Polymeric peptide pigments with sequence-encoded properties

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Melanins are a family of heterogeneous polymeric pigments that provide ultraviolet (UV) light protection, structural support, coloration, and free radical scavenging. Formed by oxidative oligomerization of catecholic small molecules, the physical properties of melanins are influenced by covalent and noncovalent disorder. We report the use of tyrosine-containing tripeptides as tunable precursors for polymeric pigments. In these structures, phenols are presented in a (supra-)molecular context dictated by the positions of the amino acids in the peptide sequence. Oxidative polymerization can be tuned in a sequence-dependent manner, resulting in peptide sequence-encoded properties such as UV absorbance, morphology, coloration, and electrochemical properties over a considerable range. Short peptides have low barriers to application and can be easily scaled, suggesting near-term applications in cosmetics and biomedicine.

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To shed more light on the organization of the tripeptides, including those for which crystal structures could not be obtained under the assembly conditions, we used Fourier transform infrared spectroscopy (FTIR) (Fig. 2D). FYD, YDF, and FFD did not show evidence of periodically organized intermolecular interactions, as indicated by broad bands at 1652 cm⁻¹ (internal amide) and 1672 cm⁻¹ (terminal amide) in the FTIR spectrum, which is in agreement with disorder observed by TEM. For the assembled DFY and DFF peptides, these bands redshifted to 1620 to 1640 cm⁻¹ and 1658 cm⁻¹, respectively, which suggests a β-sheet-like organization. The YFD spectrum implies a different packing geometry, with additional narrow, redshifted bands in the amide region, but an additional shift of the aspartate carboxylate band from 1580 cm⁻¹ to 1560 cm⁻¹; this suggests intramolecular salt bridge formation with the amine group of the N terminus, in agreement with the crystal structure (Fig. 2C), which helps to stabilize paired aromatics in the syn configuration.

The six peptides showed variable crystallinity (Figs. S9 to S14). FYD, YDF, and DFF formed highly crystalline materials, and DFF exhibited lower crystallinity, evidenced by the peak intensity and broadness. In contrast, FYD and YDF formed amorphous materials. However, all the peptides shared some common features in terms of molecular stacking, reflected by the peaks in the ranges of 4.4 to 4.8 Å and 2.9 to 3.2 Å. In addition, the diffraction patterns of FYD and DFF were similar, indicating the structural resemblance of these two peptides.

To examine the relative stability of the different conformations of the monomers, molecular dynamics simulations were carried out (25, 26) (Fig. 2, E and F). The results demonstrate that the six peptides have different preferential conformations, which is reflected both in the assembled state and in solution, depending in a pairwise manner on the position of the aspartic acid. The following relationships exist: Tyrosine and phenylalanine residues are presented in anti (DXX) or syn (XXD) configuration, which is in agreement with the crystal structures obtained. When the aspartic acid is in the central position (DXX), the preferred conformations have dihedral angles of –90°, which limits the potential for extended stacking.

We subsequently investigated whether the pairwise sequence-dependent supramolecular order of the peptides influences enzymatic oxidation and further polymerization pathways. Wide-angle x-ray scattering (WAXS) and solid-phase FTIR data showed loss of order, with the strongest effect observed for oxidized DXX (DXXox) and less for oxidized XDX (XXDox), whereas oxidized XDX (XXDox) remained disordered (Fig. 3, A to C). The peptides lost supramolecular order (Fig. 3, A and B, and Figs. S9 to S14), retaining few structural features corresponding to the molecular packing, according to the peaks at about 4.5 and 2.9 Å. The FTIR spectra (Fig. 3C) showed narrow, redshifted absorptions of the amide group in FYD, YDF, DFF, and DFF that disappeared following oxidation in favor of broad absorptions at 1650 to 1675 cm⁻¹. Additionally, tyrosine-specific ring modes were lost (e.g., 1516 cm⁻¹), and a new band absorption assigned to quinone was observed around 1680 cm⁻¹, confirming catechol oxidation.

High-performance liquid chromatography (HPLC) analysis showed (near-)complete conversions of peptides to oxidation products for both the disordered (XDXox) and highly ordered (DXXox) peptides, with lower conversions observed for XDXox (YDFox giving lower conversion than YDFox) (Table S2). Under the conditions examined, peptide assembly had a more pronounced effect on oxidation and polymerization than did the position of the aspartic within the tripeptides. Early-stage conversions were higher for XDXox peptides than for the assembling counterparts (Fig. S15). However, early-stage kinetics were similar for the non-assembling FYDox and YDFox. We conclude that the overall polymerization process is dictated more by the supramolecular order of the precursors and less by enzyme affinity.

Liquid chromatography–mass spectrometry (LC-MS) data obtained after 24 hours of oxidation revealed the expected catechol and quinone, as well as a wide range of dimeric and trimeric species with different connections (Fig. 3, D to F, and Table S3). A pairwise relationship is again clear from these data, with XDXox peptides giving rise to complete conversion of the precursors to oligomers and polymers, XDXox giving medium conversion with intermediate polymerization, and DXXox peptides giving rise to formation of extensive oligomeric and polymeric species (table S3 and Fig. 3D). In each case, the ultraviolet (UV) absorbance of the polymeric species was substantially redshifted from precursors, as would be expected for an extensive catechol-quinone network.

The polymers had distinct morphologies as observed by optical microscopy (Fig. 4A). Although YDFox maintained a 1D morphology, it formed much larger fibers, suggesting a degree of lateral aggregation that may be facilitated by the positioning of 2D sheets of reactive aromatic species at interfaces, as seen in the crystal structure (Fig. 2C). DFXox polymerized into extended 2D sheets, whereas DFXox formed spheres. These morphologies, and the amorphous structures of the other oxidized peptides, were also observed by TEM (Fig. S16). Whereas the DFXox sheets and YDFox fibers were in the solid phase, the DFXox spheres remained dispersed in the aqueous buffer.

The greatest contrast among structures was evident in DXXox tripeptides, which, starting from similar molecular packing of the precursors, showed high levels of polymerization accompanied by loss of order. For these peptides, a subtle difference in sequence dictated the initial (crystalline fibers versus supramolecular fibers) and oxidized (spheres versus sheets) morphologies. For DFXox, we propose that the anti conformation of aromatic side chains is favorable for polymerization along the length of the β-sheet but also laterally between neighboring fibrils, eventually resulting in loss of supramolecular structures (fibrils) and formation of extended, micron-scale 2D sheets. Time-course TEM analysis of DFXox (Fig. S17) supports this mechanism for the fiber-to-sheet transition.
revealing the formation of dark layers on the fibrils' surface (4 hours); these layers further assembled and polymerized into 2D sheets that extended from the fiber surface (1 week). For DYF, a different orientation of tyrosine gave rise to an additional stabilizing interaction (Y-Y) within the crystal lattice (27) (Fig. 2B). Oxidation of tyrosine eliminated hydrogen bonding in these residues, thereby disrupting the crystalline fiber and reconfiguring the peptides into spherical assemblies. These data are in agreement with the loss of the original packing and subsequent polymerization observed for both DFY_{ox} and DYF_{ox} by FTIR, WAXS, and LC-MS (Fig. 3).

The results show that supramolecular order in peptide precursors can be systematically converted into disordered polymeric pigments, resulting in variable characteristics that relate to their functionality (Fig. 4). UV-visible (UV-Vis) measurements showed different broadband spectra, with DFY_{ox} showing absorption throughout the visible region (420 to 650 nm) and high absorption observed for FYD_{ox}, possibly contributed by highly scattering aggregates (Fig. 4B). The observed maximum around 340 nm for YFD_{ox} together with the LC-MS (Fig. 3, D and E) and HPLC analyses (fig. S15D), suggest that the N-terminal positioning of the catechol results in a lower degree of connectivity and cross-linking.

Oxidized peptides were configured into cathodes in aqueous half-cell configurations. The charge storage capacity can provide an estimate of the concentration of redox-active components, and the shape of the discharge curve can provide insight into the distribution of morphological...
phases. For this purpose, electrodes were fabricated by compacting peptide melanin powders into a stainless steel support mesh (Fig. 4C) (28). For all systems tested, the potentials became monotonically more negative during discharge, which confirms that these materials are largely disordered. DFYox 2D sheets exhibited the highest specific charge storage capacity, followed by DYFox (Fig. 4, D and E), which is attributed to an increase in the concentration of redox-active tyrosine-based derivatives (2, 29) and is confirmed by cyclic voltammetry (CV) (fig. S18). Capacitive storage is the likely source of differential capacities in cathodes composed of YFDox versus FYDox, which are otherwise largely devoid of redox behavior as assessed by CV. The specific capacity of DFYox is comparable to that measured in natural eu-melanin cathodes and less than that of the synthetic melanin-based cathodes (fig. S19). CV of DFYox-based cathodes showed multiple redox peaks that are not commonly observed in other types of natural and synthetic melanin-based pigments (30), which are attributed to the presence of multiple types of polyphenols with a variety of redox behaviors. Electron paramagnetic resonance (EPR) suggests that DFYox sheets exhibit the highest gravimetric concentration of radical content.

Fig. 3. From order to disorder in polymeric peptide pigments. (A and B) WAXS analysis including 1D (A) and 2D (B) patterns of tripeptides before [black in (A)] or after [red in (A)] 24 hours of enzymatic oxidation. (C) FTIR absorption spectra of tripeptides before (solid lines) or after (dashed lines) 24 hours of enzymatic oxidation. (D) LC-MS chromatograms at 280 nm (black) and 350 nm (red) of the soluble fraction of tripeptides oxidized for 24 hours. Numbers refer to (F). (E) Summed mass/charge (m/z) intensities of soluble higher-molecular-weight polymers composed of heterogeneously connected monomers (“4” in (F)] eluted between 8 and 10 min. (F) Chemical structures of the nonoxidized peptides (“1”) and the oxidation products 3,4-dihydroxyphenylalanine (“2”) and 3,4-quinone (“3”) in the context of tripeptides, “4,” connectivity of potential aryl cross-linked and Michael addition products (supplementary materials).
among the polymeric peptide pigments (fig. S20 and table S4). We propose that relatively higher semiquinone concentrations correspond to not only higher overall concentrations of catechols, but also molecular configurations that permit supramolecular interactions. The attenuated EPR signal observed in the polymeric peptide pigments is consistent with this model, owing to the smaller overall catechol concentrations and dihedral barriers to application and can be easily scaled, they offer great promise for a variety of uses. We demonstrate the ability to leverage different assembly strategies with emerging optical microscopy. Scale bars for FYDox, YFDox, and DFyox, 20 μm; for FYFyox, 10 μm. (B) UV-Vis absorption spectra of solution fractions of polymeric peptide pigments and oxidized tyrosine. (C) Macroscopic image of polymeric peptide pigment electrode and schematic illustration of the electrochemical cell used for discharge measurements. (D) Electrochemical potential profiles and (E) average specific capacity of polymeric peptide pigments. Error bars, standard errors (n = 3). MSE, mercury/mercurous sulfate electrode; Ah, ampere hour.

Fig. 4. Morphology, UV-Vis absorption, and electrochemical properties of polymeric peptide pigments. (A) Structures formed by the polymeric peptide pigments at the micron scale, observed using optical microscopy. Scale bars for FYDox, YFDox, and DFyox, 20 μm; for FYFyox, 10 μm. (B) UV-Vis absorption spectra of solution fractions of polymeric peptide pigments and oxidized tyrosine. (C) Macroscopic image of polymeric peptide pigment electrode and schematic illustration of the electrochemical cell used for discharge measurements. (D) Electrochemical potential profiles and (E) average specific capacity of polymeric peptide pigments. Error bars, standard errors (n = 3). MSE, mercury/mercurous sulfate electrode; Ah, ampere hour.

REFERENCES AND NOTES
28. We used the solid-phase sheets for DFyox, the dispersed solution phase for spherical FYFyox, and the solution phases for FYDox and YFDox, because of the presence of substantial starting materials in the solid phase (fig. S16 and table S2).

ACKNOWLEDGMENTS
The research leading to these results has received funding from the U.S. Air Force Office of Scientific Research (grant FA9550-15-1-0392). A.L is funded by the Planning and Budget Committee of the Israel Council for Higher Education. P.W.J.M.F. is funded by the Netherlands Organization for Scientific Research (Veni program, grant number 722.015.005). The authors are grateful for support from the Materials Research Science and Engineering Center program of the National Science Foundation (NSF) under award numbers DMR-0820341 and DMR-1420073 and for the assistance of C.-H. (J.) Chen at the University of Indiana and Y.-S. Chen at the ChemMatCARS Sector 15 of the Advanced Photon Source (APS), which is principally supported by the NSF (grant number CHE-1460572). Use of the APS, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under contract no. DE-AC02-06CH11357. We thank A. Bykov (Department of Physics, City College of New York) for help with WAXS analysis and J. Gu and V. M. Mxoxon (Department of Physics, City College of New York) for help with UV-Vis analysis. Hunter Mass Spectrometry is supported by the City University of New York, the NSF, and the National Institute on Minority Health and Health Disparities (NIMHD) of the NIH. Results were obtained using the Engineering and Physical Sciences Research Council-funded ARCHIE-WeSt High Performance Computer (www.archie-we.st.ac.uk; grant number EP/K000986/1). The City University of New York has filed a provisional patent application (serial number 62/385,544) for technology related to this work.

SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/356/6342/1064/suppl/DC1 Materials and Methods Figs. S1 to S20 Tables S1 to S4 References (31, 32) Movies S1 and S2 Data S1 29 November 2016; accepted 8 May 2017 10.1126/science.aal5005
Designing molecular disorder

Melanins are a group of natural pigments that are the primary factor affecting skin color. Lampel et al. examined a family of melanin-inspired materials based on tripeptides containing tyrosine as precursors for polymeric pigments. They found that the supramolecular organization of the tripeptide assembly is the most important factor for the enzymatic oxidation, with the position of the tyrosine residue playing a dominant role. Thus, simply juggling the order of the peptides allowed tuning of the optical and electrical properties of the resulting polymers.

Science, this issue p. 1064