Synthetic chromo/fluorogenic receptors for the recognition of biomolecules and ions

Project K108752, final report

The aim of our project has been the development of novel fluorescent molecular sensors for the detection of non-fluorescent biomolecules and ions (amino acids, biogenic amines, nucleotides). Two types of sensors have been created: direct sensors – with covalently bound receptor and indicator units and fluorescence indicator displacement (FID) assays. With respect to the potential applications, water-solubility, high photostabilty and favorable spectroscopic properties have been important aspects. The preliminary design of the new compounds and the evaluation of the spectroscopic results have been supported by theoretical chemical calculations.

The results of the project have been published in 21 papers in international journals. Students' research work had a substantial contribution to the results: MSc and PhD students were co-authors in all of our papers. Two MSc students were awarded 1st and one MSc student a 2nd prize at the National Scientific Students' Associations Conferences. Márton Bojtár, a participant of the project, received his PhD in 2017, another participant, Zoltán Szakács submitted his PhD thesis this year.

The main expenditures paid from our project have been direct materials costs: the costs of materials for the synthetic work, spectroscopic solvents, buffer materials, pipettes, cuvettes, office supplies. As instruments, UV lamps, photodiodes, diode lasers, a conductometer and two computers have been aquired. The personnel costs afforded the short-time (2-3 months) employment of three PhD students. The participation of two of these students at international conferences has also been covered from the budget.

Scientific results

1. 3-Hydroxyflavones: photophysics, photochemistry, synthesis of a novel ATP sensor

3-Hydroxyflavone (3HF) and many of its derivatives are dually fluorescent compounds, due to their intramolecular excited-state proton transfer (ESIPT) from their hydroxyl to their carbonyl group. Utilizing ESIPT, many 3HF-based ratiometric fluorescent sensors have been constructed. With respect to these applications, the photostability of 3HF-s is a key requirement. In our theoretical work, we have calculated the Gibb's free energy profiles for the major photodegradation reactions of the 3HF parent compound, which are its photooxygenation with triplet oxygen into O-benzoyl salicylic acid, its photosensitized oxygenation (3HF + singlet oxygen) into the same product and its photorearrangement into an indane-1,2-dione derivative.[1] The various mechanisms proposed in the literature, hypothesized on the basis of spectroscopic experiments, have been analyzed in the light of our theoretical results.

3HF-s substituted with a dialkylamino group in their 4'-position are versatile fluorescent probes: the positions and intensities of their N* and T* fluorescence bands depend on the polarity and hydrogen bonding nature of their local environment. In addition, they bind ATP selectively over the other nucleotides and indicate the reaction via the shift and enhancement of their N* fluorescence. We experienced that the diethylamino derivative, EtHF shows some decomposition under strong UV irradiation, which has to be taken into account in the applications of this compound in microscopic and analytical spectroscopic techniques. A detailed study on the photoreactions of EtHF has been carried out.[2] It was clarified that

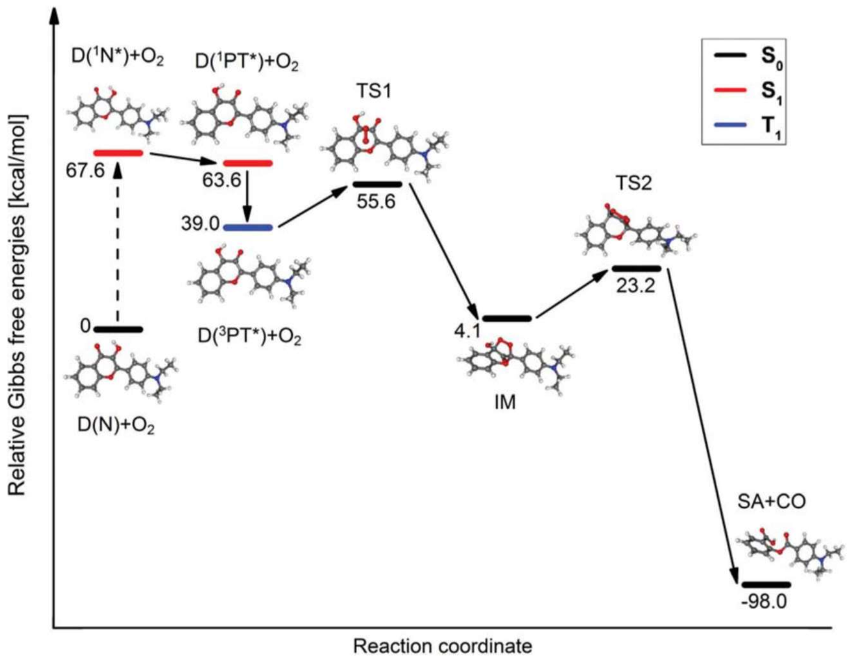

Fig. 1. Reaction path of the photoxygenation of EtHF. The product is the salycilic acid derivative SA.

photooxygenation takes place, with O-4-diethylaminobenzoyl salicylic acid as the main product. The photorearrangement into the respective indan-1,2-dione, shown by the parent compound 3HF, has not been observed. Theoretical calculations confirmed that the photooxygenation of EtHF (P.E.S is shown in Fig. 1) is more favorable than the rearrangement reaction, the reaction Gibbs energy and the energy barrier are lower for the former reaction.

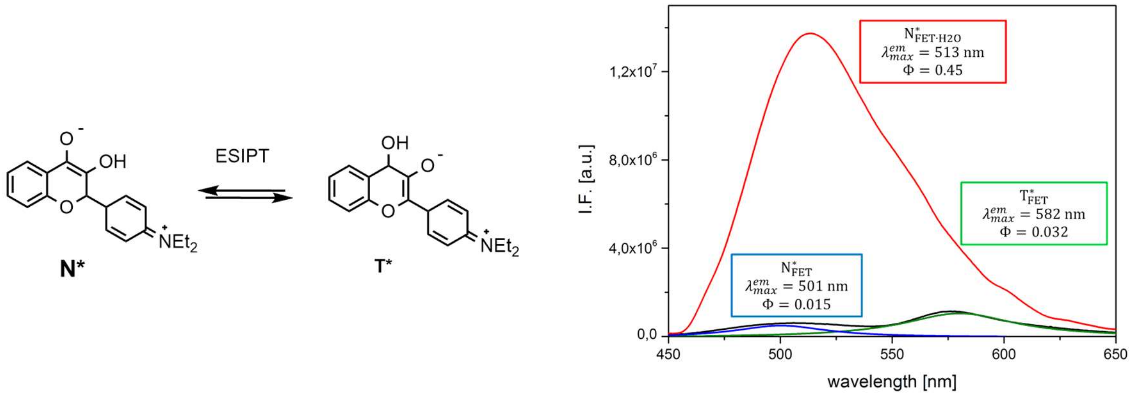

Fig. 2. Excited state proton transfer (ESIPT) in 4'-diethylamino-3-hydroxyflavone leads to dual fluorescence in acetone, ESIPT is blocked in the water complex.

4'-Dialkylamino-hydroxyflavones, as probes sensitive to the polarity and hydrogen bonding ability of their local environment, are frequently applied in aqueous systems (proteins dissolved in aqueous buffers, micelles, vesicles dispersed in water). With respect to such applications, we carried out a detailed study on the hydrogen bonding effects on the fluorescence properties of EtHF in water and acetone water mixtures.[3] It has been established that at low water contents the dye forms a 1:1 complex with water, which shows emission only from the normal excited form (ESIPT is blocked in the complex), the fluorescence quantum yield of the complex is high (see Fig. 2). At higher water concentrations, the interaction of EtHF with the hydrogen-bonded water clusters results in fluorescence quenching. In neat water the fluorescence quantum yield fell to ~0.001. Theoretical calculations showed that ESIPT is hindered both thermodynamically and kinetically in the water complex.

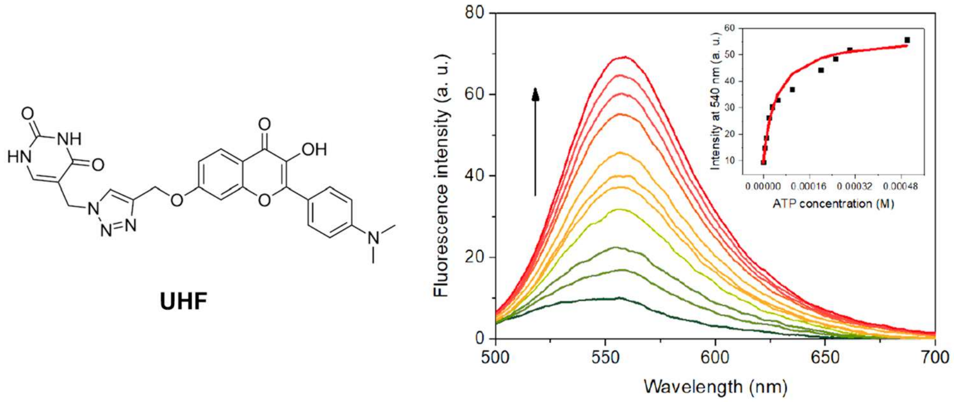

Fig. 3. Variation of the fluorescence spectrum of UHF upon the addition of ATP.

A new fluorescent ATP sensor has been designed (UHF in Fig. 3) in which an uracyl unit was coupled to 7-dimethylamino-3HF.[4] It was hoped that the interactions between the uracyl group of the sensor and the adenine unit of the ATP analyte will be analogous as in RNA-s, resulting in the enhancement of the selectivity. The synthesis was performed using click chemistry and the nucleotide recognition properties of the probe were evaluated using fluorescence spectroscopy. An excellent selectivity was observed over other nucleotides that might be the result of the beneficial effect of the complementary nucleobase.

2. Fluorescent indicator displacement assays with pillararene macrocyclic hosts

The fluorescence indicator-displacement (FID) assay is a leading approach in the field of molecular recognition. They are chemically supramolecular complexes of a fluorescent indicator and a synthetic receptor. The analyte - a non-fluorescent biomolecule in our cases - replaces the indicator in the complex. The differences in the optical properties of the free and complexed forms of the dye allows then the detection of the analyte.

The FID assays studied first in this project consisted of three stilbazolium dyes as guests and the anionic water-soluble carboxylato-pillar[5]arene as host.[5] Of the three dyes, 4-dimethylaminostyryl-N-methylpyridinium iodide (DAST) was found to exhibit a remarkable fluorescence enhancement and color change upon complexation by the pillararene. The potential application of such systems for the determination of the pesticide paraquat, as a non-fluorescent analyte has been demonstrated.

The same macrocycle, WP5 has also been applied in our FID assays for the fluorescent detection of basic amino acids.[6] The indicators in these assays were 4-amino-naphthalimides with an ‘anchor’ unit attached to their amino groups (see Fig. 4). As shown by the NMR spectra of the complexes, the anchor moieties penetrated into the cavity of the pillararene host, whereas the naphthalimide unit remained at the rim of the macrocycle. The fluorescence of the indicators was strongly quenched in their complexed forms, and was recovered when the indicators were displaced from the complexes by lysine and arginine. The effect has been found selective for these two analytes over the other essential acids.

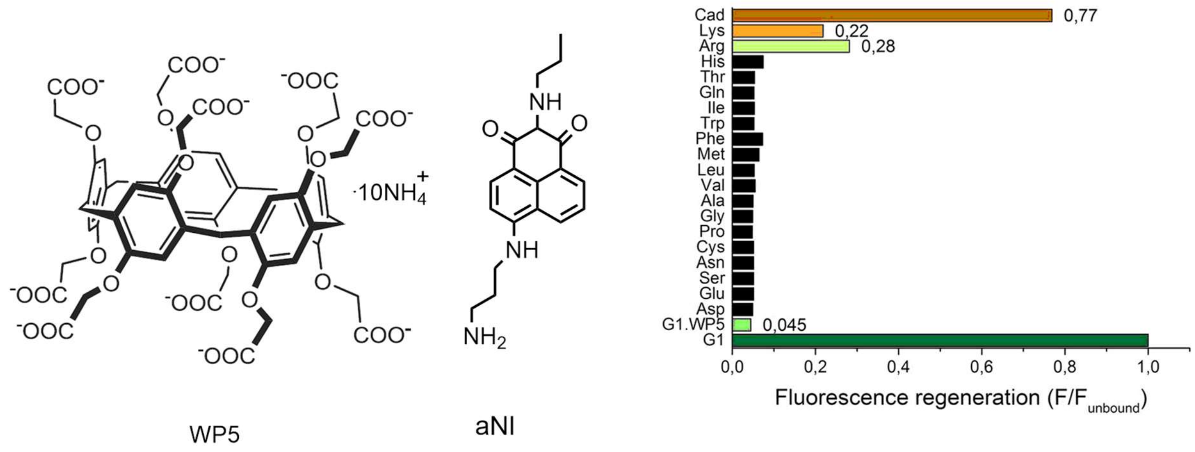

Fig. 4. Fluorescence regeneration values of cadaverine and various amino acids using aNI-WP5 indicator displacement assay. [aNI] = 3μM, [WP5] = 45 μM, [analyte] = 3mM.

A novel FID assay has been developed for the detection of ATP.[7] The assay was a host-guest type complex of the cationic macrocycle ammonium pillar[6]arene (AP6 in Fig. 5) with the anionic fluorescent dye Dapoxyl sodium sulfonate (DSS). The fluorescence of DSS enhanced dramatically upon binding to WP6. The addition of ATP to this system resulted in a strong fluorescence quenching ('turn-off' sensor), and the effect was selective over other nucleotides (AMP, ADP, GTP). To our knowledge, this has been the first AP6-based FID system and the first cationic pillararene-based FID assay for an analyte with biological importance.

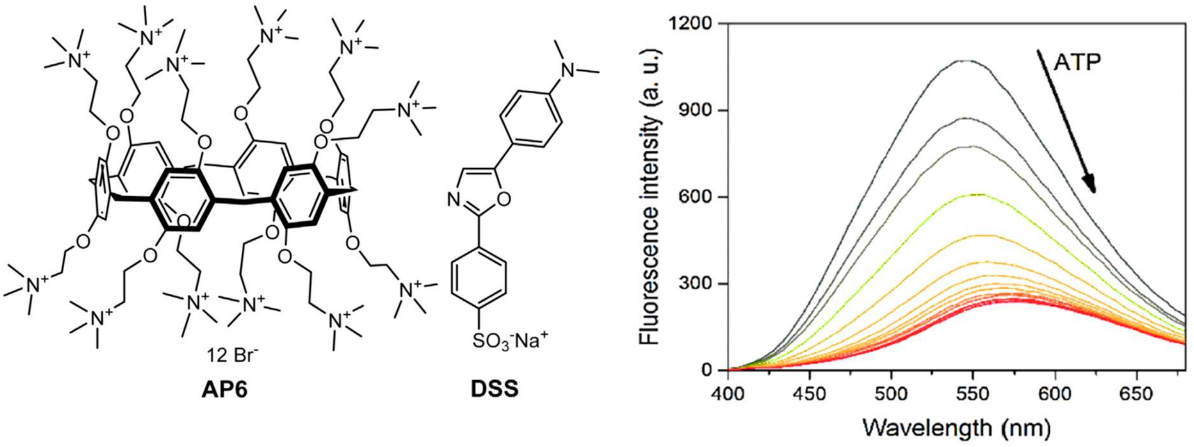

Fig. 5. Fluorescence spectra of assay DSS-AP6 in the presence of 0-200 equiv. of ATP.

Förster resonance energy transfer (FRET) is an energy transfer mechanism occurring between an excited donor and a ground state acceptor fluorophore. FRET is widely used in fluorescence sensing and imaging techniques. We synthesized two stilbazolium dyes (D and A in Fig. 6) exhibiting opposite fluorescence responses upon complexation with anionic, water soluble carboxylato-pillar[5]arene (WP5). [8] Then A and D were covalently linked via click chemistry, yielding DA, a FRET-capable ditopic indicator. In water, DA formed a pseudorotaxane with WP5, which was accompanied by a strong fluorescence enhancement combined with the modulation of the FRET process. We are currently working on the potential application of this new type of supramolecular system in ratiometrically sensing FID assays.

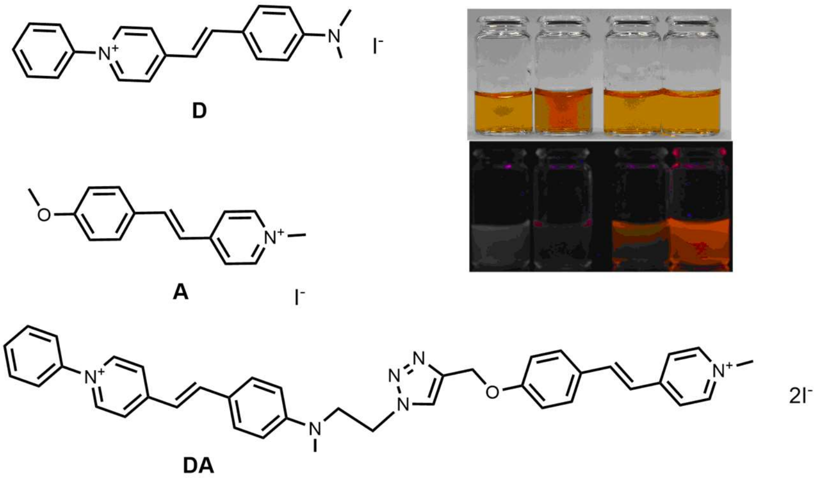

Fig. 6. FRET effect in dyad DA and its modulation by complexation with WP5. The vials contain (from left to right) D + A; D + A + WP5; DA; D + A + WP5. They are exposed to (top) daylight and (bottom) UV irradiation.

3. Functionalization of a water-soluble pillararene

There are several existing methodologies for the selective functionalization of pillararenes, however, the functionalization of the water-soluble derivatives remained still unexplored. The functionalized, water-soluble derivatives can be coupled with a signaling group making direct sensing possible. We have developed a novel synthetic strategy to address this problem.[9] The key step of our synthesis is the Claisen rearrangement, which can lead to the appropriately substituted key derivative. We synthesized the first orthogonally functionalized pillararene with a 10+1 substitution pattern capable of coupling through O, N, S, atoms. This could be an important starting material towards the expansion of the current applicability of this macrocycle family.

4. Spectroscopic characterization of phosphorous heterocycles

We determined the stereostructure of chiral phospholene oxides and a tetrahydrophosphinine derivative by analyzing their ECD spectra on the basis of theoretical calculations. [10,11,12,13,14,15] This was a part of a cooperative project coordinated by Professor G. Keglevich on the synthesis, detailed structural characterization and catalytic activity of chiral phosphorous heterocycles and their metal complexes. As a continuation of the project, the development of novel fluorescent dyes with phosphorus heterocycles may be considered – this is a promising new trend in the chemistry of fluorescent probes.

5. Chiral fluorescent crown-ether sensors

Two new chiral glucose-based azacrowns, a 15-crown-5 and a 21-crown-7, incorporating acridine fluorescent signaling units were synthesized by Prof. P. Bakó et al.[16] We studied their enantioselectivity towards chiral ammonium salts by absorption and fluorescence spectroscopy. The ligand with 21-memebered ring formed more stable complexes and exhibited chiral discrimination in case of four ammonium salt guest molecules. The highest enantioselectivity (K(R)/K(S) >3) was observed with the enantiomers of phenylethyl ammonium perchlorate.

Further glucose-, mannose- and altrose-based azacrowns, synthesized also by Prof. Bakó, were tested as chiral catalysts in asymmetric Michael reactions. Our contributions to the latter works have been the determination of the absolute configurations of prototype products in combined ECD spectroscopic and theoretical studies.[17,18]

6. Bioorthogonally applicable fluorogenic dyes

We participated in the design of new, bioorthogonally applicable azide quenched fluorogenic labels, the project co-ordinated by Dr. P. Kele.[19] With preliminary theoretical calculations we screened the possible compounds proposed by our organic chemist collaborators. The most promising ones were synthesized and tested in vitro and in vivo experiments, and their applicability has been proven. We also provided theoretical insights into the nature of such azide-quenched systems. These results can be used in the design of further fluorogenic dyes with better properties.

7. Solvation and protonation of Coumarin 102 (C102) in aqueous media

C102 is used as a fluorescent model drug for studying drug-protein interactions and drug delivery systems As a weak base, C102 is also utilized to probe the local acidity in proton-transfer membranes and at water-micelle interfaces. Motivated by such applications of the dye in aqueous systems, we have studied the solvation and protonation of C102, as two essential interactions occurring in the aqueous solutions, by optical spectroscopic experiments and theoretical calculations.[20] $pKa = 1.61$ was obtained for dissociation of the ground state dye and $pKa^* = 2.19$ for the dissociation of the excited species, from the absorption and fluorescence spectra, respectively. These values were closely reproduced by theoretical calculations via a thermodynamic cycle, using an implicit-explicit solvation model. The theoretical calculations revealed the dramatic changes of the strength of the solute-solvent hydrogen bonds upon excitation. A comprehensive analysis of fluorescence decay data has been performed, which concluded that a quasi-equilibrium of excited state proton transfer is reached only in strongly acidic solutions. From the equilibrium constants and photophysical data the stationary and time-resolved fluorescence spectra of this dye probe can be calculated as a function of *p*H and excitation wavelength.

8. Excited state processes in a naphtalimide fluorescent probe

1,8-Naphthalimides (NIs) with an electron donor substituent in the 4-position, constitute an important class of push–pull type fluorescent compounds. Their fluorescence quantum yields are highly sensitive to the local environment, due to a photoinduced electron transfer (PET) process. We carried out a detailed study on the excited state properties of 4-piperidino-1,8-naphthalimide, carrying out time-resolved fluorescence spectroscopic experiments and theoretical calculations.[21] The results unanimously proved the occurrence of an additional photoinduced process, the formation of a dark twisted intramolecular charge transfer (TICT) state from the emissive charge transfer (CT) species, the direct product of excitation. The temperature dependence of the kinetic data (see. Fig. 7) was consistent with a kinetic barrier consisting of three terms, the inherent barrier of the reaction, and the contributions of the solute–solvent interactions related to the solvent viscosity and polarity. The phenomenon can be utilized in the construction of polarity and viscosity sensitive fluorescent probes and aggregation-induced emission type materials.

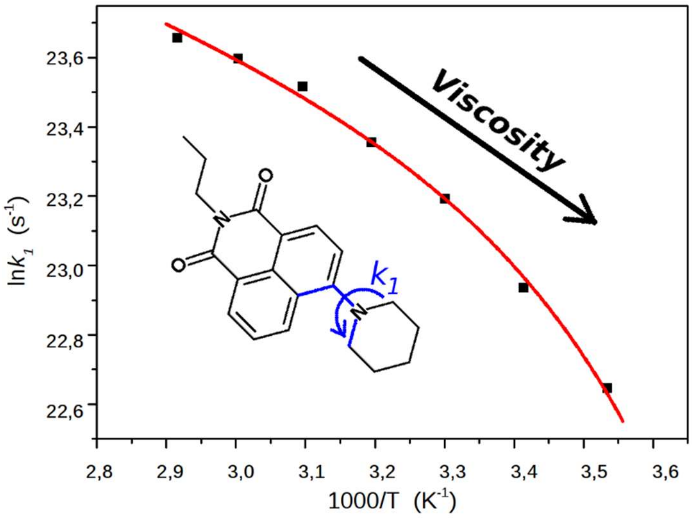

Fig. 7. The Arrhenius plot of the rate coefficient of TICT of 4-piperidino-1,8-naphthalimide in ethylene glycol is non-linear since the kinetic barrier varies with the temperature dependent solute-solvent interactions.

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