

**Analysis of crystallographic structures and DFT calculations reveal a new structural arrangement in proteins involving lysine NH<sub>3</sub><sup>+</sup> group and carbonyl.**

*Nikolai R. Skrynnikov, Purdue University, West Lafayette, IN*

*Sergei A. Izmailov, St. Petersburg State University, St. Petersburg, Russia*

*Lyudmila V. Slipchenko, Purdue University, West Lafayette, IN*

*Olga N. Rogacheva, St. Petersburg State University, St. Petersburg, Russia*

Lysine side-chain NH<sub>3</sub><sup>+</sup> group is capable of forming three conventional hydrogen bonds. This arrangement creates a “sweet spot” for the fourth electron-rich ligand, such as carbonyl oxygen. Specifically, carbonyl O atom often occupies the position on the (three-fold) symmetry axis of the NH<sub>3</sub><sup>+</sup> group at a distance 2.7-3.0 Å from the nitrogen atom. The analysis of high-to-medium-resolution protein structures in the PDB finds more than 10,000 such occurrences, corresponding to a distinct cluster on the phase-space probability density map. Our DFT calculations confirm that this arrangement, which is termed “linear NH<sub>3</sub><sup>+</sup>-carbonyl interaction”, offers a stabilizing energy of up to 2 kcal/mol that is mainly of electrostatic origin. Among many biologically relevant examples, linear interaction involving conserved lysine is consistently found in the P-loop of numerous NTPase domains (including Ras and Ras-like proteins), where it stabilizes the substrate-binding conformation of the P-loop. In addition to carbonyl, oxygen atoms of crystallographic water molecules as well as oxygen atoms from Ser, Thr and Tyr side-chain hydroxyl groups can also form a similar linear arrangement with ε-ammonium ion. Curiously, carboxylic oxygens do not engage in linear interactions. It appears that COO<sup>-</sup> can usually force its way into a more favorable hydrogen-bonded position by displacing a weaker acceptor, such as carbonyl. As a result, COO<sup>-</sup> ··· NH<sub>3</sub><sup>+</sup> salt bridges almost invariably occur in a form of hydrogen bonds.

*The research was supported by RSF grant 15-14-20038.*