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 inhibitors 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. A key to the success of such applications is the quality of molecular mechanics force fields (**MMFFs**). **However, nowadays it is still very challenging to accurately predict the free energies of such interactions and processes.** **The major goal of this project is to expand the chemical space and improve the quality of the existing as well as to develop a new generation of the general AMBER force fields (GAFF) for studying biomolecule-ligand interactions.** The new general-purpose MMFF (referred to here and thereafter as **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. 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. The new set of 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 to facilitate computer-aided drug discovery. The large-scale FF assessment will be accelerated by utilizing GPUs in both MD simulations and alchemical free energy calculations.

**Aim 1: Expand the chemical space and improve the quality of the Second Generation of GAFF Force Field (GAFF2).** We propose to develop force field 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. The ultimate goal is to make GAFF2 a universal force field covering most elements in the periodic table except lanthanoids and actinoids. We also plan to significantly improve the quality of GAFF2 by re-parameterizing the existing and introducing new parameters. For torsional angle parameters, we’ll 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. Next, we’ll examine how well rotational profiles generated by GAFF2 compare to those generated by *ab initio* calculations at the MP2/aug-cc-pVTZ//MP2/6-31G\* level. New torsional angle parameters will be introduced if necessary.

**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. 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. Next, van der Waals parameters will be adjusted to reproduce the experimental values of two pure liquid properties (density and heat of vaporization) and high-level *ab initio* intermolecular energies. Last, the bonded force field parameters will then be derived following the same parameterization strategies in 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 develop a new sampling strategy, 𝞴-SAMS, with GPU acceleration 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, even thousands of actives and decoys to evaluate the “Screening Power” and the “Docking Power” of the GAFF2/GAFF3 based MM-PB/GBSA models.

**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. GAFF3 in combination with the GAFF3-compatiable biomolecular force fields will enable us to accurately model biosystems and calculate binding free energies of protein-ligand and nucleic acid-ligand binding, which will in turn greatly improve the successful rate of computer-aided drug design.