様式３

論　文　内　容　の　要　旨

氏　　名　（　　粟津　利邦　　）	
論文題名	A STUDY ON ETERNAL SINGLE-MOLECULE TRACKING UNAFFECTED BY PHOTOBLEACHING: LIMITS OF EXISTING FLUORESCENT PROBES AND EVALUATION AND OPTIMIZATION OF NOVEL FLUORESCENT PROBES （永遠にフォトブリーチングの影響を受けない単一分子追跡に関する研究： 既存の蛍光プローブの限界と新規蛍光プローブの評価と最適化）

論文内容の要旨

Fluorescence microscopy has been widely used in biology, especially after the introduction of genetically encodable fluorescent probes such as green fluorescent protein (GFP). Fluorescent probes, however, are bleached by illumination light during imaging, which has posed a serious limitation on the application. For example, a single molecule of GFP or other fluorescent probe molecules can be imaged by using total internal reflection fluorescence microscopy (TIRF), but each molecule can be observed only for a short time due to photobleaching. Many efforts have been made to prevent or delay photobleaching, but single-fluorescent-molecule imaging tracking for 10 minutes or longer is still challenging. In this study, I tested ″StayGold,″ a recently developed green fluorescent protein that has garnered considerable attention for its exceptional photostability, the highest among fluorescent proteins. Nevertheless, tracking single-molecule fluorescence imaging for periods exceeding 10 minutes proved to be difficult.

Therefore, in this doctoral thesis, I propose a new approach to break this barrier. The proposed system has two components: a receptor protein and its fluorogenic ligand. The ligand is designed to bind reversible to the receptor and to turn on fluorescence upon binding. If ligand exchange is fast enough, the target protein can be labeled repeatedly by fresh dye and observed indefinitely. Since rapid binding and dissociation kinetics are required to materialize this idea, I measured the kinetics by single molecule imaging. I found that one system showed rapid binding and dissociation kinetics with kon = 1.8 ± 0.4μM-1s-1 and koff = 10.2 ± 1.7 s-1, respectively. These values imply that the mean binding time of the ligand is about 100 ms, and a new ligand re-binds in about 50 ms at the ligand concentration of 10 micro M. Namely, a fresh dye will be replenished faster than video rate.

As a proof of this concept, I have expressed this receptor protein as a fusion protein to kinesin, a microtubule motor protein. The tagged kinesin molecules were introduced into the flow cell where microtubules were immobilized on its cover glass surface. At lower concentration of ligand, tagged kinesin molecules showed blinking fluorescent signals on the microtubules under our custom-built TIRF microscope. This blinking behavior was consistent with the binding and dissociation kinetics. With 10 micro M ligand, blinking was much faster the frame rate (100 ms/frame), and each kinesin molecule appeared as a spot continuously for more than 1,000,000 frames (24 hours).

These results do not only demonstrate that each single molecule can be tracked indefinitely. The receptor protein can be genetically expressed as a fusion protein with the target protein, just like GFP. The ligand is membrane-permeable and can stain intracellular proteins. Thus, the target protein inside the cell can be labeled fluorescently and observed indefinitely without photobleaching. Therefore, I believe that this system has a wide range of application in cell biology, and will push the fluorescence live imaging to the next stage.

様式 7

論文審査の結果の要旨及び担当者

氏　名（　粟津　利邦　）		
	（職）	氏　名
論文審査担当者	主査　招へい教授	岡田　康志
	副査　教授	上田　昌宏
	副査　教授	石井　優
	副査　教授	近藤　滋

論文審査の結果の要旨

蛍光一分子イメージング法は、生体分子の動態を一分子レベルで計測することができる優れた手法である。しかし、その計測時間は蛍光色素の褪色に制限され、高々数分程度に留まっていた。本論文では、高速に蛍光色素分子を交換する新規蛍光標識法を開発し、蛍光色素の褪色による影響を無視できる蛍光一分子イメージング法を実現し、同一分子の24時間連続イメージングによりその有効性を実証した。さらに、本標識法をドナーとする一分子FRET計測へと発展させることで、酵素タンパク質分子の遅い構造変化のキネティクスの直接計測に成功した。本標識法は、蛍光一分子イメージング法の最大の制約の一つを解決し、蛍光一分子イメージング法の適用範囲を大きく拡げる画期的なものである。このため、博士の学位を授与するに値するものと認める。なお、チェックツール“iThenticate”を使用し、剽窃、引用漏れ、二重投稿等のチェックを終えていることを申し添えます