



## **Assessment of Altered Fucosylation of Serum $\alpha$ -1-Acid Glycoprotein in Hepatocellular Carcinoma Patients by Gold-Nanoparticle Aggregation Immunoassay**

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### **Abstract**

Gold nanoparticles have attracted considerable attention due to their unique properties and potential applications as optical probes. Proteins that are adsorbed on gold nanoparticles get cross-linked by any interaction specific to that protein, the size of the aggregates increases and this enhancement of size have been used for sensitive, convenient and powerful tool to monitor the presence of the specific cross-linkers. Dynamic light scattering (DLS) is a technique that is routinely used for detecting aggregation in macromolecular solutions. In this work, we first applied DLS to identify specific glycoprotein–lectin interactions exclusively present in the serum of hepatocellular carcinoma patients compared to healthy controls that showed the altered fucosylation of a serum protein, Serum  $\alpha$ -1-Acid glycoprotein. Further, based on the DLS data a simple, rapid, serological assay was developed based on antibody coated gold nanoparticle and fucose binding lectin (Aleuria aurantia lectin) as linker to assess the level of fucosylation of  $\alpha$ -1-acid glycoprotein. As a consequence of the triggered aggregation of the GNP probes in presence of lectin, plasmon band was shifted from red to blue, which colorimetrically reported the enhanced fucosylation of  $\alpha$ -1-acid glycoprotein and formed the basis of a rapid visual assay for hepatocellular carcinoma.



### **Article History**

Received: 22 November 2018

Accepted: 12 December 2018

### **Keywords:**

Aleuria Aurantia Lectin,  
Biomarker,  
Fucosylation,  
Gold Nanoparticle,  
Hepatocellular Carcinoma

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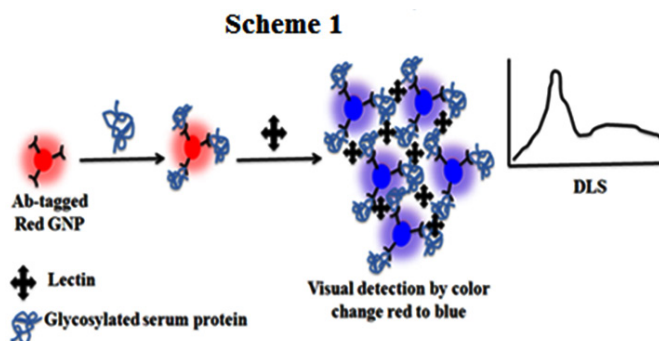
Doi: <http://dx.doi.org/10.13005/msri/150309>

## Introduction

Glycosylation is one of the most common modifications of proteins; it regulates protein-protein interactions, cell-cell recognition, adhesion, and motility. Development of disease and its progression are very often associated with alterations in glycosylation on tissue proteins and/or serum proteins. The relationship between serum glycoproteins, their uncontrolled synthesis and clearance in liver suggests that liver abnormalities are closely associated with aberrant microheterogeneity of serum protein N/O-glycoforms found in diverse liver diseases especially in cancer. The increase of fucosylation on certain serum glycoproteins, such as haptoglobin,<sup>1</sup> cholinesterase<sup>2</sup>,  $\alpha$ -1-acid glycoprotein (AGP),<sup>3,4</sup>  $\alpha$ -fetoprotein (AFP)<sup>5</sup> and  $\alpha$ -1-antitrypsin (AAT) was observed in liver cirrhosis and hepatocellular carcinoma (HCC). Assessments of glycosylation of serum proteins by lectins have been employed in diverse form to detect new glycosylation biomarkers for various liver diseases. Microheterogeneity of serum transferrin was determined in alcoholic liver disease using concanavalin A affinity method.<sup>6</sup> Among the viral liver diseases, the hepatitis B virus (HBV) infection has been the most extensively investigated method with respect to its associated changes in glycosylation in different stages of the infection. Alteration in glycosylation between HBV and HCC showed many similarities, because of the high correlation of long-term HBV infection and the increased risk of HCC. Our previous works has established the clinical relevance of aberrant glycosylation of different serum proteins by thoroughly determining their lectin-based interactomics in this regard.<sup>4,5</sup> In our current

endeavor we monitored alteration in the lectin-carbohydrate interaction of a specific biomarking serum glycoprotein (AGP) with the utilization of dynamic light scattering (DLS) and on the basis of that a rapid and easy visual assay for the relevant clinical diagnosis of HCC has been introduced.

Gold nanoparticles (GNPs) have received considerable attention due to their ease of synthesis, flexible surface modification, bioconjugation and optical properties. Formation of aggregates caused a change in color of GNP suspension from red to blue or violet due to the blue shift of surface plasmon band in aggregated GNPs.<sup>7</sup> GNPs have a large scattering cross section in the surface plasmon resonance wavelength region allowing the size distribution of the GNP probes to be measured by DLS. The strategy adopted in this work for detection of aberrant glycosylation pattern of a biomarking serum protein by its interaction with antibody functionalized GNP and their subsequent aggregation triggered by added lectin are depicted in Scheme 1. A key feature of this approach was to fish out the targeted serum glycoprotein from sera to the specific antibody coated GNP surface that leads to trigger the aggregation of the GNPs. It has been documented in literature that the binding of glycoproteins to GNP surfaces promoted protein unfolding.<sup>8</sup> We experienced that in our strategy also; the arrest of serum protein on the GNP surface would make glycan-lectin interactions more favorable by partially exposing the carbohydrate ligands that eventually would enhance the sensitivity of the protocol. Subsequent addition of a lectin, specific to the biomarking glycosylation pattern of the



**Scheme 1: Schematic representation of the estimation of glycosylation in a specific serum glycoprotein by lectin-GNP aggregation immunoassay**

glycoprotein will trigger cross-linking among the glycoprotein-bound GNPs that causes aggregation to change scattering efficiency and optical properties of the solution with the characteristic visual change in color from red to blue. Recently, immunoassay using gold nanoparticles and dynamic light scattering (DLS) analysis have been reported for sensitive detection and accurate analysis of serological cancer biomarkers.<sup>9</sup> In this study we have investigated the change in fucosylation of serum AGP using GNP aggregation. Initial optimization of lectin crosslinking triggered GNP aggregation was performed with a commercial model protein (Haptoglobin; Hp) and a non-specific lectin Concanavalin A and the method was subsequently applied for the detection of the fucosylation of serum AGP, an established biomarker for HCC. The change in fucosylation level was monitored using a fucose specific lectin, Aleuria aurantia lectin (AAL). The interaction of AAL with serum AGP that already bound to antibody coated GNP was monitored using DLS, TEM and UV-visible absorbance measurements.

## Materials and methods

### Materials

N-Hydroxy succinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC),

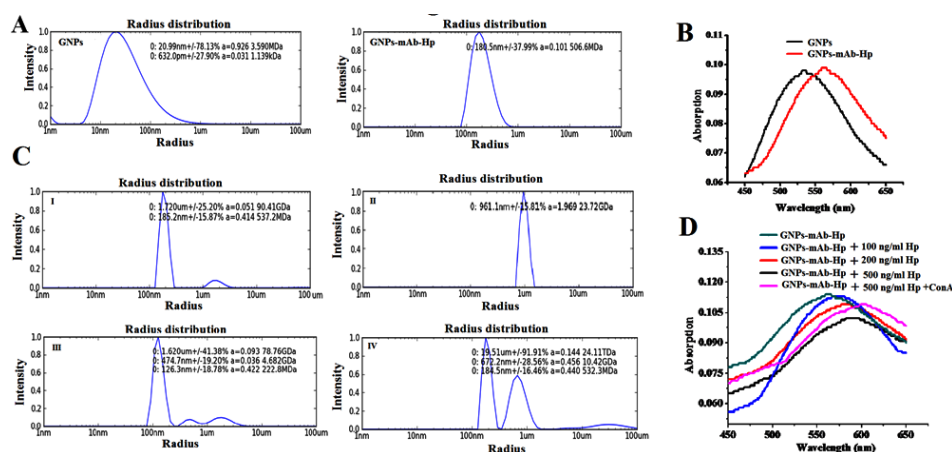
chloroauric acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), lipoic acid (LPA), haptoglobin (Hp), Concanavalin A (ConA), monoclonal anti-human AGP and Hp (mAb-AGP and mAb-Hp) were purchased from Sigma-Aldrich. Aleuria aurantia lectin (AAL) was purchased from Vector Laboratory. All other analytical grade reagents were locally procured.

### Subjects and ethics statement

Serum samples from 20 hepatocellular carcinoma (HCC) patients (study group) were collected from the outpatient clinic of Hepatology, the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The sera from 20 age and sex-matched healthy individuals were taken as healthy controls. All subjects were diagnosed by serological-radiological detection according to the standard medical practice (Table 1) and were recruited in this study with their informed consent. The ethical committee of the PGIMER approved this study (Micro/2010/1092/3rd Mach 2010).

### Deglycosylation of monoclonal anti-AGP (mAb-AGP)

Deglycosylated mAb-AGP was prepared by sodium periodate oxidation.<sup>7</sup> Monoclonal antihuman AGP and monoclonal antihuman Hp was treated with 50



**Fig. 1:** (a) DLS measurement of GNPs and anti-Hp functionalized GNPs (GNPs-mAb-Hp). (b) UV/Vis spectra of GNPs and anti-Hp functionalized GNPs solution (GNPs-mAb-Hp). (c) Measurement of size dependent increase of hydrodynamic radius of gold nanoparticles aggregates after immune reaction with different concentration (I) 100 ng/ml, (II) 200 ng/ml and (III) 500 ng/ml of Hp (IV) 500 ng/ml of Hp + Concanavalin A (1 µg/ml) by DLS; (d) UV/Vis spectra of different concentration of Hp after binding with antibody functionalized GNPs (GNPs-mAb-Hp) in presence of added Concanavalin A.

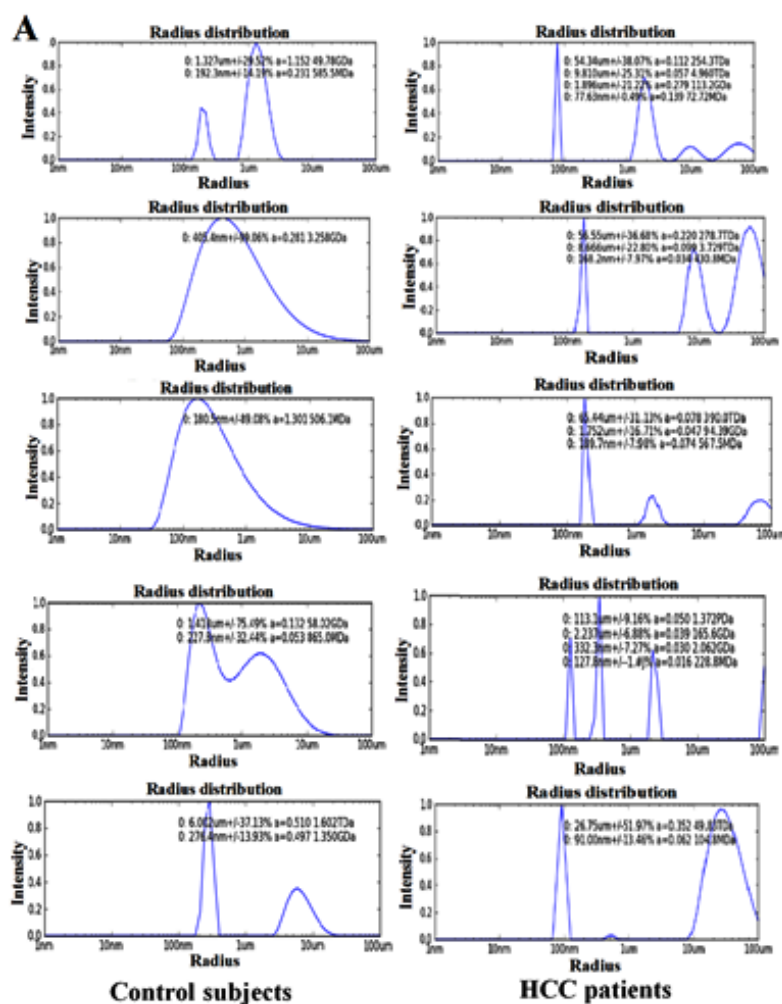
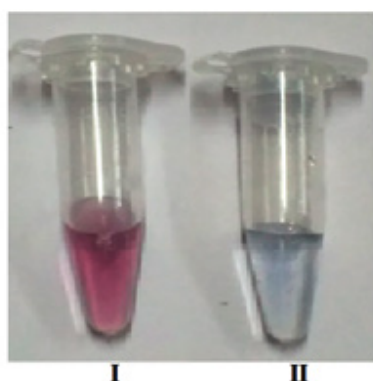
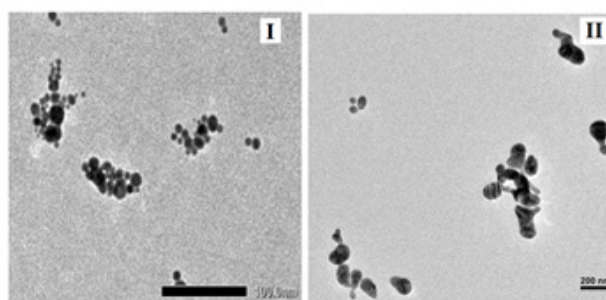
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Fig. 2: (a) The particle size distribution curves of assay solution from 5 HCC patients and 5 control subjects analyzed by DLS measurement. (b) Visual colour of (I) antibody-functionalized GNPs (GNPs-mAb-AGP) and (II) antibody functionalized GNP bound with serum AGP and cross-linked by AAL. (c) TEM images of (i) non-aggregated red particles and (ii) aggregated blue particles for anti-AGP tagged GNP and the test solution of patient 1 after AAL cross-linking, respectively.

mM sodium periodate in 50 mM sodium acetate, pH 4.0 followed by incubation for 2 h in the dark at 4°C. Afterwards, the reaction mixture was dialyzed into 10 mM PBS (pH 7.4) overnight with three changes of the buffer prior to use in the following experiments.

#### Preparation of gold nanoparticles (GNPs)

Gold nanoparticles were synthesized by Turkevich method.<sup>10</sup> 6 mL of 0.5% trisodium dihydrate citrate solution was rapidly added with continuous stirring to 25 ml double distilled water with 24  $\mu$ l of 500 mM HAuCl<sub>4</sub> solution at 70-80 °C. The mixture was boiled and stirred till color turned into red wine color and then mixture was cooled to room temperature.

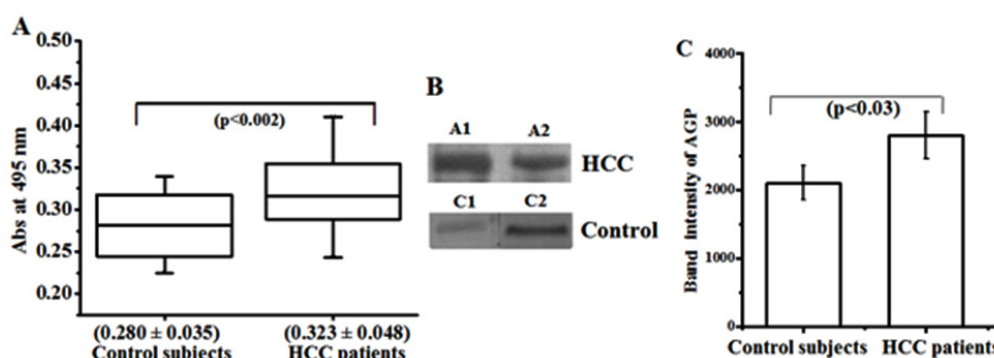
#### Preparation of antibody-gold nanoparticle conjugates

The citrate-stabilized gold nanoparticles were centrifuged (10 min, 9500 g) and dispersed in sodium phosphate buffer (pH 7). The surface modification of GNPs using  $\alpha$ -lipoic acid (LPA) was done according to Nietzold *et al.*<sup>11</sup> Briefly, GNPs and LPA (400  $\mu$ M) in phosphate buffer were gently mixed and kept at room temperature for 1 h in stirring condition. After removing the excess of LPA by centrifugation, the SAM (self assembled monolayer) layered gold nanoparticles were dispersed in sodium phosphate

buffer (pH 7.5). Then 1% EDC (in water) was added to the LPA coated GNPs solution and after gentle mixing, 1% NHS (in DMSO) was added and the reaction was continued for 15 min followed by centrifugation. After this, monoclonal anti-Hp (20 g/ml in 5 mM PBS, pH 7.5) was added to functionalize GNPs. The reaction was continued for 30 min at room temperature and the antibody functionalized GNPs (GNPs-Ab) were centrifuged for 5 min to remove unbound antibody. Finally, the antibody functionalized gold nanoparticles (GNPs-mAb-Hp) were resuspended in PBS buffer. Same procedure was followed for the preparation of antibody functionalized gold nanoparticles (GNPs-mAb-AGP) using monoclonal anti-AGP.

#### Immune assays with commercial Hp

The colloidal gold-immunocomplex with Hp was prepared by mixing the antibody functionalized GNPs (GNPs-mAb-Hp) and Hp (Commercial Hp, Sigma) in a ratio of 9:1 (v/v). Different concentrations (100, 200, 500 ng/ml) of Hp was added in antibody functionalized GNPs and the mixture was incubated for 10 min at 25 °C. After centrifugation, the size of the immunoaggregates and absorption maximum was measured by DLS and UV/Vis spectrophotometer respectively. DLS and UV/Vis spectra were recorded



**Fig. 3.** (a) Determination of AGP fucosylation level in control subjects and HCC patients' group using AAL. Each horizontal line indicates the mean value of lectin binding with serum AGP. Data was presented as mean  $\pm$  SD. Statistical analysis was done using Student's t test and  $p < 0.005$  was considered as statistically significant. (b) Lectin blotting of serum AGP with AAL from HCC patients' group and control subjects. In control ( $n=20$ ), C1 and C2 represent pool consisted of 10 samples each. Similarly, in HCC patients' group ( $n=20$ ) A1 and A2 represent pool consisted of 10 samples each (c) Mean density of AAL reactive AGP band in control subjects and HCC patients' group were plotted on Y-axis. Statistical analysis was performed using One way Anova test ( $p < 0.05$ ).

after addition of Concanavalin A (Con A, 1 µg/ml) with Hp (500 ng/ml), which was bound to anti-Hp-antibody-GNPs (GNPs -mAb-Hp).

#### Enzyme linked lectin sorbent assays (ELLSA) of patient sample using gold nanoparticles

In this assay system, the diluted sera from HCC patient groups and control subjects (AGP concentration adjusted to 500 ng/ml) was added to the antibody functionalized gold nanoparticles (GNPs-mAb-AGP) in a ratio 1:9. The mixture was incubated for 10 min at 25 °C followed by addition of *Aleuria aurantia* lectin (AAL, 1 µg/ml) to the reaction mixture. After incubation another 10 min, the solution was centrifuged and resuspended in buffer. The size of the immunoaggregates and spectral shift in solution was measured by DLS and UV/Vis spectrophotometer.

#### Fucosylation level of serum AGP by classical ELISA

The fucosylation level of AGP was assessed by ELISA using a fucose binding lectin, *Aleuria aurantia* lectin (AAL).<sup>12</sup> The wells of the microtiter plate were coated with 100 µl (2 µg per well) of monoclonal anti-AGP in bicarbonate buffer (0.01 M Na<sub>2</sub>CO<sub>3</sub> and 0.035 M NaHCO<sub>3</sub>, pH 9.6). The plates were kept at 4 °C for 24 h, washed with 100 µl 0.01 M PBS, pH 7.4, and containing 0.05% Tween-20 and incubated with 100 µl of PBS containing 1% BSA at 37 °C for 1 h. To each well, 100 µl of diluted sera of

HCC patients and control groups were added (AGP concentration was adjusted to 500 ng/ml with proper dilution in PBS). The plates were incubated at 25 °C for 1 h and then 100 µl of biotinylated AAL (1: 1000 in blocking buffer) was added. On further incubation for 1 h and washing, 100 µl of streptavidin–HRP conjugate (1:10000 in blocking buffer) were added and incubated at room temperature for 1 h. After that 0.1% O-phenylenediamine dihydrochloride (OPD) (100 µl) and 0.05% H<sub>2</sub>O<sub>2</sub> in 0.05 M citrate phosphate buffer (pH 5.0) were added to each well. The plate was left for 30 min at room temperature. The absorbance of each well was measured at 490 nm by ELISA Reader. All experiments were done in triplicate.

#### Lectin blotting

After purification of serum AGP by anti-AGP affinity chromatography from HCC patients groups and healthy groups was transferred from SDS-PAGE gel to the NC membrane.<sup>13</sup> The membrane was blocked with 5% BSA prepared in PBS containing 0.1% Tween-20 (PBST) for 1 h at room temperature. After washing, the membrane was treated with 1 µg/ml of biotinylated *Aleuria aurantia* lectin (AAL) prepared in PBST at room temperature for 1 h. The membrane was washed again 3 times with PBST and HRP conjugated streptavidin (dilution 1:10,000) was added and kept for 1 h at room temperature. The blots were developed using developing solution, which contained diaminobenzidine and 0.01% H<sub>2</sub>O<sub>2</sub>.

**Table 1. Clinical features of hepatocellular carcinoma (HCC) patients and control subjects**

| Groups               | Control (n=20) | HCC (n=20) |
|----------------------|----------------|------------|
| Age (years)          | 25–64          | 30–70      |
| Gender (M/F)         | 15/5           | 15/5       |
| AFP (ng/ml)          | 26±2           | >450       |
| ALT (IU/l)           | 5–40           | 40–275     |
| AST (IU/l)           | 5–40           | 35–305     |
| ALP (IU/l)           | 35–125         | 97–825     |
| Bilirubin (g/dl)     | 3.6–5.0        | 0.45–2.2   |
| Albumin (g/dl)       | 3.5–5.5        | 2.4–4.5    |
| Prothrombin time (s) | 12–16          | 14–17.2    |
| HBsAg                | -              | +          |
| Anti-HCV             | -              | +          |

in sodium acetate buffer (pH 5). The image analyses of four differentially expressed protein spots were calculated by image J software.

#### **UV-Vis analysis**

UV-Visible absorbance measurements were carried out by using a UV/Vis spectrophotometer (Shimadzu 1800) using a quartz cuvette of 1 cm path length. The absorption spectrum was measured from 650 to 400 nm against PBS buffer.

#### **Dynamic light scattering measurements**

The hydrodynamic diameter of aggregated (before and after cross-linking) was evaluated on Xtal SpectroSize 300 analyzer at the 90° scattering angle and the wavelength was 660 nm. The hydrodynamic diameters of all samples were recorded at 20 °C. One hundred microliter of the prepared sample was transferred to a cuvette for DLS. In DLS, each sample was measured 10 times (10 seconds).

#### **Characterization GNP by transmission electron microscopy (TEM)**

The aggregation state of GNPs obtained after the assay was determined by TEM analysis. Briefly, 10 µl of antibody functionalized GNP (GNPs- mAb-AGP) and antibody functionalized GNP bound with serum AGP and cross-linked by AAL from the reaction mixtures were dropped on a carbon coated copper grid (300 mesh). Then the grid containing the drop of the sample solution was dried under vacuum. All the TEM measurements were performed in JEOL 2010 under an accelerating voltage of 100 kV.

#### **Results and Discussion**

Glycosylation carries out a diverse modification on proteins, mainly found in extracellular environments, including cell surface-membrane proteins, and secreted proteins. These proteins are easily accessible for diagnostic purposes. The analyses of the glycosylation patterns have potential to identify disease associated glycosylation isoforms that increased the clinical performance of glycoproteins in diagnosis. Most relevant marker proteins are often present in low abundance. The analytical sensitivity of conventional glycosylation analysis platforms including chromatography, or electrophoretic methods is not sufficient for discovery, validation and monitoring of the glycosylation changes between different disease states in clinical samples. Moreover,

to validate different biomarking glycosylation changes in a cohort of HCC, a sensitive and high throughput screening platform is necessary to conclude the global screening and monitoring of the stages of the fatal diseases. Liver biopsy which is the gold standard method in the diagnosis of HCC is highly invasive and can not be used as HCC monitoring. Thus, for follow up and other routine monitoring of HCC, development of a rapid, sensitive and specific detection tool is a requirement for better clinical management of the fatal disease.

It has already been demonstrated that during the progression of HBV/HCV to HCC overall fucosylation of AGP were enhanced and that had introduced the serum AGP concentration and overall fucosylation of AGP as a sensitive monitoring biomarkers for HCC.<sup>14</sup> In our design we used fucose specific lectin AAL to trigger cross-linking among the immune arrested AGP on GNP (coated with anti-AGP antibody) that eventually promoted the aggregation as monitored by DLS (Scheme 1). We employed antibody (anti-AGP) tagged GNP that arrested the AGP from sera and on interaction with GNP surface there had been a putative exposure of glycan part of bound AGP which made the subsequent fucose-AAL crosslinking interaction more feasible and enhance the sensitivity of the plasmonic assay. In current assay we had used same AGP concentrations for all the clinical samples tested and that ensured the propensity of AAL-triggered aggregation to be linear with the abundance of fucose in bound AGP, which could reflect the stage of HCC of the corresponding patients.

#### **Characterization of the GNP aggregates**

For detailed characterization of nanoparticle aggregates, first GNPs were prepared by the classical reduction of the chloroauric acid with trisodium citrate. In order to stabilize the negative charge on the particle surface, a ligand LPA was used. The functional groups of LPA can be exploited for the conjugation of capture antibody that can be reacted with primary amines by means of EDC mediated condensation reaction to yield amide bonds. The size distribution of the GNP and antibody functionalized GNP (GNPs-mAb-Hp) were determined using DLS. The average hydrodynamic diameter of the GNPs as measured by DLS was 21 nm and after antibody conjugation the average size of the particles was

increased to 180 nm (Fig 1a). UV–visible absorbance analysis was also used to evaluate the conjugation of antibody to the GNPs. Unmodified GNPs exhibit a characteristic absorbance peak ( $\lambda_{\text{max}}$ ) at 533 nm due to the surface plasmon resonance of the colloidal gold, whereas the  $\lambda_{\text{max}}$  value of antibody-modified GNPs shifted to 562 nm (Fig 1b). The 29-nm shift was likely due to the changes in the refractive index at the GNP surface upon antibody conjugation and was consistent with those reported for the adsorption of proteins to colloidal gold.<sup>15</sup>

#### **Optimization of immunosorbent assays with Hp**

Upon addition of commercial Hp to the Hp-antibody functionalized GNPs, Hp molecules were arrested on the surface of GNP. In order to evaluate the formation of nanoparticle aggregates and its dependence on the concentration of protein (Hp), measurements of the hydrodynamic radius of aggregates were performed with different concentrations of Hp (100, 200 and 500 ng/ml). The measurements confirmed the formation of immune complexes by absorption on the GNP surface via sandwich of antibodies and Hp. The size analysis of the aggregates by DLS demonstrated that there was an increase in size of nanoparticles from 180 nm to 185 nm for 100 ng/ml Hp, 961 nm for 200 ng/ml and  $\sim 2 \mu\text{m}$  in case of 500 ng/ml Hp. Further size increase ( $\sim 20 \mu\text{m}$ ) was observed when Concanavalin A ( $1 \mu\text{g/ml}$ ) was added with 500 ng/ml Hp (Fig.1c) that reflected the lectin triggered cross-linking between the Hp-carbohydrates on different GNPs. Concanavalin A is a lectin with very broad specificity, because of that it was used in the optimization step to ensure crosslinking via glycans on the Hp molecules. This change has also been reflected by UV/Vis spectroscopy and the absorption maximum was shifted with the increasing concentration of the protein. The functionalized GNPs cross-linked by Hp showed a shift of the plasmon adsorption. The plasmon absorption band at 562 nm for the antibody-functionalized nanoparticles was found to be shifted to 573 nm for the concentration of 100 ng/ml, 586 nm for 200 ng/ml and 591 nm for 500 ng/ml of Hp and further shift to 605 nm after addition of Concanavalin A with Hp that reflected the formation of cross-linked aggregates having enhanced sizes. The corresponding spectra are shown in Figure 1d.

#### **Analysis of patient serum AGP fucosylation by lectin triggered GNP aggregation**

After establishing the protocol for the assay system with the commercial protein Hp, the system was modified to detect AGP glycosylation pattern in clinical samples of patients suffering from HCC and compared with the healthy controls as aberrant fucosylation of serum AGP is an established serum biomarker for HCC. For this assay, same GNP immobilization procedure was followed as done in case of Hp. After addition of diluted sera from both patient and control groups with GNPs-mAb- AGP, fucose specific lectin-AAL was added to the reaction mixture. The binding of serum AGP to antibody-functionalized nanoparticles and subsequent binding with AAL was confirmed by DLS measurements. A significant increase in the average particle diameter was observed after immune reaction of HCC patient groups. The particle distribution curves of the assay solution revealed significant differences on the complex/aggregates level between patient and control subjects. The particle size distribution in the test solution was significantly higher in radius and polydispersed for HCC patients groups (Fig. 2a) whereas nearly monodispersed radius distribution was recorded in control subjects. This change of particle size distribution pattern reflected the crosslinking between AAL and fucose on the AGPs arrested on different neighboring GNPs (Scheme 1). There was an associated visual color change also from red to blue after 10 min upon increase in aggregation in patient's sample due to enhanced fucosylation and more cross-linking and this may be utilized for a development visual clinical analysis of AGP fucosylation trend in patient's sample (Fig. 2b). The transmission electron microscopy images in Fig 2c illustrated the morphological differences and the aggregation state of GNPs as non-aggregated, non-cross-linked (quasi-spherical) and cross-linked (irregular) with an increase in size.

#### **Validation of AGP fucosylation by ELISA and lectin blot**

To assess the extent of fucosylation level of AGP in the sera of HCC patients as well as normal subjects, sandwich ELISA using monoclonal anti-AGP antibody and biotinylated AAL were performed. The sera was diluted such that the amount of AGP added

per well was 500 ng, both for HCC patient groups and control groups and this allowed to compare the fucosylation status of AGP for patients and controls directly from the OD values of the corresponding wells. The extent of AGP-fucosylation was found to be significantly high ( $p < 0.002$ ) in the HCC patient group ( $0.323 \pm 0.048$ ) than control groups ( $0.280 \pm 0.035$ ) for healthy controls (Fig. 3a).

Next, we studied the same AAL activity of the serum AGP, i.e. the extent of fucosylation by lectin blot analysis. The fucosylation status of serum AGP by lectin blotting showed a significantly enhanced binding in the HCC patient group ( $p < 0.03$ ) as compared to healthy controls (Fig. 3b). Densitometric analysis of blot by Image J software also revealed a substantial difference in the lectin (AAL) reactivity with of serum AGP between HCC patients and control groups (Fig. 3c) that substantiate the DLS results and the result for colorimetric assay for the corresponding subjects.

### Conclusion

In this study, a simple, rapid and sensitive method has been developed for the detection of change

in fucosylation of serum AGP, a serum biomarker for HCC, based on the aggregation of antibody-functionalized GNPs. This protocol is unique for the detection of glycosylation change in other serum glycoprotein biomarkers through the formation of crosslinked aggregate of GNP in presence of low sample marker concentration (ng/ml), as is required for clinical diagnosis. The antibody-functionalized GNPs cross-linked by AGP upon addition of AAL have shown a visual color change from red to blue of the plasmon absorption due to the presence of high level of fucosylation, which is having the diagnostic implication of a visual analysis of AGP-fucosylation in HCC samples.<sup>7</sup> The results established a generic platform for the rapid, sensitive and visual detection of glycosylation change by the use of antibody-functionalized GNPs for determining the levels of serum glycoprotein biomarkers.

### Acknowledgement and Funding Source

PPB thanks the Amity Institute of Applied Sciences, Amity University, Noida, India. Authors declare no conflict of interest in this work.

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