Use of a fluorescence assay to determine relative affinities of semisynthetic aminoglycosides to small RNAs representing bacterial and mitochondrial A sites

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Supplementary material

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Figure S1. Aminoglycosides binding to the fluorescein-tagged bacterial A-site duplex RNA (wt-bac) construct. Binding curves of fraction bound (Fr) as a function of neomycin (A), paromomycin (B), 4'-*O*-ethylparomomycin (C), and propylamycin (D) concentration fitted to Equation 3 are shown ($R^2 = 0.97, 0.99, 0.97, and 0.95$, respectively).



Figure S2. Aminoglycosides binding to the fluorescein-tagged mitochondrial A-site duplex RNA (wt-mt) construct. Binding curves of fraction bound (Fr) as a function of neomycin (A), paromomycin (B), 4'-*O*-ethylparomomycin (C), and propylamycin (D) concentration fitted to Equation 3 are shown ($R^2 = 0.93$, 0.98, 0.94, and 0.94, respectively).



Figure S3. Aminoglycosides binding to fluorescein-tagged mutant mitochondrial A-site RNA (mut-mt) construct. Binding curves of fraction bound (Fr) as a function of neomycin (A), paromomycin (B), 4'-*O*-ethylparomomycin (C), and propylamycin (D) concentration fitted to Equation 3 are shown ($R^2 = 0.99, 0.97, 0.91$, and 0.92, respectively).



Figure S4. Aminoglycosides binding to fluorescein-tagged bacterial A-site duplex RNA (wt-bac) construct. Change in volume corrected fluorescence intensity of fluorescein-tagged RNA upon addition of aminoglycosides are shown.



Figure S5. Aminoglycosides binding to fluorescein-tagged mitochondrial A-site duplex RNA (**wt-mt**) **construct.** Change in volume corrected fluorescence intensity of fluorescein-tagged RNA upon addition of aminoglycosides are shown.



Figure S5. Aminoglycosides binding to fluorescein-tagged mutant mitochondrial A-site duplex RNA (mut-mt) construct. Change in volume corrected fluorescence intensity of fluorescein-tagged RNA upon addition of aminoglycosides are shown.