

**Monitoring of successive phosphorylations of thymidine using free and immobilized human nucleoside/nucleotide kinases by Flow Injection**

**Analysis with High-Resolution Mass Spectrometry**

Justine Ferey<sup>1</sup>, David Da Silva\*<sup>1</sup>, Cyril Colas<sup>1,2</sup>, Reine Nehmé\*<sup>1</sup>, Pierre Lafite<sup>1</sup>, Vincent Roy<sup>1</sup>, Philippe Morin<sup>1</sup>, Richard Daniellou<sup>1</sup>, Luigi Agrofoglio<sup>1</sup>, Benoît Maunit<sup>1</sup>.

<sup>1</sup> *Univ. Orléans, CNRS, ICOA, UMR 7311, F-45067 Orléans, France.* <sup>2</sup> *CNRS, CBM, UPR 4301, Univ-Orléans, F-45071, Orléans, France*

\* **Corresponding authors:** project and mass spectrometry: Dr David DA SILVA,  
capillary electrophoresis: Dr Reine NEHME

**Address:** Institut de Chimie Organique et Analytique (ICOA) UMR 7311, Université d'Orléans  
CNRS. Rue de Chartres, 45067 Orléans cedex 02 – France

**E-mail address:** david.da-silva@univ-orleans.fr, reine.nehme@univ-orleans.fr

**Tel:** +33 238492411. **Fax:** +33 238417281

## 1 In-source fragmentation

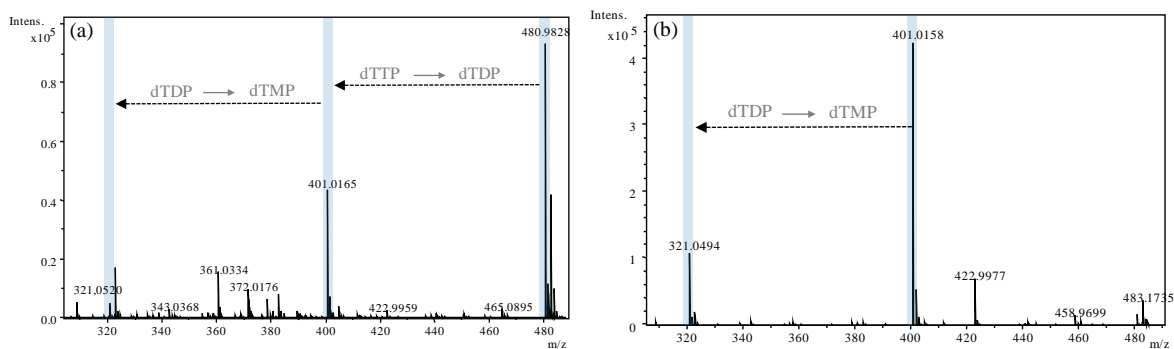


Figure S1: Mass spectra of (a) dTTP and (b) dTDP (capillary voltage – 4 kV). In-source fragmentation of phosphate.

## 2 Mass spectra of dTMP conversion into dTDP

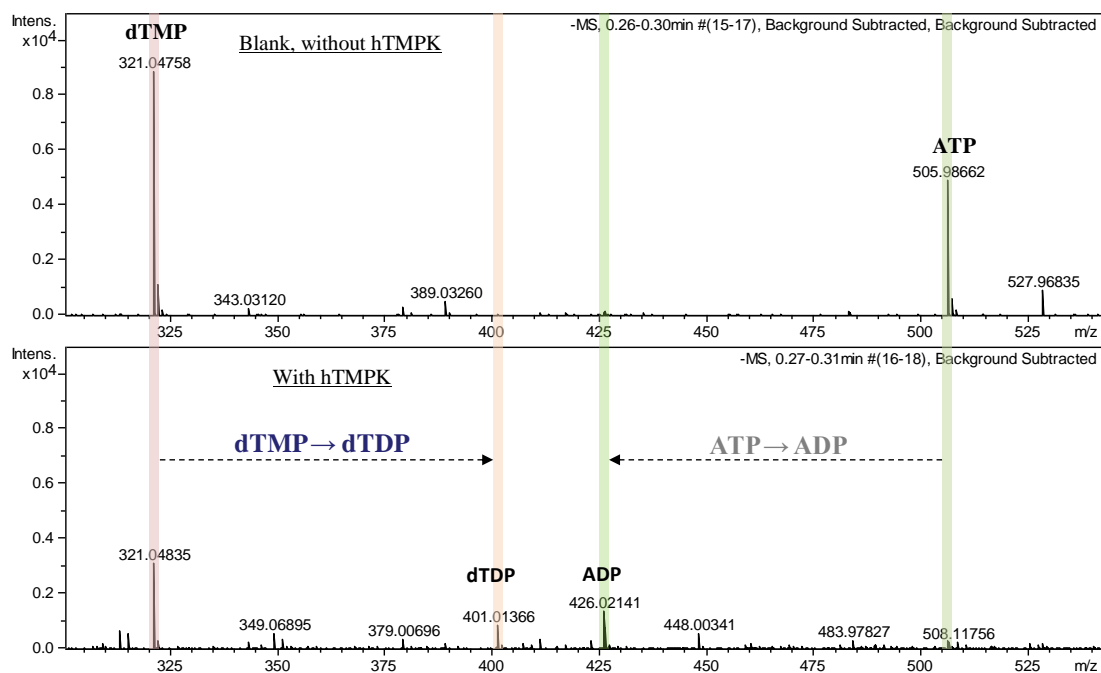


Figure S2: Mass spectra of dTMP conversion into dTDP. Analyses conducted by FIA-HRMS.

## 3 Calibration curves (FIA-HRMS)

Four solutions of dTMP and dTDP (from 5 to 40  $\mu\text{M}$ ) and five solutions of dTTP (from 5 to 50  $\mu\text{M}$ ) were prepared in ammonium acetate buffer (50 mM ionic strength, pH = 7) containing 200  $\mu\text{M}$   $\text{MgCl}_2$ .

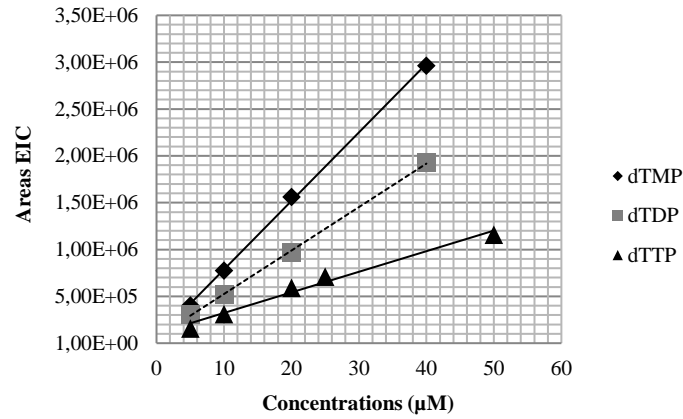


Figure S3: Calibration curves of dTMP (m/z 321.0482, diamonds,  $y = 73164x + 55415$ ,  $R^2 = 0.999$ ), dTDP (m/z 401.0145, squares,  $y = 46403x + 60855$ ,  $R^2 = 0.999$ ) and dTTP (m/z 480.9808, triangles,  $y = 21986x + 102720$ ,  $R^2 = 0.982$ ).

#### 4 Monitoring of product accumulation

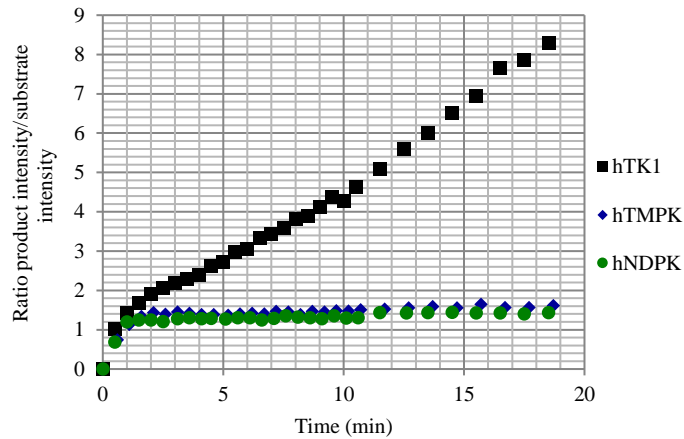


Figure S4: Monitoring of substrate phosphorylation (dT, dTMP and dTTP, 25 µM). Reaction catalyzed by free hTK1 (0.48 g/L), free hTMPK (0.04 g/L) and free hNDPK (0.04 g/L). Analyses conducted by FIA-HRMS.

Figure S4 shows product accumulation (dT, dTMP and dTTP) in function of time. hTMPK and hNDPK catalyzed reactions show fast conversion within 2 min whereas hTK1 catalyzed reaction shows linear trend. Reaction time was set at 30 min to reach plateau conversion for hTMPK and hNDPK.