BL-5A/2012G519

# Crystal structure of the bacterial A site in complex with *β*-fluoro neomycin analog

Hiroki Kanazawa[1], Juan Pablo Maianti[2], Stephen Hanessian[2] and Jiro Kondo[1,*]
[1]Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan, [2]Department of Chemistry, Université de Montréal, CP 6128 Succ. Centre-Ville, Montréal Québec, H3C3J7, Canada

## 1 Introduction

Aminoglycosides (AGs) comprise a diverse group of potent broad-spectrum natural and semi-synthetic bacterial antibiotics used in clinical practice worldwide. Kidney toxicity has known as side effect of AGs and correlates with the number of protonated and non-protonated amino groups on the AG sugar scaffolds, yet these features are also a requirement for potent antibacterial action. Before the present study, we have designed and synthesized some fluorinated novel AGs. Compound JPM5177 (Fig. 1), one of these AGs, has smaller kidney toxicity through decreasing the p*K*a of amino groups caused by fluorination. And surprisingly, the bactericidal effects of JPM5177 against several bacteria are remarkably increased. In the present study, we have performed X-ray analysis of the bacterial rRNA molecular switch in complex with the JPM5177 to reveal a role of the fluorine in the AG binding [1].

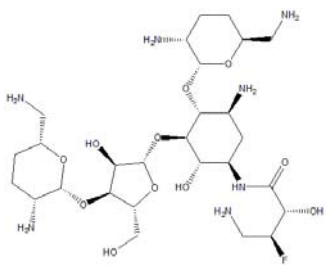

Fig. 1: Chemical structure of JPM5177

## 2 Experiment

The RNA oligomer designed to fold as a double helix containing two asymmetrical internal loops of the bacterial A site was chemically synthesized. Crystallizations of the RNA/JPM5177 complex were performed by the hanging-drop vapor diffusion method. X-ray data were collected at 100K with synchrotron radiation at the structural biology beamline BL-5A in the Photon Factory. Initial phase of the complex crystal was derived with the molecular replacement program *AutoMR* from the *Phenix* suite. The atomic parameters of the crystal structure was refined using the program *CNS*.

## 3 Results and Discussion

The bacterial A-site molecular switch forms an A-form duplex and takes the “on” state with two bulged-out adenines (Fig. 2a). JPM5177 binds to the A-site switch. Ring A of JPM5177 stacks over G1491 and forms a pseudo-pair with A1408. Ring B fixes the molecular switch in the “on” state through three hydrogen bonds. These interactions are common among AG series and are required when they bind to the A-site molecular switch. The hydroxy-aminobutyric acyl group (HABA) observed in the RNA/JPM5177 and RNA/amikacin complexes [2] are shown in Fig. 3. The unmodified HABA of amikacin makes three hydrogen bonds with RNA. On the other hand, the HABA with a fluorine atom of JPM5177 makes two additional hydrogen bonds. Although the original purpose is controlling the p*K*a of amino groups by the fluorination, we found that the fluorine itself contributes to the direct interactions with the RNA molecule.

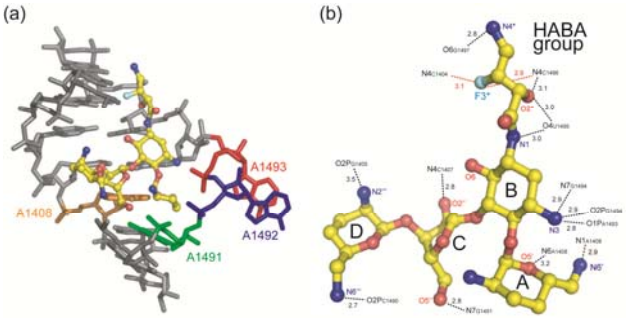


Fig. 2: Binding (a) and detailed interactions (b) of JPM5177 with the bacterial A site.

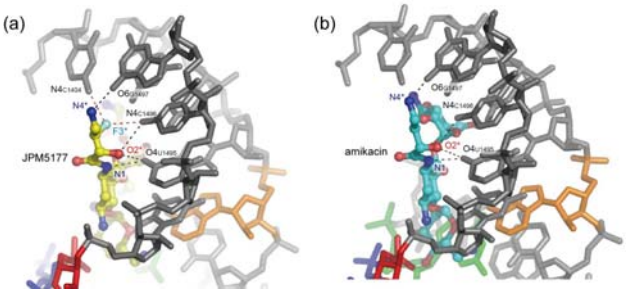


Fig. 3: Interactions between the HABA groups of (a) JPM5177 and (b) amikacin [2] and the bacterial A-site molecular switch.

## References

[1] J. P. Maianti *et al.*, *ACS Chem. Biol.* **9**, 2067-2073 (2014)
[2] J. Kondo *et al., Biochimie.* **88**, 1027-1031 (2006)

* j.kondo@sophia.ac.jp