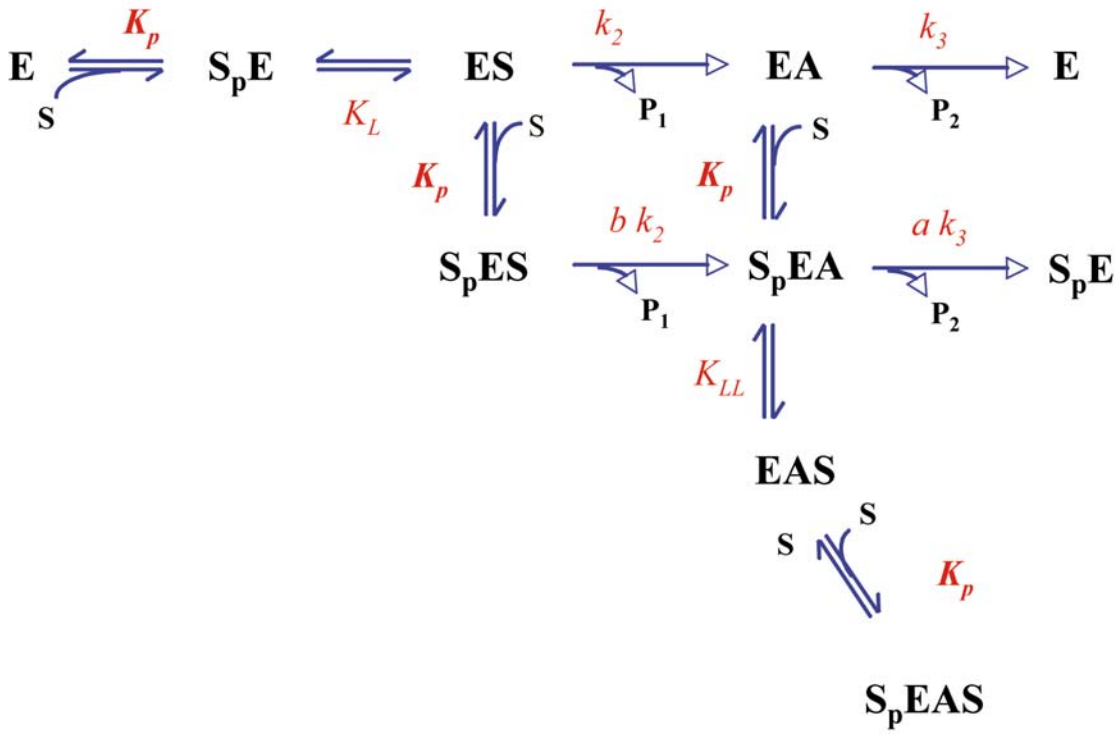
Kinetics of ATCh hydrolysis and choice of crystals soaking concentrations

The rate of hydrolysis of ATCh by *Tc*AChE as a function of ATCh concentration (Figure 6) was analyzed by multiple non-linear regressions, using the model (Scheme SM1) and equation of Stojan and colleagues (Stojan *et al.*, 2004) and the program GOSA (www.bio-log.biz). This model is the one that best describes the experimental data. The values obtained from the fit for the different kinetic parameters are shown in Table SM1. These parameters were used to estimate the relative proportions of the first and the second substrate-inhibited species as a function of ATCh concentration (Fig. SM2). The first substrate-inhibited species (S_PES) accumulates due to the hampering of choline exit by a substrate molecule at the peripheral anionic site. The second species (S_PEAS) results from the binding of two substrate molecules in the active-site gorge of the acetylated enzyme. The first substrate-inhibition phenomenon has its maximum at ~30 mM ATCh. At 100 mM ATCh, both substrate-inhibited species are populated equally. Substrate concentrations below (20 mM) and above (500 mM) 100 mM were chosen for the crystal soaks, in order to preferentially accumulate either the first or the second substrate-inhibited species.



Scheme SM1 : Kinetic model for ATCh hydrolysis and inhibition in AChE (reproduced from Stojan *et al.*, 2004).

Kinetic parameter	Value	Uncertainty (σ^2)
k_2	65300 $sec.^{-1}$	± 48700
k_3	872 $sec.^{-1}$	± 49
K_P	136 μM	± 32
K_L	11.2	± 2.5
K_{LL}	644	144
a	1	0.08
b	0.008	0.002

Table SM1 : Kinetic parameters obtained from fitting the experimental pS curve of *Tc*AChE by use of the model and equation described by Stojan and colleagues (Stojan *et al*., 2004).

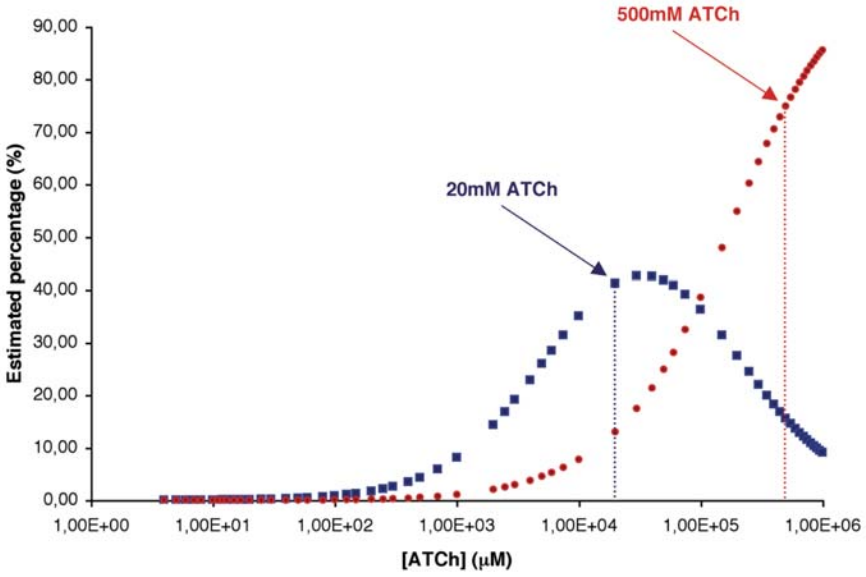


Figure SM2 : Calculated proportions of the two substrate-inhibited species of *Tc*AChE as a function of ATCh concentration (blue squares: S_PES, red circles: S_PEAS).