Supporting information

1. Pressure-Induced Transitions in Lipid Phase and Scaffold Protein of Nanodiscs

In order to probe the stability of Nanodiscs under pressure and possible pressure-induced phase transitions in the lipid phase we investigated the effect of hydrostatic pressure on the tryptophan fluorescence of the scaffold protein and on the fluorescence of 4-(dicyanovinyl)julolidine (DCVJ, Fig. S1) incorporated into Nanodiscs devoid of CYP3A4. The fluorescence of DCVJ, which is known to be sensitive to microviscosity of the environment (41), was used as a probe of a pressure-induced phase transition in the lipid phase of Nanodiscs.

Experimental

Incorporation of DCVJ into the Nanodiscs was achieved by 15 min incubation of 50 μM solution of Nanodiscs in Na-Hepes buffer, pH 7.4, 1 mM DTT, 1 mM EDTA at room temperature with a 3-fold molar excess of DCVJ added as a 20 mM solution in acetone. Excess label was removed on Bio-Beads resin (Bio-Rad Laboratories, Hercules, CA) followed by concentration on a Sartocon-1 (MWCO 20,000) concentrator (Sartorius AG, Goetingen, Germany) and gel filtration on a BioGel P-6 column (Bio-Rad Laboratories, Hercules, CA). The resulting preparation had approximately one mole of DCVJ per two moles of Nanodiscs, as estimated from the absorbance spectra using the extinction coefficient of 61.5 mM⁻¹cm⁻¹ for DCVJ absorbance at 460 nm (41). Pressure perturbation fluorometric experiments were performed using a MC2000-2 multi-channel CCD rapid scanning spectrometer (Ocean Optics, Inc., Dunedin, FL) connected to a fluorescence-monitoring window of the high-pressure cell.

Results

Effect of hydrostatic pressure on the fluorescence of the scaffold protein. The effect of hydrostatic pressure on the tryptophan fluorescence of the CYP3A4-containing Nanodiscs is illustrated in Fig. S2. As seen from this figure, increase in hydrostatic pressure results in a gradual decrease in tryptophan fluorescence, which reveals a process with ΔV° of -16 ml/mol (Fig S2b). These pressure-induced changes in tryptophan fluorescence, which apparently represent some pressure-induced conformational transition in the scaffold protein, were completely and immediately reversible upon decompression from 4.8 kbar (Fig S2a, dashed line). We may conclude therefore that the application of hydrostatic pressures up to 4.8 kbar does not result in any denaturation of the proteins of the CYP3A4-containing Nanodiscs and does not affect their structural integrity.

Effect of hydrostatic pressure on the fluorescence of DCVJ incorporated into Nanodisks. As shown in Fig. S3a, a remarkable increase in the intensity of the fluorescence of DCVJ is observed at high hydrostatic pressures. This finding suggests a transition in the lipid phase of the Nanodiscs (ΔV° = -21 ml/mol, P½ = 4.3 kbar) that results in an increase in the microviscosity of the probe environment. This pressure-induced increase in DCVJ fluorescence was completely reversible upon decompression from 4.8 kbar (Fig. S3a, dashed line), suggesting that the structural integrity of the Nanodiscs is not affected by hydrostatic pressure in the range studied. The extent of this transition in the region of interest for the effect of pressure on substrate binding and spin equilibrium of CYP3A4 (1-2500 bar) does not exceed 18% of its maximal amplitude (Fig. S3b). Therefore, the transition in the lipid phase does not interfere with the barotropic behavior of CYP3A4ND described in our study.

Fig. S1 4-(dicyanovinyl)julolidine (DCVJ)
**Fig S2.** Pressure-induced changes in the fluorescence of the scaffold protein of CYP3A4-containing Nanodisks. Panel *a* shows a series of emission spectra recorded at 1, 200, 400, 600, 800, 1600, 2400, 3200, 4000 and 4800 bar. The spectrum shown by a dashed line was recorded at 1 bar after decompression from 4.8 kbar at the end of the experiment. Panel *b* illustrates the changes in the integral intensity of fluorescence. The line represent the approximation of the data with eq. 3. Conditions: 1 μM solution of CYP3A3ND in 0.1 M Na-Hepes buffer, pH 7.4, 1 mM DTT, 1 mM EDTA. Excitation at 284 nm with 18 nm bandwidth.

**Fig S3.** Pressure-induced changes in the fluorescence of DCVJ incorporated into bare Nanodiscs. Panel *a* shows a series of emission spectra recorded at 1, 600, 1200, 1600, 2000, and 2400 bar. The spectrum shown by a dashed line was recorded at 1 bar after decompression from 4.8 kbar at the end of the experiment. Panel *b* illustrates the dynamics of the changes in the intensity of integral fluorescence. The data shown in this panel were scaled according to the result of fitting to equation 3 (solid line) to represent the relative extent of the pressure-induced transition. Conditions: 10 μM solution of bare Nanodisks with incorporated DCVJ probe in 0.1 M Na-Hepes buffer, pH 7.4, 1 mM DTT, 1 mM EDTA. Excitation at 440 nm with 40 nm bandwidth.
2. Applicability of the Complexes of 1-PB and Testosterone with 2-Hydroxypropyl-β-Cyclodextrin in Studies of Pressure-Induced Transitions in Cyp3A4

In order to extend the range of our studies of CYP3A4 interactions with 1-PB and testosterone to higher substrate concentrations we used the complexes with 2-hydroxypropyl-β-cyclodextrin (HPCD). As shown in Fig. S4 the results of spectral titrations using the HPCD complexes are very similar to the data obtained with the acetone solutions of these substrates.

The effect of hydrostatic pressure on the complexes of 1-PB with HPCD is illustrated in Fig S5. An increase in hydrostatic pressure results in a decrease in the amplitude of the peaks of fluorescence 1-PB at 376, 394 and 416 nm with a concomitant increase in the broad band of fluorescence of 1-PB excimers around 500 nm. This change signifies a pressure-induced dissociation of 1-PB complexes with HPCD. However, since the parameters of the interactions of HPCD complexes of 1-PB and testosterone with CYP3A4 are similar to those of the free substrates (Fig. S4), gradual dissociation of these complexes at increasing pressure would not appear to have any effect on the observed barotropic behavior of the enzyme.

Fig S4 Effect of HPCD on the interactions of CYP3A4 with 1-PB (a) and testosterone (b). Titration curves shown in circles and solid lines were obtained with the HPCD-complexed substrates, while the curves shown in squares and dashed lines show the results of titrations with acetone solutions. Lines show the results of the fitting of the data sets with the Hill equation with the following parameters: $S_{50} = 12.5$ μM, $n = 1.50$, $\Delta$(High spin)$_{\text{max}} = 42\%$ (a, circles); $S_{50} = 13.4$ μM, $n = 1.58$, $\Delta$(High spin)$_{\text{max}} = 43\%$ (a, squares); $S_{50} = 74.8$ μM, $n = 1.18$, $\Delta$(High spin)$_{\text{max}} = 43\%$ (b, circles); $S_{50} = 107$ μM, $n = 1.25$, $\Delta$(High spin)$_{\text{max}} = 40\%$ (b, squares). Titrations were performed using 1.5-2 μM solutions of CYP3A4 in 0.1 M Na-Hepes buffer, pH 7.4, 1 mM DTT, 1 mM EDTA.
Fig. S5  Effect of hydrostatic pressure on the fluorescence of a 12 μM solution of the 1-PB complex with HPCD. The spectra shown were recorded at 1–3200 bar with 400 bar increments. The inset shows the dynamics of the first principal component of the changes scaled according to the results of fitting to eq. 3 to represent the relative extent of the pressure-induced transition ($\Delta V^o = 18.7$ ml/mol, $P_{1/2} = 3080$ bar).