Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Figure S1: MS/MS spectrum of DiART labeled phosphopeptide of alpha casein TVDMESpTEVFTK (Mass shift in Da, caused by DiART labeling is indicated in parentheses). A) CID was used to identify peptide with high confidence (Xcorr 4.76, phosphoRS phosphorylation probability at S6, 98.3%). B) PQD spectrum, in the low mass range m/z 110-120 showing distinct DiART reporter ions. These were used to calculate reporter ion ratios and compared to theoretical values (in brackets).

Figure S2: A schematic of spike-in experiments used in the study. A cell lysate of *A.nidulans* was trypsinized, tagged with DiART reagents 114, 115, 118 and 119 and mixed 1:1:1:1. DiART labelled Mixture-1 or Mixture-2 was diluted 10 fold with the above mixture, subjected to TiO_2 enrichment followed by LC MS/MS.

Figure S3: Ultra-long gradient used for separating complex phosphopeptide mixtures. A cell lysate of *A.nidulans* tagged with DiART reagents was enriched using TiO_2 and subjected to ultralong this reverse phase gradients coupled to MS/MS analysis. The y-axis indicates the m/z values of all identified peptides, during the gradient. The concentration of acetonitrile in the mobile phase is displayed along the plot.