

# KinITC

Subjects: Thermodynamics

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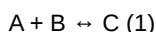
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## 1. What Is *kinITC* ?

The method *kinITC* for *kinetic Isothermal Titration Calorimetry* is an extension of the classical **ITC** technique in view of obtaining kinetic information in addition to thermodynamic information. It has been described in full in,<sup>[1]</sup> and in a simplified and less general form in.<sup>[2]</sup> In short, *kinITC* allows obtaining with a single technique both the information obtained with classical **ITC** and with a technique like **Surface Plasmon Resonance (SPR)**. In situations where a chemical reaction proceeds clearly through two successive kinetic steps, the major interest of *kinITC* is of being potentially more informative alone<sup>[1]</sup> than ITC and SPR used jointly, but in a classical way. The experimental limitation for its successful application comes from the response time of the instrument, which prevents from obtaining kinetic information if the reaction proceeds too rapidly. It turns out that this is not so much a severe limitation for many reactions occurring with biological macromolecules. The *kinITC* method is thus quite appropriate in biology. Note that, as pioneered long time ago by J. Sturtevant, **ITC** has already been used for deriving kinetic information in biochemistry, but only in the particular situations of enzyme kinetics.<sup>[3]</sup>

## 2. Basic Reminder on ITC

Classical ITC is a typical thermodynamic technique allowing to measure directly the heat evolved during a reaction that can be described by:



At constant pressure, the heat evolved during the formation of one mole of the product C is nothing else than  $\Delta H$ , the molar variation of enthalpy of the reaction. By considering a titration experiment during which small amounts of the compound B are injected at regular intervals into a measurement cell containing the compound A, one can determine the equilibrium constant  $K_a$  of the reaction (in  $M^{-1}$ ) or, equivalently, the dissociation constant  $K_d = K_a^{-1}$  (in M). From this, the standard Gibbs free energy of the reaction may be obtained:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = RT \ln K_d \quad (2)$$

and thus also  $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$ . As important the four quantities  $\Delta H^\circ$ ,  $\Delta G^\circ$ ,  $\Delta S^\circ$  and  $K_d$  may be, particularly for drug design,<sup>[4]</sup> it is important to emphasize that they are typical thermodynamic quantities that do not say anything about the kinetics of the reaction.

## 3. How to Overcome This Limitation ?

An important feature of **ITC** is that the instrument does not measure directly the total heat  $Q$  evolved during the reaction, but its rate of production (or absorption) during the reaction. Therefore, the primary signal is the heat power:

$$P_s(t) = dQ/dt \quad (3)$$

and this is only by integrating  $P_s(t)$  that  $Q$  can be obtained. By definition of  $\Delta H$ , one has therefore a direct link between  $P_s(t)$  and  $d[C]/dt$ , the rate of production of the compound C:

$$P_s(t) = V_{\text{cell}} \Delta H d[C]/dt \quad (4)$$

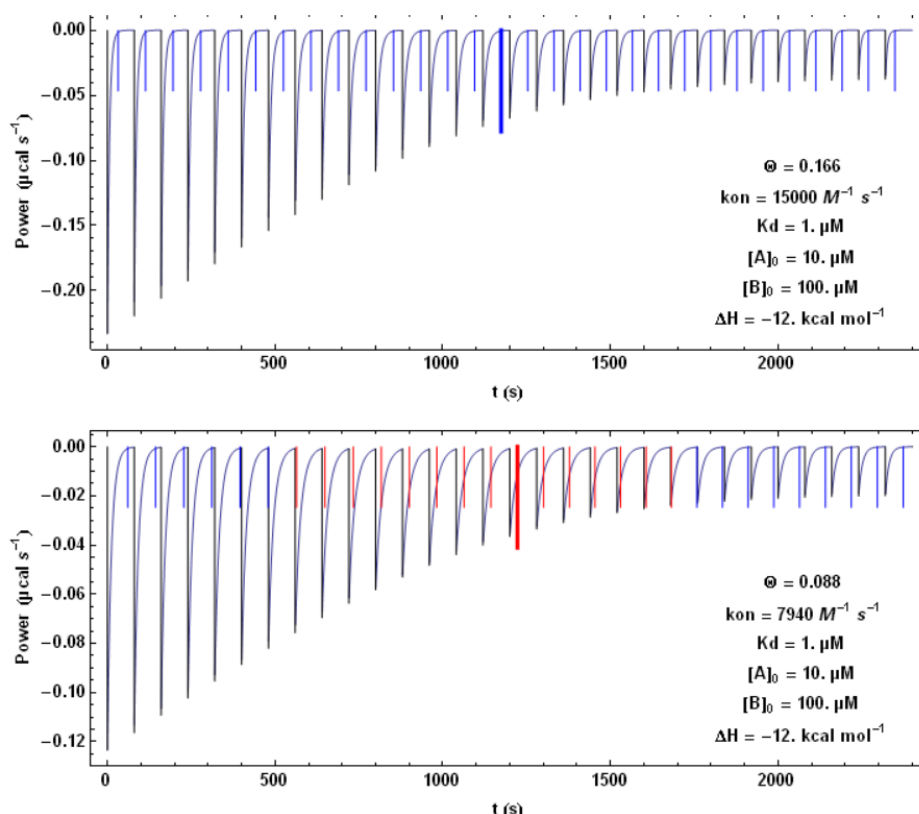
where  $V_{\text{cell}}$  is the volume of the cell where the reaction takes place. Therefore, knowing  $P_s(t)$  yields  $d[C]/dt$ , which opens the way for obtaining kinetic information.

## 4. Simplified Analysis for the Evaluation of $D[C]/dt$

The kinetics of the bimolecular reaction  $A + B \leftrightarrow C$  is governed by the two parameters  $k_{\text{on}}$  and  $k_{\text{off}}$  which leads to:

$$d[C]/dt = k_{\text{on}} [A] [B] - k_{\text{off}} [C] \quad (5)$$

This equation may be complemented with two conservation equations:  $[A] + [C] = [A]_0$  and  $[B] + [C] = [B]_0$ ,  $[A]_0$  and  $[B]_0$  being the total concentrations of the compounds A and B at the beginning of the reaction. These two concentrations  $[A]_0$  and  $[B]_0$  vary during each injection of compound B (which typically lasts more than 0.5 s and less than 3 to 4 s), but are constant between two successive injections (separated by much longer times). We consider here a simplified situation where the short injection time is neglected, which means that  $[A]_0$  and  $[B]_0$  are constant during each titration step. In addition, the problem of the finite response time of the instrument is also neglected. It is explained in the following how these limitations can be suppressed. Within this simplified frame, an analytical solution to equation (5) can be obtained. This is illustrated with Fig. 1 highlighting two simulations with two different values of  $k_{\text{on}}$ .



In Fig. 1, each step following the injection of 1.3 µl of compound B lasts 80 s. <https://handwiki.org/wiki/index.php?curid=1633980>

The vertical bars mark the theoretical value of the time needed to reach equilibrium at each titration step. It is seen that with the higher  $k_{\text{on}}$  value of 15000 M<sup>-1</sup> s<sup>-1</sup> (upper figure) the time interval of 80 s is always sufficient for returning to equilibrium, whereas with the lower  $k_{\text{on}}$  value of 7940 M<sup>-1</sup> s<sup>-1</sup>, it becomes too short (red bars) for some injections. The thickest and longest bar marks mid-titration, that is the titration step closest to a unit stoichiometry ( $[A] = [B]$ ). It can be shown that this corresponds to the step with the slowest return to equilibrium.

## 5. The Practical Problems

The previous analysis is a simplified one because the power signal  $P_s(t)$  given by equation (4) does not correspond to the actually measured power  $P_m(t)$ . There are two reasons for that. First, neither the injection, nor the mixing of compound B are instantaneous. Second, the ITC instrument has also a finite response time  $\tau_{\text{ITC}}$ , which implies that a sudden heat signal  $P_s(t)$  delivered into the measurement cell will in fact give rise to an actually measured signal  $P_m(t)$  decreasing exponentially with a characteristic time  $\tau_{\text{ITC}}$ . Such a response is named the *impulse response*. These two complications can nevertheless be dealt with by using standard methods in signal processing. Essentially, the actual signal is obtained by a Convolution of the ideal signal with the impulse response function. This is exemplified with the influence of  $\tau_{\text{ITC}}$  on the shape of the actually measured signal  $P_m(t)$  (Fig. 2). The influence of all parameters can be visualized on kinITCdemo.

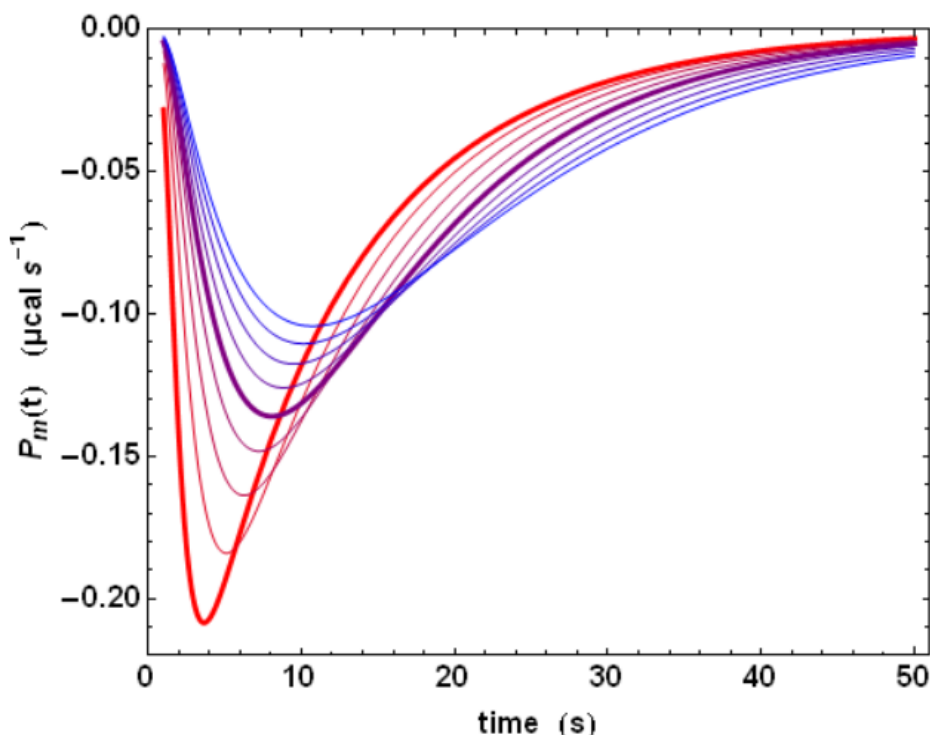


Fig. 2: Influence of  $\tau_{ITC}$ . <https://handwiki.org/wiki/index.php?curid=1288913>

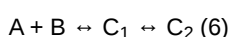
The practical consequence of such a convolution is a smoothing of the actual signal (compare the sharp ideal response curves in Fig. 1 with the realistic response curves in Fig. 2). Importantly, the convolution operation "dilutes" the power response curve, but does not change its time-integral, which corresponds to the total heat evolved during the titration step.

## 6. Obtaining the Kinetic Parameters $K_{on}$ and $K_{off}$ in Simple Situations

When one is able to simulate accurately all response curves of a titration experiment, it becomes possible to fit the experimental response curves and, in turn, to derive the kinetic parameters  $k_{on}$  and  $k_{off}$  that influence the return to equilibrium (see Fig. 1). This is obviously possible for reactions that are correctly described with one single kinetic step. Examples of such fits are shown on *kinITCdemo*. It is important to emphasize that a convincing result is obtained by considering experiments at different temperatures and by fitting all titration steps of all experiments with a single set of thermodynamic and kinetic parameters. Two features are particularly important for the success of the method. First, the kinetic parameters  $k_{on}$  and  $k_{off}$  are linked at all temperatures to the equilibrium 'constant' through  $K_a = k_{on}/k_{off}$  (or  $K_d = k_{off}/k_{on}$ ) and, second, the evolution of these equilibrium 'constants' with the temperature is rigorously governed by the Van 't Hoff equation:  $\partial \ln K_a / \partial T = \Delta H / RT^2$  (or  $\partial \ln K_d / \partial T = -\Delta H / RT^2$ ). Since the enthalpic term  $\Delta H$  is the primary result obtained by ITC, the experimental method itself dictates the evolution of  $K_a(T)$ , that is also of  $k_{on}(T)/k_{off}(T)$ . Therefore, either  $k_{on}(T)$  or  $k_{off}(T)$  needs to be considered as unknown, since their ratio is known. It is useful to parameterize the unknown temperature-dependence of this kinetic parameter with the Arrhenius equation. If, for example,  $k_{off}(T)$  is considered as unknown, the determination of only two parameters, namely the value of  $k_{off}$  at a reference temperature  $T_0$ , and an activation energy  $\Delta H^\ddagger_{off}$ , should allow fitting all titration steps of experiments at all temperatures. How many different temperatures should be considered? In practice, experiments at two different temperatures may be sufficient if there is a linear variation of  $\Delta H$  vs.  $T$ , i.e. if  $\partial \Delta H / \partial T = \Delta C_p = \text{constant}$ , which is often (but not always!) the case. Note that, indeed, at variance with a common assumption in many textbooks,  $\Delta H$  is most often variable with the temperature ( $\partial \Delta H / \partial T = \Delta C_p \neq 0$ ), and this has to be taken into account for obtaining  $K_a(T)$  from the Van 't Hoff equation.

## 7. The Interest of *kinITC* for More Complex Situations

If, for example, a chemical process cannot be represented by equation (1), but needs to be represented by:



all usual experimental methods used alone yield information on the global process  $A + B \leftrightarrow C_2$ , which ignores the intermediate step. On the contrary, as shown in,<sup>[1]</sup> *kinITC* used alone is able to dissect such a composite process both thermodynamically and kinetically. This means that *kinITC* allows dissecting the overall enthalpic term  $\Delta H_{1+2}$  (obtained by

a classical use of ITC) into  $\Delta H_1 + \Delta H_2$ , where  $\Delta H_1$  pertains to the first step  $A + B \leftrightarrow C_1$ , and  $\Delta H_2$  pertains to the second step  $C_1 \leftrightarrow C_2$ . Furthermore, the two steps are also dissected kinetically, which means that the kinetic parameters  $k_{on}$  and  $k_{off}$  for the first step, as well as  $k_{1,2}$  and  $k_{2,1}$  for the second step, are also obtained. Obviously, on one hand, obtaining such a wealth of information has a price in terms of the number of experiments that should be performed at different temperatures (at least three, but the exact number depends on the quality of the data and on whether or not  $\Delta C_p$  is constant). On the other hand, one is rewarded by a complete knowledge of all parameters, comprising their temperature dependence.

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