Systems Chemistry

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Indirect Optical Analysis of a Dynamic Chemical System**

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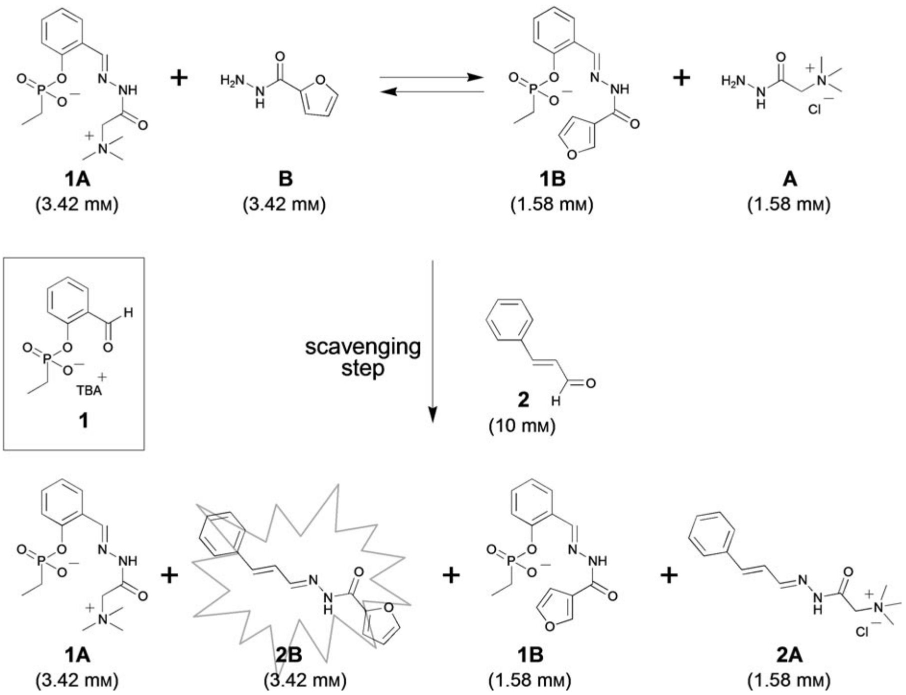
Dynamic covalent chemistry involves reversible covalent bond formation; these reactions are typically under thermodynamic control.[1] Dynamic covalent chemistry is now widely applied in the fields of nanotechnology,[2] dynamic combinatorial chemistry,[3,4] materials chemistry,[5] and more recently in systems chemistry.[6] A particular area concerns bimolecular reactions in which reversible covalent bond formation is flanked by a noncovalent interaction, referred to as dynamic covalent capture.[7] Here, the thermodynamic stability of the product is determined by the strength of the noncovalent interaction, which renders dynamic covalent capture a powerful analytical tool for measuring weak interactions.[8–10] Recently, we have shown that it allows for the self-selection of functional groups that are able to stabilize the transition state of a chemical reaction and are able to accelerate the chemical conversion.[11] The critical point in these studies is the determination of the equilibrium constant for the dynamic covalent equilibrium reaction. In general, the equilibrium constant is determined by measuring the concentration of the target compound at equilibrium, taking advantage of a characteristic property (e.g. chemical shift, ionization potential, or polarity). Consequently, this determination often requires an adaptation and optimization of the analytical protocol for each specific compound under investigation. This method is therefore less attractive when large numbers of compounds are to be screened. Herein, we report a new approach for determining the composition of a dynamic covalent equilibrium. Rather than directly measuring the concentration of the target compound, it is indirectly determined by quantifying the concentration of a reporter molecule. This method has the advantage that the equilibrium composition is translated into a unique UV/Vis signal that is independent of the target molecule.



Scheme 1. Detection protocol for the analysis of the thermodynamic equilibrium between hydrazones **1A** and **1B** based on the quantitative conversion of hydrazides **A** and **B** into **2A** and **2B**, respectively, using the scavenging agent **2**. The concentration of **2B** can be determined unequivocally from an absorbance measurement at 348 nm, and the concentration of all other species can be determined using mass balance.

Previously we have shown that the thermodynamic stability of hydrazone members of a dynamic library increases when stabilizing noncovalent interactions occur between the functional groups present in the aldehyde and the hydrazide components.[12] For example, in hydrazone **1A** stabilizing interactions occur between the phosphonate group of **1** and the ammonium group of **A** (Scheme 1). The screening protocol refers to the determination of the thermodynamic stabilities of hydrazones relative to a reference hydrazone lacking any particular functional groups (for example, **1C**, Figure 3). Recently, we have shown that ultrafast ^1H–^{13}C HSQC NMR spectroscopy is a very powerful tool to assess in a single experiment all kinetic and thermodynamic parameters of an equilibrating system.[13] Herein we describe a UV/Vis-based methodology which renders the parallel screening of hydrazides straightforward, fast, and inexpensive.[14,15]

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The strategy presented herein was developed based on two general considerations. First, since all hydrazides are screened against the same reference hydrazide, it is more attractive to focus on the determination of the concentration of the reference rather than the target. Second, since no particular structural properties are required for the reference, it can be chosen based on its ability to generate a unique signal. In fact, our choice to use hydrazide **B** as a reference was motivated by the observation of Herrmann, Lehn et al.[16] that the reaction of **B** with *trans*-cinnamaldehyde (**2**) results in the formation of hydrazone **2B**, which has a highly characteristic UV/Vis absorption spectrum. This results from its conjugated push–pull system (Figure 1) and allows for the quantification of the concentration of **2B** in the presence of other species, simply by measuring the absorbance at 348 nm.

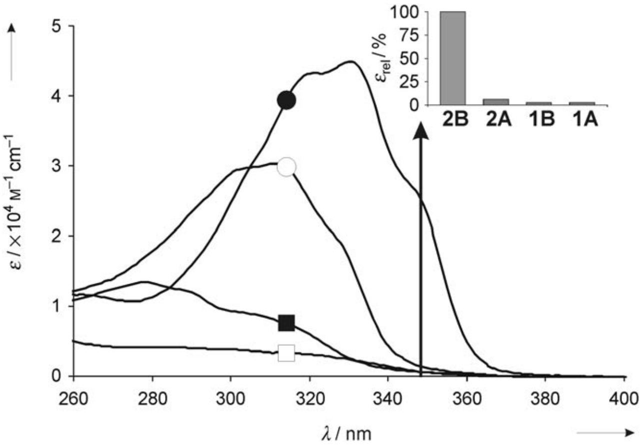

Figure 1. UV/Vis spectra for hydrazones **1A** (■), **1B** (□), **2A** (○), and **2B** (●) in a mixture of $CD_3OD/[D_6]DMSO/CD_3CN$ (70:15:15). Inset: relative molecular extinction coefficients (ε_{rel}) at 348 nm.

A two-step protocol was used involving an initial equilibration between hydrazones **1A** and **1B** followed by a scavenging step in which all free **B** is converted into hydrazone **2B** (Scheme 1). Mixing aldehyde **1** and hydrazides **A** and **B** in a 1:1:1 ratio (5 mM of each) in a 70:15:15 $CD_3OD/[D_6]DMSO/CD_3CN$ mixture at 40°C in the presence of a catalytic amount of TFA (0.1 equiv) results in the quantitative formation of hydrazones **1A** and **1B**. Owing to the presence of an excess of hydrazides **A** and **B**, these hydrazones interconvert and reach a thermodynamic equilibrium in which **1A** and **1B** are present in a ratio of 68:32. Kinetic ^{1}H NMR measurements showed that it took about two hours before thermodynamic equilibrium was reached. The law of mass dictates that at equilibrium, hydrazone **1A** has a concentration of 3.42 mM, equal to the 'left-over' concentration of hydrazide **B**. Subsequent addition of *trans*-cinnamaldehyde (**2**; 10 mM, 2 equiv) resulted in the quantitative conversion of hydrazides **A** and **B** into hydrazones **2A** and **2B**. The products were formed within 2 h, and benefits from the addition of two equivalents of **2**. Addition of only one equivalent of **2** slowed down the reaction kinetics due to depletion of the compound **2**. The scavenging reaction had no effect on the concentrations of **1A** and **1B** (Figure 2). In fact, even after 12 additional hours at 40°C no change in the concentration of any of the four hydrazones was observed. In the final mixture hydrazones **2B** and **1A** were present in an equimolar ratio, as determined by ^{1}H NMR spectroscopy. Importantly, measurement of the absorbance of the mixture at 348 nm after a 100-fold dilution and comparison with a calibration curve for **2B** gave a concentration of 3.37 mM, which is in excellent agreement with the concentration obtained for **2B** (and thus **1A**) by NMR analysis. This agreement illustrates that the protocol is simple, fast (4 h), and accurate.

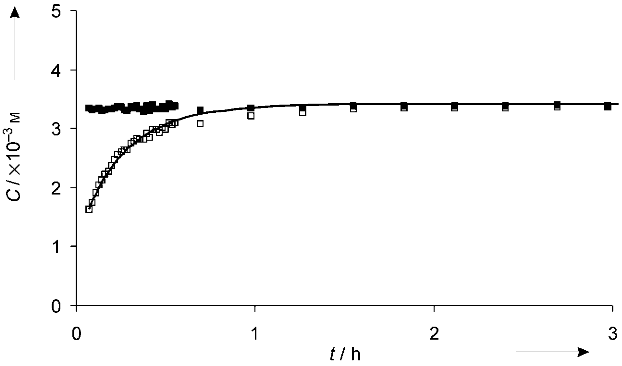

Figure 2. Concentrations (*C*) of **1A** (■) and **2B** (□) according to ^{1}H NMR (300 MHz) analysis as a function of time upon the addition of two equivalents of **2** to a 1:1:1 mixture of **1**, **A**, and **B** at thermodynamic equilibrium. Spectra were recorded in a 70:15:15 mixture of $CD_3OD/[D_6]DMSO/CD_3CN$ at 40°C.

The main advantage of this screening methodology is that the structure of the target has no influence on the output signal. To illustrate the advantage, we reexamined hydrazides **C**–**F** that were previously shown to form hydrazones of different thermodynamic stabilities with aldehyde **1** (Figure 3).[11] All hydrazides were subjected to the previously described reaction conditions and the concentrations of the corresponding hydrazones with **1** were determined directly from the ^{1}H NMR spectra and indirectly from a UV/Vis readout at 348 nm. The excellent correspondence between both values demonstrates the validity of this screening method.

This approach has a general applicability for analyzing product formation in competitive reactions. These reactions include not only the determination of dynamic covalent equilibria as described herein, but also covalent reactions or noncovalent reactions that lead to the formation of kinetically inert complexes. For a successful application of the method, one should consider the following. First, it is absolutely essential that the scavenging reaction does not affect the equilibrium composition. This condition implies that the scavenging reaction should occur on a faster time scale than the exchange reaction to prevent a shift in the equilibrium that might occur as a result of the changes in the concentration of the building blocks.[17] Second, it is necessary to scavenge the complete 'left-over' concentration of the building blocks to circumvent the selective scavenging of one building block as a result of its higher reactivity.[18] A third point is related to maximizing the output signal. As shown

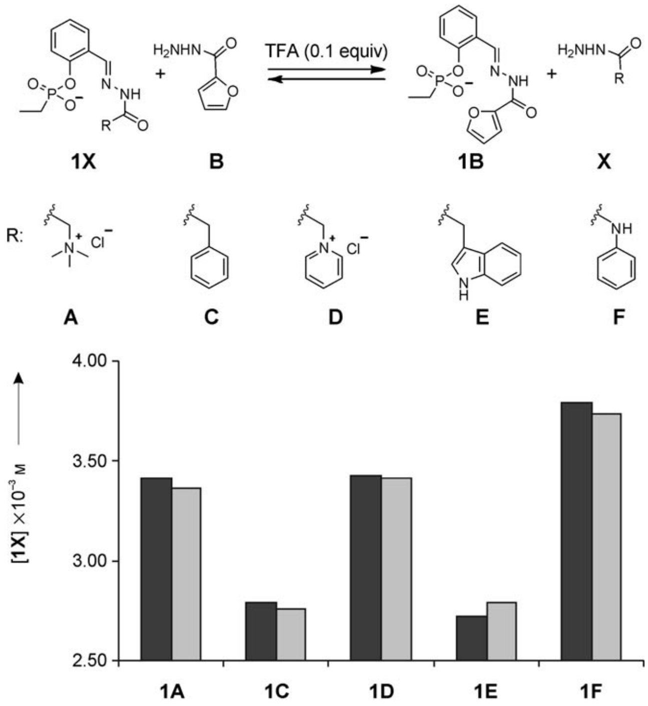

Figure 3. Equilibrium concentrations of hydrazones **1A**, **1C**–**1F** obtained from a direct measurement using ^{1}H NMR spectroscopy (dark bars) and an indirect measurement using a UV/Vis readout at 348 nm (light bars) after scavenging with **2**. All samples were prepared and analyzed as described in the experimental section.

earlier, the thermodynamic composition of a dynamic covalent equilibrium depends on the excess of the competing building blocks that are present.[12] The equilibrium concentration of **1A** follows the profile (Figure 4) obtained from a simulation of a hypothetical equilibrium in which **1A** has a 100-fold higher thermodynamic stability compared to **1B** (assuming $K_{eq} = 0.01$ for the equilibrium in Figure 3). A small excess of hydrazides statistically favors the formation of **1B** because of depletion of **A**. The maximum amount of **1A** is obtained only when at least six equivalents of hydrazides are present.[19] This observation indicates that under these conditions the absolute concentration of **1A** shows the strongest variations, which is beneficial for the generation of a stronger signal. However, this comes at the cost of a much stronger background signal owing to the fact that all hydrazides must be scavenged, as discussed before.[20] A plot of the maximum relative change in concentration of **B** as a function of the amount of **B** added (Figure 4) indicates the best working conditions for this protocol. An optimal response is maintained up to about 2.8 equivalents of hydrazides, whereupon the increase in concentration of **1A** no longer compensates for the increase in the background signal.

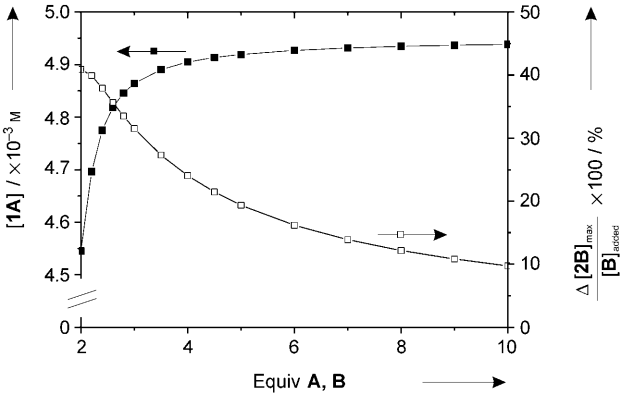

Figure 4. Calculated equilibrium concentrations of **1A** as a function of the equivalents of **A** and **B** in the case of a 100-fold higher thermodynamic stability of **1A** with respect to **1B** (■). The ratio between the maximum difference in the concentration of **2B** and the amount of **B** added as a function of the equivalents of **A** and **B** (□). Simulations were performed using 5 mM as the initial concentration of **1** (Supporting Information).

Finally, we were interested whether this approach could be used for the analysis of a complex chemical system, as recently anticipated in a theoretical contribution by Sanders, Otto, et al.[21] The main target of such an experiment is to obtain the relative thermodynamic stabilities of hydrazones **1A**–**1F** from the analysis of a mixture. This approach would be highly advantageous, as it would eliminate the necessity of requiring the building blocks in pure form. A 12-membered chemical system (6 hydrazones and 6 hydrazides) composed of 15 different equilibrium reactions was conceived by adding a mixture of hydrazides **A**–**F** to aldehyde **1** (Figure 5a).[22] Although complex, for screening purposes only the equilibria that involve reporter hydrazide **B** (which can be quantified using the scavenging reaction with **2**) are relevant. In fact, the system can be conceptually reduced to a simple equilibrium between hydrazone **1B** and all other hydrazones **1X** ($\mathbf{X} = \mathbf{A} + \mathbf{C} + \mathbf{D} + \mathbf{E} + \mathbf{F}$; Figure 5b).[23] Determination of the relative thermodynamic stability of the hydrazones necessarily requires the study of a series of mixtures selectively enriched in one of the hydrazones **1A** or **1C**–**1F**. This study shows the capacity of that given hydrazone to increase the overall 'apparent' equilibrium constant K_{system}. Thus, the higher the thermodynamic stability of the hydrazone **1A**, **1C**–**1F**, the higher the concentration of the free hydrazide **B** and, consequently, the higher is the absorbance at 348 nm after scavenging with **2**. The use of one hydrazide in excess for each experiment also facilitates the analysis by establishing favorable reaction conditions as described previously (Figure 4). Accordingly, five mixtures were prepared containing **1** and **B** in a 1:1 ratio (5 mM of each), 0.2 equivalents of four of the five hydrazides **A**, **C**–**F**, and 0.6 equivalents of the fifth hydrazide (either **A**, **C**, **D**, **E**, or **F**). The mixtures were equilibrated overnight under the conditions described before, after which **2** (15 mM) was added. After 6 h, the absorbance of each mixture was measured at 348 nm after dilution (Figure 5c, dark gray bars). A simple glance at the observed values for the absorbance is sufficient to rank the hydrazones in terms of thermodynamic stability ($\mathbf{1F} > \mathbf{1A} \approx \mathbf{1D} > \mathbf{1C} \approx \mathbf{1E}$). This observation is in perfect accordance with that obtained from the separate competition experiments (Figure 3). The analysis of the mixture presented herein is successful because this system behaves as a linear combination of the individual equilibria.[24] In fact, a calculation of the expected absorbance values for each mixture based on the equilibrium constants

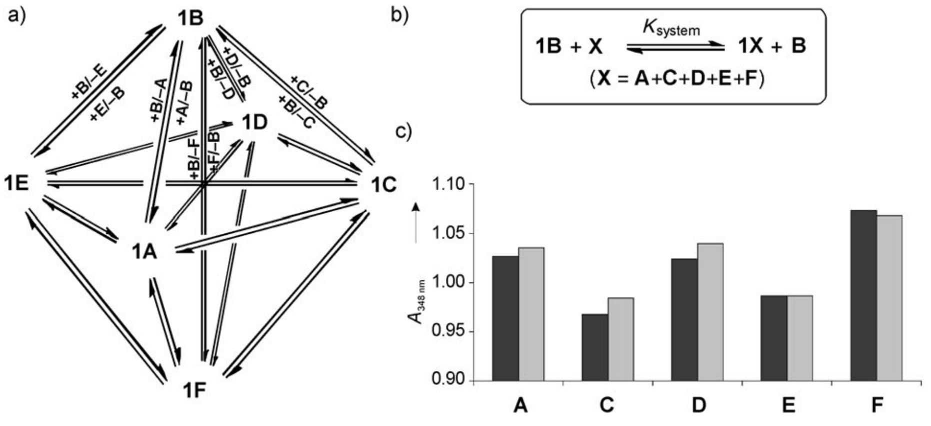

Figure 5. a) Equilibria present in the system obtained from mixing **1** and **A**–**F**. For clarity, hydrazides are indicated only for the equilibria including reporter hydrazide **B**.[22] b) Interpretation of the chemical system as a thermodynamic equilibrium between hydrazone **1B** and hydrazones **1X** (**X** = **A** + **C** + **D** + **E** + **F**) which interconvert through hydrazide exchange. c) Measured absorbance values (dark gray bars) at 348 nm (originating from **2B**) of the five mixtures selectively enriched in either **A**, **C**, **D**, **E**, or **F** after scavenging with **2** and the calculated absorbance values (light gray bars) for the same mixtures based on the individually obtained equilibrium constants (Supporting Information). Crude absorbance data have been reported without conversion into concentrations (as in Figure 3) to show the immediate information obtained from the experiments.[26]

obtained individually for each hydrazone (Supporting Information) showed an excellent correspondence with the experimental values (Figure 5c, light gray bars). We envision that this approach will be very useful for the rapid evaluation of the potential of the guest molecules to alter the composition of a dynamic combinatorial library. In cases when strong interactions are identified, 'dynamic' deconvolution procedures, as reported by Lehn et al.[25] can then be used to identify the responsible members of the library.

In conclusion, we have demonstrated a straightforward methodology for the analysis of dynamic covalent equilibria, which can also be extended towards competitive reactions that yield covalent products or kinetically inert noncovalent complexes. The stronghold of the approach is the disconnection between the molecular structure of the target and the output signal, which allows for an independent optimization of the latter. Herein, a UV/Vis signal is presented as an output, although the use of fluorescence or catalytic readouts are equally feasible. Thus, the simple output makes this methodology very attractive for parallel screening of competitive reactions in a microtiter plate format. Additionally, we have shown that the protocol can be applied for the analysis of a complex chemical system, which may be particularly valuable for rapid assessment of the adaptation potential of dynamic combinatorial libraries to external stimuli.

Experimental Section

The synthesis and characterization of hydrazones **1X** (**X** = **A**, **C**–**F**)[11] and **2B**[16] has been reported before. The syntheses and characterization of hydrazones **1B** and **2A** are given in the Supporting Information, together with the calibration curve for **2B** and a detailed description of the theoretical simulations.

In a typical experiment, a 1:1 mixture of hydrazides **X** (**X** = **A**, **C**–**F**) and **B** (5 mM each) was prepared in CD_3OD in an NMR tube. $[D_6]$DMSO (15%) and trifluoroacetic acid (0.1 equiv) were added and equilibration was started by the addition of 1 equivalent of aldehyde **1** from a stock solution in CD_3CN. The final solvent mixture was composed of CD_3OD, $[D_6]$DMSO, and CD_3CN in a 70:15:15 ratio. The mixture was kept at 40°C for 2 h after which 2 equivalents of *trans*-cinnamaldehyde (**2**; 10 mM) were added from a stock solution in CD_3CN. After an additional 2 h at 40°C, the composition of the mixture was determined from ^{1}H NMR analysis. The UV/Vis absorbance at 348 nm was measured after a 100-fold dilution in the identical solvent mixture. Comparison of the measured absorbance with a calibration curve resulted in the concentrations of **2B**.

For the simultaneous screening of all hydrazides **A**–**F**, mixtures were prepared containing **1** and **B** in a 1:1 ratio (5 mM of each) and hydrazides **A**, **C**–**F** (0.2 equivalents of each, except for one at 0.6 equivalents). The five mixtures were equilibrated overnight, after which **2** (15 mM, 3 equivalents) was added. After 6 h the absorbance was measured at 348 nm after dilution in the same mixture.

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[17] The formation of hydrazone **2B** from **2** and **B** has a second-order rate constant ($k_{2+B} = 0.12\,L\,mol^{-1}\,s^{-1}$), which is about 40-fold higher than the formation of **2B** using the exchange reaction ($k_{2A+B} = 2.93 \times 10^{--3}\,L\,mol^{-1}\,s^{-1}$). It should be noted that the rate of exchange progressively decreases as the concentration of free hydrazide drops upon scavenging. This decrease makes the initial scavenging period most critical.

[18] If the scavenging reaction is a reversible reaction, which by itself is not a prerequisite, a complete scavenging avoids the installment of a second equilibrium involving the scavenging agent, *trans*-cinnamaldehyde (**2**).

[19] We have observed previously that the amplification drops when very large equivalents (> 10) of hydrazides are used, as under those conditions the intermolecular recognition event may become competitive with respect to the intramolecular interaction. In these simulations we have ignored this competition, because this approach gives an optimal response when the concentration of the excess hydrazides is small.

[20] For example, the use of 10 equivalents of **A** and **B** (25 mM of each) will lead to a maximum difference in concentration of **1A** of 2.44 mM between 100-fold and no stabilization with respect to **1B** (equilibrium concentrations of **1A** are 4.94 mM and 2.50 mM, respectively). Correspondingly, the difference in 'leftover' concentration of hydrazide **B** (and thus **2B** after scavenging) will vary between 24.94, and 22.50 mM, however, implying a change in signal intensity of only 10%.

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[24] This is not trivial as additional equilibria may be present in the system that are not possible in the individual equilibrium reactions (as in the case of dimerization between two hydrazones). Such 'systems behavior', however, would emerge from a study of the system composition as a function of concentration.

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