ON THE FEATURES OF MULTI-CHARGED *meso*-PORPHYRINS BINDING TO NUCLEIC ACIDS

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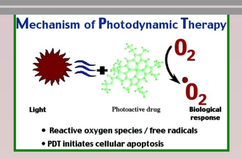
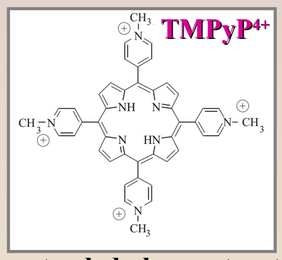
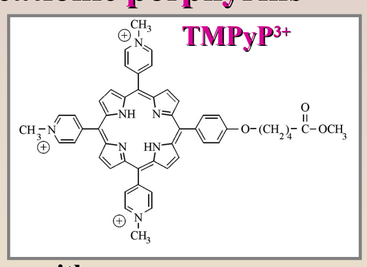
The porphyrins are macrocyclic compounds with unique spectroscopic and photophysical properties, a high anticancer and biological activity. They are widely used as probes for the structure and dynamics of nucleic acids, as photosensitizers in anticancer photodynamic therapy, anti-viral and antimicrobial agents, as a carrier of antisense oligonucleotides for their delivery, stabilizers of G-quadruplexes.



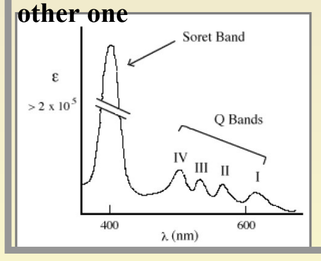
Binding of two multicharged cationic *meso*-porphyrins to synthetic double-stranded polynucleotides of different base composition and secondary structure including DNA (B-form), RNA (A-form), and DNA·RNA hybrids (A-form) has been studied in neutral aqueous buffered solutions without and with low and near-physioplogical NaCl content in a wide range of molar phosphate-to-dye ratios, *P/D*, using various spectroscopic techniques. The types of the porphyrin binding to the polynucleotide depending on *P/D* ratio were determined, and the spectroscopic properties and features of the complexes formed were established.

Water-soluble cationic porphyrins



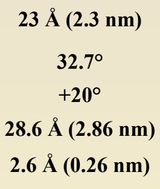
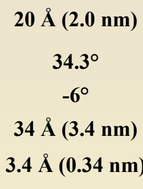


- extended planar structure with
- highly-conjugated electronic system
- selective accumulation in tumor cells
- high extinction coefficient in its red region where the transparency of tissues to light increases considerably
- photosensitizer for PDT of cancer
- cationic group facilitate their binding to nucleic acids
- G-quadruplex binding ligands and stabilizers
- human telomerase inhibition IC_{50} = 6.5 μM
- side chain give the possibility to conjugate the dye with other one



Porphyrin visible absorption bands ($\pi \rightarrow \pi^*$)
Soret band: $S_0 \rightarrow S_2^*$
Q-bands: $S_0 \rightarrow S_1^*$

Double-stranded polynucleotides

Parameter	A-form (*ds*-RNA and DNA·RNA hybrids)	B-form (*ds*-DNA)
		
Helix sense	Right-handed	Right-handed
Diameter	23 Å (2.3 nm)	20 Å (2.0 nm)
Helix rotation on per base pair	32.7°	34.3°
Base pair tilt to axis	+20°	-6°
Helix pitch (rise per turn)	28.6 Å (2.86 nm)	34 Å (3.4 nm)
Helix rise per base pair	2.6 Å (0.26 nm)	3.4 Å (0.34 nm)
Base pairs per turn	11	10.5
Glycosyl bond angle	Anti	Anti
Sugar pucker	C3′-endo	C2′-endo
Major groove	Narrow and Deep	Wide and Deep
Minor groove	Wide and Shallow	Narrow and Deep
EXAMPLES of the ds-polynucleotides	poly(A)·poly(U) poly(G)·poly(C) poly(rA)·poly(dT) poly(rG)·poly(dT)	poly(dA)·poly(dT) poly(dG)·poly(dC)

Most widely used spectroscopic techniques

Absorption spectroscopy: registration of changes in the intensity (hypochromism (H) or hyperchromism), shape and position of the porphyrin absorption bands maximum (mainly, in the Soret band).

Polarized Fluorescence Spectroscopy: registration of changes in the shape, position and intensity of the porphyrin emission band, as well as of its fluorescence polarization degree.

Fluorimetric titration: the dye sample was added with increasing amounts of the concentrated polymer stock solution containing the same porphyrin content, whereupon fluorescence intensities and polarization degree were measured versus molar phosphate-to-dye ratio, *P/D*. For example, C_{dye} = 10 μM, λ_{exc} = 500 nm, λ_{obs} = 680 nm.

Resonance Light Scattering (RLS) is a highly sensitive and selective method for studying chromophore arrays with strong electronic coupling between chromophores. It was proposed in 1993 by R.F. Pasternak [Pasternack et al., J. Am. Chem. Soc. 1993 Vol. 115, P. 5393-5399. // R.F. Pasternack et al., Science 1995 Vol. 269 P. 935-939] as an effective tool for studying the aggregation of porphyrin dyes. It gives an information on the size, shape, and aggregation number of supramolecular aggregates of organic dyes (including heteroaggregates) and does not require additional devices. RLS experiments are usually performed at wavelengths away from absorption bands, but for species that aggregate, enhancements in light scattering of several orders of magnitude can be observed at wavelengths characteristic of these species. For example, $\lambda_{exc} = \lambda_{obs}$ = 500 nm.

Induced Circular Dichroism (ICD) is a powerful tool to detemine the porphyrin-DNA(RNA) binding type. The porphyrins represent chiroptical conformational probes. The type of ICD signals in the Soret region provides precise diagnostic insights into binding mechanisms and molecular interactions

POPRHYRIN - DNA(RNA) BINDING MODES AND THEIR FINGERPRINTS

Low *P/D* ratios

EXTERNAL BINDING OF PORPHYRIN TO DNA/RNA PHOSPHATE BACKBONE

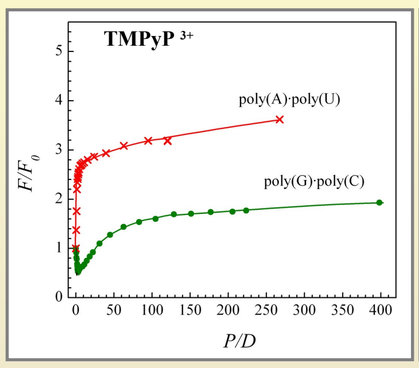
It depends strongly on the Na^+ content in the solution

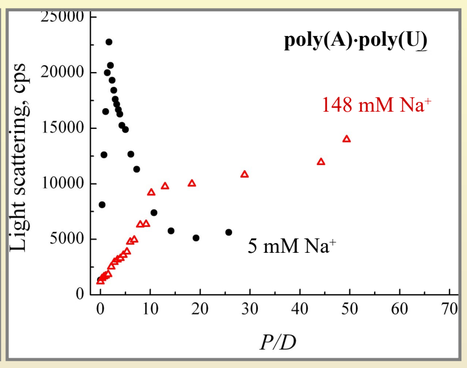
WITH SELF-STACKING (highly cooperative process) | **WITHOUT SELF-STACKING**

Examples:

TMPyP^{3+} + poly(G)·poly(C) [2] | **TMPyP^{3+} + poly(A)·poly(U) [1]**

TMPyP^{4+}+poly(dG)·poly(dC) [7] | **TMPyP^{4+}+poly(dA)·poly(dT) [7]**

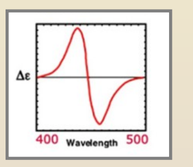


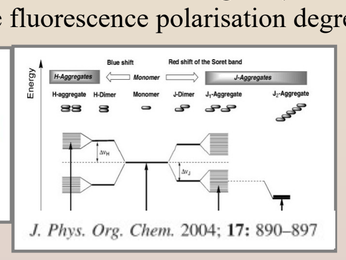


Quenching of the porphyrin fluorescence

Enhancement of the porphyrin emission

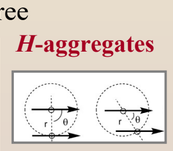
- substantial hypochromism (H) and red shift of Soret absorption band
- splitting of the emission band
- increase of the fluorescence polarization degree
- strong resonance light scattering due to formation of the dye aggregates
- bisignate ICD band in the Soret region (near 440 nm)
- increase in the fluorescence polarisation degree





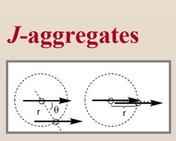
J. Phys. Org. Chem. 2004; **17:** 890–897

***H*-aggregates**



54.7° ≤ θ < 90°

***J*-aggregates**



0 ≤ θ < *54.7°*

Fluorescence enhancement upon binding at low *P/D*	Fluorescence quenching upon binding at *low P/D*
TMPyP^{3+} + poly(A)·poly(U) [1]	TMPyP^{3+} + poly(G)·poly(C) [2]
	TMPyP^{3+} + poly(G) [2]
TMPyP^{4+} + poly(A) [9]	TMPyP^{3+} + poly(P) [10]
	TMPyP^{4+} + poly(P) [11]
TMPyP^{4+} + poly(A)·poly(U) [5]	Zn^{2+}TMPyP^{4+} + poly(A)·poly(U) [12]
TMPyP^{4+} + poly(dA)·poly(dT) [7]	TMPyP^{4+} + poly(dG)·poly(dC) [7]

Stabilization of porphyrin aggregates:
electrostatic forces
π-π stacking
H-bonding,
van der Waals forces
hydrophobic interaction

Possible applications of porphyrin aggregates:
- design of new photonic materials
- light- harvesting systems
- molecular electronics
- solar batteries
- nonlinear optics
- chemotherapeutics

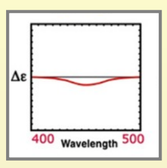
Example of natural porphyrin aggregates:
light-harvesting complexes of the plants (200 chlorophyll molecules)

High *P/D* ratios

INTERCALATION OF PORPHYRIN BETWEEN NUCLEIC BASE PAIRS

was detected for GC-containing deoxypolynucleotides

For example, **TMPyP^{4+} +poly[(dG-dC)]$_2$ [3]**
TMPyP^{4+} + poly(G)·poly(C)

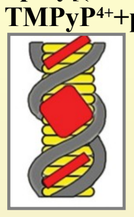


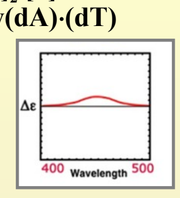
For DNA duplexes (B-form)
- Great changes in Soret absorption band maximum:
  H > 40%, red shift Δλ > 15 nm
- Fluorescence quenching
- Strong increase in the fluorescence polarization degree
- Negative ICD band in the Soret region (at ≈ 440 nm)
- Lengthening of DNA (RNA)
- Rise of the solution viscosity
- Energy transfer between DNA and porphyrin → 2-4-fold rise of fluorescence quantum yield upon excitation at 260 nm

PORPHYRIN MONOMERIC INCORPORATION IN DOUBLE HELIX GROOVE

was detected for AT-containing deoxypolynucleotides

For example: **TMPyP^{4+} +poly[(dA-dT)]$_2$ [3]**
TMPyP^{4+}+poly(dA)·(dT)





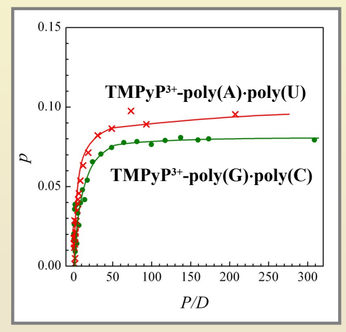
For DNA duplexes (B-form)
- No or minor changes in the porphyrin absorption spectra
- No or minor changes in the porphyrin fluorescence spectrum
- No energy transfer between porphyrin and DNA
- No lengthening of DNA
- Moderate increase in the fluorescence polarization degree, *p*.
- Positive ICD band in the Soret region (at ≈ 440 nm)
- Relatively low value of binding constant

EMBEDDING OF PORPHYRIN DIMERS IN THE DOUBLE HELIX GROOVE

was detected for RNA duplexes

For example:
TMPyP^{3+} + poly(G)·poly(C) [2]
TMPyP^{3+} + poly(A)·poly(U) [1]

For RNA duplexes (A-form)
- Substantial changes in Soret absorption band maximum:
  H > 22 % red shift Δλ > 15 nm
- Moderate increase in the fluorescence polarization degree, *p*.
- Exciton coupled bisignate ICD band in the Soret region (at ≈ 440 nm)



Porphyrin + Polynucleotide	Reference
TMPyP^{3+} + poly(A)·poly(U)	[1] Ryazanova O. et al. *J. Fluoresc.* doi:10.1007/s10895-024-04000-4
TMPyP^{3+} + poly(G)·poly(C)	[2] Ryazanova O. et al. *Methods Appl Fluoresc.* 4(3) (2016) 034005.
TMPyP^{3+} + [poly(dA-dT)]$_2$	[3] Andrews K. et al. *Biochemistry* 47(4) (2008) 1117–1125.
TMPyP^{3+} + [poly(dG-dC)]$_2$	
TMPyP^{4+} + poly(A)·poly(U)	[4] Uno T. et al. *Inorg. Chem.* 36(8) (1997) 1676–1683.
TMPyP^{4+}+ poly(G)·poly(C)	
TMPyP^{4+} + poly(rA)·poly(dT)	
TMPyP^{4+} + poly(rG)·poly(dC)	
TMPyP^{4+} + poly(A)·poly(U)	[5] Tolstykh G. et al. *J. Mol. Str.* 1098 (2015) 342-350.

Porphyrin + Polynucleotide	Reference
TMPyP^{4+} + poly(dA)·poly(dT)	[6] Pasternack R. et al. *Biochemistry* 22(10) (1983) 2406-2414.
TMPyP^{4+} + poly(dG)·poly(dC)	
TMPyP^{4+} + poly(dA)·poly(dT)	[7]. Kelly J.M. et al. *Nucleic Acids Res.* 13(1) (1985) 167–184.
TMPyP^{4+} + poly(dG)·poly(dC)	
TMPyP^{4+} + [poly(dA-dT)]$_2$	[3] Andrews K. et al. *Biochemistry* 47(4) (2008) 1117–1125.
TMPyP^{4+} + [poly(dG-dC)]$_2$	
TMPyP^{4+} +DNA (B-form)	[8] Oh Y.S. et al. *ACS Omega* 3 (1) (2018) 1315-1321.
TMPyP^{4+} + DNA (A-form)	

CONCLUSIONS

TMPyP^{3+} and TMPyP^{4+} porphyrins bind to the polynucleotide duplexes via a three competitive binding modes:
(i) external ligand binding with or without self-stacking dominates at *P/D* < 4;
(ii) intercalation of the porphyrin chromophore between the nucleic bases of GC-containing *ds*-deoxypolyribonucleotides (B-DNA) was observed at *P/D* > 30;
(iii) embedding of the porphyrin monomers or partially stacked porphyrin *J*-dimers into the biopolymer groove prevails at *P/D* >30.

TMPyP^{3+} discriminates between polynucleotide duplexes containing A·U (A·T) and G·C base pairs at low *P/D* ratios

In contrast to TMPyP^{4+}, large bathochromic shifts of the TMPyP^{3+} Soret band at high *P/D* don't depend on the polynucleotide base composition and type of helical structure.

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