Peptide Nucleic Acid Films and Capsules: Assembly and Enzymatic Degradation[a]

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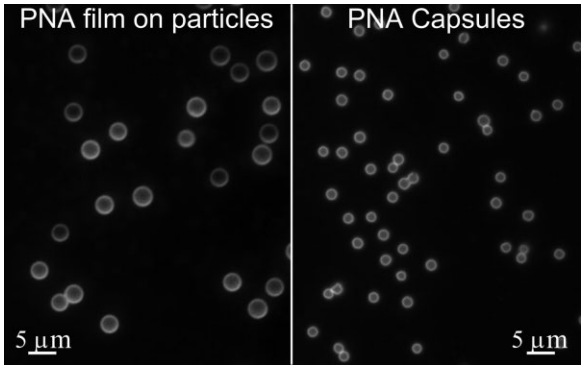
Sequence-directed hybridization of nucleic acids provides a high level of control for the bottom-up assembly of nanostructured materials. Altering the DNA sequence affords control and versatility over the film structure, but is limited by the chemical and physical properties of DNA. Here, we use DNA analogues, peptide nucleic acids (PNAs), to introduce new properties to multilayered thin films and retain the advantages of sequence-directed assembly. Thin films, formed by the layer-by-layer (LbL) assembly of PNA strands, were assembled from short PNA sequences on planar and colloidal substrates. In the case of PNA-coated particles, hollow capsules were obtained following removal of the sacrificial particle template. The PNA films were stable to both nuclease and protease degradation, and the nuclease degradation rate could be tuned by varying the amount of DNA incorporated into the films. These thin films may find use in biomedical applications.



Introduction

Nucleic acids are useful building blocks for materials assembly because of their high specificity, which allows for programmable interactions, and because their behaviour and chemistry are well understood. Nature has provided a toolbox of enzymes for the specific ligation, cleavage, replication and transcription of DNA and RNA. This level of control is ideal for the bottom-up synthesis of nanostructured materials. However, the properties of these materials are limited to the inherent properties of DNA (e.g., susceptible to nuclease degradation), which may limit in vivo applications of the films. To overcome this, nucleic acid analogues, such as peptide nucleic acids (PNAs), can be employed as building blocks to limit degradation and increase the stability of engineered materials, while retaining the specificity of sequence-directed assembly.

Some of the earliest DNA structures were prepared by Seeman and coworkers. Starting with the creation of a three-way junction,[1] it is possible to design a large number of two-dimensional network structures from DNA. These include geometric shapes, a smiling face and a map of the world.[2] These structures highlight the exquisite specificity of DNA self-assembly. DNA hybridization has also been used for the ordered aggregation of gold nanoparticles upon the addition of a specific DNA sequence,[3] and has been extended to use PNA for the aggregation of the nanoparticles.[4] Hydrogel networks of DNA, PNA and protein have been assembled into micro- and nano-sized microgel particles.[5] Hollow micron-sized DNA particles (capsules)

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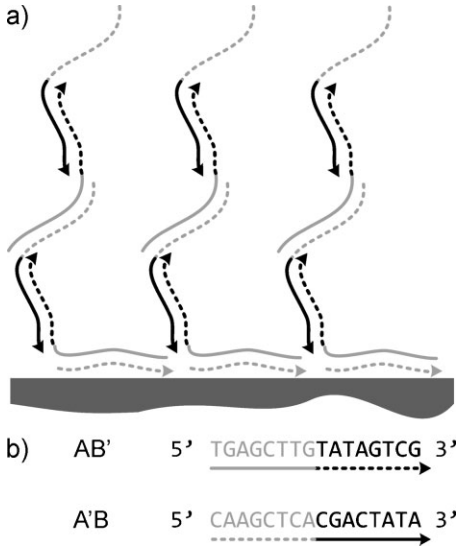
and supported DNA thin films[6] have been assembled using the layer-by-layer (LbL) technique.[7–9] When the DNA films are assembled on particles, subsequent dissolution of the templates[10,11] results in the formation of free-standing structures known as hollow DNA capsules.[6,12–14]

For LbL assembly using DNA, two complementary strands are alternately hybridized to the film. The selection of DNA sequences is crucial to successful LbL growth. Each DNA strand contains two distinct sequence regions that have negligible internal hybridization. However, the order and direction of the sequences is such that hybridization is not limited to two DNA strands but can occur infinitely, forming longer DNA chains. Hybridization of each sequence into the film leaves one block available for the hybridization of the next layer (Scheme 1). The stability of the film after template removal is presumably from entanglement and cross-hybridization of the DNA chains grown from the surface. The programmable nature of sequence-specific hybridization provides an additional level of control for LbL films. For example, because DNA is a directional molecule, reversing the direction of the sequence changes the growth and stability of the film.[12] For DNA films formed on colloids, the degree of capsule shrinkage after core removal was dependent on the sequence of the final layer and on the directionality of the sequence used.[13] The stability of the films in low salt solutions was increased by increasing the length of the sequences[14] and by incorporating oligonucleotide cross-linkers.[15] Although the stability and shrinkage of the films can be controlled by altering the sequence and length of the DNA, these films are susceptible to nuclease degradation.[16] In this work, a synthetic DNA analogue, PNA, is used to form nuclease-resistant thin films and hollow capsules. The assembly of DNA/PNA hybrid materials allows tuning of the rate and degree of film/capsule degradation. Additionally, the use of PNA reduces the electrostatic repulsion between the nucleic acid strands, permitting shorter nucleic acid sequences to be used in the assembly of stable PNA-based films.



Scheme 1. (a) Schematic representation of idealized LbL assembly of diblock PNA or DNA strands. The arrows correspond to the direction of the sequence (from the 5′ to the 3′ end). This scheme does not represent the actual hybridization of the strands. (b) Sequence of each diblock strand. Within the strand name, each letter represents a sequence block and the complementary sequence is indicated by a prime (′).

PNA is a DNA analogue that replaces the phosphate backbone with a peptide-mimicking *N*-(2-aminoethyl)glycine backbone. The directionality of the backbone is determined by the terminal carboxy (c) and amine (n) groups like peptides, rather than the terminal 5′ phosphate and 3′ hydroxyl groups like DNA. The c-terminus corresponds to the 5′ end of DNA, and the n-terminus corresponds to the 3′ end. It hybridizes with both DNA and RNA by standard Watson–Crick base-pairing, predominantly in an antiparallel conformation; however, PNA can hybridize with DNA in both parallel and antiparallel conformations.[17] There is no electrostatic repulsion from the uncharged pseudo-peptide backbone, resulting in rapid and stronger hybridization compared to a normal DNA helix. The modified structure of the backbone restricts hydrolysis and degradation by proteases and nucleases.[18] Thus, films that incorporate PNA may have improved stability, particularly against enzymatic degradation.

In the current work, sequence-directed LbL assembled films were formed using two alternating PNA strands to create a PNA film, and also by alternating PNA and DNA to create hybrid films with intermediate properties. The films were also formed on colloids and the core was dissolved, leaving PNA and PNA/DNA capsules, which may find application as drug delivery vehicles. Finally, the resistance of PNA and PNA/DNA films to degradation by nucleases and proteases was examined. PNA films assembled on planar and colloidal templates were resistant to enzymatic degradation.

Experimental Part

Materials

PNA was custom synthesized by Biosynthesis Inc. (Lewisville, USA) and DNA was custom synthesized by Geneworks (Adelaide, Australia). The detailed sequences can be found in Table 1, and are termed AB′ and A′B. Concentrated stock solutions were made to 150×10^{-6} M in water. DNase I was purchased from Genesearch Pty. Ltd. (Arundel, Australia). SiO_2 particles (3 μm diameter) were purchased from MicroParticles GmbH (Berlin, Germany) as a $50\ g \cdot L^{-1}$ suspension and were used as received. Protease from *Streptomyces griseus*, poly(ethyleneimine) (PEI, 25 000 $g \cdot mol^{-1}$), tris-hydroxymethylaminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), sodium citrate, sodium acetate buffer, ammonium fluoride (NH_4F), hydrofluoric acid (HF) and 3-aminopropyl-

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Table 1. Details of PNA and DNA diblock sequences used in LbL assembly.

Name	Sequence (5′ to 3′)
$PNA_{AB'}$ [a)]	Glu-TGAGCTTG TATAGTCG-Glu
$DNA_{AB'}$	TGAGCTTG TATAGTCG
$PNA_{A'B}$	Glu-CAAGCTCA CGACTATA-Glu
$DNA_{A'B}$	CAAGCTCA CGACTATA
DNA_{BB}	CAAGCTCA CAAGCTCA

[a)]The letters A and B represent a block within the strand. A prime (′) indicates the complementary sequence.

triethxoysilane (APS) were purchased from Sigma–Aldrich (Sydney, Australia). Ammonia (30% w/w), sodium chloride, ethanol, sulfuric acid and hydrogen peroxide were obtained from Merck. Picogreen and degradation buffer [Tris (10×10^{-3} M, pH 7.6) with $MgCl_2$ (2.5×10^{-3} M) and $CaCl_2$ (0.5×10^{-3} M)] were purchased from Invitrogen (Melbourne, Australia). Picogreen was diluted 50 times in TE buffer [TE buffer consisted of Tris (10×10^{-3} M, pH 7.5) with EDTA (1×10^{-3} M)]. SSC buffer consisted of sodium citrate (50×10^{-3} M, pH 7) with NaCl (0.5 M). High-purity water with a resistivity greater than 18 MΩ cm was obtained from an inline Millipore RiOs/Origin system.

Amine Modification of SiO_2 Particles

A suspension of 3 μm diameter SiO_2 particles in 1 mL of ethanol was reacted with 250 μL of APS and 50 μL of ammonia (30% w/w) for 2 h. After this time, the particles were washed several times in ethanol and then three times in water. The resulting particles had a ζ-potential of 72 ± 3 mV in sodium acetate buffer (10 mM, pH 4), or 8 ± 5 mV in TE buffer.

Analysis of Layer-by-Layer (LbL) Assembly by QCM-D

Gold-coated 5 MHz AT-cut crystals were cleaned by immersion in Piranha solution (70% sulphuric acid/30% hydrogen peroxide) for 15 min followed by rinsing in water. This process was repeated twice. *Caution! Piranha solution is highly corrosive. Extreme care should be taken when handling Piranha solution and only small quantities should be prepared.* The crystals were dried under a stream of nitrogen and placed in a UV ozone cleaner for 20 min. QCM-D measurements were performed using a Q-Sense E4 device equipped with flow cells (Q-Sense AB, Västra Frölunda, Sweden). The temperature was kept constant at 23.4 °C during the experiments. After initially depositing a layer of PEI ($1\ g \cdot L^{-1}$) in 0.5 M NaCl, PNA (2.5 μM) and DNA (2.5 μM) in TE buffer or SSC buffer were adsorbed to the film for 10 min. After each adsorption step, the film was washed with TE buffer or SSC buffer. For DNase I degradation, the films were washed into degradation buffer for 10 min, followed by the addition of 10 U of DNase I ($50\ U \cdot mL^{-1}$) or protease ($50\ U \cdot mL^{-1}$). The films were incubated overnight and washed into TE or SSC buffer for the final measurements. All measurements were recorded using the same buffer conditions: either TE buffer or SSC buffer.

Layer-by-Layer (LbL) Assembly on Particles

Thin film formation on particles was initiated by incubating 25 μL of 3 μm diameter amine-modified SiO_2 particles ($0.5\ g \cdot L^{-1}$) with the priming sequence BB (10 μM) in TE buffer for 15 min. After three wash/centrifuge cycles ($2\,000 \times g$ for 30 s) with TE buffer, sequence AB′ (5 μM) was adsorbed for 15 min. Sequences AB′ and A′B were added sequentially as described until 10 layers were deposited. Hollow capsules were formed by removal of the silica core particles with HF. *Caution! HF and NH_4F are highly toxic. Extreme care should be taken when handling HF solution and only small quantities should be prepared.* The HF readily reacted with the SiO_2, forming SiF_6^{2-}, which can diffuse through the polymer film. The particles were suspended in 50 μL of TE buffer to which 250 μL of HF (2 M) in NH_4F (8 M, pH 5) was added. The resulting capsules were washed four times into TE buffer via centrifugation ($4\,000 \times g$ for 4 min).

Melting Temperature

In a quartz cuvette, the PNA and/or DNA sequences of interest were mixed with 3 mL of TE buffer or SSC buffer at a final concentration of 25 nM. The absorbance of the solution was monitored at 260 nm as the temperature was increased from 20 to 95 °C at $0.5\ °C \cdot min^{-1}$.

Nuclease and Protease Degradation on Particles

PNA- and DNA-coated particles ($1\ \mu L$, $0.5\ g \cdot L^{-1}$) were added to 200 μL of degradation buffer containing 2.5 U of DNase I or 2.5 U of protease, in addition to a control sample containing no enzyme. After incubation in enzyme solution overnight, the core was dissolved using HF (2 M) in NH_4F (8 M, pH 5). The capsules were stained with picogreen and observed by fluorescence microscopy.

Results and Discussion

Layer-by-Layer (LbL) Assembly of PNA Films

PNA thin films were assembled using two diblock strands: one block had a sequence that was complementary to the adsorbed layer, and the second was complementary to one block on the incoming layer. Both PNA and DNA strands were used for film assembly. The sequences of strands used are detailed in Table 1 and are termed AB′ and A′B. Each strand contains two 8-mer blocks (total 16-mer) of randomly-arranged bases with negligible internal hybridization. Our previous work has shown that the length of the DNA strand affects the amount of material deposited,[14] and that the melting temperature of 5-mer DNA blocks was too low for film growth. However, for the same sequence, PNA duplexes have higher melting temperatures than DNA because of the uncharged backbone. Thus, the

PNA strands used for multilayer growth can be shorter than DNA strands. This is important because the uncharged backbone of PNA renders it hydrophobic and poorly soluble, and this property increases with strand length. To ensure the strands were soluble, the length was minimized and the sequence was designed to be random with equal amounts of purine and pyrimidine bases. Typically, positively charged lysine residues are added to the end of the PNA sequence to improve solubility; however, the strands used in this work contain a single glutamine acid residue at each end. This confers a negative charge on the PNA at physiological conditions, increasing its solubility and ensuring that multilayer growth was the result of base-pair hybridization without the possibility of electrostatic interactions.

After adsorption of the priming layer DNA_{BB}, the first strand adsorbed was AB′. This was followed by A′B and the cycle was repeated until four bilayers were deposited. These films were assembled using combinations of DNA and PNA strands in TE buffer. Formation of the films was investigated in situ using QCM-D; a decrease in the QCM-D frequency indicates an increase in mass on the surface of the sensor, including associated water. The rate of growth of the films made of $PNA_{AB'}/PNA_{A'B}$, $PNA_{AB'}/DNA_{A'B}$ and $DNA_{AB'}/PNA_{A'B}$ generally decreased with layer number (Figure 1a). The effect was more pronounced in $DNA_{AB'}/DNA_{A'B}$ films. This suggests that the strands become less available for additional hybridization after incorporation into the film. The total amount of growth for each of the films depended on the chemical nature of the AB′ strand (i.e., DNA vs. PNA, Figure 1a). $PNA_{AB'}$ strands resulted in films with lower frequency (and hence increased mass), which may result from the increased PNA hybridization strength. Additionally, PNA duplexes and PNA/DNA duplexes have different helical structures to DNA duplexes.[19] As the AB′ strand is the first layer adsorbed onto the priming strand, the structure of this initial layer may affect the hybridization of subsequent layers. These data indicate that the chemical properties of the PNA and/or DNA strands used in LbL assembly control the growth (and thickness) of multilayered films.

Each of the films had a similar dissipation value in TE buffer, which increased slightly with layer number (Figure S1a, Supporting Information). The dissipation values (0.1×10^{-6} per 10 Hz) were significantly lower than the random sequenced diblock DNA films previously used in LbL assembly (1×10^{-6} per 10 Hz),[12] indicating that the PNA films are more rigid. The low salt conditions and hydrophobic nature of the films result in decreased hydration. This can be seen in the ratio of dissipation to frequency for each of the films, which indicates that the films containing PNA are less viscoelastic than the films made solely of DNA (Figure S1b, Supporting Information).

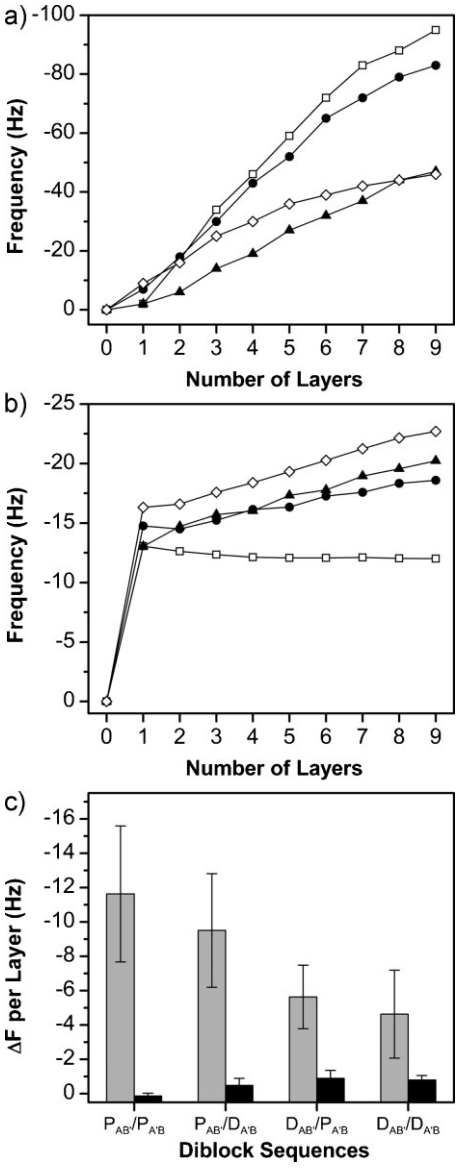

Figure 1. QCM-D analysis of LbL-assembled films from AB′/A′B diblocks in (a) TE buffer, and (b) SSC buffer. $PNA_{AB'}/PNA_{A'B}$ (□), $PNA_{AB'}/DNA_{A'B}$ (●), $DNA_{AB'}/PNA_{A'B}$ (▲) and $DNA_{AB'}/DNA_{A'B}$ (◇). Layer 1 is a priming layer, DNA_{BB}. The even numbers represent AB′ layers; the odd numbers represent A′B layers. (c) Average incremental change in frequency per layer in TE buffer (grey) and SSC buffer (black).

When LbL assembly of the same PNA and DNA sequences was performed in SSC buffer, significantly reduced growth was observed (Figure 1c). Indeed, no growth was apparent in the sample containing only PNA sequences (Figure 1b). To further understand the hybridization of the sequences, melting temperatures were probed experimentally.

The absorbance of PNA duplexes in TE buffer did not display a sharp transition, but rather a linear increase in

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absorbance as the temperature increased (Figure S2, Supporting Information), thus no T_m is reported. The linear behaviour was probably because the hybridization is not between two strands, but rather between a long chain of strands. For the PNA strands in SSC, a decrease in absorbance was recorded as opposed to the increase expected when nucleotide strands melt (Figure S2, Supporting Information). This suggests that the PNA was aggregated or unavailable for hybridization in the high salt buffer. This is in agreement with reports of an inverse effect of salt on PNA/DNA hybridization in solution.[20] The high dissipation compared to the frequency indicates that the films assembled in SSC buffer (Figure S3b, Supporting Information) were more viscoelastic than the films assembled in TE buffer, and was similar to other DNA films.[12] This is probably due to increased hydration of the films.

To create hollow capsules that may have potential as carrier vehicles, LbL films of PNA and DNA were assembled on amine-functionalized silica particles. After the deposition of four bilayers of PNA or DNA, picogreen was used to visualize the films. Picogreen, a dye that undergoes a significant increase in fluorescence when intercalated with double-stranded DNA,[21] also showed enhanced fluorescence upon interaction with PNA. Films were observed on particles from all PNA and DNA films studied (Figure 2). Further proof of successful LbL assembly on particles was the formation of free-standing capsules after core removal (Figure 2 and Table 2). $PNA_{AB'}/PNA_{A'B}$ films formed stable, shrunken capsules after core removal (Figure 2b). The deposition of only one bilayer was required to form stable capsules. The four bilayer PNA capsules shrunk to around 25% of the original surface area. This is similar to DNA multilayered films using random sequenced diblock strands of increased length,[13] although DNA capsules formed from homopolymeric reverse diblock sequences did not shrink to the same degree.[13] For DNA homopolymeric reverse diblock sequences, we previously showed that the outer layer governed the degree of capsule shrinkage and that the amount of shrinkage was independent of the number of layers deposited.[13] Capsules formed from both $DNA_{AB'}/PNA_{A'B}$ strands (Figure 2f) shrunk more than the capsules made entirely of PNA (Figure 2b). It was surprising that no stable capsules were formed from $PNA_{AB'}/DNA_{A'B}$, as these films had a larger frequency change (indicating the deposition of more material) than $DNA_{AB'}/PNA_{A'B}$ films as measured by QCM-D (Figure 1a). However, higher film mass does not necessarily correlate to increased film stability.[12] The instability of the $PNA_{AB'}/DNA_{A'B}$ capsules may be due to hybridization of fewer than the eight bases per block. Alternatively, there may be less cross-hybridization between strands. Both possibilities would lead to more material in the film but less stability. Films formed entirely from DNA deconstructed on removal of the template particle (Figure 2h). Although DNA capsules have been formed using other DNA sequences,[6] the deconstruction of these films was anticipated because the length of the sequences used (16-mer) was very close to the minimum length required for successful LbL assembly,[14] and the theoretical melting temperature of the 8-mer blocks is close to room temperature (24 °C).[22] The stable PNA capsules highlight the improved strength of the PNA/PNA hybridization compared to DNA/DNA hybridization.

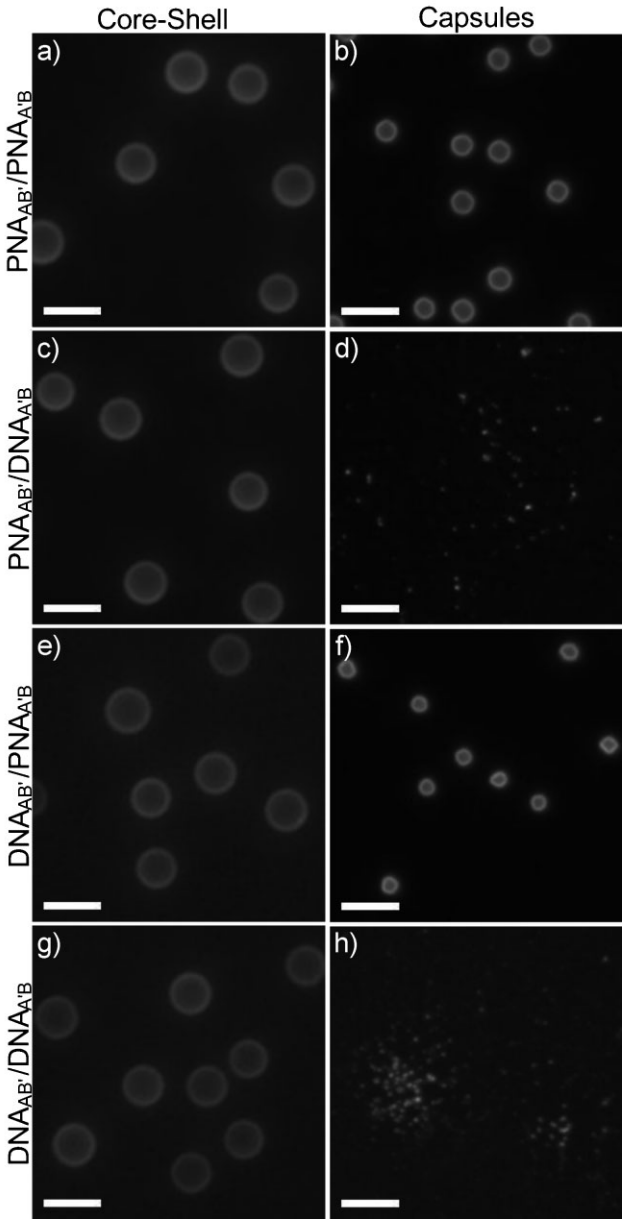

Figure 2. Fluorescence microscopy images of PNA and DNA films on particles: (a) $(PNA_{AB'}/PNA_{A'B})_4$, (c) $(PNA_{AB'}/DNA_{A'B})_4$, (e) $(DNA_{AB'}/PNA_{A'B})_4$, (f) $(DNA_{AB'}/DNA_{A'B})_4$; and after core removal: (b) $(PNA_{AB'}/PNA_{A'B})_4$, (d) $(PNA_{AB'}/DNA_{A'B})_4$, (g) $(DNA_{AB'}/PNA_{A'B})_4$, (h) $(DNA_{AB'}/DNA_{A'B})_4$. Films were stained with picogreen. Scale bars are 5 μm.

Table 2. Measured capsule diameters[a] after core removal using HF/NH_4F.

Number of layers	$PNA_{AB'}/PNA_{A'B}$	$PNA_{AB'}/DNA_{A'B}$	$DNA_{AB'}/PNA_{A'B}$	$DNA_{AB'}/DNA_{A'B}$
1	–[b]	–[b]	–[b]	–[b]
2	1.4 ± 0.2	–[b]	–[b]	–[b]
3	1.7 ± 0.2	–[b]	–[b]	–[b]
4	1.7 ± 0.2	–[b]	–[b]	–[b]
5	1.8 ± 0.2	–[b]	1.1 ± 0.2	–[b]
6	1.6 ± 0.2	–[b]	1.4 ± 0.1	–[b]
7	1.7 ± 0.2	–[b]	1.4 ± 0.2	–[b]
8	1.9 ± 0.2	–[b]	1.5 ± 0.4	–[b]

[a]Size of core–shell particles was 3.6 ± 0.2 μm; [b]Dashes (-) indicate when no capsules were formed.

Protease and Nuclease Degradation

DNA, and therefore DNA-containing films, are susceptible to degradation by nucleases. Although this can be an advantage for triggered cargo release and film removal, for some applications it may be desirable to reduce or hinder nuclease degradation. For example, the use of nucleases to degrade drug delivery vehicles may also result in degradation of the therapeutic nucleic acid cargo. The stability of the DNA, DNA/PNA and PNA films to DNase I was investigated by observing the material lost using QCM-D (observed by an increase in the film frequency). After the deposition of eight layers of diblock sequences, the films were washed into a buffer for optimal DNase I activity and incubated overnight either with or without 10 U of DNase I. One unit of DNase I will completely degrade 1 μg of DNA in 10 min at 37 °C. The material lost from the film was expressed as a percentage of the frequency of the film without enzyme. Films incubated only in buffer were stable over 14 h, retaining 98% of their mass (Figure S4, Supporting Information). When incubated with DNase I, the film made entirely of DNA strands ($DNA_{AB'}/DNA_{A'B}$) was slowly degraded (Figure 3) (≈25% film loss in 14 h), whereas the film made entirely of PNA strands remained intact (≈2% film loss in 14 h). This was attributed to the susceptibility of the DNA backbone to hydrolysis by DNase I, whereas the modified PNA backbone was not recognized by DNase I. The two hybrid films containing both DNA and PNA showed some susceptibility to degradation by the DNase I, but the degradation occurred at a significantly slower rate than the DNA film (Figure 3).

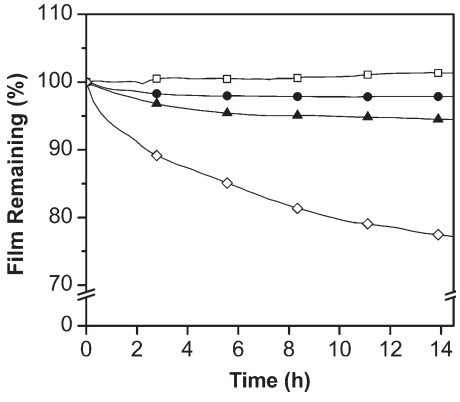

Figure 3. DNase I degradation of $PNA_{AB'}/PNA_{A'B}$ (□), $PNA_{AB'}/DNA_{A'B}$ (●), $DNA_{AB'}/PNA_{A'B}$ (▲) and $DNA_{AB'}/DNA_{A'B}$ (◇) films, normalized to the control films without enzyme.

The degradation rate of the film can be controlled by varying the amount of PNA in the film. Figure 3 shows that an increase in the PNA content reduced the rate of degradation. DNase I is a non-specific endonuclease and it is capable of degrading DNA when it is in a duplex with RNA; thus it is also expected to degrade DNA when in a duplex with PNA, as was observed here. The decreased degradation rate of the hybrid films was probably because the structure of a DNA/PNA double helix is different to a DNA/DNA double helix,[19] and is therefore more difficult to fit into the DNase I active site.

Similarly, to determine the susceptibility of DNA, DNA/PNA and PNA films to protease, the films were incubated overnight in solutions with or without 10 U of a non-specific protease. One unit of protease will hydrolyse casein to produce the equivalent to 1 μmol of tyrosine per min at pH 7.5 at 37 °C. The $PNA_{AB'}/PNA_{A'B}$ film displayed an initial increase in the mass that saturated within several hours (Figure 4). Protease from *S. griseus* is a non-specific protease that causes hydrolysis of the peptide bond. The proteases may recognize the peptide-like backbone of PNA, resulting in their initial accumulated adsorption. However, the hydrolysis of the PNA backbone is not possible due to the modified structure. The slow rate of degradation observed in the DNA-containing films (Figure 4) could be due to electrostatic and hydrophobic interactions with the protein causing defoliation of the film by interrupting the hydrogen bonding. In solution, PNA oligomers have been shown to be stable in human blood serum and in extracts of *Escherichia coli*, *Micrococcus luteus*, and mouse Ehrlich ascities tumour

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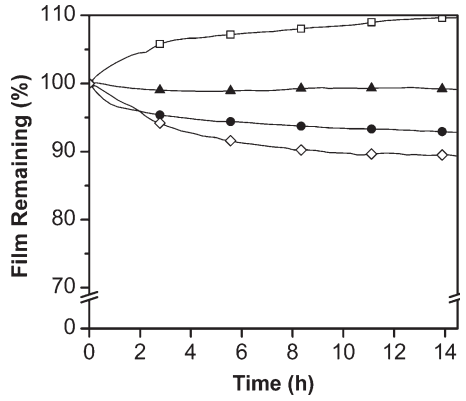

Figure 4. Protease degradation of $PNA_{AB'}/PNA_{A'B}$ (□), $PNA_{AB'}/DNA_{A'B}$ (●), $DNA_{AB'}/PNA_{A'B}$ (▲) and $DNA_{AB'}/DNA_{A'B}$ (◇) films, normalized to the control films without enzyme.

cells.[18] These data indicated that PNAs were not degraded by nucleases and proteases present inside cells.

The stability of the films on particles to nucleases and proteases was investigated by incubating the core–shell particles in enzymatic solutions. After 24 h incubation, the films were tested for capsule formation. As expected, capsules were formed from the film made from $PNA_{AB'}/PNA_{A'B}$ after both DNase I and protease treatment (Figure 5c and e). This indicated that the film was stable to both DNase I and protease, and is in agreement with the QCM-D experiments. The $DNA_{AB'}/PNA_{A'B}$ film was the only other film to form stable capsules in the control buffer (Figure 5b). It was also stable to protease, forming capsules (Figure 5f). Moreover, in further agreement with the results obtained on planar films using QCM-D, this film was not stable to DNase I, as indicated by the absence of capsules and the presence of debris in solution (Figure 5d).

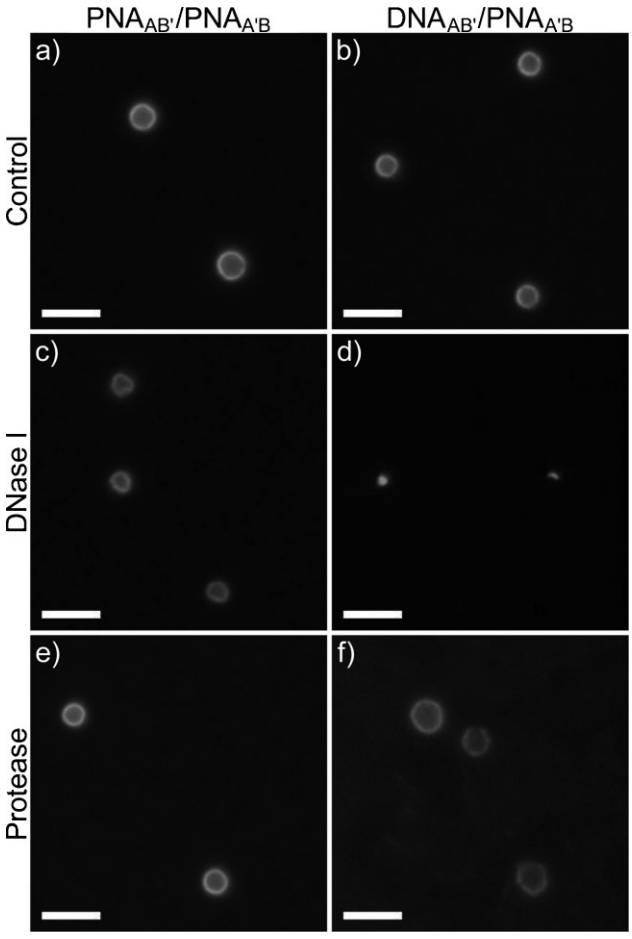

Figure 5. Fluorescence microscopy images of (a) $PNA_{AB'}/PNA_{A'B}$ and (b) $DNA_{AB'}/PNA_{A'B}$ capsules formed after treatment with DNase I (c), (d) and protease (e), (f). Films were stained with picogreen. Scale bars are 5 μm.

Conclusion

Sequence-directed hybridization was used for the LbL assembly of PNA and DNA multilayered films. PNA films and hybrid films were assembled in TE buffer; however, the high salt concentration of SSC buffer restricted growth of the PNA films. For hybrid PNA/DNA films, the growth depended on the chemistry of the first strand adsorbed (i.e., PNA or DNA). Films were formed on colloidal particles; however, hollow capsules were formed only from PNA/PNA films and the DNA/PNA hybrid system. The shrinkage of the capsules after core removal was consistent with other similar sequenced DNA capsules. The PNA films were resistant to nuclease degradation, unlike DNA films and the DNA/PNA hybrid films displayed slower degradation kinetics. This provides a mechanism for controlling the degradation properties of the films by varying the PNA content. After incubation with nuclease, the DNA/PNA film on particles was degraded and capsules were not formed after core removal. However, all of the PNA and hybrid films on planar surfaces and particles were resistant to proteases. These data indicate that films with non-DNA properties can be formed, while still taking advantage of the sequence-directed control. Moreover, additional properties can be introduced into DNA films, which may improve their suitability for specific applications. One of the most interesting properties of PNA is its ability to form a sequence-specific triplex with double-stranded DNA. Although plasmids have already been incorporated into many LbL films by electrostatic interactions with polycations,[23] in the future the use of PNA may allow the incorporation of uncomplexed plasmids into films.

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