Ratiometric Detection of ATP by Fluorescent Cyclophanes with Bellows-Type Sensing Mechanism

Aleksandr M. Agafontsev,[a, b] Tatiana A. Shumilova,[b] Aleksandr S. Oshchepkov,[b] Frank Hampel,[c] and Evgeny A. Kataev*[c]
Supporting Information

Ratiometric detection of ATP by fluorescent cyclophanes with harmonica-like sensing mechanism

Aleksandr M. Agafontsev, Tatiana A. Shumilova, Aleksandr S. Oshchepkov, Frank Hampel, and Evgeny A. Kataev

General
All the solvents were dried according to standard procedures. Reactions were performed in oven-dried round bottom flask. Crude products were purified by column chromatography on silica gel 100-200 mesh. TLC plates were visualized by exposure to ultraviolet light and/or by exposure to acidic ethanolic solution of ninhydrin followed by heating (<1 min) on a heat gun (~250 °C). Organic solutions were concentrated on rotary evaporator at 35–40 °C. NMR Spectra: Bruker Avance 600 Mhz. The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants are given in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = double doublet. Integration is determined as the relative number of atoms. The solvent used is reported for each spectrum. Mass Spectra: Finnigan MAT TSQ 7000 (ESI). Melting Point: Melting Points were determined on Büchi SMP or a Lambda PhotometricsOptiMelt MPA 100.

Synthesis of compounds
1,6-Dibromopyrene 1,8-dibromopyrene synthesized according to literature methods without modifications.[1] Already published procedures were used to synthesize pyrene-1,6-dicarbaldehyde or pyrene-1,8-dicarbaldehyde.[2]

The general conditions for the dialdehyde synthesis are the following: A solution of n-butyllithium in hexane (2.5M, 6 equiv) was added dropwise to a solution of a mixture of 1,6- and 1,8-dibromopyrene (1:1 molar ration obtained from the previous reaction) in anhydrous THF under an atmosphere of argon at –50°C. Reaction was stirred for 20 min at this temperature and then warmed to 25°C and stirred for additional 1 h. The reaction mixture was cooled to ~40°C and DMF (6 equiv) was added dropwise to the solution. The mixture was stirred at room temperature for 12 h before water was added. THF was removed under reduced pressure. The resulting residue was suspended in 100 ml of H2O and 200 ml DCM and the aqueous phase was extracted three times with 200 ml of CH2Cl2. The organic phases were combined and dried over Na2SO4. Solvents were removed under reduced pressure. The residue was purified by a column chromatography on silica gel using eluent CH2Cl2-hexane 1:1 with decreasing hexane portion to pure DCM. The first fraction contained pyrene-1,6-dicarbaldehyde (25% yield), the second fraction contained pyrene-1,8-dicarbaldehyde (21% yield).
The general method for the synthesis of pyrene-macrocycles

The synthesis of macrocyclic receptors was adapted from the method described by Fabbrizzi.\[^3\] Pyrene-1,8-dicarbalddehyde or pyrene-1,6-dicarbalddehyde (3 mM, 774 mg) was added to a round bottom flask, then 400 ml of acetonitrile and 40 ml of methanol were added. The flask was placed in an oil bath and heated to 50°C with stirring, until the dialdehyde was completely dissolved. The appropriate amine (3 mmol) was dissolved in 100 ml of acetonitrile. The resulting solution was slowly added from the dropping funnel to the pyrene-1,8-dicarbalddehyde or pyrene-1,6-dicarbalddehyde solution with stirring and heating at 50°C. The reaction was achieved by heating at 50°C for 72 h. The solvent was removed under reduced pressure without heating. To the residue was added 300 ml of methanol and 30 mmoles of sodium borohydride, the flask was placed in an oil bath and heated to 50°C for 3 hours, then the reaction was kept overnight at room temperature. Methanol was removed under reduced pressure. The resulting solid was suspended in 100 ml of H$_2$O and 100 ml mixture chloroform-ethanol 10:100 and the aqueous phase was extracted with three portions 100 ml of mixture chloroform-ethanol 10:100. The organic phases were collected and dried with Na$_2$SO$_4$. Solvents were removed under reduced pressure. The residue was purified by chromatography on silica gel using eluent ethanol-chloroform 1:1 to ethanol-chloroform-ammonia 100:100:5.

6,16-dioxa-3,9,13,19-tetraaza-1,11(1,6)-dipyrenacycloicosaphane

The product is a pale yellow powder. Yield: 43%. M.p. 176-179°C. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.96 (d, 4H), 7.57 (d, 4H), 7.48 (d, 4H), 7.38 (d, 4H), 4.33 (s, 8H), 3.76 (t, 8H), 3.10 (t, 8H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 133.2, 129.8, 128.7, 127.4, 126.7, 124.6, 124.5, 121.9, 70.7, 52.2, 49.6. HRMS (ESI-TOF) m/z: [M+H]$^+$ calcd for C$_{44}$H$_{45}$N$_4$O$_2$, 661.3537, found 661.3541.
Figure S1. $^1$H and $^{13}$C NMR spectra of compound 4 measured in CDCl$_3$.

6,16-dioxa-3,9,13,19-tetraaza-1,11(1,8)-dipyrenacycloicosaphane

The product was obtained after chromatography as a pale yellow powder. Yield: 43%. M.p. 192-195°C. $^1$H NMR (600 MHz, CD$_3$OD, δ, ppm) 7.72 (s, 4H), 7.40 (s, 4H), 7.24 (m, 4H), 7.05 (d, 4H), 3.92 (s, 8H), 3.37 (t, 8H), 2.68 (t, 8H). $^{13}$C NMR (151 MHz, CD$_3$OD) δ 131.2, 130.2, 127.3, 126.7, 125.8, 125.8, 124.3, 124.2, 122.1, 68.8, 50.2. HRMS (ESI-TOF) m/z: calcd for C$_{44}$H$_{45}$N$_4$O$_2$ [M + H]$^+$ 661.3537, found: m/z = 661.3539.
Figure S2. $^1$H and $^{13}$C NMR spectra of compound 2 measured in CD$_3$OD.
**3,6,9,13,16,19-hexaaza-1,11(1,6)-dipyrenacycloicosaphane**

The product was obtained after chromatography as a pale yellow powder. Yield: 56%. M.p. 180-183°C. 

$^1$H NMR (600 MHz, CDCl$_3$, δ, ppm) 8.15 (d, 4H), 7.65 (d, 4H), 7.62 (d, 4H), 7.48 (d, 4H), 4.28 (s, 8H), 2.95 (t, 8H), 2.76 (t, 8H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 133.8, 130.2, 129.2, 127.5, 126.9, 125.1, 124.6, 122.8, 52.2, 49.5, 49.0. HRMS (ESI-TOF) m/z: calcd for C$_{44}$H$_{47}$N$_6$ [M + H]$^+$ 659.3856, found 659.3857.
Figure S3. $^1$H and $^{13}$C NMR spectra of compound 3 measured in CDCl$_3$.

3,6,9,13,16,19-hexaaaza-1,11(1,8)-dipyrenacycloicosaphane

The product was obtained after chromatography as a pale yellow powder. Yield: 29%. M.p. 158-161°C. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$, ppm) 8.25 (s, 4H), 7.82 (s, 4H), 7.79 (d, 4H), 7.72 (d, 4H), 4.25 (s, 8H), 2.79 (t, 8H), 2.65 (t, 8H). $^{13}$C NMR (151 MHz, CDCl$_3$, $\delta$, ppm) 133.8, 130.8, 128.6, 127.2, 126.7, 125.3, 124.7, 123.4, 51.8, 48.8, 48.7. HRMS (ESI-TOF) m/z: calcd for C$_{44}$H$_{47}$N$_6$ $[M + H]^+$ 659.3856, found 659.3859.
Figure S4. $^1$H and $^{13}$C NMR spectra of compound 1 measured in CDCl$_3$. 
Figure S5. COSY and ROESY spectra of compound 1 measured in DMSO-\textit{d6}.
**Potentiometric studies of receptor 1.**

All solutions were prepared in 0.05M NaCl solution with ca. 0.5 mM concentration of compounds in deionised water. For titrations standard 0.1M solution of NaOH was used. The potentiometric titrations were carried out on a Mettler Toledo G20 Titrator equipped with a DGi 102-Mini pH-electrode. The electrode was calibrated with standard calibrating solutions from Mettler Toledo. The reaction vessel was kept at constant temperature 23°C. The value of $K'w$ was determined from data obtained in the alkaline range of the titration, and found to be equal to 13-14.00 in our experimental conditions. The titration experiment was carried out as follows: in the reaction vessel was placed a solution of a compound (and calculated amount of HCl); after stirring the solution for 5 minutes the titrations was started. The experiment was repeated 3-5 times. For the experiment in the presence of an anion, the corresponding amount of its solution was added prior to the titrations. The obtained data was imported to the HYPEQUAD 2008 program and fitted to obtained protonation constants.[4]

---

**Figure S6.** ROESY spectra of compound 1 measured in a 50 mM MES buffer pH 6.2.
Figure S7. Distribution diagram of protonated forms of receptor 1 depending on the pH of the solution.

Fluorescence studies

Relationship between pH and fluorescence intensity

The solution with a desired pH value were prepared by adjusting 50 mM solution of acetic acid with an appropriate amount of sodium hydroxide. Receptor concentration $10^{-5}$ M was achieved by addition of a receptor in DMSO to an aqueous solution with the fixed pH. Content of DMSO in the final solution was 6% vol. Excitation at 350 nm, slit 2:1.

Figure S8. Fluorescence spectra of receptor 1 depending on the pH of the solution.

Competitive binding of ATP

The competition experiment was conducted as follows: $10^{-5}$ M solution of the receptor in a buffered solution (50 mM MES, pH 6.2, 6% DMSO) was treated first with excess ATP (50 equiv) dissolved in the same buffer and fluorescence was measured before and after addition. The ratio of intensities ($I/I_0$) represents the relative fluorescence increase after addition of ATP (marked and “None” below in Figure). The following experiments were done with a competing nucleotide and a mixture of a competing nucleotide with ATP. For example, the fluorescence the receptor was measured a) in the presence of 100 equiv of GTP and b) in the presence of 100 equiv of GTP together with 50 equiv of ATP (see Figure below).
Figure S9. Competitive experiment for receptor 1 for ATP detection in the presence of other nucleotides. “None” corresponds to fluorescence changes after addition of 50 equiv of ATP. “GTP” corresponds to fluorescence changes after addition of 100 equiv. of GTP. “GTP+ATP” corresponds to fluorescence changes after addition 100 equiv. of GTP and 50 equiv. of ATP.

Fluorescence titration of receptors with nucleotides
The receptors (0.01 mM) were dissolved in a buffered solution (50mM MES, pH 6.2, 6%DMSO) and then titrated with nucleotides (0.02M) followed by fluorescence measurements of each titration point.
Figure S10. Fluorescence changes for 1 induced by addition of NTPs and other nucleoside mono- and diphosphates.
Figure S11. Fluorescence changes for 3 induced by addition of NTPs and other nucleoside mono- and diphosphates.
Figure S12. Fluorescence changes for 2 induced by addition of NTPs and other nucleoside mono- and diphosphates.
Figure S13. Fluorescence changes for 4 induced by addition of NTPs and other nucleoside mono- and diphosphates.
UV-Vis studies

UV-Vis titrations were carried out in the same manner as the fluorescence titrations. To a receptor solution with $10^{-5}$ M concentration, a solution of the receptor ($10^{-5}$ M) and a nucleotide (0.02 M) was added in portions. At each step a spectrum was measured. All the spectra were combined by using HypSpec program and fitted to yield the binding constants.

**Figure S14.** UV-Vis spectral changes upon dilution of receptor 1 with linear Lambert-Beer dependence, which indicates the absence of receptor aggregation in aqueous solution. Conditions: 50 mM MES buffer, pH 6.2, 6% DMSO.
Figure S15. UV-Vis changes and fitting curves for receptor 1 with selected nucleotides. The calculated binding constants are:

ATP: log$K_{11}$=5.50, log$K_{12}$=4.12
GTP: log$K_{11}$=5.82, log$K_{12}$=4.43
CTP: log$K_{11}$=5.06, log$K_{12}$=3.71
UTP: log$K_{11}$=5.05, log$K_{12}$=3.82
TTP: log$K_{11}$=5.05, log$K_{12}$=3.82.
ITC titration of 1 with ATP.

The ITC experiments were evaluated by the help of the program SupraFit [C. Hübler, Institut of Organic Chemistry, TU Freiberg. Suprafit https://github.com/conradhuebler/SupraFit 02.2020]. The calculated results are in good agreement with those obtained by the program NanoAnalyze™ (TA Instruments). In the fitting average n values is 1.5.

**Figure S16.** ITC titration: heat vs. time plots for addition of Na$_2$C$_2$O$_4$ and NaClO$_4$ together with fitting graphics.

DFT calculations

Coordinates of the adenosine complex with 1H$_4$$^{4+}$

```
MOL> cartesian
MOL> 7 3.86129517 1.02124969 -2.87970200
MOL> 7 -1.46119457 6.40528557 -2.23717088
```
|   |   |   |   |   |   |
|---|---|---|---|---|---|
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |
X-ray studies

Single clear light colourless plate crystals of 19Kat_OSH01_2 recrystallised from a mixture of methanol and TCM by solvent layering. A suitable crystal with dimensions 0.39 × 0.25 × 0.11 mm³ was selected and mounted on a mylar loop in perfluoroether oil on a SuperNova, Dual, Cu at home/near, Atlas diffractometer. The crystal was kept at a steady T = 152.95(10) K during data collection. The structure was solved with the ShelXT (Sheldrick, 2015) solution program using dual methods and by using Olex2[5] as the graphical interface. The model was refined with ShelXL 2018/3 (Sheldrick, 2015) using full matrix least squares minimisation on $F^2$.

Crystal Data. C₂₈H₂₁N₂O₂₂, $M_r$ = 859.10, monoclinic, $P2_1/c$ (No. 14), $a = 17.8077(5)$ Å, $b = 14.2917(4)$ Å, $c = 8.9714(3)$ Å, $\beta = 96.735(3)$°, $\alpha = \gamma = 90°$, $V = 2267.49(12)$ Å³, $T = 152.95(10)$ K, $Z = 2$, $Z' = 0.5$, $\mu$(Cu K$_\alpha$) = 0.691, 6870 reflections measured, 3613 unique ($R_{int} = 0.0228$) which were used in all calculations. The final $wR_2$ was 0.2295 (all data) and $R_1$ was 0.0720 (I > 2I).
A clear light colourless plate-shaped crystal with dimensions 0.39 × 0.25 × 0.11 mm³ was mounted on a mylar loop in perfluoroether oil. Data were collected using a SuperNova, Dual, Cu at home/near, Atlas diffractometer equipped with a Cryojet Oxford Instruments low-temperature device operating at T = 152.95(10) K.

Data were measured using ω scans using Cu Kα radiation. The diffraction pattern was indexed and the total number of runs and images was based on the strategy calculation from the program CrysAlisPro (Rigaku, V1.171.40.53, 2019). The maximum resolution that was achieved was Θ = 63.693° (0.86 Å).

The diffraction pattern was indexed and the total number of runs and images was based on the strategy calculation from the program CrysAlisPro (Rigaku, V1.171.40.53, 2019)The unit cell was refined using CrysAlisPro (Rigaku, V1.171.40.53, 2019) on 2494 reflections, 36% of the observed reflections.

Data reduction, scaling and absorption corrections were performed using CrysAlisPro (Rigaku, V1.171.40.53, 2019). The final completeness is 96.30 % out to 63.693° in Θ. A gaussian absorption correction was performed using CrysAlisPro 1.171.40.53 (Rigaku Oxford Diffraction, 2019) Numerical absorption correction based on gaussian integration over a multifacetted crystal model Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. The absorption coefficient μ of this material is 0.691 mm⁻¹ at this wavelength (λ = 1.54184Å) and the minimum and maximum transmissions are 0.536 and 1.000.

The structure was solved and the space group P2₁/c (# 14) determined by the ShelXT (Sheldrick, 2015) structure solution program using using dual methods and refined by full matrix least squares minimisation on F² using version 2018/3 of ShelXL 2018/3 (Sheldrick, 2015). All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model. Most hydrogen atom positions were calculated geometrically and refined using the riding model, but some hydrogen atoms were refined freely.

_diffrn_special_details: The asym unit contains two molecules of H2O and two molecules of MeOH

_exptl_absorpt_process_details: CrysAlisPro 1.171.40.53. Numerical absorption correction based on gaussian integration over a multifacetted crystal model Empirical absorption correction using spherical harmonics implemented in SCALE3 ABSPACK.

CCDC Deposition Number 1985471.

References

[1] M. V. Ivanov, K. Thakur, A. Boddeda, D. N. Wang, R. Rathore, J Phys Chem C 2017, 121, 9202-9208.
[2] Y. Niko, S. Sasaki, K. Narushima, D. K. Sharma, M. Vacha, G.-i. Konishi, J Org Chem 2015, 80, 10794-10805.
[3] L. Fabbrizi, M. Licchelli, N. Marcotte, F. Stomeo, A. Taglietti, Supramol Chem 2002, 14, 127-132.
[4] P. Gans, A. Sabatini, A. Vacca, Talanta 1996, 43, 1739-1753.
[5] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, J Appl Crystallogr 2009, 42, 339-341.