Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Mechanism-based Crosslinking as a Gauge for Functional Interaction of Modular Synthases

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SUPPORTING INFORMATION

1. Crosslinking FabF and ACP

Compounds **1** and **2** were synthesized as described previously.[i] To a 50 mM potassium phosphate, pH 7.0, buffer with 50 mM $MgCl_2$ and 24 mM ATP, were added carrier protein (10 µg, ≥ 15-fold excess), CoaA (0.5 µg), CoaD (2.5 µg), CoaE (5 µg), and *B. subtilis* Sfp (0.5 µg). To the mixture, we added pantetheine analog **1** or **2** (0.1 mM), and the mixture was incubated at 37°C for 5 minutes. To the negative controls with pantetheine analog missing was added DMSO (2.5%). To all samples, we added FabF (3 µg), and the reaction was allowed to proceed at 37 °C

for 1 hour. The final volume of reaction was 40 μL. Samples were run on 3-8% Tris-Acetate NuPage with MOPS SDS Running Buffer (Invitrogen). Crosslinked product was detected by staining with Coomassie. Gels were photographed with a BioRad Fluor-S MultiImager, and bands were quantified using the ImageJ software provided by NIH.[ii]

2. ^{14}C-Octanoic Acid Transacylase Assay

0.15 U Acyl CoA Ligase from *Pseudomonas putida* was incubated with 150 uM CoA and 77 μM ^{14}C-Octanoate (specific act. = 54 mCi/mmol) in 50 mM sodium phosphate buffer, pH 7.0, with 50 mM $MgCl_2$, 24 mM ATP and 10 mM β-mercaptoethanol for 2 h at 37 °C. The mixture was then partitioned into different reactions for the different carrier proteins. Sfp was added along with 17-20 μM *apo*-carrier protein (65-80 μM for *apo*-OtcACP to compensate for its lower activity) to each reaction, and the reactions were incubated for 3 h at 37 °C. 1 μM FabF was then added and the reactions were incubated for the specified amount of time at 37 °C before being terminated by mixing with SDS gel loading buffer and boiling for 5 min. Reactions were run on 10% SDS-PAGE and the radioactivity in FabF bands was determined by autoradiography using a Phosphorimager (Molecular Dynamics, courtesy of the Maho Niwa Lab) and a Typhoon 8400 Variable Mode Imager (Amersham Biosciences). Band density was quantified with the ImageQuant program (Molecular Dynamics). All experiments were repeated in order to generate two independent sets of data. The initial rates of transacylation were determined from linear regression plots of the concentration of ^{14}C-labeled FabF (per mole of carrier protein) versus time. The error in transacylation rates was the standard deviation between the two runs. Facilities for using radioactivity were provided by the Gourisankar Ghosh Lab (UCSD).

3. ITC

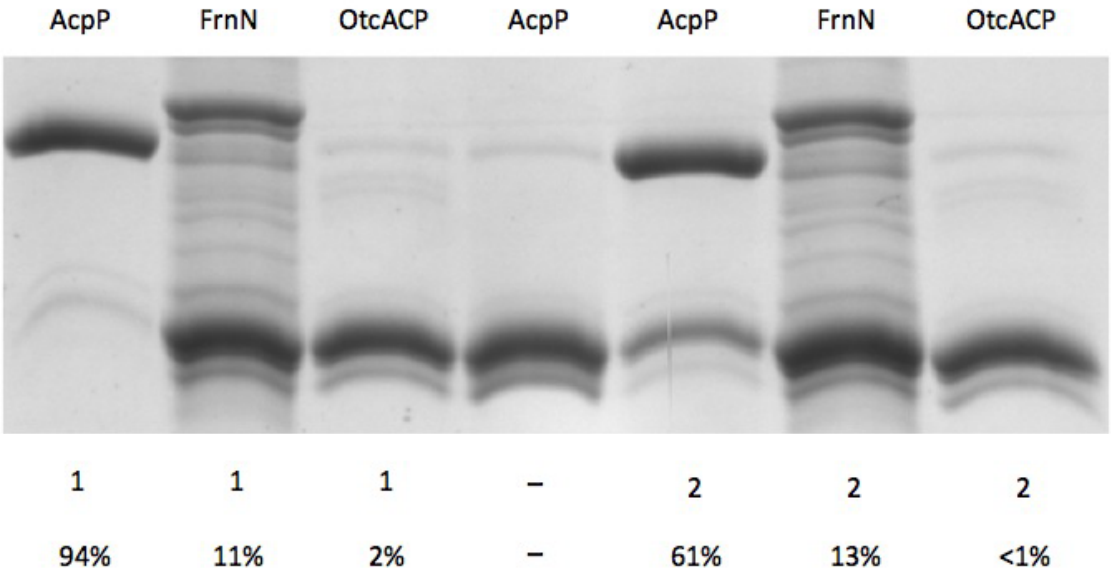
E. coli (BL21) cells were transformed with FabF/pCA24N or co-transformed with His-tagged ACP (AcpP/pET24b, FrnN/pET24b, and OtcACP/pH8 from our lab) and non-tagged Sfp (Sfp/pUC8 and Sfp/pSU20, from the Christopher Walsh lab). Cells were plated on either LB/AMP/CAM (AcpP and FrnN) or LB/AMP/KAN (OtcACP). A single colony of each transformation was grown to an OD 0.6-0.8 in 1 L LB. Cultures were induced with 1 mM IPTG and grown for an additional 5 hours. Cells were concentrated, resuspended in ITC Buffer (20 mM Tris, pH 8.0, 40 mM NaCl, 0.02% Sodium Azide), with 10 mM imidazole added, and lysed with two passages through a French Press. Lysates were cleared by multiple rounds of centrifugation. Batch binding with Ni-NTA resin (5 mL settled bed volume/ACP, 10 mL for FabF) was performed for 1 hour on ice. The presence of *holo*-ACP was verified by the absence of fluorescently labeled protein on SDS-PAGE following the one-pot incubation of ACP with bodipy-maleimide-CoA and Sfp, following the protocol of La Clair et al., 2004.[iii]

VP-ITC was run at 37 ºC using the following conditions:

Reference Power: 5, Initial Delay: 180 s, Stirring speed: 307, Volume (titration): 15 μL, Duration: 20 s, Spacing: 240 s.

FabF (50-60 μM) was incubated in the cell, and ACP (600-900 μM) was titrated. Data were analyzed using Origin 7.0.[iv] Data were fit to the one-site curve to minimize total error in the calculated calorimetric constants.

Supplementary Fig. 1



Supplementary Fig. 1 Mechanism-based crosslinking of *E. coli* KASII (FabF) and *E. coli* AcpP, *S. roseofulvus* FrnN, and *S. coelicolor* OtcPKS using analogs **1** and **2** to produce KASII-ACP; the lane marked with – is a negative control without pantetheine analog. Crosslinking efficiency is indicated on the bottom row.

i A.S. Worthington, G.H. Hur, J.L. Meier, Q. Cheng, B.S. Moore and M.D. Burkart, *Chembiochem.*, 2008, **9**, 2096

ii M. Kitagawa, T. Ara, M. Arifuzzaman, T. Ioka-Nakamichi, E. Inamoto, H. Toyonaga and H. Mori, *DNA Res.*, 2005, **12**, 291

iii J.J. La Clair, T.L. Foley, T.R. Schegg, C.M. Regan and M.D. Burkart, *Chem. Biol.*, 2004, **11**, 195

iv S. Bergqvist, M.A. Williams, R. O'Brien and J.E. Ladbury, *J. Mol. Biol.*, 2004, **336**, 829