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Investigation of Dopamine Analogues: Synthesis, Mechanistic Understanding, and Structure-Property Relationship

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ABSTRACT: Dopamine, perhaps the simplest molecule that covalently links catechol and amine, together with its derivatives, has shown impressive adhesive and coating properties with its polymers. However, the scope of the molecules is rather limited, and the polymerization mechanisms are still elusive. We designed a general synthetic scheme and successfully synthesized a series of dopamine analogues with different alkyl chain length between the catechol and amine. Taking these new dopamine analogues, together with the molecular systems that have separate catechol and alkyl amine, we show that having both catechol and amine in the molecular system, whether covalently linked via an alkyl chain or not, is sufficient to polymerize under a similar reaction condition to that of dopamine polymerization. However, the time-dependent UV-Vis characterization of the individual polymerization indicates that the polymerization for individual molecular systems likely proceeds via different reaction intermediates, depending on the length of alkyl chain and whether or not there is a covalent linkage. Interestingly, while the covalent linkage via an alkyl chain is not necessary for showing adhesive property, it is required to achieve the impressive coating property. Our results offer new insights to the design and synthesis of dopamine analogues for future applications, as well as further mechanistic understanding of the polymerization of these dopamine analogues.

INTRODUCTION

The unique moisture-resistant adhesion of marine mussels has attracted scientists' attention in recent years.[1] Studies revealed that the mussel-adhesion is mediated by five unique adhesive proteins, all of which contain a significant amount of catechol-containing amino acid, L-3,4-dihydroxyphenylalanine (L-DOPA), and amine-containing amino acids, such as lysine, histidine and arginine.[2] Unsurprisingly, fundamental understanding of the functions of these mussel adhesive proteins [2,3] has facilitated the design of synthetic small molecules/polymers to mimic the unique adhesive property of mussels.[4-6] Reciprocally, investigating these mussel protein-inspired small molecules/polymers, in particular, the structure-property relationship, has helped to identify the key functional components and the working mechanism for these unique adhesion/coating behaviors. Pioneering work by the Deming group [7] demonstrated that the catechol functionality of L-DOPA is the primary component required to replicate the moisture-resistant adhesion. Further studies have shown that catechol and its oxidized *o*-quinone form are largely responsible for the adhesive behavior through hydrogen bonding[8,9] and cross-linking via metal chelating[9,10] as well as aryl-aryl coupling,[4,11] respectively.[12] These discoveries have promoted the incorporation of catechol and its derivatives into various polymer backbones to achieve functional hydrogels and adhesives, among others.[4,5,13-17]

While the importance of having the catechol functional group to reproduce mussel's adhesive properties has been generally agreed upon, the potential involvement of the amine group in achieving mussel's adhesive properties is much less studied. Researchers suggested that amine may contribute to the mussel's adhesion via ionic bonding to negatively charged surfaces [18,19] and intermolecular cross-linking with *o*-quinones through Michael addition or Schiff-base formation.[9] Thus, having both catechol and amine would create a synergistic effect to reproduce the mussel's adhesive properties.[20] Indeed, in 2007, the Messersmith group [21] first reported that dopamine, a small molecule that covalently links both catechol and amine, could form polydopamine (PDA) under mild conditions, e.g., under a buffer condition of pH = 8.5.[21] More impressively, PDA demonstrated an almost universal adhesion/coating property to a variety of substrates, including metals, ceramics and polymers, among others.[21] Inspired by the formation mechanism of the eumelanin polymer, Messersmith and co-workers proposed a similar polymerization mechanism to explain the formation of PDA (**Scheme 1**). This proposed mechanism first involves the oxidation of dopamine to dopaminequinone, which can then cyclize to form dopaminechrome. Subsequent oxidation and rearrangement would form the pre-monomer which can then polymerize via oxidative coupling into the PDA.[21] Though a generally agreed formation mechanism of PDA and its exact chemical structure remain

elusive,[22-25] the easy synthesis of PDA and its impressive coating/adhesive property have promoted the application of PDA and its derivative materials in various fields, including energy, environment and biomedical engineering.[6]

Scheme 1. Overview of mussel-inspired materials and proposed polymer structure
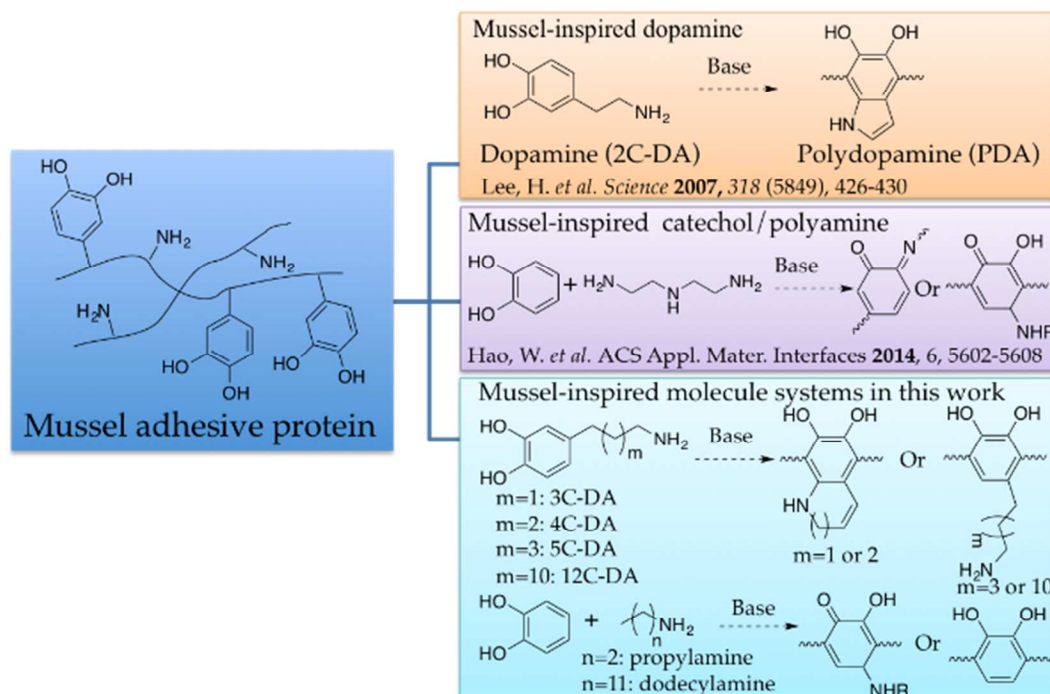
However, PDA and its related materials have their own limitations. For instance, the dark brown/black color associated with PDA and its related materials [21] are not desirable for certain clinical applications. Further structural functionalization of dopamine molecule without impacting its polymerization behavior also appears to be difficult; the only reported strategy to covalently incorporate dopamine with other polymers/organic materials is through its amine group, which, for example, can form amide with the pendent carboxylic acid from the parent polymer.[16,17,26,27] This status quo motivated us to explore new mussel adhesive inspired molecules that could maintain notable features of dopamine/PDA, including water-solubility, facile polymerization, and the extraordinary coating ability, while mitigating issues in current systems.

Based on all these previous studies, we hypothesized that any molecule that covalently links the catechol and primary amine functional groups would likely show similar polymerization and coating behavior as dopamine/PDA have demonstrated. However, the polymerization mechanism could be molecule dependent, in particular, when factoring in the proposed ring-formation process in the dopamine polymerization. In general, forming a cyclic product from a linear precursor would be affected by both entropy and enthalpy. For example, in the series of dopamine analogues shown in **Scheme 1**, 3C-DA and 4C-DA would likely undergo cyclization during their polymerization, leading to the formation of cyclic structures (e.g., six-membered ring for 3C-DA) in their polymers, similar to the proposed five-membered ring formation in the case of dopamine (i.e., 2C-DA). This is because five- and six-membered rings are the most stable cyclics. Indeed, an earlier computational study of the intramolecular amine addition to ortho-quinones [28] indicated the possibility of forming six- and

seven-membered rings. On the other hand, it is hard to imagine that 5C-DA and 12C-DA, having longer alkyl chains, would be able to undergo cyclization and form eight-membered ring (for 5C-DA) and larger ring structure (for 12C-DA). This is because the large ring formation via intramolecular ring-closure is entropically disfavored. Therefore, having all these new dopamine analogues and investigating their polymerization behaviors would help shed more light on the polymerization mechanisms of dopamine and its analogues. Furthermore, comparing the adhesive and coating properties shown by these dopamine analogues would help to disclose how these subtle structural changes (i.e., the length of alkyl chain between the catechol and the amine) would influence the adhesive/coating ability of PDA and its derivative materials.

Thus, the objective of our study is three-fold: synthesis, mechanistic investigation, and structure-property relationship. We find that all of these new dopamine analogues are able to polymerize, albeit likely via different mechanisms, based on experimental observations. While 3C-DA and 4C-DA could go through similar polymerization pathways (e.g., cyclization followed by oxidative coupling) to the one proposed for dopamine (**Scheme 1**), 5C-DA and 12C-DA appear to go through a simpler reaction pathway to polymerize (e.g., without cyclization). Importantly, the adhesive properties of polymers from these dopamine analogues (including dopamine) are tightly correlated with the amount of catechol and amine functionalities in the system, with negligible influence by the length of the alkyl chain that links catechol and amine. Furthermore, even the alkyl linkage between catechol and amine does not seem to be a prerequisite to achieve the adhesive property. In fact, non-covalently linked catechol and amine

based molecular systems (e.g., catechol and "free" propyl amine) – when subjected to the same polymerization condition – also form materials that show comparable shear adhesive strength to those achieved with covalently linked catechol and amine via an alkyl chain. On the other hand, this covalent linkage via an alkyl chain seems to be critical in achieving the coating ability of these analogous catechol-amine systems: while the covalently linked catechol and amine systems also show comparable coating ability with their polymers to that of PDA, materials formed with un-linked catechol and amine only show mediocre coating abilities.

MATERIALS AND METHODS

Materials and substrates preparation. Dopamine hydrochloride (99%), 3,4-dimethoxybenzealdehyde (99+%), cyanoacetic acid (99%), sodium borohydride (NaBH$_4$, 99%, powder), triethylamine (98%) and lithium alumina hydride (LAH, 95%, powder) were purchased from Acros Organics and used as received. Piperidine (99%), methyl trifluoroacetate (99%), 1 M boron tribromide in methylene chloride (DCM) and 3 N methanolic HCl solution were purchased from Sigma Aldrich and used as received. Hexanes, acetone, DCM, toluene, methanol (MeOH), ethyl acetate (EtOAc), tetrahydrofuran (THF), dimethylformamide (DMF) were obtained from Fisher. THF was freshly distilled over sodium before use. Poly(ethylene terephthalate) (PET), poly(tetrafluoroethylene) (PTFE), glass (Fisher), Indium tin oxide (ITO) were cleaned ultrasonically in 2-propanol (IPA) for 15-20 minutes before use. Gold (20 nm deposited onto 5 nm Ti) surface on Si-wafers was prepared by electron beam deposition.

Characterization methods. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-400 (400 MHz) spectrometers. CDCl$_3$ and DMSO-d6 were used as the solvent. Mass spectra were acquired by the University of North Carolina, Chapel Hill mass spectrometry facility. UV-Vis absorbance spectra were obtained on a UV-2600 spectrophotometer (Shimadzu Scientific Instruments, Inc.). Lap-shear testing

was conducted on an Instron 5566. For the coating experiments, different substrates were vertically immersed in a diluted aqueous solution of a given molecular system (e.g., 2 mg of dopamine or 3C-DA per milliliter of 10 mM Tris, pH=8.5) with gentle stirring for 17 hours. The substrates were then washed with distilled water and dried over nitrogen gas. X-ray photoelectron spectroscopy(XPS) was obtained on a Kratos Axis Ultra DLD X-ray photoelectron spectrometer using a monochromatic Al K alpha source (1486.8 eV).

RESULTS AND DISCUSSION

Synthesis of dopamine analogues. Due to the high reactivity of dopamine analogues that have unprotected amine and catechol, all these analogues were synthesized in their HCl salt form, similar to the commercially available dopamine (also as its HCl salt). **Scheme 2** outlines the synthetic route that successfully offered the 3C-DA in its HCl salt form. A condensation of commercially available 3,4-demethoxybenzaldehyde with cyanoacetic acid, followed by a NaBH$_4$ reduction, leads to compound (3). Decarboxylation of (3) gives 3-(3,4-dimethoxyphenyl)propylnitrile (4), which undergoes another reduction by LiAlH$_4$ to afford the key intermediate, 3-(3,4-dimethoxyphenyl) propylamine (5).[29] Multiple attempts to remove the methyl from the methoxy group (e.g., via BBr$_3$ treatment) to directly convert (5) into the targeted 3C-DA failed, possibly due to the interference of the primary amine functionality of (5). Thus we decided to protect the primary amine with a trifluoroacetyl (Tfa) group, converting (5) into (6), following a similar protocol previously reported.[30] Treating (6) with the strong Lewis acid BBr$_3$ can then smoothly remove these two methyl groups, revealing the catechol of (7). In the last step, the Tfa protecting group is readily cleaved under strong acidic condition to generate the target molecule (3C-DA), however, in its HCl salt form (8). The chemical structure of the product formed after each step was confirmed by ^{1}H NMR and ^{13}C NMR, respectively, and the chemical formula of target molecule (8) was further verified by ESI-MS.

Scheme 2. Synthesis of 3C-DA Analogue
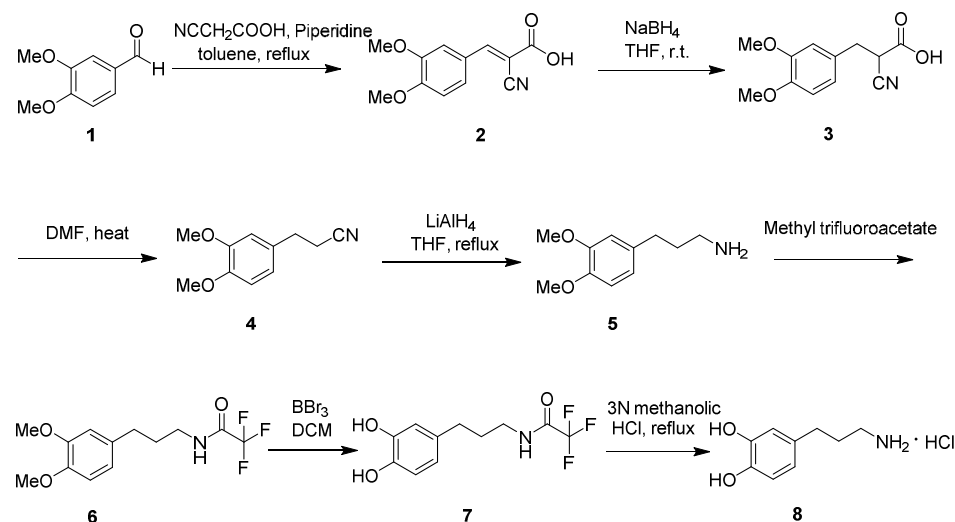
The rather general synthesis of dopamine analogues with longer alkyl linkers in between the catechol and amine (4C-DA, 5C-DA and 12C-DA) is largely similar to that of 3C-DA; the major difference lies in the preparation of 3-(3,4-dimethoxyphenyl)alkylnitrile, molecule (4') in **Scheme 3**. Specifically, a Friedel-Crafts acylation of the commercially available 3,4-dimethoxy benzene (1'), followed by a NaBH$_4$ reduction, readily affords (3'). A nucleophilic substitution of the bromide in (3') with the cyano offers (4'), which is the structural analogue to (4) in **Scheme 2**. The molecule (4') can then go through similar reactions outlined in in **Scheme 2** (i.e., from (4) to (8)) to afford the targeted molecule (8'), also in its HCl salt form.

Scheme 3. Synthesis of 4C-DA, 5C-DA and 12C-DA Analogues
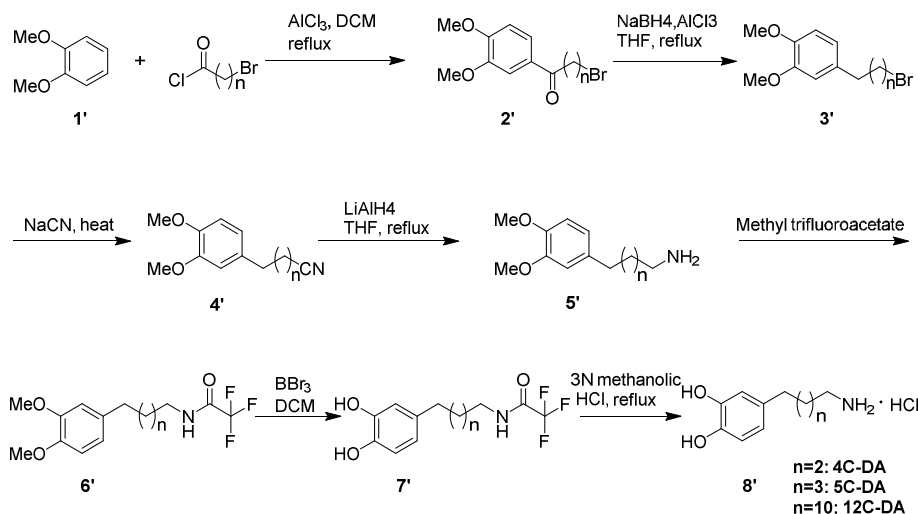
Polymerization behavior studied by UV-Vis spectra. After having obtained substantial quantity of these dopamine analogues (in their HCl form), we next explored the polymerization behavior of these newly prepared dopamine analogues, together with dopamine·HCl as the control/reference. Given the difficulty in separating reaction intermediates from the polymerization, we chose to monitor the polymerization process by UV-Vis spectroscopy, which has been previously used to track the polymerization progress of dopamine.[31,32] Though Tris buffer has been a popular medium for the polymerization of dopamine, we have noticed that Tris buffer could be incorporated into the PDA structure,[23] which could interfere with our study on the polymerizations. Thus, we simplified the polymerization condition and carried out the polymerization of different dopamine analogues in aqueous solution with excess NaOH base.[33] Through comparing the time-dependent UV-Vis absorption spectra during the polymerization to the absorption feature of known reaction intermediates,[13,31,32] we can identify plausible reaction pathways.

Experimentally, after adding excess amount of base (dopamine analogues:1 M NaOH =1:8, molar ratio) to a dilute aqueous solution (1 mM) of dopamine/3C-DA/4C-DA/5C-DA (note: 12C-DA is not soluble in water), UV-Vis absorbance spectra was recorded at different time intervals for 24 h. **Figure 1a** shows the time-dependent UV-Vis spectra of the polymerization of dopamine, which agrees well with a previous literature report.[32] The most notable feature of the UV-Vis spectra is the appearance of an absorbance peak at 420 nm immediately after the base addition, which gradually develops its intensity in the first 25 min and then attenuates. As the polymerization progresses, this absorption peak broadens and gradually decreases; and the whole spectrum becomes almost featureless but widely absorptive after 4 h, which explains the black/brown color of polydopamine (PDA). Similar time-dependent UV-Vis spectra for the polymerization of 3C-DA was observed (**Figure 1b**); however, the absorption peak position shifts to 470 nm, and the maximum peak intensity ever reached is much lower than that of the 420 nm absorption peak observed in the dopamine polymerization. Nevertheless, the absorption spectrum loses this absorption peak (~ 470 nm) as the polymerization proceeds. More interestingly, the spectrum after 4 h does not bear much intensity after 450 nm, hence the solution containing poly(3C-DA) appears almost colorless (at low concentration) or much lighter color (at high concentration) than the solution of poly(2C-DA) (i.e., PDA). Interestingly, with one more methylene in its structure than 3C-DA, 4C-DA does not show any obvious absorption peaks during its polymerization, even at the very beginning (i.e., 2 min). This could be caused by a fast polymerization rate, which would render the observation of the reaction intermediate more difficult, given the "transient" nature of such reaction intermediate. Similar to 3C-DA, 4C-DA almost loses the absorption intensity entirely in the range of 400 nm to 600 nm during its polymerization (**Figure 1c**), whereas 5C-DA regains the absorption in the same region in the UV-Vis spectrum (**Figure 1d**). Finally, the polymerization of catechol and propylamine (i.e., the non-covalently linked molecular system of 3C-DA) also has noticeable absorption within the range of 400 nm to 600 nm (**Figure 1e**), though no clearly defined peak can be identified in the same range. Nevertheless, in all cases, the UV-Vis absorption spectrum becomes almost featureless after 24 h, with much less intensity in the visible region than that of the spectrum from the polymerization of dopamine.
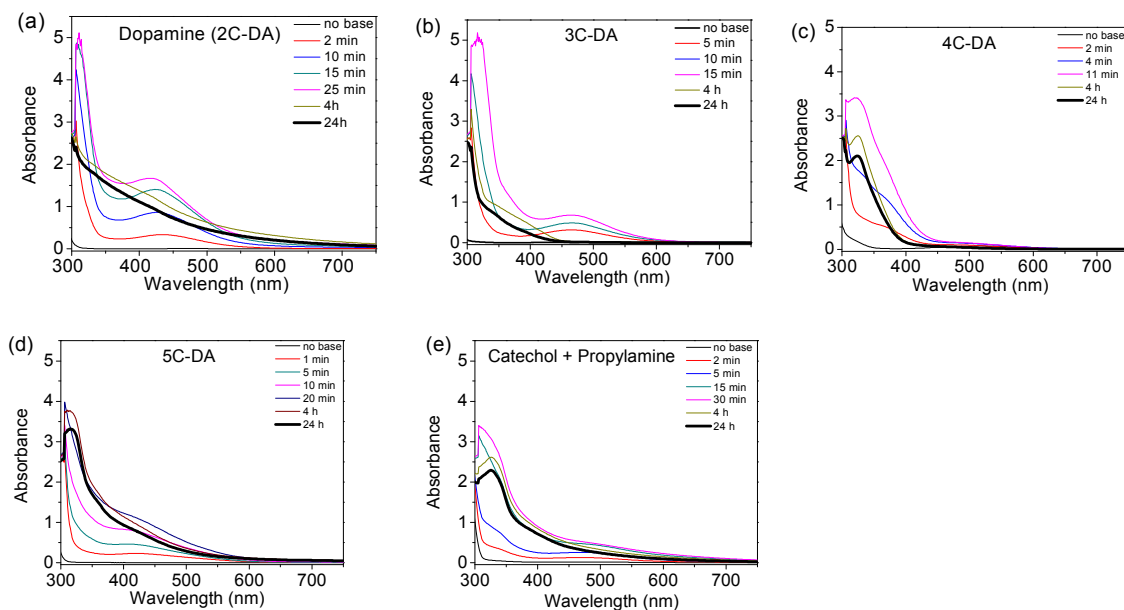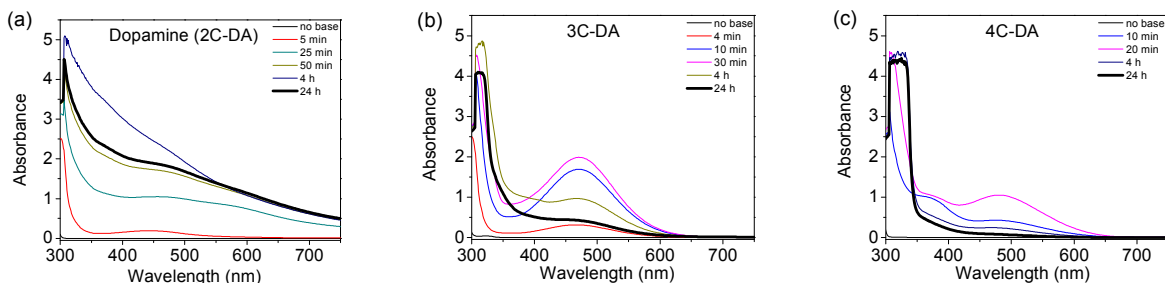
Figure 1. Time dependent UV-Vis spectroscopy for the polymerization of (a) dopamine, (b) 3C-DA, (c) 4C-DA, (d) 5C-DA, and (e) catechol and propylamine in aqueous solution after adding 1M NaOH.

Given that alcohol-water mixed solvent has been used to affect the polymerization rate of dopamine [34,35] and 12C-DA is insoluble in water but has better solubility in methanol (MeOH), we next chose methanol as the solvent to conduct the polymerization and aimed to spectroscopically capture the reaction intermediates. After adding the same excess amount of base (dopamine analogues: 1 M NaOH= 1:8, molar ratio) to a dilute MeOH solution (1 mM) of dopamine/3C-DA/4C-DA/5C-DA/12-DA, we again monitored their polymerization by UV-Vis (**Figure 2**). A few interesting observations can be noted. First, the noticeable absorption peak at 420 nm in the early stage of the dopamine polymerization in water (**Figure 1a**) loses its intensity for the same polymerization in the methanol solution (**Figure 2a**), though a hump at 470 nm can still be identified in this broad absorption. Second, the UV-Vis spectrum for the 3C-DA polymerization in the mixed solvent (**Figure 2b**) is qualitatively similar to that in water (**Figure 1b**). However, the 470 nm peak has shown a higher intensity, and the time it takes for reaching the highest absorbance intensity is twice that as in water. Interestingly, the 4C-DA polymerization in the mixed solvent develops a new absorption peak around 480 nm, which eventually disappears after 24 h (**Figure 2c**). If we assume that the ~470 (480) nm absorption peak indicates the formation of the polymerization intermediate, a shorter time to reach the maximum intensity and a faster

disappearance of this absorption peak would imply more rapid formation of the polymer (**Figure S1** in Supp. Info. and **Figure S2** in Supp. Info.). Under this assumption, it appears that the polymerization of either 3C-DA or 4C-DA in the methanol solution (MeOH:water=125:1, v/v) is slower than the corresponding polymerization in water. Third, the dopamine analogues with longer aliphatic linkers (5C-DA and 12C-DA) show qualitatively similar time-dependence UV-Vis spectra (**Figure 2d** and **2e**), with a noticeable absorption peak at 410 nm in the early stage of the polymerization. Nevertheless, in all these polymerizations of dopamine and its analogues in the mixed solvent, the UV-Vis spectra do not contain identifiable absorption peak beyond 350 nm after 24 h, similar to what we observed for the polymerizations in water. In contrast, the UV-Vis spectra for the non-covalently linked catechol/propylamine has developed an absorption peak at 326 nm, whose intensity continues to rise even after 24 h (**Figure 2f**). Increasing the alkyl chain length for the non-covalently linked system has negligible impact on the polymerization, evidenced by the fact that almost identical UV-Vis spectra were observed for catechol/dodecylamine (**Figure S2g** in Supp. Info.).
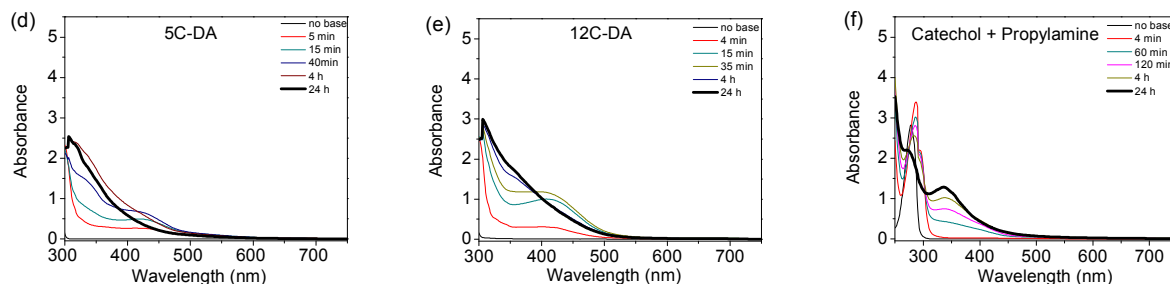
Figure 2. Time dependent UV-Vis spectroscopy for (a) dopamine, (b) 3C-DA, (c) 4C-DA, (d) 5C-DA, (e) 12C-DA, and (f) catechol and propylamine in MeOH (1mM) after adding 1M NaOH (MeOH:water=125:1, v/v).

Combining our time-dependent UV-Vis data in both H_2O and MeOH with literature proposals on the polymerization mechanism of dopamine,[22-25] we propose plausible mechanisms for the observed polymerizations as follows. First, the absorbance peak of ~ 470 nm (480 nm) (**Figure 1b, 2b, 2c**), appearing in the early stage of the polymerization of 3C-DA and 4C-DA agrees well with the absorbance of dopachrome, which has been previously identified during the biosynthesis of eumelanin from L-DOPA and has a λmax of 473 nm.[13] Therefore, we tentatively assign this peak of 470 nm (480 nm) to the formation of 3C-dopachrome and 4C-dopachrome (**Scheme 4**) via a similar formation process for dopachrome, i.e., intramolecular cyclization and then oxidation. On the other hand, for entropic reasons, it would be difficult for similar cyclization occurring during the polymerization of 5C-DA or 12C-DA, both having longer aliphatic chains between the catechol and the amine. Thus, we tentatively ascribe the 410 nm absorption peak which appeared during the 5C-DA and 12C-DA polymerization (**Figure 2d** and **2e**) to the presence of 5C-dopaquinone and 12C-dopaquinone (**Scheme 4**). In fact, this 410 nm peak agrees well with the ~400 nm absorption peak for dopaquinone.[11] In all cases (3C- to 12C-DA), the intensity of the absorption peak would decrease after a short period of time, because cross-coupling between the structural units of catechol and/or quinone would increasingly dominate the reaction progress to form the polymer (**Scheme S3** in Supp. Info.).
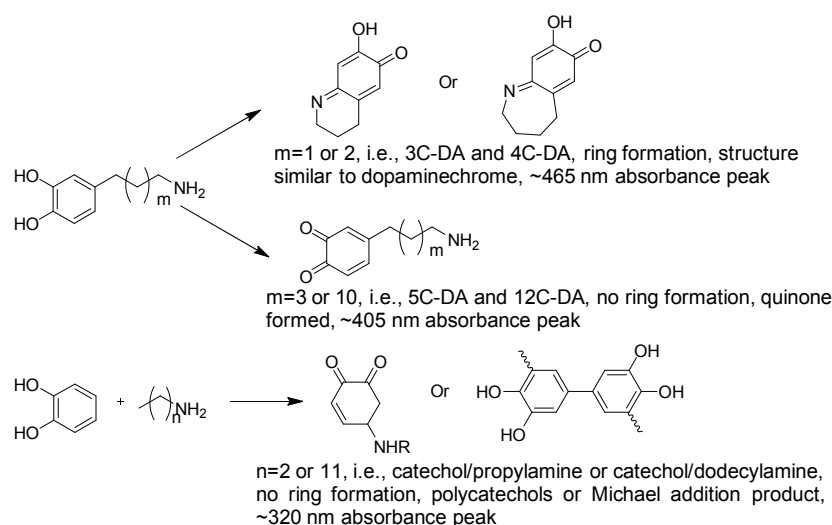
However, for dopamine polymerization, the UV-Vis spectrum is more complicated. First, in H_2O/NaOH, we did not observe any absorbance peak at ~ 470 nm (**Figure 1a**), which was believed to indicate the formation of dopaminechrome, a known reaction intermediate for dopamine polymerization. This could be caused by the high pH (8 mM NaOH) and thereby rapid spectral evolution, which could mask the initial ~470 nm peak.[36] Furthermore, we speculate the observed unique absorption peak at ~ 420 nm(**Figure 1a**), which has also been observed by others,[32] might indicate the formation of some oligomers (e.g., coupling of phenols) in the early stage of the dopamine polymerization. Interestingly, when we decreased the polymerization rate for dopamine polymerization with MeOH-H_2O (v/v=125:1), we observed a broad absorption

over the entire spectra range (**Figure 2a**) with a hump near 470 nm, which implies the formation of dopaminechrome. Nevertheless, the broad absorption which rendered the dark color of PDA is believed to be a result of its complicated, presumably cross-linked structure analogous to that of eumelanin.[21] It is very likely that PDA is a mixture of oligomers/polymers with various monomeric components (e.g., dopamine, dihydroxyindole, indoledione, etc.), tied together via cross-linking and non-covalent interactions.[24,25] In contrast, the much lighter color of these new dopamine analogues after polymerization, supported by the much attenuated absorption in the visible region after 24 h (**Figure 2b** to **2e**), suggests simpler structures that have less conjugation than PDA. Given the structural similarity between dopamine and these dopamine analogues, further investigation of the polymerization mechanism of these dopamine analogues – ideally, together with other dopamine analogues – can offer more insights on a clearer picture of the formation of PDA.

On the other hand, when the amine is not covalently linked with the catechol, the UV-Vis spectra of polymerizations with these molecular systems (i.e., catechol/propylamine and catechol/dodecylamine) are distinctly different from those observed with covalently linked ones (**Figure 2f** and **Figure S2g** in Supp. Info. vs. **Figure 2a** to **2e**). In particular, the absorption peak at 326 nm gains more intensity as polymerization continues and becomes much more visible after 24 h. We tentatively assign this absorbance peak at 326 nm to the Michael addition product between the quinone and amine or phenol coupling (**Scheme 4**). The polymerization of such noncovalently linked systems could undergo the coupling among semiquinone and/or quinones as well as products from Michael addition to form the polymers.[37]

Lastly, please note that the proposed mechanisms are largely postulated based on the time-dependent UV-Vis absorption data for the studied molecular systems in the mixed solvent (MeOH:water = 125:1, v/v). The noticeable difference of UV-Vis spectra for the same molecular system in different solvents imply that the polymerization mechanism could also depend on the given solvent system, in particular, the water dominant system vs. the methanol dominant system.

Scheme 4. Proposed reaction intermediates during polymerization

m=1 or 2, i.e., 3C-DA and 4C-DA, ring formation, structure similar to dopaminechrome, ~465 nm absorbance peak

m=3 or 10, i.e., 5C-DA and 12C-DA, no ring formation, quinone formed, ~405 nm absorbance peak

n=2 or 11, i.e., catechol/propylamine or catechol/dodecylamine, no ring formation, polycatechols or Michael addition product, ~320 nm absorbance peak

Adhesive property studied by lap-shear testing. Many mussel-inspired materials have shown significant adhesive properties;[5,14,21] since dopamine and the analogues in this study are also inspired by mussel, we next investigated the bulk adhesive properties of all molecular systems in our study. Specifically, we measured the lap shear adhesion of two glass substrates sandwiching the polymer, following a protocol established by the Wilker group.[14] We first dissolved some monomers (including 2C-DA, 3C-DA, 4C-DA, 5C-DA and catechol/propylamine) in water, or all monomers (2C-DA, 3C-DA, 4C-DA, 5C-DA, 12C-DA, catechol/propylamine and catechol/dodecylamine) in isopropanol/water (1:2, v/v). Please note that isopropanol (IPA) was used here instead of methanol in order to mitigate the solubility issue of 12C-DA, since the IPA/water (1:2, v/v) is still a water dominant solvent system. In fact, these two solvent systems appear to offer similar polymers via similar polymerization mechanisms for each studied molecular system, since UV-Vis spectra for the polymerization in the mixed solvent where H_2O was dominant (IPA-H_2O, v/v=1:2) (**Figure S3** in Supp. Info.) are very comparable to those obtained in H_2O (**Figure S1** in Supp. Info.), for each studied molecular system.

We then added excess base (100 μL 1M NaOH aqueous solution) to induce the polymerization. After 5 minutes, we applied 30 μL of the polymer solution onto one side of the glass slide, which was then covered with another identical glass slide with an overlapping area of 1 inch × 1 inch. A binder clip was applied to maintain this sandwich structure for the next 48 hours to ensure the completion of the polymerization (**Figure 3a**, left). This binder clip was removed before the sandwich structure was tested via an Instron (**Figure 3a**, right). After measuring three maximum load at break point for each polymer, adhesion strength was calculated by maximum load/sandwich area, i.e., 1 inch × 1 inch in our case. A similar load vs extension curve was obtained for each sample to the literature results,[14] (**Figure S8** in Supp. Info.) indicating the complete removal of the solvent. In addition, we measured the UV-Vis spectra of the polymers sandwiched in between the glass substrates. The UV-Vis spectrum of the polymer does not contain characteristic absorption features of the reaction intermediate (for each studied system), and the overall spectrum is very similar to the final UV-Vis spectrum (i.e., measured after 24 h) of the same molecular system obtained in H_2O. These results confirmed that it was the polymers that were tested in the lap shear testing (**Figure S9** in Supp. Info.).
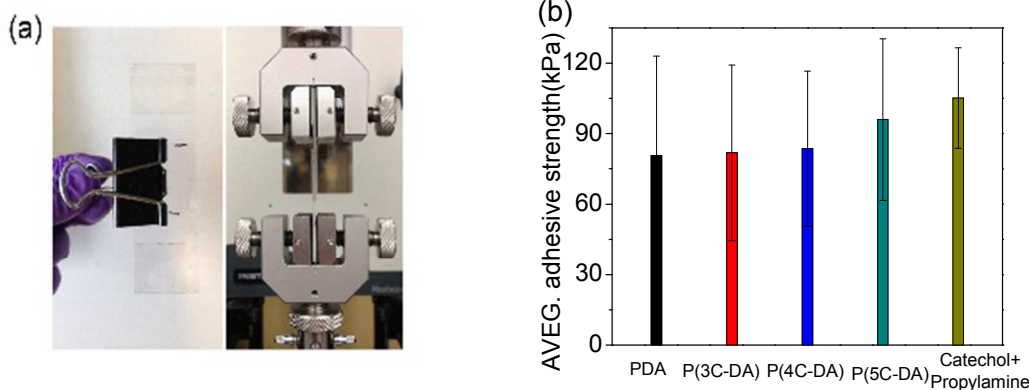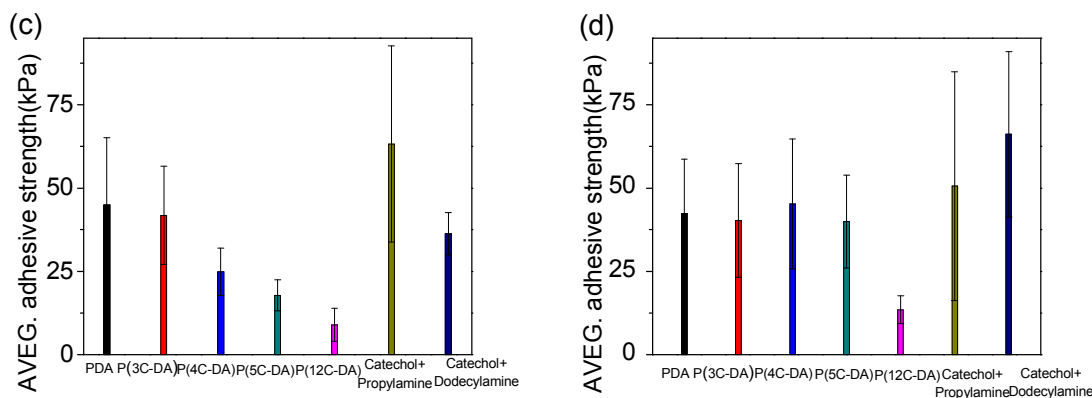
Figure 3. (a) Image of test sample (left) and main part of Instron machine (right) for lap-shear testing; (b) results for adhesive strength for PDA, poly(3C-DA), poly(4C-DA), poly(5C-DA) and catechol/propylamine in water at 6.7 mg/mL;(c) results for adhesive strength for PDA, poly(3C-DA), poly(4C-DA), poly(5C-DA), catechol/propylamine and catechol/dodecylamine in IPA:H_2O at 6.7 mg/mL (IPA:H_2O=1:2, v/v);(d) results for adhesive strength for PDA, poly(3C-DA), poly(4C-DA), poly(5C-DA), catechol/propylamine and catechol/dodecylamine in IPA:H_2O at 33.3 mM (IPA:H_2O=1:2, v/v).

As shown in **Figure 3b**, polydopamine (i.e., PDA), poly(3C-DA), poly(4C-DA) and poly(5C-DA) have demonstrated comparable adhesive strength (~ 90 kPa) from the identical polymerization in water (i.e., same mass concentration of 6.7 mg/mL), indicating that the increment of the aliphatic chain length between the amine and the catechol only negligibly influence the adhesive property of the polymer. Interestingly, the polymer from reacting catechol/propylamine under the same condition (i.e., catechol and propylamine together at 6.7 mg/mL with 1:1 molar ratio) demonstrated almost identical adhesion strength as that of covalently-linked dopamine analogues, implying that the covalent bonding (i.e., the alkyl linkage) between the amine and the catechol is not necessary to achieve the adhesive strength of the polymer.

Again, since 12C-DA is not soluble in water, we switched to the mixed solvent, IPA:water (1:2, v/v). For comparison with the results obtained with polymers synthesized in water, we used the same mass concentration (6.7 mg/mL) in the mixed solvent as well. Surprisingly, for each of dopamine and its analogues with longer aliphatic chains, the adhesive strength of the polymer created in the mixed solvent is significantly smaller than that of the counterpart synthesized in water. We offer two plausible explanations. First, the mixed solvent (IPA:H_2O, v/v=1:2), which has a different solvent evaporation rate that that of pure H_2O, might lead to incomplete film formation under our experimental condition. Alternatively, pure H_2O may generate more polymers than the mixed solvent of IPA:H_2O (though the structure of the polymers could be identical, regardless of the solvent system, as we previously discussed). Furthermore, the adhesive strength of the polymer decreases monotonically from dopamine to 12C-DA (**Figure 3c**). However, when the same polymerizations were done with an identical molar concentration (i.e., 33 mM) in the mixed solvent, the adhesive strength from the different polymers becomes comparable (**Figure 3d**), except the polymer from 12C-DA. We tentatively ascribe the weaker adhesive strength demonstrated by the polymer from 12C-DA in the mixed solvent to its limited solubility (even in the mixed solvent), which would significantly impede its polymerization. Nevertheless, these results indicate that the amount of catechol and amine in the system is proportional to the adhesive strength of polymers

from these dopamine analogues in the mixed solvent. This conclusion is further supported by the fact that the polymers created from the non-covalently linked analogues (i.e., catechol/propylamine and catechol/dodecylamine) also show comparable adhesive strength to that of their covalently linked counterparts, when the same molar concentration (33 mM) was used for the polymerization in the mixed solvent (**Figure 3d**). This observation also supports the previous conclusion that the covalent bonding between the amine and the catechol is not a prerequisite for achieving the adhesive strength of the polymer.

Coating property studied by XPS. The most impressive property of polydopamine (PDA) is its ability to coat almost any substrates, effectively serving as an ad-layer to change the surface property of the coated substrate and allow further functionalization. Thus, our last task was to investigate the coating ability of these newly prepared polymers. To compare the coating ability of our new synthesized dopamine analogues to that of PDA, we followed a protocol reported earlier by the Messersmith group, i.e.,10 mM Tris buffer, pH=8.5.[21] To investigate whether the Tris buffer solution would have any impact to the polymerization when compared with the NaOH solution, we conducted a similar time-dependence UV-Vis study of these dopamine analogs in the Tris buffer solution. Comparing the additional data (**Figure S4** in Supp. Info.) with the UV-Vis data acquired in H_2O/NaOH (**Figure S1** in Supp. Info.), we did not discern any significant difference. Thus, we believe the polymerization mechanisms (and related polymers) in the Tris buffer condition would be comparable to those under the H_2O/NaOH condition.

We chose PTFE, which is known for its anti-fouling ability, as our target to study the coating ability of these polymers. Experimentally, PTFE substrate was immersed into dilute aqueous solutions of 3C-DA, 4C-DA, 5C-DA and catechol/propylamine, respectively (i.e., 2 mg/mL in 10 mM Tris buffer, pH=8.5), which were then gently stirred overnight. A control experiment with dopamine·HCl was also carried out in parallel after it was coated with the polymer (**Figure 4a**). While the PTFE substrate coated with PDA has a black brown/grey color in appearance, the ones coated with poly(3C-DA), poly(4C-DA) and poly (5C-DA) show lighter

color instead. More importantly, X-ray photoelectron spectroscopy (XPS) characterization of the substrates coated with these dopamine analogues reveals a complete absence of the signals specific to the substrate (e.g., fluorine) after the coating, demonstrating a similar "shielding" effect as PDA has shown[21] (**Figure 4a** and **Figure S11** in Supp. Info.). Further chemical analyses (via XPS) of the surface of these PTFE substrates after coating with these new polymers disclose that the nitrogen/carbon ratio is very close to the theoretical value of each dopamine analogue (**Figure 4b**, rightmost column).

This further supports a complete surface coating of the PTFE substrate by polymers from these dopamine analogues, similar to the coating ability of PDA.[21] However, the non-covalently linked system, i.e., catechol and amine, is not able to achieve the complete coating of the PTFE substrate, as the fluorine signal (from the PTFE substrate) is still present after the coating (**Figure 4a**). These results suggest that the covalent bonding between the amine and the catechol might play an important role to maximize the coating ability of these dopamine analogues.
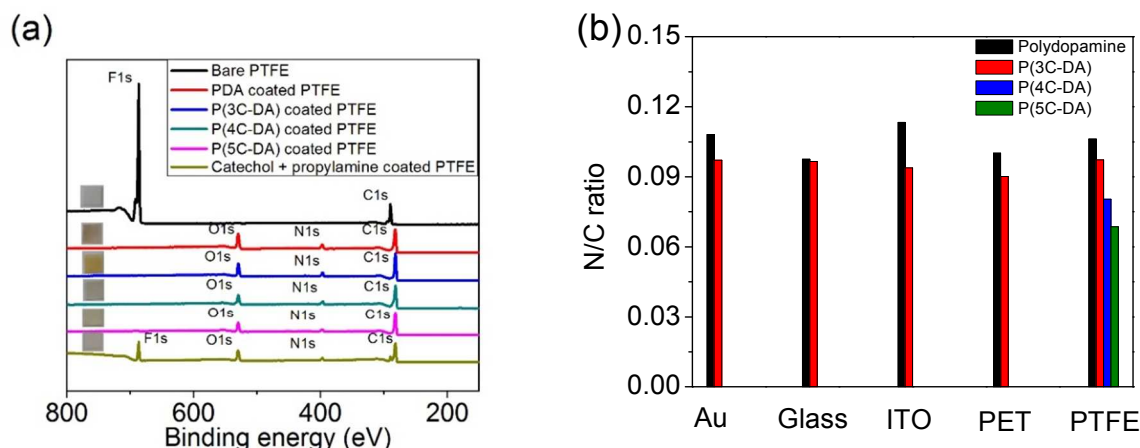

Figure 4. (a) XPS spectra of bare PTFE substrates, PDA coated PTFE and poly(3C-DA) coated PTFE, poly(4C-DA) coated PTFE, and poly(5C-DA) coated PTFE; (b) the quantitative analysis of nitrogen/carbon (N/C) ratio of different substrates coated with PDA and poly(3C-DA) as indicated by the black bar and red bar, respectively. Blue bar and green bar represent the N/C ratio of PTFE substrate coated with poly(4C-DA) and poly(5C-DA), respectively.

To further investigate whether the excellent coating ability on PTFE demonstrated by these new dopamine analogues – similar to that of PDA – is also universal as PDA has shown,[21] we chose 3C-DA as the example to test the coating of its polymer on other substrates. For comparison, dopamine was also included in this set of experiments. Four different substrates having representative surface properties, gold, indium tin oxide (ITO), glass, and poly(ethyleneterephalate) (PET), were coated with poly(3C-DA) or PDA, following the same protocol as illustrated above. Not surprisingly, there is a visible change in the color appearance of the substrate after it was coated with the polymer (i.e., PDA or poly(3C-DA)) (**Figure S10a** in Supp. Info.). XPS characterization of the surface of these substrates after coating reveals a complete disappearance of substrate signals, demonstrating comparable coating ability of poly(3C-DA) to that of PDA (**Figure S10b** in Supp. Info.). In addition, the nitrogen/carbon ratio, ranging from 0.902 to 0.972 on these coated substrates, is very close to the theoretical value of 3C-DA (0.1111) (**Figure 4b**, red bar), suggesting a complete surface coverage on all these substrates by poly(3C-DA), similar to that achieved by PDA (**Figure 4b**, black bar). These results strongly suggest that the 3C-DA, after polymerization, offers a similar coating ability and general applicability to those of dopamine/PDA.

CONCLUSION

In summary, through a series of molecular systems that are structurally analogous to dopamine, we demonstrate that the intriguing polymerization behaviors of dopamine are not

unique to dopamine; in fact, it appears that all molecular systems that contain catechol and amine – covalently linked via an alkyl chain or not – are able to polymerize. However, the exact polymerization mechanism for individual molecular system appears to be dependent on (a) whether or not the catechol and amine are covalently linked via alkyl chain, and (b) the length of the alkyl chain that links the catechol and amine. Furthermore, the solvent system chosen to carry out the polymerization also seems to have an impact on the polymerization mechanism. It appears conditions where water is dominant (i.e., IPA:H$_2$O, H$_2$O and Tris buffer) should offer similar polymers via similar mechanism, whereas the polymerization mechanism in methanol is different. Further mechanistic studies on these molecular systems and other related systems are needed to elucidate the polymerization mechanisms.

Furthermore, our results indicate that covalent attachment of catechol and amine (e.g., as in the case of dopamine) appears to have only negligible influence on the adhesive property shown by polydopamine; almost all studied molecular systems show comparable adhesive strength when normalized with the amount of catechol and amine. However, such covalent linking between catechol and amine via an alkyl chain is crucial in reproducing the impressive coating ability that polydopamine has shown; non-covalently linked catechol and amine only show mediocre coating behaviors. All these findings offer valuable guidelines in selecting and designing mussel adhesive protein-inspired materials for specific applications.

ASSOCIATED CONTENT

Supporting Information

Proposed polymerization mechanism for dopamine and dopamine analogues, synthesis procedures of dopamine analogues, ^{1}H and ^{13}C NMR spectra, UV-Vis spectra, adhesive data, coating images and XPS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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