Electrochemical high throughput screening of transketolases activity

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Since the 1950s, cases of pathogens that developed multi-drug resistance increases notably. One of these typical cases is the group of bacteria called ESKAPE, described for the first time in 2009 : *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species.* Currently, few antibiotics are still efficient against them.

According to the World Health Organization in 2015, deaths by infection is still the first cause of mortality in the world. Faced with the lack of efficient antibiotics and antifungals, the future health challenge will be to find new molecules against these pathogens. For this purpose, two conditions are required. i) Identify a good target such as hub protein. ii) Screen thousand of molecules that can cause the desired effect.

We are looking for new potential inhibitor of protein target by a new electrochemical High Throughput Screening (HTS) method.

Transketolase as a potential target

Transketolase (TK) is an enzyme of the non-oxidative part of the pentose phosphate pathway found in all living organisms. It is implied indirectly in the synthesis of nucleic acid bases, NADPH and aromatics amino acids. This hub protein is an appropriate target for drug development versus pathogens.

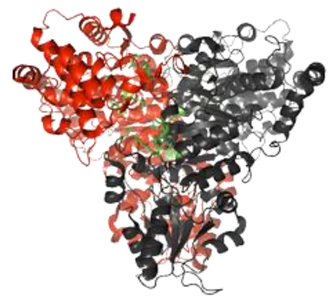

Fig. 1 (right): Crystal structure of *Mycobacterium tuberculosis* TK (*mtTK*), PDB code : 3RIM.
Each TK is an composed of two subunits of 75kDa in black and red. This enzyme has got two active sites on each face, visible in green. Thiamine pyrophosphate (TPP) is the cofactor present in the active site and allows two transfer two carbon units from a ketose donor to an aldehyde acceptor.

Intermittent Pulse Amperometry

Although all HTS methods, intermittent pulse amperometry (IPA) [1, 2] is a new approach to follow electrochemical reactions. IPA is: fast, sensible, allow the parallelization, limit trials price, and causes little variation in environmental changes. Potential application allow to following reactions recording current variations at the electrode surface. Thanks to IPA, it is possible to have a 96 well design to follow up to 96 enzymatic reactions quasi-simultaneously.

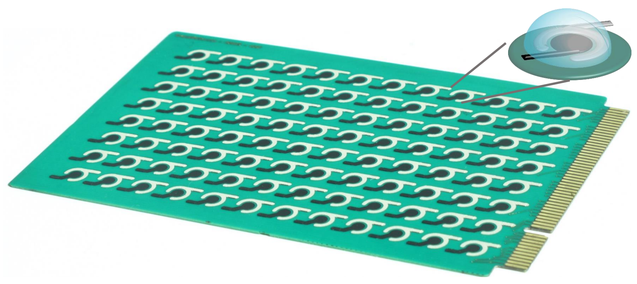

Fig. 2: 96 electrodes for IPA assay. Working electrode made of carbon and counter/ref is an Ag/AgCl electrode. In our assay, we applied a potential of + 500mV vs Ag/AgCl with a pulse of 82ms, at 1Hz frequency during 2min. Each electrode is covered by a drop of 35µL buffer and reagents.

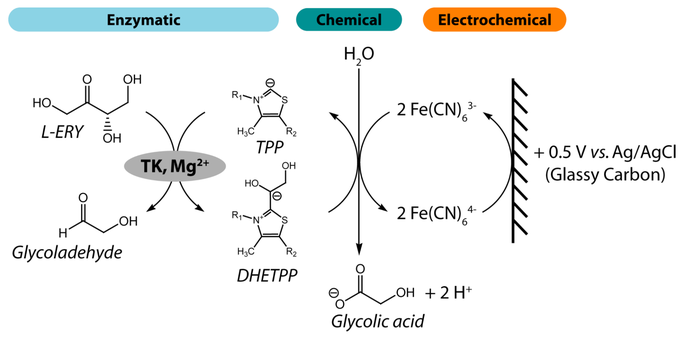

Fig. 3: Electrochemical assay for measure TKs acitivty by IPA. TK enzyme catalyzes CC bond breaking of L-erythrulose (L-ery). Two carbons units are transferred onto thiamine pyrophosphate (TPP) to generate dihydroxyethylthiamine pyrophosphate (DHETPP) intermediate. This reaction step is followed by the release of glycoladehyde as the side product, and DHETTP is oxidized back to TPP by ferricyanide ($Fe(CN)_6^{3-}$) [3]. Then, $Fe(CN)_6^{4-}$ is oxidized at the electrode surface. Current variation is representative of TK activity [1, 2].

Results

TK of *Escherichia coli* (*ecTK*) was already expressed [1]. Recently, we have expressed *mtTK* in order to perform inhibition assays on this enzyme by IPA.

Fig 4. Schematic representation of *mtTK* expressed in pET21b(+) vector. In order to remove the His tag, TEV protease cleavage site is include between TK sequence and His tag.

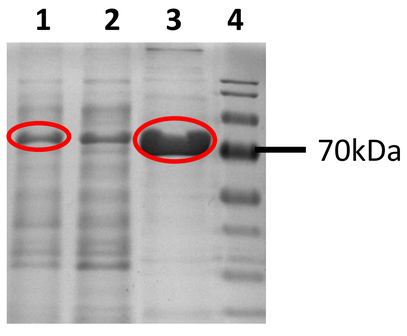

Fig 5. SDS-PAGE analysis of *mtTK* the purification by IMAC.
Lane 1: bacterial lysis supernatant, lane 2: unbounded proteins, lane 3 : *mtTK* after purification with imidazole, lane 4 : molecular weight marker. *mtTK* is circled in red.

ecTK and *mtTK* are expressed with specific activities of 0.066 and 0.398 µmol. $min^{-1}.mg^{-1}$ respectively.

Inhibition assays of *ecTK* were previously obtained by IPA [1]. Comparison is done with *mtTK*. All IPA assay need only 15µg *ecTK* or 3.5µg *mtTK* with 200µM TPP, 1mM L-ery, 0.1mM ferricyanide, 1mM $MgCl_2$, 50mM Hepes 100mM KCl pH 7.0 buffer.

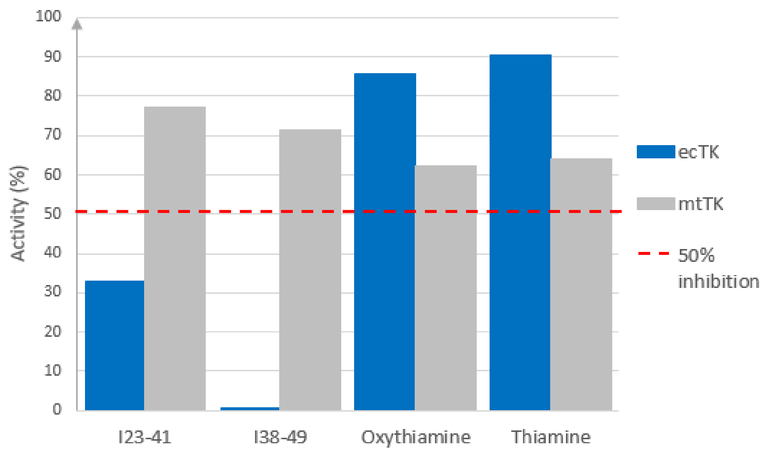

Fig. 6: Inhibition assays of *ecTK*[1] and *mtTK* by IPA. IPA inhibition assays were performed with 200µM of each inhibitor (n=1).

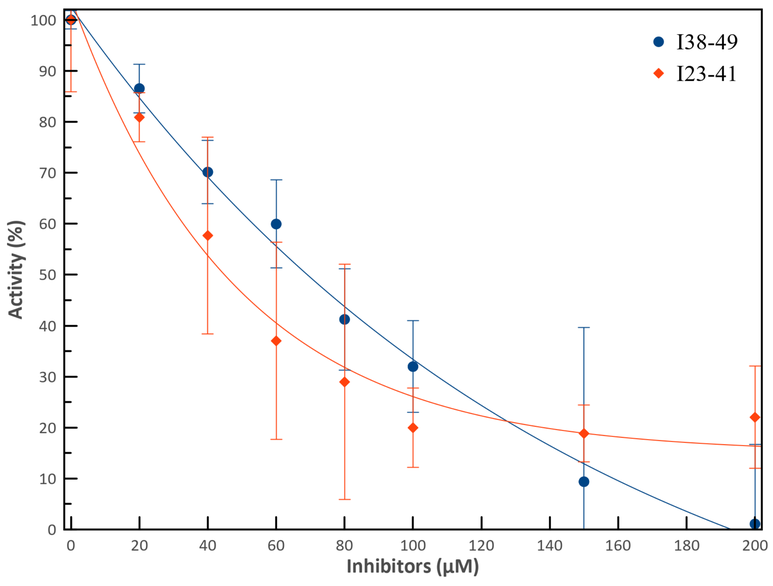

Fig 7: Determination of IC_{50} for I38-49 and I23-41 on *ecTK* [1]. Results are determined in quadruplicates.

A new specific inhibitor of *ecTK* is discover by IPA: 2-(4-ethoxyphenyl)-1-(pyrimidin-2-yl)-1H-pyrrolo[2,3-b]pyridine, also call I38-49 (IC_{50} = 63µM).
No potential inihibitors of *mtTK* has been identify in this early stage of the project.

IPA could be easily applicate in research, medical and pharmacological fields particularly for molecules screening. It is already allow us to determined a new inhibitor for *ecTK*. This strategy will be applied soon to screen a broader range of TK, from bacteria to multicellular pathogens, in order to discover new drug candidates.

	Time for 42 samples (min)	Mass of mtTK per well (µg)	Volume (µL)
IPA	11	3.5	35
Spectrophotometry	210	14	140

Table 1. Time, amount of mtTK and reaction volume for IPA or spectrophotometry assay.

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