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Protein separation and purification using inorganic oxide materials: from surface chemistry to biochemical applications

Shogo Kanoh^{a, b}, Kentaro Shiraki^b, Katsuya Kato^c and Atsushi Hirano^{a, b*}

^a*Research Institute of Core Technology for Materials Innovation, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8565, Japan*

^b*Institute of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan*

^c*Chubu Center, National Institute of Advanced Industrial Science and Technology (AIST), Nagoya, Aichi 463-8560, Japan*

Corresponding Author

Atsushi Hirano - Research Institute of Core Technology for Materials Innovation, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8565, Japan; Institute of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan; Email: hirano-a@aist.go.jp

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Abstract

Inorganic oxide materials are widely used for protein and peptide separation because their surfaces support multiple types of interactions, including metal-centered coordination, electrostatic interactions, and both hydrophilic and hydrophobic interactions. This review summarizes the binding mechanisms between proteins/peptides and representative inorganic oxides— ZrO_2 , TiO_2 , SiO_2 , Fe_3O_4 , Al_2O_3 , ZnO , CeO_2 , SnO_2 , Nb_2O_5 , Ta_2O_5 , and Ga_2O_3 . Particular emphasis is placed on the comparative analysis of adsorption mechanisms across these inorganic oxides and on factors such as crystal structure, surface acidity, and surface functionalization that influence adsorption selectivity. The review further discusses the effects of surface chemistry on purification performance and their combined effects on multi-step purification workflows. Recent advances in surface engineering and hybrid material design are highlighted to provide perspectives for the development of selective and mild protein purification processes. This framework offers guidance for the rational selection of inorganic oxide materials, enabling reproducible, selective, and mild purification of diverse proteins and peptides in biopharmaceutical applications.

Introduction

Proteins and peptides are central to medicine, biotechnology, and fundamental research [1–3]. They serve as therapeutic agents—such as monoclonal antibodies and peptide drugs—as well as industrial enzymes, diagnostic reagents, and model molecules used in molecular and cellular biological research [3,4]. However, their production processes typically generate complex mixtures containing host cells and cellular debris, host cell proteins (HCPs) and DNA, protein aggregates or cleavage products, and residual medium components supporting cell growth [4]. Efficient separation and purification are thus essential to ensure the safety, reproducibility, and cost-effectiveness of biopharmaceuticals [2,5]. High-yield and robust purification strategies are indispensable for both industrial bioprocessing and laboratory workflows that require well-defined protein preparations [1,5]. This demand drives the development of advanced materials and platforms for separation and purification.

Organic polymer-based media, such as agarose, are widely used in conventional protein purification [1,6]. Common formats include ion-exchange, reversed-phase, size-exclusion, and affinity chromatography, which exploit electrostatic, hydrophobic, and specific ligand–protein interactions [1,7]. They are widely used at both laboratory and industrial scales, but organic polymer-based media have several limitations. For example, their chemical and mechanical stability may be insufficient under harsh conditions, reducing reusability and long-term performance [8–10]. Additionally, gel swelling in solution can affect column packing, flow rate, and pressure [11]. Thus, alternative platforms with greater chemical robustness and operational durability, are needed for reliable and reproducible protein purification.

Inorganic oxides are promising candidates for versatile and robust alternatives. Representative materials such as ZrO_2 , TiO_2 , SiO_2 , and Fe_3O_4 exhibit high chemical and mechanical stability and can withstand oxidative and other harsh conditions that would damage conventional organic polymer-based medium [12–14]. Most of these inorganic oxides possess surface hydroxy groups and acid–base active sites, enabling multiple interaction modes with adsorbates [15]. TiO_2 and ZrO_2 offer selective interactions with proteins and peptides through high-density metal centers [16]. SiO_2 provides complementary selectivity for proteins and peptides through hydroxy-mediated electrostatic interactions and hydrophobic interactions arising from surface siloxane regions [17]. Magnetic oxides, such as Fe_3O_4 , enable rapid and straightforward recovery of adsorbed proteins and peptides, facilitating high-throughput purification workflows

[18]. Collectively, these characteristics of inorganic oxides offer versatile and robust platforms for protein and peptide purification across diverse biological and industrial applications.

This review provides a comprehensive overview of inorganic oxide materials for protein and peptide separation and purification, with particular emphasis on structure–function relationships, material-specific adsorption mechanisms, and selective enrichment strategies. While previous studies have primarily focused on individual materials or separation applications, comprehensive comparisons of adsorption mechanisms, surface chemistry, and separation performance across different inorganic oxides remain limited. This review provides a comparative perspective on inorganic oxide materials for protein and peptide separation and purification, including a comparison of key factors governing adsorption behavior, such as crystal structure, surface acidity, and surface functionalization. Particular emphasis is placed on elucidating how these factors govern protein and peptide adsorption selectivity. The discussion includes the advantages, limitations, and application scopes of representative inorganic oxides for phosphorylated peptide enrichment, tagged protein purification, antibody purification, and other biomolecule purification processes. By integrating mechanistic insights with recent advances in surface engineering and hybrid material design, this review provides guidance for rational material selection and offers perspectives for purification workflow development in future protein and peptide purification applications.

General principles of protein and peptide adsorption on inorganic oxides

Inorganic oxides provide chemically diverse interfaces that enable protein and peptide adsorption through multiple interaction modes. Their surface metal centers act as Lewis-acidic sites with varying affinities for oxygen-donor ligands, while surface hydroxy groups impart amphoteric character through protonation and deprotonation equilibria. These properties generate surface charge distributions that depend on solution pH and ionic strength, thereby modulating adsorption strength and reversibility. Surface modification and particle morphology further regulate the accessibility and density of adsorption sites, defining the overall adsorption landscape in oxide-based separation systems. For comparison across materials, Table 1 summarizes the representative sample formats, surface properties, functional groups, and target biomolecules of major inorganic oxides.

Lewis acidity is a key factor governing the adsorption behavior of solutes on inorganic oxide surfaces. Surface metal centers such as Ti^{4+} and Zr^{4+} act as Lewis-acidic electron-pair acceptors that coordinate oxygen-donor ligands, which function as Lewis bases. TiO_2 and ZrO_2 are representative inorganic oxides widely used for coordination-mediated adsorption and separation because of their strong Lewis-acidic surface sites [19–21]. Similar coordination behavior has also been reported for other inorganic oxides containing Lewis-acidic metal centers, including Fe_3O_4 , Al_2O_3 , CeO_2 , ZnO , Nb_2O_5 , Ta_2O_5 , SnO_2 , and Ga_2O_3 [22–32]. In contrast, SiO_2 generally exhibits weak or negligible Lewis acidity compared with many transition-metal oxides [33]. Among Lewis-basic ligands, phosphate groups readily interact with coordinatively unsaturated metal centers on oxide surfaces through metal–oxygen coordination. These Lewis acid–base interactions underpin phosphate-related adsorption on inorganic oxide surfaces and contribute to the selective enrichment of phosphorylated biomolecules.

Surface charge on inorganic oxide surfaces is commonly governed by protonation and deprotonation equilibria of surface hydroxyl groups and is commonly characterized by the point of zero charge (PZC). SiO_2 generally exhibits low PZC values because of the acidic nature of surface silanol groups, whereas Al_2O_3 , ZnO , and CeO_2 are often reported to exhibit relatively high PZC values [34,35]. TiO_2 and ZrO_2 typically exhibit intermediate PZC values [34]. Fe_3O_4 also exhibits amphoteric surface behavior [36]. Consequently, inorganic oxide surfaces can exhibit positive or negative surface charge depending on the solution pH relative to the PZC. These pH-dependent surface charge properties contribute to electrostatic interactions with charged biomolecules during adsorption and desorption processes. Notably, PZC values can vary with crystal phase, synthesis route, surface treatment, and measurement conditions; therefore, careful interpretation is required when comparing different materials or studies [34].

Surface hydroxy groups constitute key interfacial functional groups on inorganic oxide surfaces and act as hydrogen-bond donors and acceptors involved in the formation of interfacial hydration structures at oxide–water interfaces [37]. Interfacial water molecules interact with these hydroxyl groups through hydrogen bonding, and the resulting water organization depends on hydroxyl group density, surface termination, and crystal structure [37–40]. TiO_2 and ZrO_2 surfaces undergo water-induced hydroxylation under aqueous conditions, where coordinatively unsaturated metal centers with hydroxy groups interact with interfacial water molecules through hydrogen bonding [41,42]. SiO_2 surfaces present siloxane networks bearing silanol groups, which

govern the heterogeneity of hydrogen-bonding sites available for interfacial water [37]. Differences in hydroxy group distribution and surface termination lead to variations in interfacial water structure at oxide–water interfaces, which in turn modulate the hydration-dependent adsorption of biomolecules, including proteins and peptides.

Surface functionalization introduces additional interaction modes beyond intrinsic oxide surface chemistry. Metal-ion immobilization, organic ligand grafting, phosphate group modification, polymer coatings, and biomolecular conjugation enable electrostatic, coordination-mediated, hydrophobic, and affinity-based adsorption mechanisms [43–48]. SiO_2 and Fe_3O_4 are widely utilized as functionalization platforms because of their chemical stability, synthetic versatility, and compatibility with diverse surface modification strategies [44–50]. ZrO_2 is employed as a platform for phosphate-functionalized surface modification because of the strong affinity between zirconium oxide surface sites and phosphate moieties [43,51]. Such surface engineering approaches expand the applicability of inorganic oxides to selective purification systems for proteins and peptides.

Crystal structure and morphology of inorganic oxides also influence adsorption behavior through their effects on surface-site density, pore accessibility, and interfacial structure, even among chemically identical materials. For example, anatase- and rutile-phase TiO_2 exhibit different phosphate adsorption characteristics [52]. Particle size (e.g., nanoparticles) and porosity (e.g., mesoporous structures) influence protein adsorption kinetics through differences in accessible surface area and transport properties [53–55]. In Fe_3O_4 -based systems, the inverse spinel structure provides magnetic responsiveness, enabling rapid separation as an operational advantage [56].

Material-specific applications in protein and peptide separation

ZrO_2

Surface chemistry and fundamental binding mechanisms of ZrO_2

ZrO_2 surfaces are amphoteric, possessing both acidic and basic sites [19,57,58]. Surface zirconium centers function as Lewis acids that coordinate oxygen donors, whereas surface hydroxyl groups act as Brønsted acid and base sites [19]. The nature and distribution of these surface sites depend strongly on the crystal phase [19]. Among ZrO_2 polymorphs, the monoclinic form is thermodynamically stable under ambient conditions and exhibits a relatively high surface density of hydroxy groups [59,60]. Because of these characteristics, monoclinic ZrO_2 is widely used for biomolecule

purification and adsorption studies in aqueous media.

The ZrO_2 surface exhibits versatile interactions due to the coexistence of Lewis- and Brønsted-acidic and basic sites [19,61]. In particular, coordinatively unsaturated zirconium centers ($\equiv\text{Zr}^{4+}$) possess high charge density and strong oxophilicity [51]. A well-characterized example is the interaction with phosphate species: Zr^{4+} centers preferentially coordinate with phosphate oxygen atoms, which act as Lewis bases, thereby forming stable Zr-O-P coordination bonds [51]. This coordination underpins the selective adsorption of phosphorylated peptides [62]. In addition, phosphate groups can be chemically immobilized on the ZrO_2 surface, introducing additional negatively charged sites [63]. These phosphate functionalities interact electrostatically with basic amino acid residues such as lysine, arginine, and histidine [64]. Such interactions underpin the selective adsorption of proteins containing localized basic regions, thereby extending the applicability of ZrO_2 materials beyond phosphorylated peptide enrichment.

Phosphorylated peptide enrichment using ZrO_2

Various ZrO_2 materials, including nanoparticles, mesoporous beads, and microtips, have been developed for phosphorylated peptide enrichment [62,65–67]. ZrO_2 is widely employed as a separation medium in phosphoproteomic analysis because of its affinity for phosphate groups [62,65,67]. In a typical workflow, phosphorylated peptide enrichment using ZrO_2 is performed after proteolytic digestion [67], where peptide mixtures are loaded onto ZrO_2 particles under acidic conditions. These conditions suppress non-specific interactions of cationic peptides with the particle surfaces while simultaneously promoting selective coordination of phosphorylated moieties to surface Zr^{4+} centers [67]. Non-phosphorylated peptides are removed during washing steps, whereas bound phosphorylated peptides are eluted using phosphate-containing buffers, alkaline solutions, or other competitive ligands that disrupt Zr-O-P interactions [67]. In addition, the use of surface modifiers, such as aliphatic hydroxy acids, as well as phosphonate-functionalized ZrO_2 , effectively reduces non-specific adsorption and improves enrichment efficiency, particularly for multiply phosphorylated peptides [68,69]. These procedures enable reproducible and efficient isolation of phosphorylated peptides from complex peptide mixtures. Because of its high chemical stability, ZrO_2 can be regenerated and reused even after harsh washing processes [66]. Recent studies have developed hybrid ZrO_2 materials for efficient phosphorylated peptide enrichment

from limited sample amounts [70]. Collectively, these properties—high surface area, tunable selectivity, and chemical robustness—make ZrO_2 suitable for reproducible and efficient phosphorylated peptide and protein enrichment.

Phosphate-modified zirconia for protein purification

Phosphate-modified zirconia ($\text{ZrO}_2\text{-P}$) materials have been developed as separation media for protein purification. $\text{ZrO}_2\text{-P}$ refers to zirconia surfaces modified with phosphate- or phosphonate-containing compounds, which are immobilized through Lewis acid–base interactions between phosphate groups and surface zirconium sites [43,63]. Negatively charged phosphate groups immobilized on ZrO_2 surfaces facilitate protein adsorption through electrostatic interactions with basic amino acid residues [64]. The bound proteins can be eluted by increasing the ionic strength or by mild pH adjustment, allowing purification under relatively gentle conditions [43,63,71–73].

Since proteins containing histidine (His) residues can interact with phosphate groups through electrostatic interactions [64], histidine-rich proteins can be effectively adsorbed onto $\text{ZrO}_2\text{-P}$ surfaces. In particular, proteins containing polyhistidine tags (His-tag) exhibit enhanced affinity toward $\text{ZrO}_2\text{-P}$ due to multivalent interaction with surface phosphate groups [71]. Such interactions enable the selective capture of recombinant proteins as an alternative to conventional immobilized metal affinity chromatography (IMAC) using heavy metal ions [74]. In comparison, conventional cation-exchange resins exhibit minimal binding of His-tagged proteins under the same conditions, whereas $\text{ZrO}_2\text{-P}$ shows strong and controllable adsorption and desorption of these proteins (Fig. 1) [71]. Immunoglobulins, which contain localized basic amino-acid-rich regions (e.g., the CL–CH1–hinge region in immunoglobulin G), can also electrostatically interact with surface phosphate groups [64]. These interactions facilitate the adsorption of immunoglobulins onto $\text{ZrO}_2\text{-P}$ particles [64]. Based on these adsorption properties, $\text{ZrO}_2\text{-P}$ is employed for the purification not only of immunoglobulin G (IgG), which is widely used in therapeutic antibody products [43,73,75,76], but also of other immunoglobulin classes, such as IgM and IgA, which are being explored for therapeutic and prophylactic applications [63,72,73,77]. In general, affinity protein-based columns, such as Protein A column and Protein L column, are widely used for antibody purification, but these ligands are sensitive to harsh conditions [78,79]. Repeated purification processes can reduce ligand activity [78,79]. Phosphate-functionalized ZrO_2 particles have the potential to serve as an

alternative column filler for an initial enrichment step, allowing antibodies to be captured gently. This pre-purification reduces contaminants and improves the quality of subsequent affinity protein-based purification in terms of antibody purity, yield and activity.

TiO₂

Surface chemistry and fundamental binding mechanisms of TiO₂

TiO₂ surfaces contain both acidic and basic surface sites—under-coordinated Ti⁴⁺ cations act as Lewis-acidic centers, while surface oxygen atoms and hydroxy groups exhibit weak basic character [20,21]. The density and distribution of these acid–base sites can be influenced by factors such as crystal structure and surface hydration [80]. TiO₂ mainly occurs in anatase and rutile crystal phases, both exposing Ti–O surface terminations but differing in lattice structure and surface hydroxylation behavior [81,82]. Surface hydroxy groups undergo protonation and deprotonation depending on pH, thereby modulating the surface charge [80]; the resulting electrostatic interactions govern protein adsorption behavior and further depend on solution conditions such as ionic strength [83].

TiO₂ is available in multiple formats, such as anatase nanoparticles, mesoporous beads, and microtips [62,84–86]. These formats differ in surface area and accessibility, thereby affecting the capture efficiency of proteins and peptides [87]. Anatase surfaces tend to form abundant hydroxy groups, which can directly coordinate with oxygen-containing ligands such as carboxylate groups [88] and phosphate groups [89]. In particular, Ti–O–P linkages underlie the selective adsorption of phosphorylated peptides and proteins and are widely employed in TiO₂-based metal oxide affinity chromatography (MOAC) for phosphorylated peptide enrichment [84]. Anatase surfaces exhibit strong affinity for water molecules and form structured interfacial hydration layers under aqueous conditions [42,90]. These hydration layers can modulate the approach and adsorption of amino acids to the surface [91,92]. Variations in solution conditions alter the stability and organization of these hydration layers, which can be exploited to modulate protein–surface interactions [93].

Enrichment of phosphorylated peptide using TiO₂

TiO₂-based phosphorylated peptide enrichment is widely used in phosphoproteomic workflows [84,85,94,95]. This material is chemically stable and maintains consistent performance across repeated experiments, ensuring reliable recovery of phosphorylated peptides from complex biological samples [96]. In addition, enrichment can be performed in a single tube, providing a simple and convenient workflow suitable for high-throughput applications (Fig. 2) [97,98]. The enrichment process is typically performed after proteolytic digestion [94], where phosphorylated peptides are loaded under acidic conditions to suppress non-specific binding, thereby promoting specific phosphate-TiO₂ interactions [16]. For example, organic acids such as 2,5-dihydroxybenzoic acid (DHB) are used to acidify the solution, thereby enhancing phosphate-specific binding to TiO₂ [16,99]. Bound phosphorylated peptides are eluted from TiO₂ using phosphate-containing buffers or alkaline solutions (e.g., NH₄OH at pH 10.5) without degrading phosphorylation modifications [94]. Additives such as glycerol, secondary amines, and bis-Tris propane improve phosphorylated peptide recovery, particularly for hydrophilic, acidic, and longer peptides [100]. Specifically, glycerol enhances selectivity for singly phosphorylated peptides, whereas secondary amines and bis-Tris propane broaden the applicability of TiO₂ to peptides with diverse lengths and compositions [100]. In addition, amine functionalization of TiO₂ surfaces can tune surface Lewis acidity and hydrophilicity, thereby enhancing selectivity for phosphorylated peptides [85]. Other surface modifiers, such as aliphatic hydroxy acids and phosphonates, can reduce non-specific adsorption and improve enrichment efficiency, particularly for multiply phosphorylated peptides [68,69]. Such optimization of solution conditions enhances overall recovery efficiency and supports robust downstream mass spectrometric analysis.

Protein purification via interfacial water on TiO₂

Interfacial water at anatase surfaces is strongly adsorbed due to abundant surface hydroxy groups [42,90]. Water molecules in the vicinity of the TiO₂ surface form structured hydration layers that mediate hydrogen bonding and solvent organization, thereby modulating solute-surface interactions [91,92]. This interfacial phenomenon can be exploited for the selective separation of proteins, even those with similar sizes and net charges [93]. The introduction of ionic liquids on TiO₂ creates a heterogeneous local environment composed of cations and anions [93]. This environment alters the structure of interfacial water and, in turn, modulates protein-surface interaction [93]. As

a result, the hydration environment on the TiO₂ surface in the presence of ionic liquids differs from that in their absence, leading to distinct adsorption and retention behaviors. This approach enables selective separation while preserving the intrinsic surface chemistry and stability of TiO₂.

SiO₂

Surface chemistry and fundamental binding mechanisms of SiO₂

Amorphous SiO₂ presents a hydroxylated surface composed of silanol groups ($\equiv\text{Si-OH}$) and siloxane bridges ($\equiv\text{Si-O-Si}\equiv$) [101]. Since surface silanol groups exhibit amphoteric behavior depending on their protonation state [102], they can participate in hydrogen bonding and electrostatic interactions with proteins and peptides [103]. In particular, deprotonation of silanol groups generates negatively charged $\equiv\text{Si-O}^-$ sites under neutral to alkaline aqueous conditions, enabling electrostatic interactions [104]. In contrast, siloxane bridges ($\equiv\text{Si-O-Si}\equiv$) are generally chemically inert under typical conditions [105].

Amorphous SiO₂ is widely used in diverse separation systems due to its high specific surface area and high silanol density [106,107]. Porous structures enhance accessibility to internal surface area, facilitating efficient interactions with biomolecules [106]. Owing to these characteristics, SiO₂ is widely used as stationary phase for protein and peptide separation. The negatively charged silica surface interacts electrostatically with positively charged amino acid residues, such as lysine [108]. Therefore, proteins with high isoelectric points exhibit strong adsorption under suitable pH conditions, whereas proteins with lower isoelectric points remain weakly bound [109]. Although protein adsorption on SiO₂ surfaces is predominantly governed by electrostatic interactions, other interactions, such as hydrophobic interactions, also contribute [17,110]. pH and ionic strength modulate these electrostatic interactions, thereby controlling adsorption and desorption behavior and improving adsorption selectivity for protein purification.

SiO₂ materials also exist in crystalline polymorphic forms. α -quartz is a representative crystalline form of SiO₂ that shares the Si–O tetrahedral framework of amorphous SiO₂ but differs in surface silanol density and spatial arrangement, thereby modulating biomolecular interactions. Compared with amorphous SiO₂, α -quartz exhibits a lower silanol density and a more ordered arrangement of surface oxygen atoms. These characteristics can be exploited in tag-based purification systems (e.g., Si-tag and CotB1-tag), where selective adsorption of engineered peptide sequences is

achieved through controlled silica–protein interfacial interactions [111–114].

Surface silanol groups serve as reactive sites for chemical functionalization, such as covalent coupling [110,115]. Organosilane reagents, which react with $\equiv\text{Si-OH}$ groups, form stable Si-O-Si-R linkages, thereby introducing functional groups onto the SiO_2 surface. The functional moieties include metal-chelating ligands and organic small molecules [44,116]. Such functionalization enables diverse interaction modes, including electrostatic attraction, coordination bonding, and hydrophobic interaction [44–46]. Additional strategies, such as polymer coating and ligand immobilization, can further enhance protein and peptide selectivity by providing denser or more specific binding sites [47,48]. Collectively, these approaches establish SiO_2 as a versatile platform for constructing tailored interfaces in protein and peptide separation systems.

Purification of proteins using bare SiO_2

Bare SiO_2 particles enable protein purification through electrostatic and hydrophobic interactions [17,109]. Industrial-scale studies using preparative SiO_2 gels have demonstrated the effectiveness of SiO_2 columns for the purification of recombinant proteins [17]. Notably, single-step purification on SiO_2 can replace conventional multi-step processes, including hydrophobic interaction chromatography (HIC), ultrafiltration/diafiltration (UF/DF), and cation-exchange (CEX) chromatography [17]. Bare SiO_2 particles also exhibit pH-dependent competitive adsorption of proteins with different isoelectric points, enabling the fractionation of proteins such as lysozyme, myoglobin, and cytochrome c [109]. This approach provides a cost-effective and high-performance purification platform, achieving high purity and recovery while reducing process complexity.

Bare SiO_2 has also been applied to the purification of fusion-tagged proteins because it interacts specifically with certain peptide tags. For example, the Si-tag, derived from bacterial ribosomal protein L2, binds tightly to SiO_2 surfaces. Si-tagged proteins bound to these surfaces can be eluted by adding divalent cations [111,112]. Notably, the interaction between the Si-tag and SiO_2 surfaces remains effective even in the presence of protein denaturants, allowing Si-tagged proteins to be purified using SiO_2 even if they are expressed as insoluble aggregates [111]. Cationic residue-rich tags (such as the CotB1p tag and (RH)4 tag) mediate selective adsorption through electrostatic interactions between their positively charged arginine residues and the negatively charged SiO_2 surface [113,117]. The Car9 tag, originally identified as a peptide that

bind to carbonaceous materials, also shows strong affinity for SiO₂ [118,119]. Car9-tagged proteins can be purified under mild conditions with moderate pH shifts and the addition of lysine [118,119]. Bare SiO₂ can also be used for the purification of His-tagged proteins, which are widely employed in recombinant protein production, with elution achieved by the addition of lysine and arginine [114]. These properties of bare SiO₂ thus provide a versatile platform for both tag-free and tag-mediated protein purification workflows.

Enrichment of phosphorylated peptides and purification of proteins using functionalized SiO₂

Surface modification expands the functionality of SiO₂, enabling selective protein purification based on tailored interfacial interactions. Chemical modification introduces defined binding motifs and converts SiO₂ into an affinity platform. Representative strategies include the immobilization of metal ions, the introduction of organic small molecules, coating with functional polymers, and the immobilization of proteins and biomolecular ligands.

Immobilization of metal ions on SiO₂ enables coordination-driven purification through metal–ligand interactions. Chelating ligands, such as iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA), covalently introduced onto SiO₂ surfaces can coordinate transition metal ions including Cu²⁺ and Co²⁺ [44,120]. The surface-bound metal centers further coordinate with electron-donor groups, particularly histidine residues, thereby enabling IMAC-based purification [44]. For example, Co²⁺-modified SiO₂ particles are used for the purification of His-tagged proteins under mild conditions [44]. Cu²⁺-modified SiO₂ capillaries exhibit selective retention of certain proteins via coordination bond to Cu²⁺ centers [120]. Metal oxide coatings further extend this coordination framework. Specifically, ZrO₂- and TiO₂-coated SiO₂ surfaces present Lewis-acidic metal sites that strongly interact with phosphate groups, allowing the selective enrichment of phosphorylated peptides [121]. These inorganic modification strategies are widely applicable to tag-based and post-translational modification-specific protein purification [44,120,121].

Organic small molecule functionalization offers defined interaction motifs for protein purification. Tetrazole- and morpholine-modified SiO₂ can function as cation- or anion-exchange media for protein purification [45,122]. Cholesterol- and octadecylsilyl (C18)-bonded SiO₂ materials exhibit hydrophobicity and hence are used in

reversed-phase chromatography for protein separation [46,123]. In particular, cholesterol-bonded SiO₂ has also been used for protein purification coupled with protein refolding because of its moderate hydrophobicity [123]. Dye molecules that mimic enzyme cofactors and substrates offer selective binding to corresponding enzymes. Cibacron Blue F3G-A immobilized on SiO₂ acts as a pseudo-affinity ligand [116]. Because this dye interacts with nucleotide-binding proteins, the functionalized SiO₂ can be used for affinity purification of enzymes such as dehydrogenases [116]. Other small molecules immobilized on SiO₂ surfaces also provide different selectivities. For example, glutathione-modified SiO₂ selectively enriches N-linked glycopeptides through hydrophilic interactions, thereby enabling the purification of GST-tagged proteins [124,125]. Boronic acid immobilized on SiO₂ forms reversible covalent interactions with cis-diol groups, which can be applied to the purification of glycoproteins [126].

Polymer-modified SiO₂ provides high ligand density and multivalent interactions, leading to high binding capacity and separation efficiency. Cationic polymers introduce high-density positive charges to the SiO₂ surfaces, functioning as anion-exchangers in protein purification [47]. In contrast, anionic polymers act as cation-exchangers [127]. Polymer modification can thus control interaction strength, selectivity, and binding capacity in protein purification.

Immobilization of specific proteins and peptides on SiO₂ offers highly specific affinity interactions. For example, magnetic porous SiO₂ (Mag(SiO₂)) microspheres can be functionalized with protein A and enable the selective capture of immunoglobulin G (Fig. 3) [128]. Protein A immobilized on mesoporous SiO₂ selectively binds to immunoglobulin G, allowing selective antibody purification [48]. Lectins such as concanavalin A immobilized on SiO₂ recognize glycan structures, enabling the enrichment of glycoproteins and glycopeptides [129]. Affinity peptides can also be introduced onto SiO₂ surfaces to purify target antibody through specific peptide–protein interactions [130]. These protein- and peptide-based ligand strategies offer particularly high specificity among the various SiO₂ modification strategies and are suitable for the purification of structurally defined proteins.

Fe₃O₄

Surface chemistry and fundamental binding mechanisms of Fe₃O₄

Fe₃O₄ surfaces have both acidic and basic functionalities [36]. Coordinatively

unsaturated Fe sites on the surfaces act as Lewis-acidic centers for coordinating oxygen-donor ligands [22,36]. Surface hydroxy groups, formed spontaneously in aqueous systems, contribute to the acid–base character through protonation and deprotonation depending on pH [36]. Fe₃O₄ surface acid–base site density also varies with crystal phase [36]. These structural and chemical properties render Fe₃O₄ suitable for selective adsorption of proteins and peptides.

Fe₃O₄ surfaces have coordination bonds of oxygen- and nitrogen-donor groups of biomolecules [131–134]. Typically, oxygen-donor groups, such as carboxylates and phosphates, coordinate to Fe centers [133,134]. Phosphate groups form particularly stable multidentate Fe–O–P linkages [133]. Nitrogen atoms of imidazole groups, as in histidine residues, can also coordinate to Fe sites [132]. Such Fe–ligand coordination, especially with phosphate and imidazole groups, together with electrostatic interactions, governs the selective adsorption of proteins and phosphorylated biomolecules.

γ-Fe₃O₄ nanoparticles exhibit ferrimagnetism because of their inverse spinel crystal structure [135,136]. In this structure, Fe²⁺ and Fe³⁺ ions occupy tetrahedral and octahedral sites with antiparallel spin alignment [135,136], producing a net magnetic moment and hence exhibiting characteristic magnetization [135,136]. Strong magnetization combined with nanoscale size allows the particles to respond quickly to external magnetic fields. These intrinsic magnetic features can be used for the magnetic separation and purification of proteins and peptides bound to the nanoparticles.

Enrichment of phosphorylated peptide and purification of protein using bare Fe₃O₄

Bare Fe₃O₄ nanoparticles exhibit selective interactions with phosphorylated peptides and His-tagged proteins [18,137,138]. The formation of Fe–O–P linkages enables preferential binding of phosphorylated peptides over non-phosphorylated counterparts [137]. This binding can be optimized by pH and buffer composition, thereby enhancing both purity and recovery [137]. Additionally, the magnetic properties of Fe₃O₄ allow rapid separation of the enriched fraction from complex mixtures [137]. Together, these features provide a simple, ligand-free approach for phosphorylated peptide enrichment prior to mass spectrometric analysis.

Bare Fe₃O₄ nanoparticles can also capture His-tagged recombinant proteins directly from crude lysates [18]. The imidazole groups of histidine residues coordinate with metal ions, similar to immobilized metal ion affinity chromatography (IMAC) [139]. Proteins lacking His tags exhibit minimal binding, whereas His-tagged proteins are

selectively adsorbed onto Fe_3O_4 surfaces and subsequently eluted using imidazole-containing buffers [18]. In addition, peptide tags such as His-tag, CotB1p-tag, and Car9-tag, which have affinity for both Fe_3O_4 and SiO_2 , enable sequential workflows that involve magnetic capture followed by purification using silica surfaces, or vice versa [138].

Enrichment of phosphorylated peptides and purification of protein using modified and coated Fe_3O_4

Surface modification and coating enhance the specificity of Fe_3O_4 nanoparticles toward peptides and proteins. Various functional groups, including chelating ligands and boronic acids, provide selective binding sites for target proteins [49,50,140–143]. Inorganic coatings further confer affinity for proteins and peptides while preserving the magnetic core [50,144–146].

Ni^{2+} ions immobilized via polymer layers on Fe_3O_4 nanoparticles provide selective binding sites for His-tagged proteins and His-rich proteins [49,140,143]. A core-shell architecture of $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{Ni}$ microspheres was developed along with a workflow for magnetic capture and selective enrichment of His-rich proteins (Fig. 4) [143]. Boronic acid moieties introduced on the Fe_3O_4 surface capture glycopeptides and glycoproteins through cis-diol interactions [50,141]. Immobilization of protein A on Fe_3O_4 affords high-affinity binding to antibody Fc regions [142]. The resulting Fe_3O_4 nanoparticles retain magnetic separability, supporting efficient enrichment workflows.

Inorganic materials such as SiO_2 , TiO_2 , and ZrO_2 are used to form core-shell structures with Fe_3O_4 cores [50,144–146]. In particular, SiO_2 -coated magnetic nanoparticles can be functionalized with boronic acid ligands for glycopeptide and glycoprotein enrichment and with proteolytic enzymes such as trypsin for on-particle digestion [50,147]. TiO_2 and ZrO_2 coating layers on Fe_3O_4 exhibit affinity for phosphorylated species [144,145]. These materials enable the enrichment of phosphorylated peptides from complex peptide mixtures through magnetic separation.

More recently, aptamer-functionalized Fe_3O_4 nanoparticles have been developed for the selective capture of extracellular vesicles, extending magnetic enrichment strategies from protein- and peptide-level targets to nanoscale biological assemblies through sequence-specific molecular recognition [148]. These advances suggest that Fe_3O_4 -based platforms can serve as versatile tools for multiscale biological separations and integrated bioanalytical workflows.

Other materials

Al₂O₃

Al₂O₃ surfaces exhibit both acidic and basic sites [23]. Surface aluminum centers (Al³⁺) function as Lewis acids, and surface hydroxy groups can undergo reversible protonation and deprotonation [23,149]. The density and distribution of these sites depend on crystal structure and can be further controlled by surface hydroxylation and preparation conditions such as calcination temperature and pretreatment conditions [150]. For example, γ -Al₂O₃ exhibits a greater density of coordinatively unsaturated Al³⁺ sites compared with other common polymorphs such as α - and θ -Al₂O₃ [150–153]. These Al³⁺ sites can bind oxygen-donor groups, such as carboxylate and phosphate moieties, through coordination bonds [154,155]. Such binding is also influenced by pH-dependent protonation of surface hydroxy groups [154,155]. These physicochemical characteristics govern the adsorption behavior of peptides and proteins on Al₂O₃.

The combination of Lewis-acidic Al³⁺ sites and modifiable surface hydroxy groups enables Al₂O₃ to exhibit electrostatic interactions [154]. In fact, unmodified γ -Al₂O₃ has been used in high-performance liquid chromatography (HPLC) to separate proteins through these electrostatic interactions, where the retention depends on the difference between a protein's isoelectric point (pI) and the point of zero charge (PZC) of γ -Al₂O₃ [156]. Specifically, proteins carrying charges opposite to the surface are preferentially retained. Modulating pH and ionic strength allows fine-tuning of both retention and selectivity [156]. In addition, Lewis-acidic Al³⁺ sites serve as specific coordination sites for phosphate groups, enabling selective capture of phosphorylated peptides [157]. This mechanism has been exploited in Al₂O₃-coated magnetic core-shell microspheres, which allow rapid and highly specific enrichment of phosphorylated peptides [157]. Compared with commercial TiO₂-based materials, Al₂O₃-coated systems exhibit higher selectivity while minimizing non-specific adsorption [157].

Surface modification of Al₂O₃ is effective for protein separation based on differences in hydrophobicity. Octadecyl- and polybutadiene-bonded Al₂O₃ are used in reversed-phase HPLC of proteins and peptides while maintaining the structural advantages of the Al₂O₃ support [158,159].

ZnO

ZnO surfaces are amphoteric and contain Lewis acidic zinc sites (Zn^{2+}) [24,160]. The surface charge of ZnO can thus be controlled by pH, affecting biomolecule adsorption [160,161].

ZnO surfaces adsorb proteins, including bovine serum albumin (BSA), lysozyme, and cytochrome c [161–163], and adsorption behavior depends on pH [163]. Electrostatic interactions predominantly contribute to adsorption, while hydrogen bonding and van der Waals interactions may also be involved [164,165]. Although ZnO has primarily been studied for protein adsorption, it also holds potential as a material for protein and peptide purification with appropriate control of solution conditions.

CeO₂

CeO₂ exhibits amphoteric surface properties and contains Lewis-acidic cerium sites (Ce^{4+}) [25,26,166]. Phosphate and carboxylate groups adsorb onto CeO₂ surfaces; this adsorption is influenced by surface properties such as the density of hydroxy groups and the strength of Lewis acidity [167–170]. These properties of CeO₂ are applied to the enrichment and isolation of phosphorylated peptides and proteins [171,172]. In addition, CeO₂ microspheres exhibit peroxidase-like activity and have been proposed as nanozyme platforms for the colorimetric quantification of phosphoproteins in complex samples [172].

CeO₂ surfaces can also be used for adsorption of non-phosphorylated proteins. Some proteins, including bovine serum albumin (BSA) and lysozyme, are reported to adsorb onto CeO₂ nanoparticles [161,162,173]. The adsorption can be controlled by changing the solution pH [173,174]. Proteins may undergo local conformational changes upon binding to CeO₂ in some cases [161,162]. Although CeO₂ has not been systematically studied for protein purification, its pH-dependent adsorption may be leveraged for this purpose, provided that conditions are carefully controlled to minimize protein denaturation.

SnO₂, Nb₂O₅, Ta₂O₅, Ga₂O₃

SnO₂, Nb₂O₅, Ta₂O₅, and Ga₂O₃ contain Lewis-acidic metal centers (Sn^{4+} , Nb^{5+} , Ta^{5+} , Ga^{3+}) [27–32]. These centers coordinate with phosphoryl oxygen atoms. SnO₂ and Nb₂O₅ act as affinity materials for phosphorylated peptides and are employed in proteomic analysis [175–177]. Phosphorylated peptides retained on the oxide surfaces under acidic conditions are eluted by a drastic change in pH or by the introduction of

phosphate-containing buffers [175–177]. SnO_2 showed phosphorylated peptide enrichment performance comparable to that of TiO_2 and enabled selective adsorption of phosphorylated peptides under simple solvent conditions [176]. In some cases, SnO_2 also provided higher recovery of singly phosphorylated peptides than TiO_2 [177]. Nb_2O_5 exhibits selectivity toward phosphorylated peptides that differed from that of TiO_2 , suggesting its potential complementary use for phosphorylated peptide enrichment [175]. Similar phosphorylated peptide adsorption behavior is observed for Ta_2O_5 - and Ga_2O_3 -coated oxide materials on magnetic nanoparticles [178,179].

Comparative discussion and integration

Metal oxides exhibit differences in Lewis acidity, amphotericity, and hydroxy group density, which influence their interactions with proteins and peptides. TiO_2 and ZrO_2 possess high-valent metal centers (Ti^{4+} and Zr^{4+}) that form coordination bonds with oxygen-donor groups such as phosphate groups [51,89]. Fe_3O_4 contains moderate Lewis-acidic $\text{Fe}^{2+}/\text{Fe}^{3+}$ sites, exhibiting pH-dependent surface charge and magnetic separability [22,135,136]. SiO_2 exhibits negligible intrinsic Lewis acidity; instead, its surface silanol groups mediate electrostatic interactions. Across these inorganic oxides, crystal phase and hydroxylation affect site accessibility, governing the balance among coordination bonds, electrostatic interaction, and hydration-mediated interaction.

TiO_2 and ZrO_2 are among the most widely used materials for phosphorylated peptide enrichment, as they bind phosphate groups via surface Lewis acid sites [67,94]. TiO_2 exhibits particularly high selectivity for multiply phosphorylated peptides compared with singly phosphorylated peptides [62]. In contrast, ZrO_2 tends to preferentially capture singly phosphorylated peptides rather than multiply phosphorylated ones [62]. The complementary selectivities of TiO_2 and ZrO_2 can be exploited in combined workflows to broaden the range of phosphorylated peptides recovered [62,180]. Various materials, such as Fe_3O_4 , Al_2O_3 , SnO_2 , Nb_2O_5 , Ta_2O_5 , CeO_2 , and Ga_2O_3 have also been reported to enrich phosphorylated peptides [157,171,172,175–179].

Affinity purification of tagged proteins is essential for high-purity purification of recombinant proteins. Typically, His-tagged proteins can be selectively captured on inorganic and hybrid materials, offering distinct advantages. Phosphate-functionalized zirconia particles enable purification of His-tagged proteins under near-neutral pH by adjusting phosphate concentration, providing a mild and efficient workflow [71]. SiO_2

with surface silanol groups is also used for His-tagged protein purification. A remarkable advantage of these materials is that they do not contain toxic transition metal ions [114]. An additional advantage of SiO_2 is its applicability to other tags, including Si-tag, Car9-tag, CotB1p-tag, and GST-tag, demonstrating its versatility [111–113,117,118,124]. Magnetic iron oxide (Fe_3O_4) nanoparticles also capture His-tagged proteins [18]. Both silica and Fe_3O_4 surfaces can be further modified with chelating agents such as nitrilotriacetic acid (NTA) loaded with Co^{2+} , Cu^{2+} , or Ni^{2+} to perform immobilized metal affinity chromatography (IMAC) [44,49,120,140]. This modification provides tunable selectivity, reduces non-specific binding, and, particularly for Fe_3O_4 , combines the advantages of magnetic handling. Dual-affinity strategies using both Fe_3O_4 and SiO_2 surfaces have been developed for multi-step workflows [138]. These strategies enable sequential capture, separation, and elution of recombinant proteins [138]. Taken together, inorganic oxides provide complementary options for tagged protein purification under mild conditions. They also have the potential to adapt to other tag systems through tailored surface modifications.

Outlook and future perspectives

Inorganic oxides and their hybrid materials offer diverse strategies for protein and peptide purification. The surface chemistry of these materials modulates their interaction modes, influencing selectivity and efficiency. Optimizing workflows requires the careful choice and combination of these materials based on their complementary properties. For example, Fe_3O_4 and SiO_2 support affinity purification of tagged proteins, whereas TiO_2 and ZrO_2 enable selective phosphorylated peptides enrichment. Dual-affinity peptide tags, including Car9-derived sequences, further allow protein capture on both Fe_3O_4 and SiO_2 , demonstrating the feasibility of integrated workflows (Fig. 5) [138]. Li et al. demonstrated that $\text{Fe}_3\text{O}_4@\text{TiO}_2\text{-ZrO}_2$ core-shell microspheres combine the magnetic separation capability of Fe_3O_4 with the distinct phosphate affinities of TiO_2 and ZrO_2 to efficiently enrich phosphorylated peptides from complex mixtures [180]. These examples demonstrate that combining inorganic oxides enables more efficient and versatile purification than single-material approaches. For instance, ZrO_2 enables ligand-free antibody capture under mild conditions for initial sample cleanup [43], whereas Fe_3O_4 and SiO_2 functionalized with affinity ligands provide high selectivity for subsequent polishing steps [48,142]. This sequential use

exploits complementary properties, including ligand-free capture, magnetic separation, and surface modification versatility, to improve purification performance. Thus, the combination of inorganic oxides based on their complementary properties enables flexible workflows tailored to specific purification requirements, leading to shorter processing times, higher recovery, and greater reusability.

A understanding of protein–material and peptide–material interactions remain essential. For example, phosphorylated peptides enrichment has been widely examined using inorganic oxides, including ZrO_2 , TiO_2 , SiO_2 , Fe_3O_4 , Al_2O_3 , ZnO , CeO_2 , SnO_2 , Nb_2O_5 , Ta_2O_5 , and Ga_2O_3 , each showing distinct selectivity and binding behavior; however, a systematic framework that links surface chemistry, site accessibility, and solution conditions to enrichment performance is still lacking, as direct comparisons across materials remain limited. Establishing standardized comparative evaluation methods and design principles would deepen the understanding of the underlying fundamental interaction mechanisms, thereby contributing to the rational development of efficient, reproducible, and versatile purification workflows.

Novel purification strategies continue to emerge, including new surface engineering approaches. SiO_2 phases modified with *N*-methylimidazolium ionic liquids provide multi-interaction surfaces that enhance selectivity and resolution for acidic proteins under combined reversed-phase and ion-exchange conditions [181]. Macroporous SiO_2 functionalized with imidazole-octyl ligands achieves selective isolation of proteins such as ovomucoid and ovotransferrin through electrostatic and hydrophobic interactions [182]. In addition, thermoresponsive polymer interfaces have been developed for temperature-triggered control of protein purification. By reversibly altering surface hydrophilicity and electrostatic properties in response to temperature changes, these systems regulate protein–surface interactions and enable controllable adsorption and desorption under mild conditions [183]. Further exploration of functional polymers, chelating groups, and inorganic oxides for surface modification will be essential, as this will promote the development of versatile purification strategies that expand the range of target proteins while achieving high yield, high purity, and preserved activity.

Conclusion

Inorganic oxides provide versatile platforms for protein and peptide separation and purification due to their distinct surface chemistry and interaction modes. TiO_2 and

ZrO₂ efficiently enrich phosphorylated peptides through surface Lewis-acidic sites, with TiO₂ favoring multiply phosphorylated sequences and ZrO₂ favoring singly phosphorylated sequences. Phosphate-functionalized ZrO₂ can be used for antibody pre-enrichment prior to subsequent purification steps. Fe₃O₄, which enables rapid magnetic separation, can be used for IMAC-like purification. SiO₂, with both metal-free and metal-ion-mediated affinity, can be tailored with ligands for antibodies, His-tags, and other fusion proteins. Combining these materials in multi-step workflows exploits their complementary properties and enables high-purity recovery and efficient fractionation. Expanding the evaluation of emerging oxides, such as Al₂O₃, CeO₂, SnO₂, Ta₂O₅, Nb₂O₅, and Ga₂O₃ could further enhance selective enrichment strategies. These approaches provide flexible and effective solutions for phosphorylated peptide enrichment, tagged protein purification, and broader applications in proteomics and biopharmaceutical research.

Disclosure statement

The authors declare no conflicts of interest associated with this manuscript.

ORCID

Shogo Kanoh

<https://orcid.org/0009-0007-7454-7825>

Kentaro Shiraki

<https://orcid.org/0000-0003-3438-4076>

Katsuya Kato

<https://orcid.org/0000-0001-8981-8359>

Atsushi Hirano

<https://orcid.org/0000-0002-4138-0308>

Author contributions

Shogo Kanoh drafted the manuscript. Atsushi Hirano, Katsuya Kato, and Kentaro Shiraki contributed to manuscript revision. All authors approved the final manuscript.

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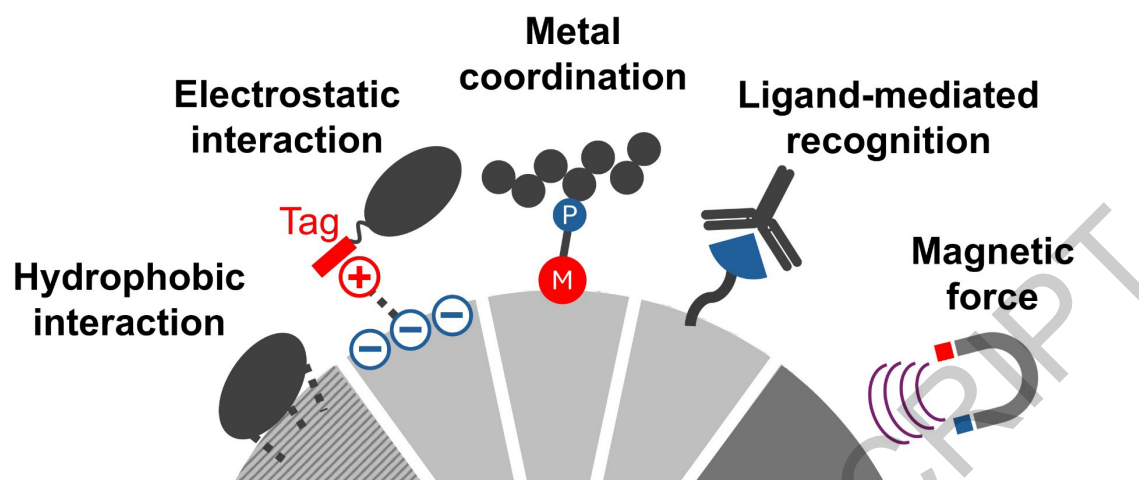
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Figures



Graphical Abstract: Inorganic oxide materials interact with diverse biomolecular targets, including phosphorylated peptides, antibodies, tagged proteins, and other proteins, through multiple surface mechanisms such as metal coordination, electrostatic interactions, hydrophobic effects, and ligand-mediated recognition. These interaction modes can coexist on a single surface and collectively control adsorption behavior. In the schematic, M represents surface metal ions, while P denotes phosphate groups in phosphorylated peptides, illustrating metal–phosphate coordination. Magnetic responsiveness further enables rapid separation under an external magnetic field. The schematic highlights the tunable and versatile nature of inorganic oxides for selective biomolecular capture and purification.

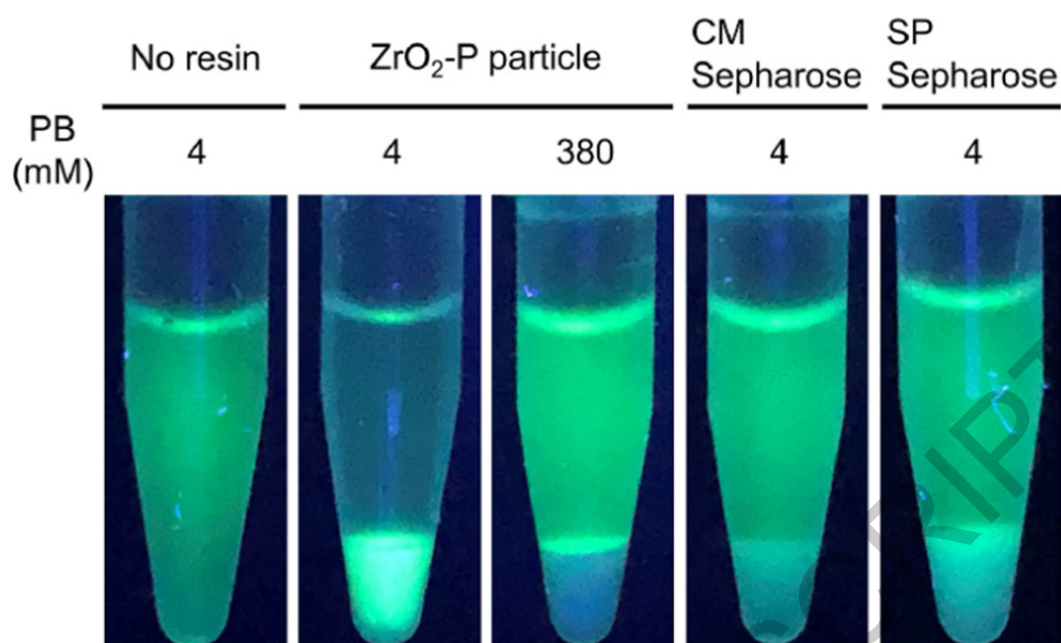


Fig. 1. Fluorescence images of GFP–His–expressing lysates after incubation with ZrO₂-P particles, CM Sepharose, or SP Sepharose and brief centrifugation. GFP–His localization reflects adsorption to the particles/resins under 4 mM PB (green fluorescence under 254-nm light). Reproduced from Kanoh *et al.*, *J. Chromatogr. A*, 1703 (2023) 464112, with permission from Elsevier.



Fig. 2. Schematic workflow of single-tube phosphorylated peptide enrichment using metal oxide particles. The procedure involves (1) wetting and conditioning of the particles, (2) sample loading, (3) washing to remove unbound peptides, and (4) elution of bound Phosphorylated peptides. This batch-wise approach enables simple, scalable, and controllable enrichment suitable for downstream proteomic analysis. Reproduced from Pocsfalvi, G., *Methods in Enzymology*, 457 (2009) 81–96, with permission from Elsevier.

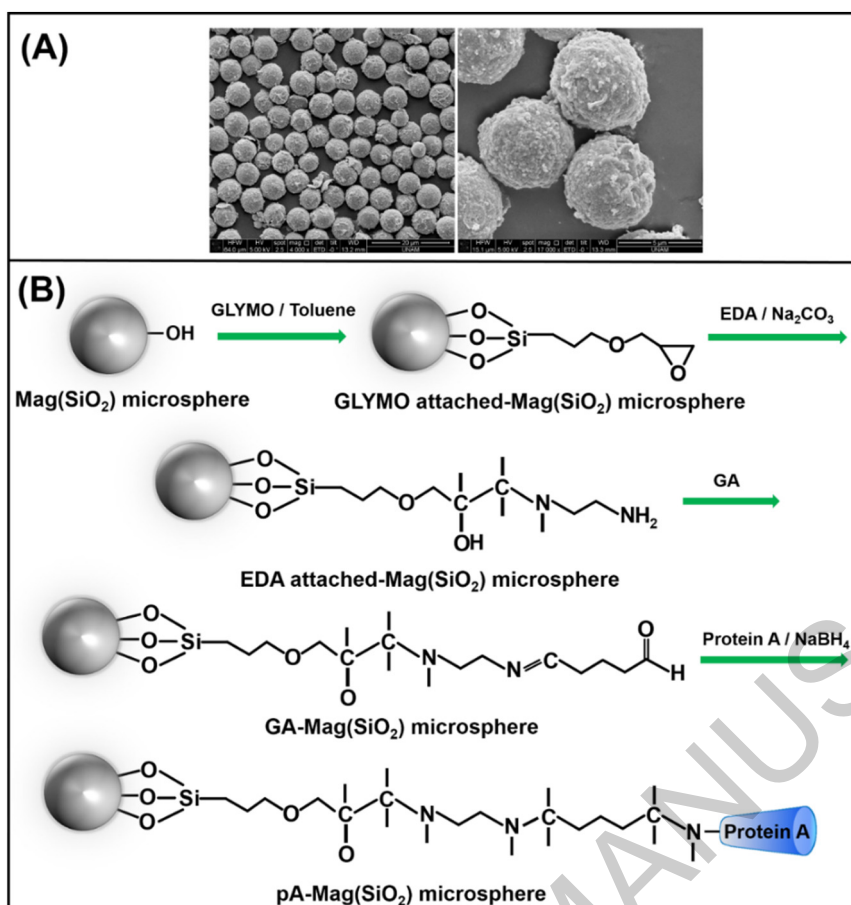


Fig. 3. Schematic illustration of protein A immobilization on magnetic porous silica (Mag(SiO₂)) microspheres. Surface hydroxyl groups of the silica allow covalent attachment of protein A, enabling selective capture of immunoglobulin G. Reproduced from Salimi et al., *Int. J. Biol. Macromol.*, 111 (2018) 178–185, with permission from Elsevier.

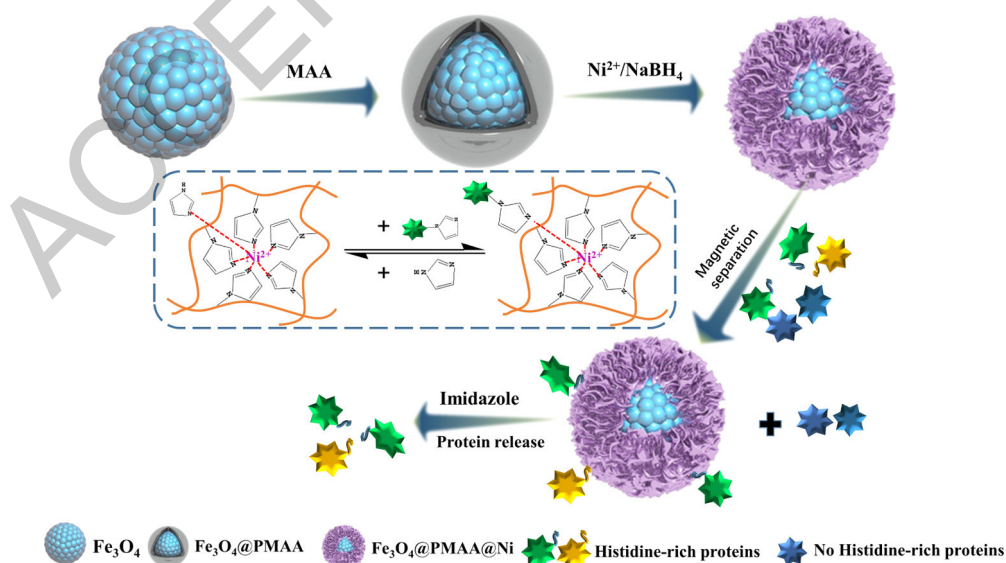


Fig. 4 Preparation of Core-Shell $\text{Fe}_3\text{O}_4@\text{PMAA}@\text{Ni}$ Microspheres and Their Application for Efficiently and Selectively Separating His-rich proteins. Reproduced from Wang et al., *ACS Appl. Mater. Interfaces*, 13 (2021) 11166–11176, with permission from ACS.

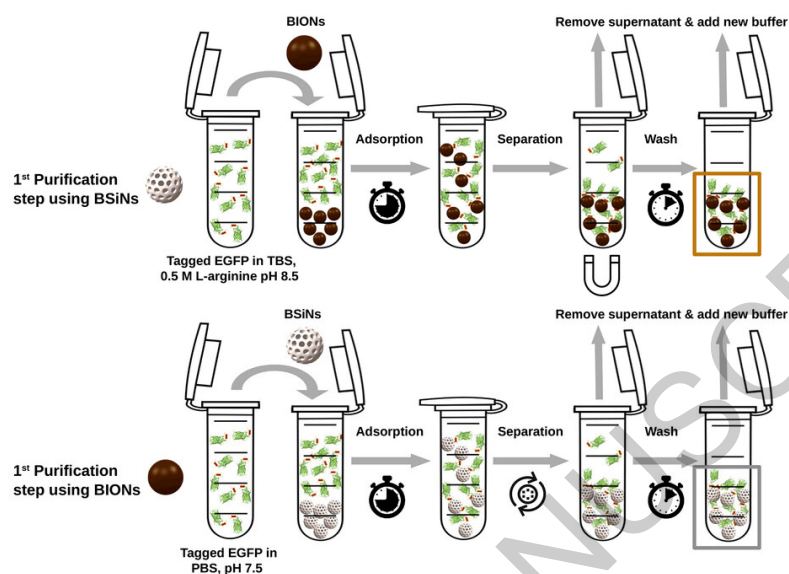


Fig. 5. Workflow of affinity-tagged proteins using Fe_3O_4 (BIONs) and SiO_2 (BSiNs) particles. Reproduced from Aguiar and Domingues, *Biotechnol. J.*, 18 (2023) 2300152, with permission from Wiley.

Table. 1 Representative formats, surface properties, functional groups, and target biomolecules of inorganic oxides.

	<i>Sample formats</i>	<i>Surface properties</i>	<i>Functional groups</i>	<i>Target biomolecules</i>
ZrO ₂	Monoclinic phase [43,63,71]/ Nanoparticles [65]; Mesoporous beads [66,67]; Microtips [62]	Lewis-acidic Zr ⁴⁺ surface sites [19] Interfacial hydration layers [41]	Aliphatic hydroxy Acid (e.g., β -hydroxypropanoic acid) [68] Phosphonate compounds (e.g., ethylenediamine tetra(methylene phosphonic acid) (EDTMP)) [43,69,71] Phosphate compounds (e.g., Phosphate) [63]	Phosphorylated peptides [62,65–68] His-tagged proteins [71] Antibodies (IgG, IgM, IgA) [43,63,72,73,75–77]
TiO ₂	Anatase phase [69,85,87,93]/ Nanoparticles [85]; Mesoporous beads [86,87]; Microtips [62]	Lewis-acidic Ti ⁴⁺ surface sites Interfacial hydration layers [42,90]	Amine [85] Aliphatic hydroxy Acid (e.g., Lactic acid) [68] Phosphonate (e.g., alendronate sodium trihydrate,	Phosphorylated peptides [16,68,69,85–87,96,98–100] Highly Similar Proteins (separation of proteins which similar sizes and net charges)

			nitrilotri(methylphosphonic acid) (ATMP)) [69]	[93]
SiO ₂	Amorphous silica [17,109,118,121,125]; α -quartz [111–114]/ Porous silica gel [17]; Porous particles [109,116,123,124,128]; Nanoparticles [121,184]; Core-shell magnetic particles [44]	Comparatively low intrinsic Lewis acidity High silanol density Deprotonatable silanol groups Hydrophilic hydroxylated surface Hydration-induced apparent hydrophobicity	IDA/NTA-metal complexes (Co ²⁺ , Cu ²⁺) [44,120] ZrO ₂ /TiO ₂ coatings [121,184] Ion-exchange ligands (e.g., morpholine, tetrazole) [45,122] Hydrophobic ligands (e.g., C18, cholesterol) [46,123] Hydrophilic ligands (e.g., glutathione) [124,125] Dye ligands (e.g., Cibacron Blue F3G-A) [116] Boronic acid compounds [126] Polymer coatings (e.g.,	Recombinant proteins/ model proteins (e.g., Lysozyme, cytochrome C) [17,47,109,116,122,123,127,18 5] Tagged proteins (e.g., Si-tag, His-tag) [44,111–114,117–120] Phosphorylated peptides [121,184] Glycopeptides / glycoproteins [124–126,129] Antibodies (IgG) [48,128,130]

			cationic/anionic polymers) [47,127] Affinity biomolecules (e.g., protein A, concanavalin A, affinity peptide) [48,128–130]	
Fe ₃ O ₄	Inverse spinel structure [137]/ Nanoparticles [18,137,138,140–142]; Core-shell microspheres [50,143–146]	Lewis-acidic Fe ²⁺ /Fe ³⁺ surface sites Amphoteric surface hydroxyl groups Magnetic separability [18,49,50,138,140,141,145,146]	Metal ion-coordinating polymer layers [49,140,143] boronic acid ligands [50,141] SiO ₂ /TiO ₂ /ZrO ₂ coatings [50,144–146] Affinity biomolecules (e.g. protein A, enzyme) [142,147]	Phosphorylated peptides [144,145] His-rich proteins [143] Tagged proteins (e.g., CotB1p- tag, His-tag) [18,49,138,140,146] Glycopeptides/proteins [50,141] Antibodies (IgG) [48,142]

Al ₂ O ₃	γ-Al₂O₃ / Packed chromatographic particles [156]; Magnetic core-shell microspheres [157]	Lewis-acidic Al³⁺ surface sites [23] Amphoteric surface hydroxyl groups [149]	Octadecyl and polybutadiene coatings [158,159]	phosphorylated peptides [157] Strongly basic proteins [156]
ZnO	Wurtzite ZnO [165] / Nanoparticles [161–165]	Amphoteric surface hydroxyl groups [160] Hydrogen bonding and van der Waals contributions [164,165]	–	–
CeO ₂	face-centered cubic phase [172] / Nanoparticles [161,162,171]; monodisperse-porous microspheres [172]	Lewis-acidic Ce⁴⁺ surface sites [25,26] Amphoteric surface hydroxyl groups [166]	–	Phosphorylated peptides/proteins [171,172]
SnO ₂	Not specified /	Lewis-acidic Sn ⁴⁺ surface sites	–	Phosphorylated peptides

	Porous microspheres [176,177]	[28]		[176,177]
Nb ₂ O ₅	Not specified / Powder [175]	Lewis-acidic Nb ⁵⁺ surface sites [27]	–	Phosphorylated peptides [175]
Ta ₂ O ₅	Not specified / Core-shell magnetic microspheres [178]	Lewis-acidic Ta ⁵⁺ surface sites [29]	–	Phosphorylated peptides [178]
Ga ₂ O ₃	Not specified / Core-shell magnetic particles [179]	Lewis-acidic Ga ³⁺ surface sites [30,32]	–	Phosphorylated peptides [179]

Statement of Novelty

This review summarizes frameworks for proteins and peptides purification using inorganic oxide materials. Surface chemistry, adsorption mechanisms, and workflow design are integrated to establish these frameworks including single-step and multi-step purification processes. These frameworks provide selective and robust protein and peptide purification.