

**Supplemental data table 5S: Criteria for selection of synthetic peptides subjected to TiO<sub>2</sub> enrichment**

The following criteria were considered to select five exemplary synthetic arginine phosphorylated peptides to subject them to TiO<sub>2</sub> enrichment.

criterion	explanation
molecular weight	molecular weight of phosphorylated isoform should cover a wide range (between ~1000 and ~2000 Da)
peptide length	length of phosphorylated isoform should cover a wide range (peptides with 8 – 16 amino acids were selected)
sequence characteristics	amino acids with different chemical properties around the phosphosite (group of hydrophilic, hydrophobic, positively or negatively charged amino acids)
peptide concentration	different initial peptide concentration simulated high and low abundant phosphoproteins
peptide of biological interest	conserved phosphosite between <i>B. subtilis</i> and <i>S. aureus</i> or peptide belonging to regulator proteins were selected
peptide stability	stability of arginine phosphorylation is determined at 25 °C, very instable peptides (half-life period < 180 minutes) were omitted
retention time	Peptides were selected to avoid chromatographic coelution as far as possible