Fluorescent Molecular Photoswitches for the Generation of All-Optical Encryption Keys

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Fluorescent Molecular Photoswitches for the Generation of All-Optical Encryption Keys

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Herein, we report a tri-component photochromic molecular cocktail that can be used to encrypt and decrypt information. The time-dependent fluorescent response of this cocktail is highly non-linear with respect to the set of inputs used (concentrations of the three photochromic components, excitation- and emission wavelengths), a property required for the generation of so-called encryption keys. The all-optical system can generate more than 80 million unique fluorescence responses by applying different input combinations and is operated using a conventional fluorimeter.

Introduction

Encryption and decryption are part of our daily lives.[1] Internet banking is an obvious example, but activities like watching TV and blipping your bus pass also implies that the transmission of information is secured in a similar manner. Encryption/decryption of data is conventionally done by highly complex mathematical algorithms and so-called encryption keys. A key is typically a 128-bit number that is used to scramble the information in the encryption process, orchestrated by the algorithm. Each individual key scrambles the information in a unique way, and to decrypt the encrypted information back to the original form again, the same key is needed. Considering that there is a total of 3.4×10^{38} different 128-bit numbers, it is easily realized that it is *practically impossible* for an intruder to find out how the plain text (the original information) is related to the cipher text (the encrypted information). Thus, the secret lies in the encryption key, as it is *practically impossible* to decrypt information without knowing it.

Molecules can perform encryption/decryption too, but without the need for complex algorithms and processor power. What is needed is a molecular system with properties that makes it *practically impossible* to predict how the system responds when exposed to external stimuli of different kind. These systems are referred to as molecular encryption keys. Typically, the chemical response is interrogated by fluorescence means (changes in the emission intensity or the emission color) following the exposure to the stimulus (e.g., chemicals,[2] light,[3] heat,[4] mechanical force,[5] or multiple stimuli[5–6]). Several reviews summarize the collective efforts in this field.[7] Using terminology from data security, the external stimuli and the resulting chemical response can be compared to the *challenge* and the *response*, respectively, and they constitute a so-called challenge-response pair (CRP).[1,8] To exemplify, we can use the well-known pH indicator Bromothymol blue (BTB) as a molecular encryption key. Here, the challenge could be the BTB concentration and the pH, whereas the corresponding response is the absorbance values at 453 nm and 616 nm (the absorption maxima of the yellow and the blue forms of BTB).[9] Thus, the challenge "0.04, 4.8" would imply that the user prepares a 0.04 wt.% solution of BTB at pH 4.8 and records the absorption spectrum to get the response. This trivial key offers very limited security, as the challenge and the response are related by the Beer-Lambert[10] and Henderson-Hasselbalch[11] equations. Thus, it is *not* practically impossible to predict the response from a given challenge with access to a sufficiently large number of CRP:s. Instead, molecular systems must be devised such that there is a highly non-linear and unpredictable relation between challenge and response. Herein, we present such a system that is based on a cocktail[12] of three fluorescent molecular photoswitches in fluid solution. This molecular system displays a highly non-linear time-dependent fluorescence response upon photoexcitation using conventional steady-state fluorimeters, offering on the order of 82×10^6 unique CRP:s.

Results and Discussion

The molecular cocktail consists of three different compounds: **Dasy**,[13] **DAE9**,[14] and the **FG-DTE** triad.[15] **Dasy** and **DAE9** are monomers displaying intense fluorescence only in one of the two isomeric forms. The **FG-DTE** triad consists of one **DTE** photoswitch covalently linked to two identical **FG** photoswitches, offering a total of four isomeric forms (considering

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only the forms where the **FG** photoswitches are in the same isomeric form) out of which only one displays intense emission. The synthesis and the photophysical characterization of each photoswitch has been previously reported.[13b,14–15] Figure 1 shows the structures and the isomerization schemes of all compounds. Briefly, all investigated photoswitches exist in an open and a closed isomeric form, indicated by index (o) and (c) respectively. The open isomers absorb almost exclusively in the UV region, whereas the colored isomers display absorption also in the visible region. Isomerization from (o) to (c) is triggered by UV exposure, whereas the reverse reaction is driven by visible light in the wavelength region where the respective closed isomer absorbs light. Thermal isomerization reactions are very slow, occurring on the time scale of several days or slower. Thus, during readout of the fluorescence responses (see below), the thermally induced isomerization reactions can be safely ignored, why temperature variations do not affect the performance of the herein described systems. **FG(c)**, **DAE9(c)** and **Dasy(o)** display moderate or intense emission (see below for fluorescence quantum yields), whereas all other isomers display no or only very weak fluorescence. All spectra are shown in Figure 2.

The thermally stable form of all four photoswitches (**Dasy**, **DAE9**, **FG**, and **DTE**) is the open colorless isomer. Hence, the initial state of the cocktail consists of **Dasy(o)**, **DAE9(o)**, and

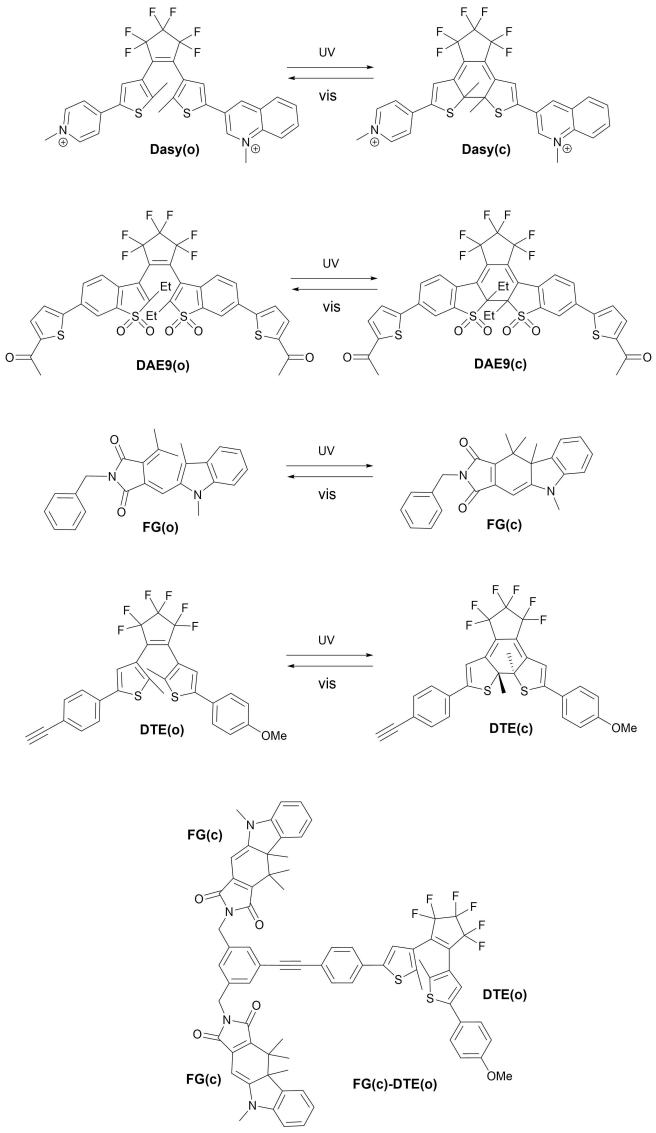

Figure 1. Structures and isomerization schemes for **Dasy**, **DAE9**, **FG**, and **DTE**. Shown is also the structure of the only fluorescent form of the **FG-DTE** triad, **FG(c)-DTE(o)**.

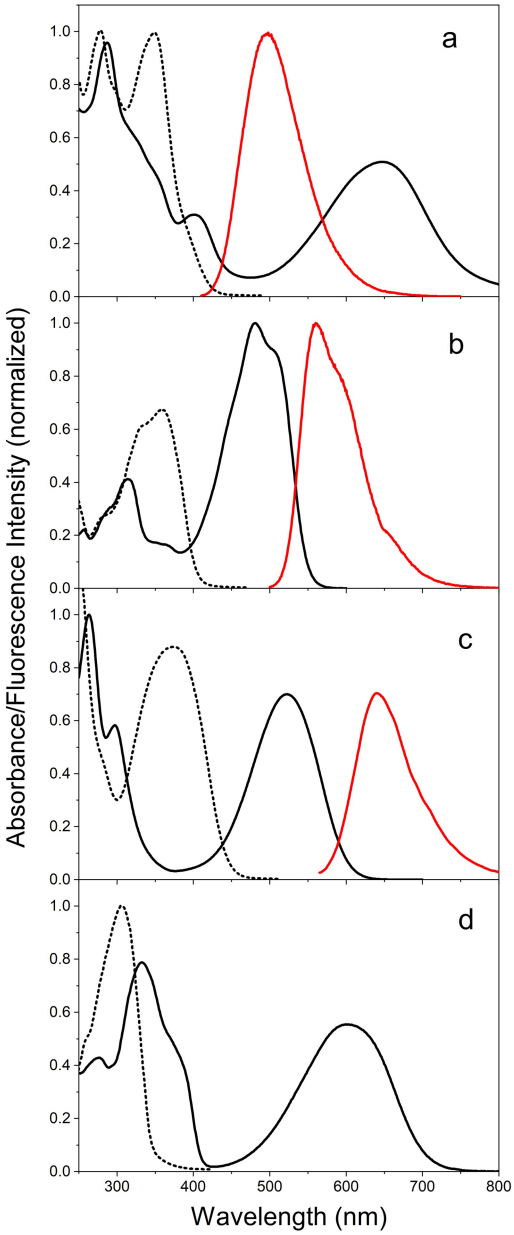

Figure 2. Absorption and emission spectra of the four photoswitches in acetonitrile solution. a) **Dasy**, b) **DAE9**, c) **FG**, and d) **DTE**. Dotted black lines: Absorption spectra of the open isomeric forms. Solid black lines: Absorption spectra of the closed isomeric forms. Red solid lines: Emission spectra of the respective fluorescent isomeric form (**Dasy(o)**, **DAE9(c)**, and **FG(c)**, all other forms display no or only very weak emission). Optically diluted solutions were used (i.e., Abs < 0.1), and excitation wavelengths for emission readout were 380 nm, 480 nm, and 540 nm for **Dasy**, **DAE9**, and **FG**, respectively. a, b): Adapted from Ref. [13b], copyright 2020, published by the Royal Society of Chemistry.

FG(o)-DTE(o). Exposure to UV light triggers the isomerization of all open forms to the closed forms, until reaching the photostationary state, highly enriched in the closed form for all photoswitches. Monitoring the emission of the three fluorescent photoswitches during the isomerization, the following is expected: (1) Fluorescent **Dasy(o)** ($\Phi_F = 0.11$)[13b] is isomerized ($\Phi_{iso} = 0.57$)[13b] to non-fluorescent **Dasy(c)** described by monoexponential kinetics. (2) Non-fluorescent **DAE9(o)** is isomerized ($\Phi_{iso} = 0.13$)[13b] to fluorescent **DAE9(c)** ($\Phi_F = 0.37$)[13b] described by monoexponential kinetics. (3) Non-fluorescent **FG(o)** is isomerized ($\Phi_{iso} = 0.13$)[16] to the intrinsically fluorescent form **FG(c)** ($\Phi_F = 0.01$).[17] However, the emission spectrum of **FG(c)** overlaps the absorption spectrum of **DTE(c)**, implying that the fluorescence from **FG(c)** is quantitatively quenched by **DTE(c)** in an efficient FRET-process. Thus, in the **FG-DTE** triad, the only fluorescent isomer is **FG(c)-DTE(o)**. Upon UV irradiation of thermally stable **FG(o)-DTE(o)**, the emission intensity will initially rise as a result of the increase in the concentration of the fluorescent isomer **FG(c)-DTE(o)**. Subsequently, the emission intensity will decrease, as **FG(c)-DTE(o)** is further isomerized to the non-fluorescent isomer **FG(c)-DTE(c)**. A biexponential rise-and decay process of the fluorescence intensity will therefore result. Please note that in the examples above, the UV light is used both to trigger the isomerization processes and for emission readout. The time-dependent emission profiles of the three compounds are sketched in Figure 3.

In the tri-component cocktail, the overall kinetic trace is a linear combination of the three individual traces, assuming optically dilute solutions and no intermolecular communication. This overall kinetic trace is thus expected to be described by a multi-exponential function containing up to four sets of rate constants and pre-exponential factors (amplitudes). The rate constants are proportional to the intensity of the excitation light, the molar absorption coefficient of **Dasy(o)**, **DAE9(o)**, **FG(o)**, and **DTE(o)** at the excitation wavelength, and the isomerization quantum yield to the respective closed isomer. The corresponding amplitudes are proportional to the intensity of the excitation light, the concentration of the respective compound, the fluorescence quantum yield of the fluorescent isomers, and the emission wavelength. The intensity of the excitation light will influence all rate constants and all amplitudes equally much, why the shape of the kinetic trace remains unchanged by excitation intensity alterations. Thus, when recording the overall kinetic trace in the fluorimeter, the user can influence the shape of the overall kinetic trace by varying the following parameters:

1) The concentrations of the three compounds
2) The excitation wavelength
3) The emission wavelength

where the concentrations influence the amplitudes, the excitation wavelength influences both the amplitudes and the rate constants, and the emission wavelength influences the amplitudes. Points 1–3 above thus represent the challenge, whereas the resulting kinetic trace is the response.

To experimentally demonstrate the notions described above, three kinetic traces are displayed in Figure 4, each representing a response to a specific challenge. Here, the challenge consists of the three different concentrations, the excitation wavelength, and the emission wavelength. The concentrations are given in μM and are allowed to vary between 0 and 20 with an increment of 1 μM, whereas the excitation wavelength is in the range 250 nm–425 nm with 2 nm increment, and the emission wavelength is in the range 500 nm–750 nm with 2 nm increment. The blue kinetic trace shown in Figure 4 was recorded according to the following:

- [**Dasy**] = 2 μM (02 μM in the two-digit notation)
- [**DAE9**] = 3 μM (03 μM in the two-digit notation)
- [**FG-DTE**] = 1 μM (01 μM in the two-digit notation)
- Excitation wavelength = 300 nm
- Emission wavelength = 520 nm

Thus, the challenge behind the blue response in Figure 4 is 020301300520. The corresponding challenges for the other two responses are indicated in the Figure legend. It is very

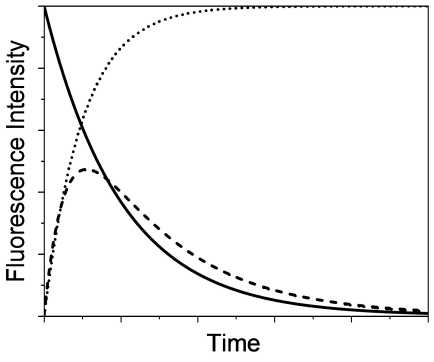

Figure 3. Simulated individual time-dependent emission profiles (kinetic traces) for **Dasy** (solid line), **DAE9** (dotted line), and **FG-DTE** (dashed line). Note that in the initial state at t = 0, all compounds are in their thermally stable isomeric forms **Dasy(o)**, **DAE9(o)**, **FG(o)**, and **DTE(o)**. The rate constants and the amplitudes of the kinetic traces are arbitrarily chosen in the figure.

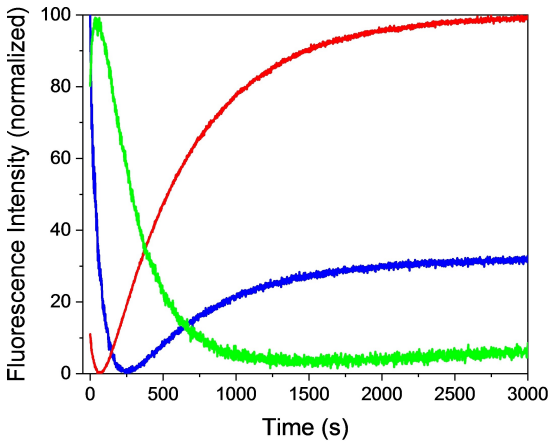

Figure 4. Kinetic traces (responses) for three different samples in acetonitrile solution. Red line: [**Dasy**] = 2 μM, [**DAE9**] = 3 μM, [**FG-DTE**] = 1 μM, excitation wavelength = 300 nm, emission wavelength = 540 nm, yielding a challenge = 020301300540. Blue line: [**Dasy**] = 2 μM, [**DAE9**] = 3 μM, [**FG-DTE**] = 1 μM, excitation wavelength = 300 nm, emission wavelength = 520 nm, yielding a challenge = 020301300520. Green line: [**Dasy**] = 1 μM, [**DAE9**] = 0 μM, [**FG-DTE**] = 9 μM, excitation wavelength = 425 nm, emission wavelength = 580 nm, yielding a challenge = 010009425580.

encouraging to note the dramatically different responses represented by the red and the blue lines in Figure 4, resulting from merely shifting the emission wavelength by 20 nm while keeping the concentrations and the excitation wavelength constant. This observation serves as a testament to the "sensitivity" of the molecular cocktail used to generate the keys (see below). Despite this pronounced sensitivity of the responses to small changes in the challenge, the responses display excellent reproducibility when one and the same challenge is used. This is manifested by the very similar kinetic traces generated by two individual samples with identical concentrations, excitation- and emission wavelength (see Supporting Information).

To convert the responses to numerical values, the emission intensities can, for example, be read at predetermined times, preferably after normalization of the emission intensities (0–100) and rounding down to the nearest whole value using the two-digit notation. Multiexponential fitting could potentially be applied in order to reduce the effect of noise (see Supporting Information). To exemplify, if the emission intensities are to be read at times 0, 500, 1000, 1500, 2000, 2500, and 3000 s the numerical response of the blue trace shown in Figure 4 would be 99082127303132 (fluorescence intensity = 99, 08, and 21 at t = 0 s, 500 s, and 1000 s, etc.) Alphanumerical methods can be used in the actual encryption/decryption processes, converting the plain text to the cipher text.[2g] This task, however, is relatively trivial. The heart of the overall encryption/decryption protocol presented herein is instead the strongly non-linear relation between the challenge and the response.

Given that (i) the excitation- and emission wavelength windows span 250 nm–425 nm and 500 nm–750 nm, respectively, with an increment of 2 nm and (ii) the concentration of each compound can vary between 0 and 20 μM with an increment of 1 μM, there are a total of 101860000 different ways of selecting the challenge. Taking out challenges with equivalent concentration ratios (see Supporting Information) 82643000 combinations remain. With few exceptions (where the ratio of the molar absorption coefficients or emission intensities of all four photoswitches are very similar at two or more wavelengths) all these different challenges are expected to result in unique responses. We base this notion on the "sensitivity" of the system, shown in the Supporting Information.

Responses in the form of time-dependent fluorescence traces at single wavelengths[3e,12a,18] has one main advantage compared to approaches where instead the fluorescence spectra are used: The response does not depend on the wavelength-sensitivity of the detector used, implying that the response should be identical using any fluorimeter after normalization. It is a known problem that the correction files used to compensate for the wavelength-sensitivity are inexact, why the responses would typically vary from fluorimeter to fluorimeter when the response is extracted from spectral information. On the downside, the time-dependent traces depend on the intensity of the excitation light, even if normalization of the response is being used. This is due to that the isomerization rates increase with increasing intensity of the excitation light, thus also influencing the resulting fluorescent traces. Calibration of the lamp spectrum to light-flux could solve this problem.

Finally, we would like to emphasize that the herein reported scheme can be used also as an authentication protocol. Suppose that Alice wants to share sensitive information with Bob, and thus wants to assure herself that it is really Bob on the other side of the communication channel. Given that both Alice and Bob have access to the cocktail molecules and a fluorimeter, Alice can pose a challenge to Bob, that is, concentrations, excitation wavelength, and emission wavelength. Bob records the response and sends it back. Alice compares the response generated by Bob to the response that Alice also generates. If they match, Bob is authenticated.

Conclusions

We have presented a molecular cocktail consisting of three different fluorescent photochromic compounds that is capable of performing encryption and decryption of data. In essence, the only requirement for a molecular platform to perform this task is that the system should display highly non-linear (unpredictable) physicochemical changes upon exposure to external stimuli (e.g., addition of chemicals or light irradiation). Our system displays strongly non-linear time-dependent fluorescence changes in response to light exposure at different wavelengths. The system can be used using conventional steady-state fluorimeters.

Experimental section

The synthesis of all compounds has been described previously.[13b,14–15] Spectroscopic grade acetonitrile was used as solvent. Ground state absorption spectra were recorded on a Cary 50 UV/vis spectrometer. Corrected fluorescence spectra and time-based fluorescence traces were recorded on a SPEX Fluorolog-3 spectrofluorometer. The light from the fluorimeter-lamp was used to trigger both isomerization and excitation for emission readout when recording the time-dependent emission profiles. The slit widths of the fluorimeter were set to 0.5 mm on both the excitation and the emission side, corresponding to a resolution of 2 nm.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Photoswitches · energy transfer · photochromism · encryption key · molecular logic

[1] W. Buchanan, *Cryptography*, 1 ed., River Publishers, **2017**.
[2] a) A. C. Boukis, K. Reiter, M. Frolich, D. Hofheinz, M. A. R. Meier, *Nat. Commun.* **2018**, *9*, 1439; b) S. S. Fan, D. F. Wang, J. Cheng, Y. Liu, T. Luo, D. X. Cui, Y. G. Ke, J. Song, *Angew. Chem. Int. Ed.* **2020**, *59*, 12991–12997; c) X. S. Hou, C. F. Ke, C. J. Bruns, P. R. McGonigal, R. B. Pettman, J. F. Stoddart, *Nat. Commun.* **2015**, *6*, 6884; d) X. Li, Y. J. Xie, B. Song, H. L. Zhang, H. Chen, H. J. Cai, W. S. Liu, Y. Tang, *Angew. Chem. Int. Ed.* **2017**, *56*, 2689–2693; e) O. Lustgarten, R. Carmieli, L. Motiei, D. Margulies, *Angew. Chem. Int. Ed.* **2019**, *58*, 184–188; f) D. Margulies, C. E. Felder, G. Melman, A. Shanzer, *J. Am. Chem. Soc.* **2007**, *129*, 347–354; g) T. Sarkar, K. Selvakumar, L. Motiei, D. Margulies, *Nat. Commun.* **2016**, *7*; h) S. Shoshani, R. Piran, Y. Arava, E. Keinan, *Angew. Chem. Int. Ed.* **2012**, *51*, 2883–2887; i) Z. Xu, D. Gonzalez-Abradelo, J. Li, C. A. Strassert, B. J. Ravoo, D. S. Guo, *Mater. Chem. Front.* **2017**, *1*, 1847–1852.
[3] a) J. Andréasson, S. D. Straight, T. A. Moore, A. L. Moore, D. Gust, *Chem. Eur. J.* **2009**, *15*, 3936–3939; b) A. D. Erlich, N. P. Dogantzis, L. Al Nubani, L. A. Trifoi, G. K. Hodgson, S. Impellizzeri, *Phys. Chem. Chem. Phys.* **2021**, *23*, 25152–25161; c) J. Li, M. Tian, H. F. Xu, X. K. Ding, J. B. Guo, *Part. Part. Syst. Charact.* **2019**, *36*; d) Z. Q. Li, H. Z. Chen, B. Li, Y. M. Xie, X. L. Gong, X. Liu, H. R. Li, Y. L. Zhao, *Adv. Sci* **2019**, *6*, 1901529; e) J. Wang, J. Ma, J. C. Zhang, Y. Fan, W. X. Wang, J. K. Sang, Z. D. Ma, H. H. Li, *ACS Appl. Mater. Interfaces* **2019**, *11*, 35871–35878.
[4] J. H. Du, L. Sheng, Y. Xu, Q. N. Chen, C. Gu, M. J. Li, S. X. A. Zhang, *Adv. Mater.* **2021**, *33*, 2008055.
[5] Y. Jiang, J. H. Ma, Z. Y. Ran, H. Q. Zhong, D. H. Zhang, N. Hadjichristidis, *Angew. Chem. Int. Ed.* **2022**, *61*, e202208516.
[6] G. Lee, M. Kong, D. Park, J. Park, U. Jeong, *Adv. Mater.* **2020**, *32*, 1907477.
[7] a) J. Andréasson, U. Pischel, *Chem. Soc. Rev.* **2015**, *44*, 1053–1069; b) J. Andréasson, U. Pischel, *Chem. Soc. Rev.* **2018**, *47*, 2266–2279; c) J. Andréasson, U. Pischel, *Coord. Chem. Rev.* **2021**, *429*; d) R. Arppe, T. J. Sorensen, *Nat. Chem. Rev.* **2017**, *1*; e) A. P. de Silva, S. Uchiyama, *Nat. Nanotechnol.* **2007**, *2*, 399–410; f) P. Kumar, S. Singh, B. K. Gupta, *Nanoscale* **2016**, *8*, 14297–14340; g) K. Szacilowski, *Chem. Rev.* **2008**, *108*, 3481–3548.
[8] H. C. A. van Tilborg, S. Jajodia, *Encyclopedia of Cryptography and Security*, Springer, Boston, MA, **2011**.
[9] E. Klotz, R. Doyle, E. Gross, B. Mattson, *J. Chem. Educ.* **2011**, *88*, 637–639.
[10] https://doi.org/10.1351/goldbook.B00626.
[11] https://doi.org/10.1351/goldbook.H02781.
[12] a) J. Andréasson, S. D. Straight, G. Kodis, C. D. Park, M. Hambourger, M. Gervaldo, B. Albinsson, T. A. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2006**, *128*, 16259–16265; b) S. A. de Silva, K. C. Loo, B. Amorelli, S. L. Pathirana, M. Nyakirang'ani, M. Dharmasena, S. Demarais, B. Dorcley, P. Pullay, Y. A. Salih, *J. Mater. Chem.* **2005**, *15*, 2791–2795; c) P. Paul, M. Karar, A. Mallick, T. Majumdar, *J. Mater. Chem. C* **2021**, *9*, 11229–11241.
[13] a) G. Naren, W. Larsson, C. Benitez-Martin, S. M. Li, E. Perez-Inestrosa, B. Albinsson, J. Andréasson, *Chem. Sci.* **2021**, *12*, 7073–7078; b) G. W. Naren, S. M. Li, J. Andréasson, *Chem. Commun.* **2020**, *56*, 3377–3380.
[14] K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka, M. Irie, *J. Am. Chem. Soc.* **2011**, *133*, 13558–13564.
[15] J. Andréasson, S. D. Straight, T. A. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2008**, *130*, 11122–11128.
[16] Y. C. Liang, A. S. Dvornikov, P. M. Rentzepis, *J. Mater. Chem.* **2000**, *10*, 2477–2482.
[17] P. Remon, M. Bälter, S. M. Li, J. Andréasson, U. Pischel, *J. Am. Chem. Soc.* **2011**, *133*, 20742–20745.
[18] M. Bälter, S. M. Li, J. R. Nilsson, J. Andréasson, U. Pischel, *J. Am. Chem. Soc.* **2013**, *135*, 10230–10233.

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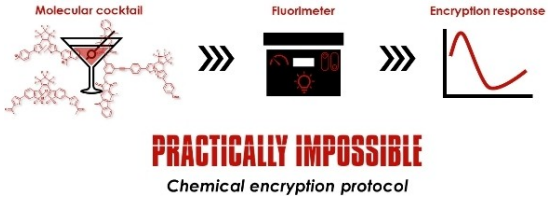

A molecular cocktail consisting of three fluorescent photochromic compounds is demonstrated as a molecular encryption key. It is operated by all-photonic means and the function relies on highly non-linear time-based fluorescence in response to excitation and emission readout at different wavelengths.

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