aminoglycosides will result in unfavorable steric and/or electrostatic interactions, causing a reduction in the ability of the antibiotic to bind to the RNA.

The phosphorylation of AGA hydroxyl groups upon the action of APHs introduces a negative charge into the molecule, which results in a dramatic change in its ability to bind to the A-site in the ribosome, due to electrostatic repulsion between the modified AGA and the RNA backbone. Examples are APH(2″) kinase enzymes, which phosphorylate the 2-hydroxyl in the C-ring in gentamycin-related AGAs (Figure 1.3a). Upon binding to the antibiotic, the kinase and its GTP cofactor (the phosphate donor) transition from a stable, inactive form to an activated form that triggers modification of the 2″-hydroxyl in 4,6-disubstituted AGAs. This transition, which Caldwell et al. named *catalytic triphosphate switch* (2016), is driven by conformational changes of the enzyme that bring into contact distinct regions of the protein, namely, the distal helical subdomain (of the C-terminal lobe) and the so-called Gly and B loops (of the N-terminal lobe), closing the APH(2″) kinase's active site (Figure 1.3a). In this site, the acceptor 2″-hydroxyl of the AGA stays directly in line to react with the GTP phosphate. The 4,6-disubstituted AGA is held in place, through its neamine-like moiety, by ionic, hydrogen, and ring stacking interactions with the protein residues. The second type of AMEs, acetyl-CoA-dependent AACs, promotes acetylation of amino groups in aminoglycosides (Figure 1.3b), affecting binding to rRNA. As observed by analysis of the crystal structures of AAC(6′)-Ig, AAC(6′)-Ih, and AAC(6′)-Iy (Stogios et al. 2017), the binding site of AAC(6′)-Ig can accommodate either 4,6-substituted (e.g. tobramycin; Figure 1.3b), 4,5-substituted (e.g. ribostamycin), or 4,1-substituted (e.g. amikacin) AGAs, which are held in place by stacking interactions with the central 2-deoxystreptamine ring (ring II). The protein residues that promote the stacking, a tryptophan and a tyrosine in alternate chains (Figure 1.3b), are also involved in the enzyme's dimerization. Further ionic and hydrogen contacts are done with rings I and III and the 6′-amine (to be acetylated). Although not visible in the crystal structures, the authors could assign a pocket to be occupied by acetyl-CoA (Figure 1.3b). Addition of the acetyl group to the 6′-amine position impairs hydrogen binding to the N1 of adenine 1408 (h44) in the 30S subunit of rRNA. The third type of AMEs, the ANTs, catalyzes the transfer of an AMP group from the co-substrate ATP to a hydroxyl group in the AGA (Figure 1.3c). The presence of a bulky group, such as AMP, prevents accommodation of the modified AGA in the 16S rRNA A-site, affected by steric clashes with guanine 1405. According to the work by Cox and colleagues (2015), ANT(2″)-Ia can accommodate and modify 4,6-disubstituted 2-deoxystreptamine-based AGAs, such as gentamycin, tobramycin, and kanamycin (Figure 1.3c), without major conformational changes of the polypeptide chain upon AGA binding. The antibiotic occupies a large cleft (in the frontier between the N- and C-terminal domains) with its central ring (ring II) facing the interior of the protein and rings I and III facing the solvent. Close to the