

SUPPORTING INFORMATION

Well Plate Circular Dichroism Reader for the Rapid Determination of Enantiomeric Excess

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Experimental Details

Materials and Methods

All reagents were purchased from commercial sources and used as received with the exception of aldehyde **1**, which was purified by sublimation under high vacuum with mild heat (60-70°C) using a cold finger.

Recovery of CD in the presence of small container anisotropy

A general Mueller matrix M has the following elements,

$$M = \begin{bmatrix} M_{00} & M_{01} & M_{02} & M_{03} \\ M_{10} & M_{11} & M_{12} & M_{13} \\ M_{20} & M_{21} & M_{22} & M_{23} \\ M_{30} & M_{31} & M_{32} & M_{33} \end{bmatrix}$$

The Mueller matrix of a material having only small linear dichroism and small linear birefringence has the form given in equation (1), M_{anis} , in the text. Therefore, the normalized values of the Mueller matrix have the following symmetry,

$$\begin{bmatrix} 1 & A & B & -C \\ A & D & E & -F \\ B & E & D & -G \\ C & F & G & H \end{bmatrix}$$

If an anisotropic container influences the light after the sample, the Mueller matrix measured by the instrument is calculated as the matrix product $M_{anis}M_{sol}$, and has values,

$$M = \begin{bmatrix} 1 & A & B & -C \\ A & D & E & -F \\ B & E & D & -G \\ C & F & G & H \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 & a \\ 0 & b & c & 0 \\ 0 & -c & b & 0 \\ a & 0 & 0 & 1 \end{bmatrix} = \begin{bmatrix} 1-aC & Ab-Bc & bB+Ac & a-C \\ A-aF & bD-cE & cD+bE & aA-F \\ B-aG & bE-cD & bD+cG & aB-G \\ C+aH & bF-cG & cF+bG & aC+H \end{bmatrix}$$

The values of M can be normalized by dividing by the M_{00} element, as polarimeters deliver the normalized matrix. We solve for a , the solution circular dichroism, by constructing a set of linear equations from the normalized values of M . The minimal set of equations is,

$$M_{03} = \frac{(a-C)}{(1-aC)}, M_{30} = \frac{(C+aH)}{(1-aC)}, M_{33} = \frac{(aC+H)}{(1-aC)}$$

Solving the equations for a yields the relation $a = \frac{M_{03} + M_{30}}{1 + M_{33}}$. Positioning the anisotropic perturbation before the sample corresponds to the matrix product $M_{sol}M_{anis}$. The resulting M differs from $M_{anis}M_{sol}$ as matrix multiplication is generally non-commutative, but following the same approach, one can verify that the same relation for a results. We find that the correction is valid so long as the linear dichroism and linear birefringence is small ($< \sim 0.5$ rad) and homogeneous. The correction does not apply to anisotropy both before and after the sample.

In testing, CD was recovered from measurements of a Cu(II)-D-tartrate solution with various anisotropic objects placed before or after the cuvette (Fig. 2 and S1-2). For glass having small amounts of strain birefringence (S1 Strained Glass) M_{03} and M_{30} differ only slightly and CD is easily recovered. We expect typical amounts of strain in glass containers to be less than this, however, fused quartz containers can have larger strain (not shown). For glass with strain LB ~ 0.45 rad, (S1, highly stained glass) the correction gives a noticeable improvement. To test whether CD could be recovered from a container with both LD and LB, a glass slide was rubbed with methylene blue unidirectionally, and then placed after the cuvette. Elements M_{03} and M_{30} differed noticeably, and the correction is closer to the CD than either raw element. The complete

raw Mueller matrices for the measurements are given in Figure S2, with the same color indexing as Fig. S1.

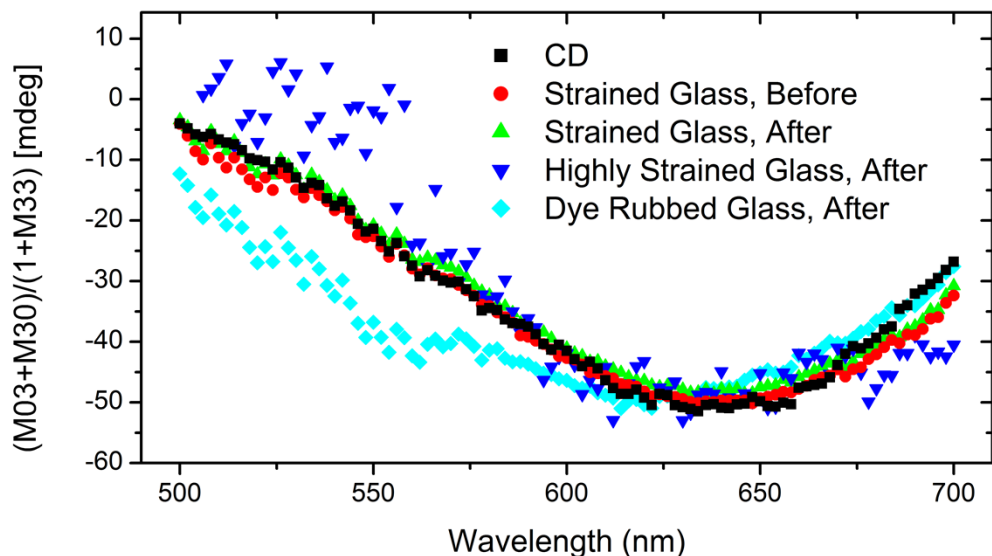


Figure S1. CD spectra of a Cu(II)-D-tartrate solution calculated from the Mueller matrix. The same solution, placed in a 1mm glass cuvette, was used for all measurements. In all but “CD”, an anisotropic material was placed before or after the cuvette.

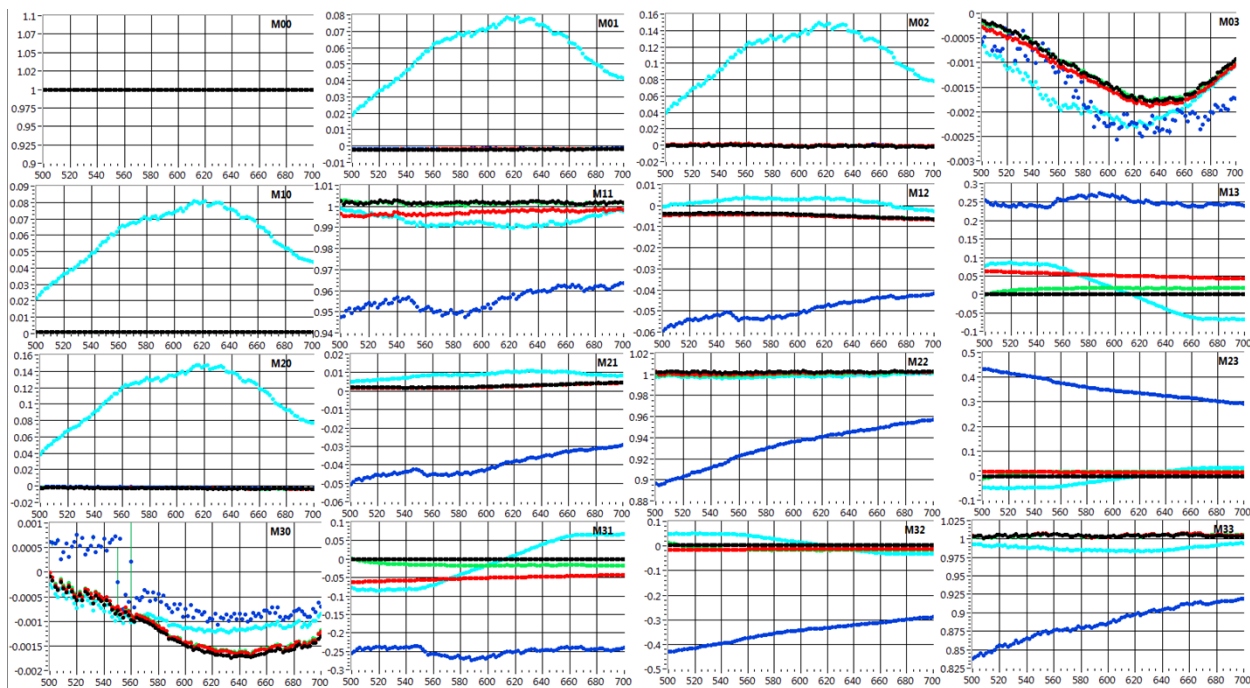


Figure S2. Mueller matrix spectra used for the calculations in Fig. S1 using the 4PEM. The measurements at each wavelength are 3 averages of 1.2 second acquisitions at 448 KHz sampling. In some instances, the sign of M_{30} can be ill-determined at very small values.

CD Calibration Curves and Test Sample Experimental

Solutions were prepared as follows: 100 μL of the appropriate amine combination (obtained by mixing 3 mM HPLC grade acetonitrile solutions of the two enantiomers of each analyte in prescribed ratios) were mixed with 100 μL of a 3 mM solution of aldehyde 1 for 10 minutes. Then 100 μL of a 1 mM solution of $\text{Fe}(\text{OTf})_2$ was added to the mixture and the system was allowed to equilibrate for 2 hours, effectively making the concentration of host-guest complex 0.33 mM. Upon completion of the reaction, 30 μL of each solution was placed in each well of the 96 well plate. Each well was filled fully with an additional 270 μL of HPLC grade acetonitrile, leaving the final concentration of the active complex at 0.033 mM. Comparatively dilute samples were used to account for the increased optical path (12 mm) as compared with 10 mm cuvettes.

Plots in main text were prepared with OriginLab and linear fits were computed using the least squares method. Supporting figure plots were prepared with LabView or Microsoft® Excel® and the physically relevant roots of the corresponding third-degree polynomial equations calculated *via* Wolfram Mathematica software.

The standard solution of Cu(II)-tartrate was prepared from copper(II) sulfate pentahydrate (Aldrich 98%, 0.1049g) and *D*-tartaric acid (Aldrich 99%, 97%ee, 0.0327g) dissolved in distilled deionized water (10.0 mL) and brought to a pH of ca. 9 using several drops of aq. NaOH.

Additional Figures

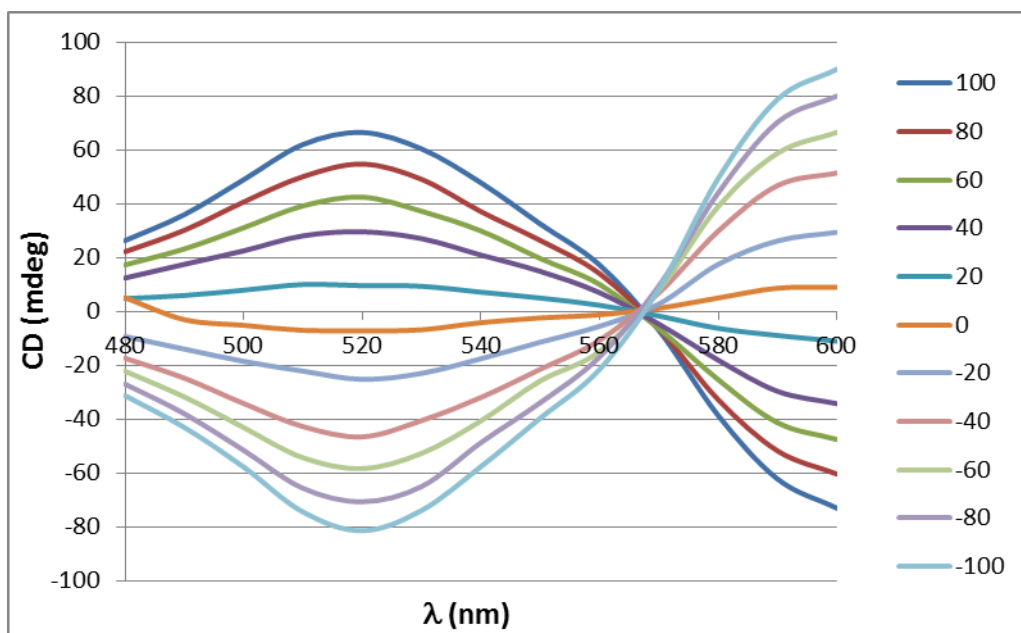


Figure S3. CD spectra for the assembly with **5**.

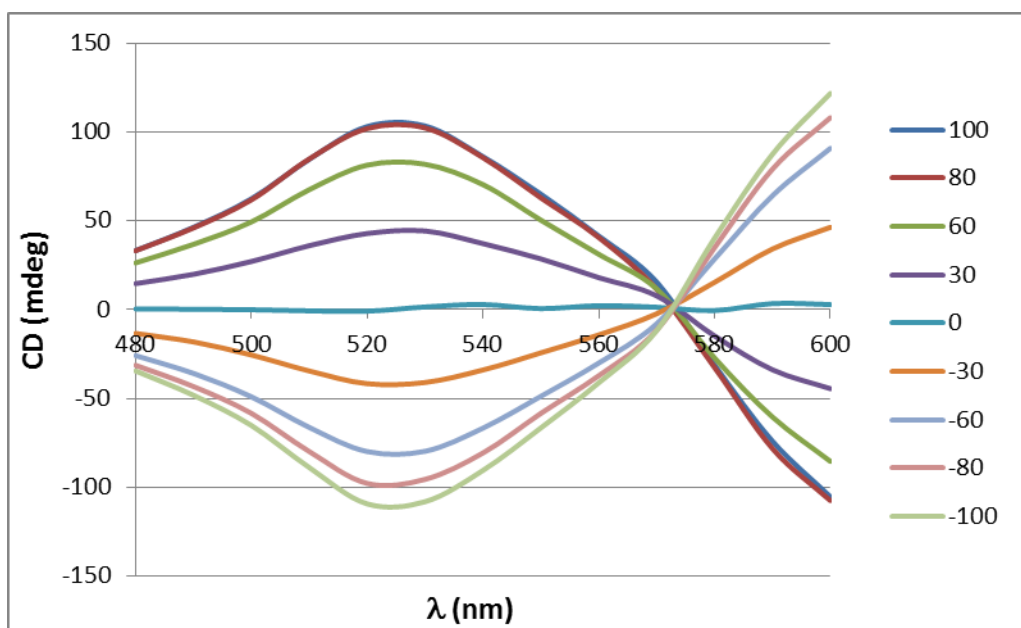


Figure S4. CD spectra for the assembly with **6**.

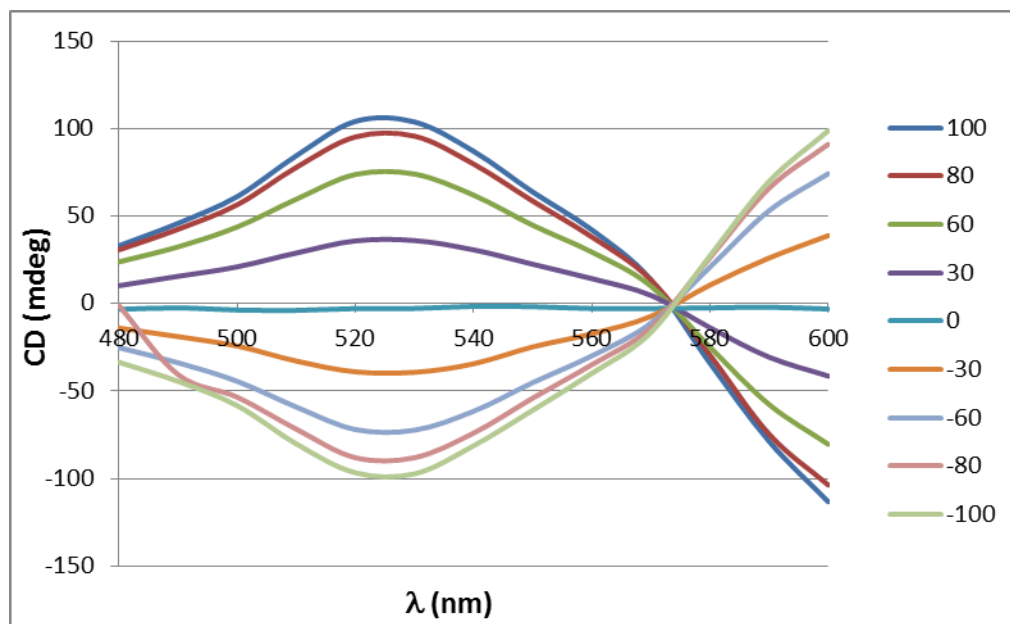


Figure S5. CD spectra for the assembly with 7.

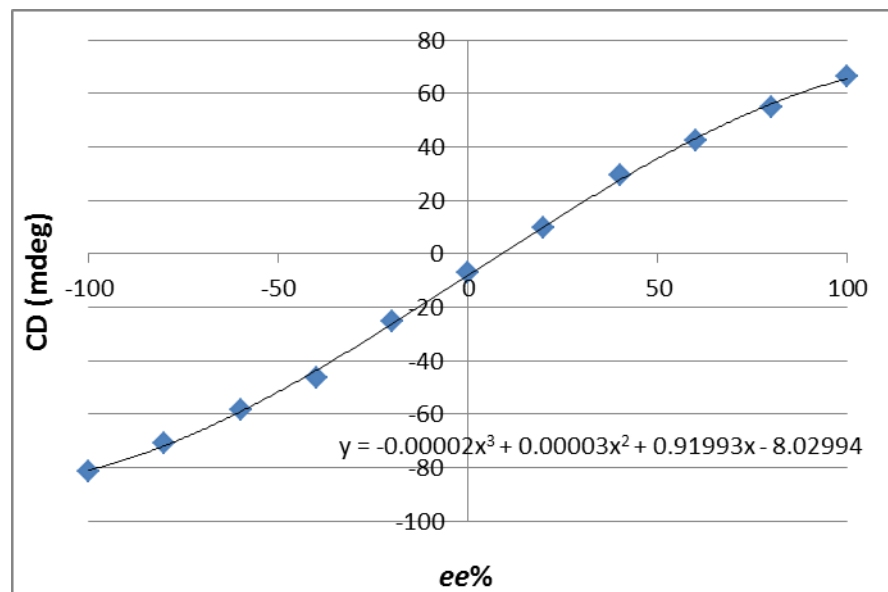


Figure S6. Calibration curve for the assembly with 5 at 520 nm.

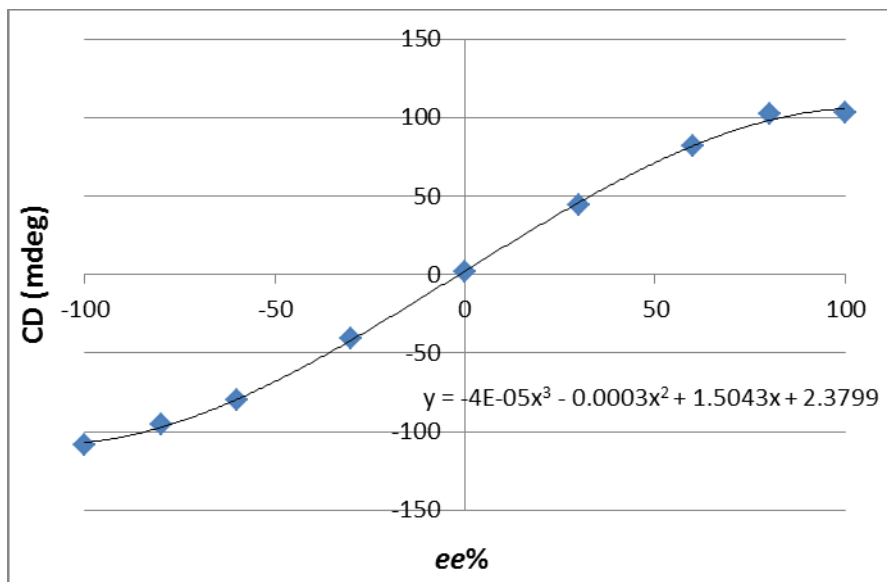


Figure S7. Calibration curve for the assembly with **6** at 530 nm.

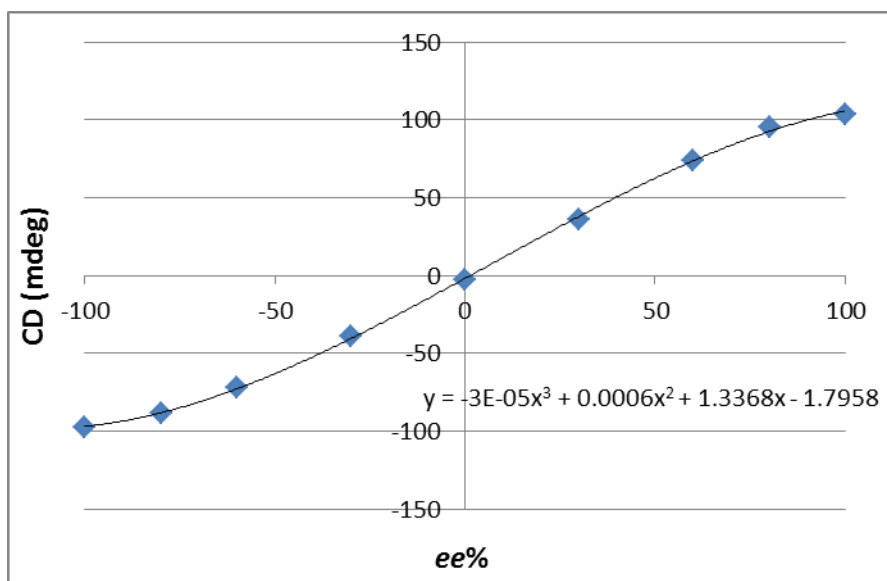


Figure S8. Calibration curve for the assembly with **7** at 530 nm.

Analysis of Anisotropic of Polystyrene Well Plate

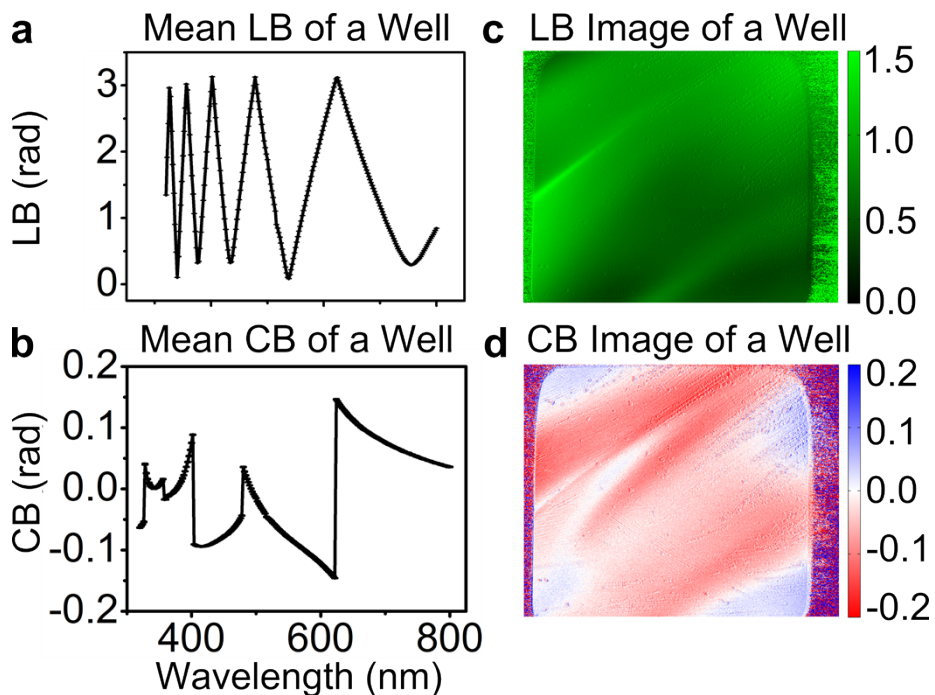


Figure S9. Measurements of polystyrene BRANDplates® PureGrade® 96-well plates showing poor optical quality. (a) The measured linear birefringence (LB) of a single well as a function of λ . Discontinuities at 0 and π show several orders of LB. (b) Artfactual circular birefringence (CB) which is a consequence of heterogeneities in linear birefringence ($n_x - n_y$) along the optical path, (L). Image of (c) LB and (d) CB showing large spatial heterogeneities within a single well.