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The bacterial transcriptional regulator RutR is controlled by lysine acetylation.

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The bacterial transcriptional regulator RutR was shown to be lysine acetylated at five distinct sites in the DBD and LBD. However, how lysine acetylation affects RutR function is not known. Applying genetic code expansion using a synthetically evolved acetyl-lysyl-tRNA-synthetase (AcKRS3)/tRNACUA (*MbPylT*)-pair from *Methanosarcina barkeri*, we produced site-specifically lysine-acetylated RutR proteins in yield and purity suitable for biophysical studies including X-ray crystallography.

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