BINDING of PHEOPHORBIDE-*a* AND ITS DERIVATIVES TO BIOPOLYMERS OF DIFFERENT COMPOSITION AND SECONDARY STRUCTURE: a SPECTROSCOPIC STUDY

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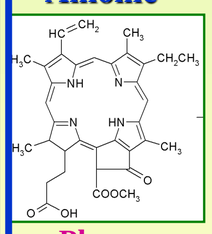
Binding of Pheophorbide-*a* (Pheo-*a*), its cationic (CatPheo-*a*) and neutral (MePheo-*a*) derivatives, Pheo-*a* conjugate with cationic intercalative aminophenazinium dye (Pheo-Pzn) to inorganic polyphosphate, poly(P), poly-L-lysine, pLL, and nucleic acids of various primary and secondary structures (*ds*-poly(A)·poly(U), *ds*-poly(G)·poly(C), *fs*-poly(G), antiparallel telomeric DNA quadruplex Tel22) were studied in a wide range of molar phosphat-to dye ratios by various spectroscopic methods in solutions of low (1 mM Na^+) and near-physiological (0.15 M Na^+) ionic strengths.

The aim: to determine the types of the dye binding to the biopolymers *vs P/D* ratio; to establish the spectroscopic properties and features of the complexes.

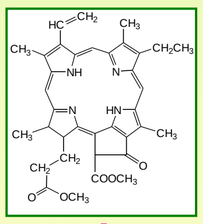
Dyes

Anionic



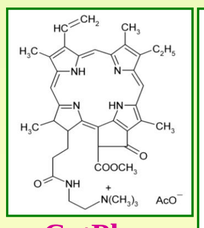
Pheo-*a*

Neutral

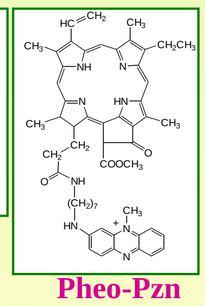


MePheo-*a*

Cationic



CatPheo-*a*



Pheo-Pzn

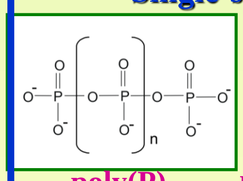
Pheophorbide-a - macrocyclic anionic chlorine derivative

- selectively accumulates in tumor cells
- extended planar aromatic structure
- high extinction coefficient in the red region where the transparency of tissues to light increases considerably
- photosensitizer for PDT of cancer
- G-quadruplex binder

- Pheo moiety is supposed to be stabilizer of G-quadruplexes
- Pzn moiety is supposed to be intercalated into double-stranded regions (additional stabilization)
- positively charged group will facilitate Pheo binding to nucleic acids

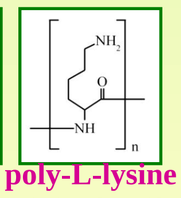
Biopolymers

Single-stranded



poly(P)

Sodium polyphosphate with polymerisation degree of 75 takes planar or helix conformation where negatively charged phosphate groups are arranged along two oppositely situated rows so that the intercharge distance become equal to *ca.* 0.42 nm for the planar conformation and 0.36 nm for the helix one



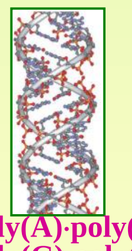
poly-L-lysine

cationic polypeptide with antimicrobial action

Conformations:
- random coil (at low and neutral pH)
- α-helix (at pH > 10.6)
- β-sheet (at pH > 10.6 after heating)

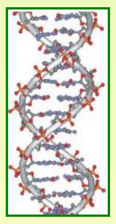
Such arrangement of the negative charges on the polymer promotes formation of two extended porphyrin stacking-aggregates on opposite sides of the poly(P) chain.

Double-stranded



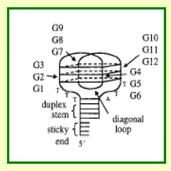
poly(A)·poly(U)
poly(G)·poly(C)

double-stranded RNA helices of A-type

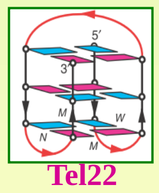


DNA

double-stranded helice of B-type



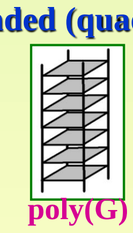
Four-stranded (quadruplex)



Tel22

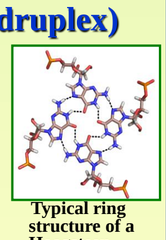
intramolecular G-quadruplex formed by 5'-d[$AG_3(T_2AG_3)_3$]-3' oligodeoxynucleotide of human telomeric repeat

Telomeres are long repetitive G-rich nucleotidic sequences located at the end of chromosomes of most eucariotic organisms



poly(G)

RNA polymer, it can adopt four-stranded helical arrangements with the G-tetrads stacked on one another. It can be a model of G-quadruplex regions of telomeric DNA.

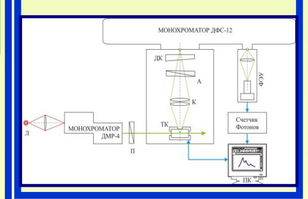


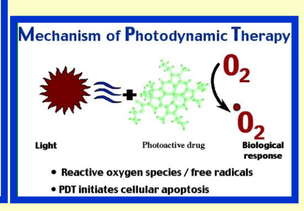
Typical ring structure of a Hoogsteen paired **G-quartet**

Cheong C., Moore P.B. Biochemistry (1992). 31 (36): 8406–14.

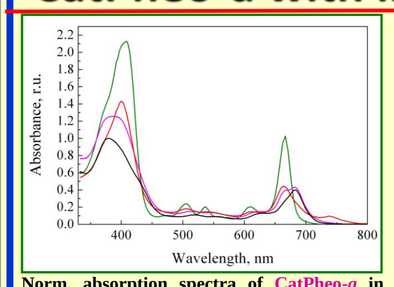
Techniques

- Absorption spectroscopy
- Polarized fluorescence spectroscopy
- Fluorimetric titration
- Abs and Flu melting

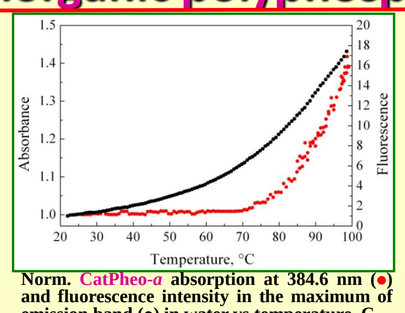




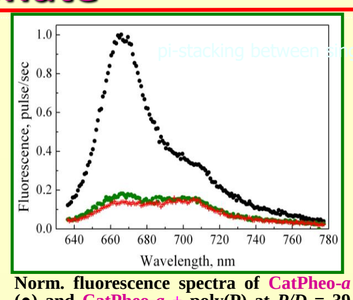
CatPheo-a with inorganic polyphosphate



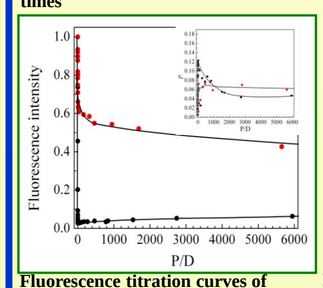
Norm. absorption spectra of CatPheo-*a* in ethanol (—) and water (—) at 20 °C, C_{dye}= 1.3·10^{-5} M, data in water C_{dye}= 1.3·10^{-6} M at 20 °C (—) and 85°C (—) are multiplied by 10 times



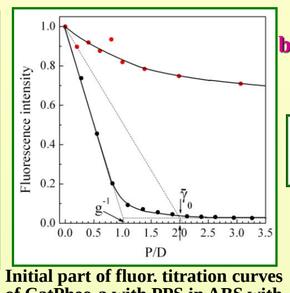
Norm. CatPheo-*a* absorption at 384.6 nm (●) and fluorescence intensity in the maximum of emission band (●) in water vs temperature, C_{dye} = 1.3·10^{-5} M, λ_{exc} = 633 nm.



Norm. fluorescence spectra of CatPheo-*a* (●) and CatPheo-*a* + poly(P) at *P/D* = 39 (+) and 1560 (●) in ABS with 2 mM Na^+, λ_{ex} = 633 nm, C_{dye} = 1.3·10^{-5} M.



Fluorescence titration curves of CatPheo-*a* with PPS in ABS with 2 mM Na^+ (●) and 0.15 M Na^+ (●), C_{dye} = 1.3·10^{-5} M, λ_{exc} = 633 nm.



Initial part of fluor. titration curves of CatPheo-a with PPS in ABS with 2 mM Na^+ (●) and 0.15 M Na^+ (●), C_{dye} = 1.3·10^{-5} M, λ_{exc} = 633 nm.

Parameters of cooperative binding estimated by Schwarz's method *[Eur. J. Biochem. 12 (1970) 442-453]*

$$K = \frac{1}{\bar{\gamma}_0 \cdot C_T}$$

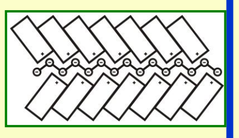
$$K = qK^+$$

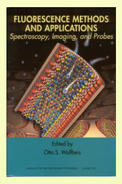
g = 1.00
A number of binding sites per monomer unit of poly(P)

K^+ = 1800 M^{-1}
for binding of monocationic dyes to polyanionic lattice

q ≈ 1000
cooperativity parameter

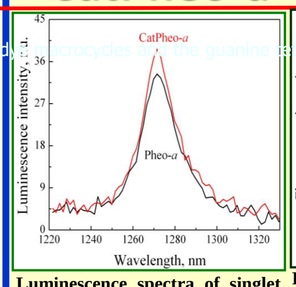
cooperative binding constant
K ≈ 2·10^6 M^{-1} (at 2 mM Na^+)



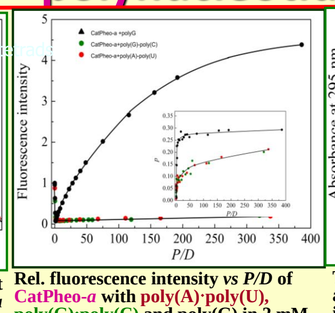


***P/D* < 3 –** highly cooperative electrostatical outside binding with chromophore self-stacking
high *P/D* – electrostatically bound dye dimers

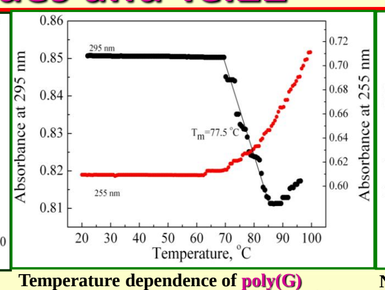
CatPheo-a + polynucleotides and Tel22



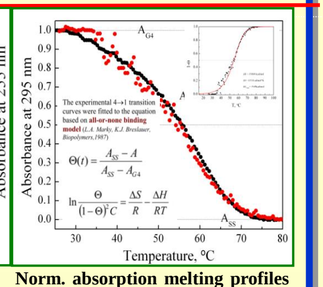
Luminescence spectra of singlet oxygen generated by CatPheo-*a* and Pheo-*a* in ethanol, λ_{ex}=665 nm.



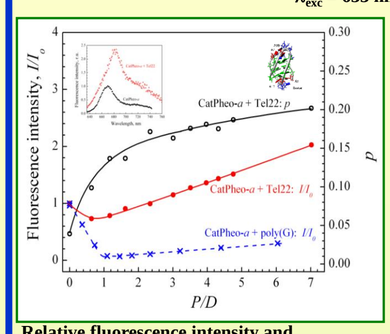
Rel. fluorescence intensity *vs P/D* of CatPheo-*a* with poly(A)·poly(U), poly(G)·poly(C) and poly(G) in 2 mM ABS with 6.6% of ethanol, C_{dye} = 10 μM, λ_{exc} = 633 nm, λ_{obs} = 670 nm.



Temperature dependence of poly(G) absorption at 295 nm (●) and 255 nm (●) in ABS with 2 mM Na^+, $C_{polymer}$ = 3·10^{-4} M



Norm. absorption melting profiles at 295 nm for Tel22 (●) and Tel22+CatPheo-*a* at *P/D* = 0.7 (●) in ABS with 0.1 M NaCl, C_{Tel22} = 30 μM (in strands).



Relative fluorescence intensity and polarisation degree in emission band maximum *vs P/D* for CatPheo-*a* with Tel22 and poly(G) in ABS with 1 mM Na^+, C_{dye} = 10 μM, λ_{exc} = 633 nm, λ_{obs} = 677 nm.

Mechanisms of photosensitizer deactivation:

Type I: radical transfer
$^3SENS + O_2 \rightarrow {}^1SENS_{ox} + O_2^-$
$^3SENS + SH \rightarrow {}^1SENS^0 + S^0$ (H-transfer)
$^3SENS + S \rightarrow {}^1SENS^- + S^+$ (e-transfer)
or
$^3SENS + S \rightarrow {}^1SENS^+ + S^-$ (e-transfer)

Type II: energy transfer
$^3SENS + O_2 \rightarrow {}^1SENS + {}^1O_2$

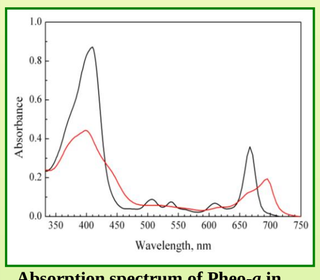
Thermodynamical parameters of G4 formation

Compound	T_m,°C	C, μM (in strands)	*P/D*	-ΔH, kcal/mol	-ΔS, cal/mol·K	-ΔG[a], kcal/mol	K_{eq}[a], M^{-1}
Tel22	55.6	17.5	0.0	52	134.9	10.15	1.4·10^7
CatPheo-*a* + Tel22	54.7	30.0	0.7	51.8	135.8	9.69	6.7·10^6

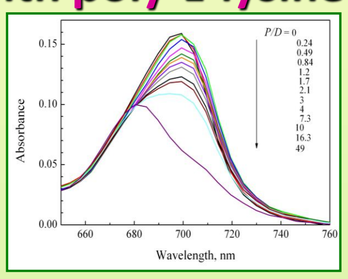
[a] *Data were calculated at t = 37 °C using equations* $\Delta G = \Delta H - T\Delta S$ $K_{eq} = e^{-\Delta G/RT}$

***P/D* < 1 –** highly cooperative electrostatical outside binding of CatPheo-*a* to polynucleotide phosphate groups with chromophore self-stacking
high *P/D* – intercalation of the dye chromophore between AU and GC base pairs and guanine quartets of poly(G), pi-stacking between single dye macrocycles and the guanine tetrads electrostatically bound dye dimers
CatPheo-*a* slightly destabilizes the quadruplex structure Tel 22 decreasing T_m of quadruplex → single strand (4→1) conformational transition by approximately 1 °C.
In ethanol solution CatPheo-*a* exhibits 15% higher efficiency of singlet oxygen generation as compared to parent Pheo-*a* compound that suggests its improved photodynamical activity.
So CatPheo-*a* can be better photosensitizer for PDT of tumors

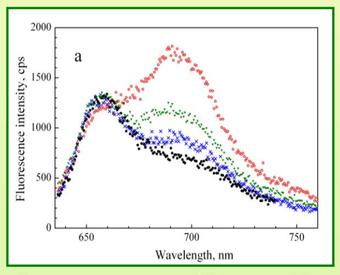
Pheo-a with poly-L-lysine



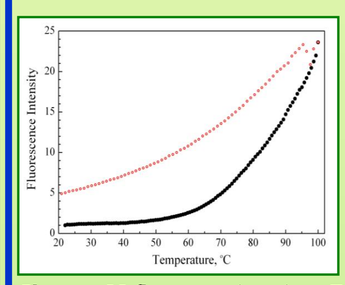
Absorption spectrum of Pheo-*a* in ethanol (—) and in 1 mM aqueous buffered solution + 5.34% of ethanol (—), C_{dye}=10 μM, L = 1 cm.



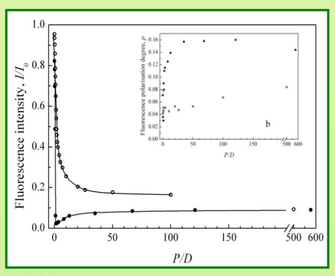
Longwave absorption band of Pheo-*a* and Pheo-*a* + PLL, *P/D* = 0 – 49, in ABS with 2.4 % of ethanol, C_{dye} = 3 μM, L= 2 cm.



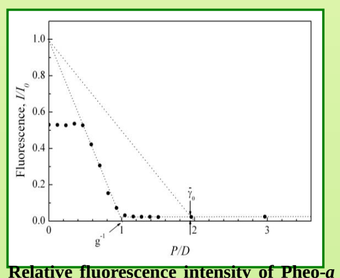
Fluorescence spectra of Pheo-*a* + pLL in 1 mM ABS with 5.9% of ethanol *P/D* = 10000 (o), 7333 (▼), 5000 (×), 3500 (●), C_{dye} = 19.5 μM, λ_{exc} = 633 nm.



Pheo-*a* + pLL fluorescence intensity *vs* T upon dissociation (●) and association (o) the complex *P/D* = 500 in ABS with 1 mM Na^+, C_{dye} = 1.95·10^{-5} M, λ_{exc} = 633 nm, λ_{obs} = 657 nm.



Rel. fluorescence intensity and polarisation degree of Pheo-*a* + pLL *vs P/D* in ABS with 5.6% of ethanol and 1 mM Na^+ (●) and 0.15 M Na^+ (o), C_{dye} = 1.9·10^{-5} M, λ_{exc} = 633 nm, λ_{obs} = 655 nm.



Relative fluorescence intensity of Pheo-*a* + pLL *vs P/D* in 1 mM ABS with 5.9% of ethanol, C_{dye} = 19.5 μM (●), λ_{exc} = 633 nm, λ_{obs} = 655 nm. The dashed lines are used for determination of parameters *g* and *K*.

Parameters of cooperative binding estimated by Schwarz's method *[Eur. J. Biochem. 12 (1970) 442-453]*

$$K = \frac{1}{\bar{\gamma}_0 \cdot C_T}$$

$$K = qK^+$$

g = 1.00
A number of binding sites per monomer unit of pLL

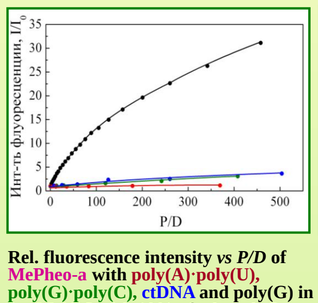
q ≈ 1000
cooperativity parameter

K^+ = 1800 M^{-1}
for binding of monocationic dyes to polyanionic lattice

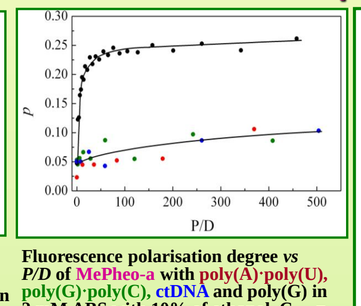
Cooperative binding constants:
K ≈ 2.1·10^6 M^{-1} (at 1 mM Na^+)
K ≈ 1.4-1.8·10^5 M^{-1} (at 0.15 M Na^+)

***P/D* < 10 –** highly cooperative electrostatical outside binding of Pheo-*a* to pLL with chromophore self-stacking
high *P/D* – electrostatically bound small aggregates of the dye (dimers, trimers, tetramers).

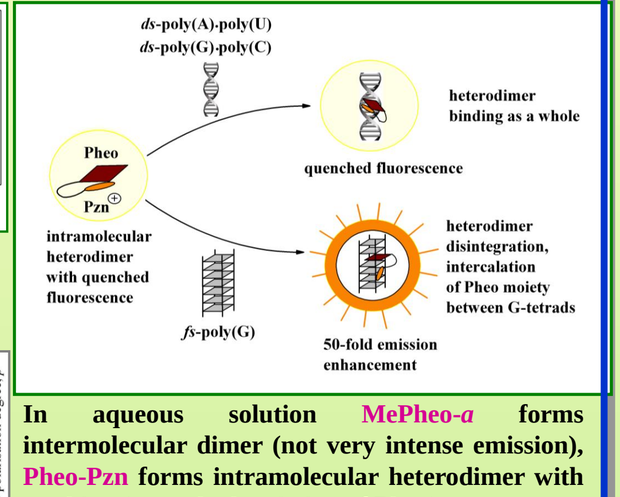
MePheo-*a* and Pheo-Pzn with polynucleotides



Rel. fluorescence intensity *vs P/D* of MePheo-a with poly(A)·poly(U), poly(G)·poly(C), ctDNA and poly(G) in 2 mM ABS with 10% of ethanol, C_{dye} = 5 μM, λ_{exc} = 633 nm.



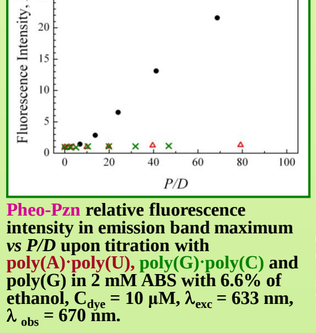
Fluorescence polarisation degree *vs P/D* of MePheo-a with poly(A)·poly(U), poly(G)·poly(C), ctDNA and poly(G) in 2 mM ABS with 10% of ethanol, C_{dye} = 5 μM, λ_{exc} = 633 nm.



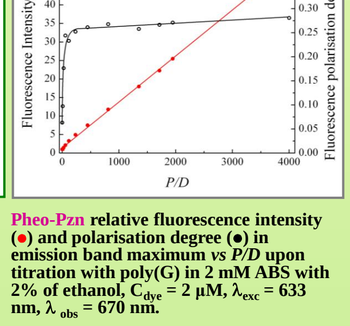
In aqueous solution MePheo-*a* forms intermolecular dimer (not very intense emission), Pheo-Pzn forms intramolecular heterodimer with stacking (quenched emission of Pheo).

MePheo-*a* + DNA, poly(A)·poly(U), poly(G)·poly(C) – quenched emission

Pheo-Pzn + poly(A)·poly(U) and poly(G)·poly(C) – quenched emission



Pheo-Pzn relative fluorescence intensity in emission band maximum *vs P/D* upon titration with poly(A)·poly(U), poly(G)·poly(C) and poly(G) in 2 mM ABS with 6.6% of ethanol, C_{dye} = 10 μM, λ_{exc} = 633 nm, λ_{obs} = 670 nm.



Pheo-Pzn relative fluorescence intensity (●) and polarisation degree (●) in emission band maximum *vs P/D* upon titration with poly(G) in 2 mM ABS with 2% of ethanol, C_{dye} = 2 μM, λ_{exc} = 633 nm, λ_{obs} = 670 nm.

MePheo-*a* and Pheo-Pzn +poly(G) at high P/D ratios – 48- and 50-fold enhancement of Pheo emission, rise of *p* to 0.26 and 0.3, substantial red shift of fluorescence band maximum → intercalation of MePheo-*a* and Pheo moiety of Pheo-Pzn between G-tetrads of poly(G) is supposed.

MePheo-*a* and Pheo-Pzn can be proposed as a fluorescent light-up probes for G-quadruplex structure.

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