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.

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 to detect antibodies and their aggregates in serum. BITC member companies are using this instrument to determine whether their therapeutic antibodies form aggregate serum after injection: the drug development and formulation divisions in Genentech, Roche, Amgen, Abbott, Boehringer-Ingleheim, Pfizer, and Johnson & 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 exceed billions of dollars annually. 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 times 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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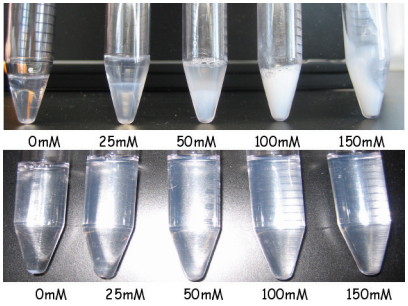
Making Biopharmaceuticals Less Expensive



The top photo shows a protein that forms a white precipitate as the salt concentration increases. Protein drugs with precipitates cannot be injected because doing so can lead to patient death. The bottom photo shows a second protein drug that does not precipitate as the salt concentration increases. This protein was therefore a suitable candidate for injection. The first protein had to be abandoned from further development, even though $100M already had been spent in its development.

In order for proteins to remain soluble they must carry a net charge that blocks aggregation. Only those antibodies with a high net charge are good candidates for formulation. This BITC research demonstrated that: charge must be measured with protein drugs; charge can and should be measured early; and a prototype device in an academic lab could make accurate measurements. Researchers developed methods for accurately determining protein charge. The charge measurement can be made non-destructively on tiny quantities of the protein (significant since only small quantities of candidate molecules may be available).

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

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. For example, companies now know that their charge estimates were too often incorrect, which is why the drugs were aggregating.

The biochemistry of cells and tissues takes place at very high protein concentrations. 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.

BITC put up the funds to help develop a commercial version of this prototype. A small company was founded to make the instrument. Workshops were held to teach researchers how to make the measurements.

> **Economic Impact:** It is estimated that the development of a protein drug costs over $1 million a day. By instituting charge determinations as part of the drug development process, pharmaceutical companies are now able to prevent drug aggregation prior to expensive clinical testing. By making the charge measurements early in the development cycle, it is now possible to weed out bad candidate molecules earlier, thus saving a tremendous amount of time, effort, and money. Because drugs will remain aggregate free up to very high concentrations, it is more feasible for patients to administer them at home, thus saving clinic costs. All BITC member companies (Genentech, Roche, Johnson & 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. They now measure the charge on candidate therapeutic monoclonal antibodies before embarking on multi-million dollar drug development projects.

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