Evaluation of Nanomedicine Drug Release and Pharmacokinetics

Stable Isotope Tracer Ultrafiltration Assay (SITUA)

Great for assessing bioequivalence of generic nanomedicines/nanosimilars

NCL Nanotechnology Characterization Laboratory

The SITUA for Nanomedicine Drug Release and PK

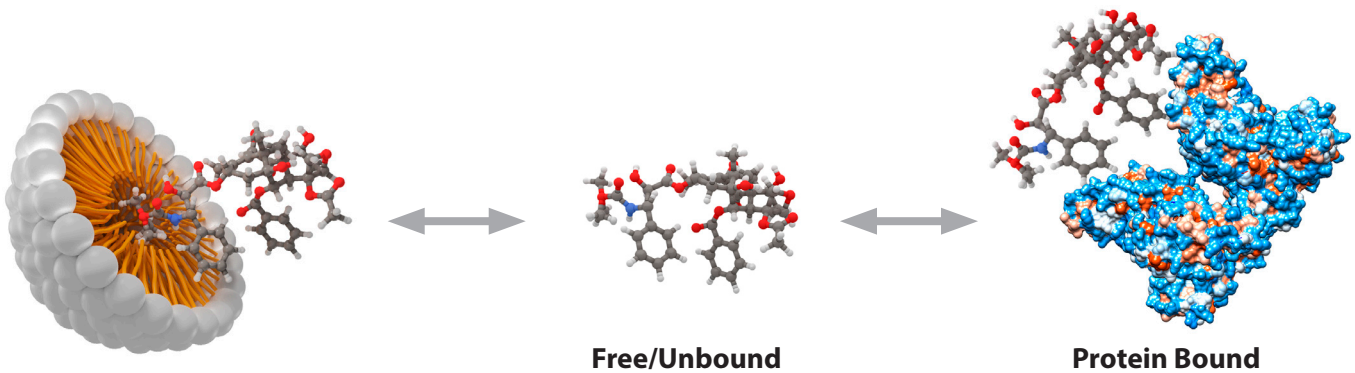
The complexity of nanomedicine drug formulations poses unique scientific challenges. In contrast to conventional small molecule formulations, the active pharmaceutical ingredient (API) in a systemically administered nanomedicine formulation exists in several forms: (a) nanomedicine-encapsulated, (b) unencapsulated, free/unbound, and (c) unencapsulated, protein-bound. While the free, unbound form is considered the only biologically active form of the API, all three fractions are important in characterizing a nanomedicine's pharmacokinetics—especially in evaluation of bioequivalence (BE) (pharmacokinetic similarity). Existing methods to measure these various nanomedicine fractions (e.g. solid phase extraction, conventional ultrafiltration, etc.) are not ideal due to a variety of shortcomings. NCL has developed a novel bioanalytical technique to fractionate the various subpopulations of a nanomedicine in plasma, in an effort to fulfill this unmet need and facilitate regulatory review of generic nanomedicines/nanosimilars—the Stable Isotope Tracer Ultrafiltration Assay (SITUA). This method is now available as a service to nanomedicine developers. Developers can select from one of two studies, designed to further advance the preclinical development of their formulation.

Two services are available to evaluate nanomedicine drug release and pharmacokinetics:

NCL-01: An in vitro drug release study in human plasma using the SITUA. The assay will provide concentrations of encapsulated, free/unbound, and protein-bound drug and is ideal for **formulation optimization** and **lot release**.

NCL-02: A pharmacokinetic study in rats using the SITUA. Blood samples will be collected at various timepoints for analysis of encapsulated, free/unbound, and protein-bound drug concentrations, as well as noncompartmental pharmacokinetic analysis to complement **preclinical bioequivalence evaluation**.

For more information on the Technical Services, please click here.



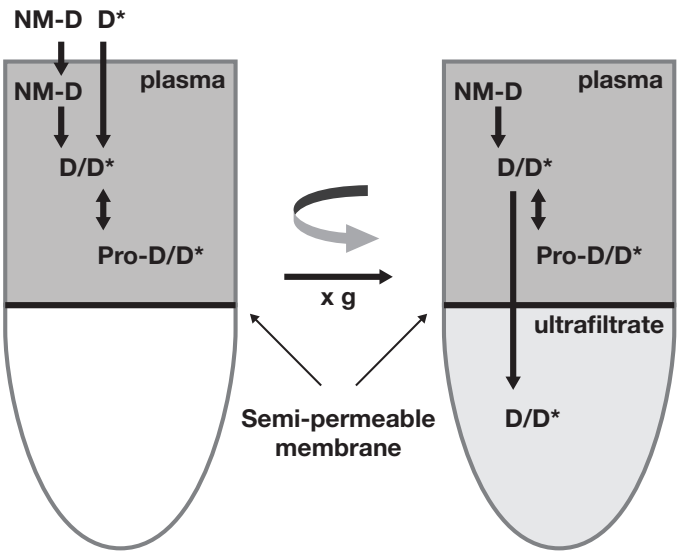
How The Assay Works

The assay uses a stable isotopically labeled version of the nanomedicine-encapsulated drug (D*) and an ultrafiltration technique. When spiked into plasma or other matrix, D* equilibrates with protein (Pro) and nanomedicine (NM) identical to the unlabeled, normoisotopic drug (D). Therefore, the ultrafiltrate fraction of the isotopically labeled drug represents a reliable measurement of the free drug fraction. The plasma protein bound, unencapsulated, and encapsulated nanomedicine fractions can then be easily calculated:

$$\textbf{\% Bound D*} = \frac{([\text{Total D*}] - [\text{Ultrafilterable D*}]) * 100}{[\text{Total D*}]}$$

$$\textbf{[Unencapsulated D]} = \frac{[\text{Ultrafilterable D}]}{(1 - (\%\text{Bound D*}/100))}$$

$$\textbf{[Encapsulated D]} = [\text{Total D}] - [\text{Unencapsulated D}]$$



More details on the SITUA can be found in the following publications:

J Control Release, 2015;220(Pt A):169-174
Methods Mol Biol. 2018;1682:223-239

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Frequently Asked Questions

Q: Who will benefit from this service?

A: Both preclinical and early-stage clinical developers of nanomedicines/complex drugs. The SITUA is ideally suited to assess the in vitro or in vivo bioequivalence of a generic nanomedicine/nanosimilar to that of a reference formulation. The SITUA is also well suited for formulation optimization and lot release.

Q: What do I get/How are the results reported?

A: Data will be supplied in a written report, sent electronically. The report will contain 1) a description of the method, 2) raw data, and 3) analysis and interpretation of the results. Results are prepared for efficient method transfer to GLP or GMP laboratories to perform studies in support of regulatory filings, clinical bioequivalence testing, and lot release.

Q: When can I expect results?

A: The time for study completion and delivery of the final report is dependent upon the Technical Service purchased. NCL-01, drug release assessment in human plasma, is anticipated to take 5 weeks for completion of the assay and preparation of the report. NCL-02, the rodent PK study, is expected to take 8 weeks.

Q: What are the requirements?

A: Developers will be expected to supply at least 20 mg of the API equivalent of their nanomedicine formulation, 10 mg of the free API, and 1 mg of a stable isotopically labeled API. Ideally, the stable isotope should be ≥3 atomic mass units (amu) greater than the normoisotopic API, and use ^{13}C labeling (or other stable isotope) as opposed to deuteration.

Q: Is this via an application process, similar to the NCL Assay Cascade characterization program?

A: No. These assays are available as a fee-for-service to nanomedicine developers. This is not part of NCL's Assay Cascade program and no application is required.

Q: How do I access the service?

A: Interested parties simply contact the NCL to complete the necessary paperwork and arrange for payment.

For questions or more information, please contact the NCL at **ncl@mail.nih.gov**

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