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Detection of Paracetamol Binding to Albumin in Blood Serum using 2D-IR Spectroscopy

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Paracetamol Justification

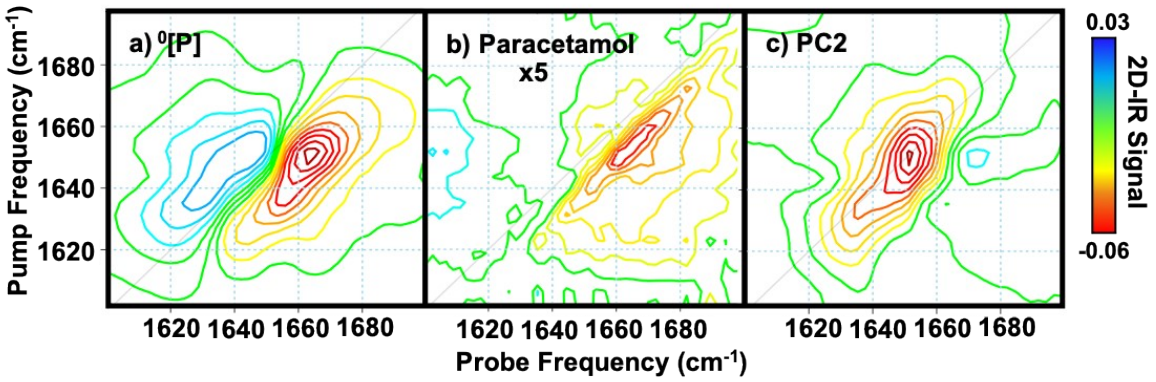

Figure S1: 2D-IR spectra of (a) serum only (^{0}[P]), (b) 10 mg/mL (66 mM) solution of paracetamol in H_2O, amplified by a factor of 5 for clarity, (c) The second principal component loadings from principal component analysis discussed in the main article.

Figure S1 compares 2D-IR spectra of serum and a solution of paracetamol in aqueous buffer solution. The bands in the 2D-IR spectrum of paracetamol do not coincide with those in the serum spectrum (Figure S1(a)). The diagonal paracetamol signal (Figure S1(b)) peaks at a probe frequency of 1665 cm^{-1}. In contrast, the changes to the serum spectra observed upon addition of paracetamol are represented by the PC2 loadings described in the main text (Figure S1(c)). This contains a distinct negative peak at a probe frequency of 1652 cm^{-1}, indicating that the changes observed in our serum dataset are not due to free paracetamol molecules. It is noted that the paracetamol signal shown in Figure S1(b) is amplified by a factor of 5 so that it can be plotted on the same scale as the serum only spectra in panel (a). The free paracetamol 2D-IR signal seen in Figure S1(b) is probed at a concentration of 66 mmol/dm^3 which is ~40 times greater than the highest levels used (1.8 mmol/dm^3) in this study. Based on this, the signals expected from the paracetamol concentrations used in this study would be well below the detectable limit.[1] Moreover, as a percentage of the paracetamol will bind to the serum albumin, the free fraction of paracetamol would be further reduced. Taking these together, we conclude that the spectral changes reported in the main text are not due to the free paracetamol fraction, but instead due to structural changes of the serum albumin upon binding.

Protein-Paracetamol Binding Curve Analysis

The albumin-paracetamol binding curve was modelled for comparison with the curve observed in our spectroscopic data. The association constant for paracetamol and albumin was taken from another study which determined the value to be $1x10^4$ mol/L.[2] Using the drug concentrations used in this study (7 µm to 1.8 mM) and the ligand binding equations,[3] the following binding curves were obtained for 100% availability of serum albumin (Figure S2). The binding curves for 10% and 20% highlighted in the main text were obtained by reducing the amount of serum albumin in the binding equations.

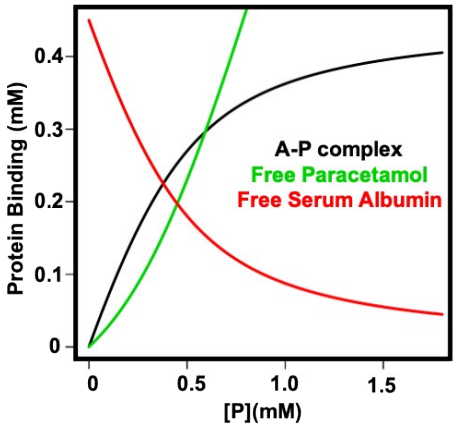

Figure S2: Binding curves for serum albumin and paracetamol. The black curve shows the formation of the albumin – paracetamol (A-P) complex with increasing paracetamol [P] concentration. The red curve indicates the free, unbound serum albumin left in solution and the green line shows the concentration of free, unbound paracetamol.

References

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