Supplemental Information

in vivo cross-linking MS reveals conservation in OmpA linkage to different classes of β-lactamases

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Experimental

in vivo cross-linking of AB5075

AB5075 cells were cultured in LB media (37 °C, 220 rpm shaking) and harvested at OD_{600}=1. The cells were washed twice with PBS, pelleted, and resuspended in a minimum amount of cross-linking buffer (170 mM Na_2HPO_4, pH 8). *In vivo* cell cross-linking reactions were conducted by adding 10 mM PIR cross-linker, d_0- or d_8- biotin-aspartate proline-n-hydroxyphthalimide (BDP-NHP)[1-3] to the cell suspension and shaking the reaction mixture at 800 rpm at room temperature for 1 hr. For each biological replicate, cell suspension collected from ~ 250 mL LB culture was cross-linked. The reaction was quenched by adding 1 mL of 0.1 M NH_4HCO_3. The cross-linked cells were pelleted, washed, lysed by heating at 95 ^oC in cell lysis buffer (4% SDS, 100 mM Tris, pH8). DNA was sheared by ultrasonication. The cell lysates were reduced, alkylated, and then buffer exchanged against 8 M urea in 0.1 M NH_4HCO_3 (pH=8) with 30 kDa molecular weight cut-off filter (MilliporeSigma). After removing SDS, the proteins were resuspended in 0.1 M NH_4HCO_3 buffer and digested overnight with trypsin (1:200 enzyme/protein mass ratio).

LC-MS analysis of cross-linked peptides

The digested samples were desalted with C18 SPE column and fractionated by strong cation exchange (SCX) HPLC (250 × 10 mm, Luna 5 μm diameter, 100 Å pore size particles, Phenomenex, Torrance, CA). The peptides were separated with a binary solvent gradient (Solvent A, 7 mM KH_2PO_4 (pH 2.6)/ 30% ACN; Solvent B, 7 mM KH_2PO_4 (pH 2.6)/350 mM KCl) at 1.5 mL/min mobile phase flow rate (0−2.5 min, 0% B; 2.5−7.5 min, 0−5% B; 7.5−47.5 min, 5−60% B; 47.5−67.5 min, 60−100% B; 67.5−77.5 min, 100% B). Five fractions (42.5-

52.5min, 52.5-57.5 min, 57.5-62.5 min, 62.5-67.5min, 67.5-87.5 min) were collected and further concentrated by vacuum centrifuge. Sample from each fraction was dissolved with 0.1 M NH_4HCO_3 buffer, pH adjusted to 7.5, and then incubated with around 200 µL of 50% UltraLink monomeric avidin beads suspension (Thermo Pierce, Rockford, IL) for 30 minutes. The cross-linked peptides were eluted with 70% ACN and 0.5% formic acid. The samples were dried and resuspended in 0.1% formic acid for duplicate nano LC−MS analysis. Cross-linked peptides from each SCX fraction were analyzed by a Waters NanoAcquity UPLC coupled to a Thermo Velos Fourier transform ion cyclotron resonance mass spectrometer implemented with ReACT[4]. The cross-linked peptide pairs were separated by a 40 cm × 75 µm C8 column (5 µm diameter, 100 Å pore size MichromMagic beads) with a 4 hour 10–40% B gradient (solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile, flow rate 300 nL/min). High resolution full MS and CID MS/MS scans for ions baring more than 3 charges were acquired in the ICR cell with resolving power set to 50 k to examine cross-linked peptide pair candidates in real-time. In the MS/MS spectra, the presence of reporter ion mass (m/z 752.4129) along with fragment ion masses satisfying the PIR mass relationship (precursor mass = reporter ion mass + peptide α mass + peptide β mass) within 20 ppm of mass tolerance among the top 500 fragment ions in the MS/MS spectra suggests the precursor ion as potential candidate of cross-linked peptide. Real-time discovery of PIR mass relationship triggers low resolution CID MS/MS/MS analysis of two linear peptides released from the cross-linked peptide pair candidate ion ‘on-the-fly’. Two MS/MS/MS scans were acquired by the linear ion trap for each released peptide ion from one cross-linked peptide pair precursor. Normalized collision energy was set at 25 for MS/MS scans, and 35 for MS/MS/MS scans.

Identification of cross-linked peptides

MS3 spectra from ReACT analysis were searched against AB5075 proteome database containing both forward and reverse protein sequences (7720 total sequences) using Comet for identification of linear peptide sequences released from a cross-linked peptide pair. The linear peptide sequences were then assembled to cross-linked peptide pairs according to the mass relationships recorded during the ReACT data acquisition. Comet search settings were the same as those used for DDA proteomics except: fragment ion mass tolerance 1.005 Da with 0.4 Da offset; variable modifications including Met oxidation (15.9949 Da) and BDP stump modification (197.0324 Da or 201.0597 Da) of Lys and protein N-termini, where 'stump' refers to the residual group from the cross-linker attached to lysine side chain or peptide N-terminus after CID cleavage; up to three missed tryptic cleavages allowed. All the target and decoy MS3 PSMs containing an internal Lys modified by BDP stump and passing through e-score value cutoff 0.2 were mapped back to the cross-linked relationships with a 20 ppm tolerance. The global false discovery rate (FDR) of cross-linked peptide pair identification was estimated to be 0.88%, from the ratio of number of decoy cross-linked peptide pairs (either one or both peptides are decoy sequences passing the MS^3 e score 0.2 threshold) to the total number of identified cross-linked peptide pairs.

Table S1 Cross-linked peptide-spectra-matches for inter-protein crosslinks between two β-lactamases and OmpA C-terminus.

References

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