Catch Me If You Can!

Detecting Untraceable Synthetic Drugs Using NanoBiT® β-Arrestin Recruitment Assays

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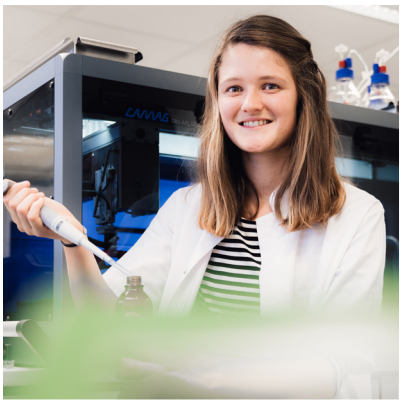

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Challenge: Synthetic drugs are a growing human health hazard. These drugs rapidly change formulation, making them difficult to detect with targeted methods.

Solution: GPCR activation assays based on β-arrestin 2 recruitment use NanoBiT® reporter technology to provide an untargeted approach for sensitive detection of synthetic drugs in relevant biological samples.

Dr. Christophe Stove is a professor in the Laboratory of Toxicology at Ghent University with roles in education, toxicology testing and research. His laboratory research includes investigating molecular mechanisms that control G-protein-coupled receptor (GPCR) signaling, studying the role of related protein:protein interactions (PPI), and developing novel bioassays to determine the biological activity of diverse compounds in biological matrices. His lab has recently focused on the development of screening assays for synthetic "designer" drugs. Here, Annelies Cannaert, a former graduate student and current post-doctoral fellow in Dr. Stove's lab, describes her work using NanoBiT® PPI Assay to measure the GPCR/β-arrestin 2 interaction as a marker of GPCR activation to develop novel untargeted screening assays for the detection of synthetic opioids and cannabinoids.

Understanding the Challenge of Designer Drugs

Since their first documented appearance in 2008, synthetic drugs (also called 'designer drugs' and 'new psychoactive substances') have shown explosive growth as a new segment of the illegal drug market. By December 2017, more than 800 substances have been reported to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory. Synthetic drugs are typically created by modifying the chemical structure of illegal drugs or prescribed medications to generate substances that are not covered by international drug controls. These newly synthesized drugs are characterized by high-market dynamics and make up a broad range of substances that can be abused with similar effects as cannabis, cocaine, heroin, LSD, MDMA ('ecstasy') and methamphetamines.

Due to the ever-changing formulas of these synthetic substances, lawmakers find it difficult to pass all-encompassing legislation that makes these new drugs illegal. And with these altered formulas come new chemical combinations that are unknown, unstudied and potentially hazardous. The synthetic drugs also pose a challenge for detection, as it is hard to find a substance if you don't know what it might look like.

Screening for Synthetic Drugs: A New Approach

"We developed a novel 'untargeted' concept to tackle the issue of synthetic drug detection."

Most of the current approaches in drug detection are 'targeted'. That means you are looking at specific parts of the substance's chemical structure. These approaches typically involve using antibodies to recognize the substances of abuse in immunoassays or chromatographic techniques, which are libraries with a fixed list of compounds. Due to the fluctuations of supply and demand, the content of the synthetic drugs is rapidly changing, resulting in a cat and mouse game between the authorities and the drug producers. Every time a new compound becomes traceable, a novel analog hits the market, bypassing the drug tests and often the law.

The rapid proliferation of designer drugs has sparked considerable interest in the development of so-called 'untargeted' screening strategies. This means that knowing the chemical structure of the substance of abuse is not required. For example, untargeted screening can be done by sophisticated equipment (e.g., employing high-resolution mass spectrometry). However, due to the expensive and time-consuming character of this technique, high-resolution mass spectrometry is not routinely implemented in most clinical and forensic laboratories.

At the Laboratory of Toxicology of Ghent University, we developed a novel 'untargeted' concept to tackle the issue of synthetic drug detection. Instead of looking at the 'unknown' chemical structure of the drug ('targeted' approach), we examine what the drugs does in the body as a way to trace the substances. This alternative 'untargeted' activity-based screening method may offer a solution for the current expensive and time-consuming 'untargeted' techniques, functioning as a first-line screening tool and complementing the conventional targeted and untargeted detection methods.

Monitoring GPCR Activation for Untargeted Drug Detection

Our lab mainly focuses on detecting synthetic cannabinoid receptor agonists ('synthetic cannabinoids') and synthetic opioid receptor agonists ('synthetic opioids'). These are recently emerged compounds, distinct from the natural psychoactive compound(s) in cannabis and opium. Synthetic cannabinoids are one of the largest classes of designer drugs (32%), while the class of the synthetic opioids is the most deadly subgroup. Drugs exert their activity by interacting with specific proteins in the human body. For the synthetic cannabinoids and opioids, these are the cannabinoid and opioid receptors, respectively, which are G-protein coupled receptors (GPCRs), a large family of membrane proteins with seven transmembrane domains.

To identify the presence of synthetic cannabinoids or opioids in a biological matrix (e.g., blood, urine or vitreous), we looked at the activity of these drugs, and more specifically the GPCR activation. When activated, GPCRs interact with several cytosolic proteins, including G-proteins and β-arrestin 2 (βarr2).

To assess cannabinoid and opioid receptor activation, we chose to use the NanoBiT® Protein:Protein Interaction (PPI) System to create reporter assays that monitor the βarr2 and GPCR interaction. This was accomplished by fusing βarr2 and the specific GPCR to two nonfunctional NanoBiT® subunits, Large BiT (LgBiT) and Small BiT (SmBiT). When the GPCR is activated by a ligand, βarr2 is recruited to the receptor, bringing together the complementary NanoBiT® peptides and creating a functional enzyme that generates a bioluminescent signal in the presence of the substrate furimazine (Figure 1).

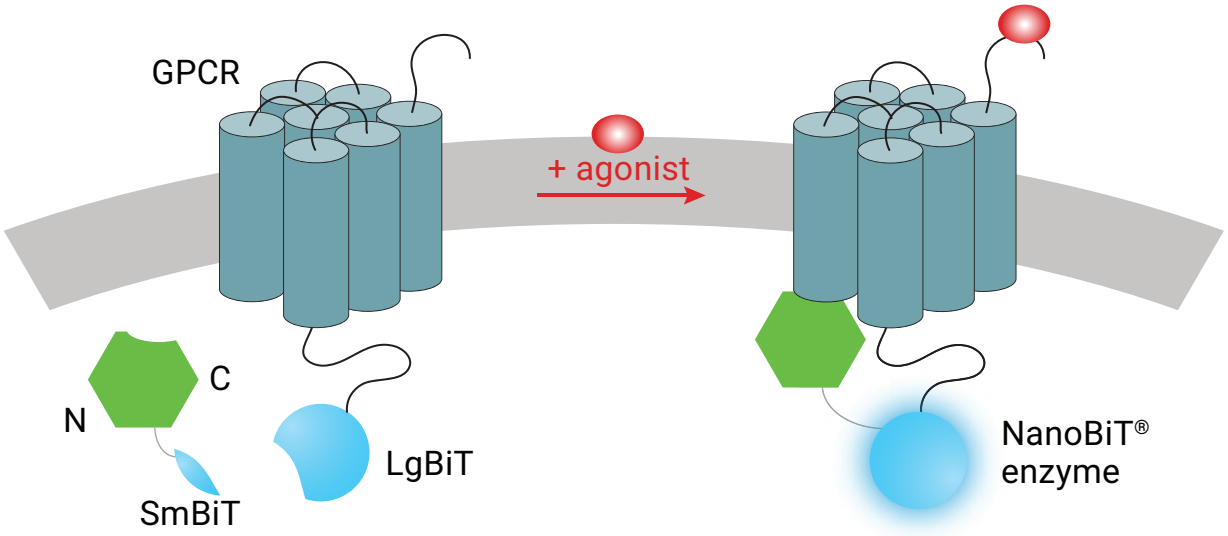

Figure 1. Example set up of the G-protein-coupled receptor activation (GPCR) assay using NanoBiT® Assay Technology.

Developing the NanoBiT® GPCR/β-Arrestin 2 Assays

The 2016 paper from our lab is the first discussion of the live-cell-based cannabinoid reporter assays. Mammalian cell systems were set up to transiently express one of the cannabinoid receptors (CB1 or CB2) together with βarr2. We demonstrated the suitability of these newly developed CB1 and CB2 bioassays for monitoring cannabinoid activity by applying several synthetic cannabinoids and their main phase I metabolites to these reporter systems. By doing so, we showed that several major metabolites retain their activity at cannabinoid receptors, which is consistent with reports by others. This was an important finding because synthetic cannabinoids are strongly metabolized and almost no parent compound is found in the urine. The presence of these active metabolites in urine offers a longer observation window for detecting synthetic drug use. The cannabinoid reporter assays were used as a proof-of-concept to test cannabinoid activity in an authentic urine sample (1).

In a follow up paper in 2017, the transient cannabinoid reporter assays were improved by generating stable cell systems. The advantages of the stable bioassays compared with the initial transient format include (i) a reduced workload, (ii) higher reproducibility within experiments and (iii) a stability control using co-expressed markers. The stable bioassays were demonstrated as a screening method for detecting the presence of synthetic cannabinoids in urine using a relatively large set of authentic urine samples (n = 74; 2).

We next aimed at improving the sensitivity of the cannabinoid reporter assays because lower concentrations of synthetic cannabinoids are expected in serum or plasma samples. For this purpose, two C-terminal βarr2 truncated mutants were evaluated. The idea to truncate βarr2 was based upon its prominent role in GPCR desensitization and signaling. This approach resulted in improved stable cell systems, which were successfully used to screen for cannabinoid activity in a set of authentic serum (n = 45) and plasma (n = 73) samples (3). These screening results were further confirmed and validated using an even larger set of patient samples (n = 471; 4).

Strengthened by the promising results we obtained for activity-based detection of synthetic cannabinoids, a similar concept was developed for activity-based screening of biofluids for the presence of opiates and synthetic opioids. Here, adding G-protein-coupled receptor kinase 2 was necessary to promote βarr2 recruitment to the opioid receptor. We demonstrated suitability of the opioid reporter bioassay using a set of 107 authentic blood samples (5). Also, in a case report involving a fatal intoxication with the extremely potent opioid carfentanil, the opioid bioassay was applied successfully (6).

Real-Time Monitoring with Easy-to-Use Sensitive Detection

"The reporter assays that we developed using NanoBiT® Technology are currently the only ones that have been successfully applied on biological matrices as an 'untargeted' screening strategy."

Whilst there is a multitude of commercially available assays for monitoring GPCR activation, the reporter assays that we developed using NanoBiT® Technology are currently the only ones that have been successfully applied on biological matrices as an 'untargeted' screening strategy (7). A key factor is the sensitivity of the system because some drugs are only found in trace amounts (sub- to low-ng/ml) in biofluids like blood or urine.

Another key advantage of the NanoBiT® Technology for this work was the capability to perform experiments in live cells. The mechanics of other available βarr2 recruitment assays require either long incubation times or do not allow real-time monitoring of the interaction. Also, the NanoBiT® assays are read on a standard multimode reader, which is easily adaptable for high-throughput screening.

Citations

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