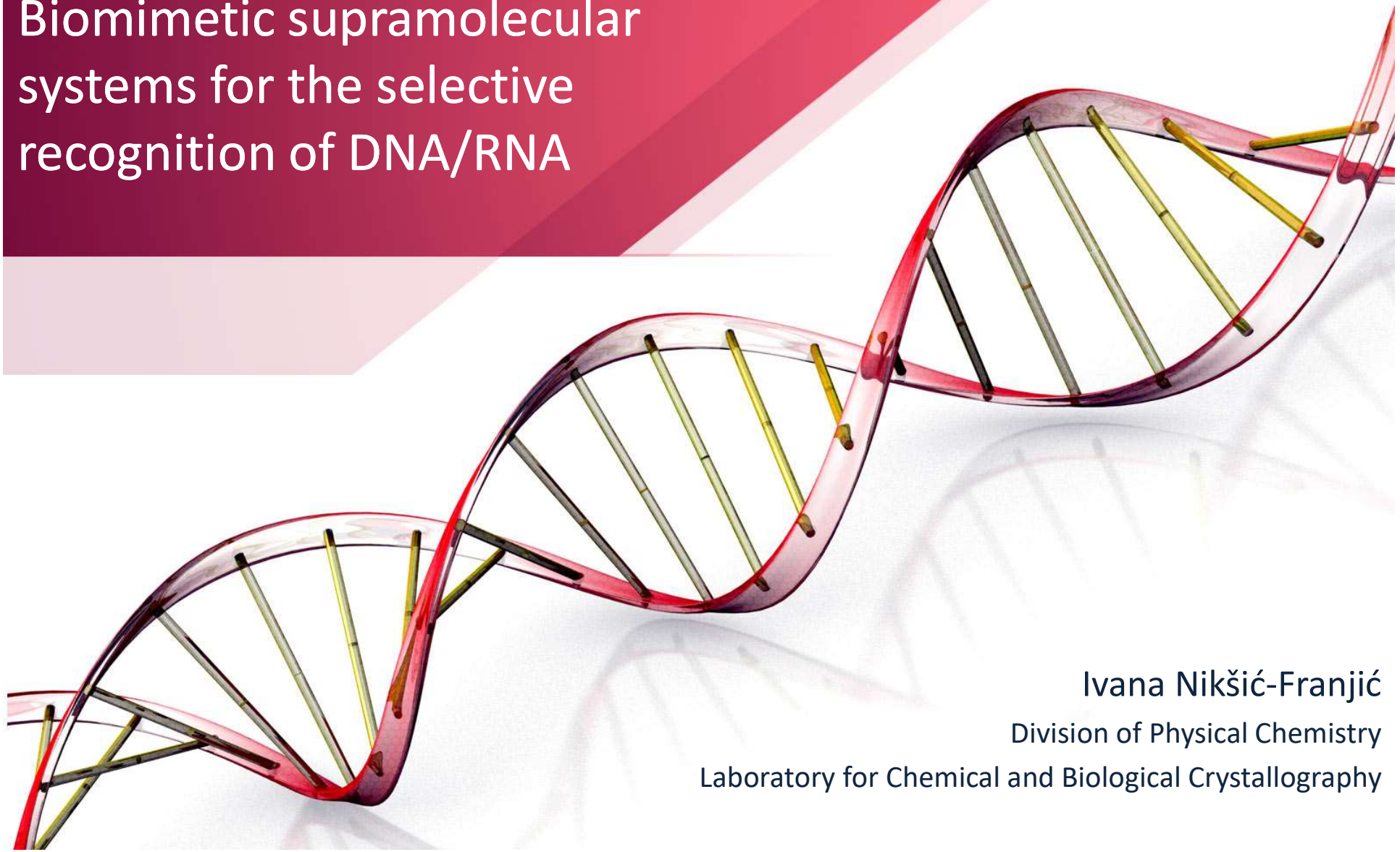
Biomimetic supramolecular systems for the selective recognition of DNA/RNA



Ivana Nikšić-Franjić

Division of Physical Chemistry

Laboratory for Chemical and Biological Crystallography

CalixDNA project

- **CalixDNA project** - Biomimetic „funnel" and „bowl" supramolecular systems for selective recognition of DNA/RNA

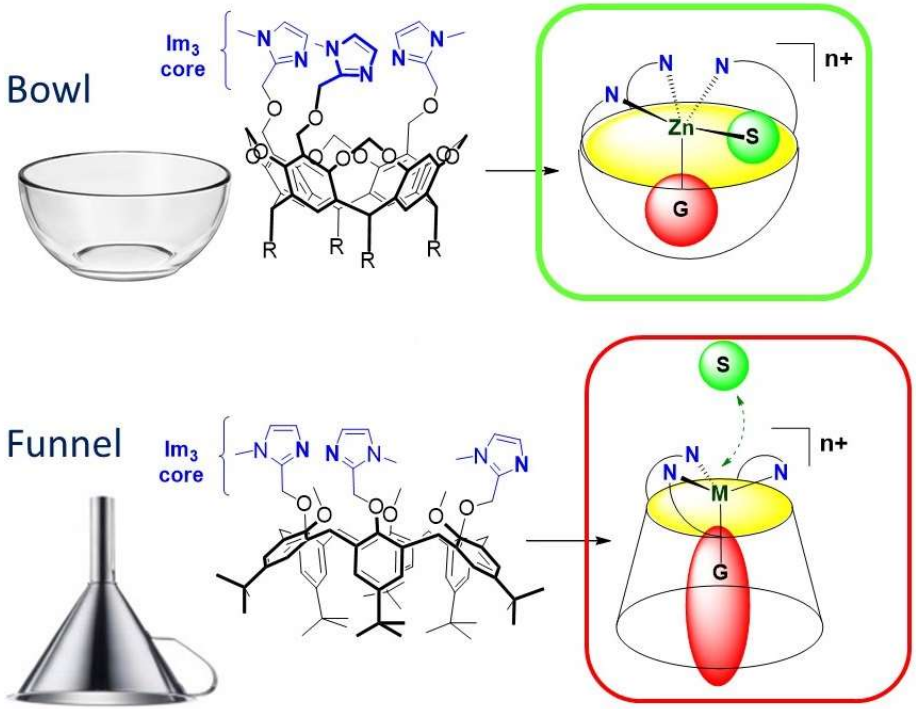


Figure 1. Examples of our supramolecular binders: resorcinarene based (up) and calixarene based (down).

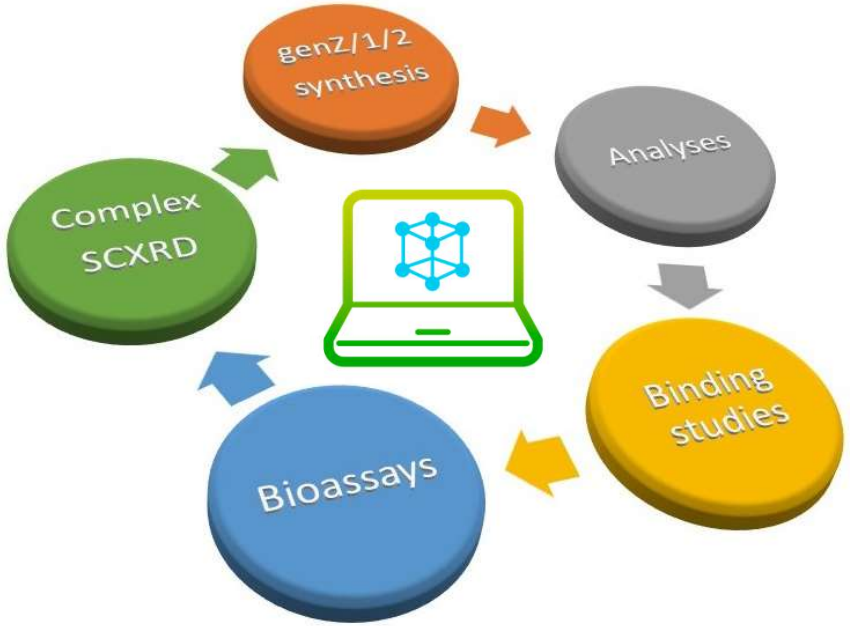


Figure 2. Schematic representation of the calixDNA research cycle.

Results

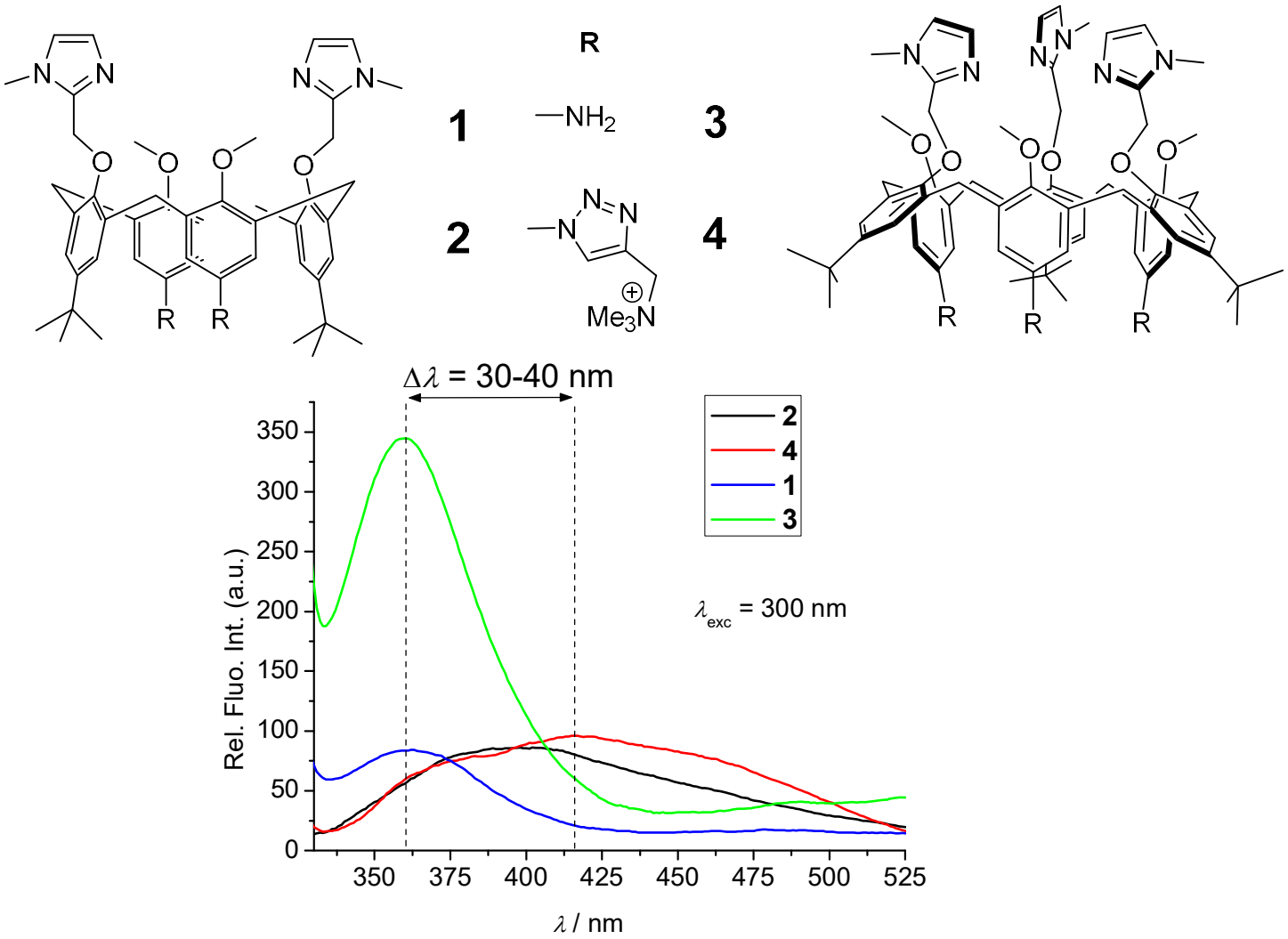
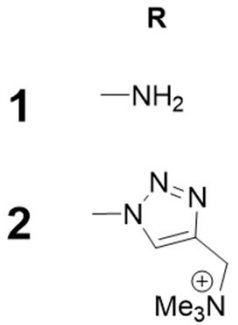


Figure 3. The fluorescence spectra of **1**-**4** (c = 2 × 10^{-6} mol dm^{-3}), λ_{exc} = 300 nm, at pH 7.0, sodium cacodylate, I = 0.05 mol dm^{-3}.

I. Krošl, E. Otković, I. Nikšić-Franjić, B. Colasson, O. Reinaud, A. Višnjevac and I. Piantanida, **Impact of positive charge and ring-size on the interaction of calixarenes with DNA, RNA and nucleotides**, *New J. Chem.*, 2022, **46**, 6860-6869.

Results



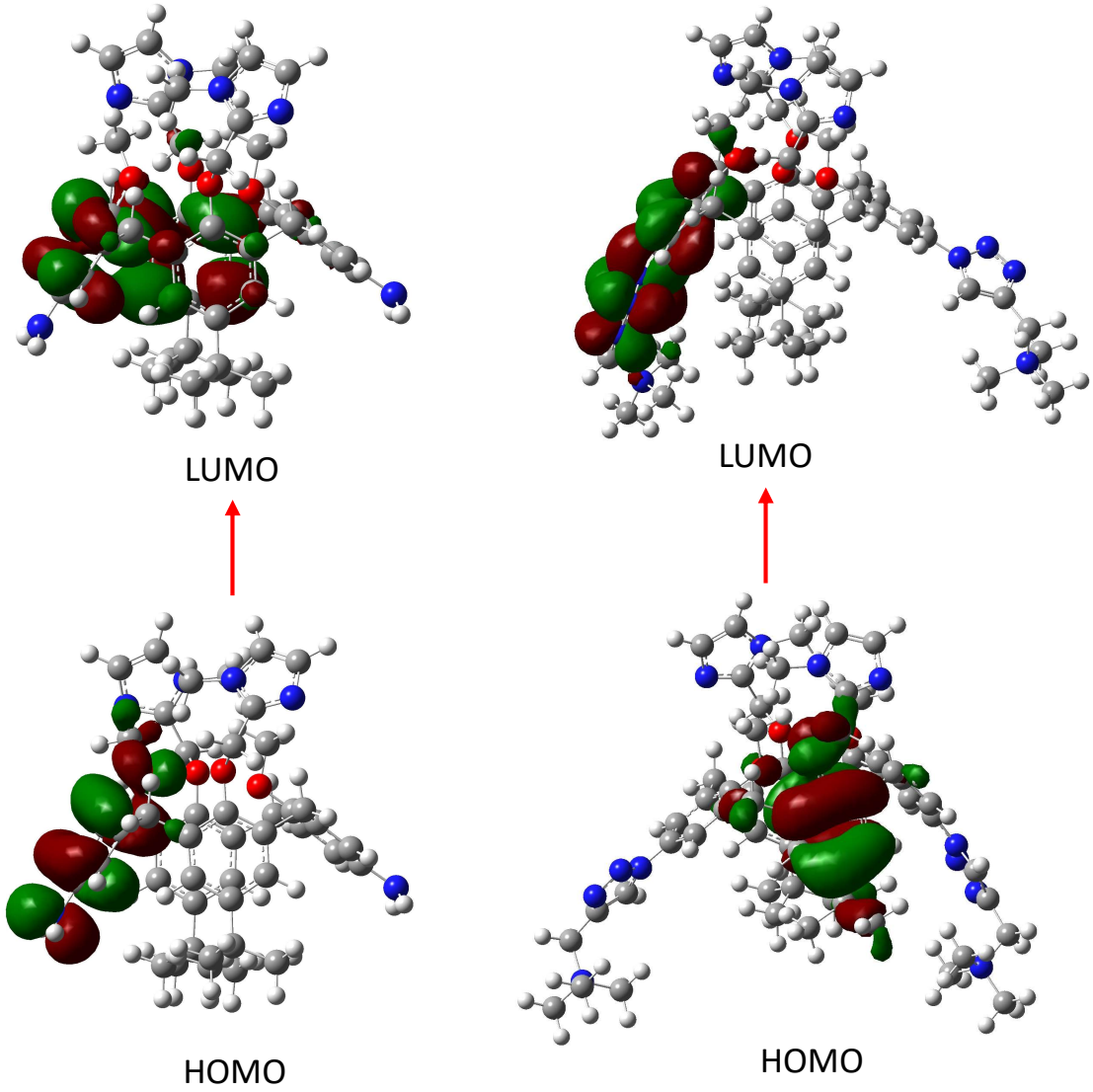


Figure 4. The optimized excited state structures of calix[4]arene **1** (left) and **2** (right) and the emission mechanisms predicted with TD-DFT/B3LYP/6-31G(d).

I. Krošl, E. Otković, I. Nikšić-Franjić, B. Colasson, O. Reinaud, A. Višnjevac and I. Piantanida, **Impact of positive charge and ring-size on the interaction of calixarenes with DNA, RNA and nucleotides**, *New J. Chem.*, 2022, **46**, 6860-6869.

Results

CMP

AMP

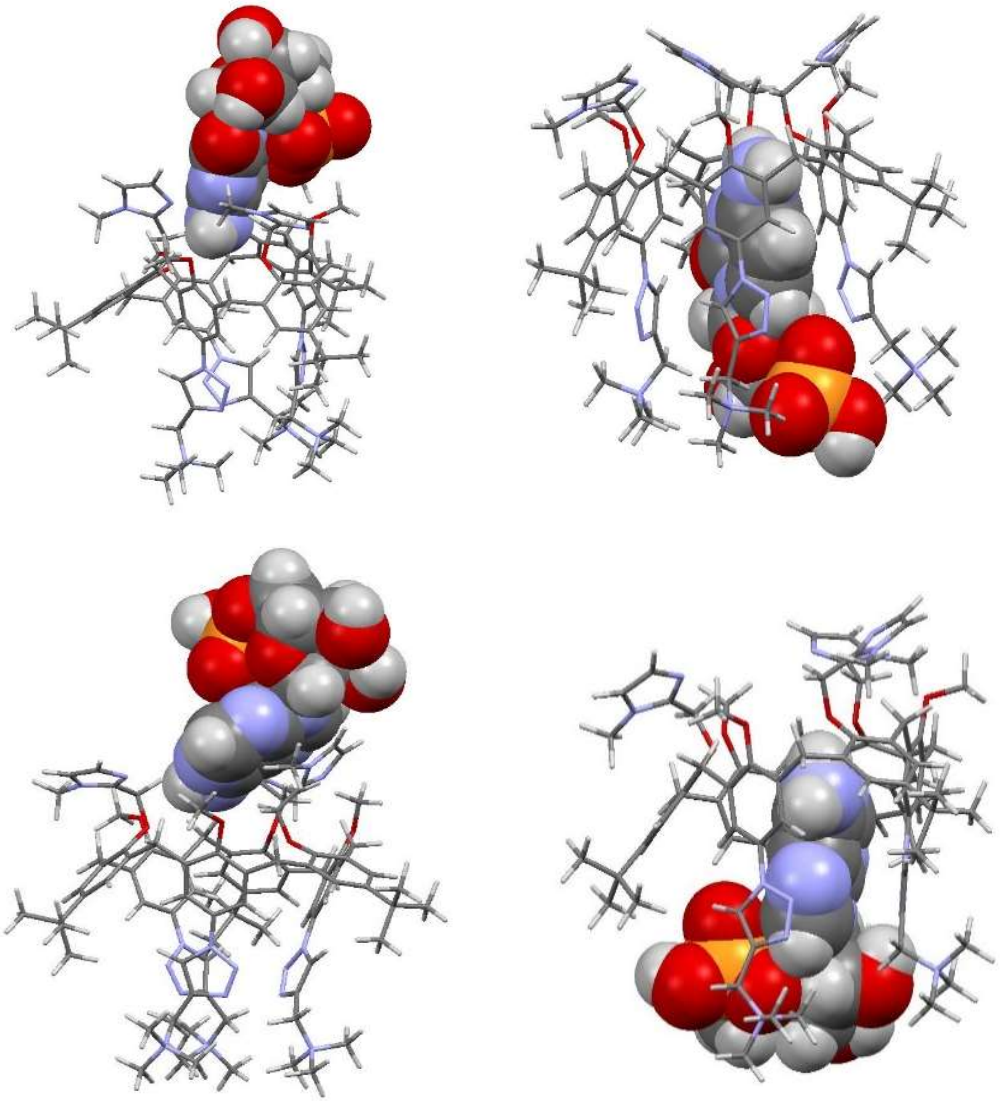
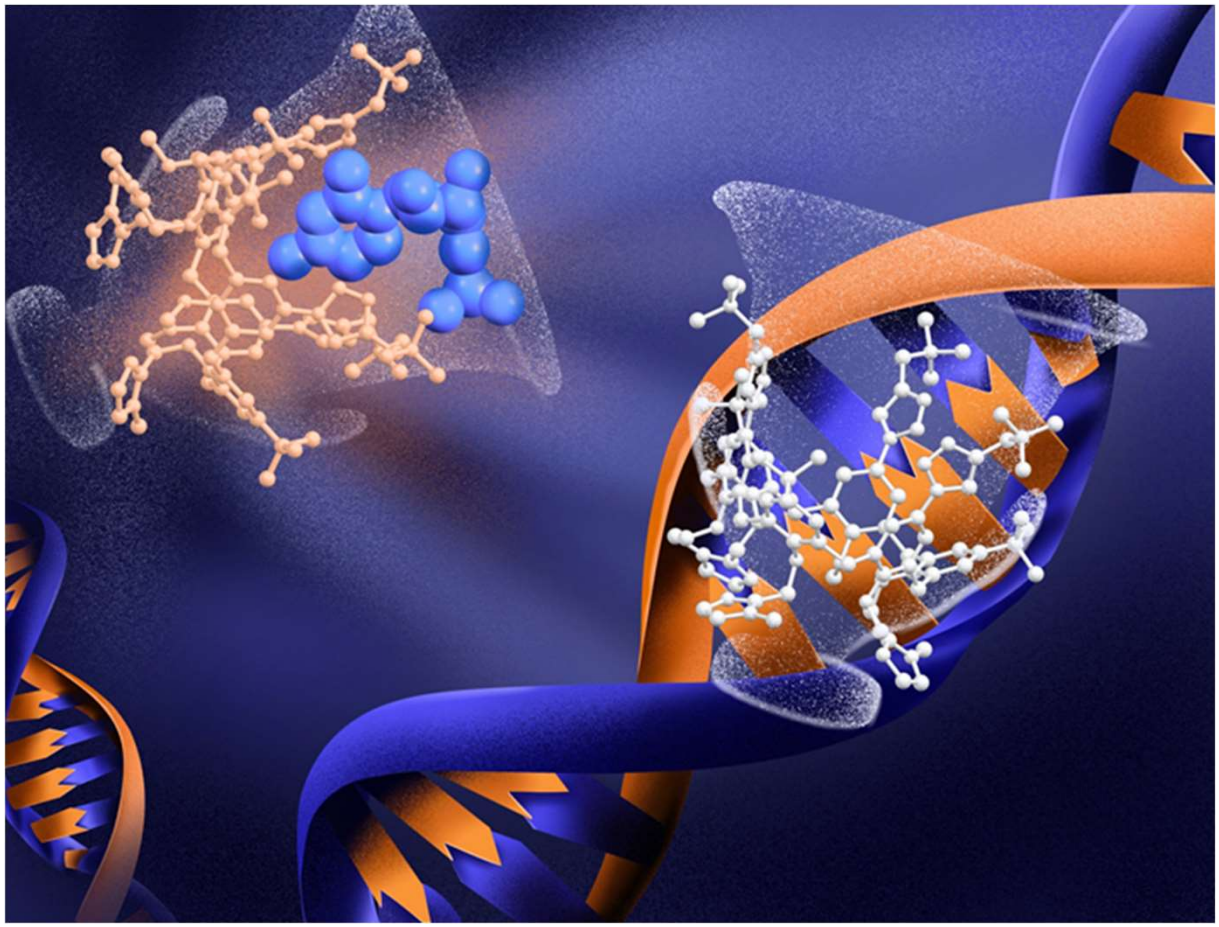


Figure 5. MD simulations of calixarene **4** complexes (capped sticks style) with nucleotides (space fill) inserted between imidazoles (left) and triazoles (right), *T* = 300 K, 10 ns.

I. Krošl, E. Otković, I. Nikšić-Franjić, B. Colasson, O. Reinaud, A. Višnjevac and I. Piantanida, **Impact of positive charge and ring-size on the interaction of calixarenes with DNA, RNA and nucleotides**, *New J. Chem*., 2022, **46**, 6860-6869.

Results

Coverpage of New Journal of Chemistry, 2022, Issue 15

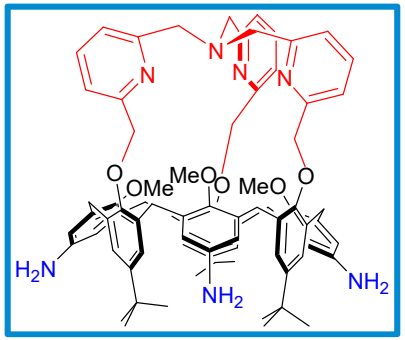


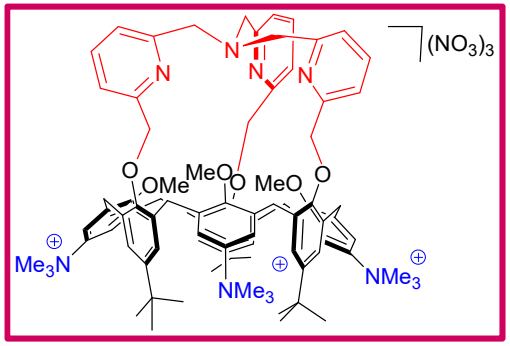
I. Krošl, E. Otković, I. Nikšić-Franjić, B. Colasson, O. Reinaud, A. Višnjevac and I. Piantanida, **Impact of positive charge and ring-size on the interaction of calixarenes with DNA, RNA and nucleotides**, *New J. Chem.*, 2022, **46**, 6860-6869.

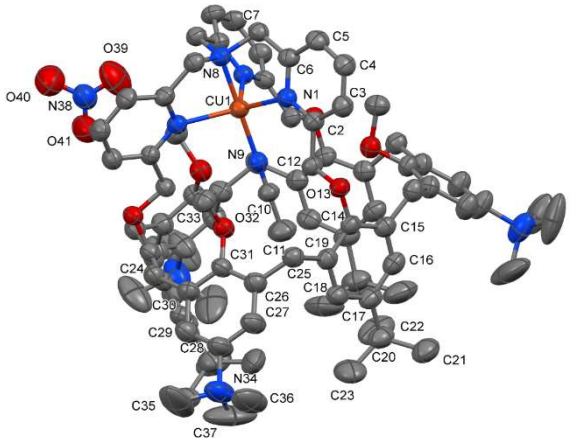
Non-published results

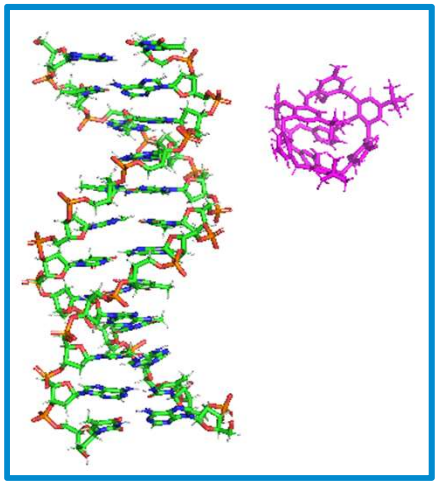
TMPA (Tris(2-pyridylmethyl)amine) capped calixarenes

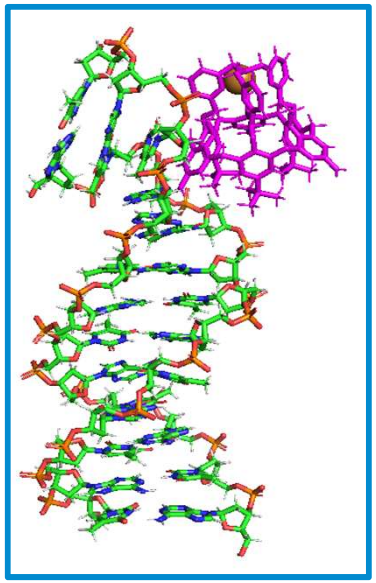
Corentin Thouzé

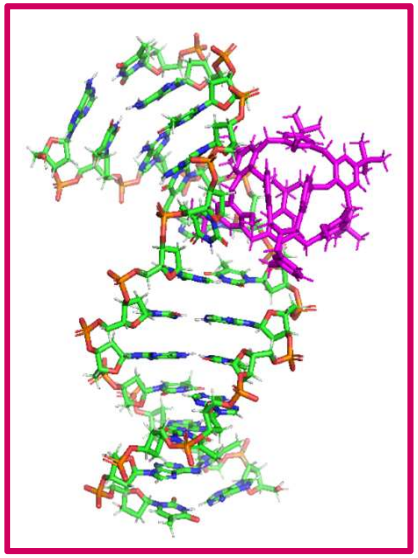












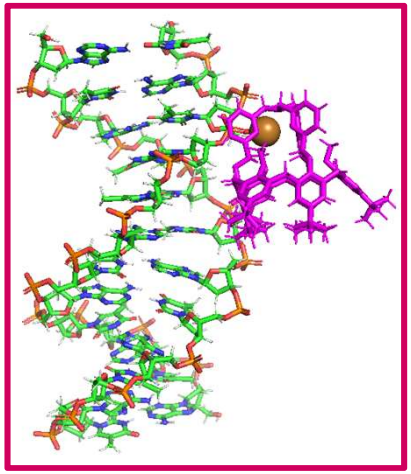
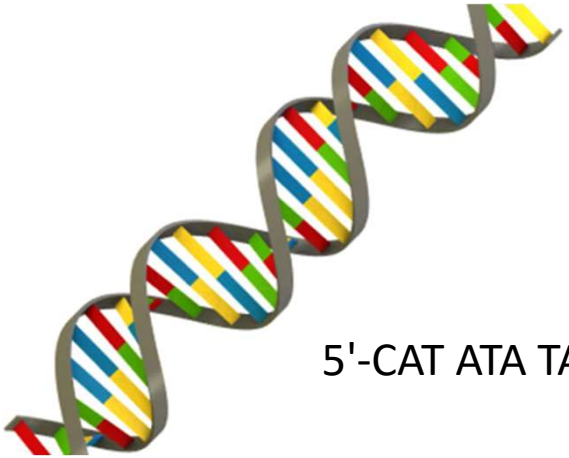


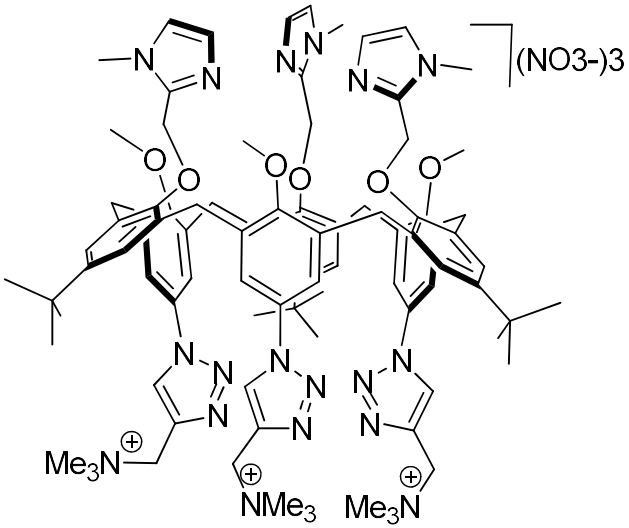
Figure 7. MD simulations of TMPA-capped calixarenes and Cu^{2+}-complexes with $p(dAdT)_2$ at T = 300 K after 50 ns. Water molecules and Na^+ ions are omitted for clarity.

Crystallization experiments

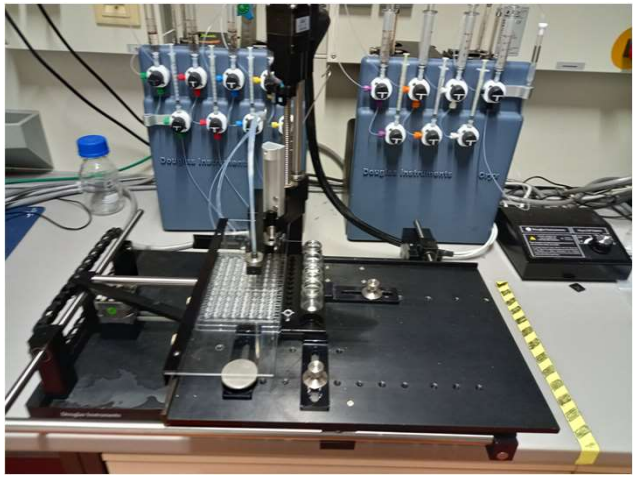


5'-CAT ATA TAT G-3'

Institute for Biomolecular Sciences, Graz, Austria



4, X6Me_3ImMe_3(TZMeNMe_3^+)$_3$

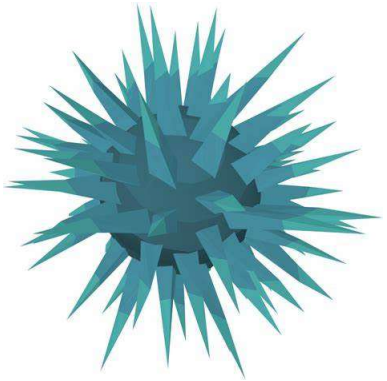


Oryx 8, crystallization robot

Crystallization experiments

Observation	Crystallization screens
No crystals	Morpheus III, SG1Eco
No diffraction of crystals	JCSG+, Morpheus I
Salt crystals	Index, Memstart + Memsys, Wizard Classic 1+2
Crystal precipitate	Midas
„Sea urchin” crystals	Morpheus II

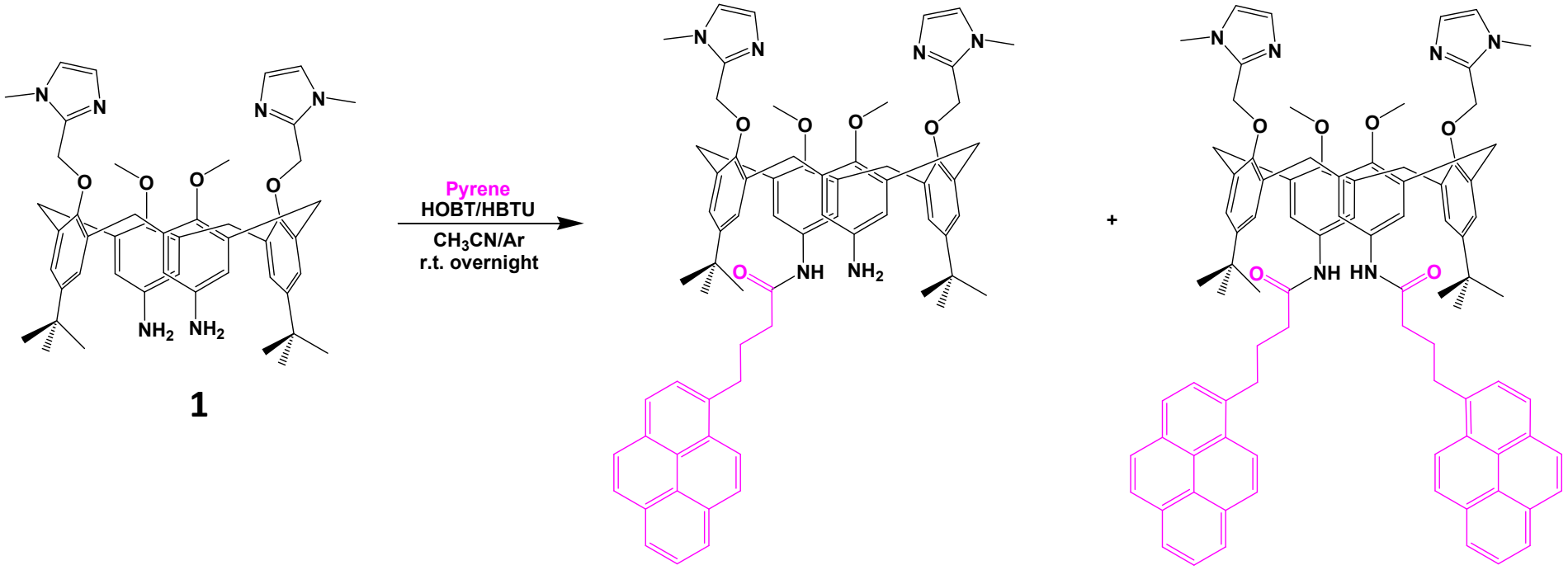
Morpheus II screen	Salt	Buffer	Precipitate
H5	-	0.1 M BES/TEA pH 7.5, 0.01 M polyamine	15 % (w/v) PEG 3K, 20 % (v/v) 1,2,4-butanetriol, 1 % (w/v) NDSB 256
H6	-	0.1 M BES/TEA pH 7.5, 0.01 M polyamine	12.5 % (w/v) PEG 4K, 20 % (v/v) 1,2,6-hexanetriol



Pyrene-calix[4]arene Derivatives as Highly Sensitive Sensors for Cations, Nucleotides, DNA and RNA

Ivana Nikšić-Franjić, Benoit Colasson, Olivia Reinaud, Aleksandar Višnjevac, Ivo Piantanida and Dijana Pavlović Saftić

NEW SYNTHESIS



1

5: *mono*-pyrene

6: *bis*-pyrene

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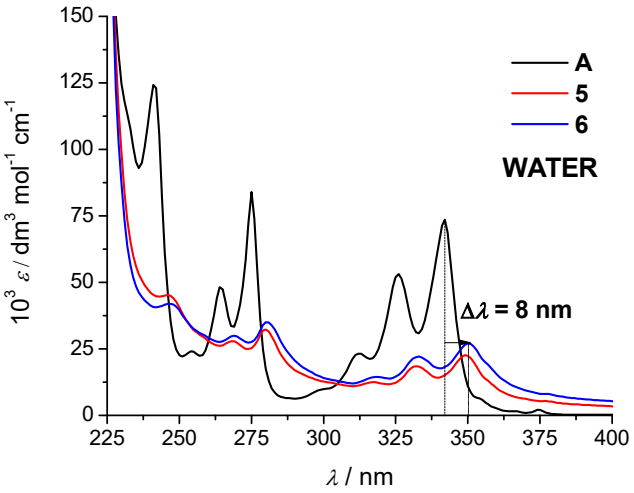
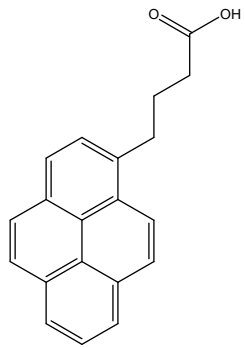
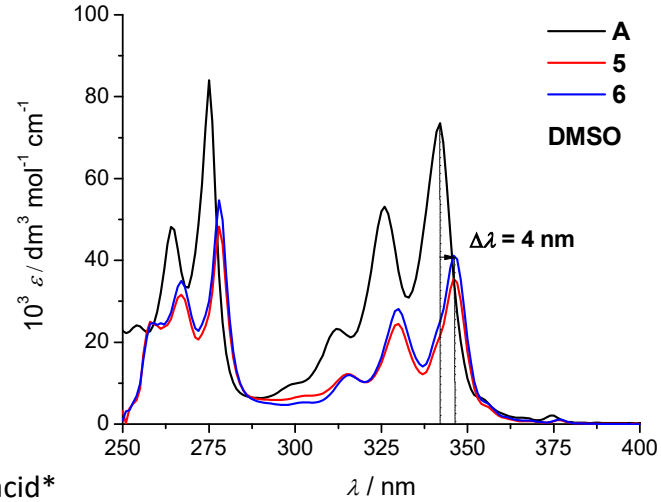
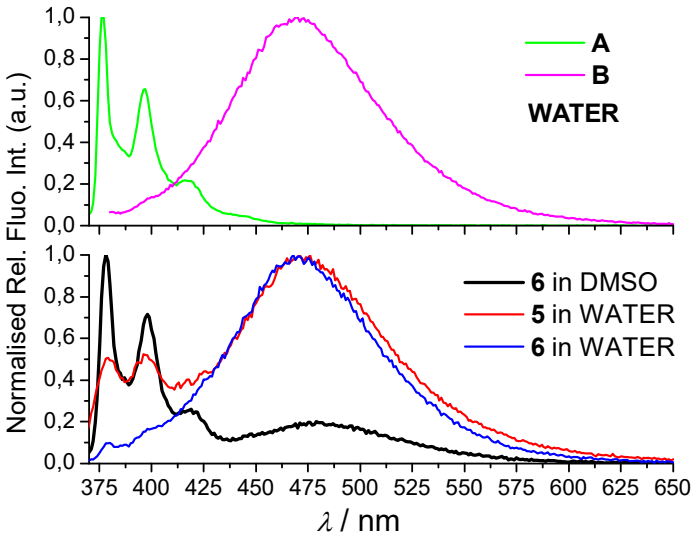
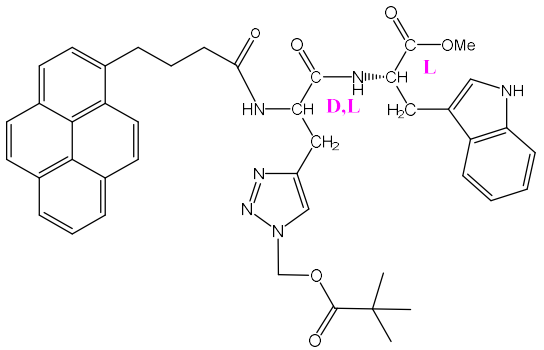




A = 1-pyrenebutyric acid*







B = PyrL-Ala(Triazole-R)-Trp*

* I. Krošl, M. Košćak, K. Ribičić, B. Žinić, D. Majhen, K. Božinović and I. Piantanida, *Int. J. Mol. Sci*. 2022, **23**, 7006.

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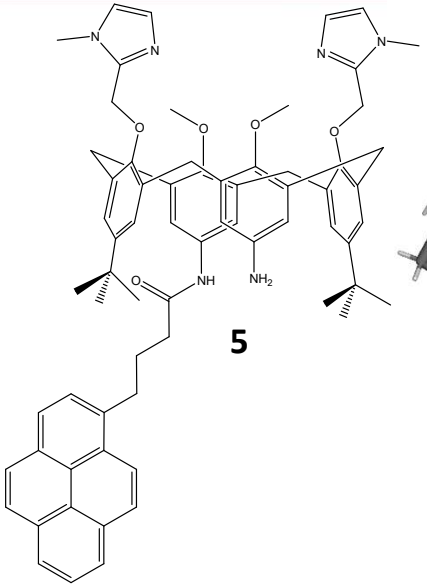
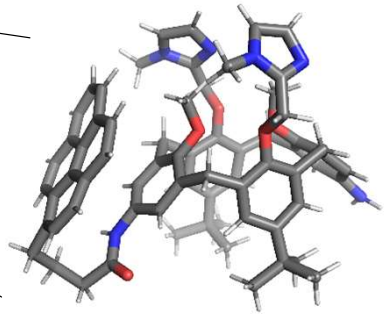
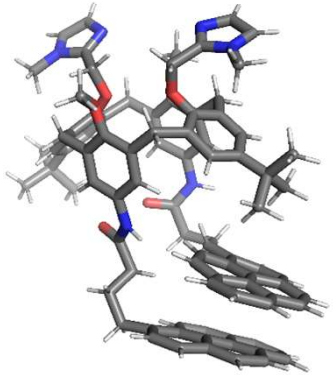
MOLECULAR DYNAMICS SIMULATIONS

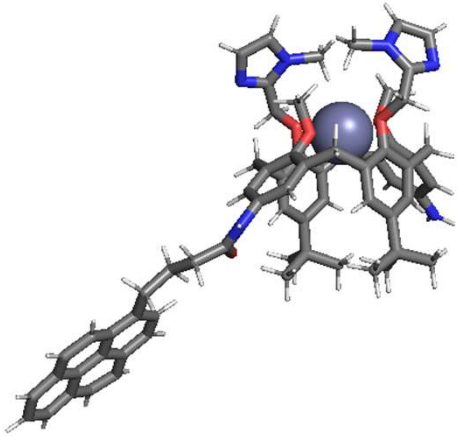


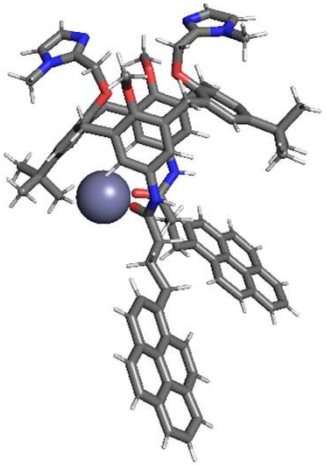
5



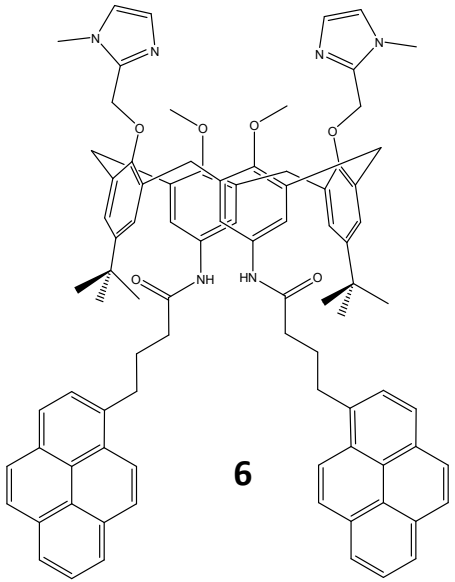


(a) calixarene **5** (left) and **6** (right),





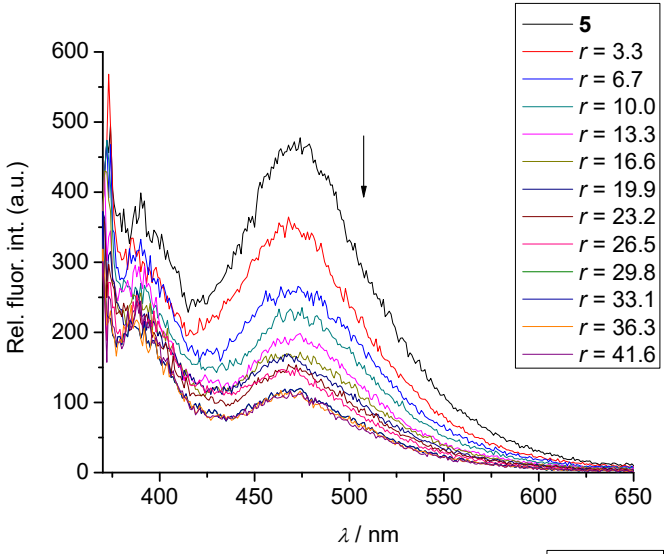
(b) metal complexes: Zn**5** $^{2+}$ (left) and Zn**6**$^{2+}$ (right).



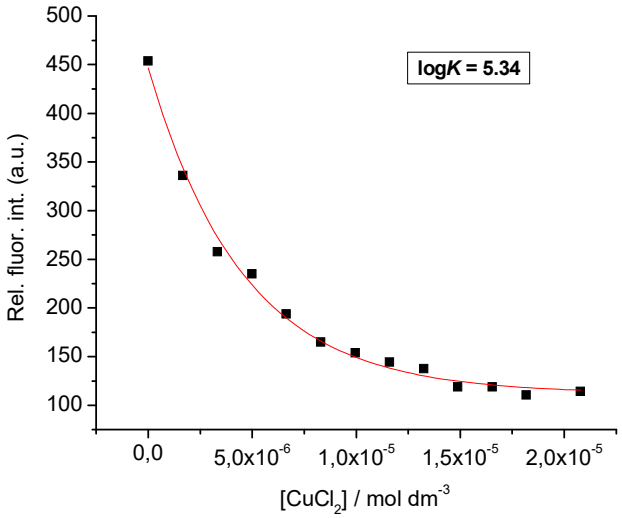
6

Figure 8. Structures of: (a) calixarenes **5** and **6**; and (b) their Zn^{2+}-complexes; after 10 ns at *T* = 300 K. Water molecules are omitted for clarity.

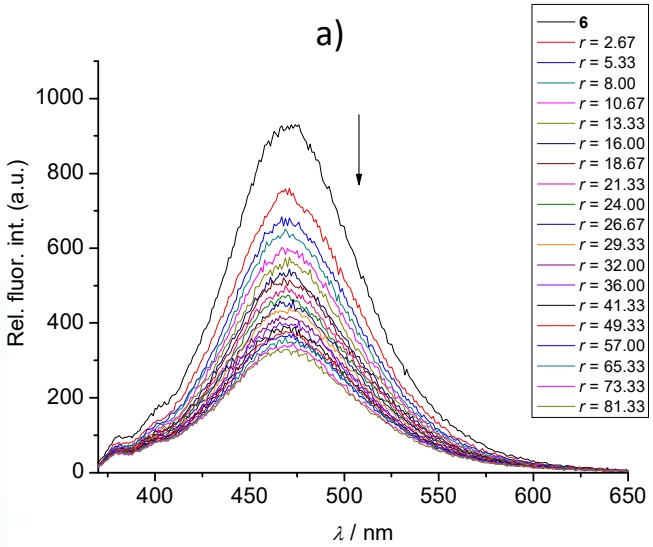
INTERACTIONS WITH CATIONS



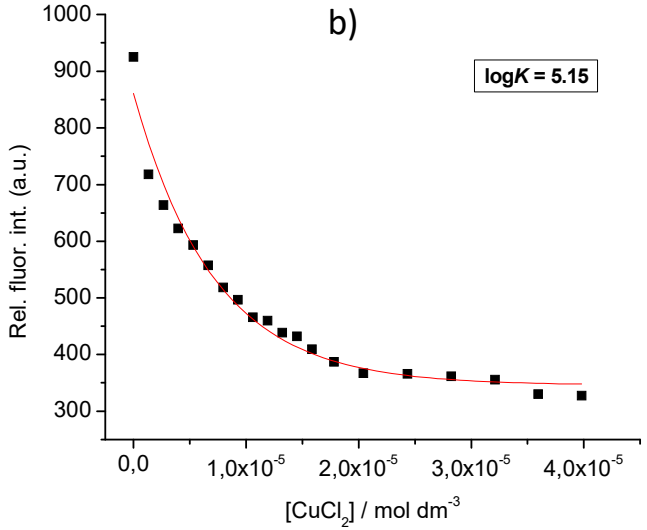
a)



b)



c)



d)

Figure 9. a) Fluorimetric titration of **5** (a) and **6** (c) ($c = 5 \times 10^{-7}$ mol dm^{-3}; λ_{exc}= 350 nm) with $CuCl_2$ ($c = 2 \times 10^{-3}$ mol dm^{-3}) and dependence of fluorescence at λ_{max}= 475 nm on $c(CuCl_2)$ for **5** (b) and **6** (d). Done in water; r = [$CuCl_2$] / [compound].

Pyrene-calix[4]arene Derivatives as Highly Sensitive Sensors for Cations, Nucleotides, DNA and RNA

Ivana Nikšić-Franjić, Benoit Colasson, Olivia Reinaud, Aleksandar Višnjevac, Ivo Piantanida and Dijana Pavlović Saftić

Table 1. Binding stability constants (log*K*) and emission quenching efficiency (ΔInt[a], in parentheses) for **1**, **5** and **6** with cations determined fluorimetrically. Done in water.

Cation	Anion	1	5	6
Cu^{2+}	Cl^-	5.04 (0.3)	5.34 (0.3) 6.69 (0.3)*	5.15 (0.4) 6.49 (0.1)** 7.52 (0.12)*
Cu^{+}	I^-	> 8 (1.4)	5.74 (0.3)	5.58 (0.5)
Cd^{2+}	Cl^-	4.15 (1.2)	6.17 (0.4)	6.12 (0.6)
Co^{2+}	Cl^-	3.76 (3)	6.88 (0.8)	6.92 (0.6)
Ag^{+}	NO_3^-	No spectroscopic change	7.29 (0.6)	7.04 (0.6)
Zn^{2+}	Ac^-	6.30 (2.5)	7.76 (0.6)	7.77 (0.6) 7.61 (0.6)*
Zn^{2+}	Cl^-		-	7.84 (0.6)
Hg^{2+}	Cl^-	6.80 (4)	8.01 (0.6)	7.80 (0.7)

[a] ΔInt = Int (100% complex)/Int_0

*measured in sodium cacodylate buffer (pH = 7.0, *I* = 0.05 mol dm^{-3})

**measured in NaCl aqueous solution (*I* = 0.05 mol dm^{-3})

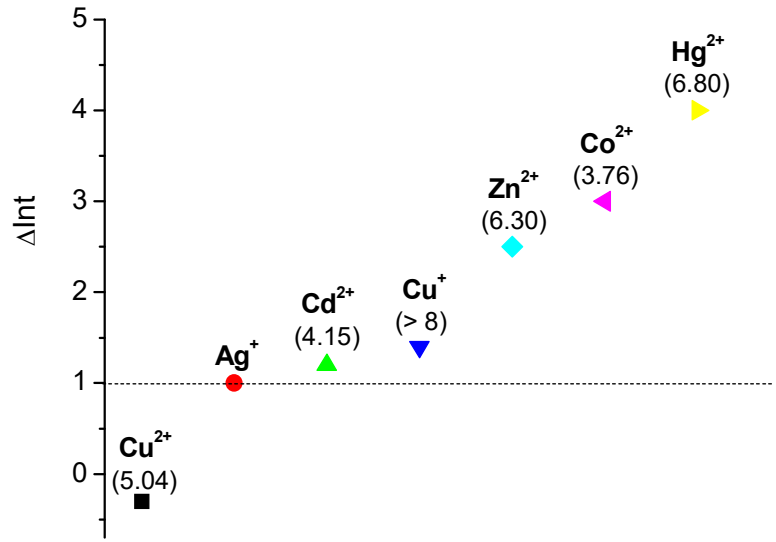


Figure 10. Comparison of relative intensity values (ΔInt = Int(100% complex)/Int_0) from fluorimetric titrations of **1** with different cations in water. Stability constants (log*K*) are given in parentheses.

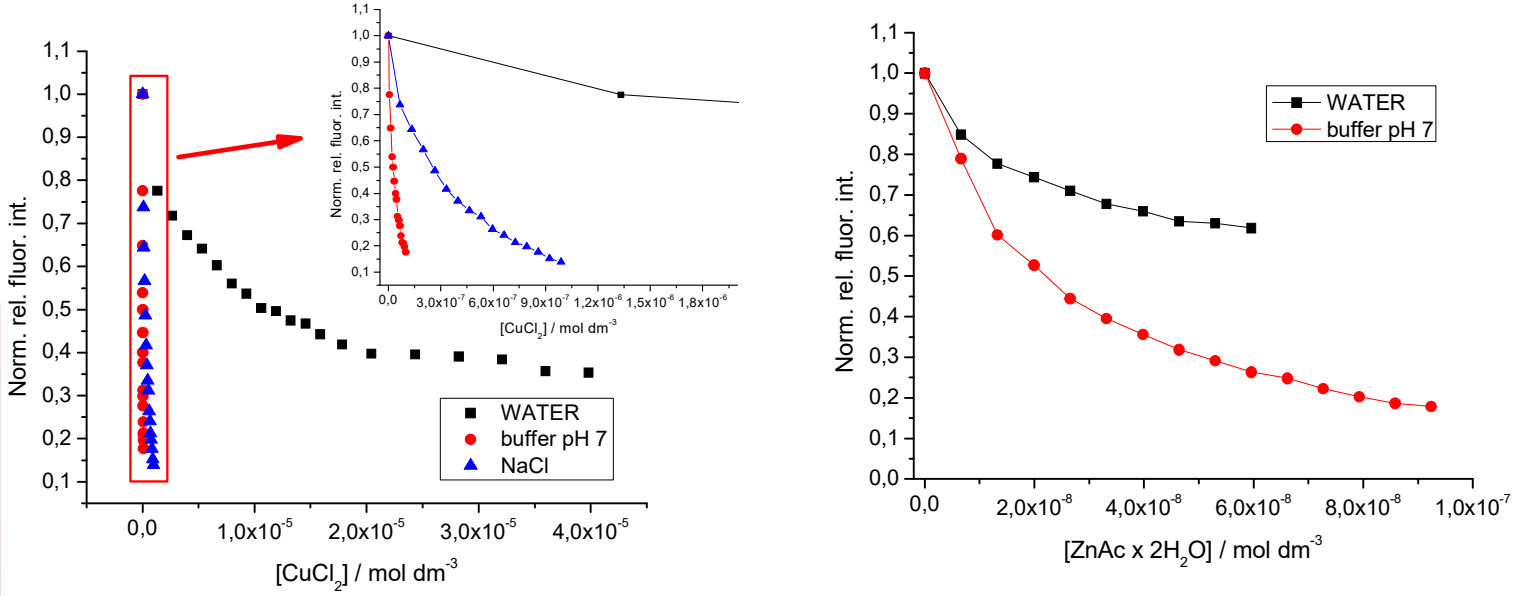


Figure 11. Fluorimetric titration of **6** ($c = 5 \times 10^{-7}$ mol dm^{-3}; λ_{exc}= 350 nm) with: a) $CuCl_2$ ($c = 10^{-4}$ mol dm^{-3}) and b) ZnAc × $2H_2O$ ($c = 10^{-5}$ mol dm^{-3}) in water (■), buffer pH = 7, I = 0.05 mol dm^{-3} (●) and NaCl aqueous solution, I = 0.05 mol dm^{-3} (▲).

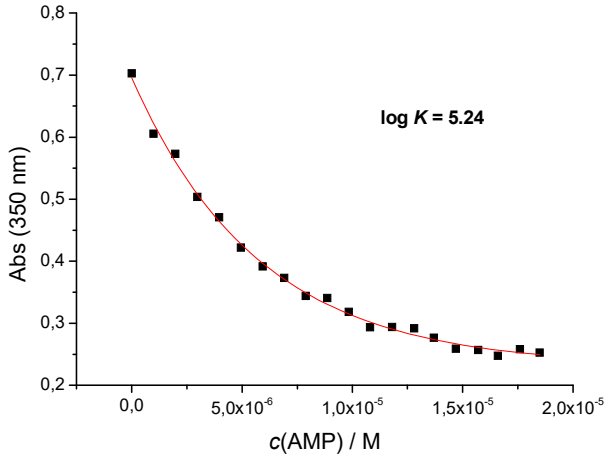
INTERACTIONS WITH MONONUCLEOTIDES AND DNA/RNA

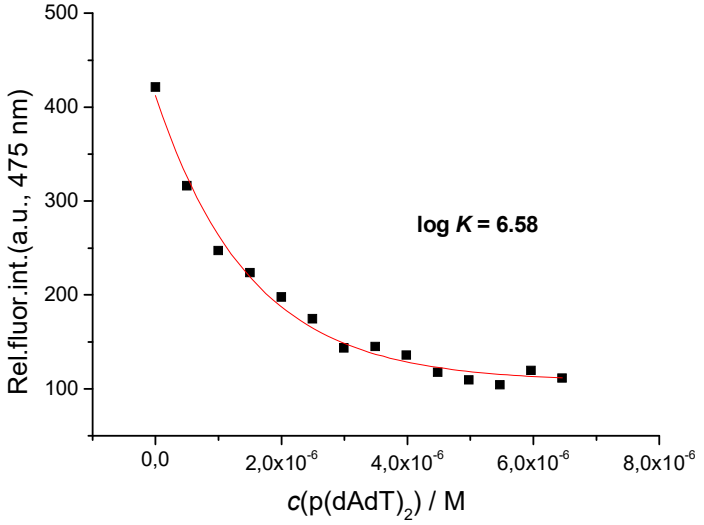
Marine Legras

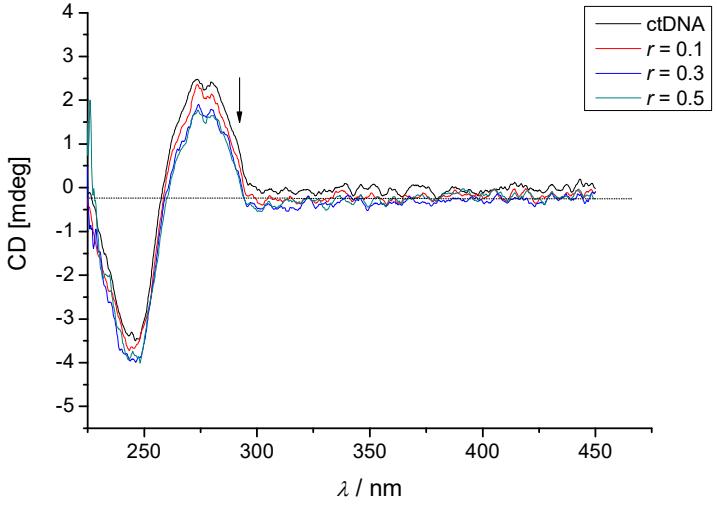
Malorie Wallet

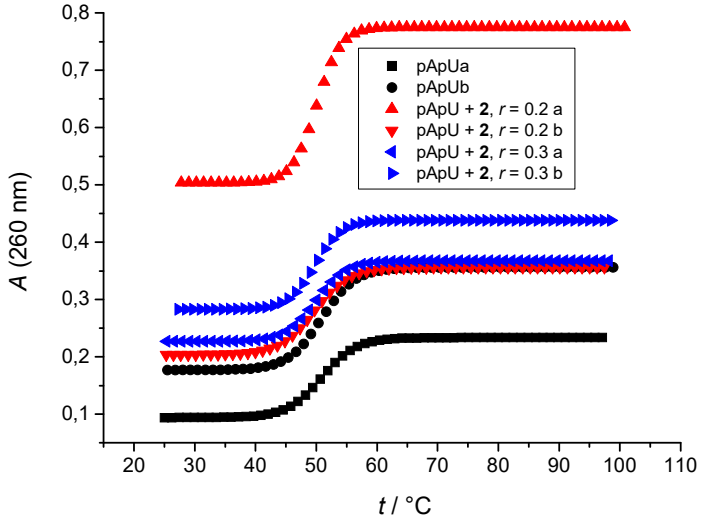
AMP
UMP
CMP
GMP

ctDNA
$p(dAdT)_2$
$p(dGdC)_2$
pApU









INTERACTIONS WITH MONONUCLEOTIDES AND DNA/RNA: STABILITY CONSTANTS

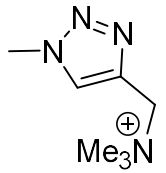
Table 2. Binding stability constants (log*K*) and emission quenching efficiency (ΔInt, in parentheses) for **1**, **2**, **5** and **6** with mononucleotides and ds-DNA/ds-RNA determined spectrophotometrically and fluorimetrically. Done in sodium cacodylate buffer (pH = 7.0, I = 0.05 mol dm^{-3}).

	1[c]	2[c]	5	6
CMP	-	3.1	6.6[a] 5.8 (0.7)[b]	6.4[a] 5.6 (0.3)[b]
AMP	5.0	4.3	6.3[a] 5.9 (0.7)[b]	6.4[a] 5.6 (0.3)[b]
UMP	4.1	2.5	6.7[a] 5.7 (0.2)[b]	6.6[a] 5.0 (0.7)[b]
GMP	4.7	4.8	6.7[a] 5.8 (0.2)[b]	6.2[a] 5.3 (0.6)[b]
ctDNA	-	5.2	6.7[a] 7.6 (0.3)[b]	6.6[a] 6.2 (0.1)[b]
p(dAdT)$_2$	-	5.0	5.8[a] 7.4 (0.2)[b]	6.3[a] 6.6 (0.3)[b]
pApU	-	5.1	7.8[a] 6.4 (0.2)[b]	7.4[a] 7.2 (0.2)[b]
p(dGdC)$_2$	-	5.0	6.3[a] 5.7 (0.3)[b]	6.1[a] 6.9 (0.2)[b]

[a] UV-VIS at 350 nm
[b] Fluorimetrically
[c] published data: *New J. Chem.*, 2022, **46**, 6860-6869.
ΔInt = Int(100 % complex)/Int_0

1 —NH_2

2



5: *mono*-pyrene

6: *bis*-pyrene

Pyrene-calix[4]arene Derivatives as Highly Sensitive Sensors for Cations, Nucleotides, DNA and RNA

Ivana Nikšić-Franjić, Benoit Colasson, Olivia Reinaud, Aleksandar Višnjevac, Ivo Piantanida and Dijana Pavlović Saftić

THERMAL DENATURATIONS

Table 3. ΔT_m values for ctDNA and pApU with **5** and **6** and their complexes with Cu^{2+} and Zn^{2+} at different ratios. Done in sodium cacodylate buffer (pH=7.0, I = 0.05 mol dm^{-3}); r = [compound] / [DNA/RNA].

		ctDNA	pApU
r = 0.2	**5**	-2	0
	6	-2	-1
r = 0.3	**5**	-	+1
	6	-1	-1
	Cu^{2+}	+2	+2
	Zn^{2+}	0	0
	$Cu\mathbf{5}^{2+}$	-3	+2
	$Cu\mathbf{6}^{2+}$	+1	-1
	$Zn\mathbf{5}^{2+}$	-2	0
	$Zn\mathbf{6}^{2+}$	0	-1

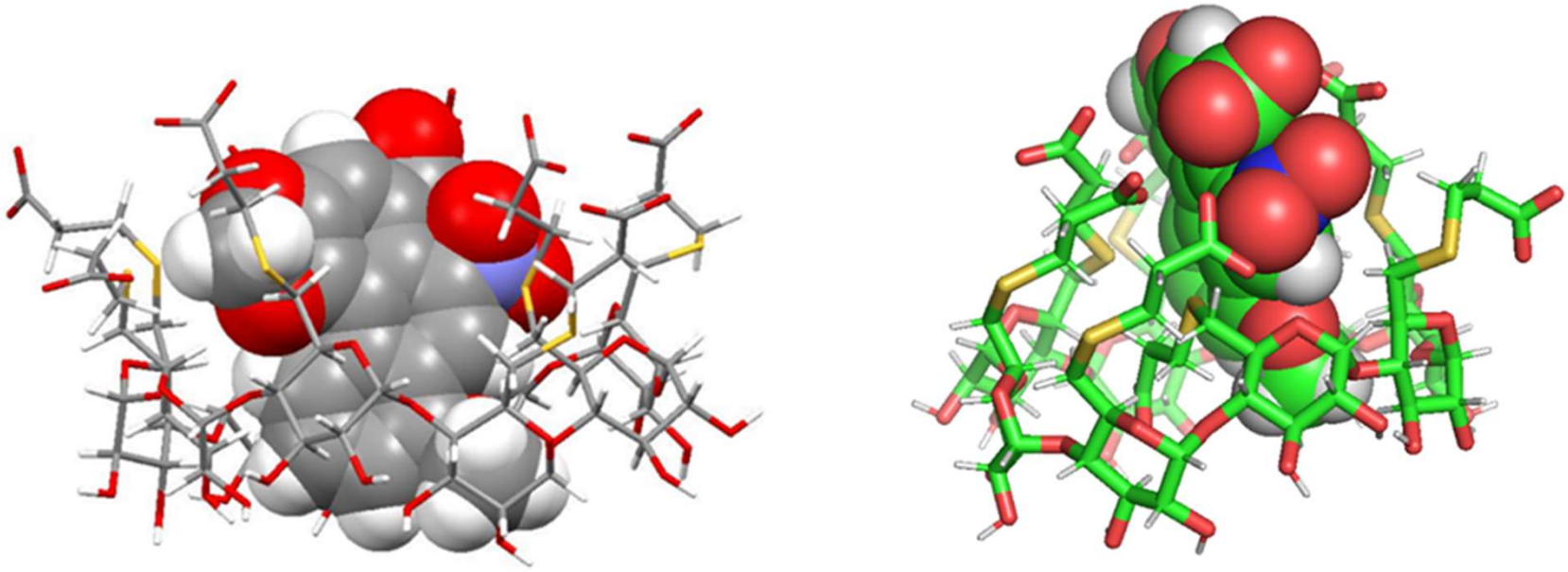
$\Delta T_m = T_{m,poly+compound} - T_{m,poly}$

CIRCULAR DICHROISM

- Conjugates **5** and **6** achiral
- No induced CD bands > 300 nm
- No significant influence of **5** and **6** on helicity of polynucelotides

N. Gazdek, I. Zonjić, I. Nikšić-Franjić, L. Frkanec and I. Piantanida, Competitive binding of aristolochic acid between various cyclodextrins and serum albumin as a model for acute poisoning detoxification, *New J. Chem.*, 2022, **33**, 569-577.

MD SIMULATIONS: CYCLODEXTRINES

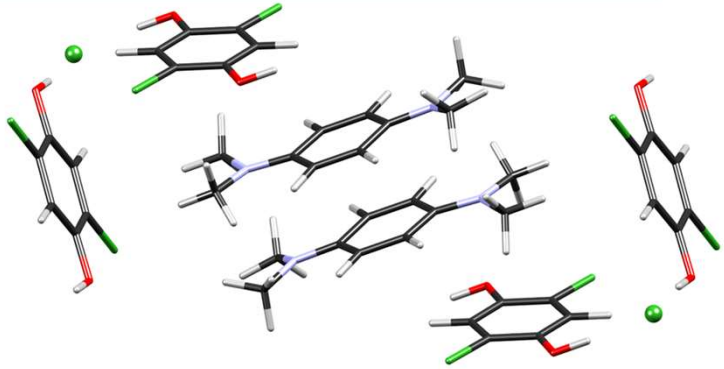


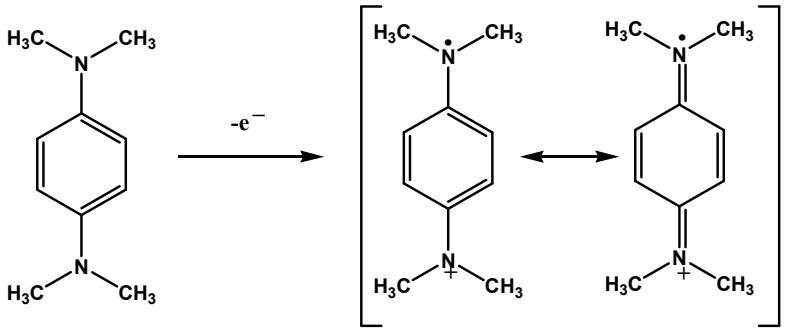
a) b)

Figure 8. a) Molecular docking optimized energy structure of the **ARI**(VdW balls) / SMX(sticks) complex; b) molecular dynamics optimized structure in water, after 5 ns at T = 300 K, p = 1 atm. Water molecules are omitted for clarity.

P. Stanić, I. Nikšić-Franjić and K. Molčanov, **Pancake-bonded dimers of semiquinone radical cations of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (Wurster's blue)**, accepted manuscript in *Cryst. Growth Des.*

TMPD · 2,5-dichlorohydroquinone





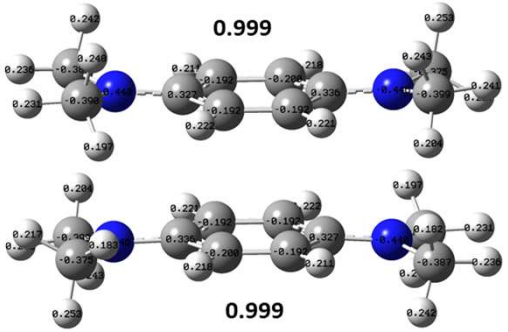
$E_{S\text{-}T} = E_{tot}(\text{triplet}) - E_{tot}(\text{singlet})$

TMPD radical cations: -3.24 kcal mol^{-1}

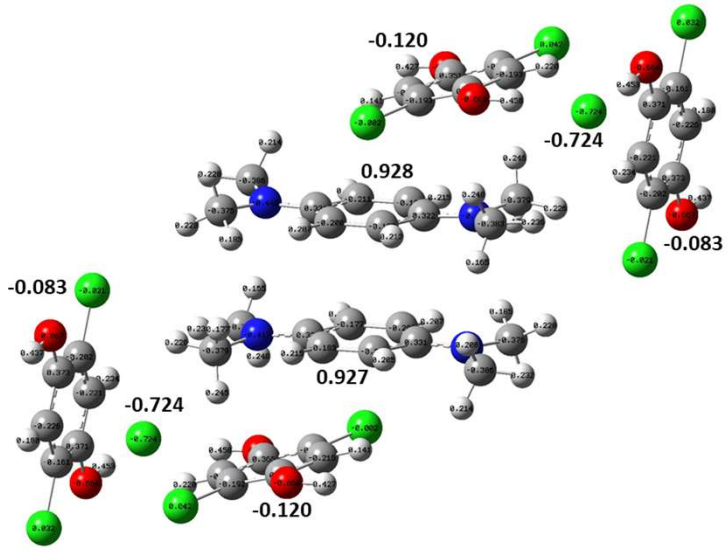
Semiquinone radical anions:

Stacks of equidistant radicals (-2.9 kcal mol^{-1})

Pancake-bonded dimers (-9.4 kcal mol^{-1})



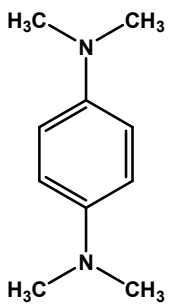
TMPD radical cations



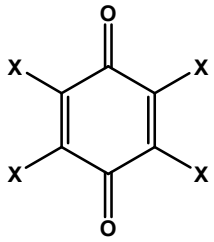
TMPD · 2,5-dichlorohydroquinone

*M06-2X-D3/6-311+G(2d,p), surface isovalue: 0.01 a.u., density 0.0004 a.u.

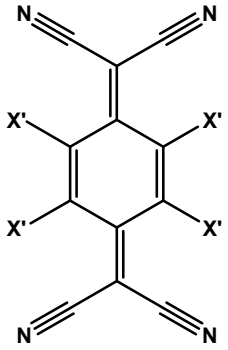
P. Stanić, I. Nikšić-Franjić, L. Pavić and K. Molčanov, **Two electron multicentre bonding (pancake bonding) between electron donors and acceptors in charge-transfer compounds of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and (semi)quinoid electron acceptors**, accepted manuscript in *Cryst. Growth Des.*



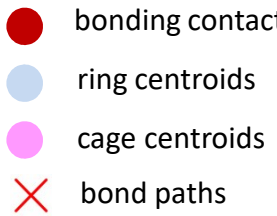
TMPD

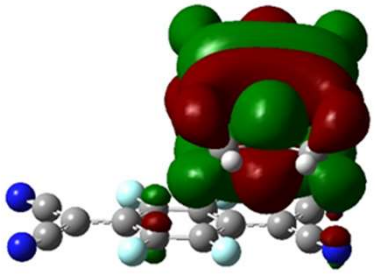


F_4Q: X = F
Cl_4Q: X = Cl
Br_4Q: X = Br



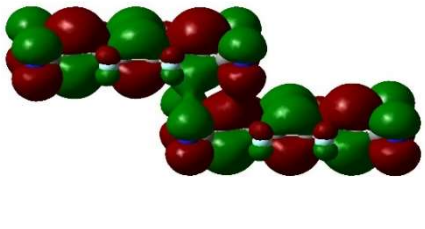
TCNQ: X' = H
F_4TCNQ: X' = F





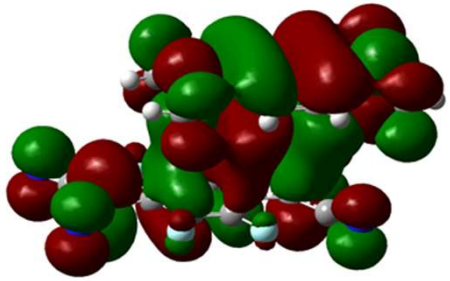
HOMO

TMPD · F4TCNQ



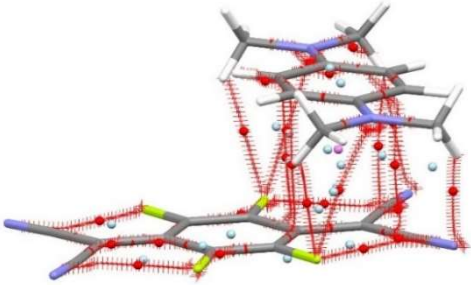
HOMO

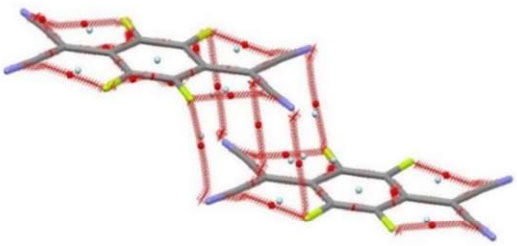
F4TCNQ · F4TCNQ

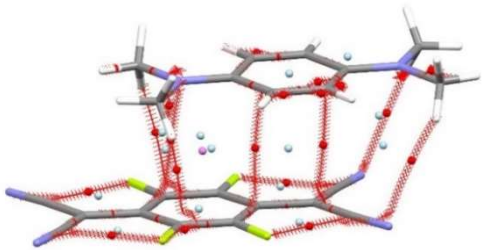


HOMO

Reoriented TMPD · F4TCNQ

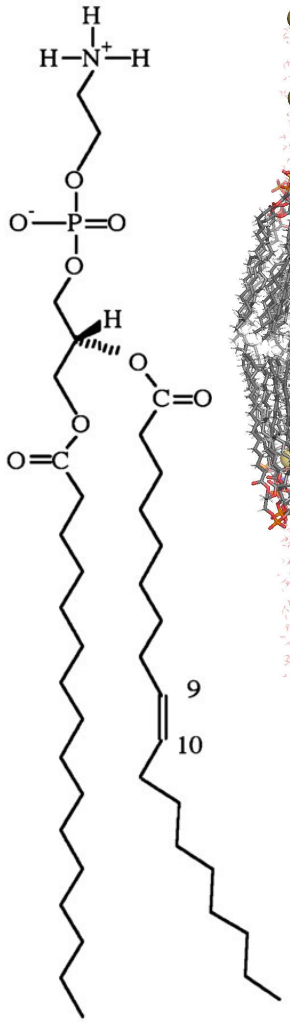


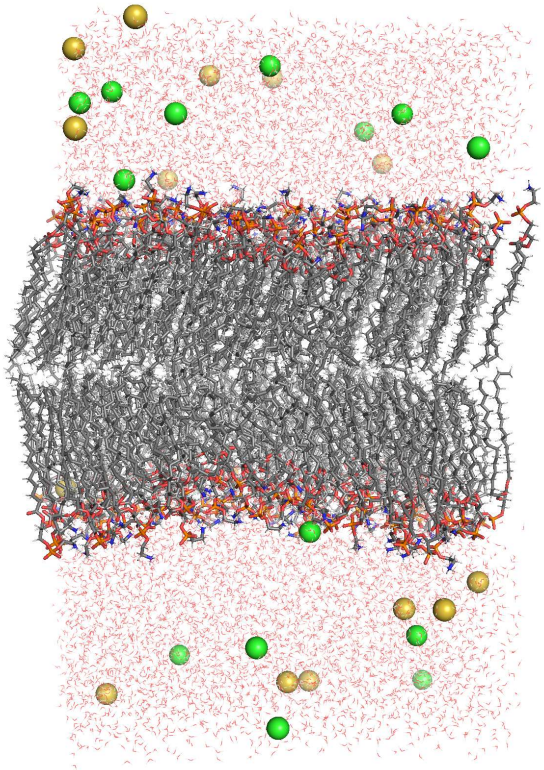


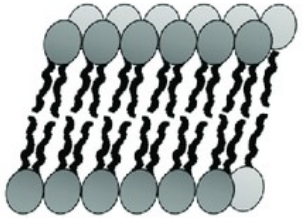


Non-published results: POPE lipid

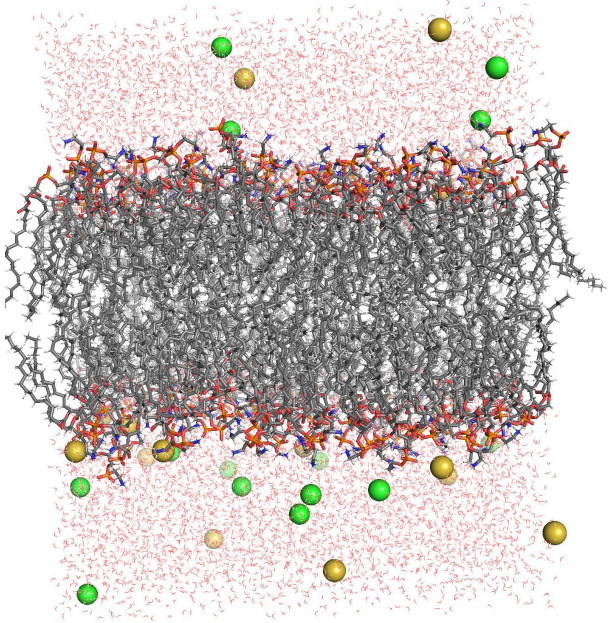
Dr. Danijela Bakarić

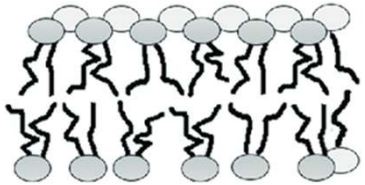




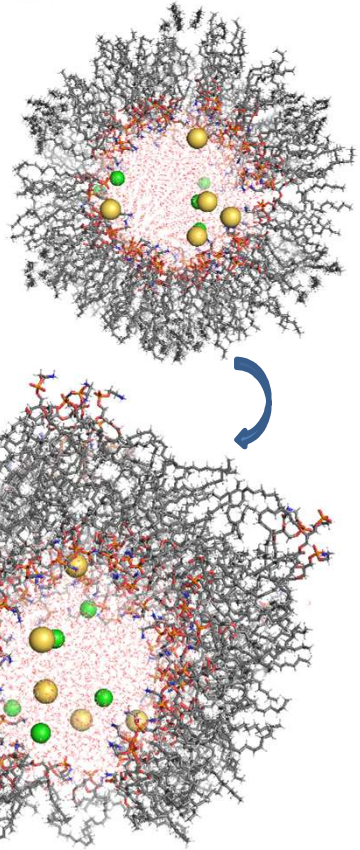


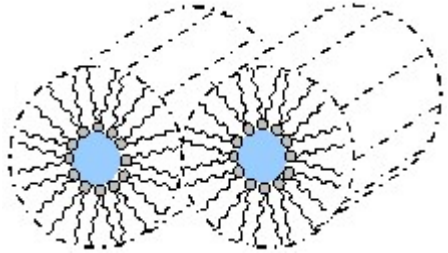
Gel phase (15 °C)





Fluid phase (50 °C)



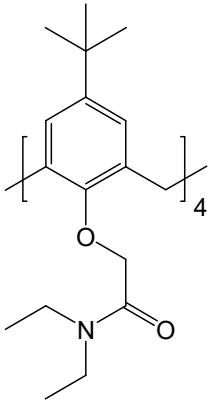


Inverse hexagonal phase (85 °C)

1-palmitoyl-2-oleoyl phosphatidylethanolamine

Non-published results: Fluorescent calixarenes

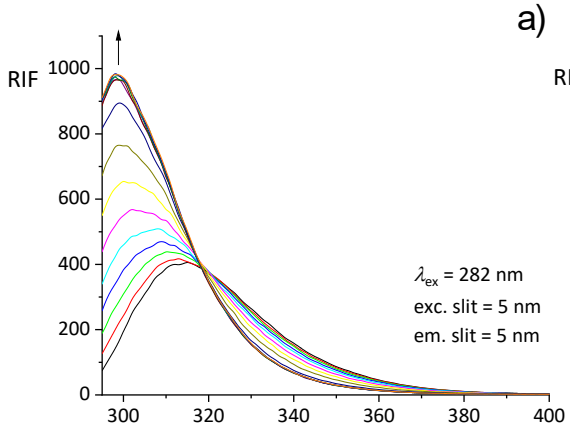
Prof. Tomišić & Prof. Piantanida & Prof. Došlić

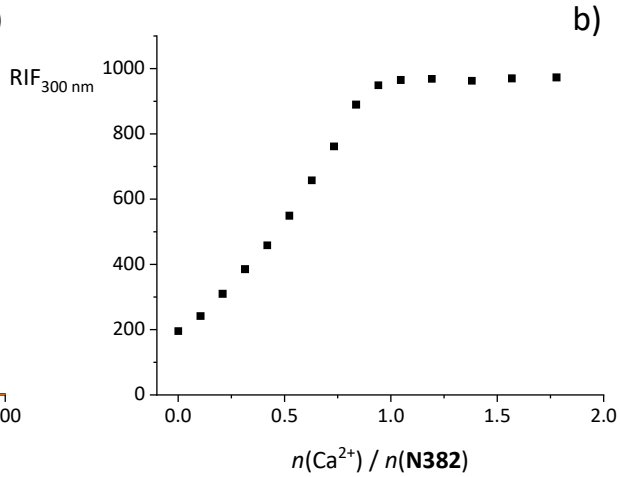


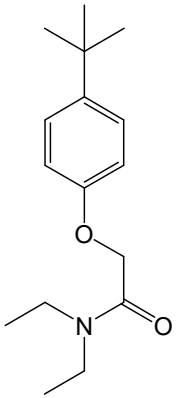
N382

Tertiary amide

N382, MeCN



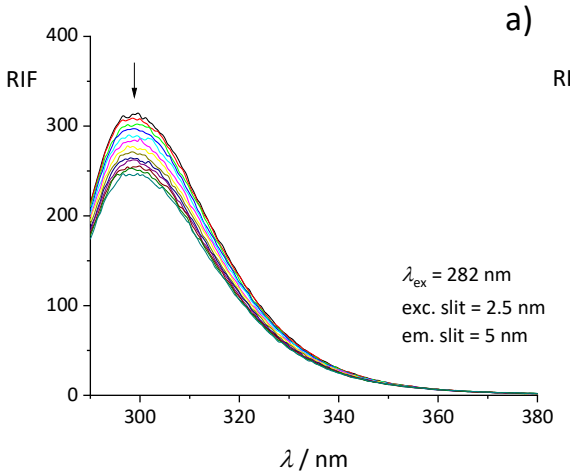


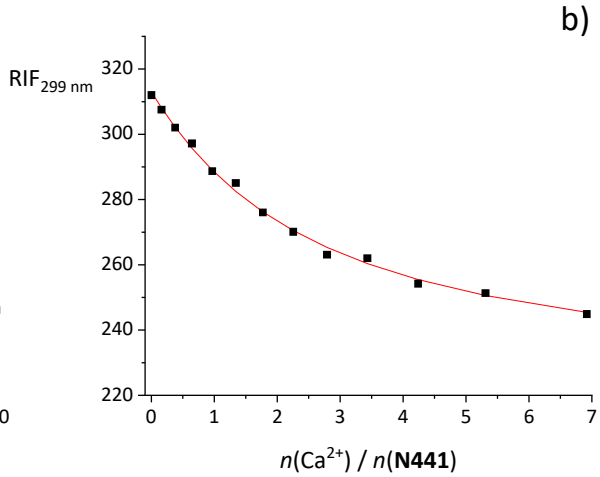


N441

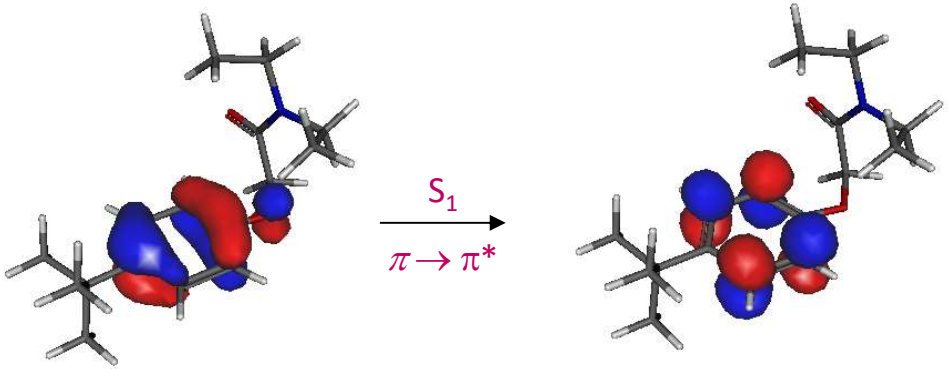
Amide monomer

N441, MeCN



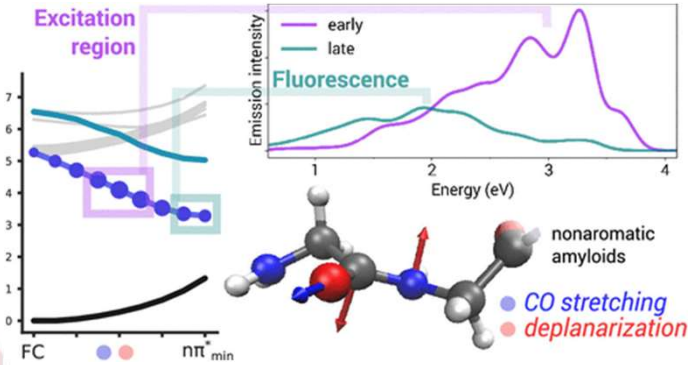


NON-PUBLISHED RESULTS: MONOMER



S_1: 4.42 eV, f = 0.0214

S_1: 4.80 eV, f = 0.0168



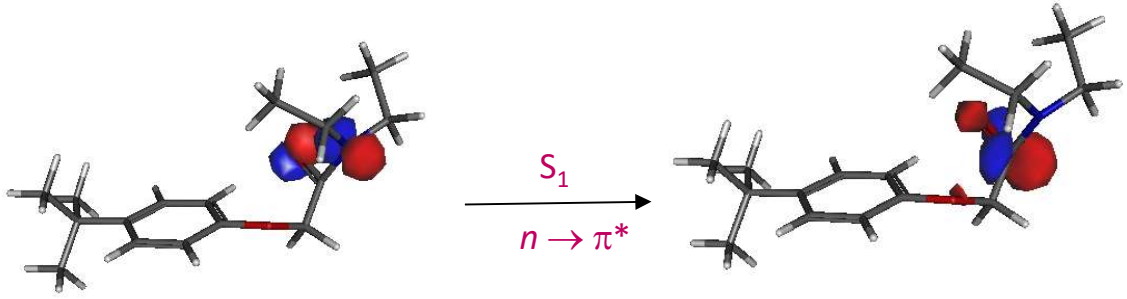
N. Došlić *et. al*. *JACS*, 2020, 142, 42, 18042-18049.

hv

S_0/S_1

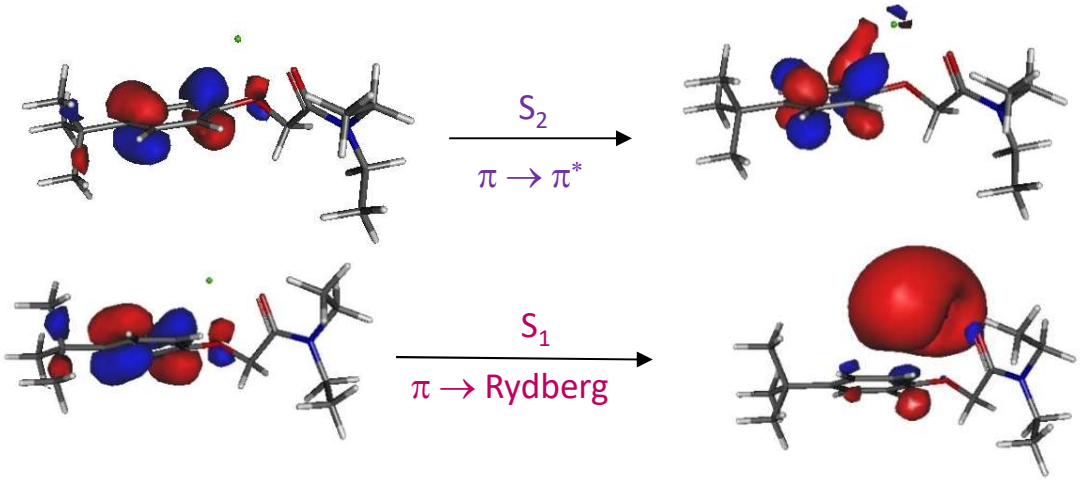
S_1: 1.40 eV, f = 0.0019

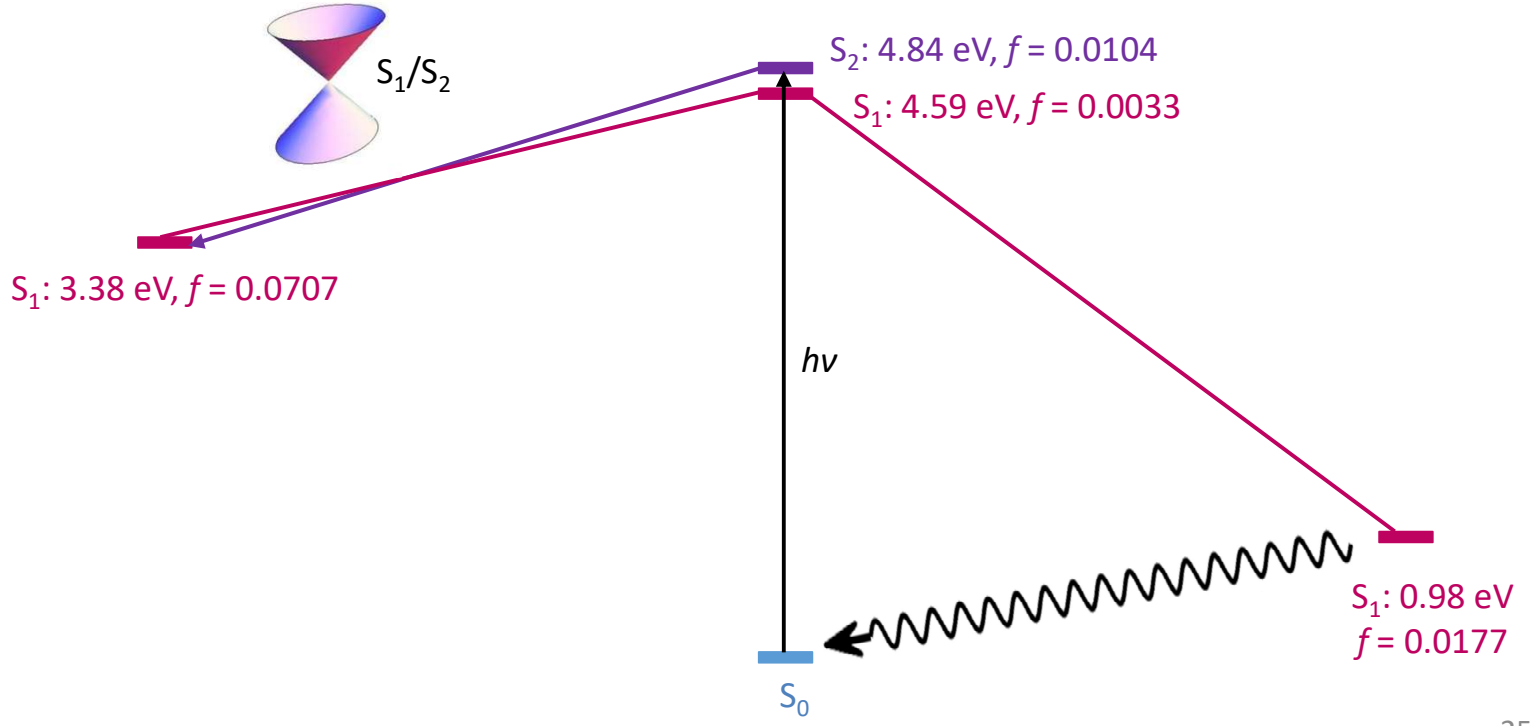
S_0



SOS-ADC(2)/cc-pVDZ

NON-PUBLISHED RESULTS: MONOMER -Ca

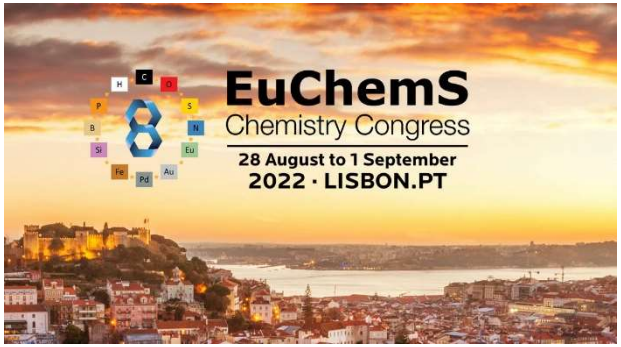




SOS-ADC(2)/cc-pVDZ (def2-SVP for Ca)

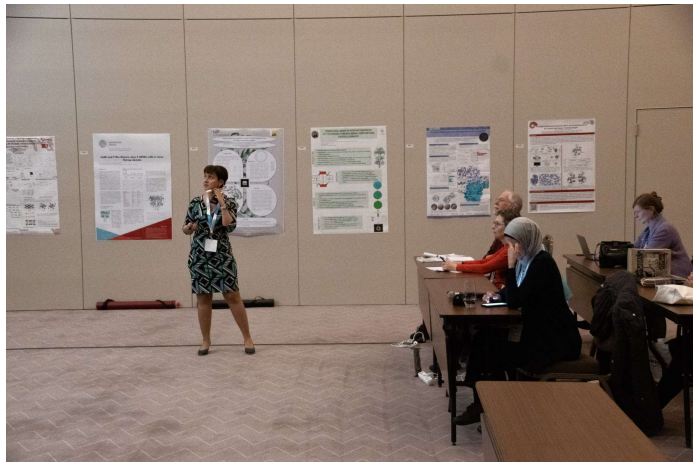
CONFERENCES, COURSES AND FOREIGN VISITS

17th European Powder Diffraction Conference – EPDIC17



Computational Chemistry Day 2022
September 24th, 2022
Ruđer Bošković Institute
Zagreb, Bijenička 54

Hot Topics in Contemporary Crystallography – HTCC5



Acknowledgment


Dr. Aleksandar Višnjevac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Dr. Dijana Pavlović Saftić


Prof. Dr. Olivia Reinaud


Prof. Dr. Benoit Colasson


Prof. Dr. Karl Gruber


Dr. Krešimir Molčanov


Dr. Leo Frkanec


Prof. Dr. Vladislav Tomišić

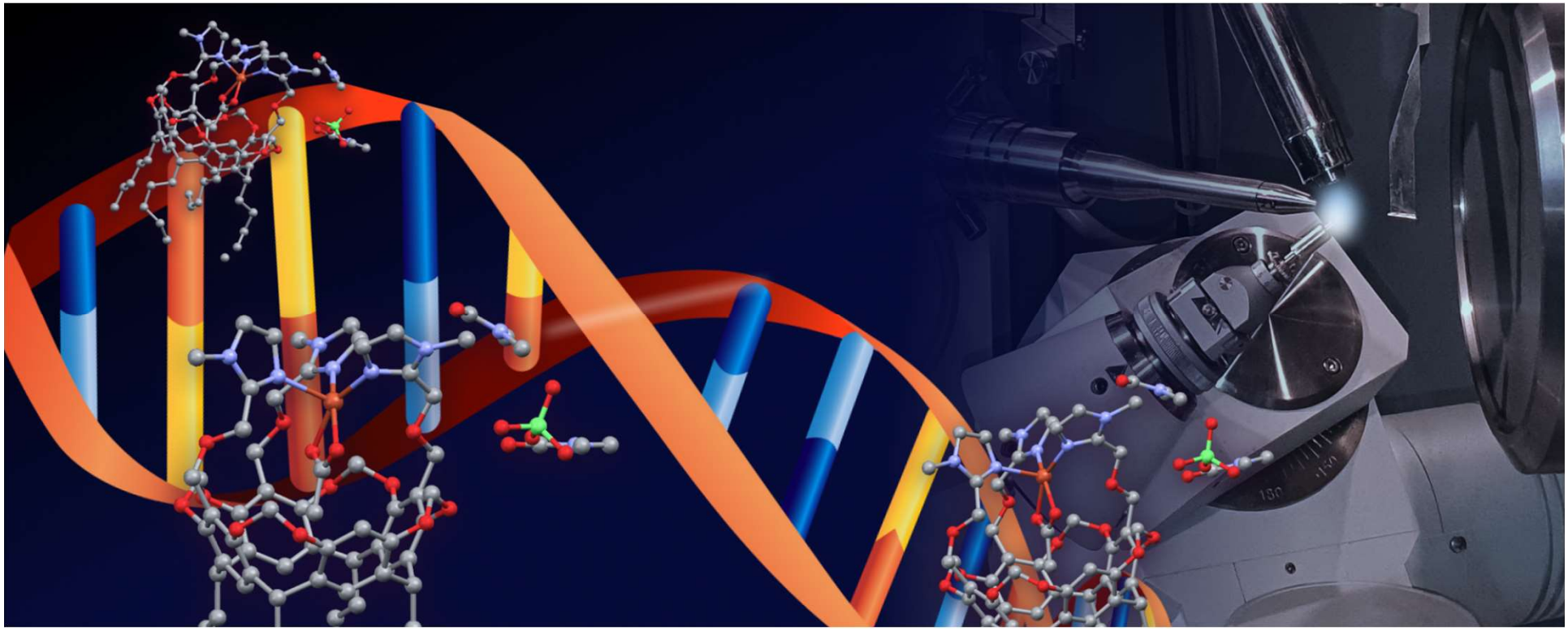

Dr. Danijela Bakarić


Prof. Dr. Nađa Došlić


Dr. Darko Babić


Dr. Zlatko Brkljača

Thank you for listening!



CalixDNA

RB

HrZZ Croatian Science Foundation