Supporting Information for

Electron transfer control in soluble methane monooxygenase

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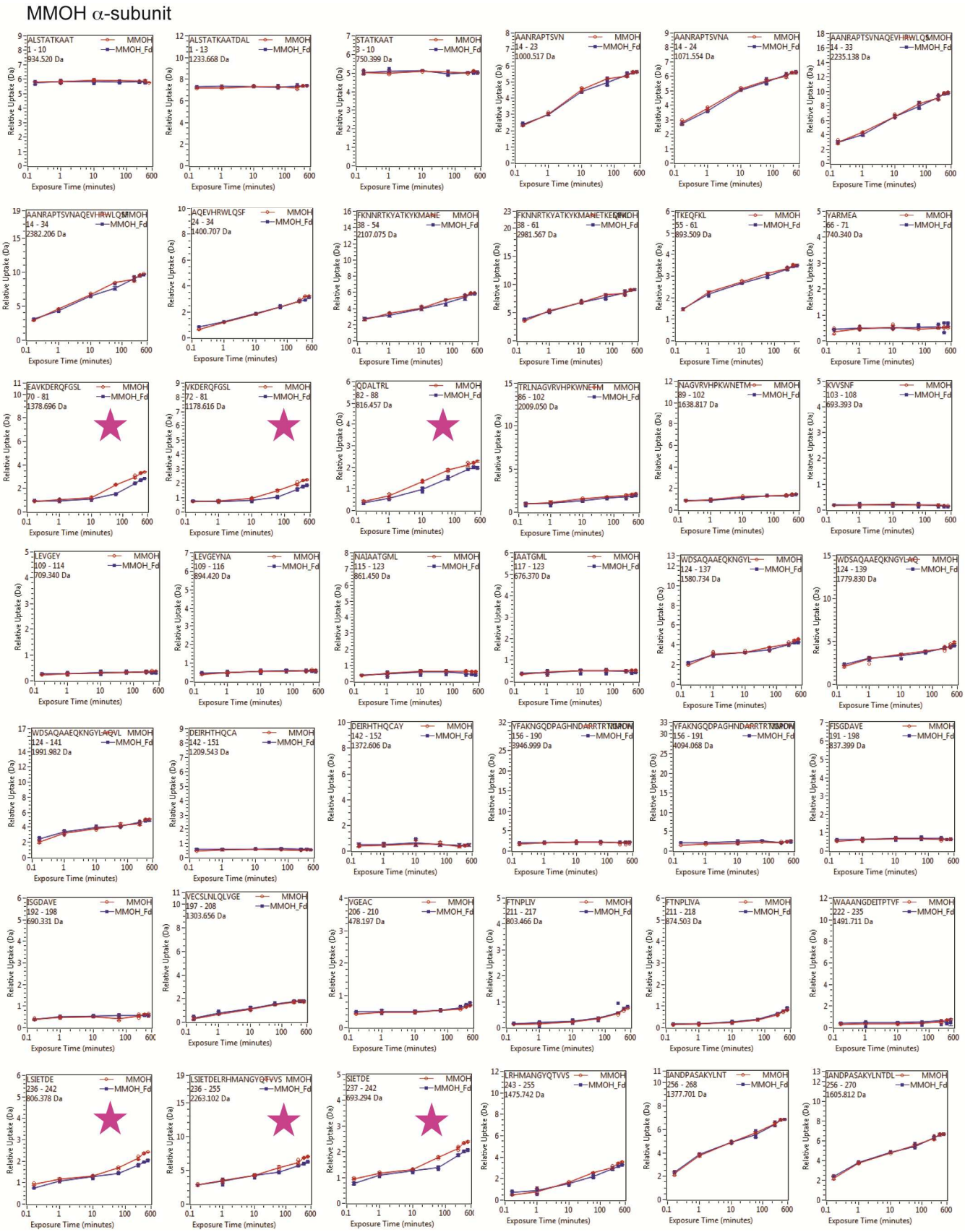
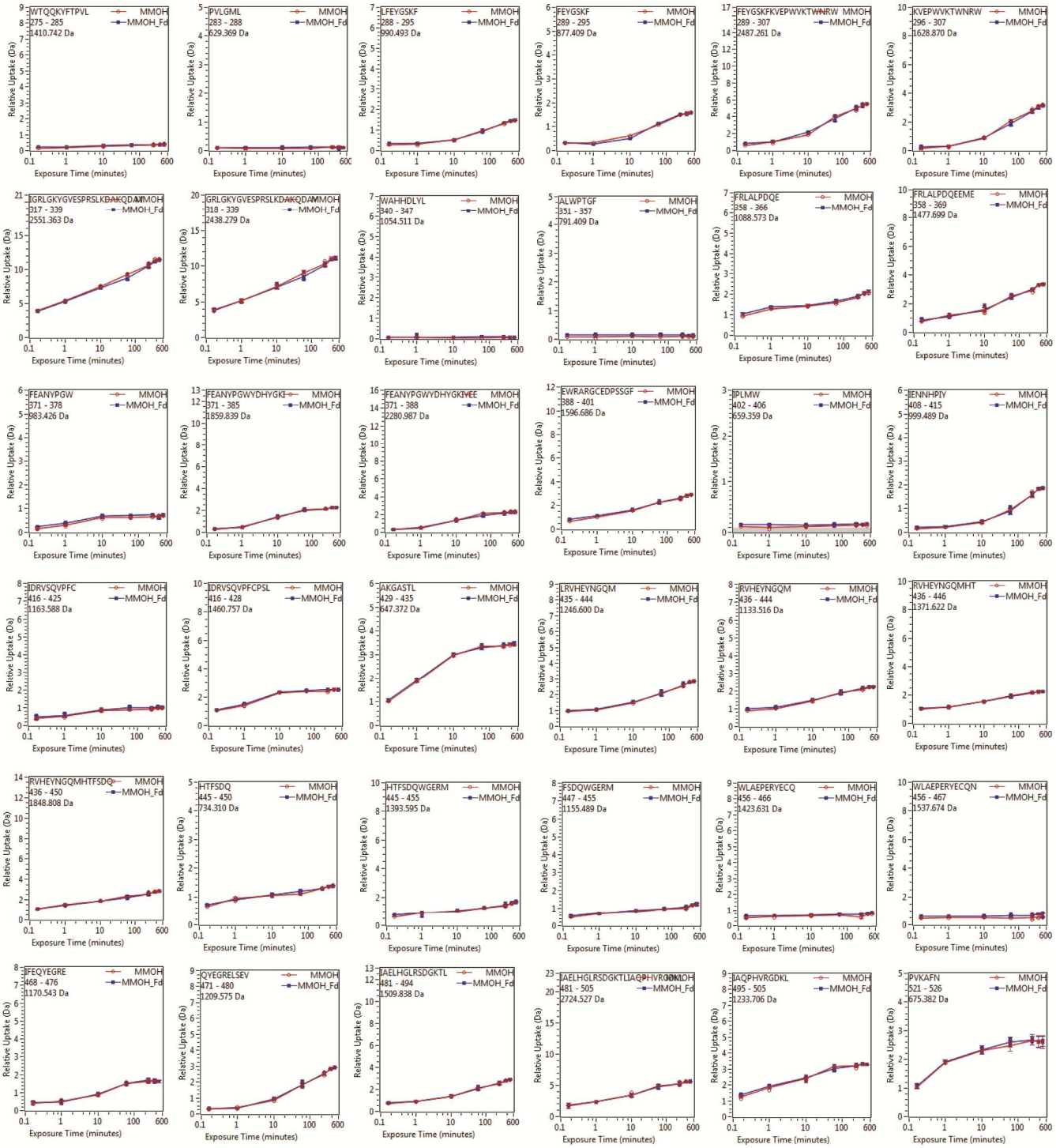
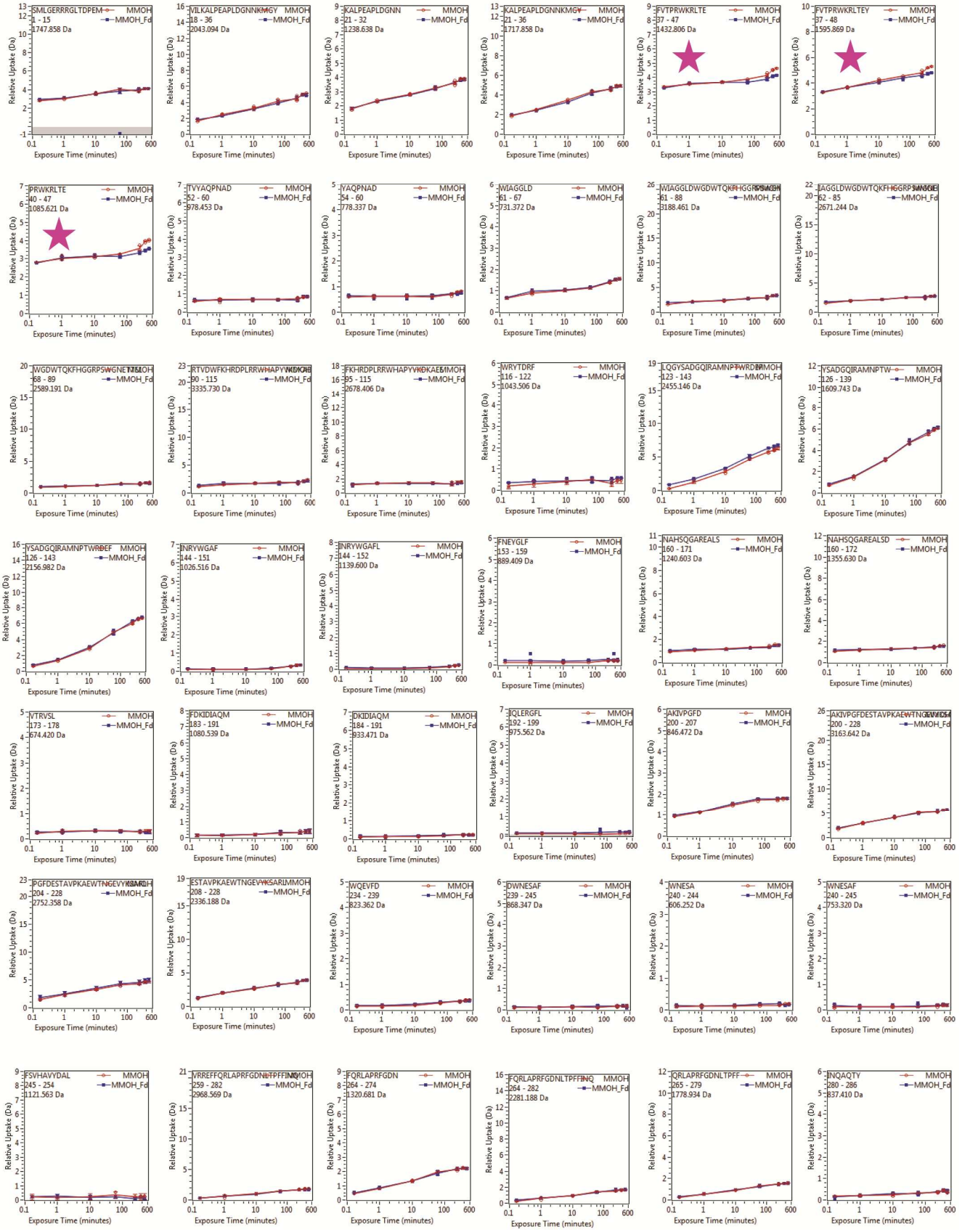
Figure S1. Amino acid sequence and peptic peptide coverage for MMOH. Each colored bar under the sequence corresponds to a peptic peptide that was identified and followed by HDX MS analysis. A total of 93.9% linear coverage was obtained for the α-subunit, 93.5% for β-subunit and 96.4% for the γ-subunit. This protein coverage map was generated using the DynamX 2.0 software package (Waters Corp).


MMOH α-subunit
ALSTATKAAT 1 - 10 934.520 Da
ALSTATKAATDAL 1 - 13 1233.668 Da
STATKAAT 3 - 10 750.399 Da
AANRAPTSVN 14 - 23 1000.517 Da
AANRAPTSVNA 14 - 24 1071.554 Da
AANRAPTSVNAQEVHRWLQS 14 - 33 2235.138 Da
AANRAPTSVNAQEVHRWLQSF 14 - 34 2382.206 Da
AQEVHRWLQSF 24 - 34 1400.707 Da
FKNNRTKYATKYKMANE 38 - 54 2107.075 Da
FKNNRTKYATKYKMANETKEQVKDH 38 - 61 2981.567 Da
TKEQFKL 55 - 61 893.509 Da
YARMEA 66 - 71 740.340 Da
EAVKDERQFGSL 70 - 81 1378.696 Da
VKDERQFGSL 72 - 81 1178.616 Da
QDALTRL 82 - 88 816.457 Da
TRLNAGVRVHPKWNETM 86 - 102 2009.050 Da
NAGVRVHPKWNETM 89 - 102 1638.817 Da
KVVSNF 103 - 108 693.393 Da
LEVGEY 109 - 114 709.340 Da
LEVGEYNA 109 - 116 894.420 Da
NAIAATGML 115 - 123 861.450 Da
IAATGML 117 - 123 676.370 Da
WDSAQAAEQKNGYL 124 - 137 1580.734 Da
WDSAQAAEQKNGYLAQ 124 - 139 1779.830 Da
WDSAQAAEQKNGYLAQVL 124 - 141 1991.982 Da
DEIRHTHQCA 142 - 151 1209.543 Da
DEIRHTHQCAY 142 - 152 1372.606 Da
YFAKNGQDPAGHNDARRTRTDAPQN 156 - 190 3946.999 Da
YFAKNGQDPAGHNDARRTRTDAPQN 156 - 191 4094.068 Da
FISGDAVE 191 - 198 837.399 Da
ISGDAVE 192 - 198 690.331 Da
VECSLNLQLVGE 197 - 208 1303.656 Da
VGEAC 206 - 210 478.197 Da
FTNPLIV 211 - 217 803.466 Da
FTNPLIVA 211 - 218 874.503 Da
WAAANGDEITPTVF 222 - 235 1491.711 Da
LSIETDE 236 - 242 806.378 Da
LSIETDELRHMANGYQTVVS 236 - 255 2263.102 Da
SIETDE 237 - 242 693.294 Da
LRHMANGYQTVVS 243 - 255 1475.742 Da
IANDPASAKYLNT 256 - 268 1377.701 Da
IANDPASAKYLNTDL 256 - 270 1605.812 Da
MMOH
MMOH_Fd
Relative Uptake (Da)
Exposure Time (minutes)

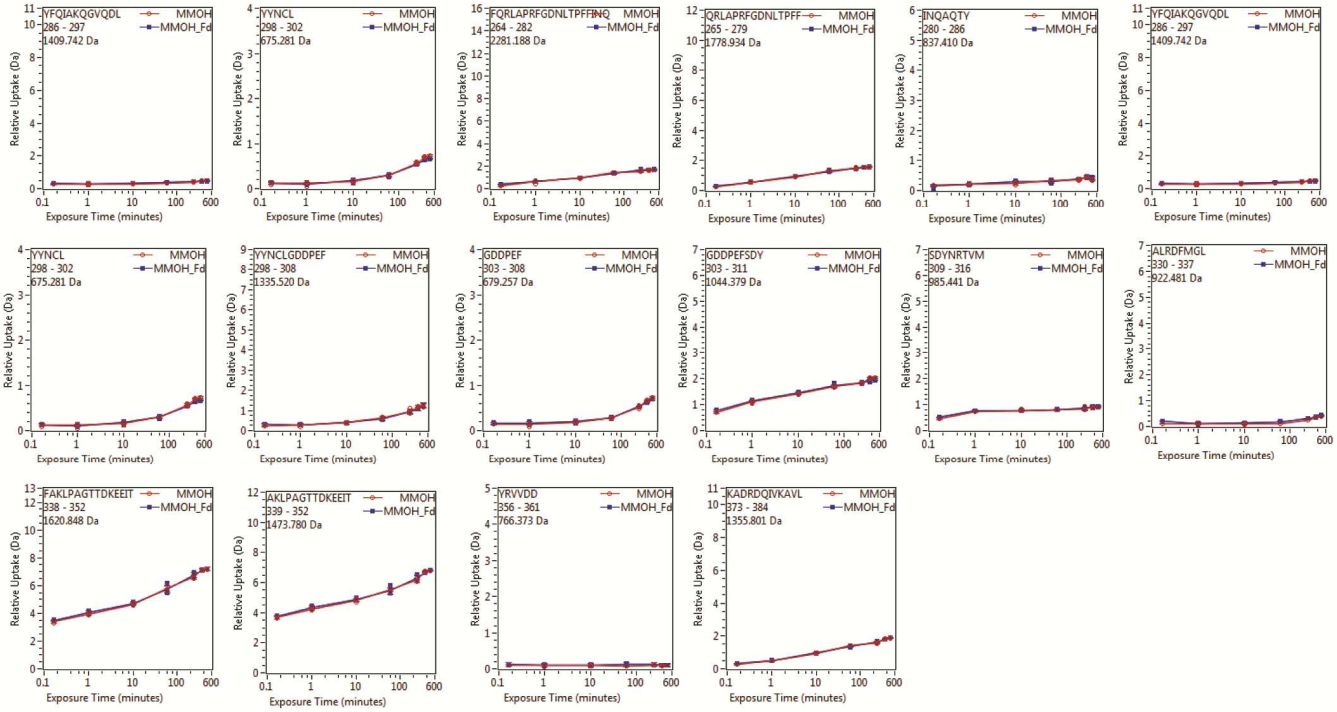
MMOH α-subunit, continued



MMOH β-subunit



MMOH β-subunit, continued



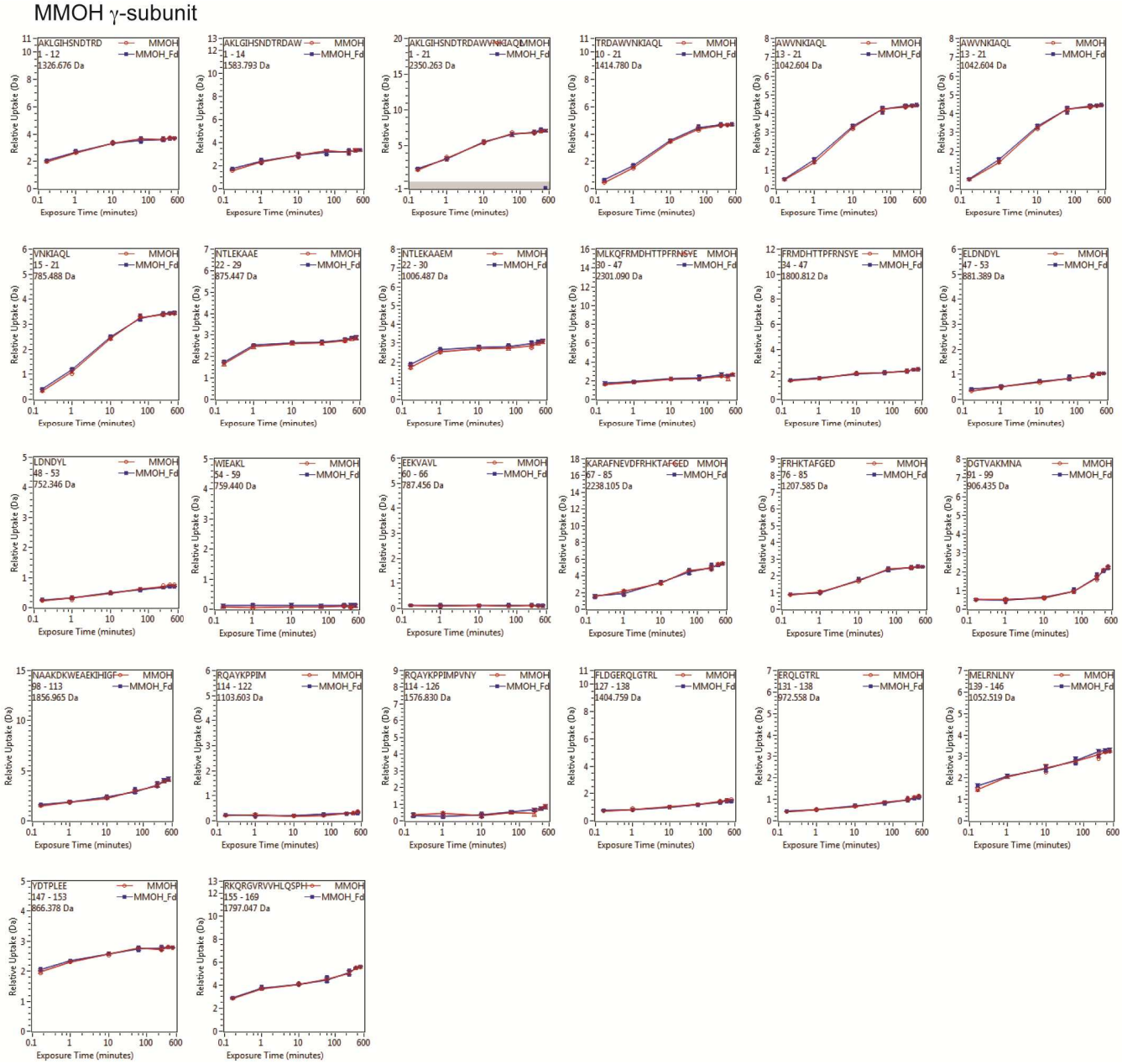


Figure S2. Deuterium incorporation graphs for the peptic peptides that were followed by HDX MS for MMOH free (red) and bound to MMOR-Fd (blue). Each data point represents the average of three independent measurements. The magenta stars indicate the regions where differences in deuterium uptake were observed between free MMOH and Fd-bound. The graphs were produced by DynamX 2.0 software (Waters Corp.).

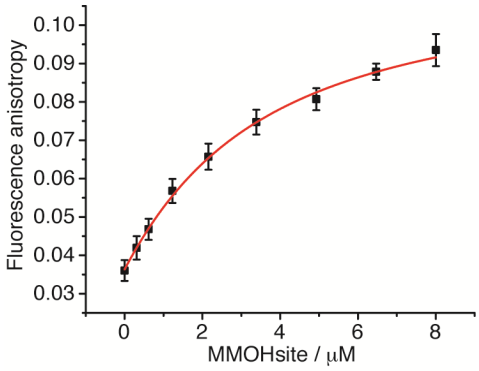


Figure S3. Fluorescence anisotropy titration of MMOH into MMOB Δ2-33 labeled with IAEDANS, for K_d determination.

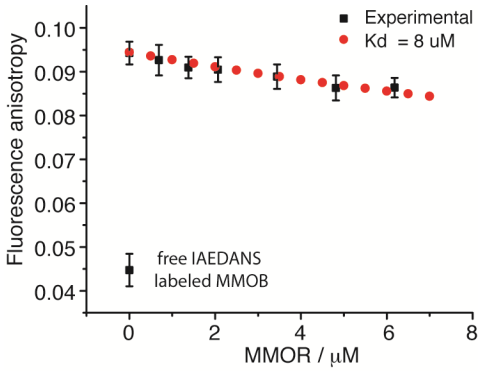


Figure S4. Fluorescence anisotropy titration of MMOR into a mixture of 1 μM MMOH and 1 μM IADEANS labeled full length MMOB.

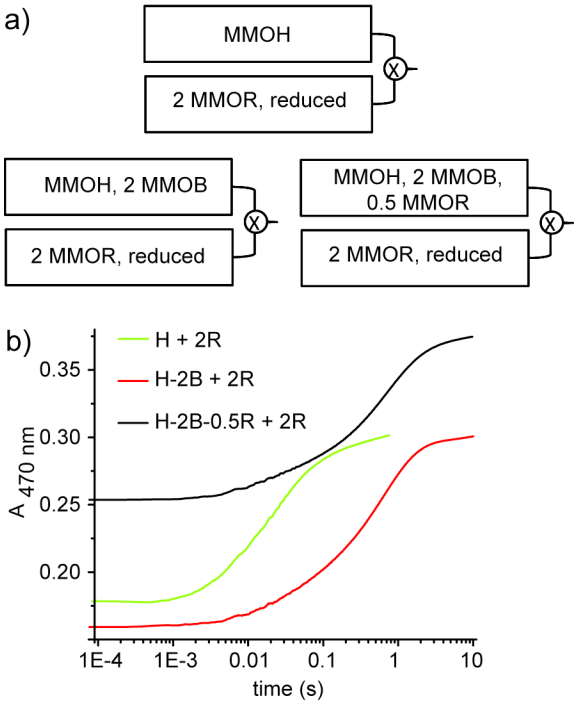


Figure S5. MMOB inhibits electron transfer using chemically reduced full length MMOR as the electron source. a) Schematic diagrams for the experimental setups. b) Electron transfer kinetic curves. Wavelength monitored, 458 nm; T = 15 °C.

Table S1. Primers used for making mutant proteins.

mutant	Primers	
MMOR ferredoxin domain (1-107)	Forward	5- GTT TTG GTG AGGTCG GCT AGT AGG AGG CGG AGGTCG -3
	Reverse	5- CGA CCT CCG CCT CCT ACT AGC CGA CCT CAC CAA AAC -3
MMOB Δ2-33 D36C	Forward	5- GGA GGT ATT ACG ATG GAA AGC GAC ACG GTC GTT C -3
	Reverse	5- GAA CGA CCGTGT CGCTTT CCATCG TAATAC CTC C -3