Electronic Supplementary Information (ESI)

For

Electrochemical detection of protein glycosylation using direct protein-gold affinity interactions

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Western blot for protein captured by Immunoprecipitation:

The eluted samples were used for western blot to verify the presence of ovalbumin protein after Immunoprecipitation. Twenty micrograms of sample was mixed with sample buffer and loaded on SDS-PAGE gel along with the protein ladder and run at 100 volt for 1 hour. The gel was then transferred on PVDF membrane. After washing the membrane with Tris-buffered saline with 0.05% Tween 20 (TBST) the membrane was blocked with blocking buffer (5% milk in TBST) followed by wash and incubation with rabbit anti ovalbumin antibody (Sigma; 5503, diluted 1:1000 in blocking buffer) and kept overnight on roller at 4^{o}C. The membrane was washed thrice with TBST and the secondary antibody (anti rabbit HRP diluted 1:3000 in blocking buffer) was added and kept for 2 hours on roller at 37^{o}C. SuperSignal™ West Pico Chemiluminescent Substrate was used for final detection as per manufacturer's instructions and the final image was taken on FUJI Medical X-ray film with an exposure time of 30 seconds.

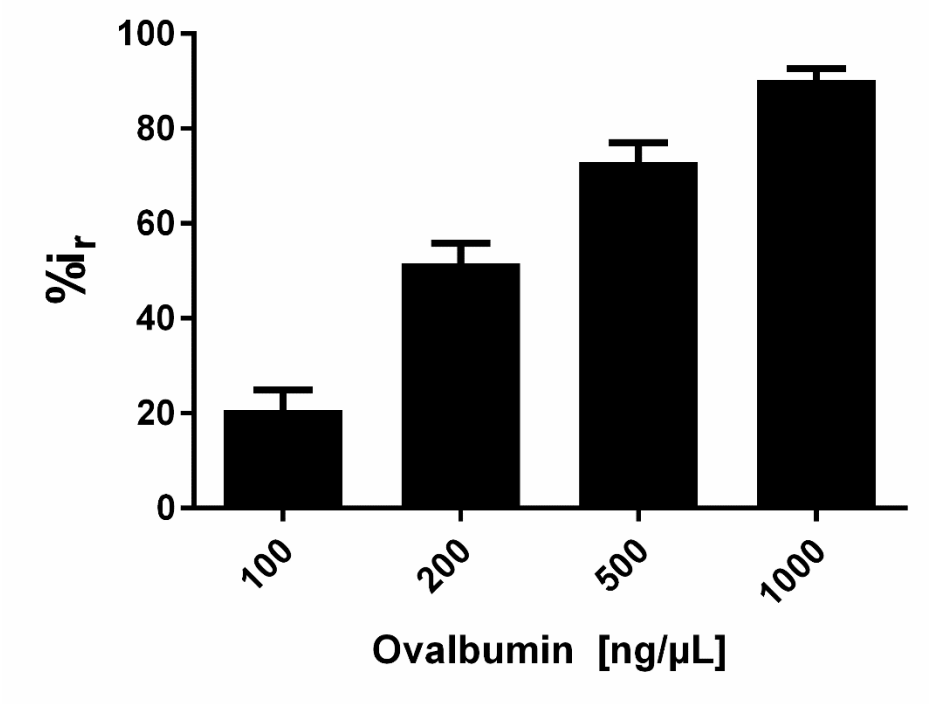

Fig. 1. Mean values of the percentage current difference ($\%i_r$ ^{protein}) for the adsorption of different concentration of ovalbumin at pH 7 over 20 mins period.

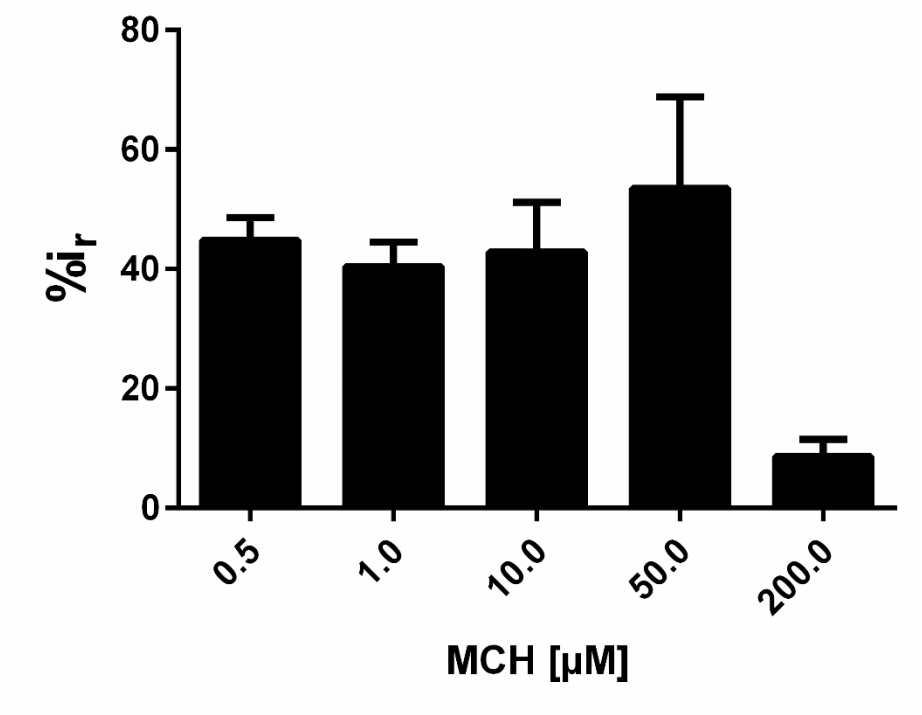

Fig. 2a. Mean values of the percentage current difference ($\%i_r$) .The optimization of MCH concentration using streptavidin (negative control). At 200 µM MCH, streptavidin gives negligible signal.

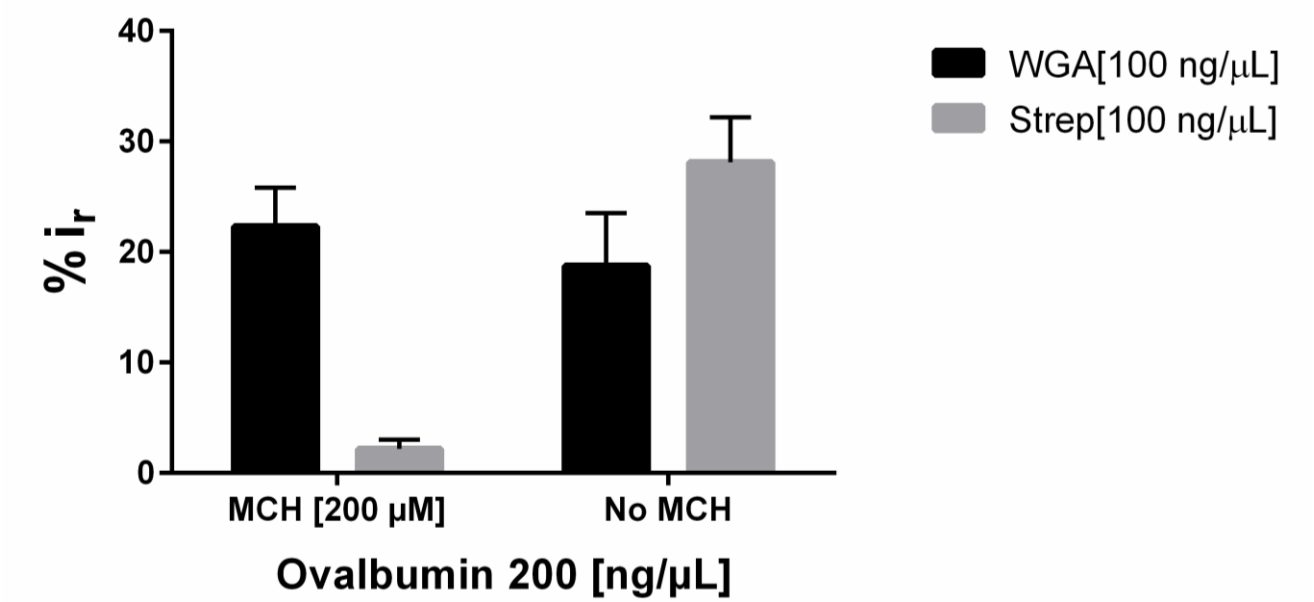

Fig. 2b. Mean values of the percentage current difference ($\%i_r^{Lectin}$) for WGA and Streptavidin with and without using MCH

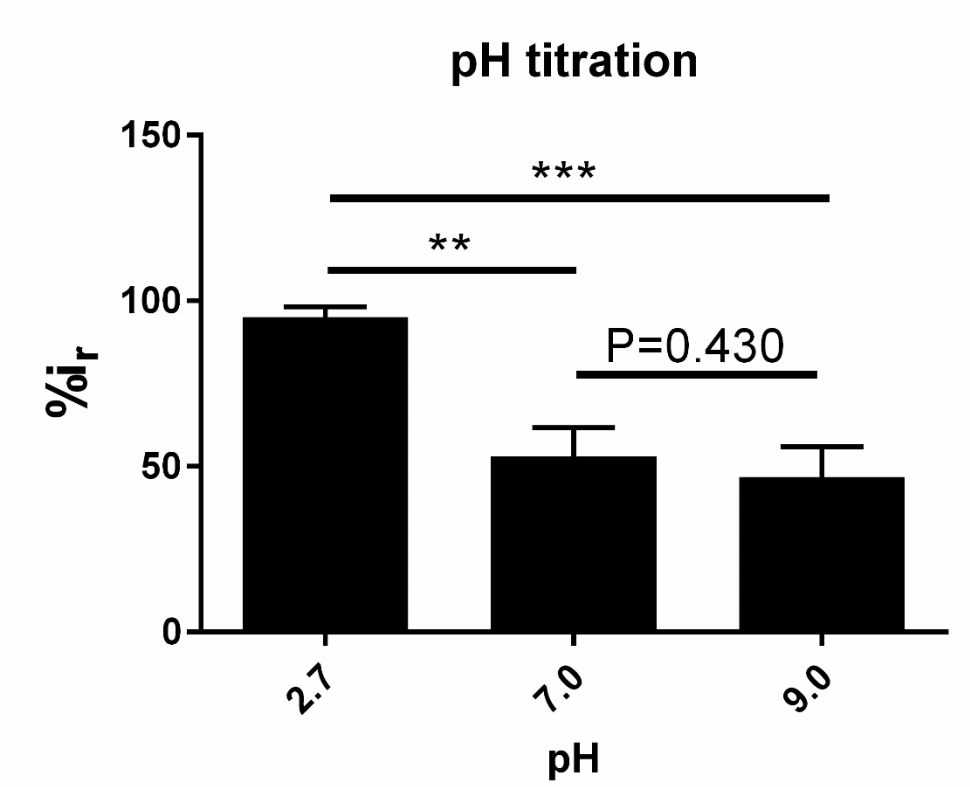

Fig. 3. Mean % i_r ^{protein} values for adsorption of 100 ng/µL Ovalbumin at three different pH, adsorption time 55 minutes. At pH 2.7 (acidic) Ovalbumin shows highest adsorption compared to neutral or alkaline pH. Statistical significance was determined by pairwise comparisons between 2 conditions using student's t-test. *, p = 0.005 to 0.05; **, p = 0.0005 to 0.005, ***, p = 0.00005 to 0.0005.

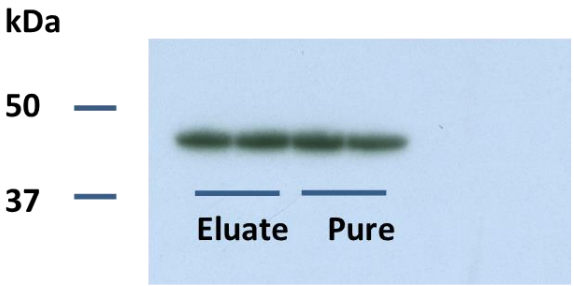

Fig. 4. Western blot analysis of eluted sample after immunoprecipitation using magnetic beads showed the presence of ovalbumin (45 kDa) confirming specific capture of the protein by biotinylated antibody and streptavidin beads. Two immunprecipitations were performed and analysed on the same western.

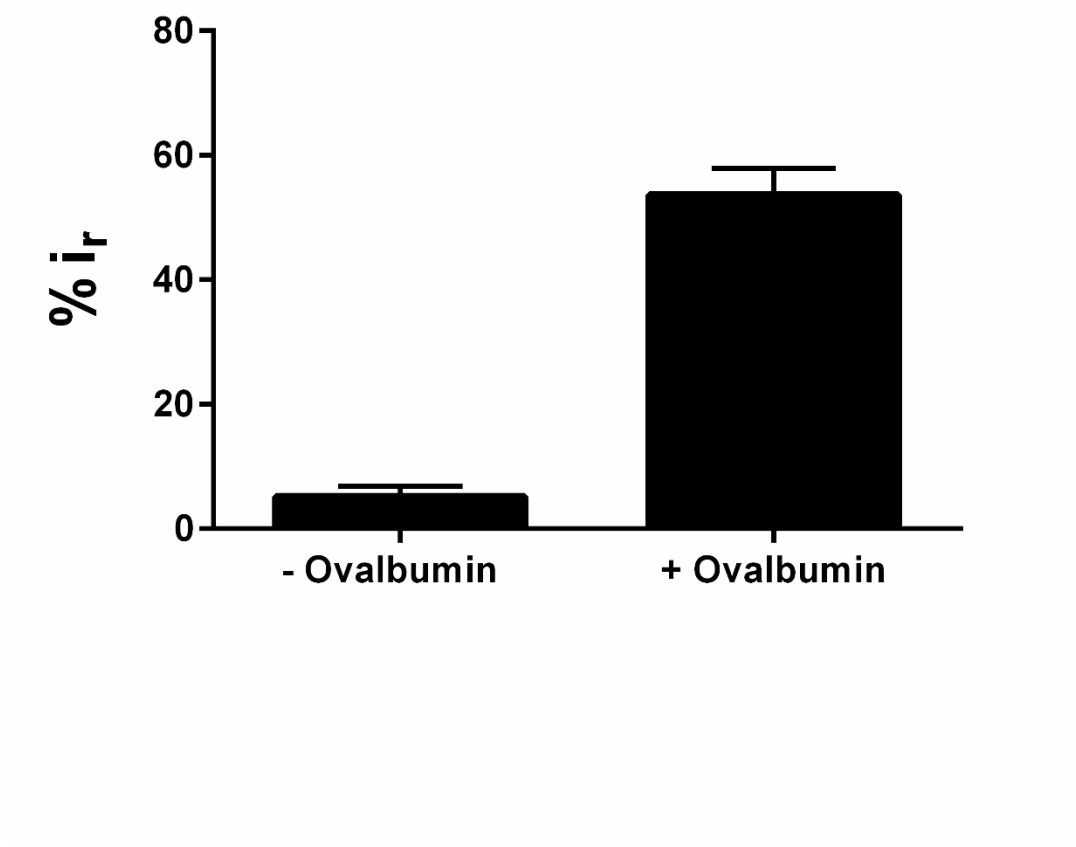

Fig 5. Mean values of the percentage current difference $\%i_r^{protein}$ for the adsorption of eluted sample from the serum using streptavidin magnetic beads. Magnetic beads were first introduced into serum (both ovalbumin spiked and not spiked) following the elution protocol, eluate from both the magnetic beads was then diluted to 5ng/μL and adsorbed on the gold surface for 15 minutes showing significant difference in the adsorption between spiked and non-spiked serum. Bars show standard deviation from 3 independent experiments.