Parameterization Protocol

The geometry parameters of drugs (bond lengths, angles, torsions) were taken as it is presented in crystallographic data. The position of undefined hydrogen atoms were obtained by *hbuild* utility implemented in CHARMM program[1] (Figure S1).

Electrostatic parameters are crucial to reproduce the binding affinities of drugs to the protein receptor. The methodology of charge fitted to the electrostatic map was employed to obtain the charge distribution. This scheme mostly known as RESP scheme is implemented in the FITCHARGE module of CHARMM package. This procedure requires the molecular electrostatic potential to be calculated at *ab initio* level. Since non-polarizable models would be used for the following molecular simulations, the only one electrostatic map was calculated based on x-ray geometry of 2q72 and 2q6h. Cartesian coordinates for ESP grid were created by CGRID program[2]. Then Cartesian coordinates of drug and grid were used as an input for ESP calculations by Gaussian[3] program at B3LYP/6-31G* level. The data for calculated electrostatic maps were extracted from Gaussian output and used for charge fitting inside CHARMM program.

Initial guesses for fitting procedure were CHARMM non polarizable force field charges. The summary charges for the molecules were equal zero. The fitting algorithm with charges to be restrained to their initial valued (parabolic penalty function switched on by PARA keyword) were used. There restrain force were 10^{-4} $Å^{-2}$. The flat well potential were applied to the penalty function keeping the deviation of charges to be zero under and above the parabolic function, in other words forcing them to be restrained by only restrain penalty function. Some equivalents for chemically similar hydrogens and carbons were evoked as well. The final charges obtained by this scheme along with the initial charges are presented in Tables S1.

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[1] CHARMM: Brooks, B. R., et. al. 1983. CHARMM: a program for macromolecular energy minimization and dynamics calculations. *J.Comp.Chem.* 4:187-217.

[2] In more details this program was described by V. Anisimov et al. at J. Chem. Theory Comput. 2005, 1, 153-168

[3] Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

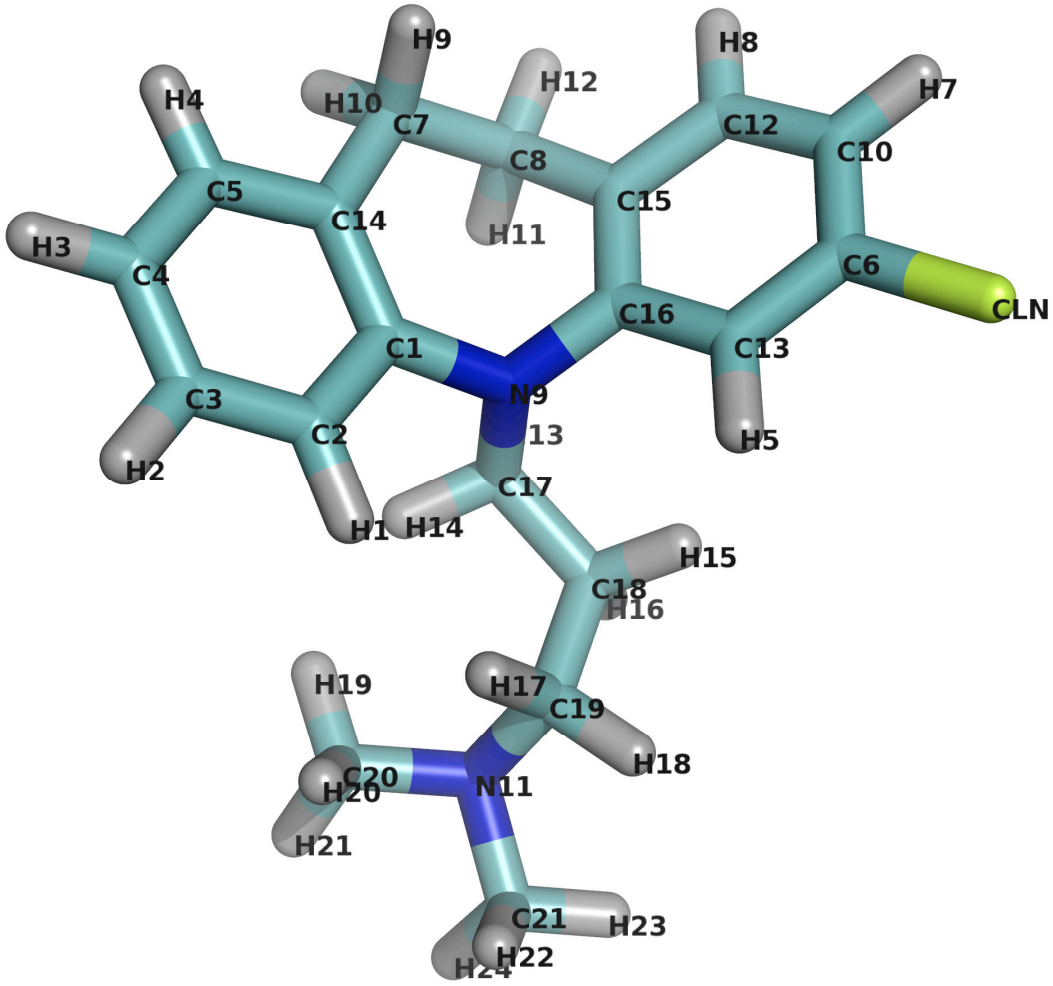

Figure S1. The geometry of clomipramine (from PDB ID 2Q6H) with atom numbering and nomenclature.

Table S1. Fitted charges for 2q6h (non-polarazable CHARMM force field).

atom	Partial Charge
C1	0.167
C2	-0.138
C3	-0.138
C4	-0.138
C5	-0.138
C14	0.167
H1	0.109
H2	0.109
H3	0.109
H4	0.109
C15	0.167
C16	0.167
C13	-0.138
C6	-0.138
C10	-0.138
C12	-0.138
H5	0.109
CLN	0.034
H7	0.109
H8	0.109
C7	-0.198
H9	0.073
H10	0.073
C8	-0.198
H11	0.073
H12	0.073
N9	-0.509
C17	-0.027
H13	0.113
H14	0.113
C18	-0.133
H15	0.128
H16	0.128
C19	-0.133
H17	0.128
H18	0.128
N11	-0.559
C20	-0.051
H19	0.078
H20	0.078
H21	0.078
C21	-0.051
H22	0.078
H23	0.078
H24	0.078

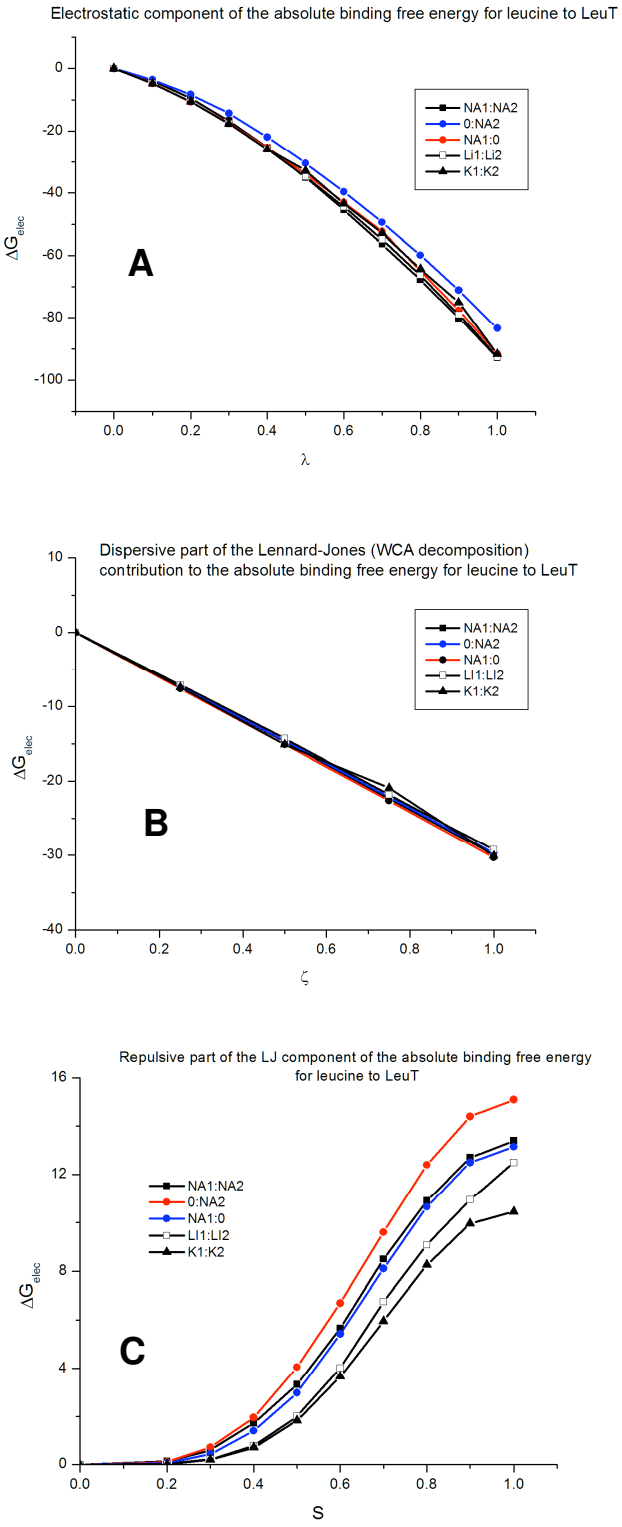

Figure S2. The leucine absolute binding free energy decomposition as function of from the FEP simuilation with different ion types and ion occupancies.

A. Free energy of uncharging (electrostatic component) B. Dispersive part of the Weeks-Chandler-Anderson (WCA) decomposition of the Lennard-Jones interactions. C. Repulsive component from the WCA analysis. For further information please refer to the original Deng and Roux papers (see below)

Wang, J. Y., Deng, Y. Q., Roux, B. 2006. *Biophysical Journal* 91:2798-2814.

Deng, Y.Q., and B. Roux. 2006. *J.Chem.Theor.Comp.* 2:1255-1273.