1. **Salvatore Nesci, PhD**
The mitochondrial F1FO-ATPase is kinetically dependent on the dithiol redox state in modulating the permeability transition pore

2. **Kun Yuan, PhD Candidate**
Applications of sulfur–triazole exchange (SuTEx) chemistry for protein and inhibitor discovery

3. **Anthony Ciancone, BS**
Discovery of a Cell-Active SuTEx Ligand of Prostaglandin Reductase 2

1. ***The mitochondrial F1FO-ATPase is kinetically dependent on the dithiol redox state in modulating the permeability transition pore***

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The phenylarsine oxide (PAO) and dibromobimane (DBrB) have an opposite effect on the catalysis of F1FO-ATPase. PAO increase ATP hydrolysis by 20% at 50 µM and 150 µM in presence of the natural cofactor Mg^{2+} and Ca^{2+}, respectively. The PAO-activated F1FO-ATPase is reverted to basic activity by 50 µM DTE reducing thiols reagent. Conversely, DBrB has an inhibitory effect on the Mg^{2+}- and Ca^{2+}-dependent F1FO-ATPase by 25% and 50% decrease of enzyme activity at 300 µM with Mg^{2+} and Ca^{2+}, respectively. Moreover, the F1FO-ATPase inhibition by DBrB is insensitive to DTE. The mitochondrial permeability transition pore (mPTP) formation is related to Ca^{2+}-dependent F1FO-ATPase. mPTP opening is sensitized by PAO, whereas is desensitized by DBrB. The results on the F1FO-ATPase kinetic reaction in presence of the Ca^{2+} cofactor dependent on the cross-link of different thiol groups uncover the role of enzyme links to the mPTP modulation.

2. Applications of sulfur–triazole exchange (SuTEx) chemistry for protein and inhibitor discovery

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Recently, we introduced sulfonyl-triazoles as a reactive group for covalent modification of tyrosines on proteins through sulfur–triazole exchange (SuTEx) chemistry. SuTEx achieves covalent reaction with protein sites through binding and irreversible adduct group (AG) modification of a nucleophilic residue upon departure of a leaving group (LG). The introduction of a heterocyclic leaving group such as the triazolide introduces unique capabilities for medicinal chemistry optimization of reactivity and chemoselectivity of the sulfur electrophile. Such tunability has enabled our group and others to evaluate thousands of functional tyrosine sites in lysates and live cells by chemical proteomics. Our discoveries, to date, include identification of tyrosines with enhanced nucleophilicity as well as catalytic and non-catalytic sites with propensity for covalent modification. Here, we describe our efforts to include elaborated binding elements on the AG of SuTEx compounds to guide selective probe development. Given the larger size and hydrophobic nature of the resulting covalent adduct, a customized LC-MS/MS strategy was developed for direct target site identification of complex, targeted covalent probes and inhibitors. Collectively, we highlight the versatility of SuTEx chemistry as a new reactive group for chemical proteomic investigations of tyrosine reactivity, pharmacological tractability, and functional state.

3. *Discovery of a Cell-Active SuTEx Ligand of Prostaglandin Reductase 2*

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The Hsu lab recently introduced sulfur-triazole exchange chemistry (SuTEx), which deploys a sulfonyl-triazole reactive group for covalent modification of functional tyrosine (and to a lesser degree lysine) sites. SuTEx chemical probes enrich binding to protein domains involved with RNA binding and recognition, which have been historically difficult to target.[1, 2] Here, we describe the application of SuTEx for fragment-based ligand discovery ('FBLD') in live cells to develop a prostaglandin reductase 2 (PTGR2) ligand. PTGR2 reduces 15-keto-PGE_2 into 13,14-dihydro-15-keto-PGE_2, lipids which are critical for inflammatory regulation and have been implicated as important for a variety of cancer types.[3, 4] PTGR2 was identified as a target of interest because SuTEx probes liganded tyrosine-100 (Y100), which is located in the active site.[5] We combined medicinal chemistry and SuTEx chemical proteomics for the optimization of more selective ligands (e.g. HHS-0701) to target PTGR2 Y100. We utilized the 1,2,4-triazole scaffold as the basis of our ligands, as our lab previously found that this moiety enhances the tyrosine-to-lysine selectivity of SuTEx chemical probes and ligands.[1, 6] Future work will involve derivatizing HHS-0701 to decrease the number of off-target proteins and increase the potency against PTGR2 with potential site selectivity against Y100.

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