A CHIMERA MODEL OF Rel_{Seq} PRE-CATALYTIC STATE

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Bacteria use nucleotides as second messengers to regulate diverse cellular processes in response to environmental stimuli. For instance, intracellular accumulation of guanosine tetra- or pentaphosphate ((p)ppGpp) leads to a temporary antibiotic-tolerant phenotype called persister, playing a starring role in chronic and recurrent infections [1]. (p)ppGpp is synthesized and/or hydrolyzed by proteins belonging to the RelA/SpoT-Homologue (RSH) superfamily. The multi-domain 'long' RSH enzymes, such as Rel_{Seq} [2] (*S. equisimilis*), have both a synthetase (SYNTH) and a hydrolase (HD) domain. Along with long RSHs, shorter and specialized RSHs that carry either the SYNTH or the HD domain have been identified. Recently, the X-ray crystal structure of the pre-catalytic state of the *S. aureus* single synthetase RelP was reported [3]. The structural homology between the SYNTH domains of these two proteins led us to consider the pre-catalytic binding site of RelP as a reliable template for building the pre-catalytic state of Rel_{Seq}.

Here we report a composite homology model of the bifunctional enzyme generated using both the X-ray structure of Rel_{Seq} (to model the HD domain) and RelP (to model the SYNTH pre-catalytic state) as templates. Molecular dynamics simulations were then performed to assess the stability of the model.

[1] K. Gerdes and E. Maisonneuve, *Cell*, **2014**, *2*, 539-548

[2] T. Hogg *et al.* ,*Cell*, **2014**, *117*, 57–68

[3] M. C. Manav et *al.*, *J. Biol. Chem.*, **2018**, *293(9)*, 3254-3264