Supplemental Material and Methods

Data collection, processing and structural determination of the amidase domain of LytA and of the choline-binding domain of prophage LytA

Data collection from crystals of $LytA^{AMI}$ was performed under cryogenic conditions using beam line ID23-1 at ESRF (Grenoble, France). Crystals were soaked in a cryoprotectant solution containing 25 % glycerol before freezing in liquid nitrogen. The best crystal diffracted to 1.05Å resolution and a data set consisting of 130 images with 0.3° oscillation per frame was processed and scaled with iMOSFLM (1) and SCALA (2) from the CCP4 suite (3). The space group was $P3_2$ with unit cell parameters $a = b = 50.44$ and $c = 72.6$ Å. Crystal solvent content estimations suggested one $LytA^{AMI}$ monomer in the asymmetric unit with 53% of solvent.

The crystal structure of $LytA^{AMI}$ was determined by single wavelength anomalous dispersion (SAD) method using the anomalous signal derived from a single bound Zn ion. Although no Zn was added to $LytA^{AMI}$ during purification and crystallization, X-ray fluorescent spectrum recorded from the native crystal at beam line ID29 (ESRF, France) (4) revealed a strong peak corresponding to Zn^{2+} in addition to smaller peaks for Ni^{2+} and Cu^{2+} which arose from the purification procedure with His-tag. The SAD dataset from the $LytA^{AMI}$ crystal was collected at ID29 at the wavelength 1.28226 Å corresponding to the peak of Zn^{2+} absorption. A 1.5 Å dataset, collected with cell dimensions almost identical to the first crystal, was processed with XDS (5). POINTLESS and SCALA were used for scaling and merging, respectively (2). The Autorickshaw server was used to determine the crystal structure of $LytA^{AMI}$ (6). The site of the single Zn^{2+} ion was established using SHELXE (7) implemented within Auto-Rickshaw and the derived phases allowed tracing of about 80% of all $LytA^{AMI}$ residues.

Crystals of the C-terminal CBD of prophage LytA grew after two months incubation of full-length prophage LytA in hanging drops containing 0.7 M sodium citrate tribasic dihidrate, 0.1 M bis-Tris propane, pH 7.0. A mixture of 1µl of a 20 mg/ml protein solution with 1µl of well solution was equilibrated against 200 µl crystallization reservoir solution at 20°C. Crystals were soaked in crystallization solution supplemented with 25 % glycerol before freezing in liquid nitrogen. Data collection was performed on beamline ID29 at ESRF (Grenoble, France). Diffraction data were processed and merged with XDS (5) and SCALA (2), respectively. The crystal, which belongs to space group $P2_1$ with unit cell parameters $a =$ 40.58 Å, $b = 113.4$ Å, $c = 40.64$ Å and $\beta = 94.91°$, diffracted to 2.6 Å.

The rotation function calculated by MOLREP (8) identified a strong peak (82% of origin) at $\theta = 132°$ and $\varphi = 0°$, corresponding to a non-crystallographic two fold axis. However, the size of the asymmetric unit was too small to include two full-length p-LytA molecules, with a calculated solvent content of only 8%. Combined SDS gel and mass-spectrometry analyses of the contents from several crystals from the same crystallization drop revealed that all crystals contained only $p\text{-}LytA^{CBD}$. We were not able to establish neither why only crystals of the CBD were formed nor how the amidase domain was spliced and/or degraded. One possibility is that the full-length LytA protein could be auto-catalytically cleaved in the prevailing buffer conditions. The crystal structure of $p\text{-}LytA^{CBD}$ was determined by molecular replacement using the program PHASER (9), and the coordinates of a monomer from the crystal structure of the shorter CBD of LytA (PDB 1GVN) (10) as a search model.

Ab initio shape determination and molecular modeling

Evaluation of the overall parameters of the solute, low resolution ab initio shape determination and rigid body modeling was performed using ATSAS programs (13). The 'shape scattering' curve was used to generate low resolution ab initio shapes of each protein

using DAMMIF (11). An assembly of densely packed beads represents the particle shape and simulated annealing is employed to construct a compact interconnected model that fits to the experimental data Iexp(s). Ten DAMMIF runs were performed to check solution stability, resulting in well superimposable models. The obtained models were further analyzed to determine common structural features using DAMAVER (12) and SUPCOMB (13). Two arbitrary low- or high-resolution models, each represented by an ensemble of points, were aligned using SUPCOMB by minimizing a dissimilarity measure called normalized spatial discrepancy (NSD). Discrepancies are added and normalized against the average distances between the neighboring points for the two models. NSD values close to unity indicate similarity between the two models. Finally, DAMAVER generated an average model of the superimposed structures with the lowest average NSD for all models.
The discrepancy to the experimental data $I_{exp}(s)$ is

$$\chi^2 = \frac{1}{N-1}\sum_j \left[\frac{I_{\exp}(s_j) - cI_{calc}(s_j)}{\sigma(s_j)} \right]^2 ,$$

where N is the number of experimental points, c is a scaling factor and $I_{calc}(s)$ and $\sigma(s_j)$ are the calculated intensity and the experimental error at the momentum transfer s_j, respectively.

The scattering from the high-resolution model of p-LytA^{CBD} was calculated with CRYSOL (14) which, based on the atomic coordinates, minimizes fit discrepancy to the experimental intensity by adjusting the excluded volume of the particle and the contrast of the hydration layer. Rigid body refinement of the structure of full-length LytA was performed using SASREF (15). Scattering amplitudes were pre-computed by CRYSOL using the high-resolution models of LytA^{AMI} and p-LytA^{CBD}. Simulated annealing was used to establish non-overlapping interconnected domain configurations that fitted to the experimental data.

Circular dichroism (CD)
Far-UV CD measurements were performed using a J-810 spectropolarimeter (Jasco, Easton, MD). Purified wt-LytA and cysteine to alanine-substituted LytA proteins were dialyzed against 20 mM sodium phosphate buffer, pH 7.2, 150 mM NaCl, 5 mM choline cloride and 1µM $ZnCl_2$. Protein concentrations were determined spectrophotometrically at 280 nm and corrected to 6 mg ml^{-1}. Prior to analysis proteins was further diluted 30-fold in milli-Q water to give an experimental concentration of 0.2 mg/ml. Spectra were recorded from 190 to 260 nm at 20°C with 0.5 nm steps. A total of five scans were averaged for each protein. The optical activity of the buffer was subtracted from the protein spectra.

Microscale thermophoresis
Binding of muropeptides to LytA was assessed using microscale thermophoresis (MST) (16). Although the 15 tryptophan residues present in LytA allowed for the use of the label-free approach, the maleimide-NT-547 dye was used also to label solvent-accessible Cys60 in LytA. The inactive mutant LytA-H133A, devoid of the zinc-binding histidine, was used in all experiments to exclude the cleavage of the substrate. LytA-H133A in a 0.5 µM solution in PBS with 10 mM choline chloride and 0.05% of Tween was incubated with M5P, GM5P or di(GM5P) in serial dilutions ranging from 1mM to 30 nM. Binding assays were performed using Monolith NT LabelFree (NanoTemper Technologies, Munich, Germany) at 21°C (LED power 20%, IR laser power 60%) for the label-free experiments and Monolith NT115 for the labeled LytA (LED power 50%, IR laser power 60%). Data were processed by Nano Temper Analysis package software to estimate the K_D values. Experiments were performed at least in triplicates. Label-free and NFS-labeled experiments gave comparable results.

Supplemental References

1. **Battye, T.G., Kontogiannis, L., Johnson, O., Powell, H.R. and Leslie, A.G.** 2011. iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr. D Biol. Crystallogr*. 67:271-281.
2. **Evans, P.** 2006. Scaling and assessment of data quality. *Acta Crystallogr. D Biol. Crystallogr*. 62:72-82.
3. The CCP4 suite: programs for protein crystallography. 1994. *Acta Crystallogr. D Biol. Crystallogr*. 50:760-763.
4. **Leonard, G.A., Sole, V.A., Beteva, A., Gabadinho, J., Guijarro, M., McCarthy, J., Marrocchelli, D., Nurizzo, D., McSweeney, S. and Mueller-Dieckmann, C.** 2009. Online collection and analysis of X-ray fluorescence spectra on the macromolecular crystallography beamlines of the ESRF. *J.Appl. Crystallogr.* 42:333-335.
5. **Kabsch, W.** 2010. Xds. *A Acta Crystallogr. D Biol. Crystallogr*. 66:125-132.
6. **Panjikar, S., Parthasarathy, V., Lamzin, V.S., Weiss, M.S. and Tucker, P.A.** 2005. Auto-rickshaw: an automated crystal structure determination platform as an efficient tool for the validation of an X-ray diffraction experiment. *Acta Crystallogr. D Biol. Crystallogr*. 61:449-457.
7. **Sheldrick, G.M.** 2008. A short history of SHELX. *Acta Crystallogr. A*, 64:112-122.
8. **Vagin, A. and Teplyakov, A.** 2010. Molecular replacement with MOLREP. *Acta Crystallogr. D Biol. Crystallogr*. 66:22-25.
9. **McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C. and Read, R.J.** 2007. Phaser crystallographic software. *J. Appl. Crystallogr.* 40:658-674.
10. **Fernandez-Tornero, C., Lopez, R., Garcia, E., Gimenez-Gallego, G. and Romero, A.** 2001. A novel solenoid fold in the cell wall anchoring domain of the pneumococcal virulence factor LytA. *Nat. Struct. Biol*. 8:1020-1024.
11. **Franke, D. and Svergun, D.I.** 2009. DAMMIF, a program for rapid ab-initio shape determination in small-angle scattering. *J. Appl. Crystallogr.* 42:342-346.
12. **Volkov, V.V. and Svergun, D.I.** 2003. Uniqueness of ab initio shape determination in small-angle scattering. *J. Appl. Crystallogr.* 36:860-864.
13. **Kozin, M.B. and Svergun, D.I.** 2001. Automated matching of high- and low-resolution structural models. *J. Appl. Crystallogr.* 34:33-41.
14. **Svergun, D., Barberato, C. and Koch, M.H.J.** 1995. CRYSOL - A program to evaluate x-ray solution scattering of biological macromolecules from atomic coordinates. *J. Appl. Crystallogr.* 28:768-773.
15. **Petoukhov, M.V. and Svergun, D.I.** 2005. Global rigid body modeling of macromolecular complexes against small-angle scattering data. *Biophys. J.* 89:1237-1250.
16. **Wienken, C.J., Baaske, P., Rothbauer, U., Braun, D. and Duhr, S.** 2010. Protein-binding assays in biological liquids using microscale thermophoresis. *Nat. Commun.* 1:100.