# Gold Nanoparticles Decorated with Oligo(ethylene glycol) Thiols: Enhanced Hofmeister Effects in Colloid−Protein Mixtures

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Oligo(ethylene glycol) (OEG) thiol self-assembled monolayer (SAM) decorated gold nanoparticles (AuNPs) have potential applications in bionanotechnology due to their unique property of preventing the nonspecific absorption of protein on the colloidal surface. For colloid−protein mixtures, a previous study (Zhang et al. *J. Phys. Chem. A* **2007**, *111*, 12229) has shown that the OEG SAM-coated AuNPs become unstable upon addition of proteins (BSA) above a critical concentration, $c^*$. This has been explained as a depletion effect in the two-component system. Adding salt (NaCl) can reduce the value of $c^*$; that is, reduce the stability of the mixture. In the present work, we study the influence of the *nature* of the added salt on the stability of this two-component colloid−protein system. It is shown that the addition of various salts does not change the stability of either protein or colloid in solution in the experimental conditions of this work, except that sodium sulfate can destabilize the colloidal solutions. In the binary mixtures, however, the stability of colloid−protein mixtures shows significant dependence on the nature of the salt: chaotropic salts (NaSCN, $NaClO_4$, $NaNO_3$, $MgCl_2$) stabilize the system with increasing salt concentration, while kosmotropic salts (NaCl, $Na_2SO_4$, $NH_4Cl$) lead to the aggregation of colloids with increasing salt concentration. These observations indicate that the Hofmeister effect can be enhanced in two-component systems; that is, the modification of the colloidal interface by ions changes significantly the effective depletive interaction via proteins. Real time SAXS measurements confirm in all cases that the aggregates are in an amorphous state.

## Introduction

Charge effects are ubiquitous in nature. Under biologically relevant solution conditions (i.e., in water and in the presence of salt ions), biomolecules are generally surrounded by charges, which are crucial for their structure, function, and interactions. The understanding of the subtle effects induced by different types of ions is a challenging and still unsolved issue. The Hofmeister effect is a very important phenomenological concept.[1] It is a classification of ions in order of their ability to change water structure and was initially studied in the context of protein solubility in various salt solutions. It has since been studied in many other systems, including the phase behavior and self-assembly of surfactants in solution, but the mechanisms are still not fully understood.[1−8] A representative Hofmeister series can be given for anions and cations as follows:

$$\text{citrate}^{3-} > SO_4^{2-} > PO_4H^{2-} > F^- > CH_3COO^- > Cl^- > Br^- > I^- > NO_3^- > ClO_4^- > SCN^- \quad (1)$$

$$NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > H^+ > Ca^{2+} > Mg^{2+} \quad (2)$$

Ions on the left-hand side of the series precipitate (salt out) solutes, whereas ions on the right dissolve or denature (salt in) solutes. Note that the specific binding effect of the multivalent ions in lists 1 and 2, especially in biological systems, can make the Hofmeister effect more complicated. The Hofmeister effect has been explained in terms of structure-making and structure-breaking abilities of these ions with water. Salts have been described as kosmotrope or chaotrope depending on their interaction with water.[3,4] Kosmotropes interact with water strongly, and water molecules surrounding the salt ions are more structured relative to bulk water. Chaotropes break up the structure of the surrounding water molecules because of the large size of the ion and its weak interaction with water. For example, the specific ion effects on protein solubility were described in terms of the ability of ions to "salt in" the polar peptide group and "salt out" the nonpolar side chains.[9] The Hofmeister effect on the solubility of proteins has also been related to the protein−water interfacial tension.[10]

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Despite the practical importance of the Hofmeister effect, the interplay of the underlying interactions is still not fully understood. Molecular dynamics simulations suggest that the ion effects could reflect differences in the hydration of ions near surfaces compared to the bulk solution.[11] On the other hand, the effects of salt on the structure and self-assembly of surfactant in solution were interpreted as an adsorption and depletion effect.[12] It was further shown that kosmotropic salts desorb, whereas chaotropes adsorb on the monolayer of the microemulsion phase.[12] Kosmotropic anions will compete for water with the ether groups of PEO-based surfactants, thus leading to lower cloud points. Anions are known to depress the critical micelle concentration of nonionic surfactants and to increase the attractive interactions between micelles.[6] The Hofmeister effect in the restabilization of IgG−latex particles studied by López-León et al. has shown that the hydration force is responsible for the stability of colloids at high ionic strengths.[13−17] The origin of this non-DLVO (Derjaguin−Landau−Verwey−Overbeek) interaction and deviation from the mean-field picture is related to the local structure of the water molecules located at the surface and the structure of the water molecules involved in the hydration of the ions that surround the colloidal particles.[13,18]

Importantly, although the Hofmeister effect on the stability of various colloidal systems has been extensively studied, not much has been reported on how this affects the stability of a *two*-component colloidal system. In our colloid−protein system, the main driving force for the instability of the system is the depletion effect as discussed in previous work.[19,20] The short-range repulsive force on the protein molecules exerted by the OEG capped colloidal particles leads to an effective attraction between the colloids when a certain protein concentration is exceeded. This depletion effect can be explained in terms of the entropy of the entire system: by the formation of colloidal aggregates, with an overlap of the regions inaccessible for the proteins, the total accessible volume is increased and is thus the entropically favored state. It is therefore *a priori* not obvious how the Hofmeister series affects the stability of these complex systems.

Monolayer-protected colloidal gold has many unusual properties which have been widely used for biodiagnostics, bio- and chemical sensors, drug delivery, and biomolecular recognition purposes.[21−28] Among these, oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) at the liquid−solid interface have attracted much attention due to the prevention of nonspecific protein absorption.[29−42] Experiments have suggested that the tightly bound water layer at the interface could form a physical barrier to prevent direct contact between the protein and the interface.[30,40,41] Our recent work also provides direct evidence for water penetration into the OEG SAMs.[38] Further studies using chemical force spectroscopy on the direct interactions between a hydrophobic probe and OEG SAMs at various pH values and with several different added salts indicate that not only the bound water but also electrostatics has an effect on the interactions.[29,31,33−36,42,43] The charge at the SAM/water interface was suggested to be due to the adsorption of the hydroxyl ions from the aqueous solution.[34,36] It is known that various ions in the Hofmeister series have a different influence on the structure of water and that they are able to react specifically with ethylene glycol units within OEG SAMs and, hence, change the interface properties.[2−6,9,34]

In this work, we study how the *nature* of the salt influences the stability of colloid−protein mixtures, which provides information how the nature of salt modifies on the SAM/water interfaces. We show that the phase behavior of mixtures of OEG SAM-coated colloidal gold and proteins with addition of various salts can be used to understand the ion−SAM interactions. The system studied here is very complex due to the interplay of several interactions: protein−protein interaction, colloid−colloid, colloid−protein, and ion interactions with all other components. The effect of the nature of the salt on both proteins and OEG thiol-coated colloids is important, but in different ways. We show that there is an enhanced Hofmeister effect observed in these two-component systems; that is, the nature of the salt affects the OEG-decorated colloidal gold differently, which significantly affects the effective colloid interactions via proteins, and the stability of the protein−colloid mixtures.

## 2. Experimental Section

**2.1. Materials.** Citrate-stabilized gold colloids in aqueous solution with mean size of approximately 20 nm were purchased from British BioCell International (BBI) and were used as received. Hexa (ethylene glycol)-terminated thiol, $HS(CH_2)_{11}(OCH_2CH_2)_6OMe$ (lot 1506), and $HS(CH_2)_{11}(OCH_2CH_2)_6OH$ (lot A0311) abbreviated as EG6OMe and EG6OH, respectively, were purchased from ProChimia, Poland and were used as received. Bovine serum albumin (BSA) (product no. A7638) was purchased from Sigma-Aldrich. This is a lyophilized powder with a molecular weight of ∼66 kDa and was used as received. The following salts were used as received: $NaNO_3$ (Sigma-Aldrich), $Na_2SO_4$, NaCl, $NaClO_4$, $NH_4Cl$ (Merck), NaSCN (AlfaAesar), and $MgCl_2$ (Fluka).

**2.2. Preparation of Surface-Modified Gold Colloids.** The colloidal gold was modified by directly adding OEG thiols (∼2 mg) (EG6OH or EG6OMe) to the colloid solution (10 mL) without stirring. The modified colloids were incubated at room temperature for more than 4 h, and the stability of the modified colloids was examined by monitoring the UV−vis spectra while varying pH, ionic strengths and temperature. The modified gold colloids were found to be stable in a wide range of temperature (5−70 °C), pH (1.3−12.4), and ionic strength (NaCl, 0−4.0 M). The colloidal solution was further concentrated (by a factor of ∼10) by using a rotation−evaporation instrument at ∼10 mbar at 40 °C for ∼50 min. For the following real-time UV−vis and SAXS studies, 0.05 mL of concentrated colloid solution was mixed with a fixed amount of protein solution with various protein and salt concentrations. The dilution of the colloid with the protein and salt solution was completed to return the colloid, in the final solution, to the same concentration as the original solution. This was determined by the absorption intensity (1.09) at a wavelength of 525 nm in the UV−vis spectrum, which corresponds to a number density of about $7 \times 10^{11}$ particle/mL, as provided by the manufacturer. Details of the preparation method have been described in previous publications.[19,20]

***2.3. Methods.***

***2.3.1. Real Time UV−Visible Spectroscopy.*** UV−visible absorption and kinetic measurements were performed at room temperature using a Cary 50 UV−visible spectrophotometer (Varian Optical Spectroscopy Instruments). Quartz and disposable PE UV cuvettes with an optical path length through the sample of 1.0 cm were used to contain the sample while collecting the spectra in the wavelength range from 300 to 800 nm. The concentrated colloid (0.05 mL) was quickly mixed with protein and salt solution, and then the real time UV−vis measurements were started within 1 min.

The flocculation parameter, $P$, was used to compare the stability of the mixture of colloid and protein with addition of various salts following the approach of refs 26 and 20. The flocculation parameter is defined as

$$P = \int_{600}^{800} I_{Abs}(\lambda)\, d\lambda \quad (3)$$

It is the integral of absorption intensity between 600 and 800 nm. Theoretical modeling and comparison to TEM observations indicate that the formation of gold colloid aggregates results in additional absorption bands at wavelengths much longer than the absorption bands exhibited by the isolated colloids. These bands are concentrated above 600 nm in the visible spectrum.[26,44] In our previous work,[20] we have used this parameter to follow the aggregation kinetics of OEG SAM-coated colloidal gold caused by the depletion attraction. We have further defined the maximum flocculation parameter, $P_{max}$, which can be used to compare the degree of aggregation in the mixtures.[20]

***2.3.2. Small-Angle X-ray Scattering (SAXS).*** Small-angle X-ray scattering measurements were carried out at station 6.2 of the Synchrotron Radiation Source (SRS) at the Daresbury Laboratory, Warrington, UK.[45] The beam energy was 8.8 keV, corresponding to a wavelength of 1.51 Å. The scattered intensity was measured with a 200-mm-radius quadrant detector located 3.3 m from the sample. The accessible $q$ range was thus from 0.008 to 0.25 $Å^{-1}$. The detector response was calibrated using the scattering from water. The angular scale was calibrated using the scattering peaks of wet rat tail collagen.

Protein or colloid solutions were filled into capillaries from Hilgenberg GmbH, Malsfeld, Germany. The capillaries are made of borosilicate glass with an inner diameter of 4.0 mm and a wall thickness of 0.05 mm. The scattering of water or a salt solution was measured as the background, in exactly the same way as the protein/colloidal solutions and was subtracted from the sample scattering. All measurements were carried out at room temperature. The resulting data were (electronically) converted to a 1D profile by integrating around an arc. The raw data were corrected for transmission, fluctuation of primary beam intensity, exposure time, and the geometry of the detector. The absolute intensity was calibrated using water as the standard with an estimated error of 10%. The detailed data correction and calibration has been described in previous publications.[19,46]

## 3. Results and Discussion

This section is organized as follows: First, we present the effect of the nature of salt on the stability of pure colloid solutions of both Au20EG6OMe and Au20EG6OH by UV−vis and SAXS. Second, we show the SAXS results on the protein−protein interactions in concentrated protein solutions with addition of various salts. Third, we present the real-time UV−vis spectra of colloid−protein mixtures by adding different salts, which clearly display significant changes in stability. Combining all of these observations, the influence of the nature of salt on the structure and properties of the SAM/water interface can be inferred.

**3.1. Stability of OEG SAM-Coated Gold Nanoparticle Solution with Addition of Various Salts.** Figure 1shows photographs of colloid solutions (Au20EG6OMe and Au20EG6OH) with various salts added. When Au20EG6OMe was mixed with 1.0 M aqueous solutions of NaCl, NaSCN, $Na_2SO_4$, and $MgCl_2$, respectively, the color change of the solution indicates that sodium sulfate destabilizes the colloid solution (Figure 1a). For the other salts, including $NaClO_4$, $NaNO_3$, and $NH_4Cl$, the colloid solutions are stable even for much higher salt concentrations (e.g., up to 4 M of NaCl). Similar results were obtained for Au20EG6OH. To find the critical concentration, the colloids were mixed with various amounts of sodium sulfate (Figure 1b and 1c). For Au20EG6OMe,

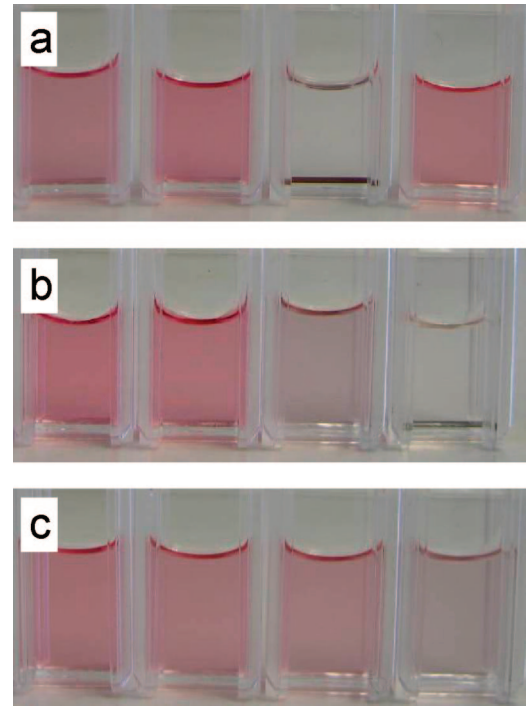


**Figure 1.** Digital photographs of OEG thiol-decorated colloidal gold with various salts: (a) Au20EG6OMe with 1.0 M NaCl, NaSCN, $Na_2SO_4$, and $MgCl_2$; (b) Au20EG6OMe with $Na_2SO_4$ of 0.1, 0.2, 0.3, and 0.4 M; and (c) Au20EG6OH with $Na_2SO_4$ of 0.1, 0.2, 0.3, and 0.4 M.

above 0.25 M of $Na_2SO_4$, the colloid solution becomes unstable and forms aggregates, whereas for Au20EG6OH, this critical concentration is about 0.35 M. This observation can be interpreted by the strong salting out effect of sodium sulfate, which reduces the solubility of OEG thiols in solution as well as the thiol-decorated colloidal gold. The different critical salt concentrations for Au20EG6OMe and Au20EG6OH are due to the different solubilities of the terminal group. The −OH-terminated thiol has a higher solubility than that of the $-CH_3$-terminated thiol in aqueous solution.

The aggregation upon adding sodium sulfate is a reversible process; diluting the solution with water results in the redissolution of the aggregates. The structure of the aggregate has been studied by SAXS in real time. SAXS results for Au20EG6OH with 1.0 M $Na_2SO_4$ for 30 min (Figure 2) reveal a scattering maximum at $q^* = 0.032$ $Å^{-1}$ in the first SAXS profile. Its position does not change with time. This peak corresponds to a center-to-center distance of 200 Å. This value is the same as observed in the aggregation of a colloid−protein mixture due to the depletion attraction in previous studies.[19] The same results were obtained for Au20EG6OMe.

Upon increasing the salt ($Na_2SO_4$) concentration further up to 1.0 M and even 2.0 M, the colloids are still observed to aggregate. However, the time required for aggregation to occur increases for higher salt concentrations. Real time UV−vis spectra of AuEG6OH (Figure 3) show that the aggregation process slows down with increasing salt concentration. In contrast to the precipitates at low salt concentration, where the precipitates are blue or black and sediment to the bottom of the cuvette, with 2.0 M $Na_2SO_4$, the aggregates are red and float on top of the solution. This may be due to the increased hydration of OEG SAMs at high salt concentration, which slightly enhances the stability of the colloidal solution. Studies on protein-coated latex complexes have reported that the hydration force under high salt concentration can restabilize the system.[14−17]

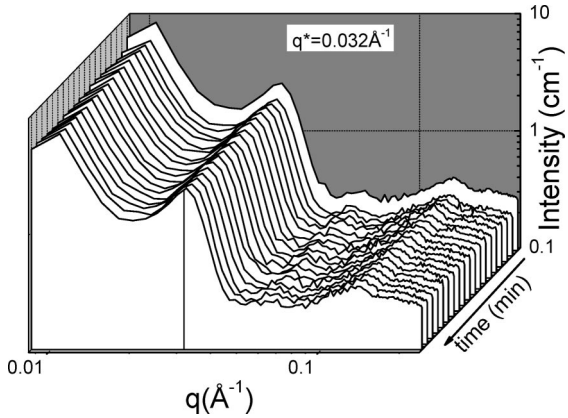


**Figure 2.** Real time SAXS results of Au20EG6OH with 1.0 M $Na_2SO_4$. A peak at $q^* = 0.032$ Å$^{-1}$ has already appeared after 1 min of sample preparation, which corresponds to the interparticle distance within the cluster about 200 Å.

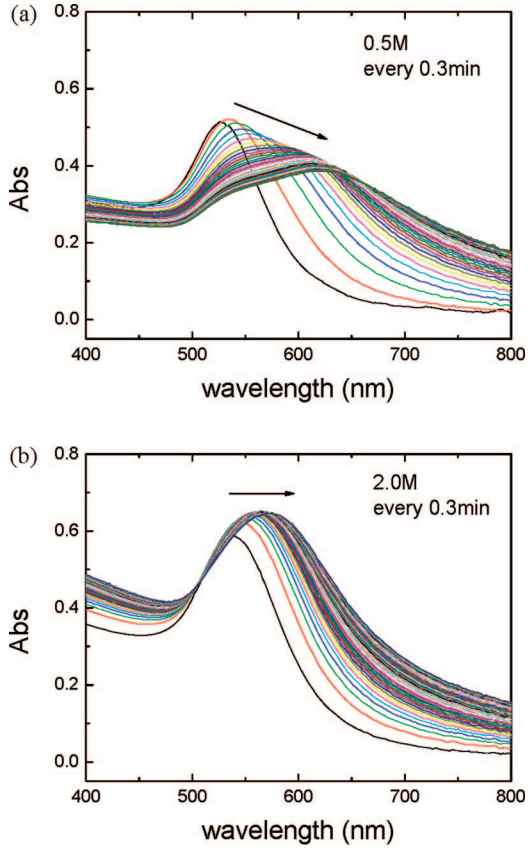


**Figure 3.** Real time UV−vis spectra of Au20EG6OH with (a) 0.5 M and (b) 2.0 M $Na_2SO_4$ show that both the red shift and the absorption intensity between 600 and 800 nm are reduced with increasing salt concentration.

It should be noted that although the SAM-coated colloid solutions appear to be stable with various salts except sodium sulfate up to 2.0 M, it is not clear whether the salts further stabilize the colloids or change the interfacial interactions. Changes in stability and interfacial interactions can be observed by adding proteins to such solutions, and the results of this are discussed below (Section 3.3). However, to understand this complex mixture of salt, protein, and colloid, it is essential first to understand the effect of various salts on the protein−protein interactions.

**3.2. Effect of the Nature of Salt on the Effective Protein−Protein Interaction and Further Effect on the Colloid−Colloid Interaction.** The effect of the nature of the salt on protein interactions is very complex and widely studied.[47−54] Studies on concentrated solutions of cytochrome *c* with addition of various salts by Baglioni et al. reported that the proteins form clusters at high concentrations of sodium thiocyanate and sodium sulfate.[47] Multivalent counterions can lead to a reentrant condensation in protein solution.[48] Therefore, addition of salt may significantly change the protein interactions in addition to the screening effect. We have studied the effect of the nature of salt on the effective protein−protein interaction (in BSA solutions) as a function of protein and salt concentrations.[55] Here, we present results for high protein concentrations. It was found that up to 2.0 M concentrations of various salts, including NaCl, NaSCN, $Na_2SO_4$, and $MgCl_2$, at pH ∼ 7.0, the protein solutions are stable and clear in all cases. The high solubility and stability of BSA at high salt concentration is due to the strong hydration effect.[46,56] SAXS results for BSA at 400 mg/mL with salt concentration (NaCl) ranging from 0 to 2.0 M (Figure 4a) reveal that, in all cases, there is a pronounced correlation peak and an identical scattering intensity in the high *q* range. The only difference appears in the low *q* region, where the scattering intensity increases with increasing ionic strength. It has been shown in our previous work that increasing the ionic strength reduces the repulsive electrostatic interactions due to electrostatic screening.[46] However, for high protein concentration (400 mg/mL, volume fraction 23%), the protein−protein interaction is dominated by the excluded-volume (hard-sphere) effect. Therefore, we observe a correlation peak, even though the ionic strength is 2.0 M, where the surface charge is

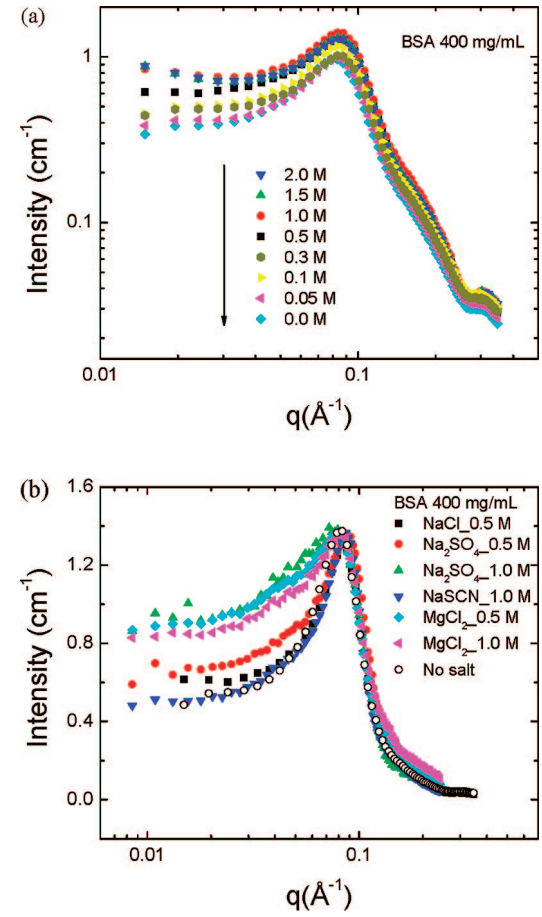


**Figure 4.** (a) SAXS results of BSA 400 mg/mL with various NaCl concentrations, adding salt slightly reduces the repulsive interactions, and (b) SAXS results of BSA 400 mg/mL with various salts.

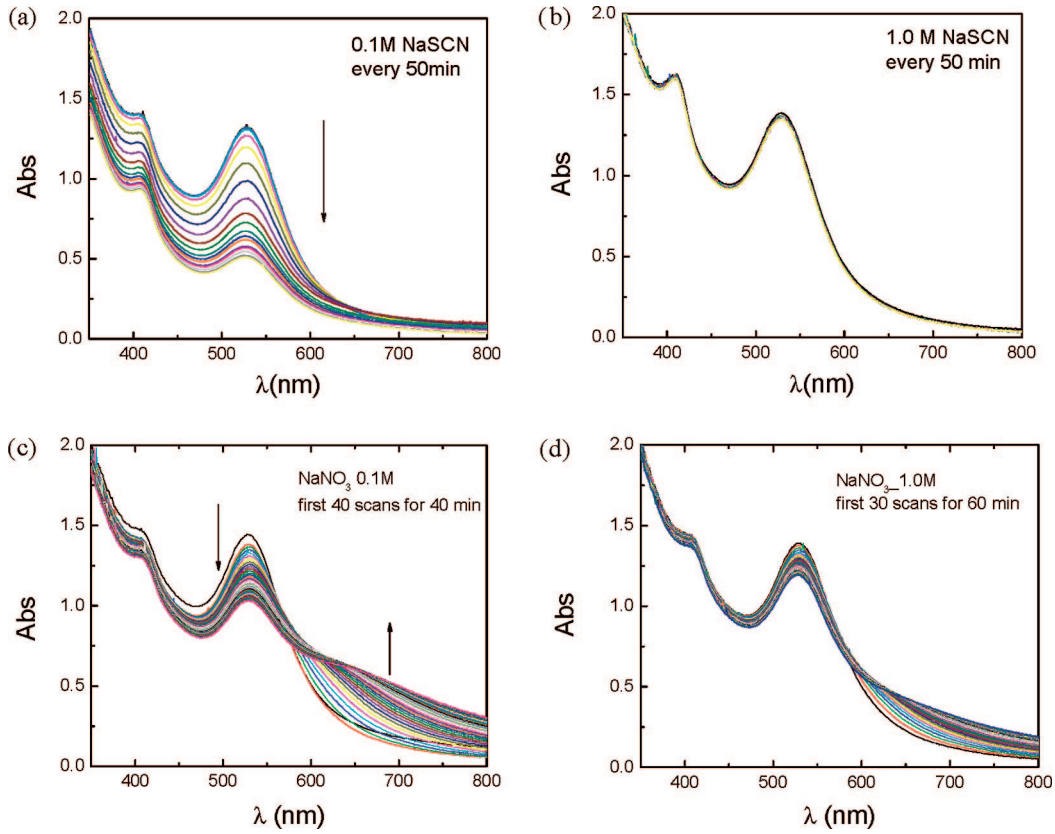


**Figure 5.** Realtime UV−vis spectra for Au20EG6OMe with BSA 400 mg/mL by adding (a) 0.1 M and (b) 1.0 M NaSCN and Au20EG6OH with BSA 400 mg/mL by adding (c) 0.1 M and (d) 1.0 M $NaNO_3$.

completely screened. The slight increase in intensity at low $q$ may be caused by impurities (the remainder globulins) in the BSA samples and is not observed for high purity BSA samples.[55] Generally, an increase in the scattering intensity at low $q$ may also be due to the progressive onset of an attractive interaction. Effects similar to sodium chloride are observed for other salts. In Figure 4b, we show the SAXS results for BSA at 400 mg/mL with high concentrations of NaCl, NaSCN, $Na_2SO_4$ and $MgCl_2$. For comparison, BSA at 400 mg/mL without added salt is also presented. It is clear from the strong correlation peak at $q = 0.05$ Å$^{-1}$ that in all cases, the protein−protein interactions are dominated by the excluded-volume effect. The response to the different salts is only slightly different in the low $q$ range.

Previous studies have demonstrated that protein in the colloid−protein mixture plays the role of a depletion agent due to the excluded-volume effect.[19,20] Adding salt to the mixture only screens the surface charge of the protein and enhances its hydration (which is a short-range effect). As described above, the role of protein in the mixtures (as a depletion agent) as well as the stability of protein solutions does not change significantly with the addition of various salts. Hence, if there is a significant change in the stability of the protein/colloid/salt mixture for different salts, it must be caused by interactions between the salt and the OEG SAM-coated AuNPs.

**3.3. Effect of the Nature of Salt on the Stability of the Mixtures.** The stability of colloid−protein mixtures on addition of various salts was studied by real time UV−vis spectroscopy. Figures 5−7 show the spectra of Au20EG6OMe with 400 mg/mL BSA on adding various salts. Note that in the absence of salt, 400 mg/mL BSA is slightly above the critical protein concentration for colloid aggregation, and on addition of NaCl, this critical concentration is reduced.[19] Without addition of salt, the aggregation is very slow, and only a fraction of the colloids

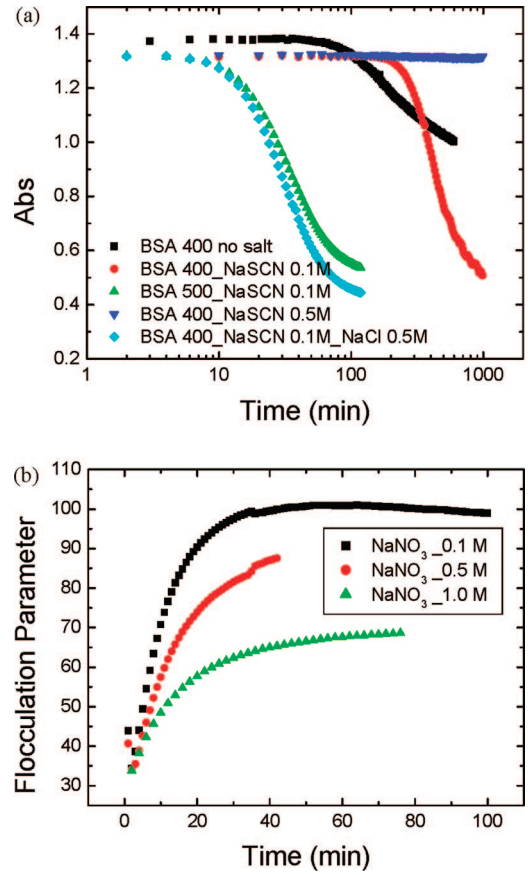


**Figure 6.** (a) Plots of absorption peak intensity as a function of time for adding NaSCN at various conditions and (b) plots of flocculation parameter calculated on the basis of eq 3 for adding $NaNO_3$ at various salt concentrations.

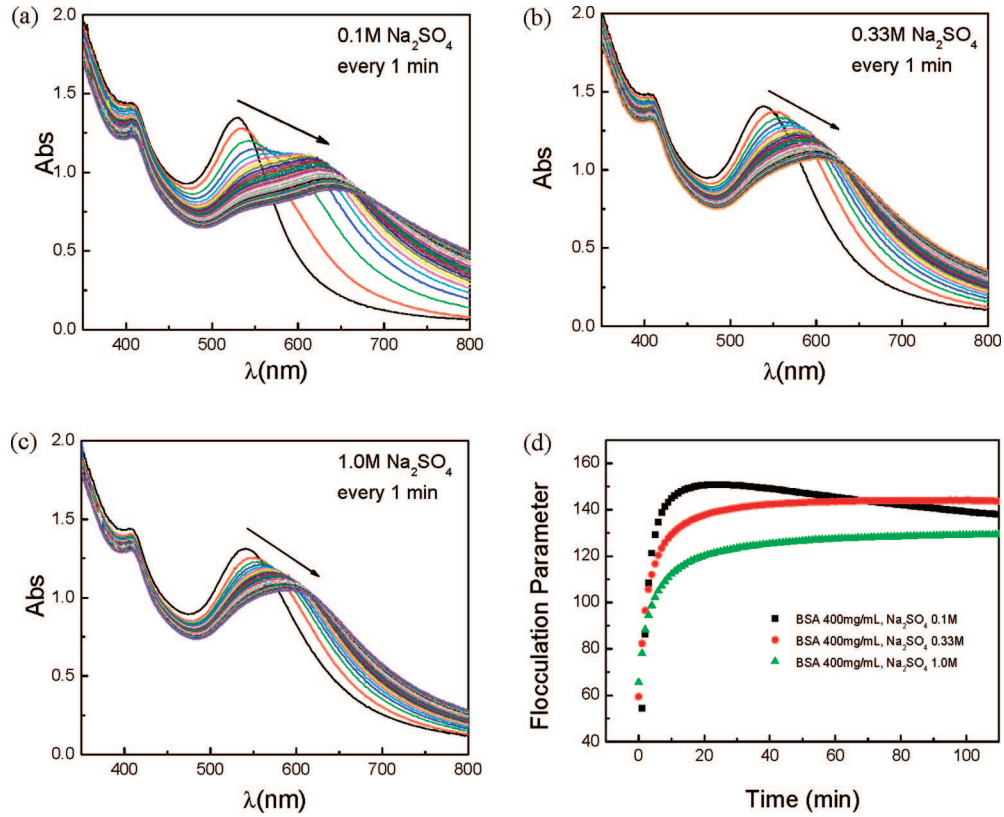


**Figure 7.** Realtime UV−vis spectra for Au20EG6OMe with BSA 400 mg/mL by adding (a) 0.1 M, (b) 0.33 M, and (c) 1.0 M $Na_2SO_4$ and the plots of $P(t)$ as a function of time from panels a to c.

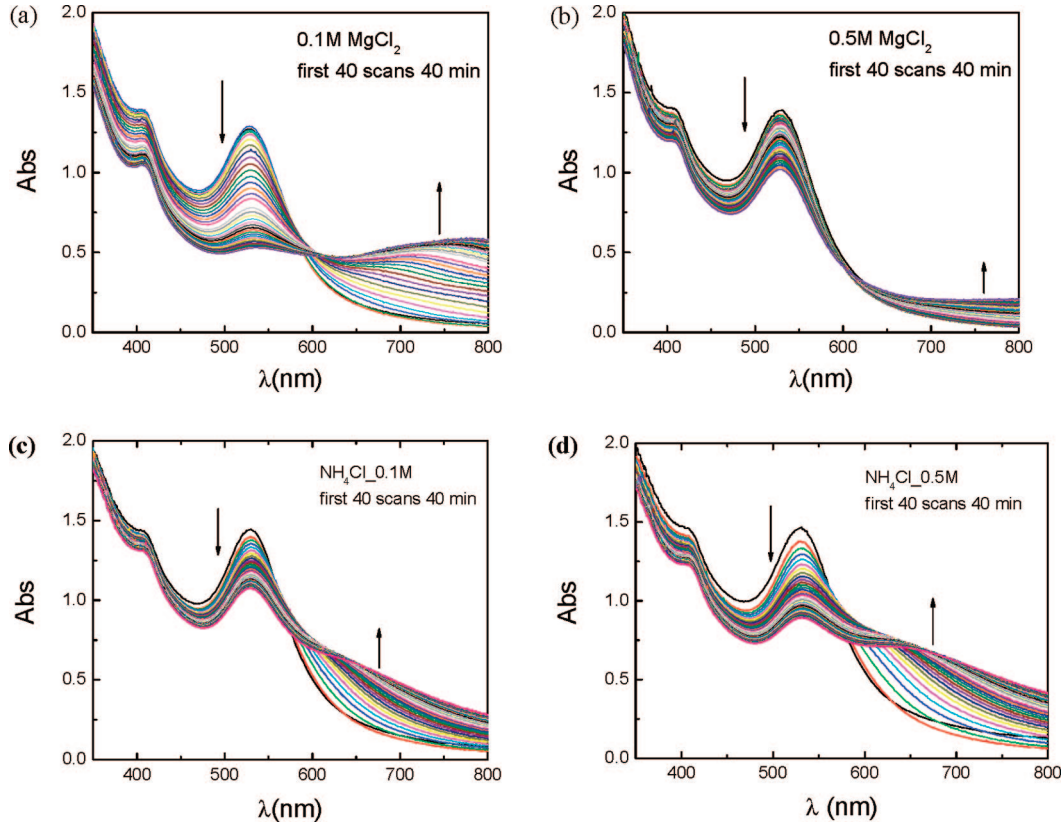


**Figure 8.** Realtime UV−vis spectra for Au20EG6OMe with BSA 400 mg/mL by adding (a) 0.1 M and (b) 0.5 M $MgCl_2$ and Au20EG6OH with BSA 400 mg/mL by adding (c) 0.1 M and (d) 0.5 M $NH_4Cl$.

forms aggregates and precipitates out of the solution. The stability of these mixtures on addition of sodium chloride has been established in a previous study.[20] In general, increasing the salt concentration increases the aggregation rate. Here, we present the data for various anions and cations in the Hofmeister series. For sodium chloride, both anion ($Cl^-$) and cation ($Na^+$)

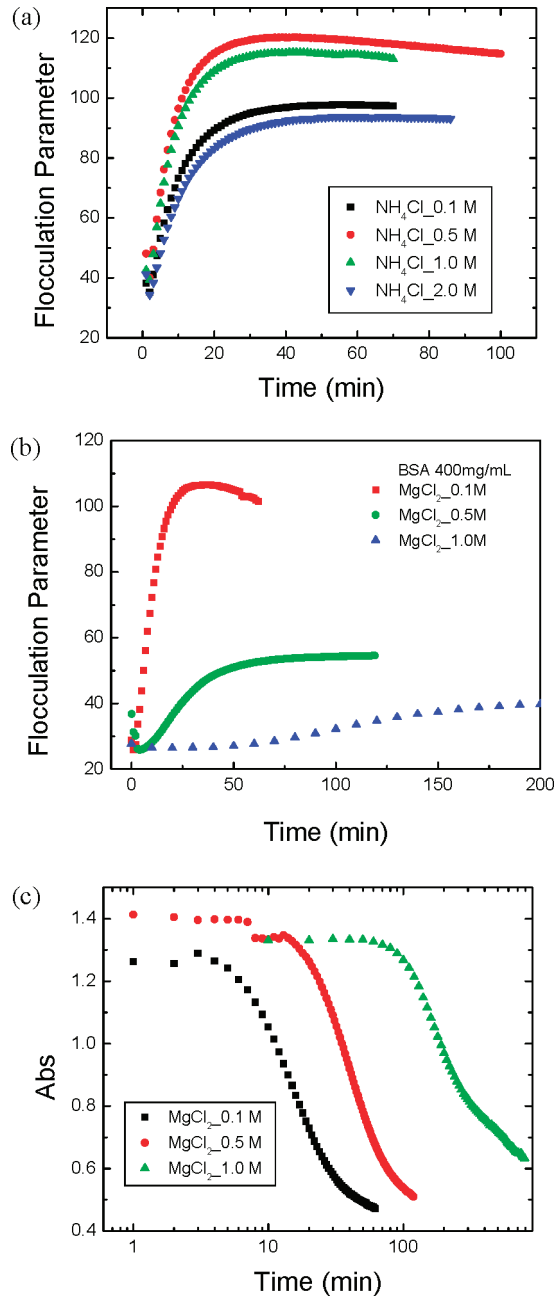


**Figure 9.** Plots of flocculation parameter for (a) $NH_4Cl$ and (b) $MgCl_2$ and (c) plots of absorption intensity at 525 nm for $MgCl_2$ as a function of time, which indicate the enhanced stability with increasing salt concentration.

are located in the middle of the Hofmeister series, indicating the unspecific property of the ions, which is used to compare and specify the effect induced by ions located at opposite sides of the Hofmeister series.

We first studied the anions located at the right-hand side of the Hofmeister series 1. Real time UV−vis spectra of the mixtures with sodium nitrate and sodium thiocyanate are displayed in Figure 5. The stability of the mixtures is enhanced with increasing salt concentration (Figure 5). With 0.1 M sodium thiocyanate, the aggregation of the colloids becomes very slow. Above 0.1 M NaSCN, the spectra remain almost constant, even after several months; hence, the system appears to be very stable. The addition of sodium nitrate also enhances the stability of the mixtures, but this enhancement is less pronounced compared to that of thiocyanate. These effects can be illustrated further

**TABLE 1: Summarized Salt Effects on the Sability of OEG SAM-Coated Colloidal Gold and Its Mixture with Proteins**

| salts | pure colloid (SAM coated AuNP) | mixture of colloid and protein |
|---|---|---|
| $Na_2SO_4$ | unstable above a critical salt concentration | enhance the flocculation significantly, the enhancement decreases slightly at high salt concentration |
| NaCl | stable | enhance the flocculation |
| $NaNO_3$ | stable | enhanced stability with increasing salt concentration |
| $NaClO_4$ | stable | enhanced stability with increasing salt concentration |
| NaSCN | stable | enhanced stability with increasing salt concentration |
| $NH_4Cl$ | stable | enhance the flocculation, the enhancement decreases slightly at high salt concentration |
| $MgCl_2$ | stable | enhanced stability with increasing salt concentration |

by the plot of absorption peak intensity at 525 nm for the case of addition of sodium thiocyanate (Figure 6a). The flocculation parameter in this case is not suitable because there is no significant change in the spectra (Figure 5) between 600 and 800 nm. For comparison, the plot for a solution with no added salt is also presented, where the absorption intensity asymptotically decreases to ~1.0. With addition of 0.1 M NaSCN, the absorption intensity shows a longer induction time before decreasing to ~0.4. Upon further increasing the salt concentration, the absorption intensity stays constant for a very long time (>1000 min). It is interesting to see that the enhanced stability by addition of 0.1 M NaSCN can be disrupted by increasing protein concentration or adding sodium chloride (Figure 6a). Upon increasing the protein concentration to 500 mg/mL or addition of 0.5 M sodium chloride, the plots shift significantly to the short time region. An increasing protein concentration simply increases the strength of the depletion force, whereas the added sodium chloride screens the surface charge of protein more efficiently than sodium thiocyanate. Both effects reduce the stability of the mixtures, as demonstrated in our previous work.[19] In the case of sodium nitrate, the plots of the flocculation parameter (Figure 6b) show that the maximum value of the flocculation parameter ($P_{max}$) decreases with increasing salt concentration, indicating the enhanced stability of the mixture.

Anions located at the left-hand side of the Hofmeister series typically have a salting-out effect. As described in the previous section, sodium sulfate destabilizes the OEG SAM-coated colloidal gold in solution above a critical salt concentration. Therefore, it is reasonable to expect that adding this salt to the protein−colloid mixture will dramatically speed up the aggregation process. Indeed, the aggregation is increased significantly (compare Figure 7a−c and Figure 5). However, it is interesting to see that the aggregation slows down with increasing salt concentration. A similar trend was also observed for pure SAM-coated colloid (Figure 3). Therefore, the added sodium sulfate, especially at high concentration, plays a similar role on the colloid in both pure colloid solution and the mixtures with proteins. The $P_{max}$ values increase first for salt concentration less than 0.33 M and decrease upon further increasing salt concentration (Figure 7d).

We further studied a pair of cations located at either end of the Hofmeister cation series 2; that is, magnesium and ammonium. Magnesium chloride (Figure 8a, b) at a concentration of 0.1 M

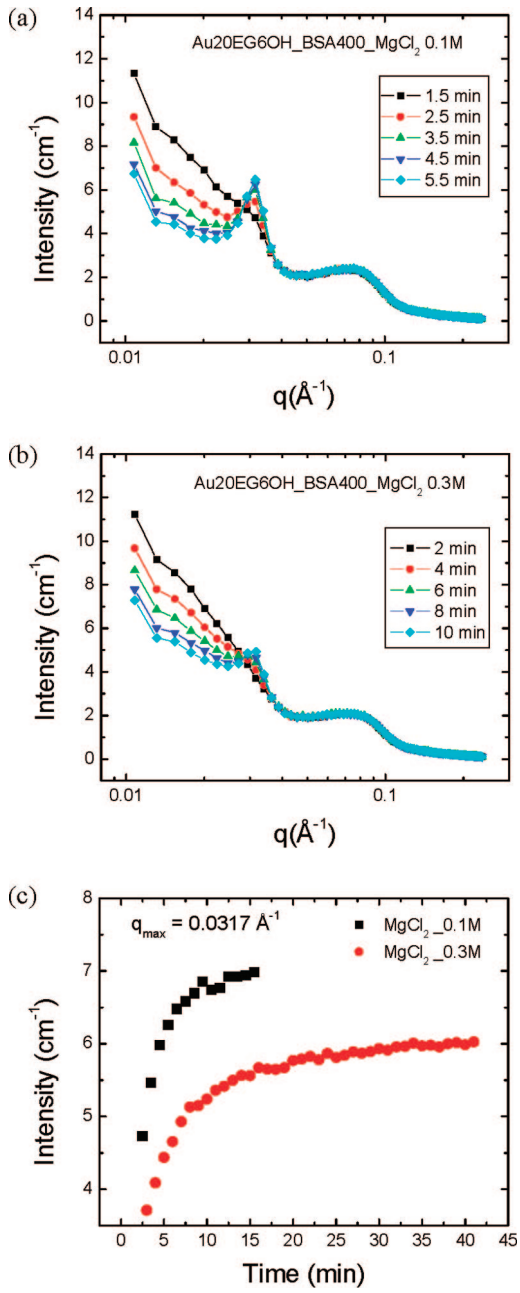


**Figure 10.** Real time SAXS profiles of Au20EG6OOH with 400 mg/mL BSA plus 0.1 M $MgCl_2$ (a) and 0.3 M $MgCl_2$ (b). Only the first five profiles are plotted. The scattering intensity at $q = 0.032$ Å$^{-1}$ as a function of time for both cases a and b is presented in c.

causes the mixture to lose its stability faster than without salt. This is due to the fact that the divalent cation has a much stronger screening effect than monovalent ions. However, on increasing the $MgCl_2$ concentration to 0.5 M, the aggregation process clearly slows down, giving a smaller $P_{max}$ (Figure 9b) and a longer induction time (Figure 9c). On further increasing the salt concentration to 1.0 M, only a very small change is observed in the spectra after 600 min (data not shown). This observation is very similar for salt-in salts with anions of nitrate and thiocyanate (Figure 5), which indicates the similar effect of cations and anions on the same side of the Hofmeister series.

In contrast, when ammonium chloride is used, the aggregation process speeds up first with increasing salt concentration (Figure 8c, 8d) up to 0.5 M, and then slows down upon further increasing the salt concentration (data for 1.0 M salt is not shown). The $P_{max}$ of the flocculation parameter has a similar salt concentration dependence (Figure 9a) which is consistent with the real time UV−vis spectra. The effect of ammonium chloride is very similar to what we observed for sodium sulfate. The difference is that the ammonium chloride does not destabilize the pure SAM-coated colloid solution, as was observed for sodium sulfate (Figure 2).

The salts used in this work have either the unspecific anion $Cl^-$ or cation $Na^+$; therefore, the observations described above really reflect the chemical nature effect of the other ions in the Hofmeister series. When comparing these effects for anions and cations (Figures 5−7 vs Figures 8,9) it is interesting to see that the anions and cations on the same side of the Hofmeister series give a similar effect as seen for thiocyanate, nitrate vs magnesium and sulfate vs ammonium. These results are summarized in Table 1.

As has been shown by studying the protein−protein interaction as a function of ionic strength, the main effect of adding sodium chloride at concentrations below 0.5 M is the screening of the surface charge of the proteins. Above 0.5 M, the aggregation rate still increases with salt concentration. In this case, the screening effect alone cannot explain this acceleration; instead, the contribution of the salt ions in the depletion driven attraction becomes important. According to Kuehner et al., colloids (proteins) in a concentrated salt solution have an attractive interaction caused by the excluded-volume effect of the ions, which is a short-ranged attractive potential.[57] This contribution can be used to explain the observation, because NaCl is an unspecific salt without any specific interaction with OEG SAMs. For NaCl > 0.5 M, the salt ions continue to contribute to the interactions causing the depletion driven aggregation, and hence, the aggregation rate continuously increases with NaCl concentration. This observation also implies that there is no surface accumulation of the unspecific ions. Compared to the unspecific ions, thiocyanate and nitrate are typical salting-in anions: they tend to accumulate at the SAM/water interface, which creates an energy barrier for colloid aggregation, and increase the solubility of the OEG thiol and implicitly that of the thiol decorated colloid. The stronger the salting-in effect of the anions, the more stable the colloid−protein mixtures become (Figure 5). On the other hand, the salting-out anion sulfate interacts strongly with bulk water, which reduces the solubility of OEG-protected colloid in water. Divalent magnesium cations interact with water strongly because of their high charge density.[22] However, due to the positive charge nature, they can physically adsorb onto the −O− group within the SAMs. This behavior is similar to the accumulation of salting-in anions at the SAM/water interface, which enhances the stability of the mixtures, resulting in a long induction time because the $Mg^{2+}$ has to be removed from the interface before the aggregates can grow. Ammonium shows a salting-out effect being similar to sulfate reducing the solubility of OEG SAM-coated colloid in solution. Similar trends on addition of the various salts described in this work are obtained for both Au20EG6OMe and Au20EG6OH, although in general, the stability of the latter colloid is better than the methyl terminated SAM protected colloid (Figure 2).

**3.4. Structure of Aggregates Studied by Real-Time SAXS.** Our previous work has demonstrated that the aggregates are in an amorphous state and that adding sodium chloride into the mixture speeds up the colloid aggregation dramatically and the growth rate increases with salt concentration.[20] In this study, we show that the aggregation speed also depends on the nature of the added salt. UV−vis results indicate that adding $MgCl_2$ reduces the aggregation speed (Figure 8). Real-time SAXS measurements of colloid−protein mixtures with addition of salts (Figure 10) are consistent with this observation. From the first 5 SAXS profiles with $MgCl_2$ of 0.1

and 0.3 M (Figure 10a and b), it is obvious that the evolution of the scattering maximum decreases with increasing salt concentration. It is worth noting that in both cases, the peak position is at $q = 0.032$ Å$^{-1}$. The scattering intensity at $q = 0.032$ Å$^{-1}$ is plotted as a function of time for both cases (Figure 10c), which further demonstrates that the colloidal aggregation procedure is suppressed with increasing salt concentration. These observations are consistent with our previous work and suggest that the colloid aggregates are in an amorphous phase, which does not depend on the nature of the added salt.[20]

## 4. Conclusions

In this work, we have reported that protein (BSA) solutions with the addition of any salt used in this work (sodium chloride, sodium nitrate, sodium thiocyanate, ammonium chloride, and magnesium chloride) up to 2.0 M are always stable. For OEG SAM-coated colloidal gold, they are stable in aqueous solutions with salt up to 1.0 M, except for $Na_2SO_4$; that is, the colloid starts to aggregate with $Na_2SO_4$ above a critical concentration. This observation indicates that the nature of added salts (i.e. the Hofmeister effect) does not change the stability of either the OEG SAM-decorated colloidal gold or BSA in solution. On the other hand, there is a strong dependence of the stability of colloid−protein mixtures on the nature of salts. Anions and cations on the same side of the Hofmeister series give a similar effect: thiocyanate, nitrate, and magnesium enhance the stability of the mixture with increasing salt concentration, whereas sulfate and ammonium lead to the aggregation of colloids. Compared to the effects on the one-component systems (protein or colloid solution), an enhanced Hofmeister effect is observed in two-component systems. The structure of the aggregates was studied by real-time SAXS measurements. No Bragg peak could be observed in both the scattering profiles and the evaluated effective structure factor, which indicates that the resulting aggregates are always in an amorphous phase.

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