Communication

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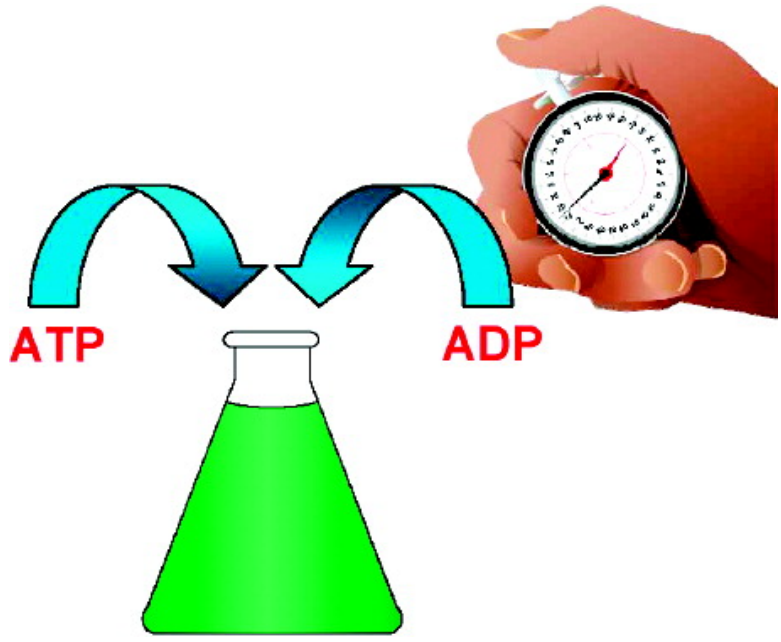

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Indicator Displacement Assays as Molecular Timers

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Indicator displacement assays (IDAs) have emerged as powerful analytical tools.[1] These assays are based on dyes or fluorescent ligands, which compete with analytes for the binding to synthetic receptors. Recent applications include the determination of catecholamines in urine samples,[2] the threshold detection of malate in Pinot noir grapes,[3] the sensing of nitric oxide,[4] and the monitoring of glucose oxidase activity in blood serum.[5]

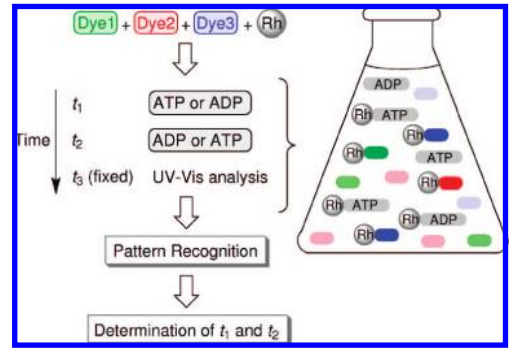
So far, IDAs have primarily been used to determine the identity and/or the quantity of certain analytes. In the following we demonstrate that IDAs can also be used to obtain information about *the history of analyte variations*. In particular, we will show that a simple mixture of commercially available dyes and a rhodium complex can be used to time the addition of ADP and ATP with good resolution.

In IDAs, the signaling unit is bound to the receptor via noncovalent interactions. As a consequence, fast exchange reactions and short analyte response times are generally observed.[6] A photochemical sensor, whose optical status is determined by the time at which certain analytes are added, however, needs to operate under kinetic control. To realize a molecular timer with IDAs, we therefore had to find receptor-dye combinations that undergo sufficiently slow exchange reactions.

For the receptor, we decided to use the Rh^{III} complex $[Cp^*RhCl_2]_2$. When $[Cp^*RhCl_2]_2$ is dissolved in water, air-stable Cp*Rh-aqua complexes are formed. The three coordination sites opposite to the π-ligand display a high affinity to N/O-donor ligands. We had previously shown that $[Cp^*RhCl_2]_2$ can be used to build IDAs for biologically interesting analytes.[7] Importantly, these studies had revealed that the 4d transition metal complex Cp^*Rh^{III} shows significantly slower exchange reactions than what is typically found for complexes of 3d transition metal ions such as Cu^{II} or Zn^{II}. We therefore reasoned that the competitive complexation reaction of a certain analyte-dye pair to the Cp*Rh-complex should display a characteristic rate profile due to specific exchange kinetics.

Searching for suited dyes, we first investigated the binding kinetics of the dye Azophloxine. We had previously shown that this dye forms a strong 1:1 complex with Cp^*Rh^{III} in buffered aqueous solution ($K = 3.2 \times 10^7$ M^{-1}).[7c] The complexation is accompanied with a pronounced decrease in absorption in the region of 450−500 nm. When a buffered solution (MOPS, pH 7.4) of complex $[Cp^*RhCl_2]_2$ (final Rh concn: 75 μM) was added to a solution of Azophloxine (final concn: 75 μM) at room temperature, a fast complexation reaction was observed with a half-life of $t_{1/2} \approx$ 1.3 min. For an application as a timer on the 0−10 min time scale, this rate was too fast. Looking for means to slow down the reaction without lowering the concentrations, we found that the addition of pyrophosphate (PPi) resulted in significantly slower complexation rates. When the above-mentioned experiment was repeated in the presence of 0.25 mM of PPi, the initial rate of the complexation reaction was reduced by a factor of 3. A likely explanation is that the Cp*Rh complex forms a complex with PPi, which slows down the complexation with the dye.

Scheme 1. A UV−Visible Analysis after Time t_3 in Combination with a Multivariate Analysis Can Be Use to Determine the Times t_1 and t_2, When ADP and ATP Were Added to a Solution Containing a Cp^*Rh^{III} Complex and the Dyes Azophloxine, Glycine Cresol Red, and Naphthol Blue Black



To further increase the discriminatory power of our system, we wanted to use simultaneously several dyes with distinct colors and binding properties.[7a] A screening of commercially available dyes revealed two other dyes with suitable properties. Under similar conditions as described above, glycine cresol red and naphthol blue black bind to the Cp*Rh complex with comparable kinetics. Importantly, these dyes show very distinct changes in different regions of the UV−vis spectrum upon binding to the Rh complex (see Supporting Information).

The three dyes were then used to construct an assay, which can be employed to determine the time at which the analytes ADP and ATP were added. The basic concept is shown in Scheme 1. The analytes ADP and ATP are added at times t_1 and t_2 to a freshly prepared mixture of the dyes and the metal complex. The two analytes compete with the three dyes for the complexation to the metal. Since the free and the metal-bound dyes show different colors, a characteristic UV−vis spectrum is obtained. A UV−vis analysis at time t_3 followed by a multivariate analysis then provides the determination of the addition times t_1 and t_2.

In a first set of experiments, we have calibrated the timer. The analytes ([ADP] = [ATP] = 250 μM) were added between 0 and 10 min to a freshly prepared solution containing the Rh complex, PPi, and the three dyes (for details see Supporting Information). For the calibration, 25 samples with variable ADP- and ATP-addition times were analyzed by measuring the absorption at 375, 500, 525, 550, and 620 nm after t_3 = 15 min. Each sample was measured five times using a microplate reader. The dimensionality of the data set was reduced to two dimensions with the help of a LDA.[8] This data preprocessing did not result in any significant loss of information and facilitated further analyses. The resulting values

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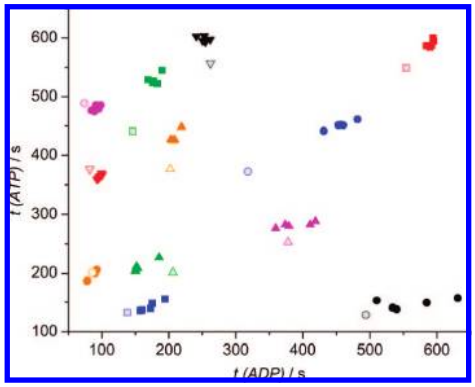

Figure 1. The ADP and ATP addition times determined by the molecular timer in comparison with the real addition times for 12 test samples. The predictions are shown as filled symbols (five measurements each) and the real addition times are indicated by empty symbols.

were then used to train an artificial neural network (ANN) employing a standard multilayer perceptron model.[9] The predictive power of the trained ANN was evaluated with 12 test samples with randomly chosen addition times between 0 and 10 min for ADP and ATP. Analogous to the calibration samples, the test samples were analyzed by UV−vis spectroscopy after 15 min (five repetitions). The predictions of the ANN for the five measurements in comparison to the real addition times of ADP and ATP are shown in Figure 1. On average, the deviation of the prediction for the addition time of ADP is 36 s and for the addition time of ATP 34 s. This correlation is quite remarkable, given that the timer has to distinguish submillimolar concentrations of two structurally very similar analytes.

The determination of two independent addition times requires at least a two-channel signal output. In our case, we have used the data of five different wavelengths. These data are partially correlated, since the UV−vis spectrum of the molecular timer is a composite spectrum of the various metal-dye complexes and the free dye molecules in solution.[10] However, the information content of the signal is clearly sufficient to get good temporal resolution. The simultaneous utilization of two or more analyte-specific—and therefore completely uncorrelated—IDAs would represent an interesting extension of the present concept. One should note, however, that the experimental realization of such a system would likely be much more challenging (for the analysis described above, for example, one would need two receptors that show a perfect discrimination between ADP and ATP).

In summary, we have described a multicomponent indicator-displacement assay, which can be used to determine in retrospect the time at which two bioanalytes were added to a solution. The molecular timer is based on UV−vis spectroscopy and has a 0 to 10 min time window, but it is easy to envision conceptually related systems, which operate at a different time scale, or which are based on fluorescence spectroscopy.[11] Contrary to previously described photochemical devices, which can indicate the history of a specific chemical event with a threshold-defined signal,[12] we use the time-dependent adaptation of a complex chemical network as the basis for our analysis. This "systems chemistry"[13] approach has the advantage that the timer can decode a larger number of chemical events. Molecular timers with a memory for time-dependent analyte variations should be regarded as a potential alternative to real-time measurements with chemosensors, which show a fast response time. They could be of interest for cases, where a real-time analysis is not possible. A limitation of the present system is that the signal readout has to be performed at a fixed time. For future timers this limitation could be overcome by quenching of the exchange reactions before the spectroscopic analysis by chemical (e.g., change of pH) or physical means (e.g., filtration of an immobilized receptor). Studies along these lines are pursued in our laboratory.

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Supporting Information Available: UV−visible data for the reaction of the Rh complex with the three dyes in the absence and in the presence of analytes, experimental procedures, and a description of the data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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