PNAS

Supporting Information for

De novo sequencing and construction of a unique antibody for the recognition of alternative conformations of cytochrome c in cells.

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Methods

Peroxidatic activity of cyt c. Peroxidatic activity of native cyt c, SO-M80 and NO2-Y74 was performed with minor modification as previously described(1). Assessment of the peroxidatic activity was assayed with Amplex UltraRed Reagent (Invitrogen). The fluorescence of the oxidation product, resorufin, was monitored at λem = 585 nm and λex = 570 nm. Briefly, native, nitrated and/or SO-M80 cyt c (0.5 μM) was incubated in HEPES buffer (100 mM, pH = 7; DTPA, 100 μM) in the presence of Amplex Red (50 μM). The reaction was started by the addition of H2O2 (25 μM) and the fluorescence registered using a Varioskan Flash Multi-mode Reader (Thermo). The reaction rate was determined by linear fit of the fluorescence intensity.

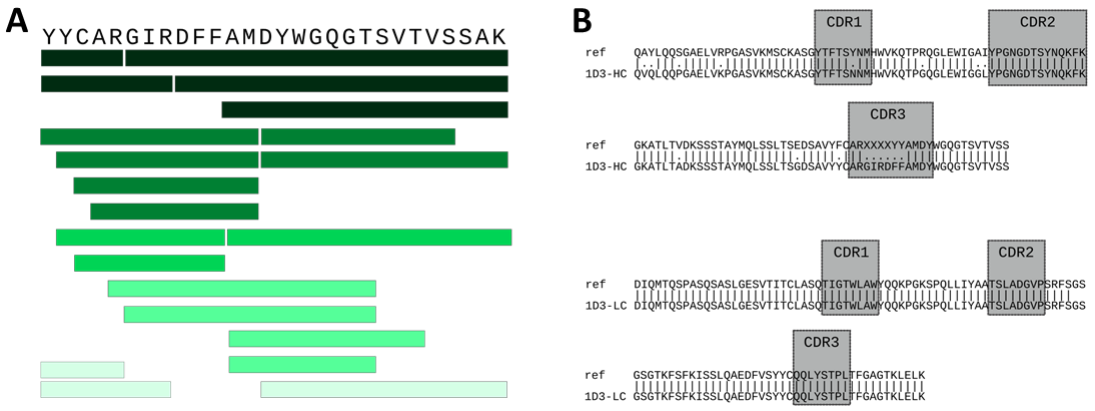
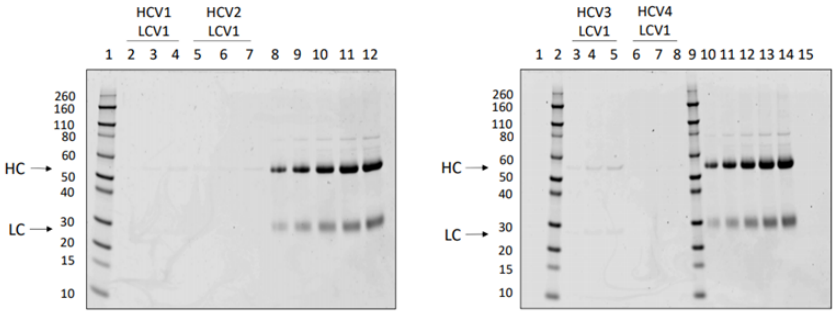


Fig. S1. Analysis of mAb 1D3 heavy chain sequence variable region. A. Unique peptide coverage of the H chain complementarity determining region 3 (CDR3). Each color corresponds to a distinct enzymatic digest in order from darkest to lightest (trypsin, subtilisin, chymotrypsin, elastase, aspn). Each enzyme provides unique and orthogonal evidence for the CDR3. B. The alignment of the mAb 1D3 H and L chain to the closest germline with CDRs noted. The L chain is completely identical to the germline sequence and could be determined largely by comparing the mass spectra to the reference sequence. In contrast, the H chain contains many mutations inside the CDR3s as well as framework regions. The character 'X' in the H chain reference is used to indicate the absence of reference sequence available for most of the CDR3. Despite the mutation load, 100% peptide coverage of both the H chain and L chain were achieved.



SAMPLE:	1:100	1:500	1:1000	1:5000	blank	2°AB only
HCV1 CHO, 0.05ug/ul	0.112	0.054	0.072	0.051	0.045	0.043
HCV2 CHO, 0.02ug/ul	0.125	0.086	0.063	0.05	0.049	0.044
HCV3 CHO, 0.02ug/ul	2.805	0.76	0.364	0.11	0.046	0.045
HCV4 CHO, 0.01ug/ul	0.08	0.059	0.051	0.049	0.046	0.046
+ ctrl (original 1D3 sample), 0.5ug/ul	3.955	3.666	1.701	1.523	0.039	0.039
no Ab	0.044	0.046	0.043	0.043	0.048	0.042
- mouse	0.047	0.048	0.043	0.043	0.04	0.04

Fig. S2. Expression of R1D3. A. SDS-PAGE 10% gels of purified R1D3 expressed in HEK293 cells. R1D3 and mAb 1D3 of purified product loaded. Lanes 8 – 12 in the gel on the left and lanes 10 – 14 in the gel on the right contain IgG standards for quantitation. **B.** ELISA results of purified 1D3 variants from CHO cells binding to antigen, cyt c. Additional expression trials using HCV3 reproduced the observed results.

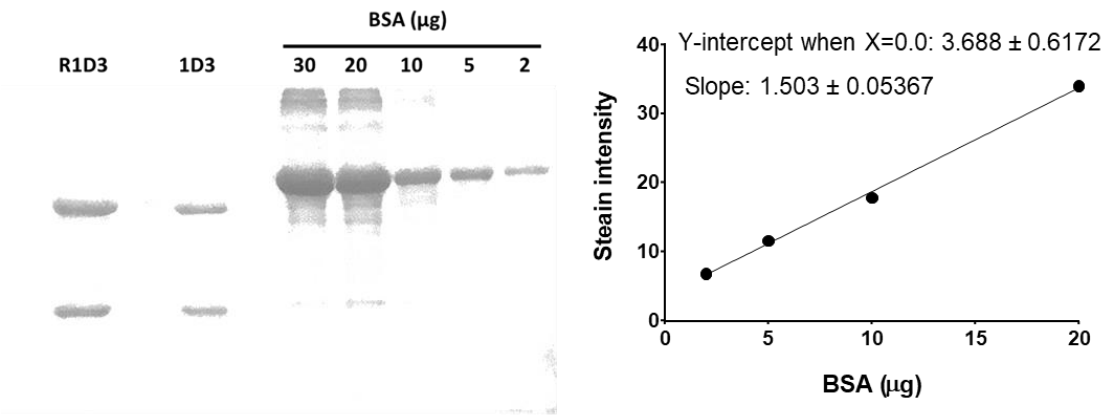


Fig. S3. **Expression of R1D3. A.** SDS-PAGE 10% gels of purified R1D3 expressed in HEK293 cells. R1D3 of purified product and mAb 1D3 loaded. Lanes 6 – 10 in the gel on the right contain BSA standards for quantitation. **B.** Calibration curve of the Intensity of each bands of monomeric form of BSA quantified by ImageJ versus amount of BSA loaded. The concentration of R1D3 was determinate using the lineal regression of the curve. R1D3 is 2.42 mg/mL and 1D3 is 1.5 mg/mL.

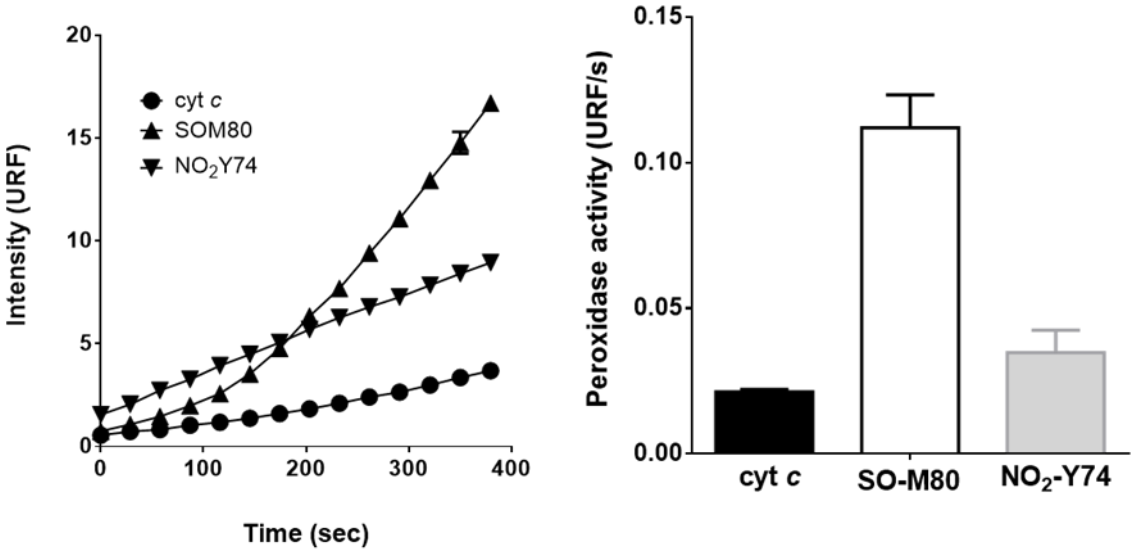


Fig. S4. Peroxidase activity. A. Peroxidase activities of cyt *c*, SO-M80 and NO_2-Y74 (0.5 μM) incubated in HEPES (10 mM plus 100 μM DTPA), pH 7, Amplex Red (50 μM) and H_2O_2 (25 μM). B. The average activity of cyt *c* and variants is summarized in the graph.

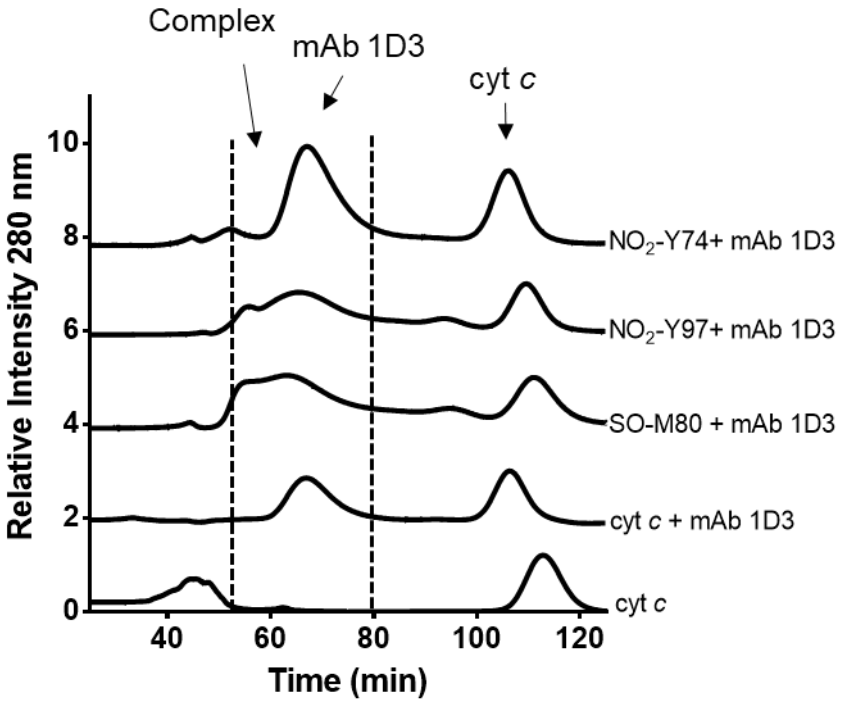


Fig. S5. Profile of cyt *c* (Abs 280 nm) chromatography on a Sephacryl S-200 column (GE Healthcare). Pre-incubated mAb 1D3 and cyt *c*/NO_2-Y74/NO_2-Y97/SO-M80 protein were loaded and eluted with phosphate buffer (50 mM, pH 7.4) containing NaCl (150 mM) at a flow rate of 0.8 mL/min. Fractions between dashed lines (50-80 min, mAb-1D3-cyt *c* complex) were collected and concentrated.

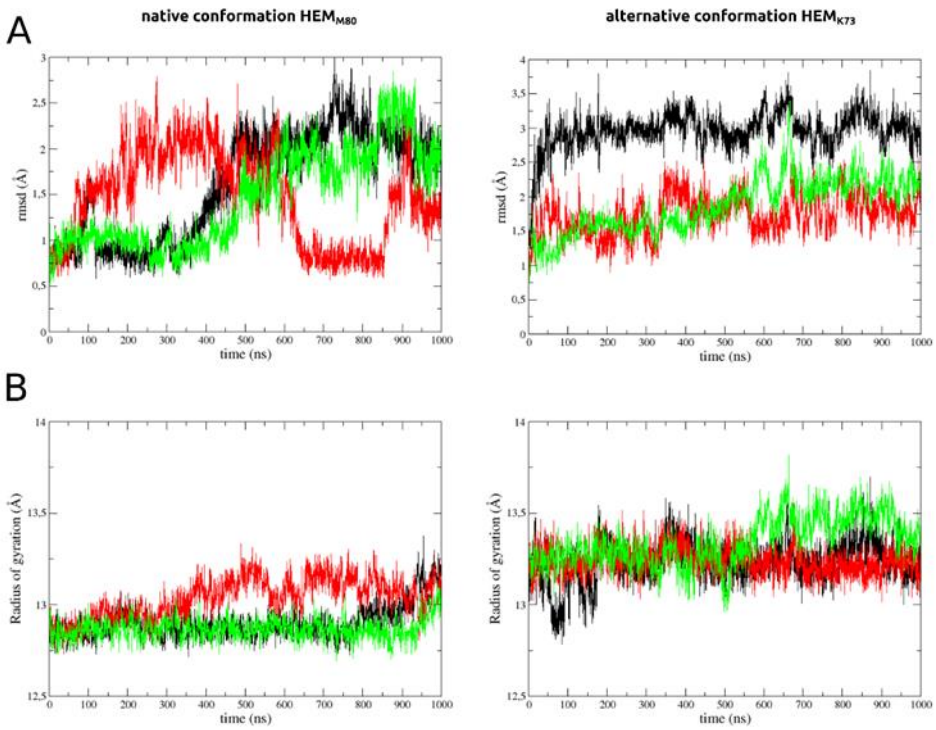


Fig. S6. Molecular dynamics simulations (MDs). Temporal courses of Cαs root mean square deviation (rmsd, Å, panel A) and protein radius of gyration (Å, panel B) calculated from each of three MD replicas of the native (left) and alternative (right) conformations of cyt *c*.

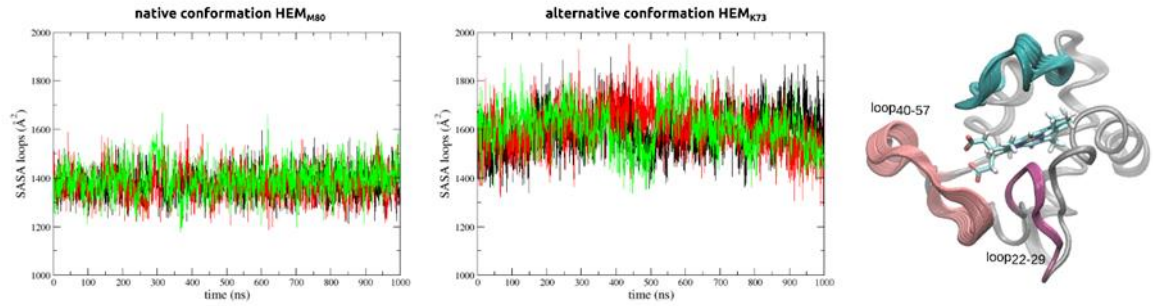


Fig. S7. Loops solvent accessibility. Comparison of the solvent accessible surface area (SASA, Å2) of the 40-57 and 22-29 loops, obtained from three MD replicas of the native (left) and alternative (right) conformations of cyt *c*.

SI References

1. D. A. Capdevila, *et al.*, Active Site Structure and Peroxidase Activity of Oxidatively Modified Cytochrome c Species in Complexes with Cardiolipin. *Biochemistry* **54** (2015).