Biomolecular Interaction Technologies Center (BITC)

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Making Biopharmaceuticals Safer

By preventing aggregation in serum, drugs will be safer and more effective.

Protein drugs, also called biopharmaceuticals, are at the forefront of modern medicine. Of particular use are antibodies, proteins that are part of the body's immune system. Therapeutic monoclonal antibodies are used in the treatment of cancers, multiple sclerosis, asthma and other life-threatening diseases. Delivery of these drugs is currently achieved by intravenous injection. Pharmaceutical companies wish to develop high-concentration versions of the drugs that can be administered by patients subcutaneously at home, similar to how insulin is administered now, thus substantially reducing costs and making treatment easier. Development of high concentration monoclonal antibody formulations is complicated by their tendency to stick to one another, making insoluble aggregates and plugging up needles. However, the aggregates are more than just a nuisance. Aggregation can result in reduced effectiveness, drug resistance and life-threatening anaphylactic shock. Even current lower-dose versions of the drugs may suffer from aggregation. It is estimated that up to 30% of patients develop drug resistance to some of the monoclonal antibody cancer treatments due to aggregate formation.

The analytical ultracentrifuge (AUC) is widely used in academic and industrial laboratories to characterize molecular interactions, including aggregate formation. BITC funding helped develop a fluorescence detection optical system (FDS) with unparalleled sensitivity and selectivity, which is now produced commercially by Aviv Biomedical, Inc (the AU-FDS). Using the AU-FDS, it is now possible for the first time ever to detect antibodies and their aggregates in serum. BITC member companies are using this instrument to determine whether their therapeutic antibodies form aggregates serum after injection: the drug development and formulation divisions in Genentech, Roche, Amgen, Abbott, Boehringer-Ingleheim, Pfizer and Johnson and Johnson either have purchased an AU-FDS to conduct these studies, or are contracting with laboratories that have an AU-FDS to make the measurements. By developing formulations that prevent aggregation in serum, drugs will be safer and more effective.

Economic Impact: Costs associated with chronic monitoring of drug effectiveness annually exceed billions of dollars. By detecting aggregate formation early in the drug development process, companies can modify or reformulate their drugs before beginning drug trials. This saves hundreds of millions of dollars annually. Lower drug development costs reduce medical costs. For example, one BITC member pharmaceutical company switched to another molecule when its original candidate molecule was discovered to aggregate in human serum. Since no clinical trials of the drug had begun, the switch to the new, non-aggregating molecule cost almost nothing and saved the company upwards of $100,000,000 in fruitless clinical trials. There are considerable cost savings to society by making non-aggregating drugs. First, by not aggregating, these molecules are far more likely to be tolerated by patients over a longer period of time. This means that patients require less medical supervision and do not need to be switched so frequently to new drugs. It is estimated that 30 to 60% of patients must switch drugs, often several time during treatment, to circumvent aggregate-related immune responses. Reduced aggregation results in lower incidence of anaphylactic shock, a life-threatening condition. The rate of severe anaphylaxis is 1 to 3 per 10,000 patients; costing an average treatment of $10,000 per incident.

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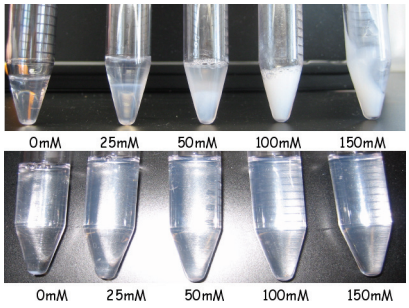
Making Biopharmaceuticals Less Expensive

Shown below are two therapeutic monoclonal antibody preparations. The sample in the top photo forms aggregates and forms a white precipitate as salt is increased to physiological concentrations. The lower photo shows the results for a different antibody which does not aggregate as salt is increased. The antibody in the top photo had to be abandoned as a possible drug due to its aggregation. Unfortunately, over $100M dollars already had been spent on its development.



The difference between the two proteins is their net charge. More charged protein remains in solution, while the low-charged protein aggregates and precipitates. Just as NaCl dissolves in water to form Na+ and Cl- ions, proteins also may be charged. BITC-sponsored research has shown that only proteins that have a high net charge remain soluble. This research has demonstrated that the current methods for calculating the net charge may be exceedingly inaccurate, misleading researchers about their solubility. That was the case for the proteins shown here.

BITC-sponsored research has developed methods for accurately determining protein charge. Workshops have been held to teach researchers how to make the measurements. Member companies now are routinely measuring the charge on candidate therapeutic monoclonal antibodies before embarking on multi-million drug development projects. All of the BITC member companies (Genentech, Roche, Johnson and Johnson, Pfizer, Amgen, Abbott, Boehringer-Ingelheim) have instituted routine screening of candidate drug proteins early in the drug development process to determine which candidates carry sufficient charge to remain soluble at high concentrations. Only those antibodies with a high net charge are good candidates for formulation.

Economic Impact: In order for proteins to remain soluble they must carry a net charge that blocks aggregation. Prior to the work by BITC, pharmaceutical companies did not have a way to measure protein charge. Instead, they calculated charge estimates based on indirect measurements. The companies were repeatedly and unpleasantly surprised to find that their therapeutic proteins were not soluble at high concentrations. Because the proteins had to be diluted, patients had to undergo long and expensive infusions in the clinic. By developing ways to determine protein charge BITC has reduced the cost of drug development substantially in two ways. First, companies now know that their charge estimates were too often incorrect, which is why the drugs were aggregating. By instituting charge determinations as part of the drug development process, they now will be able to prevent drug aggregation prior to expensive clinical testing. Second, the drugs will remain aggregate free up to very high concentrations, making it feasible for patients to administer them at home, thus saving clinic costs. One other important impact of charge determination is longer ranged and more abstract, but will have significant impact on medicine. The biochemistry of cells and tissues takes place at very high protein concentrations. However, our current understanding of biochemistry is based on measurements made in very dilute solutions. The same erroneous assumptions about protein charge made by the pharmaceutical companies have been at the foundation of our understanding of high concentration systems. It is clear that charge measurements must be made a routine part of all of biochemistry and cell biology if we are to have an accurate view of how cells function. This new knowledge will be at the heart of biological medicine, and will open up new horizons for how medicines are developed and used.

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