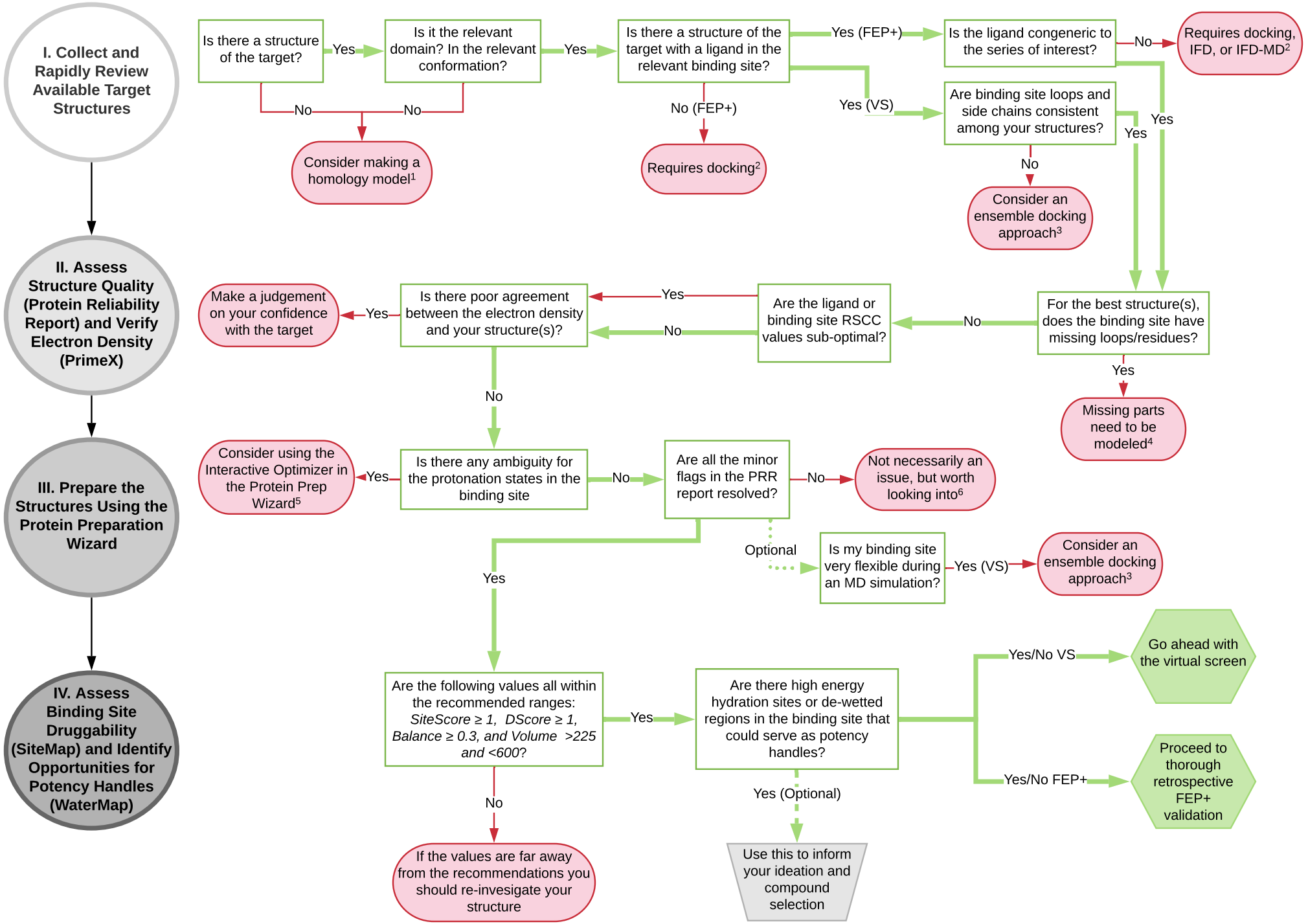
Computational Target Analysis Workflow



Appendix

[1] - The difficulty of successfully running a virtual screen (VS) without an experimental structure of the target largely depends on how similar the closest protein with an available structure is. If a high-resolution template with a high enough sequence identity is available, you can build a homology model using **Prime**. Although we've had a number of successful examples, using a homology model is considered at the limits of the domain of applicability for **FEP+**. In any case, a thorough retrospective study needs to be conducted for validation.

[2] - **FEP+** accuracy depends on the correctness of the binding pose used for the congeneric compound series studied. Therefore, in cases where good quality structures are available for the target but not for a complex with a ligand close to series of interest, the binding pose needs to be accurately modeled, for example by docking using **Glide**, **IFD** or **IFD-MD**. Here again, the pose modeled needs to be validated with a through retrospective study before proceeding to any prospective study.

[3] - In cases where the targeted binding site seems to be flexible or can adopt several distinct conformations, ensemble docking using multiple grids and weighted contributions can be an effective way to maximize the enrichment from a virtual screen. The structures used to generate docking grids can come from different X-ray structures or can be generated using an MD simulation sampling.

[4] - Missing loops and side chains can be added in the **Protein Preparation Wizard** using a knowledge-based method (note: the **Protein Preparation Wizard** can only model in missing loops with 5 or fewer residues). You can also perform energy-based, de novo loop prediction using the **Prime** Refine Loops panel or **PrimeX** (note: loop prediction is not recommended for loops greater than 15 residues). The reliability of the structure (and the chances of success of the VS) decreases rapidly with the extent of the missing parts in the binding site.

[5] - The Interactive Optimizer allows you to manually flip a residue, or adjust its protonation/tautomeric state. Challenging cases include when a residue pKa is close to the pH (resulting in ambiguous protonation states), and when the protonation state might be a function of the ligands bound in the pocket. An ensemble approach (using different grids with different protonation states for the residues in question) can help.

[6] - Sometimes, a few minor flags (yellow or red circles corresponding to secondary aspects of the structure, like unsatisfied buried donor or acceptor, or protein packing) can remain even after protein preparation. The **Protein Reliability Report** panel is interactive, and by clicking on the circles you can look closer into the various residues that contribute to the flags. Many of these flags will have little to no effect on a virtual screen (especially if they are not near the binding site) and, for **FEP+**, will be resolved during the relaxation step.