# Bringing Time into Molecular and Cellular Biology

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**Abstract:** In conjunction with the defense of a doctoral thesis on the deciphering of complex protein interactions on living cells, six scientists shared their view on time in molecular and cellular biology. This brief review takes the form of a conference report and summarizes the contributions of the speakers and the defense. Opportunities and challenges for time resolved assays in molecular and cellular biology were vividly discussed during two days with a pan-European audience. Awareness of biological timeframes and understanding the temporal aspects were claimed critical for analytical applications in biology.

**Keywords:** Protein interactions, timelapse imaging, kinetics, affinity, thermodynamics.

## INTRODUCTION

From a historic perspective, time resolved information in biochemical and cell-based assays has in many cases increased the understanding of the underlying biology and has made quantification possible. One example is the time resolved spectrophotometer, designed in the early 1940-ies for the quantification of inorganic reactions, but later applied to enzyme kinetics [1]. Enzyme efficiency could then be quantified leading to greater understanding of the enzyme biology, and ultimately resulting in enzymatic biosensors and enzyme-linked immunosorbent assays (ELISA) that are instrumental in the diagnostic industry of today. Similar examples include real-time PCR that turned PCR into a quantitative tool [2], real-time molecular interaction analysis that has been a workhorse in the laboratories for developing therapeutic proteins and other drugs [3]. These techniques now form a foundation for drug development processes and clinical studies.

Biology encompasses a huge range of timeframes. The brain activity and the motor system responds in fractions of a second in a human, and the very same human can reach an age of about 100 years. Thus, the timeframe of the biology of humans covers more than 8 orders of magnitude. Understanding the impact of timeframes, including time to onset, duration of effect, potential hysteresis, and other dynamic aspects is critical for understanding the biology.

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February 15, 2013, Hanna Björkelund defended her doctoral thesis [4] on "Novel methods for Analysis of Heterogeneous Protein-Cell Interactions". Her thesis describes a study focusing on the application of novel tools for time-resolved interaction analysis on living cells, using the EGFR system as expressed on cancer cell lines as example. In conjunction with her dissertation, six scientists from different fields within biology were invited to present their respective views on how they applied time-resolved information in their research. The conference on "Bringing time into molecular and cellular biology" attracted a wide audience with attendees travelling far to participate. This brief review takes the form of a conference report. It summarizes the contributions of the speakers and discusses the use of time-resolved assays for analytical applications in biology.

## THE IMPACT OF TIMING

Two speakers discussed the importance of timing in signal transduction. Prof. Yosef Yarden (Weizmann Institute, IL) described aspects of time-resolved regulation related to EGFR and HER2, and its relevance to cancer therapy [5]. Prof Marene Landström (Umeå University, SE) talked about the ubiquitination of key molecules in the TGFbeta-signaling pathway as a way to, in the right time, determine cellular responses related to tumor invasion [6]. In both these cases, timing is critical. One example is the cellular requirement of epithelial cells and fibroblasts, where two growth factor pulses, spaced approximately 9 hours apart, are required to make the cells cross the restriction point in the cell cycle and proceed into S phase. This finding opens up new

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possible routes for cancer therapy, wherein the timing of drug administration could be a key factor for efficacy.

## THE IMPACT OF TIME RESOLVED INFORMATION

The purpose of the thesis was to bring forward time resolved assays as means for better understanding the heterogeneity of proteins interacting with living cells, and hence most contributions were related to time resolved information per se. Dr. Marika Nestor (Uppsala University, SE) presented time from a medical imaging perspective, where a series of Fab fragments binding to CD44v6 were evaluated in a time resolved manner in biophysical (Surface Plasmon Resonance, SPR), cell-based (LigandTracer) and *in vivo* biodistribution assays [7]. Within nuclear medicine and molecular imaging, the importance of time extends far beyond the biological domains. There is a delicate balance of biological half-life, radionuclide half-life, and the time for the patient occupying a bed in a hospital to be achieved for an imaging compound to be clinically successful. Bringing time into the picture already from the start of compound development could increase the likelihood of success, since the *in vitro* binding traces of the protein-cell interactions behavior of the compounds matched the *in vivo* tumor to blood ratio.

In her thesis defense, Hanna Björkelund (Uppsala University, SE) showed how the complexity of the EGF-EGFR interaction could be resolved into underlying components. By Interaction Map analysis of the binding curves depicting how radioiodinated EGF interacts with its receptor. Dr Björkelund could separate EGF binding to monomeric and dimeric forms of the EGF receptor, and also follow the changes of interaction pattern when the cells were subjected to drug treatment [4]. To illustrate the results presented in her thesis [4], Figure **1** shows the $^{125}$I-EGF interaction with its receptor on two different cell lines (A431 and SKOV-3). The shape of the binding curve in panel A has signs of multiphasic

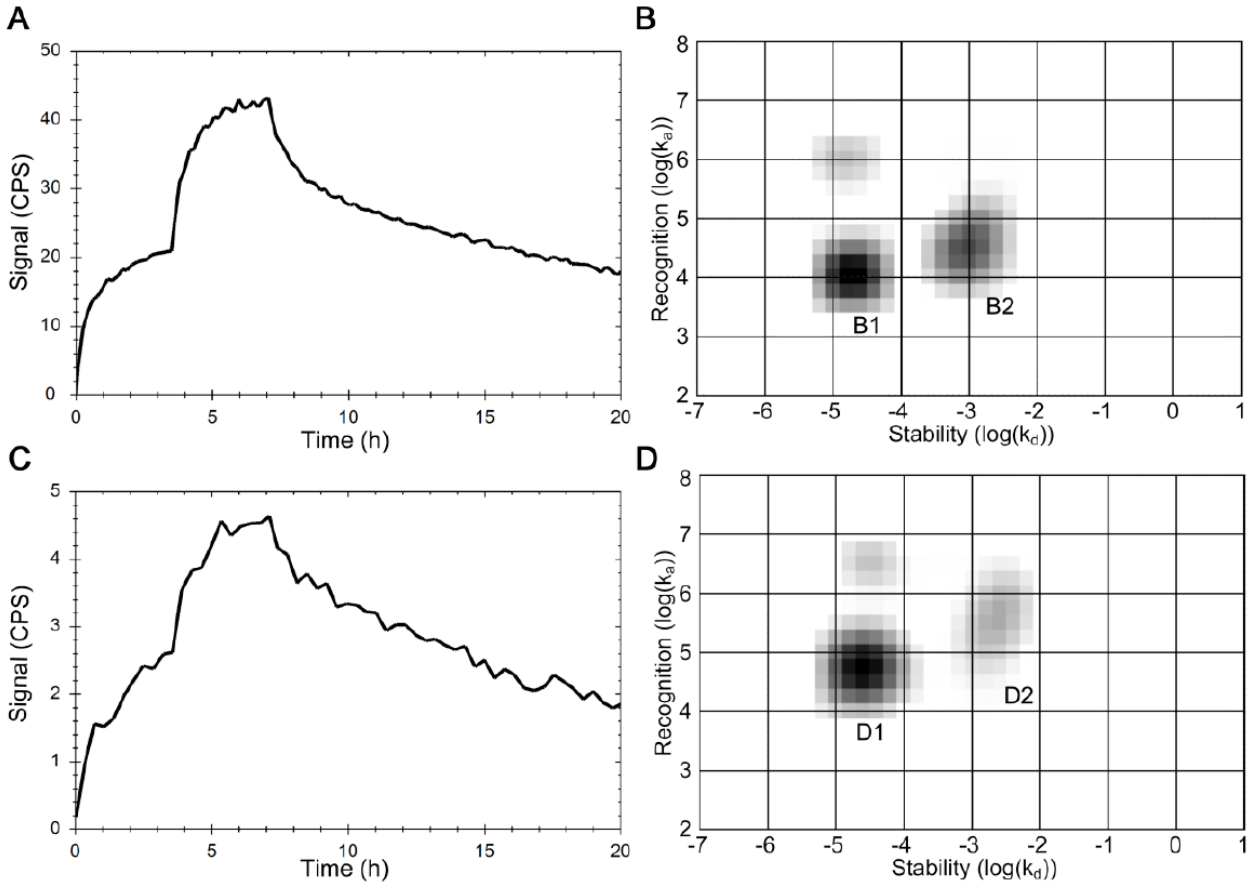


**Figure 1: A**: $^{125}$I-EGF binding to EGFR on A431 cells, initially incubating 3.5 hours of 3 nM, then 3.5 hours of 9 nM, and finally washout over night in liquid devoid of $^{125}$I-EGF. **B**: Interaction Map analysis of the data in panel **A**. Two clear interaction like processes are identified, B1 with an affinity of 1.8 nM contributing to about 47 % of the observed binding and B2 with an affinity of 33 nM contributing to about 43 % of the observed binding. **C**: $^{125}$I-EGF binding to EGFR on SKOV-3 cells, initially incubating 3.5 hours of 0.7 nM, then 3.5 hours of 2 nM, and finally washout over night in liquid devoid of $^{125}$I-EGF. **D**: Interaction Map analysis of the data in panel **C**. Two clear interaction like processes are identified, D1 with an affinity of 0.5 nM contributing to about 69 % of the observed binding and B2 with an affinity of 6.6 nM contributing to about 22 % of the observed binding.

interaction, most evidently in the dissociation phase where bound EGF is released by at least one rapid process and one slow process. Interaction Map evaluation of this binding data (Panel B) revealed that there were two different interaction like processes, and through a series of control experiment the higher affinity process (B1) was proven related to EGFR dimers while as the weaker affinity process (B2) was related to EGFR monomers. The same interaction taking place on SKOV-3 cells has a completely different signature (Panel C) and the corresponding Interaction Map (Panel D) show two interaction like events, but this time with a different balance. The higher affinity process (D1) was more abundant in the SKOV-3 data. This is most probably due to the heterodimerization of EGFR and the sibling receptor HER2, the latter being highly expressed in SKOV-3 cells. Only a shadow of the peak representing monomeric EGFR interaction (D2) is present, suggesting that the HER2 receptors consume the vast majority of EGFR to form heterodimers. The use of the temporal dimension was necessary to produce results of this kind.

Dr. Michael Schräml (Roche Diagnostics, DE) presented how Surface Plasmon Resonance (SPR) - based time resolved molecular interaction analysis combined with Interaction Map analysis could be used extract the transition state thermodynamics which in turn was suggested to predict antibody promiscuity. The observation that a positive transition state entropic burden of the antibody-antigen interaction seems to correlate with promiscuity has both a scientific and an industrial value: It contributes to the general understanding of molecular interactions and it may improve the selection of antibodies for diagnostic and therapeutic application, where cross reactivity and promiscuity may render the antibody useless.

## THE IMPACT OF NOVEL TECHNOLOGIES

Dr. Tapani Viitala (Helsinki University, FI) discussed novel use of multi-parametric SPR for the assessment of drug adsorption into living cells. SPR is commonly not applied in cell biology, but when extracting the sensor output in a multi-parametric manner, drug adsorption properties could be distinguished. The application of SPR in cell biology is a truly emerging field wherein Dr. Viitala is one of the pioneers.

Dr. Carolina Wählby (Uppsala University, SE) illustrated how image analysis could be used for tracking cells and for following the transportation of molecules. Time-lapse microscopy opens up a new field of research, where the order of events, molecular transportation between and within cells and similar properties can be characterized. The need for computerized analysis tools is obvious, and Dr. Wählby discussed the current state of the art of image analysis for cells and how it can be applied within biology.

## CONCLUDING REMARKS

In the concluding remarks of the seminar series the organizer Dr. Karl Andersson discussed the general issues of bringing time into biochemical assays. In contrast to the end-point assay where a snapshot of the system conditions is recorded, time-resolved measurements can reveal if equilibrium has occurred or if the conditions of the system are in change. Temporal aspects are difficult to grasp but potentially rewarding, put simple.

Discussions with colleagues during the two days raised a couple of interesting reflections. One of these were the thought that our human minds have a good reference points regarding solid measures like physical size and mass, whereas time is a more fuzzy magnitude. This could explain a general resistance towards the temporal dimension. Another reflection was the problem of extracting information from analytical signals that appear as a curve. How does one do so? Curves are difficult to summarize in a table and to retrieve the underlying information contained in the curve one needs to apply some kind of mathematical regression, in the simplest case an average or a linear fit and in other cases advanced differential equations. Even though there is no general and simple answer to how to summarize curve based data, it is a topic requiring the greatest attention.

In the discussions, many agreed that by following analytical signals over time more information on the process can be obtained. An interesting aspect of time resolved measurements of biological processes is that, once the process is understood, the initial time-signal curve can be used to predict the result at a later stage. This opens up the possibility for more rapid analytical methods that will be valuable in for example clinical diagnostics.

Another point that was raised was the robustness of biological systems. It seems that biological systems are robust when challenged with one event or perturbation at a time, but when combining two perturbations the system may become interrupted. The suggestion for

cancer therapy was that it is important to introduce several uncommon perturbations with defined timing in order to kill cancer cells.

The initial technical challenges include engineering the technology required to turn a traditional biochemical or cell-based end-point assay into a time-resolved assay. The first level of acceptance of a novel time resolved assay is often obtained when being used as a trouble-shooting method for the conventional end-point equivalent. Looking back on history of different technologies it is easy to see that a few courageous scientists implement the technology, and in many cases it suddenly (some years later) becomes a golden standard. Even though an initial skepticism is sound and every new technology (time-resolved or not) should be thoroughly evaluated, let us remember that the timescales of biology covers a broad range and that every attempt to describe a biological event should do so within the timeframe of its action.

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## CONFLICT OF INTEREST

Ridgeview Instruments AB (RIAB) and Ridgeview Diagnostics AB (RDAB) are spin-out companies from the department of Radiology, Oncology and Radiation Sciences, Uppsala University commercializing tools for time resolved interaction analysis. The author is a shareholder in RIAB and RDAB.

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