Biothiol Detection by Fluorescent Probes Incorporating Nanogels

Xudong Guo[1], Hua Yuan[1], Guoqiang Yang[1]

[1] *Beijing National Laboratory for Molecular Sciences, Key Laboratory of Photochemistry, Institute of Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100190, China.*

E-mail: gqyang@iccas.ac.cn

Biothiols, including cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), possess several vital biological functions. An anomalous level of biothiols in organisms is directly correlated to numerous diseases. Hence, it is essential to develop rapid and specific indicators of biothiols *in vivo* considering their potential as powerful tools for diagnosis and triage of related diseases in initial phases. A variety of hydrophilic fluorescent indicators synthesized by covalent methods have been reported[1], which exhibited decent detection performance in biology system but bore relatively prohibitive cost owing to the difficulty when hydrophilic moieties were covalently introduced. Herein, we proposed a different strategy by employing nanogels as carriers of water-insoluble probes for biothiols detection in living cells.

Two probes, DMDP-M[2] and HTBNM[3], were synthesized. The fluorescence of DMDP-M and HTBNM was both quenched by maleimide groups *via* photo-induced electron transfer (PET) mechanism. Nevertheless, the luminescence would recover once the conjugated structure of maleimide was broken by a Michael addition of thiol. DMDP-M and HTBNM, therefore, could detect thiols as "turn-on" probes.

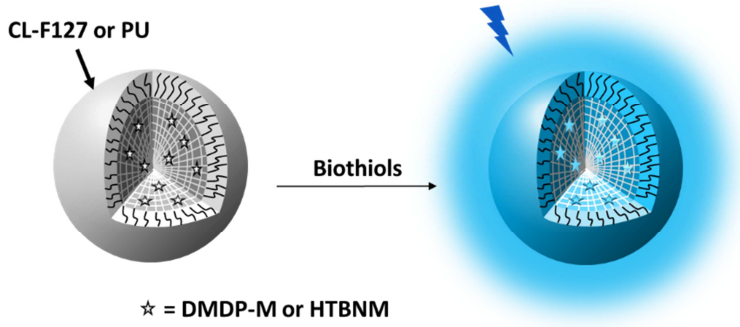

Figure 1 Mechanism of DMDP-M/CL-F127 and HTBNM/PU for selective detection of biothiols

For fluorescence microscopy imaging in living cells, two nanogels, CL-F127 (based on cross-linked Pluronic® F127) and PU (based on polyurethane) were applied as carriers of the water-insoluble probes, respectively, to form DMDP-M/CL-F127 system and HTBNM/PU system (Figure 1). In phosphate buffer (PBS, pH = 7.4), DMDP-M/CL-F127 and HTBNM/PU demonstrated dramatic emission enhancement (~25-fold and ~200-fold, respectively) upon biothiols (either Cys or Hcy) addition. Both DMDP-M/CL-F127 and HTBNM/PU could detect Cys and Hcy *in vitro* with high sensitivity, effectivity and specificity. Moreover, HTBNM/PU showed high specificity to Cys/Hcy over GSH owing to the stronger steric effect

and lipophilicity of PU than CL-F127. Both systems presented no notable effects on cell viability, indicating their good biocompatibility. After introduced in cultured NIH/3T3 fibroblasts, they could both act as *in vivo* biothiol-imaging agents under a confocal fluorescence microscopy. DMDP-M/CL-F127 could also operate under a two-photon fluorescence microscopy because of its relatively large two-photon absorption cross section. Furthermore, HTBNM/PU was applied to detection of endogenous Cys/Hcy caused by reactive oxygen species.

Funding: National Basic Research Program (2013CB834505 and 2013CB834703). National Natural Science Foundation of China (Grant No. 21627802, 91623105, 21373240 and 21233011)

References:

[1] H. Chen, Y. Tang, W. Lin, *TrAC, Trends Anal. Chem.*, **2016**, 76, 166

[2] X. Guo, X. Zhang, S. Wang, S. Li, R. Hu, Y. Li, G. Yang, *Anal. Chim. Acta*, **2015**, 869, 81

[3] X. Peng, H. Yuan, J. Xu, F. Lu, L. Wang, X. Guo, S. Wang, S. Li, Y. Li, G. Yang, *RSC Adv.*, **2017**, 7, 5549