There is a history of pioneering contributions from the Melis lab. Below is a chronological synopsis of major achievements:

**Photosystem-II (PSII) heterogeneity and the PSII damage and repair cycle in chloroplasts:**

Melis discovered and named the “alpha-beta heterogeneity” of PSII reaction centers in chloroplasts and showed this duality of reaction centers to be a consequence of a unique process, which he termed the “PSII damage and repair cycle”. He defined PSII-α centers to be the functional PSII, while PSII-β centers were those in the process of repair.

Photodamage of PSII is a dynamic phenomenon and a daily occurrence in the life of the oxygenic photosynthetic apparatus. The repair process selectively removes and replaces the photo-oxidized D1/32 kD PSII reaction center protein from the massive (>1,000 kD) multi-subunit H₂O-oxidizing and O₂-evolving PSII holocomplex. The repair mechanism is unique in the annals of biology and specific to photosynthetic organisms. Nothing analogous in complexity and specificity has been reported in other biological systems.

Melis and co-workers made several fundamental contributions to elucidating the mechanism of this unique PSII repair process. Included are the mechanism and kinetics of photodamage and repair, the transient conformation and status of photodamaged PSII, and the role of a “tetratricopeptide repeat protein” (REP27) in the repair process.

Selected publications:


Photosystem stoichiometry in oxygenic photosynthesis:

Since the 1960 hypothesis paper by Hill and Bendall (Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature 186:136-137), and the pioneering research published in 1961 by Duysens, Amesz and Kamp (Two photochemical systems in photosynthesis. Nature 190:510-511) and in 1962 by Duysens and Amesz (Function and identification of two photochemical systems in photosynthesis. Biochim et Biophys Acta 64:243-260), the field of photosynthesis research operated on the basis of two photosystems, PSII and PSI, functioning in the linear electron-transport chain and helping to pump electrons from water to ferredoxin and NADP\(^+\), in a highly overall endergonic reaction.

![Z-scheme of linear electron transport in oxygenic photosynthesis](image)

The Z-scheme of linear electron transport in oxygenic photosynthesis, depicting the pathway of electron transport and the mechanism of \(\text{H}_2\text{O}\) oxidation, ATP and NADPH generation. Electrons originate at PSII upon the photooxidation of \(\text{H}_2\text{O}\), their potential energy is elevated upon transfer to plastoquinone (PQ) in a light-mediated endergonic reaction. Electrons from the plastoquinone pool are transported via the cytochrome \(b-f\) complex (Cyt) to PSI, where their potential energy is elevated further upon transfer to ferredoxin (FD) in a second light-mediated endergonic reaction. P680, Reaction center of PSII; QA, primary quinone acceptor of PSII; PC, plastocyanin; P700, reaction center of PSI; A, primary electron acceptor of PSI; FD, ferredoxin; FNR, ferredoxin-NADP reductase.
Based on the Z-scheme of electron-transport in photosynthesis, and with the advent of time, it became a dogma in the field that stoichiometrically equal numbers of the two photosystems functioned jointly and concurrently in the linear electron transport process of all oxygenic photosynthesis. This assumption became a dogma in the field and served as the basis of further research on the functioning of the photosynthesis apparatus.

The advent of sensitive absorbance difference spectrophotometry and Melis’ own and unique laboratory-designed split-beam absorbance difference spectrophotometer, enabled him to quantify, directly and for the first time, the actual concentration of the photosystems in a variety of different photosynthetic membranes. Photosystem I reaction centers were quantified from the light-induced absorbance change at 700 nm (oxidation of the primary electron donor, P700). Photosystem II reaction centers were quantified from the light-induced absorbance change at 325 nm (reduction of the primary quinone electron acceptor, QA). Spinach chloroplasts and membrane fractions obtained by French press treatment, mature and developing pea chloroplasts, and blue-green algal membranes were initially investigated. The results showed a large variability in the ratio of system-II to system-I reaction centers (from 0.43:1 to 3.3:1) in different photosynthetic membranes. Thanks to this work, it is now established that cyanobacteria and red algae have a PSII/PSI ratio substantially lower than 1:1, whereas green plants and algae have a PSII/PSI ratio > 1:1. Chlorophyll deficient mutants and chloroplasts in the early stages of development have a PSII/PSI ratio that is substantially greater than that of the corresponding wild type or mature chloroplasts.

Melis provided evidence that plants, algae, and cyanobacteria dynamically adjust and optimize the ratio of their photosystems in response to genetic, developmental, and environmental light conditions for the purpose of maintaining a balanced and efficient electron flow through the electron transport chain. This pioneering discovery comprised a drastic departure from the accepted dogma of an inflexible photochemical apparatus of photosynthesis and was, for a period of time, a controversial and highly debated issue.

**Selected publications:**

**Photosynthetic hydrogen production research:**

Following his earlier pioneering work on the “photosystem II damage and repair cycle”, a term that he coined, Melis made a 60-year breakthrough in 1998-99, when he found that deprivation of sulfur nutrients causes a sealed culture of green microalgae to switch from evolving oxygen (O$_2$) to producing hydrogen (H$_2$) via the microalgal native enzyme hydrogenase.

Under S-deprivation, the repair of PSII from photodamage is retarded, effectively lowering the number of functional PSII-α centers and limiting photosynthetic oxygen evolution to a level below that of cellular respiration. Under these conditions, the rate of respiration is greater than that of photosynthesis, resulting in the consumption of all cellular oxygen. The ensuing anaerobiosis is necessary and sufficient to induce gene expression and activation of the cellular H$_2$ metabolism, diverting the natural flow of photosynthetic electrons toward the hydrogenase. A sustainable generation of photosynthetic H$_2$ prevails, instead of the normally evolved O$_2$.

Melis showed that the process can be sustained for several days, instead of the 90 seconds previously achieved, or as long as the cellular respiration continues to consume endogenous O$_2$. Thus, application of know-how from the PSII repair mechanism enabled Melis to bypass the long-known incompatibility in the simultaneous O$_2$ and H$_2$ photoproduction by green microalgae and constituted a long-sought breakthrough, tracing back to the initial discovery (by Gaffron and co-workers 1939 and 1942) of the ability of unicellular green microalgae to metabolize H$_2$. The S-deprivation H$_2$ photoproduction method has been adopted globally and employed by numerous laboratories, serving as a platform for further photosynthetic H$_2$-production research.

The breakthrough publication in year 2000 attracted widespread interest by the scientific news media, academia, and the public. Melis' primary publications in the field have exceeded 3,000 scientific citations.

Selected publications:

A hydrogen-producing *C. reinhardtii* culture. Hydrogen bubbles emanate toward the surface of the liquid medium. The gas is drained through a syringe (inserted in the middle of the silicone stopper) and, through teflon tubing, collected in an inverted burette and measured by the method of water displacement. Photograph courtesy of Michael Barnes, UC Office of the President. From Melis and Happe 2001.
Chloroplast sulfate transport and hydrogen production research:

Melis spearheaded further advances by applying molecular biology approaches to the hydrogen production process, including cloning and engineering of the *Chlamydomonas reinhardtii* chloroplast sulfate permease genes—a first for a photosynthetic eukaryote and a major advance in understanding the link between sulfur deprivation and hydrogen production.

*Chlamydomonas reinhardtii* strains with attenuated expression of the chloroplast sulfate permease (*SULP*) gene were able to grow photo-heterotrophically under anaerobic conditions in sealed cultures stabilizing the cellular H₂-production and its associated metabolism. Specifically, downregulation in the expression of the *SULP* gene attenuated sulfate uptake by the *Chlamydomonas reinhardtii* chloroplast, without depriving other cellular compartments (e.g. cytosol and nucleus) of sulfate nutrients. This condition enabled a constitutive H₂-production process the cell, without exerting adverse sulfur deprivation effects on the biochemistry of other cellular compartments.

Sulfate anions are transported from the environment through the cell plasma membrane sulfate transport system to the cytosol. A separate chloroplast sulfate transport system (*SULP*) is responsible for the subsequent sulfate transport from the cytosol to the chloroplast stroma. Sulfate assimilation occurs exclusively in the chloroplast of the green algae, leading to the biosynthesis of cysteine. Continuous cysteine biosynthesis is required for the D₁ reaction center protein turnover, thereby enabling normal photosynthesis, oxygen evolution, and biomass accumulation. A limitation in the supply of sulfate to the chloroplast impedes D₁ protein turnover, thereby limiting the capacity of PSII function, and lowering water oxidation and O₂-evolution activity.

**The green microalga Chlamydomonas reinhardtii**

**Selected publications:**


The microalgal H₂-production breakthrough constituted a turning point in the field of Photosynthesis Research, as it was the first experimental approach showing the generation of a useful product directly from photosynthesis. Following this work, Melis originated and subsequently applied the concept of “Photosynthetic Bioproducts”, entailing the direct application of photosynthesis for the generation of biofuels, useful chemicals, and biopharmaceuticals. According to this concept, a single photosynthetic microorganism operates both as photocatalyst and processor, consuming carbon dioxide (CO₂), synthesizing, and emitting ready-to-use commodity products.

**Natural chemicals and biopharmaceuticals:**

Melis expanded the concept of “Photosynthetic Bioproducts,” when he introduced a platform for the renewable generation of isoprene (C₅H₈) and monoterpenes (C₁₀H₁₆) hydrocarbons, derived entirely from sunlight, CO₂ and H₂O in cyanobacteria and green microalgae. He correctly predicted that small-sized hydrocarbons (C₅H₈ and C₁₀H₁₆), like H₂, would spontaneously diffuse and separate from the cell interior and the liquid culture, enabling easy harvesting of the product.

He applied metabolic engineering approaches to enhance the activity and yield of the terpenoid biosynthetic pathway in microalgae, resulting in notable increases in the yield of heterologous C₅H₈ and C₁₀H₁₆. To accomplish this, he installed the genes of the exogenous mevalonic acid (MVA) pathway along with isoprene and monoterpane synthase genes from plants in a cyanobacterium (Synechocystis) that normally uses the sluggish methylerythritol-phosphate (MEP) terpenoid biosynthetic pathway. The work employed a novel chromosomal integration and simultaneous expression of three heterologous synthetic gene operons in Synechocystis under the control of single promoters. This was the first time that an entire biosynthetic pathway with 12 recombinant enzymes had been expressed in a photosynthetic microorganism. In constituting a major addition to the genetic engineering toolkit, the work serves as a paradigm in the pursuit of approaches using sunlight for the renewable generation of high-impact products. Highly-cited publications were generated from this research.

The Photosynthetic Bioproducts approach utilizes sunlight, CO₂, and water to generate fuel and chemicals in a high capacity process. Cyanobacteria offer an opportunity to develop such technology, with the microorganism acting as a single-cell factory, capturing carbon dioxide and converting it into natural commodity products.

**Selected publications:**


Efficiency and productivity of photosynthesis in microalgae, cyanobacteria, and crop plants:

Melis conceived, designed and pioneered, and currently leads an international effort to improve, theoretically, by up to 3-fold the efficiency and productivity of photosynthesis under direct and bright sunlight conditions. Inefficient sunlight utilization in cultivated microalgae and crop canopies occurs because of a genetic tendency of all photosynthetic organisms to assemble large arrays of light absorbing chlorophyll antenna molecules in their photosystems. This is a survival strategy and a competitive advantage in the wild, where light is often limiting. Maximum competition in the wild requires capturing more light for self, even if wasted, and preventing light capture by competing neighbors. Obviously, such over-absorption and wasteful dissipation of excess sunlight is detrimental to the yield and productivity of high density cultivated monocultures, including microalgae and crop canopies under bright sunlight conditions.

He correctly predicted that genetically minimizing the size of the array of chlorophyll molecules that serve as antennae to harvest sunlight for the photosynthetic apparatus could minimize overabsorption of sunlight and the ensuing wasteful dissipation. He coined the term Truncated Light-harvesting Antenna (TLA) and showed that the TLA concept brings about enhancement in the photosynthetic productivity of microalgal cultures and, more recently, of a model crop plant, tobacco, cultivated in high density under direct sunlight. In this ground-breaking work, Melis noted that TLA cultivars show promise of increased yields, while minimizing field space needed for cultivation. Higher density planting of crop plants, in addition to greater yields per canopy, offer ancillary benefits, such as lower amounts of fertilizer and herbicide use.
Visual appearance of a pair of *Nicotiana tabacum* wild type and TLA canopies, shown at the end of their growth period and immediately prior to harvesting of the plants. The wild type tobacco leaves had a dark green coloration and the TLA tobacco leaves a lighter green coloration. The overall foliage density was greater in the TLA canopy, compared to the wild type. **Canopy characteristics:** TLA / WT total biomass ratio = 1.25:1; TLA / WT leaf biomass ratio = 1.35:1; TLA / WT stem biomass ratio = 1.05:1

**Selected publications:**


**Cultures in the Greenhouse**

High-density-cultures of *Chlamydomonas reinhardtii* for measurement of photosynthetic productivity under direct sunlight conditions. The wild type (WT) and the *tla1* antenna mutant were grown in 2.5-L size bottles having an internal diameter of about 15 cm. Photosynthetic O\(_2\) was collected through a syringe (inserted in the middle of the silicone stopper) and Teflon tubing and the volume and rate of oxygen gas produced was measured. Note the higher cell density (biomass) and the lighter green coloration attained by the *tla1* culture, as compared to the wild type. **Culture Characteristics at the time of test:**

**WT:** 6.4x10\(^6\) cells/mL, 25.6 μM Chl concentration, rate of oxygen evolution 22 mL/h.

**tla1:** 10x10\(^6\) cells/mL, 15.4 μM Chl concentration, rate of oxygen evolution 31 mL/h.

The 40% greater photosynthetic productivity of the *tla1* over WT culture is attributed to better sunlight penetration and less wasteful energy dissipation.
**Patents:** Ten patents have been issued to the University of California, with Melis as the Principal Inventor, from the above described research.

**Publications:** Melis has published more than 280 peer-reviewed Original Research Articles, Reviews, and Book Chapters.

**Invited seminars and lectures:** Owing to his research contributions, Melis has been invited as a speaker and has delivered more than 180 international and national invited lectures and seminars at academic, conference, government, and industry settings in (alphabetically) Brazil, Canada, Europe, India, Israel, Japan, Korea, Turkey, and the U.S.

**Awards and honors:** Melis has received many honors, including prestigious fellowships enabling him to do research in Europe (Universities of Lund, Leeds and Hamburg), Australia (CSIRO in Canberra) and Japan (National Institute for Basic Biology in Okazaki). He has been honored by awards from the US Department of Energy (Research Achievement Award), Daimler-Chrysler Corporation (University Research Award), and UC Berkeley (Distinguished Teaching Award). He has been elected a Fellow of the American Association for the Advancement of Science (AAAS). These honors attest both to the excellence of Melis’ research and his dedication to teaching.

**Service to the scientific community:** Melis’ contributions extend to professional service. He gives admirably of his time as an organizer and convener of conferences and workshops, as a peer-expert reviewer of manuscripts, member of grant review panels, and as a scientific journal editor. In the latter capacity, he has served as a *Plant Physiology* (ASPB) Editorial Board member, *Plant and Cell Physiology* Overseas Editor, and Associate Editor of both *Photosynthesis Research* and *Bioenergy Research*. Furthermore, he has served on the Editorial Board (since 1995) and as Editor-in-Chief (since 2002) of the prestigious international plant biology journal *Planta*. 