Signal amplification and transduction by photo-activated catalysis†

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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] ribozymes[2] or adjustable organic catalysis[3] 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-silylation 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-H$_2$**. The quantum yield of this well known process is in the order of 0.4.[11] Dihydroflavin (**1-H$_2$**) has a sufficient redox potential to reduce Cu(II) to Cu(I) 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]

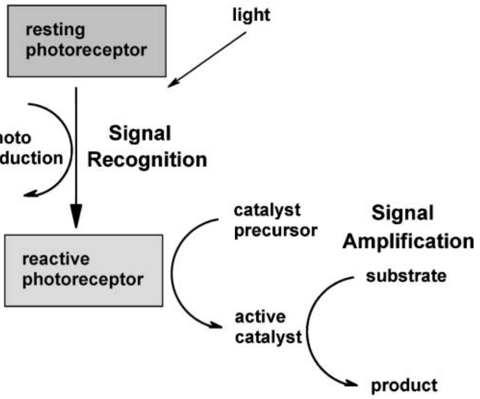
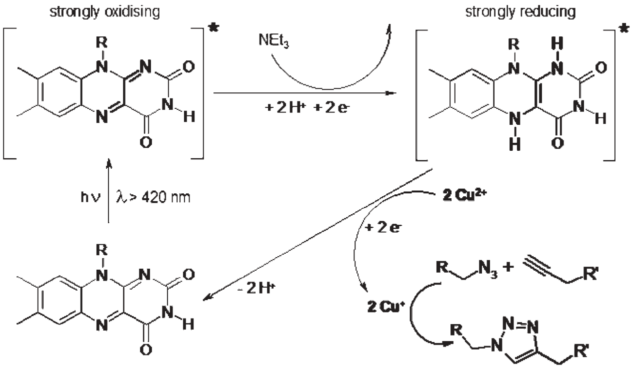


Fig. 1 General signal-transduction pathway of a photo-receptor.

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.

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† Electronic supplementary information (ESI) available: Synthesis of compound **2**, concentrations and calculations of reaction rates. See DOI: 10.1039/b610696j

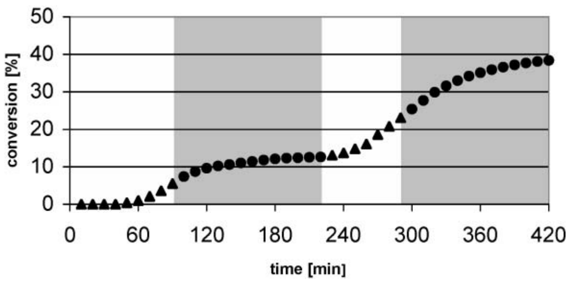


Fig. 2 Conversion of the azide-alkyne-cycloaddition reaction in water during periods of irradiation (triangle, white background) and dark periods (dots, grey background).

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 NEt$_3$ reduction of Cu^{2+} 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$_2$**[19] and decreasing concentrations of the sacrificial electron donor and CuCl$_2$.[20]

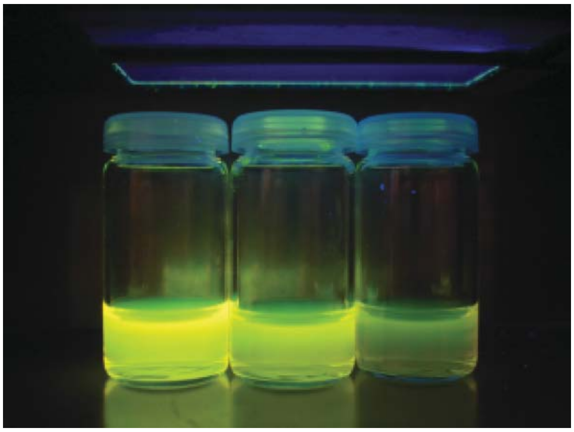


Fig. 3 Emission fading during the photoinitiated cycloaddition in acetonitrile; initial irradiation time was 90 s; from left to right: emission of the solution after 1, 10 and 20 min reaction time in the dark.

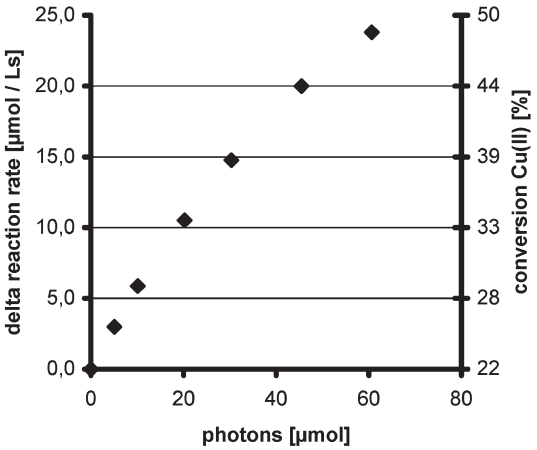
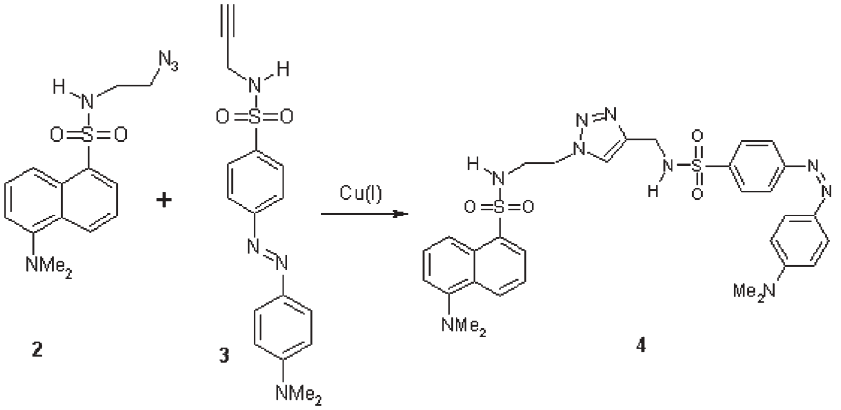


Fig. 4 Acceleration of the initial reaction rate and conversion of Cu(II) to Cu(I) in acetonitrile depending on the amount of light (data points are for irradiation times of 0, 15, 30, 60, 90, 135 and 180 s).

To 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 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 μmol, 4 mol%), $CuCl_2$ (4.3 mg, 32 μmol, 8 mol%) and 55.4 μl of NEt_3 (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 μl were taken every minute. The samples were added to a solution of 20 μl of 30% aqueous H_2O_2 in 2000 μl acetonitrile to stop the reaction. Samples of 60 μl of this solution were diluted with 3000 μl acetonitrile and emission spectra were recorded. See electronic supplementary information (ESI) for further details.†

1 A. Saghatelian, K. M. Guckian, D. A. Thayer and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2005, **127**, 7849; B. W. Matthews, *Acc. Chem. Res.*, 1988, **21**, 333; F. C. Simmel and W. U. Dittmer, *Small*, 2005, **1**, 284; E. D. Matayoshi, G. T. Wang, G. A. Krafft and J. Erickson, *Science*, 1990, **247**, 954.

2 G. A. Soukup and R. R. Breaker, *Trends Biotechnol.*, 1999, **17**, 469; G. A. Soukup and R. R. Breaker, *Curr. Opin. Struct. Biol.*, 2000, **10**, 318; R. R. Breaker, *Curr. Opin. Struct. Biol.*, 2002, **13**, 31; J. S. Hartig, I. Grüne, S. H. Najaf-Shoushtari and M. Famulok, *J. Am. Chem. Soc.*, 2004, **126**, 722; S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303; W. Tan, K. Wang and T. J. Drake, *Curr. Opin. Chem. Biol.*, 2004, **8**, 547.

3 W. Steed and J. L. Atwood, *Supramolecular Chemistry*, Wiley, Ltd., 2000; J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, Weinheim, 1995; J. P. Collman, L. S. Hegedus, J. R. Norton and R. G. Finke, *Principles and Applications of Organotransition Metal Chemistry*, University Science Books, Mill Valley, CA, 1987; L. Kovbasyuk and R. Krämer, *Chem. Rev.*, 2004, **104**, 3161; C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057; V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; L. Zhu, V. M. Lynch and E. V. Anslyn, *Tetrahedron*, 2004, **60**, 7267; 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; Q. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682; Q. Wu and E. V. Anslyn, *J. Mater. Chem.*, 2005, **15**, 2815; N. C. Gianneschi, P. A. Bertin, S. T. Nguyen, C. A. Mirkin, L. N. Zakharov and A. L. Rheingold, *J. Am. Chem. Soc.*, 2003, **125**, 10508; N. C. Gianneschi, S. T. Nguyen and C. A. Mirkin, *J. Am. Chem. Soc.*, 2005, **127**, 1644; M. S. Khoshbin, M. V. Ovchinnikov, C. A. Mirkin, L. N. Zakharov and A. L. Rheingold, *Inorg. Chem.*, 2005, **44**, 496; L. G. Mackay, R. S. Wylie and J. K. M. Sanders, *J. Am. Chem. Soc.*, 1994, **116**, 3141.

4 Anslyn recently summarized the state of the art of this nature-inspired strategy and analyzed the factors important to achieve high sensitivity and specificity: L. Zhu and E. V. Anslyn, *Angew. Chem.*, 2006, **118**, 1208 Although not all of the quoted systems fulfill the exact definition of being allosteric, at least their function corresponds to an allosteric behavior, which is defined as: A term to describe proteins that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence its biological activity. This definition can be used for artificial systems as well.

5 J. F. Hamilton, *Adv. Phys.*, 1988, **37**, 359; E. Moisar, *Chem. Unserer Zeit*, 1983, **17**, 85.

6 J. W. Grate, S. N. Kaganove and D. A. Nelson, *Chem. Innovation*, 2000, **30**, 29; B. E. Fry and D. C. Neckers, *Macromolecules*, 1996, **29**, 5306; F. D. Lewis and G. D. Salvi, *Inorg. Chem.*, 1995, **34**, 3182; L. D. Boardman, *Organometallics*, 1992, **11**, 4194; A. L. Prignano and W. C. Trogler, *J. Am. Chem. Soc.*, 1987, **109**, 3586.

7 M. A. Schroeder and M. S. Wrighton, *J. Organomet. Chem.*, 1977, **128**, 345; M. A. Schroeder and M. S. Wrighton, *J. Am. Chem. Soc.*, 1974, **96**, 6235.

8 M. Aizawa, K. Namba and S. Suzuki, *Arch. Biochem. Biophys.*, 1977, **182**, 305; T. Hohsaka, K. Kawashima and M. Sisido, *J. Am. Chem. Soc.*, 1994, **116**, 413; I. Hamachi, T. Hiroaka, Y. Yamada and S. Shinkai, *Chem. Lett.*, 1998, **6**, 537; I. Willner, S. Rubin and A. Riklin, *J. Am. Chem. Soc.*, 1991, **113**, 3321; D. G. Weston, J. Kirkham and D. C. Cullen, *Biochem. Biophys. Acta*, 1999, **1428**, 463.

9 Uncontrolled polymerisation reaction in alkyne hydrosilylation does not allow transforming the initial light signal into a certain chemical output signal. No correlation between the amount of light and the amount of active catalyst exist. In azobenzene-based systems light is the allosteric effector, but a linear correlation between the amount of light and the catalysts activity is missing.

10 The tetraacetate of vitamin B2 has a better photostability and solubility compared to the parent compound.

11 Experimentally determined.

12 $E_0(\mathbf{1}) = -660$ mV, $E_0([Cu(NR_3)_4]^{2+}) > 0$ V. The Cu^{2+}/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 Cu^{2+} 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.

13 V. O. Rodionov, V. V. Fokin and M. G. Finn, *Angew. Chem., Int. Ed.*, 2005, **44**, 221–2215.

14 Redox potentials of amines are in the range of $E_0 = +\ 730$ mV to + 1010 mV (in MeCN, *vs.* Ag/AgI), while $E_0(\mathbf{1^*})$ can be estimated to be + 1820 mV (in MeCN, *vs.* Ag/AgCl). The photoreduction of **1** by amines (*n*-butyl amine, *n*-propyl amine, ethylene diamine, diethyl amine, triethyl amine) under the reaction conditions was confirmed by UV/Vis-spectroscopy. The absorption spectrum of **1** significantly differs from **1-H$_2$**. J. T. Simpson, A. Krantz, F. D. Lewis and B. Kokel, *J. Am. Chem. Soc.*, 1982, **104**, 7155; M. Julliard and M. Chanon, *Chem. Rev.*, 1983, **83**, 425; B. König, M. Pelka, H. Zieg, T. Ritter, H. Bouas-Laurent, R. Bonneau and J.-P. Desvergne, *J. Am. Chem. Soc.*, 1999, **121**, 1681; F. Müller, *Chemistry and Biochemistry of Flavoenzymes*, CRC Press, Boston, 1991; S. Ghisla, V. Massey, J.-M. Lhotse and S. G. Mayhew, *Biochemistry*, 1974, **13**, 589.

15 W. G. Lewis, F. G. Magallon, V. V. Fokin and M. G. Finn, *J. Am. Chem. Soc.*, 2004, **126**, 9152.

16 A. Zaramella, N. Conti, M. Dal Cin, A. Paio, P. Seneci and S. Gehanne, *J. Comb. Chem.*, 2001, **3**, 410.

17 Experimental results showed that after addition of NEt_3 enough Cu^{+} was generated from $CuCl_2$ to start the cycloaddition reaction. Several amines (*n*-butyl amine, *n*-propyl amine, ethylene diamine, diethyl amine, triphenyl amine, edta) were tested to avoid the background reaction, but gave negative results as no reaction occurred.

18 F. Müller, *Chemistry and Biochemistry of Flavoenzymes*, CRC Press, Boston, 1991.

19 P. F. Heelis, *Chem. Soc. Rev.*, 1982, **11**, 15.

20 Related flavin photoreactions showed a similar behaviour: R. Cibulka, R. Vasold and B. Koenig, *Chem.–Eur. J.*, 2004, **10**, 6223; S. Fukuzumi, K. Yasui, T. Suenobu, K. Ohkubo, M. Fujitsuka and O. Ito, *J. Phys. Chem. A*, 2001, **105**, 10501.