JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Addition/Correction

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Correction to "Evaluating the Catalytic Contribution from the Oxyanion Hole in Ketosteroid Isomerase"

Jason P. Schwans, Fanny Sunden, Ana Gonzalez, Yingssu Tsai, and Daniel Herschlag*

J. Am. Chem. Soc. **2011**, *133*, 20052−20055. DOI: 10.1021/ja208050t

Supporting Information

We report that the kinetic constants for several mutants of ketosteroid isomerase (KSI) from our 2011 publication are incorrect. Jason Schwans and Daniel Herschlag, the paper's first and corresponding authors, take full responsibility for and deeply regret these errors.

Based on extensive control experiments, the errors likely arose from low levels (∼0.1−1%) of contaminating wild-type enzyme introduced during fast protein liquid chromatography (FPLC) purification; this contamination can be avoided by stringent washing of the FPLC injection loop and other plumbing with base (Supplemental text).

The main conclusions of the Communication are not affected by these errors. For clarity, we list below the central conclusions from our work and how they are or are not altered by these errors.

Also, we remeasured kinetic constants from two papers we published around the same time[1,2] and found all of those results to be fully reproducible (Supplemental Table 1).

All remeasured values are presented as Supporting Information and directly compared to those from the prior work (Supplemental Tables 2−5). Additional measurements were made with a faster-reacting substrate and with several additional mutant enzymes to extend these measurements and comparisons in light of the new findings (Supplemental Tables 6 and 7). Inhibition constants were determined for several mutants to provide evidence that the new kinetic constants correspond to the mutant activities and not wild-type contamination (Supplemental Tables 8 and 9).

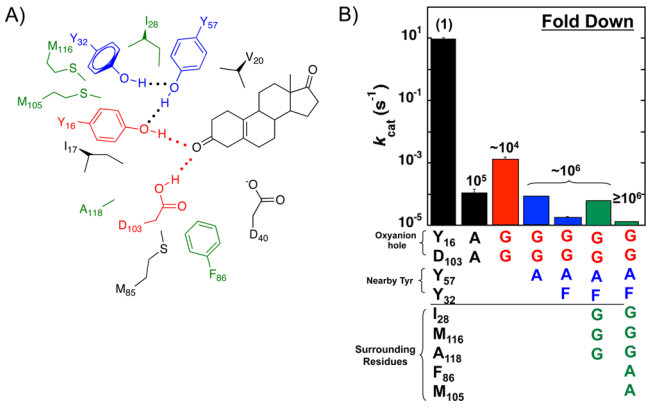


Figure 2. (Revised) "Carving out" the pKSI oxyanion hole. (A) Schematic representation of oxyanion hole hydrogen bond donors (red), tyrosine network (blue), and surrounding residues (green and black). The substrate is also shown in black. (B) Rate effects from ablating neighboring side chains in the oxyanion hole mutant background. Values are from revised Table S1. Bars and residues are colored according to panel A.

Prior and Updated Conclusions:

1. *The oxyanion hole catalytic contribution is overestimated by so-called conservative mutations.* **This conclusion holds.**

   More drastic mutations of oxyanion hole residues have smaller effects on catalysis, as originally reported (Supplemental Tables 2 and 3).

2. *More drastic mutations that create an extensive cavity surrounding the oxyanion hole have no additional deleterious effects.* **This conclusion is incorrect.**

   There are large effects from these additional mutations (Supplemental Tables 4−7). The origin of these deleterious effects remains to be determined but likely involves additional conformational rearrangements.

3. *A mutant enzyme with both oxyanion hole side chains truncated (tKSI Y16A/Y57F/D103A) shows no significant structural rearrangements.* **This conclusion holds.**

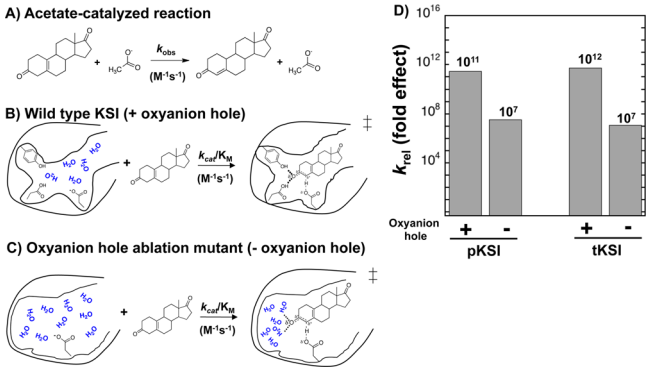
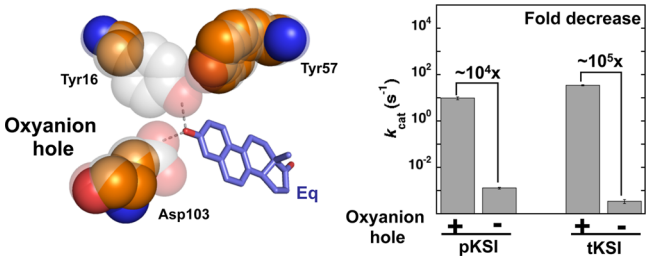


Figure 5. (Revised) Determining the catalytic contribution from the oxyanion hole in KSI. (A) The second-order solution reaction of a KSI substrate with acetate ion. (B) The second-order reaction of wild-type KSI (k_{cat}/K_M) with the same substrate. (C) The second-order reaction of the KSI oxyanion hole ablation mutant (k_{cat}/K_M). (D) Comparison of the second-order rate constant with the second-order nonenzymatic acetate-catalyzed reaction rate constant to measure the overall rate increase for KSI relative to the solution reaction that uses the same general base functionality. The values in panel D were obtained from the following: $k_{rel} = k_{enz}/k_{ac}$, for WT pKSI, 3.3×10^5 M^{-1} $s^{-1}/1 \times 10^{-6}$ M^{-1} s^{-1} = 10^{11}; for Y16G/D103G pKSI, 3.9×10^1 M^{-1} $s^{-1}/1 \times 10^{-6}$ M^{-1} s^{-1} = 10^7 (values from revised Table S1 and ref 3); for WT tKSI, 1.3×10^6 M^{-1} $s^{-1}/1 \times 10^{-6}$ M^{-1} s^{-1} = 10^{12}; for Y16G/D103G tKSI, 9 M^{-1} $s^{-1}/1 \times 10^{-6}$ M^{-1} s^{-1} = 10^7 (values from revised Table S3 and ref 3). Comparison of reaction from the KSI E·S complex relative to the acetate-catalyzed reaction is given in revised Figure S7.

DOI: 10.1021/jacs.6b04665
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

In addition to the new Supporting Information noted above, the Table of Contents graphic and Figures 2 and 5 from the original publication have been corrected; they are reproduced above and below.



■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04665.

Supplemental text and Tables 1−9 (PDF)
Supplemental Figures 1−9, which plot the data from Supplemental Tables 1−9 (PDF)
Experimental methods, Figures S1−S7, and Tables S1−S6 (revised original SI) (PDF)

■ ACKNOWLEDGMENTS

We are indebted to Filip Yabukarski, Aditya Natarajan, Vandana Lamba, and Margaux Pinney for bringing this problem to our attention and for helping collect the revised data.

■ REFERENCES

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(2) Schwans, J. P.; Sunden, F.; Lassila, J. K.; Gonzalez, A.; Tsai, Y.; Herschlag, D. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 11308−11313.
(3) Schwans, J. P.; Kraut, D. A.; Herschlag, D. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14271−14275.

DOI: 10.1021/jacs.6b04665
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX