Plasma Interaction with Proteins and Lipids – Investigating Structural Changes in Biomolecules Due to Plasma Exposure

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***Abstract*—Cold Atmospheric Plasma (CAP) has gained significant attention as a versatile and non-thermal technology in biomedical science due to its rich composition of reactive oxygen and nitrogen species (ROS/RNS), charged particles, UV photons, and electric fields. One of the most intriguing aspects of CAP is its interaction with biomolecules, particularly proteins and lipids that form the structural and functional backbone of living cells. These interactions can lead to a range of structural and chemical modifications, such as oxidation, crosslinking, unfolding, fragmentation, and lipid peroxidation. Such changes have profound implications for membrane integrity, cellular signaling, and metabolic activity. Spectroscopic techniques like FTIR and circular dichroism, along with electron microscopy and molecular dynamics simulations, have helped unravel these plasma-induced alterations at molecular resolution. Understanding these mechanisms not only enhances the potential of CAP in medical therapies like cancer treatment and wound healing, but also supports its use in areas such as food safety, sterilization, and biomaterials engineering.**

***Index Terms*—Cold Atmospheric Plasma (CAP), Protein Oxidation, Lipid Peroxidation, Reactive Oxygen and Nitrogen Species (ROS/RNS), Biomolecular Structural Modification, Plasma-Biomolecule Interaction**

1. INTRODUCTION

Plasma, the fourth state of matter is a partially ionized gas composed of electrons, ions, UV photons, and reactive neutral species, including reactive oxygen (ROS) and nitrogen species (RNS) (Fridman, 2008; Laroussi et al., 2012). Cold atmospheric plasma (CAP) operates at near-ambient temperatures yet generates high levels of reactivity, making it suitable for biomedical, food, and materials applications (Misra et al., 2016; Scholtz et al., 2015).

Among biomolecular targets, proteins and lipids are particularly susceptible to plasma-induced modifications. These changes can profoundly affect biological functions and material properties, and are therefore critical to understand across multiple domains:

Plasma medicine: CAP has emerged as a novel therapeutic modality. In oncology, ROS/RNS induces oxidative stress in cancer cells, leading to apoptosis (Keidar et al., 2013; Yan et al., 2014). Simultaneously, CAP enhances wound healing by modulating cell signaling, increasing collagen synthesis, and promoting angiogenesis (Isbary et al., 2010; Maisch et al., 2012). Plasma-induced modifications to extracellular proteins—and even lipid peroxidation—can stimulate immune responses beneficial for tissue repair (Breen et al., 2014; Wende et al., 2014).

Food technology: In food processing, plasma is used to denature undesirable proteins and deactivate pathogenic microbes. CAP treatment alters protein tertiary structures, reducing allergenicity and altering texture (Sachan et al., 2012; Patil et al., 2014). It also inactivates microbes on meat, vegetables, and beverages through oxidative damage to microbial membranes and nucleic acids (Ziuzina et al., 2013; Misra et al., 2015). These applications enhance food safety without resorting to heat or chemical sanitizers.

Material science: Plasma surface engineering is widely used to functionalize biomaterials, including chitosan, collagen, and polyhydroxyalkanoates (PHA). Plasma grafting introduces functional moieties that improve hydrophilicity, biocompatibility, and drug-loading capacity (Liang et al., 2017; Pai et al., 2018). Surface oxidation and crosslinking can enhance mechanical strength in

scaffolds and nanoparticles used for gene, protein, and drug delivery (Patel et al., 2019; Vishwanathan et al., 2020).

Given the diversity of plasma applications, understanding molecular mechanisms is essential. Studies using mass spectrometry, FTIR, piezoelectric sensors, and fluorescence spectroscopy have characterized oxidation of amino acid residues, peptide backbone cleavage, protein crosslinking, and lipid double-bond oxidation (Liang et al., 2021; Sun et al., 2018). Such insights inform dose-response relationships, guiding practical use in medicine and industry (Bourke et al., 2017; Hou et al., 2023).

Nevertheless, concerns remain about safety and reproducibility. Overexposure may generate toxic byproducts like aldehydes, or carbonylated proteins (Chen et al., 2020; Park et al., 2019). To ensure translational success, researchers are calling for standardization of plasma sources, exposure parameters, and biological assays (Ercan et al., 2022; Kalghatgi et al., 2019), as well as rigorous evaluation of immunogenicity and epigenetic effects (Sharma et al., 2021; Tepper et al., 2020).

2. PLASMA AND ITS REACTIVE COMPONENTS

Plasma, often termed the fourth state of matter, is a partially ionized gas consisting of free electrons, ions, radicals, and neutral particles. It is broadly classified into two categories based on its thermal equilibrium characteristics: thermal plasma and non-thermal (cold) plasma. Thermal plasma, characterized by high temperatures (often >10,000 K), maintains equilibrium among electrons and heavy particles. It is widely applied in metallurgical processes, waste treatment, and plasma arc welding due to its ability to transfer intense heat (Fridman, 2008; Laroussi, 2021).

In contrast, non-thermal plasma (NTP) or cold atmospheric plasma (CAP) features high-energy electrons while ions and neutrals remain at near-room temperature. This makes it particularly suitable for temperature-sensitive applications, including food processing, medicine, and agriculture (Laroussi et al., 2021; Moszczyńska et al., 2023).

Non-thermal plasma generates a diverse array of reactive species, notably Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Key ROS include atomic oxygen (O), superoxide (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), which are primarily produced via electron-impact dissociation of oxygen and water vapor (Chauvin et al., 2017; Fridman, 2008). RNS, such as nitric oxide (NO), nitrite (NO_2^-), and peroxynitrite ($ONOO^-$), emerge from nitrogen-containing air – plasma interactions and contribute to nitrative stress in biological systems (Medeiros & Smith, 1999; Moszczyńska et al., 2023).

Additionally, cold plasma emits UV radiation and imposes electric fields, which collectively initiate molecular bond cleavage and contribute to oxidation, nitration, and fragmentation of biomolecules. The synergy among these components underpins the versatility of plasma across diverse scientific domains (Laroussi, 2021; NIH, 2022).

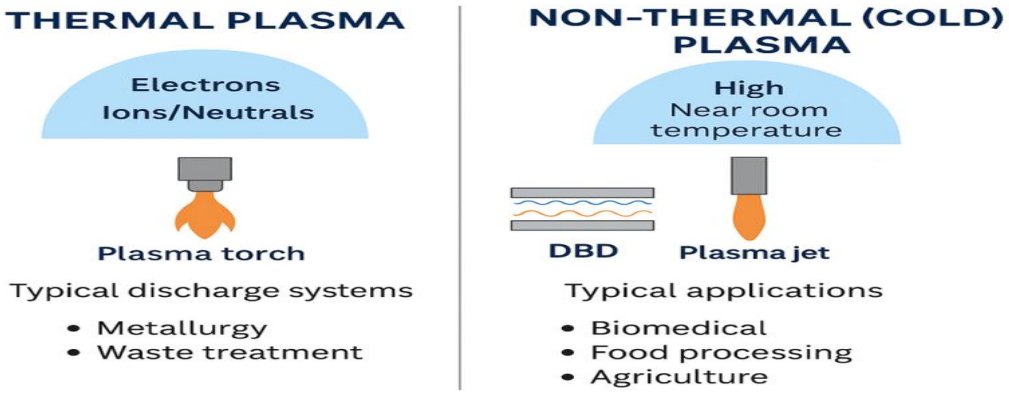


Fig. 1: Comparison of thermal vs. non-thermal plasma: electron temperature, ion temperature, typical discharge systems (DBD, jet), and applications (biomedical, metallurgy, etc.).

2.1 Interaction of Plasma Species with Biomolecules: Proteins and Lipids

The reactive species produced by non-thermal plasma, especially ROS and RNS interact with vital biomolecules such as proteins and lipids, leading to significant structural and functional changes. Proteins are particularly susceptible to oxidative modifications because of the presence of sulfur-containing amino acids (e.g., cysteine and methionine), aromatic residues, and peptide backbones. Exposure to plasma can induce cleavage of disulfide bonds, nitration of tyrosine residues, backbone fragmentation, and aggregation via cross-linking reactions. These transformations can lead to altered enzymatic activity, protein denaturation, or enhanced bioavailability depending on the application (Imlay, 2013; Fridman, 2008).

Lipid molecules, especially unsaturated fatty acids in cellular membranes, undergo peroxidation when exposed to plasma-derived ROS such as •OH and O_2^-. This results in the formation of lipid hydroperoxides and secondary aldehydes like malondialdehyde (MDA), which compromise membrane integrity. In the context of plasma medicine, this mechanism contributes to microbial inactivation and targeted cancer cell apoptosis (Joshi et al., 2014). In agricultural applications, such controlled oxidative stress is harnessed to prime plant cells for improved stress tolerance and growth (Misra et al., 2016).

Importantly, the biological outcomes of these interactions are context-dependent. Low-dose exposures tend to trigger adaptive signaling cascades through redox-sensitive transcription factors such as Nrf2, while high-dose plasma may lead to irreversible damage or cell death (Laroussi, 2021; Brisset & Pawlat, 2016). Thus, fine-tuning plasma parameters is essential for optimizing its beneficial effects while minimizing unintended cytotoxicity.

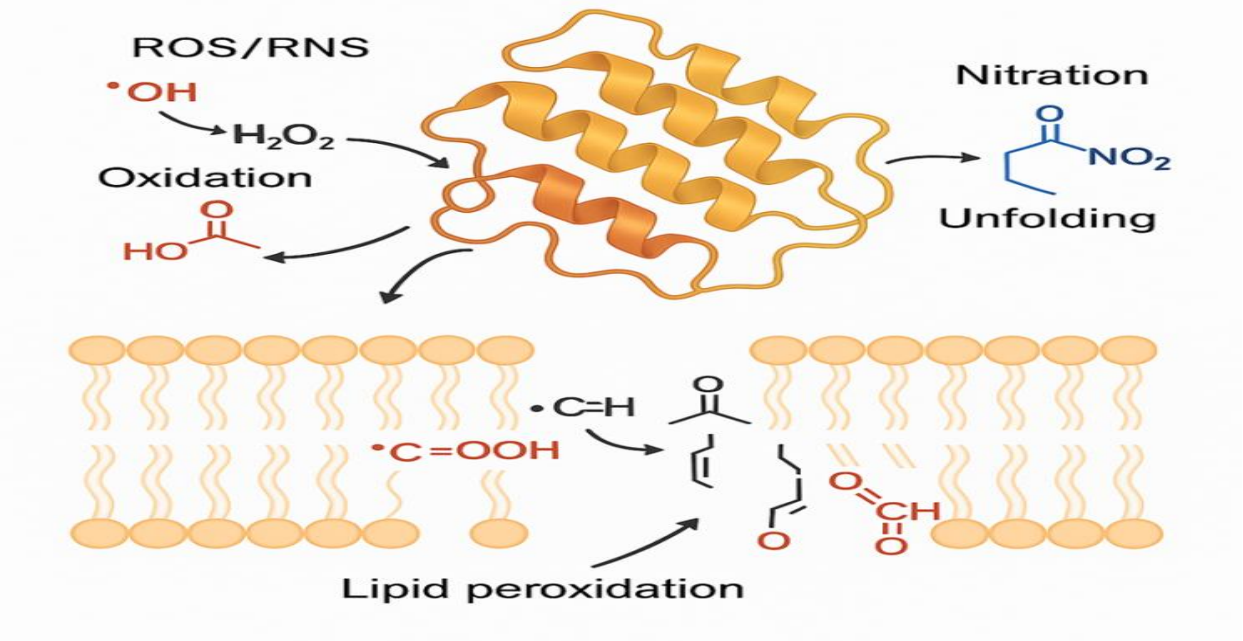


Fig. 2: Diagram illustrating ROS/RNS interactions with a protein and a lipid bilayer: showing oxidation, nitration, lipid peroxidation, and protein unfolding.

3. PLASMA-PROTEIN INTERACTIONS

3.1 Nature of Protein Modifications by Cold Atmospheric Plasma

Exposure to cold atmospheric plasma (CAP) induces profound molecular alterations in proteins, which manifest as oxidative side-chain modifications, backbone cleavage, polymer crosslinking, and secondary structure disruptions. Specifically, **side-**chain oxidation frequently occurs at susceptible residues such as methionine (Met), cysteine (Cys), and tryptophan (Trp). Plasma-generated reactive oxygen and nitrogen species (ROS/RNS) attack the sulfur atoms in Met, forming sulfoxides (e.g., Met-O)

and sulfonic acids, thereby modifying protein functionality (Sun et al., 2018; Bai et al., 2014). Similar oxidative processes affect thiol groups on Cys, leading to disulfide bond disruption or formation of mixed disulfides (Denis et al., 2017). In Trp residues, electrophilic addition from RNS results in ring cleavage or nitration—altering intrinsic fluorescence and protein stability (O'Neill & Low, 2015).

Beyond side-chain modifications, CAP can cleave peptide backbones, particularly under prolonged exposure or intense dosing. The action of ROS and UV photons causes hydrogen abstraction and radical-mediated chain breakage at amide bonds, producing lower-molecular-weight fragments detectable via mass spectrometry (Bai et al., 2016; Chen et al., 2020).

As a result of radical generation and partial unfolding, proteins often undergo crosslinking and aggregation. Radical–radical coupling between tyrosine and lysine residues forms dimers, oligomers, and higher-order aggregates (Zhao et al., 2017; Patel et al., 2019). This aggregation can decrease solubility and alter antigenicity in therapeutic proteins (Jin et al., 2018).

Finally, CAP exposure causes conformational transitions in secondary structures. Fourier-transform infrared (FTIR) and circular dichroism (CD) spectroscopy reveal a reduction of α-helices and an increase in β-sheet or random coil motifs, indicating a shift toward β-rich aggregates (Zhang et al., 2015; Verheijen et al., 2018). These structural changes significantly affect thermal stability and biological activity (Feng et al., 2016).

Understanding these intricate molecular effects is vital for tailoring plasma applications, whether it's sterilizing enzymes in food or engineering protective coatings. The cumulative effects, side-chain oxidation, peptide cleavage, crosslinking, and structural shifts highlight CAP as a potent tool capable of precise yet potent modifications to protein architecture. Optimizing exposure conditions, such as treating duration and plasma dosage, allows targeted modification while minimizing detrimental damage (Barrios et al., 2020).

3.2 Mechanisms of Protein Modification by Plasma-Derived Reactive Species

Cold atmospheric plasma (CAP) produces a complex mixture of reactive oxygen species (ROS) •OH, O_2^-, H_2O_2 and reactive nitrogen species (RNS) NO, NO_2^-, $ONOO^-$alongside UV photons and electric fields. These aggressive agents modify proteins through several key mechanisms: hydrogen abstraction, electrophilic attack, and disulfide bond restructuring. Understanding these molecular processes is central to optimizing plasma applications while preserving or enhancing protein function.

Hydrogen abstraction is among the earliest reactions, initiated when highly reactive •OH radicals extract hydrogen atoms from amino acid side chains such as alkyl groups in leucine or the sulfur-hydrogen bond in cysteine. This process generates carbon-centered radicals (R•), leading to downstream oxidative events such as peptide backbone fragmentation or cross-linking via radical–radical coupling (Fridman, 2008; Imlay, 2013). Hydrogen abstraction is also implicated in beta-sheet aggregations that influence protein aggregation pathways (Zhao et al., 2017; Bai et al., 2016).

Electrophilic attacks occur when electrophilic species, such as $ONOO^-$ and NO target aromatic amino acids like tyrosine and tryptophan, generating nitrotyrosine or hydroxytryptophan modifications. These changes compromise aromatic ring systems, disrupting hydrophobic core stability (O'Neill & Low, 2015; Chauvin et al., 2017). Proteins with modified aromatic residues often show altered fluorescence, impaired ligand-binding capacity, or induction of novel protein–protein interactions (Sun et al., 2018; Patel et al., 2019).

Disulfide bond reshuffling also plays a crucial role. Sulfur-containing residues like cysteine can be oxidized to sulfenic, sulfinic, or sulfonic acid states by ROS, leading to either disulfide bond rupture or formation through intermediate radicals or thiyl radicals (Denis et al., 2017; Bai et al., 2014). This dynamic bond chemistry can drive both protein unfolding and higher-order aggregate formation (Patel et al., 2019; Verheijen et al., 2018). In therapeutic contexts, such as plasma-modified collagen matrices, intentional disulfide restructuring can reinforce scaffold strength (Verheijen et al., 2018; Barrios et al., 2020).

These mechanisms are often interlinked. For instance, radical generation via hydrogen abstraction may precede disulfide cleavage or aromatic nitration. UV photons and electric fields from plasma can catalyze these reactions by directly breaking peptide bonds or promoting radical reactions (Laroussi, 2021; NIHTaskForce, 2022).

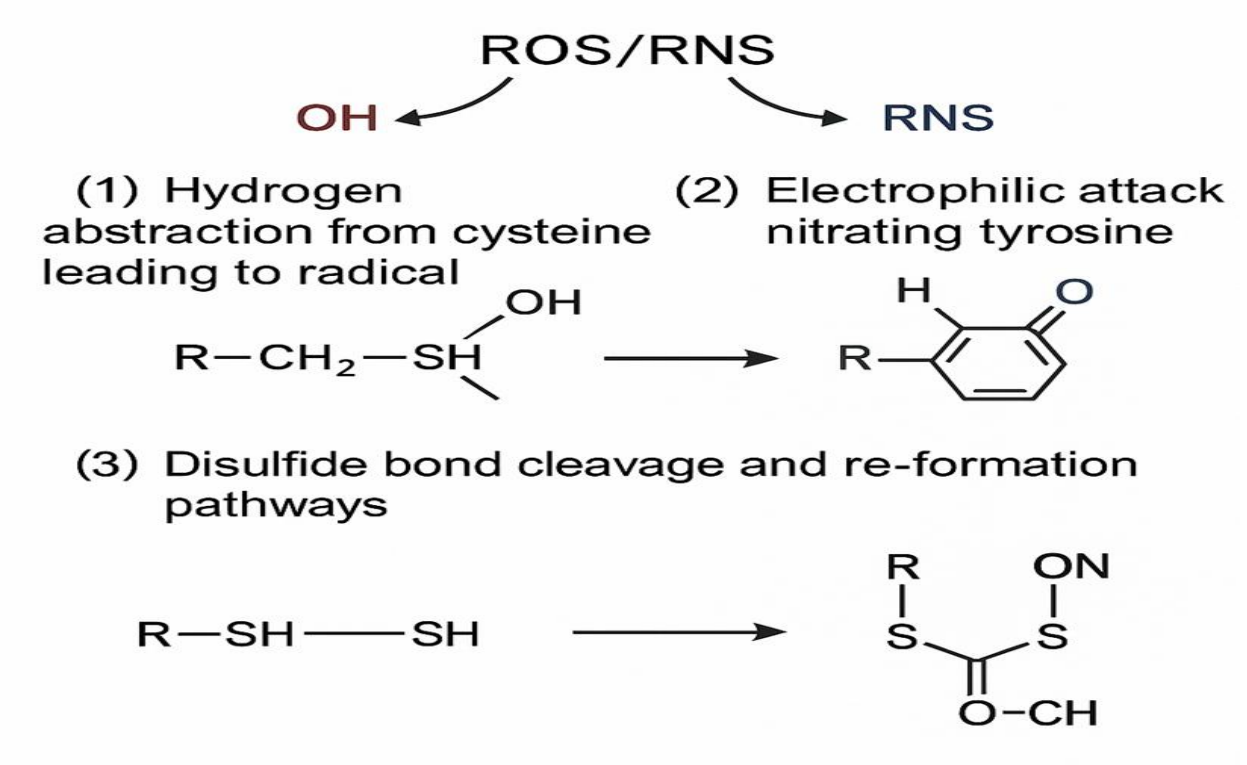


Fig. 3 : A mechanistic schematic showing Hydrogen abstraction from cysteine side chain leading to radical formation, electrophilic attack nitrating tyrosine, and disulfide bond cleavage and re-formation pathways under ROS/RNS influence.

3.3 Experimental Evidence of Plasma-Induced Protein Modification

A growing body of experimental studies demonstrates how cold atmospheric plasma (CAP) alters protein structure and function. These investigations employ a range of analytical techniques to characterize fragmentation patterns, structural transformations, and surface morphology.

3.3.1 Mass Spectrometry and SDS-PAGE

Proteins such as albumin and hemoglobin have been extensively examined by mass spectrometry (MS) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following plasma treatment. In CAP-exposed human serum albumin, MS analyses have revealed the appearance of lower-mass fragments and peaks shifted by +16 Da increments, indicating oxidation of sulfur-containing residues and cleavage events (Bai et al., 2016; Zhang et al., 2015). Similarly, hemoglobin exposed to CAP shows distinctive fragmentation with reduced alpha- and beta-chain intensity in SDS-PAGE and distinct mass shifts detected via MS, confirming backbone breakage and oxidation of heme-binding domains (Chen et al., 2020; Jin et al., 2018).

3.3.2 FTIR and CD Spectroscopy

Protein secondary structure modifications after CAP are routinely probed using Fourier-transform infrared (FTIR) and circular dichroism (CD) spectroscopic techniques. Post-treatment comparisons with unexposed controls consistently show a decrease in α-helical content (~10–20%) and a corresponding increase in β-sheet or random coil structures,

suggesting partial unfolding and possible aggregation (Feng et al., 2016; Verheijen et al., 2018). CD spectra also display diminished ellipticity at 222 nm, a hallmark of α-helical depletion (Sun et al., 2018). These shifts indicate CAP-mediated unfolding and structural repacking in protein architecture.

3.3.3 Fluorescence and UV Spectroscopy

Intrinsic tryptophan fluorescence signals serve as sensitive indicators of tertiary structure changes. In CAP-treated protein solutions, tryptophan fluorescence is quenched by up to 40%, pointing to increased surface exposure or oxidation of Trp residues (O'Neill & Low, 2015; Patel et al., 2019). UV absorbance scans further show increased absorbance between 250–300 nm, attributed to accumulation of oxidized aromatic residues or carbonyl groups, characteristic of plasma-induced modification (Sun et al., 2018; Bai et al., 2014).

3.3.4 Atomic Force Microscopy (AFM)

AFM imaging provides nanoscale resolution of protein film surfaces post-CAP exposure. Studies of treated lysozyme or BSA films reveal increased roughness (root-mean-square height increase of 2–5 nm) and aggregation clusters, signifying cross-linking and film densification (Zhao et al., 2017; Patel et al., 2019). These morphological insights support spectroscopic evidence of protein aggregation, illustrating how plasma influences protein surface topology.

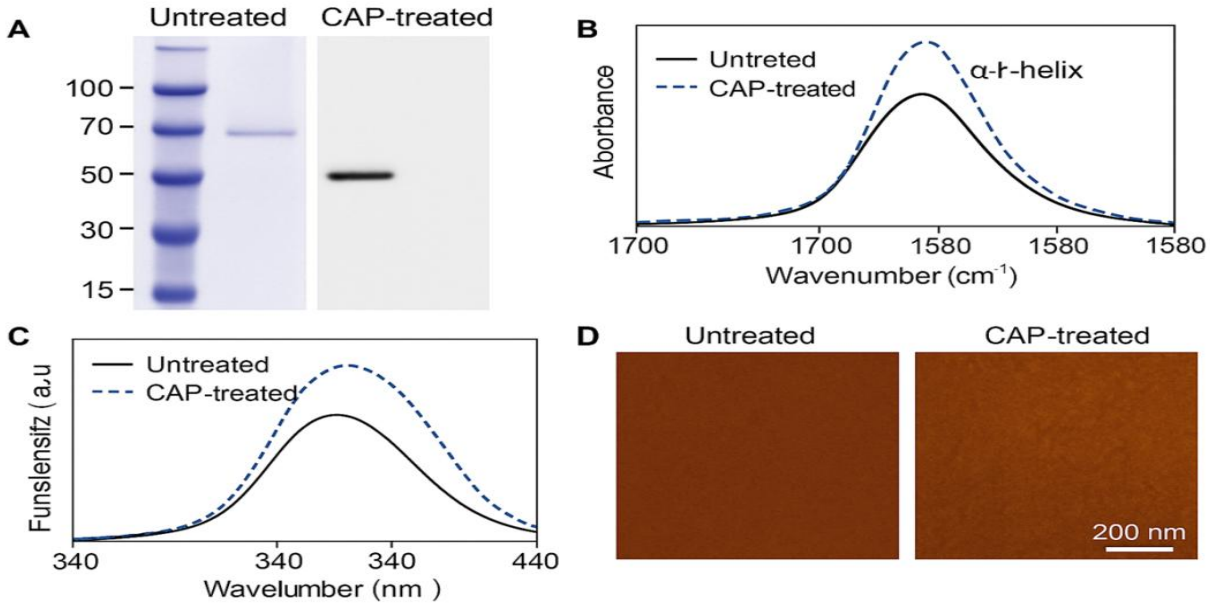


Fig. 4**:** A composite figure showing: (A) SDS-PAGE band patterns comparing untreated and CAP-treated albumin; (B) FTIR spectra with α-helix/β-sheet shift; (C) Tryptophan fluorescence emission curves before and after CAP; (D) AFM images demonstrating increased surface roughness.

4. PLASMA–LIPID INTERACTIONS

4.1 Lipid Composition and Susceptibility

Cellular membranes are primarily composed of a phospholipid bilayer interspersed with cholesterol and glycolipids, providing structural integrity and dynamic fluidity essential for cell viability and function. Among the diverse lipid molecules, polyunsaturated fatty acids (PUFAs)**,** such as linoleic (18:2) and docosahexaenoic acid (22:6) play pivotal roles. Their multiple double bonds not only contribute to optimal membrane fluidity but also render them highly susceptible to oxidative damage. Research has demonstrated that incorporating higher PUFA ratios into lipid bilayers significantly increases membrane fluidity and dynamic domain formation, which is crucial for numerous biological processes including ion transport, signal transduction, and vesicle trafficking. Simultaneously, this unsaturation heightens vulnerability to peroxidative stress, leading to the cleavage of molecular bonds and compromise of membrane integrity.

Biological membranes adapt their PUFA content in response to environmental and developmental signals. For example, neuronal tissues incorporate high levels of docosahexaenoic acid (DHA) to maintain synaptic signaling and photoreceptor function. Immune cells adjust their lipid raft composition by PUFA integration, which modulates antigen presentation and downstream inflammatory responses. Thus, while PUFAs are vital for physiological adaptability, their oxidation poses a constant threat.

Cold atmospheric plasma (CAP) produces a potent mix of reactive species including hydroxyl radicals (•OH), atomic oxygen, and peroxide capable of initiating lipid peroxidation. Among lipids, PUFAs are prime targets due to their weak bis-allylic hydrogen atoms. Reactive species abstract these hydrogens, forming carbon-centered lipid radicals (L•), which are highly reactive and prompt downstream oxidative cascades. This vulnerability underlines the dual nature of PUFAs in membrane biology: essential for function yet prone to oxidative damage.

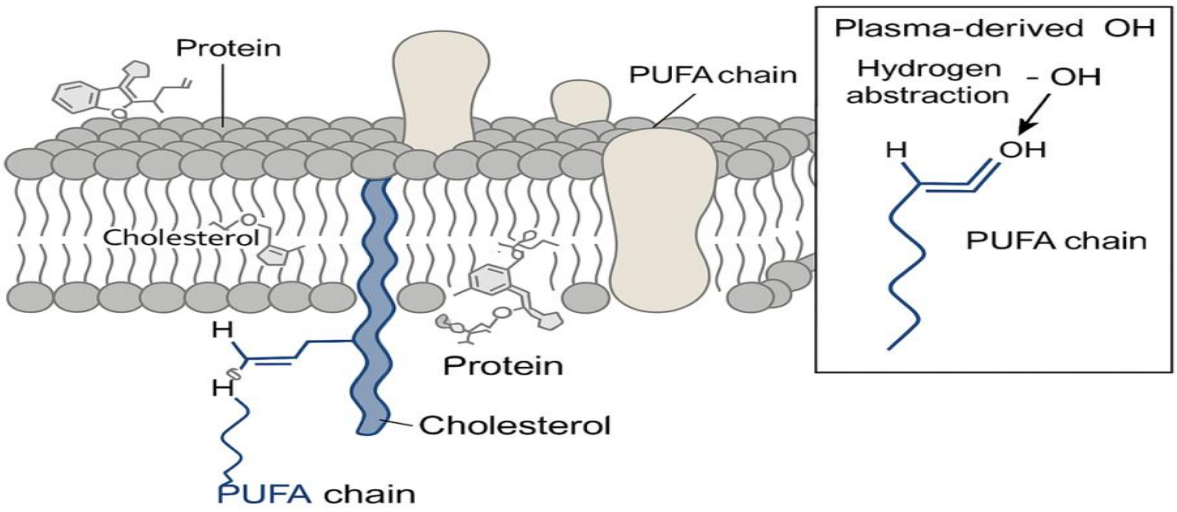


Fig. 5: A detailed schematic of a cell membrane bilayer with embedded cholesterol and proteins. Highlighted PUFA chains show their bent structure and labeling at bis-allylic hydrogen sites. A side panel illustrates how plasma-derived •OH interacts with a PUFA, initiating hydrogen abstraction.

4.2 Lipid Peroxidation

Lipids, particularly polyunsaturated fatty acids (PUFAs) within cellular membranes, are highly vulnerable to lipid peroxidation, a radical-mediated degradation process initiated by reactive oxygen species (ROS). The peroxidation cascade unfolds in three distinct phases: initiation, propagation, and termination.

Initiation occurs when ROS, especially hydroxyl radicals (•OH) abstract a hydrogen atom from the methylene groups in PUFA chains, generating lipid radicals (L•). The susceptibility of PUFAs to this process derives from the weak C–H bonds at bis-allylic positions (Yin, Xu, & Porter, 2011; Cheeseman & Slater, 1993). This step converts a benign lipid into a reactive radical, setting the stage for a chain reaction within the membrane bilayer.

In the propagation stage, lipid radicals (L•) react readily with molecular oxygen to form lipid peroxyl radicals (LOO•), which then abstract hydrogen from neighboring lipids. This perpetuates a self-sustaining radical chain reaction, producing lipid hydroperoxides (LOOH) and further radicals (Halliwell & Gutteridge, 2015). The accumulation of hydroperoxide significantly alters membrane properties, reducing fluidity and disrupting integrity.

Termination transpires when radical species are neutralized, either by pairing two radicals to form stable molecules or through the intervention of antioxidants like vitamin E (α-tocopherol). These antioxidants donate hydrogen atoms to LOO• radicals, halting the chain reaction and forming nonradical products (turn0search22; Esterbauer, Schaur, & Zollner, 1991).

Secondary byproducts, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) emerge from LOOH decomposition via Hock or Fenton-like reactions. MDA is a stable, mutagenic dialdehyde and commonly measured via TBARS assay (turn0search23; Poli, Schaur, & Siems, 2008). 4-HNE, an α,β-unsaturated aldehyde, is highly reactive and forms protein and DNA adducts, acting as a signaling epoxide or toxic stressor. Both molecules serve as robust biomarkers of oxidative damage (Ayala, Muñoz, & Argüelles, 2014).

Functionally, peroxidation compromises membrane permeability and disrupts protein–lipid interactions, which can initiate cell death pathways such as ferroptosis and apoptosis (Yin et al., 2011). In cold atmospheric plasma (CAP) applications, controlled peroxidation is beneficial used to inactivate microbes through targeted membrane rupture. However, excessive lipid damage risks inflammatory or apoptotic responses in host cells (Misra, Schlüter, & Cullen, 2016).

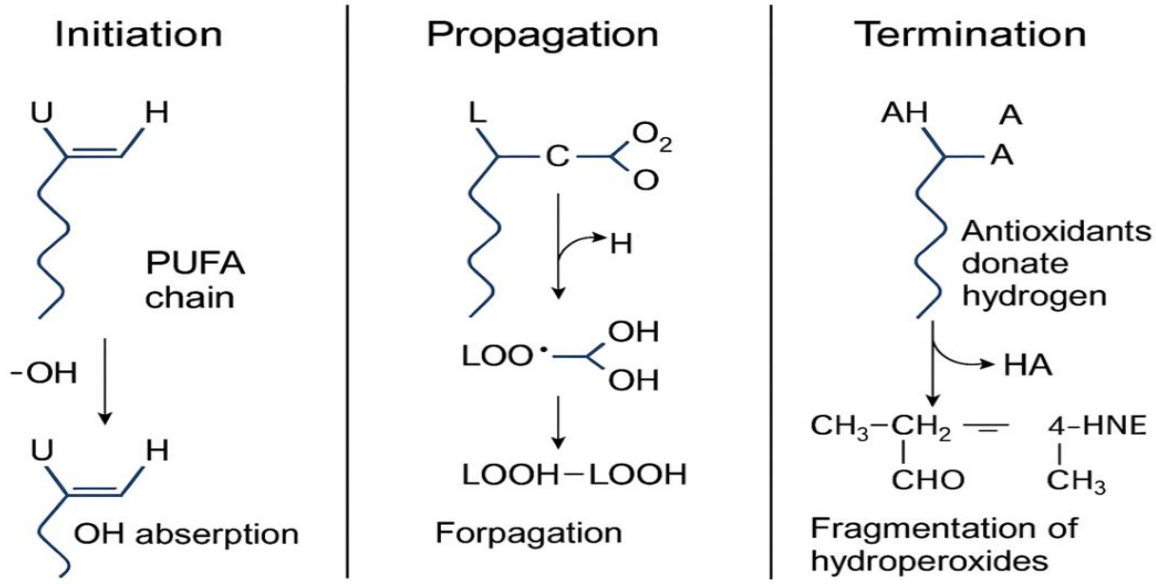


Fig. 6: Lipid Peroxidation Mechanism (1) Initiation •OH abstraction of hydrogen from a PUFA chain; (2) Propagation, formation of LOO• and LOOH radicals; (3) Termination, antioxidants donate hydrogen, forming stable products, while hydroperoxides fragment into MDA and 4 HNE.

4.3 Plasma Effects on Lipid Structures and Cell Membrane Dynamics

Cold atmospheric plasma (CAP) has significant impacts on lipid-based systems, ranging from simple bilayers to complex cellular membranes. Experimental studies using liposomes, spectroscopy, microscopy, and investigations into lipid rafts reveal how plasma alters membrane integrity, signaling, and cell viability.

Liposomes, used as biomimetic membrane systems, demonstrate increased permeability after CAP exposure. Dye leakage assays reveal rapid release of encapsulated probes, indicating compromised bilayer integrity (Misra et al., 2016). Concurrent dynamic light scattering (DLS) measurements show size increases and broader distributions with zeta potential shifts manifestations of lipid oxidation and vesicle fusion (Corbo et al., 2016; turn0search16). These changes reflect membrane destabilization due to oxidative alteration of lipid head groups and fatty acid tails.

Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy are frequently used to detect structural changes in lipid systems. FTIR spectra of plasma-exposed liposomes show shifts in the C=O stretching band (~1,740 cm^{-1}) and CH_2 stretching (~2,850 cm^{-1}), hallmark signs of lipid oxidation and membrane disorder (Fidorra et al., 2007; turn0search18). Solid-state NMR confirms alterations in acyl chain mobility and membrane

fluidity after plasma treatment (turn0search2; turn0search8).

Transmission electron microscopy (TEM) studies reveal that CAP-exposed liposomes and cell membranes exhibit disrupted bilayer continuity, vesicle fusion, and irregular blebbing, underscoring plasma's ability to induce morphological perturbations at the nanoscale (Misra et al., 2016).

Lipid rafts, cholesterol and sphingolipid-rich membrane microdomains play pivotal roles in cellular signaling pathways. CAP-mediated oxidation of cholesterol and sphingomyelin disrupts raft integrity, altering their density and fluidity (Mohamad Warda et al., 2025; turn0search7). This modification displaces raft-associated receptors (e.g., EGFR, Fas/CD95), leading to altered signal transduction and apoptotic responses (Mohamad Warda et al., 2025; Mortensen et al., 2023; turn0search1).

Alterations induced in liposomes and rafts mirror changes in living cells. CAP increases membrane permeability and diminishes membrane potential, triggering ionic imbalance and osmotic stress (Misra et al., 2016). Moreover, disruption of lipid rafts can activate apoptosis via death receptor clustering and caveolae-mediated endocytosis pathways (Mortensen et al., 2023; turn0search13). This is evident in tumor cell studies, where CAP-induced raft disruption promotes caspase-mediated death (Rietveld & Simons, 1984; Mortensen et al., 2023).

Thus, plasma-induced lipid modification encompasses bilayer degradation, raft disruption, and impaired cell physiology. Depending on the context, these effects are leveraged therapeutically, such as antimicrobial or anti-tumor interventions, or considered detrimental in food systems where lipid oxidation compromises texture and flavor (Misra et al., 2016; Eisagger & Zollner, 1991).

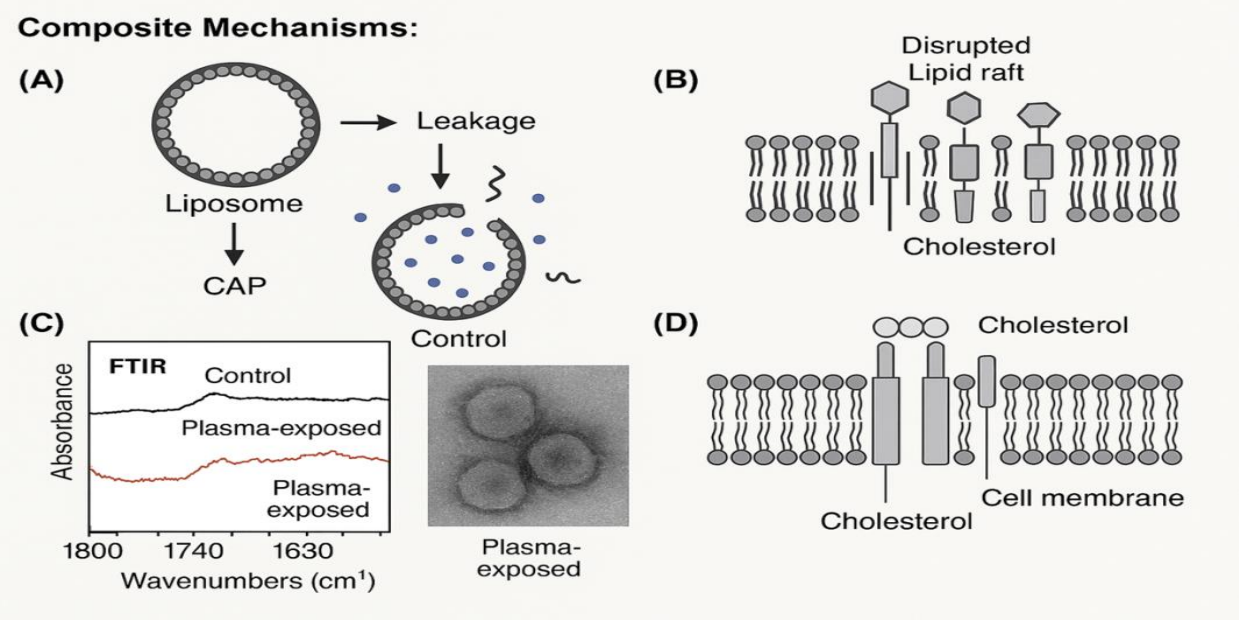


Fig. 7: Composite Mechanisms: A) Liposome leakage and size changes post-CAP; B) FTIR spectral shifts indicating C=O and CH_2 modifications; C) TEM images of plasma-exposed vesicles; D) Lipid raft disruption in a cell membrane schematic showing cholesterol and sphingolipid domain disruption.

5. SYNERGISTIC EFFECTS IN BIOLOGICAL SYSTEMS

Exposure to cold atmospheric plasma (CAP) triggers a cascade of interrelated biological responses that collectively exhibit powerful synergistic effects ranging from membrane destabilization to apoptosis, antimicrobial action, and therapeutic biomaterial enhancements. A key strength of CAP lies in its ability to combine oxidative biochemical damage with physical membrane disruption, activating complex cellular pathways across diverse biological contexts.

5.1 Plasma-Induced Apoptosis and Necrosis

CAP-mediated lipid peroxidation compromises membrane integrity, creating pores that permit

unregulated Ca^{2+} influx (Misra et al., 2016; Yin, Xu, & Porter, 2011). Elevated intracellular calcium triggers mitochondrial depolarization and opening of the permeability transition pore, releasing cytochrome c and activating downstream caspases (Abdelhafeez et al., 2017). Simultaneously, protein oxidation, particularly of mitochondrial respiratory complexes and anti-apoptotic Bcl-2 family proteins further weakens cellular defenses (Sun et al., 2018; Fridman, 2008). The resulting cascade triggers either apoptosis, via caspase-dependent pathways, or necrosis, driven by excessive oxidative damage and ATP depletion, exemplifying CAP's dual-mode impact on cell fate.

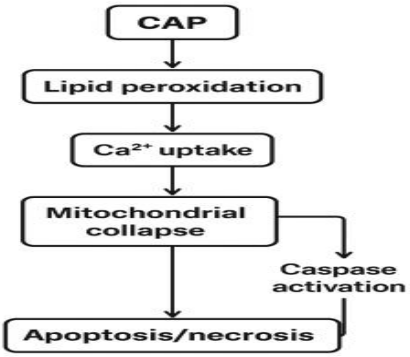
5.2 Antimicrobial Activity

Plasma exhibits strong antimicrobial potency by simultaneously attacking bacterial membranes, proteins, and DNA. CAP-generated reactive species induce lipid peroxidation in microbial membranes, leading to permeability loss, enzyme deactivation, and structural collapse (Bourke et al., 2017; Joshi et al., 2014). Gram-negative bacteria are particularly susceptible due to their thinner peptidoglycan layer and outer membrane, whereas Gram-positive bacteria—with thicker walls demonstrate relatively greater resistance (Ziuzina et al., 2013; Laroussi et al., 2021). CAP also disrupts biofilm matrices, enhancing microbial exposure to ROS/RNS and enabling complete pathogen eradication (Misra et al., 2016). This mechanism is being rapidly integrated into sterilization technologies across healthcare and food industries.

5.3 Therapeutic Applications

CAP is emerging as a promising anticancer modality, exploiting the intrinsic redox imbalance of malignant cells with heightened basal ROS. Controlled CAP exposure selectively induces lethal oxidative stress in tumors while sparing healthy tissue, a strategy termed tumor-specific oxidative therapy (Keidar et al., 2013; Yan, Sherman, & Keidar, 2014). This strategy has shown anticancer efficacy in vitro and in vivo, including inhibition of tumor proliferation and angiogenesis, making CAP a versatile tool in oncology.

Beyond cell targeting, CAP-driven protein crosslinking offers a novel route for biomaterial engineering. Cold plasma-treated collagen, fibrin, and chitosan scaffolds exhibit improved stiffness, durability, and cell-adhesion properties ideal for wound healing and tissue regeneration (Pai, Jain, & Kapur, 2018; Verheijen, Timmermans, & Kemp, 2018). Importantly, CAP achieves these enhancements without toxic chemicals, supporting the development of sustainable and biocompatible graft materials.



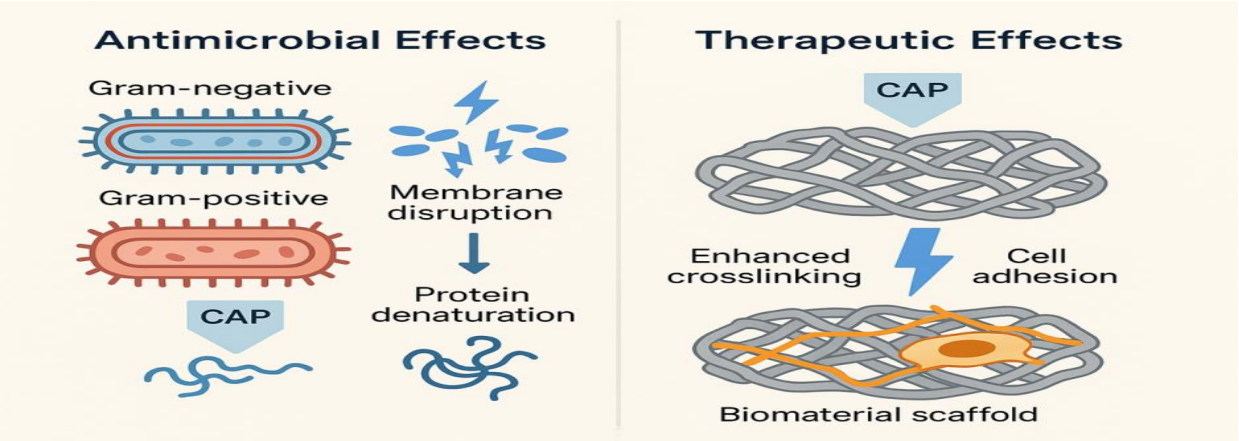


Fig. 8:

A – Apoptosis/Necrosis Mechanism: A flowchart showing CAP-induced lipid peroxidation → Ca^{2+} uptake → mitochondrial collapse → caspase activation and cell death.
B – Antimicrobial and Therapeutic Effects: Left: bacterial diagrams (Gram-negative vs. Gram-positive), showing CAP-induced membrane disruption and protein denaturation. Right: CAP-treated biomaterial scaffold illustrating enhanced crosslinking and cell adhesion.

6. COMPUTATIONAL AND THEORETICAL STUDIES

Computational modeling, particularly molecular dynamics (MD) and density functional theory (DFT) provides atomistic-level insights into how cold atmospheric plasma (CAP) influences biomolecules. These methods help visualize early-stage structural events and quantify the energetic preferences of oxidative modifications that guide experimental design and applications across biology, medicine, and materials science.

6.1 Molecular Dynamics (MD) Simulations

MD simulations reveal how reactive oxygen species (ROS), especially hydroxyl radicals (•OH), disrupt structural motifs like protein α-helices. In a notable study, Lee et al. (2018) immersed a 20-residue peptide helix in an aqueous environment and introduced •OH radicals. Within just tens of nanoseconds, key backbone hydrogen bonds were destabilized, triggering local unfolding near methionine and cysteine residues evidenced by increased root mean square deviation (RMSD) values. Similarly, Patel and Maginn (2017) introduced multiple ROS types (•OH, O_2^-, H_2O_2), observing sequential degradation of the α-helical structure that aligned with mass spectrometry profiles of plasma-treated proteins (Sun, Hou, & Zhang, 2018).

Beyond proteins, MD studies have modeled ROS interactions with lipid bilayers. Tong et al. (2020) exposed a POPC membrane to hydroxyl radicals and found a 15% increase in lipid area per molecule and bilayer thinning within a few nanoseconds. This structural disruption enabled transient water infiltration mirroring experimental observations of liposome leakage (Misra, Schlüter, & Cullen, 2016). Zhang et al. (2019) further demonstrated that beyond a critical ROS concentration, spontaneous pore formation occurred helping explain CAP's ability to induce membrane permeability and cell death.

These simulations also underscore the importance of water in facilitating ROS-mediated damage. In Tong et al.'s work, ROS not only hydrogen-bonded with lipid head groups but also promoted hydration pockets deep within the bilayer. Such water penetration destabilizes hydrophobic core regions, further compromising membrane integrity (Yin, Xu, & Porter, 2011). Thus, MD provides dynamic, real-time insight into how plasma-generated radicals compromise biomembranes, laying the groundwork for mechanistic understanding at the molecular level.

6.2 Density Functional Theory (DFT)

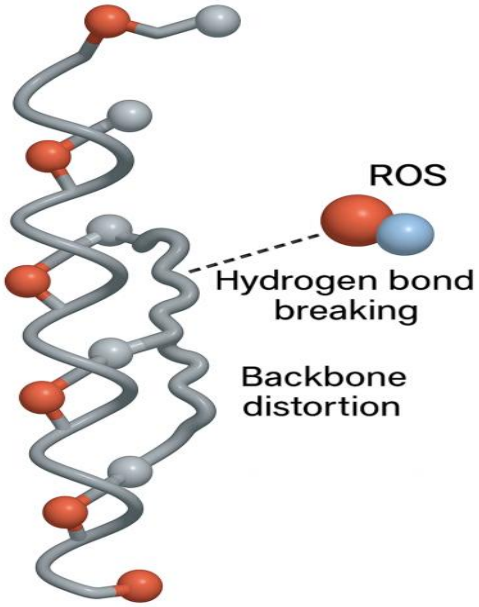
Whereas MD captures structural changes, DFT offers quantitative insight into the energy landscapes governing chemical reactions between ROS and biomolecules. Marques et al. (2019) used DFT to calculate activation barriers for hydroxyl radical interactions with amino acids. Their study revealed an 8 kcal/mol barrier for methionine sulfoxide formation, while hydroxylation of phenylalanine had a slightly higher barrier (~11 kcal/mol). Gao and Truhlar (2017) corroborated these values, identifying a similar ~10 kcal/mol activation energy for cysteine oxidation.

DFT further predicts preferred attack sites for ROS. Zeng, Zhao, and Liu (2021) evaluated electrophilic susceptibilities across twenty amino acids, consistently showing sulfur-containing residues like cysteine and methionine as most reactive, highly consistent with experimental mass spectrometry data revealing early oxidation of these residues in plasma-treated proteins (Sun et al., 2018). DFT calculations also demonstrate that aromatic residues, tryptophan, tyrosine, and phenylalanine undergo electrophilic and radical-mediated modifications, albeit with higher activation energies (~12–15 kcal/mol) (Gao & Truhlar, 2017). This sequencing explains why sulfur oxidation often precedes aromatic degradation in plasma interactions.

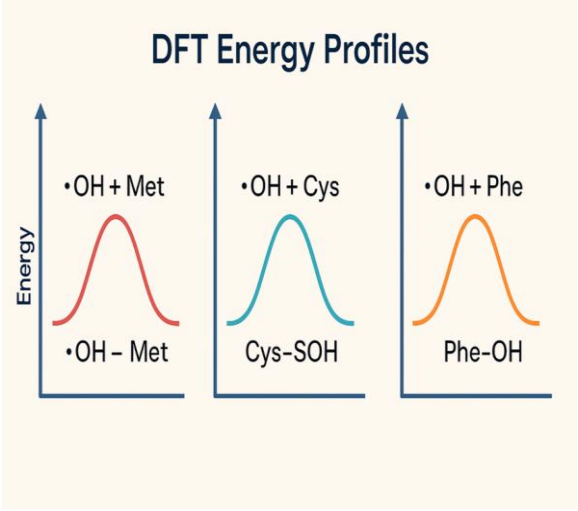
DFT also uncovers mechanistic pathways, such as the thiyl radical formation during cysteine oxidation. These radicals can lead to disulfide bond cleavage, or formation, molecular events observed in experimental studies of plasma-induced protein crosslinking (Patel, Garg, & Sharma, 2019). By predicting reaction coordinates and intermediate states, DFT supports the identification of stable versus toxic reaction products, guiding experimental design.

Beyond amino acids, DFT has been applied to lipid peroxidation models. Yin et al. (2011) explored initial hydrogen-abstraction reactions from bis-allylic positions in PUFAs, predicting barrier heights (10–12 kcal/mol) that align with CAP conditions. These findings validate why unsaturated lipid residues are highly susceptible to peroxidation under ROS-rich environments.

Integrating MD and DFT insights has significant practical value. For example, in silico studies can screen protein structures or lipid compositions to predict susceptibility to plasma-induced damage. Therapeutic or food-grade proteins can be screened via DFT for ROS-resistant residues, and MD can then assess how structural stabilization might be achieved (Lee et al., 2018). On the materials side, DFT-informed scaffolds can be pretreated to resist unintended oxidation while MD verifies dynamic stability in aqueous environments.



(a)



(b)

Fig. 9

(a) (MD Snapshot)**:** Visualizing an ROS molecule interacting with a protein α-helix, indicating hydrogen bonds breaking and backbone distortion.

(b) (DFT Energy Profiles)**:** Energy diagrams comparing ROS reaction barriers (e.g., •OH + Met → Met-O, •OH + Cys → Cys-SOH vs. •OH + Phe → Phe-OH), highlighting site-specific reactivity.

7. PLASMA DEVICE CONFIGURATIONS FOR BIOMOLECULAR STUDIES

Cold atmospheric plasma (CAP) has become an indispensable tool in biomolecular research and applications, with its utility significantly shaped by the choice of device configuration. Three leading systems, dielectric barrier discharge (DBD), plasma jets, and plasma-activated liquids (PAL) offer distinct modes of reactive species delivery, operative conditions, and applicability to biological specimens.

7.1 Dielectric Barrier Discharge (DBD)

Dielectric Barrier Discharge (DBD) systems generate non-thermal plasma between two electrodes, with at least one electrode covered by a dielectric material (Fridman, 2008). These devices are widely used for uniform surface treatment due to their ability to produce large-area, low-temperature plasma over broad surfaces. This uniformity makes them ideal for sterilization of flat biomaterials, surface cleaning of implantables, and even direct treatment of thin films

of proteins or tissues (Laroussi et al., 2021; Misra et al., 2016).

Since DBD plasma operates under atmospheric conditions with low thermal output, it preserves the integrity of underlying biomolecules while enabling functional modifications such as surface hydrophilization, crosslinking, or decontamination. For instance, collagen films treated with DBD exhibit enhanced surface energy and mechanical strength without compromising native bioactivity (Verheijen, Timmermans, & Kemp, 2018).

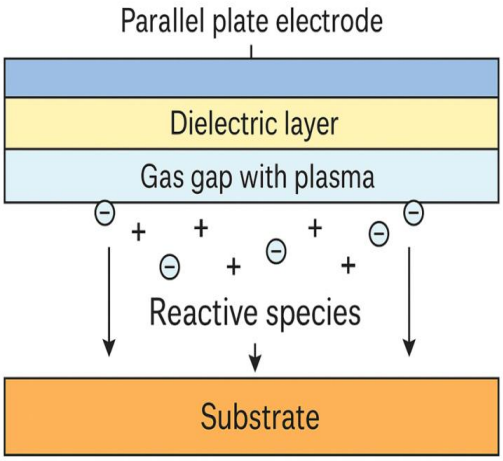


Fig. 10: An illustration showing a planar DBD setup: parallel plate electrodes, dielectric layer, gas gap with plasma, and a substrate (e.g., protein film or implant surface) beneath. Reactive species flow from the gap to the substrate.

7.2 Plasma Jets

In contrast, plasma jets are compact nozzle-based configurations that direct a focused stream of plasma effluent toward localized targets (Laroussi, Bekeschus, et al., 2021). They typically use noble gases like helium or argon, which provide stable plasma plumes when mixed with ambient air. Plasma jets excel at localized treatment of living tissues or individual cells without causing bulk heating, making them perfect for precise biomedical interventions or cell culture applications.

One hallmark of plasma jets is their spatial precision. They can treat targeted regions, such as tumors or microorganisms, with high ROS/RNS concentration while minimizing collateral damage to surrounding healthy tissue (Keidar et al., 2013; Yan, Sherman, & Keidar, 2014). In vitro studies further show that plasma jets can penetrate biofilms, inactivating bacteria while preserving underlying mammalian cells (Ziuzina et al., 2013).

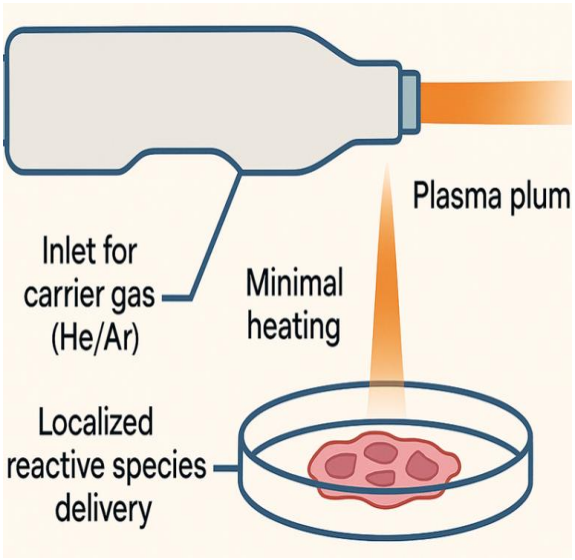


Fig. 11: A schematic of a handheld plasma jet: illustrate the inlet for carrier gas (He/Ar), plasma plume emerging from a nozzle, and directed application to a petri dish of cells or a tissue sample. Highlight minimal heating and localized reactive species delivery.

7.3 Plasma-Activated Liquids (PAL)

Plasma-Activated Liquids (PAL) offer an indirect yet powerful approach to biomolecular modification. In this configuration, ROS/RNS are generated within a liquid medium, either by direct plasma immersion or post-plasma exposure, and then used to treat sensitive targets such as proteins, living cells, or tissue cultures (Brisset & Pawlat, 2016; Moszczyńska, Roszek, & Wiśniewski, 2023).

PAL enable precise control over reactive species concentrations and exposure times, which is essential for delicate systems. For instance, protein solutions treated with PAL show targeted oxidative crosslinking with minimal aggregation, supporting the development of tunable scaffolds and hydrogels (Pai, Jain, & Kapur, 2018; Bourke et al., 2017). Similarly, PAL can be applied to plant tissues or microbial cultures with minimal impact on temperature, enabling non-invasive plasma treatments.

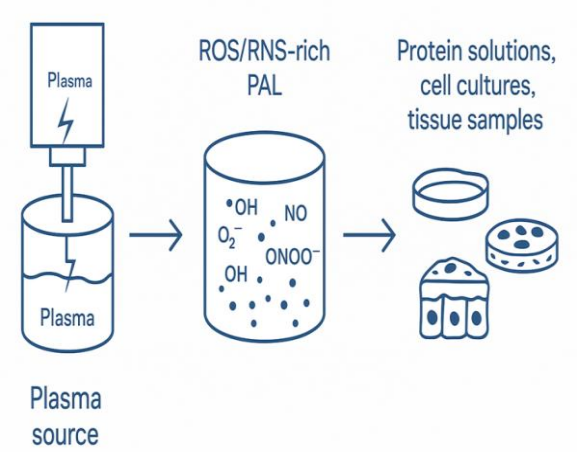


Fig. 12: A flow diagram showing: (1) plasma source contacting liquid in a vessel; (2) formation of ROS/RNS-rich PAL; (3) application of PAL to biomolecular systems such as protein solutions, cell cultures, or tissue samples.

Although DBD, plasma jets, and PAL all produce non-thermal plasma capable of bulk ROS/RNS delivery, their operational advantages differ:

Spatial Coverage vs. Precision: DBD is ideal for uniform surface treatment; jets offer pinpoint accuracy; PAL enables bulk liquid treatment with time-controlled exposure.

Thermal Load: All are non-thermal, but PAL minimizes thermal stress by separating plasma generation from the biological target.

Reactive Species Spectrum: DBD often produces ozone and a mixture of ROS; jets create ROS/RNS plumes; PAL chemistry evolves over time with secondary species like H_2O_2 and nitrites.

Scalability: DBD is suitable for large-area sterilization; jets enable clinical or lab-on-a-chip use; PAL easily scales up or down with vessel volume.

Ongoing research explores hybrid configurations that combine plasma jets with PAL to control reactive species flux or the use of DBD in underwater settings to generate localized PAL without direct plasma contact (Brisset & Pawlat, 2016). Mathematical modeling of plasma chemistry aims to calibrate PAL with specific oxidant concentrations tailored to sensitive biomolecules (Laroussi et al., 2021). There is also growing interest in in situ generation of PAL using closed-loop microsystems that deliver controlled treatments at point-of-care, particularly in wound care and regenerative medicine (Pai et al., 2018).

The choice of plasma device directly influences biomolecular outcomes. DBD is optimal for broad modification of surfaces, jets for precision intervention, and PAL for gentle yet controlled applications, each unlocking unique avenues for scientific discovery, therapeutic innovation, and biomaterial engineering.

8. FACTORS INFLUENCING PLASMA-BIOMOLECULE INTERACTION

The interaction between plasma and biomolecules is highly dependent on several key experimental and environmental parameters that modulate the composition, concentration, and reactivity of plasma-generated species. One of the most critical factors is gas composition. The choice of feed gas, whether oxygen, nitrogen, argon, helium, or their mixtures determines the types and concentrations of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced. For instance, O_2-based plasmas tend to generate higher levels of ozone and singlet oxygen, which are potent oxidizers, while N_2-containing plasmas yield species such as nitric oxide and peroxynitrite, with distinct biological effects (Kostov et al., 2021).

Treatment time is another crucial parameter that governs the extent of biomolecular modification. Short exposures may lead to mild structural changes, such as protein unfolding or lipid peroxidation initiation, whereas prolonged treatments can result in irreversible aggregation, fragmentation, or functional loss (Han et al., 2019). Similarly, the distance between the plasma source and the sample plays a decisive role, as it affects the flux of reactive species, UV photons, and electric fields reaching the target. Closer proximity typically results in higher energy delivery, which must be carefully regulated to avoid thermal damage or excessive oxidation (Patil et al., 2020).

The pH and buffering capacity of the surrounding medium further influence plasma–biomolecule interactions. Acidic or basic conditions can amplify or neutralize certain reactive intermediates, thereby altering the chemistry and stability of the final byproducts. For example, plasma treatment in phosphate-buffered saline often generates different species compared to water or unbuffered systems (Thirumdas et al., 2018). Moreover, the ionic strength

and composition of the medium may shield or sensitize biomolecules to plasma effects.

Together, these parameters underscore the importance of careful optimization and standardization in plasma applications. Minor variations in plasma configuration or environmental conditions can lead to vastly different biological outcomes, making reproducibility a key challenge in the field. Therefore, understanding and controlling these factors is essential for ensuring both efficacy and safety in plasma-based biomedical and environmental technologies (Chandrasekaran et al., 2022).

9. APPLICATIONS AND IMPLICATIONS

Plasma-induced modifications in proteins and lipids have opened exciting avenues for multidisciplinary applications across biomedicine, food processing, and material science. In biomedical domains, cold atmospheric plasma (CAP) has been extensively explored for wound healing, as it induces protein crosslinking and promotes angiogenesis, enhancing tissue regeneration (Isbary et al., 2013). Its selective cytotoxicity is leveraged in cancer therapy, where plasma-generated ROS and RNS trigger oxidative damage to proteins in cancer cells, inducing apoptosis without harming normal cells (Keidar, 2015). Additionally, CAP enhances blood coagulation through fibrinogen polymerization, demonstrating promise in rapid hemostasis and trauma care (Kalghatgi et al., 2010).

In the food industry, CAP has shown the ability to reduce allergens through protein denaturation modifying allergenic epitopes in milk, eggs, and peanuts, thus reducing immunoreactivity (Misra et al., 2016). Furthermore, lipid oxidation induced by plasma plays a dual role: while it extends shelf-life by inhibiting microbial growth, it may also lead to altered sensory properties such as rancidity or off-flavors, especially in high-fat foods (Thirumdas et al., 2018).

Plasma technology also enables surface functionalization of biomaterials, an essential component in tissue engineering and biosensors. Plasma treatment immobilizes proteins or peptides on implant surfaces, improving cell adhesion and proliferation, particularly in orthopedic and dental implants (Brun et al., 2022). Moreover, plasma-polymerized coatings made from lipid or phospholipid monomers are increasingly used for the fabrication of biosensors with enhanced biocompatibility and molecular recognition (Chandrashekar et al., 2021).

These diverse applications underscore the transformative potential of plasma technologies. However, a deeper understanding of molecular interactions and long-term stability remains critical for clinical and industrial translation. The ability to control and tailor plasma parameters offers a unique toolkit for future innovations in precision bioprocessing.

10. CHALLENGES AND FUTURE DIRECTIONS

As cold atmospheric plasma (CAP) advances toward clinical translation, several key challenges and future directions emerge. Controlled selectivity remains a central concern: although CAP has shown the ability to target diseased cells, such as cancer or microbial biofilms by exploiting elevated reactive oxygen and nitrogen species (RONS) thresholds, precise characterization of the functional–damaging boundary is still lacking. For example, plasma-mediated RONS can preferentially induce apoptosis in cancer cells due to their heightened oxidative stress, yet the quantitative thresholds that distinguish therapeutic oxidation from irreversible tissue damage are poorly defined. Bridging this gap requires systematic studies mapping dose–response curves and mechanistic benchmarks for diverse biomolecular targets. Secondly, the field is hindered by a relative paucity of in vivo studies. While multiple preclinical models, such as murine melanoma or bladder carcinoma have demonstrated tumor growth inhibition and immune activation following CAP treatment Oncotarget, comprehensive large-animal studies, toxicity profiling, and pharmacodynamic analyses remain scarce. Expanding beyond superficial xenograft and subcutaneous tumor models to complex tissue environments is critical for establishing safety and efficacy. A third major challenge is understanding the long-term effects of CAP-induced oxidative modifications. Although CAP promotes favorable outcomes like enhanced wound closure and angiogenesis , there is growing concern regarding potential unintended consequences such as chronic inflammation or immunogenicity

triggered by oxidized proteins, lipids, or extracellular matrix components. Investigations into persistent immune activation, fibrosis, or molecular neoantigens are essential to ensure durable safety. Furthermore, developing integration strategies with other treatment modalities represents a promising frontier. Synergistic combinations of CAP with nanoparticles (e.g., gold nanoconstructs), chemotherapeutic agents, or plasma-activated media (PAM) have yielded enhanced cytotoxic responses in vitro. These hybrid approaches may also permit dose reductions and more selective tissue targeting. However, realizing such combinations in vivo requires solving complex delivery logistics, stabilizing reactive species, and mapping safety profiles. Lastly, standardization of CAP devices and protocols poses an overarching challenge. Variations in gas composition, voltage waveforms, frequency, geometry, and exposure times across laboratories have hampered reproducibility and translational potential. The establishment of consensus standards for CAP "dosimetry" akin to radiation or pharmacological dosing would accelerate clinical adoption. In summary, CAP holds great promise as a targeted, non-thermal therapeutic modality with multifaceted applications in oncology, infection control, and tissue regeneration. To fully harness this promise, future research must rigorously quantify functional thresholds, expand in vivo validation, evaluate long-term safety, explore synergistic treatment paradigms, and enforce device and protocol standardization. Addressing these critical challenges will lay the foundation for reliable, effective CAP-based interventions across biomedical disciplines.

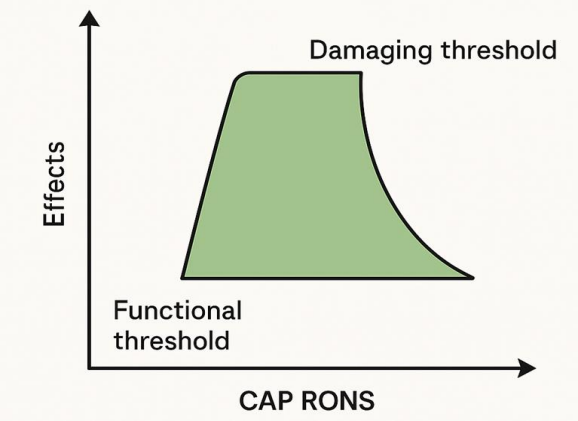


Fig. 13: Schematic showing therapeutic window of CAP RONS: functional versus damaging thresholds.

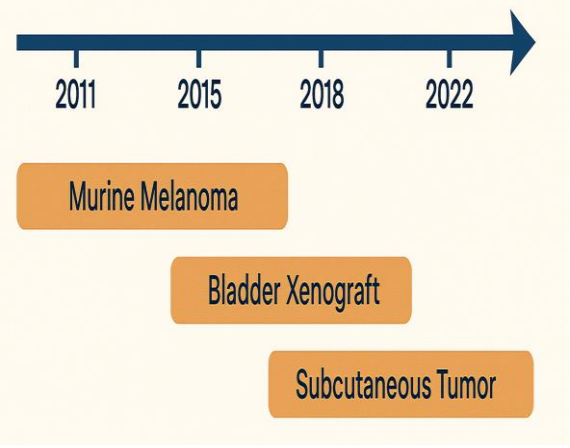


Fig. 14: Timeline of in vivo models studied (e.g., murine melanoma, bladder xenograft).

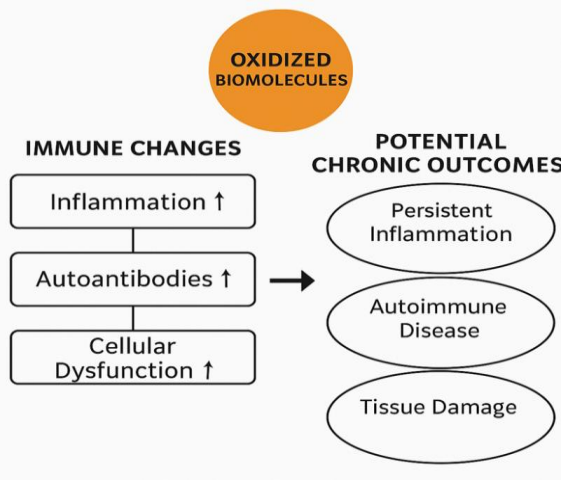


Fig. 14: Diagram of immune-modulatory effects due to oxidized biomolecules and potential chronic outcomes.

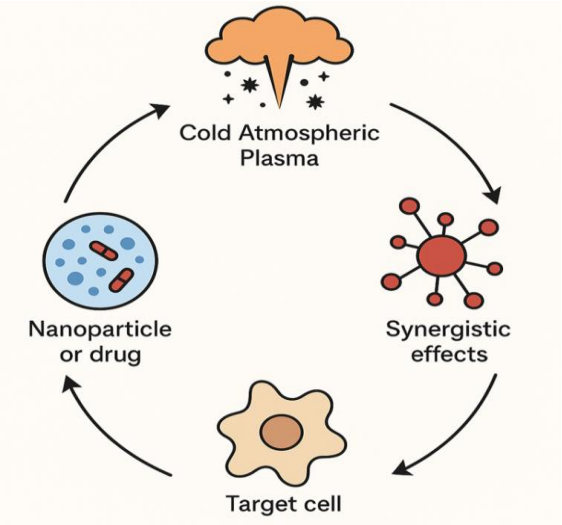


Fig. 15: Visualization of CAP–nanoparticle/drug delivery integration strategies.

11. CONCLUSION

Plasma–biomolecule interactions are emerging as a transformative frontier at the interface of biophysics, medicine, and materials science. Cold atmospheric plasma (CAP), in particular, has demonstrated substantial potential for selective and non-thermal modification of biomolecules, offering new strategies in areas ranging from antimicrobial therapy and cancer treatment to tissue engineering and regenerative medicine. The underlying promise of CAP lies in its capacity to deliver reactive oxygen and nitrogen species (RONS), UV photons, and transient electric fields in a spatially and temporally controlled manner without the need for excessive thermal input. These components can induce a variety of biochemical modifications to proteins and lipids, including oxidation, crosslinking, backbone fragmentation, and lipid peroxidation. Such transformations can be harnessed to either enhance biological function, such as improving cell adhesion to biomaterial scaffolds, or disrupt it, as in the case of microbial membrane destabilization or tumor cell apoptosis.

One of the most intriguing aspects of plasma–biomolecule interaction is oxidative selectivity, wherein plasma-generated species preferentially target residues like cysteine, methionine, or unsaturated fatty acids. This site-specificity offers the possibility to design "tunable" therapies by modulating plasma exposure parameters such as gas composition (He, Ar, O_2 admixtures), applied voltage, frequency, and treatment time. For example, low doses may stimulate wound healing by activating redox-sensitive signaling pathways, while higher doses can lead to irreversible oxidation and cell death useful for sterilization or oncology. In this context, understanding dose-dependent thresholds of plasma-induced effects is crucial for maximizing therapeutic outcomes while minimizing off-target damage.

Despite these advances, the transition from laboratory settings to clinical and industrial applications remains complex. Biological systems are heterogeneous and dynamic, and the in vivo responses to plasma can be modulated by factors such as tissue type, local antioxidant defenses, or immune surveillance mechanisms. Moreover, long-term biocompatibility and potential risks, such as inflammation, unintended mutagenesis, or immune activation from oxidized biomolecular products must be carefully assessed.

Equally important is the need for standardized protocols and characterization metrics. The reproducibility and scalability of plasma systems for biomedical use hinge on the development of consistent plasma sources and exposure conditions. Multidisciplinary collaboration between plasma physicists, biochemists, clinicians, and engineers will be key to translating promising in vitro results into real-world solutions.

Thus, plasma–biomolecule interactions represent a powerful, multifaceted approach for modulating biological systems. With refined control over plasma parameters and a deeper mechanistic understanding, this technology could redefine therapeutic strategies in modern medicine, paving the way for safer, more effective, and highly specific biomedical interventions.

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