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Bottom-up strategies for reconstitution of multi-protein complexes using the baculovirus expression system

The production of a homogeneous protein sample in sufficient quantities is an essential prerequisite not only for structural investigations but represents also a rate-limiting step for many functional studies. In the cell, a large fraction of eukaryotic proteins exists as large multicomponent assemblies with many subunits, which act in concert to catalyze specific activities. Genome editing allows to isolate native protein complexes produced from their natural genomic contexts but their limited natural abundance is often limited and so recombinant expression and reconstitution are then required. The *baculovirus* expression vector system (BEVS) has turned out to be particularly powerful, unlocking the structure and mechanism of many important complex assemblies that had remained inaccessible to detailed analysis beforehand. Here, we will comment on current developments and their potential to accelerate protein complex research: Use of Lambda red recombination in *E. coli* for manipulation and improvement of the baculoviral genome, vector development for parallel expression/co-expression screening and assembly of multi-gene constructs from synthetic biology approaches. As model systems, we will use human multi-protein complexes invoved in the regulation of gene expression such the pTefb cdk/cyclin pair, nuclear hormone receptor complexes or the 10 subunits transcription/DNA repair complex TFIIH. We will describe state-of-art strategies for the efficient production of multiprotein complexes using the baculovirus/insect cell expression system. Here, we will comment on current developments and their potential to accelerate protein complex research: Use of Lambda red recombination in *E. coli* for manipulation and improvement of the baculoviral genome, vector development for parallel expression/co-expression screening and assembly of multi-gene constructs from synthetic biology approaches. As model systems, we will use human multi-subunit transcription factors such as Cdk/cyclin pairs, nuclear hormone receptor complexes or the 10 subunits transcription/DNA repair complex TFIIH.

Speaker Biography

Poterszman Arnaud, after studying at ENS Cachan, completed his PhD from Strasbourg University and joined the CNRS one year later. He holds a CNRS Research Director position and performs his studies at the Department of Integrated Structural Biology at IGBMC, Illkirch France. He has a dual expertise in Structural and Molecular Biology, with insights on expression technologies and sample preparation. His research is focused on eukaryotic multi-protein complexes involved in transcription regulation and DNA repair by nucleotide excision, particularly, the transcription/DNA repair factor TFIIH and its partners. He has around 50 publications in Pubmed, h-index 21.

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