

IECB
2024
Conference



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

A LAB-ON-PAPER BIOSENSOR FOR ATP QUANTIFICATION VIA A CHEMILUMINESCENT DNA NANOSWITCH ASSAY

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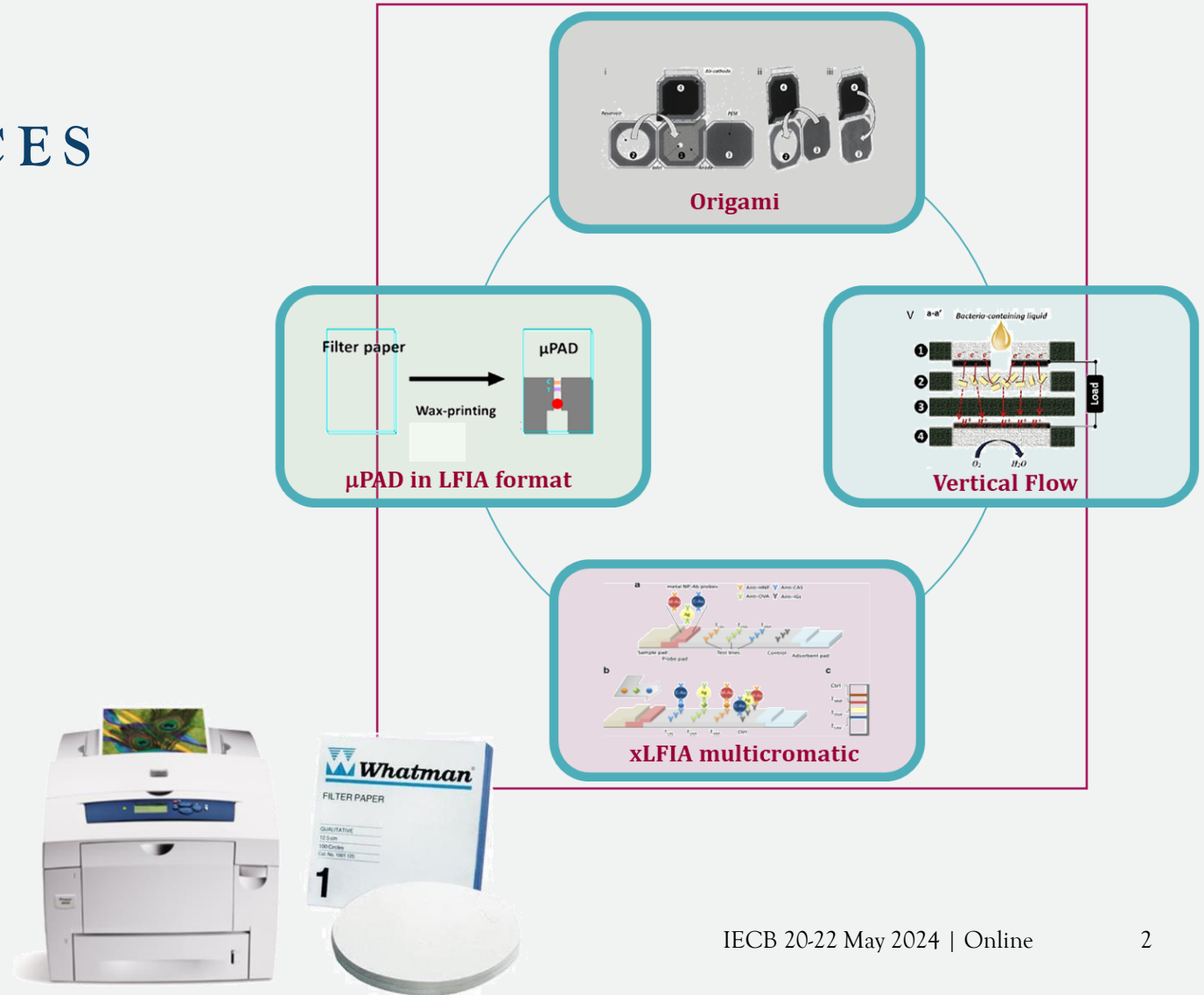
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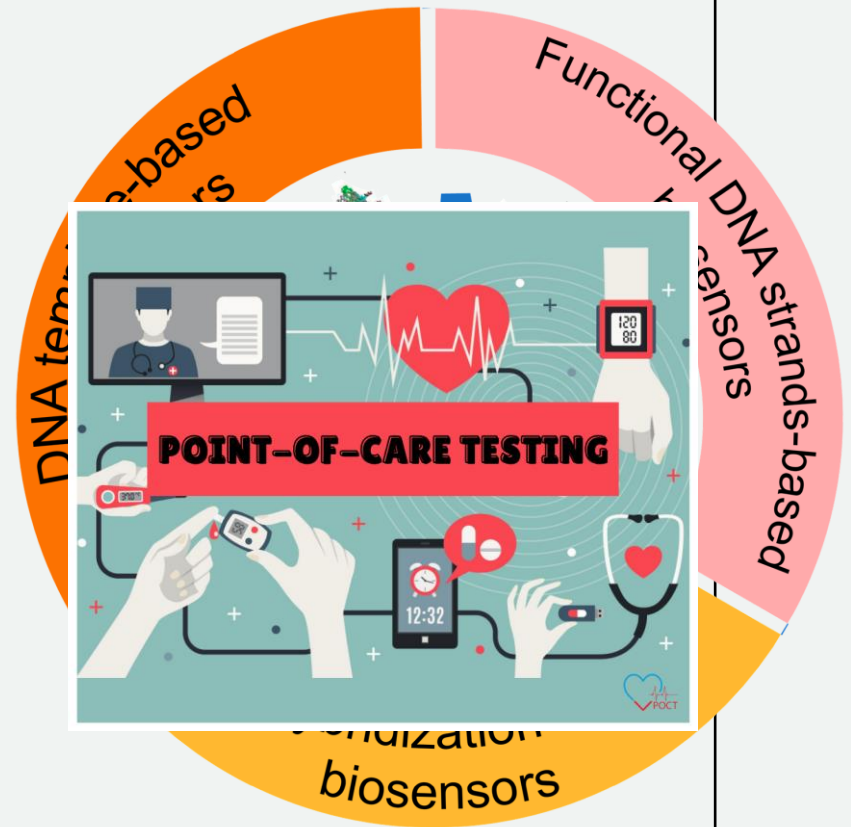
The 4th International Electronic Conference on Biosensors
(IECB 2024)

PAPER-BASED MICROFLUIDIC ANALYTICAL DEVICES (μ PADs)

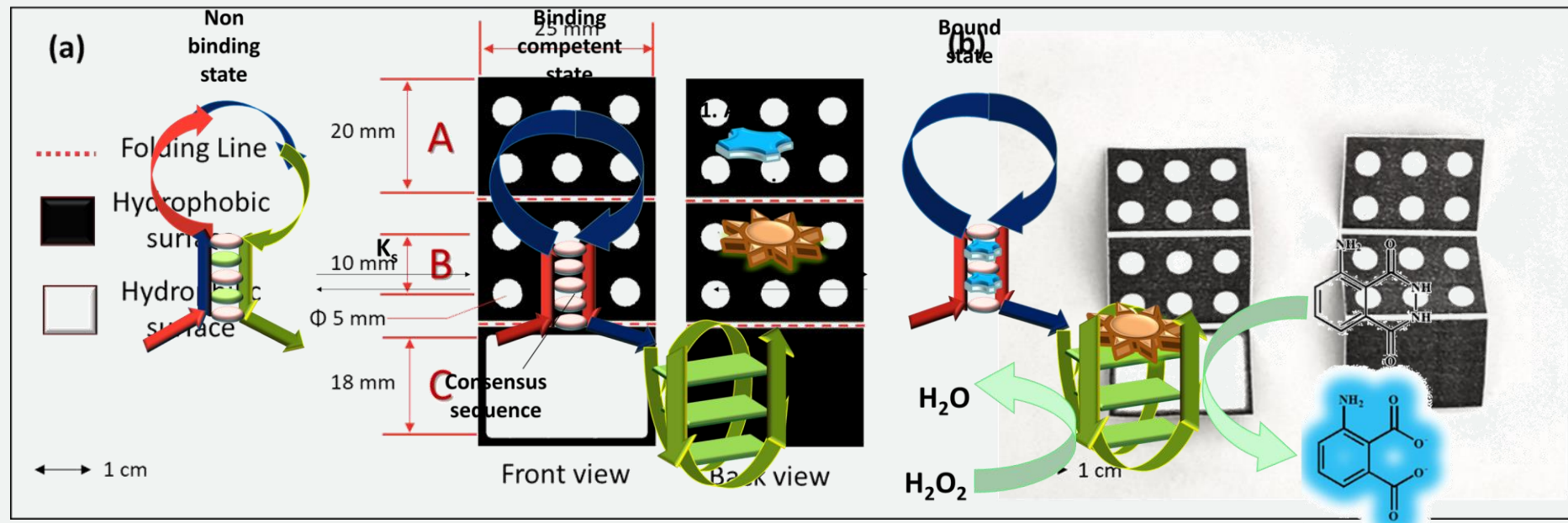
- Cheap
- Biodegradability
- Controlled porousness and capillarity
- Simple fabrication
- Easy disposal
- Point of Care (POC) applications



DNA NANOTECHNOLOGIES

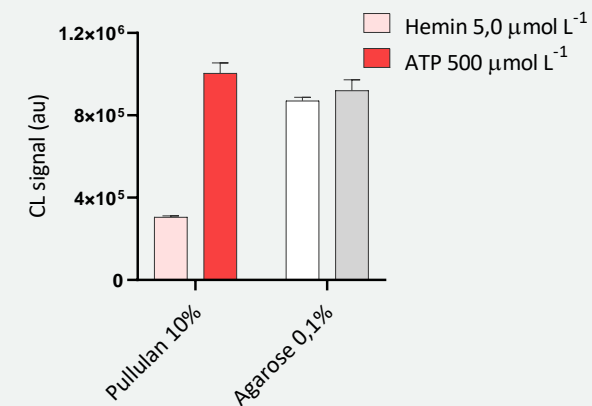
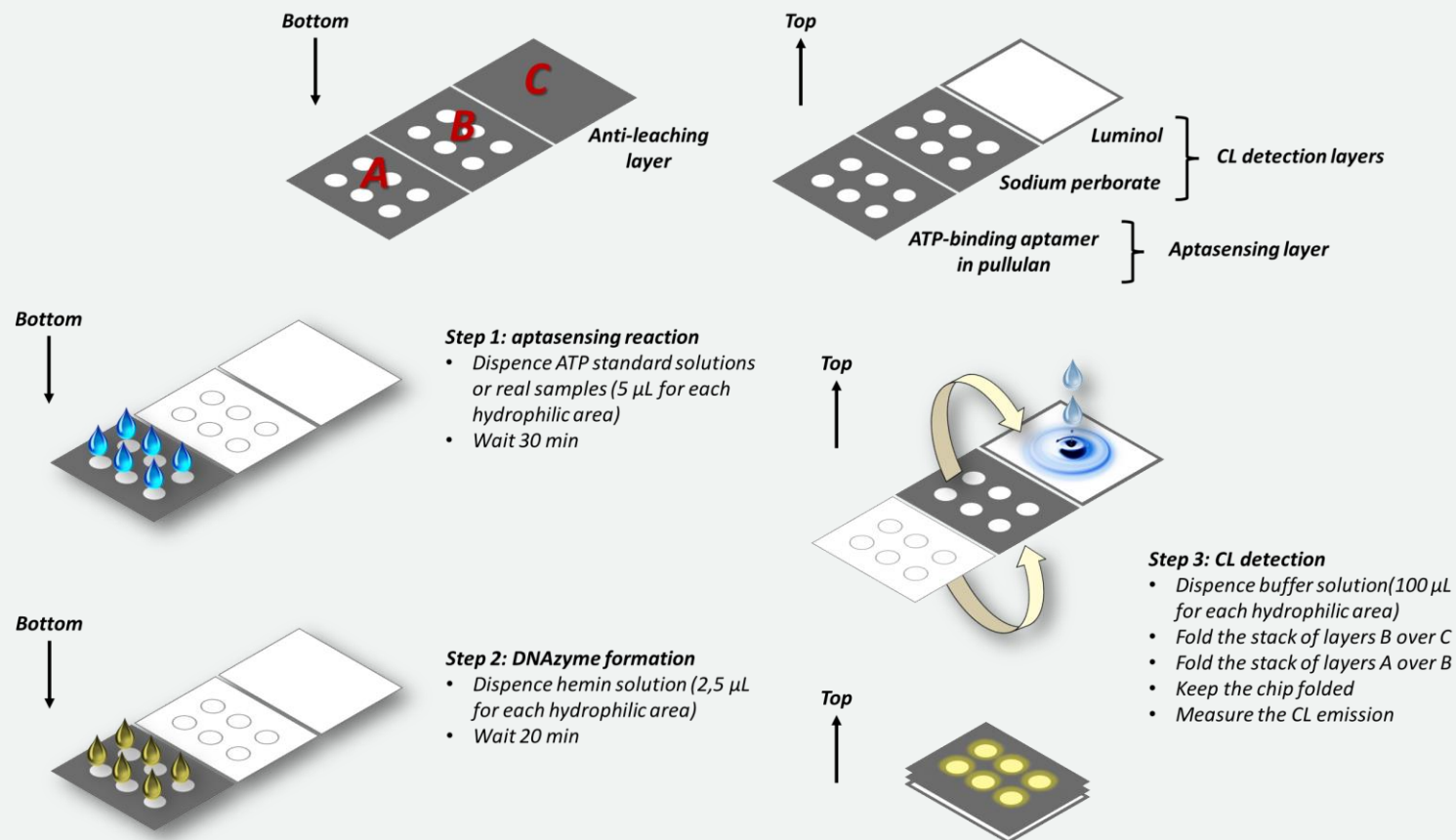


DNA NANOSWITCH LAB-ON-PAPER DEVICE FOR ATP DETECTION

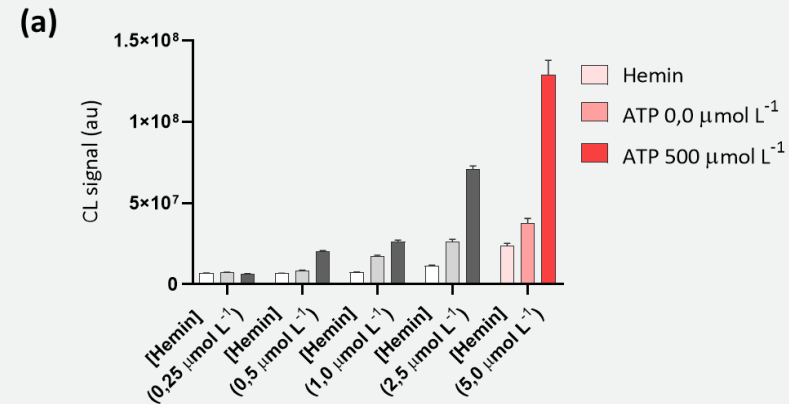


Switch: 5'-*ACCTGGGGGAGTAT*CCAACCC*TGCGGAGGAAGGT*TTTGGGTGGGTGGGTGGGT-3'

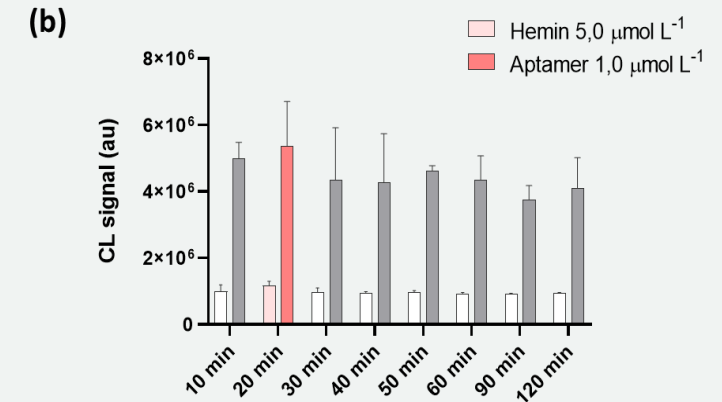
ANALYTICAL PROCEDURE



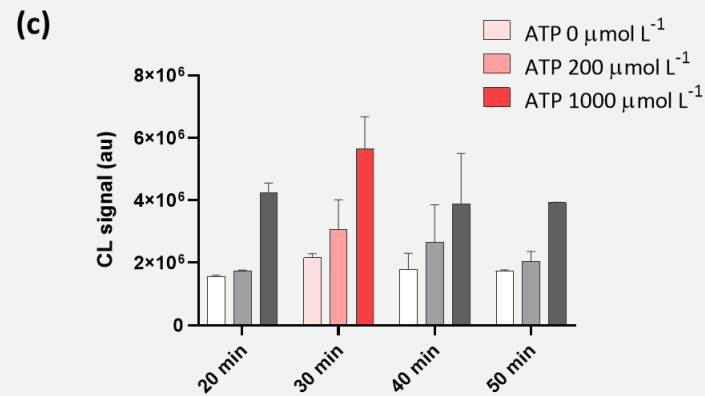
DETERMINATION OF EXPERIMENTAL PARAMETERS



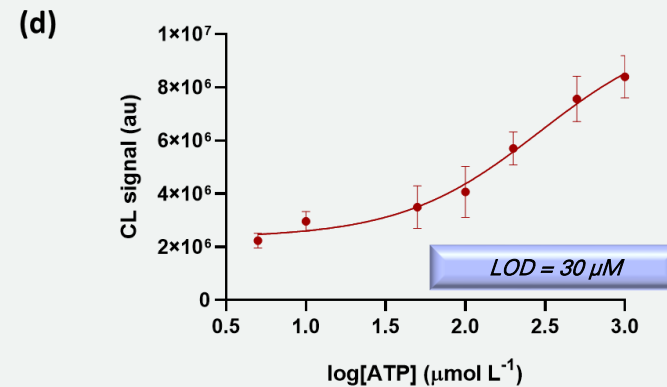
Determination of the optimal concentration of hemin, keeping constant concentration of DNA (1 μM).



Optimization of the incubation time of the DNA nanoswitch with hemin in the presence of a minimal concentration of ATP (100 nM) monitoring the best CL signal-to-noise ratio.



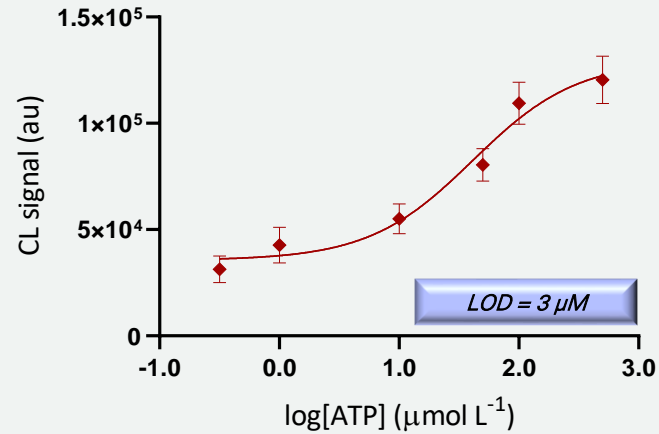
Optimization of incubation time of the DNA nanoswitch with ATP, keeping constant the concentration of hemin.



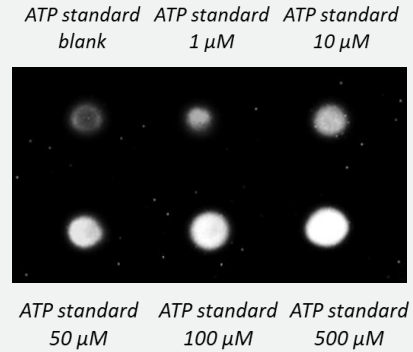
Calibration curve generated by combining the results obtained by analyzing ATP standard solutions.

PEPER-BASED ANALYTICAL TEST

(a)



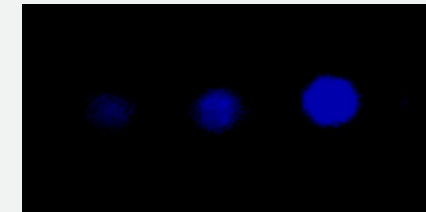
Calibration curve generated by combining the results obtained by analysing ATP standard solutions with different μ PAD biosensors.



CMOS (Complementary Metal Oxide Semiconductor)

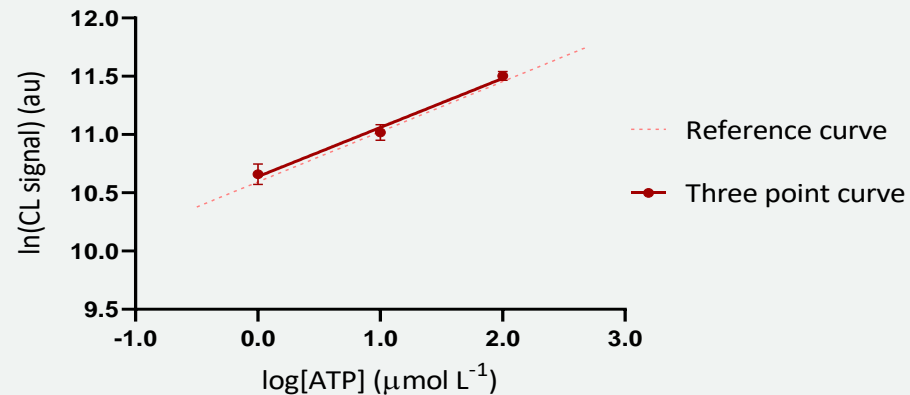


ATP standard 1 μM ATP standard 10 μM ATP standard 100 μM

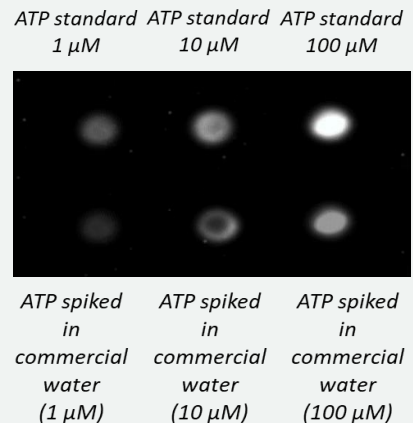


Imaging of CL signal corresponding to three-point calibration curve acquired by mobile BI-CMOS camera of smartphone Samsung S20 (Samsung, Seoul, South Korea).

(b)



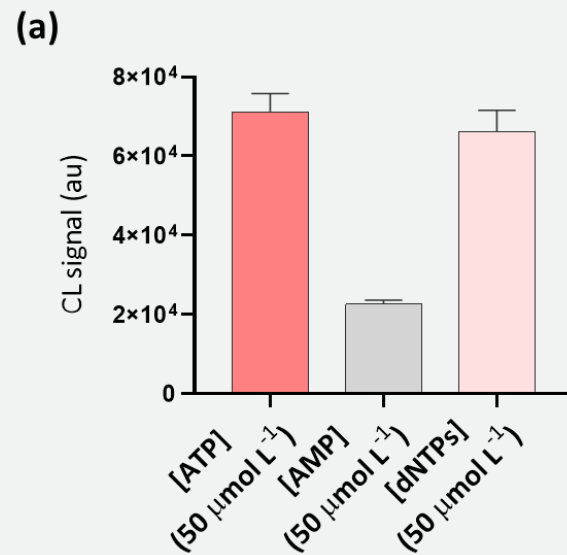
Comparison of slope between reference and three-point calibration curve.



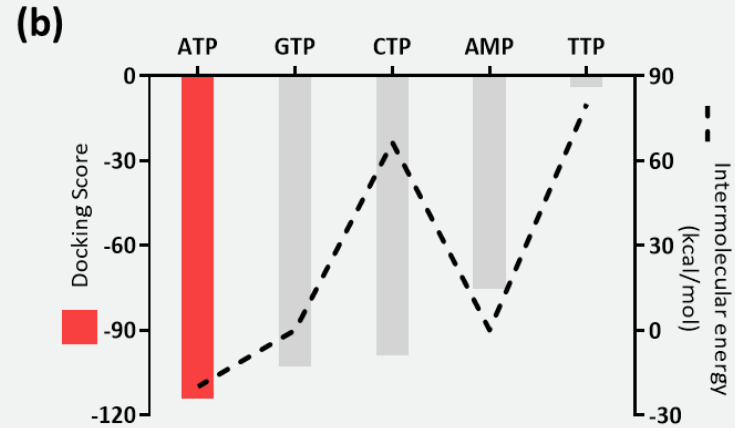
CCD (Charge Coupled Device)



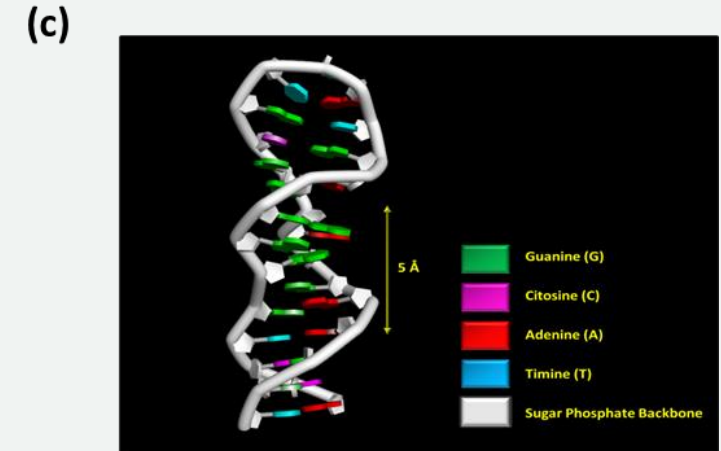
ASSAY SPECIFICITY



CL signals measured in the origami μPAD for 50 μM standard solutions of ATP and the potentially interfering nucleotides AMP, dNTPs.



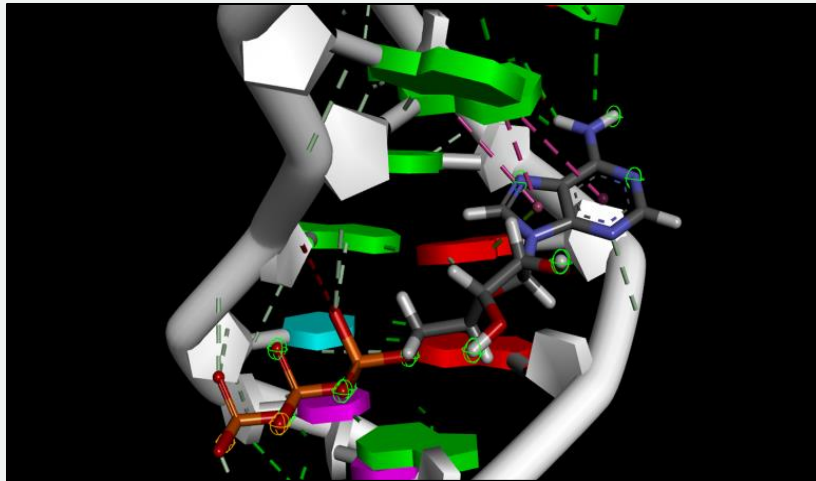
Results of molecular docking study: comparison between docking score values and intermolecular energy (kcal/mol) (abalone software) for each nucleotide docked with the aptamer.



Crystallographic structure of aptamer specific for ATP.

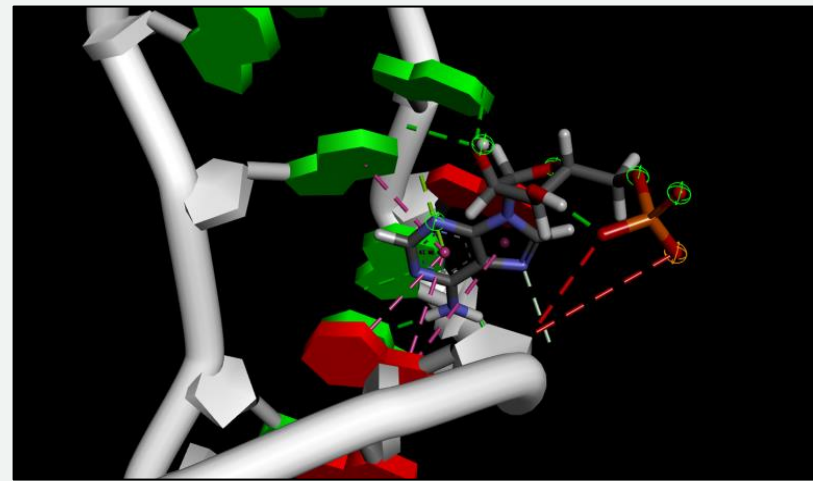
ASSAY SPECIFICITY

(e)



Most probable 3D structures obtained by molecular docking simulations
for the complexes of **ATP** with aptamer.

(d)

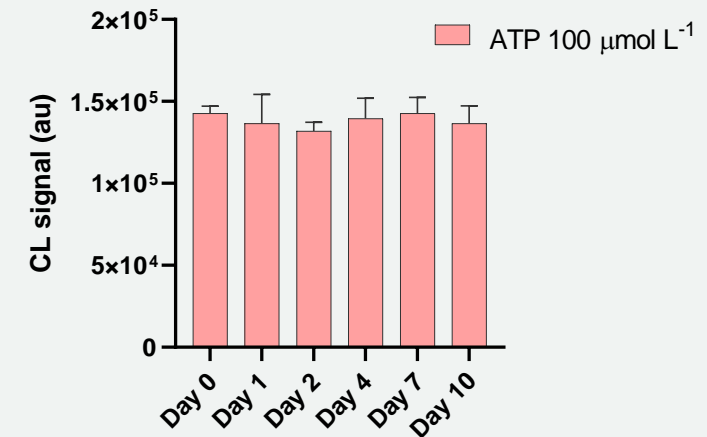


Most probable 3D structures obtained by molecular docking simulations
for the complexes of **AMP** with aptamer.

ACCURACY AND STABILITY

	Concentration of ATP Spiked ($\mu\text{mol L}^{-1}$)	Concentration of ATP Measured ($\mu\text{mol L}^{-1}$)	Recovery (%)
Sample 1	1	$0,99 \pm 0,04$	99 ± 4
Sample 2	10	$10,1 \pm 0,7$	101 ± 7
Sample 3	100	112 ± 14	112 ± 14

Results of the recovery study of the origami μPAD biosensor performed on a commercial drinking water sample spiked with known amounts of ATP.



Monitoring of stability over time of paper-based device preserved at $+4^\circ\text{C}$ in the dark by CL signal detection, testing a solution of ATP standard at concentration of $100 \mu\text{M}$.

CONCLUSIONS



The same approach could be used for other biomolecules as **toxins** and **clinical markers** of interest.

Implementation of a **smartphone's camera method** (CMOS) and dedicated application for data elaboration to further improve assay portability and widespread applicability.

An Origami μ PAD biosensor for ATP quantification relying on DNA nanoswitch aptasensing, followed by CL detection has been proposed.

Immobilization on paper **improved the analytical sensitivity** and the **portability** of the system.

AKNOWLEDGMENTS

Analytical and Bioanalytical
Chemistry Group

Chemistry department
«G. Ciamician»,
University of Bologna





THANK YOU
FOR YOUR KIND
ATTENTION