From biologically inspired physics to physics inspired biology

The conference “From DNA inspired physics to physics inspired biology” (1-5 June 2009, International Center for Theoretical Physics, Trieste, Italy, that myself and two former presidents of American Biophysical Society - Wilma Olson (Rutgers University) and Adrian Parsegian (NIH) , with the support of local ICTP team (Ralf Gebauer and Doreen Sauleek),have organized was intended to establish stronger links between the two communities on the DNA front. The relationships between them where never easy. In 1997, Adrian published a paper in Physics Today “Harness the Hubris”) summarizing his thoughts about the main obstacles for a happy marriage. The bottom line of that article was that physicists must seriously learn biology before going into it and even having an interpreter friend/co-worker, who will be cooperating with you and translating the problems of biology into a physical language, may not be enough. He starts his story with a joke about a physicist asking a biologist:
“I want to study brain. Tell me something about it!”
Biologist: “First, the brain consists of two parts, and …”
Physicist: “Stop. You told me too much.”

Adrian listed few direct avenues where the physicist contributions may be particularly welcome. This gentle and elegantly written paper caused however a stormy reaction of Bob Austin (Princeton), published together with Adrian’s notes, accusing Adrian in forbidding physicists to attack big questions in biology straightaway. 12 years have passed and many new developments took place in biologist – physicist interaction. This was something I covered in my conference opening speech, with my position lying somewhere in-between Parsegian’s and Austin’, and, perhaps, it could make sense to briefly outline it here. I will recall first certain precepts or ‘dogmas’ that fly in the air as Valkyries, poisoning those relationships.

Since early seventies when I was a first year PhD student at the Frumkin Institute in Moscow attending hot theoretical seminars chaired by Benjamin Levich (1917-1986, a pupil of Landau and the founding father of physical – chemical hydrodynamics) , I particularly remember one of his many jokes he used to spice his seminar. When some overly enthusiastic speaker was telling us with 100% confidence how the electron transfers between atomic moieties in a solvent near an electrode, and what the molecules exactly do to promote the transfer, he used to ask the speaker: “How do you know it? Have you been there?”

Today this is no longer a question or even a joke. We have plenty of experimental tools to ‘get there’. The list of such techniques will be too long, name just FIONA (Fluorescence Imaging with Nanometer Accuracy) which allows to trace the motion of myosin on actin and similar aspects of protein motility in vivo and in vitro (Fluorescence methods where in the center of the Biological and Molecular Machine Program at Kavli ITP, Santa Barbara, where the founders of that techniques where teaching us what we can learn using them) or visualizing the positions of adsorbed counterions on DNA by synchrotron radiation,
So, here comes -

<u>Dogma 1</u>: “Seeing is believing”. Once, I have asked an Assistant Professor from one of the top US Universities, who was preaching such methods, had he tried to treat his data in some coordinates, where I would have expected his data lying on the straight line. The answer was, “Come on, what you speak about is the XX century science; no longer interesting!” I am afraid he was not unique in his generation, voting for what I would call “MTV-science.” This science does make you dance, but alone is not sufficient without a deep theoretical analysis of what you actually see. Otherwise, “what you see is what you get”…and not more.

Dogma 2: “A theory must contain not more than exponential functions, logarithms and alike. Otherwise the job should be left with computers. No Bessel functions, please!”

This point of view was advocated by my office mate at KITP, Rob Philips, Professor of Applied Physics and Molecular Biophysics at Caltech, who came to his new love - biology – from solid state theory. We had hot arguments about it. And my strongest was that it was the math of the now famous W. **C**ochran, F. H. C. **C**rick, and V. **V**and, 1952, Acta Crystallogr. 5, 581) paper that allowed Watson and Crick to decipher the roentgenograms of Franklin and Gosling, and Morris Wilkins. The CCV formula for the X-ray scattering intensity fully explained the structure of the famous cross of the scattering maxima on the (k_z, K)-map, where k_z and K are, respectively, the components of the scattering wave-vector transfer in the direction along the main axis of the columnar array of the DNA molecules and in the perpendicular plane. From the distance and the position of the darkest spots on that pattern it was possible to deduce that the studied DNA has a shape of a double helix, to find its radius, the width of the minor and major groove, vertical rise between base pairs and the helical pitch. There were still some features in that pattern which have not been noticed, which were understood half a century later [Kornyshev A.A., Lee D.J., Leikin S., and Wynveen A..--Structure and interactions of biological helices -- *Rev.Mod.Phys*. **79**, 943-996 (2007); A.Wynveen; D.J. Lee; A. A. Kornyshev; S. Leikin, Helical coherence of DNA in crystals and solution, *Nucleic Acids Research* **36**, 5540-5551 (2008)], but those were not essential for the structure of DNA itself, but rather for the understanding of DNA-DNA interactions and their effect on more subtle aspects of DNA structure, which are an issue today. And the ‘Bessel function’ was the key player in the CCV equation.

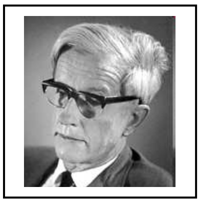
Dogma 3 is that “this happened once. Unlikely this will ever happen again” [from a conversation with Dan Herschlag (Stanford) on that revolution made by physics in biology]. A common opinion it is. Note, that of four discoverers of the DNA structure, three were physicists and only Watson was a biologist, and the key secret in that discovery was the ‘chemistry’ between an enthusiastic biologist (Watson) and physicist (Crick) that helped them to find common language, and as a result discover not only the structure but also the ‘function’ of DNA. Now we know that the machinery of DNA replication is very complex, promoted by motor proteins such as DNA helicase, polymer-rase, ligases etc., but the complimentary principle of synthesis of two identical DNA on the unwound complimentary single strands as temp-lates remains the same as mentioned in the famous phrase (“It did not escape our attention…”) of the first Watson-Crick paper.

Francis Crick James Watson Maurice Wilkins Rosalind Franklin

Dogma 4 (From a letter from Don Roy Forsdyke, Biochemistry Professor at Queens Ontario). “Biologists will not read a paper with formulae. Biological literature is vast. Biologists have too many papers to read and too many experiments to make. They will leave aside any reading that looks difficult”. If this is true, and I think it is, we are in big trouble, bring us to the next dogma.

Dogma 5: (Catch 22)
It is impossible to publish a serious theoretical paper in a biological journal. Physicists, particularly, theorists need derivations to prove validity of their findings. But with the derivations in the script, the paper will be rejected. If you still publish it in a physical journal it will not be read by those to whom it is addressed.

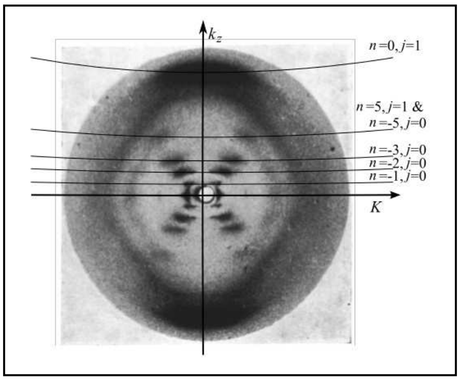
Dogma 6. Physicists are too ignorant to offer biologists anything useful. Perhaps, some new spectroscopic method or apparatus for force measurement, but that's about it…. Leave biology to professionals. Full stop. I make no comments about this extreme point of view, referring the reader to the dispute between Parsegian and Austin, which is still quite actual today.

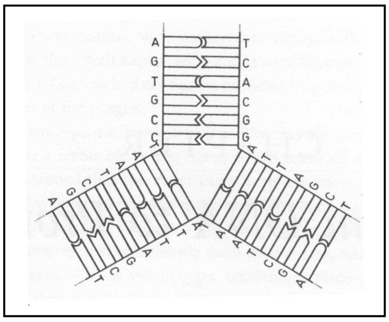


Next, a wisdom of a theoretical physicist, Nobel Laureate in Physiology and Medicine, Max Delbrück (Caltech), formulated in his 1949 lecture in Copenhagen: the principles on which organism of today is based must have been determined by a couple of billion years of evolutionary history; "*you cannot expect to explain so wise an old bird in a few simple words*". It is indisputably so, but it is followed by two other competing sub-dogmas:

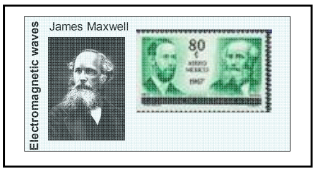
Dogma N6a: Physics wants to simplify and unify things, as much as possible, biology resists the reductionist approach and is happy about diversification and complexity.

To my opinion all these dogmas have been beaten by this icon, understanding of which gave rise to the idea of DNA replication and all the following principles of molecular biology. Not only 'this will happen again' but on a smaller scale this happens all the time.

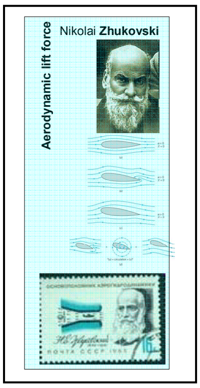




Generally, through centuries, physics and mathematics has changed our life completely. In a short article one cannot give a list of such achievements from the Aristotle times, but name just a few of the summits of the last two centuries. Ernest Rutherford (who was, by the way, Nobel Laureate in *Chemistry*) was also famous by an extreme (an definitely outdated) statement: "All science is either stamp collecting or physics.". Let, us paraphrase him and collect some stamps.



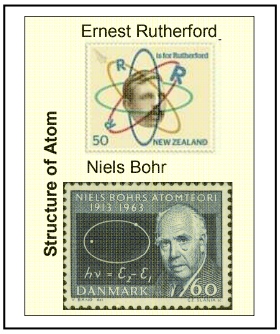
I have no room to stop on Faraday-Ampere laws of stationary electricity (who cares, electric current comes from a plug would be the answer of most of the people unfamiliar with physics, and… forget about electricity that is supplied to biological laboratories). So, let us go straightaway to James Clerk Maxwell. He derived four equations that related electricity and magnetism and, as the legend tells us, it has taken him seven years to write the fourth equation to complete the set with four unknown variables. The story of the fourth Maxwell equation is one of the most dramatic stories in the history of science [I. S. Shapiro*,* "On the History of the Discovery of *Maxwell's Equations*", Sov. Phys. Uspekhi, 15 (1973), 651; www.iop.org/EJ/article/0038-5670/15/5/A08/PHU_15_5_A08.pdf -.]. As a solution of that set he obtained relativistically-invariant electromagnetic waves, which no one ever saw at that time. Hertz understood how to generate them, Thomson how to receive them, and now we have the World all connected on line.



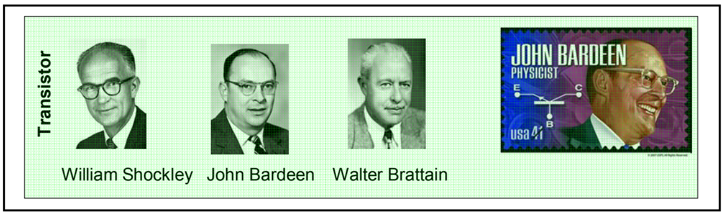
My next stamp goes to the Zhukovski equation of the hydrodynamics of the wing, which explained how to get the aerodynamic lift force. And now we can get from London to Washington in a third of a day, essentially due to that equation.

Of many things that Einstein discovered his energy-matter relation has led us to atomic power, whether we like it or not .

Rutherford and Bohr unraveled the structure of atom and all our material science followed from it.



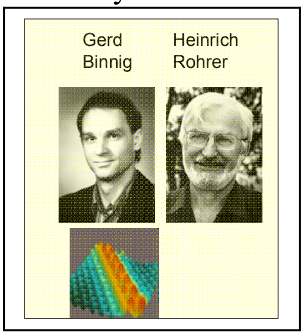
Discovery of a transistor made the world of electronics and computers possible,

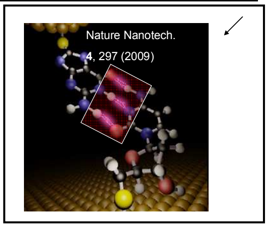


and, again - whether we like it or not - most of us spend daily many ours looking into the computer screen.

Crick’s equations and Franklin-Wilkins observations (made possible by Roentgen’s discovery that I missed to mention after Maxwell) gave rise to the world of molecular biology which is also easy to forget about in public, if not our ever grateful forensic experts.

Just two more milestones of much more ‘modest’ caliber. This is the discovery of lasers which are massively used for communication, in medicine and spectroscopy, including the biological research. Next, I mention the discovery of scanning probe techniques, which allowed us to see individual atoms. For these two I even did not find post stamps, but I am sure they must exist somewehere. The STM has just led Stuart Lindsey’s team (University of Arizona) to the some first steps towards ultrafast sequencing of DNA using functionalized STM tips.





At the Abdus Salam International Center for Theoretical Physics there is no need to convince anyone that involved mathematics and physics is needed. But neither we need to explain anyone there that applications of physics may be equally exciting as its fundamentals**.** The appreciation of massive achievements of physical methods of DNA research made it possible to host and massively sponsor this DNA conference at the ICTP. The conference was generously co-sponsored by the Welcome Trust (UK). It comprised of about 60 talks on topically focused sessions devoted to:

- DNA mechanics
- DNA structure, interactions and aggregation
- Recognition of homologous genes
- Conformational dynamics, supercoiling and packing
- DNA compactization in viruses
- DNA-protein interaction and recognition
- DNA in confinement (pores and vesicles)

• Smart DNA (robotics, nano-architectures, switches, sensors and DNA electronics)

The success of the conference was in that it was not a meeting of a club of physicists interested in biology, but a meeting of physicists, doing important works widely published not only in physical but also biological journals, with the leading biologists who, personally, were keenly interested in learning what new can physical methods and existing knowledge could offer them. They were equally eager to explain to physicists and mathematicians the most challenging paradigms of the molecular biology research. The conference was opened by two inspiring broad-impact talks, of a Director of the European Molecular Geneticists Center in Trieste, Arturo Falaschi , the Editor of HFSP, and by a scientist of next generation, Lynn Zechiedrich, Professor of Baylor Medical School and former co-worker of late Nick Cozzarelly. Both were showing astounding manifestations of polymeric behavior of DNA, where physics is just awaited as rain in the desert. But through the whole conference about 40% of lectures were delivered by biologists. In this short article it is not possible to cover even the most exciting presentations, and I refer interested readers to the website [http://cdsagenda5.ictp.trieste.it/full_display.php?ida=a08164] where one can get some valuable information about it. I will outline below just a couple of issues.

The conference revealed big progress in understanding the details of DNA mechanics, including its local sequence dependent elastic properties. Progress was achieved in understanding of the role of electrostatic interactions with ions and charged moieties that can influence the shape and elasticity of DNA, highlighted particularly in the studies of Jim Maher (University of Minnesota). Generally, the role of helical structure dependent, so called 'helix-specific' interactions on which the lecture of Sergey Leikin (NIH) was focused was unequivocally found to play a crucial role in the interaction, aggregation and assembly of DNA –from liquid crystals to intracellular compartments, as well as viral capcids.

One of the hottest sessions was devoted to the 'last great enigma' of genetic recombination: its 'zero' stage - the recognition of homologous genes. The big picture was overviewed in biological terms by Adi Barzel (following his 2008 'manifesto' article with Martin Kupiec in Nature Reviews). New experiments were then reported that showed that DNA can recognize its homology from a distance *without* unzipping and local base pair formation. The reported published experiments of an Imperial-NIH team [G.Baldwin et al, Duplex DNA recognize sequence homology in protein free environment, *J. Phys.Chem.B* **112**, 1060-1064 (2008)] widely discussed last year under a controversial notion of DNA-"telepathy" (in quotes, of course) were based on the direct observation of spontaneous segregation of homologous DNA in cholesteric liquid crystals. The reported, yet unpublished, beautiful experiments of the Harvard team (Mara Prentiss et al) were more involved and were based on application of magnetic beads technique (purely physical methods). These have unambiguously demonstrated the homology pairing at the double stranded DNA level, also giving evidence of unimportance of defect-based Watson and Crick pairing in this phenomenon. Both kinds of experiments supported the expectations of an electrostatic snapshot recognition mechanism behind the intact, double stranded DNA homology pairing [A.Kornyshev, S.Leikin, Sequence recognition in the pairing of DNA duplexes, *Phys.Rev.Lett* **86***,* 366 (2001); A.A.Kornyshev and A.Wynveen, The homology recognition well as an innate property of DNA structure, *Proc. Natl. Acad.Sci USA,* **106**, 4683 (2009)]. But none of them has yet systematically studied its various features, after which one could consider the mentioned mechanism experimentally confirmed. Discussions at breakout meetings referred to the experiments to be done, that might finally turn down the last presumption of molecular biology that only the Watson and Crick pairing can provide recognition, i.e. that the recognition between intact double stranded DNA is impossible. Notable the suggested electrostatic snap-shot recognition mechanism is also based on the helical structure of DNA and correlation of the structure with the text of the sequence.

DNA packing in chromatine and cromatine dynamics were in the center of attention of the conference. Andrew Travers (Ecole Normale Superiore de Cachan), exposed the problem in all its biological complexity, followed by the physical insight into its modeling, overviewed by Helmut Schiessel. Using different kind of single molecule pulling experiments Jörge Langowski (University of Heidelberg) and David Bensimon (Laboratoire Physique Statistique, Paris) exposed invaluable insights into the nucleosome opening and the role of remodeling factors. Jim Kadonaga (UCSD) reported a discovery of a new ATP driven motor-protein, exhibiting annealing/reverse helicase activity. Lars Nordensiöld (Singapore Nanyang TU) has established the sequence of counterions promoting DNA compactization in chromatin, and so on.

Another class of astounding results was related with the structure of DNA phases, coils and toroids in viral capcids, understanding of which at the nanoscopic level is instrumental for the development of antiviral therapies. Bell Gelbart (UCLA) and Avi Ben Shaul (Hebrew University of Jerusalem) highlighted various aspects of packing inside the capcids, as well as how viral DNA or RNA can get in and out. Amazing observations of Francoise Livolant has shown the local liquid crystalline structure of DNA in that dense packing. The experiments of her group have unambiguously demonstrated azimuthal correlations between the densely packed double strands, in agreement with similar effects detected earlier in wet DNA fibers described on the physical level in the Leikin's talk. [A.A.Kornyshev, D.J.Lee, S.Leikin, A.Wynveen, S.Zimmerman, Direct observation of azimuthal correlations between DNA in hydrated aggregates, *Phys.Rev.Lett.* **95** , #148102, 1-4 (2005)].

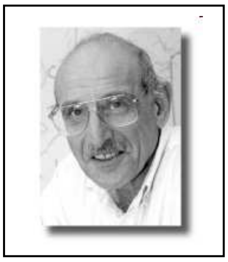
No matter which aspect of DNA research was discussed at the conference, the physical chemistry of solution, particularly the role of counterions, was found to be extraordinarily important. Loren Williams (Georgia Tech) presented decisive synchrotron x-ray 3d-maps of distribution of the most important class of adsorbed counterions between the major and minor groves of DNA or phosphates. Purely physical methods were used to obtain them with the results crucial for understanding the resulting charge patterns of DNA (including the adsorbed counterions) that determine DNA physical behaviour and DNA-DNA helix specific forces.

The conference has shown a substantial progress in characterization, understanding of physics, geometry and topology of DNA-supercoiling as well as its biological implementations, and set of lectures was devoted to its modeling and experimental characterization.

New techniques were also in a center of attentions, such DNA transport through solid-state pores. In particular Serge Lemay (Kavli Institute, TU Delft) has shown a number of new developments related to a combination of magnetic tweezers techniques and transport, allowing to precisely characterize the trapping of DNA in the pores and what can be learned from it. Amit Meller (BU) was reporting an intriguing result showing that DNA capture rate increases with its length for medium long DNA whereas there is no length dependence for longer ones. Good statistical physics of polymers was needed to explain this, revealing also a crucial role of electrostatics. Creation of salt gradients across the pore was providing a tool that increases the sensitivity of this popular new method by the order of magnitude. Unique single molecule technique for the study of the effect of RNA polymeraze backtracking, using dual trap optical tweezers assay, was reported by Stephan Grill (Max-Plank Institute, Dresden).

Many theoretical models reported at the conference were elegant but most importantly closely related with experimental findings.

On the first day we were able to celebrate Adrian's 70th birthday. A worldwide renowned figure in modern biological physics, its distinguished veteran, Adrian worked at NIH for four decades and in the two last ones was leading there a vibrant Structural and Physical Biology Laboratory created by him. Adrian did a lot for physicists and biologists coming closer together. This summer, full of his ever young energy -an example for many young scientists -- he is moving to build a new research team as a Professor at the University of Massachusetts. Happy birthday, Adrian!



My feeling is that something is moving in difficult interactions between the physical and biological communities, the progress noticeable at least at the scale 140 people present in Trieste. Few years ago, Paul Selvin, physicist University of Illinois who has made crucial contributions into visualization and characterization of biomolecular motility, suggested that if Rutherford was alive today, he would have possibly concluded that "All Science is either …biology or tool-making for biology... or not fundable…". Generally, the 'pride and prejudice' today is no longer on the side of physicists. But in order to overcome the barrier of skepticism we, physicists, not only should not be shy about what we were able to demonstrate in the test tube, but also have to think how we could show that our 'beautiful physical effects' equally work inside the cell! This is much more difficult.

Many of us will not be able to do it alone without finding a match. Crick was not only a great mind, he was also lucky to meet *his biologist*. But Crick himself was *very* serious about real biology rather than just 'biologically inspired physics'. And this is what Adrian advised all of us to do in his old Physics Today paper. But in support of his opponent, Bob Austin, I wish to quote the conclusion from the Steven Hawking's talk at the White House: "The greatest discoveries of the XXI century will be where we don't expect them". So, physics will bring surprises to biology, and the conference left no doubt about it.

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