THE BIOLOGICAL PHYSICIST

The Newsletter of the Division of Biological Physics of the American Physical Society

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In this Issue

From the Editor

Dear DBP Members,

Your editor is moving! This won't interrupt publication of THE BIOLOGICAL PHYSICIST, but it will shift my contact coordinates. As of May 1, my new mailing address will be

Dr. Sonya Bahar, PhD
Department of Neurological Surgery
Weill Cornell Medical College
New York Presbyterian Hospital
525 East 68th St., Box #99
New York, N.Y. 10021

My Cornell email & office phone haven't been set up yet, but thanks to the wonderful the APS email alias system, I will still be reachable at bahar@neurodyn.umsl.edu. I will be in transit during the last two weeks of April, so don't expect quick responses to email between 4/17 and 5/1. For editorial emergencies during this time I can be reached by cell at (314) 713-8200. The next issue of the newsletter will come out in late June.

On a personal note, I want to thank everyone in at the Center for Neurodynamics at the University of Missouri – St Louis, particularly Frank Moss, for making the last two and a half years a wonderful experience!

Sonya Bahar

DNA NANOTECHNOLOGY: LIFE'S CENTRAL PERFORMER IN A NEW ROLE

Dr. Nadrian C. Seeman

Since Watson and Crick's determination of its structure nearly 50 years ago, DNA has come to fill our lives in many areas, from genetic counseling to forensics, from genomics to deliberate genetic engineering. These, and other ways in which DNA affects human activities, are related to its function as genetic material, not just our genetic material, but the genetic material of all living organisms. Here I wish to ignore DNA's role in the biological context; rather, I will discuss how the properties that make it so successful in performing as genetic material also make it a convenient and logical molecule to use for constructing new materials on the nanometer scale. The well-known B-DNA double helix is about 20 Å wide, and its helical repeat is 10-10.6 nucleotide pairs for a pitch of 34-36 Å. Thus, constructions made from DNA will have nanoscale features.

We are all aware that the DNA found in cells is a double helix consisting of two antiparallel strands held together by specific hydrogen-bonded base pairs; adenine (A) always pairs with thymine (T), and guanine (G) always pairs with cytosine (C). This arrangement of the two molecules leads to a linear helix axis, linear not in the algebraic sense of being a straight line, but in the topological sense of being unbranched. It is worth pointing out that the complement of a DNA strand is only unique and well-defined if the complement contains no branches [1], so linearity may be regarded as a necessity for faithful replication. Genetic engineers discovered in the 1970's how to splice together pieces of DNA to add new genes to DNA molecules [2], and synthetic chemists worked out convenient syntheses for short pieces of DNA (up to ~100-150 units) in the 1980's [3]. Regardless of the impact of these technologies on biological systems, hooking together linear molecules leads only to longer linear molecules, although circles, knots and catenanes may result from time to time.

The missing element to make DNA the basis for interesting materials is a branch point in the helix axis. However, branch points aren't missing at all! They occur as ephemeral structures in cellular DNA metabolism, most prominently in the Holliday [4] structure, a 4-arm branched intermediate in the process of genetic recombination. It is fairly easy to design sequences of DNA molecules that lead to stable synthetic variants of Holliday junctions, other branched molecules, and more complex motifs [5], to synthesize their constituent strands, and to get them to

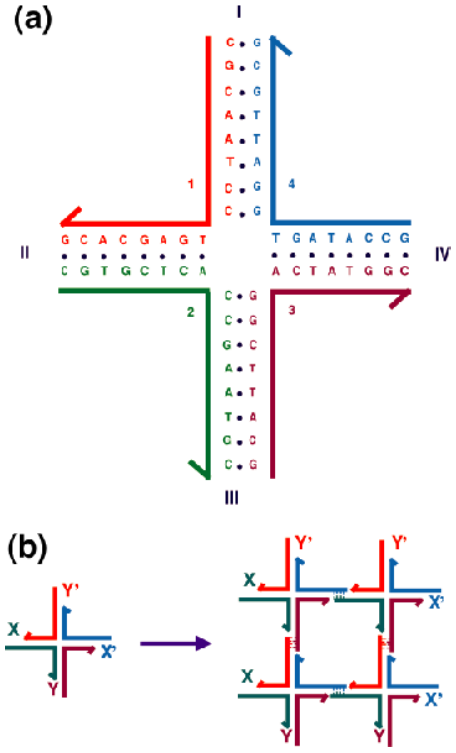

Figure 1. *(a) A Branched Molecule with Four Arms. The four strands labeled with Arabic numerals combine to produce four arms, labeled with Roman numerals. Arrowheads indicate strand polarity. (b) Formation of a Two-Dimensional Lattice from a Four-Arm Junction with Sticky Ends.* ***X*** *is a sticky end and* ***X'*** *is its complement. The same relationship exists between* ***Y*** *and* ***Y'****. Four of the monomeric junctions on the left are complexed in parallel orientation to yield the structure on the right. Note that the complex has maintained open valences, so that it could be extended by the addition of more monomers.*

self-assemble into target systems. The use of branched intermediates allows us to make N-connected objects [6] from DNA, as well as periodic [7] and aperiodic [8] arrays. Similarly, branched DNA motifs have been the basis for several nanomechanical devices [9].

How does this all work? A simple four-arm branched junction is shown in Figure 1a. The system maximizes the base pairing between its four component strands by forming the structure shown. In addition to having strands that are completely paired with one another, it is also possible to have one strand a little longer than its complement, leading to an overhang. This overhang, called a 'sticky end', is the way in which genetic engineers direct double helices to associate. Likewise, it is possible to direct branched molecules to associate by using sticky ends. This is shown in Figure 1b. The complementary sticky end pairs {**X**, **X'**} and {**Y**, **Y'**} are shown to cohere so as to produce a quadrilateral on the right. In addition, there are sticky ends on the outside, so that a crystalline array of junctions might be formed. Sticky ends are typically 4-8 bases long, and cohere with good fidelity; the ability to direct cohesion through sticky-ended complementarity is straightforward. However, there is a second key feature to sticky ended cohesion: Sticky ends form B-DNA when they cohere [10], so that the local geometry of the cohesive system is known without performing a new experiment (e.g., a crystal structure determination) every time a new sticky end is designed. Thus, the use of sticky ends is convenient because the intermolecular structures formed are predictable, because complementarity is easy to program, and because there is a great diversity of possible sequences available.

Why would anyone wish to produce such target species? We expect these systems can be applied to several practical ends: The initial motivating goal for this research is that spatially periodic networks are crystals. If we can build stick-figure crystalline cages on the nanometer scale, they could be used to orient other biological macromolecules as guests inside those cages, thereby rendering their 3D structures amenable to diffraction analysis. Of course, the same types of crystalline arrays could be used to position and orient components of molecular electronic devices with nanometer-scale precision [11]. Furthermore, nanomechanical devices can lead to a nanometer scale robotics. In addition, DNA nanotechnology creates motifs that are likely to be useful for DNA-based computation and algorithmic assembly of materials [8].

What are the reasons to use DNA for these purposes? The key reason has been described above: In the area of intermolecular recognition, DNA is the champion molecule, from the perspectives both of predictable affinity and predictable local product structure. However, the field has been accelerated by the presence of convenient automated chemistry [3], applicable both to 'vanilla' DNA and to exotic bases. The existence of commercially-available modifying enzymes, such as ligases (to join cohesive ends covalently), restriction endonucleases (to cut DNA at specific 4-8 base-long sequences), exonucleases (to digest failure products that do not contain cyclic strands) and topoisomerases (to change and analyze the topological linking of DNA strands) have all contributed to the growth of the field. Even when it is base-paired, the sequence of DNA can still be read from the outside [12], so points on a construct can be recognized uniquely. DNA is also a stiff molecule; its persistence length is about 500 Å, about 15 double helical turns, under standard conditions [13], and we work with lengths of double stranded DNA about 2-3 turns long. If one were to compare DNA to spaghetti, whose persistence length is about 15 mm, the DNA lengths used correspond to pieces of spaghetti 2-3 mm long, a distance over which its axis bends very little. The high functional group density on DNA is also an asset; large DNA tiles can be derivatized down to

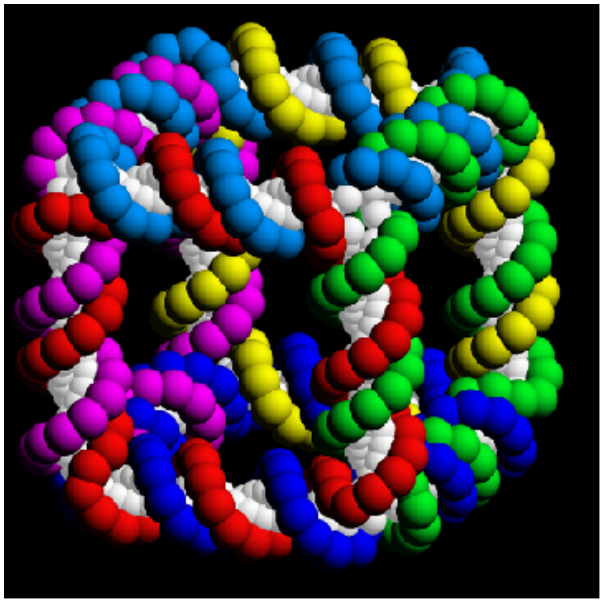

Figure 2. *A DNA Molecule with the Connectivity of a Cube. The six backbone strands are represented by the colored balls, and the bases are all drawn in white. Note that the molecule is a hexacatenane, with the six strands linked to each other. Each single strand corresponds to a face of the molecule.*

separations of a single base unit, 3.4 Å. Current work with DNA focuses on conventional molecules, particularly because of the enzymes available to trouble-shoot experiments. However, the gene therapy enterprise has produced a large numbers of backbone and base variants that may be better suited to particular applications [14].

How far has this effort progressed? The first clear success of DNA nanotechnology was the

construction of a 3-connected DNA molecule whose helix axes were connected like the edges of a stick cube [6]. This object is shown in Figure 2. It consists of six cyclic single strands of DNA, one for each face of the polyhedron. Each edge consists of two turns of double helical DNA. The red strand corresponds to the front face; owing to the helical character of DNA, it is linked twice to the green strand on the right, the cyan strand on the top, the magenta strand on the left and the dark blue strand on the bottom. It is linked only indirectly to the yellow strand at the rear. Individual branched junctions are floppy, so only their topology can be designed (or analyzed), not their geometry. In this case the topology was established by electrophoretic analysis of cubes cleaved by specific restriction endonucleases; every edge contained a unique site for cleavage by a restriction enzyme.

We have been successful in producing a number of specific two-dimensional arrays. Components with greater structural integrity are needed to generate periodic matter, and these have come from DNA molecules containing multiple branch points that link unbent helices. An example is

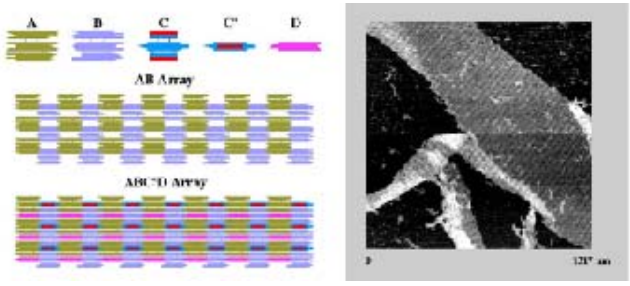

Figure 3. *A Two-Dimensional Crystalline Array of Triple Crossover Molecules. The four components of this array are shown at the top of the schematic on the left.* ***A***, ***B***, ***C***, *which are TX molecules, and* ***D***, *which is a conventional double helix. Sticky ends are represented geometrically.* ***A*** *and* ***B*** *can be connected 1-3 to tile the plane, which is shown below the components. This arrangements leaves gaps that can be filled by a single DNA double helix, such a* ***D***. *However, if another TX molecule is rotated by three units from planarity (102˚), it, too can fit in the gap.* ***C'*** *is the* ***C*** *TX molecule that has been rotated in this fashion. Its middle domain fits in the* ***AB*** *plane, with its other helical domains extending both above and below this plane. The AFM image on the right shows this feature clearly.*

shown in the top portion of Figure 3, which shows a motif known as a DNA triple crossover (TX). Three helices are linked to each other, twice each, in an arrangement as coplanar as possible. Two TX molecules (or tiles), **A** and **B** are shown at the upper left. Below them is a lattice formed by connecting them 1-3, so that there are helix-wide gaps in the

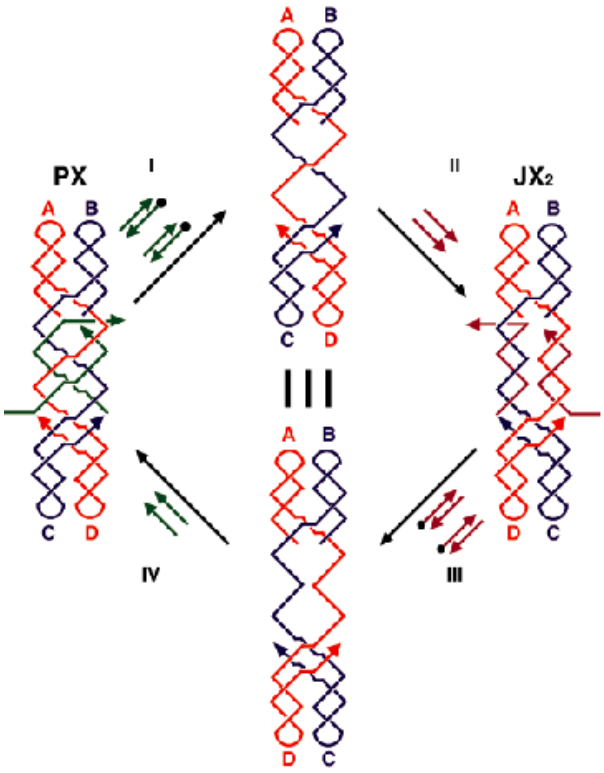

Figure 4. *A Sequence-Dependent Device. This device uses two motifs, PX and* JX_2. *The labels* ***A***, ***B***, ***C*** *and* ***D*** *on both show that there is a 180˚ difference between the wrappings of the two molecules. There are two green strands at the center of the PX motif, and two purple strands at the center of the* JX_2 *motif; in addition to the parts pairing to the larger motifs, each has an unpaired segment. These 'set' strands can be removed and inserted by the addition of their total complements (including the segments unpaired in the larger motifs) to the solution; these complements are shown in processes I and III as strands with black dots (representing biotins) on their ends. The biotins can be bound to magnetic streptavidin beads so that these species can be removed from solution. Starting with the PX, one can add the complement strands (process I), to produce an unstructured intermediate. Adding the set strands in process II leads to the* JX_2 *structure. Removing them (III) and adding the PX set strands (IV) completes the machine cycle. Many different devices could be made by changing the sequences to which the set strands bind.*

array. The gaps can be filled with a double helix, **D**. They can also be filled by a TX molecule, if it is rotated three helical positions, to be about 102° from its original position, roughly perpendicular to the **AB** array. This molecule is shown as **C**, and its rotated version is shown as **C'**. The **ABC'D** array can be seen at the bottom of the figure. At the right is an atomic force micrograph (AFM) showing features that result from the **C'** molecule extending out of the **AB** plane. We have demonstrated with other 2D arrays that we can produce specific patterns from double crossover (DX) molecular tilings and we can build arrays containing cavities with tunable dimensions from parallelograms made from branched junctions [7]. Aperiodic self-assemblies corresponding to logical computations have also been produced [8].

The other area to which DNA nanotechnology is making a contribution is nanomechanical devices, with ultimate applications in nanorobotics [9]. The first such device contained two DX molecules separated by a DNA shaft containing a segment that could be converted to left-handed Z-DNA by adding $Co(NH_3)_6Cl_3$ to the solution. The rotary motion had a maximum displacement of 60 Å, and was demonstrated by fluorescence resonance energy transfer (FRET). To produce an effective nanorobotics, devices must be embedded in an array matrix, and they must be addressable individually, not by the addition of a small molecule to the solution. Therefore, we developed a new device, using a principle originated by Yurke *et al.*[9]. The machine cycle of this device is shown in Figure 4. The device is based on a motif called PX, in which two helices are joined by strands crossing over at all possible places. A related motif, JX_2, is similar, except that it lacks two crossovers in the middle. Note that the ends of PX molecule, on the left of Figure 4, and those of the JX_2 molecule, on the right, are the same on the top (**A** and **B**), but rotated 180° on the bottom (**C** and **D**). The green strands in the PX molecule have small (horizontal) extensions on them. When the complete complement, including the extensions, is added to the PX molecule, the green strands are removed from the PX molecule (process I), to leave the intermediate at the top. When the purple strands are added to the intermediate (process II), the JX_2 molecule is formed, rotating the bottom of the molecule. Processes III and IV restore the original PX conformation. The operation of this device has been demonstrated by electrophoresis and by AFM. A variety of devices can be developed by changing the sequences of the green strands, the purple strands and the sections of the device to which they bind.

Where is DNA nanotechnology going? The achievement of several key near-term goals will move DNA nanotechnology from an elegant structural curiosity to a system with practical capabilities. First among these goals is the extension of array-making capabilities from 2D to 3D, particularly with high order. Likewise, heterologous molecules must be incorporated into DNA arrays, so that the goals of both orienting biological macromolecules for diffraction purposes and of organizing nanoelectronic circuits may be met. A DNA nanorobotics awaits the incorporation of the PX-JX_2 device into arrays. Algorithmic assembly in 3D will lead ultimately to very smart materials, particularly if combined with nanodevices. The development of self-replicating systems using branched DNA appears today to be somewhat oblique [15], but it nevertheless represents an exciting challenge that will significantly economize on the preparation of these systems. Ultimately, DNA nanotechnology must advance from the biokleptic to the biomimetic, not just using the central molecules of life, but improving on them, without losing the inherent power they possess.

ACKNOWLEDGEMENTS

The science described above represents the efforts of many individuals, largely my co-authors; I wish to thank all of them for their contributions to the founding of DNA Nanotechnology. This work has been supported by grants GM-29554 from the National Institute of General Medical Sciences, N00014-98-1-0093 from the Office of Naval Research, grants CTS-9986512, EIA-0086015, DMR-01138790, and CTS-0103002 from the National Science Foundation, and F30602-01-2-0561 from DARPA/AFSOR.

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Minutes Of the Division of Biological Physics
Executive Committee Meeting
March 18th 2002

Attendees: Mark Spano, Robert Austin, Raymond Goldstein, Robert Eisenberg, Dan Gauthier, Sergey Bezrukov, Lewis Rothberg, Ken Dill, Sonya Bahar, Andrea Markelz.

6:30 pm, Cameral Room, Indianapolis Westin Hotel.

Minutes taken by Andrea Markelz.

[Minutes are reproduced in the newsletter in a condensed version.]

The meeting began with the acceptance of the minutes from last year's meeting.

Marty Blume, editor-in-chief of the Physical Review Journals, stopped by to discuss Physical Review Letters and Physical Review E. He noted that the recent accomplishment of including all of Physical Review journals on the Medline database will greatly enhance the presence of these journals in the biological physics community. He discussed the virtual journals now available through the APS. Currently the biological physics virtual journal appears biweekly. The next online effort is to have everything internally online as well: refereeing and editing of the submissions. He mentioned that the Physical Review Journals can not yet accept MSWord documents, mainly due to so many different Word versions, however they do accept PDF documents. They are also currently working on including more multimedia in the journals such as movies.

Mark Spano discussed the DBP Treasurer's report. This year's expenses include a $4000 projector for the March meeting and a $4200 journal budget bill, leaving the net balance at 35,000. Mark Spano discussed the Biological Physics Prize, and attempts to increase the current prize money from $5000 awarded every two years to $10,000 awarded yearly. Mark Spano and Bob Austin discussed attempts to create an endowment for this prize. APS's strategy for allocating money to different divisions was discussed. Mark stated that allocation of money is proportional to membership; the number of symposia for next year is determined by the number of contributed talks at the current APS meeting.

The discussion then turned to the website. The current links to biophysics programs need to be updated. Dan Gauthier, DBP Website Coordinator, suggested that he contact those investigators currently listed and suggest that their continued listing requires membership in the division. It was also suggested to obtain a complete listing of biophysics programs and contact the PIs within these programs, again to both increase membership and add links to web page.

Margaret Foster, Assistant Editor *at Physical Review E*, then stopped by to discuss PRE. It was proposed that biological physics articles in the current issue of PRE be automatically included and updated on the DBP website. The discussion again turned to how to increase the presence of PRE in the biophysical community.

APS Executive Officer Judy Franz then joined the discussion, which turned to the question of membership. There are two separate issues: 1.) recruitment of APS members who do biophysics and yet do not check off the DBP box on their membership form and 2.) recruitment of non-APS members. Membership in the Biophysical Society on the other hand is increasing at a much higher rate. There was discussion of the functions and perceptions of the Biophysical Society compared with the perception of DBP.

Among the concrete suggestions to increase membership were:
-Contact all new faculty in biophysics.
-Ask the editors for PRE and those appointed by DBP for the Biophysical J. to suggest to both referees and submitting authors to consider membership in the DBP.
-Have a booth at the Biophysical Society's Annual Meeting as well as other biological physics related conferences.
-Increase activities such as the currently planned fall workshop, "Opportunities for physicists in biology conference," for prospective graduate students. This workshop will be held in Boston,

Sept. 27-29, 2002 with a maximum attendance of 250.
-Offer joint membership in both the Biophysical Society and DBP/APS for ½ price.

APS Treasurer Thomas McIlrath discussed the financial prospects of APS for the coming year, including financial concerns stemming from the shift from paper to web-based journals.

Mark Spano discussed the executive committee members' duties. He said that six member-at-large positions exist without clear duties. Bylaw revisions have been suggested which would eliminate 1/2 of the number of members at-large and switch these positions over to appointed positions. Appointed positions would include Web "guru", newsletter editor, membership coordinator and/or a public affairs post. However, it was pointed out that members-at-large posts do actually serve as representatives of the community. The possibility of a public affairs liaison was discussed. The appointment of journal editors for the Biophysical Journal was discussed.

Minutes Of the Division of Biological Physics Business Meeting March 20th 2002.

Room 209, Indianapolis Convention Center, 5:30 PM, Wednesday March 20.

Minutes taken by Sonya Bahar.

[Minutes are reproduced in the newsletter in a condensed version.]

Mark Spano called the meeting to order and asked for a motion re reading last year's business meeting minutes. Several participants moved the minutes not be read, and they weren't.

Mark Spano presented an update on DBP activities over the past year. He said DBP has grown 10% or more over the past year, and is in the process of restructuring the leadership and rewriting bylaws. A new Executive Committee position, Membership Coordinator, is being considered.

DBP membership growth was discussed. The future of the Biological Physics Prize was discussed. Currently the prize is $5000 given every two years. APS wants to double the amount, and DBP would like to award the prize yearly. DBP is looking for a $200K endowment for the prize. Current plans for changing the bylaws, which include eliminating some of the member-at-large positions and replacing them with specifically named posts, were discussed.

The relationship between DBP and the Biophysical Society and Biophysical Journal was discussed.

Mark Spano said that the division is financially healthy aside from the Biological Physics Prize, and reviewed reports made from the APS leadership at the Executive Committee meeting Monday night. The main concern of the APS leadership, he said, is DBP membership.

Margaret Foster encouraged support of more biological physics PRE submissions and more DBP members volunteering to referee articles.

Peter Hanggi asked what the current membership in DBP is. Mark Spano and Ray Goldstein said the number is about 1700, or 3.5% of total APS membership. Ray Goldstein said 1700, and mentioned that the division is doing well in terms of number of invited symposium speakers, which is proportional to the number of contributed talks at the previous year's meeting.

Bob Austin was recognized for his role as DBP Program Chair for this year's March Meeting.

A complaint was made about too many parallel sessions. Bob Austin responded that some of our topics, such as protein structure, have been covered by DPOLY and GSNP, and that when he found this out he arranged that these become joint DPOLY/DBP or GSNP/DPB sessions. Mark Spano mentioned that there are more and more DBP sessions, which makes parallel sessions unavoidable.

Ray Goldstein mentioned that Judy Franz solicited comments on distribution of invited symposia. He said that perhaps we should ask for 9 or 10 invited symposia instead of 8, and urged everyone to solicit contributed talks for the 2003 March Meeting.

Margaret Foster mentioned that Poster Sessions are often neglected because they are in

parallel with oral sessions. She suggested that posters be left up longer.

There was an extensive discussion of the role of biophysics in PRE and PRL, and of the article review process in these journals.
It was mentioned that until a few years ago biological physics was not even listed as a topic in PRL, and that coverage in MedLine has increased visibility for biological physics.

Peter Hanggi commented that PNAS is not listed in the Virtual Journal of Biological Physics Research (http://www.vjbio.org/bio/). Bob Austin said he is working to get PNAS included.

It suggested that DBP sponsor prizes for graduate student and postdoc publications.

Mark Spano recognized Dan Gauthier for his work as Website Coordinator and Sonya Bahar for her editorship of the DBP newsletter. He reported that a motion was approved at the Executive Committee meeting that Gauthier and Bahar will continue in these posts for the coming year.

Sonya Bahar asked for suggestions for articles in the newsletter over the coming year, and mentioned that she wanted to give exposure to research by postdocs, graduate students and young faculty.

Mark Spano read the citations for the new APS fellows:

Thomas Harrison Foster, of the University of Rochester, for his unique and successful applications of physical concepts to the understanding and advancement of magnetic resonance imaging, photodynamic therapy and diffuse-light optical studies of tissues.

J. Raul Grigera, of IFLTSIB, for his role in developing the SPC/E model of water, which is perhaps the most widely used one in computer simulation of biological systems, and for his application to unveiling the structure of hydrated biomolecules.

Ned S. Wingreen, of the NEC Research Institute, for contributions to the fundamental understanding of protein folding and design, including theoretical insights into the selection of protein structures.

Mark Spano asked for nominations for new APS fellows for next year, and introduced the new DBP leadership. The new Chair Elect is Ray Goldstein, the new Vice Chair is Phil Nelson, the new Chair is Bob Austin, and Mark Spano is the new Past Chair. New Members-at-Large are Andrea Markelz and Leon Glass.

Mark Spano then turned the Chairmanship of the Division over to Robert Austin.

Photo by Dr. Jan W. Kantelhardt, Indianapolis, March 2002