Supporting Information for

Substrate Channeling Between the Human Dihydrofolate Reductase and Thymidylate Synthase

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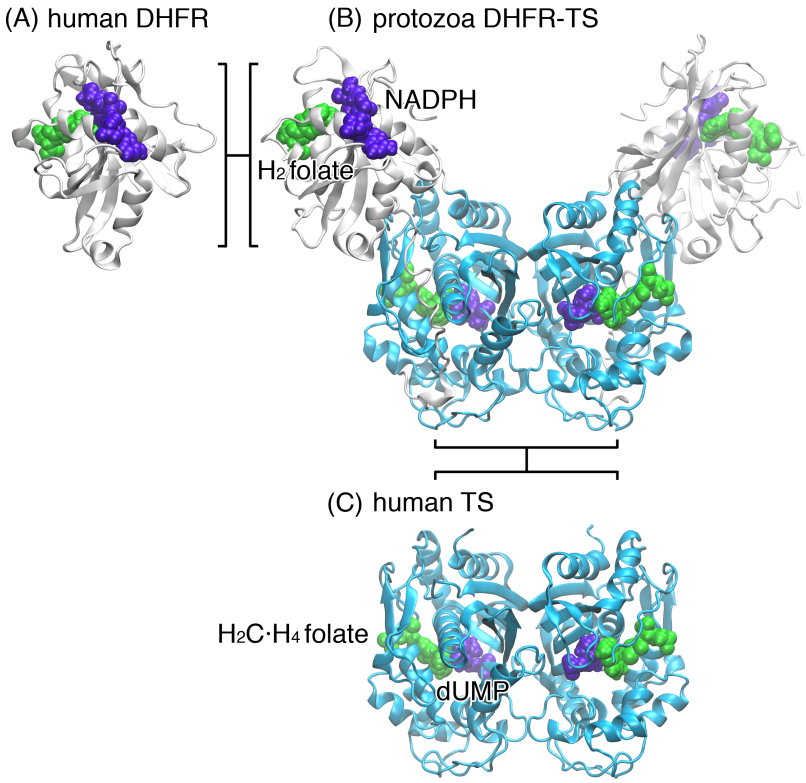

Figure S1. The three dimensional structures of the human and protozoa *Leishmania major* DHFR and TS enzymes. DHFR and TS are represented by the white and cyan cartoons respectively. The substrates are shown in the van der Waals representation in purple and green. The *Leishmania major* DHFR-TS (Ref. 19 in the main text), (B), is a single covalently connected bifunctional enzyme; two *Leishmania major* DHFR-TSs form a homodimer joined through the TS domains. The human DHFR (PDB ID: 1DHF) and TS (PDB ID: 1HVY), (A) and (C), are individual enzymes; DHFR is a monomer, TS is a homodimer. The human DHFR and TS have the same overall tertiary structures compared to that of the protozoa.

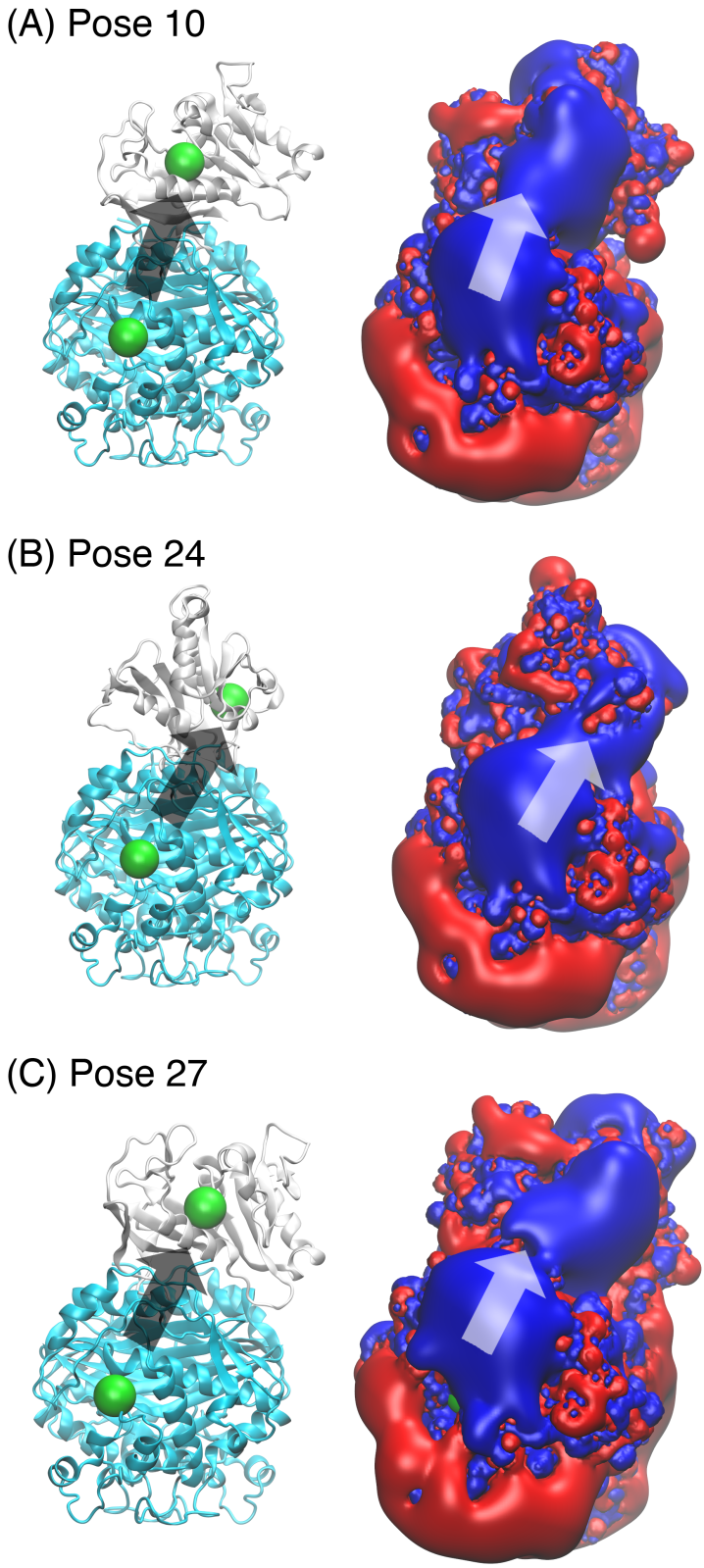

Figure S2. Three more examples of the "electrostatic highways" formed in the 30 ClusPro docking poses. All of the coloring and representations are the same as the main text Figure 2, only that the yellow contours for the "electrostatic highways" are not drawn. Since it has not been mentioned, the pose shown in the main text Figure 2 is the 9th pose.

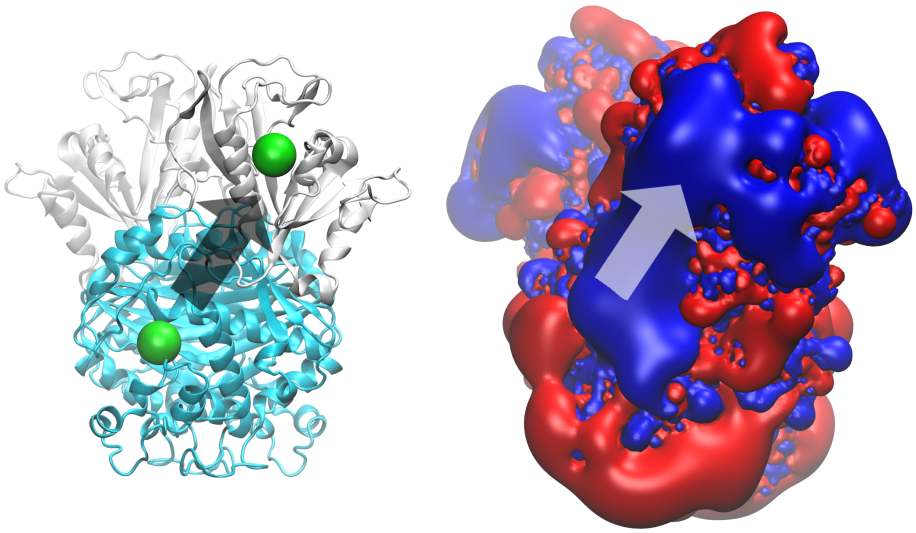

Figure S3. The bound human DHFR-TS is capable of forming an "electrostatic highway" in the *Leishmania major* binding conformation. All of the coloring and representations are the same as the main text Figure 2. The human DHFR and TS are aligned onto the *Leishmania major* binding conformation, the resulting structure is shown on the left; the electrostatic potential of this structure is calculated and shown on the right.

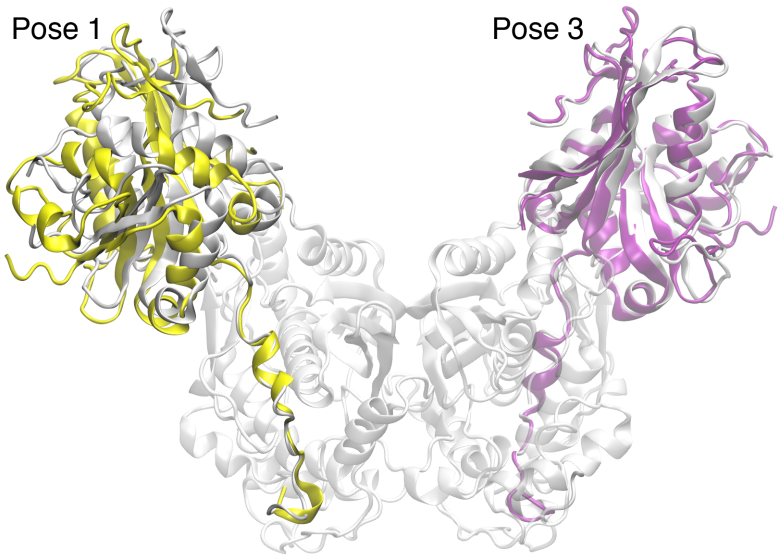

Figure S4. The partial proof of validity of ClusPro. The *Leishmania major* DHFR domain is manually separated from its TS and re-docked onto the *Leishmania major* TS. ClusPro gives 30 poses; the first (yellow) and third (pink) ranked docking poses correctly predict the two DHFR binding conformations on TS; the protein colored in white represents the native structure of the *Leishmania major* DHFR-TS. The RMSDs between the native and docking pose α-carbon atoms are less than 5Å.

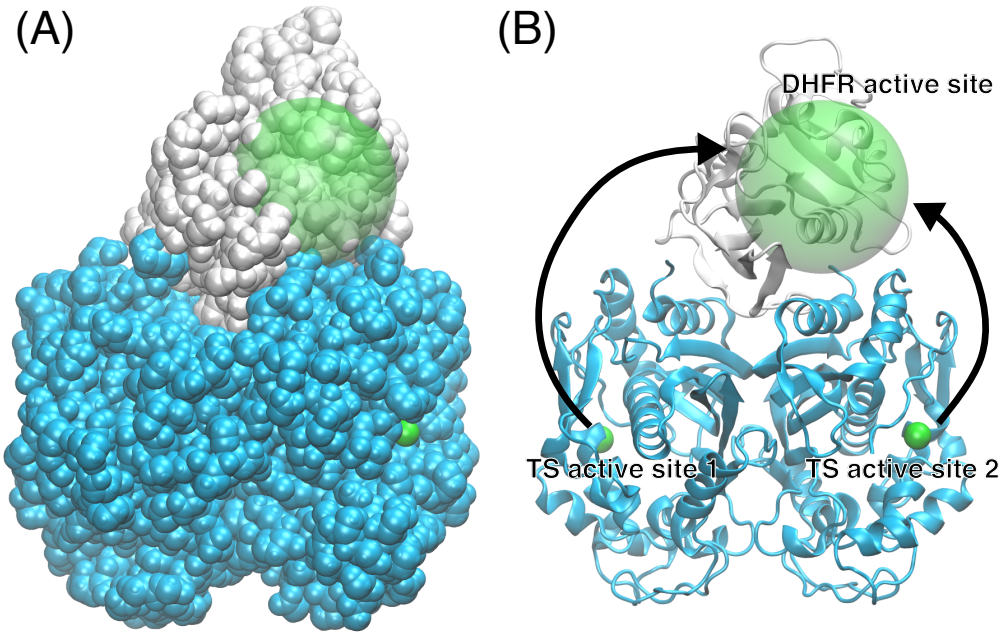

Figure S5. The BrownDye setup. The protein structure shown is one of the 30 ClusPro docking poses. (A) The van der Waals representation. (B) The cartoon representation. Both the substrate and the protein are considered as rigid bodies. The atomic structure of the protein is used, the charge and radius of the protein atoms are taken from CHARMM27 force field. Specifically, the atomic radii are the $r_{min}/2$ values in the Lennard-Jones potential in CHARMM27. The substrate H_2folate is approximated by a 2Å sphere with -2e charge (solid green spheres). The starting position of the substrate, the "active site" of a TS monomer, is a point displaced by 2Å from the geometrical center of the H_2folate binding conformation in TS. The displacement is made along the line passing the geometrical center of that TS monomer and the geometrical center of H_2folate in the direction away from the TS center. The reaction of the substrate with the DHFR active site is defined as the substrate entering a spherical region of radius 12.5Å (transparent green sphere) centered around the "active site" of DHFR, which is a point defined in the same manner as the TS "active site". The escape of the substrate from the system is defined in Figure S6. Most of these settings are taken from the previous work (Ref. 31 in the main text) and the channeling efficiency of protozoa *Leishmania major* calculated using these settings is 73.6%, in agreement with the previous publication (Ref. 31 in the main text). Note that for the docking poses, the channeling efficiency between each TS active site and the single DHFR active site is calculated. But for the native *Leishmania major* structure, due to symmetry, only one TS active site is chosen, and both of the DHFR active sites (there are two DHFRs, not one, in the native *Leishmania major* DHFR-TS) accept substrates.

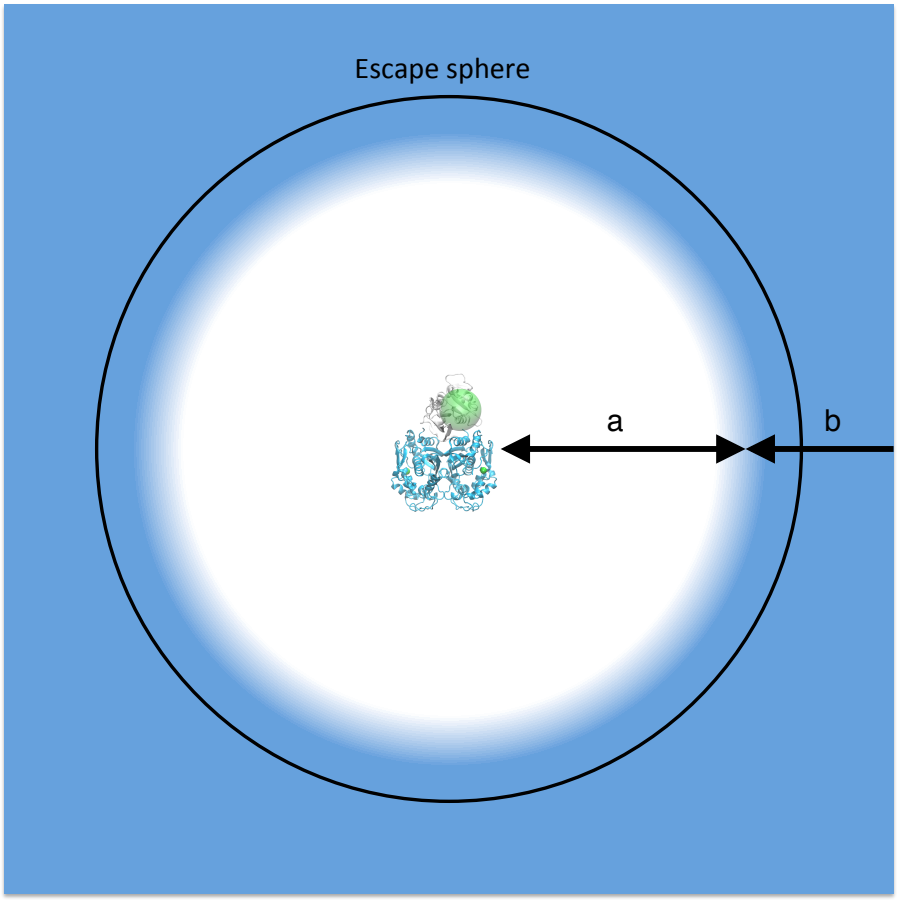

Figure S6. The definition of substrate escape. Figure S5B shows the path that the substrate takes in a successful reaction, however, the substrate always has a possibility to diffuse away from the protein and escape into the bulk solution. **a** (white) represents the immediate region around the protein where the electrostatic forces exerted by the protein on the substrate is dependent on the relative orientation between the two. **b** (blue) represents the space away from the protein where the electrostatic forces that the substrate experiences from the protein can be approximated as orientation-independent. The "escape sphere" is set in the **b** region. Once the substrate reaches the escape sphere, there is a probability that the substrate escapes forever, otherwise the substrate diffuses back to the **a** region. The mathematical expression of this probability is described in "Diffusive reaction rates from Brownian dynamics simulations: Replacing the outer cutoff surface by an analytical treatment" by Luty, McCammon and Zhou, J. Chem. Phys. 97, 5682 (1992). In our simulations, when the substrate reaches the escape sphere, it will escape (and the trajectory will be terminated) according to this probability.

Section S7. The APBS input file example

```
read
    mol pqr enzyme.pqr
end
elec
    mg-auto
    dime 385 385 385
    cglen 240 240 240
    fglen 190 190 190
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    fgcent mol 1
    mol 1
    lpbe
    bcfl sdh
    pdie 2.0000
    sdie 78.5400
    srfm smol
    chgm spl2
    sdens 10.00
    srad 1.40
    swin 0.30
    temp 298.15
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    ion charge -1 conc 0.15 radius 2.27
    calcenergy total
    calcforce no
    write pot dx enzyme-pot
end
quit
```