



Single-molecule fluorescence system for determination of biogenic amines in biological samples

Mikhailova A.M., Makedonskaya M.I., Shekhovtsova T.N., Veselova I.A.

Department of Chemistry, M. V. Lomonosov Moscow State University,

** +7 495 939 4675; e-mail: a.mikhaylova.chem@gmail.com*



Biogenic amines

Matrix of hydrogel

Fluorescent system

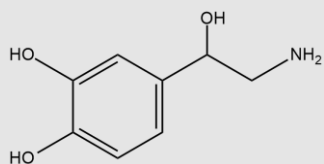
Immobilization of the system

Toxicity for cells

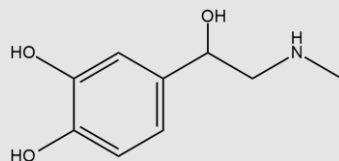
"sandwich" system

Neurotransmitters

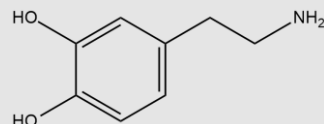
Biomarkers of neuroendocrine and neurodegenerative diseases



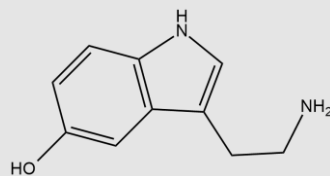
Adrenaline



Noradrenaline



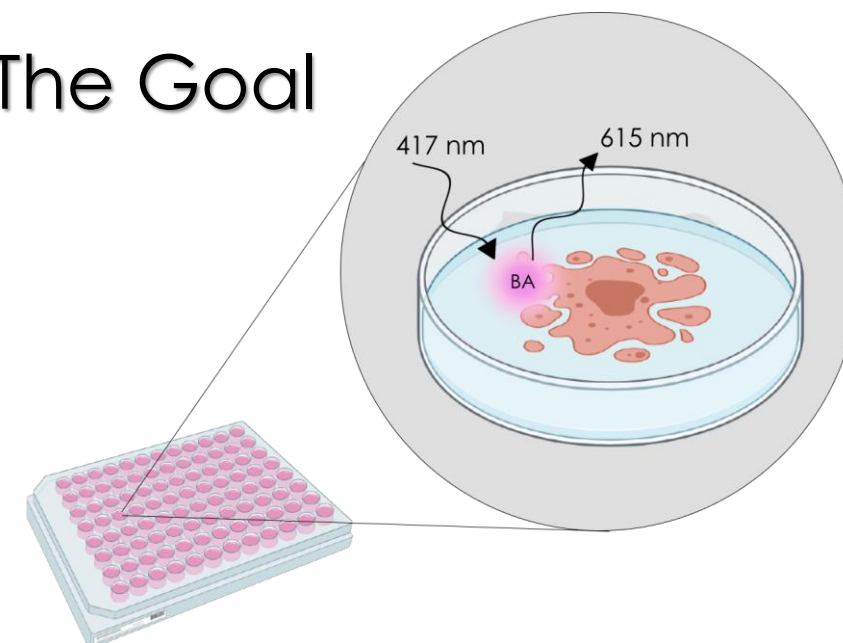
Dopamine



Serotonin

Early diagnosis of neurodegenerative diseases can be based on the determination of decreased content of Biogenic amines in cell cultures.

The Goal



Immobilization of the fluorescent system for biogenic amines (BA) determination in hydrogels matrixes as the part of detecting system and the medium for cells growing.



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Cell cultures growing medium

NATURAL HYDROGELS

- ✓ Rigid 3D matrices
- ✓ Increasing the quantum yield of fluorescence by complex stabilizing

- ✓ *Ex vivo* mammalian cell cultivation for studying the physiology of cells and tissues, to diagnose and predict diseases.
- ✓ 3D growing imitating natural cells of living organisms

Fluorescent systems immobilization matrixes



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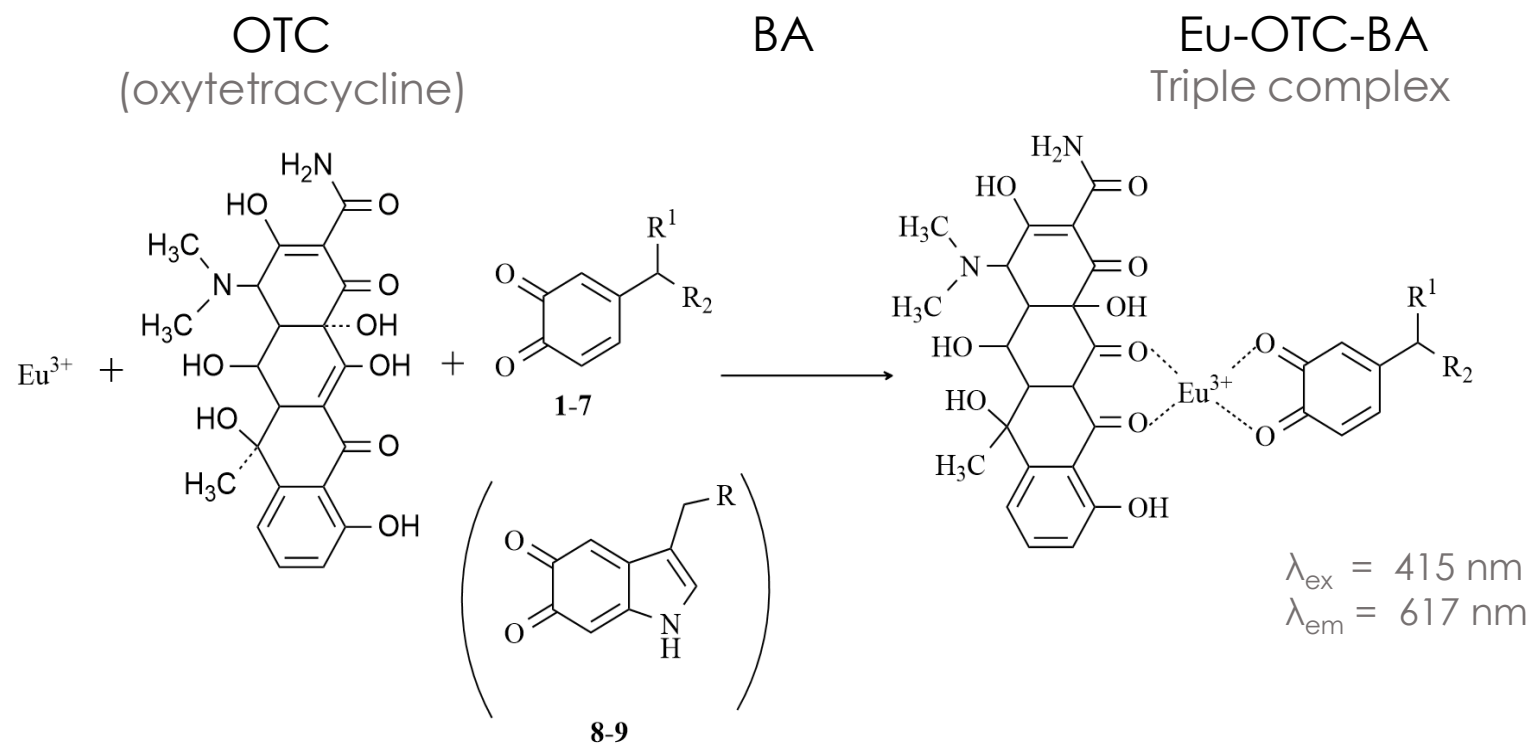
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Biogenic amines (BA) determination by the formation of highly fluorescent triple complexes Eu^{3+} -OTC-BA in micellar solution in the single molecular level



1 NE: $\text{R}^1 = -\text{OH}$; $\text{R}^2 = -\text{CH}_2\text{-NH}_2$

2 EP: $\text{R}^1 = -\text{OH}$; $\text{R}^2 = -\text{CH}_2\text{-NH-CH}_3$

3 DA: $\text{R}^1 = -\text{H}$; $\text{R}^2 = -\text{CH}_2\text{-NH}_2$

4 VMA: $\text{R}^1 = -\text{OH}$; $\text{R}^2 = -\text{COOH}$

5 HVA: $\text{R}^1 = -\text{H}$; $\text{R}^2 = -\text{COOH}$

6 NMN: $\text{R}^1 = -\text{OH}$; $\text{R}^2 = -\text{CH}_2\text{-NH}_2$

7 L-DOPA: $\text{R}^1 = -\text{H}$; $\text{R}^2 = -\text{CH}(\text{NH}_2)\text{COOH}$

8 Serotonin: $\text{R} = \text{CH}_2\text{-NH}_2$

9 5-HIAA: $\text{R} = \text{COOH}$



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Alginate hydrogel

Collagen hydrogel

Good results

Conc. range, pM	LOQ, pM	S _r (n = 4, P = 0.95)
500 – 5000	300	0.008
5 – 50	2	0.007
5 - 50	3	0.009

Conc. range, pM	LOQ, pM	S _r (n = 4, P = 0.95)
100 – 1000	60	0.030
50 – 500	25	0.044
100 – 1000	70	0.037

Dopamine
Adrenaline
Noradrenaline

✓ Good reproducibility

✓ Highest compatibility with living cellular systems

Idea: to combine these two advantages

✗ Very dense

✗ Alkali environment

	Conc. range, pM	LOQ, pM	S _r (n = 4, P = 0.95)	Conc. range, pM	LOQ, pM	S _r (n = 4, P = 0.95)
Dopamine	500 – 5000	210	0.010	500-5000	280	0.006
Adrenaline	100-500	30	0.012	10-100	65	0.009
Noradrenaline	100-500	30	0.015	5-50	3	0.011

Bad results

Gelatin hydrogel

Albumin hydrogel

Do not suit for live cells growing



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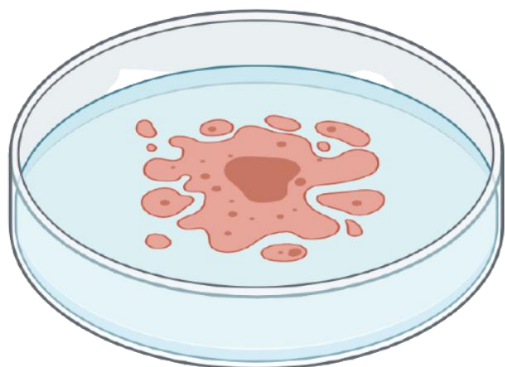
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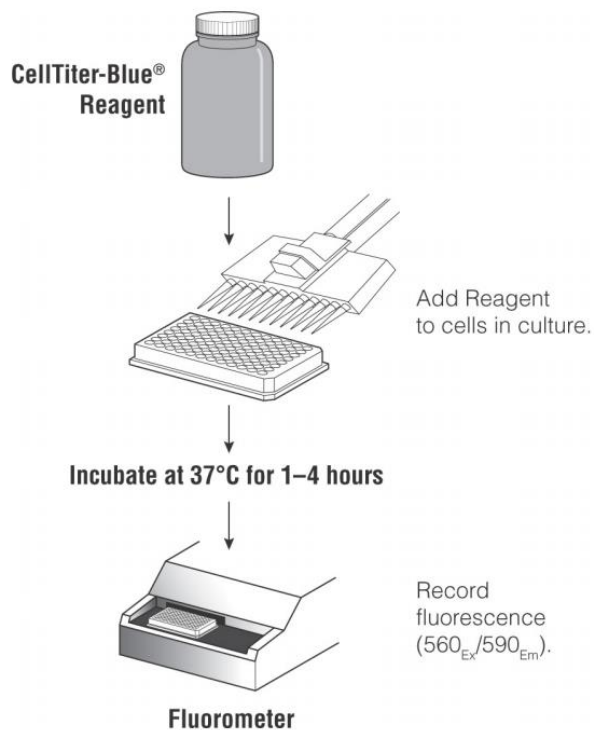
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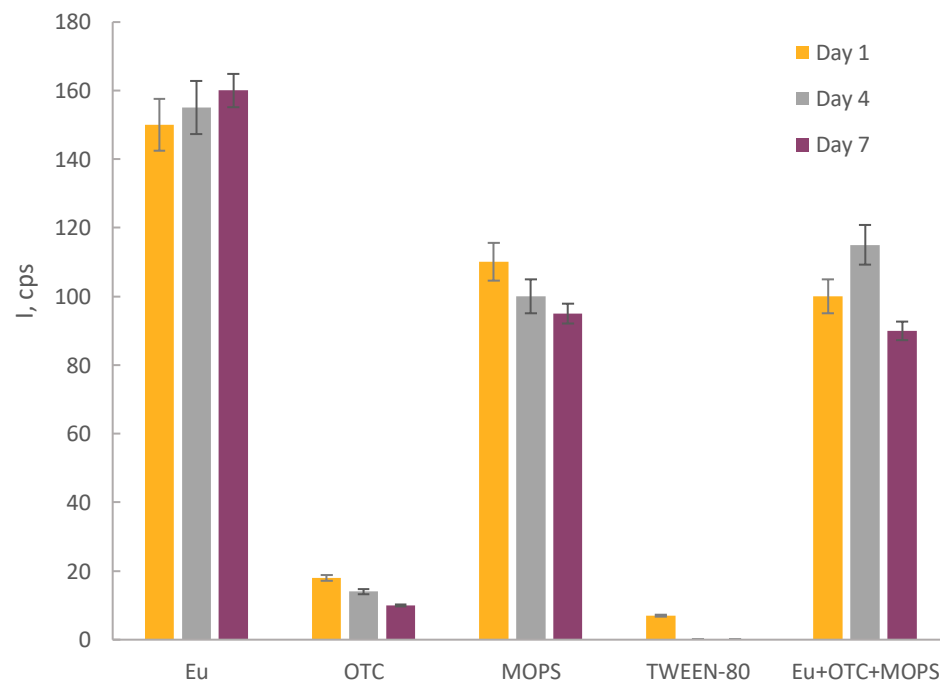


PC 12 cells are cloned from pheochromocytoma, a tumor of the chromaffin tissue of the adrenal glands, and are similar in their ability to synthesize, accumulate and excrete neurotransmitters with chromaffin cells.



Determination of the cell viability using the fluorometric method «CellTiter-Blue® Cell Viability Assay (Promega)»

The toxicity study of the components of the indicator reaction (Eu³⁺, OTC, MOPS, TWEEN 80 and Eu³⁺ + OTC + MOPS) for the viability of PC12 cells.





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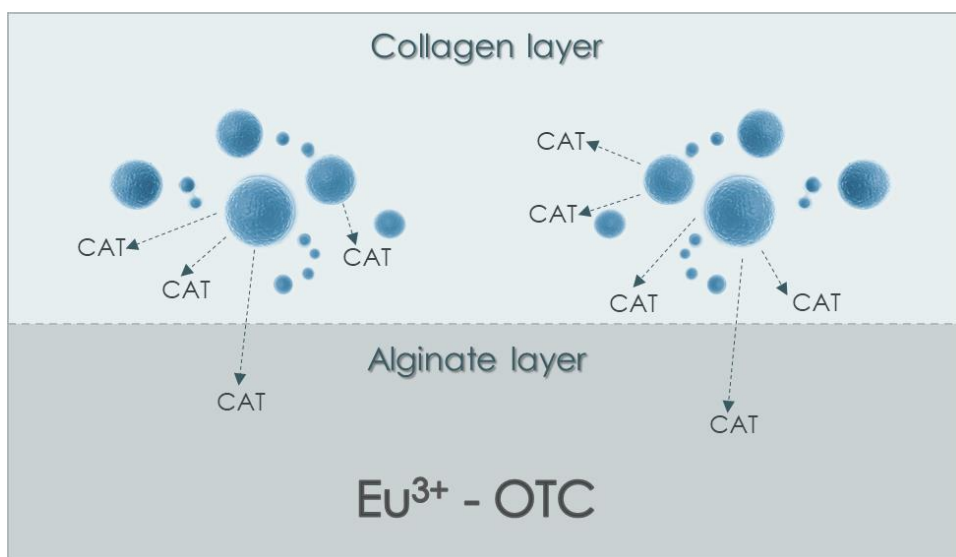
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Spatial separation of toxic components of the reaction and living cell cultures



Reducing the detection limits

Analyte	Conc. range, pM	LOQ, pM	S_r (n = 4, P = 0.95)
Alginate hydrogel			
Dopamine	500 – 5000	300	0.008
Adrenaline	5 – 50	2	0.007
Noradrenaline	5 - 50	3	0.009
Alginate-collagen sandwich			
Dopamine	90 – 400	83	0.013
Adrenaline	2.5 – 15	1.0	0.008
Noradrenaline	2 – 25	0.8	0.030

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