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Label-free detection of small molecules in clinical samples. Aptamers take the lead

Anna Ferretti, Enrico Tenaglia, Fabio Spiga, Carlotta Guiducci

Laboratory of Life Science Electronics (CLSE) - École Polytechnique Fédérale de Lausanne – Switzerland

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

The Context

Therapeutic Drug Monitoring (TDM): the determination of circulating drugs' and correction of the dosage if the concentration is outside the therapeutic range.

Problems

Costs: TDM implies costly equipment (e.g. mass spectrometers) and specialistic expertise

Restricted availability: only facilities possessing the adequate instrumentation (non Point Of Care - POC)

Limited availability of detection platforms for small molecules' TDM

Limited application: only a fraction of treatments that would benefit from it currently undergo TDM

Approach - Questions - Goals

1. Employ **cheaper and easier methods** to detect small molecules in complex matrices (e.g blood/serum)

nucleic acid based[1]

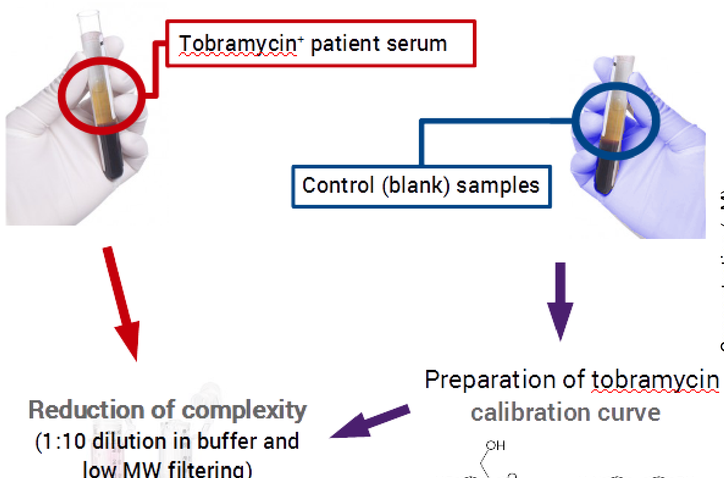
+

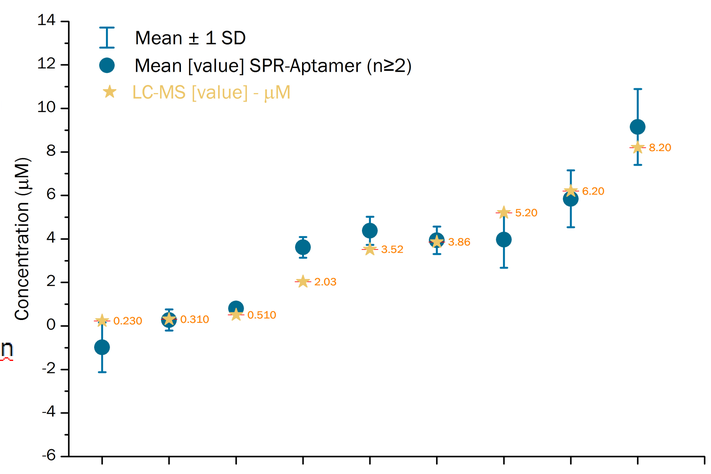
Optical-based and label-free translatable to a portable device for smart POC/self monitoring2

→ **How comparable to standard of clinical practice? How performing?** → **Characterize pitfalls and potentials of aptamer based detection of small molecules in complex matrices through optical strategies**

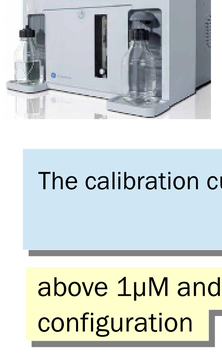
2. Develop **novel DNA aptamers** for small molecule biosensing → **SELEX strategy to obtain a beacon DNA aptamer binding Imatinib, an anticancer drug requiring TDM**

1. Label-free SPR & beacon aptamers assessment of clinical tobramycin concentrations in patients' sera





patient mg/L	µM	Avg (µM)	StDev	Error %	Δ %
1.07	0.23	-0.92	1.04	454.3%	501.9%
1.45	0.31	0.33	0.46	148.1%	6.0%
1.54	0.33	0.57	0.50	151.9%	72.9%
1.59	0.34	-0.38	n.a.	n.a.	212.6%
2.38	0.51	0.96	0.36	71.4%	88.9%
2.80	0.60	1.15	0.84	139.5%	92.2%
4.58	0.98	1.00	n.a.	n.a.	1.7%
4.90	1.05	1.43	1.03	98.5%	36.2%
7.61	1.63	2.13	n.a.	n.a.	30.6%
9.48	2.03	2.80	0.75	37.0%	38.0%
9.81	2.10	0.02	n.a.	n.a.	99.2%
16.44	3.52	3.50	0.89	25.1%	0.5%
18.03	3.86	2.97	0.98	25.4%	23.0%
19.15	4.10	1.88	n.a.	n.a.	54.0%
24.28	5.20	3.50	1.43	27.5%	32.6%
28.95	6.20	5.17	1.00	16.1%	16.6%
38.29	8.20	8.07	1.26	15.4%	1.6%
Average			0.86	68.7%	50.4%
Average above 1µM - 4.7mg/L			1.05	35.0%	33.2%



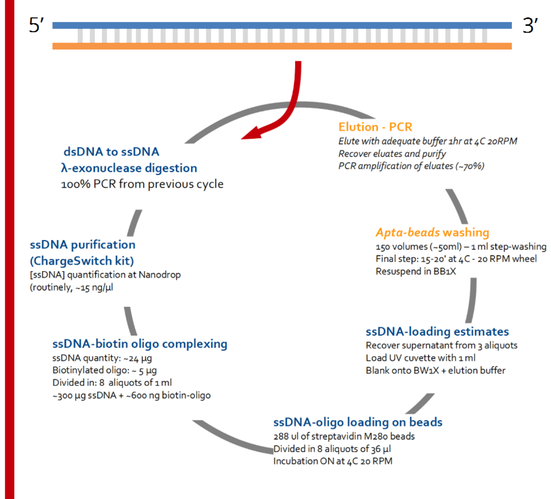
CHUV [Tobramycin] in clinical serum samples from tobramycin-treated patients undergoes **LC-MS quantification** at the CHUV.

EPFL The same samples are processed to reduce their complexity and **analyzed at the SPR** taking advantage of a custom tobramycin-specific DNA beacon aptamer

The calibration curve method allows to obtain concentration values reflecting the ones obtained through LC-MS...

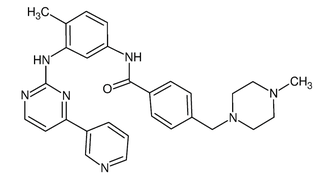
...With an **accuracy** that reaches 33.2% when dealing with concentrations above 1µM and a **precision** of 35% in the same concentration range, all that in a simple label-free SPR configuration

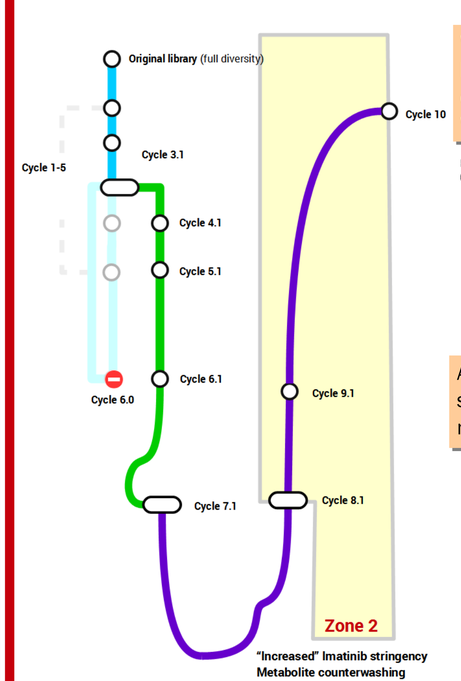
2. Towards a beacon DNA aptamer for Imatinib (Gleevec)



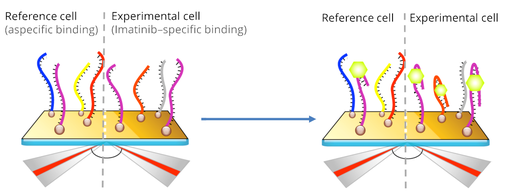
We developed an interative procedure to **produce** DNA beacon aptamers against, potentially, any small molecule of choice *(Spiga et al. 2015)*.

We validated it by generating an aptamer binding **tobramycin**, an antibiotic commonly undergoing therapeutic drug monitoring.

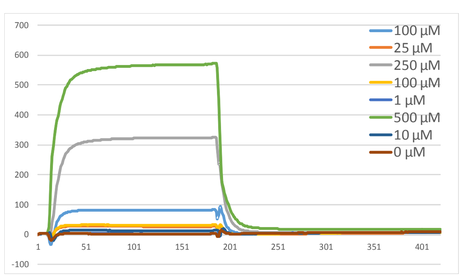




We applied the **SELEX protocol** to obtain an **aptamer binding Imatinib**, a molecule undergoing TDM and a candidate for systematic TDM approach[3].
We test its affinity for the molecule using SPR



After A 10-step selection we appreciate the biding of selected DNA aptamer pools against the target small molecule



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