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Phosphate-binding proteins and peptides: from molecular mechanisms to potential applications

Brooke K Mayer[1], Justin M Hutchison[2], Eric S McLamore[3,4], Maria Torres[5] and Kaushik Venkiteshwaran[6]

Selective binding of phosphate is vital to multiple aims including phosphate transport into cells and phosphate-targeted applications such as adsorption-based water treatment and sensing. High-affinity phosphate-binding proteins and peptides offer a nature-inspired means of efficiently binding and separating phosphate from complex matrices. The binding protein PstS is characterized by a Venus flytrap topology that confers exceptional phosphate affinity and selectivity, and is effective even at low phosphate concentrations, all of which are essential for applications such as phosphate sensing, removal, and recovery. The binding event is reversible under controlled conditions, making it germane to catch-and-release objectives that advance phosphorus sustainability. Peptides such as the P loop motif are also promising for such applications. Future advances in protein/peptide design can contribute to increased implementation in engineered systems.

Addresses
[1] Department of Civil, Construction and Environmental Engineering, Marquette University, Milwaukee, WI, USA
[2] Department of Civil, Environmental & Architectural Engineering, University of Kansas, Lawrence, KS, USA
[3] Environmental Engineering and Earth Sciences, Clemson University, Clemson, SC, USA
[4] Agricultural Sciences, Clemson University, Clemson, SC, USA
[5] Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, USA
[6] Department of Civil, Coastal and Environmental Engineering, University of South Alabama, Mobile, AL, USA

Corresponding author:
Mayer, Brooke K (Brooke.Mayer@marquette.edu)

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Bio-inspired materials leveraging phosphate-binding proteins and peptides have the potential to advance phosphorus sustainability

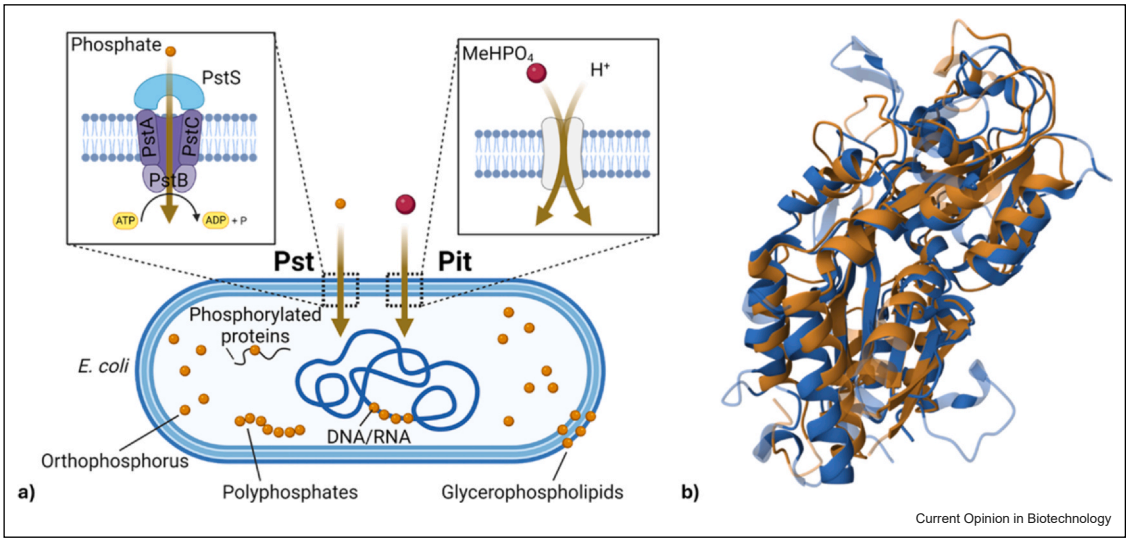
Phosphorus is a critical element underpinning all life with essential roles in cellular structures and the storage and transfer of energy and information [1,2]. Since phosphate (orthophosphate) "can do almost everything…and no alternative is obvious" [3], cells have evolved elegant mechanisms of acquiring it. Leveraging this natural functionality yields opportunities to design and implement bio-inspired materials that can help address the phosphorus paradox, wherein phosphorus is a finite, nonsubstitutable resource needed for agriculture, yet excess discharges of phosphorus to environmental waters stimulate eutrophication. To address this formidable challenge and establish a sustainable phosphorus cycle, all possibilities must be considered [1,4]. This includes designing and implementing phosphate-binding protein- and peptide-based technologies to efficiently and reversibly capture phosphate with remarkable affinity and selectivity, even when phosphate is present at low levels. Here, we describe foundational work establishing protein and peptide phosphate-binding mechanisms. We then focus on advances in protein and peptide phosphate-binding applications in phosphate removal/recovery from water and real-time sensing and decision support.

Molecular mechanisms of phosphate-binding proteins and peptides

All living organisms rely on dedicated transport systems to uptake critical nutrients, such as phosphorus, that cannot diffuse through cellular membranes [1,5]. The ATP-binding cassette (ABC) superfamily is a large class of transporters that includes ABC importers, which feature two nucleotide-binding domains, two transmembrane domains, and a substrate-binding protein [5,6]. Substrate-binding proteins vary in size and sequence [7], but their overall three-dimensional structural folding pattern is highly conserved with a Venus flytrap topology wherein two structurally conserved globular domains are connected by a flexible hinge region that is open when empty but closes upon binding the substrate in the cleft [8].

The high-affinity phosphate-specific ABC transporter Pst is one of two major phosphate transport systems in bacteria

Figure 1



(a) Illustration of *E. coli*'s phosphate transport systems Pst and Pit and the cell's phosphorus inventory (not drawn to scale). The high-affinity Pst ABC transporter features a hinged Venus flytrap phosphate-binding mechanism utilized at low phosphate concentrations. Pit is a low-affinity phosphate transporter operating at high phosphate concentrations and consists of a single constitutively synthesized transmembrane protein that symports a proton and a soluble, neutral metal-phosphate complex ($MeHPO_4$, where Me is a divalent cation) [1,9]. (created with Biorender.com) **(b)** Phosphate-binding proteins (e.g. PstS) are characterized by similar tertiary structure, as illustrated by the comparison between *E. coli* PstS (PDB ID: 1IXH, orange) and the human phosphate-binding protein (PDB ID: 2V3Q-1, blue) [15,16].
(a) Modified after Blank [1]

(Figure 1a). Pst is induced at low phosphate concentrations and has approximately 100 times greater phosphate affinity than the other major transporter, Pit [9], making Pst highly efficient and phosphate-specific, even when phosphate is initially present at low concentrations. The periplasmic phosphate-binding protein PstS serves as the high-affinity phosphate receptor in Pst. PstS offers exquisite phosphate specificity [10], readily distinguishing phosphate from other highly similar tetrahedral oxyanions, for example, sulfate (discriminated by ~5 orders of magnitude) and arsenate (discriminated by ≥500-fold) [7,11]. This specificity derives from ion-dipole hydrogen bonding between phosphate and PstS [12]. Recent molecular dynamics simulation of *Escherichia coli* PstS showed that 15 amino acid residues have significant interactions with phosphate [13], 8 of which match the 12 hypothesized hydrogen bonds found in the crystal structure [7]. Additionally, phosphate is protonated in common pH ranges, whereas sulfate is fully deprotonated; this lends further selectivity since PstS contains negatively charged amino acids that only accommodate protonated oxygen [12]. Although arsenate and phosphate have nearly identical pKa values, arsenate is still strongly discriminated against by PstS, ostensibly due to a unique low-barrier hydrogen bond between phosphate and the aspartate side chain [11,14].

There is no precedence for the existence of eukaryotic PstS homologs, wherein phosphate transport occurs via DING phosphate-binding proteins. DING proteins are less well-characterized compared to PstS, with the exception of the human phosphate-binding protein, whose tertiary structure strongly resembles PstS (Figure 1b) [6].

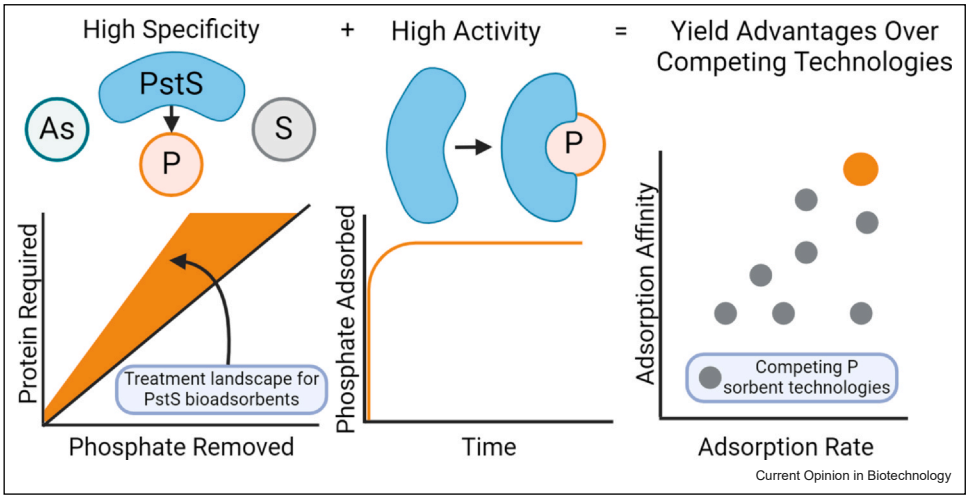
Outside of phosphate transport proteins, the most common structural motif identified among enzymes that catalyze phosphorus bonds is a generic P loop, GXXX structure. Several motifs in the catalytic domain of ABC include the Walker's A ((GXXXXGK(T/S)), which contains a conserved lysine residue. The P-loop motif wraps around phosphate to optimize the formation of hydrogen bonds between the sequence's backbone atoms and phosphate [17,18].

Beyond high phosphate affinity, selectivity, and binding efficiency at low phosphate levels (all of which characterize an excellent phosphate adsorbent), phosphate binding by PstS is reversible to enable the membrane protein to shepherd phosphate into the cell (in cells, phosphate release from PstS is triggered by ATP binding) [19,20]. Controlled binding reversibility is likewise critical for technologies designed to both capture/remove and release/recover phosphate.

Phosphate-binding proteins and peptides for phosphate removal and recovery from water

Depletion of mineable phosphorus, together with the challenge of aquatic phosphorus pollution, drives the exploration of innovative methods to improve phosphorus removal and

Figure 2



Graphical representation of the advantages offered by protein- and peptide-based adsorbents for environmental applications. The high specificity and activity of the proteins for phosphate offer advantages compared to other P-capture technologies.
Information adapted from Hutchison et al. [23] and Venkiteshwaran et al. [24].

recovery from water (including wastewater) [21]. Among these methods is the use of phosphate-binding proteins and peptides, which have tremendous promise as selective phosphate adsorbents able to facilitate recovery via pH-dependent phosphate release (Figure 2) [22,23].

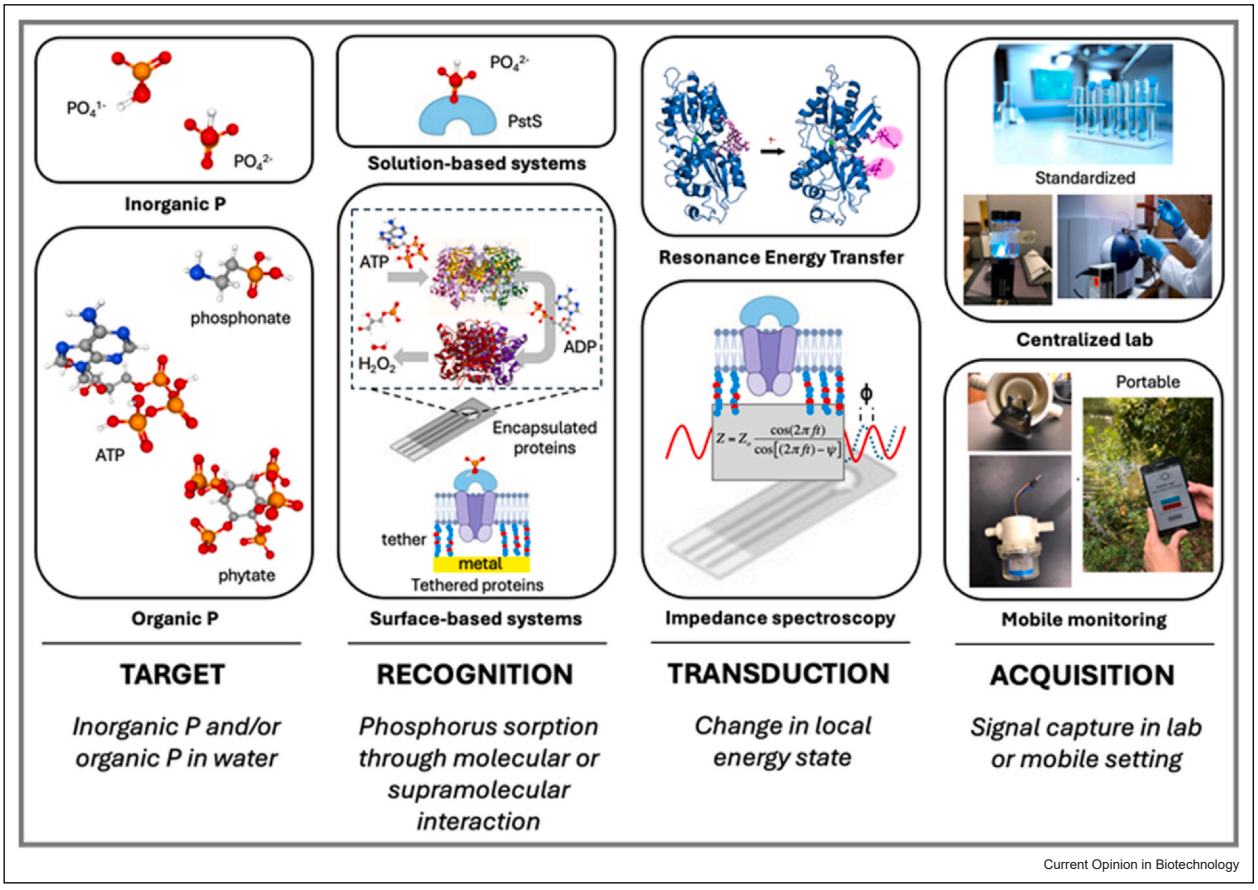
Extracellular immobilized PstS can remove phosphate to ultra-low levels (< 100 µg/L), even when phosphate is initially present at low concentrations, for example, 15 µg/L [25,26,24,27]. Lab-scale tests demonstrated superior performance in phosphate adsorption kinetics ($k = 4.9\times10^{3}$ g immobilized PstS/(mg P-PO_4-min)) and affinity (K_L = 220 ± 52 L/mg-P-PO_4) compared to ion exchange and metal oxide-based reversible adsorbents [28]. Immobilized PstS also discriminates against arsenate; this selectivity is crucial for recovering high-purity phosphorus suitable for agricultural reuse [29].

Lab-scale research has also been performed using genetically engineered bacteria overexpressing PstS or displaying PstS on the cell surface for use as a bioadsorbent to enhance phosphate removal [30–32]. Although phosphate removal was observed, lower removal and recovery efficiency was observed versus immobilized PstS configurations. Additionally, the deployment of genetically modified organisms in environmental applications may be limited due to regulations.

Optimizing recovery conditions and long-term reusability of PstS adsorbents is crucial for reducing costs and environmental impacts for water treatment systems [22]. More than 85% of phosphate was desorbed from lab-scale immobilized PstS using pH ≈12 without any impact on phosphate adsorption efficiency over 10 adsorption (circumneutral pH) and desorption (pH ≈12) cycles [24]. Consistent performance over multiple cycles highlights the robustness of the immobilized proteins in water treatment applications.

Phosphate-binding peptides may offer a means of further improving phosphate adsorption bio-inspired technologies [13,22]. Peptides are short amino acid sequences that encompass only the portion of proteins responsible for a particular function. In the case of phosphate binding, the P-loop motif has been the target for developing peptides to recover phosphate. The binding site found in ATPase, which is characteristic of the Walker's A P-loop motif (GXXXXGK(T/S)), has been tested as a peptide (sequence SGAGKT). The peptide shows pH-dependent phosphate sorption and desorption, making it ideal for phosphate recovery and peptide reuse in environmental systems [33]. While the conserved lysine is critical for catalytic activity, it may not be necessary for phosphate binding [34]. Dynamic force spectroscopy showed selective peptide binding of phosphate beginning at pH 8, indicating a preference for HPO_4^{2-}, with selective phosphate binding over arsenate, characteristic of many phosphate-binding proteins [35]. At higher phosphate concentrations and when the peptide was attached to a hydrophobic tail with a spacer, the peptide bound > 90% phosphate at pH 5 to 8 [35]. Phosphate-binding peptides may be inhibited by NaCl ionic strength $> 10^{-3}$ µM [33], requiring careful consideration of their application in phosphate recovery from environmental matrices. Peptides that facilitate calcium-P precipitation have been pursued extensively for applications such as remineralization of dental caries [36].

Figure 3



The R-T-A working mechanism for sensor design is based on *target recognition (R)*, *signal transduction (T)*, and *signal/data acquisition (A)*. High-affinity proteins/peptides used to target phosphorus (P) using either solution-based or surface-based approaches. Two common transduction systems (fluorescent/electrochemical) are shown as examples. Images courtesy of PubChem (ID: 1003; ID: 3681305; ID: 6326969; ID: 5957; ID: 890) and PDB (ID: 1B05; ID: 2RGH). Select components created using BioRender.com.

Despite current advances, several challenges must be addressed to achieve widespread adoption of protein- or peptide-based phosphate removal, including further consideration of material stability, design configuration, and production scaling. Scaling laboratory-based protein/peptide systems to industrial applications presents technical and economic challenges. The cost of producing and immobilizing proteins or peptides at large scales must be optimized to compete with existing technologies [22]. Additionally, the longevity and stability of immobilized biomaterials in wastewater and environmental conditions, with varying water compositions, flow rates, and potential fouling agents, must be investigated to fully leverage PstS proteins and/or peptides for phosphate recovery.

Sensing applications using phosphate-binding proteins and peptides

Biosensors are analytical technologies that employ high-specificity biomaterials to facilitate rapid analysis [37]. Materials such as PstS, Pit, or peptides have high affinity in the presence of other compounds and thus are ideal for optical and/or electrical sensing. Examples of the logic for biosensor design are shown in Figure 3, which is the basis for decision support systems based on biosensors [38]. To realize this ambition, sensors should have high target affinity, operate without reagents/cofactors, and be reversible.

Many solution-based protein biosensors have been developed using optical transduction techniques, reviewed in detail by Edwards [39]. Most PstS biosensors of this type are focused on Förster resonance energy transfer (FRET), which takes advantage of conformational protein changes to transduce signal. Yu et al. [40] extended this concept by developing a bioluminescence system (BRET) that uses luciferase as a donor. The BRET system does not depend on hardware for excitation of the acceptor, which is a step towards improving autonomy. Both FRET and BRET systems have detection

limits in the 1 µg/L range under laboratory conditions [39] but are not commonly employed in field studies.

Anchored/encapsulated protein biosensors may offer continuous monitoring if desorption is controlled. Electrochemical biosensors based on proteins encapsulated in polymeric gels (e.g. kinase-to-oxidase cascade) are robust for measuring molecules such as ATP [41], with detection limits in the 1 µg/L range. In the last decade, nanopore sensors have emerged for quantifying charged species, for example, a nanopore system for detecting organic phosphorus using a phosphonate-binding protein [42]. Exposure to 2-aminoethylphosphonate or ethylphosphonate induced conformational changes (Venus flytrap), which in turn transduced an electrical signal (detection limits in the 125 µg/L range).

Peptides are capable of selective interaction that has been used to fabricate sensors targeting phosphorus. For example, Fowler [43] developed a peptide-based system for quantifying orthophosphate (HPO_4^{2-}) using a micelle system. The label-free system was based on aggregate-induced fluorescence triggered by interaction with a phosphate-binding sequence (limit of detection as low as 1 mg/L). Although nanopore systems to date have not targeted phosphate, tethered peptide systems (nanopore sensors) are an excellent platform. The transduction system is rooted in noncovalent intermolecular interaction and/or peptide self-assembly. Zhang et al. [44] provide an excellent review of an emerging concept known as pore-in modification that serves as an opportunity for phosphate-sensitive peptides in selective sensing.

Most P biosensors are based on the Venus flytrap behavior of PstS, where protein conformational changes transduce local photonic and/or electronic behavior to drive a signal. BRET and FRET sensors based on PstS are generally reagent-free and perform well compared to the standard colorimetric method based on Keggin ion formation. These techniques have the potential to compete with the colorimetric Keggin ion system, which has been the standard for more than 60 years. Nanopore protein sensors and tethered peptide sensors are potentially reusable (if on/off states are controlled), but limits of detection (approximately 100 µg/L or greater) need to improve.

Future opportunities in phosphate-binding protein and peptide biotechnology

Phosphorus biotechnology "should from the start consider the potential application" [1]. Bio-inspired protein and peptide-based systems have tremendous promise to advance phosphorus sustainability, including in the realms of water treatment and sensing. Beyond these demonstrated applications, phosphate-binding bio-inspired materials may have promise in laboratory analytics as solid-phase extraction materials (e.g. for phosphate concentration or removal to improve the detection of other analytes). Additionally, Zhao et al. [45] observed the occurrence of PstS in cyanophage P-SSM2, which was able to interact with the host to enhance the rate of phosphate uptake by the cyanobacteria, introducing the possibility of influencing oceanic phosphorus cycling via picocyanobacteria. Increased implementation and expanded applications may increase the importance of optimized phosphate-binding protein and peptide design.

Environmental selective pressures yielded exquisitely selective PstS; however, these selective pressures may not have produced a protein design optimal for biotechnology applications. As such, the design of new proteins and peptides with improved functionality will be required. Machine learning approaches to improve existing or design novel proteins and peptides with improved characteristics (e.g. stability and resistance to inactivation) are emerging. The development of such tools comes with challenges, including the availability of adequate high-quality data [46], as well as implementation challenges in design tradeoffs, for example, enhanced thermal stability versus solubility. Several tools have been created to predict protein structure (i.e. Alphafold 3 [47]), improve thermal stability (i.e. ThermoMPNN [48]), and design novel tertiary structures (i.e. RoseTTAFold Diffusion [49]), which could yield improvements to proteins and peptides for environmental applications.

CRediT authorship contribution statement

Brooke K Mayer: Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Visualization. **Justin M Hutchison:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Eric S McLamore:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision, Visualization. **Maria Torres:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Kaushik Venkiteshwaran:** Conceptualization, Writing – original draft, Writing - review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as

potential competing interests: B.K. Mayer and K. Venkiteshwaran are co-inventors on a patent application entitled "Process for controlled adsorption and desorption of phosphate from liquids using phosphate-selective proteins". The authors declare no other potential conflicts of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Blank LM: **The cell and P: from cellular function to biotechnological application**. *Curr Opin Biotechnol* 2012, **23**:846-851.

2. Elser JJ: **Phosphorus: a limiting nutrient for humanity?** *Curr Opin Biotechnol* 2012, **23**:833-838.

3. Westheimer FH: **Why nature chose phosphates**. *Science* 1987, **235**:1173-1178, https://doi.org/10.1126/science.2434996

4. • Elser J, Call D, Deaver J, Duckworth O, Mayer BK, McLamore E, Rittmann BE, Mahmood M, Westerhoff P: **The phosphorus challenge: biotechnology approaches for a sustainable phosphorus system**. *Curr Opin Biotechnol* 2024, (In Press).
Discussed avenues for phosphorus biotechnology to contribute to a sustainable phosphorus future, including harnessing biological systems as biosensors, in the production of protein substitutes as alternatives to current animal production systems, bioadsorbents, polyphosphate accumulating organisms and anaerobic wastewater treatment, and biomineralization.

5. Rees DC, Johnson E, Lewinson O: **ABC transporters: the power to change**. *Nat Rev Mol Cell Biol* 2009, **10**:218-227.

6. Berna A, Bernier F, Chabrière E, Perera T, Scott K: **DING proteins; novel members of a prokaryotic phosphate-binding protein superfamily which extends into the eukaryotic kingdom**. *Int J Biochem Cell Biol* 2008, **40**:170-175.

7. Luecke H, Quiocho FA: **High specificity of a phosphate transport protein determined by hydrogen bonds**. *Nature* 1990, **347**:402-406.

8. Mao B, Pear MR, McCammon JA: **Hing-bending in L-Arabinose-binding protein: the Venus's-flytrap model**. *J Biol Chem* 1982, **257**:1131-1133.

9. van Veen HW: **Phosphate transport in prokaryotes: molecules, mediators and mechanisms**. *Antonie Van Leeuwenhoek* 1997, **72**:299-315.

10. Quiocho FA: **Atomic basis of the exquisite specificity of phosphate and sulfate transport receptors**. *Kidney Int* 1996, **49**:943-946.

11. Elias M, Wellner A, Goldin-Azulay K, Chabriere E, Vorholt JA, Erb TJ, Tawfik DS: **The molecular basis of phosphate discrimination in arsenate-rich environments**. *Nature* 2012, **491**:134-137.

12. Davidson AL, Dassa E, Orelle C, Chen J: **Structure, function, and evolution of bacterial ATP-binding cassette systems**. *Microbiol Mol Biol Rev* 2008, **72**:317-364.

13. • Hussein FB, Cannon AH, Hutchison JM, Gorman CB, Yingling YG, Mayer BK: **Phosphate-binding protein-loaded iron oxide particles: adsorption performance for phosphorus removal and recovery from water**. *Environ Sci Water Res Technol* 2024, **10**:1219-1232, https://doi.org/10.1039/D4EW00052H.
Used experimental data, machine learning, and theoretical calculations to describe the need for parallel improvements in the surface area-to-mass ratio of protein/peptide immobilization material together with identification of phosphate-specific peptide binding sequences to further advance design and implementation of bioadsorbents for water treatment.

14. Qi R, Jing Z, Liu C, Piquemal J-P, Dalby KN, Ren P: **Elucidating the phosphate binding mode of phosphate-binding protein: the critical effect of buffer solution**. *J Phys Chem B* 2018, **122**:6371-6376.

15. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov I N, Bourne PE: **The Protein Data Bank**. *Nucleic Acids Research* 2000, **28**:235-242, https://doi.org/10.1093/nar/28.1.235

16. Sehnal D, Bittrich S, Deshpande M, Svobodová R, Berka K, Bazgier V, Velankar S, Burley SK, Koča J, Rose AS: **Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures**. *Nucleic Acids Research* 2021, **49**:W431-W437, https://doi.org/10.1093/nar/gkab314

17. Kinoshita K, Sadanami K, Kidera A, Go N: **Structural motif of phosphate-binding site common to various protein superfamilies: all-against-all structural comparison of protein–mononucleotide complexes**. *Protein Eng* 1999, **12**:11-14.

18. Hirsch AKH, Fischer FR, Diederich F: **Phosphate recognition in structural biology**. *Angew Chem Int Ed Engl* 2007, **46**:338-352.

19. Brune M, Hunter JL, Howell SA, Martin SR, Hazlett TL, Corrie JET, Webb MR: **Mechanism of inorganic phosphate interaction with phosphate binding protein from *Escherichia coli***. *Biochemistry* 1998, **37**:10370-10380.

20. Rice AJ, Park A, Pinkett HW: **Diversity in ABC transporters: type I, II and II importers**. *Crit Rev Biochem Mol Biol* 2014, **49**:426-437.

21. Mayer BK, Baker LA, Boyer TH, Drechsel P, Gifford M, Hanjra MA, Parameswaran P, Stoltzfus J, Westerhoff P, Rittmann BE: **Total value of phosphorus recovery**. *Environ Sci Technol* 2016, **50**:6606-6620.

22. Hutchison JM, Hussein FB, Mayer BK: **Evaluating sustainable development pathways for protein- and peptide-based bioadsorbents for phosphorus recovery from wastewater**. *Environ Sci Technol* 2023, **57**:16317-16326.

23. •• Hutchison JM, Mayer BK, Vega M, Chacha WE, Zilles JL: **Making waves: biocatalysis and biosorption: opportunities and challenges associated with a new protein-based toolbox for water and wastewater treatment**. *Water Res X* 2021, **12**:100112.
This work identified and prioritized research goals needed to advance protein- and peptide-based removal and recovery of phosphate from environmental systems.

24. Venkiteshwaran K, Pokhrel N, Hussein F, Antony E, Mayer BK: **Phosphate removal and recovery using immobilized phosphate binding proteins**. *Water Res X* 2018, **1**:100003.

25. Kuroda A, Kunimoto H, Morohoshi T, Ikeda T, Kato J, Takiguchi N, Miya A, Ohtake H: **Evaluation of phosphate removal from water by immobilized phosphate-binding protein PstS**. *J Biosci Bioeng* 2000, **90**:688-690.

26. Brune M, Hunter JL, Corrie JET, Webb MR: **Direct, real-time measurement of rapid inorganic phosphate releasue using a novel fluorescent probe and its application to actomyosin subfragment 1 ATPase**. 1994, **33**:8262-8271.

27. Solscheid C, Kunzelmann S, Davis CT, Hunter JL, Nofer A, Webb MR: **Development of a reagentless biosensor for inorganic phosphate, applicable over a wide concentration range**. *Biochemistry* 2015, **54**:5054-5062.

28. Venkiteshwaran K, Wells E, Mayer BK: **Kinetics, affinity, thermodynamics, and selectivity of phosphate removal using immobilized phosphate-binding proteins**. *Environ Sci Technol* 2020, **54**:10885-10894.

29. Venkiteshwaran K, Wells E, Mayer BK: **Immobilized phosphate-binding protein can effectively discriminate against arsenate during phosphate adsorption and recovery**. *Water Environ Res* 2021, **93**:1173-1178.

30. Li Q, Yu Z, Shao X, He J, Li L: **Improved phosphate biosorption by bacterial surface display of phosphate-binding protein utilizing ice nucleation protein**. *FEMS Microbiol Lett* 2009, **299**:44-52.

31. Hussein FB, Venkiteshwaran K, Mayer BK: **Cell surface-expression of the phosphate-binding protein PstS: System development, characterization, and evaluation for phosphorus removal and recovery**. *J Environ Sci* 2020, **92**:129-140.

32. Yang Y, Ballent W, Mayer BK: **High-affinity phosphate-binding protein (PBP) for phosphorous recovery: proof of concept using recombinant *Escherichia coli***. *FEMS Microbiol Lett* 2016, **363**:fnw240.

33. Zhai H, Qin L, Zhang W, Putnis CV, Wang L: **Dynamics and molecular mechanism of phosphate binding to a biomimetic hexapeptide**. *Environ Sci Technol* 2018, **52**:10472-10479.

34. Saraste M, Sibbald PR, Wittinghofer A: **The P-loop — a common motif in ATP- and GTP-binding proteins**. *Trends Biochem Sci* 1990, **15**:430-434.

35. Fowler WC, Deng C, Griffen GM, Teodoro OT, Guo AZ, Zaiden M, Gottlieb M, De Pablo JJ, Tirrell MV: **Harnessing peptide binding to capture and reclaim phosphate**. *J Am Chem Soc* 2021, **143**:4440-4450.

36. Ding L, Han S, Wang K, Zheng S, Zheng W, Peng X, Niu Y, Li W, Zhang L: **Remineralization of enamel caries by an amelogenin-derived peptide and fluoride in vitro**. *Regen Biomater* 2020, **7**:283-292.

37. Wongkaew N, Simsek M, Griesche C, Baeumner AJ: **Functional nanomaterials and nanostructures enhancing electrochemical biosensors and lab-on-a-chip performances: recent progress, applications, and future perspective**. *Chem Rev* 2019, **119**:120-194.

38. McLamore ES, Datta SPA: **A connected world: system-level support through biosensors**. *Annu Rev Anal Chem* 2023, **16**:285-309.

39. Edwards KA: **Periplasmic-binding protein-based biosensors and bioanalytical assay platforms: advances, considerations, and strategies for optimal utility**. *Talanta Open* 2021, **3**:100038.

40. Yu J, Zhang Y, Zhao Y, Zhang X, Ren H: **Highly sensitive and selective detection of inorganic phosphates in the water environment by biosensors based on bioluminescence resonance energy transfer**. *Anal Chem* 2023, **95**:4904-4913.

41. Vanegas DC, Clark G, Cannon AE, Roux S, Chaturvedi P, McLamore ES: **A self-referencing biosensor for real-time monitoring of physiological ATP transport in plant systems**. *Biosens Bioelectron* 2015, **74**:37-44.

42. Bernhard M, Diefenbach M, Biesalski M, Laube B: **Electrical sensing of phosphonates by functional coupling of phosphonate binding protein PhnD to solid-state nanopores**. *ACS Sens* 2020, **5**:234-241.

43. • Fowler WC: **Intrinsic fluorescence in peptide amphiphile micelles with protein-inspired phosphate sensing**. *Biomacromolecules* 2022, **23**:4804-4813.
Characterized the phosphate detection performance of a biosensing system based on peptide amphiphile micelles incorporating a protein-derived phosphate-binding peptide sequence and featuring aggregation-induced fluorescence emission.

44. Zhang X, Dai Y, Sun J, Shen J, Lin M, Xia F: **Solid-state nanopore/nanochannel sensors with enhanced selectivity through pore-in modification**. *Anal Chem* 2024, **96**:2277-2285.

45. F. Zhao X. Lin K. Cai Y. Jiang T. Ni Y. Chen J. Feng S. Dang C-Z. Zhou Q Zeng Biochemical and structural characterization of the cyanophage-encoded phosphate-binding protein: implications for enhanced phosphate uptake of infected cyanobacteria Environmental Microbiology 24 3037 3050 doi: 10.1111/1462-2920.16043.

46. Goshisht MK: **Machine learning and deep learning in synthetic biology: key architectures, applications, and challenges**. *ACS Omega* 2024, **9**:9921-9945.

47. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, *et al.*: **Accurate structure prediction of biomolecular interactions with AlphaFold 3**. *Nature* 2024, **630**:493-500, https://doi.org/10.1038/s41586-024-07487-w

48. Dieckhaus H, Brocidiacono M, Randolph N, Kuhlman B: **Transfer learning to leverage larger datasets for improved prediction of protein stability changes**. *PNAS* 2024, **121**:e2314853121, https://doi.org/10.1073/pnas.2314853121

49. •• Watson JL, Juergens D, Bennett NR, Trippe BL, Yim J, Eisenach HE, Ahern W, Borst AJ, Ragotte RJ, Milles LF, *et al.*: **De novo design of protein structure and function with RFdiffusion**. *Nature* 2023, **620**:1089-1100.
Developed methodology (RoseTTAFold Diffusion) that provides novel tertiary protein architecture around known active sites and binding pockets.