A New Method for Detecting Nitroxyl Inspired by Natural Reactivity of HNO with Proteins

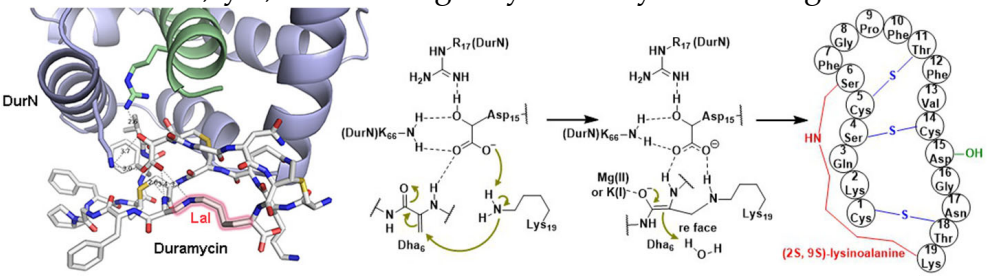
Nicholas W. Pino and Jefferson Chan

Nitroxyl (HNO) is the one-electron reduced relative of nitric oxide (NO), a well-known biological signaling molecule. Owing to its high reactivity and short biological lifetime, the physiological and pharmacological roles of HNO remain unclear. This is exacerbated by a lack of effective detection methods with the requisite sensitivity. This work addresses these challenges by developing a novel HNO-detecting strategy utilizing the rapid and selective reaction between HNO and reactive moieties found in nature. Using physical organic chemical phenomena, the trigger moiety and a custom fluorescein-based dye were rationally designed to synthesize a probe with which we aim to detect endogenous HNO—a currently unachieved task across the field. Our trigger applies to both *in vitro* and *in vivo* imaging modalities which we aim to employ to better understand the biological function of HNO.

Substrate-assisted Catalysis of Lysinoalanine Formation in Duramycin

Linna An, Dillon Cogan, Claudio D. Navo, Gonzalo Jiménez-Osés, Satish K. Nair, and Wilfred A. van der Donk

Duramycin is an antibiotic containing a (2*S*,9*S*)-lysinoalanine (Lal) which is required for duramycin antimicrobial activity. Lal is installed in a stereospecific fashion by a 13-kDa protein, DurN, following dehydration at Ser6 by the bifunctional dehydratase-cyclase enzyme, DurM. We reconstituted DurN activity *in vitro*, demonstrating (2*S*,9*S*)-Lal formation in the dehydrated, partially cyclized and Asp15 hydroxylated peptide substrate. Mutational studies of DurN suggest residues important for DurN homodimer formation or substrate binding are critical for DurN activity. Surprisingly, in the cocrystal structure of DurN and duramycin, Lal is exposed to solvent while the hydroxylated Asp15 residue in the substrate is hydrogen bonded to Lys66 and Arg17 from DurN. Substrate without β-hydroxyl at Asp15 and substrate mutant Asp15Ser still bind to DurN, yet, are no longer cyclized by DurN. Together with other data, we propose that DurN confines the substrate and brings Lys19 close to Dha6, while the substrate itself assists Lal formation with its own hydroxylated Asp15.



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