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Targeted Nanoparticles for Imaging and Treatment of Prostate Cancer Metastases in Lymph Nodes

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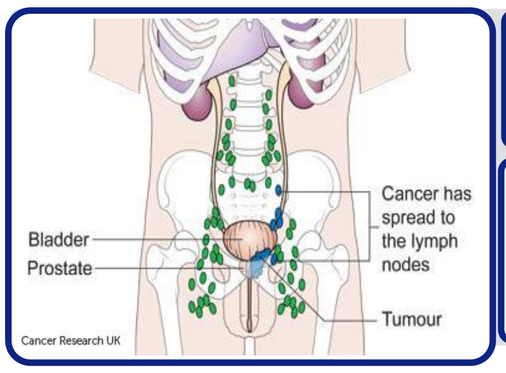


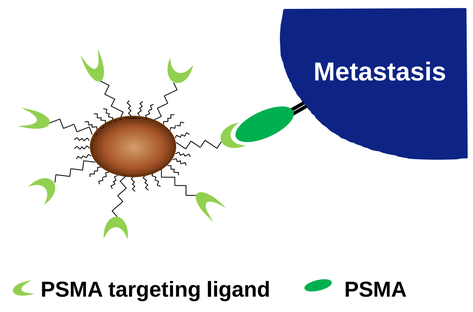
> "Prostate cancer most commonly spreads to lymph nodes in other parts of the body or bone." Cancer Research UK

Early detection of **metastatic spread** represents a critical step in **cancer staging** and consequent treatment planning. Removal of lymph nodes for staging often leads to lymphedema (build-up of lymph fluid due to lack of drainage).

CHALLENGE

OUR APPROACH

We aim to develop **nanoparticles** targeting **prostate cancer** cells for non-invasive **detection** and **treatment** of lymph node **metastases.**



Nanoparticle core

Iron oxide nanoparticles (IONs)

- Shorten T2 relaxation of surrounding tissue → **MRI contrast agent**
- Dissipate heat when exposed to an alternating magnetic field → **Hyperthermia**

Combining IONs ability for hyperthermia and MR imaging is highly valuable for **theranostic** (therapy and diagnostic) applications.

The IONs are coated with different biocompatible small molecules.

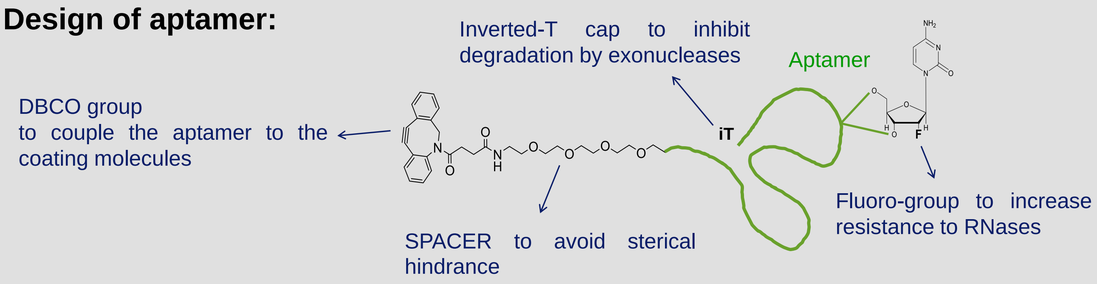
Targeting ligands

The coated IONs are then functionalized with different ligands [1-2]:

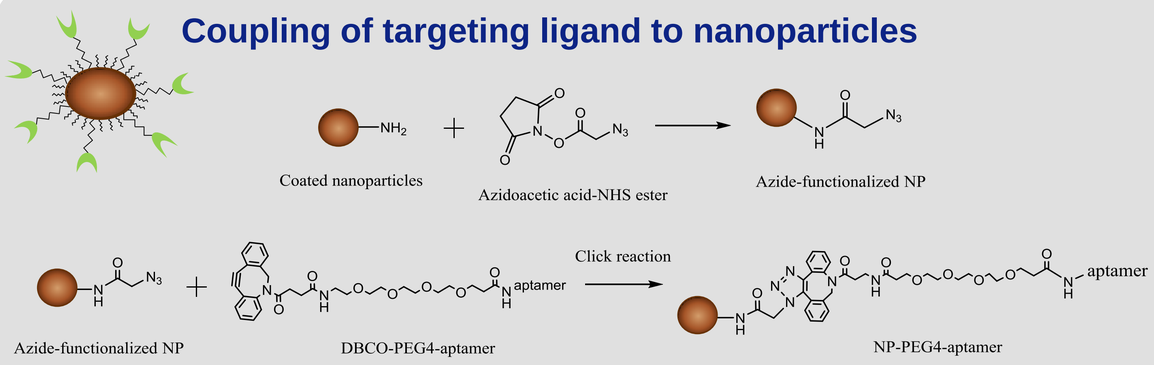
Small molecule	**Aptamer**	Antibody
320 Da	18000 Da	150000 Da

Aptamers are short segments of DNA or RNA binding to a specific target. Their smaller size compared to antibodies, which allows **faster target recognition**, and **higher binding affinity** compared to small molecules [3] make them interesting for specific targeting:

Design of aptamer:



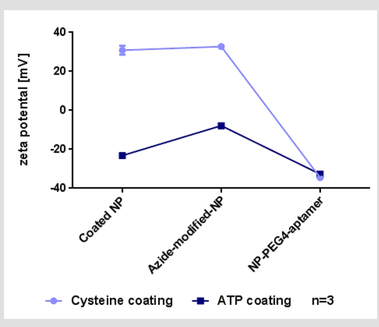
Coupling of targeting ligand to nanoparticles



Cu-free click chemistry

- ✓ highly selective and efficient
- ✓ mild reaction conditions (aqueous medium, RT)

With a zeta potential of ~ -30 mV and a **size** of **less than 100 nm**, aptamer-functionalized IONs show **promising poperties** for lymph node targeting.



Zeta potential after each reaction step

Conclusions and Outlook

- ✓ Aptamer specifically binds PSMA+ cancer cells
- ✓ Aptamer has affinity for a rat cancer cell line that can develop prostate cancer metastases *in-vivo*
- ✓ Successful functionalization of IONs with a PSMA-targeting aptamer
  - Nanoparticles are also functionalized with a PSMA-targeting small molecule and antibody

Nanoparticles' binding ability and selectivity are now compared *in-vitro.*

Target

The **prostate specific membrane antigen** (PSMA) is a transmembrane receptor highly overexpressed in prostate cancer.

Targeting PSMA – *In-vitro* studies

Fluorescence microscopy imaging: The **aptamer** is binding to **PSMA-positive LNCaP cells** (human prostate cancer cells from lymph node metastasis) and not to **PSMA-negative PC3 cells** (human prostate cancer cells):

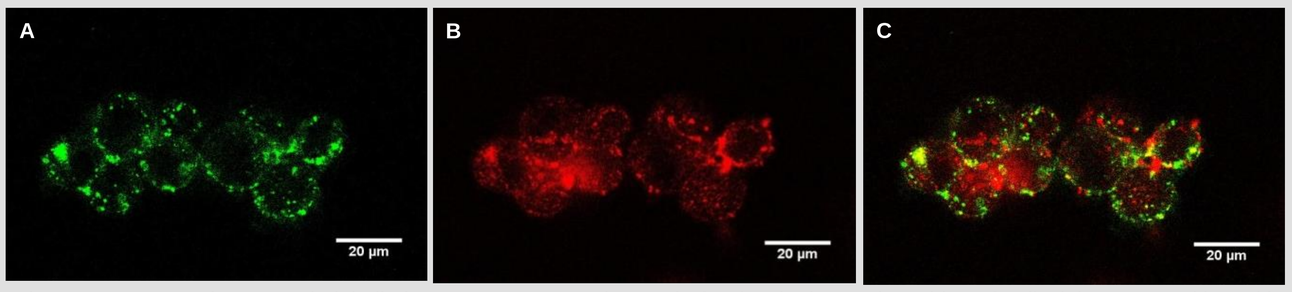


*Figure 1: **LNCaP**: Fluorescently labelled aptamer-Cy5 was detected after incubation and washing of cells (B). Superposition with image A (fluorescently stained cell membrane) shows colocalisation of the aptamer and the cell membrane.*

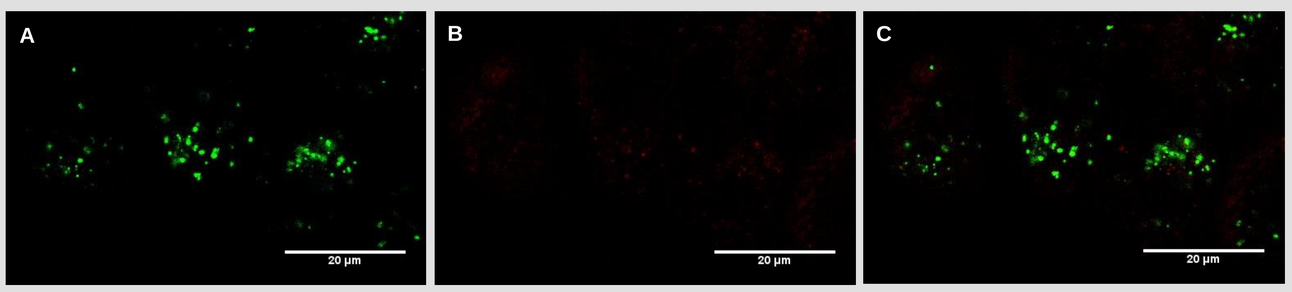


*Figure 2: **PC3**: Beside the fluorescently stained cell membranes (A), almost no aptamer-Cy5 was detected (B, superposed: C) for PSMA-negative PC3 cells.*

Testing of cell line for *in-vivo* studies

The aptamer is also binding to and internalized in the metastatic rat prostate cancer cells **MAT-LyLu** (PSMA status unknown) which are of high interest as ***in-vivo*** **model** for detection of lymph node metastases (confocal scanning laser microscopy):

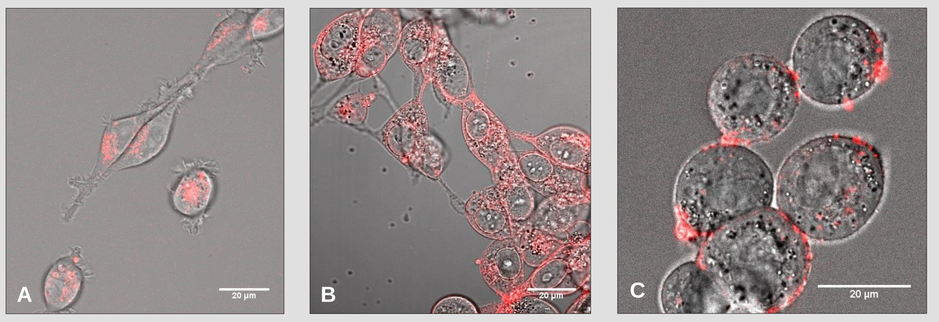
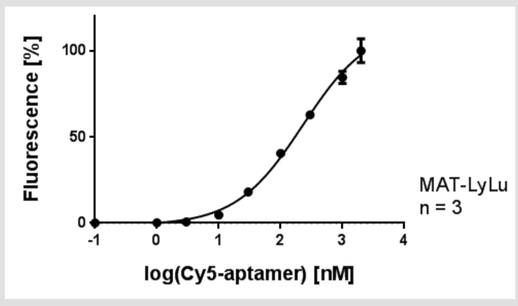


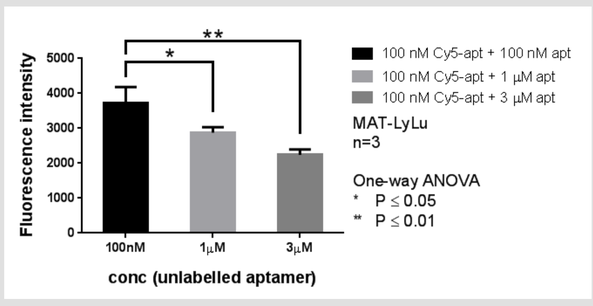
Figure 3: Z-stacks show internalization of aptamer-Cy5 in MAT-LyLu cells (A after 1h, B after 4h) at 37° C.

C: LNCaP cells with aptamer-Cy5 bound on the surface and slightly internalized at 20° C.

Binding of the aptamer to a specific binding site on the surface of MAT-LyLu cells was confirmed by **saturation binding** (K_D=105.8 nM) and **competitive binding** assays:



Saturation binding assay: Incubation of MAT-LyLu cells with increasing concentrations of Cy5-labelled aptamer



Competitive binding assay: Incubation of MAT-LyLu cells with Cy5-labelled aptamer and increasing concentrations of unlabelled aptamer.

ACKNOWLEDGMENTS

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REFERENCES

[1] Lupold SE et al. Cancer Res 2002; 62: 4029-33
[2] Maresca KP et al. J Med Chem 2009; 52: 347-57
[3] Keefe AD et al. Nat Rev Drug Discov 2010; 9: 537-50