Malignant tumors metabolize glucose through glycolysis to lactic acid and produce ATP at an accelerated rate when compared to normal cells, a phenotype detected clinically using Positron Emission Tomography (PET). Out of the four available isoforms, Hexokinase 2 (HK2) is the major expressing isoform in cancers specially those that metastasize and kill their human host. In malignant cells, HK2 not only improves the cell’s energy supply but also protects cancer cells against apoptosis through direct interaction with the mitochondria and Voltage Dependent Anion Channel 1 (VDAC1). Here we report the 3D crystal structure of the human HK2 in complex with glucose and glucose-6-phosphate, the first enzyme in the glycolytic pathway. The N- and C-terminal domains are catalytically active and linked by a long seven turn α-helix. Each domain contains an active site that is sandwiched between a small and large sub-domain. Cancer patients with high HK2 gene expression level showed a worse prognosis and aggressive character. Therefore, HK2 is an ideal therapeutic target to inhibit cancer proliferation and tumor destruction. Very limited literature describes HK2 and detailed understanding of the HK2 structure and function is needed to characterize its mechanism, to understand its role in cancer metabolism and to develop new drug treatments to slow the rate of cancer proliferation.