Isolation of Biomolecules Using MXenes

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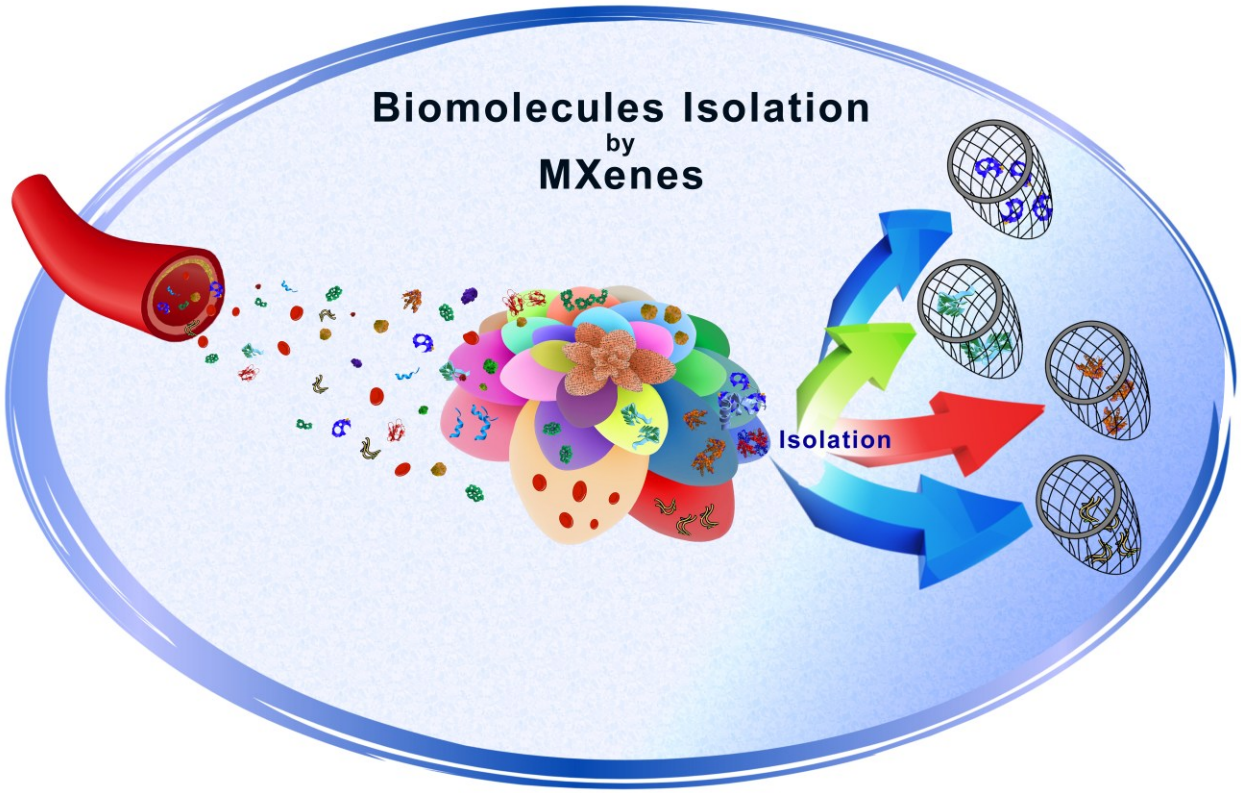
Abstract

Biomolecule isolation is a crucial process in diverse biomedical and biochemical applications, including diagnostics, therapeutics, research, and manufacturing. Recently, MXenes, a novel class of two-dimensional nanomaterials, have emerged as promising adsorbents for this purpose due to their unique physicochemical properties. These biocompatible and antibacterial nanomaterials feature a high aspect ratio, excellent conductivity, and versatile surface chemistry. This timely review explores the potential of MXenes for isolating a wide range of biomolecules, such as proteins, nucleic acids, and small molecules, while highlighting key future research trends and innovative applications poised to transform the field. We provide an in-depth discussion of various synthesis methods and functionalization techniques that enhance the specificity and efficiency of MXenes in biomolecule isolation. Additionally, we elucidate the mechanisms by which MXenes interact with biomolecules, offering insights into their selective adsorption and customized separation capabilities. This review also addresses recent advancements, identifies existing challenges, and examines emerging trends that may drive the next wave of innovation in this rapidly evolving area.

Graphical Abstract



1. Introduction

Recent advancements in two-dimensional (2D) nanomaterials have been largely attributed to the exfoliation of graphene and other layered materials. The integration of 2D layered nanomaterials into other materials has significantly advanced medical grade compounds.[1–5] Among them, graphene,[6,7] MoS_2,[8] phosphorene,[9] and their derivatives are the most extensively and successfully studied.[9–11]

To fully realize the potential of MXenes, a family of 2D nanomaterials, it is crucial to comprehend their fundamentals, from synthesis and properties to applications. Despite initial breakthroughs, numerous scientific questions remain unresolved, especially related to technical challenges in scaling up, reproducibility in production, and process integration. As a young family of nanomaterials, MXenes[12] are still relatively underexplored.[13,14]

A wide range of MXene-based medical-grade materials have been developed. These include nanomedicines,[15][16] biosensors,[17] scaffolds for tissue engineering,[18,19] wound healing,[20] and antibacterial compounds.[21,22] Also, there has been a particular interest in the use of MXenes as adsorbents in biomimetic media to purify biomolecules.[23–25] Biomolecules isolation by MXenes has seen substantial growth, with studies focusing on various aspects of the field. This burgeoning interest is evident in the increasing number of publications and the attention from researchers, underlining its importance and potential. High-impact journals have featured recent papers on this subject, reflecting their significance and widespread appeal.[26–28] This trend indicates a recognition of the area's potential to address critical challenges and drive future technological advancements. Hence, there are still many unexplored avenues of research despite the research conducted so far, and there is a need for putting the latest progress into perspective.[29]

1.1. Importance of biomolecule isolation

Applications of biomolecule isolation in biotechnology and medicine. A wide range of biomolecules, such as nucleic acids, proteins, peptides, lipids, and carbohydrates, regulate the activities of animals, plants, and microorganisms. Nanotechnology, materials science, analytical instruments, and biotechnology have contributed to the understanding of these biomolecules' structures, functions, and mechanisms in recent decades, allowing the creation of intelligent biomimetic systems as well as providing insights into the basic principles of life. Furthermore, for diagnosing diseases, it is crucial to detect abnormal biomolecule expression, and drug development increasingly focuses on biomolecules that target specific diseases. However, complex biological fluids contain a multitude of molecules, and for omics, structural analysis, drug purification, and clinical diagnostics, intended biomolecules must be isolated (Figure 1).[30]

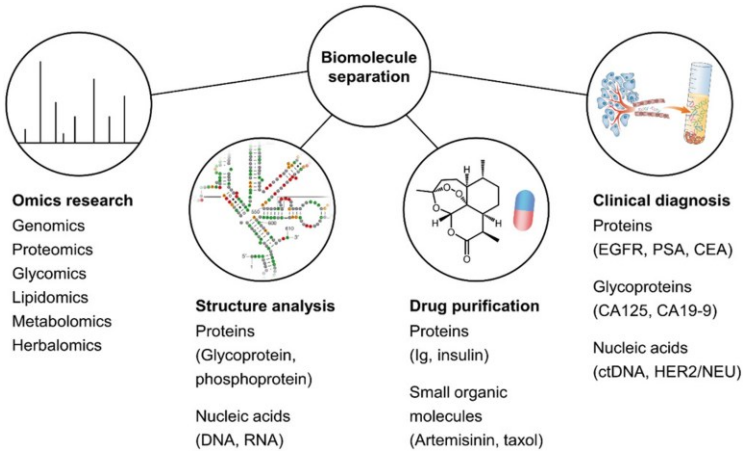


Figure 1. Application fields of biomolecule isolation. Reproduced with permission.[30] 2022, John Wiley & Sons.

First of all, omics fundamental research relies on workflows linking identification, separation, and database that is essential for glycomics,[31] genomics,[32] lipidomics,[33] metabolomics,[34] proteomics,[35] and herbalomics.[36] Second, high-purity biomolecules are crucial for subsequent

functional analysis and high-resolution structural identification,[37,38] especially for those with secondary or tertiary structures, such as proteins,[39] RNA,[40] and DNA.[41] Thirdly, producing high-pure drugs from complex precursor samples demands numerous separation methods and multi-step refinement processes since they often coexist with multiple biomolecules. For instance, immune globulin (Ig) for inflammatory and autoimmune diseases is prepared via the recombinant monoclonal antibody technology or is separated from animal plasma.[42] pancreatic extract isolation or the recombinant DNA technology is used to prepare insulin for diabetes treatment. Taxol for cancer treatment is extracted from the yew tree and Artemisinin is separated from Artemisia annua for malaria treatment.[30,43]

Lastly, the separation and analysis of trace molecular biomarkers is a promising strategy for liquid biopsy in clinical screening, monitoring, and staging.[30,44,45] The FDA (Food and Drug Administration) has approved several glycoproteins, nucleic acids, proteins, and carbohydrates as molecular biomarkers for various cancers, including carcinoembryonic antigen (CEA) and epidermal growth factor receptor (EGFR) from colon cancer, prostate-specific antigen (PSA) from prostate cancer, CA125 from ovarian cancer, CA19-9 for pancreatic cancer, and HER2/NEU from breast cancer. Similarly, for cancer monitoring, a potential molecular biomarker is circulating tumor DNA (ctDNA) that is found in the blood. Amyloid-β peptide as a neurotoxic peptide, is implicated in the Alzheimer's disease pathogenesis.[30,46]

Current methods and their limitations. A wide range of technologies and materials have been developed over the past several decades to achieve effective biomolecule isolation. It has been possible to separate highly selective and specific biomolecules using a variety of techniques, including isoelectric precipitation,[47] organic solvent precipitation method,[48] dialysis,[49]

ultrafiltration,[50] chromatography,[51] electrophoresis,[52,53] molecular imprinting,[54] microfluidic chip,[55] magnetic separation,[56] reverse micelles (RM),[57] crystallization,[58] and adsorption.[59,60]

As a isolation method, adsorption has various advantages and provides high-resolution separation compared to other methods.[61] Besides being versatile, this technique can also be applied to small molecules,[62] macromolecules,[63] hydrophilic,[64] hydrophobic,[65] positively charged,[66] and negatively charged[67] ones. Adsorption is scalable and, suitable for both large-scale production and small-scale analysis.[68] The most commonly used adsorbents in separation processes include nanoporous silica-based structures[69] and carbon-based materials.[70]

Meanwhile, the rapidly growing demand for biomolecules in medical institutions, research, and industry mandates adsorbent structures with higher efficiency, selectivity, and high separation kinetics. A variety of nanomaterials have been developed in recent years for biological separation.[71] Among them a diverse range of structures such as molecularly imprinted polymers (MIPs),[72] 2D materials such as graphene oxide (GO),[73] metal-organic frameworks (MOFs),[74] covalent organic frameworks (COFs),[75] Magnetic nanoparticles (MNPs)[76,77], and MXenes[29] have been investigated. A schematic illustration of metal decorations on magnetic nanoparticles for selective protein adsorption is shown in Figure 2, and recent studies on biomolecules adsorption by novel nanostructures are listed in Table 1.[76]

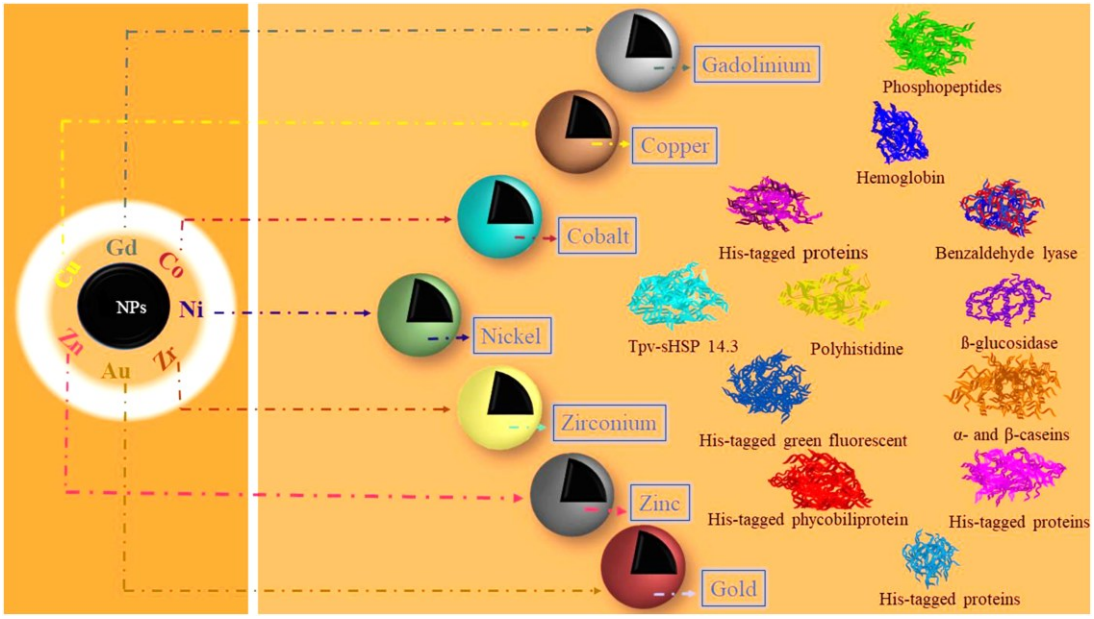


Figure 2. Protein adsorption on magnetic nanoparticles decorated with different metals. Reproduced with permission. [76] 2021, Elsevier.

Table 1. Recent studies on biomolecules adsorption by novel nanostructures.

Adsorbent Name	Adsorbed Biomolecule Name	Biomolecule Category	Adsorption Mechanism	Aim of Adsorption	Results of Adsorption	Ref.
MIL-101 MOF	Denatured proteins (e.g., lysozyme, carbonic anhydrase B)	Denatured proteins	Selective adsorption of denatured proteins through narrow apertures of MIL-101 MOF nanopores	Purification of protein mixtures by removing denatured fraction	Selective adsorption of denatured proteins by MOF Achieved native purity of over 99% in protein mixtures. Simplified and scalable approach for protein purification	[78]
SPION@CDs nanoparticles	Calf thymus	Nucleic acids	Electrostatic isolation and release at different pH values	Efficient DNA separation with high extraction capacity and biocompatibility	Maximum DNA adsorption capacity of 125.12 µg/mg Biocompatibility demonstrated by MTT assay (cell viability > 90% at 500 µg/mL)	[79]
Gold nanoparticles	ss(ds)DNA	Nucleic acids	Noncovalent adsorption; dsDNA acts as anchor for fixation on GNP surface	Understanding noncovalent interaction of dsDNA with GNPs	High-affinity motifs in dsDNA function as anchor for fixation on GNP surface. Competitive adsorption of ssDNA on GNPs	[80]
SiO_2@MNPs	RNA	Nucleic acids	Hydrogen bonding	Efficient RNA separation for clinical applications	Adsorbent shows highest RNA binding efficiency	[81]
NH_2-Th-Tz COF	Tyrosine enantiomers	Amino acids	Hydrogen bond interactions between chiral COFs and tyrosine	Enantioselective adsorption of tyrosine enantiomers	Adsorbent shows enantioselective adsorption toward tyrosine. Maximum enantiomeric excess (ee) value of 25.20% achieved	[82]
Fe_3O_4@SiO_2 @Azo-COOH	LDL, total cholesterol, triglycerides	Lipoproteins, cholesterol, lipids	π-π* and n-π* electronic transition Hydrogen bonding	Selective removal of LDL and other lipids from hyperlipemia patient blood	Efficient reduction in serum LDL content in dynamic perfusion experiments Potential for clinical application in blood perfusion therapies	[83]
Fe_3O_4–Cu NR	His-rich proteins (e.g., bovine hemoglobin)	Proteins	Hydrogen bonding	Highly selective enrichment of His-rich proteins from complex biological samples	Adsorbent with ring-like structure offers high Cu^{2+} binding sites for His-rich proteins. Adsorption capacity for bovine hemoglobin: 932.2 mg/g	[84]

Mesoporous UiO-66	EasyTaq	Nucleic acids	Electrostatic and hydrogen bonding	High-throughput DNA isolation for meat adulteration detection	High-throughput DNA isolation method for quantitative identification of meat adulteration Optimization of lysis buffer volume	[85]
Fe_3O_4/PPy/GO	Deoxyribonucleic acid	Nucleic acids	π–π and hydrogen bonding interactions	Efficient DNA isolation with high biocompatibility	DNA adsorption capacity: 142.71 mg/g High biocompatibility confirmed by MTT assay	[86]
p(MAA)/GOA	Dopamine, l-tyrosine	Neurotransmitters	π–π and hydrogen bonding interactions	Quantitative identification of dopamine and l-tyrosine in urine samples	Linearity ($r > 0.9990$) for dopamine (0.075–2.0 μg mL^{-1}) and l-tyrosine (0.75–20.0 μg mL^{-1}) Limit of detection: 0.018–0.048 μg mL^{-1}	[87]
Graphene oxide-lignin biopolymer	Biomolecules with variable molecular weight	Protein	Hydrophilic 2D channels	High water permeability	99% rejection for biomolecules	[88]

By offering unique properties and functionalities, these materials can enhance biomolecule separation efficiency and specificity. However, developing and applying them to biomimetic media is still a major challenge. For example, separation of complex mixtures of biomolecules effectively requires sufficient selectivity and specificity. A majority of adsorbent materials cannot separate biomolecules with similar structural or physicochemical properties, limiting their utility in high-purity biomolecule isolation applications.[30,77]

Developing reproducible and scalable biomolecule nanosorbents can also be challenging. Besides, differences in synthesis and preparation processes can affect biomolecule purification consistency, affecting adsorbent reliability and performance. Moreover, adsorbents' durability and stability under different environmental conditions, such as changing pH levels, temperatures, and solvents, pose additional challenges to adapt to diverse environments.[89–91]

Furthermore, integrating adsorbents into practical biomolecule separation workflows, such as automated sample handling and processing systems, remains a significant challenge. Implementation of effective biomolecule purification methods in research settings is also crucial to streamlining processes and enabling high-throughput applications.[92–94]

Among the studied adsorbents, we explore these challenges and discuss recent advances in the development and application of MXenes as novel adsorbents for biomolecule isolation. Figure 3 illustrates examples of nanoporous adsorbents for isolating biomolecules.[30]

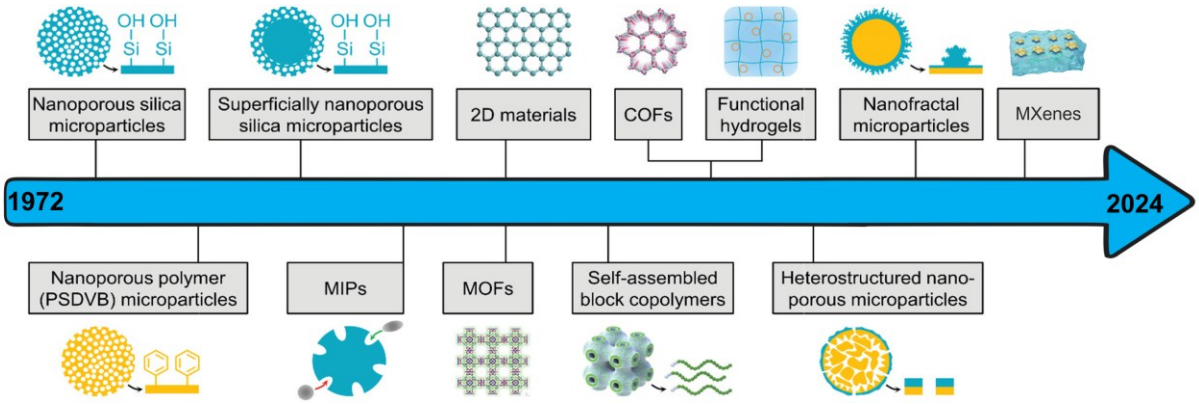


Figure 3. Examples of nanoporous structures for biomolecule isolation over years. Reproduced with permission.[30] 2022, John Wiley & Sons.

1.2. Why MXenes for biomolecule absorption

MXenes are well-suited for biomolecule adsorption due to their unique combination of structural and chemical properties. They possess a high surface area, which provides a large number of easily tailorable active sites for the adsorption of biomolecules. Their layered structure allows for the easy modification of surface chemistry, enabling selective interaction with specific biomolecules. The presence of functional groups, such as hydroxyl, oxygen, or fluorine, on their surfaces can be tailored to enhance binding affinity to proteins, nucleic acids, or small molecules. This tunability, combined with MXenes' high electrical conductivity, allows for the development of responsive surfaces capable of detecting and binding biomolecules in a highly controlled manner.

Additionally, MXenes exhibit excellent biocompatibility and biodegradability, making them ideal for use in biological and medical applications. Their compatibility with aqueous environments, often necessary for biomolecular interactions, allows them to maintain stability

while interacting with complex biological systems. The ease of functionalizing MXenes further enhances their suitability, as they can be engineered to display specific chemical groups that promote selective adsorption of target biomolecules. Moreover, the high mechanical strength and stability of MXenes ensure that they can withstand the dynamic conditions of biological environments without degrading or losing functionality, making them excellent candidates for applications such as biosensing, drug delivery, and tissue engineering.[29,95–97]

1.3. Purpose and scope of the review

MXenes, as a class of 2D nanomaterials, are characterized by their unique combination of physical and chemical properties making them ideal for various applications in the biomedical area.[98–100] Recent advances in MXenes research have enhanced their ability to isolate biomolecules through modifying their surface properties and implementing integration strategies.[29,101]

This review provides a comprehensive summary of recent advances in MXenes capabilities for biomolecule isolation. We begin by introducing MXenes' diverse types and unique properties. Following this, we delve into the specifics of MXenes interactions with different biomolecules, tracking the adsorption mechanisms in contact with proteins, nucleic acids, and small biomolecules. Our study highlights how MXenes' surface chemistry and structural features contribute to their ability to isolate critical biomolecules from complex biological matrixes. Furthermore, we discuss the limitations and weaknesses of MXenes in biomolecule isolation and propose potential solutions to address these issues. Finally, we explore the future potential of MXene-based materials, discussing upcoming trends, innovations, and their role as emerging stars in biomolecule isolation. We conclude with insights into how continued research and development could further enhance MXenes applications in biomedical research, clinical diagnostics, and therapeutic development (Figure 4).

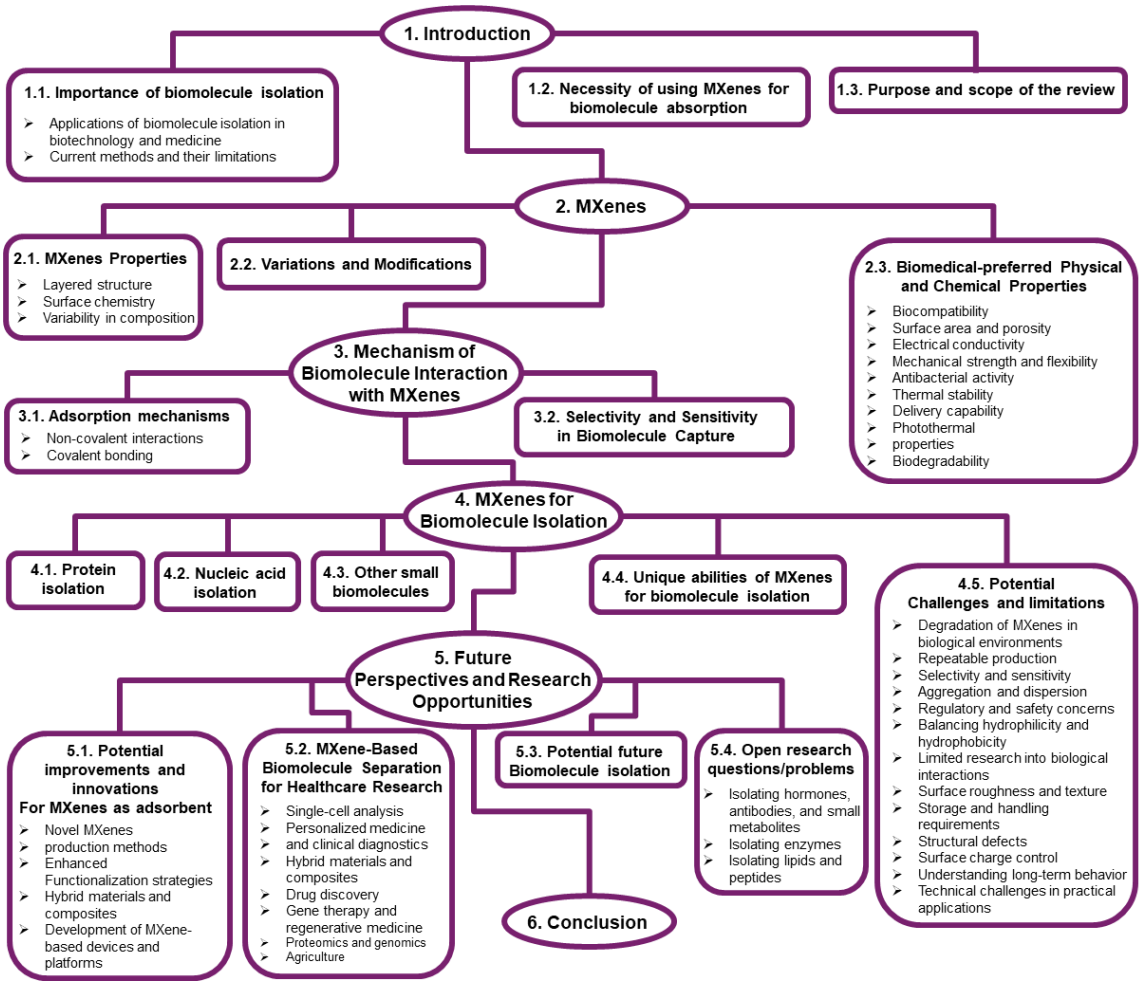


Figure 4: Flowchart depicting the organization of this review.

2. MXenes

MXenes are 2D layered materials derived from transition metal nitrides, carbides, or carbonitrides, e.g., Ti_3C_2.[102] MXenes do not have a natural 3D precursor in nature,[103] compared to phosphorene[104] and graphene.[105] Typically, MXene multilayer flakes are obtained by selectively removing the A layers from MAX phases, such as Al from Ti_3AlC_2.[106] Delamination of MXene nanosheets can be accomplished through intercalation agents like DMSO and LiF.[107] Research on MXenes began with experimental testing of lithium-ion batteries for energy storage,[108] and theoretical computational chemistry soon led to predictions of new members of their family.[109,110]

It is important to comprehend the structures of MXenes by considering their origins, from the MAX phase to the isolated 2D layer discussed in the Supplementary Information (Figure 5).[111–113]

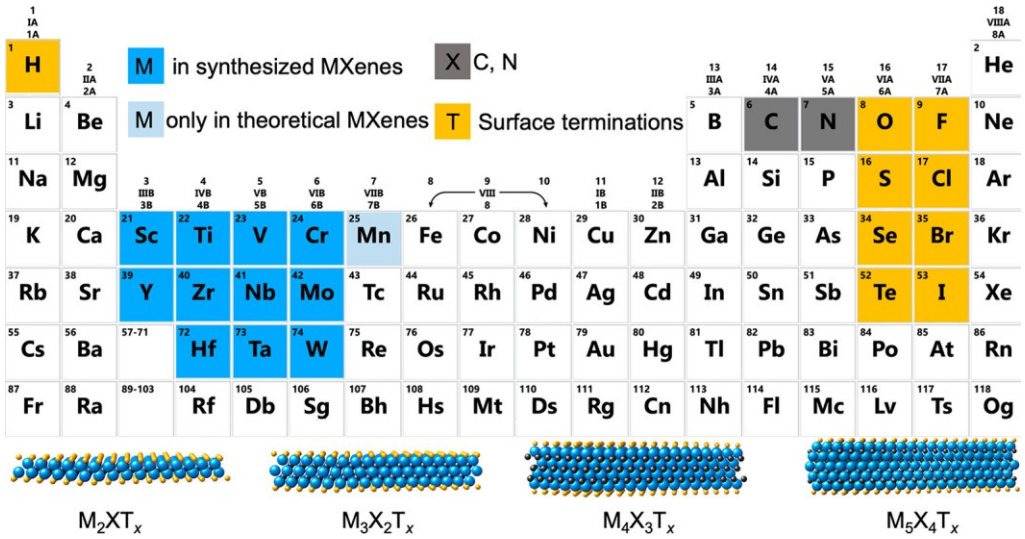


Figure 5. Periodic table showing compositions of MXenes. Elements used to build MXenes are color-coded. The schematics of four typical structures of MXenes are presented at the bottom. Reproduced with permission.[113] 2021, American Chemical Society.

2.1. MXenes Properties

MXenes are known for their remarkable hydrophilicity, metallic conductivity, and exceptional mechanical properties (Figure 6).[12,114–118] Some MXenes have demonstrated electron mobilities as high as 10^6 $cm^2\ V^{-1}\ s^{-1}$, which are comparable to or even exceed that of graphene (~2.5×10^5 $cm^2\ V^{-1}\ s^{-1}$). The metallic conductivity of $Ti_3C_2T_x$ films is around 6500 S cm^{-1}, surpassing carbon nanotubes and graphene. Competing 2D materials like graphene oxide, clay, and layered double hydroxides are hydrophilic but insulating. Additionally, MXenes possess high strength, wear resistance, hardness, and bending rigidity (D ~ 49.55 eV), significantly higher than that of graphene (D ~ 2.3 eV) and MoS_2 (D ~ 9.61 eV). According to molecular dynamics simulations, M_2X MXenes exhibit superior strength and stiffness to M_3X_2 and M_4X_3 structures (Figure 7).

Recent research has revealed a Young's modulus of 0.33 ± 0.03 TPa for single-layer (SL) $Ti_3C_2T_x$, the highest measured value among all other solution-processed 2D materials, such as graphene and MoS_2 (~0.27 TPa) and oxide (GO, ~0.21 TPa).[119–121]

Several studies have investigated the reactivity of Ti-based MXenes, notably hydrolysis and oxidation.[122,123] As a result of the reaction between air and water, Zhang et al.[124] observed that a colloidal solution of water and $Ti_3C_2T_x$ MXene degrades within five days when stored in air at room temperature. In contrast, MXene solution was found to remain stable for over two years when kept at 5°C in an argon atmosphere, since dissolved oxygen cannot be formed.[125,126]

MXenes are generally less thermally stable than graphene and MoS_2. Atomistic simulations suggested that the melting points of graphene and MoS_2 are 4500 K and 3700 K, respectively[127] [128]. In contrast, Ti_2C, Ti_3C_2, and Ti_4C_3 have significantly lower melting points, indicating that they may not be suitable for high-temperature applications such as nanoelectronics.[129]

Unlike graphene oxide,[130,131] $Ti_3C_2T_x$'s contact angle with water is 21.5° and remains stable even after vigorous shaking in water. Hence, MXenes are highly suitable for water purification,[132] desalination,[133] membrane sequestration,[134] and other water-based applications.[135,136] Recently, researchers have successfully removed phosphorus,[137] and lead,[138] copper,[139] mercury,[140] and chrome[141] ions from water using MXenes. Since MXene membranes have a small interlayer spacing, they can reject ions/molecules larger than the interlayer spacing.[126,142]

There are many applications for MXenes, including energy storage,[143] water purification,[144] transparent conductive coatings and films,[145] gas separation,[146,147]

electrochemical biosensors,[148] structural composites,[108,149] wearable medical sheets,[150] cancer protection,[15] electromagnetic shielding,[151] electrocatalysts,[152] hydrogen storage,[153] and antibacterial membranes.[154] Moreover, applications of MXenes in other fields are being explored.[155,156] Various research fields from robust industrial applications to delicate medical applications have benefited from MXenes' tunable and multifunctional properties.[100,157]

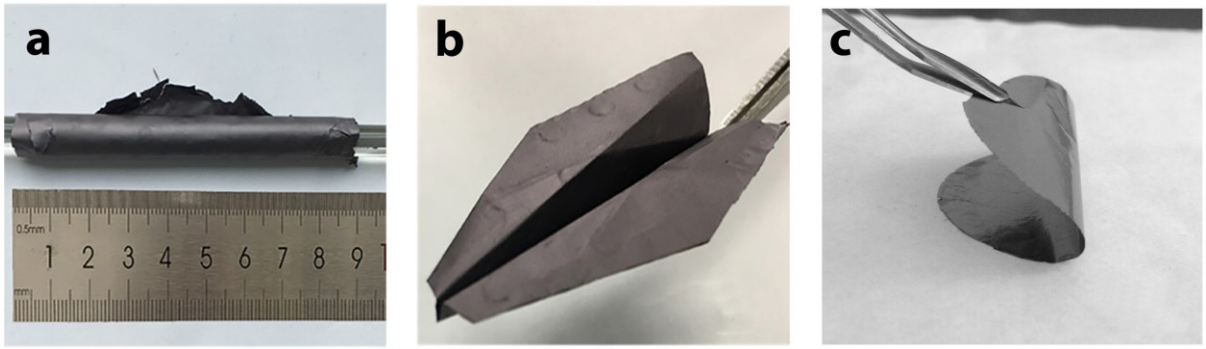


Figure 6. a) Digital image of the wrapping of a freestanding $Ti_3C_2T_x$/CNTs/CuS film on a 5 mm-diameter glass rod. Reproduced with permission.[117] 2022, American Chemical Society. b) The corresponding plane-shaped film folded by hand. Reproduced with permission.[117] 2022, American Chemical Society. c) Digital image showing the flexibility of a 3D macroporous $Ti_3C_2T_x$ film. Reproduced with permission.[118] 2017, John Wiley & Sons.

Layered structure. Typically, MXenes are produced by selectively etching the A layers from their MAX phase counterparts, which are layered ternary carbides and/or nitrides with $M_{n+1}AX_n$ (n = 1–4). M stands for an early transition metal (such as Zr, V, Sc, Ti, Nb, Cr, or Mo), A represents a group 13 or 14 element, and X indicates carbon and/or nitrogen. The crystal structure of a typical M_3AX_2 phase consists of hexagonal layers with P63/mmc symmetry, featuring intergrown hexagonal M_3X_2 layers and planar A atomic sheets stacked alternately along the C-axis. The M-X bonds exhibit a mixed covalent/metallic/ionic character, while the M-A bonds are metallic. The weaker interlayer M-A bonds and interatomic A-A bonds render the A layers more chemically reactive. Consequently, 2D MXenes can be derived by removing the A atoms from their parent M_3AX_2 phase.[158–160]

Surface chemistry. Surface terminations of MXenes typically contain –OH, –O, and –F groups and their chemical formulas are $M_{n+1}X_n(OH)_xO_yF_z$. For simplicity, this is often expressed as $M_{n+1}X_nT_x$, where T refers to the surface termination. MXenes without termination have not yet been synthesized. Recent computational studies include surface terminations in MXene properties evaluations. Mixed termination MXenes were also considered in computational studies. Surface terminations are evolving with the prediction of the most stable arrangement of T atoms with neighboring M and X atoms. As an exception, T atoms may sit directly on top of X atoms in order to gain more electrons.[158–160]

Researchers have examined the surface terminations and flake stacking of certain MXenes using neutron scattering, electron energy-loss spectroscopy, and NMR spectroscopy. According to these studies, MXene surfaces are characterized by random terminations by specific atoms or groups[161,162]. The atomic stacking aligns with DFT predictions, where F and OH are directly connected to MXene flake surfaces, and water molecules are hydrogen-bonded to OH groups. Additionally, there are no neighboring –OH terminations. The results of these studies have provided a realistic map of the terminations on the surface of MXene sheets, allowing property predictions to be made using DFT.[162,163]

Variability in composition. By tuning the chemical nature of the metal or functional groups on the surface, MXenes can exhibit a high degree of variability in composition and band gap. Transition metals, carbon/ nitrogen, and functional groups combine to create a diverse range of compositions and structures. Theoretically, more than 100 stoichiometric compositions have been predicted, and over 50 types of MXenes have been synthesized experimentally. MXene compositions can be classified as mono-transition metal, solid solution, ordered double-transition metals, and ordered divacancies. Aside from being highly attractive for their chemical diversity

and property variability, MXenes also introduces significant complexity. As the popularity of these materials grows among researchers from diverse scientific and engineering disciplines, the need for deep fundamental understanding of processes that govern the properties of these nanomaterials grows.[109,111,164]

2.2. Variations and Modifications

MXenes possess high electrical conductivity, large specific surfaces, hydrophilicity, and abundant surface functional groups, which make them suitable for applications such as energy storage, catalysis, sensors, and electromagnetic interference (EMI) shielding. Despite extensive research, they have inherent drawbacks such as restacking, oxidation susceptibility, poor flexibility, and poor electrochemical performance, limiting their use in various applications. Several modifications have been made to MXenes to overcome these limitations, including hybridization with polymers, heteroatom doping, and cation/organic intercalation. For example, MXene conductivity can be enhanced through heteroatom doping without nonconductive additives.[165–169] The interlayer spacing of MXene can also be expanded through cation and organic molecular intercalation without requiring additional nonconductive agents. Expanding the interlayer improves accessibility and enhances key properties. By leveraging reversible intercalation, new species are avoided. Hybridizing MXenes with polymers leads to synergistic effects that improve overall performance. While polymers may impact conductivity, they significantly enhance flexibility, which can be tailored to specific applications. These functionalized MXenes exhibit improved physical and chemical properties, making them potentially useful in next-generation advanced applications.[168]

2.3. Biomedical-preferred Physical and Chemical Properties

MXenes have been found to have appealing properties for many biomedical applications. This section describes extensively the key biomedical properties and potential applications of these nanomaterials (Figure 7).[170]

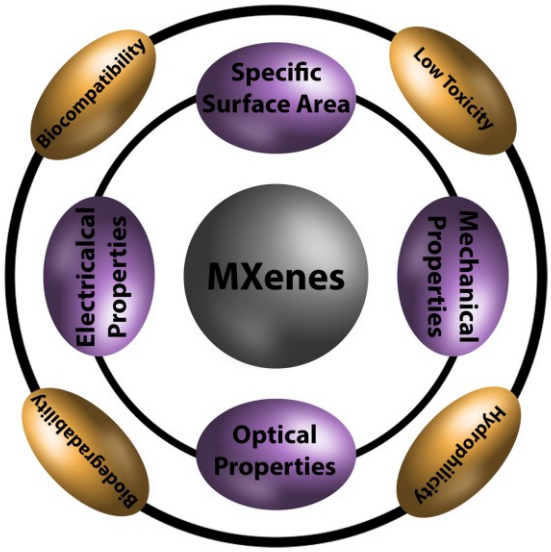


Figure 7. Biotechnology-related properties of MXenes and MXene-based materials.

Biocompatibility. MXenes exhibit excellent biocompatibility, which is an essential property for their safe use in biomedical devices and compounds. In biology, biocompatibility refers to the ability of a material for performing its intended function without triggering an adverse immune response. Biomedical applications, including implants, drug delivery systems, and tissue engineering scaffolds, can benefit from this property. As a result of their surface terminations (e.g., –OH, –F, –O), MXenes can be tolerated towards biological molecules and cells. The biocompatibility of MXenes has been extensively examined by researchers, highlighting their potential for clinical application.[171–173]

Surface area and porosity. With high surface areas and porosities, MXenes are suitable for biomedical applications such as biomolecule isolation for drug delivery. By loading agents

efficiently and controlling their release, porosity promotes cell adhesion, migration, and proliferation.[99,174] Using surface functionalization and morphology control, MXene-based materials can be tailored to have specific surface properties to enhance their interactions with biological molecules and cells.[175,176] In wound healing, tissue regeneration, and controlled drug delivery, where effective interactions with biological systems are vital for therapeutic effectiveness, these properties are crucial.[177,178]

Electrical conductivity. A major advantage of MXenes is their excellent electrical conductivity which makes them ideal for bioelectronics,[179] and neural interfaces.[180] This ability enables MXenes to efficiently transmit electrical signals, making them suitable for applications involving electrical stimulation and sensing in biomedical devices.[181,182] MXene-based electrodes and sensors have shown promise in neural implants,[183] biosensors,[184] and cardiac pacemakers,[173] where precise and reliable electrical communication with biological systems is critical. MXenes' high electrical conductivity also supports their use in bioelectronics and electrochemical biosensors, contributing to advancements in medical diagnostics and monitoring.[185]

Mechanical strength and flexibility. In terms of mechanical strength and flexibility, MXenes are ideal for tissue engineering and implantable medical devices.[171] In physiological environments, these materials are capable of sustaining mechanical stress without fracturing or losing structural integrity.[186] MXene-based scaffolds and implants provide mechanical support to tissues and organs, promoting proper integration and functionality.[187] The mechanical properties of MXenes can be tailored through material design and processing methods to match the requirements of specific biomedical applications, ensuring long-term stability and performance in

vivo. The combination of mechanical strength and flexibility makes MXenes-based compounds suitable for load-bearing applications and dynamic tissue environments.[188,189]

Antibacterial activity. A number of MXenes exhibit inherent antibacterial properties, which can be applied to biomedical applications to prevent infections.[21,190,191] MXenes can disrupt bacterial cell membranes and inhibit bacterial growth, making them suitable candidates for antimicrobial coatings on implants and wound dressings. The antibacterial ability is particularly substantial for reducing the risk of implant-associated infections and supporting healing in biomedical settings.[192,193] MXene-based antimicrobial coatings have shown effectiveness against a wide spectrum of pathogens, highlighting their potential to enhance the safety and longevity of medical devices.[194,195]

Thermal stability. MXenes exhibit excellent thermal stability, enabling them to withstand sterilization processes such as autoclaving. This thermal stability ensures that MXene-based materials can be effectively sterilized without compromising their structural integrity or performance.[196,197] It also makes them suitable for applications in hyperthermia treatments and other therapies involving exposure to elevated temperatures.[19,198] MXene-based materials can maintain their functionality and structural integrity under physiological conditions, contributing to their reliability and longevity in biomedical applications.[199] MXenes exhibit high thermal stability, making them ideal candidates for durable and reliable applications.[200]

Delivery capability. As versatile drug delivery platforms, MXenes can be functionalized to enhance loading and release capabilities. Therapeutic agents can be delivered efficiently from functionalized MXenes, allowing controlled and targeted delivery. This capability is crucial for developing personalized medicine approaches and improving therapeutic efficacy and

safety.[201,202] The ability to tune drug release kinetics and bioavailability of MXene-based carriers makes them attractive for advancing drug delivery technologies.[203]

Photothermal properties. Several MXenes have photothermal properties that enable them to convert near-infrared (NIR) light into heat. This feature can be harnessed for photothermal therapy (PTT), a non-invasive cancer treatment modality. MXenes can selectively heat and destroy cancer cells while sparing healthy tissue, making them promising candidates for therapeutic applications.[204] Photothermal properties of MXenes have been explored in cancer treatment and imaging, demonstrating their potential for precise and targeted therapies.[205]

Biodegradability. MXenes' biodegradability under ambient conditions makes them desirable for biomedical applications requiring them to absorb and metabolize over time. Biodegradability ensures that MXene-based nanostructures will be naturally removed from the environment without leaving residues or causing long-term damage.[95,206] This capability is particularly critical for bioresorbable medical implants,[207] drug delivery systems,[208] and temporary biomedical scaffolds designed to provide therapeutic benefits. MXene-based materials can be engineered to degrade at controlled rates, offering a versatile platform for developing bioresorbable medical technologies.[209,210]

3. Mechanism of Biomolecule Interaction with MXenes

3.1. Adsorption mechanisms

Biomolecules with reactive functional groups, such as thiols or amines, can undergo chemical reactions with surface groups on MXenes under appropriate conditions. According to the nature of the MXenes surface termination and the functional groups present in the biomolecules, they interact through covalent and non-covalent mechanisms. Some biomedical applications require the

isolation and tailoring of surface interactions with biomolecules to maximize performance and functionality. Figure 8 illustrates examples of DNA and MXenes surface.[101]

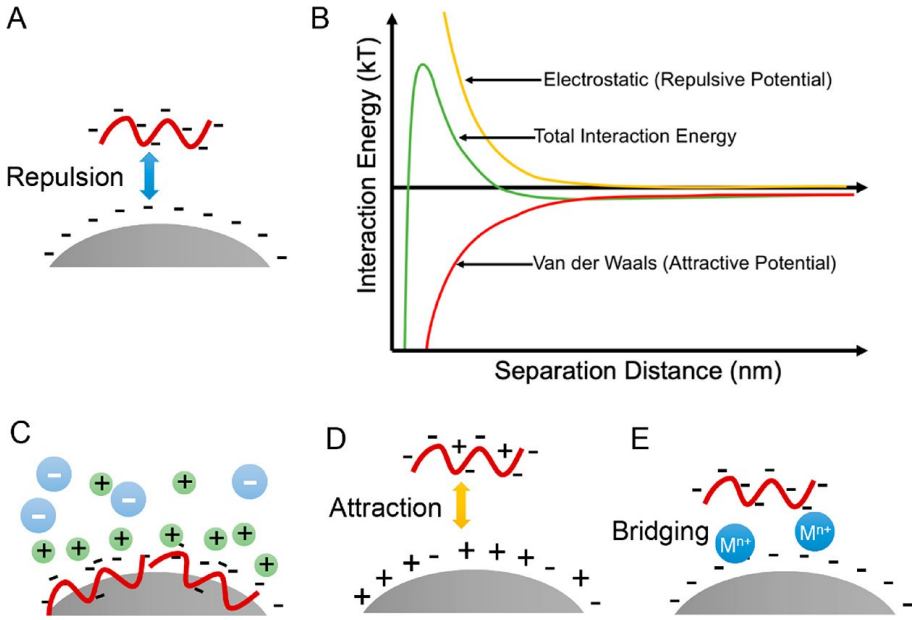


Figure 8. Schematics show the repulsion and attraction of DNA on negatively charged adsorbent. (A) Charge repulsion between DNA and a negatively charged nanoparticle. (B) Distance-dependent intermolecular and surface forces. With strong attraction forces such as Van der Waals (VDW) and non-VDW forces, DNA can be stably adsorbed once passing the repulsion barrier indicated by the positive green peak. DNA adsorption promoted by (C) increasing ionic strength to screen charge repulsion, (D) adding acids to lower pH, and (E) adding polyvalent metal ions. Reproduced with permission.[101] 2020, American Chemical Society.

Non-covalent interactions. Non-covalent interactions are fundamental to MXene binding with biomolecules, with van der Waals forces being the predominant mode of interaction. Van der Waals forces occur between the MXene surface and biomolecule functional groups such as amino acids, proteins, and DNA. MXenes terminated with surface groups like –OH or –O facilitate these weak interactions due to their unique 2D structure and high surface area. These interactions are crucial, as they allow biomolecules to adsorb onto the MXene surface without forming permanent bonds, thus maintaining the integrity and functionality of both the MXenes and the biomolecules.[211–214]

In addition to their weak yet highly versatile nature, van der Waals forces are particularly important in dynamic biological systems, whose ability to temporarily bind and release biomolecules without altering their structure is critical. Hence, MXenes with transient interactions are a good candidate for biosensing and diagnostic applications, where these types of interactions with biomolecules such as proteins and nucleic acids are essential for accurate identification and analysis. Furthermore, MXenes have a large surface-to-volume ratio, allowing them to have a greater Van der Waals binding capacity, allowing them to efficiently interact with a wide range of biomolecules, particularly in high-throughput biological assays.[215–222]

Hydrogen bonding also plays a significant role in the interaction between MXenes and biomolecules. MXenes have functional groups, such as hydroxyl (–OH) groups, form hydrogen bonds with various biomolecules. For example, the nitrogen atoms in the amino groups of amino acids can hydrogen-bonded with the MXene surface. This type of interaction is weak and reversible, which is beneficial for applications requiring the easy attachment and detachment of biomolecules, such as biosensors and drug delivery systems. The ability to form multiple hydrogen bonds enhances the stability and specificity of biomolecule adsorption on MXenes.[26,211,213,223]

Hydrogen bonds can provide a controlled mechanism for fine-tuning the interaction strength between the MXene surface and biomolecules in biosensors and targeted drug delivery. Modulating the binding affinity by the density of hydroxyl groups on the MXene surface allows for precise control of adsorption and desorption kinetics. Through tailored hydrogen bonds, biomolecule delivery timing and dosage can be regulated in controlled release systems. Moreover, hydrogen bonding's reversible nature facilitates the development of reusable platforms. Hence, the same MXene substrate can be used for multiple cycles, improving efficiency and sustainability.[202,224,233–235,225–232]

Electrostatic interactions are another critical mechanism by which MXenes bind to biomolecules. MXene surfaces are often negatively charged, which attracts positively charged biomolecule functional groups. For instance, proteins or peptides with cationic groups interact electrostatically with the anionic MXene surface. This interaction is particularly useful in separating and concentrating biomolecules from complex biological mixtures, as it provides an additional layer of selectivity and binding strength.[26,27,213,236] Specifically, electrostatic interactions are effective for biomolecule purification and selective targeting, where charge-based discrimination allows adsorbents to isolate specific biomolecules even from highly complex biological fluids. MXenes' surface charge tunability through surface functionalization or pH adjustments further enhances their charge-selective binding capacity. Due to their customized electrostatic surface, MXenes are ideal candidates for membrane-based filtration systems and electrochemical biosensors. Besides ensuring high purity in biomolecule isolation, this level of selectivity also reduces non-specific binding, a common challenge in conventional separation techniques.[237–245]

The combination of van der Waals forces, hydrogen bonding, and electrostatic interactions allows MXenes to bind a wide range of biomolecules with high specificity and efficiency. MXenes' versatility and tunability further enhance their potential in creating advanced biomimetic systems and innovative therapeutic solutions.

MXenes are able to function in multifunctional platforms that capture, detect, and deliver biomolecules simultaneously due to the synergistic effect of these non-covalent interactions. This versatility is particularly promising for the development of next-generation biomedical devices, such as wearable biosensors, real-time diagnostic tools, and therapeutic delivery systems. The tunability of MXenes' surface properties enables smart, adaptive biomaterial systems to respond

dynamically to biological conditions by developing interfaces that are highly responsive to environmental stimuli.[99,246,255,247–254]

Covalent bonding. MXenes are typically terminated with groups like –O or –OH, which are not readily covalently bonded to biomolecules functional groups, but certain chemical reactions can be created between them. Under specific conditions, biomolecules containing nitrogen atoms can form covalent bonds with titanium atoms in the MXene lattice. For example, glycine has been shown to form Ti-N bonds with $Ti_3C_2T_x$, where the amino group nitrogen binds covalently to the titanium atom on the MXene surface. This interaction is stronger and more stable than non-covalent interactions and can significantly alter the electronic properties of the MXene surface.[256]

Molecular orientation and binding strength can be enhanced by site-specific covalent modification in functionalization chemistry. Covalent bonds are essential for developing adsorbent platforms, where the efficiency of adsorption is highly dependent on how active sites are stable on the surface. For instance, by forming Ti-N bonds, MXenes are able to enhance their charge transfer properties, improving their performance in biomedical applications. Using these chemical bonds, MXene surface can also be engineered with hydrophilic or hydrophobic characteristics.[256–261] The ability to form strong covalent bonds opens new avenues for the functionalization of MXenes with a wide variety of biomolecules that expands their application scope in biotechnology and materials science. The robust nature of these covalent interactions ensures that the functionalized MXenes maintain their integrity and performance even under harsh conditions, making them ideal for use in special biomedical applications such as diagnostics.

Developing long-lasting biosensors requires the stability imparted by covalent bonds between MXenes and biomolecules, which provides consistent performance over repeated cycles. Due to their chemical robustness, MXenes can remain sensitive to biomarkers even after prolonged

exposure to biological fluids. Moreover, this stability translates into improved shelf life for MXene-based diagnostic devices, enhancing their commercial viability.[262–264] Covalently attaching targeted ligands or agents to MXenes in therapeutic applications allows for the development of site-specific delivery systems, where therapeutic agents can be delivered precisely to target cells or tissues, reducing off-target effects. By covalently functionalizing MXenes, multifunctional platforms can also be produced to serve a range of functions simultaneously, including therapeutic carriers, imaging agents, and drug release platforms. By combining covalent binding with stimuli-responsive release mechanisms, these multimodal systems can control delivery based on environmental triggers such as pH, temperature, or magnetic fields, which makes them ideal for cancer treatment.[202,265–268]

Covalent bonding can also be used to develop bioinspired interfaces on MXenes for applications such as tissue engineering. Growth factors and cell-adhesion biomolecules could be attached covalently to MXenes to promote cell differentiation, proliferation, and tissue regeneration, allowing them to help develop bioactive tissues. By focusing on active biomaterials that can modulate cellular functions in regenerative medicine, MXenes extends beyond passive materials.[172,269–272]

3.2. Selectivity and Sensitivity in Biomolecule Capture

Providing a versatile platform for selective biomolecule capture requires MXenes' specific interactions with biomolecules. Non-covalent interactions play an important role in reversible adsorption, which is crucial for biosensing and drug delivery applications. Surface terminations of MXenes can form weak interactions with biomolecule functional groups that enable selective binding to various proteins, nucleic acids, and small molecules. An additional mechanism for capturing biomolecules with high specificity is hydrogen bonding between nitrogen atoms in

amino acids and the MXene surface. Furthermore, covalent interactions, such as Ti-N bonds, allow for a stronger and more stable bond, which is especially valuable for long-term applications, such as diagnostics and therapeutics. MXenes are able to adapt to different surface chemistries through these bonding mechanisms.[256,273–275]

MXene surface functionalization can be optimized in term of selectivity by incorporating metal ions, antibodies, or aptamers to enhance capture specificity. By neutralizing the negative charges on the MXene surface, metal ions like Mn^{2+} or Ca^{2+} can facilitate stronger electrostatic interactions with biomolecules like DNA. The grafting of antibodies or aptamers onto MXene surface allows for highly selective binding to target proteins and nucleic acids. Cancer biomarkers have been detected with remarkable sensitivity using MXene-based biosensors functionalized with antibodies that selectively bind to disease-specific proteins. By utilizing this strategy, diagnostic accuracy and specificity are improved by reducing interference from non-target molecules.[213,276–282]

To improve sensitivity, surface modifications that minimize non-specific bindings are essential. Functionalizing MXenes with biocompatible coatings or passivating agents can prevent unwanted interactions that result in noise and reduced accuracy due to non-specific bindings in biosensing applications. A combination of surface terminations and flake size allows researchers to optimize MXene selectivity in biological media and sensitivity. Smaller adsorbent flakes have a larger surface-area-to-volume ratio that facilitates adsorption and activation of reactants. Therefore, target molecules even at low concentrations can be detected more sensitively with MXene nanoflakes. This requires MXene flakes not to aggregate, which blocks active sites and reduces interaction efficiency. The aggregation can also decrease MXenes' electronic properties, reducing their utility in applications such as electrochemical biosensors.[283–290]

Due to their excellent electrical conductivity, MXenes have demonstrated robust potential in sensitive biosensing applications after isolation of target species. MXene-based sensors have shown high adsorption capacities, making them suitable for detecting trace amounts of biomolecules, such as in cancer diagnostics. A powerful tool for early detection was provided by MXenes modified with aptamers that isolated and identified specific cancer biomarkers with enhanced selectivity. Electrochemical sensing and immunoassays benefit from MXenes' conductive properties, making them excellent materials for real-time biomolecular interaction monitoring.[291–297]

Developing advanced functionalization techniques is essential for many applications of MXenes. To enhance their selectivity and performance, MXenes can be combined with polymers, metal nanoparticles, or other nanostructures. Using hybrid systems, smaller biomolecules can be selectively isolated, such as hormones, peptides, and enzymes, while maintaining high detection sensitivity. Surface coatings could provide additional specificity, allowing MXenes to distinguish between molecules with similar structures. [26,29,256,298–300]

Ultimately, MXenes' surfaces must be optimized for precise biomolecule isolation applications. Refining functionalization strategies and balancing hydrophilicity *vs.* hydrophobicity can help capture biomolecules with greater precision. Specifically, tailoring MXene surface properties to specific biomolecule types will improve their selectivity, sensitivity, and performance in real-world applications.[211,301–305]

4. MXenes for Biomolecule Isolation

This section provides a comprehensive review of the current literature, categorized into three main parts, proteins, nucleic acid, and other biomolecules. The categorization aims to offer a clear

understanding of the state-of-the-art MXenes' applications in isolating biomolecules. Table 2 compares key points of these reported projects.

Table 2. Overview of research projects investigating the applications of MXenes in adsorption of biomolecules

Biomolecule Type	MXenes' Name	Modification Type	Studied Biomolecule	Project's Aim	Adsorption Mechanism	Project's Results	Ref.
Protein	Ti_3C_2 and Ti_2C	None (intrinsic properties studied)	Lysozyme (cationic protein)	To investigate the interactions of MXenes with lysozyme	Electrostatic interactions	Ti_3C_2 showed higher adsorption capacity for lysozyme compared to Ti_2C;	[236]
Protein	$Ti_3C_2T_x$	Phenylboronic acid modification and Fe_3O_4 particles	Catecholamines (dopamine, norepinephrine, epinephrine, isoprenaline)	To investigate adsorption performance towards catecholamines in urine samples	Van der Waals forces, hydrogen bonding, π-π stacking	High adsorption capacity for dopamine (319.6 μmol g^{-1}); Successful application to clinical samples from volunteers and Alzheimer's patients	[211]
Protein	Ti_3C_2	Chitosan modification via unidirectional freeze-casting	Bovine serum albumin (BSA) and bovine hemoglobin (BHb)	To evaluate adsorption performance for protein separation	Facilitating film mass transfer and porous diffusion	High specific surface area (103.09 $m^2 \cdot g^{-1}$); high permeability (1.94×10^{-12} m^2)	[29]
Protein	$Ti_3C_2T_x$	Functionalized with −O, −OH, and −F	Lysozyme (cationic protein)	To investigate lysozyme adsorption on different functionalized $Ti_3C_2T_x$ surfaces using PTMC and MD simulations.	Electrostatic and van der Waals interactions	Indicating good biocompatibility; interfacial water layer influenced adsorption	[212]
Protein	$Ti_3C_2T_x$	None (intrinsic properties studied)	Bilirubin (BR)	To investigate the removal efficiency of bilirubin for treating hyperbilirubinemia	Physical adsorption and chemical adsorption	High adsorption capacity (1192.9 mg g^{-1}) for bilirubin, significantly higher than traditional activated carbon; Good blood compatibility	[306]
Protein	$Ti_3C_2T_x$	Different etching methods for synthesis	Human plasma proteins	To evaluate $Ti_3C_2T_x$ nanosheets interaction with human plasma proteins	Hydrogen bonding, steric hindrance, and hydrophobic interactions	Complement activation and coagulation cascades were involved in the protein corona; Electrostatic attraction had little effect on the interaction	[223]
Protein	$Ti_3C_2T_x$	PLA functionalization	Bilirubin	To develop Ch/MX/PLA composite aerogel spheres for bilirubin adsorption in hemoperfusion	Chemisorption (monolayer and multilayer adsorption)	High bilirubin adsorption capacity (596.31 mg/g); Good biocompatibility and hemocompatibility	[300]
Protein	Ti_3C_2	None	Bilirubin	To design Ch/MX composite aerogel spheres for efficient bilirubin removal	Hydrogen bonding and electrostatic interactions	High adsorption capacity (521.95 mg/g), strong anti-interference; Good blood compatibility; Shortened adsorption equilibrium time.	[28]
DNA	Ti_2C	None (discussing various materials)	DNA oligonucleotides	To promote DNA adsorption on nanoparticles by lowering pH and adding polyvalent metal ions	Adding polyvalent metal ions to create additional interactions	DNA adsorption improved by reducing charge repulsion and creating attraction forces; Polyvalent metal ions and low pH conditions facilitate DNA binding on nanoparticles.	[101]

DNA	Ti_2C	None (unmodified; promoted with Mn^{2+})	DNA oligonucleotides	To investigate the promotion of DNA adsorption on Ti_2C using Mn^{2+} and compare it with Ca^{2+} and Mg^{2+}	Electrostatic interactions mediated by Mn^{2+}; minimal van der Waals and hydrogen bonding	Mn^{2+} neutralized the negative charge of Ti_2C MXenes; Enabling high DNA adsorption capacity (298 nM on 20 μg/mL Ti_2C)	[213]
DNA	Ti_2C	Probe DNA, Ag/Pt nanohybrids	HBV DNA	To develop a CRISPR-Cas12a based colorimetric biosensor for target HBV detection	Electrostatic interactions	High sensitivity and specificity for HBV DNA detection with sub picomolar limits; Successful application in human serum samples; Smartphone integration for user-friendly	[27]
DNA	$Ti_3C_2T_x$	Cy^3-CD63 aptamer	Exosomes	To develop a self-standard ratio metric FRET nanoprobe for quantitative detection of exosomes	Hydrogen bonding, metal chelate interaction, FRET	High sensitivity with a detection limit of 1.4 × 103 particles mL^{-1}, over 1000× lower than ELISA; Potential for use in biological fields	[301]
Amino Acid	Ti_3C_2	Surface-termination with OH group and Ti-vacancy	Dopamine (DA) and serine (Ser)	To investigate the adsorption of DA and Ser on Ti_3C_2-MXene using DFT calculations	Electrostatic interactions and hydrogen bonding	Pristine Ti_3C_2 showed strong adsorption with Ser (-3.960 eV) and DA (-2.244 eV); OH-termination significantly reduced adsorption energies (-0.097 eV for DA and -0.330 eV for Ser).	[26]
Amino Acid	Ti_2CO_2	O-terminated	Six amino acids (aspartic acid, cysteine, glycine, phenylalanine, histidine, serine)	To predict binding energies and ground-state configurations of selected amino acids	Van der Waals forces and weak bonds	Most amino acids prefer N-bonded adsorption with bond lengths around 2.35 Å; Moderate adsorption energies and structural integrity suggest Ti_2CO_2 as a reusable biosensor.	[214]
Amino Acid	$Ti_3C_2T_x$	Glycine functionalization	Glycine	To prevent restacking of MXenes layers through glycine functionalization	Ti-N bonding evidenced by shared electrons between Ti and N atoms	XRD and XPS confirmed increased interlayer spacing and possible Ti-N bonding. Flexible freestanding films of $Ti_3C_2T_x$/glycine hybrids showed improved rate and cycling performance.	[256]

4.1. Protein isolation

MXenes have shown remarkable potential for protein isolation due to their unique physicochemical properties. This section delves into recent advancements in this area focusing on the mechanisms of interaction and surface modifications to enhance specificity, and practical applications.

Rozmysłowska-Wojciechowska *et al.* examined the surface-property changes of 2D MXenes flakes (Ti_3C_2 and Ti_2C) upon interaction with the model cationic protein lysozyme using time-resolved dynamic light scattering and zeta potential measurements (Figure 9a)[236]. MXenes

interaction with lysozyme was evaluated based on their structure, stoichiometry, surface chemistry, and concentration of exposure. Ti_3C_2 MXene, characterized by higher surface porosity, demonstrated significantly higher lysozyme adsorption capacity in comparison to Ti_2C MXene. According to the study, the MXene surface could release lysozyme under basic pH conditions. This adsorption was concentration-dependent and mainly driven by electrostatic interactions. MXenes' interactions with biomacromolecules and underlying adsorption mechanisms must also be evaluated before developing drug delivery systems. Additionally, the dramatic changes in surface physics and chemistry during protein adsorption may have a significant impact on separation, future delivery, and purification systems that target specific proteins.

Zhao *et al.*[212] employed parallel tempering Monte Carlo and molecular dynamics methods to investigate lysozyme adsorption on different functionalized $Ti_3C_2T_x$ surfaces (–O, –OH, and –F) (Figure 9b). The simulation results revealed that lysozyme effectively adsorbs on $Ti_3C_2T_x$ surfaces, with the interaction strength following the order of $Ti_3C_2O_2$ > $Ti_3C_2F_2$ > $Ti_3C_2(OH)_2$. During the adsorption process, electrostatic and van der Waals interactions were found to be important. Based on Coulombic repulsion between positively charged hydrogens and lysozyme, the orientation distributions of lysozyme on $Ti_3C_2O_2$ and $Ti_3C_2F_2$ surfaces were more concentrated than those on $Ti_3C_2(OH)_2$. The study also indicated minimal conformational changes in lysozyme upon adsorption, suggesting an acceptable biocompatibility of $Ti_3C_2T_x$. Furthermore, the distribution and structure of the interfacial water layer on $Ti_3C_2T_x$ surfaces influence lysozyme adsorption. This work provides valuable theoretical insights into the biocompatibility of 2D $Ti_3C_2T_x$ materials and offers guidance for engineering their surfaces for bio-related applications.

Hu et al.[211] prepared the Fe_3O_4@$Ti_3C_2T_x$-BA magnetic multilayer porous MXenes composite by depositing magnetic nanoparticles with in situ growth on phenylboronic acid

modified $Ti_3C_2T_x$ nanosheets. This magnetic composite presented excellent selectivity for catecholamines and achieved a high adsorption capacity of up to 319.6 μmol g^{-1} for dopamine. The unique 2D layered structure of $Ti_3C_2T_x$ facilitated the rapid adsorption of urinary catecholamines within 2.0 minutes, reducing diffusion paths and improving molecule transport. Multilayer adsorption as well as synergetic interactions involving borate affinity, hydrogen bonding, and stacking also contributed to adsorption. In urine samples, a sensitive method for determining catecholamines was demonstrated by coupling magnetic boronated affinity composites with liquid chromatography and fluorescence detection, which demonstrated good linearity and low detection limits (0.06-0.16 ng mL^{-1}). Catecholamines were successfully analyzed in urine samples from 15 healthy volunteers and 16 patients with Alzheimer's disease using a methodology with high accuracy and recovery rates (88.14-112.3%). Fe_3O_4@$Ti_3C_2T_x$-BA composites exhibited excellent selectivity, high regeneration abilities, and capability for saccharides, glycoproteins, and RNA extraction. They also stated that future studies will focus on enhancing the method's precision for the simultaneous analysis of multiple neurotransmitters (Figure 9c).

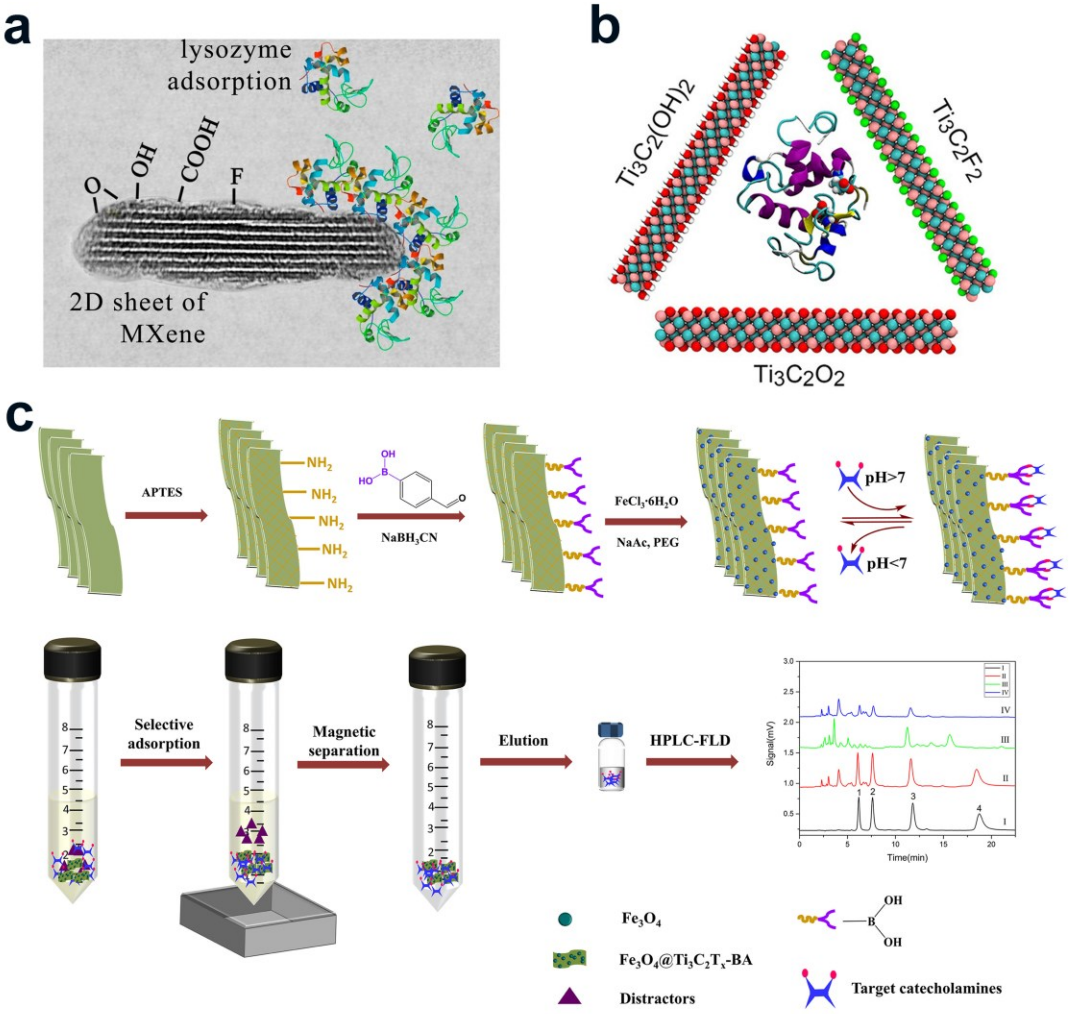


Figure 9. a) Surface interactions between 2D Ti_3C_2/Ti_2C MXenes and lysozyme. Reproduced with permission.[236] 2022, Elsevier. b) Lysozyme adsorption on different functionalized MXenes. Reproduced with permission.[212] 2021, American Chemical Society. c) Magnetic borate-modified MXene, a highly affinity material for the extraction of catecholamines. Reproduced with permission.[211] 2021, Elsevier.

An aligned porous Chitosan/Ti_3C_2 MXenes monolith was synthesized by a facile, versatile, and repeatable unidirectional "freeze-casting" method in 2023 by Ai *et al.* (Figure 10a)[29] This innovative monolith featured aligned micropores (2.53 nm), macropores (21 μm), and a high specific surface area (103.09 $m^2 \cdot g^{-1}$). Compared to conventional monoliths, the prepared structure's permeability (1.94×10^{-12} m^2) was approximately ten times higher, enhancing mass transfer and chromatographic performance. Batch adsorptive experiments demonstrated

significantly elevated adsorptive capacities for bovine serum albumin (91.16 $mg \cdot g^{-1}$) and bovine hemoglobin (292.33 $mg \cdot g^{-1}$) due to proteins' high affinity for embedded Ti_3C_2 MXenes. By facilitating film mass transfer ($[kLa]_f$) and increasing global mass transfer ($[kLa]_g$), the monolith is suitable for continuous protein separation in large-scale production. These findings highlight the potential of aligned porous CTS/Ti_3C_2 MXenes monoliths for improving chromatographic performance and their broad application in protein separation processes.

Sun *et al.*[306] discovered that 2D $Ti_3C_2T_x$ sheets exhibit an ultra-high removal capability for bilirubin (BR), showing 47.6 times higher removal efficiency compared to traditional activated carbon absorbents (Figure 10b). The study investigated the effect of $Ti_3C_2T_x$ on the removal rate of BR in solutions containing different concentrations of bovine serum albumin (BSA). An adsorption capacity reduction of 15% was found with BSA at a concentration of 5 g L^{-1}. SEM, FT-IR, and XPS characterization of $Ti_3C_2T_x$ before and after adsorption confirmed its stability and effectiveness as a bilirubin adsorbent. Furthermore, $Ti_3C_2T_x$ beads demonstrated higher adsorption capacities for bilirubin than coconut shell charcoal within two hours when they were subjected to hemoperfusion simulation experiments. Adsorption kinetics indicated that both physical and chemical adsorption processes contribute to high capacity, with chemical adsorption dominating. The study concluded that $Ti_3C_2T_x$, with its large specific surface area and active sites, coupled with high blood compatibility, holds promise as a hemoperfusion adsorbent for the treatment of hyperbilirubinemia.

The influence of protein coronas on $Ti_3C_2T_x$ nanosheets synthesized by different etching methods was investigated by Wu *et al.*[223] (Figure 10c). They studied the physicochemical characteristics of $Ti_3C_2T_x$ nanosheets before and after exposure to human plasma (HP). They found that $Ti_3C_2T_x$ nanosheets with protein coronas tended to aggregate more than pristine $Ti_3C_2T_x$. The

$Ti_3C_2T_x$ variants show high overlap in protein types and functions based on LC-MS/MS proteomics analysis, but significant differences in relative protein abundances. Immunoglobulins and coagulation proteins were highly enriched in the coronas, while albumin was depleted compared to its abundance in original HP. According to the study, hydrogen bonding, steric hindrance, and hydrophobic interactions drive HP protein adsorption on $Ti_3C_2T_x$, while electrostatic attraction plays little role. This work provided insights into the colloidal stability of $Ti_3C_2T_x$ nanosheets and their interaction with human plasma proteins, offering valuable information for the development of biomedical applications.

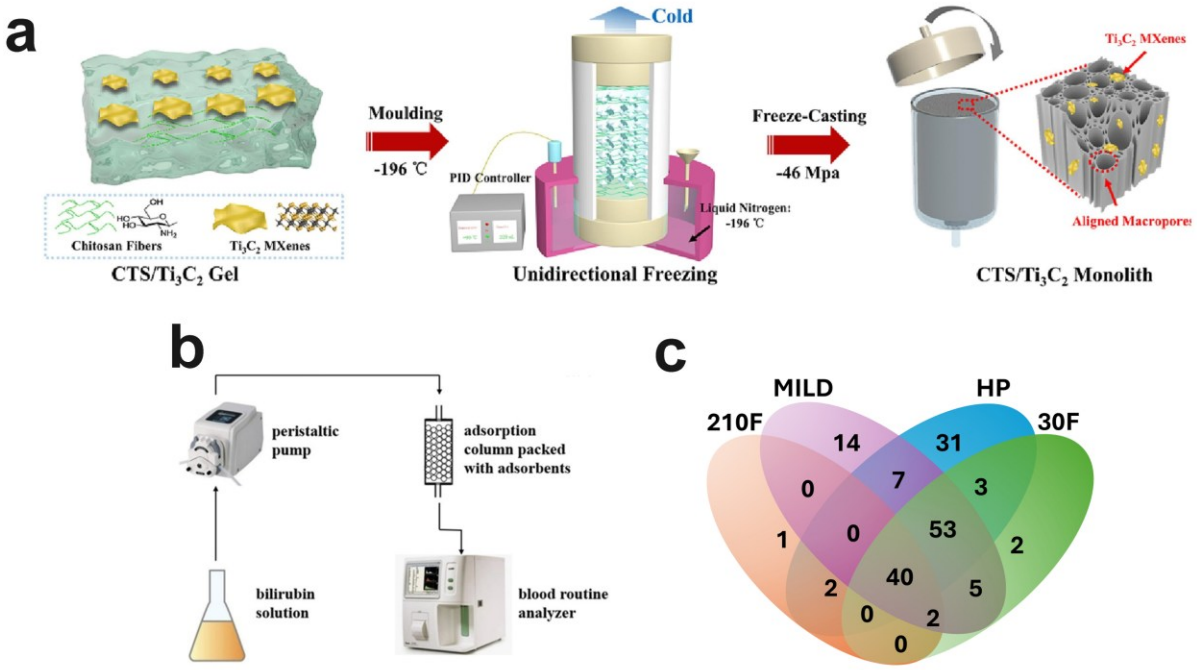


Figure 10. a) Aligned porous Chitosan/Ti_3C_2 MXenes monolith as a high-performance fixed-bed separation medium toward globular albumin separation. Reproduced with permission.[29] 2023, Elsevier. b) Highly efficient adsorption of Bilirubin by $Ti_3C_2T_x$ MXene. Reproduced with permission.[306] 2021, John Wiley & Sons. c) Venn diagrams of the number of the identified proteins of all the prepared samples. (MK: marker, 10F: 10F-$Ti_3C_2T_x$@corona, 30F: 30F-$Ti_3C_2T_x$@corona, MILD: MILD-$Ti_3C_2T_x$@corona, HP: human plasma proteins).

Yao *et al.*[300] investigated advanced solutions for hemoperfusion, a critical clinical treatment for toxin removal from the blood (Figure 11a). To avoid non-specific adsorption of blood

proteins, hemoperfusion devices must balance high adsorption capacity with biocompatibility. Addressing the urgent need for specific bilirubin adsorbents to treat hyperbilirubinemia, Yao et al. also introduced poly(L-arginine) (PLA) into chitin/MXene (Ch/MX) composite aerogel spheres using supercritical CO_2 technology. This innovative Ch/MX/PLA composite demonstrates superior mechanical properties, enduring up to 50,000 times its own weight. In vitro hemoperfusion tests reveal an impressive adsorption capacity of 596.31 mg/g, a 15.38% improvement over Ch/MX alone. Furthermore, the composite exhibits excellent biocompatibility and hemocompatibility, as demonstrated by hemolysis rate and CCK-8 testing. Although preclinical evaluation in patient blood is still required, the Ch/MX/PLA composite shows promising potential for mass production and clinical application in the treatment of hyperbilirubinemia.

According to Zhang *et al.*,[28] patients can suffer irreversible brain and nervous system damage as a result of bilirubin removal from blood (Figure 11b). To achieve safe, rapid, and efficient bilirubin adsorption, they developed chitin/MXene composite airgel spheres using supercritical CO^2 technology. To enhance the mechanical strength and adsorption capacity of airgel spheres, Ti_3C_2 MXene was uniformly dispersed within chitin fibers as a monolayer nanofiller. Ti_3C_2 MXene was tightly connected to chitin as a monolayer nanofiller. The Ch/MX aerogel spheres demonstrated remarkable stability, capable of withstanding 25,000 times their own weight. Compared to chitin aerogel spheres, the Ch/MX aerogel spheres exhibited a 40% increase in adsorption capacity and a 33% reduction in equilibrium time for bilirubin adsorption. Even in the presence of high BSA protein concentrations (up to 80 mg/mL), Ch/MX aerogel spheres maintained a high adsorption capacity of 142.86 mg/g of bilirubin. Compared to commercial products, Ch/MX aerogel spheres showed superior adsorption capacity and interference resistance. Moreover, Ch/MX aerogels required lower amounts of functional materials, achieved higher

bilirubin adsorption rates, and demonstrated better biocompatibility than chitinous/graphene spheres. This study highlights the potential of MXene as a nanofiller for enhancing bilirubin adsorption and suggests Ch/MX aerogel spheres as promising candidates for clinical applications in hyperbilirubinemia treatment.

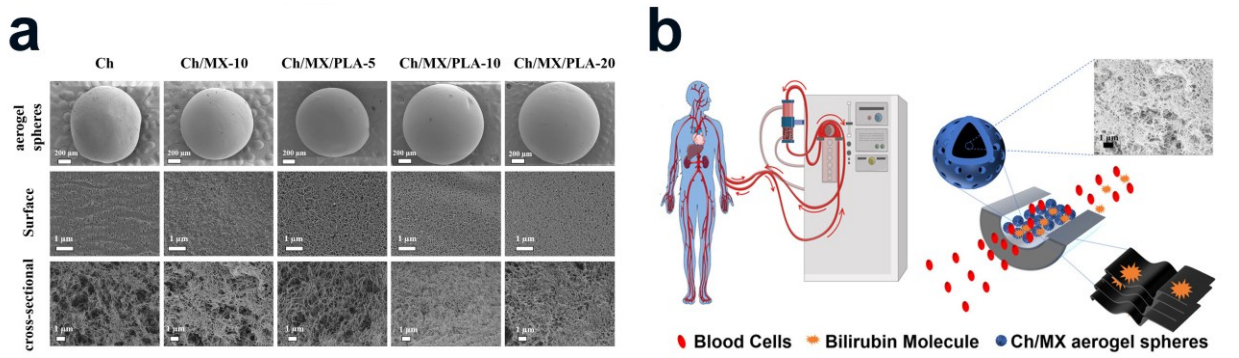


Figure 11. a) SEM images of different ratio of chitin/MXene/poly(L-arginine) in composite aerogel spheres. Reproduced with permission.[300] 2023, Elsevier. b) MXene nanosheet-enhanced chitin aerogel spheres for Bilirubin adsorption. Reproduced with permission.[28] 2022, American Chemical Society.

4.2. Nucleic acid isolation

MXenes are also promising candidates for efficient nucleic acid isolation due to their unique structural and chemical properties. In this section, we discuss recent progress in nucleic acid isolation using MXenes, including their mechanisms of interaction, surface functionalization techniques to enhance selectivity, and their potential applications.

Kushalkar *et al.*[101] discussed the challenges and strategies in enhancing DNA adsorption onto nanoparticles, critical for developing DNA-based biosensors, drug delivery systems, and smart materials (Figure 12a). They highlighted that, as a polyanion, DNA is typically electrostatically attracted to negatively charged nanoparticles, the predominant type of nanomaterial. Normally, charge repulsion is mitigated by adding NaCl, but this approach lacks

additional forces that cause nanoparticle aggregation at high concentrations. As an alternative, they proposed lowering pH and introducing versatile metal ions, particularly transition metal ions. DNA bases and nanoparticle surfaces are protonated at low pH, reducing charge repulsion, and potentially enhancing attraction, although DNA folding can be adversely affected. Alternatively, versatile metal ions promote additional adsorption interactions, overcoming pH adjustments' limitations. This article suggests a number of future research directions, including further exploration of metal ions and a better understanding of adsorption and desorption kinetics, both of which are key to optimizing the stability and practical application of hybrid materials.

Huang et al. studied Ti_2C MXenes' interaction with DNA[213] (Figure 12b). MXene's surface is negatively charged, repelling DNA. A significant finding of this study is that Mn^{2+} neutralizes the negative charges on Ti_2C MXene, which in turn allows for electrostatic interactions with DNA. Additionally, Mn^{2+} displays non-quenching properties, which makes it attractive for applications, unlike Ca^{2+} and Mg^{2+}. The study also revealed that inorganic phosphate ions can desorb DNA from Ti_2C MXene delayed due to manganese phosphate crystals. Interestingly, DNA desorption induced by complementary DNA and random DNA was similar in MXene, which contrasts with graphene oxide. The difference can be attributed to the distinct surface chemistry of MXene and graphene oxide, highlighting MXene's unique interaction mechanisms with DNA.

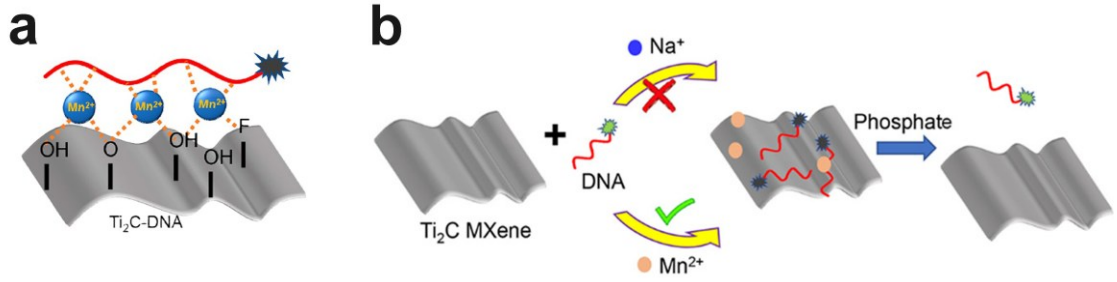


Figure 12. a) Promoting DNA adsorption by Mn^{2+}-assisted Ti_2C MXene. Reproduced with permission.[213] 2019, American Chemical Society. b) Mn^{2+}-assisted DNA oligonucleotide adsorption on Ti_2C MXene nanosheets. Reproduced with permission.[213] 2019, American Chemical Society.

The MXene-probe DNA-Ag/Pt nanohybrids can detect HBV DNA highly sensitively using a CRISPR-Cas12a-based colorimetric biosensor reported Tao *et al.*[27] (Figure 13). By utilizing MXene-based nanohybrids as a catalyst, this nanosensor degrades DNA probes in the presence of HBV DNA by activating Cas12a's trans-cleavage activity. This process inhibits DNA metallization, and the peroxidase-like activity enhances DNA adsorption on the MXene, resulting in significantly reduced catalytic activity. In human serum samples, MXene-probe DNA-Ag/Pt nanohybrids demonstrated excellent sensitivity and specificity, as well as high accuracy and stability. Colorimetric sensing could also be integrated with a smartphone platform for visible, sensitive detection of target DNA in resource-constrained circumstances. A dual-amplified colorimetric method is rapid, cost-effective, and user-friendly to diagnose HBV DNA, demonstrating the versatility of MXene-based nanomaterials.

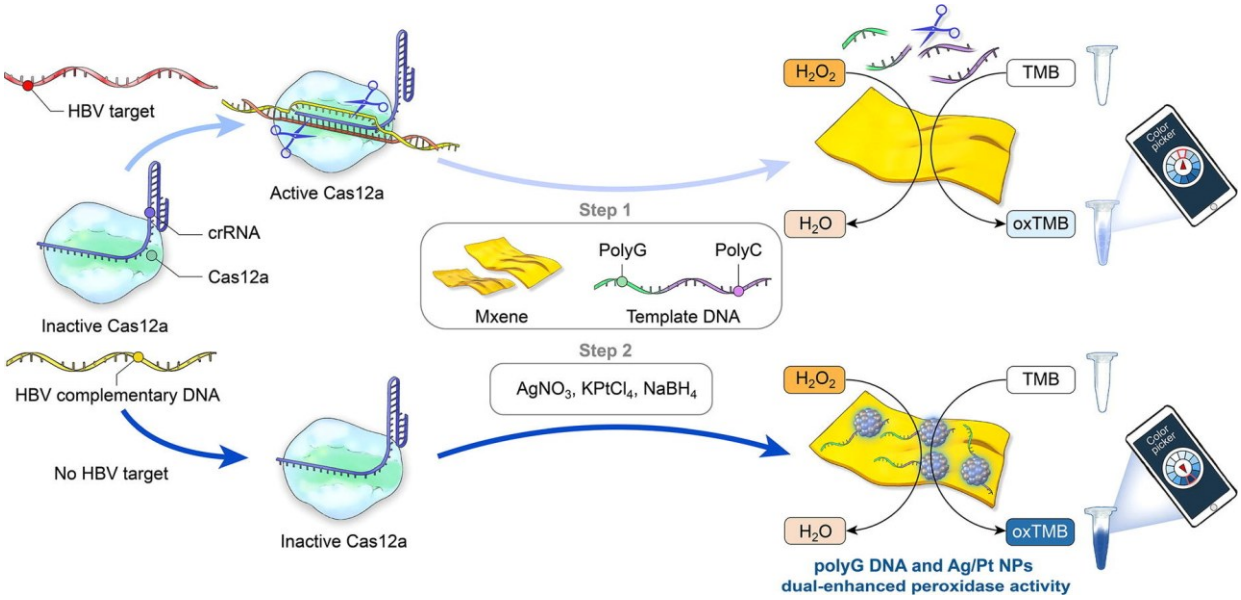


Figure 13. CRISPR-Cas12a-regulated DNA adsorption and metallization on MXenes as enhanced enzyme mimics for sensitive colorimetric detection of hepatitis B virus DNA. Reproduced with permission.[27] 2022, Elsevier.

In another study by Zhang et al.[301], MXene nanosheets were utilized in a novel self-standard ratiometric fluorescence resonance energy transfer (FRET) biosensing platform for quantitative detection of exosomes (Figure 14). This study employed a Cy3-labeled CD63 aptamer/Ti_3C_2 MXene nanocomplex in which Cy3-CD63 aptamer selectively absorbs onto Ti_3C_2 MXene nanosheets via hydrogen bonds and metal chelates. As a result of the FRET interaction between Cy3 and MXenes, fluorescence quenching occurred. Combined with exosomes, which have a high affinity for CD63 protein and specifically bind to the aptamer, Cy3 fluorescence is greatly increased. FRET biosensing platform showed a detection limit of 1.4×10^3 particles mL^{-1} for exosomes, which is over 1000 times lower than conventional ELISA. This platform is also capable of identifying several biomarkers on the surface of exosomes as well as diverse types of exosomes, demonstrating its versatility for biological applications.

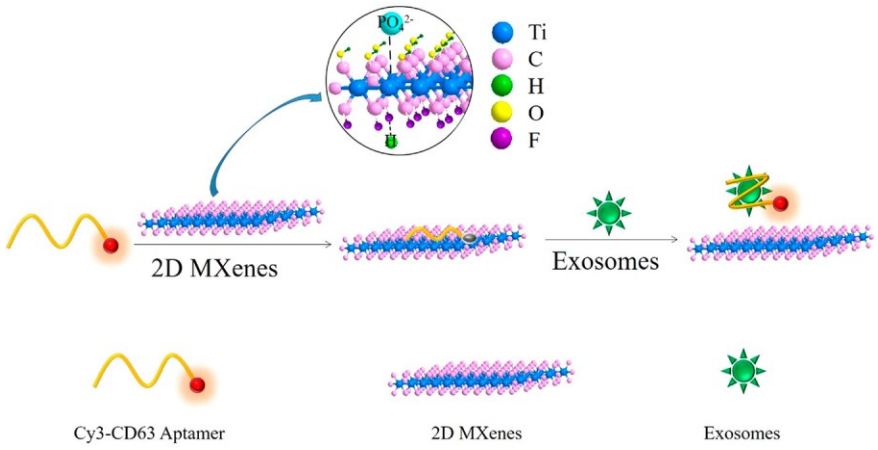


Figure 14. Universal Ti_3C_2 MXenes based self-standard ratiometric fluorescence resonance energy transfer platform for highly sensitive detection of exosomes. Reproduced with permission.[301] 2018, American Chemical Society.

4.3. Other small biomolecules

Beyond proteins and nucleic acids, MXenes have also been investigated for the isolation of various other biomolecules, such as dopamine (DA) and serine (Ser), and small amino acids. This section

discusses the interactions, functionalization strategies, and potential applications of MXenes in other small biomolecule separation.

Recently Ozdemir et al.[26] carried out a density functional theory (DFT) investigation of the interactions between a Ti_3C_2-MXene monolayer and the biological molecules dopamine (DA) and serine (Ser), acting as neurotransmitters and amino acids, respectively (Figure 15). In this study, previous literature findings regarding the optimized Ti_3C_2 monolayer structure were confirmed and DA and Ser molecules were found to bind to the Ti_3C_2 surface with adsorption energies of -2.244 eV and -3.960 eV, respectively. Serine's adsorption resulted in the dissociation of one hydrogen atom, while electronic density of states analyses revealed minimal changes in the Ti_3C_2-MXene monolayer's electronic properties upon biomolecule adsorption. The researchers further explored the effects of surface modifications, specifically OH-functional groups, and Ti-vacancies, on adsorption behavior. They found that surface termination –OH groups significantly reduced adsorption energies to –0.097 eV for DA and –0.330 eV for Ser, suggesting weaker binding, whereas Ti-vacancies increased adsorption energies to –3.584 eV for DA and –3.856 eV for Ser, indicating stronger interactions. The results highlight the potential of Ti_3C_2-MXene in biosensing and biomedical applications, emphasizing its sensitivity to surface modifications. Future studies could leverage these insights to develop advanced biomaterials and devices for biomedical technologies such as biosensors and biofilms.

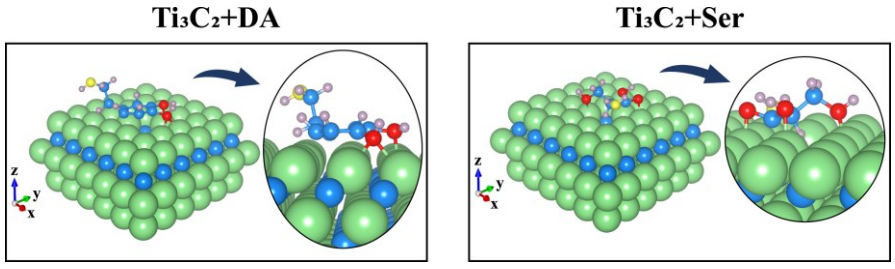


Figure 15. Adsorption of dopamine (DA) and serine (Ser) on Ti_3C_2 MXenes. Reproduced with permission.[26] 2024, Elsevier.

Gouveia *et al.*[214] explored the diverse applications of bioinorganic lamellar nanomaterials with a particular focus on T_2C MXenes (Figure 16). They stated that biocompatibility, pharmacological applicability, energy storage performance, and potential as single-molecule sensors are some of the advantages demonstrated by MXenes, such as O-terminated Ti_2CO_2. Gouveia *et al.* reported first-principles predictions based on density functional theory, investigating the binding energies and ground-state configurations of six selected amino acids adsorbed on Ti_2CO_2. Their findings indicate that most amino acids prefer to adsorb via their nitrogen atom, forming a weak bond with a surface Ti atom at a distance of approximately 2.35 Å. However, serine and histidine tend to adsorb parallel to the MXene surface, with their α carbon approximately 3 Å away. According to the calculated adsorption energies, ranging from 0.5 to 1 eV, van der Waals forces dominate adsorption. The minimal structural deformation of both amino acids and the MXene surface upon adsorption suggests Ti_2CO_2 as a potential reusable biosensor for amino acids.

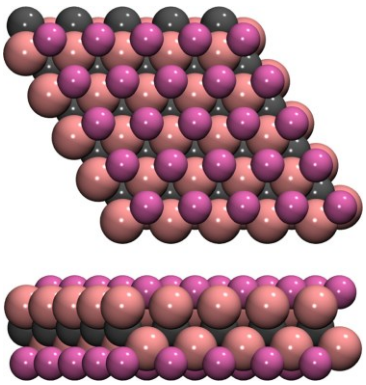


Figure 16. Top and side views of the Ti_2CO_2 supercell. Reproduced with permission.[214] 2020, American Chemical Society.

Chen *et al.*[256] addressed the issue of restacking in two-dimensional (2D) materials, particularly $Ti_3C_2T_x$ MXene, which limits ion accessibility and hinders performance in energy

storage applications (Figure 17). Glycine was proposed as a strategy for preventing restacking and maintaining open interlayer structures. A combined theoretical and experimental approach was used to investigate the interaction between $Ti_3C_2T_x$ and glycine. Based on first-principal calculations, a stable glycine configuration is predicted on the $Ti_3C_2O_2$ surface, indicating Ti-N bonding. By synthesizing $Ti_3C_2T_x$/glycine hybrid films, X-ray diffraction and X-ray photoelectron spectroscopy revealed increased interlayer spacing and Ti-N bonding, respectively. Expanding the MXene structure led to an increase in rate capabilities and cycling stability over pristine $Ti_3C_2T_x$. This study highlighted the potential of organic molecule functionalization in optimizing MXene materials.

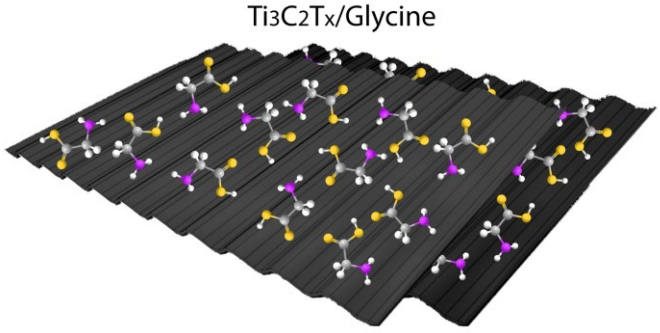


Figure 17. Glycine-functionalized $Ti_3C_2T_x$ film.

4.4. Unique properties of MXenes for biomolecule isolation

MXenes exhibit a distinct set of properties that make them exceptionally well-suited to biomolecule isolation, fulfilling critical requirements in biomedical and biotechnological applications. They are distinguished by their remarkable surface properties, including a tunable surface chemistry,[307] which significantly enhance biomolecule isolation efficiency and selectivity. MXenes' large surface area provides abundant binding sites for biomolecules, while their tunable

surface chemistry allows for precise functionalization with specific ligands or polymers. This functionalization enhances the affinity and selectivity of MXenes for target biomolecules such as proteins, DNA, RNA, and metabolites. Through mechanisms like electrostatic interactions, hydrogen bonding, and van der Waals forces, MXenes can selectively adsorb biomolecules,[28,213,308] enabling the precise capture and separation of complex biological matrices. These characteristics are crucial for applications in biomedical diagnostics, therapeutics, and biotechnological processes.[308]

Compared to carbon-based nanomaterials (e.g., graphene, carbon nanotubes), conventional nanomaterials (e.g., silica or alumina), and metal-organic frameworks (MOFs), MXenes offer several unique advantages. The partially inert surface of graphene and the limited surface chemistry of silica and alumina often restrict their functionalization, making it difficult to achieve selective binding.[309–312] In contrast, MXenes possess naturally tunable surfaces, characterized by abundant termination groups such as –OH, –O, and –F, which enhance their functional versatility.[313,314] Moreover, MXenes exhibit superior hydrophilicity compared to graphene, facilitating easier dispersion in aqueous solutions.[315,316]

When compared to MOFs, MXenes stand out due to their high electrical conductivity and mechanical flexibility, making them particularly suitable for electrochemical biosensing, where real-time monitoring and biomolecule isolation are critical.[317,318] Additionally, MXenes can be produced on a larger scale than MOFs, which are more challenging to synthesize and process. [190,319] Unlike carbon nanotubes, which often face aggregation issues in aqueous environments due to their hydrophilic surfaces, MXenes disperse readily in water. This makes them more reliable for use in biological fluids, where stable dispersion is essential for effective biomolecule isolation. [320,321]

Moreover, MXenes' unique surface characteristics facilitate the rapid and straightforward isolation of biomolecules. MXenes exhibit high adsorption capacities and rapid kinetics, allowing for the quick capture and release of biomolecules and significantly reducing overall isolation times compared to traditional methods.[302] The ease of functionalizing MXene surfaces to optimize interactions with target biomolecules further streamlines isolation. This capability is particularly advantageous in clinical settings and biopharmaceutical production, where speed and efficiency in biomolecule purification are critical. Additionally, MXenes can be integrated into automated and high-throughput systems, enhancing their utility in industrial and research applications.[322,323]

In addition to their efficiency and speed, MXenes are known for their robustness and stability under various environmental conditions, including biological and chemical environments. They can be reused multiple times without significant loss of performance, reducing operational costs and minimizing environmental impact. Functionalization can enhance MXenes' stability and resistance to degradation, ensuring prolonged functionality in repeated biomolecule isolation cycles. This reusability not only contributes to MXenes' economic feasibility but also supports sustainable practices in biomolecule purification processes. Their stability under physiological conditions ensures reliable performance in biomedical applications, such as biosensory, drug delivery, and tissue engineering.[95,324]

Furthermore, MXenes have demonstrated excellent biocompatibility, making them suitable for a wide range of biological and medical applications. Their inert nature and minimal cytotoxicity ensure compatibility with biological samples and cells, minimizing adverse effects during biomolecule isolation procedures. MXenes can be further modified to enhance biocompatibility, ensuring safe interactions with biological systems. Moreover, their stability in physiological environments ensures reliable biomedical performance. These properties make MXenes highly

attractive for biomolecule isolation in clinical diagnostics, therapeutic delivery systems, and biotechnological research.[325,326]

Another significant advantage is that MXenes are produced through relatively straightforward synthesis methods, contributing to their scalability for industrial applications. The precursor MAX phases are abundant and cost-effective, enhancing scalability and reducing production costs. The high yield during synthesis and potential for large-scale production make MXenes economically viable for biomolecule isolation on an industrial scale. This scalability and cost-effectiveness position MXenes as promising candidates for commercial applications in the biotechnology and biomedical industries, facilitating the development of advanced biomolecule isolation technologies.[327,328]

In summary, the combination of enhanced efficiency and selectivity, rapid and straightforward isolation, reusability and stability, biocompatibility and safety, and scalability and cost-effectiveness underscore the potential of MXenes as revolutionary materials for biomolecule isolation. These properties enable MXenes to meet the stringent demands of modern biomedical and biotechnological applications, driving innovations in diagnostics, therapeutics, and beyond.

4.5. Potential challenges and limitations

Degradation of MXenes in biological environments. One of the primary challenges facing MXenes in biomolecule isolation is their susceptibility to degradation in biological environments. While MXenes are generally stable under many chemical and physical conditions, they can undergo oxidation or hydrolysis reactions in the presence of certain biological fluids or enzymatic environments.[123,329] MXenes can also be chemically unstable under different pH conditions[330] and ionic strengths found in biological samples. Furthermore, functional groups on MXenes can

degrade over time, especially under harsh biological or chemical conditions. This degradation can lead to changes in their surface chemistry and structural integrity, potentially compromising their effectiveness in biomolecule isolation over time.[331,332] Strategies to address this challenge include the development of surface modifications and coatings to enhance MXenes' stability and durability in biological settings. Additionally, understanding the mechanisms of degradation and identifying protective strategies are crucial for improving MXenes' long-term performance in biomedical applications.[333]

Repeatable production. Achieving consistent and reproducible MXene production is essential for their widespread application in biomolecule isolation. Besides, having uniform functionalization across the entire surface of MXenes and controlling narrow distribution for size and shape of MXene particles can be critical to their performance in biomolecule isolation.[97,334] MXenes are usually synthesized by selective etching of MAX phases, which can be influenced by a range of factors such as precursor material quality, etchant composition, and synthesis conditions. Variability in these parameters can result in differences in MXene properties, including surface chemistry, layer thickness, and morphology, which affect their biomolecule isolation performance. Developing standardized synthesis protocols and robust quality control measures is critical to ensure consistent MXene production with uniform properties. This will enable reliable and predictable performance in biomolecule isolation processes, supporting their integration into biomedical diagnostics and therapeutic applications.[319,335]

Selectivity and sensitivity. The high surface area of MXenes can result in non-specific binding of biomolecules, reducing the selectivity and specificity of isolation processes. Developing methods that minimize adverse interactions and increase compatibility with certain biomolecules is crucial. To improve selectivity, MXenes need to be functionalized, making preparation more

complicated. Ensuring that these functionalized MXenes maintain their binding specificity and stability in biological environments is critical for effective biomolecule isolation. Specific molecules or ligands that selectively bind target biomolecules can be grafted onto the MXene surface to enhance performance. Covalently attached antibodies[336], aptamers[337], or small molecules[338] can be suitable ways to achieve efficient capturing agents that improve selectivity by uniquely recognizing and binding to the target molecules. Modifying MXenes surface by metal ions, can also enhance sensitivity in biomolecule isolation by minimizing non-specific interactions.[175,339–341]

Aggregation and dispersion. MXenes tend to aggregate in aqueous solutions, reducing their effective surface area and hindering their ability to interact with target biomolecules. Effective dispersion methods are needed considering maintaining functional properties. To ensure stable MXenes dispersibility in various biological media requires optimization of conditions such as pH, ionic strength, and the use of suspension agents[342,343] Developing surfactants or polymers that can stabilize MXenes in suspension and prevent aggregation can be efficient in this regard. MXenes can also be dispersed after breaking up aggregation through ultrasonication and other mechanical methods. Furthermore, combining MXenes with hydrophilic polymers or other 2D materials that increase dispersion stability can enhance biomolecule isolation performance. Tailoring the synthesis process to produce MXenes with improved dispersibility, such as optimizing flake size and surface chemistry, can also enhance their performance in biomolecule isolation (Figure 18).[290,344–346]

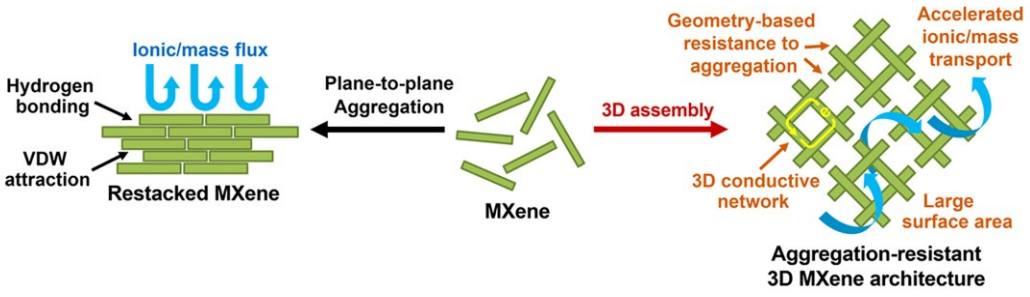


Figure 18. Substantial merits of aggregation-resistant 3D MXene architecture. Reproduced with permission.[290] 2018, American Chemical Society.

Regulatory and safety concerns. MXenes may face regulatory challenges in biomedical applications due to possible safety concerns. Evaluating the environmental impact of MXenes, particularly their long-term stability and potential toxicity in biological and ecological systems, is crucial. Assessing the environmental impact of MXenes and developing disposal and recycling strategies can help mitigate potential risks. To meet regulatory requirements, it is necessary to conduct comprehensive biocompatibility and toxicity studies.[347] Collaboration with regulatory agencies early in the development process can help identify and address potential hurdles, ensuring a smoother path to approval. Testing protocols and guidelines are necessary to assess the safety and efficacy of MXenes in biomedical applications. Furthermore, greener synthesis methods and biodegradable MXene composites should be explored to reduce the environmental footprint of MXenes. Hence, MXenes must be safe for humans and the environment in order to be used for biomolecule isolation and other purposes.[170,347–349]

Balancing hydrophilicity and hydrophobicity. Achieving the right balance between hydrophilicity and hydrophobicity is crucial for effective biomolecule isolation, as different biomolecules have varying affinities to surfaces. Surface properties of MXenes can be tailored by functionalizing them with specific groups in order to tune their hydrophilicity or hydrophobicity. For instance, attaching hydrophilic polymers or hydrophobic alkyl chains can modify surface

characteristics to suit specific applications. Optimal biomolecule isolation can be achieved by fine-tuning the functionalization process (Figure 19).[350–354]

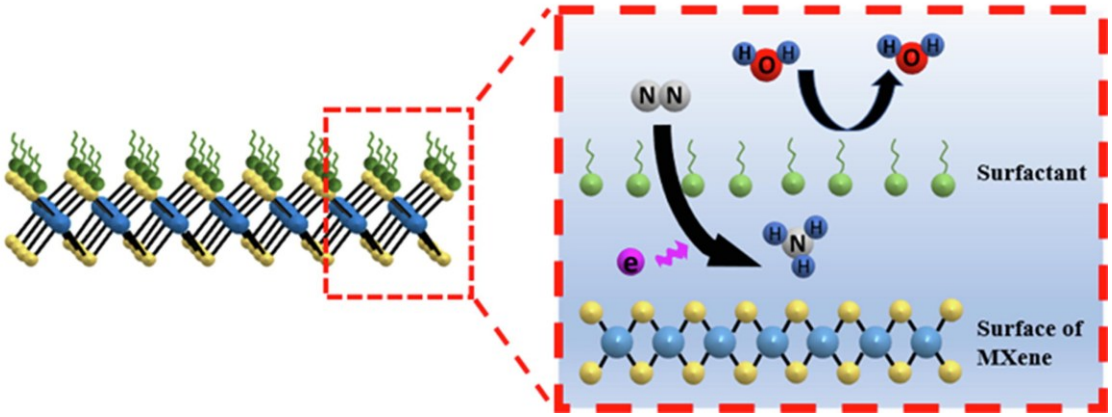


Figure 19. Schematic illustration of hydrophobic modification in a nitrogen-reduction-reaction process. Reproduced with permission.[354] 2023, Elsevier.

Limited research into biological interactions. While MXenes show significant potential, there is limited research focused on specific applications in biomolecule isolation. Understanding the interactions of MXenes with various biomolecules, such as proteins and nucleic acids is still relatively unclear. To address this, researchers are conducting detailed studies to uncover these interactions at the molecular level. Advanced characterization techniques, such as atomic force microscopy (AFM) and surface plasmon resonance (SPR), can be used to study binding interactions and dynamics between MXenes and biomolecules. Collaborating with biologists and medical researchers can also provide deeper insights into MXenes' biological interactions and potential applications.[26,27,300]

Surface roughness and texture. Surface roughness and texture of MXenes can significantly influence their interaction with biomolecules, affecting binding efficiency, selectivity, and overall performance in biomolecule isolation. Rough surfaces can increase the surface area

available for interactions, potentially enhancing biomolecule binding capacity. However, excessive roughness can create irregularities that prevent consistent biomolecule attachment and cause nonspecific binding.[135,355–357] MXene surface morphology can be precisely controlled by optimizing the synthesis and functionalization processes. By fine-tuning parameters such as etching time, temperature, and the choice of etching agents, it is possible to achieve the desired surface roughness and texture. Additionally, post-synthesis treatments such as annealing or chemical modification can be employed to further refine surface characteristics. Improved control over surface morphology can lead to more efficient and selective isolation processes, increasing the utility of MXenes in various biomedical and biotechnological fields.[135,358,359]

Storage and handling requirements. Maintaining MXene stability and functionality requires specific storage and handling conditions, which can be challenging in practice. The properties of MXenes can be highly sensitive to changes in temperature, pH, light, pressure, and chemical environment. To address this, MXenes nanostructures can be stored in protective storage environment, such as encapsulating MXenes in inert matrices or using vacuum-sealed packaging. Maintaining them in a suitable chemical media can prevent their properties from changing. Providing clear guidelines for MXene storage and handling can also help users maintain their quality and performance.[330,360]

Structural defects. MXenes with structural defects can affect their performance and consistency in biomolecule isolation. Researchers are working on optimizing the synthesis process to minimize defects and produce high-quality MXenes with uniform properties. Advanced characterization techniques, such as transmission electron microscopy (TEM) and X-ray diffraction (XRD), are used to identify and quantify defects. Post-synthesis treatments and

synthesis method improvement such can also reduce defects and improve the structural integrity of MXenes (Figure 20).[361–367]

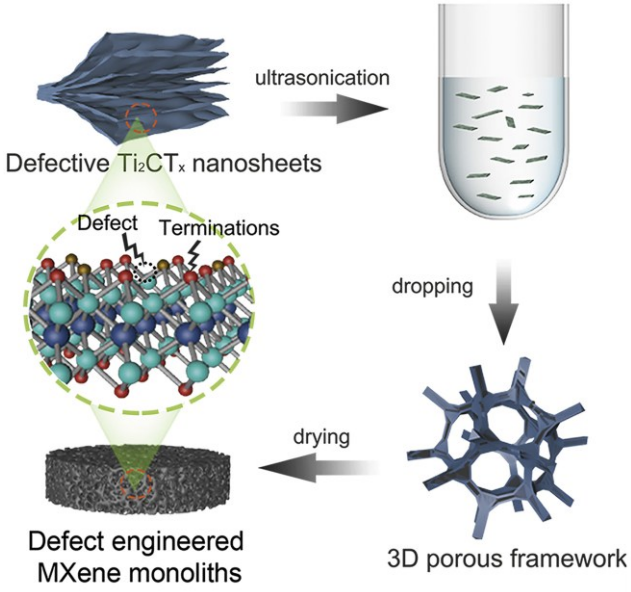


Figure 20. Synthesis of defective MXene monoliths. Reproduced with permission.[367] 2022, Cell Press Elsevier.

Surface charge control. Managing the surface charge of MXenes is challenging, but essential for selective biomolecule isolation, as it influences interactions with charged biomolecules. Biomolecules such as proteins, nucleic acids, and other macromolecules often possess inherent charges that affect their affinity for surfaces based on electrostatic interactions. Biomolecule isolation processes can be enhanced by correctly tuning the surface charge of MXenes.[175,368,369] Developing methods for precisely functionalizing MXenes with charged groups can be effective in controlling surface charge. According to the desired biomolecule interaction, these groups may be positively or negatively charged. For example, attached amino groups can provide a positive charge and carboxyl groups can generate a negative charge.[140,370] Additionally, adjusting the environment pH can also influence MXene surface charge. The pH can affect the ionization state of the functional groups on the MXene surface, altering its overall

charge.[175,371] Systematic studies to understand the relationship between surface charge and biomolecule binding are crucial for optimizing functionalization and isolation processes. Techniques such as zeta potential measurements and surface plasmon resonance (SPR) can provide insights into how surface charge influences biomolecule interactions (Figure 21).[242,368,372,373]

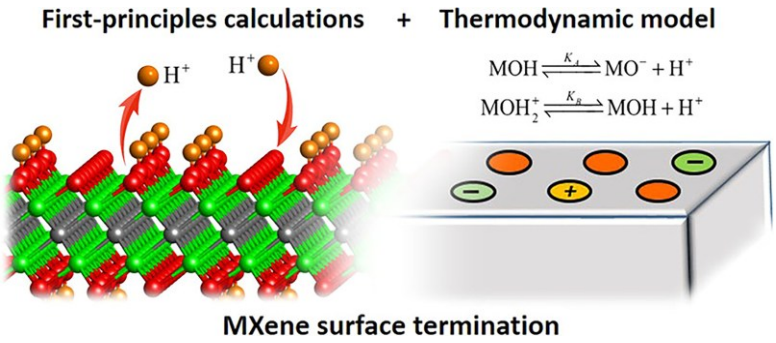


Figure 21. Theoretical insights into MXene termination and surface charge regulation. Reproduced with permission.[373] 2021, American Chemical Society.

Understanding long-term behavior. MXenes' long-term behavior, including interaction with biological systems over extended periods, is not well understood. Biological alterations pathways of MXenes are not completely understood, which can affect their long-term risks. Addressing this unpredictable behavior requires comprehensive long-term studies to evaluate MXene performance and stability in various environments. Predictive models and simulations can also be helpful to better understand and anticipate the long-term behavior of MXenes in biological media. These efforts can provide valuable insights into MXene durability and reliability for biomolecule isolation and other applications.[333,374,375]

Technical challenges in practical applications. Despite their promising properties, MXenes face several technical challenges when applied to practical biomolecule isolation applications. Integrating MXenes into existing biomolecule isolation technologies, such as microfluidic devices or biosensor platforms, presents technical challenges related to compatibility,

scalability, and system integration. MXenes need to be incorporated in a way that allows them to interact effectively with target biomolecules while being compatible with the fluid dynamics and other operational aspects of integrated system. Addressing these challenges requires interdisciplinary collaboration involving material scientists, chemists, biologists, and engineers to advance MXene-based technologies towards practical and impactful biomedical applications.[376–378] By addressing these additional weaknesses and developing targeted solutions, the potential of MXenes for biomolecule isolation and other biomedical applications can be further enhanced. Continued research and innovation will help overcome these challenges and unlock new opportunities for MXenes in various fields.

5. Future Perspectives and Research Opportunities

Recent advancements in biomolecule isolation using MXenes have significantly contributed to the understanding of general abilities of these nanomaterials for this application. [26,300,306] Future research should refine these techniques and explore these applications in a wider range to improve robustness and provide versatile solutions.

MXene-based biomolecule isolation is still in its infancy, presenting numerous research opportunities in the future. Looking ahead, there are several exciting possibilities for future research. For instance, new methods can be developed to improve the chemical and physical properties of MXenes. These directions will address current limitations and open up new applications in isolating several types of biomolecules. MXenes have the potential to become a key technology in biomolecule isolation, leading to innovations in diagnostics, therapeutics, and beyond. Navigating these future research directions will be accompanied by integrating related areas in interdisciplinary approaches.

5.1. Potential improvements and innovations for MXenes as biomolecule adsorbents

Novel MXenes production methods. MXene research advancements will enable the development of novel MXene materials tailored to specific biomolecule isolation applications. The novel etching method can be used to produce MXenes with unique surface chemistry, enhanced stability, and enhanced biocompatibility. The more efficient types of MXenes can be produced using molecular engineering and structural nanoarchitecture. These newly developed MXene types will allow precise control over MXene surface properties. Due to these advancements, MXenes can be customized for selective biomolecule adsorption and separation, contributing to their expansion into biomedical diagnostics and biotechnology (Figure 22a).[212,379]

Enhanced functionalization strategies. Future research in MXene functionalization aims to significantly enhance their specificity and affinity for biomolecules, thereby advancing their utility in biomolecule isolation applications. Advanced functionalization techniques will focus on precise control over surface chemistry, to tailor interactions with specific biomolecules. Strategies may include the development of novel ligands, polymers, or biomimetic coatings that optimize MXene surfaces for biomolecule recognition and binding. Additionally, improving the stability and durability of MXene functionalization in biological environments will be crucial for their practical application in biomolecule isolation (Figure 22b).[380–382]

Hybrid materials and composites. The development of MXene-based hybrid materials and composites is poised to drive innovation in biomolecule isolation technologies. Integrating MXenes with nanoparticles,[383] polymers,[384] or other 2D materials[385] can synergistically enhance their mechanical, chemical, and biological properties. These hybrid materials can be engineered to achieve improved biomolecule adsorption, selectivity, and stability under diverse environmental conditions. Future research will explore novel synthesis methods, such as in-situ

growth or layer-by-layer assembly, and advanced characterization techniques to optimize the design and performance of MXene-based hybrid materials. This approach holds promise for applications in precision medicine, environmental monitoring, and biotechnological processes (Figure 22c).

Development of MXene-based devices and platforms. MXene-based devices and platforms represent a significant advancement in biomolecule isolation and biomedical applications. Researchers will design and fabricate novel MXene-based sensors, membranes, and functional coatings tailored to biomolecule detection, separation, and delivery. MXene-based membranes, for example, exhibit high permeability, selectivity, and stability, making them suitable for biomolecules isolation. These advancements will drive the development of portable, point-of-care isolation devices and advanced therapeutic systems, revolutionizing the healthcare and biotechnological industries (Figure 22d).[306,386–388]

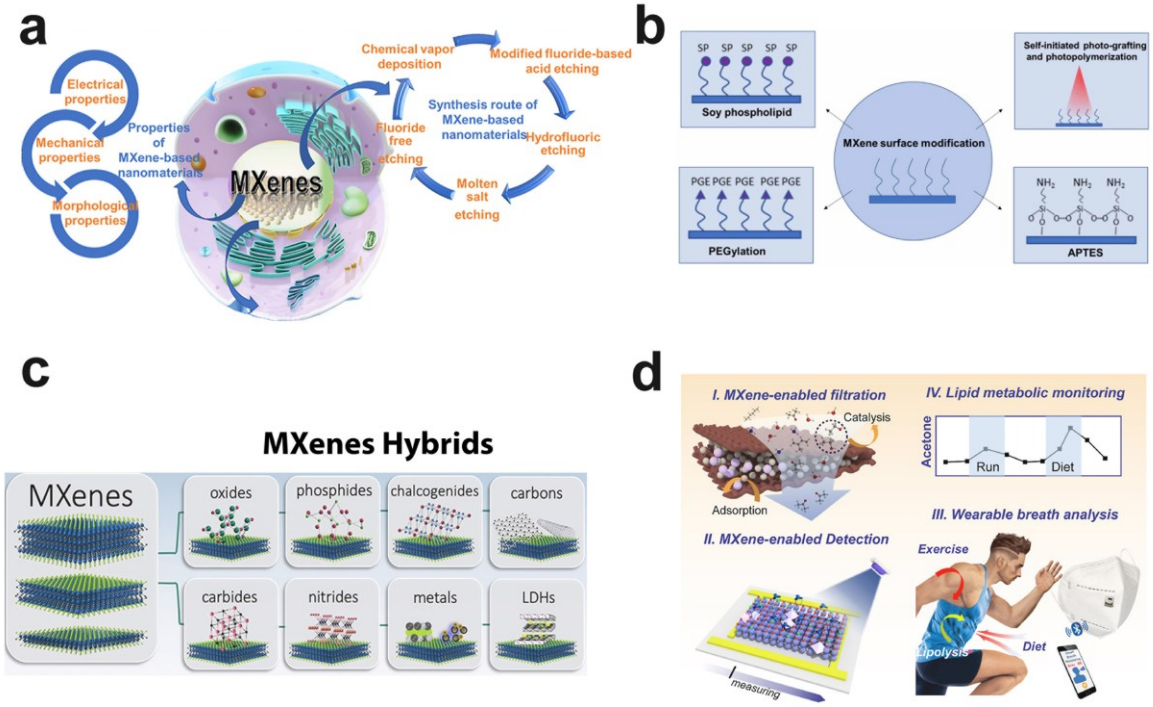


Figure 22. a) Novel production methods and properties of MXene-based nanomaterials. Reproduced with permission.[379] 2022, Elsevier. b) Surface modification and functionalization strategies of MXenes. Reproduced under the terms of the Creative Commons Attribution 4.0.[382] 2020, IOP Publishing. c) Various MXene-based hybrids. Reproduced with permission.[389] 2020, American Chemical Society. d) MXene-based wireless facemask enabled wearable breath acetone detection for lipid metabolic monitoring. Reproduced with permission.[390] 2023, Elsevier.

5.2. MXene-Based Biomolecule Separation for Healthcare Research

Single-cell analysis. Single-cell analysis involves studying individual cells' molecular characteristics. This technique is crucial for studying cellular heterogeneity, which plays a key role in many biological processes and diseases.[391–393] MXenes, with their exceptional properties, can be used to efficiently isolate and analyze biomolecules from individual cells, enhancing disease mechanisms understanding. For instance, in cancer research, single-cell analysis utilizing MXenes can help identify cancer stem cells and track tumor evolution, leading to better-targeted therapies.[394,395] In developmental biology, discovering how individual cells contribute to tissue and organ development can reveal insights into congenital disorders[396,397] In immunology,

analyzing immune cell populations at the single-cell level using MXene-based isolation techniques an improve immune responses and autoimmune diseases (Figure 23a).[398–400]

Personalized medicine and clinical diagnostics. Genetic and biomolecular information is often used in personalized medicine to adjust treatment to each patient's characteristics.[401,402] By isolating patient-specific biomarkers, MXenes can play a critical role in personalized medicine. Their ability to selectively capture and detect biomolecules can lead to more accurate diagnoses and tailored treatment plans, resulting in better health outcomes. Pharmacogenomics, the study of how genetic variations affect drug responses, can be greatly advanced through the use of MXene-enhanced isolation techniques, leading to optimized medication regimens.[403,404] Additionally, isolating patient-specific biomarkers with MXenes can help predict disease progression and treatment outcomes, further personalizing patient care (Figure 23b).[405,406]

Drug discovery. Drug discovery is the process of identifying new candidate medications. Isolation of biomolecules plays a crucial role in this process. MXenes can be used to isolate and identify potential therapeutic compounds with high efficiency, accelerating the development of new drugs. In drug discovery, MXenes can aid in target identification by selectively isolating specific proteins or nucleic acids, thanks to their customizable surface properties.[407–409] Understanding how drugs interact with isolated biomolecules can be elucidated using MXene-based platforms, improving drug design and efficacy (Figure 23c).[410–412]

Gene therapy and regenerative medicine. By isolating specific biomolecules, MXenes can aid in the development of novel therapies with unprecedented precision and efficiency. In gene therapy and gene editing, isolating nucleic acids using MXenes can help correct genetic defects, leading to new treatments for genetic disorders.[413] Immunotherapy can benefit from the isolation and analysis of immune-related biomolecules with MXenes, leading to the development of new

cancer treatments and therapies for autoimmune diseases.[414,415] In regenerative medicine, isolating growth factors and other molecules using MXenes can enhance tissue engineering approaches, promoting the regeneration of damaged tissues and organs (Figure 23d).[416–418]

Proteomics and genomics. MXenes can facilitate the isolation and analysis of proteins and nucleic acids with high efficiency and specificity, advancing research in proteomics and genomics. Analyzing proteins and genes with MXenes can provide deeper insights into the underlying mechanisms of diseases, leading to new treatment approaches. Discovering new proteins and genes involved in disease can be accelerated with the use of MXenes, leading to the development of novel therapeutic targets (Figure 23e).[39,419–422]

Agriculture. In agriculture, biomolecule isolation can be used to study plant health, disease resistance, and other factors important for crop production. MXenes can be used to isolate and analyze biomolecules with high sensitivity and specificity, leading to the development of more resilient crops.[423–425] Understanding plant-pathogen interactions at the molecular level can be improved with MXenes, enhancing pest and disease management strategies.[425–428] Identifying biomarkers associated with soil health and crop nutrition can be greatly advanced using MXene-based platforms, promoting sustainable farming practices and enhancing crop yields.[429,430] MXenes can contribute to significant advancements in agricultural research, leading to improved crop production and food security (Figure 23f).[425,431]

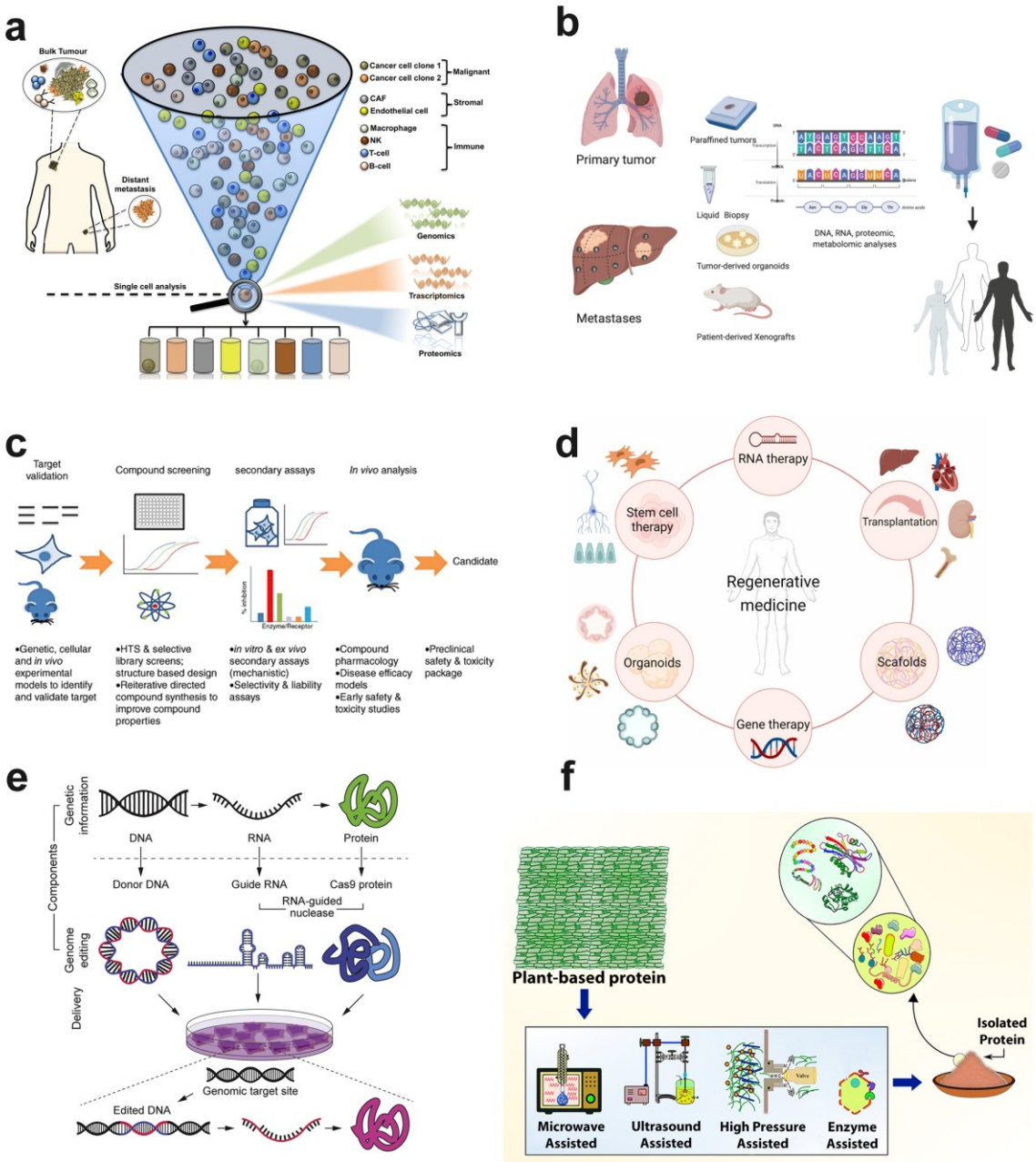


Figure 23. a) Single cell analysis to dissect molecular heterogeneity and disease evolution in metastatic melanoma. Reproduced under the terms of the Creative Commons Attribution 4.0.[400] 2019, Springer Nature. b) Personalized treatment: an integrated precision approach. How to personalize cancer treatment from the molecular evaluation of primary tumor or metastases by evaluating liquid biopsy, tumor-derived organoids, and tumor-derived xenografts. Reproduced under the terms of the Creative Commons Attribution 4.0.[406] 2020, Multidisciplinary Digital Publishing Institute. c) Overview of drug discovery screening assays. Reproduced with permission.[412] 2011, John Wiley & Sons. d) Major technologies used in regenerative medicine at present. Reproduced under the terms of the Creative Commons Attribution 4.0.[418] 2023, Elsevier. e) DNA, RNA, and protein tools for editing the genetic information in human cells. Reproduced with permission.[422] 2018, Cell Press Elsevier. f) Illustration of various techniques for plant protein extraction. Reproduced with permission.[432] 2021, Elsevier.

5.3. Potential future biomolecule isolation

Isolating hormones, antibodies, and small metabolites. Future innovations in MXene-based biomolecule isolation are expected to be on selective isolation of specific biomolecules such as hormones, antibodies, and small metabolites from complex biological samples. Tailoring MXene surface properties and functionalization strategies will enable precise and efficient adsorption and separation of these biomolecules. These advancements are critical for applications in clinical diagnostics, personalized medicine, and pharmaceutical development, where accurate and rapid biomolecule analysis is essential. Enhanced selectivity and sensitivity in isolating these biomolecules can lead to improved diagnostic tools, better therapeutic monitoring, and more effective drug development processes (Figure 24a).[433–436]

Isolating enzymes. MXene-based enzyme isolation represents a burgeoning research area with substantial potential. MXenes' high surface area, tunable surface chemistry, and biocompatibility make them ideal candidates for developing enzyme isolation platforms. Innovations in these areas will facilitate advancements in enzyme biocatalysis, molecular diagnostics, and genetic research, supporting applications in biopharmaceutical production, bioremediation, and biomedical research. By improving enzyme stability and activity through MXene-based immobilization, we can enhance biochemical processes' efficiency, leading to more sustainable and cost-effective industrial and medical applications (Figure 24b).[437–439]

Isolating lipids and peptides. Isolating lipids and peptides using MXene-based materials presents a promising frontier in bio-separation technology. MXenes can be engineered to interact selectively with various lipids and peptides, enabling their efficient separation from complex mixtures. This capability is crucial for studying Lipidomic and Peptidomic, which are essential for

understanding cellular functions, disease mechanisms, and developing novel therapeutics. Advances in MXene functionalization will allow for the targeted isolation of specific lipid and peptide classes, facilitating detailed molecular analyses and high-throughput screening processes. This can significantly impact areas such as metabolic research, drug discovery, and the development of lipid- and peptide-based biomarkers and therapeutics (Figure 24d).[440–442]

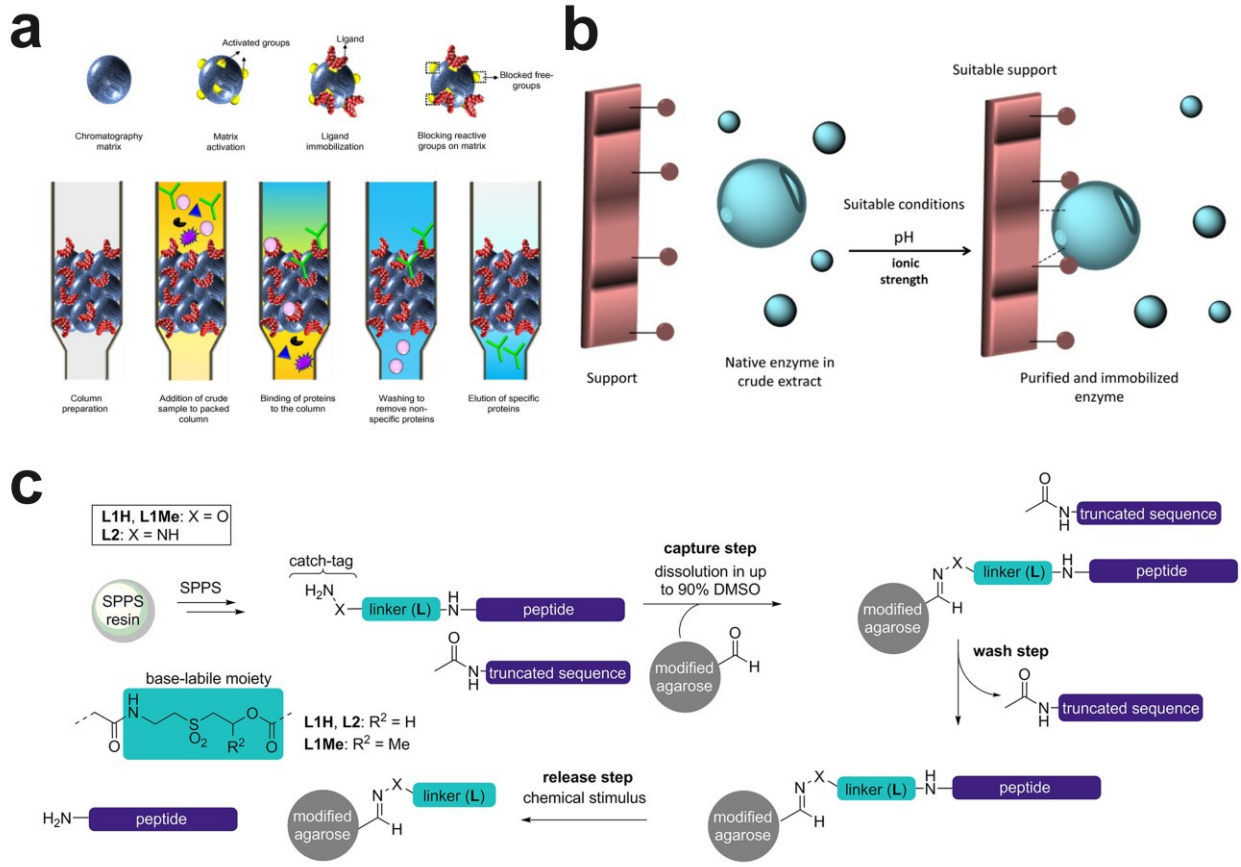


Figure 24. a) Steps involved in antibody purification using affinity chromatography. Reproduced with permission.[436] 2012, Elsevier. b) Immobilization and purification of enzymes by control of the support and/or immobilization conditions. Reproduced with permission.[439] 2015, John Wiley & Sons. c) The oxime/hydrazone-based catch and release purification of synthetic peptides by a base-labile linker, coined peptide easy clean (PEC). Reproduced with permission.[442] 2015, John Wiley & Sons.

5.4. Open research questions/problems

MXenes for biomolecule isolation have made considerable progress, but several critical questions remain.

- How can MXenes be integrated with advanced separation techniques, such as microfluidics or lab-on-a-chip systems, to enhance the efficiency and throughput of biomolecule isolation?[443]
- What strategies can be employed to create multifunctional MXenes that combine isolation, detection, and therapeutic delivery capabilities in a single platform?[444]
- How can self-healing coatings or stimuli-responsive polymers be integrated with MXenes to improve their reusability for repeated biomolecule isolation cycles?[445]
- What are the effects of specific transition metal compositions in MXenes (e.g., Ti, Nb, V) on their interaction profiles with biomolecules?[446]
- How can regulatory and safety challenges be addressed to facilitate the clinical translation of MXene-based biomolecule isolation process?[447]

By overcoming the current challenges and expanding the capabilities of MXene-based materials, we can unlock new opportunities for innovation and improve the quality of advanced medical grade materials in biomedical applications.

6. Conclusion

This review provided a comprehensive examination of MXenes' potential for biomolecule isolation. Their versatility and efficacy in biomedical applications stem from their unique medical grade properties.

Synthesis, characterization, and functionalization methods of MXenes were explored in depth, highlighting their capabilities in selectively adsorbing and isolating biomolecules such as proteins, nucleic acids, and small biomolecules. These capabilities are driven by tunable surface chemistry and interaction mechanisms. Advances in functionalization techniques have notably improved the specificity and efficiency of MXenes in targeting and isolating biomolecules, positioning them as superior alternatives to conventional materials.

MXenes offer several inherent advantages over traditional adsorbents, including excellent electrical conductivity, antibacterial properties, biocompatibility, biodegradability, and ability to undergo diverse functionalization enhancing the precision and efficiency of biomolecule isolation processes. Tailoring MXenes for specific interactions with target biomolecules has led to the development of highly sensitive and selective extraction techniques, pivotal for the accurate detection and analysis of biomolecules in complex biological samples. Such precision improves diagnostic assay reliability but also facilitates targeted therapeutics through isolated biomolecules.

The integration of MXenes into biomedical research and applications has the potential to accelerate progress by enhancing performance, reducing processing times, and increasing throughput. However, several challenges must be addressed to fully realize their potential. MXenes are susceptible to degradation in biological environments, making them challenging to isolate biomolecules. Even though MXenes are generally stable, certain biological fluids and enzyme environments can cause them to oxidize or hydrolyze. MXenes are synthesized by selective etching of MAX phases, and their widespread application in biomolecule isolation requires consistent and reproducible production. A variety of factors can affect the synthesis process, including precursor quality, etchant composition, and synthesis conditions. Surface chemistry, layer thickness, and morphology can vary due to these parameters. Biomolecule isolation can be

affected by these differences. Aggregation in aqueous solutions can reduce the effective surface area and interaction with target biomolecules, so effective dispersion methods are essential. The interactions of MXenes with various biomolecules should also be clearly understood. Furthermore, structural defects, surface charges, surface roughness, storing problems, and a lack of hydrophilicity/hydrophobicity balance can affect biomolecule isolation efficiency. In addition to the challenges listed above, addressing regulatory and safety concerns, and discovering MXenes' long-term behavior requires comprehensive studies.

The growing body of research and increasing interest from the scientific community underscore the importance of MXenes in advancing biomedical applications. To improve MXene's specificity and efficiency in isolation of biomolecules, future research should focus on optimizing functionalization strategies. MXenes can be used to isolate a wider range of biomolecules by exploring new surface modification techniques and functionalization chemistries.

Furthermore, interdisciplinary collaborations are essential for driving innovation and translating laboratory findings into practical applications. Collaboration among materials scientists, chemists, biomedical engineers, and clinicians is crucial for developing and implementing MXene-based technologies.

As part of future interdisciplinary collaborations, MXene's capabilities in biomolecule isolation should be maximized in several key areas, including biomedical labs-on-a-chip devices, personalized medicine, clinical trials, real-world applications, single-cell analysis, point-of-care diagnostics, personalized medicine, and drug discovery. Consequently, MXenes can continue to drive innovation and open new frontiers in biomolecule isolation, contributing significantly to biomedical research.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M.S. reports that financial support was provided by U.S. National Science Foundation; and H.V. declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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