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Riding the chlorophylls: From photosynthetic energy conversion to cancer therapy

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1. Utilization of novel bacteriochlorophyll (Bchl) derivatives in vascular targeted photodynamic therapy (VTP) of tumors and other diseases (with Yoram Salomon, Department of Biological Regulation)

Research Objective: Synthesis of Bchl derivatives that induce rapid and selective occlusion of the tumor vasculature upon in-situ illumination of the tumor suspected domain and development of corresponding treatment protocols.

Research achievements: *Synthesis of new therapeutic candidates (Alex Brandis, Varda Belkin, Yahel vakrat-Hagalili, Efrat Rubinstein, Ilia Lichman).* A simple platform for the synthesis and preliminary examination of metal substituted Bchl derivatives with different side groups was developed, tested for light induced ROS generation, cellular and antitumor activity. Of particular interest are charged (negative or positive), compounds and compounds that specifically homed at vascular targets, stay in the circulation, clear fast from the body, have intermediate affinity to serum albumin and generate oxygen radicals in aqueous solutions.

VTP with TOOKAD-from the laboratory bench to phase II clinical trials (Vakrat-Hagilli et al). TOOKAD (WST11), the first novel VTP reagent synthesized in our laboratory and developed for clinical use by Steba-Biotech NV, recently entered phase II clinical trials in patients with recurrent localized prostate cancer in eight centers world wide. (see Yoram Salomon and ref therein).

WST11, A Novel Water-Soluble Bacteriochlorophyll Derivative: (Mazor et al) WST11 is the water soluble aminolysis product of TOOKAD and 2-aminomethyl sulfonate. It has a limited affinity to serum albumin, short half-life time in the circulation and practically clears from the body in ~2h. WST11, presently under development by Steba-Biotech for application in age related macular degeneration (AMD) and liver tumors (with D. Shouval, hadassa, Jerusalem), causes complete and selective occlusion with no significant perforation of tumor blood vessels in a few minutes after illumination leading to a selective tumor necrosis (Figure 1).

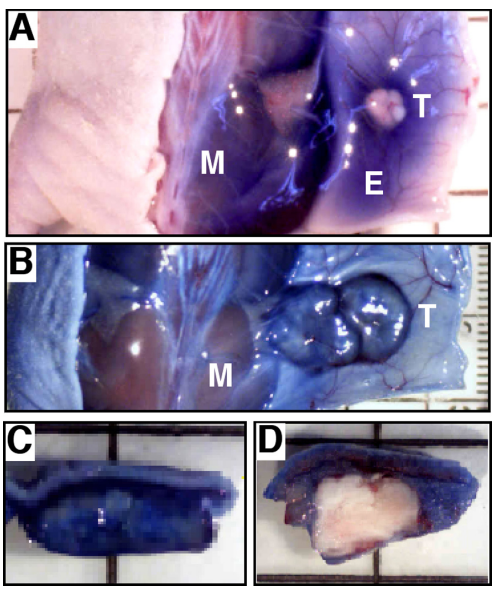

Fig. 1 *Blood flow stasis and tumor necrosis in HT-29 Human colon carcinoma xenograft but not in the surrounding normal tissue of mice after VTP with WST11 (from Mazor et al, 2004). (A,D) After treatment; (B,C) untreated. The vital dye, evans blue, was injected after treatment. Uncolored area represent vessel shutdown. T=tumor; M=muscle; E-edema*

2. Principles that underlay charge migration among molecules and molecular fragments and their utilization in molecular tools

Research objectives: Definition, measurement and utilization of fragmental charges in biological and synthetic molecular tools.

Research achievements: *An experimental look into sub-electron charge flow (Yerushalmi*

et al). The prediction and measurement of charge distribution and fragmental charge flow between interacting chemical entities in complex environments is a major challenge and an urgent need for modern chemistry, biology, material sciences, and other rapidly developing molecular disciplines. Using Ni substituted bacteriochlorophyll (NiBchl), we could estimate the fragmental charge flow to the metal center from different ligands. The experimental system provides a bench- mark for the evaluation of different computational approaches. The results helped suggesting a mechanism for redox transitions in methyl dehydrogenase (F430). Macromolecular assemblies based on metal substituted Bchl derivatives were initiated for constructing molecular machines (Yerushalmi et al, Granot et al, in collaboration with M. Van der Boom, WIS).

Control of redox transitions and oxygen species binding in Mn centers by biologically significant ligands; Model Studies with [Mn]-bacteriochlorophyll a (Ashur et al)

We showed that the Mn(III) center encourages protonation of superoxide radical in an aprotic environment containing residual water molecules, while promoting its oxidation in the presence of basic ligands. Similar coordination and stabilization of the OOH radical by the Mn center may be key steps in the enzymatic dismutation of superoxide radicals by Mn-SOD. Ongoing studies aim at defining the pH control of the model catalytic reaction.

3. Resolving the underlying principles of membrane protein conformational stability and gating.

Research objectives: Deciphering protein motifs that allow conformational flexibility and gating in membrane proteins using bioinformatics, mutagenesis and biophysical techniques.

Achievements: *Membrane Protein Folding: Centrality of Backbone-Mediated Interhelical Hydrogen Bonds (Golberg and samish).* Utilizing different algorithms we characterized backbone-mediated interhelical hydrogen bonds in available membrane protein structures. These widespread bonds are laterally clustered in the central and conserved part of the helix, pointing to the highly buried face of the helix, and are dominated by small amino acids. Furthermore, their distribution among amino acids correlates with the packing value scale, providing biophysical insight to this phenomenological characterization. Hence, the positioning of weak interhelical hydrogen bonds may be crucial in membrane protein folding and conformational flexibility, thus providing new parameters for structure and function prediction.

Regulation of Protein-Gated Electron Transfer by Intersubunit Hydrogen Bonding (Kerner-Shlik, Samish, Kaftan et al). Combinatorial mutgenesis *in silico* and *in vivo*, follwed by biophsical charactezation were proformed. A conserved inter-subunit hydrogen bond (HB) and a packing motif found at the middle of the reaction center (RC) core complex of all photosynthetic organisms are suggested to construct the gating switch between active and inactive RC conformations for electron transfer from one quinon to the other.

Selected Publications

Koudinova, N, Pinthus, J.H. , Brandis, A., Brenner, O., Bendel, P., Ramon J., Eshhar, Z., Scherz, A, Salomon, Y. (2003). Intl. J. Cancer,104, 782-789.

Yerushalmi, R., Baldridge, K., and Scherz, A. (2003). J. Amer. Chem. Soc. 125, 12,706-12,707

Yerushalmi, R., Scherz, A. and Baldridg, K. (2004). J. Amer. Chem. Soc. In press

Ashur I. Brandis, A., Greenwald, M., Vakrat, Y., Rosenbach-Belkin, V. Scheer, H. and Scherz, A. (2003) J. Amer. Chem. Soc. 125 8852-8861

Goldberg, E., Samish, I.& Scherz A. (2004) J. Mol. Biol. Submitted

Vakrat-Haglili, Y., Weiner, L., Brumfeld, V. Brandis, A. Pawlak, A. Rozanowska, M. Mcllroy, B., Salomon, Y., Wilson, B., T. Sarna, Scherz, A. (2004). J. Amer. Chem. Soc. Sumbitted.

Mazor, O. Brandis, A. Plaks, V. Rosenbach-Belkin, V. Salomon, Y. and Scherz, A. (2004) Photochem. Photobiol. Submitted

Kerner, O., Samish, I., Kaftan, D., Holland, N., Maruthi Sai, P.S. Kless, H., Scherz, A. (2004) In preparation.

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