DNA-driven Nanoparticle assemblies tailored to early detection of Prostate Cancer

Giulia Matteoli

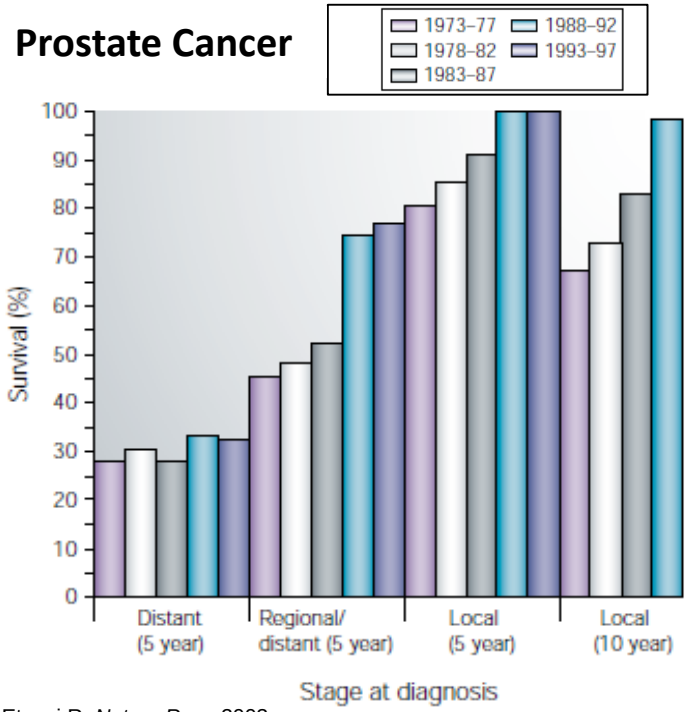
20/10/2020

Internal Supervisor (@NEST): Prof. S. Luin

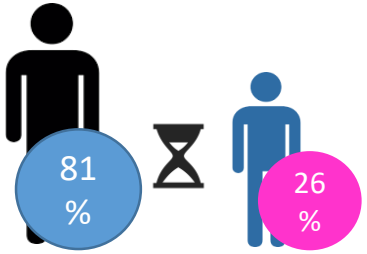
External Supervisor (@FPS): Dr. G. Signore

Importance of early cancer detection and limitations in current diagnostic

Prostate Cancer



Etzoni R. *Nature Rev.*, 2003



Early diagnosis (STAGE I+STAGE II)

Late diagnosis (STAGE III+STAGE IV)

Cancer research UK, 2013

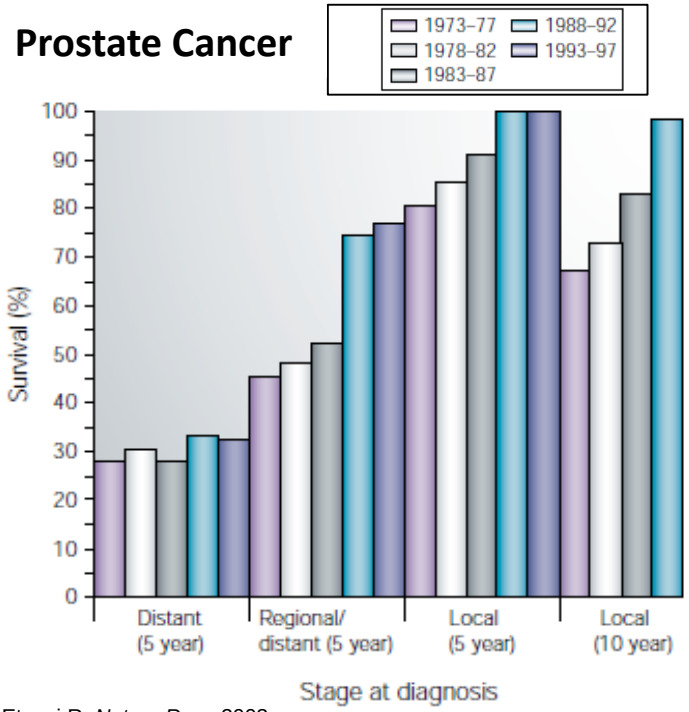
Limitation in current diagnostics

Chemical equilibrium
Sensitivity depends on K_{eq} independent by volume

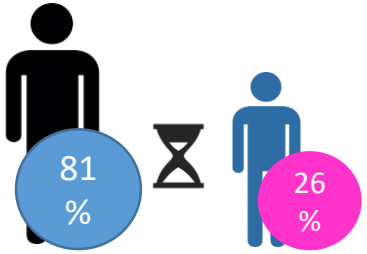
Irreversible recognition
Sensitivity depends also on absolute number of target molecule and is dependent by volume

Importance of early cancer detection and limitations in current diagnostic

Prostate Cancer



Etzoni R. *Nature Rev.*, 2003



Early diagnosis (STAGE I+STAGE II)

Late diagnosis (STAGE III+STAGE IV)

Cancer research UK, 2013

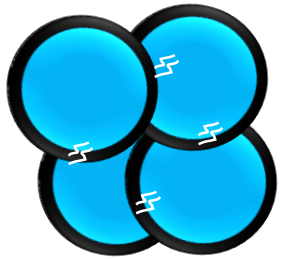
Limitation in current diagnostics

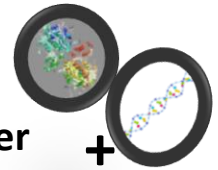
Chemical equilibrium
Sensitivity depends on K_{eq} independent by volume

Irreversible recognition
Sensitivity depends also on absolute number of target molecule and is dependent by volume

The sensor working principle

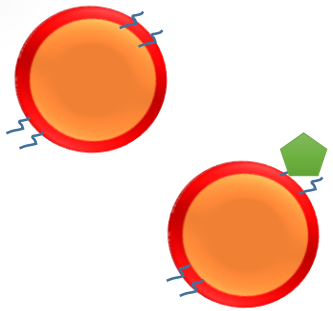
DNA-driven Nanoparticle assembly





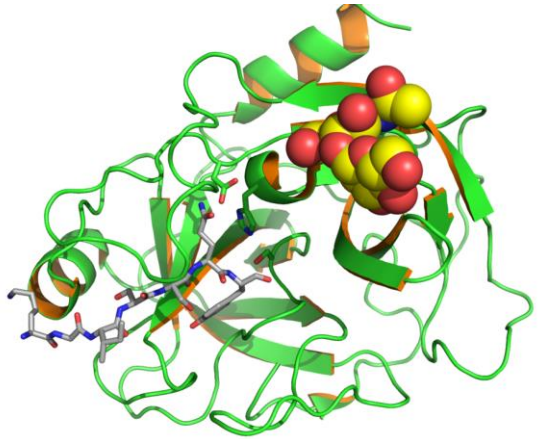
biomarker +

Biomolecule induced disaggregation (irreversible step)



Prostate Cancer, target of choice

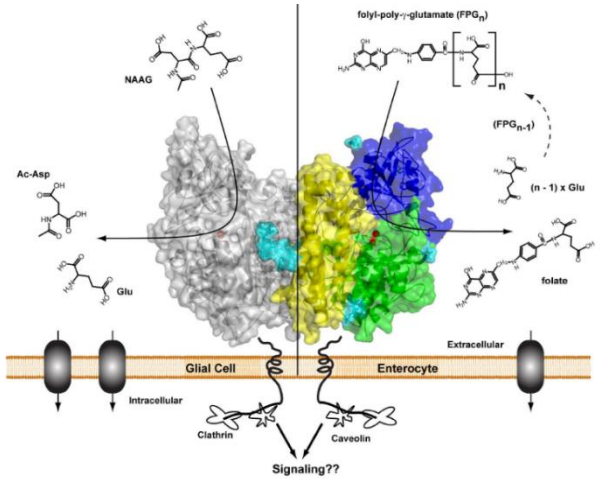
Prostate Specific Antigen (PSA)



PSA is a **secreted protein**

Serum Level
zero in non-recurrent resected patients

Prostate Specific Membrane Antigen (PSMA)

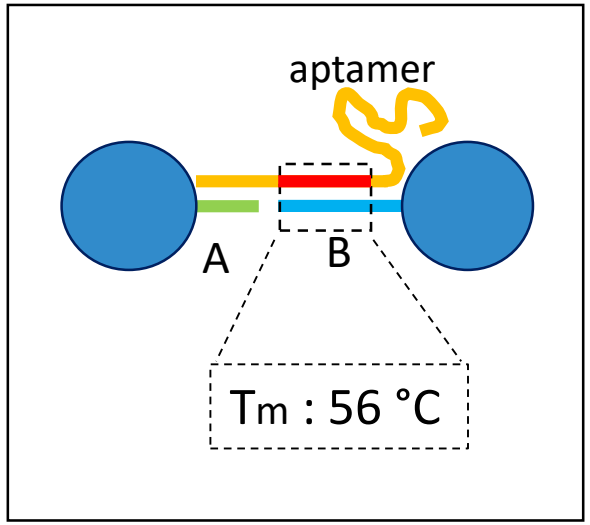


PSMA is enriched in PCa tissue, **exosomes** and in aggressive PCa

Serum level
- 6 nM patients with PC
- 2 nM physiologic level
- 50 pM in resected patients

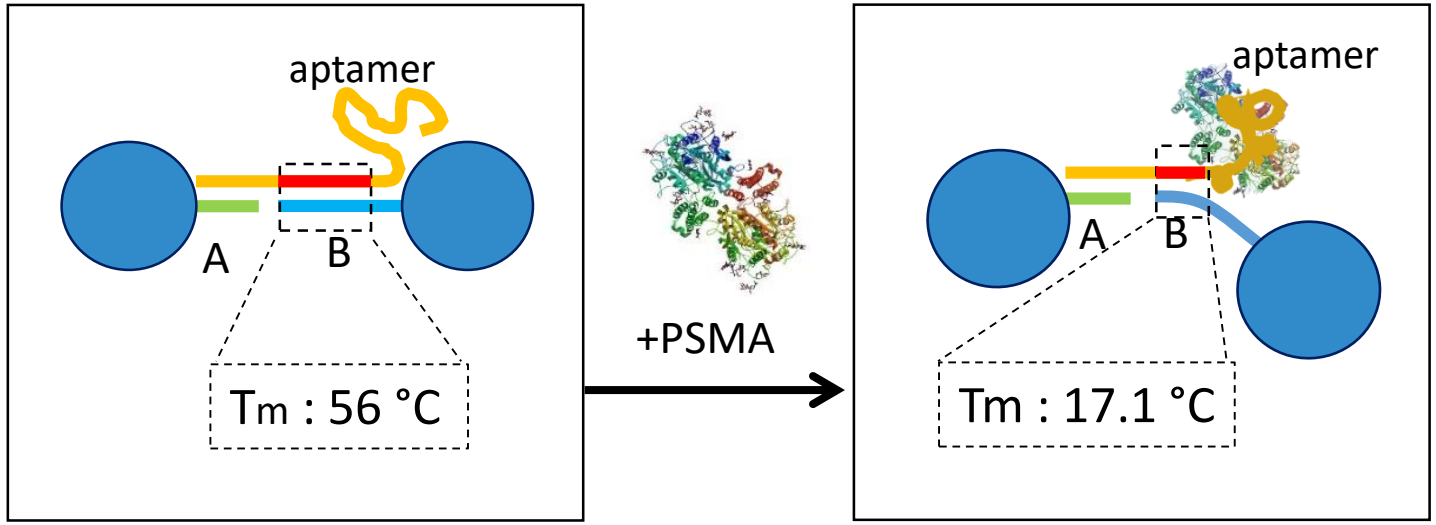
Exosomes (urine)
PSMA positive exosomes detected only in PCa urine samples

Nanoparticle aggregates: the working principle



NPs are represented with a single strand per particle to illustrate the dissassembly principle

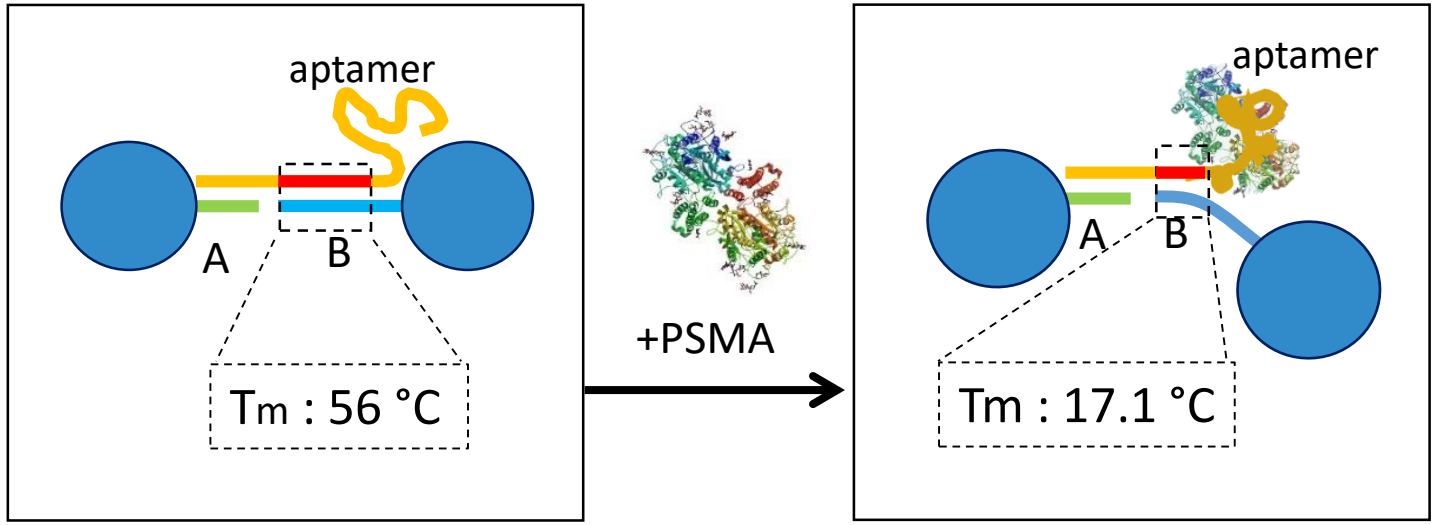
Nanoparticle aggregates: the working principle



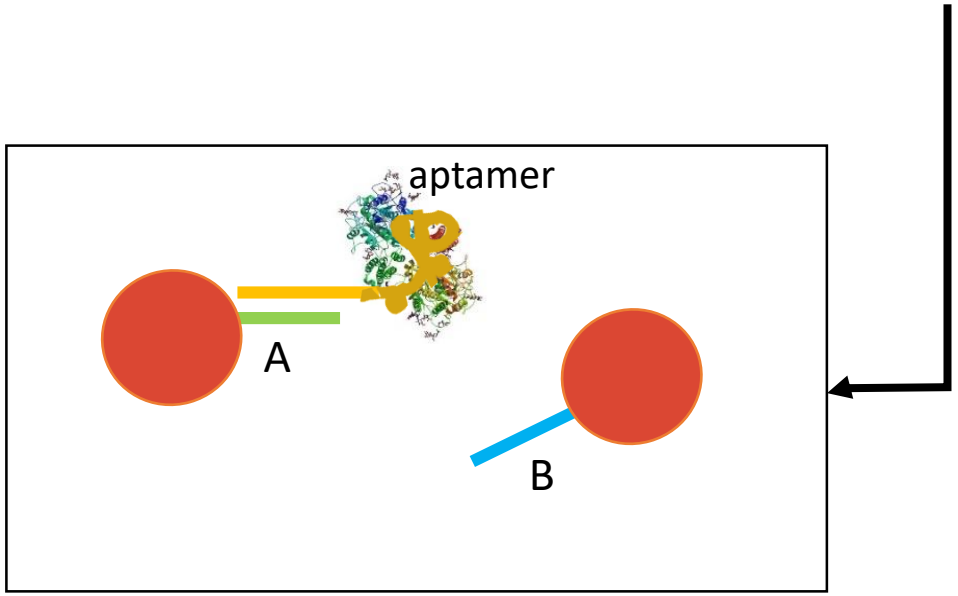
Aptamer recognition of the protein lowers down melting temperature between B strand and red portion of aptameric strand

NPs are represented with a single strand per particle to illustrate the dissassembly principle

Nanoparticle aggregates: the working principle



Aptamer recognition of the protein lowers down melting temperature between B strand and red portion of aptameric strand

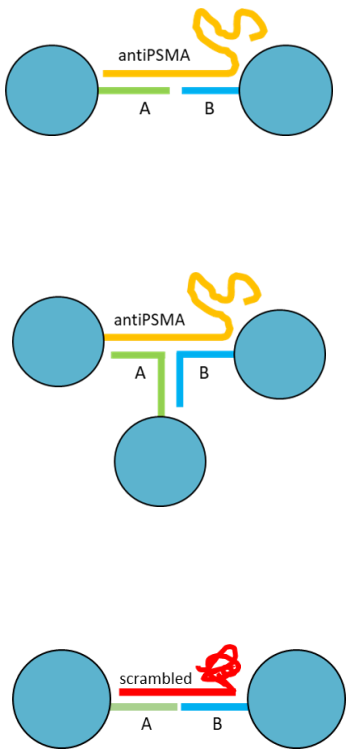


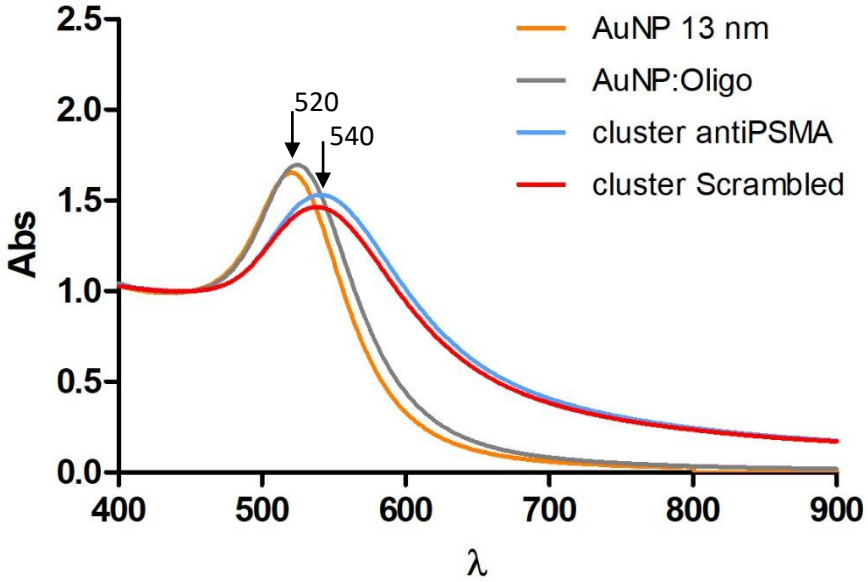
Disassembly of nanoparticles

NPs are represented with a single strand per particle to illustrate the dissassembly principle

PSMA responsive Gold NanoClusters

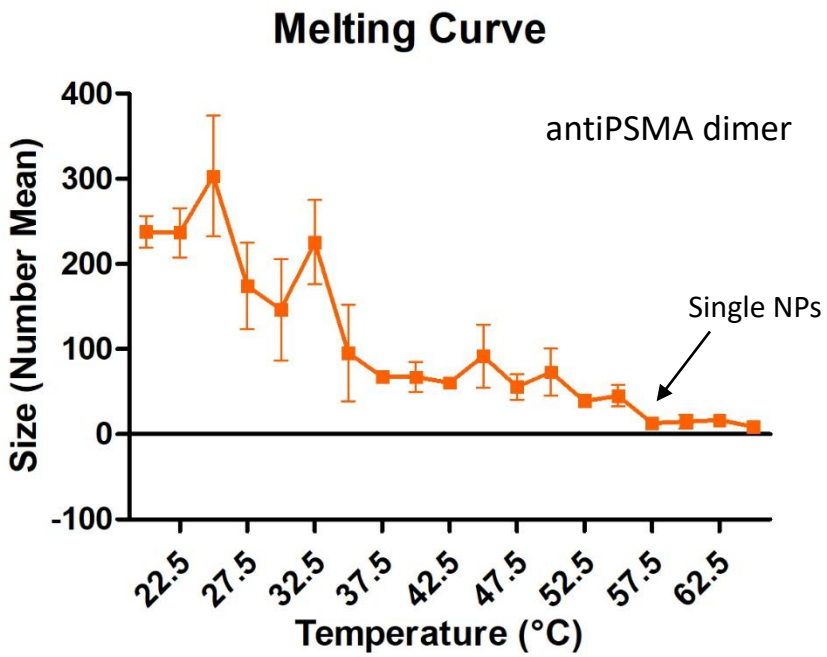
Cluster assembly





	Number mean	Number st. Dev
AuNP 13 nm	12.96	0.61
AuNP 13 nm:Oligo	31.63	0.15
Cluster antiPSMA	431.90	55.89
Cluster ScrambledPSMA	692.90	54.14

Melting Temperatures

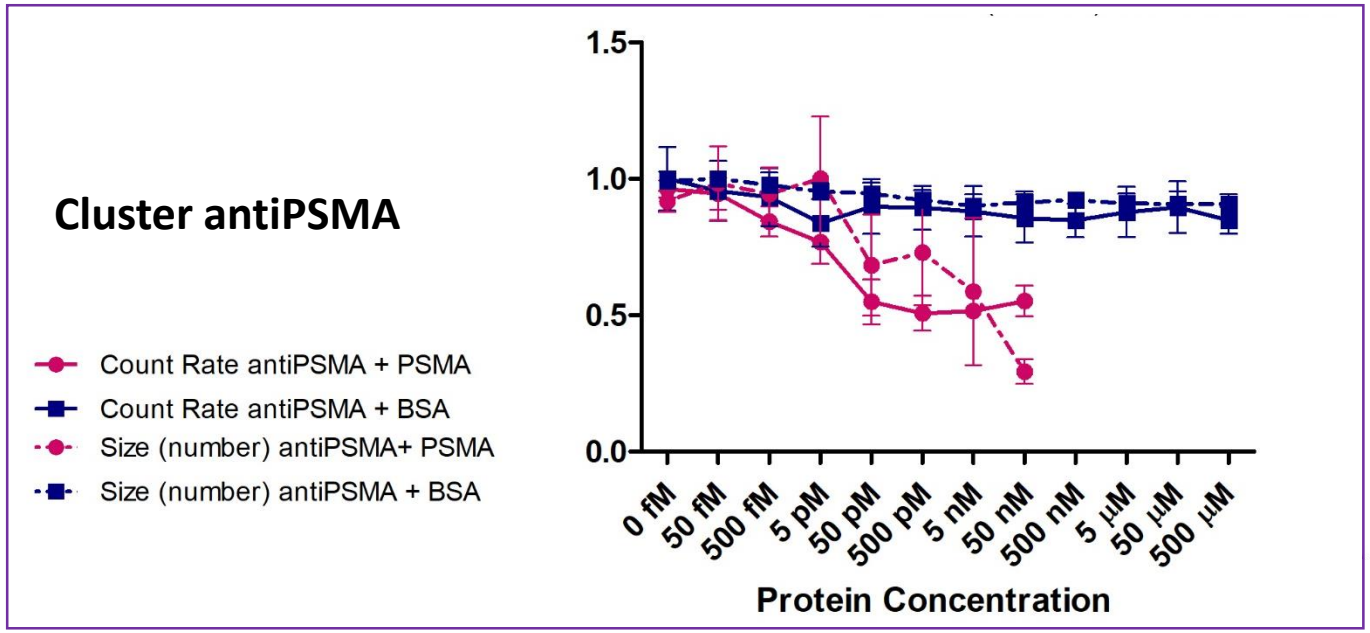


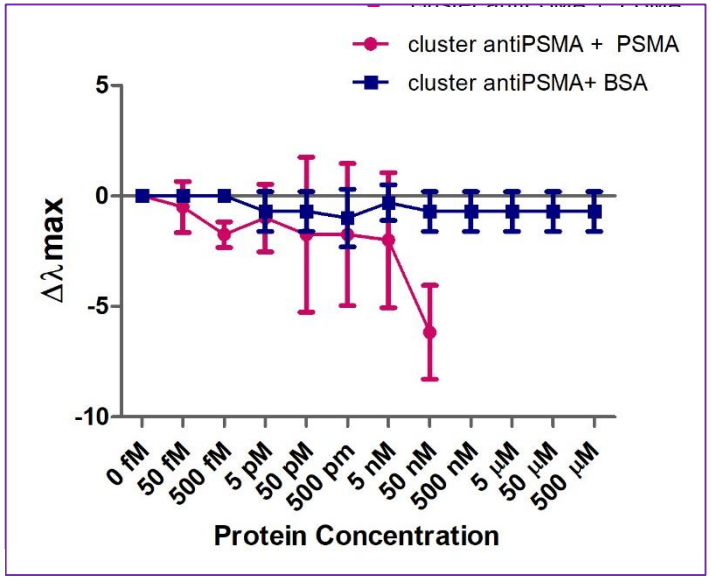
Sequence	*Tmelt In silico (DINAMelt)*	*Tmelt In vitro (260 nm abs)*	*Tmelt Nanoscluster (DLS)*
antiPSMA dimer	56 °C	53 °C	52.5°C
antiPSMA trimer	46.5°C	41 °C	35°C
Scrambled	56°C	58°C	50°C

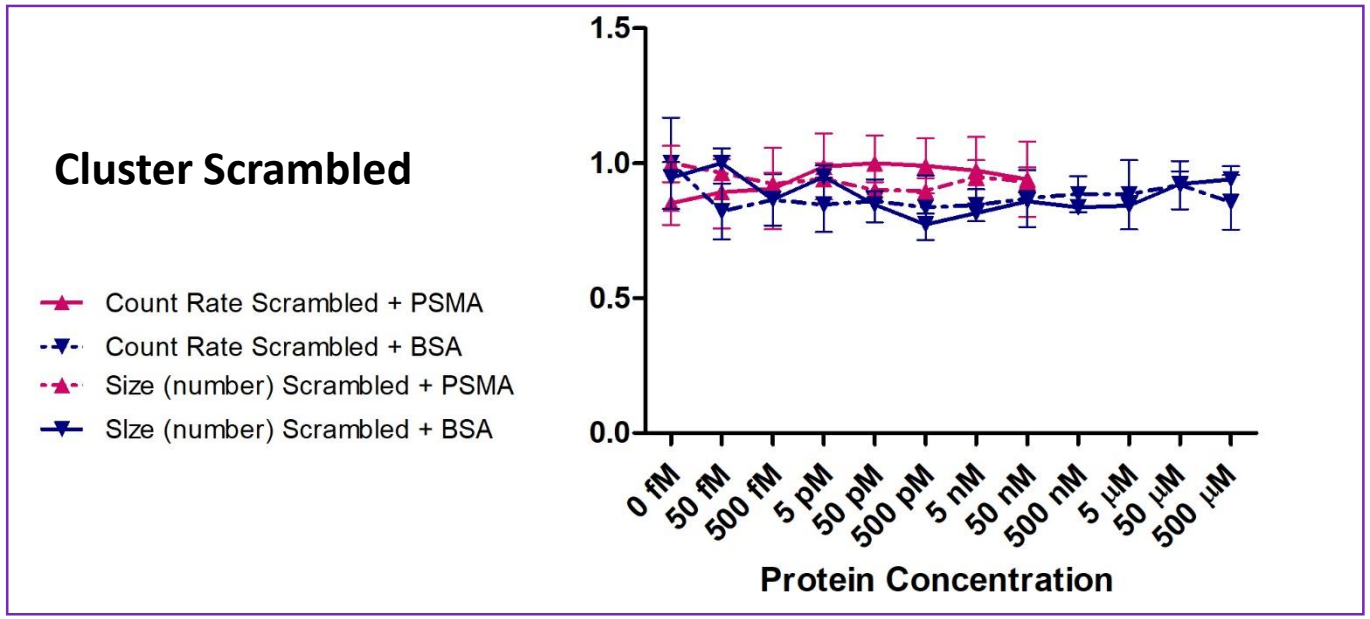
Cluster sensing response to purified protein

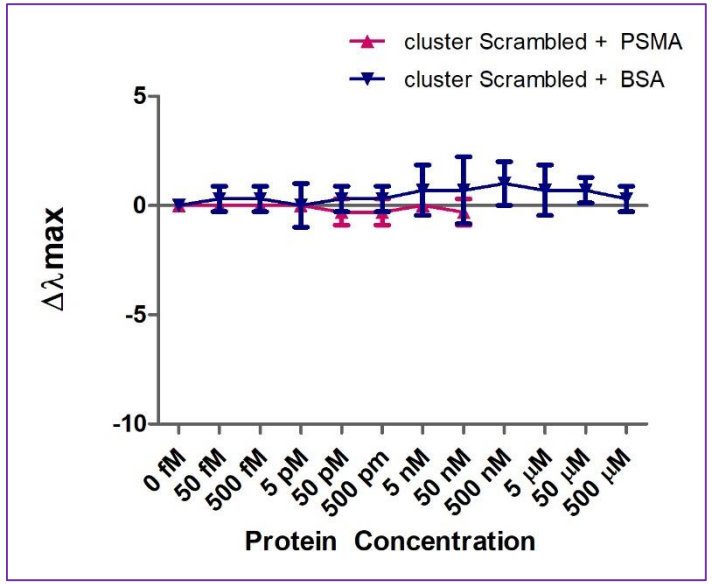
DLS size measurment (Count Rate and Size are normalized)

UV-Vis (plasmon peak variation)

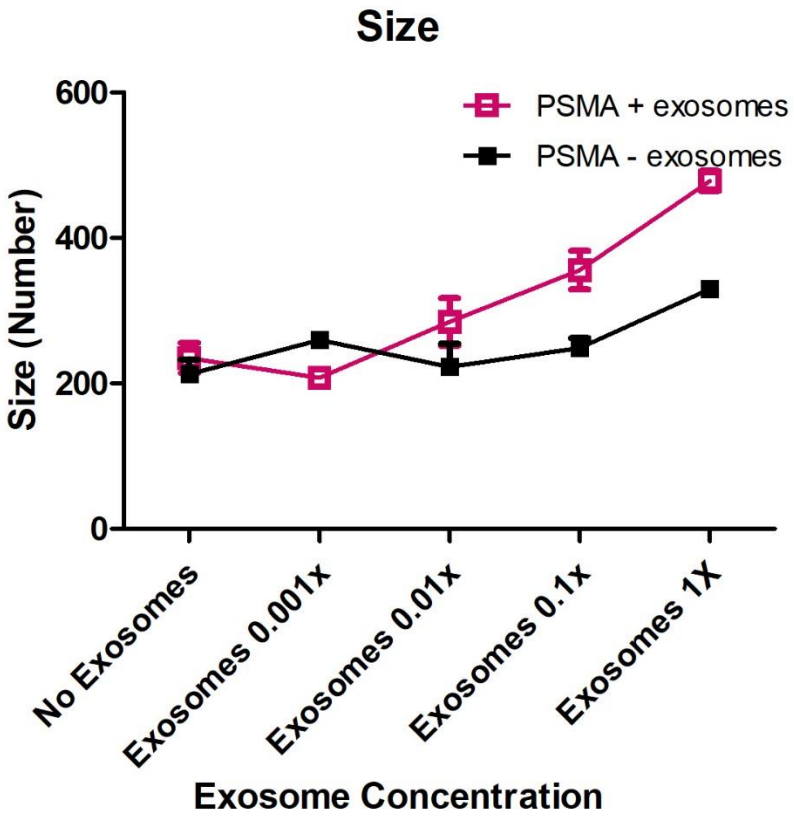


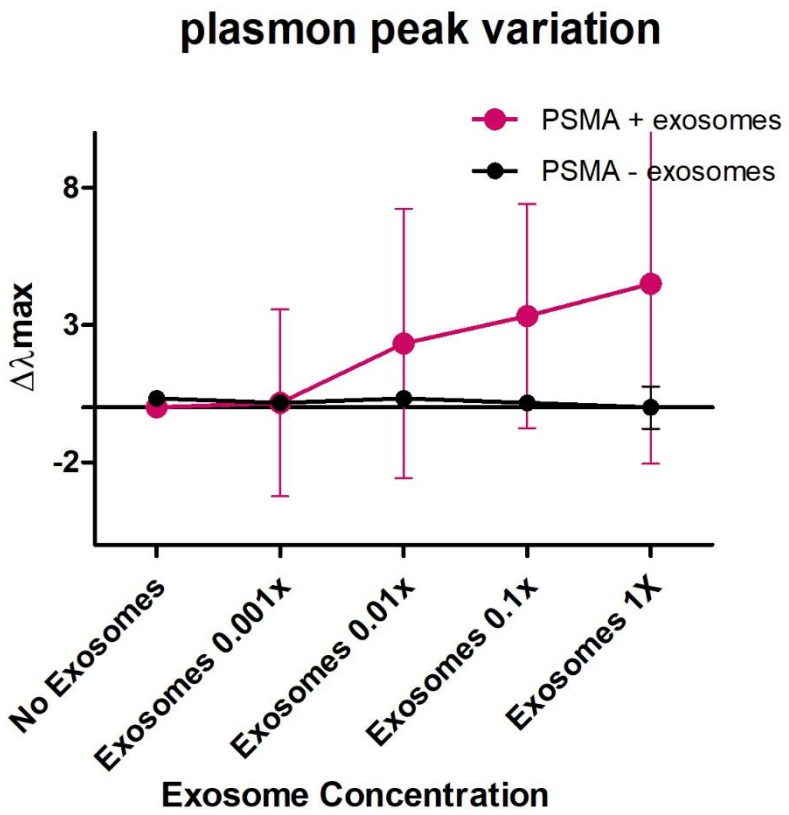


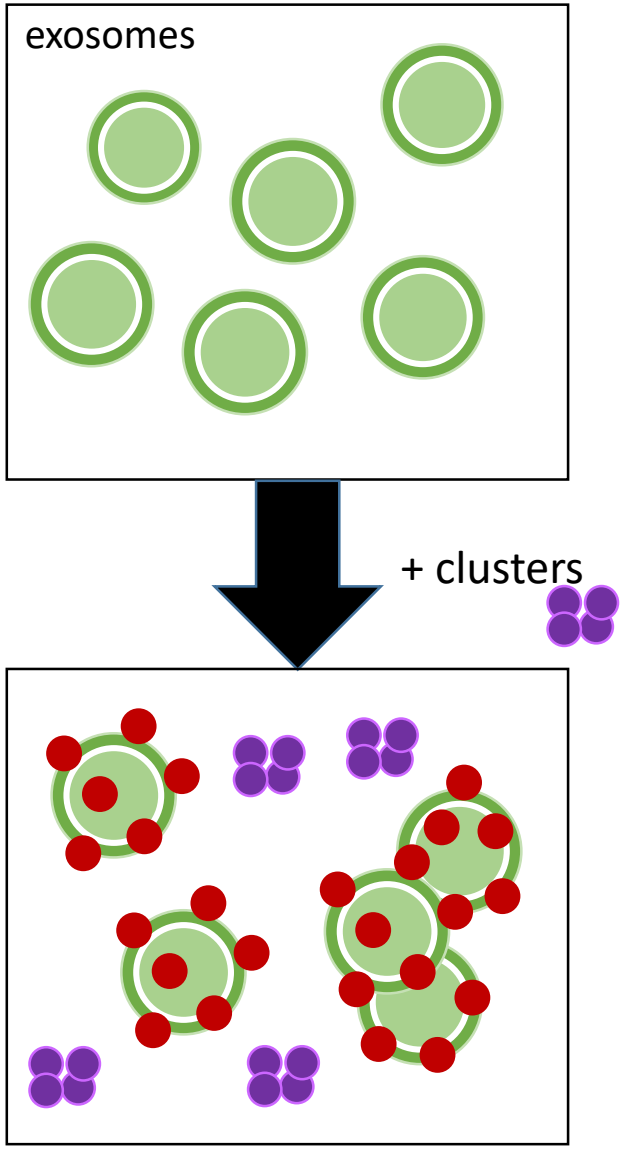




PSMA responsive Clusters were incubated with LNCaP and PC3 derived exosomes

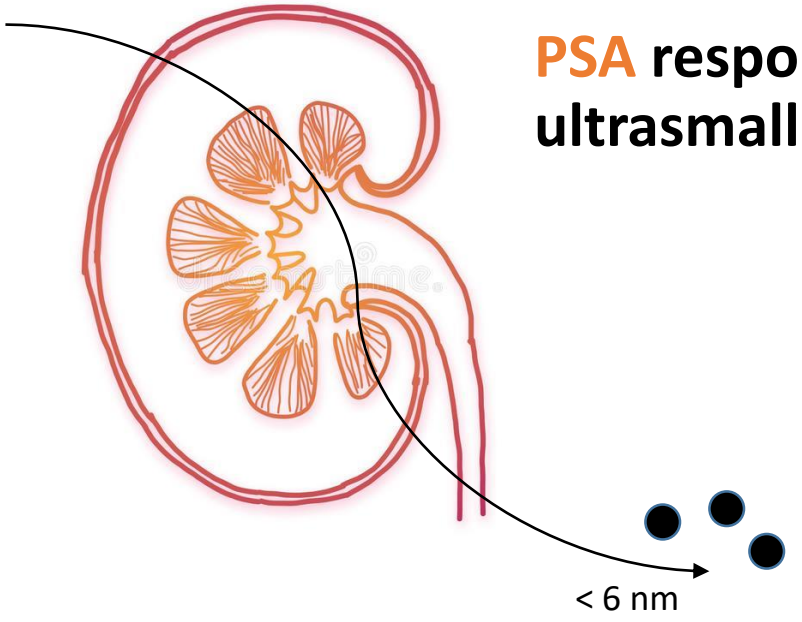


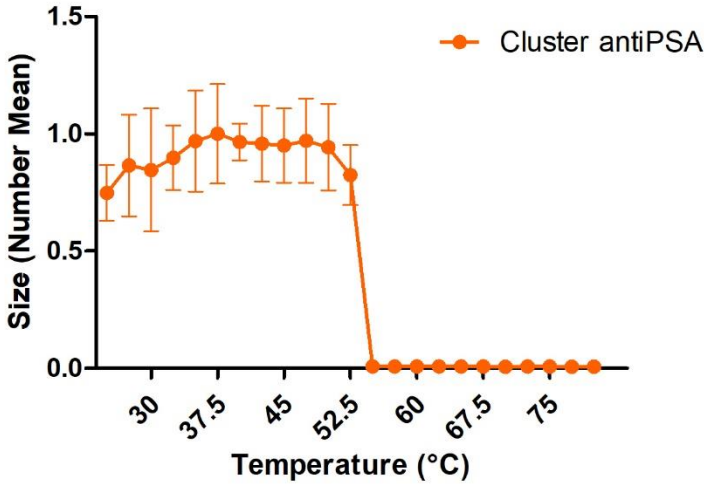


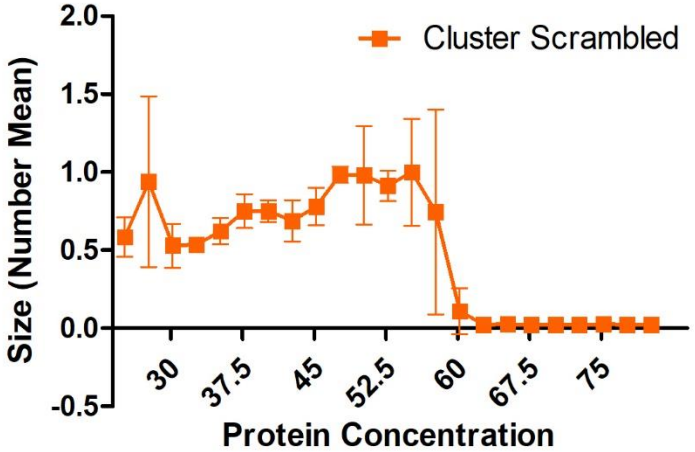


Exosomes 1x (stock solution)= 10^{12} exosomes/ml

PSA responsive Clusters, ultrasmall and renal clearable







Size normalized

- ✓ Melting temperatures
- ✓ Chemico-physical caracherisation of Clusters

- ❑ *In cuvette* PSA sensing response

Sequence	*Tmelt In silico (DINAMelt)*	*Tmelt In vitro (260 nm abs)*	*Tmelt Nanocluster (DLS)*
antiPSA	55.6	58 °C	55°C
Scrambled	55.6°C	58°C	57.5°C

	Diameter (number) (nm)	St. Dev.
AuNP 2 nm	6.1	0.4
AuNP 2 nm: oligo	13.2	1.7
Scrambled cluster	263.9	125.2
aPSA cluster	151.7	64.6

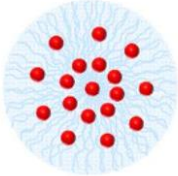
Last year prospect

✓ **PSMA sensing with 13 nm Gold NPs clusters**

○ **Ongoing experiments**

Implementation of the sequences on Ultrasmall (Clearable) Nanostructures

- 2 nm Gold NPs
- Polymeric NPs



○ **Writing thesis**

This work has been presented with an oral presentation at ANNIC2019

In Preparation

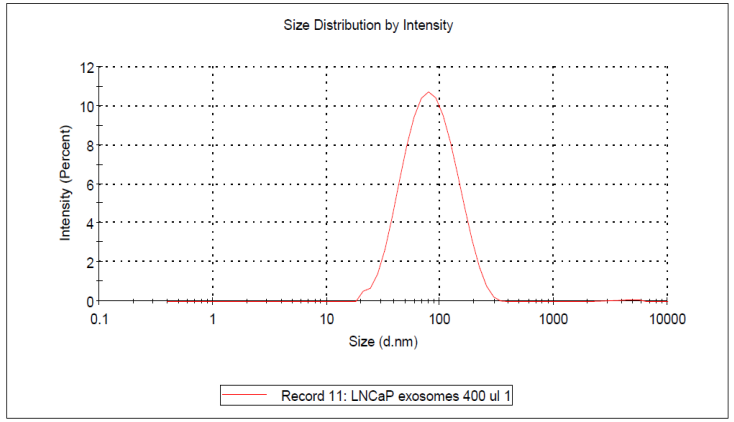
Review: "Tumor early detection and localization: an assessment of the role of nanomedicine"

Paper: " Prostate Cancer early detection with PSMA responsive NanoClusters " (submission in December 2020)

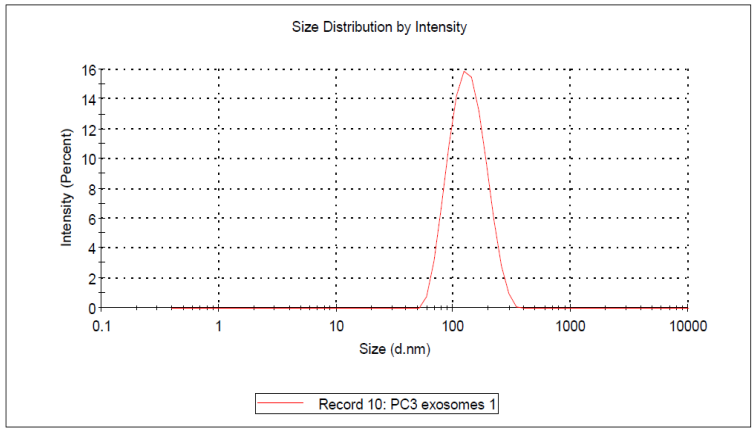
PCa Exosome Isolation and Characterisation

Exosome characterisation DLS

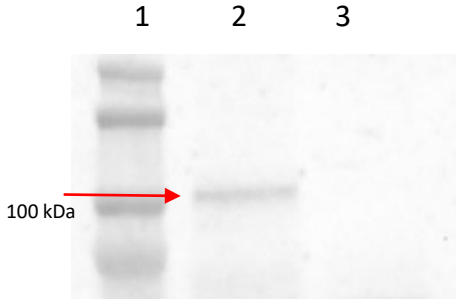
LNCaP exosomes (PSMA



PC-3 exosomes

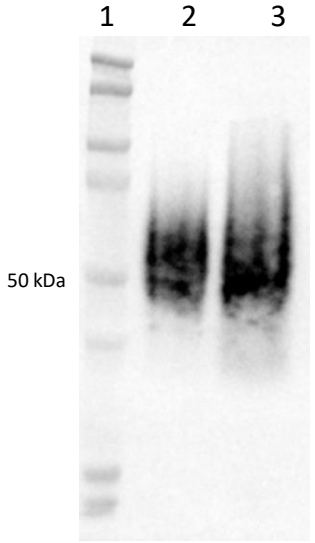


Exosome characterisation WESTERN BLOT



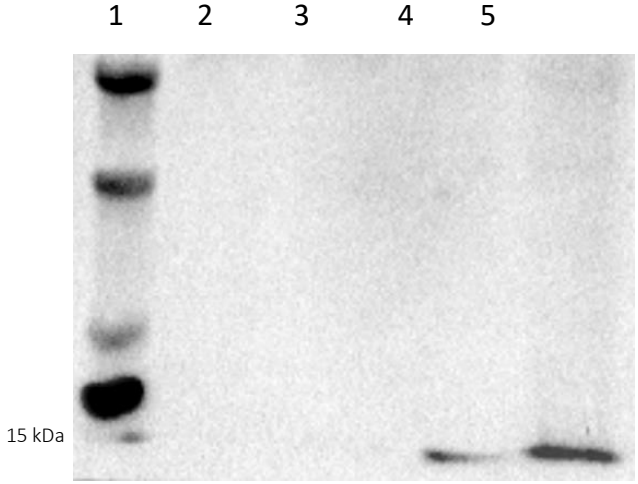
antiPSMA

1: Ladder
2: LNCaP exosome
3: PC-3 exosome



antiCD63

1: Ladder
2: LNCaP exosome
3: PC-3 exosome



antiTOM20

1. Ladder
2. PC-3 exosome
3. LNCap exosome
4. PC-3 cells
5. LNCaP cells