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主 論 文 の 要 旨

論文題目 Quantum Dot Peptide Nanobioassembly for Biosensing
(バイオセンシングに向けた量子ドット・ペプチドナノ会合体の開発)

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論 文 内 容 の 要 旨

This thesis proposes the biosensing application of a quantum dot peptide nanobioassembly. The objective in the research consist of three parts 1) bioconjugation of quantum dots (QDs) with non fluorescent black hole quencher-1(BHQ-1) molecule to construct a nanobioassembly and the formation of an array of large number nanobioassembly on a mesoporous silica nanoparticle surface (chapter 2-3) 2) Establishment of Förster resonance energy transfer (FRET) signal transduction between QD donor and BHQ-1 acceptor by bridging the two through a matrix metalloproteinase-2 (MMP-2) targeting short peptide.(chapter2-3) 3) Sensing the MMP-2 presence in solution as well as cancer cells extra cellular matrix (ECM). (chapter 4).

Chapter 2 describes the bioconjugation of CdSe/ZnS Quantum dot (QDs) and Black Hole Quencher-1 (BHQ-1) through bridging MMP-2 targeting short peptide GPLG↓VRGK and the resultant formation of QD-peptide-BHQ-1 nanobioassembly. Result and discussion describes the steady state and time resolved photoluminescence measurement of QDs and QD-pep-BHQ-1 nanobioassembly. The FRET efficiency as well as the quenching efficiency of the QD-pep-BHQ-1 system has estimated. A signal transduction channel between QDs and the peptide conjugate is established primarily

through energy transfer between the QDs and the peptide conjugate, here the QD functioning as energy transfer donor and the peptide conjugated with BHQ-1 on its surface act as acceptor.

Chapter 3 describes the conjugation of QD-pep-BHQ-1 nanobioassembly on a mesoporous silica nanoparticle scaffold and hence the formation of MSN- $\{QD-(pep-BHQ-1)_n\}_N$ nanobioassembly. Transmission Electron Microscopic images (TEM) of MSN as well as MSN- $\{QD-(pep-BHQ-1)_n\}_N$ measured. The morphology confirms the formation of MSN- $\{QD-(pep-BHQ-1)_n\}_N$ nanoassembly. Result and Discussion- described the steady state photoluminescence measurement describes the spectral overlap integral calculation and the dipole orientation factor K^2 in MSN- $\{QD-(pep-BHQ-1)_n\}_N$ nanoassembly. The distance between the QD and BHQ-1 molecule has approximately calculated by modeling the streptavidin-biotin-GPLGVRGK peptide in the extended position on the QD surface using UCSF Chimera software. The static and dynamic quenching factors have explained in the Stern-Volmer analysis part. Both the static and dynamic factors on luminescence quenching of QDs at the initial steps of titration experiments were explained. The dynamics of the slope at each data point has also plotted in order to make the data points statistically distinguishable. The slope vs concentration of acceptors shows maxima around 80 nM biotin-pep-BHQ-1 acceptor molecules. The time resolved experiment describes quantitative measurement of the resonance energy transfer mediated photoluminescence quenching in the MSN- $\{QD-(pep-BHQ-1)_n\}_N$.

Chapter 4 describes the detection of MMP-2 in solution as well as extra cellular matrix of H1299 cancer cells. The control experiments were performed to check the pro-MMP-2 inactivity on QD-pep-BHQ-1 nano assembly as well as the temperature sensitivity of QD-pep-BHQ-1 nanoassembly. Interestingly the QD-pep-BHQ-1 detected activated MMP-2 at the limit of detection of 1 ng (15 pM). Further studies revealed the sensing of MMP-2 at the extra cellular matrix (ECM) of H1299 cancer cells. In conclusion, the QD-pep-BHQ-1 has successfully sensed MMP-2 at a limit of detection of 1ng/mL by showing a clear luminescence recovery as well as MMP-2 sensed and imaged successfully at the extra cellular matrix (ECM) of H1299 cancer cells through high speed multiphoton confocal microscope.

Finally chapter 5 describes the conclusion and future perspectives.