Integrated structural biology of enacyloxin polyketide synthase

S. Kosol¹, A. Gallo¹, D. Griffiths¹, T.R. Valentí², J.L. Masschelein², M. Jenner¹, E.de los Santos³, L. Manzi⁴, D. Rea², V. Fülöp,³, N.J. Oldham⁴, S.-C. Tsai², G.L. Challis¹ and J.R. Lewandowski¹

¹Department of Chemistry, University of Warwick, Coventry, UK, ²Departments of Molecular Biology and Biochemistry, Chemistry, and Pharmaceutical Sciences, University of California Irvine, Irvine, USA, ³Department of Life Sciences, University of Warwick, Coventry, UK, ⁴School of Chemistry, University of Nottingham, Nottingham, UK, j.r.lewandowski@warwick.ac.uk

Many structurally complex antibiotics are synthesized inside bacterial cells by large modular multienzymatic assembly lines such as polyketide synthases (PKS) or non-ribosomal peptide synthases (NRPS). Because the components of these assembly lines that are responsible for a single synthesis step are often not encoded in one polypeptide, the quality of the product relies on the flawless interplay between different modules and enzymes [1,2]. The interactions between different proteins require a high degree of specificity and molecular recognition must be fast and efficient [3]. One example of a system relying on such principles for biosynthetic control is a hybrid PKS/NRPS that produces an antibiotic enacyloxin IIa shown to be active against a common hospital-acquired multidrug resistant pathogen A. baumannii. Here we use an integrated structural biology approach combining solution and solid state NMR, carbene foot printing and X-ray crystallography and molecular dynamics to elucidate the atomic details of interactions between a peptidyl carrier protein (PCP; 11 kDa) and a stand-alone condensation domain (C; 57 kDa) in a pivotal chain termination step of enacyloxin biosynthesis [4]. We show that intrinsically disordered docking domain located on the C-terminus of PCP is essential for initiation of the interaction and consequently successful substrate transfer to the condensation domain. Solid-state NMR helps us to tease out the atomic resolution details of PCP in the richly dynamic complex with C domain. Our findings suggest an intriguing dynamically regulated allosteric mechanism for condensation domain activity and provide a basis for rational engineering approaches of biosynthetic pathways to yield novel antibiotics.

References