Next generation, sequentially assembled ultrathin films: beyond electrostatics

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Over the last 15 years, the layer-by-layer (LbL) assembly technology has proven to be a versatile method for surface modification. This approach is likely to find widespread application because of its simplicity and versatility; however, the conventional use of highly charged materials with limited responsive behaviour presents some key limitations. In this *tutorial review*, the formation of multilayer thin films prepared through non-electrostatic interactions is reviewed. We discuss the assembly of films *via* a number of different methodologies, with particular emphasis on those that provide enhanced orientational control, stimuli-responsive behaviour, and improved film stability.

1 Introduction

Controlling interfacial properties is key in the preparation of advanced materials with specific and tailored functions. In fields as diverse as optics, biomaterials and corrosion, the ability to tailor the surface properties of a material on the nanoscale offers exciting possibilities. For instance, the interfacial properties of biomaterials (*e.g.*, in implants, drug delivery systems or dental materials) make a vast difference in how these materials interact *in vivo*. In recent years this has led to intense interest in the development of methods that can be used to engineer surface properties. A versatile approach for modifying interfaces is the layer-by-layer (LbL) assembly method, in which oppositely charged species are adsorbed alternately to a surface, either planar or colloidal, resulting in the formation of a nanoscale thin film (see Fig. 1).[1–12] Depending on the specific materials used in this process, the properties of the film (and thus the interfacial properties) can be varied considerably. The initial reports in this area focused on the use of synthetic, charged polymers (polyelectrolytes) for the assembly of LbL films. The LbL method has also been expanded to include a host of different materials, including

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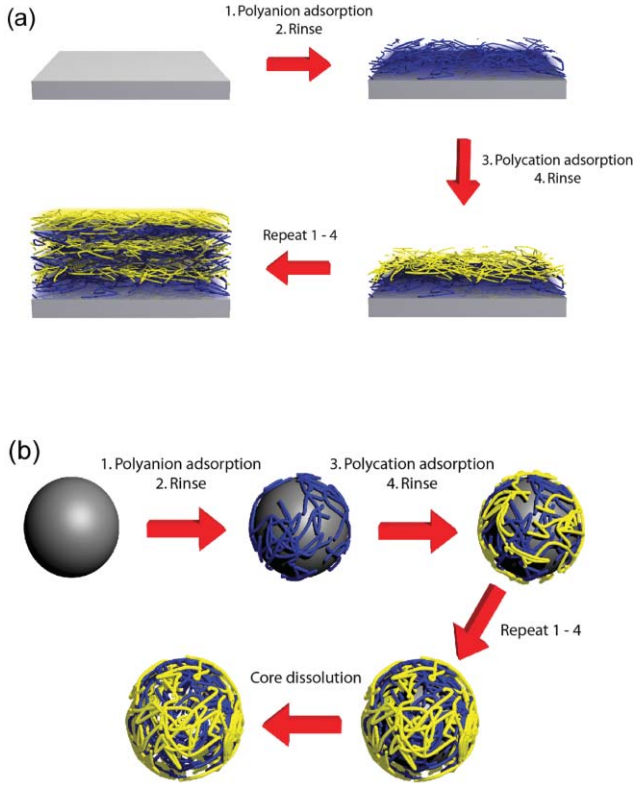

Fig. 1 Layer-by-layer (LbL) assembly of polyelectrolytes on (a) planar and (b) colloidal substrates. A substrate with inherent charge is first exposed to an oppositely charged polyelectrolyte, followed by thorough rinsing. Reversal of the surface charge then facilitates further adsorption steps. The process is continued until the desired layer number (or thickness) is achieved. In (b), the core can be dissolved to give hollow polyelectrolyte capsules.[9–12]

(but not limited to) metal and inorganic nanoparticles, dyes, peptides, oligonucleotides, proteins, and enzymes.[3] This demonstrates the utility of the approach for preparing films with diverse chemical and biological function. Further, the need to prepare multilayer films from an even wider array of components, and particularly polymers that are drawing interest for biomedical applications, has seen multilayer films constructed on the basis of a range of different, non-electrostatic interactions. These have included hydrogen bonding,[13–27] DNA hybridisation,[28–31] sequential chemical reactions,[32–39] metal–ligand complexation,[40] and hydrophobic interactions.[41] The result is that an ever-expanding suite of ultrathin films can be fabricated. Application of the LbL technology to systems that can be assembled using non-electrostatic driving forces has seen the process evolve into an almost universal approach for tailoring surface properties and the assembly of nanostructured materials with defined properties. Such materials are expected to underpin important developments in the biomedical field, including drug and gene delivery, implants and diagnostics.

2 Layer-by-layer assembly: a versatile approach to preparing thin films

First reported by Decher *et al*. for polyelectrolytes,[1] the LbL approach offers a number of key advantages when compared to other methods for surface modification. An important feature is the precision with which the layer thicknesses can be controlled. This control can be achieved by varying (a) the specific materials being used, (b) the number of layers assembled, and/or (c) the specific adsorption conditions used. For instance, by using variables such as the temperature of adsorption,[42] the ionic strength of the adsorption and rinse solutions,[43] and the solvent polarity,[43,44] the thickness of each adsorbed layer can be tuned to within nanometre resolution. This makes the LbL method ideally suited for applications in which the thickness of the adsorbed layer needs to be reproducibly controlled to within a few nanometres. A second advantage of the LbL approach is that it can be readily transferred from planar to colloidal substrates,[7–12] and is largely independent of the geometry of the initial surface. This

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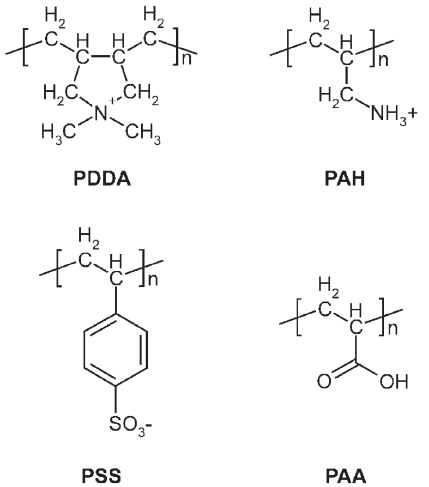

Fig. 2 A selection of polyelectrolytes commonly used in LbL systems: poly(diallyldimethylammonium chloride) (PDDA), poly(allylamine hydrochloride) (PAH), poly(styrenesulfonate) (PSS) and poly(acrylic acid) (PAA).

has given rise to a host of core–shell particles[7,8] and, following dissolution of the core, hollow capsules (Fig. 1(b)).[9–12] Such nanostructured materials are ideal candidates for catalytic, biomedical and optical applications, where the need to tightly control the film or particle properties is an important requirement. Given that the layer number, adsorption conditions and constituent materials can be used to tune these characteristics with relative ease, LbL assembly represents an excellent method for preparing the next generation of highly functional materials.[2–12]

Initially, LbL research was focused primarily on the use of commercially available polyelectrolytes for constructing thin films (see Fig. 2).[1,2] However, subsequent work has shown that many other materials can also be used in the preparation of these films: the approach can be applied to virtually any multiply-charged species.[3] Further, it has been demonstrated that, in addition to electrostatics, a number of different driving forces for multilayer buildup can be exploited. Such versatility in the driving force means that *the LbL method is not restricted to charged materials*. This observation is of critical importance as many functional polymers are uncharged, and therefore, electrostatically driven LbL assembly does not permit the formation of thin films from these polymers. For instance, poly(ethylene oxide) (PEO) is commonly used for biological applications: the PEO surface is highly resistant to protein and lipid deposition and PEO itself is non-toxic. Similarly, poly(*N*-isopropylacrylamide) (PNiPAAm) is an important polymer because of the sharp volume transitions that occur in the temperature range useful for biomedical applications (lower critical solution temperature, LCST = 32 °C, variable by copolymerisation with a hydrophilic or hydrophobic comonomer). PNiPAAm is uncharged, and therefore the use of secondary, non-electrostatic interactions is vital for its LbL assembly into functional films with nanoscale precision.

This review focuses on a range of significant approaches for constructing multilayer films on the basis of non-electrostatic interactions. In particular, the use of hydrogen bonding and hybridisation for assembling films that are responsive to changes in the local chemical environment are examined. Further, the use of covalent linkages formed *via* sequential chemical reactions in LbL multilayer assembly is presented. Future research in LbL materials is likely to draw increasingly on these "secondary" interactions, particularly as a means for assembling films from high functionality polymers, such as those frequently used in biomedical applications.

3 LbL assembly based on hydrogen bonding

3.1 Early studies on hydrogen-bonded multilayers

One of the most commonly studied, non-electrostatic interactions used in LbL assembly to date is hydrogen bonding. By exploiting this interaction, a host of different (often uncharged) materials have been successfully incorporated into multilayer films (Fig. 3). This possibility arises because many polymers incorporate moieties that can act as hydrogen bonding donors and acceptors. Examples include PEO, in which the oxygen atoms on the polymer backbone can be hydrogen bonding acceptors, or PNiPAAm, in which both an acceptor (carbonyl) and donor (amide) are present. The seminal studies in LbL multilayer assembly based on hydrogen bonding were reported independently by Stockton and Rubner[13] and Zhang and co-workers.[14] in 1997. The study by Stockton and Rubner examined the use of polyaniline (PAni) in alternation with a variety of water-soluble macromolecules, such as poly(vinylpyrrolidone) (PVPON), poly(vinyl alcohol) (PVA), polyacrylamide (PAAm), and PEO.[13] It was established that hydrogen bonding was the driving force for the assembly using Fourier transform infrared (FTIR) spectroscopy,[13] and in particular by examining the N–H stretching peaks at *ca.* 3300 cm^{-1}. A peak present at 3380 cm^{-1} arises from higher energy, non-hydrogen-bonded N–H, whereas another peak at 3310 cm^{-1} is due to lower energy, hydrogen-bonded N–H stretching. The emergence of pronounced peaks at 3304 and 3300 cm^{-1}, for PVPON/PAni and PEO/PAni multilayers, respectively, indicates that a large proportion of the N–H groups in the polyaniline are involved in hydrogen bonding. Hydrogen bonds were also observed in solution-cast blends of PVPON/PAni, but not of similarly prepared blends of PEO/PAni. This attests to the particularly strong interactions that are present between PVPON and PAni.

Additionally, the assembly of hydrogen-bonded PAni films was followed using ellipsometry and UV-visible spectrophotometry. These studies demonstrated that the deposition pH and molecular weight of the polymers used have a significant effect on the film buildup. In the case of PVPON adsorbed alternately with PAni, variation of the molecular weight of the PVPON from 5 kDa to 1000 kDa was shown to have a substantial effect on the amount of material adsorbed, with higher molecular weights leading to a considerably larger amount of material being incorporated into the film. At PVPON molecular weights below 25 kDa, no multilayer growth was observed. This was compared to PAni multilayers prepared *via* electrostatic interactions (with sulfonated polystyrene, SPS), in which it was observed that molecular weight had very little influence on the thickness of the films formed. Interestingly, the Rubner study also reported variations in the

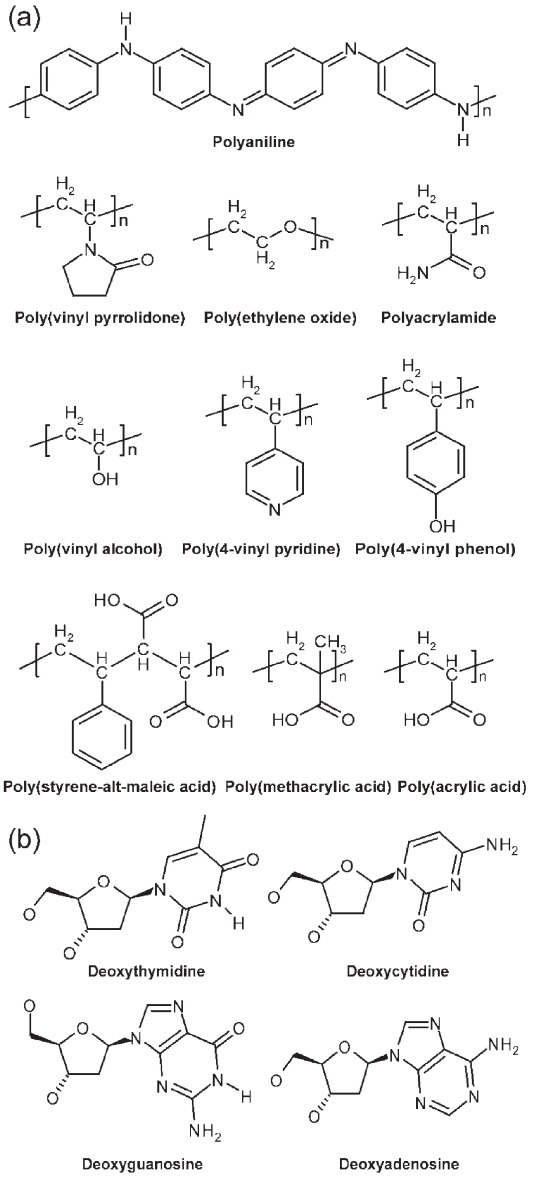

Fig. 3 Chemical structures of (a) polymers used to assemble LbL films based on hydrogen bonding, and (b) deoxyribose building blocks used to form oligonucleotides that can be assembled into films based on hybridisation.[28–31]

thickness of the films formed with the pH of the polymer solution (PAni was held constant at pH 2.5, while the alternate polymer was varied).[13] In the case of PVPON, PAAm and PVA, a decrease in the pH of the adsorption solution led to an increase in the amount of material incorporated into the film, while in the case of PEO the opposite was observed: an increase in pH led to an increase in film thickness. The origin of these effects in this study was not fully elucidated, though several possibilities were raised: (a) variations in the doping and dedoping of polyaniline over a wide pH range; (b) ionisation or protonation of sites on the hydrogen bonding polymers; and (c) OH^- ions interfering with the formation of hydrogen bonds at elevated pH. Nevertheless, the study demonstrated that the formation of hydrogen-bonded multilayer films was possible over a wide pH range.[13] Further work has demonstrated that, in certain cases, it is possible to erode hydrogen-bonded multilayers at a given pH.[15,16] This important development will be discussed in more detail below.

Also in 1997, Zhang and co-workers examined the buildup of multilayers from poly(acrylic acid) (PAA) and poly(4-vinylpyridine) (P4VP).[14] In this system, the pyridine rings in the P4VP molecules act as hydrogen bonding acceptors, while the protonated carboxylic acid groups on the PAA molecules act as donors. Adsorption of the PAA layers was performed from methanolic solutions at low pH (3.28): under these conditions the ionisation of the carboxylic acid moieties is largely suppressed. Adsorption of the P4VP was performed from either methanolic or ethanolic solutions. It should be noted that these particular hydrogen-bonded multilayers could only be constructed under conditions where the carboxylic acid groups of the PAA are largely protonated (*i.e.*, when there are hydrogen bonding donors available and electrostatic forces are minimised). Multilayer formation was followed using UV-visible spectrophotometry (λ_{max} = 256 nm), and linear film buildup was observed up to 16 layers. X-Ray diffraction studies were also conducted on the resulting films, and indicated that a layered structure was present within the film. Further, analysis of the surface morphology revealed a relatively smooth surface with a mean surface roughness of 0.64 nm, as measured over a 1 μm × 1 μm area. Combined with the linear film buildup, the smooth surface obtained indicates that the multilayer films constructed from PAA and P4VP are highly uniform. As in the Stockton and Rubner study,[13] infrared spectroscopy was used to verify that the multilayer buildup was driven by hydrogen bonding, with a shift observed in the position of the carbonyl in the carboxylic acid from 1709 to 1723 cm^{-1}. Further, there was no change observed in the spectrum from 1650–1300 cm^{-1}, suggesting that there was little ionisation of the carboxylic acid groups, which may have given rise to electrostatic interactions.

The first reports of LbL assembly *via* hydrogen bonding had a profound impact on research in the area. Importantly, it was demonstrated that polymers previously assumed to be beyond the scope of the LbL methodology were in fact suitable, and could be readily incorporated into films. Of further interest was the fact that a number of the polymers used in these initial reports (*e.g.*, PVPON, PEO and PVA) are ideal candidates for biomedical applications, with well-characterised biocompatibility. Subsequent investigations have demonstrated that hydrogen bonding also provides the opportunity to render films responsive to different chemical and physical stimuli, allowing the preparation of so-called ''intelligent'' materials.

3.2 Hydrogen-bonded films: a pathway to stimuli-responsive nanomaterials

The requirement of suppressed ionisation for the formation of hydrogen-bonded multilayer films presents the possibility of pH-tuneable film disassembly. This characteristic was exploited by Granick and Sukhishvili to prepare ''erasable'' multilayers by assembling either PAA or poly(methacrylic acid) (PMAA) in alternation with PEO or PVPON.[15,16] In these cases, the polyacid (PAA or PMAA, termed P(M)AA hereafter) acts as the hydrogen bonding donor, while the

PVPON or PEO acts as the acceptor. When the ionisation of the P(M)AA reaches a certain level, the bonds are no longer sufficient to maintain multilayer integrity, and the film disassembles rapidly. Since the ionisation of these polyacids is directly related to the local pH, the films can be disassembled by elevating the pH. That is, when the pH is increased sufficiently, a critical charge density in the polyacid is reached, at which point the hydrogen bonding is unable to keep the multilayer film together. Importantly, Sukhishvili and Granick demonstrated that the critical pH at which deconstruction takes place varies for different polymer pairs. They determined the critical pH to be 6.9 for PMAA/PVPON, 4.6 for PMAA/PEO, and 3.6 for PAA/PEO. This critical pH could be elevated when there was also a higher ionic strength in the solution (the critical pH for PMAA/PEO increases from 4.6 to 5.15 when 0.4 M NaCl is present in the solution). This provides an opportunity to tailor the disassembly pH for certain requirements.

These studies[15,16] also showed variations in the buildup profiles of hydrogen-bonded multilayers, depending on the polymers used in the film assembly. Layer growth was followed *in situ* by using attenuated total reflectance FTIR spectroscopy, and the values measured were compared with dry measurements made *via* ellipsometry. Typically, films assembled from polymers incorporating PVPON were shown to grow linearly, in contrast to films that incorporated PEO. In the latter case, the mutlilayers grew exponentially, and were characterised by much rougher surfaces.[16] The origin of this "exponential" growth was originally attributed to the enhanced surface roughness increasing the area available for adsorption, although diffusion of adsorbing polymer into the highly hydrated polymer network can also lead to similar effects.[44]

Another aspect of this work[16] was to demonstrate the usefulness of hydrogen bonding in release applications. This was achieved by loading the film (during assembly) with a small organic molecule (Rhodamine 6G). Extensive rinsing with buffer solution at pH 4 did not dislodge the Rhodamine 6G from within the multilayer. However, upon elevation of the pH to 5.5, it was demonstrated that Rhodamine 6G could be released (coinciding with disassembly of the multilayer).[16] More recently, we demonstrated that the widely used thermo-responsive polymer, PNiPAAm, could be assembled in alternation with PAA based on hydrogen bonding complexation.[17] The film mass was shown to be dependent on the temperature at which the PNiPAAm adsorption was performed, with multilayers assembled close to the LCST of PNiPAAm (32 °C) containing 30–40% more material than those prepared at room temperature. This also led to considerable variation in the film morphology, with smoother films being prepared at elevated temperatures (surface roughness of 1–2 nm compared with 5–8 nm at lower temperatures). The resulting multilayers were loaded with Rhodamine B, which was then released at a rate dependent on the temperature of the surrounding solution. Further, we also demonstrated loading/release behaviour of Rhodamine B from within multilayers formed from the hydrophilic–hydrophobic alternating copolymer poly(styrene-*alt*-maleic acid) (PSMA), assembled in alternation with PEO.[18] These multilayer films were loaded to a significant level (40 wt%) with Rhodamine B. Temperature-dependent release rates were observed over 60 min at elevated temperature. Rhodamine B leakage from these films was considerably lower than that for PNiPAAm/PAA multilayers, with only 10% of the loaded dye lost within 1 hour at 21 °C. These studies demonstrate the utility of hydrogen-bonded multilayers for release applications, either facilitated by film disassembly, or slow release of a guest molecule as the concentration equilibrates with the ambient solution.

3.3 Stabilisation of hydrogen-bonded multilayer films

While the disassembly of hydrogen-bonded multilayers provides interesting possibilities for release applications, there is also a need to stabilise the multilayers under certain conditions. For instance, physiological conditions of pH 7.35–7.45 and 145–160 mM NaCl would be sufficient to disassemble all the polymer pairs investigated by Sukhishvili and Granick,[15,16] thus limiting the applications of such films. To this end, a number of research groups have explored strategies for the stabilisation of hydrogen-bonded multilayers at elevated pH, particularly at moderate pH values that are useful in biomedical applications (*e.g.*, pH 5–8). Early work was conducted by Yang and Rubner, who used two different approaches for crosslinking multilayers assembled from PAA and PAAm.[19] Firstly, the temperature of the assembled film was elevated to 175 °C, which caused two crosslinking reactions to proceed: a thermal imidization reaction between the amide groups of the PAAm and the carboxylic acid groups of the PAA, and an anhydride formation between carboxylic acid groups. While the anhydride linkages were shown to hydrolyze almost immediately upon re-exposure to water at pH 7, the imide groups were stable for prolonged periods and proved effective for rendering the multilayer stable at elevated pH. The second approach used by Yang and Rubner[19] was to terminate film assembly with a layer of PAA functionalised with a UV-crosslinkable moiety (an α-hydroxybenzoyl group). Under UV irradiation, this moiety generates a free radical, which then facilitates radical reactions within the multilayer assembly, crosslinking the film. Ultimately, it was demonstrated that these approaches could be applied in surface patterning applications. By inducing selective area photo- and thermal-crosslinking between PAAm and PAA multilayers, and then disassembling the non-crosslinked areas *via* elevating the pH (*i.e.* rinsing in water), surfaces could be readily patterned with features on the micrometre scale.[19]

Photo-crosslinking was also explored by Chen and Cao as a route to preparing stable hydrogen-bonded assemblies.[20] In this case, films were assembled using a polymer with a pendant azo functionality (diazoresin, DR) adsorbed in alternation with phenol formaldehyde resin (PR). The multilayer buildup is facilitated by the interaction of the hydroxyl hydrogen on the phenol moiety with the lone pair on the azide. This was verified by examining shifts in the peaks associated with hydrogen groups in the IR spectrum of the films. Multilayer buildup was followed using sequential measurements on a UV-visible spectrophotometer. In this case the diazonium peak gives a strong absorption centred at 384 nm, while the aromatic groups in the PR give a peak at 284 nm. Under

UV radiation, the diazonium groups facilitate crosslinking *via* phenolic ether formation. It was demonstrated that while the films assembled can be completely disassembled in polar solvents (dimethylformamide) before UV radiation, exposure after crosslinking has little impact on the film. These results attest to the usefulness of photocrosslinking as a means of preparing stable assemblies from hydrogen-bonded multilayers.

Further, in our research group we recently demonstrated that hydrogen-bonded PNiPAAm multilayers could be stabilised at intermediate pH values (7.1) by incorporating a dual H-bonding/electrostatic copolymer in the assembly, and then infiltrating a multivalent ion into the film structure.[21] This was achieved by adsorbing PNiPAAm in alternation with poly(styrenesulfonate-*co*-maleic acid). The carboxylic acid groups (from the maleic acid moiety) undergo hydrogen bonding with the amide groups of the PNiPAAm, while the styrenesulfonate groups remain uncomplexed in the multilayer assembly. Subsequent infiltration of a multivalent ion, such as Ce^{4+} or Fe^{3+} into the multilayer assembly then leads to ionic interactions with some of the free styrenesulfonate groups. This infiltration into the multilayers improved the stability of the films to variations in the pH of the surrounding solution: multilayers, which previously disassembled at pH 7.1 swelled, but did not disassemble under these pH conditions. Further, conversion of the Fe^{3+} into Fe^{2+} *via* reduction with I^-, followed by pH elevation, was also used to trigger disassembly of the multilayer films. These results demonstrate the ability of hydrogen-bonded multilayer films to respond to a variety of external stimuli, by exploiting their inherent pH response in concert with other chemical reactions.

3.4 Hydrogen-bonded films: formation on colloidal supports

Transferring LbL hydrogen-bonded assembly onto colloidal supports allows the possibility of exploiting the pH and temperature sensitivity of these films in particle technologies and controlled release applications. In particular, when deposited onto sacrificial colloidal particles, the multilayers can form a stable membrane that will constitute a hollow capsule after dissolution of the core particle (see Fig. 1(b)). However, the adsorption of polymers onto colloidal particles presents a number of challenges when compared to the buildup of LbL films on planar substrates, the most serious of which is pronounced, irreversible aggregation of particles. In the case of polyelectrolytes (such as PSS or PDDA), such aggregation is prevented by the inherent charge of the polymers, and overcompensation of this charge at each consecutive polyelectrolyte deposition, in addition to steric and electrosteric effects. However, in the case of hydrogen-bonded multilayers the polymers are, in many cases, non-charged, and in the absence of long range repulsive forces the coated particles can lose colloidal stability and (irreversibly) aggregate. Decreasing particle size increases this tendency to aggregate, and this phenomenon can become a key limitation in many systems. This has limited the number of hydrogen bonding polymer pairs that have been successfully used in coating colloidal particles.

The first example of hydrogen-bonded multilayer assembly on colloidal particles involved the use of PVPON and *m*-methylphenol–formaldehyde resin (MPR) as hydrogen acceptor and donor, respectively. The films were assembled from methanolic solutions on sacrificial templates of either polystyrene (PS) or SiO_2 particles.[22] While no capsules were obtained when removing the PS core with tetrahydrofuran (THF), intact capsules were prepared when the silica templates were dissolved with hydrofluoric acid. In this case, five bilayers were deposited to yield stable capsules, which exhibited no shrinkage compared to the parent template particles. Further, the FTIR spectrum of the capsules was identical to that of an equivalent multilayer film deposited on a planar substrate. The multilayer assembly was also characterised by a linear growth of the membrane, with a thickness increment of 1.5 nm observed for each polymer layer. This thickness increment is similar to that previously reported for the common electrostatic LbL pair of PAH and PSS assembled from 0.5 M NaCl solutions (1–1.5 nm per layer).[7] It was also demonstrated that MPR/PVPON multilayer capsules exhibited a tendency to aggregate in methanol. To overcome this, an additional bilayer of PAA/PAH was deposited on the particle surface, which suppressed the aggregation and also prevented the particle coating from dissolving in water. The final particles remained stable in acidic and neutral conditions and deconstructed under alkaline conditions due to ionisation of the phenolic groups of the MPR. As discussed earlier, such deconstruction of hydrogen-bonded multilayers in response to ionisation of one of the constituent polymers is a signature of hydrogen-bonded multilayers.

When polycarboxylic acids are employed as hydrogen donors in aqueous solutions, the instability of the hydrogen-bonded complex at elevated pH (*e.g.* above 6.9 for PMAA/PVPON) requires assembly to be performed at acidic pH. For instance, in the first example of PMAA assembled in alternation with either PEO or PVPON on colloidal particles, the assembly was performed at pH 3.5 on $CdCO_3$ particles.[23] Deposition of five bilayers and removal of the core particles with an aqueous solution buffered to pH 1.1 yielded stable capsules with a wall thickness of 16 ± 3 nm (for PMAA/PEO) or 18 ± 4 nm (for PMAA/PVPON). Again, these values are comparable to those observed in electrostatically bonded systems, though they are somewhat thinner than the analogous hydrogen-bonded films on planar substrates. Polycarboxylic acid–based hydrogen-bonded capsules exhibited disintegration threshold pH values similar to those established for the corresponding multilayers on planar substrates; pH 4.6 for PMAA/PEO and pH 6.9 for PMAA/PVPON. Clearly, the disintegration pH is highly dependent on the particular pair of polymers used, and reflects their potency as a donor or an acceptor. As in the case on planar substrates, the PMAA/PVPON system is the most resistant to deconstruction, that is, it has the highest threshold value. Importantly, the assembly of this polymer pair may be performed at relatively moderate pH values, which is advantageous in applications where encapsulation of pH sensitive materials may be required. Nevertheless, the disintegration of PMAA/PVPON still occurs below physiological conditions, and as a result studies have focused on endowing these hydrogen-bonded capsules with stability at elevated pH.[23,24] As is the case for the membranes assembled on planar substrates, this can be achieved *via* chemical

crosslinking of the polymers. The major difference, however, is that the dry state chemistry used on films supported on planar substrates is not readily applicable to colloidal systems, and therefore the crosslinking must be performed in solution.

The most common crosslinking technique applied to hydrogen-bonded capsules to date is the use of carbodiimide chemistry to facilitate the formation of amide linkages. That is, by using 1-ethyl-(3-dimethylamino)propyl)carbodiimide hydrochloride (EDC) and a diamine linker, (such as ethylenediamine), a fraction of PMAA carboxylic groups are crosslinked within the hydrogen-bonded membrane.[25] It has been demonstrated that crosslinked PMAA/PVPON capsules prepared *via* this approach could be stored for several months at pH = 10 with no sign of capsule disintegration, retaining all of the PMAA within the capsule wall.[23] Similarly, the PMAA/PEO counterparts were also stable at elevated pH, yet in this case approximately 50% of the PMAA chains were lost from the membrane.[23] The crosslinking could be performed either on the original core–shell particles or on the capsules; however, the former route gives consistently better capsules (morphology, smoothness *etc*).[24] Importantly, such crosslinking chemistry may be engineered to utilise only one of the two multilayered polymers, or (as applied to the PAA/PAAm multilayers by Rubner and co-workers) to chemically link both constituent polymers.[25] In the absence of crosslinks, the membrane disintegrates at elevated pH, while the crosslinked counterpart remains intact in a highly water swollen state, that is, as a hydrogel. Such materials are attractive for a number of applications, since they exhibit reversible swelling-shrinking behaviour.[24]

We have recently reported the first example of reversible stabilisation of hydrogen-bonded capsules, which was achieved using polymers linked *via* disulfide bonds.[26] PMAA/PVPON films were assembled using PMAA pre-functionalised with thiol moieties (PMAA-SH), and were shown to have a similar disassembly pH threshold to films assembled with pristine PMAA (pH $<$ 7). Converting the thiol groups into disulfide linkages *via* oxidative crosslinking (*e.g.*, with hydrogen peroxide or Chloramine T) resulted in films that remained intact when exposed to neutral or alkaline solutions. However, since the films were stabilised solely *via* disulfide linkages, they could be deconstructed using a common thiol–disulfide exchange reagent such as dithiothreitol. Thiol–disulfide exchange reagents convert the disulfide linkages into thiol moieties, thereby eliminating the crosslinks from the system. At elevated pH, the hydrogen bonding no longer stabilises the film, and as the disulfide crosslinks are ''unzipped'', the capsules disintegrate (see Fig. 4). This makes these capsules particularly attractive for biomedical applications such as drug delivery, where triggered release is highly desirable. Further, the intracellular environment will favour conversion of the disulfide bonds into thiols, thereby allowing release to occur only upon internalisation of the particles. In the extracellular environment the disulfide bonds will preserve the capsule integrity. Additionally, we successfully loaded the PMAA-SH/PVPON capsules with both fluorescent protein[26] and oligonucleotide sequences.[27] To our knowledge, these are the first reports on hydrogen-bonded capsules filled with model drug molecules. In the case of oligonucleotide loading, we utilised

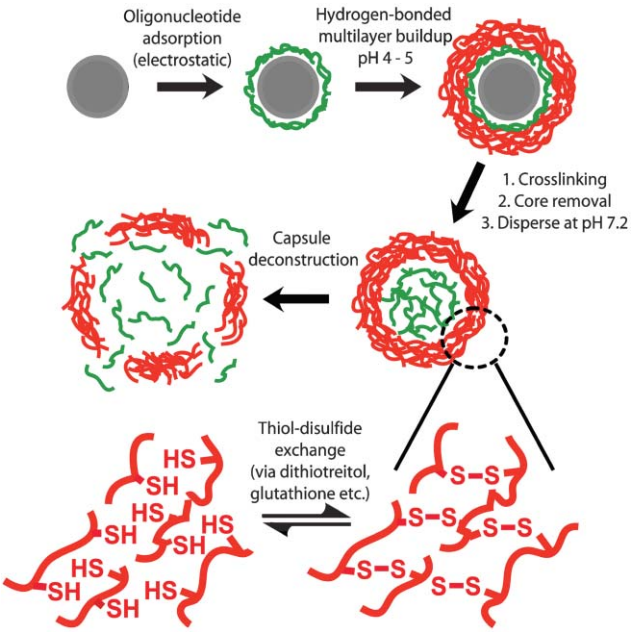

Fig. 4 Encapsulation of DNA oligonucleotides within biodeconstructible, disulfide-crosslinked, hydrogen-bonded multilayer capsules. The oligonucleotides are first adsorbed onto an oppositely charged silica particle template, upon which a PVPON and thiol-functionalised PMAA multilayer film is then assembled. Crosslinking of the thiol moieties to yield disulfide linkages, followed by dissolution of the silica template, results in encapsulated DNA. The thiol linkages can be deconstructed to release the DNA in the presence of a thiol-disulfide exchange reagent.

1 μm amine-functionalised silica particles to adsorb the oligonucleotides *via* electrostatic interactions. Subsequent multilayer build-up of PMAA-SH/PVPON was driven *via* hydrogen bonding and, importantly, caused no measurable loss of DNA from the particle surface. Upon dissolution of the silica core, the DNA oligonucleotides remained confined within the capsules, which had a negatively charged wall (Fig. 4). The key benefits of this DNA encapsulation approach include the high loading and essentially quantitative incorporation of the DNA from aqueous solution into the final preparation.

4 LbL assembly by DNA hybridisation

An extension of hydrogen-bonded multilayer films is exploiting the base pairing of DNA nucleotides to facilitate film assembly.[28–31] DNA has been used as a polyelectrolyte in numerous systems, where the incorporation of DNA into the film is driven by electrostatic interactions.[46,77] However, incorporating DNA in this way does not take advantage of the tailored interactions between base pairs which can be used to finely engineer the structure of the film. Additionally, DNA is biocompatible and biodegradable, which makes it an attractive building block for forming multilayer films.

4.1 Assembly of DNA multilayer films from engineered sequences

Hybridisation of DNA to form a double helix occurs naturally, where the base adenosine (A) pairs with thymidine (T) and

cytosine (C) pairs with guanine (G). The driving force for forming double stranded (ds) DNA is a combination of the hydrogen bonds formed between the bases and π–π stacking of the aromatic rings contained in the bases (Fig. 3(b)). As the formation of dsDNA is dependent on the correct recognition of base pairs, the structure of the film can be manipulated by altering the sequence of bases, as well as by modulating the strength of the hydrogen-bonding and hydrophobic interactions, which can be achieved by controlling the concentration of salt and urea in solution.

When forming a multilayer film solely from DNA, the first layer deposited onto the surface is typically adsorbed *via* electrostatic interactions, although covalent interactions (such as disulfide chemistry) may also be used. Multilayer films can be assembled from short DNA sequences (oligonucleotides) if the oligonucleotide contains 'blocks' of nucleotides that recognise a complementary 'block' of DNA in the film (see Fig. 5). It is important that the hybridising oligonucleotides only hybridise to the film and not to the other oligonucleotides in solution, as this would limit the number of oligonucleotides in solution that would be free to hybridise to the film. In the simplest case, the nucleotides in the block of the oligonucleotide are all the same (*i.e.* homopolymeric),[28] however, specific sequences can be incorporated into the oligonucleotides as well.[29,30] If an oligonucleotide with only a *single block* is used to assemble the film, only limited assembly occurs. This is because when hybridisation occurs between the first two layers, there is not sufficient free single-stranded (ss) DNA to allow appreciable hybridisation with subsequent layers. If, however, a di-block system is used, where one block of the oligonucleotide hybridises to the film and the other block is free for hybridisation of subsequent layers, a multilayer film can be assembled (see Fig. 5).

Homopolymeric blocks of DNA are the simplest building blocks for forming a multilayer film. Using a basic di-block oligonucleotide pair of $A_{15}G_{15}$ (15 units of adenosine followed by 15 units of guanine) and $T_{15}C_{15}$, stable multilayer films have been formed on planar, colloidal and fibre surfaces.[29,31] When a sacrificial colloidal template is used to prepare the multilayer film, hollow capsules[29] or fibres[31] consisting solely of DNA are obtained. More complicated oligonucleotide systems, such as tetra-blocks (*e.g.*, poly$T_{15}C_{15}T_{15}C_{15}$/poly$A_{15}G_{15}A_{15}G_{15}$), can be used to form multilayer films where more than one region of the hybridising oligonucleotide can hybridise to the film.[29] Additionally, specific blocks with a predefined sequence (*i.e.*, non-homopolymeric blocks) can be used to assemble films with particular structures or to incorporate specific DNA sequences that may be of interest.[29,30] Varying the complementary species in each block may lead to considerable control over the properties of the multilayers formed. For instance, when comparing the homopolymeric poly$T_{15}C_{15}$/poly$A_{15}G_{15}$ film with a multilayer hybridised from blocks of specific sequences ($X_{15}Y_{15}$/$X'_{15}Y'_{15}$), vastly different properties are obtained: the multilayers employing specific sequences tend to dissipate energy much less effectively, reflecting increased film rigidity.[29] DNA nanotubes have also been assembled by the sequential deposition of complementary oligonucleotides with specific sequences, although in this instance an outer layer of

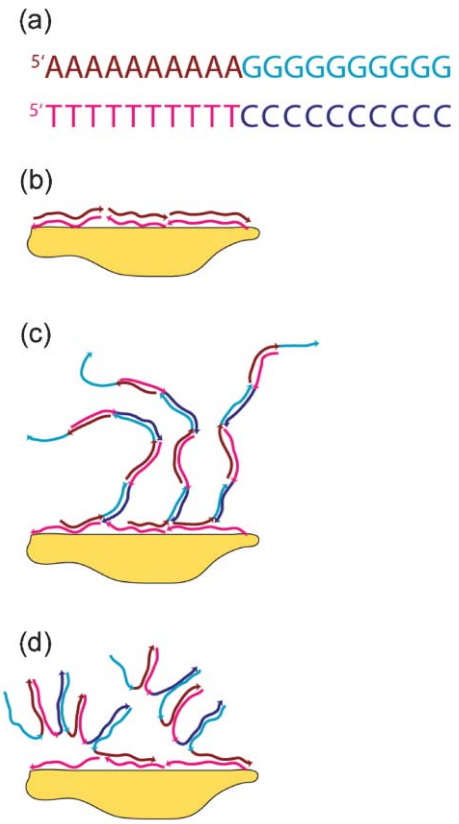

Fig. 5 Assembly of LbL films using DNA hybridisation. (a) An example of a homopolymeric di-block oligonucleotide pair suitable for use in LbL assembly, $A_{10}G_{10}$ and $T_{10}C_{10}$. (b) Adsorption of single-block oligonucleotides. In this case, hybridisation does not leave sufficient bases to facilitate subsequent hybridisation. Little film growth is observed after the first two layers.[29] (c) LbL assembly using di-block oligonucleotides. In each step one block of the oligonucleotide hybridises to the film while the other remains free (single stranded) and available for hybridisation with the next incoming oligonucleotide. Hybridisation occurs in the classical anti-parallel orientation. (d) LbL assembly using reverse di-block oligonucleotides. LbL assembly occurs as in (c), except the direction of the helix must change after the hybridisation of each layer.

α,ω-diorganophosphate Zr(IV) was required to provide structural integrity.[30]

Control over the structure of the film can be achieved by engineering the pairing of the nucleotide base pairs. Unlike thin films assembled from synthetic polymers *via* electrostatic and/or hydrogen bonding interactions, the use of DNA in multilayer films allows control over the direction and orientation of the molecules adsorbed at each layer. When electrostatic or hydrogen bonding interactions are used to assemble the film, the polymers are adsorbed onto the surface in a random conformation and there is little or no control over their orientation. DNA, however, is a directional molecule and the DNA strands can only interact in specific ways.[29] The directionality of the DNA molecule stems from the position of the phosphodiester linkage on the deoxyribose sugar. The linkages of the nucleotides in the oligonucleotide are through the 5′ and 3′ positions on the deoxyribose sugar ring, and the orientation of the molecule is designated by convention by

which end of the ribose ring is uncoupled. In a double helix, the two molecules run anti-parallel, so that if one oligonucleotide is orientated 5′ to 3′, the other is orientated 3′ to 5′. This feature of DNA can be used to control the ordering of the film (Fig. 5). By changing the direction of the hybridising oligonucleotides, the structural properties of the film can be altered, as reflected in changes in the film buildup profiles and energy dissipation.[29] This control of the order of molecules within the film is not exhibited in other polymer systems, and provides new opportunities for controlling material properties such as permeability, modulus and density.

The pairing of the DNA bases can be also used to control the structure of the film. The formation of a CG pair corresponds to the formation of three hydrogen bonds, whereas formation of an AT pair only results in the formation of two hydrogen bonds. This means that CG forms a more stable interaction than AT. By controlling the ratio of A and T to C and G in an oligonucleotide block, the stability of the hybridised oligonucleotides can be controlled. The stability of the hybridised strands (and hence the films) can also be controlled by altering the length of complementary base pairs – a higher number of complementary base pairs will lead to more stable, hybridised oligonucleotides.

4.2 Controlled degradation of LbL DNA films

When DNA forms a double helix, the two strands of DNA are held together through a balance of the attractive forces of hydrogen bonding and stacking of the aromatic rings on the bases and the electrostatic repulsion of the negatively charged phosphate backbone. As films assembled from DNA consist solely of negatively charged polyelectrolyte, the structure of the film can be manipulated by altering the salt concentration of the solution. At high salt concentrations (typically either NaCl or $MgCl_2$), the positive salt ions shield the negative charge of the phosphate backbone. This increases the stability of the helix. Conversely, low salt concentrations increase the repulsion of the film and, in some instances, this repulsion can cause film disassembly.[29] For instance, exposure of a $polyA_{15}G_{15}/polyT_{15}C_{15}$ film to 100 mM NaCl led to an increase in the dissipation factor of the film, but little change in the measured frequency.[29] This reflects an increase in repulsion between oligonucleotide layers and therefore some softening of the film. In the case of the tetra-block film treated with 100 mM NaCl, an apparent loss of approximately 64% of the film mass was observed. In this case, after further reducing the [NaCl] (to 0 M), only 12% of the mass was found to remain. These results demonstrate that the response of DNA multilayers to ambient salt conditions is highly dependent on the specific oligonucleotides used to construct the film.

The stability of DNA films may also be controlled by adding denaturing substances, such as urea, which disrupt the hydrogen bonding of the base pairs. Addition of urea to LbL DNA films has been shown to disassemble the films.[29] Interestingly, the extent to which films are influenced by urea treatment is highly dependent on their constituent oligonucleotides. For instance, a tetra-block film loses approximately 55% of its mass when exposed to 6 M urea, whereas the di-block and $X_{15}Y_{15}/X'_{15}Y'_{15}$ lose 10% and 0%, respectively, although there is significantly more material in the tetra-block and di-block films initially.[29] Further, morphological variations as a consequence of urea treatment have also been observed.[28]

DNA is also subject to degradation by a range of enzymes, such as exonucleases or endonucleases. Exonucleases hydrolyse the phosphodiester bond in DNA from either the 5′ or the 3′ end of the oligonucleotide, whereas endonucleases cleave the phosphodiester bond within an oligonucleotide chain. Endonucleases may cleave an oligonucleotide randomly, or they may cleave DNA only if a specific sequence is present in the oligonucleotide, which the enzyme recognises (such as in a restriction enzyme). Incorporation of such enzyme-responsive sequences into the films opens new avenues to controlled degradation of the DNA films, as films with an engineered 'cut site' will degrade in the presence of the appropriate enzyme, whereas the films without the appropriate nucleotide sequence will remain intact (A. P. R. Johnston and F. Caruso, unpublished data).

5 LbL assembly based on covalent bonding

The use of covalent bonds to assemble LbL films has been a more recent area of investigation. However, for some applications such films can provide significant advantages to traditional assembly methods. In particular, they have high stability due to the covalent bonds formed, and therefore do not disassemble with changes in pH or ionic strength. Bergbreiter, Crooks and co-workers performed the first example of sequential covalent assembly of polymers, using a copolymer of maleic anhydride reacted in alternation with a polyamidoamine dendrimer. After four reaction cycles, the film reached a thickness of 40–50 nm.[32] Blanchard and co-workers[32–35] also investigated approaches to prepare multilayer films using a sequential covalent strategy. Unlike the majority of LbL reports that use preformed polymers to form multilayer films, these studies use a different approach whereby the polymer is effectively grown from the interface in a stepwise fashion, adding one molecular subunit per cycle. To this end, one of their key approaches utilises urea interlayer linking chemistry. Their first paper[33] investigated two variations on this theme: firstly the alternate reaction of diisocyanates and diamines (Fig. 6) and secondly, reaction of isocyanate in the presence of a small amount of water. In the second approach, the isocyanate groups were hydrolysed into amine moieties, thereafter reacting with other isocyanates to form urea linkages. The two layering methods yielded films with identical chemical structures; however, the first method was significantly more controlled with only a single monomer unit added per cycle. Linear growth of films assembled using the first approach was observed by ellipsometry, with a total thickness of 9.8 nm over 7 bilayer cycles. There was some deviation from linearity observed for the films assembled *via* the second approach, although the films obtained were considerably thicker: 62.5 nm for 7 bilayer cycles. These thicknesses are comparable to those that can be prepared using conventional, electrostatic LbL processes. In related work,[34] the same group demonstrated the assembly of layers *via* the interchange between building the layers based on ionic and

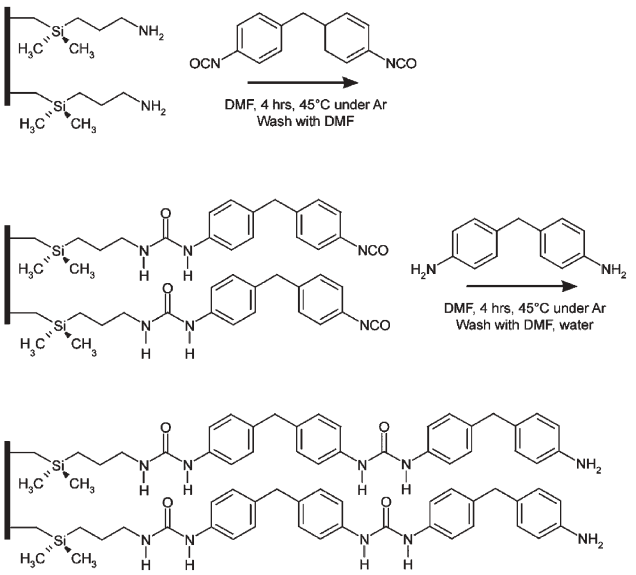

Fig. 6 Assembly of multilayer films *via* stepwise urea linkage formation, as utilised by Kohli and Blanchard.[32] An amine functionalised surface is initially reacted with a diisocyanate, yielding a surface with isocyanate moieties. Subsequent stepwise reaction with (i) a diamine and (ii) a diisocyanate leads to film buildup through the formation of urea linkages.

covalent interaction, demonstrating the compatibility of covalent LbL assembly with other interactions. Further investigations were also made into assembling an alternating hydroxyl phenyl maleimide and vinyl ether polymer with adipoyl chloride.[35] In this case, the adipoyl chloride reacts with the hydroxyl functionality to form covalently bonded layers. Linear buildup of the films was shown using several techniques, with a measured thickness of 1.6 nm per bilayer. The vinyl ether component of the polymer contained isopropylphosphonates, which could be deprotected after assembly and then used for metal absorption. These results show the utility of covalent multilayer assembly for preparing high functionality films.

Akashi and co-workers have also contributed significantly to the development of multilayer assemblies based on covalent interactions.[36,37] In early work,[36] they used the copolymer poly(vinylamine-*co*-vinylisobutyramide) P(VAm-*co*-NVIBA) in combination with poly(acrylic acid) (PAA) to construct LbL films. The amine groups within the P(VAm-*co*-NVIBA) were covalently coupled to the acrylic acid groups using EDC chemistry, yielding a final film stabilised through amide linkages. They demonstrated linear buildup with P(VAm-*co*-NVIBA) containing 39% VAm units in the polymer. These films were effectively ultrathin hydrogels due to the presence of ionisable carboxylate and amino groups present in the assembly. This was supported by atomic force microscope (AFM) measurements, which demonstrated that the thickness of a 16-step assembly in water (320 nm) was more than double that in air (150 nm). In later work,[37] the same group demonstrated the assembly of similar films under a wider range of conditions. A copolymer of poly(acrylic acid-*co*-isopropylacrylamide), P(AA-*co*-NiPAAm), was used with poly(vinylamine) and, again, EDC coupling chemistry was used to link the layers together *via* reaction of the amines with the acid functionality. The results demonstrated the successful layer buildup for P(AA-*co*-NiPAAm) with 5, 10 and 15% AA groups incorporated into the copolymer. It was shown that in these systems the lower percentage of poly(acrylic acid) resulted in thicker layers, due to conformational changes in the layers. As with the earlier systems, these films exhibited the properties of a hydrogel, and it was shown that both pH and ionic strength could be used to change the swelling ratio of the systems. In addition, the permeability of the film could be controlled with temperature by exploiting the thermoresponsive characteristics of PNiPAAm. This work highlights the benefits of the covalent approach, showing that a range of conditions can be easily varied without impacting on film stability.

The sequential covalent approach has also been used to assemble poly(*p*-phenylenevinylene) polymers, which have potential application as conducting materials.[38] This was achieved by synthesising two poly(*p*-phenylenevinylene) derivatives with amino and activated ester side chains. The alternation of the two materials facilitated a condensation reaction between the two functional groups, allowing layers to be formed. It was suggested that there was also some hydrogen bonding occurring in the system. Linear film buildup was observed using both UV absorption and ellipsometry, the latter revealing an individual layer thickness of approximately 0.8 nm. The stability of these films was demonstrated by sonication in tetrahydrofuran for several hours without loss of material. This technique was then applied to the generation of patterned surfaces.

A new approach to the formation of covalent layers was introduced recently by our group.[39] This approach utilises click chemistry, which is a covalent linking methodology that has generated significant research interest in the last few years due to its efficiency, inertness to functional groups, and mild reaction conditions. The most commonly used example involves the reaction between an alkyne and an azide functionality to form a 1,2,3-triazole linkage (*i.e.* the 1,3-Huisgen polar cycloaddition). We demonstrated that linear growth of covalent layers could be achieved using alternate PAA polymers with a 14% component of azide and alkyne functionality (Fig. 7). These systems were assembled in aqueous conditions and were stable to both a wide range of pH and various organic solvents. In the example studied, layer growth was shown to be regular up to eight bilayers, as measured using both infrared spectroscopy and UV-visible spectrophotometry. A thickness increment of 4.6 nm per bilayer was measured using ellipsometry, and the final film thickness obtained by this method agreed well with that determined by atomic force microscopy. Click chemistry is a highly generalisable method to assemble covalent layers, as the azide and alkyne functionality can be added to a range of polymers, either by simple copolymerisation with functional monomers or by post polymerisation modification of the polymer backbone. Further, the reaction can be performed in a number of solvents, including water. Click chemistry also has the benefits of being relatively insensitive to the incorporation of other functionalities (such as the carboxylate groups in acrylic acid) and being applicable to biological systems.

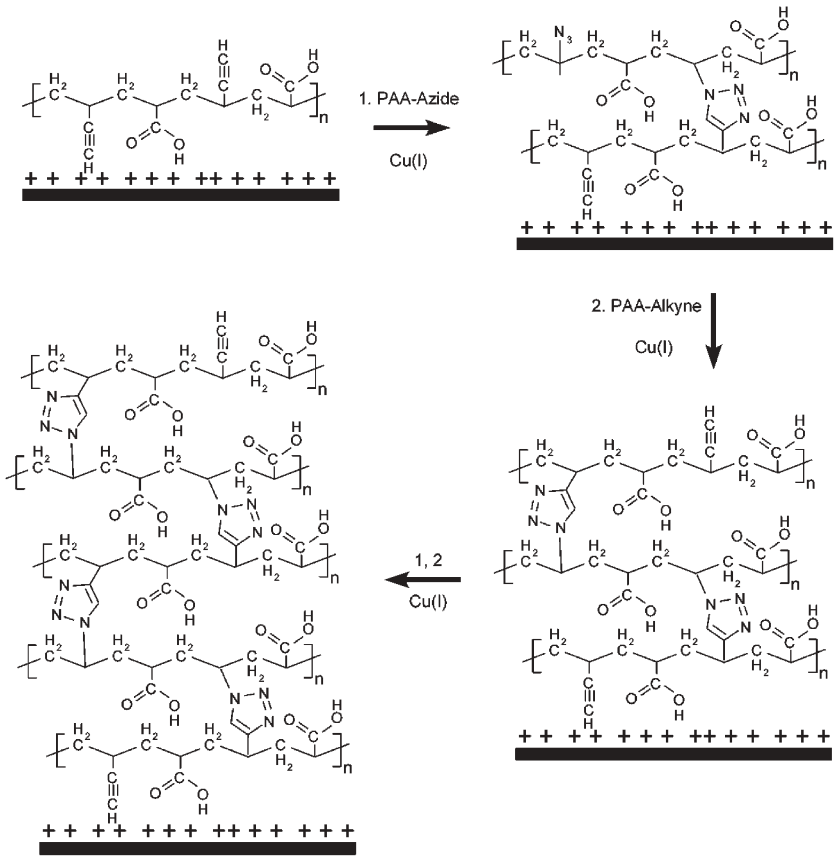

Fig. 7 LbL assembly of multilayer films *via* stepwise triazole formation (a so-called click reaction).[38] Poly(acrylic acid) functionalised with either (i) alkyne or (ii) azide pendants is reacted alternately in the presence of Cu(I). The initial layer was adsorbed through electrostatic interactions.

There is still a great deal of untapped potential in the use of covalent bonds to assemble LbL films. A simple, generalisable method, such as click chemistry,[39] could provide unprecedented opportunities for preparing high functionality polymer films, which could not be assembled using classical LbL routes. Utilising covalent bonds to assemble polymers that are biologically inert is of particular interest. Further, most of the current work in covalently assembled LbL materials has focused on films, although obvious advantages lie in adapting this technology for particles in order to maximise the impact in colloid- and interface-based technologies, such as catalysis, sensing and drug delivery.

6 Conclusions and outlook

The versatility and ease of LbL assembly has underpinned interest in this approach for surface modification and the preparation of nanostructured materials over the last 15 years. However, in order for the technique to find widespread application in emerging areas such as nanomedicine and nanobiotechnology, the move away from electrostatically bound materials is a welcomed step in the life cycle of LbL technology. The investigations of many research groups in moving beyond electrostatics have been instrumental in paving the way for a number of interesting applications. It is envisaged that as LbL assembly finds further use in systems with direct, practical applications, "secondary" interactions such as hydrogen bonding, hybridisation and sequential covalent reactions will come to the fore as excellent methods to assemble tailored, nanostructured materials.

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