Inspirative Questions and Answers

EVROPSKÁ UNIE
Evropské strukturální a investiční fondy
Operační program Výzkum, vývoj a vzdělávání

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PCR primers are short oligonucleotides (approx. 20 bp). The DNA sequence is conventionally written from the 5' to 3' strand. The complementary sequence can be obtained in two steps. First, replace A→T, T→A, C→G and G→C. Second, put the resulting sequence into the reverse order.

The forward primer can be obtained by simply taking a short sequence from the left side of the template.

The reverse primer can be obtained by taking a short sequence from the right side of the template and making a complementary sequence.

Primers must fulfill several other properties such as length, melting temperature, low self-complementary etc. There are special tools available for primer design. Many companies provide custom primer synthesis. Often you can analyze primers on their web site before ordering them.

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5′–GCATGTGCAGACGGCGATATGCGACGTACGATCGATCGACTATCACGCAACGGCACGACCGCACT–3′
 5′–CATGTGCAGACG–3′                                          3′–CCGTGCTGGCGTG–5′
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In the first cycle of the PCR the DNA polymerase indeed does not know where to terminate the DNA synthesis. This leads to a product with variable length (its 5’ terminus is defined by the primer, but its 3’ terminus is variable). However, this product is synthesized directly from the template and its concentration therefore grows linearly. PCR usually uses very small amount of the template. In a PCR with 20 cycles you can reach 20x concentration of the template, which is still low concentration.

The product with the fixed length (its both termini are defined by primers) is produced either from the product with variable length or from another molecule of the product with the fixed length. After the step 1 there is no product with the fixed length. After the step 2 there is the same concentration as the template, after the step 3 it is 2x, after the step 4 it is 4x and after the step 20 it could be concentration 524,288x higher than template.

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Most NAD(P)$^+$ dependent enzymes use this molecule as a coenzyme, i.e. a substrate that is present in the cell in low concentrations and is permanently recycled by various reactions.

S-Adenosylhomocystein hydrolase is different. It uses NAD$^+$ to oxidize the substrate and goes to NADH. Hydrolysis of the oxidized substrate is easier. After hydrolysis the oxidized product is reduced. NADH is oxidized back to NAD$^+$ in this step. NAD$^+$ therefore exits the catalytic cycle unchanged. It is an integral part of the catalyst and can be considered prosthetic group (caution: some researchers use the term prosthetic group to refer to covalently bound cofactors).

Unlike other NAD$^+$-dependent enzymes (dehydrogenases) we cannot assay S-adenosylhomocystein hydrolase activity by measuring absorbance at 340 nm. If we isolate pure S-adenosylhomocystein hydrolase saturated by NADH/NAD$^+$ and we remove free NAD$^+$ or NADH we can use absorbance to measure NADH/NAD$^+$ ratio in the active site under various conditions such as substrate concentration. By this we can learn important facts about the mechanism of the reaction.

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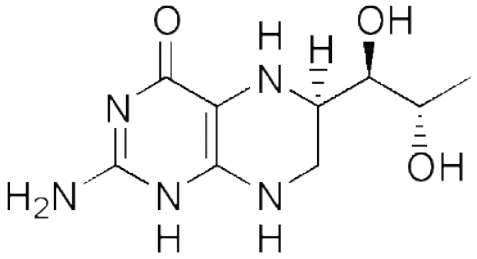
The name phenylketonuria indicates abnormally high concentration of phenylketones in urine. It is inborn error of metabolism caused by lack of phenylalanine hydroxylase activity. Patients cannot metabolize phenylalanine to tyrosine. Instead they produce phenylpyruvate, phenylacetate and other metabolites that cause physical problems. There are many mutations that may cause low or no activity of this enzyme. Many of them are destabilizing enzyme. Tetrahydrobiopterin is coenzyme of this enzyme. It can bind to mutation-destabilized enzyme, stabilize it in the native state and thus rescue its activity.



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Typically, one molecule of A may activate multiple molecules of B, each B may activate multiple C and so forth. As the result, one molecule of A may activate a huge number of molecule Z. The signaling is therefore very sensitive and rapid.

This is example of GPCR signaling, apoptosis, blood coagulation and many other processes.

The compound 2,4-dinitrophenole was used to treat obesity in 1933-1938, but was discontinued due to its toxicity. The compound can freely diffuse through a membrane in the protonated $(O_2N)_2$-C_6H_4-OH as well as in deprotonated form $(O_2N)_2$-C_6H_4-O^-. It was found that it reduces production of ATP. Try to explain how 2,4-dinitrophenole works.

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2,4-Dinitrophenole can freely diffuse through membranes in both forms. It can therefore get protonated in the intermembrane space of mitochondria and then pass into the mitochondria, lose its proton and again diffuse out of mitochondria. This eliminates the proton motive force and stops production of ATP. The cell catabolizes nutrients but anabolism is reduced.

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The term homotropic allosteric effect means that the same impulse (such as compound binding) is the cause as well as the outcome of the effect. For example, binding of oxygen to hemoglobin influences its affinity to other molecules of oxygen.

Hemoglobin is the example of the positive homotropic allosteric effect, because binding of oxygen enhances affinity to other oxygen. The physiological consequence is that it binds oxygen strongly at high concentrations (in lungs) and weakly at low concentrations (in the rest of body). Oxygen can be efficiently loaded in lungs and most of it is released in the body.

Let us imagine a fictive hemoglobin without allostery. Strong binding in lungs can be achieved by strong affinity to oxygen. However, such hemoglobin would release only a small fraction of oxygen cargo in the body and oxygen would circulate in blood without being used. Release of oxygen in body can be achieved by weak affinity to oxygen. However, it means low saturation in lungs.

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Negative homotropic allosteric effect is bit more complicated than the positive one. We can find it, for example, in some bacterial represors such as catabolic activator protein (CAP). CAP binds cAMP with negative homotropic allosteric effect. In fact, binding of cAMP to CAP causes that CAP does not bind to DNA and transcription is activated. As the result, production of mRNA is proportional to cDNA concentration at the similar manner as a positive homotropic allosteric effect. In other words, cAMP **negatively** influences binding of another cAMP, but **positively** influences production of mRNA.

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GPCRs are mostly studied as drug targets. Binding of natural as well as artificial ligands is therefore the most explored mechanism of GPCR activation. However, GPCRs can be activated by other mechanisms. The one covered by slides is activation of rhodopsin by light. There are reports of GPCRs activated by proteolytic cleavage, change of temperature or mechanically. GPCR signaling by ligands can be also influenced by membrane potential.

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Cocaine is a small molecule (303.353 g/mol) and the immune system does not produce antibodies against small molecules. Production of antibodies requires presentation of antigen using some MHC and small cocaine cannot be presented. Organism may used other mechanisms to get rid of cocaine, for example cytochrome P450.

If you want to produce antibody against cocaine you can bind it covalently onto some mouse protein and inject it back to mouse. Mouse does not produce antibodies against the protein because it is a mouse protein, but it produces antibodies against its modification, i.e. against cocaine. Such polyclonal antibody can be used in cocaine assay.

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Indeed, there are exceptions. T cells contain the gene for the T cell receptor. This gene differs among the population of T cells in the body. This is achieved by shuffling short segments of the DNA in the gene. This makes a large repertoire of T cells receptors that can bind almost any antigen. Adaptive immunity is based on the fact that the cell with the right gene can be selected.

There are some other exceptions, for example one use in brain development to avoid self junctions of neurons.

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Blood contains approximately concentration of NaCl equal to 0.15 mol/l. Blood cells and other cells in the body contain similar salt concentrations (composition of slats may depend on the cell type). Lower concentration of salts would cause high osmotic pressure on cell membranes. Cells would tend to absorb water and increase its size. This causes cell disruption, in particular hemolysis.

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Citruses, and especially grapefruits, contain high concentrations of furanocoumarins that inhibit the main drug metabolizing enzyme in the body, which is cytochrome P450 (in particular its isoenzyme 3A4). This causes that metabolism of a drug is slower and its effect may be stronger and/or longer. This may influence the results of a clinical trial. Patients using some drugs may be also instructed not to consume grapefruits due to interaction with the drug (interaction in the pharmacological meaning of the word).

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Testosterone is a small molecule, steroid hormone, highly nonpolar and barely soluble in water. When applied as a gel on arms and legs it can distribute into muscles, penetrate cell membranes, go to nuclei, bind to nuclear receptors and regulate transcription.

In contrast, EPO is a protein (21 kDa). It must be therefore delivered by injection. Moreover, EPO acts on blood cell formation.

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Maple tree is a C3 plant. Sugar cane is a C4 plant. Isotopic composition of carbon dioxide in the air is more or less constant. However, rates of enzymatic reactions (and chemical reactions in general) might be slightly dependent on isotope composition. Enzymatic reactions can slightly enrich some isotopes in their products. Carbon dioxide fixation is done by different reactions in maple and cane, so the isotopic composition is different and can be resolved by mass spectrometry.

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This compound resembles acetoin (CH_3-C(=O)-CH(OH)-CH_3), which is together with biacetyl and butandiol a side product of pyruvate decarboxylase reaction. These compounds can be found in alcoholic drinks and other food products, sometimes favored and sometimes disfavored. Pyruvate decarboxylase decomposes pyruvate to carbon dioxide and acetaldehyde. As a side product, the already formed molecule of acetaldehyde may condense with a nascent acetaldehyde molecule to form acetoin. When the first molecule of acetaldehyde is replaced by benzaldehyde it produces the above written molecule. This biotechnology was intensively used in Czechoslovakia to produce ephedrine and is responsible for today dominance of the Czech Republic in illegal metamphetamine production.

Activity of pancreatic lipase can be assayed using a fluorogenic substrate. This substrate is composed of glycerol esterified by two fatty acids and one glutaric acid. On the second carboxylic group of the glutaric acid there is a covalently bound fluorescent compound (not fluorescent when bound). The substrate is not fluorescent. Lipase can hydrolyze the bond between the glycerol and glutaric acid. The resulting conjugate of glutaric acid with the fluorescent compound decomposes and releases the fluorescent compound. However, lipase can hydrolyse the two fatty acids as well, maybe even at higher rate. How is it possible that this substrate can be used in clinical biochemistry despite the fact that it measures only a fraction of real enzymatic activity?

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When some washing powder developer wants to add lipase into a washing powder he/she might be interested in the “real” activity of the enzyme, i.e. how much of lipids it can decompose in the washing cycle under conditions of washing machine. On the other hand, clinical biochemist is usually not interested in the “real” activity of the enzyme. The fact that some enzyme is present in blood indicates that some organ was damaged and the enzyme leaked. The activity of the enzyme is used in clinical biochemistry as a measure of enzyme concentration (under stable conditions such as pH, temperature etc. enzyme activity is linearly dependent on enzyme concentration). It does not matter that the substrate measure only for example 10 % of the “real” activity if it is enough for accurate measurement and if it is always 10 %.

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If you want to use some immunochemistry technique, for example ELISA, to detect concentration of some protein you can use mouse antibody against this protein and label it by enzyme, for example by horseradish peroxidase. However, conjugation of enzyme and antibody is quite difficult job. It make sense to do it for a commercial kit but not for single laboratory experiment.

Instead it is possible to use another antibody (referred to as secondary antibody) to detect the first antibody. The secondary antibody, for example goat anti-mouse antibody labeled by horseradish peroxidase, is commercially available and already labeled from the factory.

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The wavelength 340 nm falls to UV range. It is possible to use special plastic disposable cuvettes, which is important when working with infectious material. Moreover, most blood components do not absorb at 340 nm. DNA and RNA absorb at 260 nm, proteins at 280 nm. Molar extinction coefficient is reasonably high, which makes these assays sensitive. Finally, it is one wavelength for many assays, so it is possible to use one equipment for multiple purposes.