**Figure 1. Global protein structural and abundance changes during multiple cellular responses in yeast and *E. coli.***(a) The experimental systems used in this work. We studied *E. coli* adapting to 7 nutrient sources (from growth in glucose) and yeast acutely responding to heat or osmotic shock. We monitored protein abundance and structural changes using LiP-MS and assessed the functional information content of both types of readout. (b-e) Protein changes during response to acute stress in yeast. (b) The number of proteins changing in abundance or structure under heat or osmotic shock. (c) Functional categories (GO biological processes) enriched among proteins changing abundance and structure under heat or osmotic shock. (d) Structural changes in yeast responding to heat stress. The Venn diagrams (left) show the number of proteins of the indicated category that are structurally altered after heat stress (inner circle) relative to all detected proteins in that category (outer circle). Specific chaperones that show structural alterations in heat or osmotic stress are indicated (right) and are labeled by subcellular location. (e) Structural changes in ATPase chaperones in response to heat. Two LiP peptides from Hsp104 and three LiP peptides from Kar2 (orange) that change during the response to heat shock are mapped to the respective enzyme structures (Hsp104 PDB ID: 6n8t, Kar2 PDB ID: 3qfu). For HSP104, the hexameric structure is shown and the cartoon below the structural model shows the positions of the the LiP peptides identified in the NBD1/2 (AAA+ nucleotide-binding domain 1 and 2) and MD (predicted coiled-coil middle domain) domains. Metabolites binding to the chaperone catalytic site (ATP and ADP, respectively) are depicted in cyan. See also Figure S1 and S3.

**Figure 2.** **Structural changes capture multiple regulatory events in yeast responding to osmotic shock.** A schematic of the yeast HOG-MAPK pathway and its link to the glycolytic and glycerol biosynthesis pathways is shown. Proteins undergoing structural alterations (as detected by LiP-MS) upon osmotic shock are indicated with orange labels. The changing LiP peptide or peptides (orange) are mapped onto the 3D protein structures of yeast protein-metabolite complexes or evolutionary conserved holo-complex structures obtained by homology modelling; metabolites positioned in allosteric or active sites are indicated in green. For Hog1 and Gpd1, relative locations of LiP peptide (orange) and phosphorylation sites (blue) on the linear sequence of the proteins are shown. The allosteric regulator Fructose 2, 6-bisphosphate (F2,6bP) is depicted in red. The models shown are built from structural data available from the PDB database: Pfk1 (PDB ID 3o8o), Fba1 (PDB ID 3qm3), Ste20 (PDB ID 4zlo), Hog1 (PDB ID 5ci6), Tpi1 (PDB ID 1nf0), Gpd1 (PDB ID 6e9o), Gpp1 (PDB ID 2qlt), Tdh2 (PDB ID 3pym), Pgk1 (PDB ID 1qpg), Gpm (PDB ID 1qhf), Eno2 (PDB ID 1ebh), Pyk2 (PDB ID 1a3x). See also Figure S2.

**Figure 3. Global protein structural and abundance changes during nutrient adaptation in *E. coli.*** (a) The plot shows the number of proteins changing in structure or abundance under the indicated nutrient conditions, relative to glucose. (b) Relative abundance of known nutrient transporters and uptake regulators during nutrient adaptation relative to the reference condition (growth in glucose media). The schematic (above) shows the known regulators for different nutrient sources. The heat map (below) shows abundance changes of these proteins relative to glucose under the indicated nutrient conditions. (c) Functional analysis. The plot shows functional categories (GO biological processes) enriched among proteins changing abundance and structure under the indicated nutrient conditions, relative to glucose. See also Figure S1 and S4.

**Figure 4. Structural changes reflect functional flux alterations of *E. coli.* metabolic enzymes**. (a) 13C-based metabolic flux maps for *E. coli* grown in the seven indicated nutrient conditions accordingly to the data reported in Gerosa *et.al*. (2015). In all cases metabolic fluxes measurements were normalized to the fluxes values measured in glucose. The thickness of the black arrows indicated the flux fold changes relative to the glucose growth condition. Proteins with changes in abundance, structure, or both, are indicated for each growth condition. (b) The schematic (far left) shows the glycolytic enzymes for which we detect a correlation between LiP peptide levels and metabolic flux (red circles). The plot (left) shows a linear regression between levels of the indicated LiP peptide derived from Pgk, and relative flux values through pgk, across all nutrient conditions. The plot (right) shows LiP-MS analysis of recombinant pgk spiked into an *E. coli* lysate. The level of the best correlating LiP peptide with increasing 3-phosphoglycerate (ie substrate, 3PG) concentration is plotted. In the 3D structure of pgk (far right, PDB ID 1zmr), LiP peptides that correlate with LiP peptide levels and with substrate levels *in vitro* (orange) are mapped onto the structure of pgk. 3PG bound to the enzyme is indicated in cyan.

**Figure 5. Structural changes capture allosteric regulators of *E. coli* metabolic enzymes.** (a) A depiction of *E. coli* Central Carbon Metabolism (CCM) showing the 32 enzymes with a significant correlation between LiP peptide levels and regulatory metabolite levels (gray dots). Red outlines indicate interactions supported by previous data. Metabolites are represented within rectangular boxes, and the points of entry of different nutrient sources are shown. (b) Correlations between levels of metabolites (rows) and CCM enzyme-derived LiP peptides (columns) in a linear regression analysis across all nutrient conditions. All metabolites with at least one significant correlation are plotted. (c) Structural alterations map to metabolite binding sites. LiP peptides (orange) from pfkA and pts1 that correlate with relevant metabolite levels are mapped onto the respective enzyme structures (pfkA PDB ID: 1pfk; ptsI PDB ID: 2hwg). Bound metabolites are depicted in cyan. The structures shown were the only ones for which a 3D structure with the relevant metabolite was available.

**Figure S1. Coverage of the analysis in the experimental systems used in this study.** (a) The plot shows the number of *E. coli* proteins detected using either a protein abundance or a LiP workflow under the indicated conditions. (b) The number of yeast proteins detected after the indicated stress are shown.

**Figure S2. Functional analysis of proteins that show structural and abundance changes during yeast response to acute stress.** Functional categories (GO Molecular Functions and Cellular Components) enriched among proteins changing in abundance or structure under the indicated stress conditions are shown.

**Figure S3. LiP-MS detects phosphorylation events in yeast responding to acute osmotic stress.** (a): The schematics depict the yeast HOG1-MAPK pathway, including its links to the glycolysis and glycerol biosynthesis pathways. Proteins with altered structure as detected by LiP-MS (yellow) and proteins with altered phosphorylation as detected by phosphoproteomics (blue) during acute osmotic stress are indicated*. b:* Examples of structural alterations associated with phosphorylation. For Hog1 and Gpd1, the altered LiP peptide (yellow) is overlapping or near the known phosphorylation sites (blue) in the linear sequence. For Tdh3, the LiP peptide (orange) is near the phosphoryation site (green) in 3D space.

**Figure S4. Structural and abundance changes in proteins of central carbon metabolism (CCM) during nutrient adaptation in *E. coli.***The heat maps show which E coli CCM proteins change in either structure or abundance under the indicated nutrient conditions, relative to glucose. Proteins are arranged according to the CCM pathway to which they belong. (TCA= tricarboxylic acid cycle, GS = glyoxylate shunt, PPP = pentose phosphate pathway, ED = Entner-Doudoroff pathway)

**Figure S5. Raw LiP-MS data captures both abundance and structural changes**. The plot shows functional categories (GO biological processes) enriched among proteins changing in the indicated nutrient conditions relative to glucose. Proteins showing only abundance changes, only structural changes (measured by normalized LiP-MS), both abundance and structure changes and proteins detected as changing based on the raw LiP-MS data, are plotted separately.