**Supporting Information for:**

# *Quantum dot loaded liposomes as new fluorescent labels for immunoassay*

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*Synthesis of water-soluble CdSe/CdS/ZnS core/shell/shell QD*

CdSe/CdS/ZnS core/shell/shell QD were prepared according to Beloglazova et al. [41]. The obtained QD were hydrophobic and were transferred to aqueous solutions by covering with amphiphilic polymer. The polymer was synthesized from PMAO and Jeffamine M-1000 polyetheramine (molar ratio was 1:1). For QD encapsulation, QD and the synthesized amphiphilic polymer (molar ratio was ~ 1:100) were mixed in chloroform and stirred overnight at room temperature. Afterwards, chloroform was slowly evaporated under vacuum and aqueous KOH (pH 12) was added. The mixture was sonicated for 20 min, to obtain a clear solution, followed by the addition of chloroform/water (1:1) and centrifugation to separate the two phases. The excess of amphiphilic polymer was precipitated between the two phases and the aqueous layer was carefully taken. The aqueous solution of QD was concentrated using Amicon Ultra-15 centrifugal filter units with an Ultracell-100 membrane. The obtained hydrophilic QD were

stable in aqueous solutions at pH levels > 3.5 and could further be conjugated with biomolecules by carboxyl groups of the amphiphilic polymer.

*Analytical performance of the column tests*

The cut-off level of the column tests was defined as the lowest ZEN concentration which did not result in appearance of the test layer luminescence. The absence of luminescence was considered as a positive result (analyte concentration equal or above the cut-off level), presence of luminescence, independent on its brightness, as a negative result (analyte concentration below the cut-off level). Samples with a ZEN concentration equal or above (or below) the cut-off level, which resulted in negative results (or positive results) were considered to be false negative (or

*LC-MS/MS procedure*

The method described by De Boevre *et al.* [45] was used for the LC-MS/MS determination of ZEN. A Waters Acquity UPLC system coupled to a Quattro Premier XE mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray interface (ESI) was used. Chromatographic separation was performed, applying a ZORBAX Eclipse XDB C18 column (3.5 mm, 100 mm x 4.6 mm) (Agilent Technologies, Diegem, Belgium).

*Gel-based immunoassay*

Sepharose 4B gel coupled with rabbit anti-mouse antibody (coupled gel) and gel with blocked active groups (blocked gel) were prepared as recently described [41]. The coupled (0.5 mL) and blocked (1 mL) gels were mixed with addition of the anti-ZEN antibody (25 µL for ZEN-QD, 5 µL for ZEN-LSQD and 2 µL for ZEN-LIQD). Excess of PBS was removed, and the obtained gel was washed twice with PBS, and then suspended with PBS (1.5 mL). A volume of 200 µL was placed between two polyethylene frits inside an empty 1-mL Bond Elut cartridge. The column was washed with a PBS solution and kept at 4 °C until use.

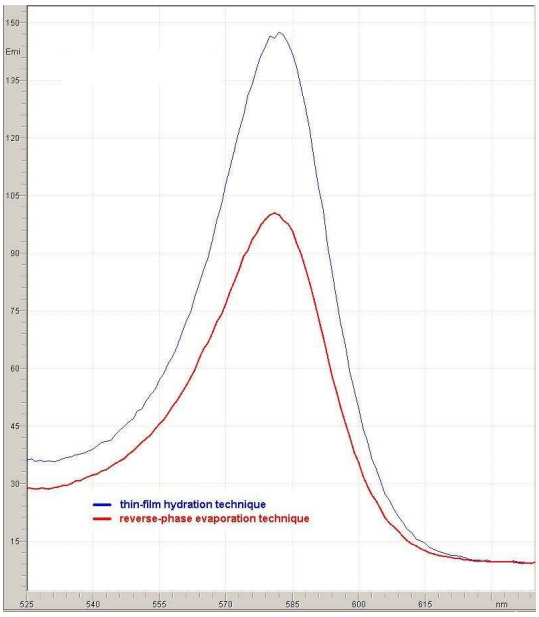


**Fig. S-1.** Luminescence spectra of the QD-loaded liposomes, obtained by the thin-film hydration and reverse-phase evaporation. $\lambda_{ex}$=380 nm.

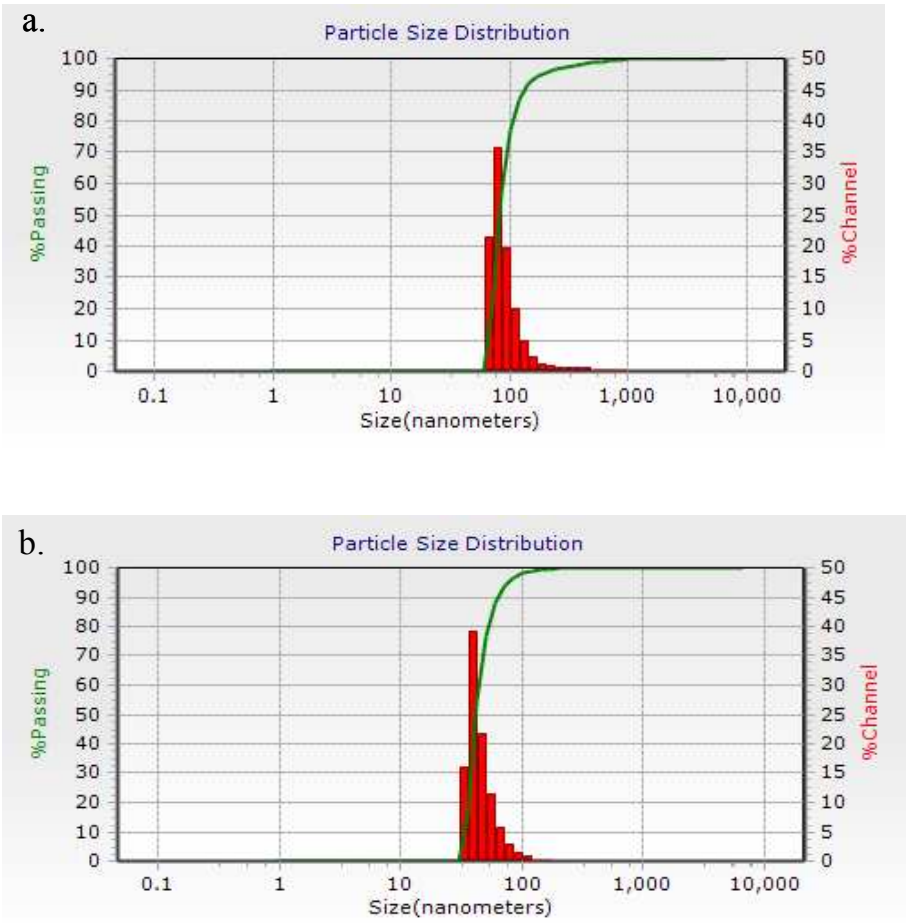


**Fig. S-2.** Size distribution of the water-insoluble QD-loaded liposomes, obtained from Lipoid S75 by thin-film hydration immediately after preparation (a) and after 5 min-sonication (b). All measurements were carried out at 25 °C.

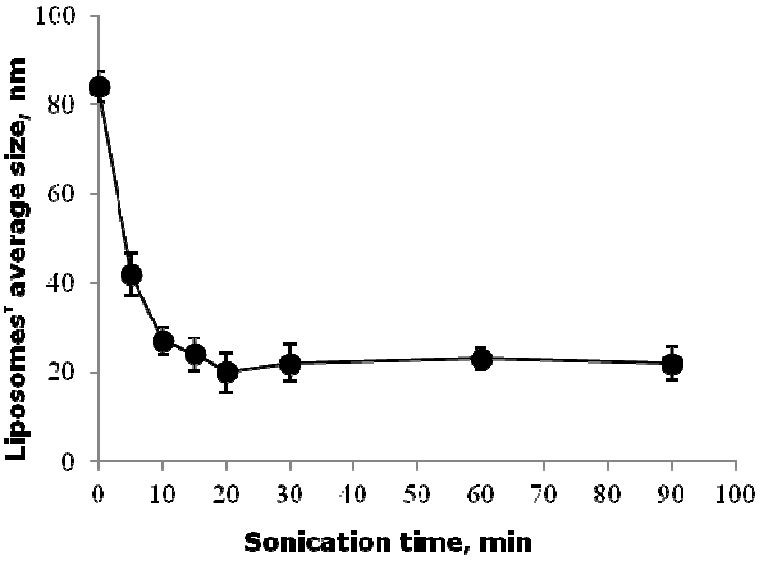


**Fig. S-3.** Liposomes' average size as a function of sonication time