Supplementary Data

CW-EPR studies revealed different motional properties and oligomeric states of the integrin β_{1a} transmembrane domain in detergent micelles or liposomes

Lu Yu^{1,2,#}, Wei Wang^{1,#}, Shenglong Ling¹, Sanling Liu², Liang Xiao¹, Yanlong Xin¹, Chaohua Lai¹, Ying Xiong¹, Longhua Zhang¹, Changlin Tian^{1,2,*}

 ¹ Hefei National Laboratory of Microscale Physical Sciences, School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230027, P. R. China;
² High Magnetic Field Laboratory, Hefei institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, 230031, P. R. China.

- [#] These authors contributed equally in this work.
- * To whom correspondence should be addressed (email clian@ustc.edu.cn)



Supplementary Figure S1: SDS-PAGE analysis of integrin β_{1a} -TMC in detergent micelles and lipid liposomes. (a) Integrin β_{1a} -TMC samples in detergent micelles. Lane 4: integrin β_{1a} variant G747C; Lane 8: integrin β_{1a} variant L747C; Lane 13: integrin β_{1a} variant L749C; Lane 1-3, 5-7 and 10-12 were samples collected during protein purification (whole cell, supernatant, etc.); Lane 9: protein molecular size marker. (b) Integrin β_{1a} -TMC samples in liposomes. Lane 1: integrin β_{1a} variant L749C; Lane 3: protein molecular size marker. Lane 2 is not used because a small fraction of sample in Lane 1 was leaked into Lane 2.



Supplementary Figure S2: CD spectra of integrin β_{1a} -L749R1 in LDAO micelles collected at pH 8.0 and at a temperature of 298 K.

β1a	717	VVENPECPTGPDIIPIVAGVVAGIVLIGLALLLIWK	LL <mark>MI</mark> IHDR <mark>REFAKF</mark>	766
β1d	717	VVENPECPTGPDIIPIVAGVVAGIVLIGLALLLIWK	LL <mark>MI</mark> HDR <mark>REFAKF</mark>	766
β3	681	VVEEPECPKGPDILVVLLSVMGAILLIGLAALLIWK	LL <mark>IT</mark> IHDR <mark>K</mark> EFAKF	730
β1a	767	<mark>F</mark> K <mark>BKMNAKWDT</mark> GEN PIYK SAVTTVVNPKYEGK	798	
β1d	767	EK <mark>BKMNAKWDTQENPIYK</mark> SPINNFKNPNYGRKAGL	801	
β3	731	EE <mark>BRA</mark> R <mark>AKWDT</mark> ANNPLYKEAT <mark>ST</mark> FTNITYRGT	762	

Supplementary Figure S3: Sequence alignment between β_{1a} , β_{1d} and β_3 demonstrated high sequence homology in the transmembrane domain and the cytoplasmic domain.

Sequence homology analysis among integrin transmembrane and cytoplasmic domains of $\beta_{1a},\,\beta_{1d}$ and β_3



Supplementary Figure S4: Plot of the splitting of the outer hyperfine extrema $(2A'_{zz})$ of the EPR spectrum versus the residue number in micelles (black squares) and in liposomes (red dots). $2A'_{zz}$ was measured as shown in the inset.

Splitting of the outer hyperfine extrema (2A'zz) of EPR spectra

Interactions of the spin label with its environment (micelles or liposomes) lead to various degrees of immobilization¹. In the absence of motion (in frozen solution), the principal value of the hyperfine tensor (A_{zz}) is readily measured by splitting of the outer hyperfine extrema ($2A_{zz}$; Supplementary Figure S4, inset)². For the EPR spectra of spin labeled residues with well-resolved hyperfine extrema at ambient temperature (298K), changes in the overall splitting between the extrema ($2A'_{zz}$, Supplementary Figure S4) provide a measure of changes in mobility, where decreases or increases in $2A'_{zz}$ reflect an increase or decrease in mobility, respectively.

- 1 Kusnetzow, A. K., Altenbach, C. & Hubbell, W. L. Conformational states and dynamics of rhodopsin in micelles and bilayers. *Biochemistry* **45**, 5538-5550, (2006).
- 2 Gross, A., Columbus, L., Hideg, K., Altenbach, C. & Hubbell, W. L. Structure of the KcsA potassium channel from Streptomyces lividans: a site-directed spin labeling study of the second transmembrane segment. *Biochemistry* **38**, 10324-10335, (1999).