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Single-step detection of norovirus tuning localized surface plasmon resonance-induced optical signal between gold nanoparticles and quantum dots

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1 **Single-step detection of norovirus tuning localized surface**
2 **plasmon resonance-induced optical signal between gold**
3 **nanoparticles and quantum dots**

4

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31 **Abstract**

32 A new method of label free sensing approach with superior selectivity and sensitivity
33 towards virus detection is presented here, employing the localized surface plasmon resonance
34 (LSPR) behavior of gold nanoparticle (AuNPs) and fluorescent CdSeTeS quantum dots (QDs).
35 Inorganic quaternary alloyed CdSeTeS QDs were capped with L-cysteine via a ligand exchange
36 reaction. Alternatively, citrate stabilized AuNPs were functionalized with 11-
37 mercaptoundecanoic acid to generate carboxylic group on the gold surface. The carboxylic
38 group on the AuNPs is subjected to bind covalently with the amine group of L-cysteine capped
39 CdSeTeS QDs to form CdSeTeS QDs/AuNPs nanocomposites. The fluorescence of CdSeTeS
40 QDs/AuNPs nanocomposite shows quenched spectrum of CdSeTeS QDs at 640 nm due to the
41 close interaction with AuNPs. However, after successive addition of norovirus-like particles
42 (NoV-LPs), steric hindrance-induced LSPR signal from the adjacent AuNPs triggered the
43 fluorescence enhancement of QDs in proportion to the concentration of the target NoV-LPs. A
44 linear range of 10^{-14} to 10^{-9} g mL⁻¹ NoV-LPs with a detection limit of 8.2 fg mL⁻¹ was obtained.
45 This method was further applied on clinically-isolated norovirus detection, in the range of 10^2
46 – 10^5 copies mL⁻¹ with a detection limit of 87 copies mL⁻¹, which is 100-fold higher than
47 commercial ELSA kit. The superiority of the proposed sensor over other conventional sensors
48 is found in its ultrasensitive detectability at low virus concentration even in clinically-isolated
49 samples. This proposed detection method can pave an avenue for the development of high
50 performance and robust sensing probes for detection of virus in biomedical applications.

51 **Keywords:** Biosensor; CdSeTeS; Gold nanoparticle; Localized surface plasmon resonance;
52 Norovirus detection; Quantum dots.

53 **1. Introduction**

54 The current progress in search of optically active nanocomposite has driven the
55 development of variety of applications in diverse fields ranging from biomedical engineering
56 to environmental safety (Dutta Chowdhury and Doong, 2016; Ganganboina et al., 2017; Hsu
57 et al., 2016; Kuila et al., 2011; Lee et al., 2007). Although several developments of optical
58 biosensors have been experienced an exponential growth during the last decade due to the
59 incorporation of nanotechnology for the direct, real-time and label-free detection of many
60 chemical and biological substances (Anh et al., 2017; Chowdhury et al., 2012; Dutta
61 Chowdhury et al., 2017), but there are very few reports which come to appear in real
62 applications. Fluorometric assays are the most often applied methods on optical sensing and
63 comes in a variety of schemes due to its easy technique and reliable outcomes (Al-Ogaidi et
64 al., 2014; Huang et al., 2014). Parameters that are being analyzed in such sensors include
65 fluorescence intensity, decay time, quenching efficiency and regeneration of fluorescence or
66 luminescence energy transfer. Among these, the most innovative and recently evolved optical
67 biosensors are those based on surface plasmon resonance (SPR) properties using different gold
68 nanocomposites (Kawaguchi et al., 2008; Lee et al., 2015; Singh and Strouse, 2010; Yeom et
69 al., 2013). These biosensors have been widely used in the fields for detection of infectious
70 diseases related with cells, bacteria or viruses. Generally, fluorescent quantum dots (QDs) have
71 been widely applied as fluorescence reporters in various LSPR based biosensor whereas the
72 SPR generated AuNPs plays the crucial role to influence the fluorescence signal depending on
73 the size, shape and distance (Lee et al., 2015; Takemura et al., 2017). Triggering with the
74 analyte, it can alter the position as well as distance between these two nanocomponents (QDs
75 and AuNPs), resulting in the variation of signal detection.

76 Inorganic quantum dots (QDs) are semiconductor nanocrystals whose electrons and holes
77 are quantum-confined from all dimensions. Due to their unique physical and optical properties

78 with respect to the corresponding bulk materials such as conventional organic dyes or
79 fluorescent proteins, QDs have emerged as a new class of fluorescent agents for biomedical
80 applications (Ahmed et al., 2016; Anderson and Chan, 2008; Lee et al., 2015; Tian et al., 2012).
81 However, poor bio-conjugating ability and high toxicity restricts their potential use for the
82 development of biosensor. To overcome this, lots of efforts have been suggested to improve
83 the performance of QDs in biomedical applications through proper surface modifications. Few
84 chalcogenides such as ZnS, TeS etc. are widely used to shell-coat the QD cores to improve the
85 physiochemical and optical performance of the QDs as well as their biocompatibility in
86 biological system (Adegoke et al., 2015; Liang et al., 2009; Liang et al., 2010).

87 There are many attempts on optical sensing which are reported on virus detection as the
88 conventional diagnostic systems still have certain limitations. Induced antibody detection on
89 serological analysis can lead to false negative or false positive misguided data interpretation.
90 Most authentic viral culture analysis is time consuming whereas immunofluorescence assays
91 are limited to their sensitivity. Therefore, there is an utmost need for the development of rapid,
92 highly sensitive and selective diagnostic sensor for the virus detection. Pang et al., (2015)
93 reported a fluorescent aptasensor system for the sensitive detection of influenza virus H5N1 in
94 human serum by guanine-enriched anti-rHA aptamers immobilized on the surface of the
95 Ag@SiO₂ nanoparticles which performed as a metal-enhanced fluorescence sensing platform.
96 Similarly, Wu et al., (2015) developed an enzyme-induced bi-functional magnetic
97 electrochemical immunosensor to detect Influenza virus A (H7N9) in complex media. In our
98 previous work, detection of influenza virus A (H1N1) has been reported on a combination of
99 LSPR-induced optical transduction from antibody-labeled AuNPs and the fluorescence signal
100 generated from adjacent antibody-conjugated CdSeTeS QDs (Takemura et al., 2017).
101 However, using several antibodies or aptamer-conjugated antibodies is making those systems
102 complicated and expensive. Moreover, the high fluorescence signal of background also

103 hampers the enhancement of surface plasmon signal, which is disadvantage of this method. To
104 overcome this, here we have developed a new method of LSPR-induced optical transduction
105 between AuNPs and CdSeTeS QDs with a single step process to detect NoV-LPs. The covalent
106 attachment between AuNPs and CdSeTeS QDs forms the rigid sensing probe of CdSeTeS
107 QDs/AuNPs which can sufficiently decreases the nonspecific interaction, resulting the
108 increasing sensitivity. Norovirus (NoV) which is mostly common causes for gastroenteritis
109 disease, generally transmitted through shellfish consumption and food and waterborne routes.
110 As the levels of enteric viruses in bivalve mollusk or in mussels are generally in very low
111 concentration, the high sensitive technique is in demand for its early detection. To establish the
112 detection technique, initially we have taken the NoV-LPs as a target analyte, because there is
113 no robust cell culture system for the NoV propagation to date. The anti-Nov antibody-
114 conjugated CdSeTeS QDs are covalently linked with AuNPs, quenching the fluorescence of
115 CdSeTeS QDs/AuNPs nanocomposites which has been used as the sensing probe for a single
116 step label free NoV-LPs detection. The detection mechanism of the biosensor involves the
117 regeneration of quenched fluorescence of CdSeTeS QDs/AuNPs due to LSPR while the
118 attached NoV-LPs create steric hindrance between two nanomaterials as depicted in Scheme
119 1.

120

121 **2. Methods and Materials**

122 **2.1. Materials**

123 PBS buffer, sodium citrate, polyoxyethylen (20), sorbitan monolaurate (Tween 20),
124 hydrogen peroxide, sulfuric acid, methanol, potassium hydroxide (KOH), tri-sodium citrate,
125 chloroform and acetone were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan).
126 Tetramethylbenzidine (TMBZ) was purchased from Dojindo (Kumamoto, Japan). H₂AuCl₄, N-

127 (3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide
128 (NHS), bovine serum albumin (BSA), 11-mercaptoundecanoic acid (MUDA), 1-octadecene,
129 cadmium oxide (CdO), tellurium (Te), *L*-cysteine, hexadecylamine (HDA), trioctylphosphine
130 oxide (TOPO), trioctylphosphine (TOP), selenium (Se) and sulfur (S) were purchased from
131 Sigma Aldrich Co., LLC (Saint Louis, MO, USA). Oleic acid was purchased from Nacalai
132 Tesque Inc. (Kyoto, Japan). Goat anti-rabbit IgG- horseradish peroxidase (HRP) was purchased
133 from Santa Cruz Biotechnology (CA, USA). Anti-Nov monoclonal antibody against NoV was
134 purchased from Abcam Inc. (Cambridge, UK). Zikavirus and influenza virus A (H3N2) for
135 selectivity test were kindly provided by Professor K. Morita of Institute of Tropical Medicine
136 Nagasaki University and Dr. C. Kawakami of the Yokohama City Institute of Health
137 (Yokohama Japan), respectively.

138 **2.2. Synthesis of CdSeTeS QDs**

139 Organometallic hot-injection synthesis of quaternary-alloyed CdSeTeS QDs was carried
140 out according to our previously reported method using CdO, Se, S as the basic precursors
141 (Adegoke et al., 2015).

142 **2.3. Capping of CdSeTeS QDs**

143 To make the hydrophilic QDs from the synthesized hydrophobic CdSeTeS QDs and to
144 functionalize its surface with amine group, the *L*-cysteine was conjugated via a ligand exchange
145 reaction. A methanolic-KOH-*L*-cysteine solution was prepared by dissolving 3 g of KOH in 40
146 mL of methanol and 2 g of *L*-cysteine. The hydrophobic QDs in chloroform solution were
147 added to the methanolic-KOH-*L*-cysteine solution, and an appropriate volume of ultrapure
148 deionized (DI) water was added to precipitate the hydrophilic QDs from solution. The solution
149 was stirred for several mins and was allowed to stand overnight for complete separation of the

150 organic phase from the water-soluble phase. The QDs were repeatedly purified using acetone
151 and chloroform.

152 **2.4. Synthesis of AuNPs**

153 For the preparation of AuNPs, 35 μL of 2 mM HAuCl_4 and finally 300 μL of 100 mM tri-
154 sodium citrate were added into 25 mL of pure boiling water under vigorous stirring condition
155 (Zhao et al., 2008). The whole solution was boiled and stirred for 15 min until the color changes
156 to pink.

157 **2.5. Functionalization of AuNPs with 11-mercaptoundecanoic acid**

158 The AuNPs was attached with the MUDA to generate carboxylic acid group in to the
159 surface. The AuNPs solution was stirred for 2 h with 0.1 mM of MUDA at pH 3 where the
160 thiol group has been covalently linked with the AuNPs via soft acid soft base interaction. After
161 successful synthesis of the AuNP-MUDA, the nanoparticle was washed several times with DI
162 water and centrifuged at $6000 \times g$ to obtain excess MUDA free AuNPs.

163 **2.6. Synthesis of sensing probe**

164 Initially, the anti-NoV antibody was conjugated with the free carboxylic group of L-
165 cysteine capped CdSeTeS QDs via EDC/NHS covalent chemistry. Then, the MUDA
166 functionalized AuNPs was covalently linked with the free amine group of L-cysteine capped
167 antibody-linked QDs via EDC/NHS reaction (Valeur and Bradley, 2009). In brief, EDC is
168 mixed with the carboxylic functionalized AuNPs and then further activated with NHS for 30
169 min before addition of the antibody conjugating QDs (Ab-QDs). The conjugate mixture was
170 stirred overnight at 7°C to form antibody-conjugating nanocomposites (Ab-CdSeTeS
171 QD/AuNPs) which were purified by centrifugation ($3000 \times g$) for 5 min and subsequently
172 dissolved in 2 mL of ultrapure DI water.

173 **2.7. Enzyme linked immunosorbent assay (ELISA)**

174 Antibody conjugated CdSeTeS QD/AuNPs nanocomposite before and after NoV-LP
175 addition along with its negative controls of bare QDs, AuNPs, BSA and DI water were taken
176 (100 μ L) in different well of a nonsterile polystyrene 96-well flat-bottom microtiter plate
177 (Becton Dickinson Labware, NJ, USA) to perform ELISA test. 100 μ L of 5 % skim milk
178 solution was added as a blocking agent after washing 3 times with PBS buffer, containing 0.1
179 % Tween 20. After blocking, the 5% skim milk was removed by washing 3 times with the
180 buffer. After that, anti-rabbit IgG-HRP was diluted to 1:4000 with 2 % BSA, and 100 μ L of
181 this solution was added to the well and was incubated for 1 h. 3,3',5,5'-tetramethylbenzidine or
182 TMB (100 μ L) was then added to the each well which was initiated by its characteristic blue
183 coloration. The reaction was then stopped by adding 50 μ L of 10 % H₂SO₄, which changed the
184 color of the solution from blue to yellow due to its diimine formation. The absorbance of the
185 solution was measured using a microplate reader at 450 nm with a reference filter of 655 nm.

186 **2.8. Physicochemical analysis**

187 To check the size and surface morphology, transmission electron microscopy (TEM)
188 images were obtained using a TEM (JEM-2100F; JEOL, Ltd., Tokyo, Japan) operated at 100
189 kV. UV-Vis absorption and fluorescence emission measurements were carried out using a
190 filter-based multimode microplate reader (Infinite® F500; TECAN, Ltd, Männedorf,
191 Switzerland). Powder X-ray diffraction (PXRD) analysis was carried out using a RINT
192 ULTIMA XRD (Rigaku Co., Tokyo, Japan) with a Ni filter and a Cu-K α source. Dynamic light
193 scattering (DLS) measurements were performed using a Zetasizer Nano series (Malvern Inst.
194 Ltd., Malvern, UK). Conjugation of the antibody to the Ab-QDs and Ab-CdSeTeS QD/AuNPs
195 nanocomposites were confirmed using a plate reader from Bio-Rad (Model 680; Hercules,
196 USA).

197 **2.9. Preparation of NoV-LPs and clinically-isolated NoVs**

198 NoV-LPs were prepared according to the standard method of VLP preparation (Ahmed et
199 al., 2017; Jiang et al., 1992). Clinically isolated NoVs were collected from fecal samples of the
200 patients with infectious gastroenteritis, including foodborne illness, by inspections based on
201 laws and ordinances. This NoV sampling was carried out according to the guideline, after
202 getting the approval by Ethics Committee of Environment and Hygiene Institute in Shizuoka
203 Prefecture (September 14, 2016).

204 **2.10. Fluorometric sensing of NoV-LPs and clinically-isolated NoVs using the CdSeTeS** 205 **QD/AuNPs sensing probe**

206 CdSeTeS QD/AuNPs nanocomposite was mixed in different concentration of 20 μL
207 volume of the target NoV-LPs as well as clinically-isolated NoVs and incubated for 1 min
208 before fluorescence measurements were acquired. The detection of NoV-LPs in the
209 concentration range of $10 \text{ fg mL}^{-1} - 100 \text{ ng mL}^{-1}$ was carried out in DI water. The sample
210 solution was excited at 450 nm, and the fluorescence intensity was measured in a range of 500
211 – 700 nm. The human serum was diluted 10 times before to spike the NoV-LPs in to it.
212 Clinically isolated NoVs were also detected with the sensor probe in the similar way to measure
213 the fluorometric response.

214 **2.11. Quantification of clinically-isolated NoVs using real-time PCR**

215 NoV RNAs were extracted from 10 % fecal suspension in PBS by using QIAamp Viral
216 RNA Mini Kit (QIAGEN, Tokyo Japan), and after treated with recombinant DNase (RNase-
217 free) (TaKaRa Bio Inc., Shiga, Japan), reverse transcription was performed by using Prime
218 Script RT Reagent Kit (Perfect Real Time) (TaKaRa Bio Inc.). Obtained cDNAs were detected
219 and quantified by real-time PCR technique by using Premix EX Taq (Probe qPCR) (TaKaRa

220 Bio Inc.) in accordance with the notice of the Ministry of Health, Labor and Welfare, Japan
221 (2003).

222

223 **3. Results and discussion**

224 The central theme in this work is to build a new and simple method to detect virus directly
225 without any pretreatment of analytes. Here, we have successfully synthesized an Ab-CdSeTeS
226 QD/AuNPs sensing probe which is able to detect the NoV-LPs by measuring the fluorescence
227 intensity after 1 min of the sensor probe addition (as depicted in Scheme 1). Due to the covalent
228 attachment between CdSeTeS QDs and AuNPs, it causes strong fluorescence quenching of the
229 QDs, initially. After addition of different virus concentration, the Ab-CdSeTeS QD/AuNPs
230 bind with the target due to the presence of monoclonal antibody in between the QDs and
231 AuNPs. This antibody-antigen interaction induces steric hindrance, which causes the optimum
232 distance for LSPR between these two nanoparticles, resulting fluorescence enhancement. The
233 enhancement is proportionated with the concentration of the target NoV-LPs as well as in real
234 NoV analytes, confirming proficient detection ability of the proposed nanobiosensor. Unlike
235 other conventional methods of LSPR detection, here, the sensor nanocomposite is conjugated
236 with a single antibody and capable to detect the virus, without any pre-treatment just after
237 addition in to the sensing sample.

238 **3.1. Synthesis of sensing probe and characterizations**

239 The morphology as well as size distribution of two individually synthesized nanoparticles
240 was examined first. The shape and morphological properties of the CdSeTeS QDs were
241 analyzed using TEM. A monodisperse particle distribution is observed while the particle shape
242 is consistently spherical across the entire TEM image (Fig. 1A). The particle size distribution

243 has been given in the inset of Fig. 1A where the highly homogenous distribution is found in
244 the range of 3 – 9 nm with the average particle size of 5.9 ± 0.6 nm. The UV-Vis spectrum of
245 the synthesized CdSeTeS QDs is given in Fig. S1, showing the signature absorption hump of
246 CdSeTeS QDs even after the antibody conjugation which confirms the successful synthesis.
247 Similarly, the citrate stabilized AuNPs are also evenly distributed in the range of 7 – 15 nm
248 with the average particle size of 11.4 ± 0.5 nm (Fig. 1B). A single TEM image of an isolated
249 AuNP is given in the inset of Fig. 1B where it is clearly seen the exact spherical nature of the
250 AuNPs. After incorporation with the MUDA, the agglomeration has been reduced to some
251 extent due to the coating of the organic layer on the surface. The capping of the organic layer
252 is verified by the UV-Vis spectra, presented in the supplementary information (Fig. S2). After
253 successful preparation of the Ab-CdSeTeS QD/AuNPs with anti-Nov antibody conjugation,
254 the nanocomposite was further characterized by TEM. In Fig. 1C, it is clearly observed the
255 close conjugation of the QDs with the AuNPs due to their covalent attachment. The antibody
256 conjugation with the CdSeTeS QD/AuNPs nanocomposites was confirmed by ELISA. The
257 absorbance peak in ELISA of the bare CdSeTeS QDs is negligible as expected. The antibody
258 loading is increased to the highest level in case of Ab-CdSeTeS QD/AuNP, confirming the
259 successful antibody conjugation. However, after the NoV-LPs loading, the ELISA signal is
260 decreased obviously due to the less availability of the active site of the NoV-LPs covered Ab-
261 CdSeTeS QD/AuNPs nanocomposites. Overall, the ELISA of different stages of CdSeTeS
262 QD/AuNPs supports the successful conjugation of antibodies with the nanocomposites.

263 The CdSeTeS QD/AuNPs nanocomposites were further characterized by XRD spectra to
264 illustrate the crystal nature of the QD nanocrystals. The diffraction pattern of the QDs indicates
265 that the QDs are crystalline and cubic in nature (Fig. 2A), exhibiting three characteristic peaks
266 at 2theta of 24.9° , 42.3° and 50.6° for (111), (220) and (311) crystal planes (Adegoke et al.,
267 2015; Li et al., 2016; Yang et al., 2013). The position of all these peaks remains unchanged

268 after functionalization with AuNPs, indicating that the attachment only takes place in the
269 functional groups of the CdSeTeS QD nanocomposites without affecting the crystal structure.
270 In addition, a small but clear peak at $2\theta = 37.9^\circ$ has been introduced due to the incorporation
271 of the (111) plane of AuNPs on the nanocomposites (Krishnamurthy et al., 2014), supporting
272 the successful formation of the CdSeTeS QD/AuNPs nanocomposites.

273 The hydrodynamic diameter as well as the dispersity of CdSeTeS QD/AuNPs
274 nanocomposites along with its individual components was determined by DLS (Fig. 2B). The
275 distribution of the AuNPs and MUDA-AuNPs are shown particle size of 8.5 ± 1.1 nm and 11.2
276 ± 1.2 nm, respectively. It proves the monodisperse nature of AuNPs which is not altered even
277 after conjugation with MUDA. Similarly, the cysteine capped CdSeTeS QDs and antibody-
278 conjugated QDs show the hydrodynamic diameter of 29.4 ± 2.3 and 54.2 ± 3.4 nm respectively
279 which differs from the size distribution finding in TEM images. This may be due to the fact
280 that being a small sized and charged particles, the QDs have a strong tendency to agglomerate
281 in aqueous medium, increasing the hydrodynamic radius (Reghuram et al., 2015). However,
282 after the conjugation of these two nanoparticles, the Ab-CdSeTeS QD/AuNPs nanocomposite
283 shows the diameter of 102.1 ± 3.2 nm which is increased up to 107 ± 2.2 nm after the NoV-
284 LPs attachment, confirming the agglomerated distribution, further supported by TEM image,
285 later.

286 **3.2. Optimization of CdSeTeS QD/AuNPs sensor and its mechanism of sensing**

287 After proper characterizations, the Ab-CdSeTeS QD/AuNPs nanocomposite has been used
288 as the fluorometric sensor probe for NoV-LPs detection. Being a strong inorganic QDs with
289 high quantum yield of 0.57 (Takemura et al., 2017), the bare Ab-CdSeTeS QDs show a strong
290 fluorescence signal at 640 nm at the excitation of 450 nm (Fig. 2C). After the covalent
291 attachment with AuNPs, the spectral intensity of Ab-CdSeTeS QDs has been quenched more

292 than 65 % due to the close interaction with AuNPs. The spectral overlap between the surface
293 plasmon spectrum of AuNPs and the emission spectrum of CdSeTeS QDs confirms the
294 quenching interaction, given in Fig. S3. However, after successive addition of NoV-LPs on the
295 nanocomposites, a significant enhancement of the fluorescence signal has been observed due
296 to the enhanced distance between AuNPs and QDs which is the key mechanism of virus
297 detection. It is well known fact that the LSPR properties between any two nanoparticles are
298 highly dependent on their distance and sizes (Guo et al., 2015). Here, the size of QDs and
299 AuNPs are always kept constant at ~6 nm and 12 nm respectively. Therefore, the distance
300 between these two nanoparticles plays the major role of altering fluorescence intensity of QDs.
301 Due to the covalent attachment through a small organic chain of MUDA, the AuNPs and the
302 CdSeTeS QDs are situated in a very closely packed structure (within 6 nm distance), resulting
303 in the initial quenching. However, after the NoV-LPs addition, the NoV-LPs are bound to the
304 Ab-CdSeTeS QD/AuNP due to the antibody-NoV-LPs conjugation. The large size of NoV-
305 LPs compared to those of AuNPs and QDs, induces strong steric repulsion which creates larger
306 distance between two nanoparticles, initiating the LSPR mediated fluorescence enhancement.

307 In most cases of virus detection methods on LSPR, the nanomaterials are conjugated with
308 different specific bio-markers or antibodies, corresponding to the analyte. After analyte
309 addition, the nanomaterials get close towards each other using analyte as a bridge molecule. In
310 this case, QDs are existed and shows high fluorescence intensity before inducing LSPR in the
311 detection system, which is background fluorescence intensity of detection system. In spite of
312 using more than one costly biomarkers or antibodies, this antibody/aptamer linked analyte
313 bridged system highly suffers from non-specific interaction between two nanomaterials,
314 resulting high background signal hence lowering sensitivity. However, here we have made the
315 system rigid by covalent bonding between two nanomaterials, initially which causes strong
316 fluorescence quenching of the QDs. Then, creating steric repulsion, induced by analyte

317 conjugation promotes the LSPR interaction. To optimize the exact size for best results, we have
318 varied the sizes of interacting AuNPs from 5 to 100 nm, maintaining the constant size of
319 CdSeTeS QDs for the detection of 1 ng mL^{-1} NoV-LPs. As shown in Fig. 2D, smaller sized
320 AuNPs have better quenching effects on CdSeTeS QDs. However, evaluating the enhancement
321 as well as quenching factor, the 10 – 12 nm AuNPs has shown best performance for this work.

322 **3.3. Fluorometric sensing of NoV-LPs using the Ab-CdSeTeS QD/AuNPs sensing probe**

323 Detection of the NoV-LPs was carried out to demonstrate the performance of the sensor
324 probe. The LSPR-induced immunofluorescence enhancement for the detection of NoV-LPs
325 and its calibration curve is given in Figs. 3A and B, respectively. The fluorescence at 640 nm
326 of CdSeTeS QDs has been monitored as sensing signal whose intensity is quenched and
327 thereafter enhanced by adjacent AuNPs. At increasing concentrations of NoV-LPs, progressive
328 enhancement of the fluorescence has been achieved without any notable peak shift, providing
329 evidence that the QDs were highly stable during the detection period. The response time is
330 around 1 min after the addition of the target NoV-LPs. The corresponding linear calibration
331 curve is shown in Fig. 3B where the limit of detection (LOD) is found of 8.2 fg mL^{-1} , based on
332 $3 \times$ standard deviation of lowest concentration divided by the slope of calibration curve (Dutta
333 Chowdhury et al. 2017).

334 To verify the LSPR behavior from AuNPs influences, the sensitivity of the biosensor was
335 further carried out by a control test using Ab-CdSeTeS QD. Instead of covalently bonded
336 AuNPs, the AuNPs was only physically mixed with the Ab-CdSeTeS QDs for the detection of
337 the targeted NoV-LPs. As shown in Fig. S4, the fluorescence emission of the Ab-CdSeTeS
338 QDs after addition of AuNPs by only physical mixing was almost unaffected, indicating that
339 without LSPR signal, the target virus cannot be detectable. The changes of fluorescence
340 intensity of the Ab-CdSeTeS QD/AuNPs have been also observed in naked eye in the 450 nm

341 UV light chamber (Fig. 3C). The highly flourished bare Ab-CdSeTeS QDs is significantly
342 quenched after the formation of CdSeTeS QD/AuNPs nanocomposites. However, after
343 addition of 1 ng mL^{-1} NoV-LPs, the enhancement of fluorescence is also observed which
344 confirms the LSPR induced phenomenon. The TEM images of CdSeTeS QD/AuNPs
345 nanocomposites are given in Fig. 3D where the agglomerated CdSeTeS QD/AuNPs
346 nanocomposites are situated clearly on $\sim 40 \text{ nm}$ NoV-LPs surface. The higher magnification of
347 the image of an isolated particle (inset of Fig. 3D) clearly demonstrates the formation of the
348 NoV-LPs conjugated nanocomposites which support our hypothesis. From the aspect of the
349 wide detection range, low LOD and short response time, our nanobiosensor shows much better
350 performances compared with recently published reports, especially in the low detection range,
351 listed in Table 1. In our previous study of LSPR detection, interaction of the target virus with
352 Ab-conjugated AuNPs and other Ab-conjugated QDs induces an LSPR signal from adjacent
353 AuNPs to trigger fluorescence-enhancement changes in the QDs in proportion to the
354 concentration of the target virus. Though the excellent linearity have been achieved in that
355 case, however due to the existence of free QDs in detection solution, the background signal is
356 quite high, resulting higher LOD of 0.03 pg mL^{-1} . In this present study, the covalent attachment
357 between two nanomaterials initially exhibited quenched fluorescence, which could able to
358 show fluorescence enhancement even in presence of small number of virus particles, ensuing
359 low detection limit of 8 fg mL^{-1} . In addition, the rapid detection with high sensitivity of this
360 proposed sensor displays clear advantages over the conventional methods of enzyme
361 immunoassays (EIAs), which requires $\sim 15 - 20 \text{ min}$, and RT-PCR, which requires several
362 hours for detection.

363 **3.4. Selectivity test of the sensor**

364 Selectivity is one of the most important parameters for real sensing application. The
365 selectivity of the nanobiosensor for the detection of the target NoV-LPs was compared with

366 Influenza virus A (H3N2) and Zika viruses. Two percent BSA solution, human serum and 10
367 fold diluted human serum samples were treated as negative control to judge the matrix effect
368 of the biosensor (Fig. 3E). Except the 100 % serum sample, the matrix effects are almost
369 negligible. Due to the presence of huge interfering agents, the sensor cannot perform well in
370 presence of 100 % human serum samples. Therefore, for further investigation in serum samples
371 later, we have used 10 % diluted serum samples. Due to the structural similarities, the sensor
372 has shown almost 32 % signal enhancement for influenza virus. However, the nanobiosensor
373 for the targeted NoV-LPs is greater than that of other viruses, demonstrating the sufficient
374 specificity of our biosensor for the target virus. In addition, some amino acids (2 mM mL^{-1})
375 and metal ions ($100 \mu\text{g mL}^{-1}$) which are common interferences for the real or clinical sample
376 analysis are also investigated in higher concentrations and found ignorable for the detection
377 analysis.

378 **3.5. Sensing in serum sample**

379 Human serum of 10 % was used as a detection medium to demonstrate the ability of the
380 biosensor in a complex biological medium. The biosensor shows a similar trend of detection
381 in the range of the spiked concentration of NoV-LPs which confirms the applicability of the
382 sensor for the real sample monitoring (Fig. 4A). The calibration curve found from the NoV-
383 LPs detection is plotted in Fig. 4B where the slope of linearity is little flattered with respect to
384 the detection found in DI water samples, presented in Fig. 3B. Though the small interference
385 of the serum matrix has lowered the slope of the calibration, decreasing of LOD value to 15.6
386 fg mL^{-1} however the sensitivity is quite appreciable with respect to other reports for its real
387 application.

388 **3.6. Detection of clinically-isolated NoV**

389 The spiked amount of RNA of NoV from clinical sample was also detected by the
390 CdSeTeS QD/AuNPs nanocomposites. The fluorescence intensity was gradually changed as a
391 function of the NoV concentration, following the similar trend as NoV-LPs (Fig. 5A). A linear
392 calibration curve was obtained in the range $10^2 - 10^4$ copies mL^{-1} (Fig. 5B) and the detection
393 limit was 87 copies mL^{-1} . This implies that the NoV from clinical sample was successfully
394 detected by this proposed technique without compromising the efficiency. However, in case of
395 higher NoV concentration of 10^6 copies mL^{-1} , the enhancement turns to quenching of QDs
396 fluorescence (data not shown). This may be due to the fact that in presence of excess virus
397 particles, some viruses themselves can entrap on the QDs surface, resulting quenching. This
398 limits the detection range up to 10^5 copies mL^{-1} , however which is enough for its practical
399 application.

400 For further confirmation, the three spiked samples of clinically-isolated NoV along with
401 two NoV-LPs are tested by commercial ELISA kit (Denka Seiken Co Ltd., Model No. 324603,
402 Niigata, Japan). It is clearly shown in Fig. 5C that the commercial NoV detection kit is unable
403 to detect the virus as well as VLPs concentration in lower range though it is useful for higher
404 concentration range of $10^4 - 10^6$ copies mL^{-1} . In contrast, our proposed sensor shows excellent
405 detectability in the low NoV concentration of $10^2 - 10^5$ copies mL^{-1} .

406 **4. Conclusion**

407 In this study, we have successfully synthesized a new class of nanocomposites which can
408 detect NoV in a single-step and rapid fluorescence based technique. In Ab-CdSeTeS
409 QDs/AuNPs nanobiosensor, the adjacent AuNPs initially quench the fluorescence signal of the
410 CdSeTeS QDs whereas after successful attachment of target NoV-LPs or NoV via antibody-
411 antigen interaction, it triggers the fluorescence enhancement of QDs. The steric repulsion
412 induced by the analyte causes the required distance replacement for the LSPR interaction which

413 is the key reason for obtaining higher sensitivity over other conventional LSPR based
414 biosensors. The enhancement is proportionated with the concentration of the target NoV-LPs,
415 maintaining a linear relationship from 10^{-14} to 10^{-9} g mL⁻¹ with a LOD of 8.2 fg mL⁻¹ in DI
416 water and 15.6 fg mL⁻¹ in human serum, confirming proficient detection of the NoV-LPs. The
417 clinically-isolated NoV from NoV-infected patients was also investigated, and the
418 corresponding sensitivity was found 87 copies mL⁻¹. The easily applicable method of this
419 proposed biosensor can be applied not only for the detection of NoV but also can be served as
420 a general platform by changing the entrapped biomolecules, in the wide variety of other sensing
421 application in future.

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429 **Appendix A: Supplementary data**

430

431 **References**

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530

531 **Table 1.** Comparison of the LSPR-based CdSeTeS QD/AuNPs biosensor with recently
532 reported other virus detection methods in term of limit of detection, range of detection and
533 response time.

534

535 **Figure Captions:**

536 **Scheme 1.** Schematic diagram for the preparation of CdSeTeS QD/AuNPs nanocomposites
537 and its sensing mechanism towards NoV-LPs detection. The close covalent attachment of
538 AuNPs with CdSeTeS QDs effectively quenched the fluorescence signal which has been
539 recovered after NoV-LPs entrapment.

540 **Fig. 1.** (A) TEM image of CdSeTeS QDs (inset: particle size distribution where n=30), (B)
541 TEM image of AuNPs (inset: particle size distribution where n=30 and a single AuNPs), (C)
542 CdSeTeS QDs/AuNPs nanocomposites and (D) ELISA of CdSeTeS QDs, CdSeTeS
543 QDs/AuNPs before and after NoV-LPs addition.

544 **Fig. 2.** (A) XRD of CdSeTeS QDs and CdSeTeS QDs/AuNPs nanocomposites, (B)
545 Hydrodynamic diameter of AuNPs, AuNPs-MUDA, CdSeTeS QDs, Ab-CdSeTeS QDs, Ab-
546 CdSeTeS QDs/AuNPs and NoV-LPs loaded Ab-CdSeTeS QDs/AuNPs, (C) Fluorescence
547 spectra of Ab-CdSeTeS QDs/AuNPs nanocomposites in comparison with bare CdSeTeS QDs
548 and NoV-LPs loaded Ab-CdSeTeS QDs/AuNPs, (D) Effect of different size of AuNPs on the
549 quenching and LSPR effect of Ab-CdSeTeS QDs/AuNPs nanocomposites.

550 **Fig. 3.** (A) Fluorescence emission spectra showing the detection of NoV-LPs in the
551 concentration range of 10 fg mL^{-1} to 100 ng mL^{-1} using the LSPR-induced Ab-CdSeTeS
552 QDs/AuNPs nanobiosensor, (B) Corresponding fluorescence calibration curve for detection of
553 the NoV-LPs. Error bars denote standard deviation of 3 replicate measurements, (C)

554 Fluorescence images of (i) bare CdSeTeS QDs, (ii) Ab-CdSeTeS QDs/AuNPs and (iii) 10 ng
555 mL⁻¹ NoV-LPs loaded Ab-CdSeTeS QDs/AuNPs nanocomposites in normal light and the UV
556 lamp of 450 nm (D) TEM image of NoV-LPs loaded Ab-CdSeTeS QDs/AuNPs
557 nanocomposites (inset: isolated NoV-LP in higher magnification), (E) Selectivity test of the
558 Ab-CdSeTeS QDs/AuNPs nanobiosensor with 30 µg mL⁻¹ of Influenza, 10⁴ PFU mL⁻¹ of Zika
559 viruses and other common amino acids and interfering metal ions.

560 **Fig. 4.** (A) Fluorescence emission spectra showing the detection of the NoV-LPs using Ab-
561 CdSeTeS QDs/AuNPs biosensor in 10 % human serum and its (B) corresponding calibration
562 curve in presence of calibration line (black dots) found in DI water (shown in Fig. 3B). Errors
563 bars denote standard deviation of 3 replicate measurements.

564 **Fig. 5.** (A) Fluorescence emission spectra showing the detection of clinically-isolated NoV in
565 the concentration range of 10² copies mL⁻¹ to 10⁶ copies mL⁻¹ using the LSPR-induced Ab-
566 CdSeTeS QDs/AuNPs nanobiosensor, (B) Corresponding calibration curve for detection of the
567 NoV, (C) Comparison of detection ability of the proposed method (red line) with commercial
568 ELISA kit (blue line) (Lot No. 395121).

569

570 **Table 1.** Comparison of the CdSeTeS/AuNPs biosensor with recently reported LSPR-based
 571 other virus detection in term of limit of detection, range of detection and response time.

Analyte	Linear range	LOD	Response time	References
Influenza	10 – 100 pg mL ⁻¹	0.03 pg mL ⁻¹	5 min	Takemura et al., 2017
Influenza	1 ng – 10 µg mL ⁻¹	1 ng mL ⁻¹	-	Ahmed et al., 2016
Influenza	5 – 50 ng mL ⁻¹	13.9 ng mL ⁻¹	15 min	Chang et al., 2010
Norovirus	$1.58 \times 10^5 - 7.9 \times 10^7$ copies mL ⁻¹	9.5×10^4 copies mL ⁻¹	10 min	Han et al., 2016
Dengue	5 – 500 ng mL ⁻¹	5.2 ng mL ⁻¹	45 min	Linares et al., 2013
NoV-LPs	$10^{-14} - 10^{-9}$ g mL⁻¹	8.4 fg mL⁻¹	1 min	This work
Norovirus	$10^2 - 10^5$ copies mL⁻¹	87 copies mL⁻¹		

572