

Supporting Information

Inhibition of the urea-urease reaction by the components of the zeolite imidazole frameworks-8 and the formation of urease-zinc-imidazole hybrid compound

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Activity measurements of the U-Zn-Met hybrid

To determine the activity of the U-Zn-Met, we used the protocol of Sigma-Aldrich [1]. We prepared a solution with urea (30 mg/mL) and bovine serum albumin (0.5 mg/mL) dissolved in 750 mM sodium phosphate solution (set to pH 7.0 with sodium hydroxide). 10 mg of urease enzyme or washed U-Zn-Met were dissolved in a cooled 20 mM sodium phosphate buffer (set to pH 7.0 with sodium hydroxide). The urea-albumin solutions (4×1 mL) were thermostated to 25.0 °C and vigorously stirred. We added 0.1 mL urease suspension to three of the samples and waited five minutes. After the reaction, we added 0.2 mL p-Nitrophenol (4.0 mg/mL) indicator solution and titrated it immediately with 100 mM standardized hydrochloric acid solution until the yellow color disappeared. In a “blank measurement”, the sample was titrated directly after the addition of urease and indicator. The activity of urease (one micromolar unit will liberate 1.0 μ mole of NH_3 from urea per minute at pH 7.0 and 25 °C) was calculated through the following formula

$$\frac{\text{Units}}{\text{mg solid}} = \frac{(\text{HCl molarity}) \cdot (\Delta V_{\text{Test}} - V_{\text{Blank}}) \cdot 1000 \cdot \text{df}}{(5) \cdot (0.10) \cdot \frac{\text{mg solid}}{\text{mL}} \text{enzyme}},$$

where $\Delta V_{\text{Test}} - V_{\text{Blank}} = \Delta V_{\text{HCl}}$ is the total volume (in milliliters) of HCl used in titration, 1000 is a conversion factor from millimoles to micromoles as per unit definition, df is the dilution factor (if applicable), 5 is the time of assay (in minutes) as per unit definition, and 0.10 is the volume (in milliliters) of enzyme solution used.

Numerical Simulations

The full set of ordinary differential equations derived from the reaction rates (RE) and (R1) – (R13) reads:

$$\begin{aligned}\frac{d[S]}{dt} &= -R \\ \frac{d[NH_3]}{dt} &= 2R + k_2 [NH_4^+] - k_{2r} [NH_3] [H^+] \\ \frac{d[NH_4^+]}{dt} &= -k_2 [NH_4^+] + k_{2r} [NH_3] [H^+] \\ \frac{d[CO_2]}{dt} &= R - k_3 [CO_2] + k_{3r} [H^+] [HCO_3^-] \\ \frac{d[HCO_3^-]}{dt} &= k_3 [CO_2] - k_{3r} [H^+] [HCO_3^-] - k_4 [HCO_3^-] + k_{4r} [CO_3^{2-}] [H^+] \\ \frac{d[CO_3^{2-}]}{dt} &= k_4 [HCO_3^-] - k_{4r} [CO_3^{2-}] [H^+] - k_9 [CO_3^{2-}] [Zn^{2+}] + k_{9r} [ZnCO_3] \\ \frac{d[H^+]}{dt} &= k_2 [NH_4^+] - k_{2r} [NH_3] [H^+] + k_3 [CO_2] - k_{3r} [H^+] [HCO_3^-] + k_4 [HCO_3^-] - k_{4r} [CO_3^{2-}] [H^+] + \\ &+ k_5 - k_{5r} [H^+] [OH^-] + k_6 [HA] - k_{6r} [A^-] [H^+] + k_7 [MImH_2^+] - k_{7r} [MImH] [H^+] + k_8 [MImH] + \\ &- k_{8r} [MIm^-] [H^+] \\ \frac{d[OH^-]}{dt} &= k_5 - k_{5r} [H^+] [OH^-] - k_{10} [Zn^{2+}] [OH^-] + k_{10r} [Zn(OH)^+] - k_{11} [Zn(OH)^+] [OH^-] + \\ &k_{11r} [Zn(OH)_2] - k_{12} [Zn(OH)_2] [OH^-] + k_{12r} [Zn(OH)_3^-] - k_{13} [Zn(OH)_3^-] [OH^-] + k_{13r} [Zn(OH)_4^{2-}] \\ \frac{d[HA]}{dt} &= -k_6 [HA] + k_{6r} [A^-] [H^+] \\ \frac{d[A^-]}{dt} &= k_6 [HA] - k_{6r} [A^-] [H^+]\end{aligned}$$

$$\begin{aligned}
\frac{d[\text{MImH}_2^+]}{dt} &= -k_7 [\text{MImH}_2^+] + k_{7r} [\text{MImH}] [\text{H}^+] \\
\frac{d[\text{MImH}]}{dt} &= k_7 [\text{MImH}_2^+] - k_{7r} [\text{MImH}] [\text{H}^+] - k_8 [\text{MImH}] + k_{8r} [\text{MIm}^-] [\text{H}^+] \\
\frac{d[\text{MIm}^-]}{dt} &= k_8 [\text{MImH}] - k_{8r} [\text{MIm}^-] [\text{H}^+] - k_{14} [\text{Zn}^{2+}] [\text{MIm}^-]^2 + k_{14r} [\text{Zn(MIm)}_2] \\
\frac{d[\text{Zn}^{2+}]}{dt} &= -k_9 [\text{CO}_3^{2-}] [\text{Zn}^{2+}] + k_{9r} [\text{ZnCO}_3] - k_{10} [\text{Zn}^{2+}] [\text{OH}^-] + k_{10r} [\text{ZnOH}^+] - k_{14} [\text{Zn}^{2+}] [\text{MIm}^-]^2 + \\
&\quad k_{14r} [\text{Zn(MIm)}_2] \\
\frac{d[\text{ZnCO}_3]}{dt} &= k_9 [\text{CO}_3^{2-}] [\text{Zn}^{2+}] - k_{9r} [\text{ZnCO}_3] \\
\frac{d[\text{ZnOH}^+]}{dt} &= k_{10} [\text{Zn}^{2+}] [\text{OH}^-] - k_{10r} [\text{ZnOH}^+] - k_{11} [\text{ZnOH}^+] [\text{OH}^-] + k_{11r} [\text{Zn(OH)}_2] \\
\frac{d[\text{Zn(OH)}_2]}{dt} &= k_{11} [\text{ZnOH}^+] [\text{OH}^-] - k_{11r} [\text{Zn(OH)}_2] - k_{12} [\text{Zn(OH)}_2] [\text{OH}^-] + k_{12r} [\text{Zn(OH)}_3^-] \\
\frac{d[\text{Zn(OH)}_3^-]}{dt} &= k_{12} [\text{Zn(OH)}_2] [\text{OH}^-] - k_{12r} [\text{Zn(OH)}_3^-] - k_{13} [\text{Zn(OH)}_3^-] [\text{OH}^-] + k_{13r} [\text{Zn(OH)}_4^{2-}] \\
\frac{d[\text{Zn(OH)}_4^{2-}]}{dt} &= k_{13} [\text{Zn(OH)}_3^-] [\text{OH}^-] - k_{13r} [\text{Zn(OH)}_4^{2-}] \\
\frac{d[\text{Zn(MIm)}_2]}{dt} &= k_{14} [\text{Zn}^{2+}] [\text{MIm}^-]^2 - k_{14r} [\text{Zn(MIm)}_2]
\end{aligned}$$

Table S1. Kinetic constants used for the simulation of the urea-urease reaction in the absence of ZIF-8 reactants (Fig. 3a of the main manuscript). Enzymatic constants were taken within the interval reported in Refs[2–6]; equilibrium rate constants were derived from the pK_a according to Refs[6–8].

Enzymatic		pH equilibria		
k_1 (u^{-1} mL M s^{-1})	2.5×10^{-5}	<i>forward</i> (s^{-1})		<i>reverse</i> ($M^{-1} s^{-1}$)
K_m (M)	3×10^{-3}	k_2	24	4.3×10^{10}
K_{es1} (M)	2.6×10^{-5}	k_3	3.7×10^{-2}	7.9×10^4
K_{es2} (M)	3.4×10^{-8}	k_4	2.8	5×10^{10}
K_S (M)	3	k_5	1×10^{-3} ($M^{-1} s^{-1}$)	1×10^{11}
K_P (M)	2×10^{-3}	k_6	7.8×10^5	4.5×10^{10}

Table S2. Kinetic constants used for the simulation of the urea-urease reaction in the presence of 2-Methylimidazole (Fig. 3b of the main manuscript). The equilibria constants involving 2-Methylimidazole were taken from Ref[9].

Enzymatic		pH equilibria		
k_1 (u ⁻¹ mL M s ⁻¹)	2.5×10^{-5}	<i>forward</i> (s ⁻¹)		<i>reverse</i> (M ⁻¹ s ⁻¹)
K_m (M)	3×10^{-3}	k_2	24	4.3×10^{10}
K_{es1} (M)	1.3×10^{-5}	k_3	3.7×10^{-2}	7.9×10^4
K_{es2} (M)	3.4×10^{-8}	k_4	2.8	5×10^{10}
K_S (M)	3	k_5	1×10^{-3} (M ⁻¹ s ⁻¹)	1×10^{11}
K_P (M)	4.2×10^{-2}	k_6	7.8×10^5	4.5×10^{10}
		k_7	1.38×10^{-7}	10
		k_8	7.94×10^{-16}	1

Table S3. Kinetic constants used for the simulation of the urea-urease reaction in the presence of 2-Methylimidazole and Zn^{2+} (Fig. 4 of the main manuscript). The equilibria constants involving Zn^{2+} were taken from Ref[9].

Enzymatic		pH equilibria		
k_1 ($\text{u}^{-1} \text{ mL M s}^{-1}$)	2.5×10^{-5}	<i>forward</i> (s^{-1})		<i>reverse</i> ($\text{M}^{-1} \text{ s}^{-1}$)
K_m (M)	3×10^{-3}	k_2	24	4.3×10^{10}
K_{es1} (M)	1×10^{-7}	k_3	3.7×10^{-2}	7.9×10^4
K_{es2} (M)	7×10^{-5}	k_4	2.8	5×10^{10}
K_S (M)	3	k_5	$1 \times 10^{-3} (\text{M}^{-1} \text{ s}^{-1})$	1×10^{11}
K_P (M)	2×10^{-3}	k_6	7.8×10^5	4.5×10^{10}
		k_7	1.38×10^{-7}	10
		k_8	7.94×10^{-16}	1
		k_9	1	1.4×10^{-11}
		k_{10}	1.10×10^5	1
		k_{11}	1.99×10^3	1
		k_{12}	3.10×10^5	1
		k_{13}	2.14×10^4	1
		k_{14}	2.51×10^{13}	1

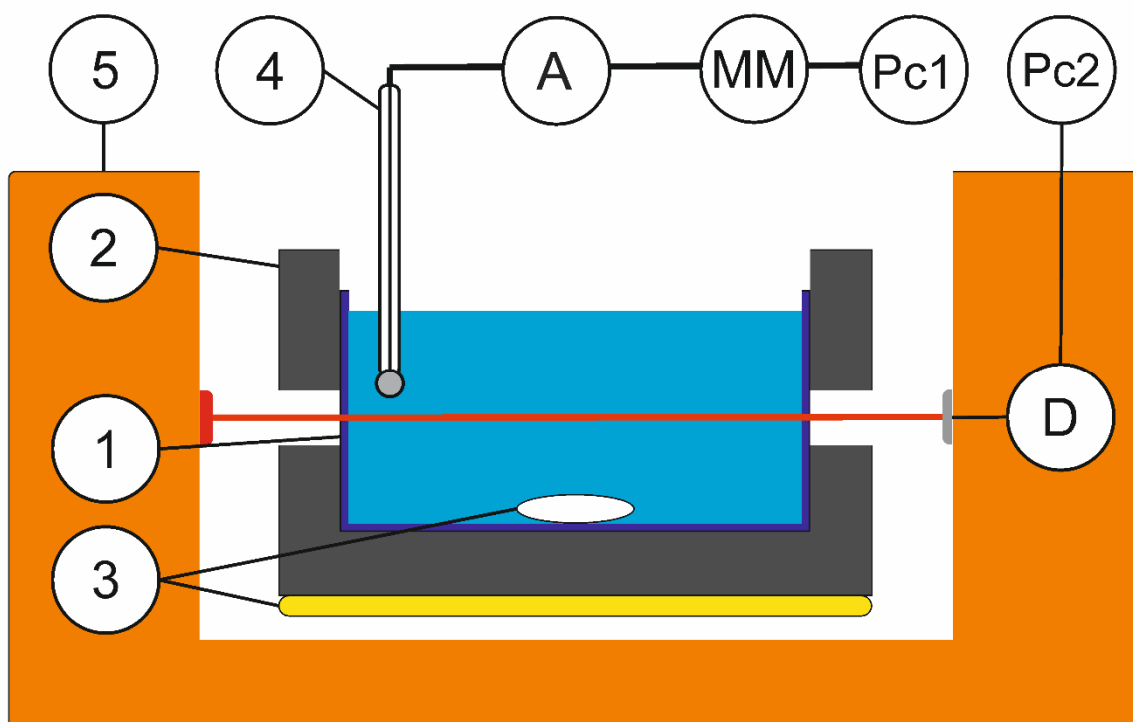


Figure S1. The scheme of the kinetic measurements. The aqueous reaction mixture was placed in a cuvette with a total volume of 100 mL (1) thermostated with a metal block (2) and stirred with a magnetic stirrer (3). The pH change was monitored with a pH electrode (4). The system is placed in a UV-Vis spectrophotometer (5). D: detector; A: amplifier; MM: multimeter; Pc1-2: computers.

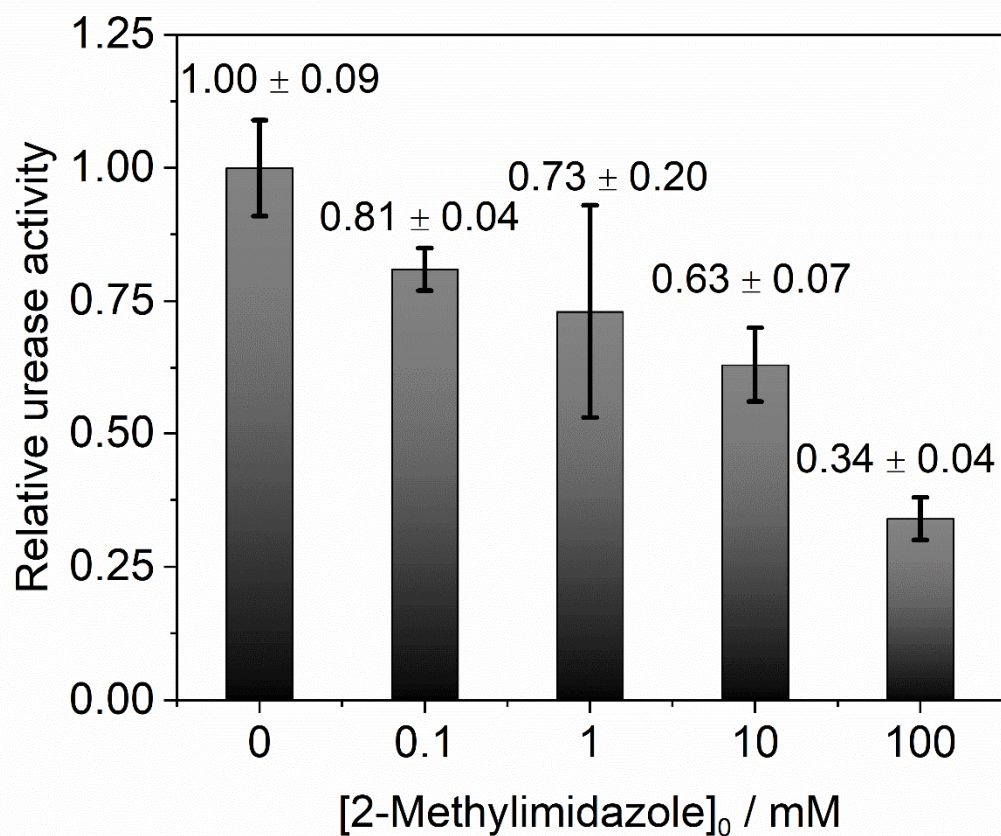


Figure S2. Inhibition effect of the 2-Methylimidazole on the activity of the urease enzyme. The activity of the urease enzyme in the absence of 2-Methylimidazole was 8.8 U mL^{-1} . The relative activity was calculated as the enzymatic activity in the presence of 2-Methylimidazole divided by the enzymatic activity in the absence of 2-Methylimidazole. We used the protocol for the activity measurement provided in Ref[1].

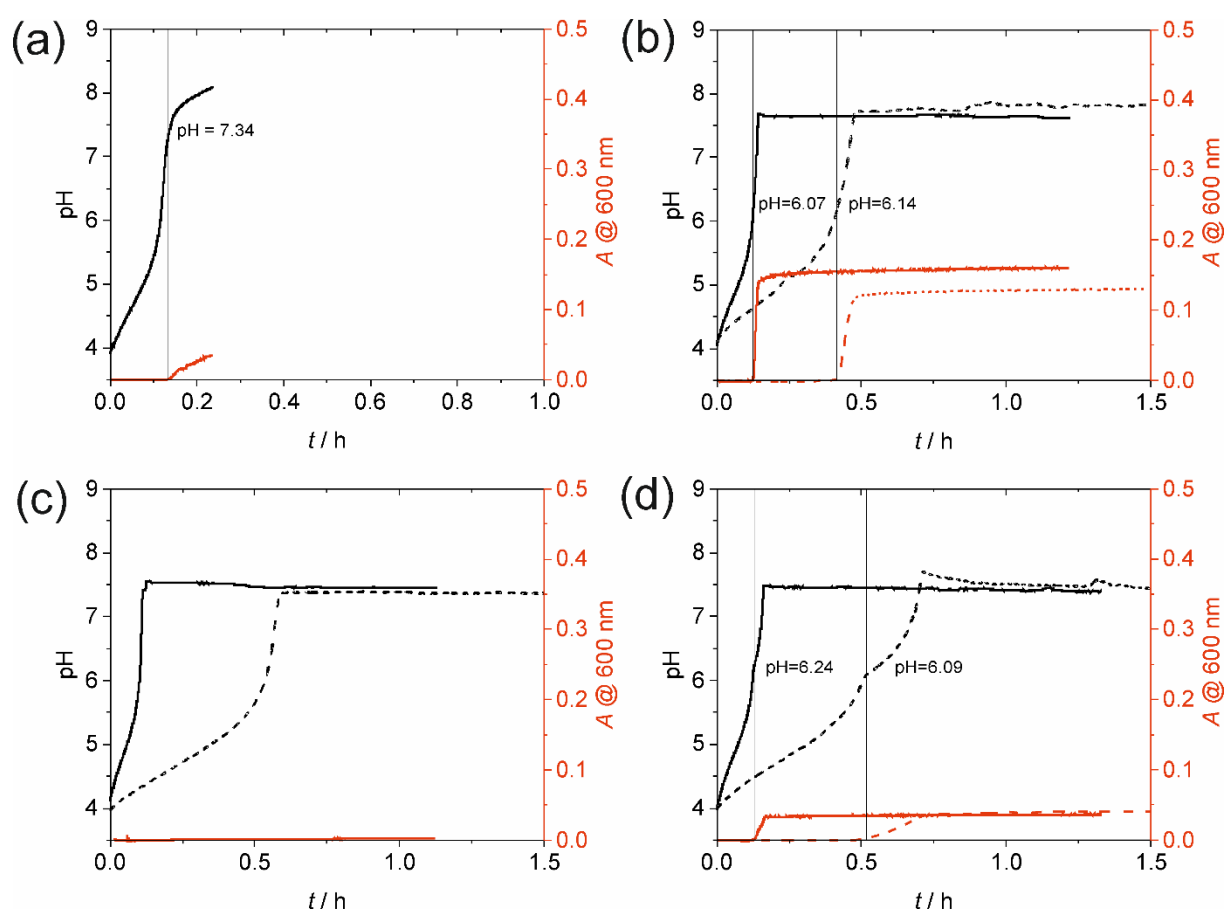


Figure S3. Titration of (a) Zn^{2+} (b) Zn^{2+} with 2-Methylimidazole in the presence of urease enzyme (c), Zn^{2+} with 2-Methylimidazole in the presence of urea and (d) Zn^{2+} in the presence of urease enzyme with sodium hydroxide solution

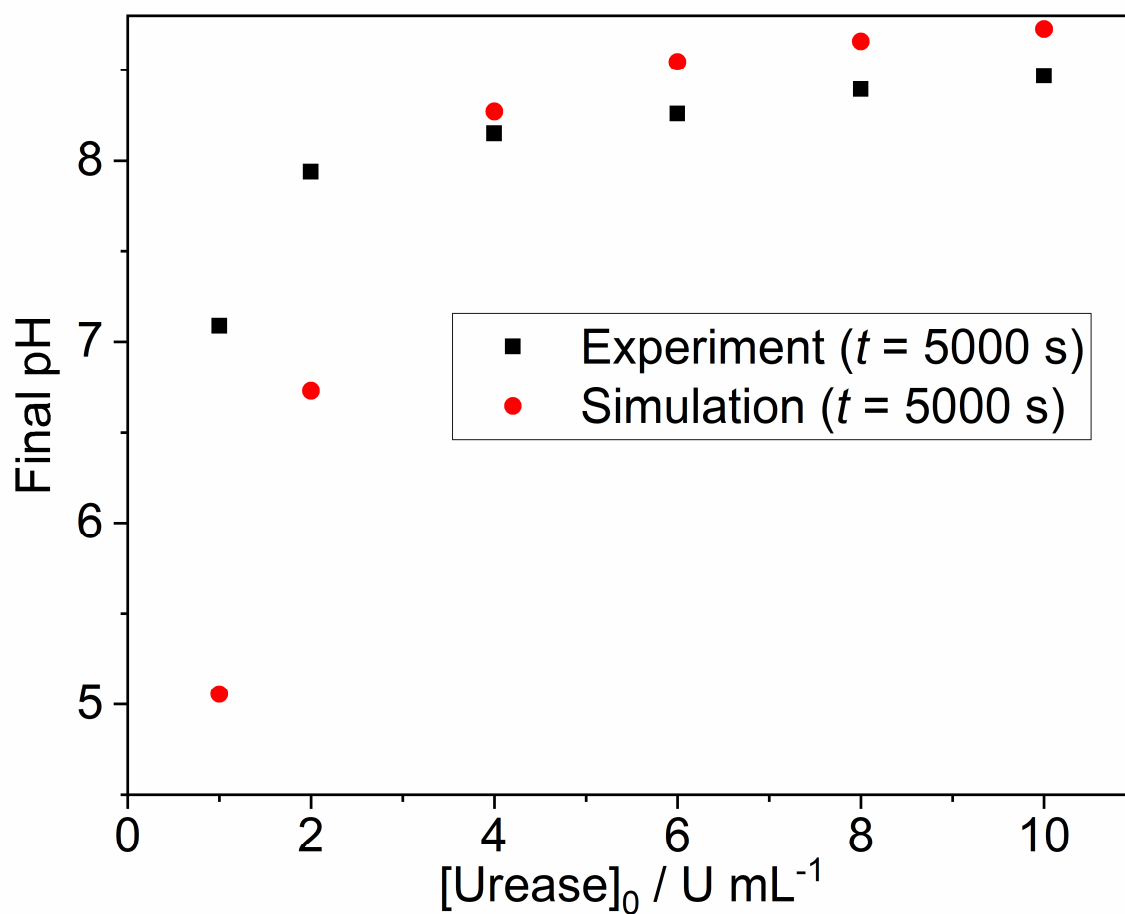


Figure S4. Simulated final pH of the urea-urease reaction with $[2\text{-Methylimidazole}]_0 = 30.0$ mM, $[\text{urea}]_0 = 390$ mM and $[\text{CH}_3\text{COOH}]_0 = 80.9$ mM.

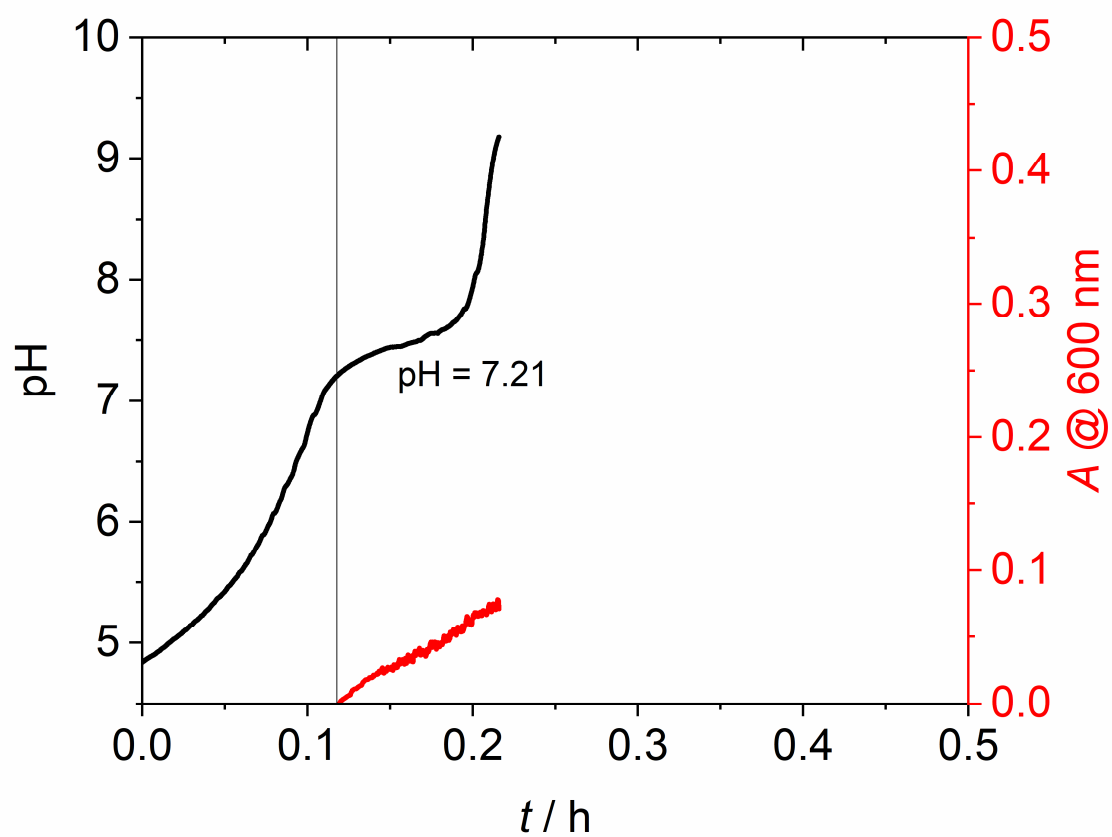


Figure S5 Titration of 1 mM Zn^{2+} in the presence of 1 mM potassium carbonate with concentrated sodium hydroxide solution.

Supporting references

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