Supplementary Information
Materials and methods

Materials

Native AAG (Sigma, cat# G9885, lot# 018K7535), sirolimus (LC Laboratories, cat# R-5000, lot# ASW-112), and tacrolimus (LC Laboratories, cat# F-4900, lot# ATH-109) were used as supplied. The F1/S and A genetic variants of AAG were separated following the method of Hervé et al.\(^1\) as described previously.\(^2\) The molar ratio of the F1/S:A genetic variants in the commercial native AAG mixture was 75:25.

Preparation of drug and AAG solutions

Stock solutions of sirolimus and tacrolimus were prepared freshly in HPLC grade ethanol. The volume of ethanol added into AAG solutions never exceeded 3% (v/v) and caused negligible effects on the protein CD spectrum. AAG was dissolved in physiological Ringer buffer, pH 7.4 (8.1 mM Na\(_2\)HPO\(_4\)·12H\(_2\)O, 1.5 mM KH\(_2\)PO\(_4\), 137 mM NaCl, 2.7 mM KCl, 0.8 mM CaCl\(_2\), 1.1 mM MgCl\(_2\)). AAG concentration was calculated for a molecular mass of 40,000.

Circular dichroism and UV absorption spectroscopic measurements

CD and UV absorption spectra were recorded on a JASCO J-715 spectropolarimeter at 25 ± 0.2 °C in a rectangular quartz cell of 1 cm optical path length (Hellma, USA). Temperature control was provided by a Peltier thermostat equipped with magnetic stirring. Each spectrum represents the average of three scans obtained by collecting data at a scan speed of 100 nm/min. Absorption spectra were obtained by conversion of the high voltage (HT) values of the photomultiplier tube of the CD equipment into absorbance units. CD and UV curves of drug-AAG mixtures were corrected by blank protein solution. JASCO CD spectropolarimeters record data as ellipticity (‘\(\Theta\)’) in units of millidegrees (mdeg). The
quantity of ‘Θ’ is converted to ‘Δε’ values using the equation
\[ \Delta \varepsilon = \Theta/(33982c) \], where ‘Δε’ is the molar circular dichroic absorption coefficient expressed in M\(^{-1}\)cm\(^{-1}\), ‘c’ is the molar concentration of the ligand (mol/L), and ‘l’ is the optical pathlength expressed in cm.

**Calculation of the AAG binding parameters of sirolimus**

The stoichiometry of sirolimus-AAG complexes was determined by linear regression analysis of the linear parts of the CD titration curves. Intersection of linear extrapolations of the first and plateau phase of the titration data provides the number of binding sites (\(n\)) per a protein molecule (Fig. S2, ESI). The obtained values were taken into account in calculation of the association constants.

Drug-Protein binding can be quantified by the association constant (\(K_a\)):

\[
D + P \rightleftharpoons DP; \quad K_a = \frac{[DP]}{[D][P]} \quad (1)
\]

It is evident that

\[
[D] = c_d - [DP] \quad (2)
\]

and

\[
[P] = c_p - [DP] \quad (3)
\]

where \(c_d\) and \(c_p\) are the total molar concentrations of the drug and the protein, respectively.

Since the formation of sirolimus-AAG complexes is responsible for the ICD signal measured at 270 nm, it can be written that

\[
ICD (mdeg) = k [DP] \quad (4)
\]

where \(k\) is a constant. Using equations 1-4, we obtain

\[
ICD (mdeg) = \frac{k}{2} \left( c_p + c_d + K_a^{-1} - \sqrt{(c_p + c_d + K_a^{-1})^2 - 4c_p c_d} \right)
\]

Non-linear regression analysis of the ICD values using NLREG\(^\circledast\) (statistical analysis program, ver. 3.4) was performed to obtain the value of \(K_a\).
References


Supplementary Figure 1

Comparison of CD spectra of tacrolimus measured in the absence and presence of native AAG (mixture of the genetic variants). Inset shows the chemical structure of the drug.
Supplementary Figure 2

Non-linear regression analysis of the ellipticity changes of sirolimus obtained by titration of AAG solutions (solid lines are the results of curve fitting procedures). Association constants \( (K_a) \) estimated for the native protein and the F1/S genetic variant are shown. The number of the binding sites per a protein molecule \( (n) \) was estimated by linear regression analysis of the linear parts of the CD titration curves (red lines).