FACULTY OF
PHARMACEUTICAL SCIENCES

DEPARTMENT OF BIOANALYSIS
LABORATORY OF TOXICOLOGY

ALTERNATIVE SCREENING TOOLS FOR THE DETECTION OF NOVEL PSYCHOACTIVE SUBSTANCES USING AN ACTIVITY-BASED APPROACH

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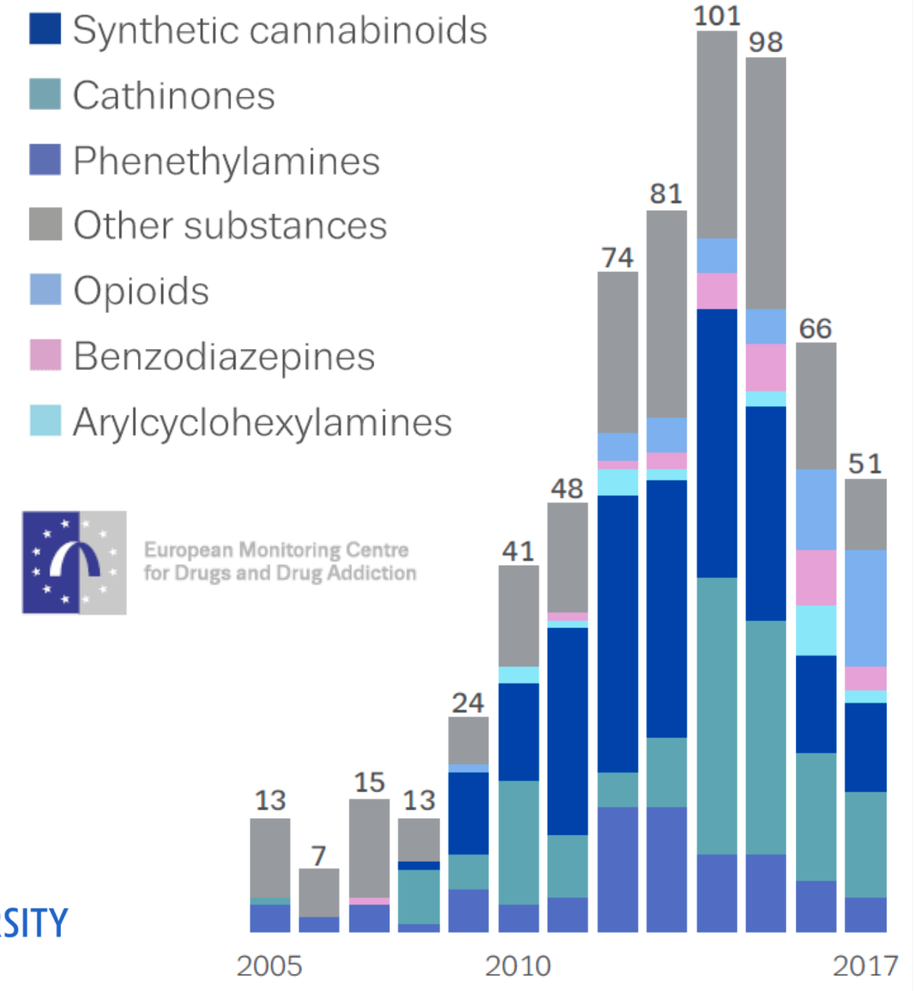
NICC
INCC

NOVEL PSYCHOACTIVE SUBSTANCES

"Substances of abuse, either in pure form or a preparation, that are not controlled by the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" (UNODC)



- High numbers
- High diversity in compounds
- High market dynamics

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PROBLEM WITH NPS DETECTION

TARGETED APPROACH:

- Immunoassays: possibility of **little or no cross-reactivity**

- LC-MS(/MS): **Not picked up in targeted screening** if mass spectral library is not up-to-date

NON-TARGETED APPROACH:

- GC-MS/LC-HRMS drug screening: **Sensitivity** might be issue (due to low [ ])

- **Time consuming** and **expensive**

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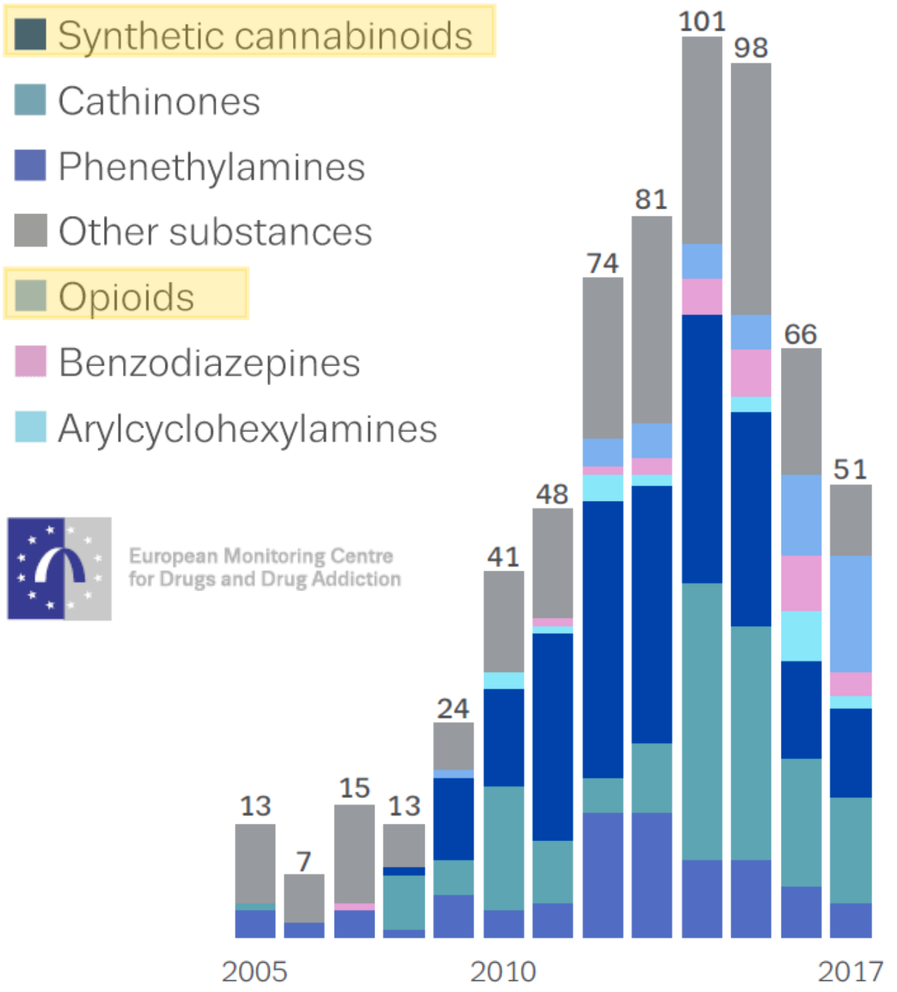
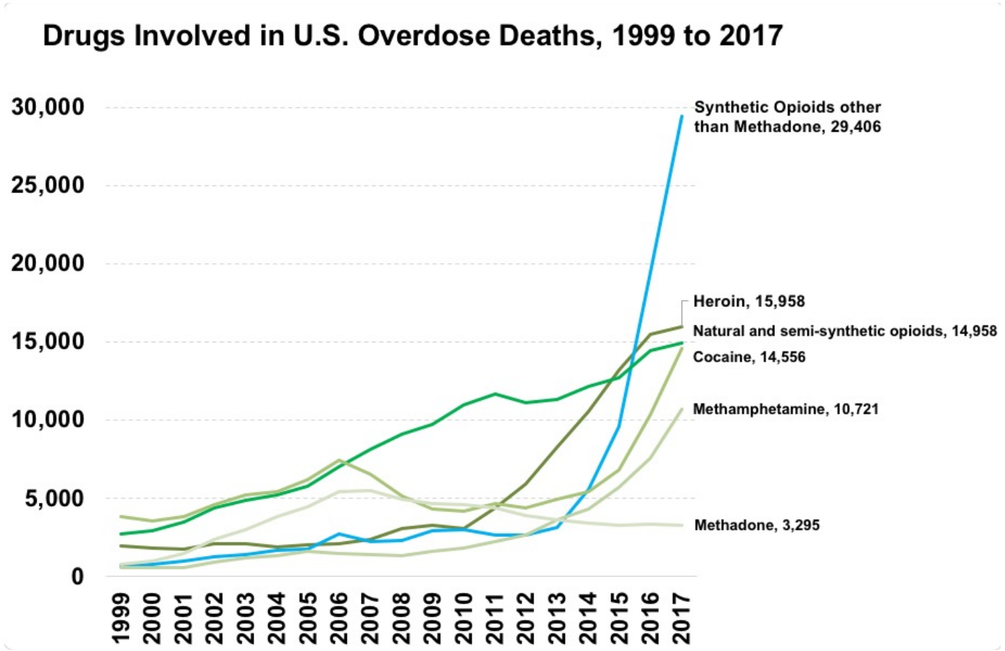
New, simple, non-targeted screening assay: based on **activity** of the drugs in the human body



Synthetic cannabinoid receptor agonists (SCRAs):
one of the largest groups within the NPS

Synthetic opioids:
one of the deadliest groups within the NPS



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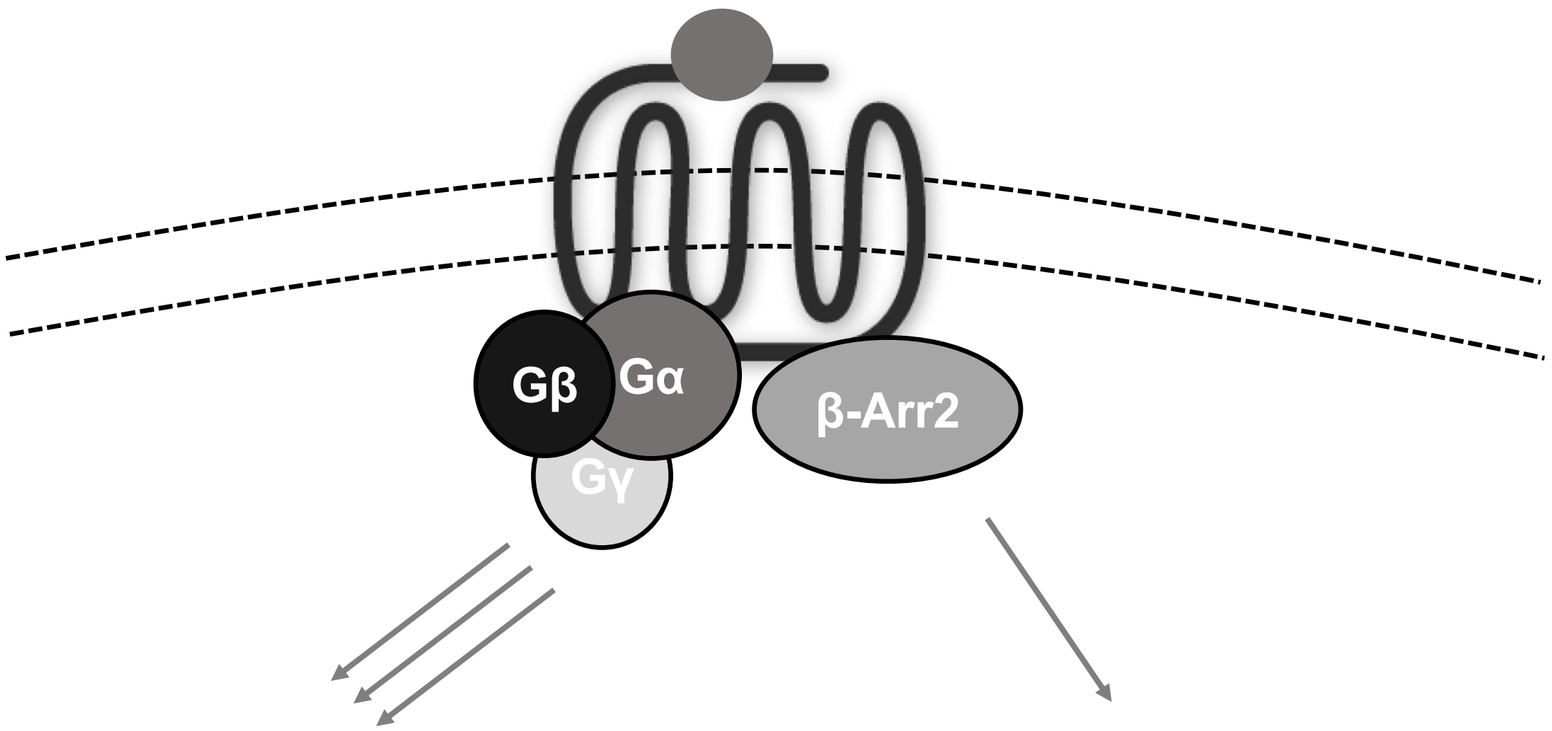
https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates

G-PROTEIN COUPLED RECEPTORS



Monitoring this interaction to evaluate receptor activation using the NanoBiT Technology

- The μ-opioid receptor and cannabinoid receptors are GPCRs.
- Upon activation, different signal transduction pathways start.
- β-arrestin 2 is recruited to the activated receptor to internalize the receptor.

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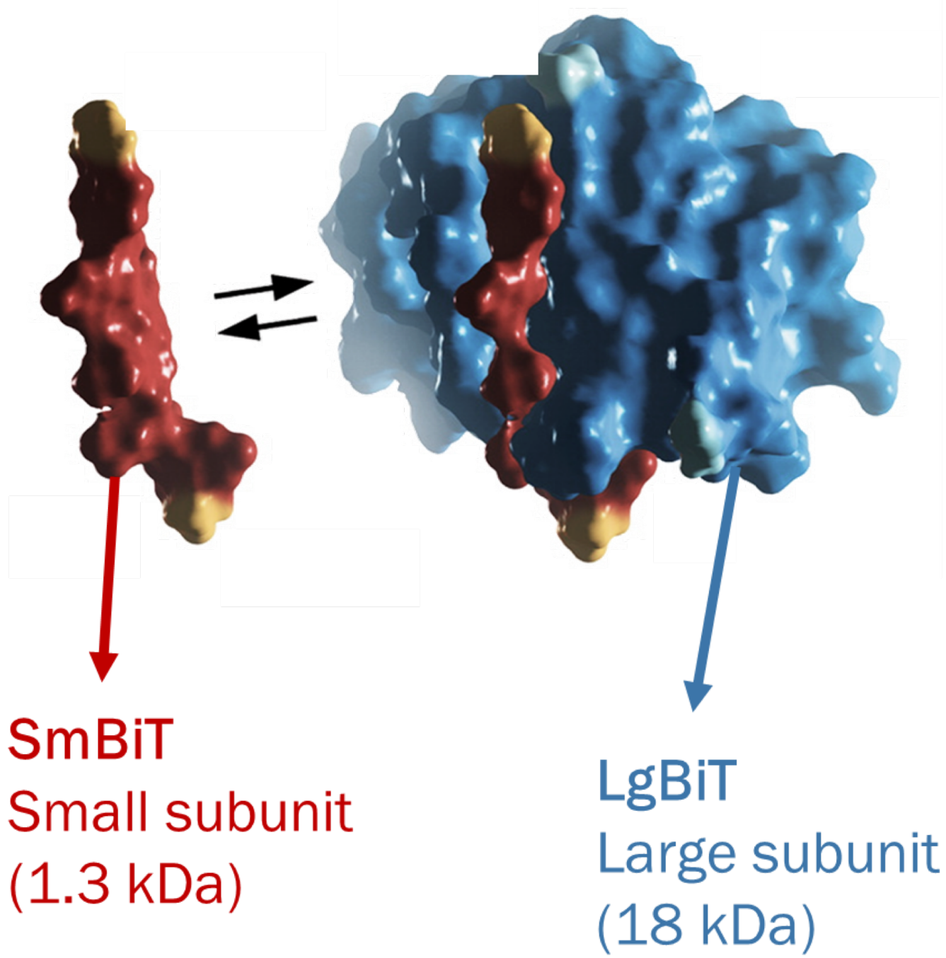
NANOBIT TECHNOLOGY



Based on structural complementation of Nano Luciferase (NanoLuc) for detecting protein interactions.

Both subunits are attached to the proteins of interest.

In our case:

- GPCR
- β-arrestin 2

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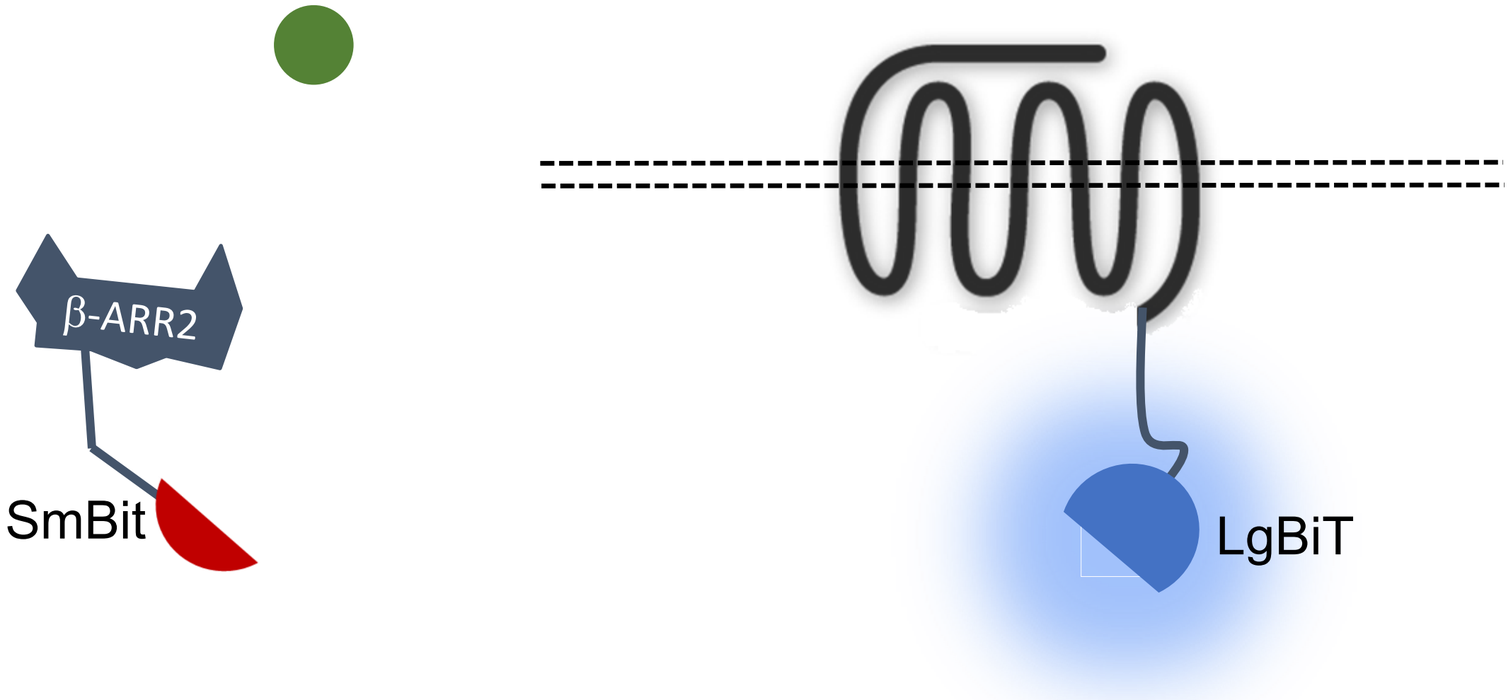
NANOBIT TECHNOLOGY



– Interaction of GPCR with drug will lead to a functional complementation of NanoLuc.

Bioluminescence

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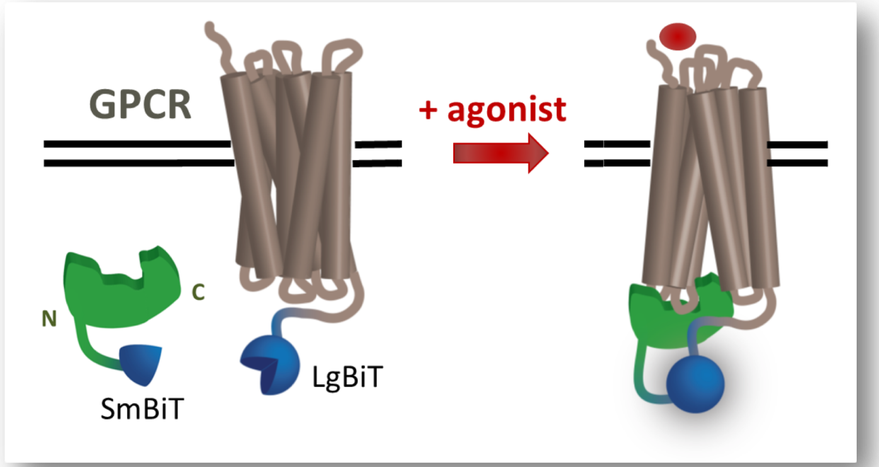
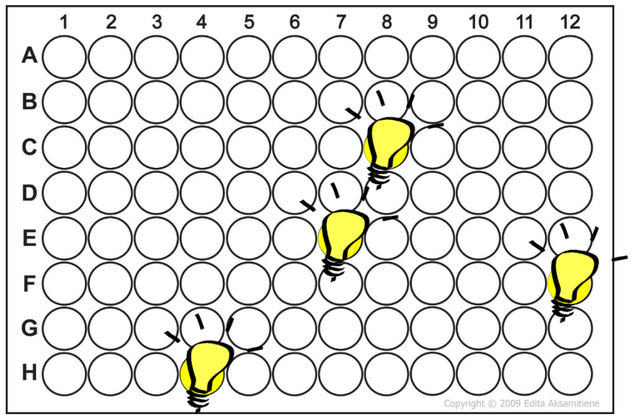
THE BIOASSAY IN PRACTICE

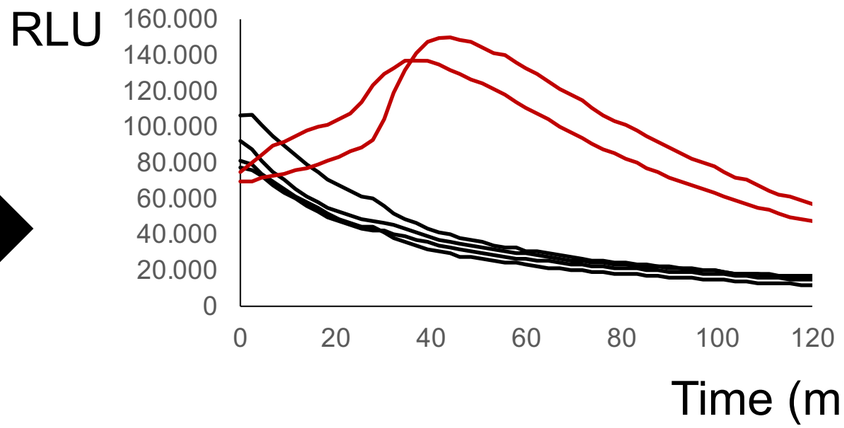
Seed in 96-well plate: **stably transfected cells**



Bioassay

- Wash cells twice with serum free medium
- Add 90/100 µl of serum free medium
- Add 25 µl of luciferase substrate
- Equilibrate 15-20 min
- Add **10/20 µl of extract**
- 2h read-out on standard luminometer





Cannaert et al. *Clin Chem* (2018)

Evaluation of the bioassay's applicability for synthetic cannabinoid receptor agonists

471 serum samples from drug-related Emergency Department visits in London

Guy's and St Thomas' NHS Foundation Trust

Compared to declared use + HR-MS/MS analysis

LGC

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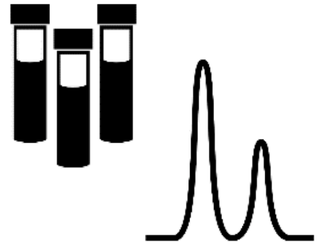
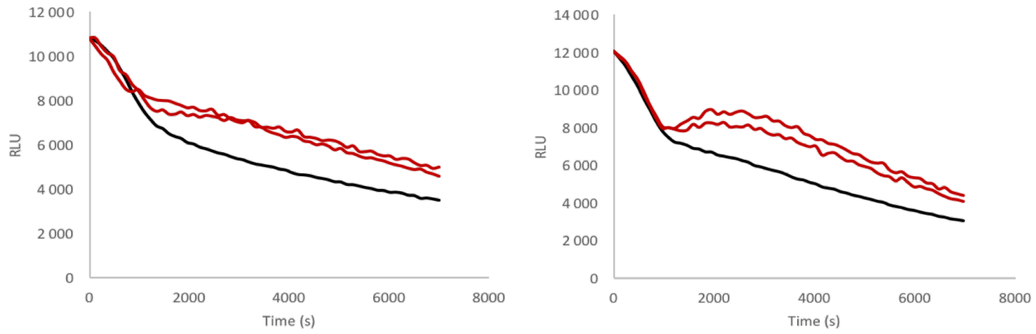
Cannaert et al. *Clin Chem* (2018)



	Standard protocol (≥ 300 µL sample volume) n = 371	Reduced sample volume (100 µL sample volume) n = 96
Sensitivity	100% (52/52)	88.9% (16/18)
Specificity	96.2% (330/343) 97.9% (330/337)*	96.6% (56/58)

* Reduction of 6 THC positives



Also successfully applied on urine samples!

Cave: presence of active metabolites

+ 2 “false positives” with claimed SCRA-use…

Evaluation of the bioassay's applicability for synthetic opioids

107 blood samples from US

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Compared to LC-MS/MS and QTOF analysis

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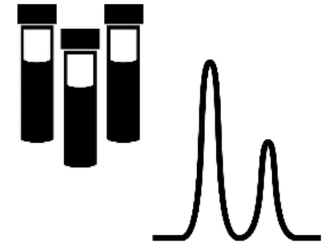
Cannaert et al. *Clin Chem* (2018)

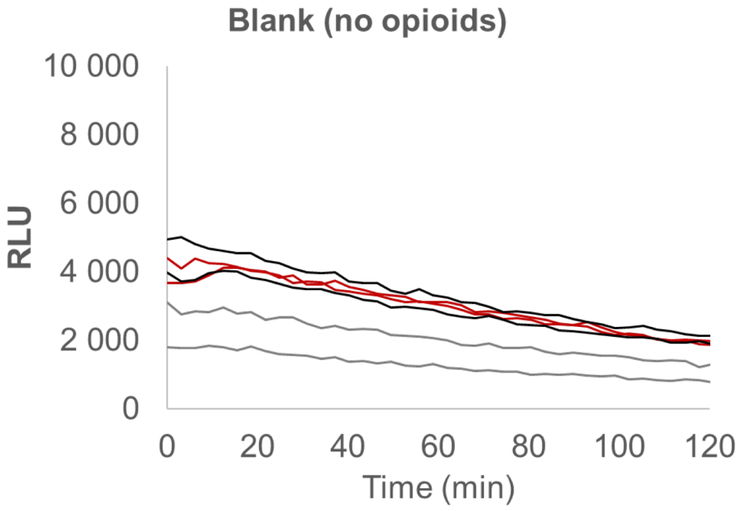


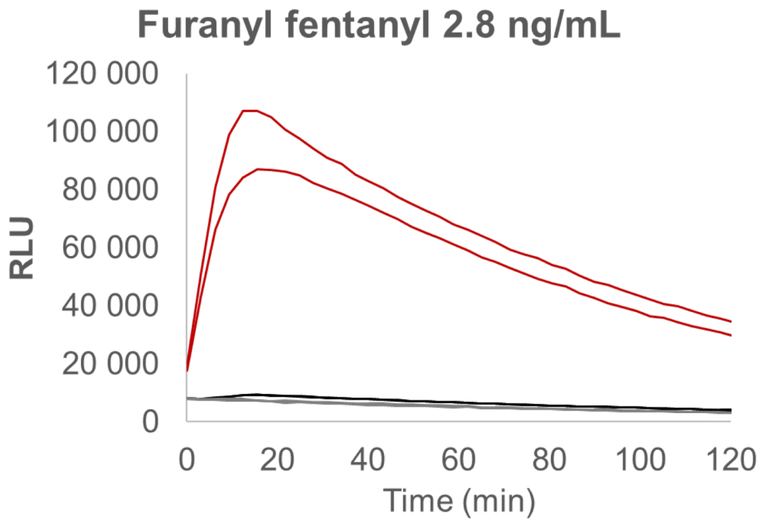
	Standard protocol (500 µL sample volume) n = 107
Sensitivity	100% (8/8 for U-47700)* 95% (21/22 for furanyl fentanyl)*
Specificity	93% (55/59)

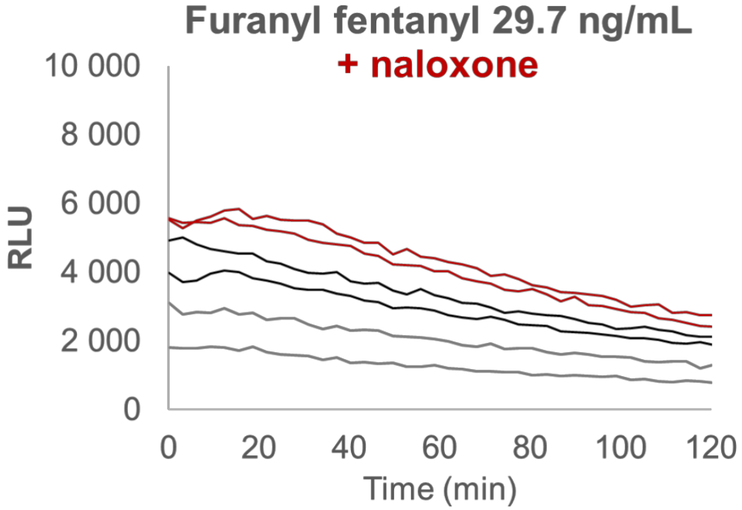
* 8 naloxone-positive samples not included

Additional samples that were opiate/opioid positive: scored positive!

Effect of MOR antagonist naloxone







CONCLUSIONS

- Alternative approach for detection of NPS in biological matrices.
  - Structure independent detection
  - Easily obtain information on the potency of new compounds

- Limitations:
  - Only screening for activity, still need mass spectrometry for confirmation for identification of the drug
  - Antagonists (e.g. naloxone, used as an antidote in opiate intoxications) block the receptor activation.
  - Requirement for active compound in biological matrix (e.g. metabolites in urine)

analytical chemistry

Article

pubs.acs.org/ac

Detection and Activity Profiling of Synthetic Cannabinoids and Their Metabolites with a Newly Developed Bioassay

Annelies Cannaert,[†] Jolien Storme,[†] Florian Franz,[‡] Volker Auwärter,[‡] and Christophe P. Stove*[,†]

Activity-Based Detection of Consumption of Synthetic Cannabinoids in Authentic Urine Samples Using a Stable Cannabinoid Reporter System

Annelies Cannaert,[†,§] Florian Franz,[‡,§] Volker Auwärter,[‡] and Christophe P. Stove*[,†]

frontiers in Pharmacology

CASE REPORT
published: 15 May 2018
doi: 10.3389/fphar.2018.00486

Activity-Based Detection and Bioanalytical Confirmation of a Fatal Carfentanil Intoxication

Annelies Cannaert[1,2†], Lars Ambach[1†], Peter Blanckaert[3] and Christophe P. Stove[1*]

Clinical Chemistry 64:8
1221–1229 (2018)

Drug Monitoring and Toxicology

Activity-Based Concept to Screen Biological Matrices for Opiates and (Synthetic) Opioids

Annelies Cannaert,[1,2] Lakshmi Vasudevan,[1] Melissa Friscia,[3] Amanda L.A. Mohr,[3] Sarah M.R. Wille,[2] and Christophe P. Stove[1*]

Activity-Based Detection of Cannabinoids in Serum and Plasma Samples

Annelies Cannaert,[1,2†] Jolien Storme,[1†] Cornelius Hess,[3] Volker Auwärter,[4] Sarah M.R. Wille,[2] and Christophe P. Stove[1*]

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Validation of Activity-Based Screening for Synthetic Cannabinoid Receptor Agonists in a Large Set of Serum Samples

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