Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Ultrasensitive and Unambiguous Bacterial Pathogen Detection through Super Selective interactions between Multivalent Supramolecular Immuno-Nanoparticles (SINs)

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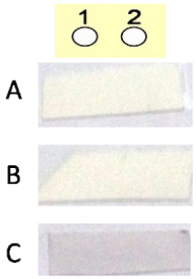
SUPPORTING FIGURES & TABLES

Figure S1. Evidence for secondary antibody never binds non-specifically to bacterial cells

Two different strains were spotted on the membrane (10^6 cells/spot). Blocked with BSA and incubated in different dilutions of direct secondary antibody (A- 1:5000 Secondary antibody, B- 1:2500 Secondary antibody, C- 1:1000 Secondary antibody)



No spots were observed in any of the membrane which implies that the secondary antibody does not bind non-specifically to the bacterial membrane.

Table S2. Details of strains used

S.No	Strain code	Strains	S.No	Strain code	Strains
1.	EP1	*E.coli*	2.	UPEC 129	*Uropathogenic E.coli*
3.	T56	*E.coli*	4.	Proteus 487	*Proteus*
5.	EC1021	*E.coli*	6.	E.coli 171	*E.coli*
7.	Pseudo	*Pseudomonas aeruginosa*	8.	UPEC 475	*Uropathogenic E.coli*
9.	Acint	*Acinetobacter*	10.	UPEC 149	*Uropathogenic E.coli*
11.	Shi.f	*Shigella flexneri*	12.	Klebsiella 4483	*Klebsiella pneumonia*
13.	M8	*Staphylococcus aureus*	14.	S.aureus 3160	*Staphylococcus aureus*
15.	T113	*Klebsiella pneumonia*	16.	E.coli 422790	*E.coli*
17.	M4	*Salmonella entericia*	18.	S.dysentrieae 1031	*Shigella dysentrieae*
19.	M11	*Proteus mirabilis*	20.	UPEC 139	*Uropathogenic E.coli*
21.	M30	*Shigella dysentrieae*	22.	S.flexneri 1457	*Shigella flexneri*
23.	B.C	*Bacillus cereus*	24.	Proteus 282	*Proteus*
25.	E.coli 723	*E.coli*	26.	S.dysentrieae 1021	*Shigella dysentrieae*
27.	Klebsiella 340053	*Klebsiella pneumonia*	28.	Klebsiella 3384	*Klebsiella pneumonia*
29.	Proteus 7002	*Proteus mirabilis*	30.	E.coli 422790	*E.coli*
31.	UPEC 478	*Uropathogenic E.coli*	32.	S.flexneri 9543	*Shigella flexneri*
33.	S.dys 1311	*Shigella dysentrieae*	34.	S.aureus 25923	*Staphylococcus aureus*

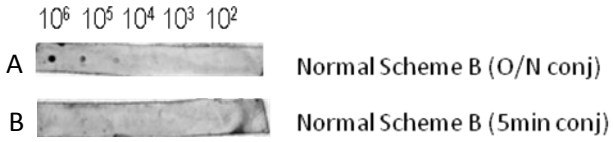
Table S3.Negative control experiment in ELISA

ELISA experiment was performed with two different primary SINs – specific primary SINs (rabbit antibody) and inert or non-specific primary SINs (chicken antibody), one that does not bind to our secondary antibody (Anti rabbit antibodies).

Secondary antibody dilution	Scheme B (Full Non-specific)	Scheme D (Full Non-specific)
0.004	0.167	0.057
0.002	0.044	0.024
0.001	0.015	0.018
0.0005	0.023	0.007
0.00025	0.004	0.013
0.000125	0.006	0.019
6.25E-05	0.018	0.015
3.13E-05	0.011	0.012
1.56E-05	0.008	0.017

Figure S4.Surface density experiment-5min conjugation of primary antibody to GNP

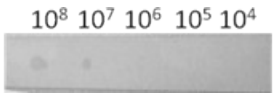
10^6 to 10^2 CFU of bacterial cells were spotted on the membrane and dot blot assay was carried out with 5min conjugated primary SINs (B) and overnight conjugated primary SINs as control (A).



When the antibodies were conjugated for only 5min to GNPs, no spots were observed on the membrane in none of the dilution of cells spotted. This can be due to the insufficient binding of primary antibodies to the GNPs due to short time incubation.

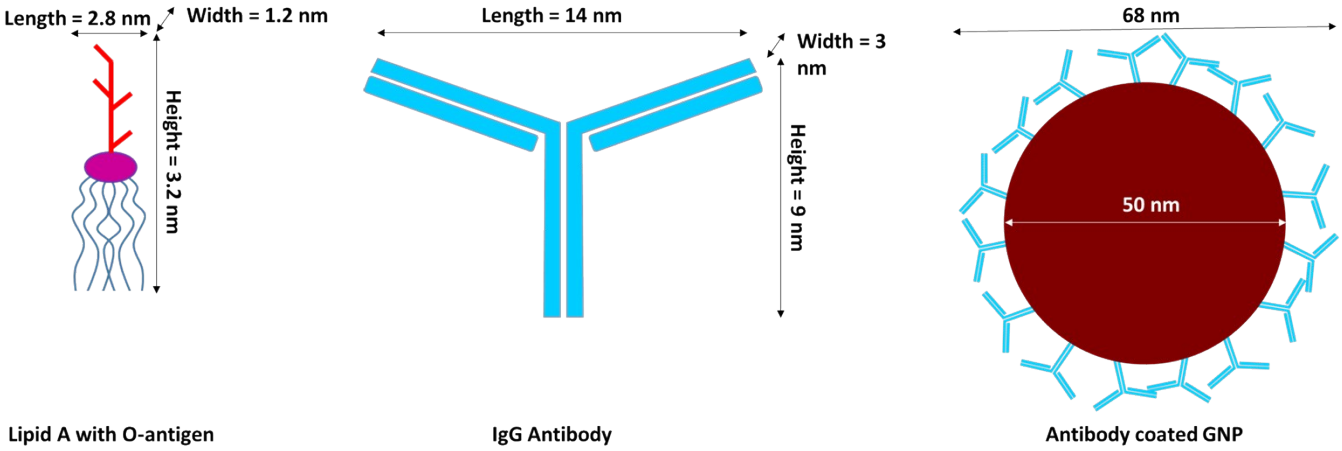
Figure S5. NBT/BCIP (ALP substrate) direct reaction with bacterial cells

10^8 – 10^4 Cells/µl/Spot on nitrocellulose membrane were allowed to react with ALP substrate (NBT/BCIP) directly without primary or secondary antibody



Spot was observed on membrane for 10^8 and10^7 CFU cells indicating that bacterial endogenous ALP was reacting directly to the substrate. Hence, cells numbering 10^6 CFU or less than 10^6 CFU should be spotted on the membrane for the experiment.

Figure S6. Dimensions of various entities used for calculations



Calculations:

Lipid A with O-antigen area (A_O) = Length x Width = 3.36 x 10^{-18} m^2
Antibody area (A_{Ab}) = Length x Width = 4.2 x 10^{-17} m^2

Assuming 90% of the outer membrane is composed of Lipid A,
Number of O-antigens buried under a primary antibody = SA_{Ab}/SA_O = 11

Antibody-coated GNP surface area (SA_{SIN})= 4 x ∏ x r^2 = 1.45 x 10^{-14} m^2
Antibody-coated GNP circular area (CA_{SIN})= ∏ x r^2 = 3.63 x 10^{-15} m^2

Maximum number of Antibodies on a GNP = SA_{SIN}/SA_{Ab} = 346
number of O-antigens buried under a SIN = CA_{SIN}/A_O = 972

SUPPORTING MATERIALS AND METHODS

M1.Flocculation Assay

In a typical assay, the suitable proportion of protein to be conjugated was identified by mixing different concentrations of antibody to equal amount of gold nanoparticles and kept at room temperature for 30min, followed by addition of 10µl of 10% NaCl. The color change was monitored via absorbance spectra and the gold nanoparticles aggregation can be seen with a peak shift towards 580nm in the samples which has less amount of protein needed to stabilize the fixed amount of nanoparticles. When the antibody molecules are enough to stabilize the gold nanoparticles, then the color change will not take place even after addition of salt.

M2.Dot blot assay for detection of mixed cell culture

Nitrocellulose membrane were cut into small strips (4 No's) for each scheme and six spots (1-6) were spotted in the following order, each with different combination of cells on each strip.

Spot No.	Combination of cells (Total 10^6 cells)
1	10^6 pure positive cells
2	10^5 positive cells+10^1 ghost spot negative cells
3	10^4 positive cells+10^2 ghost spot negative cells
4	10^3 positive cells+10^3 ghost spot negative cells
5	10^2 positive cells+10^4 ghost spot negative cells
6	10^6 Ghost spot negative cells

The experimental procedure was carried out as same as that of pathogen detection for all four schemes

M3. Simulated Non-specific Surface density dependence of the SINs (Scheme B and Scheme D)

S.No	Scheme B		Scheme D	
1	Fully specific primary SINs	100ng/well	Fully specific primary SINs	100ng/well
2	1(Specific primary SINs) :100 (Non-Specific primary SINs)	100ng/well (1ng+99ng)	1(Specific primary SINs) :100 (Non-Specific primary SINs)	100ng/well (1ng+99ng)
3	1(Specific primary SINs) :1000(Non-Specific primary SINs)	100ng/well (0.1ng+99.9ng)	1(Specific primary SINs) :1000(Non-Specific primary SINs)	100ng/well (0.1ng+99.9ng)

The primary SINs were coated on ELISA plates as mentioned in the table above and kept overnight at room temperature, followed by blocking and subsequent washing steps which are mentioned in the methods for Scheme B and scheme D.

M4. Dot-blot assay cost analysis

Nitrocellulose membrane:

50piece (80mm dia circle) = $60

1piece = $1.2

For one reaction (10 samples) we need 1/16th of a piece = **$0.07**

Primary Ab:

3ml Serum = $60

For one reaction we require 3ml of 1:1000 diluted primary antibody = **$0.06**

BSA:

100g = $90

For one reaction we need 15ml of 0.1% (wt/vol) solution = **$0.01**

Secondary Ab:

1ml = $225

For one reaction we require 3ml of 1:10000 diluted secondary antibody = **$0.07**

GNP:

1g = $105

For conjugation we are using only 100µl of 1mM solution (0.04mg of GNP) = **$0.04**

Total cost per reaction (10 samples) = **$0.25**