Supplemental Material

METHODS:

Coarse-grained model of PGK and Ficoll 70:
A coarse-grained off-lattice side-chain Cα model (SCM) (1, 2), where amino acids (except glycine) are represented by two beads, was built based on an all-atomistic model of yeast Phosphoglycerate kinase (PGK) from the Protein Data Bank (PDB ID: 1QPG (3)). The mutation R65Q of this pdb structure was reverted to the original, and three additional mutations were made at positions Y122W/W308F/W333F to match the experiments exactly. Mutations were made using the mutate function of VMD (4). After the coordinates of the mutated residues were automatically assigned, energy minimization by the conjugate gradient method was applied to avoid steric clashes.

The potential energy of this system E is, E= Ep+Ecc+Epc, where Ep, Ecc, and Epc are the potential energies of the protein, interactions between crowding agents, and interactions between the protein and crowding agents, respectively (2). The crowding agent (crowder) Ficoll 70, a polymer that adopts a semi-rigid sphere shape as found in experiments(5, 6), was approximated as hard spheres with a radius of 55Å (2) to represent the excluded volume effect.

The potential energy of the protein is Ep=E_s+E_nonbonded. The structural energy, E_s, is the sum of the bond-length potential, the bond-angle potential, the dihedral potential, and the chiral potential. Each term was described in previous studies(2), and the equilibrium values for each term are taken from the crystal structure of PGK. The nonbonded term, E_nonbonded, includes the side chain-side chain interaction (E_ss), the side chain-backbone interaction (E sb), and the backbone-backbone interaction (E bb). The solvent-mediated interaction between pairs of side chains, \( \varepsilon_{ij} \), was based on the Betancourt-Thirumalai statistical potential (7). The Betancourt – Thirumalai statistical potential addresses sequence variations where the reference interaction, \( \varepsilon = 0.6 \) kcal/mol, is based on the Thr-Thr pairwise interactions.

\[
E_{ss}^{\gamma} = \varepsilon_{ij} \left[ \frac{\sigma_{ij}}{r_{ij}} \right]^{12} - 2 \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6
\]

where \( \sigma_{ij} = f(\sigma_i + \sigma_j) \) and \( \sigma_i \) and \( \sigma_j \) are the van der Waals (vdW) radii of the side chains. To avoid clashes between bulky side chains, \( f = 0.9 \).

E_bb is the multiplication of a Lennard-Jones term similar to the equation above and an angular term, A(\( \rho \)), that assesses major secondary structures. \( \rho \) is the pseudo-dihedral angle that defines the alignment of a pair of backbone hydrogen bond by four Cα beads.

Repulsive interactions are used for E_ss, E_cc, and E_pc. The repulsive potential between two particles \( i \) and \( j \) at a distance \( r \) follows:

\[
E^{\theta} = \varepsilon \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12}
\]
where $\sigma_{ij} = f(\sigma_i + \sigma_j)$; $\sigma_i$ and $\sigma_j$ are vdW radii of interacting beads. $\varepsilon = 0.6$ kcal/mol.

**Simulation details**

An in-house version of AMBER6 (8) was used, in which the Langevin dynamics equations of motion in a low friction limit are integrated (9). The integration time step is $10^{-3}$ $\tau_L$, where $\tau_L = (m\sigma^2 / \varepsilon)^{0.5}$. Methods for preparation of equilibrated periodic boxes of crowders and protein can be found in previous studies (10, 11). To enhance the sampling efficiency, the Replica Exchange Method (REM) was implemented (12, 13). Replica exchanges to speed thermodynamic convergence were attempted every 800 $\tau_L$. Molecular simulations were run at 20 different temperatures (0.9 < $k_B T / \varepsilon$ < 1.6, where $\varepsilon$ is the solvent mediated interaction, 0.6 kcal/mol). Exchanges between neighboring replicas $i$ and $j$ are accepted based on the Metropolis criterion (14) with a probability of acceptance:

$$P_{\text{acc}} = \min \left\{ 1, \exp \left[ \left( \beta_i - \beta_j \right) \left( U(r_i) - U(r_j) \right) \right] \right\}$$

(1)

where $\beta = 1/k_B T$, and $U(r)$ represents the potential energy of the system. Each replica generates $\sim 40,000$ statistically significant conformations for data convergence, where the time separation of sampling is greater than one correlation time. Thermodynamic properties were analyzed with the weighted histogram analysis method (WHAM) (15).

**Definition of PGK domain orientation**

To calculate the orientation between the N and C domains of PGK, we defined an angle $\theta$ formed by two vectors, one from each lobe of the protein. The N-lobe vector is defined between the $C_\alpha$ beads of residues 50 and 74, while the C-lobe vector is defined between the $C_\alpha$ beads of residues 287 and 360. The specific residues were selected because $\theta$ in the crystal structure is $0^\circ$ and it is sensitive to the overall alignment of two subdomains in a broad selection of structures.
FIGURES

Figure S1. Conformational, thermal stability and kinetics trends of the PGK construct as a function of sucrose concentration. (a) Donor acceptor ratio at 22.3±0.5 °C (open circles). (b) Folding time constant. (c) Melting temperature. (d) Cooperativity from eq.(1) in the main text. All error bars are ±1 standard deviation.

Figure S2. PGK enzymatic activity in buffer, 100 mg/ml Ficoll 70 and 100 mg/ml sucrose. The activity is slowed in sucrose and drastically enhanced in Ficoll 70.
Figure S3. (a)-(b) Cartoon representation of PGK in the crystal and Sph state respectively. The coloring of each protein model ranges from N-terminus (red) to C-terminus (blue). Residues 50, 74, 287 and 360 are colored in purple, yellow, green and red van der Waals sphere representation. The angle $\theta$ formed by the two vectors defined by residues 50-74 and 287-360 (shown in gray) help monitor the orientation of the two PGK lobes in each conformation.(c)-(d). The probability distribution of the angle $\theta$ defined by the two vectors shown in (a) and (b) for the crystal and Sph states, respectively.
Figure S4. Probability of contact formation for Hydrogen Bond contacts (left column) and side chain contacts (right column). The x and y axes of both panels are residue numbers. Upper triangles present contacts found in the crystal state (Native), while lower triangles contacts that are not found in the crystal state (Nonnative). The top row (A,B) represents the contacts of the crystal structure (labeled C in the right) the middle row (C,D) the contacts of the collapsed crystal (labeled CC) state while the bottom (E,F) the contacts of the spherical (labeled Sph) state. Areas in green rectangles represent formation of new contacts between the nonnative pairs while areas in orange rectangles represent a lost in native contact found in the crystal structure (C state).
REFERENCES: