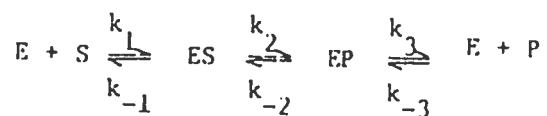


The Steady State Derivation for the Mechanism



1. The Slow Way:

Assume a steady state derivation

$$\text{i.e., } \frac{d(EX_i)}{dt} = 0$$

$$\begin{aligned} \frac{d(ES)}{dt} &= k_1 (E)(S) + k_{-2} (EP) - (ES)(k_{-1} + k_2) \\ &= 0 \end{aligned}$$

$$ES = \frac{k_1 (E)(S) + k_{-2} (EP)}{k_{-1} + k_2} \quad (1)$$

By analogy

$$EP = \frac{k_{-3} (E)(P) + k_2 (ES)}{k_{-2} + k_3} \quad (2)$$

Substitute the expression for EP with ES ((2)  $\rightarrow$  (1))

$$ES = \frac{k_1 (E)(S) + k_{-2} \left\{ \frac{k_{-3} (E)(P) + k_2 (ES)}{k_{-2} + k_3} \right\}}{k_{-1} + k_2}$$

Then solve for ES

$$ES = \frac{k_1 (k_{-2} + k_3) (E)(S) + k_{-2} k_{-3} (E)(P)}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \quad (3)$$

In the same way

$$EP = \frac{k_{-3} (k_{-1} + k_2) (E)(P) + k_1 k_2 (E)(S)}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \quad (4)$$

Conservation of mass states

$$E_0 \text{ (total enzyme)} = E + ES + EP$$

Using the values for ES and EP ((3) and (4)) gives

$$E_0 = E \left( 1 + \frac{k_1 S (k_2 + k_{-2} + k_3) + k_{-3} P (k_{-1} + k_2 + k_{-2})}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \right) \quad (5)$$

The velocity of the reaction,  $v$ , is

$$\frac{-dS}{dt} = k_1(E)(S) - k_{-1}(ES)$$

Substituting (3) here gives

$$v = \frac{-dS}{dt} = E \left( \frac{k_1 k_2 k_3 S - k_{-1} k_{-2} k_{-3} P}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \right) \quad (6)$$

Substituting the expression for E (from equation (5)) into (6) gives

$$v = \frac{(E)_0 (k_1 k_2 k_3 S - k_{-1} k_{-2} k_{-3} P)}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3 + k_1 S (k_2 + k_{-2} + k_3) + k_{-3} P (k_{-1} + k_2 + k_{-2})}$$

and dividing by the denominator term which does not contain S or P

$$= \frac{E_0 (k_1 k_2 k_3 S - k_{-1} k_{-2} k_{-3} P) / (k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3)}{1 + k_1 S \left\{ \frac{k_2 + k_{-2} + k_3}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \right\} + k_{-3} P \left\{ \frac{k_{-1} + k_2 + k_{-2}}{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3} \right\}} \quad (7)$$

If  $[S]$  and  $[P] \gg [E]_0$ , then replace S by  $S_0$  and P by  $P_0$ .

Equation (7) can be rearranged to

$$v = \frac{\frac{V_s}{K_s} S - \frac{V_p}{K_p} P}{1 + \frac{S}{K_s} + \frac{P}{K_p}}$$

with  $V_s = k_2 k_3 (E)_0 / (k_2 + k_{-2} + k_3)$

$V_p = k_{-1} k_{-2} (E)_0 / (k_{-1} + k_2 + k_{-2})$

$$K_s = \frac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_1(k_2 + k_{-2} + k_3)}$$

$$K_p = \frac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_{-3}(k_{-1} + k_2 + k_{-2})}$$

At equilibrium

$$v = 0, \text{ and } K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{k_1 k_2 k_3}{k_{-1} k_{-2} k_{-3}} = \frac{V_s K_p}{K_s V_p} = \text{Haldane relation}$$

Equation (7) can also be rearranged to

$$v = \frac{V_s}{1 + \frac{K_s}{S} \left(1 + \frac{P}{K_p}\right)} - \frac{V_p}{1 + \frac{K_p}{P} \left(1 + \frac{S}{K_s}\right)}$$

$$= v_f - v_r$$

If  $v_r \ll v_f$  and  $P/K_p \ll 1$

$$v_o = \frac{V_s}{1 + \frac{K_s}{S}}$$

2. A Faster Way - involving a different assumption.

If the system is in rapid equilibrium except for  $ES \xrightleftharpoons[k_{-2}]{k_2} EP$

$$K_s = \frac{(E)(S)}{(ES)}; \quad K_p = \frac{(E)(P)}{(EP)}$$

$$v = k_2 (ES) - k_{-2} (EP)$$

$$= \frac{k_2 (E)(S)}{K_s} - \frac{k_{-2} (E)(P)}{K_p} = E \left\{ \frac{k_2 S}{K_s} - \frac{k_{-2} P}{K_p} \right\}$$

$$E_0 = E + ES + EP = E \left( 1 + \frac{S}{K_s} + \frac{P}{K_p} \right)$$

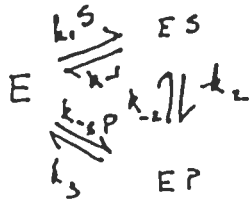
$$v = \frac{E_0 \left\{ k_2 \frac{S}{K_s} - k_{-2} \frac{P}{K_p} \right\}}{1 + \frac{S}{K_s} + \frac{P}{K_p}}$$

which has the same form as (7)

3. A Faster Way - involving the steady state assumption. (King-Altman, J. Phys. Chem., 60, 1375 (1956)).

For  $n$  different enzyme containing species (and  $n-1$  non-homogeneous linear equations).

The mechanism is written as a cycle, i.e.

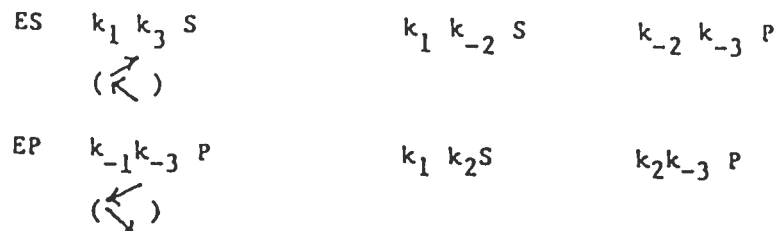


The method yields the relative concentration of each species by summing the product of  $n-1$  rate (and concentration) terms as defined by a given set of patterns. For this case the patterns are



and the terms leading to each species for each pattern is given as

$$\begin{array}{ccc}
 E & k_{-1} k_3 & k_{-1} k_{-2} & k_2 k_3 \\
 & \langle & & 
 \end{array}$$



$$v = -\frac{dS}{dt} = \frac{dP}{dt} = k_3(EP) - k_{-3}(E)(P) = \frac{E_0 \{ k_3(EP) - k_{-3}(E) \}}{E + ES + EP}$$

(Since  $E_0 = E + ES + EP$ )

$$v = \frac{E_0 k_3 [k_{-1}k_{-3}P + k_1k_2S + k_2k_{-3}P] - k_{-3}P [k_{-1}k_3 + k_{-1}k_{-2} + k_2k_3]}{(k_{-1}k_3 + k_{-1}k_{-2} + k_2k_3) + (k_1k_3S + k_1k_{-2}S + k_{-2}k_{-3}P) + (k_{-1}k_{-3}P + k_1k_2S + k_{-2}k_{-3}P)}$$

E

ES

EP

Collecting terms in S and P yields equation (7).

KING-ALTMAN METHOD  
J. Phys. Chem. 60, 1375 (1956)

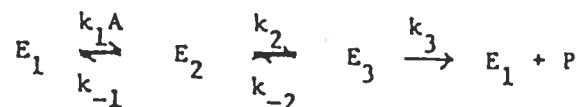
Basic Statements:

1. A mechanism involving n different enzyme containing species (including free enzyme) gives n-1 differential equations.
2. The ratio of EX<sub>i</sub> to the total enzyme concentration (EX<sub>i</sub>/E<sub>0</sub> = distribution equation) is a quotient of two summations of terms, each term being the product of n-1 rate constants (and concentration terms, if appropriate).
3. Each numerator term involves the rate constants (and concentrations, if appropriate) associated with steps leading individually or in sequence to a particular EX<sub>i</sub>. All possible combinations of the n-1 rate constants which conform to this requirement are in the numerator.
4. The denominator is the sum of all numerator terms.

NOTE: This procedure can be done by computer and the program is available.

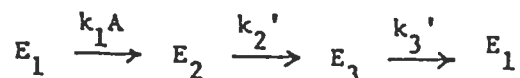
The Net Rate Constant Method  
Cleland, Biochemistry, 14, 3220 (1975)

For this derivation, the mechanism is written



The net rate constant is that constant which would produce the same flux through a particular step if that step were irreversible.

The above mechanism becomes



and the velocity of the reaction is

$$v = \frac{E_T}{\frac{1}{k_1'} + \frac{1}{k_2'} + \frac{1}{k_3'}}$$

The net rate constant for a given step is defined as the true rate constant for that step times the net rate constant for the following step divided by the sum of the latter rate constant and the true rate constant for the reversal of the step under consideration, i.e.

$$k_1' = \frac{k_1 k_2'}{k_2' + k_{-1}}$$

To work out all the net rate constants, one starts at the right of the mechanism and works back.

Thus since  $E_3 \rightarrow E_1$  is irreversible,

$$k_3' = k_3$$

$$k_2' = \frac{k_2 k_3}{k_3 + k_{-2}}$$

and

$$k_1' = \frac{k_1 \frac{k_2 k_3}{k_3 + k_{-2}}}{\frac{k_2 k_3}{k_3 + k_{-2}} + k_{-1}} = \frac{k_1 k_2 k_3 A}{k_2 k_3 + k_{-1} k_3 + k_{-1} k_{-2}}$$

To obtain  $V_{\max}$ , let  $A \rightarrow \infty$ . Thus  $\frac{1}{k_1'} \rightarrow 0$

$$\begin{aligned}
 v &= \frac{E_t}{\frac{1}{k_1'} + \frac{1}{k_2'} + \frac{1}{k_3'}} = \frac{E_t}{\frac{1}{k_2'} + \frac{1}{k_3'}} = \frac{E_t}{\frac{k_3 + k_{-2}}{k_2 k_3} + \frac{1}{k_3}} \\
 &= \frac{k_2 k_3 E_t}{k_2 + k_{-2} + k_3} = V_{\max}
 \end{aligned}$$

To obtain  $V/K_A$ , let  $A \rightarrow 0$  since, at low  $A$ ,  $\frac{v}{A} = \frac{V}{K_A}$

Under this condition,  $\frac{1}{k_1'}$  becomes dominant

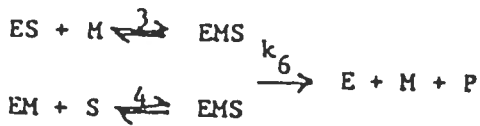
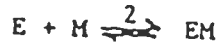
$$v = \frac{E_t k_1 k_2 k_3 A}{k_2 k_3 + k_{-1} k_3 + k_{-1} k_{-2}}$$

and

$$\frac{v}{A} = \frac{V}{K_A} = \frac{k_1 k_2 k_3}{k_2 k_3 + k_{-1} k_3 + k_{-1} k_{-2}}$$

The Single Substrate Single Modifier Case

Rapid Equilibrium Derivation



$$v_o = k_5(ES) + k_6(EMS)$$

$$v_o = \frac{k_5(E)(S)}{K_1} + \frac{k_6(E)(M)(S)}{K_1K_3(\text{or } K_2K_4)}$$

$$= E \left\{ \frac{k_5 S}{K_1} + \frac{k_6(S)(M)}{K_1K_3} \right\}$$

$$E = \frac{E_o}{1 + \frac{S}{K_1} + \frac{M}{K_2} + \frac{(S)(M)}{K_1K_3}}$$

$$v_o = \frac{E_o \frac{S}{K_1} (k_5 + k_6 \frac{M}{K_3})}{1 + \frac{S}{K_1} + \frac{M}{K_2} + \frac{(S)(M)}{K_1K_3}}$$

which is rearranged most conveniently to

$$v_o = \frac{E_o k_5 (1 + \frac{k_6 M}{k_5 K_3})}{1 + \frac{M}{K_3} + \frac{K_1}{S} (1 + \frac{M}{K_2})}$$

or

$$\frac{E_o k_5 (1 + \frac{k_6 M}{k_5 K_3}) / (1 + \frac{M}{K_3})}{1 + \frac{K_1}{S} \left\{ \frac{1 + M/K_2}{1 + M/K_3} \right\}}$$

(general equation)



For Competitive Inhibition

$$K_3 \text{ (or } K_4) = \infty \text{ since ESM cannot form}$$

$$k_6 = 0$$

then

$$v = \frac{k_5 E_o}{1 + \frac{K_1}{S} \left(1 + \frac{M}{K_2}\right)}$$

Uncompetitive (either activation or inhibition)

$$1/K_2 = \frac{k_6}{k_5 K_3} \quad \text{For complete inhibition } k_6 = 0, \quad K_2 = \infty$$

or

$$\frac{K_3}{K_2} = \frac{k_6}{k_5}$$

then

$$v_o = \frac{k_5 E_o (1 + M/K_2) / (1 + M/K_3)}{1 + \frac{K_1}{S} \left\{ \frac{1 + M/K_2}{1 + M/K_3} \right\}} = \frac{E_o k_5 a}{1 + \frac{K_1 a}{S}}$$

i.e., The apparent  $V_{\max}$  and  $K_m$  are always changed by same amount.

## Two Substrate Cases

Rapid Equilibrium

$$\text{Random } v = \frac{V}{1 + \frac{K_A}{A} + \frac{K_B}{B} + \frac{K_{AB}}{(A)(B)}}$$

$K_A, K_B$  are dissociation constants

$K_{AB}$  is not necessarily the product of two dissociation constants

Steady State

Ordered

$$v = \frac{V}{1 + \frac{K_A}{A} + \frac{K_B}{B} + \frac{K_{AB}}{(A)(B)}}$$

with one  
ternary complex

$$V = k_3 k_4 E_0 / (k_3 + k_4)$$

$$K_A = k_3 k_4 / k_1 (k_3 + k_4)$$

$$K_B = (k_{-2} + k_3) k_4 / k_2 (k_3 + k_4)$$

$$K_{AB} = k_{-1} (k_{-2} + k_3) k_4 / k_1 k_2 (k_3 + k_4)$$

with no ternary  
complex

$$V = k_3 E_0$$

$$K_A = k_4 / k_1$$

$$K_B = k_4 / k_2$$

$$K_{AB} = \frac{k_{-1} k_4}{k_1 k_2}$$

("Theorell-Chance"  
Mechanism)

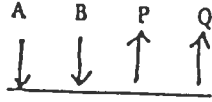
## Nomenclature for Two Substrate Cases

	Used initially	Bloomfield, Peller and Alberty, JACS <u>84</u> , 4367 (1962)	Cleland, BBA <u>67</u> , 104 (1963)	Dalziel, Acta Chem. Scand. <u>11</u> , 1706 (1957)
$V_{\max}$ i.e. $A = B = \phi$	$V$	$V_{AB}$	$V_1$	$e/\phi_0$
Turnover number	$V/(E)_0$	$V_{AB}/(E)_0$	$V_1/(E)_t$	$1/\phi_0$
Michaelis Const. for A ( $B = \phi$ )	$K_A$	$\frac{K_{AB}}{K_B}$	$K_a$	$\phi_1/\phi_0$
Michaelis Const. for B ( $A = \phi$ )	$K_B$	$\frac{K_{AB}}{K_A}$	$K_b$	$\phi_2/\phi_0$
Dissociation constant for EA complex	$\frac{K_{AB}}{K_B}$	$K_A$	$K_{ia}$	$\phi_{12}/\phi_2$
	(1)	(2)	(3)	(4)

## Reciprocal Equations

$$\begin{aligned}
 1) \quad \frac{(E)_0}{v} &= \frac{1}{V} + \frac{K_A}{(A)V} + \frac{K_B}{(B)V} + \frac{K_{AB}}{(A)(B)V} \\
 2) &= \frac{1}{V_{AB}} + \frac{K_{AB}}{(A)K_B V_{AB}} + \frac{K_{AB}}{(B)K_A V_{AB}} + \frac{K_{AB}}{(A)(B)V_{AB}} \\
 3) &= \frac{1}{V_1} + \frac{K_a}{(A)V_1} + \frac{K_b}{(B)V_1} + \frac{K_{ia} K_b}{V_1 (A)(B)} \\
 4) &= \phi_0 + \frac{\phi_1}{A} + \frac{\phi_2}{B} + \frac{\phi_{12}}{(A)(B)}
 \end{aligned}$$

Full Expression for Ordered Bi Bi (Steady State)



$$v = \frac{v_1 v_2 \left( AB - \frac{PQ}{K_{eq}} \right)}{K_{ia} K_b v_2 + K_b v_2 A + K_a v_2 B + v_2 AB}$$


---


$$+ \frac{K_q v_1 P}{K_{eq}} + \frac{K_p v_1 Q}{K_{eq}} + \frac{v_1 PQ}{K_{eq}}$$


---


$$+ \frac{K_q v_1 AP}{K_{ia} K_{eq}} + \frac{K_a v_2 BQ}{K_{iq}} + \frac{v_2 ABP}{K_{ip}} + \frac{v_1 BPQ}{K_{ib} K_{eq}}$$

## Product Inhibition Patterns for Selected Cases

P is the first product off in sequential mechanisms

C = competitive

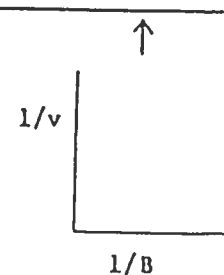
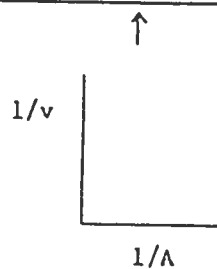
U = uncompetitive

NC = mixed or noncompetitive

- = no effect

Abortive complexes not included

	Inhibitory Product	Variable S = A		Variable S = B	
		Not sat'd with B	Sat'd with B	Not sat'd with A	Sat'd with A
Ordered Bi Bi	P	NC	UC	NC	NC
	Q	C	C	NC	-
Theorell-Chance	P	NC	-	C	C
	Q	C	C	NC	-
Random Bi Bi (Rapid Equil.)	P or Q	C	-	C	-
Random Bi Bi (Steady State)	P or Q	noncompetitive or mixed		noncompetitive or mixed	
		Possible nonlinearity			



Steady State

Random

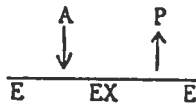
Has  $A^2$ ,  $B^2$ ,  $AB^2$  and  $BA^2$  terms and may give non-linear reciprocal plots.

Ping-Pong

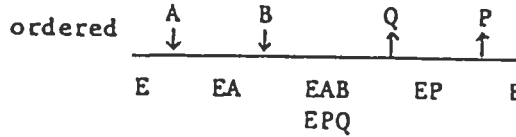
$$v = \frac{V}{1 + \frac{K_A}{A} + \frac{K_B}{B}}$$

Cleland Nomenclature, BBA, 67, 104, 173, 188 (1963)

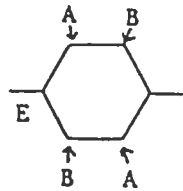
Uni-Uni = one substrate  
one product



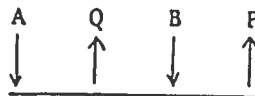
Bi Bi = two substrates  
two products



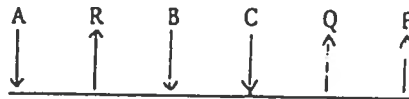
random



ping-pong



Uni Uni Bi Bi Ping Pong



Koshland Equation (n = 4: Square Geometry)

$$n\bar{Y} = \frac{4K_{AB}^2 [K_S K_t(S)] + 4(K_{AB}^4 + 2K_{AB}^2 K_{BB}) [K_S K_t(S)]^2 + 12K_{AB}^2 K_{BB}^2 [K_S K_t(S)]^3 + 4K_{BB}^4 [K_S K_t(S)]^4}{L + 4K_{AB}^2 [K_S K_t(S)] + (2K_{AB}^4 + 4K_{AB}^2 K_{BB}) (K_S K_t(S))^2 + 4K_{AB}^2 K_{BB}^2 [K_S K_t(S)]^3 + K_{BB}^4 [K_S K_t(S)]^4}$$

where  $K_{AB}$ ,  $K_{BB}$  define interactions between subunits.

These equations of Koshland are described in general by

$$N_S = \frac{aS + bS^2 + cS^3 + dS^4}{1 + aS + \frac{b}{2}S^2 + \frac{c}{3}S^3 + \frac{d}{4}S^4}$$

while those of Monod are

$$\frac{aS + bS^2 + cS^3 + dS^4}{1 + aS + 2bS^2 + \frac{4}{3}cS^3 + dS^4}$$

Hill Equation:

$$\bar{Y} = \frac{n}{1 + \frac{K_A}{A^n}}$$

where  $\bar{Y}$  is the fraction of saturation,  $K_A$  the dissociation constant and  $n$  the interaction coefficient.  $n$  is obtained from a plot of

$$\log \frac{\bar{Y}}{1-\bar{Y}} \text{ vs } \log A.$$

General Interaction Equation (4 sites)

$$\frac{\bar{Y}}{n} = \frac{\frac{A}{K_A} [1 + \frac{3iA}{K_A} + 3i^2j(\frac{A}{K_A})^2 + i^3j^2k(\frac{A}{K_A})^3]}{1 + 4\frac{A}{K_A} + 6i(\frac{A}{K_A})^2 + 4i^2j(\frac{A}{K_A})^3 + k^3j^2k(\frac{A}{K_A})^4}$$

where  $i$  is the factor by which the remaining 3 sites change affinity for  $A$  when one site occupied,  $j$  the remaining two sites, etc.

Simple Monod Equation:

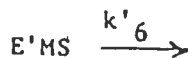
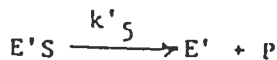
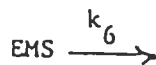
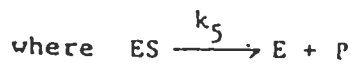
$$\bar{Y} = \frac{\alpha(1 + \alpha)^{n-1} + Lc\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L(1 + c\alpha)^n}$$

where  $\alpha = \frac{A}{K_A}$ ;  $L$  is the equilibrium constant for the  $E \rightleftharpoons E'$  step ( $L =$

$E'/E$ ) and  $c$  is the ratio of dissociation constants of  $A$  to the two forms. For cooperative effects, the initial concentration of  $E \ll E'$  and  $A$  binds preferentially to the  $E$  form.

More General Monod Equation:

$$\frac{\bar{Y}}{n} = \frac{(k_5 + k_6\mu)\alpha(1 + \alpha)^{n-1} (1 + \mu)^{n-1} + (k_5' + k_6'd\mu) Lc\alpha(1 + c\alpha)^{n-1} (1 + d\mu)^{n-1}}{(1 + \mu)^n(1 + \alpha)^n + L(1 + d\mu)^n (1 + c\alpha)^n}$$





Monomer-Dimer case: (Monod type assumption)

$$\frac{Y}{n} = \frac{\alpha(1 + \alpha)^{n-1} + 2 K_{eq} (E)d\alpha(1 + d\alpha)^{2n-1}}{(1 + \alpha)^n + 2 K_{eq}(E) (1 + d\alpha)^{2n}}$$

where  $K_{eq} = E_2/E^2$  and  $E$ , the free monomer concentration is

$$= \frac{-(1 + \alpha)^n + \sqrt{(1 + \alpha)^{2n} + 8 E_0 K_{eq} (1 + d\alpha)^{2n}}}{4 K_{eq} (1 + d\alpha)^{2n}}$$

References to the Original Allosteric Papers:

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