## CritiRes summary

CritiRes detects critical binding residues in proteins by considering evolutionary conservation and enthalpic/entropic contributions to the binding free energy, $\Delta G^{bind}$. Critical binding residues should be *conserved* and contribute significantly to the $\Delta G^{bind}$. They can enhance favorable enthalpic contributions to the $\Delta G^{bind}$ through hydrogen bonds or van der Waals (vdW) contacts across the protein's interface, rendering them energetically *unstable* in the absence of the protein's binding partner and solvent. Alternatively, they can minimize unfavorable entropic loss upon protein binding if they are not highly flexible, but are energetically *stable*, maintaining an optimal binding scaffold. The pre-organized local environment of these stable/unstable residues can further tune protein binding and function by providing additional favorable enthalpic contributions or minimizing unfavorable entropic contributions.[1] Based on these considerations, CritiRes uses the free protein structure to identify PPI-hot spots as (**1**) conserved and unstable residues (orange), (**2**) conserved and stable residues (green), and (**3**) residues in vdW contact with (**1**) or (**2**) that are most conserved or most unstable/stable (magenta), as shown in Figure 1.

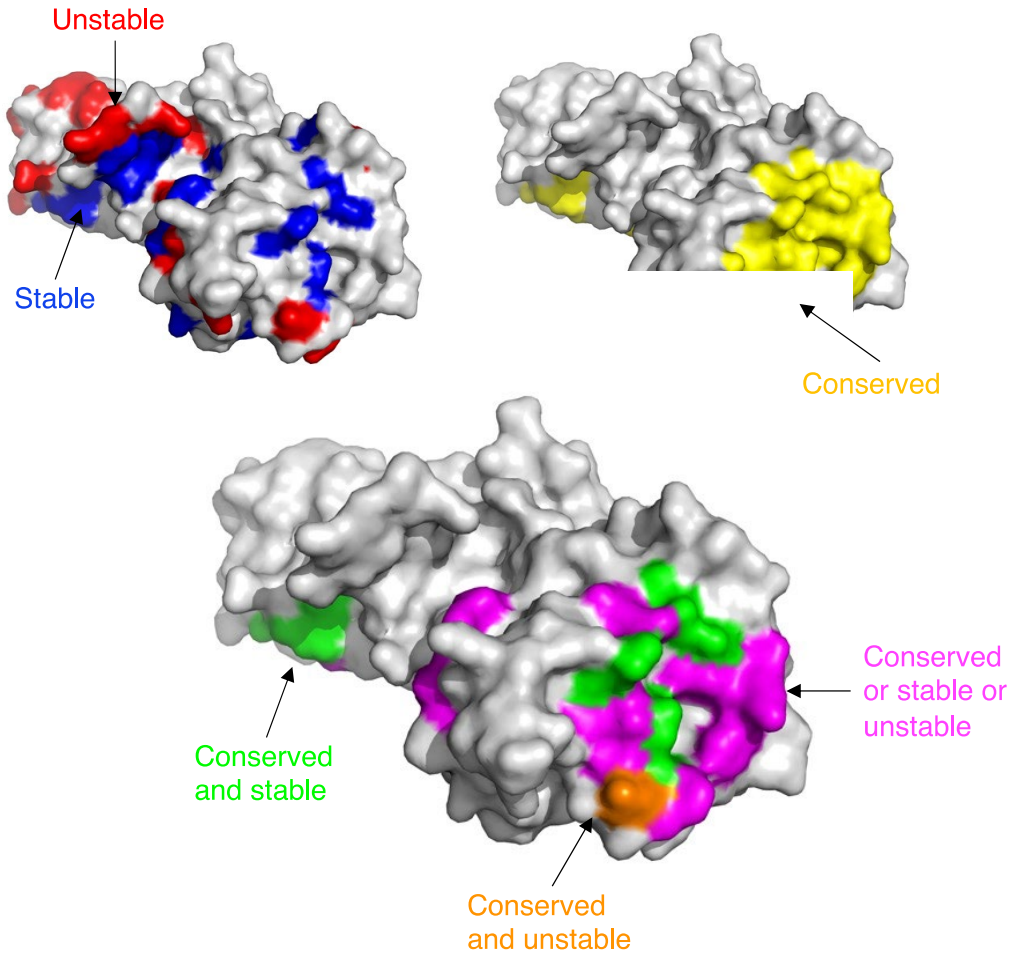


**Figure 1.** Illustration of CritiRes predictions based on the free structure of AP-2 complex subunit alpha-2 (PDB 1QTS-A). Red, unstable residues; blue, stable residues; yellow, highly conserved residues; orange, conserved and unstable residues (**1**); green, conserved and stable residues (**2**); magenta, residues in vdW contact with (**1**) or (**2**) that are the most conserved or the most unstable or the most stable.

## How CritiRes Works

CritiRes uses the free protein structure to rank the stability and conservation of each residue. It ranks the relative stability of residues using $k^E$, an integer ranging from 1 (the least stable) to 10 (the most stable), and the degree of conservation of residues by the Consurf score ($k^C$),[2] an integer ranging from 1 (the most variable) to 9 (the most conserved). It first outputs conserved residues that are either the most unstable or the most stable. Next, it examines solvent-accessible residues located within 4 Å of any atom belonging to a conserved, unstable, or conserved, stable residue and outputs those that are the most energetically stable/unstable or the most conserved (see Figure 2). For example, using the free structure of AP-2 clathrin adaptor a subunit (PDB 1QTS-A) to compute the $k^E$ and $k^C$ scores and SASA of each residue, CritiRes predicted altogether 35 PPI-hot spots namely, E718, E729, K744, E790, K824, R839, E849, D881, R905, R916, R920 with the largest $k^E$+$k^C$ ($k^E$ = 10, $k^C$ = 9); Q782, N883, S922, F938 with the smallest $k^E$-$k^C$ ($k^E$ = 1, $k^C$ = 9); and Q723, K727, F730, G735, G742, D835, F837, W840, K841, Q848, Q851, K857, H858, P882, N886, K896, G901, E907, K923, and E936 with $k^C$ = 9 or $k^E$ = 1 or $k^E$ = 10 that are in vdW contact with the largest $k^E$+$k^C$ or smallest $k^E$-$k^C$ residues.

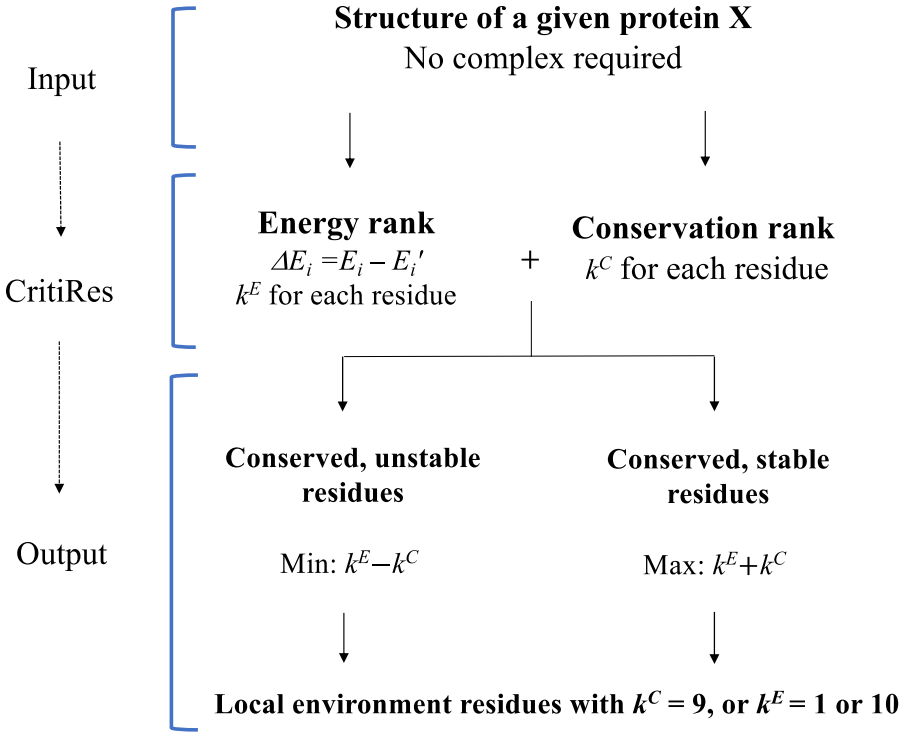


**Figure 2. How CritiRes Works**. Given the free protein structure, CritiRes computes each residue's energy rank ($k^E$) based on the energy difference ($\Delta E_i$) between the gas-phase energy of residue *i* in the native protein ($E_i$) and its energy in an extended reference state ($E_i'$). The top 10% stable residues were assigned $k^E = 10$, the next 10% stable residues were assigned $k^E = 9$, whereas the 10% least stable residues were assigned $k^E = 1$.

1. Mazmanian, K.; Sargsyan, K.; Lim, C., How the Local Environment of Functional Sites Regulates Protein Function. *J. Am. Chem. Soc.* **2020,** *142* (22), 9861-9871.
2. Glaser, F.; Pupko, T.; Paz, I.; Bell, R. E.; Bechor-Shental, D.; Martz, E.; Ben-Tal, N., ConSurf: Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic Information. *Bioinformatics* **2003,** *19* (1), 163-164.