Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Development and Application of a High Throughput Assay System for the Detection of Rieske Dioxygenase Activity

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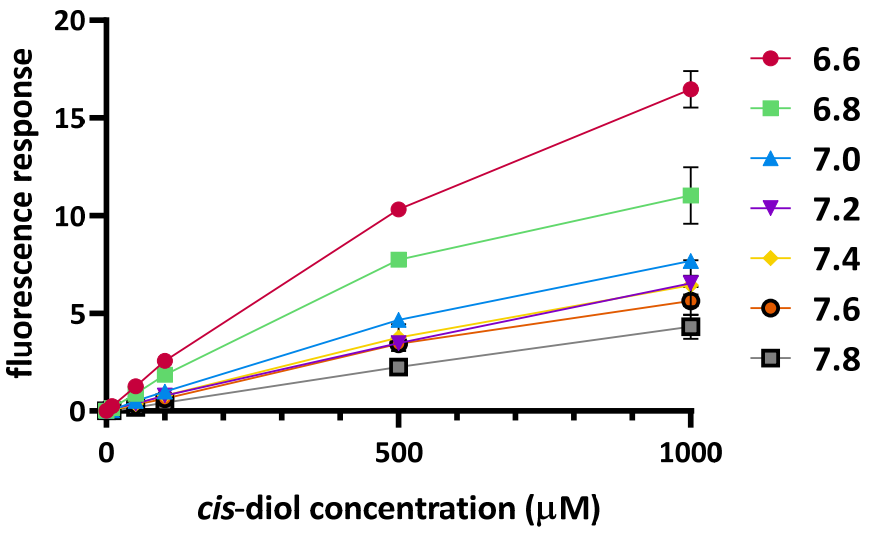

Figure S1: Assay Optimization Study; Fluorescence response in 0.1 M phosphate buffer at varied pH levels. All studies were performed in triplicate, with 0.1 mM fluoresceinamine and 10 mM $NaIO_4$. Fluorescence responses normalized to negative control ($[I - I_0] / I_0$).

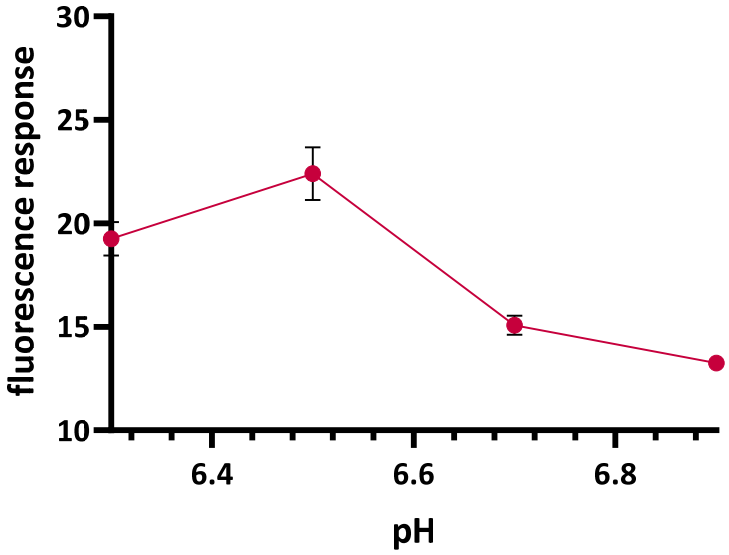

Figure S2: Assay Optimization Study; Fluorescence response in 0.1 M HEPES buffer (pH 7.2) which was adjusted to varied pH levels with acidified fluorescent probe solution. All studies were performed in triplicate with 1 mM *cis*-diol, 0.1 mM fluoresceinamine and 10 mM $NaIO_4$. Fluorescence responses normalized to negative control ($[I - I_0] / I_0$).

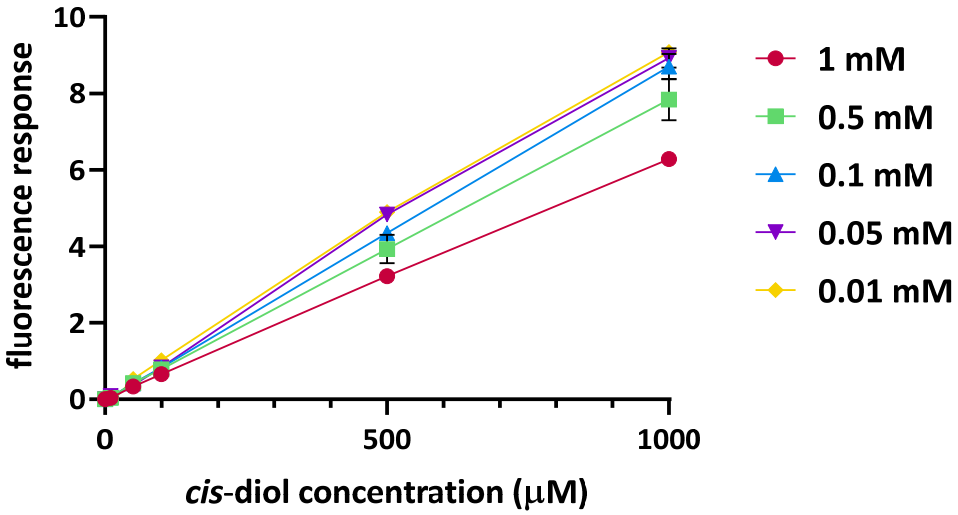

Figure S3: Assay Optimization Study; Fluorescence response in 0.1 M HEPES buffer (pH 7.2) at varied fluoresceinamine levels. All studies were performed in triplicate, with 10 mM $NaIO_4$. Fluorescence responses normalized to negative control ($[I - I_0] / I_0$).

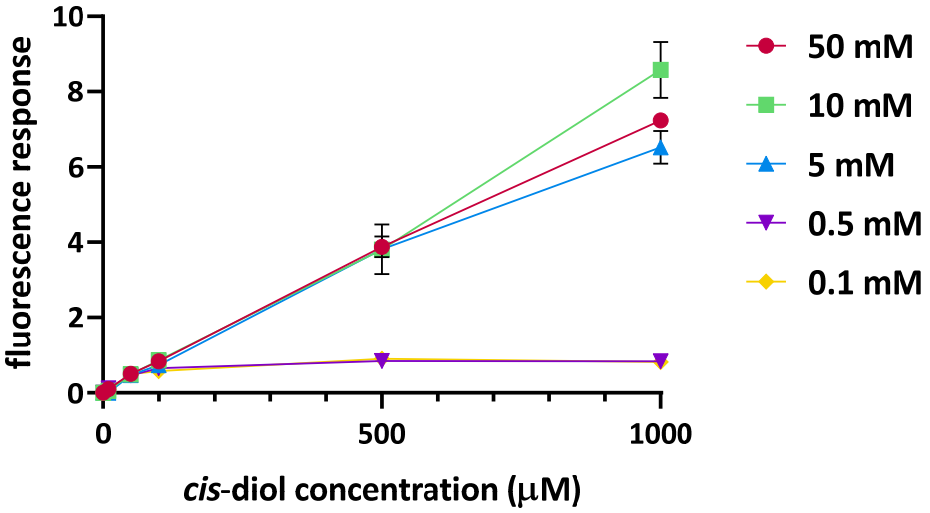

Figure S4: Assay Optimization Study; Fluorescence response in 0.1 M HEPES buffer (pH 7.2) at varied $NaIO_4$ levels. All studies were performed in triplicate, with 0.1 mM fluoresceinamine. Fluorescence responses normalized to negative control ($[I - I_0] / I_0$).

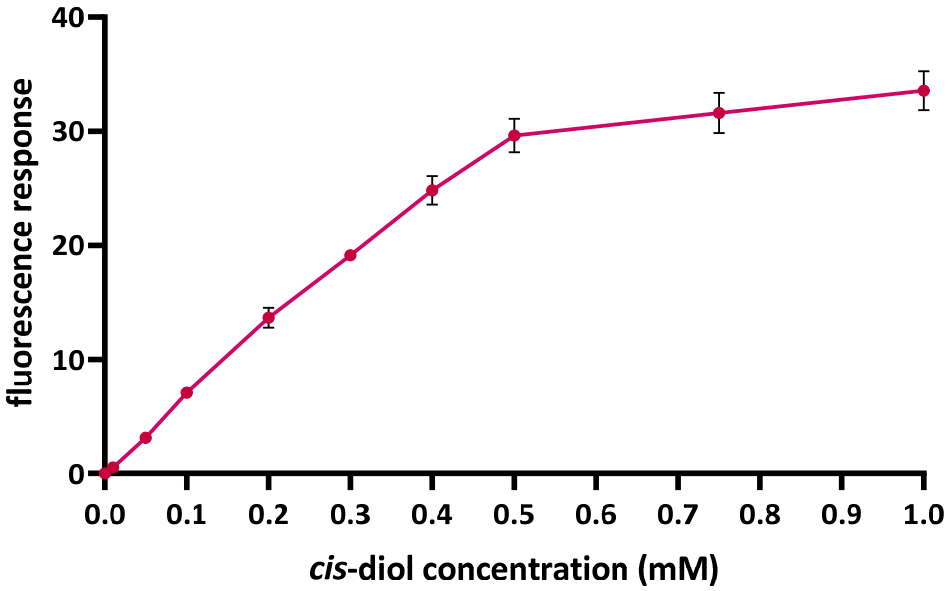

Figure S5: Concentration-response assay for the determination of linear range; purified *cis*-diol dissolved in "spent" minimal media analyzed in triplicate with 10 mM $NaIO_4$ and 0.1 mM fluoresceinamine (R^2 (0-0.5 mM) = 0.996). Fluorescence responses normalized to negative control ($[I - I_0] / I_0$).

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TDO_C1_parent     721  GAAATGGCCGACCTTGCTCCGCCGACAGTTGGCAAGCAGTACCGTGCGTCATGGGGCGGA
TDO_C1_A1         721  GAAATGGCCGACCTTGCTCCGCCGACAGTTGGCAAGCAGTACCGTGCGTCATGGGGCGGA
TDO_C1_A2         721  GAAATGGCCGACCTTGCTCCGCCGACAGTTGGCAAGCAGTACCGTGCGTCATGGGGCGGA
TDO_C1_U1         721  GAAATGGCCGACCTTGCTCCGCCGACAGTTGGCAAGCAGTACCGTGCGTCATGGGGCGGA

TDO_C1_parent     781  CATGGAAGTGGCTTCTATGTCGGCGACCCCAATCTGATGCTTGCCATCATGGGGCCAAAG
TDO_C1_A1         781  CATGGAAGTGGCTTCTATGTCGGCGACCCCAATGTTATGCTTGCCATCATGGGGCCAAAG
TDO_C1_A2         781  CATGGAAGTGGCTTCTATGTCGGCGACCCCAATTTCATGCTTGCCATCATGGGGCCAAAG
TDO_C1_U1         781  CATGGAAGTGGCTTCTATGTCGGCGACCCCAATCCGATGCTTGCCATCATGGGGCCAAAG

TDO_C1_parent     841  GTCACCAGCTACTGGACCGAAGGCCCCGCGTCGGAAAAGGCGGCCGAACGTCTGGGTAGC
TDO_C1_A1         841  GTCACCAGCTACTGGACCGAAGGCCCCGCGTCGGAAAAGGCGGCCGAACGTCTGGGTAGC
TDO_C1_A2         841  GTCACCAGCTACTGGACCGAAGGCCCCGCGTCGGAAAAGGCGGCCGAACGTCTGGGTAGC
TDO_C1_U1         841  GTCACCAGCTACTGGACCGAAGGCCCCGCGTCGGAAAAGGCGGCCGAACGTCTGGGTAGC
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Figure S6: Sequence alignment for isolated toluene dioxygenase L272 mutants. Image generated with boxshade software (A1 = L272V, A2 = L272F, U1 = L272P).

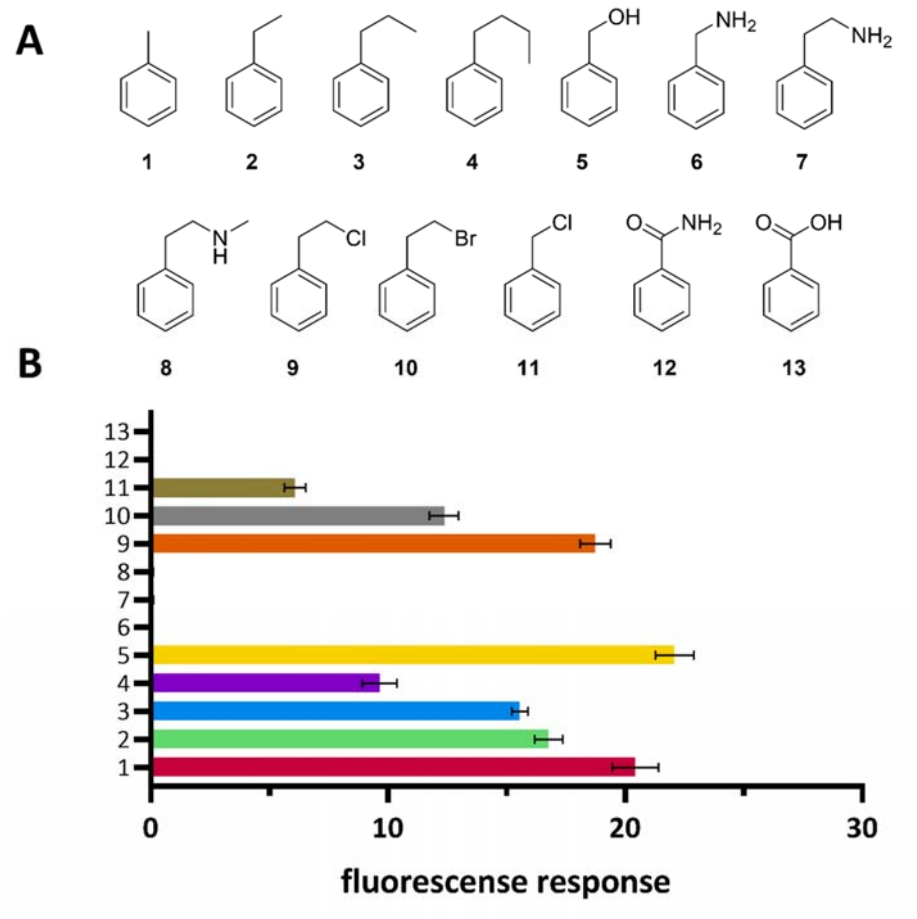

Figure S7: Toluene dioxygenase L272F mutant substrate scope is analyzed using the designed assay system (n = 4 for each substrate). Fluorescence responses normalized to negative control ($[I - I_0] / I_0)$].

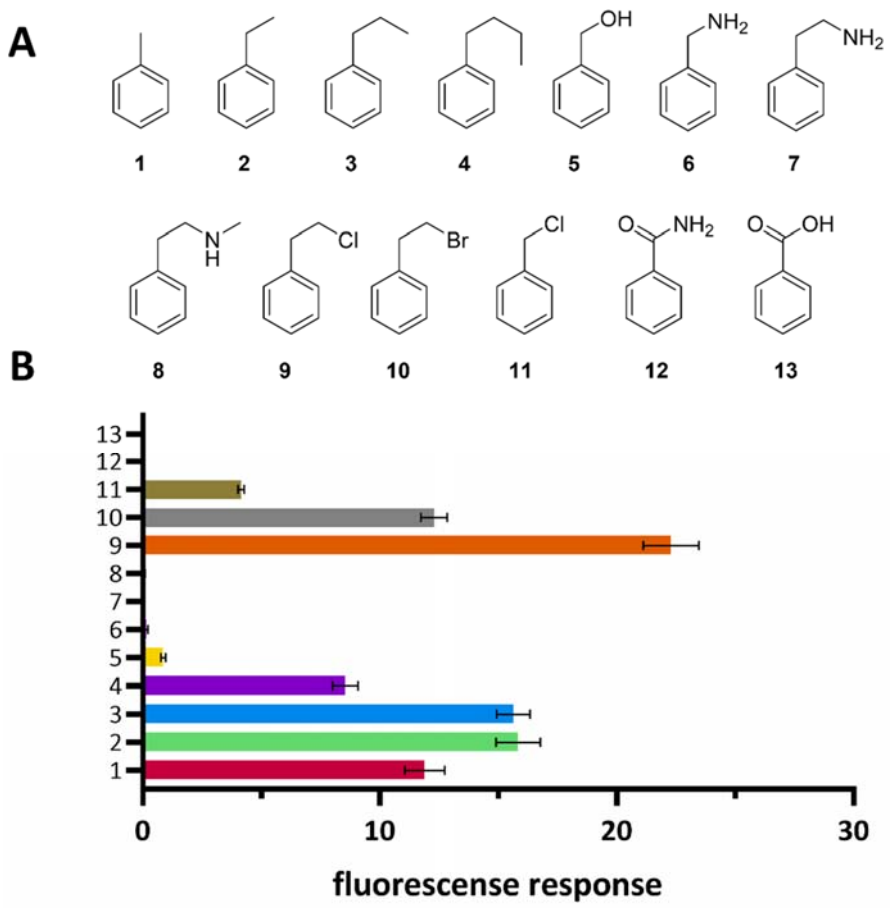

Figure S8: Toluene dioxygenase L272V mutant substrate scope is analyzed using the designed assay system (n = 4 for each substrate). Fluorescence responses normalized to negative control ($[I - I_0] / I_0$)].