

Design and Biological Evaluation of Photo-Switchable Inhibitors

Melanie Füllbeck¹

melanie.fuellbeck@charite.de

Elke Michalsky¹

elke.michalsky@charite.de

Ines Stephanie Jaeger¹

ines.jaeger@charite.de

Peter Henklein²

peter.henklein@charite.de

Hartmut Kuhn²

hartmut.kuehn@charite.de

Karola Rück-Braun³

krueck@chem.tu-berlin.de

Robert Preissner¹

robert.preissner@charite.de

¹ Berlin Center of Genome Based Bioinformatics, 3D Dataminig Group, Institute of Molecular Biology and Bioinformatics, University Medicine Berlin-Charité (CBF), Arnimallee 22, D-10117 Berlin, Germany

² Institute of Biochemistry, University Medicine Berlin-Charité, Monbijoustr. 2, D-10117 Berlin, Germany

³ Institute of Chemistry, Technical University Berlin, Strasse des 17. Juni 135, D-10623 Berlin, Germany

Abstract

Photo-switchable compounds are becoming increasingly popular for a series of biological applications based on the reversible photo-control of structure and function of biomolecules. Three applications for the usage of BODTCM and hemithioindigo as photo-reactive compounds are described here. The structure of the villin headpiece was modified by replacing a part of the backbone with hemithioindigo, aiming at induction of the folding process by irradiation with a defined wavelength. The E-isomer of BODTCM was applied as potential inhibitor of the 12/15-lipoxygenase (12/15-LOX), which is implicated in the pathogenesis of inflammatory diseases.

A required death domain for the binding of proapoptotic proteins (e.g. Bak) to the hydrophobic groove of antiapoptotic proteins is the BH3 helix. Inserting hemithioindigo into this short peptide, stabilization towards proteolytic degradation is achieved.

Such photo-reactive compounds might be developed as potential drugs for a great variety of diseases.

Keywords: photo-switchable compounds, lipoxygenase, BH3, apoptosis, villin

1 Introduction

One of the fastest chemical reactions in nature is a photo-induced isomerization of a chromophore [4]. Rhodopsins, which are involved in the conversion of light to chemical energy and phototaxis in bacteria, or vision in higher organisms, contain retinal as their chromophore. The activation of rhodopsins is initiated by a photo-induced isomerization of retinal around a specific double bond [18].

Nowadays photo-switchable compounds have been employed in various biological functions such as biocatalysis [1, 42], ion transport [5, 27], cell adhesion [17] and protein folding [2, 3, 7, 30, 31, 36]. Thereby, the biological activity of this compound is altered by light-triggering [40]. Most investigations concerns chemical modification of nucleotides, peptides, proteins, and lipids employed azobenzenes as photo-switchable chromophores [1, 2, 3, 5, 7, 21, 27, 30, 31]. Random-chemical substitution to undefined sites of biomolecules was the strategy of the past [40], whereas the novel concepts embark on the strategy of site specific incorporation and conformational transitions of structural elements of proteins [12, 23]. Therefore, hemithioindigos came into the focus because of their frequent reversibility

of light-induced isomerization or thermal stability of photochromic states [10, 20, 29, 33, 37, 41]. This paper demonstrates three different applications of photo-switchable compounds for various biological problems.

As a first example, the photo-activation of an inhibitor of the 12/15-lipoxygenase (LOX) pathway [19] was investigated. LOX, a heterogeneous family of lipid peroxidizing enzymes, catalyze the dioxygenation of polyenoic fatty acids to their corresponding hydroperoxides [6, 16]. The mammalian LOXs are classified according to the reaction specificity of arachidonic acid oxygenation [6]. The presented 12/15-lipoxygenase exhibits a dual positional specificity. The molecular oxygen is introduced at the carbon atoms 12 and 15 of the arachidonic acid backbone [6]. Several inhibitors have been developed [15, 24] for the biological relevant LOX isomers, but the mechanism of action and the specificity of the inhibitors still remains unclear. *In silico* screenings with known LOX inhibitors [32, 35, 39] as lead compounds revealed a high similarity to a photo-isomerizable compound.

The second application depicts the stabilization of α -helical peptide derived from the proapoptotic protein Bid [38] using a hemithioindigo photo-switchable compound. Bid belongs to the Bcl-2 protein family containing pro- and antiapoptotic proteins, which are characterized by up to four conserved Bcl-2 homology (BH) domains. Antiapoptotic proteins (e.g. Bcl-2, Bcl-X_L) offer a high sequence similarity in the four BH domains (BH1-BH4) whereas the proapoptotic members are divided into the multidomain proteins (e.g. Bax and Bak, containing BH1-BH3 domains) and the BH3-only proteins (e.g. Bid and Bad) [34]. The BH1 and BH2 domains can be found in all antiapoptotic, but not in all proapoptotic proteins of the Bcl-2 family. Essential residues in these domains are required for the survival of the death suppressors Bcl-2 and Bcl-X_L and for the interaction of these proteins with death promoters such as Bax and Bak [22]. All proteins of the Bcl-2 family contain the BH3 domain. This domain is required for the binding of the proapoptotic proteins to the hydrophobic groove of the antiapoptotic Bcl-2 proteins. Subsequently, the inhibition of these proteins and the triggering of apoptosis follows. A loss of the BH4 domain can diminish or abrogate the antiapoptotic function and proteins such as Bcl-2 might be converted into death promoters [9, 22]. Multidomain members affect predominantly the outer mitochondrial membrane and the endoplasmatic reticulum, which leads to the promotion or suppression of the threshold for the release of the apoptogenic factors from mitochondrial stores. BH3-only proteins are proapoptotic and function through interactions with the multidomain Bcl-2 members [14]. Therefore the BH3 α -helical segment could be used as peptide to manipulate physiological processes.

The third application aims at the design of photo-switchable mini-proteins, whereas folding of the protein can be induced by irradiation. Object of investigation was the villin headpiece. Villin is an F-actin bundling protein [11] whose activity is essential for the formation of microvilli in the absorptive epithelia. The so-called villin headpiece domain contains an autonomously and rapidly folding 35-residue subdomain (H35) at its C-terminus. This subdomain is the shortest autonomously folded sequence yet characterized that contains only naturally occurring amino acids [26]. It has been shown that the isolated H35 subdomain retains the conformation found in the intact headpiece [25]. Small proteins which fold rapidly are used as starting point for investigations of the protein folding process, particularly of the minimal requirements for a folded structure. There is evidence that the transition between unfolded and folded state of fast folding proteins is not two-state and folding might be more complex. Induction of folding by a photoswitch would allow the first measurements of folding kinetics. To realize this for the villin headpiece, we inserted a photoswitch into the main chain of the H35 subdomain.

2 Method and Results

To find and insert the photo-switchable compounds for the three applications, different strategies have been applied.

2.1 Lipoxygenases

Ebselen is a known inhibitor of the 12/15-LOX. An *in silico* screening was performed with Ebselen as a lead structure against a selection of photo-isomerizable compounds. The similarity score bases on the Tanimoto-coefficient, which compares two compounds by their chemical properties. The presence or absence of common functional groups such as alcohols or ring systems, e.g. pyrimidins, was investigated. Each substructure element was assigned to one bit of a Boolean array. Bits set in both structures (BitsAB) and bits, which were only set in one structure (BitsA and BitsB) were taken into consideration. The value varies between 0 (differentmolecules) and 1 (equalmolecules):

$$\text{Tanimoto-coefficient}(TC) = \frac{\text{BitsAB}}{\text{BitsA} + \text{BitsB} - \text{BitsAB}} \quad [8].$$

The calculated 2D similarity of 80% of Ebselen and (2Z)-2-(3-benzylidene)-3-oxo-2,-dihydrobenzo[*b*]thiophene-7-carboxylic acid methyl ester (BODTCM) suggests a similar biological activity. Whereas the E-isomer resembles the structure of Ebselen, the corresponding Z-isomer was structurally different, suggesting distinct biological activities.

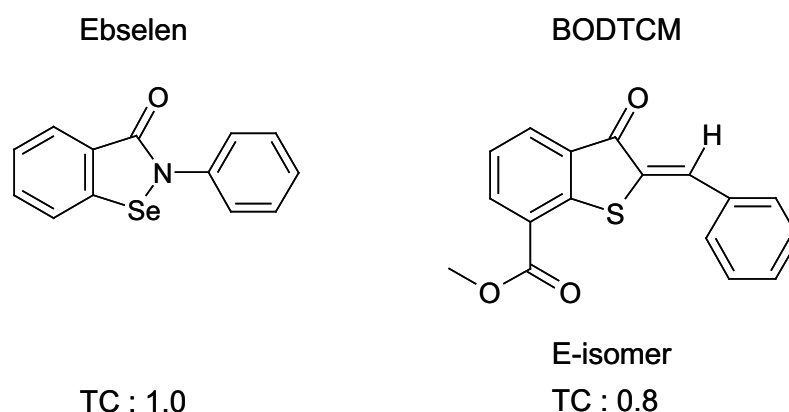


Figure 1: Structures of Ebselen, a 12/15-LOX inhibitor and the Z-isomer of the photo-switchable compound (2Z)-2-(3-benzylidene)-3-oxo-2,-dihydrobenzo[*b*]thiophene-7-carboxylic acid methyl ester (BODTCM). The calculated Tanimoto-coefficient (TC): Ebselen: 1.0, BODTCM: 0.8.

In further steps, the inhibitory effect of BODTCM was investigated using a kinetic assay. The fatty acid oxygenation (linoleic acid or arachidonic acid) by native rabbit 12/15 LOX was performed oxygraphically. After a short pre-incubation (30 s) of the enzyme with the inhibitor, the reaction was started by addition of a methanolic stock solution of the fatty acid substrate in the dark at room temperature. The LOX products were analyzed by RP-HPLC [19]. First, the IC₅₀-value of the synthesized Z-isomer was determined by using the native 12/15-LOX, which was prepared from rabbit reticulocytes as described before [19]. In Figure 2 the dose response curves for the BODTCM isomers are given. The synthesized Z-isomer of BODTCM was irradiated with a wavelength of 405 nm, which leads to the Z- to E-isomerization and offered the isomeric mixture in the photo-stationary state (pss). The isomer ratio was analyzed by ¹H-NMR-spectroscopy and the pure E-isomer was obtained by preparative RP-HPLC with an isomeric purity of > 95 % [19]. An IC₅₀ value of 0.7 mM exhibits that the Z-isomer of BODTCM is a rather weak LOX inhibitor, but the results with the E-isomer showed an improvement of the IC₅₀ value to 21 μM, which is a 33 fold higher inhibitory potency than that of the Z-isomer and indicates a geometric isomer specificity.

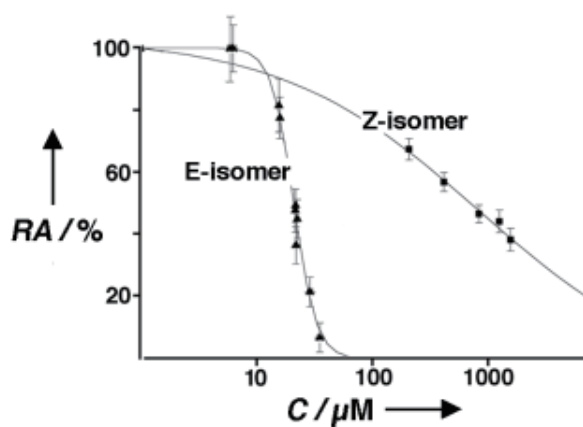


Figure 2: Dose-response curves for inhibition of native rabbit 12/15-LOX by BODTCM isomers [19]. Pure Z-isomer and pure E-isomer were used. The enzymatic activity was assayed oxygraphically using pure native 12/15-LOX prepared from rabbit reticulocytes (150 μ M linoleic acid as substrate, 81 nM 12/15 LOX). X-axis: inhibitor concentration (C) in μ M, y-axis: Residual enzymatic activity (RA) in %.

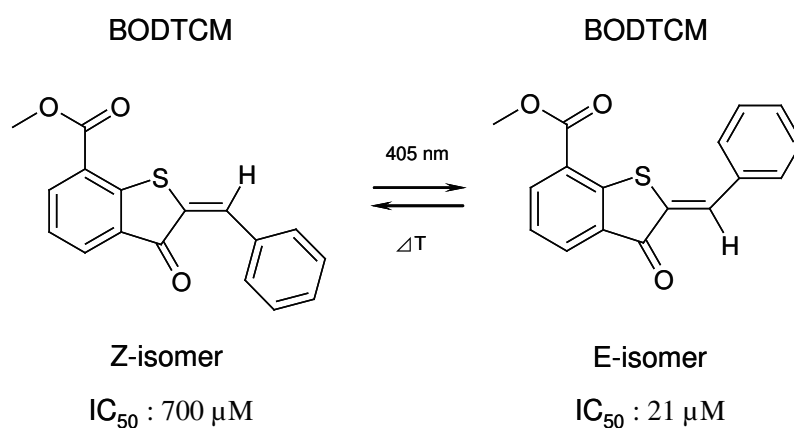


Figure 3: Structure of photo-reactive compound (2Z)-2-(3-benzylidene)-3-oxo-2,3-dihydrobenzo[b]thiophene-7-carboxylic acid methyl ester (Z-BODTCM) and the E-isomer of BODTCM. The Z-isomer is only a poor 12/15-LOX inhibitor ($IC_{50} : 700 \mu\text{M}$), but photo-activation that induces Z- to E-isomerization increases the inhibitory potency (E-isomer: $IC_{50} : 21 \mu\text{M}$).

2.2 BH3 Helix

The BH3 domain of the proapoptotic Bid is required for the binding to the hydrophobic groove of the antiapoptotic family members. As peptides are proteolytically degraded in cells, a BH3 helix with an inserted stabilizing hemithioindigo photo-switchable compound (Figure 4) was designed. The aim is the design of a peptide-like compound which mimics the function of a proapoptotic protein. It is known that a particular helix of the BH3 domain of Bid is responsible for binding to antiapoptotic Bcl-2 family members. The original sequence of the peptide was altered at two positions. Glutamine at position 94 was changed to diaminopropionic acid (DAP) and serine at position 98 was replaced with aspartic acid. The sequence alterations are necessary for the binding to the hemithioindigo compound. A peptide bond was formed between the carboxyl group and amino group of hemithioindigo and DAP or aspartic acid and hemithioindigo. In order to retain the binding properties, positions 94 and 98 were chosen for attaching the photoswitch because they are situated on that side of the helix opposite to the binding region. Moreover, these residues have an appropriate spatial distance to be able to attach hemithioindigo. DAP and aspartic acid were chosen as they provide the necessary chemical properties for the synthesis, and moreover are not too flexible, in order to achieve the desired stabilization effect.

Initial modeling was carried out with the protein visualization and modeling software Discovery Studio from Accelrys® First, the side chains at positions 94 and 98 were altered. Then they were connected to the photoswitch. Finally the structure was relaxed using the cleaning function of the software.

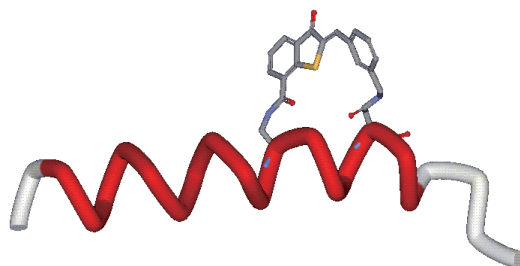


Figure 4: Structure of α -helical BH3 peptide with inserted hemithioindigo photo-switchable (Z-isomer) compound. Sequence alterations: Q94DAP and S98D.

The insertion of the photo-isomerizable compound stabilizes the BH3 helix towards proteolytical degradation, but by inducing a Z- to E-isomerization through irradiation, the helix should be destabilized and the amount of apoptotic cells should decrease.

As a first step, an apoptosis assay with the BH3 helix containing the inserted photo-switchable compound and without it was carried out in a T-cell lymphoma cell line (Jurkat A3) with increased concentrations of the peptide (0.5 – 50 μ M). After an incubation time of 20 h, an annexin-V-FITC / PI double staining was performed. Annexin V binds to phosphatidyl serine externalized to the outer leaflet of the plasma membrane bilayer during initial stages of apoptosis. To measure cell staining by annexin V the substance was labeled with FITC (fluorescein isothiocyanate). Simultaneously the cells were stained with PI. By the double staining the test discriminates between intact (FITC⁻/PI⁻), apoptotic (FITC⁺/PI⁻) and necrotic cells (FITC⁺/PI⁺). After harvesting and washing with PBS, cells were resuspended in 100 μ l annexin V binding buffer (containing 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂). Subsequently, cells were incubated with 5 μ l of FITC-conjugated

annexin V for 20 min at room temperature. After annexin-V-FITC staining, 400 μl of annexin V binding buffer containing PI (2.5 $\mu\text{g}/\text{ml}$) were added and the cells were analyzed by flow cytometry within 1 hour after staining.

First results indicate, that at a concentration of 50 μM the amount of apoptotic cells is only 18.4 % by using the helix without the stabilizing hemithioindigo compound, whereas the amount of apoptotic cells increases up to 45 % when the BH3 helix with the inserted photo-isomerizable compound is used (Figure 5).

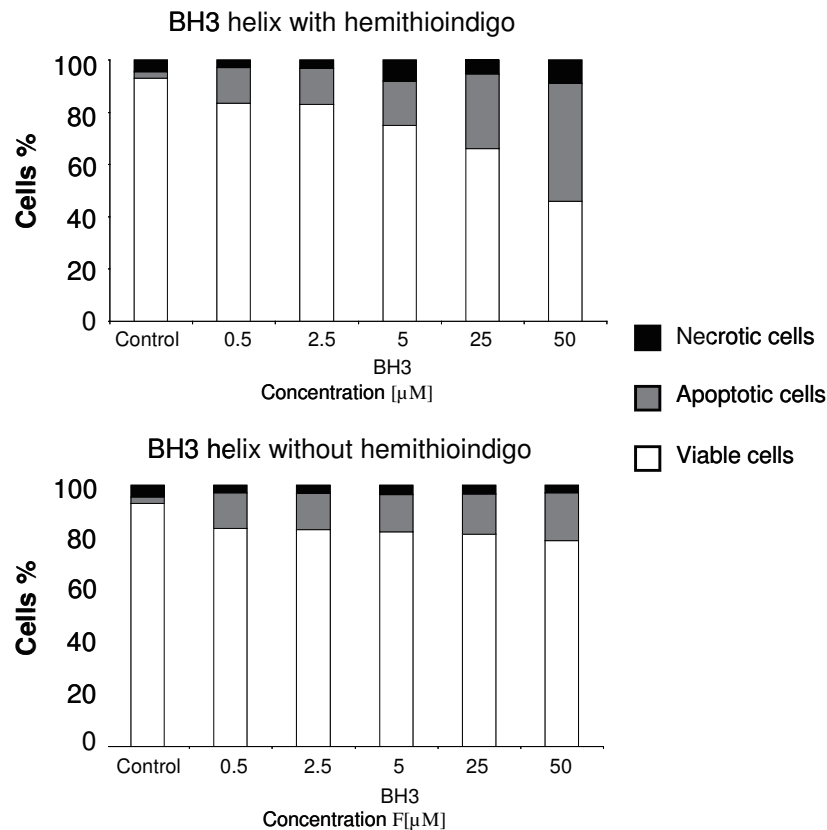


Figure 5: Jurkat A3 cells were cultured in medium or with the BH3 helix with or without the inserted photo-switchable compound (0.5, 2.5, 5, 25 or 50 μM). After 20h cell death was measured at the single-cell level by labeling cells with annexin-V-FITC and counterstaining with propidium iodide (PI). The bar chart describes the percentual distribution of necrotic, apoptotic and viable cells after treatment with the BH3 helix.

2.3 Villin Headpiece

In order to be able to induce folding of the H35 subdomain of the villin headpiece by irradiation, a possibility was searched to replace parts of its main-chain by a photoswitch without changing the overall structure of the subdomain. For this purpose, the software SuperMimic was used [13]. Given the 3D structure of a protein, it enables automatic searches for positions suitable for insertion of a given small molecule.

The small molecule is represented by several conformers, which are spatially superposed with the protein [28]. In this way, the whole protein can successively be searched for positions fitting the small molecule. Searches were performed with hemithioindigo and azobenzol. A possibility to replace three residues (Gly52-Thr54) of the first loop with hemithioindigo has been found (Figure 6).

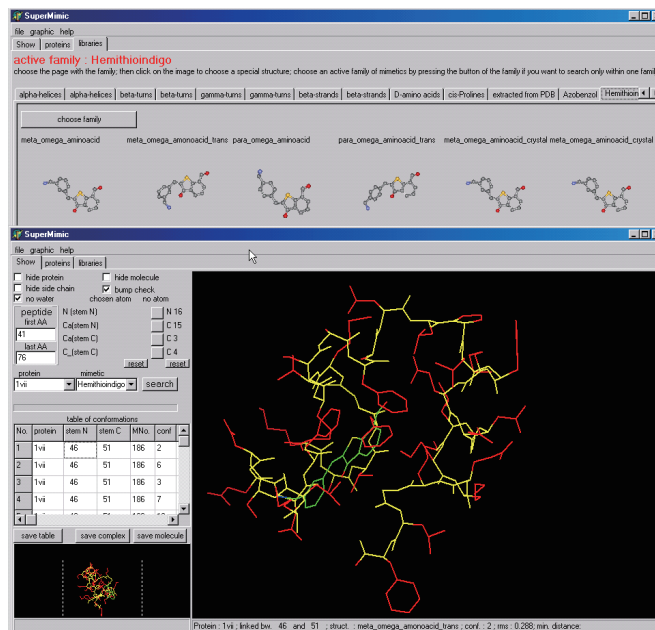


Figure 6: Screenshot of the program SuperMimic. The upper part shows the library containing different variants of hemithioindigo. In the lower part the graphical user interface presents the searching results for the villin headpiece.

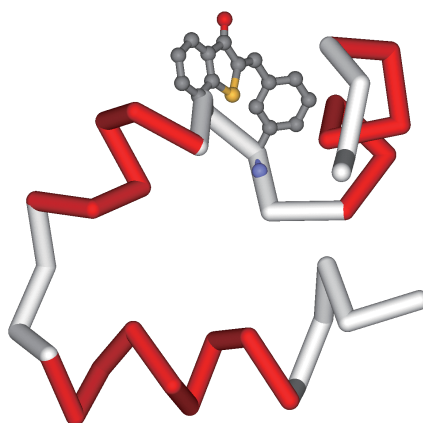


Figure 7: Villin headpiece with hemithioindigo fitted to the loop residues 52-54. During the synthesis, hemithioindigo was integrated into the protein main chain replacing these residues.

The original and the protein with inserted hemithioindigo were synthesized (Figure 7). To assess whether the proteins adopt an ordered structure, further experiments are in progress.

3 Discussion

Photo-switchable compounds comprise novel opportunities to evoke or influence biological effects or processes, because of their controllable activation at a defined spot by irradiation. Less side-effects and less stress of the whole organism could be achieved by specific therapies, e.g. chemotherapy. Furthermore, investigations of basic processes such as the protein folding process could be possible via the design of artificial proteins. We refer to a variety of utilizations: e.g.

1. The photo-switchable compound is used as an inhibitor for proteins.
2. The photo-switchable compound binds to amino acid side chains of a peptide chain, triggering the effect of a modulator.
3. The photo-switchable compound is inserted into the peptide backbone.

First experimental results show that photo-switchable compounds are suitable as ligands for proteins and the binding affinity can be influenced by photo-isomerization. BODTCM was used as a photo-sensitive compound for the inhibition of 12/15-LOX. The E-isomer of BODTCM exhibits an IC_{50} value in the lower micromolar range, whereas the Z-isomer was virtually inactive, which indicates a strong isomer specificity of inhibition.

Further investigations described in this paper concern the modification of peptides by insertion of photo-switchable compounds are a first step towards the design of switchable peptides. Preliminary results for the BH3 helix imply a stabilization of the helical structure and a decreased susceptibility to degradation by proteases. This leads to an increasing amount of apoptotic cells.

Continuative experiments show that it is in principle possible to design whole artificial proteins by directed insertion of photo-switchable compounds that adopt a native protein structure.

In general, photo-reactive compounds are promising candidates for application on diseases where strictly tissue-specific effects are desired as in inflammatory or neoplastic affection of the skin.

4 Acknowledgments

This work is supported by the BMBF funded Berlin Center for Genome Based Bioinformatics (BCB). We thank PD Dr. Clemens Mügge and Dr. Hardy Weißhoff for their NMR support.

References

- [1] Asanuma, H., Liang, X., Yoshida, T., Yamazawa, A., and Komiyama, M., Photocontrol of triple-helix formation by using azobenzene-bearing oligo (thymidine), *Angew. Chem. Int. Ed. Engl.*, 39(7):1316–1318, 2000.
- [2] Behrendt, R., Renner, C., Schenk, M., Wang, F. Q., Wachtveitl, J., Oesterhelt, D., and Moroder, L., Photomodulation of the conformation of cyclic peptides with azobenzene moieties in the peptide backbone, *Angew. Chem. Int. Ed.*, 38(18):2771–2774, 1999.
- [3] Behrendt, R., Schenk, M., Musiol, H. J., and Moroder, L., Photomodulation of conformational states. synthesis of cyclic peptides with backbone-azobenzene moieties, *J. Pept. Sci.*, 5(11):519–529, 1999.

- [4] Birge, R. R., Nature of the primary photochemical events in rhodopsin and bacteriorhodopsin, *Biochim. Biophys. Acta*, 1016(3):293–327, 1990.
- [5] Borisenko, V., Burns, D. C., Zhang, Z. H., and Woolley, G. A., Optical switching of ion-dipole interactions in a gramicidin channel analogue, *J. Am. Chem. Soc.*, 122(27):6364–6370, 2000.
- [6] Brash, A. R., Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate, *J. Biol. Chem.*, 274(34):23679–23682, 1999.
- [7] Cattani-Scholz, A., Renner, C., Cabrele, C., Behrendt, R., Oesterhelt, D., and Moroder, L., Photoresponsive cyclic bis(cysteiny)lpeptides as catalysts of oxidative protein folding, *Angew. Chem. Int. Ed.*, 41(2):289, 2001.
- [8] Chen, X., and Reynolds, C. H., Performance of similarity measures in 2D fragment-based similarity searching: comparison of structural descriptors and similarity coefficients, *J. Chem. Inf. Comput. Sci.*, 42(6):1407–1414, 2002.
- [9] Cheng, E. H., Kirsch, D. G., Clem, R. J., Ravi, R., Kastan, M. B., Bedi, A., Ueno, K., and Hardwick, J. M., Conversion of Bcl-2 to a bax-like death effector by caspases, *Science*, 278(5345):1966–1968, 1997.
- [10] Eggers, K., Fyles, T. M., and Montoya-Pelaez, P. J., Synthesis and characterization of photo-switchable lipids containing hemithioindigo chromophores, *J. Org. Chem.*, 66(9):2966–2977, 2001.
- [11] Finidori, J., Friederich, E., Kwiatkowski, D. J., and Louvard, D., In vivo analysis of functional domains from villin and gelsolin, *J. Cell Biol.*, 116(5):1145–1155, 1992.
- [12] Flint, D. G., Kumita, J. R., Smart, O. S., and Woolley, G. A., Using an azobenzene cross-linker to either increase or decrease peptide helix content upon trans-to-cis photoisomerization, *Chem. Biol.*, 9(3):391–397, 2002.
- [13] Goede, A., Michalsky, E., Schmidt, U., and Preissner, R., SuperMimic-fitting peptide mimetics into protein structures, *BMC Bioinformatics*, :11, 2006.
- [14] Goldsmith, K. C., Liu, X., Dam, V., Morgan, B. T., Shabbout, M., Cnaan, A., Letai, A., Korsmeyer, S. J., and Hogarty, M. D., BH3 peptidomimetics potently activate apoptosis and demonstrate single agent efficacy in neuroblastoma, *Oncogene*, 2006.
- [15] Gorins, G., Kuhnert, L., Johnson, C. R., and Marnett, L. J., (Carboxyalkyl)benzyl propargyl ethers as selective inhibitors of leukocyte-type 12-lipoxygenases, *J. Med. Chem.*, 39(25):4871–4878, 1996.
- [16] Grechkin, A., Recent developments in biochemistry of the plant lipoxygenase pathway, *Prog. Lipid. Res.*, 37(5):317–352, 1998.
- [17] Haiqian, Z., Hiroaki, S., Ning, G., Hiroshi, S., and Masahiko, S., Cell adhesion inhibition by RGD peptides linked with a photoisomerizable nonnatural amino acid, *J. Southeast Univ.*, 17(2):22–26, 2001.
- [18] Hayashi, S., Tajkhorshid, E., and Schulten, K., Molecular dynamics simulation of bacteriorhodopsin’s photoisomerization using ab initio forces for the excited chromophore, *Biophys. J.*, 85(3):1440–1449, 2003.
- [19] Herre, S., Schadendorf, T., Ivanov, I., Herrberger, C., Steinle, W., Ruck-Braun, K., Preissner, R., and Kuhn, H., Photoactivation of an inhibitor of the 12/15-lipoxygenase pathway, *ChemBiochem*, 7(7):1089–1095, 2006.

- [20] Ichimura, K., Seki, T., Tamaki, T., and Yamaguchi, T., Fatigue-Resistant Photochromic Hemithioindigoes, *Chem. Lett.*, 19(9):1645–1646, 1990.
- [21] James, D. A., Burns, D. C., and Woolley, G. A., Kinetic characterization of ribonuclease S mutants containing photoisomerizable phenylazophenylalanine residues, *Protein Eng.*, 14(12):983–991, 2001.
- [22] Kelekar, A., and Thompson, C. B., Bcl-2-family proteins: the role of the BH3 domain in apoptosis, *Trends Cell Biol.*, 8(8):324–330, 1998.
- [23] Kunita, J. R., Smart, O. S., and Woolley, G. A., Photo-control of helix content in a short peptide, *Proc. Natl. Acad. Sci. USA*, 97(8):3803–3808, 2000.
- [24] Malamas, M. S., Carlson, R. P., Grimes, D., Howell, R., Glaser, K., Gunawan, I., Nelson, J. A., Kanzelberger, M., Shah, U., and Hartman, D. A., Azole phenoxy hydroxyureas as selective and orally active inhibitors of 5-lipoxygenase, *J. Med. Chem.*, 39(1):237–245, 1996.
- [25] McKnight, C. J., Doering, D. S., Matsudaira, P. T., and Kim, P. S., A thermostable 35-residue subdomain within villin headpiece, *J. Mol. Biol.*, 260(2):126–134, 1996.
- [26] McKnight, C. J., Matsudaira, P. T., and Kim, P. S., NMR structure of the 35-residue villin headpiece subdomain, *Nat. Struct. Biol.*, 4(3):180–184, 1997.
- [27] Osman, P., Martin, S., Milojevic, D., Tansey, C., and Separovic, F., Optical modulation of the insertion of gramicidin into bilayer lipid membranes, *Langmuir*, 14(15):4238–4242, 1998.
- [28] Preissner, R., Goede, A., Rother, K., Osterkamp, F., Koert, U., and Froemmel, C., Matching organic libraries with protein-substructures, *J. Comput. Aided Mol. Des.*, 15(9):811–817, 2001.
- [29] Reamonn, L. S. S., and Osullivan, W. I., Configuration of 2-Arylmethylene-2,3-Dihydro-5-Methylbenzo[B]Thiophen-3-Ones, *J. Chem. Soc. Perkin. Trans. 1*, (9):1009–1012, 1977.
- [30] Renner, C., Behrendt, R., Heim, N., and Moroder, L., Photomodulation of conformational states. III. water-soluble bis-cysteiny-peptides with (4-aminomethyl) phenylazobenzoic acid as backbone constituent, *Biopolymers*, 63(6):382–393, 2002.
- [31] Renner, C., Cramer, J., Behrendt, R., and Moroder, L., Photomodulation of conformational states. II. Mono- and bicyclic peptides with (4-aminomethyl)phenylazobenzoic acid as backbone constituent, *Biopolymers*, 54(7):501–514, 2000.
- [32] Schewe, C., Schewe, T., and Wendel, A., Strong inhibition of mammalian lipoxygenases by the antiinflammatory seleno-organic compound ebselen in the absence of glutathione, *Biochem. Pharmacol.*, 48(1):65–74, 1994.
- [33] Seki, T., Tamaki, T., Yamaguchi, T., and Ichimura, K., Photochromism of hemithioindigo derivatives .2. Photochromic behaviors in bilayer-membranes and related systems, *Bull. Chem. Soc. Japan*, 65(3):657–663, 1992.
- [34] Sharpe, J. C., Arnoult, D., and Youle, R. J., Control of mitochondrial permeability by Bcl-2 family members, *Biochim. Biophys. Acta*, 1644(2–3):107–113, 2004.
- [35] Sies, H., Ebselen: a glutathione peroxidase mimic, *Methods Enzymol.*, 234:476–482, 1994.
- [36] Sporlein, S., Carstens, H., Satzger, H., Renner, C., Behrendt, R., Moroder, L., Tavan, P., Zinth, W., and Wachtveitl, J., Ultrafast spectroscopy reveals subnanosecond peptide conformational dynamics and validates molecular dynamics simulation, *Proc. Natl. Acad. Sci. USA*, 99(12):7998–8002, 2002.

- [37] Steinle, W., and Ruck-Braun, K., Synthesis and characterization of novel bifunctional hemithioindigo chromophores, *Org. Lett.*, 5(2):141–144, 2003.
- [38] Walensky, L. D., Kung, A. L., Escher, I., Malia, T. J., Barbuto, S., Wright, R. D., Wagner, G., Verdine, G. L., and Korsmeyer, S. J., Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix, *Science*, 305(5689):1466–1470, 2004.
- [39] Walther, M., Holzhutter, H. G., Kuban, R. J., Wiesner, R., Rathmann, J., and Kuhn, H., The inhibition of mammalian 15-lipoxygenases by the anti-inflammatory drug ebselen: dual-type mechanism involving covalent linkage and alteration of the iron ligand sphere, *Mol. Pharmacol.*, 56(1):196–203, 1999.
- [40] Willner, I., and Rubin, S., Control of the structure and functions of biomaterials by light, *Angew. Chem. Int. Ed. Engl.*, 35(4):367–385, 1996.
- [41] Yamaguchi, T., Seki, T., Tamaki, T., and Ichimura, K., Photochromism of hemithioindigo derivatives .1. preparation and photochromic properties in organic-solvents, *Bull. Chem. Soc. Japan*, 65(3):649–656, 1992.
- [42] Yamazawa, A., Liang, X., Asanuma, H., and Komiyama, M., Photoregulation of the DNA polymerase reaction by oligonucleotides bearing an azobenzene, *Angew. Chem. Int. Ed. Engl.*, 39(13):2356–2357, 2000.