

Electrostatic and Covalent Assemblies of Anionic Hydrogel-Coated Gold Nanoshells for Detection of Dry Eye Biomarkers in Human Tears

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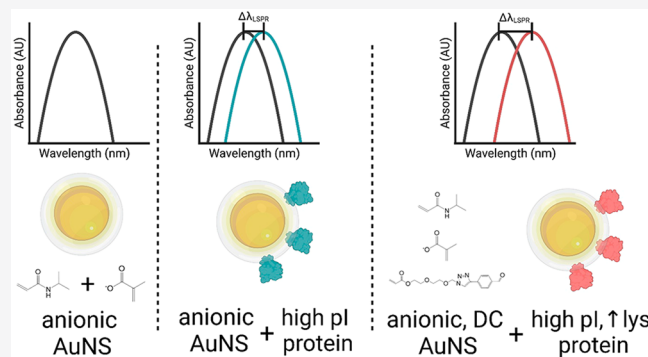
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ABSTRACT: Although dry eye is highly prevalent, many challenges exist in diagnosing the symptom and related diseases. For this reason, anionic hydrogel-coated gold nanoshells (AuNSs) were used in the development of a label-free biosensor for detection of high isoelectric point tear biomarkers associated with dry eye. A custom, aldehyde-functionalized oligo(ethylene glycol)-acrylate (Al-OEGA) was included in the hydrogel coating to enhance protein recognition through the formation of dynamic covalent (DC) imine bonds with solvent-accessible lysine residues present on the surface of select tear proteins. Our results demonstrated that hydrogel-coated AuNSs, composed of monomers that form ionic and DC bonds with select tear proteins, greatly enhance protein recognition due to changes in the maximum localized surface plasmon resonance wavelength exhibited by AuNSs in noncompetitive and competitive environments. Validation of the developed biosensor in commercially available pooled human tears revealed the potential for clinical translation to establish a method for dry eye diagnosis.

KEYWORDS: biosensor, dry eye, gold nanoshell, hydrogel, localized surface plasmon resonance, tears



The use of screening and diagnostic tests is critical for decision making in the clinical milieu.¹ However, the expense associated with such tests can affect patient compliance, especially when the costs are out of pocket. In the era of value-based healthcare, tests must be affordable and possess value in a clinical setting.¹ In the symptomatic case of dry eye (frequently associated with many diseases, a side effect of several medications, and a condition related to many factors), patients with this indication can make for a difficult diagnosis if the symptom is associated with the onset of disease. Specifically, in the case of Sjögren's syndrome (a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands), patients are often unaware that this disease may be present until they suffer from extreme dry eyes and mouth.²

Despite the high prevalence of dry eye, it is frequently under-recognized and can result in the impairment of a patient's visual function and quality of life.³ Currently, the diagnosis of dry eye is based on a clinical examination, accompanied by several tests to evaluate the ocular surface and tear film production.^{3–5} While these tests are standard and necessary diagnostic approaches for dry eye, many clinicians

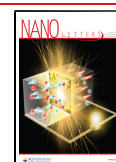
and researchers have reported the need for the identification, validation, and detection of tear biomarkers to assist in reaching a conclusive diagnosis, especially in cases where symptoms are related to the presence of autoimmune diseases.^{4,6,7}

Commercially available assays that are utilized in the clinic to monitor select protein concentrations in tears for dry eye diagnosis include enzyme-linked immunosorbent assays (ELISAs),⁴ the TearScan Microassay System,⁸ and InflammDry.⁹ While these tests are needed to reach a diagnosis, all either require specialized equipment or antibodies that are difficult to manufacture, often have poor environmental stability, or are relatively high in cost.^{10,11} To overcome some of the downsides associated with antibody use, lower-cost synthetic materials, such as various polymeric micro- and

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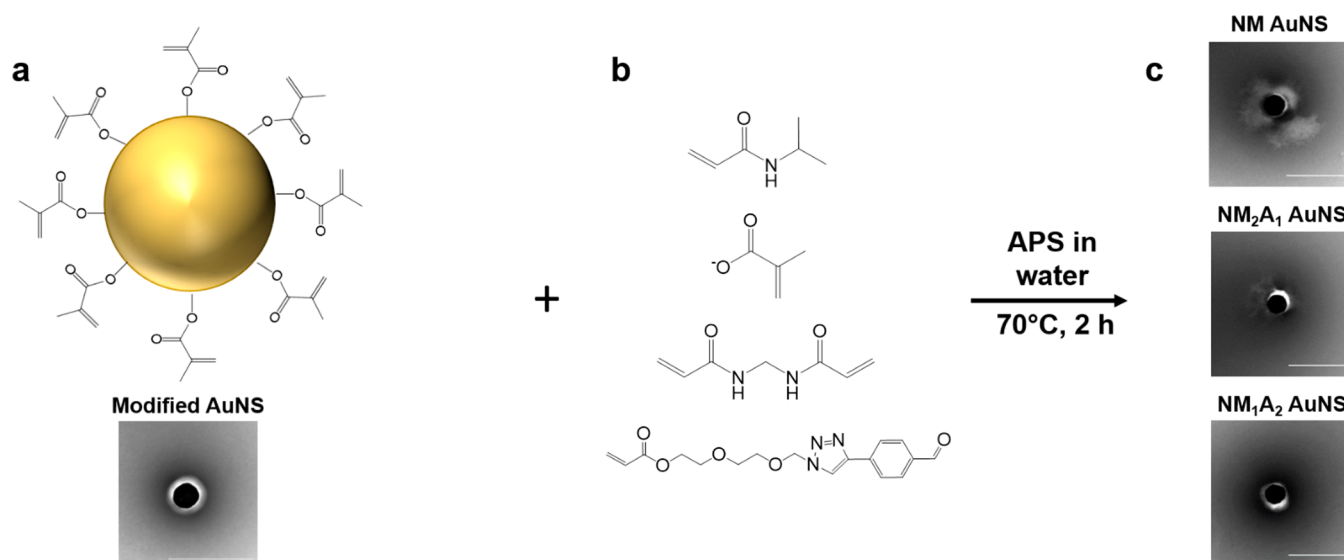


Figure 1. Synthesis of hydrogel-coated gold nanoshells. (a) Methacrylate-modified silica core gold nanoshells (AuNSs) were combined with (b) monomers (*N*-isopropylacrylamide, methacrylic acid, *N,N'*-methylenebis(acrylamide), and aldehyde-functionalized oligo(ethylene glycol)acrylate) in water. The monomers in solution with AuNSs were purged with nitrogen while heating under constant stirring. The polymerization was initiated with ammonium persulfate (APS) in water and carried out at 70 °C for 2 h. The resulting hydrogel-coated AuNSs were dialyzed and washed with water. (c) Transmission electron micrographs were obtained to determine the hydrogel coating modification on the surface of AuNSs. Abbreviations: NM, P(NIPAM-*co*-MAA); NM₂A₁, P(NIPAM-*co*-MAA₂-*co*-Al-OEGA₁); NM₁A₂, P(NIPAM-*co*-MAA₁-*co*-Al-OEGA₂). Subscripts in hydrogel-coated AuNS formulations refer to the MAA:Al-OEGA ratio included in the polymerization. Hydrogel-coated AuNSs were stained with 2% phosphotungstic acid in water (pH 7.0). Scale bar: 500 nm.

nanogels, have been engineered with molecular recognition properties for the detection of disease biomarkers.¹² Although these materials do not rival the selectivity of antibodies, they do provide promising alternatives for use in multiplex biosensors, which are of high interest in the biosensing field due to their high tunability, use of inexpensive materials, and environmentally robust stability.^{13–16}

Our laboratory has previously demonstrated that poly(*N*-isopropylacrylamide-*co*-methacrylic acid) P(NIPAM-*co*-MAA) nanogels have the ability to serve as signal transducing agents through increases in turbidity associated with increases in refractive index.¹⁷ In addition, these P(NIPAM-*co*-MAA) nanogels could be used as high isoelectric point tear protein receptors¹² or protein protective agents.¹⁸ When a custom aldehyde-functionalized oligo(ethylene glycol)acrylate (Al-OEGA) monomer (which enables the dynamic formation of covalent bonds with amines or solvent-accessible lysine residues on the protein surface¹⁹) was incorporated into the P(NIPAM-*co*-MAA)-based polymer, no difference in protein binding was observed in comparison to anionic nanogels alone, indicating that electrostatic interactions are the main driving force behind protein–polymer interactions at the bulk nanogel level.¹²

However, it is known that plasmon-based sensing, through the use of noble metals, can be utilized to achieve increased protein recognition and binding. Specifically, noble-metal nanomaterials with longer (i.e., red-shifted) localized surface plasmon resonance (LSPR) wavelengths have enhanced sensitivity to the local refractive index.²⁰ One method to red-shift the LSPR wavelength is to increase the aspect ratio of the nanomaterial.²¹ While materials such as gold nanorods and nanostars have high aspect ratios and exhibit red-shifted LSPR wavelengths, these materials are anisotropic.^{22,23} Thus, plasmonic hot spots arise in areas where the plasmon resonance is stronger and more sensitive. In relation to use

for investigation of protein binding, the LSPR absorbance wavelength will be highly dependent on the location of the nanomaterial that the protein binds. To overcome this, isotropic, spherical gold nanoshells can be utilized to promote a more uniform sensing response.²⁴ In addition, gold nanoshells also exhibit longer LSPR wavelengths, in comparison to gold colloids, which are useful for a variety of biosensing applications.^{25,26}

Moreover, gold-P(NIPAM) composite materials have been explored extensively for a variety of applications such as photothermal therapy,²⁷ catalysis,^{28,29} and temperature sensing.³⁰ Although P(NIPAM)-based hydrogels possess properties that favor protein binding, the use of gold-P(NIPAM) composites for protein biosensing would benefit from further elucidation. For these reasons, the synthesis method to fabricate hydrogel-coated, silica core gold nanoshells (AuNSs), previously described by Culver et al.,²⁴ was adapted to include a custom Al-OEGA monomer to enhance protein recognition through the formation of dynamic covalent (DC) imine bonds with solvent-accessible lysine residues present on the surface of select tear proteins.³¹ Our results showed that hydrogel-coated AuNSs, composed of monomers which form ionic and DC bonds with select tear proteins, greatly enhance protein recognition on the basis of the LSPR exhibited by AuNSs. The protein binding results indicated that, once the hydrogel coating is added to the AuNS surface, the presence and amount of Al-OEGA increased protein binding of high isoelectric point proteins with increased solvent-accessible lysines, in comparison to results obtained from hydrogel-coated AuNS containing only MAA, which is only capable of forming electrostatic interactions with charged proteins in a buffer.

To further validate the developed biosensor, the synthesized hydrogel-coated AuNSs composed of monomers capable of forming multiple noncovalent and covalent interactions with

Table 1. Properties of Proteins Used in Binding Studies and Concentrations Present in Healthy and Dry Eye Patients^a

protein	isoelectric point	molecular wt (kDa)	no. of solvent-accessible lysines	concentration (mg/mL)	
				healthy	dry eye
lysozyme	11.3	14.3	5	2.0 ± 1.0	0.7 ± 0.5
lactoferrin	8.7	80	46	2.0 ± 1.1	0.7 ± 0.5
lipocalin-1	5.3	19	11	1.7 ± 0.5	1.0 ± 0.5
IgA	4.5–6.5	385	N/A ^b	1.7 ± 0.7	0.8 ± 0.4
β -lactoglobulin ^c	5.2	18	15	N/A	N/A

^aThe solvent-accessible surface area (SASA) of select proteins was quantified using the PDBEPIA (Protein Data Bank in Europe, Proteins, Interfaces, Structures and Assemblies) SASA tool available online through the European Molecular Biology Laboratory. Table adapted from Culver et al.²⁴. ^bThe solvent-accessible lysine count is not available due to the absence of secretory IgA protein information in the PDBEPIA data bank. ^cBovine β -lactoglobulin was used in place of lipocalin-1 due to cost but is similar in isoelectric point, molecular weight, and the number of solvent-accessible lysines.

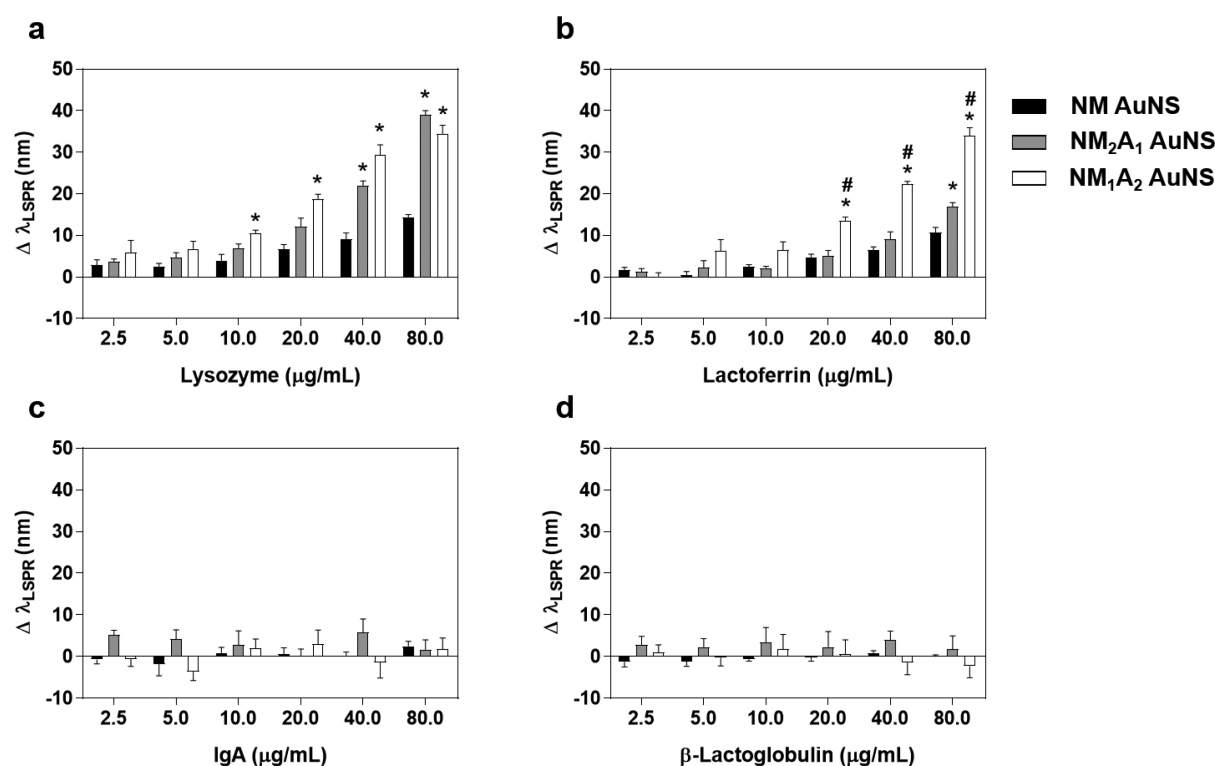


Figure 2. Quantification of hydrogel-coated AuNS $\Delta\lambda_{\text{LSPR}}$ values for high (a, b) and low isoelectric point (c, d) proteins in buffer. $\Delta\lambda_{\text{LSPR}}$ results of hydrogel-coated AuNS upon incubation with (a) lysozyme, (b) lactoferrin, (c) IgA, and (d) β -lactoglobulin in 0.1X PBS. Abbreviations: NM, P(NIPAM-*co*-MAA); NM₂A₁, P(NIPAM-*co*-MAA₂-*co*-Al-OEGA₁); NM₁A₂, P(NIPAM-*co*-MAA₁-*co*-Al-OEGA₂). Subscripts in hydrogel-coated AuNS formulations refer to the MAA:Al-OEGA ratio included in the polymerization. Protein binding was performed using three independent batches of each formulation of hydrogel-coated AuNSs and repeated in duplicate. Data are presented as mean $\pm$ standard error of the mean and were analyzed according to Student's *t* test. * indicates *p* < 0.05 in comparison to NM AuNS. # indicates *p* < 0.05 in comparison to NM₂A₁AuNS.

select tear proteins were used as label-free receptors to detect changes in biomarker concentrations in diluted, commercially available pooled human tears. The human tear fluid was diluted in buffer, not only to expand the sample volume (as individuals with dry eye due to low tear production have decreased tear volumes), but also to modulate the conditions of the aqueous medium. Through this step, the pH and ionic strength can be controlled to promote the optimal conditions for a specific analyte interaction with the receptor. The studies reported herein further elucidated the semiselectivity of the synthesized hydrogel-coated AuNS formulations in pooled human tears.

RESULTS AND DISCUSSION

Synthesis and Characterization of Hydrogel-Coated Gold Nanoshells. P(NIPAM-*co*-MAA) (NM)-coated AuNSs were synthesized as previously described by Culver et al.,²⁴ however, a custom aldehyde-functionalized oligo(ethylene glycol)acrylate (Al-OEGA) monomer was included in the polymerization (Figure 1) to amplify the recognition of proteins that have increased numbers of surface-exposed lysine residues (Table 1). The MAA:Al-OEGA monomer feed ratios were varied (Table S1), resulting in formulations noted as NM, NM₂A₁, or NM₁A₂ AuNSs, where subscripts indicate the proportion of MAA:Al-OEGA included in the polymerization.

Transmission electron microscopy images revealed hydrogel-coated AuNSs that are porous in structure (Figure S1). It is important to note that the NM coating was the most porous in

structure, resulting in flowerlike morphologies similar to those observed by Culver et al.²⁴ This is likely due to NM nucleation in solution and subsequent reaction with the methacrylate groups on the hydrophobically modified, poly(maleic anhydride-*alt*-1-octadecene)-*g*-poly(ethylene glycol) methacrylate encapsulated AuNS surface, resulting in multiple nanogels anchored to each AuNS utilizing this graft-to approach. However, as MAA incorporation into the polymerization decreased, less porous morphologies and thinner coatings were observed (Figure S1).

Prior to use in protein binding studies, all NM-coated AuNSs were further characterized by dynamic light scattering (DLS) to determine the composite material's hydrodynamic diameter, polydispersity index, and ζ potential (Table S2). The synthesis of NM AuNSs resulted in the largest hydrodynamic diameter (842 ± 233 nm) in comparison to either NM₂A₁ AuNSs or NM₁A₂ AuNSs (650 ± 223 and 487 ± 49.0 nm, respectively). As MAA incorporation into the hydrogel coating decreased, the hydrodynamic diameter also decreased. Furthermore, the ζ potential was obtained and used as an indirect measure of MAA incorporation (Table S2). NM AuNSs and NM₂A₁ AuNSs resulted in the most negative ζ potentials obtained in 0.1X PBS (-27.9 ± 3.76 and -29.2 ± 1.65 mV, respectively), indicating increased MAA incorporation into the hydrogel coating. An analysis of NM₁A₂ AuNSs resulted in a more positive ζ potential (-20.8 ± 3.74 mV) due to the decrease of MAA in the hydrogel coating.

Evaluation of Hydrogel-Coated Gold Nanoshells as Tear Protein Receptors. Protein binding studies were performed with select human tear proteins, including lysozyme, lactoferrin, and IgA (Table 1). Bovine β -lactoglobulin was used as a model protein in place of lipocalin-1 due to cost but is similar in isoelectric point (pI), molecular weight, and the number of solvent-accessible lysines (Table 1). The proteins tested are further classified as high pI proteins (i.e., lysozyme and lactoferrin) or low pI proteins (i.e., IgA and β -lactoglobulin). Controls were hydrogel-coated AuNSs combined in buffer in the absence of protein. The absorbance spectra of hydrogel-coated AuNSs and protein samples were measured (399–999 nm, using a microplate reader), and each background-subtracted spectrum collected was fit to an eight-term Gaussian in MATLAB (refer to the Supporting Information).²⁴ The LSPR wavelength (λ_{LSPR}) was taken as the average of the centers of the Gaussians. The maximum LSPR value from each sample combination was obtained and subtracted from that of the respective controls to yield $\Delta\lambda_{\text{LSPR}}$ values.

When all hydrogel-coated AuNS formulations were incubated with each high pI protein individually in 0.1X PBS, concentration-dependent red-shifted $\Delta\lambda_{\text{LSPR}}$ values were observed (Figure 2). When NM₂A₁ AuNSs were incubated with lysozyme (Figure 2a), significant increases in $\Delta\lambda_{\text{LSPR}}$ values were observed at 40 and 80 $\mu\text{g/mL}$ in comparison to those observed from NM AuNSs. In addition, when NM₁A₂ AuNSs were incubated with lysozyme, a significant increase in $\Delta\lambda_{\text{LSPR}}$ values was observed at concentrations of 10 $\mu\text{g/mL}$ and above in comparison to those observed from NM AuNSs. However, no difference in $\Delta\lambda_{\text{LSPR}}$ values was observed between either AuNS formulation containing the Al-OEGA monomer on incubation with lysozyme, indicating that electrostatic interactions predominantly drive these protein recognition events.

When NM₁A₂ AuNSs were incubated with lactoferrin in 0.1X PBS, a significant increase in $\Delta\lambda_{\text{LSPR}}$ values was observed at concentrations of 20 $\mu\text{g/mL}$ and above, in comparison to those observed from either NM AuNSs and NM₂A₁AuNSs (Figure 2b). The increase in $\Delta\lambda_{\text{LSPR}}$ values obtained from NM₁A₂ AuNSs is a result of the recognition event taking place between the increased aldehyde content in the hydrogel coating and the solvent-accessible lysines present on the surface of lactoferrin. The significant difference in $\Delta\lambda_{\text{LSPR}}$ values observed between Al-OEGA-containing AuNSs for lactoferrin, but not for lysozyme, is due to its increased number of solvent-accessible lysines. Lactoferrin contains 46 solvent-accessible lysines, while lysozyme only contains 5 (Table 1). Increased $\Delta\lambda_{\text{LSPR}}$ values observed for lysozyme are attributed primarily to electrostatic interactions, while for lactoferrin, increased $\Delta\lambda_{\text{LSPR}}$ values are primarily related to the formation of DC interactions (i.e., imine formation) between the hydrogel coating and the protein. When all hydrogel-coated AuNS formulations were incubated with each low pI protein (i.e., IgA and β -lactoglobulin) individually in 0.1X PBS, minimal to no $\Delta\lambda_{\text{LSPR}}$ values (<10 nm) were observed (Figure 2c,d).

Incorporation of the DC Al-OEGA monomer in the AuNS hydrogel coating resulted in increased sensitivity and detection of select high isoelectric point proteins in buffer at low concentrations (<80 $\mu\text{g/mL}$, Figure 2a,b). While the formation of electrostatic interactions was the predominant factor that governed protein–polymer interactions at the bulk nanogel level,¹² translation of this hydrogel coating on the surface of gold nanoshells revealed DC protein–polymer interactions to increase signal sensitivity in noncompetitive environments (in buffer alone). Our results showed that hydrogel-coated AuNSs composed of monomers that form ionic and DC bonds with select tear proteins greatly affect protein recognition on the basis of the LSPR exhibited by AuNSs. Specifically, when Al-OEGA (which enables the dynamic formation of imine bonds with lysine residues on the protein surface¹⁹) was included in the hydrogel-coated AuNSs, increased sensitivity of the biosensor was achieved through the detection of proteins present in buffered conditions at lower concentrations. The protein binding results obtained indicated that, once the hydrogel coating is added to the AuNS surface, the presence and amount of Al-OEGA increased protein binding of high-isoelectric-point proteins with increased solvent-accessible lysines, in comparison to results obtained from hydrogel-coated AuNSs containing only monomers capable of forming electrostatic interactions with select proteins.

Analysis of Total and Individual Protein Concentrations in Pooled Human Tears. Lysozyme, lactoferrin, IgA, and lipocalin-1 make up the majority of the protein content (70–85%) in tears according to previous literature reports.^{32,33} Using a standard total protein assay (Figure S2) and protein-specific enzyme-linked immunosorbent assays (ELISAs) (Figure S3), the total protein concentration and individual protein concentrations present in commercially available pooled human tears were determined (Table S3). Moreover, the percentage of lysozyme, lactoferrin, IgA, and lipocalin-1 present in each pooled tear lot was calculated on the basis of the total protein concentration in the sample (Table S4). The results obtained indicate that the four proteins analyzed make up 29–35% of the protein composition in the commercially available pooled tear lots tested. While the percentage of these proteins in tears greatly differs from those

obtained from previous literature reports, it is important to note that these differences can be attributed to the collection method used to obtain tears (collection from crying/watering eyes), evaporative concentration during processing or handling, or other unknown variables (such as age and health of donors).²⁴

Hydrogel-Coated Gold Nanoshells for Sensing of Dry Eye Biomarkers in Pooled Human Tears. While the individual concentrations of the four proteins analyzed were similar between pooled tear lots (Table S3), $\Delta\lambda_{\text{LSPR}}$ values of hydrogel-coated AuNS formulations were investigated upon incubation with diluted tear samples to determine any differences between tear lots. Of the four proteins investigated (namely, lysozyme, lactoferrin, IgA, and lipocalin-1), lysozyme and lactoferrin are high pI proteins, while IgA and lipocalin-1 have isoelectric points below neutral (Table 1). On the basis of the results obtained in Figure 2, all hydrogel-coated AuNS formulations will preferentially interact with lysozyme and lactoferrin due to their high isoelectric points and presence in tears at high concentrations (Table 1). Thus, IgA and lipocalin-1, present in tears at high concentrations but possessing low isoelectric points (Table 1), are not expected to interfere with protein binding or significantly contribute to the LSPR signal. For this reason, pooled tear lots were diluted in PBS to obtain a starting concentration of 150 $\mu\text{g/mL}$ based on the combined concentrations of lysozyme and lactoferrin in tears obtained from ELISA results (Table S5). Serial dilutions of the tear samples were further performed to obtain total concentrations of lysozyme and lactoferrin ranging between 2.35 and 150 $\mu\text{g/mL}$.

When the hydrogel-coated AuNS formulations were incubated with diluted pooled tear samples at various total lysozyme and lactoferrin concentrations, concentration-dependent $\Delta\lambda_{\text{LSPR}}$ values were observed (Figure S6 and Figure 3). The results obtained in diluted tear samples from lot 1

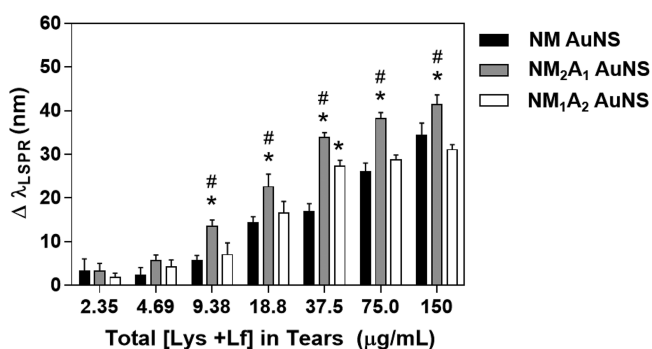


Figure 3. Quantification of hydrogel-coated AuNS $\Delta\lambda_{\text{LSPR}}$ values for pooled human tears diluted in buffer. Diluted tear concentrations are based on the combined concentrations of lysozyme (Lys) and lactoferrin (Lf) obtained from ELISA results. Abbreviations: NM, P(NIPAM-co-MAA); NM₂A₁, P(NIPAM-co-MAA₂-co-Al-OEGA₁); NM₁A₂, P(NIPAM-co-MAA₁-co-Al-OEGA₂). Subscripts in hydrogel-coated AuNS formulations refer to the MAA:Al-OEGA ratio included in the polymerization. Protein binding was performed using three independent batches of each formulation of hydrogel-coated AuNSs and repeated in duplicate for each tear lot. Data from two individual lots (lot 1 and lot 2) of commercially available pooled human tears are combined and presented as mean $\pm$ standard error of the mean. Data were analyzed using multiple two-way ANOVAs. * indicates $p < 0.05$ in comparison to NM AuNS. # indicates $p < 0.05$ in comparison to NM₁A₂ AuNS.

(Figure S6a) revealed statistically significant differences in the $\Delta\lambda_{\text{LSPR}}$ values between Al-OEGA-containing AuNSs at total lysozyme and lactoferrin concentrations above 9 $\mu\text{g/mL}$. In addition, $\Delta\lambda_{\text{LSPR}}$ values from NM₂A₁ AuNSs resulted in statistically significant increases in total lysozyme and lactoferrin concentrations above 37 $\mu\text{g/mL}$ in comparison to NM AuNSs. The results obtained from diluted tear samples from lot 2 showed statistically significant increases in the $\Delta\lambda_{\text{LSPR}}$ values from NM₂A₁ AuNSs in comparison to NM AuNSs at concentrations greater than 9 $\mu\text{g/mL}$ (Figure S6b). Differences in $\Delta\lambda_{\text{LSPR}}$ values between both Al-OEGA-containing AuNS formulations were observed at total lysozyme and lactoferrin concentrations of 75 $\mu\text{g/mL}$ and above, with NM₂A₁ consistently outperforming NM₁A₂ due to the importance of electrostatic interactions.

When data from the two pooled human tear lots tested were combined, concentration-dependent $\Delta\lambda_{\text{LSPR}}$ values were observed with increasing concentrations of total lysozyme and lactoferrin present in human tears diluted in 0.1X PBS (Figure 3). The highest $\Delta\lambda_{\text{LSPR}}$ values observed upon AuNS incubation with diluted tears were obtained using NM₂A₁ AuNS (Figure 3). Furthermore, statistically significant increases in the $\Delta\lambda_{\text{LSPR}}$ values were obtained when NM₂A₁ AuNSs were incubated with diluted tears at concentrations greater than 9 $\mu\text{g/mL}$ in comparison to results obtained from either NM AuNSs or NM₁A₂ AuNSs (Figure 3). Again, this increase in $\Delta\lambda_{\text{LSPR}}$ is likely due to the interactions between the increased number of solvent-accessible lysines present in lysozyme and lactoferrin (Table 1) with the Al-OEGA component of the NM₂A₁ AuNSs.

CONCLUSION

Several P(NIPAM-co-MAA)-based hydrogel-coated AuNS formulations composed of monomers capable of forming both covalent and noncovalent interactions with select tear proteins were synthesized. The identity of the ionizable and covalent groups within the AuNS hydrogel coating affected their swelling behavior, charge character, and consequently their protein binding behavior. Concentration-dependent $\Delta\lambda_{\text{LSPR}}$ values were observed when hydrogel-coated AuNSs were incubated with high isoelectric point tear proteins: specifically, lysozyme and lactoferrin. The protein binding results obtained indicated that, once the hydrogel coating is added to the AuNS surface, the presence and amount of the Al-OEGA monomer increased protein binding of high isoelectric point proteins, in comparison to results obtained from hydrogel-coated AuNSs only containing a monomer (specifically, methacrylic acid) capable of forming electrostatic interactions with select proteins. Incorporation of monomers that form ionic and DC bonds with select tear proteins greatly affects molecular recognition on the basis of the $\Delta\lambda_{\text{LSPR}}$ values obtained from hydrogel-coated AuNSs. In addition, the inclusion of monomers into the hydrogel coating that can form covalent bonds with select amino acid residues on the protein surface significantly increase the sensitivity of the developed biosensor through the detection of proteins at lower concentrations. The results obtained in this study facilitate the process of developing low-cost biosensors for detection of dry eye biomarkers. Further development of this biosensor and validation in healthy and diseased human tears would confirm the potential for clinical translation as a rapid and affordable screening test for dry eye and associated diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.nanolett.1c02941>.

Materials, synthesis of hydrogel-coated AuNSs, characterization of hydrogel-coated AuNSs, ELISA analysis, total protein analysis, and sensing of biomarkers in human tears (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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