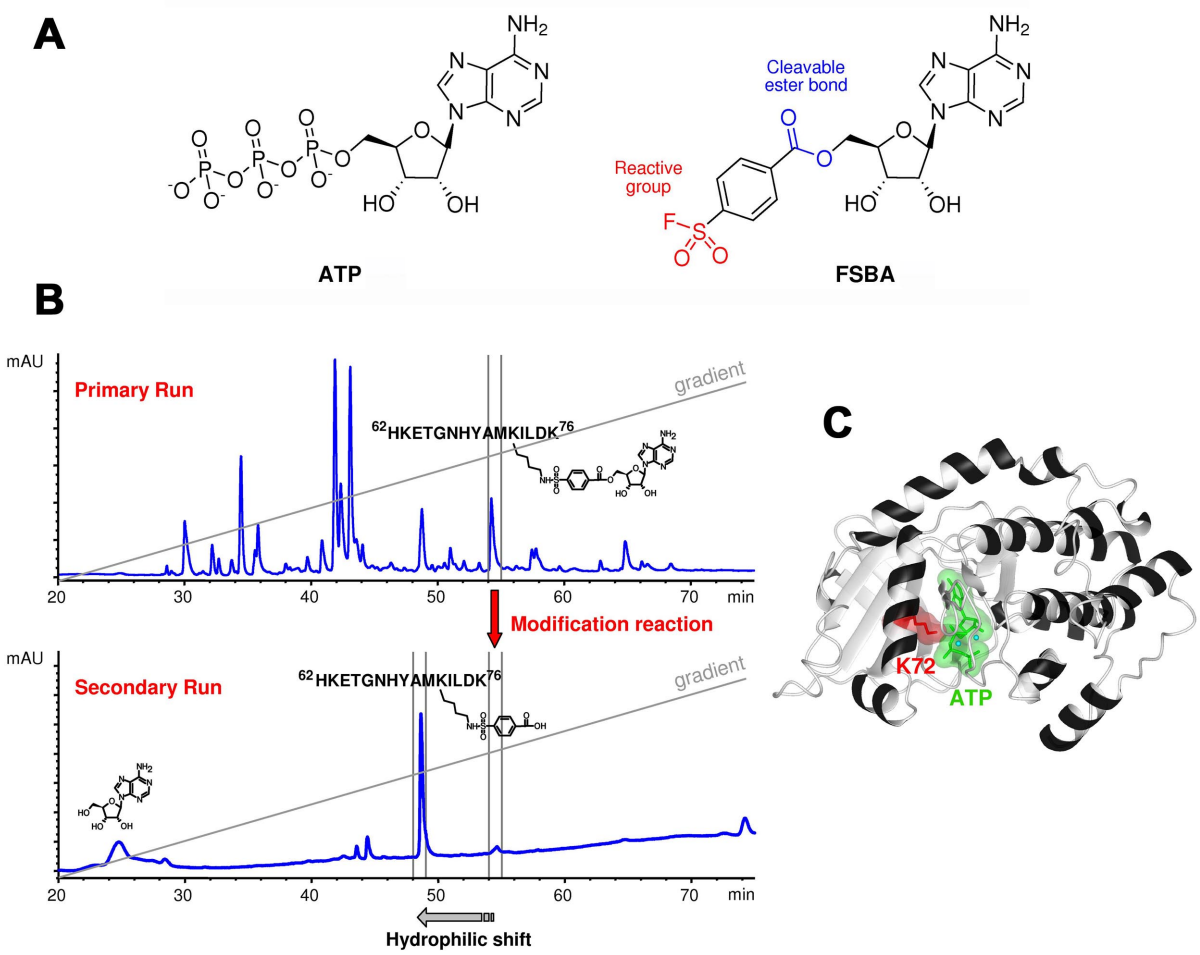
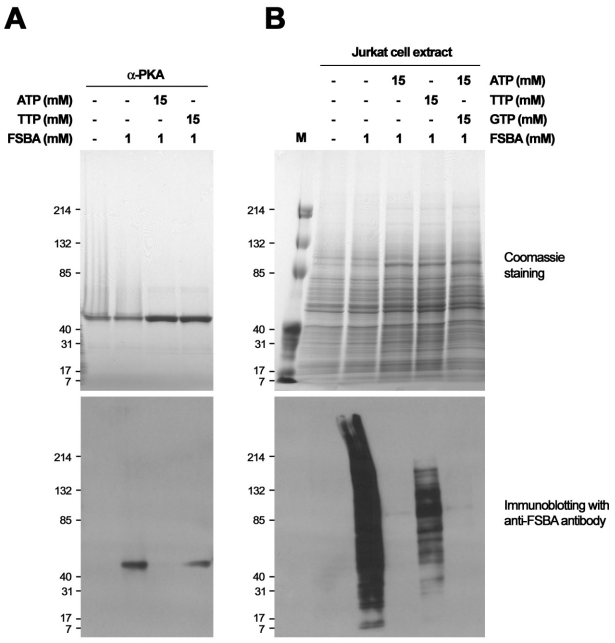
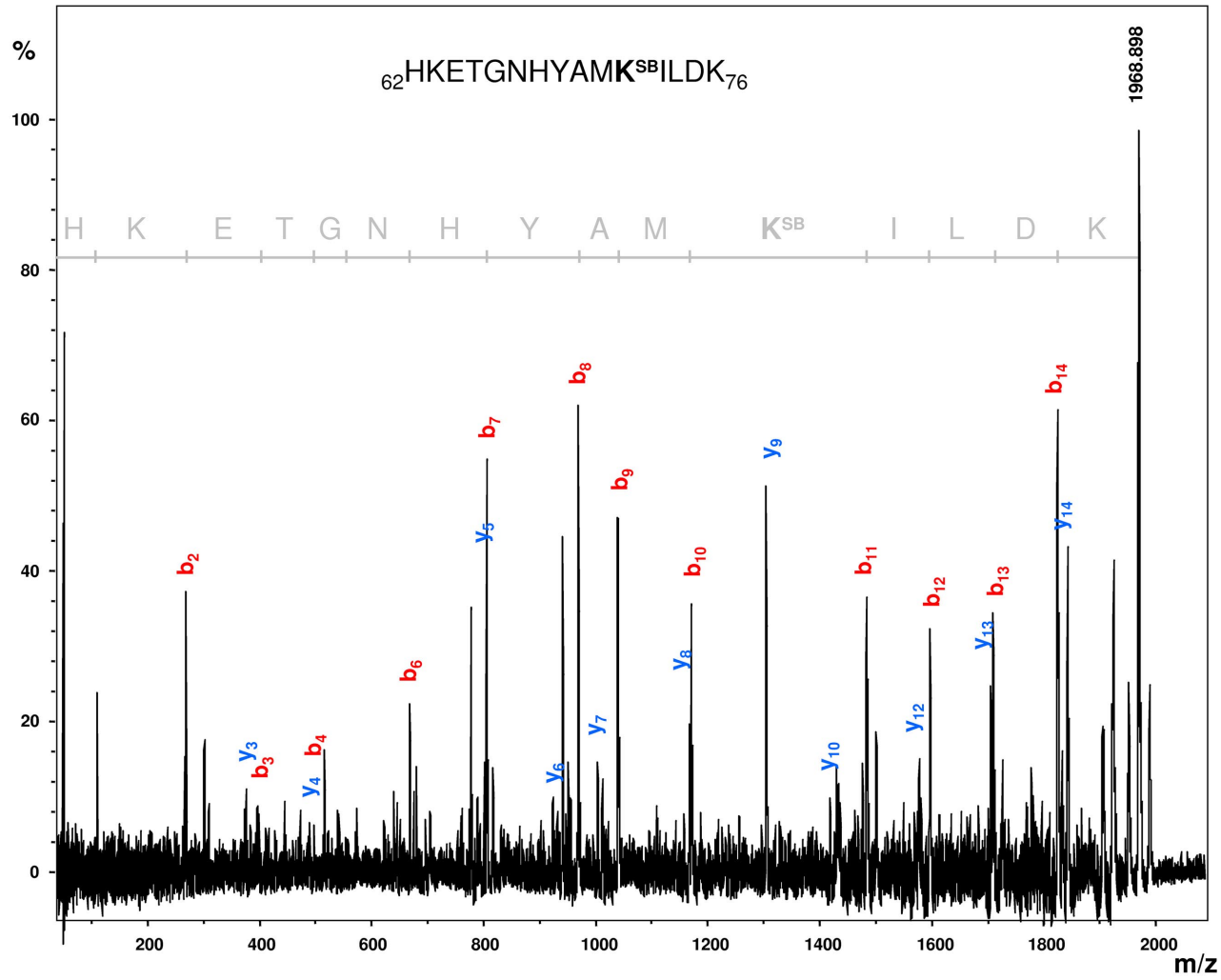
SUPPORTING FIGURES



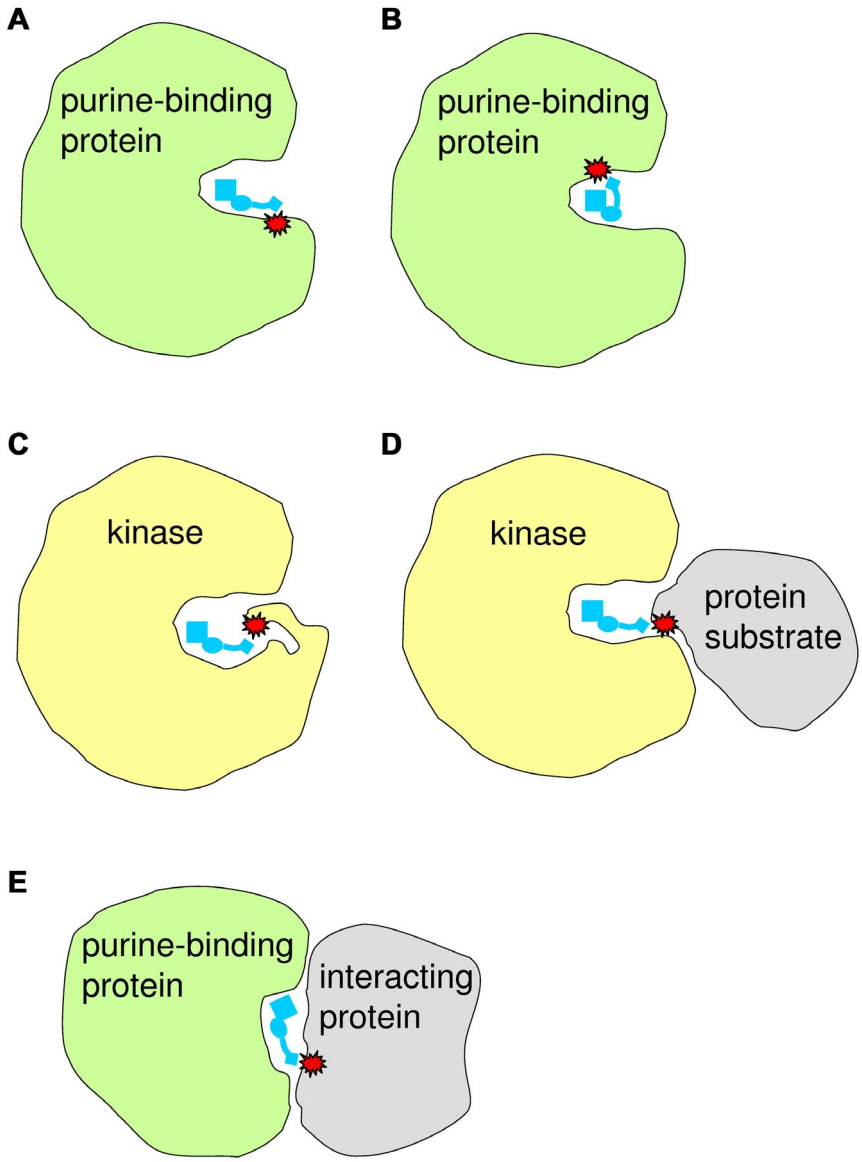
Supporting Figure 1. FSBA-COFRADIC: proof of concept study on αPKA. (A) Chemical structures of adenine nucleoside triphosphate, ATP and 5'-*p*-fluorosulfonylbenzoyladenosine, FSBA, the activity based probe for nucleotide-binding proteins. The reactive group (fluorosulfonyl) which reacts with nucleophile side chains of amino acids is shown in red and the pH sensitive ester bond in blue. (B) FSBA-COFRADIC on αPKA. The UV traces (absorbance at 214 nm) of the primary and secondary HPLC runs are shown. The cleavage of adenosine group after the primary run (SBA-peptide) induced a hydrophilic shift during the secondary run (SB-peptide) of the PKA peptide (62-76) that was labeled by FSBA. (C) Three dimensional structure of αPKA complexed with ATP (in green) (PDB Number 1ATP)[24]. The FSBA-labeled residue Lys-72 is shown in red.



Supporting Figure 2. FSBA labeling specificity. The recombinant catalytic subunit of PKA (A) or a Jurkat cell extract (B) was incubated with FSBA after pre-incubation with or without an excess of nucleotides. The samples were separated by SDS-PAGE and detected by Coomassie staining (upper panels) or by immunoblotting with an anti-FSBA monoclonal antibody[33] (lower panels). The molecular weights are in kDa.

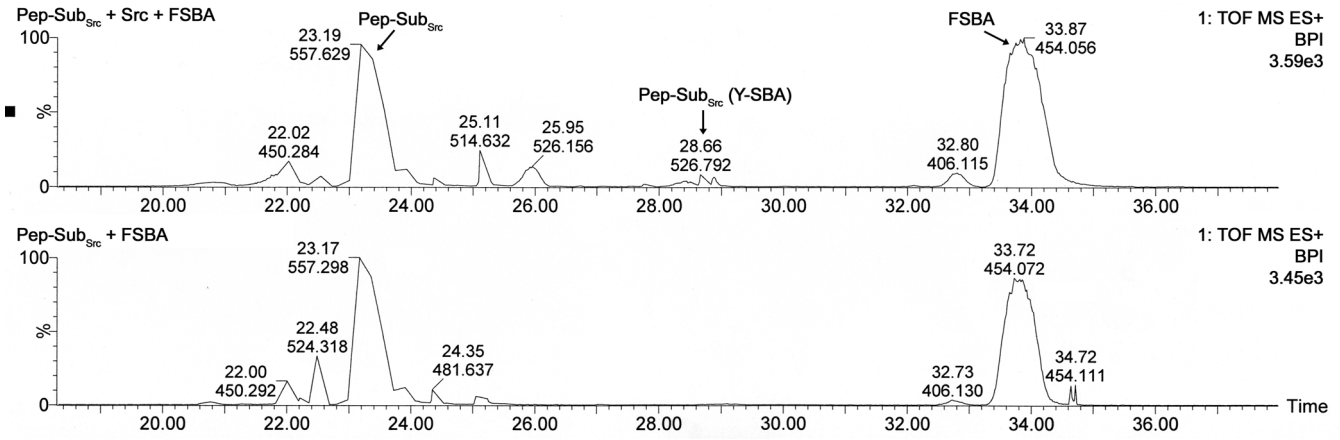


Supporting Figure 3. MS/MS spectrum of the α-PKA peptide that was FSBA-labeled. The 1968.898 Da peptide was identified as ^{62}HKETGNHYAM**K**$^{\mathbf{SB}}$ILDK^{76} based on the b- (in red) and y-ions (in blue) series. The FSBA modification occurred on the lys-72 as proved by the 443.11 Da distance between the b_{10} and b_{11} ions.



Supporting Figure 4. Different types of FSBA-labeling of proteins in complex biological samples. FSBA may bind, by affinity, purine-nucleotide binding proteins in an extended (A) or in a stacked (, B) conformation and then react ( ) with a nucleophilic side chain which is close to the reactive fluorosulfonyl group. In the case of protein kinases, FSBA may non-covalently bind the ATP-binding pocket and instead of reacting with a residue in this pocket (C) it rather reacts with a residue located on a substrate (D). If the kinase carries autocatalytic activity, the modified residue is part of the kinase itself (C, i.e. phosphorylation in an activation loop) otherwise the FSBA-labeled residue is on the protein substrate (D). Proteins interacting with purine-nucleotide binders may be FSBA labeled without

being purine binders themselves if the nucleotide is located at the interface between the two partners (E).



Supporting Figure 5. FSBA labeling of Pep-Sub$_{Src}$ in presence or absence of Src. Reaction mixtures with (upper panel) or without (lower panel) Src were analyzed by LC-MS on an ESI-Q-TOF. The y-axis of the spectra represents the base peak intensity of ions. The unmodified substrate peptide eluted at 23.19 min. ($[M+H]^+$ 1669.91 Da; $[M+3H]^{3+}$ 557.31 Da); the modified peptide (Y-SBA) eluted at 28.66 min. ($[M+H]^+$ 2102.98 Da; $[M+4H]^{4+}$ 526.50 Da) and non-reacted FSBA eluted at 33.8 min.($[M+H]^+$ 454.07 Da).