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Polymerization mechanism of dopamine resolved:

A story of strong π stacking

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The polymerisation mechanism of dopamine in aqueous solutions has puzzled the scientific community for a couple of decades.¹⁻⁴ After initial cyclisation, dopamine undergoes a rapid polymerization by forming a black precipitate called polydopamine (PDA) found to be insoluble in all organic solvents.⁵ This has prevented the resolution of polydopamine's structure as solid-state characterization techniques have not been conclusive in their findings. Interest in polydopamine originates from its ability to exhibit strong antimicrobial and adhesive properties, making it a versatile coating material for corrosion prevention, implanted medical devices and even drug delivery.^{4,6,7} Recently, select ionic liquids (ILs) were discovered to dissolve polydopamine, thus enabling the use of liquid-phase characterization techniques to decipher its structure.^{8,9} In this study we identify the mechanism of dopamine polymerisation in a series of ionic liquids using *in situ* solution-phase ¹H

NMR spectroscopy. We show that after the 5,6-dihydroxyindole (DHI) monomer is formed with the aid of basic IL anions, it undergoes rapid dimerization via the 2-2', 2-4' and 2-7' routes on the indole ring forming covalently bonded dimers. The dimers were observed to aggregate via strong π stacking interactions forming cylindrical shapes of 36 Å in length supported by *ab initio* calculations, FTIR and SAXS measurements. These findings conclusively confirm that covalently bonded dimers of DHI are the essential building blocks of PDA. Previously proposed models of PDA's structure can be ruled out, suggesting that unique adhesive properties of PDA stem from strong π stacking forces of the indole moiety. Ionic liquids containing basic anions have demonstrated their ability to initiate and slow down the polymerization of dopamine, thus opening a new chapter for discovery of polymerisation pathways of catecholamines and enabling the synthesis of organic surface coatings with superior properties.

Main text

PDA is one of the most researched bio-mimetic polymers that was originally envisioned to mimic strongly adhesive properties of proteins found in mussel foot pads.^{2,5} PDA is easily produced through polymerization of dopamine (DA), a neurotransmitter belonging to the catecholamine family, in slightly basic aqueous solutions. The polymerisation is sporadic, leading to PDA's precipitation in the form of a black powder, which is completely insoluble in all traditional aqueous and organic solvents. Despite numerous studies, the mechanism of PDA's polymerisation is largely unresolved because the structure resolution of insoluble PDA precipitate is hindered by the lack of concrete experimental evidence.^{10–16} Recently, Singh *et al.* used 1-ethyl-3-imidazolium acetate IL, [C₂mim][OAc], to not only solvate PDA but also demonstrate evidence of polymerisation of several catecholamines, including DA,^{8,9} suggesting that select ILs may be used for *in situ* reaction monitoring of polymerisation of DA in liquid state via ¹H NMR spectroscopy. Rapid autoxidation of DA was confirmed to form the first building block of the resulting PDA identified as 5,6-dihydroxyindole

(DHI).¹⁷ The polymerisation mechanism is proposed to undergo several 2-electron, 2-proton oxidation steps.¹⁵ (Figure 1).

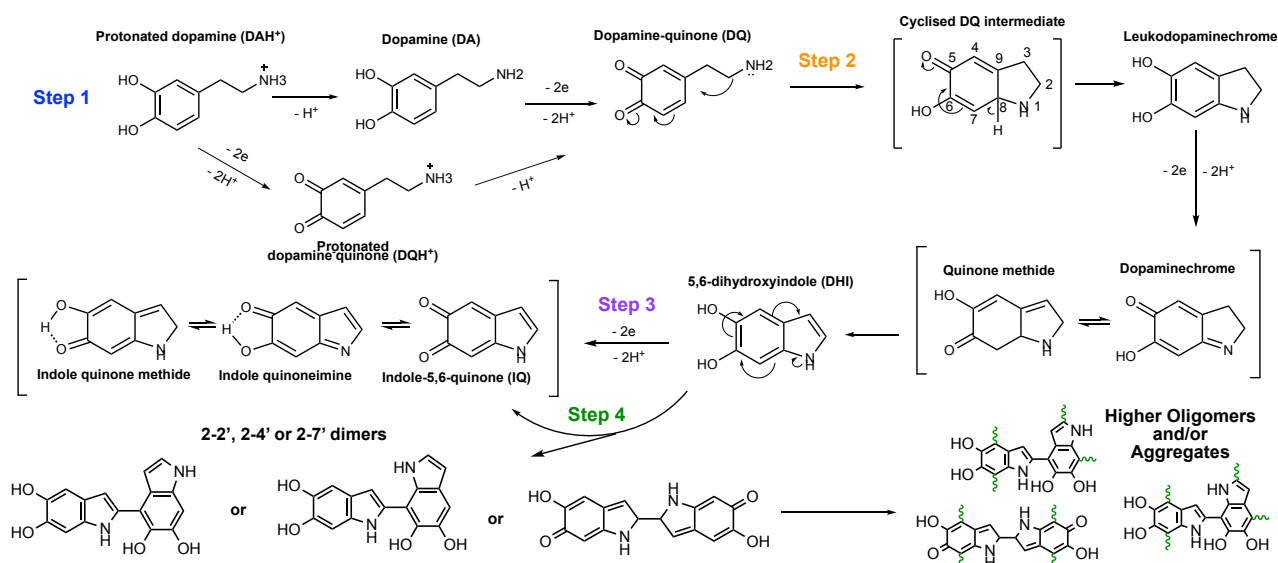


Figure 1: Proposed mechanism of potential pathways of dopamine's polymerisation. The four steps marked Step 1, Step 2, Step 3 and Step 4 are thoroughly studied in this work.

Since DA is used as a hydrochloride salt,¹⁸ its protonated form (DAH^+) dominates aqueous solutions below pH of 8.^{19,20} Before intramolecular cyclisation of DA, the deprotonation of the ethylammonium group in DAH^+ is followed by the oxidation of DA into dopamine-quinone (DQ)^{10,21} Several kinetic studies suggest that cyclisation is the rate-limiting step at pH below 5,^{16,22} but proceeds rapidly at pH above 7,²² not involving any radical species.¹² Although the formation of DHI was experimentally confirmed,²³ other possible steps of polymerisation could not be conclusively identified due to rapid precipitation of insoluble PDA. In this study we hypothesized that polymerisation of DA proceeds via four steps (Figure 1). The formed DHI undergoes autooxidation to generate indole-5,6-quinone (IQ), with DHI and IQ dimerising via the three possible routes: 2-2', 2-4' and 2-7', thus forming covalent dimers that continue polymerisation via the 4 and 7 positions of the catechol moiety, potentially forming higher order oligomers and eventually aggregates (Figure 1). To detect the formation of 2-2', 2-4' and 2-7' dimers and higher-order oligomers, solution-phase polymerisation of dopamine was monitored via ^1H NMR spectroscopy in nine ILs consisting of combinations of two cations (C_2mim^+

and $C_4\text{mpyr}^+$) and six anions (OAc^- , $\text{N}(\text{CN})_2^-$, OTf^- , HCOO^- , $\text{PO}_2(\text{OEt})_2^-$ and Cl^-). Out of these ILs, only three - $[C_4\text{mpyr}][\text{OAc}]$, $[C_2\text{mim}][\text{OAc}]$ and $[C_2\text{mim}][\text{HCOO}]$ - led to polymerization of DA and formed soluble PDA (Figure S1). Reaction monitoring (Figure 2) by ^1H -NMR measurements in tandem with DFT calculations (Figure 3) were used to investigate each of the four proposed steps leading to the formation of PDA (Figure 1).

Step 1: Deprotonation of dopamine and dopamine-quinone. Evidence of DA's cyclisation followed by polymerization was confirmed after 72 hours of reaction time at 70 °C by the disappearance of the neat DA signals in the aliphatic (the ethylamino group) and aromatic (the catechol moiety) regions in ^1H NMR spectra as recorded in DMSO-d_6 (Figure S1). $[C_2\text{mim}][\text{OTf}]$, $[C_4\text{mpyr}][\text{OTf}]$, $[C_2\text{mim}][\text{PO}_2(\text{OEt})_2]$ and $[C_2\text{mim}][\text{Cl}]$ did not facilitate the DA polymerisation. Although a dark brown or black colour was observed in all other ILs, only in ILs with the most basic anions such as $[C_2\text{mim}][\text{OAc}]$, $[C_2\text{mim}][\text{HCOO}]$ and $[C_4\text{mpyr}][\text{OAc}]$, do NMR signals characteristic of DA completely disappear. Several new proton environments appear in both acetate-based ILs, whereas the lack of similar peaks in $[C_2\text{mim}][\text{HCOO}]$ suggests advanced aggregation resulting in their broadening and subsequent disappearance, typical in large molecular assemblies such as proteins.^{24,25} Loss of distinct proton environments is also observed in both acetate based ILs after several days of polymerisation. The interaction energy of $[C_2\text{mim}][\text{HCOO}]$ with IQ was previously calculated to be weaker in comparison, suggesting a reduced ability to prevent aggregation.²⁶ Interestingly, the two standout doublets at 6.44 and 6.49 ppm recorded in $[C_2\text{mim}][\text{OAc}]$ and $[C_2\text{mim}][\text{HCOO}]$, appear to grow at the same rate as a quartet (2H, 3.62 ppm), singlet (3H, 3.21 ppm) and triplet (3H, 1.24 ppm) in the aliphatic regions (Figure S2-3). These can be attributed to the side reaction with $C_2\text{mim}^+$. Although imidazolium cations have a pK_a of ~ 22 , N-heterocyclic carbene (NHC) species can form spontaneously in imidazolium acetate ILs due to the proton transfer (PT) reaction between the cation and anion.^{27,28} NHCs have a short lifetime in ILs due to their high reactivity as confirmed in computational studies.^{29,30} The work by Stoppelman *et al.* suggested that the $C_2\text{mim}$ carbene could

exist at ppm concentrations in $[C_2mim][OAc]$.³¹ We also confirmed carbene formation using CO_2 addition experiments (Figure S4). Anion basicity appears to offer control in DA's polymerisation by facilitating the deprotonation of the ethylammonium group. In this study, DFT calculations performed on clusters of dopamine with a single ion pair (IP) of ILs (Figure S5 & Tables S1-5) showed that the PT from the NH_3^+ group could indeed occur in $[C_2mim][OAc]$ and $[C_4mpyr][OAc]$, requiring as little as +9.0 and +2.6 kJ mol⁻¹ of energy, respectively (Figure 3 – Step 1). In $[C_2mim][HCOO]$, the PT process could only be facilitated by the IP after the oxidation of DAH^+ to DQH^+ as suggested in previous reports.^{11,13} These results further highlight that the IL can promote the PT in DAH^+ in a similar fashion to water, thus priming DA for autooxidation to DQ and subsequent cyclisation.

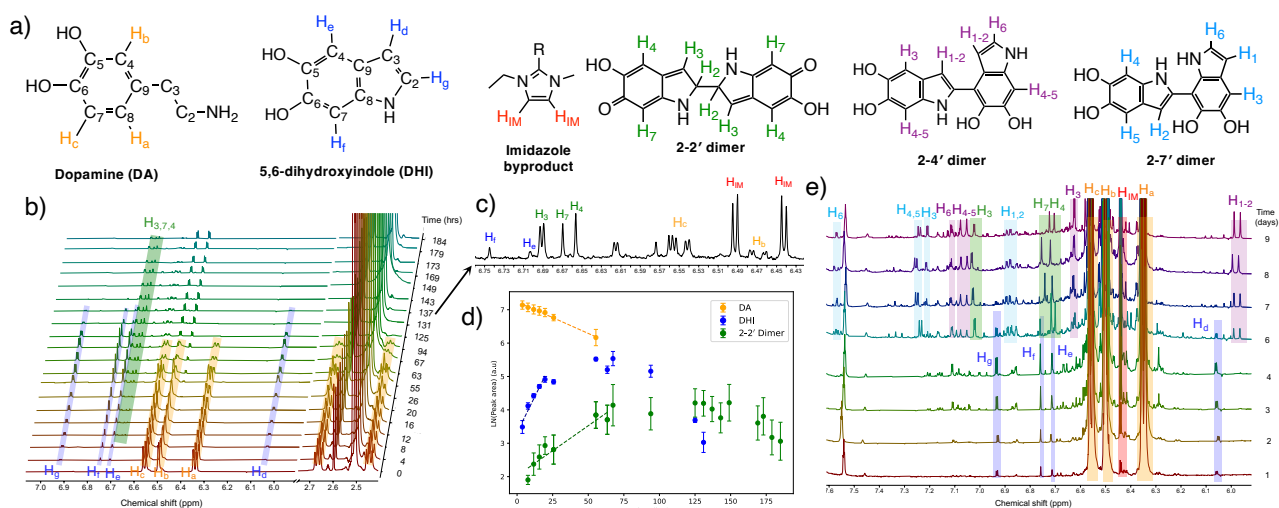


Figure 2: Summary of the liquid-state ^1H NMR spectra studies in this work. Highlighted areas and coloured proton assignments denote the following species: orange = DA; dark blue = DHI; red = C_2mim -based by-product; green = 2-2' dimer; purple = 2-4' dimer; light blue = 2-7' dimer. **a)** Molecular structures labelled with their relative proton environments observed in the ^1H NMR spectra. **b)** Full stacked spectrum of *in-situ*, RT ^1H NMR reaction monitoring of DA in $[C_2mim][OAc]$ with the **c)** zoomed aromatic region and resultant proton assignments after 137 hours. **d)** Natural logarithm of a peak area vs. time for peaks in *in situ* spectrum associated with DA ($R^2 = 0.9923$), DHI ($R^2 = 0.9492$), and the 2-2' covalent dimers ($R^2 = 0.8945$). Errors correspond to one standard deviation from the average normalized peak area for each proton signal. **e)** Zoomed aromatic region of the *ex-situ* ^1H

NMR reaction monitoring (80 °C) of 160 mg/mL dopamine polymerization in [C₂mim][OAc] at over 9 days.

Step 2: Formation of 5,6-dihydroxyindole. The cyclisation of DQ can be split into two steps: formation of the cyclised DQ intermediate via an intramolecular Michael addition, followed by subsequent rearrangements to form leukodopaminechrome (LDC) and finally, DHI (Figure 1). Cyclisation is suggested to be the rate-limiting step¹⁰ proceeding quickly under basic conditions²² and is accompanied by removal of a proton from the amine group. To create LDC from the cyclised DQ intermediate, a proton must be removed from the C8 carbon. Umek *et al.* calculated the activation-free energy of the cyclisation of DA to be 87.9 kJ mol⁻¹ using the hydroxyl anion as an explicit proton acceptor.¹⁴ Even without the inclusion of any explicit proton acceptor, our DFT calculations predicted the cyclisation of DQ to have a barrier of 164.0 kJ mol⁻¹, thus confirming it as the rate-determining step (Figure S6-7, Table S6), which can be overcome at elevated temperatures. Inclusion of an explicit acetate anion in the cyclised DQ system in DFT calculations following the cyclisation leads to a barrierless PT from the amine group to the acetate (Figure S8). Autoxidation of LDC to DHI (Figure 1) is assumed to be rapid and therefore, it is highly unlikely that any other species apart from DHI could be conclusively observed in the initial stages of DA polymerisation.

In our work, the formation of DHI was conclusively confirmed with *in situ* solution-phase ¹H NMR measurements (Figure 2). The use of ILs as a reaction medium allowed us to significantly slow down polymerisation from mere minutes in aqueous solutions to hours in ILs through lowering reaction temperatures. We ran a series of DA polymerisation in two ionic liquids, [C₂mim][OAc] and [C₄mpyr][OAc], and analysed the appearance of DHI and LDC *in-situ* ¹H NMR spectra in intervals of four hours (Figure 2). Results for [C₄mpyr][OAc] can be found in Figure S9. Analysis of the ¹H-NMR spectra in Figure 2b clearly shows evidence of DHI forming rapidly, in agreement with previously published results.³² The LDC intermediate was not detected in the spectra (absence of two distinct

aliphatic peaks in the region of 2.5 to 3.5 ppm), thus suggesting that once the cyclisation of DA is complete, the formation of DHI is almost instantaneous.

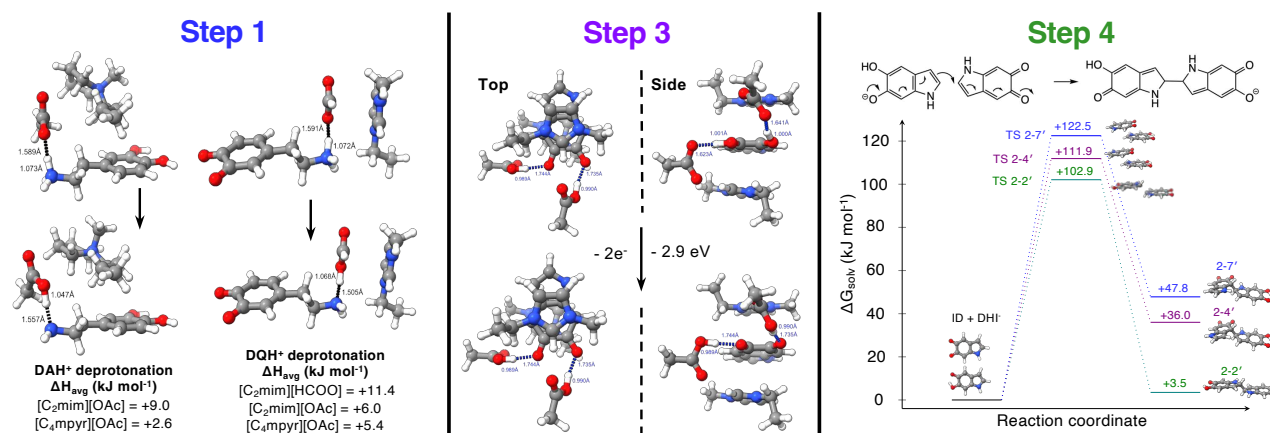


Figure 3: Summary of DFT calculations presented in this work. **Step 1:** Examples of optimized structures (before and after the PT) of protonated DAH⁺ with a single IP of [C₄mpyr][OAc] (configuration 1) and protonated dopamine-quinone (DQH⁺) with a single IP of [C₂mim][OAc] (configuration 2). Systems successful deprotonation in DA and DQ are also noted with their relative ΔH_{avg} energies (kJ mol⁻¹) noted. **Step 3:** Example of an optimised DHI system with PT to two formate anions in [C₂mim][HCOO] (stacked configuration), following the removal of two electrons. **Step 4:** Free energy barriers of the dimerization of deprotonated 5,6-dihydroxyindole (DHI⁻) and indole-5,6-quinone (IQ).

Step 3: Oxidation of 5,6-dihydroxyindole. As two DHI monomers are required for the formation of dimers, the two-electron, two-proton oxidation of DHI to indole-5,6-quinone (IQ) (Figure S10), the most stable quinone tautomer,^{33,34} is perhaps the most important next step in the formation of PDA. It enables dimerization of DHI and IQ to form covalent dimers identified as the predominant component of PDA.⁹ For the catechol moiety in DHI to be oxidised, the protons on both hydroxyl groups must be abstracted by a proton acceptor, the IL anion in this study. It is well known that the autoxidation of DA requires an oxidant to proceed^{12,16} and oxygen species are suggested to assume this role when performing polymerisation in open air.^{10,12,35} We confirmed that under an N₂ atmosphere in deoxygenated [C₂mim][OAc], DA polymerisation did not occur even after 72 hours at 80 °C (Figure

S11). After exposing the medium to air for 24 hours, the characteristic dark brown colour was observed, thus suggesting that an external oxidant such as oxygen must be present to initiate the polymerisation of dopamine in ILs. A similar observation was reported by Carra *et al.* studying the oxidation of tyrosine by Os(III)bpy₃³⁺, which was accelerated by proton-acceptor bases such as OAc⁻ and Tris.³⁶ In this multi-site proton-coupled electron transfer reaction, the electron removal by superoxide was confirmed to drive the deprotonation of catechols in ILs.^{37,38}

To model Step 3 computationally, deprotonation of DHI was examined using stacked and non-stacked clusters consisting of two IPs of [C₂mim][OAc] and [C₂mim][HCOO] (Figure 3 - Step 3 and Figures S12-S14). Without electron removal, neither single nor double PT processes were located. To simulate the two-electron removal, the optimized geometry of each cluster was assumed to have an overall charge of +2. A double PT from DHI to two IL anions was found in all clusters, demonstrating the barrierless nature of DHI's deprotonation in these ILs. The process was thermodynamically driven releasing 2.78 eV of energy in [C₂mim][HCOO] and 2.91 eV in [C₂mim][OAc] (Table S7-8). For comparison, when two IPs were replaced with two H₂O molecules to mimic conventional polymerization of DA (Figure S15), removal of two electrons produced PT for only one water molecule requiring an energy of 10.52 eV. It appears that ILs lower the energy requirements for the PT to produce IQ, thus facilitating the formation of covalent dimers.

Step 4: Formation of covalent dimers and higher order structures. The final step in the puzzle of DA polymerisation is determining the final composition of PDA. Previously, Singh *et al.* observed the presence of 2-2' dimers in PDA polymerised in aqueous Tris-buffer solution.⁹ Along with 2-2' dimers, there is evidence to suggest that 2-4' and 2-7' dimers may also exist within PDA.^{39,40} We confirmed the possible formation of these three 2-2', 2-4', and 2-7' covalent dimers with DFT calculations (Figure 3 – Step 4 and Table S9), with the 2-2' covalent dimer being identified as the most kinetically and thermodynamically preferred dimer due to the least extent of steric hindrance.

Polymerisation was monitored over a period of 184 hours in 4 hour intervals at room temperature using the *in situ* ^1H NMR monitoring method (see Methods) at 70 °C in $[\text{C}_2\text{mim}][\text{OAc}]$ to further slow down the polymerisation rate and detect covalent dimers.⁸ The resultant full stacked spectra (Figure 2b) show the polymerisation beginning with DA (highlighted in orange), with its intensity of both aromatic and aliphatic peaks decreasing at 100 hours. Results for $[\text{C}_4\text{mpyr}][\text{OAc}]$ can be found in Figure S7. The four-characteristic aromatic DHI peaks (highlighted in blue) begin to emerge at four hours and continue to grow up to the 70-hour mark, before disappearing over time. There are no new peaks observed in the aliphatic region, suggesting that any saturated intermediates (like LDC) are rapidly oxidised to DHI. A new set of peaks consisting of 2 singlets (6.66 and 6.67 ppm) and a doublet (6.69 ppm) (highlighted in green) begin to form at the 12-hour mark. After 137 hours, these peaks are more resolved (Figure 2c) and attributed to dimerization. The doublet (6.69 ppm) is assigned to the H3 protons due to coupling to their relative H2 protons in the 2-2' dimer, whilst the singlets belong to H4 and H7 protons.

The peak area of each proton environment was then tracked throughout the polymerisation and averaged throughout each species. Figure 2d shows the natural logarithm of a peak area vs time graph for dopamine, DHI, and the 2-2' covalent dimer (for more detail see Figure S16-23). The rate of DA consumption and DHI production follows first-order kinetics in first 20 hours with an R^2 of 0.9923 and 0.9492, respectively. A first-order kinetic growth is also observed for the 2-2' covalent dimer over the first 75 hours ($R^2 = 0.8945$), with both DHI and the 2-2' covalent dimer gradually decreasing over time. After 200 hours, all well-defined PDA peaks disappear due to signal broadening, which is often observed in ^1H NMR spectra of aggregated molecular species.^{24,41} In PDA, the most obvious cause is aggregation of DHI and covalent dimers, supported by previous calculations showing that clusters of stacked monomeric DHI and IQ units had an interaction energy of -500 kJ mol^{-1} due to strong π stacking.⁹

Due to a reduced probability of the formation of the 2-4' and 2-7' dimers compared to that of the 2-2' dimer, the *ex situ* 80 °C ¹H NMR monitoring method was employed to improve resolution of the proton environment (Figure 2e, for higher resolution Figure S24-25). Figure 2e begins at the point when only DA and DHI (and trace amounts of the imidazole by-products) are present in the sample at day 1. Over the course of 59 hours, several new proton environments emerge. Due to overlap of dimers peaks, their identification relied on the comparison of the rate of growth. Standalone peaks with the greatest resolution were selected and their integration over time was tracked (Figure S26, Table S10-12). A heat map of Pearson correlation coefficients (R^2) between each peak was generated to ascertain any similarities regarding their rate of growth, and the resulting number of peaks with R^2 above specific thresholds was identified (Figure S27, Table S13). This analysis highlighted two more sets of correlated peaks - shown as purple and light blue regions (Figure 2e). Using the threshold for an R^2 at 0.90, a strong correlation was found between three 2H signals: two singlets at 6.70 and 6.73 ppm, and a doublet ($J = 2.2$ Hz) at 7.02 ppm (green set), matching the pattern for the 2-2' dimer. The remaining peaks in the set (highlighted light blue) include four doublets at 6.88, 6.89, 7.21, and 7.57 ppm ($J = 1.8$ -2.2 Hz), and two very close peaks at 7.26 and 7.27 ppm, consisting of a singlet and a doublet ($J = 0.8$ Hz), respectively, closely matching the predicted splitting pattern for the 2-7' dimer (see Table S13). The purple set included the four 1H singlets at 5.97, 5.99, 7.05 and 7.08 ppm, a 2H (due to signal overlap) doublet at 6.62 ppm ($J = 2.2$ Hz), and a 1H doublet at 7.13 ppm ($J = 2.3$ Hz), closely matching the splitting pattern in the 2-4' dimer. The two upfield singlets (5.97 and 5.99 ppm) located in a similar region as the DHI's peak at 6.04 ppm (H_d proton) suggest that can be assigned to the 2-4' H_{1-2} protons. In theory, this would result in one of the peaks becoming a doublet due to coupling to the neighbouring H_6 proton. The lack of coupling could indicate abstraction of the H_6 proton due to the formation of higher-order oligomers. However, at this resolution, doublets with such small coupling constants can be difficult to discern.

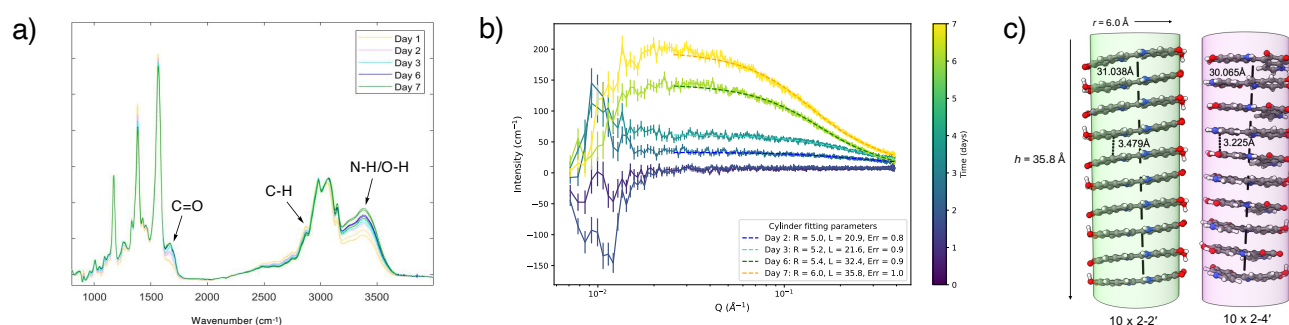


Figure 4: a) FTIR and b) SAXS spectra obtained in the first seven days of dopamine polymerization in [C₂mim][OAc] (80 mg/mL). c) Cylinder ($r = 6.0 \text{ \AA}$, $h = 35.8 \text{ \AA}$) overlaid on FMO-optimized theoretical aggregates consisting of 10 stacked 2-2' (green) and 2-4' (pink) dimers. Displayed full cluster height ($30.65 \text{ \AA}$) is measured between C2 positions of the top and bottom dimer, whilst separation between the 4th and 5th dimer ($3.23 \text{ \AA}$) is measured between C2 and C2 positions (2-2') C8 and C5 positions (2-4'), respectively.

The formation of higher-order oligomers was impossible to detect in ¹H NMR due to broadening of the signals associated with polymerized intermediates and products and their subsequent disappearance, suggesting aggregation.

To observe the nature and shape of aggregated species, FTIR and SAXS measurements were performed (Figure 4 and Figure S28-29). The FTIR results revealed an increase in signal intensity of the C=O stretch (1680 cm^{-1}), N-H stretch (3150 cm^{-1}) and O-H stretch (3383 cm^{-1}), but a decrease in the C-H stretch (2869 cm^{-1}). To put these results into the context of polymerisation, the intensity of the C-H stretches decreases with every step of the polymerisation route due to cyclisation of DA and a series of subsequent deprotonation steps (Figure 1 and Figure S30-32). There is a clear relationship between the rate of growth of C=O and O-H stretch intensities (Figure 4) and the extent of polymerisation, with dimerization of DHI (two hydroxyl groups) and IQ (two C=O groups) dominating the reaction space. The band positions of the C=O and O-H stretches do not shift position over time, suggesting the formation of stacked aggregates. Our previous work found that π stacked interactions between DHI and IQ monomers were preferred by 60 kJ mol^{-1} compared to in-plane hydrogen bonding

between adjacent carbonyl (IQ) and hydroxyl (DHI) groups.⁹ Since in-plane hydrogen bonding would undoubtedly result in the weakening of the O-H bond, causing a red shift,⁴² the formation of hydrogen bonds within the aggregates of PDA seems unlikely, thus favouring the stacking mechanism.

The SAXS traces for days 0 and 1 of the polymerization exhibit essentially no change in scattering intensity as a function of scattering vector Q from neat [C₂mim][OAc]. From day 2 onwards when covalent dimers start to form, a cylindrical model provides the best fit for the SAXS data (based on errors from a reduced χ -squared test) in comparison to a sphere or ellipsoid (Table S14). The parameters from the cylindrical fit indicate that stacks of approximately 5-7 Å in radius increase in height until they reach 35.8 Å by day seven, thus further suggesting that covalent dimers are very likely to engage in π stacking as they are formed. Our high-level quantum chemical calculations support this finding through the prediction of thermodynamically stable structures of both 10 stacked 2-2' and 2-4' covalent dimers (Figure 4c and Figure S33). These aggregates measure 6.3 and 6.1 Å in radius, 31.9 and 31.1 Å in height, respectively, whilst both clusters have an average distance of 3.5 Å between dimer centre of masses (Table S15-16). This does not rule out the possibility of forming high-order oligomers including trimers, although their concentration is expected to be low.

The use of ILs allowed us to perform *in situ* reaction monitoring of DA's polymerization via ¹H NMR spectroscopy and thereby identify the formation of three types of covalent dimers for the first time. Based on the presented results we can confidently conclude that the polymerisation mechanism of DA in ILs follows the four steps outlined in Figure 1. The initial cyclisation of DA was confirmed to be the rate-determining step in the whole polymerisation mechanism. Cyclisation is rapidly followed by the formation of DHI, the prerequisite for the formation of covalent dimers. Autoxidation of DHI in the presence of oxygen in air produces indole-5,6-quinone, thus facilitating its dimerization with DHI to form covalent dimers via the first-order kinetics and the three possible routes: 2-2', 2-4' and 2-7', whose presence was confirmed by ¹H NMR measurements. As their combined concentration accumulates in the reaction medium, they appear to undergo aggregation via π stacking, resulting in

the broadening of the ^1H NMR signals and their complete disappearance over the course of reaction. The competing aggregation slows down the rate of production of both DHI monomers and covalent dimers. When the reactants are fully consumed (DA and DHI), aggregation completely overtakes any other potential reaction, and the peak area decreases rapidly. FTIR and SAXS data suggest that aggregation of covalent dimers and monomers is the dominant process, resulting in the formation of aggregates best described via long cylindrical shapes. Higher-order oligomers may still be formed even after the aggregation process starts to dominate the dispersion solution. Due to the broadening of ^1H NMR signals these could not be observed.

This report has demonstrated that PDA polymerised in ILs predominantly consists of aggregated covalent dimers. Crucially, all three dimer types could be conclusively detected. The presented findings finally clarify the structure of PDA that has puzzled the scientific community for over 20 years. The control that ILs provide in slowing down polymerization and maintaining polymerised products in soluble form opens exciting new avenues for discovery of organic coatings based on catecholamines using a very simple one-pot synthetic approach. Chemical modification of coatings can be achieved by incorporating catecholamines of varying functionality from hydrophobic, hydrophilic to other specific functional groups in the same polymer without the need to apply any expensive kinetic or functional group protection tricks. PDA has already demonstrated its strong adhesive and antimicrobial properties. This report will enable the synthesis of myriad catecholamine-based coatings with superior properties for applications ranging from coated valves for medical surgeries to electrodes in organic batteries.

Methods

General procedures. Dopamine hydrochloride and $[\text{C}_2\text{mim}][\text{OAc}]$ (> 95%) was purchased from Sigma-Aldrich. All other ILs; $[\text{C}_2\text{mim}][\text{Cl}]$ (> 95%), $[\text{C}_2\text{mim}][\text{HCOO}]$ (97%) $[\text{C}_2\text{mim}][\text{N}(\text{CN})_2]\}$ (> 98%), $[\text{C}_2\text{mim}][\text{PO}_2(\text{OMe})_2]$ (> 98%), $[\text{C}_2\text{mim}][\text{PO}_2(\text{OEt})_2]$ (> 98%), $[\text{C}_2\text{mim}][\text{OTf}]$ (99%),

[C₄mpyr][OAc] (> 97%), [C₄mpyr][OTf] (99%) and [C₄mpyr][N(CN)₂] (> 98%); were purchased from IoLiTec. Nuclear magnetic resonance spectra were collected using a Bruker AVIII 600 MHz (with a TCI cryoprobe) or Bruker Neo 400 MHz, with signals reported in ppm and referenced against TMS. A Bruker NanoSTAR II small angle X-ray scattering (SAXS) instrument was used for SAXS measurements. The instrument was equipped with a copper rotating anode (0.3 × 3 cm filament) operating at 45 keV and 110 mA, generating Cu K α radiation with a wavelength of 1.54 Å. The incident beam was focused by Montel single-bounce multilayer optics to achieve low divergence (<1 mrad). The beam was further collimated using two pinholes of 0.55 mm diameter. SAXS data were collected with a Vantec 2000 2D detector with a pixel size of 68×68 μm^2 and an active area of 140×140 mm². The detector was located 720 mm away from the sample, allowing for simultaneous scattering vector (Q) range of $0.01 < Q < 0.35 \text{ \AA}^{-1}$. Detector images were reduced under the assumption of radially anisotropic scattering to 1D plots for model fitting. Model fits were generated using the SASView V. 6.0 software. ATR-FTIR data was collected using Bruker Alpha II spectrometer, equipped with an Attenuated Total Reflection (ATR) sampling device, a single bounce diamond internal reflection element, a globar source, KBr beam splitter and a deuterated triglycine sulphate detector. All spectra were collected with a spectral resolution 4 cm⁻¹ and in the spectral range of 4000-900 cm⁻¹. 64 scans were co-averaged for the background and sample, with background collected every 3 measurements. For each sample, spectra of 3 technical replicates were acquired and averaged.

Polymerisation of dopamine in ionic liquids (Step 1). 10 mg of dopamine hydrochloride was dissolved in 1 mL of IL ([C₂mim][OAc], [C₂mim][HCOO], [C₂mim][OTf], [C₂mim][Cl], [C₂mim][PO₂(OEt)₂], [C₂mim][N(CN)₂], [C₄mpyr][N(CN)₂], [C₄mpyr][OAc] and [C₄mpyr][OTf]) and left heating at 70 °C, uncovered for 72 hours. ¹H NMR spectra were always recorded using 20 μL of IL solution with 580 μL DMSO-d₆ as the deuterated solvent and processed using MestReNova. For more detail into the ratio see Reaction Monitoring below.

Table 1: Polymerization procedures for each experiment in this work.

Experiment	Ionic Liquid	DA/IL Concentration (mg/mL)	Temperature (°C)	Time taken
Step 1: Polymerisation of DA in ILs	[C ₂ mim][OAc], [C ₂ mim][HCOO], [C ₂ mim][OTf], [C ₂ mim][Cl], [C ₂ mim][PO ₂ (OEt) ₂], [C ₂ mim][N(CN) ₂], [C ₄ mpyr][N(CN) ₂], [C ₄ mpyr][OAc], [C ₄ mpyr][OTf]	10	70	72 hours
<i>In situ</i> reaction monitoring	[C ₂ mim][OAc] (Figure 2a-d), [C ₄ mpyr][OAc] (Figure S9)	80	RT	184 hours; 108 hours
<i>Ex situ</i> reaction monitoring	[C ₂ mim][OAc]	80 (Figure S37-38) 160 (Figure 2e) 250 (Figure S39-40)	80	9 days; 9 days; 6 days
CO ₂ absorption (Figure S4)	[C ₂ mim][OAc]	80	80	6 days
Polymerisation under N ₂ atmosphere (Figure S11)	[C ₂ mim][OAc]	25	70	4 days
FTIR and SAXS measurements (Figure 4)	[C ₂ mim][OAc]	80	100	7 days

Absorption of CO₂ in PDA/[C₂mim][OAc] and neat [C₂mim][OAc]. 1 g of dry ice was added to 200 μ L of either neat [C₂mim][OAc], or 80 mg/mL DA/[C₂mim][OAc] solution (polymerised at 80 °C for 6 days). A lid was placed on the vial and removed after complete sublimation (30 minutes). In both cases, when CO₂ was added (as a pellet of dry ice) to both PDA/[C₂mim][OAc] (80 mg/mL polymerised at 80 °C for 14 days) and neat [C₂mim][OAc], resonance signals matching Seo et al.'s⁴³ were formed (Figure S4). This did not affect the intensity of the two doublets at 6.44 and 6.49 ppm, suggesting they are not simply a product of leaving the polymerisation open to the atmosphere.

Reaction Monitoring. Recording of ¹H NMR spectra during reaction monitoring in ILs requires a detailed discussion of several important factors that were considered when designing such experiments to achieve clean, high-quality spectra. Firstly, ILs are usually very viscous and produce very intense peaks in ¹H NMR. Because of their high concentration in mixtures with DA, small peaks associated with DA's chemical transformation are hard to detect, even when shimming techniques are applied. In

this study, we used a standard approach of measuring ^1H NMR spectra by combining the IL with a deuterated solvent such as DMSO- d_6 . This polar solvent was previously found to be well miscible with ILs^{44,45} and able to prevent aggregation of polymerisation intermediates and products.⁹ At every time interval an aliquot of 20 μL of the polymerization mixture was combined with DMSO- d_6 to record a ^1H NMR spectrum. It has to be noted that polymerization of DA does not proceed in neat DMSO.⁸ In our results, we noticed that the ^1H NMR spectrum of a mixture of $[\text{C}_2\text{mim}][\text{OAc}]$ shows a single broad signal that shifts downfield (by up to 4 ppm) when increasing the amount of $[\text{C}_2\text{mim}][\text{OAc}]$ (FigureS35). Zhang *et al.* saw the same effect in mixtures of $[\text{C}_2\text{mim}][\text{OAc}]$ and a cellulose/DMSO- d_6 solution, and consequentially suggested it was due to the rapid exchange between cellulose hydroxyls and imidazolium's acidic proton.⁴⁶ We established a linear relationship between the position of this broad peak and $\%(\text{v/v})$ IL (Figure S36), thus identifying the ideal ratio of IL to DMSO- d_6 (20 μL $[\text{C}_2\text{mim}][\text{OAc}]$ to 580 μL DMSO- d_6 for the constant total volume of 600 μL) to move this peak to 4.05 ppm to avoid overlap with other signals of interest in aromatic region between 6 and 8 ppm.⁴⁷ Secondly, due to the intensity of the IL signals in ^1H NMR spectra, the quality of the signals of the polymerisation products is extremely sensitive to DA's concentration. It was found that the optimal concentration was in the range of 10 to 160 mg/mL (on a 600 MHz magnet), as this produced better quality signals that can be tracked over a reasonable time frame (and further improved with an increased scan rate). Higher concentrations of DA were found to decrease the rate of DA's cyclisation and reduce the quality of ^1H NMR spectra (see Figures S37-340). Finally, the rate of polymerisation is heavily affected by temperature, informing the number and frequency of time points.⁸ DA will not polymerise in an acetate-based IL at room temperature but will proceed slowly at 40 °C. Our previous work showed that at low concentrations (10mg/mL) 80 °C, the polymerisation can be completed in approximately 3 days. However, when mixed with DMSO, it is found that the polymerisation can proceed to a certain extent at room temperature. It is important to note that it does not proceed in neat DMSO alone, and any evidence of polymerisation is purely due to the interaction between DA and the

IL. Therefore, there are two ways that the reaction can be monitored: automatic sampling *in situ* at room temperature; or at an elevated temperature (at or above 70 °C), with intermittent manual (*ex situ*) sampling.

The automatic *in situ* method involves mixing 20 μL of the initial DA/IL (80 mg/mL) mixture with 580 μL DMSO- d_6 and allowing the polymerisation to proceed in the NMR tube. In contrast, the *ex situ* method allows the polymerisation to occur in neat IL (DA/IL 160 mg/mL), whilst 20 μL samples (then mixed with 580 μL DMSO- d_6) are intermittently taken to provide a snapshot of the system at the specific time point. Although aggregation is likely to be more prominent in the *in-situ* method due to the low fraction of IL to DMSO, it eliminates errors related to the sample variation. However, the *ex-situ* method offers better resolution of higher-order structures before aggregation. The polymerisation can also be ‘paused’ when refrigerated at approximately 4 °C.

To monitor with FTIR and SAXS, 160 mg of dopamine hydrochloride in 2 mL $[\text{C}_2\text{mim}][\text{OAc}]$ was polymerised at 80 °C for 7 days (uncovered) with 200 μL samples taken after 1, 2, 3, 6 and 7 days. Mass spectrometry proved ineffective in analyzing mixtures, even at low extents of polymerization, presumably due to the insolubility of even the dimers considered. The relative ^1H NMR for each sample can be found in Figure S28.

^1H NMR data. Dopamine in DMSO (Fig S34): ^1H NMR (600 MHz, DMSO- d_6) δ 8.84 (s, broad), 8.02 (s, broad), 6.68 (d, $J = 8.0$ Hz, 1H), 6.62 (d, $J = 2.1$ Hz, 1H), 6.48 (dd, $J = 8.0, 2.1$ Hz, 1H), 3.35 (s, 2H), 2.90 (t, $J = 7.5\text{-}8.4$ Hz, 2H), 2.69 (t, $J = 7.5\text{-}8.4$ Hz, 2H).

Ex situ 160 mg/mL dopamine in $[\text{C}_2\text{mim}][\text{OAc}]$ after 1 day at 80 °C (DA peaks): ^1H NMR (600 MHz, DMSO- d_6) δ 6.57 (d, $J = 7.9$ Hz, 1H), 6.51 (d, $J = 2.2$ Hz, 1H), 6.36 (dd, $J = 7.9, 2.2$ Hz, 1H), 2.69 (t, $J = 7.5\text{-}8.4$ Hz, 2H), 2.48 (t, $J = 7.5\text{-}8.4$ Hz, 2H).

Ex situ 160 mg/mL dopamine in $[\text{C}_2\text{mim}][\text{OAc}]$ after 1 day at 80 °C (DHI peaks): ^1H NMR (600 MHz, DMSO- d_6) δ 6.94 (d, $J = 3.0$ Hz, 1H), 6.77 (s, 1H), 6.72 (d, $J = 0.9$ Hz, 1H), 6.04 (dd, $J = 3.0, 0.9$ Hz, 1H).

Ex situ 160 mg/mL dopamine in [C₂mim][OAc] after 7 days at 80 °C (dimer peaks): ¹H NMR (600 MHz, DMSO-d₆) δ 7.58 (d, *J* = 1.8 Hz, 1H), 7.27 (d, *J* = 0.8 Hz, 1H), 7.26 (s, 1H), 7.22 (d, *J* = 2.1 Hz, 1H), 7.12 (d, *J* = 2.3 Hz, 1H), 7.09 (s, 1H), 7.06 (s, 1H), 7.04 (d, *J* = 2.2 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.89 (d, *J* = 2.2 Hz, 1H), 6.75 (s, 2H), 6.72 (d, *J* = 0.8 Hz, 2H), 6.64 (d, *J* = 2.2 Hz, 2H), 6.00 (s, 1H), 5.97 (s, 1H).

DFT calculations. Geometries of individual species (molecules and ions) were optimized at the ωB97XD⁴⁸/aug-cc-pVTZ^{49,50} level of theory using the Gaussian16 software package.⁵¹ Their structure was taken from previously found lowest energy conformations in our previous work.⁵²

Step 1: One IP clusters containing one cation (C₂mim⁺ or C₄mpyr⁺), one anion (OAc⁻, HCOO⁻, N(CN)₂⁻, or OTf⁻), and one protonated dopamine molecule were created in Avogadro.⁵³ In one configuration (labelled as configuration 1), the cation sat above the plane of the catechol ring, whilst the anion was positioned above the NH₃⁺ group (Figure S5a). The anion was kept in a similar position in the alternative configuration (labelled as configuration 2), but the cation was moved behind the anion, such that it did not interact with dopamine (Figure S5b). These structures were inspired by the lowest energy configurations found in single ion pairs of imidazolium and pyrrolidinium-based ionic liquids.⁵² These structures then underwent a geometry optimization at the ωB97XD/aug-cc-pVDZ with the SMD solvation model⁵⁴ and ethanol as solvent. Zero-point vibrational energies were calculated at the same level of theory, using the numerical frequencies in ORCA.⁵⁵ To simulate a PT (in DA and DHI systems), the proton was forced to form a 1.00 Å bond with the proton acceptor atom (on the anion), along the donor-proton-acceptor axis. These new structures underwent the same geometry optimization as the initial structure. Single point energies (*E_{elec}*) were calculated at the SRS-MP2⁵⁶/aug-cc-pVDZ level of theory and the SMD solvent model with ethanol as solvent. Deprotonation energies (ΔH) for each configuration were then calculated using the following equation:

$$\Delta H = (ZPVE + E_{elec})_{deprotonated} - (ZPVE + E_{elec})_{protonated}$$

Boltzmann weighting (298.15 K) was used to determine the final deprotonation energy ($\Delta H(\text{ave})$) using both energetically preferred configurations:

$$\Delta H(\text{ave}) = \frac{\sum_{i=1}^N \Delta E_{DP,i} \times e^{\frac{-\Delta E_{DP,i}}{RT}}}{\sum_{i=1}^N e^{\frac{-\Delta E_{DP,i}}{RT}}}$$

where N is the number of configurations (2) and i refers to the individual configuration.

Step 2: Gibbs free energy barriers were calculated using a similar methodology to our previous work.⁹ Both ground and transition state geometries (Figure S6-7) were optimised at the M06-2X⁵⁷/cc-pVDZ level of theory using an SMD ethanol solvation model in Gaussian16. Frequencies were also calculated at the same level of theory, from which enthalpies (H), entropies (S), thermal corrections (TC) and zero-point vibrational energies (ZPVE) could be extracted. Single point energies (E_{elec}) were then calculated at the ω B97XD/aug-cc-pVTZ level of theory, with the SMD solvation model corrections approximated using M05-2X⁵⁸/6-311+G**⁵⁹ and ethanol as solvent. Reaction barriers were calculated using the following equation:

$$\Delta H^\ddagger = (ZPVE + E_{\text{elec}} + TC)_{TS} - (ZPVE + E_{\text{elec}} + TC)_{\text{reactants}}$$

The total entropy (S^{Total}) of each molecule is given as a sum of electronic, vibrational, rotational and translational terms, and is used in the following equation to calculate entropic contributions:

$$\Delta S^\ddagger = S_{TS}^{\text{Total}} - \sum S_{\text{reactants}}^{\text{Total}}$$

The Gibbs free energy (at 298.15 K) was then calculated using the following equations:

$$\Delta G_{\text{solv}}^\ddagger = \Delta G_{\text{gas}}^\ddagger + \Delta \Delta G_{\text{solv}}$$

where

$$\Delta G_{\text{gas}}^\ddagger = \Delta H^\ddagger(\omega\text{B97XD/aug-cc-pVTZ}) - T\Delta S^\ddagger(\omega\text{B97XD/aug-cc-pVTZ})$$

and

$$\Delta \Delta G_{\text{solv}} = \Delta G_{\text{solv}}(\text{M05-2X/6-311+G**}) - \Delta G_{\text{gas}}(\text{M05-2X/6-311+G**})$$

The first part of this step investigated the barrier of the cyclization of DQ to produce the protonated cyclised DQ intermediate ($R_1\text{-NH}_2^+\text{-R}_2$ rather than $R_1\text{-NH- R}_2$). The removal of this proton from the cyclised DQ intermediate was investigated by introducing an acetate molecule. Geometry optimization of this cluster led to an immediate PT from the cyclised DQ intermediate to the acetate, indicating a barrierless PT (Figure S8).

Step 3: In both two IP systems, the anions were positioned to engage in hydrogen bonding with the hydroxyl groups on DHI. In the stacked configuration, the DHI molecule is sandwiched by the two imidazolium cations (Figure S13b), whereas the imidazolium cations are placed behind the anions (minimising interactions with DHI) in the non-stacked configuration (Figure S13c). These structures then underwent a geometry optimization at ω B97XD/aug-cc-pVDZ with the SMD solvation model⁵⁴ and ethanol as solvent. Zero-point vibrational energies were calculated at the same level of theory, using the numerical frequencies in ORCA.⁵⁵ In the two-electron removal in two ion-paired systems, the original two ion-paired geometry was simply re-optimised with a +2 charge and singlet multiplicity. Single point energies (E_{elec}) were calculated at the SRS-MP2⁵⁶/aug-cc-pVDZ level of theory and the SMD solvent model with ethanol as solvent. The energy required to remove the two electrons (ΔH_{2e} shown in Figure 3 – Step 2) was calculated using the following equation:

$$\Delta H_{2e} = (ZPVE + E_{elec})_{+2 \text{ charge}} - (ZPVE + E_{elec})_{0 \text{ charge}}$$

HOMO-LUMO gaps were taken from HF/cc-pVTZ single-point calculations performed on optimized geometries (Figure S14, Table S7). For the one-electron removal calculations, the single IP DHI clusters (Figure S13a) were re-optimised with a +1 charge and doublet multiplicity. As it is a radical state, the ω B97XD/aug-cc-pVQZ level of theory (with SMD ethanol solvation model) was used to calculate single point energies. The energy required to remove one electron (Table S8) was calculated using the following equation:

$$\Delta H_{1e} = (ZPVE + E_{elec})_{+1 \text{ charge}} - (ZPVE + E_{elec})_{0 \text{ charge}}$$

Step 4: Gibbs free energy barriers were calculated using the same methodology as Step 2. The relevant data can be found in Table S9.

Geometry optimization of 10-dimer aggregates:

For the geometry optimization of 10-dimer aggregates, the Fragment Molecular Orbital (FMO) method⁶⁰ was employed at the ω B97XD⁴⁸/cc-pVDZ⁴⁹ level of theory. Ionic liquid solvation was modelled implicitly with the conductor-like polarizable continuum model,⁵⁴ where ethanol was the chosen solvent to mimics the dielectric constant of ionic liquids.⁵² Each covalent dimer was considered to be a single fragment, with up to two-fragment effects were considered, otherwise referred to as FMO2. Distance cutoffs were set for two-fragment interactions, firstly by calculating the cartesian distance (r_{ij}) between atoms i and j , belonging to different fragments, scaled by their Van der Waals radii^{61,62} (R_{vdw})

$$R_{ij,vdw} = \frac{r_{ij}}{R_{i,vdw} + R_{j,vdw}}$$

After calculating $R_{ij,vdw}$ for all atom pairs between two fragments, if the minimum value was below 5, then the two-fragment interaction was calculated. This corresponds to about 14 Angstroms for the stacked dimers, meaning that each dimer had its interaction with its nearest 4 neighbours in both directions considered. All FMO calculations were performed with GAMESS⁶³ software, specifically the R2 version from 2019, using the RUNTYP=OPTFMO keyword. The SciPy⁶⁴ Python module was used to analyse cluster dimensions by computing the centre of mass of each dimeric fragment (Table S15-16).

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Author contributions.

All authors discussed the results and contributed to the final manuscript. S. A. C. designed the experimental work, carried out majority of the experimental work and data analysis and interpretation, performed majority of the quantum chemical calculations and wrote the original draft of the manuscript. W. D. carried out part of NMR experiments. P. H. performed large-scale FMO calculations. J. B. H. contributed to designing the project and supervision of the project. K. K. performed FTIR measurements and their analysis. R. N. R. performed SAXS experiments and their analysis. R. F. T. helped analyse SAXS experiments. A. M. provided technical support with NMR measurements. B. D. F. contributed to designing the project and supervision of the project. A. J. R. contributed to experimental data analysis and supervision of the project. E. I. I. conceived the project, supervised the project, and revised the manuscript.

Additional information. Supplementary Information is available for this paper. Correspondence and requests for materials should be addressed to katya.pas@monash.edu. Reprints and permissions information is available at www.nature.com/reprints.

Bibliography.

1. Hemmatpour, H. *et al.* New insights in polydopamine formation via surface adsorption. *Nat. Commun.* **14**, 664 (2023).
2. Ryu, J. H., Messersmith, P. B. & Lee, H. Polydopamine Surface Chemistry: A Decade of Discovery. *ACS Appl. Mater. Interfaces* **10**, 7523–7540 (2018).
3. Liebscher, J. Chemistry of Polydopamine – Scope, Variation, and Limitation. *Eur. J. Org. Chem.* **2019**, 4976–4994 (2019).

4. Liu, Y., Ai, K. & Lu, L. Polydopamine and Its Derivative Materials: Synthesis and Promising Applications in Energy, Environmental, and Biomedical Fields. *Chem Rev* **114**, 5057–5115 (2014).
5. Lee, H., Dellatore, S. M., Miller, W. M. & Messersmith, P. B. Mussel-Inspired Surface Chemistry for Multifunctional Coatings. *Science* **318**, 426–430 (2007).
6. Sahiner, N., Sagbas, S., Sahiner, M., Blake, D. A. & Reed, W. F. Polydopamine particles as nontoxic, blood compatible, antioxidant and drug delivery materials. *Colloids Surf. B Biointerfaces* **172**, 618–626 (2018).
7. Ball, V. Polydopamine films and particles with catalytic activity. *Catal. Today* **301**, 196–203 (2018).
8. Singh, A. *et al.* Ionic Liquid Facilitated Solvent-Phase Polymerization of UltrasMOOTH Coatings of Polycatecholamines. *Macromol Chem Phys* **223**, 2200313 (2022).
9. Singh, A. *et al.* Structural elucidation of polydopamine facilitated by ionic liquid solvation. *Phys Chem Chem Phys* (2023).
10. Tse, D. C., McCreery, R. L. & Adams, R. N. Potential oxidative pathways of brain catecholamines. *J Med Chem* **19**, 37–40 (1976).
11. Young, T. E. & Babbitt, B. W. Electrochemical study of the oxidation of .alpha.-methyldopamine, .alpha.-methylnoradrenaline, and dopamine. *J. Org. Chem.* **48**, 562–566 (1983).
12. Herlinger, E., Jameson, R. F. & Linert, W. Spontaneous autoxidation of dopamine. *J. Chem. Soc. Perkin Trans. 2* 259–263 (1995) doi:10.1039/P29950000259.
13. Hermida-Ameijeiras, Á., Méndez-Álvarez, E., Sánchez-Iglesias, S., Sanmartín-Suárez, C. & Soto-Otero, R. Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: role of ferrous and ferric ions. *Neurochem. Int.* **45**, 103–116 (2004).
14. Umek, N., Geršak, B., Vintar, N., Šoštarič, M. & Mavri, J. Dopamine Autoxidation Is Controlled by Acidic pH. *Front. Mol. Neurosci.* **11**, 467 (2018).

15. Lin, Q., Li, Q., Batchelor-McAuley, C. & Compton, R. G. Two-Electron, Two-Proton Oxidation of Catechol: Kinetics and Apparent Catalysis. *J. Phys. Chem. C* **119**, 1489–1495 (2015).
16. Salomäki, M., Marttila, L., Kivelä, H., Ouvinen, T. & Lukkari, J. Effects of pH and Oxidants on the First Steps of Polydopamine Formation: A Thermodynamic Approach. *J. Phys. Chem. B* **122**, 6314–6327 (2018).
17. Raper, H. S. The tyrosinase-tyrosine reaction: production from tyrosine of 5: 6-dihydroxyindole and 5: 6-dihydroxyindole-2-carboxylic acid—the precursors of melanin. *Biochem J* **21**, 89 (1927).
18. Cruickshank, L., Kennedy, A. R. & Shankland, N. Tautomeric and ionisation forms of dopamine and tyramine in the solid state. *J. Mol. Struct.* **1051**, 132–136 (2013).
19. Sánchez-Rivera, A. E. *et al.* Spectrophotometric study on the stability of dopamine and the determination of its acidity constants. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **59**, 3193–3203 (2003).
20. Corona-Avendaño, S. *et al.* New Insights on the Nature of the Chemical Species Involved during the Process of Dopamine Deprotonation in Aqueous Solution: Theoretical and Experimental Study. *J. Phys. Chem. B* **111**, 1640–1647 (2007).
21. Cooper, J. R., Bloom, F. E. & Roth, R. H. *The Biochemical Basis of Neuroparmacology*. (Oxford University Press, USA, 2003).
22. García-Moreno, M. *et al.* Effect of pH on the oxidation pathway of dopamine catalyzed by tyrosinase. *Arch. Biochem. Biophys.* **288**, 427–434 (1991).
23. Hong, S. *et al.* Non-Covalent Self-Assembly and Covalent Polymerization Co-Contribute to Polydopamine Formation. *Adv. Funct. Mater.* **22**, 4711–4717 (2012).
24. Foster, M. P., McElroy, C. A. & Amero, C. D. Solution NMR of large molecules and assemblies. *Biochemistry* **46**, 331–340 (2007).

25. Bellomo, G. *et al.* Aggregation kinetics of the A β 1–40 peptide monitored by NMR. *Chem. Commun.* **54**, 7601–7604 (2018).
26. Crouch, S. A., Mason, T. G., Freeman, B. D., Robinson, A. J. & Izgorodina, E. I. Understanding the aggregation properties of polydopamine with polarizable forcefield molecular dynamics. *ChemRxiv* **2026**,.
27. Amyes, T. L., Diver, S. T., Richard, J. P., Rivas, F. M. & Toth, K. Formation and stability of N-heterocyclic carbenes in water: the carbon acid p K a of imidazolium cations in aqueous solution. *J Am Chem Soc* **126**, 4366–4374 (2004).
28. Chiarotto, I., Feroci, M. & Inesi, A. First direct evidence of N-heterocyclic carbene in BMIm acetate ionic liquids. An electrochemical and chemical study on the role of temperature. *New J. Chem.* **41**, 7840–7843 (2017).
29. Holloczki, O. *et al.* Carbene formation in ionic liquids: spontaneous, induced, or prohibited? *J Phys Chem B* **117**, 5898–5907 (2013).
30. Salvitti, C. *et al.* Free N-heterocyclic carbenes from Brønsted acidic ionic liquids: Direct detection by electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **36**, e9338 (2022).
31. Stoppelman, J. P. & McDaniel, J. G. N-Heterocyclic Carbene Formation in the Ionic Liquid [EMIM+][OAc−]: Elucidating Solvation Effects with Reactive Molecular Dynamics Simulations. *J. Phys. Chem. B* **127**, 5317–5333 (2023).
32. Lyu, Q., Song, H., L. Yakovlev, N., Siew Tan, W. & L. Chai, C. L. In situ insights into the nanoscale deposition of 5,6-dihydroxyindole-based coatings and the implications on the underwater adhesion mechanism of polydopamine coatings. *RSC Adv.* **8**, 27695–27702 (2018).
33. díschia, M., Napolitano, A., Tsiakas, K. & Protá, G. New intermediates in the oxidative polymerisation of 5, 6-dihydroxyindole to melanin promoted by the peroxidase/H₂O₂ system. *Tetrahedron* **46**, 5789–5796 (1990).

34. Il'ichev, Y. V. & Simon, J. D. Building Blocks of Eumelanin: Relative Stability and Excitation Energies of Tautomers of 5,6-Dihydroxyindole and 5,6-Indolequinone. *J. Phys. Chem. B* **107**, 7162–7171 (2003).
35. Kalyanaraman, B., Felix, C. & Sealy, R. Semiquinone anion radicals of catechol (amine) s, catechol estrogens, and their metal ion complexes. *EHP* **64**, 185–198 (1985).
36. Carra, C., Iordanova, N. & Hammes-Schiffer, S. Proton-coupled electron transfer in a model for tyrosine oxidation in photosystem II. *J Am Chem Soc* **125**, 10429–10436 (2003).
37. Tyburski, R., Liu, T., Glover, S. D. & Hammarström, L. Proton-Coupled Electron Transfer Guidelines, Fair and Square. *J. Am. Chem. Soc.* **143**, 560–576 (2021).
38. Weinberg, D. R. *et al.* Proton-coupled electron transfer. *Chem. Rev.* **112**, 4016–4093 (2012).
39. Pezzella, A. *et al.* Short-lived quinonoid species from 5, 6-dihydroxyindole dimers en route to eumelanin polymers: integrated chemical, pulse radiolytic, and quantum mechanical investigation. *J Am Chem Soc* **128**, 15490–15498 (2006).
40. Pezzella, A. *et al.* 5,6-Dihydroxyindole Tetramers with “Anomalous” Interunit Bonding Patterns by Oxidative Coupling of 5,5',6,6'-Tetrahydroxy-2,7'-biindolyl: Emerging Complexities on the Way toward an Improved Model of Eumelanin Buildup. *J. Org. Chem.* **72**, 9225–9230 (2007).
41. Sattler, M. & Fesik, S. W. Use of deuterium labeling in NMR: overcoming a sizeable problem. *Structure* **4**, 1245–1249 (1996).
42. Santis, G. D. & Xantheas, S. S. Extending Badger's rule. II. The relationship between energy and vibrational spectra in hydrogen bonds. *J. Chem. Phys.* **162**, 244121 (2025).
43. Seo, S., DeSilva, M. A. & Brennecke, J. F. Physical properties and CO₂ reaction pathway of 1-ethyl-3-methylimidazolium ionic liquids with aprotic heterocyclic anions. *J Phys Chem B* **118**, 14870–14879 (2014).

44. Mohan, M., Banerjee, T. & Goud, V. V. Effect of protic and aprotic solvents on the mechanism of cellulose dissolution in Ionic liquids: A combined molecular dynamics and experimental insight. *ChemistrySelect* **1**, 4823–4832 (2016).
45. Roos, E., Gradaus, C., Sebastiani, D. & Brehm, M. A force field for the solubility of cellulose in DMSO/Ionic liquids. *Cellulose* **31**, 4793–4815 (2024).
46. Zhang, J. *et al.* NMR spectroscopic studies of cellobiose solvation in EmimAc aimed to understand the dissolution mechanism of cellulose in ionic liquids. *Phys Chem Chem Phys* **12**, 1941–1947 (2010).
47. Ren, F. *et al.* Dissolution of Cellulose in Ionic Liquid–DMSO Mixtures: Roles of DMSO/IL Ratio and the Cation Alkyl Chain Length. *ACS Omega* **6**, 27225–27232 (2021).
48. Chai, J.-D. & Head-Gordon, M. Long-range corrected hybrid density functionals with damped atom–atom dispersion corrections. *Phys Chem Chem Phys* **10**, 6615–6620 (2008).
49. Dunning Jr, T. H. Gaussian basis sets for use in correlated molecular calculations. I. The atoms boron through neon and hydrogen. *J Chem Phys* **90**, 1007–1023 (1989).
50. Grimme, S. Improved second-order Møller–Plesset perturbation theory by separate scaling of parallel-and antiparallel-spin pair correlation energies. *J Chem Phys* **118**, 9095–9102 (2003).
51. Frisch, M. J. *et al.* Gaussian~16 Revision C.01. (2016).
52. Izgorodina, E. I. *et al.* Importance of dispersion forces for prediction of thermodynamic and transport properties of some common ionic liquids. *Phys Chem Chem Phys* **16**, 7209–7221 (2014).
53. Hanwell, M. D. *et al.* Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *J Cheminform* **4**, 17 (2012).
54. Marenich, A. V., Cramer, C. J. & Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J Phys Chem B* **113**, 6378–6396 (2009).

55. Neese, F. The ORCA program system. *Wiley Interdiscip Rev Comput Mol Sci* **2**, 73–78 (2012).
56. Tan, S., Barrera Acevedo, S. & Izgorodina, E. I. Generalized spin-ratio scaled MP2 method for accurate prediction of intermolecular interactions for neutral and ionic species. *J. Chem. Phys.* **146**, 064108 (2017).
57. Zhao, Y. & Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor Chem Acc* **120**, 215–241 (2008).
58. Zhao, Y., Schultz, N. E. & Truhlar, D. G. Design of Density Functionals by Combining the Method of Constraint Satisfaction with Parametrization for Thermochemistry, Thermochemical Kinetics, and Noncovalent Interactions. *J. Chem. Theory Comput.* **2**, 364–382 (2006).
59. Ditchfield, R., Hehre, W. J. & Pople, J. A. Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules. *J. Chem. Phys.* **54**, 724–728 (1971).
60. Kitaura, K., Ikeo, E., Asada, T., Nakano, T. & Uebayasi, M. Fragment molecular orbital method: an approximate computational method for large molecules. *Chem. Phys. Lett.* **313**, 701–706 (1999).
61. Bondi, A. van der Waals Volumes and Radii. *J. Phys. Chem.* **68**, 441–451 (1964).
62. Mantina, M., Chamberlin, A. C., Valero, R., Cramer, C. J. & Truhlar, D. G. Consistent van der Waals Radii for the Whole Main Group. *J. Phys. Chem. A* **113**, 5806–5812 (2009).
63. Schmidt, M. W. *et al.* General atomic and molecular electronic structure system. *J. Comput. Chem.* **14**, 1347–1363 (1993).
64. Virtanen, P. *et al.* SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat. Methods* **17**, 261–272 (2020).

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