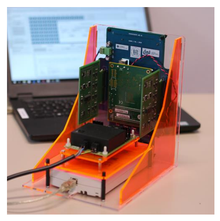
# New multiplexed electrochemical system for chemical libraries screening

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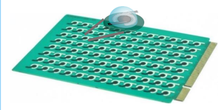

**Human pathogens are becoming increasingly multi-drug resistant (MDR). The list of priority bacterial pathogens published by the World Health Organization in 2017 are all MDR and are responsible for 1.29 million deaths in 2019. It is becoming necessary to find new biochemical targets for these pathogens in order to develop effective new drugs.[1,2]**

**Transketolases (TK) are ubiquitous proteins of the pentose phosphate pathway (PPP) essential for nucleic acids (DNA and RNA), aromatic amino acids and NADPH (involved in the regulation of oxidative stress) synthesis. TK controls 70% of PPP making it a prime target. Identifying new molecules able to inhibit this enzyme could lead to discover new antibiotics. A novel inhibitor of *E.coli* TK (TK*ec*) was identified in a previous study using an the AndCare 9600 device.[3] The latter is a 96-well electrochemical reader operating by intermittent pulsed amperometry (IPA) but is no more supported.**

**In order to screen a new chemical library by IPA, it is necessary to validate a new and more precise IPA device and to rapidly analyze the data produced by a new in-house program called Electrochemical Tracer Analysis System (ECTASy). At present, 54 molecules of this chemical library are screened using 11 TK from human pathogens including the *Homo Sapiens* TK (*hs*TK).**

## Validation of the experimental approach

IPA is an electrochemical method that applies a potential for a given time and then the electrical circuit is opened. This is repeated several times. In this study, IPA is applied on 96 screen-printed carbon electrodes at + 550 mV vs Ag | AgCl in order to detect $Fe(CN)_6^{4-}$ potential.[3, 4] This multiplexing capability facilitates the screening of a chemical library.

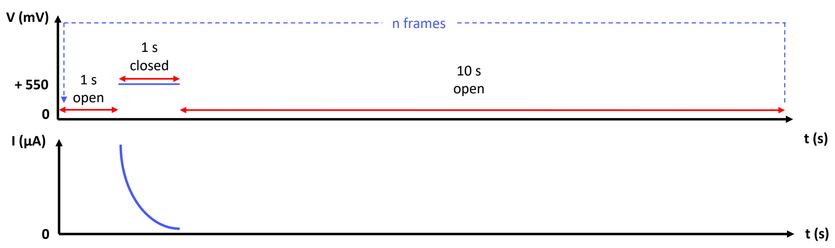


**Fig. 1. IPA protocol on a single electrode.** Potential is applied for one second affording a I = f(t) plot. During the other 11 seconds, the circuit is open and no current is recorded. This is repeated n times (n frames).

The pulses generated by IPA can be analyzed by fitting a mathematical equation to the experimental data (400 points/pulse).

The Cottrell equation **(1)** is modified to the empirical equation **(2)** to obtain fitted parameters ($k_1$, $k_2$ and $I_0$) Theoretically they are representative of the concentration of $Fe(CN)_6^{4-}$ at the electrode surface:

$$I(t) = n \,.\, F \,.\, A \,.\, C \,.\, \sqrt{\frac{D}{\pi \,.\, t}} \qquad \textbf{(1)}$$

$$I(t) = k_1 \,.\, t^{-1/2} + k_2 \,.\, t^{-1} + I_0 \qquad \textbf{(2)}$$

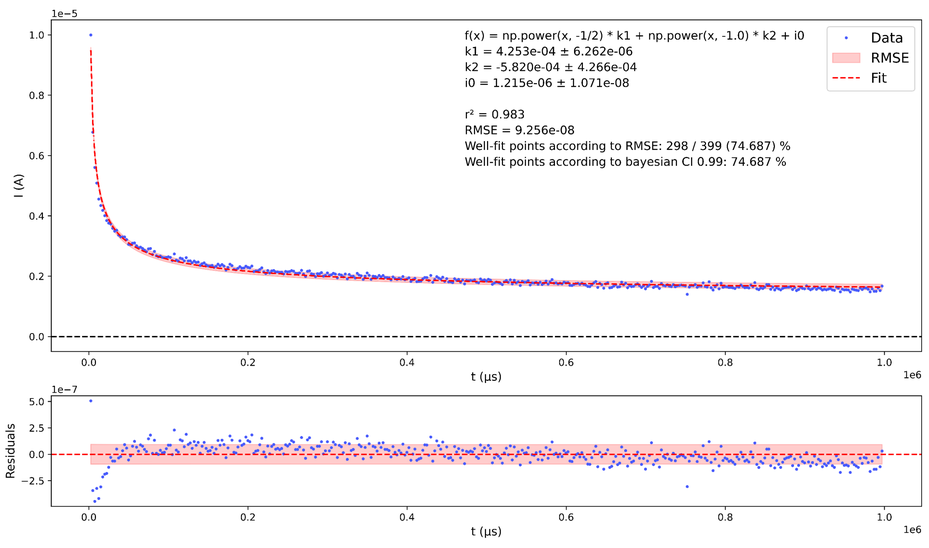


**Fig. 2. Example of curve fitted with the empirical equation (2) modified from Cottrell's equation.** Residuals are calculated as the difference between curve fitting and experimental pulse points.

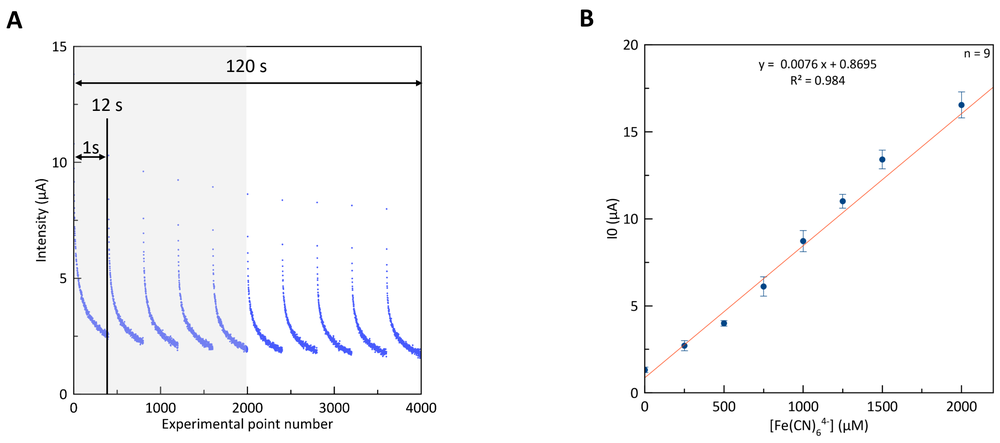


**Fig. 3. A. Pulses recorded during ferrocyanide oxidation on the same electrode.** Assays were performed with $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ (total concentration of 2 mM in 50 mM Hepes buffer pH 7.0, 100 mM KCl). Ten pulses of 1 sec are applied and spaced by 12 sec. The first five pulses show inconstant profiles (colored in grey) and are excluded of the analysis. **B. Ferrocyanide calibration curve.** Each pulse was fitted equation **(2)**. $I_0$ adjusted parameter is proportional with $Fe(CN)_6^{4-}$ concentration.

Ten pulses are generated on each electrode in 2 min, the last five pulses are reproducible. By adjusting the value of the parameter $I_0$, the signal obtained is proportional to the concentration of $Fe(CN)_6^{4-}$ present on the electrode surface.

The 72 electrodes used to obtain the $Fe(CN)_6^{4-}$ calibration curve lead to a total of 360 pulses. During a screening, several thousand pulses will be generated. We need to filter them automatically using statistical criteria.

$R^2 \geq 0.7$
$RMSE \leq 2.0$ µA

360 pulses → 345 pulses

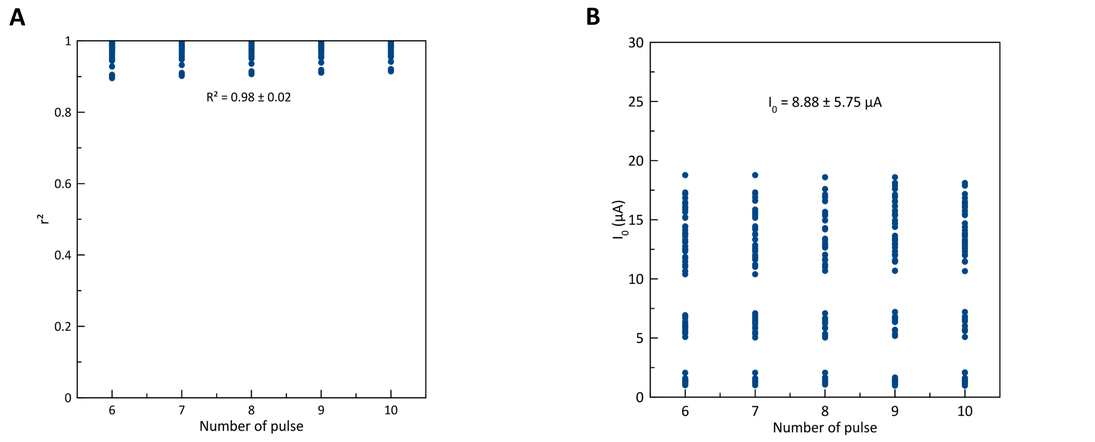


**Fig. 4.A. Validation of 345 pulses among 360 after fitting and filtering using $R^2$ and RMSE using a home made software.** $R^2$ is calculated after fitting the empirical equation. Average RMSE calculated to be 0.73 ± 0.51 µA which ensure that 68.7 ± 10.8 % of the data points are closed to the nominal value ($CI_{Bayesian}$ = 0.99). **B. $I_0$ adjusted value.** $I_0$ values are homogenously dispersed and follow $Fe(CN)_6^{4-}$ concentrations.

The experimental protocol and data analysis method enable to monitor the ferrocyanide concentration. The parameter $I_0$ is proportional to the concentration. It is now possible to screen a chemical library with TKs using IPA in order to identify new inhibitors.

## Chemical library screening by TK

The screening of 54 molecules from a chemical library by 11 TKs leads to 660 experimental conditions with controls. Each plate of 96 electrodes is measured after 15 min enzymatic reaction, corresponding to 26 400 pulses or 10 056 000 experimental points for a total of 1.2 Go of data. Reducing analysis time is therefore crucial. This why ECTASy is developed.

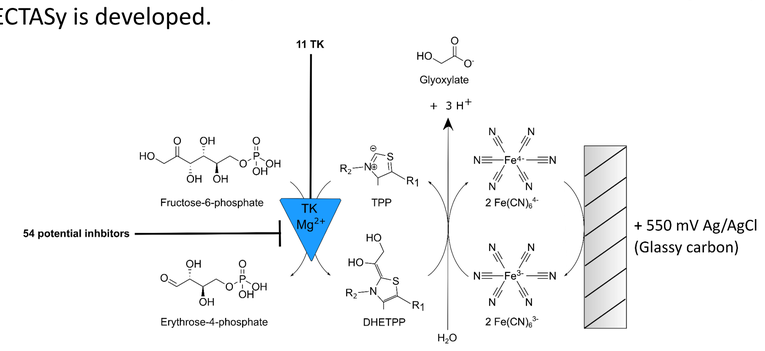


**Fig. 5. Reaction of TK measurable by electrochemistry.** TK can break $C_2$-$C_3$ bond on fructose-6-phosphate releasing erythrose-4-phosphate and transforming TPP into DHETPP. The latter is oxidized by back to TPP with two $Fe(CN)_6^{3-}$ and one water molecules releasing two $Fe(CN)_6^{4-}$ and one glyoxylate molecules. Ferrocyanide is oxidized by applying a potential of + 550 mV vs Ag | AgCl on carbon electrodes.[5,6]

n = 4 — Phylogenetic distance from *hs*TK — Inhibition

| Inhbiteur | *hs*TK | *cg*TK | *vv*TK | *ec*TK | *hi*TK | *sp*TK | *ef*TK | *ab*TK | *pf*TK | *mt*TK | *sa*TK |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 20,4 | 17,5 | 31 | 0 | 19 | 6,5 | 23,6 | 53,5 | 84,2 | 0 | 37,3 |
| 4 | 23,8 | 22,8 | 42,2 | 4,9 | 11 | 15,1 | 0 | 73,3 | 100 | 2,2 | 61,4 |
| 6 | 27,4 | 29,9 | 51,2 | 0 | 17,8 | 19,2 | 0 | 65 | 100 | 0 | 51,4 |
| 7 | 8,5 | 12,9 | 38,7 | 0 | 22 | 25,2 | 1,6 | 71,7 | 100 | 0,3 | 21,7 |
| 8 | 22,2 | 31,1 | 46,4 | 12,5 | 25,6 | 23,1 | 0 | 64,6 | 100 | 0 | 39,8 |
| 10 | 21,8 | 31,8 | 66,8 | 0 | 29,1 | 31,6 | 2,1 | 64,5 | 46 | 13,4 | 62,6 |
| 11 | 42,8 | 34,3 | 68,6 | 0 | 29,8 | 32,5 | 0 | 25,1 | 55,5 | 15,7 | 41 |
| 12 | 15,4 | 27,2 | 67,3 | 1,5 | 0 | 3,8 | 0 | 69,5 | 35,9 | 0 | 54,8 |
| 13 | 20,9 | 14 | 65,9 | 5 | 0,6 | 27,1 | 0 | 74,5 | 48,5 | 0 | 82 |
| 14 | 6,4 | 34,4 | 50,3 | 5,3 | 0 | 2,4 | 0 | 69,8 | 90 | 0 | 36 |
| 16 | 45,1 | 100 | 100 | 76,4 | 84,4 | 87,7 | 52,3 | 100 | 100 | 74,6 | 100 |
| 20 | 41,8 | 79,9 | 77 | 13,9 | 59,5 | 100 | 0 | 77,2 | 100 | 53,6 | 100 |
| 22 | 35,6 | 31,8 | 87,4 | 24,8 | 32,5 | 57,7 | 0 | 75,9 | 86,8 | 50,7 | 48 |
| 24 | 32,8 | 41,7 | 73,7 | 28,6 | 64,5 | 100 | 4 | 98,8 | 71,2 | 70,9 | 82,7 |
| 27 | 22,8 | 34,3 | 51,1 | 13,6 | 33,5 | 0 | 4,3 | 55,4 | 42,3 | 34,2 | 54,6 |
| 30 | 6,9 | 71 | 44,1 | 13 | 28,2 | 0,1 | 20,5 | 39,2 | 77,8 | 15,9 | 100 |
| 32 | 0 | 57,2 | 21,3 | 0 | 6,3 | 8 | 14,1 | 49,9 | 19,7 | 11,3 | 89,8 |
| 33 | 0 | 42,7 | 0 | 0 | 21 | 4,3 | 35,2 | 20,7 | 64,7 | 12,7 | 68,8 |
| 42 | 42,3 | 0 | 68,6 | 11,1 | 100 | 46,8 | 0 | 99 | X | 27,4 | 16,1 |
| 43 | 22,1 | 0 | 68,6 | 3,8 | 95,3 | 47,2 | 0 | 100 | X | 25,9 | 0 |
| 45 | 34,6 | 7,3 | 28,8 | 0 | 92,4 | 15,5 | 0 | 100 | X | 53,5 | 5,8 |
| 47 | 0,1 | 14,3 | 6,4 | 0 | 76,4 | 36,7 | 0 | 68 | X | 24,8 | 27,6 |
| 48 | 10,5 | 23,1 | 0,5 | 0 | 71,6 | 34,3 | 0 | 0 | X | 8,5 | 33,9 |
| 49 | 0 | 47,1 | 0 | 8,9 | 87,8 | 44,8 | 0 | 83,3 | X | 12,8 | 48,6 |
| 50 | 14 | 70,1 | 0 | 0 | 52,4 | 0 | 0 | 0 | X | 0 | 34,9 |
| 51 | 0 | 49 | 0 | 0 | 54,4 | 18,1 | 0 | 20,9 | X | 0 | 31,7 |

100 % / 50 % / 0 %

**Fig. 6. Heatmap of chemical library screening for TK inhibitors.** Assays are done with 11 TK (16 µg/assay), 2 mM $Fe(CN)_6^{3-}$, 0.2 mM TPP, 2 mM $MgCl_2$ and F6P at the $Km_{app}$ value of corresponding TK. Screening analysis is performed in just 5 min 38 s. Only molecules inhibiting *hs*TK to less than 50% will be retained.

The heatmap of the screening by IPA identifies a cluster of molecules that inhibit some TK of human pathogenic organisms without inhibiting *hs*TK by more than 50%. Other molecules are more specific to certain enzymes. Further spectrophotometric studies will confirm these findings.

**The new IPA device allows to perform 96 multiplexed assays using screen-printed electrodes. It can detect nanoamperes and it is cheaper than others electrochemical devices. ECTASy is a homemade software greatly speeding up analysis and allowing to fit any mathematical equation on pulses. The new device and the analysis by the homemade program are validated and allow the screening of chemical libraries in a reliable way.**
**IPA screening of 54 molecules from a chemical library identified a number of potential TK inhibitors with varying degrees of specificity for this enzyme.**
**Verification by spectrophotometry will enable these new inhibitors to be validated for future hits.**


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