

## Using NMR to Identify binding regions for N and C-terminal Hsp90 inhibitors using Hsp90 Domains

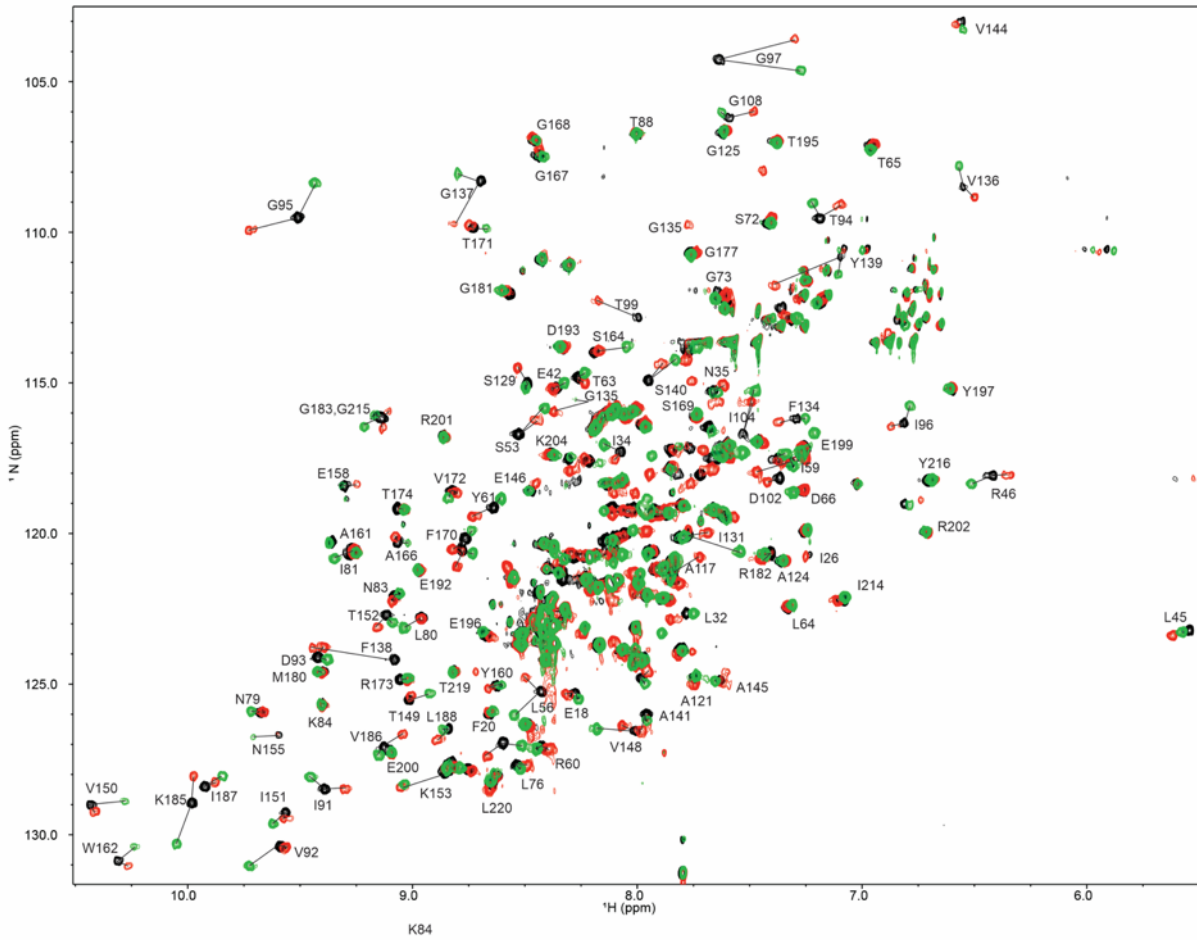
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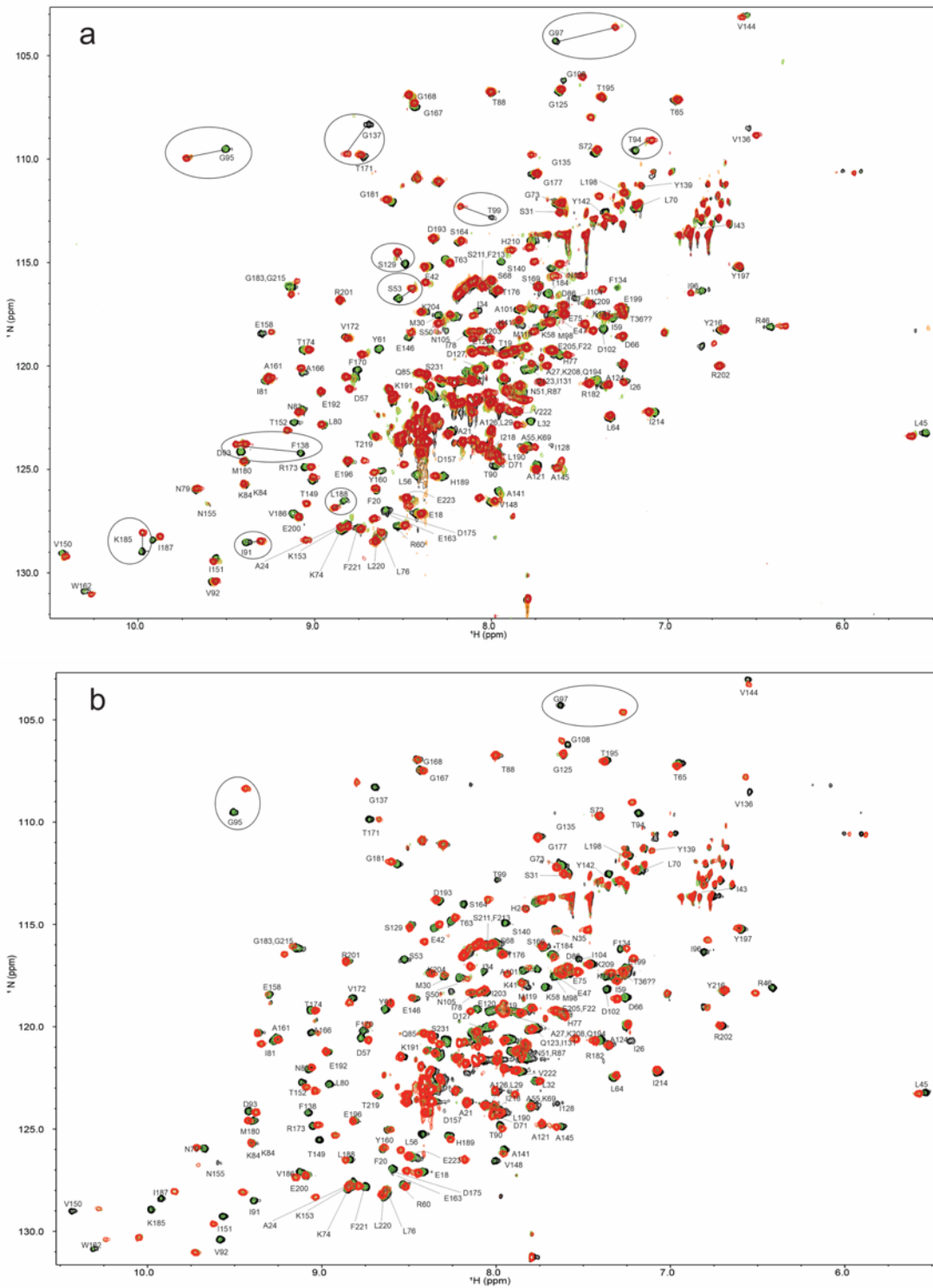
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## Supplementary Data Figure S1



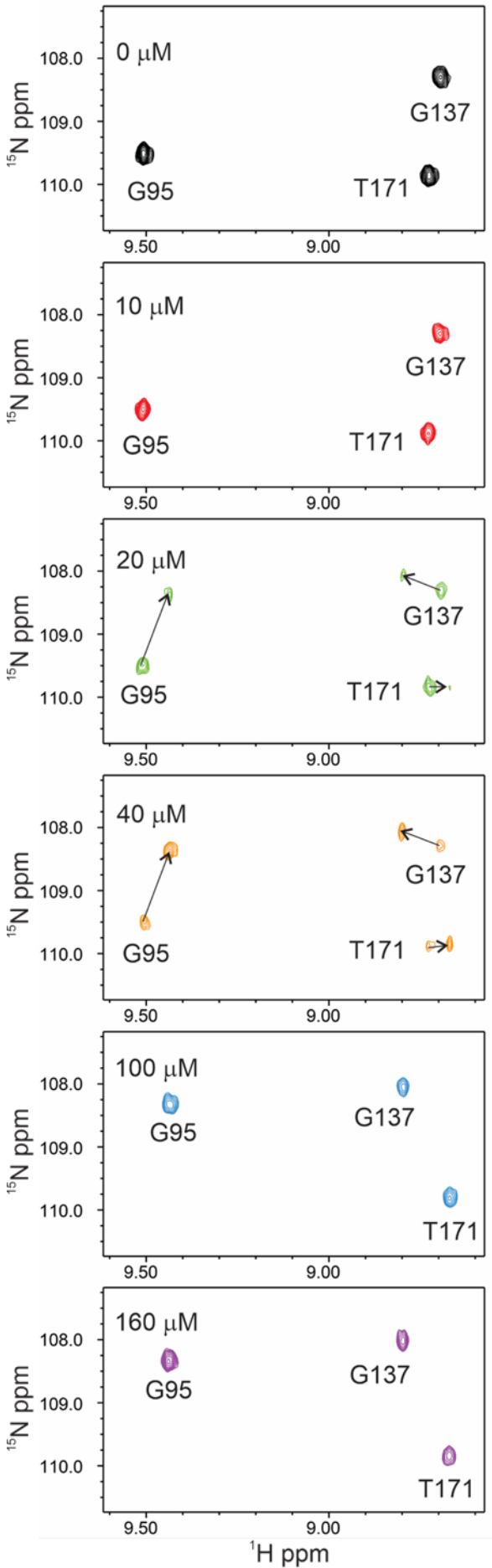
**Figure S1.** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  TROSY spectrum of  $^{15}\text{N}$ -labelled Hsp90 N domain (residues 15-235) (black) with the N domain spectrum in the presence of a 1:1 ratio of **17-AAG** (red) and of **AUY922** (green). Resonance assignments for the free protein<sup>1,2</sup> are shown, with lines joining corresponding cross peaks inferred for the complexes.

## Supplementary Data Figure S2



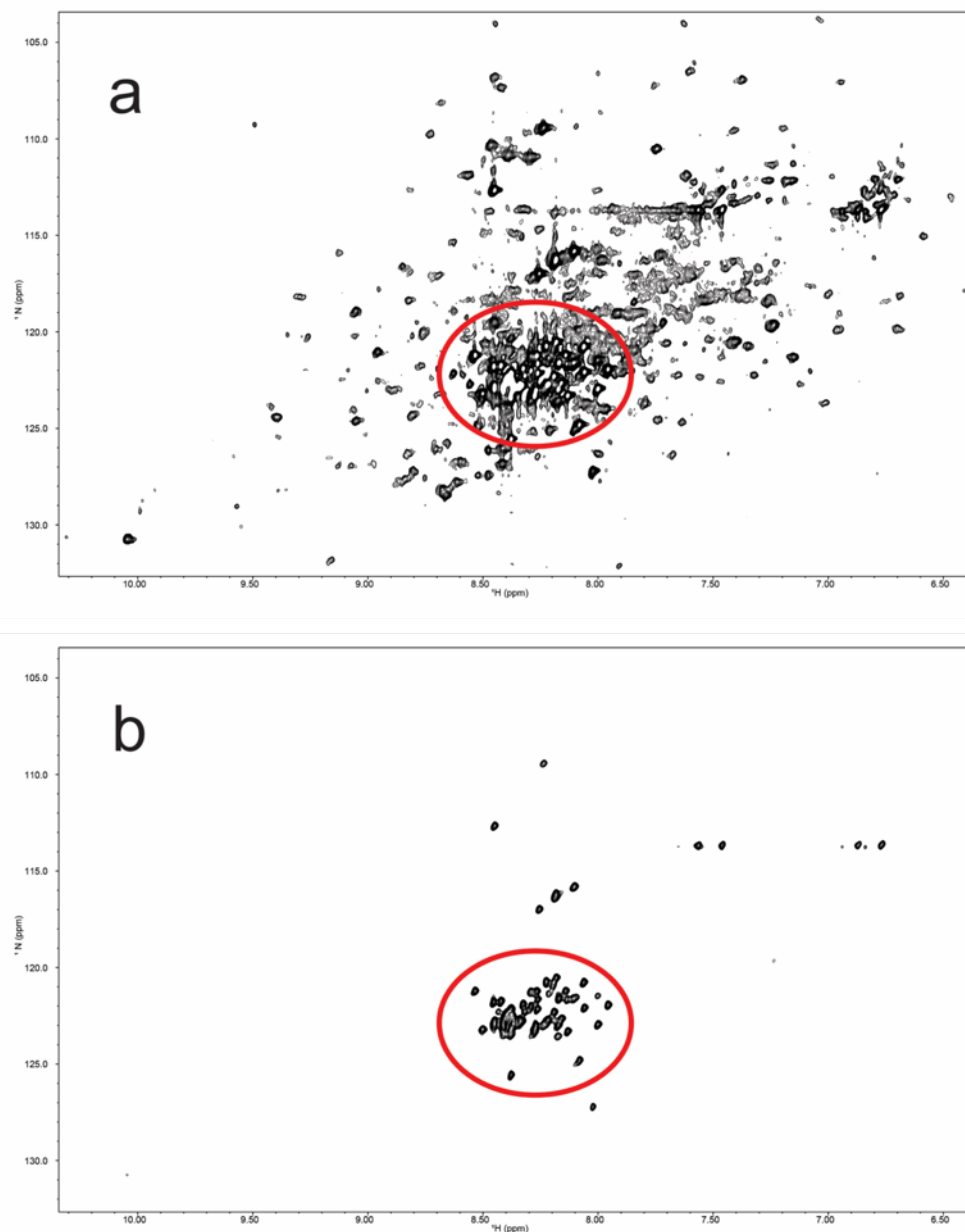
**Figure S2.** Titration points for the addition of a) **17-AAG** and b) **AU Y922** into  $^{15}\text{N}$ -labelled Hsp90 N domain. Ratio protein:inhibitor black 1:0; green 1:0.4; orange 1:0.8; red 1:1.0.

### Supplementary Data Figure S3



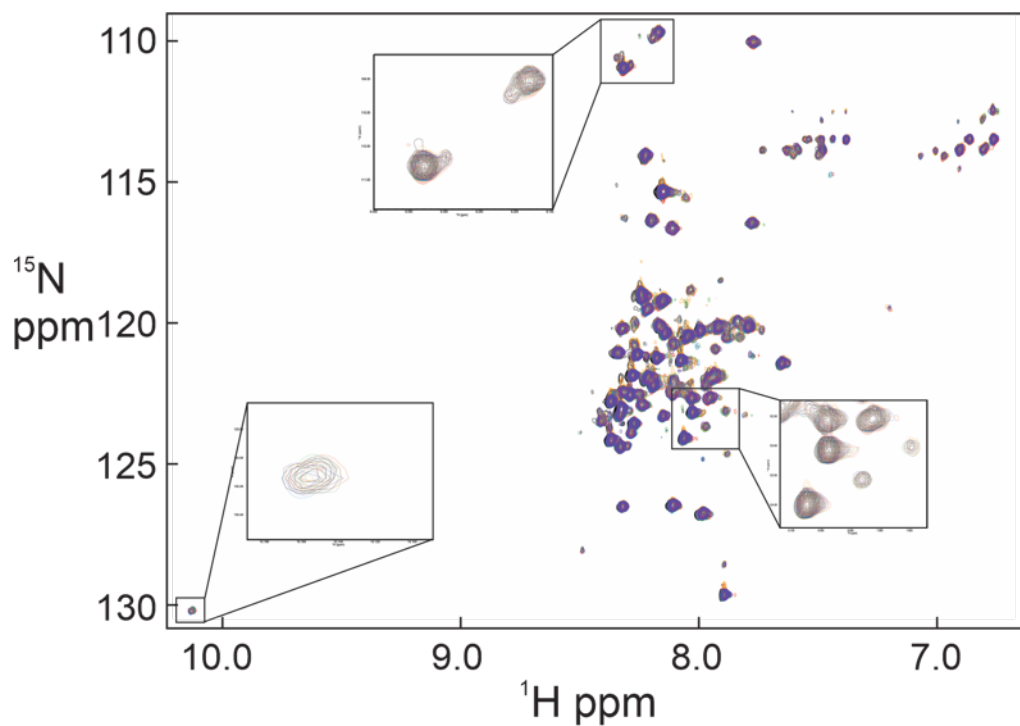
**Figure S3.** Illustration of the effects of addition of a ligand (AU922) that binds to  $^{15}\text{N}$ -labelled Hsp90 N domain in slow exchange. The intensity of the cross peak of the free protein decreases and a new cross peak rises at a different position.

## Supplementary Data Figure S4



**Figure S4.**  $^1\text{H}$ - $^{15}\text{N}$  TROSY of  $^{15}\text{N}$ -labelled Hsp90 NM (residues 15-554).

- Lowest contour level 0.5 (shows weak peaks of folded domains as well as strong peaks of the disordered linker)
- Lowest contour level 4.3 (shows only the strong peaks of the disordered linker).



**Figure S5.** The  $^1\text{H}$ - $^{15}\text{N}$  TROSY-HSQC spectrum of the  $^{15}\text{N}$ -labeled C domain (black) is superimposed with the spectrum that contains the C domain and **SM253** in ratios of 1:0.1 ratio (red), 1:0.2 (green), 1:0.4 (blue), 1:0.8 (orange) and 1:1.0 (purple) of C-terminal domain: **SM253**.

### References

1. Park, S. J.; Borin, B. N.; Martinez-Yamout, M. A.; Dyson, H. J., The client protein p53 adopts a molten globule-like state in the presence of Hsp90. *Nat. Struc. Mol. Biol.* **2011**, *18* (5), 537-541.
2. Park, S. J.; Kostic, M.; Dyson, H. J., Dynamic interaction of Hsp90 with its client protein p53. *J. Mol. Biol.* **2011**, *411*, 158-173.