

**Supplementary Table 1. SAXS parameters across the AtzF and AtzF<sub>467</sub> concentration series.**

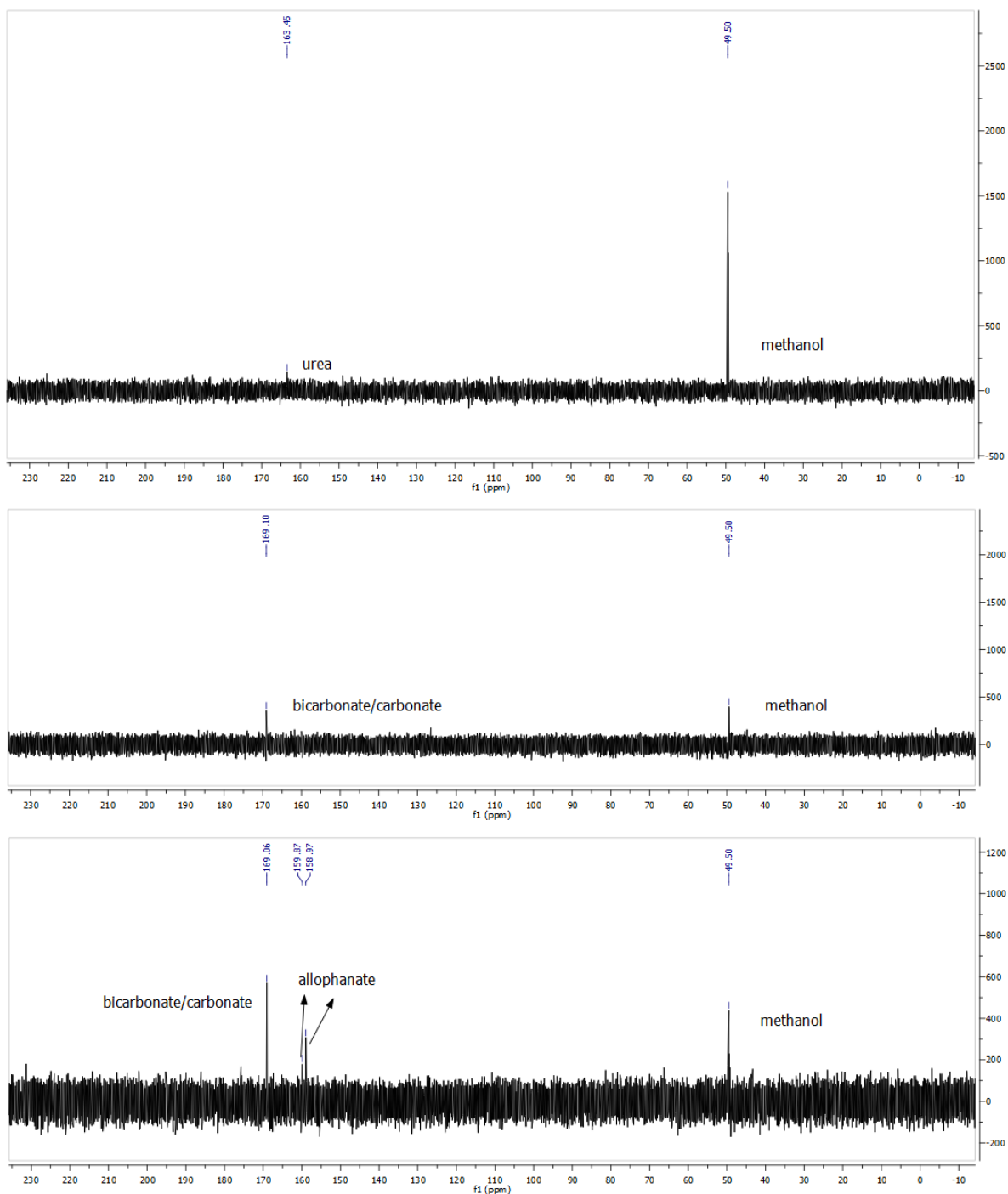
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AtzF				
Conc (mg/ml)	I(0)	MW (Kda)	Rg	No. subunits*
0.4	0.065	249.3	50.6	3.8
0.7	0.132	253.2	51.1	3.8
1.5	0.267	256.1	51.2	3.9
2.9	0.521	249.8	51.2	3.8
5.8	0.993	238.1	51.4	3.6

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AtzF <sub>467</sub>				
protein conc (mg/ml)	I(0)	Mol. Weight (Kda)	Rg (Å)	No. subunits*
0.8	0.071	119.8	37.7	2.3
1.6	0.132	111.4	37.2	2.2
3.3	0.268	113.1	37.0	2.2
6.6	0.506	106.7	35.8	2.1
13.1	0.919	96.9	36.5	1.9

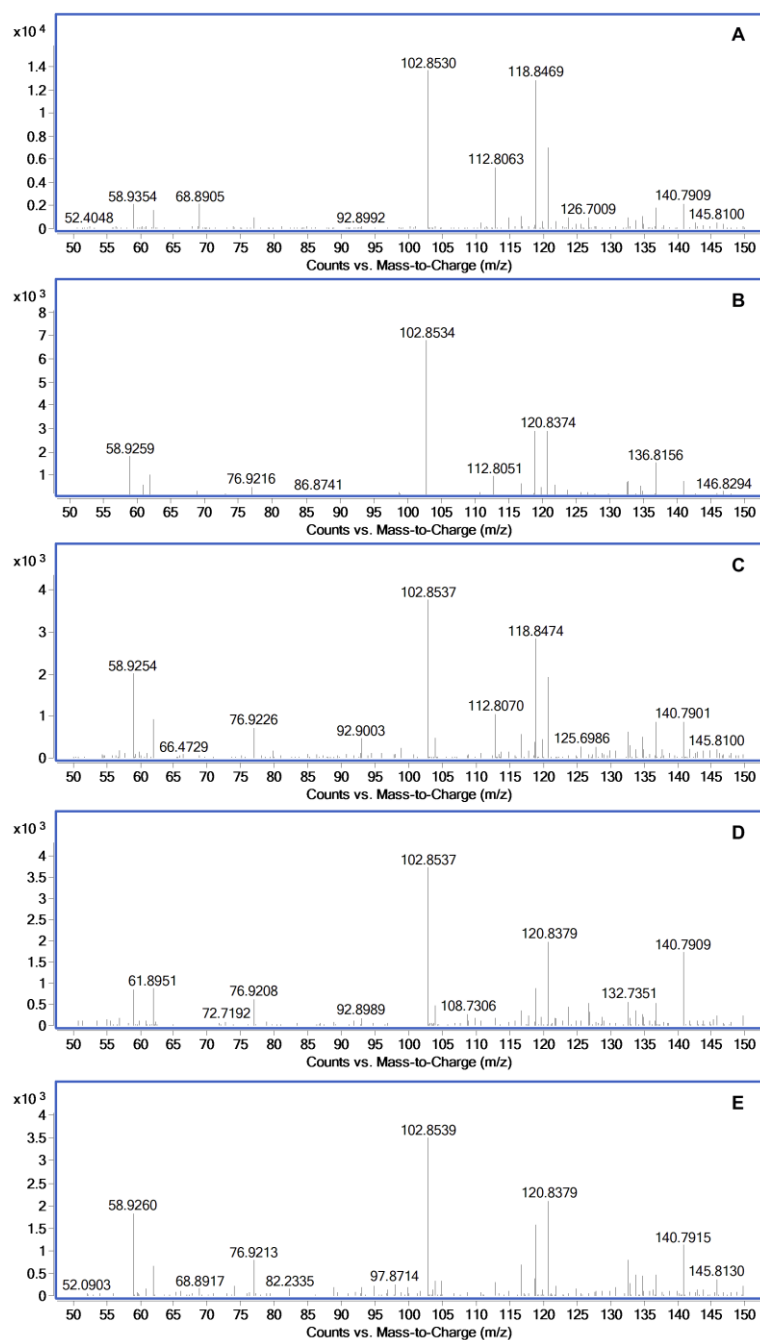
**Supplementary Table 2. Effect of temperature and pH on the catalytic properties of AtzF, AtzF<sub>467</sub>, and AtzFH488A.**

Enzyme	Temp (°C)	pH	$K_M$ (μM)	$k_{cat}$ (s <sup>-1</sup> )	$k_{cat}/K_M$ (M <sup>-1</sup> .s <sup>-1</sup> ) x10 <sup>4</sup>
AtzF	28	7.0	120.0 ± 21.4	8.6 ± 0.4	7.1 ± 1.3
AtzF <sub>467</sub>			114.6 ± 17.2	7.4 ± 0.3	6.5 ± 0.1
AtzF H488A			127.6 ± 13.6	7.8 ± 0.2	6.1 ± 0.7
AtzF	28	7.5	149.5 ± 15.7	11.6 ± 0.3	7.7 ± 0.8
AtzF <sub>467</sub>			168.6 ± 21.9	11.2 ± 0.4	6.7 ± 0.9
AtzF H488A			156.1 ± 11.2	11.6 ± 0.2	7.5 ± 0.5
AtzF	28	8.0	115.7 ± 8.8	13.3 ± 0.2	11.5 ± 0.9
AtzF <sub>467</sub>			109.3 ± 4.2	11.8 ± 0.1	10.8 ± 0.4
AtzF H488A			113.9 ± 4.7	13.1 ± 0.1	11.5 ± 0.5
AtzF	28	8.5	78.6 ± 4.6	13.2 ± 0.2	16.8 ± 0.1
AtzF <sub>467</sub>			71.0 ± 4.7	7.3 ± 0.1	10.2 ± 0.7
AtzF H488A			72.6 ± 5.0	12.0 ± 0.2	16.6 ± 1.0
AtzF	28	9.0	74.0 ± 4.6	10.7 ± 0.1	14.4 ± 0.9
AtzF <sub>467</sub>			174.9 ± 26.0	3.9 ± 0.2	2.2 ± 0.3
AtzF H488A			82.2 ± 5.9	9.0 ± 0.1	10.9 ± 0.8
AtzF	4	7.0	380.2 ± 62.0	0.7 ± 0.04	0.19±0.03
AtzF <sub>467</sub>			363.3 ± 61.0	0.7 ± 0.04	0.19±0.07
AtzF H488A			305.5 ± 46.9	0.7 ± 0.03	0.2 ± 0.01
AtzF	4	7.5	146.3 ± 5.4	2.6 ± 0.02	1.8 ± 0.1
AtzF <sub>467</sub>			164.0 ± 12.7	2.6 ± 0.05	1.6 ± 0.1
AtzF H488A			124.1 ± 10.9	2.4 ± 0.06	1.9 ± 0.2
AtzF	4	8.0	44.6 ± 3.3	4.2 ± 0.05	9.4 ± 0.7
AtzF <sub>467</sub>			57.4 ± 4.8	3.1 ± 0.05	5.4 ± 0.5
AtzF H488A			40.4 ± 2.5	4.0 ± 0.04	9.9 ± 0.6
AtzF	4	8.5	50.8 ± 3.9	4.8 ± 0.06	9.5 ± 0.7
AtzF <sub>467</sub>			54.6 ± 4.3	3.7 ± 0.05	6.8 ± 0.5
AtzF H488A			47.4 ± 3.7	4.8 ± 0.06	10.1 ± 0.7
AtzF	4	9.0	59.6 ± 4.9	4.4 ± 0.07	7.3 ± 0.6
AtzF <sub>467</sub>			66.0 ± 5.2	2.6 ± 0.04	3.9 ± 0.3
AtzF H488A			54.5 ± 3.2	4.3 ± 0.05	8.0 ± 0.5

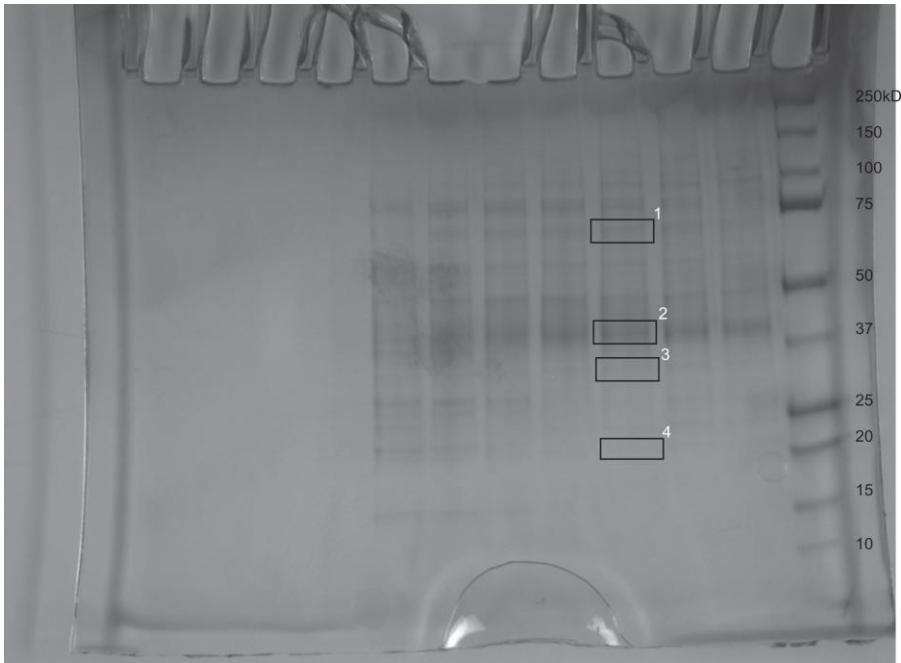


**Supplementary figure 1.  $^{13}\text{C}$  NMR of allophanate, urea and sodium bicarbonate.**

$^{13}\text{C}$  NMR shifts of urea, sodium bicarbonate and potassium allophanate in 1M potassium hydroxide prepared in  $\text{D}_2\text{O}$ . First two spectra are of pure urea and bicarbonate/carbonate, which were expected to be present as in allophanate sample as a result of autodecarboxylation, whereas, the third spectra correspond to allophanate sample.



**Supplementary Figure 2. Allophanate and N-carboxycarbamate in AtzF catalyzed allophanate deamination reactions.** Reactions were conducted at pH 9.0 and 4 °C to promote stabilization of N-carboxycarbamate (mass  $\approx$  104). An ion consistent with the mass of allophanate ( $\approx$  103) is observed in all reactions. A) Allophanate only, 0 minutes incubation, B) allophanate only at 45 minutes, C) allophanate with AtzF<sub>468</sub> (0.96 $\mu$ M) after 45 minutes, D) allophanate with AtzF<sub>468</sub> (0.48 $\mu$ M) after 45 minutes, and E) allophanate with AtzF (0.48 $\mu$ M) after 45 minutes.



**Supplementary figure 3. Gel fragments used in mass spectroscopy**

Gel fragments used for mass spectroscopy of ~660kD complex that showed cyanuric acid hydrolase, biuret hydrolase and allophanate hydrolase activity and peptides.