



HAL
open science

Polydopamine at biological interfaces

Maria Laura Alfieri, Tanja Weil, David Yuen Wah Ng, Vincent Ball

► **To cite this version:**

Maria Laura Alfieri, Tanja Weil, David Yuen Wah Ng, Vincent Ball. Polydopamine at biological interfaces. *Advances in Colloid and Interface Science*, 2022, 305, pp.102689. 10.1016/j.cis.2022.102689 . hal-03666941

HAL Id: hal-03666941

<https://hal.science/hal-03666941v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Polydopamine at Biological Interfaces

Maria Laura Alfieri^a, Tanja Weil^b, David Yuen Wah Ng^b, Vincent Ball^{c,d}

^a Department of Chemical Sciences, University of Naples Federico II, Via Cintia 21, I-80126
Naples, Italy

^b Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

^c Université de Strasbourg, Faculté de Chirurgie Dentaire, 8 rue Sainte Elizabeth, 67000
Strasbourg, France.

^d Institut National de la Santé et de la Recherche Médicale. Unité mixte de recherche 1121. 1 rue
Eugène Boeckel. 67084 Strasbourg Cedex. France.

*corresponding author: vball@unistra.fr

Abstract: In the last years coating of surfaces in the presence of dopamine or other catecholamines in oxidative conditions to yield “polydopamine” films has become a popular, easy and versatile coating methodology. Polydopamine(s) offer(s) also a rich chemistry allowing to post-functionalize the obtained coatings with metal nanoparticles with polymers and proteins. However, the interactions either of covalent or non-covalent nature between polydopamine and biomolecules has only been explored more recently. They allow polydopamine to become a material, in the form of nanoparticles, membranes and other assemblies, in its own right not just as a coating. It is the aim of this review to describe the most recent advances in the design of composites between polydopamine and related eumelanin like materials with biomolecules like proteins, nucleotides, oligosaccharides and lipid assemblies. Furthermore, the interactions between polydopamine and living cells will be also reported.

Keywords: catecholamines, polydopamine, biomolecules, nanocomposites

1. Introduction

The need for surface functionalisation of materials has long been of great importance in human technology, because the first interactions between materials and their surrounding occur *via* their surfaces [1,2]. Nowadays, the protection of metals against corrosion without the release of heavy metal cations within the anticorrosive paint, the functionalization of nanoparticles for their colloidal stabilization and for their targeting to specific tissues, the design of self-cleaning materials, and many other examples require stable and controlled functionalization of those materials. However, specific chemical strategies and processes are required for the functionalization of various materials, for example, the well-known modification of noble metals with molecules carrying a thiol moiety [3,4], the grafting of siloxanes onto the surface of oxides [5], among others. One of the most challenging surface modifications is the coating of polymeric materials without reactive groups, such as polytetrafluoroethylene. These very specific and highly demanding chemical methodologies often require organic solvents or harsh conditions, which has been a major limitation. In their seminal discovery, Messersmith, Lee et al. have reported a universal surface coating strategy of almost all known materials by simple oxidation of dopamine in the presence of dissolved oxygen at slightly basic pH values [6] (**Fig. 1**). The unique features of the dopamine precursor that deposits as a versatile and strongly adhesive film on solid surfaces is due to its molecular structure: the presence of a catechol and an amine group. Dopamine has similarities to the modified amino acid L-DOPA and L-tyrosine that appear in the amino acid sequence of the *mefp* proteins, which are responsible for the strong and irreversible adhesion of mussels to many different kinds of materials under wet conditions [7–9].

Other catecholamines (**Fig. 1**) such as norepinephrine [10,11] and the hydroxylated L-tyrosine, namely L-DOPA [12] as well as their derivatives or oxidation products [13] have been used to coat materials that also allow post-functionalization with either polymers [10,14], biomolecules [15,16], or metallic nanoparticles (which is possible owing to the presence of remaining non-oxidized catechol groups) [6]. All these chemical strategies and the applications of polydopamine based materials have been intensively reviewed in the literature [17–24] and they will not be the subject of this review. However, in the past years, major efforts have been devoted to expand the surface deposition methods of polydopamine, such as spray deposition [25], electrochemical deposition [26–29], microfluidic technology [30], UV and microwave irradiation [31,32], plasma and hydrothermal treatments [33,34], solid phase deposition using oxidants in the gas phase [35], and to investigate the role of different oxidation pathways [36–

40] on the properties of the obtained films. From these investigations, it became apparent that there are as many “polydopamines” as there are methods for their production. In consequence, all these different structures provide a large variety of surface properties, which opens access to diverse applications. For example, the hydrophilicity of polydopamine or related coatings can be varied from superhydrophilic to slightly hydrophobic by just changing the nature of the used oxidant [38].

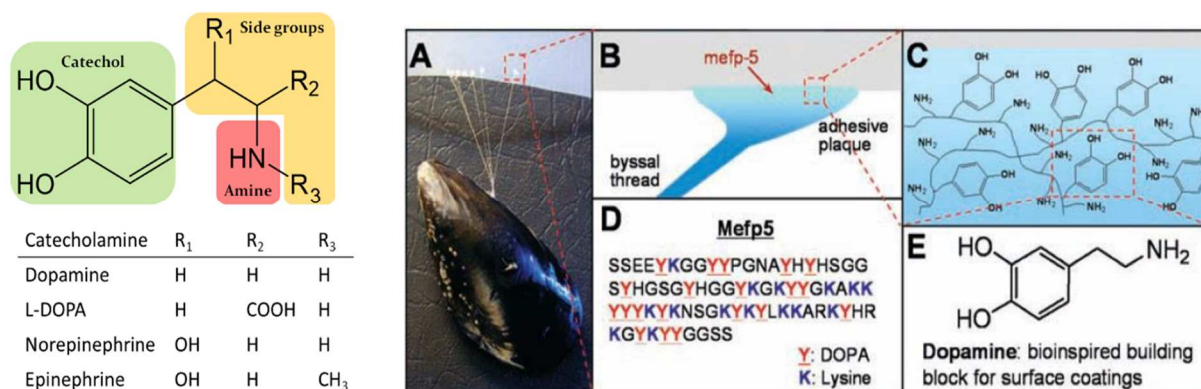


Fig. 1. Generic structure of catecholamines. The side groups of a few natural catecholamines are given in the table below the structure. A-E. From mussels to dopamine. (A): Photograph of a mussel attached to commercial PTFE. (B and C): Schematic illustrations of the interfacial location of a mussel foot protein (Mefp-5) and a simplified molecular representation of characteristic amine and catechol groups. (D): The amino acid sequence of Mefp-5. (E): Dopamine contains both amine and catechol functional groups found in Mefp-5 and was used as a molecular building block for polymer coatings. Adapted with permission from “Lee et al. Mussel-Inspired Surface Chemistry for Multifunctional Coatings. *Science*. 2007, 318(5849), 426-4301 doi: 10.1126/science.1147241.” Copyright (2007) The American Association for the Advancement of Science.

In recent years, only little progress has been made in elucidating the mechanism of polydopamine formation, which is based on a very complex covalent structure [41–43] in combination with the self-assembly of oligomeric oxidation products of 5-6-dihydroxyindole (DHI) [44]. In these structures, cation- π stacking [45] seem to play an important role (Fig. 2). The analogy to eumelanin [46], the brown-black pigment of the skin, has already been emphasized, which appears in the form of clustered nanoparticles that are surrounded and stabilized by a strongly bound protein shell, which complicates its chemical analysis. Replacement of nitrogen by sulfur can impart more regioregularity to eumelanin structure through modification of visible absorption properties and the average redox state [47]. New polydopamine-based nanomaterials have been designed based on the structure and function of eumelanin [48,49]. However, a systematic overview of the diverse biomolecule-polydopamine

interactions is still elusive, which could afford entirely new biohybrid nanomaterials for diverse applications like improved targeting of cancer cells. We will review the interactions between polydopamine(s) and the different classes of biomolecules, such as proteins, nucleotides, oligosaccharides and lipids and we will highlight their unique features and potential applications.

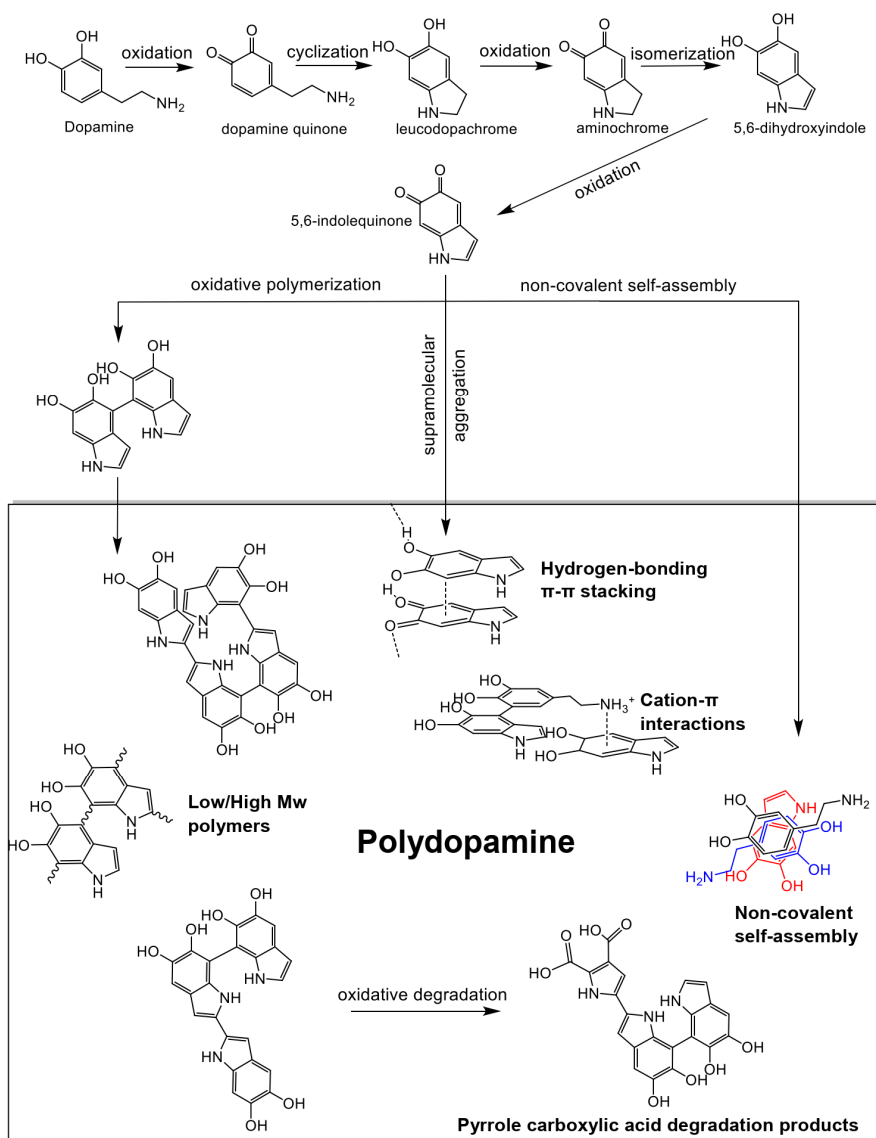


Fig. 2. Proposed mechanism of PDA formation.

2. Polydopamine and polypeptides/proteins

2.1 Protein adsorption and grafting on polydopamine nanostructures

Eumelanin in the skin is naturally surrounded by proteins. Since polydopamine (PDA) is a biomimetic analogue of this black natural photoprotective material, its ability to adsorb and covalently interact with proteins has been investigated and used for various applications. Proteins adsorb to PDA films *via* various supramolecular interactions such as electrostatic, van der Waals or hydrogen bonds [16]. However, also covalent binding between proteins such as trypsin or other model polypeptides and PDA has been demonstrated [15]. Covalent bioconjugation to proteins could proceed when the catechol groups are oxidized to quinones that are susceptible to nucleophilic attack by amines and thiols through Michael addition and Schiff base reactions. The PDA material readily reacts in a mildly alkaline solution with primary amine or thiol groups on proteins (**Fig. 3**). The adsorbed or covalently bound proteins on PDA surfaces retain at least some of their enzymatic activity, as demonstrated in the case of alkaline phosphatase [50]. Unfortunately, to our knowledge, the fraction of immobilized/grafted enzymes remaining active on PDA has not yet been determined. This is of paramount importance because adsorbed or grafted proteins usually undergo important conformational changes depending on the hydrophilicity/hydrophobicity of the surface. However, in some polar and confined environments, proteins can even increase their conformational stability, and PDA films could constitute such an environment. Similarly, silk fibroin proteins adsorbed on PDA films retain their biological activity *in vitro* [51].

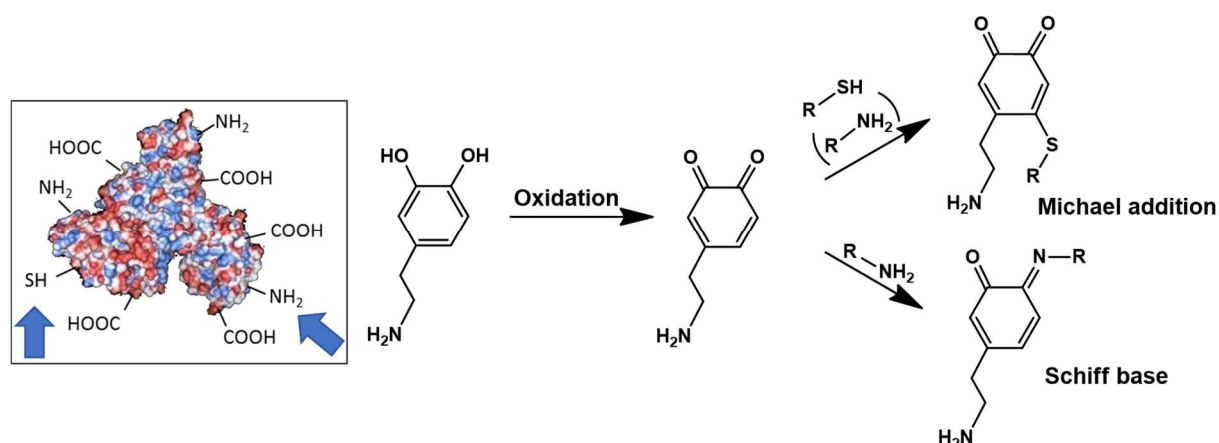


Fig. 3. Possible reactions of proteins with PDA. Most abundant reactive surface groups of the amino acid side chains are shown exemplarily.

Similar to melanin-like materials, PDA films serve as efficient fluorescent quenchers [52] due to their broad absorption spectrum. This concept has been used for biosensing applications to

record binding of fluorescently labelled proteins [52]. The adsorption and immobilization of proteins on PDA also allowed the design of molecular imprints. As an example, a PDA film deposited on an electrospun network covered with lysozyme has generated an imprinted and reusable material suitable for lysozyme binding with a capacity of about 500 mg of protein per gram of polymer scaffold. This imprinted matrix could be reused at least six times, and the released protein retained its activity as demonstrated in the bacterial cell wall decomposition of *Micrococcus Lysodeikticus* [53].

We have recently reported the biotemplated synthesis of amyloid-like polypeptide nanofibers as a bioactive scaffold for the controlled growth of a very thin PDA layer that supported the adhesion and growth of neuronal cells. The PDA-coated peptide nanofibers enhanced the development of the neuronal growth cone over that of bare nanofibers or PDA alone (**Fig. 4**). Moreover, the pH sensitive loading and release of boronic acid containing cargo onto the PDA surface was realized. Such biohybrid materials provide potential applications as neuronal implants and for tissue engineering [54].

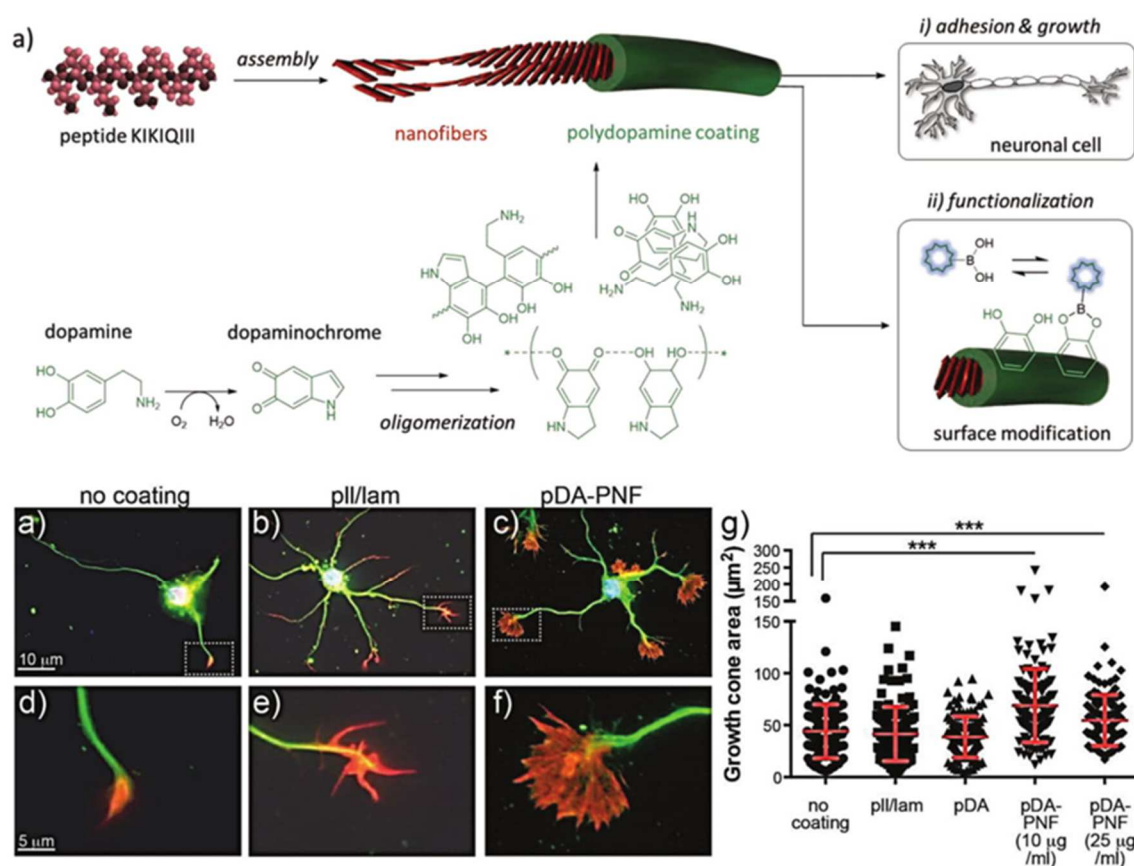


Fig. 4. Enhanced neuronal cell growth and adhesion of PDA coated fibrils. (A) Schematic representation of the formation of polydopamine-coated peptide nanofiber hybrids. Mouse primary hippocampal neurons were plated on the indicated substrates, followed by labeling for

microtubules (green) and F-actin (red); the latter is used to label growth cones. (B,E) Growth cone area of neurons plated on glass only (no coating) was comparable to (C,F) neurons grown on coverslips coated with polylysine/laminin. (D,G) By contrast, the growth cone area of neurons cultured on PDA-PNF was enhanced as was the number of finger-like filopodia. (E–G) are higher magnifications of dashed areas in (B–D). (H) Quantification of growth cone area. Adapted with permission from “Water-Dispersible Polydopamine-Coated Nanofibers for Stimulation of Neuronal Growth and Adhesion. *Adv. Healthc. Mater.* 2018, 7 (11).” Copyright John Wiley & Sons, Inc.

2.2 Peptide motifs support the formation of PDA nanoparticles with controlled size

Polydopamine usually precipitates from dopamine solution in the presence of an oxidant and the resultant material has a heterogeneous size in the micrometer range. In contrast, narrowly dispersed melanin nanoparticles are formed in the presence of alcohols which also modify the deposition of PDA films [55] and within melanocyte human skin cells or within cells of *Sepia officinalis* [56]. It has been shown that these nanoparticles are surrounded by stabilizing proteins, which prompted several studies on the role of proteins in the oxidation and self-assembly process of PDA. The first investigations performed in this direction were of trial-and-error type. Proteins of similar molecular mass and dimensions as hen egg white lysozyme or α -lactalbumin with isoelectric points of 11.4 and 4.5, respectively, have no impact on the formation of PDA films and on the precipitation of PDA from dopamine containing solutions [56]. However, when Human Serum Albumin (HSA) is added to high concentrations of dissolved dopamine (at 2.0 mg mL⁻¹, Tris buffer, pH = 8.5, dissolved O₂ as the oxidant), the average hydrodynamic diameter of the resultant PDA particles decreases with increasing the HSA/dopamine ratio. The hydrodynamic diameter of the polydopamine nanoparticles reaches values as low as 20–30 nm for an HSA concentration of 2.0 mg/mL⁻¹ corresponding to a protein/dopamine molar ratio as low as 3 × 10⁻³ [57]. In addition, the obtained nanoparticles are stable in Tris buffer for months and their cytotoxicity is negligible.

This important finding required some understanding by which mechanism some proteins take an active role in the self-assembly and polymerization processes of PDA in solution whereas others are inactive. The human adrenal gland secretes chromaffin vesicles rich in both catecholamines and proteins, mainly Chromogranins [58]. Hence it was believed that such vesicles could constitute a biological laboratory to investigate the fate of catecholamines in the presence of a large set of proteins. The proteins present in the chromaffin vesicles undergo enzymatic cleavage resulting in the formation of various bioactive peptides such as antimicrobial peptides. Dopamine was hence mixed with a large set of such peptides generated from the enzymatic degradation of Chromogranin A. Some of these peptides allowed the

production of PDA with controlled sizes, which was demonstrated by size exclusion chromatography, whereas others did not prevent uncontrolled aggregation and polymerization of PDA resulting in the formation of insoluble precipitates. Interestingly, all the peptides involved in the controlled dopamine oxidation and polymerization contained adjacent L-lysine (K) and L-glutamic acid (E) amino acids within their sequence. Control experiments with very short synthetic peptides containing either the KE diad or similar peptides with one or two glycine spacers revealed a similar effect on polydopamine size, whereas peptides without adjacent K and E could not control the sizes of the obtained PDA nanoparticles. Proteins containing K and E as adjacent amino acids in their primary sequence could all stabilize PDA nanoparticles with controlled dimensions when added to dopamine solutions at pH = 8.5 and no precipitate was formed that usually occurs in the absence of these proteins (and alcohols). Moreover, PDA deposition on the walls of the reaction beaker was almost completely inhibited and colloiddally well dispersed solutions were obtained [59]. These findings suggest some sequestration of dopamine by the protein surface, with dopamine oxidation and self-assembly (or polymerization) of dopamine preferentially occurring near the protein surface or in the vicinity of the water-accessible KE diad. Figure 5 A-E shows the particular influence of alkaline phosphatase, as a KE containing protein, on the control of PDA formation. High resolution TEM micrographs suggest core-shell nanoparticles. But the formation of a composite structure, in which the protein is dispersed throughout the particle volume could not be ruled out. Clearly, some proteins in their native and enzymatically active conformation are present on the nanoparticle surface, as demonstrated by their retained enzymatic activity [60]. In another related study, where PDA nanoparticle formation was stabilized by a poly(ethyleneglycol) polymer chain with two terminal primary amino groups, polymer and PDA were observed throughout the nanoparticle volume as shown by Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), indicating that also composite structures composed of PDA nanodomains could be formed [61].

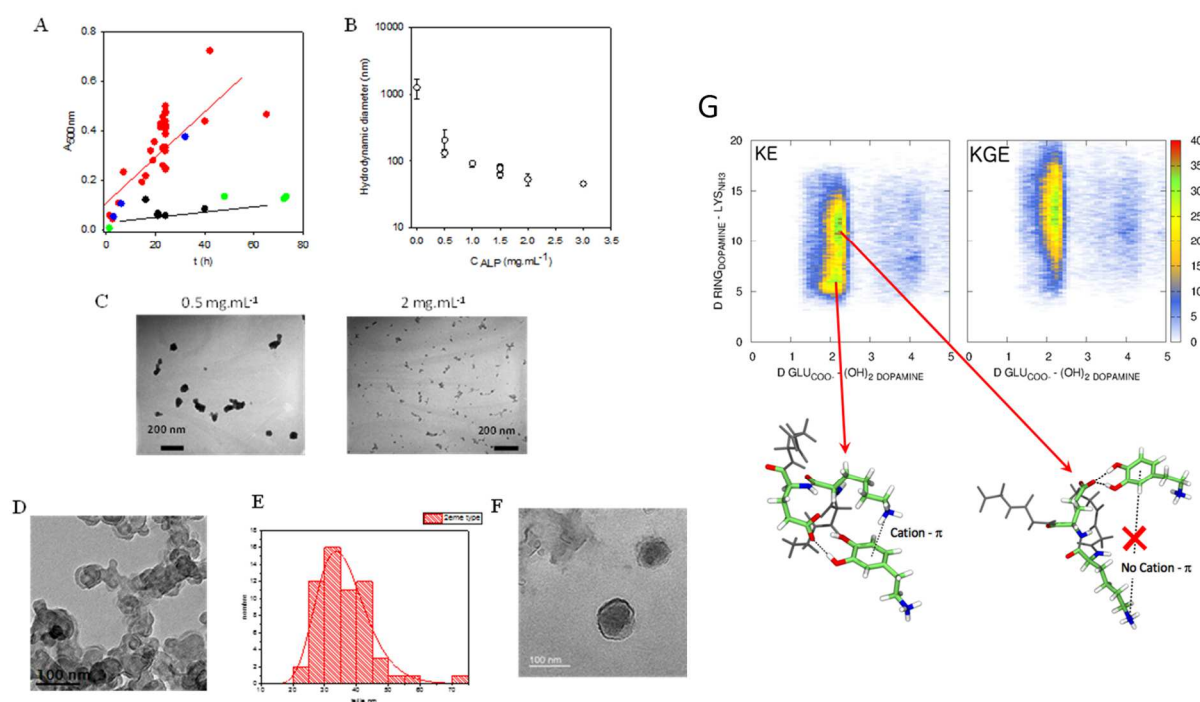


Fig. 5. (A) Influence of the alkaline phosphatase concentration on the deposition of PDA on quartz slides as measured by UV-Vis spectroscopy. The concentration in alkaline phosphatase was equal to: 0 (red disks), 0.1 (green disks) and 0.5 (black disks) mg mL⁻¹ respectively. The blue disks correspond to alkaline phosphatase pre-adsorbed from a 1 mg mL⁻¹ solution during 1 h on the quartz slides before contact with the dopamine containing solution. (B) Change in the hydrodynamic radius of PDA particles (after 24 h of oxidation from O₂ dissolved in Tris buffer at pH = 8.5) as function of the concentration of added alkaline phosphatase, the concentration of dopamine being held constant at 2 mg mL⁻¹. (C) TEM micrographs of the PDA nanoparticles obtained in the presence of alkaline phosphatase at 0.5 and 2 mg mL⁻¹ after 24 h oxidation (the initial dopamine concentration is equal to 2 mg mL⁻¹). (D) Magnification of a region in the TEM picture of panel C. (E) Size distribution (n = 322 particles) of the PDA nanoparticles obtained in the presence of alkaline phosphatase at 2 mg mL⁻¹. (F) High resolution TEM image of an individual nanoparticle suggesting a core-shell structure. (G) Results from molecular dynamics simulations showing that dopamine interacts preferentially with the carboxylic group of L-glutamic acid (E) *via* hydrogen bonds and *via* cation- π interactions with the amino group on L-lysine (K). The presence of a glycine spacer group between K and E, dramatically reduces the interaction between dopamine and the peptide sequence. Adapted with permission from: "Mimicking the Chemistry of Natural Eumelanin Synthesis: The KE Sequence in Polypeptides and in Proteins Allows for a Specific Control of Nanosized Functional Polydopamine Formation. *Biomacromolecules* 2018;19: 3693–3704." Copyright American Chemical Society.

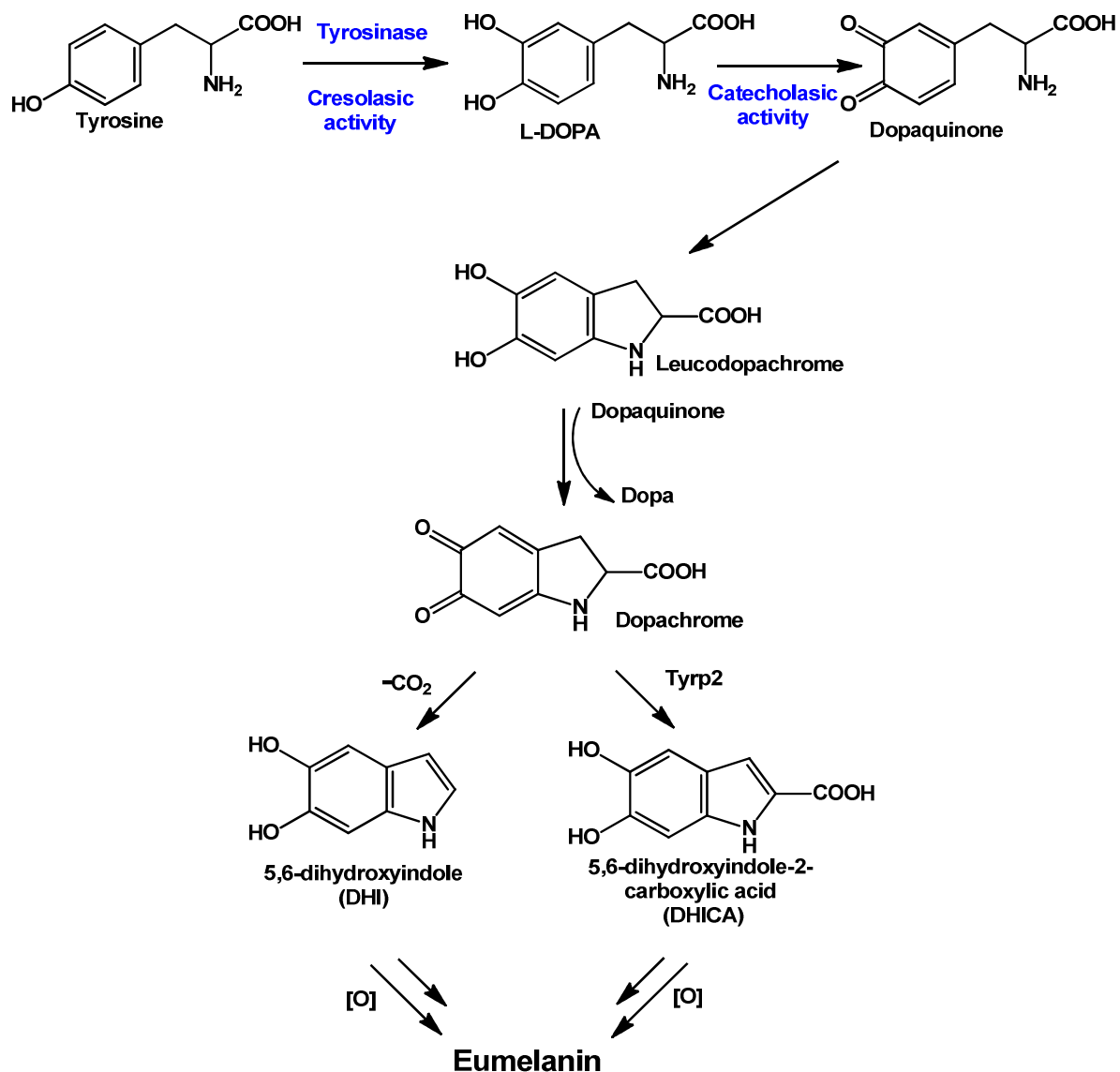
In order to elucidate the influence of the K and E amino acids on dopamine and polydopamine formation, molecular dynamics simulations have been performed to investigate the interaction modes of dopamine with K and E as well as the influence of a glycine spacer between K and E. It was found that the oxygen atoms of the carboxylic acid function of E form strong hydrogen

bonds with the two vicinal diols on dopamine whereas the protonated amino group on K (pK_a around 10) adopts a cation- π interaction with the aromatic ring of dopamine. The introduction of a spacer glycine between K and E considerably weakens these interactions (**Fig. 5G**) [59].

In addition to the KE sequence, L-tyrosine containing tripeptides also stabilized PDA nanoparticles under oxidative conditions [62]. In fact, the oxidative polymerization, initiated for example by the enzymatic oxidation of tyrosine residues, can be tuned by the peptide sequence that self-assembles into supramolecular nanostructures with sequence-encoded properties, through non-covalent interactions [62].

2.3 Enzymes to catalyze the oxidations of phenols, other catecholamines, and their use in film deposition

Considering the great potential of polyphenol-based coatings for materials design (*e.g.*, for metal-phenolic networks, universal coatings and underwater adhesives), for manipulating biological entities (including living cells), in biomedical and medicinal applications, several strategies have been used to develop a method that involves catecholamines as coating precursors. PDA film formation critically relies on the oxidation of catechol to 1,2-benzoquinone and involvement of the amino group in the reaction and deposition. In the natural process of melanogenesis [63], the enzyme tyrosinase catalyzes both the hydroxylation of L-tyrosine and the subsequent dehydrogenation of catechol to *o*-quinone, as well as the oxidation of 5-6-dihydroxyindole to indolequinone to form eumelanin (Scheme 1). Inspired by this natural process, enzymes have been used to catalyze the oxidative polymerization of phenols and catecholamines (*e.g.*, tyramine, dopamine, norepinephrine, and DOPA) at roughly neutral pH.



Scheme 1. Tyrosinase catalysed oxidation of tyrosine to eumelanin formation: hydroxylation of monophenol to *o*-diphenol (cresolasic activity) and dehydrogenation of catechol to *o*-quinone (catecholasic activity).

Enzymes provide several outstanding characteristics such as unique substrate specificity, regio-, chemo-, and stereoselectivity. In addition to increase the reaction rate, many enzymes are able to function in a non-physiological environment, can be used not only to activate or modify a substrate *via* direct catalysis but they can also be immobilized or integrated directly into materials [64].

For example, the enzyme laccase activates the catechol groups of fourteen different naturally occurring catecholamines or polyphenols for subsequent polymerization [65]. As demonstrated by Dhand *et al.*, their oxidative products were effective in improving surfaces, mechanical and the thermal properties of polyvinyl alcohol (PVA) films (**Fig. 6**). Interestingly, some of these

reinforced films, especially those obtained from pyrogallol and epinephrine, displayed excellent antimicrobial properties, inhibiting the growth of pathogenic Gram-positive and Gram-negative bacteria and yeasts strains for potential health care applications.

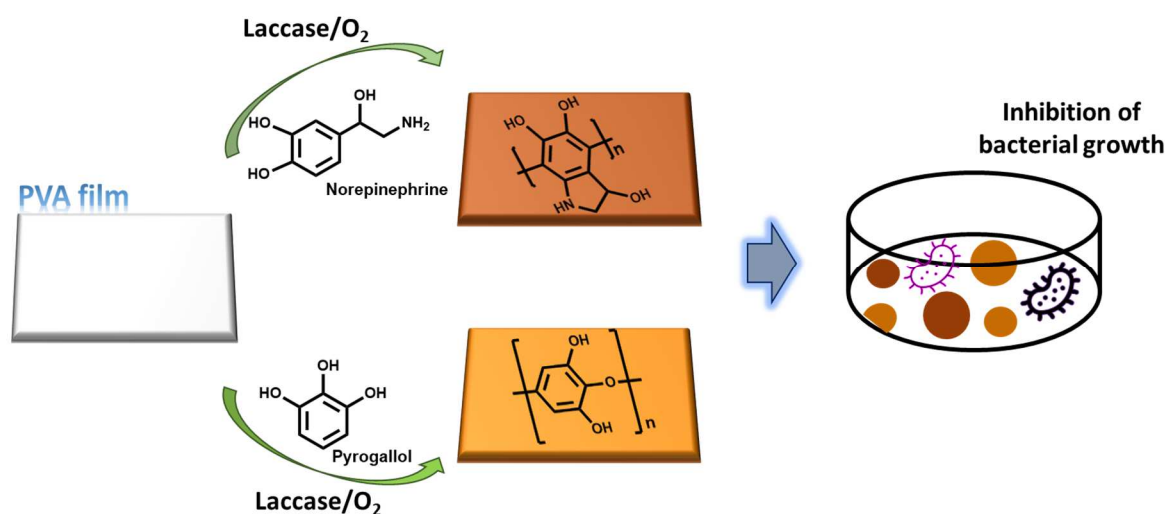


Fig. 6. PVA films endowed with antimicrobial properties prepared by laccase-catalyzed polymerization of pyrogallol or norepinephrine in aqueous solution at room temperature for 24 h. Adapted with permission from: “Multifunctional Polyphenols- and Catecholamines-Based Self-Defensive Films for Health Care Applications. ACS Applied Materials and Interfaces 2016;8:1220–32.” Copyright American Chemical Society.

Laccase-catalyzed polymerization of dopamine was also proposed as a new and efficient biomacromolecule-immobilization platform for electrochemical biosensing and biofuel cell applications. In several cases, enzymes bound to the film surface were stabilized and maintained high catalytic activity. Such biocomposite materials exhibited better performance toward hydroquinone (HQ) biosensing if compared with other laccase-entrapped polymeric nanocomposite modified electrodes (*e.g.*, aniline, *o*-phenylenediamine, *o*-aminophenol) [66]. Similarly, horseradish peroxidase (HRP)-based enzymatic polymerization of L-DOPA, in the presence of hydrogen peroxide, has also been used to immobilize mono-/bi-enzymes for HQ-mediated amperometric biosensing of H₂O₂ and uric acid [67].

Alternatively, poly(L-DOPA)-based enzyme electrodes, prepared by casting an aqueous mixture of L-DOPA and tyrosinase on a carbon electrode, exhibited improved biosensing performance and higher mass-specific bioactivity of the immobilized tyrosinase than those based on similarly biosynthesized polydopamine and poly(L-tyrosine) as well as chitosan and Nafion, commonly used for enzyme immobilization and biosensing [68]. Interestingly, the localized deposition of dopamine and pyrocatechol at the target catalytic sites of HRP allowed the fabrication of biosensors with improved sensitivity in biological detection and remarkably

concentrated colorimetric signal for spatial biomarker labeling [69]. However, HRP-labeled biomarker still requires multiple inconvenient washing steps, which represents one of the major limitation for commercialization.

The use of tyrosinases was also recently reported as a simple enzyme-mediated strategy for assembling various monophenols, including small molecules and biopolymers (*e.g.*, peptides, proteins) on various surfaces [70–72] (**Fig. 7**). The active site of tyrosinase, in fact, catalyzes the oxidative hydroxylation of the phenol moiety to the catechol, which is central in the following molecular assembly for film deposition accomplished by the adhesion between the catechol moieties and the substrate *via* covalent and coordination cross-linking. Compared to PDA coating, this method was fast and efficient and uniform films were formed with various functions depending on the original monophenol-containing building blocks (*e.g.*, radical scavenging, enzymatic catalysis, fluorescence, cytocompatibility). Importantly, the resulting coatings providing various functional groups, could act as a platform for surface-confined reactions to further tailor the surface properties leading for example to metal oxide films, multimetal-protein coatings and covalently conjugated films (*e.g.*, N-hydroxysuccinimide (NHS)-containing molecules such as Alexa Fluor 488TM (AF488) or 633 NHS ester (AF633)) (**Fig. 7**). Moreover, the confinement or immobilization of tyrosinase into alginate gels represents a mild, versatile, efficient and clean protocol for the generation of adhesive PDA-type films starting from tyramine, with considerable material savings and without undesirable substrate-consuming autoxidation processes, which limit cost-effectiveness and may interfere with the film deposition procedure [70] (**Fig. 7**).

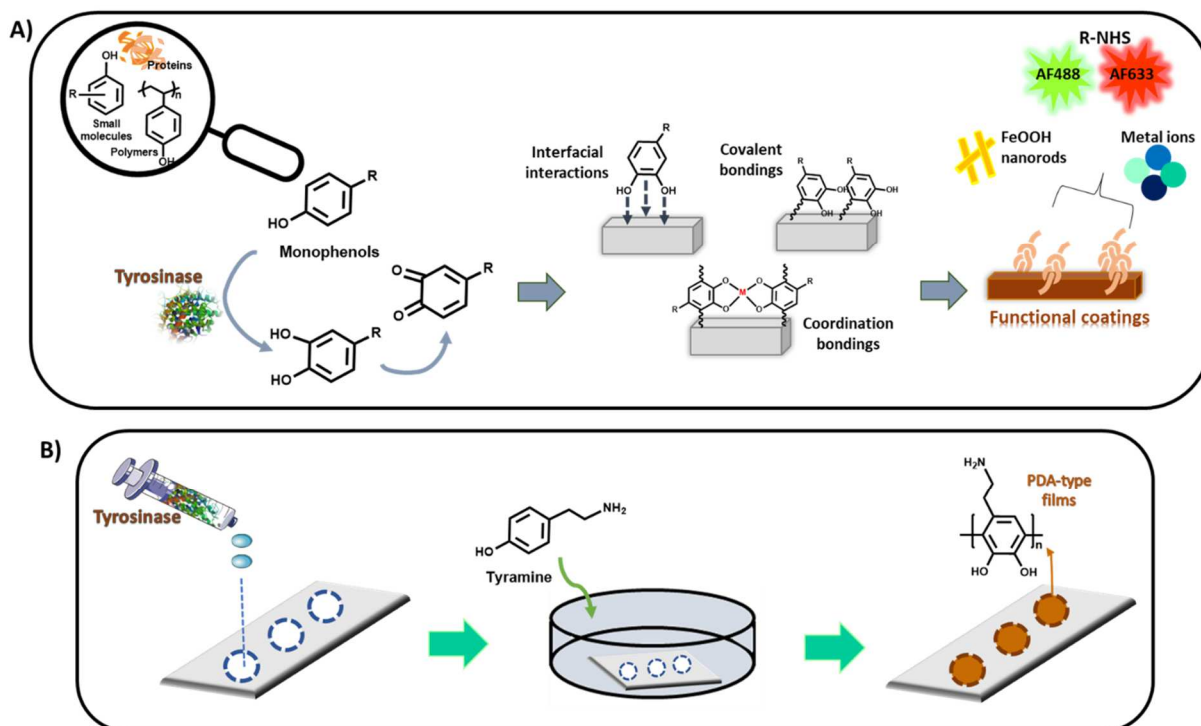


Fig. 7. (A) *In situ* conversion of a wide range of monophenols into catechols *via* tyrosinase. The films formed on various surfaces *via* covalent and coordination cross-linking represent chemically reactive platforms for secondary surface-confined reactions (*e.g.*, biomineralization, Cu and Al ion chelation and N-hydroxysuccinimide containing molecules conjugation). (B) Site-specific enzymatic deposition of PDA-type film by tyrosinase catalyzed oxidation of tyramine in phosphate buffer at pH 6.8.

The Layer-by-Layer (LbL) technique, based on the consecutive deposition of oppositely charged polyelectrolytes onto a surface, was also proposed as a general and versatile tool for the chemo-physical immobilization of tyrosinase on a large variety of surfaces. The immobilized LbL enzyme retained about 87 % of its catalytic activity and was applied for the efficient and selective synthesis of bioactive catechol derivatives by oxidation, under mild conditions, of a large panel of substituted phenols [73].

Additionally, the combination of glucose oxidase (GOx) and HRP has recently been reported [74] as a simple and fast approach that enables film formation of amine group-absent *o*-diphenols, such as protocatechuic aldehyde and pyrocatechol without requiring multiple deposition steps of enzymes and polyphenols as in the LbL technique [73,75]. First, in the presence of molecular oxygen, GOx catalyzes the oxidation of D-(+)-glucose generating hydrogen peroxide among other things. Then, HRP oxidizes *o*-diphenols leading to the formation of thin, self-adherent melanin-like species films and shells (**Fig. 8**). Moreover, the encapsulation of individual cells in these artificial shells endows the cell with exogenous

properties such as UV filtration, immunogenic shielding, and enhances their resistance to external aggressors.

Notably, hydrogen peroxide, produced in the GOx-catalyzed reaction of glucose, accelerated the oxidation of Fe^{2+} to Fe^{3+} in the Fe^{2+} -tannic acid complex providing a facile route for surface functionalization [76]. Moreover, the concomitant incorporation of GOx, with retained catalytic activity during film formation also allowed the catalytic single-cell nanoencapsulation.

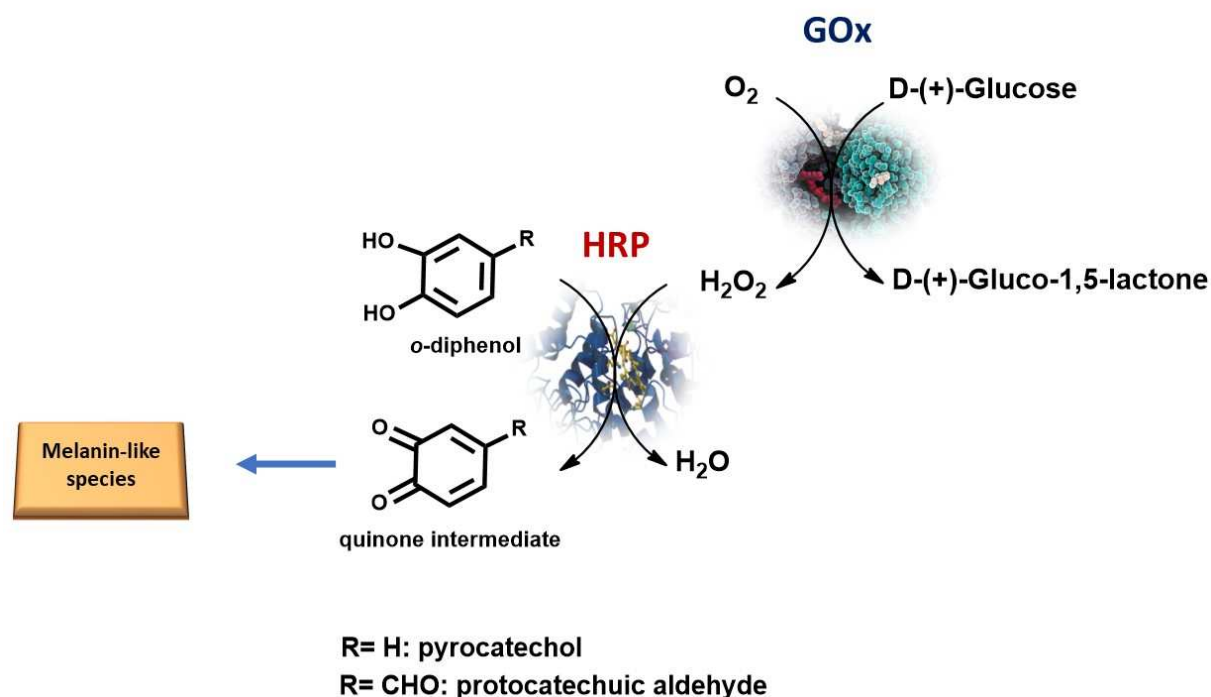


Fig. 8. Schematic representation for melanin-like species synthesis from o-diphenols by glucose oxidase (Gox)/horseradish peroxidase (HRP) mediated oxidative reactions.

3. Polydopamine and oligonucleotides/DNA or RNA

Nucleic acids, the genetic database of Nature, possess characteristic molecular features such as their polyanionic character, π - π nucleobase interactions and programmable molecular recognition, thus making them attractive candidates to impart complex functionalities into synthetic materials. Each of these molecular venues can be exploited by PDA to form a synergistic relationship where their combination would result in a unique nano-bio interface for a wide variety of applications in nanotechnology, sensing and biomedicine (**Fig. 9A**).

As PDA typically consists of assembled oligomeric species, where π -interactions govern the interaction of the oligomeric aggregates into a supramolecular cross-linked material, it can possess a wide range of affinities towards the π -conjugated nucleobases of DNA. The affinity arises due to spatial complementarity where the inter-assembly spacing of PDA ($\sim 3.4 - 3.7 \text{ \AA}$)

[46] closely matches that of the duplex rise of DNA helices (3.4 Å) [77]. Using these interactions, Ding *et al.* described the capability to direct the assembly of PDA aggregates with DNA to form nanoplatelets (**Fig. 9B**) [78]. The deposition of PDA on DNA may also be dependent on the incorporation of positive counteranions at the interface between both anionic materials.

The mechanism for the two-dimensional control uses the duplex grooves of DNA to pre-arrange the PDA aggregates such that the supramolecular polymerization is favored along the DNA axis. An increase in concentration leads to the assembly of micrometer size sheets whereas the sheet thickness (~10 nm – 25 nm) is dependent on the weight ratio of DNA/dopamine. The precision of arranging PDA at the nanoscale can be enhanced by initiating the polymerization of dopamine directly at the DNA interface (**Fig. 9C**). Instead of promoting oxidative polymerization through ambient oxygen in basic solutions, dopamine molecules can be activated by reactive oxygen species produced using DNAzymes *in situ* [79–81]. A typical DNAzyme consist of a G-quadruplex sequence (5'-TTGGGTAGGGCGGGTTGGG-3') embedded either with a metal-porphyrin complex (*e.g.* hemin) or photosensitizer (protoporphyrin IX, Eosin Y) [82]. Depending on the catalytic center, reactive oxygen can be produced with H₂O₂ (hemin) or the irradiation of light corresponding to the photosensitizer. As dopamine molecules oxidize in the proximity of the DNAzymes into PDA aggregates, the affinity between PDA and DNA is simultaneously created, causing them to immediately assemble with the DNA duplex. By patterning DNAzymes on DNA-origami, a DNA folding technique to access different geometric nano-objects, the growth of PDA can be programmed to assimilate into different shapes such as crosses, lines, rings and even numbers [80,81,83]. The challenge of stopping PDA formation and aggregation in many studies can be alleviated by photochemical control, where polymerization only proceeds upon a specific color of light (**Fig. 9D**) [79]. As a direct consequence, photo-controlled systems provide the possibility to accurately manipulate the extent of PDA deposition and thus the dimensions of the object in high resolution.

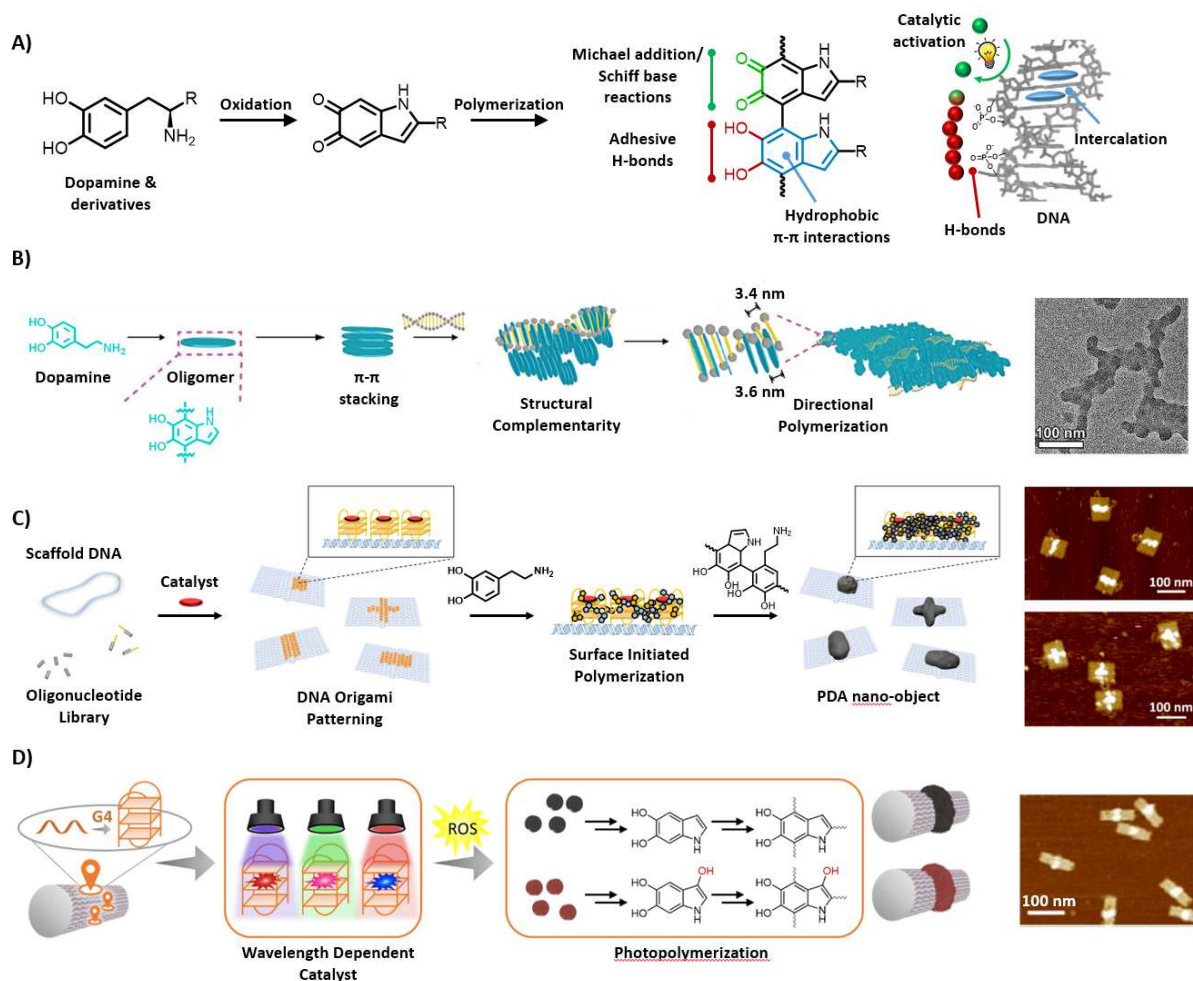


Fig. 9. (A) General schematic and points of interactions of PDA (and derivatives) with DNA. (B) DNA imparts directionality into oligomeric PDA sheets such that the growth axis of PDA is preferentially aligned [78]. Adapted with permission from “Ding T, Xing Y, Wang Z, Guan H, Wang L, Zhang J, et al. Structural Complementarity from DNA for Directing Two-Dimensional Polydopamine Nanomaterials with Biomedical Applications. *Nanoscale Horiz.* 2019;4:652–7.” Copyrights 2018 Royal Society of Chemistry. (C) Formation of a G-quadruplex containing DNA origami where the oxidation capabilities of the catalytic center initiates dopamine polymerization [81]. Adapted with permission from “Tokura Y, Harvey S, Chen C, Wu Y, Ng DYW, Weil T. Fabrication of Defined Polydopamine Nanostructures by DNA Origami-Templated Polymerization. *Angew. Chem.* 2018;57:1587–91.” Copyrights 2018 The Authors. (D) Controlling PDA growth at the nanoscale with light of specific wavelengths paired with specific photosensitizers within the G-quadruplex [79]. Adapted with permission from “Winterwerber P, Whitfield CJ, Ng DYW, Weil T. Multiple Wavelength Photopolymerization of Stable Poly(Catecholamines)-DNA Origami Nanostructures. *Angew. Chem. Int. Ed.* 2022:e202111226.”

Non-specific π - π interactions between PDA and DNA can additionally be used to functionalize PDA coated materials with DNA as a facile manner to create DNA-appended nanomaterials. As such, complex DNA based biofunctions such as that of tailor made DNAzymes using rolling circle amplification can be attached onto nanomaterials easily without the need of typical conjugation chemistry [84]. Similarly, the attachment of aptamers, short DNA sequences capable of exhibiting customized binding and recognition properties, have enabled efficient biosensing of changes in renal interstitial fibrosis (**Fig. 10A**). In addition to π - π interactions, Cai and coworkers interestingly demonstrated that mesopores formed from a modified PDA synthesis pathway are able to host short ssDNA chains within [85]. Functioning as a nano-containment device, the nanopores facilitate the hybridization of the ssDNA towards its complementary partner with greater selectivity and efficiency, reaching a dissociation constant in the femtomolar range. Intuitively, such methods have been particularly attractive for the delivery of smart nanoparticle systems where several components *i.e.* drugs, biomolecules, polymers and metals can be assembled together with relative ease [86]. The consequence of such a strategy commonly achieves combinatorial effects, where properties of several components exhibit cooperative functions to achieve a therapeutic aim. In this respect, Shen and coworkers integrated the photothermal behaviour of PDA nanospheres with *S. aureus* targeting fluorescent aptamer that self-reports its specific bacteriacidal effects on persistent biofilms (**Fig. 10 B, C**) [87]. The manifold functions are easily assembled by standard PDA coating techniques coupled with the coating's intrinsic non-covalent interactions with the DNA aptamer. For drug delivery applications in cancer, PDA was recently found to enable lysosomal escape, which is crucial for efficient delivery of drugs into the cytosol [88]. Hence, by taking advantage of this capability, small interfering RNA (siRNA) functionalized onto the PDA surface was delivered into the cytosol exhibiting efficient gene silencing (~65%). Combined with the photothermal effects of PDA, anti-cancer effects can be compounded to reach improved therapeutic efficacy [88].

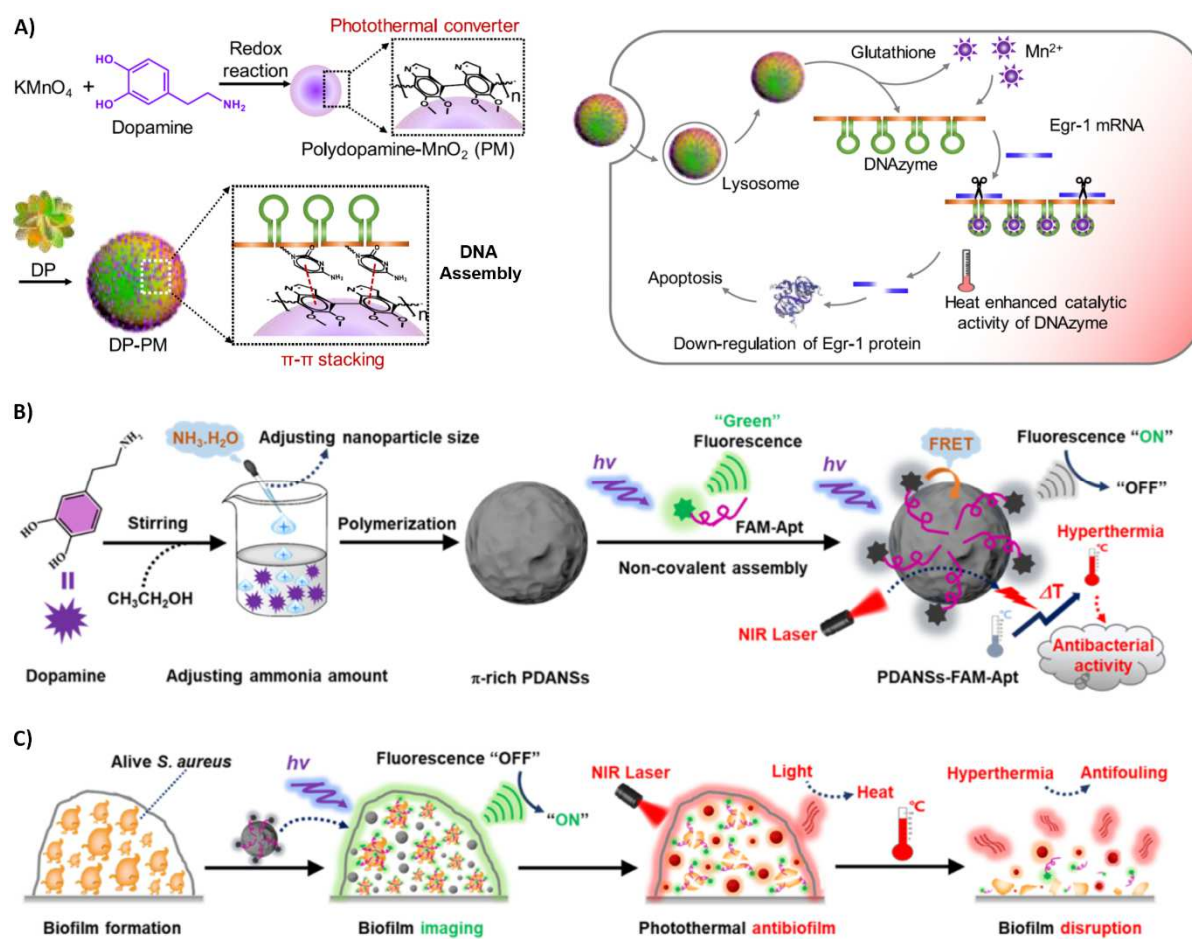


Fig. 10. (A): Functionalization of DNAzymes onto PDA-coated MnO₂ nanoparticles, demonstrating lysosomal release, photothermal effects and DNA recognition properties [84]. Adapted with permission from “Zhao H, Zhang Z, Zuo D, Li L, Li F, Yang D. A Synergistic DNA-Polydopamine-MnO₂ Nanocomplex for Near-Infrared-Light-Powered DNAzyme-Mediated Gene Therapy. *Nano Lett.* 2021;21:5377–85.” Copyrights 2021 American Chemical Society. (B) Assembly of fluorescent DNA aptamers onto PDA nanospheres as a self-reporting system, taking advantage of PDA’s quenching effect on conjugated fluorophores [87]. Adapted with permission from “Ye Y, Zheng L, Wu T, Ding X, Chen F, Yuan Y, et al. Size-Dependent Modulation of Polydopamine Nanospheres on Smart Nanoprobes for Detection of Pathogenic Bacteria at Single-Cell Level and Imaging-Guided Photothermal Bactericidal Activity. *ACS Appl. Mater. Interfaces* 2020;12:35626–37.” Copyrights 2020 American Chemical Society. (C): Mechanism of biofilm destruction using PDA nanosphere-aptamer particles [87]. Adapted with permission from “Ye Y, Zheng L, Wu T, Ding X, Chen F, Yuan Y, et al. Size-Dependent Modulation of Polydopamine Nanospheres on Smart Nanoprobes for Detection of Pathogenic Bacteria at Single-Cell Level and Imaging-Guided Photothermal Bactericidal Activity. *ACS Appl. Mater. Interfaces* 2020;12:35626–37.” Copyrights 2020 American Chemical Society.

Polydopamine and other biomolecules: polysaccharides and lipid assemblies

4.1 PDA and polysaccharides

In comparison to proteins and oligonucleotides, polysaccharides have been much less investigated with respect to their interactions or blending with polydopamine. Chitosan films have been functionalized with PDA [89] and some composite membranes have been formed between polydopamine and nanocellulose for applications in wastewater treatment [90].

4.2 PDA and lipids

Lipid bilayers could be easily formed on PDA films by vesicle adhesion and rupture as on many hydrophilic materials [91].

In a reverse way, PDA can be deposited on a phospholipid bilayer separating two aqueous compartments either using dissolved oxygen or sodium periodate as an oxidant with the interesting finding to markedly improve the bilayer stability against voltage pulses [90]. When dopamine was oxidized with dissolved O₂ at pH = 8.5, the current flowing across the single porin membrane protein embedded in the lipid bilayer decreased to the noise level in between 4 to 5 h, which resulted in a total closure of the protein's channel. However, when dopamine was oxidized at pH = 5.0 in the presence of a much stronger oxidant, NaIO₄, the same effect was obtained in less than 2h. Moreover, the lipid bilayer coated with the PDA-NaIO₄ films was more prone to the appearance of voltage induced defects than the lipid bilayer coated with PDA-O₂ for transmembrane voltages larger than 350 mV [92]. These preliminary investigations suggest that lipid bilayers coated with PDA on only one face of the membrane could be an excellent alternative to other lipid bilayer stabilization methods like the coating with an actin network or *S layer* proteins.

4.3 PDA-containing biomembrane composites

Polyelectrolyte multilayer films prepared by the layer-by-layer deposition method can display an exponential growth of their thickness as a function of the number of layer pairs deposited. Such films are highly hydrated and can be used for many applications such as cell encapsulation and drug delivery but are intrinsically soft displaying extremely low Young moduli and are hence difficult to handle [93]. To solve this challenge, it has been found that exponentially growing films made from the alternate deposition of hyaluronic acid (HA) and poly-L-lysine (PLL) can be detached from their substrate as self-standing membranes after 24 h of contact in dopamine solution at pH = 8.5. Fluorescence recovery after photobleaching

experiments were used to estimate the diffusion coefficient and the fraction of mobile fluorescence labelled PLL in the polyelectrolyte multilayer film. The diffusion coefficient and fraction of mobile PLL chains were markedly decreased after PDA formation most probably due to covalent bond formation between amino groups from PLL and quinones in PDA via a Michael addition or Schiff base reaction [94]. It was assumed that the presence of reactive PDA aggregates generated *in situ* in the polyelectrolyte multilayer architecture was responsible for its ability to be detached from the glass substrate as a free-standing membrane in the presence of sodium hydroxide solutions, a treatment which induced dissolution of the film in the absence of PDA.

Recently, ultrathin free-standing membranes composed of PDA were achieved by electropolymerization [95]. These freestanding PDA films remained stable even at the air/air interface with very high elastic moduli in the GPa range. The PDA film thickness was within the range of cellular membranes, which allowed the incorporation of protein nanopores, so-called “nanodiscs” as functional entities. These nanodiscs contained phospholipids and the transmembrane domain of glycophorin A, an antigen-presenting membrane protein found at the surface of human erythrocytes [95]. These films revealed semi-permeable properties and transport through the film was controlled by the embedded protein nanodiscs.

Moreover, these few-nanometer thick PDA membranes revealed very fast light-to-motion conversion stimulated by visible light. Light-induced heating of PDA leads to desorption of water molecules and very fast contraction of the membranes within micro seconds, whereas switching off light leads to a spontaneous expansion due to heat dissipation and water adsorption. These multi-responsive pristine PDA membranes could serve as robust building blocks for soft, micro, and nanoscale actuators stimulated by light, temperature, and moisture level [92]. The interest of the PDA membranes produced at the water-air interface stems also from their lamellar structure, their analogy with carbon-based materials and their outstanding mechanical properties with a Young modulus (measured by nanoindentation) and hardness equal to (13 ± 4) GPa and (0.21 ± 0.03) GPa respectively [96].

4. Polydopamine and living cells

In the last years, intense research has been directed to increase cellular stability and to control the interactions of cells with synthetic extracellular matrices [97]. Encapsulation of

individual living cells with reactive, functionalizable, organic shells increases the stability and durability of living cells, also in harsh environments, and provides controllability in the cell cycle, reactivity for cell-surface modification and protection against foreign aggression. For example, encapsulation of yeast (such as *Saccharomyces cerevisiae*) with either one or two deposition steps of polydopamine (**Fig. 11**), formed by covalent bonding between PDA and thiol or amine moieties of glycoproteins in the cell wall, allowed to prolong the duration of the quiescent stage preceding the division of the yeast cells up to 86 h and also to protect them against degradation by lyticase [98]. Moreover, the active catechol and amine groups in PDA allow further functionalization of yeast cells with biomolecules (*e.g.* avidin, exogenous flavin mononucleotide) [98,99]. In a study by Wang *et al.*, PDA nanocoatings of *Rhodotorula glutinis* significantly enhanced native cells' performance about their biocatalytic activity, stability and reusability in asymmetric reduction [100]. Liu and co-workers have besides demonstrated that *S. xiamenensis*-PDA coating could improve the transfer of electrons during extracellular respiration and could also promote mediated electron transfer by adsorbing self-secreted flavin molecules [99]. In addition, living electroactive bacteria could be easily encapsulated by PDA (50-200 nm film thickness) without affecting their activity, biofilm growth and biological electron transfer [101]. It is worth to mention that PDA coatings could protect bacteria from organic solvents, sonication, heat treatment and especially extreme acid shocks (pH 0.5 and 1.5) that generally cause damage to cell structures [101].

Mammalian cells like bone marrow derived mesenchymal stem cell (MSC) [102,103], human fibrosarcoma (HT1080) [104], mouse preosteoblasts (MC3T3-E1) [104] and mouse fibroblasts (NIH-3T3) [104] can selectively adhere, maintaining their morphology, to polydopamine strips (100 μm wide) patterned on poly-(dimethylsiloxane) (PDMS) substrates known to be hydrophobic and highly resistant to the cells' attachment. In addition, the cell nuclei tend to adopt a preferential orientation parallel to the direction of the PDA layer not observed on unpatterned polydopamine deposited on PDMS [102,104]. Various films consisting of PDA and poly(L-lysine) [105] as well as PDA coated on a polystyrene-based standard tissue culture plate (TCP) [106] and PDA incorporated into hydroxyapatite collagen calcium silicate materials [107], were found to aid endothelial cells adhesion [105] and proliferation, to enhance MSC attachment, proliferation and differentiation effective in *in vitro* and *in vivo* bone [107], and to boost fibronectin deposition enhancing thus migration ability mediated by integrin [106]. Noteworthy, as recently demonstrated by Deng *et al.*, PDA-coated substrates could significantly lower the levels of intracellular total reactive oxygen species (ROS) and superoxide in the cell microenvironment for 7 days if compared with uncoated materials and

also reduce the mitochondrial damage in replicative senescent MSCs [108]. ROS accumulation in stem cells, in fact, leads to proliferation and differentiation arrest and loss of homeostasis. In particular, after a 2 or 24 h coating times, PDA/TCP coated-substrates, with 0.1 or 0.5 mg/mL dopamine concentrations, were able to prevent cellular senescence up to 2 weeks as demonstrated through the quantification and staining of the senescence-associated β -galactosidase activity, the control of the levels of senescence-associated inflammatory components (such as interleukin 6) and the regulation of cellular senescence-specific genes (*e.g.*, p53 and p21) [108].

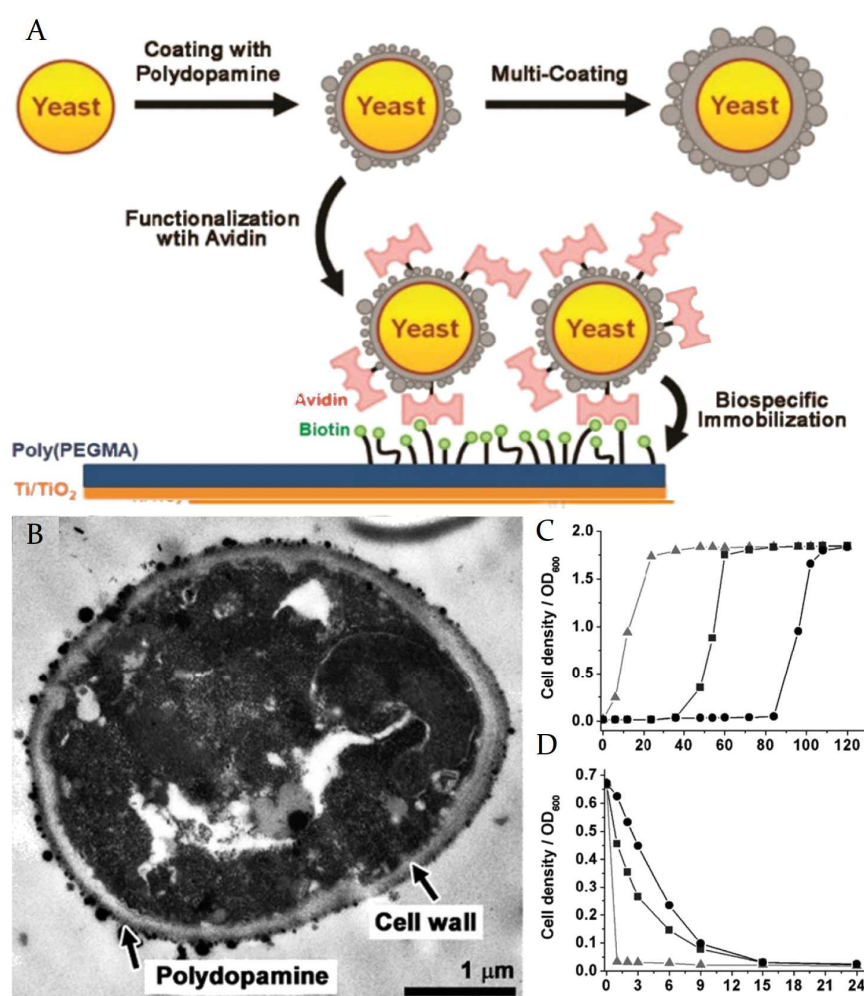


Fig. 11. PDA coating of individual yeast cells. (A): Immobilization of avidin functionalized PDA-encapsulated yeast cells. (B): TEM micrograph of microtome-sliced PDA-encapsulated yeast cell. (C): Growth curve of native yeast (\blacktriangle), single-layer PDA-encapsulated yeast cells (\blacksquare), and multi-layer PDA-encapsulated yeast cells (\bullet). (D): Survival of native yeast (\blacktriangle), single-layer PDA-encapsulated yeast cells (\blacksquare), and multi-layer PDA-encapsulated yeast cells (\bullet) in the presence of lyticase. Adapted **with permission** from: “Mussel-Inspired Encapsulation and Functionalization of Individual Yeast Cells. *J. Am. Chem. Soc.* 2011, 133 (9), 2795–2797.” Copyright (2011) American Chemical Society.

Furthermore, PDA coating on tobacco mosaic virus nanoparticles decreases the interactions with specific antibodies and allows for a prolonged stability in the organism when compared to the uncoated virus [109].

Neonatal rat cardiomyocytes were seeded in gelatin methacrylate hydrogels doped with low concentration of PDA-graphene oxide (rGO) (from 0.5 to 1.5 mg/mL). The conductivity and Young modulus of the hydrogels increased upon the addition of the PDA-rGO material (0.012-0.023 MPa) and allowed for excellent cell viability, improved alignment of α -actinin and a significant increase of the cardiomyocytes beating velocity when compared to the pristine gelatin based hydrogels [110]. Young's modulus was further increased to *ca.* 2.8 MPa in polyurethane scaffolds coated with PDA or PDA/porcine skin t gelatin A. Notably, this system significantly improved the adhesion and spreading of human umbilical vein endothelial cells colonizing the entire PDA-coated surfaces within 1-3 days post-endothelialization [111].

Conclusion and perspectives

The possibility to produce new functional materials during the oxidation of dopamine or other catecholamines in the presence of biomolecules has emerged in the last few years. Of major interest is the possibility to use specific peptide sequences to modify the self-assembly of polydopamine to yield new bioactive nanomaterials. There is strong evidence that the elemental composition and structure of polydopamine films produced in the absence of biomolecules is strongly influenced by the used oxidant and by the nature of the substrate itself. We cannot exclude, at this level, that the same holds true for the polydopamine colloidal material produced in the presence of biomolecules. For instance, concerning polydopamine produced in the presence of KE containing peptide or proteins the question remains open if the peptide or protein form a shell around polydopamine or is uniformly distributed in the obtained nanoparticles.

The interaction of oligonucleotides with polydopamine during its polymerization /self-assembly process has also been highlighted. The stability of lipid assembly seems to be notably increased in the presence of polydopamine formation offering the possibility to use polydopamine as a substituent for *S layer* proteins to produce stable lipid-based assemblies. The same holds true for polydopamine–cell assemblies where the metabolic fate of many cell types can be strongly altered and controlled in the presence of polydopamine. Despite these successful investigations and possible applications, many efforts are still required to understand

the interactions between nucleotides, polysaccharides and specific peptide sequences with polydopamine along its reaction pathway in oxidative conditions. The use of biomolecule conjugates, for instance peptide-oligonucleotide conjugates seems also of the highest interest to provide new opportunities in terms of applications. In addition, owing to the low number of investigations aimed to simulate the self-assembly or polymerization of polydopamine in the presence of biomolecules, it is of urgent interest to implement theoretical calculations at a multiscale level to better understand the structure-properties relationships of this eumelanin like material grown in an environment of biological interest.

Declaration of competing interests

The authors declare that they have no known competing financial interests or relationships that could have influenced the writing of this article.

References

- [1] Ball V. Self-Assembly Processes at Interfaces. *Interface Science and Technology* 2018;1–47.
- [2] Butt H-J, Graf K, Kappl M. *Physics and chemistry of interfaces*. Wiley-VCH; 2006.
- [3] Yao Q, Wu Z, Liu Z, Lin Y, Yuan X, Xie J. Molecular Reactivity of Thiolate-Protected Noble Metal Nanoclusters: Synthesis, Self-Assembly, and Applications. *Chemical Science* 2021;12:99–127.
- [4] Parker DM, Lineweaver AJ, Quast AD, Zharov I, Shumaker-Parry JS. Thiol-Terminated Nanodiamond Powders for Support of Gold Nanoparticle Catalysts. *Diamond and Related Materials* 2021;116:108449.
- [5] Glosz K, Stolarczyk A, Jarosz T. Siloxanes—Versatile Materials for Surface Functionalisation and Graft Copolymers. *International Journal of Molecular Sciences* 2020;21:6387.
- [6] Lee H, Dellatore SM, Miller WM, Messersmith PB. Mussel-Inspired Surface Chemistry for Multifunctional Coatings. *Science* (1979) 2007;318:426.
- [7] Tiu BDB, Delparastan P, Ney MR, Gerst M, Messersmith PB. Cooperativity of Catechols and Amines in High-Performance Dry/Wet Adhesives. *Angewandte Chemie* 2020;59:16616–24.
- [8] Krogsgaard M, Nue V, Birkedal H. Mussel-Inspired Materials: Self-Healing through Coordination Chemistry. *Chemistry – A European Journal* 2016;22:844–57.
- [9] Lee BP, Messersmith PB, Israelachvili JN, Waite JH. Mussel-Inspired Adhesives and Coatings. *Annual Review of Materials Research* 2011;41:99-132.
- [10] Kang SM, Rho J, Choi IS, Messersmith PB, Lee H. Norepinephrine: Material-Independent, Multifunctional Surface Modification Reagent. *Journal of the American Chemical Society* 2009;131:13224–5.
- [11] Hong S, Kim J, Na YS, Park J, Kim S, Singha K, et al. Poly(norepinephrine): Ultrasoother Material-Independent Surface Chemistry and Nanodepot for Nitric Oxide. *Angewandte Chemie International Edition* 2013;52:9187–91.

- [12] Jaber M, Lambert JF. A New Nanocomposite: L-DOPA/Laponite. *Journal of Physical Chemistry Letters* 2009;1:85–8.
- [13] Cui J, Iturri J, Paez J, Shafiq Z, Serrano C, d’Ischia M, et al. Dopamine-Based Coatings and Hydrogels: Toward Substitution-Related Structure–Property Relationships. *Macromolecular Chemistry and Physics* 2014;215:2403–13.
- [14] Zhu B, Edmondson S. Polydopamine-Melanin Initiators for Surface-Initiated ATRP. *Polymer (Guildf)* 2011;52:2141–9.
- [15] Lee H, Rho J, Messersmith PB. Facile Conjugation of Biomolecules onto Surfaces via Mussel Adhesive Protein Inspired Coatings. *Advanced Materials* 2009;21:431–4.
- [16] Bernsmann F, Frisch B, Ringwald C, Ball V. Protein Adsorption on Dopamine–Melanin Films: Role of Electrostatic Interactions Inferred from ζ -Potential Measurements Versus Chemisorption. *Journal of Colloid and Interface Science* 2010;344:54–60.
- [17] Ball V, del Frari D, Michel M, Buehler MJ, Toniazzo V, Singh MK, et al. Deposition Mechanism and Properties of Thin Polydopamine Films for High Added Value Applications in Surface Science at the Nanoscale. *BioNanoScience* 2011 2:1 2011;2:16–34.
- [18] Lyngø ME, van der Westen R, Postma A, Städler B. Polydopamine—a Nature-Inspired Polymer Coating for Biomedical Science. *Nanoscale* 2011;3:4916–28.
- [19] Dreyer DR, Miller DJ, Freeman BD, Paul DR, Bielawski CW. Perspectives on Poly(Dopamine). *Chemical Science* 2013;4:3796–802.
- [20] Ball V. Physicochemical Perspective on “Polydopamine” and “Poly(Catecholamine)” Films for their Applications in Biomaterial Coatings. *Biointerphases* 2014;9:030801.
- [21] Liu Y, Ai K, Lu L. Polydopamine and Its Derivative Materials: Synthesis and Promising Applications in Energy, Environmental, and Biomedical Fields. *Chemical Reviews* 2014;114:5057–115.
- [22] d’Ischia M, Napolitano A, Ball V, Chen CT, Buehler MJ. Polydopamine and Eumelanin: From Structure–Property Relationships to a Unified Tailoring Strategy. *Accounts of Chemical Research* 2014;47:3541–50.
- [23] Li H, Jiang B, Li J. Recent Advances in Dopamine-Based Materials Constructed Via One-Pot Co-Assembly Strategy. *Advances in Colloid and Interface Science* 2021;295:102489.
- [24] Qiu WZ, Yang HC, Xu ZK. Dopamine-Assisted Co-Deposition: An Emerging and Promising Strategy for Surface Modification. *Advances in Colloid and Interface Science* 2018;256:111–25.
- [25] Hong SH, Hong S, Ryou MH, Choi JW, Kang SM, Lee H. Sprayable Ultrafast Polydopamine Surface Modifications. *Advanced Materials Interfaces* 2016;3:1500857.
- [26] Li S, Wang H, Young M, Xu F, Cheng G, Cong H. Properties of Electropolymerized Dopamine and Its Analogues. *Langmuir* 2018;35:1119–25.
- [27] Stöckle B, Ng DYW, Meier C, Paust T, Bischoff F, Diemant T, et al. Precise Control of Polydopamine Film Formation by Electropolymerization. *Macromolecular Symposia* 2014;346:73–81.

- [28] Bernsmann F, Voegel JC, Ball V. Different Synthesis Methods Allow to Tune the Permeability and Permselectivity of Dopamine–Melanin Films to Electrochemical Probes. *Electrochimica Acta* 2011;56:3914–9.
- [29] Li Y, Liu M, Xiang C, Xie Q, Yao S. Electrochemical Quartz Crystal Microbalance Study on Growth and Property of the Polymer Deposit at Gold Electrodes During Oxidation of Dopamine in Aqueous Solutions. *Thin Solid Films* 2006;497:270–8.
- [30] Wang S, Wannasarit S, Figueiredo P, Li J, Correia A, Xia B, et al. Superfast and Controllable Microfluidic Inking of Anti-Inflammatory Melanin-Like Nanoparticles Inspired by Cephalopods. *Materials Horizons* 2020;7:1573–80.
- [31] Sheng W, Li B, Wang X, Dai B, Yu B, Jia X, et al. Brushing up from “Anywhere” under Sunlight: A Universal Surface-Initiated Polymerization from Polydopamine-Coated Surfaces. *Chemical Science* 2015;6:2068–73.
- [32] Lee M, Lee SH, Oh IK, Lee H. Microwave-Accelerated Rapid, Chemical Oxidant-Free, Material-Independent Surface Chemistry of Poly(dopamine). *Small* 2017;13:1600443.
- [33] Wang Z, Xu C, Lu Y, Wei G, Ye G, Sun T, et al. Microplasma-Assisted Rapid, Chemical Oxidant-Free and Controllable Polymerization of Dopamine for Surface Modification. *Polymer Chemistry* 2017;8:4388–92.
- [34] Zheng W, Fan H, Wang L, Jin Z. Oxidative Self-Polymerization of Dopamine in an Acidic Environment. *Langmuir* 2015;31:11671–7.
- [35] Pezzella A, Barra M, Musto A, Navarra A, Alfè M, Manini P, et al. Stem Cell-Compatible Eumelanin Biointerface Fabricated by Chemically Controlled Solid State Polymerization. *Materials Horizons* 2015;2:212–20.
- [36] Zhang C, Ou Y, Lei W-X, Wan L-S, Ji J, Xu Z-K. $\text{CuSO}_4/\text{H}_2\text{O}_2$ -Induced Rapid Deposition of Polydopamine Coatings with High Uniformity and Enhanced Stability. *Angewandte Chemie* 2016;128:3106–9.
- [37] El Yakhlifi S, Alfieri ML, Arntz Y, Eredia M, Ciesielski A, Samorì P, et al. Oxidant-Dependent Antioxidant Activity of Polydopamine Films: The Chemistry-Morphology Interplay. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2021;614:126134.
- [38] Ponzio F, Barthès J, Bour J, Michel M, Bertani P, Hemmerlé J, et al. Oxidant Control of Polydopamine Surface Chemistry in Acids: A Mechanism-Based Entry to Superhydrophilic-Superoleophobic Coatings. *Chemistry of Materials* 2016;28:4697–705.
- [39] Du X, Li L, Li J, Yang C, Frenkel N, Welle A, et al. UV-Triggered Dopamine Polymerization: Control of Polymerization, Surface Coating, and Photopatterning. *Advanced Materials* 2014;26:8029–33.
- [40] Alfieri ML, Panzella L, Oscurato SL, Salvatore M, Avolio R, Errico ME, et al. The Chemistry of Polydopamine Film Formation: The Amine–Quinone Interplay. *Biomimetics* 2018;3:26.
- [41] Della Vecchia NF, Avolio R, Alfè M, Errico ME, Napolitano A, d’Ischia M. Building-Block Diversity in Polydopamine Underpins a Multifunctional Eumelanin-Type Platform Tunable Through a Quinone Control Point. *Advanced Functional Materials* 2013;23:1331–40.

- [42] Liebscher J, Mrówczyński R, Scheidt HA, Filip C, Haidade ND, Turcu R, et al. Structure of Polydopamine: A Never-Ending Story? *Langmuir* 2013;29:10539–48.
- [43] Alfieri ML, Micillo R, Panzella L, Crescenzi O, Oscurato SL, Maddalena P, et al. Structural Basis of Polydopamine Film Formation: Probing 5,6-Dihydroxyindole-Based Eumelanin Type Units and the Porphyrin Issue. *ACS Applied Materials and Interfaces* 2018;10:7670–80.
- [44] Hong S, Na YS, Choi S, Song IT, Kim WY, Lee H. Non-Covalent Self-Assembly and Covalent Polymerization Co-Contribute to Polydopamine Formation. *Advanced Functional Materials* 2012;22:4711–7.
- [45] Hong S, Wang Y, Park SY, Lee H. Progressive Fuzzy Cation- Assembly of Biological Catecholamines. *Science Advances* 2018;4:7457–64.
- [46] d’Ischia M, Napolitano A, Pezzella A, Meredith P, Sarna T. Chemical and Structural Diversity in Eumelanins: Unexplored Bio-Optoelectronic Materials. *Angewandte Chemie International Edition* 2009;48:3914–21.
- [47] Iacomino M, Mancebo-Aracil J, Guardingo M, Martín R, D’Errico G, Perfetti M, et al. Replacing Nitrogen by Sulfur: from Structurally Disordered Eumelanins to Regioregular Thiomelanin Polymers. *International Journal of Molecular Sciences* 2017;18.
- [48] Ball V. Polydopamine Nanomaterials: Recent Advances in Synthesis Methods and Applications. *Frontiers in Bioengineering and Biotechnology* 2018;6:109.
- [49] El Yakhlifi S, Ball V. Polydopamine as a Stable and Functional Nanomaterial. *Colloids and Surfaces B: Biointerfaces* 2020;186:110719.
- [50] Ball V. Activity of Alkaline Phosphatase Adsorbed and Grafted on “Polydopamine” Films. *Journal of Colloid and Interface Science* 2014;429:1–7.
- [51] Xu C, Xia Y, Wang L, Nan X, Hou J, Guo Y, et al. Polydopamine-Assisted Immobilization of Silk Fibroin and its Derived Peptide on Chemically Oxidized Titanium to Enhance Biological Activity in Vitro. *International Journal of Biological Macromolecules* 2021;185:1022–35.
- [52] Chen D, Zhao L, Hu W. Protein Immobilization and Fluorescence Quenching on Polydopamine Thin Films. *Journal of Colloid and Interface Science* 2016;477:123–30.
- [53] Amaly N, El-Moghazy AY, Sun G. Fabrication of Polydopamine-Based NIR-light Responsive Imprinted Nanofibrous Membrane for Effective Lysozyme Extraction and Controlled Release from Chicken Egg White. *Food Chemistry* 2021;357:129613.
- [54] Sieste S, Mack T, Synatschke C v., Schilling C, Meyer zu Reckendorf C, Pendi L, et al. Water-Dispersible Polydopamine-Coated Nanofibers for Stimulation of Neuronal Growth and Adhesion. *Advanced Healthcare Materials* 2018;7:1701485.
- [55] Bernsmann F, Ponche A, Ringwald C, Hemmerle J, Raya J, Bechinger B, et al. Characterization of Dopamine-Melanin Growth on Silicon Oxide. *Journal of Physical Chemistry C* 2009;113:8234–42.
- [56] Clancy CMR, Simon JD. Ultrastructural Organization of Eumelanin from *Sepia officinalis* Measured by Atomic Force Microscopy. *Biochemistry* 2001;40:13353–60.

- [57] Chassepot A, Ball V. Human Serum Albumin and Other Proteins as Templating Agents for the Synthesis of Nanosized Dopamine-Eumelanin. *Journal of Colloid and Interface Science* 2014;414:97–102.
- [58] Crivellato E, Nico B, Ribatti D. The Chromaffin Vesicle: Advances in Understanding the Composition of a Versatile, Multifunctional Secretory Organelle. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology* 2008;291:1587–602.
- [59] Bergtold C, Hauser D, Chaumont A, El Yakhlifi S, Mateescu M, Meyer F, et al. Mimicking the Chemistry of Natural Eumelanin Synthesis: The KE Sequence in Polypeptides and in Proteins Allows for a Specific Control of Nanosized Functional Polydopamine Formation. *Biomacromolecules* 2018;19:3693–704.
- [60] El Yakhlifi S, Ihiawakrim D, Ersen O, Ball V. Enzymatically Active Polydopamine @ Alkaline Phosphatase Nanoparticles Produced by NaIO_4 Oxidation of Dopamine. *Biomimetics* 2018;3:36.
- [61] Harvey S, Ng DYW, Szelwicka J, Hueske L, Veith L, Raabe M, et al. Facile Synthesis of Ultrasmall Polydopamine-Polyethylene Glycol Nanoparticles for Cellular Delivery. *Biointerphases* 2018;13:06D407.
- [62] Lampel A, McPhee SA, Park HA, Scott GG, Humagain S, Hekstra DR, et al. Polymeric Peptide Pigments with Sequence-Encoded Properties. *Science (1979)* 2017;356:1064–8.
- [63] Prota Giuseppe. *Melanins and Melanogenesis*. San Diego, CA: Academic Press; 1992.
- [64] Richter M, Schulenburg C, Jankowska D, Heck T, Faccio G. Novel Materials through Nature's Catalysts. *Materials Today* 2015;18:459–67.
- [65] Dhand C, Harini S, Venkatesh M, Dwivedi N, Ng A, Liu S, et al. Multifunctional Polyphenols- and Catecholamines-Based Self-Defensive Films for Health Care Applications. *ACS Applied Materials and Interfaces* 2016;8:1220–32.
- [66] Tan Y, Deng W, Li Y, Huang Z, Meng Y, Xie Q, et al. Polymeric Bionanocomposite Cast Thin Films with in Situ Laccase-Catalyzed Polymerization of Dopamine for Biosensing and Biofuel Cell Applications. *Journal of Physical Chemistry B* 2010;114:5016–24.
- [67] Dai M, Huang T, Chao L, Xie Q, Tan Y, Chen C, et al. Horseradish Peroxidase-Catalyzed Polymerization of L-DOPA for Mono-/Bi-Enzyme Immobilization and Amperometric Biosensing of H_2O_2 and Uric Acid. *Talanta* 2016;149:117–23.
- [68] Dai M, Huang T, Chao L, Tan Y, Chen C, Meng W, et al. Tyrosinase-Catalyzed Polymerization of L-DOPA (Versus L-Tyrosine and Dopamine) to Generate Melanin-Like Biomaterials for Immobilization of Enzymes and Amperometric Biosensing. *RSC Advances* 2016;6:17016–22.
- [69] Kim S, Hong S. Nature-Inspired Adhesive Catecholamines for Highly Concentrated Colorimetric Signal in Spatial Biomarker Labeling. *Advanced Healthcare Materials* 2020;9:2000540.
- [70] Alfieri ML, Panzella L, Arntz Y, Napolitano A, Ball V, d'ischia M. A Clean and Tunable Mussel-Inspired Coating Technology by Enzymatic Deposition of Pseudo-Polydopamine (ψ -PDA) Thin Films from Tyramine. *International Journal of Molecular Sciences* 2020;21:4873.
- [71] Kim JY, Kim W il, Youn W, Seo J, Kim BJ, Lee JK, et al. Enzymatic Film Formation of Nature-Derived Phenolic Amines. *Nanoscale* 2018;10:13351–5.

- [72] Zhong Q, Richardson JJ, Li S, Zhang W, Ju Y, Li J, et al. Expanding the Toolbox of Metal–Phenolic Networks via Enzyme-Mediated Assembly. *Angewandte Chemie* 2020;132:1728–34.
- [73] Guazzaroni M, Crestini C, Saladino R. Layer-by-Layer Coated Tyrosinase: An Efficient and Selective Synthesis of Catechols. *Bioorganic & Medicinal Chemistry* 2012;20:157–66.
- [74] Kim N, Lee H, Han SY, Kim BJ, Choi IS. Enzyme-Mediated Film Formation of Melanin-Like Species from Ortho-Diphenols: Application to Single-Cell Nanoencapsulation. *Applied Surface Science Advances* 2021;5:100098.
- [75] Allais M, Meyer F, Ball V. Multilayered Films Made from Tannic Acid and Alkaline Phosphatase with Enzymatic Activity and Electrochemical Behavior. *Journal of Colloid and Interface Science* 2018;512:722–9.
- [76] Lee H, Nguyen DT, Kim N, Han SY, Hong YJ, Yun G, et al. Enzyme-Mediated Kinetic Control of Fe³⁺-Tannic Acid Complexation for Interface Engineering. *ACS Applied Materials and Interfaces* 2021;13:52385–94.
- [77] Griffith JD. DNA Structure: Evidence from Electron Microscopy. *Science* (1979) 1978;201:525–7.
- [78] Ding T, Xing Y, Wang Z, Guan H, Wang L, Zhang J, et al. Structural Complementarity from DNA for Directing Two-Dimensional Polydopamine Nanomaterials with Biomedical Applications. *Nanoscale Horizons* 2019;4:652–7.
- [79] Winterwerber P, Whitfield CJ, Ng DYW, Weil T. Multiple Wavelength Photopolymerization of Stable Poly(Catecholamines)-DNA Origami Nanostructures. *Angewandte Chemie International Edition* 2022:e202111226.
- [80] Winterwerber P, Harvey S, Ng DYW, Weil T. Photocontrolled Dopamine Polymerization on DNA Origami with Nanometer Resolution. *Angewandte Chemie International Edition* 2020;59:6144–9.
- [81] Tokura Y, Harvey S, Chen C, Wu Y, Ng DYW, Weil T. Fabrication of Defined Polydopamine Nanostructures by DNA Origami-Templated Polymerization. *Angewandte Chemie* 2018;57:1587–91.
- [82] Ma L, Liu J. Catalytic Nucleic Acids: Biochemistry, Chemical Biology, Biosensors, and Nanotechnology. *iScience* 2020;23:100815.
- [83] Zhang M, Wang S, Li L, Li M, Cheng X, Shi Y, et al. Digital Numbers Constructed by Fine Patterned Polydopamine on DNA Templates. *Macromolecular Rapid Communications* 2021;42:2100441.
- [84] Zhao H, Zhang Z, Zuo D, Li L, Li F, Yang D. A Synergistic DNA-Polydopamine-MnO₂Nanocomplex for Near-Infrared-Light-Powered DNAzyme-Mediated Gene Therapy. *Nano Letters* 2021;21:5377–85.
- [85] Wang Z, Zhang J, Chen F, Cai K. Fluorescent miRNA Analysis Enhanced by Mesopore Effects of Polydopamine Nanoquenchers. *Analyst* 2017;142:2796–804.
- [86] Xie X, Tang J, Xing Y, Wang Z, Ding T, Zhang J, et al. Intervention of Polydopamine Assembly and Adhesion on Nanoscale Interfaces: State-of-the-Art Designs and Biomedical Applications. *Advanced Healthcare Materials* 2021;10:2002138.

- [87] Ye Y, Zheng L, Wu T, Ding X, Chen F, Yuan Y, et al. Size-Dependent Modulation of Polydopamine Nanospheres on Smart Nanoprobes for Detection of Pathogenic Bacteria at Single-Cell Level and Imaging-Guided Photothermal Bactericidal Activity. *ACS Applied Materials and Interfaces* 2020;12:35626–37.
- [88] Wang Z, Wang L, Prabhakar N, Xing Y, Rosenholm JM, Zhang J, et al. CaP Coated Mesoporous Polydopamine Nanoparticles with Responsive Membrane Permeation Ability for Combined Photothermal and siRNA Therapy. *Acta Biomaterialia* 2019;86:416–28.
- [89] Brooks AK, Imran M, Pradhan S, Broitman JM, Yadavalli VK. Facile Fabrication and Nanoscale Assembly of Polydopamine-Functionalized, Flexible Chitosan Films: *Journal of Bioactive and Compatible Polymers* 2021;36:380–93.
- [90] Gholami Derami H, Jiang Q, Ghim D, Cao S, Chandar YJ, Morrissey JJ, et al. A Robust and Scalable Polydopamine/Bacterial Nanocellulose Hybrid Membrane for Efficient Wastewater Treatment. *ACS Applied Nano Materials* 2019;2:1092–101.
- [91] Nirasay S, Badia A, Leclair G, Claverie JP, Marcotte I. Polydopamine-Supported Lipid Bilayers. *Materials* 2012;5:2621–36.
- [92] Neveshkin A, Citak F, Ball V, Winterhalter M. Polydopamine Coating to Stabilize a Free-Standing Lipid Bilayer for Channel Sensing. *Langmuir* 2017;33:7256–62.
- [93] Lavallo P, Voegel JC, Vautier D, Senger B, Schaaf P, Ball V. Dynamic Aspects of Films Prepared by a Sequential Deposition of Species: Perspectives for Smart and Responsive Materials. *Advanced Materials* 2011;23:1191–221.
- [94] Bernsmann F, Richert L, Senger B, Lavallo P, Voegel JC, Schaaf P, et al. Use of Dopamine Polymerisation to Produce Free-Standing Membranes from (PLL-HA)_n Exponentially Growing Multilayer Films. *Soft Matter* 2008;4:1621–4.
- [95] Marchesi D'Alvise T, Harvey S, Hueske L, Szelwicka J, Veith L, Knowles TPJ, et al. Ultrathin Polydopamine Films with Phospholipid Nanodiscs Containing a Glycophorin A Domain. *Advanced Functional Materials* 2020;30:2000378.
- [96] Coy E, Iatsunskiy I, Colmenares JC, Kim Y, Mrówczyński R. Polydopamine Films with 2D-like Layered Structure and High Mechanical Resilience. *ACS Applied Materials and Interfaces* 2021;13:23113–20.
- [97] Ariga K, Fakhrullin R, Ariga K. Nanoarchitectonics on Living Cells. *RSC Advances* 2021;11:18898–914.
- [98] Yang SH, Kang SM, Lee KB, Chung TD, Lee H, Choi IS. Mussel-Inspired Encapsulation and Functionalization of Individual Yeast Cells. *J Am Chem Soc* 2011;133:2795–7.
- [99] Liu SR, Cai LF, Wang LY, Yi XF, Peng YJ, He N, et al. Polydopamine Coating on Individual Cells for Enhanced Extracellular Electron Transfer. *Chemical Communications* 2019;55:10535–8.
- [100] Wang L, Hu ZY, Yang XY, Zhang BB, Geng W, van Tendeloo G, et al. Polydopamine Nanocoated Whole-Cell Asymmetric Biocatalysts. *Chemical Communications* 2017;53:6617–20.
- [101] Du Q, Li T, Li N, Wang X. Protection of Electroactive Biofilm from Extreme Acid Shock by Polydopamine Encapsulation. *Environmental Science and Technology Letters* 2017;4:345–9.

- [102] Sharma D, Jia W, Long F, Pati S, Chen Q, Qyang Y, et al. Polydopamine and Collagen Coated Micro-Grated Polydimethylsiloxane for Human Mesenchymal Stem Cell Culture. *Bioactive Materials* 2019;4:142–50.
- [103] Chuah YJ, Koh YT, Lim K, Menon N v., Wu Y, Kang Y. Simple Surface Engineering of Polydimethylsiloxane with Polydopamine for Stabilized Mesenchymal Stem Cell Adhesion and Multipotency. *Scientific Reports* 2015 5:1 2015;5:1–12.
- [104] Ku SH, Lee JS, Park CB. Spatial Control of Cell Adhesion and Patterning through Mussel-Inspired Surface Modification by Polydopamine. *Langmuir* 2010;26:15104–8.
- [105] Zhang Y, Lynge ME, Teo BM, Ogaki R, Städler B. Mixed Poly(Dopamine)/Poly(L-Lysine) (Composite) Coatings: from Assembly to Interaction with Endothelial Cells. *Biomaterials Science* 2015;3:1188–96.
- [106] Deng Z, Wang W, Xu X, Ma N, Lendlein A. Polydopamine-Based Biofunctional Substrate Coating Promotes Mesenchymal Stem Cell Migration. *MRS Advances* 2021;6:739–44.
- [107] Lee DJ, Lee YT, Zou R, Daniel R, Ko CC. Polydopamine-Laced Biomimetic Material Stimulation of Bone Marrow Derived Mesenchymal Stem Cells to Promote Osteogenic Effects. *Scientific Reports* 2017 7:1 2017;7:1–14.
- [108] Deng Z, Wang W, Xu X, Nie Y, Liu Y, Gould OEC, et al. Biofunction of Polydopamine Coating in Stem Cell Culture. *ACS Applied Materials and Interfaces* 2021;13:10748–59.
- [109] Nkanga CI, Chung YH, Shukla S, Zhou J, Jokerst J v., Steinmetz NF. The in Vivo Fate of Tobacco Mosaic Virus Nanoparticle Theranostic Agents Modified by the Addition of a Polydopamine Coat. *Biomaterials Science* 2021;9:7134–50.
- [110] Li XP, Qu KY, Zhou B, Zhang F, Wang YY, Abodunrin OD, et al. Electrical Stimulation of Neonatal Rat Cardiomyocytes Using Conductive Polydopamine-Reduced Graphene Oxide-Hybrid Hydrogels for Constructing Cardiac Microtissues. *Colloids and Surfaces B: Biointerfaces* 2021;205:111844.
- [111] Kopeć K, Wojasiński M, Eichler M, Genç H, Friedrich RP, Stein R, et al. Polydopamine and Gelatin Coating for Rapid Endothelialization of Vascular Scaffolds. *Materials Science and Engineering: C* 2021:112544.

