Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information

1. pH Dependent and colour dependent tests for urease activity measurement.

The amine group of the enzyme responsible for bonding with GNPlts is not the active site of enzyme and is not affecting its activity. To confirm we conducted some experiments to check the activity of the hybrid. The tests include pH Dependent and colour dependent approach for urease activity measurement.

A. pH dependent activity measurement of urease: In the pH based study, urea solutions (0.1, 0.2 and 0.3M) were taken and then urease (1 mg/ml) and GNPs-urease (1 mg/ml) were added in them separately. Increase in pH was observed, which is due to generation of ammonium ions upon hydrolysis of urea by urease[1] as tabulated below.

Table S1: pH Readings for different concentrations of urea solutions.

S.No	Urease Solutions	pH Readings on addition of varying urea concentration		
		0.1 M	0.2M	0.3M
1	Urease solution	5.6	6.0	6.4
2	GNPs conjugated urease	5.5	5.9	6.2

B. Colour dependent approach: Indothymol test:

The ammonium ions were produced during the hydrolysis of urea by urease. Indothymol is an ammonium ions specific reagent[2] which gives colour to reaction solution. Four samples were prepared by adding a) Urea (0.1 M) b) Urease (1mg/ml) c) Urea (0.1 M) + Urease (1mg/ml) d) Urea (0.1 M) + GNPlts-Urease (1mg/ml) respectively in Indothymol reagent [Thymol reagent (1ml) + Sodium Hypochlorite (1ml)]. In Sample a) & b) no change in colour was observed due absence of ammonium ions, whereas in Sample c) and d) greenish blue colour was observed which ensures that urease is active in both unconjugated and conjugated form (as shown in the Figure S1).

Hence After conjugation with GNPlts the urease is still active.

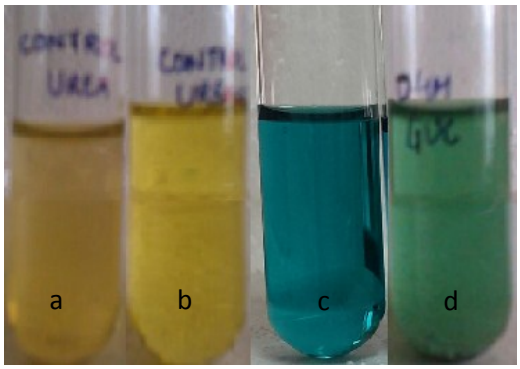

Fig.S1. Test samples a) Urea - control b) Urease - control c) Urea + Urease d) Urea + GNPlts-Urease respectively in Indothymol reagent.

2. I/V responses for blank electrodes without GNP (without urease), and drop-casted GNP (without urease as control experiments.

The experiments were carried out at lowest to highest concentration of urea on blank electrodes (CNT-SPE) and GNPlts/CNT-SPE hybrid electrodes. As expected the blank electrodes without GNPlts (without urease) gave insulator type response Fig a). While after drop casting GNPlts (without urease) gave Ohmic characteristics with no 0V response.

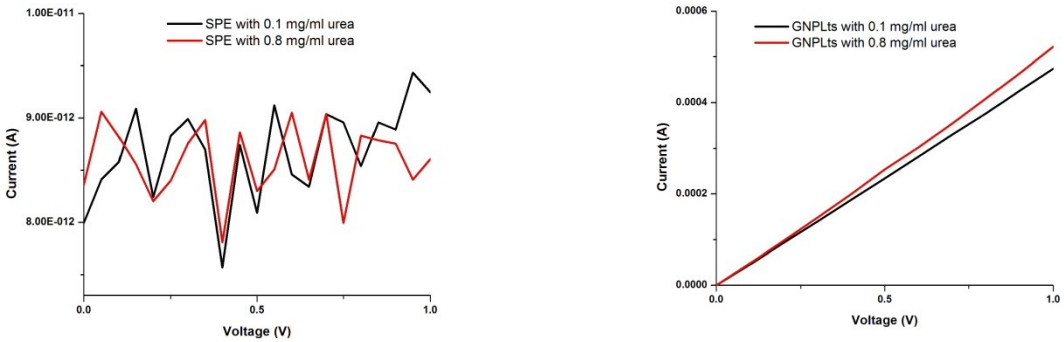

Fig. S2. Lowest to highest concentration of urea on **a)** blank electrodes (CNT-SPE), **b)** GNPlts/CNT-SPE hybrid electrodes.

References

1 B. Krajewska, R. V. Eldik, and M. Brindell, J. Biol. Inorg. Chem., 2012, 17, 1123.
2 C. Molins-Legua, S. Meseguer-Lloret, Y. Moliner-Martinez, and P. Campı´ns-Falco, Trends Anal. Chem., 2006, 25, 282.