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Supplementary Information for

Molecular characterization of a complex of Apoptosis Inducing Factor 1 (AIFM1) with cytochrome c oxidase of the mitochondrial respiratory chain

Johannes F. Hevler[1,2], Riccardo Zenezeni Chiozzi[1,2], Alfredo Cabrera-Orefice[3], Ulrich Brandt[3,4], Susanne Arnold[3,4] and Albert J.R. Heck[1,2*]*

[1] Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht,
Padualaan 8, 3584 CH Utrecht, The Netherlands
[2] Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands
[3] Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands
[4] Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50931 Cologne, Germany.

*Correspondence:

Susanne Arnold, sarnold2012@googlemail.com; Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands
Albert Heck, a.j.r.heck@uu.nl; phone: +31302536797; address: Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

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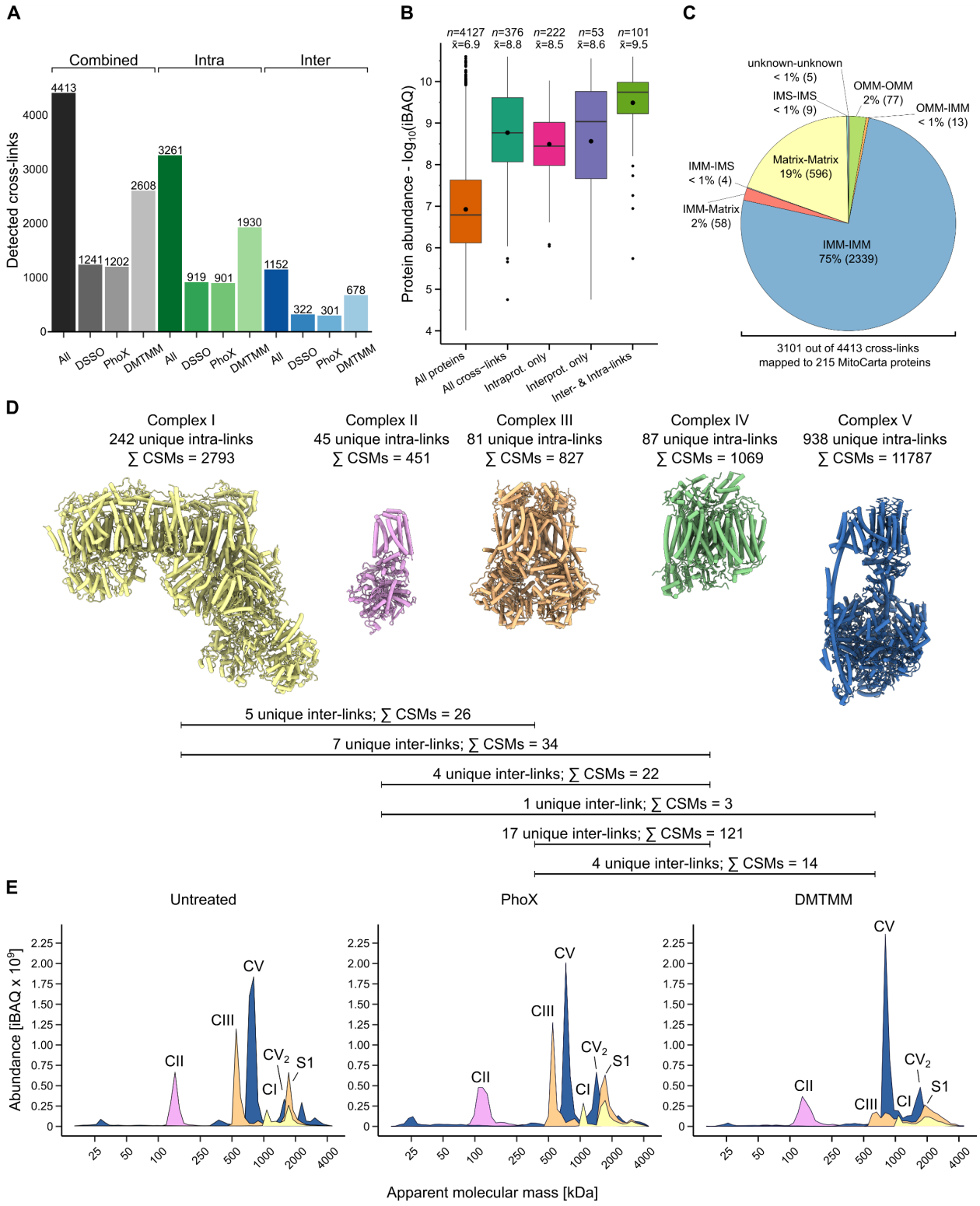

Fig. S1. Overview of cross-linking and complexome profiling of bovine heart mitochondria (BHM). BHM were cross-linked in parallel with three different cross-linkers (DSSO, PhoX and DMTMM) and subjected to XL-MS analysis or complexome profiling. (*A*) Overview of the number of unique cross-links, identified for each of the cross-linkers used (DSSO, PhoX, DMTMM) and in combination (all). (*B*) Observed cross-linked proteins are generally more abundant. Boxplot showing protein abundances for all identified proteins, all cross-linked proteins and proteins that have either only inter-, intra- or both (inter

and intra) links. The number of proteins and the median iBAQ is indicated on top of each box. (C) Pie chart showing the sub-mitochondrial localization of the cross-linked proteins identified based on their MitoCarta 3.0 annotation. (*D*) Numbers of obtained cross-links for OXPHOS complexes I-V. Complexes are heavily cross-linked (~35% of all detected cross-links). Cross-links are observed within subunits of the same complex (intra cross-links) but also between subunits of different OXPHOS complexes (inter cross-links). For structural representation, deposited structural models were chosen (PDB: 5LNK, 1ZOY, 1NTM, 1V54, 5ARA). (*E*) Averaged migration profiles of the OXPHOS complexes CI, CII, CIII and CV without cross-linker treatment and after treatment with either the PhoX or DMTMM cross-linker using a 4-16% gradient BN gel. The profiles were obtained by plotting the relative abundance of the averaged subunits of each complex against the respective molecular mass. Peaks are annotated based on the molecular masses of CI, CII, CIII, CV and the supercomplex S1 (CI-$CIII_2$-CIV). Upon addition of cross-linker, the OXPHOS complexes largely maintain their overall migration profile, and thus structural integrity.

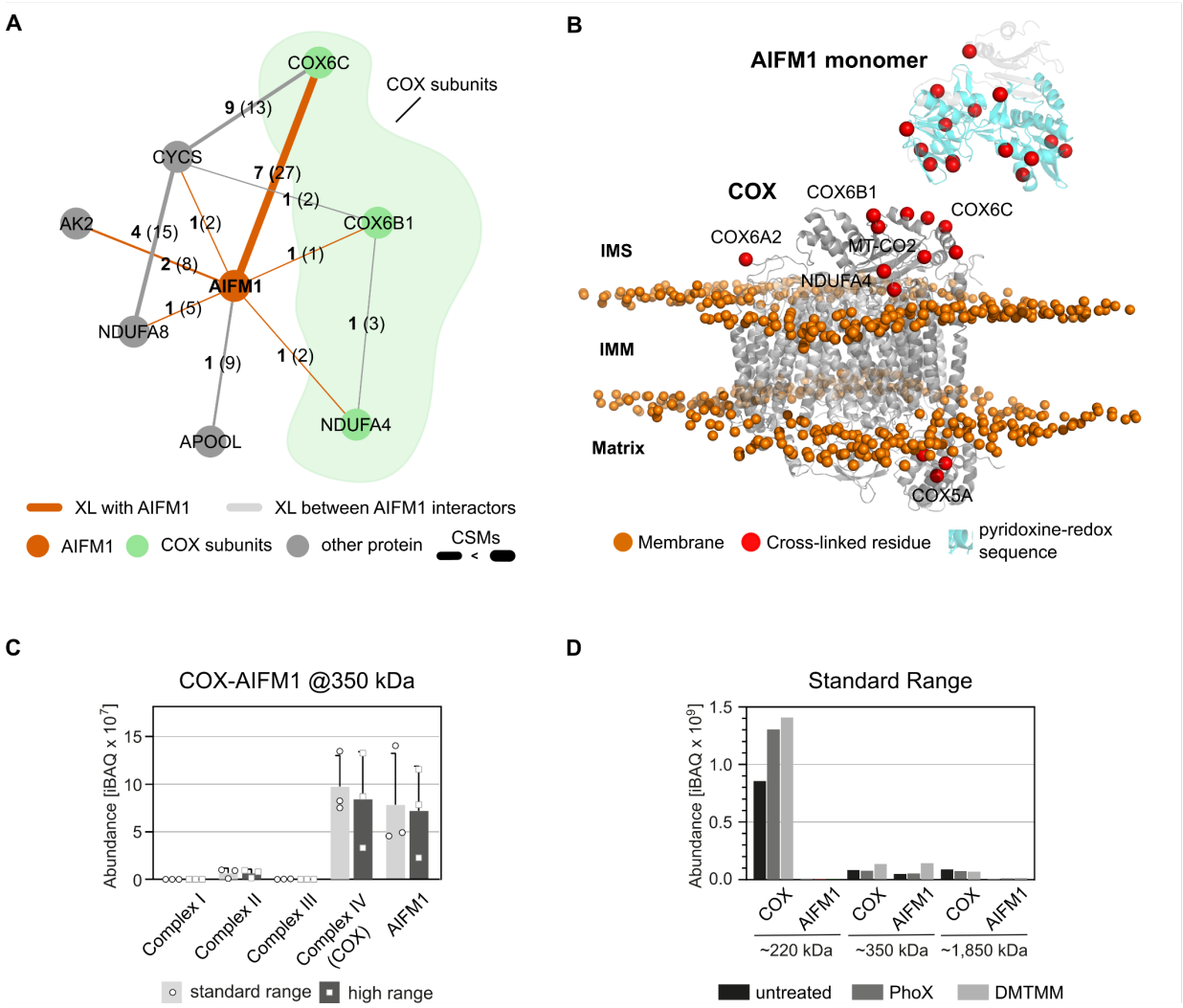

Fig. S2. COX-AIFM1 interactions. *(*A*)* The AIFM1 interactome as observed by XL-MS in intact mouse heart mitochondria adapted from (Liu et al., 2018). Bold numbers indicate the observed cross-links for each interaction and thickness of lines indicate the cumulative evidence (CSMs) for each interaction (number in parentheses). CSMs were summed from triplicates. *(*B*)* Visualization of cross-linked residues (red spheres) of COX (gray) and truncated AIFM1 monomer (gray with residues of the Pyr-redox sequence being cyan) in our data on BHM. Orange spheres indicate the phosphate groups of a simulated lipid bilayer (IMM) which was structurally aligned based on the simulation for bovine monomeric COX (PDB: 6JY3) obtained from the MemProtMD server. *(*C*)* Comparison of the amounts of respiratory chain complexes I to IV and AIFM1 at ~350 kDa representative of the COX-AIFM1$_2$ complex. Complexome profiling was performed using a standard range BN-gel (4-16%) and a high range BN-gel (3-10%). The average of individual values ± SD from all three conditions (untreated, PhoX and DMTMM cross-linked) is shown. *(*D*)* Comparison of COX and AIFM1 abundances at ~220 kDa, ~350 kDa and ~1,850 kDa in complexome profiles using a standard BN-gel (4-16%) of BHM as prepared (untreated) and after cross-linking with PhoX and DMTMM. In *(*C*)* and *(*D*)*, iBAQ values of AIFM1 are divided by two to account for the dimer and the average of subunits of the respective complexes at the indicated approximate apparent masses in the migration profiles were taken as a measure for their abundance.

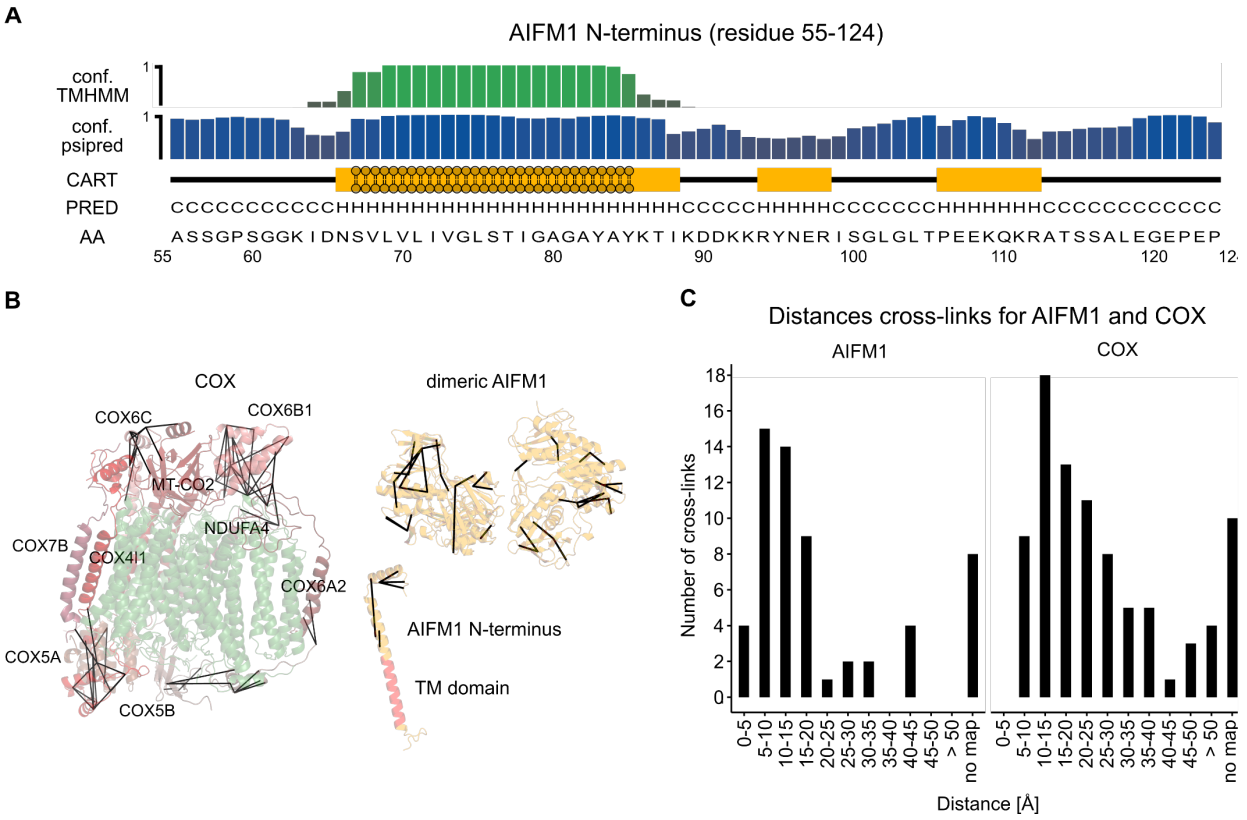

Fig. S3. Structural properties of the N-terminal region of AIFM1 and cross-link distances in the COX and AIFM1 structures used for docking. *(A)* Secondary structure prediction of the N-terminal region of AIFM1 (res 55-124; sequence indicated under AA). The upper bar plot shows the confidence of residues being transmembrane residues (Score = 1) or not within a membrane (Score < 0.6). The second bar plot shows the confidence of the secondary structure prediction (1 = highest, 0 = lowest) for each residue which is indicated under PRED as letter code (C = coil, H = helix) und visualized as cartoon under CART. *(B)* Detected cross-links (intra and inter domain) mapped onto the structural models of COX, AIFM1 dimer as well as the de-novo modelled N-terminal domain of AIFM1. The COX structure (green, with cross-linked subunits colored in different shades of red as indicated) was based on a previously resolved structure (PDB: 1V54) supplemented with NDUFA4 (structurally aligned based on PDB: 5Z62). Dimeric AIFM1 (orange) was modelled based on the previously resolved human homologue (PDB: 4BUR, res 128-516, 551-613). The N-terminal domain of AIFM1 (orange) (res 55-124) was generated using trRosetta. The predicted transmembrane (TM) domain is highlighted in red. *(C)* Distance histogram of mapped cross-links (combination of DSSO, PhoX and DMTMM) for COX and AIFM1. AIFM1 includes distances for cross-links mapped on the AIFM1 dimer and the N-terminal region of AIFM1. For both structures, a number of cross-links are obtained within or attaching to missing regions of AIFM1 (N-terminus (55-127) and residue 517-550) and COX (N-terminus COX4l1).

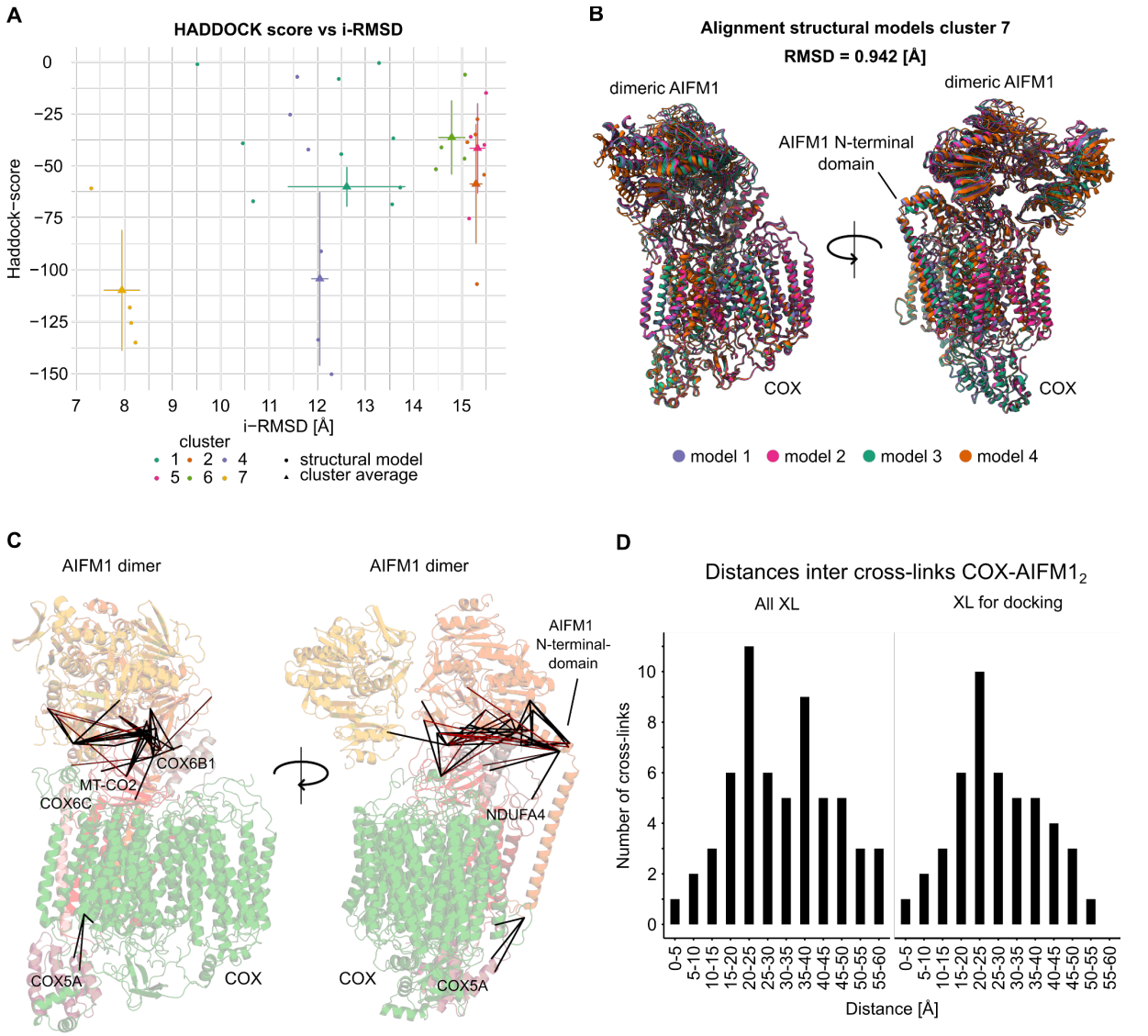

Fig. S4. Haddock cluster validation and inter cross-link distances in the COX-AIFM1$_2$ model. *(A)* Plot of HADDOCK score of the chosen clusters (note: cluster 3 was disregarded due to a positive Haddock score) as a function of their RMSD from the lowest energy structure. Circles represent individual structures of each cluster and triangles correspond to the cluster averages with standard deviation indicated by bars. *(B)* Structural alignment of individual structures of cluster 7. A low average RMSD (0.942 Å) indicates high cluster precision. Different models are colored as indicated. *(C)* Visualization of cross-links between COX and AIFM1 which were used for structural docking of the COX-AIFM1$_2$ complex. COX subunits are colored green, while AIFM1 protomers are colored orange. Cross-links with a Cα-Cα distance ≤ 35 Å are colored in black, while cross-links with a mapped Cα-Cα distance ≥ 35 Å are colored in red. Cross-linked subunits of COX are colored in different shades of red as indicated. *(D)* Cross-links (combination of DSSO, PhoX and DMTMM) used for structural docking and all observed cross-links for COX-AIFM1$_2$ interaction were mapped onto the best model of the complex. For AIFM1, all obtained cross-links can be mapped onto the final structure of AIFM1, containing two AIFM1 protomers (res 128-516, 551-613) and one N-terminal region of one protomer (res 55-124).

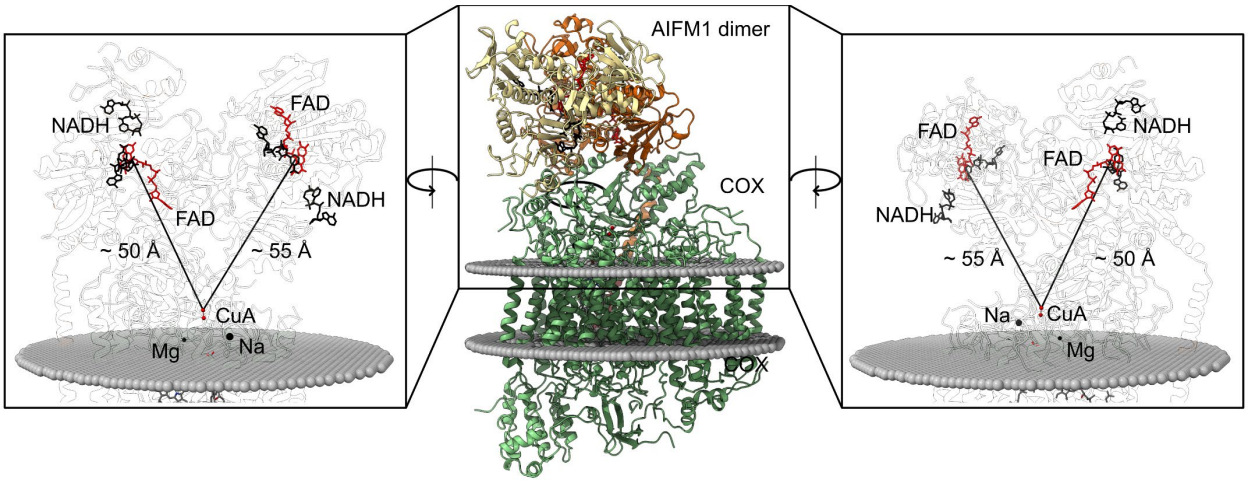

Fig. S5. Mapping the distance from the isoalloxazine moiety of FAD in AIFM1 to the CuA center of COX. Cofactors were structurally aligned based on structures of AIFM1 (PDB: 4BUR) and COX (PDB: 1V54). AIFM1 protomers are orange and COX subunits are green (middle); red sticks represent FAD, black sticks represent NADH, Cu_A, and Cu_B are red (left and right). Boundaries of the IMM are indicated as gray spheres.