Faecal indicator sensing – toward a faster method for marine & freshwater

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Outline

- Drivers
- Background
- Results
- Rapid method-initial tests.
- Conclusions/Impact

Drivers

➢2006/7/EC BWD

Inland waters					
	Parameter	Excellent quality	Good quality	Sufficient quality	Reference methods of analysis
1	Intestinal *Enterococci* (cfu/100mL)	200(*)	400(*)	330(*)	ISO 7899-1 or ISO 7899-2
2	*Escherichia coli* (cfu/100mL)	500(*)	1,000(*)	900(**)	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95-percentile evaluation, (**) Based on a 90-percentile evaluation.

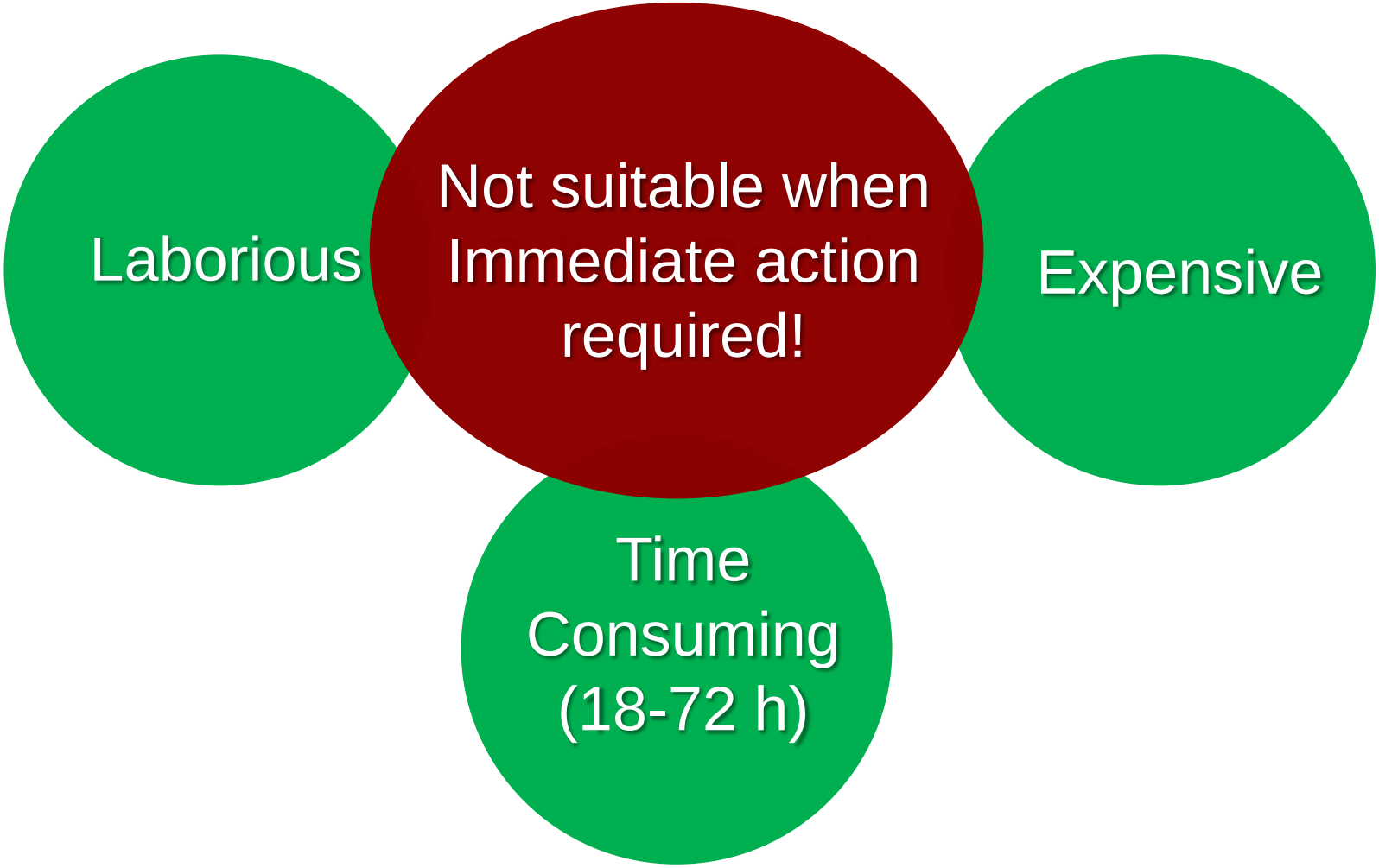
Coastal and transitional waters					
	Parameter	Excellent quality	Good quality	Sufficient quality	Reference methods of analysis
1	Intestinal *Enterococci* (cfu/100 mL)	100(*)	200(*)	185(*)	ISO 7899-1 or ISO 7899-2
2	*Escherichia coli* (cfu/100mL)	250(*)	500(*)	500(**)	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95-percentile evaluation, (**) Based on a 90-percentile evaluation.

Drivers

- Public protection

Standard Methods – *E. Coli*

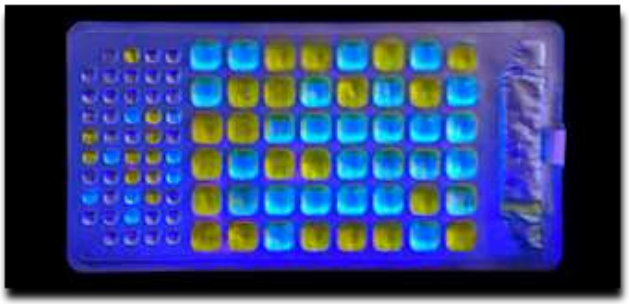


Enzyme based methods

β-D-Glucuronidase (GUD)

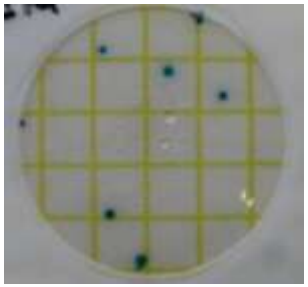
GUD extraction

GUD activity measurement



Colilert 18

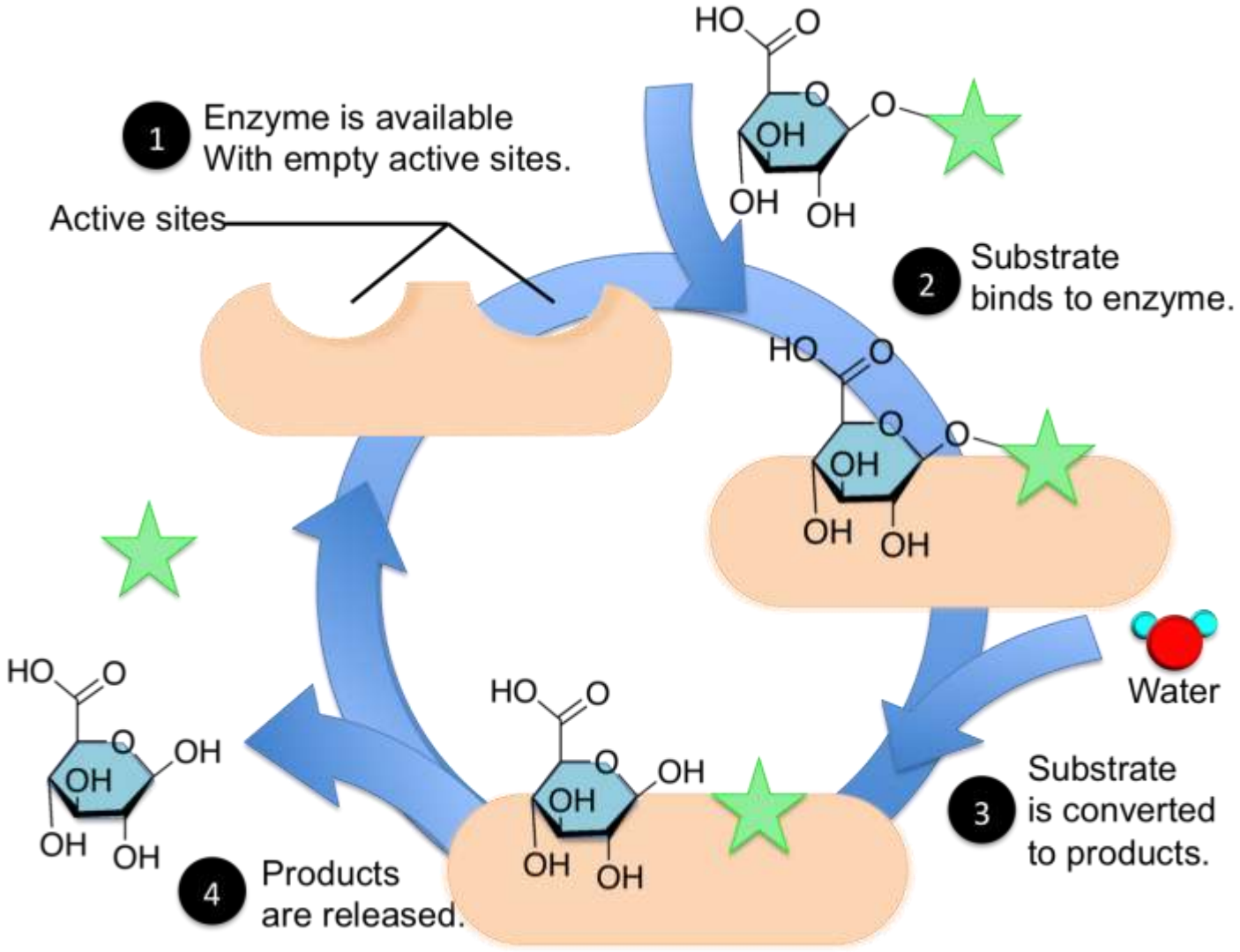
Chromocult



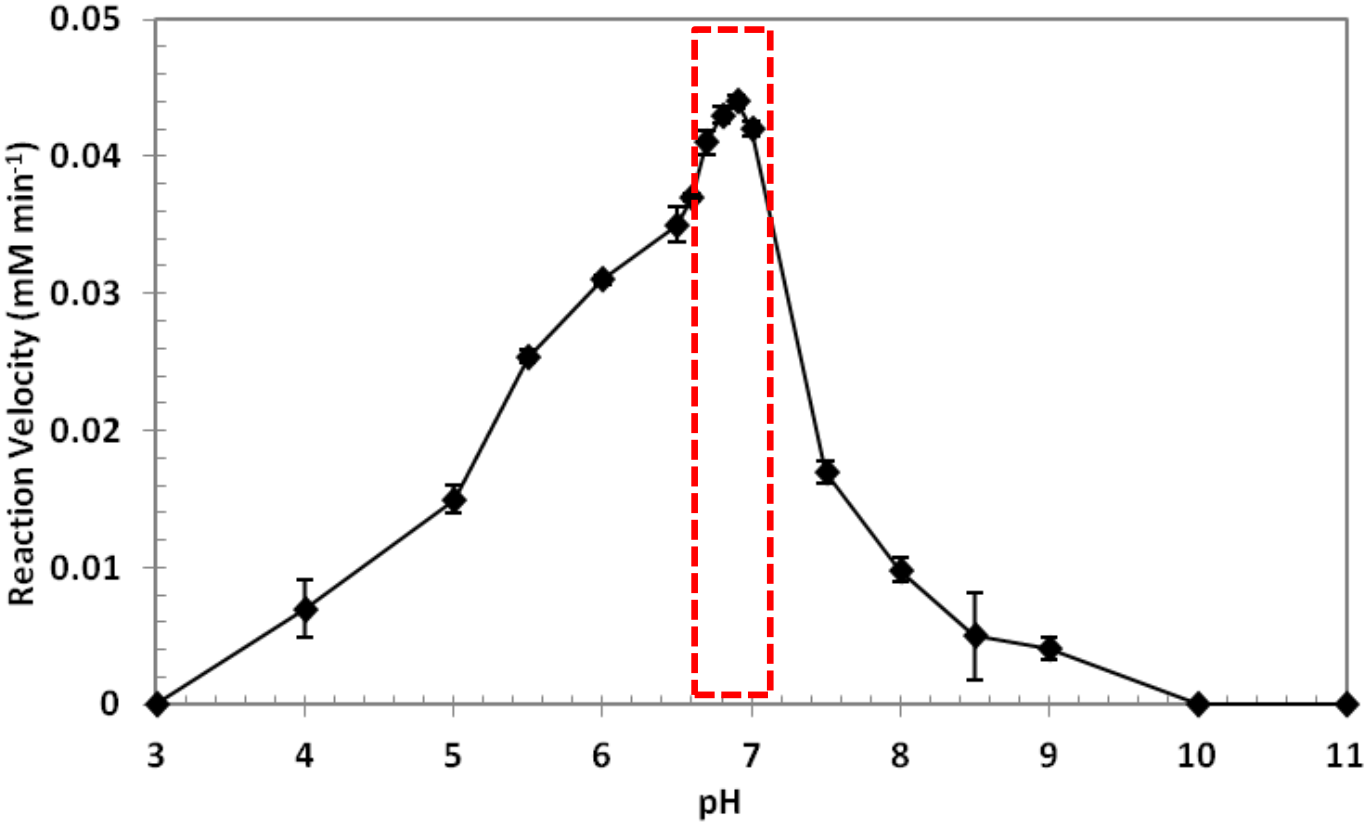
3M Petrifilms

Substrate uptake

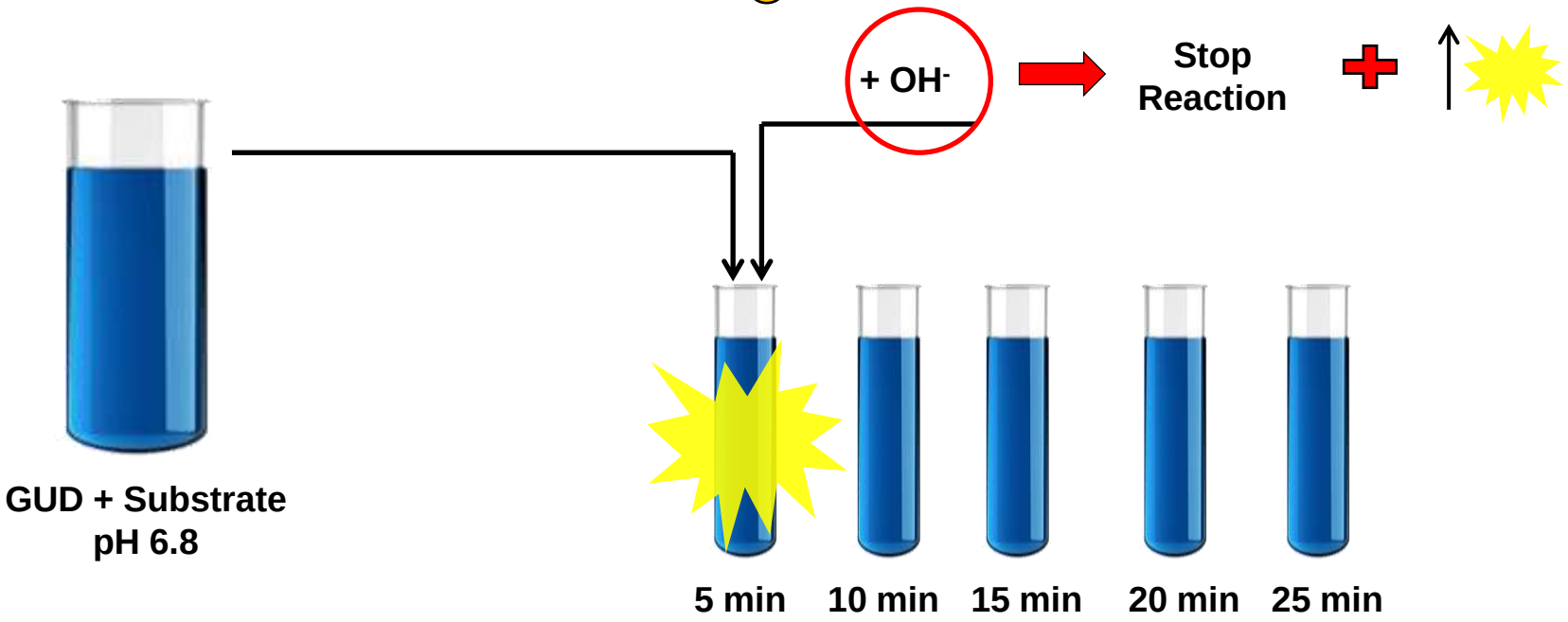
Background: How this works?

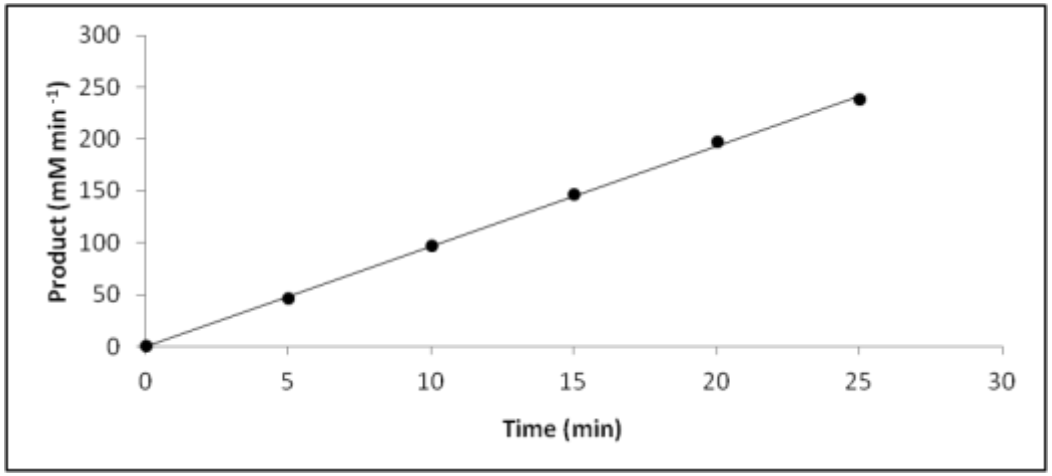


Background: GUD pH optimum



Background: Discontinuous Methods

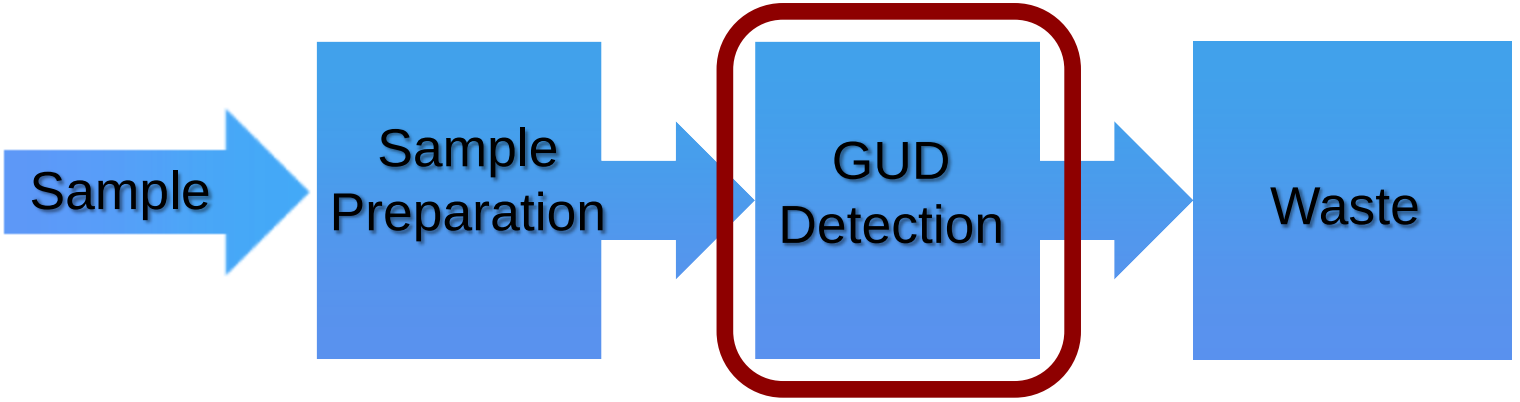




Aim:

- Develop a continuous fluorometric method for the detection of GUD activity.

- Develop a sensing platform for *E. Coli* detection in environmental waters.



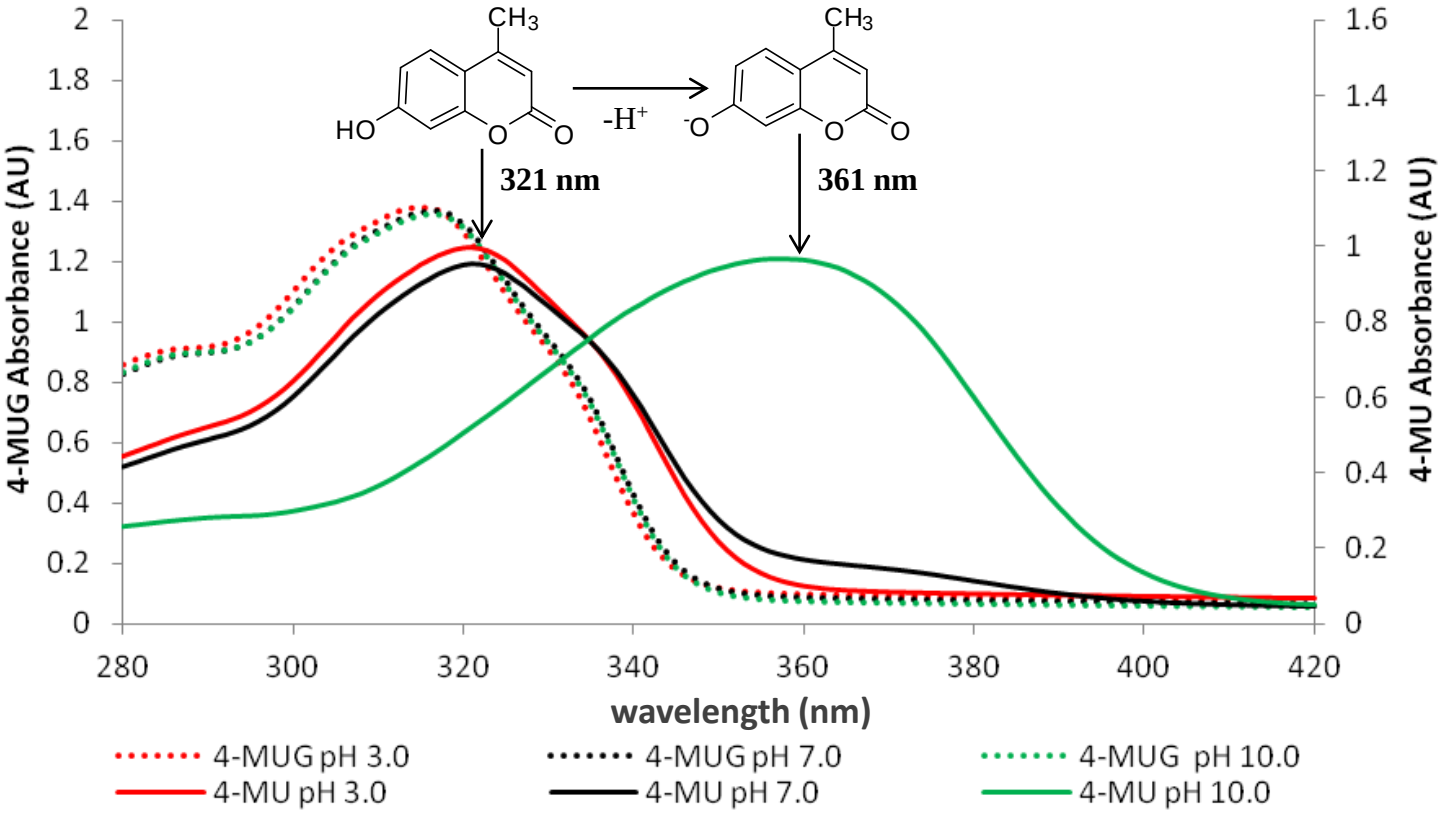
Results

- Comparison of 3 substrates for the measurement of GUD activity:
  - Ex/Em optimisation
  - GUD substrate kinetics

- 4-MUG/4-MU
- 3-CUG/3-CU
- 6-CMUG/6-CMU

UV-VIS Characterization

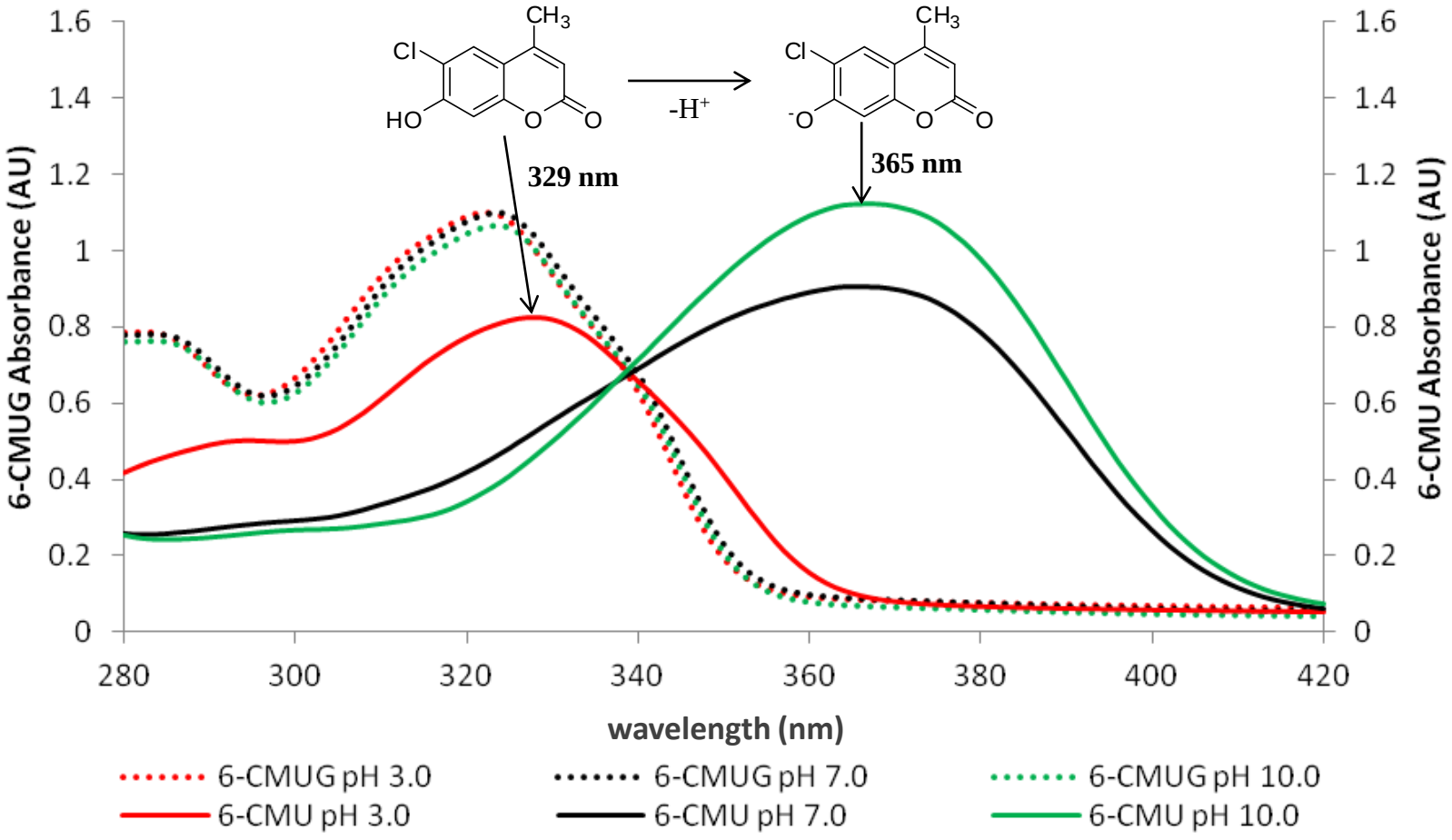
- 4-MUG / 4-MU



Absorption spectra of 100 μM 3-MUG and 50 μM 4-MU in acidic, neutral and alkaline conditions.

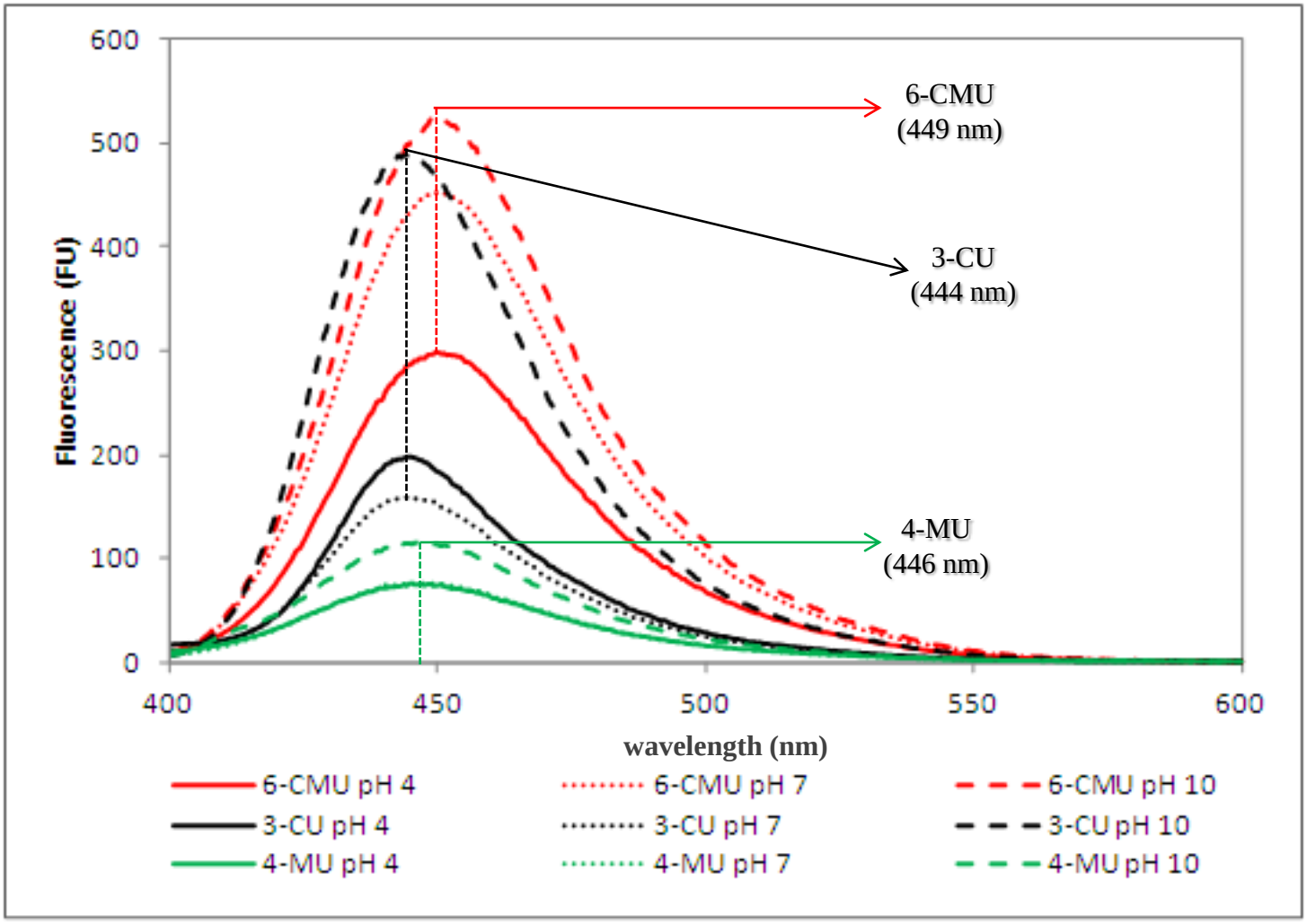
Results – UV-VIS Characterization

❑ 6-CMUG / 6-CMU



Absorption spectra of 100 μM 6-CMUG and 50 μM 6-CMU in acidic, neutral and alkaline conditions.

Ex/Em optimisation



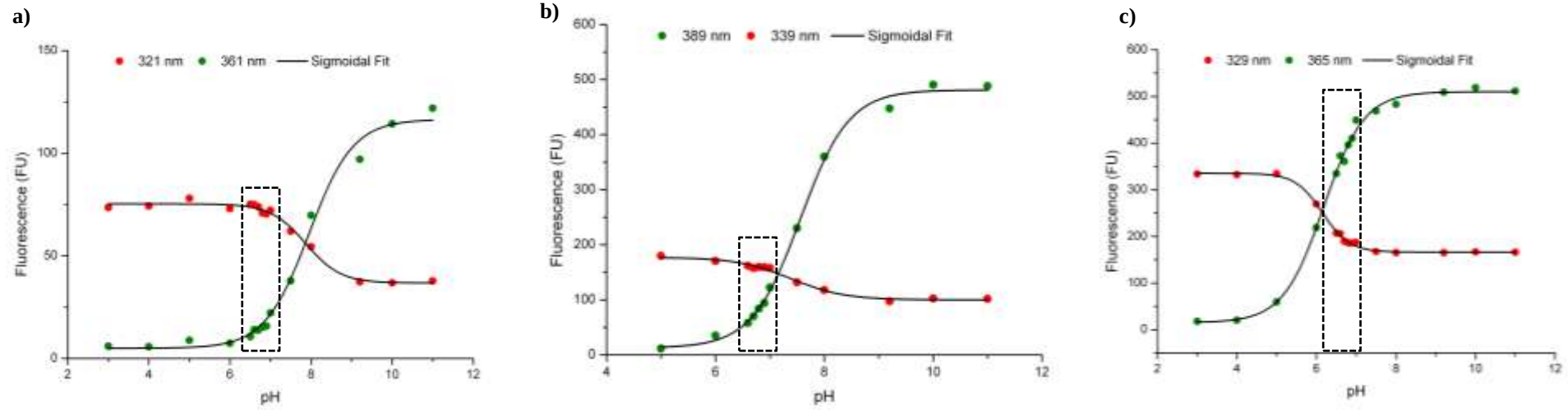
Emission spectra of 0.1 µM 4-MU, 3-CU and 6-CMU solutions in acidic, neutral and basic pH conditions. Each fluorophore was excited at the maximum excitation wavelength for the corresponding N and A^- ground states.

Ex/Em optimisation

4-MU pKa= 7.86 ±0.6

3-CU pKa= 7.38 ± 0.6

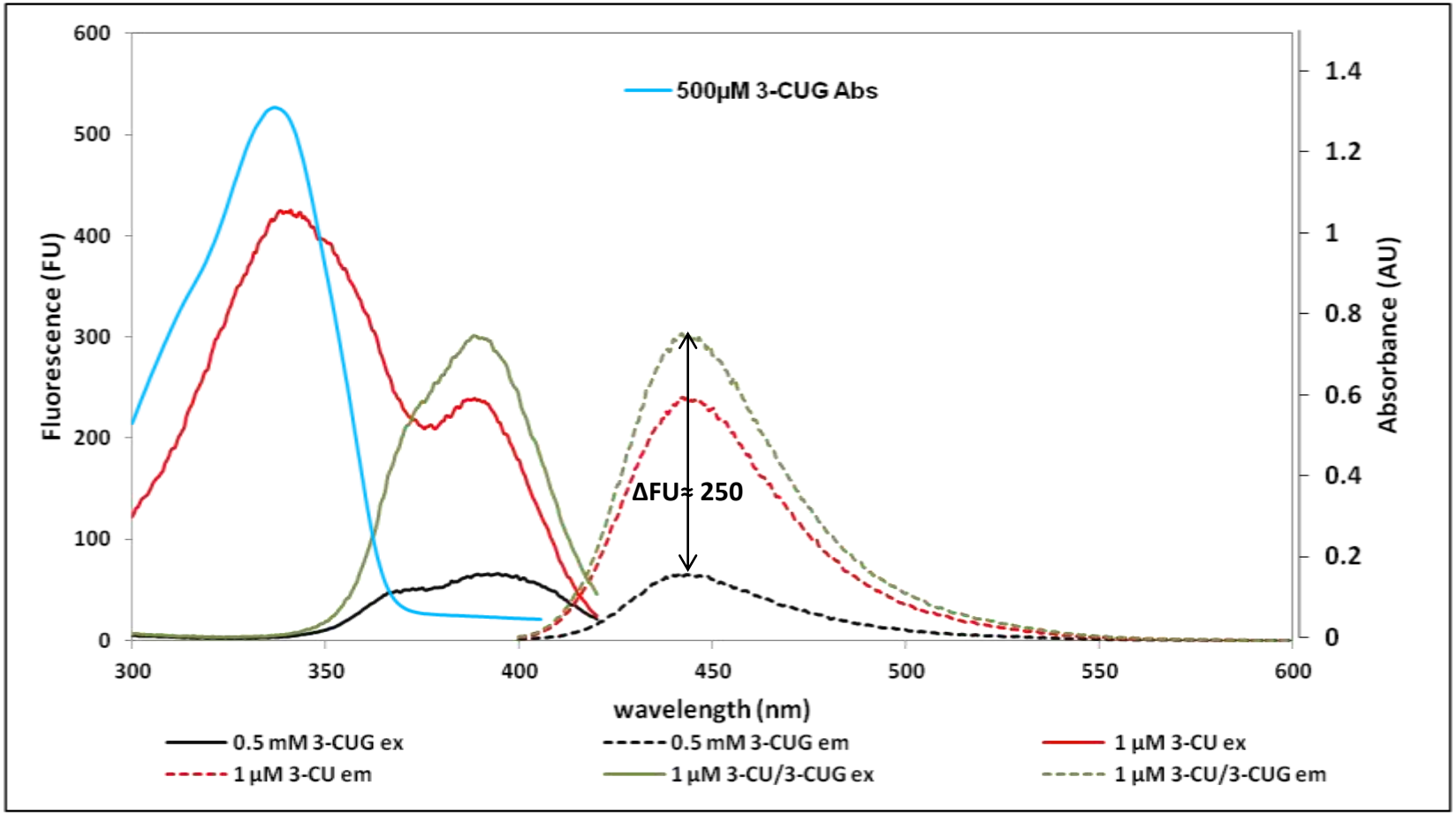
6-CMU pKa= 6.18 ± 0.3



Nonlinear regression fitting of the experimental data to Boltzman Sigmoidal model; Experimental data and model line for 4-MU (a), 3-CU (b) and 6-CMU (c). Excitation wavelengths are shown in the legend.

Ex/Em optimisation

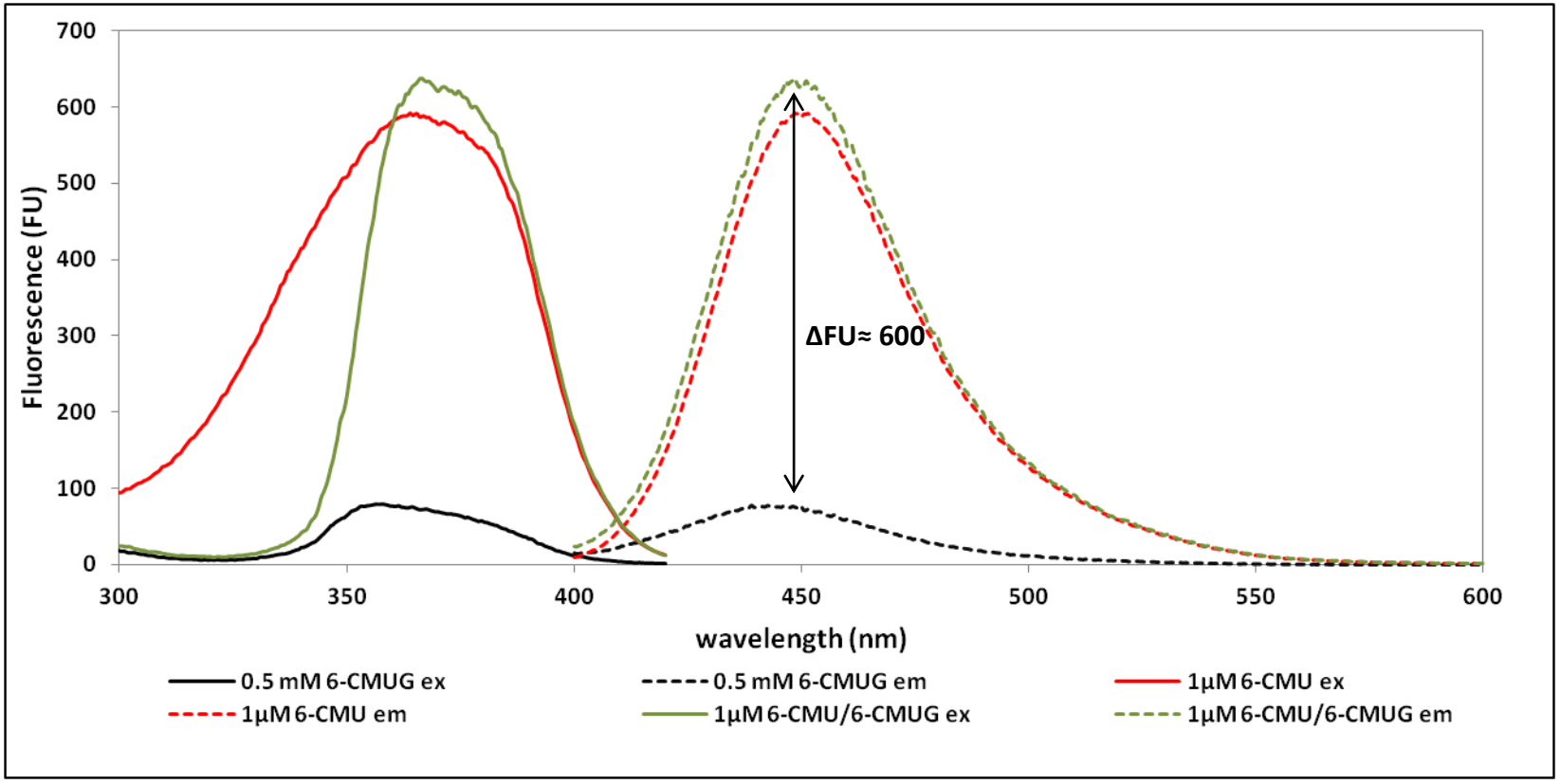
- 3-CUG / 3-CU



Excitation and emission spectra of 3-CU as influenced by the presence of 3-CUG in Phosphate buffer pH 6.8

Ex/Em optimisation

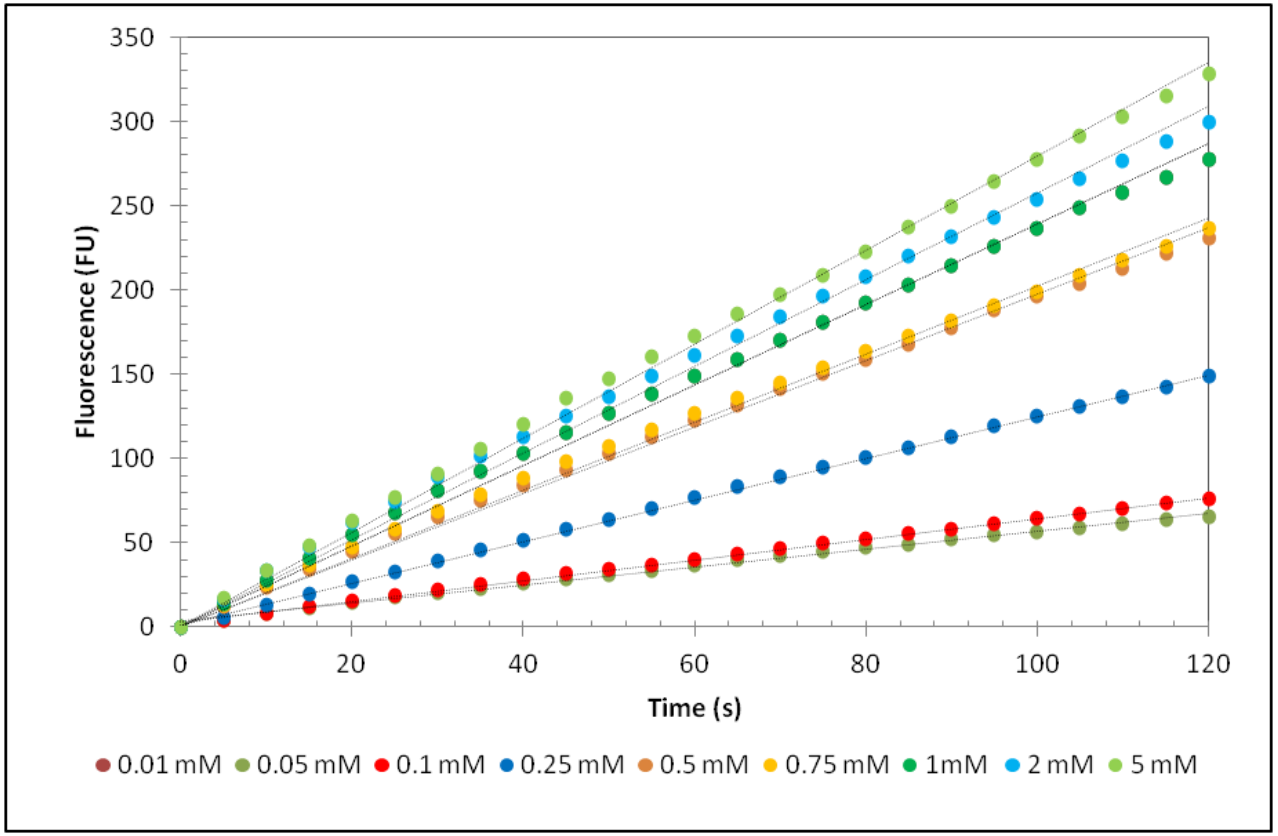
- 6-CMUG / 6-CMU



Excitation and emission spectra of 6-CMU as influenced by the presence of 6-CMUG in Phosphate buffer pH 6.8

GUD-Substrate kinetics

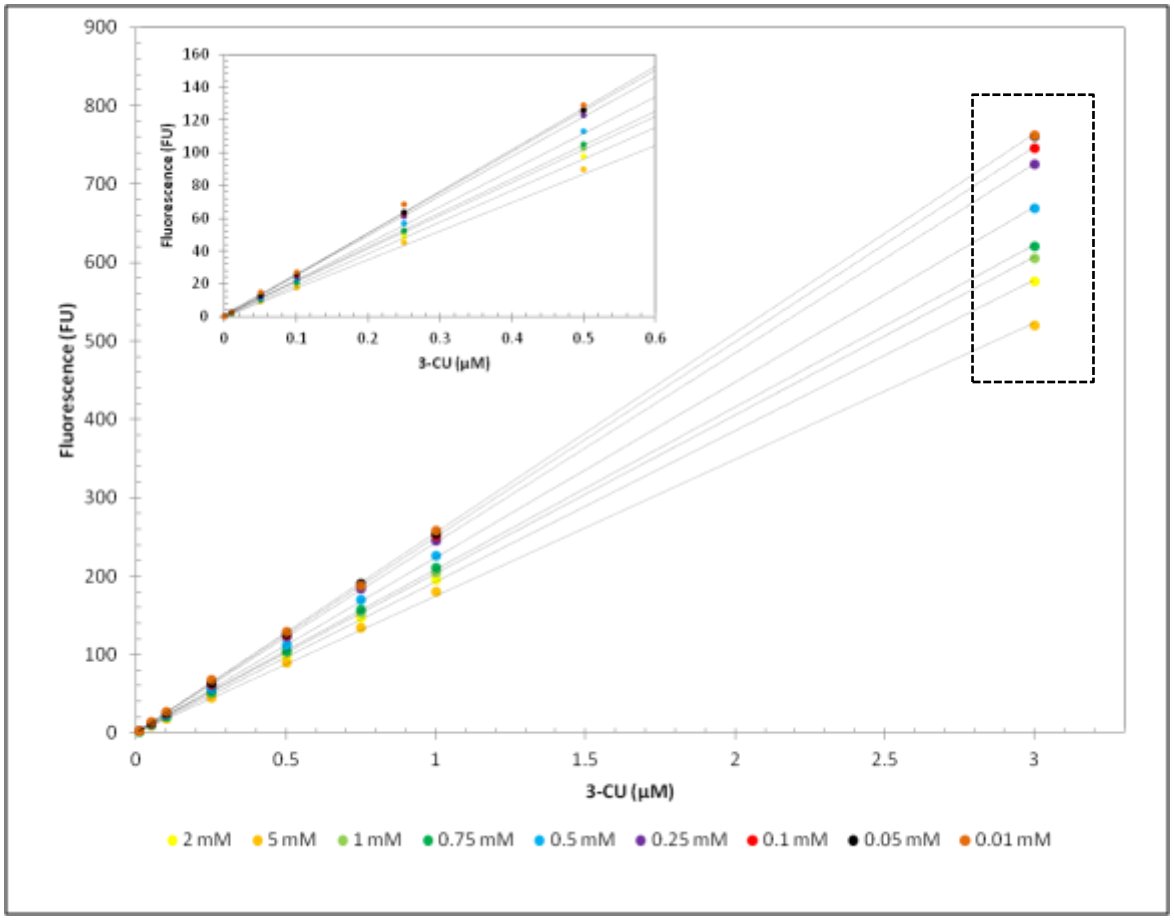
- Reaction progress curves



Reaction progress curves for GUD catalysed hydrolysis of different 3-CUG concentrations (shown in the legend); λex= 389 nm, λem 444 nm; slit widths: 5 nm (ex), 2.5 nm (em); GUD was added at a concentration of 500 ng mL^{-1}.

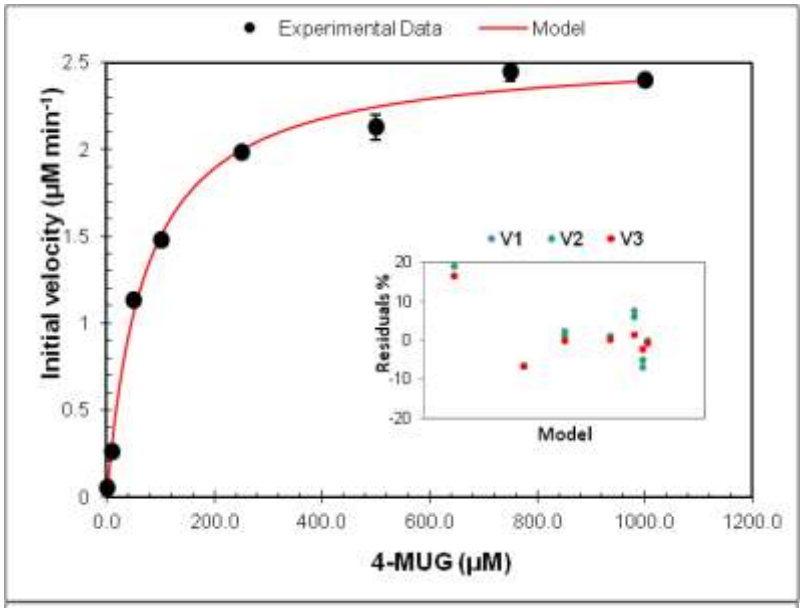
GUD-Substrate kinetics

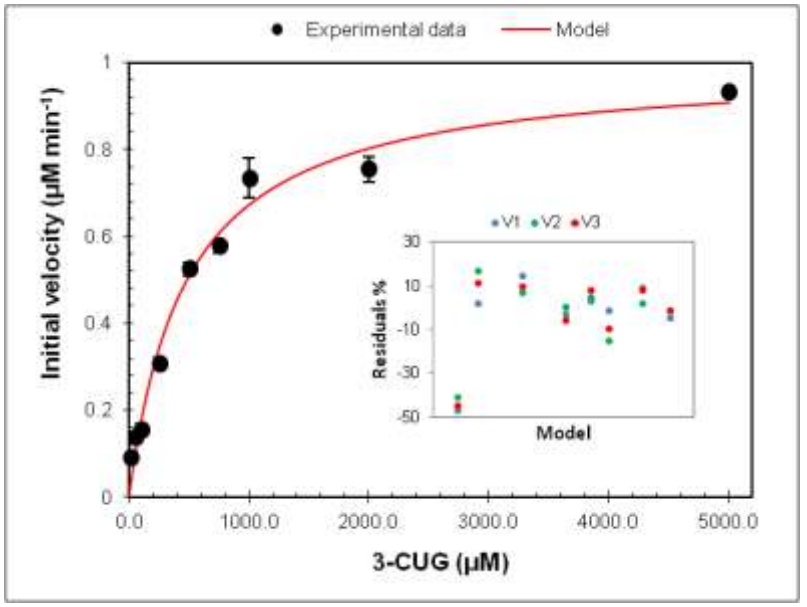
- Calibration

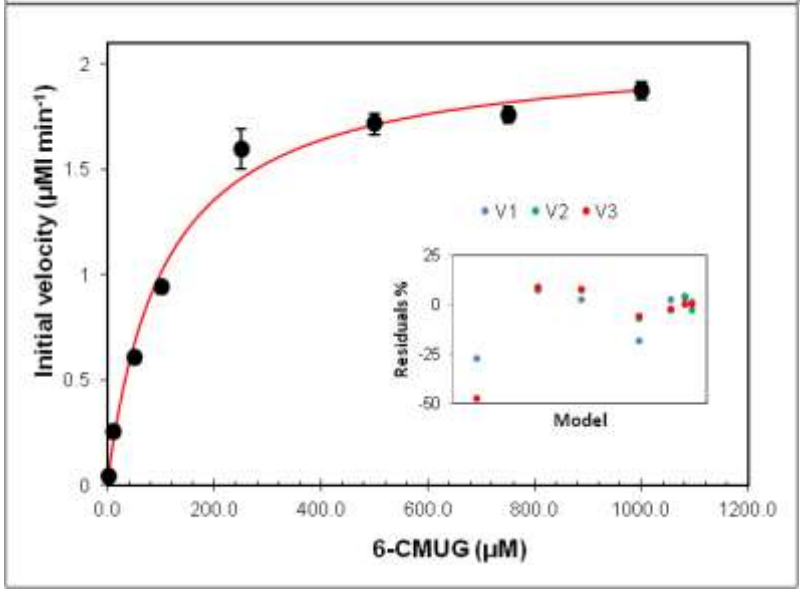


Calibration curves for 3-CU in the presence of different 3-CUG concentrations (shown in the legend); λex= 389 nm, λem 444 nm; slit widths: 5 nm (ex), 2.5 nm (em);

GUD-Substrate kinetics







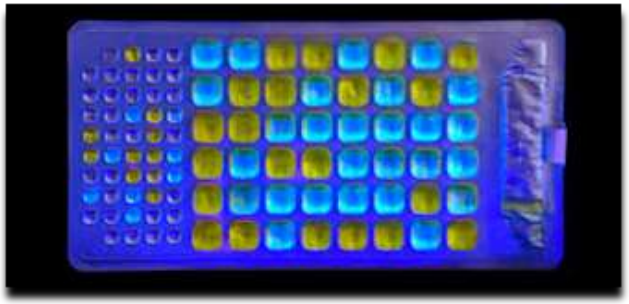
Michaelis-Menten kinetic parameters (20 °C, pH 6.8)

Substrate	K_m (µM)	V_{max} (µM min^{-1})	V_{max} / K_m
4-MUG	70.82	2.56	0.031
3-CUG	479.28	0.99	0.002
6-CMUG	106.88	2.07	0.019

Rapid *E. Coli* detection.Procedure

100 mL

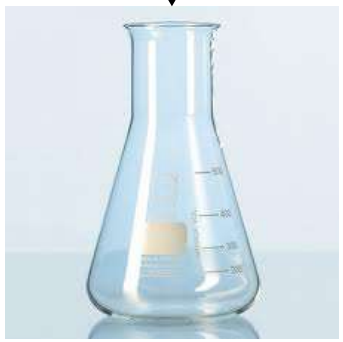
Culture based method



18-24 h

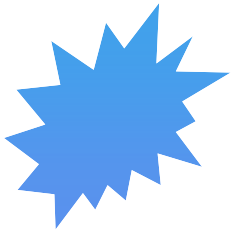
GUD Assay
10-300 mL

10mL GUD extraction buffer (pH 6.8)



1 mL

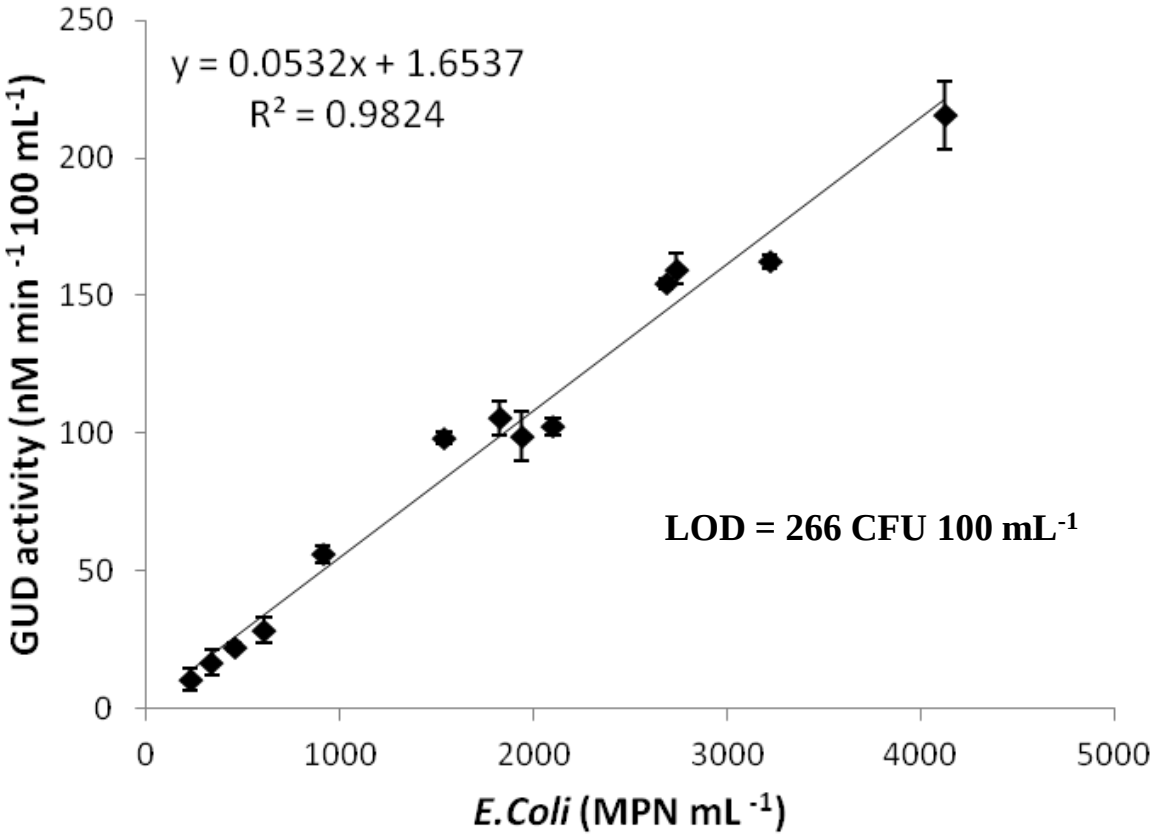
1 mL 6-CMUG 1mM



20 min + 30 min + 10 min = 1 h

Rapid *E. Coli* detection

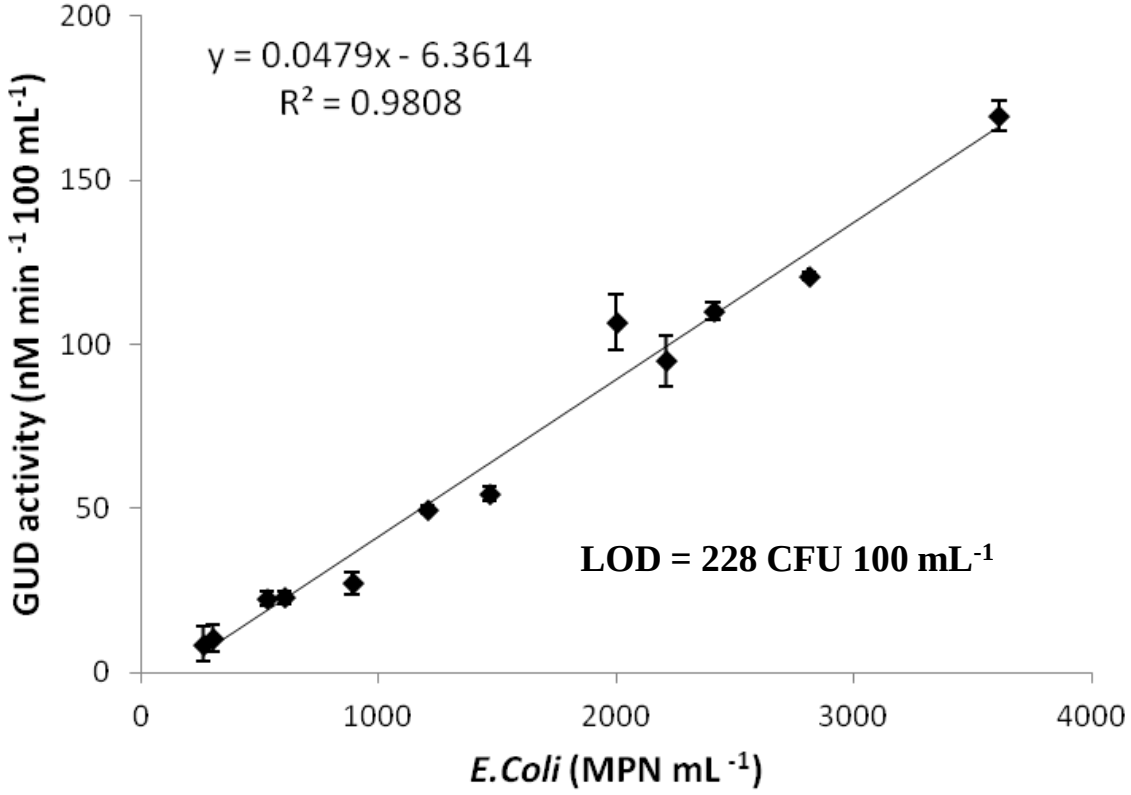
- Sea water



Linear regression between GUD activity and *E. Coli* concentrations in seawater. Error bars represent the SD of n=3 GUD activity measurements taken for each *E. Coli* concentration;

Rapid *E. Coli* detection

- Fresh water



Linear regression between GUD activity and *E. Coli* concentrations in river water. Error bars represent the SD of n=3 GUD activity measurements taken for each *E. Coli* concentration;

Conclusions/Impact:

❑Continuous fluorometric method for the determination of GUD activity has been developed.

❑Advantages
- ✓reagent consumption is minimised
- ✓possible to follow reaction kinetics only when a small amount of sample is available.
- ✓straightforward approach, prompt evaluation of kinetic data
- ✓minimal sample manipulation, experimental error reduced
- ✓potential for the implementation into an autonomous sensing platform

❑The method was applied for the detection of *E. Coli from* environmental water samples and was successful in predicting *E. Coli* concentrations below the EU threshold for “excellent quality” , in 1h.

❑Better management of bathing areas.

Acknowledgements

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Thank you all very much for your attention!

Questions?