

*Article*

# Rational design of a thermostable 2'-deoxyribosyltransferase for nelarabine production by prediction of disulfide bond engineering sites

Guillermo Cruz <sup>1</sup>, Javier Acosta <sup>1</sup>, Jose Miguel Mancheno <sup>2</sup>, Jon Del Arco <sup>1</sup>, and Jesús Fernández-Lucas <sup>1,\*</sup>

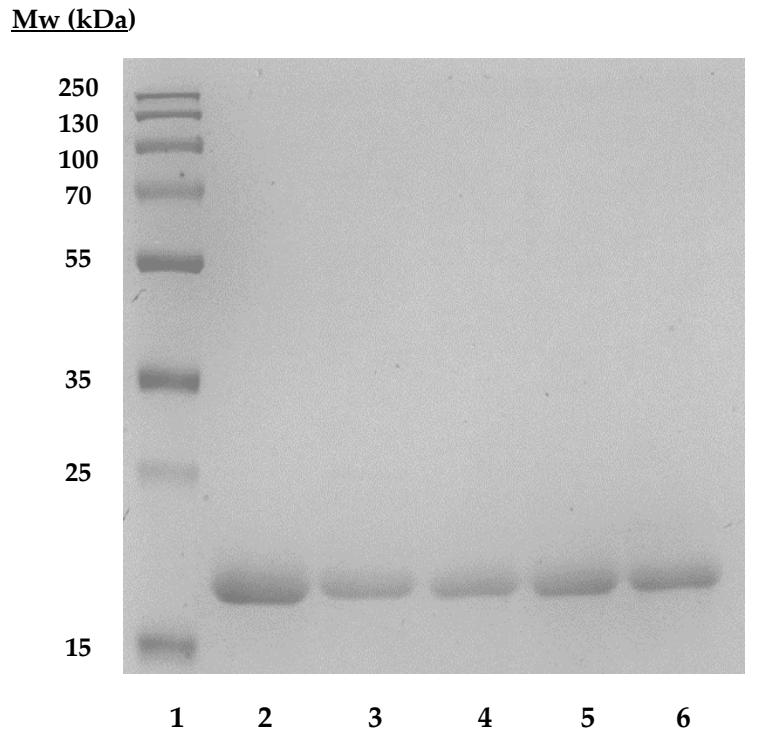
<sup>1</sup> Applied Biotechnology Group, Universidad Europea de Madrid, Urbanización El Bosque, Calle Tajo, s/n, 28670 Villaviciosa de Odón (Madrid), Spain. guille.sle@icloud.com (G. C), jacosta19.ja97@gmail.com (J. A.), jon.delarco@universidadeuropea.es (J. D.)

<sup>2</sup> Department of Crystallography and Structural Biology, Institute Rocasolano (CSIC), Serrano 119, 28006 Madrid, Spain, jm.mancheno@csic.es (J. M.)

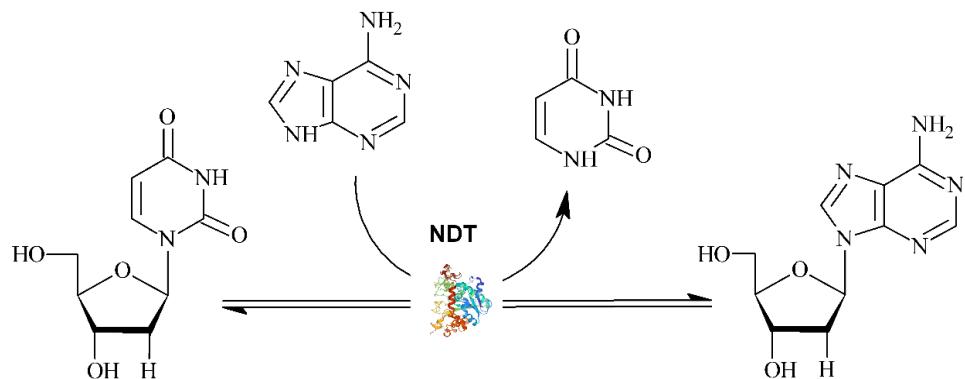
\*Correspondence: jesus.fernandez2@universidadeuropea.es (J. F.)

## Electronic Supplementary Information

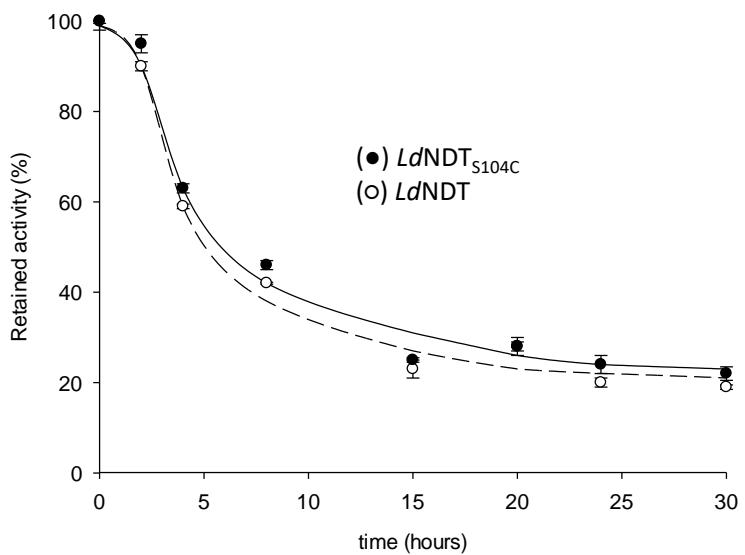
## Figures



**Figure S1.** SDS-PAGE analysis of pure *LdNDTwt* and mutant enzyme samples. **Lane 1.** Prestained standard proteins (Thermoscientific used as molecular weight markers. **Lane 2.** Pure fraction of *LdNDTwt*. **Lane 3.** Pure fraction of *LdNDTv64C*. **Lane 4.** Pure fraction of *LdNDTv93C*. **Lane 5.** Pure fraction of *LdNDTS104C*. **Lane 6.** Pure fraction of *LdNDTv93C/S104C*. *LdNDT*: NDT from *Lactobacillus delbrueckii*.



**Figure S2.** Enzymatic synthesis of 2'-deoxyadenosine (dAdo) from 2'-deoxyuridine (dUrd) and adenine catalyzed by 2'-deoxyribosyltransferases (NDTs).



**Figure S3.** Thermal inactivation profile of  $LdNDT$  and  $LdNDT_{S104C}$  at 55 °C in 10 mM sodium phosphate, pH 7.0, in the presence of 1 mM DTT. All determinations were carried out in triplicate and the standard error of the mean is calculated using the standard deviation.