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[16.30–16.50]
Ribosome display for antibody engineering
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Abstract: In vitro evolution of antibody variable regions using ribosome display is a powerful tool to improve the potency of antibodies. The benefits of using this technology will be exemplified by data from several *in vitro* evolution studies. In the drug discovery context, the practice of engineering therapeutic antibodies from nanomolar to picomolar potency is crucial in allowing more candidates to progress into preclinical and clinical studies. Furthermore, sequence hotspot data generated from antibody evolution studies can pinpoint the important structural changes during the evolution process. When taken in conjunction with the mapping of antibody:antigen interaction surfaces it is possible to reveal trends in the structure-activity relationship of candidate drugs.

In addition the talk will touch on some more recent work using ribosome display to optimise the potency of agonist antibodies. This model system allows an exploration of the assumption that improving antibody affinity is directly linked to improvements in potency.

[16.50–17.10]
Generation of high antibody producing NS0 cell lines by site-specific recombination
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Mammalian cells offer significant advantages over bacteria or yeast for antibody production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed antibodies. Stable, high-level expression systems are routinely produced by introducing recombinant genes to competent cells through insertion of recombinant gene at random locations in the host cell genome. The approach requires several rounds of selection and clonal expansion to produce an acceptable expression system. Moreover, this process must be repeated every time an expression system for a new antibody is sought. As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration.

We developed an efficient method to overcome the random nature of antibody cell line generation. We generated stable antibody-producing NS0 cell line using FLP-FRT site-specific recombination system. Site-specific recombination with powerful positive and negative selection ensures that the gene of interest always goes to the same high expressing site. First, we generated high antibody-producing NS0 cell lines using a vector containing unique FRT sites surrounding the antibody A expression cassette and selection marker. This vector helped us to identify a site "hot-spot" in the NS0 genome that is transcriptionally highly active, i.e., a site at which gene expression levels are high. Then we transfected antibody A expressing NS0 cells with an exchange vector containing antibody B expression gene and positive-negative selection markers. The resulting cells produce antibody B at a high and stable level. Genetic characterization showed that the gene for antibody A was replaced by the gene for antibody B. In summary, this site-specific recombination system enables us to quickly generate a cell line that consistently produces high levels of antibody.

[17.10–17.30]
RapMAT, a fast track for selecting high affinity HuCAL GOLD® antibodies
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High affinity is most often a desired feature of therapeutic antibodies. HuCAL GOLD® is a known and validated tool for the generation of fully human antibodies with high affinities and proven efficacies against a large variety of targets.

Here were present recent developments to accelerate the antibody optimization process. In comparison to standard procedures, the affinity optimization step is integrated into the process of antibody selection with only minimal additional effort. Utilizing the modular design of HuCAL® combined with an efficient means of CDR exchange, the sampled sequence space is drastically broadened. Numerous examples will be given in which Fab fragments with affinities down to 5 pM were selected. Implications on diversity, library size and selection conditions will be discussed.

[17.30–17.50]
Antibody mammalian display system
David Shen
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This talk will present a novel antibody display system. In contrast to phage and yeast display systems, this system allows display of bivalent, full length IgGs, instead of monovalent, antibody fragments. It allows us to directly select antibodies in full length IgG format with desirable affinity and biological activities during screening. This system also allows selecting agonistic antibodies directly on displayed mammalian cells. Therefore, it is a very efficient system for antibody screening, agonistic antibody selection, affinity maturation, humanization, and antibody stability and expression selection. Data for some of these applications will be presented.

[17.50–18.10]
A new approach to antibody maturation through DNA deamination in a retroviral system
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In the biological world, the cellular deaminase enzymes AID and APOBEC are broadly distributed. They play an essential role in diverse enzymatic pathways that deaminate cytosine in the context of RNA and/or DNA. By converting cytosine to uracil, the deaminase enzymes revise DNA and/or RNA sequences, which have a significant effect on various physiological functions, especially innate and adaptive immunity.

APOBEC3 proteins act to deaminate cytidine in the plus strand DNA of retroviral replication intermediate. This is a major strategy for defence against retrovirus. APOBEC deaminase activity could be used to mutate antibody in a retroviral genome. By using retroviral constructs carrying scFv genes, retroviral genes and the genes of A3G, A3F and A3C deaminases were cotransfected to introduce a substantial load of mutations on the antibodies. By coupled this to a selection process, we would be able to mutate and evolve antibody and other proteins over a series of iterative rounds to a desired endpoint,

We show that by using the APOBEC family members, APOBEC 3G, 3F and 3C, we can introduce a substantial load of mutations in scFv. Using repeated rounds of mutation and selection, we are attempting to improve the affinity of a scFv fusion protein. The experiment should allow us to evaluate whether this protein evolution strategy will be generally applicable for the antibody industry.