



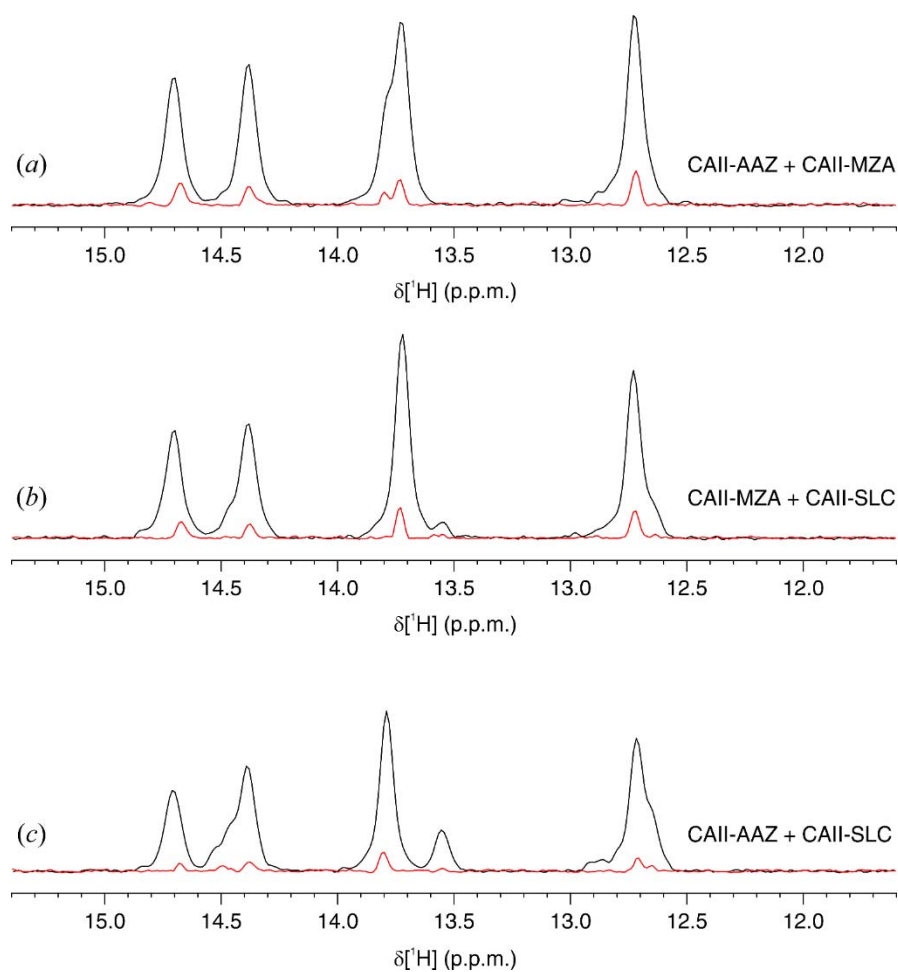
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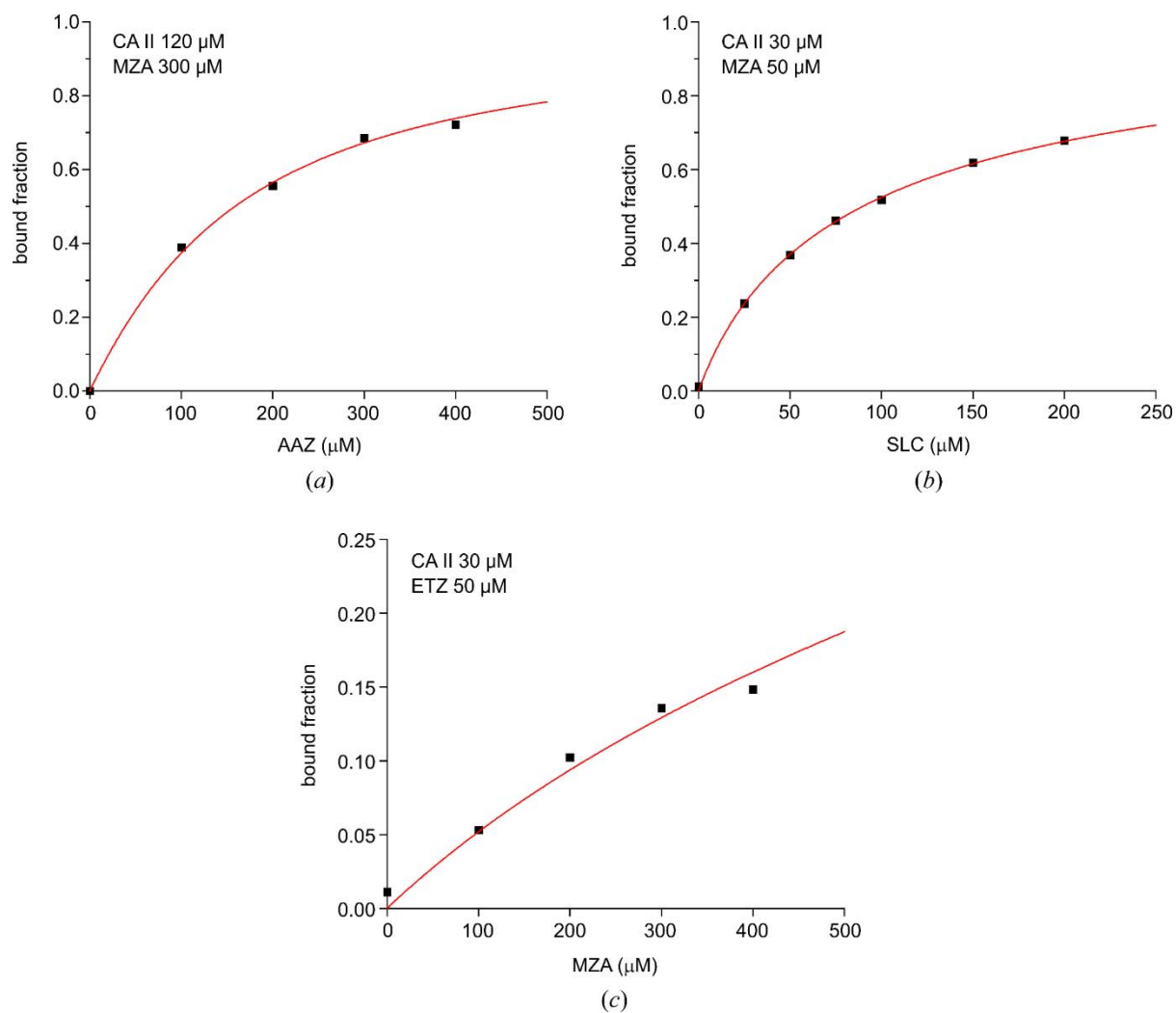
**Supporting information for article:**

**Determination of intracellular protein–ligand binding affinity by competition binding in-cell NMR**

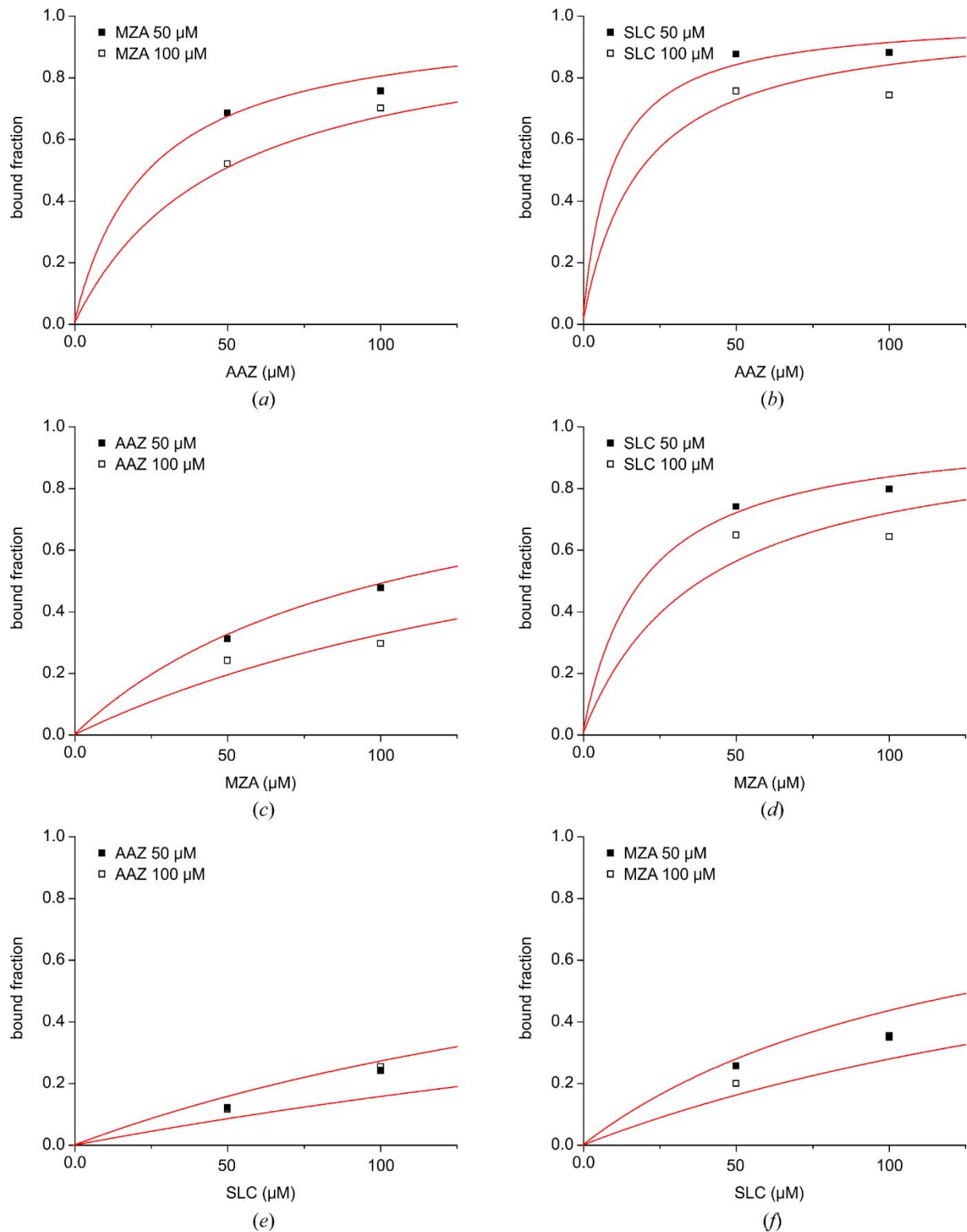
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**Figure S1**  $^1\text{H}$  projections of  $^1\text{H}$ - $^{15}\text{N}$  NMR spectra recorded on intact cells in closed tube (black) treated with (a) 50  $\mu\text{M}$  AAZ + 100  $\mu\text{M}$  MZA; (b) 50  $\mu\text{M}$  MZA + 100  $\mu\text{M}$  SLC; (c) 50  $\mu\text{M}$  AAZ + 100  $\mu\text{M}$  SLC, and on the corresponding supernatants (red) after  $\sim 1$  hour of in-cell NMR experiments. Protein leakage, estimated by total peak area, amounts to 8% (a), 5% (b), and 7% (c).



**Figure S2** Non-linear curve fitting of competition binding experiments *in vitro*. (a) fraction of CA II bound to AAZ in the presence of MZA; (b) fraction of CA II bound to SLC in the presence of MZA; (c) fraction of CA II bound to MZA in the presence of ETZ.  $K_d$  values obtained from the fittings are reported in Table 3.



**Figure S3** Global non-linear curve fitting of competition binding experiments by closed-tube in-cell NMR (see Figure 3). (a,b) fraction of CA II bound to AAZ in the presence of MZA (a) or SLC (b); (c,d) fraction of CA II bound to MZA in the presence of AAZ (c) or SLC (d); (e,f) fraction of CA II bound to SLC in the presence of AAZ (e) or MZA (f).  $K_d$  values obtained from the fittings are reported in Table 3.