Supplemental Information

Observing Selected Domains in Multi-Domain Proteins via Sortase-Mediated Ligation and NMR Spectroscopy

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Native, full length MecA (MW=25764)
MEIERINEHTVKFYMSYGDIEDRGFDREEIWYNRERSEELFWEVMDEVHEEEEFAVEGPLWIQVQALD
KGLEIIVTKAQLSKDGQKLE**LPIPE**DKKQEPASEDLDALLDDFQKEEQAVNQEEKEQKLQFVLRFGDFE
DVISLSKLNVNGSKTTLYSFENRYYLYVDFCNMTDEEVENQLSILLEYATESSISIHRLEEYGKLIISEHA
LETIKKHFAS

Double mutant, full length MecA ("MecAmut", for sortase ligation, MW=25695Da)
MEIERINEHTVKFYMSYGDIEDRGFDREEIWYNRERSEELFWEVMDEVHEEEEFAVEGPLWIQVQALD
KGLEIIVTKAQLSKDGQKLE**LPITG**DKKQEPASEDLDALLDDFQKEEQAVNQEEKEQKLQFVLRFGDF
EDVISLSKLNVNGSKTTLYSFENRYYLYVDFCNMTDEEVENQLSILLEYATESSISIHRLEEYGKLIISEH
ALETIKKHFAS

Triple mutant, full length MecA ("MecAmut2G", MW=25752Da)
MEIERINEHTVKFYMSYGDIEDRGFDREEIWYNRERSEELFWEVMDEVHEEEEFAVEGPLWIQVQALD
KGLEIIVTKAQLSKDGQKLE**LPITGG**DKKQEPASEDLDALLDDFQKEEQAVNQEEKEQKLQFVLRFGD
FEDVISLSKLNVNGSKTTLYSFENRYYLYVDFCNMTDEEVENQLSILLEYATESSISIHRLEEYGKLIISEH
ALETIKKHFAS

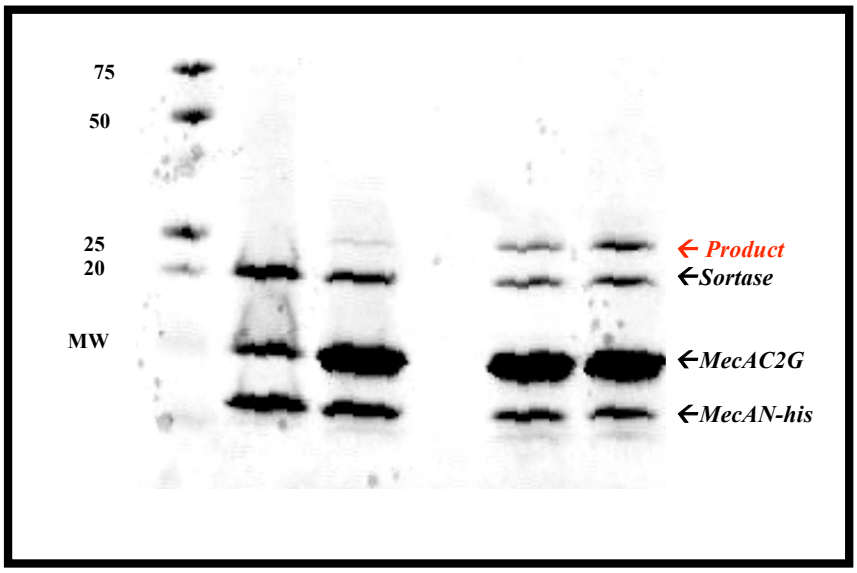
Modified, N-terminal MecA ("MecAN", MW=11106Da)
MEIERINEHTVKFYMSYGDIEDRGFDREEIWYNRERSEELFWEVMDEVHEEEEFAVEGPLWIQVQALD
KGLEIIVTKAQLSKDGQKLE**LPITG**

Modified, N-terminal MecA with C-terminal his ("MecAN-His", MW=11929Da)
MEIERINEHTVKFYMSYGDIEDRGFDREEIWYNRERSEELFWEVMDEVHEEEEFAVEGPLWIQVQALD
KGLEIIVTKAQLSKDGQKLE**LPITG**HHHHHH

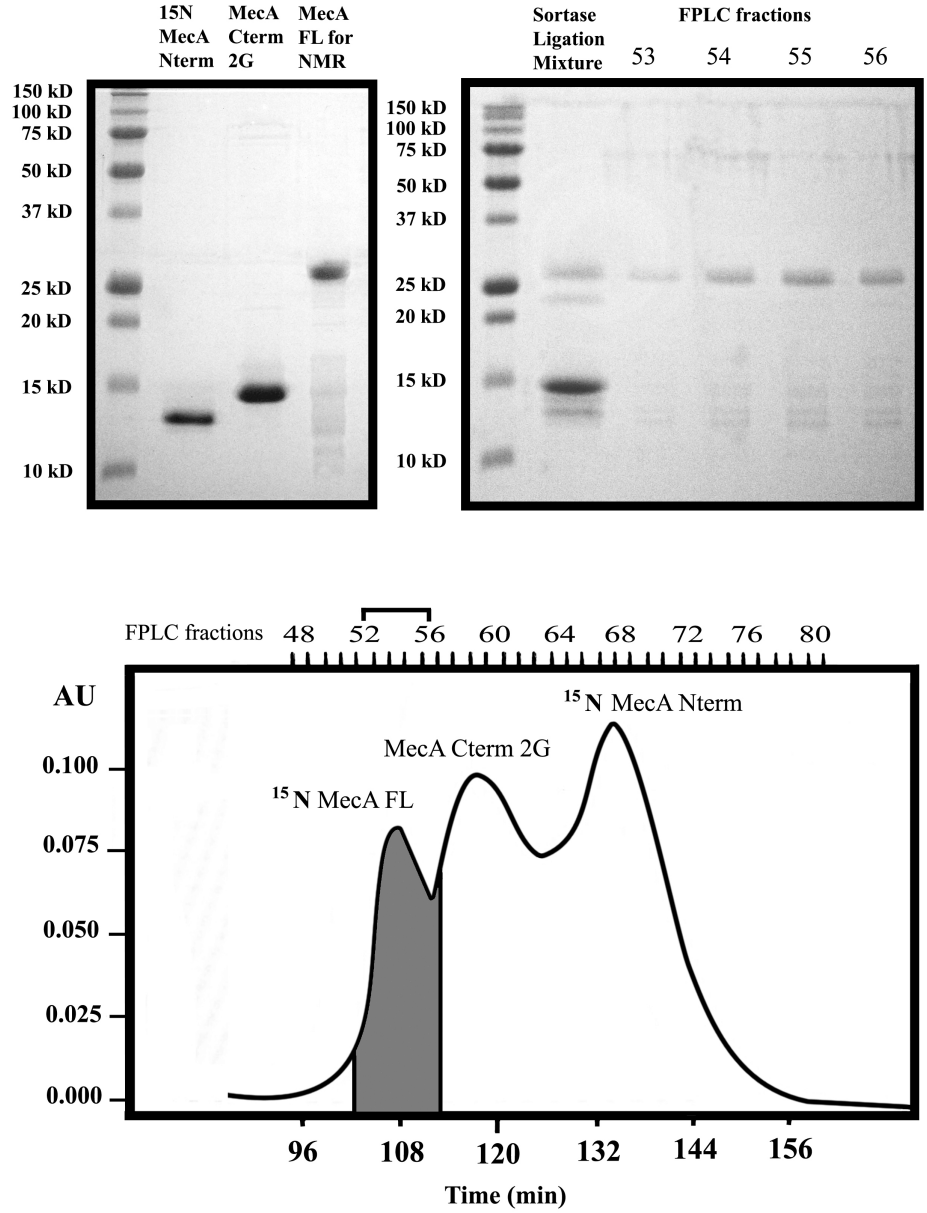
C-terminal MecA ("MecAC", MW=14664Da)
GDKKQEPASEDLDALLDDFQKEEQAVNQEEKEQKLQFVLRFGDFEDVISLSKLNVNGSKTTLYSFENR
YYLYVDFCNMTDEEVENQLSILLEYATESSISIHRLEEYGKLIISEHALETIKKHFAS

C-terminal MecA ("MecAC2G", MW=14739Da)
GGDKKQEPASEDLDALLDDFQKEEQAVNQEEKEQKLQFVLRFGDFEDVISLSKLNVNGSKTTLYSFEN
RYYLYVDFCNMTDEEVENQLSILLEYATESSISIHRLEEYGKLIISEHALETIKKHFAS

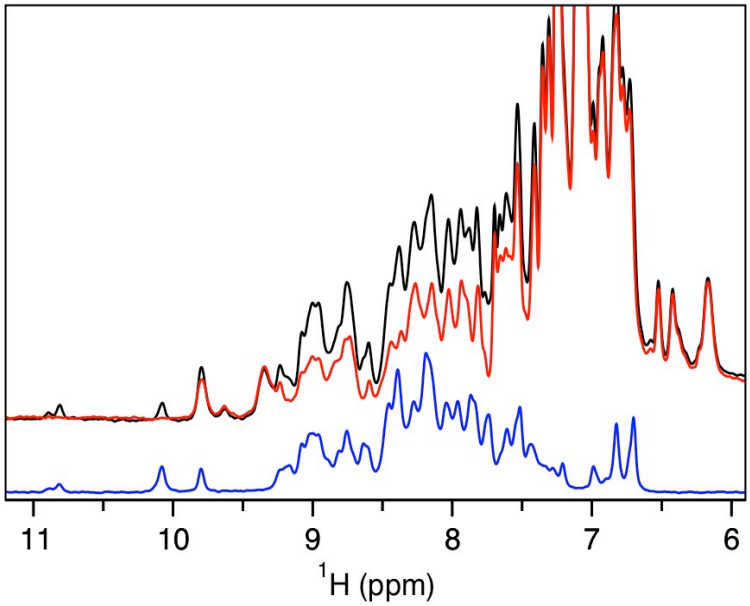
Supplemental Figure 1: The amino acid sequences corresponding to various forms of MecA and its domains that were constructed, employed or made for this sortase ligation study are indicated above. The linker residues (83-93) are indicated in red. Residues that were modified or added for optimal sortase ligation are underlined.



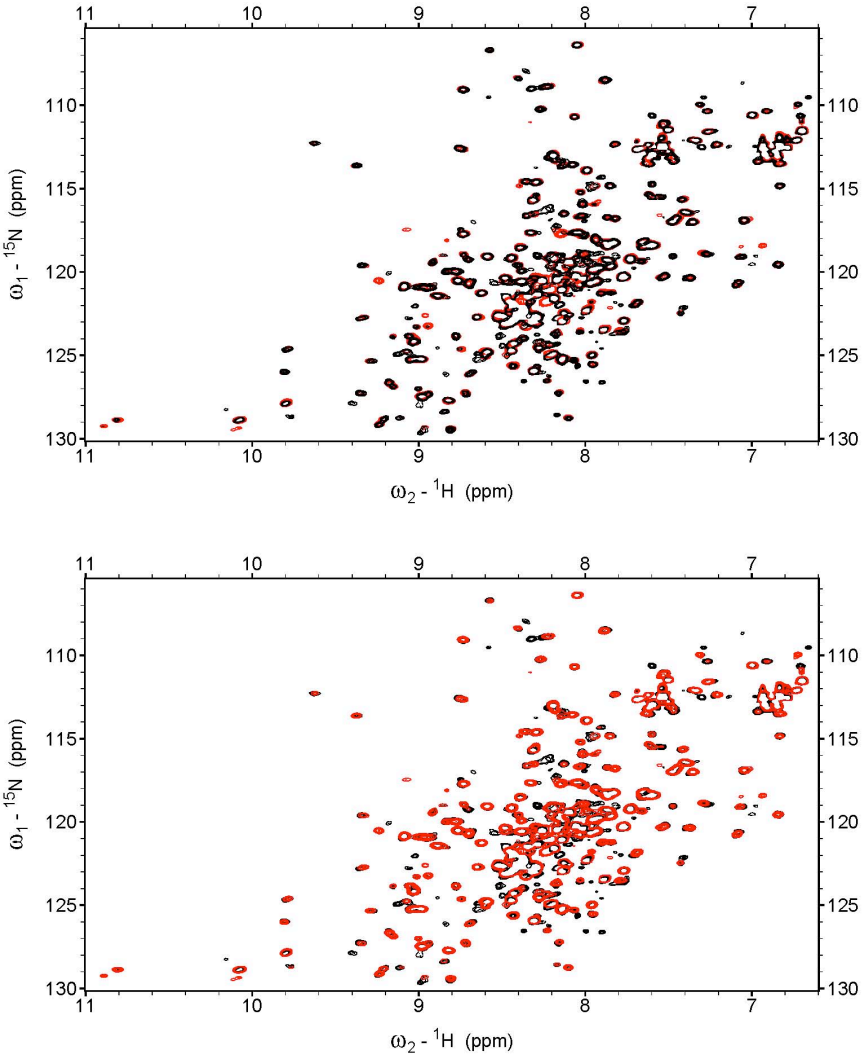
Supplemental Figure 2: SDSPAGE analysis of the large scale, sortase ligation reaction. From left to right of this Coomassie-stained 20% SDSPAGE Phastgel: molecular weight markers, 0hour, 24hour, empty, 48hour and 72hour ligation reaction time points. The product band is indicated by red arrow.



Supplemental Figure 3: Purification of ^{15}N MecAN-MecAC2G protein conjugate (MecAmut2G). Bottom: FPLC chromatogram (tube fractions indicated above) of the segmentally ^{15}N-labeled MecAN conjugate. Top right: A Coomassie-stained 15% SDSPAGE comparing the final NMR product (last lane) against the substrates MecAC2G (third lane), ^{15}N MecAN-His (second lane) and molecular weight markers (first lane). Top left: 15% SDSPAGE comparing the proteins present in FPLC fractions. Markers are in lane 1, the reaction mixture is in lane 2, lanes 3-6 correspond to the indicated FPLC fractions.



Supplemental Figure 4: 600MHz one-dimensional ^{1}H spectra (downfield region) acquired from the ^{15}N-MecAN-MecAC2G segmentally labeled protein conjugate (MecAmut2G). A spectrum including all observable amide proton resonances of the conjugate is shown in black. The ^{15}N-filtered spectrum, in which ^{15}N-coupled proton signals have been suppressed, is shown in red (drawn to the same vertical scale as the unfiltered spectrum). A spectrum showing only ^{15}N-coupled proton resonances is drawn in blue (drawn with an expanded vertical scale relative to the other spectra). Spectra were acquired on a Varian Inova spectrometer at a temperature of 303K and sample concentration of 0.5 mM. For all spectra, ^{15}N-decoupling was employed during acquisition. The lowest field signals near 11 ppm in the ^{15}N-edited spectrum are reduced in intensity due to roll-off of a band-selective 180 degree pulse employed in the pulse sequence.



Supplemental Figure 5: Bottom: Overlay of 2D ^{1}H-^{15}N HSQC of fully ^{15}N-labeled MecAmut (red) on top of ^{15}N-labeled native MecA protein (black). Top: The reverse order of the HSQC overlay is shown. Spectra were recorded at 303K on a Varian Inova 600MHz spectrometer.

Gene Cloning, Protein Expression and Purification

Sortase A plasmid (obtained from H. Mao, Ansata Pharmaceuticals), expressing $SrtA_{\Delta N59}CHis_6$ and conferring carbenicillin resistance, was transformed into E. coli BL21/DE3 cells. A single colony was selected and grown in LB at 37C (50ug/ml carbenicillin) to 0.5=OD_{600nm} prior to induction with 0.2mM IPTG. The harvested cells were resuspended using PBS containing protease inhibitors and DNAse I. The cell suspension was incubated on ice for 30minutes before cell lysis using a French Press. The cell lysate was clarified by centrifugation (16,800 X g, 1 hour) after which large DNA was removed via streptomycin sulfate precipitation. The His-tagged sortase enzyme was purified via elution from a Ni Sepharose 6 Fast Flow (GE Healthcare) column. The sortase was dialyzed into a 20% glycerol, pH 7.5, 50mM Tris, 150mM NaCl, 2mM $CaCl_2$ and 2mM BME buffer.

To generate the MecAmut plasmid, the *mecA* gene in pET28a was mutated from LPIPE to LPITG using the Stratagene Quickchange PCR method. The N-terminal domain of MecA including amino acids 1-93 was cloned into pET28a and the thrombin cleavage site was

replaced with a TEV cleavage site (ENLYFQG). In addition, a C-terminal 6 histidine tag was added to generate pMecA Nter His. The C-terminal domain of MecA, amino acids 94-218, was cloned into pET28a with a TEV tag containing either one or two extra G residue (ENLYFQGG(G)) to make pMecA Cterm G or pMecA Cterm G, respectively.

All MecA proteins were prepared by transformation of BL21 DE3 pRIL cells with the appropriate protein plasmid. A single colony was picked and used to inoculate a starter 5ml LB culture (50 ug/ml kanamycin and 34 ug/ml chloramphenicol) that was grown overnight at 37^{o}C. This was expanded in 1liter of LB and grown to an OD_{600} of ~1.8. Prior to induction, 6g/l of glucose was added as well as 10X NaH_2PO_4, pH 7.4 to a final 50 mM phosphate concentration. The temperature was dropped to 20^{o}C and the cells induced with 0.2 mM IPTG. After ~18 hours, the cells were harvested and resuspended in 30 ml of 50 mM Tris, pH 8.0, 500 mM NaCl, 25 mM imidazole, and 2 mM BME before freeze thawing the cells at -80^{o}C. The cells were then incubated for 10minutes on ice with DNaseI before French pressing them at 20,000 psi followed by centrifugation at 33,000 x g (45 minutes). The supernatant was then applied to Nickel Sepharose 6 Fast Flow resin and this slurry was rotated overnight at 4^{o}C. The slurry was then reapplied to a column and washed with binding buffer. The proteins were eluted with imidazole and the appropriate fractions then dialyzed overnight at 4^{o}C into 25 mM Tris, pH 7.4, 150 NaCl, 1 mM EDTA, and 1 mM BME. The fractions were analyzed by SDS PAGE and quantitated by Bradford reagent (Bio-Rad). Preparation of ^{15}N Labeled MecA Nter His differed in that the LB starter culture was expanded into 1 liter of pre-warmed minimal media (prepared with ^{15}N NH_4Cl containing 50ug/ml kanamycin and 34 ug/ml chloramphenicol) instead of LB. All relevant protein fractions that were eluted off of the Ni resin were then concentrated and FPLC-purified using a Superdex 75 sizing column. The MecAC and MecAN-His proteins were then dialyzed into a 50 mM Tris,150 mM NaCl, 2 mM BME, 2 mM $CaCl_2$ for use in sortase reactions.