

Supplementary Information

Exploring the Catalytic Mechanism of the 10-23 DNAzyme: Insights from pH-Rate Profiles

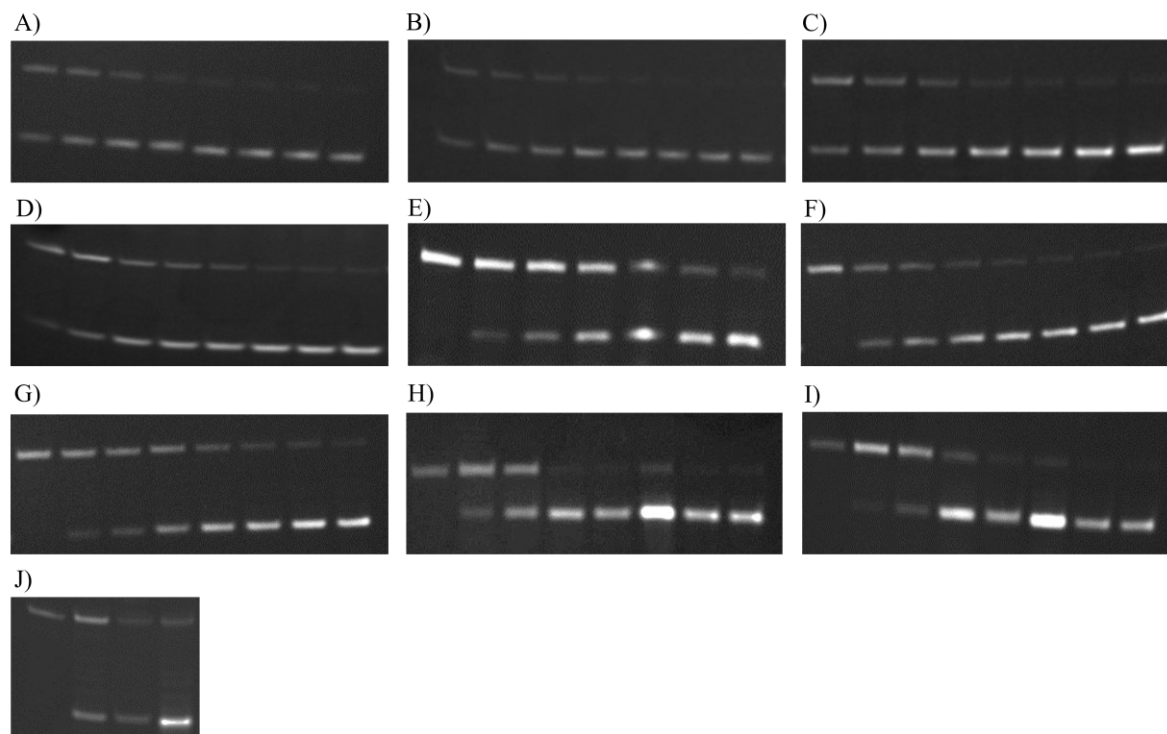
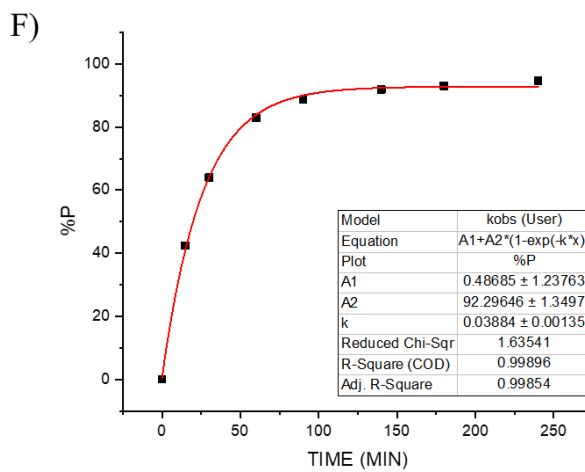
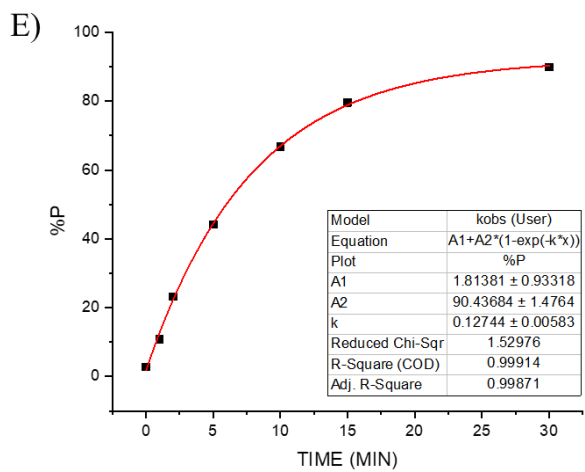
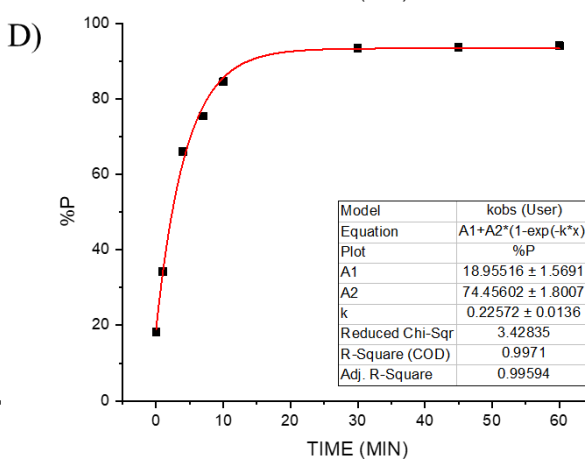
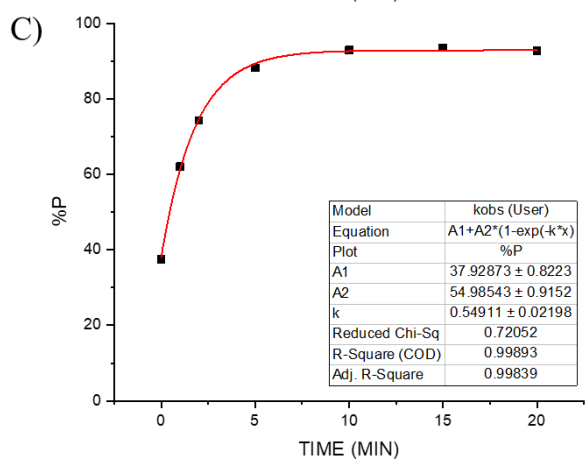
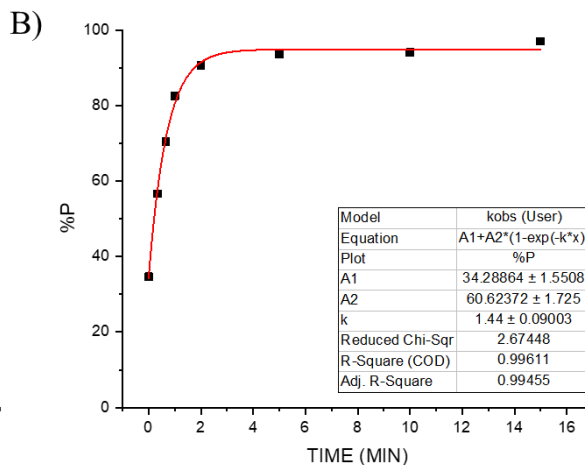
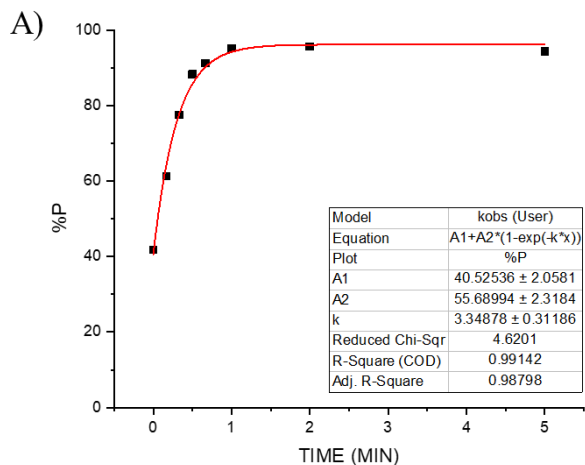


Figure S1. Representative PAGE image of activity assays of the 10-23 DNAzyme with its 5'-FAM labelled substrate with 10 mM Mg^{2+} , measured at different pH values. A) pH 9.0, B) pH 8.5, C) 8.0, D) 7.5, E) 7.0, F) 6.5, G) 6.0, H) 5.5, I)5.0 and J) 4.5.

All gel images were capture in the gel doc system XRQ G:Box Chemi (Synegen) and quantified with the Genesys Tools software.



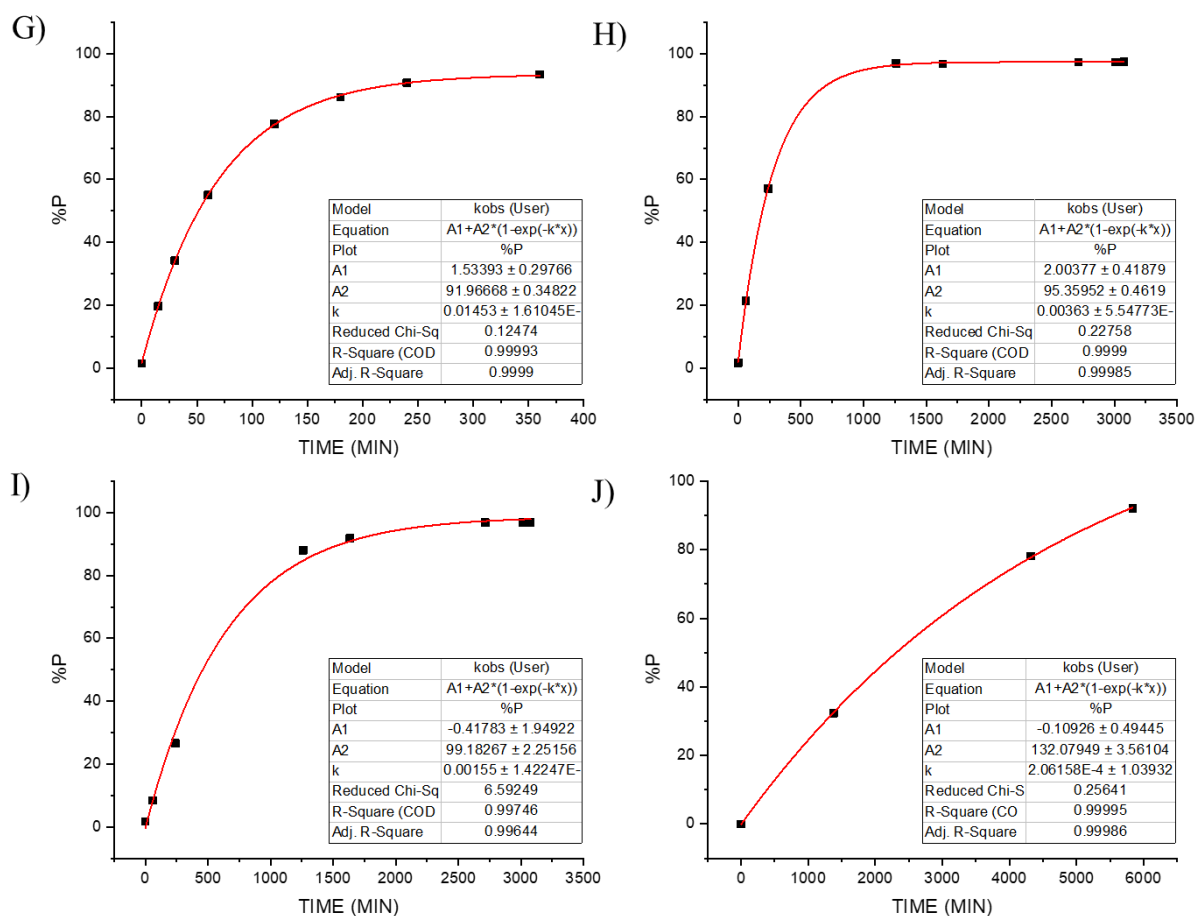


Figure S2. Representative activity assays of the 10-23 DNAzyme with its 5'-FAM labelled substrate with 10 mM Mg²⁺, measured at different pH values. Every point is calculated as the % of product formed for each time collected. Data are quantified from the PAGE image shown in Figure S1. A) pH 9.0, B) pH 8.5, C) 8.0, D) 7.5, E) 7.0, F) 6.5, G) 6.0, H) 5.5, I) 5.0 and J) 4.5.

Kinetic plots were fitted according to the equation (1): $P = \% P_0 + \% P_\infty (1 - e^{-kt})$, where $\% P_0$ is the initial amount of product (at time, $t = 0$), $\% P_\infty$ is the amount of product formed at the endpoint plus the initial amount of product of the reaction (at $t = \infty$), P is the amount of product at time t , and k is the observed constant rate.

Table S1. Observed rate constant obtained from the activity assays of the 10-23 DNase in presence of 10 mM Mg²⁺, measured at different pH values. Average value and standard deviation are provided.

pH	k_{obs}	Average k_{obs}	Standard deviation
4.50	0.000295	0.000251	0.0000629
	0.000206		
5.00	0.00092	0.00146	0.000313
	0.00168		
	0.00148		
	0.00167		
	0.00155		
5.50	0.00401	0.00344	0.000667
	0.00423		
	0.00363		
	0.00239		
	0.00313		
	0.00323		
6.00	0.0145	0.0139	0.00110
	0.0126		
	0.0145		
6.50	0.0380	0.0388	0.00085
	0.0397		
	0.0388		
7.00	0.1183	0.1192	0.00876
	0.1274		
	0.1234		
	0.1220		
	0.1046		
7.50	0.2257	0.2198	0.01482
	0.2231		
	0.2005		
	0.2029		
	0.2370		
	0.2297		
8.00	0.5806	0.5958	0.03625
	0.6216		
	0.6415		
	0.5491		
	0.5863		
8.50	1.3039	1.4187	0.13854
	1.2649		
	1.5659		
	1.5953		
	1.4400		
	1.3424		
9.00	2.9903	3.9753	0.81198
	5.0155		
	4.3663		
	3.4812		
	3.3488		
	4.6497		

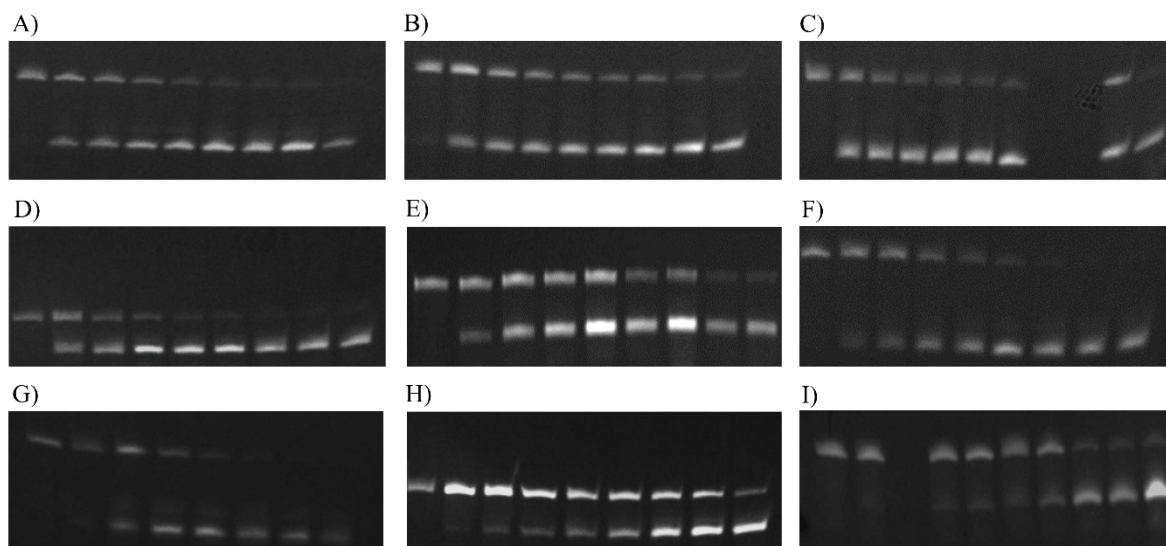
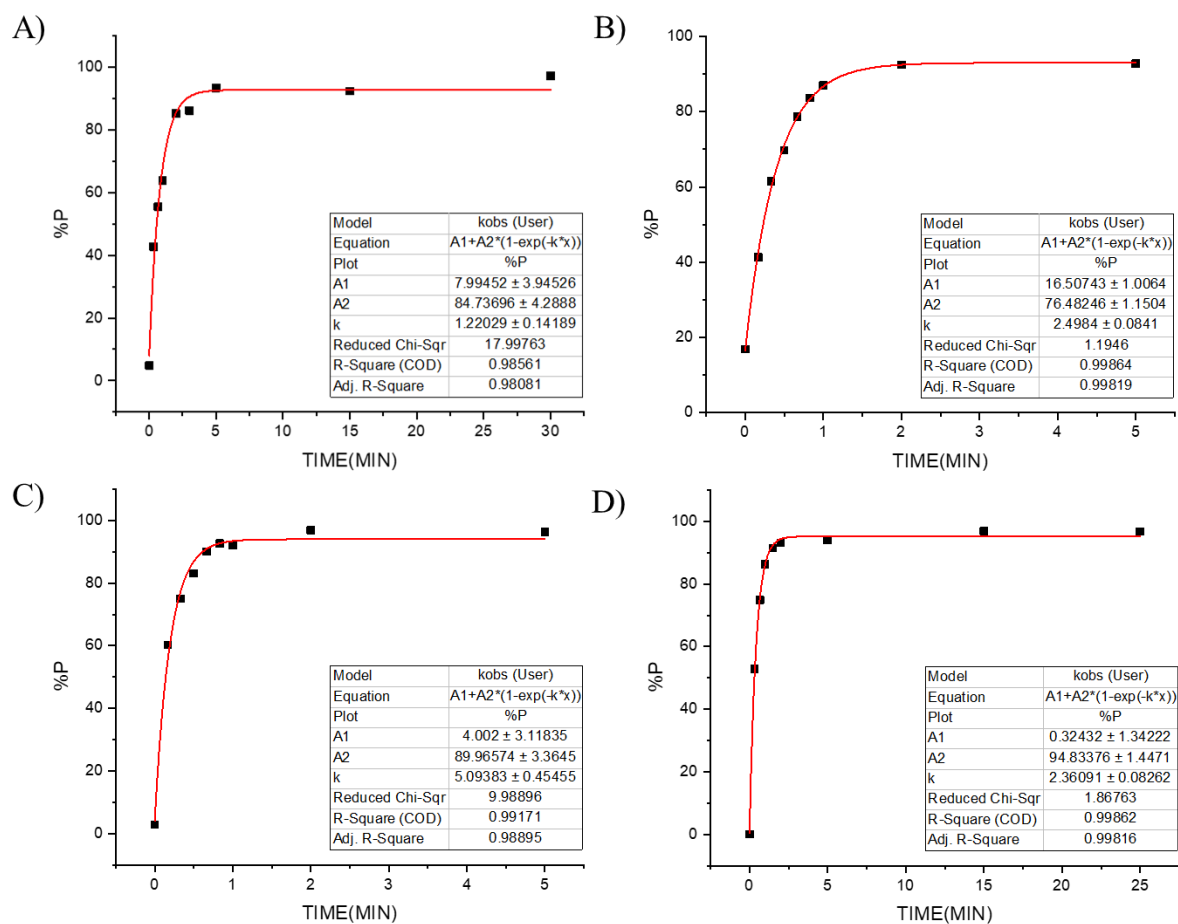


Figure S3. Representative PAGE image of activity assays of the 10-23 DNazyme with its 5'-FAM labelled substrate with $10 \mu\text{M Pb}^{2+}$, measured at different pH values. A) pH 8.5, B) 8.0, C) 7.5, D) 7.0, E) 6.5, F) 6.0, G) 5.5, H) 5.0 and I) 4.5.



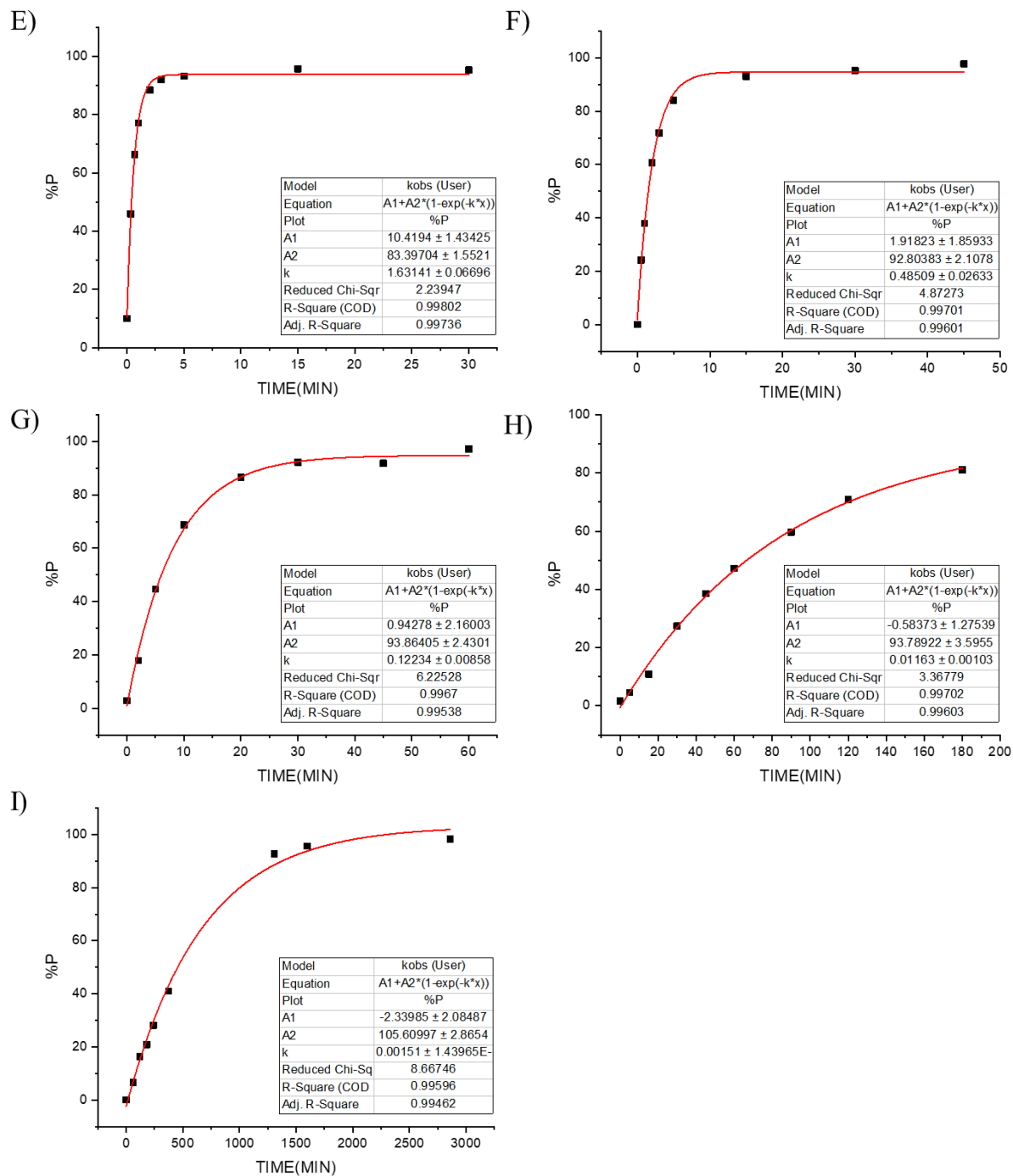


Figure S4. Representative activity assays of the 10-23 DNazyme with its 5'-FAM labelled substrate with $10 \mu\text{M Pb}^{2+}$, measured at different pH values. Every point is calculated as the % of product formed for each time collected. Data are quantified from the PAGE image shown in Figure S3. A) pH 8.5, B) 8.0, C) 7.5, D) 7.0, E) 6.5, F) 6.0, G) 5.5, H) 5.0 and I) 4.5.

Table S2. Observed rate constant of the 10-23 DNase in presence of 10 μM Pb^{2+} , measured at different pH values. Average value and standard deviation are provided.

pH	k_{obs}	Average k_{obs}	Standard deviation
4.50	0.00118	0.00133	0.000141
	0.00127		
	0.00151		
	0.00136		
5.00	0.0116	0.0115	0.000141
	0.0114		
5.50	0.0700	0.0880	0.0298
	0.0715		
	0.1223		
6.00	0.565	0.551	0.0607
	0.485		
	0.604		
6.50	1.843	1.830	0.1486
	1.991		
	1.853		
	1.631		
7.00	3.514	3.434	0.1131
	3.354		
7.50	2.106	5.038	2.0606
	6.488		
	6.465		
	5.094		
8.00	1.919	2.289	0.2619
	2.523		
	2.498		
	2.403		
	2.428		
	1.913		
8.50	2.339	1.235	0.0110
	1.229		
	1.248		
	1.229		

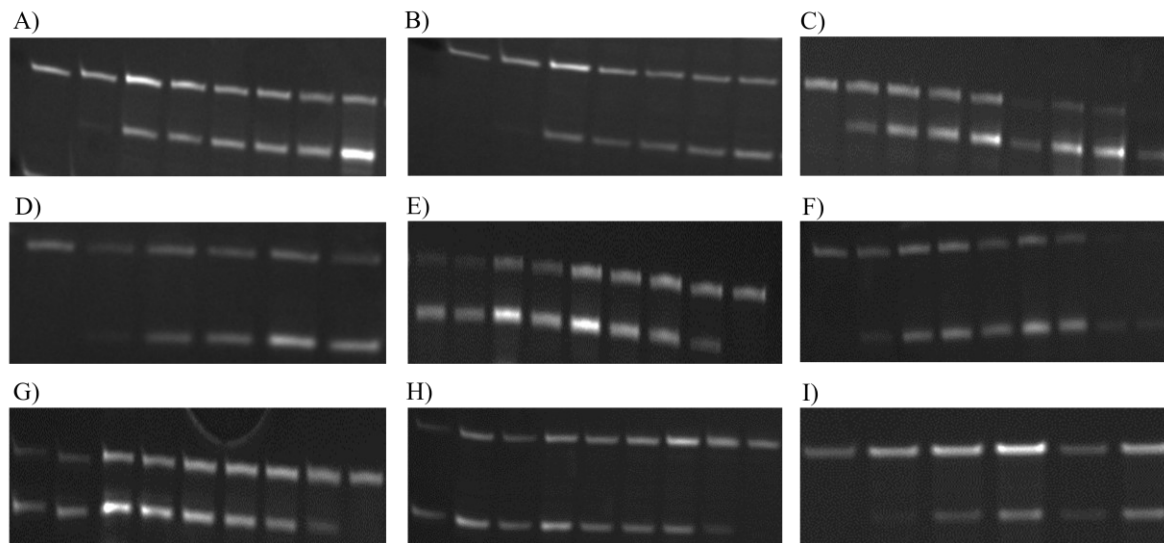
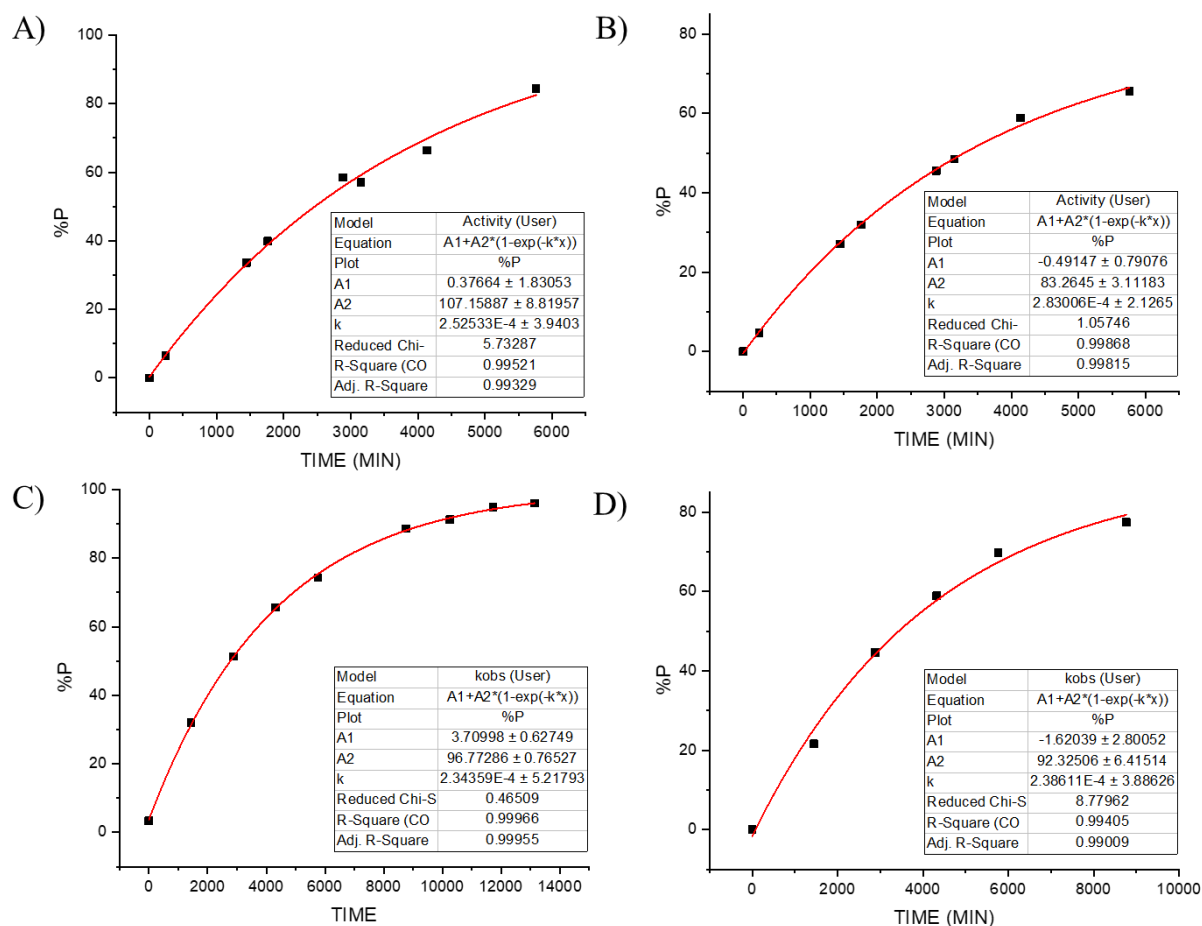


Figure S5. Representative PAGE image of activity assays of the 10-23 G14AP DNAzyme variant with its 5'-FAM labelled substrate with 10 mM Mg²⁺, measured at different pH values. A) pH 8.5, B) 8.0, C) 7.5, D) 7.0, E) 6.5, F) 6.0, G) 5.5, H) 5.0 and I) 4.5.



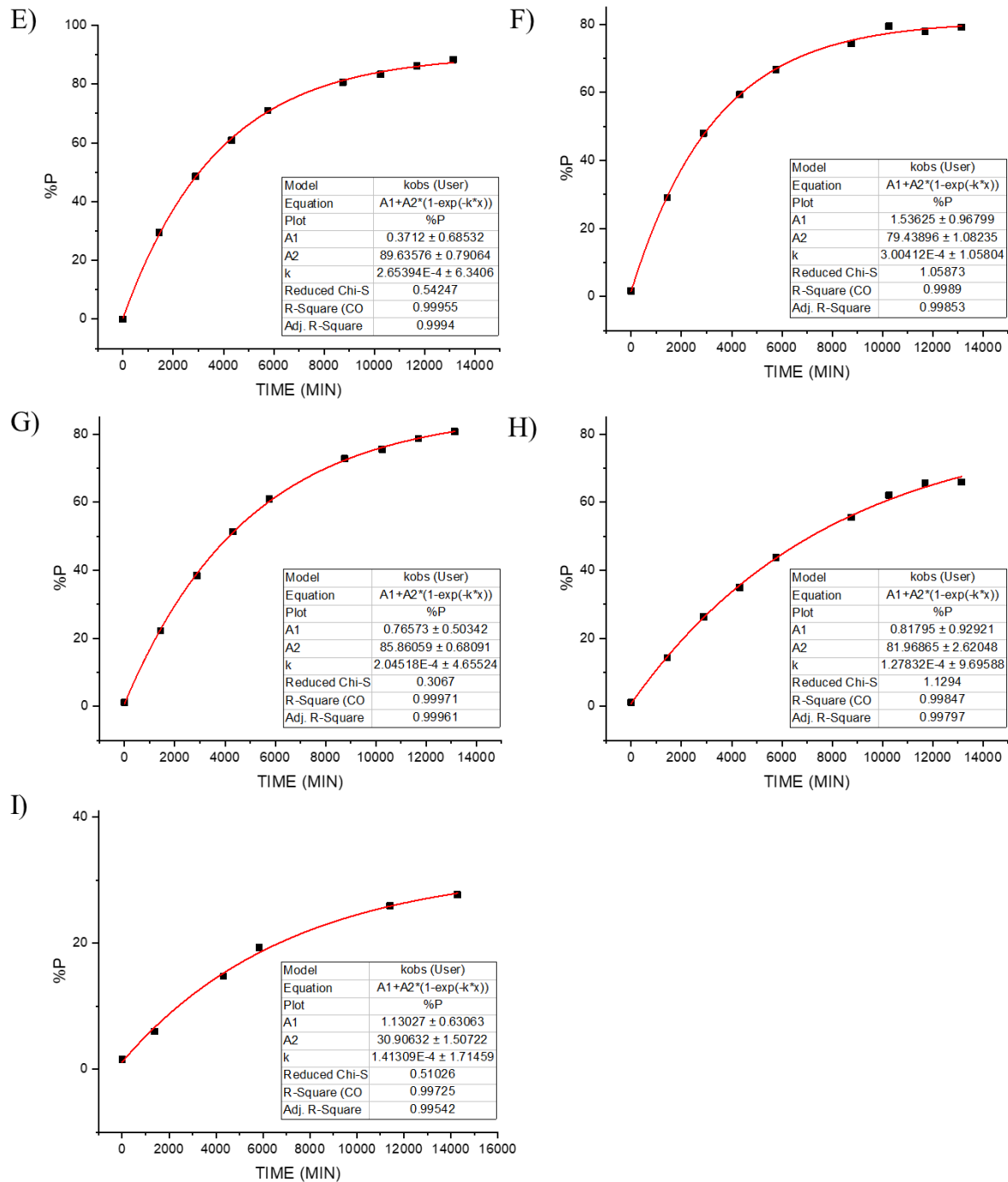


Figure S6. Representative activity assays of the 10-23 G14AP DNase variant with its 5'-FAM labelled substrate with 10 mM Mg^{2+} , measured at different pH values. Every point is calculated as the % of product formed for each time collected. Data are quantified from the PAGE image shown in Figure S5. A) pH 8.5, B) 8.0, C) 7.5, D) 7.0, E) 6.5, F) 6.0, G) 5.5, H) 5.0 and I) 4.5.

Table S3. Observed rate constant of the 10-23 G14AP DNAzyme variant in presence of 10 mM Mg^{2+} , measured at different pH values. Average value and standard deviation are provided.

pH	k_{obs}	Average k_{obs}	Standard deviation
4.50	3.31E-05	8.90E-05	5.40E-05
	9.26E-05		
	1.41E-04		
5.00	1.20E-04	1.24E-04	5.66E-06
	1.28E-04		
5.50	2.06E-04	2.05E-04	7.07E-07
	2.05E-04		
6.00	1.90E-04	2.56E-04	4.66E-05
	2.67E-04		
	2.66E-04		
	3.00E-04		
6.50	2.67E-04	2.65E-04	6.03E-06
	2.56E-04		
	2.70E-04		
	2.65E-04		
7.00	2.46E-04	2.51E-04	1.05E-05
	2.55E-04		
	2.39E-04		
	2.63E-04		
7.50	3.05E-04	2.58E-04	3.61E-05
	2.68E-04		
	2.26E-04		
	2.34E-04		
8.00	2.83E-04	2.60E-04	2.02E-05
	2.52E-04		
	2.45E-04		
8.50	2.60E-04	2.70E-04	2.36E-05
	2.53E-04		
	2.97E-04		

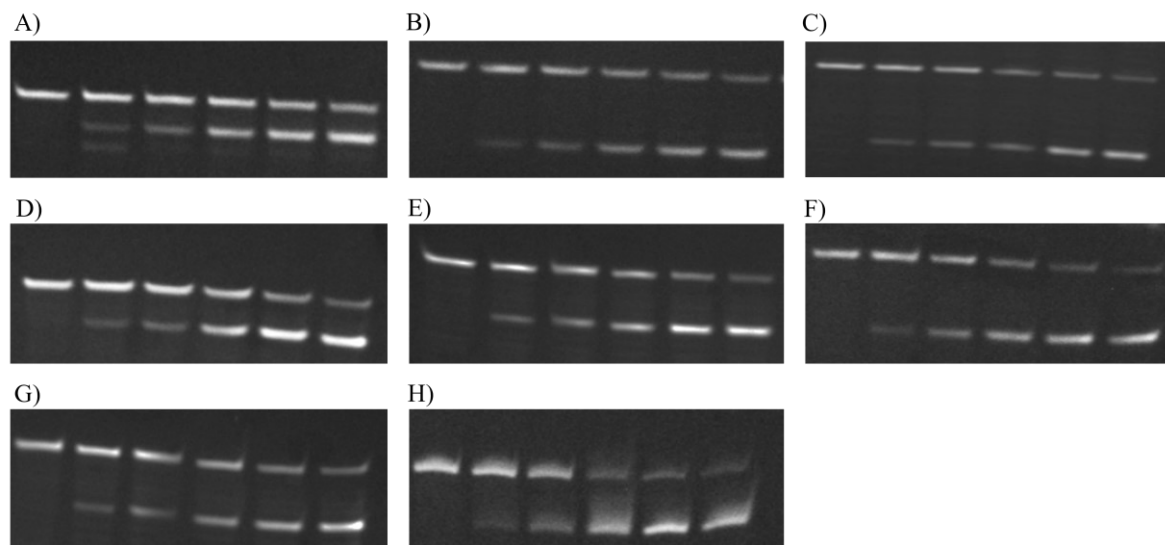
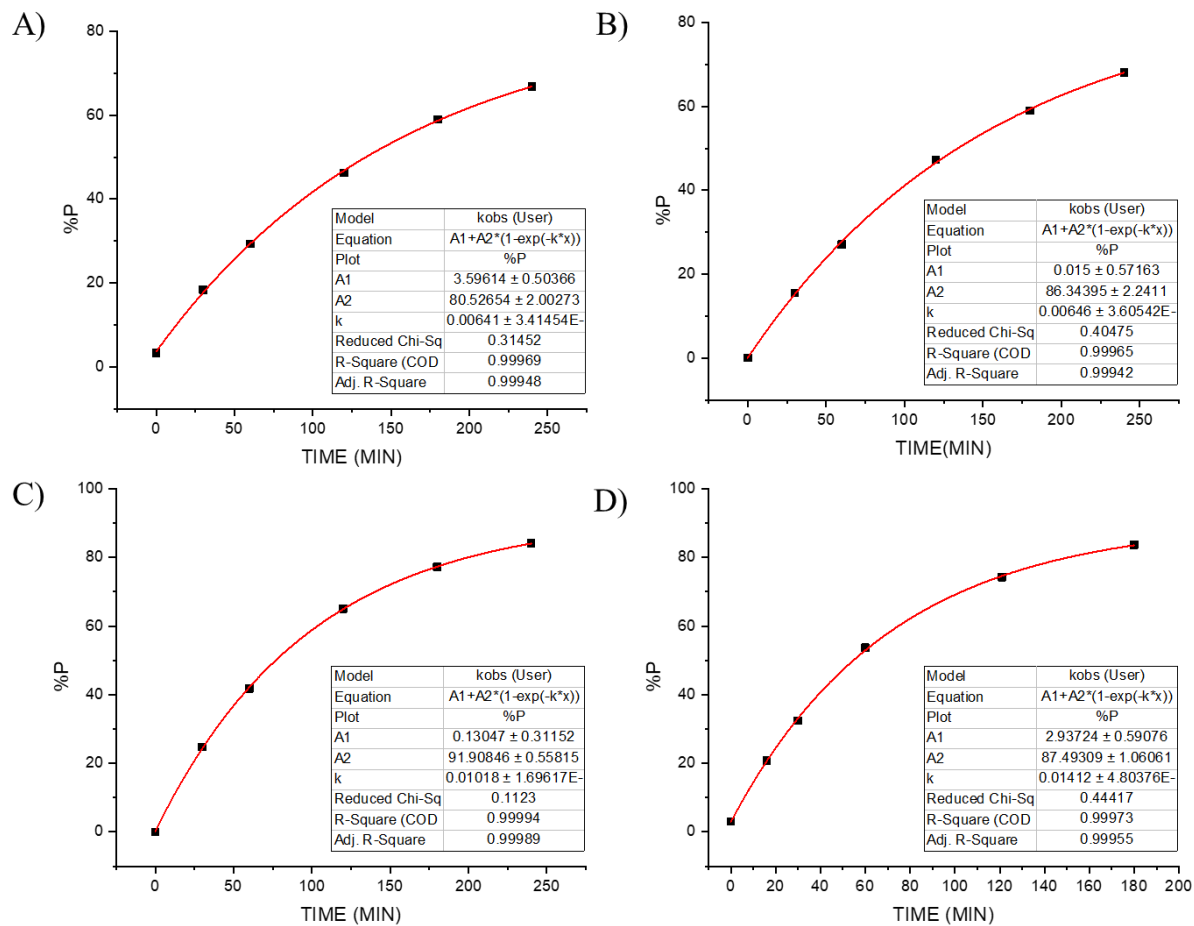


Figure S7. Representative PAGE image of activity assays of the 10-23 G14AP DNAzyme variant with its 5'-FAM labelled substrate with 25 μM Pb^{2+} , measured at different pH values. A) pH 7.5, B) 7.25, C) 7.0, D) 6.5, E) 6.0, F) 5.75, G) 5.5 and H) 5.0.



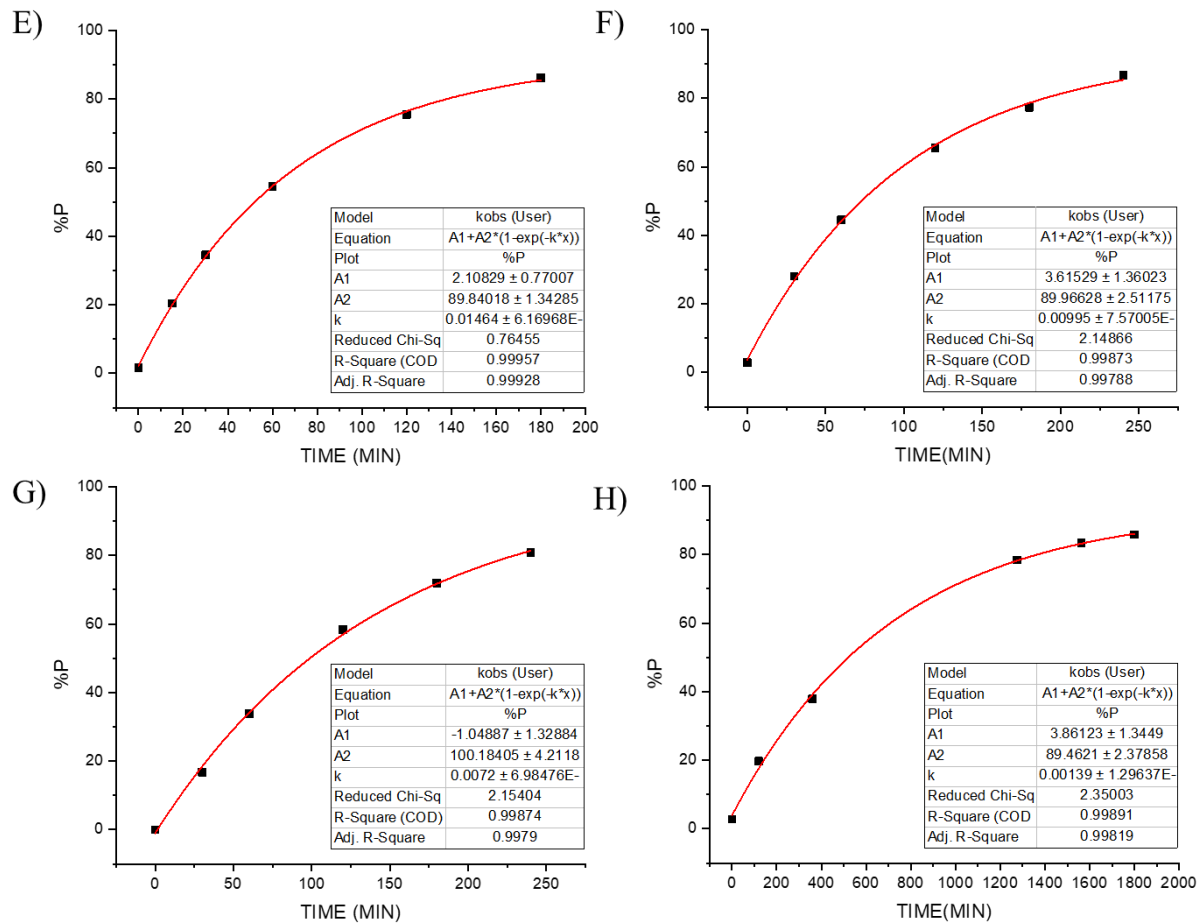


Figure S8. Representative activity assays of the 10-23 G14AP DNAzyme variant with its 5'-FAM labelled substrate with 25 μM Pb^{2+} , measured at different pH values. Every point is calculated as the % of product formed for each time collected. Data are quantified from the PAGE image shown in Figure S7. A) pH 7.5, B) 7.25, C) 7.0, D) 6.5, E) 6.0, F) 5.75, G) 5.5 and H) 5.0.

Table S4. Observed rate constant of the 10-23 G14AP DNase variant in presence of 25 μM Pb^{2+} , measured at different pH values. Average value and standard deviation are provided.

pH	k_{obs}	Average k_{obs}	Standard deviation
5.00	0.00133	0.00130	0.000104
	0.00116		
	0.00139		
	0.00123		
	0.00140		
5.50	0.00720	0.00880	0.00140
	0.00978		
	0.00942		
5.75	0.00995	0.01023	0.000671
	0.00941		
	0.01073		
	0.01082		
6.00	0.01464	0.01185	0.00238
	0.01140		
	0.01246		
	0.00890		
6.50	0.01131	0.01203	0.00140
	0.01144		
	0.01412		
	0.01124		
7.00	0.01077	0.01052	0.000487
	0.01004		
	0.01018		
	0.01107		
7.25	0.00663	0.00762	0.00107
	0.00646		
	0.00903		
	0.00806		
	0.00794		
7.50	0.00641	0.00586	0.000867
	0.00583		
	0.00464		
	0.00654		

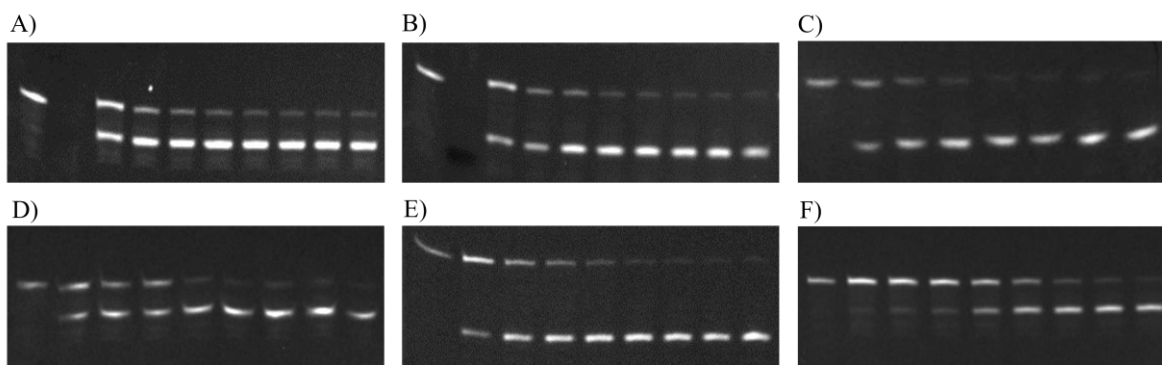
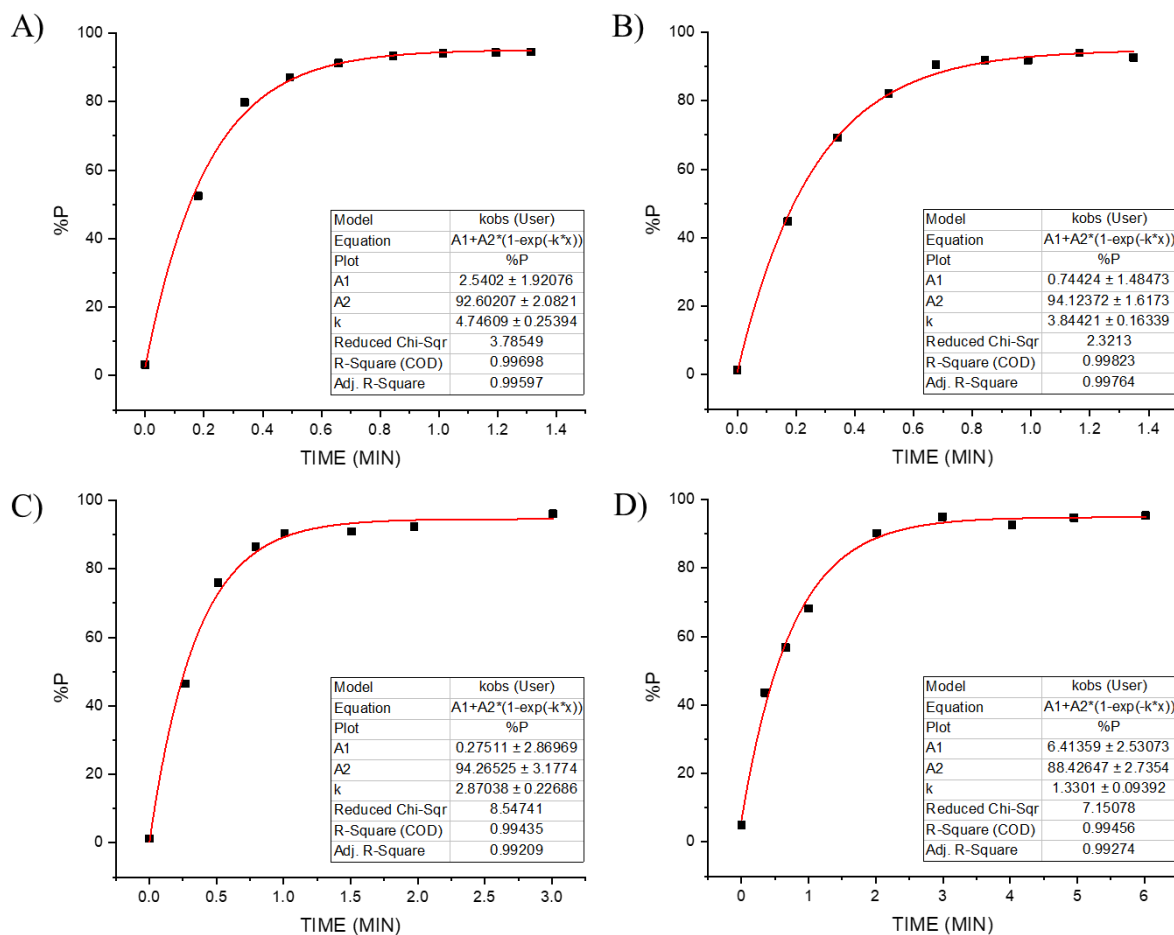


Figure S9. Representative PAGE image. Activity assays of the 10-23 DNase with its 5'-FAM labelled substrate with A) 400 μM Pb^{2+} , B) 250 μM Pb^{2+} , C) 150 μM Pb^{2+} , D) 75 μM Pb^{2+} , E) 25 μM Pb^{2+} and F) 10 μM Pb^{2+} .



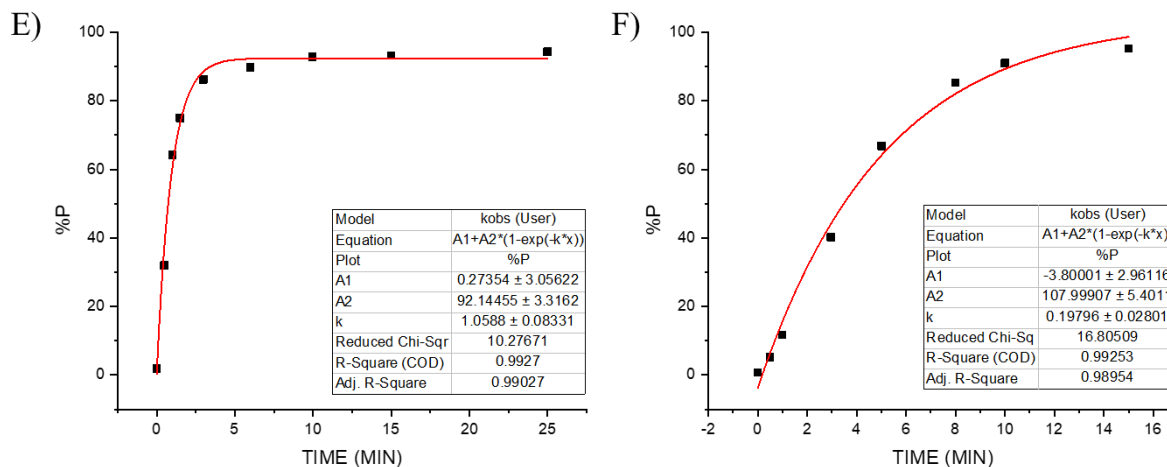


Figure S10. Representative activity assays of the 10-23 DNase with its 5'-FAM labelled substrate at pH 6.0, measured at different Pb²⁺ concentration values. Every point is calculated as the % of product formed for each time collected. Data are quantified from the PAGE image shown in Figure S9. A) 400 μM Pb²⁺, B) 250 μM Pb²⁺, C) 150 μM Pb²⁺, D) 75 μM Pb²⁺, E) 25 μM Pb²⁺ and F) 10 μM Pb²⁺.

Table 1. Observed constant rate as a function of the Pb²⁺ concentration, measured at pH 6.0. The average and standard deviation are provided.

[Pb ²⁺]	k _{obs}	Average k _{obs}	Standard deviation
400 μM	4.13	4.44	0.2533
	4.46		
	4.44		
	4.75		
250 μM	4.01	3.93	0.1202
	3.84		
150 μM	2.85	2.87	0.0153
	2.87		
	2.88		
75 μM	1.14	1.93	0.6225
	1.33		
	2.01		
	2.78		
	2.40		
25 μM	1.26	1.14	0.0823
	1.23		
	1.10		
	1.06		
	1.11		
	1.09		
10 μM	0.27	0.29	0.1042
	0.44		
	0.20		
	0.25		