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Interfacial Supramolecular Structures of Amphiphilic Receptors Drive Aqueous Phosphate Recognition

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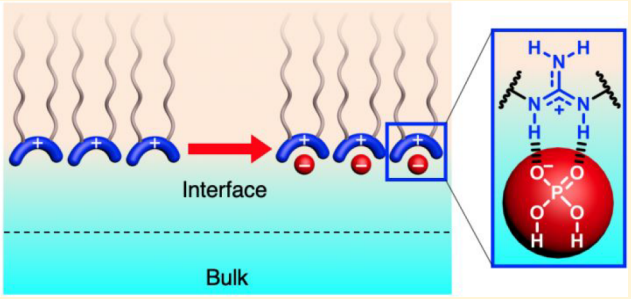
Supporting Information

ABSTRACT: Phosphate remediation is important for preventing eutrophication in fresh waters and maintaining water quality. One approach for phosphate removal involves the utilization of molecular receptors. However, our understanding of anion recognition in aqueous solution and at aqueous interfaces is underdeveloped, and the rational design of surface-immobilized receptors is still largely unexplored. Herein, we evaluated the driving forces controlling phosphate binding to elementary amphiphilic receptors anchored at air−water interfaces. We designed biologically inspired receptors with neutral thiourea, positively charged guanidinium, and thiouronium units that all formed Langmuir monolayers. Phosphate binding was quantitatively examined using surface pressure−area isotherms and infrared reflection−absorption spectroscopy (IRRAS). The receptors within this homologous series differ in functional group, charge, and number of alkyl chains to help distinguish the fundamental components influencing anion recognition at aqueous interfaces. The two charged receptors bearing two alkyl chains each displayed strong phosphate affinities and 10^3- and 10^1-fold *anti*-Hofmeister selectivity over chloride, respectively. Neutral thiourea and the single-chain guanidinium receptor did not bind phosphate, revealing the importance of electrostatic interactions and supramolecular organization. Consistently, charge screening at high ionic strength weakens binding. Spectroscopic results confirmed phosphate binding to the double alkyl chain guanidinium receptor, whereas surface pressure isotherm results alone showed a minimal change, thus emphasizing the importance of interfacial spectroscopy. We found that the binding site identity, charged interface created by the electrical double layer, and supramolecular superstructure all affect interfacial binding. These detailed insights into phosphate recognition at aqueous interfaces provide a foundation to develop efficient receptors for phosphate capture.



INTRODUCTION

The development of receptors capable of overcoming the high hydration energies and inherent difficulties of aqueous phosphate recognition is a necessary challenge for a modern sustainable society.[1−4] Demand for phosphate fertilizers grows in conjunction with the global population.[5,6] Consequently, phosphate runoff from agricultural nonpoint sources threatens fresh water supplies,[7−10] and phosphate-driven eutrophication in natural waters can generate harmful algal blooms which negatively impact aquatic life.[7,9,11−13] Furthermore, phosphate rock is a nonrenewable resource, and all losses have negative impacts on long-term food security. Thus, there is a continued need to understand the principles of recognition and underlying physical−organic interactions to design synthetic phosphate receptors for use in sensing and remediation applications.[14] Challenges of phosphate recognition in bulk water consist of the high energetic cost of phosphate dehydration (ΔG_{hyd} = −465 kJ/mol),[15] the large dielectric constant (ε) of bulk water (ε = 80) that screens all electrostatic interactions,[16] the large size-to-charge ratio of the phosphate guest,[17,18] and the acid−base properties of phosphate[1,17,19] (pK_{a1} = 2.16, pK_{a2} = 7.21, pK_{a3} = 12.32).[20] We seek to exploit the unique and technologically relevant environment present at aqueous interfaces, akin to those of remediation membranes, to overcome some of the inherent disadvantages of phosphate recognition in bulk solution.

The air−water interface is a unique microenvironment. It has a much lower dielectric constant than bulk water (ε < 40),[21,22] thus amplifying electrostatic interactions[16] to increase phosphate binding. In addition, Langmuir monolayers provide a confined local environment and have the advantage of preorganization,[23,24] which is expected to enhance recognition.[2,25] The long hydrophobic tails of the receptor are well ordered upon compression, while the hydrophilic component (the binding site) is anchored down into the water and available for phosphate binding. Once anchored, they can also benefit from the lower dielectric in the interfacial microenvironment to boost affinity.

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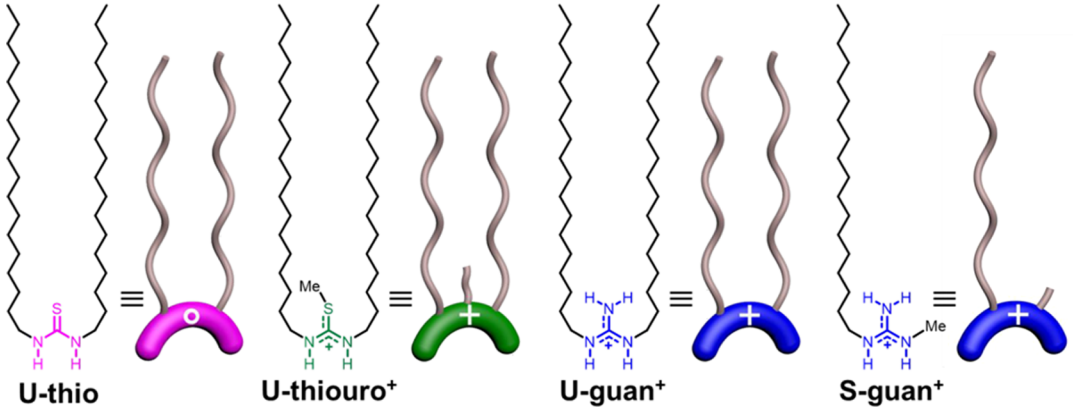

Figure 1. Structures of the octadecyl-based, U-shaped neutral receptor **U-thio**, the U-shaped and charged receptors **U-thiouro**$^+$ and **U-guan**$^+$, and the single-chain and charged **S-guan**$^+$ receptor for phosphate binding at the air−water interface.

Pioneering work by Kunitake on interactions between monolayers of guanidinium amphiphiles, bearing a single hydrophobic tail, and aqueous solutions of adenosine triphosphate revealed that affinity is greatly enhanced at the monolayer interface and by multivalency.[26−29] A recent study showed that the interfacial binding between a monolayer of organophosphate and aqueous guanidiniums could be studied in situ. The affinity was also significantly enhanced at the air−water interface relative to aqueous solution (>10 000 times) in the model system composed of the phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid (DPPA) and the amino acid arginine.[30] Although the enhancement in binding at the air−water interface is promising, the governing principles of interfacial binding are underexplored, which has hindered the rational design of effective surface-bound receptors.

Molecular and Supramolecular Design. We explored a series of elementary and biologically inspired amphiphilic receptors to help elucidate the design principles of phosphate recognition. The guanidinium and thiourea motifs are well known to display binding selectivity toward phosphate,[28,31−35] with only a few studies extended to interfacial environments for guanidinium,[26] thiouronium,[31] and thiourea.[33] We expect the monolayer-organized receptors with these binding sites will retain selectivity for phosphate binding over chloride at interfaces.

We tuned the structures of the homologous receptor series by modulating the binding sites and alkyl chains and evaluated their binding to aqueous phosphate in the subphase using in situ methods.[30] Octadecyl chains ($C_{18}H_{37}$) were chosen to facilitate monolayer formation, and the number of alkyl chains was varied to determine how the organization of the monolayer affects phosphate affinity. For the binding motif, the charged guanidinium and thiouronium moieties are almost isosteric while the charge-neutral thiourea group was chosen to investigate how different chemical and physical driving forces contribute to phosphate recognition. Four elementary receptors were synthesized (Figure 1) to test phosphate binding: bis-octadecyl-substituted guanidinium where the two chains are directed upward to make a U-shape (**U-guan**$^+$), the similar thiouronium (**U-thiouro**$^+$), and thiourea (**U-thio**), as well as mono-octadecyl-substituted guanidinium with a single chain (**S-guan**$^+$).

The guanidinium functional group of **U-guan**$^+$ and **S-guan**$^+$ has a trigonal planar geometry with a positive charge formally distributed across three nitrogen atoms by resonance stabilization and several possible hydrogen-bonding donor sites.[36−38] Thus, the guanidinium moiety typically binds to phosphate with two charge-assisted hydrogen bonds complementary to the structure of phosphate's hydrogen-bond acceptor sites. **U-thiouro**$^+$ also has the advantage of directed, planar hydrogen-bonding interactions.[39−41] Thiouronium binding sites are largely unexplored in supramolecular chemistry but nevertheless have been shown to have strong binding affinity to oxyanions[42−46] arising from the charge on the large polarizable sulfur atom, providing charge-assisted hydrogen bonding. While the thiourea binding site of **U-thio** has the advantage of hydrogen bonding, it does not bear the positive charge. In addition to the chemical features of these receptors, differences in the binding may be modulated by the number of alkyl groups.

Interfacial binding of preorganized Langmuir monolayers was evaluated using the surface-sensitive techniques of surface pressure−area isotherms (Π−*A*) and infrared reflection absorption spectroscopy (IRRAS). Although a few other in situ surface techniques (vibrational sum frequency generation spectroscopy,[47] Brewster angle microscopy,[30] fluorescence[48] imaging, and X-ray reflectometry)[49,50] would also be useful, Π−*A* isotherms and IRRAS were the preferred tools because of their high-throughput capabilities. Ex situ methods require Langmuir−Blodgett transfer of the monolayer to a solid substrate before further analysis by X-ray photoelectron spectroscopy, UV−vis spectroscopy, or other spectroscopic techniques.[26,31]

The use of IRRAS in this study enabled a phosphate binding response for the charged **U-guan**$^+$ and **U-thiouro**$^+$ receptors to be recorded, verifying the effectiveness of the technique for detecting these binding interactions. Although Π−*A* isotherms have been used extensively in the literature to demonstrate binding between ions of salts[51] and organic compounds,[30,52,53] our isotherm results often showed a null response to binding. We attribute this outcome to the two alkyl chains impeding macroscopic changes in the molecular area of the monolayer despite phosphate binding at the interface. Competitive binding between phosphate and chloride revealed that monolayers of the two charged **U-guan**$^+$ and **U-thiouro**$^+$ receptors are selective toward phosphate. This behavior is the opposite from that expected from the Hofmeister series, which highlights the benefit of binding at the interface where there is only partial dehydration. However, at a constant ratio of phosphate to chloride, the ionic strength weakens the

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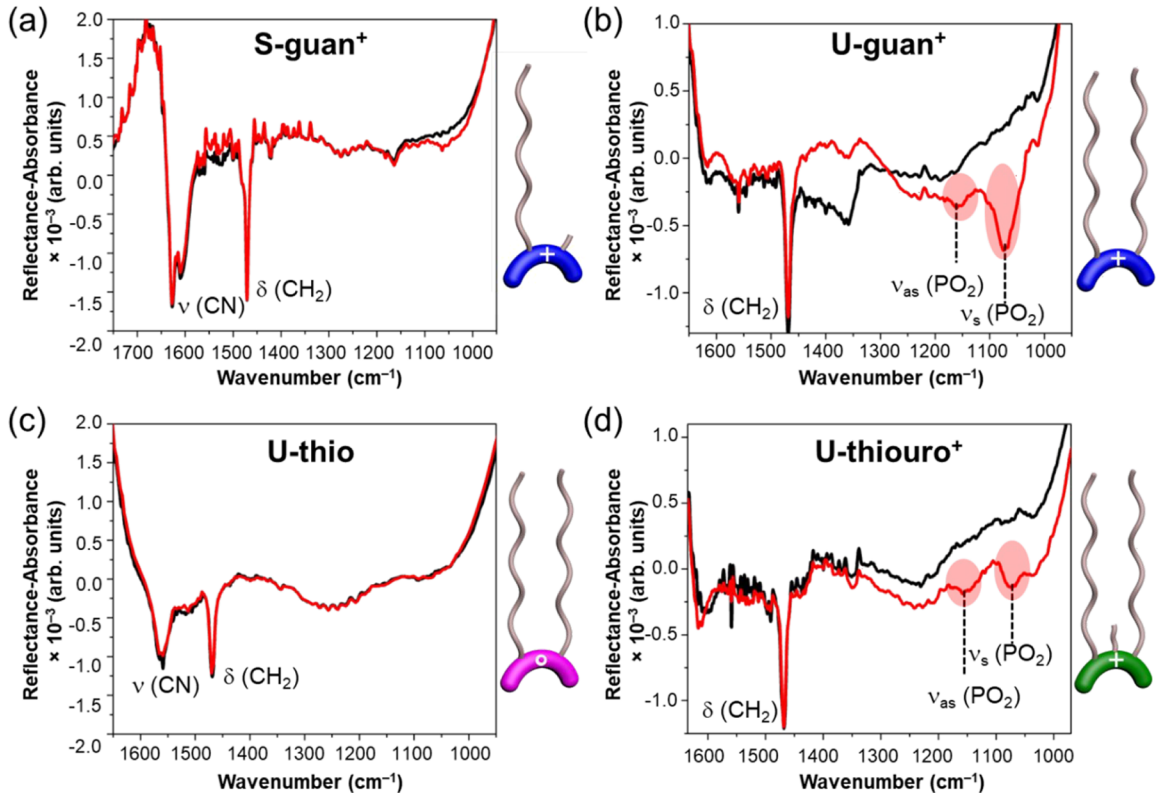

Figure 2. IRRAS spectra in the condensed phase (Π = 40 mN/m) for (a) **S-guan**$^+$, (b) **U-guan**$^+$, (c) **U-thio**, and (d) **U-thiouro**$^+$ on water (black trace) and 10 mM phosphate (red trace) reveal that phosphate is interacting with the **U-guan**$^+$ and **U-thiouro**$^+$ receptors. Symmetric and asymmetric stretches from bound phosphate are marked.

observed selectivity; at high ionic strength with significant charge screening there is a weakening of electrostatic interactions. We found that a constant chloride concentration, <10 mM in the bulk aqueous subphase, is necessary to limit screening of the electrostatic potential of the receptors. Our findings suggest that the nature of the binding site (chemical), the contribution of electrostatics (physical), and the organization of the receptor in the molecular monolayer (supramolecular superstructural) all affect phosphate binding affinity. These results provide some of the first fundamental understanding of rational supramolecular design and of how anion recognition is influenced by the local environment at the air−water interface.

RESULTS AND DISCUSSION

Identification of Receptor−Phosphate Binding at the Air−Water Interface. Spectroscopic measurements by IRRAS proved to be a valuable approach to interrogate the associated binding interaction between the molecular monolayer and the aqueous phosphate. IRRAS spectra in the low-frequency binding region (1600−950 cm^{-1}) were collected across the receptor series to elucidate whether the **S-guan**$^+$, **U-guan**$^+$, **U-thio**, and **U-thiouro**$^+$ receptors (Figure 1) interact with aqueous phosphate ions. The IRRAS spectra were collected at a surface pressure (Π) of 40 mN/m, which is in the condensed phase of the monolayer for all receptors. It is apparent from these spectra that the charged **S-guan**$^+$ receptor (Figure 2a) bearing only one alkyl anchor and the neutral **U-thio** receptor (Figure 2c) have negligible spectral changes with phosphate. The two U-shaped and charged receptors **U-guan**$^+$ (Figure 2b) and **U-thiouro**$^+$ (Figure 2d) both show significant changes upon phosphate addition.

The IRRAS spectra show clear signatures from the interfacial binding with phosphate. Specifically, new peaks appear in the spectra for **U-guan**$^+$ (Figure 2b) and **U-thiouro**$^+$ (Figure 2d) at approximately 1158 and 1074 cm^{-1}, which we assign to the phosphate's asymmetric (ν_{as} PO_2) and symmetric (ν_s PO_2) stretching modes.[54,55] The presence of these phosphate peaks strongly supports phosphate binding to the receptor molecules. According to the IRRAS equation, $-\log(R/R_0)$, where R is reflectivity and R_0 is the solution prior to depositing the monolayer, the phosphate modes are represented in both the numerator and the denominator of the equation. Therefore, the phosphate modes should only be present when there is binding. A series of control experiments further confirms that the two **U-guan**$^+$ and **U-thiouro**$^+$ receptors are indeed binding to phosphate (for details, see the Supporting Information).

The presence of phosphate modes in the IRRAS spectra of **U-guan**$^+$ and **U-thiouro**$^+$ verifies that these receptors bind phosphate. Conversely, the absence of phosphate peaks with **S-guan**$^+$ and **U-thio** receptors show that they have no interaction with phosphate. Furthermore, the phosphate modes in the **U-guan**$^+$ and **U-thiouro**$^+$ spectra are blue shifted relative to aqueous phosphate. For the **U-guan**$^+$ and **U-thiouro**$^+$ receptors, changes from hydrated phosphate to less hydrated phosphate leads to blue shifts of approximately 20 and 5 cm^{-1} for the ν_{as} PO_2 and ν_s PO_2, respectively (Figure 2, S8a). We propose from literature precedence[56−58] that the blue shift correlates with decreased hydrogen bonding. The logical interpretation of the blue shift, therefore, is that the net hydrogen bonding from the receptor is weaker than from the

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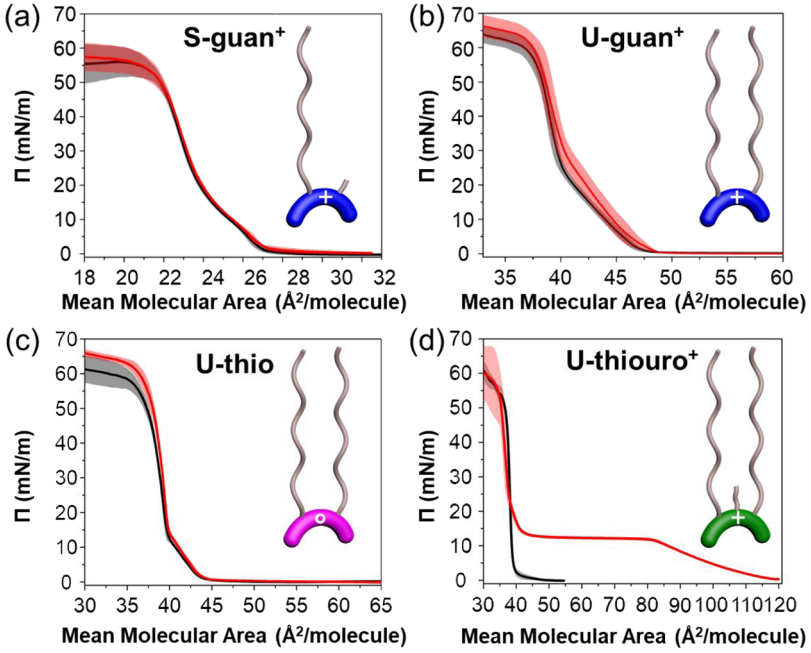

Figure 3. Π–*A* isotherms of **S-guan**$^+$ and **U-thio** (a and c) on water (black trace) and on a 10 mM sodium dihydrogen phosphate solution (red trace) do not produce significant changes in the surface pressure with phosphate. (b) **U-guan**$^+$ shows a minimal expansion, and (d) **U-thiouro**$^+$ exhibits a large monolayer expansion on phosphate, strongly suggesting binding. Shaded regions represent one standard deviation above and below the mean.

displaced portion of the hydration sphere. Weaker hydrogen-bonding interactions stemming from the receptor are likely compensated by the stabilizing nature of the ion–ion interactions at the air–receptor interface. In addition to the phosphate modes there is further spectroscopic evidence to verify the extent of phosphate binding. In the **U-guan**$^+$ spectra (Figure 2b, black), we tentatively assign the peak at approximately 1362 cm^{-1} to the asymmetric N–C–N stretch (ν_{as} NCN) based on tetramethyl guanidine.[59] This mode decreases in intensity upon phosphate addition in a way that correlates with phosphate interaction. The IRRAS spectra of **U-thiouro**$^+$ on 10 mM phosphate (Figure S9) with *p* polarization and *s* polarization shows a blue shift (~12 cm^{-1}) and narrowing of the 1600 cm^{-1} peak upon phosphate addition, providing additional support for binding. This mode is assigned to the CN stretch (ν CN)[60] with additional contribution from the N–H bend (δ N–H).[61]

Surface pressure–mean molecular area (Π–*A*) compression isotherms were also recorded to test for phosphate recognition. However, Π–*A* isotherms of **S-guan**$^+$, **U-guan**$^+$, **U-thio**, and **U-thiouro**$^+$ receptors (Figure 3a, 3b, 3c, and 3d, respectively) on water and 10 mM sodium dihydrogen phosphate do not always show changes in the presence of phosphate. Π–*A* isotherm results of the **U-thio** and **S-guan**$^+$ receptors on 10 mM phosphate were identical to trials on water (within one standard deviation), and the **U-guan**$^+$ receptor showed only a minimal expansion. For these three receptors the Π–*A* isotherms did not produce a significant expansion or compression of the monolayer upon phosphate addition, giving the appearance that the receptors were not interacting with phosphate. It is important to recognize, however, that the isotherms for the U-shaped receptors composed of **U-guan**$^+$ and **U-thio** may not be informative for binding as it is possible that the large size of the two alkyl chains obscure changes associated with the headgroup region. The interfacial organization of these U-shaped receptors (Figure 4) is expected to direct the binding site (i.e., headgroup) and thus

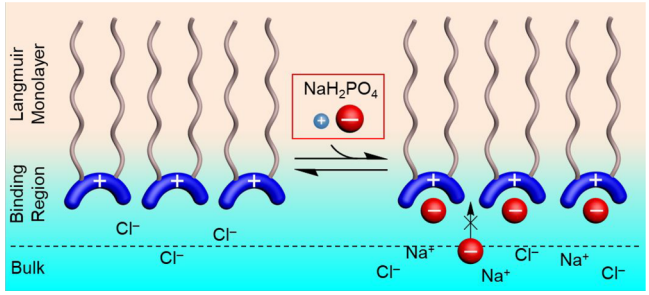

Figure 4. Schematic representation of the hypothesized binding event for the U-shaped receptors. Phosphate is binding beneath the monolayer instead of in between the molecules of the monolayer.

any bound phosphate into the region beneath the monolayer. This binding arrangement of phosphate will not necessarily influence the alkyl chain packing. With this binding mechanism in mind for these U-shaped receptors it is possible that changes in the Π–*A* packing density would be negligible.

However, for the single-chain receptor (**S-guan**$^+$), Π–*A* isotherms should be more sensitive to binding because of the higher packing density. If the **S-guan**$^+$ receptors were binding phosphate, the phosphate anions could be driven in between the receptor molecules, and a change in the mean molecular area (MMA) of the monolayer would confirm binding. However, the isotherm for the **S-guan**$^+$ receptor does not change with phosphate (Figure 3a), which suggests the **S-guan**$^+$ receptor is not interacting with phosphate.

Impact of the Supramolecular Superstructure. It is important to appreciate differences in the environment confined by the 2D air–water interface compared to host–guest recognition chemistry commonly studied in bulk

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solution. Here, the receptors are tethered to the air−water interface, and when situated in the confined space of the monolayer, increased receptor−receptor interactions can occur. With the intermolecular spacing of **S-guan**$^+$ defined by just one alkyl chain, we believe it does not separate the guanidinium headgroups from each other. They are thus free to interact with neighboring headgroups (receptor to receptor). It has been shown[62−68] that guanidinium moieties can form pairs in water despite being similarly charged and are known as "magic" arginine complexes.[69] They fall into a growing set of same-charge ions that can associate together.[70−78] The guanidinium−guanidinium interactions are consistent with our results, and it is therefore reasonable to conceive that the receptor can self-associate and contribute to the inhibition of phosphate binding.

In the condensed state of the **S-guan**$^+$ monolayer (i.e., compacted) we suggest that the close proximity of the headgroups allows for self-association. By contrast, the U-shaped receptors cannot easily form interactions with neighboring receptor headgroups on the basis of the larger intermolecular spacing defined by the alkyl chains. All our results illustrate that the charged **U-guan**$^+$ and **U-thiouro**$^+$ receptors are interacting with phosphate, yet the neutral **U-thio** receptor is not. These findings demonstrate the importance of electrostatics on the binding affinity. The thiouronium and guanidinium headgroups of **U-thiouro**$^+$ and **U-guan**$^+$ can form charge-assisted hydrogen bonding[1] to the phosphate, and the combination of electrostatics and hydrogen bonding provides greater phosphate affinity. Additionally, we propose that the low dielectric constant[30] and preorganization of the monolayers at the air−water interface[26] further stabilize this interfacial binding.

Spectroscopic results proved to be more informative than Π−*A* isotherms alone and give mechanistic insight into the receptor−phosphate interaction; nevertheless, Π−*A* isotherms still offer valuable information for the **U-thiouro**$^+$ receptor. Returning to the Π−*A* isotherms (Figure 3d), **U-thiouro**$^+$ has a considerable mean molecular expansion (MME) from 39.4 (±0.6) Å^2 on water to 99.0 (±0.7) Å^2 on 10 mM phosphate, taken at a surface pressure of 5 mN/m (Table 1). The 59.6 Å^2

Table 1. Summary of Binding Interactions Observed by Π−*A* Isotherms and IRRAS

method	U-thio	U-thiouro$^+$	U-guan$^+$	S-guan$^+$
Π−*A*	no response	large expansion	minor response	no response
IRRAS	no response	binding	binding	no response

expansion is too large to rationalize simply as coming from the binding of a single phosphate anion, and thus, a reasonable explanation for this behavior is supramolecular reorganization[50] within the monolayer.

To elucidate any reorganization, IRRAS spectra of the **U-thiouro**$^+$ receptor were taken at 5, 10, and 40 mN/m on both water and 10 mM phosphate (Figure 5a and 5b). The peak at 1468 cm^{-1} is assigned to the CH_2 scissoring (δ CH_2), and peaks at 2963, 2924, 2883, and 2850 cm^{-1} are the CH_3-asymmetric (ν_{as} CH_3), CH_2-asymmetric (ν_{as} CH_2), CH_3-symmetric (ν_s CH_3), and CH_2-symmetric (ν_s CH_2) stretching modes, respectively. The lowering of the intensity is consistent with lower molecular density. Yet, the scissoring mode at 1468 cm^{-1} remains at the same relative peak position at both low and high pressures. The existence of the single peak and at a

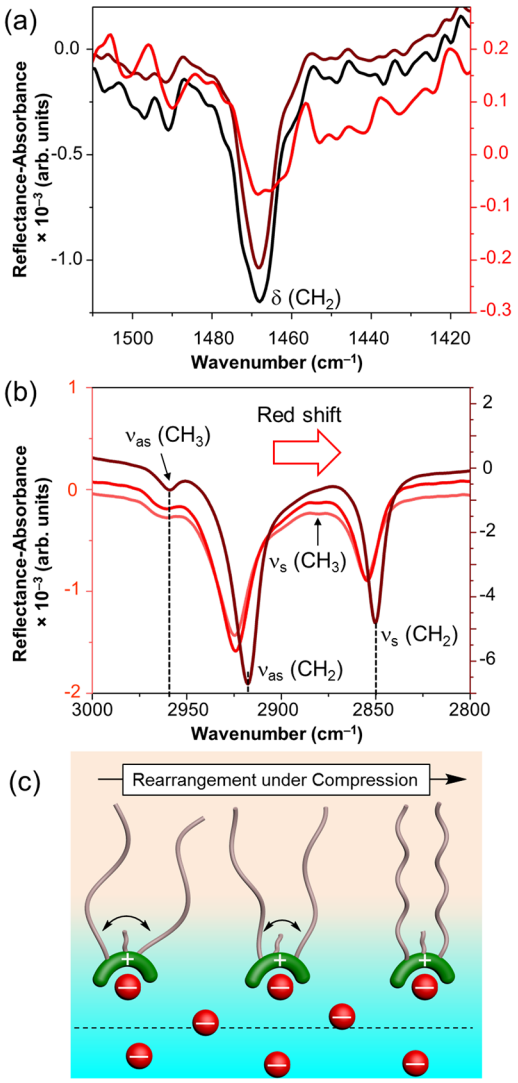

Figure 5. IRRAS spectra showing the **U-thiouro**$^+$ monolayer reorganizes upon phosphate binding. (a) CH_2 scissoring mode (1468 cm^{-1}) of **U-thiouro**$^+$ with 10 mM phosphate at 10 (red) and 40 mN/m (dark red) and without phosphate (black). (b) Alkyl stretching modes (between 3000 to 2800 cm^{-1}) with 10 mM phosphate at 5 (light red), 10 (red), and 40 mN/m (dark red). Alkyl peak positions of **U-thiouro**$^+$ on water are shown as black dotted lines for reference. (c) Schematic illustration of the **U-thiouro**$^+$ headgroup reorganizing and impacting alkyl chain conformations (see monolayer expansion in Figure 3d).

constant peak position suggests the monolayer remains in a hexagonal lattice structure throughout the isotherm (Figure 5a).[79]

Comparing the **U-thiouro**$^+$ receptor on phosphate at low surface coverage at 5 and 10 mN/m with their larger MMA to the more condensed monolayer with a smaller MMA at 40 mN/m, we observe a diagnostic red shift in the ν_{as} CH_2 and ν_s CH_2 stretching modes (Figure 5b). To emphasize this red shift, the spectra recorded at different pressures (Figure 5b) are plotted on different axis scales and the relative peak positions of **U-thiouro**$^+$ on water are included as black dotted lines. Upon phosphate binding, the molecular monolayer undergoes reorganization. The red shift of the CH_2 modes indicates greater gauche conformers in the earlier expanded regions of the monolayer (i.e., lower Π, larger MMA) with more trans

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conformers in the later stages of the isotherm (i.e., higher Π, smaller MMA). Furthermore, the presence of the methyls' symmetric stretch, ν_s CH_3 at lower pressure (5 and 10 mN/m) but not at higher pressure (40 mN/m), supports this hypothesis of reorganization.

Phase changes and reorganization induced through interfacial interactions are common in biological systems that occur when phospholipids bind to calcium,[80−82] proteins,[83,84] and cholesterol.[85−87] Interestingly, this change from a highly disordered monolayer to a more ordered one upon phosphate binding is unique to the **U-thiouro**$^+$ receptor, as we do not observe this change with the other receptors. We believe this could come as a result of the bulky methyl group residing on the positively charged sulfur atom of the thiouronium headgroup. Upon binding, this methyl may need to reorganize to accommodate the large phosphate guest (Figure 5c).

Phosphate Selectivity. It is apparent from the IRRAS results that the **U-guan**$^+$ and **U-thiouro**$^+$ receptors are promising for phosphate recognition. Therefore, it is important to determine whether these receptors have selectivity toward phosphate over competing anions. We focus here on chloride because of its prevalence in natural water (0−100 mg L^{-1}).[88] To evaluate phosphate selectivity, we recorded IRRAS spectra (Figure 6) of these receptors when the subphase was composed of a mixture of phosphate and different relative equivalents of chloride.[89] The IRRAS spectra of the **U-guan**$^+$ receptor were recorded with the two anions present at a 1:1 molar ratio at 10 and 1 mM. Under these conditions (Figure 6a) the phosphate modes are present, suggesting that the monolayer of the **U-guan**$^+$ receptor is selective for phosphate over chloride at this ratio.

The ratio of the two anions was altered to evaluate the extent of the selective recognition of phosphate over chloride. Interestingly, by increasing the relative amount of chloride (10 mM NaH_2PO_4 versus 100 mM NaCl), the phosphate modes are seen to diminish (Figure 6b). We can infer from this observation that the **U-guan**$^+$ receptor has lost selectivity toward phosphate under these conditions. At 100 mM, charge screening can retard the electrostatic interactions[90−92] between **U-guan**$^+$ and phosphate in a manner consistent with the electrical double-layer theory.[93] To evaluate this further, we tested the selectivity of **U-guan**$^+$ toward phosphate at a variety of chloride concentrations (0.1−100 mM) while maintaining the 1:10 ratio of phosphate to chloride (Figure 6b and 6c). Across these concentrations, the Debye length shrinks from ∼30 to 1 nm. Phosphate modes are present at lower chloride concentrations (0.1−10 mM Cl^-), suggesting the **U-guan**$^+$ receptor is selective for phosphate by outcompeting chloride (Figure 6b).

A plot of the absolute integration (1120−1020 cm^{-1}) of the phosphate peak ν_s $(PO)_2$ as a function of chloride concentration (Figure 6c) shows a clear trend with the phosphate integration reaching a maximal plateau when the chloride concentration is at or below 1 mM. We further characterized the phosphate selectivity of the **U-guan**$^+$ receptor using a chloride concentration of 1 mM to mimic the concentration of chloride present in natural waters[88] and to limit the negative effects of charge screening. On the basis of the presence of the phosphate modes in the IRRAS spectra (Figure 7), the **U-guan**$^+$ receptor shows selectivity toward phosphate up to a factor of 1000. It is clear (Figure 7) that the **U-guan**$^+$ receptor demonstrates high selectivity toward phosphate over chloride. This selectivity displays *anti-*

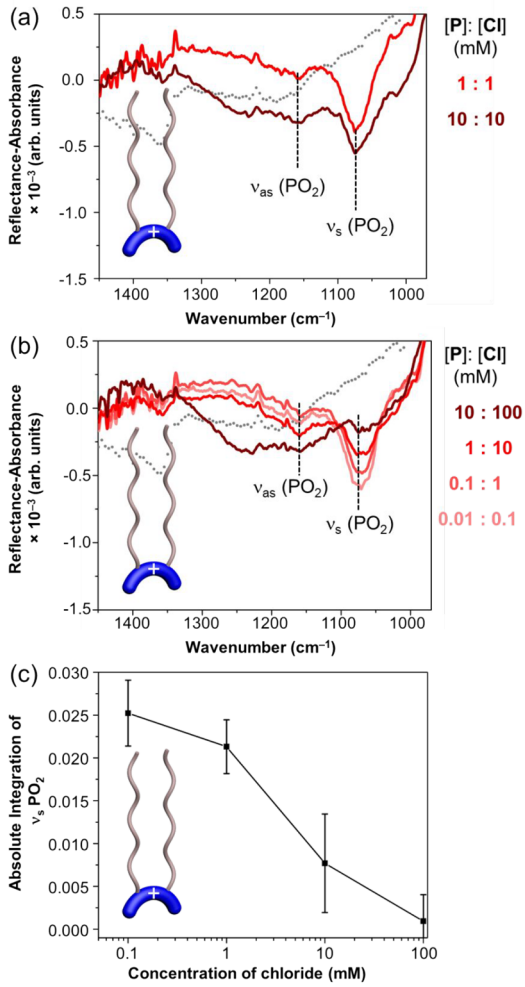

Figure 6. Competitive binding between phosphate and chloride for the **U-guan**$^+$ receptor at 40 mN/m investigated using IRRAS spectroscopy. IRRAS spectrum on water is shown as a gray dotted line for clarity. (a) **U-guan**$^+$ receptor shows good selectivity at 1:1 equivalence ($[H_2PO_4^-]$:$[Cl^-]$ = [P]:[Cl]). (b) Selectivity at 1:10 molar ratio ([P]:[Cl]) depends on the total concentration of chloride. (c) Absolute integration of the ν_s (PO_2) peak (1120−1020 cm^{-1}) of the **U-guan**$^+$ receptor with the integration of **U-guan**$^+$ on water subtracted from each integration. (d) Schematic representation of the phosphate binding with **U-guan**$^+$ at aqueous interface.

Hofmeister behavior, i.e., $H_2PO_4^-$ ≫ Cl^-.[32,94−97] Typically, Hofmeister binding selectivity suggests that chloride would have stronger binding than phosphate on account of its smaller hydration energy (−381 kJ/mol). Interestingly, the selective phosphate binding also reverses expectations from Collins' rule, which is known as "anions and cations form stable ion pair interactions only when their hydration enthalpies match".[98,99] The hydration energies are ranked as phosphate (−465 kJ/mol), sodium (−409 kJ/mol), and chloride (−381 kJ/mol), while guanidinium is much more weakly hydrated.[100] Consequently, the pairing of the most hydrated phosphate with least hydrated guanidinium is not consistent with Collins' rule.

We also evaluated the selectivity of the **U-thiouro**$^+$ receptor toward phosphate (Figure 8). The selectivity experiments for **U-thiouro**$^+$ at 1:1 phosphate to chloride (1 mM) show the presence of the phosphate modes and the selectivity of the **U-thiouro**$^+$ receptor. However, upon decreasing the phosphate

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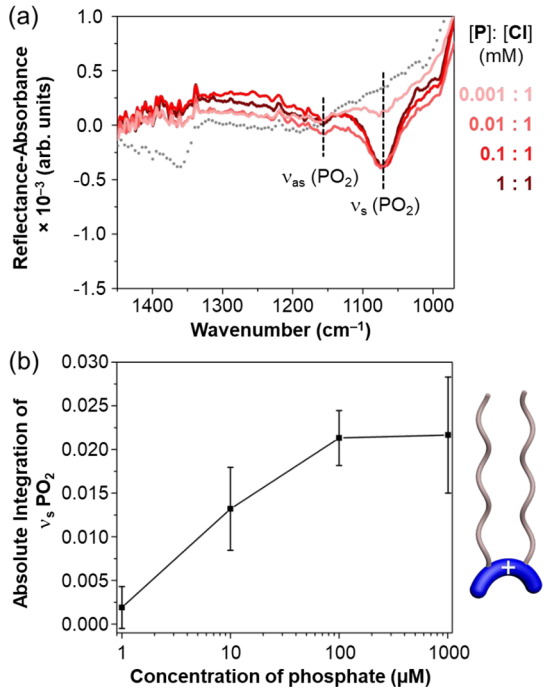

Figure 7. Maintaining a constant chloride background concentration of 1 mM NaCl (a) IRRAS spectra reveal the phosphate modes up to 1:1000 ratios of phosphate to chloride ([P]: [Cl]) and (b) integration of the ν_s (PO_2) further supports the high selectivity of the **U-guan**$^+$ receptor.

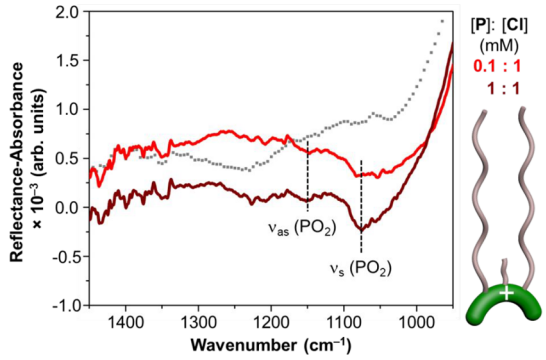

Figure 8. Competitive binding of **U-thiouro**$^+$ shows preference for phosphate over chloride at a 1:1 ratio, but there is less preference toward phosphate observed at 1:10 phosphate to chloride. IRRAS spectrum on water is shown as a gray dotted line for clarity

concentration to test a 1:10 ratio, the **U-thiouro**$^+$ receptor shows a sharp decrease in phosphate peak intensity with a loss in selectivity. We infer from this observation that the guanidinium receptor **U-guan**$^+$ is more selective than the thiouronium **U-thiouro**$^+$ receptor for phosphate (Figures 7 and 8). The exact origin is the topic of current work in our laboratories. First, the calculated electrostatic potential of the hydrogen atoms on the binding motifs (N−H) is similar for **U-guan**$^+$ and **U-thiouro**$^+$ (Figure S10). However, the guanidinium has two more potential hydrogen-bonding donors than the thiouronium. Second, the bulky methyl residing on the sulfur atom of the thiouronium is believed to undergo reorganization upon phosphate binding (Figure 5c), which would introduce a free energy penalty that needs to be paid upon binding phosphate. The organizational differences are the most probable cause for this difference.

Ultimately, the selectivity experiments support our findings that electrostatics play a dominant role in the binding affinity. **U-guan**$^+$ and **U-thiouro**$^+$ bind stronger to phosphate than **U-thio**. We also see that when electrostatics are screened by Cl^-, only the hydrogen bonding of **U-guan**$^+$ remains and is not strong enough to bind the phosphate (Figure 9a). The finding is consistent with the negligible binding seen with **U-thio**. Our results also suggest that both the charged receptors **U-guan**$^+$ and **U-thiouro**$^+$ possess selectivity toward phosphate over chloride. This finding is consistent with our hypothesis that the headgroups of **U-guan**$^+$ and **U-thiouro**$^+$ promote phosphate selectivity by charge-assisted hydrogen bonding (Figure 9b and 9c).

At the aqueous interface, the driving forces we have identified to influence recognition come from the receptor, double-layer electrostatics, and receptor's organization in the monolayer. Charges are essential for synergizing with the hydrogen bonding present in the **U-guan**$^+$ and **U-thiouro**$^+$ receptors to drive phosphate binding and selectivity. We demonstrated that screening of the double-layer charge distribution will shut down binding. We also showed that charges alone are not enough to enable phosphate binding. When using the **S-guan**$^+$ receptor, binding was not observed. Thus, we also found that the supramolecular superstructure of the monolayer influenced binding and that the binding site must be accessible to the aqueous interface, that is, the U-shaped receptor **U-guan**$^+$ was found to bind phosphate likely by directing the guanidinium binding site down toward the water subphase to enable binding. In support of this idea, Buhlmann and Umezawa[32] previously suggested that allowing phosphate to retain some of its hydration shell will enable recognition to proceed in a way defined by the binding site instead of by the steep cost of complete dehydration. We therefore believe that the supramolecular superstructure within the monolayer is critical to controlling the location of the binding site relative to the aqueous interface. In this case, the alkyl groups in the U-shaped receptors provide adequate separation to accommodate the guest and hinder any receptor−receptor interactions in the confined space of the monolayer. Of all these driving forces, the selection of the binding unit and its organization relative to the aqueous binding region are subject to molecular synthetic design and need to be controlled to enhance phosphate recognition at aqueous interfaces.

By mimicking the hydrophobic binding sites on the surface of proteins and the resulting low dielectric constant present at these locations,[39,101,102] we exploit the interface to boost affinity in our synthetic receptor system. The role of the lower dielectric constant at the interface is believed to enhance electrostatic interactions. It is, therefore, not surprising that the **U-thiouro**$^+$ and **U-guan**$^+$ are stronger receptors for phosphate recognition than **U-thio** on account of the importance of this physical characteristic of the interface. Throughout this work we have shown that chemical, physical, and supramolecular superstructure features serve as important design criteria and that each plays an influential role in phosphate recognition in these monolayer systems. These principles can be applied to the design of sensor and sequestration materials targeted for phosphate in which functional interfaces between aqueous solutions and solids are prevalent.[32,103−106]

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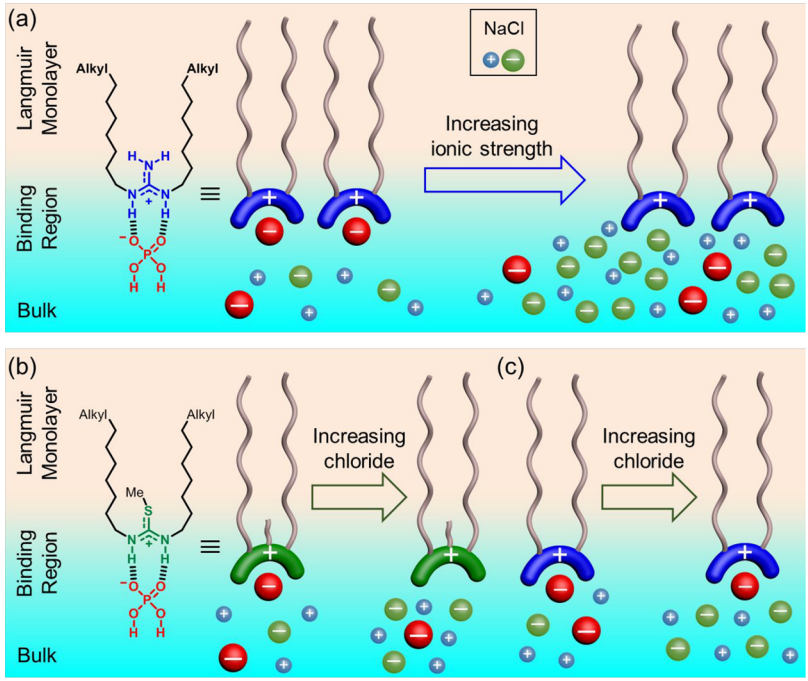

Figure 9. Schematic illustration of (a) the charge screening at high ionic strength observed for **U-guan**$^+$, (b) **U-thiouro**$^+$ outcompeting chloride to bind phosphate with 10−fold selectivity, and (c) **U-guan**$^+$ displaying even higher selectivity for phosphate at 10^3 fold.

CONCLUSION

We used a homologous series of amphiphilic receptors differing in charge (positive or neutral) and supramolecular organization (single or double U-shaped chains) to better understand the design features that impact phosphate recognition (affinity and selectivity) at monolayer−aqueous interfaces. We find the guanidinium **U-guan**$^+$ and thiouronium **U-thiouro**$^+$ receptors bind phosphate through charge-assisted hydrogen bonding in the aqueous region created beneath the larger U-shaped anchoring groups. The uncharged **U-thio** and the single-chain but charged **S-guan**$^+$ receptors do not bind phosphate. The **S-guan**$^+$ receptor does not interact with phosphate as a result of compaction of the monolayer possibly from headgroup−headgroup interactions facilitated by smaller anchoring groups. For the **U-thiouro**$^+$ receptor, phosphate binding within the monolayer is evident from changes in the ratio of gauche to trans conformers during compression of the Π−*A* isotherm. Only the **U-thiouro**$^+$ receptor underwent this structural reorganization upon binding, giving insights into the behavior of the additional bulky methyl group. Competition studies show that the **U-guan**$^+$ receptor is selective to phosphate over chloride by a factor of 1000, but careful consideration must be given to the ionic strength of the solution since charge screening of the electrical double layer (≥10 mM NaCl) can weaken binding and alter the interpretation of the results. The **U-thiouro**$^+$ receptor was also selective toward phosphate over chloride but only by a factor of 10. Our results provide mechanistic insights into phosphate binding at aqueous interfaces, advancing a deeper understanding of anion recognition and promoting the remarkable advantages of the interfacial environment over traditional homogeneous solution.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.9b02148.

Experimental materials, instrumentation details, synthetic procedures, and characterization of the **U-thio**, **S-guan**$^+$, **U-guan**$^+$, and **U-thiouro**$^+$ receptors; Raman spectroscopy calibration curve of the filtered phosphate solution; Π−*A* and IRRAS of the receptors using a filtered phosphate solution; IRRAS control experiments with eicosane, perfluorotetradecanoic acid, and tripalmitin; calculated electrostatic potential maps (PDF)

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Notes

The authors declare no competing financial interest.

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