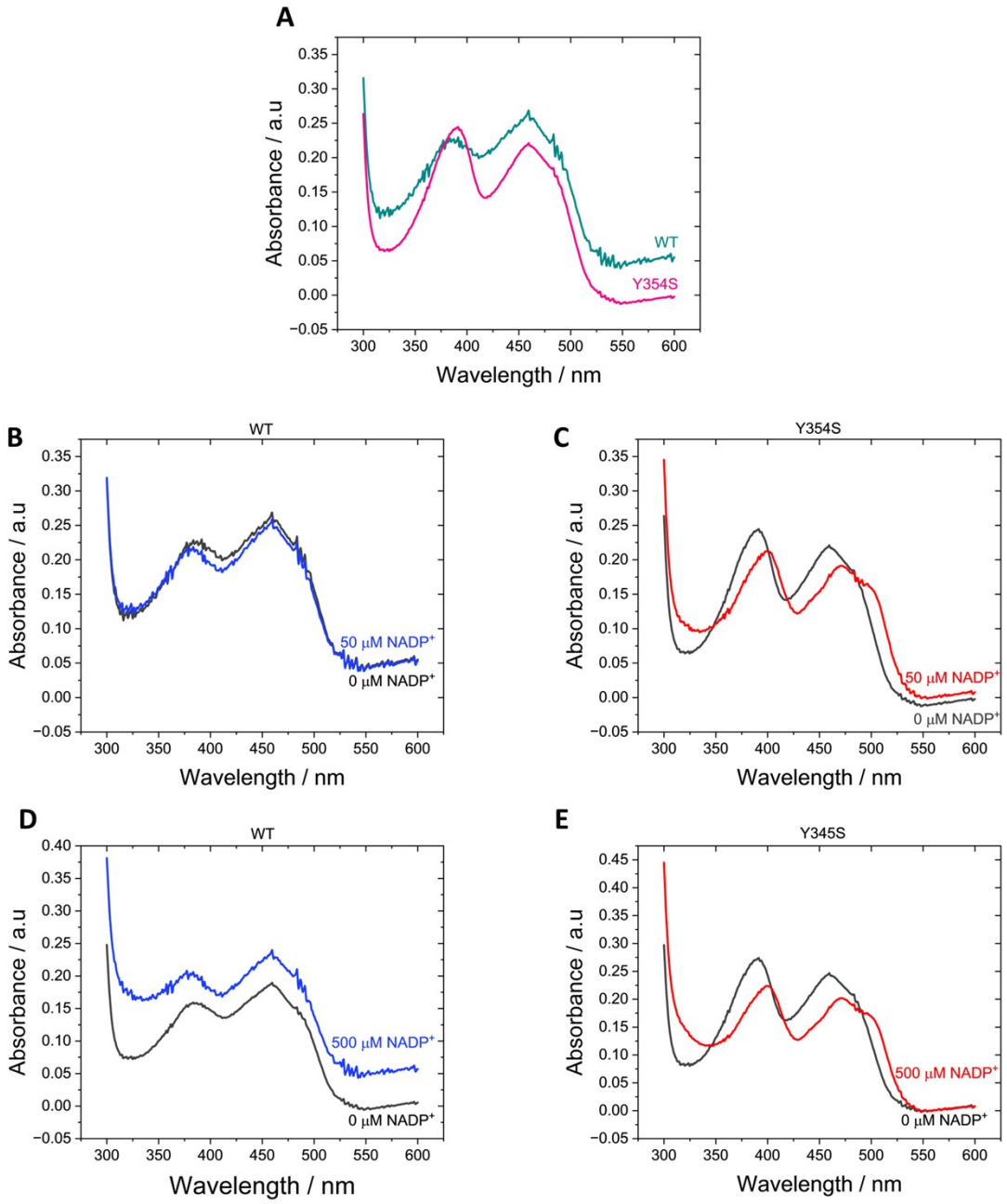
Retuning the Potential of the Electrochemical Leaf

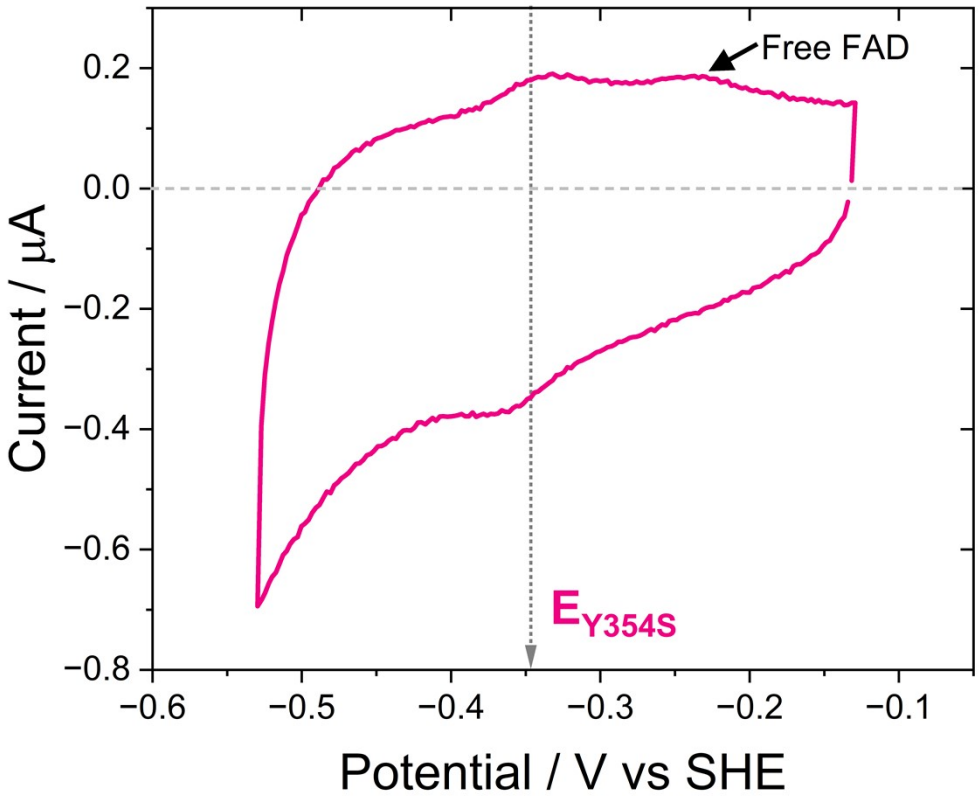
Marta M. Dolinska, [a] Adam J. Kirwan [a] and Clare F. Megarity* [a]

Supplementary Information

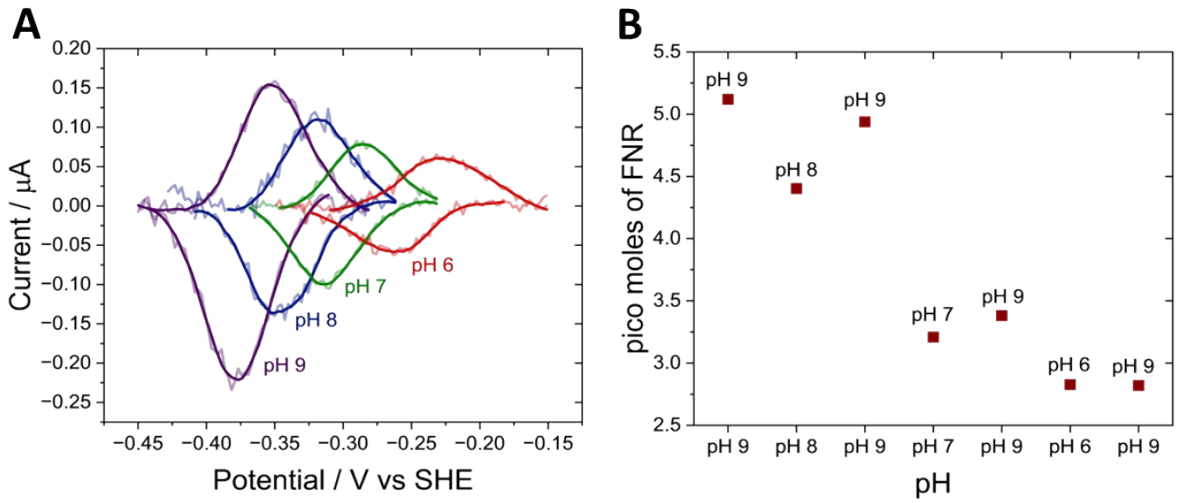
Contents



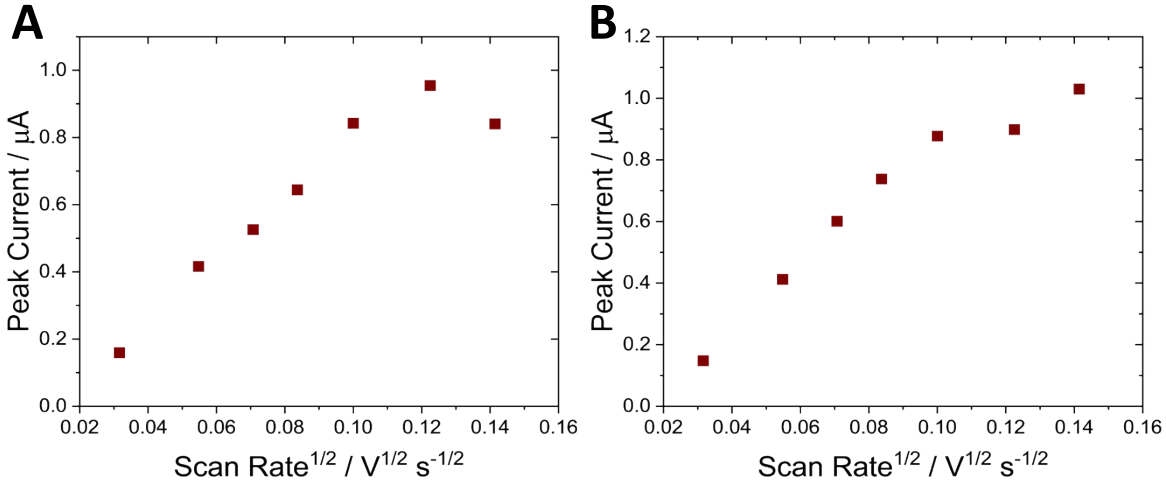
SI Figure 1: Visible spectra of the enzymes as purified. **A:** Comparison of WT FNR and the Y354S variant. **B:** WT with and without an equimolar amount of $NADP^+$ **C:** Y354S with and without an equimolar amount of $NADP^+$ **D:** WT with and without ten times excess of $NADP^+$. **E:** Y354S with and without ten times excess of $NADP^+$ ($NADP^+$ premixed with enzyme before measurement). Experiment conditions: Buffer: 50 mM MES 50 mM TAPS, pH 8; [enzyme] 50 µM in each experiment.



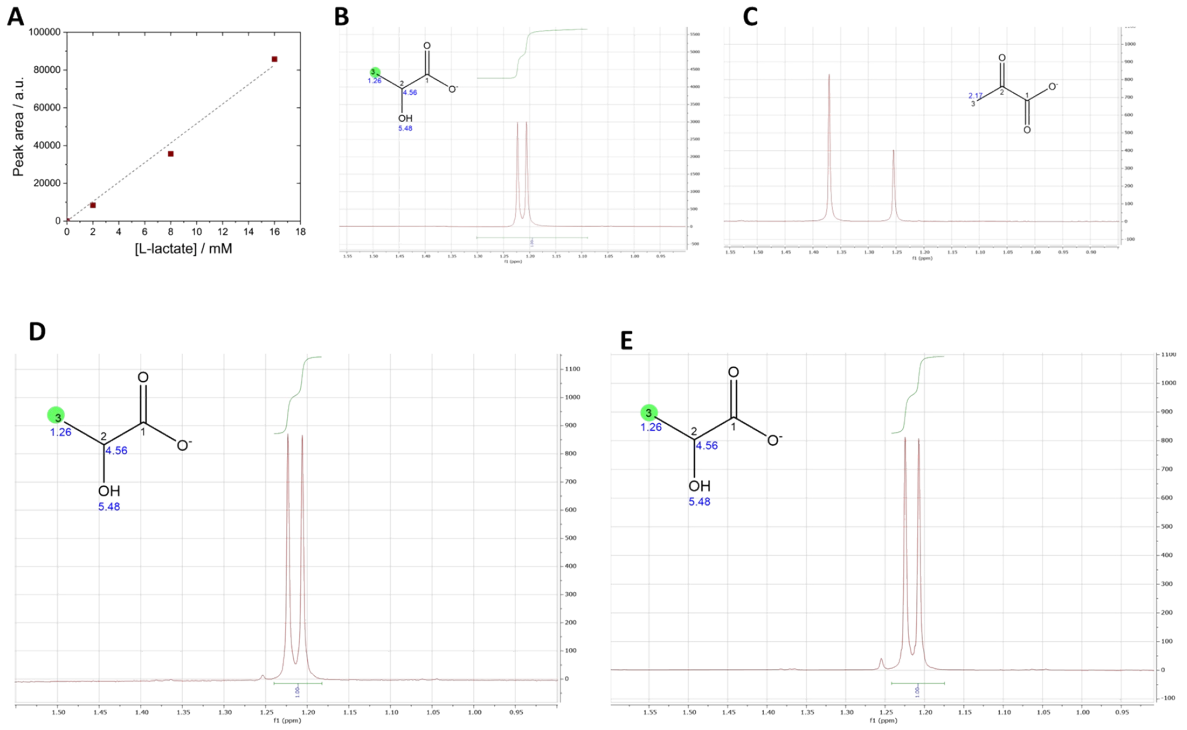
SI Figure 2: Flavin released from Y354S measured by cyclic voltammetry. Redox non turnover peaks at pH 8 corresponding to the oxidation and reduction of the FAD cofactor in the Y354S variant; the additional oxidation peak is indicated by an arrow and occurs at approximately the reduction potential unbound "free" FAD at pH 8. Experiment conditions; buffer: 50 mM MES, 50 mM TAPS pH 8; electrode Y354S@ITO/PGE; scan rate 5 mVs^{-1}; cell solution purged with argon to remove contribution to current from oxygen.



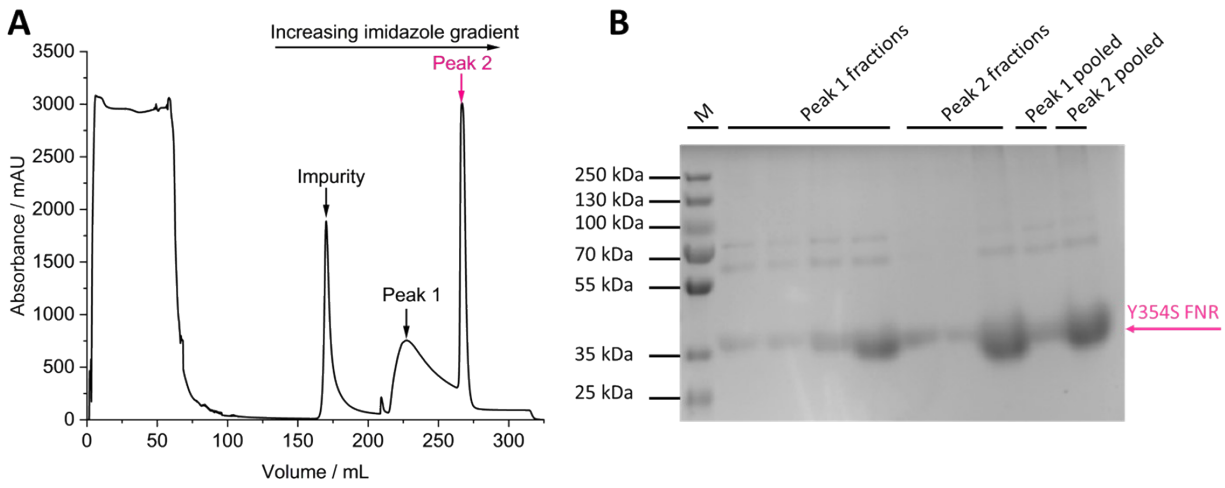
SI Figure 3. pH dependence of the Y354S FNR variant immobilised on the FNR@ITO/PGE electrode. **A:** Background subtracted non-turnover peaks of the Y354S FNR in a range of different pHs (6.0 – 9.0). **B:** Changes in Y354S FNR coverage starting at pH 9 and alternating between each pH in the order as shown. Experiment conditions: buffer: 50 mM MES, 50 mM TAPS (pH range 6-9); electrode Y354S@ITO/PGE; scan rate 10 mVs^{-1}; cell solution purged with argon to remove contribution to current from oxygen.



SI Figure 4: Scan rate dependence of reduction and oxidation of NAD^+ catalysed by Y354S immobilised in the ITO electrode pores. **A:** Peak reduction currents for NAD^+ (20 µM) against the square root of scan rate. **B:** Peak oxidation currents for NAD^+ (20 µM) against the square root of scan rate. The peak currents remained linear up to scan rate 0.03 Vs^{-1} after which it became difficult to analyse the peaks due to peak broadening. The WT enzyme showed an upward deviation from linearity over a wider range of scan rates indicating partially surface-confined behaviour of the cofactor[1] this cannot be ruled out for the variant since faster scan rates were not measurable. Experiment conditions: buffer: 50 mM MES, 50 mM TAPS pH 8; electrode Y354S@ITO/PGE; cell solution purged with argon to remove contribution to current from oxygen.



SI Figure 5: ^{1}H NMR Analysis **A:** Standard curve for the quantification of L-Lactate by ^{1}H NMR; **B:** ^{1}H NMR spectrum for a standard solution of 16 mM L-lactate in 50 mM MES, 50 mM TAPS pH 9 (the reaction buffer for the chronoamperometry experiment in the main text)- the doublet peak shown was used for quantification (assignment of the doublet shown as green circle on the molecule); **C:** ^{1}H NMR spectrum for a standard solution of pyruvate in 50 mM MES, 50 mM TAPS pH 9; D: ^{1}H NMR spectrum for the sample taken at the end of phase one (t= 5 h); **E:** ^{1}H NMR spectrum for the sample taken at the end of the experiment.



SI Figure 6: Purification of Y354S **A:** Chromatogram for the purification by nickel-affinity chromatography of the HIS-tagged Y354S variant**. B:** SDS PAGE showing selected fractions based on the peaks on the corresponding chromatogram.

References

1. C. F. Megarity, B. Siritanaratkul, R. S. Heath, L. Wan, G. Morello, S. R. FitzPatrick, R. L. Booth, A. J. Sills, A. W. Robertson, J. H. Warner, N. J. Turner and F. A. Armstrong, *Angewandte Chemie International Edition*, 2019, **58**, 4948-4952.