Supplementary figures

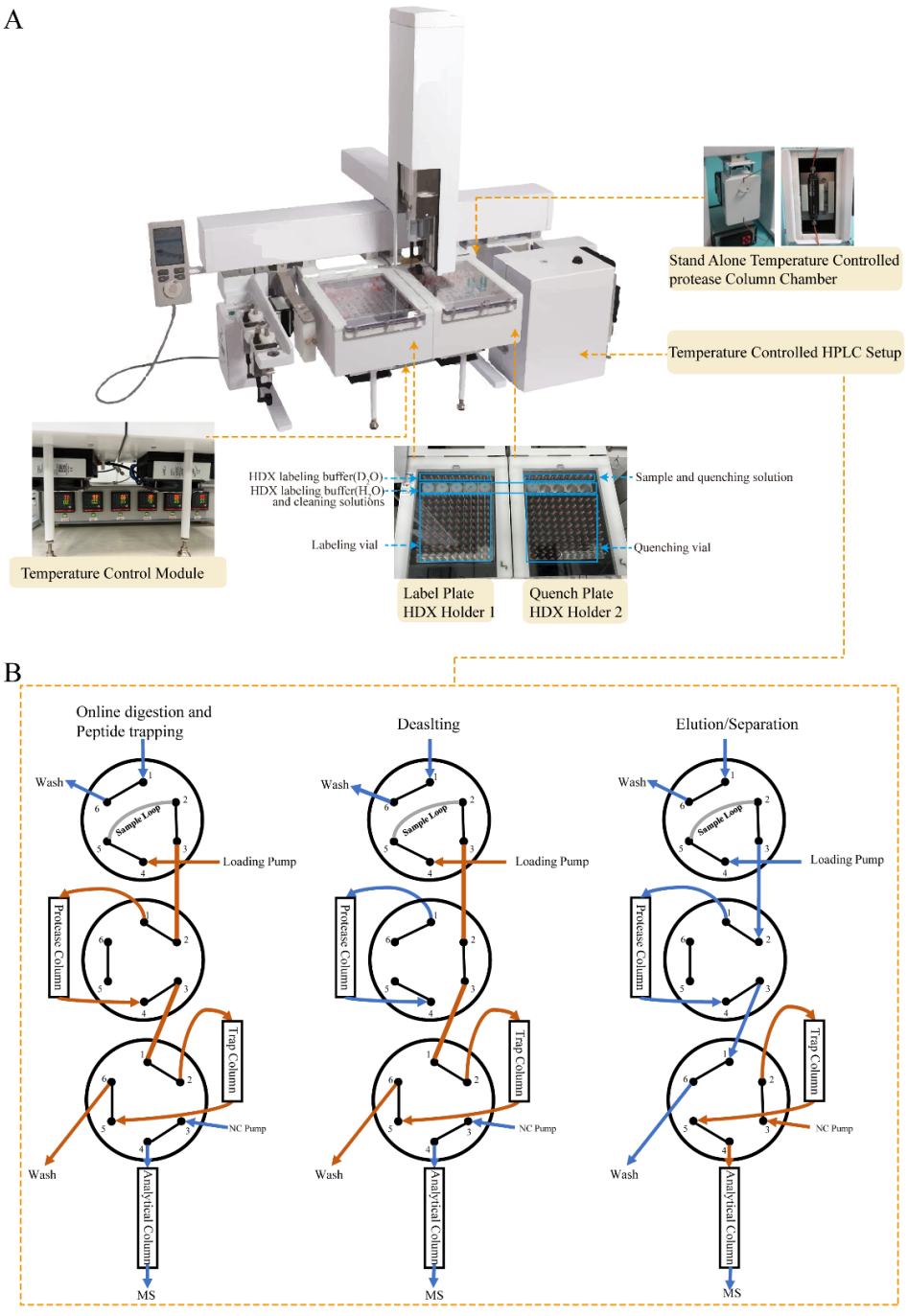

Fig. S1 Structural diagram of the PAL3 autosampler system. **A** PAL3 system and its critical hardware settings for five main parts: HDX holder 1, HDX holder 2, temperature-controlled protease column, temperature-controlled HPLC compartment and temperature control module. **B** HPLC setup for online digestion, desalting, and elution/separation

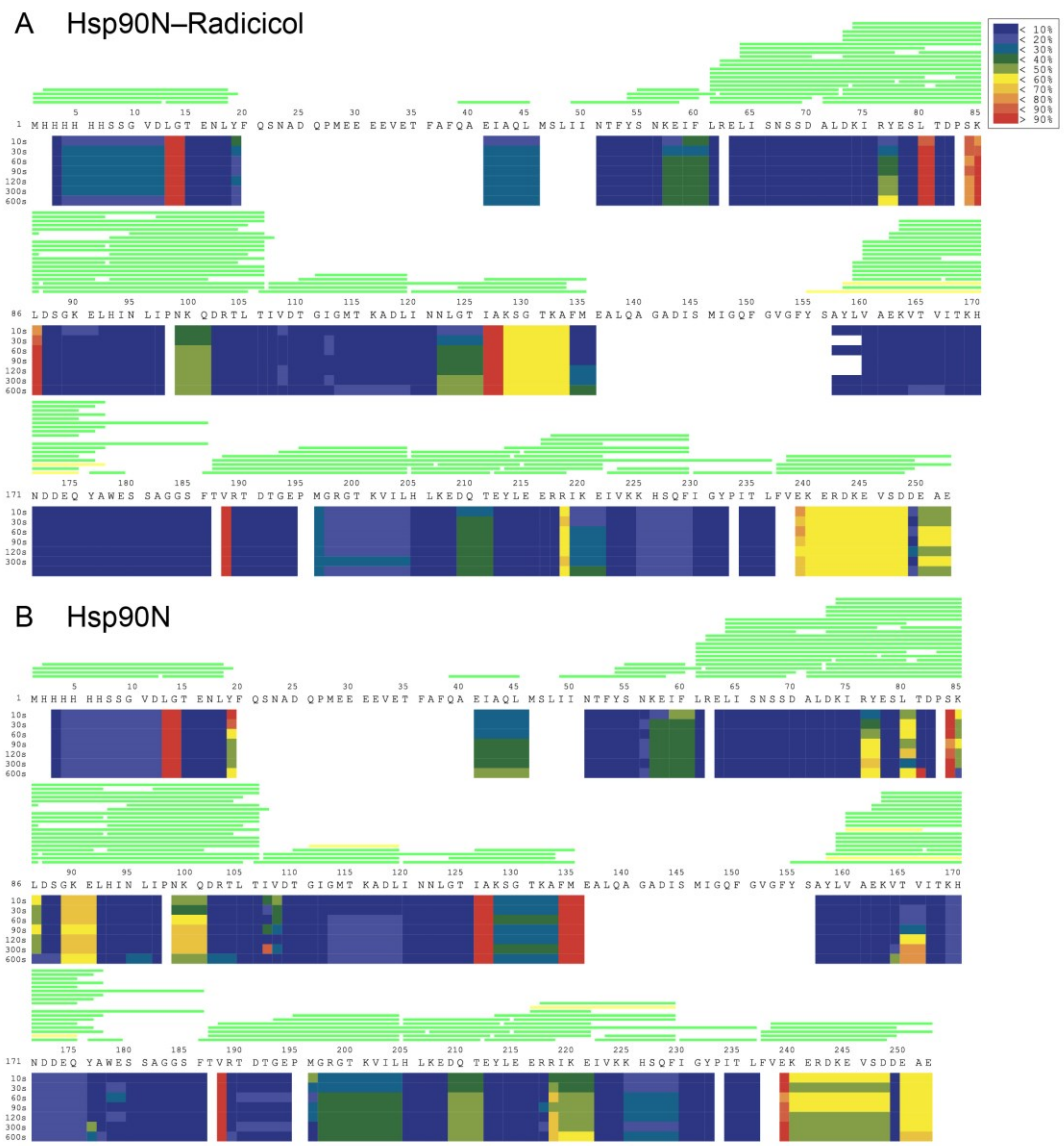

Fig. S2 HDX kinetics comparison of the Hsp90N–Ridicicol complex (**A**) and the Hsp90N protein (**B**) at the protein level. Each bar in the heat map corresponds to a peptide for which an undeuterated result was found. By default, these bars are green for high-confidence peptides and yellow for medium-confidence ones. Each row of the heat map represents one of the partially deuterated time points. Its color corresponds to the computed deuteration percentage of that area of Hsp90N proteins

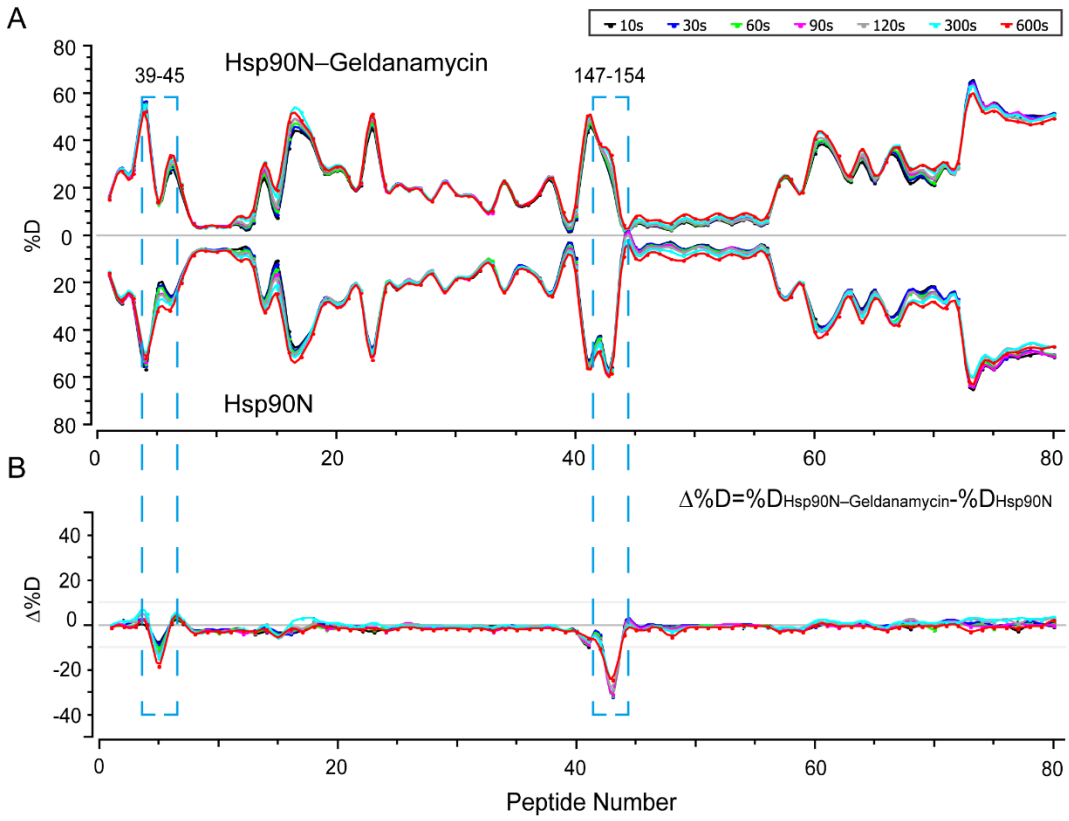

Fig. S3 HDX kinetics comparison of the Hsp90N–Geldanamycin complex and Hsp90 protein at the global peptide level. **A** Butterfly plot of the HDX kinetics of the Hsp90N–Geldanamycin complex (top) and the Hsp90N protein (bottom). The *y*-axis is the deuteration percent of individual peptides. **B** Residual plot of individual peptides from the Hsp90N–Geldanamycin complex and the Hsp90 protein. The *y*-axis is the deuteration percent difference of individual peptides calculated by equation (*i.e.*, $\Delta\%D = \%D_{Hsp90N–Geldanamycin} - \%D_{Hsp90N}$). The gray lines at *y*-axis represent ±5% threshold for identifying significant differences between the Hsp90N–Geldanamycin complex and the Hsp90 protein. Each data point is an average of three experiments. The black, dark blue, green, magenta, grey, and light blue lines correspond to data acquired at 10, 30, 60, 90, 120, 300, and 600 s of deuterium labeling, respectively, for both samples