Supporting Information for:

Stoichiometry of the Redox Neutral Deamination and Oxidative Dehydrogenation Reactions Catalyzed by the Radical SAM Enzyme DesII

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1 Determining the Stoichiometry from Fractions of Reaction.

In designing the experiments to determine the stoichiometry, x in equation 4,[1] it was reasoned that measurements of relative changes in the concentrations of SAM and the TDP-sugar substrate of interest would be both more reliable and facile than attempting to accurately measure the absolute concentrations of each species during the course of the reaction. Because relative changes in concentration are being measured, this approach should be insensitive to variations in the HPLC injection volume and any manipulations of the reactions, such as adding additional enzyme or reductant, that only affect the absolute concentrations of SAM, TDP-sugar substrate and the corresponding products but otherwise have no effect on the relative concentrations. Likewise, this approach obviates the need for a highly accurate determination of the initial concentrations of SAM and the TDP-sugar substrate, because it could be replaced by a more accurate and precise measurement of the relative initial concentrations by HPLC. This is a particularly useful consequence as commercial SAM is approximately 70% pure, and gravimetric determination of the TDP-sugar substrates is complicated by the presence of ammonium salts.

Accordingly, it was decided to determine fractions of reaction in terms of both SAM and the TDP-sugar substrate, f_{SAM} and f_{sub}, respectively. For this reason, it is necessary to find an expression relating changes in f_{SAM} versus f_{sub} that permits

[1] Equation and figure numbering follows that in the primary manuscript.

a determination of the stoichiometry of interest. We can write the stoichiometry, x, according to

$$x = |dn_{SAM}/dn_{sub}| \tag{8}$$

where n_{SAM} and n_{sub} denote the number of moles of SAM and the TDP-sugar substrate in the reaction mixture at any given point in time. As there is no reason to assume that the stoichiometry is not a constant, which is an assumption justified by the apparent linearity of the plots in Figure 2, equation 8 can just as well be written as,

$$x = \Delta n_{SAM}/\Delta n_{sub} \tag{9}$$

where we have removed the absolute value bars as both SAM and the TDP-sugar substrate are consumed in the DesII forward reaction.

The fraction of reaction for SAM can be written as,

$$f_{SAM} = -\Delta n_{SAM}/n^0_{SAM} \tag{10}$$

where n^0_{SAM} denotes the number of moles of SAM present prior to any reaction having taken place. An expression analogous to equation 10 can also be written for the TDP-sugar substrate. We can then rewrite equation 9 in terms of fractions of reaction as

$$x = (n^0_{SAM}/n^0_{sub})/(f_{sub}/f_{SAM}) \tag{11}$$

Therefore, a plot of f_{SAM} versus f_{sub} should be linear with zero intercept and a slope, β, equal to the stoichiometry modulated by the ratio n^0_{sub}/n^0_{SAM}, which directly corresponds to the correction factor γ in equations 1 and 7. This then provides

$$\beta = \gamma x \tag{12}$$

which immediately leads to equation 7. This correction is therefore necessary and intuitive. Consider, for example, the case where the stoichiometry is one-to-one. If the initial concentration of SAM is twice that of the TDP-sugar substrate, then a 100% conversion of the latter would be accompanied by only a 50% conversion of the former. Neglecting the correction of $\gamma = 1/2$, interpretation using the slope alone would lead to the erroneous conclusion that SAM is consumed at half the rate of the TDP-sugar substrate. The correction factor γ is determined according to equation 1 by recognizing that the peak areas, a, for any given species are proportional to the concentration in the injected sample. This proportionality is then the product of the extinction coefficient at the detector wavelength of 267 nm and a constant of proportionality that is unique to the chromatogram but nevertheless equivalent for all peaks therein. This latter constant therefore cancels in any ratio of two peak areas from the same chromatogram.

2 Correcting for a Contaminant Underlying the 5′-Deoxyadenosine HPLC Peak.

In the determination of f_{SAM} it is necessary to take into account that a small contaminating peak overlaps with that from the 5′-deoxyadenosine product, see Figure 1. Direct subtraction of this contaminant is complicated by the attempt to keep all measurements in terms of relative concentrations. The correction is therefore made by assuming that the concentration

of the contaminant relative to the sum of SAM and 5′-deoxyadenosine does not change during the course of the reaction. We denote this constant ratio as $\kappa - 1$ (equation 2), and find it in terms of the peak areas of SAM and the contaminant prior to any reaction, since there is no 5′-deoxyadenosine present, or least none that is a result of the reaction of interest. Using our assumption of no change with extent of reaction we then have

$$\kappa - 1 = \frac{a_{cont}}{a_{dAdo} + a_{SAM}} \tag{13}$$

The observed fraction of reaction for SAM at any given time point is given by

$$f_{SAM}^{obs} = \frac{a_{dAdo} + a_{cont}}{a_{dAdo} + a_{SAM} + a_{cont}} \tag{14}$$

Dividing equation 14 through by $a_{dAdo} + a_{SAM}$ and applying expression 13 we obtain

$$f_{SAM}^{obs} = \frac{f_{SAM} + (\kappa - 1)}{1 + (\kappa - 1)} \tag{15}$$

This expression can then be rearranged to

$$f_{SAM} = \kappa f_{SAM}^{obs} - (\kappa - 1) \tag{16}$$

which is equivalent to equation 3 providing the corrected fraction of reaction in terms of SAM.