Plasma Protein Binding:
Reliable data for the most challenging drug modalities

Hinnerk Boriss

Sovicell, Friedrich Ebert Str 16, 04109 Leipzig, Germany, hbo@sovicell.com

Abstract

Accurate assessment of plasma protein binding (PPB) for lipidated peptides and other complex biologics remains a major challenge. Molecules such as semaglutide and tirzepatide exhibit slow diffusion, strong lipophilicity, and high plasma binding, which compromise conventional approaches like equilibrium dialysis and ultrafiltration. These limitations result in poor recovery, high variability, and unreliable free fraction estimates when binding is extensive.

Here, we introduce the TRANSIL Peptide Binding Kit, an equilibrium shift assay specifically developed for PPB determination of biologics. The method uses plasma in all assay compartments to improve solubility and recovery, and quantifies the concentration of test items in the liquid phase (bound and free) versus an immobilized phase. By shifting the binding equilibrium through controlled amounts of an alternative immobilized binding phase, the assay enables calculation of binding parameters without requiring direct measurement of extremely low free fractions.

We present experimental results for semaglutide alongside ODE-based simulations of assay performance. The simulations demonstrate how assay design influences precision at very low free fractions and define the optimal conditions for reliable measurement down to 0.001% unbound fraction. Together, the data confirm that the TRANSIL Peptide Binding Kit delivers robust, reproducible, and biologically meaningful PPB estimates for lipidated peptides and related modalities, providing a practical solution for DMPK groups working on complex drug classes.

Introduction

Plasma protein binding (PPB) is a key determinant of drug distribution, clearance, and pharmacological activity. While conventional methods such as equilibrium dialysis and ultrafiltration are well established for small molecules, they often fail when applied to complex modalities like lipidated peptides, protein fragments, PROTACs, and macrocycles. Slow diffusion, high lipophilicity, and extensive binding frequently lead to low recovery and inconsistent results. Reliable methods capable of quantifying very low free fractions are therefore essential to support the development of next-generation therapeutics.

The Assay

The TRANSIL Peptide Binding Kit determines one or more dissociation constants (KD) of a test item to plasma proteins through an equilibrium shift approach. Test compounds are incubated with diluted plasma in the presence of increasing amounts of immobilized beads that serve as an alternative binding phase. Each plasma dilution generates multiple equilibrium points—for example, 2 dilutions yield 10 equilibria, 3 dilutions yield 15, and up to 25 equilibria can be obtained with 5 dilutions. These data provide a rich binding profile that is analyzed by fitting the parameters of an ordinary differential equation (ODE) model, which accurately describes the binding process and allows robust estimation of binding parameters, even at very low free fractions of 0.001% or less.

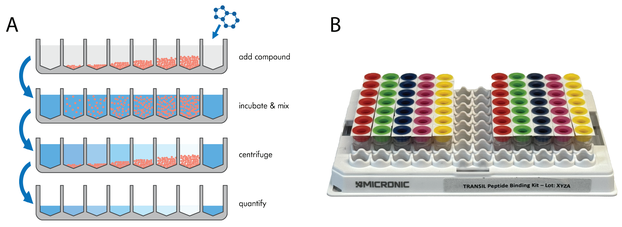

Fig. 1: A: Illustration of the assay workflow. The test item is added at a constant concentration to all wells containing plasma and varying amounts of beads. Incubation is carried out with 120 aspiration–suspension cycles to ensure equilibrium. Following centrifugation, the beads are pelleted and the supernatants collected for LC–MS/MS analysis of compound concentrations. B: picture of the assay kit in 96 well format for testing two compounds using 5 plasma dilutions.

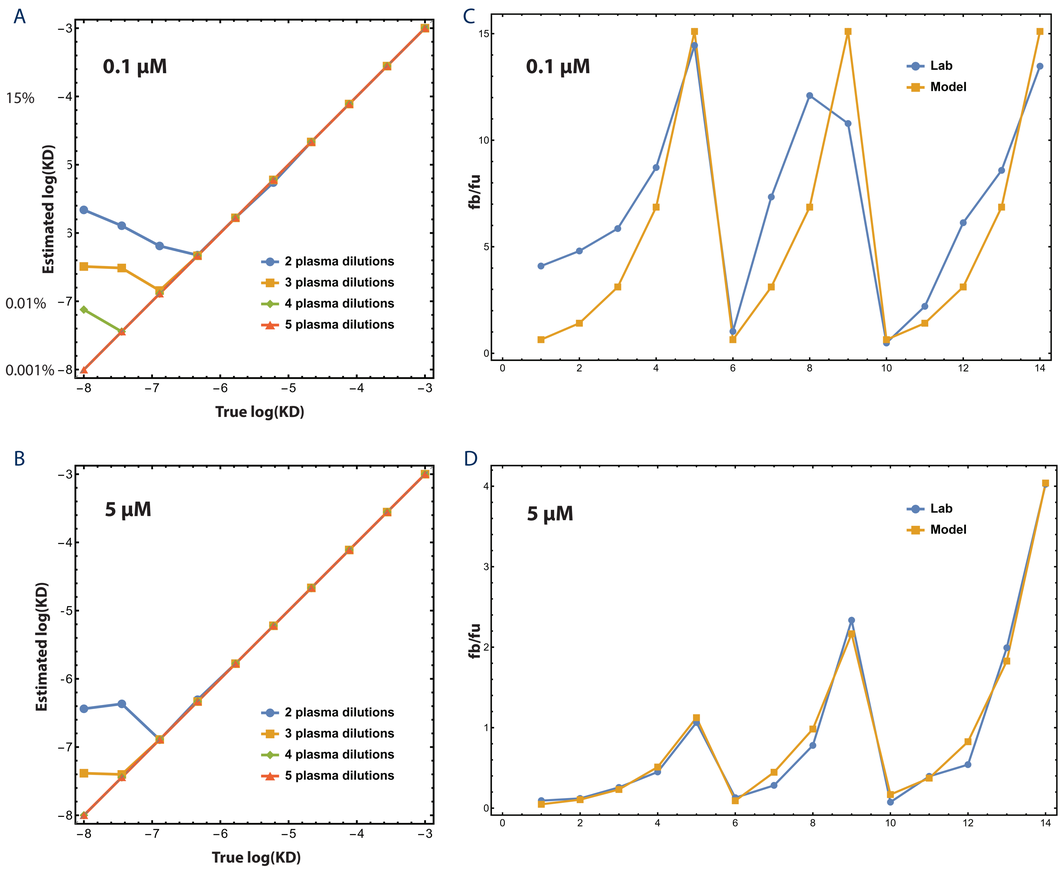

Fig. 2: Analyzing the influnce of the test item concentration on assay accuracy by simulation (A and B) and lab experiments (C and D). A: simulations with 0.1 µM test item conc. and a set of 2 to 5 plasma dilutions; B: simulations with 5 µM test item conc. and a set of 2 to 5 plasma dilutions; C: Assay data and fit for 0.1 µM semaglutide and 3 plasma dilutions; D: 5 µM semaglutide and 3 plasma dilutions.

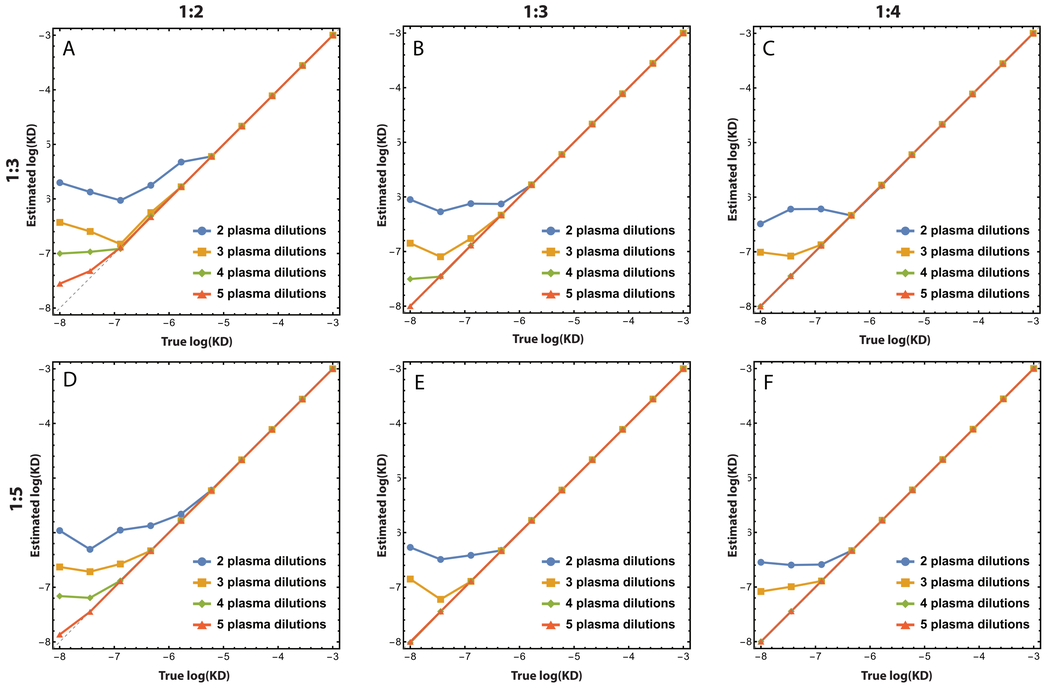

Fig. 3: Analyzing the influnce of the plasma dilution steps using the simulation model and a 5 µM test item concentration. A: initial plasma diltution: 1:3, subsequent dilutions steps 1:2; B: initial plasma diltution: 1:3, subsequent dilutions steps 1:3; C: initial plasma diltution: 1:3, subsequent dilutions steps 1:4; D: initial plasma diltution: 1:5, subsequent dilutions steps 1:2; E: initial plasma diltution: 1:5, subsequent dilutions steps 1:3; F: initial plasma diltution: 1:5, subsequent dilutions steps 1:4.

Conclusion

(1) The TRANSIL Peptide Binding Kit provides reliable plasma protein binding measurements for lipidated peptides, PROTACs, macrocycles, and small molecules.

(2) The assay enables quantification of free fractions as low as 0.001% or below.

(3) Highest accuracy is obtained when test item concentrations are ≥ 5 µM.

(4) Assay accuracy and resolution improve with greater spread of binding equilibria achieved through stronger plasma dilutions, balanced against maintaining test item solubility.

(5) Because data analysis relies on a non-linear kinetic model, binding does not need to be measured at multiple concentrations; saturation effects are captured by the model parameters and can be used to predict in-vivo behavior.

(6) For compounds with weak albumin binding but strong affinity to AGP, the non-linear kinetic model is essential to accurately estimate overall plasma protein binding

(7) Together, these results demonstrate that the TRANSIL Peptide Binding Kit offers a robust, high-resolution, and biologically relevant approach for plasma protein binding studies of complex drug modalities.

The Model

We developed a kinetic binding model that mechanistically describes time-resolved binding based on drug-specific on- and off-rates. The model captures saturation effects and nonlinearities arising from competitive interactions, providing an accurate representation of complex binding behavior. It explicitly incorporates binding to the major plasma proteins, albumin and α1-acid glycoprotein (AGP), as well as binding to the TRANSIL beads, ensuring a realistic description of the experimental system.

The model accounts for binding of the test item (S) to three key components: binding protein 1 (P1, albumin), binding protein 2 (P2, α1-acid glycoprotein), and the beads (B). Each interaction is described by its specific on-rates (m) and dissociation rate (k) constants, allowing the model to capture the full kinetics of binding across all relevant phases.

$$P_1 \underset{m_{11}}{\overset{k_{11}}{\rightleftharpoons}} P_1S$$

$$S + P_2 \underset{m_{12}}{\overset{k_{12}}{\rightleftharpoons}} P_2S$$

$$B \underset{m_b}{\overset{k_b}{\rightleftharpoons}} BS$$

$$S'[t] = m_{11}P_1S[t] - k_{11}S[t]P_1[t] + m_{21}P_2S[t] - k_{21}S[t]P_2[t] + m_bS[t]P_2[t] - k_bS[t]B[t]$$

$$\frac{dP_1}{dt} = m_{11}P_1S[t] - k_{11}P_1[t]S[t] \qquad \frac{dP_1S}{dt} = k_{11}P_1[t]S[t] - m_{11}P_1S[t]$$

$$\frac{dP_2}{dt} = m_{21}P_2S[t] - k_{21}P_2[t]S[t] \qquad \frac{dP_2S}{dt} = k_{21}P_2[t]S[t] - m_{21}P_2S[t]$$

$$\frac{dB}{dt} = m_bBS[t] - k_bB[t]S[t] \qquad \frac{dBS}{dt} = k_bB[t]S[t] - m_bBS[t]$$

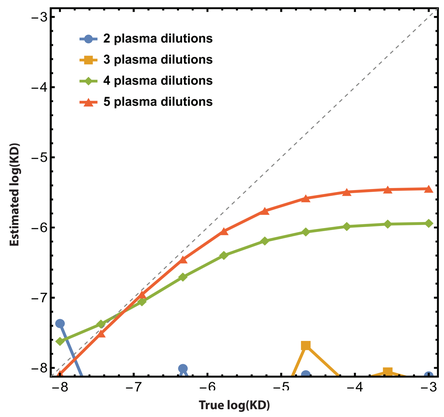

Fig. 4: Illustration of the importance of the non-linear model. Simulations show binding profiles across varying albumin dissociation constants under conditions of strong AGP binding. A simple model assuming albumin binding alone produces accurate estimates only when albumin affinity is strong; once albumin binding weakens, estimates deteriorate while AGP binding dominates.