Molecular simulations have played essential roles in biochemical and biophysical research. One of their major applications is to elucidate the molecular interactions between small molecule ligands and protein or nucleic acid targets so that rational design of high-potency agonists or antagonists can be conducted to enhance or eradicate the functions of their targets. **However, nowadays it is still very challenging to accurately predict the free energies of such interactions and processes.** A key to the success of such applications is the quality of molecular mechanics force fields (**MMFFs**). While polarizable FFs are under active developments, they still cannot replace or beat the classical additive FFs in a wide range of applications due to the serious issues of computational efficiency and parameterization difficulty. Also, there is still a great room for the improvement of the accuracy of additive FFs, such as the popular general AMBER force field (**GAFF**) which has been cited more than 6,000 times. **The major goal of this project is to significantly improve the quality and the chemical space coverage of the existing 2nd generation of GAFF (GAFF2) as well as to develop a new 3rd generation of GAFF** (**GAFF3**). GAFF3 will be based on a new charge model that is efficient, largely conformation-independent, and highly accurate for solvation free energy calculations. The developed GAFF force fields will be rigorously scrutinized and critically assessed through direct comparisons with experimental data of an extensive set of model systems. The large-scale assessment will be accelerated by utilizing GPUs in both MD simulations and free energy calculations. In the long run, a spectrum of high-quality GAFF3 compatible MMFFs will be developed for all types of biological macromolecules following a top-down force field parameterization strategy.

**Aim 1: Improve the quality and chemical space coverage of the second generation of GAFF force field (GAFF2).** We also plan to significantly improve the quality of GAFF2 by re-parameterizing the existing and introducing new parameters including van der Waals, bonds, angles, and especially torsional angles which controls the 3D conformations of molecules. For torsional angle parameters, we’ll first apply a brutal force algorithm to iteratively dissect drug molecules into fragments, and then generate model compounds for all fragments that have one rotatable bond. Last, we’ll examine how well GAFF2 rotational profiles compare to those generated by high-level *ab initio* calculations, and new torsional angle parameters will be introduced if necessary. We also propose to develop FF parameters for broader coverage of the chemical space of small molecules, going beyond the ten basic elements covered by GAFF2.0, which has been released with AMBER software package. Such expansion of the chemical space is in great need in various fields such as drug discovery.

**Aim 2: Develop a new generation of GAFF force field (GAFF3) based on a new physical charge model,** which is efficient, largely conformation-independent and highly accurate in solvation free energy (ΔGsolv) calculations. This new charge model is based on AM1-BCC, but we plan to systemically re-adjust the bond-charge-correction (BCC) parameters for various atom types and bond types of organic molecules to directly target accurate reproduction of experimental ΔGsolv values, which has never been used as the primary target in previous FF parameterizations. Considering the essential relationship between ΔGsolv and receptor-ligand binding free energy ΔGbind, the much more accurate ΔGsolv will lead to more accurate prediction of ΔGbind. The other FF terms will be derived following the successful parameterization strategies for GAFF/GAFF2 development. The successful development of GAFF3 will pave the road to develop a spectrum of biomolecular force fields which apply the GAFF3 charge method.

**Aim 3: Evaluate GAFF2/GAFF3 by studying biomolecule-ligand interactions**. We propose to conduct large scale critical evaluation of GAFF2/GAFF3 in binding free energy calculations with both the pathway and endpoint methods. For the former, we plan to apply a newly developed, GPU-accelerated algorithm, 𝞴-SAMS, to enhance the sampling efficiency, as well as to develop a set of online GUI-toolkit to facilitate setup and analysis of alchemical free energy calculations. The new force fields, sampling method, as well as toolkit will be assessed by both the benchmark and cherry-picked data sets (20-30 drug targets). For the latter, we’ll develop a set of PB/GBSA models that are consistent to the GAFF3 charge model, and then study a set of drug targets which have tens, hundreds, or even thousands of actives and decoys to evaluate the “Screening Power” and the “Docking Power” of the GAFF2/GAFF3 based MM-PB/GBSA models. Alternative general force fields such as CGenFF, 2016H66, SMIRNOFF, etc., will also be evaluated and compared with GAFF2/GAFF3.

**Expected Outcome.** We expect to release a set of GAFF2 force fields according to the roadmap; to accomplish the development of GAFF3, a new generation of general-purpose force field based on a high-quality charge model. The new set of GAFF3-compatible MMFFs will be highly transferable and self-consistent for studying protein-ligand and nucleic acid-ligand interactions, and will enable more accurate calculations of the protein-ligand and nucleic acid-ligand binding free energies, which will in turn greatly improve the successful rate of computer-aided drug design.