

Natural Deep Eutectic Solvent-Guided Engineering of Herbal Residue-Derived Carbon Dots for Endothelial Oxidative Stress Protection

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Abstract

Despite retaining valuable carbon resources and redox-active phytochemical precursors, post-extraction herbal residues remain largely underutilized. Here, a natural deep eutectic solvent (NADES)-guided hydrothermal strategy was developed to construct bioactive carbon dots (SM-PN-CDs) from *Salvia miltiorrhiza* and *Panax notoginseng* residues for redox regulation and endothelial protection. Thirty NADES formulations were initially evaluated, and betaine–lactic acid (1:1) emerged as the optimal system based on physicochemical characteristics and antioxidant performance. The resulting SM-PN-CDs exhibited uniform nanoscale morphology, abundant oxygen- and nitrogen-containing surface functionalities, and broad-spectrum free radical scavenging activity. In H₂O₂-induced oxidative injury models of human umbilical vein endothelial cells (HUVECs), SM-PN-CDs reduced intracellular ROS accumulation, restored superoxide dismutase and catalase activities, preserved mitochondrial membrane potential, and regulated Keap1/Nrf2/HO-1-related antioxidant responses. Preliminary oral administration in mice showed no evident short-term toxicity at the tested dose, as indicated by normal physiological parameters and major-organ histology. Density functional theory (DFT) and molecular dynamics (MD) simulations further indicated that cooperative hydrogen-bonding and van der Waals interactions within the NADES environment contributed to the stabilization and dispersion of representative phytochemical precursors. Together, these findings demonstrate a NADES-guided approach for generating redox-active carbon dots from herbal residues and highlight their potential as bioactive nanomaterials for endothelial oxidative stress protection.

1. Introduction

Post-extraction herbal residues represent an underutilized biomass resource that still contains carbon-rich structural components, heteroatom-containing constituents, and residual phytochemicals [1]. Conventional disposal or low-value utilization of these residues fails to fully recover their chemical and functional value and may generate additional environmental burdens. Their conversion into high-value functional products therefore offers an attractive route for improving resource efficiency and extending the value chain of medicinal-plant processing [2]. However, for the production of bioactive materials, effective upcycling requires not only the conversion of residual biomass into new products but also the preservation or reconstruction of useful chemical functionalities during processing [3]. Developing green approaches that can simultaneously promote residue deconstruction, precursor utilization, and functional material formation remains an important challenge.

Carbon dots (CDs) provide a promising platform for this purpose because their carbon cores and surface functionalities can be generated directly from biomass-derived precursors [4]. Their ultrasmall dimensions, favorable aqueous dispersibility, tunable surface chemistry, and generally good biocompatibility have stimulated growing interest in biomass-derived CDs for biomedical applications. However, precursor complexity, structural heterogeneity, and variable surface chemistry remain major obstacles to achieving reproducible biological functions [5]. Antioxidant CDs are particularly attractive because oxygen- and nitrogen-containing surface groups and conjugated carbon domains can

participate in electron transfer, hydrogen-atom donation, and radical-scavenging processes. Nevertheless, whether CDs exhibit antioxidant or pro-oxidant behavior depends strongly on their surface composition, carbon structure, and surrounding environment [6]. Rational regulation of the precursor environment during carbon-dot formation may therefore provide an effective route to engineer redox-active nanomaterials with enhanced biological functionality.

Oxidative stress provides a biologically relevant context in which to evaluate such redox-active materials. Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including $\cdot\text{OH}$, $\text{O}_2^{\cdot-}$, and nitric oxide-derived reactive intermediates, disrupts cellular redox homeostasis and damages lipids, proteins, and nucleic acids [7]. Vascular endothelial cells are particularly susceptible to oxidative injury, and persistent redox imbalance can impair endothelial homeostasis and promote endothelial dysfunction [8, 9]. Because endothelial dysfunction represents an early and functionally important event in atherogenesis, materials capable of scavenging reactive species and preserving endothelial redox homeostasis may have potential relevance to vascular protection [10].

Natural deep eutectic solvents (NADESs), assembled from naturally derived hydrogen-bond acceptors and donors, provide highly tunable molecular environments for interacting with phenolic acids, flavonoids, and other redox-active natural products [11, 12]. Beyond their conventional use in extraction, deep eutectic solvents, including NADESs, have been incorporated into hydrothermal biomass conversion and carbon-dot synthesis, where their composition can influence precursor organization and the properties of the resulting carbon materials [13, 14]. However, most reported systems employ eutectic solvents as substantial components of the reaction medium. Whether a very low loading of NADES can regulate precursor dispersion and hydrothermal transformation in a predominantly aqueous system, and thereby influence the biological functionality of the resulting carbon dots, remains insufficiently understood.

Salvia miltiorrhiza (SM) and *Panax notoginseng* (PN) are widely used medicinal plants with established cardiovascular relevance [15, 16]. In this study, we developed a natural deep eutectic solvent (NADES)-guided hydrothermal strategy to construct bioactive carbon dots from post-extraction SM and PN residues. Thirty NADES formulations were screened, and betaine–lactic acid (Bet-Lac, 1:1) was identified as the optimal modifier. The effects of NADES composition and herbal-residue combination on carbon-dot formation and radical-scavenging activity were systematically investigated. The biological functions of the resulting SM-PN-CDs were evaluated in H_2O_2 -induced HUVEC oxidative injury models by examining intracellular ROS accumulation, antioxidant enzyme activities, mitochondrial membrane potential, and Keap1/Nrf2/HO-1-related responses. Network pharmacology provided complementary biological context for SM/PN-associated phytochemicals, while density functional theory (DFT) and molecular dynamics (MD) simulations were employed to investigate the molecular interactions underlying NADES-mediated precursor stabilization and dispersion. Preliminary short-term biosafety was also assessed (Scheme 1). This work establishes a NADES-guided strategy for constructing redox-active carbon dots from herbal residues and demonstrates their potential for regulating cellular redox homeostasis and protecting endothelial cells against oxidative stress.

2. Results and discussion

2.1 Screening and characterization of NADESs

To identify suitable NADES media for the valorization of herbal residues into antioxidant carbon dots, 30 NADES combinations were prepared using two hydrogen bond acceptors and five hydrogen bond donors at different molar ratios (**Figure S1**). Among them, only nine systems formed clear and homogeneous liquids at room temperature, whereas the remaining combinations either remained crystalline or failed to form stable eutectic phases. These nine successfully formed NADESs, namely L-Pro-Glu (2:1), L-Pro-Lac (1:1), L-Pro-Lac (1:2), L-Pro-Ca (1:2), L-Pro-Mal (1:1), Bet-Lac (1:1), Bet-Lac (1:2), Bet-Ca (1:1), and Bet-Mal (1:2), were subsequently subjected to viscosity evaluation (Fig. 1a, b and Table 1). Because low viscosity is generally favorable for mass transfer, precursor release, and hydrothermal conversion, L-Pro-Lac (1:1) and Bet-Lac (1:2), which showed relatively high viscosities, were excluded, and the remaining seven systems were retained for further screening (Table 2).

Among the preselected candidates, Bet-Lac (1:1) was chosen for detailed structural characterization because of its favorable physicochemical properties and clear eutectic appearance [17]. Notably, this system was later confirmed to be the optimal NADES in the antioxidant screening experiments described in Section 2.3. FTIR analysis revealed distinct spectral changes after NADES formation. Specifically, the broad O–H stretching band of lactic acid shifted from 3380 to 3360 cm^{-1} , indicating the involvement of hydrogen bonding. Meanwhile, the characteristic C = O stretching band of lactic acid at 1702 cm^{-1} remained visible, whereas the C = O band of betaine shifted from 1625 to 1720 cm^{-1} , and the characteristic C–N stretching band of betaine at 1480 cm^{-1} was retained in the eutectic system (Fig. 1c and Table 3). These spectral features suggest that the interaction between betaine and lactic acid is dominated by noncovalent hydrogen bonding rather than covalent bond formation [18, 19].

The ^1H NMR results further supported this interpretation. After NADES formation, the proton signals associated with the hydroxyl and carboxyl groups of lactic acid showed clear chemical shift perturbations, whereas the methyl and methylene proton signals of betaine remained largely unchanged. In addition, no new proton signals were detected in the NADES spectrum relative to those of the individual components (Fig. 1d), indicating that the eutectic assembly was stabilized through intermolecular interactions without the generation of new covalent species [20, 21].

To further clarify the structural basis of Bet-Lac (1:1), three possible configurations, designated NADES-1, NADES-2, and NADES-3, were constructed and optimized. Among them, NADES-1 exhibited the lowest Gibbs free energy ($\Delta G = -45.18 \text{ kcal mol}^{-1}$), lower than those of NADES-2 ($-40.16 \text{ kcal mol}^{-1}$) and NADES-3 ($-37.02 \text{ kcal mol}^{-1}$), indicating that NADES-1 was the most thermodynamically stable configuration [22] (Fig. 1e). Electrostatic potential (ESP) analysis further revealed pronounced charge complementarity between betaine and lactic acid. Specifically, betaine displayed a strongly negative region around the carbonyl oxygen and a positive region around the quaternary ammonium group, whereas lactic acid showed negative potential around the carbonyl and hydroxyl oxygens together with a

positive region near the hydroxyl proton [23]. In the assembled NADES structure, these complementary electrostatic features were concentrated between the carbonyl oxygen of betaine and the hydroxyl hydrogen of lactic acid, further confirming the formation of a stable hydrogen-bonded eutectic complex (Fig. 1f).

To further characterize the noncovalent interactions within Bet-Lac (1:1), the independent gradient model based on Hirshfeld partitioning (IGMH) and Hirshfeld surface analyses were performed. The IGMH map showed pronounced hydrogen-bonding interactions between betaine and lactic acid together with broader van der Waals contributions (Fig. 1g). Consistently, the d_{norm} -mapped Hirshfeld surface and corresponding fingerprint plot revealed complementary donor–acceptor contacts between the two components (Fig. 1h). Together, these results indicate that hydrogen bonding, assisted by weaker van der Waals interactions, contributes to the stabilization of the Bet-Lac (1:1) assembly.

Table 1
Compositions and appearances of 30 NADESs at room temperature.

No.	Abbreviation	Type of HBA	Type of HBD	HBA/HBD ratio	Appearance at room temperature
1	L-Pro-Glu	L-Proline	D-Glucose	2:1	Transparent
2				1:1	Unsynthesized
3				1:2	Crystalline
4	L-Pro-Lac		Lactic Acid	2:1	Crystalline
5				1:1	Transparent
6				1:2	Transparent
7	L-Pro-Ca		Citric Acid	2:1	Unsynthesized
8				1:1	Unsynthesized
9				1:2	Transparent
10	L-Pro-Mal		Malonic acid	2:1	Unsynthesized
11				1:1	Transparent
12				1:2	Unsynthesized
13	L-Pro-Suc		Succinic Acid	2:1	Unsynthesized
14				1:1	Unsynthesized
15				1:2	Unsynthesized
16	Bet-Glu	Betaine	D-Glucose	2:1	Unsynthesized
17				1:1	Unsynthesized
18				1:2	Unsynthesized
19	Bet-Lac		Lactic Acid	2:1	Crystalline
20				1:1	Transparent
21				1:2	Transparent
22	Bet-Ca		Citric Acid	2:1	Unsynthesized
23				1:1	Transparent
24				1:2	Unsynthesized
25	Bet-Mal		Malonic acid	2:1	Crystalline
26				1:1	Unsynthesized

No.	Abbreviation	Type of HBA	Type of HBD	HBA/HBD ratio	Appearance at room temperature
27	Bet-Suc		Succinic Acid	1:2	Transparent
28				2:1	Unsynthesized
29				1:1	Unsynthesized
30				1:2	Unsynthesized

Table 2
Viscosities of the nine preliminarily selected NADESSs.

NADESSs	Viscosity (mPa·S)
L-Pro-Glu (2:1)	0.7
L-Pro-Lac (1:1)	87.6
L-Pro-Lac (1:2)	23.2
L-Pro-Ca (1:2)	4.7
L-Pro-Mal (1:1)	1.0
Bet-Lac (1:1)	0.9
Bet-Lac (1:2)	16.9
Bet-Ca (1:1)	0.3
Bet-Mal (1:2)	0.7

Table 3
Assignment of characteristic FTIR bands for the key functional groups.

Functional group	Vibration mode	Typical wavenumber range (cm ⁻¹)	Observed wavenumber (cm ⁻¹)
O-H stretch (lactic acid)	Stretching	3200–3600	3380
O-H stretch (after DES formation)	Stretching (H-bonded)	3200–3600	3360
C = O stretch (lactic acid)	Stretching	1700–1750	1702
C = O stretch (betaine)	Stretching	1600–1650	1625
C = O stretch (after DES formation)	Stretching (H-bonded)	1700–1750	1720
C-N stretch (betaine)	Stretching	1450–1550	1480

2.2 Preparation and characterization of SM-PN-CDs

To investigate the physicochemical characteristics of the carbon dots generated in the NADES-assisted system, the sample prepared with Bet-Lac (1:1) was selected for detailed characterization, as this NADES was later confirmed to be the optimal medium in the antioxidant screening experiments described in Section 2.3. Under the present hydrothermal conditions, 23.743 mg of dry SM-PN-CD powder was obtained, corresponding to a yield of approximately 8% based on the total mass of herbal residues (Fig. 2a). HRTEM images showed that the resulting SM-PN-CDs were well dispersed and predominantly quasi-spherical in morphology [24]. A lattice fringe spacing of 0.163 nm was observed, indicating the presence of short-range ordered domains within the carbon core. Because no bulk crystalline phase was evident, this feature was interpreted as local structural ordering rather than extensive graphitization. Statistical analysis of more than 100 particles revealed diameters ranging from 2.32 to 3.96 nm, confirming a relatively narrow size distribution and good structural uniformity [25] (Fig. 2b, c).

The optical properties of SM-PN-CDs were further examined by UV–visible absorption and fluorescence spectroscopy. A distinct absorption band centered at 282 nm was observed in the UV–visible spectrum, which can be attributed to the π – π^* transition of aromatic sp^2 carbon domains [26] (Fig. 2h). Consistently, the aqueous dispersions of SM-PN-CDs appeared pale yellow under daylight and emitted bright blue fluorescence under UV irradiation, with the fluorescence gradually weakening upon dilution (Fig. 2d), confirming the successful formation of fluorescent carbon dots. The excitation–emission contour map showed that the dominant emission region was distributed between 420 and 510 nm, with the most effective excitation window located at 360–410 nm (Fig. 2e). As the excitation wavelength increased from 300 to 400 nm, the emission maximum gradually red-shifted from 453 to 481 nm (Fig. 2f), indicating pronounced excitation-dependent fluorescence. This behavior is commonly associated with the coexistence of multiple emissive centers, including carbon core states and surface-related defect states. In agreement with this interpretation, the photoluminescence decay profile recorded at 378 nm excitation suggested that the luminescence of SM-PN-CDs originated from multiple radiative recombination pathways [27] (Fig. 2g).

To further elucidate the surface chemistry of SM-PN-CDs, FTIR and XPS analyses were performed. The FTIR spectrum displayed characteristic absorption bands at 3260, 1560, 1410, and 1090 cm^{-1} , corresponding to O–H/N–H, C = C, C–N, and C–O–C vibrations, respectively (Fig. 2i). These results indicate the presence of abundant oxygen- and nitrogen-containing surface functionalities. These heteroatom-containing groups may provide potential redox-active sites and contribute to the aqueous dispersibility of SM-PN-CDs [28]. XPS analysis further confirmed that SM-PN-CDs were mainly composed of C (64.33%), O (29.06%), and N (6.61%) (Fig. 2j). The high-resolution C 1s spectrum could be deconvoluted into C–C/C = C, C–O, and C = O/C = N components centered at 284.8, 286.3, and 288.3 eV, respectively (Fig. 2k), while the N 1s spectrum revealed graphitic N and pyrrolic N species [29, 30] (Fig. 2l). The O 1s spectrum further indicated the coexistence of C = O and C–O groups [31] (Fig. 2m).

These heteroatom-rich surface structures are expected to contribute to the aqueous dispersibility, colloidal stability, and potential redox-active sites of SM-PN-CDs.

Taken together, these results demonstrate that the obtained SM-PN-CDs possess uniform nanoscale morphology, characteristic excitation-dependent photoluminescence, and rich surface functionality, thereby providing a favorable structural basis for their broad-spectrum antioxidant activity and endothelial protective potential.

2.3 Effect of NADES on the antioxidant capacity of SM-PN-CDs

Among the SM-PN-CDs prepared using the preselected NADESs, the sample synthesized with Bet-Lac (1:1) exhibited the strongest DPPH scavenging activity (Fig. 3a). This result identified Bet-Lac (1:1) as the optimal NADES and further supported its selection for subsequent carbon dot preparation and theoretical analysis.

To clarify the contribution of NADES addition, the DPPH[•] scavenging behaviors of products obtained from different precursor systems were compared. Powder-derived CDs, residue-derived CDs, and NADES-assisted residue-derived CDs all produced time-dependent decreases in DPPH[•] absorbance, whereas NADES alone showed negligible time-dependent scavenging activity (Fig. 3c). Thus, the enhanced antioxidant response of the NADES-assisted product could not be attributed simply to the intrinsic activity of the added NADES.

Morphological observations provided further evidence that NADES addition altered the hydrothermal processing of the residues. Before treatment, the mixed SM/PN residues retained recognizable plant-tissue-like structures, whereas hydrothermal treatment caused darkening and structural disruption. This disruption was more pronounced in the NADES-assisted group than in the group prepared without NADES (Fig. 3e–g), suggesting that low-loading Bet-Lac promoted deconstruction of the residual biomass matrix. As schematically illustrated in Fig. 3d, low-loading Bet-Lac is proposed to promote disruption of the residual biomass matrix and modify the local hydrothermal environment, favoring the formation of carbon dots with abundant surface functionalities and enhanced radical-scavenging performance.

The product prepared from NADES-assisted residues exhibited DPPH[•] scavenging activity comparable to that of the powder-derived CDs and higher than that of the residue-derived CDs prepared without NADES (Fig. 3b, c, h). Under UV irradiation, all carbon-dot-containing products emitted blue fluorescence, whereas NADES alone showed negligible DPPH[•] scavenging despite its detectable fluorescence (Fig. 3i, j). These findings indicate that fluorescence intensity was not directly associated with antioxidant performance.

Collectively, these results suggest that low-loading Bet-Lac promoted residue deconstruction and altered the hydrothermal conversion environment, thereby contributing to the formation of SM-PN-CDs with

enhanced DPPH• scavenging activity.

2.4 Radical scavenging activities of SM-PN-CDs

The antioxidant activity of SM-PN-CDs was evaluated using the artificial stable radical DPPH• and three biologically relevant reactive oxygen or nitrogen species, namely $O_2^{\bullet-}$, •NO, and •OH. SM-PN-CDs produced dose-dependent responses in all four assay systems.

As illustrated in Fig. 4a, DPPH• scavenging involves electron transfer and/or hydrogen atom donation, accompanied by fading of its characteristic purple color [32]. Increasing amounts of SM-PN-CDs progressively decreased the absorbance at 517 nm and weakened the purple color of the reaction solution (Fig. 4b, c). The scavenging process was also time-dependent and gradually approached a plateau (**Figure S2**). Under the tested assay conditions, SM-PN-CDs showed stronger DPPH• scavenging responses than the SM-PN decoction and ascorbic acid comparison groups (**Figure S3**).

The $O_2^{\bullet-}$ scavenging activity of SM-PN-CDs was evaluated using the NBT assay (Fig. 4d). Increasing amounts of SM-PN-CDs progressively reduced the absorbance at 560 nm and weakened the corresponding color of the reaction solution, demonstrating dose-dependent $O_2^{\bullet-}$ scavenging (Fig. 4e, f).

The •NO scavenging activity was evaluated using the Griess reaction system (Fig. 4g). Increasing amounts of SM-PN-CDs resulted in a progressive decrease in absorbance at 540 nm and a corresponding attenuation of the pink color, indicating dose-dependent suppression of the NO-derived reaction signal (Fig. 4h, i).

The •OH scavenging activity was investigated using the Fenton reaction with TMB as the chromogenic substrate (Fig. 4j). Increasing amounts of SM-PN-CDs progressively decreased the absorbance at 652 nm and attenuated the color of oxidized TMB, demonstrating dose-dependent •OH scavenging (Fig. 4k, l).

Overall, SM-PN-CDs exhibited dose-dependent scavenging activity toward DPPH•, $O_2^{\bullet-}$, •NO, and •OH, supporting their broad radical-scavenging capacity and providing a functional basis for subsequent evaluation in a cellular oxidative-stress model.

2.5 Optimization, functional robustness, and metal ion response of SM-PN-CDs

The hydrothermal temperature had a pronounced influence on the antioxidant activity of the resulting carbon dots. Among the three tested temperatures, the sample synthesized at 180°C exhibited the strongest DPPH• scavenging activity (Fig. 5a), suggesting that this condition provided a favorable balance between hydrothermal carbonization and retention of redox-active surface characteristics. Therefore, 180°C was selected for subsequent preparation.

The influence of precursor composition was then evaluated by varying the mass ratio of PN to SM. Regardless of whether PN or SM was used individually or in mixed ratios, all derived carbon dots exhibited a gradual increase in DPPH• scavenging over time (Fig. 5b). Across the four radical-scavenging assays, increasing the proportion of SM generally resulted in stronger scavenging responses, indicating a substantial contribution of SM-derived precursors to the antioxidant activity of the resulting carbon dots (Fig. 5d–g). The corresponding photographs showed consistent color changes (Fig. 5h). These results suggest that PN and SM contribute differently to the redox properties of the resulting carbon dots, likely owing to differences in their precursor compositions and derived surface functionalities [33]. The 1:1 PN/SM system retained broad-spectrum scavenging activity while incorporating equal contributions from both herbal residues and was therefore selected as the balanced co-precursor formulation for subsequent experiments.

To further evaluate the effect of precursor integration strategy, co-hydrothermal synthesis was compared with post-synthetic physical mixing. At all three PN/SM ratios, co-synthesized carbon dots consistently exhibited higher DPPH scavenging activity than physically mixed samples (Fig. 5c). This finding suggests that in situ interactions during hydrothermal carbonization facilitate cooperative transformation of the two residue precursors derived from PN and SM, thereby producing carbon dots with superior antioxidant performance. Taken together, 180°C and a 1:1 PN/SM mass ratio were selected as the synthesis temperature and balanced co-precursor formulation, respectively, for subsequent preparation of SM-PN-CDs.

The functional robustness and short-term storage stability of SM-PN-CDs were subsequently evaluated under different temperature, pH, and storage conditions. SM-PN-CDs retained substantial DPPH• scavenging activity over a temperature range of 10–90°C, with stronger responses observed at 70–90°C (Fig. 5i). Their DPPH• scavenging performance also varied with pH, with relatively high activity under near-neutral to mildly alkaline conditions and lower activity under strongly acidic or alkaline conditions (Fig. 5j). This pH dependence may be associated with protonation and deprotonation of surface hydroxyl and carboxyl groups, which can influence their electron- or hydrogen-donating behavior. In addition, refrigerated storage and nitrogen protection better preserved the radical-scavenging activity of SM-PN-CDs than storage at room temperature under ambient conditions (Fig. 5k). Collectively, these results demonstrate functional robustness across the tested temperature and pH conditions together with favorable short-term storage stability.

Among the tested metal ions, Fe^{3+} induced the most pronounced fluorescence quenching, whereas most other ions caused smaller changes (Fig. 5l, m). These findings reveal a preliminary Fe^{3+} -responsive fluorescence behavior of SM-PN-CDs and further demonstrate the sensitivity of their surface electronic states to metal-ion interactions.

2.6 Network pharmacology context and greenness assessment

Network pharmacology analysis identified 303 targets specific to SM/PN-associated candidate phytochemical precursors, 1060 targets specific to endothelial dysfunction, and 136 shared targets, suggesting a potential molecular intersection between the candidate precursors and endothelial regulation (Fig. 6a). The protein–protein interaction (PPI) network showed extensive interactions among the shared targets, with SRC, AKT1, EGFR, STAT3, ESR1, PTPN11, MAPK1, EP300, JUN, and RELA displaying relatively high connectivity. NFE2L2 and KEAP1 were also present, suggesting potential involvement of redox-responsive regulation (Fig. 6b). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment highlighted pathways related to oxidative stress and vascular dysfunction, including chemical carcinogenesis–reactive oxygen species, fluid shear stress and atherosclerosis, lipid and atherosclerosis, HIF-1 signaling, and AGE–RAGE signaling in diabetic complications (Fig. 6c). Gene Ontology (GO) analysis further identified oxidoreductase- and peroxidase-related molecular functions, together with biological processes involving responses to H₂O₂ and oxidative stress, ROS metabolism, cellular redox homeostasis, nitric oxide biosynthesis, vasodilation, and responses to fluid shear stress (Fig. 6d) (**Table S3**). Collectively, these analyses associated the selected SM/PN-related candidate phytochemicals with redox regulation and vascular homeostasis, providing complementary biological context for the selection of subsequent cellular oxidative-stress endpoints.

Beyond the biological context, the sustainability of the preparation strategy was evaluated using AGREE and ComplexGAPI. The process achieved an AGREE score of 0.74, indicating favorable overall compliance with green chemistry principles (Fig. 6e). Consistently, the ComplexGAPI profile contained predominantly green and yellow regions, with only three red sections, reflecting generally favorable environmental performance while identifying several remaining process burdens. Moreover, no hazardous organic-solvent waste was generated within the defined assessment boundary, corresponding to a hazardous organic-solvent E-factor of 0 (Fig. 6f). These results were mainly associated with the use of herbal residues as the carbon source, water as the predominant reaction medium, and the low loading of naturally derived NADES.

2.7 Protective effects of SM-PN-CDs against oxidative stress

To evaluate the cellular relevance of the radical-scavenging activity, the cytocompatibility and protective effects of SM-PN-CDs were examined in HUVECs. Cell viability remained above 80% even at the highest tested concentration, indicating low cytotoxicity. Notably, at the working concentration of 1000 µg/mL, selected for subsequent experiments, cell viability remained above 97%, demonstrating favorable biocompatibility [34] (Fig. 7a). These results indicate that SM-PN-CDs are well tolerated by HUVECs and are therefore suitable for subsequent evaluation of their endothelial protective effects.

To establish an oxidative stress model, HUVECs were exposed to increasing concentrations of H₂O₂ ranging from 50 to 800 µM. Treatment with 400 µM H₂O₂ reduced cell viability to approximately 50%, representing a moderate yet reproducible oxidative insult (Fig. 7b). This concentration was therefore

selected for subsequent experiments. Under this condition, co-treatment with SM-PN-CDs markedly attenuated H₂O₂-induced cytotoxicity and restored cell viability. In contrast, carbon dots prepared without NADES showed only partial protection and NADES alone produced no measurable cytoprotective effect (Fig. 7c). These results suggest that NADES-guided preparation enhanced the cytoprotective performance of the resulting carbon dots, whereas NADES alone showed no measurable protection under the tested conditions.

To further elucidate the underlying protective mechanism, intracellular ROS levels together with SOD and CAT activities were examined. H₂O₂ exposure caused a marked increase in intracellular ROS and a concomitant decrease in SOD and CAT activities. This indicates severe disruption of cellular redox homeostasis. By contrast, treatment with SM-PN-CDs significantly reduced ROS accumulation and restored SOD and CAT activities toward basal levels, whereas the NADES group showed little or no protective effect (Fig. 7d–f). These findings indicate that SM-PN-CDs effectively alleviate oxidative stress by suppressing ROS buildup and reinforcing endogenous antioxidant defenses.

Because loss of mitochondrial membrane potential is an early hallmark of oxidative stress-induced apoptotic damage, JC-1 staining was used to evaluate mitochondrial function. H₂O₂ treatment resulted in a marked increase in green fluorescence, indicating mitochondrial depolarization. In contrast, SM-PN-CD treatment significantly reduced the green fluorescence signal and attenuated H₂O₂-induced mitochondrial depolarization (Fig. 7g, h). These results indicate that SM-PN-CDs alleviated H₂O₂-induced mitochondrial dysfunction.

Western blot analysis showed that H₂O₂ exposure significantly increased Keap1 protein expression while suppressing Nrf2 protein expression (Fig. 7i–k). Upon treatment with SM-PN-CDs, Keap1 expression was markedly reduced, whereas Nrf2 expression was significantly restored. RT-qPCR analysis revealed that SM-PN-CD treatment effectively reversed the H₂O₂-induced changes, downregulating Keap1 while upregulating Nrf2 and HO-1 mRNA levels (Fig. 7l–n). These protein and mRNA expression changes indicate that the protective effects of SM-PN-CDs were associated with modulation of Keap1/Nrf2/HO-1-related antioxidant responses.

Based on the chemical and cellular results, a plausible working model is proposed in Fig. 7o. The radical-scavenging activity of SM-PN-CDs may contribute to reduced cellular ROS accumulation under H₂O₂-induced oxidative stress. In parallel, the observed changes in Keap1, Nrf2, and HO-1 expression suggest modulation of endogenous antioxidant responses. These effects were accompanied by restoration of SOD and CAT activities and preservation of mitochondrial membrane potential. Together, the results support a working model in which radical scavenging and regulation of cellular redox responses jointly contribute to the protection of HUVECs against oxidative injury.

2.8 Preliminary biosafety evaluation of SM-PN-CDs

A preliminary *in vivo* biosafety assessment of SM-PN-CDs was conducted in healthy BALB/c mice. After oral administration for 1 or 7 consecutive days (Fig. 8a), no obvious abnormalities in appearance, behavior, or body weight were observed in the treated groups. In particular, mice receiving SM-PN-CDs for 7 days showed no significant difference in body weight compared with the blank control group, indicating that repeated oral administration did not induce overt systemic toxicity [35] (Fig. 8b). Serum biochemical analysis further revealed that all tested indices remained within the normal range after 1 and 7 days of administration, with no significant differences relative to the blank control group (Fig. 8c). Likewise, no significant abnormalities were detected in the major hematological parameters (Fig. 8d). Histopathological examination showed no obvious lesions, inflammatory infiltration, tissue degeneration, or structural abnormalities in the heart, liver, spleen, lung, or kidney after treatment [36] (Fig. 8e). Collectively, no overt short-term toxicity was observed at the tested dose and time points, supporting the preliminary *in vivo* biocompatibility of SM-PN-CDs.

2.9 DFT analysis of the NADES effect

To investigate the intrinsic interaction potential of Bet-Lac (1:1), DFT calculations were performed using kaempferol (Kae) and salvianolic acid B (Sal B) as representative flavonoid- and phenolic-acid-type model compounds associated with PN and SM, respectively. These compounds were selected to probe possible noncovalent interactions between representative phytochemicals and the NADES model.

ESP mapping first revealed the distribution of electron-rich and electron-deficient regions within the target molecules [37]. Both Kae and Sal B exhibited pronounced negative ESP regions around phenolic hydroxyl oxygens, carbonyl groups, and ether oxygens, indicating that these sites can serve as hydrogen bond acceptors [38]. In contrast, the positive ESP regions were mainly concentrated around hydroxyl protons, suggesting their roles as hydrogen bond donors (Fig. 9a, b). Such complementary electrostatic distributions imply that both antioxidant molecules possess multiple potential interaction sites for noncovalent association with the Bet-Lac (1:1) through noncovalent interactions.

To further characterize the nature of these interactions, IGMH analysis was performed for the Bet-Lac (1:1) itself and for the corresponding Bet-Lac (1:1)–target complexes. In the Bet-Lac (1:1) structure, distinct blue isosurfaces were observed between the hydroxyl hydrogen of lactic acid and the carbonyl oxygen of betaine, indicating that hydrogen bonding is the dominant force stabilizing the eutectic assembly (Fig. 9d). After association with Kae and Sal B, the interaction isosurfaces became broader and more diffuse, with green regions dominating the maps, suggesting that van der Waals interactions became more prominent in the NADES–solute complexes while moderate hydrogen bonding remained present (Fig. 9e, f). This interpretation was further supported by Hirshfeld surface analysis and two-dimensional fingerprint plots. The d_{norm} -mapped surfaces of both Bet-Lac (1:1)–Kae and Bet-Lac (1:1)–Sal B showed localized red regions corresponding to close intermolecular contacts associated with hydrogen bonding, whereas the broader white and green regions represented weaker van der Waals interactions [39] (Fig. 9g, h). Notably, the highlighted contacts involved both donor and acceptor sites, suggesting that the Bet-Lac (1:1) can interact with the target molecules through bidirectional hydrogen

bonding. Taken together, these results indicate that hydrogen bonding and van der Waals interactions act cooperatively in the association and stabilization of the model molecules within the NADES environment.

Frontier molecular orbital (FMO) analysis was then carried out to examine how the NADES influences the electronic structures of the target antioxidant molecules. The Bet-Lac (1:1) itself exhibited a relatively large HOMO–LUMO energy gap of 6.56 eV, indicating high intrinsic electronic stability and relatively low chemical reactivity (Fig. 9c). This result suggests that the Bet-Lac (1:1) mainly acts as a stable supramolecular environment for noncovalent association and molecular stabilization rather than as a strongly redox-active species. For Kae, the frontier orbitals became more evenly delocalized across the molecular framework after interaction with the Bet-Lac (1:1). A similar redistribution was observed for Sal B, whose frontier orbitals shifted toward broader aromatic and ester-containing regions after association with the Bet-Lac (1:1). The HOMO–LUMO energy gap decreased from 2.54 to 2.39 eV for Kae and from 3.50 to 2.41 eV for Sal B after complexation with Bet-Lac (1:1) (Fig. 9i, j). These changes indicate that NADES complexation altered the frontier-orbital distributions and electronic responsiveness of the model molecules, which may favor their participation in redox-related interactions [40]. Overall, the DFT results provide molecular insight into the cooperative hydrogen-bonding and van der Waals interactions through which Bet-Lac may stabilize representative phytochemical molecules.

2.10 MD analysis of the NADES effect

To compare the intrinsic dispersion behavior of the selected model phytochemicals in different NADES environments, Bet-Lac (1:1) was evaluated together with L-Pro-Lac (1:2) and Bet-Mal (1:2). Representative snapshots obtained at 0, 50, and 100 ns showed that both Kae and Sal B remained more uniformly dispersed in Bet-Lac (1:1), whereas more pronounced aggregation was observed in L-Pro-Lac (1:2) and Bet-Mal (1:2) as the simulation progressed (Fig. 10a, b). These observations suggest that Bet-Lac (1:1) more effectively maintained the dispersion of both model molecules throughout the simulation [41].

This trend was further supported by solvent-accessible surface area (SASA) and root-mean-square deviation (RMSD) analyses. For both Kae and Sal B, the Bet-Lac (1:1) system maintained relatively stable SASA profiles, whereas L-Pro-Lac (1:2) showed more pronounced decreases or fluctuations, consistent with reduced solvent exposure. Bet-Mal (1:2) retained relatively high SASA values in some cases but showed more evident clustering in the representative snapshots. The RMSD profiles further revealed NADES-dependent differences in the conformational behavior of the model molecules [42] (Fig. 10c, d). Overall, Bet-Lac (1:1) provided a balanced environment for maintaining the dispersion and conformational stability of both Kae and Sal B. This molecular behavior was consistent with the superior DPPH• scavenging performance of the product prepared using Bet-Lac (1:1), supporting its selection as the optimal NADES formulation.

3. Conclusion

A low-loading NADES-guided hydrothermal strategy was developed to engineer post-extraction *Salvia miltiorrhiza* and *Panax notoginseng* residues into bioactive carbon dots. Screening of 30 formulations identified Bet-Lac (1:1) as the optimal NADES modifier. The resulting SM-PN-CDs exhibited uniform nanoscale morphology, abundant oxygen- and nitrogen-containing surface functionalities, and broad radical-scavenging activity toward DPPH•, O₂•⁻, •NO, and •OH. In H₂O₂-injured HUVECs, SM-PN-CDs reduced intracellular ROS accumulation, restored SOD and CAT activities, preserved mitochondrial function, and regulated Keap1/Nrf2/HO-1-related antioxidant responses. Preliminary *in vivo* evaluation demonstrated favourable short-term biocompatibility without obvious systemic toxicity. DFT and MD analyses further suggested that hydrogen bonding and van der Waals interactions contributed to the stabilization and dispersion of representative phytochemical molecules within the Bet-Lac environment.

Together, these findings demonstrate that NADES-guided precursor regulation enables the construction of herbal residue-derived carbon dots with redox-regulating and endothelial-protective properties. Further studies should clarify the material–cell interaction mechanisms, determine the contribution of antioxidant signalling pathways, and evaluate biodistribution, long-term safety, and protective efficacy in relevant vascular disease models. This work provides a potential strategy for developing bioactive carbon nanomaterials for oxidative stress-related biomedical applications.

4. Experimental

4.1 Reagents and materials

L-Proline, betaine, D-glucose, lactic acid, citric acid, malonic acid, succinic acid, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), riboflavin, L-methionine, sodium nitroprusside (SNP), N-(1-naphthyl)ethylenediamine dihydrochloride, sulfanilic acid, and ferrous sulfate (all ≥ 98%) were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). 3,3',5,5'-Tetramethylbenzidine (TMB), phosphate-buffered saline (PBS), ethanol, and hydrogen peroxide were obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Dried roots of *Salvia miltiorrhiza* and *Panax notoginseng* were collected from Wenshan, Yunnan, and Zibo, Shandong, respectively. Ultrapure water was prepared using a Milli-Q purification system (Millipore, Billerica, MA, USA). The ROS assay kit was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). CCK-8, the JC-1 mitochondrial membrane potential assay kit, and RIPA lysis buffer were obtained from MedChemExpress (Shanghai, China). SOD and CAT assay kits were purchased from Abbkine Scientific Co., Ltd. (Wuhan, China). The BCA protein assay kit and ECL reagent were obtained from Thermo Fisher Scientific (Waltham, MA, USA) and Millipore (Burlington, MA, USA), respectively. Primary antibodies against Keap1 and Nrf2 were purchased from Cell Signaling Technology (Danvers, MA, USA), and the β-actin antibody was obtained from Aibotek Biotech Co., Ltd. (Wuhan, China). Primers for Keap1, Nrf2, HO-1, and the endogenous reference gene GAPDH were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). HUVECs were obtained from Saiye Biotechnology Co., Ltd. (Guangzhou, China).

4.2 Preparation and screening of NADESs

Thirty candidate NADES formulations were evaluated by combining two hydrogen bond acceptors, betaine and L-proline, with five hydrogen bond donors, D-glucose, lactic acid, citric acid, malonic acid, and succinic acid, at molar ratios of 1:1, 1:2, and 2:1. Each mixture was heated at 70°C under continuous stirring for 30 min and was subsequently classified according to whether a homogeneous transparent liquid was formed. The prepared NADESs were first screened according to their clarity, homogeneity, phase behavior, and apparent viscosity at room temperature. In the second screening step, each of the seven preselected NADESs was added to the herbal residues before one-pot hydrothermal treatment. The resulting carbon dots were then evaluated using the DPPH assay, and the NADES affording the strongest antioxidant performance was selected for subsequent experiments.

4.3 Preparation of herbal residues

Dried PN and SM powders (5 g each) were individually refluxed with 100 mL of 60% ethanol using an electric heating mantle (DZTW, Beijing Yongguangming Medical Instrument Co., Ltd., Beijing, China) at 150 W for 6 h. After filtration, the insoluble residues were thoroughly washed with ethanol, dried at 60°C to constant weight, ground, and passed through a 30-mesh sieve before NADES treatment.

4.4 Synthesis of SM-PN-CDs

SM-PN-CDs were synthesized using a one-pot hydrothermal method. Briefly, SM and PN residues (150 mg each) were dispersed in 30 mL of deionized water, followed by the addition of 30 μ L of the selected NADES, corresponding to a loading of approximately 0.10% v/v relative to the aqueous phase. After thorough mixing, the suspension was transferred into a 50 mL Teflon-lined stainless-steel autoclave and heated at 180°C for 12 h. After natural cooling to room temperature, the reaction mixture was centrifuged at 8000 rpm for 20 min, and the supernatant was passed through a 0.22 μ m membrane. The filtrate was dialyzed against deionized water for 24 h using a dialysis membrane with a molecular-weight cut-off of 1000 Da (Jielepu, Changsha, China), with the external medium replaced every 8 h. The purified dispersion was subsequently lyophilized for 30 h to obtain dry SM-PN-CD powder [43].

4.5 Characterization of NADESs and SM-PN-CDs

The viscosities of the preselected NADESs were measured using a rotational viscometer (NDJ-5S, Decca Precision Meter, Shenzhen, China). The optimized NADES and its individual components were characterized by ^1H NMR spectroscopy using a Bruker AVANCE III 600 MHz spectrometer (Bruker, Ettlingen, Germany) and by FTIR spectroscopy using a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA, USA). The morphology and particle size of SM-PN-CDs were examined by HRTEM using an FEI Tecnai G2 F30 microscope (FEI, Hillsboro, OR, USA) operated at 300 kV and equipped with an Oxford XPLORE EDS detector. Particle-size distributions were determined from at least 100 particles using ImageJ. UV–visible absorption spectra were recorded using a MAPADA P9 spectrophotometer (Shanghai, China), whereas photoluminescence spectra, excitation–emission matrices, and fluorescence-decay profiles were obtained using a Fluorolog-3 spectrofluorometer (Horiba Scientific, Piscataway, NJ, USA). Surface functional groups were analyzed by FTIR spectroscopy, and the elemental

composition and chemical states were determined by X-ray photoelectron spectroscopy using a Thermo ESCALAB 250Xi system equipped with an Al K α source.

4.6 *In vitro* antioxidant assays

The radical-scavenging activities of SM-PN-CDs toward DPPH $\cdot$, O $_2\cdot^-$, $\cdot$ NO, and $\cdot$ OH were evaluated using spectrophotometric methods adapted from Gao *et al.* with minor modifications [30]. For each assay, an appropriate radical or chromogenic control and a corresponding sample blank were included to correct for the intrinsic absorbance of SM-PN-CDs. All measurements were performed in triplicate.

For the DPPH $\cdot$ assay, different volumes of SM-PN-CD solution (0–500 μ L) were mixed with 2 mL of 0.1 mM DPPH solution in ethanol. After vigorous mixing, the reaction mixtures were incubated in the dark, and the absorbance at 517 nm was recorded every 30 s for 10 min. Ascorbic acid (1 mg $\cdot$ mL $^{-1}$) was used as the positive control, while a traditional decoction prepared from PN and SM (SM-PN, 10 mg $\cdot$ mL $^{-1}$) was included to compare the antioxidant activity before and after carbon-dot formation. Additional comparisons were conducted using CDs prepared from the original herbal powders, untreated residues, NADES-assisted residues, and NADES alone.

For the O $_2\cdot^-$ assay, the reaction mixture contained riboflavin (6.67 μ M), L-methionine (4.33 mM), NBT (25 μ M), and SM-PN-CDs at the indicated final concentrations in 0.01 M PBS (pH 7.4). The mixtures were irradiated under a 30 W fluorescent lamp for 10 min, after which the absorbance of the generated formazan was measured at 560 nm.

For the $\cdot$ NO assay, 100 μ L of 20 mM SNP solution and SM-PN-CDs at the indicated final concentrations were added to 2 mL of 0.1 M PBS (pH 7.4). After incubation at 37°C for 30 min, 50 μ L each of Griess reagents I and II were added, and the mixtures were further incubated for 10 min. The absorbance of the resulting azo product was measured at 540 nm.

For the $\cdot$ OH assay, H $_2$ O $_2$ (100 mM), TMB (10 mM), and SM-PN-CDs at the indicated final concentrations were mixed in 0.01 M PBS (pH 4.5) and incubated for 5 min. FeSO $_4$ was then added to a final concentration of 10 mM to initiate the Fenton reaction. The absorbance of oxidized TMB at 652 nm was recorded every 30 s for 5 min.

The scavenging rates in all four assays were calculated using the following equation:

$$\text{Scavenging rate (\%)} = [1 - (B - C)/A] \times 100\%$$

where A is the absorbance of the complete radical-generating or chromogenic control system without sample, B is the absorbance of the complete reaction system containing the sample, and C is the absorbance of the corresponding sample blank prepared without the radical-generating reagent or chromogenic reaction.

4.7 Process optimization, functional robustness, and metal ion responsiveness

The synthesis conditions of SM-PN-CDs were optimized by comparing the DPPH scavenging activities of products prepared at different hydrothermal temperatures (160, 180, and 200°C). To determine the optimal precursor combination, carbon dots synthesized individually from SM or PN residues, as well as those prepared from mixed SM/PN residues at mass ratios of 2:1, 1:1, and 1:2, were further evaluated using four radical scavenging assays. The time-dependent DPPH scavenging behavior of these samples was also examined, and the activities of co-synthesized and physically mixed carbon dots with the three SM/PN ratios were compared.

The functional robustness and storage stability of SM-PN-CDs were evaluated by monitoring their DPPH[•] scavenging activity under different pH, temperature, and storage conditions. For the pH-dependent evaluation, the DPPH[•] reaction system was adjusted to pH 2–12 using 10 mM HCl or NaOH. For the temperature-dependent evaluation, SM-PN-CDs were incubated with DPPH[•] solution at 10–90°C for 10 min. For storage stability, SM-PN-CDs were stored for 5 days under three conditions: room temperature in the dark, room temperature under nitrogen protection, and 5–8°C in the dark.

Metal ion responsiveness was investigated by recording the fluorescence changes of 10-fold diluted SM-PN-CD solution after the addition of individual metal ions (2 mM, 200 µL), including Na⁺, Mg²⁺, Al³⁺, K⁺, Ca²⁺, Zn²⁺, Pb²⁺, Cu²⁺, Hg²⁺, Ag⁺, Fe²⁺, and Fe³⁺. After incubation for 5 min at room temperature, fluorescence spectra were recorded at an excitation wavelength of 387 nm, and the emission intensity at 448 nm was used for analysis. The quenching efficiency was calculated as $(1 - I/I_0) \times 100\%$, where I and I_0 represent the fluorescence intensities in the presence and absence of metal ions, respectively.

4.8 Network pharmacology analysis

Candidate phytochemicals associated with SM and PN were retrieved from the Traditional Chinese Medicine Systems Pharmacology database using oral bioavailability $\geq 30\%$ and drug-likeness ≥ 0.18 as screening criteria and were supplemented with representative compounds reported in the literature. Their putative human targets were predicted using SwissTargetPrediction and standardized using UniProt. Endothelial dysfunction-related targets were collected from GeneCards, OMIM, and TTD, followed by removal of duplicate entries. Shared targets between the candidate phytochemicals and endothelial dysfunction were identified by Venn analysis and imported into STRING with the organism restricted to *Homo sapiens* and a minimum interaction score of 0.9. The PPI network was visualized and topologically analyzed using Cytoscape 3.10.3. GO and KEGG enrichment analyses of the shared targets were performed using DAVID with $P < 0.05$ as the significance threshold. All databases were accessed on July 2026.

4.9 Cell culture and cellular oxidative stress evaluation

HUVECs were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and vascular endothelial growth factor (VEGF) at 37°C in a humidified atmosphere containing 5% CO₂. The culture medium was replaced every 48 h. For cytotoxicity evaluation, HUVECs were seeded into 96-well plates at a density of 1×10^4 cells·mL⁻¹, with 100 µL of cell suspension added to each well, and cultured to approximately 70% confluence. The cells were then exposed to SM-PN-CDs at final concentrations of 62.5, 125, 250, 500, 1000, 1500, and 2000 µg·mL⁻¹ for 24 h. CCK-8 reagent was subsequently added, followed by incubation at 37°C for 30 min. The absorbance was recorded at 450 nm, and cell viability was calculated as follows:

$$\text{Cell viability (\%)} = [(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}})] \times 100\%$$

For cellular oxidative stress evaluation, HUVECs cultured in 6-well plates were allocated to five groups: control, H₂O₂, H₂O₂ + SM-PN-CDs, H₂O₂ + CDs prepared without NADES, and H₂O₂ + NADES. The cells were treated according to their group assignments, with all groups except the control group exposed to 0.4 mM H₂O₂. Intracellular ROS levels were evaluated using DCFH-DA staining, and fluorescence was recorded at excitation and emission wavelengths of 488 and 525 nm, respectively. Under the same treatment conditions, SOD and CAT activities were determined using commercial assay kits according to the manufacturers' instructions and normalized to the total protein content.

For mitochondrial membrane potential analysis, the treated cells were incubated with 2 µM JC-1 for 20 min at 37°C, washed with PBS, and imaged using a live-cell imaging system. For western blot analysis, cells were lysed in RIPA buffer containing protease and phosphatase inhibitors. After centrifugation, the protein concentrations of the collected supernatants were determined using the BCA assay. Equal amounts of protein (10 µg per lane) were separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk and sequentially incubated with the corresponding primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Protein bands were visualized using ECL, and their intensities were quantified using ImageJ, with β-actin used as the loading control. The primary antibodies and their working dilutions are listed in **Table S1**.

For RT-qPCR analysis, total RNA was extracted using TRIzol reagent and reverse-transcribed into cDNA. Quantitative real-time PCR was subsequently performed using the primer sequences listed in **Table S2**. The relative mRNA expression levels of Keap1, Nrf2, and HO-1 were normalized to GAPDH and calculated using the 2^{-ΔΔCt} method.

4.10 *In vivo* biosafety evaluation

Healthy BALB/c mice were randomly divided into three groups (n = 7 per group): an untreated control group, a 1-day SM-PN-CD group, and a 7-day SM-PN-CD group. Mice in the treatment groups were orally administered 100 µL of an SM-PN-CD suspension prepared from the lyophilized powder (400 µg·mL⁻¹) for 1 or 7 consecutive days, whereas the control group received no treatment. Body weight was recorded daily throughout the experimental period. At the designated endpoints, whole blood and serum were collected for routine hematological and biochemical analyses, respectively. The heart, liver, spleen, lung,

and kidney were subsequently collected and subjected to hematoxylin and eosin staining for histopathological evaluation. All animal procedures were approved by the Experimental Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2024193W3321).

4.11 Greenness assessment

The environmental profile of the SM-PN-CD preparation was evaluated using the Analytical GREENness metric (AGREE) [44], Complex Green Analytical Procedure Index (ComplexGAPI) [45], and a hazardous organic-solvent E-factor adapted from the E-factor concept [46]. The assessment boundary extended from the dispersion of the pre-obtained herbal residues to the collection of lyophilized SM-PN-CDs. The hazardous organic-solvent E-factor was calculated as the mass of hazardous organic-solvent waste generated relative to the mass of isolated SM-PN-CD product.

4.12 DFT calculations

DFT calculations were performed to investigate the interactions between the NADES model and two representative antioxidant molecules, kaempferol and salvianolic acid B. All calculations were conducted using Gaussian 16W, and molecular structures were visualized using GaussView 6.0. Geometry optimizations and energy calculations were performed at the B3LYP-D3(BJ)/6-31G(d) level. Electrostatic potential maps were generated using Multiwfn 3.8 [47] and visualized with VMD 1.9.3 [48] to examine the electronic distributions of betaine, lactic acid, the NADES complex, and the two antioxidant molecules. Interfragment noncovalent interactions were analyzed using the independent gradient model based on Hirshfeld partitioning, together with Hirshfeld surface analysis and two-dimensional fingerprint plots. Interaction-intensity distributions were visualized using Gnuplot 5.4. Frontier molecular orbital analysis was further performed to compare the HOMO and LUMO energy levels of the antioxidant molecules before and after complexation with the NADES model.

4.13 Molecular dynamics calculations

The two-dimensional structures of kaempferol and salvianolic acid B were obtained from the PubChem database, and the corresponding three-dimensional coordinates and topology files were generated using the ATB server. Molecular dynamics simulations were performed using GROMACS 2023.5 with the GROMOS 54A7 force field. Ten molecules of either kaempferol or salvianolic acid B were randomly placed in a cubic simulation box measuring $10 \times 10 \times 10 \text{ nm}^3$. Idealized solvent environments composed of Bet-Lac (1:1), L-Pro-Lac (1:2), or Bet-Mal (1:2) were then constructed. Energy minimization was performed to remove steric clashes and unfavorable interactions, followed by equilibration under the NVT and NPT ensembles for 100 ps each. A 100 ns production simulation was subsequently conducted under the NPT ensemble. Solvent-accessible surface area and root-mean-square deviation were calculated using standard GROMACS analysis tools.

4.14 Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD). Statistical significance among multiple groups was determined by one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons

test. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

Declarations

Author Contribution

K.D. and H.L. conceived the experimental design, performed data analysis, and wrote the original manuscript draft. S.C. performed experiments and contributed to data interpretation. Z.P., R.C., Z.J., and Y.J. contributed to experimental work, data analysis, and manuscript revision. X.L. and Y.C. supervised the study, provided funding support, and critically revised the manuscript. All authors reviewed and approved the final manuscript.

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Data availability

Data will be made available on request.

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Scheme

Scheme 1 is available in the Supplementary Files section.

Figures

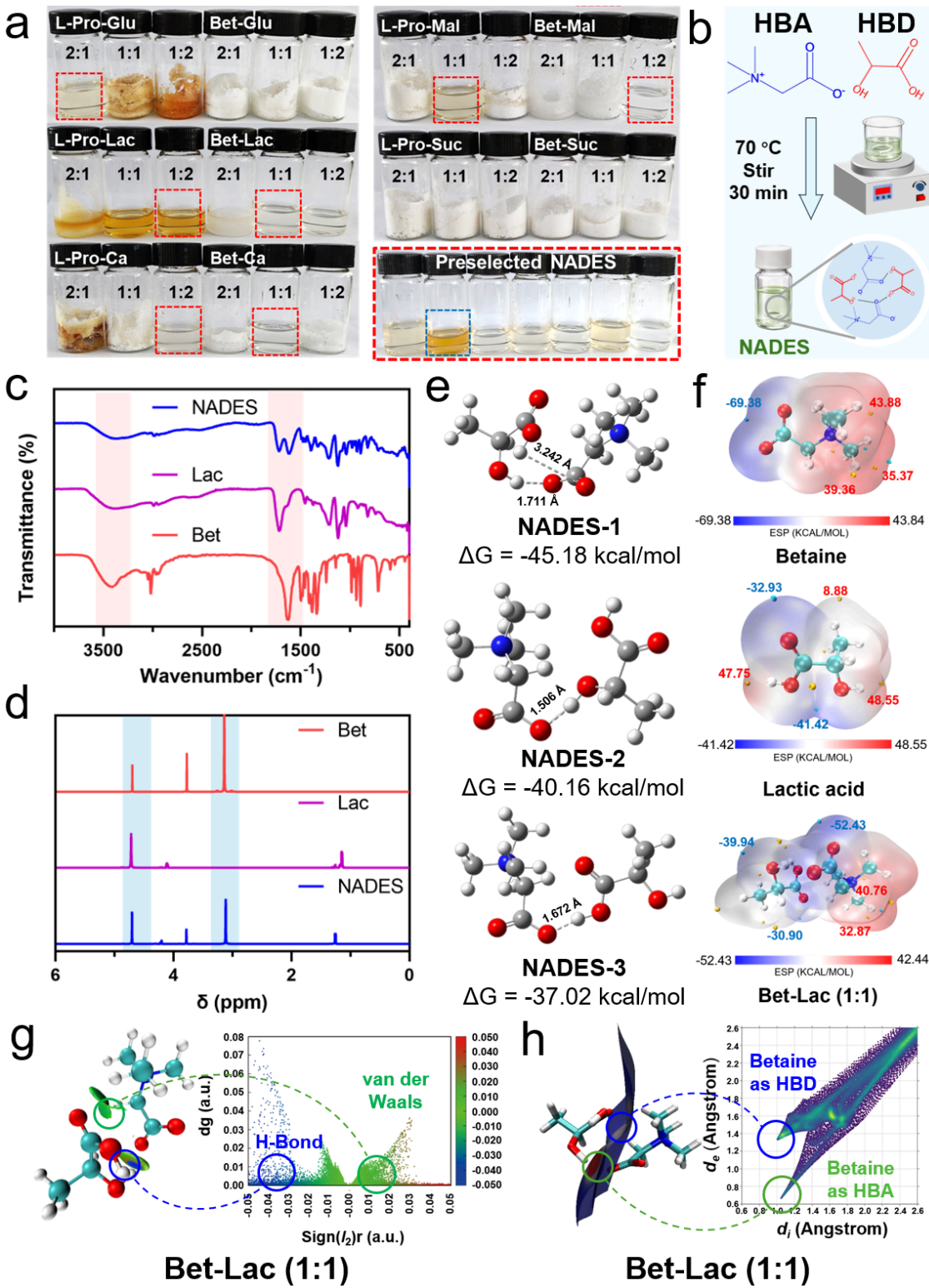


Figure 1

Screening and characterization of NADESs. (a) Photos of the prepared and preselected NADESs. (b) Schematic of NADES preparation. (c) FTIR of betaine, lactic acid, and NADES. (d) ^1H NMR of betaine, lactic acid, and NADES. (e) Optimized structures of three possible NADES configurations. (f) ESP of betaine, lactic acid, and NADES. (g) Sign (λ^2) ρ colored IGMH scatter plot and colored function δg^{inter} map of Bet-Lac (1:1) (isosurface value = 0.05 a.u.). (h) Colored maps of d_{norm} on Hirshfeld surface and corresponding fingerprint plot of Bet-Lac (1:1).

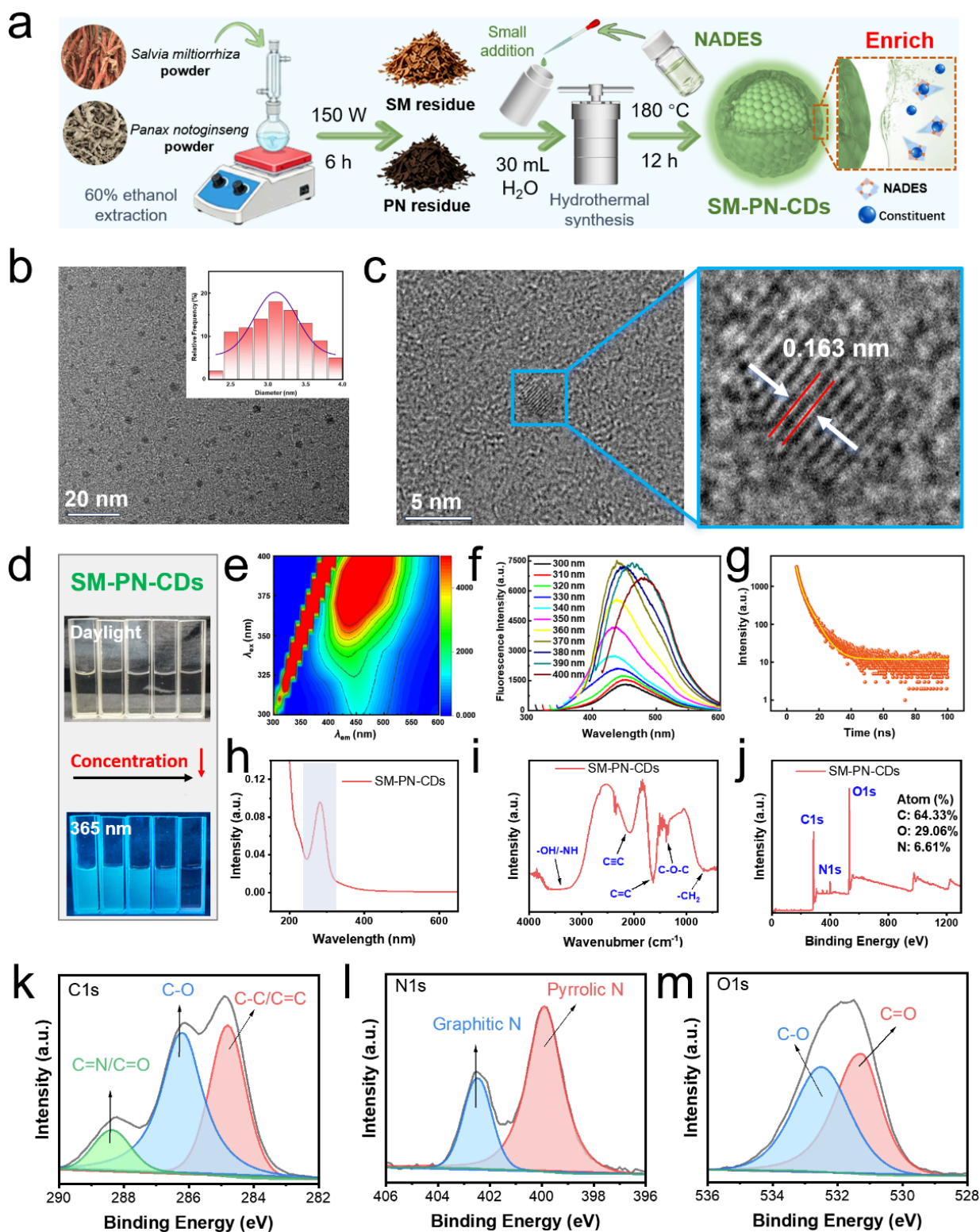


Figure 2

Preparation and characterization of SM-PN-CDs. (a) Schematic of SM-PN-CDs preparation. (b, c) HRTEM images and particle size distribution of SM-PN-CDs. (d) Photos of SM-PN-CDs at different concentrations under daylight and UV light. (e) Excitation–emission contour map of the SM-PN-CDs. (f) Fluorescence emission spectra of the SM-PN-CDs at different excitation wavelengths. (g) Fluorescence lifetime decay curve of SM-PN-CDs. (h) UV–Vis absorption spectrum of SM-PN-CDs. (i) FTIR spectrum of SM-PN-CDs. (j) XPS survey spectrum of SM-PN-CDs. (k–m) High-resolution XPS spectra of C 1s, N 1s, and O 1s, respectively.

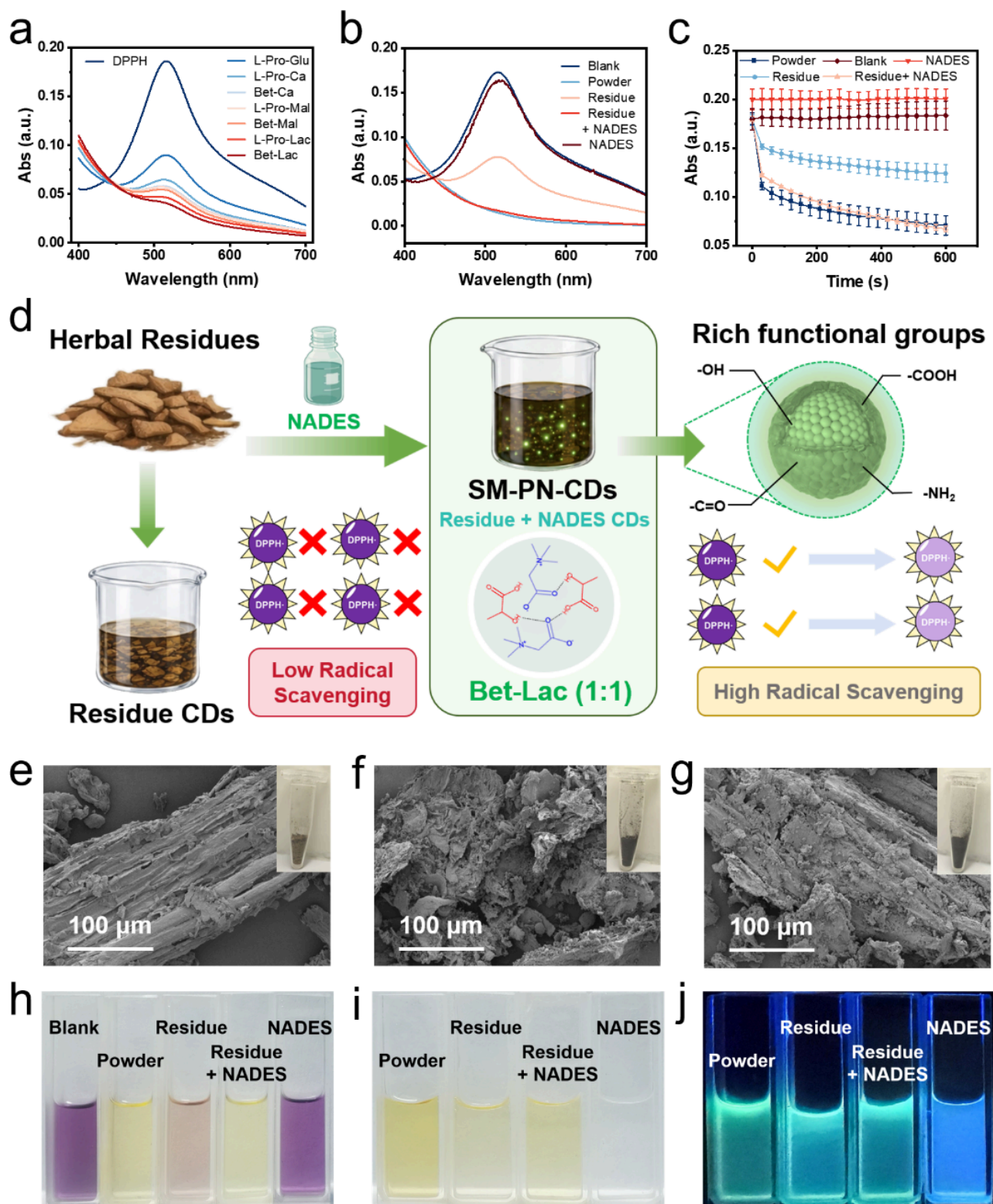


Figure 3

Effect of NADES on the antioxidant capacity of SM-PN-CDs. (a) DPPH $\cdot$ scavenging activity of SM-PN-CDs prepared using the preselected NADESs (b, c) Spectral and time-dependent changes of DPPH in the presence of powder, residue, residue + NADES, and NADES. (d) Schematic of NADES-mediated conversion of herbal residues into SM-PN-CDs. (e-g) Photos and SEM images of the SM/PN residue mixture before hydrothermal treatment, after hydrothermal treatment with NADES, and after

hydrothermal treatment without NADES. (h) Photos of the DPPH• scavenging assay solutions. (i, j) Photos of powder, residue, residue + NADES, and NADES under daylight and UV light, respectively.

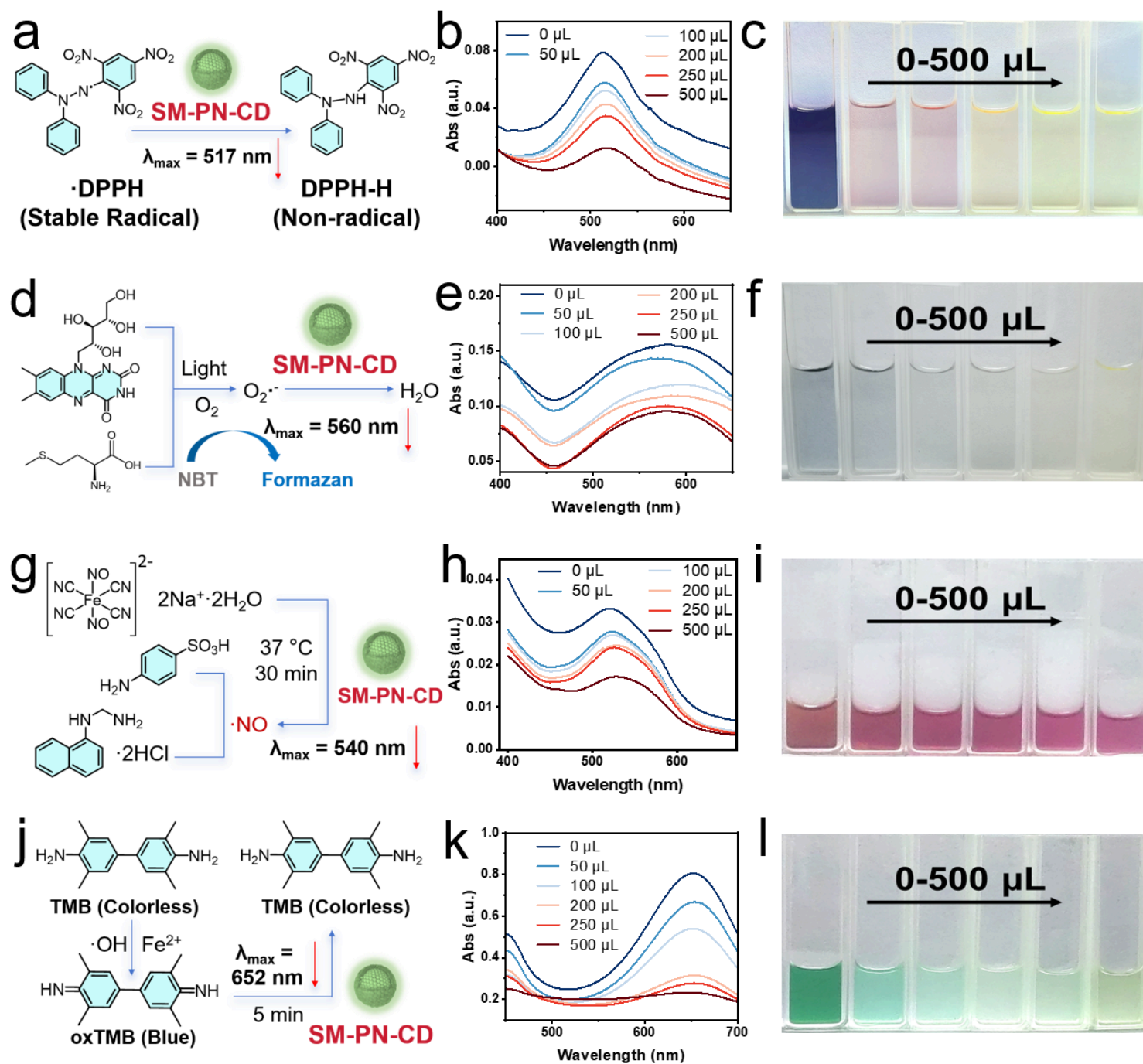


Figure 4

Radical-scavenging activities of SM-PN-CDs. (a–c) Schematic illustration, UV–visible spectral changes, and visual changes of the DPPH• scavenging assay. (d–f) Schematic illustration, spectral changes, and visual changes of the $\text{O}_2^{\bullet-}$ scavenging assay. (g–i) Schematic illustration, spectral changes, and visual changes of the •NO scavenging assay. (j–l) Schematic illustration, spectral changes, and visual changes of the •OH scavenging assay.

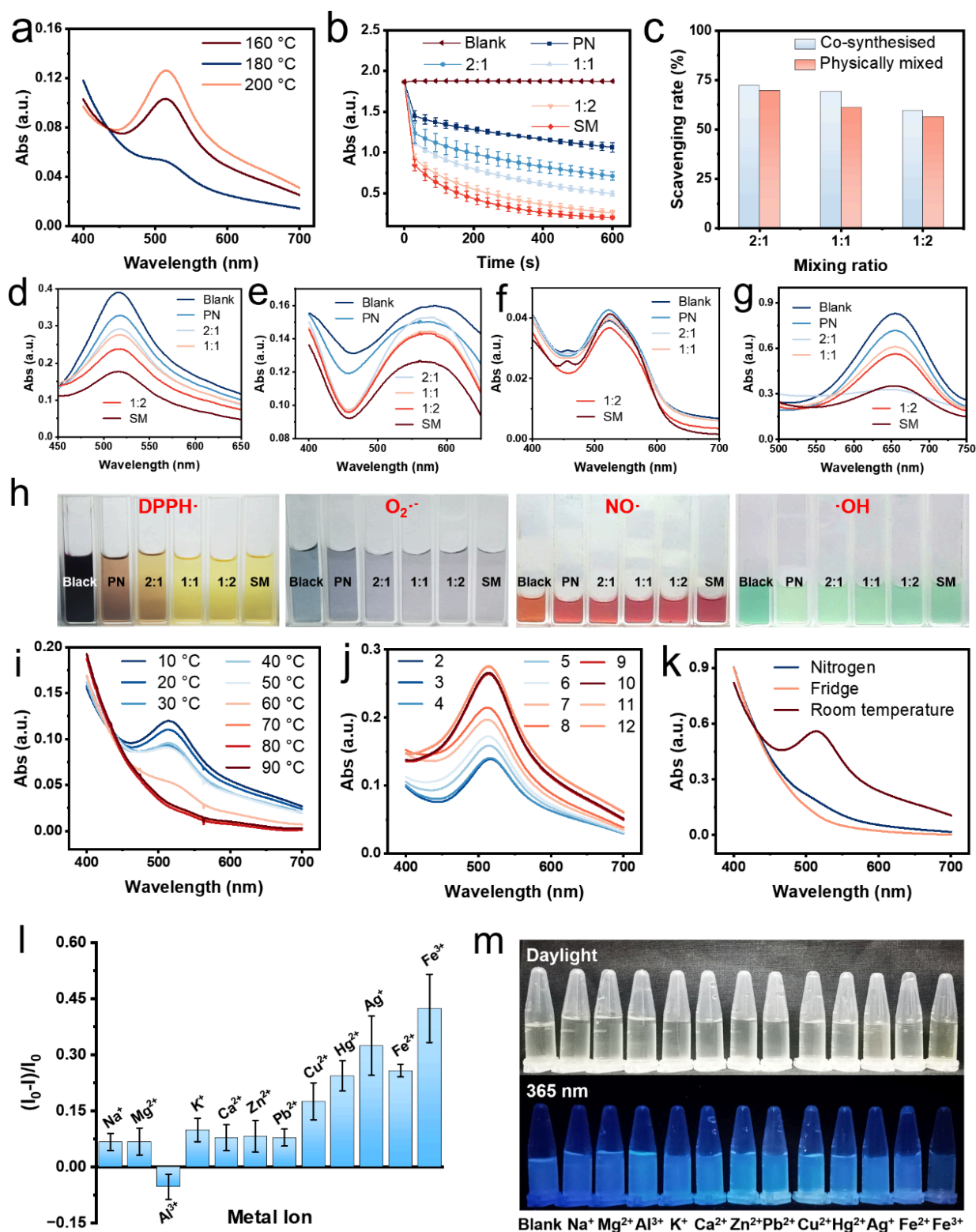


Figure 5

Optimization, functional robustness, and metal ion response of SM-PN-CDs. (a) DPPH• scavenging of SM-PN-CDs synthesized at different temperatures. (b) Time-dependent DPPH• scavenging of SM-PN-CDs from different precursor combinations. (c) Comparison of DPPH• scavenging by co-synthesized and physically mixed CDs with different PN/SM ratios. (d–g) DPPH•, O₂^{•-}, •NO, and •OH scavenging by CDs with different PN/SM ratios. (h) Photos of the four radical scavenging assays. (i,j) Effects of temperature

and pH on the DPPH• scavenging activity of SM-PN-CDs. (k) Short-term storage stability of SM-PN-CDs. (l–m) Selective quenching of SM-PN-CDs by metal ions and corresponding photos under daylight and UV light.

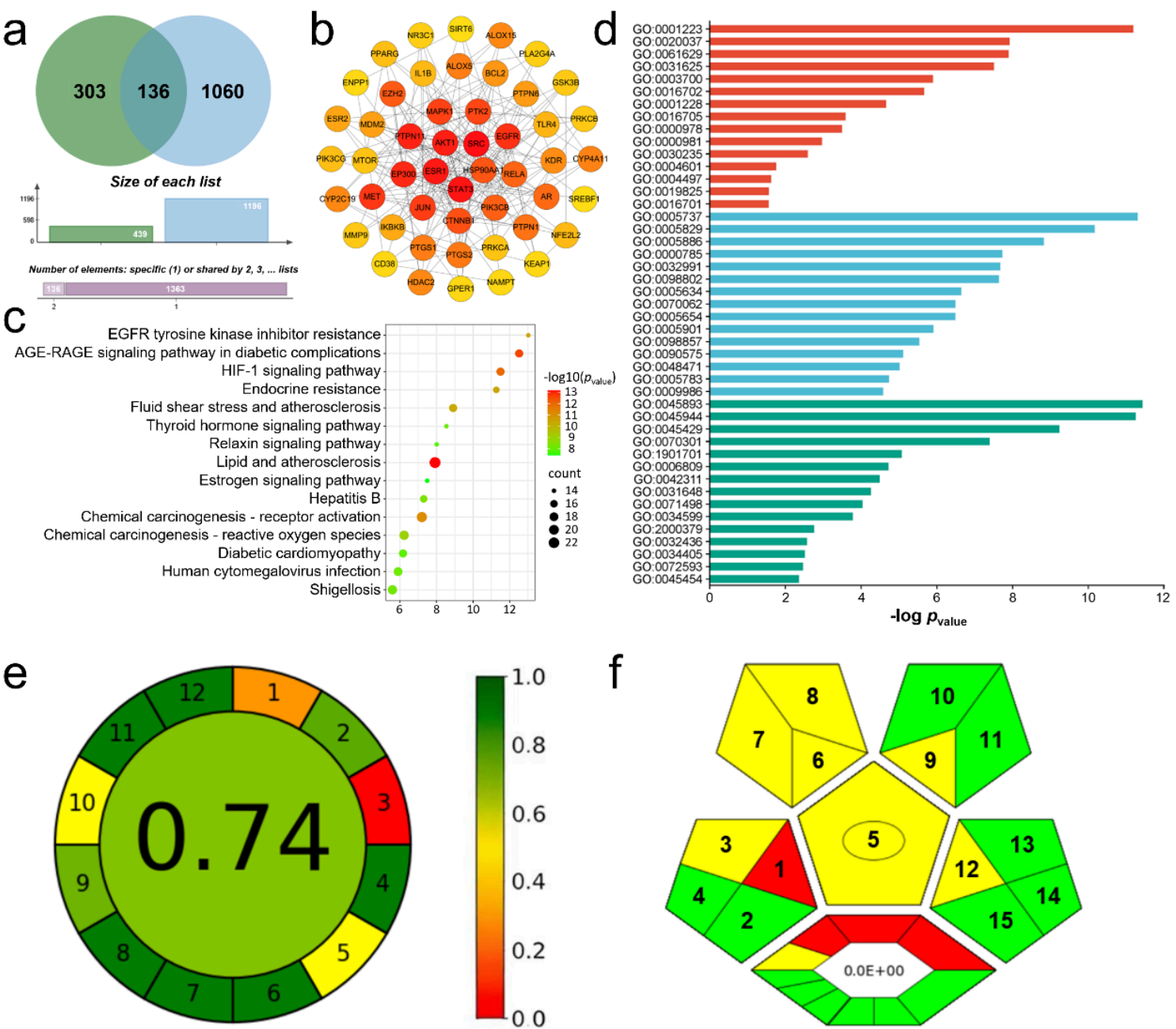


Figure 6

Network pharmacology context and greenness assessment. (a) Venn diagram of the shared targets between candidate phytochemical precursors and endothelial dysfunction. (b) PPI network of the shared targets. (c) KEGG pathway enrichment analysis. (d) GO enrichment analysis, including molecular function, cellular component, and biological process categories. (e) AGREE evaluation. (f) ComplexGAPI evaluation.

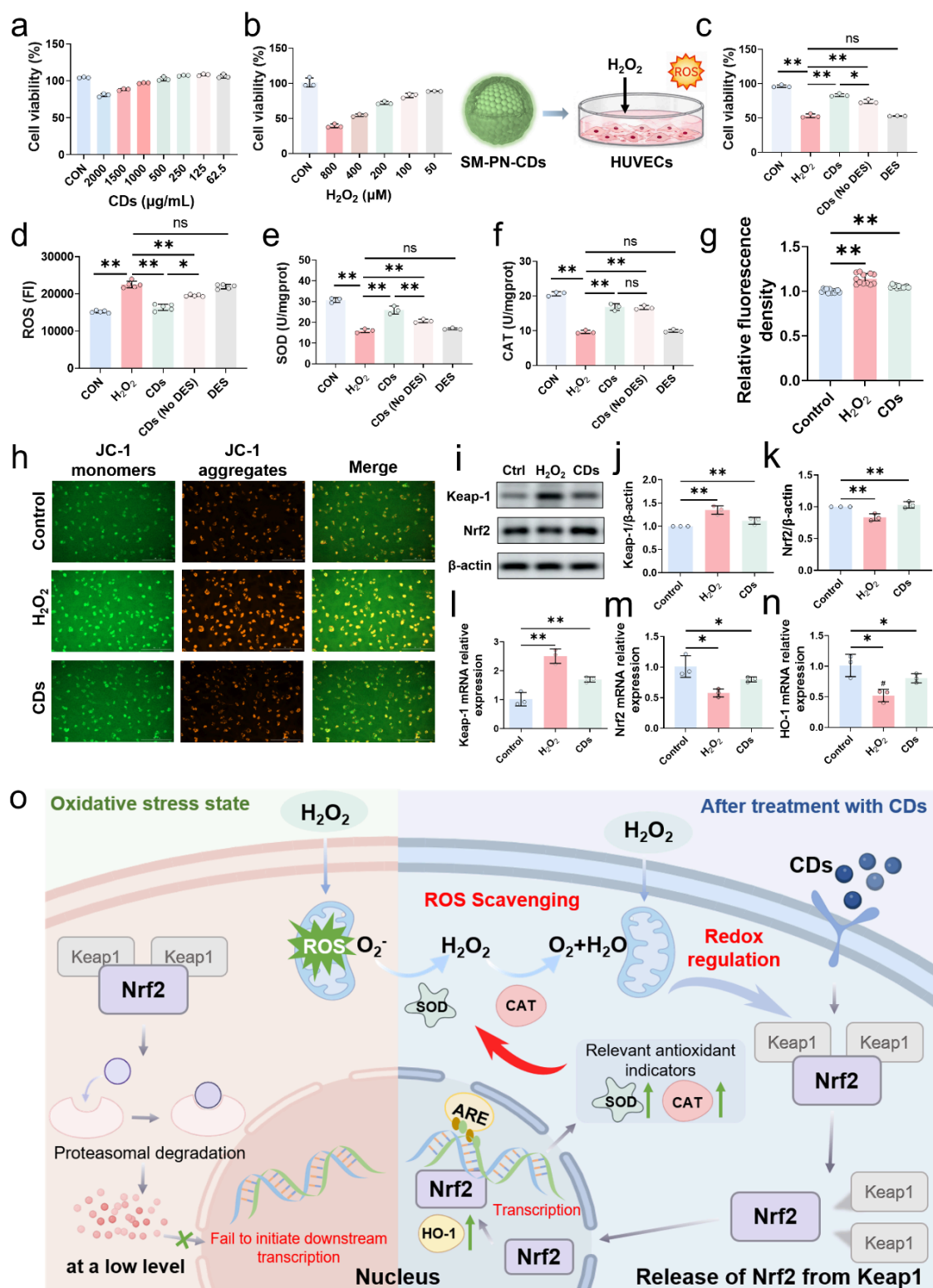


Figure 7

SM-PN-CDs protect HUVEC from oxidative stress damage. (a) Cell viability after incubation with SM-PN-CDs for 24 h. (b) Cell viability after exposure to H₂O₂ for 24 h. (c) Protective effects against H₂O₂-induced injury in HUVECs. (d–f) Intracellular ROS level and SOD and CAT activities after different treatments. (g, h) JC-1 staining and fluorescence analysis of mitochondrial membrane potential. (i–k) Western blot and relative quantitative analysis of Keap1 and Nrf2 protein expression. (l–n) Relative

mRNA expression of Keap1, Nrf2, and HO-1. (o) Proposed mechanism. The data are presented as mean $\pm$ SD ($n \geq 3$). * $p < 0.05$; ** $p < 0.01$.

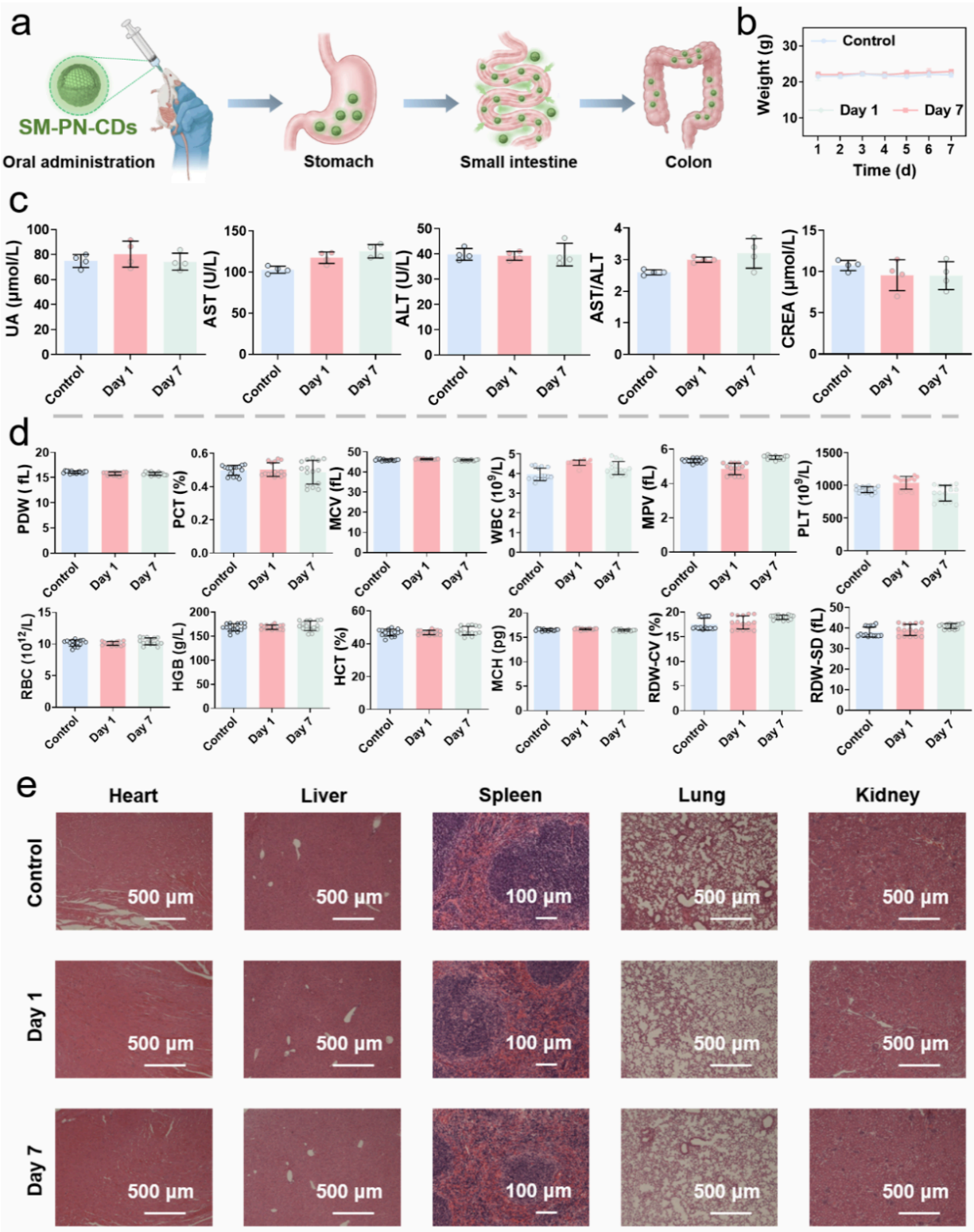


Figure 8

Preliminary biosafety evaluation of SM-PN-CDs. (a) Schematic illustration of oral administration of SM-PN-CDs and the monitoring schedule. (b) Body weight changes during the 7-day treatment period. (c)

Serum biochemical parameters of mice. (d) Hematological parameters of mice. (e) Representative H&E-stained sections of the heart, liver, spleen, lung, and kidney. Data are presented as mean $\pm$ SD ($n \geq 4$).

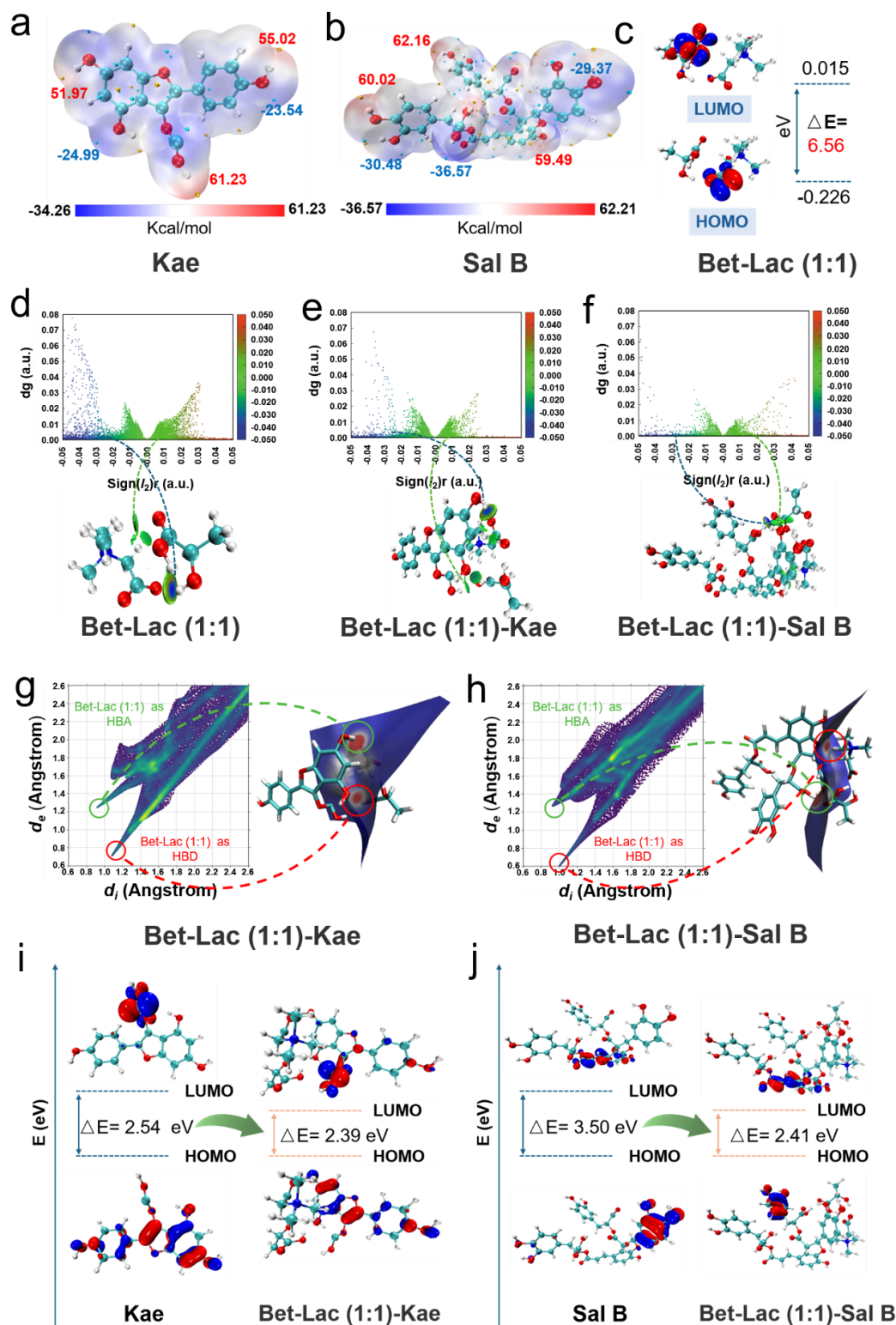


Figure 9

DFT analysis of the NADES effect. (a–b) ESP maps of Kae and Sal B. (c) LUMO and HOMO distribution of Bet-Lac (1:1). (d–f) Sign(λ^2)p colored IGMH scatter plot and colored function δg^{inter} map of Bet-Lac

(1:1)–Kae, and Bet-Lac (1:1)–Sal B (isosurface value = 0.05 a.u.). (g,h) Colored maps of d_{norm} on Hirshfeld surface and corresponding fingerprint plot of Bet-Lac (1:1)–Kae and Bet-Lac (1:1)–Sal B. (i,j) HOMO and LUMO distributions of Kae and Sal B before and after complexation with Bet-Lac (1:1).

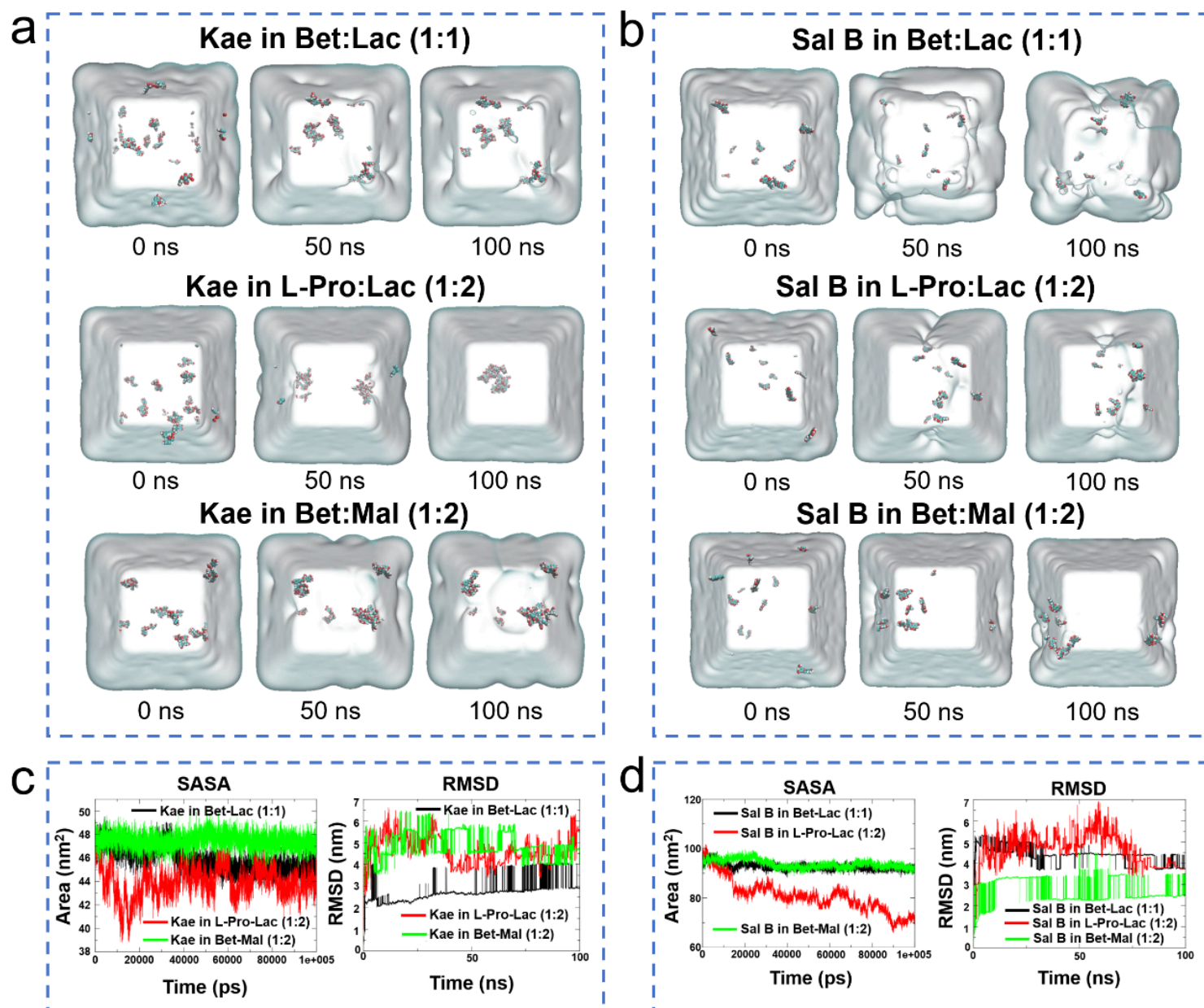


Figure 10

MD analysis of the NADES effect. (a) Representative snapshots of Kae in Bet-Lac (1:1), L-Pro-Lac (1:2), and Bet-Mal (1:2) at 0, 50, and 100 ns. (b) Representative snapshots of Sal B in Bet-Lac (1:1), L-Pro-Lac (1:2), and Bet-Mal (1:2) at 0, 50, and 100 ns. (c) SASA and RMSD profiles of Kae in the three NADES environments. (d) SASA and RMSD profiles of Sal B in the three NADES environments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.docx](#)
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