Supporting Information

Real-time quantitative in-cell NMR: ligand binding and protein oxidation monitored in human cells using multivariate curve resolution

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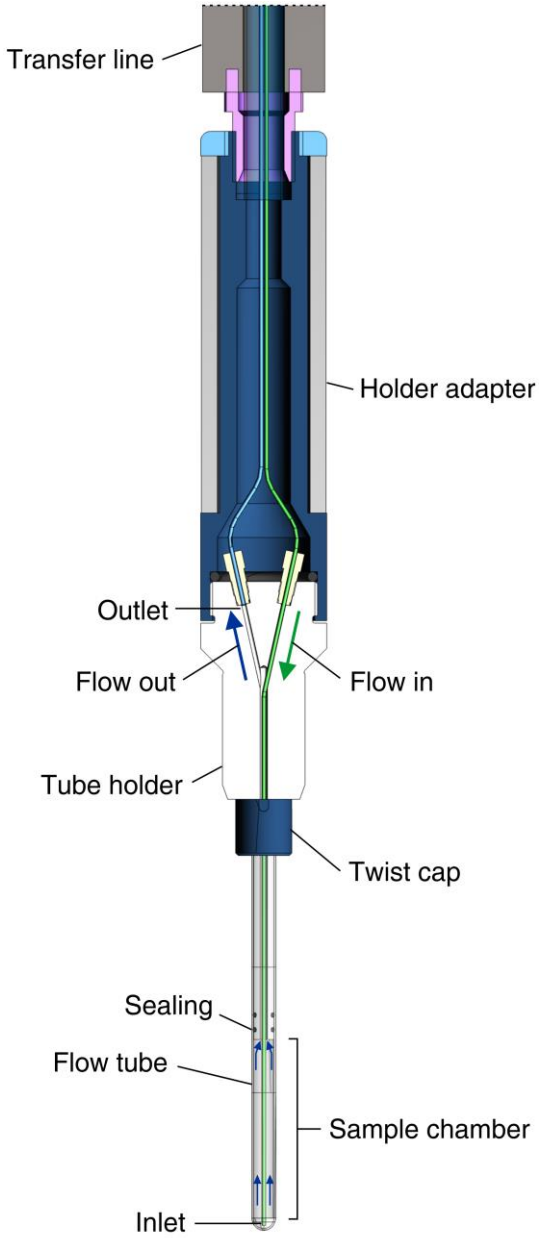


Figure S1. Cross-section view of the empty InsightMR flow unit. The inlet consists of a PEEK tubing (green) inserted down to the bottom of the glass flow tube. The medium flows upwards from the inlet (small blue arrows) and leaves the sample chamber through a cavity external to the inlet tubing. In the tube holder the cavity bifurcates and the outflow medium reaches the outlet at the top of the holder, where a PTFE outlet tubing (blue) is connected. Adapted with permission from Bruker UK Ltd.

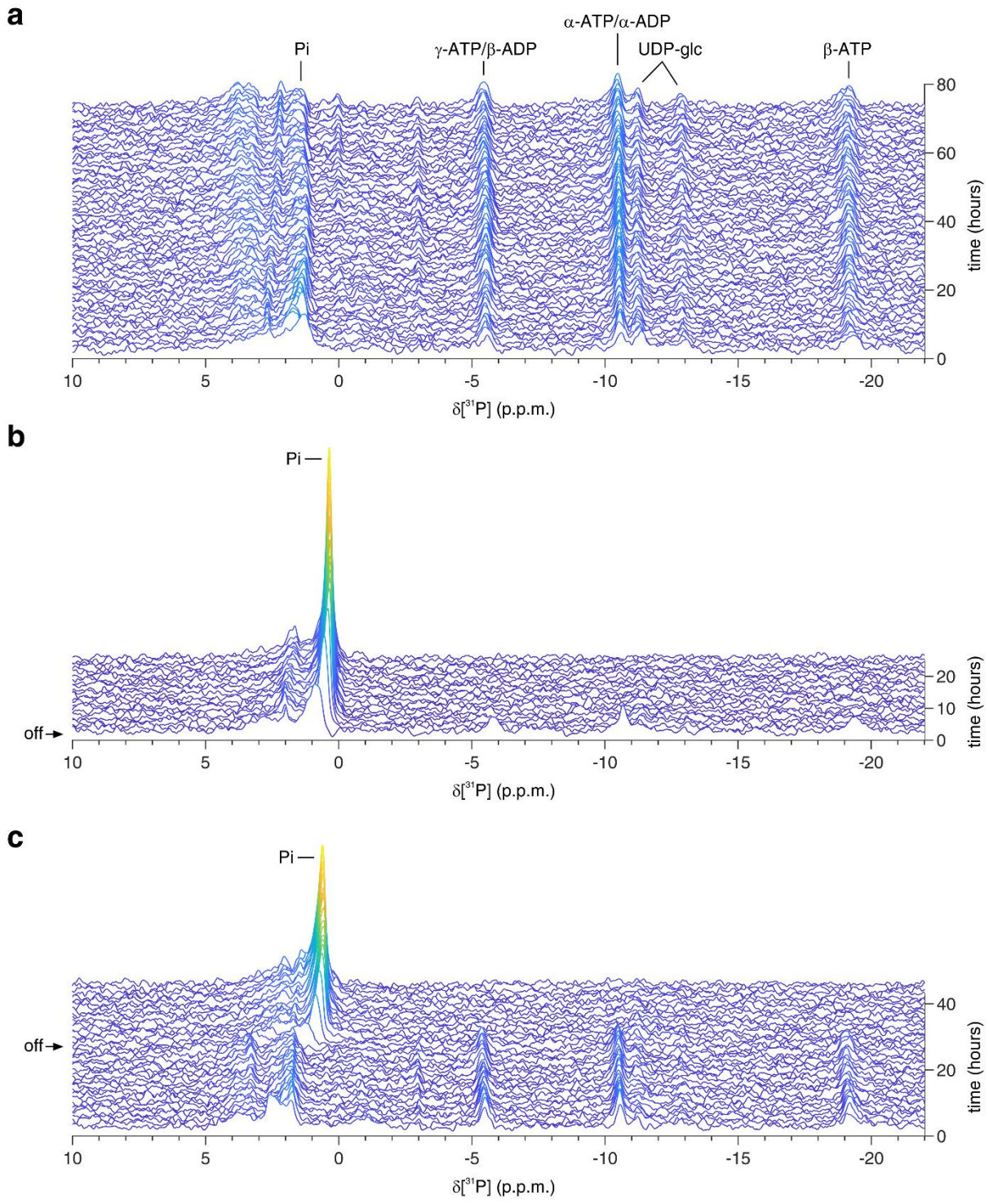
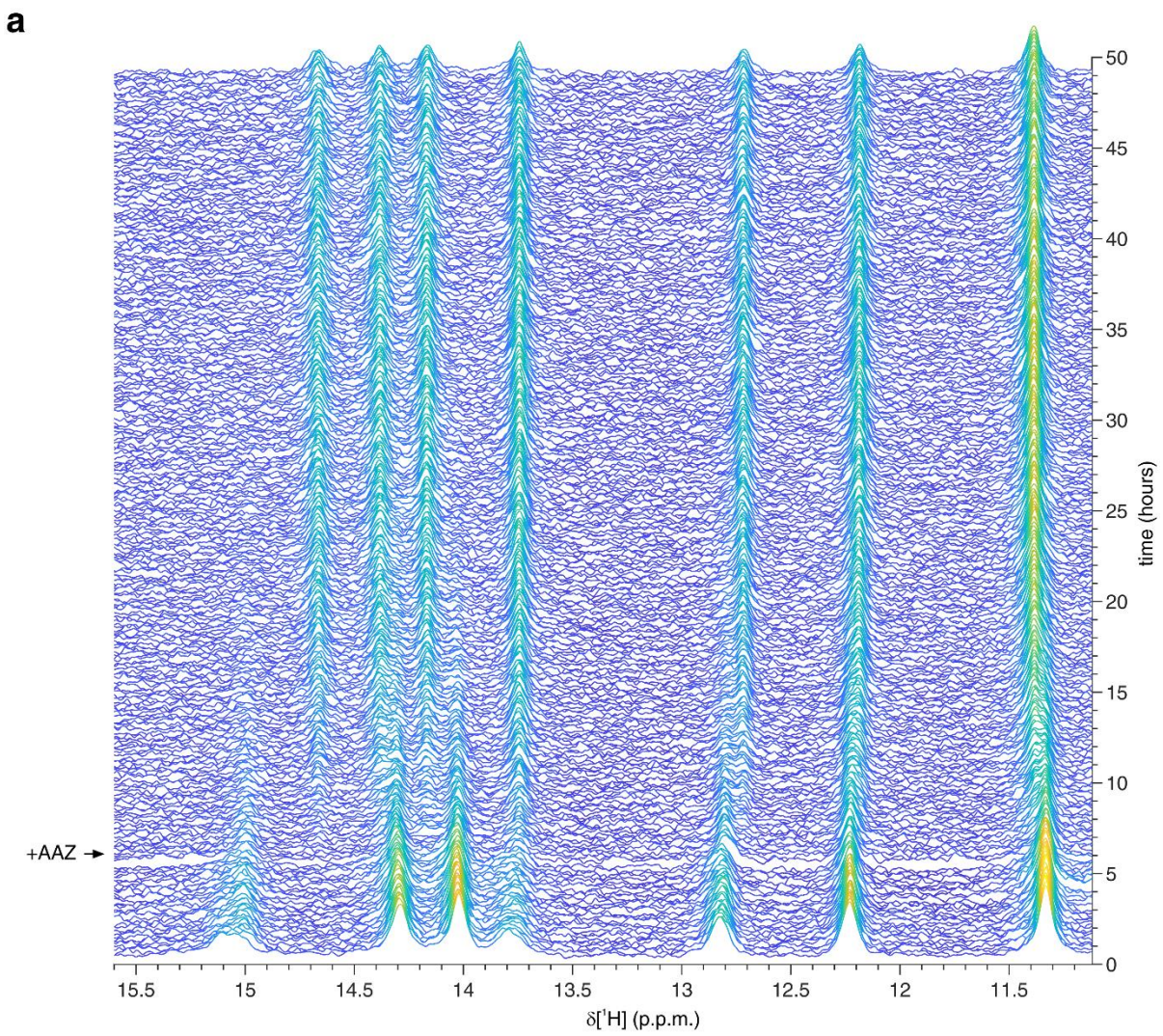


Figure S2. Real-time ^{31}P NMR spectra. Waterfall plots of 1D ^{31}P NMR spectra of HEK293T cells recorded as a function of time in the NMR bioreactor (a) with constant flow for 72 hours, (b) in the absence of flow for 24 hours, (c) for 42 hours with flow switched off after 24 hours. Spectral intensity (a.u.) is colour-coded from blue (lowest) to yellow (highest). Peaks arising from different ^{31}P metabolites are annotated.

Figure S3


a
+AAZ
50
45
40
35
30
25
20
15
10
5
0
time (hours)
15.5
15
14.5
14
13.5
13
12.5
12
11.5
δ[¹H] (p.p.m.)

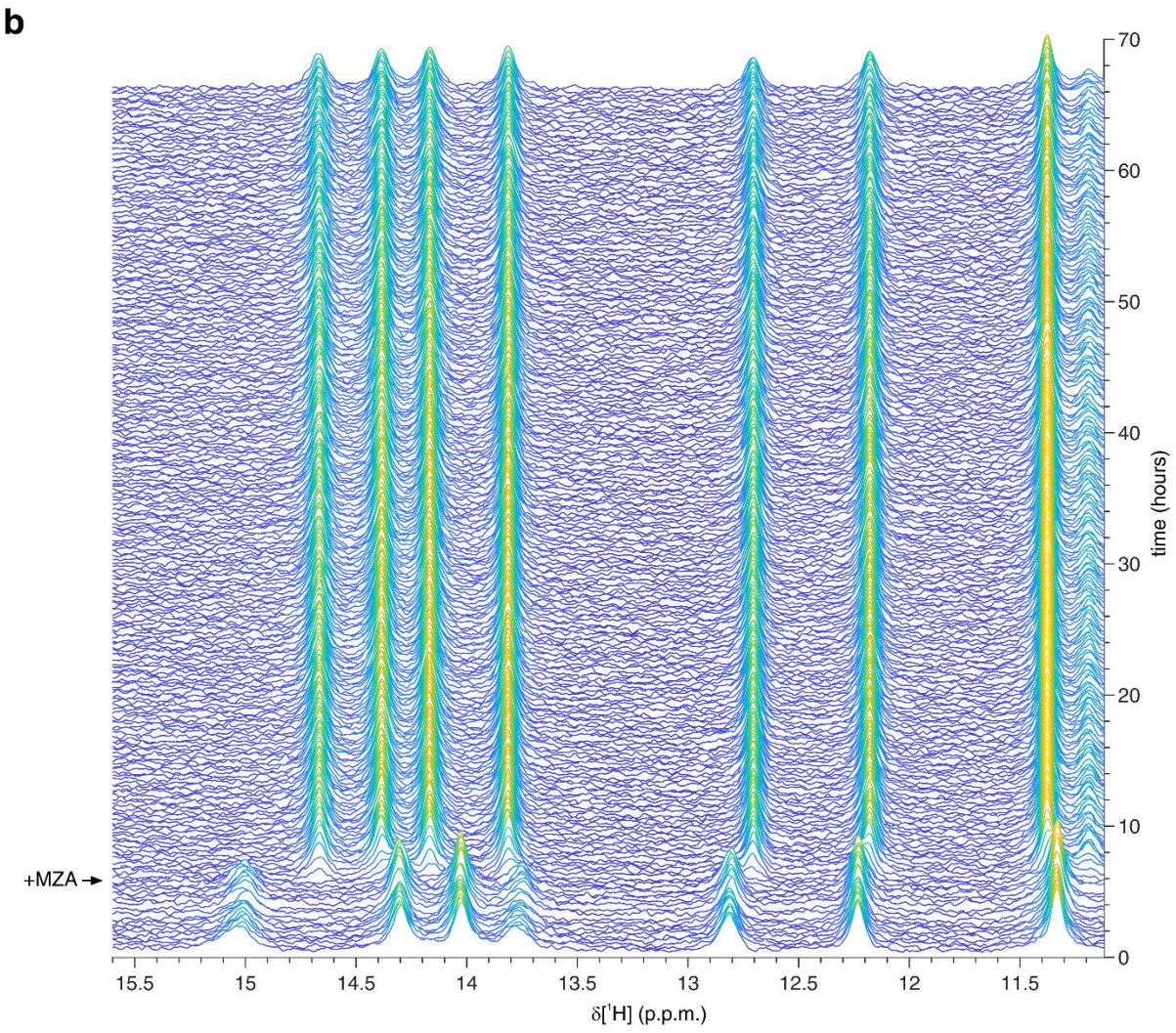


Figure S3. Real-time in-cell NMR spectra of CA2. Waterfall plots of 1D ^{1}H NMR spectra (region between 15.6 and 11.1 p.p.m.) of intracellular CA2, treated with (a) 25 µM AAZ and (b) 10 µM MZA, recorded as a function of time in the NMR bioreactor. Spectral intensity (a.u.) is colour-coded from blue (lowest) to yellow (highest).

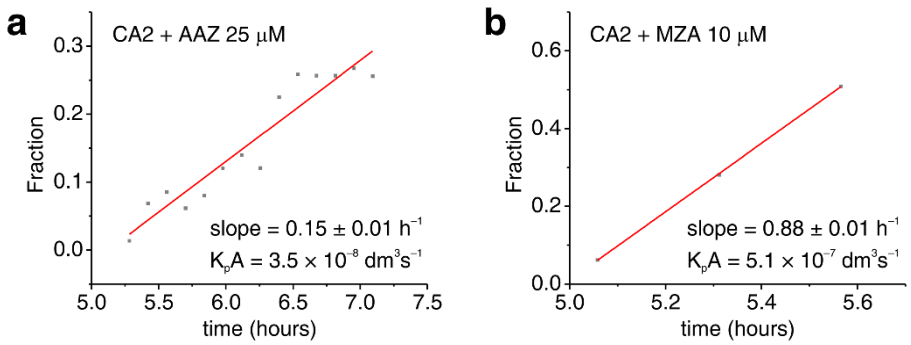


Figure S4. Permeability coefficients from linear regression of CA2 ligand binding. Linear regression (red lines) of the initial points (grey dots) of the binding curves from real-time NMR data of intracellular CA2 binding to (a) AAZ and (b) MZA. The permeability coefficients × membrane area (K_pA) calculated from the slope (see Materials and Methods) are reported in each panel.

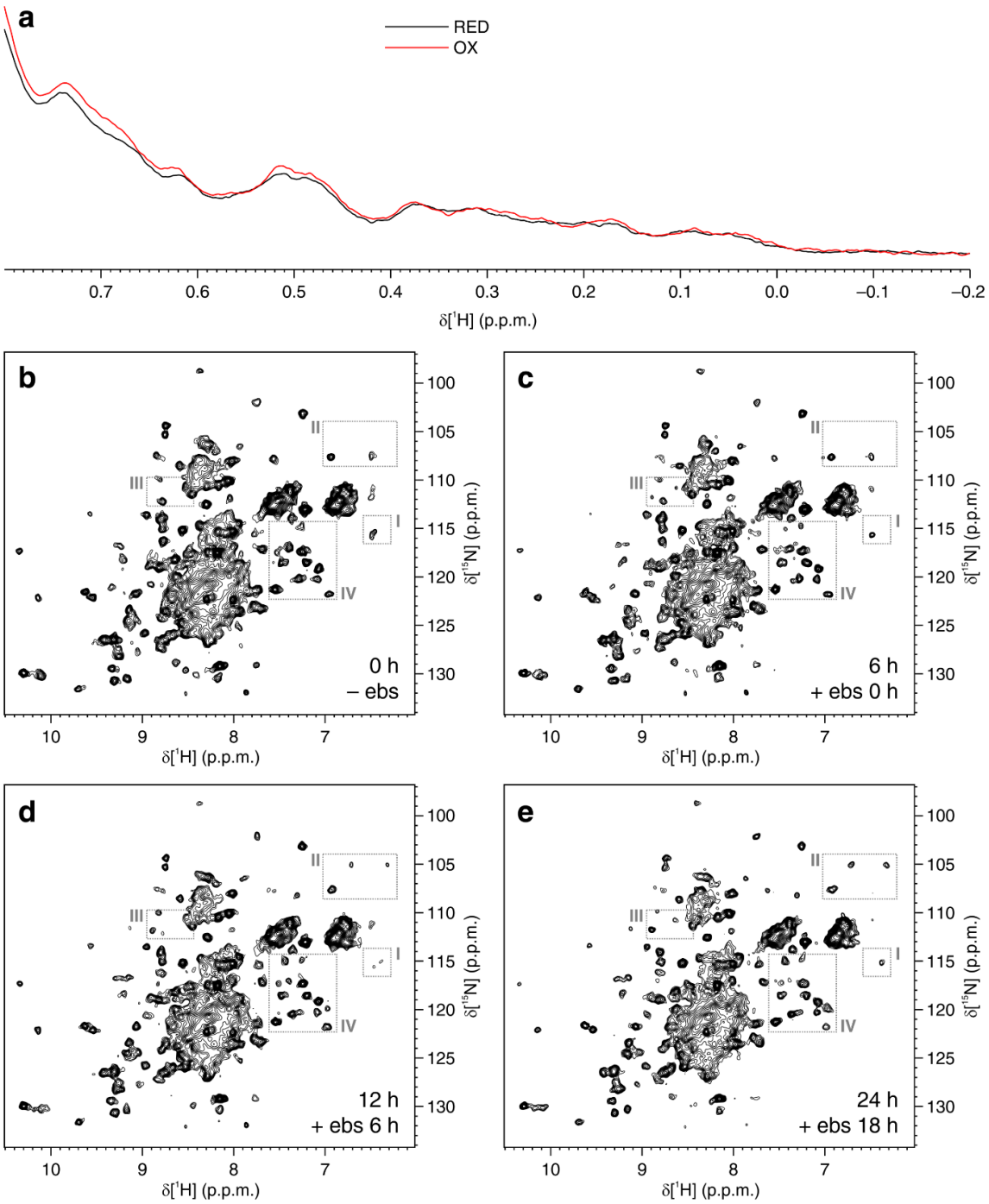


Figure S5. Real-time in-cell NMR spectra of SOD1. (a) Representative ^{1}H NMR spectra (methyl region between 0.8 and -0.2 p.p.m.) of intracellular disulfide-reduced SOD1 (black) and disulfide-oxidized SOD1 (red) in the NMR bioreactor. (b-e) ^{1}H-^{15}N NMR spectra of intracellular SOD1 in the bioreactor at (b) 0 hours, (c) 6 hours, (d) 12 hours, (e) 24 hours. Time after ebselen treatment is reported in each panel. The spectral regions (I-IV) selected for MCR-ALS analysis (see Figure 4) are indicated as grey dotted rectangles.

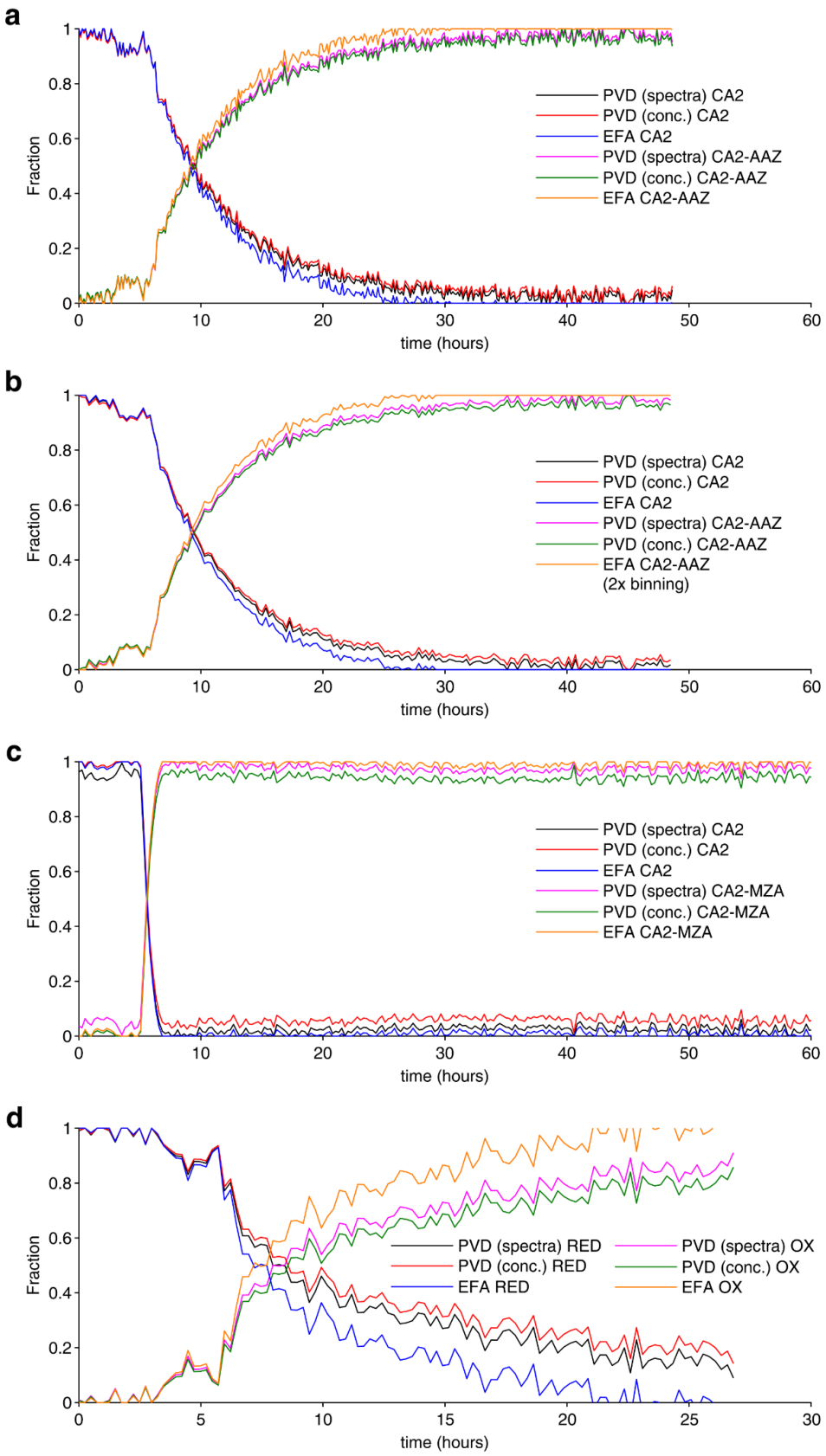

a
Fraction
time (hours)
PVD (spectra) CA2
PVD (conc.) CA2
EFA CA2
PVD (spectra) CA2-AAZ
PVD (conc.) CA2-AAZ
EFA CA2-AAZ
b
Fraction
time (hours)
PVD (spectra) CA2
PVD (conc.) CA2
EFA CA2
PVD (spectra) CA2-AAZ
PVD (conc.) CA2-AAZ
EFA CA2-AAZ
(2x binning)
c
Fraction
time (hours)
PVD (spectra) CA2
PVD (conc.) CA2
EFA CA2
PVD (spectra) CA2-MZA
PVD (conc.) CA2-MZA
EFA CA2-MZA
d
Fraction
time (hours)
PVD (spectra) RED
PVD (conc.) RED
EFA RED
PVD (spectra) OX
PVD (conc.) OX
EFA OX

Figure S6. MCR-ALS with different choice of initial components. (a-c) Relative concentration profiles of intracellular free and bound CA2 upon addition of (a) AAZ from original spectra, (b) AAZ from 2x binned spectra and (c) MZA. (d) Relative concentration profiles of intracellular disulfide-reduced SOD1 (RED) and disulfide-oxidized SOD1 (OX). The curves were obtained by MCR-ALS analysis using different methods of choice of initial pure components: Purest Variable Detection (PVD) along the 'spectra' dimension (black, magenta), PVD along the 'concentrations' direction (red, green) and Evolving Factor Analysis (EVA) (blue, orange).