**Emily Ryan**
Biology
Year in School: Sophomore
Hometown: St. Louis, Mo.
Faculty Mentor: Dr. Stefan Sarafianos, Molecular Microbiology & Immunology
Funding Source: NIH grant to S. Sarafianos

**Novel inhibition mechanism and potent antiviral activity of translocation-deficient reverse transcriptase inhibitors**
Emily Ryan, Lefteris Michailidis, Bruno Marchand, Ei-Ichi Kodama, Amelie Olga Mafotsing Fopoussi, Ali M. Sawani, & Stefan Sarafianos

Nucleoside RT inhibitors (NRTIs) are among the most potent anti-HIV agents and act as chain terminators because they lack a 3’OH. However, this feature can reduce affinity for RT compared to the analogous dNTP substrate, as well as reduced intracellular conversion to the active dNTP. To overcome this, it was shown that certain nucleosides that retain the 3’OH and have substitutions at the 4’ ribose and 2 position of the base have exceptional antiviral properties. One of these compounds, 4’-ethynyl, 2-fluoro deoxy-adenosine (4’E-2FdA) is the most potent NRTI inhibitor against wild-type and multi-drug resistant HIV viruses described to date. We have recently reported that 4’E-2FdA acts as a chain terminator despite the presence of an accessible 3’OH. We show that after 4’E-2FdA-MP incorporation, RT does not bind the next incoming dNTP. We analyzed RT translocation on different sequences terminated with 4’E-2FdA-MP, and found that even at sequences when RT is naturally found post-translocated, the inhibitor prevents translocation. This decrease in translocation efficiency explains the reduced binding of the next incoming dNTP and the termination of elongation. While the inhibitor stabilizes the pre-translocated 4’E-2FdA-MP-terminated primer, the pyrophosphate-dependent excision rate of 4’E-2FdA-MP was not very high compared to ddAMP. In conclusion, this highly potent chain termination activity arises from difficulty of the primer 3’-terminus to translocate following incorporation of the compound, and not from simple steric hindrance due to the 4’ substitution. Therefore, we propose that 4’E-2FdA is a Translocation-Deficient Reverse Transcriptase Inhibitor (TDRTI) that acts by a novel mechanism.