Electrochemical screening of chemical library of transketolases inhibitors

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High throughput screening (HTS) methods are developed in order to experiment a large set of conditions in a small time. The development of miniaturization systems allow to multiplex assays while limiting the consumption of reagents. By reducing time assays and costs it can be possible to extend experimentations. Among all **HTS methods**, some of them use **electrochemical signals** to follow **chemical** or **biochemical reactions**. One of them is the **Intermittent Pulse Amperometry (IPA)** which consists to apply a potential on an electrode during a fixed time transiently. Recording the current variation on **96 independent multiplexed electrodes** allows to follow large number of reactions in a single experiment[1].

Transketolases (TK) are ubiquitous enzymes of the pentoses pyrophosphate pathway. These hub proteins are implicated indirectly in the synthesis of nucleic acids, aromatics amino acids and NADPH. **Face of the emergence of bacterial strains resistant against a broad range of antibiotics, the screening of chemical libraries by IPA will allow to discover new inhibitors of TK and then new drugs**.

New IPA device and spectrometric assays of transketolases

With the **new IPA device** (Fig. 1) it's possible to perform rapidly numerous assays with **high sensitivity (nA range electronically)** thanks to the architecture of **96 multiplexed electrodes**. The access to all raw data allows to perform data treatment.

IPA allows to detect rapidly current intensity variations at numerous potential on several electrodes. It's possible to obtain a voltammetry like profile and to determine the oxidation or reduction potential for a given species.

It's also possible to detect **enzymatic activities** and to **screen chemical libraries** (Fig. 4) [2,3].

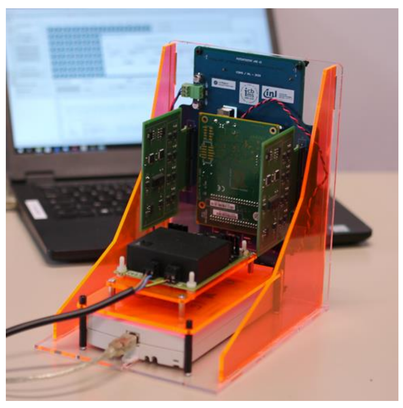


Fig. 1. New IPA device developed by INL. IPA is performed on 96 multiplexed electrodes screen printed on a PCB. Working electrodes are made of carbon and counter/ref are Ag/AgCl electrodes.

TK from *Escherichia coli* (*ec*TK), *Enterococcus faecium (ef*TK), and *Vibrio vulnificus* (*vv*TK) are produced and purified. This last fifty decades, these organisms had developed **numerous resistances against a large set of antibiotics** used in human and animal medicine. According to the World Health Organization, they are priority pathogens and can lead to **many medical and social-economic issues** in a near future .

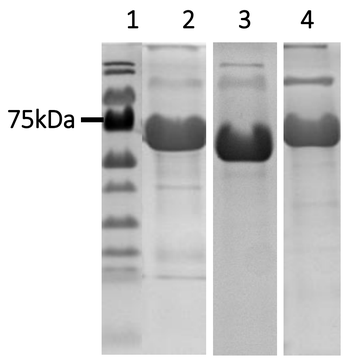
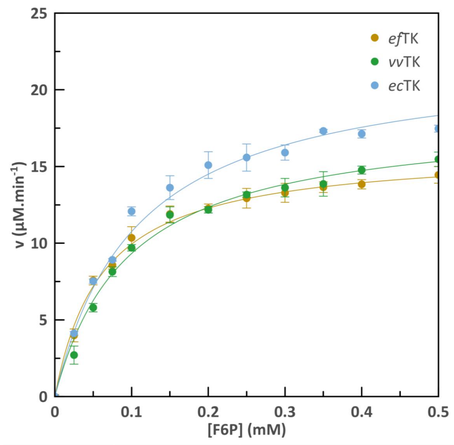


Fig. 2. SDS-PAGE of *ef*TK, *ec*TK and *vv*TK. All TK bear a 6-His-Tag and are expressed in *E. coli BL21(DE3)* They are purified by Ni-NTA affinity chromatography. Line 1 : molecular weight marker, line 2 : *vv*TK, line 3 : *ec*TK, line 4 : *ef*TK.



	K_M (mM)	Vm (µM/min)
*ec*TK	0.088	21.10
*ef*TK	0.062	16.18
*vv*TK	0.100	18.42

Fig. 3. Michaelis-Menten representation of *ec*TK, *ef*TK and *vv*TK and determination of the K_M for F6P. Assays are performed with 2 µM *ec*TK, 0.4 µM *ef*TK or 1.2 µM *vv*TK with 0-500 µM F6P, 0.2 mM TPP, 2 mM $MgCl_2$, 1 mM $Fe(CN)_6^{3-}$ in Hepes 50 mM, KCl 100 mM buffer at pH 7.0 for *vv*TK and 8.0 for *ec*TK and *ef*TK (n=3).

Bioelectrochemical assays

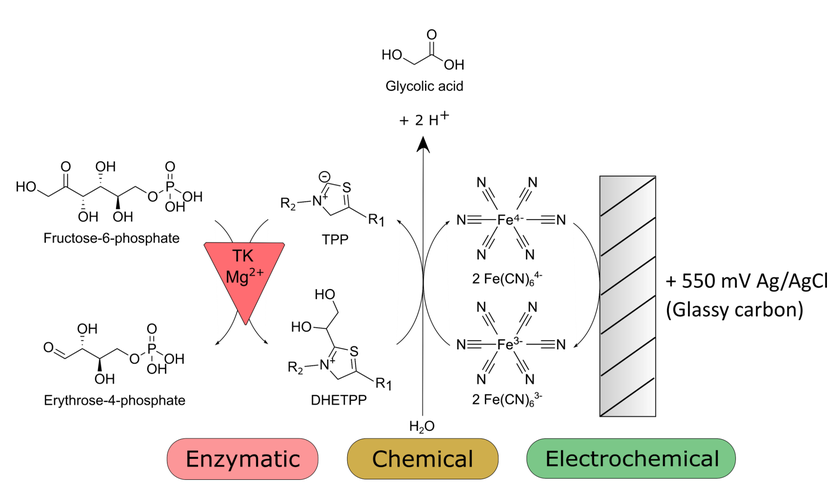


Fig. 4: Electrochemical assay for measuring TKs acitivty by IPA. TK enzyme catalyzes CC bond breaking of fructose-6-phosphate (F6P). Two carbons units are transferred onto thiamine pyrophosphate (TPP) to generate dihydroxyethylthiamine pyrophosphate (DHETPP) intermediate. This reaction step is followed by the release of erythrose-4-phosphate as product, and DHETTP is oxidized back to TPP by $Fe(CN)_6^{3-}$ [4]. Then, $Fe(CN)_6^{4-}$ is oxidized at the electrode surface. Current intensity variation is representative of TK activity [2, 3].

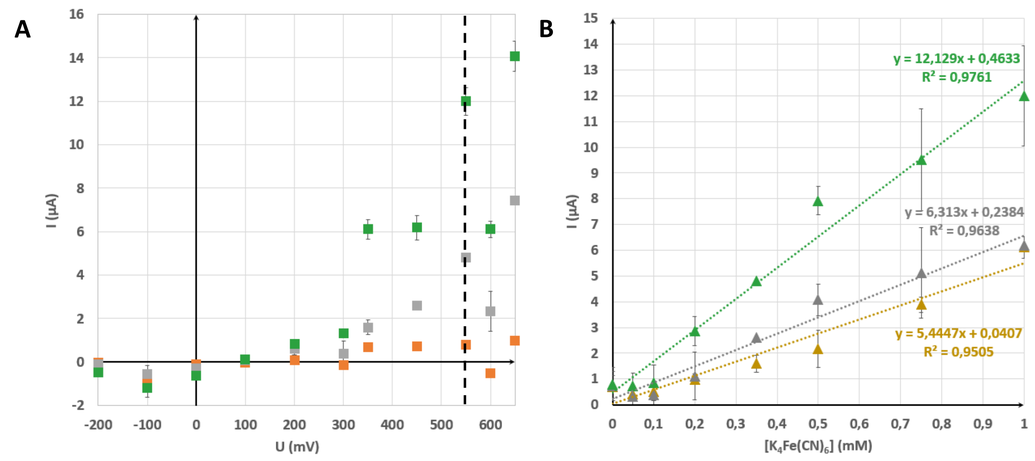


Fig. 5. Oxidation of $Fe(CN)_6^{4-}$ at several potentials and concentrations. All assays are performed with a single pulse of 5 s at 0.017 Hz in Hepes 50 mM, KCl 100 mM pH 7.0 (n = 3). **Fig. A.** Intensity detected versus applied potential applied. $Fe(CN)_6^{4-}$ concentrations is 1 mM in green, 0.35 mM in grey and 0 mM in orange. Dashed line represents the oxydation potential of $Fe(CN)_6^{4-}$ at 550 mV. **Fig. B.** Linear oxidation range of $Fe(CN)_6^{4-}$ at 550 mV in green, 450 mV in grey and 350 mV in gold.

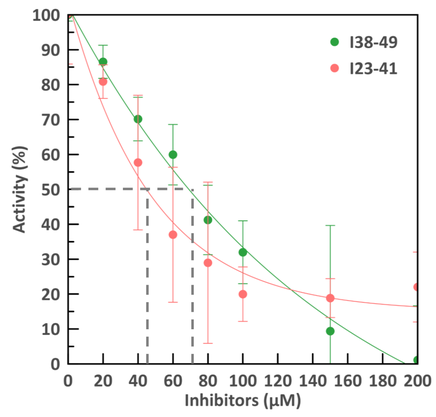


Fig. 6. Inhibition assays of *ecTK* with two TPP analogs (I38-49 and I23-41) previously obtained by screening a chemical library of more 3 000 molecules [1]. IPA assays were performed with *ec*TK (15 µg) at various inhibitors concentrations, TPP 15 µM (Km value), 1 mM L-eryrthrulose, 0.1 mM $Fe(CN)_6^{3-}$, 2 mM $MgCl_2$ in Hepes 50 mM, 100 mM KCl pH 7.0 buffer. The potential applied is +550 mV vs Ag/AgCl (n=4). Grey dash line is the IC_{50} value.

The new IPA device developed by the INL team show **high sensitivity** and provide access to all raw data. The first results indicate the possibility to **detect TK reaction** with 0.1 – 1 mM of $Fe(CN)_6^{4-}$ at 550 mV vs Ag/AgCl.
Among all **electrochemical methods, IPA** could be applied for biomedical and pharmacological projects. With the possibility of **multiplexing** it becomes possible to assay a large set of molecules from **chemical libraries** in a reasonable time [1, 2]. **The first screening of ICBMS chemical library allowed to find a new inhibitor of *ec*TK : 2-(4-ethoxyphenyl)-1-(pyrimidin-2-yl)-1H-pyrrolo[2,3-b]pyridine, also call I38-49 (IC_{50} = 63 µM) [2]. In order to enlarge the discovery of new potential TK inhbitors, screening of chemical libraries of ICCF and ICBMS will be performed soon on *ec*TK, *ef*TK and *vv*TK.**

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