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A Chemistry Highlight of 2006

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This paper discusses the March 17, 2006 article in *Science* magazine titled "Probing Gene Expression in Live Cells, One Protein Molecule at a Time." The article was written by Ji Yu, Jie Ziao, Xiaojia Ren, Kaiqui Lao, and X. Sunney Xie in the Department of Chemistry and Chemical Biology at Harvard University. Their methods, described in this paper, span several fields of study and represent many advances over several decades. These advances enabled stunning recordings of live cellular protein creation. Areas of immediate application derive from the demonstrated genetic transcriptional control and cross verification of research techniques. The key outcome of their research, however, was evident upon analysis of the results: protein synthesis is a random process justifying the use of probability distributions for mathematical characterization. Ultimately, computer simulation models can be developed to more accurately reflect quantitative and time domain factors.

Briefly, their research involved developing a strain of E. Coli with a single copy of a carefully engineered gene inserted into its chromosome to produce a highly repressed fluorescing version of the transmembrane serine receptor (Tsr) chemotaxis protein. Under the engineered high repression of this specific gene, transcription to messenger RNA fit a Poisson distribution occurring approximately once every 46 minutes or 1.2 times per cell cycle. The subsequent burst of ribosomal protein creation proceeded to completion – the protein translation, folding, membrane positioning and fluorophore maturation sequence – and was monitored as fluorescing events characterized by a geometric distribution (a single geometric decay) averaging 4.2 ± 0.5 molecules/burst. It is important to note that there were many more unmodified versions of the Tsr gene present on the chromosome; this one highly repressed gene had little impact on the overall function of the cell. The *in vivo* data collection was performed over several cell cycles using the MetaMorph Imaging Control System for both epi-fluorescent and differential interference contrast (DIC) imaging. The results were compared to statistical analysis of the same protein using *in vitro* techniques. The data was not only consistent, but was also verified at the cellular level to be stochastic.

Clearly, a team approach was required to synthesize the methods and tools for this work. For the purposes of this paper, several technical fields were explored with references cited. Considerable previous research on E. Coli bacteria provided the groundwork for this project. Cellular biology research was strongly represented by the transmembrane chemotaxis receptor selected as the protein under study and its DNA gene site lac repressor control mechanism. Key components of the research depended on molecular biology methods: RT-PCR and Western Blot Assay for protein analysis. Work done in biochemistry on fluorescing molecules for event tagging will be mentioned while the field of biophysics provided the tools for measurement: epi-fluorescent microscopy and differential interference contrast (DIC) microscopy. Computer control automated the coordination of the equipment. Finally, biostatistics was at the very core of what the research accomplished: the verification of basic protein construction as a stochastic process confirming previous molecular biology research.

Von Hippel's paper states, "The lac repressor of E. Coli and its interactions … have long been the central model system for understanding transcriptional control." Work on the control of gene expression began in 1961 by Jacob, Monod, and co-workers using the E. Coli lac operon. By 1967, Gilbert and Muller-Hill identified a DNA site near the protein promoter site where the gene

repressor molecule binds to prevent protein expression. It was fifty years later, in 2004, when Kalodimos finally filled in the last details of the molecular mechanisms involved in protein transcription regulation.

Both Western Blot Assay and Polymerase Chain Reaction [PCR] are methods routinely used in molecular biology. The Western Blot method was first described in the paper "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications" by Towbin et al.in1979. The procedure requires proteins to be extracted from cells and denatured for dimensional and quantitative analysis. Gels and electric fields are used to separate the proteins while antibody identification pinpoints the specific protein of interest. Polymerase chain reaction was developed in 1983 by Kary Mullis as a means of copying large quantities of DNA *in vitro*. Kary is quoted as saying, "[It] lets you pick the piece of DNA you're interested in and have as much of it as you want." The DNA double helix is heated in order to separate the two strands creating templates for making two new strands. Short DNA pieces called "primers" are attached to the end of each template. The DNA polymerase enzyme then starts at the primer and builds a mirror-image copy of each DNA strand. New cycles of the reaction exponentially amplify DNA up to 40 kilobases in length and up to 1 million times. There are many variations on this technique including Reverse Transcription PCR, which copies RNA segments. Both Western Blot Assay and PCR are fast, highly sensitive and versatile methods for studying proteins *in vitro*.

Fluorescence is another technique used for some time but with ever growing applications. Pennisis offers a historical perspective of this approach to viewing processes within live cells. In 1978, D. Lansing Taylor of Carnegie Mellon used fluorescent dyes to track intracellular actin movement. By the early 1990s, Roger Y. Tsien at the Univ. of California, San Diego developed fluorescing molecules that diffused through cellular membranes to track calcium activity *in vivo*. Pennisis says it was no longer necessary "to grind up the cell" to measure concentrations of a chemical. By the mid-90s, high volume fluorescing biosensors for more complex cellular messengers and more complex processes was at the forefront in the merge of molecular biology, cellular biology and protein engineering. Taylor explained the connection, "The theory is that if you can map these changes in time and space as the cell functions, then you can define the mechanism of the cell's processes."

The tools for observing the live cellular processes have been in place for some time. Georges Nomarsky began work on Differential Interference Contrast Imaging [DIC] in the 1960s coupling a modified Wollaston prism with standard phase contrast microscopy. Although it's an expensive method, it is still frequently used with fluorescence microscopy for viewing living cellular structures. This minimally invasive method is very effective in providing highly detailed three-dimensional relief images of thick, transparent structures. Polarized light is split and then passed through the specimen before remerging on the other side. Any difference in the refractive index in the specimen is evidenced as interference causing a change in the amplitude of light. Edges and lines are emphasized while areas with homogeneous refractive indexes simply appear gray. Fluorescent microscopy, first demonstrated by Reichert and Heimstadt in 1911, has also had a long history. The dichroic mirror invented by Bromberg in 1953 brought epi-fluorescent microscopy: brighter images and higher resolution at lower cost. Still, fluorescence required high intensity illumination posing a problem for living cells. Over time, continuous light radiation photobleaches the fluorescing molecule and eventually destroys the cell. When Ploem invented the epi-illumination filter cube in 1970, fluorescent images could then be combined with images obtained with transmitted light. Continuous images could be obtained with DIC imaging while epi-fluorescent microscopy periodically looked for specific events followed by a dwell or rest interval. Computer control of the processes since 2004 brought further advances detailed in the article by Rabut and Ellenberg. Automated focusing adjustments can correct for mechanical instabilities, focus drift, and temperature fluctuations over long time by tracking to selected points of integrated light intensity. Imaging can

be triggered by short, high intensity illumination intervals defined and repetitively activated through a configuration file. These capabilities are realized as the ability to monitor slow or rare processes over many hours and days or even over an entire cell cycle.

Finally, after nearly a century, the pieces were in place to merge cellular and molecular biology. The researchers compare the information on protein synthesis available through established molecular biology techniques with the data obtained by the imaging of that same protein creation during normal cellular function over several reproductive cycles. It is shown that protein synthesis is a stochastic process that can, indeed, be successfully modeled by *in vitro* methods. Statistical methods have long been used to model biological systems providing probabilistic outcomes, typically a better match for living organisms than deterministic methods. Correspondingly, the field of molecular biology has relied heavily on statistics to draw conclusions about protein research without having strict confirmation that the underlying process of protein transcription and translation were, in fact, random and independent processes. This research clears that possible stumbling block. The door has been opened to further verification of protein synthesis processes and manipulation of gene expression in living systems. According to Coates, "Molecular and cellular biology are no longer discrete subject areas but vital tools and an integrated part of …biological research."

But maybe even more significantly, protein synthesis models are being refined. Mathematical modeling of natural phenomena enhances our ability to interpret experimental data and offers an avenue to predict the outcome of subsequent experiments. Performing a quick web search of protein modeling, it is evident that computer simulation of protein construction is an active field. Based on this research, time simulation can now be added to structural simulation. The implications and applications are huge. The Institute for Mathematics and Its Applications discusses the importance of "…mathematical, statistical and computational methods … on the prediction and control of spatiotemporal molecular and cellular behavior." We simply can't obtain information out in the field or at the lab bench as quickly as it can be computed. The need to reduce the time and lower the risk of gathering knowledge drives computer simulation. For example, pharmaceutical trials can be shortened while at the same time minimizing tests on animals and humans. Proteins are the building blocks for all living things. Good models accurately predict what happens, how it happens, and how long it takes. There's no limit to the number of ways those questions can be asked.

In summary, this research reflects a long list of advancements by many people over a long time. To single out this research as more significant than others belies the effort made previously and still to be made. I would like to end my assessment of this research article with a quote I came across while preparing this paper. I think it must come from the heart of all researchers. Tsien said, "Our ultimate motivation is [to understand] the biology... the aggregate contribution is greater than what we ourselves could ever do."

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