Chapter 4

Analysis of cluster aggregation processes by dynamic light scattering

4.1 Introduction

The most important result of the previous chapter was the preferential formation of mixed aggregates of Au$_{55}$–M and Au$_{55}$–D clusters in media composed of DMSO and 1,4-dioxane.$^1$ When the DMSO/dioxane ratio was altered, the morphology of the aggregates changed systematically. It remained unclear however, why the particular strand-like morphology was formed. Most conclusions were derived from a posteriori investigations by transmission electron microscopy (TEM) of macroscopic aggregates that had deposited in these experiments. In this chapter aggregation processes will be monitored in situ by dynamic light scattering (DLS). The formation of aggregates can be observed in a non-destructive way with this technique, long before the aggregates have reached their final macroscopic size. DLS is a statistical technique, probing a number of aggregates simultaneously. The evolution of the aggregates as a function of time gives some insight in the kinetics of the process. As before, a comparison will be made of aggregation processes in DMSO/dioxane mixtures of different composition.

This chapter is organised as follows: First, a brief introduction to the field of colloid aggregation kinetics will be given. Most theories in this field have been developed for the aggregation of particles that are substantially larger than Au$_{55}$. They provide a good background for our present investigation however, and their applicability to the aggregation of Au$_{55}$ will be tested. In the next section, the applied light-scattering technique is explained. The experimental approach and its results will be dealt with

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$^1$'Dioxane' always means 1,4-dioxane in the remainder of the chapter.
in sections 4.4 and 4.5. A discussion linking these results with the general colloidal aggregation kinetics is given in §4.6.

4.2 Kinetics of colloid aggregation

There is a wide range of literature concerning the analysis of irreversible colloid aggregation [1–9]. Irreversible aggregation means that two particles remain bound, once they have been connected. The morphology of aggregates formed in this way is determined by the growth kinetics, rather than by the thermodynamically most stable final form.

The key feature of the binding of Au$_{55}$–M and Au$_{55}$–D is the intercluster hydrogen bonding, which was intended to be reversible. Several features of the structures obtained in the previous chapter indicate that the aggregation process ultimately is an irreversible process: (1) The strand-like aggregates did not show any macroscopic restructuring once they were formed, even though (2) the thinnest strands – with the largest exposed surface – were formed in the least solvating solvent mixture. Moreover, (3) attempts to redissolve aggregates in DMSO have failed. Therefore, several properties that were shown to be universal for irreversibly aggregating particles may be applicable to the aggregation of Au$_{55}$–M and Au$_{55}$–D as well. The following section will describe some of these universal features.

4.2.1 Fractal growth and limiting regimes

The most prominent feature of irreversibly grown colloidal aggregates is their relatively open spatial structure. Many of these aggregates$^2$ have a spatial structure that is to a certain degree scale-invariant. Their mass scales with their radius as $M \propto R^{d_F}$. Here $d_F$ denotes the fractal dimension. For a dense aggregate (e.g. a crystal) this dimension equals the geometrical dimension: $d_F = 3$, whereas so-called fractal aggregates have $d_F < 3$. It has been shown that the aggregation morphology is independent of the specific type of particles [10, 11], and that most aggregates have $1.75 \leq d_F \leq 2.1$ [7, 12]. This was in agreement with computer simulations (see e.g. [1, 13]). The two limiting values for $d_F$ are determined by two limiting regimes for the aggregation process which have been called diffusion limited colloid aggregation (DLCA) and reaction limited colloid aggregation (RLCA). The origin of these regimes will now be explained.

The rate of aggregation is governed by the potential energy barrier ($E_{\text{bar}}$) which has to be overcome by two approaching particles to come into close contact. Once they are in contact, the binding is irreversible (i.e. $E_{\text{bind}} \gg k_b T$). If the energy barrier is very high ($E_{\text{bar}} \gg k_b T$), the colloid is stable in the suspended state. If there is no significant energy barrier ($E_{\text{bar}} \ll k_b T$), aggregation is fast, the only rate limit being

$^2$Notice that in the colloid aggregation field aggregates are typically called clusters, their constituents being colloidal particles. For clarity, I only use cluster in its inorganic chemical meaning (cf. Ch.1.1).
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the rate at which particles and aggregates can diffuse towards each other. This is the DLCA regime. Since no structural rearrangement is possible in this scenario, it leads to relatively open and tenuous structures with values of $d_F \approx 1.75$. The characteristic average mass of DLCA-aggregates increases linearly with time, which means that the radius scales as $R \propto t^{1/d_F}$ [10].

If on the other hand the energy barrier is $E_{\text{bar}} \gtrsim k_B T$, most colliding particles will not overcome the barrier and there only is a finite chance of binding. The growth rate will be slowed down by the stability ratio [14]:

$$W = \frac{\text{(number of collisions)}}{\text{(number of collisions leading to coagulation)}} = \frac{k_{\text{DLCA}}}{k_{\text{RLCA}}} \quad (4.1)$$

Alternatively, the sticking probability is defined as $p = 1/W$ [15, 16]. In this regime, approaching particles or aggregates have the opportunity to ‘explore’ a number of mutual configurations before they bind. The resulting higher degree of interpenetration leads to denser aggregates with $d_F \approx 2.05$ [12, 16]. The time-evolution of RLCA is quite different from DLCA: $R \propto e^{Ct}$, where $C$ is a parameter depending on the exact experimental conditions [11, 16]. The continually increasing rate may be understood by the fact that larger aggregates have a larger exposed area and hence a larger overall sticking probability [12]. Since larger aggregates diffuse more slowly, and aggregate concentration decreases during the aggregation process, a cross-over to DLCA-like behaviour is expected to take place at prolonged aggregation times [12].

So far, the focus was on limiting regimes of irreversible aggregation. Intermediate behaviour between RLCA and DLCA can also be observed [7, 12], when neither diffusion nor sticking is dominant or when the aforementioned cross-over takes place. Restructuring due to a certain degree of reversibility can lead to denser structures on prolonged aggregation times [17–19]. On the other hand, denser structures may also be formed (only) in the initial stages of aggregation, if the experimental conditions cause the mean free path of individual particles to be long compared to the size scale of the forming aggregates. The particles thus seem to move along straight trajectories, resulting in denser structures. This has been called ballistic aggregation [1, 20]

4.3 Dynamic Light Scattering

Dynamic Light Scattering (DLS) can be used to determine particle size distributions. A thorough understanding of the technique is required to avoid possible pitfalls in the interpretation of the data. A brief introduction will be given here.

A DLS setup is depicted very schematically in figure 4.1. A particle-containing solution is irradiated with a laser beam. Part of the incoming light is scattered by the particles, provided they have a different dielectric constant than the medium. The

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3Note that DLS is also referred to in literature as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS).
Figure 4.1: Schematic experimental setup for Dynamic Light Scattering.

angular dependence of the scattered intensity can reveal structural information in a technique called Static Light Scattering, but that will not be employed here. In a typical DLS experiment, intensity fluctuations in the scattered light will be analysed for a fixed scattering angle. These fluctuations arise from interference effects in the scattered light, due to the (Brownian) motion of the particles. In the so-called self-beat or homodyne scattering mode, light scattered by one scatterer (particle) interferes with that scattered by another particle.

### 4.3.1 The scattered intensity

A derivation of an expression for the intensity of the light scattered by a particle is outside the scope of this work and can be found in literature [14, 21, 22]. It is worthwhile to note the following general relationships:

\[ I \propto E^2 \propto \mu^2 \propto \alpha^2 \propto m^2 \propto r^6. \]  

This states that the scattered intensity \( I \) is proportional to the square of the scattered electric field \( E \). \( E \) is proportional to the induced dipole moment \( \mu \), which depends on the polarisability. It is a general assumption in DLS analysis that the overall polarisability of a scattering object is proportional to its mass. Finally, for a compact (spherical) object, the mass is proportional to the cube of the radius. Therefore, there is a strong dependence of the scattered intensity on the mass or size of the scattered object.\(^4\) In case there is a mass or diameter distribution, contributions from the larger objects usually dominate the scattered intensity.

\(^4\)Note that the simple relationship \( m \propto r^3 \) only applies for compact objects. For fractal objects \( m \propto r^{d_f} \), but the tendency is the same.
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4.3.2 Analysis of the fluctuations

In DLS, the intensity fluctuations are analysed by means of the scattered intensity time-autocorrelation function [7, 21, p.13]:

\[ G^{(2)}(\tau) = \langle I(0)I(\tau) \rangle = \lim_{T \to \infty} \frac{1}{T} \int_0^T I(t)I(t + \tau) dt \tag{4.3} \]

It will be shown below that the relevant information is contained in the (normalised) electric field correlation function \( g^{(1)}(\tau) \), which relates to \( G^{(2)}(\tau) \) by [7]:

\[ G^{(2)}(\tau) = A(1 + b|g^{(1)}(\tau)|^2) \tag{4.4} \]

Here, \( A \) is the background (\( A = \langle I \rangle^2 \)), \( b \) is a spatial coherence factor. Relation (4.4) is valid under the assumption of a sufficient amount of statistically unrelated scattering volumes, only singly scattered photons (see [21], p.40–42) and ignoring rotatory movement of anisotropic particles [23].

In case of a single scatterer, \( g^{(1)}(\tau) \) shows exponential decay, determined by the diffusion coefficient \( D \):

\[ g^{(1)}(\tau) \propto \exp\{-q^2D\tau\} \tag{4.5} \]

Here, \( q \) is the magnitude of the scattering vector [21]:

\[ q = \frac{4\pi n}{\lambda} \sin \theta/2 \tag{4.6} \]

with \( n \) the refractive index of the medium, \( \lambda \) the wavelength of the incident light and \( \theta \) the scattering angle. \( D \) is related to the hydrodynamic radius \( R_h \) of the scatterer by the Stokes-Einstein relation [14]:

\[ D = \frac{k_B T}{6\pi \eta R_h} \tag{4.7} \]

Here, \( \eta \) is the (dynamic) viscosity of the solvent.

If there is no single scatterer, but an ensemble having a size-distribution of unknown shape and magnitude, the statistical moments describing the distribution can be obtained from the ensemble-average \( |g^{(1)}(\tau)| \) by determination of the parameters in a so-called cumulants expansion [7, 10, 21, 23]:

\[ \ln |g^{(1)}(\tau)| = \exp\{1 - \Gamma_1 \tau + \frac{1}{2} \Gamma_2 \tau^2 - \frac{1}{3!} \Gamma_3 \tau^3 + \ldots\} \tag{4.8} \]

\(^5\)The normalised electric field correlation function is defined as \( g^{(1)}(\tau) = \frac{\langle E^*(q,0)E(q,\tau) \rangle}{\langle E^*(q,0)E(q,0) \rangle} \).

\(^6\)Note that \( R_h \) is defined as the radius of the spherical particle having the same \( D \) as the particle under investigation.
The first and second moments of the distribution (\( \Gamma_1 \) resp. \( \Gamma_2 \)) relate to the z-average \( D \) and its variance respectively:\(^7\)

\[
\bar{\Gamma}_1 = q^2 \langle D \rangle_z \\
\Gamma_2 = q^4(\langle D^2 \rangle_z - \langle D \rangle_z^2)
\]

(4.9)

The polydispersity is defined as \( \Gamma_2/\bar{\Gamma}_1^2 \). The only type of \( D \)-distribution that would be fully described by \( \mathcal{D}_z \) and the polydispersity is a monomodal log-normal distribution. Other distributions have non-zero higher order moments. Note also that \( \langle R_h \rangle \) is an intensity averaged radius. Due to eq. (4.2) this means that the contribution of each \( R_h(M) \) value will be weighted by \( M^2 \), making \( \langle R_h \rangle \) a z-average radius. Strictly speaking, application of the Stokes-Einstein relation to obtain \( \langle R_h \rangle \) ignores internal motion/rotation (for non-spherical particles)\(^8\) and motion due to interparticle interactions. For the purpose of this work, the accuracy is sufficient. Hence these effects are neglected.

Gold clusters or nanoparticles have a large absorption coefficient at the wavelength of the used laser-light. Therefore it has been suggested [24] that thermal blooming should be taken into account to find the correct radii, since absorption causes local heating around the particles. In the system under investigation here, the trends in the radii are far more important than their absolute value, so this absorption correction has been omitted.

In this work, the focus will be on \( \langle R_h \rangle \) as a descriptor of the aggregates that are formed. On theoretical grounds, neither DLCA nor RLCA are expected to produce a simple log-normal distribution [10, 11, 16]. Nevertheless, \( \langle R_h \rangle \)–as obtained via eq. (4.8)–is a characteristic size measure in both cases [10, 11, 16]. Its time development therefore also is characteristic of the corresponding growth regime.

### 4.4 Experimental approach

The first goal of this work is to determine whether it is possible to follow the aggregation process of \( \text{Au}_{55}\)-M and \( \text{Au}_{55}\)-D in DMSO/dioxane mixtures. When that has been established, the effect of variation of the DMSO concentration in the aggregation media can be investigated. First the proper experimental conditions need to be found, taking into account several limitations of the DLS equipment: (1) The DLS apparatus has a theoretical detection range of \( \sim 5 \sim 5000 \text{ nm} \): Small particles are too weak scatterers, very large particles (or aggregates) move too slowly for correlation analysis. This means that individual \( \text{Au}_{55} \) clusters cannot be detected, but

\(^7\)A z-average moment is defined as the average over \( M^2 \), as in

\[
\mathcal{D}_z \equiv \frac{\sum N_i m_i^2 D_i}{\sum N_i m_i^2}
\]

where \( N_i \) is the number of aggregates with mass \( m_i \) and diffusion coefficient \( D_i \).

\(^8\)For fractal aggregates this is treated in [23], for rigid rods in [21], p.177
aggregates of clusters are detectable, until they become too large. (2) Sufficient counting statistics are required to obtain a reliable value for $\langle D \rangle_z$. The higher the initial cluster-concentration, the better the counting statistics and the shorter the required measurement time will be. (3) The process should not develop too fast with respect to the time necessary for a single measurement. The aggregation is expected to be slower when the concentration is lower. To find a balance between (2) and (3), a number of measurements was performed with solutions containing different concentrations of Au$_{55}$–M and Au$_{55}$–D (0.006, 0.025, 0.05, 0.1 mg/ml of each) but with the same dioxane/DMSO ratio (10%($v/v$) DMSO). Subsequently, the DMSO concentration was varied from 5–15%($v/v$) at one cluster concentration. Reference samples were prepared containing only one kind of cluster, at the same total cluster concentration, to compare homo- and hetero-aggregation processes.

A typical measurement sequence consisted of the following steps: the desired mixture of Au$_{55}$–M and Au$_{55}$–D stock solutions, DMSO and dioxane was prepared directly in the cuvette, immediately homogenised by shaking for 2–3 seconds and placed in the DLS apparatus. After three minutes of equilibration, the measurements started. Every measurement lasted 13 minutes (600s of actual photon counting). The interval between two measurements was 30 minutes. Every aggregating system was monitored for at least 16 hours, or until no further development was noticeable. After the measurement, the cuvette was visually checked for macroscopic aggregates and TEM-specimens of the aggregates and/or solution were prepared.

### 4.5 Results

#### 4.5.1 Analysis of a single measurement

In this paragraph the analysis of a typical single measurement will be explained. This analysis should lead to a value of $\langle R_h \rangle$, which will constitute one data point in a sequence. These sequences are presented in the next section.

Every measurement produces an autocorrelogram of the scattered intensity, summed over the total acquisition time (600s). To determine $\langle R_h \rangle$, a least squares fit of the data to eq. (4.8) is performed. A typical correlogram is shown in figure 4.2.a and b, with the corresponding fit. Here, $[\text{Au}_{55}–\text{M}] = [\text{Au}_{55}–\text{D}] = 0.025 \text{ mg/ml}$, $[\text{DMSO}] = 10\% (v/v)$ and $t = 4.4$ hour. The exponential decay can clearly be seen from the graph in figure 4.2.a with a linear correlation time scale, while the details of the correlation and the fit are best observed with the logarithmic time scale of figure 4.2.b. The relevant fit parameters $\bar{\Gamma}_1$ and $\bar{\Gamma}_2$ result in $\langle R_h \rangle = 113.3 \text{ nm}$ with a polydispersity of 0.089. The log-normal distribution that would be fully determined by these parameters is shown in figure 4.2.b. It should be stressed again, that this may not

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$^9$Depending on particle size and concentration this time may vary between a few minutes and several hours.
be the real size distribution, since this method does not take possible multimodality into account. An alternative approach is by the Contin-method [25, 26], which can be capable of resolving multimodal distributions. No significant improvement was obtained by this method. Therefore the measure of $\langle R_h \rangle$ obtained by the monomodal method has been used in the following interpretations, in accordance with procedures used in the literature (cf. §4.3.2).

### 4.5.2 Variation of the cluster concentration

Aggregation experiments were performed in DMSO/dioxane mixtures with 10% (v/v) DMSO, with $[\text{Au}_{35}-\text{M}] = [\text{Au}_{35}-\text{D}] = 0.006$, $0.050$ and $0.025$ mg/ml respectively. At the lowest concentration, the count rate was too low to obtain reasonable statistics throughout the first three hours. Both other concentrations yielded reasonable count rates and a reasonable time evolution of $\langle R_h \rangle$, as can be seen in figure 4.3 and 4.4 respectively.

The count rate is the analogon of the scattered intensity when a discrete photon counting technique is used. Since the count rate is sensitive to both the mass and the number of aggregates formed, it can give a first impression of changes taking place during the experiment, without the application of any fitting or modelling.\(^{10}\) Both samples show an initial increase in count rate followed by a more or less stable regime. At 0.050 mg/ml, a decay of the count rate sets in after about ten hours. This is attributed to sedimentation, removing material from the solution. Indeed, the formation of sedimented macroscopic aggregates was visible in the cuvette after

\(^{10}\)The count rate is expressed in units of 1000 Counts per second (kCps).
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Figure 4.3: Time development of the count rate (in kCps) during the aggregation of Au₅₅–D and Au₅₅–M at different cluster concentrations in DMSO/dioxane mixtures containing 10%(v/v) DMSO. The cluster concentrations are [Au₅₅–M] = [Au₅₅–D] = 0.050 mg/ml and 0.025 mg/ml.

Figure 4.4: Time development of the mean hydrodynamic radius ⟨Rₜₜ⟩ during the aggregation of Au₅₅–D and Au₅₅–M at different cluster concentrations in DMSO/dioxane mixtures containing 10%(v/v) DMSO. The cluster concentrations are [Au₅₅–M] = [Au₅₅–D] = 0.050 mg/ml and 0.025 mg/ml.
The development of the average cluster size as depicted in figure 4.4 shows that the behaviour of both samples is quite similar. Both show a progressive increase in average size until $\langle R_h \rangle \approx 600 \text{ nm}$ at the end of the sequence, but for a concentration of 0.025 mg/ml of each cluster the development is slower than for a concentration of 0.050 mg/ml. At the latter concentration, the aggregate size development shows a deflection after about ten hours: The rate of increase becomes smaller until $\langle R_h \rangle$ becomes more or less constant. Just as mentioned above for the change in count rate, this is attributed to the sedimentation of large aggregates, although depletion of free clusters in the solution may also play a role in slowing down the growth of the aggregates.

The concentration of $[\text{Au}_{55-}\text{M}] = [\text{Au}_{55-}\text{D}] = 0.025 \text{ mg/ml}$ seems the most suitable for further investigation. Of the investigated concentrations with detectable signals, this concentration gives rise to the slowest development and fewer effects of sedimentation or depletion.

### 4.5.3 Variation of DMSO concentration

In this section a few measurement sequences with varying DMSO concentrations will be presented. The time-evolution of $\langle R_h \rangle$ and the scattered intensity will be described in all cases. A further interpretation and comparison of these results will be treated in the discussion in §4.6.

In figure 4.5 and 4.6 the development of $\langle R_h \rangle$ and the count rate during aggregation in DMSO/dioxane mixtures containing 5, 10 and 15%($\text{v/v}$) DMSO are compared. These mixtures will be addressed as MD5a & MD5b (identical measurements in duplo), MD10 and MD15 respectively. A quick comparison already shows that the higher the DMSO concentration, the larger the aggregates that are formed. At a concentration of 5%($\text{v/v}$) DMSO there is a relatively fast stabilisation of the count rate, compared to MD10 and MD15. The average radius increases slowly (but steadily) throughout the experiment (up to $\langle R_h \rangle \approx 175 \text{ nm}$ after 27 hours in one case). The data of MD5a&MD5b do not indicate the occurrence of sedimentation at a substantial degree during the studied time frame. The two experiments that were performed with 5% DMSO show marked differences in count rate and an increasing divergence of their average radii. There are several possible explanations: It will become clear in §4.6.1, that $\langle R_h \rangle$ grows exponentially as a function of time. Therefore, minor differences in the aggregation conditions at early stages of the process will be amplified as time progresses. The magnitude of the count rate is very sensitive to the size (or actually the mass) of the aggregates, but that may not be sufficient to explain the differences.

Experiment MD10 is the same experiment as presented in §4.5.2 (cluster concentrations of 0.025 mg/ml), with a steady increase in average radius. In MD15, by far the largest aggregates are formed. As a result, the effect of sedimentation becomes obvious earlier than in the other samples: the count rate reaches a maximum after about
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Figure 4.5: Time development of the average hydrodynamic radius $\langle R_h \rangle$ during the aggregation of Au$_{55}$–D and Au$_{55}$–M in DMSO/dioxane mixtures of varying composition, where $[\text{Au}_{55}^-\text{M}] = [\text{Au}_{55}^-\text{D}] = 0.025$ mg/ml. Two experiments were performed with 5%(v/v) DMSO.

Figure 4.6: Time development of the count rate (in kCps) during the aggregation of Au$_{55}$–D and Au$_{55}$–M in DMSO/dioxane mixtures of varying composition, where $[\text{Au}_{55}^-\text{M}] = [\text{Au}_{55}^-\text{D}] = 0.025$ mg/ml.
7 hours and than falls off steadily almost until the background noise level (∼2 kCps) is reached after 44 hours. At that moment, practically all material had sedimented. The size data are only presented for the first 16 hours. After that, the values found for ⟨Rh⟩ by fitting the data, start to fluctuate strongly and therefore become unreliable. The underlying poor statistics are caused by (1) the large aggregate size compared to the range accessible with the autocorrelator, (2) the low total number of aggregates (because of their size and the sedimentation). In all experiments discussed here, the data obtained in the first 10 to 15 hours will suffice to assess the kinetics of the aggregation process.

4.5.4 Comparison with homo-aggregation

Aggregation experiments were also performed at the same DMSO concentrations with either Au55–M or Au55–D alone. The same total cluster concentration was used as in the previous part, i.e. 0.050 mg/ml. The resulting ⟨R⟩ and count rates are presented in figures 4.7 and 4.8. The behaviour of the samples containing Au55–M can be interpreted in a straightforward way. At 5%(v/v) DMSO (experiment M5) the count rate is 4.4 kCps, slightly above the background level. The fits resulting in ⟨R⟩ ≈ 100 nm are very poor, making the result questionable. If any aggregates are formed, they are too few to be detected properly. At 10%(v/v) (experiment M10) the count rate does not exceed the background level of ∼2 kCps. The fits leading to ⟨R⟩ are even poorer. A cuvette filled with only DMSO and dioxane gives the same result. Together with what was already known about Au55–M homo-aggregation from Chapter 3, this leads to the conclusion that at a DMSO concentration of 5%(v/v) too little aggregation (if any) takes place to be detectable, whereas at a DSMO concentration of 10%(v/v), no aggregation takes place at all. For that reason, higher DMSO concentrations were not investigated for Au55–M.

The situation is quite different for the homo-aggregation of Au55–D. Some kind of aggregation clearly occurred, both in mixtures containing 10 and 15%(v/v) DMSO (experiments D10 and D15). The key question therefore is whether this kind of aggregation can be distinguished from the aggregation that took place in solutions containing both Au55–D and Au55–M. D15 shows a much higher maximum count rate, yet a smaller average aggregate size compared to MD15. In D15 the count rate starts to decrease within two hours, which is much faster than in MD15. This implies that sedimentation is much faster in this case. These observations would be consistent with the clusters in the aggregates formed in D15 being packed more densely than in MD15. The scattered intensity is proportional to $M^2$ and only via that to ⟨R⟩, so denser aggregates would produce higher count rates than less dense aggregates of the same diameter. Aggregates with a higher packing density will also sediment faster. Indeed, the formation of dense spherical aggregates of Au55–D was observed in Chapter 3. A further indication of the higher density of the aggregates is the nature of the precipitate that formed in the course of two days. Unlike the precipitates
Figure 4.7: Time development of the average hydrodynamic radius ($\langle R_h \rangle$) during the aggregation of either Au$_{55}$–D or Au$_{55}$–M alone in DMSO/dioxane mixtures of varying composition, where [Au$_{55}$–M] or [Au$_{55}$–D] = 0.050 mg/ml.

Figure 4.8: Time development of the count rate (in kCps) during the aggregation of either Au$_{55}$–D or Au$_{55}$–M alone in DMSO/dioxane mixtures of varying composition, where [Au$_{55}$–M] or [Au$_{55}$–D] = 0.050 mg/ml.
formed in the MD experiments, the precipitated film could not be redispersed by mild agitation. On the other hand, the material dissolved readily in pure DMSO. This is an indication that the DTOT–DTOT binding in these aggregates is weaker than that in the MOT–DTOT aggregates. Hence, it could explain the formation of denser aggregates. D10 behaves similar to D15, with two differences: the count rate develops much more slowly and the average aggregate size is smaller. This may be explained by the fact that under these conditions the solvation of the clusters is poorer. This leads to a smaller critical nucleus size (cf. §3.8.2) and therefore to an increased probability of nucleus formation. Therefore many more nuclei are formed. Consequently, these can only grow into smaller aggregates, before complete depletion of the solution from free Au55–D clusters is attained. Super-aggregation (i.e. the aggregation of aggregates) may also be expected to proceed more slowly than for larger aggregates.\textsuperscript{11}

Summarised, homo-aggregation of Au55–D can occur in DMSO/dioxane mixtures, but it develops a clearly different aggregate morphology than the one which is formed when both Au55–D and Au55–M are present. This observation enables the possibility of competition between homo- and hetero-aggregation. This is not expected however, in mixtures with 10 or 15\%(v/v) DMSO, because in §3.7.3 the morphological effect of this competition was shown to disappear when more than 6\%(v/v) DMSO was present.

Altogether, the observations presented above confirm that Au55–D is less soluble in DMSO/dioxane mixtures than Au55–M. Nevertheless, MOT–DTOT binding is much stronger than DTOT–DTOT binding. Therefore, upon addition of dioxane to the DMSO solution, the sticking probability \( p(\text{Au55–M}—\text{Au55–D}) \) will increase in such a way that hetero-aggregation will be the favoured process, even if solvated Au55–D clusters are more destabilised than the Au55–M clusters.

### 4.5.5 Transmission Electron Microscopy

Investigation of the aggregates, formed in the experiments described, by TEM confirmed that their morphology was very similar to that presented in §3.7.2 on page 88. Figure 4.9 shows an example that was obtained from MD10. The strand-like structures that are shown here exhibit a typical diameter of 41 ± 8 nm at 10\%(v/v) DMSO. This is in quite good agreement with the trend observed in §3.7.3, where a diameter of 49 ± 9 was obtained at 10\%(v/v) DMSO. Therefore it may be concluded that the aggregation in these experiments proceeded in the same way as in §3.7.3, despite the differing cluster concentrations (0.025 mg/ml here and 0.06 mg/ml in §3.7.3).\textsuperscript{12}

\textsuperscript{11}The size-dependence of the rate of super-aggregation will depend on a balance between diffusion speed and sticking probability: \( D \propto 1/r \) whereas \( p \propto r^3 \). Therefore, the larger sticking probability of larger aggregates outweighs their slower diffusion.

\textsuperscript{12}The aggregates formed by homo-aggregation of Au55–D could not be studied by TEM, since they could not be recovered from the sedimented state in any other way than by redissolving the clusters in DMSO.
4.6 Discussion

4.6.1 Reaction limited or diffusion limited growth?

As a first approach towards further understanding of the growth behaviour, the aggregate growth presented in figure 4.5 can be modelled with the DLCA and RLCA rate laws. This is most conveniently done by fitting a straight line to the data either in a double logarithmic (DLCA) or in a single logarithmic representation (RLCA). Figure 4.10 shows fits of the data of all the samples in figures 4.4 and 4.5 to functions of the shape $\ln\langle R(t) \rangle = \ln R_0 + Ct$. The corresponding parameters are listed in table 4.1. Good fits are obtained for intervals ranging from the first eight hours for MD15 to the whole sampling period for MD5.\(^{13}\) This means that in all cases exponential growth is observed, at least up to a point where sedimentation starts to interfere. This is an indication that the aggregation process is reaction limited. It should be noted however, that it has not been worked out at this stage, which process governs this growth: cluster-aggregate growth, aggregate-aggregate growth or a combination of the two. The exponential scaling is valid for RLCA-type behaviour in all these cases [15].

The value of the scaling parameter $C$ clearly varies with either the cluster or the DMSO concentration. When the cluster concentration is increased, $C$ increases too. This can be concluded by comparison of the measurements with 10%($\nu/\nu$) DMSO and cluster concentrations of 0.050 and 0.025 mg/ml respectively, yielding $C$-values of 0.19

\(^{13}\)On top of that, it was impossible to obtain satisfactory fits of the data to a DLCA model.
Figure 4.10: Fits of $\langle R_i \rangle$ to $\ln \langle R_i \rangle(t) = \ln \langle R_i \rangle_0 + Ct$. Note that MD10–ii contains 10% (v/v) DMSO and cluster concentrations of 0.050 mg/ml.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>$\ln \langle R_i \rangle_0$</th>
<th>$\langle R_i \rangle_0$ (nm)</th>
<th>C (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD5a</td>
<td>3.99</td>
<td>54</td>
<td>0.08</td>
</tr>
<tr>
<td>MD5b</td>
<td>4.10</td>
<td>60</td>
<td>0.04</td>
</tr>
<tr>
<td>MD10</td>
<td>3.66</td>
<td>39</td>
<td>0.19</td>
</tr>
<tr>
<td>MD10–ii</td>
<td>3.86</td>
<td>47</td>
<td>0.25</td>
</tr>
<tr>
<td>MD15</td>
<td>3.53</td>
<td>34</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Table 4.1
Parameters of fitting size data to an exponential growth curve: $\ln \langle R_i \rangle(t) = \ln \langle R_i \rangle_0 + Ct$ (see text). All experiments are carried out with $[\text{Au55–M}] = [\text{Au55–D}] = 0.025$ mg/ml, except MD10–ii which had concentrations of 0.050 mg/ml.
and 0.25. The observation of faster growth at higher concentrations seems reasonable by intuition, although the data are insufficient to establish a quantitative relationship between $C$ and the cluster concentrations.

The values of $C$ for MD5a, b, MD10 and MD15 are plotted against the mole fraction of DMSO in the mixture in figure 4.11. The data seem to suggest a linear dependence of $C$ on the DMSO fraction: $C(x_{\text{DMSO}}) = C_0 + ax_{\text{DMSO}}$, with $C_0 = -0.09 \text{ hr}^{-1}$ (sic!) and $a = 2.5 \text{ hr}^{-1}$. There are no theoretical grounds to assume linear behaviour, however. Rather, the relationship has to break down somewhere on either side outside the investigated concentration range: the relationship does not make sense when $x_{\text{DMSO}} = 0$ and above a certain threshold $x_{\text{DMSO}}^c$ aggregation will no longer take place ($0.23 \lesssim x_{\text{DMSO}}^c \leq 1$).

Fullam et al. have performed similar DLS analysis with complementary functionalised silver and gold particles aggregating by means of trifold hydrogen bonding [27]. They reportedly find DLCA behaviour on a much shorter time scale (1000 sec). However, it is difficult to judge how reliable this assignment is, based on the poor fit that is presented. A different aggregation mechanism may be attributed to the fact that their work was carried out in chloroform, which does not suppress hydrogen bonding. On the other hand, all aggregation experiments presented in our work, show reaction-limited kinetics, since they are carried out in conditions where the inter-cluster hydrogen bonding is tempered. An advantage of the latter is, that reaction limited processes offer the possibility to obtain denser structures through ‘geometry optimisation’ via the probing of multiple configurations before definite irreversible bonding takes place. The composition of the solvent mixture also provides a tool for ‘fine-tuning’ of the interactions.

### 4.6.2 Nucleation and growth

In this section the difference in development between experiments MD5, MD10 and MD15 will be linked with a possible mechanism of formation of the aggregates. In Chapter 3 it was already speculated that the ease of formation of critical nuclei might be the key in understanding the trend that increasing strand diameters are observed with increasing DMSO concentrations. At lower DMSO concentrations, the solution is more destabilised and more nuclei will be formed. When more nuclei are formed, they can grow only to a smaller size before the solution is depleted of free clusters, yielding more aggregates, but with a diminished strand diameter. This does not offer any explanation for the strand-like elongation however. In this and the next section, this nucleation hypothesis will be worked out. One of its consequences will lead to a possible explanation of the strand-like elongation of the aggregates.

Based on the microscopic observations of early stages of aggregation in §3.7.4, one might assume that nucleation and the depletion of unbound clusters from solution are fast in MD5 compared to the timescale of the experiment. This could justify the assumption that the growth that is observed at a DMSO concentration of $5\%(v/v)$
is based on aggregate-aggregate mergers for all but the first few measurements. Apparently, this leads to the rather slow and small increase of $\langle R_h \rangle$ from $\sim 65$ to $\sim 175$ nm in 10 resp. 23 hours. Thus it may be concluded that aggregate-aggregate merger is a fairly slow process. There is no reason to assume that this is much different at higher concentrations of DMSO. Therefore aggregate-aggregate merger alone cannot account for the observation of much larger values of $\langle R_h \rangle$ in MD10 and MD15. The only other possible means of growth is by cluster-aggregate growth. Therefore this must substantially contribute to the growth in MD10 and MD15 in the intermediate time range (4 to 10 hours). From the observations it may be concluded that the addition of single clusters to aggregates has to play a role in the stage in which elongation of the aggregates occurs. An explanation for this elongation therefore needs to be found in the interactions governing the attachment of a single cluster to an aggregate.

4.6.3 The Bending Ligand Model

The elongated shape of an aggregate seems to arise while free clusters are added to it. This means that clusters have the tendency to bind a protrusion (‘tip’) of an irregularly shaped aggregate, rather than at its side. Since the aggregation is reaction limited, there must be a difference in sticking probability between tip and side. From a geometrical point of view the only difference between these two is their curvature, which is higher at the tip. How can curvature influence the sticking probability? This may be explained by noting a few characteristic features of the clusters: the ligands are long compared to the diameter of the clusters (their ratio is almost 1:1). The ligands are not very densely packed at their extremities, even when packing is dense close to the gold surface. Therefore the alkyl backbones of the ligands, which are flexible in nature, have some freedom to bend. It has been shown that the ligand shells of gold nanoparticles can adapt their geometry, to optimise interactions. An example is given by Boal and Rotello, where a combination of $\pi$-stacking and hydrogen bonding with a guest molecule causes rearrangement of the ligand shell [28].

When a $\text{Au}_{55} \text{M}$ and a $\text{Au}_{55} \text{D}$ cluster dimerise, it is conceivable that some ligands bend towards the other particle to maximise the interparticle hydrogen-bonding interactions. If two clusters are situated in the surface of an aggregate, the surface curvature will play a role: The lower the surface curvature, the less bending is required to maximise ligand–ligand interaction along the surface. Therefore, there will be a lower number of protruding (unbound) H-bonding moieties along the sides than at the tips of an aggregate. This situation is illustrated in figure 4.12. When a new cluster comes into contact with the aggregate and ‘probes’ the possible binding positions, a higher degree of cooperativity will, on average, be possible at the tips. This provides an explanation for the preferred tip-growth of the aggregates. This model will hen-

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14It should be noted that the observed final macroscopic aggregates for MD10 and MD15 were actually denser than for MD5. This has also been concluded in Chapter §3.7, where sedimentation was observed in mixtures containing 15%($v/v$) DMSO and floc formation in mixtures containing 5%($v/v$) DMSO.
ceforth be referred to as the ‘Bending Ligand Model’ (BLM). It may be remarked that it rests on a subtle balance between reversibility and irreversibility. The hydrogen bonding of an incoming cluster with the aggregate is still reversible, when there is only a small degree of cooperativity. At the same time, clusters firmly embedded in the aggregate by many ligand–ligand interactions are irreversibly bound. This transition from reversible to irreversible binding by increasing cooperativity determines the morphology of the clusters, where on one hand the growth exhibits kinetically determined characteristics, while the ‘fine structure’ is determined by thermodynamic factors.

At the same time, this model offers an alternative explanation for the variation in strand diameter with varying DMSO concentrations. At the surface of an aggregate, there will be a distribution of ligands engaged in inter-ligand bonding and those ‘free’ (solvated) for interactions with incoming clusters/aggregates. This distribution will depend both on surface curvature and solvation stabilisation of the protruding ligands. When the solvation is poorer, this distribution will shift towards a higher degree of bending and bonding, resulting in a more closed surface. Differences in curvature then become more important, leading to a more pronounced preference for binding at the tip. In this model, a low DMSO concentration will lead to a higher tendency to form protrusions and develop these into elongated structures and therefore into thinner strands. Such an explanation is still valid even when the assumptions that lead to the nucleation hypothesis in the preceding paragraph are abandoned, as long as specific ligand–ligand interactions are important. More experiments would be needed to establish the importance of the BLM in determining the morphology of the aggregates, but it gives a consistent explanation of the observations made so far.

4.7 Conclusions

In this chapter it was shown that DLS can be used to investigate the aggregation process of Au$_{55}$–M and Au$_{55}$–D clusters in DMSO/dioxane mixtures. The equipment used is not fast enough, nor has it a sufficiently high resolution to observe the nucleation process. Nevertheless, growth in the intermediate size range (the strand formation) can be observed. By DLS is was shown that homo-aggregation of Au$_{55}$–D can occur under the investigated conditions. As the morphology and behaviour of such aggregates can clearly be distinguished from hetero-aggregates, their formation is ruled out in the mixed aggregation experiments at 10 and 15%($v/v$) DMSO.

The average hydrodynamic radius $\langle R_h \rangle$ of the aggregates grows exponentially in time in all measurements performed in a range of 5–15%($v/v$) DMSO. This is indicative of a reaction limited growth process, with a finite sticking probability upon close encounter of clusters and/or aggregates.

The differences in aggregate size development can be explained by assuming a trend in nucleation rate and abundance with varying DMSO concentrations. That
led to the conclusion that single cluster addition is an important contribution to the aggregate growth in the assessed time interval, at least at higher DMSO concentrations (10–15%(v/v)).

The Bending Ligand Model can explain the tendency of the clusters to form elongated strands and may contribute to the trend of increasing strand diameters with increasing DMSO concentrations.

4.8 Experimental section

4.8.1 General

Au_{55–M} and Au_{55–D} clusters were prepared as described in Chapter 2. Anhydrous dimethylsulfoxide (DMSO, <50 ppm H_{2}O) was purchased from Aldrich and kept in a bottle equipped with a Sure-Seal. 1,4-Dioxane was dried on sodium and distilled. Both solvents were stored under a nitrogen atmosphere and filtrated with a 0.2µm PTFE filter (Sartorius Minisart 15) shortly before use. Small amounts of the cluster compounds were weighed using a precision balance (Mettler Toledo MT5, d=0.001mg). Small volumes of solvents and stock solutions were handled with calibrated volumetric pipettes (Gilson Pipetman) with disposable tips with volumes of 1000, 100 or 10µl and an error of ≤2%. Cuvettes were rigourously cleaned before use, by rinsing with acetone, sonication in Superdecontamine 33 (Intersciences) for at least 1hr, rinsing with demineralised water and drying in air at 150°C.

4.8.2 DLS Sample preparation

Typical stock solutions of the clusters (1.00 mg/ml) were prepared by dissolving 1.00 mg of the cluster compound in 1.00 ml DMSO. Stock solutions were filtrated with a 0.2µm PTFE filter. Stock-solutions were used within two days after preparation.

Test solutions were prepared by combining stock solution and solvents directly in a square 1.00×1.00 cm quartz cuvette (Hellma). The total volume of each solution was 1 ml. A typical DMSO/dioxane mixture containing 10%(v/v) DMSO and 0.025 mg/ml of each cluster, would be composed by adding 25 µl Au_{55–M} stock, 25 µl Au_{55–D} stock, 50 µl DMSO and 900 µl dioxane.
Immediately after addition of the dioxane, the cuvettes were thoroughly shaken for 3 seconds and placed in the DLS-apparatus.

4.8.3 DLS Measurements

Dynamic Light Scattering measurements were performed with a commercial Zetasizer 5000 (Malvern Instruments), equipped with a He-Ne laser (633 nm, 5 mW), a photomultiplier detector (detection angle: 90°) and a Malvern 7132 Multibit autocorrelator, which determines both $G^{(2)}(\tau)$ (by a discrete version of eq. (4.3)) and $\langle I \rangle$, the background intensity. The Zetasizer 5000 has an accessible size range of $\sim 5$ nm–$\sim 5 \mu$m (hydrodynamic diameter). The accompanying Malvern Instruments PCS v1.41 software package was used to perform a timed series of measurements (see below).

A measurement series starts by allowing the cuvette with the aggregating clusters to equilibrate for 3 minutes in the DLS-apparatus. A series of correlograms is obtained – each averaging a measurement time of 600s (due to data-handling this requires $\sim 13$ minutes) – with an interval of 30 minutes between every two measurements. All measurements were carried out at 25°C.

Malvern Instruments PCS is also used to perform monomodal and Contin analysis. Results are expressed as $Z_{\text{ave}}$, the mean hydrodynamic diameter ($\langle R_h \rangle = \frac{1}{2} Z_{\text{ave}}$). The viscosities of mixtures of varying DMSO/dioxane ratio were calculated by linear interpolation of the viscosities of the pure substances weighted by mole-fraction, where $\eta_{\text{DMSO}} = 1.99\text{cP}$ and $\eta_{\text{Dioxane}} = 1.10\text{cP}$ [29]. The indices of refraction $n$ of DMSO and dioxane are 1.4170 and 1.4224 respectively at 20°C [29], therefore a value of 1.42 was used for all mixtures.

4.8.4 TEM

Samples for transmission electron microscopy (TEM) were prepared by depositing a droplet of $\sim 25$-$50 \mu$l of a solution containing (aggregates of) clusters on a carbon-coated 400 mesh copper grid by a Pasteur pipette. Within seconds the droplet was removed by blotting with filtration paper, after which final drying took place with a stream of dry nitrogen. In case macroscopic aggregates had formed (and sedimented) the solution was carefully agitated, one or a few floating aggregates were picked up in a Pasteur pipette and blotting took place taking care that the aggregates would not be disrupted or blotted off.

Electron microscopic investigations were carried out with a Philips CEM 120 transmission electron microscope, equipped with a LaB$_6$ filament. The microscope was operated at 120 kV. Images were acquired with a built-in Gatan 794 CCD-camera (1024 x 1024 pixels), controlled by Gatan Digital Micrograph v3.5 software.
References


REFERENCES


Appendix to Chapter 3:
Full colour photograph of hetero-aggregates

Figure 3.9: Photograph of vials ($\approx$ 1.1 cm) containing aggregates formed in DMSO/dioxane mixtures when varying the DMSO concentrations from 2 to 20%($v/v$). This is a full colour version of the photograph at page 89.