From photosynthesis to cancer therapy-the green pathway

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Overall summary

Photosynthesis maintains life on earth by biomass formation, oxygen generation and carbon dioxide consumption. Global temperature changes and the need to extend sustainable energy resources have posted new challenges in photosynthetic research. Thus, a significant part of the research in the lab aims at understanding the underlying principles that govern adaptation of photosynthesis to the ambient temperatures of different habitats and engineering of new, thermo-stable and thermo-flexible microorganisms to be used as novel platforms for the above.

In the fundamental process of photosynthesis, the five-membered ring chlorophylls play key roles as light collectors and energy converters, therefore defined as "the pigments of life". Following our ongoing studies of photosynthesis we hypothesized that interactions among chlorophylls (Chl) from photosynthetic bacteria (Bchl) and light within animal tissues should provide novel opportunities for cancer imaging and therapy, treatment of vision threatening diseases and other pathologies. To enable this application we are designing new Bchl derivatives (Bchl-D) with higher stability, water solubility and better capacity for pharmaceutical use as radical generators. In collaboration with the lab of Prof. Yoram Salomon from the Dept. of Biological Regulation (see related abstract), we showed that illumination of tumors in animal models shortly after intravenous injection of different Bchl-D results in a very high cure rate, thereby giving a new interpretation to the Chls definition as "the pigments of life". Findings in our labs concerning the pharmacokinetics and pharmacology of the new Bchl-D helped translating the proposed treatment modality to clinical trials aiming at first to cure localized prostate cancer and age related macular degeneration with high selectivity. These studies also provided novel means to generate, at high spatial and temporal resolution, reactive oxygen species and follow their role in cellular and vascular processes. In the last four years we have focused a major part of our drug-designing, synthetic efforts and biological evaluation on a new family of Bchl-D molecules, that specifically home at the tumor vasculature and metastatic tumor cells enabling selective imaging, *in vivo* prognosis and hopefully therapy of localized and metastatic cancer lesions. Here I report highlights of the achieved progress and ongoing studies in our lab and together with collaborating labs during last year.

ſGenetically engineered cyanobacteria with high acclimation potential over a broad temperature range.

Vital growth of wild photosynthetic mesophils is confined to a narrow range of temperatures defined by the heat susceptibility of the enzymes located at the opposite ends of the photosynthetic reactions chain: the photosystem II (PSII) and the Rubisco complex. Predicted global warming by few degrees may therefore lower the primary productivity, shaking-up ecosystems worldwide and poses threat to both food and sustainable energy resources. After revealing elements that control temperature adaptation of electron transfer in PSII at mesophils and thermophils (1) we set out to generate new strains of photosynthetic organisms that function at a larger range of temperatures than the wild-types, enabling adaptation of biomass production to the forthcoming global changes. In this study we showed that mutations at the D1-212 and D1-209 sites of the PSII protein complex, render whole cells of the mesophilic Synechocystis sp. PCC6803 with stability, thermoplasticity and photosynthetic activity within the 15 - 43°C range. The sustained electron flow from the stabilized PSII enables for expression of the photosystem I and Rubisco proteins and physiological adaptation to a temperature range that is significantly broader than in the wild type. The proposed approach may provide the first step for eco-systems adaptations and for making robust primary producers grown at elevated and fluctuating temperatures for biotechnological applications (2).

ſMeasurements and significance of electrostatic potential changes on atoms in molecules

Changes in the electrostatic potentials (ESP) on atoms in molecules are proposed to play key roles in many experimentally observed processes including inter and intramolecular charge mobilization, protein folding, biotic and abiotic catalysis. Yet, up to-date direct measurement of such changes and evidences for their effect on particular reactivity are rare. Here we measure changes in the ESP on specific atoms in biologically important electron acceptors, and show for the first time, their effect on the harvesting and localization of donated electrons. Computational analysis of the examined molecules, metalated and non-metalated Bchls, provides a rational for experimental observations with their self-assembled monolayers on different surfaces. Of particular importance is the counter intuitive but experimentally observed and computationally confirmed, evolution of negative ESP on positively charged and strongly electronegative metals within these molecules. This observation is explained by electron density attraction from the metal coordinating atoms. The combined experimental and computational data suggest that Chl and Bchl in the photosynthetic machinery have been self organized to provide maximal efficiency of directed electron flow, accounting in part for the high quantum yield of these processes and providing new guidelines for man-made solar harvesting systems (3).

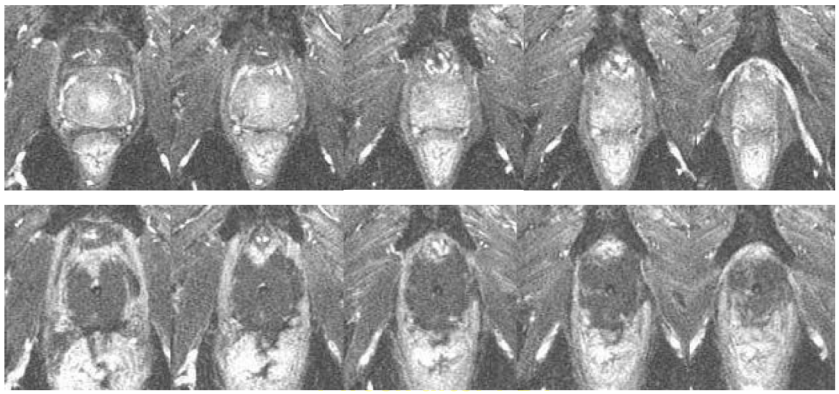

Fig. 1 *MR perfusion images of the human prostate before (upper panel) and after (lower panel) of Tookad-VTP treatment (2 mg/kg, 5 fibers). Perfusion arrest is evident by the dark area. The urethra remained intact.*

[&]Photodynamic therapy for recurrent prostate cancer after failure of radiation therapy using Tookad (WST09) in a Phase I/IIa dose escalating study

Following a previous, phase I/II study (4), this study aimed at evaluating the efficacy of Tookad-Vascular Targeted Photodynamic therapy (VTP) as a method of whole organ ablation in patients with localized prostate cancer following external beam radiation therapy. Following pre-clinical studies in our labs, a novel photodynamic protocol, where illumination starts during the sensitizer's infusion was launched. Patients received a fixed photosensitizer dose of 2 mg/kg and patient-specific light doses as determined by computer-aided treatment planning. Up to 6 cylindrical diffusing light delivery fibers were placed transperineally in the prostate under ultrasound guidance. Treatment response was assessed by serum PSA, avascular lesion formation measured on 7-day gadolinium-enhanced T1-weighted MRI and 6-month biopsy. Treatment of the whole prostate gland was possible with minimal impact on surrounding organs. Increased light dose improved tissue response, with MRI associated avascular lesions, encompassing as much as 80% of the prostate in some patients. Complete treatment response, as determined by 6-month biopsy, was observed in 8 of 13 patients who received light doses of at least 23 J/cm^2 in 90% of the prostate volume (D90 > 23 J/cm^2). Side effects were modest and self-limited in most but rectal fistulae occurred in two patients one of which closed spontaneously. In conclusions, Tookad-VTP can produce large avascular regions in irradiated prostate, and results in complete negative biopsy response at high light doses. A response rate of greater than 50% for those patients receiving the highest light doses shows the clinical potential of Tookad-VTP to manage post-EBRT recurrence of prostatic carcinoma (5).

[&]Quantitative Structure Activity Relationship models for prediction of Human Serum Albumin binding affinity of bacteriochlorophyll derivatives

Various Bacteriochlorophyll (Bchl) derivatives were developed in our lab in recent years as circulating photoactivators of vascular-targeted photodynamic therapy (VTP). Two such compounds, Tookad and WST11, have already entered into Phase II clinical trials for treatment of localized prosatet cancer (4,5) and age related macular degeneration (6), respectively. In all these cases as well as in the case of other clinically applied sensitizers, the binding affinity to serum proteins greatly influences the biodistribution, mode of action and subsequently efficacy and toxicity of the sensitizer. The binding affinity of water-soluble Bchl derivatives (Bchl-D), such as WST11, to serum albumin, the major constituent of the plasma and a well recognized carrier of many drugs, is of particular interest because they function while circulating in the blood, probably as Bchl-D/HSA non-covalent complexes (Brandis et al, 2005). Understanding the factors that underlay this affinity in terms of molecular structures and properties, namely, the construction of quantitative structure-activity relationships (QSAR) where the activity is defined as affinity to HSA, is therefore important and will enable tailoring new sensitizers to particular applications.

In the present study we constructed two QSAR models for predicting the HSA binding affinity of several Bchl-D. Both models predict the binding affinity for the training set and external set at high correlations suggesting that in addition to the hydrophobicity of the compounds the compounds electronegativity plays a key role in determining their binding affinity to HSA. This novel finding provides new guidelines for the rational design of new sensitizers tailored for imaging and VTP of lesions in relation with their host organs (7).

[&]Imaging and treatment of primary tumors and necrotic tumor domains by novel RGD-bacteriochlorophyll derivatives

Necrosis and hypoxia of primary and matastatic tumors have been strongly correlated with tumor aggressiveness and poor prognosis of cancer patients. Tumor necrosis was determined as an independent predictor of treatment outcome and recommended to be routinely reported and used for clinical assessment. Necrotic and hypoxic conditions are known to create a major problem in cancer therapy. Therefore their early recognition and *in vivo* imaging might be critical for treatment planning. There is a rising need for high resolution imaging techniques that can distinguish primary from necrotic tumor regions in cancer research and therapy.

As tumors grow they require increasing blood supply and thus switch to an angiogenic phenotype, typified by enhanced vascular integrin expression. Many members of the integrin family recognize ligands that contain an Arg-Gly-Asp (RGD) motif, making

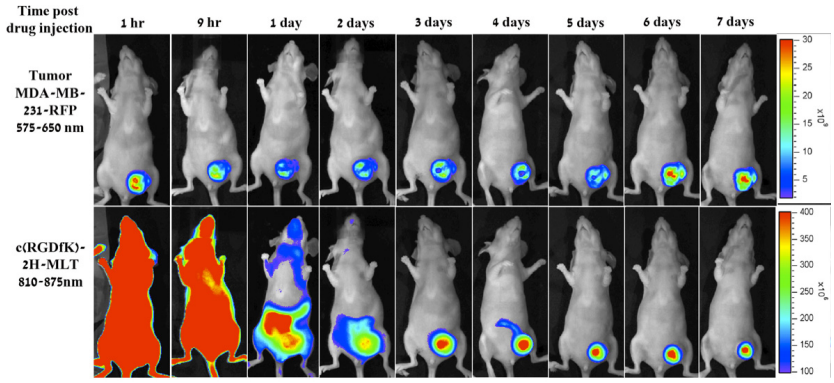

Fig. 2 *Accumulation of c(RGDfK)-2H-MLT in MDA-MB-231-RFP orthotopic tumor (tumor size ~1 cm3). The mouse was injected with c(RGDfK)-2H-MLT. Images were taken from day 1 to 7 post drug injection. Top panel –imaging of cells transfected with RFP. Bottom panel – imaging of c(RGDfK)-2H-MLT fluorescence.*

RGD containing compounds attractive vehicles for tumor targeting. Recently a new family of RGD-conjugates with Bchl was synthesized in our laboratory ((8) and *Rubinstein E, Brandis A, Salomon Y &, Scherz A (2008), Targeting endothelial cell adhesion and cytophototoxicity of bacteriochlorophyll derivatives via avidin-biotin-RGD constructs, In preparation).* Because of the intrinsic fluorescence of these molecules and their tumor homing abilities, they enable respective imaging of integrin rich tumor components.

We used human mammary carcinoma xenografts, orthotopically grafted and human ovarian carcinoma xenografts subcutaneously grafted to the mammary pad of CD1 nude mice, to explore imaging and therapy of primary and metastatic tumor lesions with the new RGD-Bchl conjugates. In this study we show (Fig. 2) that c(RGDfK)-2H-MLT (where MLT stands for the Bchl moiety in the conjugate) accumulates during the first 3-10 h after administration in the tumor viable domain, and later on migrates into the tumor necrotic domain. Here it stays for > 8 days allowing clear imaging of this region. In the first 10 h, illumination of the tumor volume resulted in tumor regression. Ongoing study aims at resolving the mechanism that underlies the RGD-MLT accumulation in the necrotic domain, development of combinational therapies and exploting the necrotic drug accumulation to novel therapeutic modalities *(Goldshaid L, Segal D, Rubinstein E, Brandis A, Eren D, Neimann K. Salomon Y & Scherz A (2008), In preparation;).*

&Accurate Description of Atomic Charges and Electrostatic Potentials at Receptor-Ligand Binding Sites; Introducing new concepts and tools for RGD-Bchl-D/integrin receptors binding

Computational docking of small molecules into structures of macromolecular targets has become a crucial component of many drug discovery programs during the last three decades and is widely used in hit identification and lead optimization. The docking process involves the prediction of ligand conformation and orientation within a binding site, where the energetic model employed is typically a molecular mechanics (MM) force field with fixed, non-polarizable atomic charges (e.g. Amber and CHARMM force fields). Since the electrostatic interactions, as described by these charges, have a major contribution to the non-bonded ligand-receptor energy, accurate ligand and binding site charges may lead to improved docking. These can be obtained by considering the polarization of the ligand and/or the binding site by their counterparts and the surrounding protein and aqueous environment, either by utilizing a polarizable force field or a quantum mechanics / molecular mechanics (QM/MM) approach.

QM/MM hybrid methods partition the system into QM and MM layers and describe the polarization of a QM-treated molecular domain due to the influence of the surrounding environment, typically modeled as fixed atomic charges according to a MM force field. In a recent study, accurate ligand charges obtained from QM/MM calculations on receptor-ligand complexes were shown to significantly improve docking results, compared with docking with fixed charges from a MM force field (OPLS). Complementarily, the binding site's charges can be rigorously calculated. In previous studies of our lab we showed experimentally and computationally, using high-level density functional theorm (DFT) calculations, that the effective charge of ligated metal is significantly smaller than its formal charge because of electronegativity equalization (9).

Inspired by these studies, and by the failure of current approaches to explicitly consider charges of metals in docking sites, we are developing methodologies for calculating accurately, QM quality, atomic partial charges and ESP at receptor binding sites, and to use these charges and ESP values to improve docking simulations. QM/MM methods will be used to account for the polarization of the binding site by the surrounding protein environment. Using resolved structures of RGD containing ligands *(Goldberg E. and Scherz A., 2008, In preparation).*

&Catalytic generation of oxygen radicals by WST11/serum albumin non-covalent complexes in aqueous solutions.

This study focuses on the impact of serum albumin on the profile of ROS generation by photoexcited WST11 in aqueous solutions. Cumulatively, our data introduce a novel concept suggesting that the WST11/SA non-covalent adduct, previously suggested by our group as the main form of circulating WST11 (Brandis et al, 2005), catalyses electron transfer from the protein matrix to the solvated oxygen molecules and thereby produces superoxide radicals. The superoxide radicals can further generate hydroxyl radicals as we previously described (Vakrat-Haglili

et al, 2005). Using optical absorption and fluorescence spectroscopy at the femto-microsecond time domains, spectro-electrochemistry, electron paramagnetic resonance with different spin traps and oxygen measurements, we: (1) followed the physical and chemical response of the hydro-solvated WST11 to photoexcitation in the presence of SA, whole serum and quenchers; (2) identified and quantified the evolved ROS; (3) monitored the rate and yield of oxygen consumption in response to photoexcitation of WST11 in the absence and presence of SA; (4) Monitored chemical modifications of the SA molecules following ROS generation. We showed that in pure aqueous solutions WST11 generates superoxide and hydroxyl radicals at comparable amounts but no detectible singlet oxygen while rapidly degrading through oxidative processes as previously observed for Tookad (Vakrat-Haglili et. al, 2005). The added SA profoundly reduces the photochemical degradation of WST11, as well as the yield of the spin trapped ROS signals. Still, about 70% of hydroxyl radicals are produced compared to solutions that do not contain SA and the rate and yield of oxygen consumption increases to ~15 per single molecule of WST11. These and other findings provide the ground for understanding the mechanism underlying vascular arrest by the circulating Bchl-D ((9) and *Ashur I, Goldschmidt R, Pinkas I, Salomon Y, Sarna T & Scherz A, 2008, In preparation).*

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Selected publications

Kerner, O. Samish, I. Kaftan, D. Holland, N. Maruthi Sai, P.S. Kless, H. & Scherz, A., (2006) Modulation of Protein Flexibility Acclimatizes Photosynthetic Energy Conversion to the Ambient Temperature. Nature, 442, 827-830

Shlyk-Kerner O, Samish I, Kaftan D, Dinamarca-Serda J. Photosynthetic organisms with mutated polypeptide of type II reaction center and compositions and methods of generating same. PCT Int. Appl. (2008), WO 2008010228, A2 20080124

Philip-Granit N, Goldberg E, Aschur I, Cohen H, Vilan A, van der Boom M and Scherz A, Electrostatic potential changes on atoms in molecules: measurements and implications (2008), Submitted

Trachtenberg J, Bogaards A, Gertner M, Weersink RA, Yue C, Scherz A, Savard J, Evans A, McLusky S, Haider MA, Aprikian A, Wilson BC, and Elhilali M (2007), Vascular-Targeted Photodynamic Therapy (VTP) with Tookad for Recurrent Prostate Cancer Following Definitive Radiation Therapy: Assessment of Safety and Treatment Response. J. Urology, 2007:178: 1974- 1979

Trachtenberg J1, RA Weersink, SRH Davidson, MA Haider, A Bogaards, MR Gertner, A Evans, A Scherz, J Savard, JL Chin, BC Wilson and M Elhilali (2008) TOOKAD-VTP (Padoporfin, WST09) for recurrent prostate cancer after EBRT failure: a light-dose escalating study, BJU, In the press

Berdugo, R. Bejjani, F. Valamanesh, M. Savoldelli, JC Jeanny 1, A. Scherz, Y Salomon, D. BenEzra, F. Behar-Cohen, (2008) Evaluation of the new photosensitizer STAKEL (WST-11) for photodynamic choroidal vessel occlusion in the rabbit and rat eye, Invest. Ophthal. & Vis Sci. (IVOS), In the press

Segal D, Brandis A, Goldshaid L, Ashur I, & Scherz A (2008) Quantitative Structure Activity Relationship (QSAR) models for predicting Human Serum Albumin binding affinity to different photosensitizers. Submitted

Scherz, A., Salomon, Y., Rubinstein, E., Brandis, A., Eren, D., Neimann, K. Conjugates of RGD peptides and porphyrin or (bacterio)chlorophyll photosensitizer and their uses. PCT Int. Appl. (2008), 191pp. WO 2008023378 A1 20080228 AN 2008:253145

Ashur, I., Goldschmidt, R., Pinkas, I., Salomon, Y., Scherz, A. (2006) Serum proteins strongly modulate the photogeneration of reactive oxygen species by novel vascular targeted photodynamic therapy reagent Free Radicl Res. 40: S165-S165

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