**Figure S1.** Stability studies of KlGk1 protein using Thermal Shift Assay (panels a and b) and Tycho (panels c and d). Panels (a) and (c) represent the raw fluorescence data, panels (b) and (d) show the corresponding first derivative.
Figure S2. Kinetic analysis of KlGlk1 activity. (a) Lineweaver-Burk linearization (reciprocal plot). Datapoints for low ATP concentrations obey linear trend and were considered for fitting of a linear model (solid line, $R^2=0.98$). $V_{max}$ was determined from the Y-intercept, X-intercept is equal to -1/Km. Error bars correspond to SD, N=3. (b) Comparative fit of classical Michaelis-Menten plot ($R^2=0.96$) and a Hill plot ($R^2=0.99$, $h=1.97$). (c) Hill Plot. Error bars correspond to SD, N=3.

Figure S3. Analysis of RALS/LALS distribution for KlGlk1. Elution profile indicates formation of a KlGlk1 dimer in the solution with calculated molecular weight of 100 kDa (second peak). Refractive index (mV) and corresponding calculated molecular weight (Da) are represented as a red and green line, respectively.
Figure S4. Complete topology of KlGlk1 protein. Side view (a) and front view (b) of KlGlk1. H indicates helices, S indicates strands.

Figure S5. 2D topology map of KlGlk1 monomer.
Figure S6. Amino acid sequence alignment of KlGlk1 and KlHxk1 glucose kinases from Kluyveromyces lactis. Amino acid sequence identity between KlGlk1 and KlHxk1 is 37%. Sequence secondary structures for both proteins are indicated.
Figure S7. Coloured representation of normalized B-factors in KlGlk1 protein structure. Red parts represent high, white middle, blue low B factors.

Figure S8. SDS-PAGE gel showing purity of the KlGlk1 after final step of purification.