Penicillin Detection by *Tobacco Mosaic Virus*-Assisted Colorimetric Biosensors

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Supplementary

Supplementary Figures:

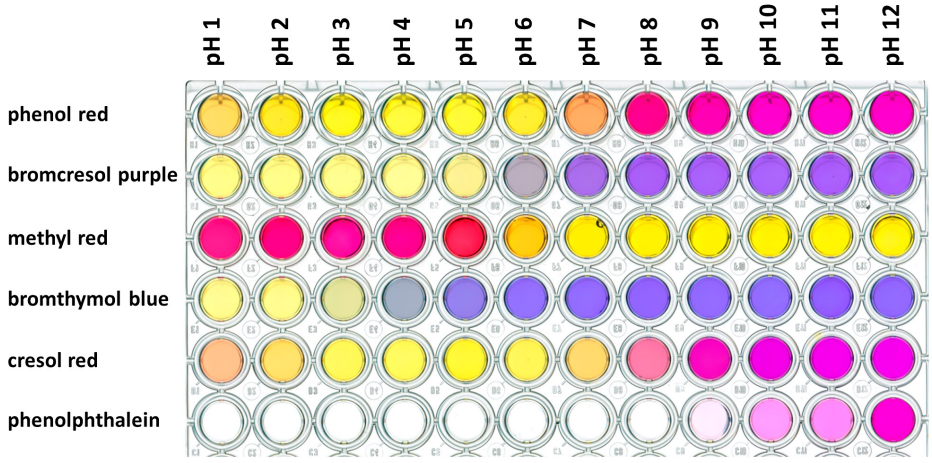

Figure S1: Color changes of pH indicators between pH 1 and pH 12.
Test solutions with pH values ranging from pH 1 to pH 12 were supplemented and mixed with the distinct dyes indicated at the left (for details, refer to the main text).

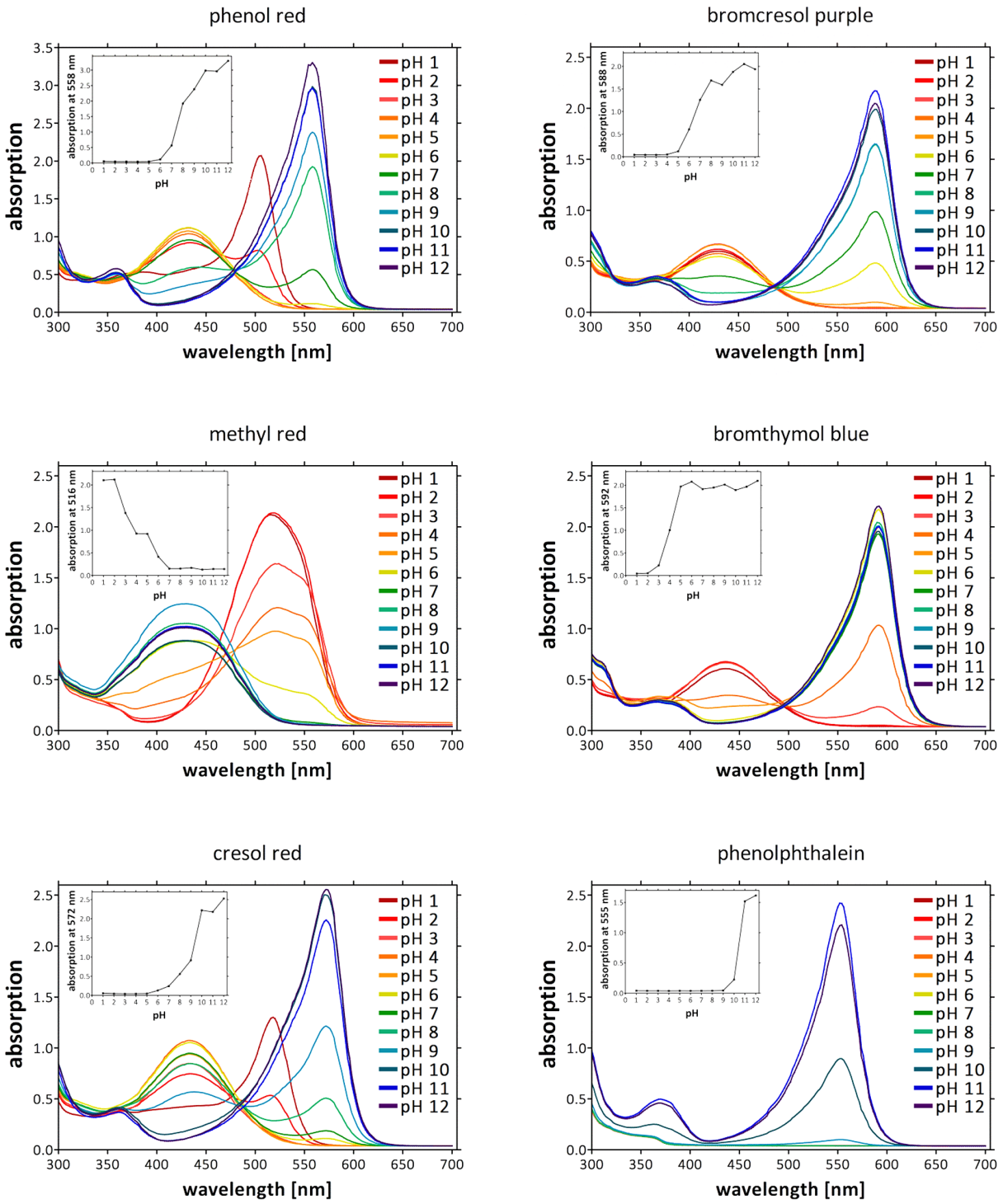

Figure S2: Absorption spectra of pH indicators.

Absorption spectra (300 nm – 700 nm) of the halochromic dyes indicated above, incubated at pH values from pH 1 to pH 12 (see Figure S1). The wavelength with maximum absorption change over the tested pH range was determined for every individual dye: phenol red: 558 nm, bromcresol purple: 588 nm, methyl red: 516 nm, bromthymol blue: 592 nm, cresol red: 572 nm, phenolphthalein: 555 nm.

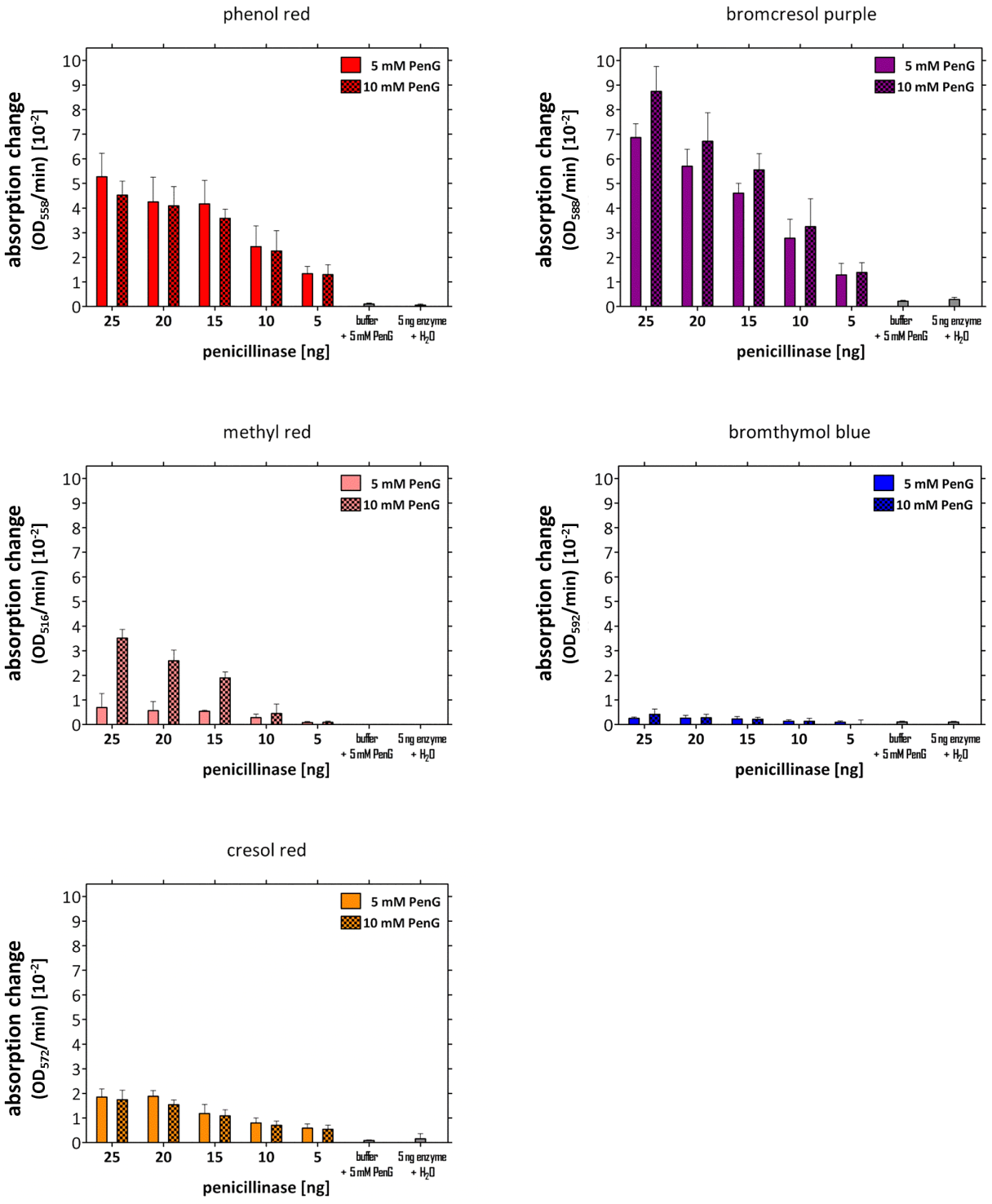

Figure S3: Absorption changes in mixtures of penicillinase, penicillin (penG) and pH indicators.
Variable enzyme amounts (5 ng ≈ 12.5 µU; 10 ng ≈ 25 µU; 15 ng ≈ 37.5 µU; 20 ng ≈ 50 µU; 25 ng ≈ 62.5 µU) blended with 5 mM penG or 10 mM penG, respectively, were mixed with pH indicator solutions. The absorption of each mixture was measured at the pH indicator-specific wavelength (see Figure S2) and the absorption change (OD/min) of each sample determined.

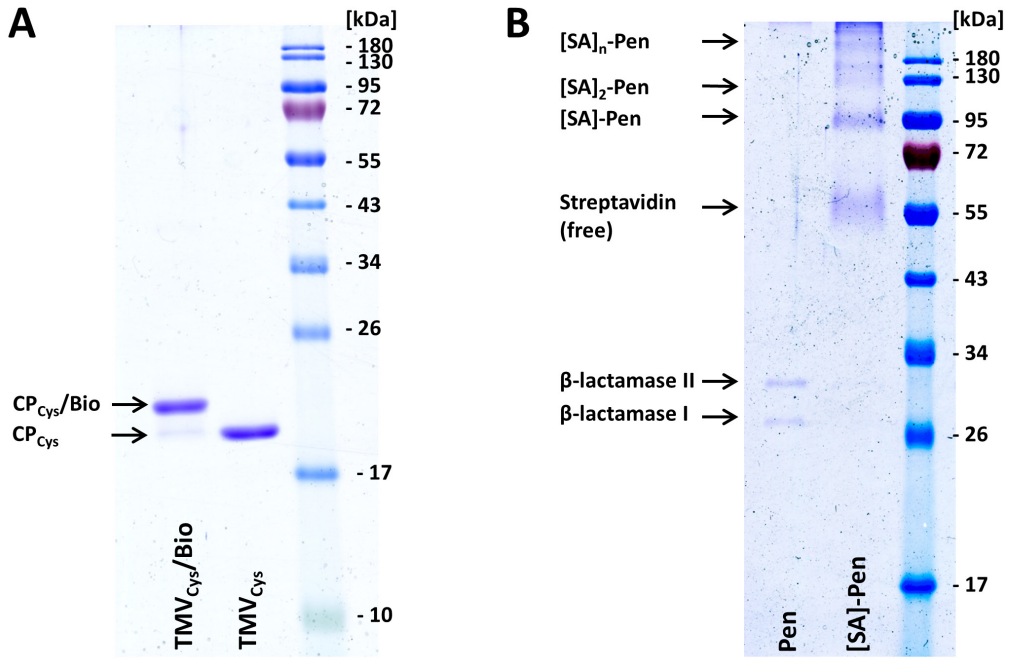

Figure S4: Functionalization of TMV_{Cys} particles and conjugation of streptavidin ([SA]) to penicillinase (Pen). **A)** Denaturing SDS-PAGE reveals a selective coupling of maleimide-PEG_{11}-biotin (Bio) linker molecules to coat protein units (CP_{Cys}) of TMV_{Cys} particles, resulting in a distinguishable mobility shift (CP_{Cys}/Bio). Image evaluation by ImageJ confirmed a biotinylation efficiency of around 95 % (see main text for details). **B)** The enzyme penicillinase from *B. cereus* is a mixture of two types of β-lactamases (E.C.3.5.2.6). To enable bioaffinity binding of the enzymes to TMV_{Cys}/Bio, penicillinase molecules were conjugated with streptavidin [SA] using a LYNX Rapid Streptavidin Antibody Conjugation Kit®, resulting in [SA]-Pen. To verify conjugation of [SA] and Pen, samples were analyzed by 12 % SDS-PAGE and stained with Coomassie Brilliant Blue R250. Successful conjugation of one or more streptavidin tetramers per penicillinase was confirmed by appearance of new band signals corresponding to $[SA]_n$-Pen, accompanied by weaker signals for bare β-lactamase.

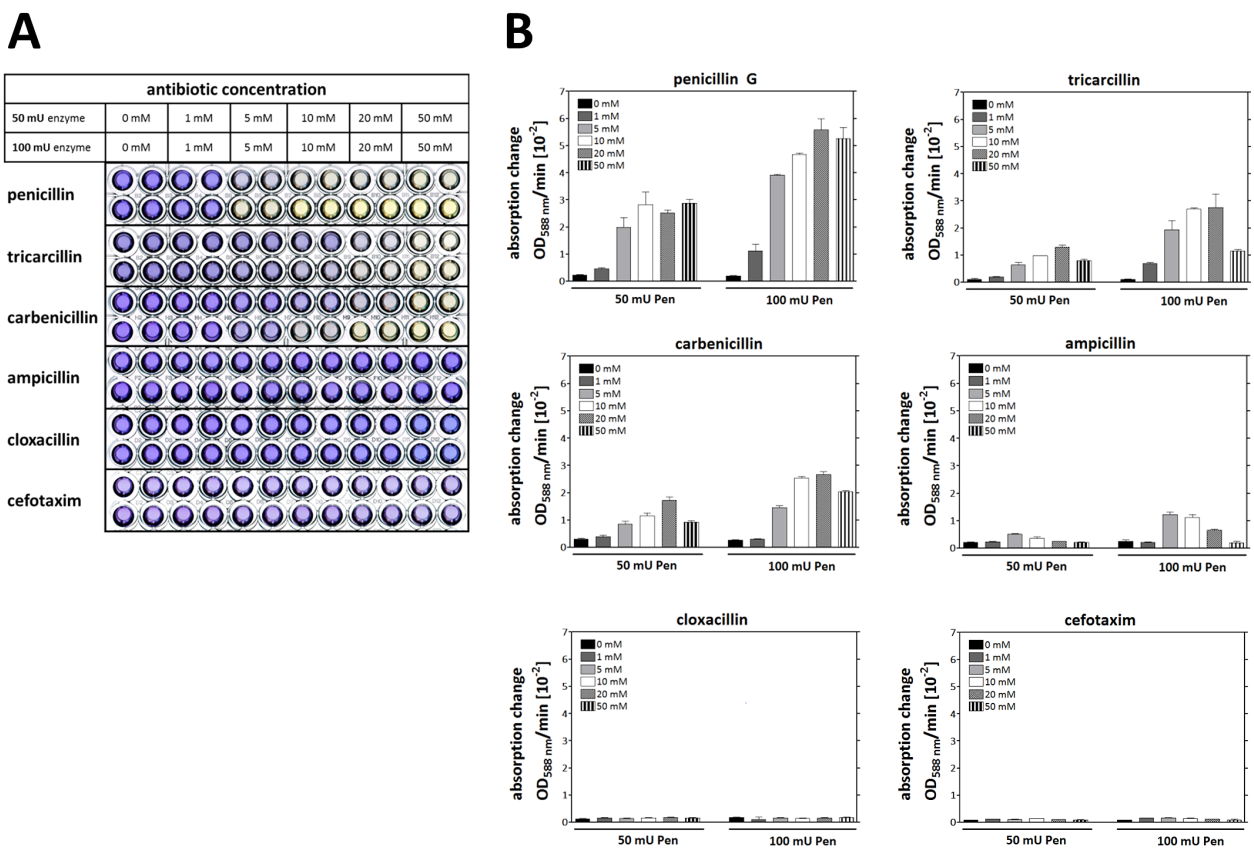

Figure S5: Detection and degradation of different β-lactam antibiotics by *B. cereus* penicillinase.
50 mU or 100 mU penicillinase were blended with analyte solutions containing variable concentrations (0 mM – 50 mM) of one out of six different antibiotics (penicillin G, tricarcillin, carbenicillin, ampicillin, cloxacillin, cefotaxim). Enzymatic degradation of the antibiotic was detected by a color change of the pH indicator. **A)** Photograph showing the color of mixtures containing penicillinase, antibiotics and bromcresol purple after 15 min incubation. **B)** The absorption of each mixture was measured at the pH indicator-specific wavelength (λ=588 nm) and the absorption change (OD/min) of each sample determined.