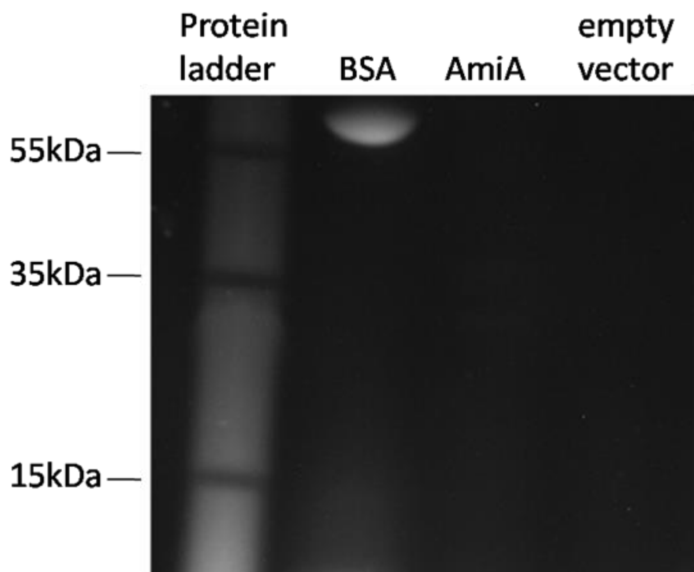
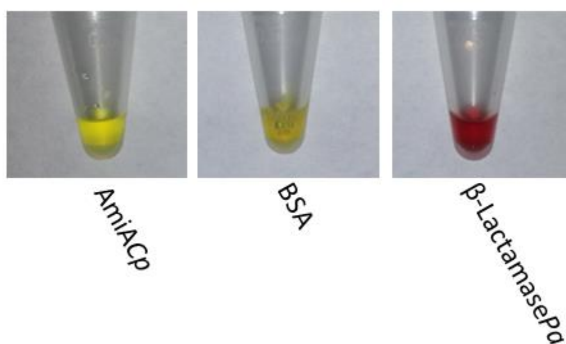
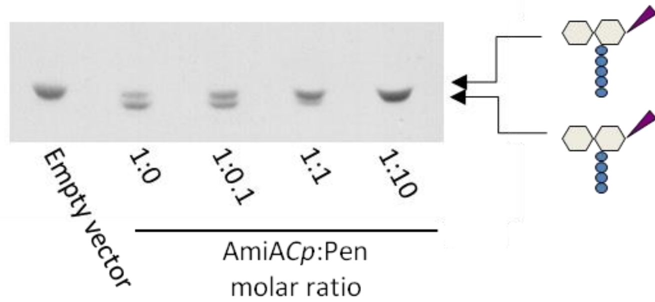
Supplementary Figures



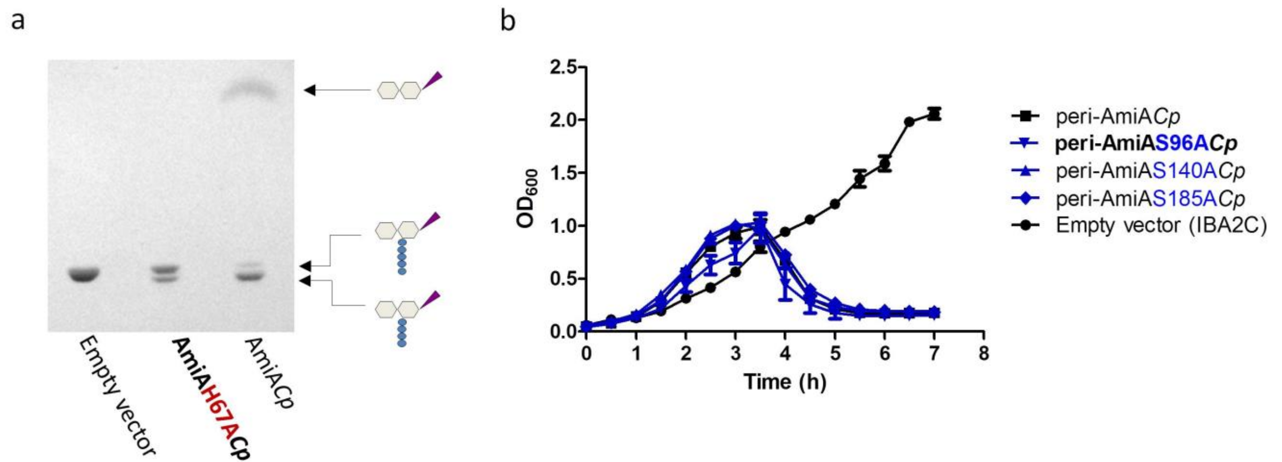
Supplementary Figure 1
Control for contaminating *E. coli* PBP DD-CPases in AmiA purifications. Serum albumin is known to bind drugs such as beta-lactams[1,2] and served as a positive control for binding of bocillin FL (bovine serum albumin (BSA, 66.4kDa)).



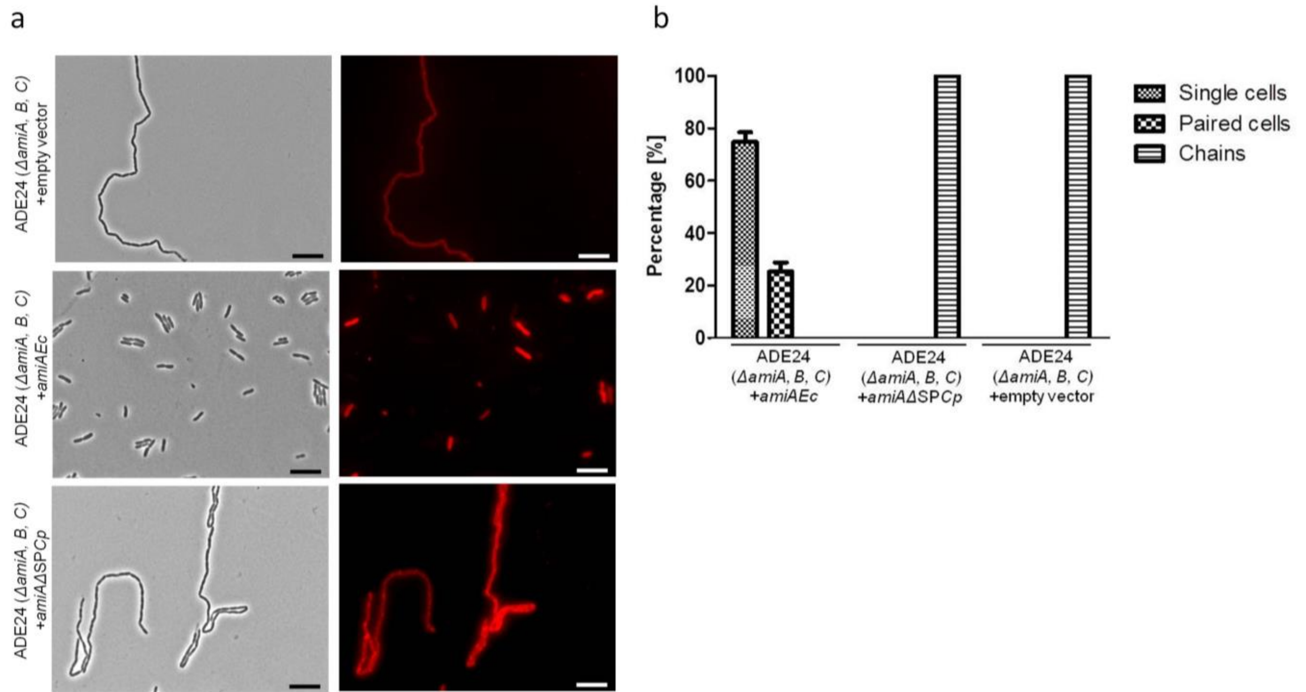
Supplementary Figure 2
Detection of beta-lactamase activity. AmiA*Cp* did not show beta-lactamase activity in a nitrocefin hydrolysis assay. β-lactamase*Pa*: beta-lactamase from *Pseudomonas aeruginosa* (Sigma Aldrich, Germany).



Supplementary Figure 3
Inhibition of AmiA*Cp* DD-CPase activity on lipid II by penicillin. TLC analysis of DD-CPase reaction products after treatment with AmiACp in the presence of varying concentrations of penicillin (titration of protein:inhibitor molar ratios ranging from 1:0 to 1:10).
DD-CPase activity was blocked in a molar ratio of 1:1.



Supplementary Figure 4
Characterization of enzymatic activies of AmiA*Cp* mutant proteins. Loss of amidase activity in the amidase active site mutant AmiAH67A*Cp* did not affect DD-CPase activity (A). Conversely, amidase activity was not impaired in DD-CPase active site mutants (B). Error bars indicate ± s.d. (n=3).



Supplementary Figure 5

Complementation experiments with *E. coli ΔamiABC* triple knockout mutant ADE24, AmiA*Ec* and AmiAΔSP*Cp*. The mutant was transformed with plasmids that allow for AHT induced expression of AmiAEC and AmiAΔSP*Cp*, respectively*;* microscopy (a) and quantative (b) analyses of the experiments: in the presence of glucose and AHT, expression of AmiC from *E. coli* was blocked and complementation of the chain forming triple amidase mutant by AmiA*Ec* resulted in separated rod-shaped cells, whereas complementation with the *C. pneumoniae* homolog that lacked its native signalpeptide (ΔSP) failed. Error bars indicate ± s.d. (n=3). Scale bar 10 µm.

Supplementary Tables

Supplementary Table 1. Strains, plasmids and primers[a] used in this study

Strain, plasmid or primer	Description	Reference or source
E. coli JM 83	Expression strain	DSM3947
E. coli W3110	Wild type, used as control for complementation assays	DSM5911
E. coli ADE24	Δ *amiA, amiB, amiC* amidase triple mutant, harboring pBAD33-amiCEc, glucose induced chain forming phenotype, used for complementation assays	This study
pBAD33-amiCEc	*amiC* from *E. coli*, chloramphenicol^R, expression of *amiC*Ec is blocked in the presence of glucose, used for complementation assay	This study
IBA2-amiACp*	*amiA* from *C. pneumoniae,* the native N-terminal signal peptide is replaced by the OmpA leader peptide, C-terminal Strep-tag, used for periplasmic overproduction and growth kinetic assay	This study
IBA3-amiACp	*amiA* from *C. pneumoniae,* C-terminal Strep-tag, used for complementation and growth kinetic assays	This study
IBA2-amiAEc	*amiA* from *E. coli,* the native N-terminal signal peptide is replaced by the OmpA leader peptide, C-terminal Strep-tag, used for complementation	This study
IBA3-amiAEc	*amiA* from *E. coli,* C-terminal Strep-tag, used for complementation	This study
IBA2-amiAH67ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAH67ACp), used for active site studies	This study
IBA2-amiAH136ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAH136ACp), used for active site studies	This study
IBA2-amiAE207ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAE207ACp), used for active site studies	This study
IBA2-amiAS96ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAS96ACp), used for active site studies	This study
IBA2-amiAS140ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAS140ACp), used for active site studies	This study
IBA2-amiAS185ACp	*amiA* from *C. pneumoniae,* C-terminal His-tag, AmiA H67A mutant (AmiAS185ACp), used for active site studies	This study
SacIamiCup	TAGGAGCTCCAGATTATGCGTCTTTCGC	This study
HindIIIamiCdw	GGCAAGCTTTTCAGCGCCTTTTTATCATC	This study
amiA-SP Cp IBA2F	ATGGTAGGTCTCAGGCCCAAACACCGAATCCTCCTCAGC	This study
amiA-SP Cp IBA3F	ATGGTAGGTCTCAAATGCAAACACCGAATCCTCCTCAGC	This study
amiA Cp IBA2F	ATGGTAGGTCTCAGGCCATGAAGCTTACCAAATATTTAAACACC	This study
amiA Cp IBA3F	ATGGTAGGTCTCAAATGATGAAGCTTACCAAATATTTAAACACC	This study
amiA +/-SP Cp IBA2/3R	ATGGTAGGTCTCAGCGCTATTTGCTTGTATCTGTGGTTTACGT	This study
amiA-H67A-sense	AGTGAGGTTATATTTATAGATCCTGGAGCCGGGGGAAAAGATCA	This study
amiA-H67A-antisense	TGATCTTTTCCCCCGGCTCCAGGATCTATAAATATAACCTCACT	This study
amiA-H136A-sense	GCGTTTGAAGAATGATTACAGGCGATGCTGATAAAGACATCCCC	This study
amiA-H136A-antisense	GGGGATGTCTTTATCAGCATCGCCTGTAATCATTCTTCAAACGC	This study
amiA-E207A-sense	GCCTGCAGTTTTGGTGGCAACCGGGTTTTTATCCA	This study
amiA-E207A-antisense	TGGATAAAAACCCGGTTGCCACCAAAACTGCAGGC	This study
amiA-S96A-sense	CTCTTGCTTTGACGGTTCAAGCTTACTTAAAGCGGATGGGTT	This study
amiA-S96A-antisense	AACCCATCCGCTTTAAGTAAGCTTGAACCGTCAAAGCAAGAG	This study
amiA-S140A-sense	GGCTGCTGCGTTTGAAGCATGATTACAGTGGATGC	This study
amiA-S140A- antisense	GCATCCACTGTAATCATGCTTCAAACGCAGCAGCC	This study
amiA-S185A-sense	TCGCAGTTTTCAAACCTCGAGCCTTCAAAATGCCATTTTTTTC	This study
amiA-S185A-sense	GAAAAAAATGGCATTTTGAAGGCTCGAGGTTTGAAAACTGCGA	This study
amiA Ec IBA2CF	ATGGTAGGTCTCAGGCCATGAGCACTTTTAAACCACTAAAAAC	This study
amiA Ec IBA2CR	ATGGTAGGTCTCAGCGCTTCGCTTTTTCGAATGTGCTTTCTG	This study
nlpD IBA2CF	ATGGTAGGTCTCAGGCCATGAATCGTAGAGACATGGTAATAAC	This study
nlpD IBA2CR	ATGGTAGGTCTCAGCGCTACGTATGCGCAACTGATCTCCAG	This study

[a] in 5‘-3‘ direction. * For penicillin inhibition, bocillin FL binding and nitrocefin hydrolysis assays the AmpR resistance marker was exchanged with CamR resistance marker to prevent potential contamination with TEM beta-lactamase.

Supplementary Methods

Detection of penicillin-binding proteins (PBPs) using bocillin FL

The detection of contaminating *E. coli* PBPs was performed with labeling assays using fluorescent penicillin bocillin FL as previously described with slight modifications[3]. 5 µM of chlamydial AmiA (produced with expression vector IBA2-amiACp containing CamR resistance marker instead of AmpR to prevent a potential contamination with TEM beta-lactamase), a mock purification or bovine serum albumin (BSA) was incubated in a final volume of 20 µl containing 50 mM MES, pH 5.5, 2 mM $MgCl_2$ and 25 µM bocillin FL for 2 h. Then the sample received 5 µl of SDS-sample buffer, was boiled for 5 min and chilled on ice for 10 min. 20 µl of the sample were analyzed by SDS-PAGE. The bocillin-labeled proteins were detected by UV-transillumination.

Detection of beta-lactamase activity

Beta-lactamase activity was tested in nitrocefin hydrolysis assays as described previously[4].

The experiments were carried out in a final volume of 50 µl containing 130 µg of the protein and 100 µM of the chromogenic cephalosporin nitrocefin.

Supplementary References

1. Nerli B, Romanini D, Picó G (1997) Structural specificity requirements in the binding of beta lactam antibiotics to human serum albumin. *Chem. Biol. Interact.* **104**:179-202.
2. Sleep D, Cameron J, Evans LR (2013) Albumin as a versatile platform for drug half-life extension. *Biochim. Biophys. Acta.* **1830**:5526-34.
3. Zhao G, Meier TI, Kahl SD, Gee KR, Blaszczak LC (1999) BOCILLIN FL, a sensitive and commercially available reagent for detection of penicillin-binding proteins. *Antimicrob. Agents. Chemother.* **43:**1124-1128.
4. Uri JV (1985) Detection of beta-lactamase activity with nitrocefin of multiple strains of various microbial genera. *Acta. Microbiol. Hung.* **32**:133-45.