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Real-time Imaging of Protein Therapeutics Using Liquid Cell EM

Lynn M. DiMemmo[1], A. Cameron Varano[2], Jonathan Haulenbeek[3], Madeline J. Dukes[4], Steven P. Piccoli[3] and Deborah F. Kelly[2].

[1.] Drug Product Science and Technology, Bristol-Myers Squibb Company, USA.
[2.] Virginia Tech Carilion Research Institute, Virginia Tech, Roanoke, VA 24016, USA.
[3.] Analytical and Bioanalytical Operations, Bristol-Myers Squibb Company, USA.
[4.] Application Science, Protochips, Inc., Raleigh, NC 27606, USA.

The use of biotherapeutics holds great promise for modulating and controlling processes in the body. Due to an increasing number of protein-based therapies in the pharmaceutical pipeline new technologies are needed to assess these products to ensure safety and efficacy for consumers. One challenge that persists in the development of biological drug formulations is the issue of protein aggregation [1]. While analytical methods are in place to identify micron-sized particulates, smaller items in the nanometer range are ideally studied using Electron Microscopy techniques. Growing concern from the FDA regarding immunogenic effects [2] demands that we better characterize drug therapies in a native environment that mimics the body. Here we present the use of Liquid Cell Electron Microscopy (LC-EM) to examine an FDA-approved drug while developing methods to image and to quantify its behavior under a variety of conditions [3].

For these experiments we used the well-characterized drug-conjugate, PEGylated Interferon α_{2a} (Pegasys®; Roche) [4]. The interferon monomer is ~40 kDa and contains branched PEGylated chains (~ 40 kDa). This size (~80 kDa) allows us to assess the protein-based therapy as a monomer, multimer, or aggregate in solution. Although Pegasys® is currently being used to treat cancer and viral diseases in human patients, it has not been evaluated at the molecular level using EM. Therefore, the product provided a model system to evaluate the temporal evolution of a biological therapy. All specimens were examined using an FEI Spirit BioTwin TEM (FEI Company, Hillsboro, OR, USA) equipped with a LaB_6 filament operating at 120 kV under low-dose conditions (0.1 – 1 electron / $Å^2$). Images were recorded using an FEI Eagle 2k HS CCD camera having a pixel size of 30 μm. For real-time acquisition, sequential images were collected at 1-sec intervals for a cumulative dose of ~6 - 8 electron / $Å^2$ for the 30 and 40-sec recordings.

We evaluated the Pegasys® formula under conditions of acid-treatment, freeze-thaw, and heat-stress. Only the heat-stress conditions induced protein aggregates, which could be imaged and quantified using LC-EM applications (**Figure 1**). The protein aggregates that formed upon heating the Pegasys® for 60 minutes at 50°C showed migration rates that were inversely proportional to the size of the aggregates. Larger aggregates moved at a slower rate than the smaller aggregates. Additional real-time imaging experiments on the heat-stressed aggregates demonstrated protein association and growth that was quantified using contour mapping (**Figure 2**). Understanding protein aggregation and growth processes in solution can provide future insight to prevent these detrimental effects and reduce immunogenic responses in patients during drug therapy.

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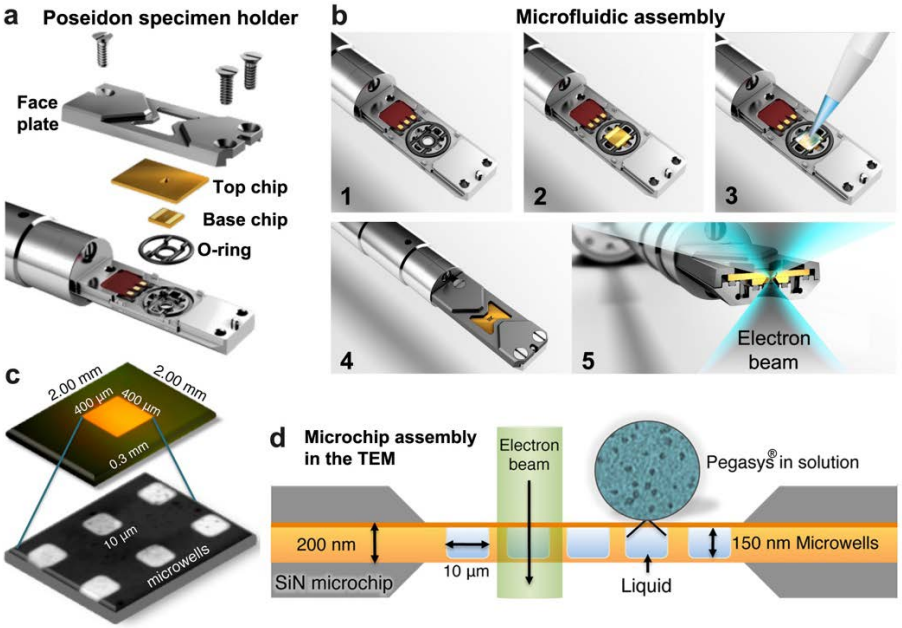

Figure 1. The Poseidon Select system for LC-EM. (a) The microfluidic chamber of the Poseidon Select holder. **(b)** The assembly base chip is placed on top of an O-ring fitting (1-2), and the liquid specimen is added to the base chip (3). The top chip is then hermetically sealed (4) prior to entering the EM (5). **(c)** The base chip contains microwells that are transparent to the electron beam. **(d)** The cross-section through the specimen assembly accommodates a solution thickness of 150 nm [3].

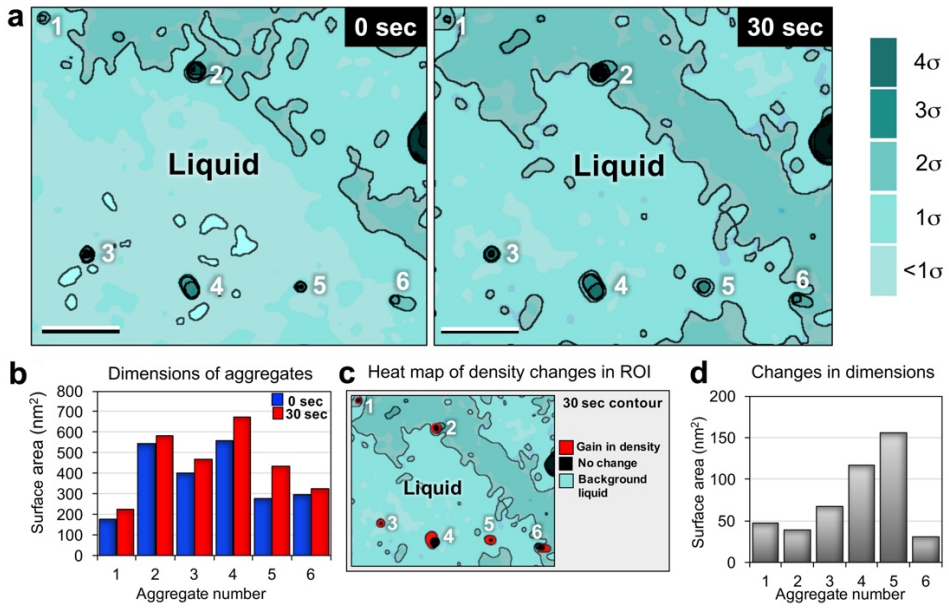

Figure 2. Protein aggregate growth in solution for the Pegasys® formulation. **(a)** Contour maps of heat-induced Pegasys® aggregates (1 – 6) at 0 and 30 sec show differences in aggregate growth within the density range from <1 to 4σ statistical differences in electron density. **(b)** The surface area of protein aggregate shows growth over 30 seconds. **(d)** Heat map indicates regions of increased (red) or unchanged (black) density compared to the background image (green). **(e)** Changes in surface area were quantified among the aggregates. Scale bar is 100 nm in (a) and (b). Adapted from DiMemmo et al, 2016 [3]

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