

Supporting Information for

Organic fluorescent probes for monitoring autophagy in living cells

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Table S1. The characters of fluorescent probes designed based on pH differences.

Probes	Monitoring process	Type	Staining targets	Design mechanisms	Advantages	Limitations	References	
Probe 1	Mitophagy	Monochromatic	Mitochondria	PET	Immobilized-mitochondria	External interferences	1	
Mtphagy Dye							2	
HQO		Ratio		Aldol conversion	1) Simultaneously staining mitochondria and autolysosomes 2) Avoiding external interferences	Lacking of immobilized mitochondria group	3	
NIR-HMA				1) REDOX reaction, 2) 1,6-rearrangement	1) NIR 2) specifically monitoring the mitophagy induced only by hypoxia		4	
CyT				Aldol conversion	1) NIR 2) Avoiding external interferences		5	
HXPI-Cl				Ratio	Aldol conversion	1) Immobilized-mitochondria 2) NIR 3) Quantitative acidification process	Anchoring mitochondria by chemical reactions	6
HcP-H						Avoiding external interference	Lacking of immobilized mitochondria group	7
Cy-NH ₂					ICT	1) NIR 2) Avoiding external interferences		8
Probe A, Probe B					TBET	2) Avoiding external interferences	9	
DALGreen , DAPGreen	Autophagy	monochromatic	Autophagosomes, autolysosomes	PET	3) Simultaneously staining autophagosomes and autolysosomes	External interferences	10	
RML			Lysosomes	Cyclization	High sensitivity to pH	1) External interferences 2) Unable to monitor whole process	11	
Lyso-MPCB		Ratio		Protonation	1) Two-photon (TP) 2) Avoiding external interferences	Unable to monitor whole process	12	

Cyto-Lyso			Lysosomes, cytoplasm	1) Aldol conversion, 2) cyclization	1) Dual-site controlled 2) Avoiding external interferences		13
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Table S2. The characters of fluorescent probes designed based on viscosity differences.

Probes	Monitoring process	Type	Staining targets	Design mechanisms	Advantages	Limitations	References
MitoAIE1, LysoAIE2	Mitophagy	monochromatic	Mitochondria (MitoAIE1), Lysosomes (LysoAIE2)	AIE	Excellent photostability	External interferences	14
NI-VIS			Mitochondria	TICT	NIR		15
BMVC			Mitochondria	Rotor	Immobilized-mitochondria		16
Lyso-NP	Autophagy		Lysosomes	Fluorescence lifetime	First probe to analyze lysosomal viscosity during autophagy based on fluorescence lifetime	Equipment expensive	17

Table S3. The characters of fluorescent probes designed based on polarity differences.

Probes	Monitoring process	Type	Staining targets	Design mechanisms	Advantages	Limitations	References
HXPI-P	Mitophagy	Ratio	Mitochondria	\	1) NIR 2) Avoiding external interferences	Lacking of immobilized mitochondria group	18
Lyso-OC	Autophagy	monochromatic	Lysosomes	solvatochromism	First TP fluorescent probe for monitoring autophagy via detecting lysosomal polarity.	1) External interferences 2) Unable to monitor whole process	19
Lyso-OSC					1) Large TP absorption cross-section 2) Deep tissue penetration depth		20

Table S4. The characters of fluorescent probes designed based on other methods.

Probes	Monitoring process	Type	Staining targets	Design mechanisms	Advantages	Limitations	References	
TPE-Py-NCS	Mitophagy	monochromatic	Mitochondria	AIE	Immobilized-mitochondria	1) External interferences 2) Damaging mitochondria	21	
MTG				\			22	
ECPI-12, IVPI-12				Long alkyl chain strategy	1) Immobilized-mitochondria 2) Reaction-free (low cytotoxicity)	External interferences	23	
AIE-Red AIE-Green			Autophagy	Mitochondria (AIE-Red) Lysosomes (AIE-Green)	AIE	Multicolor monitor	1) External interferences 2) Lacking of immobilized mitochondria group	24
Cy-B				Lysosomes	REDOX reaction	1) NIR 2) Recognizing H ₂ O ₂	1) External interferences 2) Unable to monitor whole process	25
DMOTY FMOTY 1 FMOTY 2					\	Recognizing G-quadruplex		26

References

1. M. H. Lee, N. Park, C. Yi, J. H. Han, J. H. Hong, K. P. Kim, D. H. Kang, J. L. Sessler, C. Kang, J. S. Kim, *J. Am. Chem. Soc.* 2014, **136**, 14136-42.
2. H. Iwashita, S. Torii, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, S. Shimizu, K. Okuma, *ACS Chem. Biol.* 2017, **12**, 2546-2551.
3. Y. Liu, J. Zhou, L. Wang, X. Hu, X. Liu, M. Liu, Z. Cao, D. Shangguan, W. Tan, *J. Am. Chem. Soc.* 2016, **138**, 12368-74.
4. Y. Liu, L. Teng, L. Chen, H. Ma, H. W. Liu, X. B. Zhang, *Chem. Sci.* 2018, **9**, 5347-5353.
5. L. Gui, Z. Yuan, H. Kassaye, J. Zheng, Y. Yao, F. Wang, Q. He, Y. Shen, L. Liang, H. Chen, *Chem. Commun.* 2018, **54**, 9675-9678.
6. X. Li, Y. Hu, X. Li, H. Ma, *Anal. Chem.* 2019, **91**, 11409-11416.
7. L. Q. Niu, J. Huang, Z. J. Yan, Y. H. Men, Y. Luo, X. M. Zhou, J. M. Wang, J. H. Wang, *Spectrochim. Acta. A* 2019, **207**, 123-131.
8. W. Tang, Y. Dai, B. Gu, M. Liu, Z. Yi, Z. Li, Z. Zhang, H. He, R. Zeng, *Analyst* 2020, **145**, 1427-1432.
9. S. Xia, J. Wang, Y. Zhang, N. Whisman, J. Bi, T. E. Steenwinkel, S. Wan, J. Medford, M. Tajiri, R. L. Luck, T. Werner, H. Liu, *J. Mater. Chem. B* 2020, **8**, 1603-1615.
10. H. Iwashita, H. T. Sakurai, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, K. Okuma, S. Shimizu, Y. Ueno, *FEBS Lett.* 2018, **592**, 559-567.
11. X. Wang, L. Fan, Y. Wang, C. Zhang, W. Liang, S. Shuang, C. Dong, *J. Mater. Chem. B* 2020, **8**, 1466-1471.
12. P. Ning, L. Hou, Y. Feng, G. Xu, Y. Bai, H. Yu, X. Meng, *Chem. Commun.* 2019, **55**, 1782-1785.
13. M. Tian, C. Liu, B. Dong, Y. Zuo, W. Lin, *Chem. Commun.* 2019, **55**, 10440-10443.
14. W. Chen, C. Gao, X. Liu, F. Liu, F. Wang, L. J. Tang, J. H. Jiang, *Anal. Chem.* 2018, **90**, 8736-8741.
15. Y. Zhang, Z. Li, W. Hu, Z. Liu, *Anal. Chem.* 2019, **91**, 10302-10309.
16. Z. Zou, Q. Yan, S. Ai, P. Qi, H. Yang, Y. Zhang, Z. Qing, L. Zhang, F. Feng, R. Yang, *Anal. Chem.* 2019, **91**, 8574-8581.
17. L. Hou, P. Ning, Y. Feng, Y. Ding, L. Bai, L. Li, H. Yu, X. Meng, *Anal. Chem.* 2018, **90**, 7122-7126.
18. X. Li, X. Li, H. Ma, *Chem. Sci.* 2020, **11**, 1617-1622.

19. J. Jiang, X. Tian, C. Xu, S. Wang, Y. Feng, M. Chen, H. Yu, M. Zhu, X. Meng, *Chem. Commun.* 2017, **53**, 3645-3648.
20. C. Jiang, L. Li, J. Jiang, L. Hou, G. Fang, Y. Haizhu, X. Meng, *Chinese Chem. Lett.* 2020, **31**, 447-450.
21. W. Zhang, R. T. Kwok, Y. Chen, S. Chen, E. Zhao, C. Y. Yu, J. W. Lam, Q. Zheng, B. Z. Tang, *Chem. Commun.* 2015, **51**, 9022-5.
22. S. Rodriguez-Enriquez, I. Kim, R. T. Currin, J. J. Lemasters, *Autophagy* 2006, **2**, 39-46.
23. R. Zhang, G. Niu, X. Li, L. Guo, H. Zhang, R. Yang, Y. Chen, X. Yu, B. Z. Tang, *Chem. Sci.* 2019, **10**, 1994-2000.
24. F. Hu, X. Cai, P. N. Manghnani, Kenry, W. Wu, B. Liu, *Chem. Sci.* 2018, **9**, 2756-2761.
25. H. L. Feng Xu, Qichao Yao, Jiangli Fan, Jingyun Wang, Xiaojun Peng, *J. Mater. Chem. B* 2016, **4**, 7363.
26. H. Chen, H. Sun, S. Zhang, W. Yan, Q. Li, A. Guan, J. Xiang, M. Liu, Y. Tang, *Chem. Commun.* 2019, **55**, 5060-5063.