Reply to "Comment on 'Limits on Fluorescence Detected Circular Dichroism of Single Helicene Molecules'"

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We thank Prof. Barnes and his coauthors for their comments on our paper.[1] We made some changes in our manuscript in response to comments Prof. Barnes graciously provided on a draft. We acknowledge his contribution but certainly do not intend to imply that he or his coauthors agree with our conclusions.

There is no mention of compensation for birefringence in the dichroic mirror in either refs 2 or 3. Figure 1A in ref 2 and Figure 2A in ref 3 clearly show CPL being sent into the microscope *before* the dichroic. In ref 2 the authors state "we delivered right- or left-handed circularly polarized light from a CW Ar^+ ion laser (457 nm; $\approx$100 mW nominal power) to the sample by orienting a multi-order quarter waveplate (QWP) on a rotation stage at ±45° with respect to the (horizontal) input polarization axis." Such a scheme does not allow compensation for birefringence in the dichroic mirror. On the basis of the published descriptions, we conclude that either the authors found a dichroic mirror that perfectly preserves polarization or that they had uncompensated ellipticity at the sample plane. We deem the first scenario unlikely because we have not been able to find a dichroic mirror with this property. Omega Optical does not specify any special polarization properties for dichroic XF2027-485DRLP used in the experiments of Barnes and coworkers.

As for the control experiments cited by Barnes et al., the measurements on polystyrene nanospheres, shown in refs 2 and3 are not sensitive to elliptical polarization. These nanospheres typically contain hundreds to thousands of randomly oriented dye molecules, so the linear birefringence of each dye molecule averages to zero in the ensemble. In neither paper do the authors show data taken on single-molecule control samples, so we cannot comment on the claim that these samples lacked apparent dissymmetry.

In their comment on our paper, the authors do not explain how they measured the polarization state of the light at the plane of the sample, so we cannot comment on these measurements.

In their comment, Barnes et al. claim that their result "is not grossly dissimilar from Cohen's result obtained from CPL at the sample." This is not true. We have overlaid the data from Figure 3B of our paper with the data from Figure 2B of ref 2, taking care to line up the tick marks on the *x*-axes as precisely as possible (Figure 1). Our data for both enantiomers are in bright green, the single-molecule dissymmetry factors of ref 2 are in red and blue, and the control sample of nanospheres from ref 2 is the dotted black line. The reader may judge whether our single-molecule data are more similar to the control sample or to the single-molecule data of ref 2.

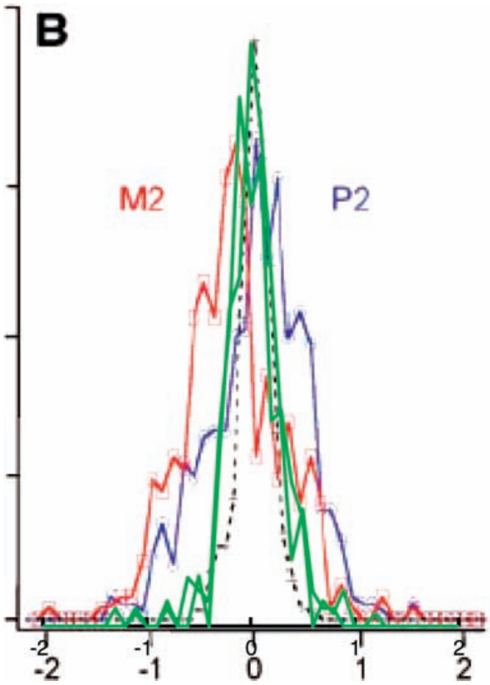

Figure 1. Comparison between the data of refs 1 and 2. See text for explanation.

The final issue raised by Barnes et al. is the question of how to select single molecules for analysis. In ref 2 they note, "Our experimental bias toward molecules with higher photostability might, however, bias the distribution of dissymmetry parameters to higher (absolute) values." We agree with this statement. Our automated procedure for selecting molecules admits no such bias.

We stand by our conclusion that fluorescence detected circular dichroism has not yet been observed from single molecules.

References and Notes

(1) Tang, Y.; Cook, T. A.; Cohen, A. E. *J. Phys. Chem. A* **2009**, *113*, 6213–6216.

(2) Hassey, R.; Swain, E. J.; Hammer, N. I.; Venkataraman, D.; Barnes, M. D. *Science* **2006**, *314*, 1437.

(3) Hassey, R.; McCarthy, K. D.; Swain, E.; Basak, D.; Venkataraman, D.; Barnes, M. D. *Chirality* **2008**, *20*, 1039.

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