# Reviewer 1

*The authors believe this is the first report of a recombinant enzyme that converts ethylene to ethylene oxide, and it may well be.  This is a known function in the alkene monooxygenases, but these enzymes have not been functionally expressed in E. coli.  Thus this report is important in that a recombinant enzyme could form the basis of a biocatalytic system in vitro or a metabolic pathway in vivo for utilization of ethylene to make ethylene oxide or further products.*

*The authors obtained wildtype TOM (toluene monooxygenase) and a panel of mutants constructed and characterized by Tom Wood, who made them in an effort to degrade thrichloroethylene. They then tested these enzymes for the ability to oxidize ethylene to ethylene oxide.  Whereas wildtype TOM has little or no activity, at least two of the mutants showed significant activity, converting 1.5% ethylene to ethylene oxide, with >99% yield.*

*The authors then go on to try to ‘explain’ the effects of the mutations using structural modeling. Because a crystal structure of the parent enzyme is not available, a homology model was made based on 3 structures of enzymes having ~65% identity.  This revealed that the effective mutations are in the active site pocket.****More is said, but should be said with at least a caveat to the reader that the detailed structural arguments may well be incorrect.***

*I am a bit torn with respect to this report.  I find it highly interesting that a mutant TOM does this reaction, but I would like to better understand how well this mutant functions.****That is a little bit difficult to do, since the enzyme presumably cannot be purified for characterization (is that [t]rue?)*** *and is instead used in whole cells.   However, it would be very informative to* ***characterize the rate of product formation (EO) using the best mutant  and compare that to the rate of product formation for wildtype TOM acting on toluene****.  Then the reader would have a meaningful comparison of activities.  I am presuming that there is significant room for improvement for activity on ethylene.  Is the mutant 1000-fold less active on ethylene?   100-fold?  10-fold?*

# Referee 2

*1)   It is not clear from the context, what toluene monooxygenase was used in this study. The authors provide several references (for instance 4 and 5) which describe two different toluene ortho-xylene monooxygenases (from Pseudomonas mendocina KR1 and from Pseudomonas stutzeri OX1). However, in the work the toluene ortho-monooxygenase (TOM) of Burkholderia cepacia G4 and its mutants were used. Please provide this information.*

*2)   Toluene monooxygenases are three-component enzymes. Please provide this information. Obviously, this multi-component nature of TOM is the reason why recombinant E. coli cells were used for ethylene oxidation and kcat values could not be provided.*

*3)   Please provide either enzyme or protein or cell concentration used in the experiments and the corresponding initial oxidation rates in nmol/min/mg of protein.*

*4)   It would be interesting to know which products can be produced from ethylene oxide.*

*5)   The reference 6 is incomplete; the correct one is as follows: K. A. Canada, S. Iwashita, H. Shim, T.K. Wood, J. Bacteriol., 2002, 184, 344–349. Please correct this one and check other references for accuracy and correctness.*

*6)   Please provide the GC program for the identification of ethylene oxide.*

# Responses

## Referee 1

We have added a caveat that our modeling requires verification in the form of crystallographic (or other structural) experimental characterization in the final paragraph before the conclusions section.

It is true that mutant TOMs cannot be purified for characterization, thus leading us to a whole-cell assay.

We agree that a comparison of the best mutant (A113F) on ethylene to WT on toluene would be very interesting. However, two problems confront us in the calculation of a rate that can be meaningfully compared to previously-published rates on toluene. First, the differences in assay conditions. Second, the physical differences between the substrates (i.e., one is an aromatic liquid and one is an aliphatic gas) cast doubt on the meaning of a rate comparison. That said, we would be excited to see this work continued and a meaningful comparison made, but believe it is outside the scope of this Communication. Our study quickly communicates the mutations necessary to transform the wild type enzyme (which has no activity on ethylene) to a modified (new?) enzyme that has activity on ethylene, and as such focuses on the relative activities of wild type and mutant enzymes.

## Referee 2

1 and 2) We have added a clause to the third paragraph specifically mentioning the origin and multi-component nature of TOM.

3) A measurement of cell concentration (OD = 10) used in our assays is provided in paragraph 5 with the experimental details. We did not measure total protein content, and while it would be possible for us to do so using the Total Protein Kit, this would require additional time and investment. Since our study elucidates the differences between TOM (which has no activity on ethylene) and the best mutants (which do), our measurements are relative to the wild type.

4) A brief mention of products that can be made from ethylene oxide has been added to the first paragraph.

5) We have corrected reference 6.

6) The GC program is now included in the supplemental material.