Plasma Protein Binding: Disentangling binding saturation with a kinetic model

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Abstract

We developed a differential equation model to simulate the competitive binding of two ligands to two plasma proteins, each with a single binding site. These two proteins can be considered representative of albumin and alpha-1-acid glycoprotein (AGP), the primary plasma binding proteins in humans. The model provides a mechanistic, time-resolved description of binding, saturation, and displacement based on drug-specific kinetic parameters and physiologically relevant protein concentrations. Simulation results show that binding saturation follows the same kinetic principles as displacement: it becomes observable only when a compound binds weakly to albumin (KD ≥ 10^4 M) and strongly to AGP (KD ≤ 10^6 M), or any other low-abundance plasma protein. Saturation becomes detectable when the drug concentration approaches 50% of the protein's plasma concentration. Similarly, displacement between two compounds only occurs when both compounds exhibit this binding profile. If either compound binds strongly to albumin or lacks measurable AGP affinity, neither saturation nor displacement is observed at pharmacologically relevant concentrations. These findings demonstrate how kinetic modeling can help distinguish true non-linear binding from experimental artifacts and guide early compound profiling in drug development.

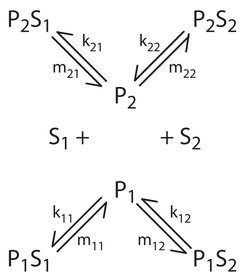
Although true plasma protein binding saturation is rare at therapeutic concentrations, it can lead to non-linear pharmacokinetics when it occurs - especially for high-affinity compounds targeting low-abundance proteins like AGP. This non-linearity can cause unpredictable increases in free drug levels, altered distribution, and changes in clearance, which may impact both efficacy and safety. A clear strategy to assess the risk of binding saturation is therefore essential during drug development.

The Model

To characterize compound properties influencing plasma protein binding and displacement, we developed a simulation model describing two ligands with distinct binding properties competing for two plasma proteins. Each protein is modeled with a single binding site that can reversibly bind either ligand, allowing competition at both sites. The conceptual structure of the model is shown in Figure 1, and the corresponding system of differential equations is presented in Figure 2. All simulations and analyses were performed using Mathematica.

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.

A



B

$$\frac{dS_1}{dt} = m_{11}P_1S_1 - k_{11}\cdot S_1\cdot P_1 + m_{21}\cdot S_1 - k_{21}\cdot S_1\cdot P_2$$

$$\frac{dS_2}{dt} = m_{12}P_1S_2 - k_{12}\cdot S_2\cdot P_1 + m_{22}\cdot S_1 - k_{22}\cdot S_2\cdot P_2$$

$$\frac{dP_1S_1}{dt} = k_{11}\cdot P_1\cdot S_1 - m_{11}\cdot P_1S_1$$

$$\frac{dP_1S_2}{dt} = k_{12}\cdot P_1\cdot S_2 - m_{12}\cdot P_1S_2$$

$$\frac{dP_2S_1}{dt} = k_{21}\cdot P_2\cdot S_1 - m_2\cdot P_2S_1$$

$$\frac{dP_2S_2}{dt} = k_{22}\cdot P_2\cdot S_2 - m_{22}\cdot P_2S_2$$

$$\frac{dP_1}{dt} = m_{11}\cdot P_1S_1 + m_{12}\cdot P_1S_2 - k_{11}\cdot P_1\cdot S_1 - k_{12}\cdot P_1\cdot S_2$$

$$\frac{dP_2}{dt} = m_{21}\cdot P_2S_1 + m_{22}\cdot P_2S_2 - k_{21}\cdot P_2\cdot S_1 - k_{22}\cdot P_2\cdot S_2$$

C

$$f_u = \frac{1}{1 + \frac{[HSA]}{K_D^{HSA}} + \frac{[AGP]}{K_D^{APG}}}$$

Fig. 1: A: Definition of the competitive binding model. Plasma proteins P_1 (=HSA) and P_2 (=AGP) each have a single binding site that can either bind ligand (drug) S_1 (test item 1) or S_2 (test item 2). The on-rates are denoted by k_{ij} while the off-rates are denoted by m_{ij}. B: Set of differential equations used to implement to model for simulation drug displacement. Ligand one is denoted by S_1 (denoted drug A in results section), ligand two is denoted by S_2 (denoted drug B in results section). HSA without ligand is denoted as P_1 and AGP without ligand is denoted P_2. HSA with ligand S_1 bound is denoted P_1S_1, AGP with ligand S_1 bound is denoted P_2S_1, HSA with ligand S_2 bound is denoted P_1S_2, AGP with ligand S_2 bound is denoted P_2S_2.
C: Linearized model.

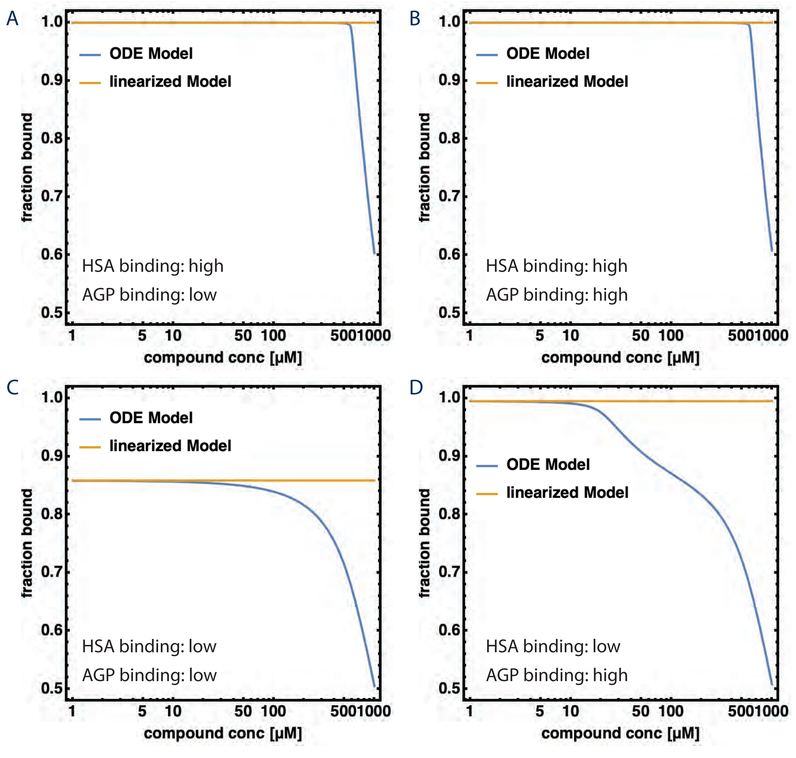


Fig. 2: Influence of compound concentration on plasma protein binding in a physiological mixture of human serum albumin (HSA) and alpha-1-acid glycoprotein (AGP).
High-affinity binding is modeled with a dissociation constant KD of 10^{-7} M, and low-affinity binding with a KD of 10^{-4} M. The standard linearized binding model significantly overestimates actual plasma protein binding when the compound binds weakly to HSA but strongly to AGP.

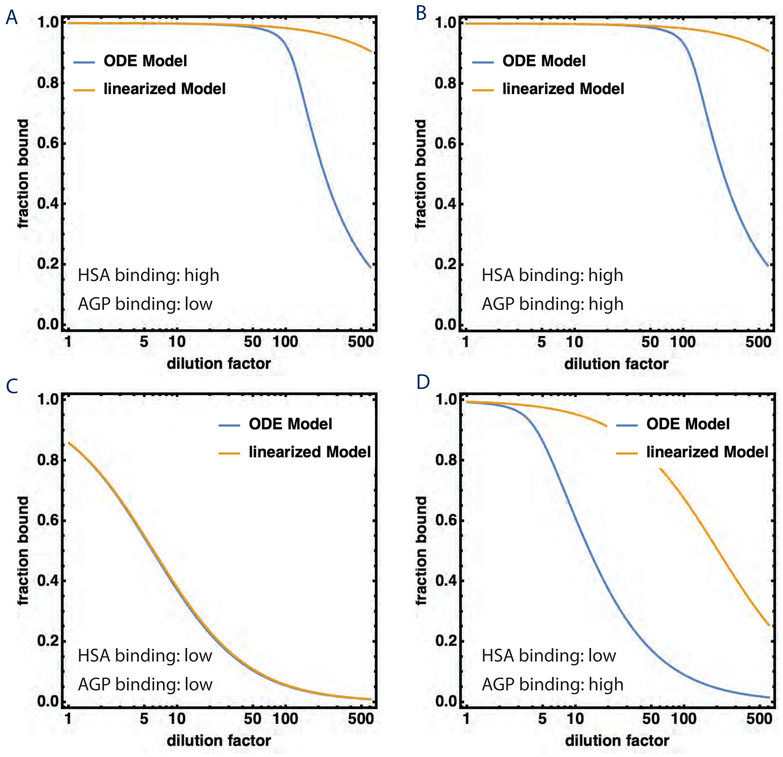


Fig. 3: Influence of plasma dilution on plasma protein binding in a physiological mixture of human serum albumin (HSA) and alpha-1-acid glycoprotein (AGP). High-affinity binding is modeled with a dissociation constant KD of 10^{-7} M, and low-affinity binding with a KD of 10^{-4} M. The standard linearized binding model significantly overestimates actual binding when the compound binds weakly to HSA but strongly to AGP.

Conclusion

(1) Saturation of plasma protein binding leads to non-linear pharmacokinetics, causing disproportionate increases in free drug levels and affecting distribution, clearance, and half-life.

(2) Drugs are only at risk of saturation at clinically relevant concentrations if they bind weakly to albumin and strongly to low-abundance proteins like AGP - unless doses are extremely high or plasma is highly diluted.

(3) Highly bound drugs with high AGP affinity and low albumin affinity are especially prone to saturation and non-linear binding behavior.

(4) Reliable prediction of plasma protein binding requires a kinetic model that accounts for on-rates, off-rates, and competition for binding sites.

(5) Standard assays often miss these effects due to methodological limitations and assumptions of linearity.

(6) Accurate prediction requires drug-specific KD values and a mechanistic kinetic model that captures time-resolved binding dynamics.

(7) The TRANSIL HSA and AGP Binding Kits provide a fast and reliable way to determine dissociation constants and assess the risk of saturation-driven non-linearity early in development.

Assay Strategy

Reliable prediction of plasma protein binding saturation requires accurate measurement of binding affinities to both major plasma proteins—albumin and AGP. The TRANSIL HSA and AGP Binding Kits offer a robust and practical strategy to obtain precise KD values under controlled, physiologically relevant conditions. Unlike conventional assays, which often require measurements at varying compound concentrations and may suffer from solubility limits or non-specific binding artifacts, the TRANSIL approach determines true thermodynamic binding parameters without the need to approach saturation experimentally. This makes it particularly well-suited for identifying compounds at risk of non-linear pharmacokinetics due to saturable plasma protein binding.

The TRANSIL HSA Binding Kit determines the dissociation constant KD of test compounds—whether small molecules or biologics—toward human serum albumin (HSA). The resulting KD values are ideally suited for non-linear pharmacokinetic models, enabling accurate, mechanistic prediction of binding saturation under both experimental and physiological conditions. Each kit supports the determination of KD values for up to 12 compounds.

Incubation requires only 20 aspiration and suspension cycles for small molecules and 120 cycles for larger biologics. Binding is measured in a pH-controlled PBS buffer that resists CO_2-induced pH shifts, ensuring high physiological relevance. The readout is based on quantitative LC-MS, UV, or fluorescence, depending on compound characteristics.

The TRANSIL AGP Binding Kit measures the dissociation constant KD of small molecules and biologics to alpha-1-acid glycoprotein (AGP) - a key plasma protein involved in the binding of basic and lipophilic compounds. These KD values are critical for predicting non-linear plasma protein binding and for identifying potential saturation effects, particularly when AGP is the dominant binding partner.

Each kit enables the profiling of up to 12 compounds with a simple incubation protocol: 20 mixing cycles for small molecules and 120 cycles for biologics. The assay is performed in a CO_2-stable, PBS-based buffer optimized for physiological relevance. Detection is compatible with LC-MS, UV, or fluorescence, depending on the test compound's properties.