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Perspectives for the Study of ^{18}O Isotope Effects of Enzymatic Phosphoryl Transfer Reactions by ESI-Orbitrap MS

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Abstract: Isotope effects play an important role in investigating enzymatic phosphoryl transfer reactions, which are central in biochemical pathways and environmental metabolism. However, resolving different isotope effects for mechanistic insights into phosphoryl transfer reactions with stable isotope analysis at natural abundance remains challenging. Current analytical methods primarily allow ^{18}O/^{16}O ratio measurements of phosphate and preclude efficient quantification at different reaction extents. Moreover, various oxygen bond changes make mechanistic interpretation of observable O isotope fractionation particularly difficult. Here, we review recent advances in electrospray ionization Orbitrap mass spectrometry for O isotope analysis in phosphate and organophosphates, and the state-of-knowledge on position-specific isotope effects in phosphoryl transfer reactions. Using a kinetic toy model, we illustrate that further development of ^{18}O/^{16}O ratio analysis with Orbitrap MS should include quantification of O isotope fractionation in organophosphate substrates and alcohol leaving groups to evaluate nucleophilic attack and leaving group departure isotope effects.

Keywords: Isotope effects · Isotope fractionation · Orbitrap MS · Oxygen isotope ratios · Phosphoryl transfer reactions

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1. Introduction

Enzyme-catalysed phosphoryl transfer reactions are fundamental to many biochemical, biological and environmental processes, including transcription, protein regulation, and metabolism.[1–3] In biology and medicine, these reactions and the involved enzymes are targets for drug design and enzyme engineering.[4,5] In earth and environmental sciences, they serve as proxies for metabolic processes and ecosystem functioning.[6,7] In both disciplines, stable isotope-based approaches are crucial for evaluating reaction mechanisms, enzyme function,[8–10] and tracing biochemical pathways. Isotope fractionation, the subtle changes in isotopic compositions, is interpreted as kinetic and equilibrium isotope effects, offering unique insights into bonding changes in reactive processes.[11–14]

Methods deriving isotope effects from isotope fractionation in reactants and/or products are valuable as they operate at natural isotopic abundance without isotopically labelled materials. These methods are well-established and have been applied to study numerous chemical and biological reactions and various isotopic elements.[15,16] However, this principle does not apply to phosphoryl transfer reactions and ^{18}O isotope effects related to oxygen bond changes due to two critical factors. First, isotope ratio mass spectrometry requires cumbersome sample preparation for ^{18}O/^{16}O ratio measurements in organophosphorus compounds and inorganic phosphate, making O isotope fractionation determination over the course of the reaction rare. Second, phosphoryl transfer reactions involve both O bond cleavage in the leaving group and bond formation from nucleophilic attack by O-containing nucleophiles, subjecting O isotope fractionation to changes in multiple oxygen bonds. These circumstances make the interpretation of O isotope fractionation particularly challenging. While sophisticated labelling schemes partially circumvent this issue,[17,18] they

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preclude studying biological processes in environmental matrices. Here, we elaborate on both the analytical procedures for $^{18}O/^{16}O$ ratio measurements in (organo)phosphorus compounds and the complexity of ^{18}O isotope effects in phosphoryl transfer reactions to discuss novel perspectives for the study of these processes using recent Orbitrap mass spectrometry developments.

2. Oxygen Isotope Ratio Analysis of Phosphate and Organophosphorus Compounds

Measurements of $^{18}O/^{16}O$ ratios in phosphate and organophosphorus compounds are currently undergoing promising developments. Until recently, $^{18}O/^{16}O$ ratios were obtained by bulk isotope ratio analysis using elemental analysis (EA) and isotope ratio mass spectrometry (IRMS),[19,20] requiring phosphate isolation in Ag_3PO_4 salts. This approach limits sample throughput and analysis of O isotope fractionation at different reaction stages. These limitations could be overcome through $^{18}O/^{16}O$ ratio analysis by electrospray ionization Orbitrap mass spectrometry (ESI-Orbitrap MS)[21,22] due to two major advantages. First, $^{18}O/^{16}O$ ratios are obtained from relative signal intensities of simultaneous mass spectrometric analysis of the two most abundant O isotopologue species, eliminating analyte conversion to a single O-containing species.[23,24] Second, $^{18}O/^{16}O$ ratios can be determined not only from phosphate isotopologues but also from its PO_3^- fragment, generated through in-source or collision cell fragmentation.[21] These findings imply that $^{18}O/^{16}O$ ratios can be determined in organophosphorus compounds if their phosphate moiety is fragmented without alteration of its $^{18}O/^{16}O$ ratio. Provided that accuracy of such measurements is established, ESI-Orbitrap MS would offer novel and comprehensive avenues for isotopic analysis of reactants and products of phosphoryl transfer reactions.

3. Isotope Effects in Phosphoryl Transfer Reactions

^{18}O Isotope effects reveal the fundamental mechanisms of phosphoryl transfer reactions and are exploited for different scientific purposes. Mechanisms of bond cleavage and formation, transition state structures, and (enzyme) kinetics uncover common catalytic strategies in biology and evolutionary relationships.[3,25–27] Additionally, the expression of ^{18}O isotope effects by various phosphoryl transfer-catalysing enzymes determines the observable changes in $^{18}O/^{16}O$ ratios in phosphate and organophosphorus compounds, allowing inferences of metabolic processes.[6]

We illustrate the relationship between ^{18}O isotope effects and mechanistic information for a simplified phosphoryl transfer of a phosphate monoester according to a concerted S_N2-type mechanism.[9,18,28] In Scheme 1, a phosphate monoester is hydrolysed by water through a synchronous transition state to orthophosphate and an alcohol leaving group. The observable kinetic isotope effect, ^{18}O KIE_{obs}, corresponds to the ratio of observable rate constants, k_{obs}, for reactants with ^{16}O and ^{18}O substitution at one of the various O atoms, as in Eqn. 1.

$$^{18}O\ KIE_{obs} = {}^{16}k_{obs}/{}^{18}k_{obs} \quad (1)$$

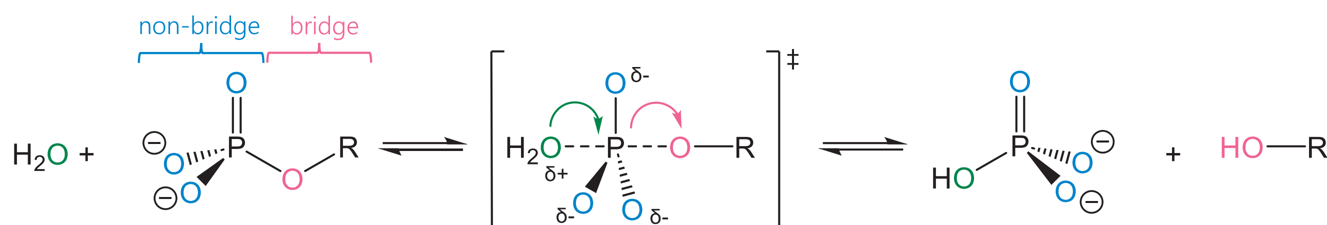
For comprehensive definitions of the isotope effects in chemical and enzymatic reactions, see Refs. [13,15]. As is apparent from Scheme 1, the ^{18}O KIE_{obs} magnitude arises from ^{18}O substitution at three chemically distinct positions, each exhibiting its position-specific ^{18}O KIE. ^{18}O KIE_{obs} is thus a combined measure of oxygen bonding changes due to nucleophilic attack (green O in Scheme 1) and leaving group departure affected by bridge O (red) and non-bridge O of the transferred phosphoryl group (blue). ^{18}O KIEs related to bond formation and cleavage are 'primary', whereas bonding changes to non-bridge O during transition state formation are 'secondary' and typically one order of magnitude smaller.[28] For simplicity, we ignore isotope effect modulation from forward/reverse commitment to catalysis[15] and minor isotope fractionation from substrate binding by these enzymes.[29]

Position-specific ^{18}O KIEs cannot be measured directly. Instead, their contribution to ^{18}O KIE_{obs} is obtained from evaluating observable O isotope fractionation in reactants and products through $^{18}O/^{16}O$ ratio measurements. The colour-coded O atoms in Scheme 1 illustrate that all three position-specific ^{18}O KIEs contribute to O fractionation in the phosphate monoester substrate. ^{18}O KIEs from nucleophilic attack are reflected primarily in $^{18}O/^{16}O$ ratio changes of orthophosphate, whereas leaving group ^{18}O KIEs are obtained mainly from isotope fractionation in the alcohol. The $^{18}O/^{16}O$ ratios of water would reflect the ^{18}O KIE of nucleophilic attack, but due to large H_2O excess, O isotope fractionation in H_2O is too small to be detected. Given these limitations, ^{18}O KIEs of phosphoryl transfer reactions have been evaluated selectively and sometimes incompletely, and we discuss the state-of-knowledge of these isotope effects before evaluating developments in $^{18}O/^{16}O$ ratio analysis by ESI-Orbitrap MS.

3.1 *Leaving Group Isotope Fffects*

Leaving group isotope effects, ^{18}O KIE_{LG}, are the most thoroughly studied isotope effects of phosphoryl transfer reactions.[9,10,18,28,30] Evidence for ^{18}O KIE_{LG} originates from laboratory experiments with isotopically labelled probe compounds,[17,30,31] most often substituted nitrophenyl- and nitrobenzylphosphates, and from computational methods.[10] While this approach cannot be applied to environmental samples, ^{18}O KIE_{LG} data allows insightful extrapolations regarding observable O isotope fractionation.

Table 1 shows selected ^{18}O KIE_{LG} values for hydrolysis of ^{18}O labelled nitrophenyl- and -benzylphosphates (NPP and NBP) catalysed by alkaline phosphatase (AlkP) wild-types and one variant.[8,10,32,33] Entries labelled 'bridge' and 'non-bridge' represent primary and secondary ^{18}O KIE_{LG}, respectively. These data reveal general trends for isotope effects in phosphoryl transfer reactions, discussed in detail elsewhere.[9,18,28,30] (i) Primary ^{18}O KIE_{LG} values of enzymatic phosphoryl transfer range from unity to 1.02, indicating P–^{16}O cleavage rates are 2% faster than those of P–^{18}O. (ii) Secondary ^{18}O KIE_{LG} values are an order of magnitude smaller and inverse, favouring leaving groups with ^{18}O at non-bridge positions over ^{16}O. For comparison, we include chemical reference data with other substrates at elevated temperatures in Table 1, which can serve as upper bounds for enzymatic isotope effects. (iii) Data for phosphate mono-, di- and triesters of NPP show that



Scheme 1. Simplified phosphoryl transfer reaction following a concerted S_N2 mechanism through a single transition state (‡) with H_2O as incoming nucleophile and R-OH as leaving group. Oxygen atom labels (nucleophile, non-bridge, bridge) are used for discussion of ^{18}O kinetic isotope effects, ^{18}O KIE_{obs}, and their consequences for the $^{18}O/^{16}O$ ratios in reactants and products.

primary $^{18}O\ KIE_{LG}$ values are largest for phosphate monoesters and decrease up to 4-fold (1.006) for phosphodi- and -triesters. (iv) Secondary $^{18}O\ KIE_{LG}$ values show the opposite trend, increasing in magnitude from phosphate monoesters to di- and triesters. Primary and secondary $^{18}O\ KIE_{LG}$ values for phosphate triesters can even be of similar magnitude (up to 1.006). Collectively, these trends suggest primary $^{18}O\ KIE_{LG}$ values are sufficiently large to cause measurable isotope fractionation despite compensation by inverse secondary effects. Such fractionation would be best observed in substrates and alcohol leaving groups. A moderate secondary $^{18}O\ KIE_{LG}$ of phosphate triesters suggest these effects might also be reflected in $^{18}O/^{16}O$ ratio variations of *ortho*-phosphate.

Table 1. Leaving group isotope effects, $^{18}O\ KIE_{LG}$, and $^{18}O\ KIE_{obs}$ for phosphoryl transfer reactions of organophosphate substrates catalyzed by alkaline phosphatase (AlkP) wild type, AlkP variant R166S and two different acid phosphatases (AcidP), and abiotic reference reactions in aqueous solution. Substrate abbreviations stand for *p*-nitrophenylphosphate (*p*NPP), *m*-nitrobenzylphosphate (*m*NBP), adenosine 5'-monophosphate (AMP), glycerolphosphate (GP), ethyl-*p*nitrophenylphosphate (EtO*p*NPP), diethyl-*p*-nitrophenylphosphate ((EtO)$_2$*p*NPP). Leaving group KIEs for bridging and non-bridging O-atoms are reported as $^{18}O\ KIE_{LG}^{non-bridge}$. $^{18}O\ KIE_{obs}$ were determined from ε_O according to Eqn. 3.

enzyme	substrate	$^{18}O\ KIE_{LG}^{bridge}$	$^{18}O\ KIE_{LG}^{non-bridge}$	$^{18}O\ KIE_{obs}$	Ref.
AlkP wt	*p*NPP	1.0003 ± 0.0004	0.9982 ± 0.0001	-	[8]
	*m*NBP	1.0072 ± 0.0007	0.9988 ± 0.0004	-	[37]
Alk P R166S	*p*NPP	1.0091 ± 0.0006	0.9925 ± 0.0011	-	[38]
	*m*NBP	1.0199 ± 0.0013	0.9933 ± 0.0004	-	[37]
Alk P (*E. coli*)	AMP	-	-	1.0338	[39]
	GP	-	-	1.0409	[39]
Acid P (potato)	AMP	-	-	1.0074	[39]
	GP	-	-	1.0100	[39]
Acid P (wheat germ)	AMP	-	-	1.0096	[39]
	GP	-	-	1.0127	[39]
(abiotic)	*p*NPP	1.0189 ± 0.0005	0.9994 ± 0.0005	-	[8]
	EtO*p*NPP	1.0042 ± 0.0009	0.9974 ± 0.0006	-	[40]
	(EtO)$_2$*p*NPP	1.0060	1.0063 ± 0.0001	-	[41]

3.2 *Isotope Effect from Nucleophilic Attack*

Unlike leaving group isotope effects, contributions from nucleophilic attack of water or O-containing nucleophiles to $^{18}O\ KIE_{obs}$ have not been evaluated so far. A phenomenological assessment of O isotope fractionation in enzymatic phosphate ester hydrolysis correlates $^{18}O/^{16}O$ ratio changes in orthophosphate upon hydrolysis of phosphate esters with water of different isotopic composition.[34,37] This relationship is shown in Eqn. 2 where $^{18}O/^{16}O$ ratios of orthophosphate, PO_4, organophosphate substrate, and water are given as isotope signatures, $\delta^{18}O$.

$$\delta^{18}O_{PO_4} = 0.75 \cdot \delta^{18}O_{substrate} + 0.25 \cdot \left(\delta^{18}O_{H_2O} + \varepsilon_O\right) \quad (2)$$

Eqn. 2 assumes (i) one of four orthophosphate O atoms stems from water, three from the organophosphate substrate (Scheme 1); (ii) due to large water excess, $\delta^{18}O_{H2O}$ remains constant during reaction; (iii) deviation of the orthophosphate isotope signature $\delta^{18}O_{PO_4}$ (after reaction completion) from the weighted average of $\delta^{18}O_{H_2O}$ and initial $\delta^{18}O$ substrate is the O isotope enrichment factor, ε_O. ε_O represents the combined isotope fractionation from different isotope effects and thus measures $^{18}O\ KIE_{obs}$. Eqn. 2 is convenient as it does not require measuring $^{18}O/^{16}O$ ratios at different reactant turnovers. Experiments with alkaline and acid phosphatase using adenosine 5'-monophosphate (AMP) and glycerolphosphate (GP) substrates revealed O isotope fractionation with ε_O up to 39.3‰.[34] Converting ε_O to $^{18}O\ KIE_{obs}$ *with* Eqn. 3 yields substantial isotope effects up to 1.0409 (Table 1). Experiments with acid phosphatase show smaller isotope fractionation and $^{18}O\ KIE_{obs}$ values, though many still exceed $^{18}O\ KIE_{LG}$.

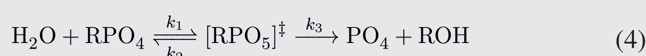
$$^{18}O\ KIE_{obs} = \frac{1}{1 + \varepsilon_O} \quad (3)$$

How position-specific ^{18}O KIEs relate to experimentally derived $^{18}O\ KIE_{obs}$ remains unclear. From phosphoryl transfer mechanisms (Scheme 1), orthophosphate isotope fractionation has two contributions: primary ^{18}O KIE of nucleophilic H_2O attack and secondary ^{18}O KIE of non-bridge O atoms. Assuming a small inverse secondary ^{18}O KIE (Table 1), nucleophilic attack ^{18}O KIE can indeed be substantial. Thus, new approaches determining isotope effects from substrates rather than products are required to elucidate origins of O isotope fractionation.

4. Perspectives for ESI-Orbitrap MS Applications

Current limitations of $^{18}O/^{16}O$ ratio analysis of phosphate and organophosphorus compounds make the complex interplay of position-specific ^{18}O KIEs in phosphoryl transfer reactions difficult to assess experimentally. Evaluating O isotope fractionation of reactants and products at different reaction progress, analogous to compound-specific isotope analysis,[14] would offer important clues for the contribution of multiple ^{18}O KIEs. This is key for interpreting O isotope fractionation of (organo-)-phosphorus compounds as reactive probes for biochemical pathways and metabolic processes.

We simulated $\delta^{18}O$ trends of reactants and products of the simplified phosphoryl transfer reaction from Scheme 1 to compare possible O isotope fractionation to current ESI-Orbitrap MS precision for resolving $^{18}O/^{16}O$ ratio changes. We implemented the reaction scheme from Eqn. 4 for ^{16}O and ^{18}O isotopologues and computed an illustrative O isotope fractionation scenario with a toy model. Parameters represent typical isotope effects from Table 1 and are listed in the caption of Fig. 1.



$$H_2O + RPO_4 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [RPO_5]^{\ddagger} \xrightarrow{k_3} PO_4 + ROH \quad (4)$$

Fig. 1 shows O isotope fractionation *vs.* fractional reaction progress, quantified as organophosphate substrate turnover. The illustrative data point represents long-term precision of phosphate $^{18}O/^{16}O$ ratio measurements by ESI-Orbitrap MS.[21] Except for water, $\delta^{18}O$ values change with reaction progress according to ^{18}O KIEs for nucleophilic attack, leaving group departure, and secondary effects of non-bridge O atoms. Trend lines show $\delta^{18}O$ varies considerably for organophosphate and alcohol whereas phosphate undergoes limited O isotope fractionation. Given the current long-term phosphate $^{18}O/^{16}O$ ratio measurement precision of 2.8‰,[21] the inverse O isotope fractionation of 1.6‰ from the assumed secondary $^{18}O\ KIE_{LG}$ of 0.995 would be undetectable. Conversely, O isotope fractionation of both organophosphate substrate and alcohol leaving group substantially exceeds the analytical uncertainty. Quantifying $\delta^{18}O$ changes in reactants and products could be achieved at recommended fractional conversion regimes of 0.6–0.8 and 0.2–0.4,[13] respectively, where analyte $^{18}O/^{16}O$ ratios differ most. Such data would provide information on nucleophilic attack and leaving group departure isotope effects, so far unquantified or only determined using isotopically labelled substances.

These toy model results reveal that characterizing phosphoryl transfer reactions could advance most effectively through $^{18}O/^{16}O$ ratio measurements of organophosphates and corresponding al-

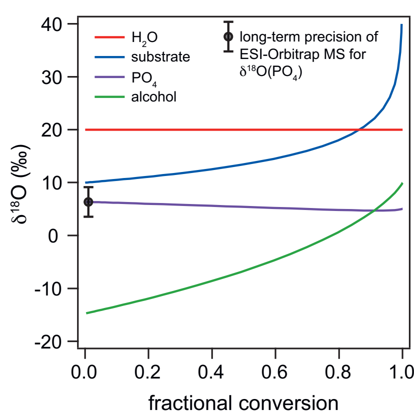


Fig. 1. $\delta^{18}O$-values of reactants and products of a generic phosphoryl transfer from Scheme 1 computed from Eqn. 4 *vs.* fractional organophosphate conversion. Toy model parameters: Initial conditions: $c_{substrate,0} = 10^{-3}$ M, $c_{H_2O,0} = 55.5$ M, $\delta^{18}O_{substrate,0} = 10‰$, $\delta^{18}O_{H_2O,0} = 20‰$. Rate constants and isotope effects: $k_1 = 1$ M^{-1} s^{-1}, $k_2 = 0.1$ s^{-1}, $k_3 = 100$ s^{-1}, primary and secondary ^{18}O KIE$_1$ were 1.030 and 1.005, respectively, primary, and secondary ^{18}O KIE$_3$ were 1.020 and 0.995, respectively, and ^{18}O KIE$_2$ was 1. Black data point and error bars indicate current phosphate $^{18}O/^{16}O$ ratio measurement precision of ±2.8‰ by ESI-Orbitrap MS.[21]

cohol leaving groups. Adding these analytes to the measurement portfolio is an important next step in establishing the utility of stable isotope analysis by ESI-Orbitrap MS.

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[1] F. H. Westheimer, *Science* **1987**, *235*, 1173 https://doi.org/10.1126/science.2434996.
[2] R. Gupta, S. Laxman, *eLife* **2021**, *10*, 1, https://doi.org/10.7554/eLife.63341.
[3] S. C. L. Kamerlin, P. K. Sharma, R. B. Prasad, A. Q. Warshel, *Q. Rev. Biophys*. **2013**, *46*, 1, https://doi.org/10.1017/s0033583512000157.
[4] V. L. Schramm, *Annu. Rev. Biochem*. **2011**, *80*, 703, https://doi.org/10.1146/annurev-biochem-061809-100742.
[5] G. Kiss, N. Çelebi-Ölçüm, R. Moretti, D. Baker, K. N. Houk, *Angew. Chem. Int. Ed*. **2013**, *52*, 5700, https://doi.org/10.1002/anie.201204077.
[6] R. E. Blake, J. C. Alt, A. M. Martini, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2148, https://doi.org/10.1073/pnas.051515898.
[7] F. Tamburini, V. Pfahler, E. K. Bünemann, K. Guelland, S. M. Bernasconi, E. Frossard, *Environ. Sci. Technol*. **2012**, *46*, 5956, https://doi.org/10.1021/es300311h.
[8] A. C. Hengge, W. A. Edens, H. Elsing, *J. Am. Chem. Soc*. **1994**, *116*, 5045, https://doi.org/10.1021/ja00091a003.
[9] W. W. Cleland, A. C. Hengge, *Chem. Rev*. **2006**, *106*, 3252, https://doi.org/10.1021/Cr050287o.
[10] D. Roston, Q. Cui, *J. Am. Chem. Soc*. **2016**, *138*, 11946, https://doi.org/10.1021/jacs.6b07347.
[11] M. Wolfsberg, A. van Hook, P. Paneth, L.P.N. Rebelo, 'Isotope Effects in Chemical, Geological, and Bio Sciences' Springer, **2010**.
[12] A. Kohen, H. Limbach, Eds. 'Isotope Effects in Chemistry and Biology', CRC Press / Taylor & Francis, **2006**.
[13] H. J. A. Dale, A. G. Leach, G. C. Lloyd-Jones, *J. Am. Chem. Soc*. **2021**, *143*, 21079, https://doi.org/10.1021/jacs.1c07351.
[14] T. B. Hofstetter, R. Bakkour, D. Buchner, H. Eisenmann, A. Fischer, M. Gehre, S. B. Haderlein, P. Höhener, D. Hunkeler, G. Imfeld, M. A. Jochmann, S. Kümmel, P. R. Martin, S. G. Pati, T. C. Schmit, C. Vogt, M. Eisner, *Nat. Water* **2024**, *2*, 14, https://doi.org/10.1038/s44221-023-00176-4.
[15] D. B. Northrop, *Annu. Rev. Biochem*. **1981**, *50*, 103, https://doi.org/10.1146/annurev.bi.50.070181.000535.
[16] M. Elsner, M. A. Jochman, T. B. Hofstetter, D. Hunkeler, A. Bernstein, T.C. Schmidt, A. Schimmelmann, *Anal. Bioanal. Chem*. **2012**, *403*, 2471, https://doi.org/10.1007/s00216-011-5683-y.
[17] M. H. O'Leary, J. F. Marlier, *J. Am. Chem. Soc*. **1978**, *100*, 2582, https://doi.org/10.1021/ja00476a070.
[18] A. C. Hengge, *Adv. Phys. Org. Chem*. **2005**, *40*, 49, https://doi.org/10.1016/S0065- 3160(05)40002-7.
[19] F. Tamburini, C. Pistocchi, J. Helfenstein, E. Frossard, *Eur. J. Soil Sci*. **2018**, *69*, 816, https://doi.org/10.1111/10.1111/ejss.12693.
[20] F. Fourel, F. Martineau, C. Lécuyer, H.-J. Kupka, L. Lange, C. Ojeimi, M. Seed, *Rapid Commun. Mass Spectrom*. **2011**, *25*, 2691, https://doi.org/10.1002/rcm.5056.
[21] N. M. Bernet, C. Soldini, T. M. O. Felder, K. Lapčíková, C. Neubauer, W.L. Queen, R. Kaegi, F. Tamburini, T. B. Hofstetter, *Anal. Chem*. **2025**, *97*, 22777, https://doi.org/10.1021/acs.analchem.5c04367.
[22] C. Neubauer, A. Crémière, X. T. Wang, N. Thiagarajan, A. L. Sessions, J. F. Adkins, N. F. Dalleska, A. V. Turchyn, J. A. Clegg, A. Moradian, M. J. Sweredoski, S. D. Garbis, J. M. Eiler, *Anal. Chem*. **2020**, *92*, 3077, https://doi.org/10.1021/acs.analchem.9b04486.
[23] K. Kantnerová, N. Kuhlbusch, D. Juchelka, A. Hilkert, S. Kopf, C. Neubauer, *Nat. Protoc*. **2024**, *19*, 2435, https://doi.org/10.1038/s41596-024-00981-5
[24] E. P. Mueller, A. L. Sessions, P. E. Sauer, G. M. Weiss, J. M. Eiler, *Anal. Chem*. **2022**, *94*, 1092, https://doi.org/10.1021/acs.analchem.1c04141.
[25] S. K. Whittier, A. C. Hengge, J. P. Loria, J. P. *Science* **2013**, *341*, 899, https://doi/10.1126/science.1241735.
[26] M. Pfeiffer, R. M. Crean, C. Moreira, A. Parracino, G. Oberdorfer, L. Brecker, F. Hammerschmidt, S. C. L. Kamerlin, B. Nidetzky, *ACS Catal*. **2022**, *12*, 3357, https://doi.org/10.1021/acscatal.1c05656.
[27] F. Duarte, J. Åqvist, N. H. Williams, S. C. L. Kamerlin, *J. Am. Chem. Soc*. **2015**, *137*, 1081, https://pubs.acs.org/doi/10.1021/ja5082712.
[28] J. K. Lassila, J. G. Zalatan, D. Herschlag, *Annu. Rev. Biochem*. **2011**, *80*, 669, https://doi.org/10.1146/ annurev-biochem-060409-092741.
[29] K. Swiderek, P. Paneth, *Chem. Rev*. **2013**, *113*, 7851, https://doi.org/10.1021/cr300515x.
[30] A. C. Hengge, *Acc. Chem. Res*. **2002**, *35*, 105, https://doi.org/10.1021/ar000143q.
[31] M. H. O'Leary, J. F. Marlier, *J. Am. Chem. Soc*. **1979**, *101*, 3300, https://doi.org/10.1021/ja00506a027.
[32] J. G. Zalatan, I. Catrina, R. Mitchell, P. K. Grzyska, P. J. O'Brien, D. Herschlag, A. C. Hengge, *J. Am. Chem. Soc*. **2007**, *129*, 9789, https://doi.org/10.1021/ja072196+.
[33] I. Catrina, P. J. O'Brien, J. Purcell, I. Nikolic-Hughes, J. G. Zalatan, A. C. Hengge, D. Herschlag, *J. Am. Chem. Soc*. **2007**, *129*, 5760, https://doi.org/10.1021/ja069111+.
[34] C. von Sperber, H. Kries, F. Tamburini, S. M. Bernasconi, E. Frossard, *Geochim. Cosmochim. Acta* **2014**, *125*, 519, https://doi.org/10.1016/j.gca.2013.10.0
[35] I. E. Catrina, A. C. Hengge, *J. Am. Chem. Soc*. **2003**, *125*, 7546, http://doi.org/10.1021/ja0340026.
[36] S. R. Caldwell, F. M. Raushel, P. M. Weiss, W. W. Cleland, *Biochemistry* **1991**, *30*, 7444, https://doi.org/10.1021/bi00244a011.
[37] Y. Liang, R. E. Blake, *Geochim. Cosmochim. Acta* **2006**, *70*, 3957, https://doi.org/10.1016/j.gca.2006.04.036.

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