Signal amplification and transduction by photo-activated catalysis[†]

Stefan C. Ritter and Burkhard König*

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A simple flavin-based catalytic system is able to transform light into chemical output with amplified response utilizing a Cu(I)-catalyzed cycloaddition reaction.

Signal amplification by allosteric catalysis (SAAC) mimics biological signal transduction processes, in which a molecular recognition event of an external signal or analyte to a receptor is transduced into catalytically amplified chemical information. Using engineered enzymes,¹ ribozymes² or adjustable organic catalysis³ the concept was applied by several groups for analytical purposes. Artificial SAAC systems reported so far converted external chemical signals into amplified chemical output.⁴ This mimics the signal transduction pathway, e.g., of a membrane bound receptor with subsequent amplification of the response. Visual response, in which light stimulates a photoreceptor to activate a signal transduction cascade resulting in amplified chemical output, is another biological model for signal amplifying catalysis. Examples of artificial light-induced signal amplifying catalysis are the classical photographic process (autocatalytic signal amplification),⁵ hydro-silvlation of alkynes catalyzed by Pt(II)-complexes after initial irradiation,⁶ by Fe(II)- or Cr(III)-complexes under continuous irradiation,⁷ or photo-adjustable catalysts and enzymes based on azobenzene isomerisation.⁸ However, only the classical photographic process allows a simple linear correlation between light signal input and chemical output.⁹ We report here the amplification and transduction of an initial photo input by light-activated Cu(I)-catalyzed cycloaddition into a quantitatively related chemical output.

Fig. 1 summarizes the general signaling pathway. Initial excitation of the chromophore in the presence of an electron donor leads to its photoreduction. Now, conversion of an inactive catalyst precursor into an active catalyst becomes possible by the reduced chromophore. The active catalyst then amplifies and converts the signal into a chemical output. The number of received photons is correlated with the amount of active catalyst generated, which translates into the reaction rate of the substrate to product conversion.

Riboflavine tetraacetate $(1)^{10}$ was used as redox-active chromophore receiving the light signal. In the presence of a sacrificial electron donor, *e.g.* benzyl alcohol or triethylamine, excited flavin 1* undergoes photoreduction to give 1-H₂. The quantum yield of this well known process is in the order of 0.4.¹¹ Dihydroflavin (1-H₂) has a sufficient redox potential to reduce Cu(II) to Cu(I)



Fig. 1 General signal-transduction pathway of a photo-receptor.

under the experimental conditions.¹² Finally, the Cu(I)-catalyzed cyclo-addition of an alkyne with an azide produces the chemical output of the cascade (Scheme 1).¹³

The presence of oxygen must be excluded to avoid formation of flavin hydroperoxides, which competes with Cu(I) formation. Amplification of the initial light signal requires the produced Cu(I) to be active and stable for some time. The choice of solvent is therefore crucial. If performed in water, the reaction cascade shows light-dependent behaviour: The cycloaddition reaction proceeds under continuous irradiation, but ceases if the light is switched off (see Fig. 2 and ESI for data†). Cu(I) is not stable in aqueous solution and disproportionates. To allow photoreduction of **1**, benzyl alcohol was used as sacrificial electron donor.

The use of tertiary amines with electron-donating groups is essential for the Cu(II) photoreduction in acetonitrile. Although several primary, secondary and tertiary amines are oxidised by excited 1^{*} ,¹⁴ only electron-rich tertiary amines allowed reduction to Cu(I) by $1-H_2$.



Scheme 1 Light depending formation of Cu(I) and following catalyzed cycloaddition.

Universität Regensburg, Universitätsstr. 31, D-93040, Regensburg, Germany. E-mail: burkhard.koenig@chemie.uni-regensburg.de; Fax: +49-941-943-1717; Tel: +49-941-943-4575

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Fig. 2 Conversion of the azide-alkyne-cycloaddition reaction in water during periods of irradiation (triangle, white background) and dark periods (dots, grey background).

In acetonitrile solution the photochemically produced Cu(I) is sufficiently stable to catalyze the cycloaddition for extended periods after short irradiation times. To facilitate reaction monitoring of the Cu(I)-catalyzed cycloaddition after short irradiation, we used a substituted alkyne and azide (see Scheme 2) described previously by Finn,¹⁵ which allowed simple optical read-out. The covalent connection between a dabsyl and a dansyl unit results in quenching of the fluorophore's emission.¹⁶ Determination of the remaining fluorescence allows calculation of the conversion (Fig. 3)[‡]

The reaction mixture was irradiated under identical conditions for various times from 0 to 180 s and the reaction rate of the initiated Cu(I)-catalyzed cycloaddition in the dark was monitored. A slow background reaction was observed without irradiation due to spontaneous NEt₃ reduction of Cu²⁺ to Cu^{+,17} However, within irradiation times of 30 to 180 s a significant and linear increase of the reaction rate is observed (Fig. 4). With shorter reaction times the background reaction becomes important, while longer irradiation leads to a non-linear response of the photoreceptor due to bleaching of **1-H**₂,¹⁸ the formation of side-products instead of **1-H**₂¹⁹ and decreasing concentrations of the sacrificial electron donor and CuCl₂.²⁰

To estimate the efficiency of the light to catalyst conversion of the reaction, the dependence of the cycloaddition reaction rate on the Cu(I) concentration under the experimental conditions was determined (see ESI for data).† A correlation with rates of the light-induced catalysis in acetonitrile gives a quantum yield of the Cu(I) generation of 0.2 and an overall quantum yield of triazole formation of up to 15 after 20 min reaction time. This corresponds



Fig. 3 Emission fading during the photoinitiated cycloaddition in acetonitrile; initial irradiation time was 90 s; from left to right: emission of the solution after 1, 10 and 20 min reaction time in the dark.



Fig. 4 Acceleration of the initial reaction rate and conversion of Cu(II) to Cu(I) in acetonitrile depending on the amount of light (data points are for irradiation times of 0, 15, 30, 60, 90, 135 and 180 s).

to a turnover number of 70 for Cu^+ after that time. UV/Vis and fluorescence titration experiments of **1** with $CuCl_2$ in acetonitrile revealed a coordination of Cu^{2+} to **1** (see ESI).† The complex formation results in changes of the photophysical and redox behaviour of **1**, making the picture of competing equilibria in the



Scheme 2 Cu(I)-catalyzed cycloaddition used as indicator reaction in acetonitrile.

reaction mixture more complex. Only a fraction of the flavin may be available for copper photoreduction.

In summary, we have described a simple photo-regulated system, which is able to convert a light signal input into a correlated chemical output with amplified response. Although its fidelity in terms of sensitivity and amplification is still far from biological models or classical photography, it illustrates how coupled chemical processes may allow the transformation and amplification of information at the molecular level.

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Notes and references

‡ All reactions were performed under an inert atmosphere using standard Schlenk techniques. Acetonitrile (HPLC-grade, Baker) was freshly distilled over sodium hydride and P_4O_{10} . Fluorescence measurements were done in acetonitrile (UV-grade, Baker) at 25 °C and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer.

General procedure: Azide 2 (127.8 mg, 0.4 mmol) was dissolved in a Schlenk-tube in 5 ml of acetonitrile. Alkyne 3 (137.0 mg, 0.4 mmol) was dissolved in a Schlenk-flask in 15 ml of acetonitrile. Both solutions were degassed and saturated with argon. Under exclusion of light 1 (8.7 mg, 16 μ mol, 4 mol%), CuCl₂ (4.3 mg, 32 μ mol, 8 mol%) and 55.4 μ l of NEt₃ (40.5 mg, 0.4 mmol) were added in this order to the azide solution. The solution was stirred in the dark (for 120 to 300 s, depending on the time of irradiation afterwards) and then irradiated (for 0–180 s). After irradiation the alkyne solution was added. The mixture was stirred well in the dark and aliquots of 20 μ l were taken every minute. The samples were added to a solution of 20 μ l of 30% aqueous H₂O₂ in 2000 μ l acetonitrile to stop the reaction. Samples of 60 μ l of this solution were diluted with 3000 μ acetonitrile and emission spectra were recorded. See electronic supplementary information (ESI) for further details.†

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- 10 The tetraacetate of vitamin B2 has a better photostability and solubility compared to the parent compound.
- 11 Experimentally determined.
- 12 E₀(1) = −660 mV, E₀([Cu(NR₃)₄]²⁺) > 0 V. The Cu²⁺/Cu⁺ redox potential strongly depends on the available ligands for copper complexation. Values were determined by cyclovoltammetry (see ESI[†]). Chelating ligands like edta or ethylene diamine can not be used as they stabilise Cu²⁺ and therefore shift the Cu(II)/Cu(I) redox potential to more negative values. B. Beekman, J. W. Drijfhout, W. Bloemhoff, H. K. Ronday, P. P. Tak and J. M. te Koppele, *FEBS Lett.*, 1996, **390**, 221; J. Hirata, F. Ariese, C. Gooijer and H. Irth, *Anal. Chim. Acta*, 2003, **478**, 1; L. Zhu, V. M. Lynch and E. V. Anslyn, *Tetrahedron*, 2004, **60**, 7267.
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