Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Photo-control of kinesin-microtubule motility using caged peptides derived from the kinesin C-terminus domain

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Movies

The rhodamine-labeled microtubules before and after UV-irradiation in the (a) absence (file name: SI-a.mpg) and (b) presence of NBs922/925 (file name: SI-b.mpg). The movies are quad speed of real time. UV-irradiation: 30 s, [NBs922/925] = 10 μM, temperature: 27ºC.

Materials and methods

Chemicals

5-(and 6)-carboxytetramethylrhodamine succinimidyl ester and paclitaxel (taxol) were obtained from Invitrogen and Sigma, respectively. All other chemicals were of the highest commercial grade and were used without further purification.

Kinesin and Microtubule

A kinesin construct consisting of the truncated human conventional kinesin heavy chain fused to the C-terminal His-tag was expressed in *Escherichia coli* and purified using a Ni-NTA column according to standard methods[1].

Tubulin was purified from porcine brain by polymerization and depolymerization cycles, followed by phosphocellulose chromatography as described in previous reports[2,3]. The obtained tubulin was fluorescently labeled with 5-(and 6)-carboxytetramethylrhodamine succinimidyl ester according to standard methods (Hyman et al., 1991). The rhodamine-labeled tubulin was polymerized for 40 min at 37 °C in polymerization buffer (PME (0.1 M pipes, 1 mM $MgCl_2$, 1 mM EGTA) containing 1mM GTP and 10% glycerol) into microtubules. After the polymerization, the microtubules were immediately stabilized in 20 μM taxol.

In vitro motility assay

The motility assays were performed using an Olympus IX71 inverted research microscope with a reflected light fluorescence IX2-RFAW (xenon lamp: U-LH75XEAPO, 14V, 75W) following standard methods[4]. The following typical procedure was employed: Kinesin buffer consisted of 10 mM Tris-acetate (pH 7.5) containing 50 mM potassium acetate, 2.5 mM EGTA, 4 mM magnesium sulfate, 0.5% β-mercaptoethanol and 0.5 mg/mL casein. The microtubule buffer and the assay buffer contained 20 μM taxol and 20 μM taxol, anti-fade reagents (3 mg/mL glucose, 20 μg/mL catalase, 0.1 mg/mL glucose oxidase), 1 mM ATP, and an inhibitory peptide plus the kinesin buffer, respectively. The kinesin solution (ca. 75 μg/mL) was perfused into a flow chamber constructed from a glass slide, a coverslip and double-sided adhesive tapes, and allowed to be absorbed onto the glass surfaces for 2 min. Subsequently, the flow chamber was rinsed with a buffer to remove any excess kinesin solution. The rhodamine-labeled microtubule solution (25-50 μg/mL) was perfused into the flow chamber and incubated for 2 min. After incubation, the assay buffer was purfused into the flow chamber, and the motility of microtubules was observed. The velocity of the microtubules was obtained from the average of over 30 measurements. The abnormal microtubules, which were partially attached or detached, waved, were excluded from the measurement.

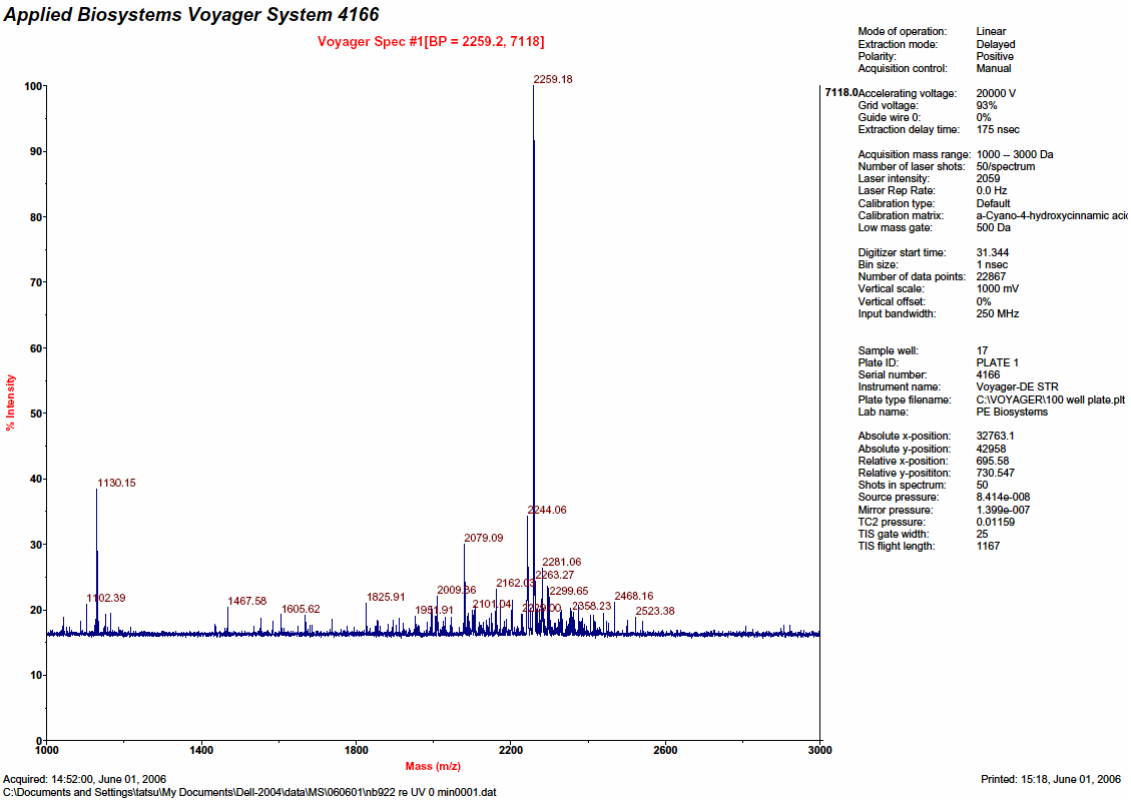
Photolysis of the caged peptides

The solution of the caged peptides (2mg/mL in water) was irradiated with UV-light of a Xenon-mercury lamp (Hamamatsu photonics, Japan, ca 20mW/cm^2 at 360 nm) for 10 min. Before and after UV-irradiation, the peptide solutions were characterized by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS): NB922 (before UV), calcd. 2257.50, obsd. ([M+H]$^+$) 2259.18; uncaged NB922 (after UV), calcd. 2078.37, obsd. ([M+H]$^+$) 2080.61; NB925 (before UV), calcd. 2229.49, obsd. ([M+H]$^+$) 2230.93; uncaged NB925 (after UV), calcd. 2050.36, obsd. ([M+H]$^+$) 2052.24; NBs922/925 (before UV), calcd. 2408.62, obsd. ([M+H]$^+$) 2409.63; NBs922/925 (after UV), calcd. 2050.36, obsd. ([M+H]$^+$) 2052.49. For reference: WT, calcd. 2078.37, obsd. ([M+H]$^+$) 2080.02; R925K, calcd. 2050.36, obsd. ([M+H]$^+$) 2052.10. See also MALDI-TOF-MS spectra (page 3–5).

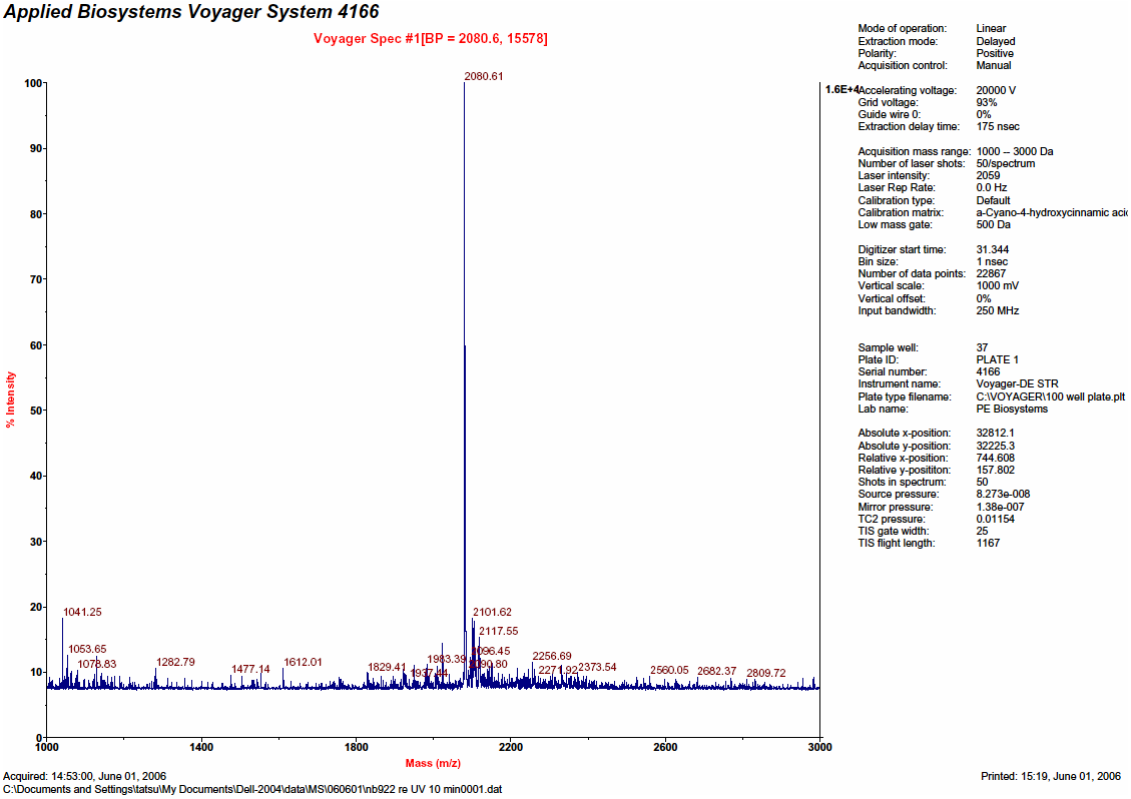
References

1) G. Woehlke, A. K. Ruby, C. L. Hart, B. Ly, N. Hom-Booher, R. D. Vale, *Cell*, 1997, **90**, 207.
2) M. L. Shelanski, F. Gaskin, C. R. Cantor, *Proc. Natl. Acad. Sci. U.S.A.,* 1973, **70**, 765.
3) M. D. Weingarten, A. H. Lockwood, S. Y. Hwo, M. W. Kirschner, *Proc. Natl. Acad. Sci. U.S.A.*, 1975, **72**, 1858.
4) R. D. Vale, T. S. Reese, M. P. Sheetz, *Cell,* 1985, **42**, 39.

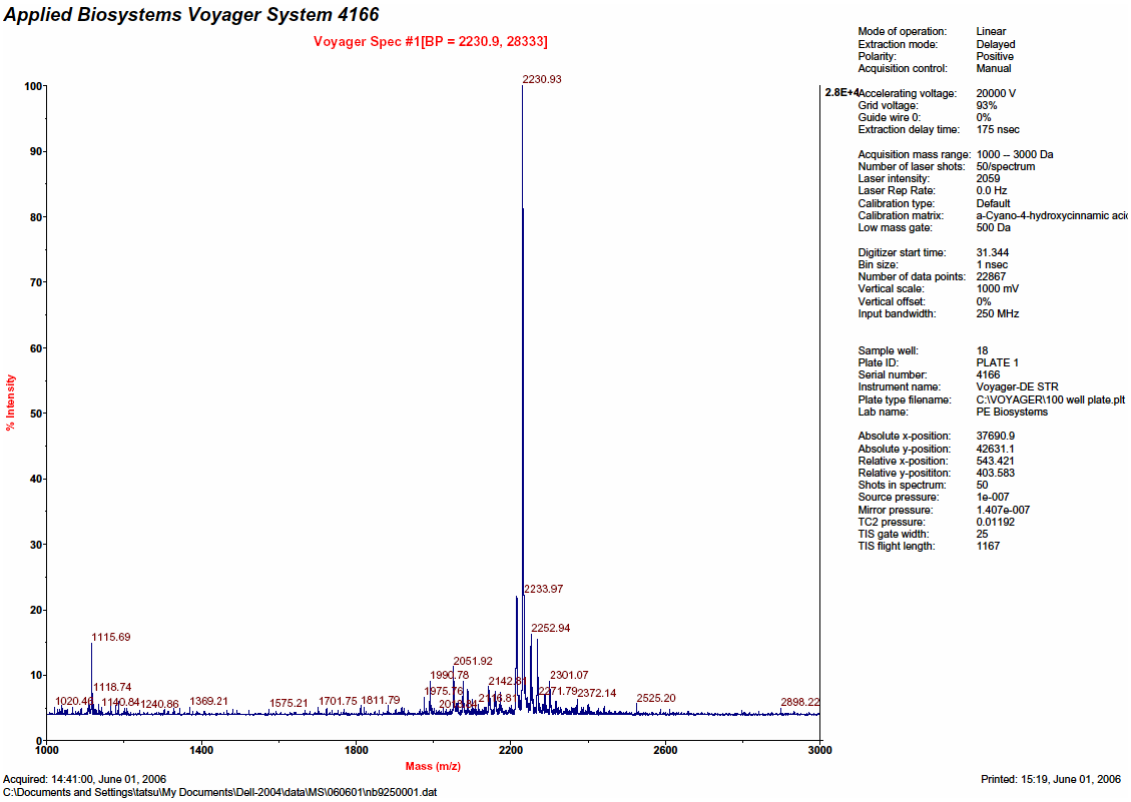
NB922 (before UV)



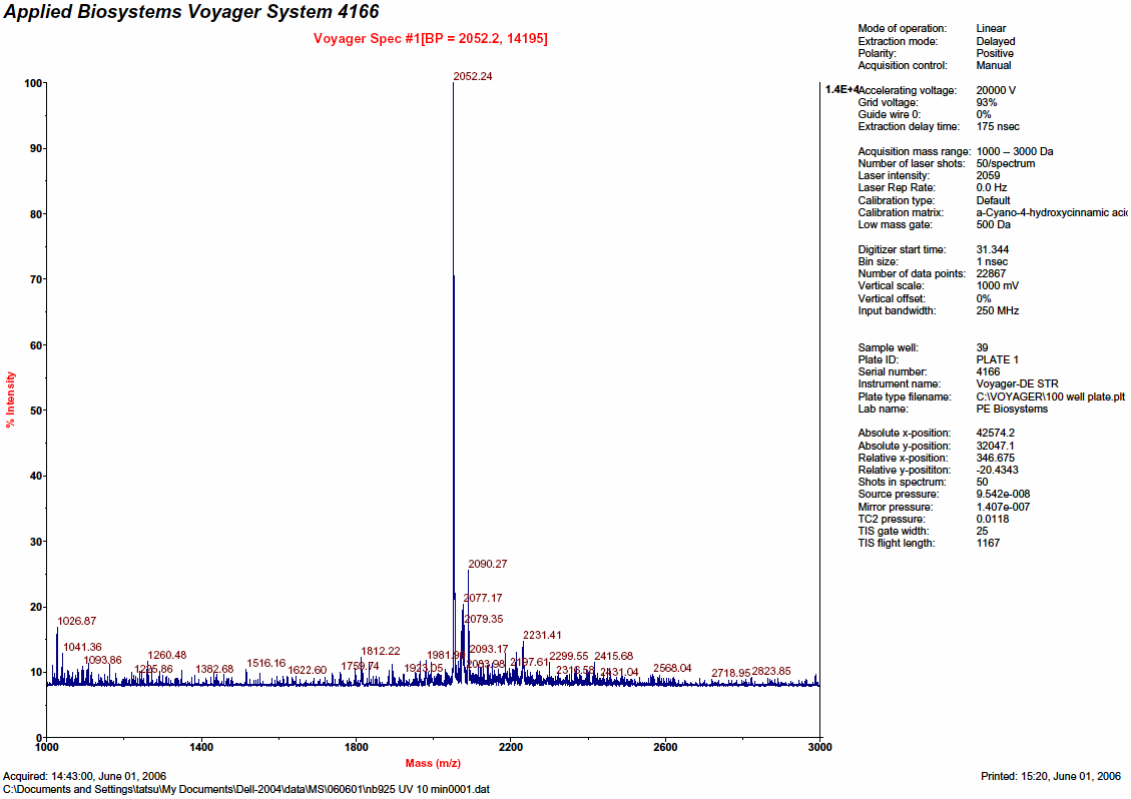
NB922 (after UV)



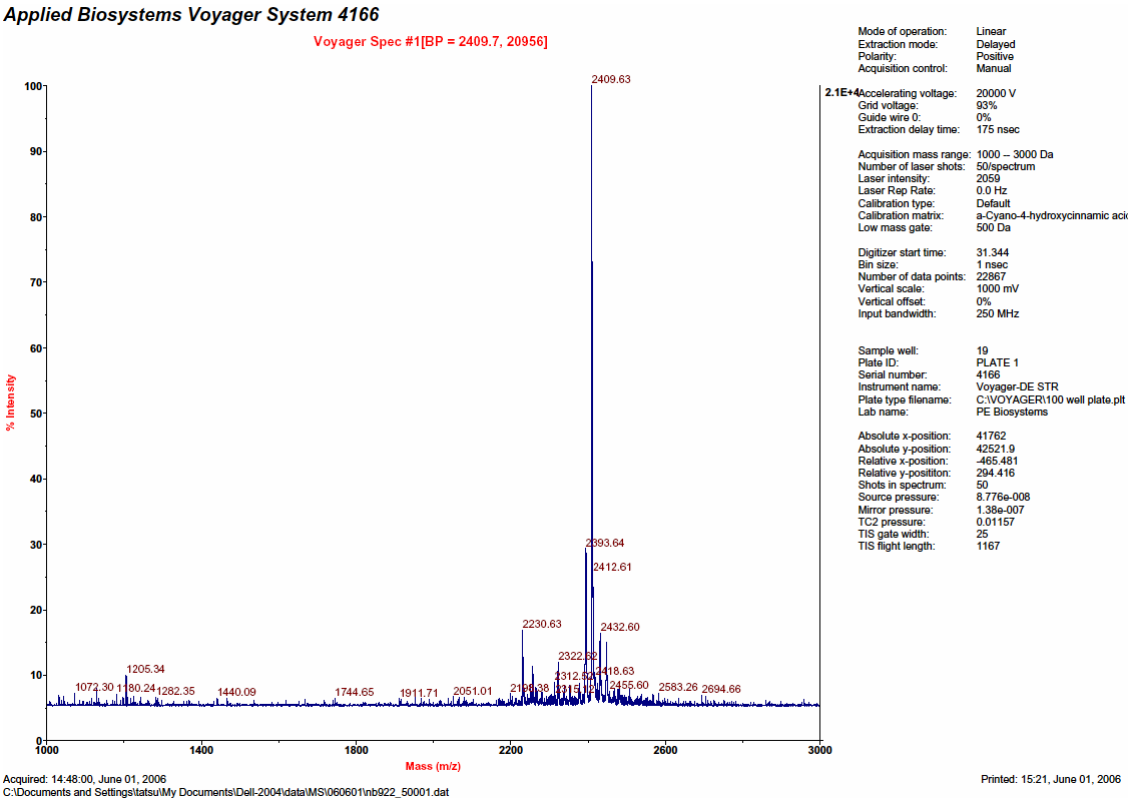
NB925 (before UV)



NB925 (after UV)



NB922/925 (before UV)



NB922/925 (after UV)