DOI: 10.1002/cbic.200900014

Blue-Light-Triggered Photorelease of Active Chemicals Captured by the Flavoprotein Dodecin

Gilbert Nöll,*[a, b] Sibylle Trawöger,[c] Madlene von Sanden-Flohe,[d] Bernhard Dick,[d] and Martin Grininger[c]

The photochemical release of caged molecules offers the possibility to control the delivery and dosing of drugs,[1] to transport and activate biological or chemical reactants,[2–4] or to initiate biological processes at a precise time and location.[3–7] Most of the molecular structures that can be used as cages or photochemically cleavable protection groups are triggered by irradiation with UV light.[1–3,5–8] Very often, 2-nitrobenzyl alcohol and its derivatives have been applied. These molecules can be cleaved by a photochemically induced intramolecular redox reaction that leads to the corresponding 2-nitrosobenzaldehydes.[6,7] Other release systems are based on benzoin or phenacyl esters, fluoren-9-ylcarboxylates, or coumaryl esters, which react by a light-induced *trans–cis* isomerization. Recently, a photorelease system consisting of dithienylethene (DTE) derivatives was presented.[9] An advantage of the DTE derivatives is that the photochemical release is stimulated with visible light and the wavelength can be varied by the substitution pattern. The use of visible light as a trigger for the photorelease of caged molecules is advantageous for biological applications, since the damage of biological tissue by high-energy irradiation can be greatly reduced.

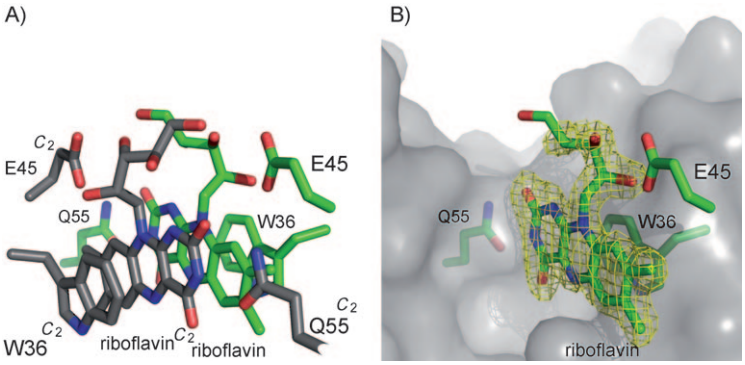
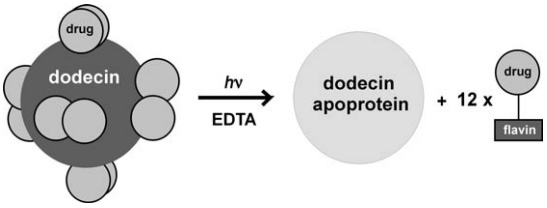


Figure 1. View of one of the dodecin binding sites with bound riboflavin (PDB ID: 2cc8).[12] A) Riboflavin ligands are arranged in a tetrad of aromatic systems (indol–isoalloxazine–isoalloxazine–indol). In the dodecin binding pocket, the flavins are anchored by their isoalloxazine substructure through interaction to W36 and Q55. Additionally, the ribityl chain forms a network of H-bonds to E45 and a single H-bond to V35 (omitted for clarity). C_2 indicates the symmetry of the binding pocket. B) Riboflavin is traced by its electron density at 1.5 σ. The ribityl chain points towards the outside of the binding pocket. Dodecin is depicted in surface presentation with the binding pocket reduced to one of the two C_2-related parts.

In this contribution, we present a blue-light-triggered photorelease system based on the riboflavin binding protein dodecin. Dodecin from *Halobacterium salinarum* is a dodecameric, hollow-spherical flavoprotein with six flavin binding sites.[10–13] In contrast to other flavoproteins, dodecin binds flavin ligands predominantly through their isoalloxazine substructure, while the aliphatic chain at the N(10) position is oriented towards the outer part of the protein (see Figure 1). Consequently, the loss of the H-bond interactions between the aliphatic ribityl chain and the protein envelope (E45 and V35) only marginally decreases ligand affinities.[14] In this approach, the artificial flavin CofC6, which has a -$(CH_2)_6$-NH_2 group as an aliphatic moiety, was used as an anchor to lock active molecules to dodecin. Based on the dodecin characteristic of only binding oxidized flavins with high affinity, reduction of the flavin can be used to trigger the dissociation of the holoprotein into apoprotein and free ligands.[14]

As flavoproteins can be reduced photochemically by irradiation with blue light in the presence of EDTA or other suitable electron donors,[15,16] we indented to use dodecin for establishing a blue-light-triggered photorelease system (Scheme 1). Any drug or active compound can be captured by dodecin after it has been linked to a flavin anchoring group. Dodecin can be loaded with up to 12 flavin–drug derivatives (two at each



Scheme 1. A blue-light-triggered molecular photorelease system based on the flavoprotein dodecin. Up to 12 flavin–drug derivatives can be captured by dodecin. The binding sites are located at the vertices of an octahedron. At any location of interest, the ligands can be released by irradiation with blue light.

[a] *Dr. G. Nöll*
Max Planck Institute for Polymer Research
Materials Science Group
Ackermannweg 10, 55128 Mainz (Germany)
Fax: (+49) 6131-379-100
E-mail: gilbert.noell@mpip-mainz.mpg.de

[b] *Dr. G. Nöll*
Present address: Organic Chemistry 1, Siegen University
Adolf-Reichwein-Strasse 2, 57068 Siegen (Germany)

[c] *S. Trawöger, Dr. M. Grininger*
Department of Membrane Biochemistry
Max Planck Institute for Biochemistry
Am Klopferspitz 18, 82152 Martinsried (Germany)

[d] *M. von Sanden-Flohe, Prof. Dr. B. Dick*
Institut für Physikalische und Theoretische Chemie
Universität Regensburg
Universitätsstrasse 31, 93053 Regensburg (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.200900014.

binding site) and transferred to any location of interest. While the ligands are in an inactive state when they are captured by dodecin, they become active after being released from the apoprotein. The release of the flavin–drug derivatives is triggered by irradiation with blue light and is based on the photochemical reduction of the dodecin-bound flavin anchor.[15–17] In flavin photochemistry, the excitation of a flavin into the singlet state is followed by fast intersystem crossing into a long-lived triplet state. The flavin, which is a better electron acceptor in its excited states than in its ground state, can be reduced by electron transfer (ET) from suitable electron donors, such as EDTA or thiols.[15–17] Flavin reduction is followed by protonation and disproportionation of two flavin semiquinone radicals into an oxidized and a doubly reduced flavin (Scheme S1 in the Supporting Information).[15,16] For the native flavins riboflavin and flavin mononucleotide (FMN), triplet quantum yields between 0.3 and 0.7 have been reported.[18–21] Therefore, photochemical reduction was considered a fast and efficient possibility to trigger the release at a precise location of active chemicals locked in the flavoprotein dodecin.

As models for flavin–drug derivatives that can be caged by dodecin and released upon irradiation, the flavin–DNA ligand **1** and the flavin–boron dipyrromethene (BDP) ligand **2** were synthesized.[14] Oligomeric DNA consisting of five bases and a blue BDP dye (purchased from Molecular Probes) were chosen as models representing different types of drugs, reagents, or active compounds.

The dissociation constants (K_d) of CofC6 and ligands **1** and **2** could be determined as 25, 690, and 470 nM, respectively, from a fluorescence-based binding assay. In the presence of the flavin-like ligand lumichrome, the affinities of ligands **1** and **2** increased to $K_d = 30$ and 50 nM, respectively. The increased holocomplex stabilities when lumichrome is added as a second ligand come from the preference of dodecin for binding flavin/lumichrome heterodimers.[14] Besides the intrinsic higher stability of the resulting mixed aromatic tetrad, saturation of the binding positions adjacent to bulky ligands with the small lumichrome might reduce steric restraints between the ligand and the dodecin apoprotein and additionally promote high-affinity binding. In Figure 2, X-ray structures of the tE-mutated[14] apododecin, tE dodecin with bound ligand **1**, and E45A-mutated dodecin with bound ligand **2** are shown. The structures could be determined with a resolution of 1.55, 1.6 and 2 Å, respectively. In both ligand structures, the flavin anchor could be traced by electron density, but not the aliphatic tail of CofC6 with the fused subunits DNA or boron dipyrromethene. This implies that the ligands are tightly anchored through their flavin moiety, while the active compound located outside the protein is flexible. Images of the dodecin crystals are given below the respective X-ray structures.

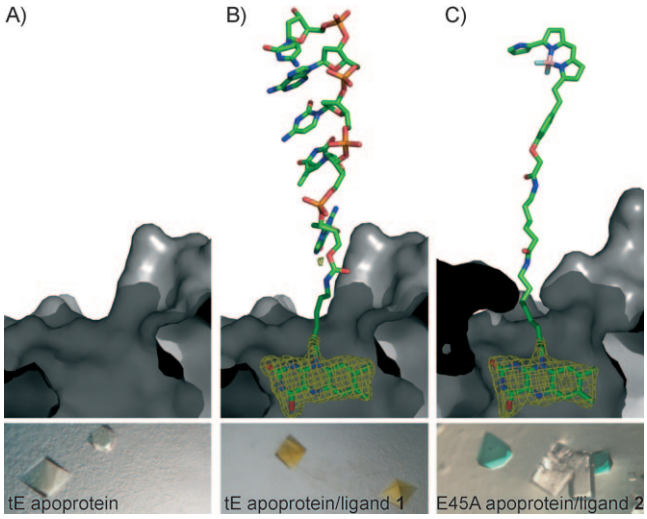
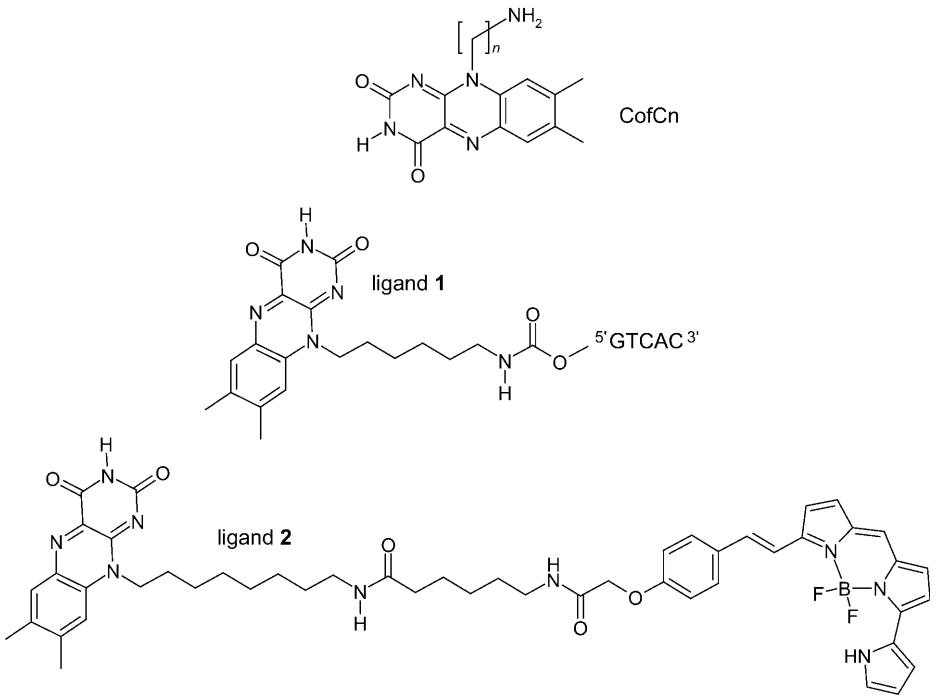


Figure 2. Structure of one dodecin binding site A) empty or saturated with B) ligand **1** or C) ligand **2**. Dodecin is depicted in surface presentation with the binding pocket reduced to one of the two C_2-related parts. For both dodecin ligand complexes, only the flavin anchor can be traced by electron density ($\sigma = 1.5$). The rest of the ligand is arbitrarily placed and has to be considered as one possible conformation. Images of the bipyramidal apododecin crystals in their mother liquor soaked with ligands are shown at the bottom. The transparent cubic structures in (C) are sodium chloride crystals.



In order to compare the photochemical reduction of free flavins in solution and captured by dodecin, we started our investi-

gations with the native flavin ligand riboflavin (vitamin B2). Although the reduction of free riboflavin was more efficient, after seconds to minutes of irradiation with blue light of 460 nm, reasonable amounts of dodecin-bound riboflavin could be reduced as well (Figure S1). At the dodecin binding site, the excited flavin states can be quenched by ET from the adjacent tryptophan.[22,23] ET from EDTA and tryptophan are competitive processes, but in contrast to ET from EDTA, which is followed by the release of CO_2,[15,16] ET from tryptophan is reversible. Next, the photochemical reduction of dodecin saturated with an artificial flavin termed CofC6 (a flavin bearing a hexylamino substituent at the N(10) position of the isoalloxazine moiety)[14] and dodecin binding the flavin–DNA ligand **1** were compared (see Figure 3). The absorption bands of the oxidized flavins with maxima at about 360 and 440 nm decrease upon ongoing irradiation.

In order to prove that protein reduction results in the release of the ligands, the protein solutions were also reduced chemically by the addition of a sodium dithionite solution followed by size-exclusion chromatography. In Figure 4, the chromatograms of dodecin saturated with ligand **1**/lumichrome before and after reduction are shown. During chromatography, the dodecin complex is stable, and only minor leakage of ligand can be observed (Figure 4 A). After reduction, the dodecin binding sites are empty, and the free ligand elutes at higher volumes of about 2.3 mL (Figure 4 B). In order to show

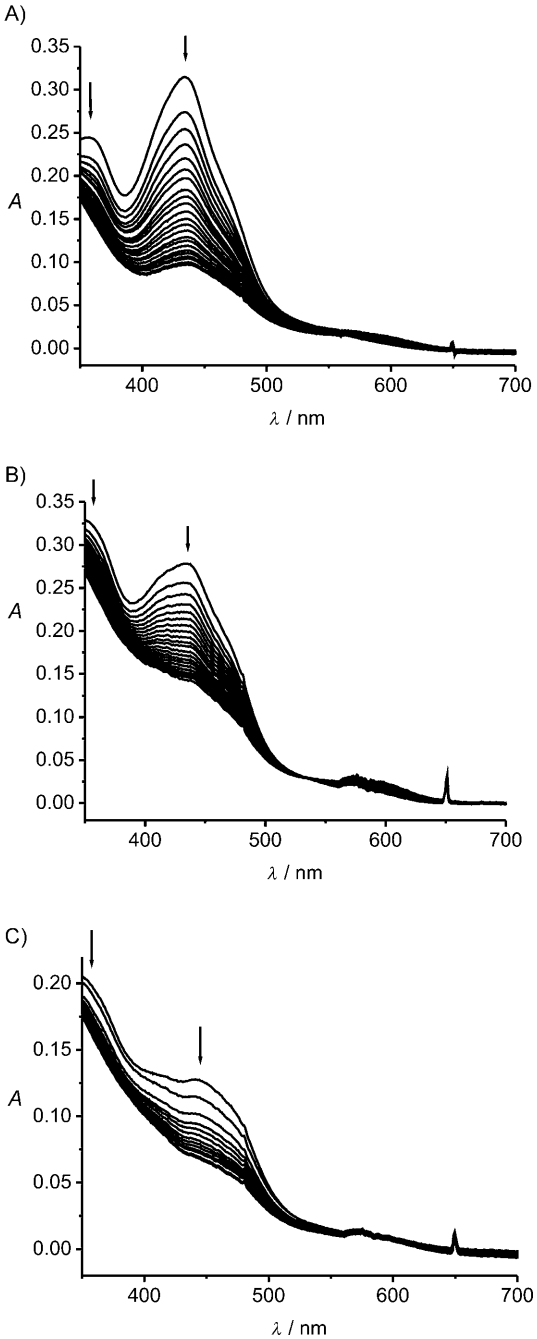


Figure 3. Photochemical reduction of dodecin by irradiation with blue light. Dodecin saturated with A) CofC6, B) CofC6 and lumichrome (1:1), or C) ligand **1** and lumichrome (1:1). Irradiation was performed in steps of 50 s. The proteins were dissolved in PBS buffer in the presence of 1 M NaCl and 50 mM EDTA in (A) and (C), and 20 mM EDTA in (B). The sharp absorption at 650 nm is an artifact of the spectrometer.

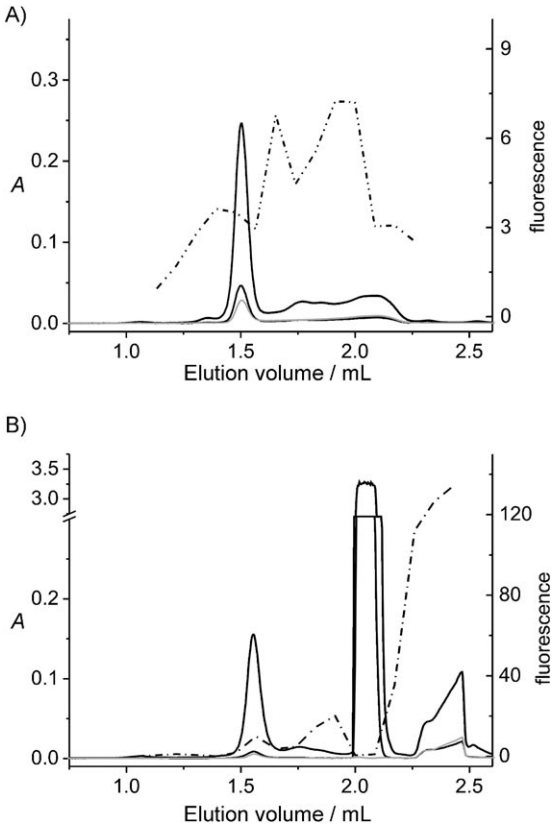


Figure 4. Size-exclusion chromatography of dodecin saturated with ligand **1** and lumichrome A) before and B) after chemical reduction. The absorption at wavelengths of 280 nm (black), 370 nm (dark gray), and 450 nm (light gray) and the flavin fluorescence (dashed line) were recorded. A) The apoprotein absorbs at 280 nm, absorption at 370 nm is caused by lumichrome and ligand **1**, and absorption at 450 nm only by ligand **1**. The dodecin holocomplex elutes at about 1.5 mL. The flavin fluorescence of ligand **1** is quenched when it is incorporated in the binding pockets. B) After reduction with sodium dithionite (50 mM), the dodecin peak shows depleted binding pockets. In line with the depleted binding pockets, dodecin elutes at higher volumes, reflecting its apoprotein state (size 90 kD). Sodium dithionite, absorbing at 280 and 370 nm, elutes at 2 mL. The free ligand elutes at about 2.3 mL. Note that in A and B the *y*-axes are scaled differently.

an additional example of the ability of dodecin to serve as a photocage, the binding and photochemical release of ligand **2** was studied. As depicted in Figure 5, dodecin saturated with ligand **2** and lumichrome could also be reduced photochemically.

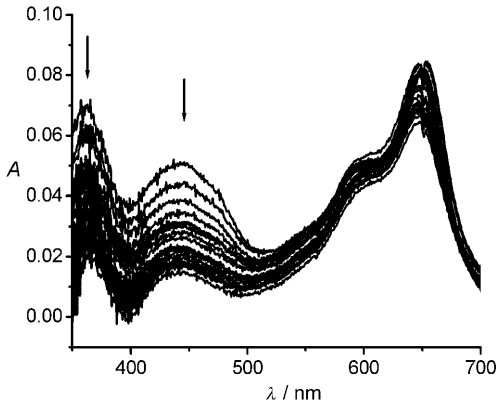


Figure 5. Photochemical reduction of dodecin tE saturated with ligand **2** and lumichrome (1:1) by irradiation with blue light in steps of 50 s in the presence of 50 mM EDTA. Experiments were performed in PBS buffer at pH 7.4 in the presence of 1 M NaCl. The solubility of ligand **2** outside dodecin is rather low. Therefore, the release of the ligand is accompanied by precipitation, which causes a shift in the absorption spectra.

In nature, riboflavin binding proteins are responsible for the transport, storage, and release of riboflavin.[24] By investigating the flavin–DNA and the flavin–BDP ligands **1** and **2** as model systems, we have shown that the flavoprotein dodecin can serve as blue-light-triggered photorelease system. For the currently available dodecin variants, the rate of photochemical release is too low to allow the determination of a quantum yield, because the excited flavin states can be quenched by ET from tryptophan.[22,23] In riboflavin binding proteins, the quenching of excited flavin states is a common process because undesired light-induced chemical reactions have to be prevented. In order to improve the efficiency of our system, further protein engineering will be necessary. The tryptophan at the dodecin binding site (the only tryptophan in dodecin) could be replaced by an artificial tryptophan derivative that does not quench the excited flavin states.[25,26] Furthermore, cysteines that can be converted into stable disulfides could be introduced close to the dodecin binding site as internal electron donors.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG Fellowship NO 740/1-1 and NO 740/3-1). G.N. and M.G. thank Prof. Dr. Dieter Oesterhelt for continuous support.

Keywords: cage compounds · DNA · electron transfer · flavins · photochemistry

[1] C. P. McCoy, C. Rooney, C. R. Edwards, D. S. Jones, S. P. Gorman, *J. Am. Chem. Soc.* **2007**, *129*, 9572–9573.
[2] D. D. Young, W. F. Edwards, H. Lusic, M. O. Lively, A. Deiters, *Chem. Commun.* **2008**, 462–464.
[3] D. D. Young, A. Deiters, *Angew. Chem.* **2007**, *119*, 4368–4370; *Angew. Chem. Int. Ed.* **2007**, *46*, 4290–4292.
[4] E. A. Lemke, D. Summerer, B. H. Geierstanger, S. M. Brittain, P. G. Schultz, *Nat. Chem. Biol.* **2007**, *3*, 769–772.
[5] S. Petersen, J. M. Alonso, A. Specht, P. Duodu, M. Goeldner, A. del Campo, *Angew. Chem.* **2008**, *120*, 3236–3239; *Angew. Chem. Int. Ed.* **2008**, *47*, 3192–3195.
[6] *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules* (Eds.: M. Goeldner, R. Givens), Wiley-VCH, Weinheim, **2005**.
[7] C. G. Bochet, *J. Chem. Soc. Perkin Trans. 1* **2002**, 125–142.
[8] A. Specht, J.-S. Thomann, K. Alarcon, W. Wittayanan, D. Ogden, T. Furuta, Y. Kurakawa, M. Goeldner, *ChemBioChem* **2006**, *7*, 1690–1695.
[9] V. Lemieux, S. Gauthier, N. R. Branda, *Angew. Chem.* **2006**, *118*, 6974–6978; *Angew. Chem. Int. Ed.* **2006**, *45*, 6820–6824.
[10] B. Bieger, L.-O. Essen, D. Oesterhelt, *Structure* **2003**, *11*, 375–385.
[11] M. Grininger, F. Seiler, K. Zeth, D. Oesterhelt, *J. Mol. Biol.* **2006**, *364*, 561–566.
[12] M. Grininger, K. Zeth, D. Oesterhelt, *J. Mol. Biol.* **2006**, *357*, 842–857.
[13] B. Meissner, E. Schleicher, S. Weber, L.-O. Essen, *J. Biol. Chem.* **2007**, *282*, 33142–33154.
[14] M. Grininger, G. Nöll, S. Trawöger, E. K. Sinner, D. Oesterhelt, *Biointerphases* **2008**, *3*, 51–58.
[15] G. Nöll, *J. Photochem. Photobiol. A* **2008**, *200*, 34–38.
[16] G. Nöll, G. Hauska, P. Hegemann, K. Lanzl, T. Nöll, M. von Sanden-Flohe, B. Dick, *ChemBioChem* **2007**, *8*, 2256–2264.
[17] K. Lanzl, G. Nöll, B. Dick, *ChemBioChem* **2008**, *9*, 861–864.
[18] L. Crovetto, S. E. Braslavsky, *J. Phys. Chem. A* **2006**, *110*, 7307–7315.
[19] A. Losi, *Photochem. Photobiol.* **2007**, *83*, 1283–1300.
[20] S. D. M. Islam, A. Penzkofer, P. Hegemann, *Chem. Phys.* **2003**, *291*, 97–114.
[21] H. Görner, *J. Photochem. Photobiol. B* **2007**, *87*, 73–80.
[22] N. Mataga, H. Chosrowjan, Y. Shibata, F. Tanaka, Y. Nishina, K. Shiga, *J. Phys. Chem. B* **2000**, *104*, 10667–10677.
[23] D. Zhong, A. H. Zewail, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11867–11872.
[24] W. S. A. Innis-Whitehouse, A. H. Merrill, Jr., D. B. McCormick in *Chemistry and. Biochemistry of the Flavoenzymes, Vol. 1* (Ed.: F. Müller), CRC, Boca Raton, **1991**, pp. 287–292.
[25] N. Budisa, *Angew. Chem.* **2004**, *116*, 6586–6624; *Angew. Chem. Int. Ed.* **2004**, *43*, 6426–6463.
[26] N. Budisa, M. Rubini, J. H. Bae, E. Weyher, W. Wenger, R. Golbik, R. Huber, L. Moroder, *Angew. Chem.* **2002**, *114*, 4238–4242; *Angew. Chem. Int. Ed.* **2002**, *41*, 4066–4069.

Received: January 8, 2009
Published online on March 2, 2009

