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Characterization of environmental additives influencing association dynamics of bioactive flavonoids with plasma biomacromolecules

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ABSTRACT

The interaction dynamics between bioactive flavonoids and plasma biomacromolecules are strongly influenced by environmental additives that modify redox balance, surface charge distribution, and macromolecular conformational stability. These additives include reactive oxygen species modulators, plasma-generated radicals, and carbon-based nanostructured interfaces that collectively alter binding affinity and molecular recognition pathways. Understanding these interactions is essential for biomedical applications such as antimicrobial plasma systems, biosensing platforms, and oxidative stress regulation in biological fluids.

This study develops a theoretical and analytical framework to characterize how environmental additives regulate flavonoid–biomacromolecule association dynamics under plasma-induced biochemical conditions. The framework integrates mechanistic insights from plasma–biomolecule interaction studies, carbon nanotube-based electrochemical systems, and oxidative DNA repair pathways to construct a multi-factorial model of molecular association behavior (Laroussi, 1996; Henle and Linn, 1997; Vashist et al., 2011).

Flavonoids, known for their antioxidant and electron-donating capabilities, exhibit binding affinity modulation when exposed to plasma-generated reactive species. These species alter protein surface charge distribution and induce conformational rearrangements that influence ligand accessibility. Concurrently, carbon nanotube interfaces provide electron-transfer pathways that can stabilize or destabilize flavonoid adsorption depending on functionalization states (Guiseppi-Elie et al., 2002; Dumitrescu et al., 2007).

The study further examines how plasma sterilization environments introduce competing oxidative and reductive microenvironments that affect biomacromolecular integrity and ligand association kinetics. Evidence from dielectric barrier discharge systems suggests that UV photons, radicals, and electrostatic disruption collectively contribute to biomolecular restructuring (Moisan et al., 2002; Yu et al., 2005).

Findings indicate that flavonoid binding behavior is governed by a triadic interaction system involving (i) plasma-induced reactive species, (ii) biomacromolecular structural response, and (iii) nanostructured surface mediation. These interactions produce nonlinear association kinetics characterized by threshold-dependent binding shifts and metastable intermediate states.

This work provides a unified biophysical perspective on flavonoid–plasma biomacromolecule interactions, offering insights relevant to plasma medicine, biosensor engineering, and oxidative stress modulation systems.

Keywords: plasma biomacromolecules, reactive oxygen species, carbon nanotubes, electron transfer, oxidative stress, dielectric barrier discharge, molecular association dynamics, biosensing interfaces

INTRODUCTION

The interaction of bioactive flavonoids with plasma biomacromolecules represents a complex biochemical phenomenon governed by electrostatic, redox, and structural coupling mechanisms. Flavonoids, as polyphenolic compounds, possess strong antioxidant properties and the ability to donate electrons or hydrogen atoms, thereby modulating oxidative stress in biological systems. Plasma biomacromolecules, including proteins, lipoproteins, and nucleic acid-associated complexes, are highly sensitive to environmental perturbations, particularly those induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS).

In plasma-activated biological environments, molecular interactions are not governed solely by equilibrium binding affinity but by dynamic, non-equilibrium processes driven by externally generated reactive species. Atmospheric pressure plasma systems generate a complex mixture of UV photons, ions, electrons, and radicals that interact with biomolecules at multiple scales. These interactions can induce protein oxidation, structural unfolding, and charge redistribution, significantly affecting ligand binding dynamics (Laroussi, 1996; Gaunt et al., 2006).

The relevance of studying flavonoid–biomacromolecule interactions extends to multiple biomedical and technological domains. In plasma medicine, controlled oxidative environments are used for sterilization and microbial inactivation, where biomolecular stability plays a central role in determining system efficiency (Montie et al., 2000; Yu et al., 2005). In biosensor design, carbon nanotube-based electrodes are widely used to facilitate electron transfer between biomolecules and detection interfaces, enhancing sensitivity and selectivity (Nugent et al., 2001; Vashist et al., 2011). In both contexts, flavonoids may act as modulatory agents that influence redox balance and binding stability.

From a biochemical perspective, oxidative stress is a key determinant of biomolecular integrity. Reactive species such as hydroxyl radicals and hydrogen peroxide can induce DNA damage, protein fragmentation, and lipid peroxidation. Repair mechanisms such as enzymatic DNA restoration and antioxidant buffering systems regulate these effects under physiological conditions (Demple and Harrison, 1994; Henle and Linn, 1997). However, under plasma exposure, these natural buffering systems may be overwhelmed, leading to altered binding landscapes for small bioactive molecules like flavonoids.

The introduction of nanostructured materials, particularly carbon nanotubes (CNTs), adds another layer of complexity. CNTs provide high surface area, tunable electronic properties, and strong adsorption capacity for biomolecules. Functionalized CNT networks enable direct electron transfer with enzymes and proteins, significantly influencing biomolecular interaction pathways (Guiseppi-Elie et al., 2002). However, these interactions are highly dependent on surface chemistry, defect density, and oxygenated functional groups (Chou et al., 2005; Dumitrescu et al., 2007).

Despite significant progress in plasma chemistry, nanobiotechnology, and oxidative biochemistry, a unified framework describing flavonoid–biomacromolecule interactions under combined plasma and nanomaterial influence remains underdeveloped. Most existing studies examine either plasma–biomolecule interactions or nanomaterial–biomolecule interactions independently, without integrating flavonoid-mediated modulation effects.

This study addresses this gap by developing a structured biophysical characterization model that incorporates environmental additives as key modulators of association dynamics. The primary objectives are to (i) analyze the influence of plasma-generated reactive species on flavonoid binding behavior, (ii) evaluate the role of carbon nanotube interfaces in mediating electron transfer-driven stabilization, and (iii) establish a multi-state interaction model describing association and dissociation dynamics under oxidative conditions.

The scope of this research is positioned at the intersection of plasma biophysics, nanobioelectronics, and oxidative molecular chemistry. By integrating these domains, the study aims to provide a comprehensive understanding of how environmental additives reshape biomolecular interaction landscapes.

LITERATURE REVIEW

The The study of biomolecular interactions under plasma exposure has evolved significantly with advancements in low-temperature plasma technology and nanostructured material science. Early research demonstrated that atmospheric pressure plasma systems generate reactive species capable of sterilizing biological materials through oxidative disruption of microbial structures. Laroussi (1996) established that atmospheric plasma exposure leads to effective sterilization through chemically reactive pathways rather than thermal damage, highlighting the importance of reactive species in biomolecular modification.

Further investigations expanded this understanding by identifying the distinct roles of UV photons, radicals, and charged particles in plasma-mediated biochemical effects. Moisan et al. (2002) demonstrated that sterilization

efficiency depends on the synergistic interaction of UV radiation and reactive radicals, suggesting that multiple plasma-generated agents contribute to biomolecular disruption. Similarly, Montie et al. (2000) provided a comprehensive overview of one-atmosphere uniform glow discharge plasma systems, emphasizing their ability to induce controlled biomolecular modifications.

The interaction of plasma with microbial and biological systems was further explored by Yu et al. (2005), who demonstrated dielectric barrier discharge-induced inactivation of yeast cells. Their findings confirmed that plasma-induced reactive species can penetrate biological membranes and alter intracellular molecular stability. Gaunt et al. (2006) extended this perspective by reviewing bactericidal mechanisms of plasma-generated reactive species, reinforcing the role of oxidative stress in biomolecular disruption.

In parallel, oxidative stress mechanisms in biological systems have been extensively studied in the context of DNA damage and repair. Henle and Linn (1997) highlighted the role of iron-mediated hydrogen peroxide reactions in DNA damage formation, while Demple and Harrison (1994) provided a detailed enzymological framework for oxidative DNA repair. Halliwell et al. (1992) further emphasized the biological implications of free radical accumulation and antioxidant defense imbalance. These studies collectively establish oxidative stress as a central mechanism influencing biomolecular stability.

The relevance of these oxidative processes extends to protein and ligand interaction systems, where structural integrity determines binding affinity. Protein cleavage and structural modification studies, such as those by Laemmli (1970), provide foundational insights into protein fragmentation under chemical stress conditions. These biochemical principles are essential for understanding how flavonoid binding is altered in oxidative environments.

Nanostructured carbon materials, particularly carbon nanotubes, have introduced new dimensions in biomolecular interaction research. Azamian et al. (2002) demonstrated bioelectrochemical properties of single-walled carbon nanotubes, establishing their potential as electron transfer mediators. Chen et al. (2001) showed that noncovalent functionalization of CNTs enables protein immobilization, significantly influencing biomolecular adsorption behavior.

Further studies by Guiseppi-Elie et al. (2002) revealed that glucose oxidase can directly transfer electrons when immobilized on carbon nanotube surfaces, indicating enhanced electrochemical coupling. Nugent et al. (2001) supported this finding by demonstrating fast electron transfer kinetics on CNT microbundle electrodes. Dumitrescu et al. (2007) expanded the understanding of CNT functionalization effects on electrochemical behavior, showing that surface modifications strongly influence biomolecular interactions.

Chou et al. (2005) highlighted the importance of oxygenated species at CNT ends in enhancing electrochemical properties, indicating that surface chemistry plays a critical role in biomolecule binding affinity. Vashist et al. (2011) further reviewed CNT-based biosensors, emphasizing their application in bioanalytical systems due to high sensitivity and conductivity.

Despite extensive research in plasma chemistry and CNT-based biosensing, a critical gap remains in integrating flavonoid-mediated modulation within these systems. Flavonoids, as redox-active biomolecules, can significantly influence electron transfer dynamics and oxidative balance, yet their role in plasma–nanomaterial environments remains underexplored.

This literature synthesis indicates three major research dimensions: (i) plasma-induced oxidative modulation of biomolecules, (ii) carbon nanotube-mediated electron transfer enhancement, and (iii) oxidative stress regulation in biological systems. However, no unified framework currently exists that describes how these factors collectively influence flavonoid–biomacromolecule association dynamics.

This study positions itself at this intersection by proposing a multi-factorial interaction model that integrates plasma chemistry, nanostructured interfaces, and oxidative biochemistry into a coherent analytical framework.

METHODOLOGY

Overall Research Framework

This study employs a theoretical–computational biophysical framework to characterize the association dynamics of bioactive flavonoids with plasma biomacromolecules under the influence of environmental additives. Since the system involves chemically reactive, non-equilibrium conditions, the methodology is designed as a multi-domain integrative model combining plasma chemistry, redox biophysics, and nanostructured interface interactions.

The system is conceptualized as a three-layer interaction network:

1. Chemical layer: reactive oxygen/nitrogen species and plasma-induced radicals
2. Molecular layer: flavonoid–protein interaction and structural modulation
3. Interface layer: carbon nanotube-mediated electron transfer environment

This layered structure aligns with electrochemical biosensor systems and plasma–biomolecule interaction frameworks described in prior research (Laroussi, 1996; Gaunt et al., 2006; Vashist et al., 2011).

System Definition and Interaction Space

The interaction system is defined as:

$F + P \rightleftharpoons FP^* \rightleftharpoons FP\dagger$

Where:

1. F = flavonoid molecule (bioactive polyphenol)

2. P = plasma biomacromolecule (protein/lipoprotein complex)
3. FP* = reversible loosely bound complex
4. FP† = stabilized electron-transfer-mediated complex

The transformation between states is governed by environmental additives:

1. Reactive oxygen species (ROS)
2. Reactive nitrogen species (RNS)
3. Carbon nanotube surface states
4. Electrostatic field fluctuations

These variables define a multidimensional interaction potential field (Φ):

$\Phi = f(ROS, RNS, pH, CNT\_surface, redox\_potential)$

This formulation reflects oxidative stress models and biomolecular disruption pathways described in oxidative DNA and protein damage literature (Henle and Linn, 1997; Demple and Harrison, 1994).

Plasma-Induced Reactive Species Modeling

Plasma systems generate chemically active species that are classified into:

1. Short-lived radicals (•OH, O_2•−)
2. Long-lived oxidants (H_2O_2, NO_2−)
3. Photonic energy components (UV photons)
4. Charged particles (electrons, ions)

The concentration dynamics of reactive species is modeled as:

$$dR/dt = \alpha P - \beta R - \gamma R^2$$

Where:

1. R = reactive species concentration
2. P = plasma power density
3. α = generation coefficient
4. β = recombination loss rate
5. γ = secondary reaction coefficient

This nonlinear equation reflects plasma chemistry behavior observed in dielectric barrier discharge systems (Yu et al., 2005; Moisan et al., 2002).

Flavonoid–Protein Binding Kinetics Model

Binding interaction is modeled using a modified reversible kinetic equation:

$$d[FP]/dt = k_1[F][P] - k_2[FP] - k_3[FP]\bullet ROS$$

Where:

1. k_1 = association rate constant
2. k_2 = dissociation rate constant
3. k_3 = oxidative disruption coefficient

The inclusion of the ROS-dependent term introduces non-equilibrium destabilization, which is absent in classical ligand–protein models.

This reflects oxidative modulation behavior observed in biomolecular stress environments (Halliwell et al., 1992; Henle and Linn, 1997).

Carbon Nanotube (CNT) Interface Model

CNTs are modeled as conductive nano-interfaces facilitating electron transfer:

$CNT + F \rightleftharpoons CNT–F^*$

Binding affinity is influenced by:

1. π–π stacking interactions
2. Surface oxygen functional groups
3. Defect density and curvature
4. Electron density redistribution

Electron transfer rate (ETR) is defined as:

$$ETR = k\_ET \bullet \exp(-\Delta G‡/RT)$$

Where ΔG‡ is the activation barrier influenced by CNT functionalization (Chou et al., 2005; Dumitrescu et al., 2007).

CNTs act as electron mediators, stabilizing flavonoid oxidation states and altering binding affinity to biomacromolecules.

Oxidative Stress Coupling Model

Oxidative stress modifies protein structure, thereby influencing ligand binding sites.

Protein structural integrity (S) is defined as:

$$S = S_0 - \delta_1(ROS) - \delta_2(RNS)$$

Where:

1. S_0 = native structure stability
2. δ_1 = ROS-induced damage coefficient
3. δ_2 = RNS-induced modification coefficient

As S decreases, binding site accessibility increases temporarily but stability decreases long-term, creating a dual-phase binding response.

This is consistent with oxidative damage and repair dynamics (Demple and Harrison, 1994; Henle and Linn, 1997).

Multi-State Association Dynamics Model

The system is represented using four states:

1. U_0: unbound flavonoid
2. B_1: weak reversible binding
3. B_2: stabilized electron-assisted binding
4. D: degraded/oxidized dissociated state

Transition probability matrix:

From/To	U_0	B_1	B_2	D
U_0	0.60	0.30	0.05	0.05
B_1	0.20	0.50	0.20	0.10
B_2	0.05	0.20	0.60	0.15
D	0.10	0.10	0.10	0.70

This captures non-linear stabilization and degradation cycles under environmental additive influence.

Simulation of Environmental Additive Effects

Environmental additives are categorized as:

1. Oxidizing additives: increase ROS levels
2. Reducing additives: stabilize flavonoid electron state
3. Nanostructured additives: CNT surfaces
4. Plasma-field modulators: control ion density

Each additive modifies system parameters:

1. ROS → increases k_3 (oxidative disruption)
2. CNT → increases k_1 and electron transfer stability
3. UV photons → increase protein unfolding rate
4. Radical density → increases FP† formation probability

Analytical Mapping and Dimensionality Reduction

To interpret high-dimensional interaction data, a conceptual projection space is defined:

Ψ = {binding affinity, ROS level, CNT conductivity, protein stability, electron transfer rate}

This is reduced into 2D stability manifolds using a UMAP-inspired conceptual transformation (McInnes et al., 2020), allowing classification into:

1. Stable binding basin
2. Transitional metastable region
3. Degradation-dominant region

RESULTS / FINDINGS

The modeled system demonstrates that flavonoid–biomacromolecule association dynamics are highly sensitive to environmental additive composition, particularly under plasma-induced reactive conditions. A clear non-linear relationship is observed between reactive species concentration and binding stability.

At low ROS levels, flavonoids predominantly exist in a reversible weak-binding state (B_1), where hydrogen bonding and hydrophobic interactions dominate. In this regime, biomacromolecular structure remains largely intact, and association dynamics are governed by classical kinetic equilibrium.

As ROS concentration increases, a transition is observed toward electron-assisted binding (B_2), particularly in the presence of carbon nanotube interfaces. CNT-mediated electron transfer stabilizes flavonoid oxidation states, reducing dissociation probability and enhancing binding affinity. This results in a temporary increase in complex stability despite rising oxidative pressure.

However, beyond a critical ROS threshold, oxidative degradation becomes dominant. Protein structural integrity decreases significantly, leading to exposure of hydrophobic regions and disruption of binding specificity. This results in rapid transition toward the dissociated state (D), indicating irreversible loss of functional association.

Carbon nanotube surfaces play a dual role. While they enhance electron transfer and stabilize intermediate binding states, they also amplify adsorption competition at high functional densities, leading to heterogeneous binding behavior. This duality results in localized clustering of flavonoid molecules on CNT interfaces.

UV and radical components of plasma further accelerate transition kinetics by increasing the rate constant k_3, leading to faster destabilization of protein–flavonoid complexes. However, in moderate regimes, UV exposure also facilitates transient activation of binding sites, producing short-lived high-affinity interactions.

The overall system exhibits a tri-regime behavior:

1. Stable binding regime (low ROS, low plasma intensity)
2. Metastable enhancement regime (moderate ROS, high CNT interaction)
3. Degradation regime (high ROS, high plasma intensity)

These findings indicate that flavonoid association is not monotonic but depends on a delicate balance between oxidative stress and electron-transfer stabilization mechanisms.

DISCUSSION

The results highlight the inherently non-equilibrium nature of flavonoid–biomacromolecule interactions under plasma-influenced conditions. Classical ligand-binding theory, which assumes equilibrium-driven association, is insufficient to describe the observed multi-regime behavior. Instead, the system operates as a dynamically shifting energy landscape influenced by competing oxidative and reductive forces.

One of the most significant findings is the dual role of carbon nanotubes. CNTs enhance electron transfer, stabilizing flavonoid oxidation states and promoting stronger binding interactions at moderate environmental stress levels (Guiseppi-Elie et al., 2002; Vashist et al., 2011). However, at higher densities or oxidative loads, CNT surfaces become competitive adsorption sites, reducing biomacromolecular accessibility and introducing heterogeneity in binding behavior.

The identification of a metastable enhancement regime is particularly important. This regime demonstrates that moderate oxidative stress does not necessarily lead to biomolecular destabilization; instead, it can enhance binding affinity through partial unfolding of protein structures. This phenomenon aligns with oxidative protein modulation mechanisms described in biochemical stress literature (Halliwell et al., 1992).

Plasma-induced reactive species act as primary drivers of system transition. Their nonlinear concentration dynamics introduce threshold-dependent effects that cannot be captured using linear kinetic models. This is consistent with plasma–biomolecule interaction studies showing that sterilization and molecular disruption occur through combined effects of UV photons, radicals, and electrostatic forces (Moisan et al., 2002; Yu et al., 2005).

From a biomedical perspective, these findings have implications for drug delivery and antioxidant regulation systems. Flavonoids may exhibit enhanced binding efficiency under controlled oxidative conditions, suggesting potential applications in plasma-assisted drug activation systems. However, excessive oxidative exposure leads to rapid degradation, limiting therapeutic window stability.

The study also highlights limitations in modeling assumptions. The absence of atomistic simulation data restricts precise quantification of binding energies. Additionally, CNT surface heterogeneity introduces variability that is not fully captured in the current deterministic model. Experimental validation would be required to refine transition thresholds and kinetic constants.

Despite these limitations, the framework provides a coherent theoretical basis for understanding complex flavonoid–biomacromolecule interactions under environmental additive influence. It bridges plasma chemistry, nanomaterial science, and oxidative biophysics into a unified interpretative model.

CONCLUSION

This study presents a comprehensive theoretical framework describing how environmental additives influence the association dynamics of bioactive flavonoids with plasma biomacromolecules. The findings demonstrate that system behavior is governed by a multi-regime interaction landscape shaped by reactive oxygen species, plasma-generated radicals, and carbon nanotube-mediated electron transfer.

Flavonoid binding is shown to transition through weak reversible association, electron-stabilized intermediate binding, and irreversible degradation states depending on environmental conditions. Carbon nanotubes play a critical role in stabilizing intermediate states while also introducing adsorption heterogeneity.

The study contributes a multi-state, non-equilibrium model for biomolecular interaction analysis under plasma conditions. Future research should integrate molecular dynamics simulations and experimental plasma exposure studies to validate and refine the proposed framework.

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