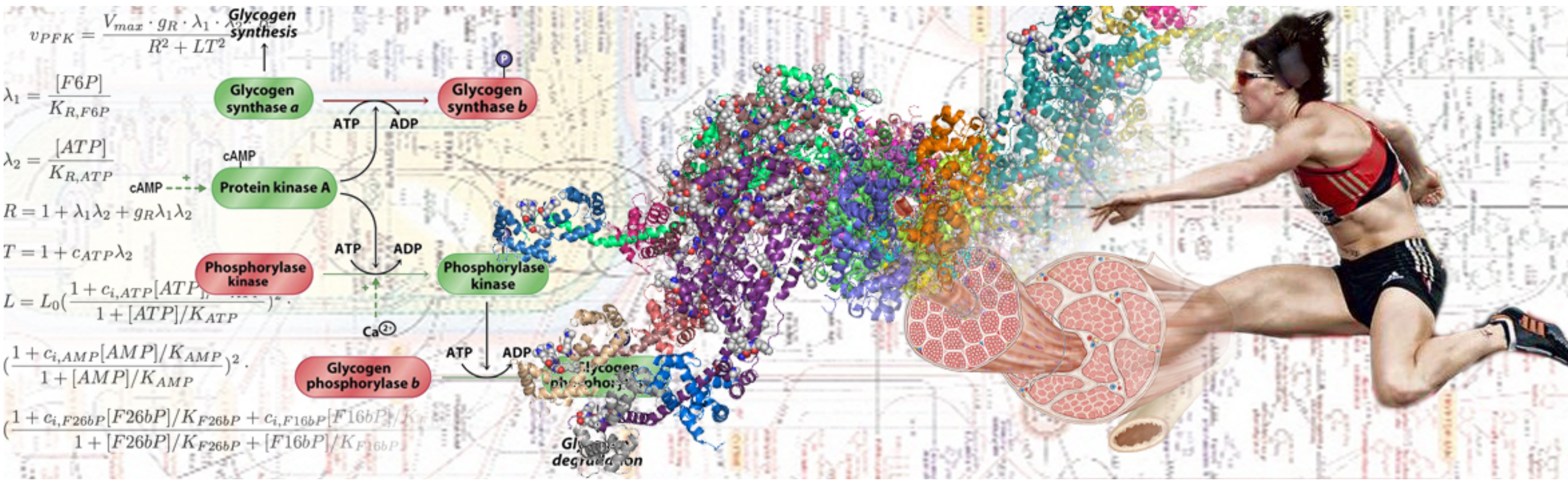
Mini-course: Molecular Systems Biology



Profs Jacky Snoep and Johann Rohwer

March 2018

Thus far

- First Lecture: Chemical kinetics
- Direction of reaction: dG, Gamma/Keq
- How far: Keq, dG^0; How fast: mass action kinetics
- Second Lecture: Enzyme kinetics
- Derivation of rate equations: equilibrium binding, steady state approximation
- Vmax, Km, saturation, cooperativity, allostery, reversibility, product inhibition
- Third Lecture: Coupled reactions
- Parameter estimation; initial rates, progress curves
- Closed, open systems; equilibrium, steady state, rate characteristics
- Fourth Lecture: Structural network analysis
- N, K, L matrix
- Steady state flux constraints, Flux analysis, Flux modes
- Flux balance analysis

Exercise

1) Derive the rate equation for a two substrate, two product
reaction, assuming rapid equilibrium binding, and a random order mechanism.

2) A substrate is delivered at a constant rate, k and is consumed by an enzyme that follows classic MM kinetics (i.e. irreversible, product insensitive). If the Vm of the enzyme is 1/10 of k, what Km would result in a substrate concentration of 1 at steady state?

Metabolic Control Analysis

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Triple J Group
Department of Biochemistry
University of Stellenbosch

MCA

- **Quantifies the importance of each of the enzymes for steady state system variables.**

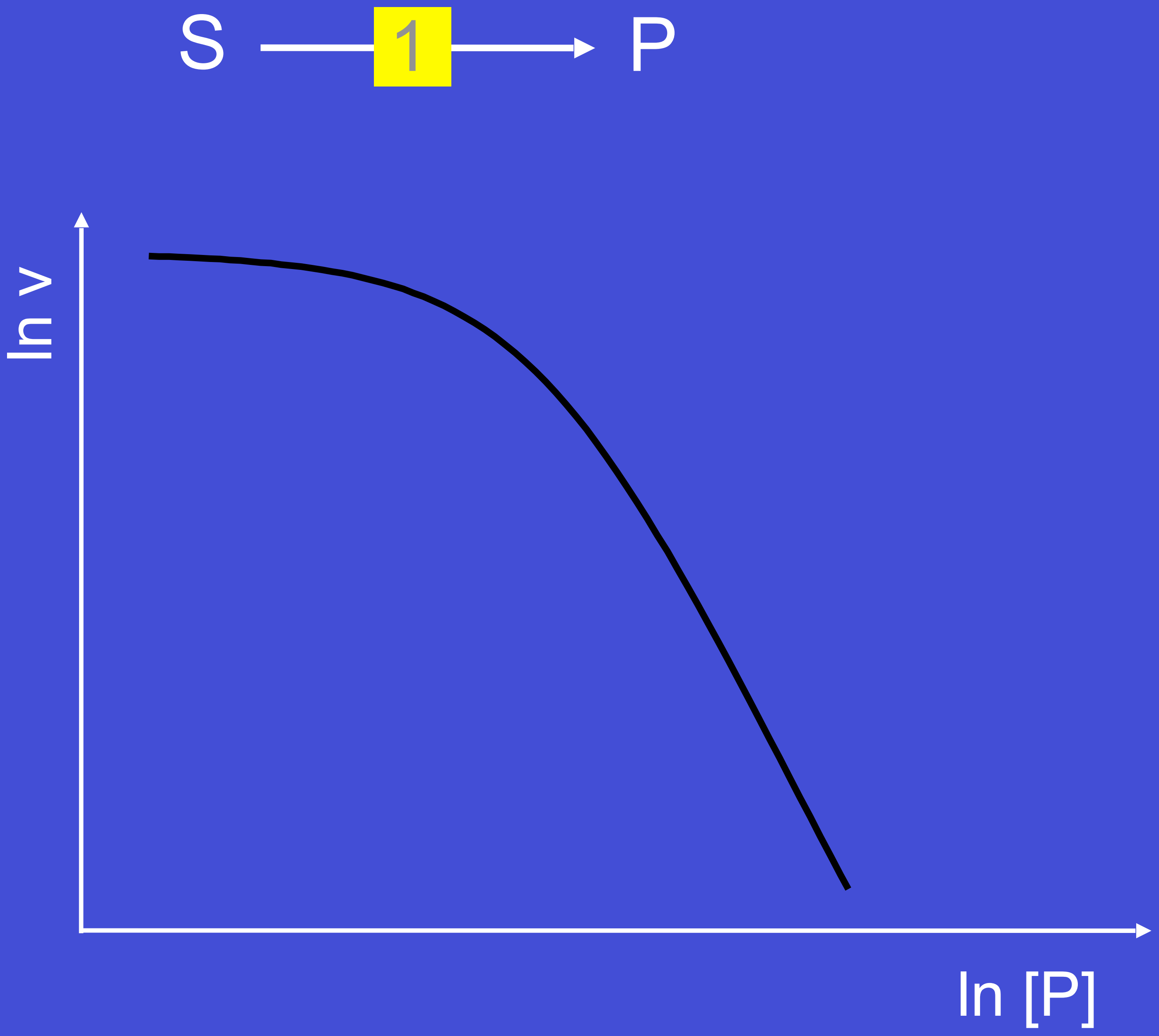
- **Relates this importance for system behavior to characteristics of the isolated components.**

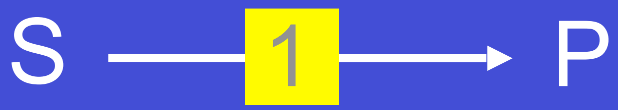
MCA

This lecture:

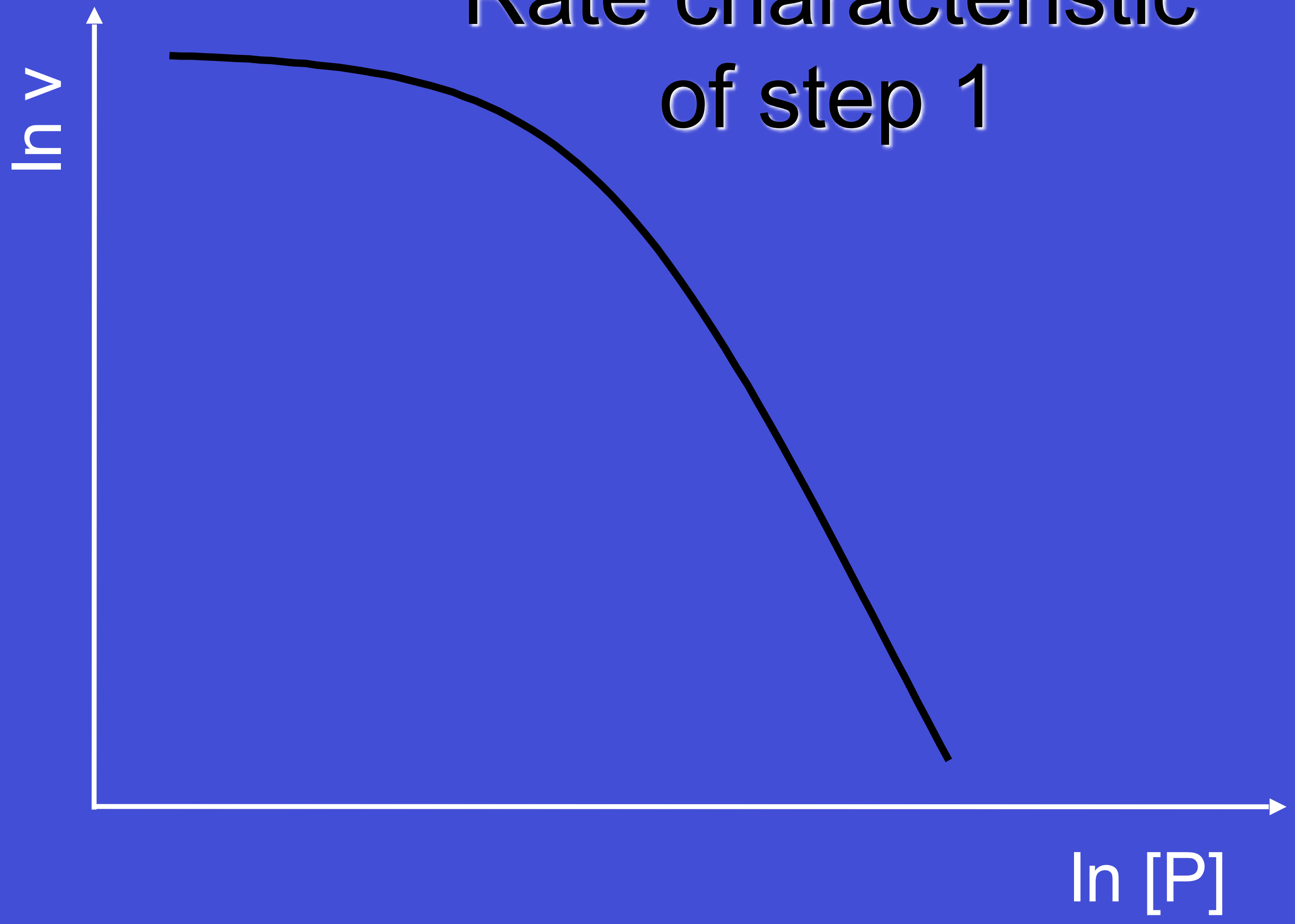
•Use a graphical method to explain the concept of MCA.

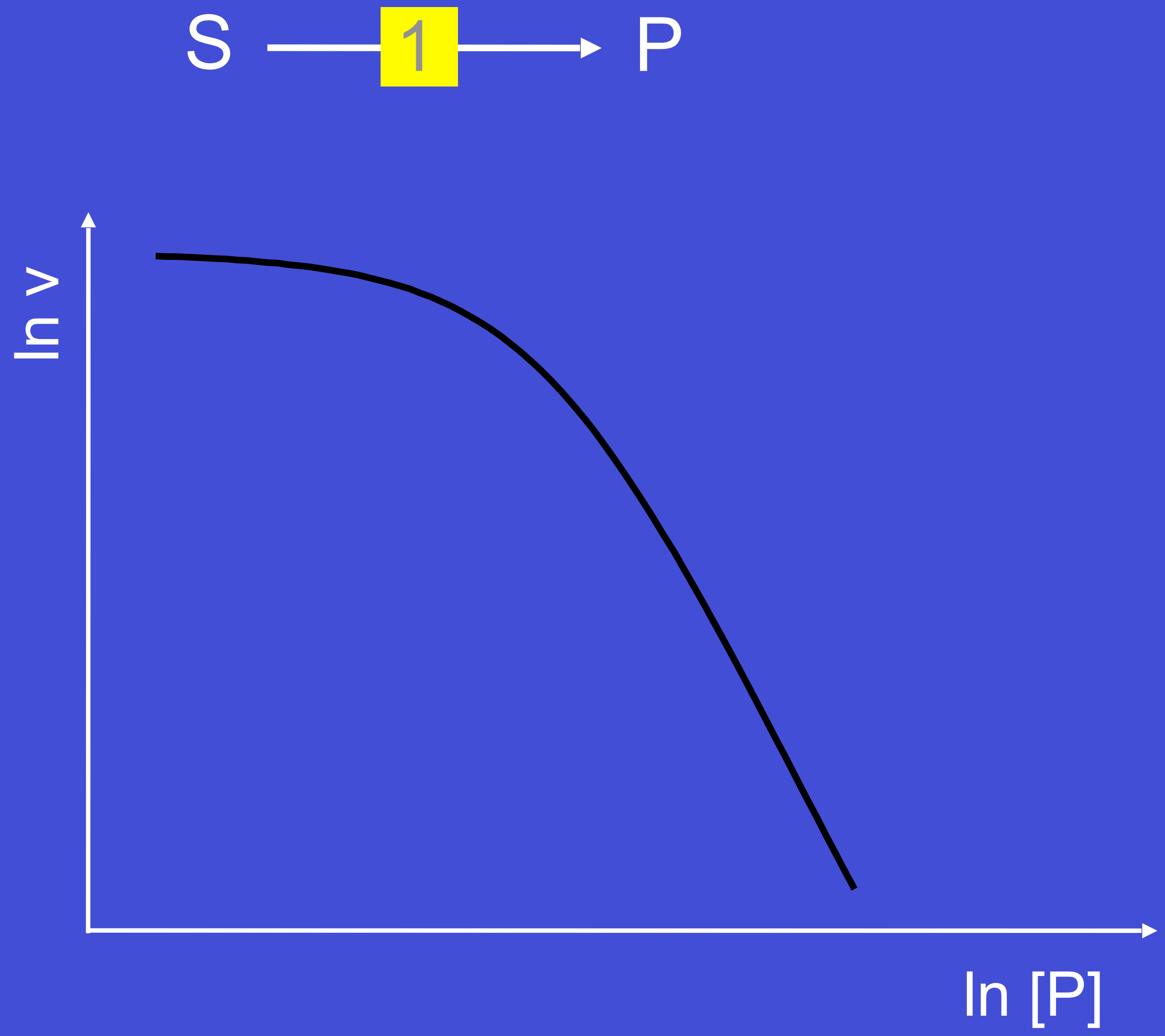
•Simple system, two enzymes and use so-called rate characteristics

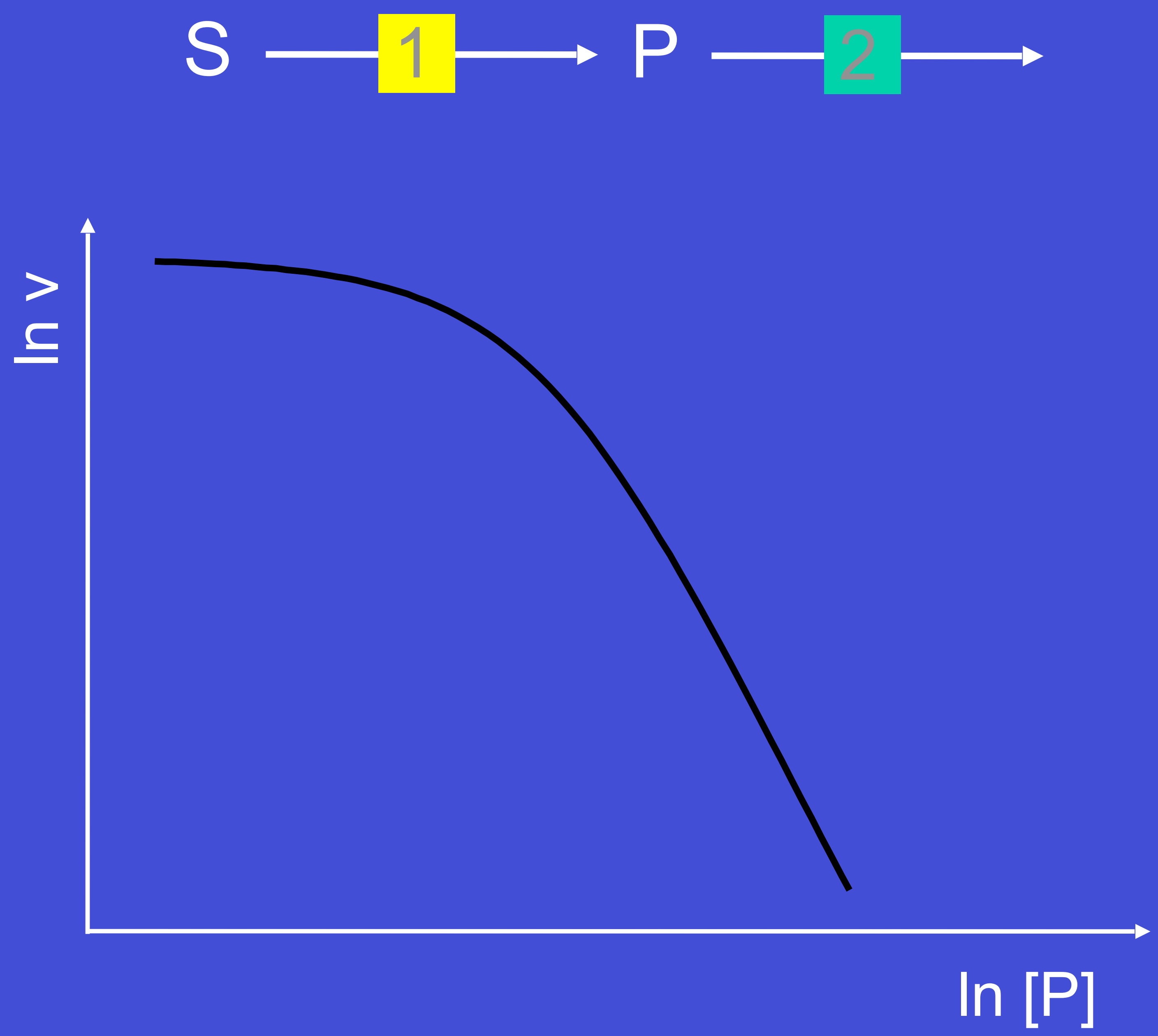

S
1
P
ln v
ln [P]

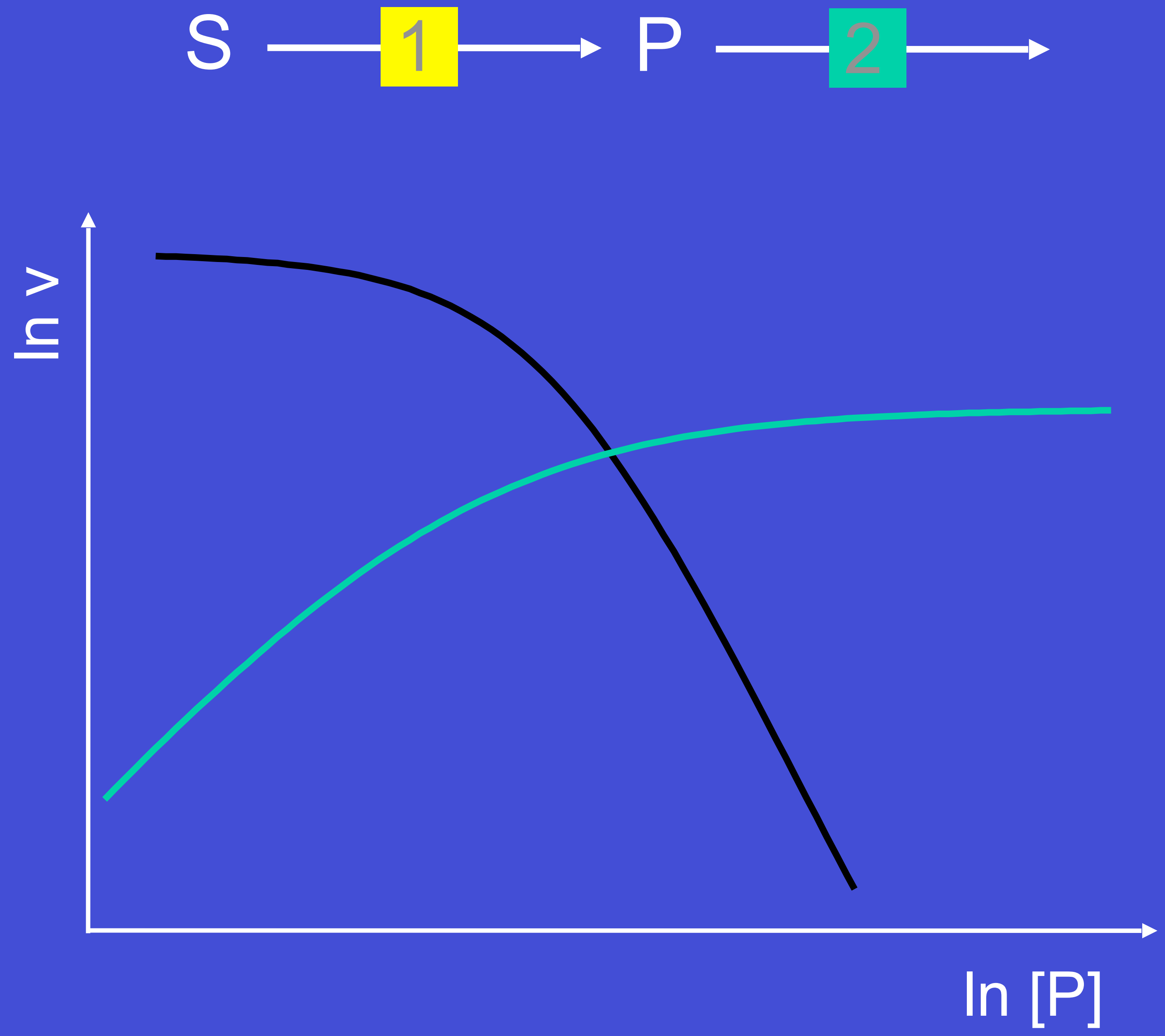


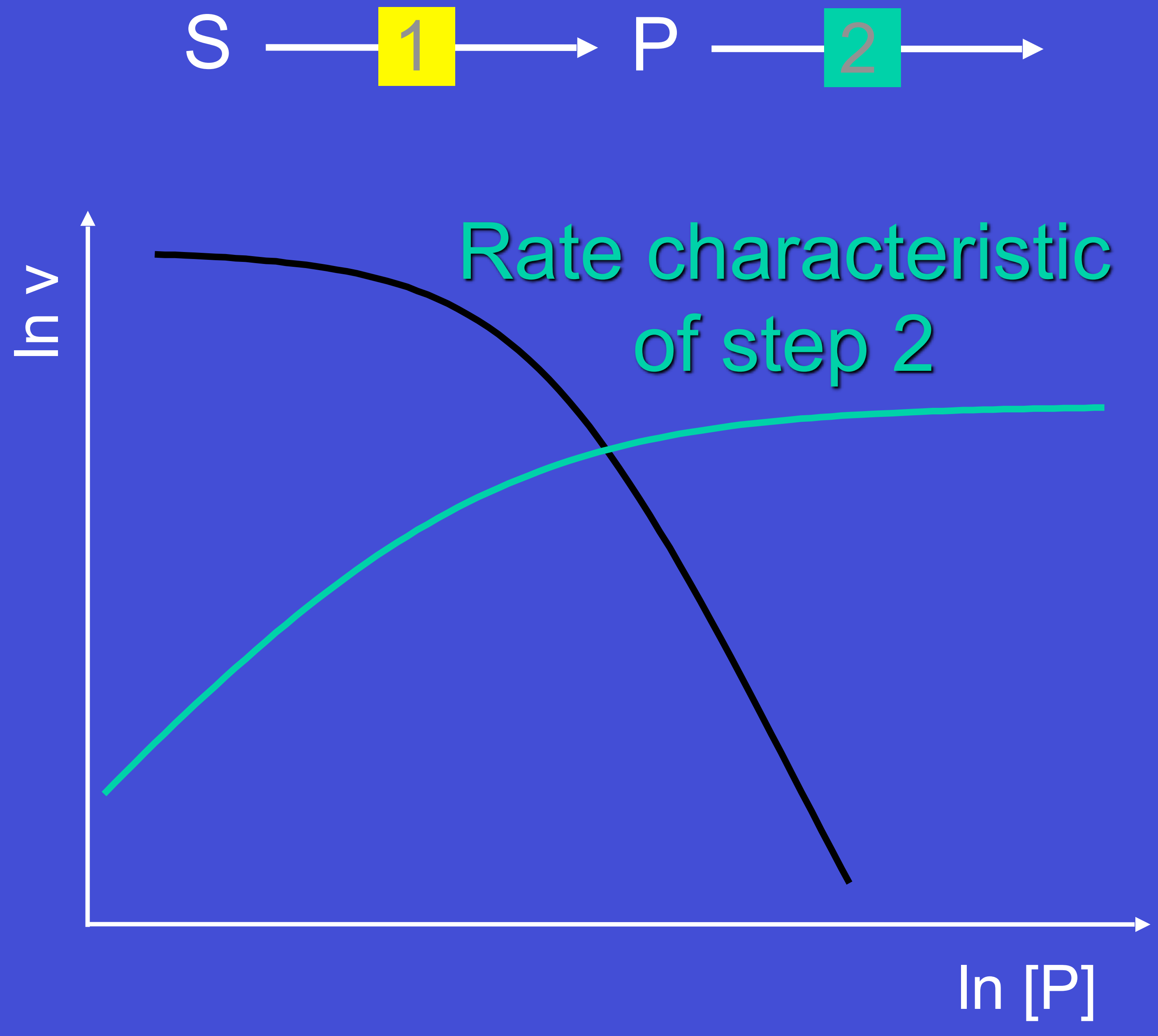
Rate characteristic of step 1

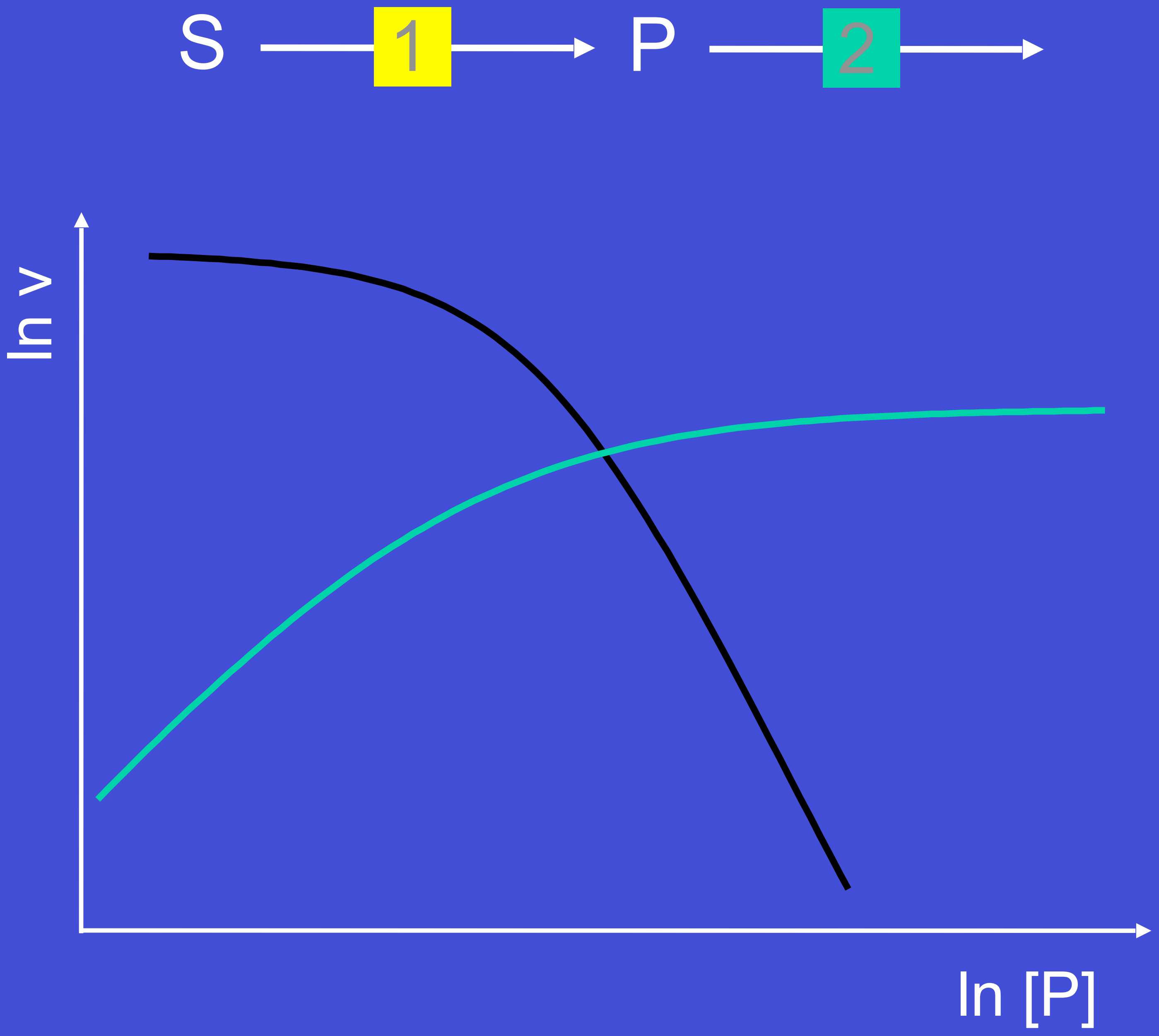



S
1
P
ln v
ln [P]

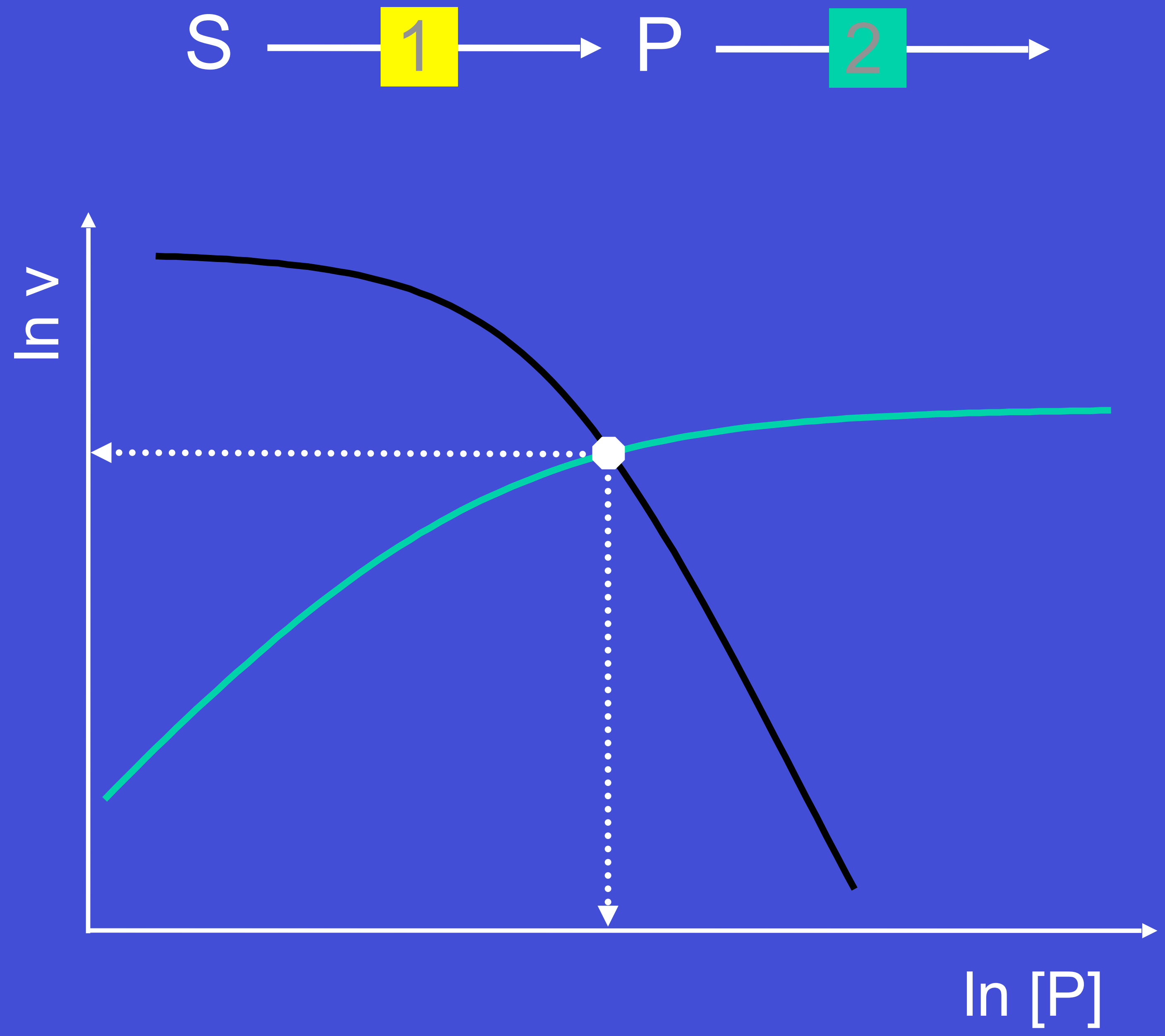

S
1
P
2
ln v
ln [P]


S
1
P
2
ln v
ln [P]

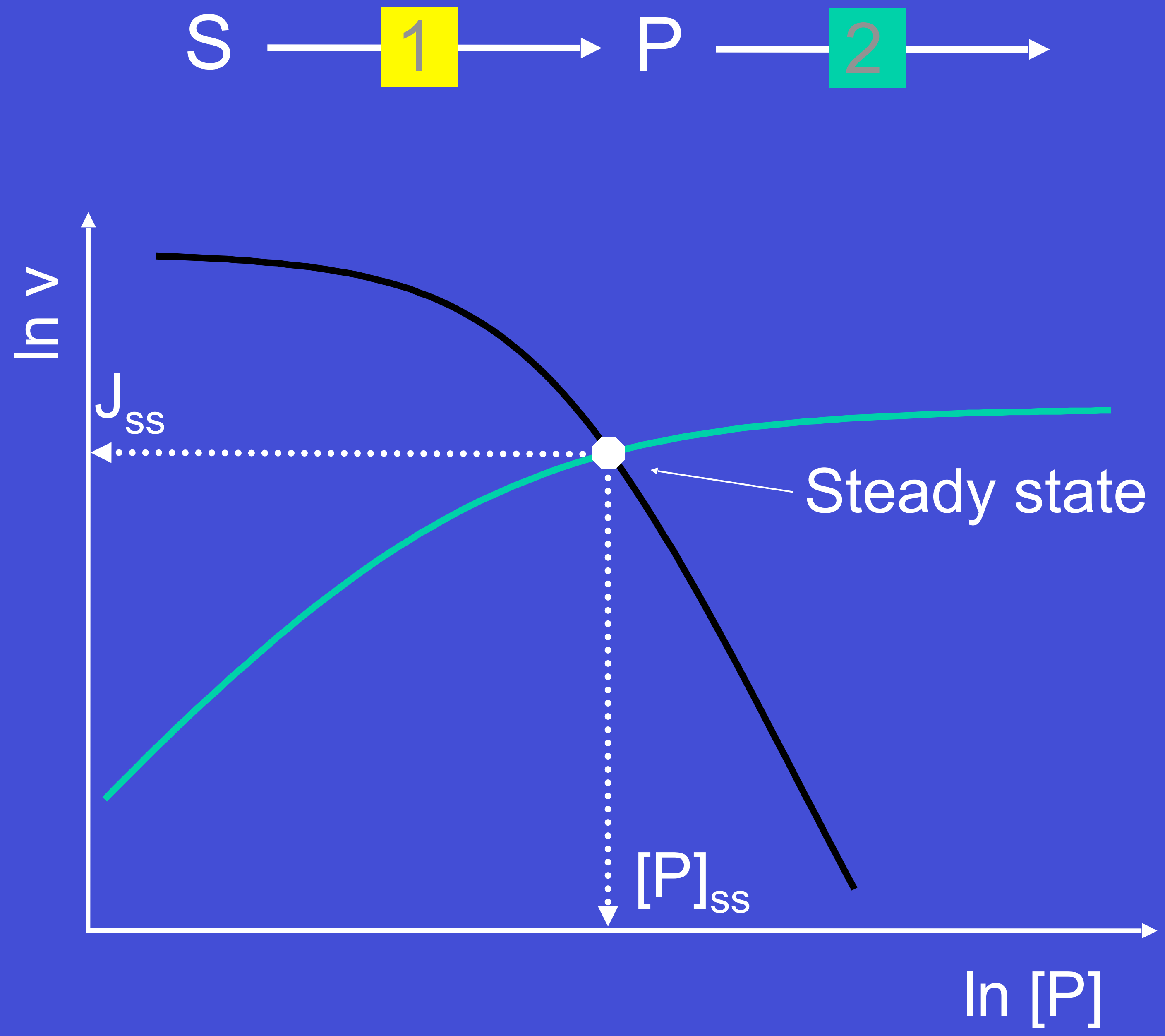

S
1
P
2
Rate characteristic
of step 2
ln v
ln [P]

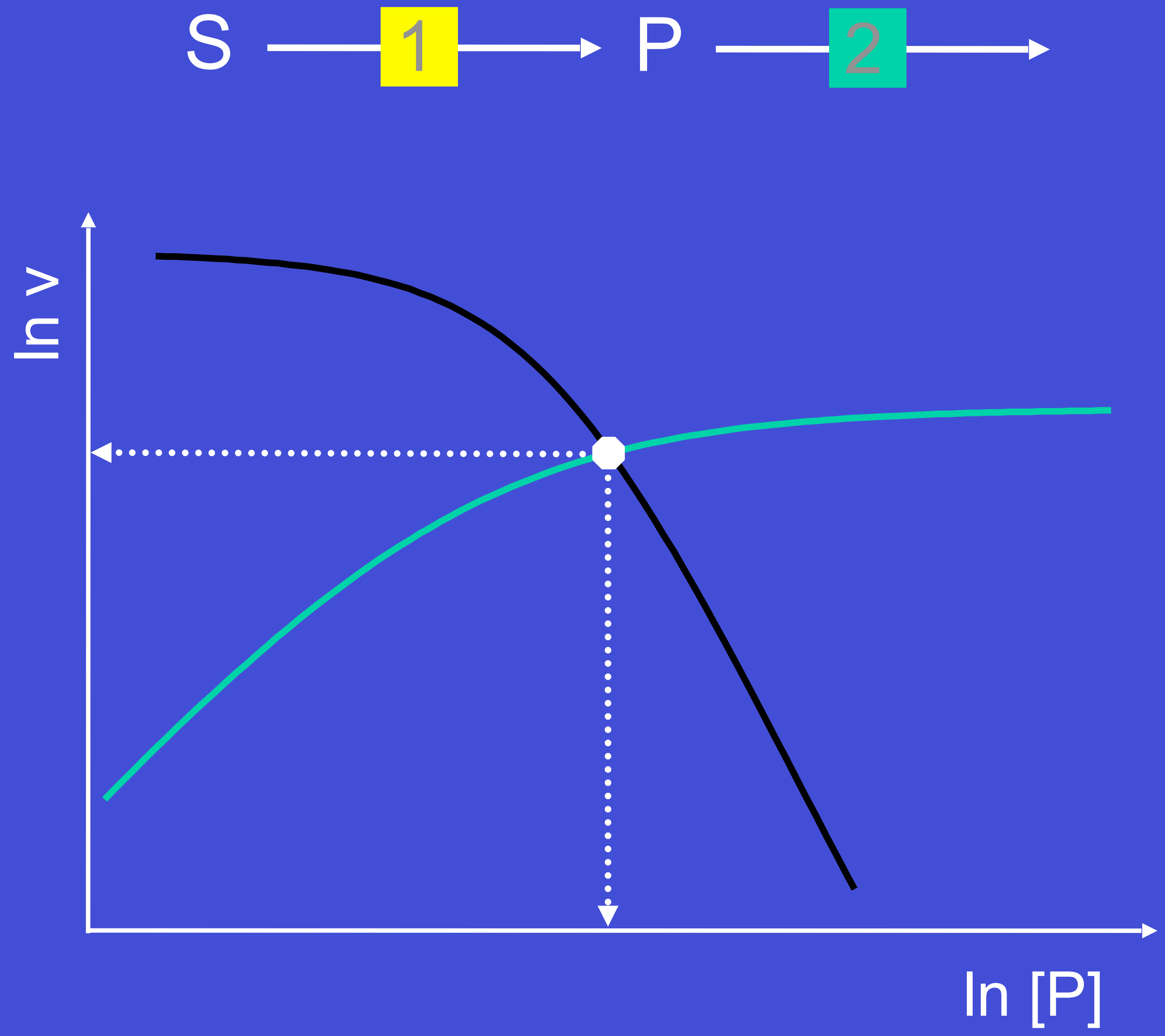


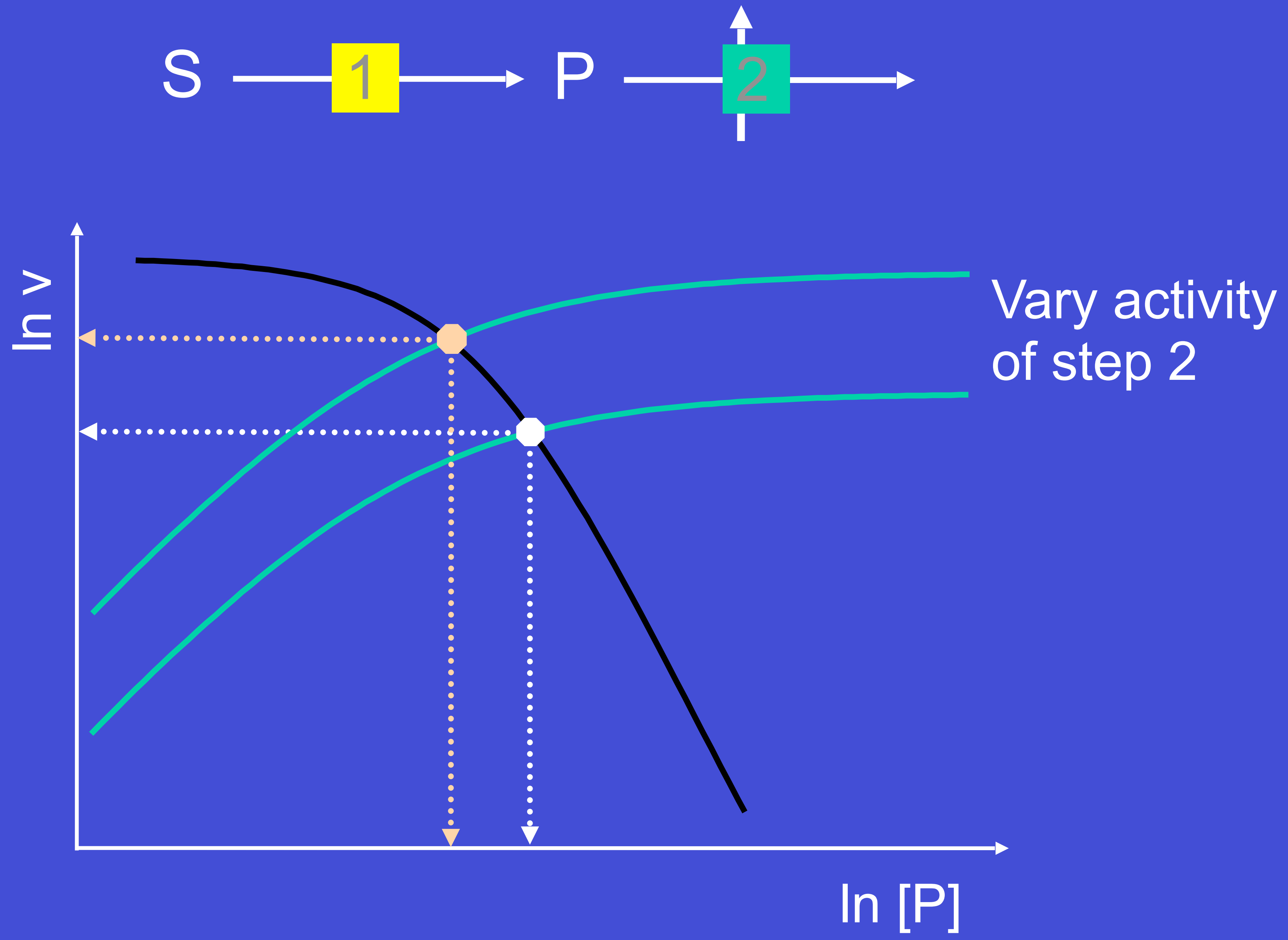
S
1
P
2
ln v
ln [P]

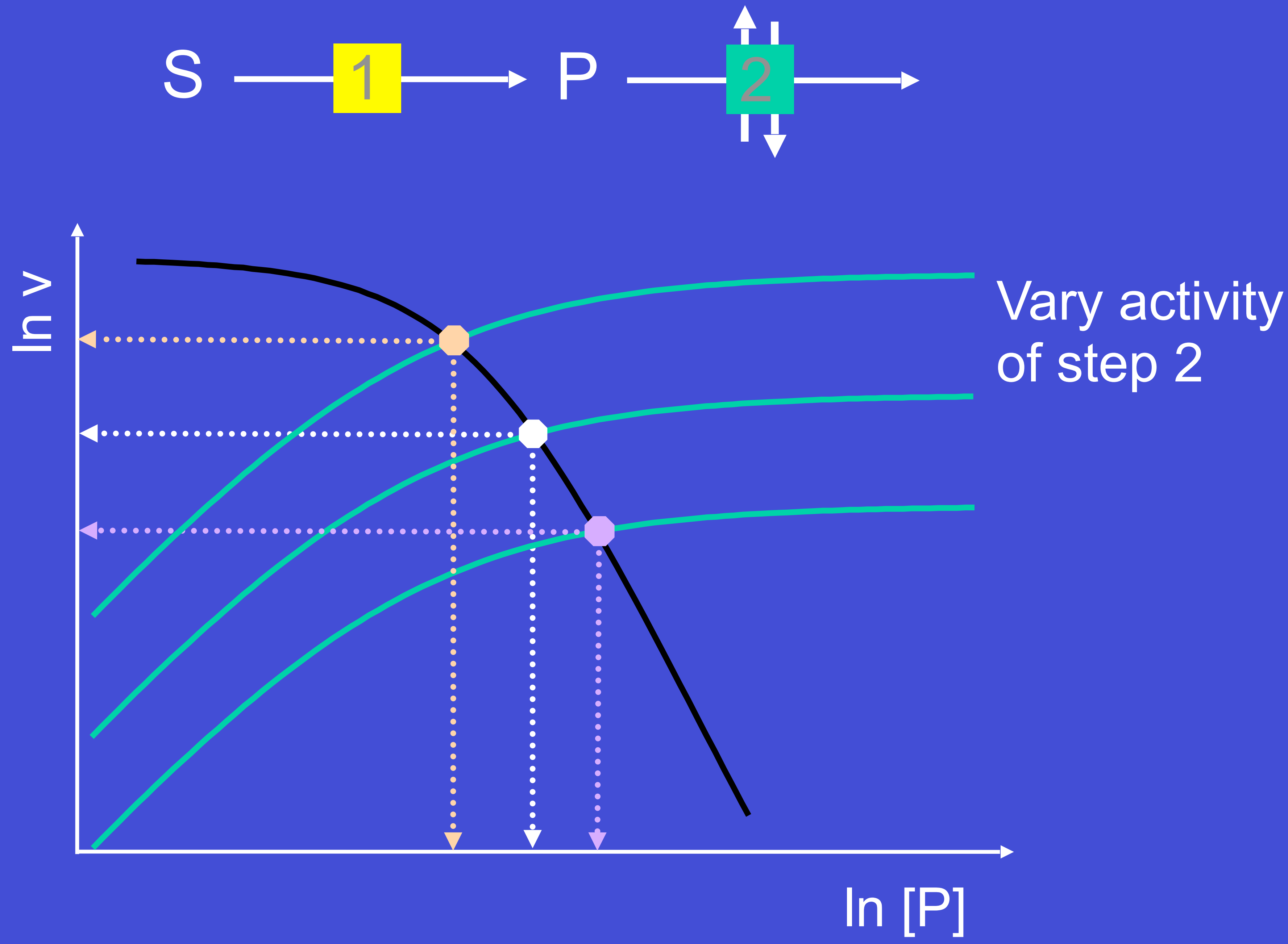


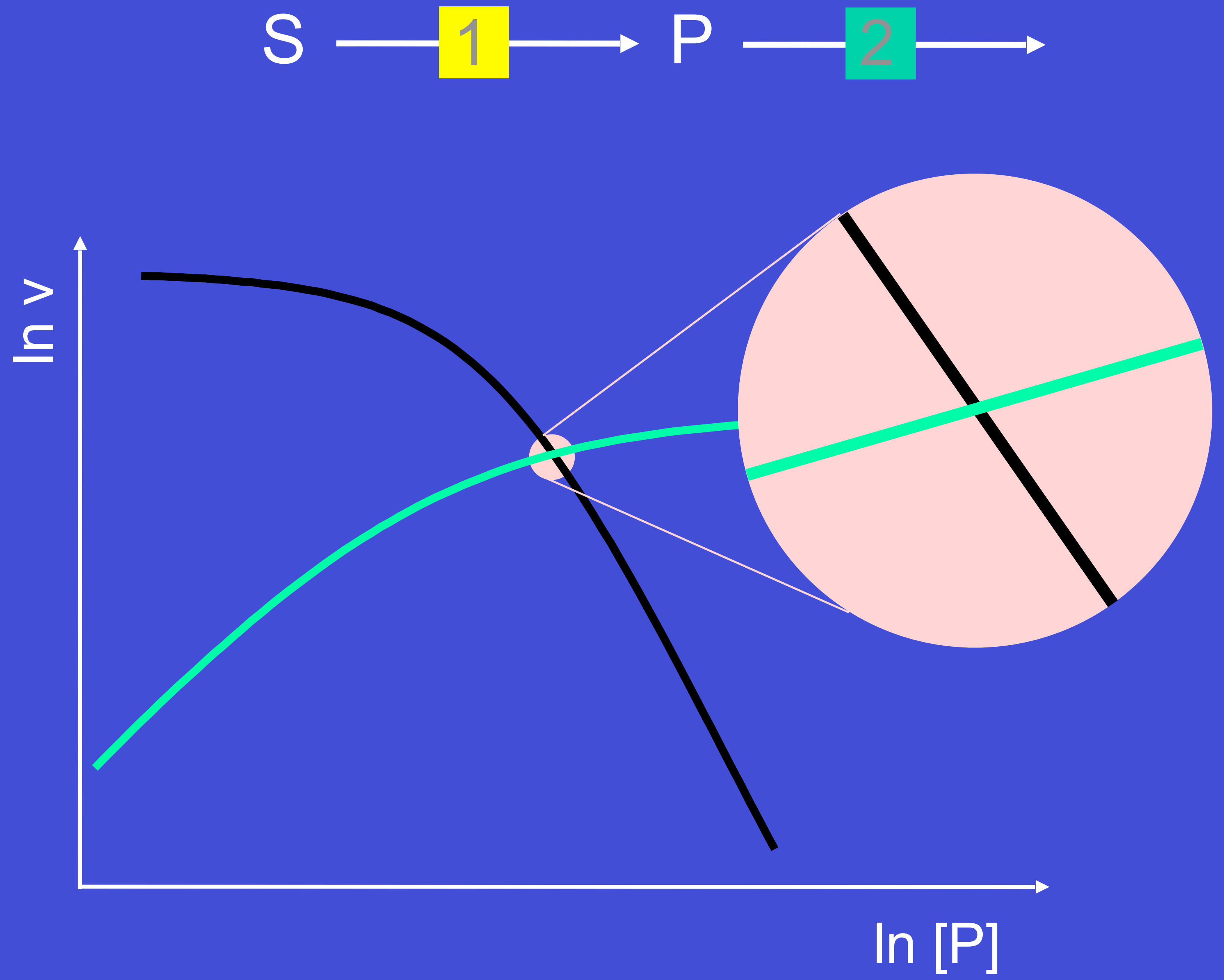
S
1
P
2
ln v
ln [P]


S
1
P
2
ln v
J ss
Steady state
[P]ss
ln [P]


S
1
P
2
ln v
ln [P]

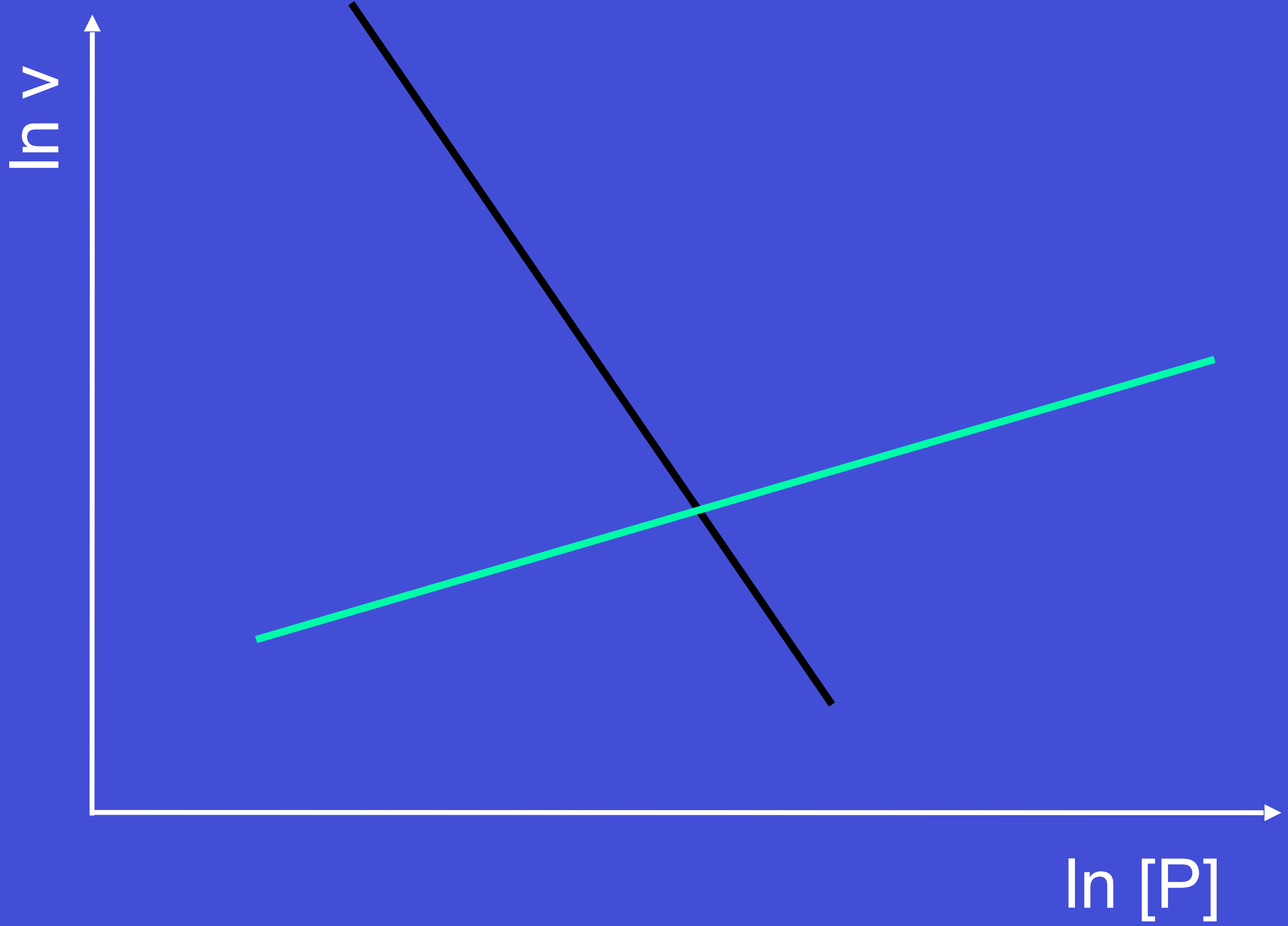

S
1
P
2
ln v
Vary activity of step 2
ln [P]

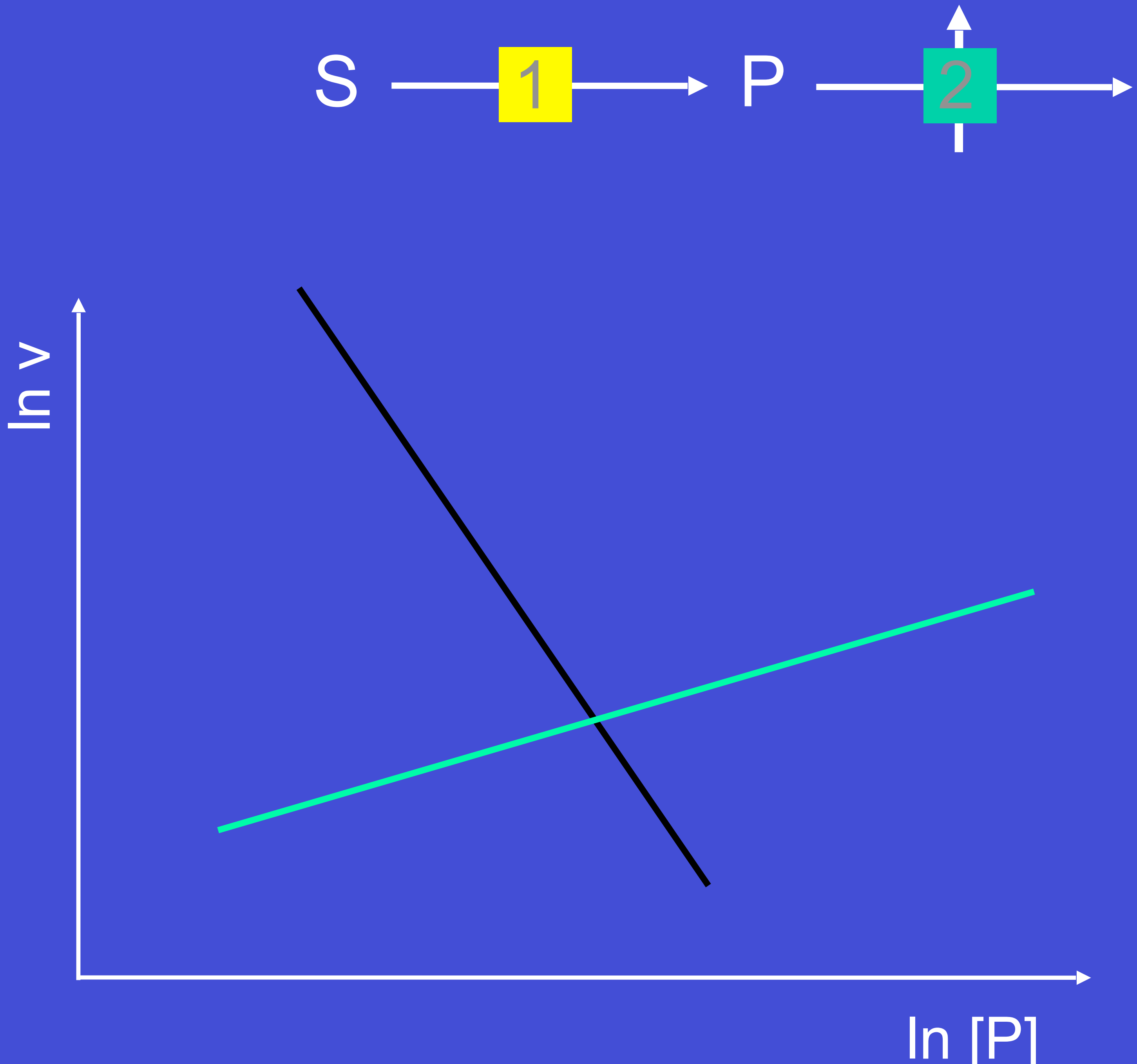

S
1
P
2
ln v
Vary activity of step 2
ln [P]



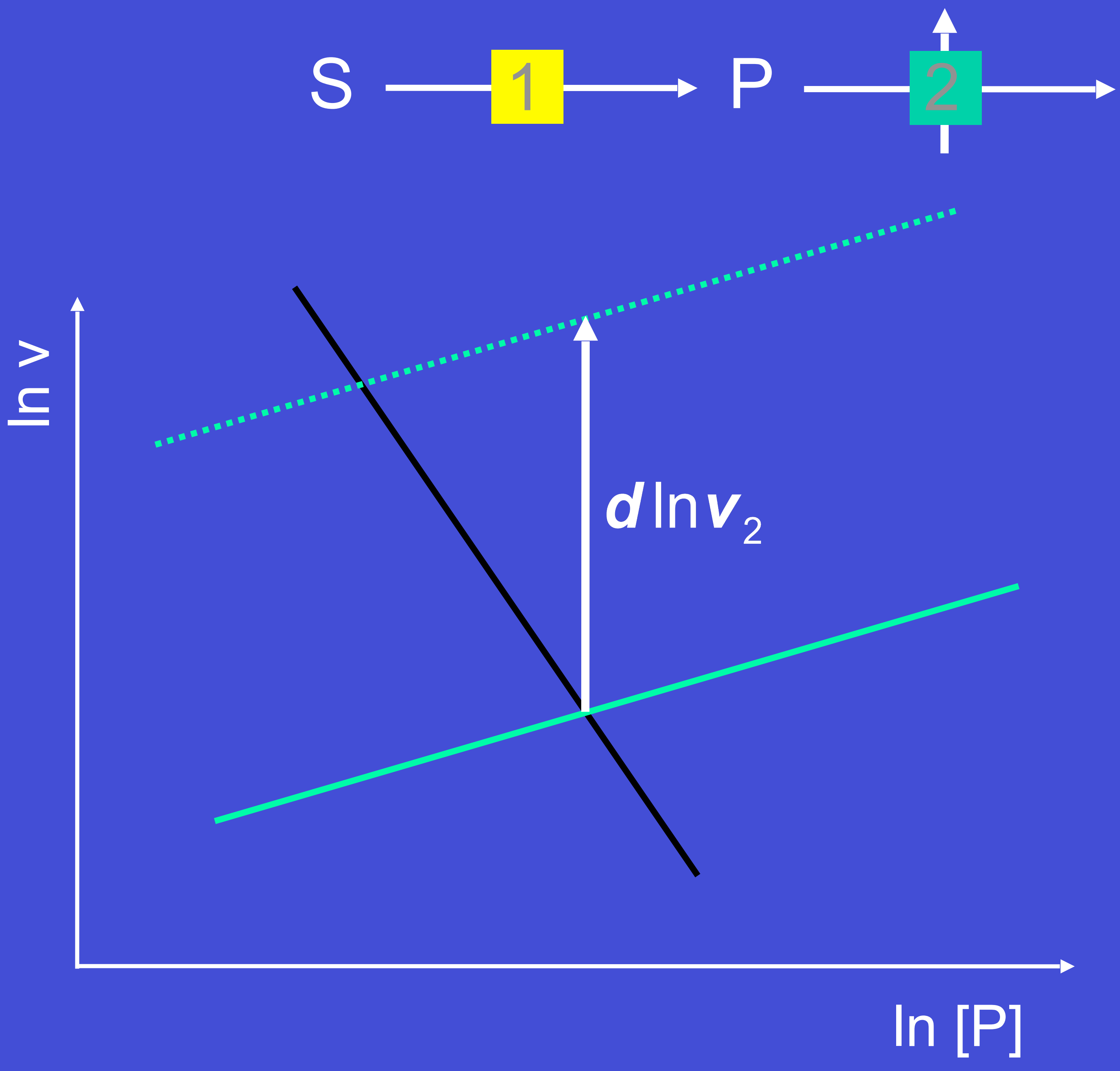
S
1
P
2
ln v
ln [P]

$$S \xrightarrow{1} P \xrightarrow{2}$$

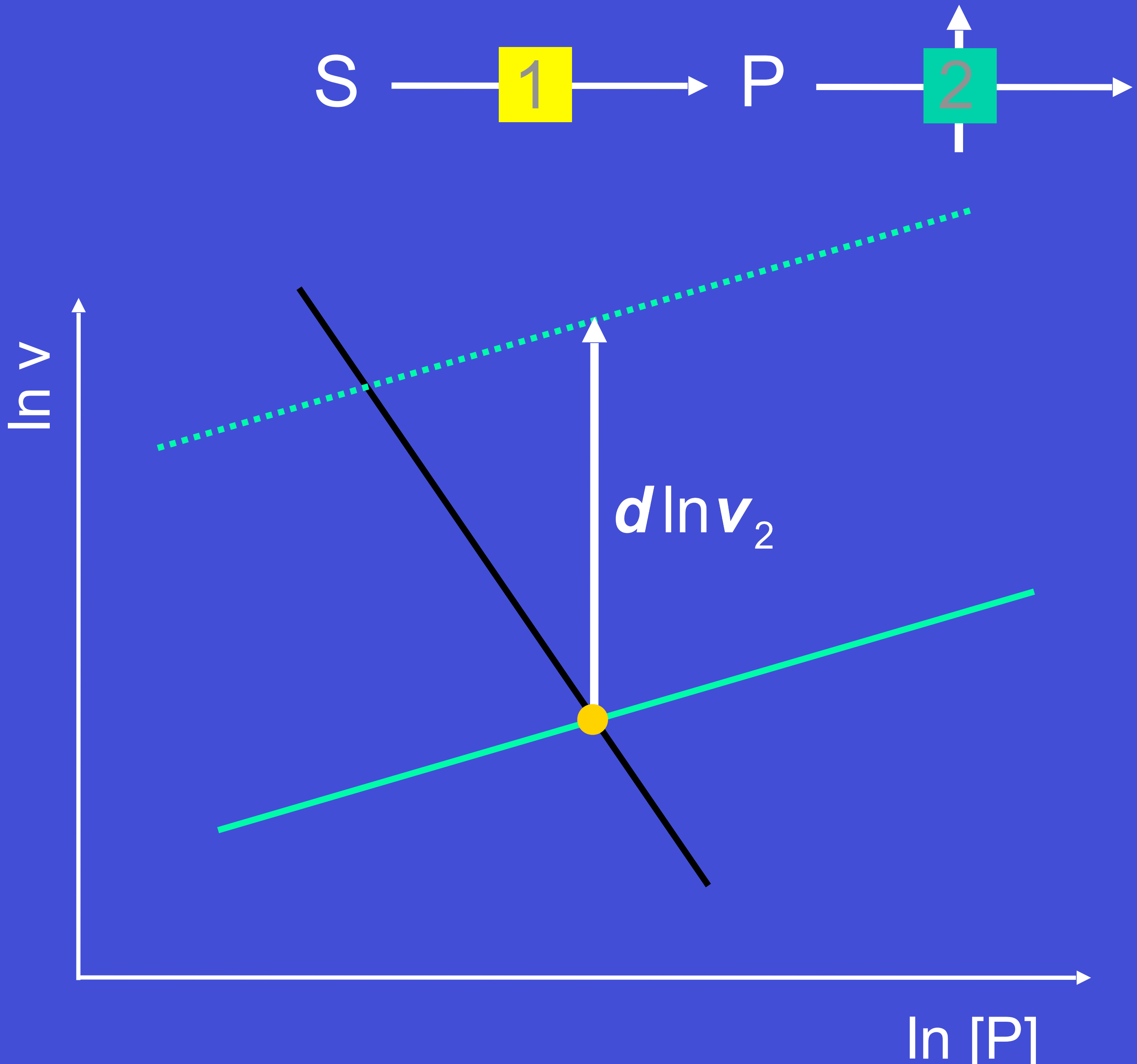




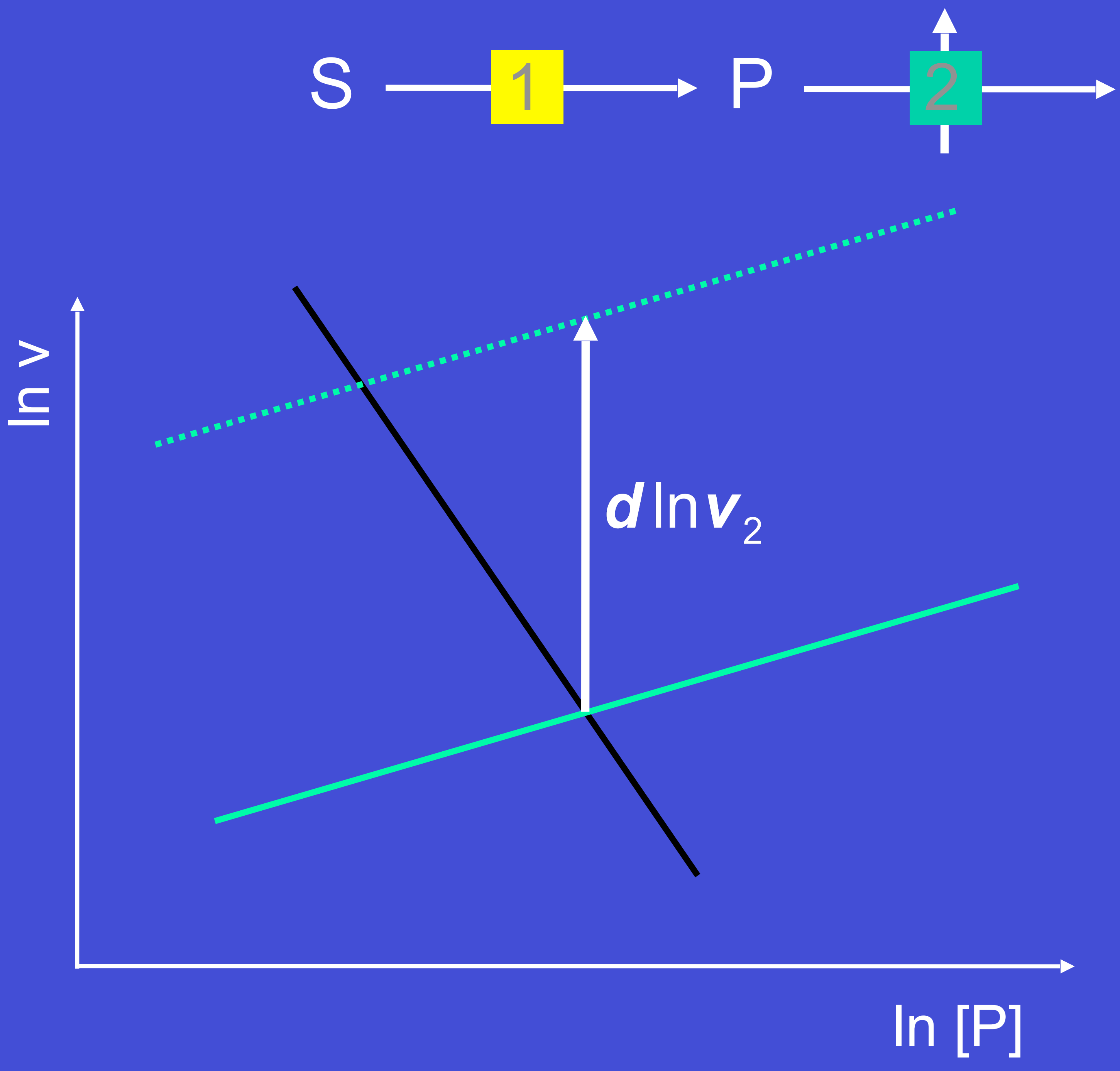
S
1
P
2
ln v
ln [P]

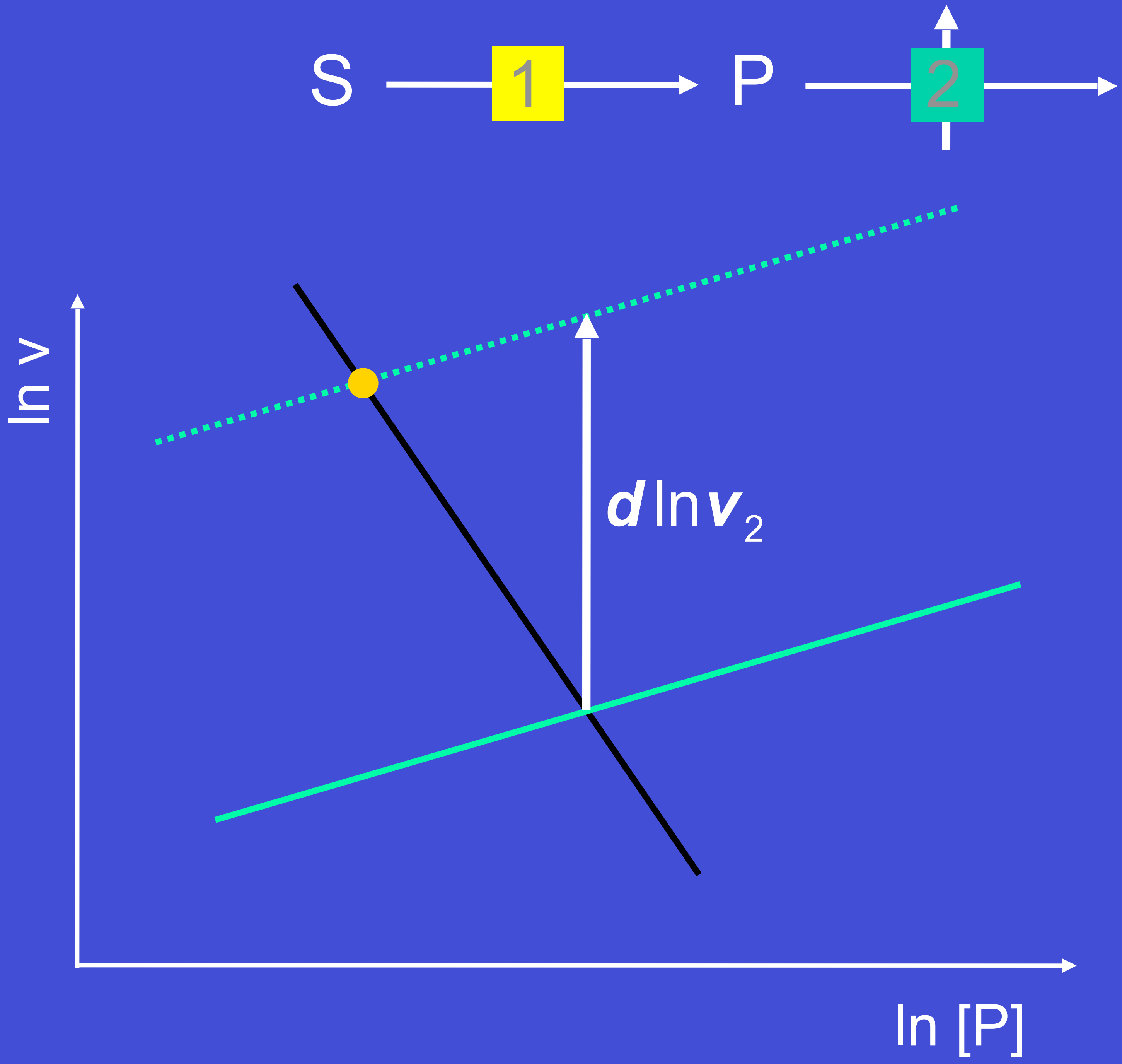


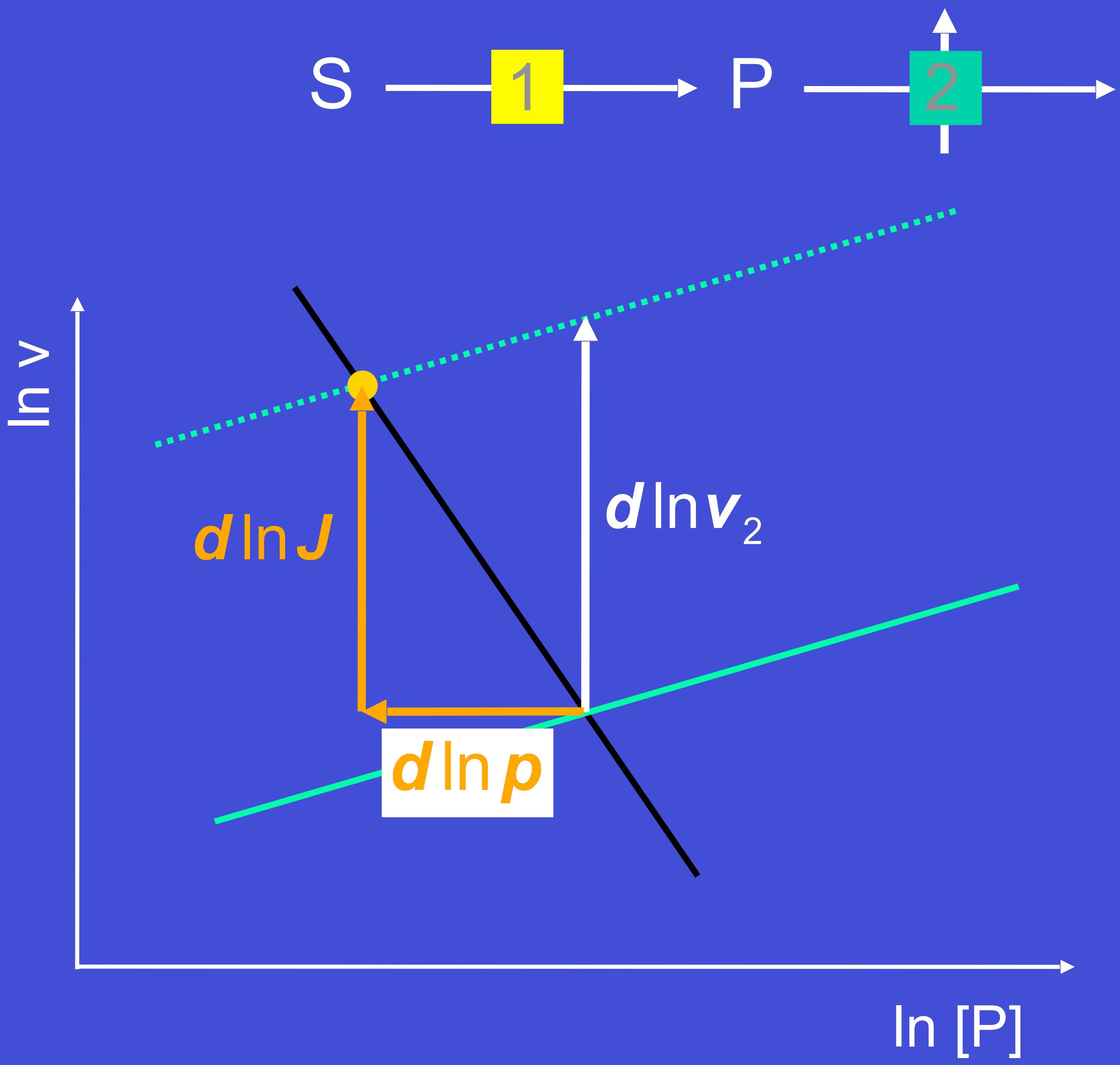
S
1
P
2
ln v
d ln v2
ln [P]

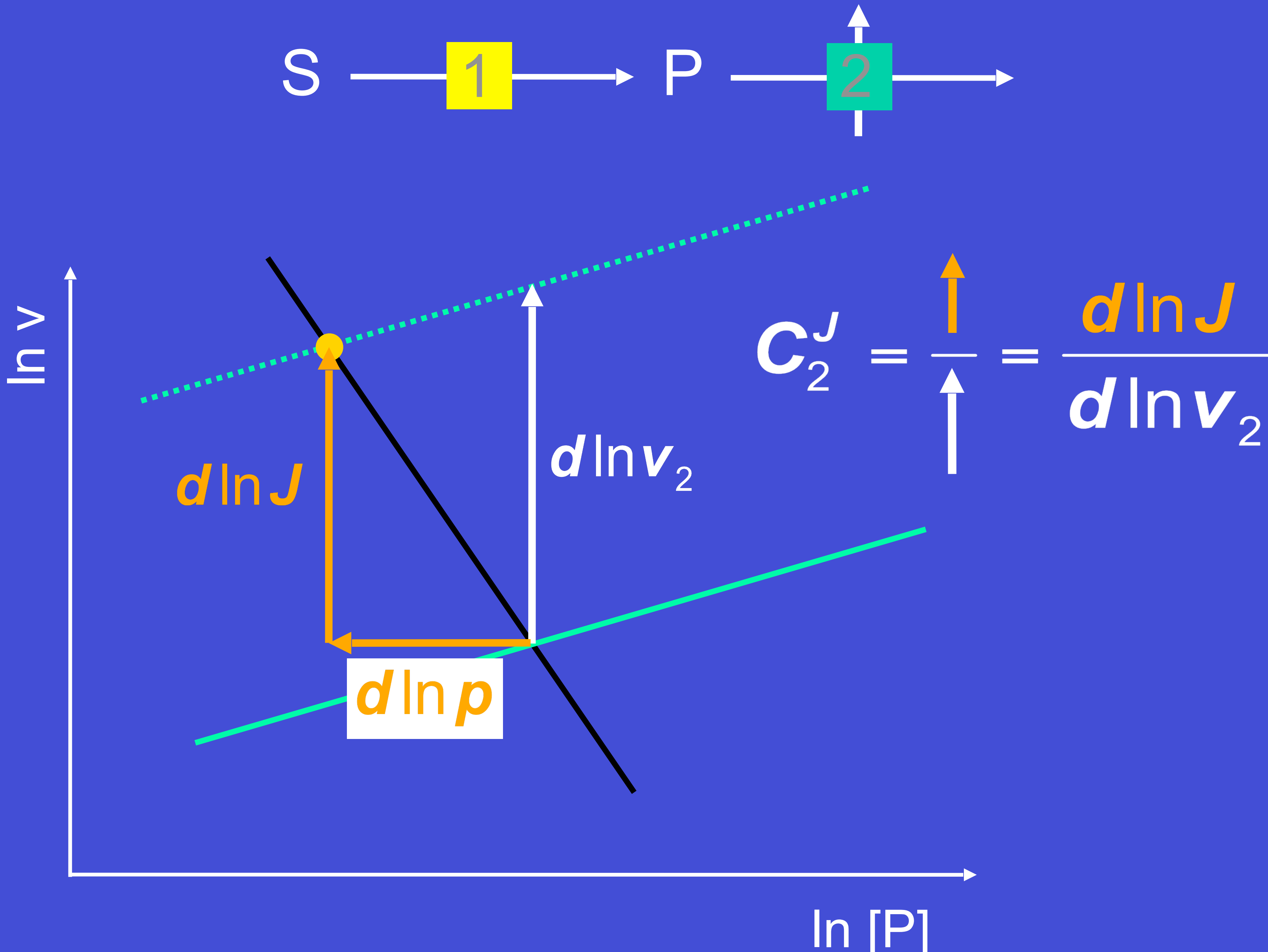


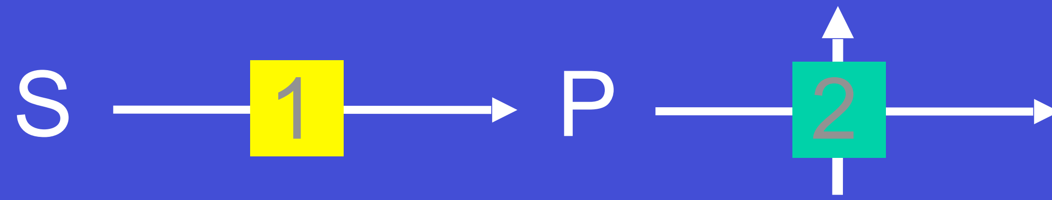
S
1
P
2
ln v
d ln v2
ln [P]

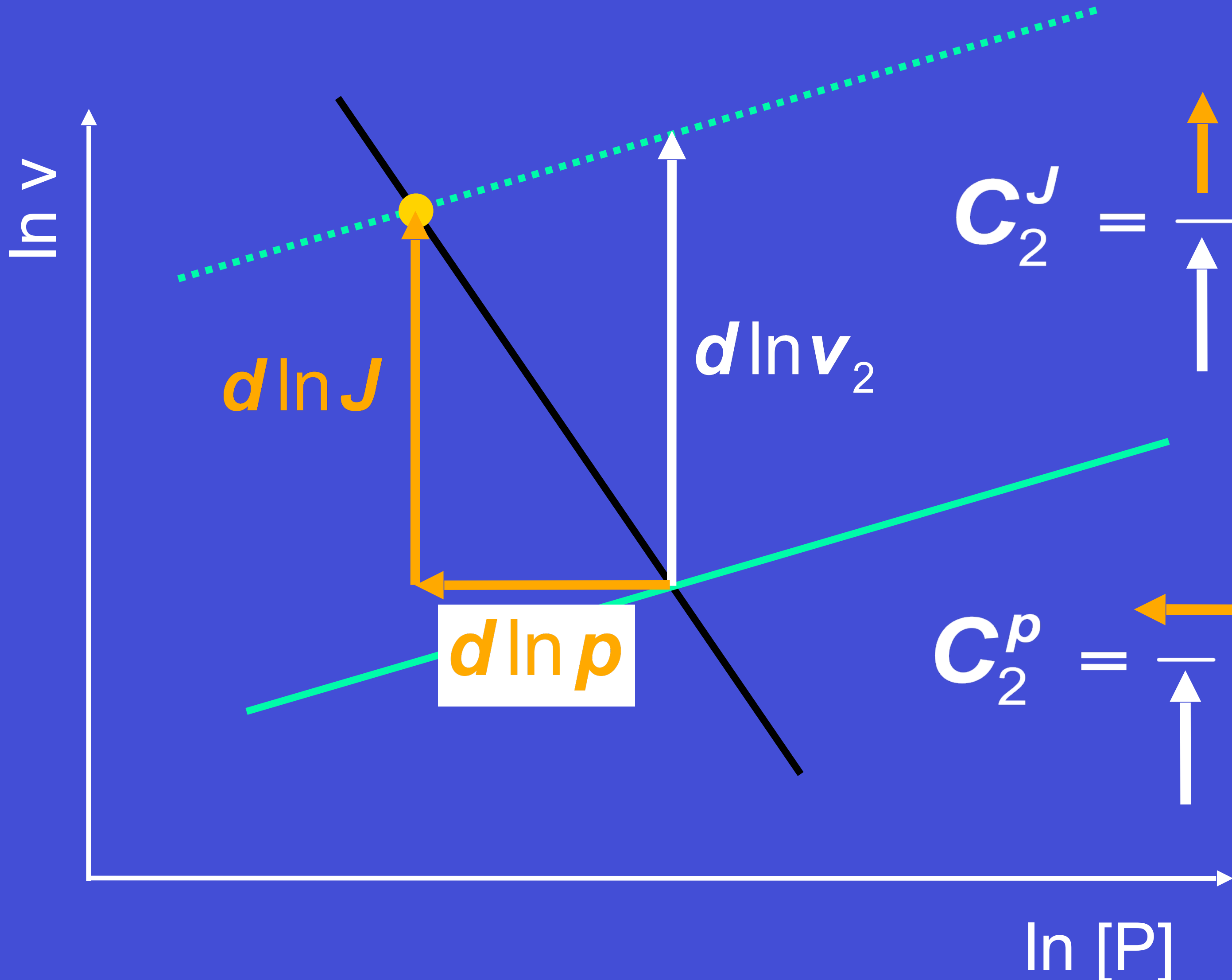

S
1
P
2
ln v
d ln v2
ln [P]


S
1
P
2
ln v
$d \ln v_2$
ln [P]


S
1
P
2
ln v
d ln J
d ln v2
d ln p
ln [P]

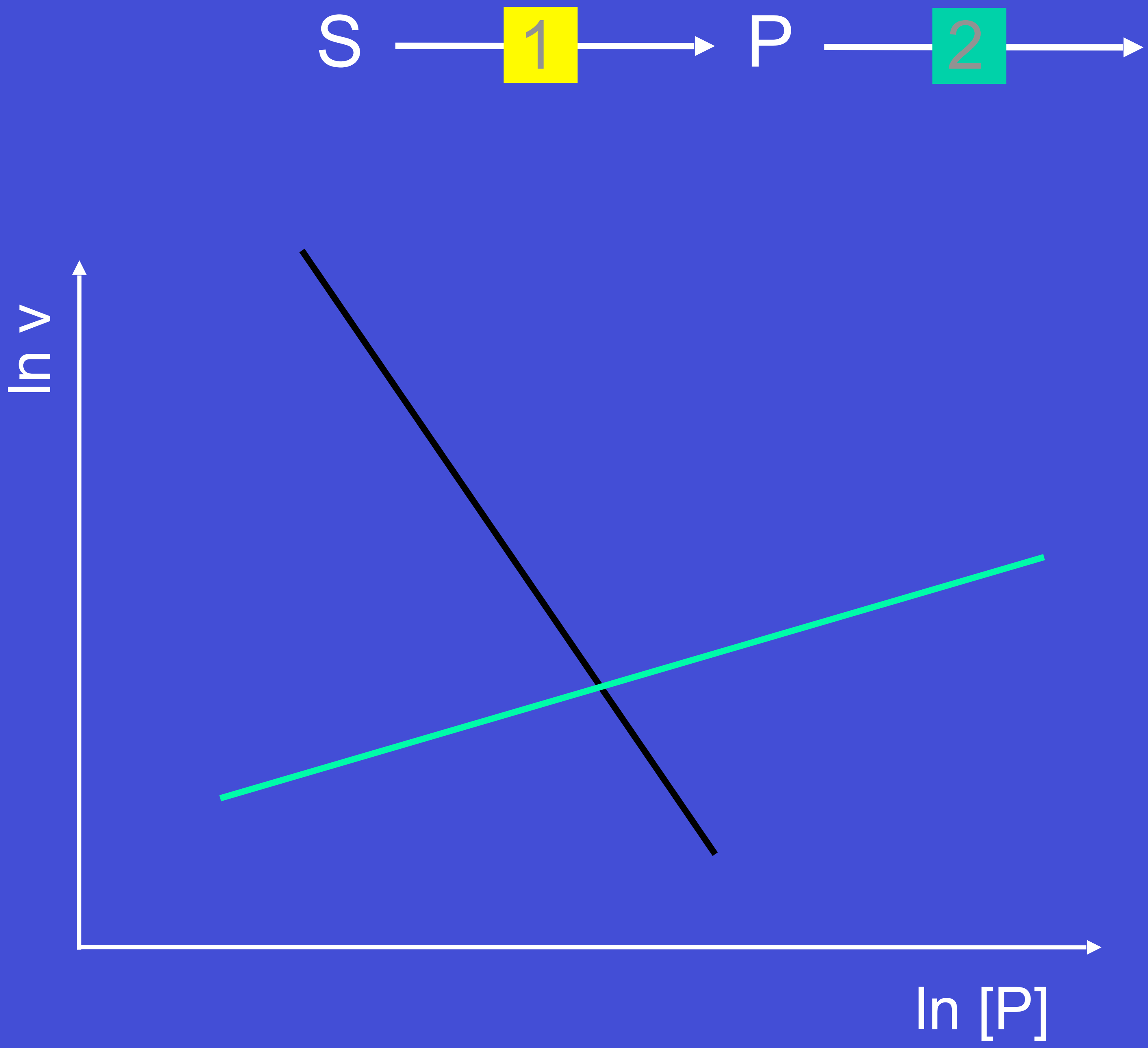

S
1
P
2
ln v
ln [P]
d ln J
d ln p
d ln v₂
$C_2^J = \frac{d \ln J}{d \ln v_2}$

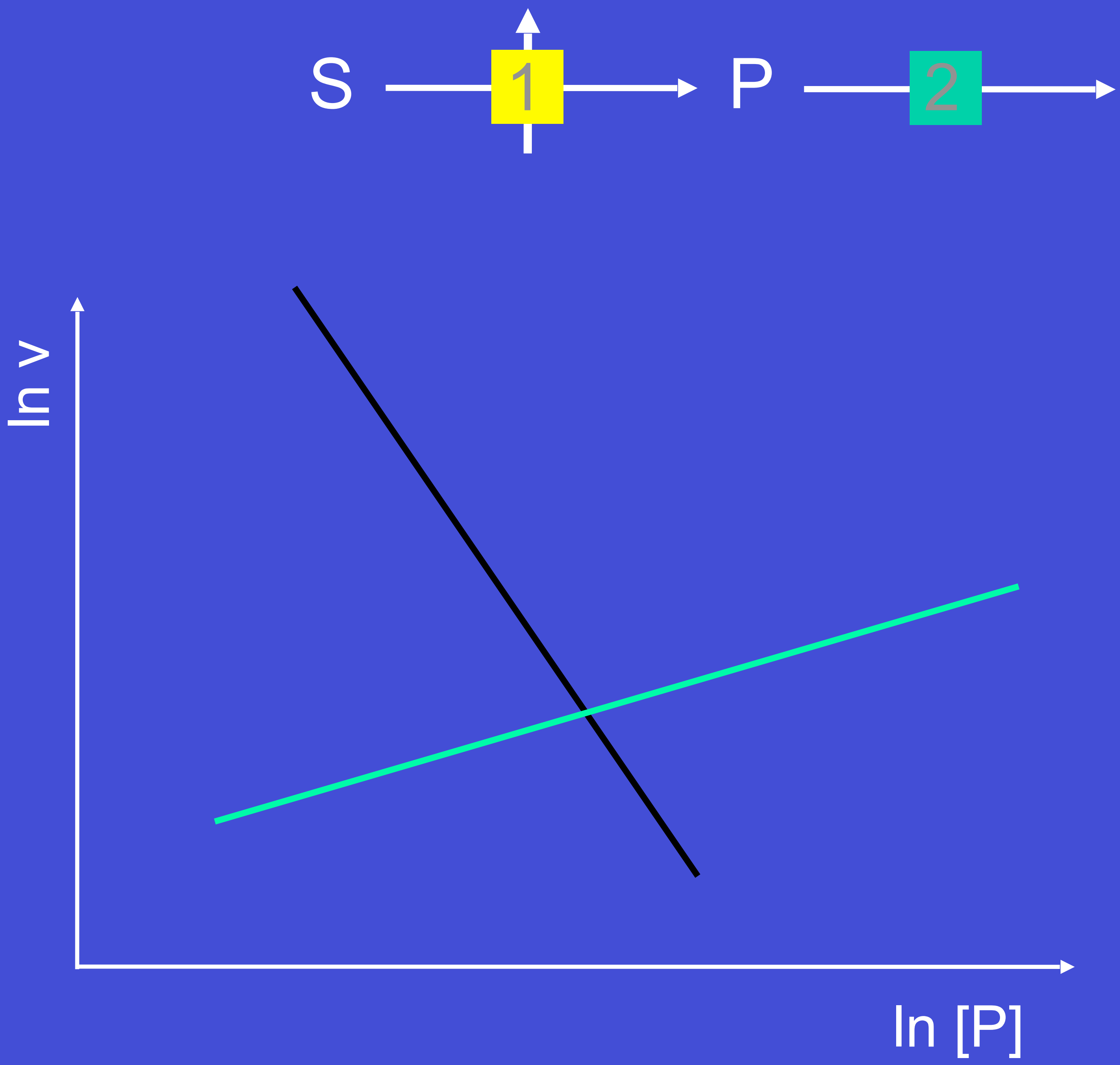


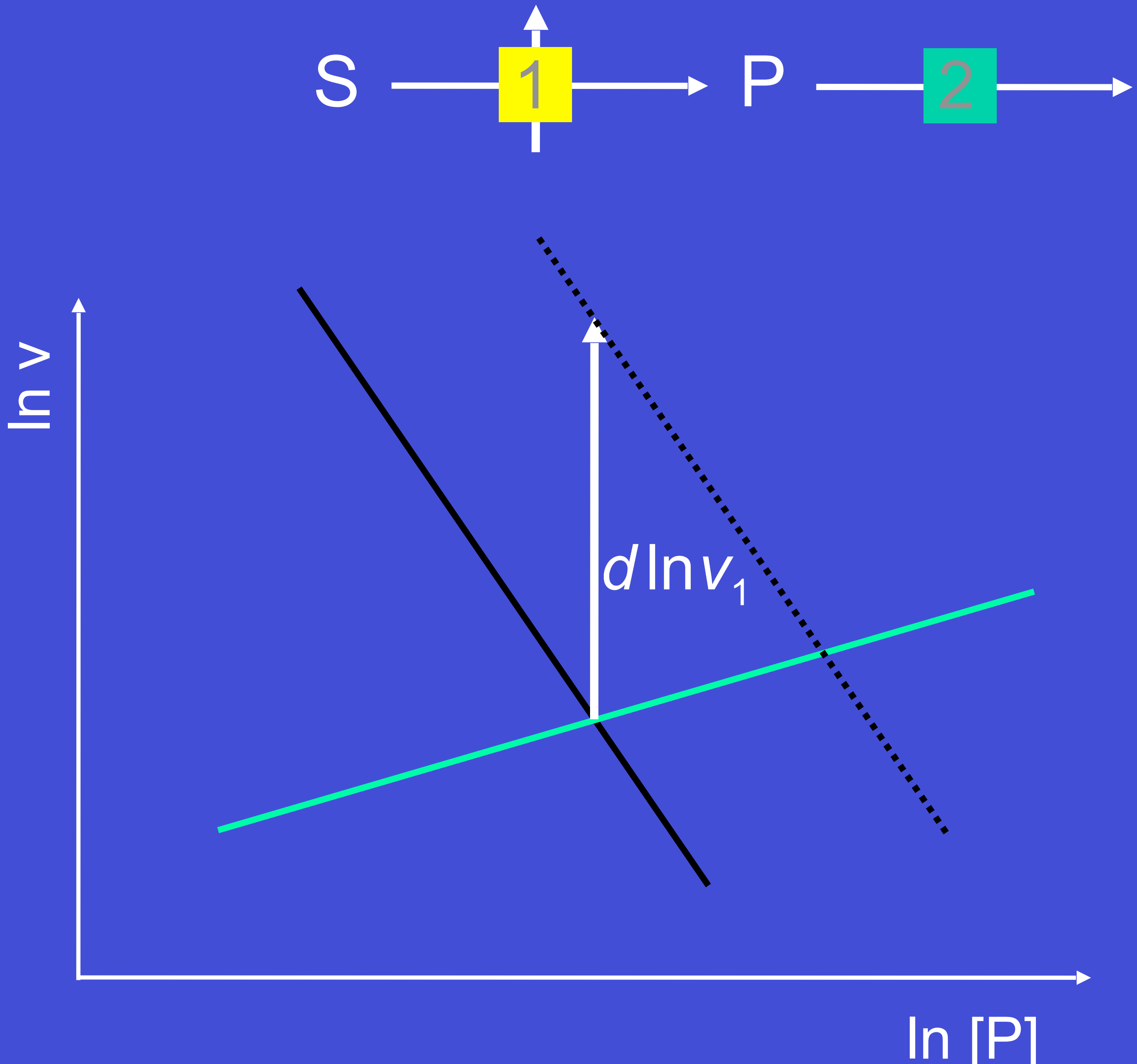


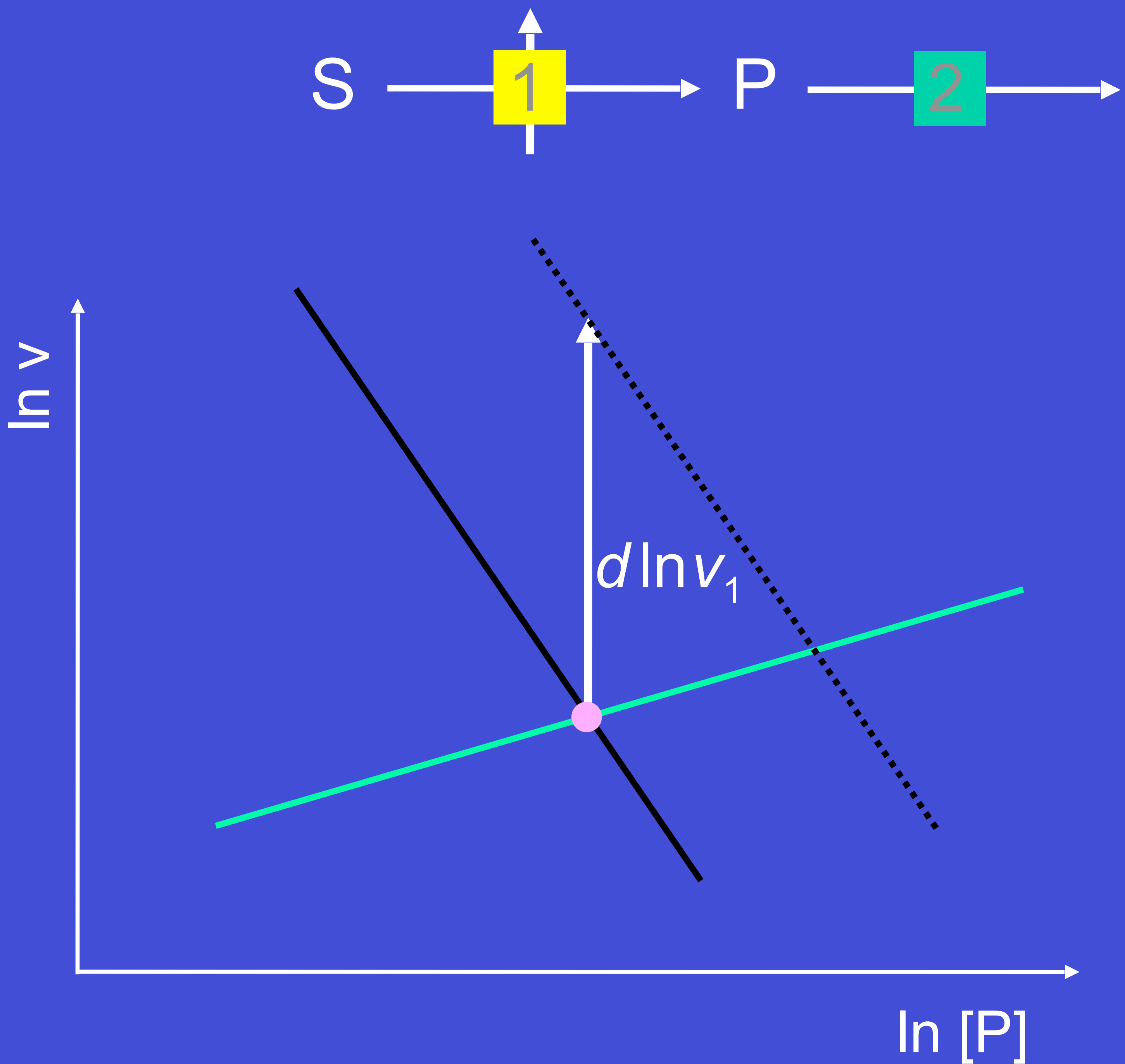
$$C_2^J = \frac{\uparrow}{\uparrow} = \frac{d\ln J}{d\ln v_2}$$

$$C_2^p = \frac{\leftarrow}{\uparrow} = \frac{d\ln p}{d\ln v_2}$$

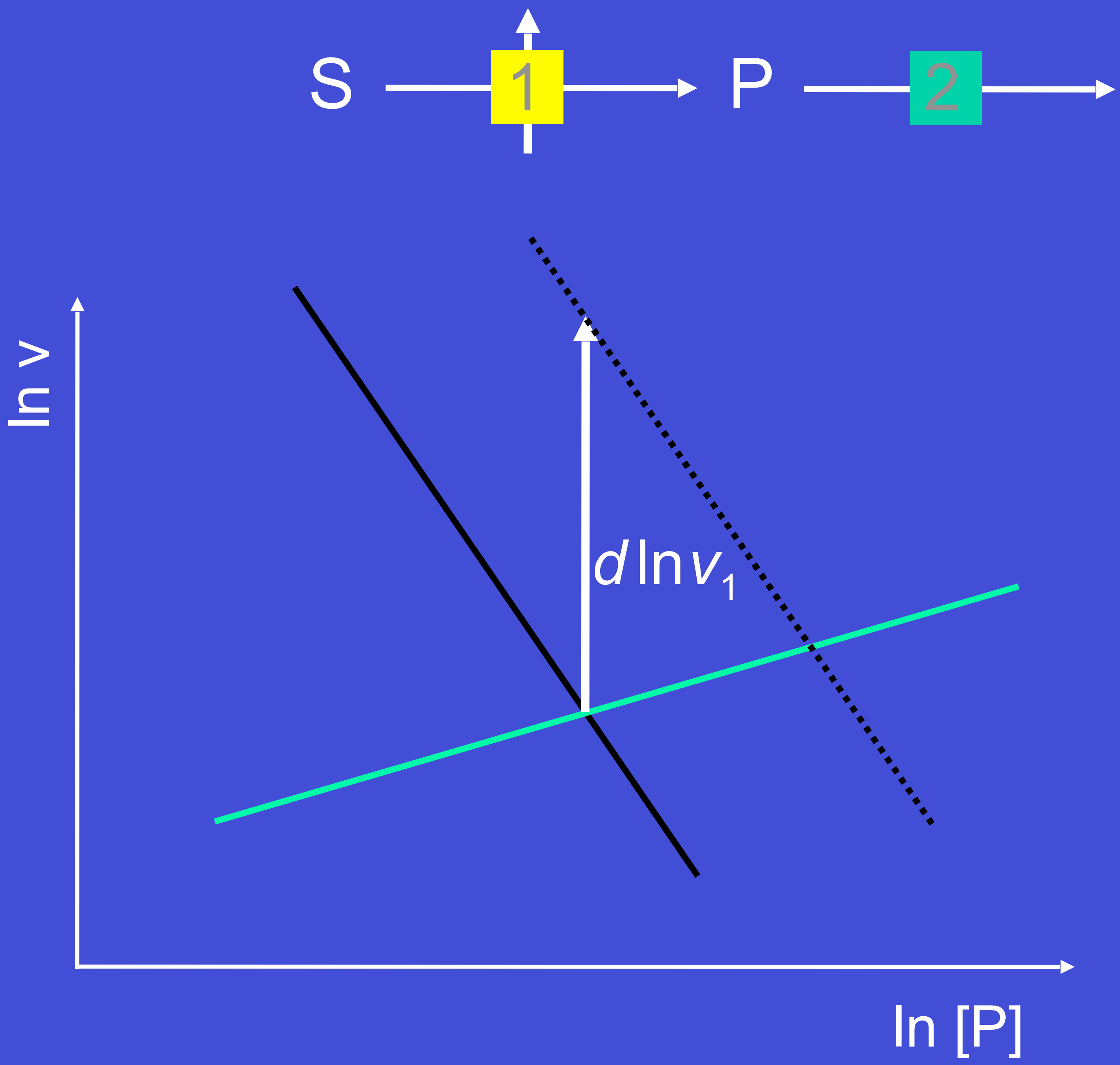

S
1
P
2
ln v
ln [P]

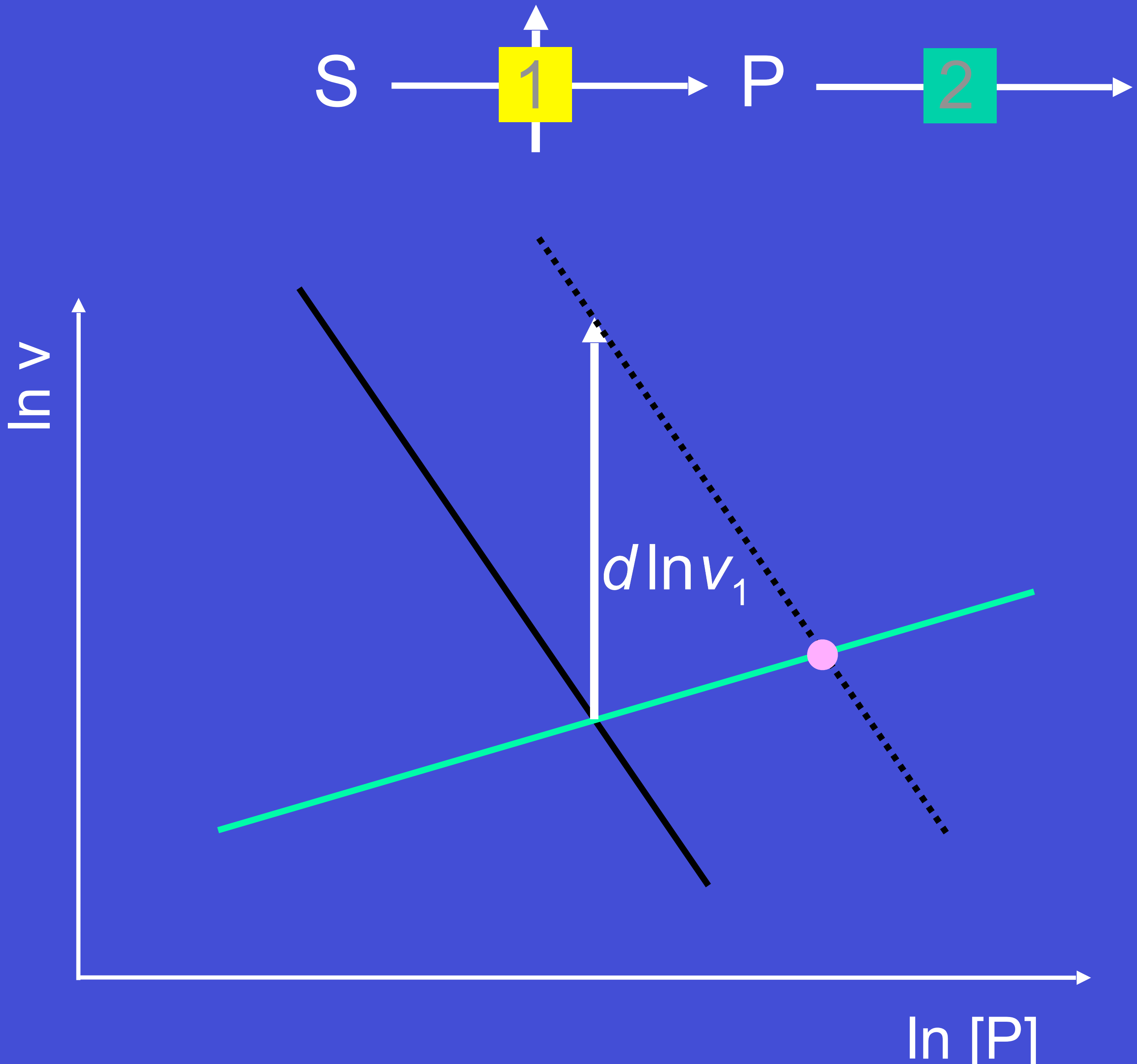

S
1
P
2
ln v
ln [P]


S
1
P
2
ln v
$d \ln v_1$
ln [P]

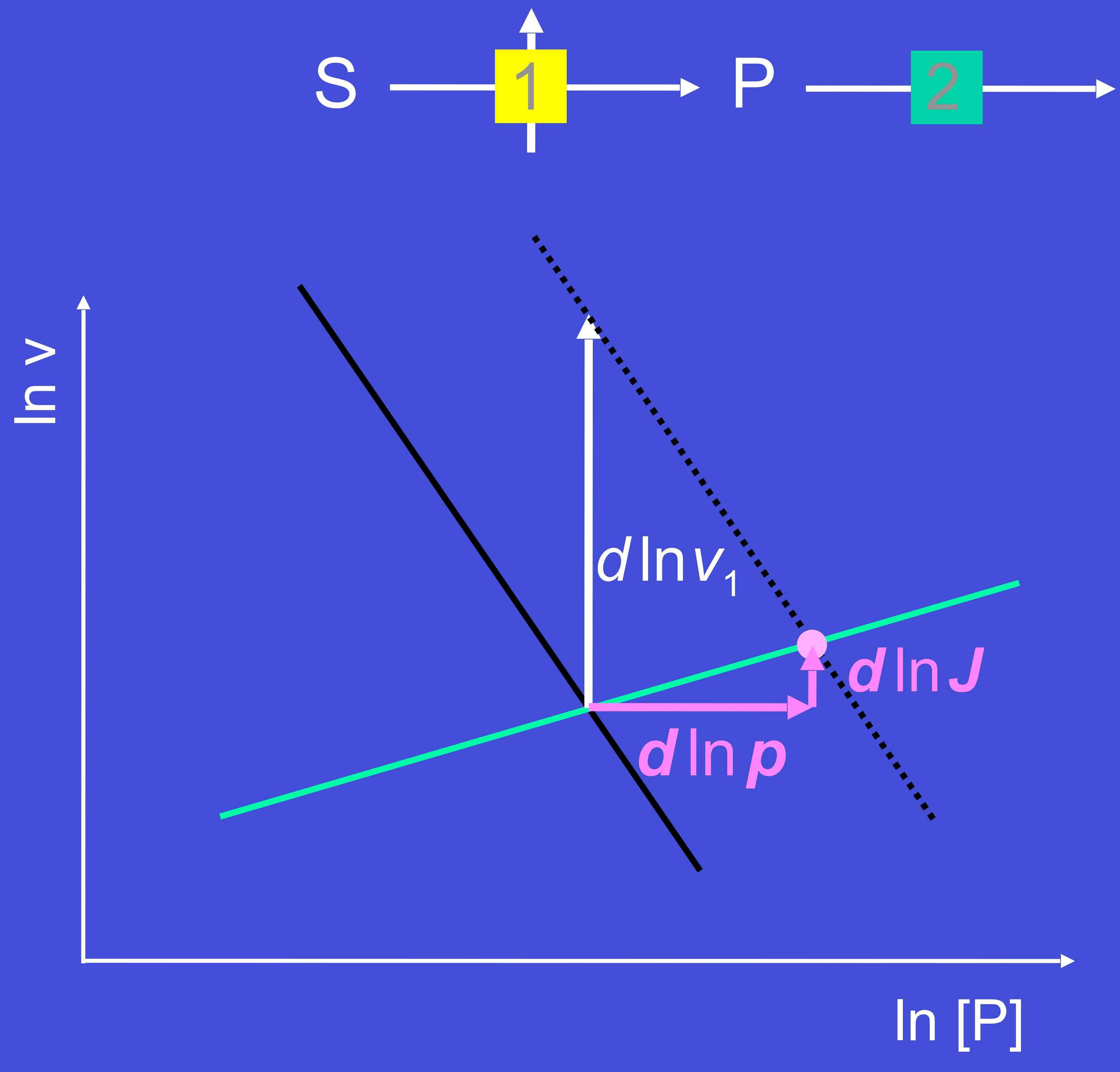


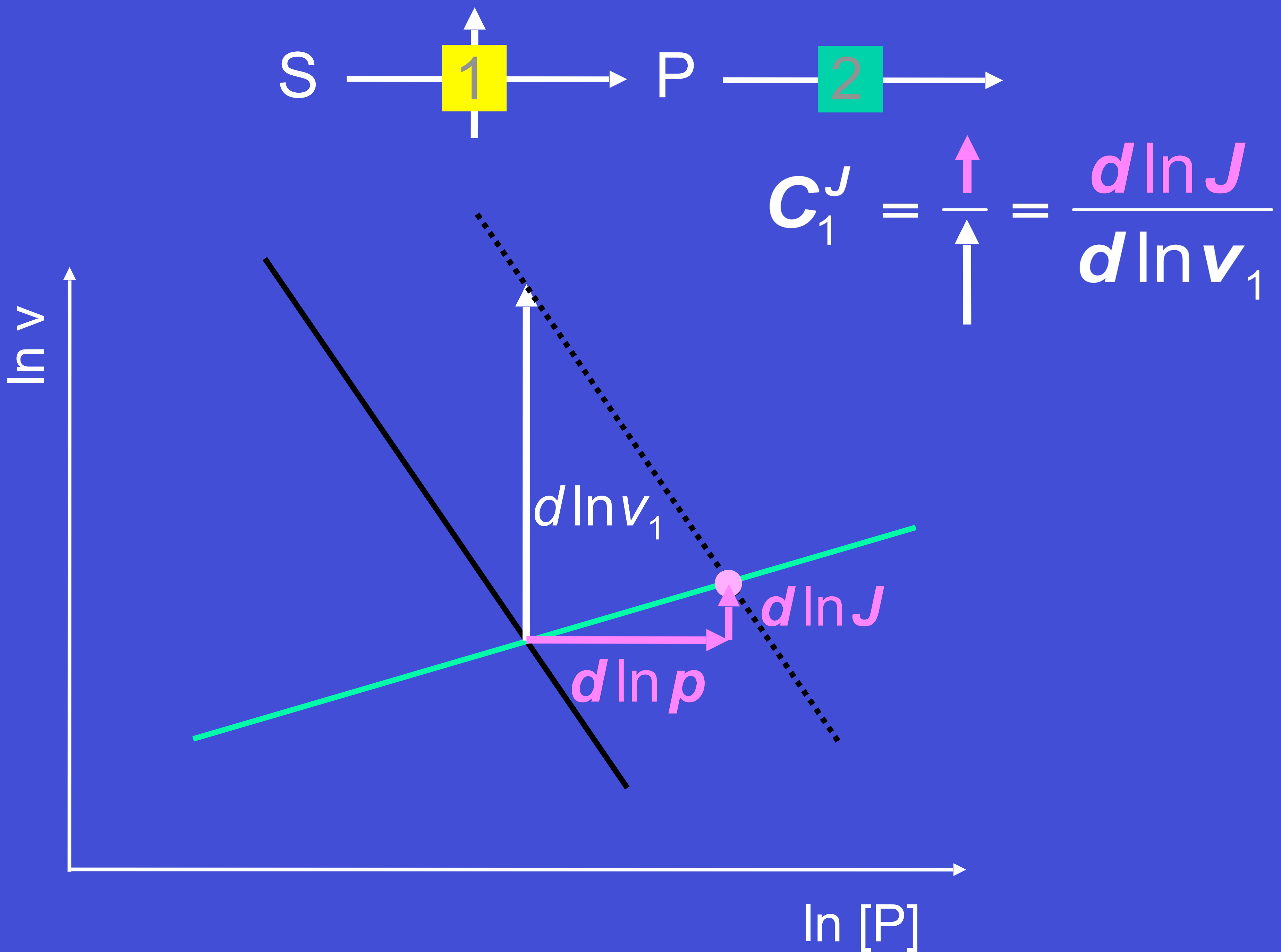
S
1
P
2
ln v
$d \ln v_1$
ln [P]

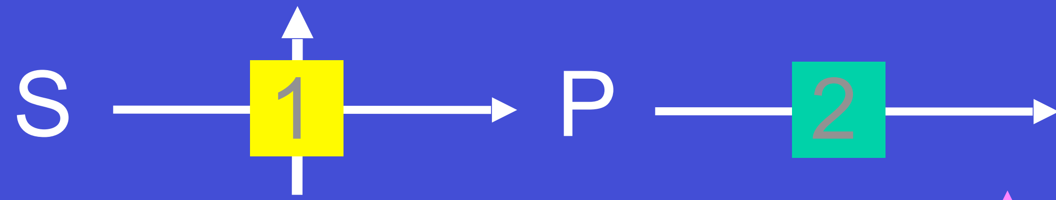

S
1
P
2
ln v
$d\ln v_1$
ln [P]



S
1
P
2
ln v
$d \ln v_1$
ln [P]

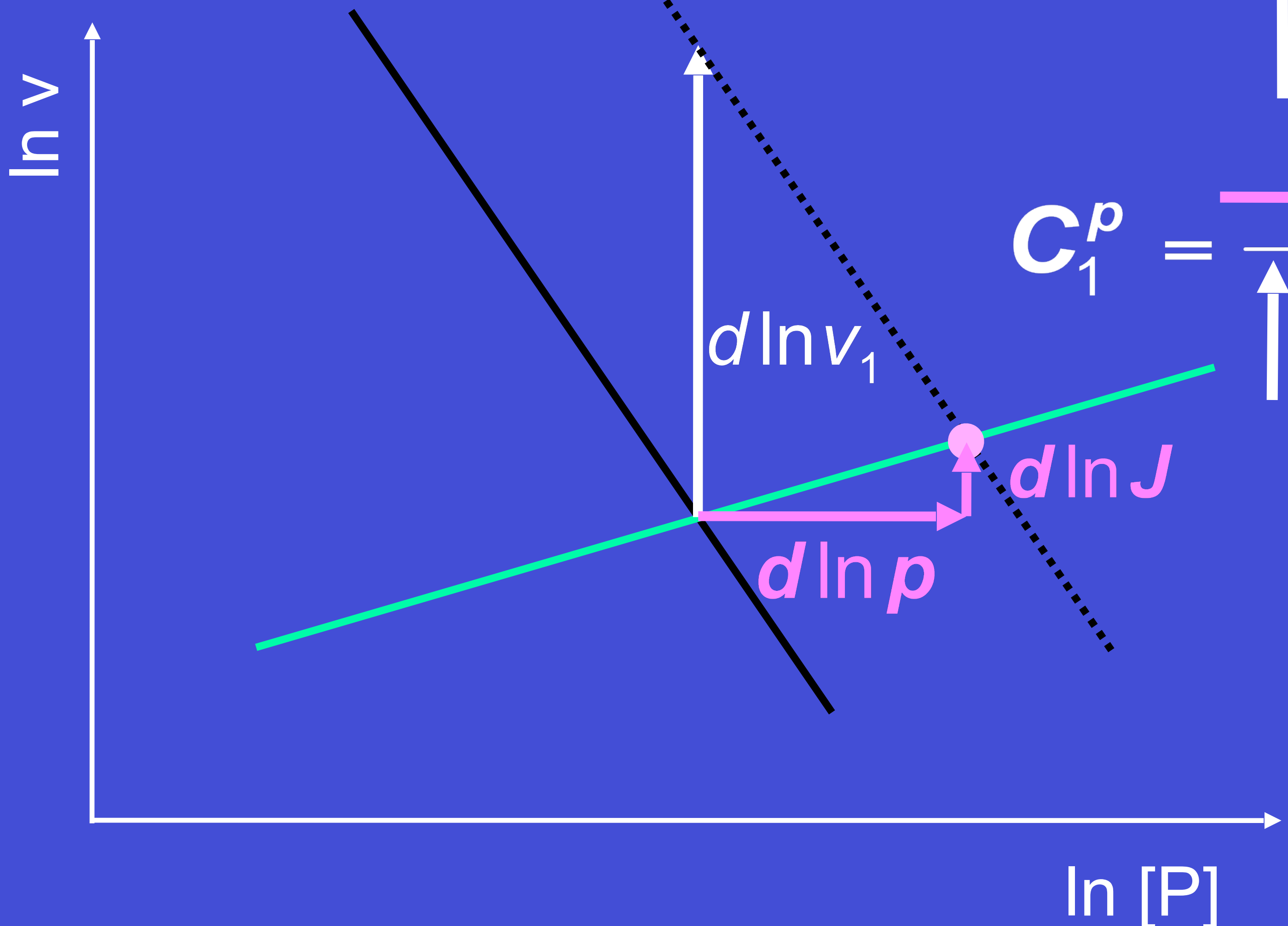

S
1
P
2
ln v
$d \ln v_1$
$d \ln J$
$d \ln p$
ln [P]


S
1
P
2
$C_1^J = \frac{\uparrow}{\uparrow} = \frac{d\ln J}{d\ln v_1}$
ln v
$d\ln v_1$
$d\ln J$
$d\ln p$
ln [P]



$$C_1^J = \frac{\uparrow}{\uparrow} = \frac{d\ln J}{d\ln v_1}$$

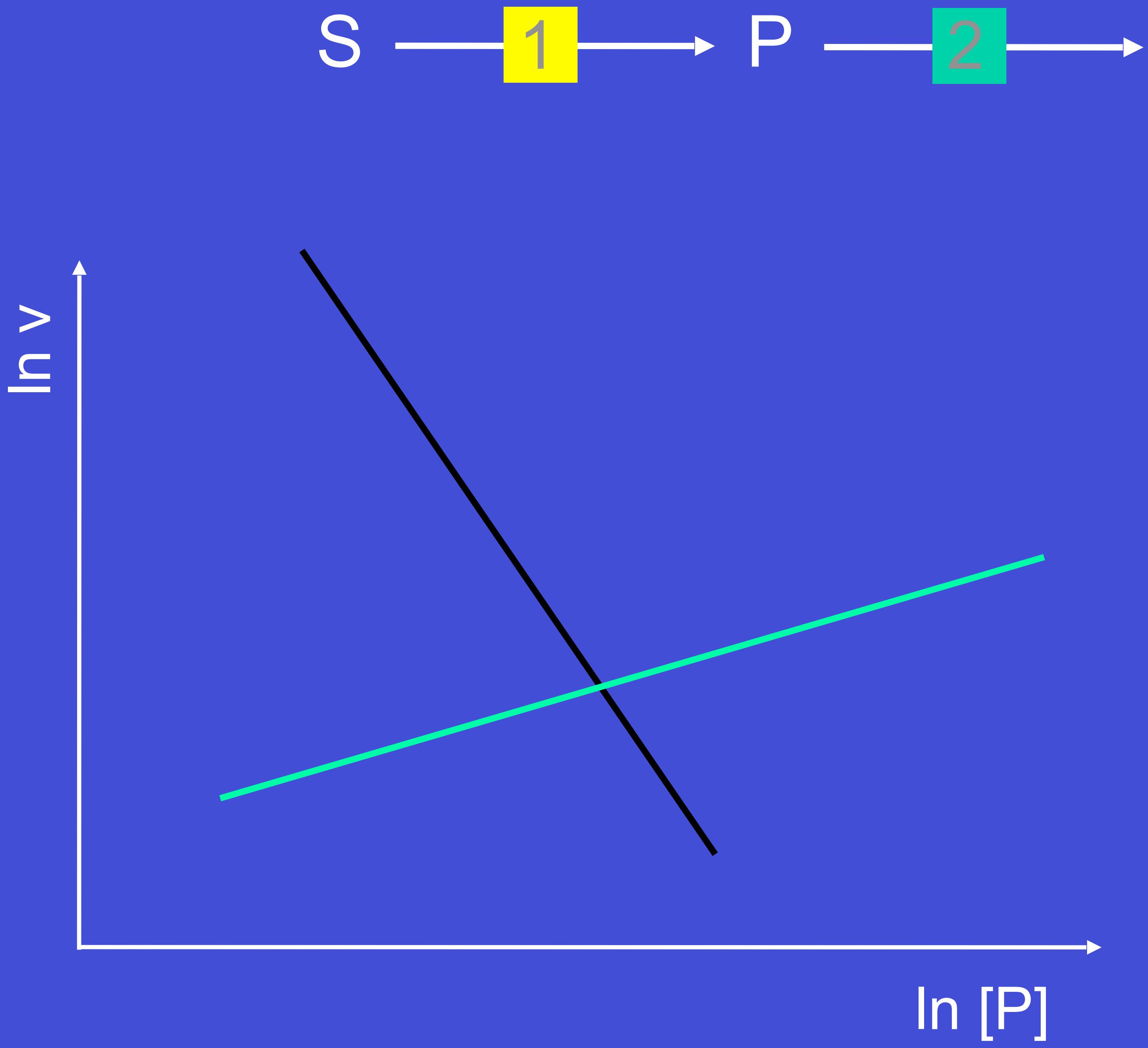
$$C_1^p = \frac{\rightarrow}{\uparrow} = \frac{d\ln p}{d\ln v_1}$$

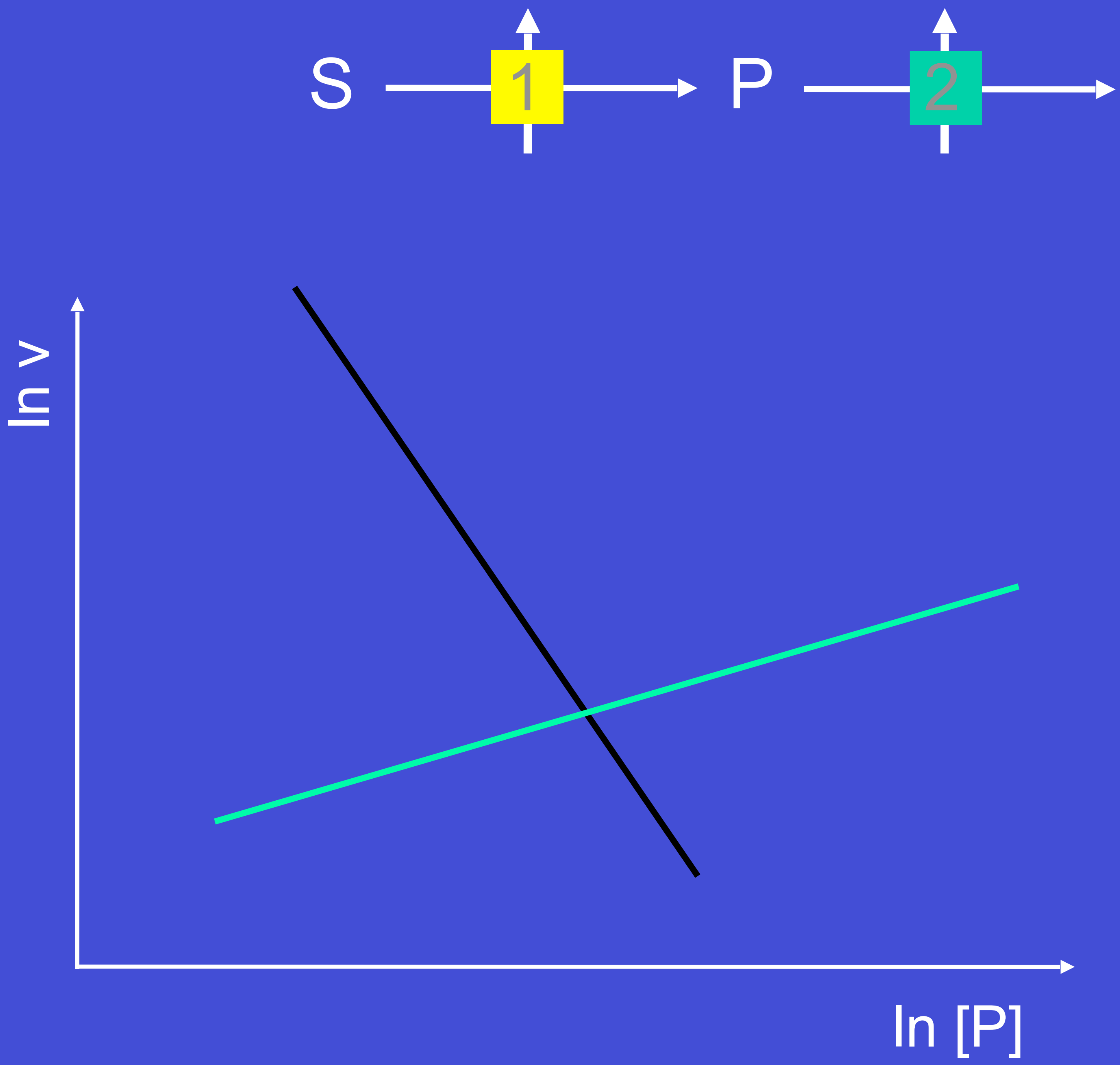


Control Coefficient

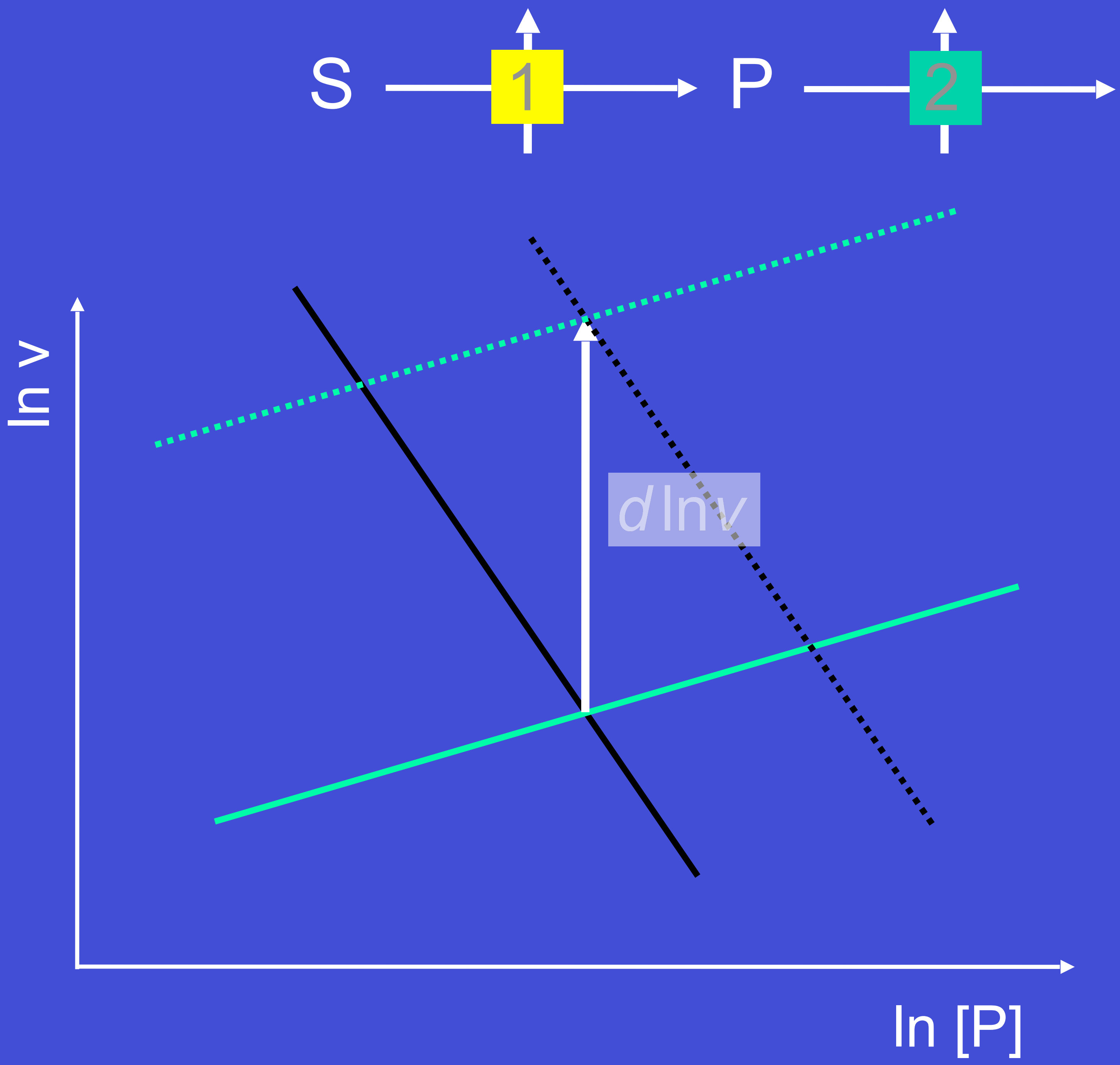
$$C^J_{v_i} = \frac{\delta J / J}{\delta v_i / v_i}$$

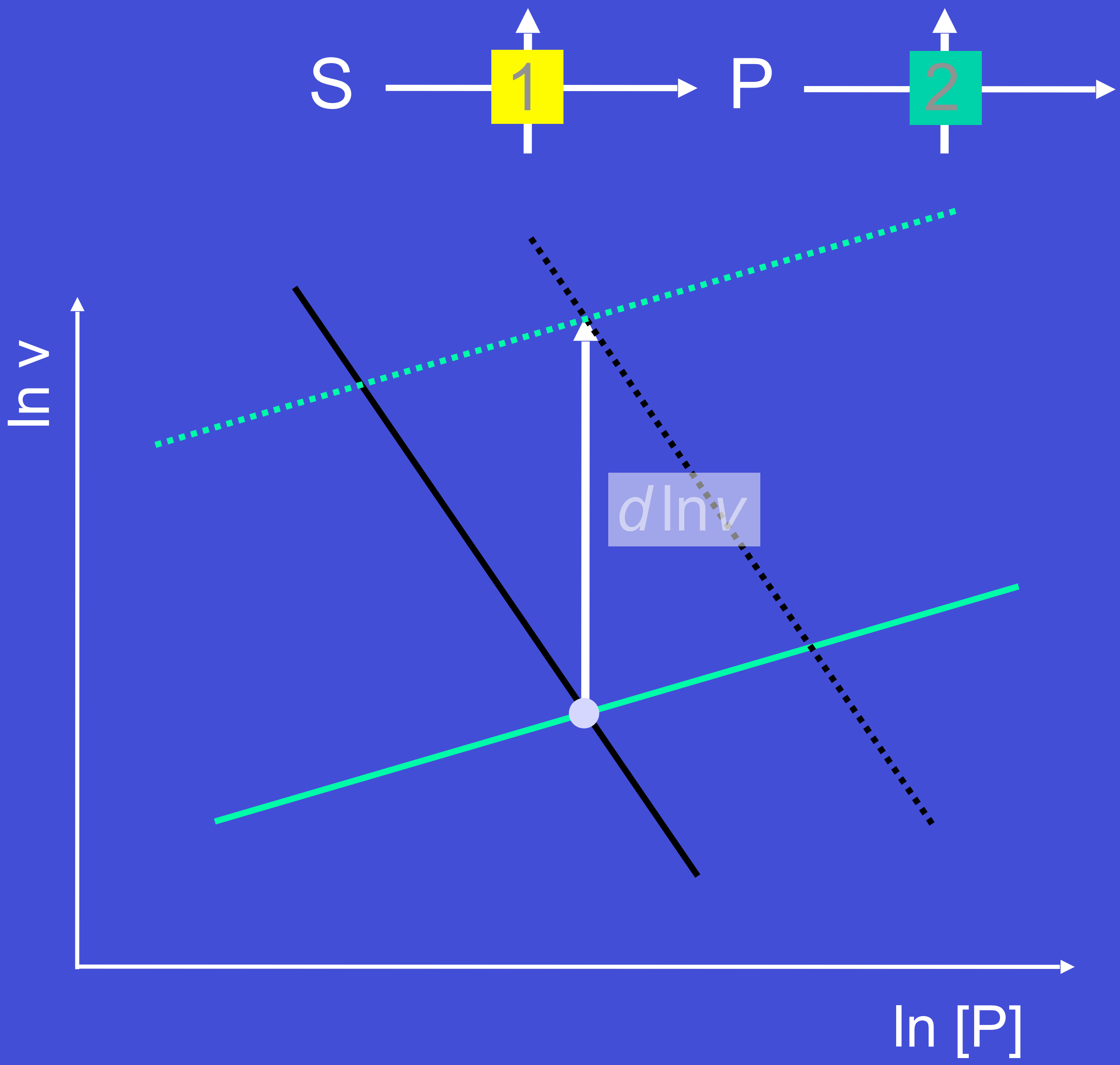
$$C^y_i = \frac{\delta y / y}{\delta v_i / v_i} = \frac{\delta y}{\delta v_i} \frac{v_i}{y} = \frac{\delta \ln y}{\delta \ln v_i}$$

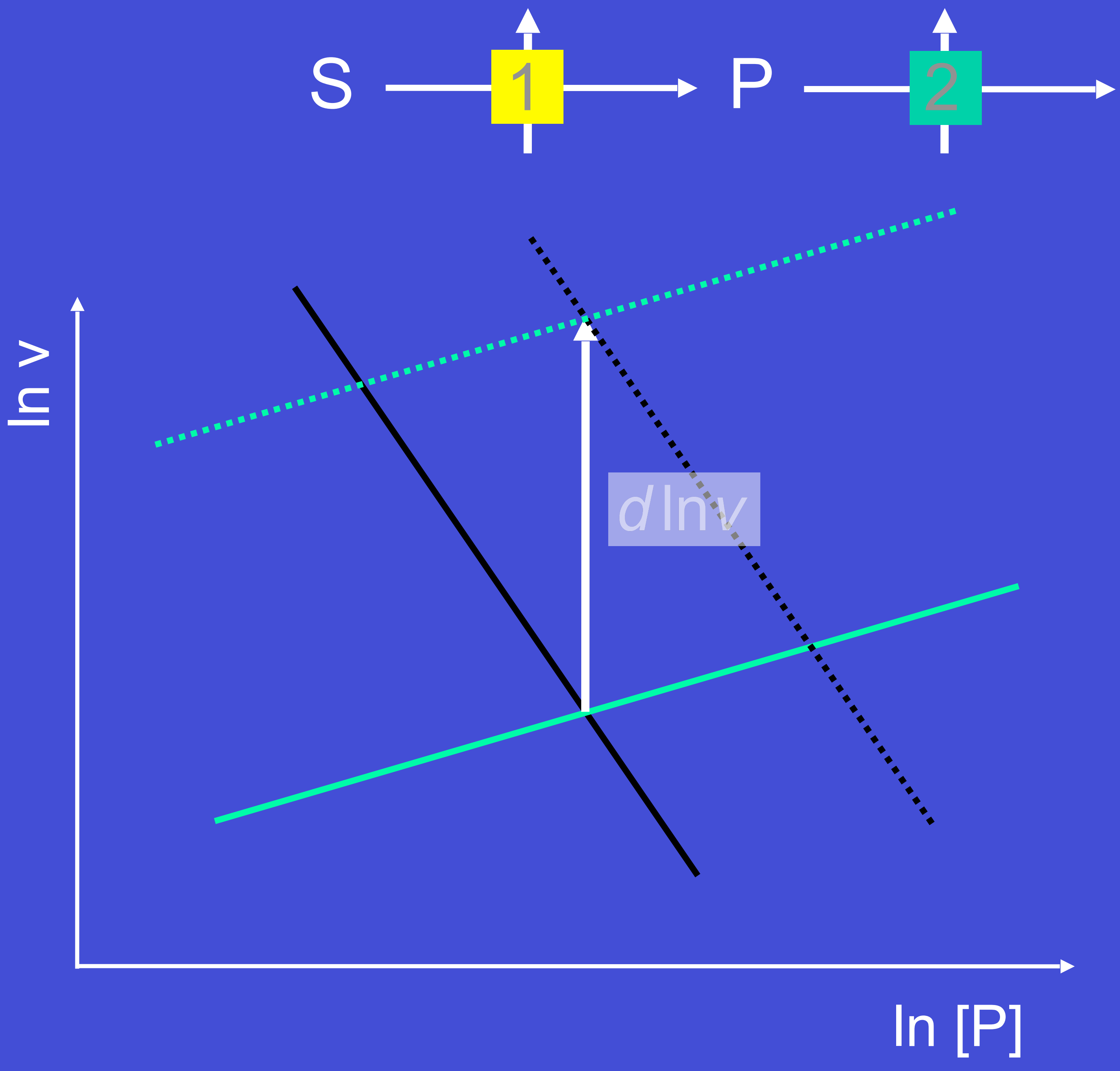

S
1
P
2
ln v
ln [P]



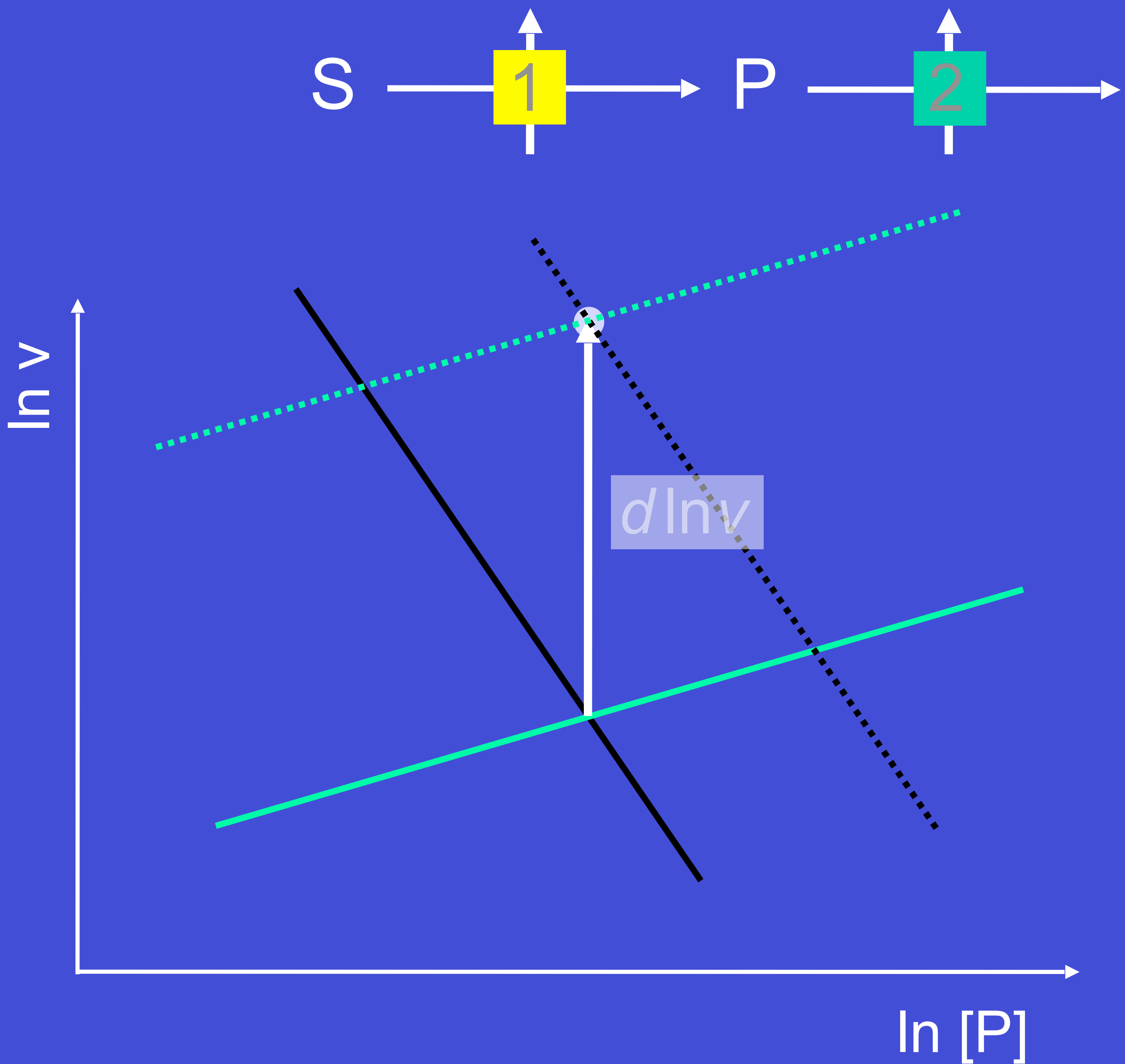
S
1
P
2
ln v
ln [P]

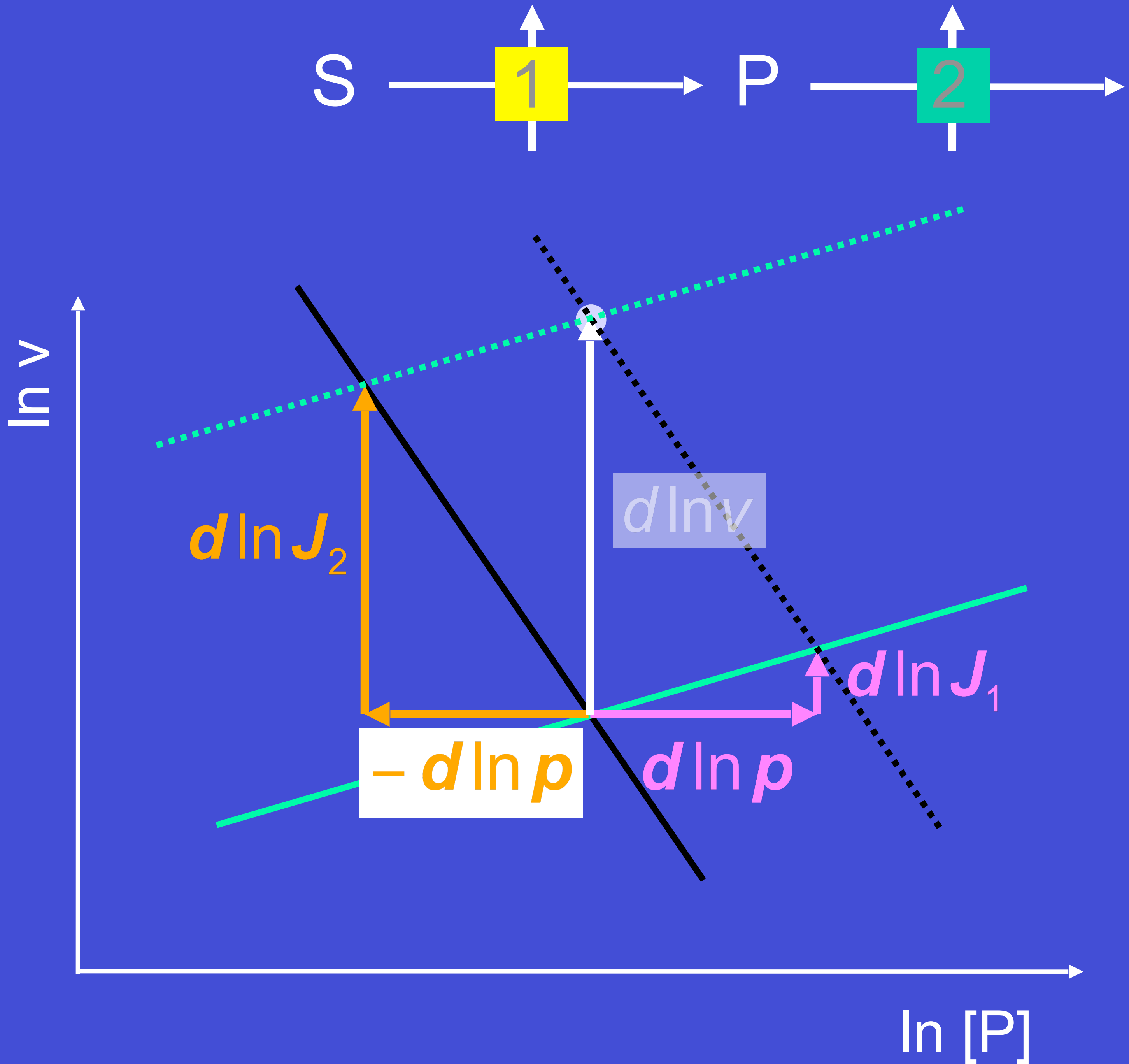

S
1
P
2
ln v
d lnv
ln [P]

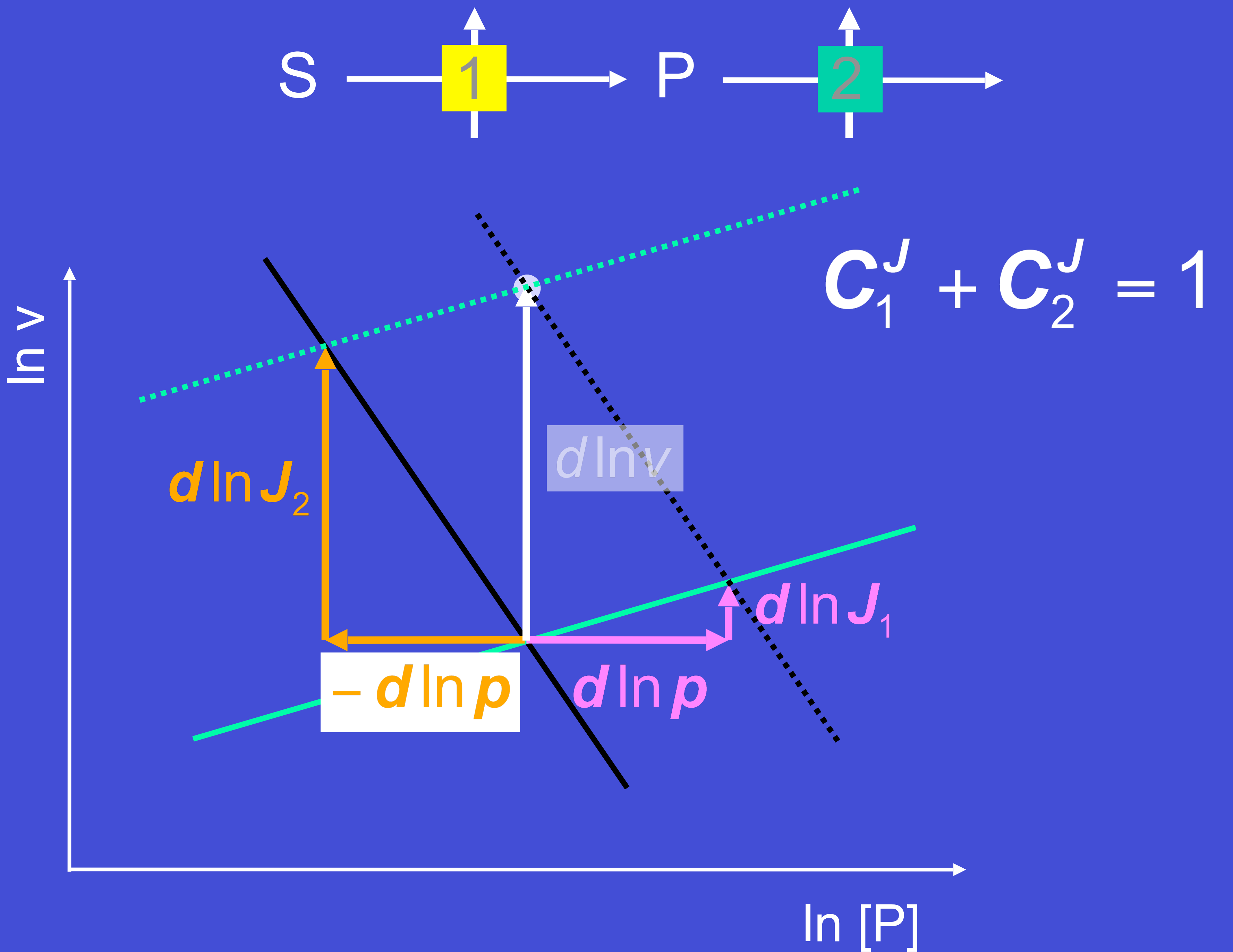

S
1
P
2
ln v
d lnv
ln [P]



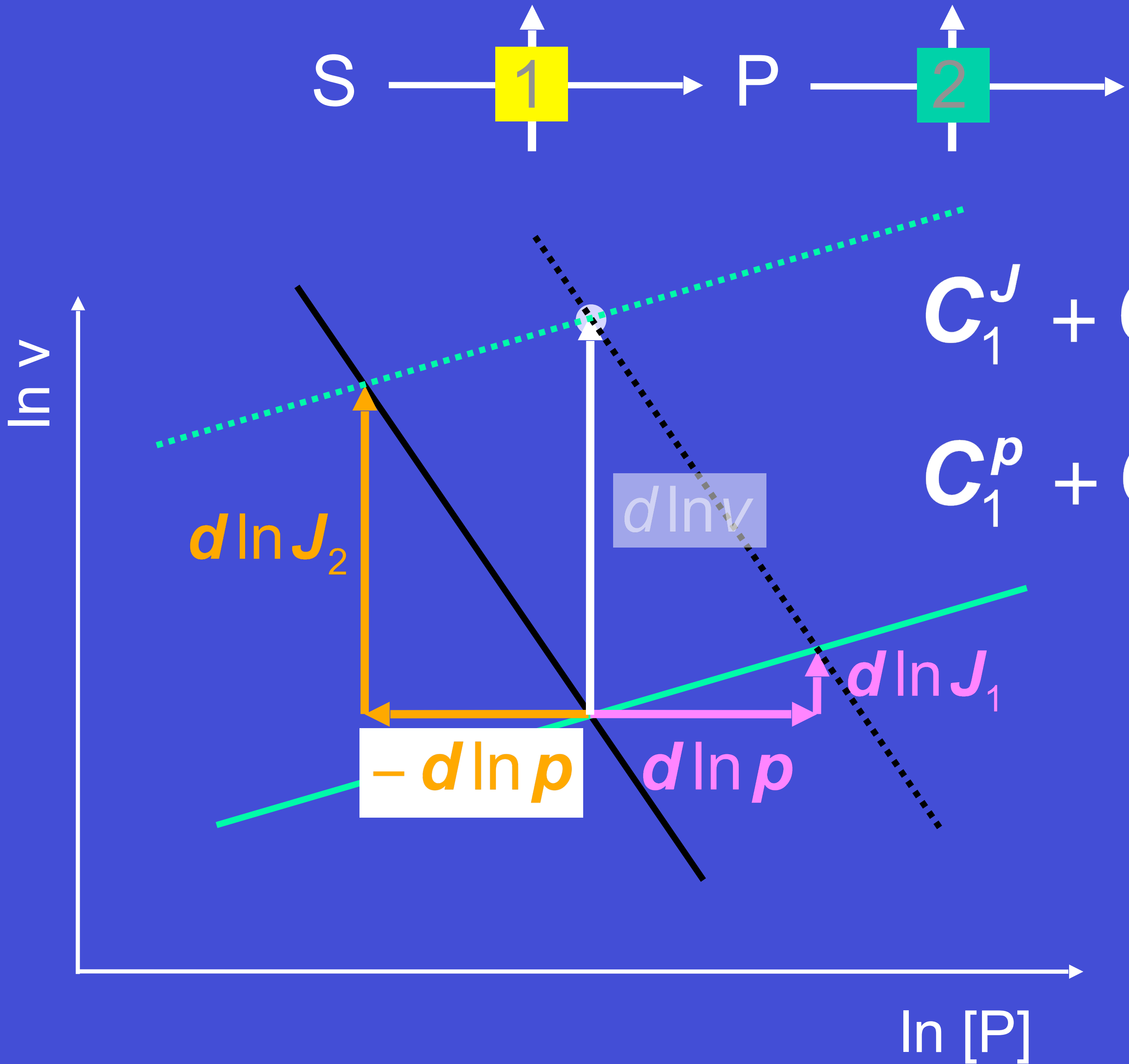
S
1
P
2
ln v
d lnv
ln [P]


S
1
P
2
ln v
d lnv
ln [P]


S
1
P
2
ln v
$d \ln J_2$
$d \ln v$
$d \ln J_1$
$- d \ln p$
$d \ln p$
ln [P]

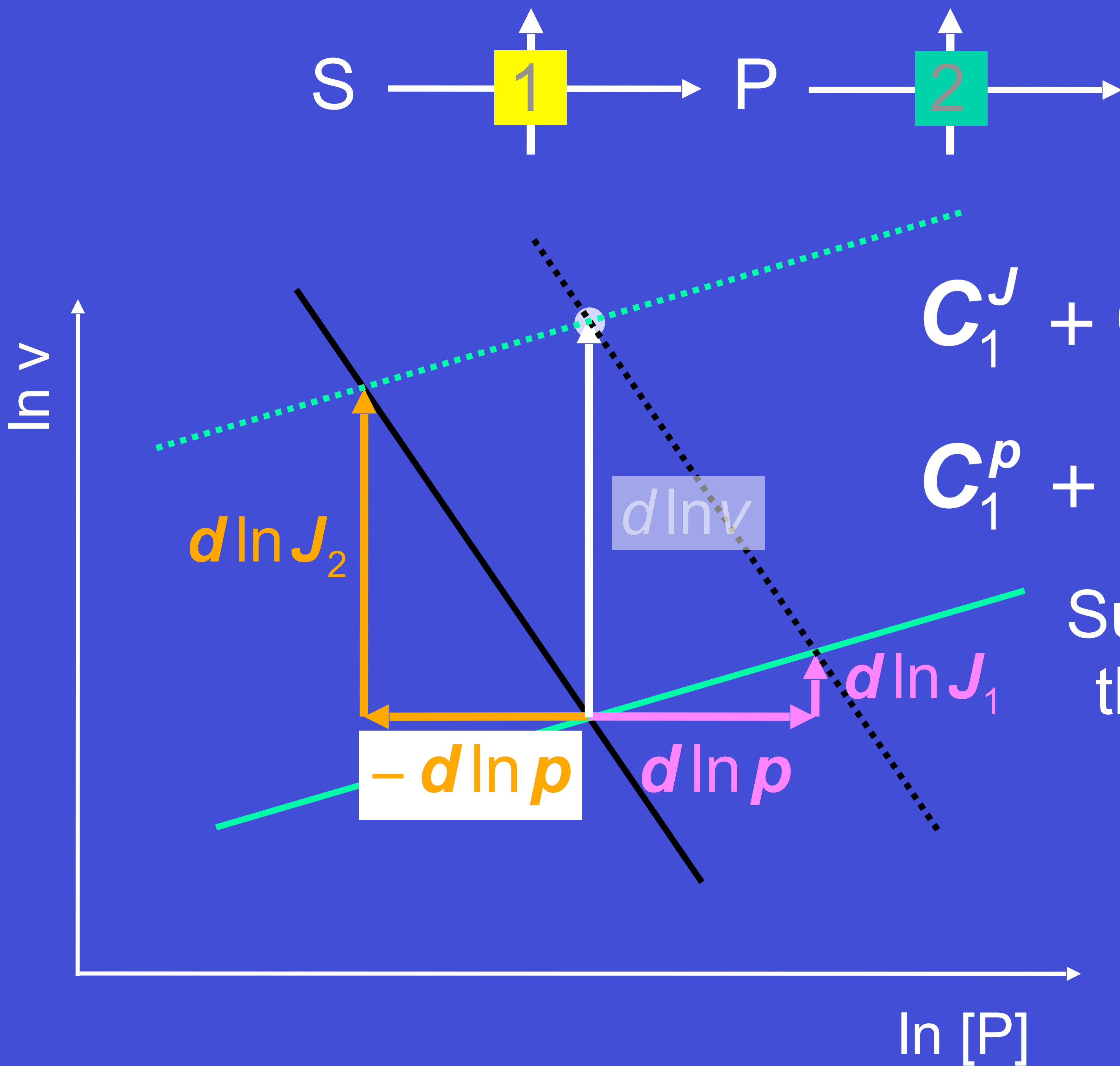


S
1
P
2
$C_1^J + C_2^J = 1$
ln v
$d \ln J_2$
$d \ln v$
$d \ln J_1$
$- d \ln p$
$d \ln p$
ln [P]



$$C_1^J + C_2^J = 1$$

$$C_1^p + C_2^p = 0$$



$$C_1^J + C_2^J = 1$$

$$C_1^p + C_2^p = 0$$

Summation theorems

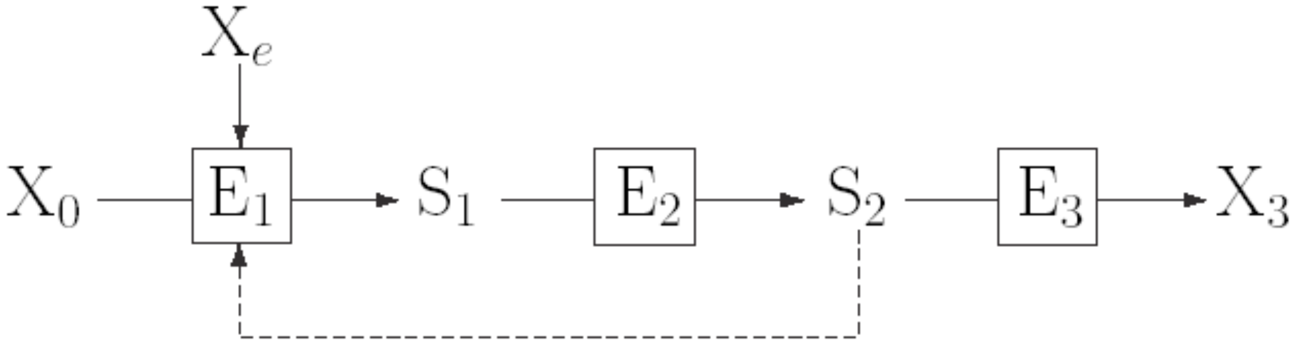


Figure 4.3: A 3-enzyme linear system with a feedback loop from S_2 onto E_1, and an external effector, X_e, that interacts with E_1.

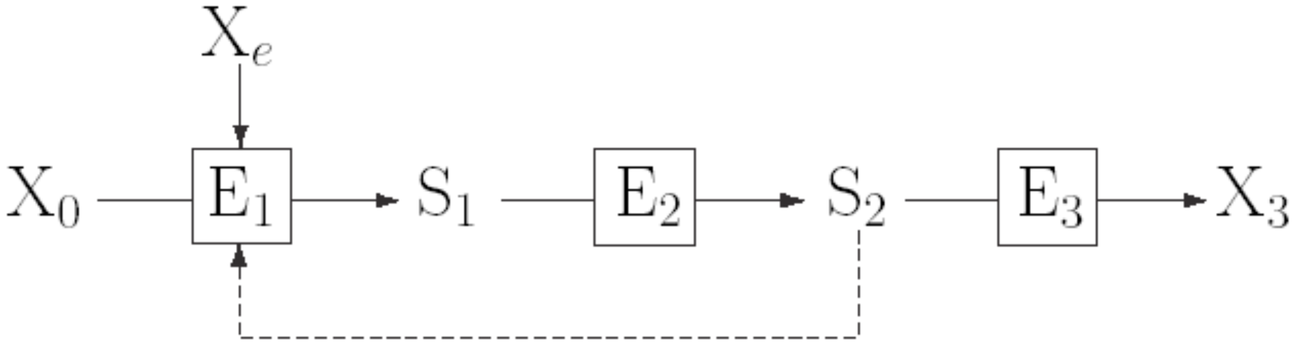
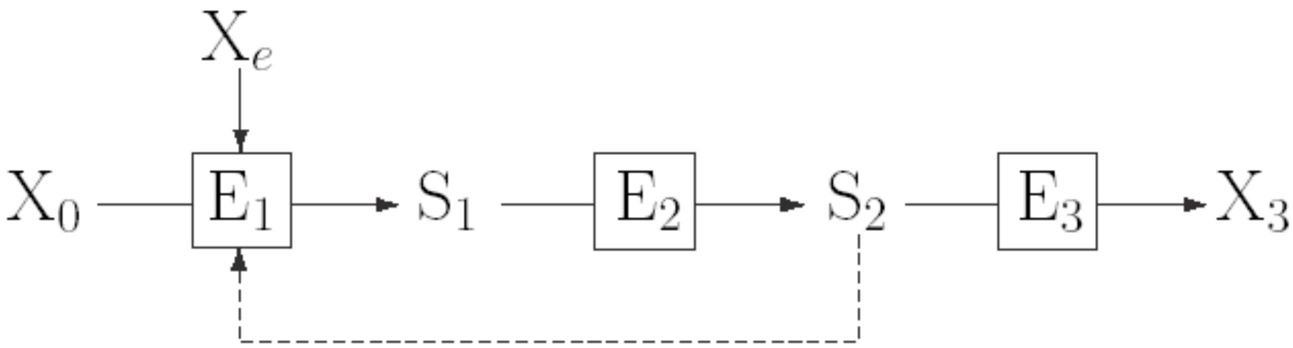


Figure 4.3: A 3-enzyme linear system with a feedback loop from S_2 onto E_1, and an external effector, X_e, that interacts with E_1.

Thought experiment: What would happen if we simultaneously made the same fractional change, α, in the local rates of all the steps in the system?, i.e. if

$$\frac{\delta v_1}{v_1} = \frac{\delta v_2}{v_2} = \frac{\delta v_3}{v_3} = \alpha \qquad (4.21)$$



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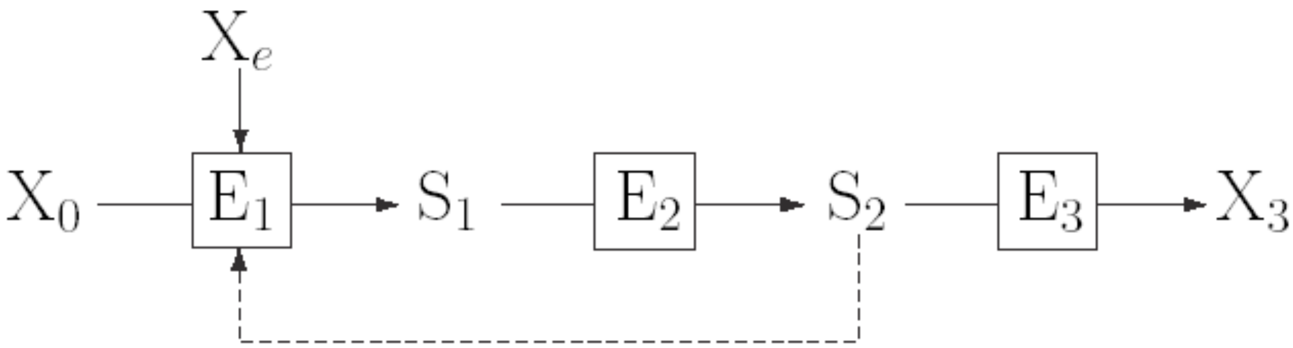

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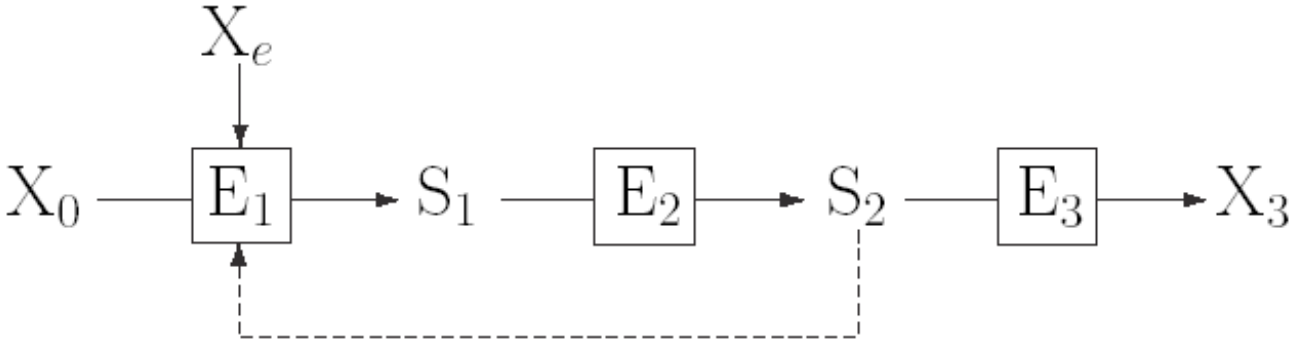
Answer: The flux, J, must increase fractionally by α, but, since all the rates increased in the same proportion, the concentrations of the variable metabolites s_1 and s_2 remain unchanged.



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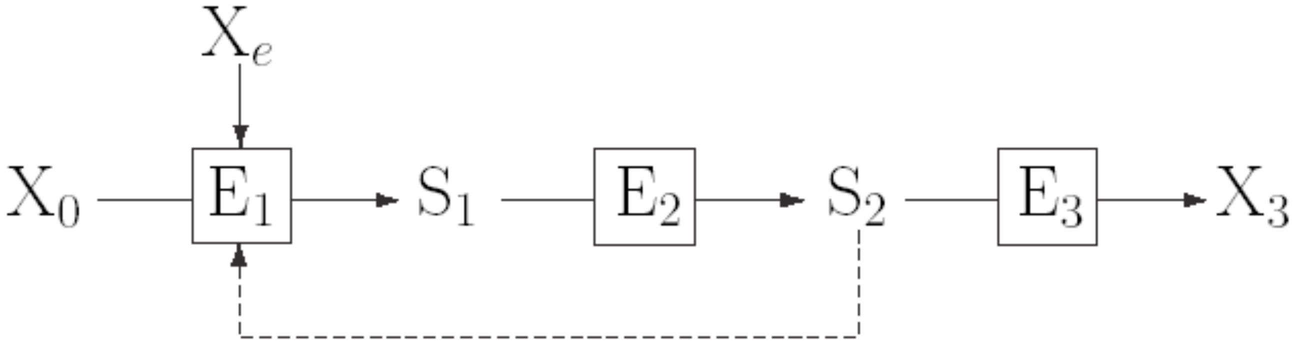
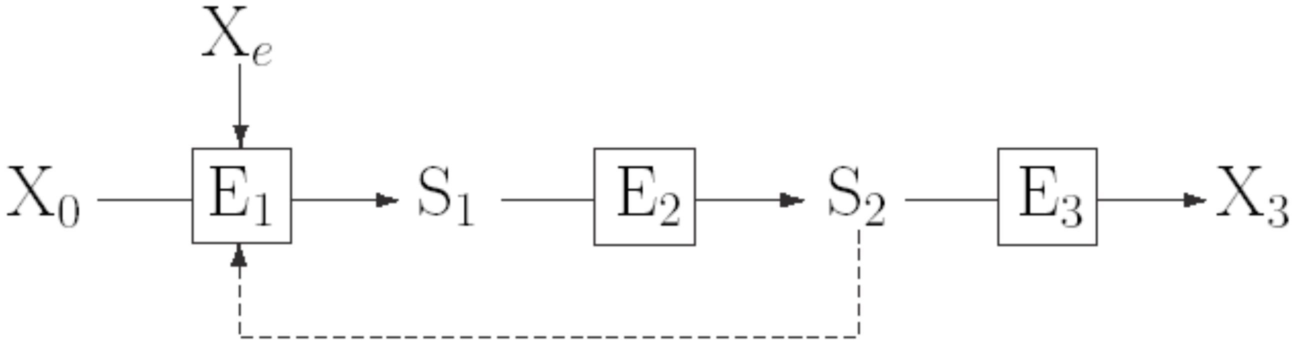


Figure 4.3: A 3-enzyme linear system with a feedback loop from S_2 onto E_1, and an external effector, X_e, that interacts with E_1.

$$\begin{aligned}\frac{\delta J}{J} &= C_1^J \frac{\delta v_1}{v_1} + C_2^J \frac{\delta v_2}{v_2} + C_3^J \frac{\delta v_3}{v_3} \\ \alpha &= \alpha(C_1^J + C_2^J + C_3^J)\end{aligned}$$

$$C_1^J + C_2^J + C_3^J = 1$$



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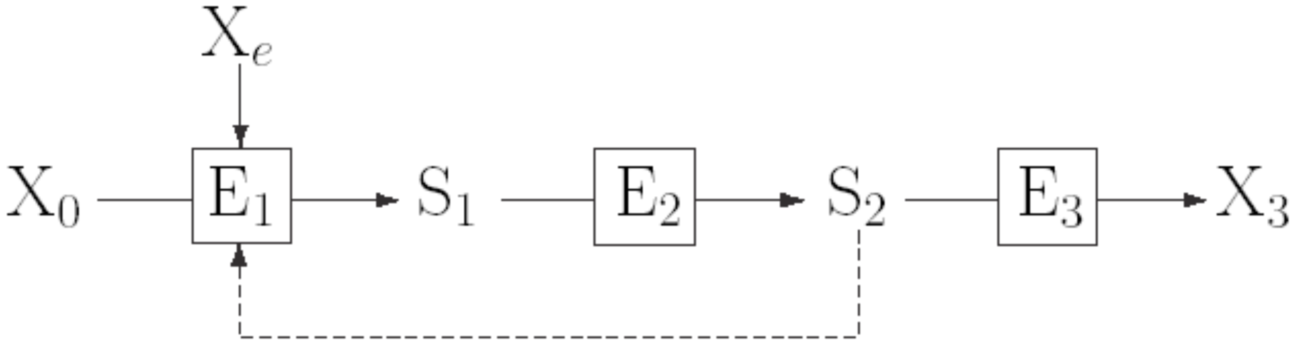


Figure 4.3: A 3-enzyme linear system with a feedback loop from S_2 onto E_1, and an external effector, X_e, that interacts with E_1.

$$
\begin{aligned}
\frac{\delta s_1}{s_1} &= C_1^{s_1}\frac{\delta v_1}{v_1} + C_2^{s_1}\frac{\delta v_2}{v_2} + C_3^{s_1}\frac{\delta v_3}{v_3} \\
0 &= \alpha(C_1^{s_1} + C_2^{s_1} + C_3^{s_1})
\end{aligned}
$$

$$C_1^{s_1} + C_2^{s_1} + C_3^{s_1} = 0$$

$$C_1^{s_2} + C_2^{s_2} + C_3^{s_2} = 0$$

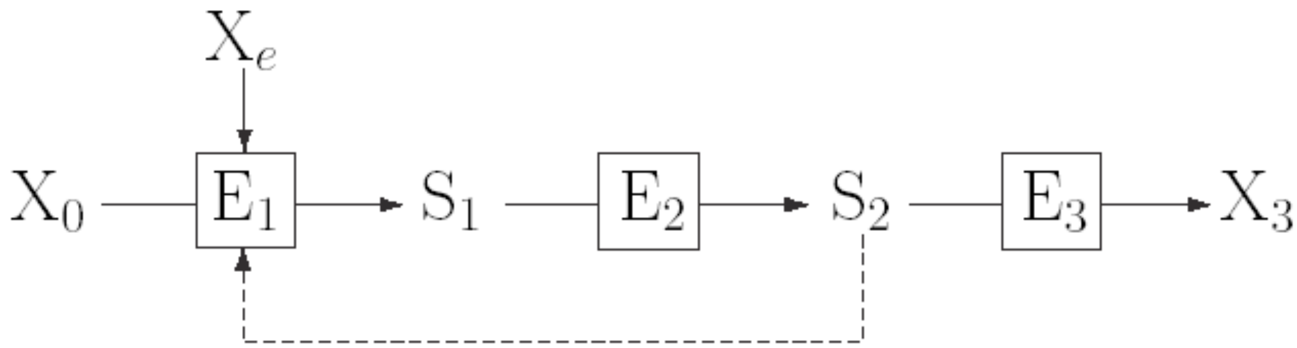


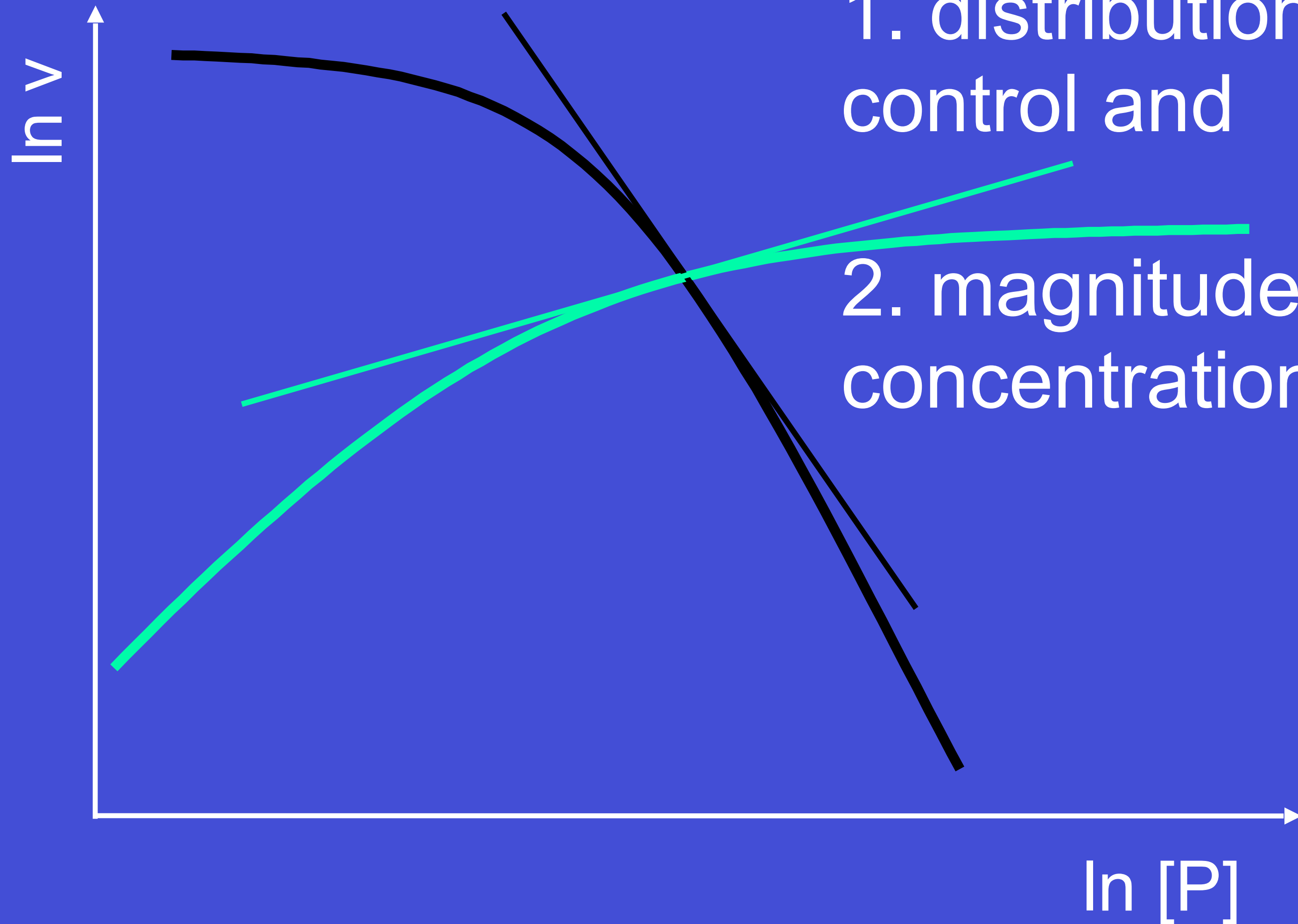
Figure 4.3: A 3-enzyme linear system with a feedback loop from S_2 onto E_1, and an external effector, X_e, that interacts with E_1.

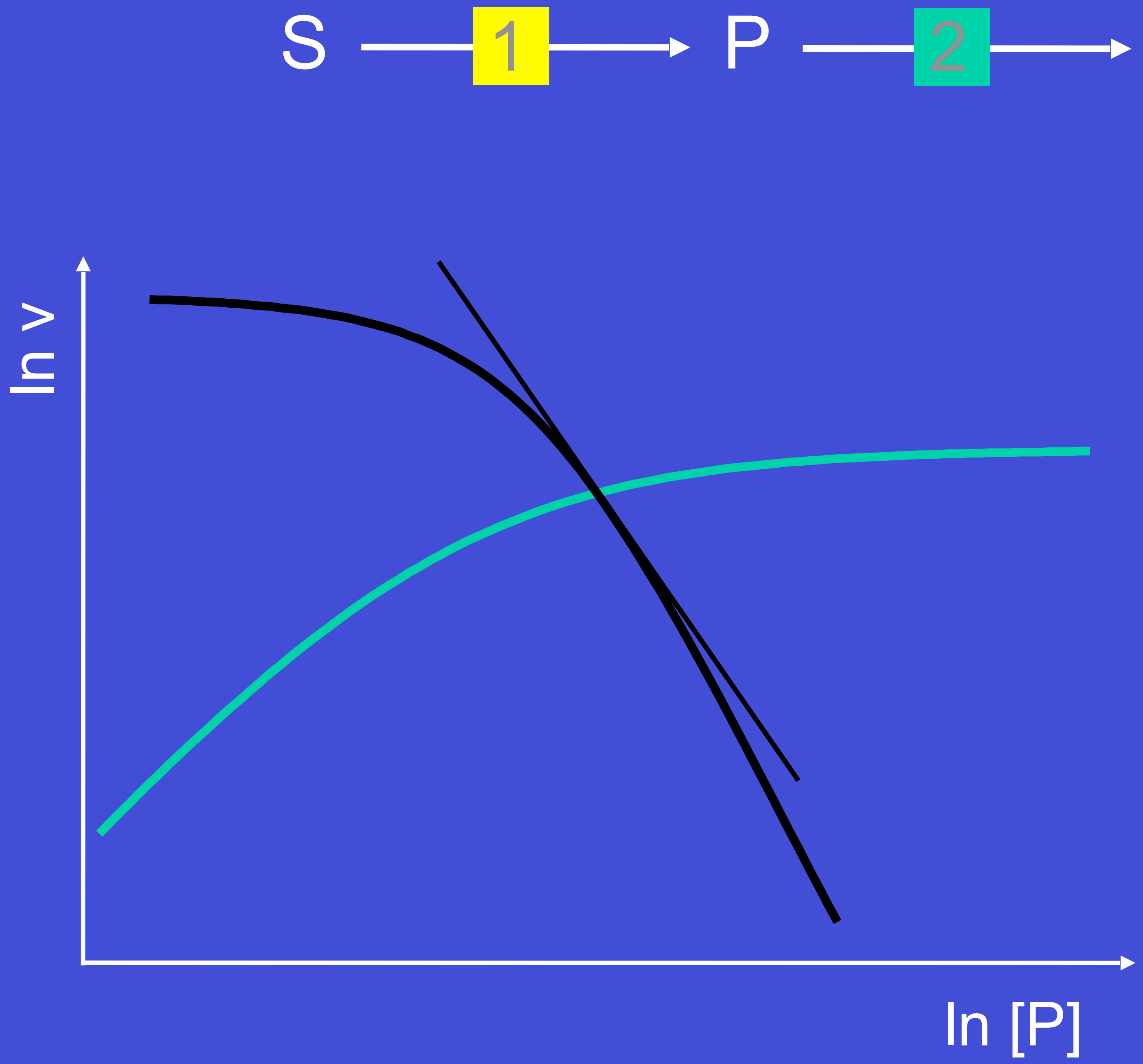
$S \xrightarrow{1} P \xrightarrow{2}$

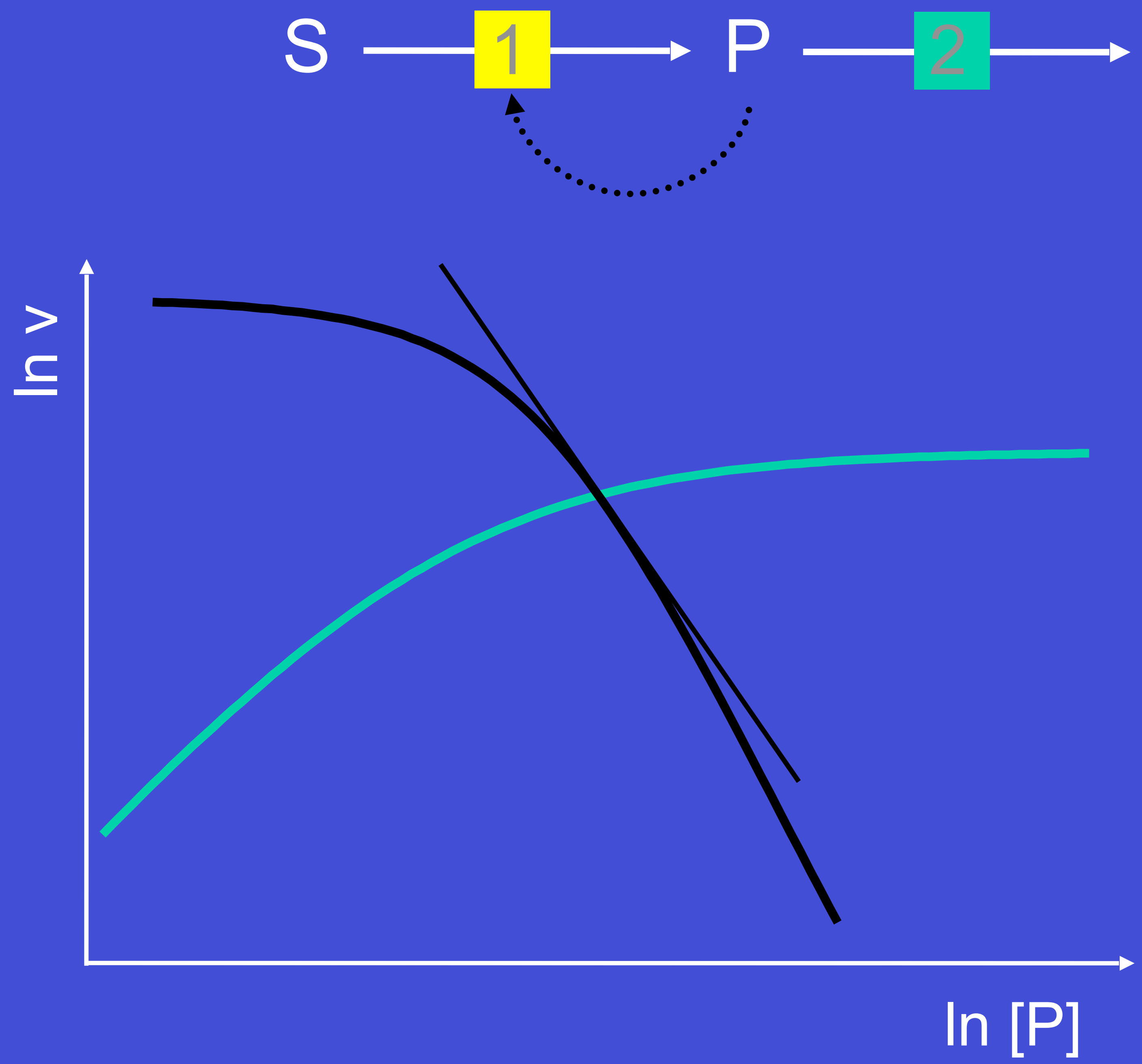
Slopes determine:

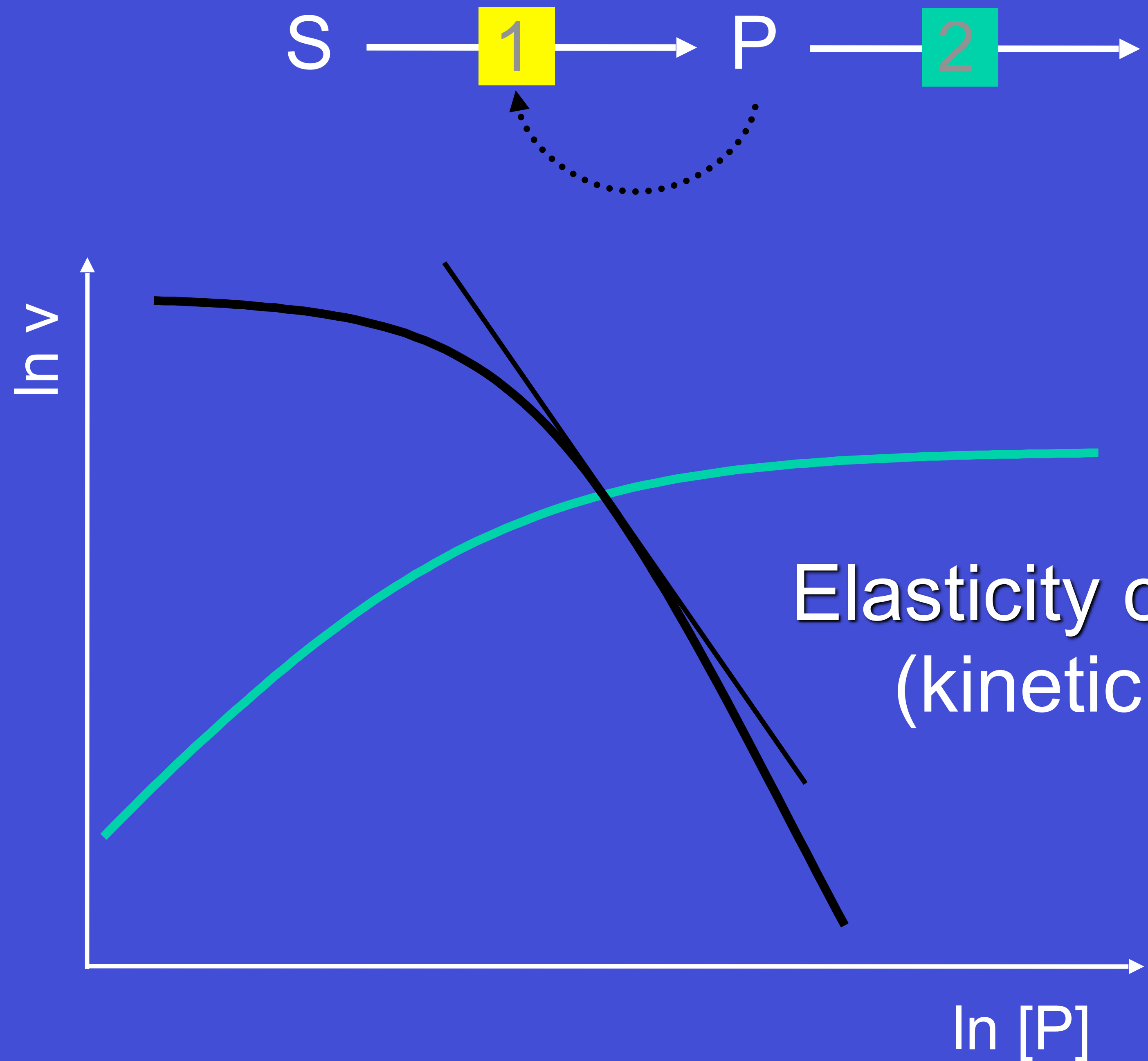
1. distribution of flux control and

2. magnitude of concentration control

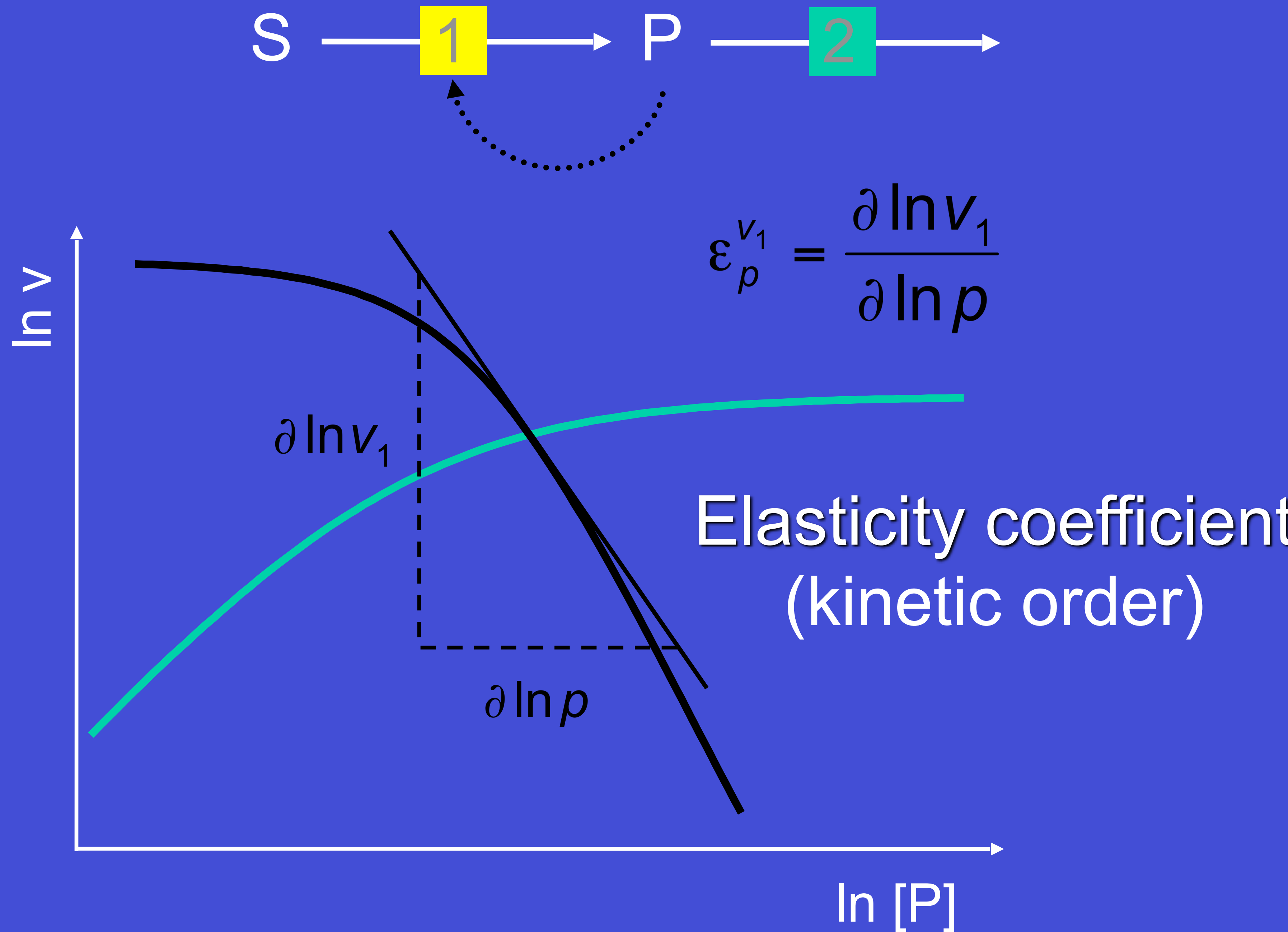


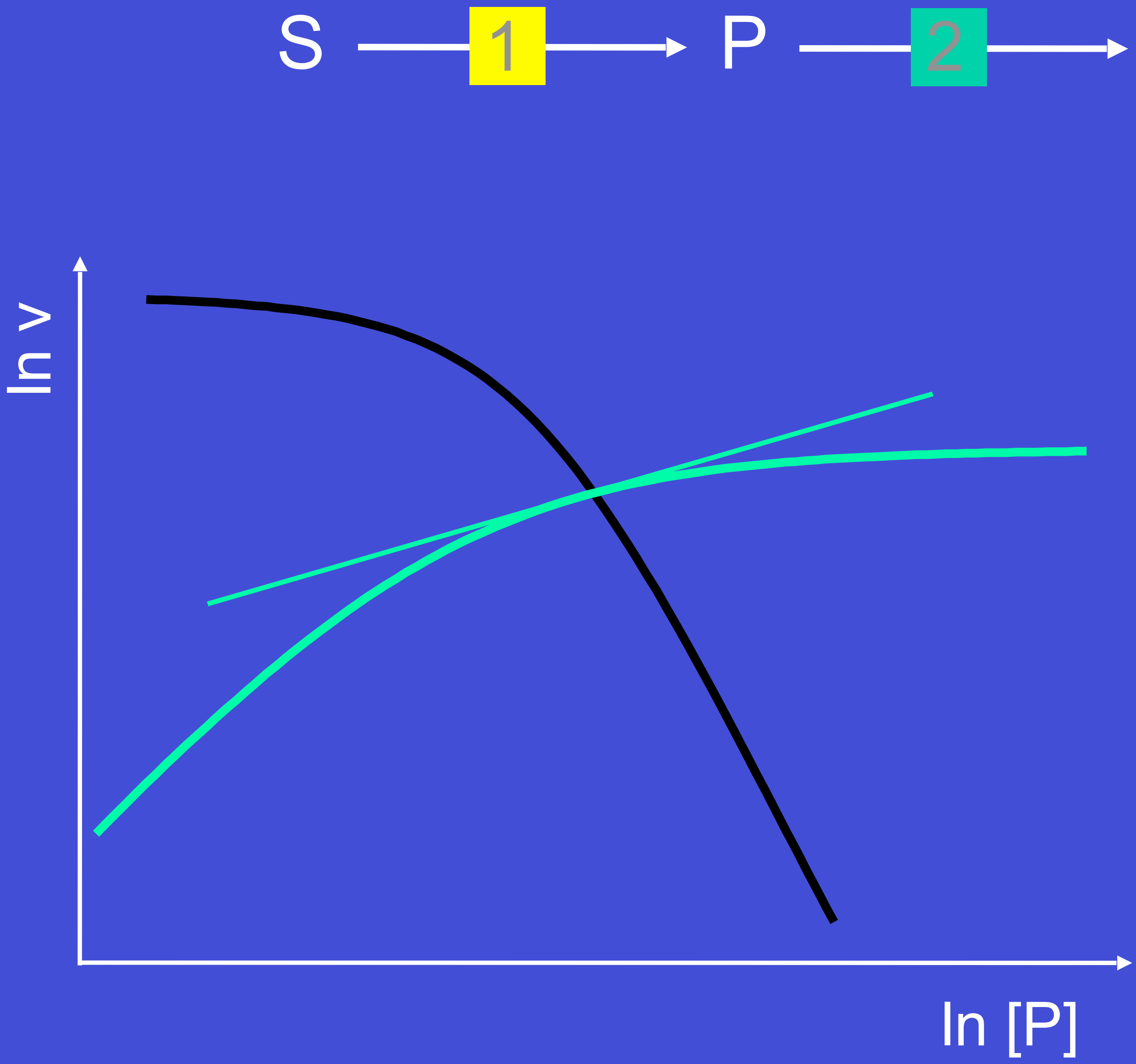

S
1
P
2
ln v
ln [P]

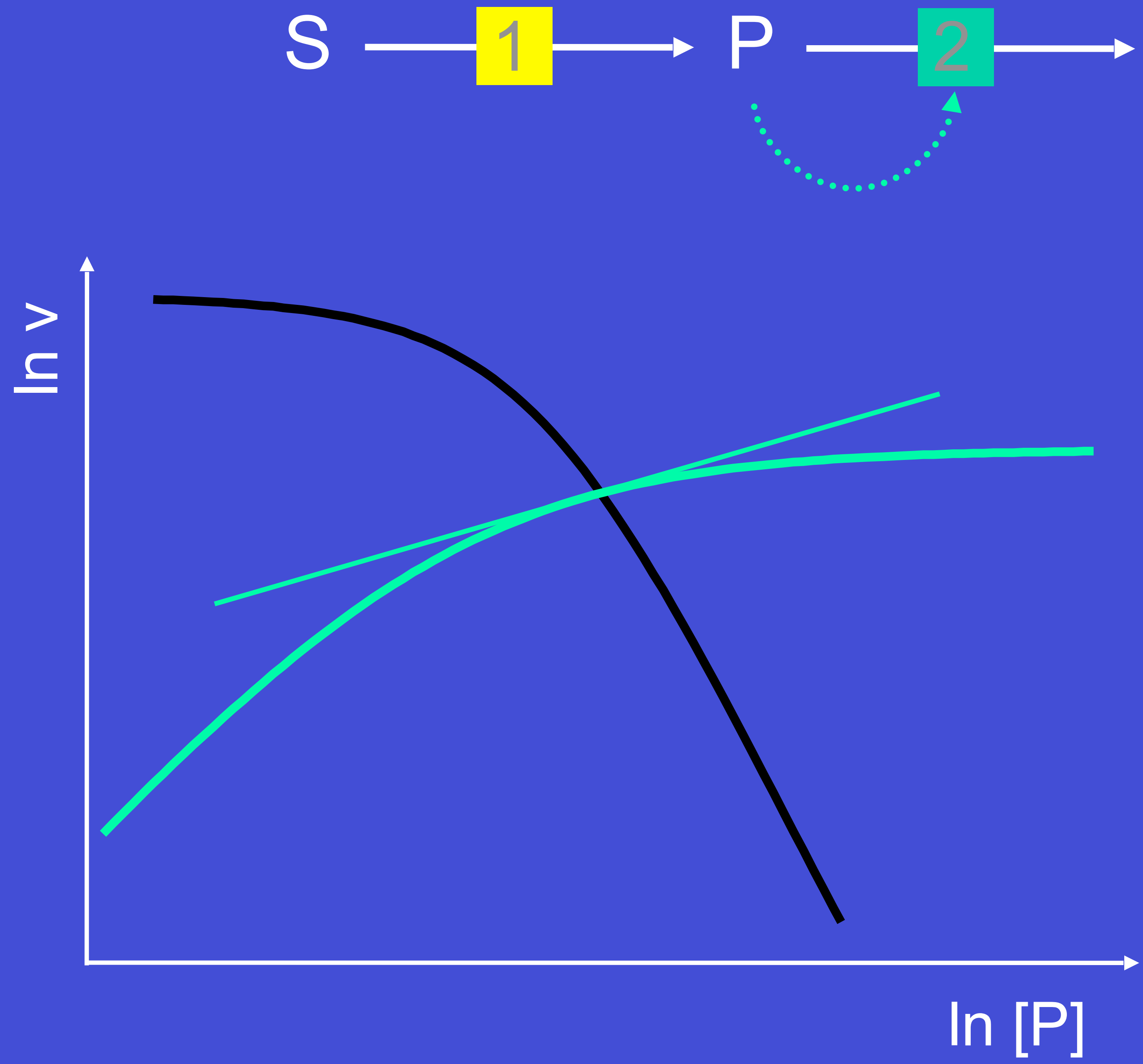

S
1
P
2
ln v
ln [P]



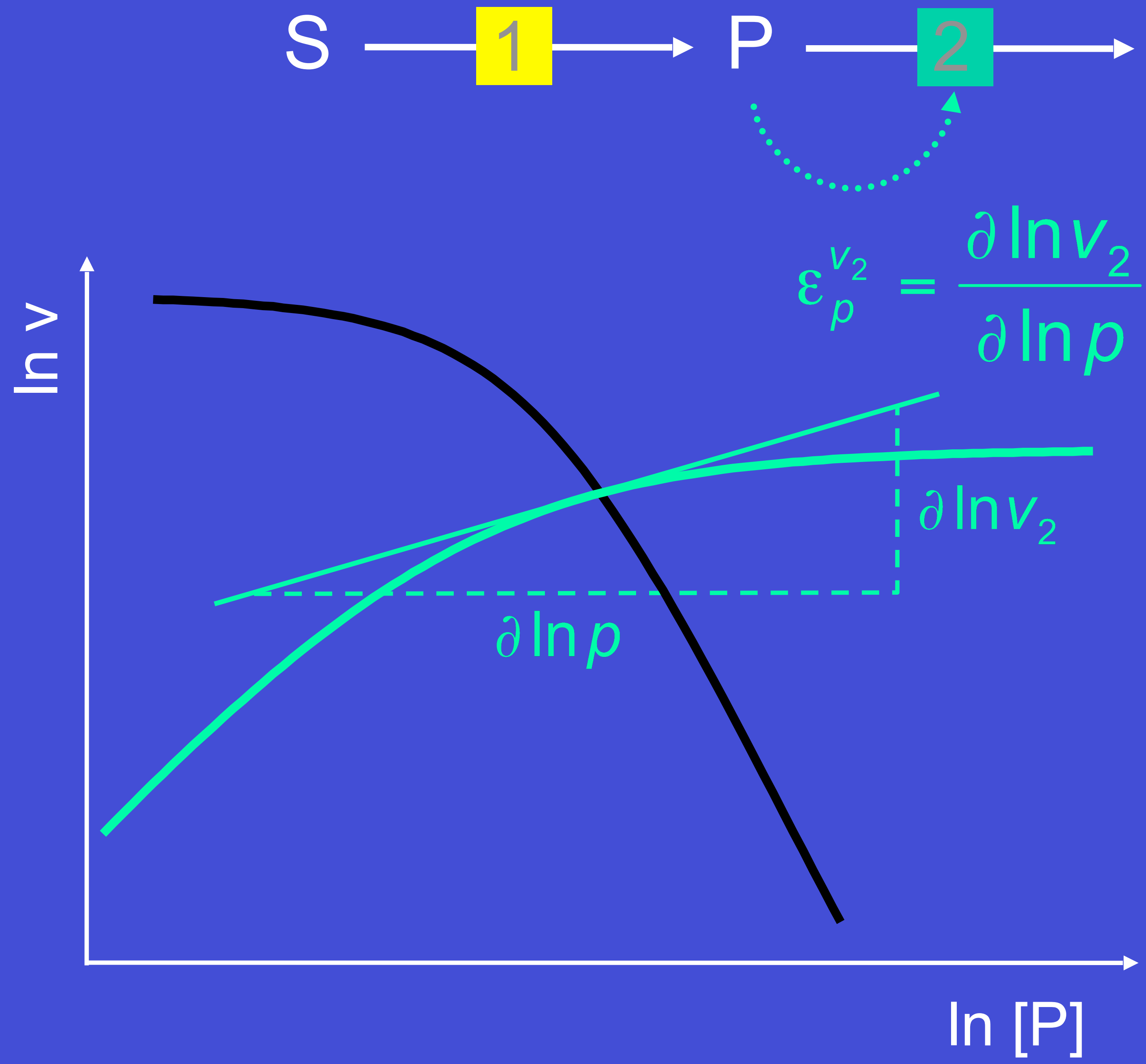
Elasticity coefficient
(kinetic order)


S
1
P
2
ln v
ln [P]
$\partial \ln v_1$
$\partial \ln p$
$\varepsilon_p^{v_1} = \frac{\partial \ln v_1}{\partial \ln p}$
Elasticity coefficient
(kinetic order)


S
1
P
2
ln v
ln [P]



S
1
P
2
ln v
ln [P]



S
1
P
2
$\varepsilon_{p}^{v_2} = \frac{\partial \ln v_2}{\partial \ln p}$
ln v
$\partial \ln v_2$
$\partial \ln p$
ln [P]

Elasticity coefficient

$$\varepsilon^v_s = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

1. The ratio of the fractional change $\delta v/v$ in the local rate of enzyme and the fractional change $\delta s/s$ in the concentration of a metabolite that interacts directly with the enzyme at a specified value of s (and, of course, specified values of all the other metabolites that directly affect v).

Elasticity coefficient

$$\varepsilon^v_s = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

2. Multiplying both fractional changes by 100 will give the percentage change in each, so that we can also describe the elasticity coefficient as the % change measured in the rate of an enzyme in response to a 1% change in a metabolite concentration.

Elasticity coefficient

$$\varepsilon^v_s = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v/v}{\delta s/s}$$

In words, the elasticity coefficient can be described in five ways:

3. The product of s/v and the slope of the tangent $\delta v/\delta s$ at a specified value of s on a plot of v against s.

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v/v}{\delta s/s}$$

In words, the elasticity coefficient can be described in five ways:

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

4. An even more elegant way of expressing elasticity coefficients takes into account that, mathematically speaking, $\delta x/x = \delta \ln |x|$. This means that we can also define the elasticity coefficient as

$$\varepsilon_s^v = \frac{\delta \ln v}{\delta \ln s} \tag{4.2}$$

in other words, the slope of the tangent to the curve obtained by plotting $\ln v$ against $\ln s$. This is shows explicitly in Fig. 4.1

Elasticity coefficient

$$\varepsilon^v_s = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v / v}{\delta s / s}$$

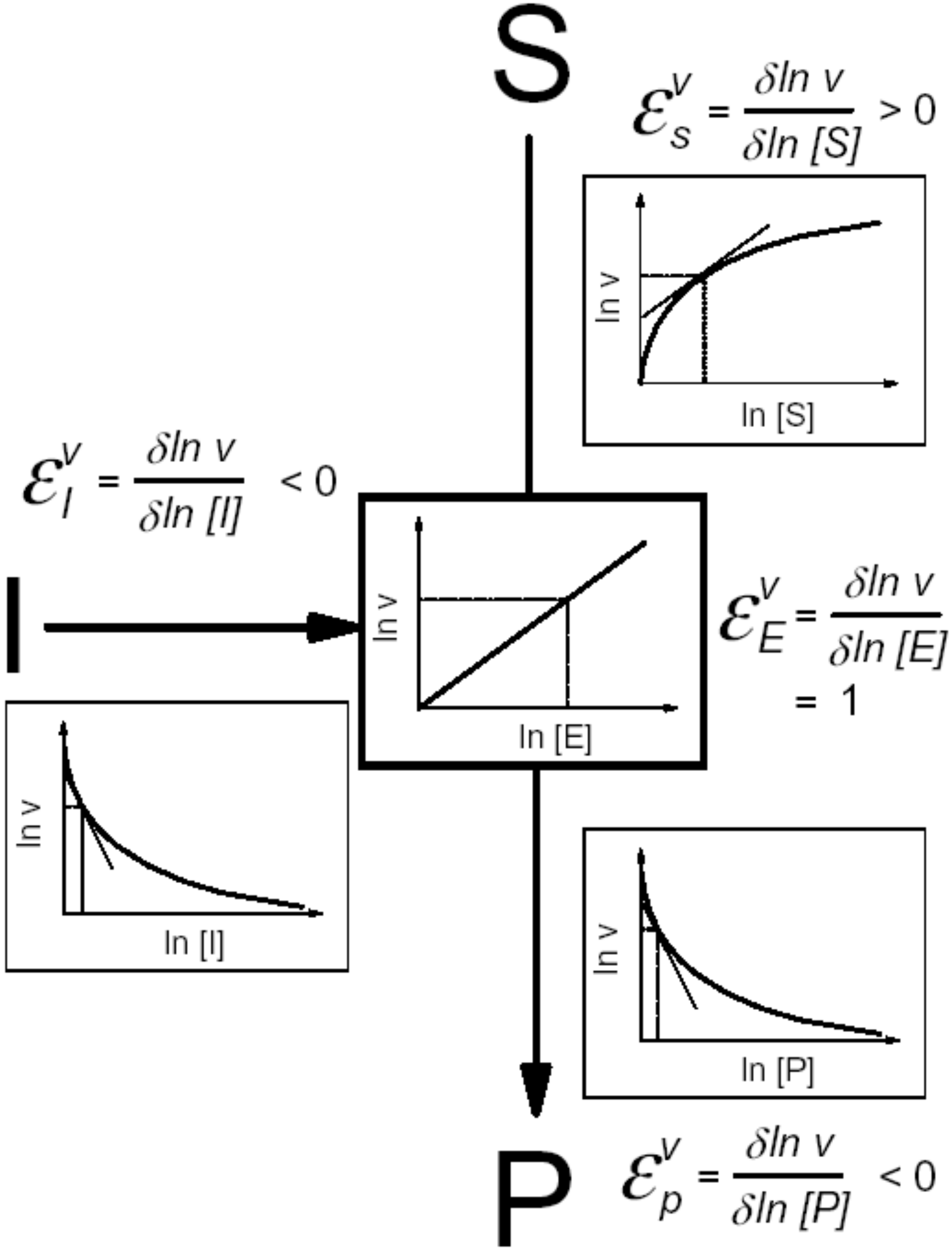
In words, the elasticity coefficient can be described in five ways:

5. The *apparent kinetic order* of the rate with respect to s at a specified value of s. This description implies that an elasticity coefficient is a variable enzymological property; like all other enzymic properties (K_M, V, etc.) its value is constant for a prescribed set of conditions but will vary as conditions vary.

Elasticity coefficient

$$\varepsilon_s^v = \frac{\delta v / v}{\delta s / s}$$

In words, the elasticity coefficient can be described in five ways:



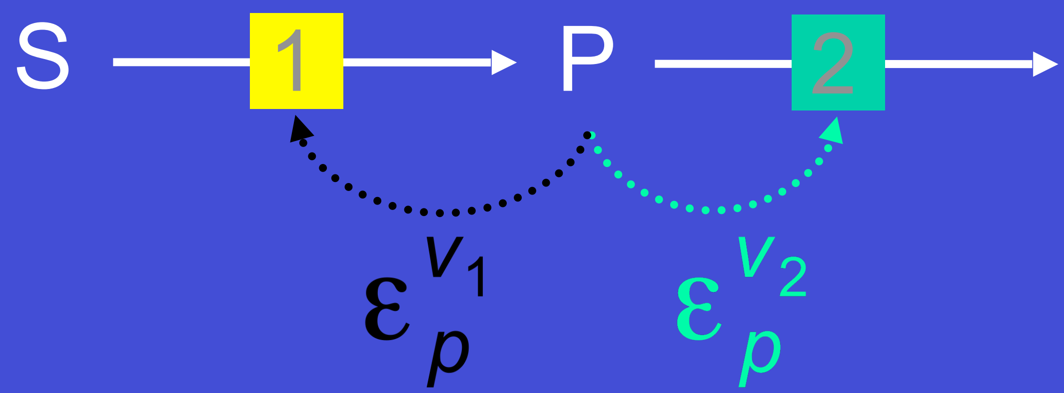
$$\mathcal{E}^{v}_{s} = \frac{\delta \ln v}{\delta \ln [S]} > 0$$

$$\mathcal{E}^{v}_{I} = \frac{\delta \ln v}{\delta \ln [I]} < 0$$

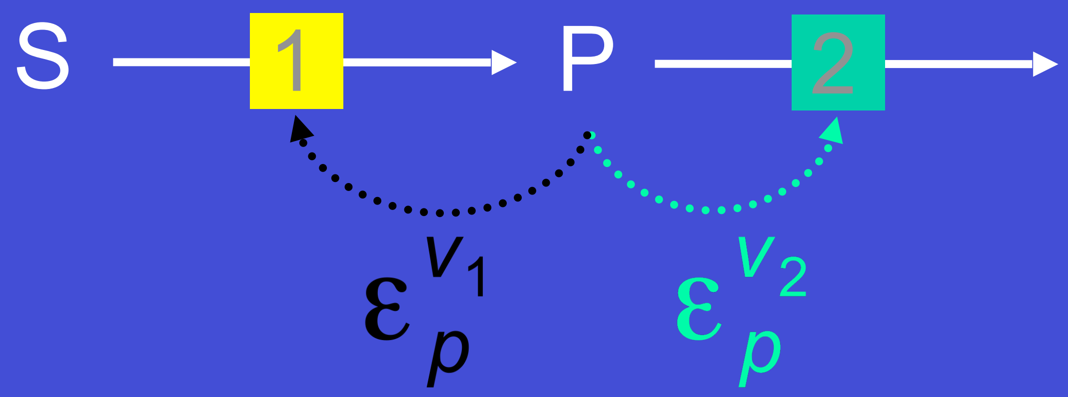
$$\mathcal{E}^{v}_{E} = \frac{\delta \ln v}{\delta \ln [E]} = 1$$

$$\mathcal{E}^{v}_{p} = \frac{\delta \ln v}{\delta \ln [P]} < 0$$

Summary of Control Properties



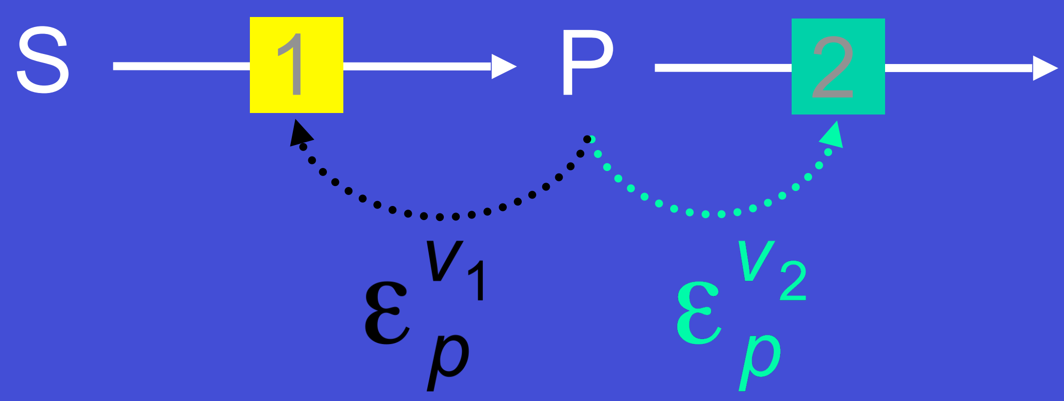
Summary of Control Properties



Summation

Flux $$C^J_1 + C^J_2 = 1$$

Summary of Control Properties

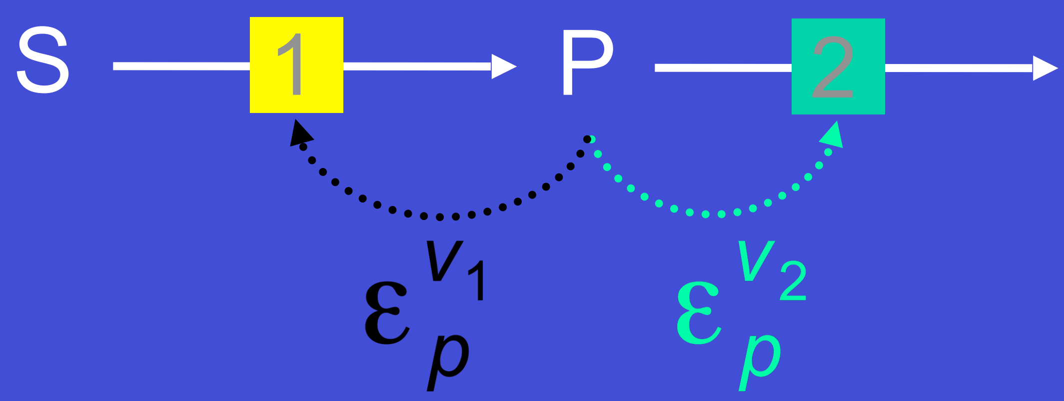


Summation

Flux $$C^J_1 + C^J_2 = 1$$

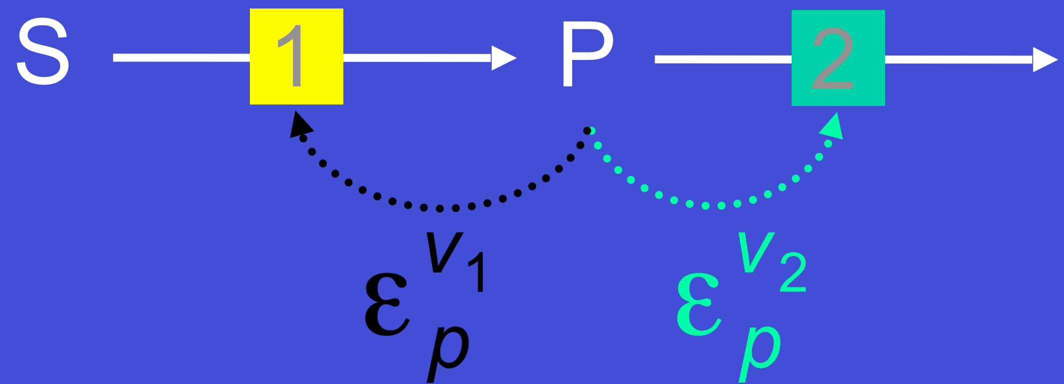
Concen-tration $$C^p_1 + C^p_2 = 0$$

Summary of Control Properties



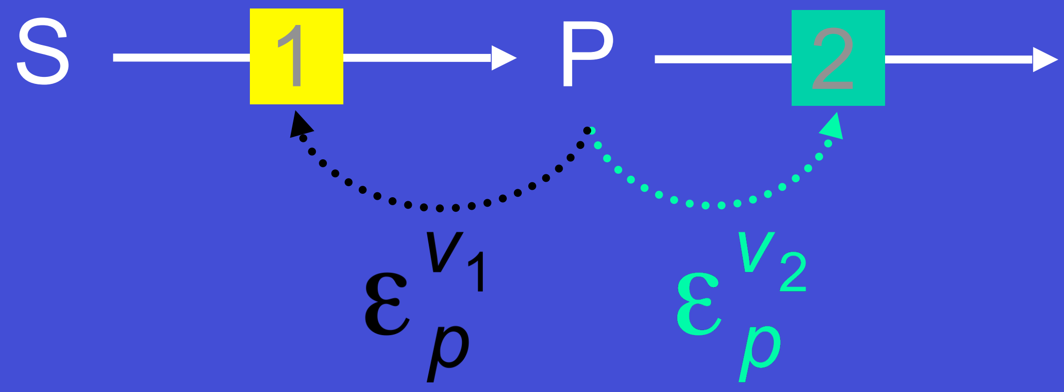
	Summation	Connectivity
Flux	$C_1^J + C_2^J = 1$	$C_1^J \varepsilon_p^{v_1} + C_2^J \varepsilon_p^{v_2} = 0$
Concen-tration	$C_1^p + C_2^p = 0$	

Summary of Control Properties



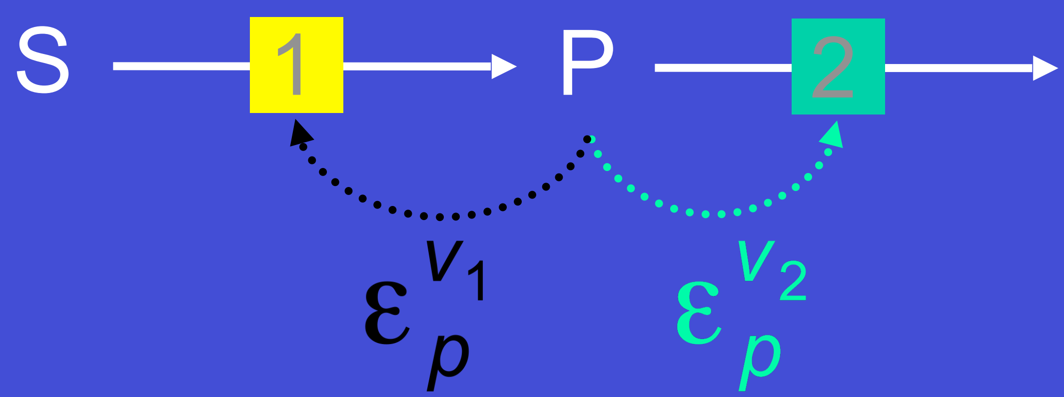
	Summation	Connectivity
Flux	$C_1^J + C_2^J = 1$	$C_1^J \varepsilon_p^{v_1} + C_2^J \varepsilon_p^{v_2} = 0$
Concen-tration	$C_1^p + C_2^p = 0$	$C_1^p \varepsilon_p^{v_1} + C_2^p \varepsilon_p^{v_2} = -1$

Control Matrix Equation



$$\begin{bmatrix} C^J_1 & C^J_2 \\ C^p_1 & C^p_2 \end{bmatrix} \begin{bmatrix} 1 & -\varepsilon^{v_1}_{p} \\ 1 & -\varepsilon^{v_2}_{p} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

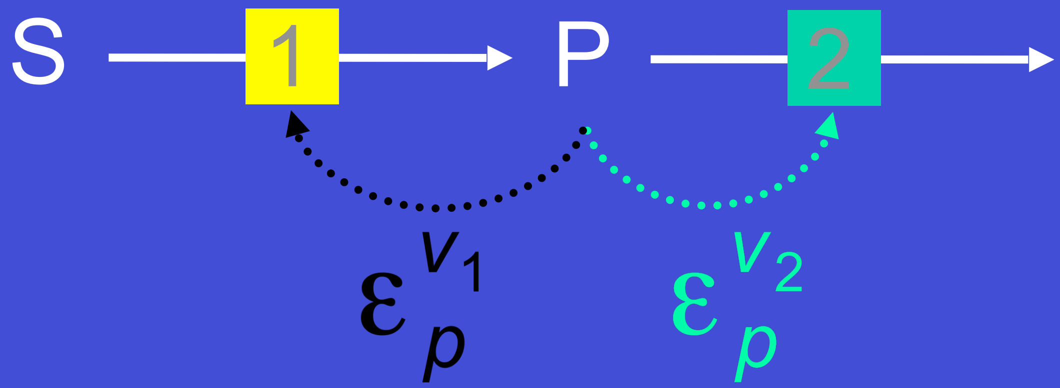
Control Matrix Equation



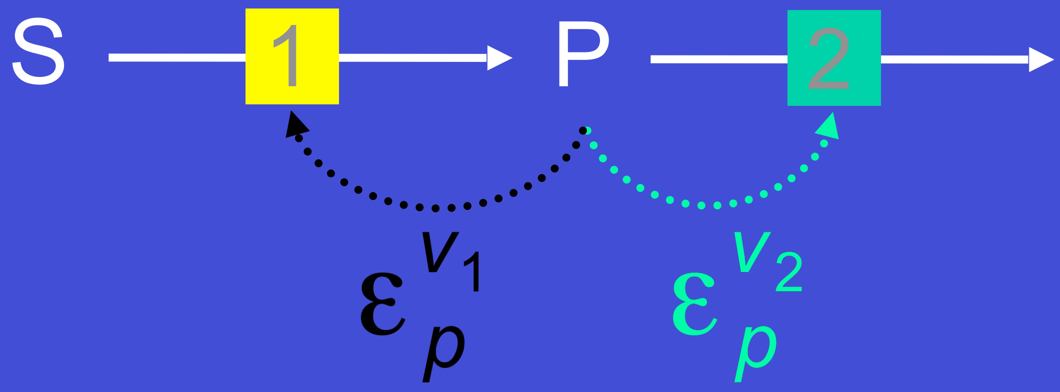
$$\begin{bmatrix} C_1^J & C_2^J \\ C_1^p & C_2^p \end{bmatrix} \begin{bmatrix} 1 & -\varepsilon_p^{v_1} \\ 1 & -\varepsilon_p^{v_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

$$\begin{bmatrix} C_1^J & C_2^J \\ C_1^p & C_2^P \end{bmatrix} = \begin{bmatrix} 1 & -\varepsilon_p^{v_1} \\ 1 & -\varepsilon_p^{v_2} \end{bmatrix}^{-1}$$

Control analytic expressions



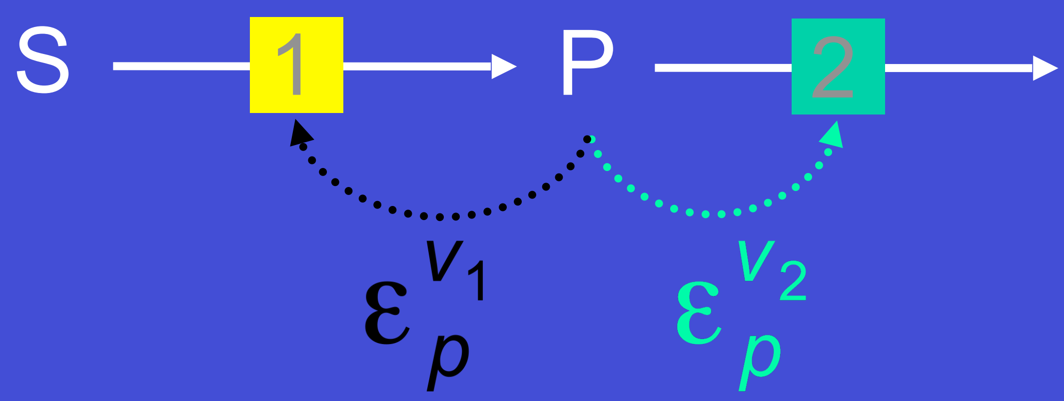
Control analytic expressions



$$C^J_1 = \frac{\varepsilon^{v_2}_p}{\varepsilon^{v_2}_p - \varepsilon^{v_1}_p}$$

$$C^p_1 = \frac{1}{\varepsilon^{v_2}_p - \varepsilon^{v_1}_p}$$

Control analytic expressions



$$C_1^J = \frac{\varepsilon_p^{v_2}}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

$$C_2^J = \frac{-\varepsilon_p^{v_1}}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

$$C_1^p = \frac{1}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

$$C_2^p = \frac{-1}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

Combined Response

$$R^{y}_{e_i} = C^{y}_{i} \varepsilon^{v_i}_{e_i}$$

Combined Response

$$R^{y}_{e_i} = C^{y}_{i} \varepsilon^{v_i}_{e_i}$$

$$R^{y}_{p} = \sum_{i=1}^{n} C^{y}_{i} \varepsilon^{v^i}_{p}$$