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Application of Electronic Circular Dichroism in the Study of Supramolecular Systems

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Abstract

Chiral supramolecular architectures constitute crucial structural and functional elements in living systems and have been long mimicked by chemists to synthesize new artificial systems endowed with desired properties and functions. Among several techniques to study noncovalent chiral assemblies or aggregates, Electronic Circular Dichroism (ECD) plays a key role because many mechanisms responsible for the appearance of ECD bands occur through space, and therefore are intrinsically sensitive to intermolecular interactions, from short to long-range.

The aim of this *tutorial review* is to emphasize the different kinds of information which can be obtained specifically when chiral supramolecular species are characterized by means of ECD spectroscopy. We will survey several typical applications of ECD in the context of supramolecular chemistry, ranging from the simple detection of chiral aggregates or complexes, to the definition of stoichiometric ratios between the partners, the derivation of thermodynamic and kinetic parameters such as binding and rate constants, and ultimately to the refinement of the most plausible structure for the supramolecular species.

Key learning points

(1) Electronic circular dichroism (ECD) depends in a sensitive way on the intermolecular arrangement and interactions between molecules, provided that the superstructure is chiral and has observable electronic transitions

(2) The ECD signals associated with supramolecular species are usually very different from those of their individual components: often, the aggregates or complexes have moderately intense ECD bands while the individual components have vanishing or weak ones

(3) ECD is a useful analytical tool to detect supramolecular aggregates and complexes, to follow their formation and disruption, to determine stoichiometries, to measure binding parameters and kinetic constants

(4) ECD is unique in establishing the absolute sense of twist of helical supramolecular aggregates

(5) When a model of the supramolecular architecture is available, ECD calculations offer a trustful way to validate/dismiss the model or to suggest more plausible ones

INTRODUCTION

Supramolecular chemistry, that is, "chemistry beyond the molecule",[1] is one of the most fascinating and active fields of chemical research.[2] Many supramolecular architectures have been inspired from Nature, or have been designed with a biomimetic approach; not surprisingly, therefore, *chiral supramolecular systems* have attracted a special interest, as witnessed by a monograph published in 2006,[3] and more recent reviews devoted to more specific topics.[4-7]

This tutorial review follows two previous ones, where we treated some fundamental aspects of Electronic Circular Dichroism spectroscopy applied to molecular systems.[8, 9] Our present goal is to broaden the scope by also including systems held together by non-covalent interactions, i.e. to describe the application of ECD in chiral supramolecular chemistry.[10-12] We shall see that ECD is very well suited to address many relevant problems in this field, ranging from the definition of simple stoichiometric ratios between the partners giving rise to a supramolecular structure, to the derivation of thermodynamic parameters, or ultimately to the refinement of a structure for a complex or an aggregate.

For the study of single chiral molecules, ECD can be effectively accompanied and complemented with other chiroptical techniques, most notably Vibrational Circular Dichroism or VCD.[13] On the contrary, for supramolecular systems, ECD is often superior or even unique. This is due to the fact that many interactions responsible for the appearance of ECD bands occur through space, while vibrational properties are more strongly dependent on mechanical couplings, requiring covalent linkages. In some cases, our statements will apply not only to electronic states, but can be extended to vibrational ones, as well. If so and when it is relevant, we shall use the acronym CD instead of ECD.

The basic principles, the instrumental aspects and the methods of interpretation and calculation of ECD spectra will not be discussed here; we refer the reader to our two previous tutorial reviews

devoted to these topics.[8, 9] Because of its prime relevance, we shall only recall what is exciton coupling and what type of information can one deduce from it.

When two (or more) chromophores with similar excitation energies are located nearby in space and are not conjugated, they can interact through a direct dipolar interaction, which depends only on the distance between the chromophores, their relative orientations, the difference in excitation energy, and their spectroscopic properties. In the very simple case of two identical chromophores, characterized by one electronic transition (endowed with electric dipole-allowed character), as it occurs in intense π-π* absorptions, when irradiated, they give rise to a delocalized excitation. The result is a characteristic pair of ECD bands of opposite signs, known as a *couplet*. The sequence of signs (going from longer to shorter wavelengths) is diagnostic of the absolute arrangement of the electric dipole transition moments, thus a positive→negative couplet (also called a *positive couplet*) reveals that the two moments define a *positive* exciton chirality, i.e. a right-handed screw sense of the electric transition dipoles. For more complicated situations, like for example when one has more than two chromophores, when they are not identical, or when they are endowed with more than one significant absorption band, a complete treatment is highly recommended and any simple reasoning should be treated with caution.[8]

I – PRINCIPLES AND GENERAL ASPECTS

I.1 – Definitions

The basic concepts of supramolecular chemistry are available in specialized monographs[2] and in physical organic chemistry textbooks.[14] Supramolecular systems are characterized by weak intermolecular forces, i.e. the energy of each single interaction is (very) grossly comparable to kT at room temperature (2.479 kJ·mol^{-1} or 0.593 kcal·mol^{-1}). Typical non-covalent interactions in supramolecular architectures are hydrogen bonds, metal coordination, electrostatic (charge-charge,

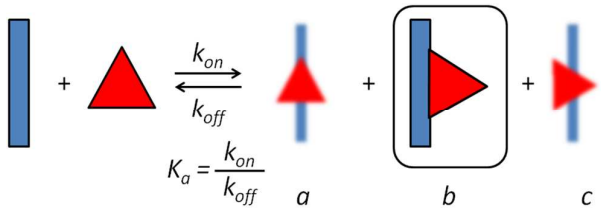
charge-dipole, dipole-dipole, multipolar and so on, interactions involving polarizable groups, including π-π, ion-π or CH-π), and hydrophobic interactions, which commonly amount to less than 20 kJ·mol^{-1}. This ensures that in solution there is at least some reversibility in the formation of supramolecular adducts, i.e. the component molecules can coexist in unbound (free) form and as aggregates (bound). In principle, these weak intermolecular interactions may lead to multiple structures of intermolecular aggregates, but the reversible equilibrium ensures that the system ends up in the most energetically favorable state (in terms of free energy, ΔG^0). This is remarkably different from an ordinary chemical reaction implying the formation/breaking of covalent bonds, where thermodynamic reversibility is often not attained and the more readily formed product (kinetic control) is usually obtained.

We can make a first broad division of supramolecular systems, based on the proportion of their component systems, into three families: a) adducts of well-defined stoichiometry, which behave in many respects very similarly to ordinary molecules; b) assembled systems where the relative proportions and/or the size of the aggregates are changeable, according to the composition, to the temperature, or to the solvent properties; c) systems where the number of constituent units is so large that it may be considered "infinite". We shall see that in these three families of supramolecular systems, ECD applications are very different and that in any case they provide relevant and sometimes unique information. For this reason, we shall organize the present review, discussing specific examples, on the basis of the above stoichiometry criterion.

In the first part (Chapter I), we shall briefly depict various situations involving chiral molecules and supramolecular chirality on a very general ground. In the second part (Chapter II), we shall discuss specific examples for each of the three families taken from recent literature.

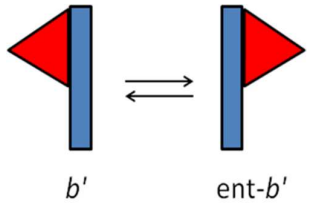
I.2 – Adducts with a well-defined stoichiometry: host-guest complexes

We shall start by representing the dynamic equilibrium described above with the following Scheme 1. Here, K_a is the complex association (binding) constant which can be expressed as the ratio of the association and dissociation apparent kinetic constants k_{on} and k_{off}.



Scheme 1. Two achiral molecules combining into a set of 1:1 supramolecular adducts: product *b* is the most stable one and dominates the mixture at equilibrium.

The assembly depicted in *b* is more stable and forms preferentially. Of course, we focus on *chirality* and we must assume that either one of the intervening molecules is chiral or that there is an external agent promoting the formation of one enantiomer over the other one. In fact, we must recall that two enantiomeric structures have the same internal energy, and – in the absence of any bias – they must be equally populated. In the example above, this would be the case if the aggregate *b* lacks improper symmetry elements (a rototranslational axis), as it happens in *b'* below (Scheme 2).

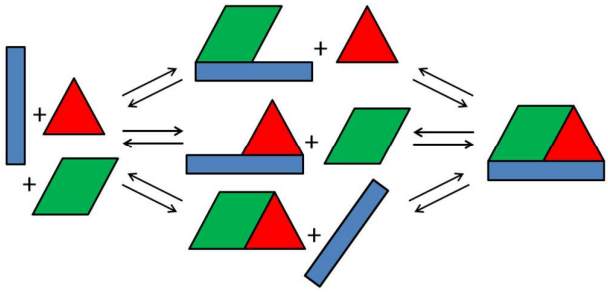


Scheme 2. The combination of two achiral molecules may result in an enantiomeric pair of chiral adducts.

The prefix *ent-* indicates the enantiomer of *b'*; in the absence of other factors, this system will be racemic and it would give rise to vanishing Circular Dichroism. In order to deracemize the system, that is, to favor one enantiomer over the other, we must invoke the intervention of a chiral entity, which either takes explicit part in the assembly, or must act as a template, i.e. at some point it puts

together the various components preferentially in the form *b'* (or *ent-b'*) and consequently CD may be observed.

In the case of multiple component systems, i.e. when the aggregate consists of three or more molecules, one may envisage that the binding proceeds stepwise, as depicted in Scheme 3.



Scheme 3. Stepwise processes leading to the formation of an adduct containing three molecules.

In this representation, each individual step has its own kinetic and thermodynamic parameters (k_{on}, k_{off}, K_a) and their detailed analysis can highlight interference phenomena like competition or cooperative binding, which have often been the object of investigation through ECD, especially in the contest of drug-protein binding.

I.2.1 – The ideal case: a chiral non-chromophoric (CN) molecule interacting with an achiral chromophoric one (AC)

The molecules intervening in supramolecular assemblies can be all different, as in the previous Scheme 3, or they may be identical, as will be discussed in §I.3 below. The conceptually simpler case of two different interacting molecules, giving rise to a well-defined adduct, is often referred to as host-guest chemistry, where intuitively one of the two partners is larger and provides a pocket, a cleft or a cavity accommodating the other one. This is very well exemplified by inclusion complexes, e.g. in cyclodextrins, calixarenes or tweezers, but it also fits very well to the substrate/receptor or drug/protein binding (§II.1.1-II.1.3). Sometimes, the concept of different size may be elusive, but we shall nevertheless make reference to host/guest terminology, which is simple and convenient.

From the point of view of ECD, a relevant situation is when one of the partners is achiral and chromophoric (AC) while the other one is chiral and non-chromophoric (CN) and we shall refer to it as AC/CN. When we say "non-chromophoric" we mean that the molecule lacks significant absorption bands in the spectral region under investigation, for example the UV-vis range above 200 nm. The reason for the special significance is that neither of the two partners alone will give rise to ECD: the AC because it is achiral, the CN because it is non-chromophoric. As soon as the complex is formed, we have a supramolecule, where the chiral host provides a dissymmetric environment around the guest chromophore, which leads to non-vanishing ECD, allowing one to *selectively* monitor complex formation. In other words, *any* ECD signal in correspondence to the guest molecule transitions demonstrates unequivocally the formation of the adduct, without any need for more or less complicated interpretation or quantification tools. This phenomenon is often referred to as *induced* CD or ICD.[10]

I.2.2 – ECD titrations and simple binding isotherms

In the AC/CN case, one may decide to follow the binding event by means of a titration experiment at constant temperature: a plot of the ECD intensity at a certain wavelength as a function of the mole ratio between host and guest provides a binding isotherm, that can be conveniently analyzed by means of mathematical treatments or available programs.[15] The principles and the softwares that are used here are the same as for ordinary spectrophotometric titrations, but ECD may offer the distinctive advantage of a *selective* response only to the aggregate: in the AC/CN case, the absorption spectrum of the complex is often similar to that of the AC component, thus selectivity also means higher *sensitivity* of the ECD signal toward the complex formation.

The quantitative analysis of the isotherm will provide data for the complex stoichiometry, the binding constant, or, if the binding isotherms are recorded at different temperatures, for the thermodynamic parameters ΔH^0 and ΔS^0, through a Van't Hoff analysis based on the equation

$$K(T) = e^{\left(\Delta S^0/R\right)} e^{\left(-\Delta H^0/RT\right)}$$

If the molecules participating in the adduct exist either in free or in bound form, there is an equilibrium between two states only, detectable by isodichroic points as typical spectroscopic signature (same as isosbestic points in absorption spectroscopy). In the ideal AC/CN case, when the free molecules have vanishing ECD spectrum, isodichroic points *must occur on the crossover points*, i.e. when ECD crosses the zero-line. If during the course of a titration we do not observe isodichroic points, we can be sure that the equilibrium is more complex than a two-state one and that we must invoke complexes with different stoichiometries. To better appreciate this point, we wish to recall and underline a notion, which is indeed common to several spectroscopic methods and not specific of ECD. Let us consider a host H with multiple non equivalent binding sites with respect to the same guest, G. Then, several 1:1 complexes will form in the initial steps of a titration of H with G, namely $(H{\cdot}G)^1$, $(H{\cdot}G)^2$, …, $(H{\cdot}G)^i$, each process is associated with a different K_a^i (here the superscript represent occupancy of site 1,2,...,*i* of the host).

The relative proportion of the various adducts $(H{\cdot}G)^1$, $(H{\cdot}G)^2$, …, $(H{\cdot}G)^i$ remains constant throughout the whole titration (indeed, $[(H{\cdot}G)^i]/[(H{\cdot}G)^j] = K_a^i / K_a^j$). The ECD spectrum will be the weighted average (with constant weights) of all the individual adducts and it will be therefore impossible to disentangle them unless recurring to other techniques (e.g. preventing the binding at one site by the introduction a mutation in a protein host or designing site-specific competition experiments).

I.2.3 – On the origin of Induced ECD: a rigid achiral chromophoric guest

We may ask ourselves what kind of mechanisms may be responsible for the appearance of induced ECD. Let's consider again the case of a 1:1 host/guest complex with a chiral non-chromophoric host and an achiral chromophoric guest. Let also first assume that the two partners (the host and the guest) are *rigid*, i.e. they do not change their conformation/structure upon binding. When the guest is bound to the host, we are in the case of a locally achiral chromophore embedded in a chiral environment. Following the definition of supramolecular system, there is no covalent interaction

between the two and through-space mechanisms will dominate, i.e. one may use a so-called *independent system approximation*,[8] where the chromophore transitions are only weakly perturbed by the environment. Sometimes this convenient approximation may not be valid, notably when there are strong specific intermolecular interactions involving π-electrons or hydrogen bonds (H-bonds), which may imply a certain degree of orbital delocalization even in the formal absence of covalent bonds.

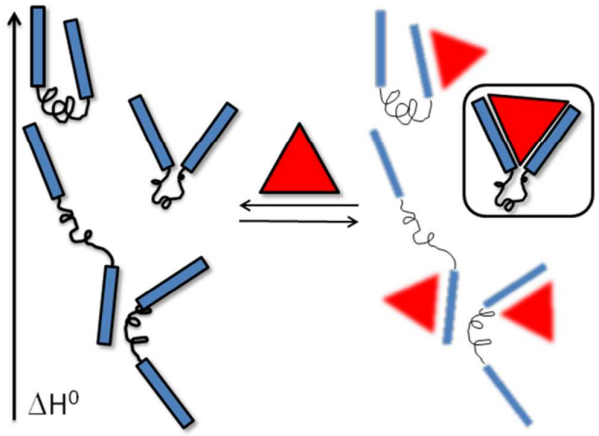
Among through-space spectroscopic interactions, we may consider **μ**-**μ** or **μ**-**m** couplings, where **μ** represents an electric dipole transition moment, and **m** a magnetic dipole transition moment. One of the two transition dipoles will reside on the chromophoric guest, while the other one (or more than one) is provided by higher energy transitions of the host. All transitions, even including σ-σ* and thus single bonds, may in principle contribute to these mechanisms. The **μ**–**μ** or coupled dipole mechanism is relevant when electric-dipole allowed transitions are involved. Electric/magnetic interference expressed by the **μ**-**m** term is especially suited for the case of ketones or of *d/f* metal ions. Any further description of these couplings is not suitable to the present discussion and here it suffices to say that nowadays they are most conveniently treated by computational tools. As a general feature, however, we may say that each transition of the isolated chromophore will correspond to one ECD band. With reference to the previous tutorial review, this will correspond to Case (1) in §1.1.[9]

If the adduct stoichiometry involves two chromophoric guest molecules, then things may become different. The host may act as a scaffold where the two guests are nailed, in a defined fashion and give rise to degenerate exciton coupling (i.e., between two equivalent transitions), which is Case (2) in §1.1 of ref. 9. From a purely spectroscopic point of view, the fact that multiple binding is associated with exciton coupling has a further relevant consequence, because this mechanism can be extremely effective and it may lead to intense ECD effects. The formation of any 1:2 (or any other higher stoichiometry) adduct *must* be preceded by more or less stable 1:1 complexes. Usually, ECD

of these latter species is monosignated (for each chromophore transition) and often it is rather weak, compared to the degenerate couplets due to the 1:2 adduct, to the point that it may even be overlooked. If so, one may misinterpret the experimental data as evidence of a strong cooperative effect, where the firstly formed 1:1 complex immediately yields to a 1:2 complex.

I.2.4 – More into ECD mechanisms: conformational flexibility and induced chiral conformations

So far we treated the intervening molecules as rigid bodies, although in general they may be flexible entities and can undergo important conformational rearrangements during binding. In Scheme 4, we represent one of the molecules (the host) as a manifold of conformers, each endowed with a different internal energy.

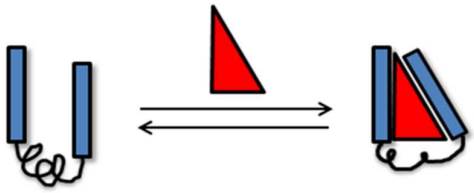


Scheme 4. A flexible host molecule (blue) interacting with a rigid guest (red).

In free form, each conformation is populated according to a Boltzmann distribution depending on its free energy ΔG^0 (practically, often one would use the enthalpy ΔH^0) and on the temperature. The binding event selects one of these conformers because it better fits the guest and drives the equilibrium to the preferential formation of one species. A perfectly equivalent situation can be found inverting the roles of the two partners: we may have a flexible guest and a (relatively) rigid host, like e.g. for drug-protein binding. The case in which both partners display multiple conformations does not really introduce any further complication. There are many very relevant

consequences of a similar process and in the following we shall discuss only some of them, obviously in their relation to ECD.

The interaction of a flexible-achiral host with a rigid-chiral guest can lead to the enforcement of a specific chiral conformation of the host, which is represented in Scheme 5.



Scheme 5. A flexible achiral host takes a preferential chiral conformation upon interaction with a chiral guest.

Once more, this very same situation is encountered in a number of examples, which at first sight may appear scattered and poorly related with one another. As a first example, the host represented in Scheme 5 recalls the porphyrin tweezers used to determine the absolute configuration of simple organic compounds, discussed in §II.1.2. A second set of cases is provided by drug-protein binding. Thus, a flavonoid like quercetin (Figure 1) is composed of two molecular moieties (a benzopyrone and a phenyl group) held together by a single bond and is achiral. All conformations beside the planar ones are chiral, but they exist in enantiomeric pairs. Once the quercetin molecule is hosted onto human serum albumin (HSA), one conformation fits better than the other ones (Figure 1).[16] In all these cases concerning the enforcement of a chiral conformation upon the formation of a supramolecular adduct, one may speak of *induced chirality*.

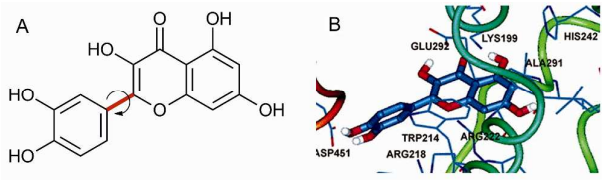


Figure 1. A: structure of the flavonoid quercetin; rotation around the evidenced bond produces chiral conformations. B: conformation assumed by quercetin bound to HSA. Reprinted from ref. 16, Copyright 2003, with permission from Elsevier.

In its free form this molecule cannot exhibit optical activity, because every chiral conformation is exactly compensated by its enantiomer, but after binding this balance is lost. We must consider this possibility as an *intrinsic* ECD of the guest, which was excluded in the section §I.2.3 above, when we treated a *rigid* achiral guest. The intrinsic contribution is mixed up with all possible intermolecular coupling effects like **μ**-**μ** or **μ**-**m**, which makes the interpretation of the spectrum rather complicated.

An extreme case of conformational selection is found when the achiral molecule by itself exists essentially in a pair of enantiomeric conformations (i.e. a pair of rapidly interconverting mirror images). This is for example the case of bilirubin interacting with serum albumin (see §II.1.3),[17] or the Pfeiffer effect in octahedral metal complexes.[18]

I.3 – Identical molecules interacting: dimers and oligomers

If the two interaction partners are identical, we can no longer speak of host/guest. Moreover, in order to observe ECD, the intervening molecules must be at the same time chromophoric *and* chiral. This means that they may display a more or less pronounced intrinsic ECD, which is usually very different from the one due to the adduct: if the individual molecules are endowed with electric dipole transitions, then in the complex they may give rise to intermolecular exciton coupling, which will dominate the spectrum of the dimer.

In this context the concept of titration becomes elusive and, in order to follow the aggregation process, one can change:

(a) the total concentration, similarly to deviations to Lambert-Beer law;

(b) the temperature (at fixed concentration), taking advantage of the entropy change associated with dimerization;

(c) the solvent composition, using mixtures of good solvent/poor solvent (in a good solvent, the molecules are well solvated and exist as monomers, while in a poor solvent they aggregate).

In these cases the ECD spectra provide evidence for the existence of the aggregate and may suggest which intermolecular forces are likely to play a role. Moreover, a fitting of the ECD data may lead to estimate thermodynamic parameters, such as the dimerization constant K and, in case (b), the associated ΔH^0 and ΔS^0, following Van't Hoff equation.

More or less detailed analysis of the ECD spectrum of the dimer possibly allows one to put forward or to validate geometrical models. In the case of exciton coupling, when the interacting chromophores have well-defined and known electric dipole transition moments, the sign of the exciton couplet can be directly related to the sense of twist between the two molecules participating in the dimer. More quantitative information may be sought through full ECD calculations on model structures.

From the point of view of ECD, there is little difference between dimers and oligomers, and indeed it may be difficult to discriminate between the two cases by chiroptical spectroscopy alone. Because of the much greater interest in large oligomeric assemblies compared to smaller dimers, we will only provide examples of the former ones in §II.1.5. Moreover, the same concepts may be transferred to infinite assemblies of a single component, discussed in §I.4.2 and for which several examples are provided in §II.3.1.

I.4 – Non-definite stoichiometries and supramolecular assemblies

So far we have considered only the case of well-defined complexes, which can also be regarded as supramolecules, i.e. as precise chemical entities held together through weak interactions.

We shall now discuss aggregates whose size and/or composition is changeable, according to the solution composition. We can distinguish at least two main classes of such processes: a) when we have a macromolecular host, able to bind a variable number of (small) guests; b) when a self-assembled supramolecular polymer is formed (it may involve either identical molecules or a set of different partners, forming together a repeating unit).

In all these cases, ECD is primarily used to demonstrate the existence of supramolecular chirality. As we have already seen several times above, a cross-interaction between (similar) chromophoric molecules brings about exciton coupling, a powerful mechanism for optical activity, which may be totally absent in the isolated molecules.

I.4.1 – Stereoregular polymeric hosts: more on binding isotherms

A typical example is the binding of achiral chromophoric guest molecules to a helical polymer as the host. During the course of a titration one observes first the onset and then the evolution of the ECD spectrum associated with the guest electronic transitions.

In an initial state the guest molecules are few and (provided they are statistically distributed) they are far apart. In this state, if the guest molecules can be represented as simple rigid chromophores, they will exhibit one or more ECD effects, each associated with one absorption band. The origin of this ECD is similar to the case of the host/guest complex described in §I.2.3.

When the loading increases, the guest molecules start to interact (spectroscopically) with each other and this gives rise to new spectral features, because of exciton coupling. Each absorption band may become associated to a more or less complex set of ECD effects (in the simple and common case an ECD couplet) and positive/negative signals will tend to compensate. In the CD jargon this feature is called *conservative spectrum*, which is a signature of exciton coupling, i.e. ultimately of close neighborhood of guest molecules. This will naturally occur at high loading, but it can happen that even at small loadings occupancy of nearby sites becomes preferred over a purely random distribution. This may be the consequence of an allosteric effect (i.e. conformational change of the host following guest binding) or of some specific chemical force between guest molecules: ECD is likely to provide immediate and clear evidence to such occurrence.

ECD lends itself to follow a titration of the type described above, and the resulting binding isotherm may be fitted to a Hill equation to derive the dimension of the binding site (i.e. how many monomers of the macromolecule are engaged in binding one guest molecule) and the so-called Hill

coefficient, which provides a measurement of the cooperativity of binding. In principle, this is not at all different from the usual treatment for ordinary spectrophotometry and we refer the reader to standard textbook literature on this topic.[14]

In the above description, we considered the macromolecular host as intrinsically endowed with a helical structure and undergoing only minor conformational rearrangements, as consequence of ligand binding. This picture applies rather well to DNA. The guest molecules can be accommodated either along its minor or major grooves or they can intercalate between the base pairs. Of course DNA presents strong absorption and dichroic bands, but they are confined below 350 nm: in the case of strongly red-shifted guests one may in a first approximation call it a "non-chromophoric host". Indeed, common systems interacting with DNA include cyanine dyes, polycondensed aromatics (including porphyrins), metal complexes, all of them characterized by absorption bands in the visible. An example of cyanine/DNA complexes will be discussed in §II.2.2.

We must also mention the possibility that the macromolecular host in its free form has a random coil, an extended, or a poorly defined conformation, which switches to a defined helix only upon binding to the host. We shall discuss an example in §II.2.1.

I.4.2 – Infinite assemblies

There are several cases where intermolecular interactions can be replicated an indefinite number of times, which leads to the formation of large aggregates, so large with respect to the standard molecular scale that the number of constituent units may considered "infinite". In order to give rise to detectable ECD, the monomeric units must be chromophoric. In the very large majority of cases, they must also be chiral, in order to ensure chirality in the supramolecular aggregate, although there are some examples of large helical architectures where achiral elements build a supramolecular assembly onto a chiral template or under the effect of some symmetry-breaking force. These will be discussed in §II.3.3 and §II.3.4. respectively.

Similarly to the case of dimers or oligomers of identical molecules (see §I.3), it is common that the individual bricks of these architectures are endowed with (weak) intrinsic ECD. Moreover, it is important to notice that while the ECD response will be dictated by the overall supramolecular structure, the ECD associated with a particular chromophore will essentially depend on its closest neighbors. In other words, the ECD signal of an infinite entity will actually correspond to the summation of the one associated with its small repeating units. Thus, the approach to the analysis of the ECD spectrum of an infinite assembly is not really different from the one followed for dimers or oligomers.

Large supramolecular assemblies of chiral molecules often provide, at the mesoscopic level, twisted and threaded superstructures which can be directly observed by means of electron or atomic force microscopies. Unfortunately, these techniques are still unlikely to capture the molecular level, which lies at least an order of magnitude below the resolution of the images. As we shall see in §II.3.1, ECD can be used to validate or discard computer-aided structural models (conformation, relative orientation, and network of hydrogen bonds). In all these cases, the orientation of the chromophore with its transition dipoles with respect to the superstructure depends strongly on the molecular conformation. Therefore, it is mandatory to have a good representation of the individual molecule within the assembled framework before attempting any interpretation of the ECD spectrum.

I.4.2.1 Hydrogen-bonded assemblies

There are innumerable systems of supramolecular assemblies held together by H-bonds, which are relatively strong, directional and at the same time are flexible enough to provide great supramolecular architectures. The component molecules may display widely different structures (especially in terms of the ratio between the molecular sizes in different dimensions) and are commonly intrinsically 3-dimensional. Most significant ECD applications are found when the

individual molecules bear some strong chromophore, possibly giving rise to exciton coupling; various examples will be given in Chapter II.

I.4.2.2 Conjugated systems and π-π interactions

A second relevant interaction is provided by electrostatic attraction between conjugated systems with extended π-orbitals. Whatever the complex nature of this force is (quadrupolar, London dispersion), it is clear that large aromatic compounds and conjugated oligo- and polymers tend to provide typical superstructures, known as more or less deformed H- or J-aggregates. In H-aggregates, two or more monomers are arranged on the top of each other, i.e. the stacking direction is roughly perpendicular to the molecular plane (Figure 2, left). In J-aggregates, the monomers are displaced with respect to each other in a direction roughly parallel the molecular plane (Figure 2, right). Naturally, the marked electron delocalization of π-conjugated systems makes them excellent chromophores, which is especially relevant for the analysis of ECD. In fact, H- and J-aggregates are most conveniently distinguished on the basis of their electronic spectra. In correspondence of the electronic π-π* transitions of the monomer, exciton theory predicts that H-aggregates will be characterized by a hypsochromic (blue-shifted) and J-aggregates by a bathochromic (red-shifted) absorption with respect to the monomer UV-vis absorption band (Figure 2). If the aggregates are chiral, both H- and J-aggregates may manifest exciton-coupled ECD spectra, with a more or less strong exciton couplet depending on the exact supramolecular arrangement.

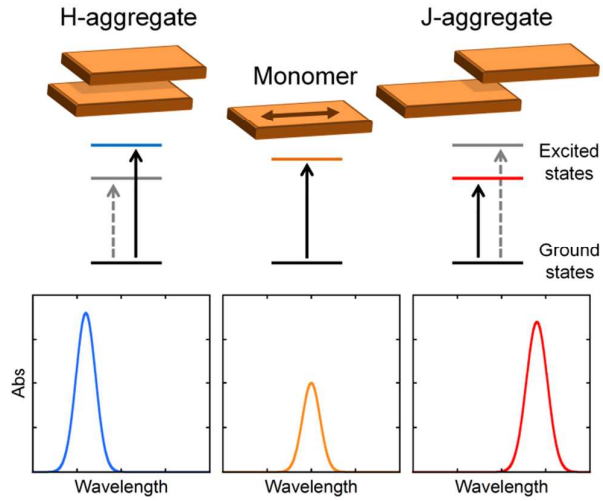


Figure 2. Typical arrangements of H- and J-aggregates (exemplified for a dimer) for a π-conjugated molecule (monomer) and correspondent effects in the absorption UV-vis spectra. Full arrows depict allowed (strong) transitions, and dashed arrows forbidden (or weak) ones. The double arrow represents the transition dipole for the monomer.

Extended π systems can be further divided into two classes, according to the aspect ratio of their component molecules. The first class is rod-like, as for example in polyenes, where the elongated chromophore has one privileged axis, and the transition dipoles of the most red-shifted transitions are aligned. The second class is disk-like, as in (polycondensed) aromatics, porphyrins or phthalocyanins. In the case of planar chromophores with an effective 3-fold symmetry or higher, such as benzene (6-fold), 1,3,5-trisubstituted benzenes (3-fold), porphyrins (4-fold) and so on, in-plane polarized π-π* transitions are represented as circular oscillators rather than as the more usual linear transition dipoles (a circular oscillator is reminiscent of ring breathing normal mode in IR spectroscopy). For example, *meso*-tetraaryl porphyrins (discussed in §II.1.2, §II.3.2 and §II.3.4) are characterized by strong absorption peaks in the 400-500 nm region, called Soret bands. The Soret bands are in fact allied with two degenerate (having the same energy) π-π* electronic transitions endowed with orthogonal transition dipoles, which globally define a circular oscillator.[19] In both rod- or disk-like systems, the chromophores are intrinsically achiral, therefore chirality must be

introduced in the side chains or groups, which are expected to provide only weak perturbations to the electronic transitions of the main chromophores. Accordingly, intrinsic ECD of isolated molecules is usually very weak and hardly detectable.

As we mentioned before, the primary and often most significant source of ECD comes from the first-neighbor's interactions, making the difference between rods and disks evident. In a very simple model (Figure 3) of twisted H-stacks, each pair of rod-like chromophores will give rise to an exciton couplet, whose sign will represent the chirality of the two transition moments and ultimately the helicity of aggregate. Thus, a positive sign of the long-wavelength ECD will witness a right-handed helix.

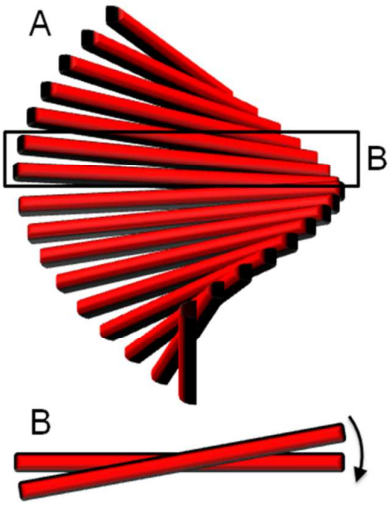


Figure 3. A twisted H-stack of rod-like molecules, defining a clockwise (positive) helicity. The close-up shown in B demonstrates the twist between two adjacent molecules.

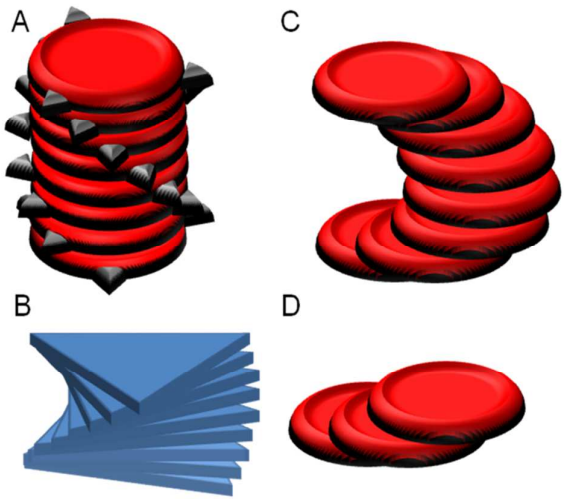


Figure 4. Various types of chiral H-stacks in disk-like systems, which must be described as circular oscillators. In A and B the molecules (the centers) are perfectly aligned and one should expect vanishing excitonic ECD. In C, a certain degree of chiral tiling ensures excitonic ECD. The close-up shown D serves to demonstrates that for disk-like and other symmetrical molecules, one needs at least 3 elements to define chirality, because a dimer of two parallel disk-like molecules is always achiral.

On the contrary, for discoid systems, things may be more complicated. The perfect piling of truly circular oscillators (like e.g. in C_3 1,3,5-trisubstituted benzenes or C_4 porphyrins) is displayed in Figure 4A, with depicted small side groups around a circular core. The overall chromophore represents a cylindrical system and it would lead to *vanishing* ECD, were it only for the interchromophoric interactions.[20] Only explicit calculations, where the role of the side groups are fully taken into account, may reproduce the ECD spectrum.

It may appear surprising or even counterintuitive, but even case B in Figure 4 gives rise to exactly the same situation. This is because, as said above, for dipole-dipole interaction, which is at the basis of exciton coupling, any symmetry higher than 3-fold appears as perfectly circular.

Panel C introduces some degree of eccentricity (a mainly H-stack, with some J-character). Here most of the symmetry is lost and at a first sight a presence of helical twist may be recognized. A

closer look into panel D, however, reveals that if we consider just a pair of chromophores, then we obtain an achiral entity, while the first chiral unit in this aggregate is a trimer. In such a system, the prediction of the ECD based on simple geometric reasoning may be tricky and a quantitative coupled-oscillator treatment would be preferred.

However, in the discussion above, the possibility for chromophore deformation causing loss of the straight rod or flat disk shape and becoming intrinsically chiral was completely neglected. It should be clear that the effect of such symmetry-lowering on the ECD may be dramatic and that intermolecular exciton coupling may lose its dominant character in determining chiroptical activity.[20]

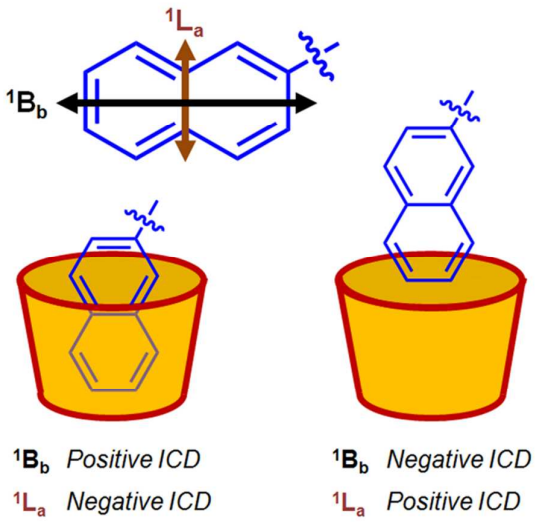
II – APPLICATIONS AND EXAMPLES

II.1 – Finite assemblies

II.1.1 – Inclusion complexes

The cyclodextrins (CyD), formed by α-D-glycopyranose units, and commonly known as α-, β- and γ-CyD (6, 7 and 8 units, respectively), are cyclic oligosaccharides with donut shape and significantly different flexibility and binding capacity. Among the three CyDs, the β-CyD is the best-known and one of the most studied chiral host molecule, capable of forming inclusion complexes with various small organic molecules and drugs. The application of ECD provides sensitive information about the complex formation, stoichiometry and orientation of the guest inside the CyD cavity. Since the free chiral CyD hosts are practically ECD-silent above 200 nm, the complexes between CyD and achiral chromophoric guests fit perfectly the AC/CN case described in §I.2.1. The observed induced CD (ICD) can reveal important structural details about the complex, since the ECD features, namely sign and intensity, are strongly dependent on the location and orientation of the guest molecule "*inside* or *outside"* of CyD cavity.[10]

Several rules relate specifically the sign of the induced Cotton effect(s) with the orientation of achiral guest in respect to CyD binding cavity. For example, according to Kodaka rule a positive induced Cotton effect means that the chromophoric guest penetrates the CyD cavity in such a way that its electric transition dipole moment **μ** is parallel to the CyD main axis, while if it is oriented perpendicular, the Cotton effect appears as negative. However if the guest is outside the CyD cavity the relationship between the sign of induced ECD and **μ** orientation is inverted (Scheme 6).[21]



Scheme 6. Kodaka's rule relating the sign of ICD and the orientation of a guest inside or outside CyD cavity illustrated for a naphthalene derivative. ^1B_b and ^1L_a are the main electronic π–π* transitions for the naphthalene chromophore, occurring respectively around 220 and 280 nm and oriented long- and short-axis. A consistent example is discussed in ref. 21.

The usefulness of ECD by providing binding and conformational details is particularly obvious in the studies of binary and ternary complex structures resulting from displacement experiments with different guests. t-Butyl derivative of chromophoric pyromellitic diimide (*t*-Bu-PMDI, **1**) forms a binary host-guest complex with β-CyD where according to the negative ICD the guest is placed outside the CyD cavity with its long axis parallel to CyD axis (Figure 5). In presence of the adamantane carboxylate derivative (ADC, **2**) the complex undergoes a transformation as a results of a displacement reaction, and the ECD almost vanishes since the only chromophoric guest *t*-

BuPMDI (**1**) is dislocated far outside the binding pocket. Conversely, the addition of adamantane-1-ammonium chloride (ADA, **3**) causes formation of a ternary complex where ECD still remains negative.[22] The binding modes were confirmed by NMR ROESY experiments.

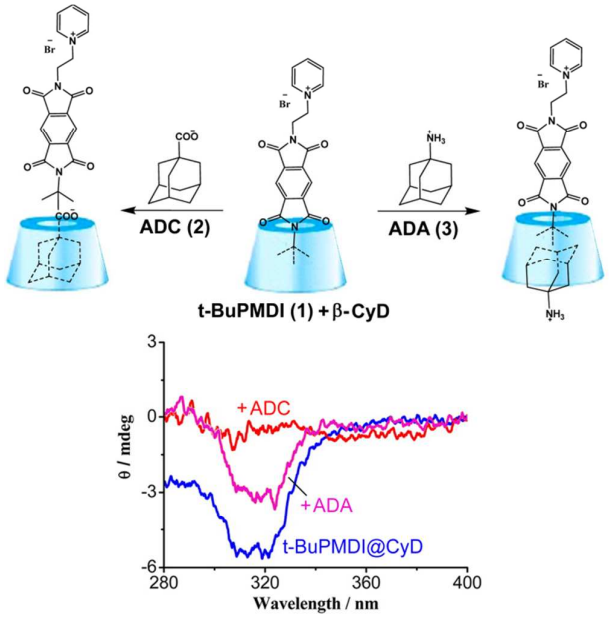
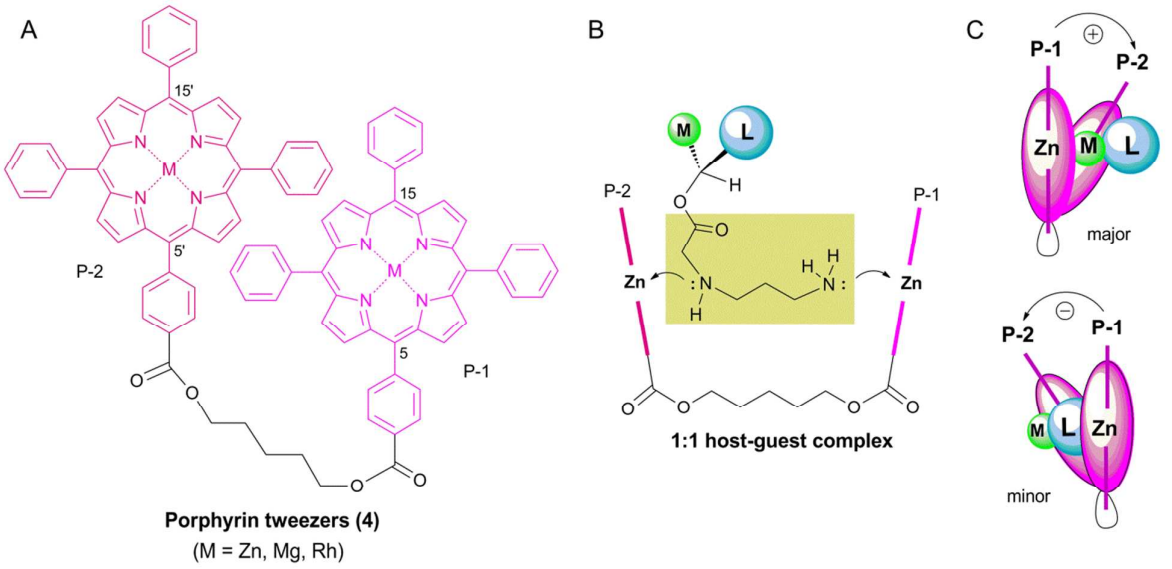


Figure 5. Top: displacement reactions of the complex between t-BuPMDI (**1**) and β-CyD with ADC (**2**) and ADA (**3**). Bottom: evolution of ECD spectra following the above reactions. Adapted with permission from ref. 22. Copyright 2012 American Chemical Society.

II.1.2 – Metal bound complexes

There are many known metal-bound supramolecular species where ECD provided a useful spectroscopic method for their structure determination, as described in several reviews.[4, 5, 23] Here, we selected to discuss the host/guest complexes based on so-called metalloporphyrin tweezers host. This represents one of the few cases where metal coordination lends itself to the absolute configuration determination of monofunctional organic molecules. The analysis by porphyrin tweezers becomes possible thanks to remarkable electronic properties of porphyrin chromophore and its metal derivatives, endowed with a very intense and red-shifted absorption band in the Soret region, as discussed in our first tutorial[8] and in other reviews.[24]

In the porphyrin tweezers (**4**, Scheme 7) two metallated (Zn, Mg or Rh) porphyrin moieties are linked with various spacers, flexible or more rigid.[25] In the free form they lack chirality and are therefore ECD-silent. However, upon mixing with a chiral bidentate guest or its derivative (conjugate) that contains two functional groups, such as amino, hydroxyl or carboxyl, the tweezers host undergoes a ditopic coordination which is stereo-controlled by the chiral guest. The process results in formation of 1:1 chiral host-guest complexes (Scheme 7). Upon complexation, instead of being mutually randomly oriented as in the free tweezers, the two porphyrin chromophores encompass the guest into the binding pocket, become mutually twisted and adopt one preferred sense of chirality between porphyrin effective electric transition dipole moments (i.e., a privileged direction within the circular oscillator, see §I.4.2.2; for a description of the concept of effective transition dipole we refer the reader to the literature).[19] As a consequence, the complex also exhibits a very intense exciton split ECD band within the porphyrin Soret region around 420 nm. In fact, this is an example of induced ECD related to adopted conformational chirality of the host upon complexation, as described in §I.2.4. In addition to the appearance of an ICD exciton couplet, the formation of host-guest complex like that shown in Scheme 7 is also supported by ^{1}H-NMR chemical shifts analysis. In particular, the changes of chemical shifts of the chiral conjugate (guest) may reflect the steric discrimination of the groups attached at the chiral center (M, L). The determination of absolute configuration of the chiral guest can be conveniently made on the basis of molecular modelling calculations on the host/guest complex. The calculations help one to establish the most probable conformation adopted by the host in the complex so that the interporphyrin helicity can be correlated with the sign of observed ECD band.[24] A recent X-ray study on some tweezers complexes with chiral diamines guests revealed the presence, in the solid state, of host-guest complexes of opposite interporphyrin helicity, whose content depended, among other factors, on the guest flexibility.[26]



Scheme 7. A: bis(*meso*-tetraphenyl)porphyrin tweezers host (**4**). B: schematic representation of the complex between the tweezers and a chiral guest obtained by derivatization of a chiral secondary alcohol; L and M indicate the relative steric size (large and medium) of the two substituents at the chiral centre. C: Accepted mechanism for chiral induction of predominant interporphyrin helicity based on guest structure.

Let us have a closer look into the example shown in Figure 6. It concerns the determination of the absolute configuration of the β-lactam (+)-**5a** with known *cis* relative configuration.[27] In order to apply the porphyrin tweezers approach, *cis*-**5a** was converted into the bidentate conjugate **5b** before submission to complexation with the porphyrin tweezers.

A very intense bisignate ECD reveals that the complexation proceeds with a high degree of stereodifferentiation and the complexes with negative interporphyrin twist are strongly preferred. In this, as well as in other cases in the same paper,[27] molecular modeling based on molecular mechanics has been found very useful in predicting preferred interporphyrin helicity. However, the following more general comment is deserved. Even when the complexation process leads to clear-cut preference of one particular interporphyrin helicity, the preferred complexes are expected to have variable value of interporhyrin twist angle and possibly slightly different location of the chiral

guest into tweezers binding pocket. Therefore, while a qualitative conformational analysis can help to rationalize the origin of ECD, it precludes a possibility for further quantitative prediction of ECD exciton couplet intensity and closer comparison with experimentally observed data.

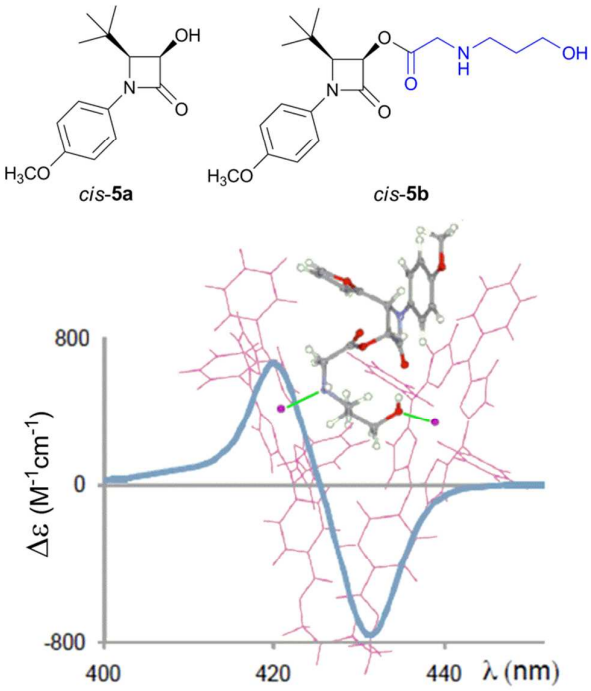


Figure 6. Top: structures of β-lactam *cis*-(+)-**5a** and its derived conjugate **5b**. Bottom: ECD spectrum (in methycyclohexane, Soret region) and MM-calculated most probable structure for the 1:1 complex between porphyrin tweezers **4** and **5b**.

II.1.3 – Protein /drug adducts

The analysis of drugs interactions with plasma and tissue proteins has been for a long time recognized as very critical for understanding the pharmacokinetic and pharmacodynamics aspects related to drug action. Among other analytical methods ECD takes a unique position since it directly reports the occurrence of stereoselective drug binding event and provides a reliable method for screening the drug-binding parameters.[28] Below we will discuss a few examples of different types of drug/protein interactions.

When the drug is achiral and chromophoric, it belongs AC/CN case, already discussed in §I.2.1.

The induced ECD signal observed upon binding can provide unique and rich information about the

stereoselectivity of binding event, the specific binding site of the protein host,[29] the stoichiometry of protein-drug complex and other details. This rather typical case is exemplified by the binding of chlorpromazine (CHLP, **6**) to human plasma α_1-acid glycoprotein (AGP), which leads to the formation of a 1:1 host guest complex and to positive induced Cotton effect at a very similar wavelength of UV band of the free drug (Figure 7).[30]

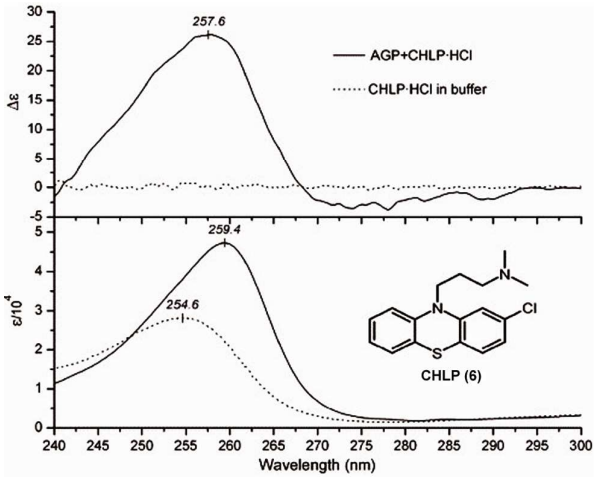
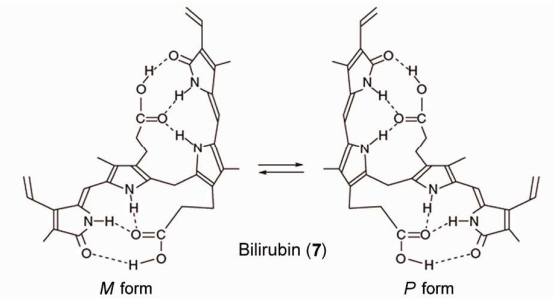


Figure 7. Absorption (bottom) and ECD spectra (top) of chlorpromazine (**6**) hydrochloride (CHLP·HCl) and its complex with human plasma α_1-acid glycoprotein (AGP) in aqueous buffer solution. Reprinted from ref. 30, Copyright 2007, with permission from Elsevier.

The fact that the π-π^* transition of the guest becomes optically active directly proves that the AGP contains a suitable binding site for this particular drug. Therefore, the sensitivity of ICD in providing a direct and convenient screening test for formation of drug/protein complex often surpasses many other alternative methods such as equilibrium dialysis, biochromatography and optical biosensor. While the nature of this type of ICD can vary from case to case, it is certain that the ICD results from non-covalent interactions with the neighbouring amino acids in the binding pocket. For further reading on the analysis of drug-protein interactions and related ICD by molecular modeling and docking calculations as well as other computational methods see the perspective article by Monti et al.[31]

A different case highlights the capability of chiral binding site of protein hosts to induce a conformational preference in the stereochemically labile bilirubin pigment molecule (**7**, Scheme 8). Bilirubin, while is free, exists as equilibrium of two chiral conformers (*P* and *M*) in 1:1 ratio, and as such, it is CD-silent. However, in presence of human serum albumin (HSA) or other albumins, which act as water-soluble chiral complexation agents, the binding event causes an asymmetric transformation of free *P/M* bilirubin mixture into the preferred and more stable bound conformer with ridge-tile shape and (*P*)-helicity (Scheme 8).[17] The resulting complex becomes easily observable by its intense induced CD exciton couplet due to two positively-twisted constituent dipyrrinone chomophores. This is another example of conformationally driven ICD in addition to that discussed in the previous paragraph and introduced in §I.2.4.



Scheme 8. Possible enantiomorphous conformations of bilirubin (**7**).

The third example discloses the remarkable sensitivity of ICD as a characteristic analytical tool for globular protein recognition, a task usually far from being trivial. The analysis relies on the application of uncharged symmetrical Co-porphyrin (Co-P, **8**, Figure 8A) as molecular probe which in aqueous solution at pH 7 and μM concentration forms a ternary 2:1 Co-P/protein complex with various proteins or peptides, such as albumin, trypsinogen, lysozyme, insulin and gramicidin.[32] By mixing Co-P (**8**) with the protein solution, two Co-porphyrin moieties come into contact with specific amino acids at the surface of protein macromolecule and yield a porphyrin sandwich structure (Figure 8B) without disrupting the protein native conformation. During the complexation process the two Co-P (**8**) moieties adjust in each case selectively on the surface for optimal amino-

acid binding. Importantly, this causes the two Co-P molecules to adopt a specific mutual orientation, easily detectable by appearance of a bisignate ECD curve, characteristic for each protein, resulting from an exciton Co-P/Co-P coupling. It is worth mentioning that the reason for the differences in overall shape of observed bisignate Cotton effects, and also for their opposite profiles and intensity, lies exclusively in the sensitivity of ECD to reflect the subtle modulation in the spatial orientation between interacting Co-P chromophores caused by selectivity in the protein binding. Figure 8C shows the positive exciton ICD obtained with gramicidin and lysozyme, and the negative one with insulin and trypsinogen.

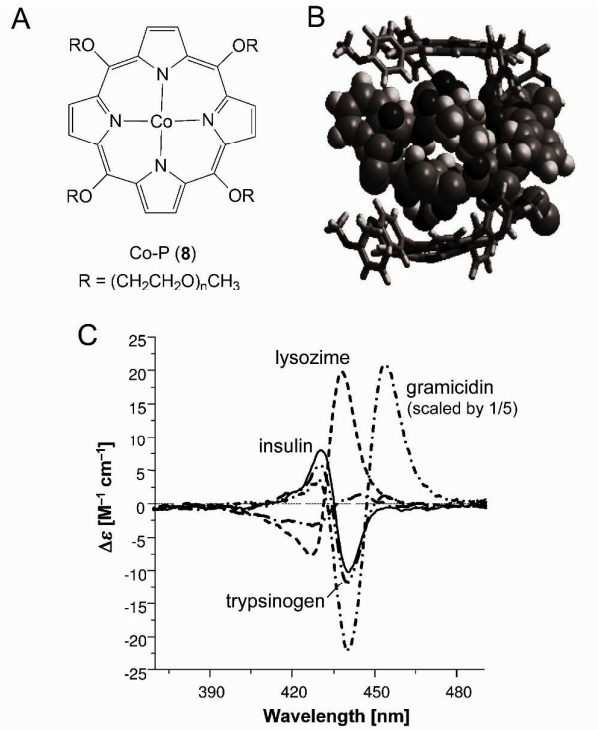


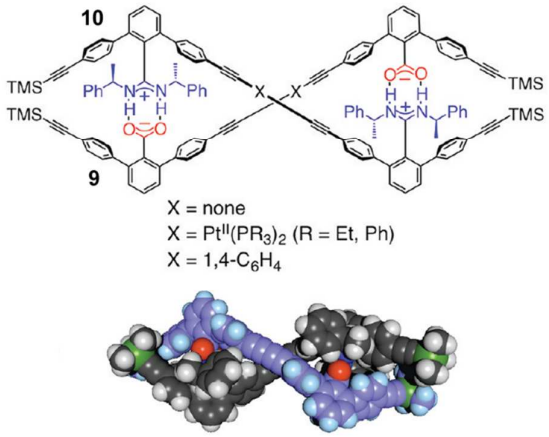
Figure 8. A: structure of the water soluble Co porphyrin Co-P (**8**). B: illustration of the Co–P/gramicidin/Co–P sandwich complex. C: ECD spectra (Soret region) in water of the 1:1 mixtures between Co-P (**8**) and some proteins or peptides. Reproduced with permission from ref. 32. Copyright 2012 Wiley-VCH.

II.1.4 – Hydrogen-bonded and salt-bridged complexes

Among the most interesting supramolecular systems based on hydrogen bonds and salt bridges studied by ECD are those folding in helical structures, usually referred to as helicates. Several chiral helicates have been investigated by Yashima and coworkers; the following example offers a very good survey of the many possible applications of ECD for quantitative studies of thermodynamics and kinetics of binding.

Achiral dicarboxylic acids (**9**) and chiral diamidines (**10**) shown in Scheme 9, when mixed in 1:1 ratio, easily form hetero-helicates **9·10** through complementary salt-bridge formation.[33] Because of the conjugated skeleton of both components, such hetero-helicates have distinct ECD spectra which can be used as a selective and sensitive analytical tool for characterizing the complexes. In fact, the ECD spectra of the double helices (**9·10**, Scheme 9) are significantly different from the one of their chiral components alone. Direct titration experiments (addition of a dicarboxylic acid **9** to a diamidine **10** solution in chloroform) were monitored by ECD at fixed wavelength and, for some of the complexes, they furnished binding curves well fitted by a simple 1:1 model yielding the relative binding (association) constants K_a (Figure 9). When binding constants are too high, however, direct titration experiments are useless because the binding curve appears as a broken line and the necessary fitting parameters cannot be determined. The upper limit of K_a measurable by titration experiments is inversely proportional to the lowest concentration detected by the instrument (around 10^{-7} M in the current case). For higher K_a's, competition experiments were thus employed, where a preformed dicarboxylate/diamidinium complex was titrated with a different diamidine. It is interesting to observe that in the above experiments the UV signal may vary only marginally (as in Figure 9), therefore the use of ECD is strongly preferable for a sensitive monitoring.[33] Kinetic studies on the double-helicates were performed by following the time evolution of a ECD signal at fixed wavelength during exchange experiments run at different temperatures. In these experiments, a preformed complex is treated with an equimolar amount of the diamidine antipode, eventually leading to helix racemization (Figure 10). The kinetic curves were analyzed through the typical

Eyring treatment, yielding the rate constants and the various activation energies, and providing a picture of the exchange mechanism. The positive activation entropy for the experiment shown in Figure 10 indicates, for instance, that the exchange reaction takes place via a two-step dissociation-exchange mechanism.[33]



Scheme 9. Top: structures of double-helix 1:1 complexes (**9·10**) between chiral diamidines **10** and achiral dicarboxylic acid **9**, with various linkers (X). Bottom: crystal structure of a diacetylene-linked double helix (X= none). Adapted with permission from ref. 33. Copyright 2012 American Chemical Society.

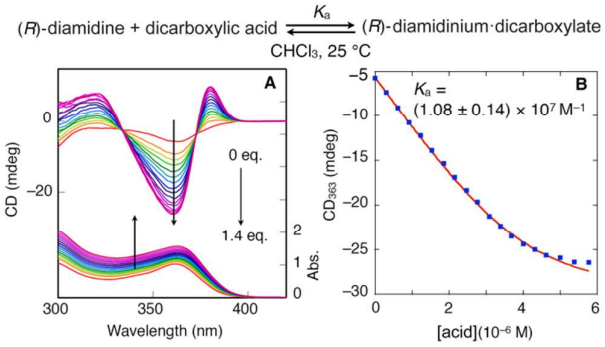


Figure 9. Left: ECD and absorption spectral changes of the chiral diamidine **10** with X = Pt(PPh$_3$)$_2$ upon addition of the dicarboxylic acid **9** with X = Pt(PPh$_3$)$_2$ (μM concentrations in CHCl$_3$, 25 °C). Right: binding isotherm obtained by plotting the ECD intensity at 363 nm versus the dicarboxylic acid concentration. Adapted with permission from ref. 33. Copyright 2012 American Chemical Society.

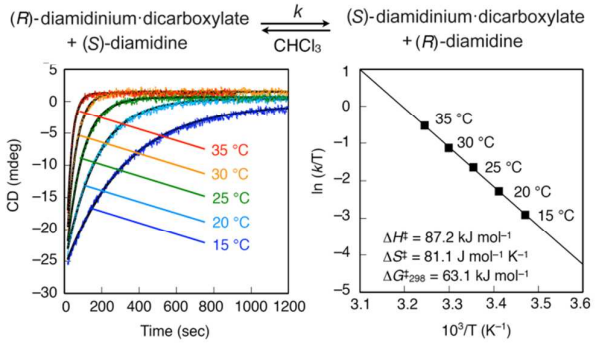
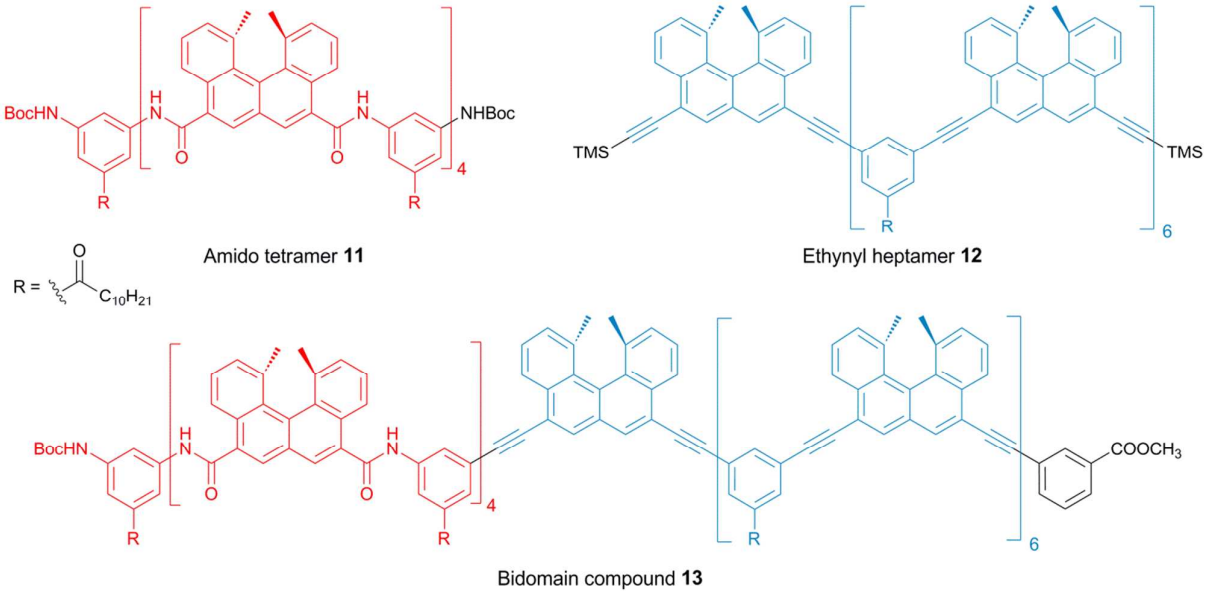


Figure 10. Left: time-dependent CD intensity changes at 355 nm of the equimolar mixtures between chiral diamidine **10** and dicarboxyxlic acid **9** with X = $Pt(PEt_3)_2$ (0.1 μM in $CDCl_3$) at various temperatures. Right: Eyring plot for the exchange rates and estimated kinetic parameters. Adapted with permission from ref. [33]. Copyright 2012 American Chemical Society.

The study of a second helicate system, reported by Yamaguchi and coworkers, demonstrates that ECD spectra may provide useful structural information even by employing the very simple method of spectra summation. The bidomain compound **13** results from the condensation between an amido tetramer **11** and an ethynyl heptamer **12** (Scheme 10).[34] The presence of stereodefinite [4]helicene cores and of the amide moieties facilitates the folding of compounds **11-13** as hydrogen-bonded dimeric helical structures. In fact, the bidomain compound **13** may exist in four different aggregate states (sketched in Figure 11), depending on the solvent: a) random coil; b) thoroughly folded (all-dimer); c) folded only in the amido portion (amido-dimer); c) folded only in the ethynyl portion (ethynyl-dimer). The four states are allied with different ECD spectra. Additionally, each of the two components **11** and **12** may exist as random-coil or folded dimer, also depending on the conditions. Therefore, by summing the ECD spectra of **11** and **12** in either of their two aggregate states, the ECD spectra of the four aggregate states of **13** were reproduced and the specific state adopted by **13** in various conditions was easily predicted.[34]



Scheme 10. Structures of the amido tetramer **11**, the ethynyl heptamer **12**, and the derived bidomain compound **13** reported by Yamaguchi and others.[34]

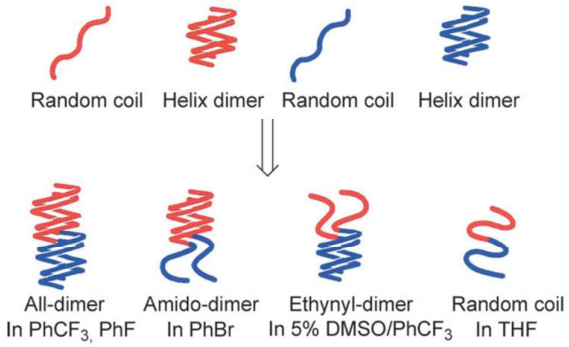


Figure 11. Pictorial representation of the various aggregate states of **11** (red, top) and **12** (blue, top), and of their bidomain conjugate **13** (bottom). Reproduced with permission from ref. 34. Copyright 2012 Wiley-VCH.

II.1.5 – Discrete complexes with higher stoichiometries

In addition to the 1:1, 2:1 and other simple stoichiometry ratios discussed previously, several other chiral supramolecular assemblies with more elaborate stoichiometries but still belonging to the family of discrete species have been reported and characterized by ECD. A notable example is provided by the so-called rosettes described by Reinhoudt and coworkers. A spontaneous assembly

of 3 calix[4]arenes (**14**) and 6 cyanurates or barbiturates (**15**) gives rise to double rosettes with overall 3:6 stoichiometry, held together by a network of 16 hydrogen bonds (Figure 12). If the cyanurate or barbiturate (**15**) component is chiral, the assembly proceeds diastereoselectively forming only one of the two possible *M* or *P* superstructures. ECD has been used to detect the formation of the rosettes and to extract thermodynamic parameters from binding curves.[35]

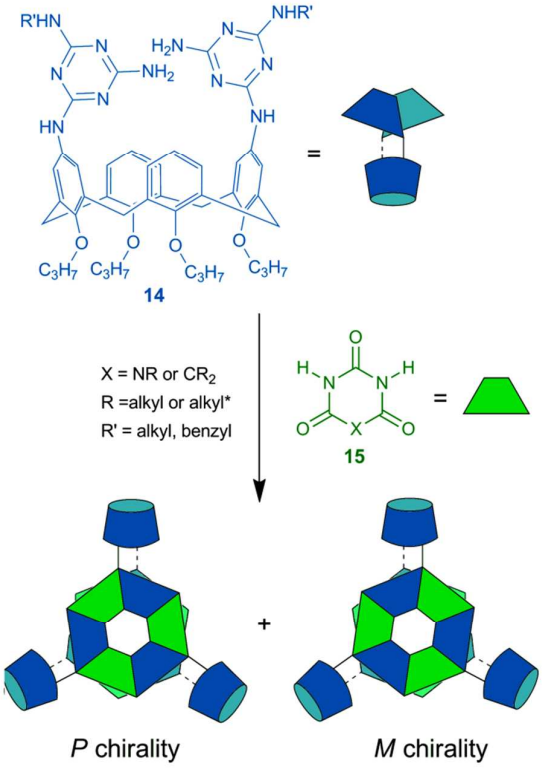
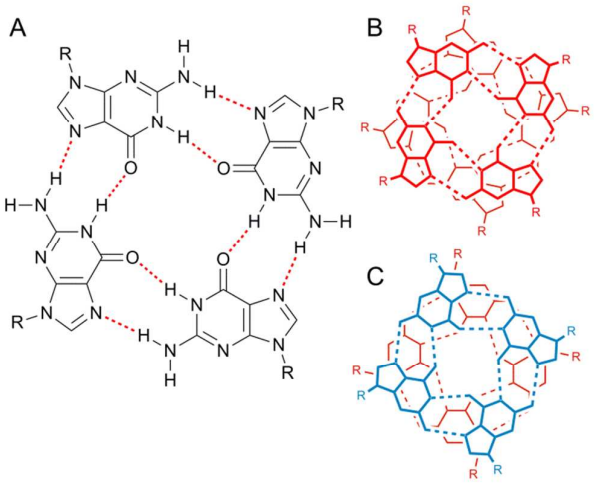


Figure 12. Self-assembly of the rosettes reported by Reinhoudt and coworkers, obtained by mixing 3 calix[4]arenes units (**14**, blue) with 6 cyanurates (X = NR) or barbiturates (X = CR_2) units (**15**, green). If R is chiral, the two enantiomers on the bottom become diastereomers.

Most of the self-assembling systems discussed in the present tutorial review have been designed either with inspiration from Nature or with bio-mimetic purposes. Biomolecules offer in fact several examples of chiral self-assemblies. From the viewpoint of ECD, one of the most interesting cases is offered by four-stranded helical DNA structures. In particular, guanine-rich DNA segments easily form G-quartets held together by 8 hydrogen bonds (Scheme 11A), which stack on the top of each

other generating so-called G-quadruplexes. Depending on the head-to-tail (Scheme 11B) or the head-to-head (Scheme 11C) arrangement between quartets, as well as on the conformation (syn or anti) around the glycosyl bond, three typical motifs of quadruplex DNA's may be obtained (designated as Groups I-III, Figure 13). The application of ECD to the study G-quadruplexes has been extensive, and is summarized in a recent review.[36] In particular, the three groups have distinctive ECD spectra and may be easily distinguished. Moreover, the transitions of the guanine chromophore are well understood, thus the ECD spectra of the quadruplexes have been rationalized in terms of the exciton coupling theory and quantitatively reproduced by coupled oscillator-type calculations.



Scheme 11. A: the G-quartet motif obtained from 4 guanine molecules. B and C: head-to-tail and head-to-head stacks between two G-quartets.

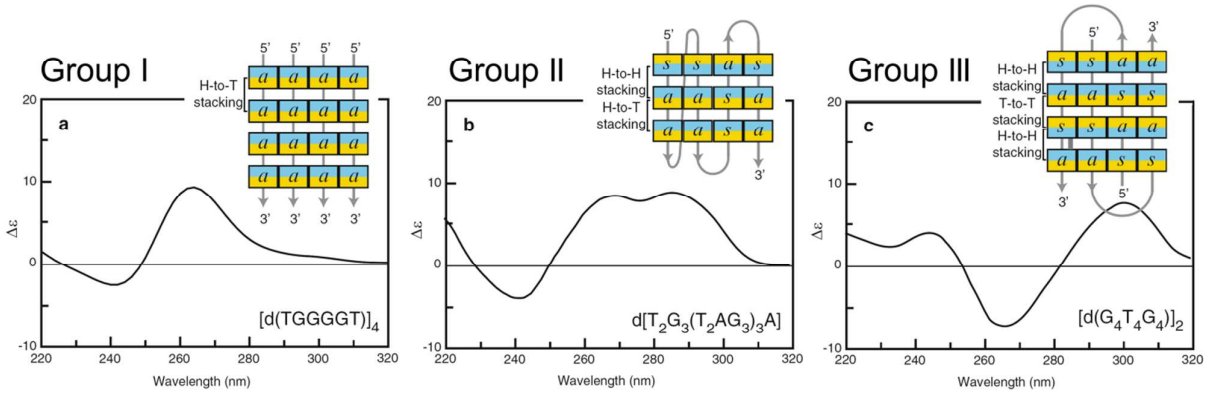


Figure 13. ECD spectra and sketches of the G-quartets arrangement for three oligonucleotids representative of the three main commonly observed "groups" I-III. In the sketches, every guanine is represented by a bi-colored rectangle and the head and the tail faces (as defined in Scheme 11) are blue and yellow, respectively; letters *s* and *a* refer to the syn and anti conformation around the glycosyl bond, respectively; the arrow represent the 5'-to-3' direction of the strand. Reproduced from ref. 36. With kind permission from Springer Science and Business Media.

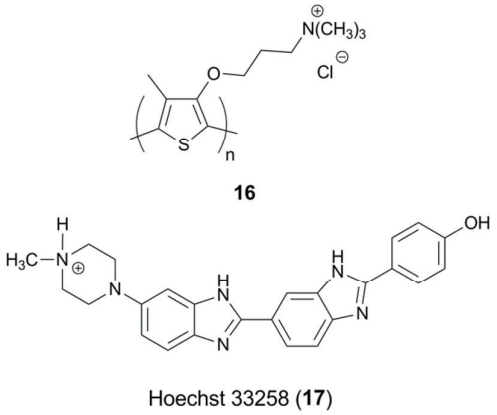
II.2 – Bio(polymer) – substrate complexes (with non-definite stoichiometry)

Here we will discuss two cases with non-definite stoichiometry, involving the interaction of a small molecule guest with a polymeric host. We shall refer to a repetitive polymer, which displays a number of sites that can possibly accommodate the guest, as opposed to a well-folded system like e.g. a protein, with definite clefts and pockets, as discussed in §II.1.3. Let's consider first an achiral polymer, which adopts a helical shape upon interaction with a chiral guest, and secondly a chiral host providing a dissymmetric environment of achiral dye molecules.

II.2.1 – Polymer/inducer systems

Polythiophenes are conjugated polymers with important applications in optoelectronic devices, which strongly depend on conformation and aggregation mode. All polythiophenes have a more or less marked tendency to form helical structures, which, in the absence of symmetry-breaking

interactions, lead to CD-inactive racemic mixtures. The cationic derivative **16** (Scheme 12) is well soluble in methanol, while it aggregates upon adding chloroform as a poor solvent.[37] This is witnessed by a color-change from orange (isolated molecules) to violet (aggregates). In low-polarity condition, i.e. in MeOH/DMSO/$CHCl_3$ 5:5:90 solvent mixture, the presence of a sugar such as galactose is immediately revealed by strong ECD bands in correspondence to the main absorption peaks. A titration with the sugar follows a smooth saturation curve.



Scheme 12. Structure of the water soluble polytiophene **16** and of the DNA grove binder known as Hoechst 33258 (**17**).

A faint inflection point around equimolarity between galactose and the single monomeric unit of **16** suggests that each substituted thiophene binds one sugar molecule. Apparently, the interaction between galactose and **16** is sufficient to impart a prevalent helicity to the polymer, which is selectively detected by ECD. Upon using mixtures of the sugar enantiomers, a perfect linear proportionality between e.e. and ECD intensity is found. Interestingly, other monosaccharides bearing five hydroxyl groups behave similarly (although inducing slightly different ECD spectra), while other substrates with different structures, including fucose and deoxygalactose (only 4 hydroxyls), as well as glycosides fail to induce a prevalent helicity (as witnessed by the lack of ECD). Finally, a puzzling relationship between the sugar optical rotation and the magnitude of induced ECD has been found.[37]

II.2.2 – Nucleic acid/dye systems

DNA minor groove provides a binding site for positively charged molecules, able to form hydrogen bonds with the nucleic acid. Among several dyes with strong affinity to this site, Hoechst 33258 (**17**, Scheme 12) is regarded as a reference compound for the development of DNA minor groove binders.

The complexes of Hoechst 33258 (achiral guest) with selected DNA sequences (chiral host) have been the object of extensive investigations: it is known that at higher guest:host ratios more than one Hoechst 33258 molecule can bind DNA, but the location of the secondary binding site has remained elusive until recently.[38] Several hypotheses have been put forward, as represented in Figure 14.

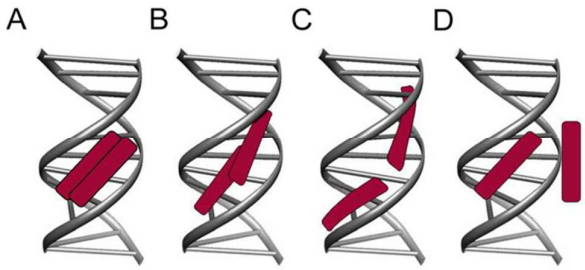


Figure 14. Schematic representation of four hypothetical binding modes of two molecules of Hoechst 33258 (**17**, represented as red bars) in a DNA dodecamer including a central A_4T_4 target. Adapted with permission from ref. 38. Copyright 2013 American Chemical Society.

Evidence of multiple binding comes from the ECD titration of A_4T_4 with Hoechst 33258 (**17**), where the lack of isodichroic points is observed together with a red-shift of the longer wavelength band associated with the dye transitions. The first model (Figure 14A) is strongly suggestive of a H-stack: this motif is common in cyanine dyes/DNA interactions, as justified by the π–π pairing of the guest molecules with extended electron delocalization. The second one (B) recalls a J-stack and similarly to the previous one should pile the two dye molecules in close vicinity to one another. The absence of the typical spectroscopic signatures (in the UV spectrum) of these stacked modes and of a strong ECD couplet allows one to dismiss both of them. The contiguous binding of the two dye

molecules (head to tail) depicted in Figure 14C fits the expected trend of the ECD spectrum: a steadily growing positive low-energy band due to the non-degenerate exciton coupling between the electronic transitions of Hoechst 33258 (**17**) and those of the nucleobases. This increase of rotational strength reaches saturation at 1:2 DNA/dye mole ratio. Above this point, a further spectral evolution with the rise of a shoulder at 330 nm is taken as proof of a non specific, external binding as suggested in model D.

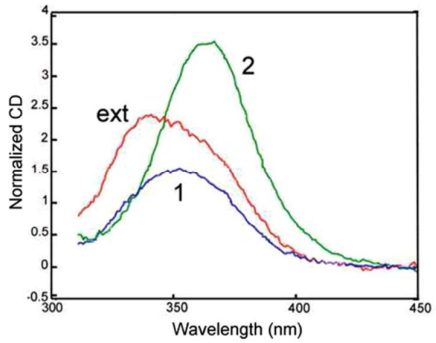


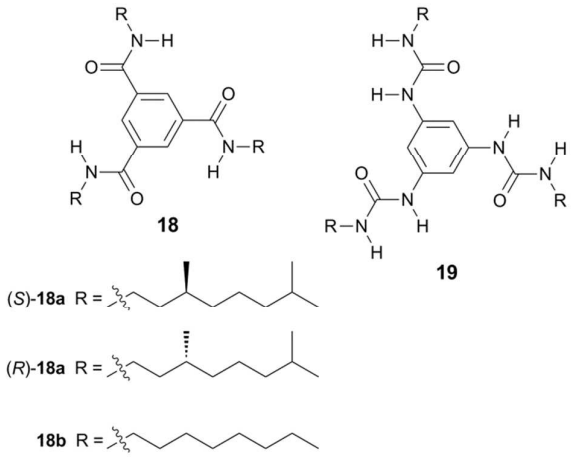
Figure 15. Reconstruction of the ECD spectra of one bound dye molecule (curve 1, violet), two bound molecules (curve 2, blue-green), a further externally bonded dye molecule (red curve, marked "ext"), obtained through a singular value decomposition (SVD) procedure, for the complexes between A_4T_4 with Hoechst 33258 (**17**). Adapted with permission from ref. 38. Copyright 2013 American Chemical Society.

The ECD spectrum measured on the whole significant spectral range at variable host/guest mole ratios were input into a singular value decomposition (SVD) procedure. This allowed the authors to reconstruct the individual spectra for the various situation of Hoechst 33258/DNA binding (Figure 15). The study underscored the sensitivity of ECD to the various modes and to the reciprocal orientation of the two molecules.[38]

II.3 – Infinite assemblies

II.3.1 – Self-assembled chiral molecules

Closely connected with the formation of infinite assemblies is the necessity of reaching a long-range supramolecular order through a cooperative self-assembly process, which in turns depends on the possibility of establishing multiple and directional non-covalent interactions, in particular hydrogen bonding and π-π interactions. The almost ideal candidate for attaining a large assembly is a discotic molecule containing a central aromatic core and various pendant groups which both introduce chirality and provide hydrogen-bonding sites. Meijer and coworkers have designed several systems of this kind based on the C_3-symmetrical benzene-1,3,5-tricarboxyamide and trisurea skeletons (respectively **18** and **19**, Scheme 13), which yield long, columnar and helical stacks as depicted in Figure 16. The discussion of the following examples will help understanding most of the possible applications and potentiality of ECD in related contexts.



Scheme 13. Structures of the C_3-symmetrical benzene-1,3,5-tricarboxyamide and trisurea discotic compounds (**18** and **19**) designed by Meijer and coworkers.

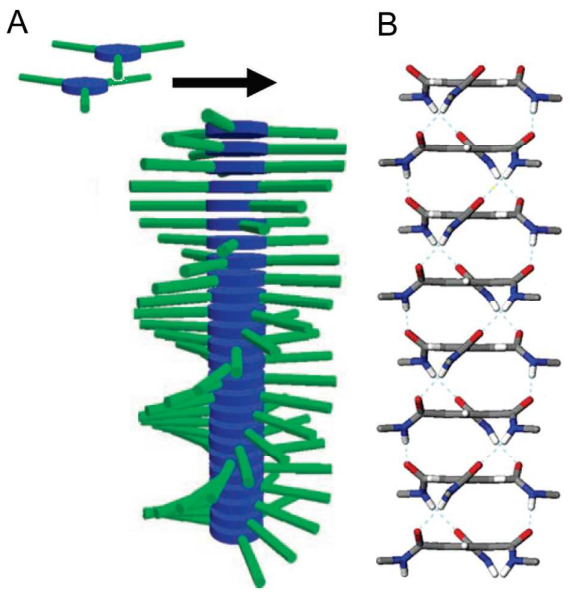


Figure 16. A: representation of the helical stack formed by self-assembly of (*R*)-**18a**. B: arrangement of the amide groups in the stack (alkyl side chains are omitted for clarity). Adapted with permission from refs. 39, 40. Copyright 2008 and 2010 American Chemical Society.

The isolated molecules of (*R*)-**18a** are practically ECD-silent. When, however, conditions are set up to promote aggregation (solvent favoring π-stacking and H-bonding, sufficient concentration, low temperature), two things happen: a) the UV maximum at 198 nm (aromatic ^1B_b transition) undergoes a hypsochromic shift indicative of a face-to-face (H-type) aggregate; b) a positive ECD signal appears with maximum at 223 nm, indicative of a right-handed (or *P*) helical columnar aggregate (as discussed in §I.4.2.2, the ECD must be due to chromophore distortion). Both effects are temperature-dependent, and by plotting the UV or ECD intensities as a function of the temperature for various concentrations ($>10^{-5}$ M), the authors obtained a series of melting curves which are clearly not sigmoidal, indicating a cooperative process. The binding isotherms were used to fit a theoretical model of the self-assembly, and to assign a nucleation-growth pathway to the aggregation process. This is similar to the aggregation mechanism of many proteins, and is characterized by a preliminary slow formation of nuclei of a critical size, followed by a fast elongation process. Among other things, the fitting furnished the temperature T_e at which the

elongation starts, as well as average stack lengths at the start and the end of the elongation (about 100 and 10'000 units, respectively).[40] The dependence of ECD intensity on the enantiomeric excess of **5** shows a distinct trend which indicates an amplification phenomenon. In fact, on decreasing the e.e. from 100% to 0% (that is, by measuring mixtures of (*R*)-**18a** and (*S*)-**18a** in various ratio), the initially positive ECD intensity remains constant until 40% e.e. is reached. In this range, the major enantiomer keeps control of the overall helicity as if no opposite enantiomer was present (majority-rule effect). Only below 40%, the ECD starts decreasing and rapidly drops to zero. In these conditions, the plot of ECD intensity vs. e.e. is obviously non-linear, and its fitting can be used to measure thermodynamic quantities such as the helix reversal penalty (HRP) and the mismatch penalty (MMP). These are the free energies penalizing, respectively, a helix reversal in a stack, and a mismatch due to the introduction of a chiral monomer in the "wrong" stack.[39] The effect of mixing between the chiral compound (*R*)-**18a** with its achiral analog **18b** will be discussed in the next paragraph (§II.3.2).

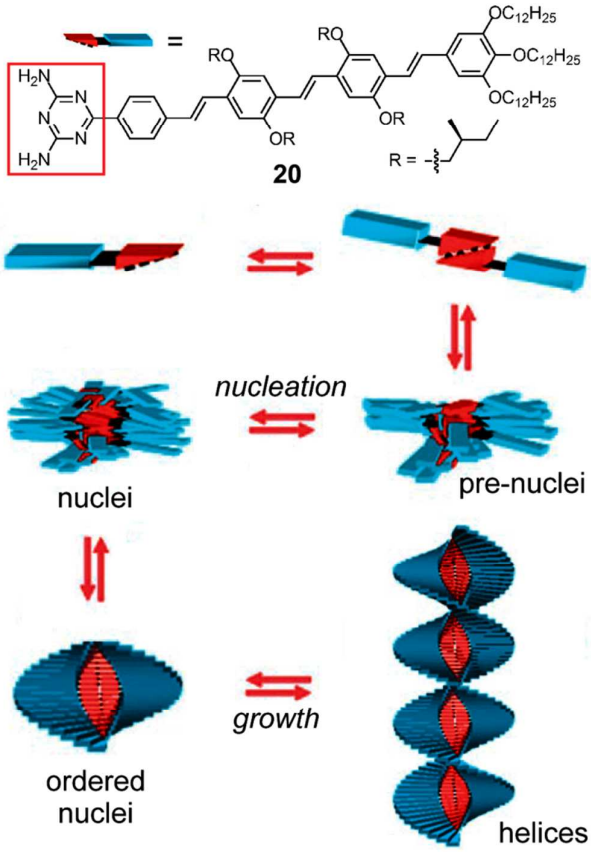
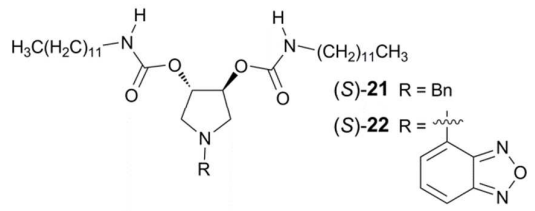


Figure 17. Schematic represenation of the hierarchical self assembly process of the oligo(p-phenylenevinylene) **20** in dodecane. Blue blocks represent the phenylenevinylene backbone and red wedges the hydrogen-bonding end groups. Reproduced with permission from ref. 41. Copyright 2007 Wiley-VCH.

In the case of compound **18a**, the melting curves obtained by following both ECD and UV signals were comparable and led to similar fittings. In general, the observation that UV and ECD are sensitive to the aggregate formation in a consistent way is not trivial. In another case described by Meijer and coworkers, the oligo(*p*-phenylenevinylene) **20** forms H-bonded dimers which undergo a step-wise aggregation mediated by π-stacking, leading eventually to chiral columnar stacks (Figure 17). The onset of the ECD signal occurs in the intermediate aggregation stage (nucleation), while the UV signals are also affected in the early stages (pre-nuclei formation). Therefore, ECD and UV

binding isotherms are different and are fitted by different thermodynamic parameters, providing selective information on the aggregation process.[42]

When molecules forming helical columnar stacks are appended with long alkyl chains, there is a good chance for these systems to entrap solvent molecules and show gelling properties. An efficient scaffold for organogelators is *N*-aryl-3,4-biscarbamate pyrrolidine, which is capable of multiple H-bonding and π-type interactions. For example, both compounds **21** and **22** form stable gels in many organic solvents (Scheme 14).[43] Dilute solutions of **21** and **22** show very weak ECD spectra, and only the gelification (attained at higher concentrations or lower temperatures) switches the ECD signal on. Similarly to Meijer's compounds just discussed, the formation of aggregates of almost ECD-silent monomers is responsible for the appearance of a relatively stronger ECD signal, which is then modulated by the specific conditions. Thus, both the appearance of the gel and its thermal stability are easily documented by ECD. Additionally, ECD offers structural information on the supramolecular species. The ECD spectrum of the gels of (*S,S*)-**22** in cyclohexane shows two strong positive ECD couplets in the 250-550 nm range (Figure 18), which are allied to the exciton coupling of two distinct π-π* transitions of furazane chromophore. The case called for the use of coupled-oscillator DeVoe calculations, a method especially well suited to calculate exciton-coupled ECD spectra of multichromophoric species.[8] Using a structure for the 24-mer of (*S,S*)-**22** generated by means of molecular-mechanics (MM) (Figure 19), DeVoe calculations reproduced the experimental ECD spectrum very satisfactorily. In this example, ECD calculations offered a quantitative proof of a MM-generated structure of the supramolecular aggregate, and in particular of the predicted right-handed helicity.



Scheme 14. Structures of the pyrrolidine-based organogelators **21** and **22**.

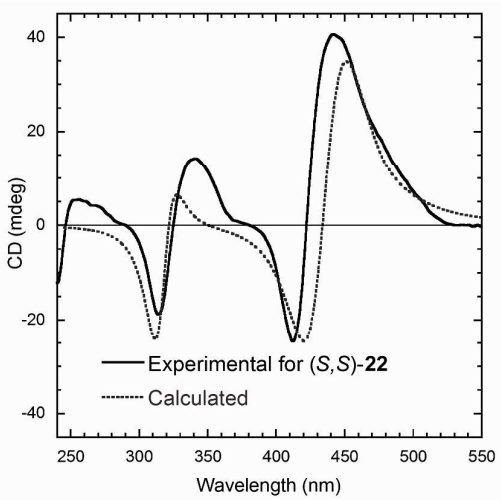


Figure 18. Experimental and calculated ECD spectra for the stable gel obtained from gelator **22** in cyclohexane. The calculated spectrum was obtained by DeVoe coupled-oscillator calculations using the MM structure shown in Figure 19. Reproduced with permission from ref. 43. Copyright 2011 Wiley-Liss.

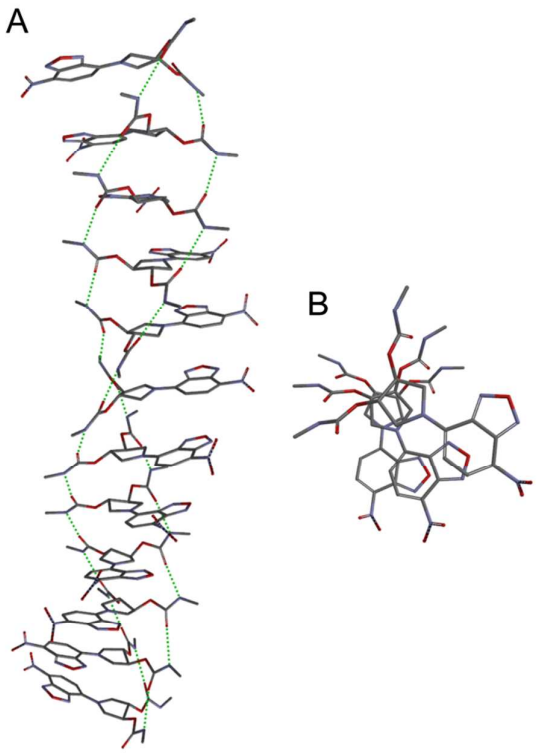


Figure 19. A: MM optimized structure for the 24-mer of (*S,S*)-**22**. B: view of three successive units highlighting the π-stacking interactions. Alkyl chains truncated for clarity; hydrogen bonds shown with green dotted lines. Reproduced with permission from ref. 43. Copyright 2011 Wiley-Liss.

Perhaps the most complex case that falls in the category of infinite supramolecular systems are the aggregates of conjugated polymers. In some sense, they present an extra dimension with respect to the systems so far considered: a monomer is first covalently polymerized in the first dimension, then the various polymeric chains aggregate in the second (and third) dimension. Conjugated polymers find many important applications as functional materials (solar cells, LEDs, sensors, transistors and so on), where they are employed in the solid state. These aggregates are also easily formed in solution, typically, by employing mixtures of good and poor solvents (solvent/non-solvent) at sufficiently high concentrations. Since it is believed that solution aggregates mimic solid-state ones, the study of the solution is helpful to get insight into the structure of the solid state. This is made possible by means of ECD if the polymers are chiral. For example, the two poly(phenyleneethynylene)s (PPE's) **23** and **24** appended with glucose units, are completely soluble in chloroform (a good solvent) at 10^{-4} M concentrations (Figure 20).[44] In these conditions, the two PPE's show the typical strong and broad absorption band around 500 nm, due to the main π-π* transition of the conjugated core. The corresponding ECD spectra are however very weak (dissymmetry factor $g = 10^{-4}$ for **23** and $3 \cdot 10^{-5}$ for **24**) because of the small perturbation exerted by the glucose units on the very large chromophore. When methanol (a poor solvent) is progressively added, both PPE's undergo aggregation, which manifests differently in the two cases. For the copolymer **24**, in fact, a typical aggregation band arises in the absorption spectrum at 470 nm; for the homopolymer **23** the UV spectrum changes less. In both cases, however, a strong and very structured ECD spectrum is observed which attains a maximum of $g = 2 \cdot 10^{-3}$ for **23** and 10^{-2} for **24** (≈300-fold increase). Thus, ECD is capable of detecting the formation of the aggregates with much larger sensitivity than UV, and of revealing the first steps of aggregation more efficiently. The

aggregate ECD spectra are due to a combination of intrinsic chirality of the chains and exciton coupling between adjacent polymer chains in the aggregates. Moreover, they are likely to be affected by vibronic factors, making a complete simulation of these spectra very complicate.[44]

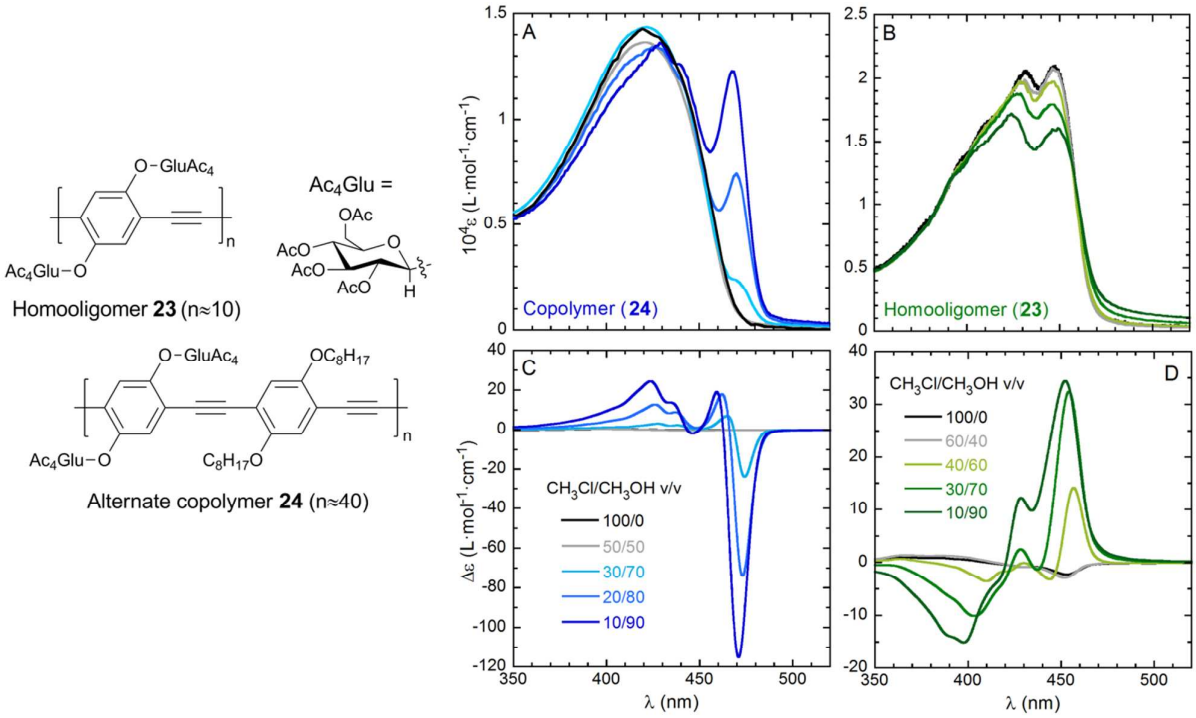


Figure 20. Left: structures of the two poly(aryleneethynylene)s **23** (a homooligomer) and **24** (a copolymer). Right: evolution of the absorption (A,B) and ECD spectra (C,D) of **23** and **24** upon addition of a "poor" solvent (methanol) to their solutions in $CHCl_3$, which promotes aggregation. Adapted with permission from ref. 44. Copyright 2012 American Chemical Society.

II.3.2 – Achiral molecules assembled with a chiral inducer

One of the most interesting properties of the mixtures between chiral and achiral molecules is the observation of chirality transfer and amplification, which are fundamental concepts in all processes involving chiral recognition, including enantioselective synthesis and enantiomer separation. Similar phenomena occur also in the field of covalent polymers, and by extension, in the field of dynamic structures of molecular aggregates.[41] When a chiroptical signal associated with a certain molecule in a mixture is observed, normally this signal is directly proportional to the content of the

chiral species. If, on the contrary, the chiroptical signal is stronger than expected, chirality has been partially transferred from one chiral species to its neighbors and, possibly, amplified.

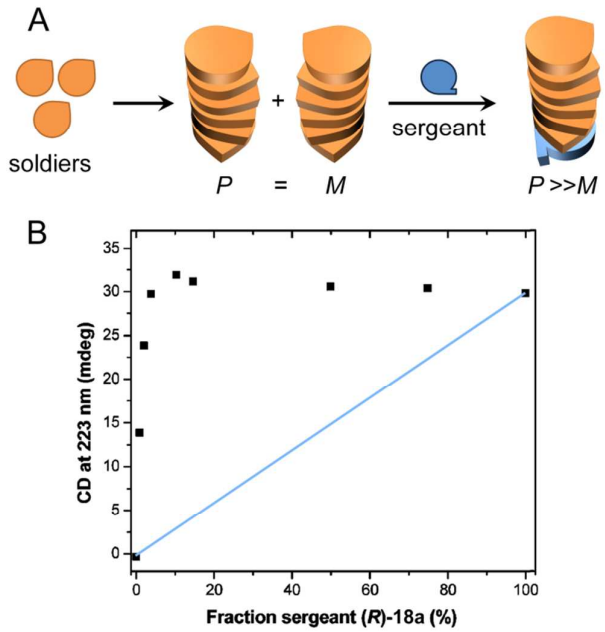


Figure 21. A: schematic representation of the sergeant-and-soldiers principle between structurally related achiral soldiers and chiral sergeant, such as **18b** and **18a**, respectively (see Scheme 13). B: evolution of the ECD signal at 223 nm for a solution of **18b** in heptane (20 μM) upon addition of (*R*)-**18a**. The blue line represents the ideal trend in the absence of any amplification phenomenon. Adapted with permission from ref. 40. Copyright 2008 American Chemical Society.

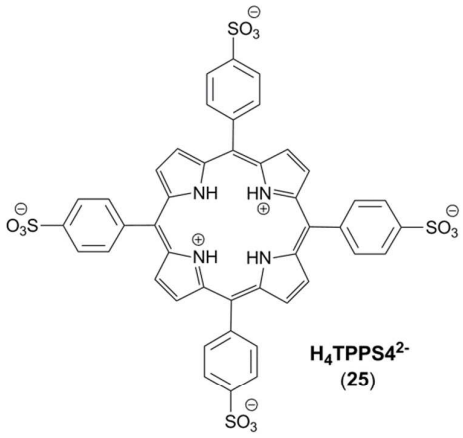
Chirality amplification is most often observed between structurally similar chiral and achiral species. In the earlier discussion of C_3-symmetric discotic molecules developed by Meijer and coworkers (§II.3.1 and Scheme 13), the aggregation mechanism was found to be similar for (*R*)-**18a** (by ECD and UV experiments) and for its achiral analog **18b** (by UV only).[40] This result indicates that, similarly to (*R*)-**18a**, compound **18b** also affords helical columnar stacks, with an equal proportion of *M* and *P* helices. The similarity requirement is important in order to observe a chirality amplification phenomenon known as sergeant-and-soldiers effect. When a small amount of (*R*)-**18a** (the "sergeant") is added to **18b** (the "soldiers"), a ECD signal is obtained similar to that

observed for (*R*)-**18a** alone. Very interestingly, as low as 4% of (*R*)-**18a** is sufficient to recover the full ECD intensity observed for enantiopure (*R*)-**18a** (Figure 21). The chiral dopant therefore acts as a sergeant able to keep control of many soldiers.[41] Contrarily to covalent polymers, in dynamic supramolecular systems the sergeant-and-soldiers effect is strongly temperature dependent. The ECD intensity of mixtures (*R*)-**18a** and **18b** decreases when raising the temperature, implying that a larger fraction of sergeant is necessary to reach a saturation of the ECD signal.

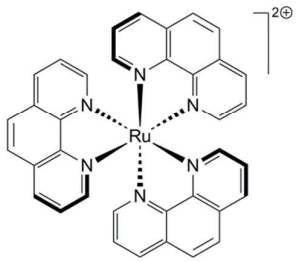
The dynamic nature of the system is also a necessary prerequisite for observing the sergeant-and-soldiers effect as depicted in Figure 21A. In normal situations, when the sergeant (or, more in general, the chiral dopant) is removed, it is expected that the soldiers will break the ranks and restore a ECD-silent equal mixture of *M* and *P* helices. In some situations however, some chiral fragments survive and retain the memory of chirality even after removal of the dopant, and thus are able to transfer their chirality to newly formed aggregates. Such an effect is referred to as *chiral memory*.[45]

Among the systems more prone to exhibit chiral memory are porphyrins. *Meso*-tetraaryl porphyrins appended with suitable functional groups have a strong tendency toward aggregation. This is favored in particular by the co-presence of a positive core (following e.g. protonation or metalation) and a negative periphery (due e.g. to donor atoms, such as pyiridine nitrogen, or negatively charged substituents, such as sulfonates). For example, the water-soluble *meso*-tetrakis(4-sulfonatophenyl)porphine (H_2TPPS4^{4-}, Scheme 15) is zwitterionic in its bis-protonated form H_4TPPS4^{2-} (**25**, at pH<3) and forms both H- and J-aggregates easily detected in the absorption spectrum. In fact, while H_2TPPS4^{4-} has a maximum at 436 nm, the H-aggregates of **25** show the characteristic blue-shifted signature (422 nm), and J-aggregates the characteristic red-shifted one (490 nm).[46] Aggregates of achiral porphyrins are of course ECD-silent. However, if an equimolar amount of the chiral propeller-shaped compound ruthenium(II) tris-(1,10-phenantroline) ($Ru(phen)_3^{2+}$, Δ-**26**, Scheme 15) is added, it transfers chirality to the porphyrin aggregates. In fact, strong ECD signals are observed not only in the phenanthrene (phen) ligand region between 250-

300 nm, but also in correspondence of porphyrin aggregate bands (Figure 22A). Very interestingly, such aggregates exhibit chiral memory. In fact, when pH is raised to 6, the porphyrin core is neutralized and ECD disappears. However, going back to pH 2.5, the aggregate ECD is entirely restored (Figure 22A). Apparently, at pH 6 small aggregated chiral seeds survive which are able to transfer their chirality to the restored aggregate, acting as refolding catalysts. Such ability is so pronounced that even when the opposite Λ-Ru(phen)$_3^{2+}$ (Λ-**26**) enantiomer is added in excess during the cycle, the handedness of the J-aggregates remains the same originally induced by Δ-Ru(phen)$_3^{2+}$ (Δ-**26**). In fact, addition of Λ-Ru(phen)$_3^{2+}$ causes a sign inversion in the phen ECD region but not in the J-aggregate one (Figure 22B) due to chiral memory effect. Here, as in a similar porphyrin system discussed below (§II.3.4), the true nature of the aggregate species is still unknown. Therefore, no clear connection is established between the aggregate geometry and the observed ECD couplets due to the exciton coupling of π-π* Soret transitions.



H_4TPPS4^{2-} (25)



Δ-Ru(phen)$_3^{2+}$ (Δ-26)

Scheme 15. Water-soluble *meso*-tetrakis(4-sulfonatophenyl)porphine in its protonated form (H_4TPPS4^{4-}, **25**) and ruthenium(II) tris-(1,10-phenantroline) $(Ru(phen)_3^{2+}$ (Δ-**26**) used in chiral memory experiments by Purrello and coworkers.

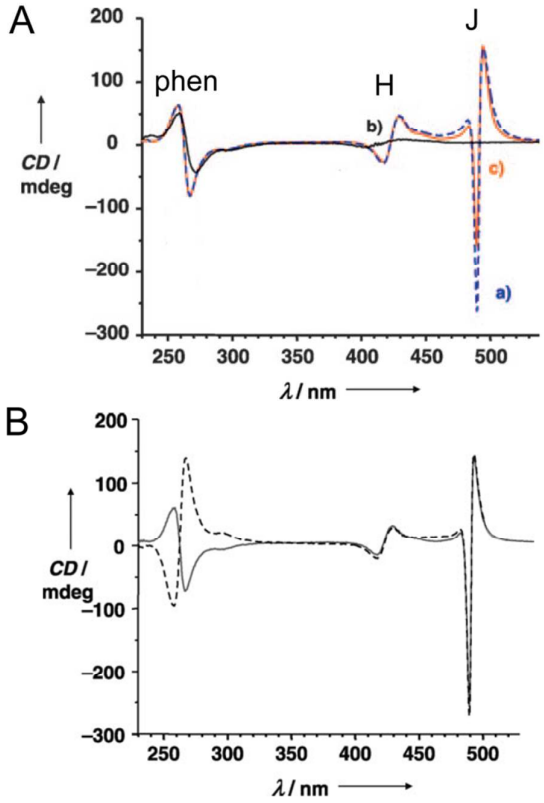
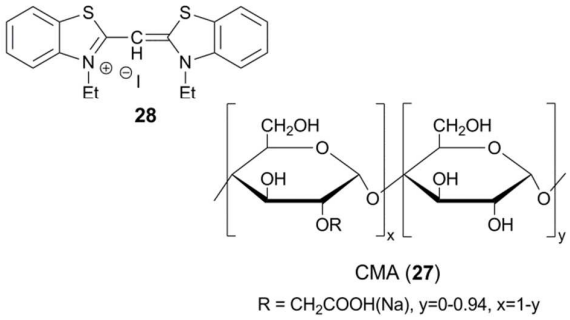


Figure 22. A: ECD spectra of an equimolar mixture of porphyrin H_4TPPS4^{4-} **25** and Δ-$[Ru(phen)_3]^{2+}$ Δ-**26** (10 μM, water) recorded: a) initially at pH 2.5 (dashed blue curve), b) then at pH 6.0 (black curve), and c) again at pH 2.5 (red curve), exhibiting chiral memory. The regions corresponding to the signals of H and J porphyrin aggregates, and of the chiral Ru complex (phen chromophore), are indicated. B: evolution of the ECD trace a) in panel A before (solid line) and after (dashed line) the addition of an excess Δ-$[Ru(phen)_3]^{2+}$ (10 μM). Notice the ECD in the phen region is reversed, while that in Soret region is not. Reproduced with permission from ref. 46. Copyright 2008 Wiley-VCH.

II.3.3 – Achiral system assembled on biomolecular templates

In this example we will discuss the interaction of a chiral biopolymer, carboxymethylated amylose (CMA, **27**), with an achiral dye molecule like the cyanine **28** in Scheme 16.[47] In its free form, CMA (**27**) exists in a random coil conformation, but when it is used as a chiral polymer template for aggregation it undergoes a conformational change into a super-helical structure. In this case the experimental ECD profile appears very informative both of the structural changes upon complexation of the chiral template and of the aggregation mode of the cyanine.



Scheme 16. Cyanine (**28**) and carboxymethylated amylose (CMA, **27**) studied by Kim et. al.[47]

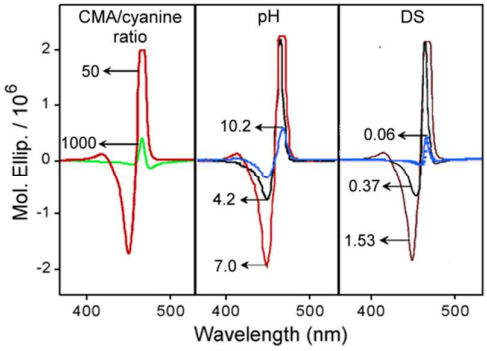


Figure 23. Dependence of ECD spectra (cyanine absorption region) of mixtures of cyanine **28** and CMA (**27**) in water in different conditions. Cyanine concentration 10 μM. Left: DS = 1.53, pH=7, at various CMA/cyanine molar ratios; middle: DS = 1.53, CMA concentration 0.5 mM, at various pH; right: CMA concentration 0.5 mM, pH=7, at various DS. DS is the degree of substitution of carboxylation. Adapted with permission from ref. 47. Copyright 2005 American Chemical Society.

First, the achiral cyanine guest **28** can form J-aggregates by itself in absence of CMA (**27**), and as expected, they are CD silent. Second, the aggregation of cyanine dye in presence of CMA, as revealed by the experimental ECD, depends strongly not only on several factors such as the CMA/dye ratio and the pH of the solution, but importantly, also on the degree of anionic loading and carboxylic degree of substitution (DS) of CMA polymer template (Figure 23).

When CMA (**27**) is mixed with cyanine **28** in a molar excess of 10 to 50, a supramolecular helical complex is born evidenced by a very intense bisignate ECD within the cyanine UV-vis absorption. The magnitude of this exciton-split ECD intensifies with the increase of DS value and pH of the solution (Figure 23). At neutral pH=7 the maximum ECD intensity is reached, as a proof that in these conditions the random coil polymer template is transformed into helix, and the achiral cyanine dye has become the most J-aggregated. The fact that the ECD maximum intensity is reached by increasing the DS value from 0.06 to 1.53, brings to mind the critical role of amylose helix twisting power on the J-aggregation process. ECD data unequivocally prove that the binding of the J-aggregates onto CMA (**27**) template is stereochemically controlled by the asymmetric environment. Once again the dependence of ECD on the structural and environmental conditions is demonstrated by the fact that at very large CMA concentration, whether the dye exists as a monomer or as a H-aggregate at pH>9, no ECD has been observed. Interestingly, the ECD response upon the cyanine J-aggregation and biopolymer template helix formation corroborate AFM and fluorescence data depicting the cyanine dye/CMA complex as a more rigid super-helix.[47]

II.3.4 – Spontaneously-resolved chiral aggregates from achiral compounds

It is a common sense that optical activity cannot be created from achiral species without the intervention of some chiral entities. Spontaneous resolution and related symmetry-breaking phenomena are extremely interesting because of their obvious connection with biological homochirality. Among this kind of phenomena, the most intriguing one concerns the chirality induction promoted by chiral flows such as stirring vortexes. The topic has been recently reviewed

by Ribó and coworkers and we will give only one example here.[48] Because of their intrinsic properties already discussed in §II.3.2, zwitterionic porphyrins are the most studied compounds in the field. Above we have seen that H_4TPPS4^{2-} (**25**, Scheme 15) has a strong tendency to form J-aggregates in solution. Apart from the use of chiral dopants such as $Ru(phen)_3^{2+}$ (**26**), chirality may be induced in these aggregates by stirring. In fact, when aggregates are allowed to form under the action of clockwise (CW) or counter-clockwise (CCW) stirring, ECD couplets of opposite signs are observed in J-aggregate region (Figure 24).[49] Other experimental evidence suggests that even in the absence of vortexes the J-aggregates adopt a helical supramolecular structure, which is biased toward one specific handedness by stirring. Moreover, the aggregates easily stack on the cuvette walls, allowing for a mechanical separation of the enantiomers: the enantiomer favored by stirring is deposited on the cuvette wall (Figure 24), the other one remains in solution. Despite the great interest in this kind of systems, their true nature has remained unknown so far, therefore the application of ECD spectroscopy is limited to detecting the chiral species and distinguishing their handedness. However, recent findings point to very large supramolecular architectures with rather elusive chirality.[50]

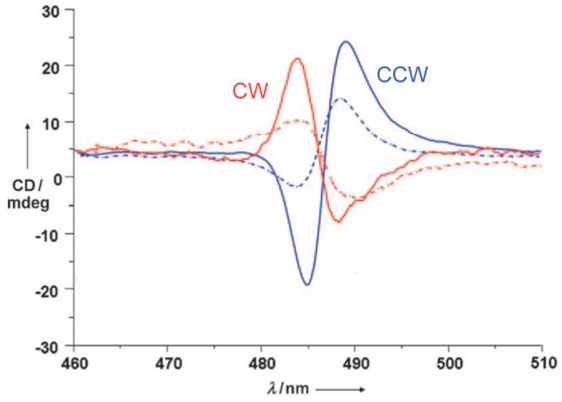


Figure 24. Continuous lines: ECD spectra in the J-aggregate Soret region of standing solutions of H_4TPPS4^{2-} **25** (10 μM in water, pH=3, NaCl 0.3 M) soon after clockwise (CW) and counter-clockwise (CCW) stirring. Dashed lines: ECD signal recorded on the empty cuvettes after removal of previous solutions. Reproduced with permission from ref. 49. Copyright 2010 Wiley-VCH.

CONCLUSIONS AND PERSPECTIVES

Chiral supramolecular species may originate from a variety of situations: we can have 1:1 host-guest complexes with one of the partners being chiral; discrete assemblies with one or more chiral components; aggregates of more or less defined size of achiral molecules put together on a chiral scaffold or template; large or even "infinite" self-assemblies of chiral molecules, assemblies of achiral molecules with chirality induced by a chiral seed or external chiral sources. Whatever the origin of supramolecular chirality and the nature of the intermolecular forces, the electronic circular dichroism (ECD) signals associated with the supramolecular species will be in many cases very different from the ones of the isolated components. In the extreme (but quite common) case, the supramolecular aggregate or complex will show ECD bands in some spectral regions where all the components are ECD-silent. Such *selectivity* and *specificity* of ECD response to supramolecular species often translate into an increased detection *sensitivity* since the signal is recorded against a zero or negligible background. This observation justifies the broad application of ECD as an analytical tool for detecting the formation of supramolecular aggregates and complexes and determining their stoichiometries and binding parameters. Additionally, ECD has the advantage that structure/spectra correlations are possible by many means, from simple exciton chirality method to full calculations based on modern quantum-mechanics methods. Thus, ECD provides unique opportunities to predict the structure of chiral supramolecular aggregates at various levels of detail, from the absolute sense of twist of helical superstructures to detailed information about intermolecular arrangements. While the analytical applications of ECD are well established, its use for quantitative structural predictions still offers much room to expand, thanks to the development of computational tools and of computer technology. These latter aspects will be especially crucial in the field of supramolecular chirality, where the relevant chemical objects are often very large and still represent a formidable task for quantum-mechanics calculations.

Abbreviations

AC	Achiral and Chromophoric (molecule)
AC/NC	Complex between an AC and a CN molecule
AGP	α_1-Acid GlycoProtein
CD	Circular Dichroism
CMA	CarboxyMethylated Amylose
CN	Chiral & Non-chromophoric (molecule)
CyD	Cyclodextrin
DS	Degree of Substitution
ECD	Electronic Circular Dichroism
G	Guest (molecule)
G-quartet	Guanine Quartet
G-quadruplex	Guanine Quadruplex
H	Host (molecule)
H-bond	Hydrogen Bond
HSA	Human Serum Albumin
ICD	Induced Circular Dichroism
M/*P* helix	Left-handed (*M*) or Right-handed (*P*) Helix
MM	Molecular Mechanics
PPE	Poly(PhenyleneEthynylene)
$Ru(phen)_3$	Ruthenium tris-(1,10-phenantroline)
TPPS4	*Meso*-Tetrakis(4-SulfonatoPhenyl)Porphine
UV	UltraViolet (range); also used as synonym for absorption spectroscopy in the UV
UV-Vis	UltraViolet-Visible (range)
VCD	Vibrational Circular Dichroism

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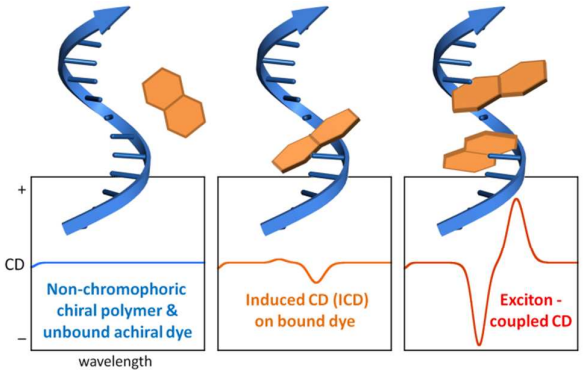
Table of contents entry

Electronic circular dichroism (ECD) is a choice technique in the analysis of chiral supramolecular systems, including their detection, determination of thermodynamic and kinetic quantities, and structural elucidation.



Biosketches

Gennaro Pescitelli received his BSc and PhD (2001) degrees in Chemistry from University of Pisa under the supervision of Piero Salvadori and Lorenzo Di Bari, and spent a postdoctoral fellowship at Columbia University in the group of Koji Nakanishi and Nina Berova. He was appointed "Ricercatore" (Lecturer) at the University of Pisa in 2006, and in 2013 he obtained the Italian national qualification as associate professor in organic chemistry. He is co-author of about 110 publications including reviews and book chapters. His research is focused on spectroscopic and computational investigations of chiral organic molecules, especially natural products, rhodopsin-like proteins, metal-based catalysts, organic crystals, organo-gelators and functional polymers.

Lorenzo Di Bari received his BSc and PhD in Chemistry from the University of Pisa and the Scuola Normale Superiore, under the supervision of C.A. Veracini. He spent a few years abroad, working with G. Bodenhausen (Lausanne, CH), J. Kowalewski and M.H. Levitt (Stockholm, SE), primarily developing NMR tools for conformational analysis of organic compounds. He returned to Pisa in 1992, where he started to work on Electronic Circular Dichroism and a long collaboration with P. Salvadori. He is mainly interested in the stereochemistry of complex systems, like flexible molecules existing as conformational manifolds, supramolecular systems, fluxional coordination compounds. His most recent activity focuses on chiral molecules for organic optoelectronic devices. He enjoys collaborating with people in Italy and abroad. He supervised many BSC and PhD theses in Chemistry and most of his former group members are now developing their independent academic careers worldwide.

Nina Berova is a Research Professor at Columbia University, New York. She received a PhD on organic chemistry and stereochemistry at Univ. Sofia, Bulgaria, and carried out there before joining Columbia in 1988 a teaching and research, as well as extensive collaborative studies on chiroptical spectroscopy with Prof. G. Snatzke at Ruhr University, Bochum (Germany). Her research is focused on stereochemistry and structural analysis, in particular, by chiroptical methods. She has been recipient of many scholarships and visiting professorship from England, Germany, France, Italy, Spain and Japan, and several awards specifically for her studies on chirality. Since 1998-present she is a co-Editor of Journal "Chirality. She was co-editor and co-author of two monographs "Circular Dichroism: Principles and Applications" (1994) & 2000), and of two-volume monograph "Comprehensive Chiroptical Spectroscopy" (2012) by John Wiley & Sons.

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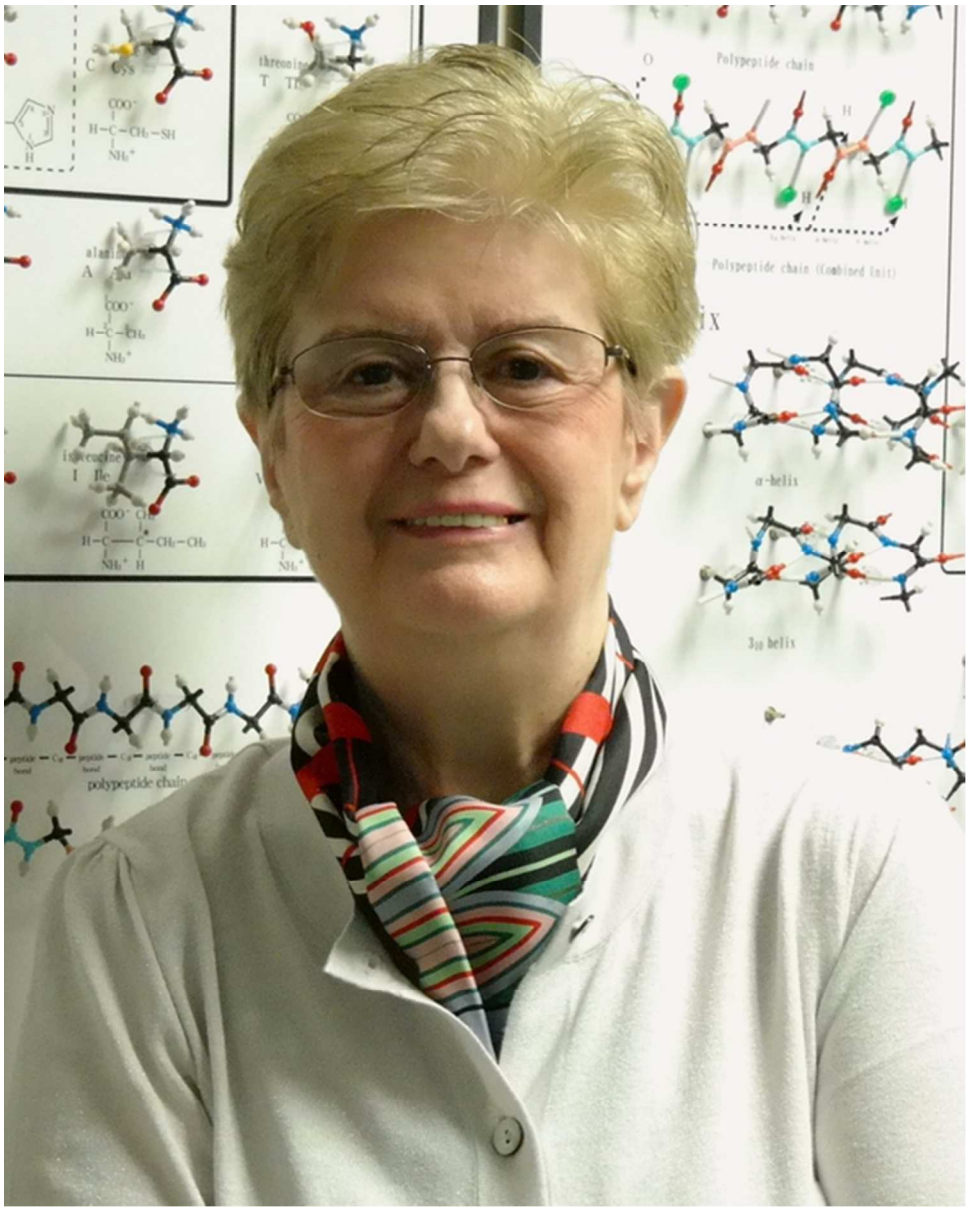

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