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Therapeutic drug monitoring on a shoestring: beacon aptamer based detection of small molecules in human serum

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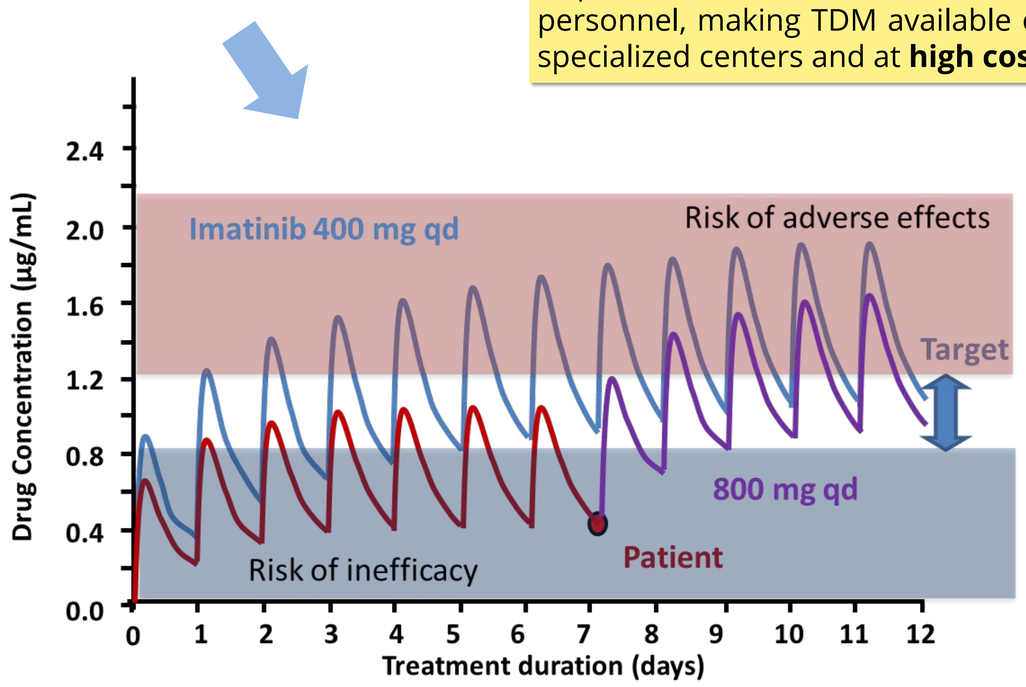
Therapeutic Drug Monitoring – TDM

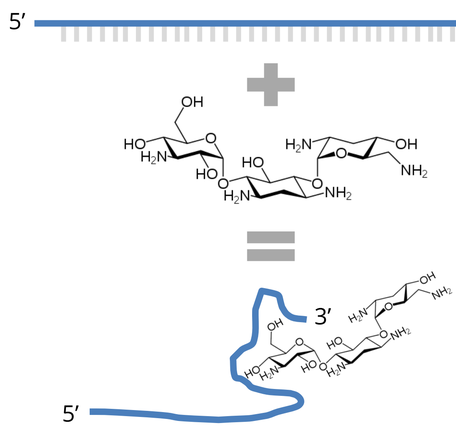
Therapeutic Drug Monitoring (TDM): determination of the drug concentration in the patient's blood and correction of the dosage if the concentration is outside the therapeutic range.

Currently TDM relies on detection techniques such as mass spectrometry or competitive immunoassays, which require dedicated facilities and trained personnel, making TDM available only in specialized centers and at **high cost.**



Adapted from T. Buclin, et al. *"Who is in charge of assessing therapeutic drug monitoring? The case of imatinib". Lancet Oncol*. 2011;12(1):9-11.

Beacon aptamers: "ideal catchers" for small molecules



DNA beacon aptamers are **single strands of DNA**...

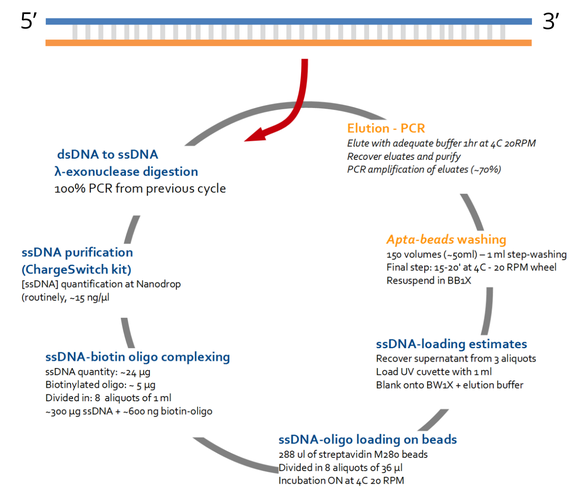
....that in the presence of a molecular ligand... (as tobramycin, depicted)

....change conformation **upon binding** their ligand

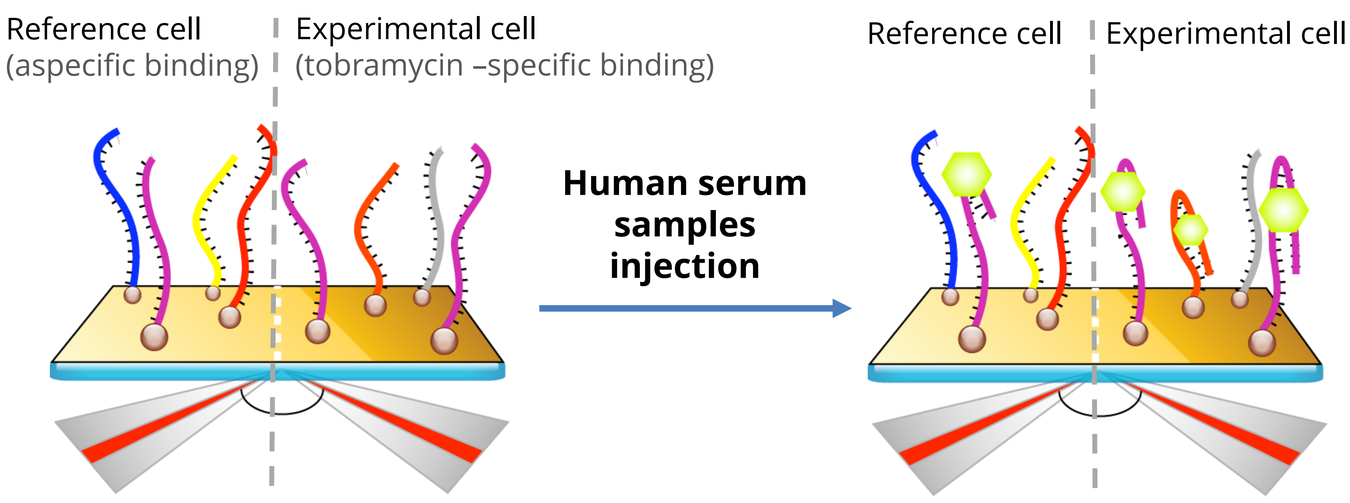
Aptamers are ideal sensors for small molecules to be employed in TDM. They can be easily raised against drugs, they are intrinsically stable in different conditions of incubation; have long shelf life, and, once the sequence is established, they can be produced **cheaply** through chemical synthesis.

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

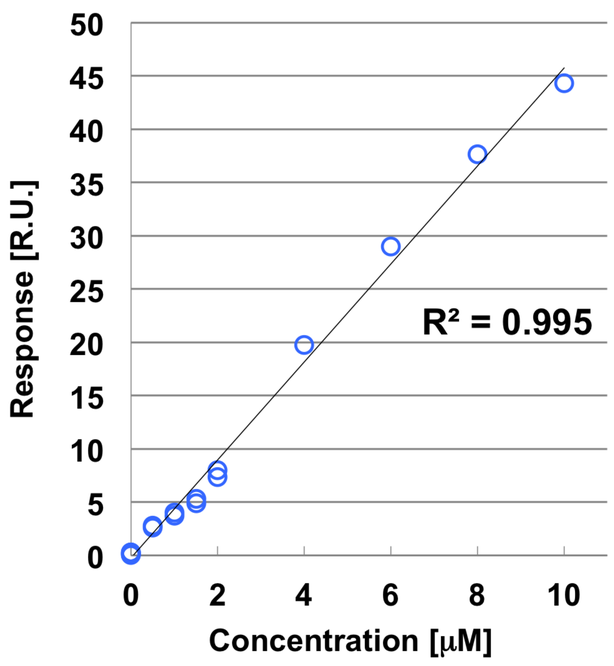
We validated it by generating an aptamer binding **tobramycin**, an antibiotic commonly undergoing therapeutic drug monitoring.



Harnessing aptamers to detect tobramycin in human serum through SPR



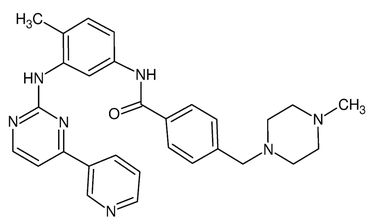
Samples are prepared by mixing 10% human serum in TE buffer with 0.01% Tween 20, then they are filtered (3 kDa cut-off) and injected in the SPR system. The **output signal** is given by the **difference** between the signal from the experimental cell and the one from the reference cell.



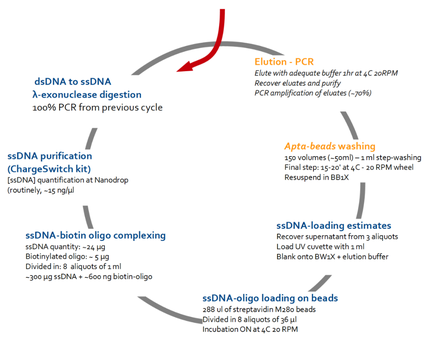
- The K_D of the binding reaction between the DNA aptamer and tobramycin is 230nM.
- The selectivity has been tested with respect to kanamycin, a molecule of the same class of antibiotics, which shows a K_D 10-fold higher, and with respect to carbenicillin, which belongs to a different class of antibiotics, for which the binding gives negligible signals.
- The linear range in serum is from **0.5 to 10µM,**
- The limit of detection attains **0.15µM**.
- With the current protocol as little as 20µl of serum are sufficient to perform a measurement in duplicate

Ongoing work (and perspectives)

Challenge the tobramycin aptamer **directly with patient blood samples** from the clinics



Define a beacon aptamer binding **Imatinib**, a major anti-tumoral drug of great interest in TDM



Adapt the aptamer selection strategy to generate sequences discriminating drugs and their major metabolites

Bibliography:

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