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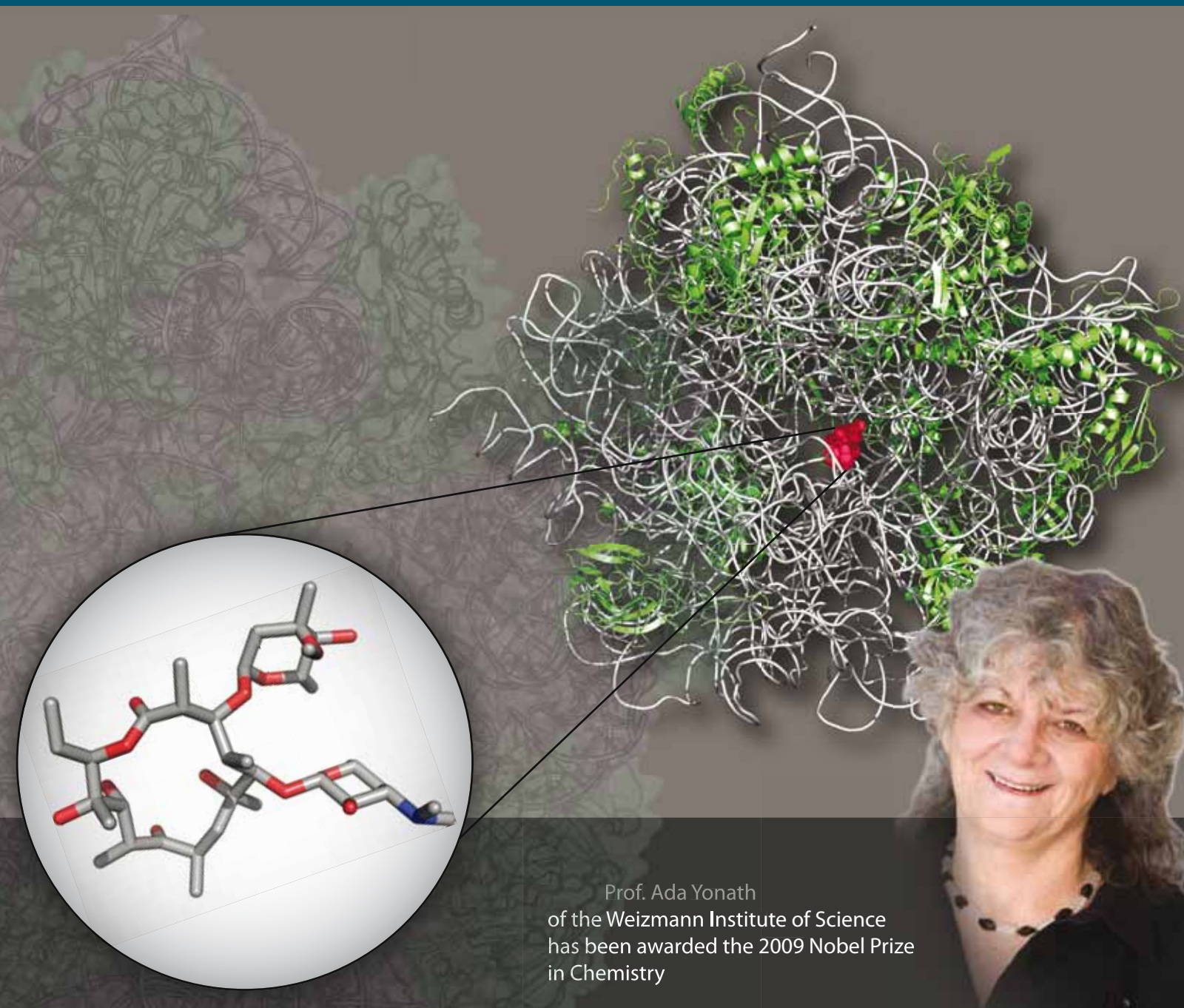
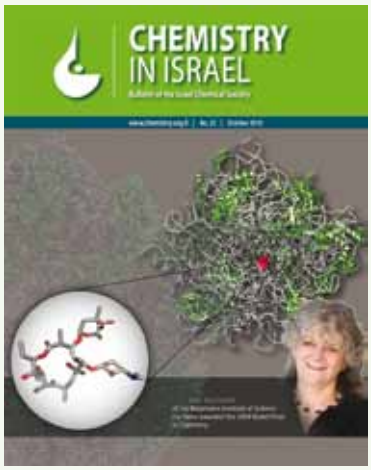


Prof. Ada Yonath
of the Weizmann Institute of Science
has been awarded the 2009 Nobel Prize
in Chemistry



Cover: Prof. Ada Yonath and an image of the ribosome together with the antibiotic molecule, erythromycin. The image, adopted from Schluenzen et al. Nature 413, 814 (2001), shows a view into the tunnel of the large ribosomal subunit from which the newly constructed chains of amino acids exit. The ribosomal proteins are shown in green, the ribosomal RNA in white and erythromycin in red. The detailed molecular structure of erythromycin is shown in the white circle.

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Letter from the president

Prof. Ehud Keinan
President of the Israel Chemical Society

Dear Colleague,

The General Assembly of the United Nations has announced 2011 as the "International Year of Chemistry" (IYC2011). Like all other chemical societies around the world, we are preparing for 2011 with special programs and public events to celebrate major achievements of the chemical sciences and their significant contributions to knowledge, medicine, environmental protection, economic development and the well-being of humankind. Prof. Avi Hofstein, of the Department of Science Teaching at the Weizmann Institute of Science, is the Chairman of our IYC2011 Committee. The Committee's main goals are to gain public awareness and appreciation of the chemical sciences, attract the younger generation to chemistry, and bolster the image of the chemical industry. Prof. Hofstein and myself would appreciate comments, ideas, documents and any other materials, which could contribute to the IYC2011. Please contact either one of us directly: avi.hofstein@weizmann.ac.il; keinan@technion.ac.il.

I am happy to announce that, among the other projects planned for next year, the Israel Philatelic Service will issue two new stamps to mark the event. The stamps, which were designed according to our proposal, will commemorate the two Nobel Prizes in chemistry awarded to Israeli scientists.

IYC2011 will also highlight the great benefits of international scientific collaboration, interdisciplinary research, and partnership between academic and industrial research. In this spirit, the ICS and the Israel Analytical Chemistry Society (IACS) have joined forces and put together an unprecedented, continuous 4-day event that will take place on February 7-10, 2011 at the David Intercontinental Hotel, Tel Aviv. This joint endeavor will comprise two consecutive scientific events: the 14th Isranalytica (February 7th-8th) and the 76th Annual Meeting of the ICS (February 9th-10th). Not only will this event include the largest-ever exhibition of scientific equipment and instrumentation, it will also provide unique opportunities for synergism and collaboration.

Following the tradition of inviting delegations of world-renowned scientists to our annual meetings, this year we shall host a team from Academia Sinica of Taiwan. The arrival of these Taiwanese scientists, as well as the Japanese delegation we hosted last year, reflect the rapidly growing importance of Asia on the world map of science and technology. The visit of this highly respected delegation will further enhance the already strong ties between the scientific communities of Taiwan and Israel.

Another exciting development is the augmentation and new format of the *Israel Journal of Chemistry*. As of 2010, the *IJC* has become the official journal of the ICS and it is being published by Wiley-VCH. This year we celebrate the publication of its 50th volume with the first issue being devoted to ribosome chemistry and honoring the 2009 Nobel Laureates in chemistry. This international, peer-reviewed journal publishes 12 topical issues a year, each of which is edited by Guest Editors and primarily contains invited review articles. The journal's Editorial Board consists of 13 Israeli chemists, and of the International Advisory Board's 30 prestigious members, 18 are Nobel Laureates.

Finally, I wish to thank Prof. Mati Fridkin for continuously strengthening the international stature of Chemistry in Israel. I thank the authors who have contributed articles to this issue and I encourage all of you to contribute future articles to this journal.

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המודעה מיועדת לנשים וגברים כאחד

Letter from the editor

Prof. Matityahu Fridkin
Editor-in-chief

The Bulletin of the Israel Chemical Society extend its heartiest and warmest congratulations to Prof. Ada Yonath of the Department of Structural Biology of the Weizmann Institute of Science, Laureate of the 2009 Nobel Prize in Chemistry. The Prize was awarded for her outstanding achievements in deciphering the secrets of the ribosomal "machinery", one of the most intricate and mysterious systems in biology. The relevant press release of the Weizmann Institute of Science is included in this issue.

The continuous increase in antibiotic resistance among pathogenic bacterial strains poses a major health threat. The design and development of novel advanced drugs is thus urgently needed. Since the ribosome represents a common drug target of many antibiotic drugs, understanding its structure has immediate consequences on drug discovery. The article by Dr. Anat Bashan, Prof. Ada Yonath and colleagues summarizes the relevant implications.

The major problem with the currently used anti-HIV drugs is the high viral mutations, which results in the emergence of drug-resistant virus strains. To cope with this problem it is important to identify new targets and new approaches for drug design. The article of Dr. Zvi Hayouka, Prof. Assaf Friedler and colleagues suggests a novel concept towards these goals.

The delicate balance between basic academic research, in particular in its early stages, and the industrial R&D, is discussed in a "case study" by Dr. Revital Green.

The highlights of the 75th ICS meeting, held in February at the David Intercontinental Hotel in Tel-Aviv, are outlined by Profs Ehud Keinan, Doron Shabat and Shmuel Carmeli, Chairman of the Organizing Committee.

Profs. Lia Addadi and Steve Weiner, winners of the 2009 ICS Prize of Excellence, summarize their studies in an article "On amorphons and crystalline phases in biomeneralization – one hundred years to answer a question".

Prof. Hanoch Senderowitz and Drs. Galia Blum and Micha Fridman provide highlights of the 8th Meeting of the Medicinal Chemistry Section of the Israel Chemical Society, held in March 16th 2010 in the Weizmann Institute of Science.

Dr. Bob Weintraub, Director of the Libraries at the Sami Shamoon College of Engineering, brings here a fabulous story on the Zionist Charles Dreyfus and his activities in the field of Yellow Dye.

We intend to publish the next issue of Chemistry in Israel early next year, which will be the International Year of Chemistry. We wish to thank very much all authors of this issue and those who will contribute to the future issues of this important bulletin.

Prof. Ada Yonath of the Weizmann Institute of Science has been awarded the 2009 Nobel Prize in Chemistry

The Weizmann Institute of Science congratulates Prof. Ada Yonath on receiving the 2009 Nobel Prize in chemistry and is proud of her scientific achievements. We are delighted that the Nobel Prize committee has recognized the significance of Prof. Ada Yonath's scientific research and awarded her this important prize.

Prof. Yonath's research is driven by curiosity and ambition to better understand the world and our place within it. This research aims high: to understand one of the most complicated "machines" of the biological system.

In the late 1970s, Prof. Yonath decided, when she was a young student at the Weizmann Institute, to take on the challenge of answering one of the key questions concerning the activities of live cells: to decipher the structure and mechanism of action of ribosomes – the cell's protein factories. This was the beginning of a long scientific journey that has lasted decades, and which required courage and devotion from the start. The journey began in a modest laboratory with a modest budget, and with the years, increased to tens of researchers under the guidance of Prof. Yonath.

This basic research, which began in the attempt to understand one of the principles of nature, eventually led to the understanding of how a number of antibiotics function, something that is likely to aid in the development of more advanced and effective antibiotics. This discovery will hopefully also help in the struggle against antibiotic-resistant bacteria, a problem recognized as one of the most central medical challenges of the 21st century.

Prof. Yonath can be considered a model of scientific vision, courage in choosing a significant scientific question, and devotion in realizing the goal to its end – which will hopefully broaden knowledge for the benefit of humanity.

Beyond the Basics

"People called me a dreamer," says Prof. Ada Yonath of the Structural Biology Department, recalling her decision to undertake research on ribosomes – the cell's protein factories. Solving the ribosome's structure would give scientists unprecedented insight into how the genetic code is translated into proteins; by the late 1970s, however, top scientific teams around the world had already tried and failed to get these complex structures of protein and RNA to take on a crystalline form that could be studied. Dreamer or not, it was hard work that brought results: Yonath and colleagues made a staggering 25,000 attempts before they succeeded in creating the first ribosome crystals, in 1980.

And their work was just beginning. Over the next 20 years, Yonath and her colleagues would continue to improve their technique. In 2000, teams at Weizmann and the Max Planck Institute in Hamburg, Germany – both headed by Yonath – solved, for the first time, the complete spatial structure of both subunits of a bacterial ribosome. Science magazine counted this achievement among the ten most important scientific developments of that year. The next year, Yonath's teams revealed exactly how certain antibiotics are able to eliminate pathogenic bacteria by binding to their ribosomes, preventing them from producing crucial proteins.

Yonath's studies, which have stimulated intensive research worldwide, have now gone beyond the basic structure. She has revealed in detail how the genetic information is decoded, how the ribosome's inherent flexibility contributes to antibiotic selectivity and the secrets of cross-resistance to various antibiotic families. Her findings are crucial for developing advanced antibiotics.

Prof. Ada Yonath's research is supported by the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly. Prof. Yonath is the Martin S. and Helen Kimmel Professor of Structural Biology.

The ribosome as drug target: lessons from 3D structures

Anat Bashan, Ella Zimmerman, Mattew Belousoff, Haim Rozenberg ,Chen Davidovich, Itai Wekselman, Tal Shapira, Miri Krupkin and Ada Yonath

Dr. Anat Bashan earned her B.Sc. degree in chemistry at the Hebrew University, Jerusalem in 1987, and received her M.Sc.(1989) and Ph.D. (1995) degrees at the Department of Structural Biology of the Weizmann Institute of Science, Israel. Between 1995 and 1998 she was a postdoctoral fellow at the Weizmann Institute, and since then she has been a staff scientist in the group of Prof. Ada Yonath at the Department of Structural Biology, Weizmann Institute. Her research activities are focused on structure and function of ribosomal components and the mode of action of ribosomal antibiotics.

Dr. Ella Zimmerman earned her B.Sc. degree in chemistry at Tel Aviv University in 1991, and received her M.Sc. (1995) and Ph.D. (2002) degrees at the Department of Structural Biology of the Weizmann Institute for Science, Israel. Between 2002 and 2006 she was a postdoctoral fellow at the Weizmann Institute, and since then she has been a staff scientist in the group of Prof. Ada Yonath at the Department of Structural Biology, Weizmann Institute. Her research activities are focused on structure and function of ribosomal components and the mode of action of ribosomal antibiotics, and in developing delicate methods for analyzing biological structures by electron microscopy.

Dr. Matthew Belousoff earned his B.Sc (Hons) in Chemistry/ Mathematics at Monash University (Australia) where he completed his Ph.D. studies at the School of Chemistry in 2008. Currently he is a post-doctoral fellow at the Weizmann Institute in the group of Prof. Ada Yonath working on synergistic pairs of ribosomal interfering antibiotics.

Mr. Chen Davidovich is a Ph.D. student in the research group of Prof. Ada Yonath, at the Weizmann Institute of Science, studying ribosomal antibiotics and resistance to them, and ribosome evolution. He earned his B.Sc. degree in Biotechnology Engineering at Ben-Gurion University in 2004. Currently he is an Adams Fellow of the Israel Academy of Sciences and Humanities.

Mr. Itai Wekselman is a Ph.D. student in the research group of Prof. Ada Yonath, at the Weizmann Institute of Science, studying the resistance to antibiotics targeting the ribosome. He earned his B.Sc. degree in Biology at Tel- Aviv University in 2005 and his M.Sc. in Chemistry at the Weizmann Institute of Science, in 2008.

Dr. Haim Rozenberg earned his B.Sc. (1988) and M.Sc. (1989) degrees in physics and DEA (1990) of Molecular Biophysics at the Universitée de Jussieu, Paris, France. In 1999, he received his Ph.D. degree at the Department of Structural Biology of the Weizmann Institute of Science, Israel. From 1999-2001, he was a postdoctoral fellow at the X-ray Crystallography Laboratory of the Weizmann Institute. Since then, he has been a staff scientist in the groups of Prof. A. Yonath and Z. Shakked at the Department of Structural Biology, Weizmann Institute of Science. His research activities are focused on ribosome crystals handling and data collection with Prof. Yonath and on structure and function of the tumor-suppressor protein p53 and its complexes with DNA with Prof. Shakked.

Ms. Miri Krupkin received a B.Sc. in Chemistry from Bar-Ilan University in 2008. She is currently working on her M.Sc. thesis in the lab of professor Ada Yonath at the Weizmann Institute of Science. She is studying the structure and function of ribosomal particles, the mechanisms of ribosomal antibiotics and the origin of the contemporary ribosome.

Dr. Ada Yonath is a structural biologist who in using X-ray crystallography. She earned her B.Sc. (1962) and M.Sc. (1964) degrees in chemistry at the Hebrew University in Jerusalem and her Ph.D. (1968) at the Weizmann Institute of Science. She conducted her postdoctoral studies at Carnegie Mellon University and at the Massachusetts Institute of Technology and in 1970 she established the first protein crystallography laboratory in Israel. She pioneered ribosomal crystallography in 1980 and together with her coworkers, some of which are coauthors of this manuscript, determined the high resolution structure of the small ribosomal subunit from Thermus thermophilus (2000) and of the large ribosomal subunit from Deinococcus radiodurans (2001). She is the Martin S. and Helen Kimmel Professor of Structural Biology at the Weizmann Inst. of Science, the Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly, a member of the Israeli, the European and the US National Academies of Sciences, and a 2009 Nobel Prize Laureate in Chemistry.

Mr. Tal shapira is a M.Sc. Student at the department of structural biology at the Weizmann Institute. He earned his B.Sc. in chemistry and biology from Tel-Aviv University. He is currently focusing on studies of ribosomal antibiotics, and crystallization of antibiotics-ribosome complexes.

ABSTRACT

The increase in antibiotic resistance among pathogenic bacterial strains poses a significant health threat. Therefore, improvement of existing antibiotics and the design of advanced drugs are urgently needed. The ribosome is the drug target for many antibiotic families. Antibiotics bind at functionally active centers, some of which are highly conserved. In several cases the ribosome utilizes its inherent functional flexibility to trigger induced fit mechanisms by remote interactions, facilitating antibiotic synergism as well as reshaping improper binding pockets. These interactions lead to antibiotic selectivity even for antibiotics that bind to conserved functional regions, as less conserved nucleotides reside in proximity to the binding pocket. Exploitation of the diverse properties of antibiotics binding and benefiting from the detailed structural information that keeps emerging, should result in significant improvement of current antibiotic treatment.

Department of Structural Biology, Weizmann Inst., 76100 Rehovot, Israel.

Introduction

Ribosomes, the universal cellular riboprotein assemblies, are the nano-machines which translate the genetic code into proteins. The translation process requires a complex apparatus composed of many components. Among them the ribosome is the key player, as it provides the framework for the proper positioning of all other components participating in the peptide bond and nascent chain elongation. Ribosomes operate continuously, in each living cell, since the constant programmed cell death implies constant proteins degradation and requires simultaneous production of proteins. Hundreds of thousands of ribosomes are present in typical mammalian cells. Fast replicating cells, e.g. liver cells, may contain a few millions ribosomes. Even bacterial cells may contain up to 100,000 ribosomes during their log period.

Within the framework of living cells, ribosomes are giant assemblies, composed of many different proteins (r-proteins) and long ribosomal RNA (rRNA) chains. The ratio of rRNA to r-proteins (~2:1) is maintained throughout evolution, with the exception of mammalian mitochondrial ribosome (mitoribosome) where almost half of the bacterial rRNA is replaced by r-proteins (consequently in mitoribosome the ratio of RNA to proteins is ~1/1). All ribosomes are composed of two unequal subunits (Table 1). In prokaryotes, the small subunit, denoted as 30S, contains an RNA chain (16S) of about 1500 nucleotides and 20–21 different proteins, whereas the large subunit (called 50S in prokaryotes) has two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and 31–35 different proteins (see more details in Table 1). In all organisms the two subunits exist independently and associate only for forming functionally active ribosomes.

The process of mRNA-encoded protein synthesis requires a complex apparatus composed of the ribosome, transfer RNA molecules (tRNA) and accessory protein factors. The mRNA chains are produced by the transcription of the segments of the DNA that should be translated. The mRNA chains carry the genetic information to the ribosomes, while tRNA molecules deliver the cognate amino acids. For increasing efficiency, a large number of ribosomes act simultaneously on a single transcript as polymerases synthesizing proteins by one-at-a-time addition of amino acids to a growing peptide chain. While translocating along the mRNA template, at ribosomes produce proteins on a continuous basis an incredible rate (namely >15 new peptide bonds per second in prokaryotes and 2-5 new peptide bonds per second in eukaryotes). While the elongation of the nascent chain proceeds, the two subunits perform cooperatively. The small subunit provides the path along which the mRNA progresses, the decoding center and the mechanism controlling translation fidelity while the large subunit provides the site for the main ribosomal catalytic function, polymerization of the amino acids and the protein exit tunnel (Figure 1).

The recent availability of the over two dozens of crystal structures of bacterial ribosome and their complexes (see below) have enabled a quantum leap in the understanding of the machinery of protein biosynthesis. These structures have shown that in each of the two subunits the ribosomal proteins are entangled within the complex rRNA conformation, thus maintaining a striking dynamic architecture that is ingeniously designed for their functions: precise decoding; substrate mediated peptide-bond formation and efficient polymerase activity. This review will focus on the ribosome as

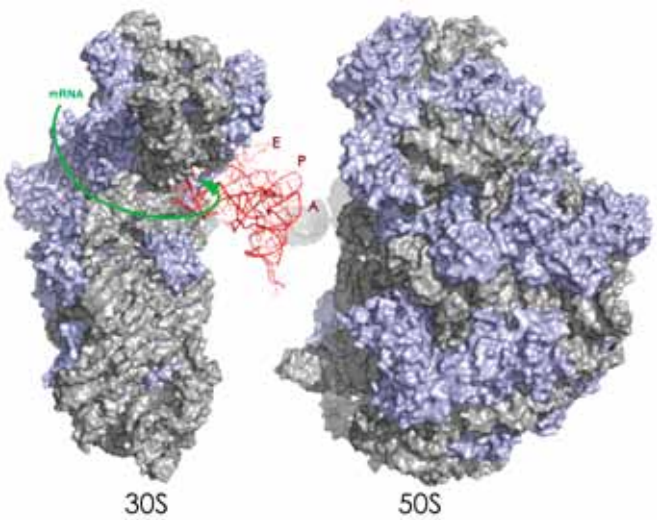

Figure1: The interface view of the crystal structures of the two ribosomal subunits, 50S from D. radiodurans and 30S from T. thermophilus. rRNA is colored grey and r-proteins are colored light blue. A, P and E site tRNA molecules as bound to the T70S subunit (PDB code 2WDM) were superimposed on the D50S structure and mRNA path is marked.

a target for antibiotics and will attempt to answer the question: "Can structures lead to improved antibiotics?"

Ribosome structure can lead to improved antibiotics

The intensive research on ribosomes has some practical aspects; one of them has clinical relevance. Owing to its key role in life cycle, namely in producing the proteins required for vitality, the ribosome is one of the major targets in the cell for antibiotics. The increasing incidence of antibiotic resistance and toxicity creates serious problems in modern medicine; combating resistance to antibiotics has been a major clinical concern in recent years. The vast amount of structural data on ribosomal antibiotics accumulated recently may supply critical information to overcome current barriers faced by antibiotics.

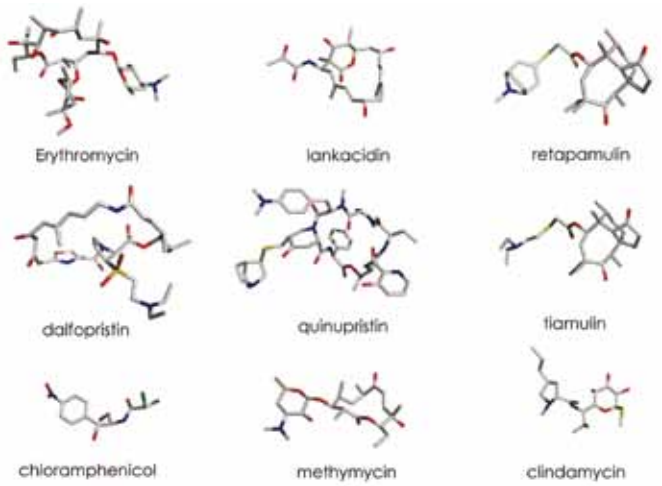

Figure 2: 3D structure of Antibiotic molecules that inhibit the large ribosomal subunit activity, represented in CPK colors.

Antibiotics are compounds used in clinical medicine for treating bacterial infections selectively by inhibiting the biological function of the bacterial ribosomes and not that of the host (Figure 2). More than 40% of the useful antibiotics interfere with the biosynthetic machinery and most of them target the ribosomes at distinct locations within functionally relevant sites. These act by diverse mechanism, many of them were revealed by analysis of crystallographic results or structural data based biochemical studies (for review see Auerbach *et al.*, 2004; Yonath and Bashan 2004; Yonath, 2005a; Tenson and Mankin, 2006; Poehlsgaard and Douthwaite, 2005; Bottger, 2006; 2007; Mankin, 2001, 2006, 2008). Numerous structural, biochemical and genetic studies provided indispensable information that illustrated the basic mechanisms of ribosomal antibiotics activity and synergism; provided the structural basis for antibiotic resistance and enlightened the principles of antibiotic selectivity, namely the discrimination between the pathogens and host, the key for therapeutical usefulness.

By its nature, X-ray crystallography should be the method of choice for investigating ribosome-antibiotics interactions. However, since X-ray crystallography requires diffracting crystals, and since so far no ribosomes from pathogenic bacteria have been crystallized, currently the crystallographic studies of antibiotics that bind the large ribosomal subunit are confined to the currently available crystals of suitable pathogen models. Currently available are high-resolution structures of antibiotic complexes with the whole ribosome or the small ribosomal subunits from the eubacterium *Thermus thermophilus* and of the large ribosomal subunits from eubacterium *Deinococcus radiodurans* (D50S), two species the ribosomes of which are suitable to serve as pathogen models. On the other hand, complexes of antibiotics with the large ribosomal subunit from the Dead Sea archaeon *Haloarcula marismortui* (H50S) that resembles eukaryotes in respect to antibiotics binding site, have been obtained.

The structures of the large ribosomal subunit D50S complexed with various antibiotics determined so far (Schluenzen *et al.*, 2001, 2004; Berisio *et al.*, 2003a and b; Auerbach *et al*., 2004; Yonath and Bashan 2004; Harms *et al.*, 2004; Pyetan *et al*., 2007; Yonath, 2005; Davidovich *et al*., 2007, 2008; Vazquez-Laslop *et al.*, 2008; Auerbach *et al*., 2009 ,2010) revealed common traits: binding sites that are composed primarily of rRNA and coincide with functionally critical centers of the ribosome. Furthermore, comparisons between these structures demonstrated that members of antibiotic families possessing common chemical elements with minute differences might bind to ribosomal pockets in significantly different modes, and that the nature of seemingly identical mechanisms of drug resistance may be dominated by the antibiotics' chemical properties. Among the modes of action of ribosomal antibiotics, are interfering with substrate binding at the PTC (Figure

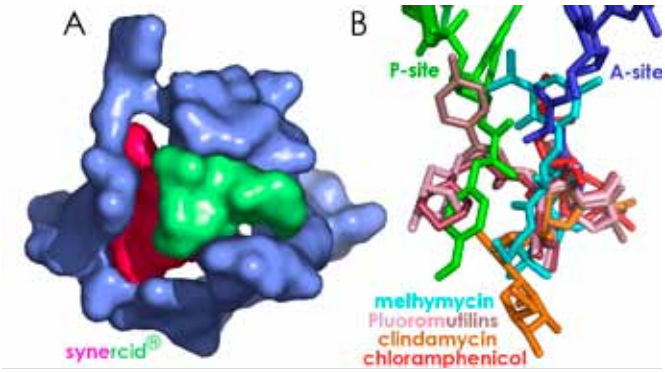

Figure 3: Antibiotics blocking the nascent peptide tunnel entrance and the PTC. **A.** The clinically used drug synercid is blocking both the PTC and the tunnel entrance using its two componants: quinupristin (in green) and dalfopristin (in pink). **B.** antibiotic molecules bound at the PTC are blocking the binding site of the A and/or P-site tRNA substrates.

3B), or blocking the protein exit tunnel (Figure 3A).

A major issue concerning the clinical usefulness of ribosomal antibiotics is their selectivity; their capabilities in the discrimination between the ribosomes of the eubacterial pathogens and those of eukaryotes. As mentioned above, although prokaryotic and eukaryotic ribosomes differ in size (~ 2.4 and 4 Mega daltons, respectively), their functional regions, which are the targets for the antibiotics, are highly conserved. Therefore the imperative distinction between eubacterial pathogens and mammals, the key for antibiotic usefulness, is achieved generally, albeit not exclusively, by subtle structural difference within the antibiotics binding pockets of the prokaryotic and eukaryotic ribosomes. A striking example for discrimination between pathogens and humans is the immense influence of the minute difference in at the rRNA position 2058 where an adenine of the eubacteria is replaced by a guanine in eukaryotes. This small difference was found to govern the binding of macrolides, a prominent antibiotics family (Lai and Weisblum, 1971) that obstructs the progression of the nascent proteins within the tunnel. However, although 2058 identity determines the antibiotic affinity, this analysis showed that the mere binding of the antibiotics is not sufficient for obtaining efficient therapeutical effectiveness. Comparisons between crystal structures of antibiotics bound to the eubacterial large ribosomal subunit, D50S to structures of complexes of the large ribosomal subunit from H50S, an archaeon sharing properties with eukaryotes, which required either extremely high antibiotics concentrations (Hansen *et al*., 2002a, 2003) or G2058A mutations, to facilitates macrolides/ketolides binding (Tu *et al*., 2005), indicated the significance of additional structural elements (Yonath and Bashan, 2004) of the binding pocket, which dictate inhibitory activity. Similar observations were made by mutagenesis in the yeast Saccharomyces cerevisiae at a position equivalent to *E. coli* A2058, which allows erythromycin binding but does not confer erythromycin susceptibility (Bommakanti *et al*., 2008).

The fine details of binding, resistance and selectivity of other members of the macrolide family and its offsprings, the azalides and ketolides, addresses additional issues, such as the sequence specificity that determines the susceptibility and the fitness cost of the ketolides (Pfister, *et al*., 2005). Another intriguing issue, which led to continuous expansion of research and to new insights, relates to the nature of the contributions of two ribosomal proteins, namely L4 and L22, to macrolides resistance. These two proteins line the exit tunnel at its constriction, and do not interact directly with most of the members of the macrolides family, yet several types of mutations at their tip acquire resistance to them (Gregory and Dahlberg, 1999; Davydova et al., 2002; Lawrence et al., 2008; Berisio *et al*., 2006; Zaman *et al*., 2007; Moore and Sauer, 2008), presumably by perturbing the rRNA structure at the tunnel walls (Gregory and Dahlberg, 1999; Lawrence *et al*., 2008).

Attempts aimed at alleviating the resistance problem include the development of synergetic antibiotics. An example is the very potent family of the streptogramins, a two-component antibiotics drug family, each of which is a rather weak drug. The impressive synergetic effect of this family can be understood by examining the mechanism exploited by the rather recent antibiotic drug, Synercid® (that is composed of 2 componants; dalfopristin and quinupristin). This mechanism is based on the binding of one of the components to the PTC that causes a dramatic alteration in the orientation of the very flexible nucleotide, U2585, which plays a principal role in the A- to P-site rotatory motion, and the fixation of the altered orientation by the second compound that binds at the tunnel entrance (Harms *et al*., 2004;

Yonath and Bashan, 2004) (Fig 2a). Another pair of new potential synergistic drug was investigated lately in our laboratory. Upon study of the structure of lanakacidin, an antibiotic that is produced by *Streptomyces rochei*, it was found to bind the PTC at the large ribosomal subunit (Auerbach *et al*., 2010). This same species also produces lankamycin, a macrolide that we recently showed is a weak protein synthesis inhibitor that seems to bind at the tunnel entrance. Biochemical work showed that there is a synergistic effect between the two drugs and crystallographic work on this new synergistic pair is underway. We anticipate that this crystallographic work will guide improved structure-based drug design to achieve a more effective antibiotic synergism.

Even subtle differences, such as the identity of nucleotide 2058 (A in eubacteria, G elsewhere) hardly exist in the PTC. Therefore, obtaining selectivity in antibiotics binding to the PTC, the core catalytic center of the ribosome, is more complex. Nevertheless, some of the antibiotics bind to the PTC of eubacterial ribosomes with high affinity and great specificity, without significant effect on the eukaryotic hosts. The crystal structures of ribosomal complexes with antibiotics indicated that the PTC provides binding sites to several clinically useful antibiotics. This super family of PTC antibiotics can shed light on general as well as specific properties of the interactions of the members of this family with their binding pockets in the PTC. This super family includes phenicols, lincosamides, pleuromutilins, streptograminsA, oxazolidinones and lankacidins. Although basically all PTC antibiotics act by blocking part or the entire PTC, they utilize different binding modes and consequently they possess various inhibitory mechanisms. Thus, chloramphenicol was found to hamper the binding of the A-site tRNA, like (Schluenzen *et al*., 2001), the pleuromutilins, linezolid and streptograminsA occupy both the A- and the P-site tRNAs (Harms *et al*., 2004; Hansen *et al*., 2003; Ippolito *et al*., 2008), and clindamycin, methymycin and lankacidin interfere with the peptide bond formation (Schluenzen *et al*., 2001, Auerbach *et al.* 2009, 2010).

Contribution of several PTC flexible nucleotides to productive binding was also observed by investigating the mode of action of the pleuromutilin family, which revealed a unique inhibitory mechanism alongside novel selectivity and resistance strategies. In particular, the elaborate pleuromutilins binding mode demonstrates how selectivity and resistance are acquired despite almost full conservation (Davidovich *et al*., 2007, 2008; Schluenzen *et al*., 2004). As all nucleotides in the immediate vicinity of the binding site are highly conserved, pleuromutilins selectivity is determined by nucleotides that are not located in the immediate vicinity of the antibiotic binding site, hence are less conserved. Thus, pleuromutilins binding triggers an induced-fit mechanism by exploiting the flexibility of the rRNA nucleotides residing in and around the PTC, as well as a network of interactions with less conserved remote nucleotides, hence allowing for drug selectivity (Davidovich *et al*., 2007; 2008). A key player in this mechanism is nucleotide 2504 that defines part of the binding surface and was observed in different conformations in crystal structures of large ribosomal particles from bacteria and archaea. This results in different network of interactions between this nucleotide and less conserved nucleotides that vary between eubacteria to archaea and eukaryotes. This mechanism for selectivity that was first observed for pleuromutilin antibiotics (Davidovich *et al*., 2007), suggested to determine selectivity to other PTC antibiotics based on a comparative study (Davidovich *et al*., 2008) and was later reconfirmed by crystal structures of the archaeal large ribosomal subunit with PTC antibiotics (Gurel *et al*., 2009). In particular, this family exploits the remote interactions that affect the positioning of the extremely flexible nucleotide U2506, as well as of U2585 that participate in navigating and anchoring the rotatory motion of the tRNA 3' end from A to P-site. These interactions partially evacuate the binding region and at the same time tighten the binding pocket on the bound antibiotic molecule. As mutations within the PTC should be lethal, resistance to pleuromutilins requires mutations or modifications of nucleotides residing either in PTC components with identity that is less crucial for ribosome function, or in the PTC environs rather than within the core of the binding pocket, therefore should occur in a relatively slow pace. Remarkably, these crystallographic studies led the way in attempts to produce advanced compounds (Lolk *et al*., 2008). Indeed, cross resistance was detected between all PTC antibiotics, regardless of their mode of binding, and the nucleotides mediating it are residing only on one side of the PTC, similar or in close proximity to those acquiring resistance to the pleuromutilins.

Attempts to overcome antibiotics resistance and increase their selectivity are going on currently (e.g. Yassin *et al*., 2005; Wilson *et al*., 2005; Bottger, 2007). These studies exploit several strategies, including the insertions of moieties that should compensate for the lost interactions of the resistant strains, benefiting from synergism of known or novel compounds possessing inhibitory properties of various levels of potency, and reviving "forgotten" antibiotics families (such as the lankacidin) (Auerbach et al., 2010). Furthermore, most eubacteria belonging to specific families utilize similar structural principles for selectivity and resistance, comprehending the factors allowing for selectivity should provide powerful tools to understand many of the mechanisms exploited for acquiring resistance. Therefore, the lessons learned from ribosome crystallography concerning combating resistance to antibiotics targeting the ribosome, are rather optimistic, as these studies have opened new paths for antibiotics improvement.

There are many revelations pertinent to antibiotic drug development that high resolution structures have provided. Firstly there is significant variability (even between drugs of the same family) of their binding modes. Secondly, there are common as well as species unique nucleobases that confer resistance. Thirdly, remote interactions in rRNA are responsible for certain induced fit binding, enabling species discrimination (even in highly conserved regions of rRNA). Combined with the identification of deleterious mutations in rRNA, there is considerable scope to exploit this knowledge for structural improvement of existing compounds as well as yielding exciting prospects for de novo drug design.

Table 1: Ribosome composition and available crystal structures

Prokaryotic ribosome: Sedimentation coefficient: 70S
small subunit: 30S
One rRNA molecule (16S with ~1600 nucleotides)
~ 20 different proteins, called S1-S21

large subunit: 50S
Two rRNA molecules (5S and 23S, with 120 and 2900 nucleotides, respectively)
~ 30 different proteins, called L1-L31, among which only L7/L12 is present in more than a single copy

Eukaryotic ribosomes: Sedimentation coefficient: 80S
small subunit: 40S
One rRNA molecule (18S with 1,900 nucleotides)
~ 30 different proteins, called S1-S33

large subunit: 60S
Three rRNA molecules (5S, 5.8S and 28S, with ~120, 160 and 4,700 nucleotides, respectively)
~ 50 different proteins, called L1-L50

Acknowledgments

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The currently available crystal structures are of native and complexed ribosomal subunits from two eubacteria and one archaeon with substrate-analogs or inhibitors (including antibiotics). These are the small ribosomal subunit from *Thermus thermophilus* (called T30S), the large subunit from *Deinococcus radiodurans* (called D50S), and the large ribosomal subunit of the archaeon *Haloarcula marismortui* (called H50S). Also available are structures of assembled ribosome complexed with their substrates from *Thermus thermophilus* (called T70S) and of empty ribosomes from *E. coli* (called E70S).

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Peptide inhibitors of the HIV-1 integrase protein

Zvi Hayouka, Aviad Levin, Abraham Loyter and Assaf Friedler

Prof. Assaf Friedler was born in Haifa, Israel, in 1971. He did his undergraduate studies in chemistry at the Hebrew University of Jerusalem, and Ph.D studies in chemistry at the Hebrew University of Jerusalem under the supervision of Prof. Chaim Gilon, in peptide chemistry and medicinal chemistry. After receiving his PhD degree, in 2000, Prof. Friedler moved to Cambridge, UK, to do his post-doctoral research at the MRC centre for protein engineering in the lab of Prof. Sir Alan Fersht, in the field of biophysical studies of protein-protein interactions. The major achievement of the post-doc was development of peptides that refold and reactivate mutants of the tumor suppressor p53. Since 2004 Prof. Friedler runs his independent research group at the institute of chemistry in the Hebrew University of Jerusalem, Israel (http://chem.ch.huji.ac.il/~assaf/). Since October 2010 he serves as the head of the school of chemistry at the Hebrew university. His major research interests are using peptides to study protein-protein interactions in health and disease, and developing peptides as drugs that modulate these interactions. Specifically, studies are focused on biological systems related to AIDS and cancer, for example: (1) development of peptide inhibitors of the HIV-1 integrase protein; (2) mapping the interaction networks of apoptosis-related proteins; (3) modulating protein oligomerization using peptides. Prof. Friedler recently won the prestigious starting grant from the ERC (European Research Council) as well as the outstanding young scientist prize by the Israeli Chemical Society.

Zvi Hayouka earned his B.sc degree in chemistry and biology at Hebrew University of Jeruslam, at 2004. In 2005, Zvi performed his M.Sc. study in the laboratories of Prof. Tuvia Sheradsky, Dept. of Organic Chemistry, and Prof. Zvi Selinger, Dept. of Biological Chemistry, The Hebrew University of Jerusalem, on the topic of Restoring the GTPase activation of mutant protein Ras. In 2010, Zvi has completed his Ph.D studies at the Institute of Chemistry, the Hebrew University of Jerusalem, in the laboratory of Prof. Assaf Friedler in collaboration with Prof. Abraham Loyter from the Dept. of Biological Chemistry. His research topic was the development of HIV-1 Integrase inhibitors, as described in the current article. Zvi has been awarded several prizes during his Ph.D studies, including the Dimitris Chorafas prize for excellent PhD students, the Chorev Award for excellent PhD students from the Israeli Medicinal Chemistry Society, and the Levine-Jortner Award for excellent PhD students from the Israeli Chemical Society.

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ABSTRACT

The HIV-1 Integrase protein (IN) mediates the integration of the viral cDNA into the host genome and is an emerging target for anti-HIV drug design. Our research in the past few years focused on the development of peptide inhibitors of IN. We designed such inhibitors using two approaches: (1) Rational design based on protein-protein interactions of IN: Peptide sequences derived from the IN-binding sites of IN-binding proteins are already optimized by nature to bind IN and have the potential to inhibit it. We designed two peptides derived from the IN-binding loops of the cellular binding partner of IN, LEDGF/p75 (LEDGF 361-370 and LEDGF 401-413) and two peptides derived from the HIV-1 Rev protein, which we found to bind IN; (2) Selection of a peptide, termed IN-1, using combinatorial library screening. All five peptides bound IN with low micromolar affinity in a cooperative mechanism as indicated by Hill coefficients around 4. The peptides inhibited the DNA-binding of IN as well as its enzymatic activity *in vitro*. The five selected peptides shifted the IN oligomerization equilibrium from the dimer towards the tetramer. According to these findings, we have proposed a new approach for inhibiting proteins by "shiftides": ligands that specifically bind an inactive oligomeric state of a protein and thus shift the oligomerization equilibrium of the protein towards it. The lead peptides penetrated cells and consequently blocked HIV-1 replication in infected cultured cells due to inhibition of integration. The most potent peptides *in vitro* and in cells were LEDGF 361-370 and Rev 13-23. LEDGF 361-370 significantly inhibited HIV-1 infection in mice model. We conclude that the five peptides, particularly LEDGF 361-370, are promising anti-HIV lead compounds for further study and development.

Introduction

The virus that causes the acquired immunodeficiency syndrome (AIDS) was first identified in 1983 and later named human immunodeficiency virus type 1 (HIV-1) [1]. Over the past 25 years, almost 60 million individuals have been infected with HIV-1 and nearly 25 million have died of AIDS. In 2008, approximately 33.4 million individuals were infected with HIV-1 worldwide, 2.5 million became newly infected and 2 million deaths occurred due to AIDS (WHO 2009 report). HIV-1/AIDS has become a major cause of death worldwide. In the last decade, there was a huge progress in the field of anti-HIV therapy, making AIDS in many cases a chronic disease rather than a lethal disease. Anti-HIV drugs block different stages of the viral life cycle that are crucial for viral replication [1]. Currently approved anti-HIV drugs inhibit HIV-1 entry into cells and inhibit the viral enzymes reverse transcriptase (RT) and protease [2]. The major problem with the currently used anti-HIV therapy is the high mutation rate that the virus undergoes, which results in the emergence of drug-resistant virus strains. To overcome this problem, it is important to identify new drug targets and to develop new approaches for the design of drugs against them. The HIV-1 integrase protein (IN) is such a novel target.

IN catalyzes the integration of the reverse-transcribed viral DNA into the host cell genome, which is an essential step in the viral replication cycle [3]. The IN-catalyzed integration proceeds in two steps [4]: (I) 3'-end processing, where IN removes a pGT dinucleotide from the 3' end of each strand of the linear viral DNA [4]. This step occurs in the cytoplasm and is carried out by two IN dimers that bind the viral cDNA at its two long terminal repeats (LTR) termini [5]; (II) Following nuclear import, the two LTR DNA-bound dimers approach each other in the presence of the cellular protein LEDGF/p75, form a tetramer and integration proceeds to the strand transfer step[6-9]. This step leads to integration of the viral cDNA into the target host genome (Figure 1). Structurally, IN is composed of three domains: an N-terminal zinc-binding domain (residues 1-50)[10], a catalytic core domain (CCD; residues 51-212)[11] and a C-terminal DNA binding domain (residues 213-280) [12]. A catalytic triad composed of residues D64, D116 and E152 in the CCD is responsible for the enzymatic activity of IN [11]. IN has no mammalian homologues, and the discovery of

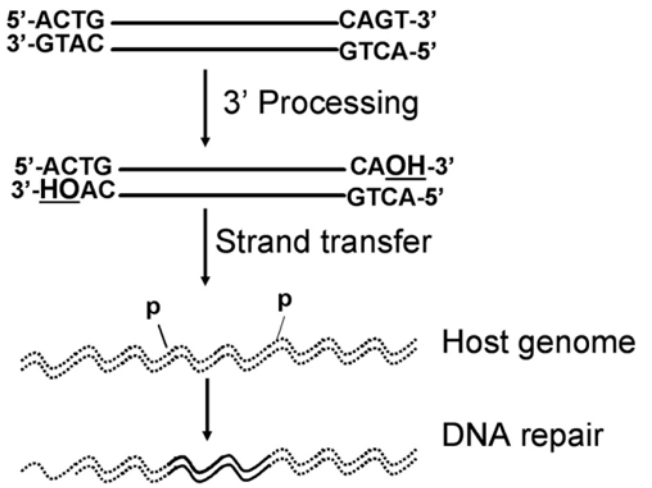

Figure 1: Mechanism of the integration reaction. The 3'-processing reaction occurs in the cytoplasm, whereas strand transfer takes place in the nucleus. The strand-transfer reaction is concerted: both viral DNA ends are inserted into the host chromosomal DNA at the same time. The DNA ends are probably bridged by host cellular factors [48].

effective IN inhibitors is a promising way for developing new, specific anti-retroviral drugs. Recently, raltegravir (Merck) was the first FDA approved IN inhibitor that came into the market [13]. Several more IN inhibitors are currently in advanced clinical trails, such as GS9137 (Gilead). The two advanced IN inhibitors are Diketo acids derivatives. The Diketo acids were first discovered from random screening and their potency and activity was significantly improved [14]. Recently the crystal structure of the Prototype Foamy Virus (PFV)-IN tetramer, which is homologous to the HIV-1 IN, was solved in the presence and absence of the two potent IN inhibitors MK-0518 and GS-9137 [15].

In the current review we will present the development of IN - inhibitory peptides performed in our labs. The peptides were designed using two approaches: (1) Rational design based on protein-protein interactions of IN [16, 17]; (2) Selection using combinatorial library screening by Yeast Two Hybrid System [18,19]. We will focus on the rational design of peptidic IN inhibitors. The combinatorial screening approach is outside the scope of the current review. For more details about it see refs. [18,19].

The Rational Design strategy for developing peptidic IN inhibitors

For developing IN-inhibitory peptides, we combined structure-based design with quantitative biophysical studies of peptide-protein interactions, enzymatic assays and structural studies using NMR. We designed IN inhibitors based on protein-protein interactions of IN and searched for peptidic sequences derived from IN-binding proteins. This is because IN-binding proteins are already optimized by nature to bind IN and thus peptides derived from their IN-binding interfaces also have the potential to bind and inhibit its activity.

Using the rational design approach we developed four peptidic IN inhibitors: two peptides derived from the IN-binding loops of the cellular binding partner of IN, LEDGF/p75 (LEDGF 361-370 and LEDGF 401-413) [16, 20], and two peptides derived from the HIV-1 Rev protein, which we found to bind IN (Rev 13-23 and Rev 53-67)[17, 21]. The sections below describe the inhibitors development in detail.

Example 1: designing inhibitors based on the cellular IN binding protein LEDGF/p75

LEDGF/p75 is the major cellular co-factor of IN, and it is essential for its activity by tethering it to the chromosomes [22]. LEDGF/p75 is a nuclear protein that binds the IN tetramer in the nucleus [8,23]. Purified recombinant LEDGF/p75 protein stimulated IN catalytic function *in vitro* [24]. The cellular functions of LEDGF/p75 remain largely uncharacterized, although initial reports have indicated a role for LEDGF/p75 in transcriptional regulation[25].

LEDGF/p75 is a member of the hepatoma-derived growth factor family, and it contains 530 residues and several functional domains. In accordance with its ability to interact with IN [21, 22, 26], an evolutionarily conserved integrase-binding domain (IBD) of ~80 amino acids (residues 347–429) was mapped to the LEDGF C'-terminus. The crystal structure of the dimeric catalytic core domain of IN in complex with the IBD of LEDGF/p75 was solved in 2005 [27]. Two inter-helical loops extend to interact with the IN core catalytic domain dimer interface and one loop also interacts with the IN N-terminal domain[27]. These loops served as basis for designing IN inhibitors. Two key features of IN that are complementary to and recognized by LEDGF/p75 IBD were observed according to the crystal structure:

(1) the specific backbone conformation of α4/5 connector residues 168–171 and (2) a hydrophobic patch accommodating the side chains of LEDGF residues Ile-365, Phe-406, and Val-408 [28]. Based on the crystal structure, we designed and synthesized two peptides derived from the IN-binding loops of LEDGF/p75 LEDGF 361-370 and LEDGF 401-413 (Table 1).

Table 1: Sources and sequences of IN inhibitors described in the current review

Peptide	Sequence	Source
LEDGF 361-370	WNSLKIDNLDV	Rational design[20,16]
LEDGF 401-413	WKKIRRFKVSQVIM	Rational design[16]
Rev 13-23	LKTVRLIKFLY	Rational design [21,17]
Rev 53-67	RSISGWILSTYLGRP	Rational design [17]
IN1	WQCLTLTHRGFVLLTITVLR	Combinatorial screening [18,19]

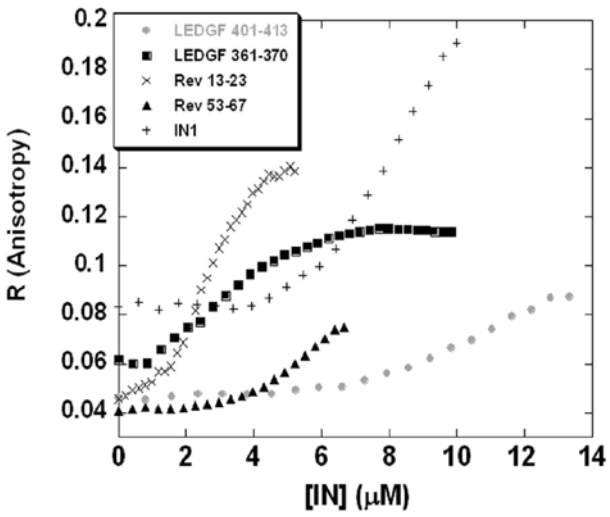

Figure 2: The five inhibitory peptides developed in our lab bind IN at the micromolar range. Shown are fluorescence anisotropy binding studies. IN was titrated into the fluorescein-labeled peptides (100 nM). Data were fit to the Hill equation for binding affinity and Hill coefficient see Table 2 [16-18, 21].

Fluorescence anisotropy was used to determine the binding affinity of IN to the LEDGF/p75 derived peptides. IN bound LEDGF 361-370 with a K_d of 4 μM and LEDGF 401-413 with a K_d of 12 μM. IN binding to the peptides was strongly cooperative, with a Hill coefficient around 4 (Figure 2 and Table 2). IN binding to a fluorescein-labeled 36-base pairs double stranded viral LTR DNA was in agreement with the previous reports [5, 29], and had a K_d of 37 nM and a Hill coefficient of 2. Using fluorescence anisotropy competition experiments we found that the LEDGF derived peptides inhibited DNA binding of IN by 3- to 6- fold (Table 2) [16].

The LEDGF derived peptides were tested for their ability to inhibit the catalytic activities of IN in a quantitative *in vitro* assay [4, 17]. LEDGF 401-413 and LEDGF 361-370 strongly inhibited IN catalytic activity *in vitro* in concentration depended manner (Figure 3).

Fluorescein-labeled LEDGF 361-370 and LEDGF 401-413 penetrated cells. These peptides inhibited HIV-1 replication in infected lymphoid cells, demonstrated by their ability to reduce the amounts of the viral p24 released by these cells (Figure 4).

To reveal the mechanism of DNA-binding inhibition, we studied whether the peptides affect the oligomerization equilibrium of IN as was implied by the cooperative binding mode of the peptides to IN. We used analytical gel filtration to study the effect of ligand binding on IN oligomerization equilibrium. Our findings indicated that IN was tetrameric in presence of the LEDGF peptides, and dimeric in the presence of LTR DNA, in agreement with our fluorescence anisotropy results. The oligomeric state of the truncated mutant IN 52-288 was not affected by binding peptides or LTR DNA, indicating that the effect is specific and that the N-terminus of IN is involved in the binding process or in the oligomerization process[16].

Example 2: peptides based on the IN binding protein HIV-1 Rev

In our labs we found a novel interaction of IN with the HIV-1 Rev protein in HIV-1 infected cells [17]. Rev is a

116-AA viral auxiliary protein that mediates the nuclear export of partially-spliced or un-spliced viral RNA[30, 31]. We showed that disruption of the Rev-IN complex by IN-derived peptides or infection by a Rev-deficient virus stimulated integration and resulted in large numbers of integration event/cell [32, 33]. Based on our results we identified Rev as a novel regulator of integration that prevents multi-integration and thus prevents genomic instability [32-35]. We performed peptide mapping using the HIV-1 Rev subtype B consensus sequence to identify the regions that mediate binding to IN. Five fluorescein-labeled peptides covering the full length of the Rev protein were synthesized and their interaction with IN was studied using fluorescence anisotropy [17]. Rev 1-30 and Rev 49-74 bound IN with K_d values at the low micromolar range, and a Hill coefficient of around 4, indicating binding of IN tetramer to the peptides. Peptides Rev 31-48, 74-93 and Rev 94-116 did not bind IN[17]. Based on screening an NIH Rev derived peptides library we selected the two Rev-derived inhibitory peptides Rev 9-23 (5993) and Rev 53-67 (6004) (Table 1) for further study. We synthesized two peptides: (1) one corresponding to 5993 (Rev 9-23 ,Table 1) but lacking its first four amino acids (DEEL) (Rev13-23, Table 1), in order to obtain a cell-permeable short peptide deficient of the negatively charged amino acids, and (2) one bearing the complete sequence of peptide 6004 (Rev 53-67, Table 1). Rev13-23 and Rev53-67 both bound IN at the low micromlar range with Hill coefficient of 4 (Figure 2, Table 2). These Rev derived peptides inhibited IN catalytic activity *in vitro* and blocked HIV-1 replication in cells [17].

Table 2: Binding affinities of the inhibitory peptides to IN and their effect on IN- DNA binding*

(a) IN binding to the inhibitory peptides

Peptide	K_d (μM) of binding to IN	Hill coefficient
LEDGF/p75 361-370	3.7 ± 0.2	3.4 ± 0.2 [20,16]
LEDGF/p75 401-413	12 ± 0.6	4.5 ± 0.6 [16]
Rev 13-23	2.8 ± 0.1	3.6 ± 0.5 [21,17]
Rev 53-67	6.9 ± 0.1	5.2 ± 0.9 [17]
IN1	8.5 ± 0.1	4.5 ± 0.3 [18,19]

(b) IN binding to the viral LTR DNA in the presence of the peptides

DNA / Peptide	K_d of binding to IN (μM)	Hill coefficient
FI′-LTR DNA only	0.034 ± 0.01	2.0 ± 0.3 [16]
FL′- LTR DNA + LEDGF 361–370	0.099 ± 0.003	2.2 ± 0.1 [16]
FL′- LTR DNA + LEDGF 401–413	0.20 ± 0.02	2.7 ± 0.4 [16]
FI′-LTR DNA + Rev 13-23	0.320 ± 0.02	1.9 ± 0.1 [21]
FI′-LTR DNA + Rev 53-67	0.300 ± 0.09	2.1 ± 0.2 [21]

*Binding studies were carried out using fluorescence anisotropy, as described in the text. Affinities and Hill coefficients are taken from refs. 16-18, 21

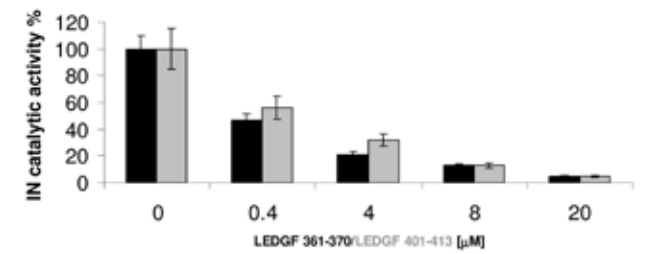

Figure 3: The peptides inhibit IN catalytic activities in vitro. IN was incubated with the designed peptides and the 3′-end processing and strand transfer enzymatic activities were monitored by ELISA based system a quantitative assay [17].

We used fluorescence anisotropy to study whether the Rev derived peptides affect the DNA binding of IN. IN bound to a fluorescein-labeled 36-bp double stranded viral LTR DNA with K_d of 34 nM and a Hill coefficient of 2 (Table 2)[16, 21]. The Rev derived peptides significantly inhibited the binding of IN to the viral LTR DNA. The affinity of IN to the DNA was reduced 10-fold from 34 nM in the absence of the Rev peptides to 320 nM and 300 nM in presence of Rev 13-23 (1μM) and Rev 53-67 (1μM), respectively (Table 2)[21].

The effect of the Rev derived peptides on the IN oligomeric state was tested using analytical gel filtration. IN was tetrameric in presence of the Rev derived peptides, but was dimeric in the presence of LTR DNA, indicating a shift in the oligomerization equilibrium in presence of the Rev derived peptides just like in the case of the LEDGF derived peptides [17, 21].

Structure Activity Relationship Studies of the HIV-1 Integrase Inhibitory Peptide LEDGF 361-370

Of the five IN inhibitory peptides that were developed (two LEDGF/p75 derived peptides, two Rev derived

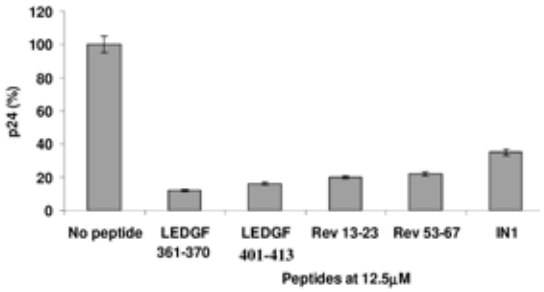

Figure 4: The designed peptides inhibit HIV-1 replication in infected cell culture. H9 T-lymphoid cells were incubated with the indicated peptides and the total amount of the released virus was estimated based on the p24 viral capsid protein content [16-18, 21].

peptides and the IN1 peptide selected from a combinatorial library), LEDGF 361-370 was the most potent according to our *in vitro* and *in vivo* assays results (See for example comparison between the ability of the peptides to inhibit HIV-1 replication in cells in Figure 4). In addition, an independent study showed that LEDGF 361-370 competes with the full length LEDGF/p75 on IN binding with K_i = 4.6 μM [36]. Thus, LEDGF 361-370 was selected by us as a lead compound for further studies.

Alanine scan of LEDGF 361-370 was performed to determine which precise residues participate in IN binding [20]. Eleven peptides were synthesized based on the LEDGF 361-370 sequence (for sequences see [20]). Fluorescence anisotropy binding studies showed that IN bound all the LEDGF 361-370-derived peptides with low micromolar affinity, similar to the parent LEDGF 361-370 peptide [20]. Since D366 was shown to be an important residue for IN binding at the protein level [27, 28], and D369 is proximate in the sequence and may have a similar effect at the peptide level, a peptide in which both D366 and D369 residues were replaced by alanine was also synthesized. The D366/369A mutant bound IN 2-fold weaker than the wild type LEDGF 361-370 [20]. NMR studies showed that almost all of LEDGF 361-370 residues contribute to IN binding, in agreement with the alanine scan results,[20].

The effect of the alanine substituted derivatives of LEDGF 361-370 on the IN catalytic activity *in vitro* was determined using the quantitative integration assay. Most of the alanine substituted peptides inhibited IN catalytic activity in the same manner as the parent LEDGF 361-370 peptide [20]. This indicated that the substitutions had almost no effect on activity, and no single residue is solely responsible for the inhibitory activity of the peptide. These results are in agreement with the fluorescence anisotropy and NMR results. LEDGF 361-370 D366A and the double mutant LEDGF 361-370 D366/369A were less potent inhibitors compared to the other substituted peptides, indicating the importance of the Asp residues for IN inhibition [20].

The fluorescence anisotropy, NMR and *in vitro* activity results indicate that all residues in the parent LEDGF 361-370 sequence contribute to IN binding and inhibition. Hence, the full LEDGF 361-370 sequence is required for further studies and development.

LEDGF 361-370 and Rev 13-23 inhibited HIV-1 infection in mice model

LEDGF 361-370 and Rev 13-23 were studied for their antiretroviral activity *in vivo* using the model of infection of mice with chimeric HIV [37, 38] (Figure 5). Mice were analyzed for normalized vif RNA (Figure 5) burdens in the spleen. LEDGF 361-370 reduced *de novo* synthesis of the single-spliced viral vif mRNA by about 80%, while Rev 13-23 had a modest activity (Figure 5) [20]. In summary LEDGF 361-370 peptide significantly inhibited *de novo* synthesis of viral RNA *in vivo*. Thus, LEDGF 361-370 may serve as a lead compound as an anti HIV-1 inhibitor for further studies.

The mechanism of action of the peptidic IN inhibitors: the shiftides approach

The inhibitory peptides described above bound preferentially to the IN tetramer and stabilized it as was shown by fluorescence anisotropy, analytical gel filtration and crosslinking experiments [16, 18, 21]. We have termed such peptides as "shiftides", since by stabilizing the IN tetramer they shift the IN oligomerization equilibrium towards it [16]. We proposed the shiftide concept, which utilizes peptides to modulate protein activity by specifically binding to an active/inactive oligomeric state of the target protein, resulting in shift of the oligomerization equilibrium and activation/inhibition of the protein respectively (Figure 6). The shiftides act in a similar manner to the allosteric model [39], according to which ligand binding can shift

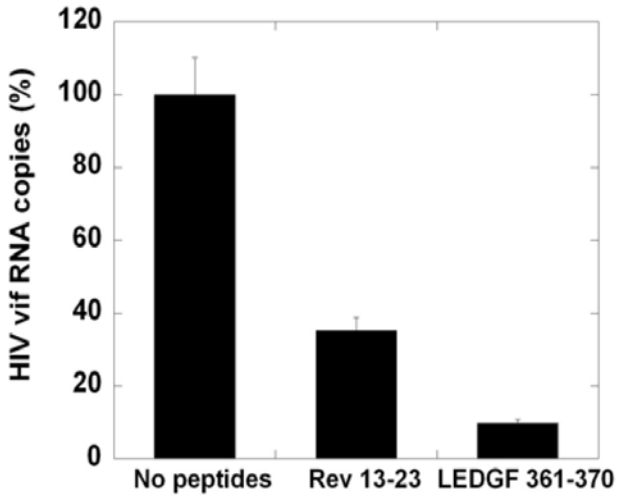

Figure 5: LEDGF 361-370 and Rev 13-23 inhibited HIV formation of HIV-1 Vif RNA in mice model. Mice were pretreated with 40 mg/kg/day of LEDGF 361-370, Rev 13-23 or vehicle for 2.5 days. Mice were sacrificed and analyzed for the normalized vif RNA [20].

this equilibrium towards R- or T- states, as in the case of hemoglobin [40-43]: The shiftides add an additional dimension to the allosteric inhibitors, since they modulate the equilibrium between various oligomeric states, and not within a given oligomer. Shiftides open new directions in the field of oligomerization inhibitors, and are advantageous over conventional dimerization inhibitors [44, 45] or ligands that covalently attach several monomers together [46]. There are intrinsic problems with competitive dimerization inhibitors, because small molecules cannot usually supply enough binding energy for the large interfaces to be targeted, and the full-length protein will bind tighter than a peptide derived from it [47]. The shiftide approach targets oligomerization by binding at a different site of the protein, in an allosteric mode. This overcomes the drawbacks of targeting a protein-protein interaction interface and presents a new way to modulate oligomerization in a non-competitive allosteric mechanism.

According to our proposed model [16], in the case of IN the inhibitory peptides shift the oligomerization equilibrium of IN in the cytoplasm from a dimer, which binds the unprocessed LTR DNA and catalyses the 3'-end processing, to a tetramer that is probably unable to bind the unprocessed DNA and catalyze this reaction. Thus, the viral DNA substrate is not ready for strand transfer, preventing the integration. Moreover, since the IN tetramer is also unable to bind directly to the processed DNA as shown by cross linking experiments [8], shifting the oligomeric state of IN towards a tetramer inhibits the strand transfer of a processed DNA template. The inhibitory peptides inhibit both integration steps, making them advantageous over strand transfer inhibitors, which inhibit only the second integration step. In the case of IN1, the inhibitory peptide that was selected by combinatorial screening, we showed that the full length peptide inhibited IN activity *in vitro* and in cells and shifted IN oligomerization towards the tetramer. Each of the two halves of the parent IN1 peptide bound the IN dimer. We suggested that each half may bind another dimer and together they induce tetramerization [19].

It is still not clear to us how the peptidic IN inhibitors induce IN tetramerization, and whether this activity is sufficient and/or necessary for inhibiting IN. To elucidate

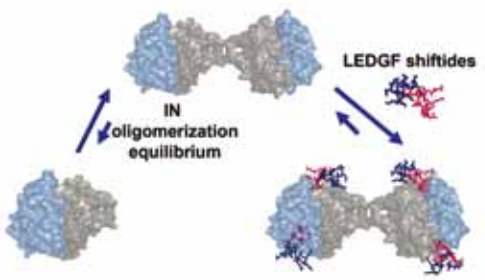

Figure 6: Mechanism of action of shiftides: Peptides or small molecules that shift the oligomerization equilibrium of proteins. Shown is the example of the IN protein and the LEDGF/p75 - derived peptides.

the structural basis for the shiftide activity, it is necessary to reveal the peptide binding sites in the IN protein at its dimeric and tetrameric forms using detailed structural analysis such as NMR or X-ray crystallography. Other projects in our lab are currently applying the shiftides approach for other biological systems

Conclusions

In this review we demonstrated the design of IN peptidic inhibitors using two approaches: (1) Rational design based on protein-protein interactions of IN; (2) Combinatorial library screening. All five peptides we developed bound IN with low micromolar affinity in a cooperative mechanism as indicated by Hill coefficients around 4. The peptides inhibited IN enzymatic activity *in vitro* and in cells and HIV-1 replication in cells and in mouse model. The five selected peptides shifted the IN oligomerization equilibrium from the dimer towards the tetramer. According to these findings, we have proposed a new approach for inhibiting proteins by "shiftides": ligands that specifically bind an inactive oligomeric state of a protein and shift the oligomerization equilibrium of the protein towards it (Figure 6).

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A RECENT DECISION BY THE FEDERAL CIRCUIT IN THE UNITED STATES MAY AFFECT INSTITUTIONS ENGAGED IN EARLY AND BASIC RESEARCH

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Revital Green was born in Tel-Aviv in 1965. She got a BSc in Chemistry, a MSc in Chemistry and Biotechnology and a PhD in organic chemistry, all in Tel-Aviv University. She is affiliated with Ehrlich & Partners, who later became Ehrlich & Fenster, since October 2000, and was licensed as a Patent Attorney in 2004. From 2004, she serves as the manager of the Chemistry and Pharma Department at Ehrlich & Fenster, and is engaged in drafting and prosecuting patent applications in a variety of fields including Biotech, Agrotech, Medicine, Pharmaceuticals, Chemistry and Medical Devices.

Introduction

According to the United States Patent and Trademark Office (USPTO), one of the requirements for obtaining a patent is the filing of a patent application that contains a full and clear disclosure of the invention. This requirement is prescribed by the 35 U.S.C. § 112, first paragraph, of the statutory law, which states that a specification of a patent application must contain a written description of the invention which "… enables a person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same". This section of the statute requires that the specification includes the following: (i) a written description of the invention, which shows that the inventor was in possession of the claimed invention at the time the invention was made; and (ii) a description of the manner and process of making and using the invention (enablement). This requirement for an adequate disclosure ensures that while an inventor of a patent is granted with exclusionary rights, the information contained in the patent becomes a part of the information available for the public for further research and development.

However, the requirements as set forth in the 35 U.S.C. § 112, first paragraph, of the statute, have for years set a difficult task for the USPTO and the U.S. Federal Circuit in balancing the interests of inventors and scientists to create an environment that encourages innovation by adequately protecting inventions, without granting overly broad patent rights. For example, while on one hand, granting exclusionary rights for an invention that is based on a theory not yet proven encourages innovation, on the other hand, such a patent may de-motivate others from exploring the related field, while at the end the public may be deprived from scientific achievements.

Another difficulty encountered by the USPTO and the Federal Circuit is the ambiguity of the 35 U.S.C. § 112, first paragraph requirement, which

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lies in defining what is regarded as "possession of the invention", for the written description requirement, and if and how the written description is separate from the enablement requirement. It is to be noted that the enablement requirement can be met also by presenting enabling data during prosecution, namely, after the filing of the patent application, whereby a written description requirement is restricted to the description of the invention in the application as originally filed. In view of this ambiguity, the 35 U.S.C. § 112, first paragraph requirement has been the subject (and challenge) of many case laws in the United States. Thus, several important decisions of the Federal Circuit deal with the written description and enablement requirements, and, particularly, with the question of whether these requirements, as prescribed in the 35 U.S.C. § 112, first paragraph, of the statute, are separate from one another. The most recent Federal Circuit ruling in this regard was issued on March 22, 2010, in Ariad Pharmaceuticals Inc. vs. Eli Lilly & Co. The Federal Circuit confirmed en banc that the 35 U.S.C. § 112, first paragraph, contains a written description requirement that is separate from the enablement requirement. This case and the decision's impact on institutions engaged in early and basic research are discussed herein.

The Holding:

On March 22, 2010, the Federal Circuit confirmed en banc that 35 U.S.C. § 112, first paragraph, contains a written description requirement that is separate from the enablement requirement [Ariad Pharms., Inc. v. Eli Lilly & Co., No. 2008-1248 (Fed. Cir. March 22, 2010)]. The lengthy (72-page) decision is based on statutory interpretation, citations of U.S. Supreme Court precedent and previous Federal Circuit decisions and attempts to provide guidance concerning what is needed to comply with the written description requirement. Importantly, although the case involved a biotechnology patent, its holding should not be viewed as in any way limited to the biotechnology area.

The Patented Technology:

The Ariad case involved the technology of gene regulation, and specifically the transcription factor NF-κB. NF-κB was first identified as playing a role in the expression of genes involved in the immune system, specifically in the regulation of the expression of the gene encoding the kappa immunoglobulin gene in B cells, which are specialized immune cells. One of the inventors of the Ariad Patent, Dr. David Baltimore, from the Massachusetts Institute of Technology, and his collaborators, have uncovered that NF-κB did much more than regulate a single immune protein, playing a crucial role in the precise control of the expression of various genes and their protein products that are responsible for the response of cells to various and disparate stimuli, including bacterial lipopolysaccharides, certain cytokines, and even sunlight. In view of these findings, NF-κB is now known to be involved in expression of proteins that are associated with many different processes, including the inflammatory response and regulation of the immune system.

In the mid-1980s, Drs. David Baltimore, Philip Sharp, Thomas Maniatis, and ten other scientists at the Massachusetts Institute of Technology, the Whitehead Institute for Biomedical Research, and Harvard University, filed several patent applications related to their work identifying and characterizing NF-κB. These separate applications, with Dr. Baltimore (at right) as the only common thread, were combined in a single application filed on November 13, 1991. Eventually, on June 5, 1995, the application that gave rise to the patent-at-issue was filed, claiming priority to this previous application and containing essentially an identical specification. The applicants, in this June 1995 application, sought claims for artificially reducing NF-κB activity in cells in order to prevent the problems when NF-κB activity runs amok.

In the end, after a lengthy prosecution, U.S. Patent No. 6,410,516 ('516) issued with broad claims directed to methods for reducing the activity of transcription factor NF-κB by reducing its binding to certain gene recognition sites, which encompassed the use of any substance that would achieve that claimed result. Notably, none of these claims indicated how NF-κB was to be reduced or altered. The specification hypothesized three types of molecules that had the potential to reduce NF-κB activity: decoy, dominantly interfering, and specific inhibitor molecules.

The litigation process:

The litigation History: The litigation history dates back to June 25, 2002, when Ariad filed its complaint

on the same day that the '516 patent issued, alleging that certain claims were infringed by two of Eli Lilly's drugs: Evista®, used for the prevention and treatment of post-menopausal osteoporosis, and Xigris®, used for the treatment of adult patients with severe sepsis who are at high risk of death. After a fourteen-day trial in April 2006, a Massachusetts jury determined that the asserted claims were not anticipated by prior art or public use, that the specification enabled and adequately described the claims, and that the use of Evista® and Xigris® infringed the asserted claims. Lilly subsequently moved for judgment as a matter of law, which the District Court denied. After a four-day bench trial in August 2006, the District Court ruled that the asserted claims were directed to patentable subject matter and that the '516 patent was not unenforceable due to inequitable conduct or prosecution latches. Lilly appealed all of these rulings except the ruling on prosecution latches.

On appeal, the Federal Circuit limited its invalidity holding to the issue of written description, and as a result did not comment on all of the other validity issues. The Federal Circuit had the opportunity to further expand its written description jurisprudence, potentially extending the rules as established in previous cases. However, the Ariad decision predominantly turned on the lack of evidence to support a finding that the inventors were in possession of the claimed invention on April 21, 1989, the effective filing date of the patent as determined by the jury. The Court concluded that because there was insufficient evidence that the '516 patent described any method for reducing NF-κB activity as of April 21, 1989, including a description of the molecules that are necessary to perform these methods, the asserted claims were invalid for failing the written description requirement.

In 2009, Ariad petitioned for en banc rehearing of the panel decision of the Federal Circuit. In its briefing to the en banc Federal Circuit, Ariad conceded the statute contained two description requirements ("what the invention is" and "how to make and use it"), but argued that compliance was "judged by whether [the specification] enables one of skill in the art to make and use the claimed invention" and that the first step of identifying what the invention is applied only to priority determinations (i.e., amended claims or later-filed claims, since original claims "constitute their own description."

Details: With respect to the claims at issue, the Federal Circuit upheld the panel's 2009 decision invalidating the asserted claims for failure to meet the written description requirement, because the original disclosure failed to describe how to accomplish the claimed result of reducing NF-κB activity, instead simply suggesting three categories of molecules that prophetically may accomplish that result. The Court noted that the jury found a relatively early priority date and that as of that date the field was "new and unpredictable" and "existing knowledge and prior art was scant," and, therefore, a more detailed description of the particular ways to accomplish the claimed result was required.

The Standard: In a 9-2 decision, the Federal Circuit held that all claims must be supported by an original disclosure that "reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter [as shown from its four corners] as of the filing date". Determination of compliance with the written description requirement was held to be a question of fact, and the level of detail required was explained as "var[ying] depending on the nature and scope of the claims and on the complexity and predictability of the relevant technology."

In response to Ariad notion that the claims do not require explicitly described compositions, the Federal Court pointed out that even if a composition is not part of a claim, the specification must demonstrate that Ariad possessed the claimed method by sufficiently disclosing molecules capable of reducing NF-κB activity to demonstrate the patentee was in possession of the invention that is claimed.

The Court further explained that the "written description" ensures that when a patent claims a genus by its function or result, the specification recites sufficient materials to accomplish that function." In other words, a patentee may not simply describe a problem to be solved and claim all later-created solutions to that problem. Similarly, the majority opinion noted that "[p]atents are not awarded for academic theories, no matter how groundbreaking...".

Judge's opinions: Judge Newman offered additional views to the court en banc decision, noting simply that, regardless of the majority's statutory interpretation, the language and policy of the patent statute requires both written description and enablement. Judge Newman noted that "[b]asic scientific principles are not the subject matter of patents, while their application is the focus of this law of commercial incentive. The role

of the patent system is to encourage and enable the practical applications of scientific advances, through investment and commerce", and that in no case has an invention of basic science been patented without even a single embodiment demonstrating its application and illustrating its breadth. Judge Gajarsa noted that he viewed the statute as a "model of legislative ambiguity" and concurred with the majority's decision solely to "provide some clarity to this otherwise conflicting area of our law".

In two spirited dissents, Judges Rader and Linn disputed both the majority's statutory interpretation and reading of prior case law. They argued that the statute was ambiguous, that prior case applied the written description requirement only to police "new matter" in amended or later-filed claims and that the case should have been remanded for resolution of enablement. According to Judge Linn, 35 U.S.C. § 112, first paragraph, should require no more than the specification enable a person skilled in the art to make and use the claimed invention and set forth the best mode for carrying out the invention.

Moreover, Judge Linn noted that because the Court decided the validity issue solely on written description, the majority failed to consider the important enablement issue raised by Lilly. Thus, the issue of whether claims that are broad enough to cover any method to achieve a particular result can ever be valid, since the specification cannot enable unknown methods, was left unresolved - an outcome that would not have occurred, Judge Linn noted, if there was not a separate written description requirement.

The Impact of the Ariad Decision:

The practical impact of Ariad to patentees is unclear, but the decision has the most potential impact on patentees who frequently file early during the research timeline, before sufficient research may have been completed to provide working examples or detailed descriptions of making and using the claimed invention. Patent prosecutors have long been aware that the USPTO and the Federal Circuit have been more closely scrutinizing the original disclosure of a patent and requiring a sufficient disclosure to support broad claims, such as the claims at issue in Ariad. However, the decision may lead to an increased focus in the courts and in the USPTO on the written description requirement. It remains to be seen in the coming months if the USPTO will issue any revised guidelines directed to the Ariad case.

If the Supreme Court does not take up the case, the only way for the Ariad holding, that written description is separate from enablement, to be overturned is for Congress to amend the statute, and any call for such amendment may depend upon the decision's impact on institutions engaged in early and basic research.

Indeed, in the Federal Circuit opinion, it was acknowledged that such institutions might suffer "some loss of incentive" to engage in such research. This is particularly true in case of academic institutions. Academic institutions rely on scientific publications, usually at early stage of research, which cannot be delayed in favor of a patenting process. Moreover, academic institutions cannot be independently engaged in advanced research and development, particularly in fields such as pharmacology and biotechnology, which require costly and complicated studies to establish the innovated technology. This may trigger a vicious cycle, as cooperation with industrial entities currently depends on the ability of the innovating entity – the academic institution – to be able to protect its rights as the owner of the intellectual property. Thus, the delicate balance between academic institutions and industrial entities may be breached, and the public's benefits from new innovations may be affected.

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Article by Jenny L. Sheafferat Mondaq (http://www.mondaq.com/unitedstates/article.asp?articleid=98246);
Ariad Pharms., Inc. v. Eli Lilly & Co., No. 2008-1248 (Fed. Cir. March 22, 2010).

On Amorphous and Crystalline Phases in Biomineralization: One Hundred Years To Answer a Question

Steve Weiner and Lia Addadi

Prof. Lia Addadi

Born in Padova, Italy (1950), Lia Addadi obtained her M.Sc. degree in organic chemistry at the Università degli Studi di Padova and earned a Ph.D. in structural chemistry from the Weizmann Institute of Science in 1979. After conducting postdoctoral studies at the Weizmann Institute and at Harvard University, she joined the faculty of Weizmann's Department of Structural Chemistry (now the Department of Structural Biology) in 1982. She was appointed associate professor in 1988 and full professor in 1993. She is the recipient of numerous prizes and honors, including the Prelog Medal in Stereochemistry from the Federal Polytechnic Institute (ETH) in Zurich, Switzerland, the Spiers Medal of the Royal Society of Chemistry UK, and this year the prize of the Israel Chemical Society.

Lia Addadi pursues a broad range of research interests that relate to crystals and biological interfaces. In collaboration with Steve Weiner, she studies biomineralization, from atoms, molecules and ions to functional materials, with emphasis on mineralization pathways and interactions between biological and inorganic components. Chirality and chiral suprastructures have been a continuing thread in her research, recently applied to the study of amyloid fibers and crystal surfaces. She also investigates antibody recognition of organized molecular arrays, including the development and characterization of antibodies recognizing specific crystal surfaces or ordered lipid domain interfaces. In collaboration with Leslie Leiserowitz, she developed means for the determination of the structures of single hydrated lipid bilayers; in collaboration with Benny Geiger, she has been investigating the mechanisms of cell adhesion to extra-cellular substrates, and in particular the mechanism of bone resorption by osteoclasts.

Prof. Steve Weiner

Steve Weiner (1948) was born in Pretoria, South Africa. He obtained a BSc degree in chemistry and geology at the University of Cape Town, an MSc in geochemistry at the Hebrew University of Jerusalem, and a PhD at the California Institute of Technology in Pasadena, USA in 1977 working in the field of mineral formation in biology. In the same year he joined the faculty of the Weizmann Institute as a post-doc, then in 1985 became an associate professor and in 1990 a full professor. He is the recipient of the 2010 prize of the Israel Chemical Society.

Steve Weiner carries out research in two fields: biomineralization (with Lia Addadi) and archaeological science. The biomineralization research focuses on mechanisms of mineral formation, as well as the relations between the structure of the mineralized tissue and its mechanical functions (with Ron Shahar, HUJ). The research in the field of archaeology is mainly on the minerals and biogenic organic molecules preserved in the archaeological record. Emphasis is placed on integrating the macroscopic record seen by the naked eye, with the microscopic record, revealed by instrumentation. He recently published a book on the latter entitled Microarchaeology: Beyond the Visible Archaeological Record. Steve Weiner established the Kimmel Center for Archaeological Science at the Weizmann Institute in 1997, with the aim of promoting this approach to archaeology.

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Introduction

In the last decade the field of biomineralization has adopted the paradigm that many mineralization processes in both invertebrates and vertebrates involve the initial deposition of a metastable amorphous mineral phase, which subsequently transforms into a more stable crystalline phase. Surprisingly, this paradigm was introduced by researchers studying mineral formation in organisms almost one hundred years ago. Did they however have the evidence?

In the early 20th century, various investigators observed that the mineral phase present in embryos producing mineralized tissues was often a less stable phase, compared to their mature adult stages. Examples are the observations of the less stable form of calcium carbonate, vaterite, in embryonic terrestrial gastropod (snail) shells, whereas the more stable polymorph aragonite is present in the adult shells [1, 2] and p313 [3]. A change from vaterite to the most stable form of calcium carbonate, calcite, was also observed in the cuticle of Crustacea (crabs etc) [4]. It can be assumed that these observations were not regarded as unexpected by these scientists, as not long before the famous chemist, Ostwald, had noted that during the process of precipitation from supersaturated solutions, the first phase to precipitate is usually not the thermodynamically most stable phase. This first-formed metastable phase transforms and eventually the most stable phase is produced [5]. This became known as Ostwald's Rule of Stages. Thus the vaterite to aragonite, or vaterite to calcite transformation, would follow Ostwald's Rule. Vinogradov's studies in the 1930's takes this one step further. Vinogradov [3] reports that an even less stable phase of calcium carbonate is present together with vaterite in young mollusks and crustaceans. This phase is called amorphous calcium carbonate (ACC) because it is isotropic when observed between crossed Nichols in a polarizing microscope. The ACC and vaterite later change into calcite or aragonite [3](translation) p455).

The question is why this paradigm never persisted, and by the mid-19th century was almost totally ignored? One obvious reason is that other investigators failed to identify vaterite in mollusk larvae. In fact it is now well established that the larval shells of mollusks are composed of aragonite [6] and not vaterite. The larval shells are however really the mature shells of the embryo. Studies in the last decade of forming embryonic mollusk shells did also not identify vaterite, but rather ACC [7, 8]. The discrepancy between the initial reports of vaterite and the recent reports of ACC may well be due to sample preparation and storage procedures. The ACC that is destined to transform into a crystalline product is inherently unstable. This unstable phase can easily transform into vaterite if the samples examined were not fresh or not maintained frozen and hydrated until their examination. Furthermore, there is always the possibility that even if ACC or for that matter vaterite is really initially formed, this phase does not transform into a more stable phase, but rather the more stable phase is subsequently deposited de novo. Thus in order to really demonstrate the transformation of an initial unstable phase into a mature stable phase, besides needing to exclude preservation artifacts, the actual transformation process has to be documented. Both these conditions are not easily accomplished even with today's technology, and were certainly not demonstrated in these early studies.

The First Demonstration of an Amorphous Precursor Phase Transformation in Biomineralization

In 1967 Towe and Lowenstam reported that during the mineralization of the outer layer in the teeth of a group of mollusks called chitons, the first-formed mineral phase is an amorphous or highly disordered iron oxide phase called ferrihydrite, and that this subsequently transforms into the more stable crystalline magnetite [9]. This could be unequivocally demonstrated because of the unique tooth production process. The chiton continuously forms its teeth on a tongue-like object called the radula. There are usually around one hundred rows of teeth. Used teeth are discarded at a rate of about a row/day, and new rows are added at the other end at the same rate in order to maintain a steady state [10]. Thus each row represents about a day's worth of tooth production activity. On the forming side of the radula, the cells responsible for tooth production first construct an organic 3-dimensional framework, and then introduce the mineral. The first mineral phase can be identified in a binocular as it has a prominent red-orange color, but after a few more rows, the color turns black [10]. Using electron diffraction in

the TEM, Towe and Lowenstam [9] identified the orange colored mineral as disordered ferrihydrite and could show that it transformed into the black magnetite. The magnetite forms the outer working surface of the tooth. Using infrared spectroscopy, it was later shown that the first-formed mineral in the inner tooth layer is amorphous calcium phosphate (ACP) that later transforms into crystalline and oriented carbonated hydroxyapatite [11]. This is the mineral present in vertebrate bones and teeth. The lesson learned from these studies is that the most amenable systems for demonstrating a dynamic change of an unstable to a stable mineral phase are those that have the mineral formation stages separated in both space and time. The problem is that such systems are extremely rare, the result being that although the chiton radular studies provided unequivocal proof of the transformation process, their relevance to other mineralizing systems could not be confirmed. It took 30 more years to demonstrate this transformation in another biological mineralization process.

Figure 1: Fully developed sea urchin embryo viewed under polarized light. The two symmetrical spicules light up under cross-polarizers, indicating that they are crystalline.

The Sea Urchin Larval Spicule

In the 1990's we were using synchrotron radiation to study the crystallographic textures of a variety of mineralized tissues, including the calcitic spicules of the sea urchin larval skeleton [12]. Sea urchin larval spicules are known to diffract X-rays as a single crystal of calcite. We noted, however, that the intensities of the reflections were much weaker than we would have expected for a calcitic object with the same volume as the spicule. We decided a few years later to investigate this further. By normalizing the intensities to a unit volume of spicule, we showed that most of the spicule was not diffracting X-rays. As we knew that the entire spicule is composed of calcium carbonate (except for a very small amount (<0.1) of organic material), we concluded that most of the calcium carbonate must be amorphous [13]. This was subsequently confirmed by direct measurements of IR, Raman and XAS, all demonstrating unequivocally that most of the mineral composing the spicule is highly disordered/amorphous [14]. It is interesting to note that the entire spicule behaves as a single crystal in polarizing light, indicating that the small amount of calcite that is present is distributed over the whole spicule (Figure 1). We then compared spicules from larvae that were the same size but at more advanced stages of maturation, and found that the proportion of calcite increased. We therefore concluded that the ACC had transformed into calcite [13, 14]. The mechanism of the transformation was, however, unknown.

We used the sea urchin larval spicule as a test case to further investigate the transformation mechanism of disordered phases into crystalline phases in biomineralization.We performed a series of studies at high resolution, using a new technique involving X-ray absorption spectroscopy measurements with synchrotron radiation [15]. The technique is called X-ray photoemission electron microscopy (X-PEEM). X-PEEM allows the acquisition of large sets of X-ray absorption spectra from regions that cover whole areas on the target object surface. Each pixel within such an area records a spectrum from a region of between 20 to 200 nm^2. Using freshly isolated spicules, we noticed the presence of three distinct phases: a highly disordered ACC 1 phase with very little short range order around the calcium ions, an intermediate phase (ACC 2) lacking long range order but having a fully ordered first coordination shell around the calcium ions, and finally calcite (Figure 2). This confirmed previous investigations performed on macroscopic samples. It also showed the presence of the three phases often in adjacent locations a few tens of nanometers apart. These regions were identified

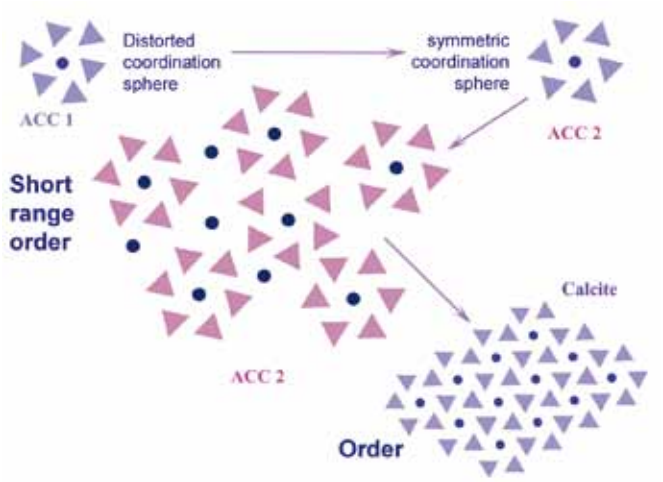

Figure 2: Schematic representation of the transformations observed in sea urchin larval spicules from a short and long range disordered phase (ACC 1) to a short range ordered/ long range disordered phase (ACC 2) to the final crystalline phase, calcite

morphologically as spherical particles, probably excreted directly into the space where the spicule is formed by the cells responsible for the spicule deposition [15].

The best description that we can provide to date for the process of spicule formation is the following: the mineral depositing cells produce and excrete discrete particles (30-70 nm in diameter) composed of a hydrated, highly disordered ACC, which transform rapidly into the more ordered ACC phase. Crystallization propagates from an initial crystal in the center of the spicule, and the atomic order of the crystal is transmitted from one particle to the next by secondary nucleation following a tortuous path, in a sort of percolation process. This explains how the whole spicule may appear at the same time amorphous and crystalline over its whole volume.

Subsequent Studies of Mineralizing Processes

Since the publication of the first paper showing the involvement of a transient amorphous phase in the deposition of sea urchin larval spicules, many other calcium carbonate mineralizing processes have been investigated. The transformation of ACC into either aragonite or calcite is now known for adult echinoderms [16], larval mollusk shells [7, 8], crustaceans [17], earthworms that produce deposits of ACC that transforms into calcite in their intestinal tract [18, 19], vertebrate eggshell formation [20], and certain cyanobacteria [21]. It has also been suggested to occur in marine sponges [22] and in corals [23]. It thus appears to be a widespread process.

A fascinating observation is that the ACC precursor phases have been shown to have different short range orders. Furthermore, the short range order of the ACC that is destined to transform into aragonite resembles the aragonite structure [8], and the ACC that will transform into calcite resembles the calcite structure [14]. Interestingly in the freshwater cyanobacteria, the ACC has a short range structure that resembles aragonite, and it was proposed that this may act as a protection mechanism against uncontrolled precipitation of calcite [21].

The Vertebrates

The mature mineral phase in vertebrate bones and teeth is carbonated hydroxyapatite. The question of whether or not this mature phase forms de novo in vivo, or via precursor phases has been the subject of much discussion in the past [24]. In vitro at around pH 8, it is known that the first precipitate from a saturated solution is amorphous calcium phosphate (ACP). ACP then transforms into the crystalline mineral phase octacalcium phosphate, having a structure that closely resembles hydroxyapatite, but has an added hydrated layer separating between apatite layers. Octacalcium phosphate (OCP) then undergoes hydrolysis to form hydroxyapatite [25]. In the in vivo environment carbonate is always present, and thus the end product will have a significant amount of carbonate substituting for phosphate, and is therefore called carbonated hydroxyapatite.

In a series of studies, Brown and his colleagues provided a variety of reasons why they thought that in vivo carbonated hydroxyapatite forms via an OCP precursor phase, and possibly also an initial ACP phase [24]. Much of the evidence was substantial, and in fact Lowenstam and Weiner [10] listed OCP as a precursor phase in mammalian vertebrate tissues. Direct evidence was provided more recently by Crane et al [26], who used Raman spectroscopy to detect in vivo the presence of an OCP-like phase in newly-formed bone. The Raman spectra also showed the possible presence of small amounts of ACP. In a study of forming enamel using X-PEEM, it was shown that the mature crystals of carbonated hydroxyapatite form via a precursor ACP phase [27]. We recently investigated the mineral formation stages in the continuously growing bone of zebrafish fin rays [28, 29] (Figure 3). The advantage

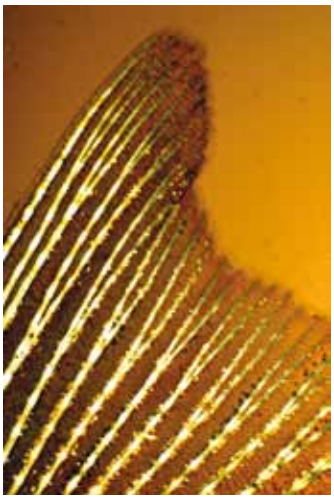

Figure 3: Polarized light micrograph of a section of a zebra fish fin: the bony rays light up under crossed-polarizers, showing their crystallinity.

of this biological system is that the thin elongated bones form fairly rapidly at the distal end, and also thicken continuously. There is thus direct access to regions of newly-formed bone. Furthermore, the organization of the crystal platelets inside the collagen fibrils is identical to that found in mammalian bone, and therefore there is no doubt that the mineralization process in these fish is relevant to that in mammals. Using both TEM imaging and electron diffraction, as well as SEM and backscattered imaging, Mahamid et al first demonstrated that much of the initially formed mineral was indeed ACP [28]. In a follow-up study using both X-ray microdiffraction and cryo-SEM (Figure 4), we showed direct evidence that the initially formed ACP does indeed transform into crystalline carbonated hydroxyapatite [29]. The above studies of a variety of different vertebrate mineralized tissues, all show that vertebrate mineralization also proceeds via an ACP precursor phase, and possibly also an intermediate OCP-like phase.

Conclusions

More than a hundred years ago Ostwald provided the conceptual basis for precipitation via disordered precursor phases. In the hundred or so years that followed many observations were made that were consistent with this process occurring in biological mineralization, but convincing evidence was absent. Only with the advent of highly resolving analytical techniques for both imaging tissues under hydrated conditions and characterizing disordered mineral phases, was it possible to provide hard evidence in support of the amorphous precursor phase strategy. It now seems that as this strategy is used by a wide variety of animal phyla, and also some phyla from other kingdoms, this is probably a widespread strategy in biomineralization.

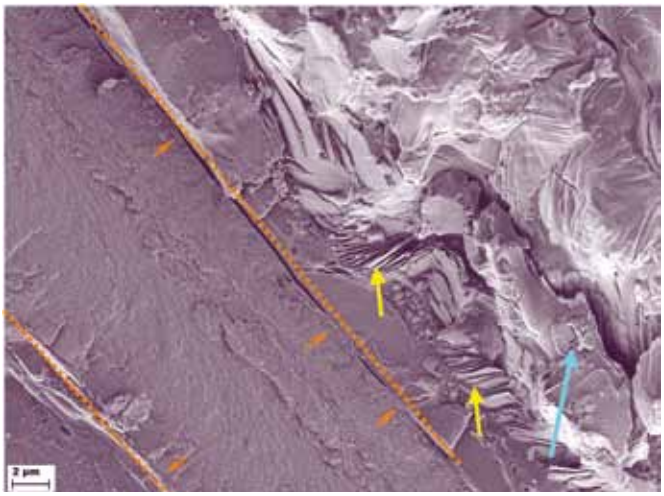

Figure 4: High-resolution cryo-SEM micrograph of zebra fish bone, after high pressure freezing and freeze fracture: the bone is delineated by orange dotted lines; orange arrows show the presence of areas where new bone is formed and where the amorphous precursor mineral phase is detected. The blue arrow indicates the layer of fish skin that lies on top of the bone. The yellow arrows show the stacks of thin guanine crystals which are responsible for the metallic iridescence of the skin. For more information on this affect, see [30, 31].

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The 75th Annual Meeting of the Israel Chemical Society*

The Japanese delegation to the 75th ICS Meeting: Standing, left to right: Prof. Hiroaki Suga, University of Tokyo; Prof. Shunichi Fukuzumi, Osaka University; Mr. Haruhisa Takeuchi, Ambassador of Japan to Israel; Prof. Daisuke Uemura, Keio University; Prof. Eiichi Nakamura, University of Tokyo; Prof. Tohru Fukuyama, University of Tokyo; Prof. Ehud Keinan, President of the ICS. Sitting, Prof. Shmuel Carmali, Chairman of the Organizing Committee.

David Intercontinental Hotel, Tel Aviv January 25-26 2010

E. Keinan,[a] D. Shabat[b] and S. Carmeli[b]

[a]The Schulich Faculty of Chemistry, Technion - Israel Institute of Technology.
[b]Sackler School of chemistry, Tel-Aviv University.

The Israel Chemical Society (ICS) has a long tradition of Annual Meetings, with almost no interruption since its establishment in 1933. Each of these colorful gathering has always been an attractive scientific event for Israeli Science, bringing together researchers from academia, chemical education, chemical industry, government laboratories, and other chemical enterprises. This year the approximately 800 participants enjoyed a broad range of over 100 lectures on diverse subjects, along with over 200 posters as well as a large commercial exhibition by vendors of research equipment and scientific instrumentation, publishing houses, offices of intellectual property, etc.

The meetings have been usually held in early February, which is the inter-semester break in all Israeli universities and the end of a short rainy season in Israel. The responsibility for each meeting has been revolving in a 6-year cycle among the 6 major research universities. For example, the 69th meeting (2004) was organized and hosted by the Sackler School of Chemistry of Tel Aviv University, the 70th meeting (2005) was organized by the Department of Chemistry of Bar Ilan University, the 71st meeting (2006) by Ben Gurion University, the 72nd meeting (2007) by the Weizmann Institute of Science, the 73rd meeting (2008) by

An expanded version of this report appears in *Israel J. Chemistry 2010, 50(3).*


Ada Yonath


Joseph Klafter


Ei-Ichi Nakamura


Lia Addadi


Steve Weiner


Uri Banin


David Avnir


Tohru Fukuyama


Daisuke Uemura


Hiroaki Suga


Shunichi Fukuzumi


Erick M. Carreira


Scott E. Denmark

the Hebrew University of Jerusalem, and the 74th meeting (2009) was organized by the Schulich Faculty of Chemistry of the Technion—Israel Institute of Technology. Thus, this year the 75th meeting began a new cycle, with a team from Tel Aviv University led by Professor Shmuel Carmeli as Chairman taking the responsibility for organizing the meeting, including the scientific program. While in earlier years each meeting was held on one of the 6 campuses, for the past several years the meetings were conveniently held at the David Intercontinental Hotel in Tel Aviv, which was the case with the 75th meeting as well.

Another unique tradition of the ICS, which has already attracted much worldwide attention and interest, has been the custom to invite to each meeting a delegation of eminent chemists from a top non-Israeli institution, to present plenary and keynote lectures. This initiative has created outstanding opportunities for many Israeli scientists, and particularly for graduate students and young scientists, to interact with top-tier chemists, thus enhancing prospects for networking, scientific collaboration, extensive exchange of ideas, and securing mutual visits of students and faculty members. Each visit of these delegations has left a long trail of postdoctoral fellowships, Sabbatical programs for Israeli scientists, joint research proposals, and other fruitful international activities. The ICS has already hosted distinguished delegations from The Scripps Research Institute (1997), California Institute of Technology (1998), University of Cambridge–UK (1999), ETH–Zurich (2000), Columbia University (2001), University of California at Santa Barbara (2006), the Max Planck Society (2009), the Chemical Society of Japan and Japan Society for the Promotion of Science (2010). At the 76th Annual Meeting, which is planned for February 9–10, 2011, the ICS will celebrate the International Year of Chemistry together with a delegation of seven scientists from Academia Sinica of Taiwan. The participation of delegations from Japan and Taiwan reflect the rapidly growing importance of Asia on the world map of science and technology.

Japan's Ambassador to Israel, Mr. Haruhisa Takeuchi, attended the opening ceremony and greeted the Japanese delegation along with the rest of the participants. The delegation of outstanding chemists, included Shunichi Fukuzumi of Osaka University, Daisuke Uemura of Keio University, and

Tohru Fukuyama, Eiichi Nakamura, and Hiroaki Suga, all of the University of Tokyo. The represented the Japanese scientific community by delivering plenary and keynote lectures. In appreciation of this event, all delegation members were awarded with a lifetime Honorary Membership in the ICS. The meeting was honored by other distinguished guests from the USA and Europe, including Erick M. Carreira of ETH–Zurich and Scott E. Denmark of the University of Illinois at Urbana.

The scientific program offered two very long and busy days, covering all facets of modern, interdisciplinary chemical research, including 9 plenary lectures, 18 symposia, and 2 poster sessions. Overall, the meeting included over 90 keynote, invited, and contributed lectures, and over 200 posters.

The annual meeting has also always been the forum in which the ICS prizes are awarded, mostly during the opening ceremony. Professors Meir Lahav and Leslie Leiserowitz, of the Weizmann Institute of Science, were awarded the 2009 ICS Medal for their unique contributions to our basic understanding and ability to control the self-assembly of organic molecules. They have developed novel stereochemical approaches that shed light on the mechanisms of crystal nucleation and growth. Among many other achievements, they have demonstrated that tailor-made inhibitors or moderators in solution can be used to control crystallization phenomena. They have also contributed to the understanding of the structure of molecular domains at the air–water interface. Their highly cited contributions have affected many scientific fields, including solid-state chemistry, materials science, surface chemistry, stereochemistry, and origin of life.

Professors Lia Addadi and Steve Weiner, of the Weizmann Institute of Science, were awarded the 2009 ICS Prize of Excellence for advancing the field of biomineralization and for the impact of their studies on the field of materials science. Professors Addadi and Weiner have developed a fundamental understanding of the mechanisms by which living organisms create crystals, showing that these processes occur by the conversion of amorphous states to crystalline arrays.

The 2009 ICS Prize of Excellence is being awarded to Lia Addadi and Steve Weiner of the Weizmann Institute of Science

Laureates of the 2009 ICS Medal: Meir Lahav (right) and Leslie Leiserowitz (left)

Dr. Michael Bendikov of the Weizmann Institute of Science was awarded the 2009 ICS Excellent Young Scientist Prize. Michael has managed to integrate synthetic chemistry, physical organic chemistry, materials science, and computational chemistry so as to make highly significant contributions to the area of conducting polymers and novel electronic organic materials.

Mrs. Pnina Shapiro of Ehad Haam High School, Petah-Tikva was awarded the Excellent Teacher Prize for her outstanding contribution to chemistry teaching in her school and on the national level, and for implementing new materials and a variety of teaching methods. Mr. Yarden Kedmi of Rogozin High School, Kiryat

The 2009 ICS Excellent Young Scientist Prize is being awarded to Dr. Michael Bendikov of the Weizmann Institute of Science

Ata, was awarded the Young Teacher Prize for his extraordinary devotion to chemistry teaching that has produced outstanding students, and for making chemistry a prestigious, highly demanded subject at his school.

A special event was related to Arab pupils participating in the Chemiada – the National Olympiad of Chemistry. Although the proportion of the Arab population in Israel is about 20%, the percentile of Arab pupils studying chemistry in high schools exceeds 40%. Nevertheless, for many years the Arab pupils have been misrepresented in the Chemiada. A joint effort to correct this situation was initiated jointly by the ICS and Mrs. Afrah Assi, a chemistry teacher of the Jaljulia High School, who won the 2008 ICS Prize for Excellent Young Teacher. Mrs. Assi has made a private donation to enhance the competitiveness of Arab pupils in the Chemiada. Accordingly, the ICS has committed a special monetary award to any Arab pupil who would reach the final stage of the Chemiada. Out of approximately 5000 pupils who participate annually in this national competition, only 250 make it for the second stage and about 25 reach the finals. An intensive educational effort led by Dr. Ibrahim Amer of the Hebrew University of Jerusalem, helped by Dr. Irena Sokoletz of the Technion and by several enthusiastic teachers and parents, has resulted in remarkable fruits. An unprecedented number of six Arab pupils have made it to the Chemiada finals, and all of them received the ICS-Assi Award in a special ceremony held during the ICS Meeting.

Ada Yonath of The Weizmann Institute of Science, who received the Nobel Prize in Chemistry one month earlier, delivered the opening lecture of the conference on "The Ribosome: Anrna Machine For Peptide Bond Formation".

Six Arab pupils who reached the Chemiada finals, receive the ICS-Assi Award

Joseph Klafter, President of Tel Aviv University, delivered the second plenary lecture on "The Ever-Fluctuating Protein". Ei-Ichi Nakamura of the University of Tokyo delivered the third plenary lecture on "Imaging Of Single Organic And Organometallic Molecules In Motion".

Lia Addadi of the Weizmann Institute of Science delivered the first ICS Prize plenary lecture: "Taking Advantage Of Disorder On Amorphous And Crystalline Phases In Biomineralization". Steve Weiner of the Weizmann Institute of Science delivered the second ICS Prize plenary lecture: "Bone, Bone Mineral And Bone Crystals: An Ongoing Saga".

The second day of the meeting started with a plenary lecture by Uri Banin of the Hebrew University of Jerusalem: "Hybrid Colloidal Nanostructures; A New Type Of Functional Materials".

Tohru Fukuyama of the University of Tokyo delivered

the seventh plenary lecture: "Development Of Nosyl Chemistry And Its Application To Total Synthesis".
Daisuke Uemura of Keio University delivered the eighth plenary lecture on "Recent Aspects Of Chemical Ecology". David Avnir of The Hebrew University of Jerusalem delivered the ninth plenary lecture on "25 Years Of Sol-Gel Research: Contributions To Chemistry".
Erick M. Carreira of The ETH, Zürich delivered a Keynote lecture on "Discovery And Surprises With Natural Products". Scott E. Denmark of University of Illinois, Urbana, IL, delivered a Keynote lecture on "Silicon-Based Cross-Coupling Reactions: Methodology, Mechanism, And Total Synthesis". Hiroaki Suga of The University of Tokyo delivered a Keynote lecture on "Genetic Code Reprogramming". Shunichi Fukuzumi of The Osaka University delivered a Keynote lecture on "Perspectives On Bioinspired Artificial Photosynthesis".
The social part of the meeting included a festive dinner at Le Relais, a nearby restaurant located in the ancient part of Jaffa. On that occasion the ICS President awarded the ICS Prize to Professors Lia Addadi and Steve Weiner. Also, all members of the Japanese delegation were awarded lifetime Honorary Membership in the ICS. Finally, Prof. Ei-Ichi Nakamura entertained the audience with an outstanding performance of ancient Japanese music played on his heavy Baroque flute, playing the tune of a very famous folk song named "Komoro-magouta", meaning "A song of the packhorse driver of Komoro".
The ICS meeting for 2011 will be the main event planned for the International Year of Chemistry. The ICS will hold its Annual Meeting in collaboration with the Israel Analytical Chemistry Society (IACS) in a continuous 4-day conference that will take place on February 7–10, 2011, at the David Intercontinental Hotel, Tel Aviv. This joint endeavor will comprise two consecutive scientific events: the 14th Isranalytica (February 7–8) and the 76th Annual Meeting of the ICS (February 9–10). This event will include an exhibition of scientific equipment and instrumentation, with over 70 exhibiting companies. At this meeting, the ICS will host a delegation from Academia Sinica of Taiwan, further enhancing the already strong ties between the scientific communities of Taiwan and Israel.

Prof. Ei-Ichi Nakamura plays traditional Japanese music on his Baroque flute (top) and proudly shows his lifetime ICS Honorary Membership card (bottom).

The 76th Annual Meeting of the Israel Chemical Society

February 9th-10th 2011

David Intercontinental Hotel, Tel Aviv

www.chemistry.org.il/76meeting/


Chi-Huey Wong


Andrew H.-J. Wang


Chung-Hsuan (Winston) Chen


Ming-Daw Tsai


Shang-Cheng Hung


Huan-Cheng Chang


Yu-Ju Chen

Dear Colleagues,

Opening the International Year of Chemistry (IYC2011), the 76th ICS meeting (February 9th-10th, 2011) will be one of the major scientific events of the Israeli Science, bringing together researchers from academia, chemical education, and industrial research.

Combined with the 14th Isranalytica meeting of the Israel Analytical Chemistry Society (February 7th-8th, 2011), the two consecutive scientific meetings will offer unique synergistic opportunities to celebrate a chemistry week (February 7th-10th, 2011). This 4-day event, which will include an outstandingly large exhibition of scientific equipment and instrumentation, will highlight the scientific and societal impacts of the chemical sciences, and reinforce the public awareness and appreciation of the chemical sciences.

This year we are proud to host a delegation of world-renowned scientists from Academia Sinica of Taiwan who will further enhance the already strong ties between the scientific communities of Taiwan and Israel. The meeting is being organized by the Department of Chemistry and Institute of Nanotechnology & Advanced Materials at Bar-Ilan University.

We look forward to seeing all of you in Tel-Aviv in February 2011!

Prof. Jean-Paul (Moshe) Lellouche
Chairman of the Organizing Committee

The ICS Prizes for 2009

ICS Medal

Prof. Meir Lahav

Prof. Leslie Leiserowitz

PROFESSOR MEIR LAHAV
Weizmann Institute of Science

For his unique contributions to our basic understanding and ability to control the self-assembly of organic molecules.

PROFESSOR LESLIE LEISEROWITZ
Weizmann Institute of Science

For his unique contributions to our basic understanding and ability to control the self-assembly of organic molecules.

Prize for Excellence

Prof. Lia Addadi

Prof. Steve Weiner

PROFESSOR LIA ADDADI
Weizmann Institute of Science

For advancing the field of biomineralization and for the impact of her studies on the material sciences.

PROFESSOR STEVE WEINER
Weizmann Institute of Science

For advancing the field of biomineralization and for the impact of his studies on the material sciences.

ICS Excellent Young Scientist

Dr. Michael Bendikov

Dr. Michael Bendikov
Weizmann Institute of Science

For his ground-breaking studies in the area of conducting polymers and novel electronic organic materials.

The Excellent Teacher

Mrs. Pnina Shapiro

Mrs. Pnina Shapiro
Ehad Haam High School,
Petah-Tikva

For her outstanding contribution to chemistry teaching in her school and on the national level, and for implementing new materials and a variety of teaching methods.

The Young Teacher

Mr. Yarden Kedmi

Mr. Yarden Kedmi
Rogozin High School
Kiryat Ata

For his extraordinary devotion to chemistry teaching that has produced outstanding students, and for making chemistry a prestigious, highly demanded subject at his school.

Lise Meitner Prize

AVITAL SHARIR - IVRY
Department of Medicinal Chemistry and Natural Products
The Hebrew University of Jerusalem
Supervisor: Dr. Avital Shurki

Ph.D. Excellent Student

SHARLY FLEISCHER
Weizmann Institute of Science
Supervisor: Prof. Yehiam Prior

DARIA BRISKER-KLAIMAN
Technion Israel Institute of Technology
Supervisor: Prof. Uri Peskin

CHARLES ELIEZER DIESENDRUCK
Ben Gurion University of the Negev
Supervisor: Dr. Gabriel Lemcoff

ASAF EILAM
Bar Ilan University
Supervisor: Prof. Arlene Gordon

ELAD GROSS
Hebrew University of Jerusalem
Supervisor: Prof. Micha Asscher

IZHAR MEDALSY
Hebrew University of Jerusalem
Supervisor: Prof. Danny Porath

ERAN SELLA
University of Tel-Aviv
Supervisor: Prof. Doron Shabat

SOPHIA LIPSTMAN
University of Tel-Aviv
Supervisor: Prof. Israel Goldberg

The 8th meeting of the Section of Medicinal Chemistry of the Israel Chemical Society

Meeting Report

Hanoch Senderowitz[1], Galia Blum[2], Micha Fridman[3]

Galia Blum
Dr. Blum received her B.Sc. in Chemistry from the Hebrew University of Jerusalem, Israel 1994. She carried out her doctoral studies at the Hebrew University of Jerusalem, in the laboratory of Professor Alexander Levitzki, for this work she was awarded the Polack prize for excellence in studies and research from the Institute of Life Sciences, The Hebrew University of Jerusalem. Her research focused on Development of potent inhibitors of Insulin-like Growth Factor 1 receptor as anti cancer drugs. Dr. Blum received her Ph.D. in the spring of 2003. She then became a postdoctoral researcher at Stanford University in the laboratory of Professor Matthew Bogyo till 2008. She worked as a Research Associate at Stanford during 2008-2009. Her work at the Bogyo lab at Stanford involved organic synthesis, protein biochemistry, enzymology and fluorescent imaging to investigate protease activity associated with human pathologies. Dr. Blum joined the Hebrew University School of Pharmacy in fall 2009 as a Senior Lecturer faculty member. Today her research lab focus is developing imaging probes for protease activity to investigate and cure cancer and atherosclerosis.

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Hanoch Senderowitz
Dr. Senderowitz received his B.Sc. in Chemistry from Tel Aviv University in 1987 (magna cum laude) and then continued his Ph.D. studies at the same institute in the laboratory of Professor Benzion Fuchs. His research focused on an interdisciplinary approach to the study of steric and stereoelectronic effects and their influence on structure and reactivity of organic molecules. Dr. Senderowitz received his Ph.D. in 1993 and subsequently became a post-doctorate Fulbright fellow at the laboratory of Professor Clark Still at Columbia University N.Y. where he worked on the development of new simulations methodologies and their application to organic and bio-organic systems. Having returned to Israel in 1997, Dr, Senderowitz worked for over a decade in the pharmaceutical industry first at Peptor Ltd. and then at EPIX Pharmaceuticals where he headed the computational development group. In the fall of 2009, Dr. Senderowitz joined the Department of Chemistry at Bar-Ilan University as an associate professor. His current research interests focus on the development of new computational methodologies for modeling the structure and mechanism of action of pharmaceutically relevant proteins and on the design of drugs targeting them and on the development of new computational methodologies for the in silico prediction of the pharmacological profile of bioactive molecules in order to design compounds with improved profiles.

Micha Fridman

Dr. Fridman received his B.A. in Chemistry from the Technion, Israel 1997. He carried out his doctoral studies at the Technion, in the laboratory of Professor Timor Baasov, for this work he was awarded the Wolff prize for excellence in studies and research. His research focused on the development of novel approaches to overcome bacterial resistance towards the clinically important aminoglycoside antibiotics. Dr. Fridman received his Ph.D. in the spring of 2005. He then joined the group of Professor Daniel Kahne at the department of Chemistry and Chemical Biology at Harvard University for a postdoctoral position until 2008. Dr. Fridman joined the School of Chemistry at Tel Aviv University in fall 2008 as a Senior Lecturer. His research focuses on studying biological processes through the synthesis of suitable organic probes and in particular Glyco-Chemistry and Glyco-Biology.

The 8th meeting of the Section of Medicinal Chemistry of the Israel Chemical Society took place at the Weizmann Institute on March 16th 2010. The Section of Medicinal Chemistry was founded in 2002 by Professor Matityahu Fridkin from the Weizmann Institute, Professor Avraham Domb from the Hebrew University and Jeff Sterling from Teva. The goal of the organization is to promote the study and research of Medicinal Chemistry in Israel. Ever since its establishment, the section of Medicinal Chemistry has an ever increasing number of members and participants in the annual meetings. This year, close to two hundred participants attended the meeting from a variety of disciplines (Chemistry, Pharmacology, Life Sciences and Medicine).

In the frame of the Medicinal Chemistry Section, Professor Michael Chorev form the Harvard Medical School established the Chorev prize which is awarded annually to two Graduate Students in Israel for their outstanding research advances and achievements in the field of Medicinal Chemistry.

The award ceremony took place during the award session and the annual Chorev prize was awarded this year to two PhD students, Mr. Igor Nudelman from the Technion and Mrs. Hadas Skaat from Bar-Ilan University, Both winners were asked to deliver short presentations describing their work.

Mr. Igor Nudelman from the Technion-Israel Institute of Technology: Mr. Nudelman is a PhD student under the supervision of Professor Timor Baasov. The focus of his study is on the design and development of aminoglycoside-based analogs that induce reading through point mutation stop codons and thus offer potential treatment for genetic disorders. By means of optimizing drug-induced suppression efficacy and toxicity; further progress in this direction may offer promise for the treatment of many genetic diseases caused by nonsense mutations.

Ms. Hadas Skaat from the department of Chemistry at Bar-Ilan University. Ms. Skaat is a PhD student under the supervision of Professor Shlomo Margel: Ms. Skaats research focuses on the synthesis and characterization of magnetic nanoparticles for the inhibition or acceleration of insulin and amyloid-β fibril formation. The nanoparticles developed by Ms. Skaat may therefore be useful for the inhibition of conformational changes of other amyloidogenic proteins that leads to neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, mad cow and prion diseases.

Two additional prizes were awarded to the winners of the best poster competitions, held this year for the first time. The winning posters were selected from among 31 posters submitted to and reviewed by the advisory board. Both winners delivered short presentations describing their work. Daniel Sherman from The Hebrew University won the prize for his poster titled "Development of new inhibitors for Jak2 protein tyrosin kinase using "click chemistry", in his work he developed potent Jak2 inhibitors that could serve as anti cancer drugs. Anat Levit from The Hebrew University won the prize as well for her poster titled "Better understanding of bitter taste combined computational and experimental study of hTAS2R14 ligand-receptor interactions". In her work she found key receptor – ligand interactions that contribute to bitter taste by the hTAS2R14 receptor.

The scientific contributions were divided into 4 sections namely, Targeting Cellular Processes, Biopolymers in

Group picture of the participants of the 8th meeting of the Section of Medicinal Chemistry of the Israel Chemical Society

Medicinal Chemistry, Computational Methods and Disease Therapy.

Targeting Cellular Processes

Presentations in this session were given by Amnon Albeck from Bar-Ilan University. The group of Prof. Albeck demonstrated induction of proteolytic activity in response to treatment of cells by KCN, a known necrosis inducer, which was partially purified and characterized. The effect of KCN mediated inhibition of the necrotic process both in-vitro and in-vivo was studied.

Ashraf Brik from Ben Gurion University presented recent development on the highly efficient and chemoselective peptide and protein ubiquitylation and on the first chemical synthesis of ubiquitin thioester. The chemical synthesis of homogeneous ubiquitylated alpha-synuclein to study the effect of ubiquitylation in Parkinson's disease was presented. Dr. Briks' results show that the entire ubiquitylation process with the E1-E3 enzymes could be mimicked using only chemical tools.

Dan Peer from Tel Aviv University presented the development of a strategy that can target different subsets of leukocytes and selectively silence genes in vivo using targeted, stabilized nanoparticles (tsNPs). The developed carriers do not induce lymphocyte activation, interferon responses or release liver enzymes and are fully degradable. Three preclinical examples inflammatory bowel disease (IBD), blood cancer and viral infection were discussed. The group of Dr. Peer demonstrated that tsNPs can be used for in vivo validation of new drug targets, for prevention of viral infection and for inducing therapeutic gene silencing in a preclinical setting.

Biopolymers in Medicinal Chemistry

Presentations in this session were given by Doron Shabat from Tel Aviv University, who presented the development of self-immolative dendrimers. These molecules are able to disassemble upon a specific triggering reaction through domino-like fragmentations. The group of Prof. Shabat designed and synthesized a novel self-immolative comb-polymer that disintegrates from head-to-tail upon a single stimulus event. Upon activation of the head group, the comb-polymer undergoes complete disassembly to release multiple copies of side-reporter group. The polymer was prepared by simple polymerization of a monomer, followed by capping of the polymer head with a trigger. This technique allowed rapid synthesis of the polymeric molecule containing a large number of reporter units. The group demonstrated that a water-soluble version of the comb-polymer can be activated

by an enzyme and thus has potential as a selective drug delivery system.

Chaim Gilon from the Jerusalem College of Engineering presented his study on methods called Cycloscan and Backbone Cyclization. In these methods conformational constraints are imposed on the parent active peptide, which reduces its conformational space while maintaining its essential pharmacophores. Gilon's group showed that the conformational space of a parent peptide can be scanned by a focused library of backbone cyclic peptides. Such libraries produce active, selective, metabolically stable and intestinally permeable peptides. Backbone cyclic peptide libraries were applied leading to the discovery of novel, orally available HIV CD4-gp120 inhibitor and a novel cell permeable substrate inhibitor of PKB/Akt. Novel synthetic achievements that allow the use of Cycloscan and Backbone Cyclization by non-specialists were presented as well.

Itzhak Ofek from Tel Aviv University presented a novel approach to enhance anti-microbial drug action by utilizing engineered peptide conjugates. The most potent conjugates bind to gram-negative lipopolysaccharides, rendering the bacterial outer membrane (OM) permeable to hydrophobic antibiotics. Structure–function studies involving substitution of key amino acids in the peptide revealed that the peptides are highly specified to bind LPS and disorganize gram-negative OM. The modified peptidesconfers chemotactic and opsonic activities upon the conjugates e.g., enhance phagocytosis and intracellular killing of the bacteria. The conjugates exhibit 8-10 times less toxicity as compared to the parent antibiotics. This work was done in collaboration with Professor Matityahu Fridkin from the Weizmann Institute.

David Aviezer from Protalix, presented an overview on the R&D of the company. Protalix has developed a proprietary plant cell culturing device with both high biological efficiency and high economical efficiency. The system has proven its efficiency with a large number of different plant species and was found to produce high plant cell mass in all cases. The plant cell culturing device could be used for secondary metabolite production as well as recombinant protein expression. Protalix is using the system for the production of pharmaceutical complex proteins. Several different protein classes were expressed in their cell culture system and all were found to be biologically active. Their lead product Glucocerebrosidase for the treatment of the Gaucher disease, is produced presently in large scale, processed to high purity of above 99% purity. This product is developed under FDA IND guidelines. The protein was found to be safe and as a result a clinical path was approved and phase I and Phase III pivotal study were terminated extension, switchover study and compassionate study are ongoing treating over 100 patients.

Computational Methods

Presentations in this session were given by Masha Niv from the Hebrew University and Stephen Todd from Accelrys. Dr. Niv presented here work on protein kinases which are key signaling enzymes and important drug targets. While the number of FDA-approved inhibitors of kinases is on the rise, ensuring inhibitor selectivity remains a major challenge. Novel approaches to this problem include inhibition of protein-protein interactions as well as targeting of kinase-specific inactive or intermediate conformations. Research done at Dr. Nivs laboratory develops computational tools geared towards such applications. Flexible peptide docking and coarse-grained conformational sampling were presented and discussed in the context of selectivity.

Stephen Todd from Accelrys presented computational approaches that can contribute crucial information in many areas, including formulation design, crystallization, permeation, nanoscale morphology and release profiles. The presentation focused on a range of modeling techniques from classical atomistic simulation to mesoscale modeling, which allow the researcher to probe the behavior of materials from the atomistic to the microstructural scale. Several approaches were introduced and illustrated by case studies relevant to the drug delivery field. Examples of case studies included the permeation in transdermal adhesives, the morphology of block-copolymer nanoparticles, and release kinetics from reservoir devices.

Disease Therapy

Avigdor Scherz from the Weizmann Institute presented his work titled "Tumor ablation using laser activation of focal vascular occluding agents (FVOA), Tookad-

soluble®". His group's work developed a non-invasive therapeutic modality that enables for the first time occlusion of the entire tumor vasculature in a few minutes of treatment. Following IV injection, Tookad-soluble® stays in the circulation until clearance (t1/2~20 minutes). Upon focal illumination an impact on the tumors' vasculature occurs, instantaneous blood flow arrest in the feeding arteries and a non-reversible obstruction of the tumor capillaries and draining veins, clots formation and perfusion arrest. Professor Scherz described worldwide Phase II clinical trials of laser activated focal vascular occlusion therapy with Tookad-soluble®-against localized prostate cancer that show good efficacy and high safety profile.

Alex Levitzki from the Hebrew University presented his study on the development of allosteric IGF-1R inhibitors, which were found to induce the phosphorylation of IRS proteins, followed by their proteolytic destruction. These tyrphostins were found to block the growth of a wide range of cancer cell lines including human ovarian cancer and melanoma in immuno-compromised mice.

The meeting was supported by Teva, Scifinder, Sigma-Aldrich, Accelrys, Bar-Gal, Di-Chem, Tel Aviv University School of Chemistry, Bar-Ilan University Department of Chemistry, Hebrew University School of Pharmacy.

On the organizing committee were: Dr. Hanoch Senderowitz, Bar Ilan University Dr. Galia Blum, Hebrew University and Dr. Micha Fridman, Tel Aviv University.

On the Advisory Board were: Prof. Matityahu Fridkin, Weizmann Institute, Prof. Amiram Goldblum, Hebrew University, Dr. Ashraf Brik, Ben-Gurion University.

Nano Spray Dryer B-90
Submicron particles of minimal powder quantities at highest yield

Dr. Cordin Arpagaus, Business Area Manager Spray Drying, Büchi Labortechnik AG, Switzerland, +41 71 394 65 08, arpagaus.c@buchi.com

1. Introduction

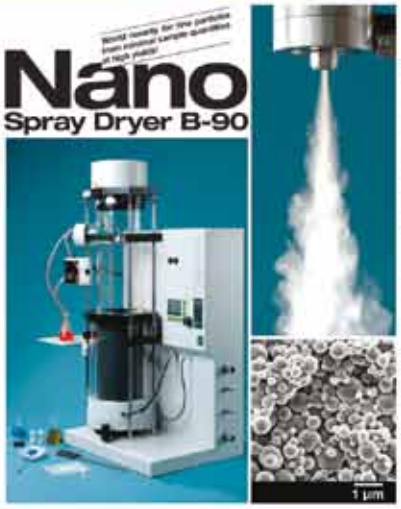
Spray drying is gaining greater attention as a **gentle, continuous** and **scalable** drying process to convert liquids to dry powders.

The new **Nano Spray Dryer B-90** is particularly suited to the needs of the **pharmaceutical, biotech, material** and **nanotech** markets. These areas show the newest application trends, focusing on effective formulation of complex and valuable drugs and nanoparticules.

Key benefits:

- Invest only a **minimal sample amount** to receive a dry powder
- Produce **submicron- or even nanoparticles** with **very narrow particle size distribution**
- Profit from minimal loss of high valuable products due to **highest yield**
- Safe process time thanks to **simple assembling, easy cleaning** and **fast product switch**

2. Nano Spray Dryer B-90 – world novelty



Technical data:	
Particle size	300 nm – 5 µm
Droplet size	3 – 21 µm, SPAN < 1.6
Typical yield	up to 90%
Evaporation capacity	max. 0.2 L/h H_2O, higher for solvents
Sample volume	1 – 200 mL
Drying gas flow rate	80 –160 L/min
Heating power	max. 1400 W
Inlet temperature	max. 220 °C
Spray head with piezoelectric vibrating mesh	
Electrostatic particle collector	
Dimensions (W x H x D)	58 x 110/150 x 56cm
Weight	65/70 kg table top

The **Nano Spray Dryer B-90** offers quick and gentle drying of aqueous and organic solutions, nanoemulsions or nanosuspensions to finest powder.

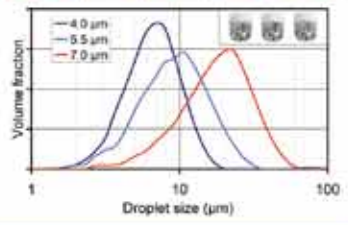
It is the ideal laboratory spray dryer **for new breakthroughs in R&D** on innovative nanomaterials.

3. Droplet generation

The Nano Spray Dryer B-90 provides finest droplets generation by a **unique piezoelectric driven spray head** incorporating a thin perforated **vibrating membrane** in a small spray cap.

The membrane features an array of precise micron-sized holes. The actuator is driven at an ultrasonic frequency, causing the membrane to vibrate, ejecting millions of precisely sized droplets with very **narrow droplet size distribution**.

Spray caps (hole size)	Mean water droplet size [4,3]	Span	Water flow rate
4.0 µm	8 µm	< 1.4	20 mL/h
5.5 µm	15 µm	< 1.6	60 mL/h
7.0 µm	21 µm	< 1.6	150 mL/h



4. Applications and SEM photographs

5. Electrostatic particle collector

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6. Table top instrument

Nano Spray Dryer B-90 in the lab. Configuration for spray drying of aqueous solutions in air with the aspirator and inlet filter.

7. Application areas

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Nanomaterials

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Charles Dreyfus (1848-1935), Yellow Dyes and the Balfour Declaration

Bob Weintraub

Charles Dreyfus:

Charles Dreyfus was born at Mulhouse, Alsace, and earned his Ph.D. in Chemistry at the University of Strasbourg. At age 21 he immigrated to England. He was active in Manchester local politics, served on the city council, was President of the East Manchester Conservative Association and was for 35 years a magistrate. Dreyfus was involved in Jewish affairs and in the Zionist movement. He became President of the Manchester Zionist Society and was provincial vice president of the English Zionist Foundation meeting in Liverpool in 1903. He was distantly related to Alfred Dreyfus, the central figure in the Dreyfus Affair.

Clayton Aniline Company:

In 1876 Dr. Charles Dreyfus together with friends founded the The Clayton Aniline Company, Ltd. Charles Dreyfus was the first managing director, a position that he held for 35 years until retirement in 1913. At that time the company employed 244 workers and a staff of 56. The company was set up next to the Manchester, Stockport & Ashton Canal. The main products at the start were benzene, toluene, and xylene for the water-proofing industry, and aniline, aniline salts and toluidines for the dyeing and printing trades. In 1911 Clayton Aniline was bought out by Ciba, later Ciba Specialty Chemicals, and operated until 2007, at which time the site was demolished to make way for a housing

Dr. Charles Dreyfus in 1906 arranged a meeting between the then British Prime Minister Arthur James Balfour and Dr. Chaim Weizmann. That meeting influenced the history of the Jewish People and the history of the world.

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development. It produced textile dyes and color formers used in carbonless copying paper and thermal paper and for other imaging products.

"During the 1880s, at this crucia1 time for Clayton, a number of discoveries were made which gave new impetus to research and development in the industry. In 1884 Böttiger, a German professor of chemistry, found that cotton could be directly dyed with a dye that he had made in the laboratory and which was later called Congo red. Before, it had always been necessary to treat cotton fabric with mordants before it could be dyed with the synthetic dyes then current. The idea of dyeing cotton without fuss using the new direct dyes at once appealed to dyers. The race among dye makers to develop and manufacture these novel colouring matters for commercial use was on. Some of them were simple variations on the theme of Congo red, others were modelled along quite different lines.

1n 1887 a young chemist of 23, Arthur George Green [later, Prof. Green, FRS], who was then working in London for Brooke, Simpson and Spiller, discovered the direct dye, primuline. It had substantivity (or power of attachment) for cotton fibres but was quite different in structure from Congo red. Moreover it could be treated on the fibre and there combined with a range of components giving azo dyes of deeper shades at will. This and other properties secured for primuline a lasting place in the dyer's range. To Green's mortification, his firm gave him no encouragement and greeted his brilliant discoveries with indifference.

Meanwhile Dreyfus, recognising at once the importance of the direct dyes, applied himself to their development with characteristic energy though with slender resources. By 1893 the Company managed to offer to the trade a small range, mainly yellows, which included Clayton yellow, oxyphenine, and cotton browns. In the same year Dreyfus patented a process for a yellow, afterwards called nitrophenine, though it did not long survive competition, having somewhat inferior dyeing properties." (E. N. Abrahart, The Clayton Aniline Company Limited, 1876-1976)

"Dr. Dreyfus, however, was always keenly interested in the establishment of a dye industry in England and, realizing the importance of continuous experimental work to that end, he endeavoured from the start to build up and maintain an active research organisation at Clayton. With this end in view, he induced A. G. Green, shortly after his discovery of Primuline, to go to Clayton and to take charge of the laboratories, and from that time the Clayton Aniline Co. steadily produced new dyes for the dyeing trade, principally yellows derived from primuline or from dinitrostilbene and their homologues. Among these developments should be mentioned the manufacture in 1898 of the first sulphur black by a synthetic method.

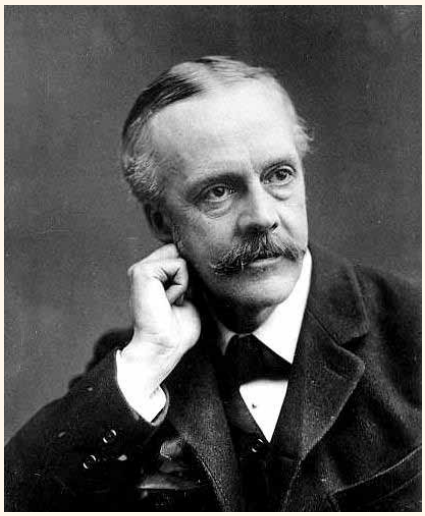
In these technical developments, Dr. Dreyfus himself played an active part. In the twenty years, 1889-1909, specifications bearing his name dealt with the manufacture of intermediates, e.g. the sulphonic acids of phenyl- and tolyl-beta-naphthylamine, aromatic sulphinic acids, sulphonyl chlorides, phenol ethers, and with the manufacture of hydrochloric, nitric, and acetic acids, the preparation of azo dyes from primuline and dehydrothio-p-toluidine sulphonic acid, lakes from basic dyes, dyes from phenolphthalein, and the preparation of indigo and numerous indigoid dyes. Other specifications relate to the vulcanization, purification and recovery of rubber; the waterproofing process; and the utilization of iron residues for the treatment of sewage." (R.Brightman, J Soc Dyers Col, 1936)

Chaim Weizmann: Uganda or Zion.

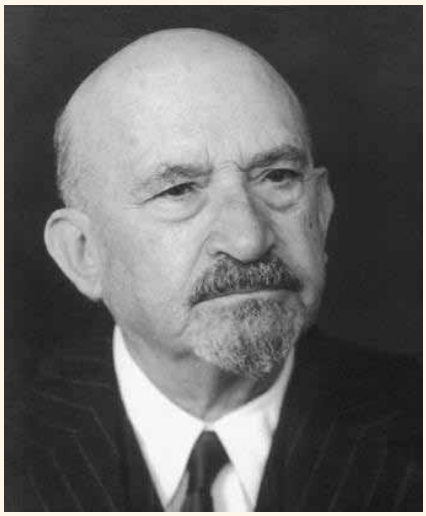
Charles Dreyfus extended kindness and friendship to Chaim Weizmann during the latter's early years in Manchester and they remained lifelong friends. Before coming to England in 1904, Weizmann had earned his Ph.D. in the area of dye chemistry and had worked in that field. In 1904 Weizmann was granted a research fellowship at Manchester University. In 1905 Dreyfus hired him on a part-time consultancy that continued until 1908. Weizmann's dye research for Clayton included work on the naphthacene quinones. (Kauffman, G.B. and Mayo, I., JCE, 1994; JCS, Bergmann, E.D., 1953)

Dreyfus was looking for new directions for the firm, one of these being the manufacture of synthetic camphor, for use in medicine and in great demand as a plasticizer in the manufacture of xylonite (or celluloid) from nitrocellulose. All the material then in use was the

Dr. Charles Dreyfus



Arthur James, First Earl of Balfour, at the time of his first meeting with Dr. Chaim Weizmann.



Dr. Chaim Weizmann

natural product imported from Japan. As a result of the Russo-Japanese War of 1904, camphor supplies were available in reduced quantities and at a higher price. Preliminary studies indicated that it might be feasible to make synthetic camphor from turpentine. Weizmann and Clayton Aniline were jointly awarded 12 patents—including patents for work on the manufacture of camphene, isobornyl esters and a process for purifying turpentine for use in the manufacture of pinene hydrochloride. A special plant was built but due to cost problems and turn of events, the project was not further developed.

Isaiah Berlin: "In 1903 public recognition at last came to the Zionist movement. The British Foreign Office, whose head was Lord Lansdowne, made a tentative approach to the Zionist leaders in England with regard to the Jewish colonization of a portion of the East African protectorate in the territory of Guas Nigishu-Platan, 5,000 square miles in extent. This proposal, the initiative for which came from the British Colonial Secretary, Joseph Chamberlain, was for the Zionists a cardinal event. It was the first time that the Jews had been recognized as a national entity by a great sovereign state-indeed, the most powerful in the Western World. Earlier efforts to obtain a territory—in British Cyprus, or in El-Arish in the Sinai Peninsula, to which the Turks could offer less resistance-had come to nothing. The Zionist movement was upset and excited. The proposed territory, commonly, if incorrectly, referred to as Uganda, was not Palestine; but it was a concrete offer. A great debate broke out at the Zionist Congress. Herzl was inclined to accept the proposal as at any rate the first stage in the great Return. Others, for the most part Russian Zionists, were against this scheme: Zionism without Zion had no meaning for them. It was to be brought back to Zion that Jews prayed thrice daily. It was only Jerusalem that could create and justify the vast uprooting that the new life involved." (in *Chaim Weizmann*, eds. Weisgal M. W. and Carmichael, J., 1963)

In 1906, East Manchester was Prime Minister Balfour's parliamentary constituency, and Balfour came to Manchester to fight for his seat. Charles Dreyfus was

Balfour's campaign chairman. The election results were a landslide victory for the liberals and a major defeat for the conservatives. In Manchester, the liberals won in every constituency and Balfour was swept out of office.

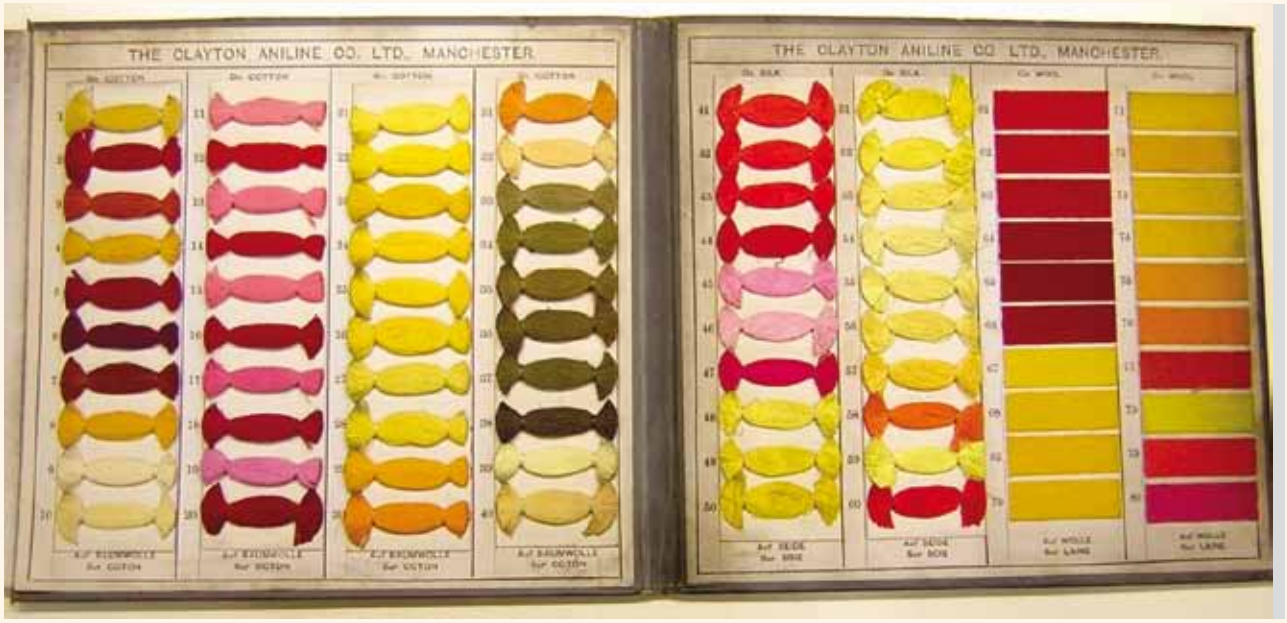
Cham Weizmann: "In the midst of the confusion and hullabaloo of the campaign, Balfour, at Dreyfus's suggestion, consented to receive me. He was interested in meeting one of the Jews who had fought against the acceptance of the Uganda offer made by his government." The meeting was scheduled to last for 15 minutes between other appointments, "Dreyfus warned me—simply to break the monotony of his routine," but lasted an hour and a quarter. "I had been less than two years in the country, and my English was still not easy to listen to. I remember how Balfour sat in his usual pose, his legs stretched out in front of him, an imperturbable expression on his face. We plunged at once into the subject of our interview. He asked me why some Jews, Zionists, were so bitterly opposed to the Uganda offer. The British Government was really anxious to do something to relieve the misery of the Jews; and the problem was a practical one, calling for a practical approach. In reply, I plunged into what I recall as a long harangue on the meaning of the Zionist movement. I dwelt on the spiritual side of Zionism, I pointed out that nothing but a deep religious conviction expressed in modern political terms could keep the movement alive, and that this conviction had to be based on Palestine, and on Palestine alone. Any deflection from Palestine, was—well, a form of idolatry. I added that if Moses had come into the sixth Zionist Congress when it was adopting the resolution in favour of the Commission for Uganda, he would surely have broken the tablets once again. We knew that the Uganda offer was well meant, and on the surface it might appear the more practical road. But I was sure that—quite apart from the availability and suitability of the territory—the Jewish people would never produce either the money or the energy required in order to build up a wasteland and make it habitable, unless that land were Palestine. Palestine has this magic and romantic appeal for the Jews; our history has been what it is because of our tenacious hold on Palestine. We have never accepted defeat and have never forsaken the memory of Palestine. Such a tradition could be converted into real motive power, and we were trying to do just that, struggling against great difficulties, but sure that the day would come when we would succeed.

I looked at my listener, and suddenly became afraid that this appearance of interest and courtesy might be nothing more than a mask. I felt that I was sweating blood and I tried to find some less ponderous way of expressing myself...Then suddenly I said: 'Mr Balfour, supposing I were to offer you Paris instead of London, would you take it?' He sat up, looked at me, and answered: 'But Dr. Weizmann, we have London.' 'That is true,' I said, 'But we had Jerusalem when London was marsh.'

He leaned back, continued to stare at me, and said two things which I remember vividly. The first was: 'Are there many Jews who think like you?' I answered: 'I believe I speak the mind of millions of Jews whom you will never see and who cannot speak for themselves, but with whom I could pave the streets of the country I come from.' To this he said: 'If that is so, you will one day be a force.' Shortly before I withdrew, Balfour said: 'It is curious. The Jews I meet are quite different.' I answered: 'Mr. Balfour, you meet the wrong kind of Jews.'" (*Trial and Error*, *The Autobiography of Chaim Weizmann, 1949*)

Balfour never forgot that meeting. Eleven years later, on November 2, 1917, Arthur James Balfour in his capacity as Foreign Minister in Lloyd George's coalition government signed the famous letter now known at the Balfour Declaration. The document declared that His Majesty's Government viewed with favour the establishment in Palestine of a national home for the Jewish People. The British Mandate over Palestine including the Balfour Declaration was formalized by the 52 governments at the League of Nations on July 24, 1922.

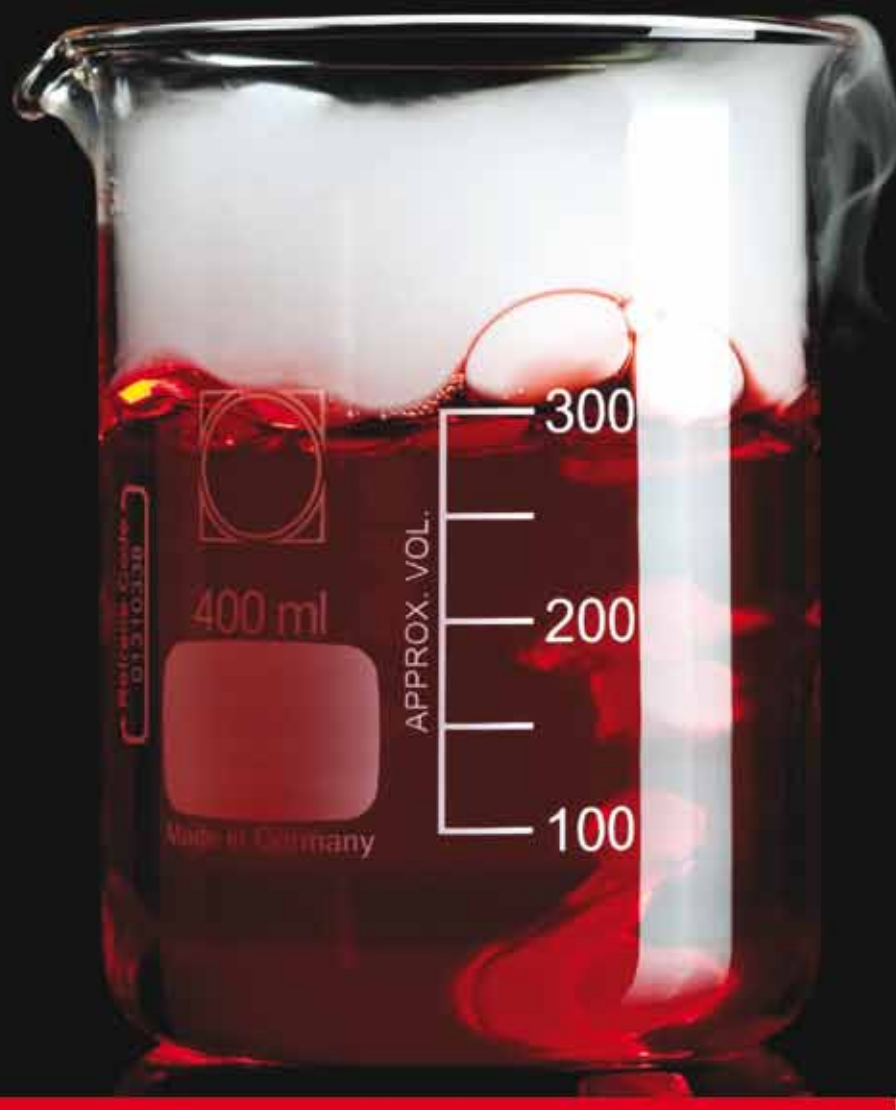
Blanche Dugdale was Balfour's niece and biographer. She worked at the Jewish Agency in London in close collaboration with Chaim Weizmann. She heard the contents of the meeting from both participants:
"It illustrates the importance of personal equations which can influence—but not determine—the course of history. Balfour once pointed to Weizmann as 'the man who made me a Zionist,' and when he said this he was referring to the Manchester conversation. But it should never be forgotten that Balfour was not the originator of the policy of the Balfour Declaration. The British Government took its decision upon that policy on more



Clayton Aniline Co., Ltd. pattern card. Aniline colours on cotton, silk and wool. 80 mounted samples of coloured threads or cloth accompanied by instructions for use. Photographed at the Edelstein Center for History and Philosophy of Science, Technology, and Medicine, Hebrew University of Jerusalem.

sufficient grounds that the opinion of one of its members, even of one who in 1917 carried the weight and prestige of Balfour in Mr. Lloyd George's government. It is only accurate to describe him as the most eager, perhaps the most persuasive advocate in the cabinet of giving the Zionists an opportunity to try out their great experiment in Palestine. He was certainly the minister who had devoted the most time to studying the subject, but the interest of the Manchester episode centers at least as much upon Weizmann as upon him, and it brings out one of the most outstanding qualities of Weizmann's genius—his power of exposition...

Here I leave the story of the friendship fraught with consequences beyond our power to trace even in the present, still less in the future. The influence of two such men does not stop short with their generation, and who shall measure their influence upon one another? Balfour was the elder by more than thirty years. It had never been his lot to knock in vain at any of life's doors. He was Prime Minister of Britain when he first met the young Jew born and bred in a poor and lonely village among the woods and waters of the Pripet region of Czarist Russia. It would seem that if these two were by a freak of chance to make contact at all, there could be no reciprocal give and take. Nevertheless, the day was to come when Balfour, talking over with me his fifty years of public service, could say: 'I think that my work for Zionism is probably the most worth while of any of it.' Humanely speaking, it appears that Balfour owed to Chaim Weizmann the comprehension of why that work was so 'worth while.' It was that work which gave him a zest and happiness that never failed or wavered." (in *Chaim Weizmann*, ed. Weisgal, M. W., 1944)



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