# Experimental and Computational Modeling of h-Bonded Uncharged (aRginine-tYrosine), or BU(RY) Dyads, in Aprotic Environments

Andrew T. Banyikwa and ***Mark S. Braiman***
Chemistry Department, Center for Science & Technology, Syracuse University, Room 1-014, Syracuse, New York 13244, United States

Email: mbraiman@syr.edu

We have shown that monoalklylguanidine free bases, purified by crystallization from aprotic solvent, are significantly more basic than previously thought. After rigorously excluding water, hydroxide, and other proton donors, the deprotonated guanidine group's spectroscopic properties [1] are strikingly different from those previously reported for aqueous arginine at high pH. Thus, addition of large amounts of $OH^-$ to arginine or other monoalklyguanidinium compounds does not substantially deprotonate them. Instead, it leads to formation of a guanidinium-hydroxide complex, with a dissociation constant near ~300 m*M* that accounts for previously-described arginine p$K_a$ values of 13-13.7. The near-ubiquitous presence of water suggests that true arginine deprotonation might not be observable even in the least polar environments present in biological systems. However, it is still theoretically possible that another strong H-bond donor, e.g. the side chain of tyrosine, might be able to displace $OH^-$ from arginine inside the most nonpolar region of a membrane or a protein, to form a strongly h-Bonded Uncharged (aRg-tYr), or BU(RY), grouping—our coinage. We have begun to model this possibility experimentally by synthesizing anhydrous crystalline dodecylguanidinium cresolate, and the corresponding covalently-linked compounds (*p*-phenolyl-alkylguanidines), and studying their properties in aprotic environments [2]. These compounds form strongly-H-bonded Lewis acid-base complexes with spectroscopic properties strikingly different from the guanidine free base itself, as well as from all previously-examined guanidinium or phenolate salts. The experimental H-bond strength of the dodecylguanidine:cresol 1:1 complex in hexane is unexpectedly large ($\Delta H$ = -70 kJ $mol^{-1}$), but agrees with *ab initio* computations *in vacuo*. In the polar aprotic solvent DMSO, analogous head-to-tail homodimer complexes of ω-(*p*-phenolyl)-alkylguanidines form H-bonds that are ~1/3 as strong. Nevertheless, the protons in these systems are shared so completely, that NMR signals of both guanidine and phenol groups simultaneously appear mostly-protonated, despite the full stoichiometric proton loss relative to (guanidinium+phenol). Conserved BU(RY) groupings should therefore be considered for potential involvement in the activated intermediates of visual rhodopsins and other GPCRs, as well as of microbial rhodopsins. It was in these systems that the Khorana lab first identified conserved membrane-buried arg-tyr neighbors as playing key mechanistic roles [3]—one of the original inspirations for the current work.

1. Banyikwa, A.; Miller, S. E.; Gao, T.; Krebs, R. A.; Xiao, Y.; Carney, C.; Braiman, M. S. (2017)Small-Molecule Models for Arginine Side Chains and Arginine-Tyrosine Pairs in Nonpolar Environments, *ACS Omega* 2. **DOI:**10.1021/acsomega.7b00281.

2. Banyikwa, A.T., Goos, A., Kiemle, D. J, Foulkes, M.A.C., Braiman, M.S. (2017) Experimental and Computational Modeling of H-Bonded Arginine-Tyrosine Groupings in Aprotic Environments, *ACS Omega* 2, 5641–5659, D**OI:**10.1021/acsomega.7b00282

3. H.G.Khorana (1988). Bacteriorhodopsin, a Membrane Protein that Uses Light to Translocate Protons, *J. Biol. Chem. 263*, 7439-7442.