Optical Analysis of Reaction Yield and Enantiomeric Excess. A New Paradigm Ready for Prime Time

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ABSTRACT: This Perspective highlights the advances of optical methods for asymmetric reaction discovery. Optical analysis allows for the determination of absolute configuration, enantiomeric excess and reaction yield that is amenable to high-throughput experimentation. Thus, the synthetic organic community is encouraged to incorporate the methods discussed to expedite the development of high-yielding, enantioselective transformations.

I. Introduction

The use of high-throughput experimentation (HTE) for catalyst design and reaction optimization in both academic and industrial asymmetric synthesis has become quite popular over the last few decades.[1-2] This approach allows screening of combinations of reaction variables (such as catalysts, solvents, bases, temperatures, concentrations, additives, etc.) in order to efficiently identify the optimal conditions for a given transformation, and has been equally successful at discovering new synthetic reactions.[3-7] In addition, the directed evolution of enzymes to create synthetically useful transformations is a form of HTE because of the ability to search protein sequence space for reaction optimization.[8-10] The HTE paradigm enables hundreds to thousands of parallel reactions to be run by a single worker over the course of a day, utilizing small volume reactions contained within multi-well plates. HTE has also allowed literature methods to be tested beyond the narrow scope of substrates for which they were originally developed, thereby pushing the techniques into the realm of structurally complex molecules typical of pharmaceutical intermediates and products.

HTE generates an extremely large number of samples, and necessarily places a significant burden on the analytical methodology required to determine reaction success.[11-12] As the number of samples grows from a few at a time to thousands per day, innovative high-throughput screening (HTS) solutions are necessary in order to prevent analysis from becoming the perpetual bottleneck in the process.[13] There are many diverse analytical techniques that have been targeted for application in determining success, both in terms of yield and enantioselectivity, of synthetic organic reactions.[14] The most commonly applied techniques include chromatography, nuclear magnetic resonance spectroscopy (NMR), and mass spectrometry (MS). A few decades ago high performance liquid chromatography (HPLC) became ubiquitous within the industrial synthetic community due to its high resolving power. More recently, supercritical fluid chromatography (SFC) has emerged as an ideal tool for faster separations due to superior diffusion rates and lower viscosity of supercritical CO_2.[15] The last few decades have seen many improvements in chromatographic instrumentation to take advantage of the speed of analysis to facilitate HTS.

The commercialization and widespread use of small particle LC columns has had a tremendous effect increasing the analytical throughput. The smaller particles, combined with instruments that can operate at significantly higher pressure, allow the same resolution for a given separation to be achieved in significantly less time. For example, a series of alkyl benzenes analytes could be separated in 15 minutes using a column with 5 μm particles. Using the same phase with smaller 1.7 μm particles, the aforementioned compounds could be resolved in only 1.8 minutes.[16-17] Ultrafast chiral separations lagged behind their achiral counterparts, but there has recently been growing interest in developing sub-2 μm particles to decrease analysis time for enantiomer resolution.[18-19] Where chiral separations often required upwards of 20 minutes in the past, we are now reaching a period where these separations can be carried out in the seconds timeframe.[20-23] A further study was carried out using a chemically diverse set of 50 racemic compounds to test the general application of this methodology, including some challenging intermediates from the pharmaceutical industry. These studies showed that it was possible to resolve 43 of the 50 members of the set under one minute of run time using either SFC or HPLC.[24] Unfortunately, all ultrafast HPLC separations mentioned above were achieved with chemically pure samples, but not with crude asymmetric reaction mixtures.

While chromatography remains the workhorse for determining the purity and enantiomeric excess for chiral compounds in regulated pharmaceutical work, the use of

optical methods in synthetic organic reactions run in parallel is rapidly gaining ground. These methods are generally less accurate than HPLC, however they offer several advantages that outweigh this limitation. Chromatography is inherently a serial process and requires new screening to be carried out for every new structure for analysis. Small changes in analyte structure can have dramatic differences in selectivity of a chiral stationary phase. Optical analysis can be performed in plates and thus has many of the advantages of a parallel process.[25-26] Numerous probe designs and optical assays that report *ee* values via circular dichroism, fluorescence, and UV absorbance have been developed and reported previously. Assays have been developed for many common functional groups created in asymmetric methodologies. The techniques are robust and ready to be employed by synthetic chemists, and advertising/popularizing these assays is the main goal of this review. We herein show via example methods for *ee* determination, *ee* and reaction yield analysis, and examples of true screening.

Thus, we offer up a new opportunity to the synthetic methodology community carrying out numerous parallel reactions - give optical analysis a try, you may not go back to your HPLC.

II. Chiral Recognition and Quantitative ee Sensing

As alluded to above, in the past two decades, significant progress has been made in the field of optical differentiation of molecular chirality, as well as quantitative *ee* determination. More recently, several methods that combine *ee* and yield analysis have emerged, and these are available to accelerate asymmetric reaction development projects. Optical methods for determining *ee,* such as UV-Vis, fluorescence, and circular dichroism (CD) spectroscopy are simple, fast, and cost-effective, allowing for them to be easily implemented in high-throughput screening protocols.

A. Chiroptical Methods The differentiation of enantiomers necessarily requires a chiral environment and circular dichroism spectroscopy is inherently suited for this task. Left- and right- circularly polarized light creates diastereomeric interactions with the chiral analyte giving rise to characteristic Cotton effects. The sign of the Cotton effect is indicative of the molecular handedness of the analyte and the magnitude of the CD signal can be correlated to the *ee* of the sample.[27-28] While most chiral molecules have small CD signals that typically appear at low wavelengths, large Cotton effects can be generated at extended wavelengths with a molecular sensor, such as metal complexes that display metal ligand charge transfer (MLCT) bands or when two chromophores in close proximity arrange in a chiral orientation, leading to exciton-coupled circular dichroism (ECCD).[29] These characteristic MLCT or ECCD signals at extended wavelengths show signals at a portion of the spectrum that is less likely to have interference from reaction components such as solvent or chiral catalyst.

i. Noncovalent Assemblies Borhan, Berova and Nakanishi developed some of the earliest chiroptical methods for chirality differentiation. They introduced zinc-porphyrin tweezers for determining the absolute configuration of chiral diamines and other analytes (Figure 1).[30] Their approach utilizes two zinc-porphyrin moieties connected via a flexible linker. Host-guest complexation between the zinc-porphyrin tweezers and a bidentate chiral analyte results in a through-space interaction between the two zinc-porphyrin units and an exciton-coupled CD signal, the sign of which is indicative of the handedness of the chiral analyte. Since the initial report in 1998, these three investigators have developed a suite of zinc-porphyrin tweezers for the determination of the absolute configuration of a wide range of chiral compounds.[31-44] In several cases, a CD spectrum can be obtained with micrograms of analyte without the need for derivatization of the chiral compound; however, a few functional groups require derivatization prior to analysis. While this approach has rarely been used to quantify *ee* values, the potential is clear.[45] The work is mentioned here primarily for the inspiration it gave to the field, setting the impetus for many others to follow suit with quantitative approaches.

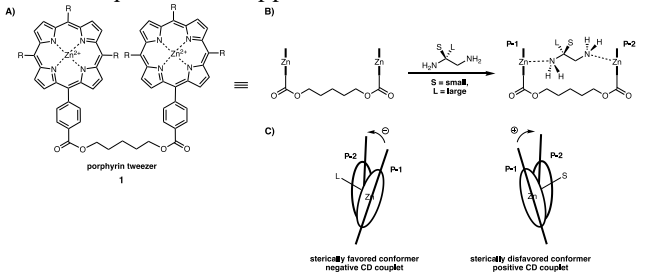
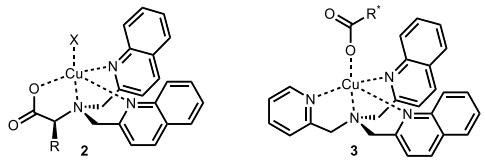


Figure 1. A) Prototypical bis-porphyrin molecular design, B) Complex formation leads to clockwise or counter clockwise twists of the porphyrins, C) The twist leads to characteristic positive or negative Cotton effects, indicative of the handedness of the stereocenter.

Inspired by this work, Canary demonstrated that derivatization of α- and β-amino acids with quinoline ligands followed by Zn(II) or Cu(II) complexation can be used for the reliable assignment of the absolute configuration via ECCD spectroscopy.[46-48] Once again, the twist of the chromophores gives rise to a CD signal, where the sign of the couplet reveals the absolute configuration of the chiral compound. Building on the strategy embodied in compound **2**, Anslyn and Canary introduced an ECCD protocol for quantitative determination of the *ee* for chiral carboxylates with the copper complex **3**.[49] In this approach, there is no need for analyte derivatization; it is a simple mix and measure protocol, reporting *ee* values with an average 3% error. With no chiral analyte present, the tripodal copper complex exists as a racemic mixture of *M* and *P* isomers. However, addition of an α- or β-chiral carboxylate results in enantiomeric complexes where one helical twist dominates over the other and a CD signal is generated. The method can be used for acetyl/Boc-protected α-amino acids and β-amino acids.[50]



In a similar approach, Buchholz and Kleij developed bis(metallosalphen) zinc complexes for the quantitative *ee* determination of chiral carboxylates, also via ECCD spectroscopy.[51] Both of these approaches are robust in measuring the *ee* values of chiral carboxylic acids with a high level of accuracy.

As discussed throughout this review, the Wolf group has made extensive contributions towards chirality sensing.[52-55] They have introduced many *ee* sensing assays and demonstrated their subsequent implementation in screening protocols. In an approach analogous to those introduced above, a stereodynamic probe **4** was shown to report *ee* values and allows the assignment of absolute configuration for amines, diamines, amino acids, amino alcohols, and α-hydroxy acids (Figure 2).[54] As with compounds **2** and **3**, substrate binding induces a distinct chiroptical signal indicative of the analyte's handedness. In this approach, the analyte and probe can simply be combined, and CD measurements can be immediately recorded for *ee* determination. The same group developed similar methods with other stereodynamic metal complexes for quantitative *ee* determination of amines, diamines, and amino alcohols.[52-53, 55] Each of these assays can be adapted for comprehensive asymmetric reaction analysis, which is discussed in Section V.

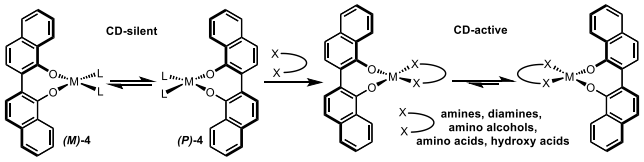


Figure 2. Stereodynamic metal probe for the sensing of chiral amines, diamines, amino alcohols, amino acids, and hydroxy acids.

Zonta and colleagues most recently introduced vanadium complex **5** carrying a tetradentate aminotriphenolate ligand for the *ee* analysis of a broad variety of chiral analytes including amides, sulfoxides, amines, amino alcohols, carboxylic acids, and α-hydroxy acids (Figure 3).[56] Coordination of the substrate to the vanadium center coincides with substantial red-shifted absorption — an attractive feature that could allow combined concentration and *ee* determination with minimized interference from impurities. Accurate *ee* determination independent of the analyte concentration using the anisotropic *g*-factor ($g=\Delta\varepsilon/\varepsilon$) was accomplished with a 5% margin of error. This major advance in the field awaits adoption by the synthetic community.

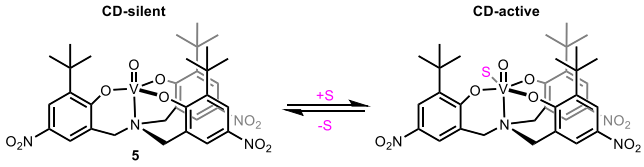


Figure 3. Vanadium complex and substrate (S) binding.

ii. Dynamic Covalent Assemblies Dynamic Covalent Chemistry (DCC) is one of the most promising strategies for rapid chiroptical reaction screening.[57] Several groups have developed chiroptical methods where the chiral analyte is incorporated into a multicomponent DCC assembly. The Wolf group has demonstrated that the 1,8-bis(3'-formyl-4'-hydroxyphenyl)naphthalene probe **6** can be used for *ee* determination of amino alcohols and *α*-amino acids (Figure 4).[58-59] In the absence of a chiral bias, the triaryl probe exists as an equal distribution of rapidly interconverting (*P,P*)- and (*M,M*)-isomers. Imine formation with chiral amines is complete within a few minutes and synergistic steric and intramolecular hydrogen bonding interactions result in chiral amplification across the chromophoric sensor. As the equilibrium between the sensor conformations is shifted to strongly favor a single isomer, a large CD signal is produced.

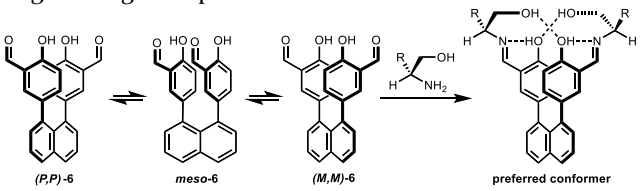
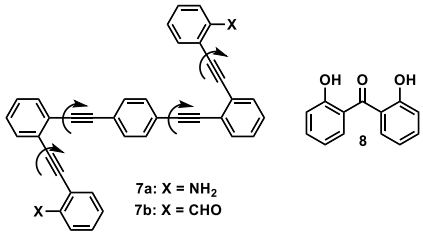


Figure 4. Stereodynamic sensor for chiral amino alcohols and amino acids.

In another approach, Wolf and colleagues used conformationally flexible arylacetylene frameworks for *ee* determination of mono- and diamines, amino alcohols and aldehydes.[60-63] The arylacetylene probes, **7a** and **b**, freely rotate about the acetylene axes and are CD silent. Diimine formation with the chiral compounds induces axial chirality, resulting in large chiroptical signals. Use of both **7a** and **b** gave *ee* values with errors ranging from 2-7%. Hong, Kim and Chin demonstrated that 2,2'-dihydroxybenzophenone, **8**, and derivatives thereof are equally powerful DCC sensors for amino acids, amino alcohols, diamines and amino esters and amides.[64-65]



The Anslyn group has developed a series of chiroptical methods for the *ee* determination of chiral amines, alcohols, and aldehydes/ketones (Figure 5).[66-71] Assays for amines and carbonyls function similarly. First, a condensation reaction yields a chiral imine or hydrazone which then undergoes complexation to an Fe(II) center to form octahedral complexes. The *Fac* and *Mer* isomers have a preferential Δ or Λ twist that induces large CD signals based on the handedness of the chiral analyte. The same group has developed ECCD methods for amines and amine derivatives based on DCC with enantiopure BINOL, *o*-formyl phenylboronic acid and the chiral analyte.[72] A three-com-

ponent assembly rapidly forms and gives a distinct chiroptical signal for *ee* determination. This system has also been used for chirality sensing via fluorescence methods.[73-74]

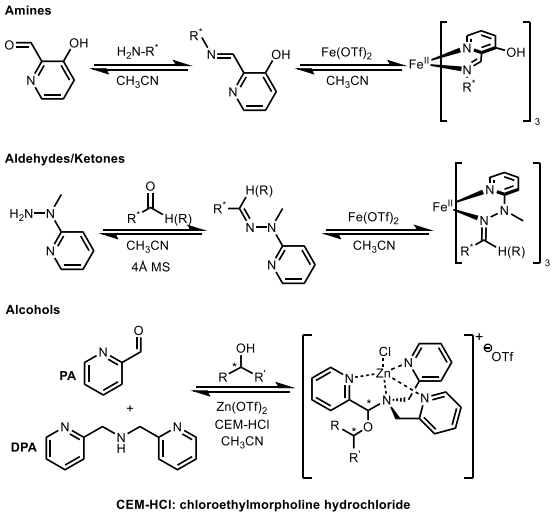


Figure 5. Dynamic covalent bond formation for *ee* determination of amines, aldehydes/ketones, and alcohols.

Importantly, the third DCC assembly shown in Figure 5 allows sensing of chiral secondary alcohols. As of yet, there has not been a secondary alcohol that did not work with this assay, although it fails with tertiary alcohols. Even simple targets such as 2-butanol give a large enough CD signal to determine *ee* values with good accuracy. The same assembly can also be used for chiral thiols, aromatic amines and secondary amines.[75] The method involves a four-component assembly: 2-pyridinecarboxaldehyde (PA), dipicolylamine (DPA), $Zn(OTf)_2$ and the chiral functional group of interest. The chiral analyte, 2-PA, and DPA combine to form a tren-like ligand that strongly binds to Zn(II). The diastereoselective assembly affords a complex with twisted pyridine rings that generate a characteristic ECCD signal. Importantly, each of the assays shown in Figure 5 were designed to operate on mono-functional chemicals, i.e. mono-ols, mono-amines, or mono-carbonyl structures. Furthermore, there is no synthesis required for any of the aforementioned assemblies; each assay functions with commercially available starting materials. The amine and alcohol *ee* assays are currently being utilized for the analysis of a wide variety of synthetic reactions.

Recently, You and Anslyn introduced the rapidly interconverting 2,2'-disubstituted biphenyl pair, **9** and **10**, to capture chiral alcohols, amines and thiols by forming diastereomeric aminals and imines (Figure 6).[76] Covalent substrate binding results in a conformational bias of the axially chiral sensor which produces a strong CD signal that allows analysis of the absolute configuration and *ee* with an error margin under 2%. This work, and those discussed in Figure 5, underscore that chiral amplification with DCC probes affords practical assays that one can directly apply to asymmetric reaction screening; successful examples of these strategies are given in Section V.

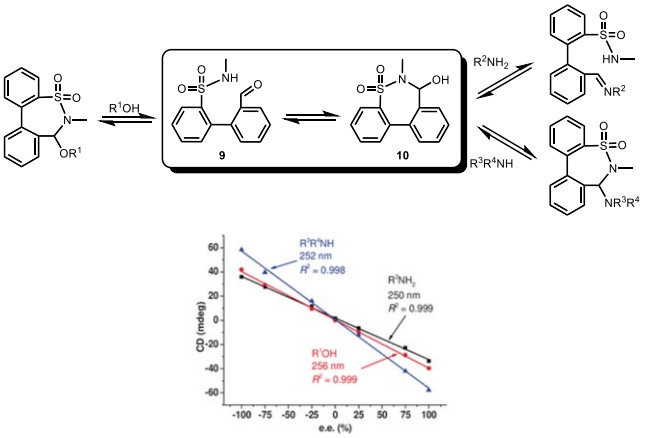


Figure 6. *Ee* sensing using the biphenyl pair **9** and **10** and the corresponding *ee* calibration curves for 2-(methoxymethyl)pyrrolidine, 1-phenylethanol, and 1-phenylethylamine. Adapted with permission from reference 76.

iii. Supramolecular Polymeric Systems Supramolecular and polymeric systems have also been investigated for chirality differentiation. Amabilino and coworkers used oligo(*p*-phenylenevinylene) aggregates for the *ee* determination of several acids.[77] The acids induce helicity in the aggregates and the enantiomeric composition can be determined via ECCD. Jiang and James have developed an ECCD protocol using perylenebisimide aggregates for the quantitative *ee* analysis of *α*-hydroxy acids.[78] Further, early studies from the Feringa group focused on liquid crystals.[79-81]

The strength of using polymers is to exploit the cooperativity properties they can impart. For example, the errors associated with optical methods for *ee* determination typically range from 3-10%, making them useful for preliminary asymmetric reaction discovery. The unique properties of helical aggregates and polymeric systems offer the advantage of increased accuracy that can rival chiral chromatography. In this regard, the Majority Rules Effect (MRE) of helical polymers has been utilized. Polymers of this kind show nonlinear responses to the enantiomeric excess of a chiral monomer. The polymer cooperatively binds monomers of the same handedness, where the handedness of the monomer dictates the twist imparted upon the polymer. Binding of the enantiomer requires a relatively large enthalpic penalty to induce helix reversal. Thus, a small enantiomeric excess of the chiral monomer can lead to an almost complete preference for one helical twist. If a chromophore is incorporated into the polymer backbone, this helical preference can be monitored via CD spectroscopy. Plotting CD intensity against the *ee* of the monomer yields a sigmoidal curve, where the change in CD intensity is highest near 0% *ee* (Figure 7).

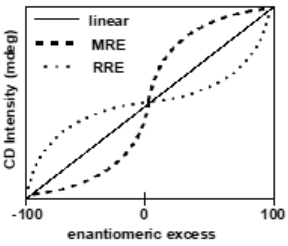


Figure 7. Typical calibration curve for linear, majority rules effect and racemate rules effect responses.

Exploiting the MRE, Jiang and Anslyn developed a CD-based method for *ee* determination of amino acids using a helical polymer.[82] A polyphenylacetylene backbone was appended with crown ether moieties and complexation of the crown ether with protonated amines induces a distinct chiroptical signal. Taking advantage of the intense slope near 0% *ee*, it was shown that adding an equivalent of the opposite enantiomer to a sample of unknown *ee* allows for the accurate determination of high *ee* values. In this manner, a sample of e.g. 97% *ee* can be converted to a sample of -1.5% *ee*, bringing the intensity of the CD sample into the most sensitive region of the CD calibration curve. This approach yielded accurate *ee* values with errors ranging from 0.2-0.7%, while retaining all the advantages of the speed of optical methods. However, there is a critical drawback to this approach. One needs to know the concentration of the unknown sample such that the correct amount of the opposite enantiomer can be added. Thus, unlike the other assays discussed herein, this particular approach is not yet amenable to true HTS.

Most supramolecular aggregates and polymeric systems exhibit either linear or MRE responses; however, Jiang and Anslyn identified supramolecular stacks that show a Racemate Rules Effect (RRE) (Figure 7).[83] There is no fundamental reason that a polymer should prefer to bind the same enantiomer, rather than alternate binding enantiomers. Thus, in a RRE system, the CD response curve generated is opposite of that observed for MRE; the CD signals of nearly racemic mixtures were lower than expected for a linear CD vs *ee* or MRE plot. Thus, this particular system cooperatively binds opposite enantiomers until the solution becomes so enriched with one enantiomer that the polymer can only bind molecules of the same handedness, which would occur with solutions with high *ee*. In such a system, the CD response for mixtures near 0% *ee* would be low because cooperative binding of opposite enantiomers would not induce a helical pitch. Only at high *ee* values will the polymer adopt a preferential helical twist giving rise to a chiroptical signal. The distinct curve associated with the RRE leads to a larger dynamic response to high *ee* values, resulting in lower errors. Furthermore, utilization of the RRE removes the requirement of adding the opposite enantiomer to dial the CD signal to the region of largest dynamic range. Although this system was only demonstrated for malate and a particular perylenebisimide aggregate, it represents an approach that would allow for the determination of high *ee* value with low error. Clearly much more work is needed to design RRE polymers to bind many chiral functional groups as a means to make the approach more general.

iv. Instrumentation As presented, substantial effort over the last decade or so has been put toward the development of CD-based assays. However, while traditional fluorescence and UV/Vis plate readers are common and ubiquitous, CD plate readers were not commercially available until quite recently. Traditional plate readers are routine instruments in biochemical laboratories, and they can be immediately adapted to HTS of *ee* values (see below). In contrast, CD spectrometers are historically interfaced with robots that transfer samples from the plate to the instrument. However, Anslyn and Kahr showed that CD plate readers can be created.[84] This has led to the recent development of a commercial system by Hinds Instruments, Inc., with distribution through Bio-Logic Science Instruments. The commercial CD well-plate reader uses a vertical light beam to measure the CD signals and *ee* values of a sample directly from the well-plate. The well-plate is moved by a computer-controlled XY stage from well to well for rapid measurements. The current commercial CD Reader, bearing the trade-mark name EKKO™, is capable of measuring CD spectra from 185 nm to 850 nm from a well-plate directly. It can also operate at a desired wavelength to read *ee* values in all 96 wells in a few minutes. In comparison to a standard CD spectrometer, which universally uses a horizontal measuring beam, the EKKO™ CD Reader eliminates the time-consuming process of transferring the content from each well of the microplate into a specific sample cell for CD measurements. It is well suited to identifying enantiomeric "hits" in HTS. Further efficiencies are possible with the 384 well microplate option.

B. Traditional Spectroscopic Methods

Irrespective of the recent advances in CD-plate readers, it is certainly true that traditional plate readers are far more common and less expensive. As such, fluorescence and UV-Vis spectroscopies are more readily adapted to be truly high-throughput on a lower budget. As with CD assays, these methods can also afford high sensitivity, rapid analysis, and the opportunity for real-time sensing. Another added benefit of traditional spectroscopic methods is that the chiral sensor may be used in the racemic form to afford a protocol for both *ee* and concentration determination. Furthermore, enantioselective fluorosensing affords high sensitivity, requiring only micrograms of analyte and sensor. As alluded to above, with well-plate readers becoming commonplace, thousands of samples can be rapidly screened via traditional enantiosensing methods. Thus, the assays described below are truly ready to be rolled out into the synthetic community.

i. Colorimetric Approaches The use of UV-Vis spectroscopy for quantitative *ee* determination is well-known.[85] A chiral analyte binds to the chiral host, which results in a change in the spectroscopic signal. In many cases, the enantioselective sensor requires multi-step synthesis, with the efficacy of the host being determined post-synthesis. Such an approach is unfavorable because a poor spectroscopic signal would necessarily require the synthesis of a

new host. This iterative process is time-consuming and introduces a bottleneck for the quantitative determination of *ee*.

In contrast, enantioselective indicator displacement assays (eIDAs) have been shown to be excellent general strategies for the discrimination of chiral compounds with a wide range of functional groups.[86] In an eIDA, there are competing equilibria involving a chiral host, an indicator and the chiral analyte of interest (*vide infra*). With this strategy, the indicator binds reversibly to the host and displays a distinct spectroscopic signal when free in solution. The guest binds to the host, displacing the indicator, which results in a change in the absorbance signal. The competition between guest and indicator can be monitored as a function of the total concentration of guest and the resulting color change. The interaction of a chiral host of an eIDA with an enantiomeric mixture of chiral guests creates competing equilibria involving diastereomeric host-guest adducts. The difference in equilibrium constants for the enantiomers with the host results in different amounts of indicator being displaced and thus, the enantiomers can be differentiated from the color of the solution. This approach is not limited to colorimetric indicators, as fluorophore indicators have also been used successfully in eIDA development.[87-88]

The Anslyn group has established the usefulness of eIDAs over the past 20 years. In one example, a simple and general method was developed for the quantitative *ee* determination of non-derivatized α-amino acids with a chiral Cu(II)-diamine complex and the indicator pyrocatechol violet (PCV).[89] It was found that D-amino acids displace the indicator more readily than L-amino acids (Figure 8). Thus, pure enantiomers gave distinct spectroscopic signals allowing for the differentiation of the enantiomers. Addition of enantiomeric amino acid mixtures gave colors that were in between the two enantiopure extremes and a calibration curve was used to quantify *ee*. It is worth noting that the ligand used in this eIDA was synthesized in one step from commercially available starting materials and required no chromatographic purification. Quantification of *ee* in the synthesis of unnatural amino acids has been of interest for decades, and thus this assay is clearly one of widespread general utility awaiting adoption.

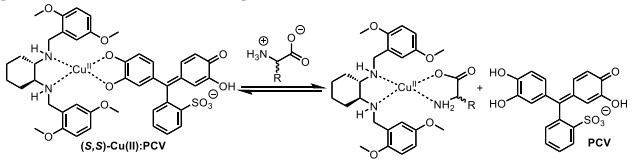
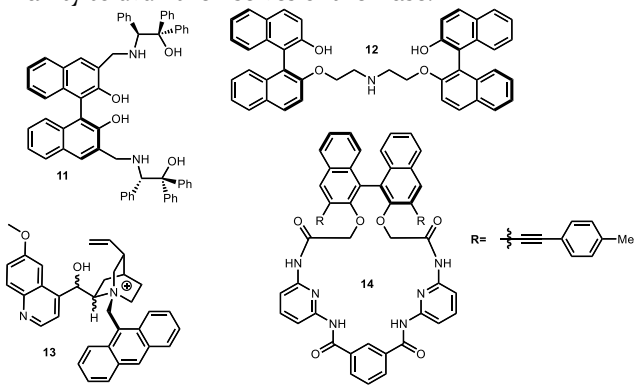


Figure 8. Enantioselective indicator displacement assay for the *ee* determination of α-amino acids.

ii. Fluorescence Methods

a. Sensing Based on Noncovalent Probe-Substrate Interactions The Pu group has pioneered the use of fluorescence methods for *ee* determination, dating back to studies in the early 2000s. Their workhorse approach utilizes enantioselective fluorescent BINOL sensors, such as **11** and **12**, for quantitative *ee* determination of α-hydroxy acids, amino alcohols, and *N*-protected amino acids.[90-99] Hydrogen bonding between the substrates and BINOL-derivatives lead to either fluorescence quenching or enhancement of the BINOL core, and the enantiomeric composition of the chiral compound can be determined. Several of the BINOL sensors can be immediately incorporated into screening protocols, and only await the synthetic community to avail themselves of their use.



In an alternative approach, the Anzenbacher group introduced the *N*-anthracenyl cinchona alkaloid **13**, which forms diastereomeric ion pairs with chiral carboxylates exhibiting characteristic fluorescence signals.[100] This group has also developed the enantioselective macrocyclic sensor **14** for chiral carboxylate sensing purposes.[101] These chiral carboxylate sensors have been incorporated into simple mix and measure procedures that are readily amenable to high-throughput screening protocols. Wolf and coworkers used 1,8-diacridylnaphthalene sensors for combined concentration and *ee* determination of chiral carboxylic acids and other compound classes which is discussed in greater detail in Section IV.[102-104]

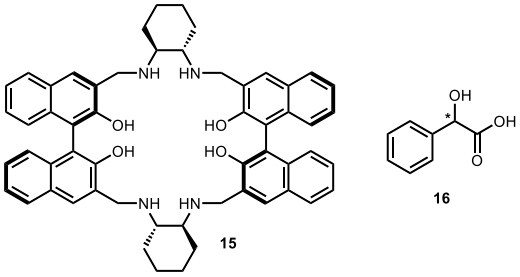
b. Covalent Substrate Binding Assays The Pu group has demonstrated the usefulness of covalent substrate binding with BINOL based probes.[105-108] In one example, a BINOL derived aldehyde was used for quantitative *ee* determination of diamines, amino alcohols, and amino acids. Imine formation and metal complexation to a Zn(II) center gave distinctive enantioselective fluorescent responses.[105] In another approach, they developed an enantioselective perfluoroalkyl BINOL-based diketone sensor for amines, diamines and amino alcohols.[106-107] Most compounds are not soluble in a fluorinated solvent without a highly fluorinated substituent. Thus, chiral recognition in the fluorous phase offers the advantage of minimizing the interference of other species in a screening protocol: i.e. catalyst, ligand, co-additive, etc., potentially allowing for the rapid screening of crude reaction mixtures. Altogether this minimizes the work-up needed prior to optical determination of *ee* values, and is a major advance in making optical *ee* assays increasingly user-friendly.

III. Pioneering Efforts Toward Real-World Sensing Application

The advances in optical methods for quantitative *ee* determination have led to their use in a variety of high-throughput screening protocols for asymmetric reaction discovery. The "proof is in the pudding", and here we

demonstrate that the protocols can truly be used to obviate the use of chiral HPLC in many instances.

In one of the earliest approaches towards optical methods for asymmetric reaction discovery, the Pu group developed macrocycle **15** for fluorescence detection of mandelic acid, **16**.[109] Using both enantiomers of the sensor, the enantiomeric composition of a reaction mixture can be determined with a calibration curve. To demonstrate the suitability of this assay for HTS, they investigated the conversion of an aldehyde to an α-hydroxy ester. A screening protocol was developed such that the asymmetric reaction product precipitated out, avoiding the need for purification prior to *ee* analysis. Subsequent hydrolysis of the methyl mandelate gave the α-hydroxy acid and the *ee* of the reaction was determined with the enantioselective fluorosensors. The optically determined *ee* values correlated well with chiral HPLC results.



Similarly, Wolf and coworkers utilized the axially chiral 1,8-diquinolylnaphthalene *N,N'*-dioxide, **17**, for the enantioselective analysis of an enzymatic reaction (Figure 9).[110] They investigated the kinetic resolution of *trans*-1,2-diaminocyclohexane with dimethyl malonate by *Candida antarctica* lipase (CAL). The 1,8-diquinolylnaphthalene *N,N'*-dioxide exhibits enantioselective fluorescence enhancement upon formation of diastereomeric hydrogen bond adducts with the diamine enantiomers. Screening of the reaction course was accomplished with small aliquots of product mixtures after fast acid/base extraction. The *ee* values were determined from a calibration curve and verified by chiral HPLC, which required a cumbersome product derivatization step prior to analysis. Thus, the optical sensing method streamlines the reaction monitoring by reducing both labor and time and is amenable to other asymmetric transformations.

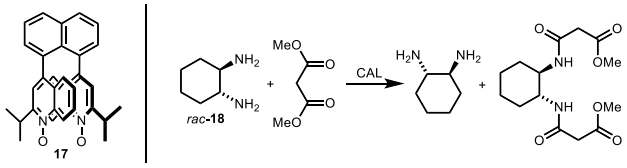


Figure 9. Enantioselective fluorosensor **17** used for the screening of the kinetic resolution of *trans*-1,2-diaminocyclohexane.

Yanagisawa developed a HTS protocol that speeds up the optimization of the $Cu(OAc)_2$-catalyzed Henry reaction between a benzaldehyde derivative and nitromethane (Figure 10).[111-112] In this approach, chiral imidazoline-amine ligands were immobilized on a solid surface and then tested. Upon completion of the reaction, the mixtures were analyzed via continuous injections into a CD spectrophotometer. Reactions with high yield/low *ee* values and low yield/high *ee* values resulted in a CD signal with low magnitude. A maximum CD signal was only observed for reactions with both high yield and high enantioselectivity. This pioneering HTS effort led to the discovery of a method that gives the desired nitroaldol product in 95% *ee*. The tandem use of combinatorial chemistry with CD spectroscopy allowed for the screening of experimental parameters that would otherwise be omitted due to the sheer number of reactions that would need to be processed. Furthermore, the use of immobilized catalysts simplifies reaction screening because any interference of potentially CD active catalysts with the optical *ee* determination is eliminated. This method is ultimately amenable to the analysis of combinatorially-derived catalysts generated on a solid-support by a split-and-pool synthesis approach. Optical methods for *ee* determination can have a large advantage over chromatographic methods in such cases. We discuss below, however, that this has become reality for homogeneous asymmetric reaction analysis as well.

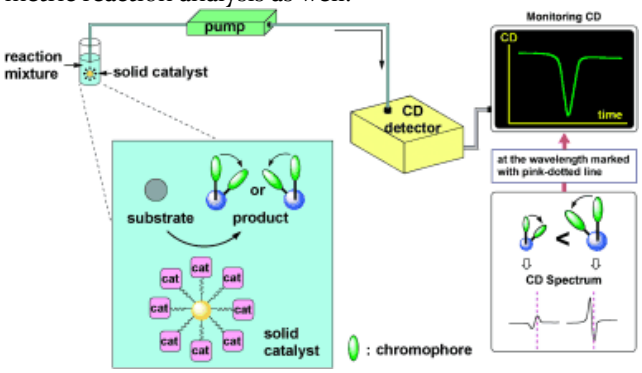


Figure 10. Schematic for HTS of heterogeneous catalysis with continuous injection CD spectrophotometry. Reproduced with permission from reference 111.

In an alternative approach, the Berkowitz lab developed enzymatic methods for the discovery of asymmetric reactions.[113-114] In one example, an *in situ* enzymatic screening (ISES) approach was applied to the kinetic resolution of propylene oxide (Figure 11).[113] A double-cuvette setup was used where the cobalt catalyst and starting material were dissolved in a common lower organic layer and the upper aqueous layer contained a reporting enzyme specific for the *R* (cuvette 1) or *S* enantiomer (cuvette 2) and an oxidizing agent ($NADP^+/NAD^+$) that acts as the chromophoric reporter. As the starting material is converted, the hydrophilic diol product diffuses from the lower organic layer into the upper aqueous layer, where an increase in absorbance at 340 nm can be monitored via UV-Vis spectrophotometry. With this approach, 49 reactions were conducted in parallel and 25 active catalysts giving *ee* values up to 65% were identified. This ISES approach is very attractive for several reasons: it allows real-time reaction monitoring without product derivatization and it relies on a readily available sensor system. We are confident such strategies will have a high impact on asymmetric reaction development if adopted widely.

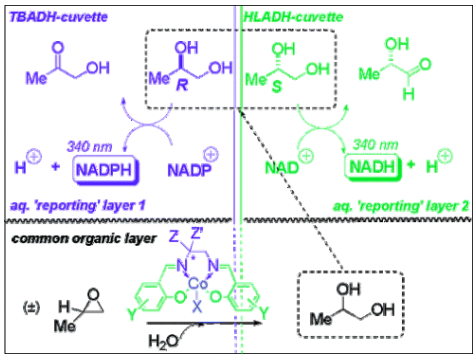


Figure 11. Principles of ISES. TBADH is an alcohol dehydrogenase from *Thermoanaerobium brockii* and HLADH is an alcohol dehydrogenase from horse liver.

IV. Combined Concentration and ee Analysis

For a long time, the primary foci within the chiroptical sensing arena were to investigate the fundamentals of chiral recognition and amplification processes, and to design probes that can be used for reliable determination of the absolute configuration of chiral compounds. Through these contributions it became apparent that optical probes can also be used for *ee* quantification, much of which is delineated above, and the first few examples of reaction analysis appeared, albeit with some practical limitations and narrow reaction scope. These pioneering efforts presented above, however, clearly demonstrated the prospect of optical assays that would allow determination of both the total amount *and* the enantiomeric composition of a chiral analyte. In recent years, several groups have come forward with innovative sensor designs and concepts that achieve this goal. These achievements can be considered key stepping stones on the path toward broadly useful optical asymmetric reaction screening. With this ultimate application in mind, we emphasize in the following discussion some of the most important features of integrated concentration and *ee* sensing: general scope, practicality and speed. We believe that high accuracy is generally desirable but less significant. The purpose of parallel analysis of hundreds of reactions is to rapidly identify the hits and general trends, that is, reaction conditions that result in yields and *ee* values above the 90% threshold and error margins of a few percent are generally considered acceptable.

A. Combined Sensing with Racemic and Enantiopure Probes Wolf and coworkers introduced the axially chiral 1,8-diacridylnaphthalenes **19** and **20** and the corresponding dioxides **21** and **22** for fluorescence sensing of a wide range of chiral compounds (Figure 12).[102-103] These probes have a fairly rigid scaffold but possess sufficient one-dimensional flexibility to accommodate analytes of varying size in the C_2-symmetric cleft. The hydrogen bond interactions with chiral carboxylic acids, hydroxy acids, diamines, and protected amino acids cause enantioselective fluorescence enhancement or quenching that can be used for quantitative concentration and *ee* analysis. First, the sensor is used in its racemic form to determine the total analyte concentration. With that information in hand, the sample *ee* is determined in a separate step by employing enantiopure probe in essentially the same assay. Altogether, the use of a single fluorosensor - first in racemic and then in enantiopure form - allows stereochemical analysis with high accuracy. For example, the concentration and *ee* of nonracemic samples of chloropropionic acid were quantified with error margins below 3% using *anti*-1,8-bis(3'-*tert*-butyl-9'-acridyl)naphthalene, **19**, as the sensor. The same principles were exploited for the sensing of diamine **18** using a chiral iron(II) complex by Feng *et al.*[115]

Inspired by Anslyn's success with indicator displacement assays, Mei and Wolf developed a ligand displacement strategy using scandium(III) complexes carrying two 1,8-diacridylnaphthalene *N,N'*-dioxide ligands (Figure 12).[87-88] The *N,N'*-dioxides are subsequently displaced from the metal center by two equivalents of a chiral analyte, including unprotected amino acids, amino alcohols, amines and carboxylic acids, and both protic and aprotic solvents are tolerated.[104] The substitution of the *N,N'*-dioxide ligands **21** or **22** by the metal coordinating analyte coincides with characteristic UV absorption and fluorescence changes at 410 and 588 nm, respectively. In analogy to the aforementioned sensing strategy, the combination of two assays is required to achieve concentration and *ee* analysis. The concentration value is first calculated from the change in the UV (or fluorescence) signal observed with a racemic Sc(*N,N'*-dioxide)$_2$ complex and the enantiomeric sample composition is then determined by using the scandium(III) complex in enantiopure form.

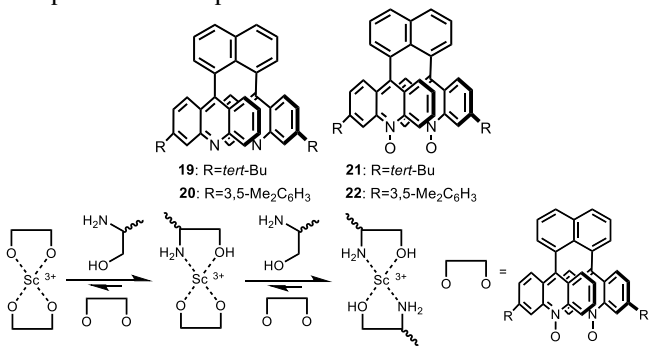


Figure 12. 1,8-Diacridylnapthalene sensor scaffolds used for combined *ee* and concentration fluorosensing and the ligand displacement reaction.

B. The One-Sensor-Double-Readout Approach: Covalent Analyte Binding The use of a sensor first in racemic and then in enantiopure form for concentration and *ee* analysis, respectively, requires two tests albeit with the same setup. Alternatively, the sensing of both unknowns can be accomplished with a single assay. Pu and Yu introduced an elegant IDA based on dynamic covalent chemistry with the BINOL derived diimine **23** (Figure 13).[116] Imine metathesis in the presence of excess of diamine **18** and zinc acetate gives a diastereomeric mixture of **25** and two equivalents of free 2-naphthylamine, **24**, which serves as the displaced indicator. The displacement thus affords **24** with a fluorescence emission maximum at 427 nm that can be correlated to the original concentration of **18** while the fluorescence intensity of **25** measured at 525 nm depends on

the chirality of **18** and provides information about the analyte *ee*. Similar results were obtained with phenylalaninol, phenylglycinol and alaninol. The free sensor shows only weak fluorescence signals under the same conditions and generates negligible background noise. Concentration and *ee* analysis of samples of **18** with this IDA gave results that deviated only by a few percent from the actual values. The same concept was exploited by Pilicer and Wolf for the development of a biomimetic IDA with an imine analogue of pyridoxal-5'-phosphate, the active form of Vitamin B_6. In this case, stoichiometric imine metathesis allows simultaneous determination of the concentration, absolute configuration and *ee* of amino acids, amino alcohols and amines via circular dichroism and fluorescence measurements.[117] These optical assays allow convenient concentration and *ee* analysis, and are considered major steps toward optical asymmetric reaction screening.

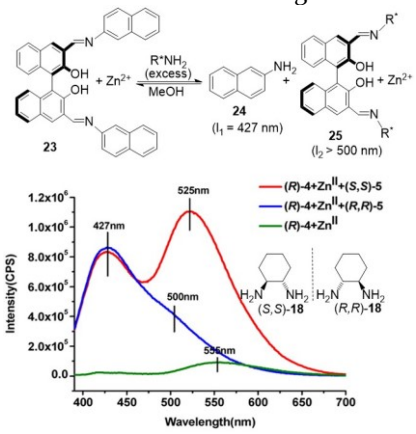


Figure 13. Enantioselective indicator displacement assay with the BINOL diimine **23**. (*R*)-**23** + $Zn(OAc)_2$ + (*S,S*)-**18** (red), (*R*)-**23** + $Zn(OAc)_2$ + (*R,R*)-**18** (blue), (*R*)-**23** + $Zn(OAc)_2$ (green).

Anslyn and coworkers demonstrated that the analytical protocol can be streamlined by combining an enantioselective IDA with a dual-chamber quartz cuvette.[118] As shown in Figure 14A, one cuvette was filled with a methanolic aqueous solution containing the colorless indicator 4-methylesculetin (ML), and the achiral boronic acid **26** as host. The other cuvette was charged with the red indicator alizarin complexone (AC), and the chiral host (*S,S*)-**27**. The reversible interactions between indicators and hosts in each cuvette were found to modulate the UV signatures of the dyes (Figure 14B). The corresponding equilibria are disturbed upon addition of nonracemic 2-hydroxy-3-phenylpropionic acid **28**, which ultimately changes the UV absorbance of the two separate systems (Figure 14C). The use of the dual-chamber cuvette allowed simultaneous collection of the optical changes by a single measurement. An artificial neural network analysis (ANN) of the UV data obtained with several samples of **28** showed that both concentration and *ee* of the analyte can be determined with error margins of no more than 2%. The use of a dual chamber cuvette is simple and is amenable to conditions of many synthetic organic reactions.

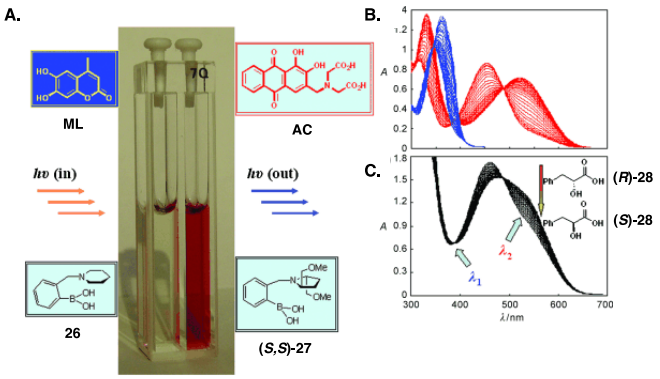


Figure 14. A) Dual-chamber cuvette containing the indicators ML (left) and AC (right) in 75% methanolic aqueous solution with 10 mM HEPES at pH 7.4. B) UV Spectra of ML in the presence of varying amounts of **26** (blue) and of AC with varying amounts of (*S,S*)-**27** (red). C) Change in the UV absorptions in the presence of hydroxy acid **28** with changing ee. Adapted with permission from reference 118.

The stereodynamic sensor **29** developed by the Wolf group carries a salicylaldehyde-derived receptor unit that undergoes fast Schiff base formation with unprotected amino acids, amino alcohols and amines (Figure 15).[119-120] The binding of a chiral analyte results in distinct central-to-axial chirality amplification across the sensor scaffold and a strong circular dichroism signal at high wavelengths. This chiroptical sensor response provides direct information about the absolute configuration and *ee* of the analyte. The binding event also affects the fluorescence of the adjacent pyridyl *N*-oxide moiety. This optical change is independent of the chirality and enantiomeric composition of the analyte, and thus provides the basis for calculation of the total concentration. This concept of stereodynamic chirality sensing and simultaneous CD/fluorescence analysis is broadly applicable. It has been successfully exploited for accurate determination of the *ee* and concentration of several compounds, and a related probe that accomplishes the same task with a CD/UV response has been reported.[121]

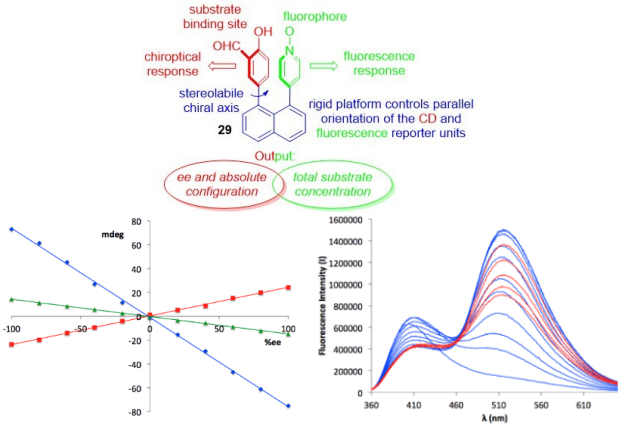


Figure 15. Illustration of the chiroptical sensing concept with **29** (left). CD Signals at 260 (blue), 290 (red), and 340 nm (green) as a function of the ee of 1,2-diphenylaminoethanol using sensor **29** at 7.50 10^{-5} M (middle). Fluorescence emission at 515 nm in the presence of substoichiometric to

equimolar amounts (blue) and excess of the analyte (red) (right).

The Pu group was able to determine the overall concentration and the *ee* of diamine **18** by a single fluorescence assay with the BINOL-derived probe **30** (Figure 16).[122] This is possible because the sensor exhibits two fluorescence outputs - one being highly enantioselective and one responding mostly to the total analyte concentration irrespective of the enantiomeric composition. The covalent substrate binding by probe **30** predominantly gives the products **31** and **32** and it coincides with a new fluorescence emission at 370 nm. This signal is nearly independent of the substrate chirality and it can therefore be correlated to the overall diamine concentration. By contrast, the intensity of a second emission at 438 nm depends on the chirality of **18** thus providing an independent means for *ee* analysis. Several samples of varying concentration and *ee* of **18** were analyzed with sufficient accuracy for HTS purposes. The integration of concentration and *ee* determination into a single measurement greatly simplifies the analytical protocol and this approach is generally useful. In fact, simultaneous concentration and *ee* analysis of diaminocyclohexane, phenylalaninol, phenylglycinol, alaninol and phenylalanine based on a similar dual fluorescence response with a probe mixture consisting of a BINOL-derived bisaldehyde, salicylaldehyde and zinc acetate was demonstrated.[123]

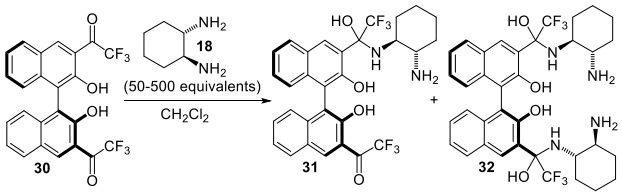


Figure 16. Sensing of diamine **18** with the BINOL-derived probe (*S*)-**30**.

C. Coordination Complexes with Dual Readouts Anslyn, Chin and coworkers realized early the potential of metal coordination chemistry for integrated concentration and *ee* sensing. They observed that vicinal diamines alter the metal-to-ligand charge transfer bands and the intrinsic CD signals in the 300-400 nm region of enantiopure [Cu(I)(BINAP)(MeCN)$_2$]PF$_6$, **33** (Figure 17).[124] The characteristic CD effects can be used for identification of individual analytes and for stereoselective sensing. Concentration and *ee* quantification with sufficient accuracy for HTS purposes were achieved based on multilayer perceptron artificial neuron network analysis. This seminal work demonstrated the practicality of chiroptical sensing with metal complexes. The same principles were exploited for fingerprint analysis and quantification of diamines with CD-silent racemic BINAP and Tol-BINAP derived Cu(I) and Pd(II) complexes.[125]

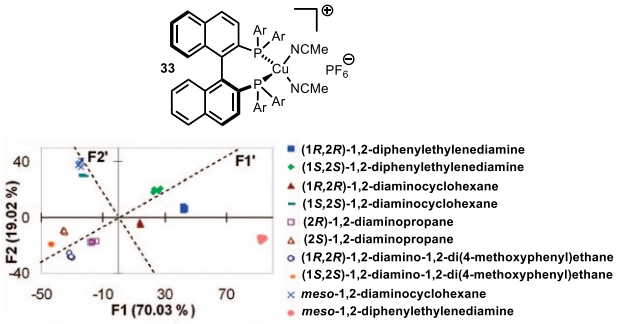


Figure 17. Structure of **33** and chemoselective response patterns with vicinal diamines based on linear discriminant analysis.

Zhang and Wolf showed that concentration and *ee* quantitation can also be accomplished with [Pd(II)(DPPF)(MeCN)$_2$](SbF$_6$)$_2$, **34**, and other stereodynamic metal complexes that carry chromophoric tropos ligands, for example **35**.[126] Coordination of a variety of diamines and amino alcohols to the CD-silent metal complex is accompanied by spontaneous imprinting of the analyte chirality onto the diphosphine ligand (Figure 18). The fast binding and chiral amplification processes generate a strong CD readout and a change in the UV absorbance of the palladium complex. The former is a function of the enantiomeric composition of the analyte and the latter correlates to its overall concentration. This sensing strategy is quite adaptable and other readily available metal probes have been utilized in the same way.[127] This quite general strategy can be immediately adopted by any investigators creating chiral diamines and amino alcohols.

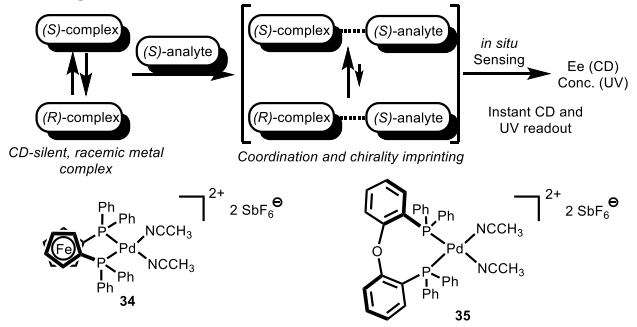


Figure 18. Principles of chirality sensing with stereodynamic palladium complexes **34** and **35**. Adapted with permission from reference 126.

Following the examples of Anslyn, Chin, and Wolf, Feng's group used a racemic *N,N'*-dioxide-iron(III) complex for determination of the absolute configuration, enantiomeric composition, and concentration of several hydroxy acids (Figure 19).[128] Metal coordination of the bidentate analyte was found to induce strong CD amplitudes above 300 nm and a significant fluorescence increase at 337 nm. These two responses were successfully used for quantitative concentration and *ee* analysis of 2-hydroxy-3-phenylpropionic acid samples. The results are sufficiently accurate for HTS purposes and underscore once more that chiroptical chemosensing with metal complexes provides

generally useful and practical means for high-throughput stereochemical analysis.

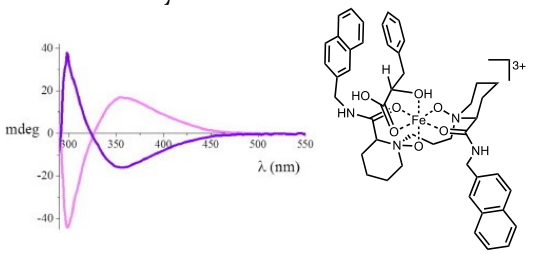


Figure 19. Cotton effects and the proposed binding model of 2-hydroxy-3-phenylpropionic acid to an *N,N'*-dioxide-iron(III) sensor. Adapted with permission from reference 128.

V. Asymmetric Reaction Screening

The examples of simultaneous concentration and *ee* determination presented in the preceding section underscore the general capability of optical sensing when nonracemic, but otherwise chemically pure, samples are used. The increased throughput, reduced labor, and the chemical sustainability benefits of optical yield and *ee* sensing are inherent features of this technology and not limited to a specific reaction type. The real power of optical HTS with regard to time, labor, energy and chemical waste reduction, however, lies in the *direct* analysis of crude microscale reaction mixtures, and by eliminating elaborate purification steps. The composition of asymmetric reaction mixtures is typically complicated and quite different from artificially prepared samples that only contain enantiomers of a single compound in varying amounts. A wide range of chemicals that could interfere with the sensing process, such as remaining starting materials, by-products, catalyst and/or additives, are generally expected to be present in the crude reaction mixture prior to work-up. Direct asymmetric reaction screening therefore requires optical assays that are robust, adaptable to microwell plate technology, and applicable to minute amounts of complex mixtures. Despite these challenges, several case studies that highlight the practicality and the advantages of optical asymmetric reaction sensing have been reported to date.

A. It Works: Optical Sensing of the Absolute Configuration, *ee*, and Yield from Crude Reaction Mixtures

The first step in this direction was made in 2009 when Anslyn and coworkers demonstrated that optical chemosensing is adaptable to yield and *ee* reaction analysis (Figure 20).[129] A tailored UV/Vis eIDA was applied to the Sharpless asymmetric dihydroxylation (AD) of *trans*-stilbene, **36**. The sensing of the isolated reaction product **37** was carried out with a combination of the boronic acid hosts **38-40** and the indicator **41**. The raw UV absorbance data collected on a 96-well plate were subjected to 3-layered MLP analysis which gave results with sufficient accuracy for HTS purposes.

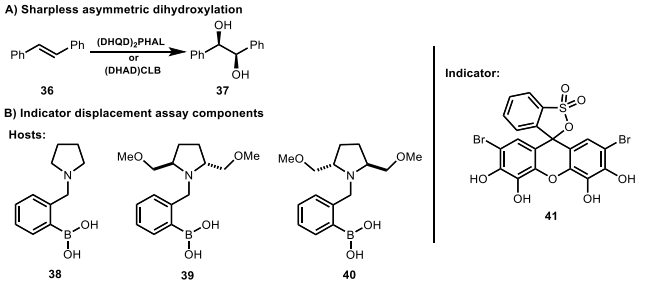


Figure 20. Sharpless asymmetric dihydroxylation and IDA components.

Essentially the same statistical treatment of metal-to-ligand charge transfer induced CD changes observed upon coordination of a pyridine-2-carboxaldehyde derived Schiff base of 1-phenylethylamine, **43**, to the (BINAP)Cu(I) complex, **33**, allowed fast determination of the *ee* and yield of the reductive amination of acetophenone, **42** (Figure 21).[130] Quantitative Schiff base formation of the reduction product **43** was achieved and the mixture was then loaded without further purification onto a 96-well plate for CD analysis with the copper complex **33**. It is important to note that the product derivatization step and the succeeding CD measurements can be easily automated. As is the case with most chiroptical assays described in this Section, this protocol eliminates the need for product isolation, typically a time-consuming and labor-intensive task. This streamlines the reaction development process and sets the stage for real high-throughput analysis.

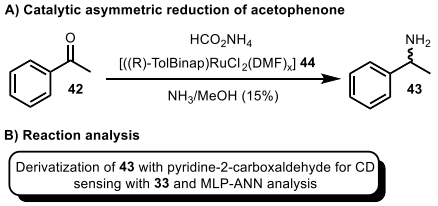


Figure 21. HTS of the asymmetric reduction of **42**.

Some of the major advantages of optical reaction analysis over traditional chromatographic methods were quantified by the Wolf group. They showed that fast chiroptical sensing of one milligram of crude reaction mixtures obtained by the Sharpless AD of *trans*-stilbene allows accurate determination of the absolute configuration, yield and *ee* of the diol product **37**.[131] The simple mix-and-measure procedure with a stereodynamic titanium complex of ligand **46** is not only adaptable to miniature-scale reactions; it also eliminates cumbersome work-up steps. Examination of four reaction samples showed that the sensing methodology significantly reduces solvent waste and analysis time compared to automated flash chromatography purification followed by gravimetric analysis and chiral HPLC *ee* determination (Figure 22). Given the serial nature of chromatography, the superior efficiency of optical sensing which is amenable to parallel data acquisition can be expected to improve even further when large numbers of samples need to be analyzed.

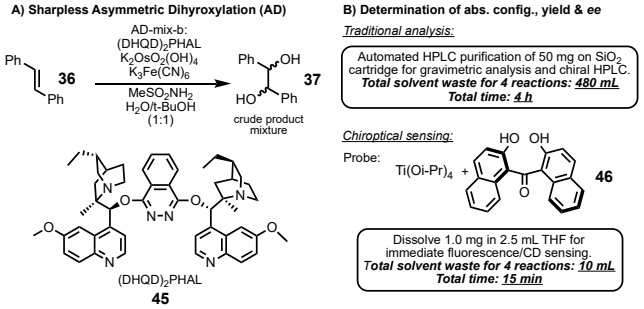


Figure 22. Fluorescence/CD sensing of one milligram of crude Sharpless asymmetric dihydroxylation mixtures.

The chiroptical Brønsted/Lewis acid receptor **47** was used to evaluate the asymmetric reduction of phenylglyoxylic acid **48** to **49** with (+)-DIP-Cl (Figure 23).[132] In this case, 0.5 milligram of the crude reaction mixture sufficed for yield and *ee* quantification which was accomplished through fast UV and CD measurements and comparison with a calibration curve. In analogy to the breakdown of the Sharpless AD sensing example shown above, only minute sample amounts were required and the total analysis time and solvent consumption were reduced to only a few percent compared to traditional reaction analysis.

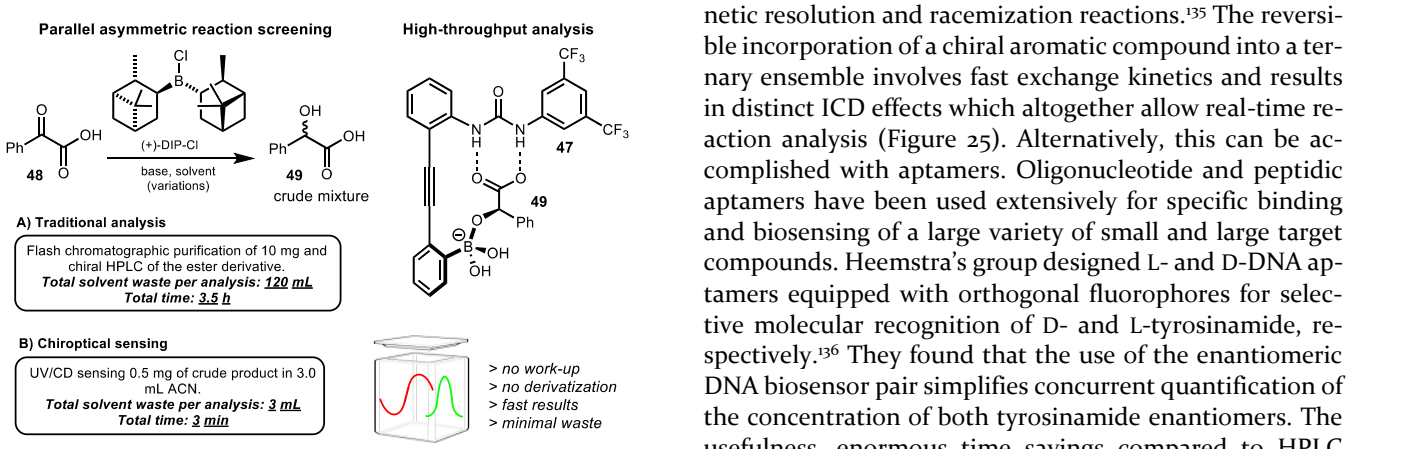


Figure 23. UV/CD sensing of crude phenylglyoxylic acid reduction mixtures.

As we have alluded repeatedly in the preceding sections, sensing assays based on DCC and the formation of supramolecular assemblies are particularly attractive for asymmetric reaction screening. The Wolf group developed a practical sensing protocol that was applied to the iridium catalyzed asymmetric hydrogenation of the iminium chloride **50** to the primary amine **43**.[133] Yield and *ee* determination was accomplished through rapid self-assembly of palladium(II) acetate, a stereodynamic phosphine ligand carrying a formyl group **51**, and a chiral compound with a primary amine function - amines, amino alcohols or amino acid can be used - that is captured as a Schiff base (Figure 24). This rugged chiroptical assay eliminates the need to purify the hydrogenation product and allows direct optical sensing of microscale amounts of crude reaction mixtures. The performance of several chiral phosphine-derived iridium catalysts in the asymmetric hydrogenation of **50** was investigated and these reactions were used to compare traditional analysis of the isolated amine product with direct optical sensing of the crude product. The absolute configuration, yield and *ee* of **43** were determined with good accuracy, and at significantly reduced cost, labor, solvent consumption and time. In a related study, a CD sensing ensemble assay was used for accelerated analysis of rhodium catalyzed asymmetric hydrogenations of imine **50**.[134]

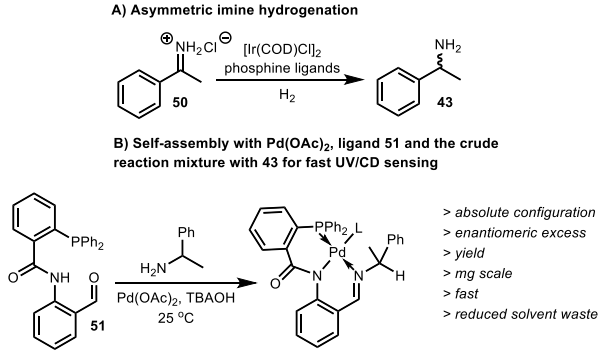


Figure 24. Chiroptical analysis of an asymmetric iminium hydrogenation reaction.

Biedermann and Nau successfully exploited noncovalent substrate binding with a cucurbit[8]uril (CB8) host and a dicationic dye to monitor the stereochemical course of kinetic resolution and racemization reactions.[135] The reversible incorporation of a chiral aromatic compound into a ternary ensemble involves fast exchange kinetics and results in distinct ICD effects which altogether allow real-time reaction analysis (Figure 25). Alternatively, this can be accomplished with aptamers. Oligonucleotide and peptidic aptamers have been used extensively for specific binding and biosensing of a large variety of small and large target compounds. Heemstra's group designed L- and D-DNA aptamers equipped with orthogonal fluorophores for selective molecular recognition of D- and L-tyrosinamide, respectively.[136] They found that the use of the enantiomeric DNA biosensor pair simplifies concurrent quantification of the concentration of both tyrosinamide enantiomers. The usefulness, enormous time savings compared to HPLC analysis, and the high accuracy of this approach which is perfectly compatible with high-throughput equipment were demonstrated by monitoring the aminolysis and concomitant racemization of the ethyl ester of D-tyrosine, **52**, to D/L-tyrosinamide, **53**, in the presence of ammonium hydroxide at various temperatures (Figure 25). While CB-hosts can be immediately exploited by any chemist because the assay is simple and CB8 is commercially available, the creation of aptamers is more specialized. Importantly, these are both extremely attractive approaches to HTS of *ee* values. This is because they demonstrate that analytes can be targeted without a focus on reactivity with a particular functional group.

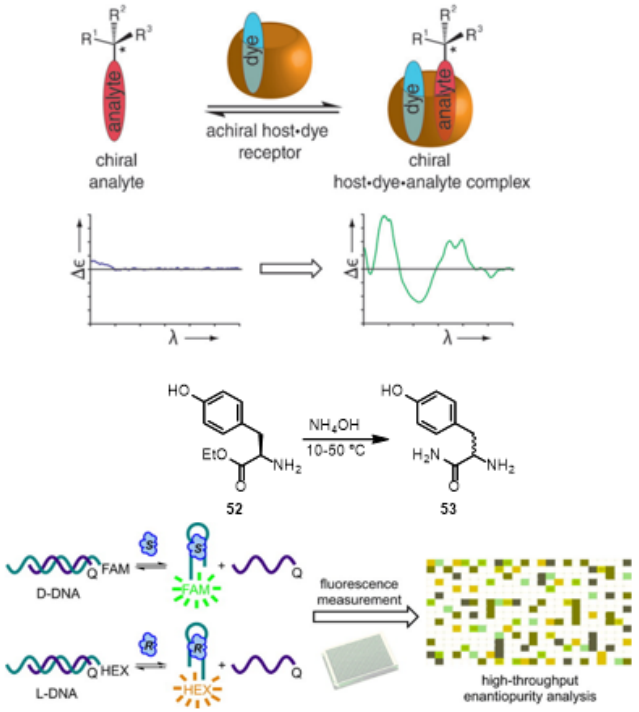


Figure 25. Top: Reaction monitoring with noncovalent supramolecular cucurbituril assemblies, Bottom: Yield/ee analysis of the aminolysis of **52** with enantiomeric DNA aptamers. Reproduced with permission from reference 135.

Anzenbacher and colleagues demonstrated that high sample throughput (*ee* determination of 20 samples in less than a minute) can be achieved by combining fluorosensing with microwell plate technology.[137] They analyzed crude reaction mixtures of the asymmetric Noyori transfer hydrogenation of benzil, **54**, with dynamic covalent assemblies obtained from the hydrogenation product, **37**, formylphenylboronic acid, **55**, and a tryptophanol fluorescence reporter **56** (Figure 26). The corresponding diastereomeric iminoboronate esters **57** have distinctive fluorescence emission profiles that were exploited for highly accurate statistical analysis of the *ee* and yield of the hydrogen transfer reaction.

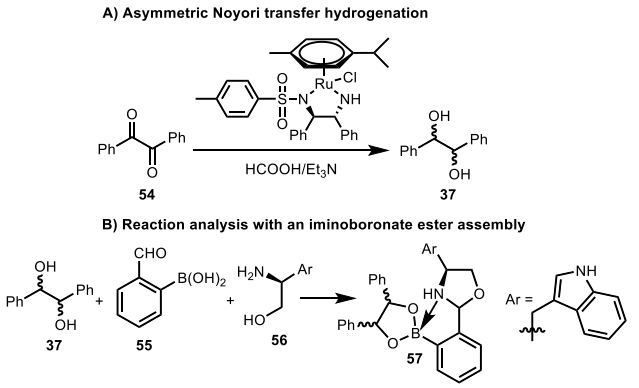


Figure 26. High-throughput sensing of the Noyori hydrogenation of benzil using a fluorescent iminoboronate ester assembly.

B. Accelerating Asymmetric Reaction Development with Hybrid Methods To date, the majority of reports on asymmetric reaction sensing have focused on general utility, accuracy, and practicality aspects with the general intent to evaluate the robustness of the assay and to compare analysis time and chemical waste production to that of traditional methods. Taken together, the examples presented above underscore that asymmetric reaction analysis with optical sensing assays has matured into a full-fledged technique that accelerates reaction analysis at reduced cost. In this regard, the Sharpless AD and other established reactions have been excellent choices to showcase the superior features of the sensing methodology over current laboratory practices. Although it is outside the scope of this review, we note that the ruggedness of optical chirality sensing assays does not only allow high-throughput screening of asymmetric reactions, it also provides exciting venues for accurate stereochemical analysis of biological targets in complex samples.[138]

The examples outlined in the preceding sections show that the time is ripe to routinely incorporate optical sensing at the reaction development stage. In other words, chiroptical sensing has matured and emerged out of its testing phase. It can now be used to expedite serendipitous screening of asymmetric reaction parameters. Realizing that high-throughput asymmetric reaction development has become reality, Miller and Anslyn applied a previously developed supramolecular CD sensing assay to speed up ongoing efforts to improve the Baeyer-Villiger oxidation shown in Figure 27.[139] They screened the performance of small peptide catalysts in the desymmetrization of the cyclic ketone **58**. For determination of the absolute configuration and *ee*, the lactone product **59** was subjected to methanolysis and a simple silica plug purification. The stereochemical analysis of the corresponding alcohol **60** was achieved by CD measurements with the self-assembled complex **61**. This approach was successful in identifying the best catalysts for this reaction and it offered significant time-savings over chiral HPLC.

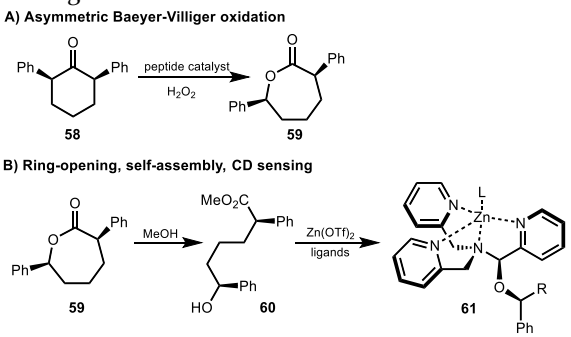


Figure 27. CD Analysis of an asymmetric Baeyer-Villiger Oxidation

Alternatively, the combination of chiroptical sensing and chromatographic analysis can increase the pace of asymmetric reaction development. Joyce introduced a fully automated HPLC/CD approach to determine the stereoselective outcome of an enzymatic transamination.[140] Krische and Anslyn further developed this idea by combining opti-

cal sensing and TLC for comprehensive screening of an enantioselective iridium-catalyzed allylation reaction (Figure 28).[141] Their TLC/CD hybrid assay enabled fast screening of over 400 reaction conditions by systematic variation of 18 preformed iridium complexes, base additives, and solvents. The reactions and purification steps were conducted in parallel using 96-well plates. Yields were determined by quantitative TLC analysis while the enantiopurity of the chiral alcohol **64** was assessed by CD sensing with the corresponding supramolecular assembly **61** as shown above in Figure 27. These HTS efforts led to the discovery of an improved catalytic method that is more cost-effective and gives higher *ee* values than previously optimized procedures.

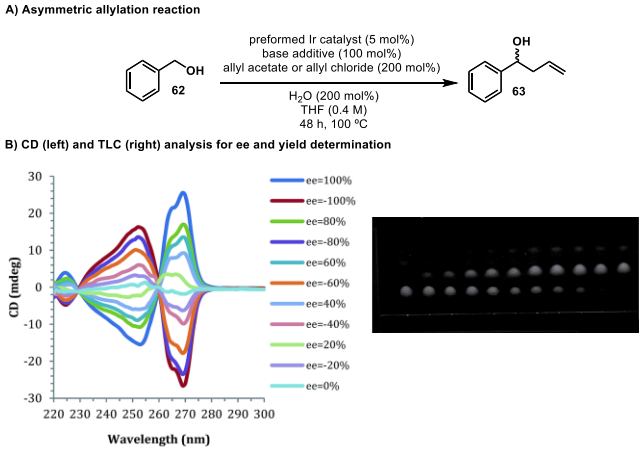


Figure 28. CD/TLC screening of the asymmetric iridium-catalyzed allylation of benzyl alcohol **63** using assembly **61**. Adapted with permission from reference 141.

VI. Outlook

During the past 15 years, optical chirality sensing has emerged from an "academic Mauerblümchen" activity into a powerful technique that allows fast determination of the absolute configuration, *ee* and yield of minute amounts of crude asymmetric reaction mixtures. Robust sensing assays are now available and have been successfully applied to a variety of reaction types, including asymmetric oxidations, reductions and C-C bond formations. Optical HTS can dramatically decrease analysis time, labor, cost, solvent consumption, and waste production compared to traditionally used techniques such as chiral HPLC. Given the compatibility of optical sensing techniques with parallel data acquisition, miniaturization and multi-well assay plate formats, the obvious cost, waste and time savings are likely to increase further when hundreds of samples are analyzed. A switch from traditional screening methods to optical chirality sensing is set to drastically accelerate asymmetric reaction discovery and development projects. We encourage our synthetic methodology colleagues to consider these methods; adopting those that exist, devising their own, or contacting one of the many investigators whose work was highlighted in this review for collaborations and further development.

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† These authors contributed equally. The manuscript was written through contributions of all authors and all authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge the following funding sources for supporting efforts towards rapid methods of *ee* determination: the National Institute of Health (‡R01GM077437), the National Science Foundation (‡GOALI 1665040, §CHE1464547, and §CHE1764135), and the Welch Foundation (‡F-0046).

REFERENCES

1. Krska, S. W.; DiRocco, D. A.; Dreher, S. D.; Shevlin, M., The Evolution of Chemical High-Throughput Experimentation To Address Challenging Problems in Pharmaceutical Synthesis. *Acc Chem Res* **2017,** *50* (12), 2976-2985.
2. Shevlin, M., Practical High-Throughput Experimentation for Chemists. *ACS Med Chem Lett* **2017,** *8* (6), 601-607.
3. Collins, K. D.; Gensch, T.; Glorius, F., Contemporary screening approaches to reaction discovery and development. *Nat Chem* **2014,** *6* (10), 859-71.
4. Jason R. Schmink, A. B., Simon Berrit Scientist-led high-throughput experimentation (HTE) and its utility in academia and industry. *Aldrichimica Acta* **2013,** *46* (3), 71-80.
5. Friedfeld, M. R.; Shevlin, M.; Hoyt, J. M.; Krska, S. W.; Tudge, M. T.; Chirik, P. J., Cobalt precursors for high-throughput discovery of base metal asymmetric alkene hydrogenation catalysts. *Science* **2013,** *342* (6162), 1076-80.
6. Li, H.; Belyk, K. M.; Yin, J.; Chen, Q.; Hyde, A.; Ji, Y.; Oliver, S.; Tudge, M. T.; Campeau, L. C.; Campos, K. R., Enantioselective Synthesis of Hemiaminals via Pd-Catalyzed C-N Coupling with Chiral Bisphosphine Monooxides. *J. Am. Chem. Soc.* **2015,** *137* (43), 13728-31.
7. Collins, K. D.; Glorius, F., A robustness screen for the rapid assessment of chemical reactions. *Nat Chem* **2013,** *5* (7), 597-601.
8. Bajaj, P.; Sreenilayam, G.; Tyagi, V.; Fasan, R., Gram-Scale Synthesis of Chiral Cyclopropane-Containing Drugs and Drug Precursors with Engineered Myoglobin Catalysts Featuring Complementary Stereoselectivity. *Angew. Chem. Int. Ed.* **2016,** *55* (52), 16110-16114.
9. Prier, C. K.; Zhang, R. K.; Buller, A. R.; Brinkmann-Chen, S.; Arnold, F. H., Enantioselective, intermolecular benzylic C–H amination catalysed by an engineered iron-haem enzyme. *Nat. Chem.* **2017,** *9*, 629.
10. Dydio, P.; Key, H. M.; Nazarenko, A.; Rha, J. Y.-E.; Seyedkazemi, V.; Clark, D. S.; Hartwig, J. F., An artificial

metalloenzyme with the kinetics of native enzymes. *Science* **2016,** *354* (6308), 102-106.

11. Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E., RNA-catalysed carbon–carbon bond formation. *Nature* **1997,** *389*, 54.

12. Traverse, J. F.; Snapper, M. L., High-throughput methods for the development of new catalytic asymmetric reactions. *Drug Discov Today* **2002,** *7* (19), 1002-12.

13. Zhong, L., High Throughput Analysis in Support of Process Chemistry and Formulation Research and Development in the Pharmaceutical Industry. In *High-throughput analysis in the pharmaceutical industry*, Wang, P. G., Ed. CRC Press: Boca Raton, FL, 2009; pp 247-278.

14. Schafer, W.; Bu, X.; Gong, X.; Joyce, L. A.; Welch, C. J., 9.02 High-Throughput Analysis for High-Throughput Experimentation in Organic Chemistry A2 - Knochel, Paul. In *Comprehensive Organic Synthesis II (Second Edition)*, Elsevier: Amsterdam, 2014; pp 28-53.

15. De Klerck, K.; Mangelings, D.; Vander Heyden, Y., Supercritical fluid chromatography for the enantioseparation of pharmaceuticals. *J. Pharm. Biomed. Anal.* **2012,** *69*, 77-92.

16. Wu, N.; Clausen, A. M., Fundamental and practical aspects of ultrahigh pressure liquid chromatography for fast separations. *J. Sep. Sci.* **2007,** *30* (8), 1167-82.

17. Abrahim, A.; Al-Sayah, M.; Skrdla, P.; Bereznitski, Y.; Chen, Y.; Wu, N., Practical comparison of 2.7 microm fused-core silica particles and porous sub-2 microm particles for fast separations in pharmaceutical process development. *J. Pharm. Biomed. Anal.* **2010,** *51* (1), 131-7.

18. Catani, M.; Ismail, O. H.; Gasparrini, F.; Antonelli, M.; Pasti, L.; Marchetti, N.; Felletti, S.; Cavazzini, A., Recent advancements and future directions of superficially porous chiral stationary phases for ultrafast high-performance enantioseparations. *Analyst* **2017**, *142*, 555-566.

19. Patel, D. C.; Wahab, M. F.; Armstrong, D. W.; Breitbach, Z. S., Advances in high-throughput and high-efficiency chiral liquid chromatographic separations. *J Chromatogr. A* **2016,** *1467*, 2-18.

20. Kotoni, D.; Ciogli, A.; Molinaro, C.; D'Acquarica, I.; Kocergin, J.; Szczerba, T.; Ritchie, H.; Villani, C.; Gasparrini, F., Introducing enantioselective ultrahigh-pressure liquid chromatography (eUHPLC): theoretical inspections and ultrafast separations on a new sub-2-mum Whelk-O1 stationary phase. *Anal. Chem.* **2012,** *84* (15), 6805-13.

21. Barhate, C. L.; Wahab, M. F.; Breitbach, Z. S.; Bell, D. S.; Armstrong, D. W., High efficiency, narrow particle size distribution, sub-2 mum based macrocyclic glycopeptide chiral stationary phases in HPLC and SFC. *Anal. Chim. Acta* **2015,** *898*, 128-137.

22. Ismail, O. H.; Antonelli, M.; Ciogli, A.; Villani, C.; Cavazzini, A.; Catani, M.; Felletti, S.; Bell, D. S.; Gasparrini, F., Future perspectives in high efficient and ultrafast chiral liquid chromatography through zwitterionic teicoplanin-based 2-mum superficially porous particles. *J. Chromatogr. A* **2017,** *1520*, 91-102.

23. Wahab, M. F.; Wimalasinghe, R. M.; Wang, Y.; Barhate, C. L.; Patel, D. C.; Armstrong, D. W., Salient Sub-Second Separations. *Anal Chem* **2016,** *88* (17), 8821-6.

24. Barhate, C. L.; Joyce, L. A.; Makarov, A. A.; Zawatzky, K.; Bernardoni, F.; Schafer, W. A.; Armstrong, D. W.; Welch, C. J.; Regalado, E. L., Ultrafast chiral separations for high throughput enantiopurity analysis. *Chem. Commun.* **2017,** *53* (3), 509-512.

25. Guo, H.-M.; Tanaka, F., A Fluorogenic Aldehyde Bearing a 1,2,3-Triazole Moiety for Monitoring the Progress of Aldol Reactions. *J. Org. Chem.* **2009,** *74* (6), 2417-2424.

26. Barder, T. E.; Buchwald, S. L., Benchtop Monitoring of Reaction Progress via Visual Recognition with a Handheld UV Lamp: In Situ Monitoring of Boronic Acids in the Suzuki–Miyaura Reaction. *Org. Lett.* **2007,** *9* (1), 137-139.

27. Leung, D.; Kang, S. O.; Anslyn, E. V., Rapid determination of enantiomeric excess: a focus on optical approaches. *Chem. Soc. Rev.* **2012,** *41* (1), 448-479.

28. Wolf, C.; Bentley, K. W., Chirality sensing using stereodynamic probes with distinct electronic circular dichroism output. *Chem. Soc. Rev.* **2013,** *42* (12), 5408-5424.

29. Berova, N.; Di Bari, L.; Pescitelli, G., Application of electronic circular dichroism in configurational and conformational analysis of organic compounds. *Chem. Soc. Rev.* **2007,** *36* (6), 914-31.

30. Huang, X.; Rickman, B. H.; Borhan, B.; Berova, N.; Nakanishi, K., Zinc porphyrin tweezer in host-guest complexation: determination of absolute configurations of diamines, amino acids, and amino alcohols by circular dichroism. *J. Am. Chem. Soc.* **1998,** *120* (24), 6185-6186.

31. Huang, X.; Borhan, B.; Rickman, B. H.; Nakanishi, K.; Berova, N., Zinc porphyrin tweezer in host-guest complexation: determination of absolute configurations of primary monoamines by circular dichroism. *Chem. - Eur. J.* **2000,** *6* (2), 216-224.

32. Anyika, M.; Gholami, H.; Ashtekar, K. D.; Acho, R.; Borhan, B., Point-to-Axial Chirality Transfer-A New Probe for "Sensing" the Absolute Configurations of Monoamines. *J. Am. Chem. Soc.* **2014,** *136* (2), 550-553.

33. Gholami, H.; Anyika, M.; Zhang, J.; Vasileiou, C.; Borhan, B., Host-Guest Assembly of a Molecular Reporter with Chiral Cyanohydrins for Assignment of Absolute Stereochemistry. *Chem. - Eur. J.* **2016,** *22* (27), 9235-9239.

34. Li, X.; Burrell, C. E.; Staples, R. J.; Borhan, B., Absolute Configuration for 1,n-Glycols: A Nonempirical Approach to Long-Range Stereochemical Determination. *J. Am. Chem. Soc.* **2012,** *134* (22), 9026-9029.

35. Tanasova, M.; Anyika, M.; Borhan, B., Sensing Remote Chirality: Stereochemical Determination of β-, γ-, and δ-Chiral Carboxylic Acids. *Angew. Chem., Int. Ed.* **2015,** *54* (14), 4274-4278.

36. Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi, K.; Berova, N., Absolute Configurational Assignments of Secondary Amines by CD-Sensitive Dimeric Zinc Porphyrin Host. *J. Am. Chem. Soc.* **2002,** *124* (35), 10320-10335.

37. Kurtan, T.; Nesnas, N.; Li, Y.-Q.; Huang, X.; Nakanishi, K.; Berova, N., Chiral Recognition by CD-Sensitive Dimeric Zinc Porphyrin Host. 1. Chiroptical

Protocol for Absolute Configurational Assignments of Monoalcohols and Primary Monoamines. *J. Am. Chem. Soc.* **2001,** *123* (25), 5962-5973.

38. Kurtan, T.; Nesnas, N.; Koehn, F. E.; Li, Y.-Q.; Nakanishi, K.; Berova, N., Chiral Recognition by CD-Sensitive Dimeric Zinc Porphyrin Host. 2. Structural Studies of Host-Guest Complexes with Chiral Alcohol and Monoamine Conjugates. *J. Am. Chem. Soc.* **2001,** *123* (25), 5974-5982.

39. Li, X.; Borhan, B., Prompt Determination of Absolute Configuration for Epoxy Alcohols via Exciton Chirality Protocol. *J. Am. Chem. Soc.* **2008,** *130* (48), 16126-16127.

40. Li, X.; Tanasova, M.; Vasileiou, C.; Borhan, B., Fluorinated Porphyrin Tweezer: A Powerful Reporter of Absolute Configuration for erythro and threo Diols, Amino Alcohols, and Diamines. *J. Am. Chem. Soc.* **2008,** *130* (6), 1885-1893.

41. Proni, G.; Pescitelli, G.; Huang, X.; Nakanishi, K.; Berova, N., Magnesium Tetraarylporphyrin Tweezer: a CD-Sensitive Host for Absolute Configurational Assignments of α-Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2003,** *125* (42), 12914-12927.

42. Proni, G.; Pescitelli, G.; Huang, X.; Quraishi, N. Q.; Nakanishi, K.; Berova, N., Configurational assignment of alpha-chiral carboxylic acids by complexation to dimeric Zn-porphyrin: host-guest structure, chiral recognition and circular dichroism. *Chem. Commun.* **2002,** (15), 1590-1.

43. Tanasova, M.; Vasileiou, C.; Olumolade, O. O.; Borhan, B., Enhancement of exciton coupled circular dichroism with sterically encumbered bis-porphyrin tweezers. *Chirality* **2009,** *21* (3), 374-82.

44. Yang, Q.; Olmsted, C.; Borhan, B., Absolute Stereochemical Determination of Chiral Carboxylic Acids. *Org. Lett.* **2002,** *4* (20), 3423-3426.

45. Zhang, J.; Gholami, H.; Ding, X.; Chun, M.; Vasileiou, C.; Nehira, T.; Borhan, B., Computationally Aided Absolute Stereochemical Determination of Enantioenriched Amines. *Org. Lett.* **2017,** *19* (6), 1362-1365.

46. Liang, J.; Canary, J. W. *Stereodynamic tripodal ligand with three different coordinating arms: Synthesis and Zinc(II), Copper(I) complexation study*. *Chirality* **2011**, *23*, 24-33,

47. Zhang, J.; Holmes, A. E.; Sharma, A.; Brooks, N. R.; Rarig, R. S.; Zubieta, J.; Canary, J. W., Derivatization, complexation, and absolute configurational assignment of chiral primary amines: Application of exciton-coupled circular dichroism. *Chirality* **2003,** *15* (2), 180-189.

48. Holmes, A. E.; Das, D.; Canary, J. W., Chelation-Enhanced Circular Dichroism of Tripodal Bisporphyrin Ligands. *J. Am. Chem. Soc.* **2007,** *129* (6), 1506-1507.

49. Joyce, L. A.; Maynor, M. S.; Dragna, J. M.; da Cruz, G. M.; Lynch, V. M.; Canary, J. W.; Anslyn, E. V., A Simple Method for the Determination of Enantiomeric Excess and Identity of Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2011,** *133* (34), 13746-13752.

50. Joyce, L. A.; Canary, J. W.; Anslyn, E. V., Enantio- and Chemoselective Differentiation of Protected α-Amino Acids and β-Homoamino Acids with a Single Copper(II) Host. *Chem. - Eur. J.* **2012,** *18* (26), 8064-8069.

51. Wezenberg, S. J.; Salassa, G.; Escudero-Adan, E. C.; Benet-Buchholz, J.; Kleij, A. W., Effective Chirogenesis in a Bis(metallosalphen) Complex through Host-Guest Binding with Carboxylic Acids. *Angew. Chem., Int. Ed.* **2011,** *50* (3), 713-716, S713/1-S713/25.

52. Bentley, K. W.; de los Santos, Z. A.; Weiss, M. J.; Wolf, C., Chirality Sensing With Stereodynamic Biphenolate Zinc Complexes. *Chirality* **2015,** *27* (10), 700-707.

53. Bentley, K. W.; Joyce, L. A.; Sherer, E. C.; Sheng, H.; Wolf, C.; Welch, C. J., Antenna Biphenols: Development of Extended Wavelength Chiroptical Reporters. *J. Org. Chem.* **2016,** *81* (3), 1185-1191.

54. Bentley, K. W.; Nam, Y. G.; Murphy, J. M.; Wolf, C., Chirality Sensing of Amines, Diamines, Amino Acids, Amino Alcohols, and α-Hydroxy Acids with a Single Probe. *J. Am. Chem. Soc.* **2013,** *135* (48), 18052-18055.

55. Irfanoglu, B.; Wolf, C., Circular Dichroism Sensing of Chiral Compounds Using an Achiral Metal Complex as Probe. *Chirality* **2014,** *26* (8), 379-384.

56. Zardi, P.; Wurst, K.; Licini, G.; Zonta, C., Concentration-Independent Stereodynamic g-Probe for Chiroptical Enantiomeric Excess Determination. *J. Am. Chem. Soc.* **2017,** *139* (44), 15616-15619.

57. Kolesnichenko, I. V.; Anslyn, E. V., Practical applications of supramolecular chemistry. *Chem. Soc. Rev.* **2017,** *46* (9), 2385-2390.

58. Ghosn, M. W.; Wolf, C., Chiral Amplification with a Stereodynamic Triaryl Probe: Assignment of the Absolute Configuration and Enantiomeric Excess of Amino Alcohols. *J. Am. Chem. Soc.* **2009,** *131* (45), 16360-16361.

59. Ghosn, M. W.; Wolf, C., Enantioselective CD analysis of amino acids based on chiral amplification with a stereodynamic probe. *Tetrahedron* **2010,** *66* (23), 3989-3994.

60. Iwaniuk, D. P.; Bentley, K. W.; Wolf, C., Enantioselective Sensing of Chiral Amino Alcohols with a Stereodynamic Arylacetylene-based Probe. *Chirality* **2012,** *24* (7), 584-589.

61. Iwaniuk, D. P.; Wolf, C., Enantioselective Sensing of Amines Based on [1 + 1]-, [2 + 2]-, and [1 + 2]-Condensation with Fluxional Arylacetylene-Derived Dialdehydes. *Org. Lett.* **2011,** *13* (10), 2602-2605.

62. Iwaniuk, D. P.; Wolf, C., A stereodynamic probe providing a chiroptical response to substrate-controlled induction of an axially chiral arylacetylene framework. *J. Am. Chem. Soc.* **2011,** *133* (8), 2414-2417.

63. Iwaniuk, D. P.; Wolf, C., Chiroptical sensing of citronellal: systematic development of a stereodynamic probe using the concept of isostericity. *Chem. Commun. (Cambridge, U. K.)* **2012,** *48* (91), 11226-11228.

64. Kim, H.; So, S. M.; Yen, C. P.; Vinhato, E.; Lough, A. J.; Hong, J. I.; Kim, H. J.; Chin, J., Highly stereospecific generation of helical chirality by imprinting with amino acids: a universal sensor for amino acid enantiopurity. *Angew. Chem. Int. Ed.* **2008,** *47* (45), 8657-60.

65. So, S. M.; Kim, H.; Mui, L.; Chin, J., Mimicking Nature to Make Unnatural Amino Acids and Chiral Diamines. *Eur. J. Org. Chem.* **2012,** (2), 229-241.

66. Barman, S.; Anslyn, E. V., Rapid determination of enantiomeric excess of α-chiral aldehydes using circular dichroism spectroscopy. *Tetrahedron* **2014,** *70* (6), 1357-1362.

67. Leung, D.; Anslyn, E. V., Rapid Determination of Enantiomeric Excess of α-Chiral Cyclohexanones Using Circular Dichroism Spectroscopy. *Org. Lett.* **2011,** *13* (9), 2298-2301.

68. Dragna, J. M.; Pescitelli, G.; Tran, L.; Lynch, V. M.; Anslyn, E. V.; Di Bari, L., In Situ Assembly of Octahedral Fe(II) Complexes for the Enantiomeric Excess Determination of Chiral Amines Using Circular Dichroism Spectroscopy. *J. Am. Chem. Soc.* **2012,** *134* (9), 4398-4407.

69. Dragna, J. M.; Gade, A. M.; Tran, L.; Lynch, V. M.; Anslyn, E. V., Chiral Amine Enantiomeric Excess Determination Using Self-Assembled Octahedral Fe(II)-Imine Complexes. *Chirality* **2015,** *27* (4), 294-298.

70. You, L.; Pescitelli, G.; Anslyn, E. V.; Di Bari, L., An Exciton-Coupled Circular Dichroism Protocol for the Determination of Identity, Chirality, and Enantiomeric Excess of Chiral Secondary Alcohols. *J. Am. Chem. Soc.* **2012,** *134* (16), 7117-7125.

71. You, L.; Berman, J. S.; Anslyn, E. V., Dynamic multi-component covalent assembly for the reversible binding of secondary alcohols and chirality sensing. *Nat. Chem.* **2011,** *3* (12), 943-948.

72. Metola, P.; Anslyn, E. V.; James, T. D.; Bull, S. D., Circular dichroism of multi-component assemblies for chiral amine recognition and rapid ee determination. *Chem. Sci.* **2012,** *3* (1), 156-161.

73. Shcherbakova, E. G.; Brega, V.; Minami, T.; Sheykhi, S.; James, T. D.; Anzenbacher, P., Jr., Toward Fluorescence-Based High-Throughput Screening for Enantiomeric Excess in Amines and Amino Acid Derivatives. *Chem. - Eur. J.* **2016,** *22* (29), 10074-10080.

74. Shcherbakova, E. G.; Minami, T.; Brega, V.; James, T. D.; Anzenbacher, P., Jr., Determination of Enantiomeric Excess in Amine Derivatives with Molecular Self-Assemblies. *Angew. Chem., Int. Ed.* **2015,** *54* (24), 7130-7133.

75. Zhou, Y.; Ren, Y.; Zhang, L.; You, L.; Yuan, Y.; Anslyn, E. V., Dynamic covalent binding and chirality sensing of mono secondary amines with a metal-templated assembly. *Tetrahedron* **2015,** *71* (21), 3515-3521.

76. Ni, C.; Zha, D.; Ye, H.; Hai, Y.; Zhou, Y.; Anslyn, E.; You, L., Dynamic Covalent Chemistry within Biphenyl Scaffolds: Reversible Covalent Bonding, Control of Selectivity, and Chirality Sensing with One Single System. *Angew. Chem. Int. Ed.* **2018**, *57*, 1300-1305.

77. Riobe, F.; Schenning, A. P. H. J.; Amabilino, D. B., Sensitive detection of enantiomeric excess in different acids through chiral induction in an oligo(p-phenylenevinylene) aggregate. *Org. Biomol. Chem.* **2012,** *10* (46), 9152-9157.

78. Wu, X.; Chen, X.-X.; Song, B.-N.; Huang, Y.-J.; Li, Z.; Chen, Z.; James, T. D.; Jiang, Y.-B., Induced Helical Chirality of Perylenebisimide Aggregates Allows for Enantiopurity Determination and Differentiation of α-Hydroxy Carboxylates by Using Circular Dichroism. *Chem. - Eur. J.* **2014,** *20* (37), 11793-11799.

79. Eelkema, R.; van Delden, R. A.; Feringa, B. L., Direct visual detection of the stereoselectivity of a catalytic reaction. *Angew. Chem. Int. Ed.* **2004,** *43* (38), 5013-6.

80. van Delden, R. A.; Feringa, B. L., Color Indicators of Molecular Chirality Based on Doped Liquid Crystals. *Angew. Chem. Int. Ed.* **2001,** *40* (17), 3198-3200.

81. van Delden, R. A.; Feringa, B. L., Colour indicator for enantiomeric excess and assignment of the configuration of the major enantiomer of an amino acid ester. *Chem. Commun.* **2002,** (2), 174-5.

82. Seifert, H. M.; Jiang, Y.-B.; Anslyn, E. V., Exploitation of the majority rules effect for the accurate measurement of high enantiomeric excess values using CD spectroscopy. *Chem. Commun.* **2014,** *50* (97), 15330-15332.

83. Chen, X.-X.; Jiang, Y.-B.; Anslyn, E. V., A racemate-rules effect supramolecular polymer for ee determination of malic acid in the high ee region. *Chem. Commun.* **2016,** *52* (85), 12669-12671.

84. Metola, P.; Nichols, S. M.; Kahr, B.; Anslyn, E. V., Well Plate Circular Dichroism Reader for the Rapid Determination of Enantiomeric Excess. *Chem Sci* **2014,** *5* (11), 4278-4282.

85. Leung, D.; Kang, S. O.; Anslyn, E. V., Rapid determination of enantiomeric excess: a focus on optical approaches. *Chem. Soc. Rev.* **2012,** *41* (1), 448-479.

86. Jo, H. H.; Lin, C.-Y.; Anslyn, E. V., Rapid Optical Methods for Enantiomeric Excess Analysis: From Enantioselective Indicator Displacement Assays to Exciton-Coupled Circular Dichroism. *Acc. Chem. Res.* **2014,** *47* (7), 2212-2221.

87. Mei, X.; Wolf, C., Determination of enantiomeric excess and concentration of unprotected amino acids, amines, amino alcohols, and carboxylic acids by competitive binding assays with a chiral scandium complex. *J. Am. Chem. Soc.* **2006,** *128* (41), 13326-7.

88. Liu, S.; Pestano, J. P.; Wolf, C., Enantioselective fluorescence sensing of chiral alpha-amino alcohols. *J. Org. Chem.* **2008,** *73* (11), 4267-70.

89. Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V., Colorimetric enantiodiscrimination of alpha-amino acids in protic media. *J. Am. Chem. Soc.* **2005,** *127* (22), 7986-7.

90. Li, Z.-B.; Lin, J.; Pu, L., A cyclohexyl-1,2-diamine-derived bis(binaphthyl) macrocycle: Enhanced sensitivity and enantioselectivity in the fluorescent recognition of mandelic acid. *Angew. Chem., Int. Ed.* **2005,** *44* (11), 1690-1693.

91. Li, Z.-B.; Lin, J.; Sabat, M.; Hyacinth, M.; Pu, L., Enantioselective fluorescent recognition of chiral acids by cyclohexane-1,2-diamine-based bisbinaphthyl molecules. *J. Org. Chem.* **2007,** *72* (13), 4905-4916.

92. Lin, J.; Hu, Q.-S.; Xu, M.-H.; Pu, L., A practical enantioselective fluorescent sensor for mandelic acid. *J. Am. Chem. Soc.* **2002,** *124* (10), 2088-2089.

93. Lin, J.; Rajaram, A. R.; Pu, L., Enantioselective fluorescent recognition of chiral acids by 3- and 3,3'-aminomethyl substituted BINOLs. *Tetrahedron* **2004,** *60* (49), 11277-11281.

94. Liu, H.-L.; Peng, Q.; Wu, Y.-D.; Chen, D.; Hou, X.-L.; Sabat, M.; Pu, L., Highly enantioselective recognition of

structurally diverse α-hydroxycarboxylic acids using a fluorescent sensor. *Angew. Chem., Int. Ed.* **2010,** *49* (3), 602-606.

95. Liu, H.-L.; Zhu, H.-P.; Hou, X.-L.; Pu, L., Highly enantioselective fluorescent recognition of serine and other amino acid derivatives. *Org. Lett.* **2010,** *12* (18), 4172-4175.

96. Pugh, V. J.; Hu, Q.-S.; Zuo, X.; Lewis, F. D.; Pu, L., Optically active BINOL core-based phenyleneethynylene dendrimers for the enantioselective fluorescent recognition of amino alcohols. *J. Org. Chem.* **2001,** *66* (18), 6136-6140.

97. Wang, Q.; Chen, X.; Tao, L.; Wang, L.; Xiao, D.; Yu, X.-Q.; Pu, L., Enantioselective Fluorescent Recognition of Amino Alcohols by a Chiral Tetrahydroxyl 1,1'-Binaphthyl Compound. *J. Org. Chem.* **2007,** *72* (1), 97-101.

98. Xu, M.-H.; Lin, J.; Hu, Q.-S.; Pu, L., Fluorescent sensors for the enantioselective recognition of mandelic acid: Signal amplification by dendritic branching. *J. Am. Chem. Soc.* **2002,** *124* (47), 14239-14246.

99. Yu, S. S.; Pu, L., One enantiomeric fluorescent sensor pair to discriminate four stereoisomers of threonines. *Sci. China: Chem.* **2013,** *56* (3), 301-306.

100. Akdeniz, A.; Mosca, L.; Minami, T.; Anzenbacher, P., Sensing of enantiomeric excess in chiral carboxylic acids. *Chem. Commun.* **2015,** *51* (26), 5770-5773.

101. Akdeniz, A.; Minami, T.; Watanabe, S.; Yokoyama, M.; Ema, T.; Anzenbacher, P., Determination of enantiomeric excess of carboxylates by fluorescent macrocyclic sensors. *Chem. Sci.* **2016,** *7* (3), 2016-2022.

102. Mei, X.; Wolf, C., Determination of enantiomeric excess and concentration of chiral compounds using a 1,8-diheteroarylnaphthalene-derived fluorosensor. *Tetrahedron Lett.* **2006,** *47* (45), 7901-7904.

103. Wolf, C.; Liu, S. L.; Reinhardt, B. C., An enantioselective fluorescence sensing assay for quantitative analysis of chiral carboxylic acids and amino acid derivatives. *Chem. Commun.* **2006,** (40), 4242-4244.

104. Mei, X. W., C., Enantioselective Sensing of Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2004,** *126*, 14736-14737.

105. Huang, Z.; Yu, S.; Wen, K.; Yu, X.; Pu, L., Zn(II) promoted dramatic enhancement in the enantioselective fluorescent recognition of functional chiral amines by a chiral aldehyde. *Chem. Sci.* **2014,** *5* (9), 3457-3462.

106. Wang, C.; Anbaei, P.; Pu, L., Highly enantioselective fluorescent recognition of both unfunctionalized and functionalized chiral amines by a facile amide formation from a perfluoroalkyl ketone. *Chem. - Eur. J.* **2016,** *22* (21), 7255-7261.

107. Wang, C.; Wu, E.; Wu, X.; Xu, X.; Zhang, G.; Pu, L., Enantioselective Fluorescent Recognition in the Fluorous Phase: Enhanced Reactivity and Expanded Chiral Recognition. *J. Am. Chem. Soc.* **2015,** *137* (11), 3747-3750.

108. Zhang, X.; Wang, C.; Wang, P.; Du, J.; Zhang, G.; Pu, L., Conjugated polymer-enhanced enantioselectivity in fluorescent sensing. *Chem. Sci.* **2016,** *7* (6), 3614-3620.

109. Li, Z.-B.; Lin, J.; Qin, Y.-C.; Pu, L., Enantioselective fluorescent recognition of a soluble "supported" chiral acid: Toward a new method for chiral catalyst screening. *Org. Lett.* **2005,** *7* (16), 3441-3444.

110. Tumambac, G. E.; Wolf, C., Enantioselective analysis of an asymmetric reaction using a chiral fluorosensor. *Org. Lett.* **2005,** *7* (18), 4045-4048.

111. Arai, T.; Watanabe, M.; Fujiwara, A.; Yokoyama, N.; Yanagisawa, A., Direct monitoring of the asymmetric induction of solid-phase catalysis using circular dichroism: diamine-CuI-catalyzed asymmetric Henry reaction. *Angew. Chem., Int. Ed.* **2006,** *45* (36), 5978-5981.

112. Arai, T.; Yokoyama, N.; Yanagisawa, A., A library of chiral imidazoline-aminophenol ligands: discovery of an efficient reaction sphere. *Chem. - Eur. J.* **2008,** *14* (7), 2052-2059.

113. Dey, S.; Karukurichi, K. R.; Shen, W.; Berkowitz, D. B., Double-Cuvette ISES: In Situ Estimation of Enantioselectivity and Relative Rate for Catalyst Screening. *J. Am. Chem. Soc.* **2005,** *127* (24), 8610-8611.

114. Dey, S.; Powell, D. R.; Hu, C.; Berkowitz, D. B., Cassette in situ enzymatic screening identifies complementary chiral scaffolds for hydrolytic kinetic resolution across a range of epoxides. *Angew. Chem., Int. Ed.* **2007,** *46* (37), 7010-7014.

115. He, X.; Zhang, Q.; Liu, X. H.; Lin, L. L.; Feng, X. M., Determination of concentration and enantiomeric excess of amines and amino alcohols with a chiral nickel(II) complex. *Chem. Commun.* **2011,** *47* (42), 11641-11643.

116. Wen, K.; Yu, S.; Huang, Z.; Chen, L.; Xiao, M.; Yu, X.; Pu, L., Rational design of a fluorescent sensor to simultaneously determine both the enantiomeric composition and the concentration of chiral functional amines. *J. Am. Chem. Soc.* **2015,** *137* (13), 4517-24.

117. Pilicer, S. L.; Bakhshi, P. R.; Bentley, K. W.; Wolf, C., Biomimetic Chirality Sensing with Pyridoxal-5'-phosphate. *J. Am. Chem. Soc.* **2017,** *139* (5), 1758-1761.

118. Zhu, L.; Shabbir, S. H.; Anslyn, E. V., Two methods for the determination of enantiomeric excess and concentration of a chiral sample with a single spectroscopic measurement. *Chem.-Eur. J.* **2007,** *13* (1), 99-104.

119. Bentley, K. W.; Wolf, C., Stereodynamic Chemosensor with Selective Circular Dichroism and Fluorescence Readout for in Situ Determination of Absolute Configuration, Enantiomeric Excess, and Concentration of Chiral Compounds. *J. Am. Chem. Soc.* **2013,** *135* (33), 12200-12203.

120. Bentley, K. W.; Wolf, C., Comprehensive chirality sensing: development of stereodynamic probes with a dual (chir)optical response. *J. Org. Chem.* **2014,** *79* (14), 6517-31.

121. De Los Santos, Z. A.; Ding, R.; Wolf, C., Quantitative chirality sensing of amines and amino alcohols via Schiff base formation with a stereodynamic UV/CD probe. *Org. Biomol. Chem.* **2016,** *14* (6), 1934-9.

122. Yu, S.; Plunkett, W.; Kim, M.; Pu, L., Simultaneous determination of both the enantiomeric composition and concentration of a chiral substrate with one fluorescent sensor. *J. Am. Chem. Soc.* **2012,** *134* (50), 20282-5.

123. Huang, Z.; Yu, S.; Zhao, X.; Wen, K.; Xu, Y.; Yu, X.; Xu, Y.; Pu, L., A convenient fluorescent method to simultaneously determine the enantiomeric composition and concentration of functional chiral amines. *Chem.-Eur. J.* **2014,** *20* (50), 16458-61.

124. Nieto, S.; Lynch, V. M.; Anslyn, E. V.; Kim, H.; Chin, J., High-throughput screening of identity, enantiomeric excess, and concentration using MLCT transitions in CD spectroscopy. *J. Am. Chem. Soc.* **2008,** *130* (29), 9232-3.

125. Nieto, S.; Lynch, V. M.; Anslyn, E. V.; Kim, H.; Chin, J., Rapid enantiomeric excess and concentration determination using simple racemic metal complexes. *Org. Lett.* **2008,** *10* (22), 5167-70.

126. Zhang, P.; Wolf, C., Sensing of the concentration and enantiomeric excess of chiral compounds with tropos ligand derived metal complexes. *Chem. Commun.* **2013,** *49* (62), 7010-2.

127. De Los Santos, Z. A.; Legaux, N. M.; Wolf, C., Chirality sensing with stereodynamic copper(I) complexes. *Chirality* **2017,** *29* (11), 663-669.

128. Peng, R.; Lin, L.; Cao, W.; Guo, J.; Liu, X.; Feng, X., A racemic N,N '-dioxide-iron(III) complex chemosensor for determination of enantiomeric excess, concentration and identity of hydroxy carboxylic acids with circular dichroism and fluorescence responses. *Tetrahedron Lett.* **2015,** *56* (25), 3882-3885.

129. Shabbir, S. H.; Clinton, J. R.; Anslyn, E. V., A general protocol for creating high-throughput screening assays for reaction yield and enantiomeric excess applied to hydrobenzoin. *Proc. Natl. Acad. Sci. U S A* **2009,** *106* (26), 10487-10492.

130. Nieto, S.; Dragna, J. M.; Anslyn, E. V., A facile circular dichroism protocol for rapid determination of enantiomeric excess and concentration of chiral primary amines. *Chem. Eur. J.* **2010,** *16* (1), 227-32.

131. Bentley, K. W.; Zhang, P.; Wolf, C., Miniature high-throughput chemosensing of yield, ee, and absolute configuration from crude reaction mixtures. *Science Advances* **2016,** *2* (2) e1501162.

132. Bentley, K. W.; Proano, D.; Wolf, C., Chirality imprinting and direct asymmetric reaction screening using a stereodynamic Bronsted/Lewis acid receptor. *Nat. Commun.* **2016,** *7*, 12539.

133. De los Santos, Z. A.; Wolf, C., Chiroptical Asymmetric Reaction Screening via Multicomponent Self-Assembly. *J. Am. Chem. Soc.* **2016,** *138* (41), 13517-13520.

134. Zhao, Q.; Wen, J.; Tan, R.; Huang, K.; Metola, P.; Wang, R.; Anslyn, E. V.; Zhang, X., Rhodium-catalyzed asymmetric hydrogenation of unprotected NH imines assisted by a thiourea. *Angew. Chem. Int. Ed.* **2014,** *53* (32), 8467-70.

135. Biedermann, F.; Nau, W. M., Noncovalent chirality sensing ensembles for the detection and reaction monitoring of amino acids, peptides, proteins, and aromatic drugs. *Angew. Chem. Int. Ed.* **2014,** *53* (22), 5694-9.

136. Feagin, T. A.; Olsen, D. P.; Headman, Z. C.; Heemstra, J. M., High-throughput enantiopurity analysis using enantiomeric DNA-based sensors. *J. Am. Chem. Soc.* **2015,** *137* (12), 4198-206.

137. Shcherbakova, E. G.; Brega, V.; Lynch, V. M.; James, T. D.; Anzenbacher, P., High-Throughput Assay for Enantiomeric Excess Determination in 1,2-and 1,3-Diols and Direct Asymmetric Reaction Screening. *Chem.-Eur. J.* **2017,** *23* (42), 10222-10229.

138. Thanzeel, F. Y.; Wolf, C., Substrate-Specific Amino Acid Sensing Using a Molecular d/ l-Cysteine Probe for Comprehensive Stereochemical Analysis in Aqueous Solution. *Angew. Chem. Int. Ed.* **2017,** *56* (25), 7276-7281.

139. Giuliano, M. W.; Lin, C. Y.; Romney, D. K.; Miller, S. J.; Anslyn, E. V., A Synergistic Combinatorial and Chiroptical Study of Peptide Catalysts for Asymmetric Baeyer-Villiger Oxidation. *Adv. Synth. Catal.* **2015,** *357* (10), 2301-2309.

140. Joyce, L. A.; Sherer, E. C.; Welch, C. J., Imine-based chiroptical sensing for analysis of chiral amines: from method design to synthetic application. *Chem. Sci.* **2014,** *5* (7), 2855.

141. Jo, H. H.; Gao, X.; You, L.; Anslyn, E. V.; Krische, M. J., Application of a High-Throughput Enantiomeric Excess Optical Assay Involving a Dynamic Covalent Assembly: Parallel Asymmetric Allylation and Ee Sensing of Homoallylic Alcohols. *Chem. Sci.* **2015,** *6* (12), 6747-6753.