Protein-protein Interaction based substrate control in the E. coli octanoic acid transferase, LipB

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Experimental Methods

Protein Purification and production Protocol

LipB was grown through overexpression in *E. coli* BL21 (DE3), cells were grown in 1L of media with 50mg/L kanamycin. Growths were started through inoculation using a 5mL starter culture grown overnight. LipB was grown until it reached an OD600~ 0.6-0.8, then induced with 1mM IPTG and incubated overnight at 16°C. After growth pelleting was performed on a Beckman floor centrifuge in a JLA-8.1 rotor at 800 RCF. Pelleted cells were frozen and stored until the days prior to the titration for purification.

Labeled AcpP was grown from a pet-22b vector with a His-tag in *E. coli* BL21 (DE3) cells. In order to label the cells they were grown in 15N supplemented M9 minimal media. 1g of 15N NH₄Cl and 8g of unlabeled glucose were added to 1L of M9 media. In order to achieve deuteration the media components were mixed in an oven dried glass graduated cylinder, followed by sterile filtration into an autoclaved and oven dried growth flask. Inoculating bacteria was carefully attenuated to the deuterated media, over the course of several growths. To begin BL21 cells were inoculated into a 25% D₂O/75% H₂O unlabeled media, these were grown overnight at 37 °C. This growth was used to then inoculate another 50% D₂O/50% H₂O media, which was grown overnight in the same conditions. This was then used to inoculate 75% D₂O/ 25% H₂O, which was grown and used to inoculate 90% D₂O media. Finally the 90% D₂O growth was used to inoculate a final starter with 100% D₂O M9 media. This final 100% D₂O starter was grown overnight at 37 °C and after confirming by eye that the media had become turbid with growth used to inoculate the labeled D₂O M9 media. This was grown at 37 °C for ~16 hours until OD600=0.7. At this point 1mM IPTG was added for induction and the growth was left to grow for 4 hours at 37 °C. After induced growth the cells were spun down on a JLA-8.1 rotor at 800 RCF. Cells were spun for 1 hour and care was taken when harvesting cells to ensure there was no loss of material.

The 15N ammonium chloride used in the labeled growth was purchased from Cambridge Isotopes laboratory. Deuterium oxide (D₂O) used in preparation of perdeuterated growth was purchased from Sigma Aldritch. All unlabeled proteins were grown on Luria broth from Teknova.

For purification, cells were re-suspended in 50mM Hepes (pH 7.4), 250 mM NaCl, and 10% glycerol. The lysed cells were spun at 10,000 RCF in a Beckman floor centrifuge equipped with a JA-20 rotor. Spun protein was checked for full clarification after 1 hour, after confirming pelleting of membrane and insoluble materials the protein was taken for purification. Clarified lysate was mixed with 2mL bed volume of Bio-Rad Ni-IMAC resin and left on a rotator in a 4 °C cold room to batch bind for 30 minutes. Washing was performed with 2.40 mL washes with 50mM HEPES (pH 7.4), 250mM NaCl, and 10% glycerol, the first wash was performed with just buffer and the second with an added 15mM imidazole. After washing elution was performed with 3 5mL volumes of wash buffer with an added 250mM imidazole. Bradford reagent was used to test the purification for protein and at the end of elution to confirm no further protein was eluted. The same purification protocol was used for the AcpP, but out of caution for losing valuable labeled protein the wash volume was lowered to 30 mL and 10mM imidazole. After purification proteins were checked by 12% SDS-PAGE to confirm successful purification. Elutions were dialyzed overnight into 50mM Tris-HCl (pH 7.4), 250 mM NaCl, 10% glycerol, and 1mM DTT. For the AcpP purification the second wash was also dialyzed but discarded once successful separation of the labeled AcpP was confirmed.
ACP Chemoenzymatic loading

After purification and dialysis, the AcpP was made uniformly apo by reaction with *Pseudomonas aeruginosa* ACPH, with an added 5mM MgCl₂ and 0.5mM MnCl₂. Reaction was performed overnight at 37 °C on a rotator. Apofication was confirmed by conformationally sensitive Urea-PAGE. After confirmation that the AcpP was fully apo chemoenzymatic labeling was carried out. The loading was performed using 3 E. coli biosynthetic enzymes CoaA, CoaD, and CoaE plus the *Bacillus subtilis* SFP. The reaction contained 12.5mM MgCl₂, 10mM ATP, 0.1µM CoaA, 0.1µM CoaD, 0.1µM CoaE, 0.2µM Sfp, 0.02% Triton X, 0.01 % Azide, 0.1% TCEP, and 0.1mM acyl mimic probe.

Purification and preparation for titrations

Samples were purified by the same means as previously published. After dialysis of the LipB or one pot chemoenzymatic loading of the AcpP the samples were collected and concentrated to 2mL on Amicon Ultra-15 spin concentrators. 3kDa and 10kDa columns were used for the AcpP and LipB respectively. After concentration AcpP and LipB were purified by size exclusion chromatography on a Superdex 75 column, 10mM potassium phosphate pH 7.4, 0.5mM TCEP, and 0.1% azide buffer was prepared and used to purify the AcpP and LipB for the experiments. In order to assure consistency, the same buffer was used for purifications and as buffer in the NMR experiments. For the first C8-AcpP titration the carrier protein and LipB were purified the day before the experiment on the FPLC. In order to assure stability of the partner protein the LipB was not concentrated until the morning of the experiment. The C8-AcpP was concentrated to 3.87 mg/mL and the LipB was concentrated to 6.1 mg/mL. These proteins were used to create a saturated NMR sample at 0.075 mM C8-AcpP and 0.113mM LipB. A zero-point AcpP sample was created with 0.075 mM C8-AcpP. In the case of C8-AcpP a 2.0 molar equivalents sample was prepared but had too poor signal to be useful. In the case of the C6-AcpP experiment the carrier protein was taken from the FPLC and concentrated to 4.1 mg/mL and the LipB was concentrated to 8.5 mg/mL, final concentrations were 0.105 mM C6-AcpP and 0.210 mM LipB in the saturated sample and 0.105 mM AcpP in the zero point sample. A 2 molar excess of partner protein was used to ensure full saturation in the non-substrate AcpP titration. In the C10-AcpP experiment the carrier protein was concentrated to a final concentration of 4.95 mg/mL and the LipB was concentrated to 7.78 mg/mL. The final concentrations were 0.1055mM C10-AcpP and 0.2112 mM LipB in the saturated sample and 0.1055 mM C10-AcpP in the zero point sample. Again the ratios were chosen to ensure full saturation at 2 molar equivalents. Approximately the same concentrations were chosen to make the experiment similar to the C6-AcpP.

NMR Experiments

All spectra collected in this experiment were collected at the UCSD Biomolecular NMR facility on their Bruker 800MHz spectrometer. Previous assignments were used for the C8-AcpP backbone HSQC assignments¹. The C6 and C10-AcpP HSQCs were assigned based on the C8-AcpP, due to the small differences between the two spectra. Assigned peaks are available to view on the BMRB. Experiments were performed at 37 °C, titrations had a total of 5 titration
points. The chemical shift perturbations were quantified using the formula below with an $\alpha$ value of 0.2. This was in order to keep the data consistent with previous work in FAB.

$$CSP = \sqrt{\frac{1}{2} (\delta_H^2 + (\alpha \cdot \delta_N^2))}$$

To perform the titrations two samples were prepared as described. A saturated sample and zero-point sample, buffers were prepared identically for both samples with only the presence of partner protein different between samples. All three sets of HSQC experiments were acquired with a 1.5 second recycle delay and 2048 data points. Between experiments samples were stored at 4°C to maintain stability, no denaturation of the labeled AcpP was seen in the spectra and no visible crashed protein was observed in any sample. Processing was performed in NMRPipe 10.9² and visualization was performed in NMRFAM-SPARKY 3.115³. After processing all figures displaying spectra were generated in Sparky, chemical shift perturbation calculations and figure generation was performed using the Matplotlib python utility⁴.

**Titan analysis**

Further analysis of the titrations was performed using the TITAN lineshape analysis program. Peaks were selected by hand across the titration before performing an initial fitting of the data. Fitting parameters were first estimated at 10μM with a $k_{off}$ of 5000 s⁻¹, following fitting the peaks were hand checked. Peaks were examined to be sure there was no errors in the cases of peaks which migrated into one another or crowded regions of the spectra which were incorrectly fit. After this an initial jackknife error analysis was performed, this gave a rough picture of the error of the calculations. After a final hand check that no peaks were fitted incorrectly the final error analysis was performed. In each titration data set 300 steps of bootstrap error analysis were performed, this took approximately 18 hours for each data set. Calculations were performed by the same protocol as previously published on fatty acid biosynthesis, a set of matched simulated and real peaks are presented.

**Docking Method**

The LipB structure was prepared by homology modeling using the 2QHS *Thermus thermophilus* lipoyltransferase with ICM Homology. The *Mycobacterial* LipB 1W66 was also considered but 2QHS had a greater sequence homology. AcpPs used for docking were taken from previous MD simulations. Before docking the LipB structure was prepared by solvation and minimization. The ICM quickflood procedure was performed to generate a water box around the LipB. Following solvation the LipB was minimized to correctly orient the amino acid side chains for interaction with the AcpP. Optimization was performed on LipB by first running the ICM optimizeHbonds and optimize HisProAsnGlnCys protocols. Molecular dynamics derived AcpP structures were used for the ACPs. The acyl chain and phosphopantetheine were preserved during the calculation to best mimic the different chain lengths. All docking was performed using the ICM – Molsoft FFT protein protein docking algorithm.

Models of the LipB•AcpP interactions were chosen based on the most stable model under 10Å RMSD form the previously published docked model. This cutoff was chosen in order to give
each chain length flexibility to adopt the most stable conformation. Over 10Å the docked conformation was so far from the active site that there was no chance for the conformation to be an active one. The chosen complexes were visualized against one another when comparing the most stable conformations. A second analysis was performed by using the stable conformation seen for the C6, C8, and C10-AcpP•LipB complex as a reference. This data set of poses with deviation from the previous model was used to map the LipB surface. Graphing of the energetics was done in Matplotlib, while visualizations were performed in PyMOL\textsuperscript{5}. 
Figure S1 Titration of dexamoyl-AcpP with the *E. coli* LipB octanoyltransferase. 5 1H-15N HSQC spectra were overlayed of the C6 AcpP interacting with increasing molar ratios of the octanoyltransferase, LipB. The titration occurs in fast exchange, with the bound and unbound state interchanging between bound and unbound rapidly and resolving as a single peak on the spectra. A) The total NMR spectra with a selection of individual peaks highlighted. B) The chemical shift perturbations of each residue in the titration. One standard deviation above the mean is colored red to highlight the most perturbed residues. C) A focus on the important serine 36 of AcpP, it should be noted the difference between this shift in the C6-AcpP titration and the other chain lengths. D) The surface of the AcpP with the CSPs colored by magnitude.
Figure S2 Titration of decanoyl -AcpP with the *E. coli* LipB octanoyltransferase. 5 1H-15N HSQC spectra were overlayed of the C10 AcpP interacting with increasing molar ratios of the octanoyltransferase, LipB. The titration occurs in fast exchange, with the bound and unbound state interchanging between bound and unbound rapidly and resolving as a single peak on the spectra. A) The total NMR spectra with a selection of individual peaks highlighted. B) The chemical shift perturbations of each residue in the titration. One standard deviation above the mean is colored red to highlight the most perturbed residues. C) A focus on the important serine 36 of AcpP, it should be noted the difference between this shift in the C10-AcpP titration and the other chain lengths. D) The surface of the AcpP with the CSPs colored by magnitude.
Figure S3 Titration of octanoyl-AcpP with the *E. coli* LipB octanoyltransferase. 4 1H-15N HSQC spectra were overlayed of the C8 AcpP interacting with increasing molar ratios of the octanoyltransferase, LipB. A fifth titration point was prepared but the signal was too weak to yield any useful data. The titration occurs in fast exchange, with the bound and unbound state interchanging between bound and unbound rapidly and resolving as a single peak on the spectra. A) The total NMR spectra with a selection of individual peaks highlighted. B) The chemical shift perturbations of each residue in the titration. One standard deviation above the mean is colored red to highlight the most perturbed residues. C) A focus on the important serine 36 of AcpP, it should be noted the difference between this shift in the C8-AcpP titration and the other chain lengths. D) The surface of the AcpP with the CSPs colored by magnitude.
Figure S4. Normalized chemical shift perturbations of three AcpPs interacting with LipB. The perturbations are normalized within their own data set, setting the largest CSP at 1.0.
Figure S5 TITAN analysis of the C6-AcpP LipB titration. Real (red) and simulated (blue) titration peaks are shown for four selected residues of the TITAN analysis. The analysis was performed using the flexible docking method, allowing flexibility in the stoichiometry. The error was analyzed using 300 seps of bootstrap error analysis. Though there is significant signal loss in the real data, the peaks overlay well demonstrating a well fit model.
Figure S6 TITAN analysis of the C10-AcpP LipB titration. Real (red) and simulated (blue) titration peaks are shown for four selected residues of the TITAN analysis. The analysis was performed using the flexible docking method, allowing flexibility in the stoichiometry. The error was analyzed using 300 seps of bootstrap error analysis. Though there is significant signal loss in the real data, the peaks overlay well demonstrating a well fit model.
Figure S7 TITAN analysis of the C8-AcpP LipB titration. Real (red) and simulated (blue) titration peaks are shown for four selected residues of the TITAN analysis. The analysis was performed using the flexible docking method, allowing flexibility in the stoichiometry. The error was analyzed using 300 seps of bootstrap error analysis. Though there is significant signal loss in the real data, the peaks overlay well demonstrating a well fit model.
Figure S8 Comparison of the E. coli LipB model to the M. tuberculosis and T. thermophilus LipB. 
A) The M. tuberculosis LipB is shown with APBS coloring generated in PyMOL. B) The T. thermophilus LipB shown with APBS coloring. C) The E. coli LipB model generated in this work shown with APBS coloring. It is interesting to note the similarities of the surfaces and electrostatics of the different LipBs. D&E) Overlays of the three LipBs, showing the similarities across the different structural models. The majority of the proteins overlay quite well, with only some loop regions showing large variations. The T. thermophilus LipB has a 51% similarity to E. coli and M. tuberculosis has a 53% similarity, as analyzed by sequence alignment in BlastP². However, the homology of T. thermophilus LipB is higher at 36%, compared to 35% for M. tuberculosis. Though, both of these values are extremely close and the models are structurally similar. F&G) The LipBs overlaid with an ACP to give context of the regions of the LipBs which are more different. It is promising that the AcpP binding surface appears to show very little variation, with the dissimilar loop beyond the binding site and active site.
Figure S9 Docking details of the C6, C8, and C10-AcpP with the *E. coli* octanoyltransferase, LipB. The docked states of the AcpPs with LipB are shown with greater detail, for each chain length docked the full 50 angstrom RMSD surface is shown. A) The C6-AcpP docking to LipB RMSD vs energy plot. The RMSD is based on the previously published model as described in the methods. B) The C8-AcpP docking to LipB RMSD vs energy plot. The RMSD is based on the previously published model as described in the methods. C) The C10-AcpP docking to LipB RMSD vs energy plot. The RMSD is based on the previously published model as described in the methods.
Figure S10 Comparison of the different chain lengths of AcpP. A) the most stable low RMSD (less than 5Å) state of the AcpP•LipB binding with C6, C8, and C10-AcpP. The complexes of C6 and C10 are significantly less stable than the C8-AcpP•LipB complex. B) The structures of the MD derived C6, C8, and C10-AcpP. The acyl chains were present during the simulations but in other figures they are not shown, as most of the chain is sequestered and it makes viewing the protein structures more difficult.
Figure S11 Visualization of CSPs onto the C8 docked orientation. The C8-AcpP docked with LipB was colored based on the CSPs observed in the titration experiment.
Table S1 C6-AcpP•LipB titration chemical shifts. The data have also been submitted to the BMRB for wide access.

| Residue Number | Residue | Nuclei | ZP chemical shift | Saturated chemical shift |
|----------------|---------|--------|-------------------|-------------------------|
| 3              | I       | H      | 8.644             | 8.647                   |
| 3              | I       | N      | 121.548           | 121.545                 |
| 4              | E       | H      | 8.579             | 8.508                   |
| 4              | E       | N      | 119.443           | 119.286                 |
| 5              | E       | H      | 7.863             | 7.865                   |
| 5              | E       | N      | 116.97            | 116.898                 |
| 6              | R       | H      | 8.342             | 8.347                   |
| 6              | R       | N      | 119.976           | 119.99                  |
| 7              | V       | H      | 8.942             | 8.942                   |
| 7              | V       | N      | 119.299           | 119.254                 |
| 8              | K       | H      | 8.213             | 8.239                   |
| 8              | K       | N      | 117.216           | 117.24                  |
| 9              | K       | H      | 8.271             | 8.258                   |
| 9              | K       | N      | 120.487           | 120.498                 |
| 10             | I       | H      | 7.647             | 7.654                   |
| 10             | I       | N      | 119.368           | 119.398                 |
| 11             | I       | H      | 8.296             | 8.287                   |
| 11             | I       | N      | 119.004           | 119.039                 |
| 12             | G       | H      | 8.451             | 8.457                   |
| 12             | G       | N      | 105.381           | 105.45                  |
| 13             | E       | H      | 8.196             | 8.208                   |
| 13             | E       | N      | 120.182           | 120.179                 |
| 14             | Q       | H      | 8.414             | 8.426                   |
| 14             | Q       | N      | 117.571           | 117.469                 |
| 15             | L       | H      | 8.043             | 8.031                   |
| 15             | L       | N      | 113.265           | 113.273                 |
| 16             | G       | H      | 7.79              | 7.785                   |
| 16             | G       | N      | 109.86            | 109.797                 |
| 17             | V       | H      | 7.867             | 7.849                   |
| 17             | V       | N      | 114.524           | 114.277                 |
| 18             | K       | H      | 8.524             | 8.545                   |
| 18             | K       | N      | 123.077           | 123.02                  |
| 19             | Q       | H      | 8.776             | 8.777                   |
| 19             | Q       | N      | 122.896           | 122.799                 |
| 20             | E       | H      | 9.415             | 9.418                   |
| 20             | E       | N      | 116.679           | 116.685                 |
| 21             | E       | H      | 7.863             | 7.865                   |
| 21             | E       | N      | 116.97            | 116.898                 |
| 22             | V       | H      | 7.527             | 7.532                   |
| 22             | V       | N      | 122.285           | 122.305                 |
| 23             | T       | H      | 7.318             | 7.295                   |
| 23             | T       | N      | 115.47            | 115.425                 |
|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
| 24 | N | H | 8.605 | 8.607 |
| 24 | N | N | 118.763 | 118.829 |
| 25 | N | H | 8.092 | 8.105 |
| 25 | N | N | 111.857 | 112.309 |
| 26 | A | H | 7.308 | 7.322 |
| 26 | A | N | 122.872 | 122.865 |
| 27 | S | H | 9.952 | 9.972 |
| 27 | S | N | 117.225 | 117.298 |
| 28 | F | H | 7.56 | 7.559 |
| 28 | F | N | 125.623 | 125.75 |
| 29 | V | N | 116.847 | 116.941 |
| 30 | E | H | 8.299 | 8.32 |
| 30 | E | N | 116.744 | 116.709 |
| 31 | D | H | 7.784 | 7.8 |
| 31 | D | N | 113.742 | 113.671 |
| 32 | L | H | 7.347 | 7.368 |
| 32 | L | N | 115.69 | 115.725 |
| 33 | G | H | 7.273 | 7.292 |
| 33 | G | N | 106.513 | 106.505 |
| 34 | A | H | 8.46 | 8.471 |
| 34 | A | N | 122.803 | 122.809 |
| 35 | D | H | 9.23 | 9.254 |
| 35 | D | N | 122.441 | 122.178 |
| 36 | S | H | 8.638 | 8.626 |
| 36 | S | N | 113.001 | 111.985 |
| 37 | L | H | 8.131 | 8.08 |
| 37 | L | N | 123.803 | 123.667 |
| 38 | D | H | 8.304 | 8.347 |
| 38 | D | N | 119.806 | 119.98 |
| 39 | T | H | 8.132 | 8.177 |
| 39 | T | N | 111.942 | 111.434 |
| 40 | V | H | 7.221 | 7.215 |
| 40 | V | N | 121.58 | 121.677 |
| 41 | E | H | 7.84 | 7.857 |
| 41 | E | N | 119.312 | 119.33 |
| 42 | L | H | 8.387 | 8.471 |
| 42 | L | N | 121.529 | 121.332 |
| 43 | V | H | 8.006 | 8.029 |
| 43 | V | N | 119.194 | 119.44 |
| 44 | M | H | 7.755 | 7.733 |
| 44 | M | N | 117.244 | 116.839 |
| 45 | A | H | 8.149 | 8.147 |
| 45 | A | N | 121.461 | 121.291 |
| 46 | L | H | 8.384 | 8.429 |
| 46 | L | N | 120.025 | 120.258 |
| 47 | E | H | 0 | 0 |
| 47 | E | N | 0 | 0 |
| 48 | E | H | 7.863 | 7.865 |
| 48 | E | N | 116.97 | 116.898 |
| 49 | E | H | 7.941 | 7.961 |
| Row | Column 1 | Column 2 | Column 3 | Column 4 |
|-----|---------|---------|---------|---------|
| 49  | E       | N       | 119.504 | 119.675 |
| 50  | F       | H       | 7.762   | 7.768   |
| 50  | F       | N       | 111.603 | 111.746 |
| 51  | D       | H       | 7.874   | 7.836   |
| 51  | D       | N       | 122.146 | 122.157 |
| 52  | T       | H       | 8.017   | 8.105   |
| 52  | T       | N       | 112.328 | 112.309 |
| 53  | E       | H       | 8.12    | 8.147   |
| 53  | E       | N       | 121.728 | 121.291 |
| 54  | I       | H       | 10.368  | 10.368  |
| 54  | I       | N       | 128.931 | 128.931 |
| 56  | D       | H       | 8.909   | 8.934   |
| 56  | D       | N       | 124.839 | 124.646 |
| 57  | E       | H       | 9.288   | 9.418   |
| 57  | E       | N       | 116.543 | 116.685 |
| 58  | E       | H       | 7.225   | 7.23    |
| 58  | E       | N       | 116.017 | 116.027 |
| 59  | A       | H       | 8.129   | 8.133   |
| 59  | A       | N       | 122.672 | 122.529 |
| 60  | E       | H       | 7.538   | 7.542   |
| 60  | E       | N       | 111.793 | 111.797 |
| 61  | K       | H       | 7.059   | 7.096   |
| 61  | K       | N       | 113.994 | 114.209 |
| 62  | I       | H       | 7.613   | 7.625   |
| 62  | I       | N       | 122.345 | 122.232 |
| 63  | T       | H       | 8.02    | 8.105   |
| 63  | T       | N       | 112.268 | 112.309 |
| 64  | T       | H       | 7.147   | 7.119   |
| 64  | T       | N       | 110.48  | 110.56  |
| 65  | V       | H       | 8.021   | 8.021   |
| 65  | V       | N       | 121.338 | 121.21  |
| 66  | Q       | H       | 8.57    | 8.545   |
| 66  | Q       | N       | 117.941 | 117.783 |
| 67  | A       | H       | 7.772   | 7.806   |
| 67  | A       | N       | 119.54  | 119.543 |
| 68  | A       | H       | 7.881   | 7.876   |
| 68  | A       | N       | 122.115 | 121.86  |
| 69  | I       | H       | 8.066   | 8.065   |
| 69  | I       | N       | 119.026 | 119.225 |
| 70  | D       | H       | 9.142   | 9.157   |
| 70  | D       | N       | 118.998 | 118.975 |
| 71  | Y       | H       | 8.21    | 8.257   |
| 71  | Y       | N       | 122.125 | 122.131 |
| 72  | I       | H       | 8.099   | 8.07    |
| 72  | I       | N       | 120.647 | 120.468 |
| 73  | N       | H       | 8.833   | 8.835   |
| 73  | N       | N       | 118.23  | 118.256 |
| 74  | G       | H       | 7.806   | 7.803   |
| 74  | G       | N       | 104.921 | 105.034 |
| 75  | H       | H       | 7.628   | 7.635   |
| 75  | H       | N       | 118.47  | 118.683 |
Table S2 C8-AcpP•LipB titration chemical shifts. The data have also been submitted to the BMRB for wide access.

| RESIDUE NUMBER | RESIDUE | NUCLEII | ZP CHEMICAL SHIFT | SATURATED CHEMICAL SHIFT |
|----------------|---------|---------|-------------------|--------------------------|
| 3              | I       | H       | 8.561             | 8.687                    |
| 3              | I       | N       | 122.666           | 121.263                  |
| 4              | E       | H       | 8.661             | 8.655                    |
| 4              | E       | N       | 118.452           | 118.452                  |
| 5              | E       | H       | 7.84              | 7.84                     |
| 5              | E       | N       | 117.526           | 116.674                  |
| 6              | R       | H       | 8.437             | 8.34                     |
| 6              | R       | N       | 121.248           | 120.288                  |
| 7              | V       | H       | 8.979             | 8.971                    |
| 7              | V       | N       | 119.3             | 119.269                  |
| 8              | K       | H       | 8.228             | 8.199                    |
| 8              | K       | N       | 116.872           | 117.166                  |
| 9              | K       | H       | 8.337             | 8.247                    |
| 9              | K       | N       | 121.898           | 120.805                  |
| 10             | I       | H       | 7.624             | 7.602                    |
| 10             | I       | N       | 118.974           | 118.937                  |
| 11             | I       | H       | 8.388             | 8.259                    |
| 11             | I       | N       | 119.968           | 118.791                  |
| 12             | G       | H       | 8.369             | 8.408                    |
| 12             | G       | N       | 104.892           | 105.041                  |
| 13             | E       | H       | 8.248             | 8.158                    |
| 13             | E       | N       | 120.95            | 119.853                  |
| 14             | Q       | H       | 8.474             | 8.426                    |
| 14             | Q       | N       | 117.519           | 117.339                  |
| 15             | L       | H       | 8.105             | 7.982                    |
| 15             | L       | N       | 114.809           | 112.847                  |
| 16             | G       | H       | 7.718             | 7.724                    |
| 16             | G       | N       | 109.869           | 109.871                  |
| 17             | V       | H       | 7.863             | 7.853                    |
| 17             | V       | N       | 114.035           | 113.954                  |
| 18             | K       | H       | 8.488             | 8.567                    |
| 18             | K       | N       | 122.864           | 122.57                   |
| 19             | Q       | H       | 8.814             | 8.818                    |
| 19             | Q       | N       | 122.342           | 122.311                  |
| 20             | E       | H       | 9.427             | 9.428                    |
| 20             | E       | N       | 116.615           | 116.566                  |
| 21             | E       | H       | 7.87              | 7.856                    |
| 21             | E       | N       | 116.696           | 116.717                  |
| 22             | V       | H       | 7.531             | 7.532                    |
| 22             | V       | N       | 122.302           | 122.252                  |
|   |   |   |   |
|---|---|---|---|
| 23 | T | H | 7.39 | 7.401 |
| 23 | T | N | 115.44 | 115.501 |
| 24 | N | H | 8.604 | 8.606 |
| 24 | N | N | 118.551 | 118.576 |
| 25 | N | H | 8.104 | 8.119 |
| 25 | N | N | 113.373 | 111.455 |
| 26 | A | H | 7.352 | 7.358 |
| 26 | A | N | 122.885 | 122.775 |
| 27 | S | H | 9.991 | 9.995 |
| 27 | S | N | 117.272 | 117.256 |
| 28 | F | H | 7.6 | 7.599 |
| 28 | F | N | 126.18 | 126.188 |
| 29 | V | H | 8.729 | 8.781 |
| 29 | V | N | 117.009 | 117.149 |
| 30 | E | H | 8.333 | 8.267 |
| 30 | E | N | 116.453 | 116.966 |
| 31 | D | H | 7.84 | 7.849 |
| 31 | D | N | 113.204 | 113.163 |
| 32 | L | H | 7.246 | 7.227 |
| 32 | L | N | 115.311 | 115.249 |
| 33 | G | H | 7.366 | 7.379 |
| 33 | G | N | 106.657 | 106.644 |
| 34 | A | H | 8.44 | 8.504 |
| 34 | A | N | 122.307 | 122.699 |
| 35 | D | H | 9.17 | 9.231 |
| 35 | D | N | 121.557 | 121.465 |
| 36 | S | H | 8.658 | 8.618 |
| 36 | S | N | 112.749 | 111.658 |
| 37 | L | H | 8.056 | 8.116 |
| 37 | L | N | 124.477 | 124.358 |
| 38 | D | H | 8.386 | 8.516 |
| 38 | D | N | 121.258 | 119.726 |
| 39 | T | H | 8.459 | 8.056 |
| 39 | T | N | 109.409 | 111.361 |
| 40 | V | H | 7.21 | 7.173 |
| 40 | V | N | 121.974 | 121.081 |
| 41 | E | H | 7.893 | 7.903 |
| 41 | E | N | 119.01 | 119.005 |
| 42 | L | H | 8.409 | 8.324 |
| 42 | L | N | 121.994 | 121.94 |
| 43 | V | H | 8.021 | 7.936 |
| 43 | V | N | 118.561 | 119.619 |
| 44 | M | H | 7.722 | 7.736 |
| 44 | M | N | 117.373 | 116.584 |
| 45 | A | H | 8.205 | 8.056 |
| 45 | A | N | 123.079 | 120.15 |
| 46 | L | H | 8.49 | 8.328 |
| 46 | L | N | 121.474 | 120.008 |
| 47 | E | H | 8.69 | 8.445 |
| 47 | E | N | 121.309 | 118.796 |
| 48 | E | H | 7.841 | 7.855 |
|   |   |   |   |   |
|---|---|---|---|---|
| 48 | E | N | 116.903 | 117.271 |
| 49 | E | H |  7.975 |  7.942 |
| 49 | E | N | 119.841 | 119.929 |
| 50 | F | H |  7.767 |  7.752 |
| 50 | F | N | 111.45 | 111.332 |
| 51 | D | H |  7.79 |  7.769 |
| 51 | D | N | 122.406 | 122.112 |
| 52 | T | H |  8.064 |  8.188 |
| 52 | T | N | 111.823 | 112.459 |
| 53 | E | H |  8.099 |  8.109 |
| 53 | E | N | 123.627 | 122.559 |
| 54 | I | H | 10.306 |  10.483 |
| 54 | I | N | 129.1 | 129.579 |
| 56 | D | H |  8.927 |  8.974 |
| 56 | D | N | 124.477 | 124.236 |
| 57 | E | H |  9.327 |  9.415 |
| 57 | E | N | 116.569 | 116.455 |
| 58 | E | H |  7.22 |  7.195 |
| 58 | E | N | 115.923 | 115.758 |
| 59 | A | H |  8.119 |  8.082 |
| 59 | A | N | 124.257 | 122.727 |
| 60 | E | H |  7.476 |  7.635 |
| 60 | E | N | 111.355 | 113.627 |
| 61 | K | H |  6.95 |  7.082 |
| 61 | K | N | 113.709 | 114.143 |
| 62 | I | H |  7.592 |  7.583 |
| 62 | I | N | 121.943 | 121.779 |
| 63 | T | H |  8.07 |  8.069 |
| 63 | T | N | 112.103 | 111.389 |
| 64 | T | H |  7.21 |  7.008 |
| 64 | T | N | 110.371 | 110.841 |
| 65 | V | H |  7.822 |  8.015 |
| 65 | V | N | 121.291 | 120.602 |
| 66 | Q | H |  8.707 |  8.352 |
| 66 | Q | N | 117.966 | 116.488 |
| 67 | A | H |  7.892 |  7.848 |
| 67 | A | N | 119.649 | 119.92 |
| 68 | A | H |  7.971 |  7.809 |
| 68 | A | N | 122.795 | 121.082 |
| 69 | I | H |  8.143 |  8.108 |
| 69 | I | N | 119.712 | 119.282 |
| 70 | D | H |  9.191 |  9.197 |
| 70 | D | N | 118.43 | 118.403 |
| 71 | Y | H |  8.196 |  8.111 |
| 71 | Y | N | 122.498 | 120.896 |
| 72 | I | H |  8.26 |  8.151 |
| 72 | I | N | 122.164 | 120.882 |
| 73 | N | H |  8.811 |  8.842 |
| 73 | N | N | 117.741 | 117.968 |
| 74 | G | H |  7.797 |  7.778 |
| 74 | G | N | 104.838 | 104.999 |
Table S3 C10-Acp-P•LipB titration chemical shifts. The data have also been submitted to the BMRB for wide access.

| Residue Number | Residue | Nuclei | ZP chemical shift | Saturated chemical shift |
|----------------|---------|--------|-------------------|--------------------------|
| 3              | I       | H      | 8.638             | 8.65                     |
| 3              | I       | N      | 121.531           | 121.495                  |
| 4              | E       | H      | 8.597             | 8.611                    |
| 4              | E       | N      | 118.707           | 118.837                  |
| 5              | E       | H      | 7.841             | 7.881                    |
| 5              | E       | N      | 117.486           | 117.486                  |
| 6              | R       | H      | 8.344             | 8.346                    |
| 6              | R       | N      | 119.755           | 119.991                  |
| 7              | V       | H      | 8.893             | 8.923                    |
| 7              | V       | N      | 119.026           | 119.141                  |
| 8              | K       | H      | 8.203             | 8.203                    |
| 8              | K       | N      | 117.172           | 117.547                  |
| 9              | K       | H      | 8.268             | 8.249                    |
| 9              | K       | N      | 120.456           | 120.478                  |
| 10             | I       | H      | 7.626             | 7.643                    |
| 10             | I       | N      | 119.423           | 118.813                  |
| 11             | I       | H      | 8.334             | 8.286                    |
| 11             | I       | N      | 118.94            | 119.115                  |
| 12             | G       | H      | 8.464             | 8.466                    |
| 12             | G       | N      | 105.384           | 105.447                  |
| 13             | E       | H      | 8.187             | 8.204                    |
| 13             | E       | N      | 120.25            | 120.197                  |
| 14             | Q       | H      | 8.419             | 8.414                    |
| 14             | Q       | N      | 117.339           | 117.425                  |
| 15             | L       | H      | 8.113             | 8.051                    |
| 15             | L       | N      | 113.507           | 113.315                  |
| 16             | G       | H      | 7.803             | 7.78                     |
| 16             | G       | N      | 109.807           | 109.817                  |
| 17             | V       | H      | 7.87              | 7.845                    |
| 17             | V       | N      | 114.711           | 114.21                   |
| 18             | K       | H      | 8.524             | 8.545                    |
| 18             | K       | N      | 123.155           | 122.979                  |
| 19             | Q       | H      | 8.776             | 8.779                    |
| 19             | Q       | N      | 122.993           | 122.803                  |
| 20             | E       | H      | 9.403             | 9.415                    |
| 20             | E       | N      | 116.551           | 116.653                  |
| 21             | E       | H      | 7.872             | 7.866                    |
| 21             | E       | N      | 116.999           | 116.912                  |
| 22             | V       | H      | 7.52              | 7.534                    |
| 22             | V       | N      | 122.311           | 122.284                  |
|    |    |    |    |    |
|----|----|----|----|----|
| 23 | T  | H  | 7.303 | 7.326 |
| 23 | T  | N  | 115.591 | 115.451 |
| 24 | N  | H  | 8.575 | 8.579 |
| 24 | N  | N  | 118.803 | 118.926 |
| 25 | N  | H  | 8.078 | 8.106 |
| 25 | N  | N  | 112.013 | 111.888 |
| 26 | A  | H  | 7.281 | 7.327 |
| 26 | A  | N  | 122.921 | 122.849 |
| 27 | S  | H  | 9.91 | 9.981 |
| 27 | S  | N  | 116.949 | 117.295 |
| 28 | F  | H  | 7.558 | 7.589 |
| 28 | F  | N  | 124.796 | 125.749 |
| 29 | V  | H  | 8.726 | 8.712 |
| 29 | V  | N  | 116.527 | 116.923 |
| 30 | E  | H  | 8.289 | 8.258 |
| 30 | E  | N  | 116.879 | 117.235 |
| 31 | D  | H  | 7.752 | 7.803 |
| 31 | D  | N  | 114.035 | 113.699 |
| 32 | L  | H  | 7.372 | 7.377 |
| 32 | L  | N  | 115.404 | 115.751 |
| 33 | G  | H  | 7.204 | 7.286 |
| 33 | G  | N  | 106.348 | 106.61 |
| 34 | A  | H  | 8.425 | 8.469 |
| 34 | A  | N  | 122.519 | 122.756 |
| 35 | D  | H  | 9.289 | 9.316 |
| 35 | D  | N  | 123.209 | 122.595 |
| 36 | S  | H  | 8.684 | 8.61 |
| 36 | S  | N  | 113.57 | 111.802 |
| 37 | L  | H  | 8.188 | 8.062 |
| 37 | L  | N  | 123.759 | 123.736 |
| 38 | D  | H  | 8.281 | 8.307 |
| 38 | D  | N  | 119.788 | 119.848 |
| 39 | T  | H  | 8.124 | 8.162 |
| 39 | T  | N  | 111.473 | 110.342 |
| 40 | V  | H  | 7.244 | 7.211 |
| 40 | V  | N  | 121.068 | 121.69 |
| 41 | E  | H  | 7.764 | 7.77 |
| 41 | E  | N  | 119.34 | 119.512 |
| 42 | L  | H  | 8.325 | 8.499 |
| 42 | L  | N  | 121.223 | 121.227 |
| 43 | V  | H  | 7.99 | 7.975 |
| 43 | V  | N  | 118.906 | 119.548 |
| 44 | M  | H  | 7.767 | 7.866 |
| 44 | M  | N  | 116.915 | 116.912 |
| 45 | A  | H  | 8.114 | 8.151 |
| 45 | A  | N  | 121.29 | 121.314 |
| 46 | L  | H  | 8.344 | 8.346 |
| 46 | L  | N  | 119.755 | 119.991 |
| 47 | E  | H  | 8.708 | 8.637 |
| 47 | E  | N  | 119.982 | 120.199 |
| 48 | E  | H  | 7.872 | 7.866 |
|   |   | | | | |
|---|---|---|---|---|---|
| 48 | E | N | 116.999 | 116.912 | |
| 49 | E | H | 7.941 | 7.93 | |
| 49 | E | N | 119.209 | 120.139 | |
| 50 | F | H | 7.76 | 7.762 | |
| 50 | F | N | 111.588 | 111.699 | |
| 51 | D | H | 7.88 | 7.878 | |
| 51 | D | N | 122.305 | 122.047 | |
| 52 | T | H | 8.011 | 8.106 | |
| 52 | T | N | 112.192 | 111.888 | |
| 53 | E | H | 8.116 | 8.115 | |
| 53 | E | N | 122.315 | 122.504 | |
| 54 | I | H | 10.368 | 10.368 | |
| 54 | I | N | 128.931 | 128.931 | |
| 56 | D | H | 8.899 | 8.956 | |
| 56 | D | N | 125.505 | 124.756 | |
| 57 | E | H | 9.229 | 9.415 | |
| 57 | E | N | 116.112 | 116.653 | |
| 58 | E | H | 7.204 | 7.218 | |
| 58 | E | N | 116.007 | 116.014 | |
| 59 | A | H | 8.106 | 8.115 | |
| 59 | A | N | 122.914 | 122.504 | |
| 60 | E | H | 7.548 | 7.553 | |
| 60 | E | N | 112.456 | 112.458 | |
| 61 | K | H | 7.026 | 7.105 | |
| 61 | K | N | 114.239 | 114.424 | |
| 62 | I | H | 7.638 | 7.638 | |
| 62 | I | N | 122.998 | 122.371 | |
| 63 | T | H | 8.011 | 8.106 | |
| 63 | T | N | 112.192 | 111.888 | |
| 64 | T | H | 7.249 | 7.11 | |
| 64 | T | N | 110.084 | 110.54 | |
| 65 | V | H | 7.951 | 7.97 | |
| 65 | V | N | 121.409 | 120.692 | |
| 66 | Q | H | 8.708 | 8.704 | |
| 66 | Q | N | 118.007 | 117.894 | |
| 67 | A | H | 7.764 | 7.825 | |
| 67 | A | N | 119.34 | 119.615 | |
| 68 | A | H | 7.948 | 7.985 | |
| 68 | A | N | 122.947 | 123.653 | |
| 69 | I | H | 8.119 | 8.106 | |
| 69 | I | N | 119.205 | 119.327 | |
| 70 | D | H | 9.096 | 9.15 | |
| 70 | D | N | 119.228 | 118.992 | |
| 71 | Y | H | 8.16 | 8.151 | |
| 71 | Y | N | 121.792 | 121.314 | |
| 72 | I | H | 8.187 | 8.204 | |
| 72 | I | N | 120.25 | 120.197 | |
| 73 | N | H | 8.833 | 8.835 | |
| 73 | N | N | 118.276 | 118.284 | |
| 74 | G | H | 7.83 | 7.804 | |
| 74 | G | N | 104.989 | 105.097 | |
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