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A Ratiometric Fluorescent On–Off Zn^{2+} Chemosensor Based on a Tripropargylamine Pyrene Azide Click Adduct

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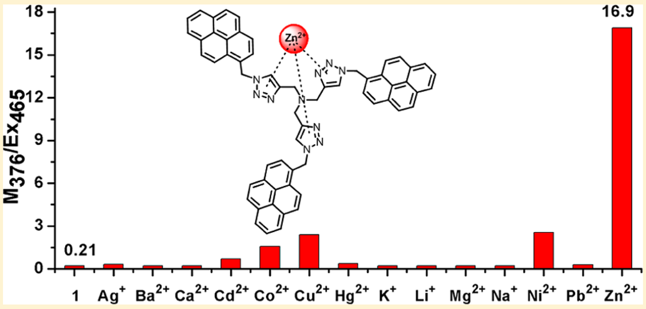
Supporting Information

ABSTRACT: A new, easy-to-prepare and highly selective pyrene-linked tris-triazole amine fluorescent chemosensor has been designed from tripropargylamine and pyrene azide using Cu(I)-catalyzed click chemistry. The fluorescence on–off sensor **1** is highly selective for Zn^{2+} displaying a ratiometric change in emission. The relative intensity ratio of monomer to excimer fluorescence (M_{376}/E_{465}) of the sensor increases 80-fold upon the addition of 10 equiv of Zn^{2+} ions (with a detection limit of 0.2 μM).



Fluorescent chemosensors for zinc ions have received wide attention because of the indispensable roles of zinc in living matter.[1] Zinc is the second most abundant transition metal in mammals. Zinc ions play vital roles in various biological processes, e.g., as a cofactor in metalloproteins, in neurotransmission, in signal transduction, and as a regulator of gene expression and cellular apoptosis.[1] Zinc is reported to be responsible for neurological disorders, developmental defects, and malfunctions.[2] The detection of zinc ions is significant for the study of its biochemical functions, the quality control of food and nutrients for zinc supplements, as well as for the assessment of environmental samples.[1,3] Some plants are adapted to higher zinc concentration and grow only on zinc-contaminated soil. The so-called "Galmei-Veilchen" (*Viola calaminaria*) found in the vicinity of Aachen, Germany, is a flower named after the mineral calamine ($ZnCO_3$). Furthermore, zinc metal plating is the most important process to avoid corrosion of steel and plays a major role in material science. Zinc complexes (in particular with $ZnCl_2$) are used as moderately strong Lewis acids in cyclization as well as in nucleophilic and electrophilic substitution reactions.[4]

Most of the fluorogenic ion sensor molecules are composed of an ion recognition unit decorated with a fluorophore. Among the various fluorophores, pyrene shows photophysical properties that make it appropriate for such purposes due to its high fluorescence quantum yield, chemical stability, and long fluorescence lifetime.[5a,b] Additionally, pyrene shows monomer–excimer dual fluorescence,[5c] and the fluorescence intensity ratio of the excimer to monomer emission (I_{Ex}/I_M) is sensitive to conformational changes of the pyrene-functionalized system.[5a,d] Consequently, pyrene sensors have been constructed for metal ion detection.[6] In view of the importance of zinc, Zn^{2+} selective sensors have been developed recently.[7] However, most of them have limitations, such as turn-off responses and interferences with other metal ions, especially with Cd^{2+}.[8] Furthermore, these sensors are often structurally complicated and require a laborious multistep organic synthesis, which causes prohibitively high costs.[9] Therefore, the development of simple and easily accessible Zn(II) chemosensor molecules is still a challenge.

The copper(I)-catalyzed Huisgen–Meldal–Sharpless cycloaddition "click" chemistry has already been used to synthesize a significant number of metal ion sensors.[10] The synthetic simplicity of the reaction and the ability of the formed 1,2,3-triazole system to act as a binding pocket makes it useful for various applications. Recently, we described oligonucleotide tripropargylamine pyrene click adducts for labeling of DNA or the formation of dendronized oligonucleotides.[11] Herein, we report on the design and synthesis of a new propeller like, highly selective turn-on fluorescent sensor for Zn^{2+} ions based on a tripropargylamine pyrene click adduct (**1**) and a bis-functionalized intermediate (**2**) which might be used for sensor modified surfaces (Figure 1).

The tripropargylamine pyrene-click adducts were synthesized by the copper(I)-catalyzed Huisgen–Meldal–Sharpless azide–alkyne cycloaddition reaction (CuAAC).[12] For this, tripropargylamine (**3**) was treated with 1-azidomethylpyrene (**4**)[8a] in presence of 2,6-lutidine and Cu(I) (1 mol %) in acetonitrile at room temperature for 3 days. Under these conditions, the reaction did not proceed to completion, and apart from the click conjugate **1** (29% yield) click conjugate **2** (25%) was

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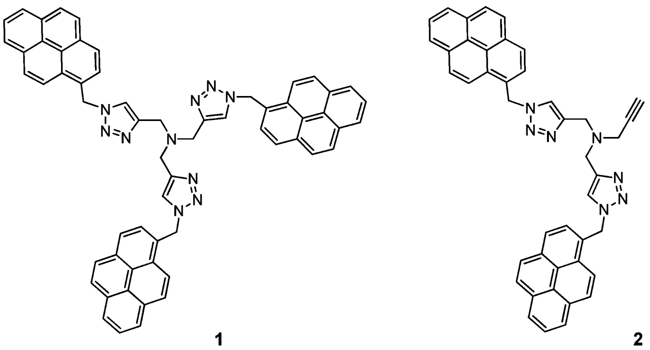


1 **2**

Figure 1. Tripropargylamine pyrene click adducts.

formed. Recently, Zhu et al. showed that the click reaction of tripropargylamine and azides proceeds in a sequential fashion due to product inhibition, forming a mono-, bis-, and finally a tris-triazole.[12d] Our reaction proceeded stepwise as well. We found that the amount of copper was less for completion of the reaction.[11c] When the alkyne/Cu(I) ratio was increased from 1 mol % to 4.5 mol %, the click conjugate **1** was the major product (62%) and click conjugate **2** was the minor compound (10%) (Scheme 1).

Compounds **1** and **2** were characterized by ^{1}H and ^{13}C NMR spectra and mass spectroscopy. ^{1}H NMR spectra of **1** revealed the disappearance of the three terminal C≡CH hydrogens, whereas the new singlet appearing around δ = 7.28 ppm was attributed to the protons of the newly formed triazole skeleton. In compound **2**, one terminal triple bond hydrogen signal was found at 2.06 ppm, and the two newly formed triazole protons gave a signal at 7.19 ppm (for details, see the Supporting Information).

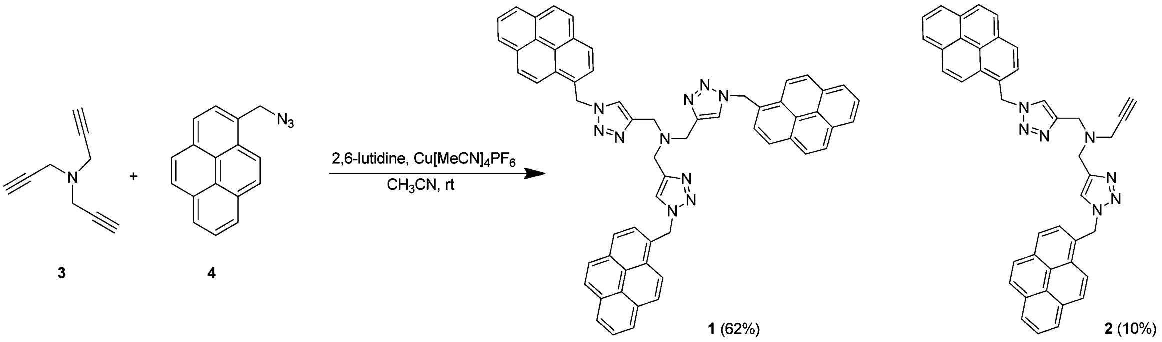
Next, the cation-binding properties of compounds **1** and **2**, both featuring triazole binding sites, were characterized by UV−vis and fluorescence spectroscopy. Spectroscopic measurements were carried out in CH_3CN/CH_2Cl_2 (1000:1, v/v) by adding aliquots of different metal cations Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Na^+, Ni^{2+}, Pb^{2+}, and Zn^{2+} (as chloride salts; for Ag^+ $AgNO_3$ was used) dissolved in water. The UV−vis absorption spectra of compounds **1** and **2** exhibit typical pyrene absorption bands in the 232−340 nm region (Figure S1, Supporting Information). Compound **1** decorated with three pyrene residues shows a higher UV absorbance at 340 nm compared to compound **2**. No differences were observed regarding the wavelength maxima, retaining similar absorption pattern. Interaction of **1** with 10 equiv of various metal ions did not result in any significant changes in the absorption spectrum (Figure S2, Supporting Information).

The fluorescence spectra of **1** and **2** show a strong excimer emission at 465 nm and a weak monomer emission at 376 and 396 nm (excitation wavelength 340 nm), with an intensity ratio of monomer to excimer emission ($I_M/I_E \approx I_{376}/I_{465}$) = 0.21 for **1** and 0.33 for **2** (Figure S1, Supporting Information). The formation of an excimer band at 465 nm indicates a strong face to face $\pi-\pi$ stacking between two pyrene units. In compounds **1** and **2**, the ratio of monomer to excimer emission is barely changed within a concentration range of 0.5 μM to 2 μM, indicating that the excimer emission results from an intramolecular excimer but not from intermolecular interactions (Figures S3 and S4, Supporting Information).

To obtain insight into the binding properties of **1** and **2** toward metal ions, we investigated the fluorescence changes upon addition of a wide range of metal cations (10 equiv) using their chloride salts in aqueous solution. As shown in Figure 2a, the addition of Zn(II) to a solution of **1** leads to a significant increase in the emission bands of the pyrene monomer fluorescence (at 376 and 396 nm), whereas the pyrene excimer emission at 465 nm declines concomitantly to show a ratiometric change. The quantum yield of the free sensor **1** is Φ = 0.004 for the monomer emission (376 nm) and Φ = 0.073 for the excimer emission (465 nm). The quantum yield of the **1**-Zn^{2+} complex is Φ = 0.03 (monomer emission). By contrast, no significant spectral changes were observed upon addition of most of the other metal ions. Both monomer and excimer emission were strongly quenched by Cu^{2+}, Co^{2+} and Ni^{2+} ions because of the heavy metal ion effect;[9a,b] in these cases the quenching is not ratiometric (Figures 2a and 2b). In case of compound **2**, the fluorescence spectral changes observed upon addition of various metal ions show that it is selective for Zn^{2+} and Cd^{2+} over other metal ions (Figure S5, Supporting Information). Similar observations were also found by Park et al. for pyrenyl-appended triazole-based calix[4]arene, where two triazole rings selectively bind Zn^{2+} and Cd^{2+}.[8a]

The relative intensity ratio of monomer to excimer emission (M_{376}/E_{465}) of the free sensor **1** was 0.21 and increased by 80-fold to 16.9 upon the addition of 10 equiv of Zn^{2+} (Figure 2b), which is attributed to formation of a **1**−Zn^{2+} complex. On the contrary, the relative intensity ratio M_{376}/E_{465} of sensor **2** increased by only 11-fold under identical conditions. We anticipitate that excimer quenching in compounds **1** and **2** is due to conformational change that take place during the binding of a zinc ion to the nitrogen atoms of the triazole ring systems. In this altered conformation, the coordination forces move the pyrene groups far away from each other inhibiting the

Scheme 1. Synthesis of the Click Adducts 1 and 2



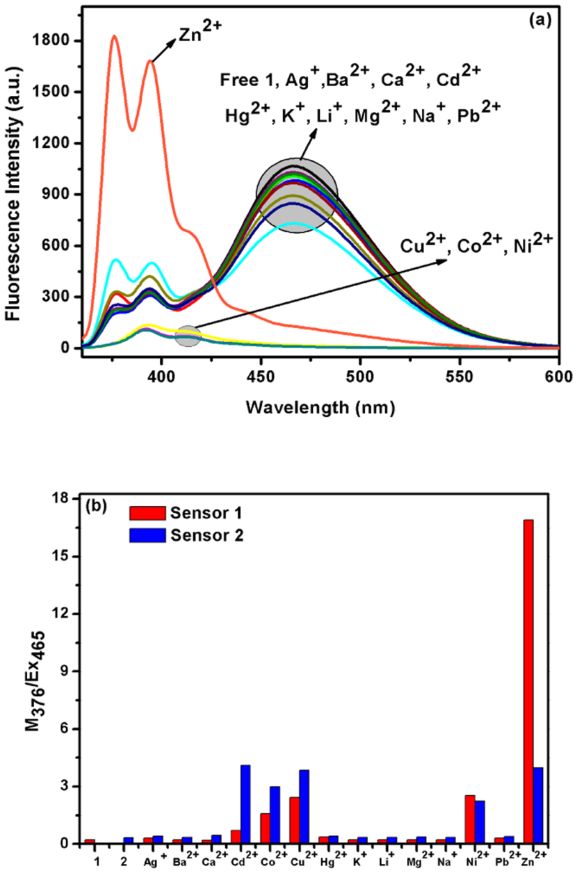


Figure 2. (a) Fluorescence spectra of compound **1** (1 μM, λ_{ex} = 340 nm); (b) ratiometric (M_{376}/E_{465}) selectivity of compound **1** (red bar) and **2** (blue bar) (1 μM) upon addition of various metal ions (10 equiv) as their aqueous solutions.

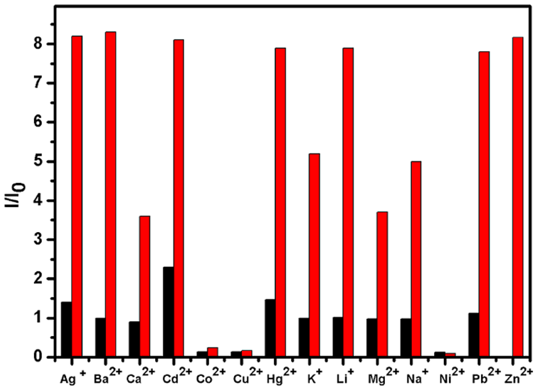


Figure 3. Fluorescence response of **1** (1 μM) in CH_3CN/CH_2Cl_2 (1000:1, v/v) to 10 μM of various tested metal ions (black bar) and to the mixture of 10 μM of tested metal ions with 10 μM Zn^{2+} ion (red bar). I_0 is the fluorescence intensity for free **1**, and I is the fluorescence intensity after addition of metal ions with an excitation at 340 nm.

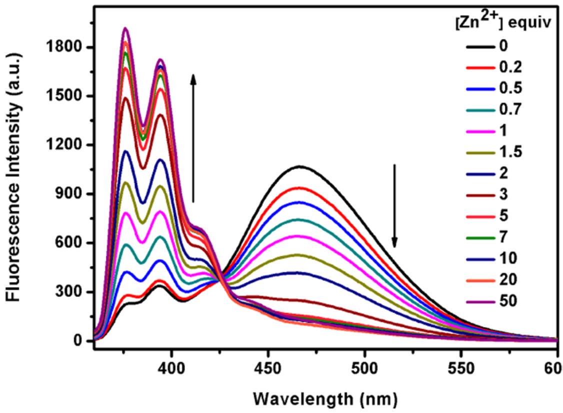


Figure 4. (a) Fluorescence spectra of compound **1** (1 μM), upon addition of an increasing concentration of Zn^{2+} ions (0−50 equiv) measured in CH_3CN/CH_2Cl_2 (1000:1, v/v). λ_{ex} = 340 nm.

$\pi-\pi$ stacking of the pyrene moieties which is necessary for the generation of excimer emission. Thus, it is likely that the three triazole units of compound **1** form a selective binding pocket for Zn^{2+} ions which is less specific for the two triazole units of compound **2**.

An important aspect of many prospective metal ion sensors is their ability to detect metal ions selectively over other competing metal ions. To utilize compounds **1** and **2** as Zn^{2+} ion-selective fluorescence sensor, competition experiments were carried out in the presence of Zn^{2+} (10 μM) mixed with 10 μM of another cation (Figure 3). As shown in Figure 3, no significant interference in detection of Zn^{2+} was observed in the presence of many competitive metal ions except for Co^{2+}, Cu^{2+}, and Ni^{2+}. However, smaller fluorescence changes were observed in the presence of Ca^{2+}, K^+, Mg^{2+}, and Na^+. On the contrary, the selective fluorescence response of sensor **2** toward Zn^{2+} is affected by a much broader range of competing metal ions (Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, Li^+, Mg^{2+}, Na^+ ,and Ni^{2+}) (Figure S6, Supporting Information). This demonstrates the exceptional selectivity of the sensor **1** for Zn^{2+}.

The fluorescence response of **1** in the presence of an increasing concentration of Zn^{2+} ions is shown in Figure 4. Addition of Zn^{2+} ions (0−50 equiv) to a solution of **1** containing three pyrene-triazoles as metal ion chelating ligands induced remarkable ratiometric changes, where the monomer emission increases as its excimer emission declines. This may be attributed to the flexibility of the triazole moieties of **1** enabling them to adopt the appropriate geometry for the binding of the Zn^{2+} ion. Meanwhile, a discernible isoemissive point was observed at 425 nm. More importantly, the ratios of the fluorescent intensities are linearly proportional to the amount of Zn^{2+} from 0 to 10 μM (Figure S7, Supporting Information). The ratiometric fluorescent measurement which is based on the ratio of two fluorescent bands, makes it possible to measure the analyte very accurately.[13] The addition of NaH_2PO_4 (0−20 equiv) to the **1**−Zn^{2+} complex leads to reversible change of the fluorescence; the monomer emission of the pyrene moiety decreases with a concomitant increase of the excimer emission (off−on) (Figure S8, Supporting Information). This results from the competition of the $H_2PO_4^-$ anion and the sensor molecule for the Zn^{2+} cation.[14] Sensor **2** also shows a ratiometric change in presence of an increasing concentration of Zn^{2+} (Figure S9, Supporting Information). However, compound **2** is less selective and recognizes Cd^{2+} almost equally well (Figure S9, Supporting Information). Moreover, the association constant (K_a) for **1** with Zn^{2+} was found to be 7.0×10^5 M^{-1}, obtained by a nonlinear curve fitting of the fluorescence titration results (Figure S10, Supporting Information). The binding constant for the **1**−Cu^{2+} complex, which is nonratiometric, was determined for comparison (Figure S11, Supporting Information). The fluorescent titration profile of **1** with Zn^{2+} (Figure S12, Supporting Information) demonstrated that the detection limit of Zn^{2+} is 2.0×10^{-7} M under the experimental conditions used here. The combination of the low detection limit and the large fluorescence dynamic range indicates that compound **1** is highly sensitive to Zn^{2+}.

In order to quantify the stoichiometry of the complex of **1** and the Zn^{2+} ion, a Job plot analysis (method of continuous variation) was carried out, in which the emission of complexes at 376 nm were plotted against molar fractions of **1** and Zn^{2+} under the conditions of an invariant total concentration. The maximum point appears at a mole fraction of 0.5, which corresponds to a 1:1 complex of compound **1** and Zn^{2+} (Figure S13, Supporting Information).

To further obtain more detailed information on the complexation mode of sensor **1** (Figure S14, Supporting Information), we measured ^{1}H NMR spectra of **1** in the presence of Zn^{2+} in $CDCl_3/CH_3CN$ (10:1, v/v) (Figure S15, Supporting Information). Upon addition of 1.0 equiv of Zn^{2+}, considerable downfield shifts of the triazole and methylene protons were noted (Table S1, Supporting Information). The metal ion-induced chemical shift changes suggest that probably the nitrogen atoms of the three triazole groups and the tertiary amino group of **1** are involved in zinc binding. This binding mode is in line with the results obtained from fluorescence spectroscopy. Fluorescence changes were attributed to conformational alterations of the triazole units upon forming a 1:1 **1**−Zn^{2+} complex. Complex formation probably enforces the separation of the two pyrene units, necessary for excimer emission, and prevents their $\pi-\pi$ stacking interactions leading to a concomitant increase of monomer fluorescence intensity as it was observed for **1**.

In conclusion, we developed a new type of fluorescent on−off sensors (**1** and **2**) based on tripropargylamine−pyrene click adducts, which display a ratiometric selectivity for the Zn^{2+} ion. Sensor **1** possesses a high affinity and selectivity for zinc ions relative to most other competitive metal ions by enhancement of monomer emission. Further, the terminal triple bond of sensor **2** can be used to immobilize this sensor on a surface by click chemistry.[15]

■ EXPERIMENTAL SECTION

Synthesis of Compounds 1 and 2. Tripropargylamine (0.025 g, 0.19 mmol) in acetonitrile (2 mL) was treated sequentially with 1-azidomethyl pyrene (0.220 g, 0.86 mmol), 2,6-lutidine (0.020 g, 0.19 mmol), and $Cu(MeCN)_4PF_6$ (4.5 mol %). Then, the reaction mixture was stirred at room temperature for 3 days, concentrated, and subjected to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 95:5). From the main zone, compound **1** (0.106 g, 62%, colorless solid) was isolated as a major product and compound **2** (0.017 g, 10%, light yellow solid) was isolated as minor product.

***N,N*-Tris[[(1-pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl]methyl]-amine (1):** mp 149−152 °C; TLC (CH_2Cl_2/MeOH 95:5) R_f 0.29; UV λ_{max} (CH_3CN/CH_2Cl_2 1000:1, v/v) (nm) 233 (ε (dm^3 mol^{-1} cm^{-1}) 163300), 242 (216900), 265 (84200), 275 (145900), 326 (82400), 340 (102300); ^{1}H NMR ($CDCl_3$, 300 MHz) (δ, ppm) 3.52 (s, 6H), 6.03 (s, 6H), 7.28 (s, 3H), 7.73 (d, J = 7.8 Hz, 3H), 7.95−8.18 (m, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm) 144.4, 132.0, 131.1, 130.5, 129.1, 128.8, 127.4, 127.2, 127.0, 126.3, 125.8, 125.7, 125.0, 124.5, 123.4, 121.9, 52.3, 47.3; ESI-TOF m/z calcd for $C_{60}H_{42}N_{10}$ [M + H]$^+$ 903.3673, found 903.3667.

***N,N*-Bis[[1-(pyren-1-ylmethyl)-1*H*-1,2,3-triazol-4-yl]methyl]-prop-2-yn-1-amine (2):** mp 225−228 °C; TLC (CH_2Cl_2/MeOH 95:5) R_f 0.32; UV λ_{max} (CH_3CN/CH_2Cl_2 1000:1, v/v) (nm) 233 (ε/dm^3 mol^{-1} cm^{-1} 96300), 242 (111000), 265 (49600), 275 (81100), 326 (40100), 340 (48700); ^{1}H NMR ($CDCl_3$, 300 MHz) (δ, ppm) 2.05−2.06 (t, J = 2.4 Hz, 1H), 3.17−3.18 (d, J = 2.4 Hz, 2H), 3.66 (s, 4H), 6.12 (s, 4H), 7.19 (s, 2H), 7.82−7.84 (d, J = 7.8 Hz, 2H), 7.99−8.23 (m, 16H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm) 144.9, 132.1, 131.2, 130.5, 129.2, 128.9, 128.3, 127.5, 127.2, 126.9, 126.4, 125.9, 125.8, 125.0, 124.9, 124.4, 122.8, 121.9, 77.2, 73.6, 52.4, 47.8, 42.0; ESI-TOF m/z calcd for $C_{43}H_{31}N_7$ [M + H]$^+$ 646.2720, found 646.2714.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra and additional spectroscopic data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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