SUPPLEMENTARY INFORMATION

Detection of ^{15}N-labeled Metabolites in Microbial Extracts using AI-Designed Broadband pulses for ^{1}H, ^{15}N Heteronuclear NMR Spectroscopy

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CONTENT:

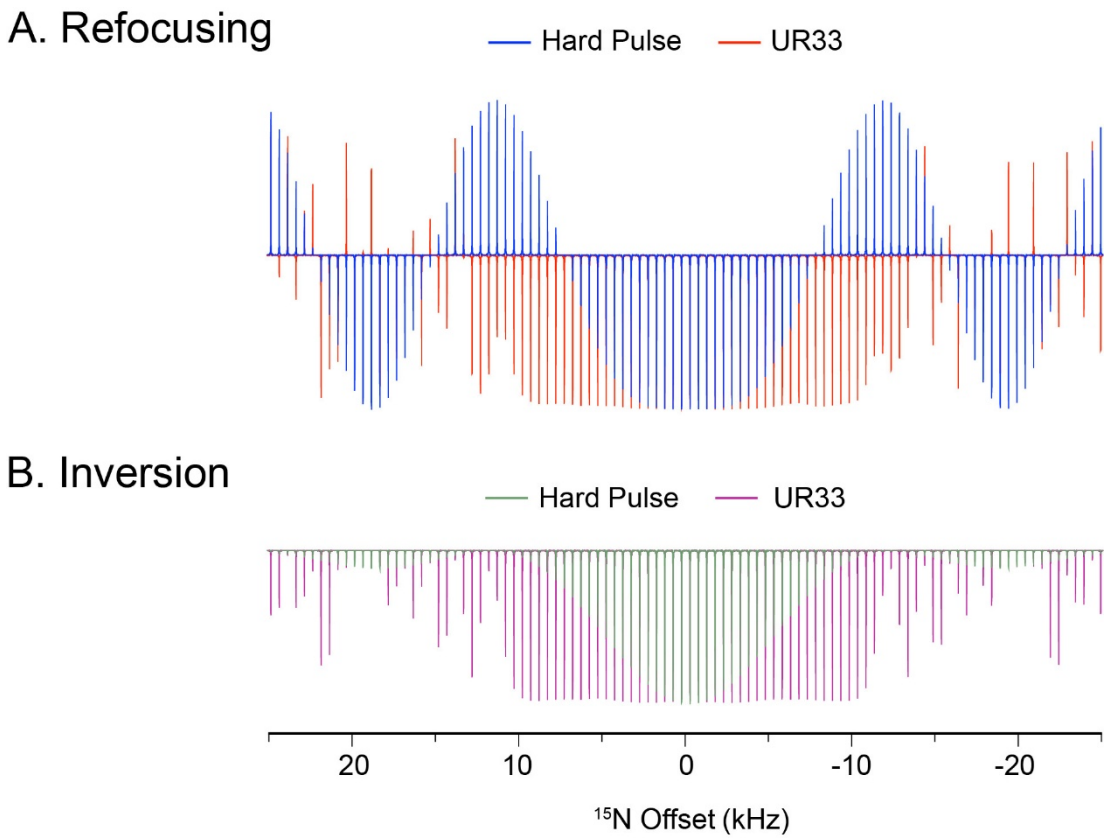

Figure S1. Offset responses for UR33 and hard pulses for refocusing and inversion operations. (A) Overlay of the frequency offset responses of refocusing operation for the UR33 pulse (blue) and hard pulse (red) across ±25 kHz offset range. (B) Overlay of the frequency offset response for inversion operation UR33 and hard pulses. The data were acquired using a sample of ^{15}N-labeled urea in DMSO. The spectra were collected with a 500 Hz interval across a ±25 kHz bandwidth. The profiles were obtained using the HSQC sequence (bbhsqcetf3gpsi2) where a single ^{15}N pulse was replaced with either the UR33 or hard pulse and its frequency arrayed to monitor the response, while maintaining all other pulses on resonance.

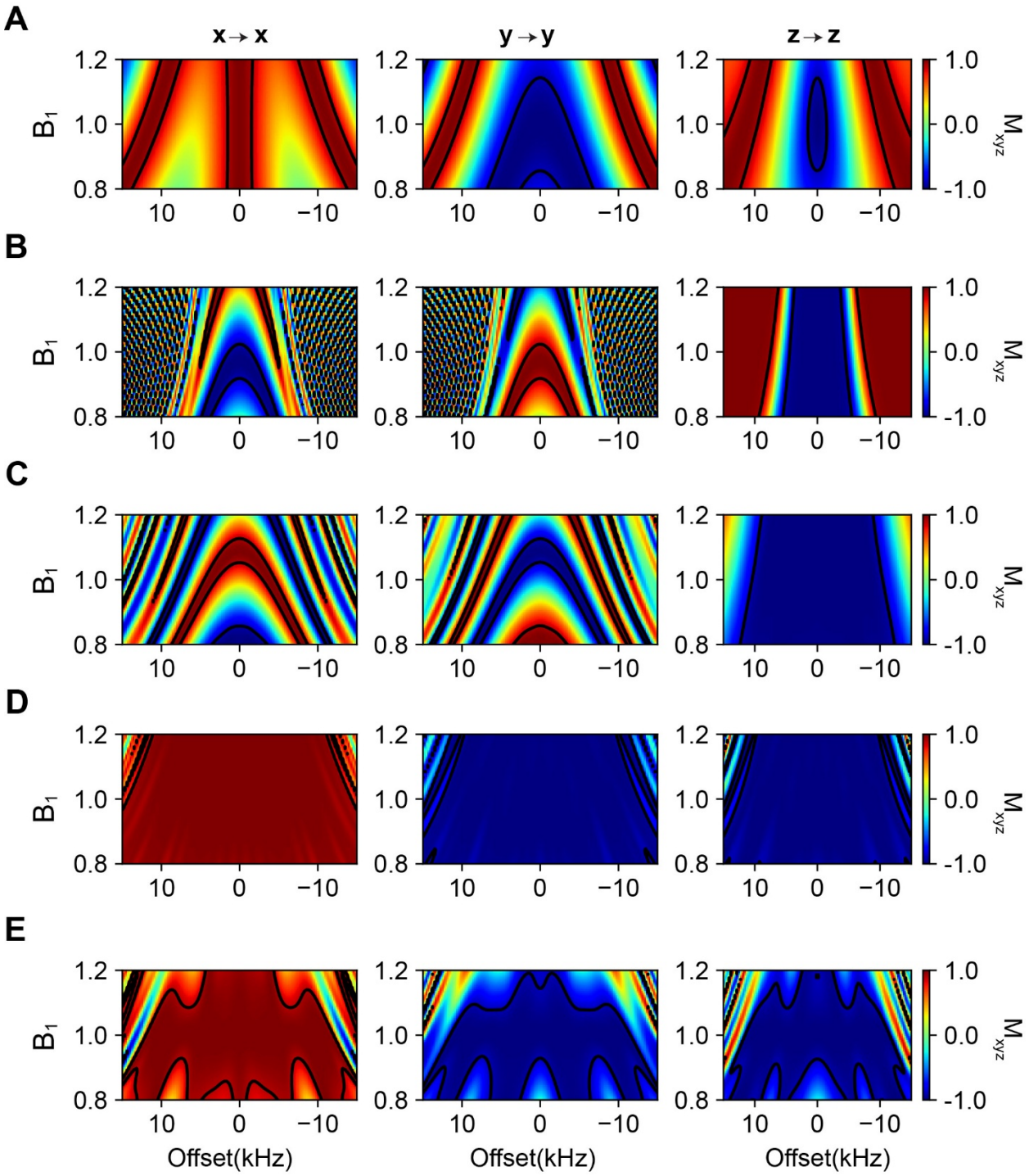

Figure S2. 2D simulated offset-B1 responses for the 180° pulses. A) hard pulse, B) HypSec.500, C) Crp20,1,40.1, D) Crp60comp.4, and E) UR33. The HypSec.500 was simulated for an RF amplitude of 7.15 kHz, and 1 ms duration; the hard π pulse with an RF amplitude of 7.62 kHz); the Crp20,1,40.1 pulse for an RF amplitude of 6.65 kHz, and duration of 600 μs; the Crp60comp.4 pulse with an RF amplitude of 6.98 kHz and duration of 2.8 ms; and the UR33 pulse for an RF amplitude of 7.61 kHz and a duration of 795 μs.

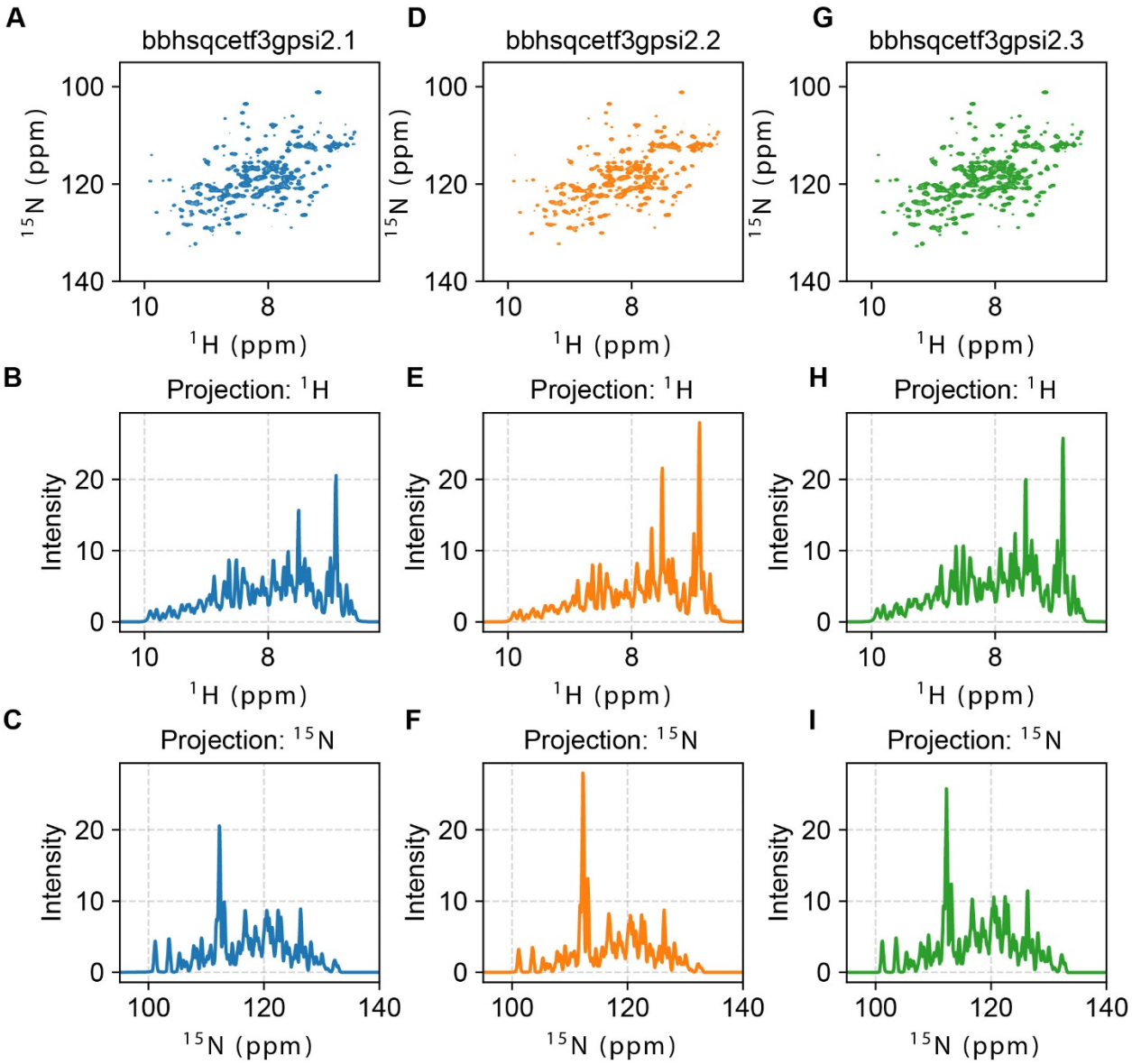

Figure S3: BB HSQC Spectra acquired with the different 180° pulses on a 0.8 mM protein sample of NRAS^{Q61R}-GTP (MW 20.3 kDa). 2D HSQC spectra and relative 1D projections for ^{1}H and ^{15}N, respectively, recorded using: (A-C) the Hypersecant pulse sequence (bbhsqcetf3gpsi2.1); (D-F) the CHIRP pulse sequence (bbhsqcetf3gpsi2.2); (G-I) the pulse sequence with the AI-generated pulses (bbhsqcetf3gpsi2.3).

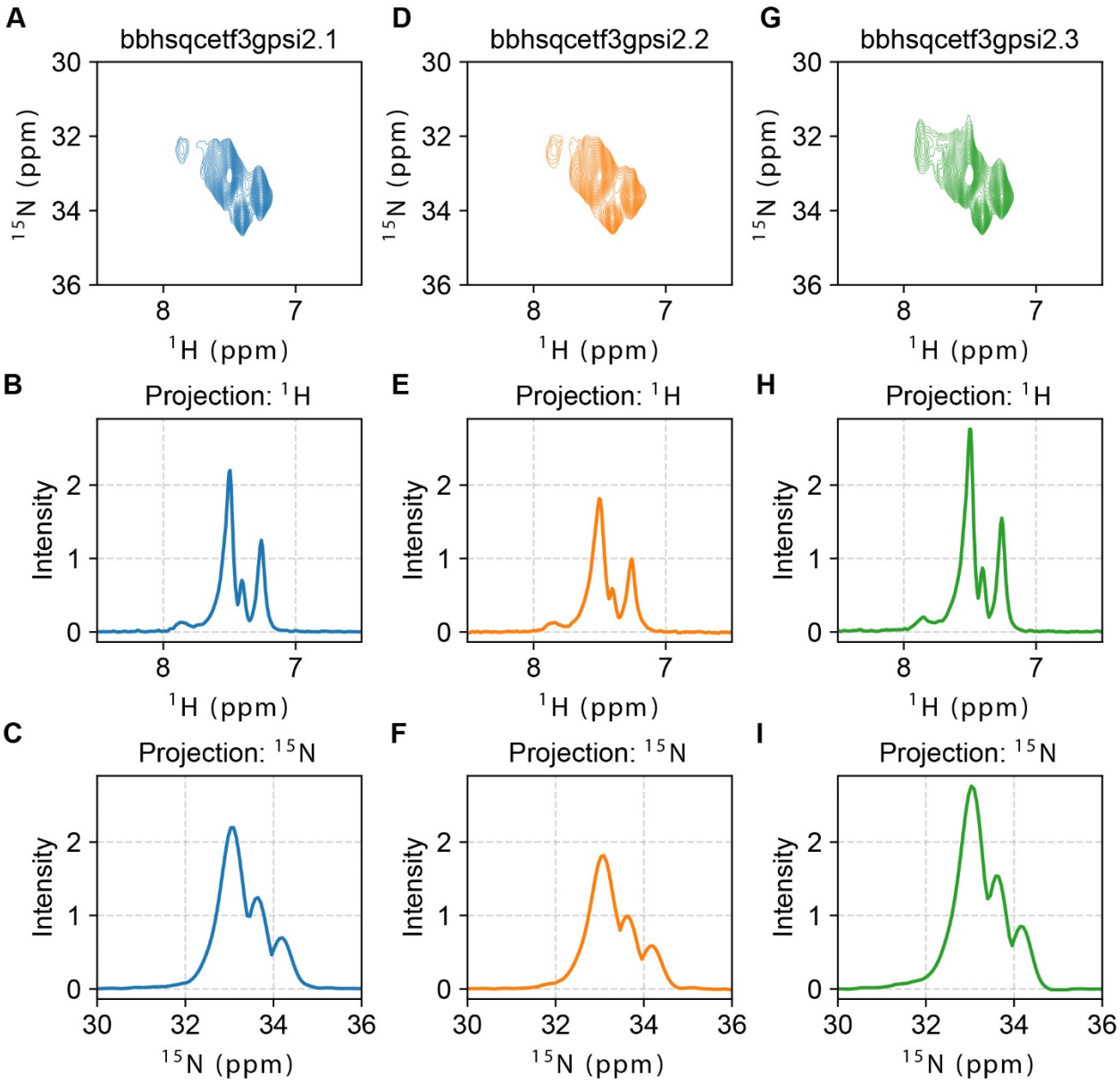

Figure S4: Portion of the BB HSQC Spectra showing the side chain resonances acquired with the different 180° pulses on a 0.8 mM protein sample of NRAS^{Q61R}-GTP (MW 20.3 kDa). 2D HSQC spectra and relative 1D projections for ^{1}H and ^{15}N, respectively, recorded using: (A-C) the Hypersecant pulse sequence (bbhsqcetf3gpsi2.1); (D-F) the CHIRP pulse sequence (bbhsqcetf3gpsi2.2); (G-I) the pulse sequence with the AI-generated pulses (bbhsqcetf3gpsi2.3).

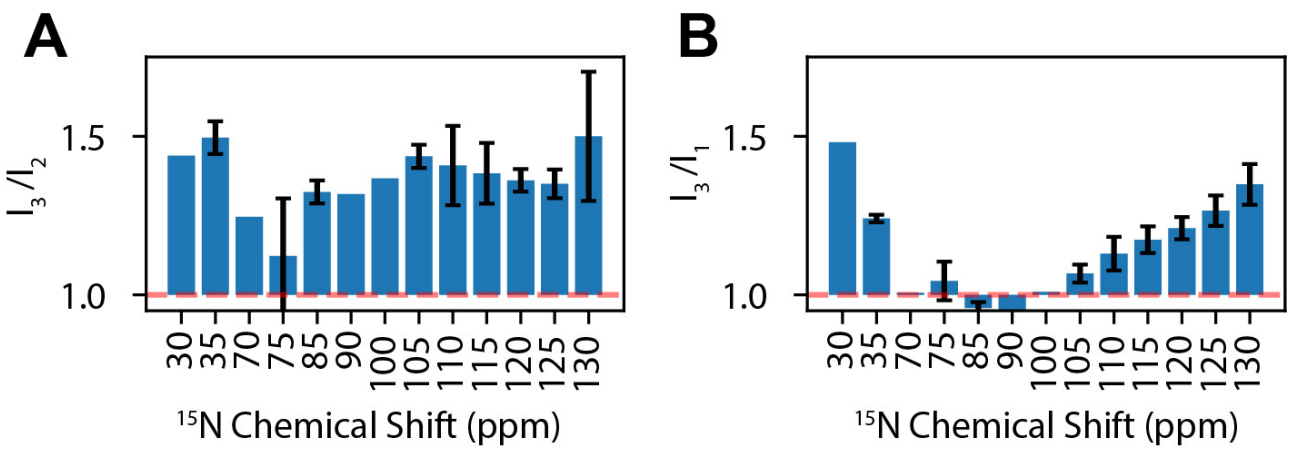

Figure S5. Gain in sensitivity measured on NRAS^{Q61R}-GTP protein spectrum. The intensity of the HSQC signals detected with the AI-generated pulses (I3, bbhsqcetf3gpsi2.3) is compared (A) to those detected using the CHIRP pulse sequence (I2, bbhsqcetf3gpsi2.2), and (B) to those detected using the Hypersecant pulse sequence (I1, bbhsqcetf3gpsi2.1).

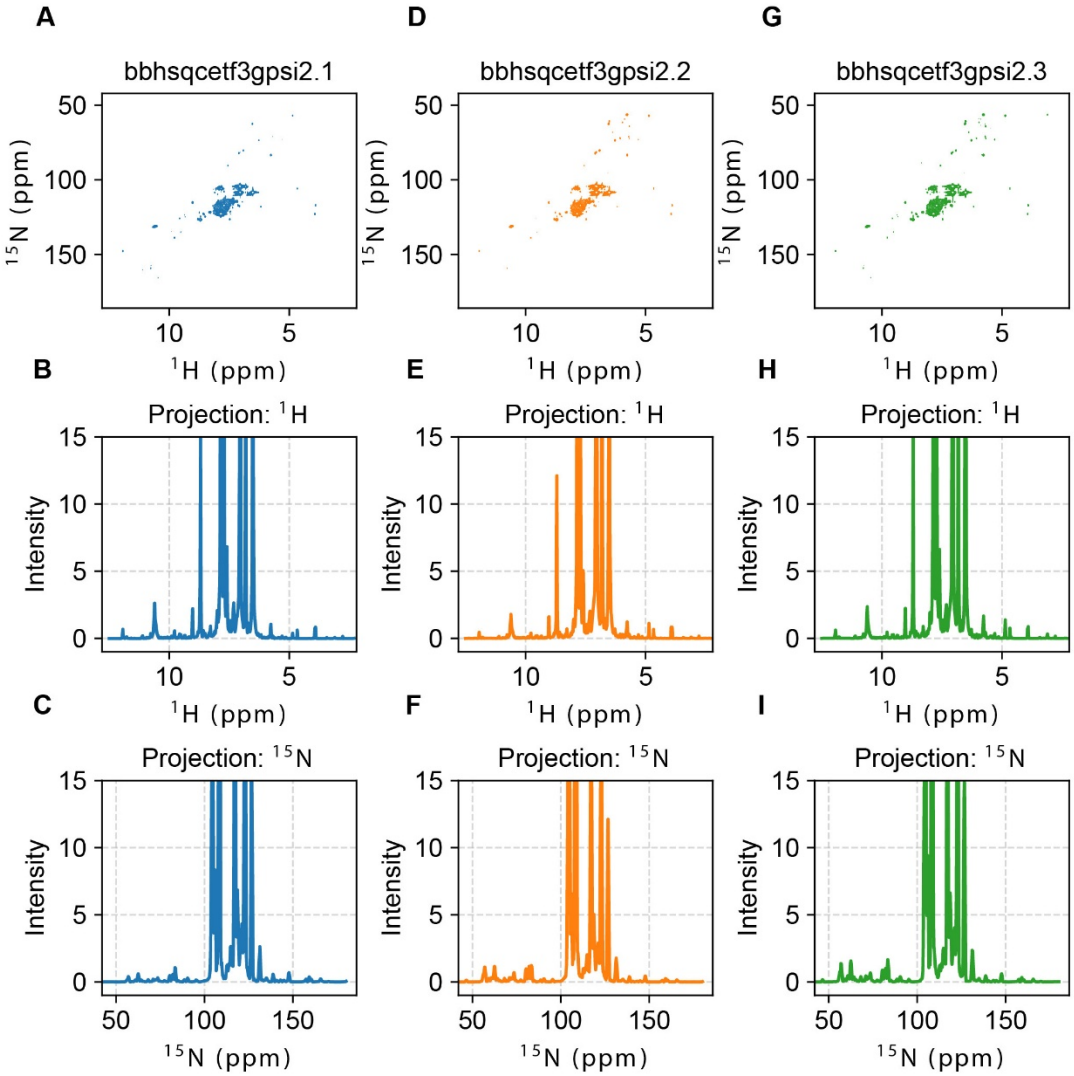

Figure S6. 2D [^{1}H,^{15}N] BB-HSQC spectra of the crude *Micromonospora* sp. WMMC264 extracts in the presence of iron. 2D HSQC spectra and relative 1D projections for ^{1}H and ^{15}N, respectively, recorded using: (A-C) the Hypersecant pulse sequence (bbhsqcetf3gpsi2.1); (D-F) the CHIRP pulse sequence (bbhsqcetf3gpsi2.2); (G-I) the pulse sequence with the AI-generated pulses (bbhsqcetf3gpsi2.3).

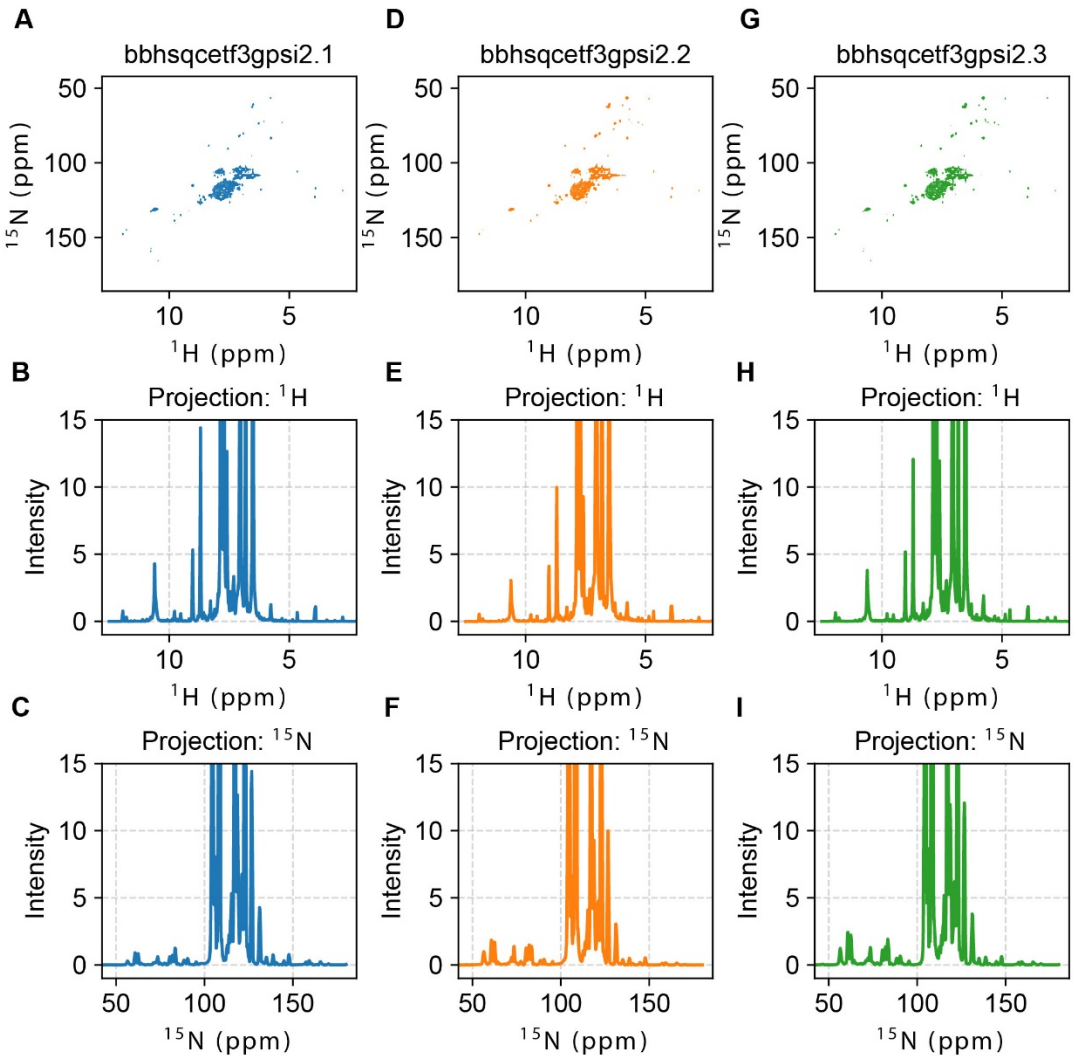

Figure S7. 2D [^{1}H,^{15}N] BB-HSQC spectra of the crude *Micromonospora* sp. WMMC264 extracts in the absence of iron. 2D HSQC spectra and relative 1D projections for ^{1}H and ^{15}N, respectively, recorded using: (A-C) the Hypersecant pulse sequence (bbhsqcetf3gpsi2.1); (D-F) the CHIRP pulse sequence (bbhsqcetf3gpsi2.2); (G-I) the pulse sequence with the AI-generated pulses (bbhsqcetf3gpsi2.3).