

Figure S1

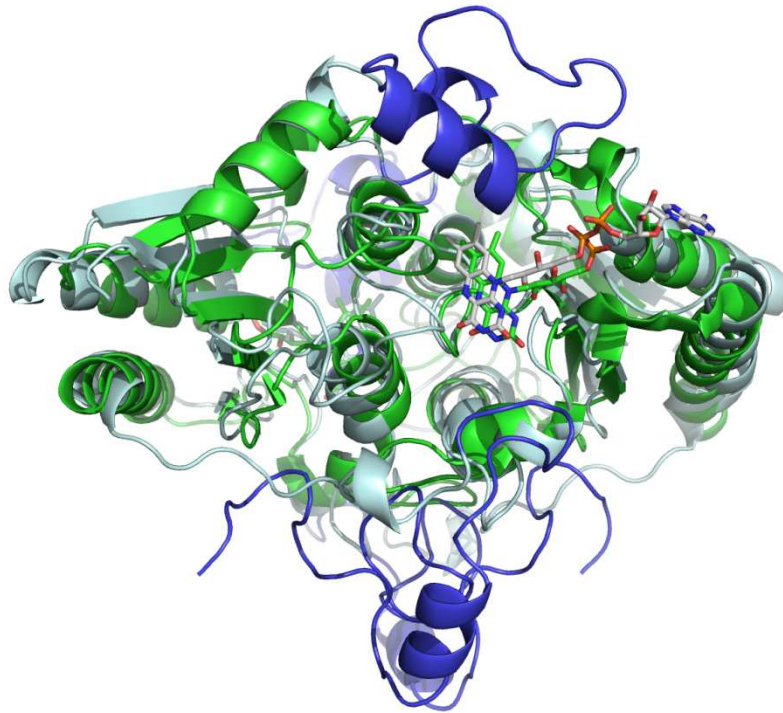


Figure S1: Structural alignment of KefF and human QR1. The crystal structure of KefF (pdb 3EYW, green) was aligned with human QR1 (pdb 1QBG, light blue) using PyMOL. QR1 has an insertion in comparison to KefF from residue 48-80 (ink blue, top), which is also found in QR2 and partially caps the active site. QR1 also has an extended C-terminus from residue 226 (ink blue, bottom) which is neither found in KefF nor in QR2.

Figure S2

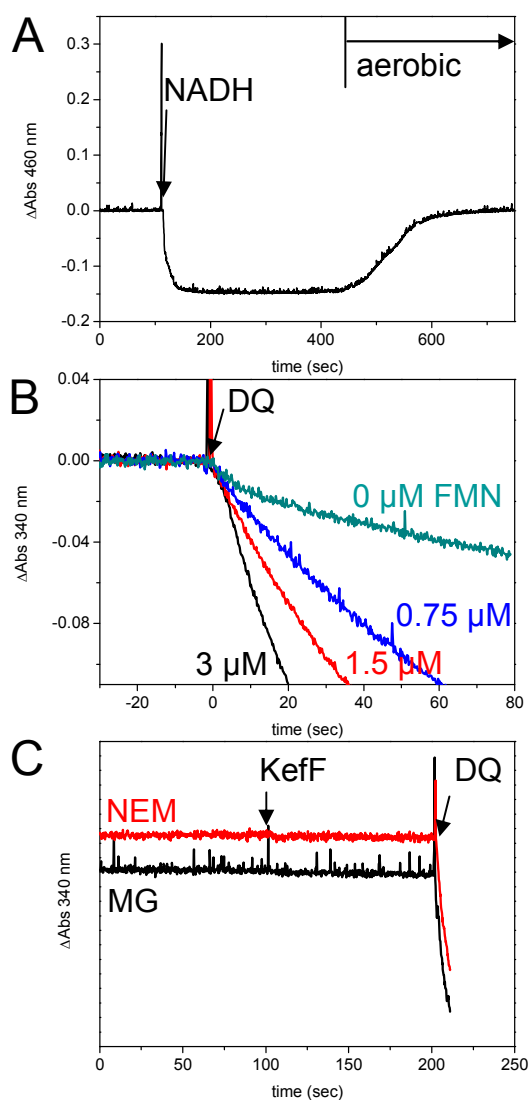


Figure S2: Catalytic properties of KefF. (A) An anaerobic solution of 19 μM KefF in a closed, stirred cuvette was reduced with 35 μM NADH. After 5 minutes, the seal was removed. The absorbance change at 460 nm was recorded. (B) KefF was incubated with different amounts of FMN in the assay buffer for 3 min before the reaction was started with duroquinone (DQ). (C) No oxidation of NADH was seen if NEM (red) or MG (black) was used as electron acceptor, while a fast reaction was seen with DQ. Traces are offset for clarity.

Figure S3

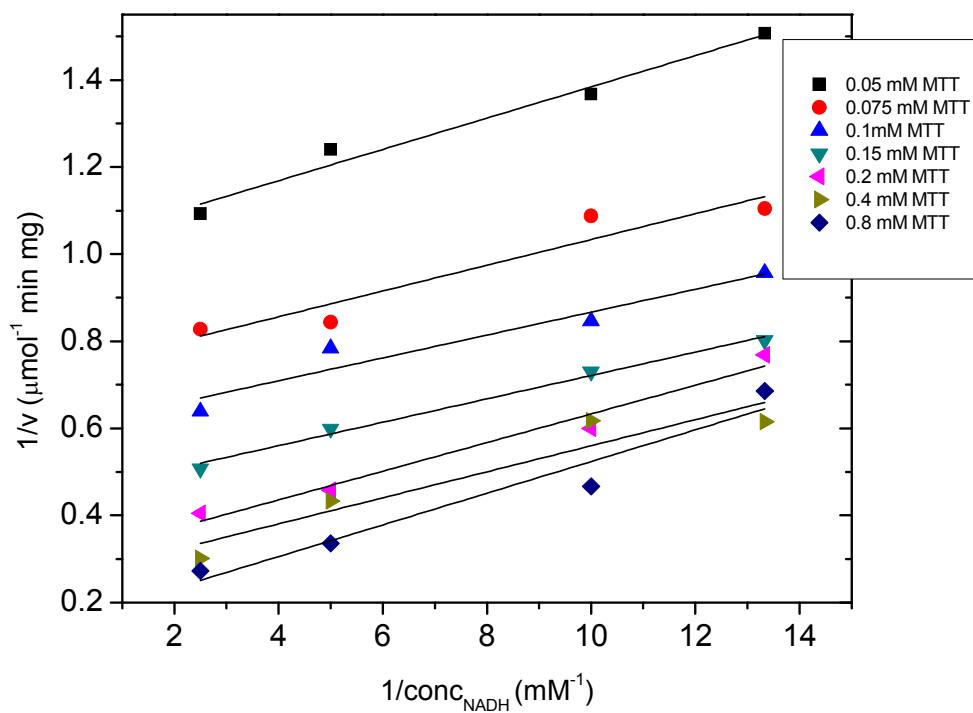


Figure S3: Lineweaver Burk plot of KefF enzymatic activity. Specific activities of KefF with NADH and MTT are plotted. Data were fitted by linear regression independently for each constant MTT concentration, respectively (lines).

Figure S4

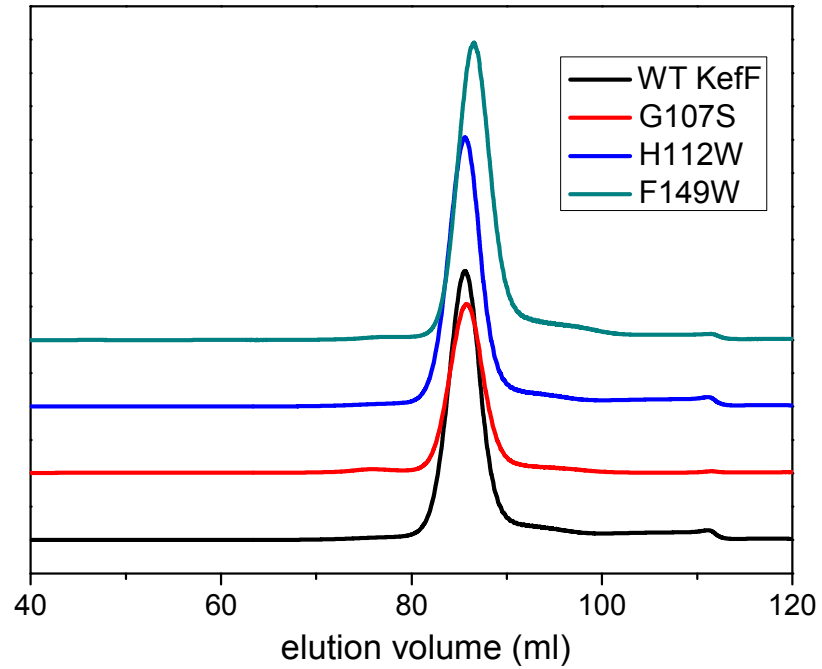


Figure S4: Size exclusion chromatography of Keff. The absorbance at 280 nm of Keff WT and mutants is shown (for clarity the baselines are offset).