3nd Solar Fuels I-CORE Workshop

September 12th-15th, 2016 * Nahsholim, Israel

POSTER'S ABSTRACTS

This workshop was supported by
The I-CORE Program of the Planning and Budgeting Committee
and The Israel Science Foundation (Grant No. 152/11)

This workshop was partially supported by
The Grand Technion Energy Program (GTEP)
Thanks to the generosity of
The Leona M. and Harry B. Helmsley Charitable Trust Program in Alternative Energy
A scientific collaboration of
The Technion – Israel Institute of Technology and The Weizmann Institute of Science

A- Biomass: Energy Crops and Algae

Growth platform-dependent and Independent phenotypic and metabolic responses of Arabidopsis thaliana and its halophytic relative, Eutrema salsugineum/Thellungiella salsuginea, under salt stress

Yana Kazachkova, Albert Batushansky, Aroldo Cisneros, Aaron Fait and Simon Barak

French Associates Institute for Biotechnology and Agriculture of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev

To investigate the natural salt tolerance of the Arabidopsis halophytic relative, Eutrema salsugineum, we performed a phenomics study of leaf growth and development under control and salt stress conditions in an in vitro plate system and a pot-based soil system. Growth responses of Arabidopsis and E. salsugineum differed depending upon growth platform. Leaf emergence was affected in a similar way in both species grown in vitro but the same effects observed in Arabidopsis occurred at higher salt concentrations in E. salsugineum. Leaf emergence of both species was unaffected on soil at all stress levels.We also unmasked a previously unobserved leaf area reduction of E. salsugineum even under mild stress on both platforms.Metabolic profiling revealed both growth platform-independent and dependent metabolic responses. For instance, E. salsugineum exhibited higher citrate and malate levels but constitutively low fumarate, galactinol and raffinose content regardless of growth platform. Such metabolic signatures could reflect core E. salsugineum stress tolerance mechanisms. Growth platform-specific metabolic differences manifested as repression of accumulation of a number of metabolites in E. salsugineum in the in vitro system compared to the soil system. These included metabolites known to be involved in stress responses. We surmise that perturbation of C:N ratio by inclusion of sucrose in the in vitro system could be responsible for this metabolic repression.

Inducible expression of Haematococcus oil globule protein in the diatom Phaeodactylum tricornutum: Association with lipid droplets and enhancement of TAG accumulation under nitrogen starvation

Zachor Shemech, Stefan Leu, Inna Khozin-Goldberg, Aliza Zarka and Sammy Boussiba

Ben-Gurion University of the Negev

The catalytic splitting of water into hydrogen and oxygen is a bio-inspired challenge for chemists seeking to find “green” alternatives for fuel. In natural photosynthesis, this process utilizes sunlight energy and a high oxidation state manganese cluster. Few synthetic high-valent manganese complexes have been applied to date as water oxidation catalysts in homogeneous and heterogeneous systems. The use of electrochemical energy for homogeneous water oxidation catalyzed by a manganese cluster, however, has not been demonstrated. Herein we present the synthesis and characterization of a unique polynuclear Mn cluster, [Mn12O12(O2CC6H3(OH)2)16(H2O)4], which has a Mn(IV)-oxo cubane core and is highly soluble and stable in water as indicated by spectroscopic studies. Electrochemical investigations in acetate buffer solutions revealed pH dependent catalytic currents at an oxidation potential as low as 1.2V (vs the NHE). O2 evolution was clearly detected by a typical reduction peak observed in cyclic voltammograms. Controlled potential electrolysis indicates that this catalyst not only performs at pH 6 with high turnover number in low potential, but also functions in seawater. We further advocate that the high electrocatalytic performance is due to the hydroxyl groups, which play an important role as proton acceptors.

The green alga Lobosphaera (Parietochloris) incisa as a source of high value LC-PUFA

Boris Zorin

Ben Gurion University of the Negev

For several decades, microalgae have been recognized as promising candidates for generating bio-products and filling the gap between expected human demands and the annual productivity of bio-sources. The unicellular green alga Lobosphaera (Parietochloris) incisa was isolated in a snowy region of Mount Tateyama, Japan and has proved to be an outstanding candidate for the efficient phototrophic production of arachidonic acid (AA), an essential ω-6 PUFA for infant brain development, and a widely used ingredient in the baby formula industry. Moreover, a mutant strain of L. incisa, which accumulates the precursor of AA di-homo linoleic acid (DGLA), was isolated and characterized in our lab. DGLA is a rare ω-6 LC-PUFA that may promote the suppression of inflammation signaling in the human body. The sequencing of genomes and transcriptome and the development of the genetic nuclear transformation system has provided us with the basis for a further step toward the development of the full molecular toolbox in this biotechnologically important organism. Based on the achieved experimental platform, we have created an engineered strain with double the amount of ω-3 PUFA. We present this organism as an efficient production platform and a superior producer of LC-PUFA with high medicinal and nutritional value.

Stability and activity of bimetallic tips as reduction co-catalysts

Eran Aronovitch[1], Maya Bar Sadan[1], Philip Kalisman[2], Lilac Amirav[2] and Lothar Houben[3]

[1]Ben-Gurion University, Beer Sheva, Israel
[2]Technion-IIT, Haifa, Israel
[3]Ernst Ruska-Centre for Microscopy, Jülich, Germany

The search for alternative clean and renewable energy source is a major pressing issue. One promising direction is the use of semiconductor nanoparticles as photocatalysts which absorb the solar radiation and produce hydrogen from water. Upon radiation, excited electrons and holes are created. They then migrate to the surface and react with the aqueous solution. Efficient photocatalysts should maintain charge separation of hole and electron and contain different sites for oxidation and reduction. Usually a small metallic particle is deposited on the semiconductor as a co-catalyst which acts as an electron sink and a reduction site for protons. Hybrid core-shell structures such as CdS@CdSe increase the charge separation and reduce the particle dissolution by confining the holes to the core and leaving the electrons delocalized over the entire structure. A bi-metallic co-catalyst composed of metals such as gold and palladium should improve the photocatalytic activity of the system. Such bimetallic particles possess the ability to attract electrons from the semiconductor and discharge them into the aqueous solution more efficiently then each of the metals on their own. Here we use the CdSe@CdS-Au\Pd system as a case study to explore the effect of the inner structure of the bimetallic tip on the photocatalytic performance. In addition we study the dynamic processes which occur during photocatalysis using both high resolution EDS imaging and online photocatalytic measurements.

Recycling of Carbon Dioxide by its Electrochemical Reduction to Fuels using Metalloporphyrin/Graphene Catalytic Systems

Yair Bochlin, Eli Korin and Armand Bettelheim

Ben-Gurion University of the Negev

The CO_2 levels in air have been increasing over the past few decades. The conversion of CO_2 back to fuels is a critical goal that would restore balance to the rising CO_2 levels. CO_2 is a very stable, linear molecule, and returning it to a useful state in the form of fuels is a challenging problem. CO_2 reduction is possible through chemical catalysis, electrochemistry, photo-chemistry and biological processes. Chemical catalytic processes generally operate at high temperatures and pressures which lead to high energy cost.

The electrocatalytic capabilities toward CO_2 reduction of some cobalt porphyrins have been reported. The present work deals with the spectroscopic, microscopic and electrochemical examination of the interactions occurring between such porphyrins and graphene derivatives, and their effect on CO_2 reduction. Such self-assembled systems formed between 5,10,15,20-Tetrakis(1-methyl-4-pyridinio) porphyrin (CoTMPyP) and graphene oxide were deposited on electrode surfaces (such as glassy carbon) by means of electrodeposition. TEM images show a homogeneously distributed CoTMPyP in the graphene sheets.

The electrodeposited system showed increased activity for CO_2 reduction compared to water reduction (1.2 mA/cm2 and 0.25 mA/cm2, respectively, at -1.2V vs. Ag/AgCl), as examined in an aqueous 0.1 M Na2CO3 solution at pH 11.5.

Polyoxometalates complexes of α-Fe2O3 cores in water

Biswarup Chakrborty

Department of Chemistry, Ben-Gurion University, Beer Sheva, Israel

An unprecedented role for metal-oxide cluster-anions (polyoxometalates, or POMs) as covalently coordinated inorganic ligands for individual hematite nanocrystals, gives isolable anionic clusters uniquely positioned between molecular macroanions and traditional colloidal nanoparticles. Sodium salts of α-PW11O397- anions serve as pentadentate “capping” ligands for complexed Fe(III) ions linked—via their sixth coordination site—to 3-5-nm α-Fe_2O_3 cores. Multiple spectroscopic methods and analytical measurements confirm the presence of POM-capping ligands, [α-PW11O39Fe-O-]n-, covalently bound to the surfaces of the hematite cores. Clear orange solutions of these unique complexes are stable in water over a wide range of pH values (2.5-8), which spans the isoelectric point of hematite (pH 5.3). Moreover, covalent attachment of the POM anions allows for repeated precipitated (by added salt), and re-dissolution in water. Raman, FTIR, EDS and XPS data show that numerous POMs are associated with each 3-4-nm hematite nanocrystal, and high-resolution TEM, cryogenic-TEM, and HAADF-STEM images clearly reveal the covalently bound POM ligands at the hematite surfaces. Differential-pulse voltammetry (DPV) was used to reveal the reversible redox chemistry of the covalently attached POM ligands. Preliminary reactivity studies of visible light driven water oxidation by the hybrid material indicate rates of O_2 formation exceeding any previously reported in literature.

Rational design of a water soluble manganese cluster for electro-catalytic water oxidation

Naama Gluz and Galia Maayan

Schulich Faculty of Chemistry, Technion - IIT

A promising approach in the search of renewable and alternative energy is to mimic nature and develop a system for artificial photosynthesis, which converts water into oxygen and hydrogen. The main and most challenging part is the development of an efficient water oxidation catalyst.

The biological water splitting process takes place at the oxygen-evolving center (OEC) of photosystem II, producing O_2, protons and electrons. The OEC is a Metallo-oxo cluster Mn4O5Ca in which Mn oxidation state varies from III to V. This cluster stores four electrons in four successive 1e- oxidation steps, with narrow potential range, followed by the generation O_2 and regeneration of the OEC in its initial oxidation state.

Our research goal is to design molecular catalysts for electrochemical water splitting, inspired by the natural OEC of PSII.

To this aim we synthesize high oxidation poly-nuclear oxide-bridged Manganese clusters, with a diversity of organic acids as protective shells. Then we test the resulting catalysts for water solubility and electrochemical performances to efficiently oxidize water.

Photoelectrochemical Reduction of CO2 with Visible Light using a di—Rhenium Molecular Catalyst Combined with a Polyoxometalate as an Electron Shuttle and Photosensitizer

Eynat Haviv [a], Linda J.W. Shimon[b] and Ronny Neumann[a*]

(a) Department of Organic Chemistry Weizmann Institute of Science, Rehovot, Israel

(b) Department of Chemical Research Support, Weizmann Institute of Science, Rehovot, Israel

The reduction of CO_2 to a higher energy species such as CO is a key transformation and important missing link towards the development of carbon-based solar fuels to remediate increasing amount of CO_2 in the atmosphere and replace finite amounts of fossil fuels. Both photochemical and electrochemical pathways are being studied. The present state of the art teaches that the CO_2 to CO reduction by

(1) a photochemical pathway requires sacrificial tertiary amines as the source of electrons and protons needed for the transformation and either low wavelength light or photosensitizers.

(2) an electrochemical pathway that still requires prohibitively high potentials, typically higher than 1.7 V versus Ag/AgNO3.

In order to overcome these two basic deficiencies, we combine a new di-rhenium molecular catalyst active for CO_2 photoreduction that also has a tether to bind a polyoxometalate via a simple acid-base interaction. The polyoxometalate is an electron reservoir that can shuttle electrons from an electrode to the molecular catalyst.

Now, in a cascade of transformations a new photoelectrochemical pathway is presented wherein a polyoxometalate, the commercially available phosphotungstic acid, H3PW12O40, is electrochemically reduced at low potential (1.3 V versus Ag/AgNO3), and low intensity visible light (60 W tungsten lamp) is used to transfer electrons from the polyoxometalate to the catalyst that is active for selective reduction of CO_2 to CO.

Design and assembly of artificial four-iron four sulfur clusters proteins

Bhanu Prakash Jagilinki and Dror noy

MIGAL-Galilee Research Institute

Proteins containing iron-sulfur (Fe-S) clusters are evolutionary old, highly abundant redox proteins. Those containing four-iron four-sulfur (4Fe4S) clusters are of particular interest to the development of alternative fuels such as molecular hydrogen due to their role as electron carriers at highly reducing potentials. Our goal is to design artificial 4Fe4S clusters protein that will serve as custom redox carriers by using computational tools. Developing an efficient system for assembling the 4Fe4S cofactors within the protein scaffold is critical to this endeavor. It is challenged by the inherent oxygen lability of 4Fe4S clusters. We are following two parallel assembly strategies, namely in-vivo assembly and in vitro reconstitution. For in-vivo assembly Iron and Sulphur containing additives were supplemented during induction and protein purification will be done strictly under anaerobic conditions inside the glove box. For in-vitro reconstitution the protein is purified aerobically and the reconstitution will be done under anaerobic conditions. We have successfully tested both these strategies on a novel protein, Coiled-coil Iron-Sulfur protein (CCIS), which was designed in-silico. The 4Fe4S assembly is further validated by various methods such as physical color, absorbance spectroscopy, CD and EPR. These strategies are currently being extended to screen other designs for the assembly of 4Fe4S clusters.

Tuning the Catalytic Properties of Ternary Nano-Flowers of Layered Materials

Vasu Kuraganti, Oren M, Lothar Houben and Maya Bar-Sadan

Ben-Gurion University of the Negev

Tuning the properties of layered materials by modifying their chemical composition and electronic structure is important for nanoelectronic and optoelectronic device applications. Alloy of 2D layered materials by metal or nonmetal atom substitution is an effective way to tune bandgap and composition. Nanostructured MoS2 and MoSe2 were promising electrode materials for electrochemical hydrogen evolution reaction (HER) and Li-ion batteries. Here by employing the colloidal synthetic route, we obtained ternary Mo1-xWxSe2 and Mo(SxSe1-x)2 alloy nanoflowers with different W concentration. The structure, composition and electrocatalytic HER activity of ternary nano-floweres were examined. X-ray diffraction (XRD) pattern revealed that the ternary Mo1-xWxSe2 and Mo(SxSe1-x)2 alloy nanoflowers crystallized into 2H-hexagonal crystal structure. Flower-like structure in Mo1-xWxSe2 and Mo(SxSe1-x)2 alloy was retained for different composition and the sizes were spanning in the range 300-500 nm. The Mo1-xWxSe2 and Mo(SxSe1-x)2 alloy nanoflowers shows tunable electrocatalytic HER activity with composition. The electrochemical over potential and Tafel slope are increased with increasing x in Mo(SxSe1-x)2 nanoflowers. MoS2 nanoflower for x=0 shows an over potential of 270 mV and Tafel slope of 32 mV/dec and these values reaches to 420mV and 46 mV/dec for MoSe2 flower of x=1. Mo1-xWxSe2 nanoflowers shows optimal minimum over potential of 320 mV and maximum Tafel slop of 56 mV/dec for x=0.5.

Using underlayers as a way to improve solar water splitting with iron oxide photoelectrodes

Hadar Mor, Barbara Scherrer, Hen Dotan and Avner Rothschild

Department of Material and Science Engineering, Technion – Israel Institute of Technology

Photoelectrochemical (PEC) water splitting is a way to convert solar energy to storable chemical energy by producing hydrogen fuel. Iron oxide (hematite) is considered as a promising photoanode candidate for water photo-oxidation. It has a favorable band gap of 2.1 eV, is chemically stable in alkaline aqueous solutions and is an abundant and low cost material. Although hematite is a promising candidate, it exhibits fast electron-hole recombination within the bulk as well as at interfaces and the surface of the photoanode. Our strategy to overcome this challenge is to modify the substrate with another oxide underlayer, in order to lower the recombination of the electrons and holes at the interface with the substrate. Herein we investigate the effect of different underlayer materials and their thicknesses on the performance of hematite thin film photoanodes. The underlayer films are deposited with atomic layer deposition (ALD) and pulsed laser deposition (PLD) with thicknesses between 0.1 to 10 nm, followed by PLD of hematite layer of 20 to 50 nm. Photoelectrochemical measurements and analysis includes measurement in solar simulated light for evaluation of the charge transport and charge transfer properties of the photoanodes. In addition, the effect of hematite and underlayer microstructure will be reported and correlated with its photoelectrochemical performance.

In vitro reconstitution of the Fenna Mathews Olson bacteriochlorophyll protein

Satyabala Neelam and Dror Noy

MIGAL - Galilee Research Institute

Typical components of artificial photosynthetic systems comprise antennas, reaction centers (RC), catalysts for fuel production and water oxidation, units for photoprotection and photo regulation. In green sulfur bacterium, sunlight is absorbed by chlorosomes, transferred to the RC through Bacteriochlorophyll (BChl a) containing protein termed as Fenna-mathew-olson (FMO) protein. FMO is a rare example of a water-soluble antenna protein, embedded in the cytoplasmic membrane that binds BChls and occupies a special place in the history of photosynthesis. Due to its key role in energy transfer it can be a very good choice to consider it in designing the artificial photosystems. FMO was a homotrimer (subunit M.wt:42 kDa) with 8 BChl a per subunit. We, for the first time are trying to reconstitute apo protein with BChls in vitro outside the membrane. We have succeeded in expressing recombinant FMO protein (E.coli system), purify stable FMO protein. We have checked the protein purity and stability using biochemical and biophysical techniques. We are implementing various strategies to reconstitute FMO with BChls. So far we were able to reconstitute FMO trimer protein to BChls in the ratio 1:1 whereas the actual ratio was 1: 8. From the structure packing of the BChls within the protein is analogous to the packing of hydrophobic residues in the core of a globular protein.We are now looking in to the ways to dissociate trimer FMO to monomer and reconstitute with maximum number of BChls.

Tunable inorganic complexes of TiO_2 nanocrystals

Manoj Raula and Ira Weinstock

Department of Chemistry and Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of Negev, Beer Sheva, Israel

POM complexes of anatase-TiO_2 cores are the first member of an emerging family of hybrid materials, with unique chemical, electrochemical or photochemical properties and reactivities. A conceptually new role for redox-active POMs is reported as covalently coordinated ligands in polyanionic “complexes” of metal-oxide nanocrystals. These new “complexes” are isolable, water-soluble nanostructures uniquely positioned between molecular macroanions and traditional—electrostatically stabilized —colloidal metal oxides. In POM complexes of TiO2 nanocrystals, for example, an iso-structural series of TiIV-substituted POM capping ligands, “[α-XW11O39Ti]–O–” (X = Al3+, Si4+, P5+) are covalently attached to ca. 6-7 nm anatase-TiO2 cores. The covalently attached POMs, which serve as photosensitizers at the surface of the metal oxide nanocrystals, control the rate of hydrogen evolution under UV-visible irradiation, with reactivity increasing in the order, X = Al3+< Si4+< P5+. The kinetic and thermodynamic basis for the enhanced reactivity will be discussed. Just as traditional ligands control catalytically active metal centers in molecular complexes, covalently coordinated POM capping ligands with tunable redox chemistries and photochemical properties now provide new options

Catalyst overlayers to assist water splitting on hematite photo electrodes

Anton Tsyganok, Daniel Grave, Mikael Leben and Avner Rothschild

Department of Materials and Science Engineering, Technion - IIT, Haifa

One of the most promising candidates for use as a photoanode for solar water splitting is α-(Fe)_2 O_3, also known as hematite. Hematite possesses several advantages such as earth abundance, cheap cost, and a bandgap suitable for effective sunlight absorption in the visible spectrum. However, α-〖Fe〗_2 O_3 also has some major drawbacks including low mobility of charge carriers, low lifetime due to bulk and surface recombination, low rate of holes reaction with solution on the interface, and high onset potential for solar driven water splitting.

One of the approaches to improve the photoanode properties is deposition of various over layers. In this work, we present the photoelectrochemical deposition of FeNiOx overlayers onto hematite photoanodes. The work will concentrate on the overlayer deposition parameters, such as current and deposition time, and their effect on the photoanode performance.

Another aspect that will be considered as a part of this work is the effect of the substrate and surface properties on the overlayer performance. For this part, surface morphology and roughness of the surface will be varied by using hematite deposited in various techniques, and on different substrates.

Supramolecular Organization of Graphene-anchored Photosynthetic Nano-arrays

Adam Weingarten[1], Zois Syrgiannis[2], Ira Weinstock[1], Maurizio Prato[2] and Marcella Bonchio[2]

[1] Ben-Gurion University of the Negev

[2] Università di Trieste

There exists a fundamental and technologically important challenge in the solar-fuels research field: How to couple molecular catalysts to photoactive domains and to drive water oxidation catalysis by visible light without the use of applied potential biases or sacrificial reagents. In a collaborative effort between Israeli and Italian research teams, we demonstrate here a new, supramolecular strategy towards achieving this end. Previously, we have visualized the non-specific organization of polyoxometalates (POMs) onto gold nanoparticle (AuNP) surfaces. Furthermore, we found cationic thiols to insert themselves between the POM monolayer and the AuNP surface. We now add functionality to this system by incorporating a tetraruthenium-based polyoxometalate water-oxidation catalyst and then inserting photoactive disulfides, bearing cationic ruthenium tris(bipyridine), into the assembly to co-localize catalyst and chromophore. Utilizing graphene's ability to bind gold nanorods, we attach AuNPs to covalently-modified graphene in a bottom-up post-functionalization scheme to integrate these nano-arrays with electrodes. The goal for these integrated electrodes is to demonstrate visible-light photoelectrochemical water oxidation without the need for sacrificial reagents.

The crystallographic structure of XynB2 reveal the crucial residues important in glycosynthesis

Oleg Chmelnik[a], Roei Dann[b], Tomer Cohen[b], Rachel Salama[a], Noa Lavid[a], Shifra Lansky[b], Alon Ben-David[a], Gil Shoham[b], Yuval Shoham[a]

[a]Technion – Israel Institute of Technology
[b]The Hebrew University of Jerusalem

Glycosynthases are catalytic mutants of mainly retaining glycoside hydrolases that catalyze the synthesis of oligosaccharides from their corresponding glycosyl-fluoride donors and suitable acceptors. In these enzymes the nucleophile is replaced by smaller non-nucleophilic residues. Enzymatic synthesis of oligosaccharides provides an attractive alternative to the classical synthetic chemical methods, since it enables a complete control over newly generated anomeric centers, and the reaction can be performed under relatively mild conditions.

β-xylosidase from Geobacillus stearothermophilus (Xyn52B2) has already proved to be useful for glycosynthesis applications. The XynB2E335G, a nucleophile-deficient mutant of XynB2, can function as an efficient glycosynthase. By use of directed evolution approach, two improved variants (V27, V29) of XynB2E335G were isolated and found to show enhanced glycosynthase activity. Recently, the three-dimensional structures of XynB2E335G and the two variants, with the glycosyl-fluoride donor and the product bound to the active site, were obtained via crystallographic analysis which revealed the crucial amino acids contributing to the improvement of the glycosynthase activity. The V27 variant includes four amino acid substitutions in addition to the E335G: F206L, I211T, C253R, and N342K. The V29 variant contains six additional amino acid substitutions: V123E, A282T, R291H, T343P, D470G and D597G. Contribution of several substitutions and their combinations to the improved glycosynthase ability were studied by structure analysis and glycosyl-fluoride donor self-condensation.

Enhancement of cellulosome-mediated deconstruction of cellulose by improving enzyme thermostability

Sarah Moraïs[1], Johanna Stern[1], Amaranta Kahn[1], Anastasia P. Galanopoulou[2], Shahar Yoav[1,3], Melina Shamshoum[1], Matthew A. Smith[4], Dimitris G. Hatzinikolaou[2], Frances H. Arnold[4], and Edward A. Bayer[1*]

[1] Department of Biomolecular Sciences, The Weizmann Institute of Science, Rehovot, Israel.

[2] Microbiology Group, Faculty of Biology, National and Kapodistrian University of Athens, Zografou Campus, Athens, Greece.

[3] Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel;

[4]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California USA

The concerted action of three complementary cellulases from *Clostridium thermocellum,* engineered to be stable at elevated temperatures, was examined on a cellulosic substrate and compared to that of the wild-type enzymes. Exoglucanase Cel48S and endoglucanase Cel8A, both key elements of the natural cellulosome from this bacterium, were engineered previously for increased thermostability, either by SCHEMA, a structure-guided, site-directed protein recombination method, or by consensus-guided mutagenesis combined with random mutagenesis using error-prone PCR, respectively. A thermostable □-glucosidase BglA mutant was also selected from a library generated by error-prone PCR that will assist the two cellulases in their methodic deconstruction of crystalline cellulose. The effects of a thermostable scaffoldin versus those of a largely mesophilic scaffoldin were also examined. By improving the stability of the enzyme subunits and the structural component, we aimed to improve cellulosome-mediated deconstruction of cellulosic substrates.

The results demonstrate that the combination of thermostable enzymes as free enzymes and a thermostable scaffoldin was more active on the cellulosic substrate than the wild-type enzymes. Significantly, "thermostable" designer cellulosomes exhibited a 1.7-fold enhancement in cellulose degradation compared to the action of conventional designer cellulosomes that contain the respective wild-type enzymes. For designer cellulosome formats, the use of the thermostabilized scaffoldin proved critical for enhanced enzymatic performance under conditions of high temperatures.

Biochemical characterization and crystal structure of a novel GH127 β-L-arabinofuranosidase

Rachel Salama[1], Shifra Lansky[2], Ruth Goldschmidt[1], Gil Shoham[2] and Yuval Shoham[1]

[1]Faculty of Biotechnology and Food Engineering, Technion-IIT, Haifa, Israel
[2]Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

The natural degradation of plant biomass is an essential step in the carbon cycle. Plant-based biomass is considered a readily available renewable energy source that can be turned into liquid biofuel without contributing much net CO2 to the atmosphere. Arabinose is one of the monosaccharides composing the plant cell wall. In its β-L-arabinofuranosyl configuration it can be found in structural glycoproteins of the plant cell wall and a terminal form of it can be found in rhamnogalacturonan-II, olive arabinan, tomato arabinoxyloglucan etc.

Geobacillus stearothermophilus, a thermophilic soil bacterium, has an extensive system for the utilization of L-arabinan. The system is composed of five transcriptional units and clustered within a 38-kb DNA segment. One of the transcriptional units encodes for eleven genes. This operon encodes for arabino-oligosaccharides transporter system, two α-L-arabinofuranosidases (Abf51A and Abf51B), and a cluster encodes for an alternative arabinose utilization pathway. The last gene in the operon encodes for GsAra127N, that hydrolyzes β-L-arabinofuranoside residues from both synthetic and natural substrates. The kcat value of GsAra127N towards pNP-β-L-arabinofuranoside in pH 6.5 is 1.5 s-1, Km is 0.57 mM and the calculated value of kcat/ Km is 2.6 s-1 · mM-1. Based on 3D structure of catalytic mutant, E311A, a Zn2+ atom is located near the catalytic site. A single replacment of Cys406 to Ala abolished metal binding and affected catalytic activity, suggesting a metal involvement in the catalytic mechanism.

Understanding GH127's enzymes mode of action is important since they are assumed to play a role in the effective degradation of plant biopolymers as well as glycoproteins.

Alternative σ Facotrs are Involved in the Regulation of Cellulose - Utilization Genes in *Clostridium thermocellum*

Andy Sand[1], Evert K. Holwerda[2], Daniel G. Olson[2], Yakir Nataf[1], Ilya Borovok[3], Abraham L. Sonenshein[4], Edward A. Bayer[5], Raphael Lamed[3], Lee R. Lynd[2], Yuval Shoham[1]

[1] Technion-Israel Institute of Technology, Department of Biotechnology and Food Engineering, Haifa, Israel

[2] Dartmouth College, Thayer School of Engineering, Hanover, New Hampshire, USA

[3] Tel Aviv University, Department of Molecular Microbiology and Biotechnology, Tel Aviv, Israel

[4] Tufts University School of medicine, Department of Molecular Biology and Microbiology, Boston, Massachusetts, USA

[5] Weizmann Institute of Science, Department of Biological Chemistry, Rehovot, Israel

Clostridium thermocellum is a gram positive, anaerobic, thermophilic soil bacterium that secretes a high molecular weight protein complex, the cellulosome, which is capable of hydrolyzing crystalline cellulose. Recently, we identified a set of seven σ and anti- σ factors that plays a role in the regulation of cellulose - utilization genes in *C. thermocellum*. These alternative σ-factors are located upstream to a gene, encoding to a trans-membrane protein (RsgI) with an intracellular anti - σ domain at its N-terminus, and at its C-terminus an extracellular polysaccharide-related function module such as carbohydrate binding modules, family 10 glycoside hydrolase domain and a PA14 module that is known to bind pectin. This arrangement provides a novel regulatory mechanism, in which the expression of the cellulosomal genes can be controlled by the composition of high molecular weight, extracellular polysaccharides. In this study, the transcriptional start sites of the seven σ factors - coding genes were mapped by primer extension analysis and the target regulated genes were found based on promoter sequence similarities. To directly test the involvement of these alternative σ factors in regulating cellulose utilization genes, we disrupted three sigI-rsgI operons and the transcript level of the putative target genes were measures by real-time RT PCR. In all these mutant strains, deletion of the rsgI gene resulted in an increased transcript level of the target cellulose utilization genes and the sigI gene itself. Taken together, these results indicate the vital role of alternative σ factors in mediating between the extracellular polysaccharides and the regulation of the expression level of cellulose utilization genes.

A Specific Oligopeptide Transporter Mediates Quorum-Sensing Regulation of the Extracellular Xylanase Gene in *Geuobacillus Stearothermophilus*

Smadar Shulami[1], Lea Feld[1] ,Abraham L. Sonenshein[2], and Yuval Shoham[1]

[1]Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Haifa, Israel

[2]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, USA

Geobacillus stearothermophilus T-6 possesses a single extracellular xylanase (Xyn10A), capable of producing short, decorated xylo-oligosaccharides from the naturally branched polysaccharide, xylan. The specific activity of the extracellular xylanase increases over 10-fold during early exponential growth, suggesting cell density regulation (quorum sensing). Addition of conditioned medium to low cell density cultures resulted in high expression of *xynA*, indicating that a diffusible extracellular *xynA* density factor (XDF) is present in the medium. XDF is heat-stable, sensitive to proteases and was partially purified using reverse phase liquid chromatography. Based on these results, it is likely that XDF is a small hydrophobic peptide or peptides. Secreted extracellular signaling peptides can be imported to the cell via specific oligopeptide (Opp) transport systems. Based on its genome sequence, G. *stearothermophilus* T-6 possesses a single Opp transport system composed of five genes (oppABCDF). Bialaphos is a toxic tri-peptide that is known to enter bacteria via oligopeptide permeases. We have isolated a bialaphos-resistant mutant of G. *stearothermophilus*, and found that the oppB gene is interrupted by an insertion element and presumably lacks a functional Opp transport system. In this bialaphos-resistant mutant the *xynA* gene does not appear to be regulated by cell density, suggesting that an intact oligopeptide transport system is required for quorum-sensing regulation of the *xynA* gene.

Facilitation of adaptive evolution through genomic amplification of antibiotic resistance genes

Idan Yelin and Roy Kishony

Faculty of Biology, Technion - Israel Institute of Technology, Haifa, Israel

Bacteria can evolve high resistance to antibiotics when grown under continuous selective pressure for only a few days in the lab. This adaptation may advance in several evolutionary paths. While paths involving accumulation of mutations in both target genes and general drug resistance mechanisms has been well characterized, the role of structural changes has received less attention. In particular, it is unknown how early structural changes can dictate adaptive potential. Here, we focus on the evolution of resistance through genomic amplification and find that early transposon translocation facilitates future adaptive amplification of resistance genes. By genotyping multiple clones isolated at different time points during the evolution of resistance to chloramphenicol in a 'morbidostat' setting we reveal newly acquired mutations, gradual copy number changes and new genomic junctions. By measuring the evolvability of clones with different genome architecture we identify a duplication step which facilitates adaptation to higher drug concentration in a rapid and highly directional route through amplification. Our results suggest that early genomic changes can dictate future evolutionary paths and that identification of such motifs may enable prediction of adaptive potential.

Evolution of Self Recognition in Bacteriophage DNA Packaging

Ning Yin, Roy Kishony

Faculty of Biology, Technion - Israel Institute of Technology

Phage terminase is a DNA-binding protein which packages phage genomic DNA into empty capsids to form mature phage particles. Its binding specificity toward a recognition site in phage genome, known as pac or cos site, is critical for phage propagation. Incidentally, in some phages such as phage P22, pac site resides within the coding sequence of the terminase. Such genetic arrangement suggests the possibility of a single mutation which changes both the pac site and the terminase protein, while maintaining the specific interaction between the two. We aim to explore the possibility of this kind of mutations in P22 by conducting experimental evolution selecting for new terminase specificity. To accomplish this, we engineered a P22 amber mutant (P22am) with a stop codon in its terminase without disrupting its pac site. Coinfection experiment of P22am and wild type P22 (P22wt) showed that terminases produced by P22wt package both genomes non-discriminately. Therefore, when we propagated P22wt populations in the presence of a large excess of mutant P22am's, a P22 strain with the aforementioned mutation in the pac site that shifts the terminase specificity will not package P22am genomes and hence has a fitness advantage over its ancestors. We therefore conducted serial transfer of 18 P22wt populations, under high multiplicity of infection in the presence of a large excess of P22am phages, aiming to select for these mutants. Over 21 transfers we saw a continuous increase of the ability of P22wt's to recognize and selectively reproduce themselves over P22am's. From the evolved phage populations, we isolated single clones and are currently phenotyping and genotyping them. In conclusion, we have conducted experimental evolution toward a specificity-shifting mutation in phage P22 terminase. Identification and characterization of mutations like that will elucidate a novel and economical route of evolution toward new protein-DNA binding specificities

Effect of growth-rate and the limiting factor on carbon-utilization genes in Clostridium thermocellum

Tal Zeltzer and Yuval Shoham

Faculty of Biotechnology and Food Engineering, Technion – Israel Institute for Technology, Haifa, Israel

Clostridium thermocellum is an anaerobic, thermophilic, soil bacterium that utilizes crystalline cellulose and ferments the cellodextrins to ethanol. Therefore, it could potentially play a part in a consolidated bioprocess, allowing a relatively low-cost conversion of biomass to biofuel. The hallmark of the cellulose degradation system is the cellulosome, an extracellular, multi-enzyme complex, which can anchor up to 63 catalytic subunits (glycoside hydrolases, GHs) at a time. Works by our group and others revealed that several cellulosomal genes are up-regulated during slow growth rates in continuous cultures under carbon limitation. Yet, it is possible that this phenomenon is derived from the carbon limitation itself and not caused by the growth rate per se. We performed RNA-seq and real-time RT-PCR, on RNA extracted from continuous cultures limited on either carbon or nitrogen, cultivated at growth rates ranging between 0.2 hr^{-1} and 0.05 hr^{-1}. Interestingly, RNA-seq and real-time PCR revealed that the alternative sigma factors *sigIW* and *sigI4* (activated upon binding of cellulose in the extracellular environment to their adjacent RsgI transmembrane protein) are significantly up-regulated (10-30 folds) in slow growth-rates. One of the genes regulated by *sigIW* is celC, a non-cellulosomal GH5, is significantly over-expressed in slow growth rates by 20 folds. The results suggest a novel growth-rate dependent regulation on carbon utilization genes in C. *thermocellum.*

Elucidating the architectural organization and diversity of the multifarious *Pseudobacteroides cellulosolvens* cellulosome system

Olga Zhivin[1], Bareket Dassa[1], Sagar M. Utturkar[2,3], Steven D. Brown[2,3], Bernard Henrissat[4], Ilya Borovok[5], Raphael Lamed[5], Edward A. Bayer[1]

[1] Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel

[2] Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, Tennessee, USA

[3] BioEnergy Science Center, Oak Ridge, Tennessee, USA

[4] Architecture et Fonction des Macromolecules Biologiques, Aix-Marseille University and CNRS, Marseille, France

[5] Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel

Pseudobacteroides cellulosolvens is an anaerobic, mesophilic, cellulolytic, cellulosome-procucing bacterium capable of utilizing cellulose and cellobiose as carbon sources. Recently, we sequenced the P. *cellulosolvens* genome, and subsequent bioinformatic analysis revealed an incredible number of cellulosome-related components, including 78 cohesin modules scattered among 31 scaffoldins and more than 200 dockerin-bearing ORFs. In terms of numbers, this potentially represents the most intricate, compositionally diverse cellulosome system yet known in nature. Surprisingly, in comparison to previously described cellulosome systems, the apparent roles of the P. *cellulosolvens* cohesins are reversed, in that the type II cohesins are located on the enzyme-integrating primary scaffoldin, whereas the type I cohesins are located on the anchoring scaffoldin. Such an organization seems to be unique to P. cellulosolvens. In addition, the dockerin-bearing ORFs include dozens of X60 modules, which are known to stabilize type II cohesin-dockerin interactions, although many of the other type II dockerins lack an adjacent X60 module. In the present work, we focused on revealing the architectural arrangement of the cellulosomal structure in this bacterium by examining numerous interactions between cohesin and dockerin modules. The results revealed two main specificities of the cohesin-dockerin interactions: type I and type II, together with three scaffoldins representing a subtype, provisionally related to type I. Cellulosomal architecture is represented by cell-anchored scaffoldins and cell-free systems. The results provide an understanding of the structure and function of one of the largest cellulosomal system known today. Deep understanding of the interactions among cellulosomal components will enable us to design high-efficiency cellulosomes for conversion of plant-derived cellulosic biomass and improved production of biofuels.

Reconstruction of surface potential from Atomic Friction Measurements

Liron Agmon[1] , Danny Yosufov[1] , Juergen Jopp[2] , Ronen Berkovich[1,2,*]

[1] Department of Chemical-Engineering, Ben-Gurion University, Beer Sheva, Israel

[2] The Ilze Katz Institute for Nanoscience and Technology, Ben-Gurion University, Beer Sheva, Israel

Friction is a phenomenon encountered in everyday life, ranging from the macro- (earthquakes, violin playing, machinery, etc.) to the nano-scales (electronic devices, biological machinery etc.), where two surfaces come into contact and move with respect to each other, resulting with an irreversibly energy dissipation. One of the key features that characterize friction is the underlying interfacial interaction potential. Understanding the role of dissipation energy and surface potential in frictional mechanisms is essential for tribology, nanoscale fabrication, catalysis, adhesion and so on. To get an adequate estimation of the free energy landscape of an experimental system, high quality data is required. This necessity is met in measurements performed with Atomic Force Microscope (AFM). The AFM enables probing nanoscale frictional forces due to its ability to approach the single-asperity level, and measure the dynamical interaction between a cantilever tip and the surface of interest, resulting with atomic stick-slip force pattern. The main hindrances to such measurements, which are capillary forces and contaminants, were successfully resolved by working in Ultra-High-Vacuum. However, an alternative approach to overcome was recently introduced by measuring the frictional interaction between the AFM tip and surfaces in ambient surrounding, which opens a new realm in probing nano-scale forces for systems that are immersed in fluid. However, despite its promising results, Friction Force Microscopy (FFM) in ambient media has been explored to very limited extent, and little is known about its dissipation mechanism. Here we perform FFM experiments of Halite (NaCl mineral) in ethanol and apply the Prandtl-Tomlinson phenomenological framework to estimate the amplitude of the surface energy corrugation, and show how it scales with the applied normal load. From this scaling we can extrapolate the surface potential in the low friction regime.