Today’s class:

Absorption Spectroscopy part 2

This lecture follows the materials from the following books

- *Physical Chemistry for Life Sciences, by PW Atkins and JD Paula, Oxford, 2006*
- *Koolman, Color Atlas of Biochemistry, 2nd Ed, 2005*

Biochemical applications of absorption spectroscopy and Beer-Lambert law

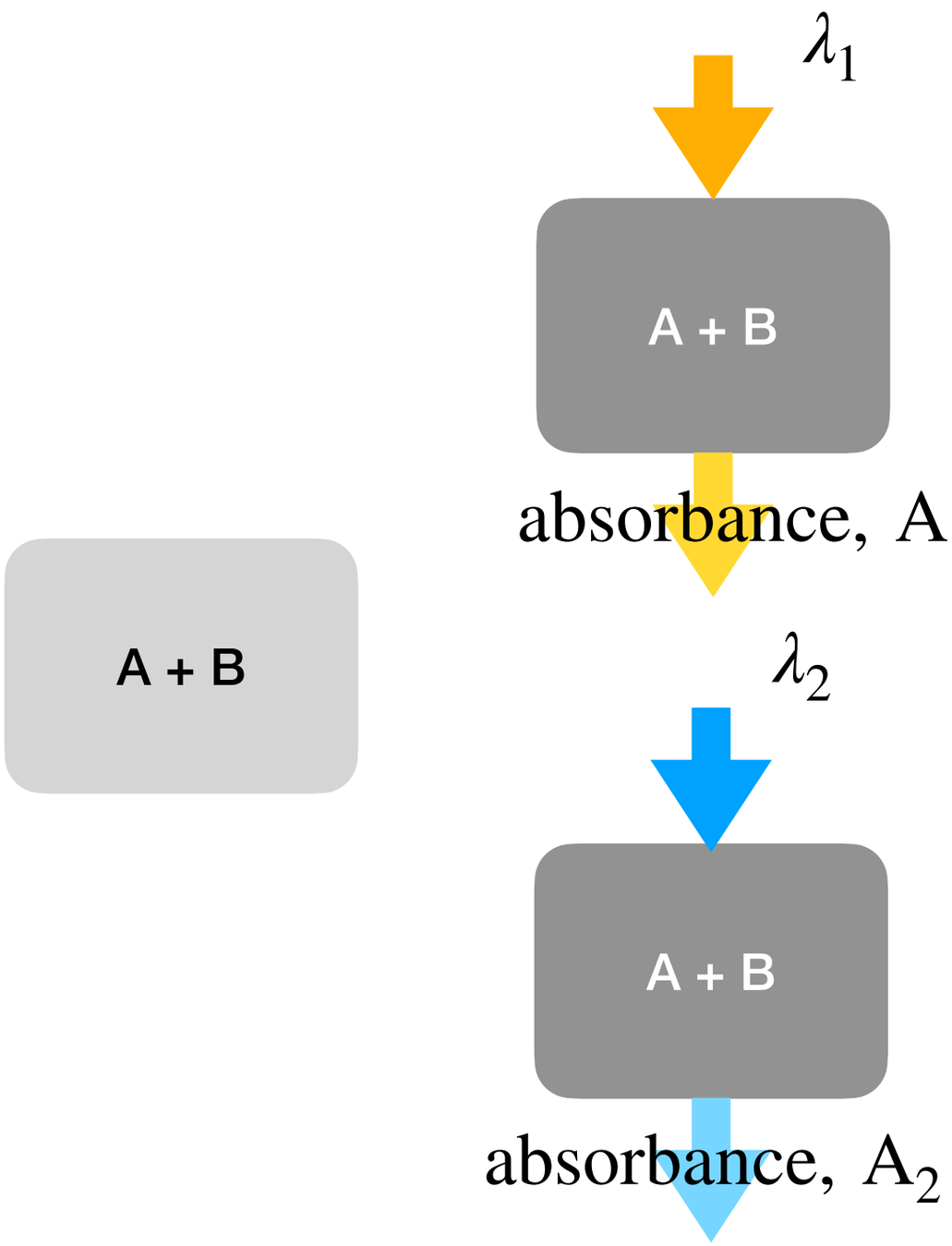
Analysis of protein mixtures

Measuring blood oxygen level

Estimation of enzymatic activity

Enzymatic determination of a colorless biomolecule

Analyzing mixtures of absorbing species



Total absorbance at a given λ

$$A = A_A + A_B$$

$$= \varepsilon_A[A]l + \varepsilon_B[B]l$$

$$= (\varepsilon_A[A] + \varepsilon_B[B])l$$

Analyzing mixtures of absorbing species …*contd*

For two wavelengths

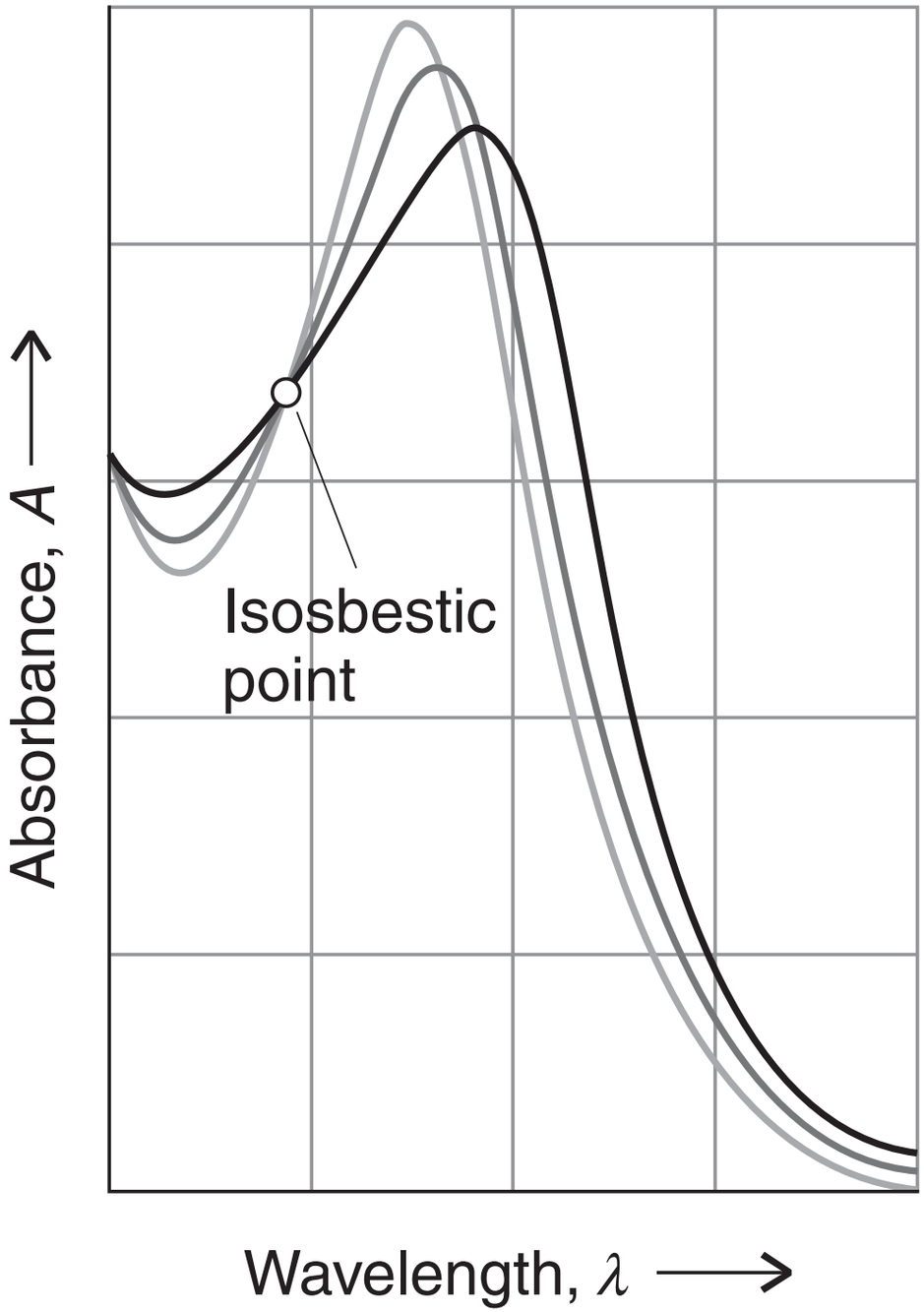
$$A_1 = \left(\epsilon_A^1[A] + \epsilon_B^1[B]\right) l \qquad A_2 = \left(\epsilon_A^2[A] + \epsilon_B^2[B]\right) l$$

Two equations, two unknowns [A] and [B]

Lets solve!

$$[A] = \frac{\epsilon_B^2 A_1 - \epsilon_B^1 A_2}{\left(\epsilon_A^1 \epsilon_B^2 - \epsilon_A^2 \epsilon_B^1\right) l} \qquad [B] = \frac{\epsilon_A^2 A_1 - \epsilon_A^1 A_2}{\left(\epsilon_B^1 \epsilon_A^2 - \epsilon_B^2 \epsilon_A^1\right) l}$$

Isobestic point



There may be a wavelength, λ° where both species in a mixture has same molar extinction coefficient ϵ°

Then we can write, total absorbance of the mixture

$$A^\circ = \epsilon^\circ([A] + [B])l$$

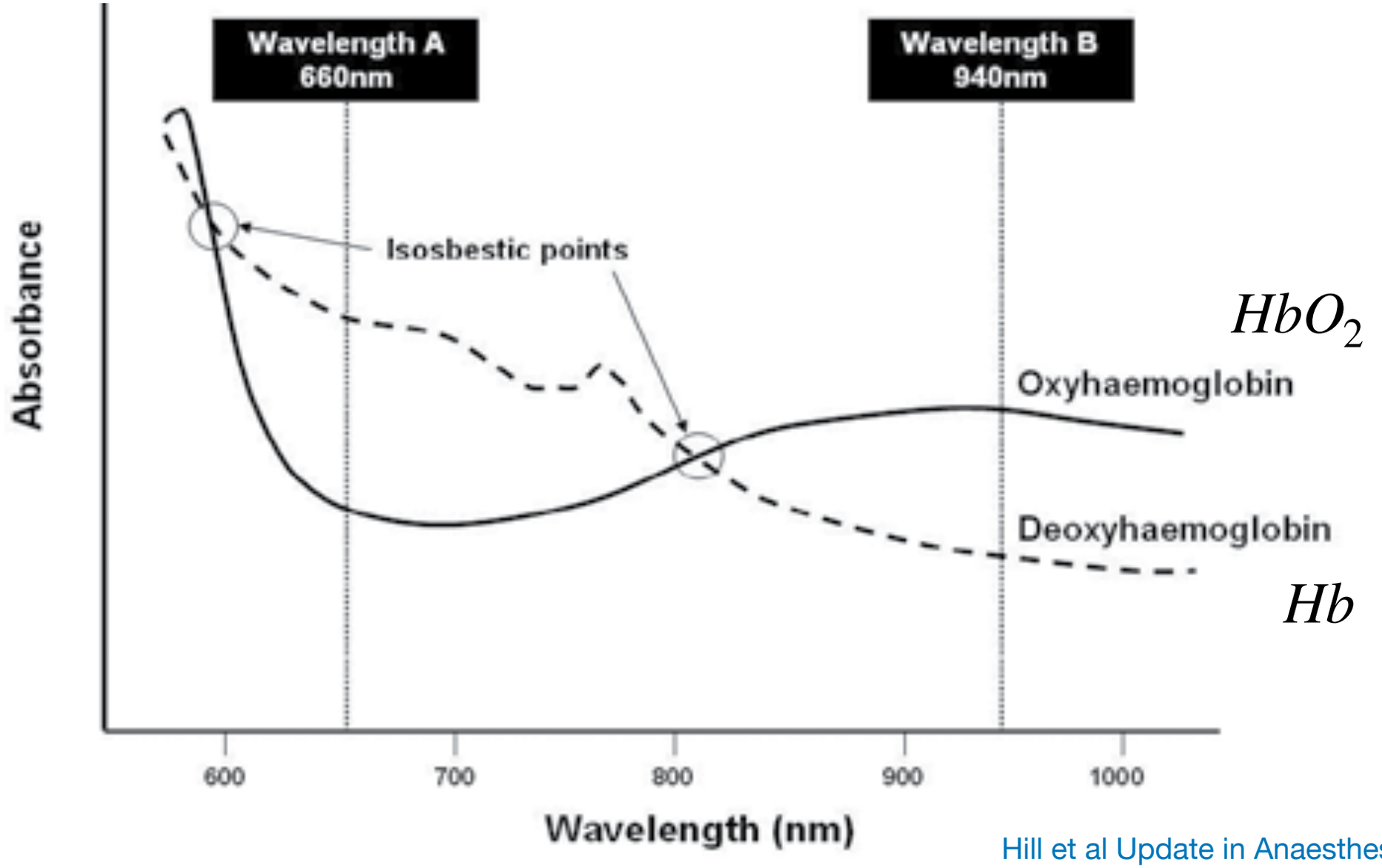
If A and B are related by interconversion

$A \rightleftharpoons B$ then [A]+[B] = constant

A° becomes an invariant quantity

This idea is employed in pulse oximetry to determine the oxygen saturation in arterial blood

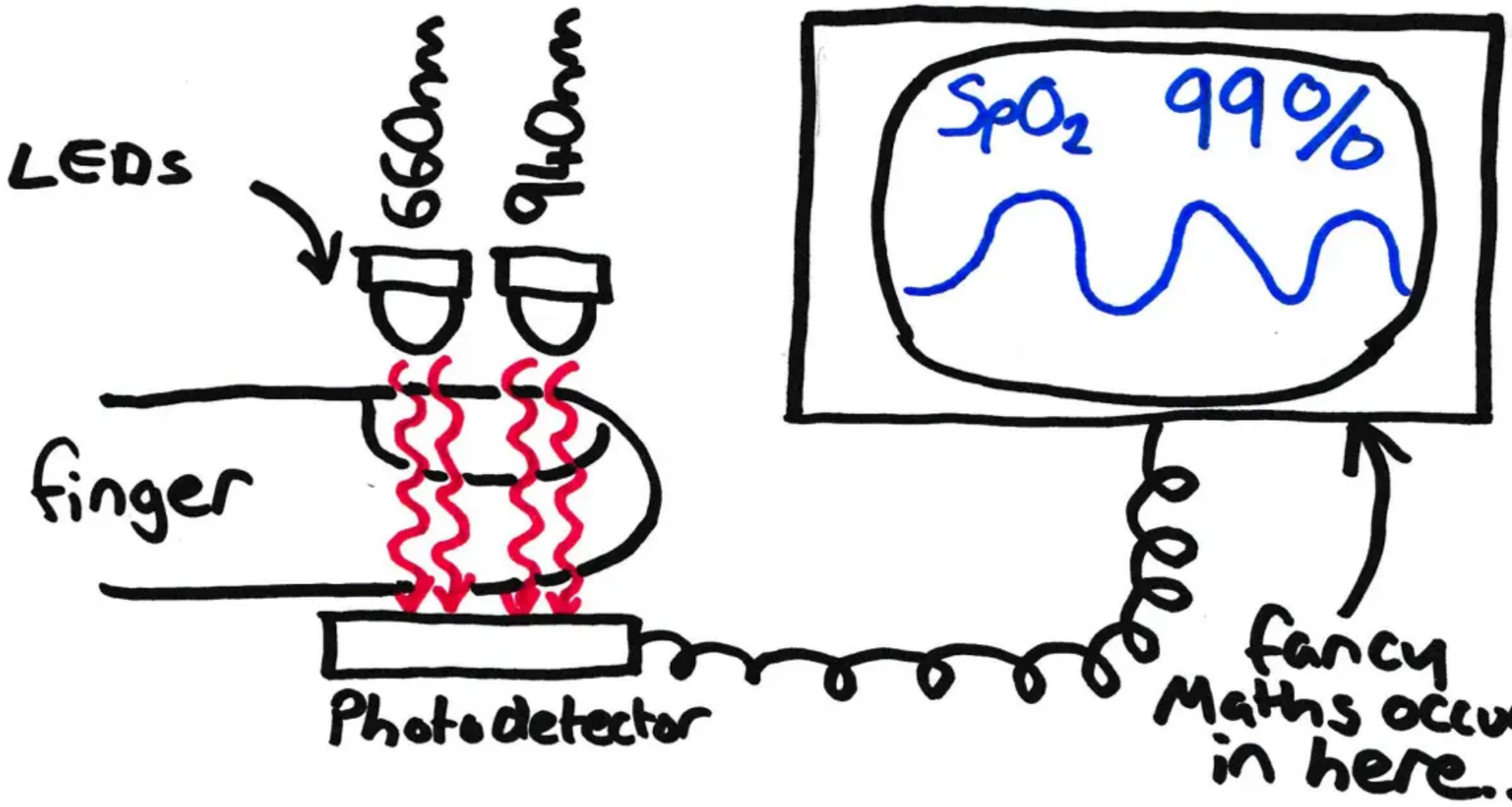
Application of isobestic point in determination of oxyhemoglobin



Hill et al Update in Anaesthesia 2000

The switching of trends around the isobestic point is leveraged by pulse oximetry for measurement of blood oxygen saturation

Basic principle of pulse oximetry



Courtesy: PHYSICS4FRCA

Here the LEDs follow this order repeatedly

- Send 660 nm
- Send 940 nm
- Turn off

→ Only the arterial pulse of blood flow - Venus blood flow subtracted and background corrected

A glimpse in to the math behind pulse oximetry

The reaction $Hb \rightleftharpoons HbO_2$

$$\left.\begin{aligned} A_{660} &\propto [Hb] \\ A_{940} &\propto [HbO_2] \end{aligned}\right\} \quad \frac{A_{660}}{A_{940}} \sim \frac{\epsilon_{Hb}(660)[Hb]}{\epsilon_{HbO_2}(940)[HbO_2]}$$

We can get $\frac{[Hb]}{[HbO_2]}$ from this ratio

From the isobestic point we get

$$A_{805} = \epsilon^{\circ}([Hb] + [HbO_2])l$$

$$\implies [Hb] + [HbO_2] = \frac{A_{805}}{\epsilon^{\circ} l}$$

This info is stored along with ϵ values as a look-up table in memory

All of these is utilized by the processor of oximeter to compute the O_2 saturation as:

$$SpO_2 = \frac{[HbO_2]}{[Hb] + [HbO_2]}$$

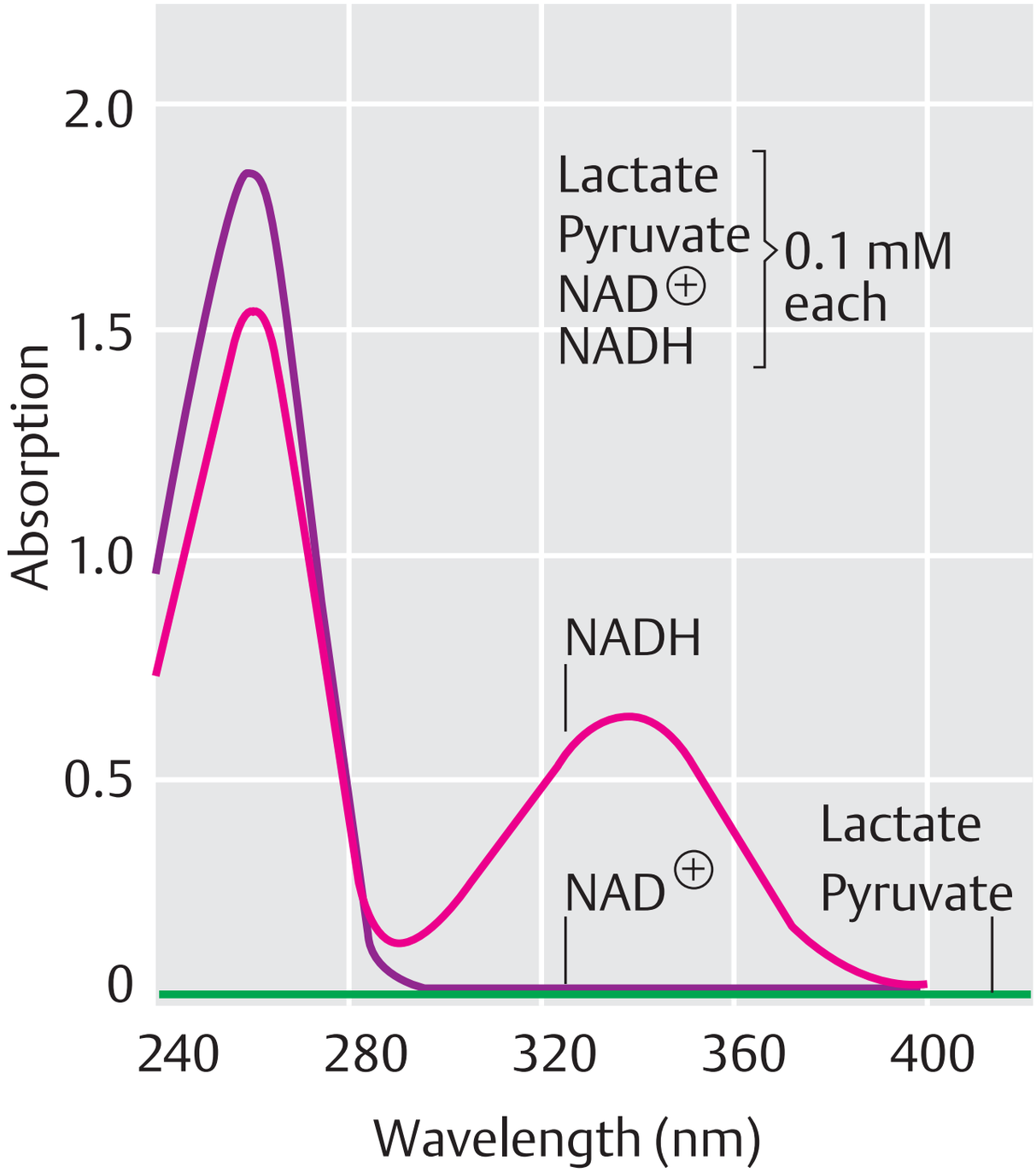
PHYSICS4FRCA

Hill et al Update in Anaesthesia 2000

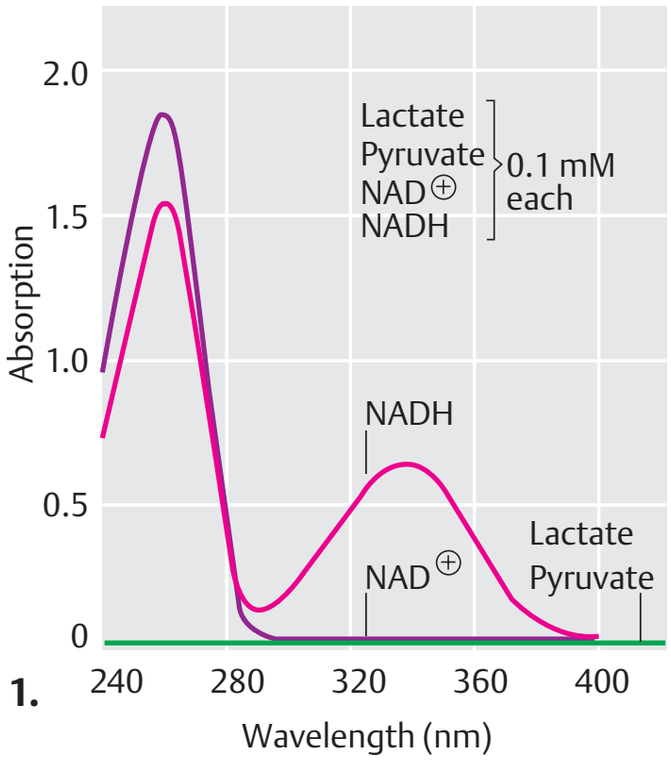
Estimation of enzymatic activity

- Lactate ↔ pyruvate is catalyzed by lactate dehydrogenase (LDH)
- Important process involved in anaerobic glycolysis
- NAD^+ and NADH shows very different absorption behaviors

This large difference in absorption behavior of NADH and NAD^+ can be utilized to monitor catalytic activity of LDH



LDH activity using Beer-Lambert law



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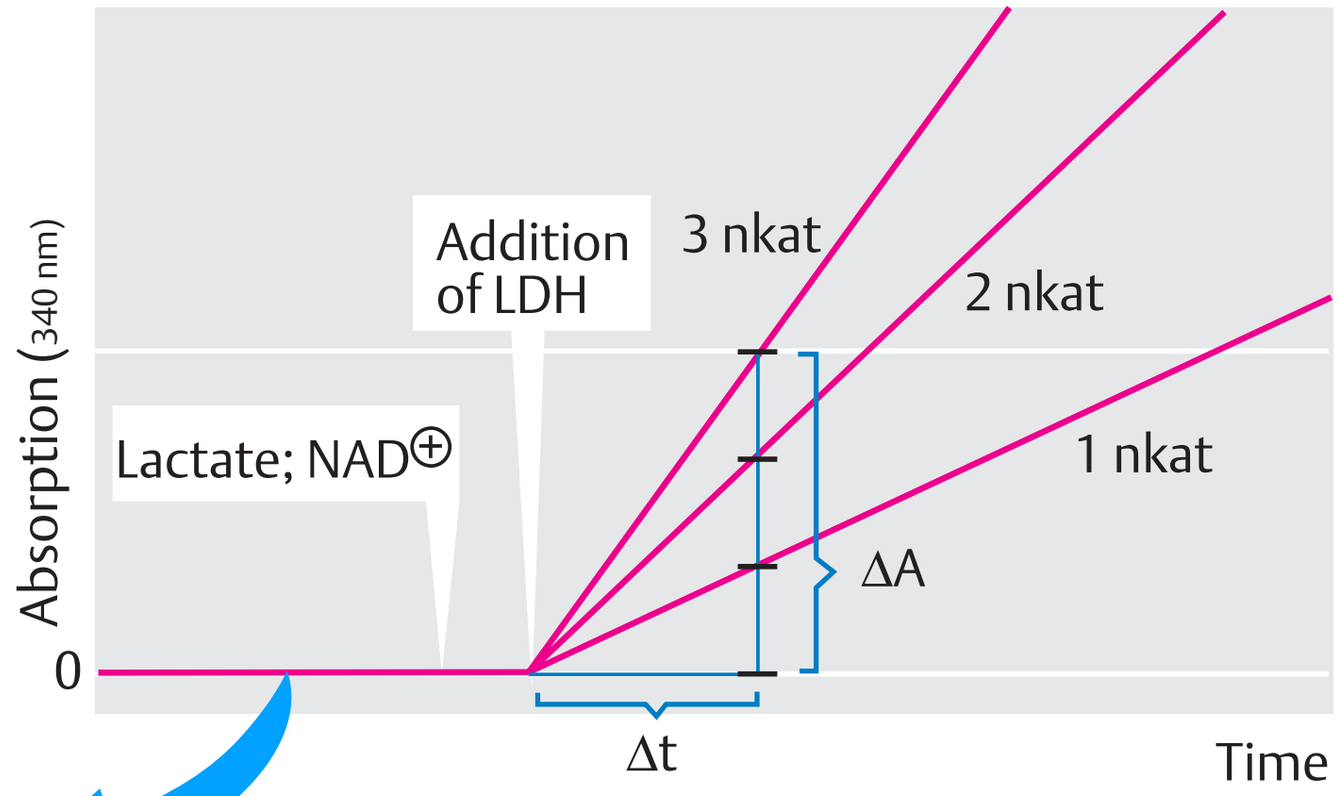


Uncatalyzed lactate+NAD reaction is very slow: almost no NADH produced

From Beer-Lambert law

$$A = \epsilon c l$$

$$\Longrightarrow \frac{dA}{dt} = \epsilon l \frac{dc}{dt}$$

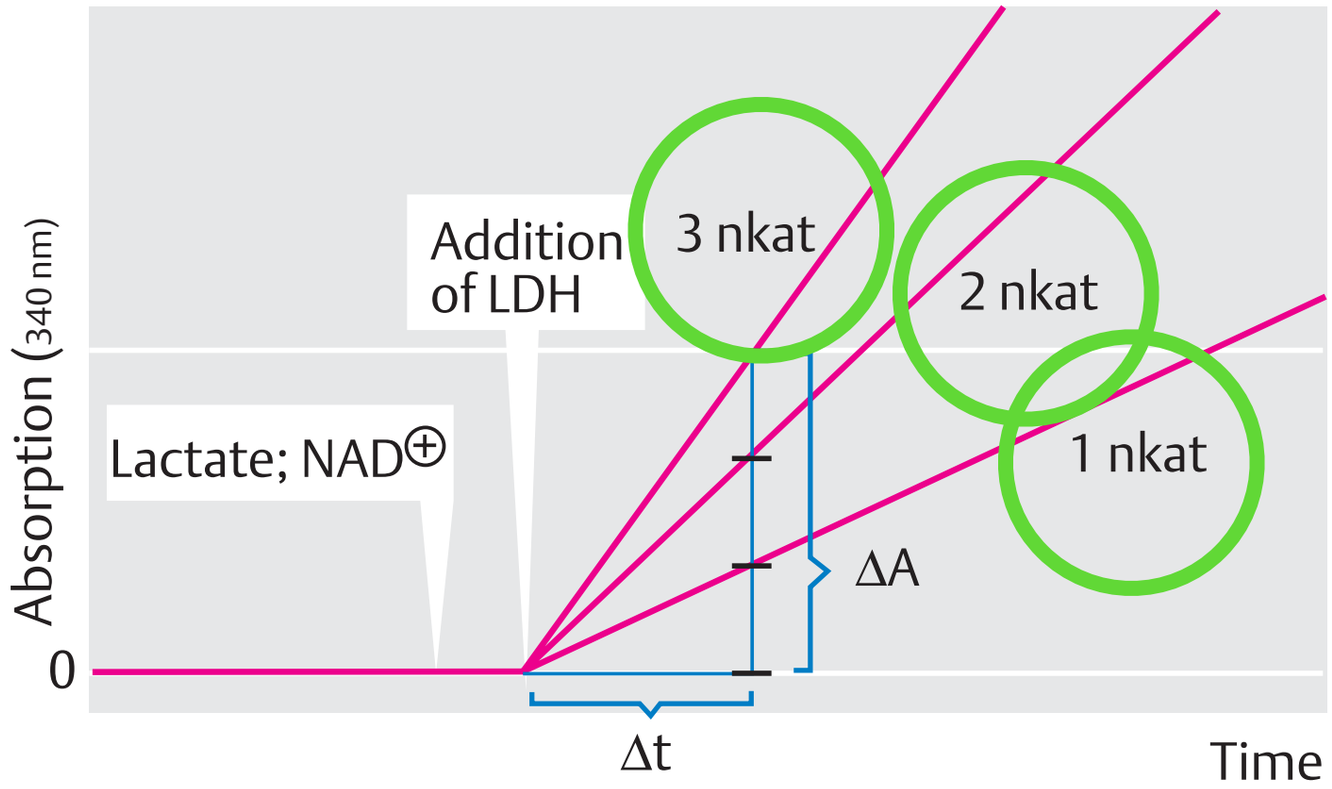
Enzymatic activity is defined as

$$\frac{dc}{dt} = v$$

So, we have

$$v = \frac{1}{\epsilon l}\frac{dA}{dt}$$

Unit of enzyme activity: kat



kat = ‘katal’

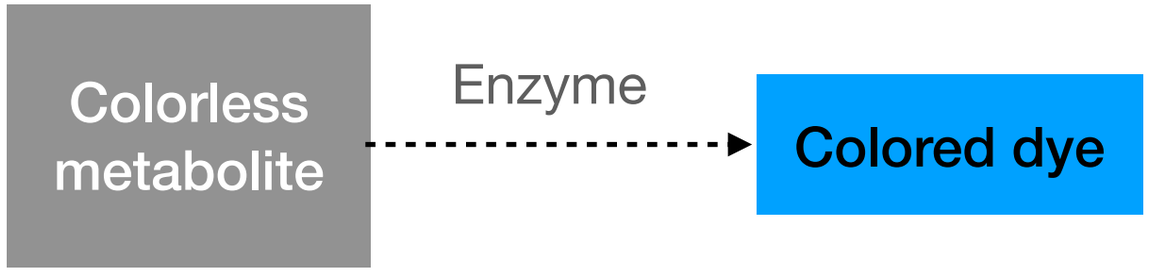
Here, $1\ \mathrm{nkat} = 1\ \mathrm{nmol/s}$ — unit of catalytic activity in intl. system of units

katal is not rate of reaction, it is a property of the catalyst.

E.g. one katal of trypsin = amount of trypsin which breaks 1 mole of peptide bonds per second under specified conditions

Enzymatic determination of a colorless biomolecule

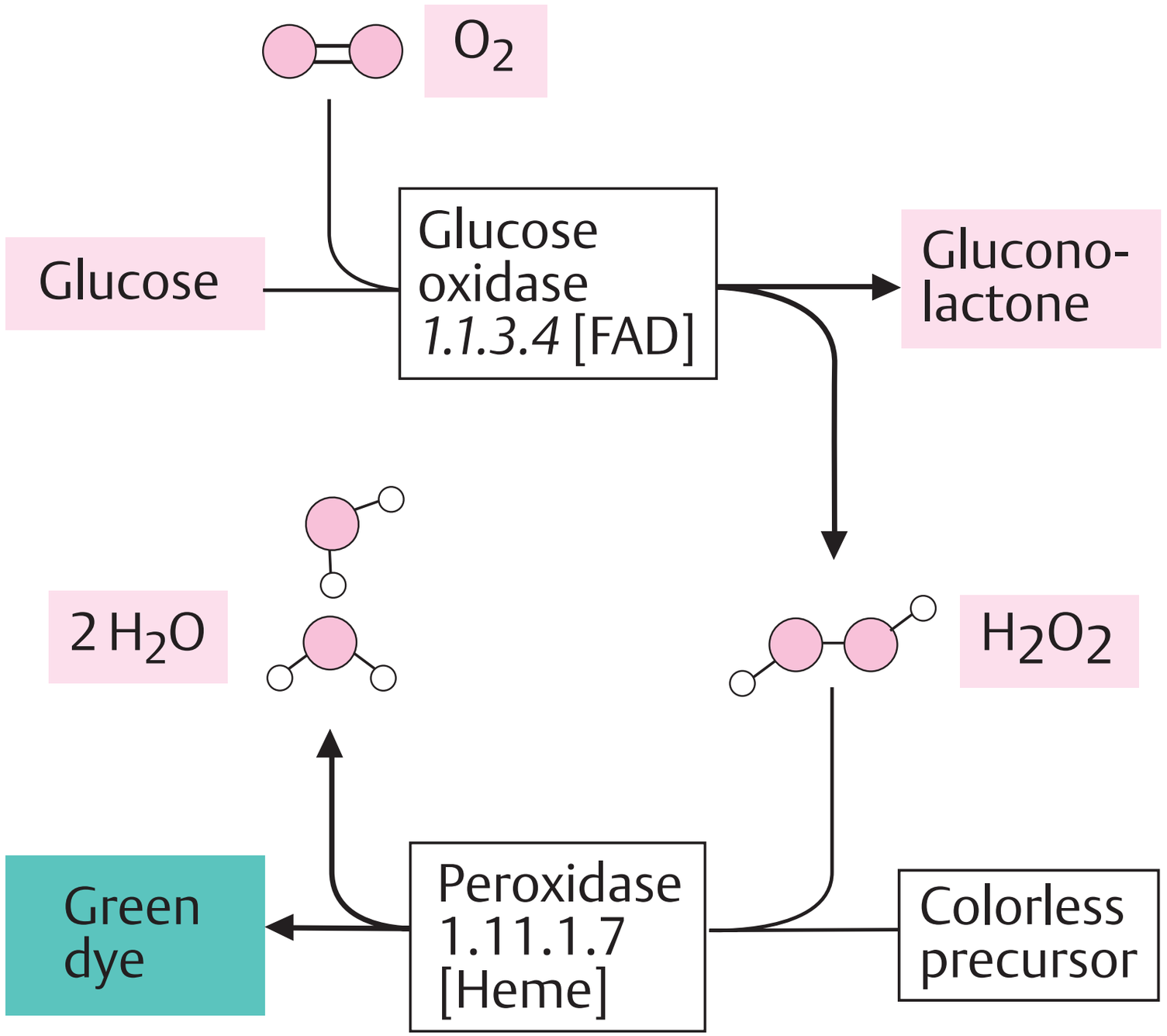
Adopted strategy



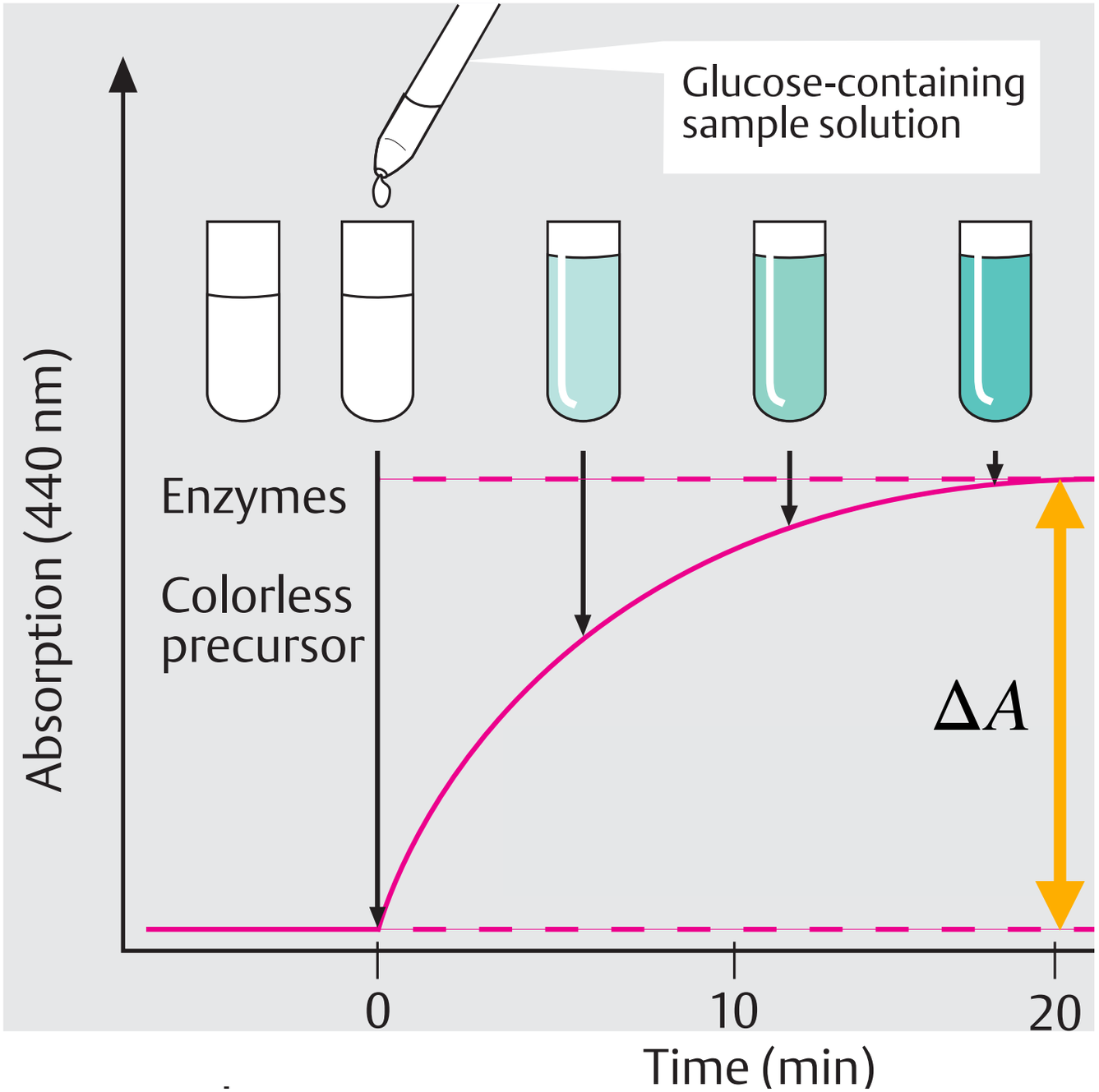
Production of the dye fully depends on the amount of the target compound present

This principle is used to monitor blood glucose levels

Enzymatic determination of blood glucose



Green dye production depends on glucose conc



Therefore, using Beer-Lambert law

Dye conc at saturation

$$[Dye]_\infty = \frac{\Delta A}{\epsilon\ l}$$

Since dye is produced only through H_2O_2 obtained from glucose

$$[\text{glucose}]_0 = [Dye]_\infty$$