DYNAMICS OF GUEST BINDING TO SUPRAMOLECULAR ASSEMBLIES

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INTRODUCTION

Supramolecular systems are formed from molecular building blocks held together by intermolecular interactions such as electrostatic interactions, hydrogen bonds, π-stacking, or the hydrophobic effect. These interactions are, in general, weaker than covalent bonds found in molecules, and consequently, supramolecular systems are inherently reversible. This reversibility is essential for some of the functions expressed by supramolecular systems, such as chemical sensing, catalysis, or transport. Reversibility and weak interactions are also responsible for the sensitivity of supramolecular systems to experimental conditions such as temperature or the solvent’s nature. The structure of a molecule, that is, the connectivity between atoms and their spatial relationship, requires large changes in temperature and solvent properties to be altered. In contrast, the structure of supramolecular systems, defined by the stoichiometry of the building blocks and their spatial relationship, can be altered with small changes to the system’s environment. For example, many molecules that are soluble and stable in polar solvents are also soluble and stable in polar hydrogen-bonding solvents. However, a supramolecular system formed primarily through hydrogen bonds can be inexistent in hydrogen-bonding solvents while being formed in polar or polarizable solvents with no hydrogen-bonding capability.
The differentiation between a molecular and a supramolecular system is not always clear-cut because a gradation can exist for the strength of the forces involved in holding atoms or molecules together. In addition, the temporal division into nonreversible and reversible systems is dictated by human experience, which establishes the timescales for “stable” and “unstable” systems. This chapter will focus on processes that are faster than a few seconds and, for this reason, require the use of fast kinetic techniques.

Each system is characterized by its structure, energetics, and dynamics. In the molecular world, primary characterization is based on structural assignments and determination of the system’s energetics, that is, thermodynamics. Dynamics are not important in the characterization of individual molecules because the vast majority of molecules are stable. Dynamics play a role in the characterization of the reactivity of molecules. Therefore, for stable molecules, structural and thermodynamic characterization can be uncoupled from kinetic aspects. The reversibility of supramolecular systems dictates that knowledge on its dynamics is as essential as structural and energetic characterization. The structure of new supramolecular systems is frequently characterized by X-ray crystallography, nuclear magnetic resonance (NMR), or imaging experiments. Thermodynamic studies reveal information on the stability of the system (e.g., equilibrium constants) and on the distribution of species with different building block stoichiometries. Rate constants for the assembly and disassembly of supramolecular systems, as well as rate constants for supramolecular function, such as transport and catalysis, are obtained from kinetics studies.

The dynamics of a system can be investigated using real-time kinetic techniques or by the measurement of relative rates. In real-time kinetic measurements, the concentration of the species of interest is followed as a function of time, and rate constants are obtained from the fit of kinetic measurements to rate laws derived for each system. Rate constants can be derived from relative rate measurements where ratios of products are related to ratios of rate constants and a rate constant for a standard reaction is known. The latter method assumes the same mechanism for the standard reaction and the reaction under study. Relative rate measurements are limited in scope when mechanistic information is not reasonably well developed, because it is difficult to assess the validity of the assumption of equal mechanisms for the reactions being compared. This is still the case for supramolecular systems where the interplay of the various intermolecular forces is not understood well enough to make predictions on their relative importance. As a comparison, in the molecular world we have a good understanding on which bonds are likely to be involved in a particular reaction, based on bond strengths and polarizabilities.

The timescale for supramolecular dynamics studies is determined by the size of the system, leading to a time–length scale relationship for the fastest reactions that can be expected. Bimolecular reactions in a solution are limited by how fast the diffusion of two reactants occurs. The dimensions of supramolecular systems span 1–100 nm, and the diffusion of a small molecule, such as glucose in water at room temperature, is 3 ns for 1 nm and 30 μs for 100 nm.
This analysis shows that the dynamics for small host–guest systems is expected to occur in microseconds or faster.\textsuperscript{10,11}

The diffusion limit for bimolecular reactions in solution establishes the fastest timescale for which one expects association processes to occur for reagents that are completely separated in solution. In an aqueous solution at $25^\circ C$ ($k_{dif} = 7.4 \times 10^9 \text{M}^{-1} \text{s}^{-1}$),\textsuperscript{12} the association process will be slower than 1 ns for reactant concentrations equal or less than 0.1 M, suggesting that kinetic studies in the nanosecond time domain and slower are required. Dissociation of a molecule from a supramolecular system or relocation of the molecule within the system is a unimolecular process, which have rate constants of $10^{12} \text{s}^{-1}$ and lower. Complete dissociation normally occurs in timescales of nanoseconds or slower, whereas femto- to picosecond processes are frequently observed for internal relocation and changes in solvation.\textsuperscript{13–16}

The requirement in supramolecular dynamics to use fast kinetic techniques in the nanosecond to microsecond timescale is ideal for the application of photophysical methods. Excited states or reactive intermediates are used to measure association and dissociation kinetics in supramolecular systems.\textsuperscript{10} This chapter covers the concepts on how photophysical techniques, that is, fluorescence, transient absorption, fluorescence correlation spectroscopy, and laser temperature jump, are employed in supramolecular dynamics studies. The advantages and disadvantages of photophysical techniques compared with other fast kinetic methods will be summarized as a comprehensive review on the applications of all these techniques has been recently published.\textsuperscript{17} Studies for small guest binding to cyclodextrins (CDs), micelles, bile salt aggregates, and DNA will be employed to exemplify on how photophysical techniques are used to gain kinetic information. This chapter focuses on the kinetic determination of the association and dissociation processes involved in supramolecular systems and does not focus on the internal mobility of guests within a confined system or changes in solvation.

**PHOTOPHYSICS IN SUPRAMOLECULAR DYNAMICS STUDIES**

Excited states and reactive intermediates in general are species that have finite lifetimes and can be formed in a fast manner by the use of lasers. The fast perturbation of a chemical system is a requirement for real-time kinetic studies. The finite lifetime of excited states compared with the time for the kinetic process of interest can be explored in different ways, that is, if the lifetimes are shorter, longer, or of the same order of magnitude as the time for the kinetic process being studied.\textsuperscript{10,18} Each of these cases will be considered below for the simplest host–guest system, where the binding stoichiometry is 1:1 (Scheme 1.1). It will be assumed that the guest is the species being excited; however, the conceptual framework is the same if the host is excited. The kinetic processes of interest are the association ($k_+$) and dissociation ($k_-$) rate constants of the guest with the supramolecular system.
DYNAMICS OF GUEST BINDING TO SUPRAMOLECULAR ASSEMBLIES

Short-Lived Excited States

A short-lived excited state will not have the time to relocate between the homogeneous phase and the supramolecular host; that is, the association/dissociation kinetics is too slow to compete with the deactivation of the excited state (Fig. 1.1). Therefore, the excited state is a probe for the environment in which it is located. Differences in excited-state lifetimes or in their emission or absorption spectra are employed to identify the excited guest in the homogeneous phase or bound to the host. Spectral or lifetime differences are explored to determine the relative concentrations of the guest bound to the host (HG) and in the homogeneous solvent (G). Absorption and emission spectra are composite spectra for all species in solution, for example, G and HG, taking into account their molar absorptivities and emission quantum yields and their relative concentrations. In contrast, in the case of excited-state lifetime measurements, each species can be identified separately provided the lifetimes for the excited states of G and HG are different.

In general, for organic molecules, the short-lived excited state is the singlet excited state, which frequently leads to fluorescence emission. From the analytical point of view, fluorescence is a much more sensitive technique than absorption. Light intensity is detected as an absolute measurement and is dependent only on the sensitivity of the detector, that is, the discrimination between noise and signal. Absorption is a relative measurement where changes in light intensities are measured, leading to lower sensitivity. Therefore, for short-lived excited states, most studies are performed using emission and not absorption measurements.

\[
H + G \xrightleftharpoons[k_+]{k_-} HG
\]

\[
K_{11} = \frac{k_+}{k_-}
\]

Scheme 1.1. Formation of a host–guest complex (HG) with 1:1 stoichiometry.

Figure 1.1. Schematic representation for the formation of the excited state of the guest (filled circles). The guest (open circles) in the homogeneous phase is in equilibrium with the host (squares) in the ground state, but no relocation occurs for the excited state.
**Long-Lived Excited States**

The formation of an excited state with a lifetime much longer than the association/dissociation kinetics can be viewed as a permanent perturbation of the system (Fig. 1.2). The formation of the excited state is equivalent to the formation of a new chemical, because excited states have properties that differ from those of their ground states, such as different geometries, longer bond lengths, and higher or lower dipole moments. These different properties will lead to a re-equilibration of the system between the excited-state guest and the host. The kinetics will only reflect this re-equilibration, that is, $k_+^*$ and $k_-^*$, because the excited state will not have time to decay back to its ground state while the re-equilibration occurs. Measurement of the kinetics for the re-equilibration using laser flash photolysis requires that the absorption for $G^*$ and $HG^*$ is different, so that changes in their relative concentrations can be measured. Fluorescence is only rarely suitable for these measurements because the singlet excited-state lifetimes are frequently shorter than the time required for the association/dissociation processes.

**Excited-State Lifetimes Comparable to the Kinetics of Interest**

Coupled kinetics are observed when the lifetime of the excited state is of the same order of magnitude as the kinetic process of interest ($k_0, k_0^H = k_+^*, k_-^*$) (Fig. 1.3). In this case, the observed rate constant ($k_{obs}$) for the kinetics will include both the re-equilibration of the excited-state guest with the host system and the decay of the excited state of the guest in the homogeneous solution ($k_0$) and inside the host ($k_0^H$). The values for the association ($k_+^*$) and dissociation ($k_-^*$) rate constants for the excited guest are determined when the excitation to $G^*$ and $HG^*$ leads to a nonequilibrium situation. These measurements require that a property of $G^*$, for example, its absorption, be different when bound to the guest ($HG^*$).
Quenching Studies

The reaction of excited states with molecules (quenchers) that lead to their deactivation can be exploited in two different ways to obtain information on the dynamics of supramolecular systems. In the case of coupled kinetics, the use of quenchers lifts the restriction that $G^*$ and $HG^*$ need to have different properties that can be followed in kinetic measurements. The introduction of a quencher leads to a competitive deactivation pathway for the excited states $G^*$ and $HG^*$ (Fig. 1.4), and information on the association and dissociation kinetics can be obtained if the quenching rate constants for the excited state in the homogeneous solution ($k_q$) and for the excited-state guest in the host ($k_q^H$) are different. For example, when $k_q \gg k_q^H$, the quenching of the guest in the homogeneous phase at high quencher concentrations leads to such a short lifetime for the free guest ($G^*$) that the rate-limiting step for the deactivation of the excited state of the guest ($G^*$ and $HG^*$) will be the guest’s exit from...
This differential quenching makes it possible to measure the association and dissociation rate constants even when the absorption of $G^*$ and $HG^*$ are the same. This quenching methodology broadens the scope of supramolecular dynamics studies because only a limited number of molecules have sufficiently different absorption spectra for $G^*$ and $HG^*$ to study their relocation kinetics without the use of quenchers.

Quenching studies can also be employed to measure the association rate constants of the quencher molecules with the host system. Short-lived excited-state guests located in the host act as probes for the host’s interior. The quenching of these excited states provides a measure of the accessibility of the quencher to the interior of the host (Fig. 1.5). Quenching corresponds to a bimolecular process, which can be viewed as a process leading to an encounter complex, followed by the intrinsic quenching rate constant ($k_{q{\text{int}}}^q$) (Fig. 1.5). A value close to the diffusion controlled limit for the quenching rate constant indicates that the intrinsic quenching rate constant ($k_{q{\text{int}}}^q$) is very high and the overall quenching process is directly related to the diffusional rate constant to form the encounter complex. In such a scenario, the quenching rate constant for the excited-state guest inside the host ($k_{qH}^q$) will be equal to the association rate constant of the quencher with the host ($k_{Q}^q$), assuming that the intrinsic quenching rate constant ($k_{q{\text{int}}}^q$) does not change because of the different environments in the host and homogeneous solution. The latter assumption can be tested by measuring the quenching of the excited guests in solvents with different polarities and viscosities. For quenching rate constants that are not diffusion controlled, the analysis for the changes in $k_{qH}^q$ needs to take into account possible changes in the intrinsic quenching rate constant for the reaction in homogeneous solution and inside the host system.

Figure 1.5. Schematic representation for the formation of the excited state of the guest (gray circles) in the singlet state. The guest (open circles) in the homogeneous phase is in equilibrium with the host (squares) in the ground state, but no relocation occurs for the excited state. Quenching (process shown by curved arrow) can occur for the excited state in the homogeneous phase and for the guest inside the host.
The techniques used to study the dynamics of supramolecular systems can be classified as perturbation and nonperturbation experiments (Fig. 1.6). The principle of perturbation measurements is to take a system away from equilibrium by inducing a perturbation that is faster than the kinetics being measured. Perturbations can be rapid changes in concentration, temperature, the simultaneous change in temperature and pressure, or changes in a chemical species. Each technique has a specific time domain for which kinetic measurements can be performed. The role of photophysics is either as an analytical tool, where absorption or emission spectroscopy is used to follow concentrations of reactants or products, or as a tool where the dynamics of excited states is employed to obtain kinetic information. The principles of each technique will be described with a focus on the time resolution between nanoseconds and seconds and the role of photophysics. Some of the techniques are suitable for kinetic measurements faster than nanoseconds or slower than seconds, but these aspects fall outside the scope of this chapter. The use of several different techniques frequently provides essential complementary information on a system, and for this reason, a description of the relevant nonphotophysical techniques is included in this chapter. More details on their applications can be found in a recent review.17

Fluorescence (Emission) Measurements

In the case of organic molecules, the most common emission from excited states is fluorescence. The principles here described can also be applied for
other emissive species, such as phosphorescence, but their use to study supramolecular dynamics has been limited. Kinetic data are obtained from time-resolved experiments. Several time-resolved fluorescence methods exist,\textsuperscript{19-23} but single photon counting is the method most commonly employed to study the dynamics of supramolecular systems (Fig. 1.7).\textsuperscript{10,18} An excitation source (flash lamps, light-emitting diodes, or lasers) with high repetition rate and pico- or nanosecond resolution is employed to create the excited state of the guest. At a suitably low detection rate ($\leq 2.5\%$ of excitation rate), the first emitted photon from the sample is detected and a histogram of the number of photons as a function of time is built. The low detection rate ensures that the time distribution for the detected photons follows a Poisson distribution. Therefore, the change in the intensity with time recorded in single photon counting experiments is equivalent to the time profile that would have been obtained if many molecules were excited in one pulse and all emitted photons were detected. The advantage of single photon counting is that a large dynamic range for the intensities is accumulated with high signal-to-noise ratios. Such a large dynamic range is essential when differentiating between emitting species that have similar lifetimes.

The perturbation of the system in time-resolved fluorescence experiments is due to the formation of the excited state. For studies involving supramolecular systems, fluorescence can be used in an analytical mode when the lifetime of the guest is short and no relocation occurs between the guest in the host and the homogeneous phase. Time-resolved fluorescence studies can also be used to obtain dynamic information when the lifetime of the excited state and the timescale for the dynamics of interest are similar. The time domain covered by time-resolved fluorescence studies is from nanoseconds to a few microseconds and is limited by the lifetime of the excited states being studied. Use of this technique requires a guest or host that has a reasonably high fluorescence quantum yield and an unreactive excited state.
Laser Flash Photolysis

A pulsed laser is used to form the excited state of the guest, and the absorption of the excited state is monitored over time (Fig. 1.8). The laser excitation pulse and the monitoring beam are usually in an orthogonal arrangement. The changes in light intensities before and after each laser pulse are measured. This change in light intensity is related to the difference in absorbance of the sample before the laser pulse and the absorbance by the transient formed during the laser pulse.

The perturbation induced in this method is the formation of a different chemical species, that is, the excited state. The time domain covered by most laser flash photolysis systems is from nanoseconds to hundreds of microseconds, because these systems were originally optimized for studies in the nanosecond time domain. The technique can in principle be extended to the millisecond time domain if the instability of common monitoring lamps is minimized. Use of laser flash photolysis studies for supramolecular dynamics requires efficient formation of an unreactive long-lived excited state.

Laser Temperature Jump

Temperature jump experiments can be employed to study any system for which the equilibrium is changed when the temperature is raised. In this experiment, increasing the temperature of the solvent causes the perturbation for which the relaxation kinetics is measured. The three methods available to change temperature in a pulsed fashion are Joule heating through an electric...
discharge, microwave heating, and heating through the absorption of laser light.  

The time resolution for Joule heating is of microseconds, and this technique requires the use of solutions with high ionic strength, which, in some cases, can lead to artifacts unless special care is taken.  

Microwave heating was not used frequently because the signals obtained were small. Laser temperature jump is the technique of choice when studying fast kinetics.  

The type of laser depends on the absorption coefficient of the solvent being heated, which was water in most cases. The typical 1064-nm laser band of Nd:YAG (neodymium-doped yttrium aluminum garnet) lasers is not suitable because the water absorption coefficient is too low at this wavelength. Excitation of water in the 1.3–2.0 μm range was implemented by either using a chemical iodine laser or Raman-shifted Nd:YAG lasers with different gases. The wavelength of the laser determines the largest volume that can be heated without leading to inhomogeneous heating. In the case of Raman-shifted lasers, the volumes heated were typically 20 μL, while for the iodine laser volumes, up to 500 μL was heated. The time resolution for the experiment is determined by how fast the laser light is transferred into thermal energy and is mainly limited by the width of the laser pulse. The longest times that can be measured are determined by the cooling rate of the volumes heated, which is ca. 10 ms for a 20-μL volume and in excess of 1 s in the case of a 500-μL volume.  

Absorption, fluorescence, or light scattering is employed to follow the relaxation kinetics after heating. The monitoring beam and detection are placed either at a 90° geometry or in a collinear fashion with respect to the laser beam. Detection by absorption or fluorescence requires that the absorption or fluorescence of the guest be different in water and when bound to the supramolecular system, and in the case of fluorescence, the excited state should be unreactive toward the components of the supramolecular system. Photophysics plays an analytical role in this technique.

**Stopped Flow**

Stopped flow is a technique in which solutions are quickly mixed by driving solutions in two (or more) syringes at high pressure through a mixing chamber and having the flow stop abruptly (Fig. 1.9). Changes in the system’s chemical composition after the flow is stopped are followed over time by detecting changes in either absorption or fluorescence. Photophysics plays an analytical role in stopped flow experiments, and the use of this technique requires that either the guest or the host have different absorption or emission properties when free in solution or as part of the HG complex. The time resolution is determined by the time required to mix the solutions and stop the flow. For most systems, the time resolution is of 1–2 ms, although shorter resolutions can be achieved with the use of cells with smaller volumes. The tradeoff in using smaller cells is the decrease in the signal-to-noise ratios for the detected signal.

Stopped flow experiments lead to a concentration jump that is employed to study the formation or disassembly of supramolecular systems. In the former
case, the host and guest are placed in different syringes, while in the latter case, the solution with the host–guest complex is placed in one syringe and the second syringe contains the solvent.

**Ultrasonic Relaxation**

Photophysics plays no role in the use of this technique, and therefore, it does not require the use of molecules that contain chromophores. A sound wave is passed through the cell containing the sample, which leads to periodic variations of pressure and temperature. Chemical equilibria, which have a nonzero $\Delta H^0$ or $\Delta V^0$, respond to the sound wave when its frequency is similar to the relaxation rate of the chemical equilibrium. The frequency of the sound waves is varied and a maximum absorption is observed for the frequency that corresponds to the relaxation rate of the chemical equilibrium being studied. When more than one equilibrium is present, an absorption maximum will be observed for each equilibrium provided the relaxation rates differ by more than a factor of 5.

**Fluorescence Correlation Spectroscopy**

This technique is a nonperturbation method where fluorescence is required as an analytical tool because of its sensitivity. The technique is sensitive to two different properties: (1) the different diffusion coefficients for the guest and the host–guest complex, and (2) different emission quantum yields for G and HG. A guest with a high fluorescence quantum yield and minimal intersystem crossing efficiency is required for these measurements.
Fluorescence correlation spectroscopy is based on the principle that intensity fluctuations for the emission of one molecule are related to events that lead to changes in the fluorescence efficiency of that one molecule. The sample volume is continuously irradiated, and fluctuations occur because of events such as diffusion of the molecule out of the detection volume, formation of a dark triplet excited state, or association/dissociation from a supramolecular system. This technique requires the detection of fluorescence from a small volume containing a small number of molecules. The concentration of fluorophores has to be low so that fluctuations in the intensity from one molecule can be discriminated from the background emission.

NMR
NMR experiments are used to measure the exchange kinetics of a system in equilibrium, and no perturbation is required for these experiments. There is no role for photophysics in NMR experiments. Relaxation processes with a time constant slower than 0.1 ms can be investigated. The biggest advantage of using NMR is the correlation between kinetic data and structural assignments.

Kinetic measurements are possible when the NMR signal for a guest or a host is different when located in the homogeneous solution or in the host–guest complex. Two limits exist for such a situation, that is, when the exchange between the host–guest complex and the homogeneous solution is slow or fast compared with the NMR relaxation processes. In the slow exchange limit, separate peaks are observed for the guest or host in the homogeneous phase and in the complex. The integration of each peak is proportional to the concentration of each species and is used for the determination of equilibrium constants. In the fast exchange limit, only one peak is observed where the position of the peak corresponds to the average values for the frequencies of the free and bound guest or host weighted by the fraction of each species present in the solution. Line broadening of the NMR signals is observed in the intermediate regime when the association/dissociation processes for the supramolecular system occur on the same timescale as the relaxation processes for the NMR experiment. The values for the association and dissociation rate constants are obtained from line-shape analysis. Line broadening is frequently induced by changes in temperature so that the system being studied changes between the slow and fast relaxation regimes.

Surface Plasmon Resonance (SPR)
SPR is used for the determination of equilibrium constants and the dynamics for complex formation. This technique has found wide use for studies with biological samples. There is no role for photophysics in the use of this technique. The principle of SPR measurements is that the surface plasmon waves traveling parallel to the interface between a metal and a dielectric are affected when the refractive index at the interface changes. The evanescent wave produced by total
reflection of an incoming beam can couple with the plasmons in the metal film at the interface. Changes in the refractive index alter this coupling, leading to changes in the intensity of the reflected light detected. One of the components of a bimolecular reaction leading to complexation (G or H) is bound to the metal surface. A solution with the other component flows over the surface, and formation of a complex changes the refractive index at the interface. The kinetics of the association process is observed until equilibration is achieved. The dissociation kinetics is measured by flowing a solution without the second reagent over the metal surface leading to the dissociation of the complex.

The advantage of SPR experiments is that small amounts of sample are required. This technique is sensitive to mass changes, and therefore, it is advantageous to immobilize the reagent with the smallest mass on the surface. Specific immobilization chemistries for each reagent have to be developed, and the surface coverage has to be kept low enough to ensure that the access of the second reagent from the solution phase is not restricted. The upper limit for the association rate constants that can be measured is $10^5 - 10^6 \text{M}^{-1} \text{s}^{-1}$, while the dissociation has to be slower than hundreds of milliseconds.

**COMPARISON OF TECHNIQUES**

The choice of a technique to study the dynamics of supramolecular systems depends on several factors, such as the timescale of the relaxation process, number of relaxation processes present, magnitude of the concentration change, and the amount of sample available.

For the simplest equilibrium possible, that is, formation of a 1:1 complex (Scheme 1.1), the relaxation rate constant ($k_{\text{obs}}$) is equal to the sum of the association and dissociation processes. Assuming that the host concentration is in excess over the concentration of the guest, the relaxation process leads to first-order kinetics where $k_{\text{obs}}$ is expressed by Equation 1.1:

\[
k_{\text{obs}} = k_+ [\text{H}] + k_-
\]  

(1.1)

The timescale for a particular study is determined by the value of $k_{\text{obs}}$. A relaxation process can be slowed down by decreasing the concentration of host, which also may lead to a decrease in the concentration of guest so as to keep the host in excess over guest. The limitation of such a strategy is that at low concentrations of host and guest, a more sensitive method, for example, fluorescence, needs to be used to measure concentration changes. The minimum value for $k_{\text{obs}}$ is determined by the dissociation rate constant; that is, the relaxation process cannot be slower than $1/k_-$. Fast processes can be studied using time-resolved fluorescence, laser flash photolysis, laser temperature jump, fluorescence correlation spectroscopy, and ultrasonic relaxation. With the exception of ultrasonic relaxation, all these techniques rely on photophysical measurements (Fig. 1.6). The widest time
range is achieved using laser temperature jump or fluorescence correlation spectroscopy. These two techniques are suitable when a system has relaxation processes that occur on different timescales. It is advantageous to measure all kinetic processes with one technique since similar experimental conditions such as concentration ratios can be used for all measurements. Fluorescence and laser flash photolysis are not suitable for kinetics measurements slower than a few microseconds and hundreds of microseconds, respectively. Stopped flow and NMR can be employed to investigate relaxation processes slower than milliseconds.

Fluorescence is the most sensitive of all the methods described and is used when the concentrations involved are low. Absorption is a relative measurement, and for this reason, it is less sensitive than fluorescence, making laser flash photolysis experiments less suitable when small concentration changes are involved. The least sensitive technique for concentration changes is NMR.

A further consideration when choosing a kinetic technique is the necessity for differentiating between relaxation processes with similar relaxation times. Time-resolved fluorescence using single photon counting is the best technique for the differentiation of similar relaxation times because of the ability to collect a large number of counts at a high signal-to-noise ratio. Laser flash photolysis, laser temperature jump, and stopped flow provide an intermediate ability to differentiate between kinetic processes with similar relaxation rate constants. Ultrasound relaxation and fluorescence correlation spectroscopy have the lowest resolution to differentiate between kinetic processes.

Ultrasound relaxation and NMR do not require the formation of an excited state, and systems can be studied that do not contain a chromophore. One requirement in using photophysical methods is to ensure that the excited states formed are unreactive toward all components of the system. This requirement is easier to meet when using fluorescence measurements because a molecule with high fluorescence quantum yield can be chosen, diminishing the likelihood of reaction from the excited state. In the case of triplet excited states, control studies are required to determine their photoreactivity because triplet excited states are, in general, more reactive than their corresponding singlet excited states.

Finally, when the amount of sample is limited, time-resolved fluorescence, laser temperature jump, and fluorescence correlation spectroscopy experiments are used because the sample volumes are small and low concentration of reactants can be employed. NMR is not a suitable technique because of the high concentrations required. In the case of stopped flow experiments, large volumes have to be employed because a number of kinetic traces are averaged.

METHODS

The various photophysical methods used to study supramolecular dynamics will be discussed in this section. Thermodynamic information, that is,
equilibrium constants and host–guest stoichiometries, is required for modeling of the kinetics. These thermodynamic measurements frequently rely on photophysical experiments, such as fluorescence, absorption, and circular dichroism. Use of these techniques for thermodynamic measurements will not be covered in this chapter, and it will be assumed that the species present at equilibrium are known before kinetic studies are performed.

**Steady-State Excited-State Detection**

Kinetic measurements using continuous irradiation of samples are employed for stopped flow, laser temperature jump, and fluorescence correlation spectroscopy measurements. For the first two techniques, the changes in concentration of chemicals can be followed by changes in absorption or fluorescence, while for fluorescence correlation spectroscopy, only fluorescence can be used as the detection mode. For all three techniques, the sample is continuously irradiated, and the lifetime of the excited state is not relevant to the experiment. In the case of stopped flow and laser temperature jump experiments, the kinetics is analyzed within the framework of relaxation processes. Each one of the exponential functions can be related a particular relaxation process. More complex equations are employed for bimolecular reactions when the concentrations of reactants are similar.

Fluorescence correlation spectroscopy measurements are based on the intensity fluctuations of single molecules in the detection volume because of events that change the molecule’s fluorescence efficiency or because of the diffusion of the molecule out of the detection volume. An autocorrelation function \( G(\tau) \) is measured, which defines the probability of detecting a photon emitted \( I \) from the same molecule at time zero and time \( \tau \). Loss of correlation at a particular time signifies that the molecule is not available for excitation. A change in the guest’s emission quantum yield because of a change in environment, such as host complexation, is detected as a change in \( I \) in the autocorrelation function. Two uncorrelated photons appear as an offset for \( G(\tau) \):

\[
G(\tau) = \langle I(t)I(t+\tau) \rangle \quad \text{(1.2)}
\]

A typical correlation curve (Fig. 1.10) shows an increase in \( G(\tau) \) at short times, called anti-bunching, which is related to the excitation of the fluorophore. The inflection points after the maximum for \( G(\tau) \) are related to events that decreases the probability of forming the singlet excited state of the molecule.

The correlation curve is analyzed by assuming a profile, normally a Gaussian one, for the excitation pulse. For a molecule in a homogeneous solution, which has an appreciable formation of its triplet excited state, the correlation function is the product of the correlation function for fluorescence \( G_F \), the triplet state \( G_T \), diffusion of the fluorophore \( G_D \), and any reaction term \( G_R \) (Eq. 1.3):
The diffusion time of the fluorophore in a solution containing a host–guest system increases as more host–guest complexes are formed at higher concentrations of host, leading to a shift to longer times for the inflection point related to the diffusion of the fluorophore. In addition, a relaxation process due to the association/dissociation of the guest becomes apparent in the correlation curve. The new inflection point that appears in the correlation curve in the presence of the host has a time constant \( \tau_R \). For a 1:1 complex between a guest and a host, the time constant for the correlation time associated with the host–guest dynamics is defined by:

\[
\tau_R = \frac{1}{k_{\text{obs}}} = \frac{1}{k_*[H] + k_-}
\]  

The correlation time shifts to shorter times as the host concentrations is raised. The amplitude for the host–guest relaxation process is largest at intermediate host concentrations when both the free guest and host–guest complexes are present is appreciable amounts.

Mechanistic assumptions are required to analyze the correlation curve for a host–guest complex. The mechanism is simplified if one works with a guest for which intersystem crossing is negligible because then one can assume that the binding dynamics is only due to reaction between the ground state of the guest and the host. The correlation function including the host–guest dynamics includes the terms \( N_G \) and \( N_{HG} \), which are the mean numbers of free guest and

\[ G(\tau) = G_F \times G_T \times G_D \times G_R \]  

(1.3)

Figure 1.10. Correlation curve showing the various processes that occur on different timescales.
complexed guest in the sample volume; the term $A_R$, which is the amplitude for the reaction term of the correlation function; and the ratio $w_{xy}/w_z$, which is the aspect ratio of the sampling volume (Eq. 1.5).\(^{38,52}\) Fitting the data of the correlation curve to Equation 1.5 leads to values of $\tau_R$ at different host concentrations, and the values of $k_+$ and $k_-$ are determined from the dependence of $1/\tau_R$ with the concentration of host (Eq. 1.4). Other mechanistic assumptions will lead to different equations for $G_R(\tau)$ from which the relaxation times $\tau_R$ are determined:

$$G_R(\tau) = \frac{1}{N_G + N_{HG}} \left( 1 + \frac{\tau}{\tau_D} \right)^{-1} \left( 1 + \left( \frac{w_{xy}}{w_z} \right)^2 \frac{\tau}{\tau_D} \right)^{-1/2} (1 + A_R e^{-\tau/\tau_R})$$

(1.5)

**Steady-State Fluorescence Measurements**

The singlet excited-state lifetimes of fluorophores are usually shorter than the association/dissociation binding dynamics of guests with hosts.\(^{10,11}\) For this reason, the excited guest does not leave the host, and it can be used to determine the accessibility of quencher molecules to the interior of the host. Steady-state quenching is observed as a decrease of the emission intensity ($I$) compared with the intensity in the absence of quencher ($I_0$). The Stern–Volmer constant ($K_{sv}$) is related to the quenching efficiency and is equal to the product of the quenching rate constant and the lifetime of the fluorophore in the absence of quencher ($\tau_0$) (Eq. 1.6). Nonlinear quenching plots are observed when more than one fluorophore with different quenching efficiencies are present (downward curvature) or when static, that is, immediate, quenching is present in addition to a bimolecular dynamic quenching reaction (upward curvature):\(^{23}\)

$$\frac{I_0}{I} = 1 + K_{sv} [Q]$$

(1.6)

Steady-state fluorescence experiments have been extensively used to characterize the binding of guests to supramolecular systems, in particular, for thermodynamic characterization, such as the determination of binding constants of host–guest complexes and aggregation numbers of micelles.\(^{18}\) Steady-state emission experiments are less useful to investigate the quencher dynamics because the measurements of rate constants is indirect. For this reason, the detailed mathematical treatment of steady-state experiments\(^{23,55}\) will not be covered in this chapter, and the focus will be on the application of time-resolved studies.

**Time-Resolved Fluorescence and Absorption Measurements**

Short-lived excited states act as probes for the environment in which they are located, that is, the homogeneous phase or within the host system. The bimolecular reaction of the excited states with the quencher provides information on the mobility of the quencher between the homogeneous phase and the host
METHODS

system\textsuperscript{18,56–63} and two separate cases will be considered: (1) The quencher deactivates the excited state in the host for every encounter between excited state and quencher ($k_{q}^{\text{int}} \gg k_{Q}^{\text{obs}}$, Fig. 1.5), and (2) the quencher has a finite probability of exiting the host without quenching the excited state bound to the host ($k_{q}^{\text{int}} \sim k_{Q}^{\text{obs}}$ or $k_{q}^{\text{int}} \ll k_{Q}^{\text{obs}}$). Scenario (1) is more realistic for host–guest complexes with confined spaces, such as CD complexes, where the quencher “entering” the host can only lead to a close contact between the guest and quencher. Scenario (2) is likely for larger hosts, such as micelles, where the quencher can be incorporated in the micelle at a distance from the excited state and an intramicellar unimolecular quenching process ($k_{q}^{\text{int}}$) competes with the exit of the guest ($k_{Q}^{\text{obs}}$).

Deactivation for Every Quencher–Excited State Encounter

The lifetimes for the excited states of the guest in the homogeneous phase and bound to the host can frequently be different, with a longer lifetime being generally observed for the guest in the host. The fluorescence decay will not follow a mono-exponential function when an appreciable amount of excited guest is present in the homogeneous solution and bound to the host, that is, when more than one emitting species with different lifetimes are present in the solution. In this case, the decay is fit to a sum of exponentials (Eq. 1.7), where $A_i$ corresponds to the pre-exponential factor of each species “$i$.” The sum of all pre-exponential factors is normalized to unity. The parameter $k_i$ is the decay rate constant for each species “$i$” and corresponds to the inverse of the lifetime for “$i$”:

$$I(t) = I_o \sum_{i=1}^{l} A_i e^{-k_i t} \quad \text{and} \quad \sum_{i=1}^{l} A_i = 1$$  \hspace{1cm} (1.7)

Quenching leads to a decrease of the observed decay rate constant of the excited fluorophore, which follows a linear relationship with the quencher concentration (Eq. 1.8). The values for the rate constant in the absence of quencher ($k_0$) and the quenching rate constant for the excited guest in the homogeneous phase ($k_q$) are obtained in an independent experiment. In the presence of the host system, the decays are fit to the sum of two exponentials where $k_{\text{obs}}^{\text{hom}}$ corresponds to the decay of the excited guest in the homogeneous phase and $k_{\text{obs}}^{\text{H}}$ corresponds to the observed lifetime of the excited guest in the host:

$$k_{\text{obs}}^{i} = k_0^{i} + k_{q}^{i}[Q]$$  \hspace{1cm} (1.8)

The values of the pre-exponential factors are related to the concentrations of excited states and the excitation efficiencies for each species. $A_i$ values can only be related to absolute concentrations if the system can be excited at an isosbestic point or if the molar absorptivity for the host–guest complex is known. In addition, the bandwidth for the excitation light has to be taken into account when calculating the excitation efficiencies when broad bandwidths are employed. The values for the pre-exponential factors are constant as the quencher concentration is raised because the excited-state guest does not
move between the host and the homogeneous phase. The assumption of non-mobile fluorophore, that is, the excited guest, is not valid if the pre-exponential factors vary and a different mechanistic model needs to be employed to analyze the data.

The values for the guest’s excited-state lifetime in the homogeneous solvent \((k_0)\) and in the host \((k_{0H})\) may not be very different and therefore a precise differentiation between \(k_{\text{obs}}^w\) and \(k_{\text{obs}}^H\) may be difficult. The values for the lifetime of the excited guest \((1/k_0^w)\) and quenching rate constant \((k_q)\) in the homogeneous solvent can be determined in an independent experiment. Therefore, the value of \(k_{\text{obs}}^w\) can be fixed for the analysis of the fluorescence decay kinetics in the presence of host. This procedure increases the precision of the \(k_{\text{obs}}^H\) values.

The reaction of the excited guest with the quencher corresponds to a bimolecular reaction in which an initial encounter complex is formed followed by the quenching reaction in the complex. In the case of the quenching for the guest bound to the host, the formation of the encounter complex is related to the association rate constant of the quencher with the host \((k_Q^+\)\), the dissociation of the quencher from the host \((k_Q^-\)\) and the intrahost quenching rate constant \((k_q^\text{int})\) (Fig. 1.5, Eq. 1.9). The quenching rate constant \((k_q^H)\) is equal to the association rate constant of the quencher \((k_Q^+\)\) when each encounter of the quencher with the guest in the host leads to quenching; that is, \(k_Q^+\) is much smaller than \(k_q^\text{int}\). In general, this condition is met for quenchers that have a diffusion controlled rate constant in solution, but this condition can also be met for lower values of \(k_q\) as long as no exit of the quencher from the host occurs before the quenching reaction:

\[
k_q^H = \frac{k_Q^+ k_q^\text{int}}{k_Q^- + k_q^\text{int}} \quad (1.9)
\]

It is important to note that time-resolved measurements are not sensitive to the situation where the guest and quencher are solubilized, prior to excitation, in the same binding site within the host and the intrahost quenching rate constant is higher than the exit rate constant of the quencher from the host. In such case, the excited guest is quenched within the excitation pulse, and no lifetime is measured for this component. However, in steady-state experiments, the guest–quencher–host complex will lead to a decrease in the emission intensity due to static quenching.\(^{64,65}\)

**Competition between Intrahost Quenching and Quencher Exit from the Host**

An intrahost quenching efficiency lower than 100% is observed when exit of the quencher from the host system competes with quenching. This situation is rare for host–guest complexes with defined binding sites, such as macrocycles, because of the limited volume within the host. However, for large self-assemblies, such as micelles, the quencher can bind in a region far from the
excited guest and intrahost migration is required for quenching to occur. The simplest model developed to analyze the fluorescence decay of excited guests in the host assumes that all guest molecules are bound to the host and that the probability of the quencher to enter and exit the host is independent of the host containing a guest molecule; that is, $k_{Q}^{+}$ and $k_{Q}^{-}$ are the same for an empty host and a host containing a guest. The decay in the absence of the quencher follows an exponential function with a rate constant $k_{0}$, while in the presence of the quencher, the nonexponential decay is fit to Equation 1.10, where parameters $A$ to $D$ are defined in Equations 1.11–1.14.\(^57,60–62\) Measurements are performed at different quencher concentrations because parameters $B$ and $C$ are dependent on the quencher concentration, leading to the recovery of all relevant rate constants:

$$I(t) = A \cdot e^{(-Bt-c[1-e^{-Dt}]])}$$  \hspace{1cm} (1.10)$$

$$A = I_0$$  \hspace{1cm} (1.11)$$

$$B = k_0 + \left[ \frac{k^{int}_q \cdot k^{Q}_r}{(k^{int}_q + k^{Q}_r) \left(1 + K^{Q}_O[H]\right)} \right][Q]$$  \hspace{1cm} (1.12)$$

$$C = \left[ \frac{(k^{int}_q)^2 \cdot k^{Q}_r}{(k^{int}_q + k^{Q}_r)^2 \cdot k^{Q}_r \left(1 + K^{Q}_O[H]\right)} \right][Q]$$  \hspace{1cm} (1.13)$$

$$D = k^{int}_q + k^{Q}_r$$  \hspace{1cm} (1.14)$$

The model described above was expanded to more complex mechanisms, such as the inclusion of the intermicellar exchange of the quencher or guest during the lifetime of the excited guest.\(^62\)

Equation 1.10 provides a general solution for quenching studies of guests in hosts, where parameters $A$, $B$, $C$, and $D$ are related differently to the rate constants relevant to different mechanisms. A theoretical analysis was performed on the identifiability of several mechanisms for guest and quencher binding to micelles.\(^66\) The mechanisms considered for the analysis of the fluorescence decays were (1) the quencher and guest are immobile, (2) mobile quencher and immobile guest, (3) immobile quencher and mobile guests, and (4) mobile guest and quencher. The models can be uniquely identified, and the minimum set of experiments required to identify each model was determined theoretically.

**Mobility of Excited-State Guests**

The association and dissociation rate constants of guests can be determined when the lifetime for the excited state guest is of the same order of magnitude as the dynamics being investigated. The lifetimes of triplet excited states of organic molecules are frequently in the tenth to hundreds of microsecond range,
which is the timescale for the host–guest dynamics involving macrocyclic host systems. The kinetics of triplet states is normally measured by laser flash photolysis experiments, where changes in the transient absorption are followed. The conceptual framework developed below is also applicable to long-lived singlet excited states, where fluorescence is the experimental observable.

Formation of the triplet excited state is a perturbation where the new chemical formed (Fig. 1.3), that is, the excited state, relaxes to a new equilibrium. If the molar absorptivities of the triplet guest in the solvent and bound to the host are different, the relocation of the guest follows a kinetic behavior that can be fit to a sum of two exponentials (Eq. 1.15), where the two exponential factors $\gamma_1$ and $\gamma_2$ are related to the deactivation rate constants of the excited guest in the solvent ($k_0$) and in the host ($k^H_0$) and the association ($k^+$) and dissociation ($k^-$) rate constants of the triplet guest with the host (Eqs. 1.16–1.19). The mathematical treatment is equivalent to that developed for the dynamics of excimer emission:

$$\Delta A = A_1 e^{-\gamma_1 t} + A_2 e^{-\gamma_2 t}$$  \hspace{1cm} (1.15)

$$\gamma_{1,2} = -\frac{1}{2} \left[ (A + B) \pm \sqrt{(A - B)^2 + 4C} \right]$$  \hspace{1cm} (1.16)

$$A = k_0 + k^+_0 [H]$$  \hspace{1cm} (1.17)

$$B = k^H_0 + k^-$$  \hspace{1cm} (1.18)

$$C = k^- k^+_0 [H]$$  \hspace{1cm} (1.19)

Parameters $A$ and $C$ in Equation 1.16 are dependent on the concentration of host and the values of $\gamma_1$ and $\gamma_2$ are determined at various host concentrations. The sum and product of the exponential factors $\gamma_1$ and $\gamma_2$ are related to the individual rate constants by Equations 1.20 and 1.21:

$$\gamma_1 + \gamma_2 = k_0 + k^H_0 + k^- + k^+_0 [H]$$  \hspace{1cm} (1.20)

$$\gamma_1 \gamma_2 = k_0 (k^H_0 + k^-) + k^H_0 k^+_0 [H]$$  \hspace{1cm} (1.21)

The analysis is simplified when the lifetime of the excited state is much longer than the association and dissociation dynamics ($k_0 \ll k^+_0 [H]$ and $k^H_0 \ll k^-$). When the $k_0$ and $k^H_0$ values are smaller by a factor of 1000 compared with the host–guest dynamics, the relaxation process levels off at a value of $A_2$ and $\gamma_2$ is equal to zero. Conceptually, the lifetime of the excited guest can be considered constant compared with the host–guest dynamics (Fig. 1.2). The value of the exponential factor $\gamma_1$ is equal to the relaxation process of the host–guest complex (Eq. 1.22):

$$\gamma_1 = k_{obs} = k^- + k^+_0 [H]$$  \hspace{1cm} (1.22)

Quenching experiments can also be employed to study the dynamics of the triplet excited-state guest with the host. This methodology is suitable when the molar absorptivities of the guest in the homogeneous phase and
bound to the host are the same. The quenching methodology can be applied when the quenching rate constant for the triplet guest in the homogeneous solution \( k_q \) is different from the quenching rate constant of the guest in the host \( k_q^H \). In general, quenchers are employed for which the quenching efficiency in the homogeneous solution is higher than the quenching efficiency for the excited guest in the host. Sufficiently high host concentrations are employed to assure that the concentration of guest in the homogeneous phase is small compared with the host–guest concentration. Under this condition, the steady-state condition is applied to the rate laws and the kinetics follows a mono-exponential decay, where the observed rate constant is given by the expression in Equation 1.23:

\[
k_{\text{obs}} = k_0^H + k_q^* + k_q^H [Q] \frac{k_q^* k_q^H [H]}{k_0 + k_q^* [Q] + k_q^H [H]}
\]

(1.23)

The dependence of \( k_{\text{obs}} \) with the quencher concentration shows a downward curvature (Fig. 1.11) because at high quencher concentrations, the lifetime of the triplet guest in the homogeneous solution becomes shorter than the exit of the excited guest from the host. Qualitatively, the larger the difference between the linear relationship in homogeneous solution with the curved quenching plot, the slower is the dynamics for the host–guest complex. The slope of the linear relationship observed for the curved quenching plot at high quencher concentrations corresponds to the quenching rate constant of the excited guest in the host. The effect of changes to the various rate constants and the host concentration on the curvature of the quenching plot have been discussed previously.10

![Figure 1.11. Linear quenching plot for the excited guest in homogeneous solution (black, Eq. 1.8) and curved quenching plot for the excited guest in equilibrium between the host and the homogeneous solution (gray, Eq. 1.23). At high quencher concentration, the linear portion of the graph corresponds to the exit rate constant of the excited guest from the host (intercept of the dashed line) and the quenching rate constant for the guest inside the host (slope).](image-url)
APPLICATIONS

The objective of this section is to present selected examples on the application of the photophysical methods described above. Four classes of host systems will be covered, that is, CDs, DNA, micelles, and bile salt aggregates. A complete survey of all the studies on the dynamics of guests with these four host systems is beyond the scope of this chapter, and a selection to cover different types of mechanisms was chosen. CDs and DNA are hosts that form host–guest complexes with defined stoichiometries, while micelles and bile salt aggregates provide larger binding regions and progressive amounts of guest can be bound to these latter hosts.

CDs

CDs are cyclic oligosaccharides (Fig. 1.12) where the internal cavity size increases with the number of glucose units (6, 7, and 8 for α-, β-, and γ-CD). Guest molecules are complexed inside the CD cavity, and the efficiency for complex formation is determined by the hydrophobicity of the guest and the steric constraints on how well the guest fits inside the relatively rigid CD cavity. The thermodynamics of guest complexation to CDs has been extensively characterized, and enthalpic and entropic factors are relevant for complex formation.

Temperature jump experiments (Joule heating) were employed in the early studies on the host–guest characterization with CDs to determine the guest binding dynamics between azo dyes 1 (Scheme 1.2) with α-CD. Binding of derivatives of 1 with α-CD led to changes in the absorption spectra of the azo dyes. Complexes with 1:1 stoichiometry were formed, and the equilibrium constants were determined from binding isotherms constructed from the changes in absorption values (Table 1.1).

![Figure 1.12. Structure of β-CD and dimensions of the various CDs.](image-url)
A linear relationship was observed between the increase in the observed relaxation rate constant and CD concentration, and the association and dissociation rate constants were obtained using Equation 1.1 (Table 1.1). The cavity of α-CD is too small to accommodate the naphthyl moiety of 1 and only the phenolate moiety can be included in the CD. The first important general observation is that, as long as the phenolate moiety fits inside the CD cavity, increases in the size of this moiety has a small effect on the equilibrium constant. However, the dynamics is significantly affected by these structural changes with changes of $10^3$ for the values of $k_+$ and $k_-$ for the phenol derivatives and $10^4$ for the phenolate derivatives (Table 1.1). These results are a good example that dynamic information cannot be obtained from thermodynamic studies. The second general feature of importance is that the values for the equilibrium constants calculated from the association and dissociation rate constants are in good agreement with the $K_{11}$ values determined from thermodynamic binding experiments. This agreement indicates that the kinetics investigated corresponds to the process leading to equilibrium, and no further slow kinetics occur beyond the time window investigated.

The increase in the bulk of the phenyl ring by substitution with methyl and ethyl groups decreased the rate constants for the association process, indicating that a tighter fit into the cavity occurred, which may require the distortion of the CD framework. However, once the complex is formed, the exit of the bulkier guests was also slowed down, leading to a small increase in the values of $K_{11}$. Di-ortho alkyl substitution for derivatives of 1 did not lead to the formation of complexes with α-CD, showing that these guests are too bulky to

---

**Scheme 1.2.** 3'-alkyl-4'-hydroxyphenylazo-1-naphthalene-4-sulfonate guests.

**TABLE 1.1. Equilibrium Constants Determined from UV-Vis Binding Isotherms ($K_{11}$), Association ($k_+$) and Dissociation ($k_-$) Rate Constants of Guests 1 with α-CD, and the Calculated Value for $K_{11}$ ($K_{11}^{calc}$) from the Kinetic Data (Scheme 1.1)**

<table>
<thead>
<tr>
<th>R</th>
<th>$K_{11}$/$\text{M}^{-1}$</th>
<th>$k_+$/10$^4$M$^{-1}$s$^{-1}$</th>
<th>$k_-$/10$^5$s$^{-1}$</th>
<th>$K_{11}^{calc}$/M$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H, phenol</td>
<td>270</td>
<td>1300</td>
<td>550</td>
<td>240</td>
</tr>
<tr>
<td>H, phenolate</td>
<td>650</td>
<td>17</td>
<td>2.6</td>
<td>650</td>
</tr>
<tr>
<td>CH$_3$, phenol</td>
<td>420</td>
<td>17</td>
<td>3.5</td>
<td>340</td>
</tr>
<tr>
<td>CH$_3$, phenolate</td>
<td>480</td>
<td>0.012</td>
<td>0.0028</td>
<td>430</td>
</tr>
<tr>
<td>CH$_2$CH$_3$, phenol</td>
<td>460</td>
<td>0.6</td>
<td>0.19</td>
<td>320</td>
</tr>
<tr>
<td>CH$_2$CH$_3$, phenolate</td>
<td>290</td>
<td>0.00028</td>
<td>0.0001</td>
<td>280</td>
</tr>
</tbody>
</table>

Data were collected at $T = 14^\circ C$, $\mu = 0.5$ M, pH 3.5 for phenol, and pH 11 for phenolate.
enter the CD cavity.\textsuperscript{75} In the case of unsubstituted 1, a significantly higher equilibrium constant was observed for the phenolate ion compared with the phenol compound. This trend seems anti-intuitive because the charged species should be more soluble in water than the uncharged guest. However, analysis of the rate constants indicated that the association process was slowed down for the phenolate derivative as would be expected for the more highly solvated species. However, the larger decrease for the dissociation rate constant for the phenolate derivative is responsible for the increase in $K_{11}$, probably because the moiety with the negative charge has to pass through the hydrophobic cavity of the CD for the guest to exit into the aqueous phase. For the bulkier methyl and ethyl derivatives of 1, the values for $k_+$ and $k_-$ are lower for the phenolate derivative than the phenol. However, the equilibrium constant for the protonated and unprotonated species is the same in the case of the methyl derivative and smaller for the ethyl derivative, showing that the bulkiness of the incorporated moiety and the presence of a charge do not act independently on the binding dynamics. This example shows that generalizations for the host–guest thermodynamics and dynamics are not straightforward.

CDs are known to form complexes with more than one guest and host. Methyl orange (2),\textsuperscript{76} pyronine Y (3),\textsuperscript{77} and pyronine B (4)\textsuperscript{78} (Scheme 1.3) were shown to form complexes with 1:1, 2:1 (guest : CD), and, in the case of 2 and 3, 2:2 stoichiometries (Scheme 1.4). Temperature jump studies showed that the formation of the 1:1 and 2:2 complexes was fast and occurred within the time

\[
\begin{align*}
2 \\
\text{3 } R = \text{CH}_3 \\
\text{4 } R = \text{CH}_2\text{CH}_3
\end{align*}
\]

\textbf{Scheme 1.3.} Structures for methyl orange (2), pyronine Y (3), and pyronine B (4).

\[
\begin{align*}
\text{G} + \text{H} & \xrightleftharpoons{K_{11}} \text{HG} \\
\text{G} + \text{HG} & \xrightleftharpoons{k_{21}^{21}} \text{HG}_2 \\
\text{H} + \text{HG}_2 & \xrightleftharpoons{K_{22}} \text{H}_2\text{G}_2
\end{align*}
\]

\textbf{Scheme 1.4.} Mechanism for the formation of host–guest complexes with multiple stoichiometries.
resolution (2–5 μs) of the equipment. The fast equilibria are not observable in the kinetic studies, but they are incorporated, in the form of equilibrium constants, into the dependence of $k_{\text{obs}}$ with the CD concentration (Eq. 1.24):

$$k_{\text{obs}} = k_{+1} K_{11} \left[ \frac{[G] ([G]+[HG]+[H])}{1+K_{11}[G]+K_{11}[H]} \right] - k_{-1} \left[ \frac{1+K_{22}[HG_2]}{1+K_{22}[H]+K_{22}[HG_2]} \right]$$

The values for the equilibrium constants and the association and dissociation rate constants for the 2:1 complex were obtained from the nonlinear fit of the data to Equation 1.24 (Table 1.2). Guests 2–4 dimerize at high concentrations in aqueous solution. The equilibrium constants for the 2:1 complex with γ-CD are much higher than for the dimer formation in water. In addition, the equilibrium constants for the 2:1 complex are much higher than for the 1:1 complex, showing that the CD cavity stabilizes the dimer of these guests. The association rate constant for the second guest to the 1:1 host–guest complex is close to the diffusion controlled limit (7.5 × 10^9 M⁻¹ s⁻¹ at 25°C), showing that the γ-CD cavity with one guest is not fully occupied and provides enough space for the entry of the second guest. The residence time of guests 2–4 is between 70 and 200 μs. These slow dissociation rate constants are an expression of the stabilization of the dimer within the γ-CD cavity.

A recent study on the binding of 3 and 4 with γ-CD agreed qualitatively with the previous investigations that γ-CD stabilizes the dimers of 3 and 4 in the 2:1 complex when compared with dimer formation in the aqueous phase. However, the new $K_{11}$ (40–70 M⁻¹ for 3 and 190 M⁻¹ for 4) and $K_{21}$ values (2 × 10⁶ M⁻¹ for 3 and 2.2 × 10⁵ M⁻¹ for 4) determined in the more detailed study are very different from the previously reported ones. This discrepancy was mainly attributed to the fact that the temperature jump experiments were performed in the presence of 1 M NaCl required for Joule heating. Xathene dye aggregation, such as 3 and 4, is known to be sensitive to the ionic strength, and the degree of aqueous dimer formation in the two sets of experiments is probably different. In addition, the assumption made in the previous work about the equality of the absorption spectra of the guest in water and in the 1:1 complex were shown to be incorrect. This example shows that rigorous thermodynamic characterization is required to establish the possible species present when performing kinetic studies. In the absence of such a characterization, the kinetics may be consistent with a mechanism proposed, but may need

<table>
<thead>
<tr>
<th>Guest</th>
<th>$K_{11}$/M⁻¹</th>
<th>$K_{22}$/10⁵ M⁻¹</th>
<th>$k_{+1}$/10⁹ M⁻¹ s⁻¹</th>
<th>$k_{-1}$/10³ s⁻¹</th>
<th>$K_{22}$/M⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2⁷⁶</td>
<td>45</td>
<td>20</td>
<td>9</td>
<td>4.8</td>
<td>6000</td>
</tr>
<tr>
<td>3⁷⁷</td>
<td>1000</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>4⁷⁸</td>
<td>430</td>
<td>1.3</td>
<td>0.82</td>
<td>6.4</td>
<td>—</td>
</tr>
</tbody>
</table>

²⁵°C for 2–4; for 2: 0.1 M Na₂PO₄, pH = 9.0; for 3: 1 M NaCl, pH = 6.1; for 4: 1 M NaCl, pH = 5.7.
reinterpretation if further experiments show that a host–guest species is missing or one has to be added.

Stopped flow experiments were employed to study the dynamics of CD host–guest complexes on a timescale slower than milliseconds. These studies include the binding of metal ions, such as Cu\(^{2+}\) to \(\alpha\)-CD,\(^{81}\) and the competition between organic guests and Ni\(^{2+}\) or Zn\(^{2+}\).\(^{82,83}\) Examples for complex kinetics studied by stopped flow are the two-step formation of \(\alpha\)-CD complexes (Scheme 1.5) with azo compounds 5 (Scheme 1.6)\(^{84,85}\) and the kinetics for the formation of 2:2 complexes between pyrene (6, Scheme 1.6) and \(\gamma\)-CD.\(^{86}\)

The binding kinetics for compound 5 with hydrogen and methyl substituents\(^{84,85}\) showed one relaxation process because these guests are sufficiently small to slip into the \(\alpha\)-CD cavity. For larger substituents with increasing chain lengths (ethyl, propyl) or branching chains (iso-propyl, iso-butyl, tert-butyl), two relaxation processes were observed, which were assigned to a sequential mechanism where an encounter complex “a” was formed followed by the internalization of the guest into the cavity in process “b” (Scheme 1.5). The rate constants for both processes were much less dependent on the size of the substituent (factors of 2–3) than observed for compound 1 containing the larger naphthyl moiety (Table 1.1). The exception is the significant slowdown observed for the tert-butyl derivative of 5, where the substituent is too large for the formation of species “a.” This latter result suggests that species “a” is not just an encounter complex but has a defined structural relationship between the CD and the guest.

Pyrene forms 1:1 and 2:2 complexes with \(\gamma\)-CD.\(^{86-88}\) The fluorescence of the pyrene monomer was observed for pyrene in water and in the 1:1 complex. An excimer-like emission was observed for the 2:2 complex, due to the pres-

\[
\begin{align*}
G + H & \overset{k^a}{\rightleftharpoons} (G - H)_a \\
& \overset{k^b}{\rightleftharpoons} (G - H)_b
\end{align*}
\]

Scheme 1.5. Sequential formation of a host–guest complex.

\[
\text{Scheme 1.6. Structures for 3'-alkyl-4'-hydroxyphenylazo-1-phenyl-4-sulfonate (5) and pyrene (6).}
\]
ence of the pyrene dimer. The excimer emission is red shifted in comparison to the monomer emission, and changes in the excimer emission intensity were used to follow the kinetics for the formation of the pyrene–γ-CD 2:2 complex from the association of two 1:1 complexes. The kinetics occurs within 0.2 s and the value for $k_{+22}$ was determined to be $6 \times 10^7 \text{M}^{-1}\text{s}^{-1}$, while the value for $k_{-22}$ was $73 \text{s}^{-1}$. The value for the association rate constant is between 5 and 40 times smaller than the rate constants observed for the formation of 1:1 complex with guests that fit into the CD cavity (see Table 1.4), while the value for the dissociation rate constant of the 2:2 complex is at least 200 times slower than the dissociation rate constants for 1:1 complexes.

In the original study of the pyrene–γ-CD system, a very slow kinetics over several seconds was assigned to the dynamics of the 1:2 (pyrene:CD) complex. A temperature annealing effect observed for the pyrene–γ-CD system showed that aggregates of γ-CD are present in aqueous solution, leading to a slowed down re-equilibration process. Preliminary studies suggested that the dynamics described for the 2:2 complex was not affected by the presence of γ-CD aggregates, but the slow dynamics assigned to the 1:2 complex is probably due to the dynamics with the small amount of aggregates present in the solution and not due to the formation of a distinct pyrene–γ-CD species.

Fluorescence correlation spectroscopy was employed to study the binding dynamics of 3 and 4 with β-CD. Two features are apparent on the correlation curves when CD was added to an aqueous solution of 3 (Fig. 1.13) or 4. The

![Figure 1.13](image)

**Figure 1.13.** Scaled ($G(6\mu s)=1.0$) and offset experimental correlation curves for 3 in water measured with increasing concentrations of β-CD (gray). The black lines correspond to the global fit of the data to Equation 1.3. Reprinted with permission from Reference 52. Copyright 2005 American Chemical Society.
diffusion term, which is characterized by the decrease of $G(\tau)$ at ca. 1 ms, shifted to longer times with the addition of CD. This trend is a consequence of the higher molar mass (ca. five times) for the host–guest complex when compared with the molar mass for the guest, which leads to a lengthening of the diffusion times of the fluorophore from 0.25 to 0.4 ms for $3$ and 0.30 to 0.45 ms for $4$. The second feature was the appearance of a new correlation term between $1$ and $10 \mu$s due to the formation of the host–guest complex. This correlation time shifted to shorter times when the $\beta$-CD concentration was raised.

The experimental data were fit to Equation 1.3 using the global analysis method, where all the experimental curves were fit simultaneously. The correlation time for the host–guest dynamics was then fit to Equations 1.4 and 1.5, and the values for $k_-$ were $5.0 \times 10^5 \text{s}^{-1}$ for $3$ and $7.6 \times 10^4 \text{s}^{-1}$ for $4$. The values for $k_+$ were calculated from the equilibrium constants and were $2 \times 10^8 \text{M}^{-1} \text{s}^{-1}$ for $3$ and $1.5 \times 10^8 \text{M}^{-1} \text{s}^{-1}$ for $4$. The association rate constants are the same for both guests and are one order of magnitude lower than the diffusions controlled limit for a bimolecular reaction in water. The difference in the binding efficiency is dictated by the dissociation rate constant, which is one order of magnitude higher for the smaller guest $3$ than for $4$. This result suggests that $4$ had the optimum size complementarity with the cavity of $\beta$-CD.

The values for $k_+$ for the binding of $3$ or $4$ with $\beta$-CD obtained from fluorescence correlation spectroscopy are the same as the $k_+$ values measured in temperature jump experiments ($1.1 \times 10^8 \text{M}^{-1} \text{s}^{-1}$ for $3$ and $4$), while the $k_-$ values were much higher for the fluorescence correlation spectroscopy measurements than determined by temperature jump ($2.6 \times 10^4 \text{M}^{-1} \text{s}^{-1}$ for $3$ and $1.5 \times 10^4 \text{M}^{-1} \text{s}^{-1}$ for $4$). The slower dissociation process in the temperature jump experiments is probably due to the presence of a high ionic strength (1 M NaCl), which is in line with the larger sensitivity of the dissociation rate constant to experimental conditions than the sensitivity for the association process.

Incorporation of guests into CDs can lead to protection of the guest’s excited state from quenchers in the solution. When the lifetime of the singlet excited state of the guest is short, no relocation occurs and the fluorescence decay corresponds to a sum of two exponentials, where one species is related to the excited guest in water and the second species is related to the $\beta$-CD–bound excited guest (Fig. 1.5). The quenching rate constants for the excited guest in the aqueous phase and bound to CD are obtained by fitting the dependencies of the observed rate constants with the quencher concentration to Equation 1.8.

The quenching of the singlet excited state of pyrene ($6$) bound to $\beta$-CD by a series of quenchers showed that the decrease in the quenching rate constants was not uniform (Table 1.3). The relative decrease in the quenching rate constants is related to the accessibility of the quencher to the guest inside the CD and to the quenching mechanism. A larger decrease of the quenching rate constant is expected for quenchers that require close contact and are relatively large, such as $\text{Tl}^+$. The excited state of 2-naphthol ($7$, Scheme 1.7) bound to $\beta$-CD is protected from the quenching by iodide anions. Analysis using Equation 1.8 showed that the quenching rate constant decreased by a factor between 3 and 4. The
fluorescence decay of this system was also analyzed using global compartmental analysis where, in addition to the quenching rate constants, the association and dissociation rate constants of the excited state of 7 with the CD were recovered (Fig. 1.4). This analysis was possible despite the short lifetime of the excited state of 7 because the lifetimes changed with the CD concentration. The quenching rate constant for the singlet excited state of 7 inside β-CD using global analysis was the same as by analyzing each lifetime separately (Eq. 1.8). The association and dissociation rate constants of the singlet excited state of 7 with β-CD were determined to be $2.5 \times 10^9 M^{-1} s^{-1}$ and $520 s^{-1}$, respectively.

The singlet excited-state lifetime of 8 is sufficiently long in water (420 ns) and D$_2$O (730 ns) for it to relocate between the aqueous phase and the host. The lifetime of 8 is shortened by a quenching mechanism involving an “aborted” hydrogen transfer, where the lifetime of the excited state is dependent on the presence of abstractable hydrogen atoms on the solvent molecules. An important feature of the quenching is that no net products are formed in contrast to the typical hydrogen abstraction reaction for excited ketones, and therefore, 8 can be used as an inert probe.

In the presence of α- and β-CD, the fluorescence decay for 8 showed two exponential terms, where the lifetime for the short-lived component was independent of the CD concentration, while the long-lived component was shortened with an increase in the CD concentration. The singlet excited state of 8 inside the CD reacts with available hydrogen atoms from the glucose units, shortening the lifetimes for 8 (33 ns for α-CD and 95 ns for β-CD). These lifetimes are too short for the excited state to exit the cavity before it is deactivated. The long-lived component is related to the bimolecular reaction between the excited state of 8 and the CDs, and corresponds to the association rate constants

### Table 1.3. Rate Constants for the Quenching of Singlet Excited State of 6 Bound to β-CD

<table>
<thead>
<tr>
<th>Quencher</th>
<th>$k_q/10^9 M^{-1} s^{-1}$</th>
<th>Water</th>
<th>β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$</td>
<td>11</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>CH$_3$NO$_2$</td>
<td>8.1</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Tl$^+$</td>
<td>6.3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>4.5</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

**Scheme 1.7.** Structures for 2-naphthol (7) and 2,3-diazabicyclo(2.2.2)oct-2-ene (8).
constant between 8 and the CDs. The values for $k_+^*$ were determined to be $1.9 \times 10^8 \text{M}^{-1}\text{s}^{-1}$, $4.0 \times 10^8 \text{M}^{-1}\text{s}^{-1}$, and $0.8 \times 10^8 \text{M}^{-1}\text{s}^{-1}$ for $\alpha$-, $\beta$- and $\gamma$-CD, respectively. These values follow qualitatively the same order as was observed for the equilibrium constants of the ground state ($K_{11}$: $50 \text{M}^{-1}$ for $\alpha$-CD, $1100 \text{M}^{-1}$ for $\beta$-CD, and $6 \text{M}^{-1}$ for $\gamma$-CD). The development of 8 for supramolecular binding studies is important because the modulation of its lifetime by aborted hydrogen transfer makes this probe uniquely suited to determine the values for the association rate constants. The requirement of using 8 as a probe to measure $k_+^*$ is that the host has abstractable hydrogens that lead to a decrease in the excited-state lifetime of 8 once the complex is formed.

The triplet excited states of guests, due to their long lifetimes, can move between the aqueous phase and the interior of the CD cavity. The quenching methodology was employed to determine the values for the association and dissociation rate constants of a series of guests with CDs. In the presence of a host, the quenching plot ($k_{\text{obs}}$ vs. [quencher]) is curved because exit of the triplet guest from the CD becomes rate limiting; that is, exit is slower than the excited-state decay in water (Fig. 1.14). The larger the difference between the curved quenching plot and the linear relationship observed in water, the slower is the binding dynamics. In the example shown in Fig. 1.15, the binding dynamics of xanthone (9, Scheme 1.8) is markedly faster than the binding dynamics for flavone (10).

Fits of the experimental data in the presence of a host to Equation 1.23 led to the determination of the $k_+^*$, $k_-^*$, and $k_q^H$ values (Table 1.4). The rate constants for the association of guests 9–11 with $\beta$-CD are lower than the limit for a diffusion-controlled reaction, and the $k_+^*$ values showed a small dependence on the structure of the guest. Compound 9 was shown to bind to the

![Figure 1.14](image-url)
rim of β-CD, while 11 is deeply included in the CD cavity, leading to a lower association rate constant for the latter guest. The dissociation of guests from CD is much more sensitive to the structure of the guest than observed for the association process. The value for the equilibrium constant of the triplet state of 9 is much lower than for its ground state, showing that the binding behavior for the excited state cannot be extrapolated from that determined for the ground state. The lower value of $K_{11}^T$ for 9 was attributed to the higher basicity and dipole moment for the excited $\pi-\pi^*$ state. However, this difference in equilibrium constants is not universal since for 11, which also has a $\pi-\pi^*$ triplet state, no significant change was observed for the ground- and excited-state equilibrium constants. Guests with the same ground-state equilibrium constants can have very different excited-state dynamics as shown for 9 and 10. In the case of 10, the phenyl ring was proposed to act as an anchor for the guest, slowing down the exit from the cavity leading to an overall slower dynamics for 10 when compared with 9.

Xanthone is, to date, the only guest for which relocation of its triplet state from the CD to the aqueous phase can be measured directly, because the triplet–triplet absorption spectrum for xanthone shifts significantly with solvent polarity. The lifetime of triplet xanthone in water is sufficiently long compared with the binding dynamics of the excited state, and Equation 1.22 can be employed for the analysis of the kinetics. The values for $k^+_+$ and $k^-_-$ recovered from the direct method ($6 \times 10^8$ M$^{-1}$ s$^{-1}$ and $8.1 \times 10^6$ s$^{-1}$) are

![Scheme 1.8. Structures for xanthone (9), flavone (10), and 2-naphthyl-l-ethanol (11).](image)

![TABLE 1.4. Values for $k^+_+$, $k^-_-$, and $k^H_T$ Determined from the Triplet-State Quenching of 9 and 10 by Cu$^{2+}$ and 11 by Mn$^{2+}$ in the Presence of β-CD](image)
somewhat lower than determined from the quenching experiments. Laser

Figure 1.15. Cartoon representation of a DNA double helix with an intercalated guest

somewhat lower than determined from the quenching experiments. Laser
temperature jump experiments, where the kinetics for the ground state was
measured, showed that the association rate constant was the same for ground
and excited states, while $k^+ \text{ increased significantly for the triplet excited state}
when compared with the ground state.\footnote{102}

**DNA**

Small molecules bind to DNA either by intercalation between base pairs of
the double helix or by binding to the minor or major grooves of the helix (Fig.
1.15). In addition, nonspecific electrostatic interactions between charged
guests and the phosphate backbone can also occur.\footnote{106} Good intercalators have
planar aromatic backbones containing heteroatoms and positively charged
flexible side chains, while guests for groove binding have a curved shape
complementary to the DNA helix with repeating aromatic units and cationic
end groups.\footnote{107} The dynamics of small guests with DNA was studied by a variety
of techniques.\footnote{17} The examples below highlight the type of mechanisms that
were proposed for DNA binding.

Ethidium bromide (\ref{12}) is a widely used staining dye for DNA because it
does not fluoresce in water but has a very high fluorescence quantum yield
when intercalated between the base pairs of DNA. The binding kinetics of \ref{12}
with DNA was extensively studied using fluorescence correlation spectroscopy, stopped flow, and temperature jump. The two mechanisms proposed for the binding of 12 with DNA are the formation of a 1:1 complex (Scheme 1.1) or the binding of 12 to two distinct binding sites in DNA ("a" and "b") with the possibility of interconversion between these two sites mediated by a second molecule of DNA (Scheme 1.10).

The binding of 12 with calf thymus DNA was studied in the original development of fluorescence correlation spectroscopy. The kinetics was analyzed as a first-order decay, despite the acknowledgement by the authors that binding would be expected to be more complex. The association process (Table 1.5) was shown to be significantly slower than a diffusion controlled process, indicating that the association had a significant activation barrier. This result contrasts with the binding to CDs (see above) and suggests that DNA needs to significantly rearrange before intercalation can occur. The residence time of 12 in DNA (37 ms) is much longer than for guests in CDs, again suggesting that once intercalation occurs, exit of the guest is hindered. Temperature jump experiments were performed parallel to stopped flow experiments in order to compare the data from both techniques. Experimental conditions were employed for which the kinetics followed a mono-exponential decay consistent with a 1:1 complexation model. In addition, the temperature jump experiments were performed at the magic angle for the detection in order to avoid artifacts that overlay the relaxation signal due to the high electric fields employed for the Joule heating of the samples. The values for the rate constants obtained from temperature jump and stopped flow measurements were the same and were comparable to those obtained from fluorescence correlation spectroscopy.
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The kinetics for the binding of 12 with DNA showed more than one relaxation process at different guest/DNA ratios. Temperature jump experiments showed three relaxation processes, where the fastest one was uncoupled from the other two processes.\textsuperscript{110} This fast process is probably related to an artifact because the detection was not performed at the magic angle. The other two relaxation processes were coupled, and the rate constants corresponding to each relaxation process varied linearly with the DNA concentration. The kinetics was consistent with parallel reactions where two different complexes were formed and the interconversion between these two complexes was mediated by a second DNA molecule (Scheme 1.10). The binding dynamics to site “a” is faster than to site “b,” and the values for the association and dissociation rate constants are within one order of magnitude of those measured for conditions where only one binding site was observed (Table 1.6). The interconversion between the two sites mediated by DNA is comparable or faster than the association process of the free guest with DNA, suggesting that the interconversion process is important when a significant amount of guest is bound to DNA compared with the fraction of free guest in the aqueous solution.

Two relaxation processes were also observed using temperature jump for the binding of proflavine (13) with calf thymus DNA.\textsuperscript{112} The slow relaxation process increased as the concentration of DNA was raised but leveled off at higher DNA concentrations, while a linear relationship was observed for the observed rate constant of the fast process with the DNA concentration. The kinetics is consistent with a sequential mechanism (Scheme 1.5) or with a parallel mechanism (Scheme 1.10). The latter was discarded because it led to an unreasonably high association rate constant for the intercalation process. The fast relaxation process was analyzed using Equation 1.1 (\(k_a = 1.4 \times 10^7 \text{ M}^{-1} \text{s}^{-1}, k_a^c = 1.3 \times 10^4 \text{ s}^{-1}, K_{11} = 1.1 \times 10^3 \text{M}^{-1}\)). The dependence of \(k_{\text{obs}}\) with the DNA concentration was fit to Equation 1.25 yielding \(k_a^b\) and \(k_a^c\) values of 6.9 \(\times 10^3 \text{ s}^{-1}\) and 4.2 \(\times 10^2 \text{ s}^{-1}\), respectively. It is important to note that the dynamics for step “a” is much faster than the dynamics observed for 12, consistent with the interpretation that step “a” corresponds to the formation of a weak complex followed by the intercalation process. The kinetics for the binding of 13 with calf thymus DNA was studied using laser temperature jump experiments at several ionic strengths (0.05–0.5 M).\textsuperscript{113} A mono-exponential decay was observed where the value for \(k_{\text{obs}}\) leveled off at high DNA concentrations. The values for \(K_{11}\)

\[\begin{array}{c|cc}
\text{Technique} & k_+ /10^6 \text{M}^{-1} \text{s}^{-1} & k_- /10^2 \text{s}^{-1} \\
\hline
\text{FCS}^{108} & 15 & 0.27 \\
\text{TJ}^{109} & 6.4 & 0.16 \\
\text{SF}^{109} & 5.4 & 0.39 \\
\end{array}\]

\textbf{TABLE 1.5. Rate Constants for the Binding Dynamics of 12 with Calf Thymus DNA Studied by Fluorescence Correlation Spectroscopy (FCS), Temperature Jump (TJ), or Stopped Flow (SF) Assuming a 1:1 Stoichiometry}
TABLE 1.6. Rate Constants for the Binding Dynamics of 12 with Calf Thymus DNA Studied by Temperature Jump (TJ) or Stopped Flow (SF) Assuming the Mechanism in Scheme 1.10

<table>
<thead>
<tr>
<th>Technique</th>
<th>$k_a^+ / 10^6 \text{M}^{-1}\text{s}^{-1}$</th>
<th>$k_a^- / 10^2 \text{s}^{-1}$</th>
<th>$k_b^+ / 10^6 \text{M}^{-1}\text{s}^{-1}$</th>
<th>$k_b^- / 10^2 \text{s}^{-1}$</th>
<th>$k_{abf} / 10^5 \text{M}^{-1}\text{s}^{-1}$</th>
<th>$k_{baf} / 10^5 \text{M}^{-1}\text{s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TJ(^{110})</td>
<td>—</td>
<td>1.6</td>
<td>0.48</td>
<td>0.35</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>TJ(^{a})</td>
<td>1.4</td>
<td>1.7</td>
<td>0.26</td>
<td>0.59</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>SF(^{109})</td>
<td>7.3</td>
<td>0.39</td>
<td>1.1</td>
<td>0.14</td>
<td>6.0</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^{a}\)Personal communication by Ryan and Crothers in Reference 111.

$([3.5 – 7.5] \times 10^3 \text{M}^{-1})$, $k_b^+ ([3.3 – 4.3] \times 10^3 \text{s}^{-1})$ and $k_b^- ([2.5 – 3.0] \times 10^2 \text{s}^{-1})$ are similar to those obtained in temperature jump experiments:

$$k_{obs} = \frac{K_{11} k_c^+[H]}{1 + K_{11}[H]} + k_b^-$$  \hspace{1cm} (1.25)

Flash photolysis experiments were performed to compare the binding dynamics of ground and triplet excited states of 13 with DNA.\(^{114}\) This qualitative study showed that the residence time for the triplet state of 13 was shorter than for its ground state. A quantitative laser flash photolysis study for the binding of triplet 13 with poly[d(A-T)] showed that binding followed a sequential mechanism as observed for the ground state.\(^{115}\) The same value was observed for the pre-equilibrium step for the ground and excited states, but the intercalation step was favored for the ground state when compared with the excited state.

Micelles

Micelles are self-assembled structures formed from surfactants that create a pseudophase within aqueous solutions into which hydrophobic guests can be solubilized. These surfactants usually have a hydrophobic chain with a charged or polar head group (Fig. 1.16). Micelles are formed when the surfactant concentration surpasses its critical micellar concentration (CMC). The exchange of individual monomers between the micelle and the aqueous phase occurs in microseconds, while micelles as a whole have lifetimes of milliseconds.\(^{116}\) Therefore, on the timescale of photophysical experiments, a micelle can be viewed as an integral host to which guest molecules are bound.

Quenching studies for guests solely solubilized in the micelle provide information on the accessibility of the quencher to the micelle interior, provided quenching inside the micelle is faster than the exit of the quencher. In this case, the fluorescence decay follows a mono-exponential function and the quenching rate constant is obtained from Equation 1.8. Micelles are formed from charged (negatively or positively) or nonionic surfactants. In the case of charged micelles and charged quenchers, electrostatic attraction or repulsion...
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plays a dominant role in determining the quencher’s accessibility to the micelle. The quenching of the singlet excited state of pyrene (6, Scheme 1.6) in the negatively charged micelles of sodium dodecyl sulfate (SDS) or in the positively charged micelle of cetyltrimethylammonium bromide (CTAB) is only modestly diminished for oxygen (factors of 2.2–2.4) or nitromethane (factors of 1.2–2.0),\textsuperscript{117} showing that these neutral quenchers diffuse readily through the micelles. On the other hand, when iodide anions were used as quenchers for 6 in SDS micelles, the quenching rate constant decreased by ca. 120,\textsuperscript{117} because of the electrostatic repulsion between the micelle and the quencher.

Equation 1.10 is employed when quenching inside the micelle is competitive with the exit of the quencher into the aqueous phase. This analysis has been extensively used to study the binding dynamics of quenchers with micelles.\textsuperscript{10,62} Studies with different quenchers showed that the association process is controlled by diffusion when electrostatic repulsion or attraction is not present. In these cases, the partition of the quencher between the micelles and the aqueous phase is determined by the dissociation rate constant of the quencher from the micelle. The more hydrophobic the quencher, the lower the dissociation rate constants and the longer the residence time in the micelle as exemplified in Table 1.7 for the quenching of the singlet excited state of 6 in SDS micelles by alkyl iodides.\textsuperscript{118}

At high concentrations of micelles, parameter $D$ (Eq. 1.14) was shown to depend on the micelle concentration.\textsuperscript{119,120} Such a dependence is inconsistent with the mechanism used to derive Equation 1.10. One proposal for the observed dependence was that at high micelle concentrations, the movement of quencher between micelles can occur due to the migration between two micelles without exit of the quencher into the aqueous phase.\textsuperscript{120} Subsequent studies showed that the dissociation rate constant for the quencher depends

Figure 1.16. Cross section of a micelle where the charged (polar) groups (white) are located at the interface with water and the hydrophobic tails are located in the interior of the micelle.
strongly on the salt concentration in solution and only moderately on the micelle concentration. This result led the authors to propose that the dissociation rate constant of ionic quenchers from ionic micelles depends on the micellar surface potential and that micellar exchange is not important. This conclusion was reached for studies with SDS and cetyltrimethylammonium chloride (CTAC) micelles.

This example shows that complementary experiments are required to validate the models employed for the fitting of fluorescence decays to complex models such as those underlying Equation 1.10.

The migration of guests between micelles was studied using a quencher that was immobile in CTAC micelles. The fluorescence decays were fit to competing mechanistic models, and global analysis was employed for the analysis of the kinetics. No migration was observed for the more hydrophobic 1-methylpyrene, while migration was observed for pyrene sulfonate.

The mobility of guests was studied for several triplet excited states using the quenching methodology (Eq. 1.23), which was originally developed to study the binding dynamics of polyaromatic hydrocarbons with SDS micelles. In the case of 1-bromonaphthalene (14, Scheme 1.11), the value for the dissociation rate constant of the triplet state \( k^- \) was determined directly from the analysis of the quenching plot \( (2.5 \times 10^4 \text{ s}^{-1}) \). A very similar value was recovered when \( k^- (3.3 \times 10^4 \text{ s}^{-1}) \) was calculated from the equilibrium constant.

### Table 1.7

<table>
<thead>
<tr>
<th>Quencher</th>
<th>( k_Q^+ / 10^9 \text{ M}^{-1} \text{ s}^{-1} )</th>
<th>( k_Q^- / 10^9 \text{ s}^{-1} )</th>
<th>( k_{\text{int}}^- / 10^9 \text{ s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl iodide</td>
<td>9.7</td>
<td>8.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Butyl iodide</td>
<td>8.8</td>
<td>1.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Hexyl iodide</td>
<td>7.8</td>
<td>0.75</td>
<td>5.4</td>
</tr>
<tr>
<td>Octyl iodide</td>
<td>6.6</td>
<td>0.4</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Scheme 1.11. Structures for 1-bromonaphthalene (14) and 10-(4-bromo-1-naphthoyl) decyltrimethylammonium bromide (15).
of the ground state and assuming a diffusion-controlled value for the association rate constant. This result showed that the binding dynamics for the ground and excited states was similar. The values for \( k^- \) for other guests were calculated from the equilibrium constants, and the dissociation was slower for the more hydrophobic guests.

The dissociation rate constants of 10-(4-bromo-1-naphthoyl)decyltrimethylammonium bromide (15) with alkyltrimethylammonium chloride (SC\(_n\)S) micelles was studied by following the phosphorescence decay of triplet 15 with the addition of ferric cyanide as quencher and by fitting the quenching plot to Equation 1.23. The values for \( k^- \) decreased as the length of the alkyl chains increased because the volume of the micelles increased. The same trend was observed at both temperatures studied (Table 1.8).

The binding dynamics of several triplet excited-state ketones with SDS micelles was investigated by using a quencher that either was immobile inside the micelle (γ-methylvalerophenone) or resided exclusively in the aqueous phase (nitrite anions). The \( k^-/k^+ \) values for the triplet state of acetophenone (7.7 \( \times 10^6 \) s\(^{-1} \)), propiophenone (3.0 \( \times 10^6 \) s\(^{-1} \)), and isobutyrophenone (1.6 \( \times 10^6 \) s\(^{-1} \)) were determined when the quencher was inside the micelle. The values for \( k^-/k^+ \) for the triplet state of acetophenone (1.6 \( \times 10^{10} \) M\(^{-1} \) s\(^{-1} \)), propiophenone (1.4 \( \times 10^{10} \) M\(^{-1} \) s\(^{-1} \)), and isobutyrophenone (1.2 \( \times 10^{10} \) M\(^{-1} \) s\(^{-1} \)) were determined from the quenching studies with nitrite anions. The studies on the dynamics of the ketones parallel the ones with polyaromatic hydrocarbons as guests, where the association rate constants were diffusion controlled while the dissociation rate constants decreased when the hydrophobicity of the guest increased. However, it is important to note that the \( k^-/k^+ \) values for the ketones are significantly higher than observed for the polyaromatic hydrocarbons, in line with the higher hydrophilicity of the ketones when compared with polyaromatic hydrocarbons.

The binding dynamics of the Rhodamine 123 cation (16) (Scheme 1.12) to micelles of Triton X-100 and Brij-35, two nonionic surfactants, was studied using fluorescence correlation spectroscopy. A cationic guest was chosen to ensure its partition between the micelle and water, while nonionic surfactants were used to eliminate the role of electrostatic interactions on the guest
binding dynamics. In the case of 16, a significant amount of triplet states is formed and the correlation time for the decay of the triplet appears in the correlation curve. Two different sets of experiments were performed to identify the correlation time for the triplet state and differentiate it from the correlation time for the host–guest dynamics. The excitation power was varied because an increase in power leads to a higher population of triplets. When the micelle concentration was varied, the correlation time for the dynamics of the host–guest complex was mainly affected; that is, at higher micelle concentrations, the correlation time for the host–guest dynamics became shorter. The correlation time for the triplet state was shown to be at longer times than for the host–guest complex, and the amplitude for the term corresponding to the triplet state was smaller than for the host–guest dynamics. The values for \( k^- \) were determined from the fluorescence correlation experiment (\( 2.2 \times 10^5 \text{s}^{-1} \) for Triton X-100 and \( 4 \times 10^5 \text{s}^{-1} \) for Brij-35), while the values for \( k^+ \) (\( 1.4 \times 10^{10} \text{M}^{-1} \text{s}^{-1} \) for Triton X-100 and \( 8 \times 10^9 \text{M}^{-1} \text{s}^{-1} \) for Brij-35) were calculated from the equilibrium constants and the \( k^- \) values. The value for the association rate constant of 16 with Triton X-100 was the same as calculated for a diffusional process, while the \( k^+ \) value for Brij-35 was slightly lower.

The redistribution of guests through micelle fusion or fragmentation was studied using the pyrene derivative 17 (Scheme 1.13) because this guest does not exit from the micelle over a period of several seconds.\(^{128,129}\) Micelle fusion is the process where two micelles collide, and the content, that is, the guests, are redistributed between the two micelles followed by the separation of the fused complex. This process is bimolecular because it requires the collision of two micelles. The fragmentation mechanism is related to the formation of two smaller micelles where these micelles then grow to the average micellar size. This reaction is unimolecular because the rate-limiting step is the fragmentation of one micelle. The concentration of 17 used was sufficiently high that a significant number of micelles contained two guest molecules, which led to the observation of the excimer emission of pyrene. Stopped flow experiments were performed in which a solution containing Triton X-100 micelles and 17 was mixed with a solution containing only the micelles. The redistribution of 17 was observed as a decrease in the excimer emission or an increase in the monomer emission because more micelles contained only one guest. Two relaxation processes were observed, one of which showed a dependence of the

**Scheme 1.12.** Structure for the Rhodamine 123 cation (16).
relaxation time on the micelle concentration and a slower process for which the relaxation time was constant when the concentration of surfactant was changed. The first process was assigned to the fusion of micelles with a rate constant of ca. $1 \times 10^6 \text{M}^{-1}\text{s}^{-1}$, while fragmentation occurred with a rate constant of ca. $12 \text{s}^{-1}$. A rigorous theoretical treatment suggested that the qualitative conclusions based on the kinetic studies using $^{17,128,129}$ were correct but a stochastic model of solubilization should be taken into account when determining the values for the rate constants.$^{130}$

**Bile Salt Aggregates**

Bile salts (Scheme 1.14) are molecules that have planar amphiphilicity, where the concave face containing hydroxyl groups is more hydrophilic than the

\[ \text{Scheme 1.13. Structure for a pyrene-substituted triglyceride (17).} \]

\[ \text{Scheme 1.14. Structures for sodium cholate (NaCh), sodium deoxycholate (NaDC), sodium taurocholate (NaTC), and sodium deoxytaurocholate (NaTDC).} \]
convex face containing the methyl substituents. This structural framework is very different from surfactants, such as those discussed in the previous section, where the hydrophilic group is at one end of the molecule and the rest of the molecule is hydrophobic. Bile salts continuously aggregate as the monomer concentration is increased, and the aggregate does not have a defined size as is the case for conventional micelles. The most widely adopted model for the structure of the aggregates is the formation of small primary aggregates at low monomer concentration, which then aggregate into larger structures at higher concentrations called secondary aggregates (Fig. 1.17). Alternate models proposed that hydrogen bonds are required for the formation of primary aggregates, or that the aggregation is stepwise.

The quenching of the singlet excited state of pyrene (6) by oxygen or nitromethane was compared with the quenching for 6 in SDS micelles. The aggregates of sodium taurocholate (NaTC) provide more protection than micelles of SDS. The quenching rate constant decreased by a factor of 2 for the quenching by oxygen, while it decreased by 25 times for nitromethane. This result shows that oxygen can have a reasonably good access to the aggregates of NaTC, while the access is more restricted for the larger nitromethane. However, the quenching rate constant for iodide anions is 14 times larger for the NaTC aggregates than for SDS micelles, showing that the charge density

**Figure 1.17.** Cartoon representation for the aggregation of bile salts where the black areas correspond to the hydrophobic face of the monomer, the white areas correspond to the face of the monomer containing the hydroxyl groups, and the gray circles are the charged head groups.
on the surface of the host, which is negative in both cases, is much higher for SDS micelles than for the NaTC aggregates.

Quenching by \(N,N\)-dimethylaniline of the singlet excited state of 6 bound to NaTC aggregates in the presence of 1 M NaCl was studied using the four-parameter analysis in Equation 1.10.\(^{139}\) This approach led to the determination of the association \((1.4 \times 10^9 \text{M}^{-1} \text{s}^{-1})\) and dissociation rate constants \((3.8 \times 10^6 \text{s}^{-1})\) of \(N,N\)-dimethylaniline with taurocholate aggregates. These values were similar to those observed for the quenching of 6 in SDS micelles by \(N,N\)-dimethylaniline, suggesting that the access of this neutral quencher is similar for micelles and bile salt aggregates.

Quenching of the fluorescence of a series of guests by iodide anions was employed to determine the protection efficiency provided by the bile salt aggregate for the bound guest.\(^{140-143}\) The values for the quenching rate constants were determined from the analysis of fluorescence decay measurements using Equation 1.8. In an aqueous solution, the quenching by iodide anions is diffusion controlled, indicating that the intrinsic quenching rate constant is very high. The negative charge on the bile salt aggregates provides a barrier for the entry of the iodide anion, and the value for the quenching rate constant \((k_q^H)\) was equated to \(k_Q^O\) since the intrinsic quenching rate constant is not expected to change with a change of the nature of the environment around the guest.\(^{142,143}\) Polyaromatic hydrocarbons, such as the naphthalenes 18–20 (Scheme 1.15), are bound to the primary aggregates of the bile salts, while introduction of a hydroxyl group (21) led to the binding of the guest to the secondary aggregates. Iodide anions quench the fluorescence of 18–21, and the quenching rate constants in the presence of different bile salts (Table 1.9) showed that the association of iodide anions to primary aggregates is much slower than observed for the secondary aggregates, since for all bile salts, the quenching rate constants for 18–20 are much lower than for 21. The quenching rate constants for 18–20 are significantly different, suggesting that the structure of the primary aggregates of sodium cholate (NaCh) change with the concentration of the guest, most likely because of the small number of monomers

![Diagram](image)

**Scheme 1.15.** Structures for naphthalene (18), 1-ethynaphthalene (19), acenaphthene (20), and 1-naphthyl-1-ethanol (21).
involved in the formation of the primary aggregates. Bile salts with a smaller number of hydroxyl groups (sodium deoxycholate [NaDC] and sodium deoxytaurocholate [NaTDC]) form more compact primary aggregates leading to a decrease of the access for the iodide anions, while the number of hydroxyl groups has a much smaller effect on the structure of the secondary aggregates, since the quenching rate constants did not vary significantly for 21.

Studies on the kinetics of the triplet excited state of guests led to information on their dissociation from bile salt aggregates. Estimates for the $k_{\ddagger}$ values for guests in NaTC aggregates were obtained for Rose Bengal ($>1 \times 10^5 s^{-1}$) in flash photolysis experiments and from the analysis of the triplet–triplet annihilation process of anthracene ($2.8 \times 10^5 s^{-1}$). The quenching methodology was employed to measure the binding dynamics of a series of guests, where the triplet states of the guests were quenched by nitrite anions and the values for $k_{\ddagger}$ and $k_{qH}$ were obtained from the analysis of the data using Equation 1.23. Qualitatively, the dynamics is slower the farther away the curved quenching plot in the presence of bile salt aggregates is from the linear quenching plot in water. The dynamics for 19 is markedly slower than for 21 (Fig. 1.18), and this result in conjunction with the singlet excited-state quenching experiments led to the proposal that 19 was bound to primary aggregates, while 21 was bound to secondary aggregates. In addition, in the case of 21, no curvature was observe for the quenching of its triplet excited state in the presence of 10 mM NaCh, a bile salt concentration at which primary aggregates are formed but no secondary aggregates are present. The dissociation rate constant for 19 was 30 times lower than for 21, showing that the residence time for the guests in the primary aggregates is much longer than in the secondary aggregates (Table 1.9).
CONCLUSION

This chapter provides the conceptual framework for using photophysical methods to measure the dynamics of supramolecular systems. The principles for the techniques and the analysis methodologies used in these kinetic studies were described and their advantages and disadvantages discussed. Examples were provided for the guest binding dynamics with CDs, DNA, micelles, and bile salt aggregates.

ACKNOWLEDGMENTS

I would like to thank the continuous support to my research program from the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of operating and equipment grants. I would also like to thank my coworkers and collaborators for their contributions in the development of the various research projects in supramolecular dynamics.

REFERENCES


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