PEGS EUROPE

Protein & Antibody Engineering Summit

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SPEAKER Q&A

On Friday, 4 November, Mario Lebendiker, from the Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem, will be presenting a case study on production of a human kinase co-expressed in *E. Coli* cells. Cambridge Healthtech Institute recently spoke to Dr. Lebendiker about his upcoming presentation, "Case Study: Human Kinase Crystallization, Phosphatase Co-Expression," taking place during the Protein Purification Technologies conference to be held 3-4 November 2016, as part of the 8th Annual PEGS Europe event in Lisbon, Portugal.

Dr. Mario Lebendiker is in charge of the Protein Purification Facilities at the Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem. He is actively involved in many collaborations for structural and biochemical studies within the Hebrew University, other Universities in Israel, as well as with biotech and pharmaceutical companies. Dr. Lebendiker received a Ph.D. in Biochemistry in 1982 from the Animal Virology Center (CEVAN), in Buenos Aires University, Argentina. Together with many other laboratories, he found the Protein Production and Purification Partnership in Europe (P4EU) network; a platform for the exchange of information, know-how and materials between core facility labs in the field of protein expression and purification.

Q: What are the challenges that kinase production presents?

The main requirements for crystallization projects are either solubility, monodisperse (homogeneous) purity, relatively high concentration and stable, on time protein batches. In the case of human kinase production in bacterial systems, once you overcome purity and solubility issues, the main obstacle is a lack of homogeneity due to random and heterogeneous auto-phosphorilation of the sample that takes place during expression.

Phosphatase co-induction can partially overcome this problem by eliminating most of the phosphate residues. However there are always some traces of phosphate groups that can prevent consistent and reproducible crystallization. So, the big challenge is to get a robust production protocol in order to achieve homogeneous protein batches.

Q: What is the importance of crystallizing your target protein? Does this process reveal crucial information?

Protein Kinases are functionally diverse gene families that function as key regulators of different cell activities. Consequently, they are extremely important therapeutic targets for different diseases. Our approach in this project is to understand how different ligands bind to our target, study their interactions, discover who their best "hits" are, learn how they work, and figure out how these ligands can be modified to get better "hits." In this sense, the information gained through crystal structure is crucial, and complements different biophysical screening techniques performed in parallel. Moreover, as was recently publish by Johannes Schiebel et al, (DOI: 10.1021/acschembio.5b01034 ACS Chem. Biol.*): the frequently applied biophysical prescreening filters deteriorate the number of possible X-ray hits while only the immediate use of crystallography enables exhaustive retrieval of a maximum of fragment structures, which represent a rich source guiding hit-to-lead-to-drug evolution. In other words, biophysical screening methods many times failed to predict at least 73% of all X-ray hits.

**Six Biophysical Screening Methods Miss a Large Proportion of Crystallographically Discovered Fragment Hits: A Case Study*

Q: What is the need that you see to establish "minimal protein quality information" in publications in order to assure reproducible results?

The connection between "minimal protein quality information" in publications and this project is very clear. The structure of protein kinase was solved a few years ago by other groups, so it was logical to assume that if we reproduce their procedure, we would arrive at the same results. And it was not so. When we consulted the author, he openly acknowledged that most of the batches he prepared were unable to crystallize, and he published the results for the batch that crystallized. Till here, all is "kosher." People want to see final results, and not the problems you have while getting to these final results. Most of the groups prefer to ignore these "negative" results, do not pay attention to little details, prefer "not to waste time" in Material and Methods descriptions, and most unfortunately, do not invest too much effort in quality control of the protein they are using. Not just researchers, but even journals themselves prefer to ignore these good practice procedures. In our case, where we wanted to crystallize our protein with many different ligands, a robust and reproducible production protocol was critical, and we saw that the protocol described by the first group was not enough to guarantee reproducible results. The protein obtained following the protocol of the first group was not homogeneous enough, and just after further optimization we succeeded to get homogeneous proteins and achieve our goal.

In this context, a group of nearly 200 specialists in protein production and biophysical characterization of biomolecules, in the most important Core facilities in Europe and Israel, have formed a joint initiative to establish guidelines on recombinant protein quality. The Production and Purification Partnership in Europe (P4EU) and the Association of Resources for Biophysical Research in Europe (ARBRE-MOBIEU) aim to develop a best practice/minimal standard for the quality control of recombinant proteins to ensure that the input material used in biophysical and biochemical research is of high quality, which, in turn, will result in optimized data quality. The prescribed tests must be both feasible for all protein production labs to perform, and, at the same time, acceptable for biophysical or structural biology labs as admission criteria. I hope that during PEGS Summit in Lisbon, together with some of our colleagues, we will be able to convince the scientific community about the importance of this initiative: minimal quality control parameters that should be tested on protein samples, and as a second task: minimal information in publications in order to be able to reproduce results.

Q: What are you most looking forward to at the PEGS Europe event?

What is going on in the Production Field, both in the Academic and Industry worlds? Where is the input now? New achievements, new technologies. Meet new people, learn from their experiences. Possible collaborations. I like to see what the exhibitors display: new products, new achievements, new technologies. And finally, PEGS event is a wonderful opportunity to meet colleagues and friends, and enjoy of a sociable atmosphere in a wonderful city.