Plastic antibodies as innovative tool for cancer imaging

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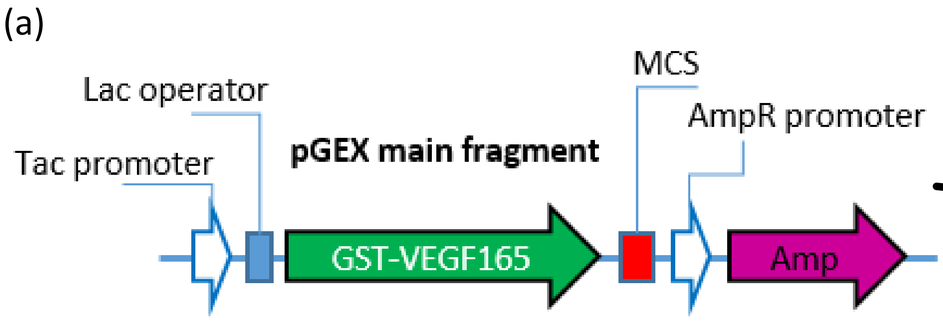
Abstract In recent years the technology for nano-molecularly imprinted polymers (n-MIPs) production has advanced significantly such that their binding affinity and specificity are now comparable to that of monoclonal antibodies, and without the activation of the immune system and related side effects. n-MIPs may be functionalised with quantum dots (QDs), to develop a multifunctional nanostructure characterized by a targeting motif and emitting in near infra-red (NIR). n-MIP embedding QDs nanostructures (QD-MIPs) are imprinted against homemade recombinant human vascular endothelial growth factor (hVEGF) and two of its epitopes (epi1 and epi2). This study shows an innovative hybrid nanostructure that emits in NIR and that can be exploited for cancer recognition and *in vivo* imaging. Ultimately, the QD-MIPs will be validated in a zebrafish model of overexpressing hVEGF cancer cell xenografts.

hVEGF production

E. coli BL21 were transfected with the plasmid pGEX (a) (optimised in Pisa), for GST-tagged VEGF165, and the restistance to ampicilline (AmpR).

(a)



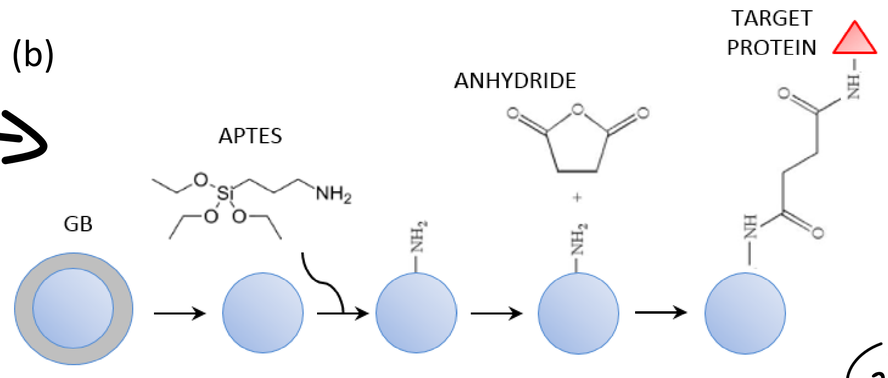
Recombinant hVEGF protein sequence inserted in pGEX plasmid

Positively transfected cells were isolated and induced to produce hVEGF. At the end of the experiment, about 2mg of hVEGF are purified by using a GSH-matrix.

Glass Beads (GBs) functionalisation

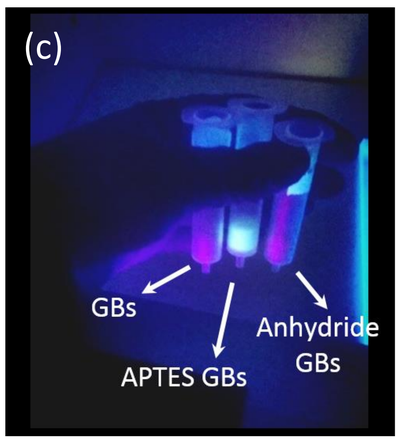
Glass beads (50-106um diameter) were deprived of the hydophobic coating and functionalised with (3-Aminopropyl)triethoxysilane (APTES) and, subsequently with glutaric anhydride (b),(c). Finally, COOH groups were activated with EDC/NHS to allow immobilising hVEGF.

(b)



Steps for GBs funuctionalisation

(c)

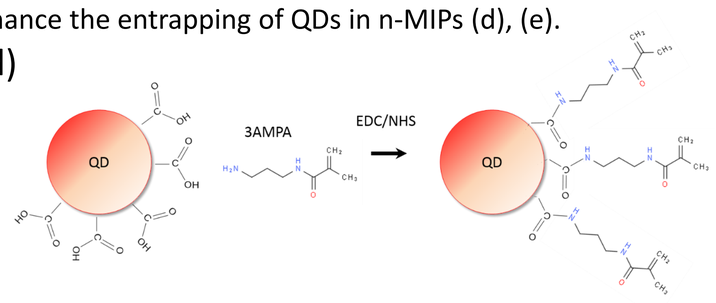


Dansyl chloride fluorescence assay

3AMPA functionalisation of QDs

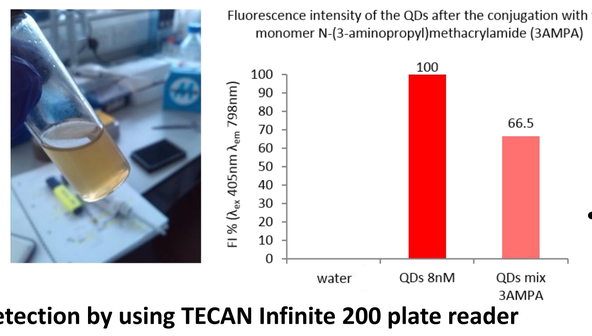
Carboxyl functionalised QDs were coupled with the functional monomer N-(3-Aminopropyl)methacrylamide (3AMPA) in order to enhance the entrapping of QDs in n-MIPs (d), (e).

(d)



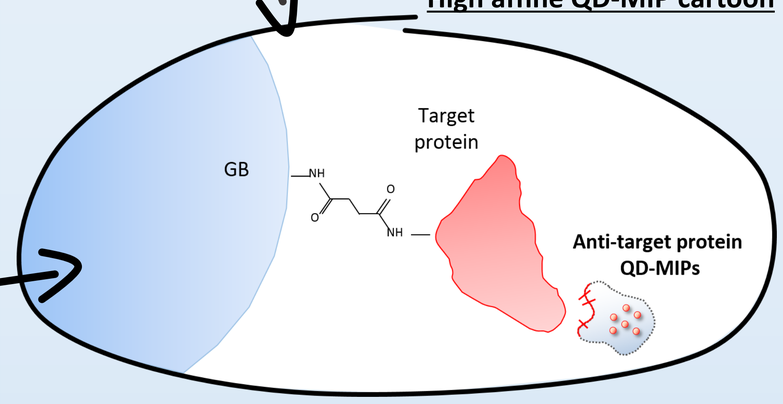
3AMPA functionalisation of commercial COOH-QDs

(e)



FI detection by using TECAN Infinite 200 plate reader

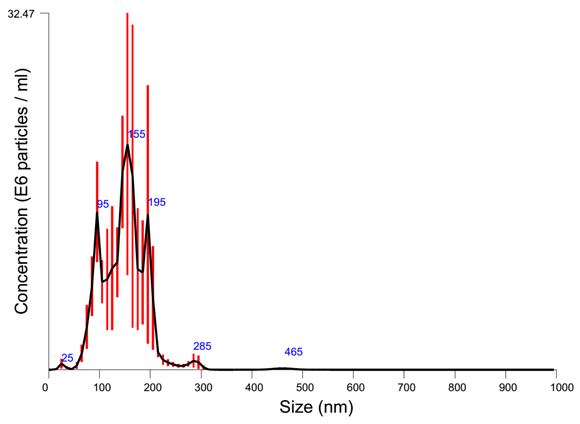
High affine QD-MIP cartoon



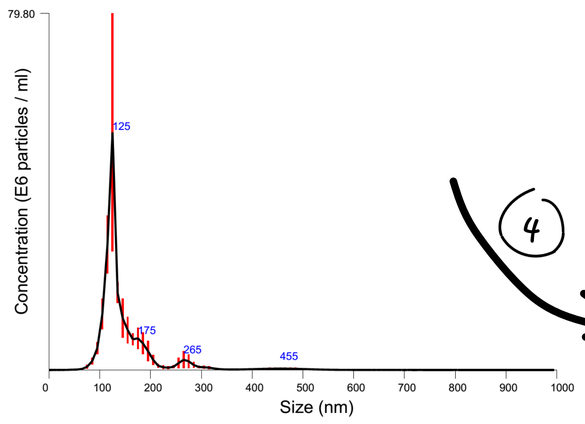
MIPs and QD-MIPs production

Evaluation of size and concentration of (g) MIPs and (h) QD-MIPs. The incorporation of QDs during the imprinting process do not affect neither the size (from 75 to 175nm diameter) nor the concentration of the n-MIPs (10^6 particle/ml).

(g)



(h)

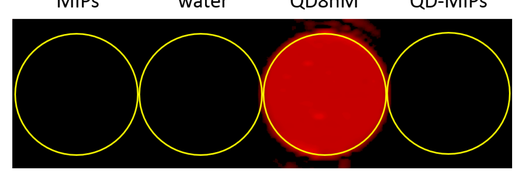


Size and concentration of the nanostructure were measured with the NANOSIGHT NS300 Malvern

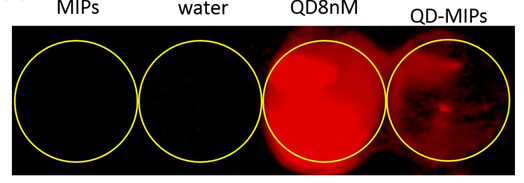
QDs-MIPs Fluorescence intensity (FI) detection

Evaluation of the FI of (i) QD-MIPs and (l) 3AMPA-functionalised QDs-MIPs.
The functionalisation with 3AMPA enhances the yield of QD embedded (m) in the n-MIPs during the polymerisation.

(i)

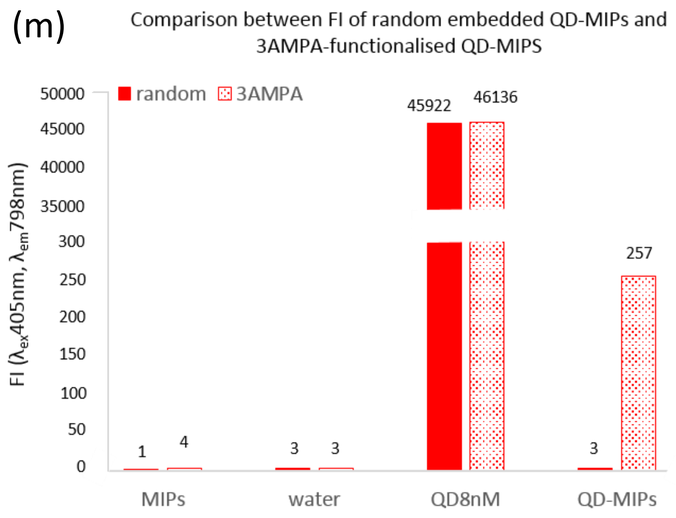


(l)



NIR signal detection by using LI-COR ODYSSEY

(m)



FI detection by using TECAN Infinite 200 plate reader

Conclusions and future perspectives

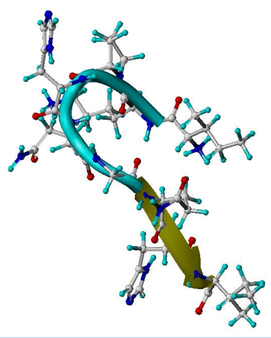
This study shows a new approach to cancer detection with the design of a «plastic antibody» coupled to monomer-functionalised NIR-QDs. By virtue of their properties, QD-MIPs represent an innovative and suitable tool for *in vivo* imaging.

What is next?

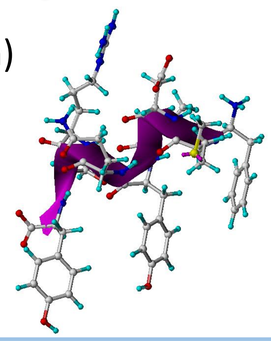
1. Optimise the polymerisation mixture composition in order to increase the signal of the QD-MIPs

2. Imprinting of (n) epi1 and (o) epi2 with an epitope-specific cocktail of monomers, chosen by running SYLBYL7.3 software and LEAPFROG algorithm

(m)



(n)



3. Cytotoxycity assay and recognition capability of QD-MIPs imprinted against hVEGF or epi1 or epi2 in a zebrafish model of overexpressing hVEGF cancer cell xenografts (p) (@University of Pisa)

(p)

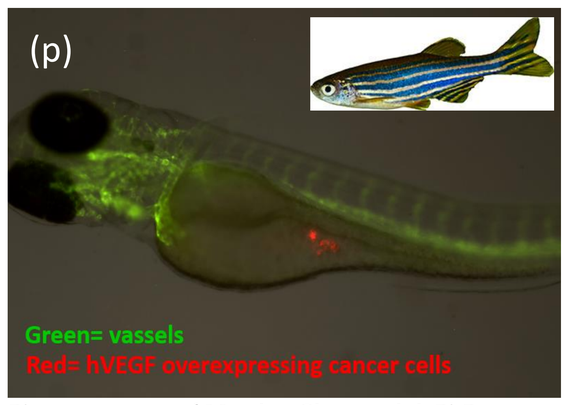


Photo courtesy of Martina Giannaccini, PhD

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Reference S. A. Piletsky et al. Molecularly imprinted polymers in clinical diagnostics-future potential and existing problems, *Med. Eng. Phys.*, vol. 28, no. 10, pp. 971–7, Dec. 2006.

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