Optical sensing system for ATP using porphyrin–alkaloid conjugates

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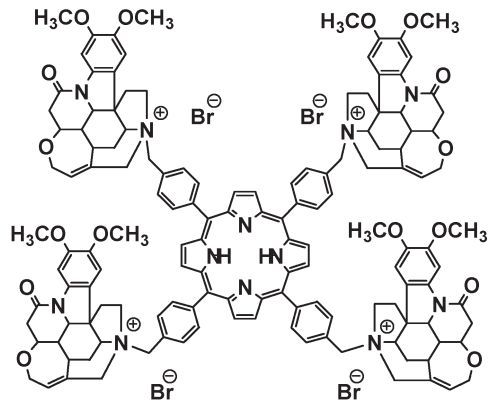
Tetrabrucin–porphyrin as a sensor for ATP was designed and tested; selectivity for ATP was proved in the presence of ADP and AMP.

The sensing of ATP† has received considerable attention in recent years[1] as ATP serves as the primary energy currency of the cell. ATP depletion is a central process in pathogenesis, in particular ischemia,[2] Parkinson's disease,[3] hypoxia and hypoglycemia.[4] ATP plays important roles in sensory transduction acting as a link from non-neural to neural cells.[5] Overabundance of ATP in blood can lead to circulatory shock.[6]

Recently, the interest in ATP sensing *in vivo* has been increasing, and several sensing systems have been reported. For measuring ATP, the luciferase–luciferin method is well known.[7] Although improved, the method still cannot be easily applied to measure ATP *in vivo*. Alternative methods based on the principle of chemosensing have been developed.[8,9] Published optical chemosensors for the detection of ATP are based on a recognition unit containing an aromatic moiety bearing cationic groups. The aromatic part of the sensor recognition unit enables a spectral change in the absorption and/or fluorescence spectrum and binds to the aromatic parts of nucleotides. Simultaneously, the cationic sites of the recognition unit bind the phosphate moieties of nucleotides. Shinkai *et al.* reported the application of cationic substituted polymers for selective ATP sensing.[9]

The quaternary ammonium salt moiety of alkaloids has been long known to have a high affinity for phosphate-containing compounds such as nucleotide phosphates. Yatsimirsky *et al.* have found that a semisynthetic alkaloid-based cyclophane strongly binds ATP.[10] The low selectivity of the cyclophane selector towards other adenosine phosphates (AMP, ADP) can be caused by the insufficient positive charge of the cyclophane. At physiological conditions, the four negative charges of ATP are not fully compensated by the two positive charges of the cyclophane. Therefore, the optimal selector structure should have four positive charges.

The tertrabrucin–porphyrin selector **1**, which we describe here as a sensor for ATP, fulfills the condition of multiple binding modes leading to selectivity for ATP under physiological conditions. Our design is based on a combination of aromatic π–π stacking (porphyrin macrocycle and/or peripheral phenyl ring moiety with adenosine) and sufficient positive charge leading to charge neutralization of the receptor–ATP complex. A porphyrin core enhances the interaction of **1** with ATP by the binding of hydroxyl groups. Moreover, the use of a porphyrin as an excellent signaling unit (chromophore and fluorophore) connected to four binding sites represents a unique selector structure.



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The synthesis and behavior of **1** in solution have been described previously.[11] The association of **1** with anionic guests (AMP, ADP, and ATP) was studied using UV-Vis spectroscopy. Because of the aggregation of **1** in water, all measurements were done in a methanol–HEPES buffer‡ mixture (50 : 50, v/v) and the concentration of **1** was kept constant. Addition of nucleotides causes a decline of the absorbance maximum of **1** (Fig. 1). During

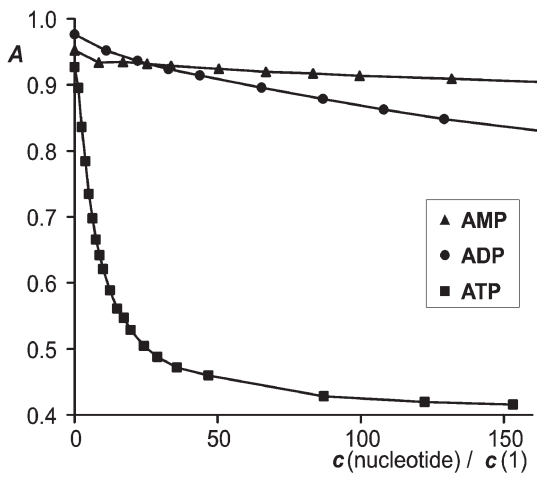

Fig. 1 Dependence of absorbance at 415 nm on the actual concentration of nucleotide. The concentration of **1** was kept constant (1.6 μmol L^{-1}).

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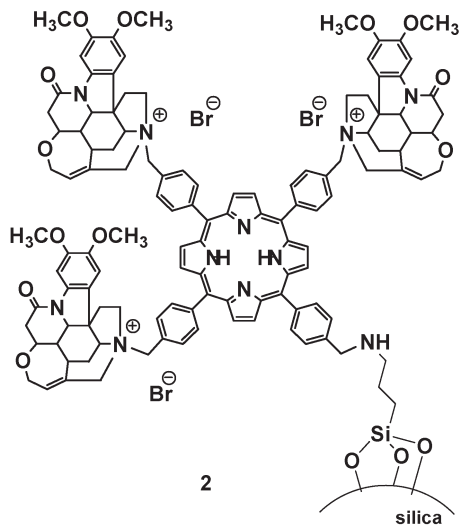
the addition of ATP (Fig. 2), all spectra measured intersect at one point (*i. e.* the isosbestic point), which indicates a 1 : 1 stoichiometry of complex **1** with ATP. The calculated values of the stability constants of 1 : 1 complexes of **1** and nucleotides were 64 000, 6700, and 2400 for ATP, ADP, and AMP, respectively (nonlinear regression of measured data pointed to the creation of a 1 : 1 complex for each analyte used). The estimated values afford evidence for the good selectivity of **1** towards ATP.

We have also used fluorescence spectroscopy in a selectivity binding study. Our data indicate that inorganic phosphate does not interfere with ATP selective binding.

For further evidence of the selective ATP binding, we have investigated the binding selectivity of an immobilized receptor. In spite of the fact that we cannot draw a direct comparison, as immobilized receptor **2** can offer only three quaternized alkaloid recognition sites, we could follow tendencies in selectivity. Thus we immobilized the trisbrucin–porphyrin conjugate on 3-aminopropyl functionalized silica (**2**). The prepared material was used as a stationary phase for HPLC separation of individual nucleotides and mixtures (Fig. 3). In contrast to experiments in solution, when the mobile phase consisted of methanol and HEPES buffer, no analytes eluted. Consequently, a mixture of acetonitrile and phosphate buffer had to be used. In spite of the very small column (amount of the sorbent 0.9 g with 20 μg per gram silica selector loading) repeated analysis gave the same differences in retention factors for the individual nucleotides. No retention of nucleotides on non-modified 3-aminopropyl silica was observed at the same elution conditions. The bottom line is that the selectivity observed in the solution (stability constants) correlates with chromatographic analysis (retention times).



2

^{1}H-NMR experiments[12] were conducted to clarify the structure of the complex. A mixed solvent (D_2O : DMSO-*d6*, 1 : 1 (v/v)) and millimolar concentrations were used to avoid precipitation. The signals of two aromatic protons of the adenine unit originally at positions 5.82 and 8.12 ppm, respectively, were slightly shifted to 5.75 and 8.05 ppm, respectively, when an equimolar mixture of ATP and **1** was measured. According to this slight up-field shift ($\Delta\delta$ = −0.07 ppm, which corresponds to $\Delta\nu$ = 21 Hz) the participation of π–π aromatic interactions between porphyrin and/or peripheral phenyl substituents and the adenine units is expected.

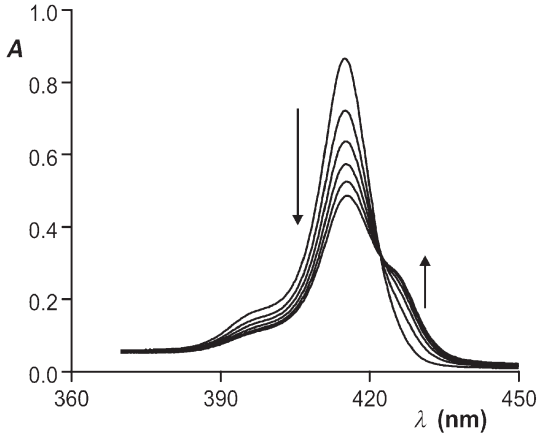


Fig. 2 UV-Vis spectra of the mixture of **1** and ATP with the number of equivalents of ATP added: *n*(ATP) = 0, 8, 15, 23, 30, 38 from the top to the lowest trace.

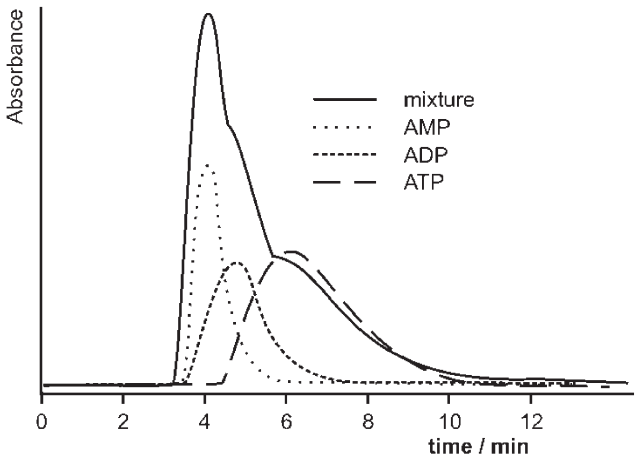


Fig. 3 Separation of a mixture of nucleotides (AMP, ADP, ATP) and individual nucleotides using **2** as a stationary phase. Mobile phase: 90% acetonitrile, 10% phosphate (10 mmol L^{-1}, pH = 8.0). Detection: 268 nm.

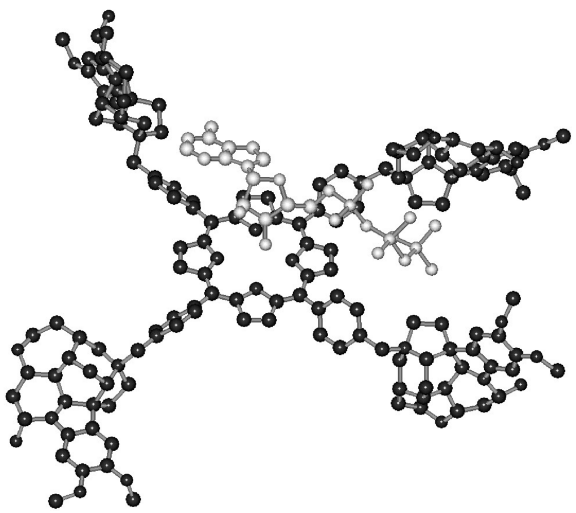


Fig. 4 Calculated structure[13] of complex of **1** with ATP.

The estimated structure of the complex calculated by a semiempirical method[13] is presented in Fig. 4.

The versatility of our design allows introduction of a central metal to the porphyrin core, permitting axial ligand binding to nucleobases. Moreover, we can change the size of the binding pocket going from *para*- to *meta*-substituted systems. These studies are under investigation.

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Notes and references

† ATP = adenosine 5′-triphosphate; ADP = adenosine 5′-diphosphate; AMP = adenosine 5′-monophosphate.
‡ HEPES buffer: 1 mmol L^{-1}, pH = 8.0.

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12 Spectrometer Varian Gemini 300 HC, working frequency 300.075 MHz, deuterium lock, temperature 298 K. All spectra were referenced to DSS (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt).
13 Gaussian program package (M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *GAUSSIAN 03*, Gaussian, Inc., Wallingford, CT, 2004) and the PM3 semiempirical method were used for the minimization of the binding energy and complex geometry.