



Vánoční výzdoba DNA aneb světýlka, elektrony a háčky ve službách biochemiků a biologů

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Biofyzikální ústav AVČR, v.v.i./CEITEC MU

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Moderní biofyzikální metody: pokročilé praktické vzdělávání v experimentální biologii (CZ.1.07/2.3.00/09.0046)



esf

evropský
sociální
fond v ČR



EVROPSKÁ UNIE

MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



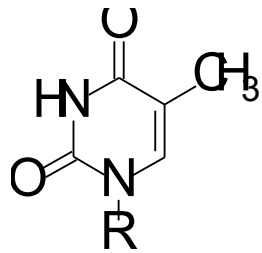
OP Vzdělávání
pro konkurenceschopnost

INVESTICE
DO ROZVOJE
VZDĚLÁVÁNÍ

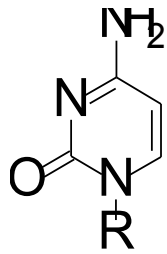


Co je to DNA?

pyrimidinové báze

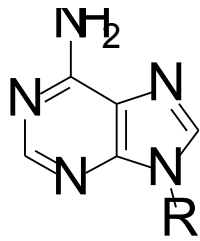


thymin (T)

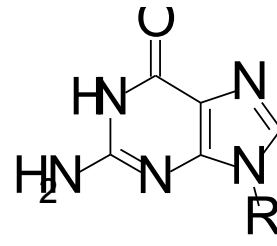


cytosin (C)

purinové báze

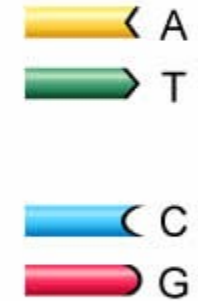


adenin (A)

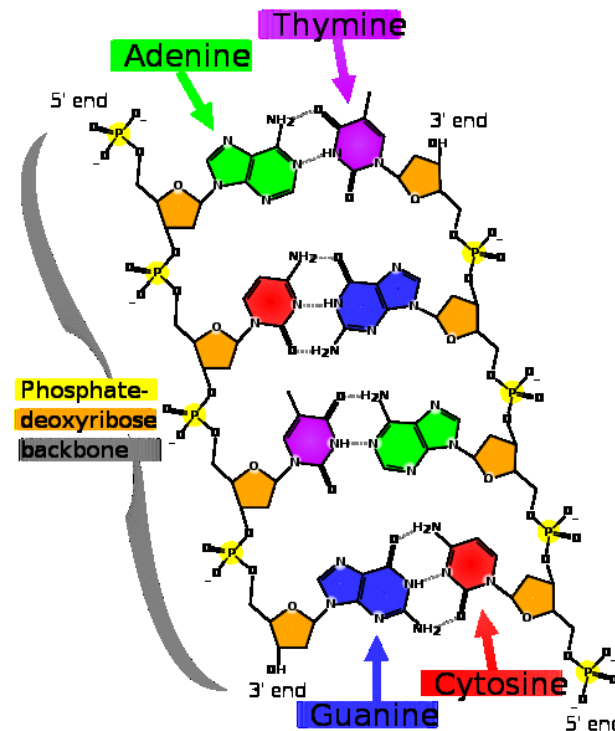
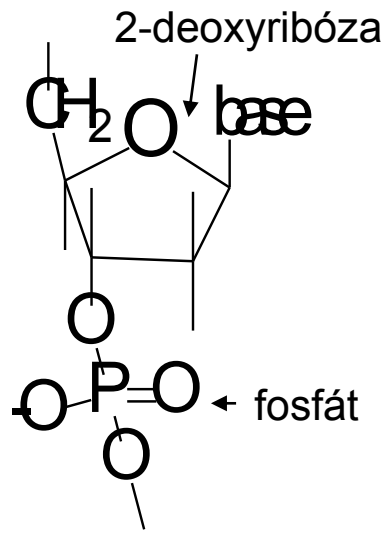


guanin (G)

dvoušroubovice

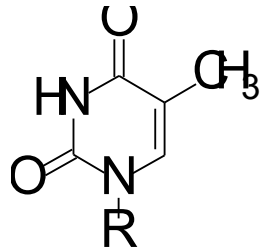


nukleotid

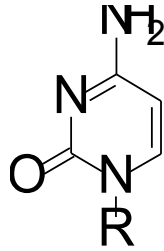


párování bází v řetězcích DNA

pyrimidinové báze

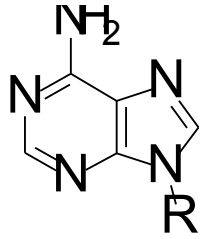


thymin (T)

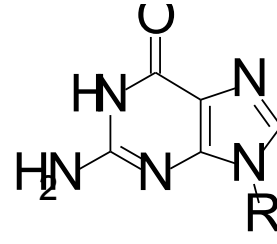


cytosin (C)

purinové báze

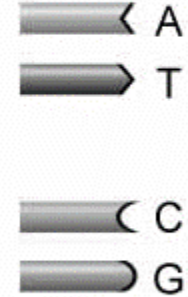


adenin (A)

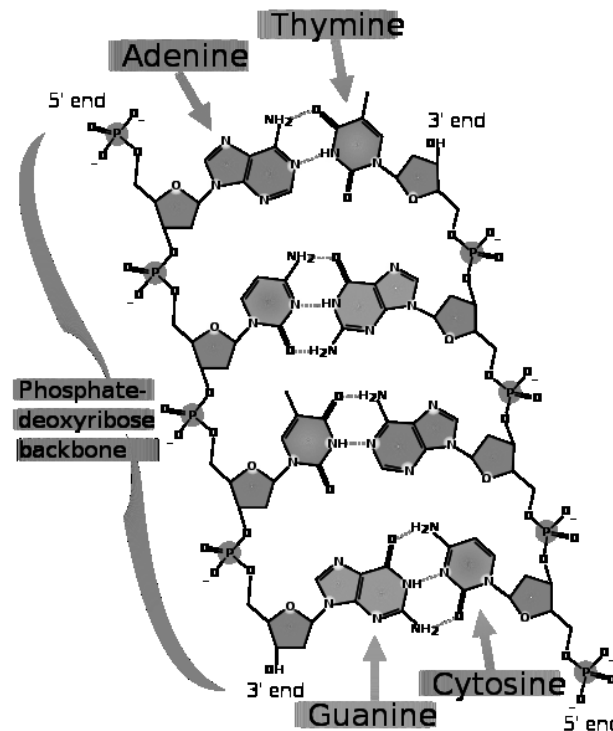
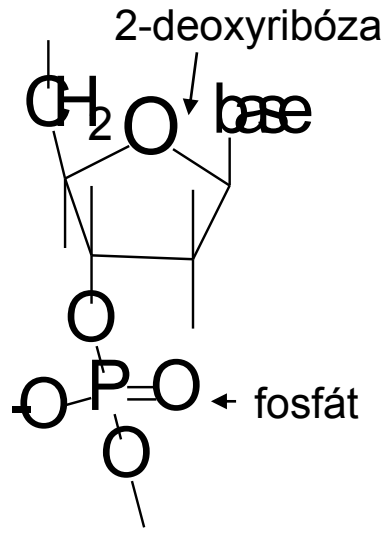


guanin (G)

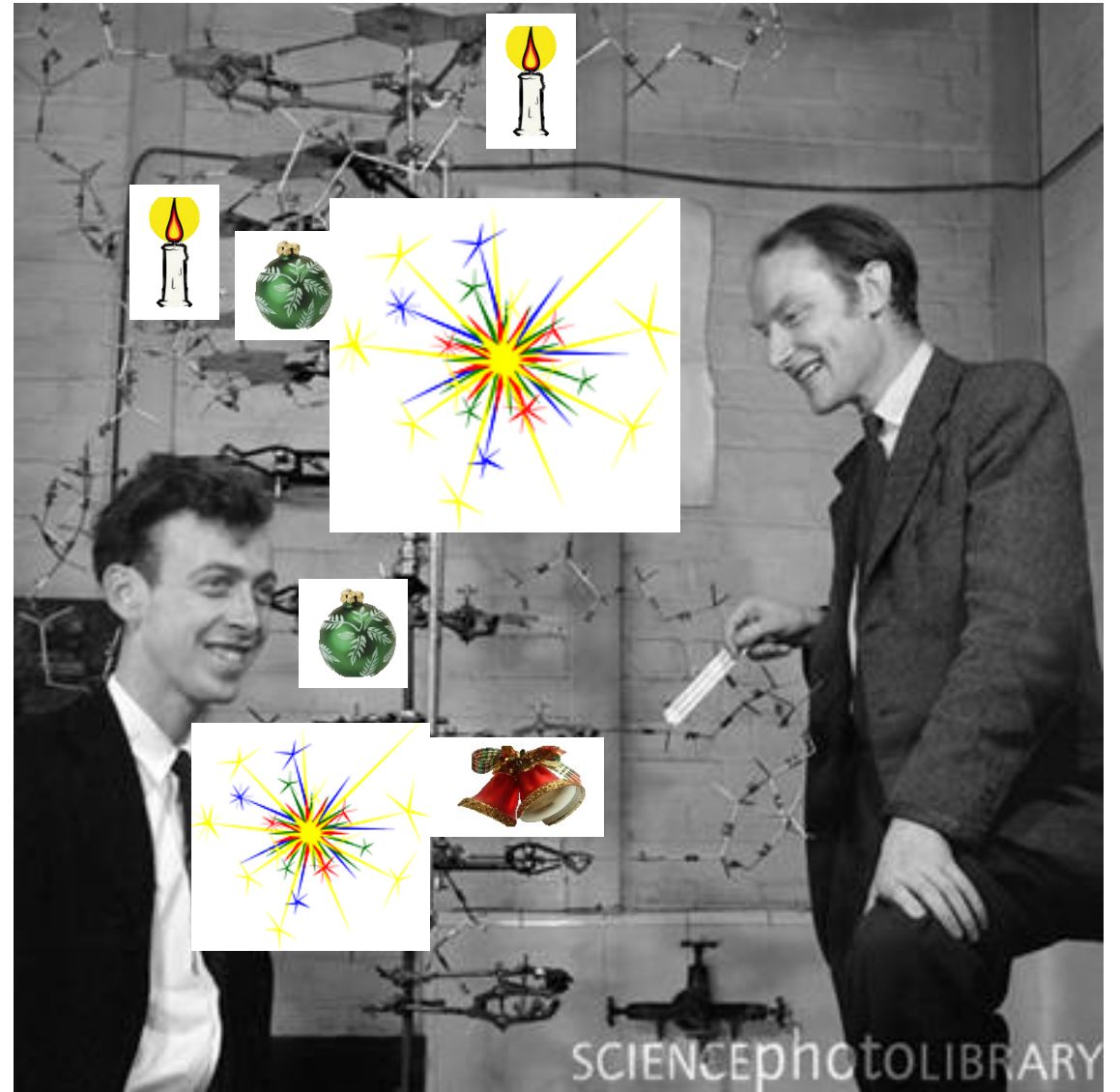
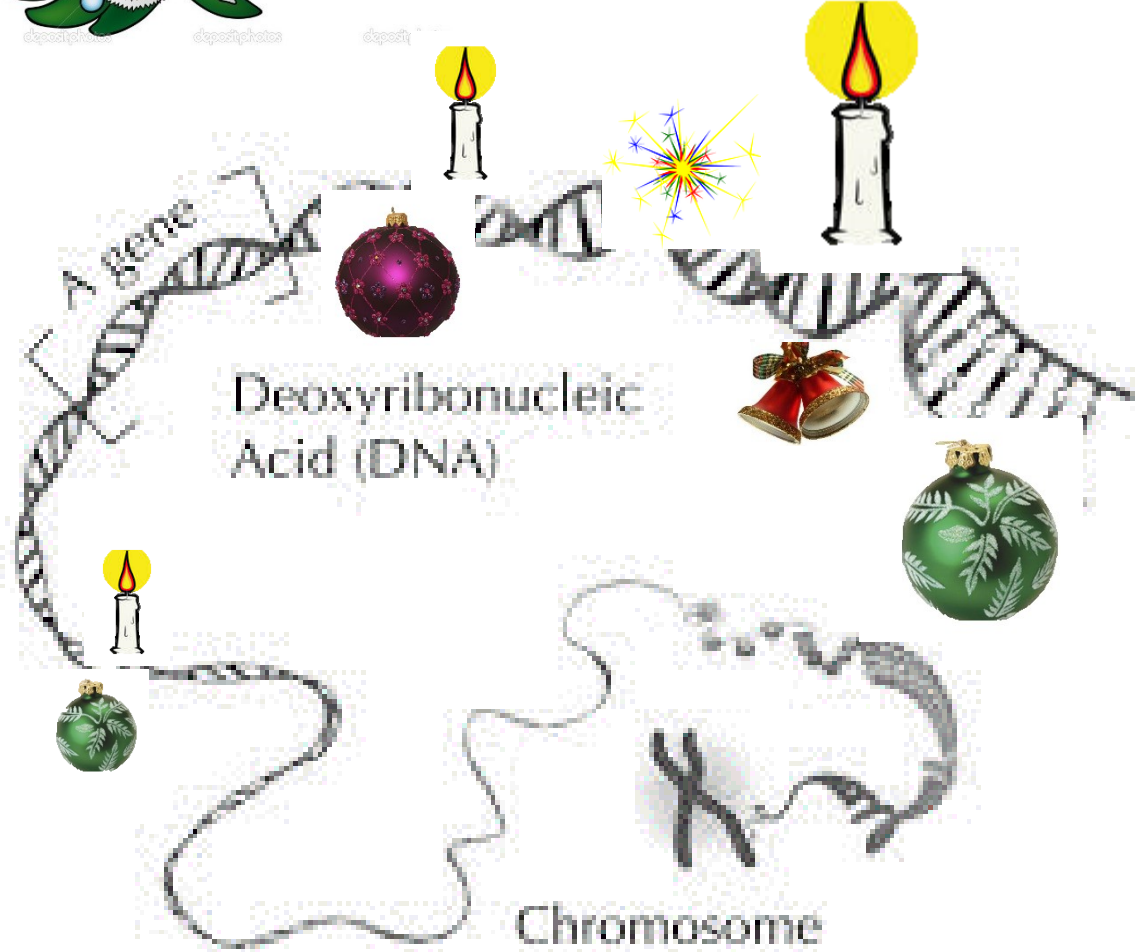
dvoušroubovice



nukleotid



párování bazí v
řetězcích DNA

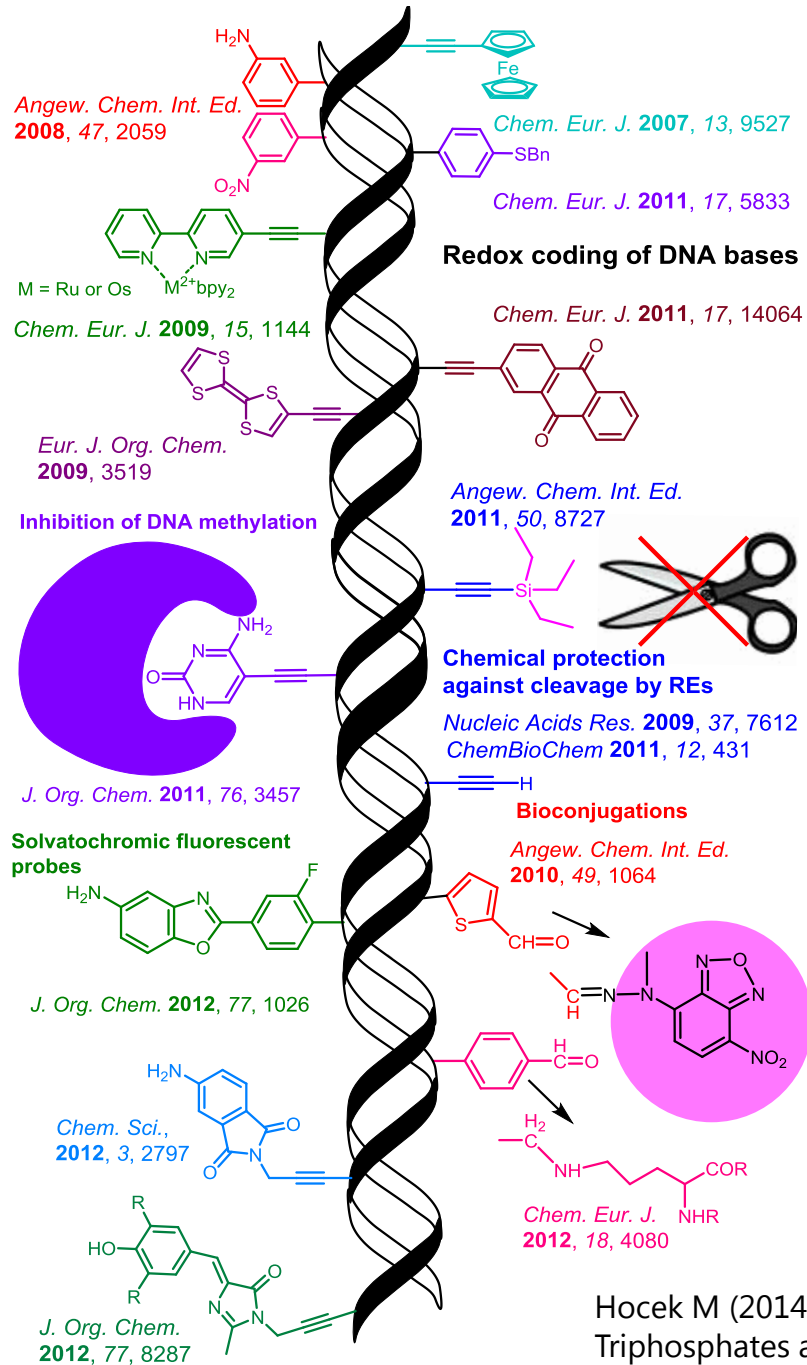




prof. Michal Hocek
(ÚOCHB AVČR Praha)

Ochranné skupiny

Fluorofory
(světýlka)



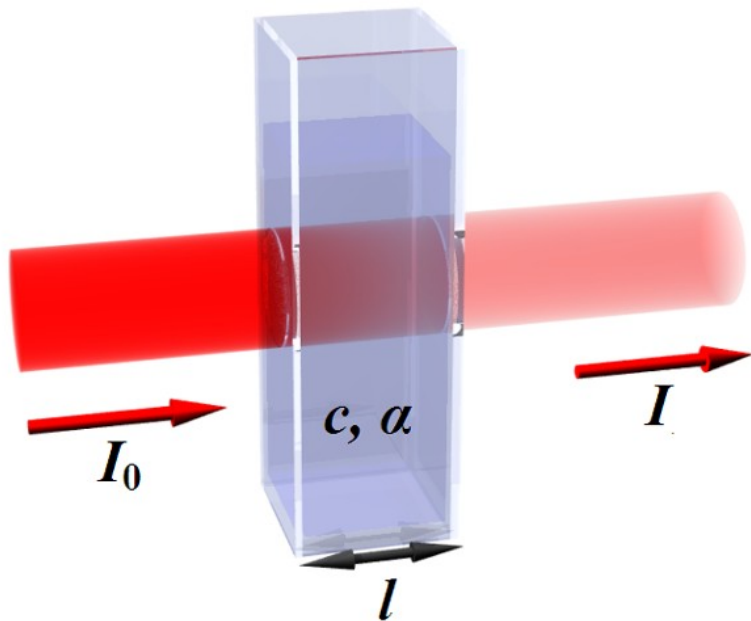
Redox značky
(hrátky s elektrony)

Reaktivní skupiny
(háčky a věšáčky)

Hocek M (2014) Synthesis of Base-Modified 2'-Deoxyribonucleoside Triphosphates and Their Use in Enzymatic Synthesis of Modified DNA for Applications in Bioanalysis and Chemical Biology. *J Org Chem* 79:9914-9921

Jak uvidět DNA?

DNA je ve viditelném světle bezbarvá



Báze DNA absorbují
ultrafialové světlo

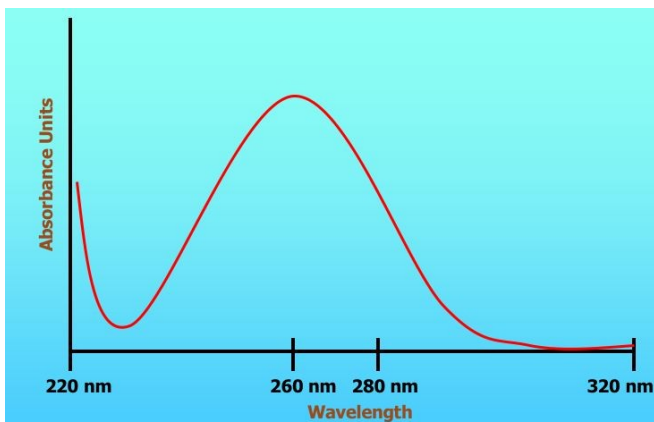
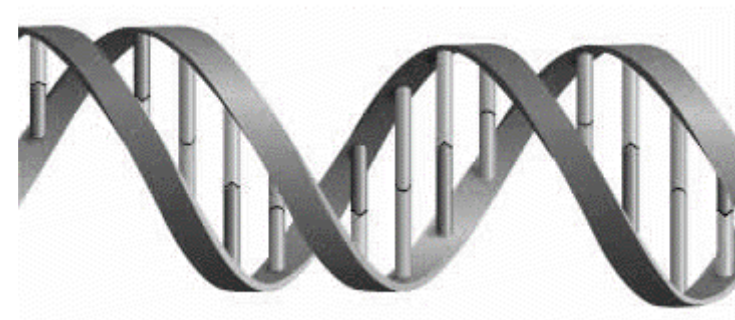
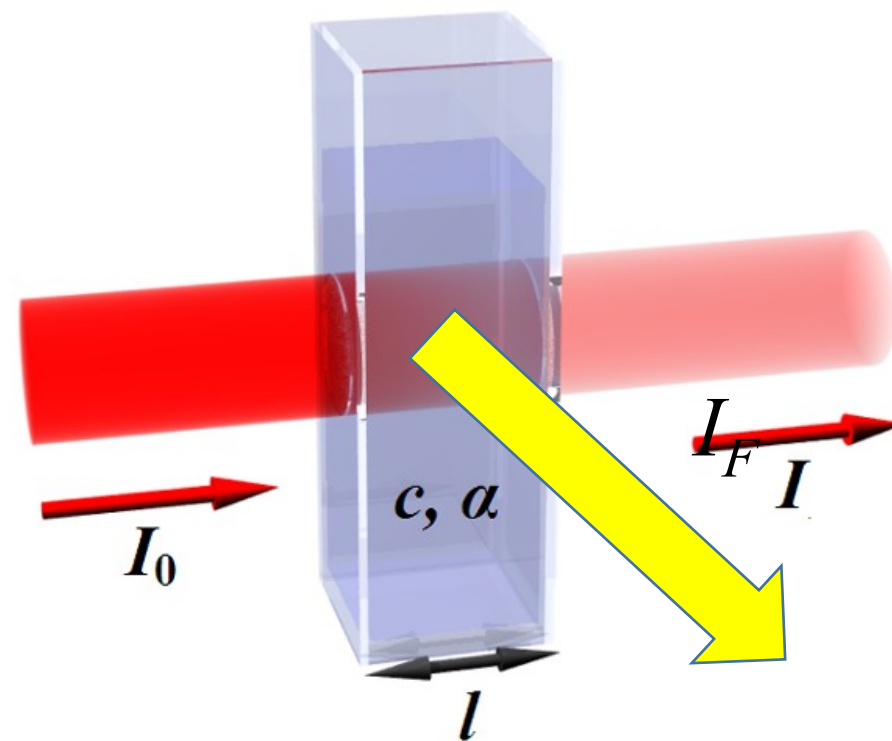
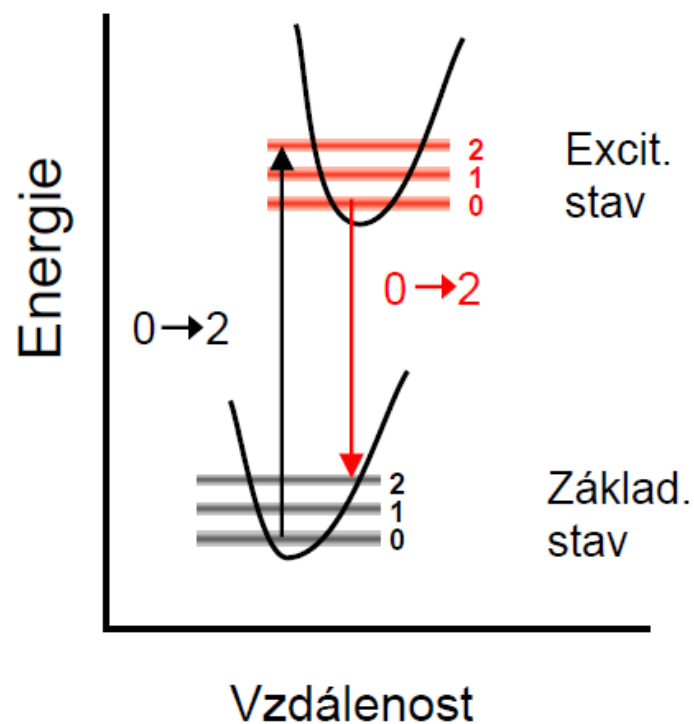


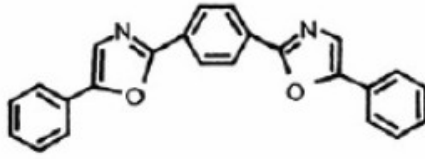
Figure 1: Spectrophotometric Scan Profile for Purified Plasmid DNA

Purified DNA typically produces a Gaussian or bell-shaped profile with the maximum peak absorbing at 260 nm. Absorbance at 220 nm increases with a significant drop between the 220 nm and 260 nm peak. This sample is relatively pure DNA.

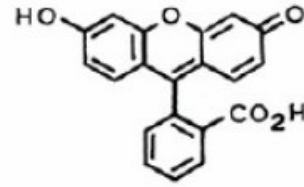
Můžeme si pomoci fluorescencí?

Fluorofory absorbují fotony UV nebo viditelného záření a poté je zase emitují (obvykle s nižší energií)

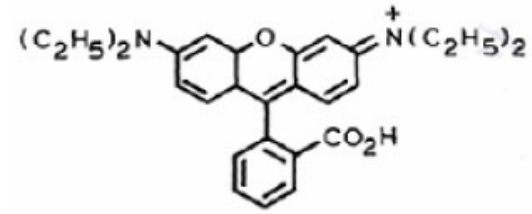




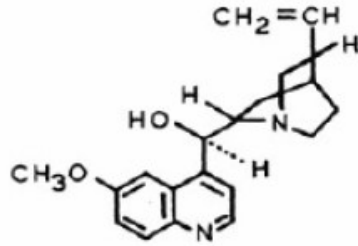
POPOP



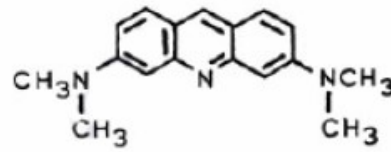
Fluorescein



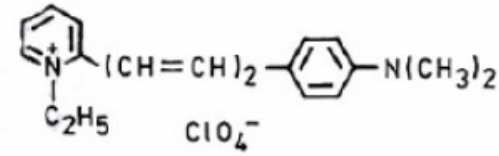
Rhodamine B



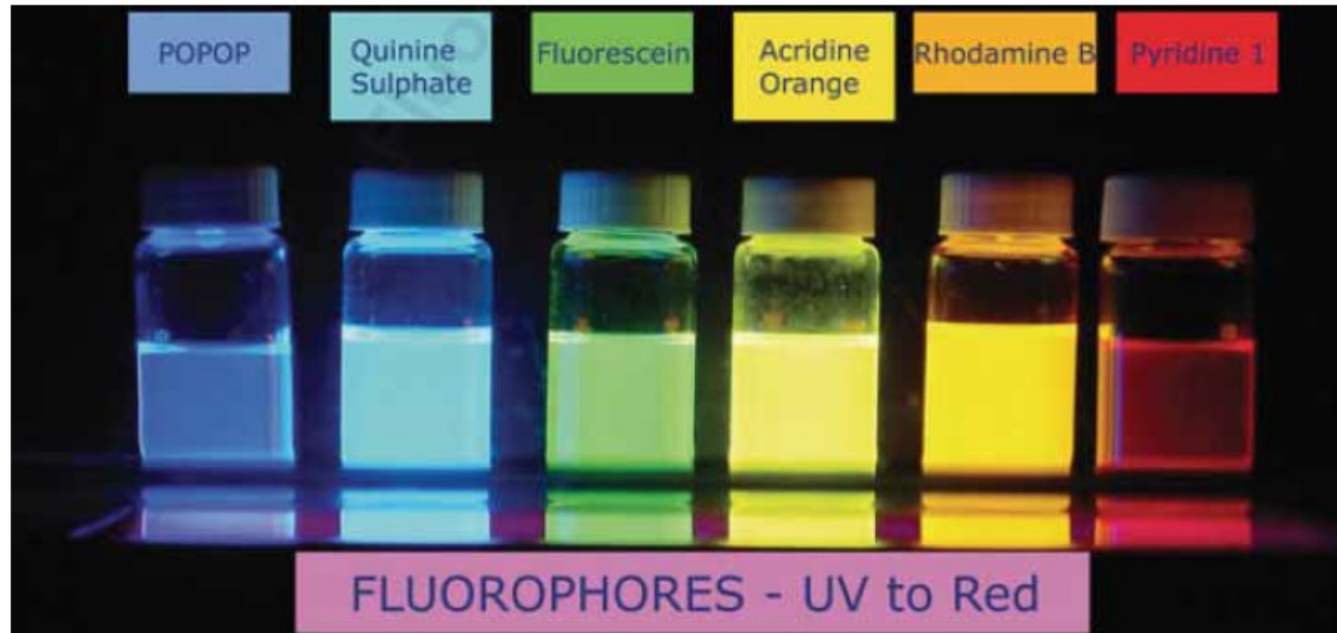
Quinine



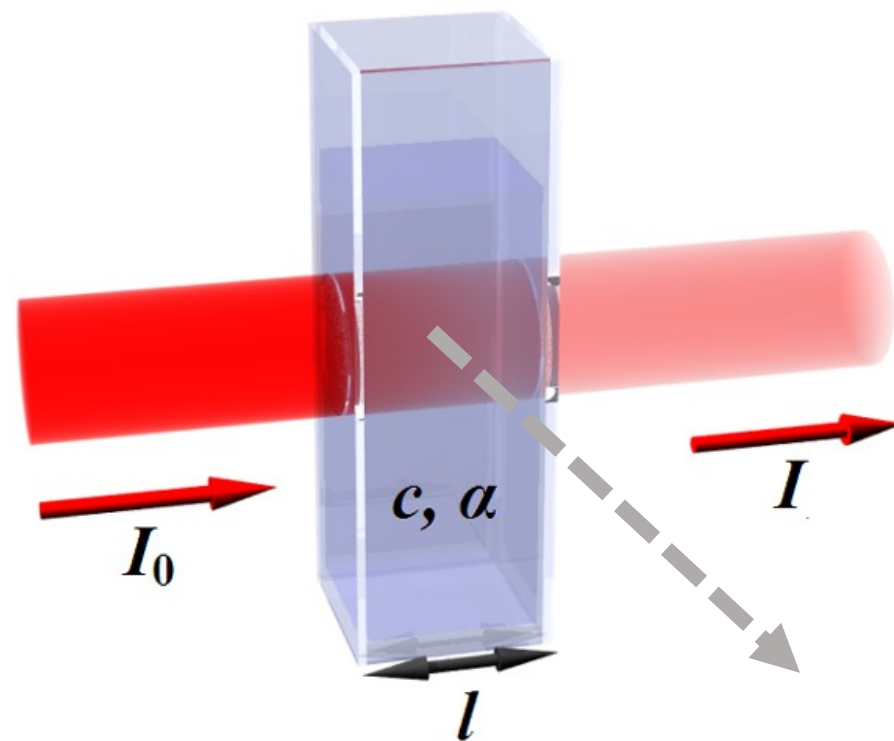
Acridine Orange



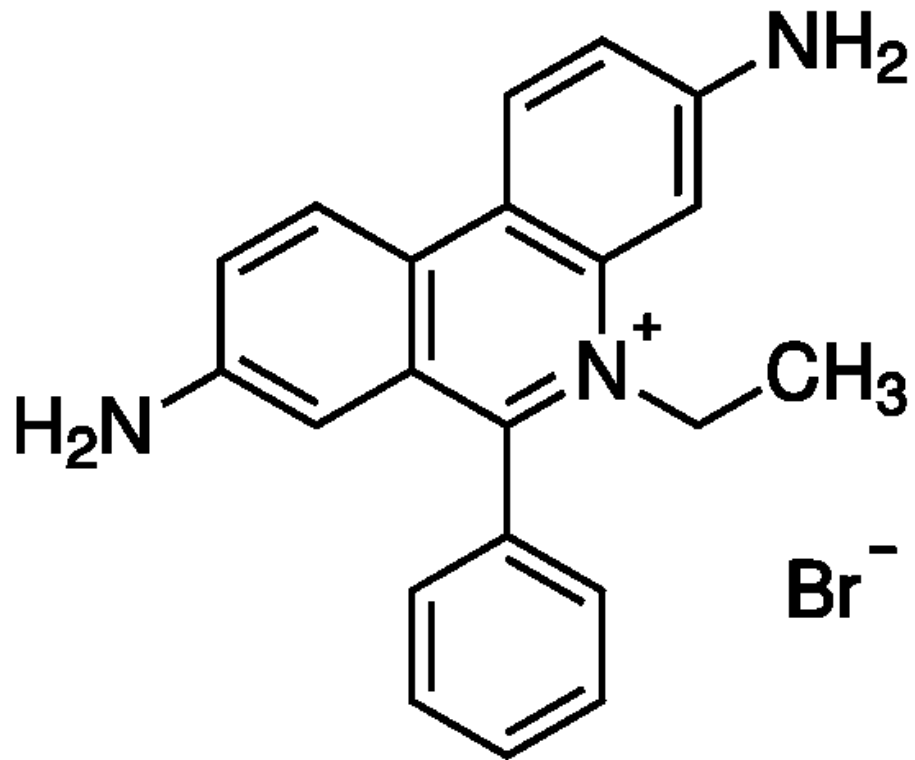
Pyridine 1



DNA nijak zajímavě nefluoreskuje...



...ale fluoreskují některé látky, když se na DNA navážou!



ethidium bromid

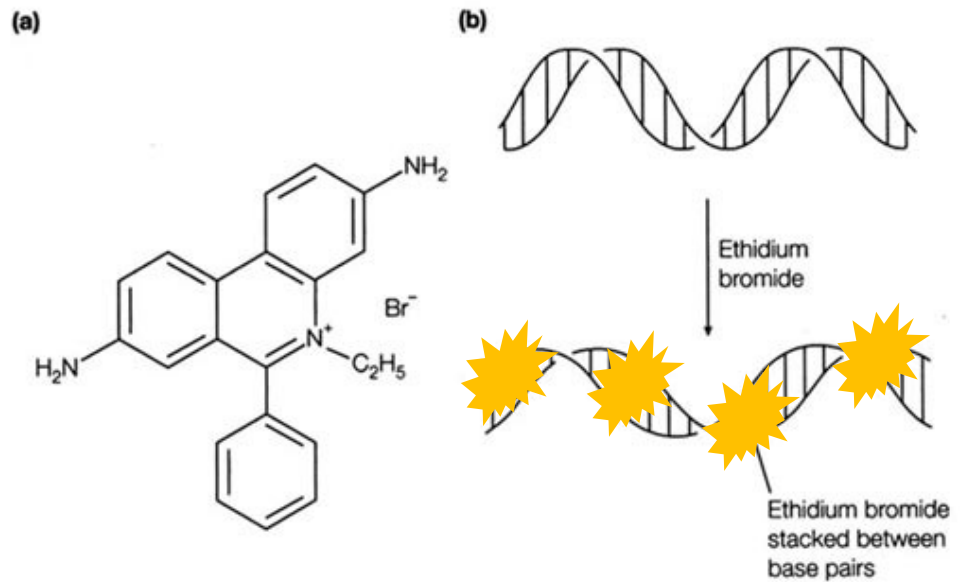
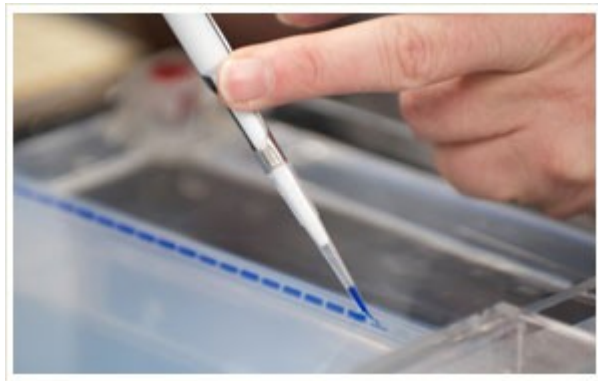
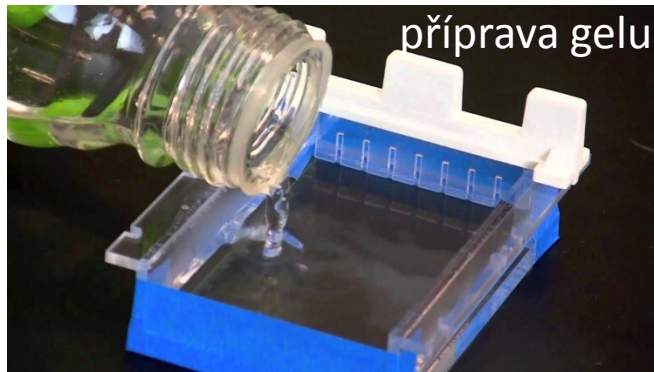
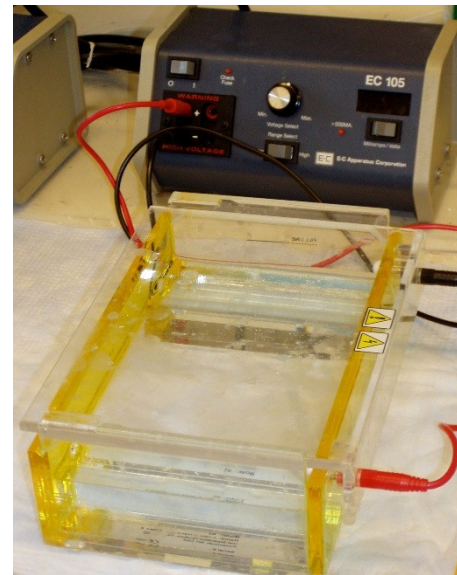


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

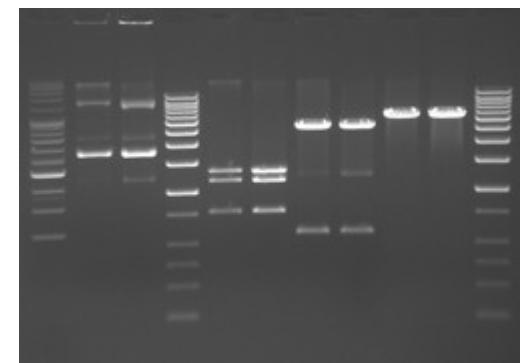
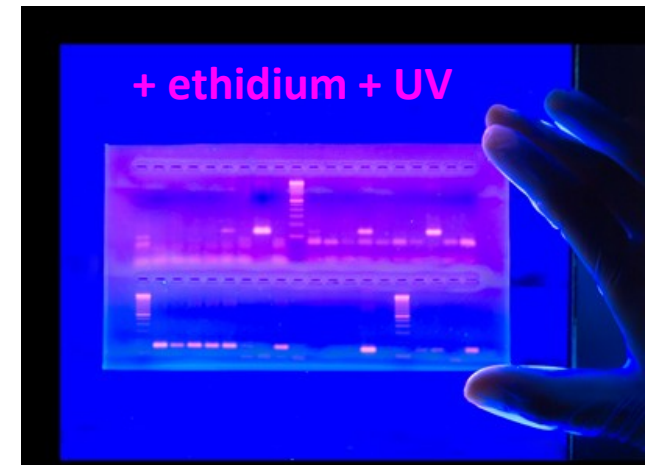
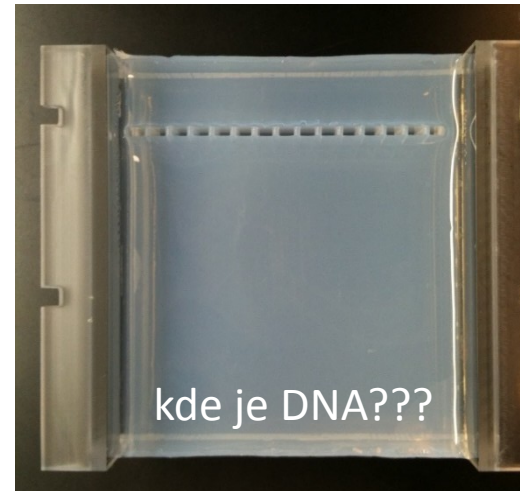
Elektroforéza DNA v agarózovém gelu: třídění molekul DNA podle velikosti



nanesení vzorků



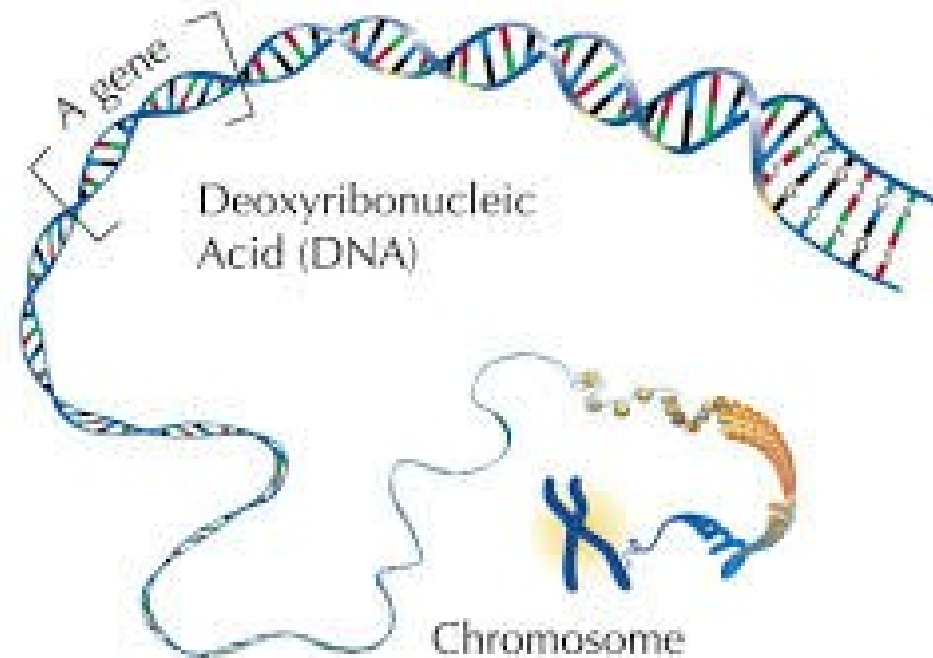
elektroforéza



foto

Je možné vidět gen?

- Gen je (ne zcela přesně řečeno) úsek DNA kódující protein
- několik stovek párů bází z několika miliard (v lidském genomu)













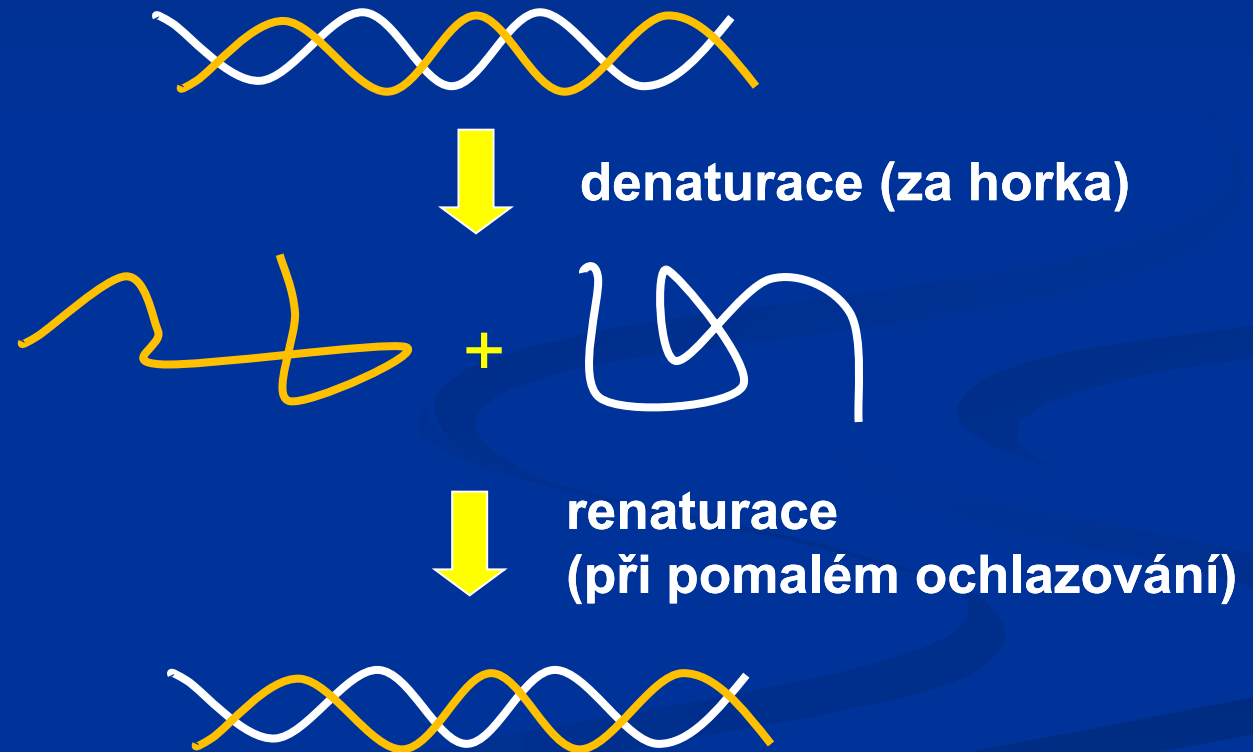


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GAAAT

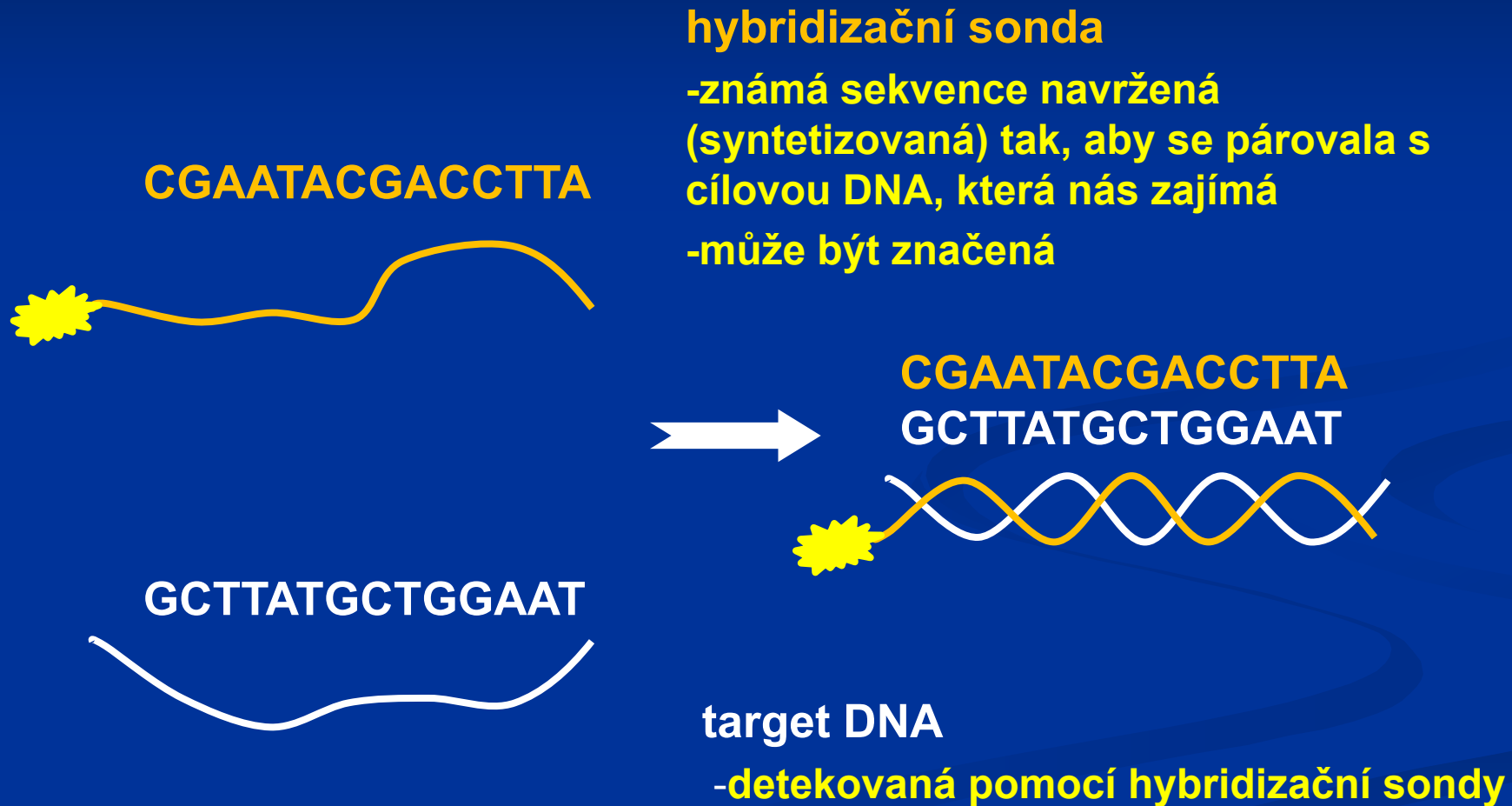
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CGCTGCCCCACCA**TGAGCGCTGCTCAGATAGCGATGGTCTGGCCCCCTCCTCAGCATC**TTATCCGA
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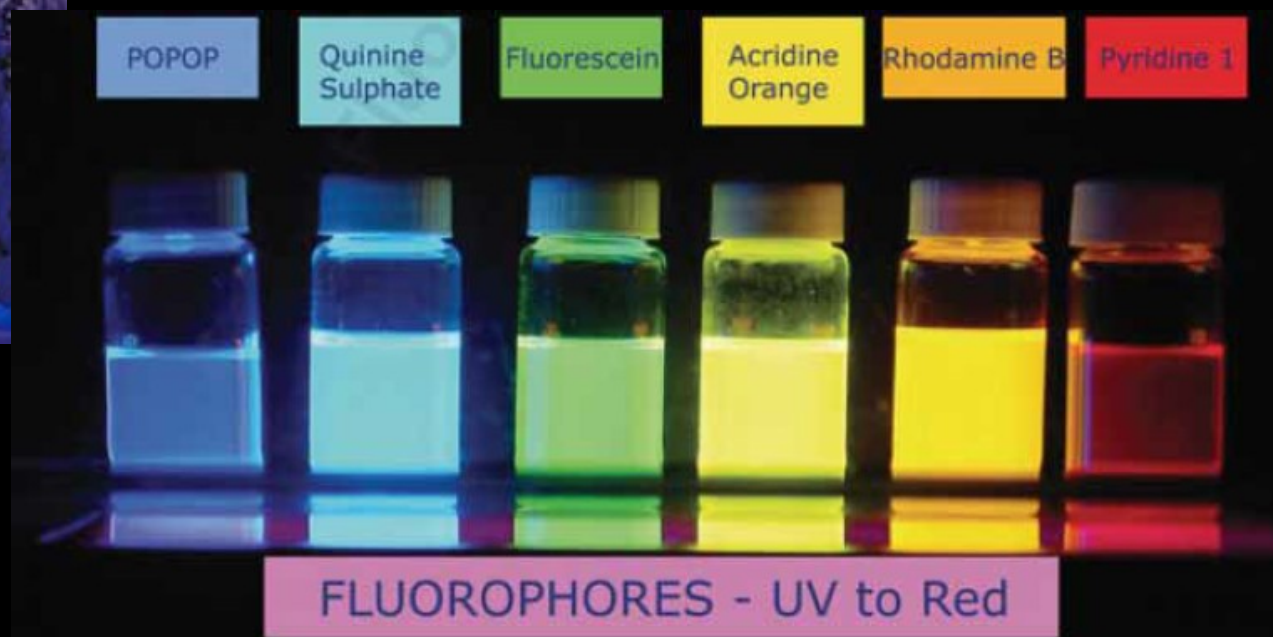
1963 Julius Marmur: renaturace DNA



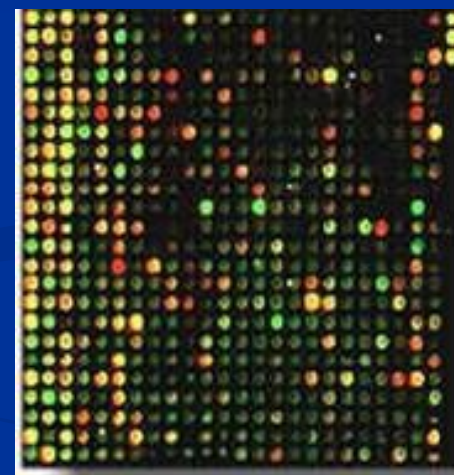
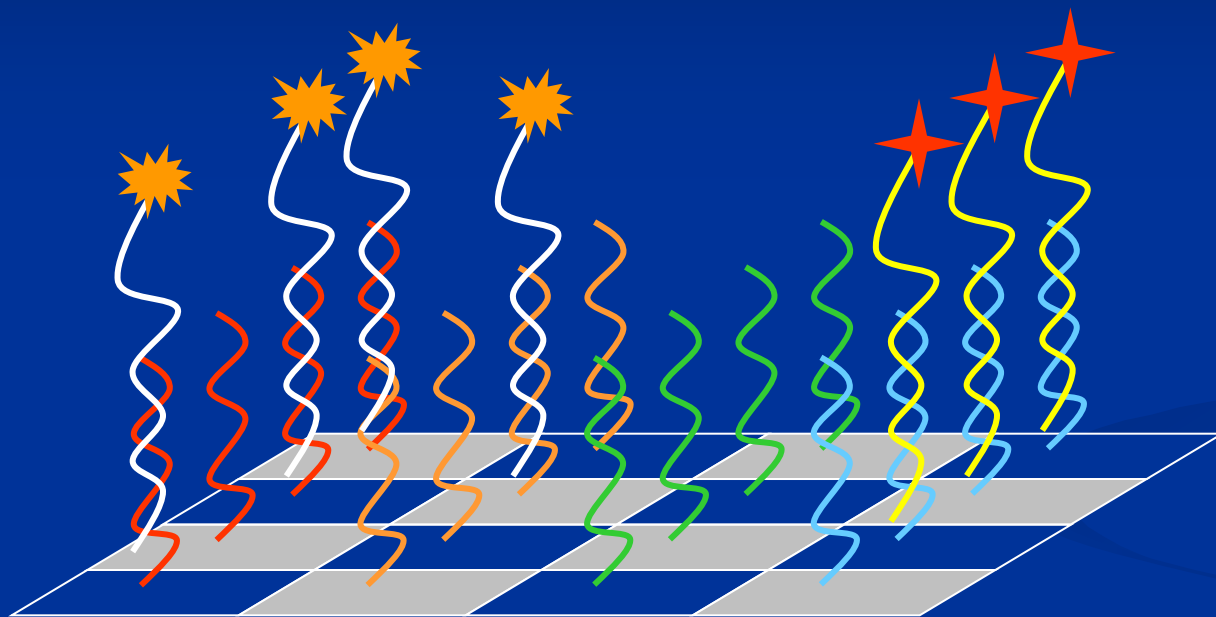
Hybridizace DNA (RNA): tvorba dvoušroubovice z řetězce sondy and komplementárního cílového řetězce



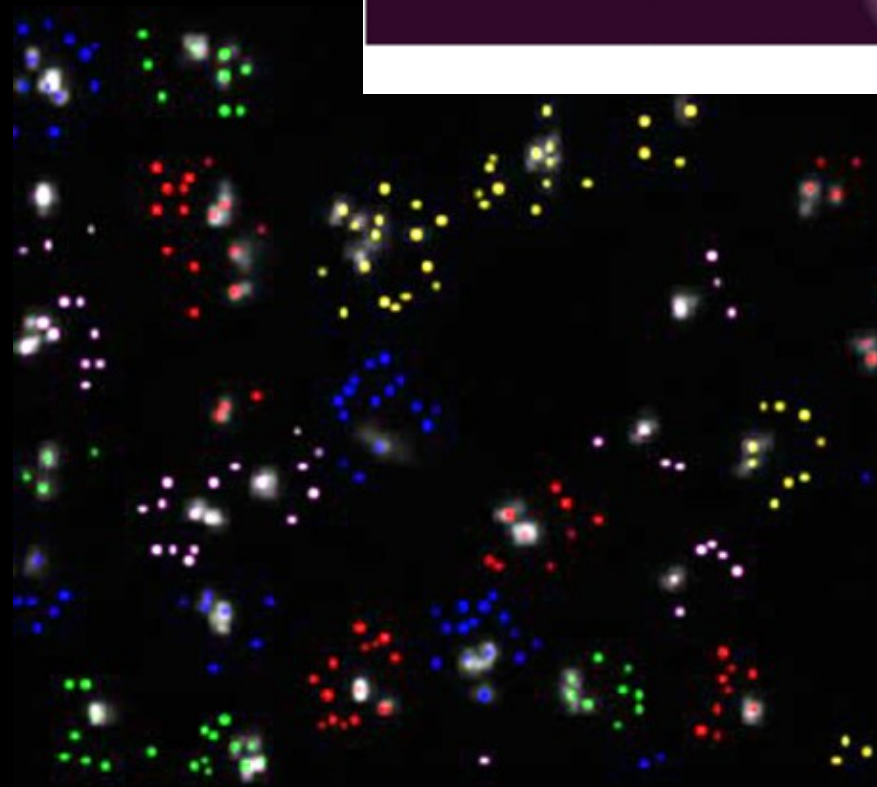
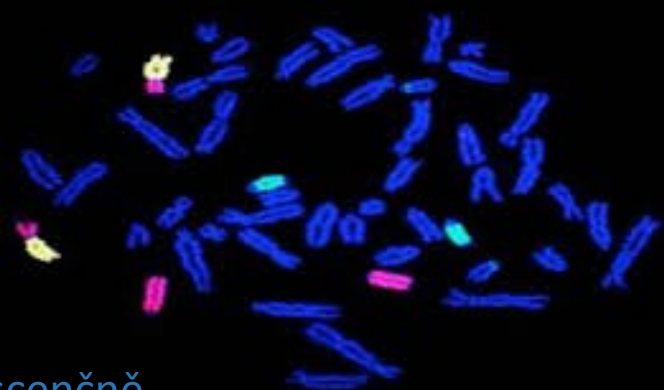
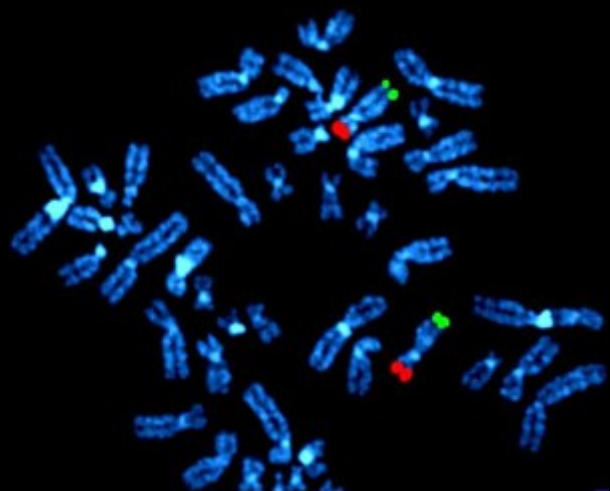
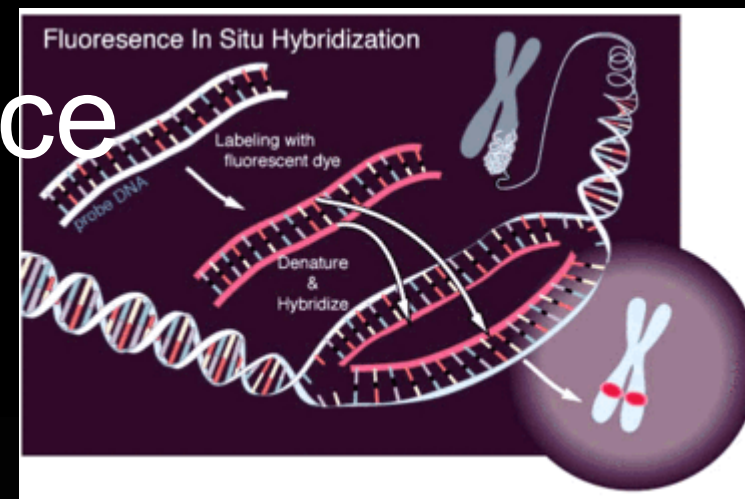
Vícebarevné značení?



Genové čipy

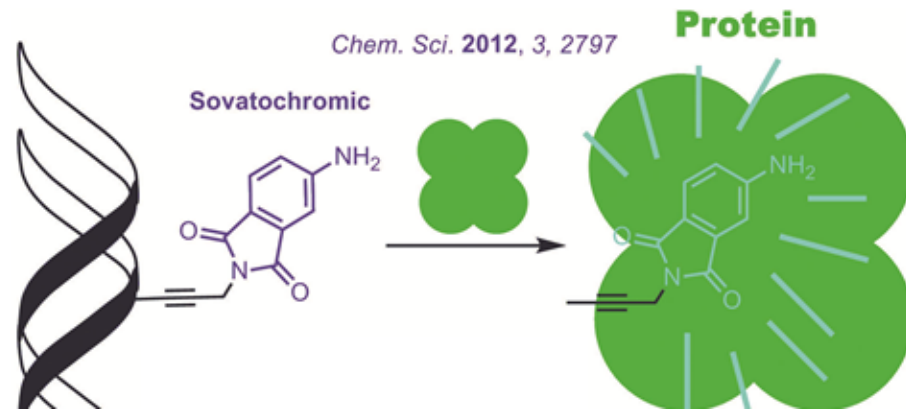


Fluorescenční in-situ hybridizace

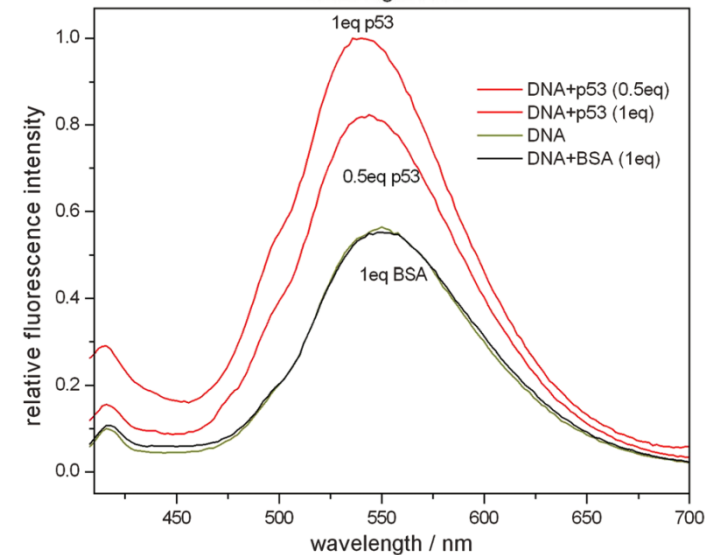
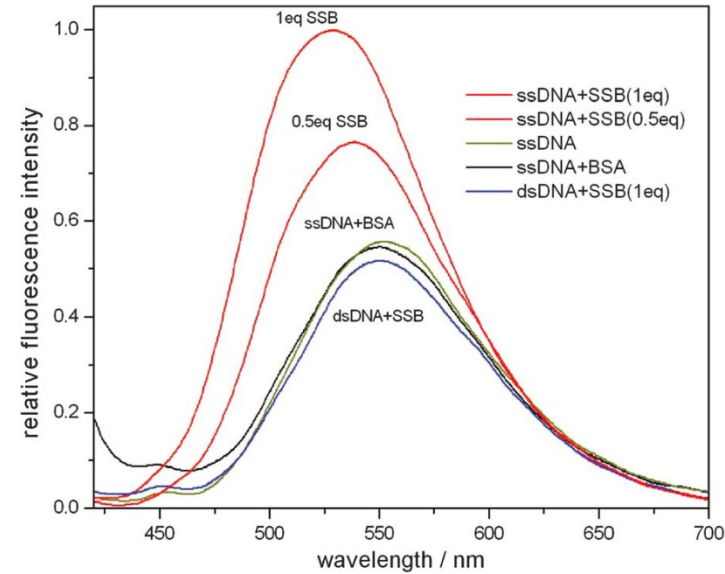


Modrá barva: fluorescenčně „obarvená“ celková DNA

Proteiny rozsvěcují světýlka na DNA



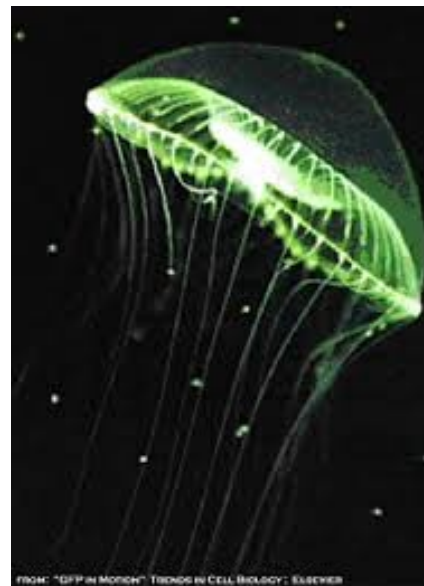
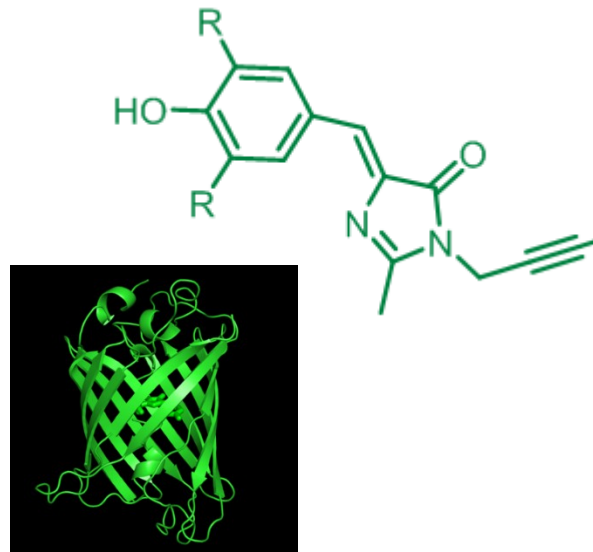
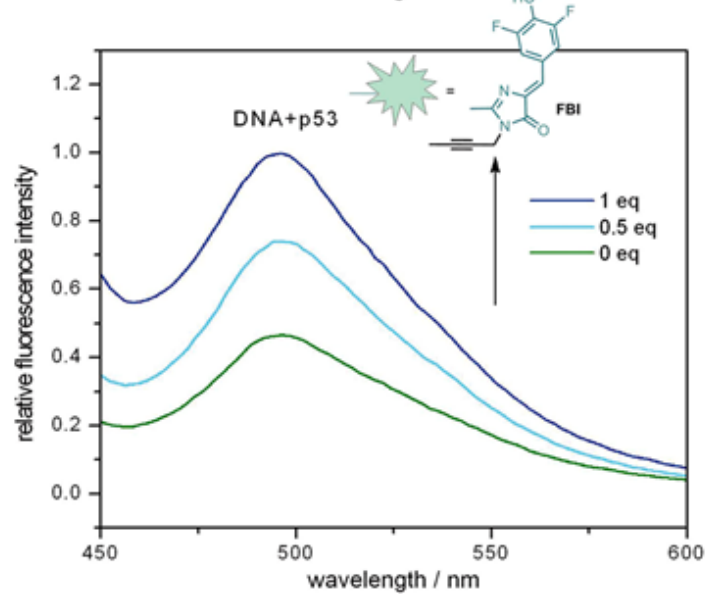
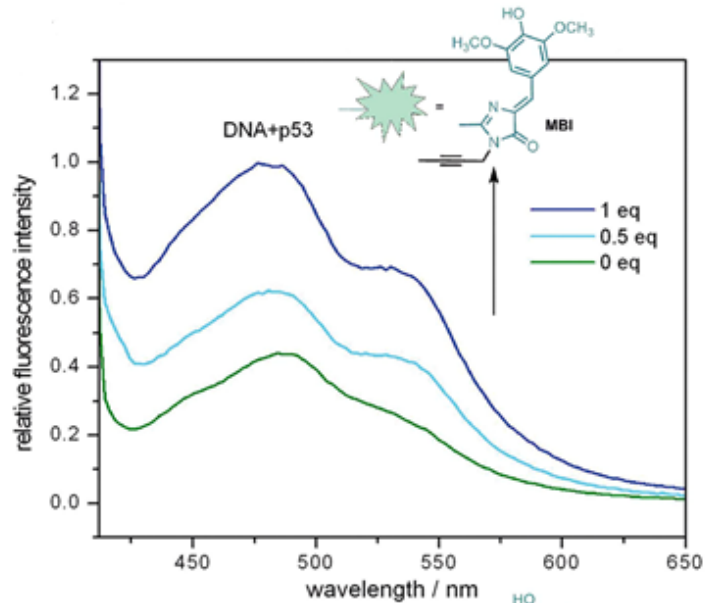
- některé fluorofory špatně „svítí“ ve vodě, ale dobře v nevodném prostředí
- navázaný protein vodu vytěsní a fluorescence se zesílí



Světlo medúzy: zelený fluoreskující protein

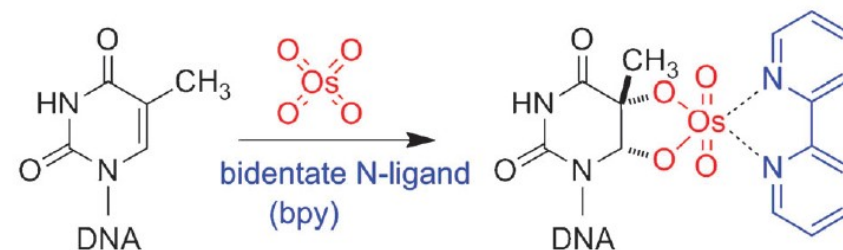
(fluorofor svítí lépe, když nemůže rotovat: např. je-li uvězněn v proteinu)

Riedl J., et al., J Org Chem 77, 8287 (2012).



Jak vánoční výzdobu DNA uskutečnit?

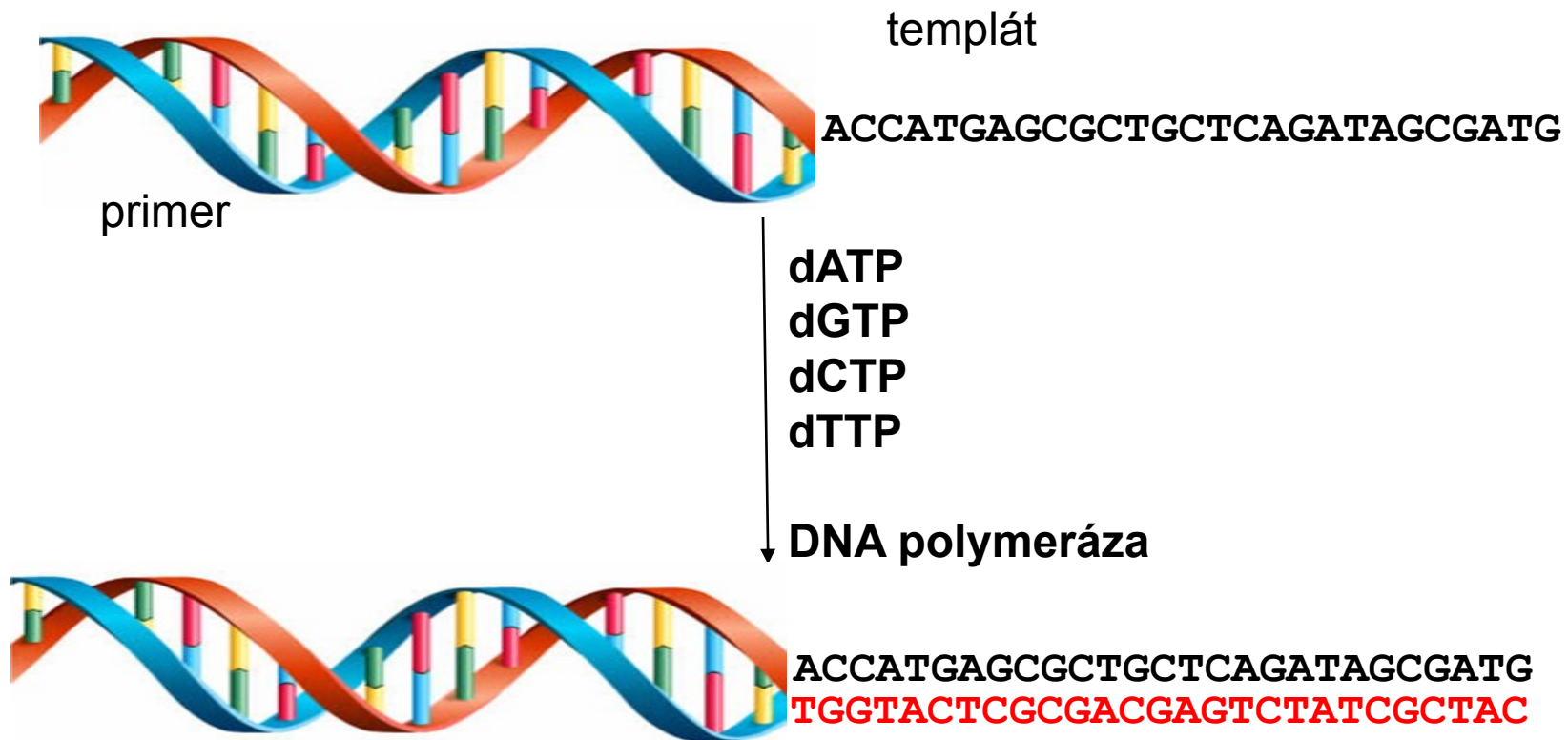
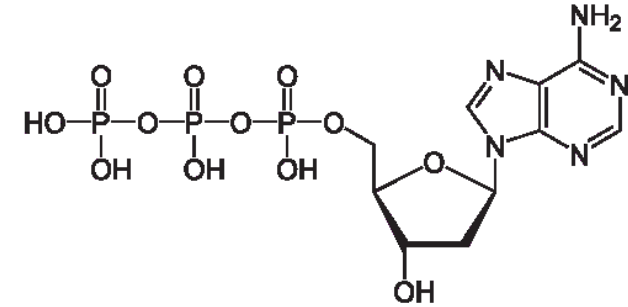
- můžeme si nechat chemicky vyrobit a koupit značenou DNA (drahé, a koho baví umělé stromečky?)
- obyčejnou DNA můžeme značit pomocí chemických reakcí



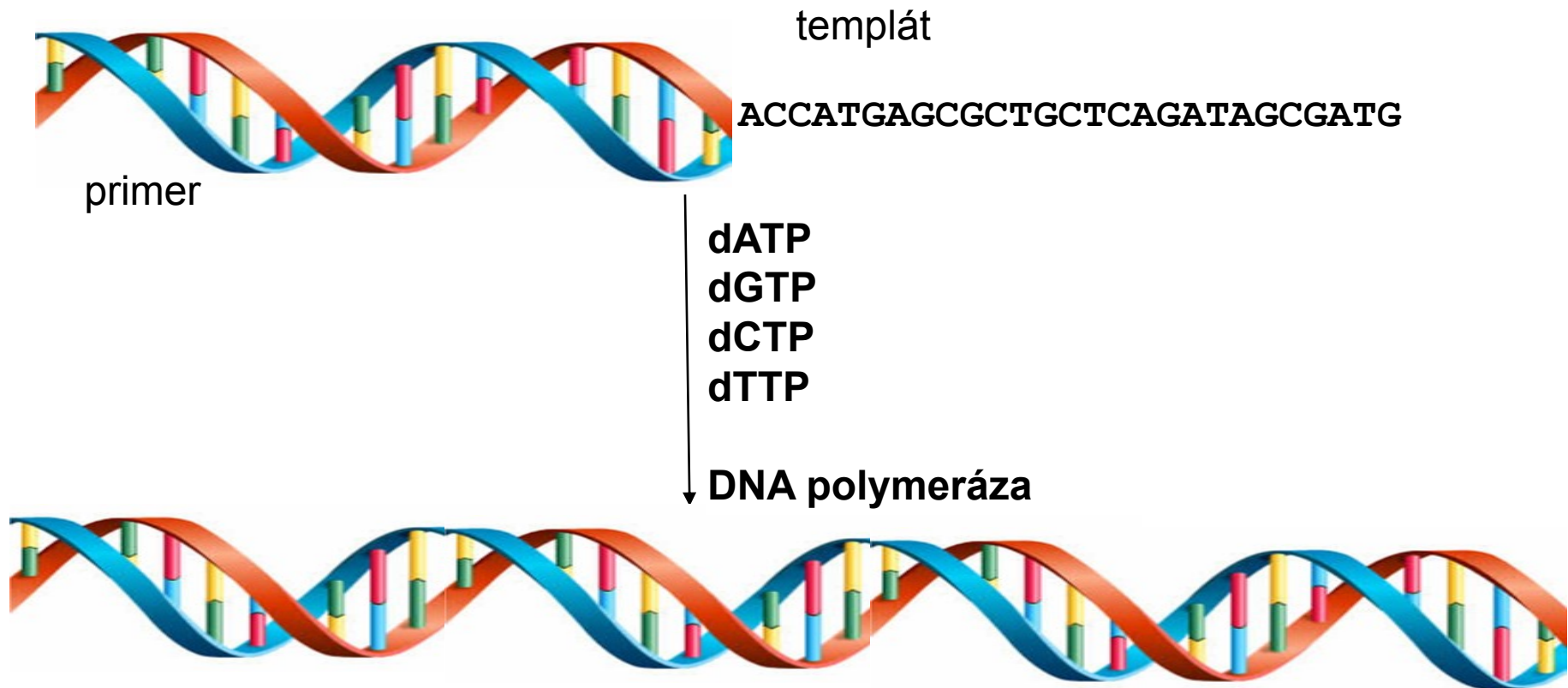
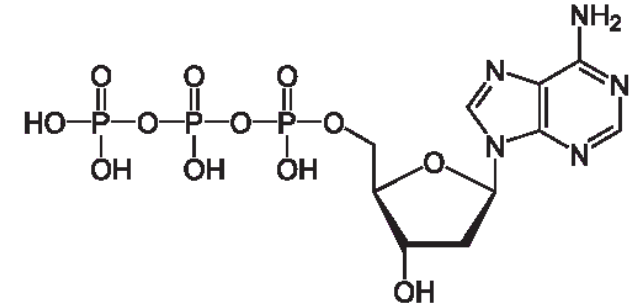
- DNA můžeme poskládat pomocí enzymů přímo ze značených stavebních kamenů

Značení DNA pomocí enzymů a
modifikovaných stavebních kamenů
– deoxynukleosid trifosfátů

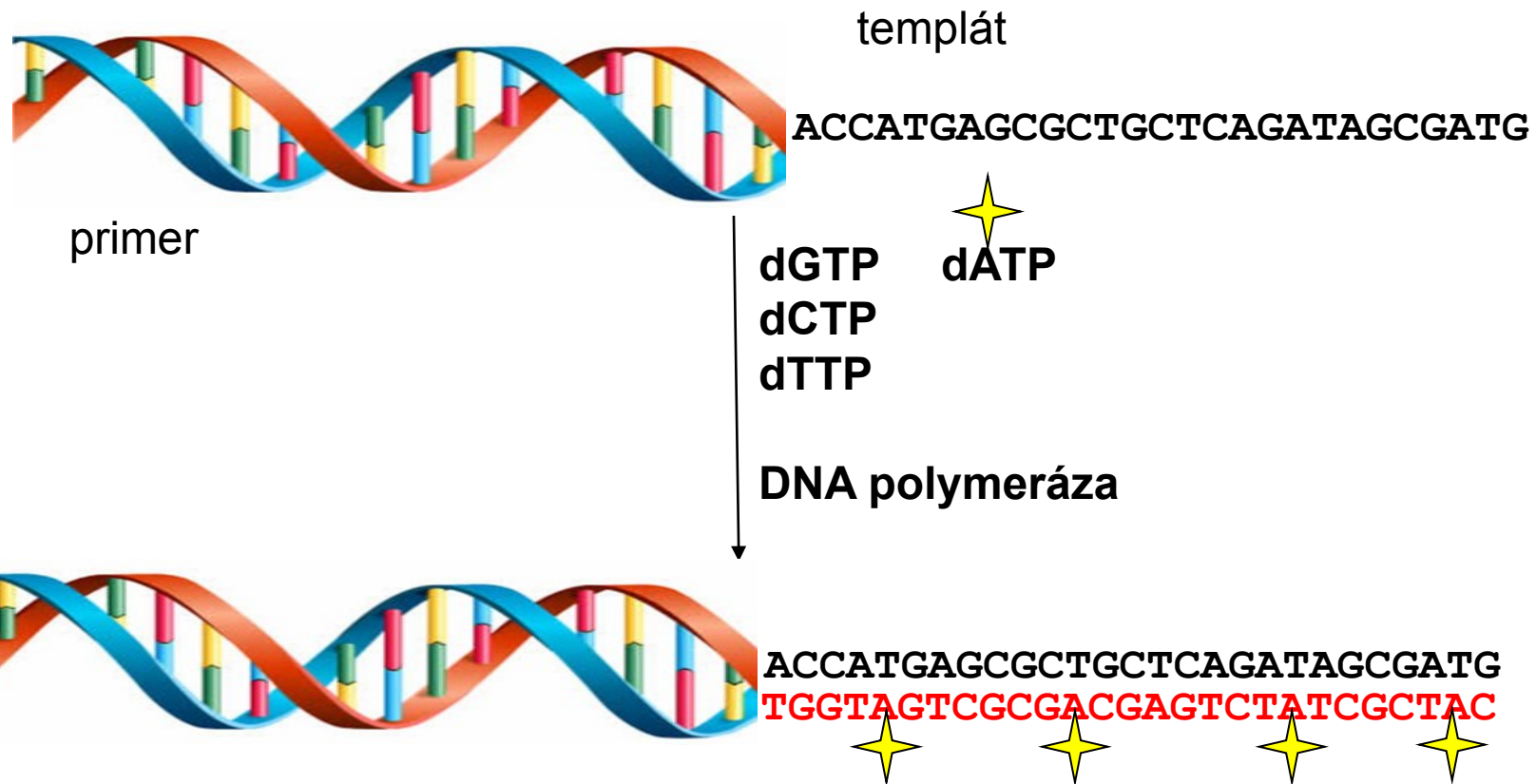
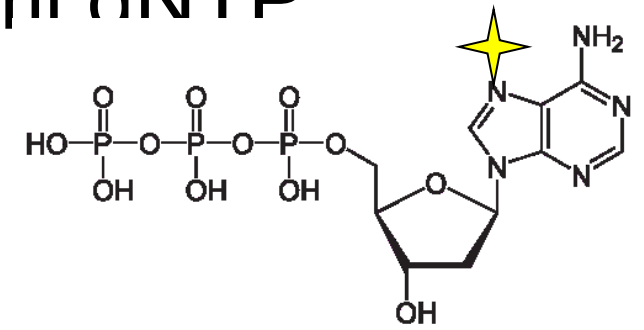
Prodlužování primeru (podstata replikace DNA)



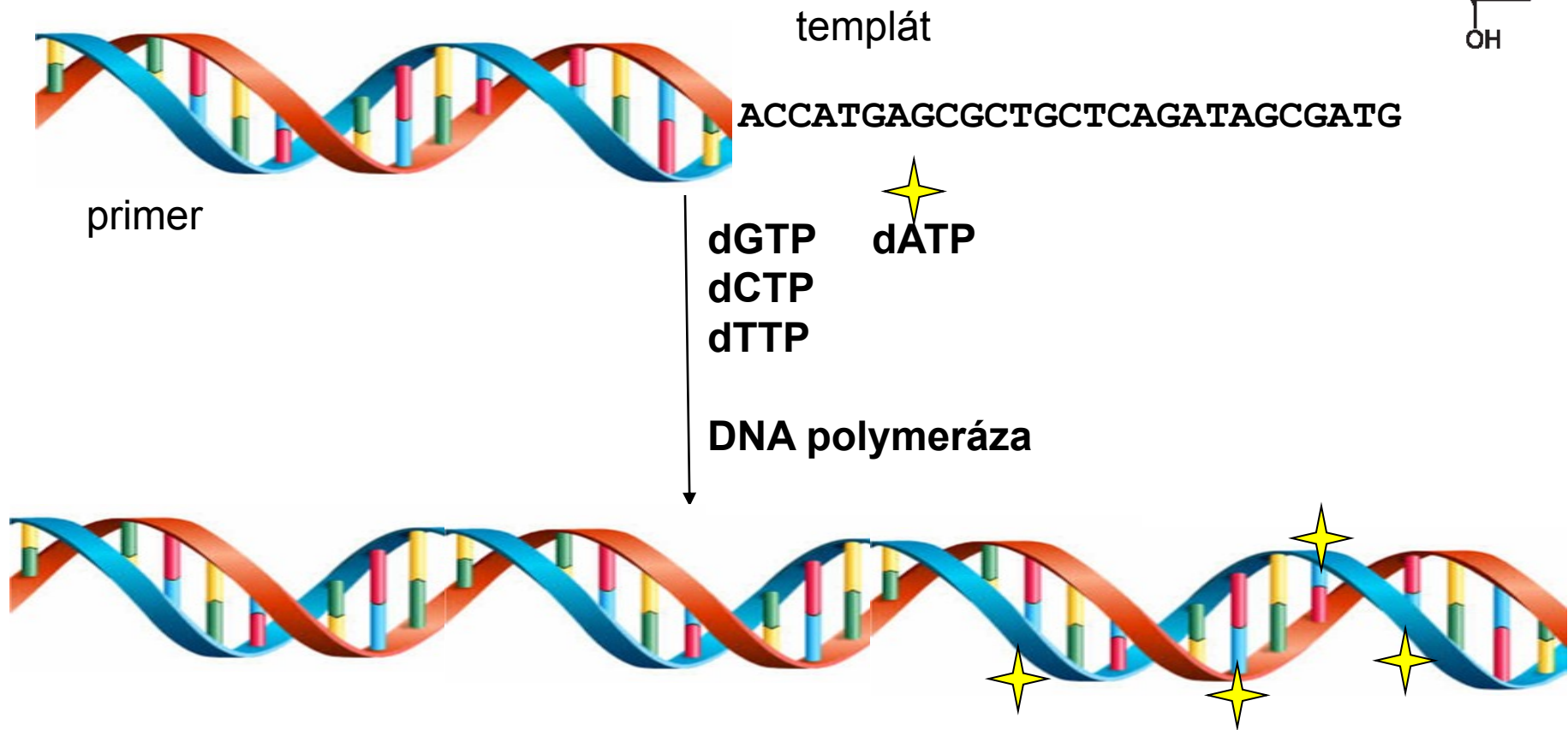
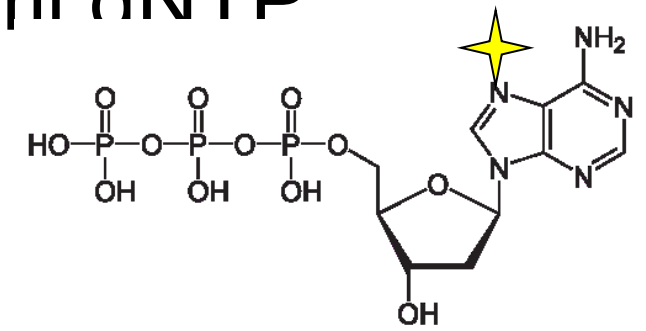
Prodlužování primeru (podstata replikace DNA)



Prodlužování primeru s modifikovanými dNTP



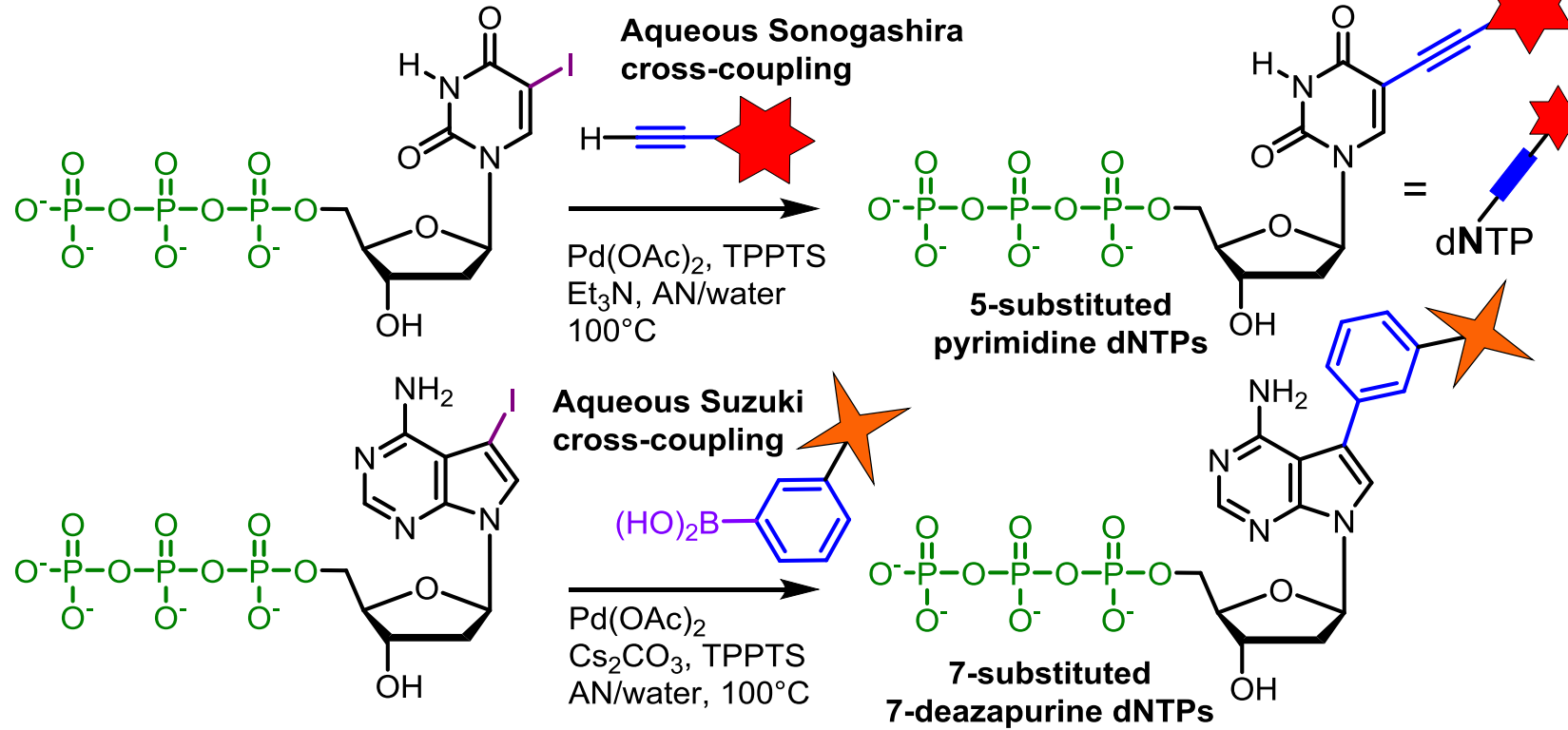
Prodlužování primeru s modifikovanými dNTP





skupina prof. Michala Hocka
syntéza modifikovaných dNTP

Synthesis of base-modified dNTPs



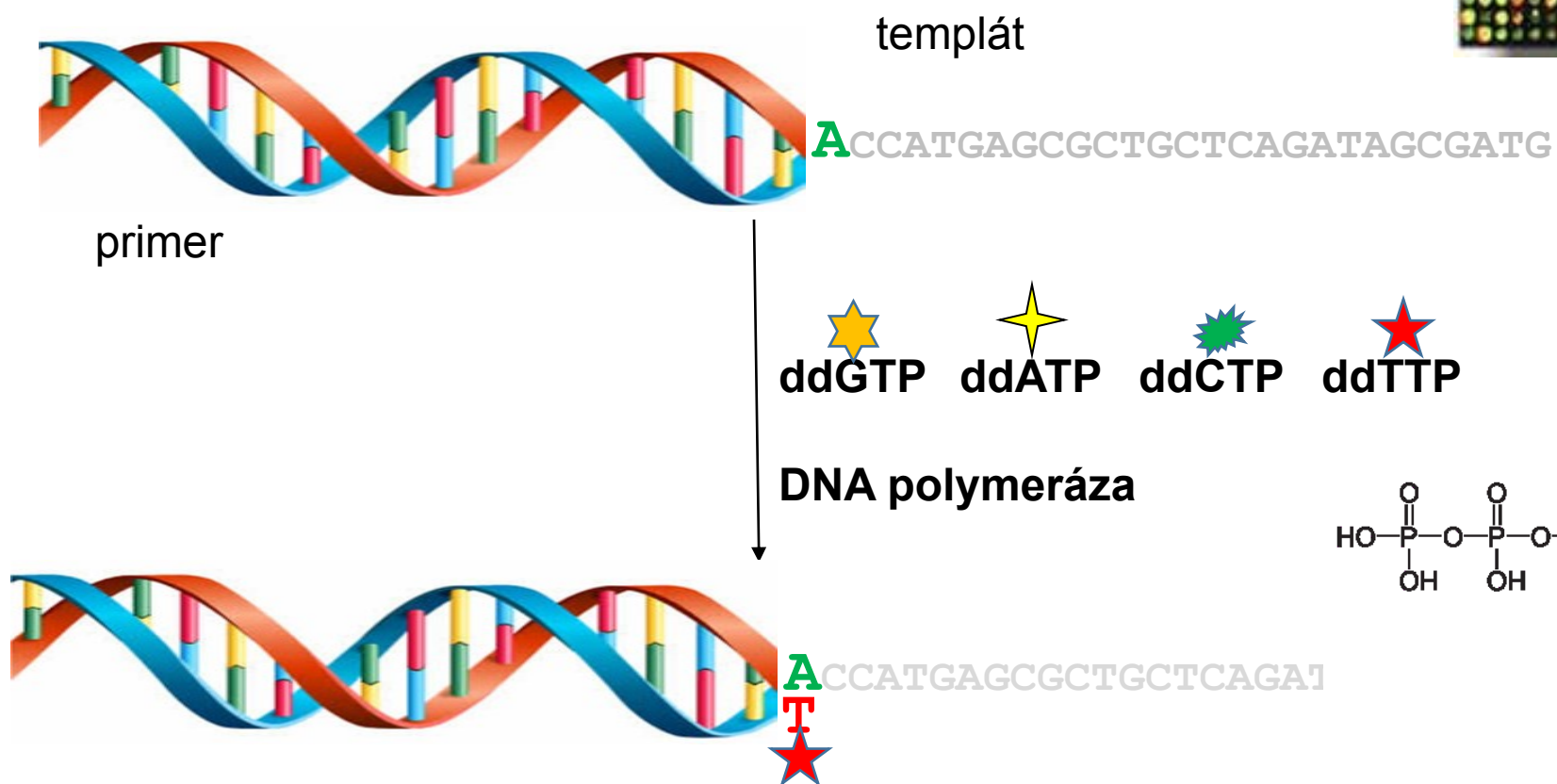
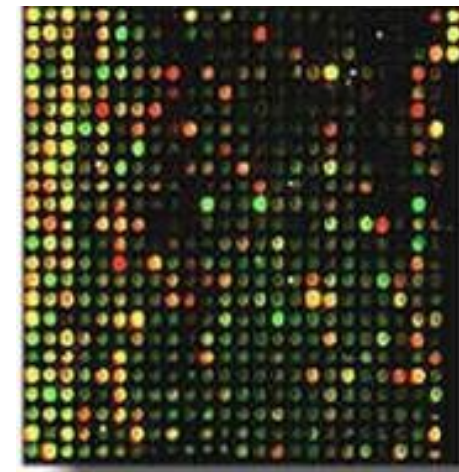
Můžeme vidět změnu jednoho
písmenka v DNA (bodovou
mutaci)?

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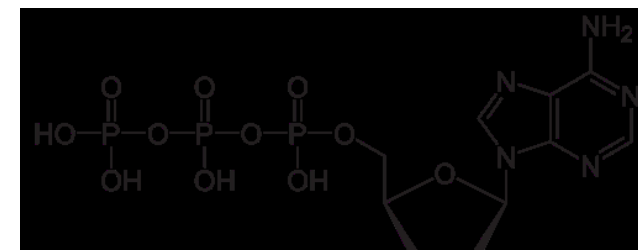
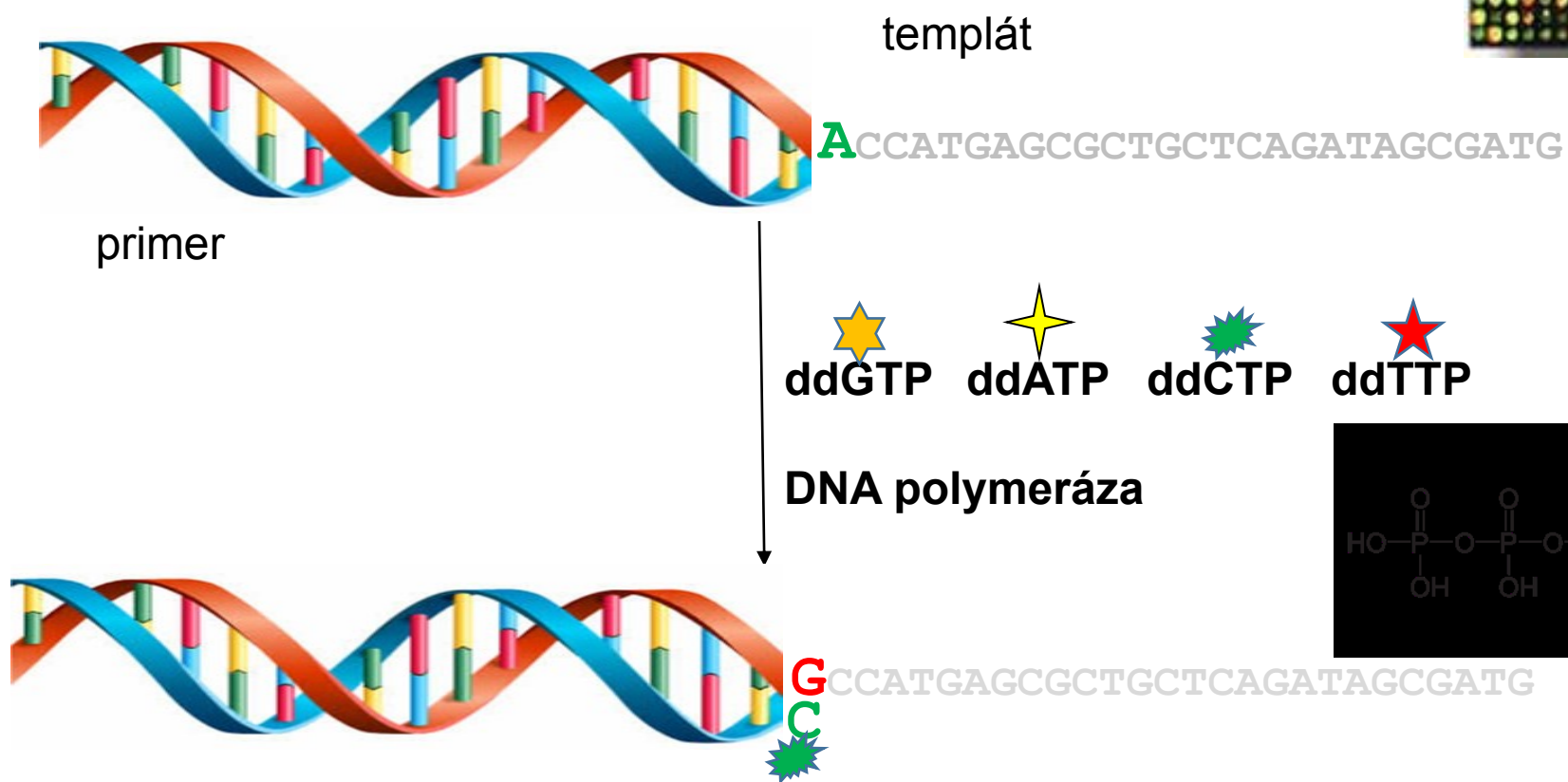
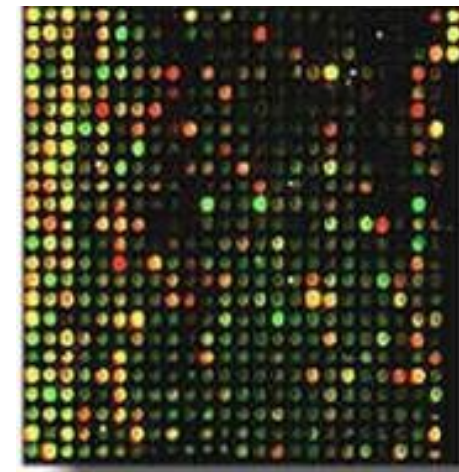
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GAAAT

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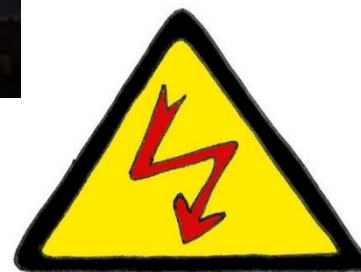
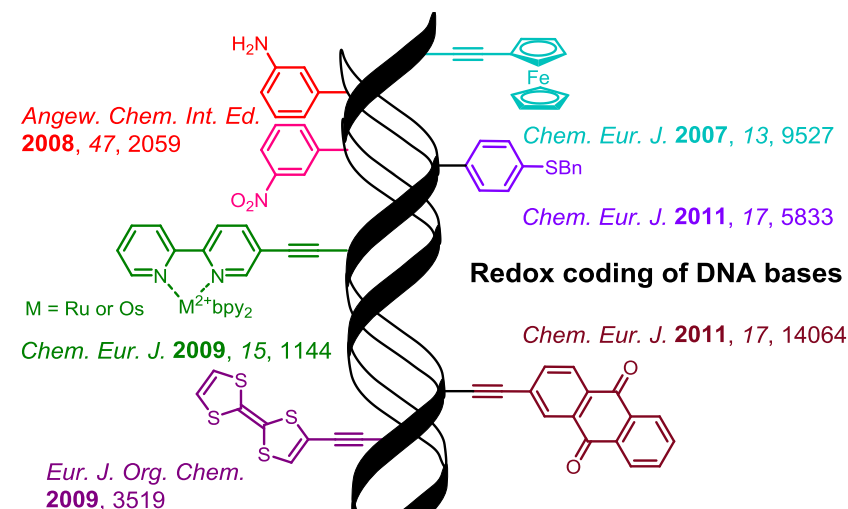
Detekce mutací



Detekce mutací



Místo světýlek elektrické proudy?



Elektrochemické metody ... polarografie

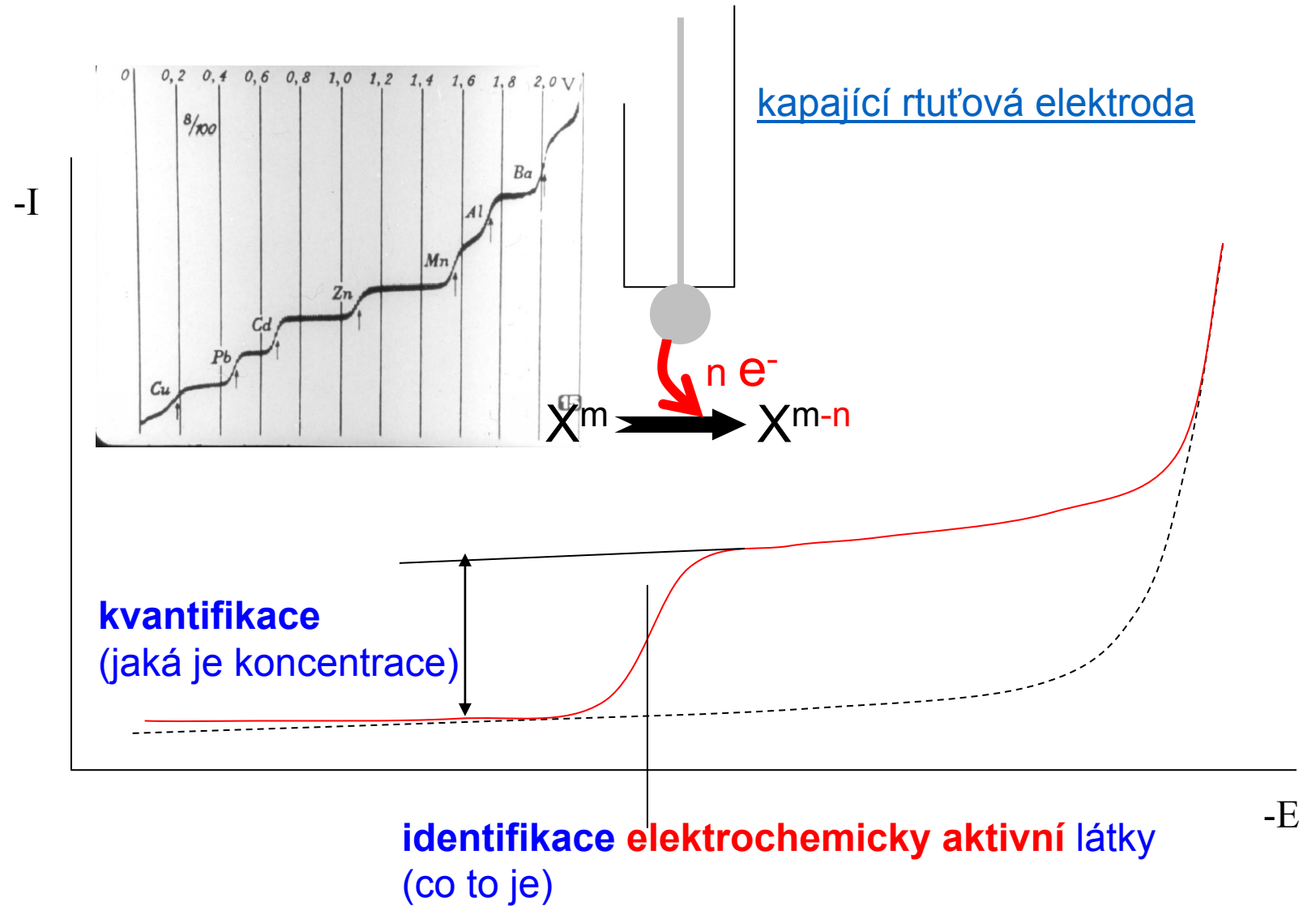
Jaroslav Heyrovský



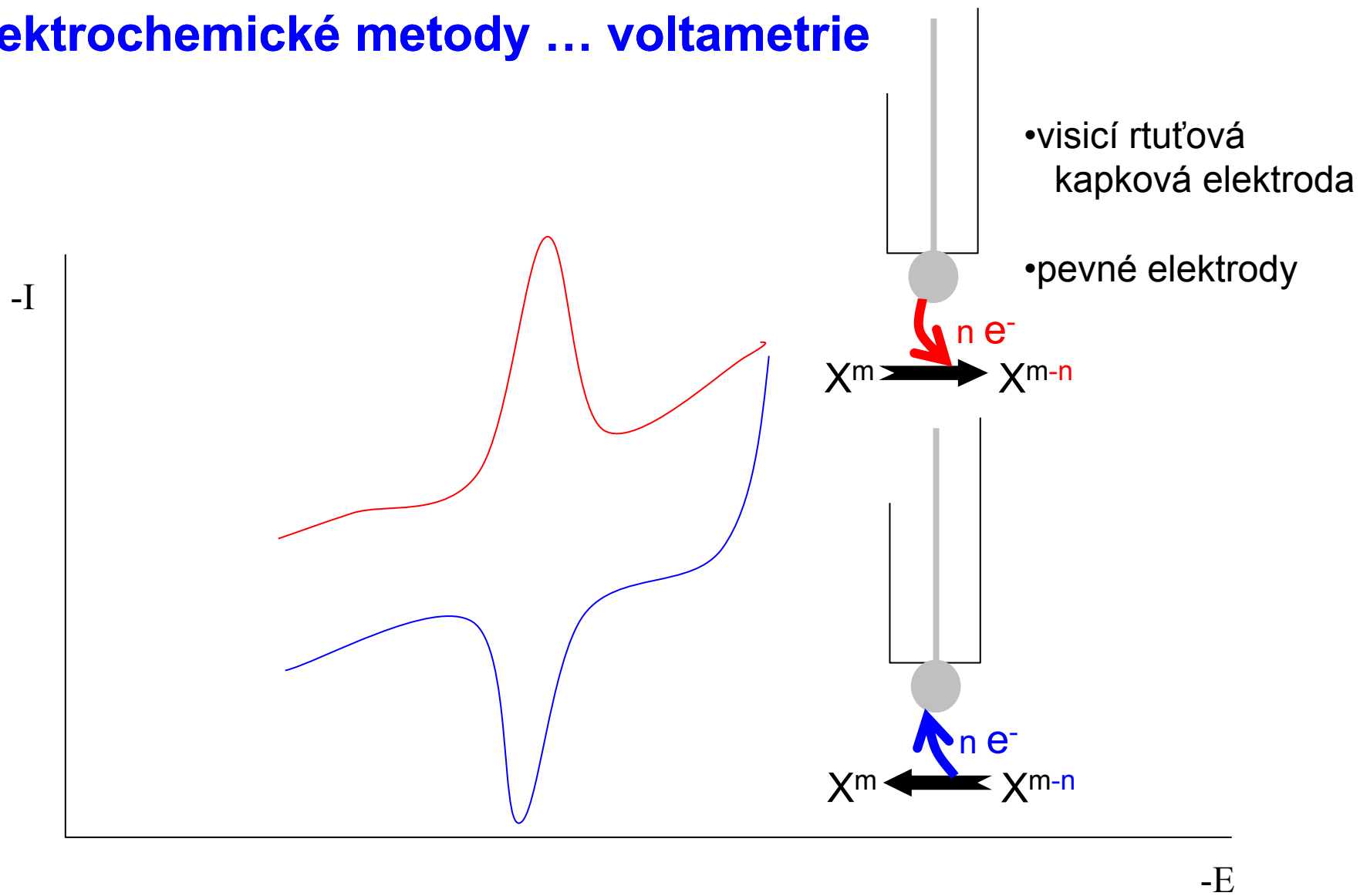
J. Heyrovský



Nobelova cena 1959



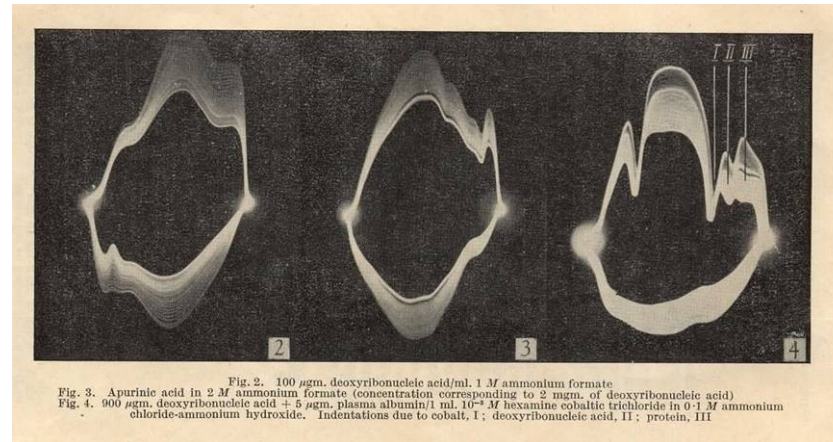
Elektrochemické metody ... voltametrie



Emil Paleček (od 50 let): polarografie DNA



Česká Hlava 2014



(Reprinted from *Nature*, Vol. 188, No. 4751, pp. 656-657, November 19, 1960)

Oscillographic Polarography of Highly Polymerized Deoxyribonucleic Acid

PROCEEDING from my finding^{1,2} that nucleotides, nucleosides and the bases of nucleic acids can be analysed by alternating current oscillographic polarography^{2,3}, I have also tried to study polymerized deoxyribonucleic acid by this method.

The apparatus used was a Polaroskop P 524 (Křížik, Praha). With this apparatus it is possible to plot dE/dt against E (Fig. 1). The analysis was carried out by means of the dropping mercury electrode in the same electrolytes as were used in my previous work^{1,2}. All measurements were carried out with specimens of deoxyribonucleic acid from calf thymus.

I have established that in a medium of molar ammonium formate, deoxyribonucleic acid shows an anodic indentation at the same potential as deoxyguanylic acid (Fig. 2). Other characteristics of both indentations are also analogous (dependence on direct voltage, temperature, concentration of the electrolyte), which appears to indicate that that due to

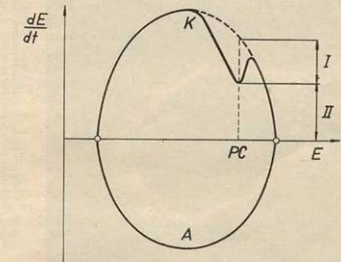
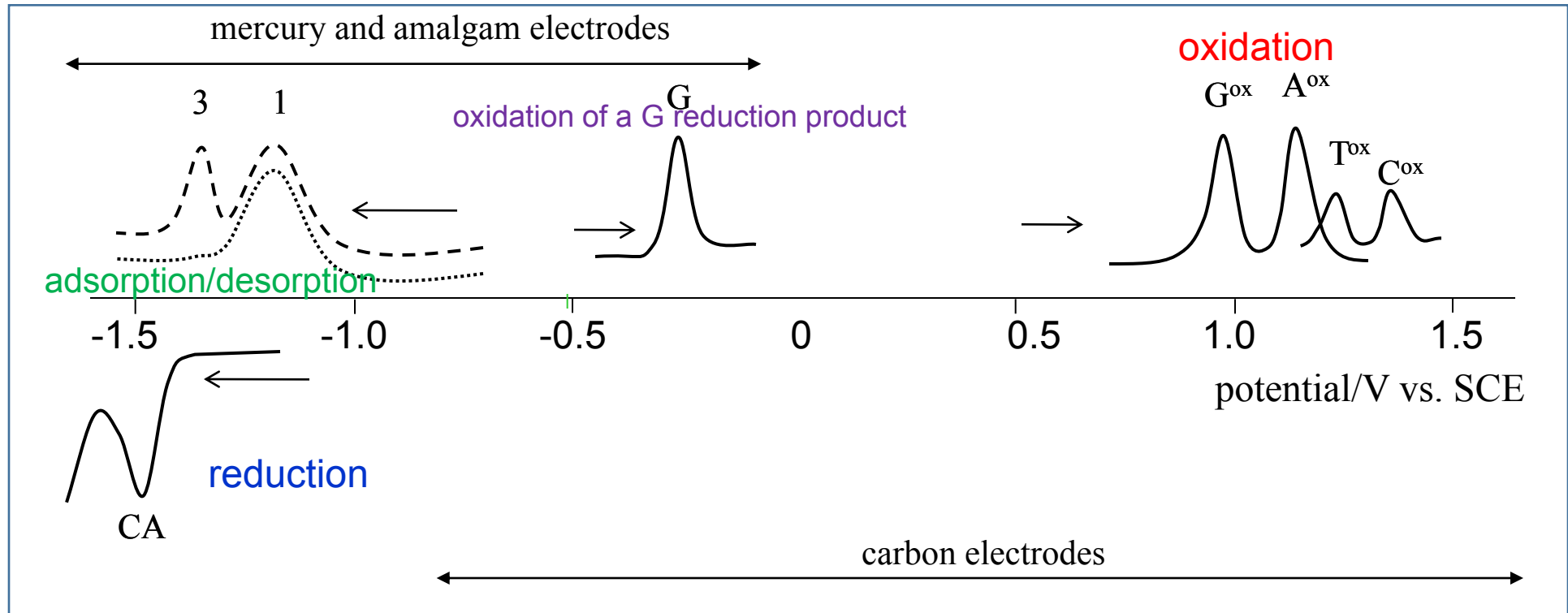
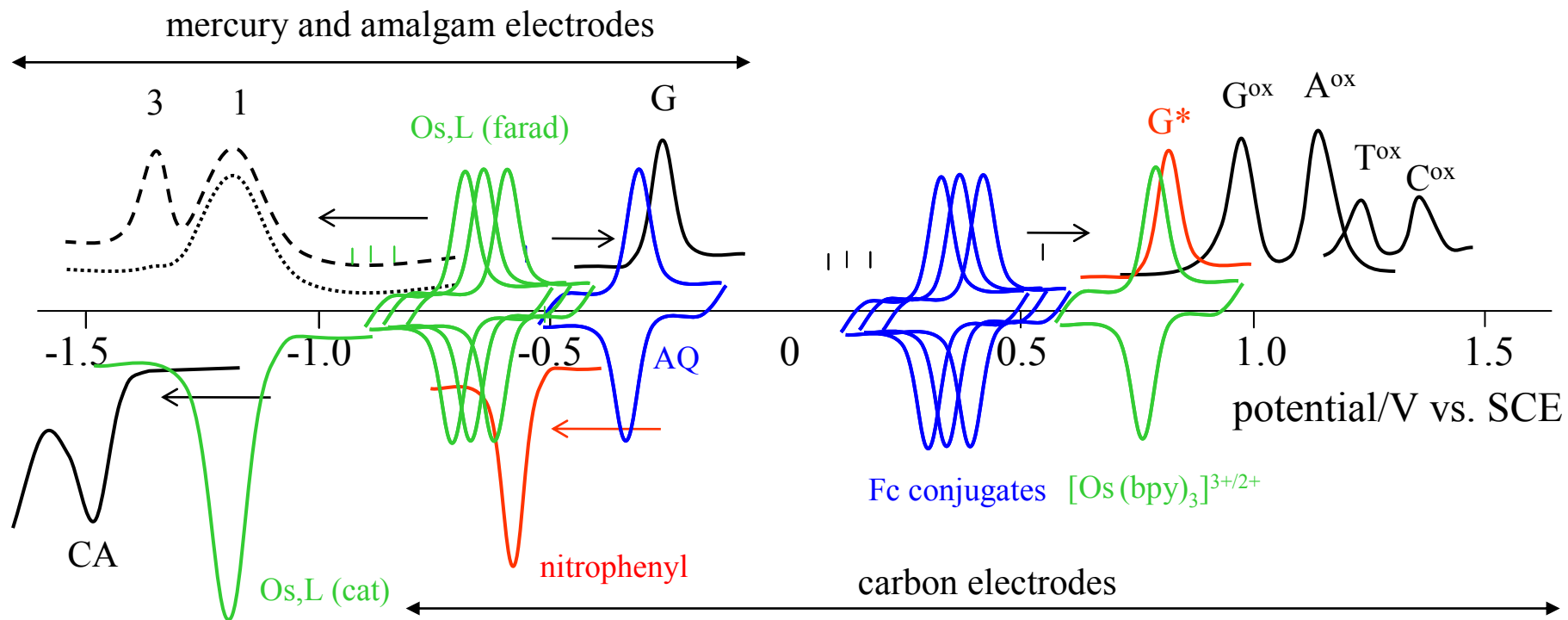


Fig. 1. Graph of dE/dt against E . The nature of the material analysed is characterized by the potential of the indentation (PC), which is somewhat similar to the polarographic half-wave potential. The quantity of the material is characterized by the depth of the indentation. For qualitative analysis, the height II, which can be measured much more easily, is generally measured. K, Cathodic part; A, anodic part

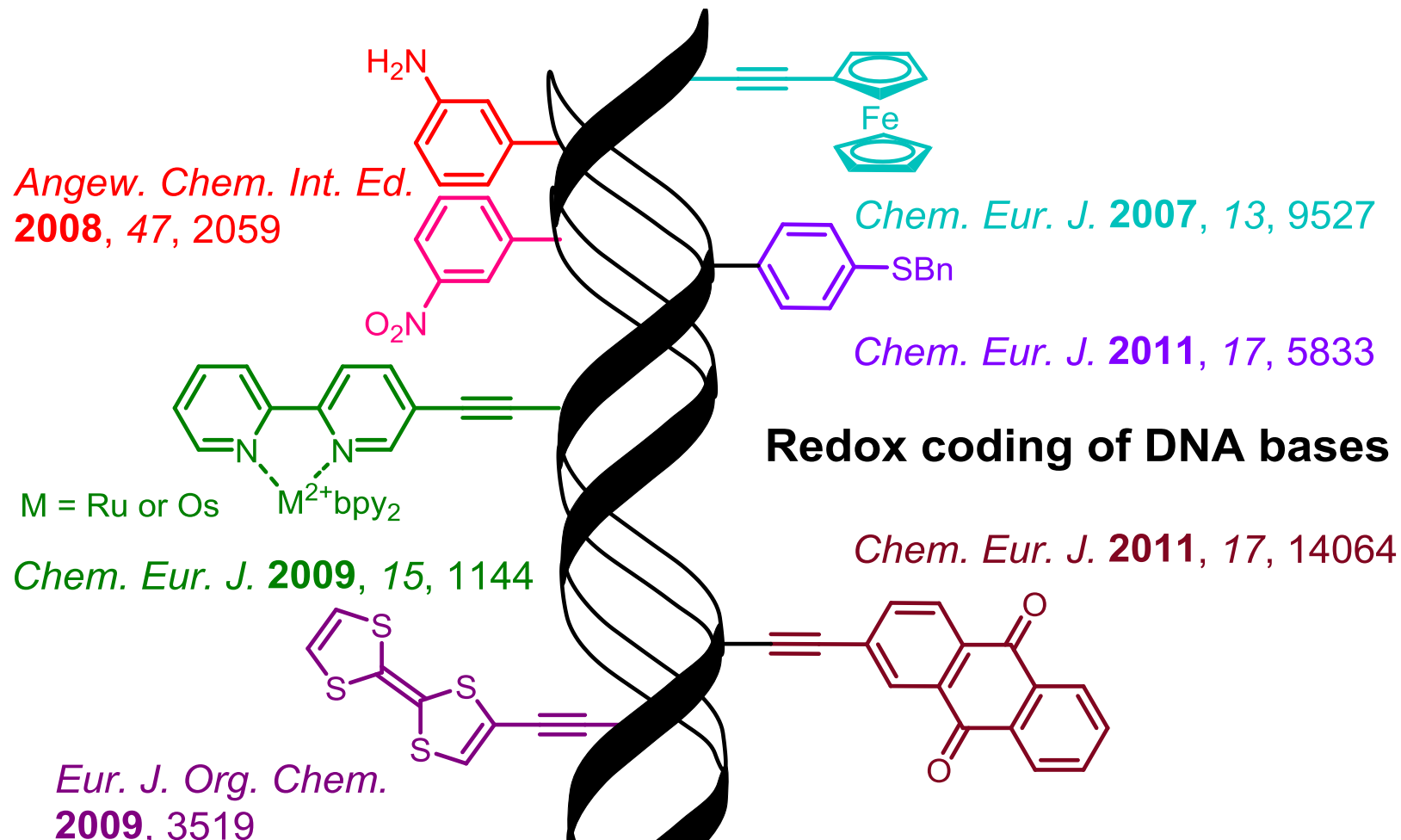
Elektroaktivita DNA



„Spektrum“ redox potenciálů různých značek

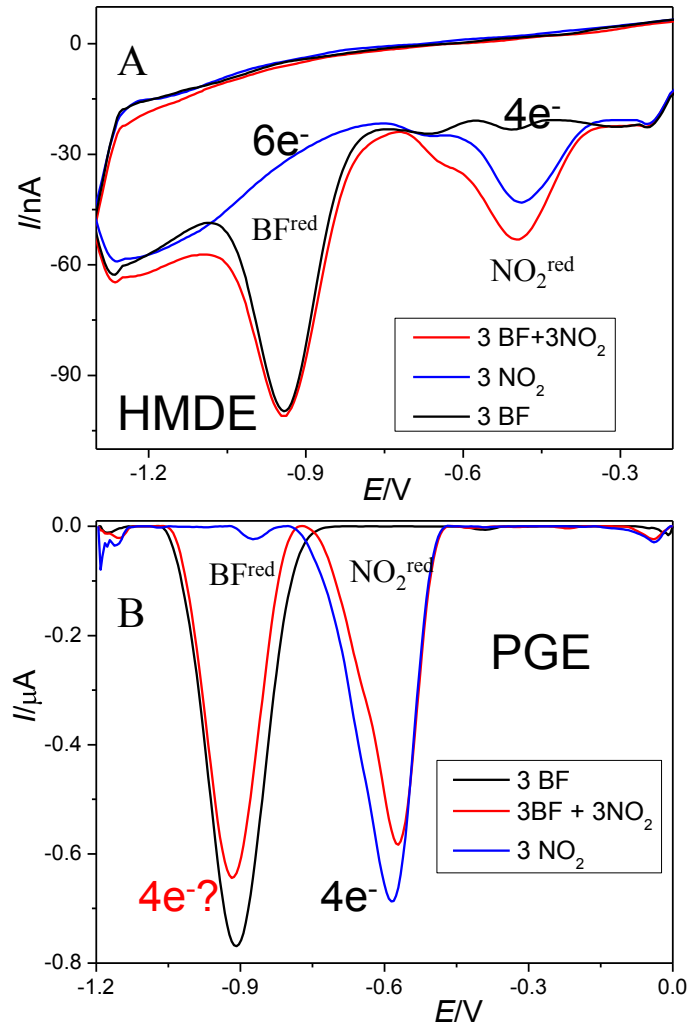


a palette of labels producing distinct electrochemical responses

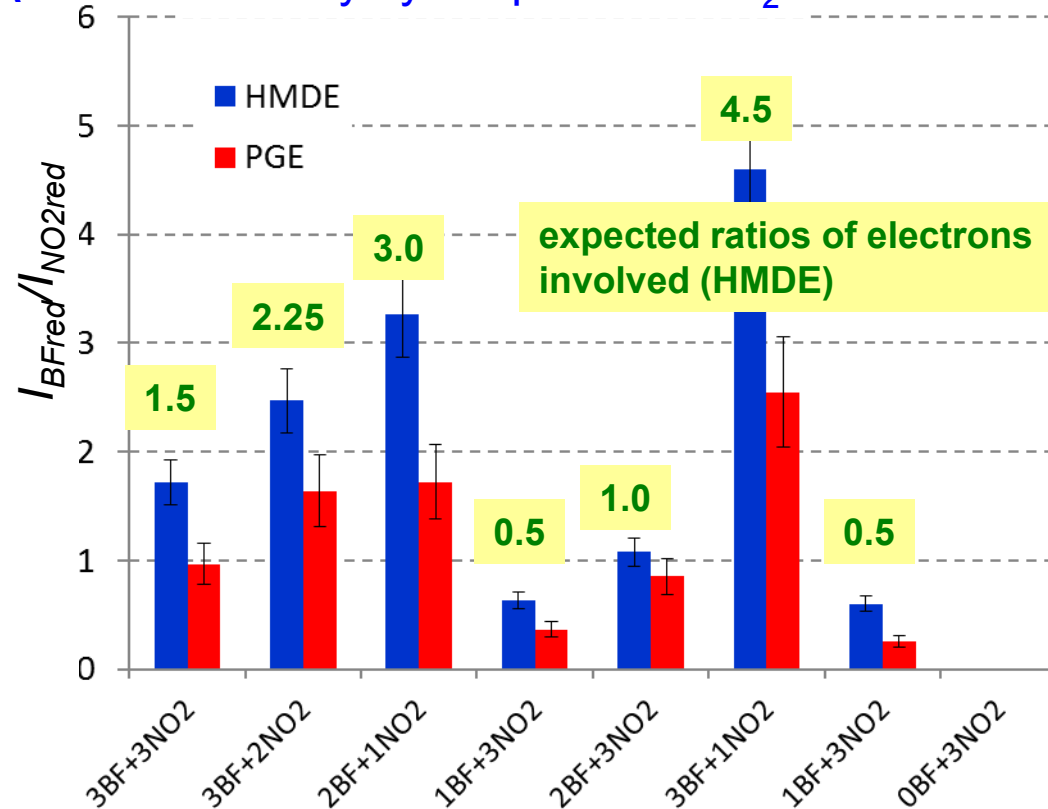


Kombinace benzofurazanu a nitrofenylu

3BF a/nebo 3NO₂ v 30-meru DNA



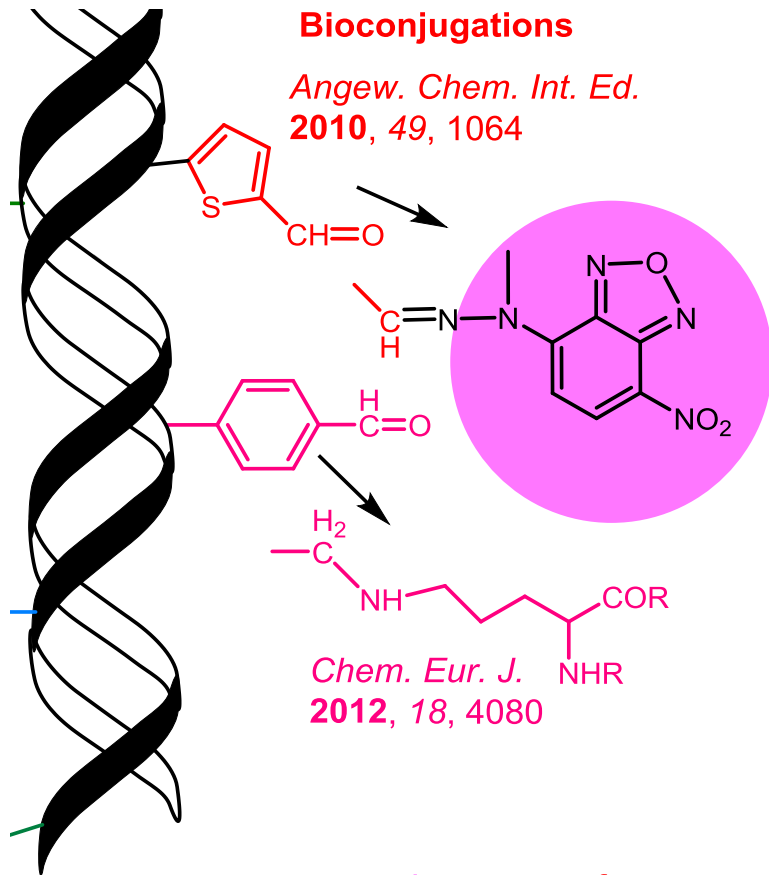
Poměry výšek píkú BF/NO₂



Přístup vhodný pro snadné sledování
relativních změn v zastoupení jednotlivých bazí
v daném úseku DNA

Háčky a věšáčky

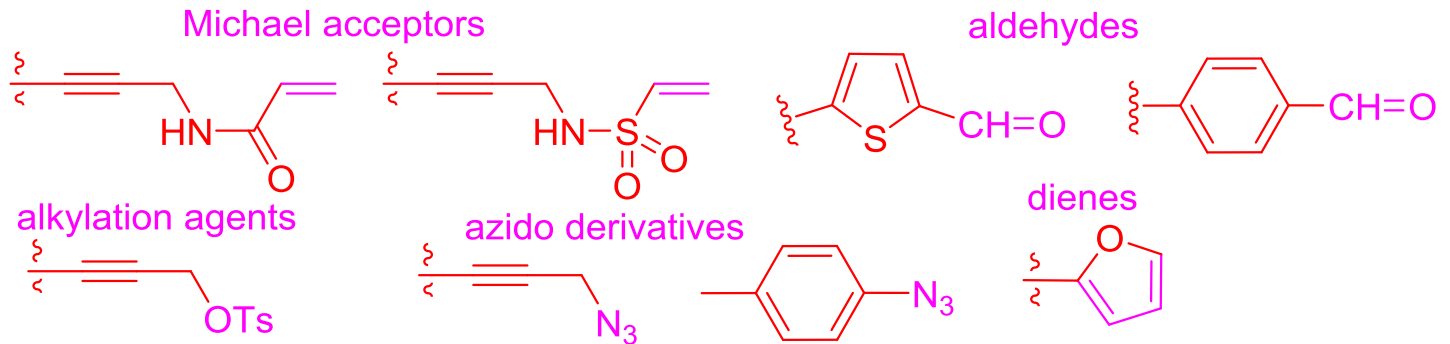




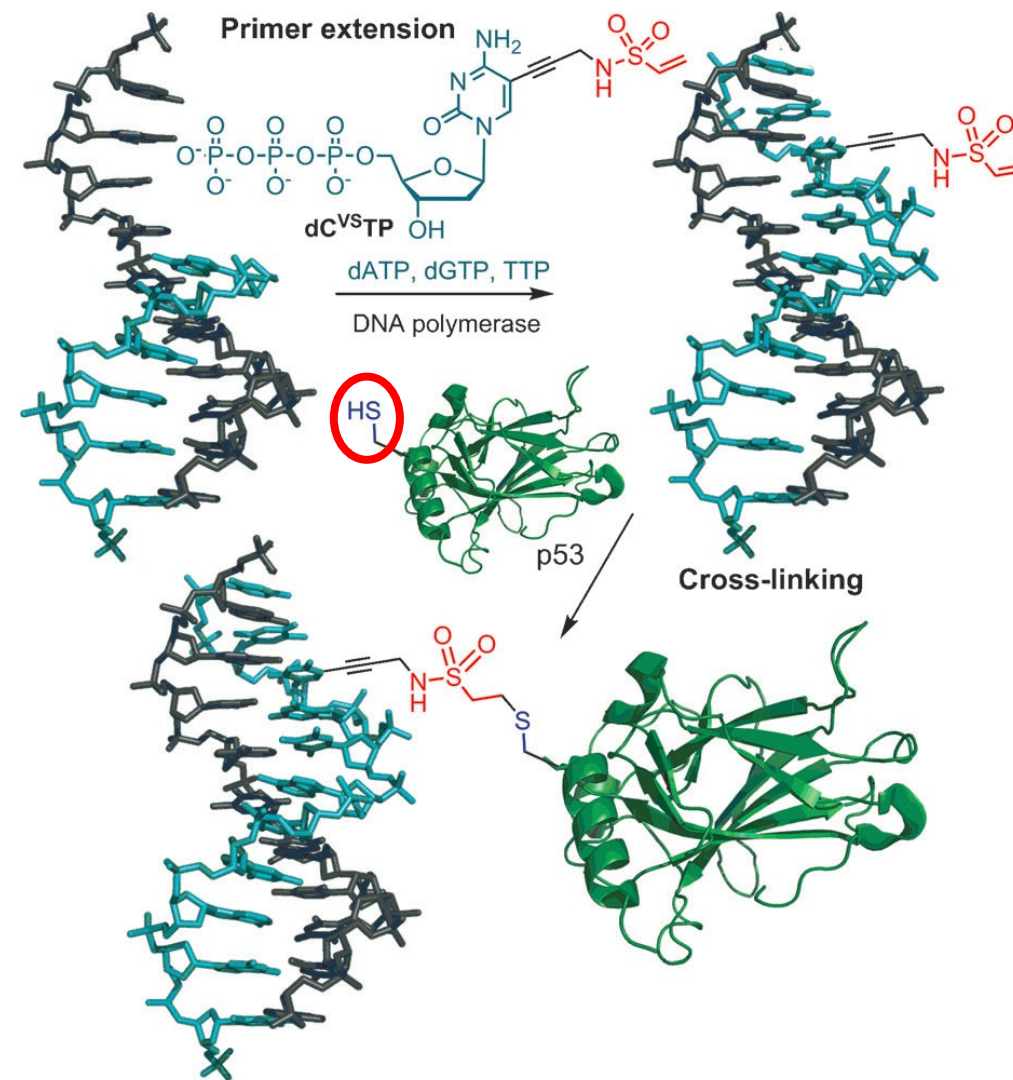
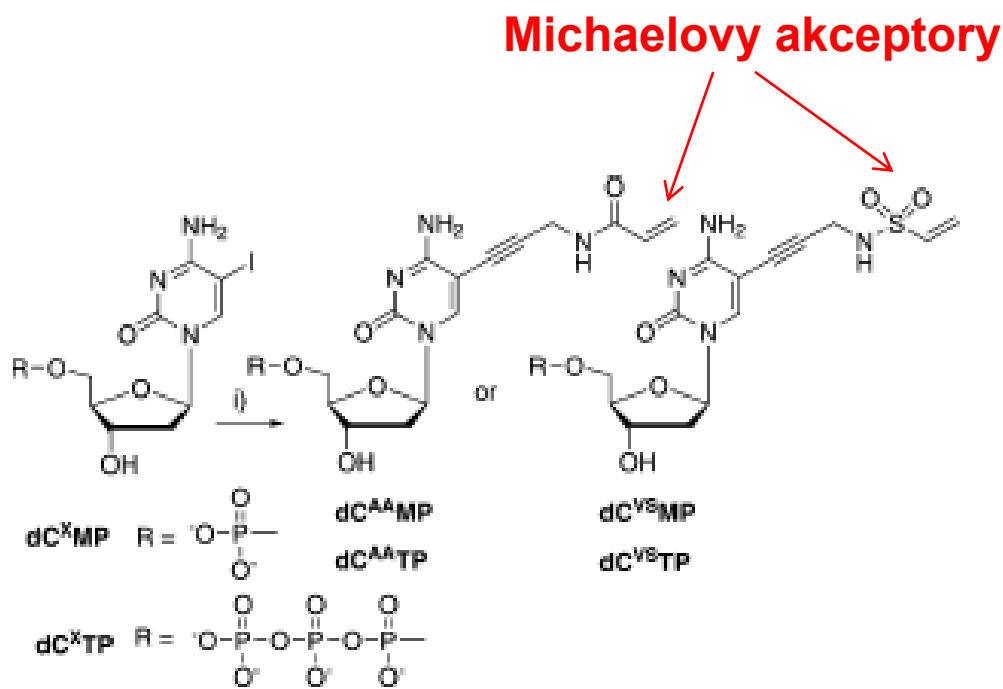
-DNA se opatří háčky při její enzymatické syntéze (malé chemické skupiny)

-k nim je pak možno připojit další molekuly

reactive groups for post-synthetic bioconjugations and cross-linking:

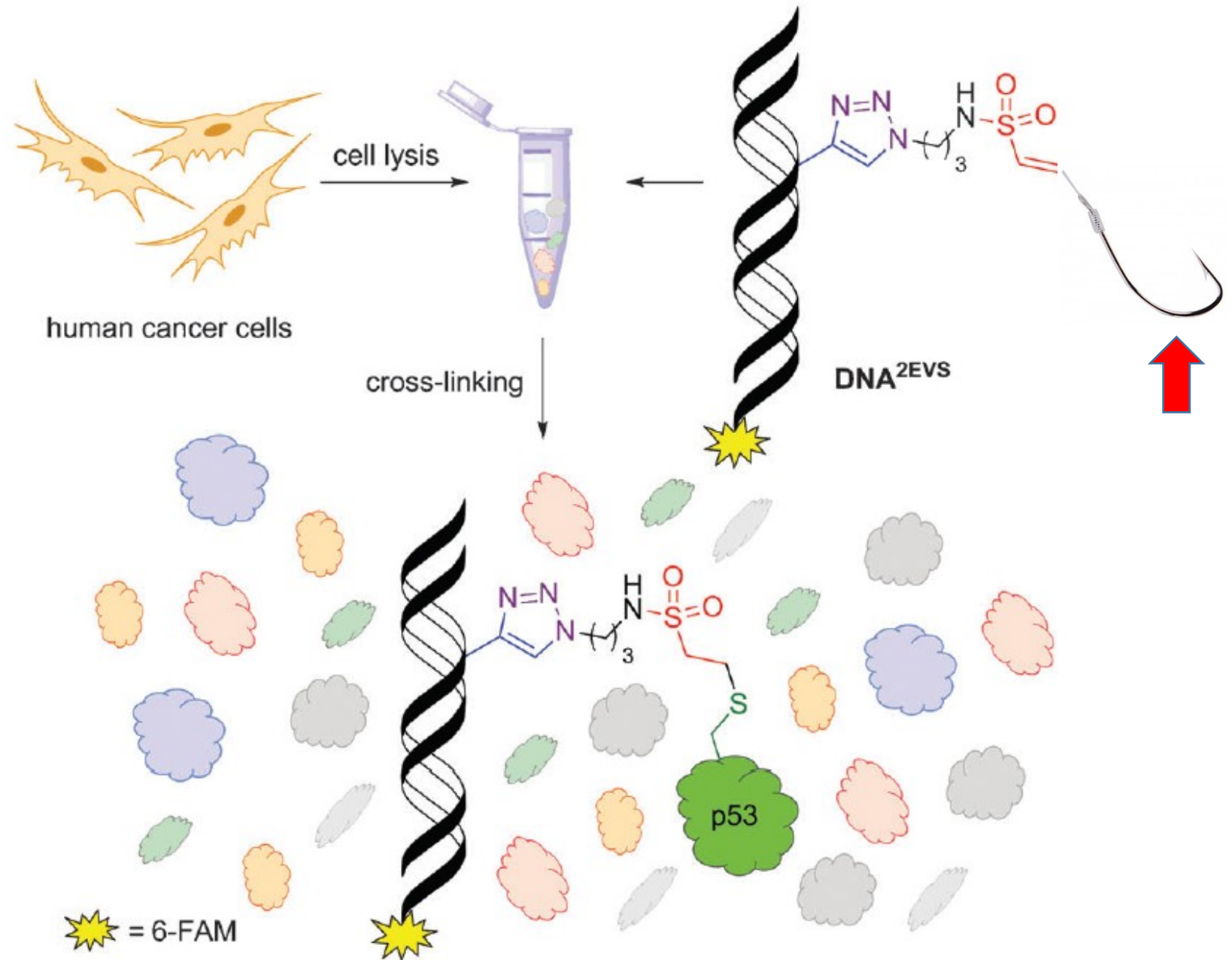


Zachycení proteinu na DNA nesoucí vhodný háček



- reakce Michaelových akceptorů na DNA s –SH skupinami proteinu
- podmínkou je správné navázání proteinu na sekvenci DNA, kterou rozpoznává, aby se obě reaktivní skupiny dostaly do vzájemné blízkosti

Molekulární rybaření



- vychytávání specifického proteinu z buněčného lyzátu



Děkuji za pozornost!

Moderní biofyzikální metody: pokročilé praktické vzdělávání v experimentální biologii
(CZ.1.07/2.3.00/09.0046)



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



**OP Vzdělávání
pro konkurenceschopnost**

INVESTICE
DO ROZVOJE
VZDĚLÁVÁNÍ