

# **Structural Insight into the Transmembrane Domain and the Juxtamembrane Region of the Erythropoietin Receptor in Micelles**

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Figure S1. Purification of the hEpoR from *E. coli*. This construct contained the TMD and JM region of the human EpoR. It was expressed and purified into 2 mM LMPC (A), 2 mM LMPG (B), 2 % bicelles (C) formed by DHPC and DMPC (q=0.33) and 15 mM DPC (D). Lane 1 is the molecular weight standard. Lane 2 is the cell lysate. Lane 3 is the supernatant of the cell lysate. Lane 4 is the pellet dissolved in the urea buffer. Lane 5 is the flow through fraction in the affinity purification. Lane 6 and 7 are the elution fractions from the Ni<sup>2+</sup>-NTA resin. E. Gel filtration analysis for the hEpoR. The hEpoR was purified in DPC micelles and followed by further purification using gel filtration chromatography. The fractions from the peak at retention volume of ~15 ml were combined and concentrated for NMR study. The gel filtration buffer contained 20 mM sodium phosphate, pH6.5, 15 mM DPC and 1 mM DTT.

Figure S2 H-D Exchange experiment of the hEpoR. The <sup>1</sup>H-<sup>15</sup>N-HSQC spectra of hEpoR in 5%, 10%, 30%, 50%, 70% and 90% D<sub>2</sub>O were collected at 313 K and processed.

Figure S3 Cross-linking study of the hEpoR (A) and mEpoR (B). Both hEpoR and hEpoR were prepared in DPC micelles and cross-linking was conducted. The samples were separated by SDS-PAGE and visualized by Commassie blue. “-” means no cross linker was added and “+” indicated that GA was added.

Figure S4 <sup>1</sup>H-<sup>15</sup>N HSQC spectra of hEpoR. A <sup>15</sup>N-labeled hEpoR was prepared in DPC micelles to obtain a sample contain 1 mM hEpoR and 50 mM DPC micelles. The HSQC spectrum of the sample (protein to DPC molar ratio was 1:50) was shown in red. DPC micelles stock (20% w/v) was added to the sample to change the molar ratio of protein to micelles to 1:200. The HSQC spectrum of the resulting sample was shown in black.

Figure S1

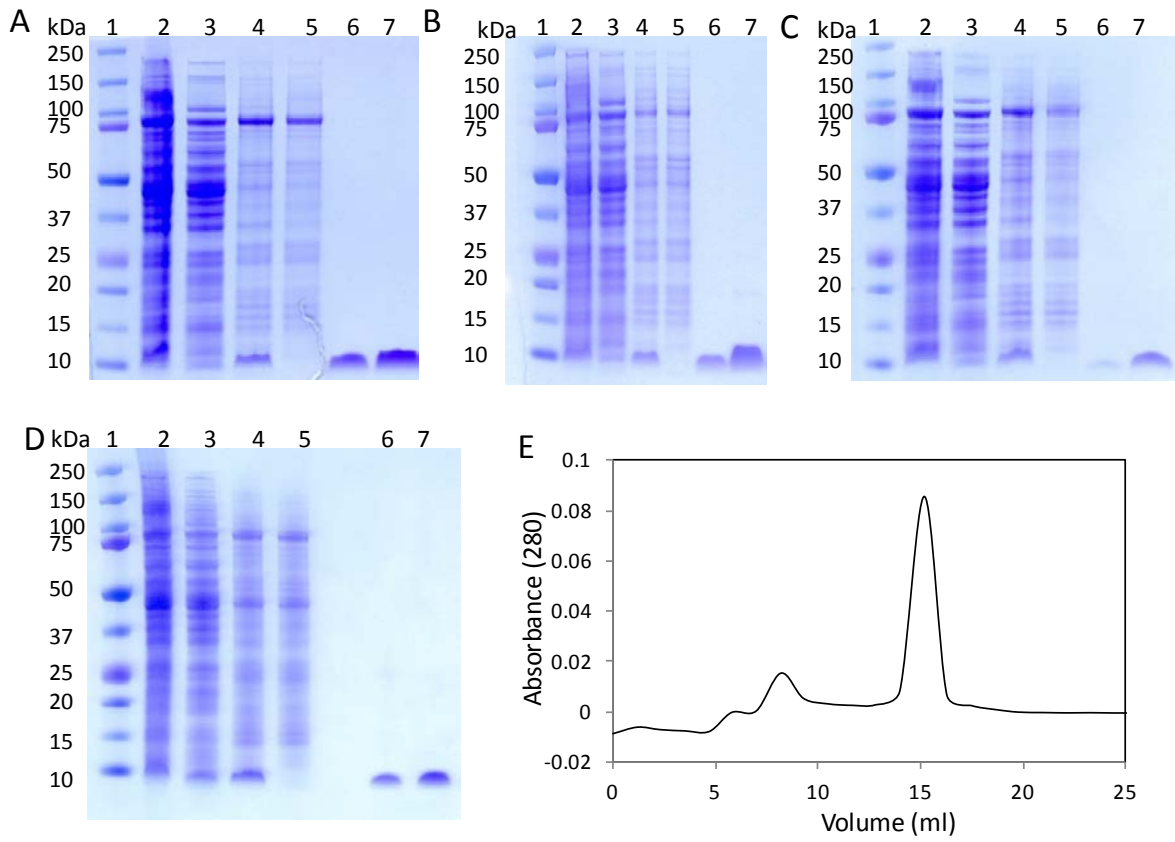


Figure S2

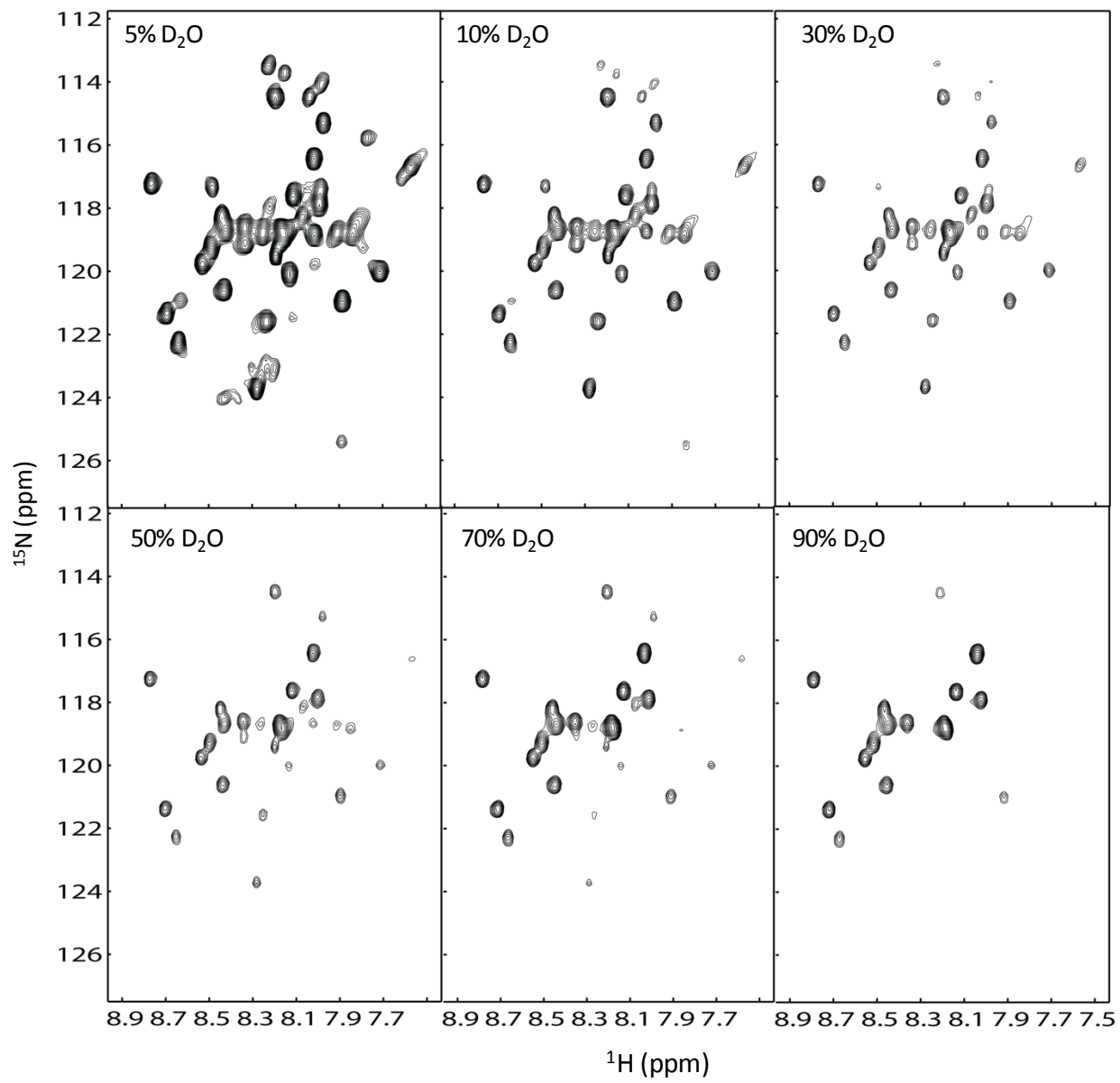


Figure S3

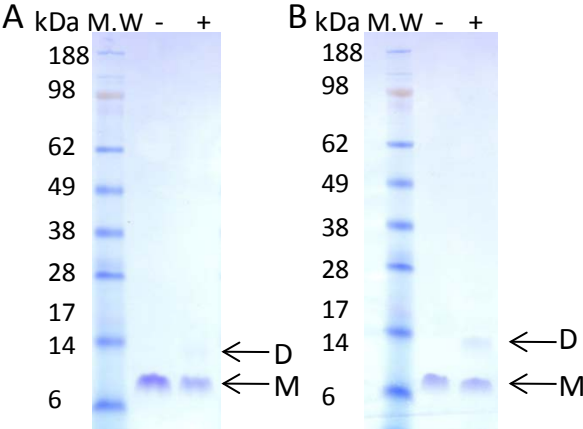
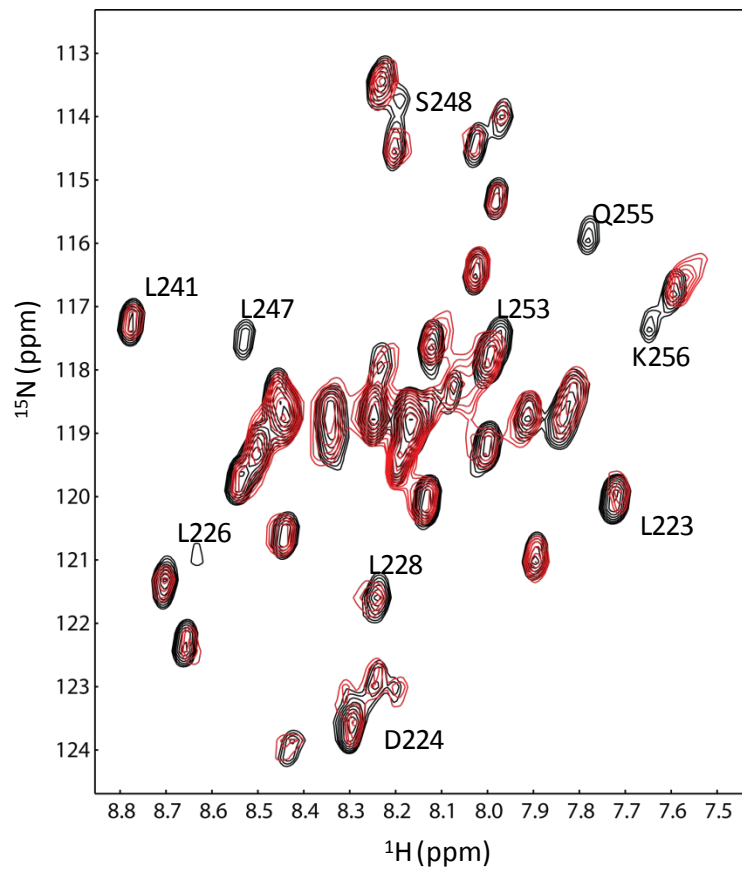


Figure S4



Supplementary Table 1 Assignment of the mEpoR in DPC micelles

Res#	Residue	N	HN	CA	CB	C
212	SER	8.31	115.79	58.96	63.84	174.05
213	GLU	8.25	121.82	55.13	29.38	174.78
214	PRO	---	---	62.76	33.9	175.99
215	ALA	8.58	126.16	53.84	19.18	178.41
216	SER	8.05	113.84	59.34	63.82	175.3
217	LEU	8.23	123.09	56.82	42.56	177.26
218	LEU	7.8	116.64	55.84	42.4	177.03
219	THR	7.82	110.68	62.07	69.75	174.81
220	ALA	8.07	124.45	53.16	19.12	177.5
221	SER	7.96	112.82	59.24	64.28	174.05
222	ASP	8.2	120.4	54.52	41.09	175.64
223	LEU	7.79	120.23	54.56	43.46	176.09
224	ASP	8.28	123.27	53.38	42.93	---
225	PRO	---	---	---	---	---
226	LEU	---	---	58.38	41.14	178.25
227	ILE	7.83	118.94	64.29	36.69	179.23
228	LEU	8.26	121.99	58.89	41.85	177.73
229	THR	7.97	115.31	68.38	---	---
230	LEU	8.31	119.4	58.55	41.84	178.24
231	SER	8.21	114.7	63.72	63	175.12
232	LEU	7.94	121.09	58.11	41.75	178.51
233	ILE	8.06	118.36	65.73	37.23	177.23
234	LEU	8.27	119.24	58.48	41.73	179.94
235	VAL	8.34	121.1	67.38	31.38	177.18
236	LEU	8.36	120.01	58.78	41.81	178.7
237	ILE	8.69	117.28	65.45	37.52	177.47
238	SER	8.17	116.72	63.85	62.72	177.16
239	LEU	8.46	124.3	58.28	41.85	178.05
240	LEU	8.31	119.39	58.5	41.75	178.57
241	LEU	8.59	117.06	58.34	41.71	178.33
242	THR	7.88	116.13	68.55	67.72	176.26
243	VAL	8.26	120.38	67.6	31.38	177.5
244	LEU	8.33	118.53	58.42	41.78	179.31
245	ALA	8.59	122.08	55.57	18.47	179.79
246	LEU	8.38	118.27	58.08	42.31	178.88
247	LEU	8.43	117.22	57.51	42.14	179.36
248	SER	8.11	113.55	61.13	63.81	175.32

249	HIS	7.97	119.17	57.06	30.42	---
250	ARG	7.79	120	58.82	29.82	---
251	ARG	---	---	58.86	29.79	178.05
252	THR	7.83	114.29	64.98	68.87	176.28
253	LEU	7.94	120.68	57.55	42.2	178.25
254	GLN	8.47	118.11	59.47	28.74	177.25
255	GLN	7.76	116.06	57.43	28.75	176.58
256	LYS	7.62	116.8	57.01	32.82	176.67
257	ILE	7.58	116.59	62.62	38.67	174.71
258	TRP	7.93	118.82	55.66	28.78	173.58