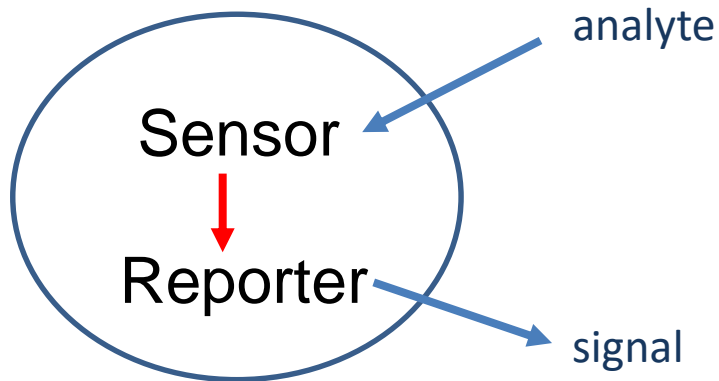


New rapid cell-based assays for beta blocker and nonsteroidal anti-inflammatory drug determination in wastewater effluents



Biosensor cell lines



Time range: **seconds**

fluorescent protein



Pharmaceuticals commonly found in the environment

Beta blocker

β -adrenergic receptor

Antihypertensives

- Metoprolol
- Propranolol
- Bisoprolol
- Atenolol
- Sotalol
- Enalapril
- Losartan
- Furosemide
-



NSAIDs

Cyclooxygenase

Analgesics

- Diclofenac
- Ibuprofen
- Naproxen
- Indometacin
- Ketoprofen
-

➔ Identification of the resulting biological effect of unknown mixtures

Target protein similarity

	LOEC
Diclofenac	µg/L

prostaglandin G/H synthase 1 isoform 1

Query: [Homo sapiens] 599 aa ([NP_000953.2](#))
 Subject: [Danio rerio] 597 aa ([AAK33030.1](#))

Score	Expect	Method	Identities	Positives	Gaps
851 bits(2199)	0.0	Compositional matrix adjust.	398/579(69%)	473/579(81%)	0/579(0%)
Query 4		SLLLWFLFLLLPPLFVLLADPGAPTIVNPPCCYPCQHQGICVRFGLDRYQCDCTRIGY			63
Sbjct 5		+ LL + + LLL P + NPCCYPCQ+QGICVR+GL+RY+CDCTRIGY			64
Query 64		SGPNCTIPGLWTLRNSLRPSPSFTHFLTHGRWFVEFVNATFIREMLRLVLTIVRSNLI			123
Sbjct 65		G NCTIP LMT + L+PSP+ H++LTH W W+ +N +F+R+ LMR VLTIVR+NLI			124
Query 124		PSPPTYN SAHYDIWESFSNVSYYTRILPSVPRKDCPTPMGTGKKQLPDAQLLARRFLLR			183
Sbjct 125		PSPPTYNS +DY++WE++SN++YYTRILP VP DCPTPMGTGKK +LPD +LL +F+LR			184
Query 184		RKFIPDPQGTINLMFAFFAQHETHQFFKTSGKMGPGFTKALGHGVDLGHIYGDNLERQYQL			243
Sbjct 185		R F DPQGTINLMFAFFAQHETHQFFKT ++G GPTK LGHGVD GHIYGD+L+RQ +L			244
Query 244		RLFKDGLKYQVLDGEMYPVVEAPVLMHYPRGIPPOQMAVGGQEVFGLLPGMLMYATL			303
Sbjct 245		RL KDGKLYQVL+G++YPP+V A V M YP +PP+ Q+A+GQEVFGLLPG +YATL			304
Query 304		WLREHNRVCDLLKAEHPTWGDEQLFQITRLILIGETIIKIVIEEYVQQLSGYFLQLKFDPE			363
Sbjct 305		WLREHNRVC+LK EHPTWGDEQLFQI RLI+IGETII+IVIEEYVQ LSGY L+L FDP			364
Query 364		LLFGVQFYQNRRIAMEFNHLYHWHPLMPDSFKVGSQESYSYQFLFNTSMLVDYGV EALVD			423
Sbjct 365		LLF QFY+NR I++EEN LYHWHPLMPDSF + Y +F+FNIS+L YG+E LV+			424
Query 424		AFSRQIAGRIGGGRNMDHHILHVAVDVIRESRMRLQPFNEYRKRFRGMKPYTSFQELVGE			483
Sbjct 425		AFS Q AG+IGGG N+ + VA VI ESRE+RLQPFNEYRKR F+KPYTSF EL GE			484
Query 484		KEMAAELEEELYGIDIDALEFYPGLLLEKCHPNISFGESMIEIGAPFSLKGLLNPNICSP EY			543
Sbjct 485		+EM+ ELEELYG IDA+EFYP LLEK P ++FGESM+E+GAPFSLKGL+GNPICSP+Y			544
Query 544		WKPSIFGGVGFNIVKTA TLKLVCLNTKICPVVSRVP			582
Sbjct 545		WKPSIFGG+ GF+IV +ATLKKLVCLNTK CPVVSF P			583



Diclofenac Target: Cyclooxygenase (*Danio rerio*)



Effects caused by compounds with same MOA

MOA (mode-of-action) based cellular assays

Reporter cell lines (reporter gene induction)

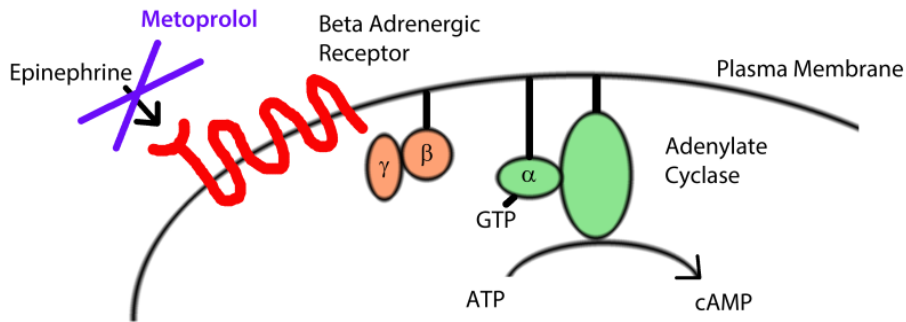
Response: 12-24 hours

Biosensor cell lines (immediate fluorescence signal)

Response: **seconds**

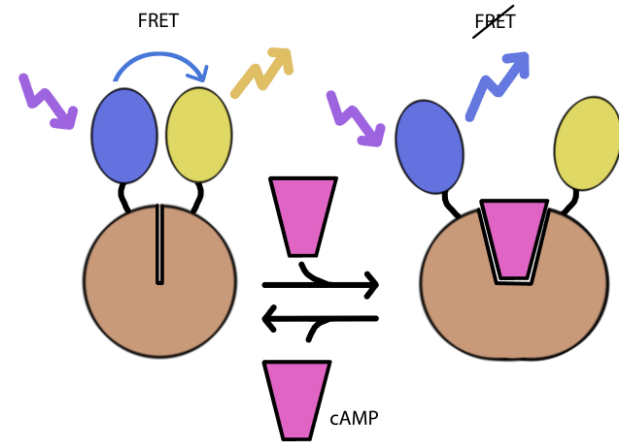
Beta blocker biosensor cell line

Sensor



↑ cAMP
 ↑ ratio (470/535)

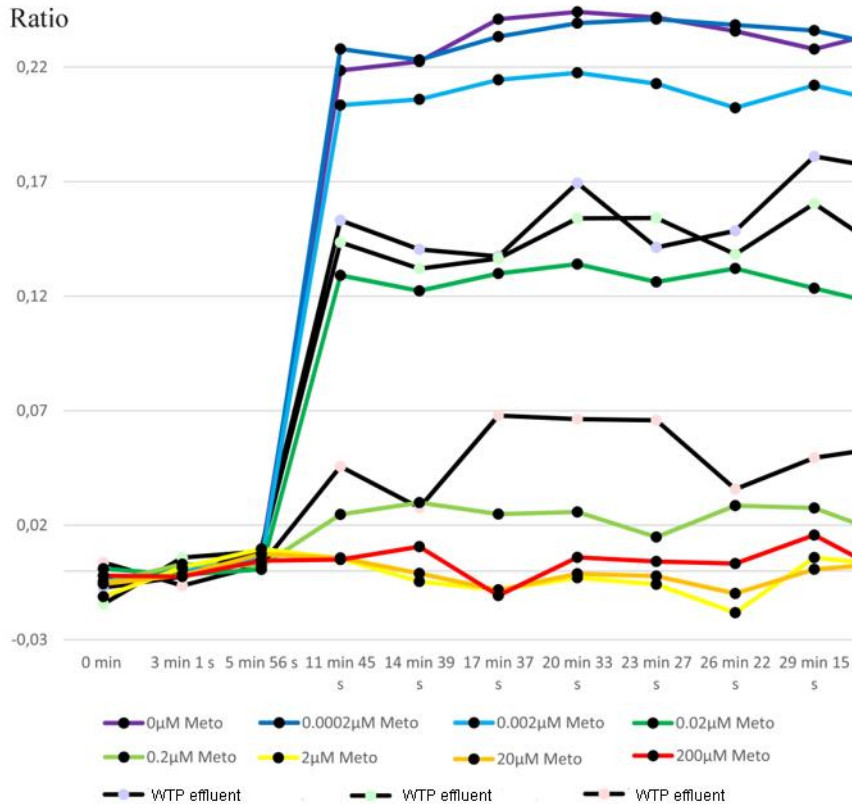
Reporter



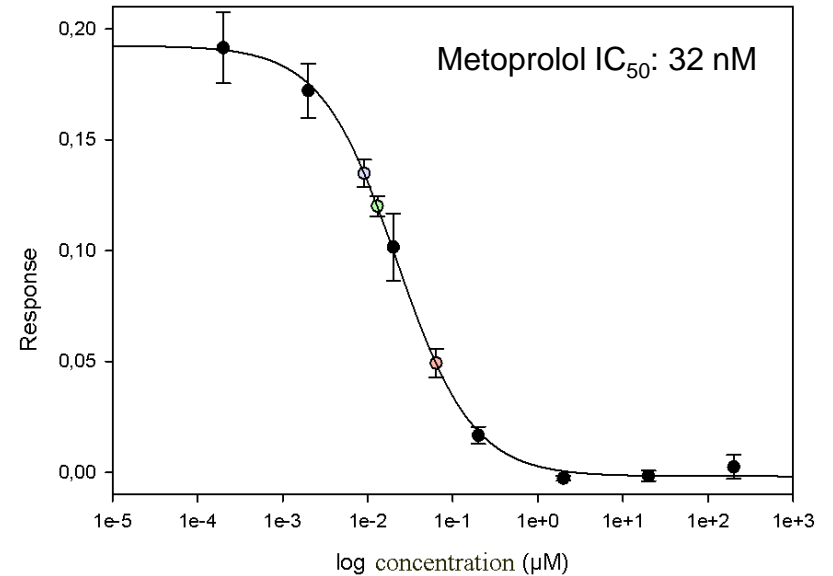
Measurement: Em470/Em535
 Excitation: 420 nm

Beta blocker *in vitro* assay

Response



Concentration-response curve



Metoprolol
GC-MS/MS

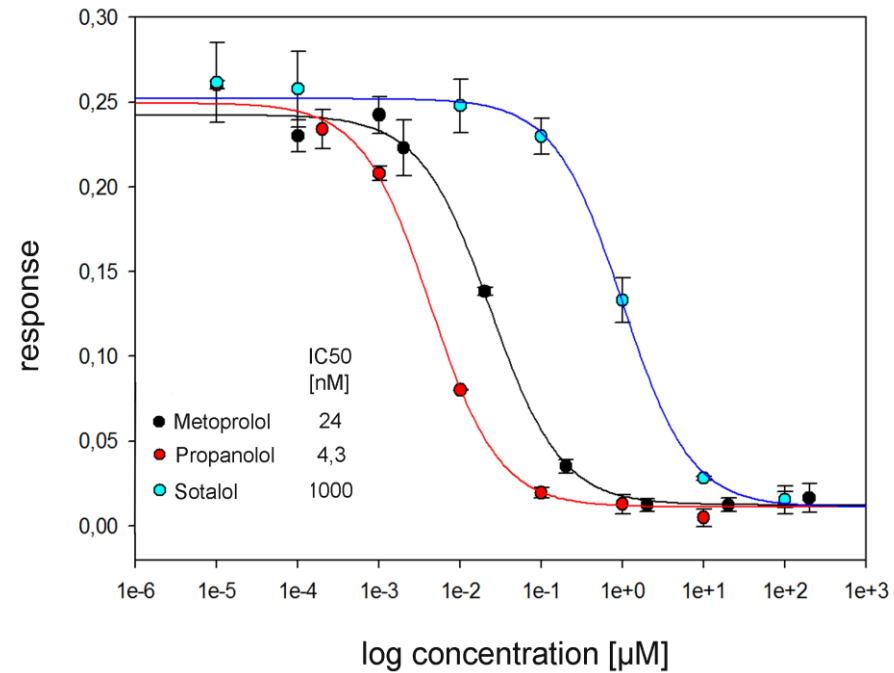
1.2 $\mu\text{g/L}$

Metoprolol equivalent quantity
(MetEQ)
in vitro Assay

4.2 $\mu\text{g/L}$

Beta blocker *in vitro* assay

Beta blocker	IC ₅₀ [nM]	Factor MetEQ
Metoprolol	32±14	1.0
Propranolol	6.5±3	5.02
Sotalol	1019±30	0.03
Bisoprolol	8.8±2,6	3.70
Atenolol	51.6±2	0.63
Metoprololsäure	426x10 ³	0.0





Wastewater treatment plant effluents Beta blocker *in vitro* Assay

WWTP effluent (SPE purified)	Metoprolol [µg/L] LC-MS/MS	MetoEQ [µg/L] <i>In vitro</i> Assay
February 2016	1.2	3.2±1.7
March 2016	1.2	4.2±0.9

WWTP effluent	Beta blocker (µg/L) LC-MS/MS	MetoEQ [µg/L]	total MetoEQ [µg/L]
February 2016	Bisoprolol (0.28)	0.85	2.05
	Metoprolol (1.2)	1.2	
	Propranolol (<BG)	0	
	Atenolol (<BG)	0	
March 2016	Bisoprolol (0.4)	1.22	2.69
	Metoprolol (1.2)	1.2	
	Propranolol (0.05)	0.23	
	Atenolol (0.07)	0.04	

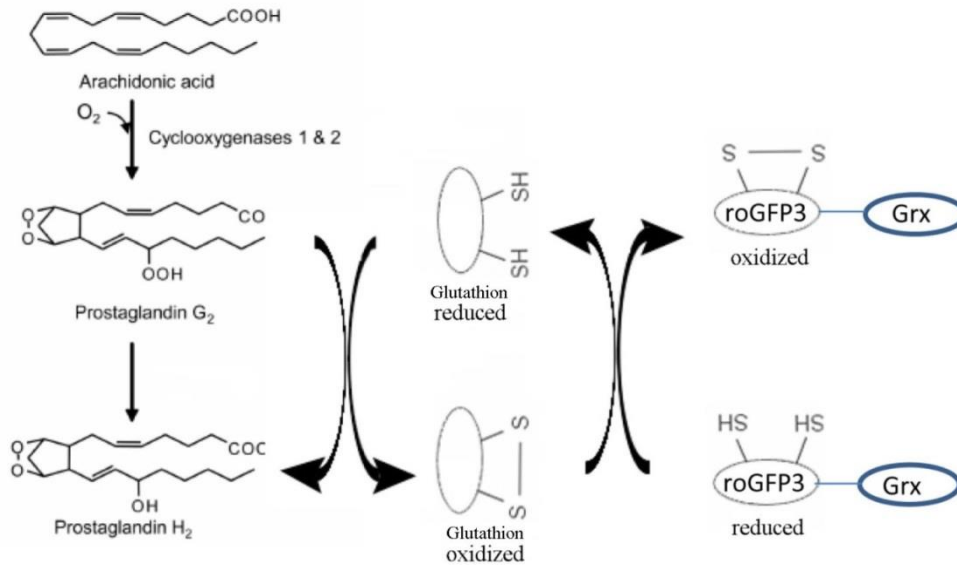
Wastewater treatment plant effluents Beta blocker *in vitro* Assay

WWTP effluent	Metoprolol [µg/L] LC-MS/MS	total MetoEQ [µg/L] LC-MS/MS	MetEQ [µg/L] <i>in vitro</i> Assay
February 2016	1.2	2.05	3.2
March 2016	1.2	2.69	4.2

- 
 MOA *in vitro* assay measures 1.6 fold beta blocker activity compared to the chemical analysis of beta blockers
- 
 Additional beta blocker compounds not analysed by chemical analysis?
Synergistic effects?

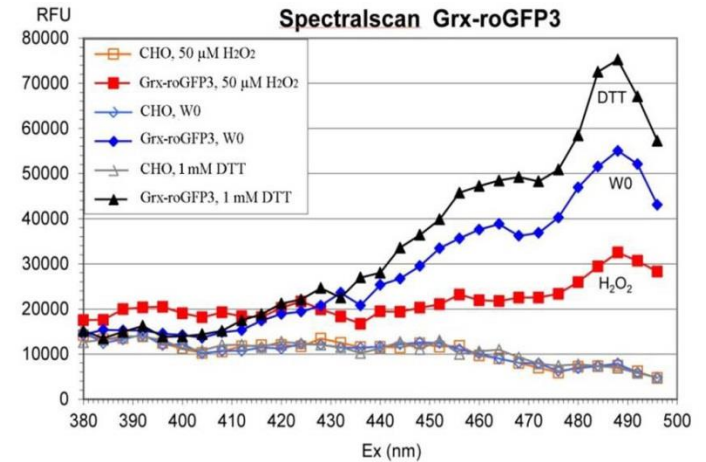
NSAID biosensor cell line

Sensor



↑ Cox-1
↑ ratio (395/485)

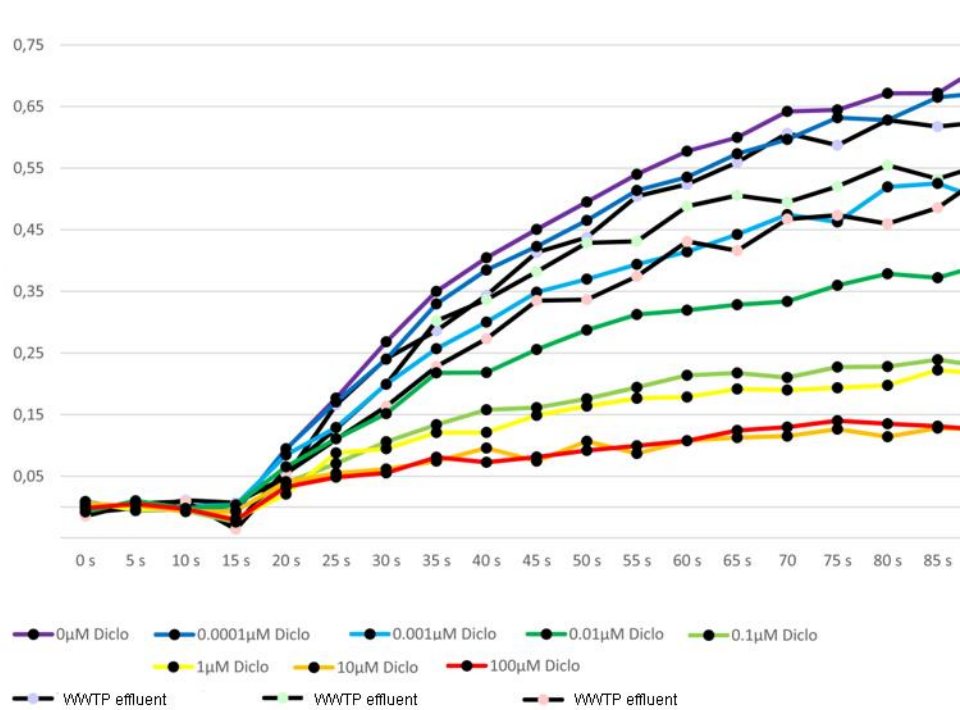
Reporter



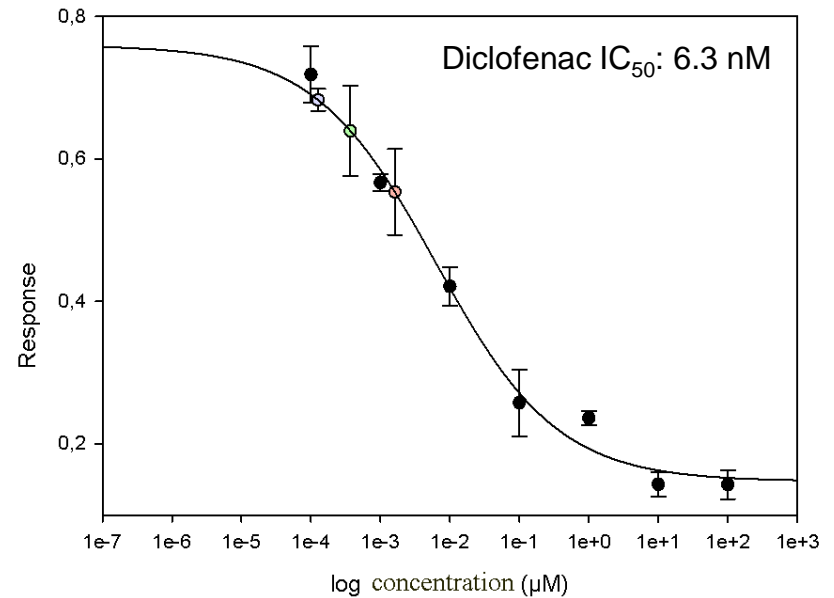
Measurement: Em528 (Excitation 395nm)
Em528 (Excitation 485nm)

NSAID *in vitro* assay

Response



Concentration-response curve



Diclofenac GC-MS/MS	Diclofenac Äquivalent <i>in vitro</i> Assay
2.2 µg/L	3.5 µg/L

Wastewater treatment plant effluents NSAID *in vitro* Assay

WWTP effluent	Diclofenac [µg/L] LC-MS/MS	DicloEQ [µg/L] <i>in vitro</i> Assay
March 2016	2.2	3.5



MOA *in vitro* assay measures 1.6 fold NSAID activity compared to the chemical analysis of diclofenac



Additional NSAID compounds not analysed by chemical analysis?

WWTP effluent	NSAID	[µg/L] LC-MS/MS
March 2016	Diclofenac	2.2
	Ibuprofen	1.1
	Naproxen	1.4

Wastewater treatment plant effluents NSAID *in vitro* Assay

NSAID	IC ₅₀ [nM]	Factor DicloEQ
Diclofenac	6	1.0
Naproxen	38	0.16
Ibuprofen	260	0.02

WWTP effluent	NSAID	[µg/L] LC-MS/MS	DicloEQ [µg/L] LC-MS/MS	DicloEQ [µg/L] <i>in vitro</i> Assay
March 2016	Diclofenac	2.2	2.2	3.5
	Naproxen	1.4	0.28	n.a.
	Ibuprofen	1.1	0.04	n.a.
	total NSAID	4.7	2.52	



MOA *in vitro* assay measures 1.4 NSAID activity compared to the chemical analysis of NSAIDs

MOA based *in vitro* assays using biosensor cell lines

- ✓ Appropriate throughput capacity to allow for routine application
- ✓ Specificity
- ✓ Sensitivity
- ✓ Robustness
- ✓ Reliability
- ✓ Matrix effects / proven suitability for environmental samples

Application: Monitoring of WWTP effluent, surface waters
 Assessment of sewage treatment technologies

Aim: Development of MOA based biosensor cell lines
 for the monitoring of additional pharmaceuticals



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**Many thanks for your interest
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