# Chemical biology: a toolbox to unlock neurochemical epigenetics?

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**The complex challenges of studying neurochemistry:** Neurochemistry comprises the molecular and biochemical roles of a vast variety of chemical compounds, including amino acids, peptides, gaseous molecules, and small molecules such as monoamines, which are crucial for the physiology of neurons, synapses and neural networks. Prominent neurochemical agents of the nervous system are the monoamines dopamine and serotonin. They are commonly associated with well-being and happiness but this perception is misleading since both molecules execute very complex and diverse physiological functions in the nervous system and in other tissues (Berger at al., 2009; Meiser et al., 2013).

The specific function of dopamine and serotonin is very difficult to isolate, extrapolate and understand because they are widespread in the organism and involved in a multitude of biological processes. In addition, their chemical properties hinder scientific interrogation. For example, dopamine is very reactive, and quickly oxidizes in aqueous solution under physiological pH (Bisaglia et al., 2010). Dopamine oxidation products participate in a convoluted series of complex reaction cascades, which gives rise to a plethora of different metabolites, including large insoluble polymers. All these metabolites are capable of interacting with biological molecules, and inducing various biomolecular responses in the cell and in the tissue.

On those grounds, studying the biological role of neurochemistry is a challenging endeavor. Today, chemical neuroscience employs a large array of different techniques and approaches that examine the function of neurochemical receptors (e.g., dopamine uptake receptors and serotonin receptors), and provide insights into the chemistry of neurochemical compounds (e.g., dopamine, serotonin) (Beyene et al., 2019). Despite these techniques, researchers still lack methods to elucidate thoughly the complex role of monoamines and other neurochemical compounds within complex biological systems. Consequently, the effects of neurochemical agents on various biological molecules and their within the cell remains largely unknown.

**Brain chemistry and neurodegenerative disorders:** A recent study highlighted the role of dopamine in the development of Parkinson's disease (PD) (Burbulla et al., 2017). PD is one of the most common age-related neurodegenerative disorders (Kalia and Lang, 2015). The hallmarks of this slowly progressing disease are loss of dopamine producing neurons and accumulation of protein aggregates in the brain.

As of now, the underlying molecular mechanism of dopaminergic cell death is unknown but the study from Burbulla et al. (2017) strengthened the link between dopamine oxidation and the loss of fundamental cellular functions in the brain of PD patients; however, the interactions of dopamine oxidation products with other biological molecules, such as proteins, remain elusive. The lack of tools and techniques to interrogate specific dopamine metabolites, especially within cellular systems, limits our understanding of the role of dopamine metabolism in the development of PD.

**Applying novel tools and techniques:** In our recent study, we sought to address this limitation and presented a chemical biology approach to investigate the role of neurochemistry in the nervous system (Farzam et al., 2020). We focused on a specific variant of oxidized dopamine—6-hydroxydopamine (6-OHDA)—that has been widely used in PD research (Hernandez-Baltazar et al., 2017). Intrastriatal administration of 6-OHDA into the brain of rodents induces death of dopaminergic neurons, and thus allows the study of the PD phenotype in the animal model.

We employed a chemically modified analog of 6-OHDA, which contained an alkyne handle that was attached to 6-OHDA's amino group via a PEG3-linker. This dopamine analog, termed 6-OHDA-PEG3-yne, allowed us to examine chemical properties of 6-OHDA, specifically autoxidation and the production of the 6-OHDA *p*-quinone. Furthermore, the alkyne handle enabled us to apply copper(I)-catalyzed azide-alkyne cycloaddition, also known as 'click chemistry', to conjugate the analog to a biomolecule for visualization and analysis. We used 6-OHDA-PEG3-yne to explore the interaction of neurochemistry and biological molecules, in particular proteins, and to elucidate the chemical reactivity of dopamine oxidation products in neurons.

We demonstrated that 6-OHDA and 6-OHDA-PEG3-yne undergo an identical autoxidation process, but 6-OHDA-PEG3-yne remained substantially more stable than 6-OHDA. We attributed this difference to the attached PEG3-linker that prevented intramolecular cyclization of 6-OHDA, and hence limited 6-OHDA's reaction cascade. Moreover, we found that abrogation of the cyclization event also abolished the formation of a black pigment, which is a polymer of 6-OHDA building blocks, and resembles naturally occurring neuromelanin. This highlights that chemical modification of neurochemical compounds has the potential to provide a deeper understanding of the role of neurochemistry in biological systems.

Furthermore, 6-OHDA-PEG3-yne allowed visualization of protein modification by quinones *in vitro* under many conditions, and enabled monitoring of its intracellular distribution in cellulo. We were able to demonstrate that proteins form adducts with 6-OHDA *p*-quinone via the thiol group of a cysteine. We performed azide-alkyne cycloaddition of biotin-azide to 6-OHDA-PEG3-yne followed by a streptavidin pulldown. Subsequently, we employed mass spectrometry in order to identify protein targets with this modification. Our analysis strongly implies that protein modification by a dopamine metabolites disrupt the thiol redox metabolism in the cell, and hence hampers cell survival. This is a remarkable finding considering that almost all research on 6-OHDA have attributed its toxicity to the induction of oxidative stress in the neuron (Hernandez-Baltazar et al., 2017).

Furthermore, we examined the biological implication of the *p*-quinone modification using protein disulfide isomerase (PDI). PDI is a key enzyme in protein folding and redox metabolism, which are two cellular processes that are disrupted in neurodegenerative diseases. Hence, PDI plays a crucial role in PD. We employed *in vitro* activity assays and demonstrated that 6-OHDA renders PDI inactive. Loss of enzyme function due to modification by dopamine metabolites is a novel and important finding, which further strengthens the emerging link between dopamine oxidation and the development of PD (Burbulla et al., 2017; Herrera et al., 2017).

**Neurochemical epigenetics:** We provide compelling evidence that there is a clandestine layer of neurochemistry contributing substantially to cellular function in the brain and in other tissues. Our data suggests that protein modification by neurochemical agents, such as dopamine metabolites, can occur in biological systems. These findings

suggest that the cell uses neurochemicals to modify biological molecules in order to alter and regulate function and activity, and even to use these compounds as epigenetic regulators for various biological processes.

A notable example of neurochemical epigenetics is serotonylation. In 2003, scientists described for the first time serotonylation as a receptor-independent signaling mechanism that regulates platelet activity by modifying small GTPases (Walther et al., 2003). A recent study also employed chemical biology techniques to demonstrate that protein modification by serotonin affects cellular function on a different scale (Farrelly et al., 2019). In this elegent study, the authors showed that serotonylation of histones alters gene expression patterns, and thus demonstrated that protein modification by neurochemical compounds can serve as an epigenetic regulator of cellular function.

The reaction mechanism described for serotonylation is different from the mechanism described for protein modification by 6-OHDA in our study; however, based on the chemical similarity between serotonin and dopamine, it is possible that dopamination occurs and executes analogous functionality as serotonylation. Moreover, it seems plausible that neurochemical epigenetics are not limited to serotonin and dopamine. The chemical biology approaches presented in our study and in the work of Farrelly et al. (2020) are applicable to other neurochemical metabolites, and will help to explore neurochemical epigenetics in the future (**Figure 1**).

**Conclusions and perspectives:** The advent of synthetic and chemical biology opens exciting new avenues for studying biological questions and phenomena that have been out of reach until now. Chemical biology provides tools and techniques to biochemists and molecular cell biologists that enable them to gain deeper insights into challenging areas of research. One such area is deciphering the role of neurochemistry in the complex physiology of the brain, and its contribution to neurodegenerative and neuropsychiatric disorders, such as dementia and depression.

Characterization of the dynamic interplay between neurochemistry and cellular function in the brain will provide a new level of understanding of the conditions that lead to the progression of neurological disorders. Ultimately, research can exploit these new findings for the development of future therapeutic strategies.

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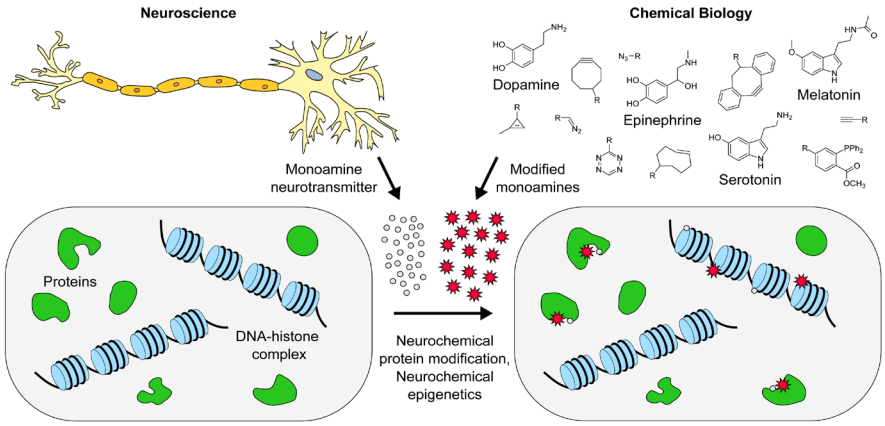


**Figure 1** | **Schematic overview of the application of chemical biology strategies in neuroscience to explore neurochemical protein modification and epigenetics.**
Neurons (top left) produce a variety of neurochemical compounds, including monoamines that serve as neurotransmitters (grey circles). Monoamines, such as dopamine, epinephrine, melatonin and serotonin, can be chemically modified with various functional groups (top right). If monoamines form covalent bonds with proteins or DNA-histone complexes, the application of monoamine analogues (red stars) enables detection and visualization of this modification. For instance, attaching a reporter molecule to the monoamine analogue via copper(I)-catalyzed azide-alkyne cycloaddition allows interrogation of biological substrates (not shown).

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