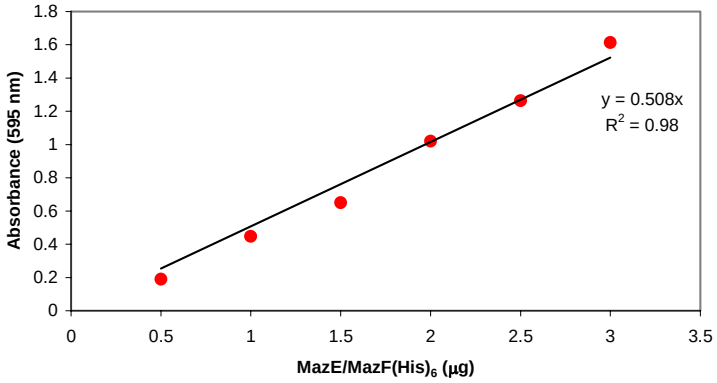
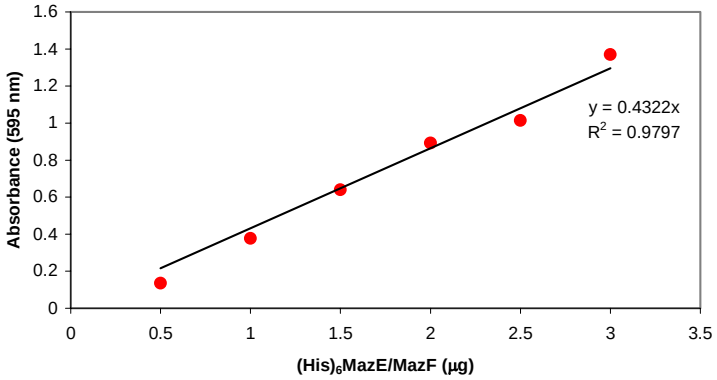
Supplementary Information

Figure 1. Bradford assay calibration curves for *A.* MazE/MazF(His)$_6$ *B.* (His)$_6$MazE/MazF *C.* MazF(His)$_6$ *D.* (His)$_6$MazE. Known amounts of lyophilized protein were resuspended in elution buffer and Bradford assays were performed obtain a relationship between absorbance at 595 nm and protein concentration.

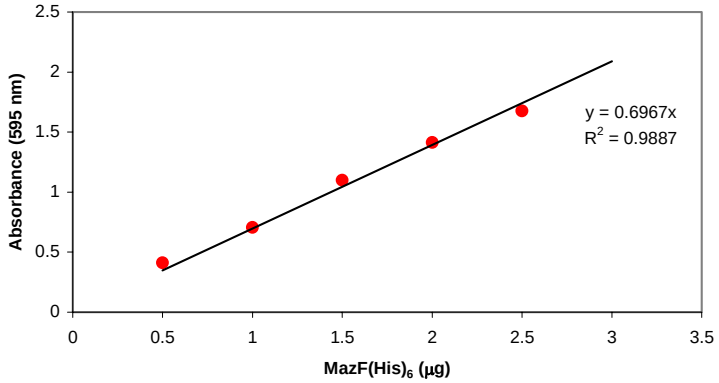
A.



B.



C.



D.

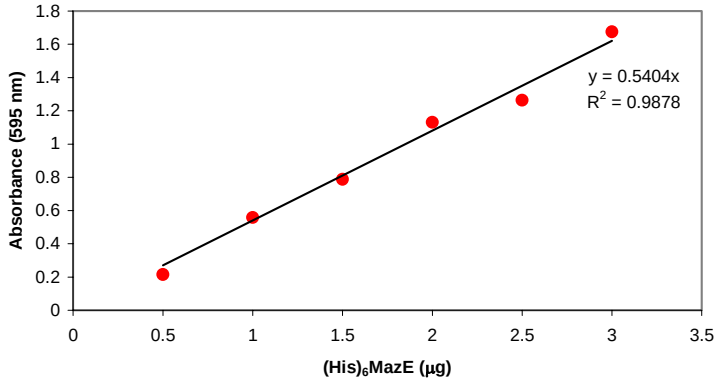
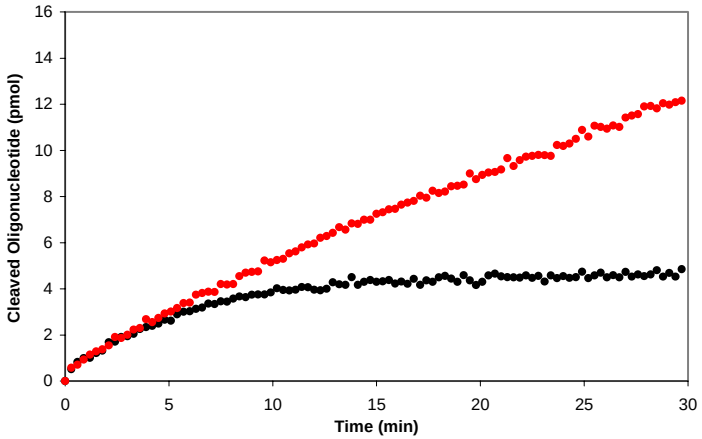


Figure 2. Initial increase in fluorescence observed when reading fluorescence (ex. 485 nm; em. 530 nm) of *A.* 20 µM oligonucleotide in the presence (red) and absence (black) of 3 µM MazF(His)$_6$ and *B.* of 100 nM 6-FAM alone

A.



B.

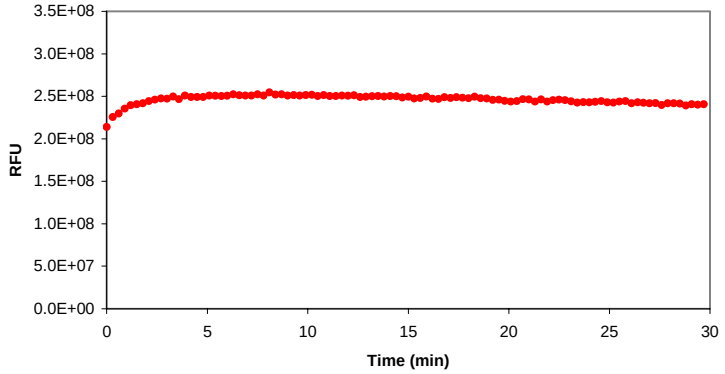


Figure 3. Reaction progress curves for three separate kinetic trials of oligonucleotide cleavage by MazF(His)$_6$

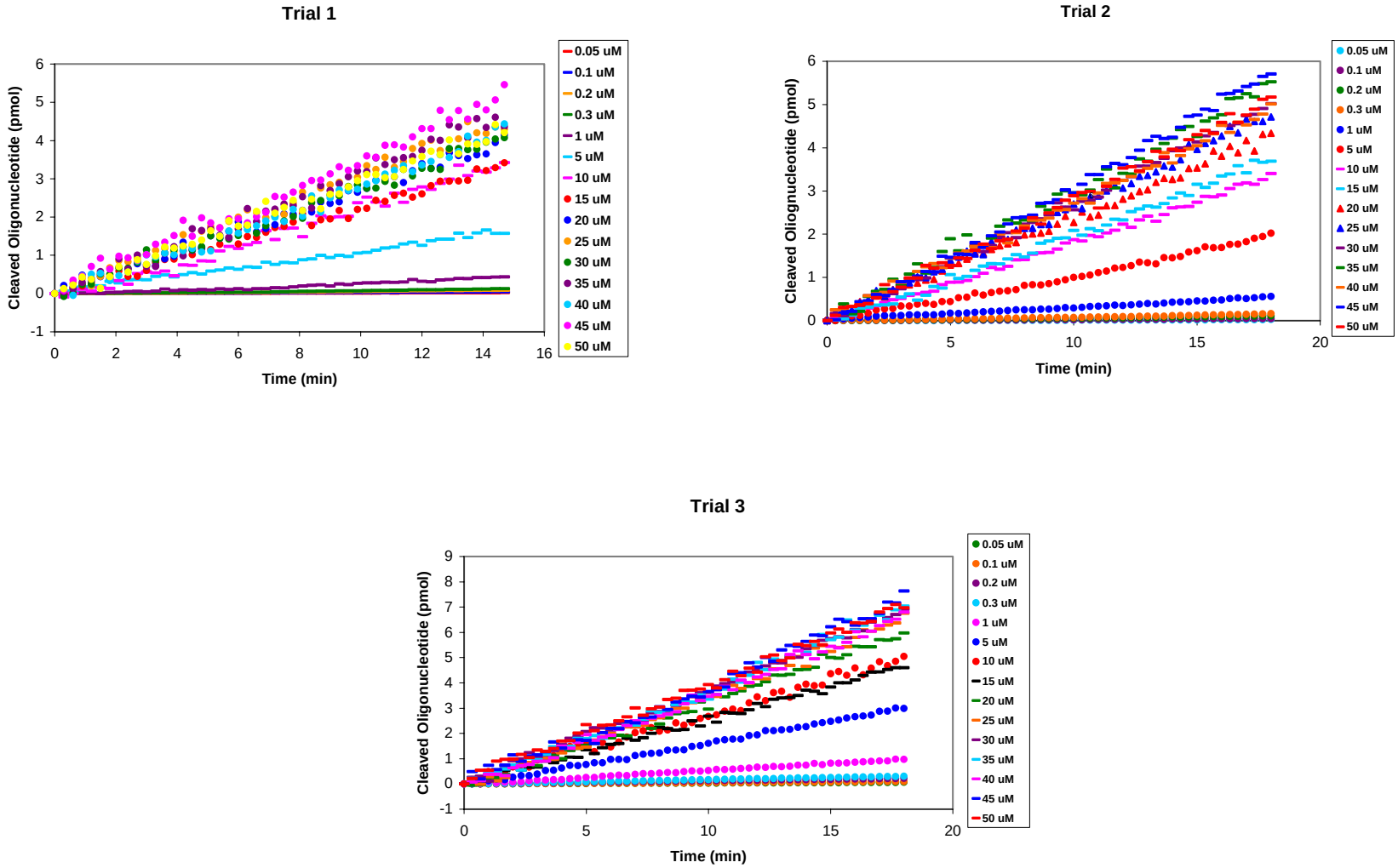
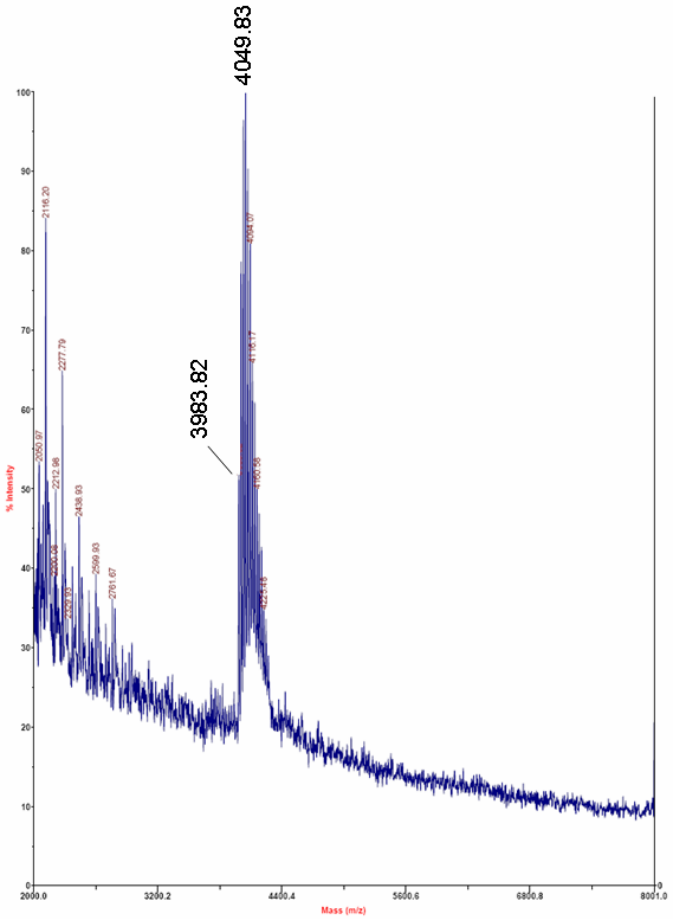
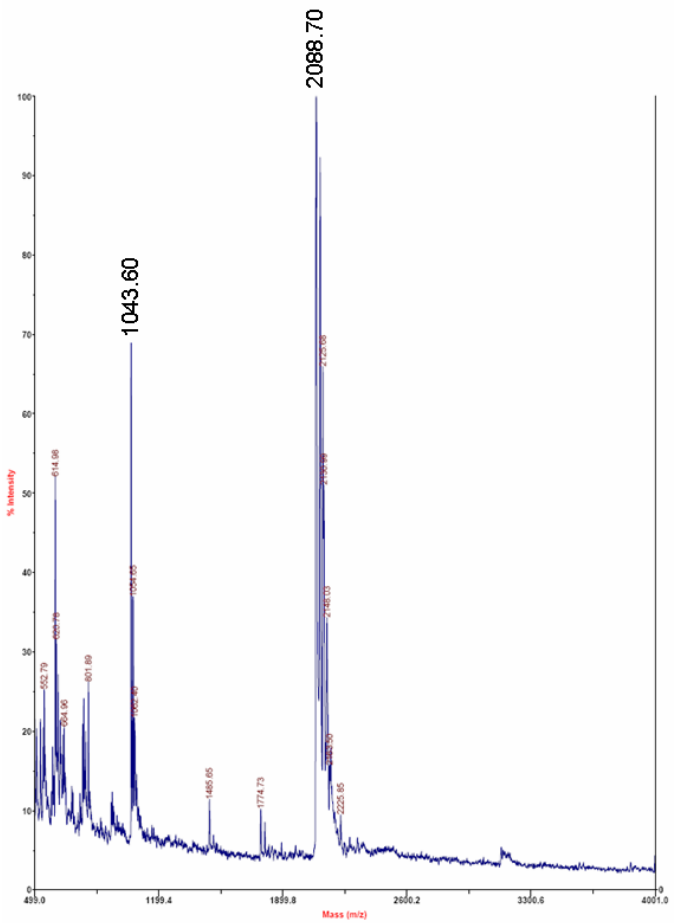


Figure 4. MALDI spectra of reaction products between oligonucleotide substrate and MazF(His)$_6$ or MazE/MazF(His)$_6$. *A-C.* Mass spectra for HPLC elutions in the reverse order that they came off the column for the reaction between oligonucleotide substrate and MazF(His)$_6$. The spectrum in *A* corresponds to the small amount of unprocessed oligonucleotide that remains, as shown in Figure 4 of the manuscript. *D.* Mass spectrum for HPLC elution forming single peak in reaction of oligonucleotide with MazE/MazF(His)$_6$.

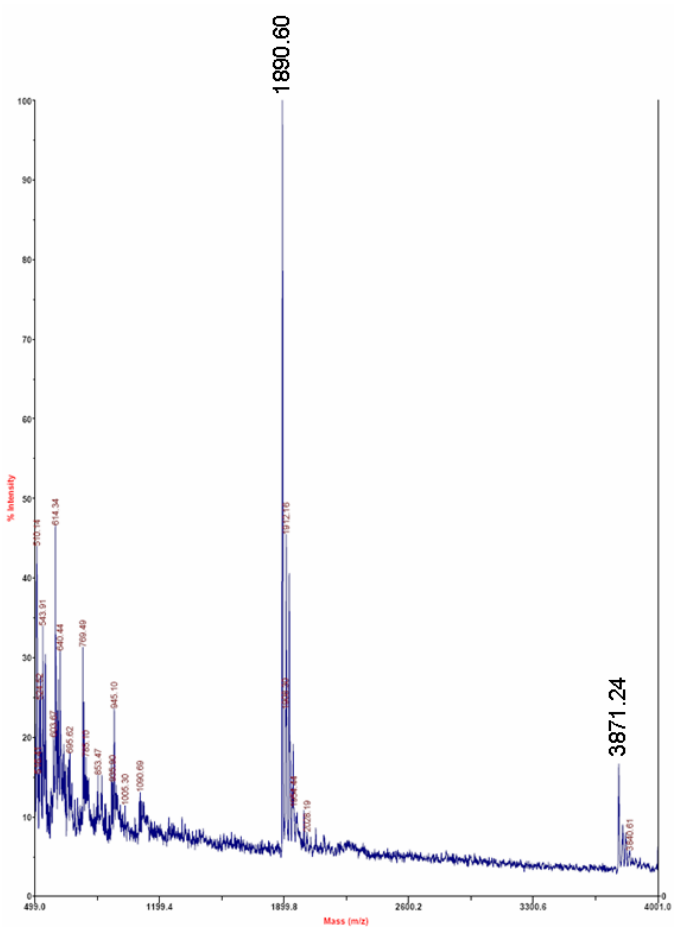
A.



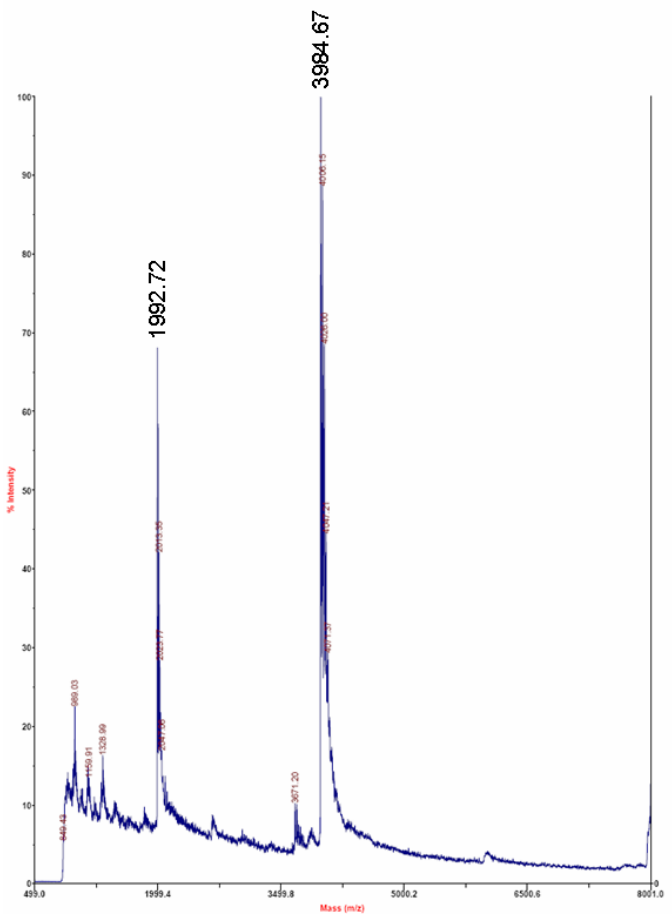
B.



C.


1890.60
3871.24
% Intensity
Mass (m/z)

D.


3984.67
1992.72
% Intensity
Mass (m/z)