# Supplementary Information

# Element Mass Spectrometry as a tool for high resolution temporal dynamics investigations of peptide phosphorylation

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## EXPERIMENTAL SECTION

**Apparatus.** *Reversed-Phase capHPLC system.* capHPLC separations were carried out using an Agilent 1100 Series (Agilent Technologies, Waldbronn, Germany) system. The reversed phase capillary column was an Agilent Zorbax SB $C_{18}$, 5 μm, 150 x 0.3 mm (pore size 80 Angstrom).

*ICPMS instrument*. A quadrupole ICPMS (Agilent 7500c, Yokogawa Analytical Systems, Tokio, Japan) equipped with a shield torch system and a collision cell (2.5 mL He $min^{-1}$), for interference attenuation in the specific detection of $^{31}P$, was used. The outlet capillary (50 μm ID, 360 μm OD x 150 mm) of the capHPLC column was inserted into a T-piece using a PEEK capillary tube (400 μm ID x 4 cm) for post-column dilution and directly connected to a microflow total consumption nebulizer (DS-5 nebulizer, Cetac Technologies, Omaha, NE, USA).

**Reagents.** Ultra pure water (18.2 MΩ $cm^{-1}$) was obtained with a Milli-Q system (Millipore, Bedford, MA). Acetonitrile and formic acid were of HPLC and analytical-reagent grade, respectively (Merck, Darmstadt, Germany). Bis (4-nitro-phenyl) phosphate, 99% (BNPP) and ammonium bicarbonate reagent plus, ≥ 99.0%, were purchased from Sigma-Aldrich (Steinheim, Germany). Bacterial alkaline phosphatase (activity/mg protein min. 30 U) was from Applichem (Darmstadt, Germany). Amino acid sequences of the standard phosphopeptides were TSTEPQpYQPGENL (peptide A, pA), N-Acetyl-DpYVPML-$NH_2$ (peptide B, pB), both purchased from Alexis Corporation (Lausen, Switzerland) and FQpSEEQQQTEDELQDK (peptide C, pC) from Sigma-Aldrich (Steinheim, Germany).

**Procedures.** *Phosphopeptide dephosphorylation using alkaline phosphatase.* A model mixture containing the three phosphopeptides (peptides A, B and C) in 50 mM ammonium bicarbonate buffer (pH 7.8) was split into different aliquots (25 μL each), which were treated with bacterial alkaline phosphatase under controlled conditions during different times to obtain a sufficient number of samples with varying degrees of phosphorylation. Original alkaline phosphatase solution (13 μL ) was diluted to 500 μL with 50 mM ammonium bicarbonate buffer (pH 7.8). 4 μL of this solution was spiked to the phosphopeptide mixtures. The molar ratio enzyme/total substrate was approximately two. The solution was incubated at different times at 37 ºC. Dephosphorylation reaction was stopped by acidification (pH<5) by addition of 0.2 μL formic acid to inactivate the phosphatase activity.

*Quantification of phosphopeptides by reversed-phase ICPMS.* The mobile phases used for capHPLC experiments were water/1 % acetonitrile/0.2 % formic acid/50 ng Ge $mL^{-1}$ (mobile phase A) and acetonitrile/0.2 % formic acid/50 ng Ge $mL^{-1}$ (mobile phase B). The sheath-flow for postcolumn dilution consisted of 40 % acetonitrile/500 ng Se $mL^{-1}$. 25 μL of the reaction mixtures were spiked with 5 μL of BNPP (9 μg P $mL^{-1}$) as internal standard, both accurately weighed. 1 μL of the resulting sample was injected into the HPLC system and separated using the gradient: 0-10.5 min 0 % B isocratic, 10.5-37 min 0-30 % B linear, 37-41 min 30-80 % B linear and 51-54 min 0-80 % B linear. capHPLC column and sheath-flow rates were 3.5 and 6 μL $min^{-1}$, respectively.

*Kinetics study*. In order to measure temporal dynamics of peptide phosphorylation, mixtures of three phosphopeptides were dephosphorylated as described above and reaction was stopped at 24 different times, from 0 to 510 min: 0, 0.2, 0.4, 0.6, 0.9, 1.1, 1.4, 1.8, 2.5, 3.5, 5.6, 10, 15, 18.1, 40, 75, 115, 155, 190, 220, 300, 350, 415 and 510 min. Quantification of the phosphopeptides content in each time point was carried out by capHPLC-ICPMS in duplicate. The ratio between the absolute concentration computed for each time point and the one at time zero gave directly the phosphorylation degree. Uncertainty associated to such phosphorylation degree was obtained taken into account the uncertainties for the absolute concentrations at the corresponding time point and time 0 min.

**Table S1:** Raw data plotted in Figure 2. Uncertainty corresponds to 1 standard deviation

| Time (min) | Phosphorylation degree (%) | | |
|---|---|---|---|
| | **pA** | **pB** | **pC** |
| 0 | 100 ± 1.9 | 100 ± 2.6 | 100 ± 2.8 |
| 0.2 | 92.5 ± 1.6 | 94.9 ± 2.1 | 98.6 ± 2.3 |
| 0.4 | 71.5 ± 1.1 | 66.2 ± 1.3 | 98.0 ± 2.0 |
| 0.6 | 65.6 ±1.2 | 54.0 ± 1.4 | 104.5 ± 2.2 |
| 0.9 | 59.2 ± 1.1 | 44.3 ± 1.5 | 99.4 ± 2.2 |
| 1.1 | 55.8 ± 1.1 | 36.5 ± 1.5 | 97.2 ± 2.3 |
| 1.4 | 52.7 ± 0.8 | 28.8 ± 0.8 | 98.4 ± 3.0 |
| 1.8 | 48.2 ± 0.8 | 21.3 ± 0.7 | 99.3 ± 2.7 |
| 2.5 | 40.7 ± 0.6 | 15.0 ± 0.5 | 98.8 ± 2.9 |
| 3.5 | 34.6 ± 0.5 | 12.1 ± 0.4 | 97.6 ± 2.9 |
| 5.6 | 24.8 ± 0.7 | 7.15 ± 0.20 | 94.8 ± 2.2 |
| 10 | 15.8 ± 0.3 | 3.85 ± 0.11 | 90.1 ± 1.8 |
| 15 | 7.39 ± 0.32 | ----- | 83.5 ± 1.7 |
| 18.1 | 2.88 ± 0.05 | ----- | 79.9 ± 1.9 |
| 40 | ----- | ----- | 66.0 ± 1.5 |
| 75 | ----- | ----- | 56.9 ± 1.3 |
| 115 | ----- | ----- | 47.7 ± 1.1 |
| 155 | ----- | ----- | 40.2 ± 0.9 |
| 190 | ----- | ----- | 33.8 ± 0.7 |
| 220 | ----- | ----- | 28.2 ± 0.6 |
| 300 | ----- | ----- | 13.6 ± 0.3 |
| 350 | ----- | ----- | 13.6 ± 0.3 |
| 415 | ----- | ----- | 7.09 ± 0.22 |
| 510 | ----- | ----- | 7.40 ± 0.22 |