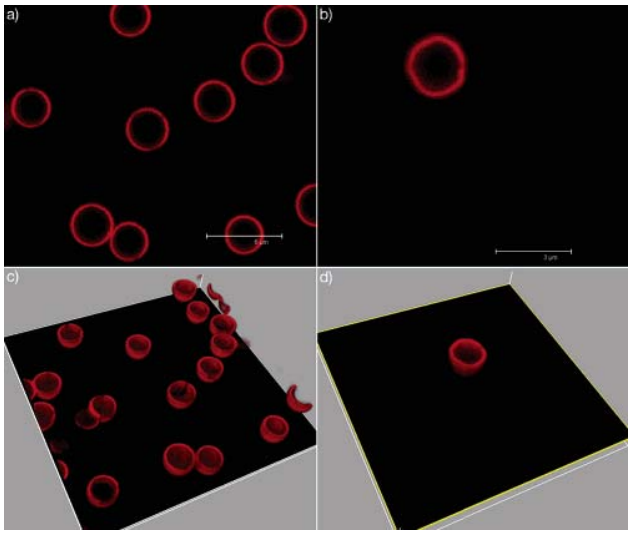


Figure 8. CLSM images of polyT$_{30}$(polyA$_{15}$G$_{15}$/polyT$_{15}$C$_{15}$)$_5$-coated particles (a and c) and hollow capsules (b and d). Panels a and b show cross-sections, and c and d show three-dimensional reconstructions from the confocal data. The capsules are ca. 2 μm in diameter.

F plot for the tetra-block system (Figure 6b) also showed a similar trend, where the dissipation of the film was significantly higher for any given frequency during the mass build-up. The XY-di-block system (Figure 6c) showed very little variation in the frequency or dissipation, indicating again that the film was stable to low concentrations of salt.

d. Urea Treatment. We have shown previously that treatment of the films with urea has a significant effect on the film morphology,[15] but interestingly, after urea treatment, the response of the films to lowering salt concentration was very similar to the films that were not treated with urea (Figure 7). The tetra-block system lost 55%[24] of the film mass (corresponding to ~300 Hz) with urea treatment, which was equivalent to the mass of the loosely associated DNA that was lost when the salt concentration was lowered to 100 mM. The di-block film lost less than 10% of the film mass (corresponding to 20 Hz) with urea treatment and the XY/X′Y′ film exhibited negligible mass loss. It would normally be expected that such a high urea concentration would completely denature the DNA.[19] It is possible that the high degree of entanglement of the DNA may keep the oligomers from completely denaturing during the urea treatment and upon exposure to the buffered solution, the DNA renatures.

e. Hollow DNA Capsules. We previously reported the formation of polyA$_{15}$G$_{15}$/polyT$_{15}$C$_{15}$ films on colloids using silica particles coated with an initial layer of PEI.[15] The PEI layer was used to impart a positive charge on the surface so the first layer of DNA could electrostatically bind to the particle. In this work, by using amine-functionalized silica particles (by reacting 3-aminopropyltrimethoxysilane onto the surface), positively charged silica particles were used to facilitate the build-up of the DNA multilayers. The use of these positively charged particles omitted the need for an initial PEI layer. Subsequent dissolution of the core using hydrofluoric acid resulted in stable hollow capsules consisting solely of DNA. The capsules were not visible using brightfield optical microscopy, so a fluorescently labeled polyT$_{15}$*C$_{15}$ oligonucleotide was incorporated into the film to facilitate visualization. Figure 8 shows the confocal laser scanning microscope (CLSM) images of the di-block film on the colloidal supports and the hollow capsules that were formed after the core was dissolved. The hollow capsules shrank in diameter to 2 μm ($\pm$0.2 μm) from the original particle size of 3 μm ($\pm$0.2 μm). This shrinkage is quite significant compared with electrostatically bound polyelectrolyte systems, such as poly(styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH), which exhibit minimal shrinkage upon capsule formation.[25,26] The shrinkage of the capsules is likely due to the loose structure of the film, as suggested by the CLSM measurements. Further evidence of the loose structure of the film was evident from the high permeability of the DNA films determined using the molecular beacon technique.[27] This technique can determine the permeability of DNA through a film and showed that films composed entirely of DNA had a significantly higher permeability than those assembled from conventional polyelectrolytes (such as PSS and PAH) (data not shown). We are currently investigating in detail the swelling and shrinking properties of these films.

Conclusions

We have shown that films consisting solely of DNA can be assembled using a variety of different DNA oligonucleotide building blocks. The morphology of the films is affected by the orientation of oligomers within the film, the ability of the DNA to hybridize in multiple positions, and the number of hydrogen bonds holding the layers together. All of the films investigated were stable in physiological salt conditions (~100 mM), although in the tetra-block system much of the weakly associated material was desorbed from the film. Both the di-block and the tetra-block systems showed a change in the viscoelasticity after treatment with reduced salt concentrations, indicating that the films undergo a morphological change when the repulsion between the layers increases. The films were stable in concentrated urea solutions that are normally used to denature double-stranded DNA. We are currently investigating the properties of hollow capsules made using these DNA layers and ways to

(25) Heuvingh, J.; Zappa, M.; Fery, A. *Langmuir* **2005**, *21*, 3165.
(26) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2201.
(27) Johnston, A. P. R.; Caruso F. *J. Am. Chem. Soc.* **2005**, *127*, 10014.

stabilize the material that is weakly associated with the film. We anticipate that these films will find applications in drug/gene delivery as well as in biomolecule sensing. These films may also find use in DNA microarrays, as they afford DNA structures with different surface-bound orientations, therefore potentially facilitating binding of the target DNA sequences.

Acknowledgment. This work was supported by the Australian Research Council under the Federation Fellowship and Discovery Project schemes and by the Victorian State Government under the STI Initiative.

LA052581H