

Supporting information

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Table S1. Residues perturbed by 20-fold excess of fragments with all three proteins.

| Fragments | Residues Perturbed for BrmGRX | Residues Perturbed for hGRX1 | Residues Perturbed for PaGRX |
|--------------------|--|--|--|
| RK207 ^a | R51, F57, G69, S70, D71, D72, L73, A75, L76, E77 | E4, F5, N7, S8, Y25, C26, R28, A29, N52, E56, I57, Y60, Q62, T65, G66, A67, R68, T69, V73, K77, G81, G82, D85, L95, L99 | V23, T28, A29, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, W76, G84, C85, D87, L88, H89, A90, L91, E92 |
| RK144 ^a | A22, A38, R51, S52, F57, G69, S70, D71, D72, L73, A75, L76, E77 | E4, F5, S8, Q11, C26, R28, A29, N52, E56, I57, Y60, Q62, G66, A67, R68, T69, V73, K77, I80, G81, G82, D85, L95, L99 | V23, T28, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, G84, C85, D87, L88, H89, A90, L91, E92 |
| RK246 ^a | R13, R51, F57, G69, S70, D71, D72, A75 | E4, F5, N7, S8, C26, R28, A29, N52, I57, Y60, G66, R68, T69, V73, K77, G81, G82, D85, L95, L99 | V23, T28, A29, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, G84, C85, D87, L88, H89, A90, L91, E92 |
| RK395 ^a | R13, Y18, C19, E32, R51, F57, G69, S70, D71, D72, L73, A75, L76, E77 | E4, S8, Y25, C26, R28, A29, N52, E56, Y60, T65, G66, R68, T69, V73, K77, G81, G82, D85, L95 | V23, T28, A29, C31, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, W76, T80, H81, V82, G83, G84, C85, D87, L88, H89, A90, E92 |
| RK104 ^a | I10, T12, R13, Y18, C19, A22, L26, E32, A38, R51, S52, F57, Q59, I62, G69, S70, D71, D72, L73, A75, L76, E77 | E4, F5, S8, Q11, Y25, C26, R28, A29, V46, N52, E56, I57, Y60, Q62, T65, G66, A67, R68, T69, V73, K77, I80, G81, G82, D85, S92, E94, L95, L99 | V23, T28, A29, C31, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, W76, T80, H81, G84, C85, D87, L88, H89, A90, L91, E92 |
| RK208 ^a | I10, T12, R13, Y18, C19, A22, L26, E32, A38, R51, S52, F57, Q59, I62, G69, S70, D71, D72, L73, A75, L76, E77 | E4, F5, S8, Q11, Y25, C26, R28, A29, V46, N52, E56, I57, Y60, Q62, T65, G66, A67, R68, T69, V73, K77, I80, G81, G82, D85, S92, E94, L95, L99, I102 | V23, T28, A29, C31, Y33, C34, I35, Q39, C53, D54, L63, L66, S69, T70, T71, V72, Z74, I75, W76, T80, H81, G84, C85, D87, L88, H89, A90, L91, E92 |
| RK192 ^a | R13, L26, A38, R51, F57, G69, S70, D71, D72, L73, A75, L76, E77 | E4, F5, S8, Y25, C26, R28, A29, N52, E56, I57, Q62, T65, G66, A67, R68, T69, V73, K77, G81, G82, D85, L95, L99 | V23, T28, C31, Y33, C53, D54, L63, L66, T71, V72, Z74, I75, G84, C85, D87, L88, H89, L91, E92 |

^a Blue letters indicates residues showing large CSPs or broadening effects.

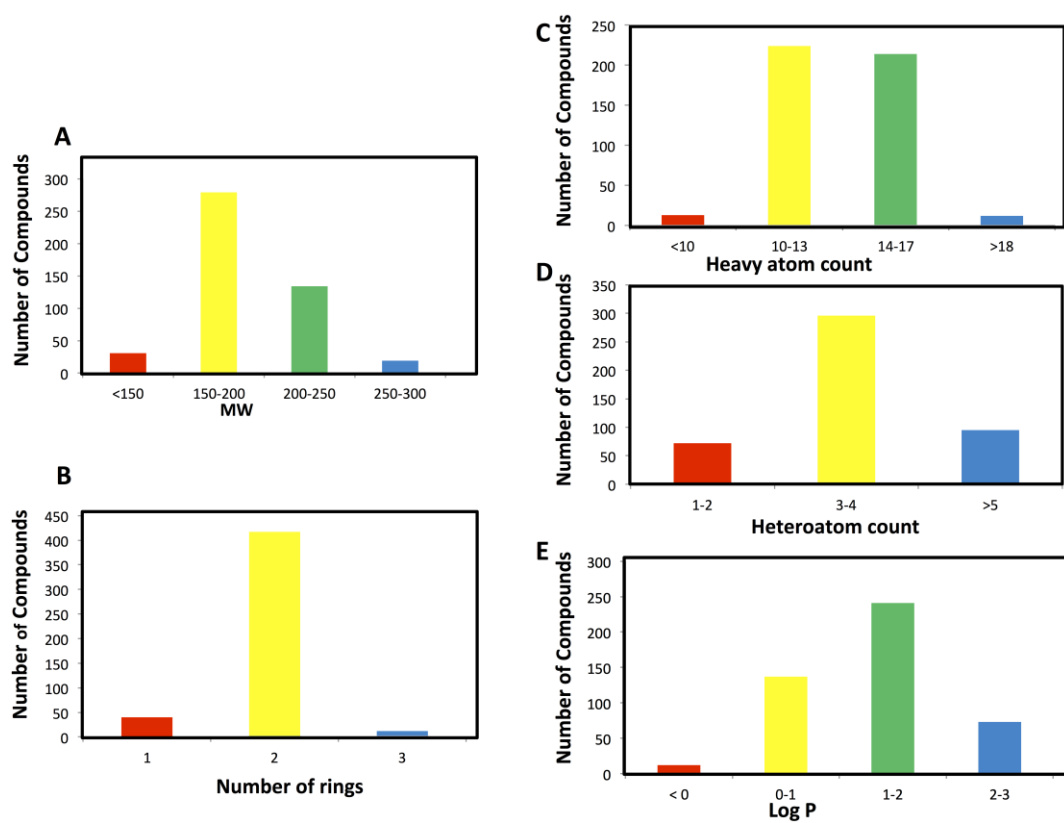


Figure S1. Diversity of different parameters in the library. Diversity of: (A) molecular weight, B) number of rings C) heavy atom count D) heteroatom count and E) partition coefficient ($\log P$) values.

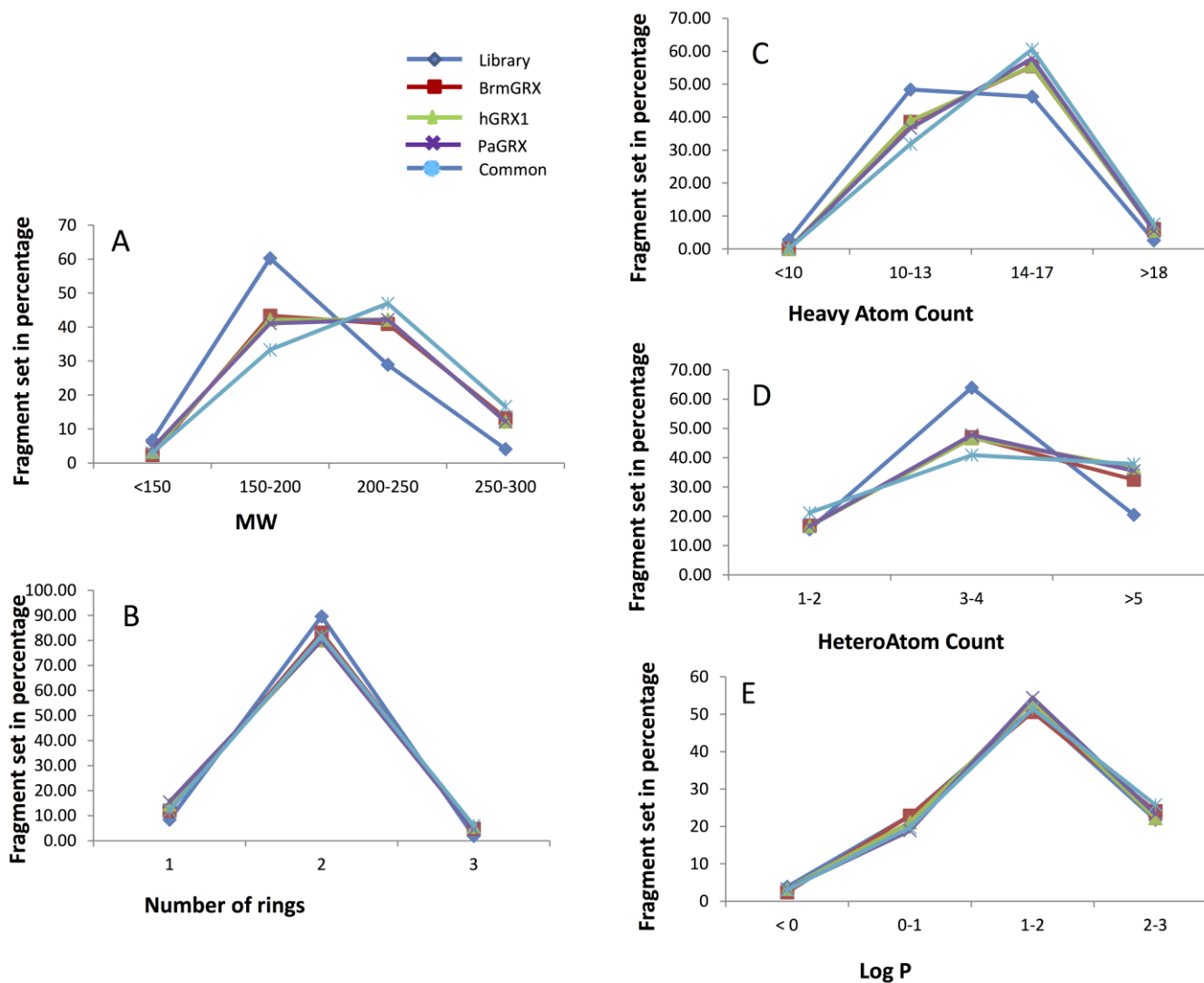


Figure S2. Diversity of average physicochemical parameters in-between hits and library: (A) molecular weight, (B) number of rings (C) heavy atom count (D) heteroatom count (E) logP; for hits of three orthologous proteins, common hits and library.

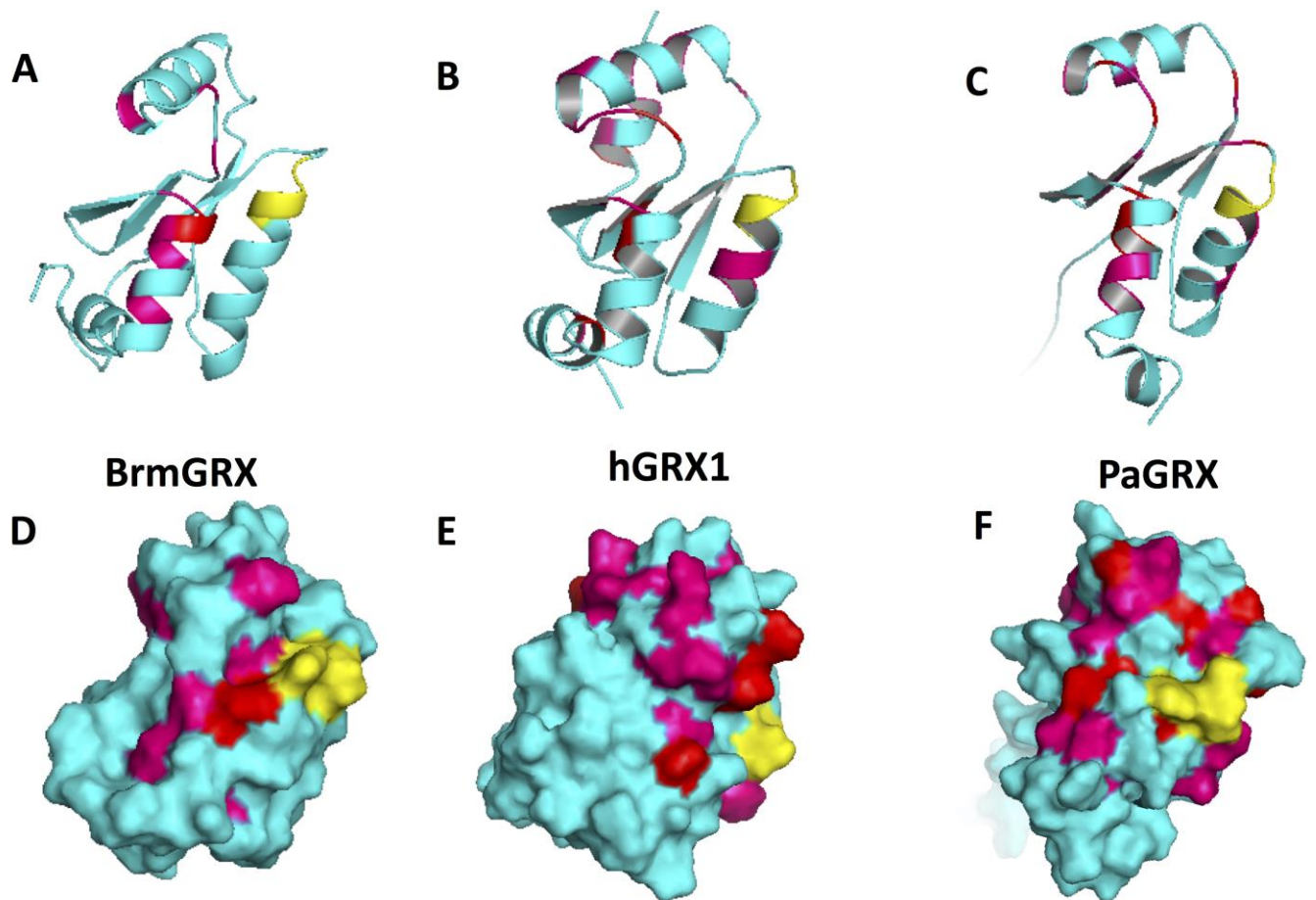


Figure S3. 2D NMR CSPs mapping. (A), (B), and (C) represents secondary structures; and (D), (E), and (F) are the surface structures with CSPs mapping for BrmGRX, hGRX1, and PaGRX, respectively. Yellow patch: conserved active site; red patches: residues with CSPs ≥ 0.1 ppm; and pink patches: residues with CSPs ≤ 0.1 ppm with large excess of fragment.

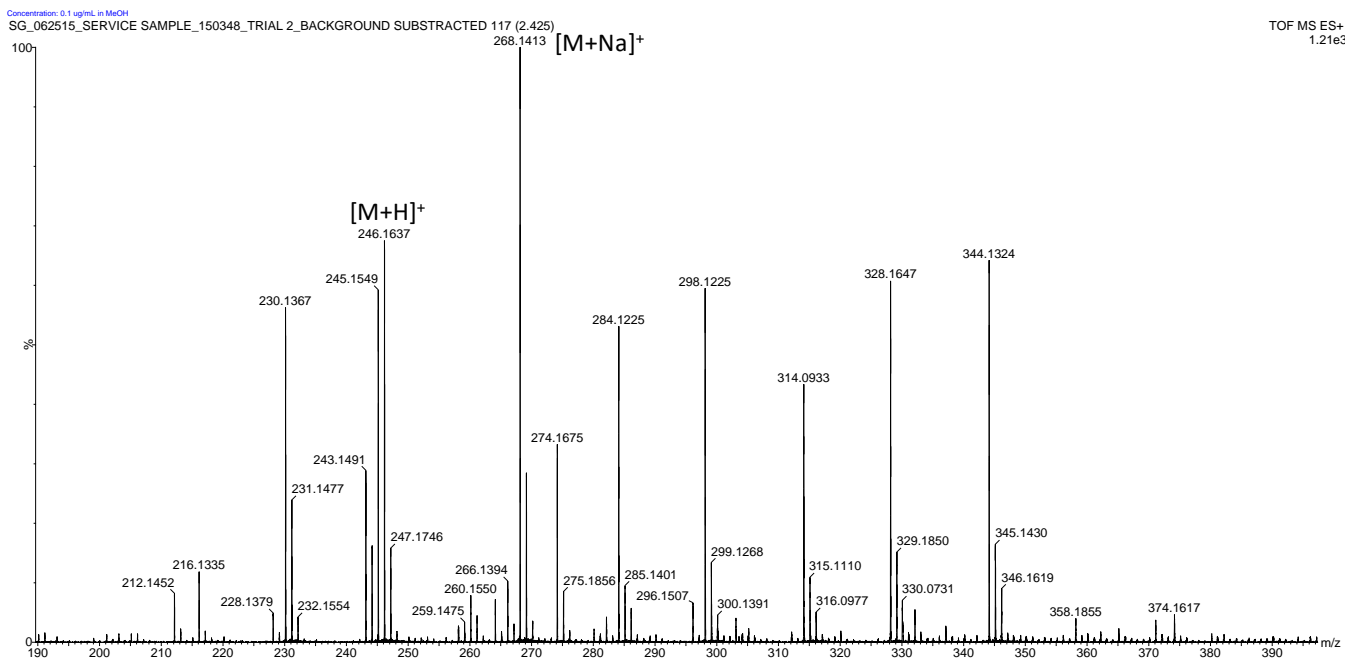


Figure S4. HRMS for RK395ACP in methanol. HRMS calculated for $C_{14}H_{19}N_3O$ 246.1606, found 246.1637 ($M + H^+$).

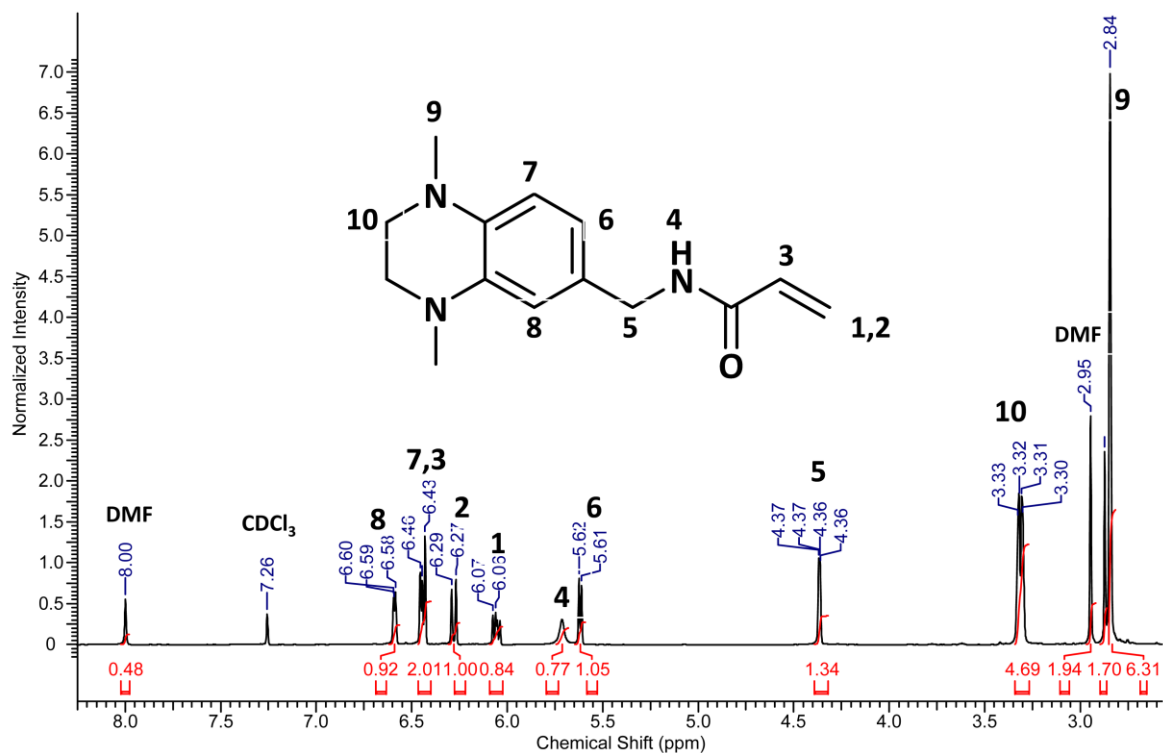


Figure 5. 1D 1H NMR for RK395ACP.

1H NMR (750 MHz, $CDCl_3$) δ 6.59-6.60 (d, 1H), 6.45-6.46 (d, 1H), 6.43 (s, 1H), 6.27-6.29 (d, 1H), 6.03-6.07 (m, 1H), 5.65 (s, 1H), 5.61-5.63 (d, 1H), 4.37 (s, 2H), 3.31-3.32 (d, 4H), 2.84 (s, 6H)

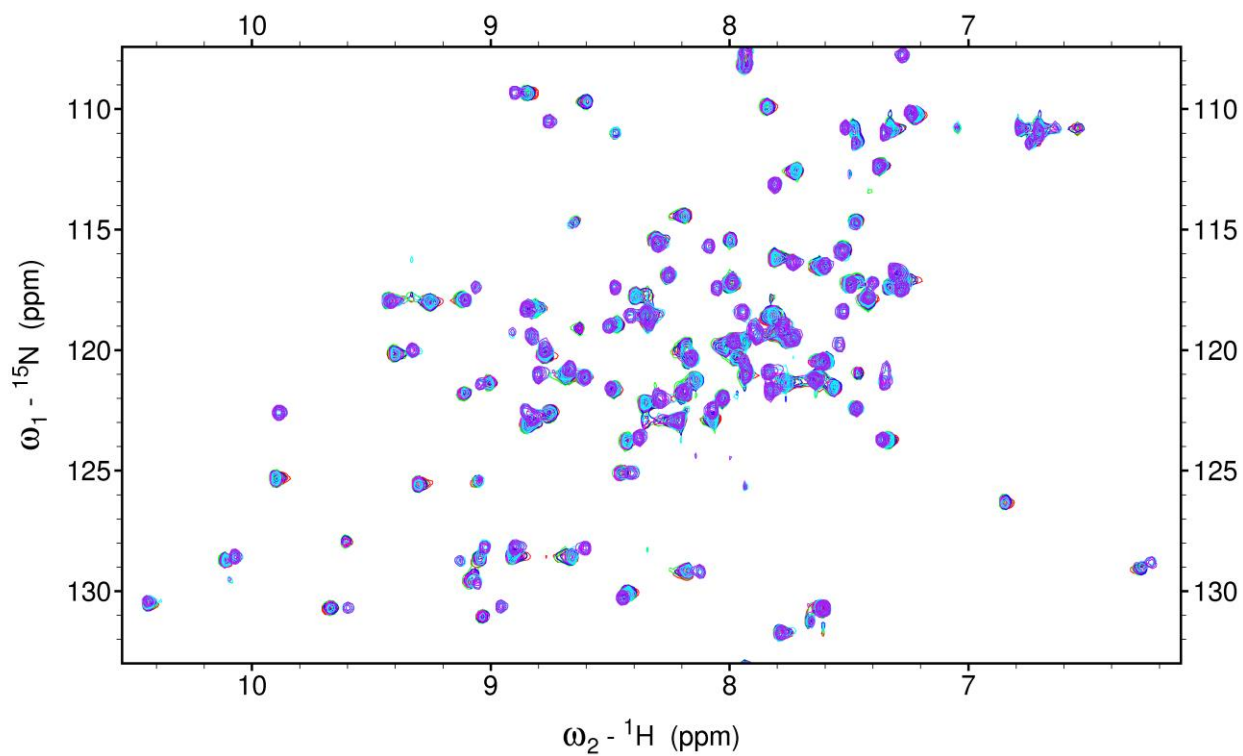


Figure S6. Spectral overlays for PaGRX reacted with RK395 ACP—Green: 0 h, blue 1 h, magenta: 2 hours, cyan: 6 h, purple: 24 h.