







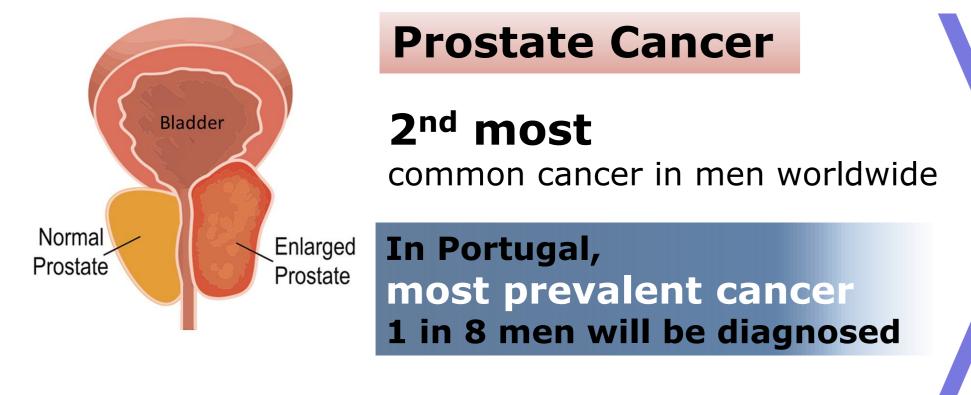


# Microfluidic Assay Based on G-Quadruplex Aptamer for Nucleolin Detection in Prostate Cancer

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### Introduction

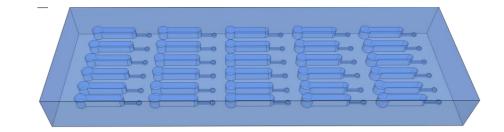


Nucleolin (NCL) is protein essential for cancer cell survival

## Overexpressed in surface of Prostate Cancer Cells

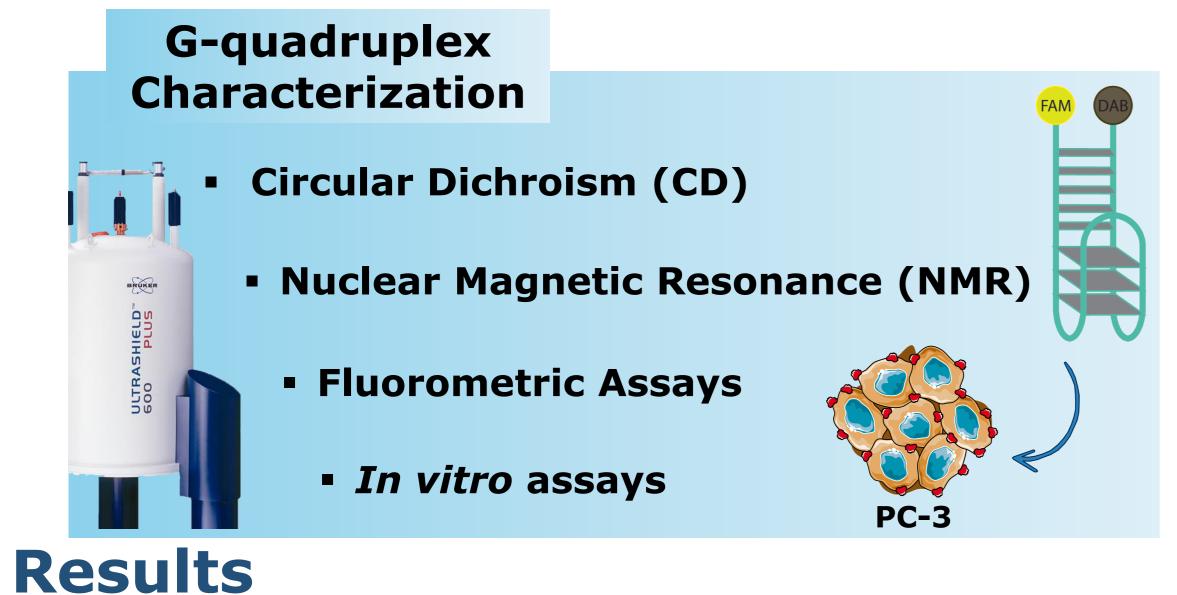
Binds specifically to DNA G-quadruplex (G4)

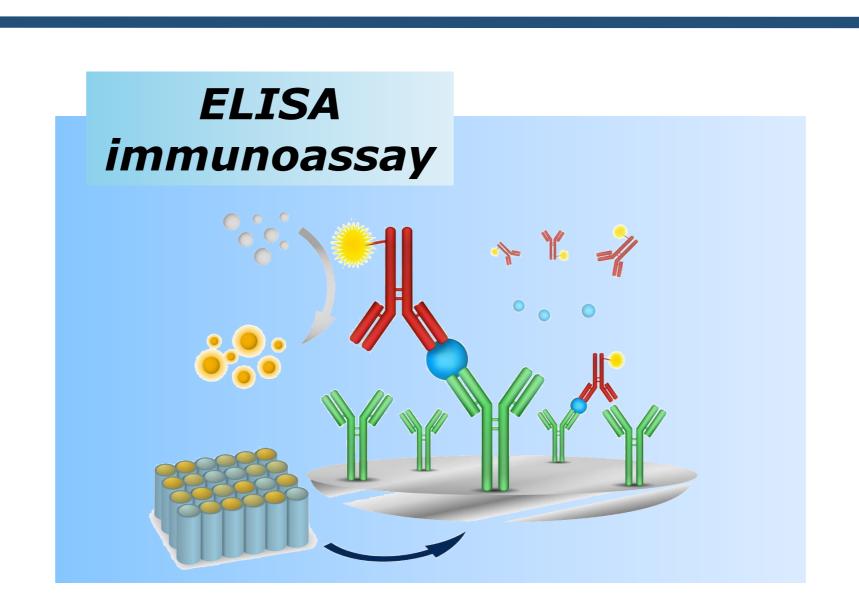
#### PDMS microfluidic detection system of NCL based on G4 aptamer

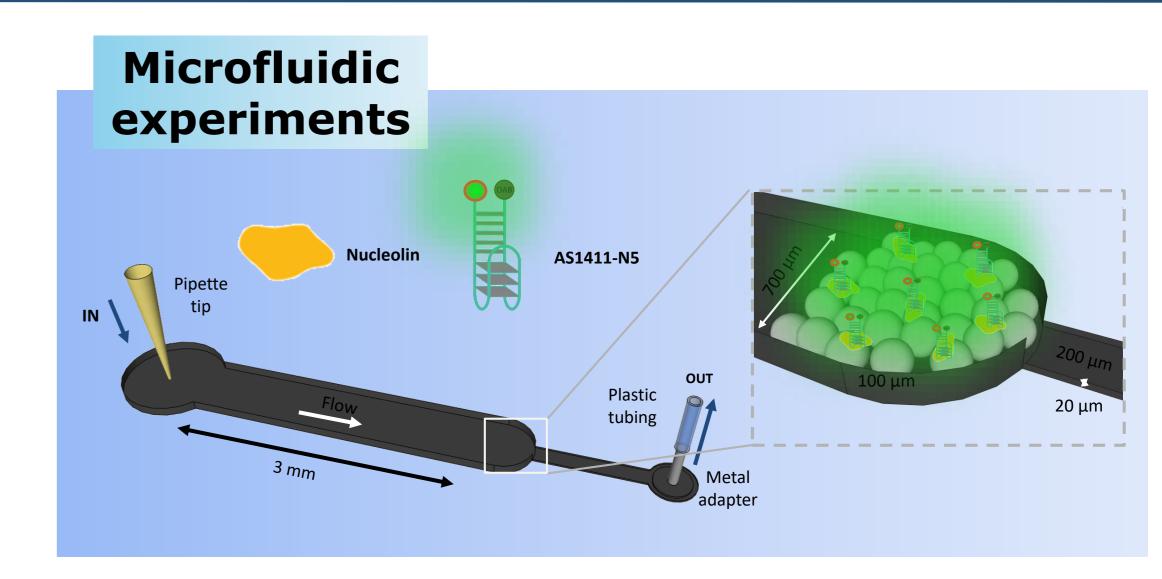


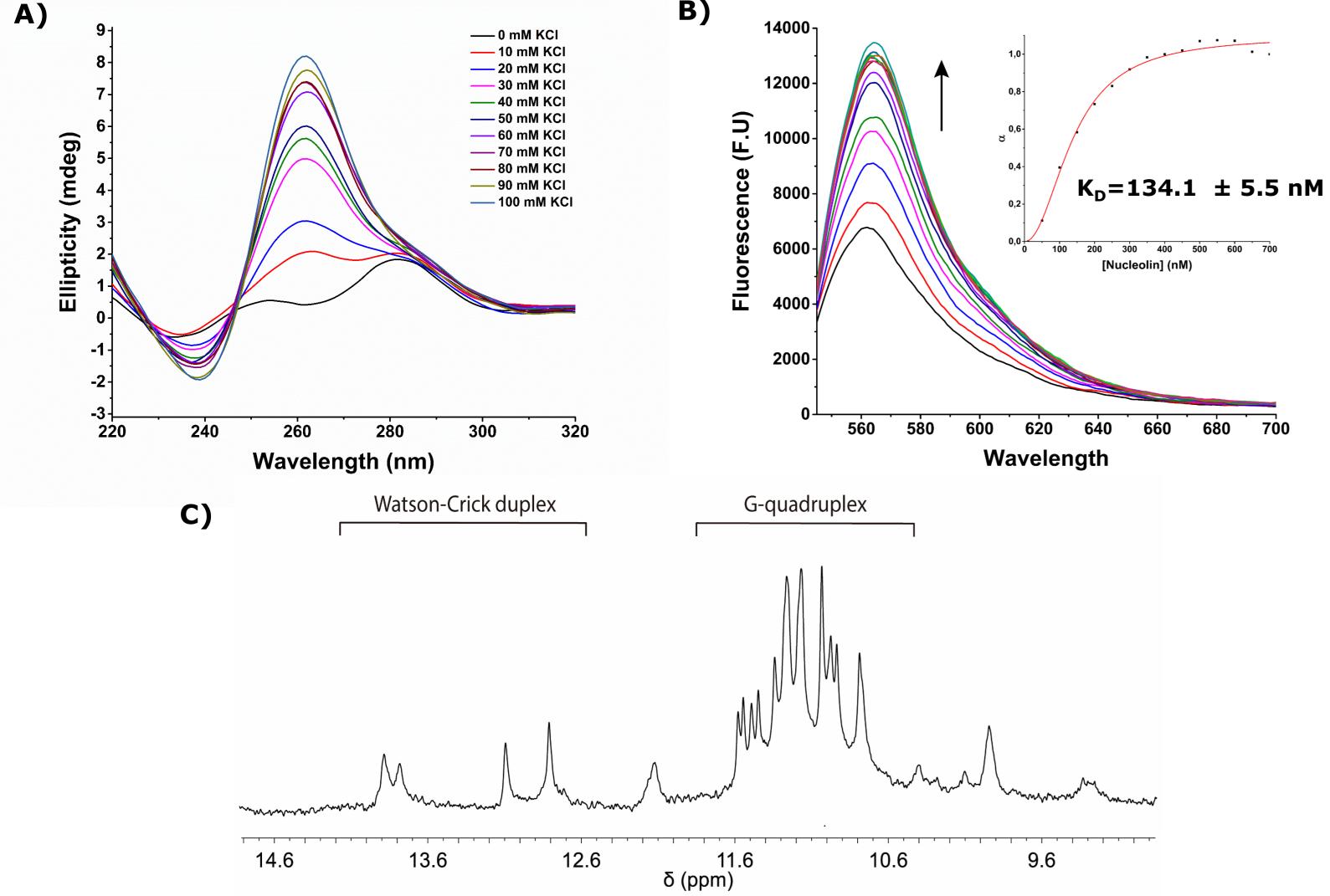
- High throughput analysis Low reagent consumption
- Potential for multiplexing Simple and Short assay time

# Methodology









CD titration of AS1411-N5 (A), quantification of target-beacon affinity, through the determination of dissociation constant ( $K_{D}$ ), by fluorometric assay (B) and NMR titration spectra of molecular aptamer beacon (C).

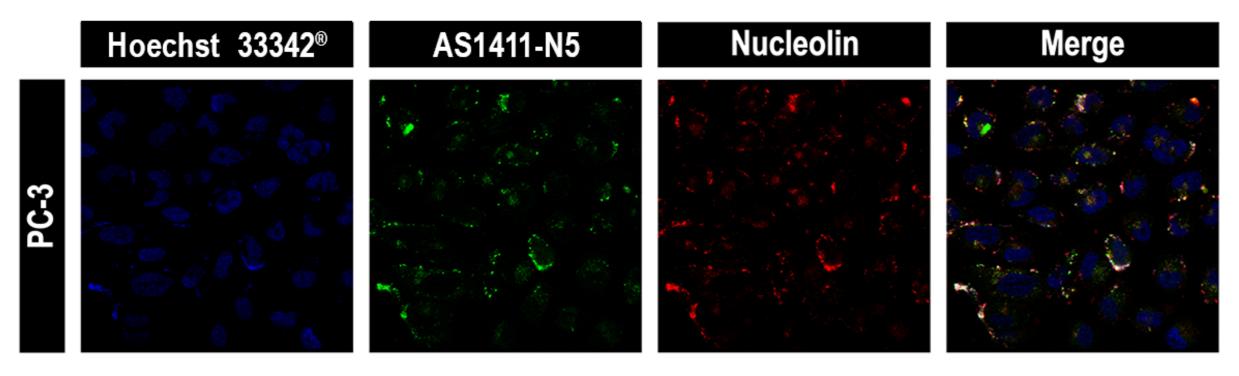


Fig. 2 - Confocal Laser Scanning Microscopy (CLSM) images of PC-3 cell line with AS1411-N5 at incubation of 24h Conclusions

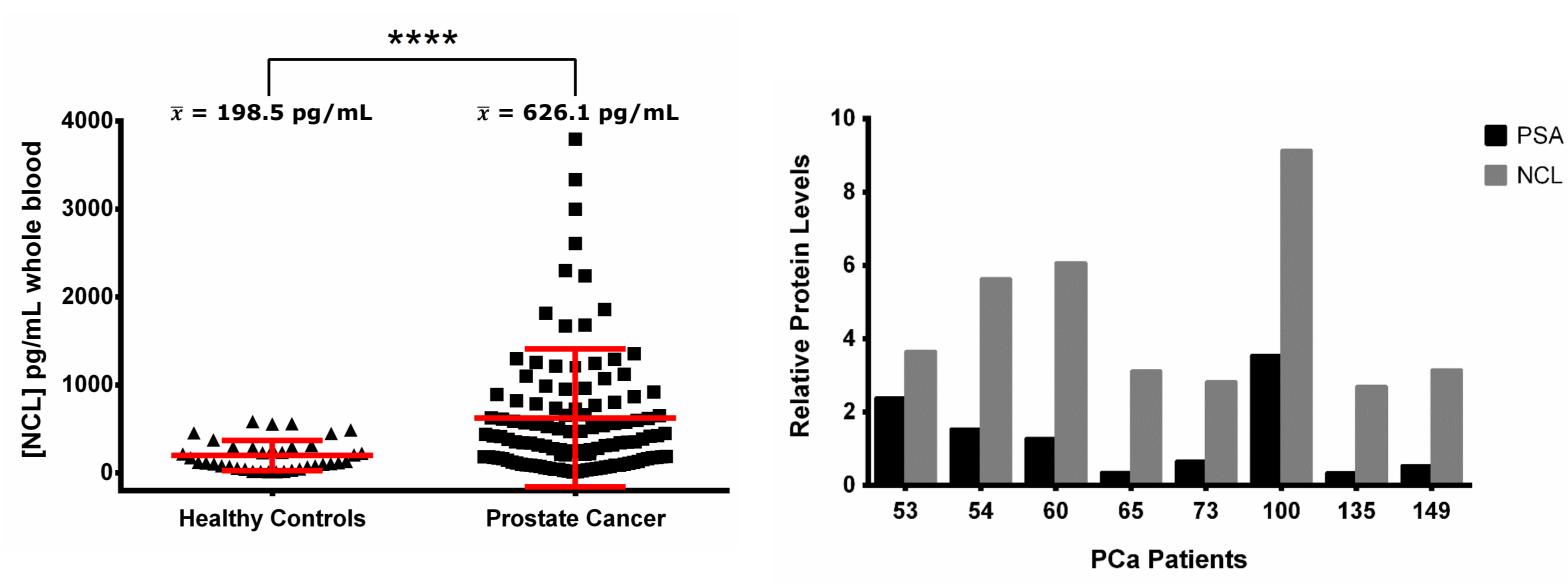


Fig. 3 – Scatter plot representation of ELISA in PCa patients (n=120) compared to healthy controls (n=38) (A) and relative PSA and NCL levels of selected patients in advanced PCa stages, obtained from the ratio between the protein levels of each patient and the healthy controls mean value.

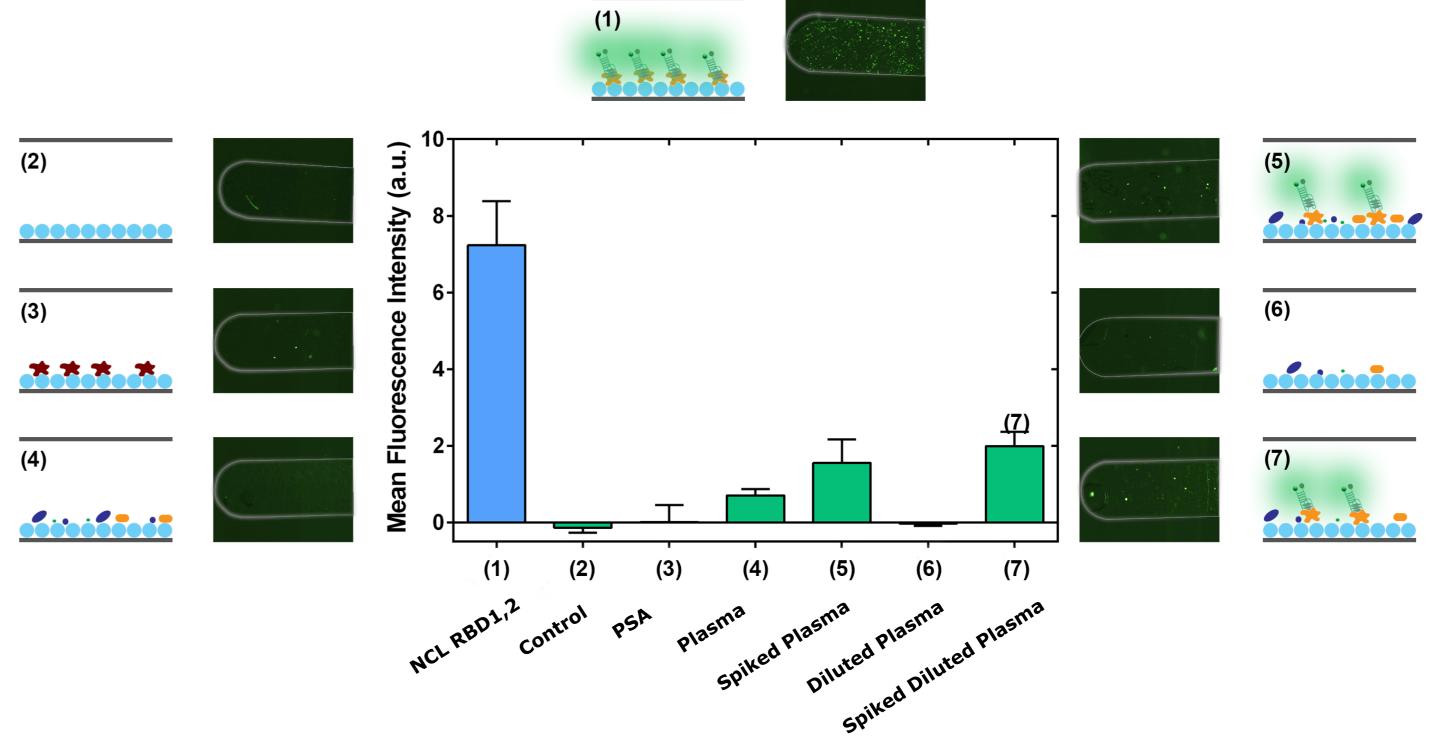


Fig. 4 – Selectivity studies for NCL measurements with AS1411-N5 by microfluidic approach in simple and complex samples

- AS1411-N5 forms a parallel G4 conformation and did not show polymorphism, suggesting a single conformation, as shown in CD and NMR spectra
- High affinity of AS1411-N5 to NCL, as evidenced by  $K_D$  determination and co-localization evidenced in vitro assays;
- Higher NCL expression in PBMC cells of prostate cancer patients when compared to healthy volunteers;
- Higher NCL levels in advanced PCa patients suggesting that NCL quantification can be used as an alternative prognosis marker;
- AS1411-N5 recognizes and detect specifically NCL protein, even in complex samples;
- AS1411-N5 revealed to be a potential candidate to NCL detection in blood samples using a suitable microfluidic system.