An activity-based fluorescent sensor for the detection of the phenol sulfotransferase SULT1A1 in living cells†

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**Human phenol sulfotransferases mediate the transfer of a sulfuryl moiety from the activated sulfate donor PAPS to hydroxy-containing substrates, altering substrate solubility and charge to affect phase II metabolism and cell signaling. Here, we present the development, computational modeling, in vitro enzymology, and biological application of STS-3, an activity-based fluorescent sensor for the SULT1A1 isoform.**

Human phenol sulfotransferases (SULT1 family) are essential phase II metabolic enzymes that mediate sulfuryl group transfer from the activated sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) primarily to hydroxy-containing small molecules.1–6 Sulfurylation enhances the water solubility to increase the clearance of xenobiotics, to recycle endogenous metabolites (e.g. estrogen, dopamine), and, in some cases, to (in)activate drugs.2–4,6,7 As such, phenol sulfotransferases are not only linked to cellular signaling in normal physiology but also in disease states ranging from cancer to neurodegeneration.1,3,6–12 Our understanding of the substrate scope and activities of these enzymes in biological contexts has been significantly advanced by parallel efforts using computational modeling, structural characterization, mechanistic enzymology, and assay methods.10,13–23

Along these lines, one of the most widely used approaches to monitor phenol sulfotransferase activity with purified protein or cell lysates relies on radiolabeling of substrates, PAP35S, or sulfate (35SO2−) coupled to chromatographic detection of the sulfurylated products.24,25 Alternatively, coupled-enzyme assays with colorimetric and fluorescent substrates provide a safer, cost-effective, and continuous readout of enzyme activity.24–28 If radiolabeling is not preferred or if the sulfurylated product does not have an optical signature, mass spectrometry or nuclear magnetic resonance spectroscopy can be used.20,24,25,29,30 A limited number of these approaches have been translated to living cells but do not provide a direct readout with a spatially and temporally-resolved map of activity.31 We envision that activity-based fluorescent sensors can address this gap.32–39 This strategy affords the ability to chemically tune and transform an enzyme’s substrate into a fluorescent imaging platform for live-cell applications. To our knowledge, activity-based sensing has not been widely exploited for phenol sulfotransferases in living cells. The SULT1 family consists of 9 isoforms, all with a high degree of sequence and structural similarity (≥55%).15 Of these isoforms, SULT1A1 is the most widely expressed in the human body and promiscuous, thus making it an ideal target for this proof-of-concept study.40,41

To generate an activity-based fluorescent sensor, we first selected the substrate 2-naphthol as it has been demonstrated to undergo sulfurylation by SULT1A1.15,18 Even though 2-naphthol and the resulting 2-naphthyl sulfate product are fluorescent in water, there are negligible differences in the emission spectra of these compounds at physiologically relevant pH.15,16 As such, our strategy to convert 2-naphthol into an activity-based fluorescent sensor relied on its structural similarity with 3-hydroxy-1,8-naphthalic anhydride (compound 1, Fig. 1). The latter can be readily functionalized with primary amines to generate naphthalimide fluorophores.42–47 Specifically, we selected three electronically distinct amines: butylamine (STS-1), 3-aminopropanoic acid (STS-2), and N,N-dimethylethlenediamine (STS-3) (Fig. 1). We reasoned that all three sensors would be weakly fluorescent because the non-bonding electrons on the oxygen atom of the phenol could quench the excited state of the naphthalimide fluorophore. However, the quenched state could be relied upon sulfurylation to generate a turn-on or ratiometric fluorescence response, as previously reported for 3- and 4-substituted-1,8-naphthalimidine-based sensors.42–44,48 We note that STS-1 has been evaluated as a fluorescent sensor for a plant glucosyltransferase in vitro,48 and STS-3 has been tested as an anti-cancer agent.45–47,49

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that both naphthalene and STS-3C were separated from the binding pocket.

In parallel, the spectroscopic properties of STS-1, STS-2, and STS-3 were evaluated with purified SULT1A1. Spectral changes were observed when 10 μM of each sensor was incubated with 0.015 μg μL⁻¹ (0.4 μM) of purified SULT1A1 and 60 μM of the PAPS cofactor at 37 °C in 50 mM Tris buffer at pH 7.4 (Fig. 3). At t = 0 min, the absorption spectrum for STS-1 was featureless, but STS-2 and STS-3 showed two broad maxima at ~340 nm and ~390 nm. Excitation at both absorption maxima resulted in no emission above the background of the buffer for all three sensors, which is consistent with a quenching mechanism. However, clear spectral changes were observed within t = 10 min of incubation with STS-2 and STS-3. Notably, the absorption maxima at ~340 nm increased in intensity whereas the absorption maxima at ~390 nm decreased in intensity, suggesting the formation of new products. Upon excitation at 340 nm, robust turn-on emission responses were observed at ~415–420 nm within t = 1 h (average ± standard deviation): STS-1 (5.7 ± 0.9), STS-2 (6.3 ± 1.7), STS-3 (7.1 ± 0.2) (Fig. 3 and Table S1, ESI†). These spectral changes did not occur in the absence of the enzyme or PAPS cofactor (Fig. S14–S22, ESI†). Similarly, co-incubation with 10 μM of 2,6-dichloro-4-nitrophenol (DCNP), a substrate inhibitor for SULT1A1 (Kᵢ = 2 μM) attenuated the turn-on fluorescence response of each sensor to varying degrees (Fig. S14–S22, ESI†). Encouraged by these results, we used liquid chromatography–mass spectrometry (LC–MS) to confirm
that the turn-on fluorescence response for each sensor was attributed to the formation of the sulfurylated product (Fig. S23, ESI†).

To better understand the differences in the observed emission responses, we determined the kinetic parameters for each sensor with SULT1A1 using a Michaelis–Menten model (Fig. S24, ESI†). The low solubility of STS-1 above 10 μM prevented accurate measurements, so it was not further evaluated. The average \( K_M \) values of STS-2 and STS-3 were determined to be 10 μM and 4 μM, respectively. Based on the \( k_{cat} \) values (average ± standard deviation), STS-3 (21 ± 5 s⁻¹) reacted faster than STS-2 (8 ± 4 s⁻¹) with SULT1A1, thus allowing for the quick buildup of fluorescence signal (Table S1, ESI†). These differences also translated to the overall catalytic efficiencies. Based on these parameters and the robust turn-on fluorescence response of STS-3, we next established the ability of STS-3 to detect SULT1A1 activity in living cells.

To validate STS-3, we selected the human SK-N-MC neuro-epithelial cancer cell line, which has been previously reported to express SULT1A1.54,55 First, SK-N-MC cells were treated with either a DMSO vehicle control or 10 μM of STS-3 for 4 h at 37 °C in serum-free media, followed by lysis and analysis with fluorescence-based high-performance liquid chromatography (HPLC) (Fig. 4 and Fig. S25–S31, ESI†). Extracts from STS-3 treated cells revealed a single fluorescent product with emission at 420 nm, whereas extracts from DMSO treated cells did not. Co-injection with the authentic sulfurylated product standard, STS-3P, confirmed that STS-3 does indeed undergo sulfurylation in living cells. Encouraged by these results, we turned to live-cell fluorescence microscopy to visualize endogenous SULT1A1 activity (Fig. 4 and Fig. S32–S35, ESI†). Cells treated with STS-3 and excited at 340 nm showed measurable levels of fluorescence signal above the autofluorescence of cells treated with DMSO. Co-incubation with the substrate inhibitor DCNP attenuated the intracellular fluorescence signal by ~51%. Moreover, this change did not arise from any cytotoxic effects of co-treatment with STS-3 and DCNP. It is not surprising that STS-3 lowered cell viability as related derivatives have been used as anti-cancer agents.46–49 Interestingly, DCNP is known to lower PAPS levels50 and inhibit the SULT1A1 isomorph, but not the SULT1A3 isomorph,15,51,57 which is also expressed in SK-N-MC cells.12,54,55 As such, this provides strong evidence that STS-3 is a reporter of SULT1A1 activity in this cell line. Indeed, the fluorescence imaging also highlights how the reported activity can vary from cell-to-cell. This could arise from differences in the uptake, distribution, or reactivity of DCNP or STS-3 with SULT1A1 or other sulfotransferases, and even cofactor availability, all of which will be the subject of future investigations.

In summary, we have presented the development, computational modeling, in vitro enzymology, and biological application of STS-3, a first-generation activity-based fluorescent sensor for SULT1A1. This proof-of-concept study sets the stage to further develop and apply activity-based fluorescent sensors to discover how phenol sulfotransferase activity can intersect competitive metabolic pathways to modify endogenous metabolites, xenobiotics, or drugs. Along these lines, efforts are currently underway to develop high-throughput screening methods with STS-3 and generate a palette of sensors with improved reaction kinetics and expanded isomorph preferences for applications in a range of cell types.

Conflicts of interest
The authors declare no competing financial interests.

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Notes and references

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