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High throughput optimization and mass spectrometric analysis of covalently labeled proteins and antibody drug conjugates

Chawita Netirojjanakul, Iain D G Campuzano, Aiko Umeda, Nelson M Carramanzana, Tisha San Miguel and **Jason Long**
Amgen, USA

The use of automated high throughput screening in large molecule discovery research still lags behind that of small molecule discovery. Recently we developed a high-throughput large molecule discovery platform to automate hundreds of bioconjugation reaction setup in one setting. In addition, given LC-MS is a widespread analytical bottleneck, we also established a high-throughput mass spectrometry (HT-MS) platform to accurately detect and rapidly quantitate protein conjugates. We showed that our HT-MS platform can be used to quantitate the extent of covalent inhibitor adducts to a cysteine-containing protein construct (~19 kDa) and of biotinylated adducts to mAbs and Fc domains (~150 and ~50 kDa, respectively). Sample acquisition time was ~20 seconds per sample, 10-50x shorter time than traditional LC-MS methods. Site-specific bioconjugation of human Fc domains with cysteine engineered at different positions were conducted under a matrix of reaction conditions varying equivalents of reductants, oxidants, and alkylating agents using the high-throughput large molecule discovery platform. Using HT-MS, 4 x 384 well plates were analyzed in ~8 hours, as opposed to ~11 days using traditional LC-MS. This approach facilitated rapid determination of DAR values for the reduced and intact huFc domains and selection of optimized conditions for different cysteine-engineered Fc constructs which will be used in preparation of Fc-peptide conjugates as therapeutic leads.

Biography

Chawita Netirojjanakul received her BSc in chemistry from MIT, conducting research in the laboratory of Prof. John Essigmann (MIT) and Prof. Steve Ley (Cambridge). After graduation, she pursued her interest in science policy and commercialization studying MPhil in Technology Policy at University of Cambridge, UK, as a Gates Scholar. She received HHMI International Student Research Fellowship to conduct PhD research under the supervision of Prof. Matthew Francis in the Chemistry Department at UC Berkeley with a focus on "development and applications of well-defined antibody and antibody fragment bioconjugates." She is a Scientist in Therapeutic Discovery Department at Amgen.

chawitan@amgen.com

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