

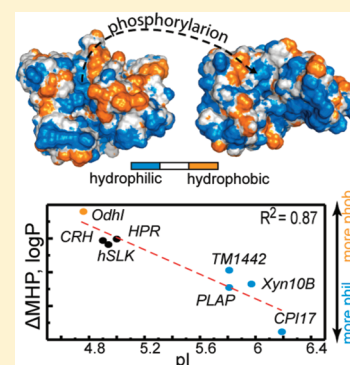
Protein Electrostatic Properties Predefining the Level of Surface Hydrophobicity Change upon Phosphorylation

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Supporting Information

ABSTRACT: We use explicit-solvent, molecular dynamics simulations to study the change in polar properties of a solvent-accessible surface for proteins undergoing phosphorylation. We analyze eight different pairs of proteins representing different structural classes in native and phosphorylated states and estimate the polarity of their surface using the molecular hydrophobicity potential approach. Whereas the phosphorylation-induced hydrophobicity change in the vicinity of phosphosites does not vary strongly among the studied proteins, the equivalent change for complete proteins covers a surprisingly wide range of effects including even an increase in the overall hydrophobicity in some cases. Importantly, the observed changes are strongly related to electrostatic properties of proteins, such as the net charge per residue, the distribution of charged side-chain contacts, and the isoelectric point. These features predefine the level of surface hydrophobicity change upon phosphorylation and may thus contribute to the phosphorylation-induced alteration of the interactions between a protein and its environment.

SECTION: Glasses, Colloids, Polymers, and Soft Matter



Phosphorylation is arguably one of the most important mechanisms of intracellular signaling and metabolic control.¹ Importantly, phosphorylation modulates physical–chemical properties of modified proteins in a highly regulated, reversible way. This not only provides a basis for a wide range of possible structural rearrangements and alterations of protein conformational dynamics^{1–3} but also affects more global aspects such as protein localization in the cell.^{4–8} The structural and dynamical consequences of phosphorylation have been addressed in a number of experimental and computational studies, which provided a solid understanding of the atomistic details of the process.^{3,9–12} At the same time, the physical–chemical principles underlying how phosphorylation might control cellular localization of proteins or perturb their interaction networks are still far from being fully understood. In particular, it is important to understand the phosphorylation-induced change in the overall protein surface hydrophobicity, especially given recent suggestions that it may be an important determinant of colocalization of binding partners in the cell.¹³

Here, we perform a set of 150 ns long explicit-solvent molecular dynamics (MD) simulations of eight different proteins in native and phosphorylated states (Table S1, Supporting Information), quantifying ensemble-averaged polar properties of their surface according to the molecular hydrophobicity potential (MHP) approach.¹⁴ The MHP formalism is based on empirical atomic hydrophobicity constants (i.e., hydrophobicity weights) derived

from partition coefficients, $\log P$, of various compounds between water and *n*-octanol. In qualitative analogy with the electrostatic Coulomb potential, at each point of the molecular surface, the algorithm sums MHP contributions of each atom, using exponential distance-dependent weighting to derive the net hydrophobicity value for each point on the surface. One can also use the sum of MHP values on the protein surface, MHP^{sas} , as a measure of its overall hydrophobicity. Negative values of $\Delta\text{MHP}^{\text{sas}}$ should be interpreted as a decrease in hydrophobicity of a protein upon phosphorylation and vice versa (more details about MHP are given in the Supporting Information). Similar techniques were used recently to estimate the hydrophobic effect of protein carbonylation.¹⁵ In the present study, we relate the surface hydrophobicity change to the proteins' electrostatic properties, such as the electrostatic potential and contact network of charged side chains. Importantly, for each protein, we analyze the impact of phosphorylation at three different levels, (1) for the modified residue only, (2) for an immediate vicinity of the modified residue, and (3) for the complete protein surface. To ensure the generality of our analysis, we chose proteins of different sizes (86–484 residues), source organisms (bacterial and eukaryotic), structural classes (α , α/β ,

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β), levels of foldedness (folded or disordered), and containing different types of modifications (pSER, pTHR, or both) (Table S1, Supporting Information).

Structural deviations of most protein structures from the initial conformations display stable behavior already after the first 15 ns of MD (Figure S2, Supporting Information). Therefore, we use all protein conformations from the last 135 ns of each trajectory for all further analyses. Interestingly, the observed structural changes in most cases are less pronounced for phosphorylated structures, agreeing well with previous MD simulations.³ A possible explanation can be that the phospho group, because of its high charge, introduces additional favorable contacts between protein residues, reducing molecular flexibility in this way. What is more, an increase in negative–negative repulsion upon phosphorylation can also rigidify a protein by decreasing the number of favorable conformations accessible to it.¹⁶

Upon phosphorylation, modified residues gain chemical properties that are not shared by any natural amino acids, including a double negative charge at physiological pH ($pK_a \approx 6.7$). Estimation of their hydrophobic properties by calculating MHP values as sums of atomic hydrophobicity constants for individual side chains reveals, expectedly, that phosphorylation makes them prominently hydrophilic (Figure 1A). For example,

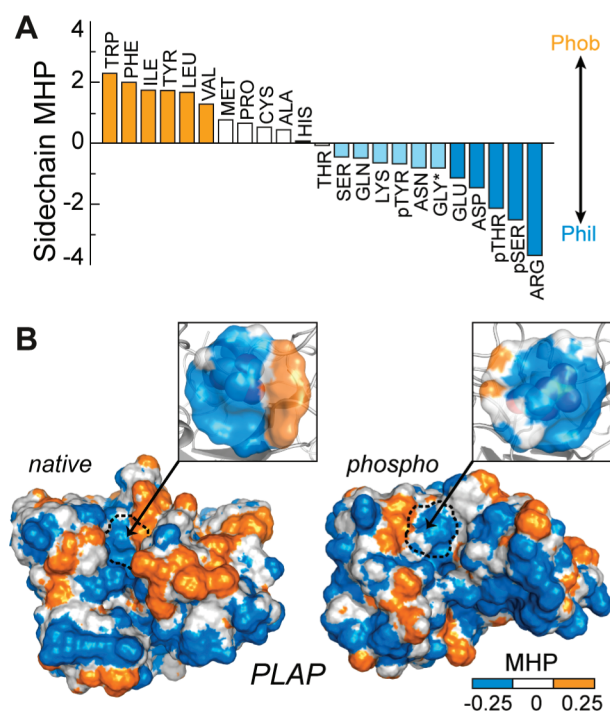


Figure 1. (A) The relative MHP scale for amino acid side chains. A value for GLY represents the hydrophobicity of the backbone. (B) MD conformations of native and phosphorylated forms of protein PLAP. The phosphosite vicinities are given in the insets. The protein's SAS is colored according to MHP values in log P units.

the predicted free energy of water/octanol partitioning for phospho-serine (pSER) is -4.37 kJ/mol, comparable in magnitude only to arginine (Figure S1, Supporting Information), with which, parenthetically, phosphorylated residues often form stable pairs in protein structures.¹¹ Following this analysis, one could expect that phosphorylation would consequently lower the overall hydrophobicity of complete

protein structures by a similar amount. Our simulations allowed us to test this hypothesis directly.

A typical protein surface exhibits a mosaic distribution of hydrophobic and hydrophilic areas (Figure 1B), whose polarity can be quantified using the MHP approach. We used the sum of MHP values over the protein solvent-accessible surface (MHP^{sas}) and its difference between phosphorylated and native structures (ΔMHP^{sas}) as a measure of the effect of phosphorylation (see the Supporting Information for details). The local decrease of hydrophobicity near phosphosites (all points within 6.5 Å of the modified residues) is approximately constant and relatively similar for all proteins (Figure 2A, open

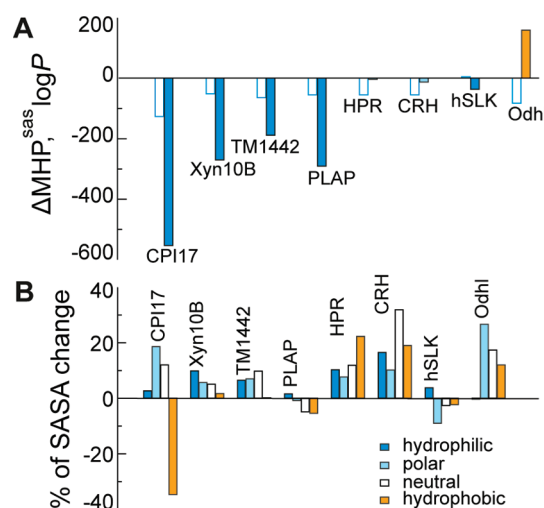


Figure 2. (A) Phosphorylation-induced changes of hydrophobicity at the phosphosites (open bars) and for the whole molecule (filled bars). (B) Relative differences in the solvent-accessible surface area (SASA) for residues of different types between phosphorylated and native protein states. Residues are grouped and colored according to their MHP values shown in Figure 1A.

bars), except for the doubly phosphorylated hSLK. On the other hand, a similar analysis for complete proteins produced unexpected results. At odds with an intuitive decrease of hydrophobicity for phosphorylated proteins, we observe a wide range of effects (Figure 2A, filled bars), a prominent drop in ΔMHP^{sas} values (CPI17, PLAP, Xyn10B, TM1442), almost no change (hSLK, CRH, HPR), and even an opposite effect (increase of hydrophobicity for Odhl), agreeing with what was also experimentally detected for some proteins.¹⁷ Importantly, our simulations gave a microscopic picture behind these changes. In general, we see a decrease in hydrophobicity as a consequence of two different types of rearrangements of the protein surface in the phosphorylated state, a burial of hydrophobic residues (CPI17, PLAP) or an exposure of prominently hydrophilic residues (Xyn10B, TM1442). Also, in some cases, strong perturbation of SASA, with compensatory changes in the exposure of hydrophobic and hydrophilic groups, displays no effect on hydrophobicity (CRH, HPR) (Figure 2B).

Phosphorylation also strongly affects local and global profiles of the electrostatic potential (Figure S3, Supporting Information), where the specific effect depends on the individual protein. Importantly, there is a strong connection between the effect of phosphorylation on the hydrophobicity change and basic protein electrostatic properties in the native state, such as the net charge per residue (Figure 3A). This protein characteristic

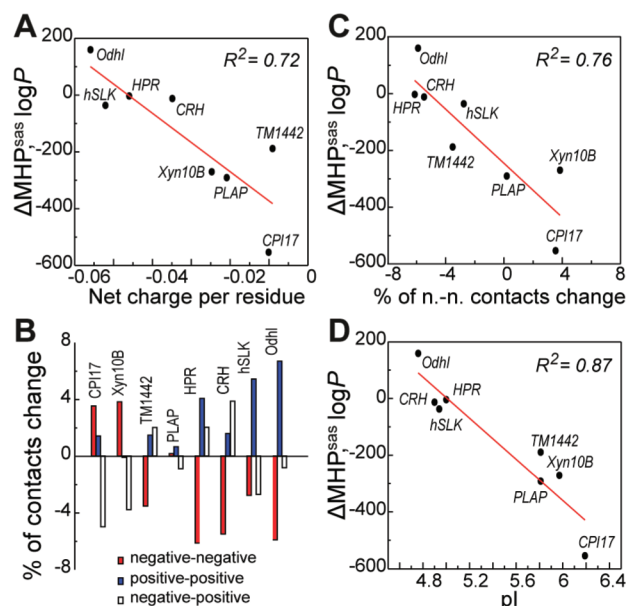


Figure 3. (A) Correlation between the hydrophobicity change upon phosphorylation ($\Delta\text{MHP}^{\text{sas}}$) and the protein net charge per residue. (B) Adjustment of the contact network of charged side chains. Relative changes in the ratio of contacts of different types between phosphorylated and native states of the proteins. (C) Correlation in the changes of protein hydrophobicity and the ratio of anionic side chain repulsion. (D) The hydrophobicity drop upon phosphorylation depends on isoelectric points of the proteins in the unphosphorylated state.

is shown to play an important role in the conformational behavior of disordered proteins.¹⁸ Here, we observe that the net charge per residue in the native structure inversely correlates with differences in the protein hydrophobicity upon phosphorylation ($R^2 = 0.72$), with no apparent connection with their structural properties as all of the proteins in our simulations follow the same trend. Because each phosphorylated residue decreases the net charge by -2 , the change in the net charge per residues upon phosphorylation depends only on protein size and varies here between -0.004 (PLAP, the biggest protein) and -0.023 (CRH, the smallest protein). Importantly, there is little correlation between the change in the net charge per residue and the change in the protein hydrophobicity; strong correlations are seen only between the latter and the absolute net charge per residue of native protein structures. A possible interpretation of the fact that the change in hydrophobicity is related to the net charge per residue in the native state is that an attachment of additional negative charge to an already highly negatively charged surface induces different structural rearrangements compared to the case of a moderately charged one. For instance, phosphorylation strongly perturbs the network of contacts between basic and acidic residues on the protein surface, reducing or increasing the extent of mutual repulsion or attraction (Figure 3B). Here, two residues are defined to be in contact if the distance between any two of their atoms is less than 6.5 \AA . Proteins with a highly negative value of net charge per residue tend to reduce the number of negative–negative contacts in order to accommodate the phosphate group. This can also be accompanied by increasing the exposure of hydrophobic side chains (HPR, CHR, Odhl) (Figure 2B). For proteins with a moderate net charge per residue, an increase of negative–negative contacts upon phosphorylation is less critical and even might correlate with strong structural stabilization, collapse of the chain,

and a prominent decrease of hydrophobicity (in the case of disordered CPI17). A similar stabilization effect of negative–negative contacts was previously also reported for other disordered proteins.¹⁶ A strong relationship between basic side-chain repulsion and surface evolution is illustrated by the high correlation ($R^2 = 0.76$) between phosphorylation-induced reduction in the number of negative–negative contacts and hydrophobicity (Figure 3C).

To generalize the relation between protein electrostatics and tuning of the hydrophobic properties upon phosphorylation, we calculated the isoelectric point (pI) for each protein from the set using a sequence-based algorithm¹⁹ and plotted these values against phosphorylation-induced hydrophobicity changes (Figure 3D). Remarkably, $\Delta\text{MHP}^{\text{sas}}$ values for proteins in our set strongly correlate with the respective pIs ($R^2 = 0.87$). Thus, the most prominent decrease in hydrophobicity is observed for the most basic protein in the set (CPI17), while the most acidic protein (Odhl) undergoes changes in the opposite direction. The proteins that become more hydrophilic upon phosphorylation have $\text{pI} > 5.5$ (PLAP, Xyn10B, TM1442), while almost no effect on hydrophobicity is observed for acidic proteins with $\text{pI} \leq 5$ (hSLK, CRH, HPR). Importantly, this relation between pI and hydrophobicity change upon phosphorylation does not depend on the protein size or source organism but seems to be dictated by its primary sequence. Thus, electrostatic properties of proteins predefine the direction of the tuning of their hydrophobic/hydrophilic properties after the attachment of the phospho group.

We speculate that modulation of physical–chemical properties (e.g., hydrophobicity) of the protein molecule induced by attachment of phospho groups gives the basis for a general mechanism of changing the character of interactions of a protein with its environment and maybe a general, nonspecific way of affecting protein localization.¹³ This process would in some ways be analogous with protein lipidation, whereby the addition and/or exposure of a lipophilic group, such as myristate, influences protein localization to the membrane.^{20,21} Importantly, these nonspecific effects would then complement the specific structural consequences of phosphorylation in affecting functional activation of proteins, fine-tuning of their interactions with binding partners, or their localization.

■ ASSOCIATED CONTENT

Supporting Information

A table with detailed description of the proteins under study, a table with MD MHP values of the proteins, a table with details of the MD protocols and analysis procedures, a figure showing correlations between MHP values for standard residues and the respective experimental values of the partition free energy, a figure with MD rmsd curves, and a figure showing changes of the electrostatic potential upon phosphorylation. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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