Host–Guest Chemistry

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# Noncovalent Chirality Sensing Ensembles for the Detection and Reaction Monitoring of Amino Acids, Peptides, Proteins, and Aromatic Drugs**

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***Abstract:*** *Ternary complexes between the macrocyclic host cucurbit[8]uril, dicationic dyes, and chiral aromatic analytes afford strong induced circular dichroism (ICD) signals in the near-UV and visible regions. This allows for chirality sensing and peptide-sequence recognition in water at low micromolar analyte concentrations. The reversible and noncovalent mode of binding ensures an immediate response to concentration changes, which allows the real-time monitoring of chemical reactions. The introduced supramolecular method is likely to find applications in bioanalytical chemistry, especially enzyme assays, for drug-related analytical applications, and for continuous monitoring of enantioselective reactions, particularly asymmetric catalysis.*

Most biological analytes are chiral, but transparent in the visible spectrum. The combination of a chiral receptor with a chromogenic signaling unit has, therefore, been the focus in the design of supramolecular chemosensing ensembles.[1] The addition of a chiral analyte leads either to a direct binding to a chiral chromophoric receptor or to the displacement of an indicator dye from a chiral synthetic host.[1] Regardless which route is taken, it is the design of the stereodiscriminating receptor which presents the main challenge, especially as analyte detection in water is required. An alternative approach to chirality sensing which has recently been receiving considerable attention exploits sensing by circular dichroism (CD) probes,[2] which undergo stoichiometric chemical reactions, typically metal ligation, with the analyte. This requires not only reactive functional groups (hydroxy or amino) to be present but also sufficiently long reaction times.

The induction of CD through noncovalent interactions of chiral analytes with achiral receptors could bypass both the design of chiral hosts and the formation of covalent adducts. Such noncovalent ICD probes have the potential to detect a broad spectrum of analytes, including nonreative ones, and to allow the monitoring of reactions, even of fast ones. Proof of principle for detectable ICD effects through noncovalent interactions with aromatic hosts exists,[2b,3] but the Cotton signals fell in the far-UV region and/or required high millimolar concentrations, both of which prevent practical application.[2a] The supramolecular chirality sensing ensembles which we introduce herein are assembled from an achiral host and achiral dyes as auxiliary chromophores (Figure 1).

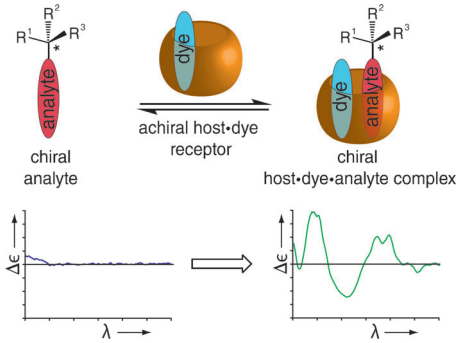


***Figure 1.*** The complexation of chiral analytes by a self-assembled, achiral host·dye receptor converts a weak intrinsic CD signal in the far-UV region into a strong ICD effect in the near-UV and visible regions.

The intrinsic CD signals of many chiral analytes, particularly amino acids, peptides, proteins, and carbohydrates as well as synthetic drugs, are weak and lie in the far-UV region (Figure 1, left). The addition of the achiral host·dye receptor results in strong ICD signals emerging as a consequence of the formation of a chiral host·dye·analyte complex (right). Our supramolecular approach to chirality sensing has the following advantages:

1) A large range of chiral aromatic compounds can be detected and their chirality differentiated through opposite ICD signals.
2) The ICD spectra, while challenging to predict,[4] are sufficiently diagnostic to distinguish different analytes, for example, peptide sequences.
3) The magnitude of the optical response allows the highly sensitive and selective (μM) detection of chiral analytes.
4) The ICD effects arise instantaneously,[5] thus allowing a real-time monitoring of (catalytic) reactions of chiral analytes.
5) The signals of the ternary complex are shifted to the near-UV or visible regions, which greatly improves compatibility with biological matrices and disposable (plastic) microtiter plates required for screening.

These assets, along with the operation in water, are essential for analytical applications.

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Host–guest complexes of the barrel-shaped cucurbit[*n*]uril macrocycles (CB*n*, $n = 5$–14)[6] have found widespread interest for analytical applications in water, because the high affinity constants allow sensitive detection.[7] The simultaneous inclusion of a dye and an analyte in the large cavity of CB8 has been utilized for the optical detection of aromatic hydrocarbons,[8] neurotransmitters,[9] and Trp/Phe-containing peptides.[10] However, none of the existing methods are capable of differentiating two enantiomers, because they have identical affinities and, therefore, afford exactly the same signals in the fluorescence, UV/Vis, ITC, and Raman spectra. Our idea was that chiral analytes should afford a strong and characteristic ICD signal with achiral CB8·dye complexes.[11] The CB8-dye-chemosensing ensemble offers a sufficiently large binding site to dock, with the desirable broad selectivity, a diversity of chiral analytes containing aromatic rings as a common recognition motif.

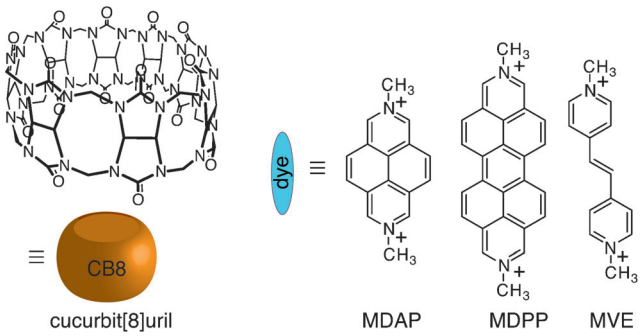


***Figure 2.*** Chemical structures and schematic representations of the macrocyclic host CB8 and the dyes.

The screening of a small library of strongly absorbing dyes (see the Supporting Information) afforded the dicationic dyes MDAP, MDPP, and MVE as prime candidates (Figure 2), because of their high binding affinity to CB8, compatibility with a large range of analytes, and, most importantly, ICD responses as strong as in previous studies (see Tables S1–S3 in the Supporting Information).[3] Although a particular dye may be best suited for a specific purpose, for example, to afford a strong ICD band at a desired wavelength, the dyes could be used interchangeably for many of the following application examples.

Figure 3 shows all the analytes studied. The amino acid derivative $NAcTrpNH_2$, for example, shows a barely detectable intrinsic CD signal near 280 nm at 40 μM (Figure 4a). When the same analyte is added to the preformed CB8·MDPP reporter pair (20 μM each) a strong ICD signal appears, which ranges up to 550 nm—the region in which the dye absorbs. In general, ICD effects are greatly enhanced by a constructive orbital overlap, which ensures exciton coupling and a conformationally confined interaction geometry.[2a,12] Evidently, both conditions are nicely fulfilled for the constrained face-to-face arrangement of analyte and reporter dye in the rigid CB8 cavity (Figure 1).[6c,13]

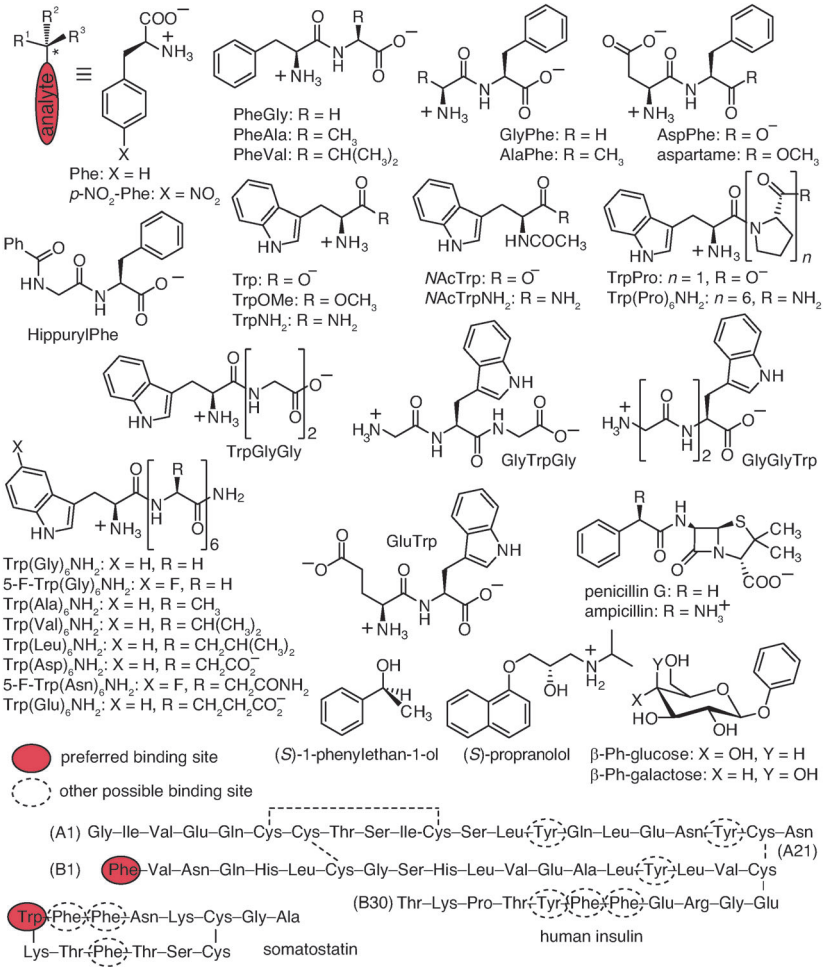


***Figure 3.*** Chemical structures of the investigated chiral analytes.

The affinities of the structurally diverse, more than 30 analytes ranged from $10^3$ to $10^6\,M^{-1}$ (see Tables S1 and S2 in the Supporting Information), which generally allowed a highly sensitive detection. Binding constants determined by ICD titrations corroborated those obtained by UV/Vis titrations. In all cases, CB8 acts as an indispensable template to assure the ICD effects; the conclusive control experiment is the destruction of the CB8·MDPP·$NAcTrpNH_2$ receptor–analyte complex through the addition of memantine, which sequesters CB8 ($K_a \approx 10^{11}\,M^{-1}$)[14] and, thereby, eliminates the ICD effect (Figure 4a). As expected, the use of an alternative reporter dye, MDAP or MVE, led to a different ICD spectrum, but at similarly low analyte concentrations (40–100 μM; see Figure S2 and Table S2 in the Supporting Information), as is desirable for sensing in water.

Most optical sensing approaches based on a single receptor afford a qualitatively similar response for structurally

  

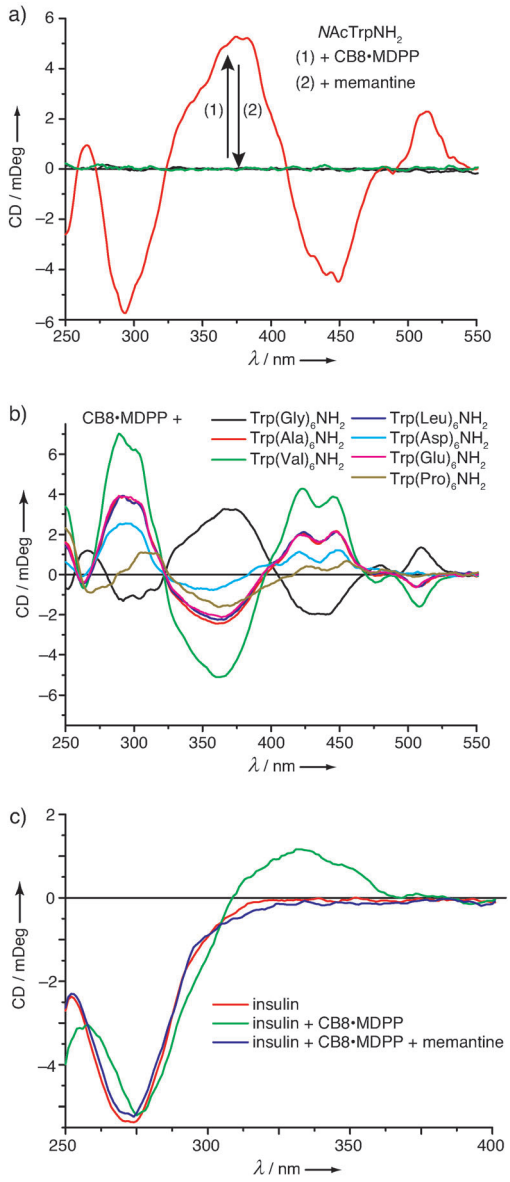


***Figure 4.*** Examples of chirality sensing. a) CD spectra of *N*AcTrpNH$_2$ (40 μM, black line), after addition of CB8·MDPP (20 μM in $H_2O$, red line), and subsequent CB8 sequestering by memantine as a competitive binder (60 μM, green line). b) CD spectra of CB8·MDPP (20 μM in $H_2O$) in the presence of different Trp(Xaa)$_6$NH$_2$ heptapeptides ($\geq$3 equiv). c) CD spectra of insulin (50 μM, pH 2.7) in the presence of CB8·MDPP (20 μM) and subsequent CB8 sequestering by memantine (60 μM).

related analytes, for example, a net fluorescence quenching, which prevents their differentiation. This is invariably the case for the popular indicator displacement assays, for which the spectral response of the liberated dye does not report on the structure of the analyte. In contrast, different derivatives of the amino acids Trp and Phe gave distinct ICD spectra with our chemosensing ensemble (see Figures S3 and S4 as well as Tables S1 and S2 in the Supporting Information). Encouraged by these results, a small library of dipeptides, tripeptides, and heptapeptides with N- or C-terminal Trp/Phe residues was investigated. Again, clearly distinguishable ICD spectra resulted (Figure 4b; see also Figures S3 and S4, and Table S1 in the Supporting Information) at micromolar concentrations. Internal Trp residues also produce characteristic ICD signatures (see Figure S3 and Table S1 in the Supporting Information). Indeed, a single Trp residue is sufficient for peptide sensing, as demonstrated for the cyclic peptidic hormone somatostatin (Figure 3). Strikingly, the ICD pattern is sufficiently characteristic, in comparison to the effects for smaller model peptides (see Figure S3 and Table S1 in the Supporting Information), to pinpoint the Trp rather than the two Phe residues as the preferred binding site in somatostatin. This is, indeed, an important piece of solution-phase structural information, which is otherwise only accessible by NMR spectroscopy at much higher concentrations. We are unaware of any other receptor that gives rise to such distinctive optical responses when binding analogous peptides. Proteomics ideas for sequence recognition and peptide fingerprinting using principle component and discriminant analysis[15] come to mind. Even without this, one can readily differentiate N-terminal Trp-functionalized heptapeptides (Figure 3) from each other at $\leq$50 μM (Figure 4b). Peptides starting with TrpGly, TrpVal, TrpAsp, and TrpPro show characteristic Cotton bands; only the starting sequences TrpAla, TrpGlu, and TrpLeu resulted in very similar ICD spectra.[16]

Carbohydrates also resist direct detection or discrimination by optical spectroscopy. Unfortunately, they do not bind to the CB8·dye complexes in their unmodified form either. Nevertheless, phenyl-β-D-galactose and phenyl-β-D-glucose are complexed tightly (see Tables S1 and S2 in the Supporting Information). These two epimers display identical spectra in the far-UV region in water; even their intrinsic CD spectra are virtually the same. However, in the presence of the CB8·MDAP receptor, their strong ICD spectra, with well-defined isodichroic points (see Figure S7 in the Supporting Information), can be readily discriminated. Interestingly, the effects for the biomolecular analytes are large, even though the stereogenic centers themselves are not immersed in the host cavity.

The vast majority (ca 80%) of oral drugs contain at least one aromatic residue,[17] which similarly serves as an anchor for our receptors. For example, the chiral naphthol-type drug propranolol (Figure 3) displays an absorption in the near-UV region, but its intrinsic CD signals are very weak, because of its conformational flexibility, which scrambles CD effects.[4,12a] Clear Cotton bands emerged in the presence of the CB8·dye reporter pair (see Tables S1 and S2 in the Supporting Information), as was the case for penicillin G and ampicillin (see Figure S4 in the Supporting Information). The method can logically be expanded to determine the enantiomeric excess of drugs because 1) racemic mixtures do not produce any ICD response (Figure 5a) and 2) because two enantiomers must display identical binding to the achiral receptor but opposite ICD effects (see Figure S3 in the Supporting Information). This offers a very important application.[2a,18]

We also tested the compatibility of the ICD sensing platform with insulin. This protein target possesses a sterically accessible N-terminal Phe residue in its B chain;[7c] its six

  

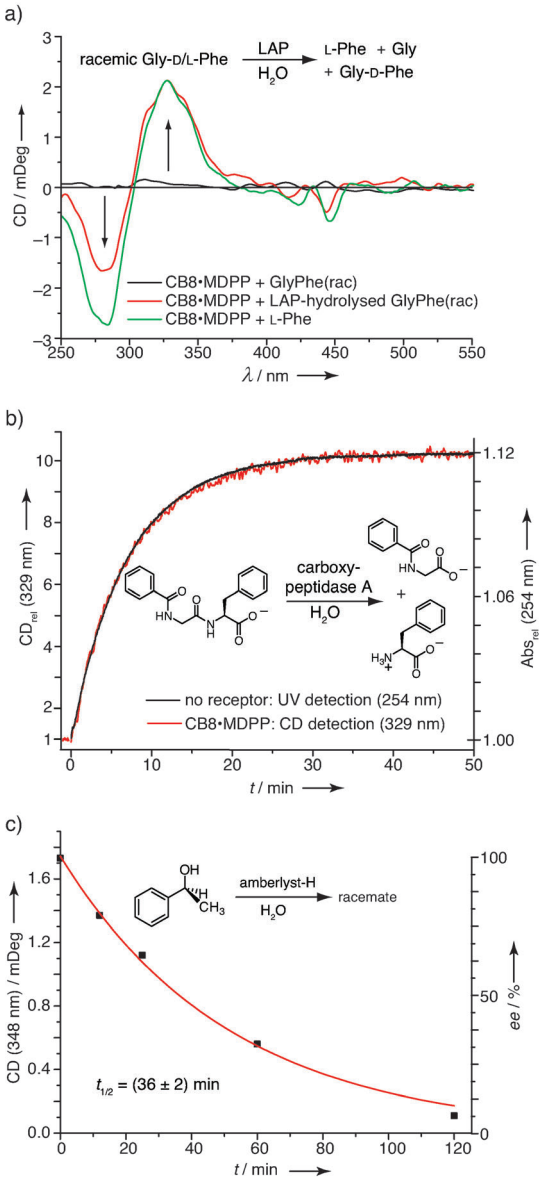


***Figure 5.*** Examples of reaction monitoring. a) CD spectra of CB8·MDPP (20 μM) in the presence of racemic Gly-D/L-Phe, before and after its enzymatic hydrolysis by leucine aminopeptidase (LAP) at pH 7.8. b) Kinetic trace for the hydrolysis of Hippuryl-Phe (160 μM) by carboxypeptidase A at pH 7.8. The reaction progress was monitored by CD (329 nm) in the presence of CB8·MDPP (20 μM, red line) and, as a control, directly by UV/Vis spectroscopy (254 nm, black line). c) Racemization of (*S*)-1-phenylethanol (0.08 M in $H_2O$) with amberlyst-H at 65 °C, monitored by the addition of reaction mixture aliquots (10 μL, 400 μM final concentration) to CB8·MVE (40 μM).

internal Phe or Tyr residues are buried inside. Addition of the CB8·MDPP receptor to insulin (50 μM) indeed led to a very distinctive ICD band at 335 nm (Figure 4c, and see Table S1 in the Supporting Information). Disassembly of the receptor–insulin complex by memantine diminished the ICD bands, and thus nonspecific competitive binding of the dye to the protein does not apply (Figure 4c). Bovine serum albumin (BSA) and lysozyme were studied as negative controls; here, no ICD effects were observed in the presence of the receptor because the aromatic amino acids of these (and most other) proteins are shielded in their hydrophobic core.

The enhanced ICD effects observed for the various analytes establish a new method for the sensitive, label-free monitoring of chemical reactions in real time, such as enzymatic conversions involving chiral metabolites. This is made possible by the fact that the noncovalent binding to the supramolecular receptor is reversible and shows fast exchange kinetics,[5] which differentiates our approach from complementary chiroptical methods involving covalent adducts.[2] The dynamic response (which probes the reaction progress through a complexation equilibrium between analyte and reporter pair) also allows nonstoichiometric amounts of the host (and even excess dye) to be used. We now provide compelling proof of this novel concept.

1) We have followed in situ the β-galactosidase-mediated enzymatic hydrolysis of phenyl-β-D-galactose. As expected, its cleavage into phenol and galactose was accompanied by a decrease in the ICD signal, as the emerging receptor·phenol complex is achiral (see Figure S7 in the Supporting Information).
2) Although BSA gives no ICD effect (see above), the addition of the protease pepsin destroys its compact tertiary structure such that the aromatic residues become accessible to the receptor. This structural and chemical change can be continuously monitored through emerging ICD effects with the reporter pair (see Figure S8 in the Supporting Information).
3) Since the ICD patterns of peptides are sequence-selective, a new line of label-free enzyme assays can be set up, which remain in high demand, for example, for exopeptidases such as carboxypeptidase A.[19] This enzyme cleaves C-terminal Phe residues preferentially, as could be directly monitored when the digestion of the model substrate hippuryl-Phe was monitored by the ICD assay (Figure 5b).
4) Likewise, leucine aminopeptidase (LAP) was successfully employed for peptide digestion, for example, of AlaPhe into Ala and Phe (see Figure S9 in the Supporting Information) or of GlyTrpGly, which was followed through both near-UV and visible ICD bands (see Figure S10 in the Supporting Information).
5) Chirogenesis can also be readily followed. When starting with a racemic mixture of a substrate in the presence of the achiral chromophoric receptor, ICD effects should arise upon any enantiospecific conversion. Indeed, enzymatic digestion of racemic Gly-D/L-Phe with LAP led to the appearance of the characteristic dichroic fingerprint of L-Phe, because only the Gly-L-Phe dipeptide was hydrolyzed (Figure 5a, and see Figure S11 in the Supporting Information).

Of major practical interest is the fact that the ICD method allowed us to monitor each of these enzymatic reactions in the near-UV region (>310 nm), thereby bypassing biological matrix effects as a result of absorbing and/or CD-active impurities or additives. The latter cannot be avoided when applying alternative far-UV assays—some of which were also performed as controls to double-check the absolute kinetics

  

obtained by the ICD assays (Figure 5b, and see Figure S8 in the Supporting Information). The agreement between both types of assays confirmed that the two additives (host and dye) did not strongly influence the enzymatic activity.

We previously introduced supramolecular tandem assays as time-resolved variants of fluorescent indicator-displacement assays.[7a,20] However, monitoring of racemization reactions or the interconversion of two enantiomers by this technique would have required the challenging design of chiral receptors, for which we have not yet been able to provide a working example.[7a,20] The ICD technique allows the use of an achiral host–dye pair to report on the degree of racemization with ease (Figure 5c), in fact for a heterogeneous catalysis. An amberlyst-catalyzed racemization was selected as a prototype reaction because of its industrial relevance for the isomerization of pharmaceutical building blocks.[21] As an additional difference, instead of measuring the racemization of (*S*)-1-phenylethanol directly in the reaction mixture, we preferred to draw aliquots and add them to a cuvette containing the sensing ensemble, the CB8·MVE pair in this case. The resulting decrease in the ICD signal at 348 nm can be directly correlated to the kinetics of the racemization reaction, thus providing a more sensitive detection tool than direct measurement of chiroptical substrate properties. This final application example illustrates that the method is not restricted to enzymatic reactions.

In conclusion, we have developed a versatile method based on self-assembled, achiral host–dye pairs that report micromolar concentrations of water-soluble chiral analytes through ICD effects in the preferred near-UV or visible range. The method can be adapted to many structurally related analytes, in our case analytes with sterically accessible aromatic recognition motifs. It is applicable to many biomolecules as well as synthetic drugs and can be transfered from simple analyte detection to the sequence recognition of peptides. Most importantly, we introduce the use of supramolecular chirality sensing ensembles for monitoring enzymatic as well as other catalytic reactions. This has broad application potential, including analyte detection in flow systems, because the chirality and affinity of the reactant or product are the only prerequisites for utilizing the method. Several achiral water-soluble hosts, including macrocycles with covalently linked chromophores,[3b,7d,22] are known, and these have a similar potential for chirality sensing.

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