Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI FAKULTET

KEMIJSKI ODSJEK

Sveučilišni poslijediplomski studij kemije

Organska kemija

# METODE PROBIRA SPOJEVA PREMA AFINITETU VEZANJA NA VIŠELANČANE I HIBRIDNE DNA/RNA STRUKTURE

(prema radu: U. Yildiz, B. Coban, *Appl Biochem Biotechnol*. **186** (2018) 547-562.)

SEMINARSKI RAD

KEMIJSKI SEMINAR I

Iva Zonjić

# Molekula DNA

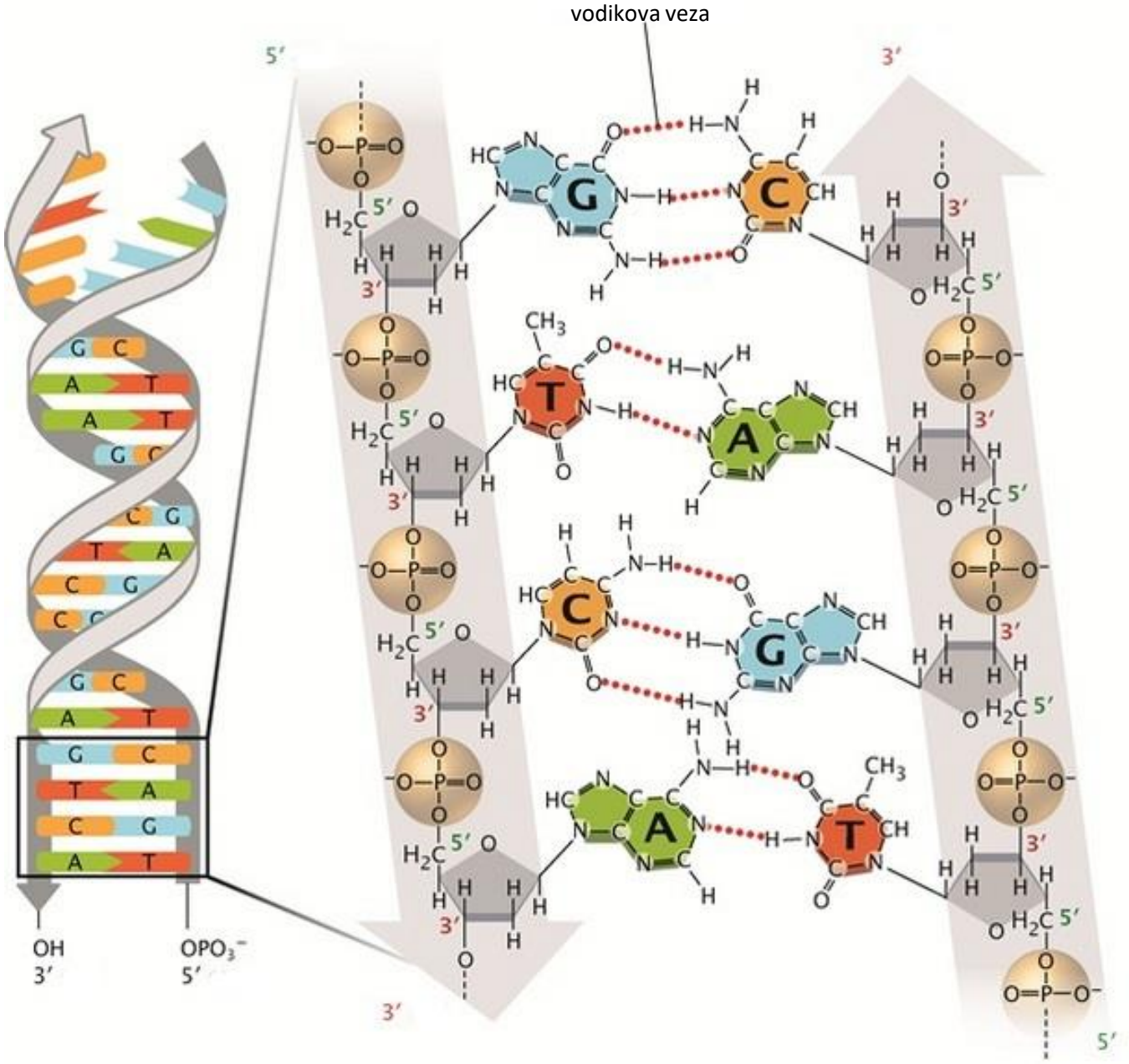
1953.-Watson i Crick

aromatske baze (purin i pirimidin)

šećeri

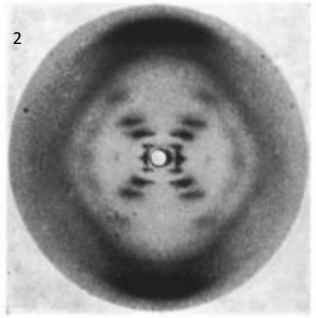
fosfatne grupe

Polimorfna molekula

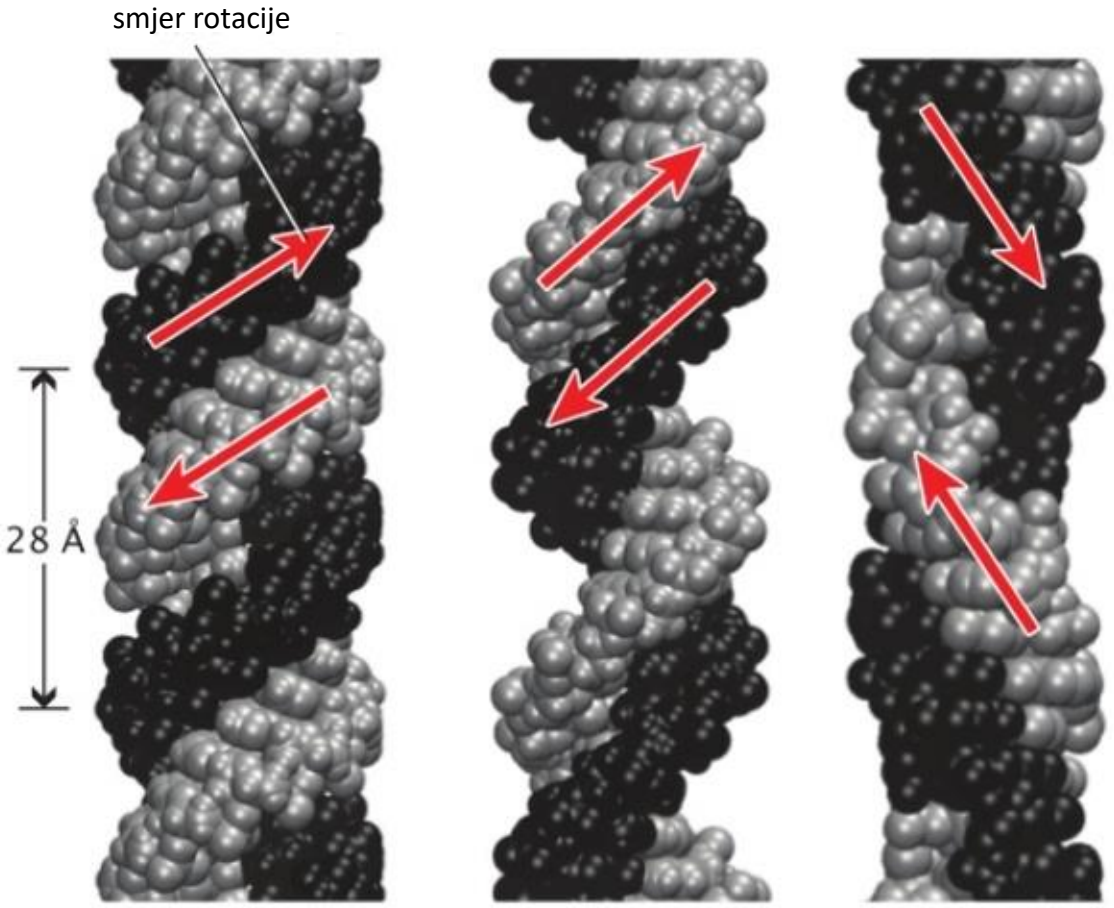



L. Pray, *Nature Education* **1** (2008) (1):100

Maurice Wilkins i Rosalind Franklin




Difrakcija X-zraka




A-uzvojnica B-uzvojnica Z-uzvojnica

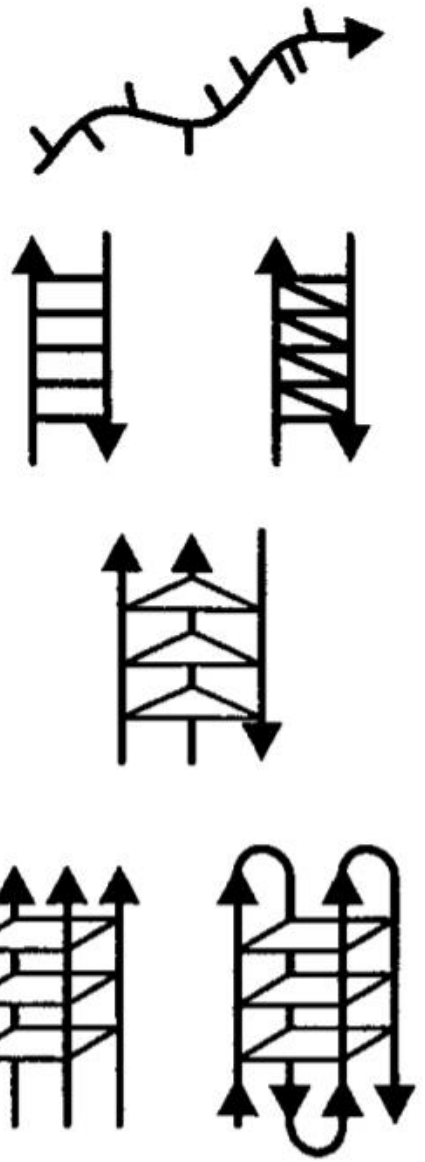
desne uzvojnice lijeva uzvojnica

Jeremy M. Berg, John L. Tymoczko i Lubert Stryer, Biokemija, Školska knjiga, Zagreb, 1, 2013., str. 107-117; str. 651-655; str. 783–788
L. Pray, *Nature Education* **1** (2008) (1):100

<u>DNA·RNA hibridi</u>
replikacija DNA i telomera
reverzna transkripcija

<u>tripleksi DNA</u>
treći lanac-Hoogsteenovo sparivanje
nestabilna struktura

<u>višelančane strukture</u>
G-kvadrupleks
proces starenja i razvoja bolesti
telomere i c-*myc* onkogen



jednolančane strukture

dvolančane strukture

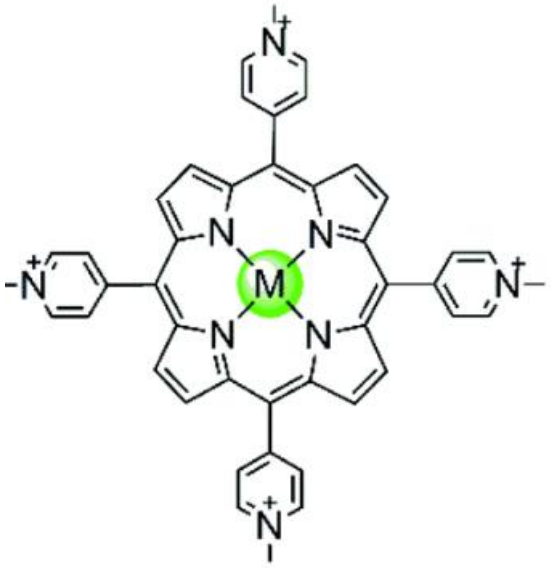
tripleksi

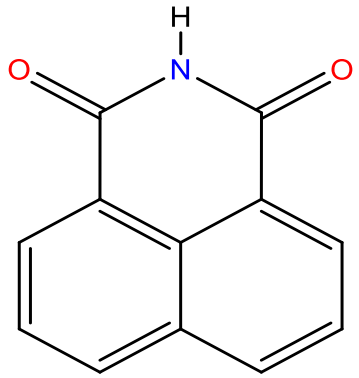
kvadrupleksi

J. Ren, J.B. Chaires, *Methods in enzymology* **340** (2001) 99-108.

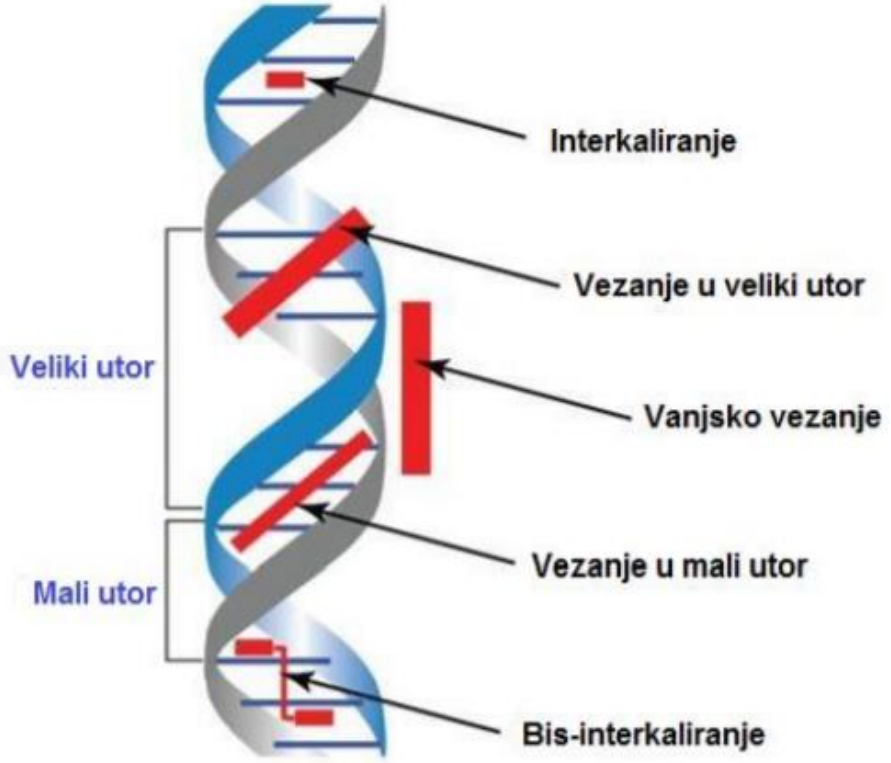
# Molekule s preferencijalnim vezanjem na G-kvadrupleks

velika, ravna, aromatska površina s protoniranim bočnim lancima



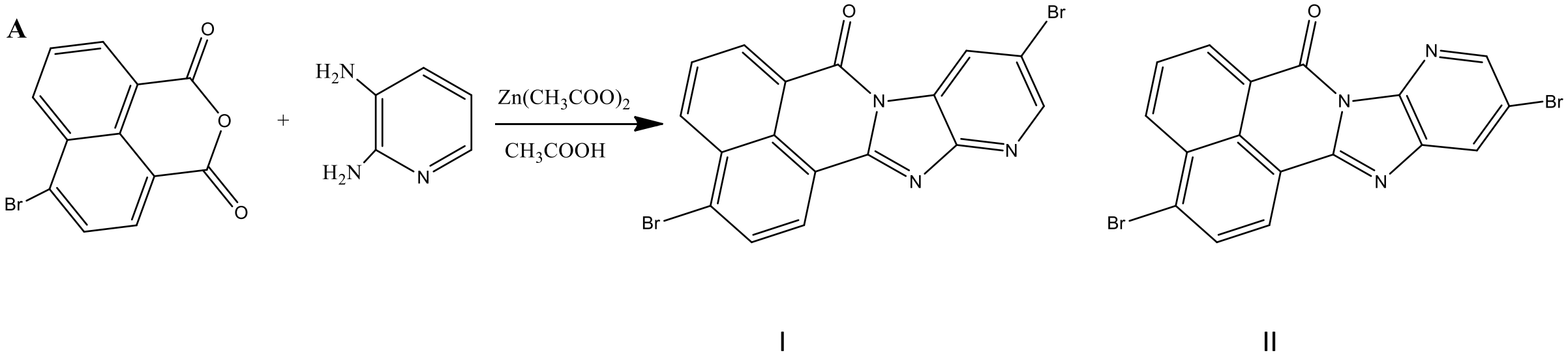

1,8-naftalimid




M. Grabar Branilović, *Eksperimentalno utemeljeno modeliranje interakcija nukleinskih kiselina i malih molekula*, Doktorski rad, Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, 2014, str. 6

Q. Li, J. Zhang, L. Yang, Q. Yu, Q. Chen, X. Qin, F. Le, Q. Zhang, J. Liu, *Journal of Inorganic Biochemistry* **130** (2014) 122-129.

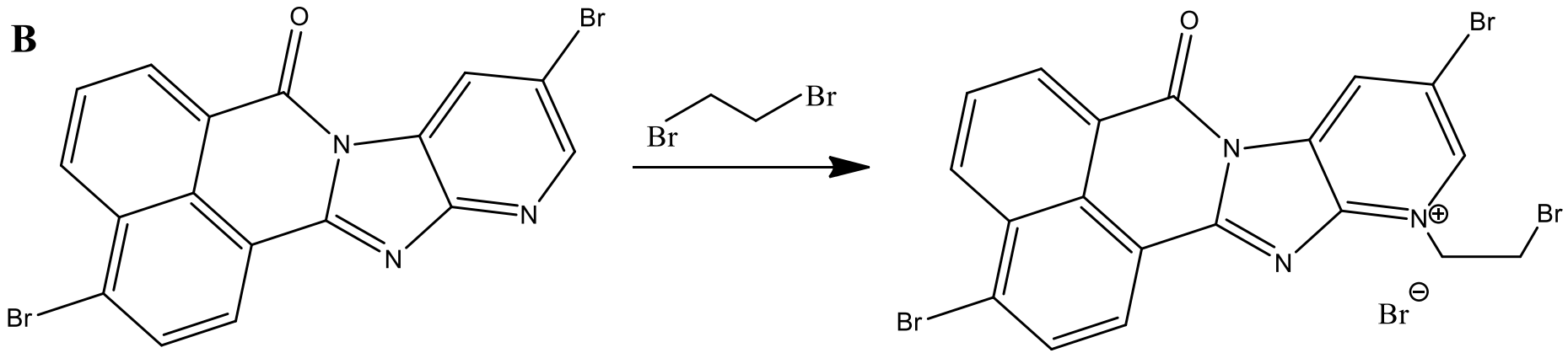
**A**




I II

3,10-dibrom-7H-benzo[de]pirido[2’,3’:4,5]imidazo[2,1-a]izokinolin-7-on

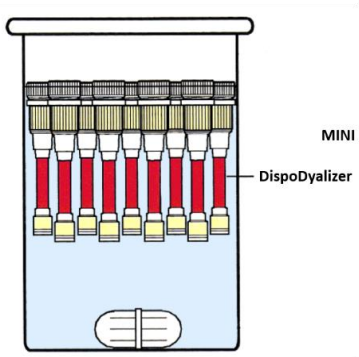
**B**

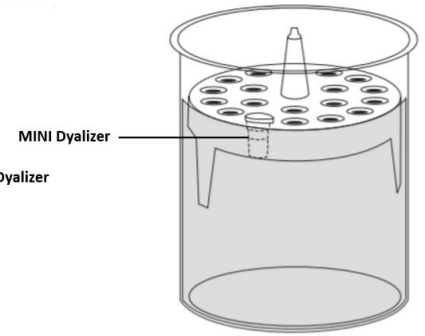


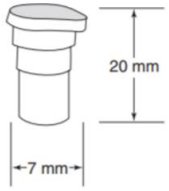
# Metoda kompeticijske dijalize

## MATERIJALI

JEDINICE ZA DIJALIZU



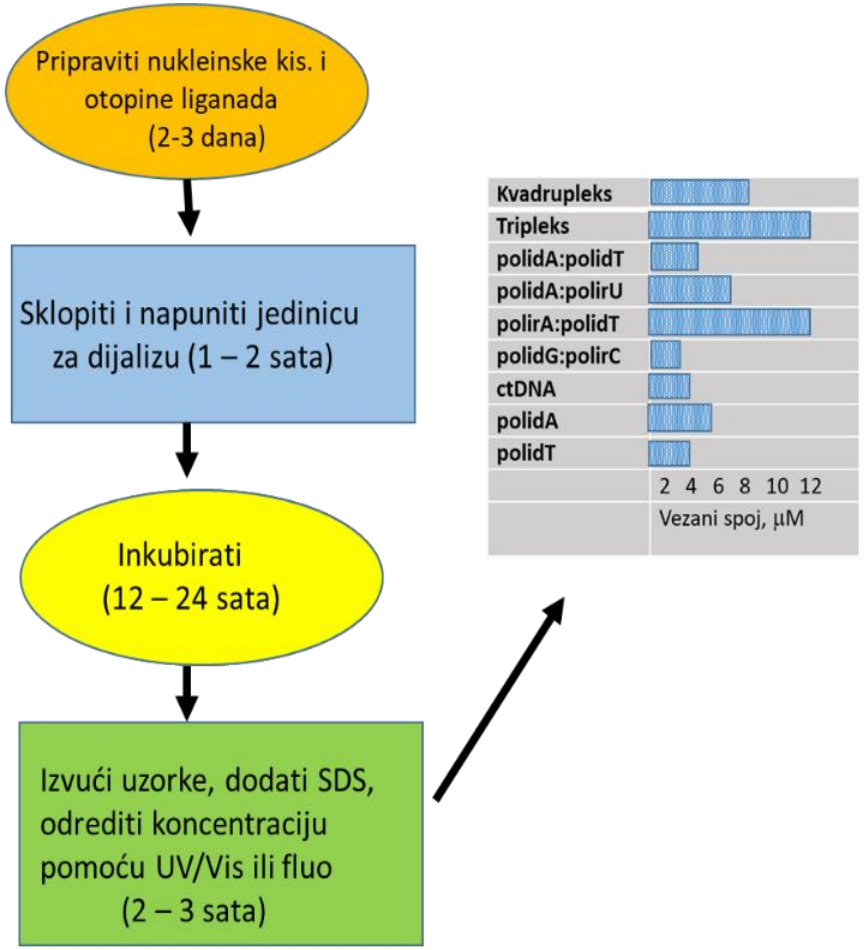




NUKLEINSKE KISELINE

LIGANDI


## METODA




P. A. Ragazzon, N. C. Garbett, J. B. Chaires, *Methods* **42** (2007) 173–182.
J. B. Chaires, *Top Curr Chem* **253** (2005) 33–53.
R. T. Wheelhouse, J. B. Chaires, *Methods Mol Biol.* **613** (2010) 55–70.
https://www.selectscience.net/products/spark-multimode-microplate-reader/?prodID=206495

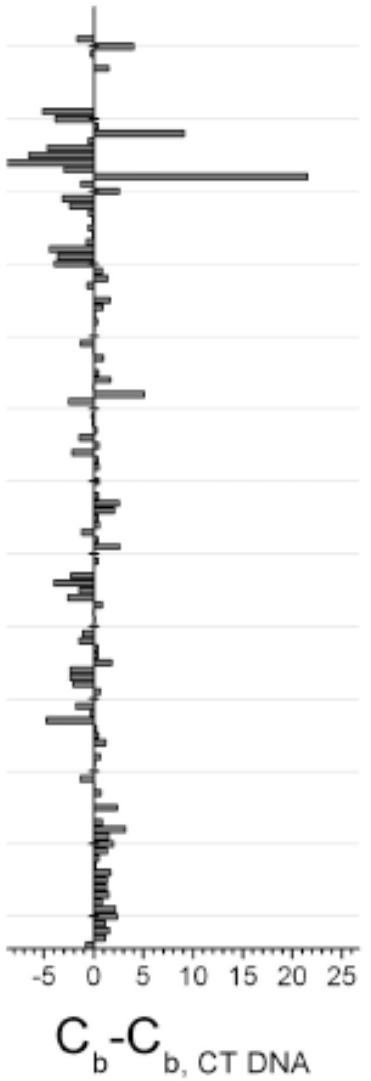
# Obrada rezultata

## Origin software

$K_{app} = C_b/\{ C_f \times ([DNA]_{total} - C_b)\}$

$C_b = C_t - C_f$
$c_t$ - ukupna koncentracija spoja;
$C_f$ - koncentracija slobodnog liganda

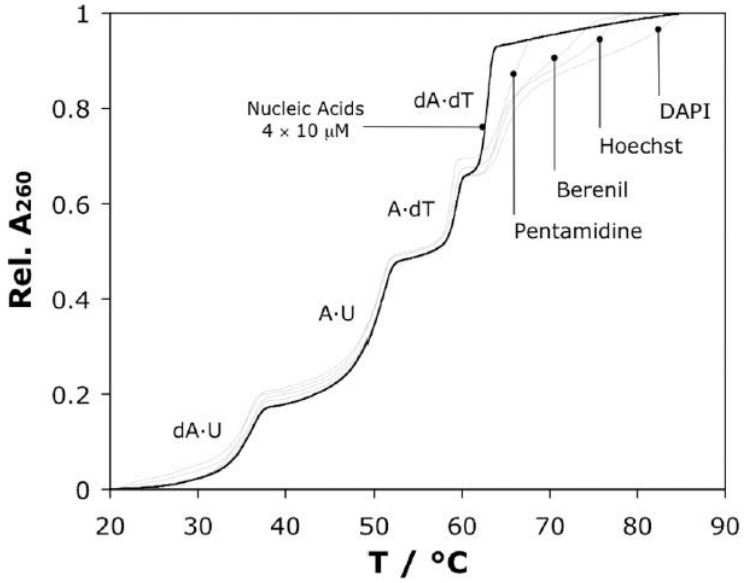
| **spoj** | **$K_{app}$ ($M^{-1}$)** | | **$C_b$ (μM)** | |
|---|---|---|---|---|
| | G-kvadrupleks | ds-DNA | G-kvadrupleks | ds-DNA |
| derivat 2 | $1{,}82 \times 10^4$ | $0{,}23 \times 10^4$ | 4,64 | 1,08 |




R. T. Wheelhouse, J. B. Chaires, *Methods Mol Biol.* **613** (2010) 55–70.

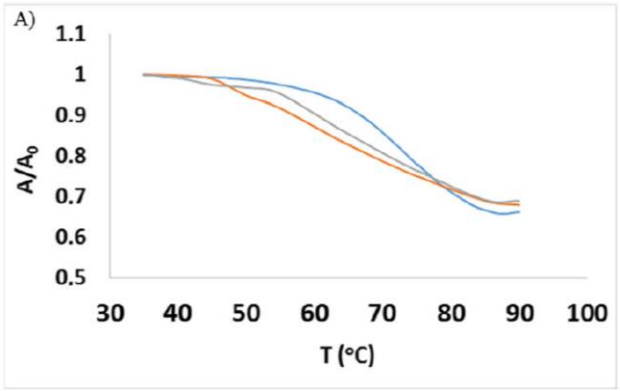
# Temperaturno mekšanje

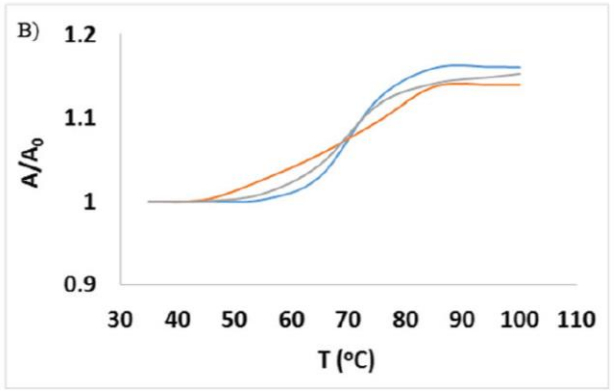
## MATERIJALI


## OBRADA REZULTATA

Origin software




| spojevi | $T_m$ za dsDNA (°C) | Δ $T_m$ (°C) | $T_m$ za G-kvadrupleks (°C) | Δ $T_m$ (°C) |
|---|---|---|---|---|
| - | 65 | - | 64,5 | - |
| 1 | 67 | 2 | 69 | 4,5 |
| 2 | 68,5 | 3,5 | 74 | 9,5 |

http://www.kefo.hr/optical-instruments-and-microscopes

R. T. Wheelhouse, J. B. Chaires, *Methods Mol Biol.* **613** (2010) 55–70.

# Metoda izmještanja fluorescentnog interkalatora

fluorescentne komponente

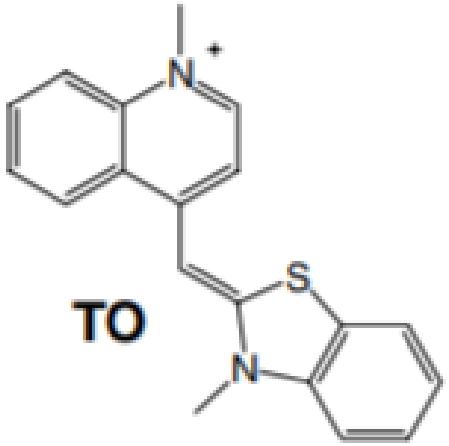
interkalatori

molekule koje se vežu u mali utor

Hoechst 33258

tiazol-narančasto

etidij bromid

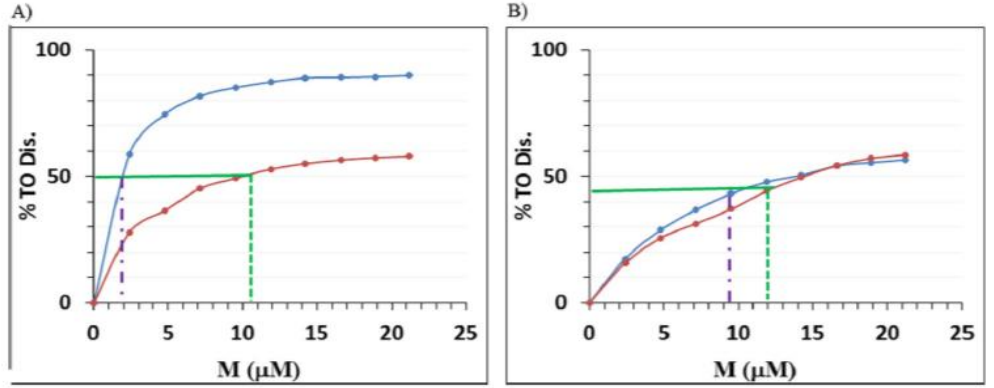




OBRADA REZULTATA

$$\%\mathrm{FID} = 100 - \left(100 \times \frac{F}{F_0}\right)$$
$$F = F_{(\mathrm{Ligand+DNA+TO})} - F_{(\mathrm{Buffer+TO})} - F_{(\mathrm{DNA+Ligand})}$$
$$F_0 = F_{(\mathrm{DNA+TO})} - F_{(\mathrm{Buffer+TO})}$$

| spoj | $DC_{50}$(μM) | | selektivnost (dsDNA/G-kvadrupleks) |
|---|---|---|---|
| | dsDNA | G-kvadrupleks | |
| **1** | 11,9 | 10,51 | 1,13 |
| **2** | 9,51 | 1.91 | 4,98 |

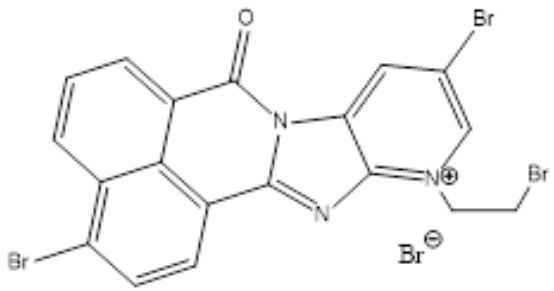




D. Monchaud, C. Allain, M. P. Teulade-Fichou, *Bioorganic & Medicinal Chemistry Letters* **16** (2006) 4842–4845.
https://www.somatco.com/fluorimeter.htm
R. del Villar-Guerra, R. D. Gray, J. O. Trent, J. B. Chaires, *Nucleic Acid Res.* **46** (2018) 1-10.

# Zaključak

- jednostavnost i kratko vrijeme izvođenja metoda
- svim metodama pokazano da **derivat 2** bolje stabilizira i selektivniji je na G-kvadrupleks
- može istisnuti interkalator TO pri nižim koncentracijama



HVALA NA PAŽNJI!