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Chapter 2

BODIPY Core as Signaling Unit in Chemosensor Design

Ana M. Costero, Margarita Parra, Salvador Gil and Pablo Gaviña

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Abstract

BODIPY derivatives possess unique photophysical properties and for these reasons, they have been used in numerous fields. Among the different applications, they are used in designing chemosensors that has increased in the last years. Here, we report several strategies and examples for detecting analytes of different characteristics: cations, anions, and hazardous and pollutant neutral molecules using BODIPY core as signaling unit.

Keywords: chemosensor, fluorescence, anions, cations, neutral molecules

1. Introduction

Supramolecular chemistry has become a coherent and alive body of concepts which has recently incorporated new areas of research [1–4]. The “classical” supramolecular chemistry has developed basic tools and concepts such as coordination of specific substrates to receptor (recognition), chemical reactivity induced by the guests (transformation), and positional controlled changes of atoms or molecules (translocation). On the other hand, another promising area of investigation is the development of “programmed supramolecular systems,” where the recognition process is coupled with a specific action.

Among these programmed systems of supramolecular background the so-called molecular chemical sensors, where the process of recognition is adapted to a process of detection, are of wide interest. The described behavior is achieved by means of the introduction in the ligand (or reactive site) of transducing units which are capable of transmitting information on the molecular recognition process through a change in its physical properties (e.g., optical or electrochemical).
There are three classical approaches for the development of chromogenic-fluorogenic sensors:

1. Binding site-signaling unit approach: In this approach, the receptor should contain two different subunits kept together by means of a covalent bond. One of such subunits is responsible for the complexation process, while the other transmits the molecular recognition process \[5\]. As it can be seen in Figure 1, the coordination of the guest induces a change of some properties of the signaling unit, that is, color (chromogenic chemosensors) or fluorescence (fluorescent chemosensors).

2. Displacement approach: This approach, as well as the former, implies the use of both, specific binding sites and signaling units. However, in this case, both subunits are not covalently linked, but forming a coordination ensemble \[6\]. In these systems, the addition of a given guest to the solution that contains this “molecular ensemble” favors the displacement reaction: the coordinating unit binds the guest, while the signaling unit is released toward the solution. If this unit shows different optical properties (color or fluorescence) depending on whether it is coordinated or in solution, its release causes a change of the signal. All these systems are based on the use of molecular receptors possessing coordination sites with size and charge distribution suitable to those of the guest (Figure 2).

3. Chemodosimeter approach: This approach involves the use of specific chemical reactions (generally irreversible) induced by the presence of certain guests that are coupled to a change of color or fluorescent emission \[7, 8\]. If the reaction is irreversible, the term sensor should not be strictly used, a more appropriate term should be a chromo or fluorogenic reagent or chemodosimeter. Figure 3 shows a scheme of this approach. The ultimate idea is to take advantage of the selective reactivity that determined guests present. The use of reactions induced by determined chemical species has the advantage of presenting a high selectivity and a cumulative effect that is directly related to the concentration.

Depending on the physical property of the signaling unit that changes in the process of complexation, one can readily have systems of different types, that is, electrochemical, fluorescent, colorimetric, etc. Among the different possibilities, fluorescent and colorimetric systems are very interesting due to their high sensibility and the advantage of a possible detection of species of interest to the “naked eye.”

Among the dyes or fluorophores that can be used as signaling unit, the BODIPY core presents several advantages due to its outstanding photophysical properties such as excitation/
emission wavelengths in the visible spectral region (∼500 nm), the relatively high molar absorption coefficients and fluorescence quantum yields, fluorescence lifetimes in the nanosecond range, and negligible triplet-state formation. On the other hand, they are relatively insensitive to pH and present good solubility, resistance toward self-aggregation in solution and robustness against light and chemicals [9, 10]. Moreover, the spectroscopic and photophysical profiles can be switched by introducing different electron releasing/withdrawing groups at the appropriate positions of the BODIPY core. Additionally, they usually show good biocompatibility that makes them useful for biological applications.

2. Fluoro- and chromogenic chemosensors and chemodosimeter based on the BODIPY derivatives

2.1. Sensors based on the binding site-signaling unit approach

Among the different substitutions in the BODIPY core, structures like these shown in Figure 4 have been widely used in probe design. There are two main reasons for this selection: (a) the presence of the methyl substituents at 1 and 7 positions of the BODIPY core hinders the free rotation or the phenyl group at 8 which enhances the fluorescence emission and (b) the substitution at 5 or 6 in structure (II) and (III) enlarges electronic delocalization giving rise to possible color changes after the interaction with the analyte.

Many examples of fluorescent sensors based on this type of BODIPY structures were summarized in 2012 by Boens, Leen, Dehaen [11]. For this reason, in the present chapter, only more recent publications will be considered.
In the field of alkaline cation sensors, compounds 1 and 2 are of interest [12] for two reasons: (a) they have been designed to act as cesium ion sensors and (b) they illustrate the influence that the position of the binding unit link to the BODIPY core has in the sensing response.

Complexation studies of 1 with potassium and cesium ions in CH$_3$CN and in a mixture of CH$_3$CN/CH$_2$Cl$_2$ 9:1 were carried out. The obtained data showed a slight bathochromic shift both in the absorption and in the fluorescence spectrum and only a small increase in fluorescence quantum yield. However, complexation with sensor 2 induced a more pronounced hypsochromic shift of absorption and fluorescence spectra. In addition, a strong increase of the fluorescence quantum yield was also observed in the presence of the cation. The different behavior is related to the structural differences between both molecules. In 1, the binding site is introduced at the meso position, while it is at the α position in compound 2. In the first case, the conjugation between oxygen atoms of the crown-ether coordination site and the BODIPY chromophore is interrupted which can explain the very slight changes in the photophysical properties of this ligand in the presence of the cations. By contrast, in 2, the BODIPY signaling unit is electronically conjugated with the oxygen system and this molecule presents a change. Therefore, important shifts in absorption and fluorescence spectra were observed in the presence of cations (Figure 5).

In relation to heavy metal cations, compound 3 is a selective sensor for Hg(II) in vitro and in vivo [13].

The sensing properties of 3 were studied using different metal ions (Li$^+$, Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Cr$^{3+}$, and Fe$^{3+}$ as perchlorates) in CH$_3$CN/PBS solution. Visual color change (from pink to orange) of compound 3 solution was observed only in the presence of Cu(II) and Hg(II) (Figure 6). However, the change of color induced by Cu(II) was less intense than the observed in the presence of Hg(II). The selectivity of 3 toward Hg(II) was established by using both UV-visible (a blue shift > 50 nm) and fluorescence (a blue shift > 20 nm and a large enhancement of the fluorescence emission) spectroscopy.

Zinc and cadmium are both elements that play many important roles in our daily life. Zinc is the second most abundant transition metal in the human body, and it is vital for the functions of a large number of enzymes, the stabilization of DNA, gene expression, and neural signal transmission. By contrast, cadmium is a dangerous poison that harms human health and the...
environment. Two BODIPY-based sensors (Figure 7) able to differentiate these two cations have been described [14].

The photophysical properties of compound 4 showed strong changes in the presence of Zn$^{2+}$ in acetonitrile solutions, whereas a large number of cations (Cd$^{2+}$, Pb$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Ag$^+$, Mg$^{2+}$, Ca$^{2+}$, Na$^+$, and K$^+$) induced small changes or no changes at all. Zn$^{2+}$ induced the appearance of a new band in the UV spectrum and a strong enhancement of the fluorescence emission. On the other hand, compound 5 showed selectivity toward Cd$^{2+}$ in phosphate buffered solution. Under these conditions, the absorption band showed a hypochromic effect upon the addition of Cd$^{2+}$ ion. In addition, the weak emission at 570 nm of compound 5 in PBS was shifted hypsochromically by 5 nm, and its intensity was greatly enhanced (the fluorescence quantum yield increased about 15-fold).

Finally, chemosensors 6 and 7 (Figure 8) based on the BODIPY scaffold have been reported for selective sensing of trivalent cations [15].
Chemosensor 6 showed, in water:CH$_2$CN (80:20 v/v), an intense absorption band at 490 nm, yet it was scarcely fluorescent. Addition of Fe$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Li$^+$, Hg$^{2+}$, and Ru$^{3+}$ did not modify the emission of 6, whereas trivalent cations Al$^{3+}$, Fe$^{3+}$, and Cr$^{3+}$ led to a very remarkable enhancement of the fluorescence emission at 515 nm. Moreover, no color modulations in the presence of metal cations were found for 6. This was an expected result bearing in mind the presence of methyl groups in the pyrrole units that most likely impose a

Figure 7. Selective sensors for Zn(II) and Cd(II).

Figure 8. Sensors for selective detection of trivalent cations.

BODIPY Dyes - A Privilege Molecular Scaffold with Tunable Properties
twist position of the phenyl ring that interrupts the conjugation between the N-methyl-N-(2-hydroxyethyl) coordination site and the signaling unit.

In contrast, the signaling unit and binding site in probe 7 are electronically connected, and therefore changes in both color and emission were found. In water:CH$_3$CN (40:60 v/v), 7 exhibited a strong absorbance with a maximum at 603 nm, and it was poorly fluorescent. Addition of Fe$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Li$^+$, Cu$^{2+}$, Hg$^{2+}$ or Fe$^{3+}$ to solutions of 7 in water:CH$_3$CN (40:60 v/v) did not induce any change neither in the UV-Vis nor in the fluorescence spectra. By contrast, in the presence of the trivalent cations Cr$^{3+}$ and Al$^{3+}$, the color of the solutions changed dramatically from blue to pink due to the appearance of a new band at 560 nm. Probe 7 also shows some color change in the presence of Fe$^{3+}$, but only when CH$_3$CN alone or mixtures with a maximum of 8% water were used. Interestingly, probe 7 also displays a remarkable strong fluorescence emission at 563 nm in water:CH$_3$CN (40:60 v/v) upon the addition of the metal cations Cr$^{3+}$ and Al$^{3+}$.

2.2. Sensors based on the displacement approach

Most of the sensors following the displacement approach are based on the complexes that in the presence of the analyte, undergoes a decomplexation process that induces strong changes in the optical properties of the system. In some cases, the fluorescence of BODIPY-based compounds is quenched when a complex with the appropriate metal ion is formed. Decomplexation induced by the analyte recovers the ligand fluorescence that can be observed. This approach allows preparing off-on fluorescent chemosensors.

In that sense, compound 8 (Figure 9) was prepared to detect nitrogen monoxide [16]. 8 in acetonitrile showed intense absorption band at 500.5 nm and an intense emission band at 520 nm. The fluorescence of the compound was quenched when Cu$^{2+}$ (as nitrate salt) was added. This behavior agreed with the coordination of the cation to the bipyridine binding group. By contrast, Cu$^+$ did not induce any change in the fluorescence of 8. Detection mechanism was based on the known ability of NO to reduce Cu$^{2+}$ to Cu$^+$. Thus, the analyte reduced the cation with the concomitant decomplexation and recovering of the ligand fluorescence.

Figure 9. Sensor of NO based on the displacement approach.
The fluorogenic sensing ability of probe 8-Cu$^{2+}$ was also observed to the naked eye. In particular, bright-green emission was clearly seen when the solutions of 8-Cu$^{2+}$ exposed to NO were illuminated at 254 nm with a conventional UV lamp. From titration studies, limits of detection (LOD) of 3 ppm were calculated. In addition, 8-Cu$^{2+}$ was recovered after the oxidation of Cu$^+$ to Cu$^{2+}$ induced by atmospheric oxygen. In particular, the regeneration of the 8-Cu$^{2+}$ probe was achieved using NO-free air. This process was repeated at least 5 times with only minimal loss of fluorescence intensity. Supported sensor using polyethylene oxide was prepared, and the sensing properties were kept in the solid state. Finally, studies of detection of NO in cells were also successfully carried out.

Based also on Cu$^{2+}$ complexes, compound 9 was prepared for detecting S$^{2-}$ in CH$_3$CN:water [17]. In this example, decomplexation was induced by the formation of the corresponding CuS salt (Figure 10). The probe works in a reversible way by adding additional amounts of cation to the solution in each step and has been tested in cells with interesting results.

Based also on the displacement approach, two complexes able to detect the V-nerve agent mimic demeton-S have been described [18]. Acetonitrile solutions of 10 showed an intense absorption band in the visible centered at 600.5 nm responsible of its blue color. Besides, solutions of 10 were nearly nonemissive. This fact was attributed to an efficient ICT quenching of the excited state of the BODIPY fluorophore from the electron-donating aniline group. In contrast, 10-Eu$^{3+}$ and 10-Au$^{3+}$ were pink and presented strong emission bands (at 572 and 573 nm for 10-Eu$^{3+}$ and 10-Au$^{3+}$, respectively) due to an inhibition of the quenching process, active in 10, upon coordination with the metal cations (Figure 11).

The behavior of 10-Eu$^{3+}$ in the presence of demeton-S was tested in acetonitrile. Addition of increasing quantities of demeton-S induced a progressive and marked decrease of the absorption at 553 nm and the appearance of a new band at 600.5 nm with a color modulation from bright pink to blue easily detectable to the naked eye. Besides, a remarkable quenching of the emission band at 572 nm was also observed. A very similar behavior was observed upon the addition of demeton-S to acetonitrile solutions of complex 10-Au$^{3+}$.

2.3. Sensors based on the chemodosimeter approach

Due to the selectivity showed by the probes designed following the chemodosimeter approach, there are a large number of applications for detecting different species.
2.3.1. Detection of anions

Following an approach that combines the chemodosimeter and the displacement mechanism, compound 11 and its Cu²⁺ complex were prepared [19]. First, it was established that the loss of Cu²⁺ from 11-Cu²⁺ led to fluorescence enhancement through the production of 11. Due to the strong affinity of Cu²⁺ to thiols, the ability of 11-Cu²⁺ to participate in the detection of various biological thiols was examined (Figure 11). Changes were displayed in the presence of thiol-containing amino acids and peptides (L-cysteine (L-Cys), homocysteine (Hcys), N-acetyl-L-cysteine (N-acetyl-L-Cys), methionine (Met), glutathione (GSH), and the remaining non-S-containing amino acids (Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in aqueous solution (CH₃OH:HEPES buffer, 30:70, pH 6.5). Significant fluorescence enhancement was only observed upon the addition of Cys. The sensor selectively recognized cysteine over other biothiols (Hcys, N-acetyl-L-Cys, Met, and GSH). The limit of detection for Cys was determined as 6.0 μM (10 mM, CH₃OH:HEPES buffer, 30:70, pH 6.5) (Figure 12).

Also, in relation to detecting biothiol compound 12 was prepared [20]. BODIPY substituted with 8-phenylmecapto group was enabled to discriminate biothiols like GSH, Cys or Hcys through a thiol-induced SNAr substitution-rearrangement cascade reaction. Discrimination among different biothiols is related to the leaving group characteristic and steric hindrance in the reactive center. In CH₃CN/PBS buffer (3:7, v/v, 10 mM, pH 7.4) at 25°C, 12 showed a main
absorption at 528 nm and displayed a main absorption at 493 nm. The addition of 10 equiv. of Hcys or GSH gave rise to a slight hypsochromic shift in the absorption maxima in addition to a small increment of intensity. The emission behaviors of 12 showed an enhancement of the fluorescence emission of 20-fold enhancement for Hcys at 543 nm, and 80-fold for GSH at 547 nm when excited at 510 nm. By contrast, no enhancement was observed in the presence of Cys. However, Cys showed a strong emission band at 493 nm when excited at 440 nm. The enhancement was around 300-fold, whereas Hcys or GSH only induced small emission changes (Figures 13).

2.3.2. Detection of neutral molecules

There are a large number of neutral compounds whose detection has been developed using BODIPY-based chemodosimeters [21–28]. In this chapter, there are summarized some probes used in detecting dangerous or strongly pollutant analytes.

2.3.2.1. Explosives

3,5-Bis(acetal) BODIPY 13 was able to detect picric acid (PA) in a chloroform solution [29]. 13 showed a strong absorption band at 508 nm with a distinct shoulder at 478 nm. The emission spectra showed a maximum located at 514 nm and a quantum yield of 0.08. The fluorescent properties were similar when water-acetonitrile mixtures were used. The sensor response in the presence of several nitroaromatic explosives (picric acid (PA), 2,4,6- trinitrotoluene (TNT), 2,6-dinitrotoluene (DNT), 1,4-dinitrobenzene (DNB), 1,4-dinitrobenzoic acid (DNBA), 2,4-dinitrophenol (DNP), 1,4-benzoquinone (BQ), and 4-nitrophenol (NP)) was studied. The absorption spectrum of compound 13 showed a red shift of around 15 nm when PA was added. The other studied explosive did not induce any change. The absorption modification could be observed by naked-eye (from pale green to bright yellow-orange). In addition, the emission spectrum also was altered in the presence of PA, with a red-shift of the band (around 20 nm) and a simultaneous increase of the emission intensity (6-fold). The emission maxima remained unaltered in the presence of the other studied analytes (Figures 14).

![Figure 12. Sensor for detecting cysteine.](image-url)
2.3.2.2. Nerve agents

Several BODIPY derivatives have been synthesized to recognize mimics and real nerve agents. In this sense, compounds 14-16 were prepared (Figures 15 and 16) [30, 31].

The sensing units in these compounds were based on the 2-(2-dimethylaminophenyl)ethanol moiety. This moiety has two nucleophilic groups, a dimethylamino group and a primary alcohol (I). The later, in the presence of some organophosphates and organophosphonates, attacks the electrophilic phosphorous atom of the nerve agent in a $S_N^2$ phosphorylation reaction. Consequently, a phosphoester good leaving group is formed (II). Assisted by preorganization factors, the dimethylamino group attacks the carbon atom holding the phosphoester in an intramolecular cyclization reaction. This yields a cyclic quaternary ammonium salt (III) that has significantly different properties to those of the dimethylamino moiety (Figure 17).
Acetonitrile solutions of 14 and 15 showed no fluorescence emission in the initial state, due to an ICT (from the dimethylamino moiety to the BODIPY core) in the excited state that efficiently deactivates the BODIPY fluorescence. Cyclization of the sensing unit after the detection reaction with the concomitant formation of the positive charge cancels the possibility of the electron pair of the dimethylamino group to travel to an ICT state. Thus, after excitation, the fastest relaxation path is via photon emission from the LE state. The orthogonal disposition of the phenyl group at the meso-position in compound 15 practically annuls the electronic conjugation between the sensing unit and the transducer. In consequence, no changes in absorption were observed for this compound. Comparative studies with other mimics (diethyl cyano-methylphosphonate (DECP), diethyl 1-phenylethyl phosphonate (DPDP), ethyl S-phenyl ethyl phosphonothiolthionate (diphonate), diethyl(methylthiomethyl)phosphonate (DMTP), malathion, diethyl(2-oxopropyl)phosphonate (DOPP), diethylchlorothiophosphate (DCTP), and compounds) present in real environments (gasoline and diesel) were studied (Figure 18).

On the other hand, the sensing unit of compound 16 was connected to the fluorophore by means of a rotation free triple bond that allows coplanarity between the two moieties. Thus, after cyclization, not only changes in emission but also a big hypsochromic shift of the main absorption band was observed (Figure 19).

Due to the probe structure, compounds 14–16 gave rise to positive false responses in the presence of acids. To overtake this problem, compound 17 was prepared [32]. This compound
possessed the necessary functionality to avoid false positives caused by adventitious acid contaminants. In fact, the pyridine moiety was more basic than the terminal amino group and was the first point of reaction in the presence of acid, thus triggering the characteristic signal response (i.e., little effect on the absorption profile but a significant change in fluorescence intensity). On the other hand, generation of the cyclic ammonium salt was the most favorable reaction in the presence of the nerve agent, thereby giving rise to the accompanying change of color. The system was designed in such a way that even in the presence of both species (i.e., nerve agent and acid), a response was reported (Figure 20).

On the other hand, compound 18 was prepared to respond differentially to Tabun and Sarin/Soman, in addition to being insensitive to acid interference (Figure 21) [33].

The chromogenic behavior of the acetonitrile solutions of probe 18 was tested in the presence of DFP and DCNP. Addition of DCNP to 18 induced a marked decrease in the band centered at 591 nm, and the appearance of new bands at 515 and 560 nm concomitantly with a clear color change from bright pink to orange. In contrast, a very different chromogenic response was observed when DFP reacted with 18. In this case, a major bathochromic shift of the visible absorption band of 18 from 590 to 715 nm was observed. This bathochromic shift was reflected in a color modulation from bright pink to light blue.
The different chromogenic responses observed upon the addition of DCNP and DFP to 18 were related with the release of CN$^-$ and F$^-$ anions upon the phosphorylation of the phenol moiety. Cyanide (the unique by-product from the Tabun simulant DCNP) reacted with the carbonyl group at the 3-position of the BODIPY core of 18 to yield an electron-rich cyanohydrin moiety. Moreover, fluoride (the unique by-product from the Sarin and Soman simulant DFP) induced the hydrolysis of the triisopropylsilyl protective group. The deprotection reaction generated a strong intramolecular charge transfer (ICT) donor phenoxide ion in full conjugation with the BODIPY core, which would reduce the energy gap for the $S_0$-$S_1$ transition, and thus resulted in a large red shift in absorbance.

Oximates have been used from the beginning in designing chemosensors for detecting nerve agents and their simulants. Following this idea, compound 19 was prepared [34].

Emission spectrum of 19 (2·10$^{-6}$ M in 0.1 mM, pH 7.4 HEPES buffer) showed a band at 508 nm. Fluorescence emission of 19 in the presence of diethylchlorophosphate (DCP) and
diethylmethylphosphonate (DEMP) decreased, but with diethylcyanophosphonate (DCNP), intensity increased. Possible mechanisms are described in Figure 22. The proposed mechanism was supported by the results obtained using model benzene derivatives bearing the same functional groups.

Compound 20 was prepared for detecting phosgene [35]. Fluorescent behavior of 20 in the presence of phosgene was investigated both in solution (acetonitrile, with 0.1% Et₃N) and supported on paper. In solution, compound 20 exhibited no emission because the PET-promoted quenching arising from the meso-amine moiety. However, upon the addition of triphosgene (1 equiv.), an intense emission band appeared at 511 nm. The PET inhibition was explained taking into account the chemical transformation of the amine group into the corresponding urea derivative. Paper supported probe was successfully used to detect the analyte by using a smart pone (Figure 23).
2.3.2.3. Pollutant gases

Nitrogen oxides are very dangerous contaminants source of severe environmental problems such as acid rain, smog formation, global warming, and ozone layer weakening. Among these compounds, NO\textsubscript{2} is one of the most prevalent and dangerous. Due to the ubiquitous presence of this gas and its health effects, the development of selective and sensitive methods for its detection and quantification has aroused a lot of interest. Thus, compounds 21 and 22 were designed to recognize this gas [36, 37]. The sensing mechanism was based on the carbonyl group regeneration in the analyte presence. In addition to the low limit of detection showed by these compounds, the simple synthesis pathway is an additional advantage (Figure 24).

Compound 22 silica nanoparticles-based material that worked following a similar protocol showed interesting chromogenic properties. In the presence of nitrogen dioxide, a change color from green to brown was observed (Figure 25).
3. Conclusions

The BODIPY core has been successfully used in the designing of chemosensors following the three more commonly used approaches: binding site-signaling unit, displacement, and chemodosimeter. Depending on the position of the reactive unit in the BODIPY core, chromogenic or fluorescent responses were achieved. In many cases, the analyte induced changes can be observed by the naked eye. Cations, anions, and neutral molecules can be detected in different media: organic or aqueous. The biocompatibility of many of these compounds allows their use in biological applications.

Author details

Ana M. Costero*, Margarita Parra, Salvador Gil and Pablo Gaviña

*Address all correspondence to: ana.costero@uv.es

Interuniversity Institute of Molecular Recognition and Technologic Development, University of Valencia-Polytechnic University of Valencia, Valencia, Spain

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