Supplementary Information

Enzymatic microfluidic sensor for β-lactam antibiotics based on cross-linked ancestral β-lactamase crystals

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**Figure S1.** Crystallization set up in a mushroom device, allowing up to 100 drops to be run **simultaneously**. Drops were prepared by mixing protein and precipitant solutions in a 1:1 ratio (2 + 2 μL) and the reservoir contained 5 mL of precipitant solution (5.0 M sodium formate, 0.1 M sodium acetate pH 4.0).

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| **Figure S2.** A) Shows the hydrolysis reaction of nitrocefin by lactamase as a function of time for the 3 consecutive cycles each composed of 10 flow rates. B) Depicted two cycles of an inhibition experiment using ampicillin and C) a single cycle of an inhibition experiment with sulbactam. Each plateau on B and C corresponds to a fix concentration of the inhibitor. |



**Figure S3.** Lactamase activity in crystalline state. A) Activity and dissolution of an ancestral lactamase crystal marked with a white arrow. In the second frame, the crystal is fully dissolved. B) Activity of βLa-CLECs, which slowly convert all nitrocefin, painting the drops pink in approximately five minutes. The single crystal is still visible at the end of the experiment when the drops are practically evaporated.