Identifying Three-dimensional Structures of Autophosphorylation Complexes in Crystals of Protein Kinases

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Protein kinase autophosphorylation is a common regulatory mechanism in cell signaling pathways. Several autophosphorylation complexes have been identified in crystals of protein kinases, with a known serine, threonine, or tyrosine autophosphorylation site of one kinase monomer sitting in the active site of another monomer of the same protein in the crystal. We utilize a structural bioinformatics method to identify all such autophosphorylation complexes in X-ray crystallographic structures in the Protein Data Bank (PDB) by generating all unique kinase/kinase interfaces within and between asymmetric units of each crystal and measuring the distance between the hydroxyl oxygen of potential autophosphorylation sites and the oxygen atoms of the active site aspartic acid residue side chain and determining whether one kinase interacts with the other in a manner resembling known substrate-kinase interactions. We identify 16 unique autophosphorylation complexes in the PDB, of which 6 complexes have not previously been described in the relevant publications on the crystal structures. These consist of tyrosine phosphorylations in the N-terminal juxtamembrane regions of CSF1R (Y561) and EPHA2 (Y594), activation loop tyrosines of LCK (Y394), IGF1R (Y1166), and EPHA2 (Y772), and a serine in a nuclear localization signal region of CLK2 (S142). Mutation of residues in the autophosphorylation complex interface of LCK either severely impaired autophosphorylation (T445D and N446D) or increased it (P447L,A,G). The P447L mutation has been previously found in a T-cell leukemia cell line and associated with activation of LCK. The 16 structures (11 Tyr and 5 Ser/Thr kinases) provide information on the phosphorylation of full-sized, folded domains, in contrast to the 20 or so peptide-kinase co-crystal structures (13 Ser/Thr and 7 Tyr kinases). The novel and previously observed autophosphorylation sites are conserved in many kinases, indicating that by homology we can extend the relevance of these complexes to many other clinically relevant drug targets.