Almost complete radiationless energy transfer from excited triplet state of a dim phosphor to a covalently linked adjacent fluorescent dye in purely organic tandem luminophores doped into PVA matrix

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Abstract

In solid PVA matrix some thiophene- and selenophene-comprising heteroaromatic compounds reveal moderate room temperature phosphorescence with emission peaks between 550 and 600 nm and possessing lifetime in millisecond range upon excitation with near-UV radiation. In tandem luminophores almost complete intramolecular interchromophore energy transfer takes place by FRET mechanism from the named phosphors in excited triplet state to adjacent covalently linked fluorescent dyes, leading to emission from the fluorophore. Variation of length of the linker connecting the chromophores enables tuning of luminescence lifetime in the range from 5 ms to 100 μs. The delayed emission spectrum of the tandem luminophores originates from the acceptor fluorophore. Triplet-singlet energy transfer outperforms other relaxation pathways of the excited triplet state even at 75 °C, making the luminescence lifetime temperature independent . Thus a universal strategy is provided for converting inherently dim organic phosphors into bright long-lifetime luminescence emitters. This discovery may open new avenues for construction of efficient purely organic light emitting devices and photoluminescent sensors for measurement of various analytes.

Introduction

Organic compounds, mostly complexes of transition metal ions, possessing room temperature phosphorescence (RTP) have been used in the design and definition of organic light emitting devices (OLEDs) [1] and sensor systems for different analytes.[2-4] Phosphorescent materials emit light from their excited triplet state and this forbidden transition leads to slow photoluminescence (PL) decay. The long-lived excited triplet state is susceptible to various radiationless relaxation processes that decrease the photoluminescence quantum yield (PLQY).[5] Efficient RTP requires strong spin-orbit coupling in the molecule, a property attributed to compounds comprising heavy atoms. Therefore among organic compounds just transition metal complexes reveal strongest RTP.[6] However, various purely organic compounds have also revealed significant RTP in solid matrixes where structural motions and interactions with quenchers are restricted.[7,8] For example, several organic compounds in crystalline state [9, 10] or doped into solid matrixes are phosphorescent at room temperature.[11-14] Inclusion of heavy atoms (Se, Te, Br, I) and carbonyl or thiocarbonyl groups into aromatic structures is an acknowledged strategy for enhancing RTP.[7,15]
We have previously shown that intensity of light emission from the excited triplet state of several sulfur- or selenium-comprising heteroaromatic purely organic phosphors could be substantially enhanced by covalent conjugation of a bright fluorescent dye whose absorption spectrum overlap with the phosphorescence spectrum of the phosphor.[16-19] Upon excitation of the phosphor moiety with a pulse of near-UV-radiation in such tandem luminophore in complexes with a protein kinase (PKs), the tandem probe exhibits long lifetime luminescence (luminescence lifetime τ in 20 - 300 μs range). In these complexes the ATP-binding pocket of PK restrains molecular motions and the protein shields the exited triplet state of the donor phosphor from quenching by dissolved molecular oxygen.[19] Compared to RTP of the distinct phosphor, the derived tandem luminophores revealed 20-1000-fold enhancement of PL.[16] This huge increase in PL intensity was ascribed to efficient

Förster-type resonant energy transfer (FRET, Figure 1) from the donor phosphor, initially in the exited triplet state, to the adjacent non-excited acceptor fluorophore through dipole–dipole coupling. This process promotes the fluorophore into the excited singlet state and leads to emission of light from the dye, whereas the emission spectrum of the tandem luminophore is that of the acceptor fluorophore. The rate and efficiency of FRET are inversely proportional to the sixth power of distance between the interacting chromophores (Equation 1) and the transfer may take place with almost 100% efficiency in case of short distances.[20]

$$E = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6} = \frac{k_{FRET}}{k_{FRET} + \sum k_x} \quad (1),$$

where E is the efficiency of ET; r is the distance between the donor and acceptor chromophores; R_0 is the Förster distance (radius), corresponding to distance between the chromophores where ET occurs with 50% of the maximal efficiency; k_{FRET} is the rate constant of FRET; and $\sum k_x$ is the sum of rate constants of all other processes depopulating exited state of the donor. Emission from the triplet state is symmetry forbidden and the corresponding transition dipole is therefore relatively small. The interchromophore triplet-singlet energy transfer (TSET) takes place slowly, in microsecond time-scale. In the case of short distances separating the interacting chromophores the rate of FRET from the excited triplet state of the donor to the acceptor can be much higher than the rate of phosphorescence of the donor, resulting in increased PLQY of the tandem luminophore compared to that of the isolated phosphor.

The feasibility of radiationless ET from a donor phosphor initially in excited triplet state, to a non-excited acceptor fluorophore, transforming the fluorophore to the excited singlet state, due to dipole-dipole interactions was first suggested by Theodor Förster in 1959.[21] Later, the realization of this phenomena was demonstrated experimentally in intermolecular processes, first in solid media at cryogenic temperatures [22,23] and later in solution at room temperature.[24] Still, this phenomena has found few practical applications, [25] Lakowicz with his co-workers showed enhanced long-lifetime PL of tandem luminophores incorporating a covalently linked transition metal complex as the PL donor and a bright fluorescent dye as the acceptor. They also introduced a comprehensive theoretical description for ET processes that took place in the described tandem luminophores in solution.[26,27]

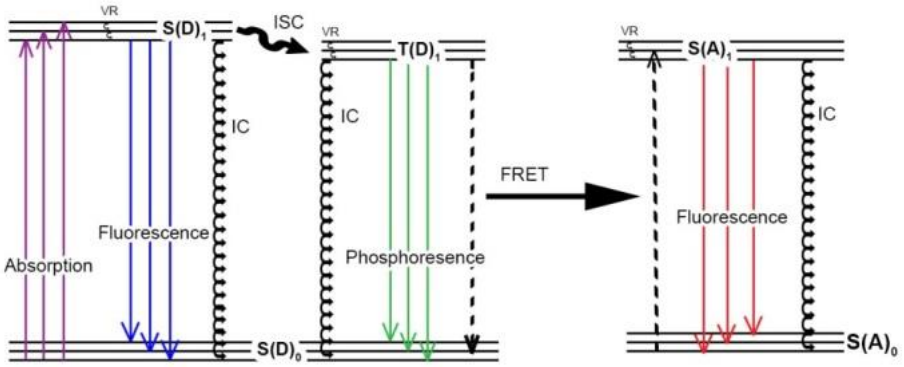

Figure 1. The Jablonski diagram of electronic transitions that take place in the tandem luminophore possessing interchromophore TSET from a donor phosphor (D) to acceptor fluorophore (A) leading to delayed emission of light from the fluorophore (phosphor-sensitized fluorescence).

Efficient TSET may be applicable for design and definition of organic light emitting devices (OLEDs). Electronic excitation generates 25% of singlet and 75% of triplet excitons.[28] Therefore compelling of both singlet and triplet excitons to emit light is decisive for construction highly efficient OLEDs. At present harvesting of light from triplet excitons is achieved via application of phosphorescent transition metal complexes[29] or by using thermally activated delayed fluorescence (TADF) of luminophores.[30] Since 2000, only a few papers have disclosed the application of interfluorophore TSET (phosphor-sensitized fluorescence) in OLED-s comprising metal complexes.[31,32] Phosphor-sensitized fluorescence in devices comprising of two purely organic compounds was demonstrated in 2013.[33] In these double-doped OLEDs intermolecular, long-range, non-radiative energy transfer from the phosphorescent sensitizer to the fluorescent dye was used to increase efficiency of the device. However, to date there is no general strategy for the application of interfluorophore TSET for construction of efficient organic triplet emitters.

After demonstrating that thiophene- and selenophene-comprising hetroaromatic compounds covalently tethered with fluorescent dyes reveal intense long-lifetime PL if associated with PKs,[16-18] we assumed that similar or even more intense PL of these compounds might occur in solid matrices where access of quenching molecular oxygen and molecular movements of the luminophores would be limited. Polyvinyl alcohol (PVA) was selected as the solid matrix for these studies as this polymer is water soluble, transparent for near-UV- and visible radiation, and can restrict diffucion molecular oxygen into the structure. Hydrogen bonds between PVA and polar molecules lead to a rigid environment that creates suitable conditions for the appearance of strong RTP of organic compounds.[12] Here we demonstrate that thiophene- or selenophene-comprising heteroaromatic compounds exhibit modest RTP with lifetimes in millisecond region. Covalent conjugation of the phosphor with a bright fluorescent dye whith absorption spectrum overlapping with the phosphorescence spectrum of the donor leads to bright luminescence emitters with μs to ms decay time. Efficiency of TSET exceeds 99% in the case adjacent positioning of the interacting chromophores.

Results and Discussion

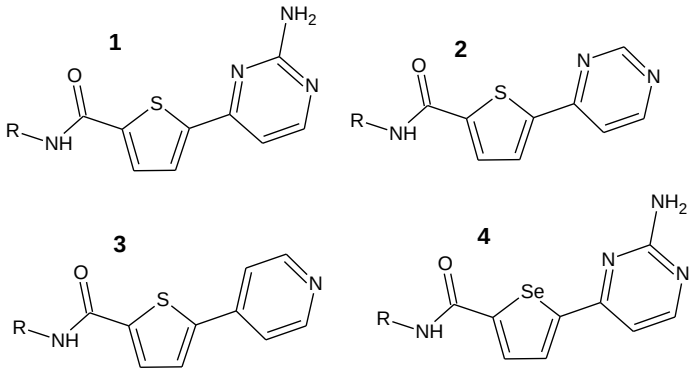

Figure 2. Chemical structures of the studied phosphorescent fragments. R = aliphatic peptide chain.

For PL measurements the compound of interest was dissolved 7% PVA solution in water. 20 μL of the solution were transferred to the bottom of well of a polystyrene-made microtiter plate or on a quartz disc and left to dry for 48 h in air and thereafter for 2h in vacuum. Delay time of 100 μs was used for recording the time-gated spectra. In the first round of study peptide derivatives of sulfur-comprising (**1 – 3**) or selenium-comprising (**4**) heteroaromatic fragments (Figure 2, SI) were tested. These compounds were available from previous studies with protein kinases.[16] Phosphorescence excitation spectra (Figure 3A) of compounds **1** - **4**

(Figure 2) coincided with their absorption spectra (Figure SI 5). Room temperature phosphorescence emission spectra of all three thiophene derivatives **1** - **3** were very similar, all compounds exhibited emission maxima at 560 nm, while the phosphorescence emission spectrum of the selenophene derivative **4** was red-shifted, exhibiting an emission maximum at 586 nm (Figure 3A). Simple carboxylic acid precursors of these peptide conjugates, compounds **1**-COOH, **2**-COOH, **3**-COOH, and **4**-COOH revealed coinciding phosphorescence excitation and emission spectra (Figure SI1) with their peptide derivatives. Thus, the structure of the aromatic heterocycle is decisive for the PL properties of the compound and the peptide moiety has no remarkable effect on its PL. In PVA matrix thiophene derivatives **1** - **3** showed long phosphorescence lifetimes of 24.9 – 43.0 ms (Table 1, Figure SI2). These lifetime were more than 10-fold longer than the lifetimes of similar compounds in complexes with PKAc protein in deoxygenated water solution and more than 100-fold longer than in complexes with PKAc dissolved in water that was equilibrated with air.[19] This result could be explained by better protection of the excited triplet state by the restriction of molecular movements and/or avoiding quenching by molecular oxygen in the PVA matrix. The selenium-comprising compound **4** showed only 2-fold longer phosphorescence lifetime (0.78 ms) in the PVA matrix as compared to the compound in the PKAc complex, in deoxygenated water solution, and about 8-fold longer lifetime than the PKAc complex in air-equilibrated buffer.[16, 19] PLQYs of luminophores in thin PVA films were determined using the previously described method.[34] Table 1 lists parameters of the phosphorescence component of PL. The compounds revealed PLQY values in the range of 1.4 - 3.2%. Interestingly, the thiophene derivative **2** showed the highest PLQY. In PK complexes, selenophene-comprising compounds like **4** generally produced a magnitude stronger phosphorescence intensity than the corresponding thiophene derivatives.[16] The PVA matrix stabilizes the excited triplet state of thiophenes more efficiently than that of the selenophene derivative, the latter phosphor also possessed substantially shorter PL lifetime.

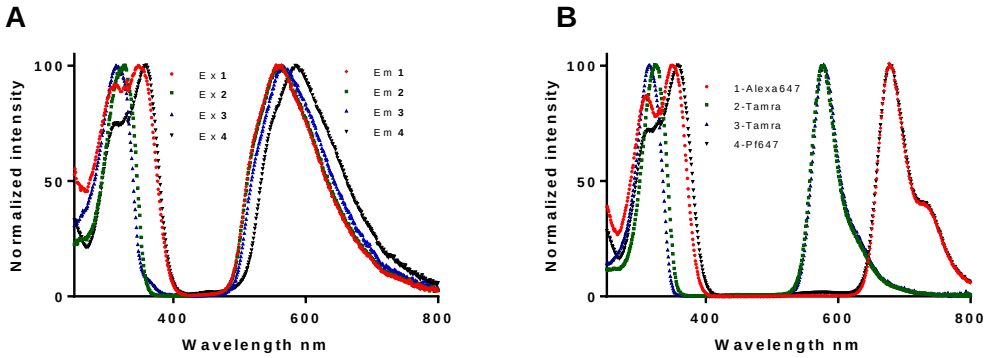

Figure 3. PL spectra of the tested compounds. A) Normalized phosphorescence excitation and emission spectra of compounds **1** (1 nmol), **2** (1 nmol), **3** (1 nmol), and **4** (0.1 nmol); all in 0.7 mg PVA. B) Normalized time-gated excitation and emission spectra of the tandem luminophores **1**-x-Alexa647, **2**-x-Tamra, **3**-x-Tamra, and **4**-x-PF647. For measurements the compounds were used in 0.02 nmol quantities in 0.7 mg of PVA. Emission spectra were measured by using excitation at the excitation maxima listed in Table 1 and the excitation spectra were measured by recording the signal intensity at emission maximum of the compound (data from Table 1 for phosphorescence spectra, 590 nm for TAMRA-labelled compounds and 670 nm for PF-647 derivatives).

Table 1. Phosphorescence properties of compounds in PVA

Compound	Excitation max., nm	Emission max., nm	Phosphorescence lifetime, ms	Phosphorescence QY, %
1	348	560	31.7 ± 0.9	2.1
2	325	561	43.0 ± 0.7	3.2
3	315	567	24.9 ± 0.7	1.4
4	358	586	0.78 ± 0.2	2.0

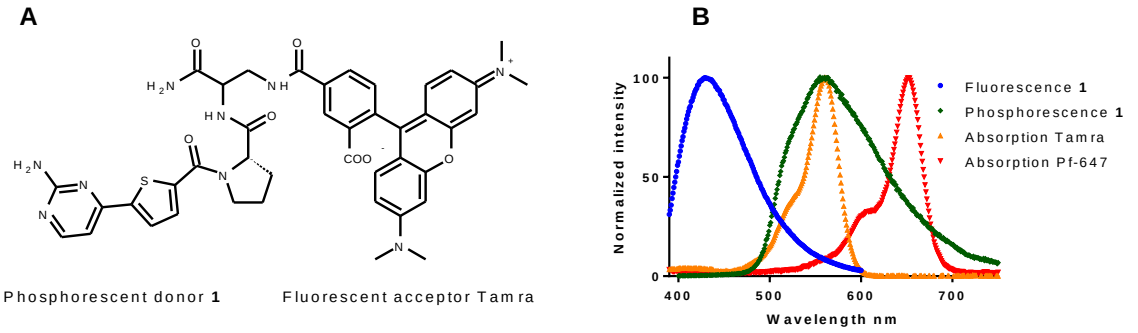

Figure 4. A) Full structure of **1**-Pro-Dap(Tamra)-NH_2 B) Fluorescence and phosphorescence spectra of donor **1** and absorption spectra of acceptors 5-Tamra and PromoFluor-647.

The tandem luminophores comprising of covalently linked organic phosphor and a fluorescent dye produces significantly more intense long-lifetime PL than the distinct isolated phosphor. Delayed PL excitation spectra of the tandem luminophores coincided with absorption spectra of the corresponding phosphorescent moieties of **1** - **4**, if the emission maximum of the fluorescent dye was monitored (Figure 3B). Direct excitation of the fluorescent dye did not contribute to long lifetime luminescence signal, although the time-gated emission spectra of tandem luminophores overlapped with the fluorescence emission spectra of the corresponding attached dyes (Figure 3B). An intense time-gated PL signal at the emission wavelength of the conjugated dye and a very weak time-gated signal at the wavelengths of the donor phosphorescence pointed to very efficient radiationless TSET from the donor phosphor in excited triplet state to the acceptor dye. This is also in agreement with good spectral overlap of the phosphorescence spectra of **1** and absorption spectra of Tamra and 647 dyes (Figure 4B). The efficiency (*E*) of ET was estimated from the ratio of PL lifetimes of the donor-acceptor conjugate (τ_{FRET}) and distinct donor (τ_D) according to Equation 2.

$$E = 1 - \frac{\tau_{FRET}}{\tau_D} \quad (2)$$

All investigated tandem compounds revealed significantly shorter PL lifetimes than the corresponding isolated phosphors, pointing to very high TSET efficiency (up to 99%). Decay curves of tandem compounds showed a clear multi-exponential shape (Figure SI3). The calculated (Eq 3 [35]) intensity-averaged mean lifetimes are listed in Table 2. The average lifetime values still provide a good analytical parameter for estimating approximate ET efficiencies. Peptide compounds are flexible and they can adapt a diverse set of fixed

conformations in the solid PVA matrix. This results in different average distances and mutual orientations of dipoles of the donor and acceptor chromophores. Thus the multi-exponential decay of long lifetime PL of these materials is an expected outcome of the described conformational diversity.

Table 2. Luminescence lifetimes τ and efficiencies E of ET from the excited triplet state of the donor phosphor to the fluorescent dye (calculated according to Equation 2) of the studied tandem luminophores in PVA

Structure	Average lifetime τ, ms	E_{FRET}
1-x-Alexa647	0.64 ± 0.17	98%
2-x-Tamra	1.7 ± 0.4	96%
3-x-Tamra	1.4 ± 0.6	95%
4-x-PF647	0.14 ± 0.02	82%
1-pip-Tamra	≤0.1	>99%
1-Pro-Dap(Tamra)-NH_2	0.34 ± 0,07	99%
1-Pro_2-Dap(Tamra)-NH_2	0.62 ± 0.09	98%
1-Pro_3-Dap(Tamra)-NH_2	1.3 ± 0.2	96%
1-Pro_5-Dap(Tamra)-NH_2	2.5 ± 0.7	92%
1-Pro_9-Dap(Tamra)-NH_2	5.3 ± 1.0	83%
1- Peptide1	31.7	-

Distance dependence of the efficiency of the intramolecular interchromophore TSET was studied with a set of compounds where the donor phosphor (fragment **1**) and acceptor dye (5-carboxytetramethylrhodamine, Tamra) were separated by an oligo-proline linker of variable length (Table 2, Figure 5). A set of compounds with the general structure of **1**-Pro_x-Dap(Tamra)-NH_2, incorporating increasing number Pro residues and a single 2,3-diaminopropanoic acid (Dap) residue between the chromophores, was used for this study. Relatively rigid oligo-proline fragments are widely used molecular rulers which enable the construction compounds with increased distance between the covalently linked moieties.[36,37] Nevertheless, the oligo-proline chain still possesses some conformational flexibility[37] and the used 2,3-diaminopropanoic acid (Dap) linker add even more uncertainty into estimated distances between the chromophores. If the number of proline residues in the linker chain was increased from 1 to 9, the average PL lifetime τ of the tandem probes increased from 0.34 ms to 5.3 ms (compared to the value of 31.7 ms determined for the distinct donor phosphor) (Table 2, Figure 5). Corresponding FRET efficiencies ranged from 99 to 83%. The compound with the structure **1**-piperazine-Tamra (SI Table 1) was constructed to position the donor and acceptor moieties as close as possible while still avoiding direct (Van der Waals) contact between the chromophores. This compound exhibited a very short PL lifetime of less than 100 μs, corresponding to almost 100% efficiency of interchromophore TSET. Extremely efficient TSET demonstrates that almost quantitative harvesting of energy of triplet excitons is possible in case of purely organic heteroaromatic phosphors.

In parallel to the phosphorescence, the donors also shows weak fluorescence with emission spectra that partially overlap with absorption spectra of the acceptor dyes (e.g., see spectra of donor **1** and fluorescent dyes in Figure 4). This leads to the possibility of Förster-type singlet-singlet energy transfer (SSET) that competes with intersystem crossing and decreases the yield of triplet state of the donor. If harvesting of all excitons is required then this is an advantage leading to higher overall emission, but decrease of the long-lifetime luminescence component might be problematic for other applications. intensity enhancement of of the long-lifetime signal could be achieved by selecting donors that show minimal fluorescence quantum yield and minimal overlap with absorption spectrum of an acceptor. Estimated

Förster distances of TSET are longer than these for SSET for all systems described in this paper. Optimal distance between the donor and acceptor for maximal long lifetime luminescence is sufficiently long to minimize SSET while still displaying very efficient TSET.
The average distances between molecules in our samples (concentration of the tandem luminophore in PVA was 20 pmol/0.7 mg) were about 30 nm that is far over the limits of the Förster-type energy transfer. Therefor we can consider the intermolecular energy transfer component to be insignificant compared to intermolecular processes.

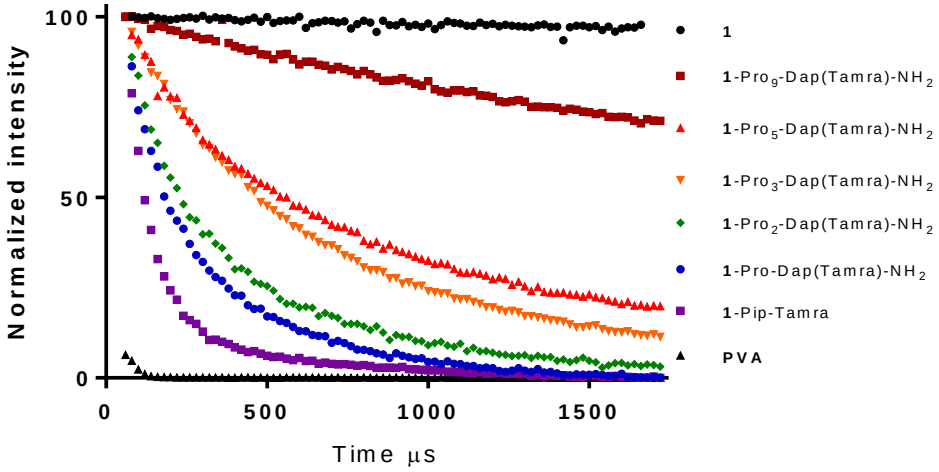

Figure 5. Dependence of PL decay rate of tandem luminophores on distance between the donor and acceptor chromophores. Signal intensity at 60 µs time-point of every curve was normalized to 100 intensity units.

PL lifetime of some luminophores was determined in PVA films in temperatures range from 25 to 75 °C (Figure SI4). Lifetime of phosphor **1** decreased remarkably (from 32.3 to 14.8 ms) when the temperature was increased from 25 to 75 °C. PL lifetimes of tandem compounds were significantly less sensitive to temperature. **1**-Pro$_9$-Dap-Tamra revealed lifetime change from 5.3 to 3.7 ms and the lifetime of **1**-Pro$_2$-Dap-Tamra was almost the same (τ = 0.6 ms) at all tested temperatures. Higher temperature leads to increased rate of radiationless decay processes of the excited triplet state and thereby to the reduction of phosphorescence lifetime. However, in case of tandem compounds showing fast and very efficient interluminophore TSET, other decay pathways play a minor role in depopulating the triplet state, even at 75 °C. The compounds showing temperature-independent slow PL might be useful for various technological applications.

Conclusions

In solid PVA polymer matrix restriction of molecular movements and protection of the phosphor moiety in excited triplet state from its quenching by molecular oxygen, led to moderate RTP with slow (millisecond scale) signal decay of thiophene- and selenophene-comprising heteroaromatic compounds. In the case of the tandem luminophore that incorporated a covalently linked phosphor and an acceptor fluorophore (e.g., Tamra dye), the excitation energy of excited triplet state of the phosphor was almost completely transferred to the adjacent fluorescent dye by radiationless dipole-dipole coupling, leading to excitation of the fluorophore and slow emission of light from the dye (so called phosphor-sensitized fluorescence). Covalent connection between the interacting fluorophores assures occurrence of efficient ET at all (even very low) concentrations of the tandem luminophore. Excellent

harvesting of energy of the triplet exited state and enhancement of PL signal of otherwise dim (possessing with low quantum yield at room temperature) organic phosphors by adjacent fluorophores seems to be a general phenomenon that may be applicable for construction of photoluminescence-based sensors for different analytes (e.g., dissolved oxygen in water). The technology for construction of tandem luminophores might also be applicable for development of efficient OLEDs, the devices that harvest energy of electrically generated triplet excitons through phosphor-sensitized fluorescence. Because of the covalent linkage between the chromophores there is no need to use the acceptor dye at high concentrations that is needed for efficient energy transfer in case of double-doped OLEDs.[32,33] Usually heavy atoms are required to get significant emission from the triplet state. Here we have shown that by using the described tandem luminophore strategy heavier atoms than sulfur were not required for construction of bright orange or red organic emitters possessing PL decay time in microsecond or millisecond range.
The discovered independence of PL lifetime on temperature of tandem luminophores is another noteworthy and remarkable property of the described materials.

Experimental Section

Chemicals and instruments

Solvents were obtained from Sigma-Aldrich, Rathburn, and Fluka. Fmoc Rink Amide MBHA resin and other peptide synthesis chemicals were from Iris Biotech, Novabiochem, and AnaSpec. 5-Tamra-NHS was from AnaSpec. Other chemicals were purchased from Sigma-Aldrich.
High-resolution mass spectra of all synthesized compounds were measured on Thermo Electron LTQ Orbitrap mass spectrometer. NanoDrop 2000c spectrometer (Thermo Scientific) was used for recording UV-Vis absorption spectra and quantification of the compounds. FluoroMax-4 (HORIBA Jobin Yvon) luminescence spectrometer was used for recording luminescence emission spectra and determination of PL lifetimes. PHERAstar (BMG Labtech) PL plate reader was used for measurement of shorter (up to 1 ms) lifetimes of the films. Synergy Neo (BioTek) multi-mode microplate reader was used to measure time-gated luminescence emission and excitation spectra. Purification of synthesized compounds was performed with Schimadzu LC Solution (Prominence) HPLC system by using the manual injector and the diode array (SPD M20A) UV-vis detector.

Synthesis of peptide conjugates

Compounds: **2** (ARC-1129), **3** (ARC-1121), **1**-x-PF647 (ARC-1182), **2**-x-Tamra (ARC-1129), **3**-x-Tamra (ARC-1122), **4**-x-PF647 (ARC-1139) and carboxylic acid precursors of **1** - **4** were synthesized and described before.[16] Compounds: **1**, **4,** and compounds of the **1**-Pro_x-Dap(Tamra)-NH_2 series were prepared by using peptide conjugate synthesis procedures that were described in [16].
Shortly: Peptide sequences were prepared by using Fmoc solid-phase peptide synthesis methods on Rink amide MBHA resin. Protected amino acids (3 equivalents) were dissolved in DMF and activated with HBTU/HOBt (2.94 eq each) in DMF/N-methylmorpholine. The solutions were added to the resin and shaken for 40 - 60 min. Reactions were monitored with Kaiser-test, which was followed by deprotection of Fmoc-group with 20% piperidine solution in DMF (20 min).
1.5 eq. of 5-(2-aminopyrimidin-4-yl)thiophene-2-carboxylic acid or 5-(2-aminopyrimidin-4-yl)selenophene-2-carboxylic acid was activated with HOBt/HBTU (1.47 eq each) in DMF/N-methylmorpholine and added to the resin and the mixture was shaken for at least 3h in a reaction vessel. Finally the resin were washed 5 times with each solvent (DMF, isopropanol,

DCE) and dried. Treatment with TFA/H_2O/triisopropylsilane solution (90/5/5, by volume) for 2 h was used for the cleavage of the peptide conjugate from the resin. The products were purified by reversed-phase HPLC and lyophilized.

Labeling of compounds with fluorescent dyes

1 eq. of an amine containing peptide conjugate and 1.3 eq. N-hydroxysuccinimide esters of the fluorescent dye were dissolved in minimum amount of DMSO and triethylamine. The solution was kept for 3h at room temperature and thereafter the solvent was removed in high vacuum. The residue was purified by reversed-phase HPLC and lyophilized to give desired products in 40 - 70% yields.

Synthesis of 1-pip-Tamra

5-(2-aminopyrimidin-4-yl)thiophene-2-carboxylic acid (1 eq), HBTU (1 eq) and DIPEA (3 eq) were dissolved in DMF. After 5 minutes piperazine (3 eq) in DMF was added and the solution was kept 3h at room temperature. The solvents were evaporated in vacuum and the residue was purified by HPLC and lyophilized. Labelling with 5-Tamra was performed as described above for other compounds.

Preparation of PVA films

PVA (Mowiol® 10-98, from Sigma-Aldrich) was dissolved in water by using overnight stirring at 80 °C. 7% solution of PVA was used in most experiments. The luminescent compound was dissolved in water and small amount of the solution was added to 7% PVA solution. In another variant of the experiment the luminescent compound was directly dissolved in 7% PVA. 20 µL of the solution was pipetted into each well of a low-volume 384-well polystyrene-made microtiter plate or on a quartz disc. Water was evaporated by keeping the samples at room temperature for 48 h. Thereafter the plate was kept under vacuum for 2 h before measurements.

20 µL of luminophore solution was pipetted to a well of a polystyrene-made microtiter plate for the recording of spectra and determination of luminescence lifetimes. 5 µL of the solution was carried to quarts discs of 1 cm diameter for measurement of PL quantum yields of compounds. 5-6 parallel measurements were performed for recording of the spectra and determination of luminescence lifetimes; two parallel measurements were performed to determine quantum yields.

Recording of time-gated PL spectra

Time-gated spectra were measured with Synergy Neo (BioTek) plate reader at room temperature and in the presence of air. High intensity excitation regime, 1 nm recording step, 100 µs measurement delay, and 2000 µs integration time (gate time) were used for all measurements. Excitation spectra were recorded in 250 – 440 nm range by using the signal registration at the emission maximum of the corresponding compound (Table 1). Emission spectra were measured in the 400 – 800 nm range by signal registration at the excitation maximum of the corresponding compound (Table 1). Spectral data from five parallel wells were summarized and normalized to present the spectra in the 0 – 100% intensity range.

Measurement of PL lifetimes

Luminescence lifetimes were measured with PHERAstar (BMG Labtech) plate reader and FluoroMax-4 (HORIBA Jobin Yvon) luminescence spectrometer. PHERAstar was the preferred instrument for measurement of shorter lifetimes because of higher sensitivity and signal stability. FluoroMax-4 was the preferred instrument for determination of longer lifetimes because of the ability to measure the signal in a wider time window. Wells of low-

volume 384-well plates were filled with compounds as described above. Lifetime measurement options of PHERAstar plate reader were applied using the module with excitation filter of 330(60) nm and emission filter of 590(50) nm. Signal intensity values in the measurement window from 50 to 1720 µs were collected and used for analyses. Preparation of PVA films containing luminescent compounds was performed on the polystyrene plates (1 cm x 2 cm x 0.2 cm dimensions), as described above. The plate was placed into the fluorescence cuvette of FluoroMax-4 at 45º angle. At least 50 µs time delay was used and maximum delay was varied depending on the properties of the compound to obtain almost complete decay of the signal. For compound **2**, the the longest measurement window of 300 ms was used. The data were fitted to the monoexponential decay model in case of distinct phosphors and to multi-exponential models in case of tandem compounds comprising an acceptor dye. Mean lifetimes were calculated from values of the mulfiexponential fit by using Equation 3

$$\overline{\tau_i} = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i} \quad (3),$$

$\overline{\tau_i}$ is the calculated mean/average lifetime, α_i proportion of the particular component of the decay and τ_i is the lifetime of this component. This property is also called an intensity-averaged lifetime.[35] Average results of measurements of 2-6 separate samples of each compound with 95% confidence intervals were presented. All lifetime measurements (expect temperature variations) were performed at room temperature (25 °C) and in the presence of air.

Measurement of PLQYs

Measurement of PLQYs was performed using the integrating sphere as outlined previously.[34] Briefly, a Fluorolog®-3 spectrometer (HORIBA Scientific) was equipped with a fiber coupled integrating sphere (Quanta Phi, HORIBA Scientific) for PLQY measurements. FluorEssence™ software (HORIBA Scientific) was used for calculation of PLQYs.

Conflicts of interest

There are no conflicts to declare.

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Keywords: phosphorescence • FRET • OLED • oligoproline • thiophene • selenophene

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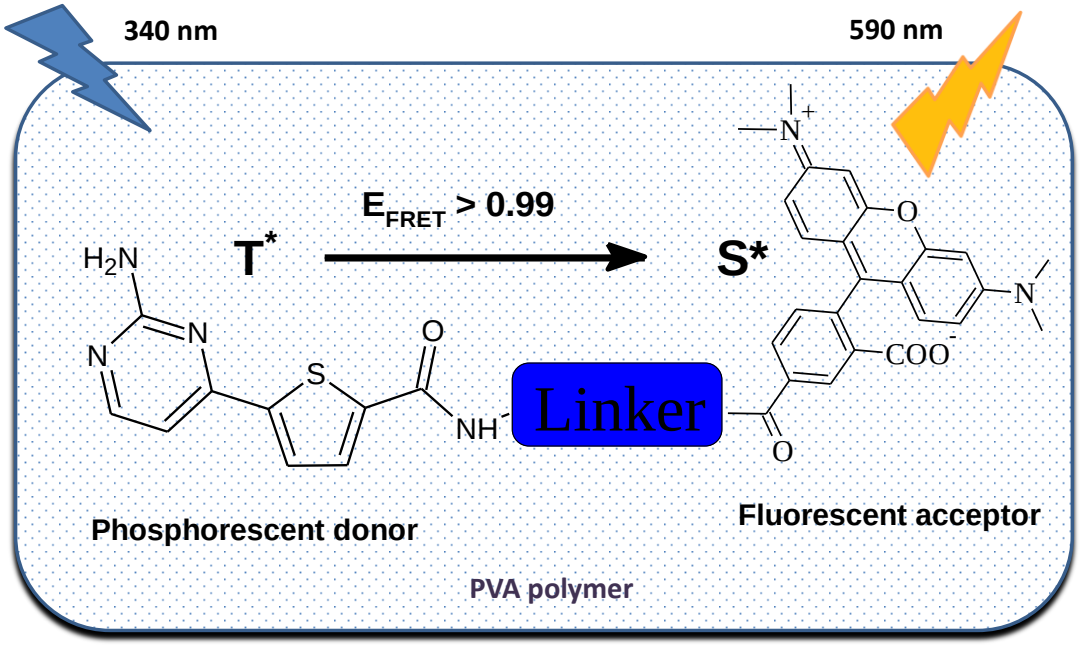
TOC


340 nm
590 nm
E_{FRET} > 0.99
T*
S*
Linker
Phosphorescent donor
Fluorescent acceptor
PVA polymer