Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Heterogeneous endotoxin detection bioassay using drug-nanoparticle bioconjugates: An optimization study

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Supplementary Information

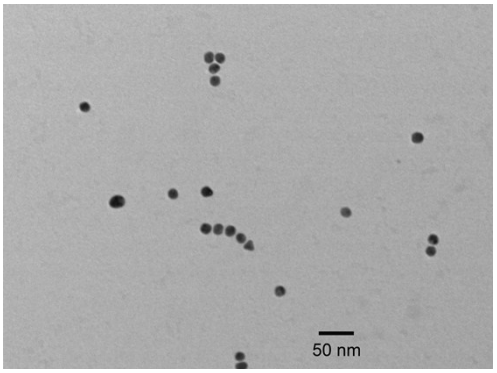

Figure S1. Transmission electron microscope (TEM) image of GNPs imaged using Jeol RM1400. The average size of the particles was estimated to be 16 ± 4 nm from the particle size distribution histogram.

Image processing

The acquired images were cropped to a uniform size of 1 inch x 1 inch and then processed using the Matlab (R2015a) software. A code was developed that had the following main components–

spot area identification, background area identification, average pixel value calculation for both regions and spot intensity normalization with respect to background. In this way, the final intensity values reported for each spot were dimensionless.

$$normalized\ intensity, I = \frac{average\ background\ intensity - average\ spot\ area\ intensity}{average\ background\ intensity}$$

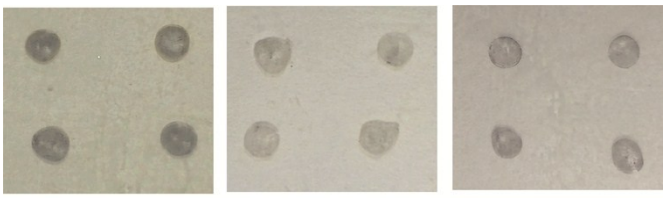

Figure S2. Bioassay spots obtained in quadrupulate on three different glasss slides A, B and C for a single LPS concentration. The overall average intensity wascalculated as

$$I_{avg} = \frac{I_{avg}(A) + I_{avg}(B) + I_{avg}(C)}{3}$$

Table S1. Results from the negative and positive controls performed in our bioassay.

Controls	**Observation**
Bare GNPs	Dark shadows on the exposed glass slide after the silver enhancement step irrespective of their silanization status. This was presumably due to electrostatic interactions between GNP and glass.
Without LPS	No spot; Clear background
Real clinical samples from sepsis patients	Similar results as those in spiked serum samples.
Icteric samples collected from non-sepsis patients	No interference of pigmentation was seen on the colorimetric response of the bioassays.
Free PMB drug along with PMB-GNPs	Concentration-dependent competitive-binding*
Free bacitracin drug along with PMB-GNPs	No change in bioassay performance*

*These two sets of experiments were performed on a modified bioassay format but using similar interaction chemistry. See Kalita S., Chaturvedula M., Sritharan V. and Gupta S., 'In vitro Flow-

Through Assay for Rapid Detection of Endotoxin in Human Sera' published in ***Nanomedicine: Nanotchnology, Biology, and Medicine,*** 13, 1483-1490 (2017) for complete details.