A "turn-off" fluorescent substrate for horseradish peroxidase detects anti-chlamydia in mouse serum by enhancing accuracy of ELISA kits by 10 fold

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Statement of Purpose: The variability in detection of antigens in human tissue samples via ELISAs is a central problem in medicine and limits diagnosis of otherwise detectable disorders. In this report we present a fluorescent "turn-off" ultrasensitive HRP substrate termed, cLiCs (**c**yanine **Li**posome **C**omplexe**s**), which increase the accuracy of protein detection using commercial ELISA kits by 20 fold because they reduce the variation due to non-specific background signal. cLiCs have been designed to decrease the diffusion distance between reactive oxygen species (ROS) enhancers and the ROS reactive cyanine dyes to nanometer scale by localizing them in a lipid bilayer, and this allows for increase in sensitivity and detection of as few as 100,000 molecules of soluble HRP in a 96 well plate. We show here that cLiCs detect anti-chlamydia antibodies in mouse serum and commercial TMB substrates could not detect these antibodies. The high accuracy of cLiCs combined with their compatibility with commercial ELISA kits gives them the ability to impact numerous areas of biology and medicine.

Methods: a) cLiCs were developed by mixing IR783 dyes with the dimyristoyl phosphotidylcholine (DMPC) bilayer lipid structures. b) 1 to 2 HRP dilutions were generated in eppendorf tubes with highest concentration of 25 x 10^{-15} M and 50 uL of the HRP solution was added to the wells of 96 well plate. 50 uL of IR783 substrate or cLiCs was added to these wells to detect the amount of HRP. c) A standard sandwich ELISA was designed and performed to detect HSP60 antibodies in mouse serum. cLiCs were compared with commercial substrate TMB for the ability to improve the accuracy of detecting anti-HSP60 antibodies in mouse serum.

Results: a) Cyanine-liposome complexes (cLiCs) are a new family of fluorescent probes for HRP which can be combined with commercial ELISAs to accurately detect antigens in human tissue samples (**Figure 1**). **b)** cLiCs are excellent substrates for horseradish peroxidase (HRP) and can detect 1 femtomolar of soluble HRP (**Figure 2**). IR783 degrade in the presence of hydroxyl radicals generated by HRP. This degradation can be detected by a conventional plate reader. **c)** cLiCs enhance the accuracy of ELISAs by 20 fold and detect anti-HSP60 antibodies in mouse serum (**Figure 3**). The average % coefficient of variation (CV) in detection of anti-HSP60 antibodies using TMB based ELISAs was 64%, where as for cLiCs based ELISAs the average % CV in detection was 6%.

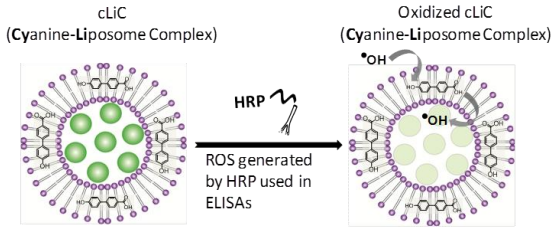

Figure 1: Assembly of cyanine liposome complexes (cLiCs) and mechanism of dye degradation is shown.

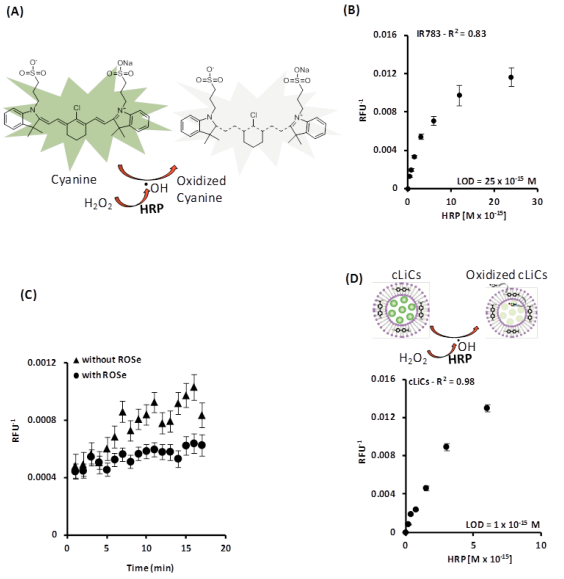

Figure 2: A) IR783 dye can get oxidized by the hydroxide radicals generated by HRP. **B)** IR783 detects 25 femtomolar levels of soluble HRP. **C)** Encapsulating IR783 in lipid bilayer enhances the stability of IR783, which is further stabilized by the ROS enhancers. **D)** cLiCs detect 1 femtomolar levels or 100,000 molecules of soluble HRP in a 96-well plate.

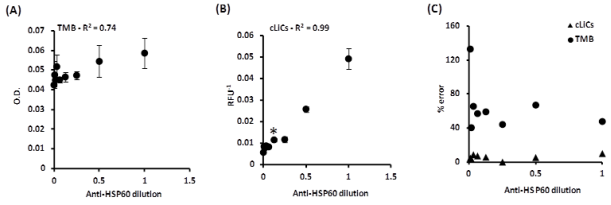

Figure 3: A) TMB based ELISAs were not able to detect anti-HSP60 antibodies ($p > 0.05$). **B)** cLiCs based ELISAs were performed on mouse serum obtained from mice inoculated with HSP60 and could detect anti-HSP60 antibodies ($*p < 0.05$). **C)** The % CV for TMB based ELISAs was 64% and for cLiCs based ELISAs the % CV was 6%.

Reference: Acharya et al. Chem Commun, 2013, 10379-81.