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Supporting Information

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for

$^{1}H\{^{19}F\}$ NOE NMR Structural Signatures of the Insulin R6 Hexamer: Evidence of a Capped HisB10 Site in Aryl- and Arylacryloyl-carboxylate Complexes

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Details of the NMR Spectroscopy and the Investigation of Spin Diffusion for the Aryl- and Arylacryloyl Carboxylate Complexes. As can be seen in Figure S1, the $^{1}H\{^{19}F\}$ NOE spectrum of compound **1** recorded using a 3.5 s recycle delay is virtually

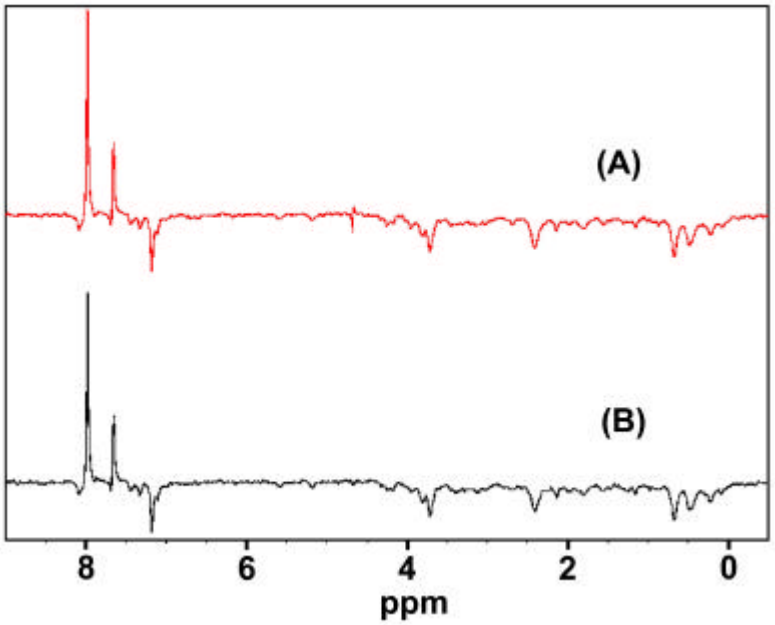

Figure S1. $^{1}H\{^{19}F\}$ NOE spectra recorded using different recycle delays A) 8 s, B) 3.5 s.

identical to a spectrum recorded using an 8 s recycle delay (>5 times the longest T_1). As the shorter recycle delay saves more than a factor of two in data collection time, it was employed throughout. The ^{19}F T_1 values for free and bound compound **1** are

1.61 and 1.24 s, respectively. Average ^{1}H T_1 values for hexameric insulin are ~1.5 s. The sequence used for collecting the $^{1}H\{^{19}F\}$ NOE spectra employs a composite pulse at the beginning before the relaxation delay that insures all ^{19}F magnetization is returned to the positive *z*-axis. Proton magnetization is maintained near saturation so that proton T_1 relaxation is largely irrelevant to the recycle time of the experiment.

To determine if spin diffusion is responsible for any of the observed NOEs, spectra were collected at mixing times of 200, 450, 700 and 900 ms (Figure S2). As can be seen, all of the major peaks are observed at the shortest mixing time. With the exception of the small peaks that appear in the 5.0 - 5.5 ppm region (900 ms mixing time), no delayed onset NOEs are observed. This result establishes that spin diffusion is insignificant.

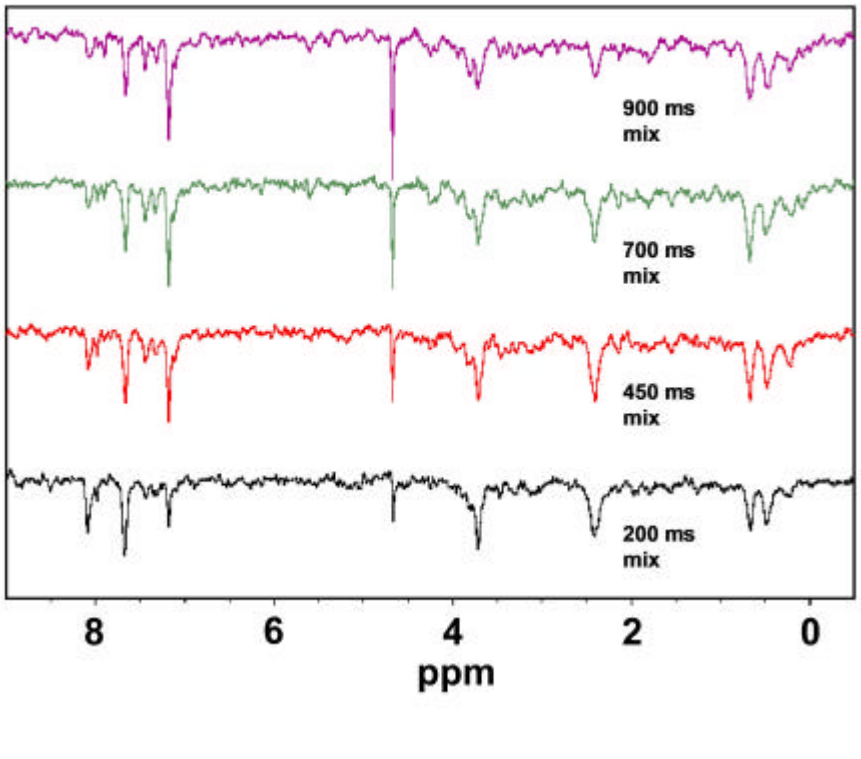

Figure S2. $^{1}H\{^{19}F\}$ NOE spectra recorded at different mixing times.

A modified version of the sequence was used to record the NOE spectrum under selective inversion of the free and bound ^{19}F peaks. Since the exchange rate is nonzero between free and bound under the experimental conditions used here, it would be expected that the hexamer protons experience some transferred NOE from

the free ligand when it is selectively inverted. Indeed this is what is observed as can be seen in Figure S3.

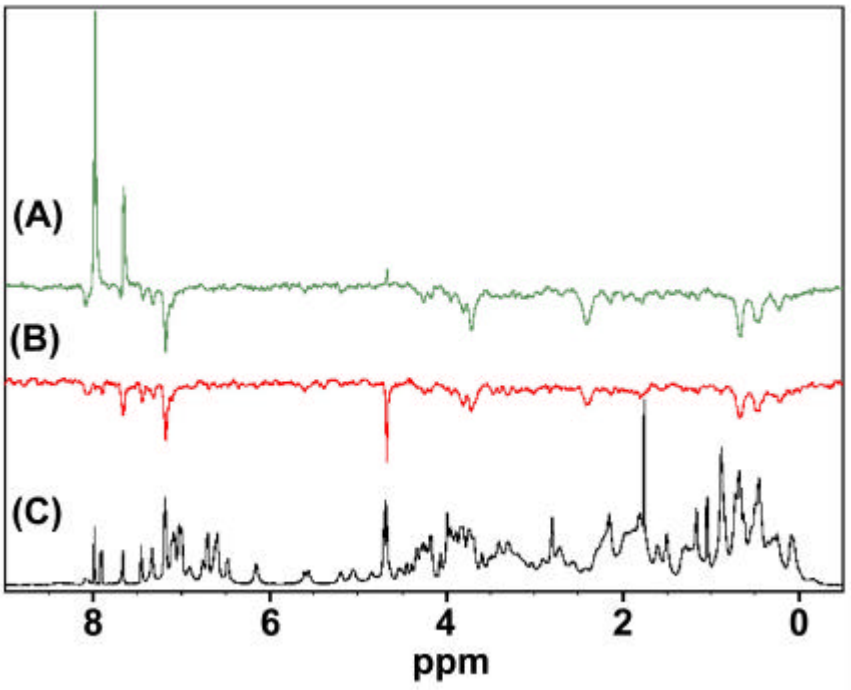

Figure S3. Comparison of the $^{1}H\{^{19}F\}$ NOE upon selective inversion of bound and free ^{19}F signal. A) Selective inversion of bound ligand. B) Selective inversion of free ligand. C) 1-D ^{1}H NMR spectrum.

Structural Models for Complexes with Compounds 1-4. Structural models also were calculated for the complexes formed between HI and compounds **2-4**. In Figure S4, these models (panels B-D) are compared with the model for the complex with compound **1** (panel A). As was found for the complex with compound **1**, these models are fully consistent with the $^{1}H\{^{19}F\}$ NOE difference spectra, which show NOEs to each of the three LeuB6, AsnB3 and PheB1 residues that form the walls of the HisB10 cavity.

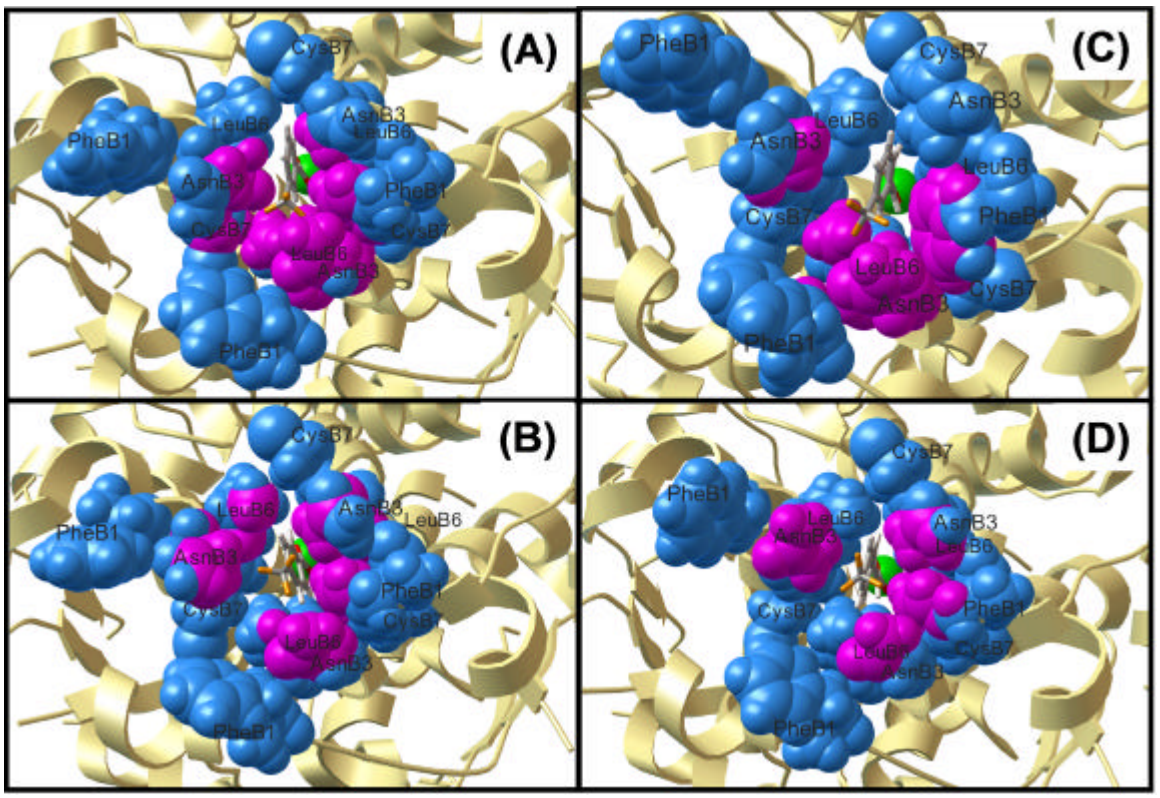

Figure S4. Comparison of the structural models for complexes between HI and compounds **1-4** (panels A-D, respectively). The models for the complexes with **2-4** (panels B-D were generated using the same approach as described for compound **1**. Amino acid residues PheB1, AsnB3, LeuB6 and CysB7 residues are shown in van der Waals spheres and the backbone of HI is shown in ribbons. Compounds **1-4** are represented in ball and sticks with CPK coloring. Color code: all protein backbone atoms, beige; F, bronze; atoms of PheB1, AsnB3, LeuB6 and CysB7 blue atoms within 5 Å of F, magenta; Zn^{2+}, green. Images created using Python Molecular Viewer.[35]