Two riboswitches with identical binding pockets and disparate RNA folds show different tolerances to equivalent mutations.

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Riboswitches are naturally occurring structured RNAs that directly "sense" the cellular levels of specific metabolites to regulate downstream genes. Although some riboswitches are being targeted for antibacterial development, others are found in bacteria that are critical for human health. For example, the Ruminococaceae family of commensal gut bacteria produce the nucleobase queuine, which is crucial for genetic decoding by tRNAs containing GUN anticodons (i.e. those that specify Asp, Asn, Tyr and His). Faecalibacterium prausnitzii (Fpr) is a member of this family that appears protective against Crohn's disease. The Fpr preQ1-III (class III) riboswitch senses the queuine precursor, preQ1, to control translation of a downstream transporter required for preQ1 salvaging. We showed previously that the class III effector binding pocket shares ten identical nucleotides with phylogenetically distinct preQ1-II (class II) riboswitches [Liberman et al. (2015) PNAS 112, E3485], even though each riboswitch class adopts a distinctive overall fold. Moreover, class II riboswitches are found in many human pathogens. Given the goal of targeting RNA motifs with small molecule therapeutics, it is important to understand how commensal class III and pathogenic class II riboswitches respond to mutations in or around their binding pockets that could lead to drug resistance or loss of health benefits for the host. Accordingly, we prepared A84G, A52G, Δ84, U8C/A8G mutations in the class III binding pocket based on our previous analysis of equivalent class II mutations, which were detrimental to function [Dutta & Wedekind (2020) JBC 295, 2555]. Interestingly, ligand binding analysis by isothermal titration calorimetry revealed that each mutation is tolerated better by class III riboswitches. X-ray crystallographic analysis of each class III mutant accounts for its ability to maintain preQ1 binding and is corroborated by chemical probing of riboswitch flexibility in solution. A take-home message is that the context of the binding pocket within the overall fold impacts the ability to tolerate mutations.