More than 37 million people are living with HIV worldwide. Despite the widespread use of antiretroviral therapy (ART), up to 70% of HIV-positive individuals suffer from cognitive and behavioral deficits known as HIV-associated neurocognitive disorders (HAND). HIV-induced damage to the dopaminergic system is a mediating factor in HAND. Because most ART cannot efficiently cross the blood-brain barrier (BBB), the brain serves as a viral reservoir that facilitates the spread of infection to susceptible cells which shed viral proteins such as Tat. Dopamine (DA) transporter (DAT)-mediated reuptake is essential for maintaining DA homeostasis. Tat plays a critical role in HIV infection-induced dysregulation of the dopaminergic system by its allosteric inhibition of DAT. Because Tat does not compete with the DA uptake site, blocking the Tat-DAT interaction will have minimal effects on normal transporter function. Cocaine, a potent DAT inhibitor, magnifies Tat neurotoxicity, which contributes to HAND severity in cocaine-abusing HIV-infected patients. We hypothesize that Tat protein inhibits DA uptake by interacting with specific recognition binding residues on DAT thereby leading to DA dysregulation observed in HAND. Targeting the Tat-DAT interaction during the early stages of HIV infection is therefore a potential therapeutic strategy to prevent HAND.

Our studies aim to characterize the binding mode of human DAT (hDAT) with Tat through molecular dynamics simulations and in vitro experimental validation. Mutations on key DAT residues attenuated Tat-induced inhibition of DAT uptake while preserving or significantly improving basal function of the transporter through alterations in phosphorylation, palmitoylation, and transporter conformation. Additionally, double and triple combinations of these mutations blocked Tat inhibition. Introducing mutations on Tat reversed the inhibitory effects of the protein, indicative of disrupted Tat-DAT binding.

Finally, to probe the therapeutic feasibility of developing allosteric modulators to block Tat and cocaine binding without interfering with normal DAT function, we utilized the SRI compounds which are novel quinazoline-based allosteric modulators. SRI-32743 pharmacologically demonstrated allosteric properties and attenuated Tat inhibition in wild-type human DAT-expressing cells. When administered systemically to inducible HIV-1 Tat transgenic mice after 14 days of Tat induction via doxycycline, SRI-32743 dose-dependently ameliorated conditioned place preference for cocaine. These results demonstrate that developing allosteric modulatory molecules which attenuate cocaine and Tat binding to DAT will aid drug discovery efforts in the search for therapeutic interventions for HIV-infected patients who are concurrent abusers of cocaine.