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SUPPLEMENTARY DATA

Fluorescent TEM-1 β-lactamase with wild-type activity as a rapid drug sensor for *in vitro* drug screening

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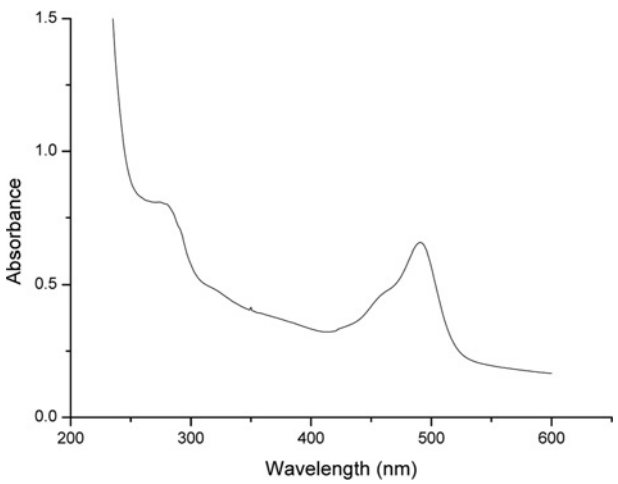

Figure S1 UV/Vis absorption spectrum of the fluorescein-labelled V216C mutant in 50 mM potassium phosphate buffer (pH 7.0)
[Labelled V216C] = 10 μM.

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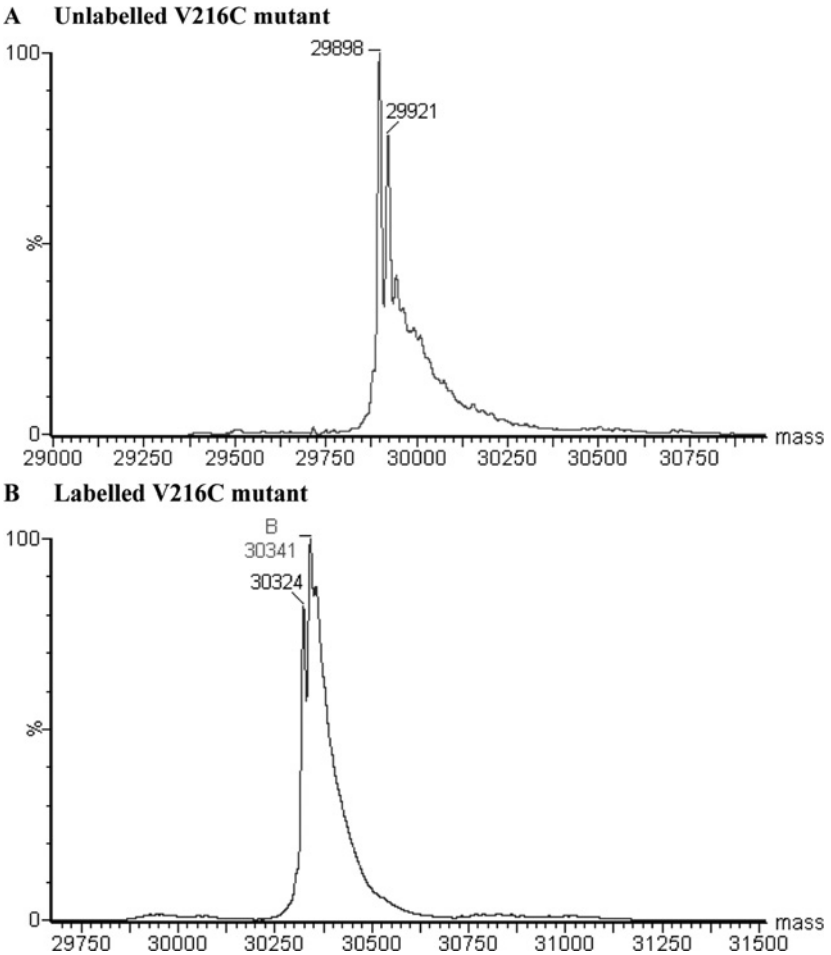

Figure S2 **ESI mass spectra of the TEM-1 V216C mutant with and without labelling with fluorescein-5-maleimide**

(**A**) Mass spectrum of the V216C mutant without fluorophore labelling. The mass peaks of 29898 and 29 921 kDa correspond to the V216C mutant and the [V216C + Na] adduct, respectively. (**B**) Mass spectrum of the V216C mutant with fluorophore labelling. The mass peaks of 30324 and 30341 Da correspond to the labelled V216C mutant and the [labelled V216C + H_2O] adduct, respectively.

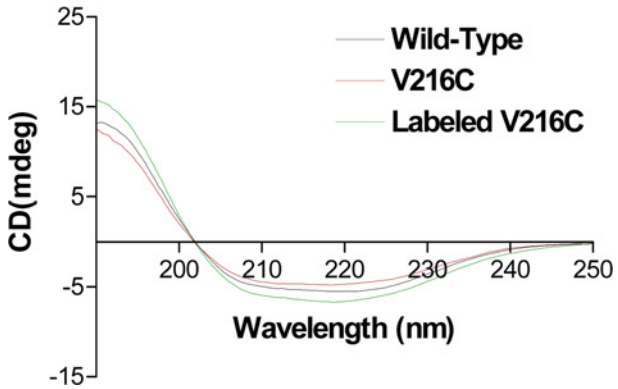

Figure S3 Far-UV CD measurements of the wild-type and mutant forms of the TEM-1 β-lactamase

Far-UV CD signals: wild-type TEM-1 (black line), unlabelled V216C (red line) and labelled V216C (green line).

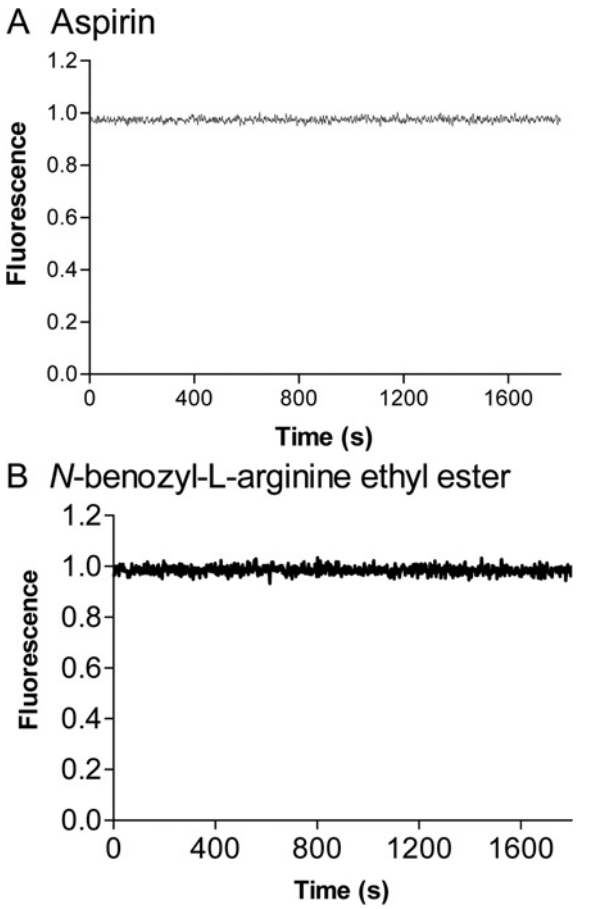

Figure S4 Time-course fluorescence measurements of the labelled V216C mutant with non-binders

(**A**) fluorescence signals of the labelled V216C mutant (20 nM) with 1 mM aspirin; (**B**) fluorescence signals of the labelled V216C mutant (20 nM) with 1 mM BAEE. Excitation: 494 nm. Buffer: 50 mM potassium phosphate (pH 7.0).

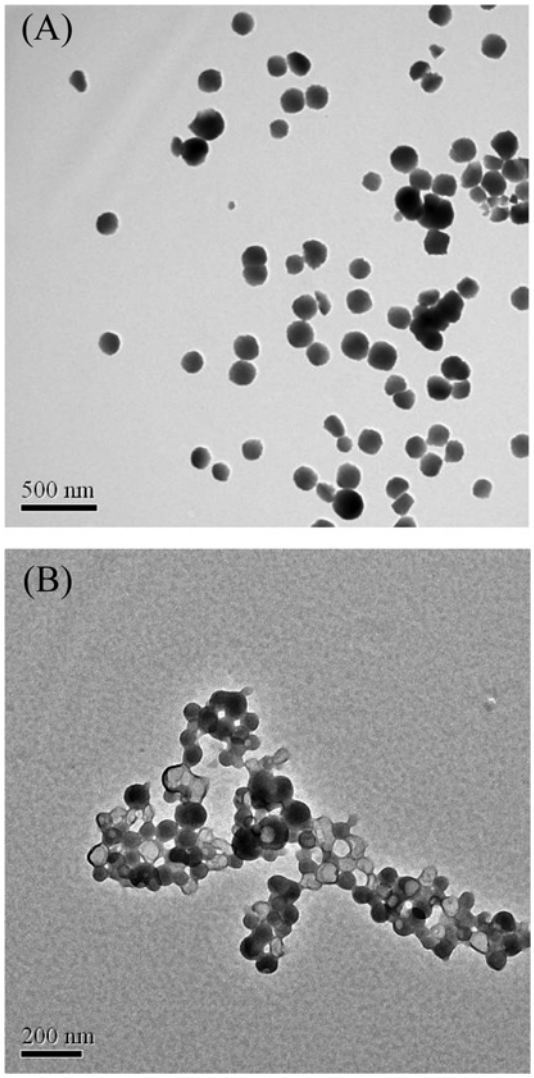

Figure S5 Transmission electron microscopic images of (A) Congo red (10 μM) and (B) TIPP (10 μM) in the presence of the labelled V216C mutant (20 nM)

Aggregates were formed by Congo red and TIPP under aqueous conditions.

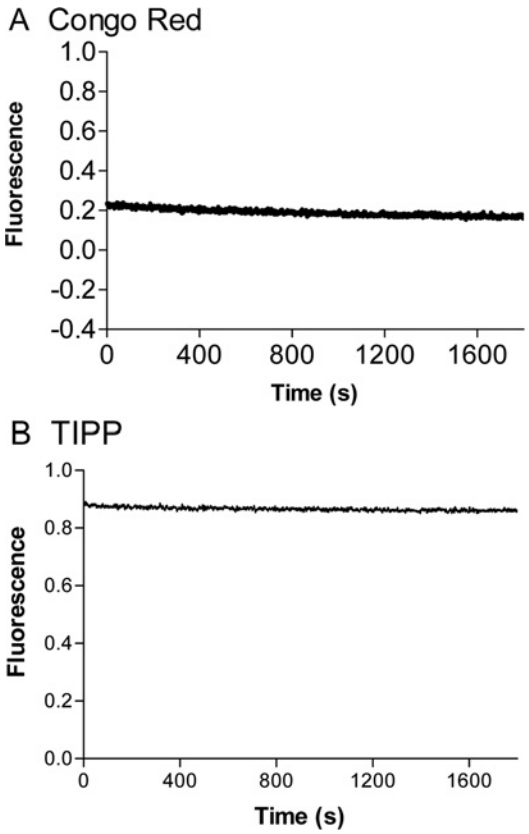

Figure S6 Time-course fluorescence measurements of the labelled V216C mutant in the presence of 'drug aggregates'

(**A**) fluorescence signals of the labelled V216C mutant (20 nM) in the presence of 10 μM Congo red; (**B**) fluorescence signals of the labelled V216C mutant (20 nM) in the presence of 10 μM TIPP. Buffer: 50 mM potassium phosphate (pH 7.0). Congo red and TIPP formed aggregates under such aqueous conditions (Figure S5).

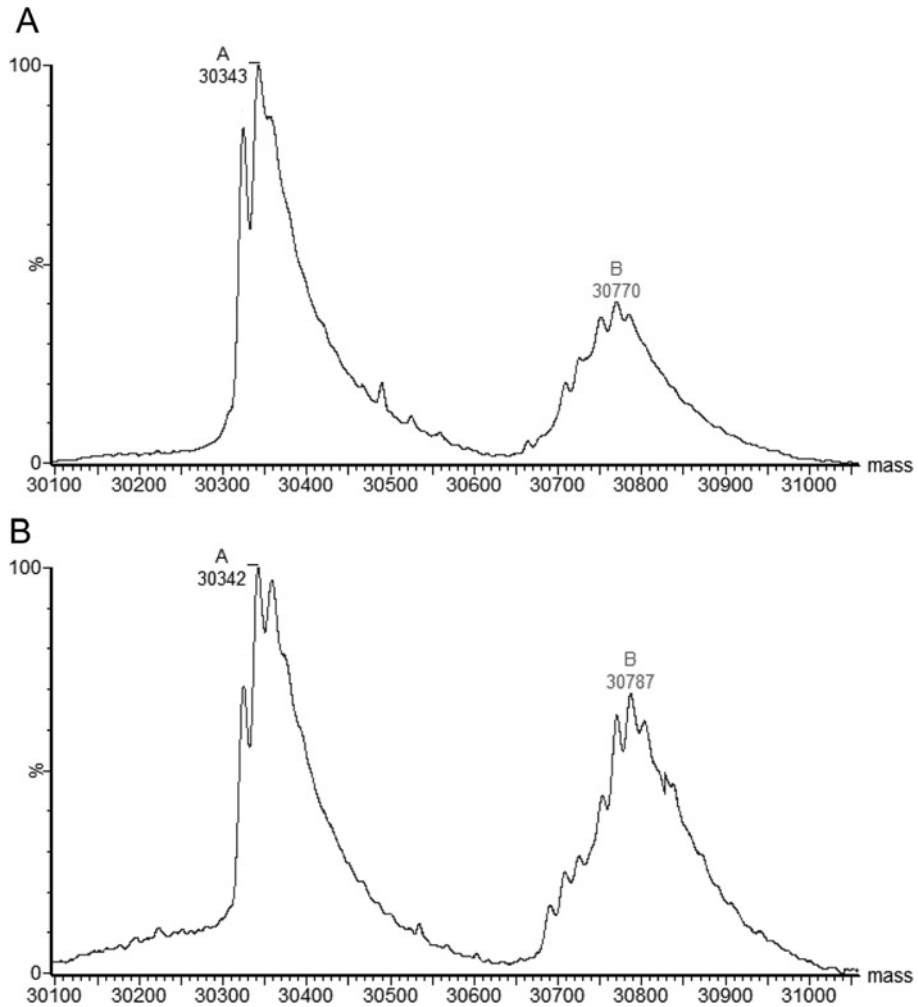

Figure S7 **ESI mass spectra of the labelled V216C mutant (5 μM) with cefoxitin (10 mM) at various time intervals**
(**A**) $t = 1$ min and (**B**) $t = 10$ min. Buffer: 20 mM ammonium acetate solution (pH 7.0). Peaks A and B correspond to the labelled V216C mutant and the covalent labelled V216C-cefoxitin complex, respectively.

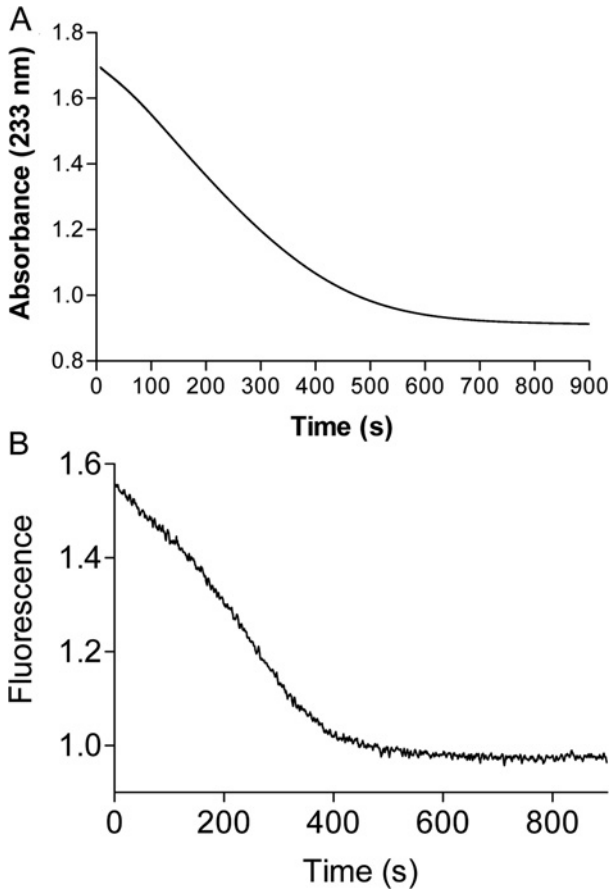

Figure S8 Time-course fluorescence and UV absorbance measurements of the labelled V216C mutant with penicillin G

(**A**) UV absorbance of penicillin G at different time intervals; (**B**) fluorescence signals of the labelled V216C mutant at different time intervals. [Labelled V216C] = 20 nM; [Penicillin G] = 1 mM. Buffer: 50 mM potassium phosphate buffer (pH 7.0).

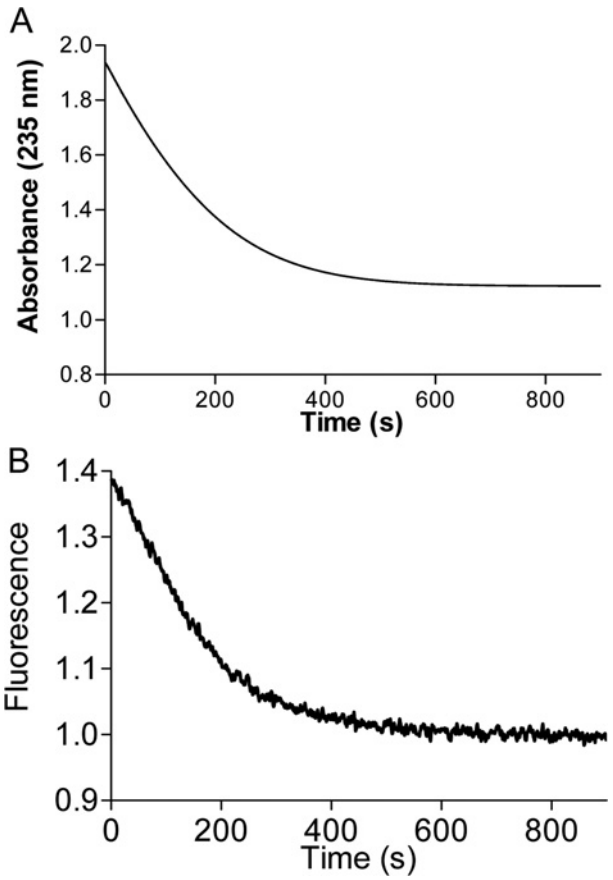

Figure S9 Time-course fluorescence and UV absorbance measurements of the labelled V216C mutant with ampicillin

(**A**) UV absorbance of ampicillin at different time intervals; (**B**) fluorescence signals of the labelled V216C mutant at different time intervals. [Labelled V216C] = 20 nM; [Ampicillin] = 1 mM. Buffer: 50 mM potassium phosphate buffer (pH 7.0).

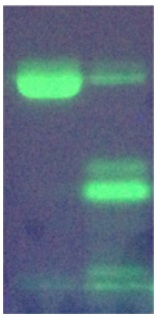

Figure S10 SDS/PAGE analysis of the labelled V216C mutant with and without trypsin digestion

The SDS/PAGE gel was illuminated with the UV light. (Left) the labelled V216C mutant without trypsin digestion; (Right) the labelled V216C mutant after trypsin digestion.

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