



Title	Structure-property relationships of photoresponsive inhibitors of the kinesin motor
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Supporting Information

Structure–property relationships of photoresponsive inhibitors of the kinesin motor

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1. Mass spectra of azo-peptides

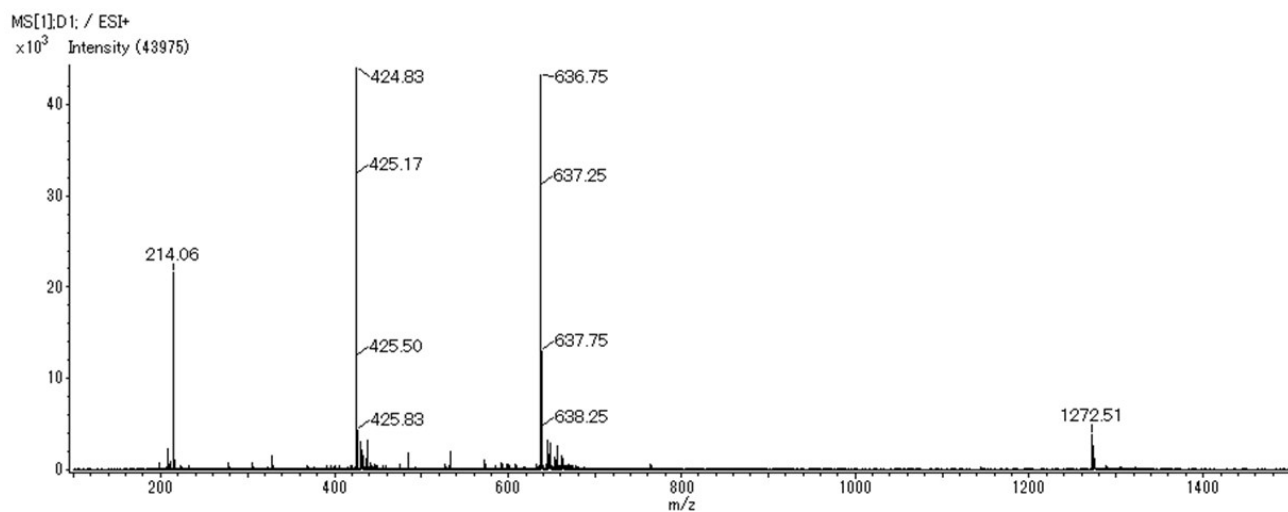


Figure S1. ESI⁺ mass spectrum of azo-peptide **2**: $m/z = 1272.51$ [M+H]⁺ (calculated mass for the most abundant isotope: 1272.65)

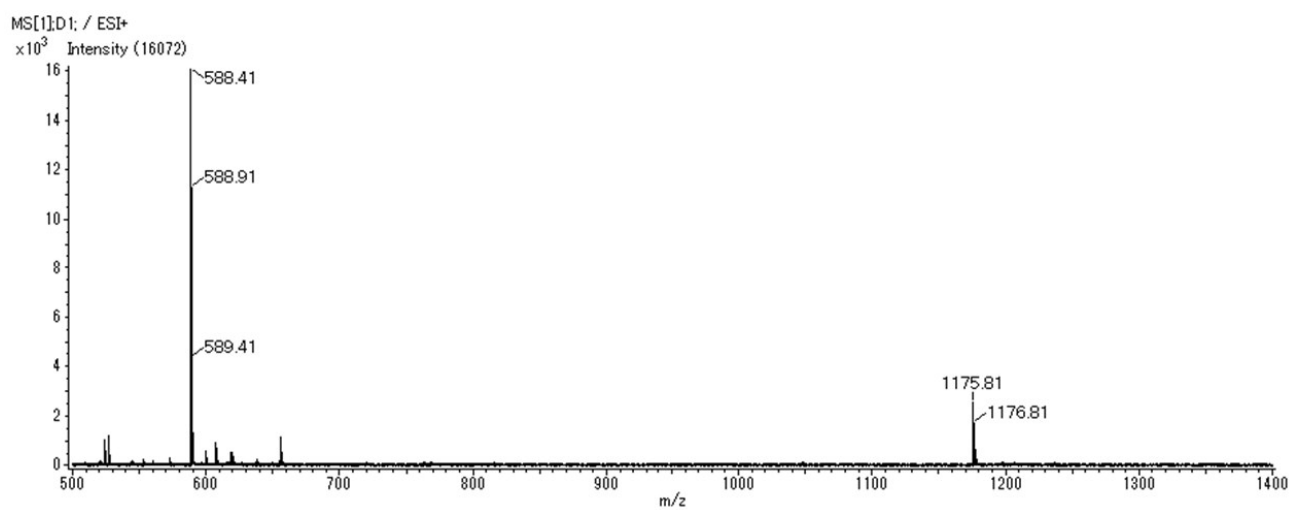


Figure S2. ESI⁺ mass spectrum of azo-peptide **3**: $m/z = 1175.81$ [M+H]⁺ (calculated mass for the most abundant isotope: 1175.6)

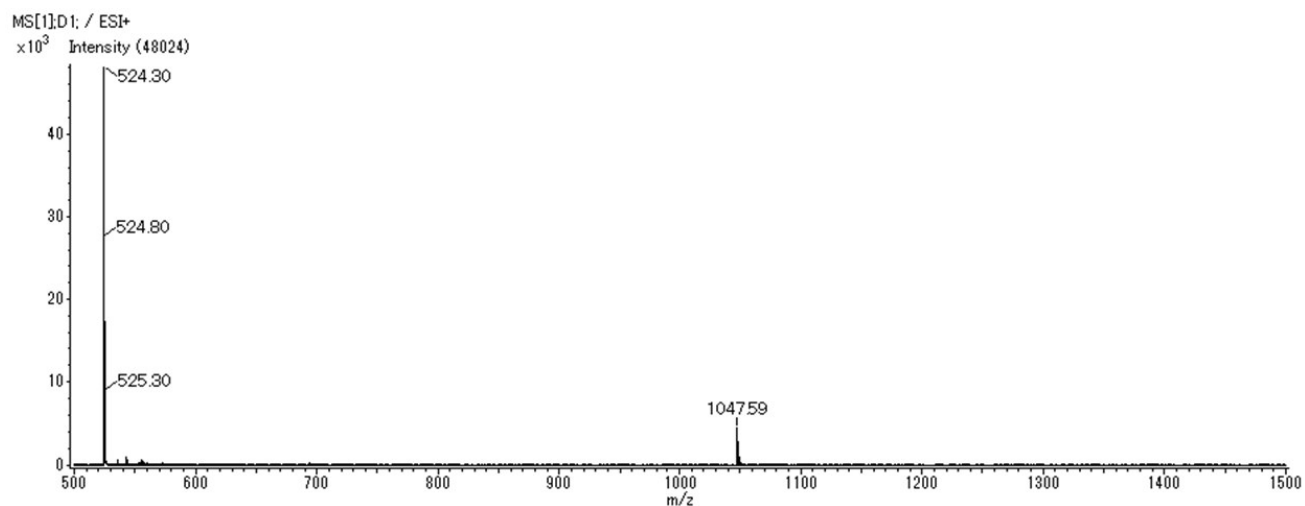


Figure S3. ESI⁺ mass spectrum of azo-peptide **4**: $m/z = 1047.59$ [M+H]⁺ (calculated mass for the most abundant isotope: 1047.50)

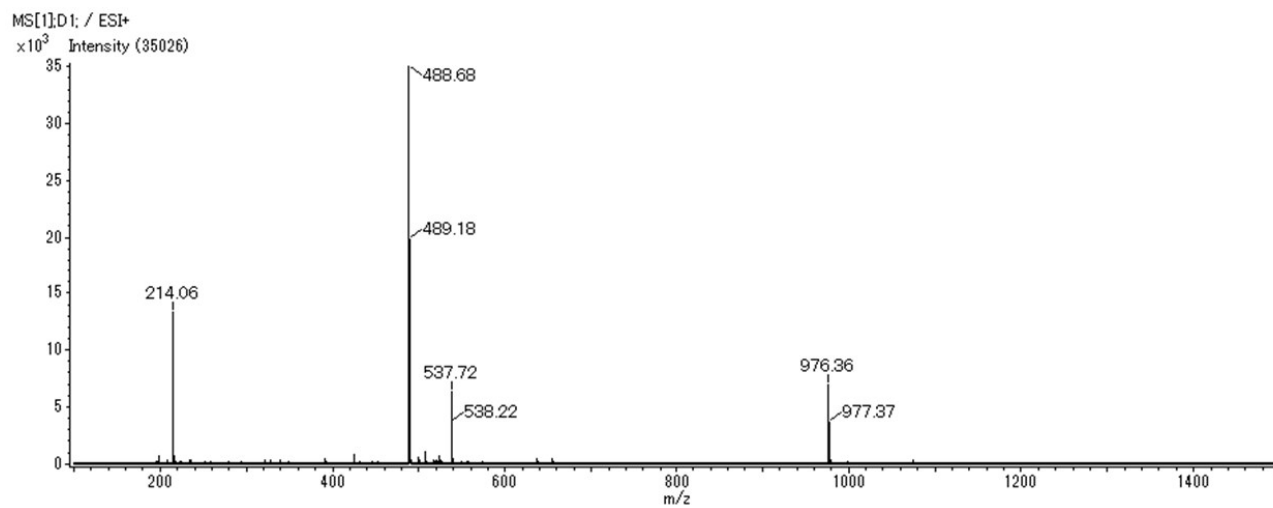


Figure S4. ESI⁺ mass spectrum of azo-peptide **5**: $m/z = 976.36$ [M+H]⁺ (calculated mass for the most abundant isotope: 976.47)

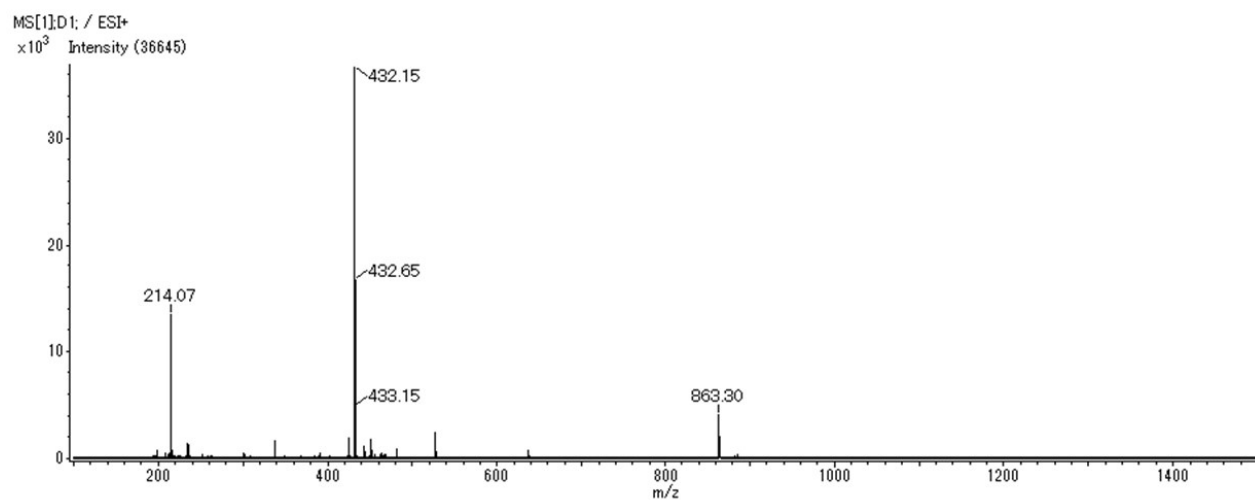


Figure S5. ESI⁺ mass spectrum of azo-peptide **6**: $m/z = 863.30$ [M+H]⁺ (calculated mass for the most abundant isotope: 863.38)

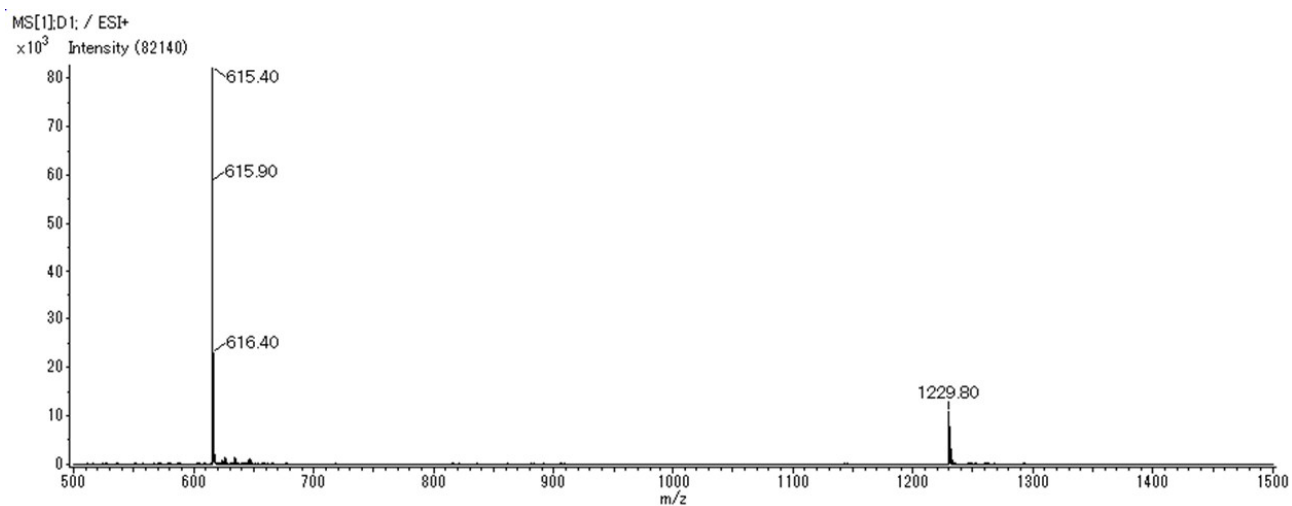


Figure S6. ESI⁺ mass spectrum of azo-peptide **7**: $m/z = 1229.80$ [M+H]⁺ (calculated mass for the most abundant isotope: 1229.64)

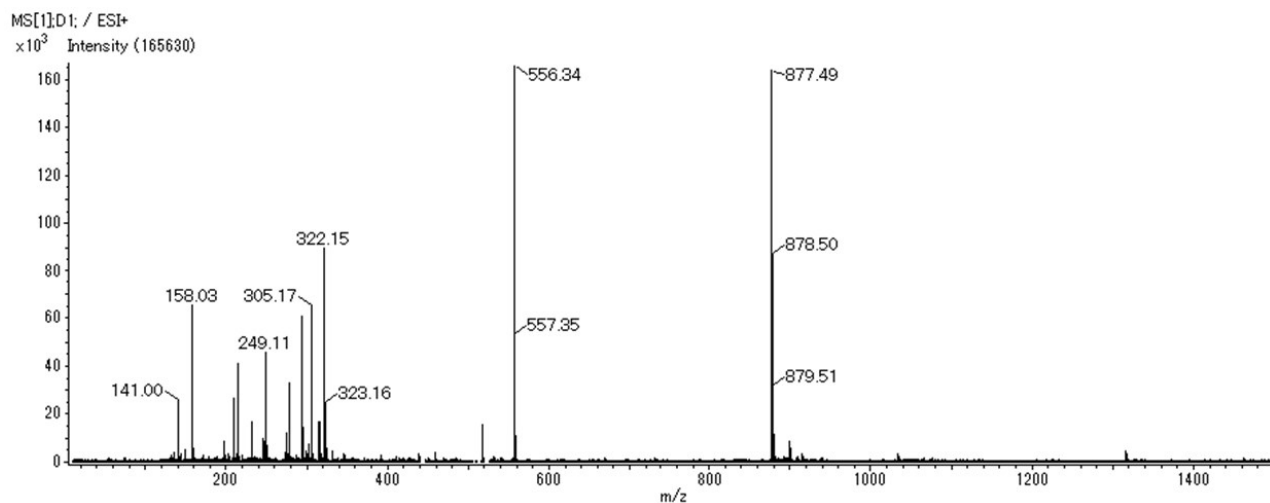


Figure S7. ESI⁺ mass spectrum of azo-peptide **8**: $m/z = 877.49$ $[M+H]^+$ (calculated mass for the most abundant isotope: 877.49), $m/z = 556.34$ $[\text{Pro-Lys-Ala-Ile-Gln}+H]^+$ and 322.15 $[\text{Azo-Ile}]^+$ (calculated mass for the fragments of the parent azo-peptide **8** cleaved at the amide linkage between the Ile and Pro : 556.34 and 322.15 respectively)

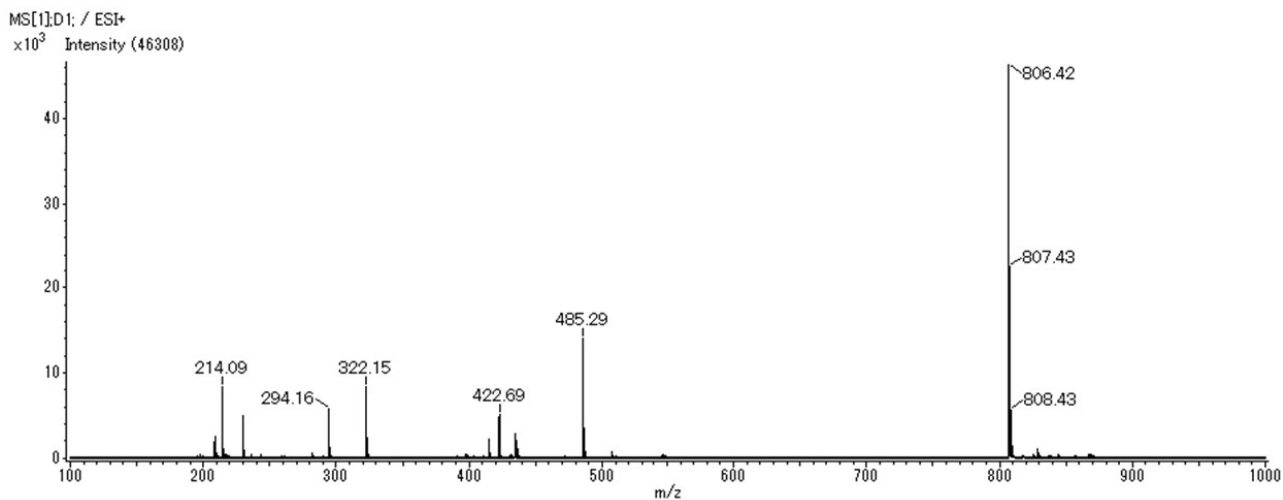


Figure S8. ESI⁺ mass spectrum of azo-peptide **9**: $m/z = 806.42$ $[M+H]^+$ (calculated mass for the most abundant isotope: 806.45), $m/z = 485.29$ $[\text{Pro-Lys-Ala-Ile-Gly}+H]^+$ and 322.15 $[\text{Azo-Ile}]^+$ (calculated mass for the fragments of the parent azo-peptide **9** cleaved at the amide linkage between the Ile and Pro : 485.30 and 322.15 respectively), $m/z = 422.69$ $[M+H+K]^{2+}$ (calculated mass for the most abundant isotope: 422.77).

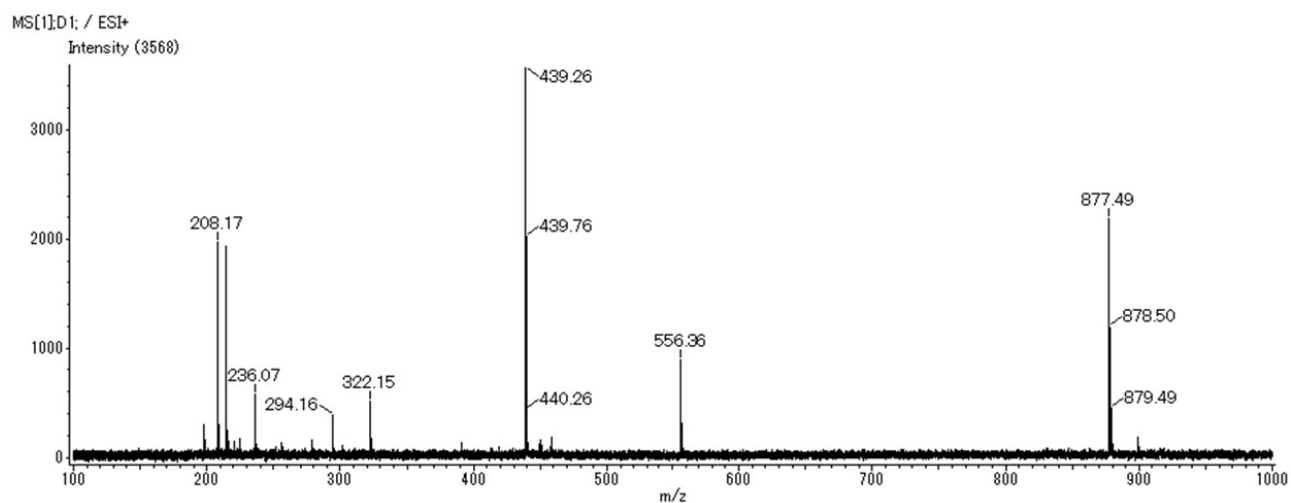


Figure S9. ESI⁺ mass spectrum of azo-peptide **10**: $m/z = 877.49$ $[M+H]^+$ (calculated mass for the most abundant isotope: 877.52)

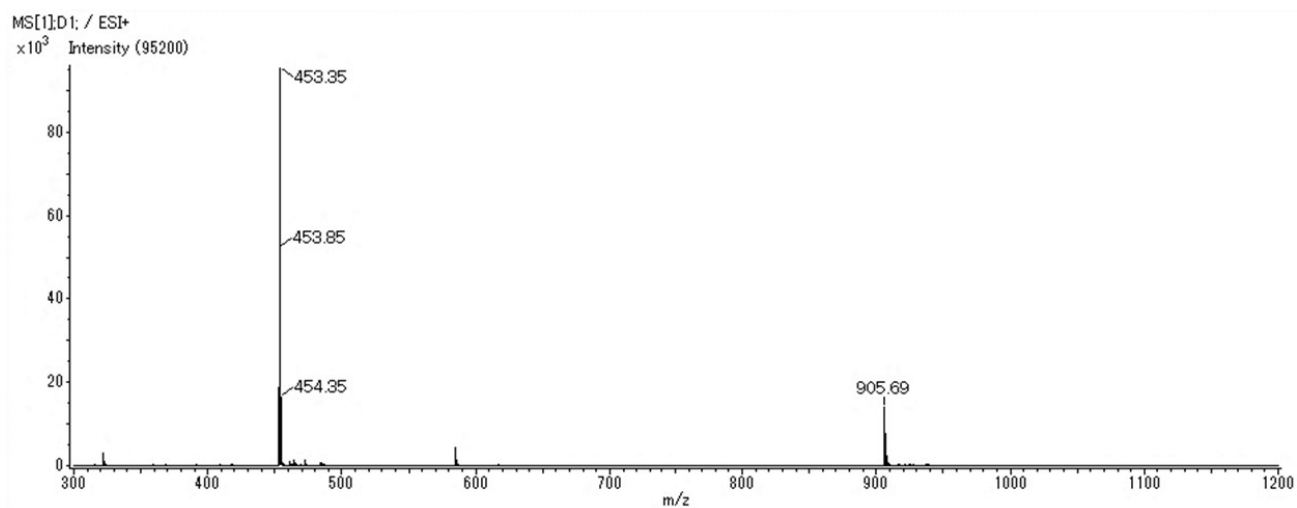


Figure S10. ESI⁺ mass spectrum of azo-peptide **11**: $m/z = 905.69$ $[M+H]^+$ (calculated mass for the most abundant isotope: 905.53)

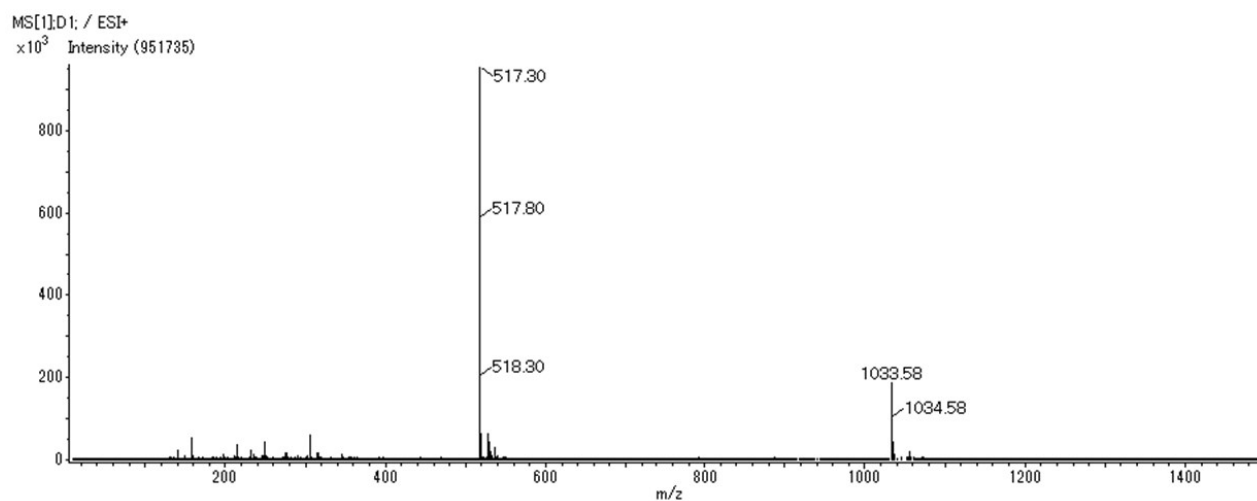


Figure S11. ESI⁺ mass spectrum of azo-peptide **12**: $m/z = 1033.58$ $[M+H]^+$ (calculated mass for the most abundant isotope: 1033.59)

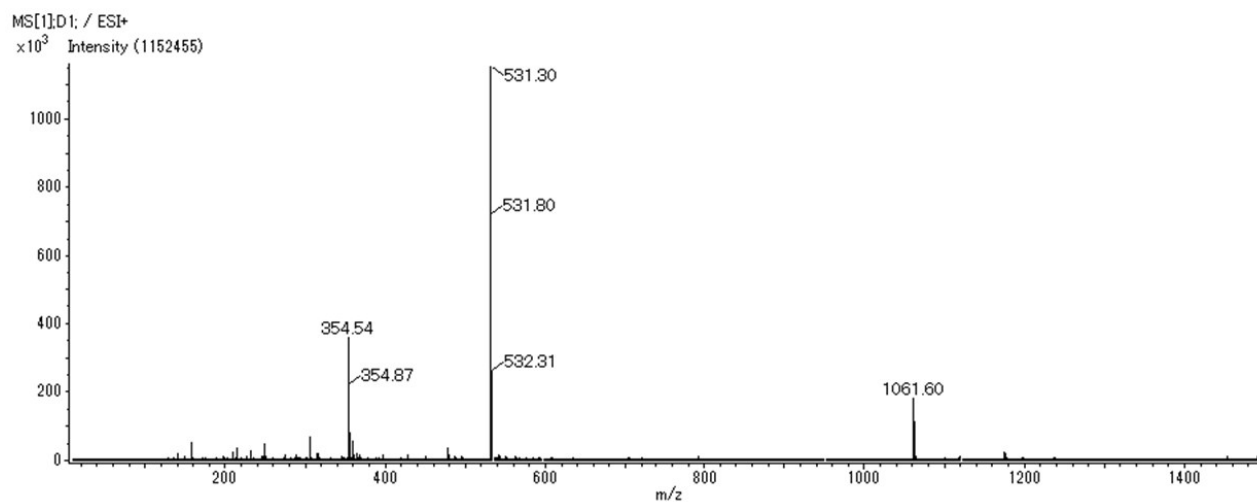


Figure S12. ESI⁺ mass spectrum of azo-peptide **13**: $m/z = 1061.60$ $[M+H]^+$ (calculated mass for the most abundant isotope: 1061.63)

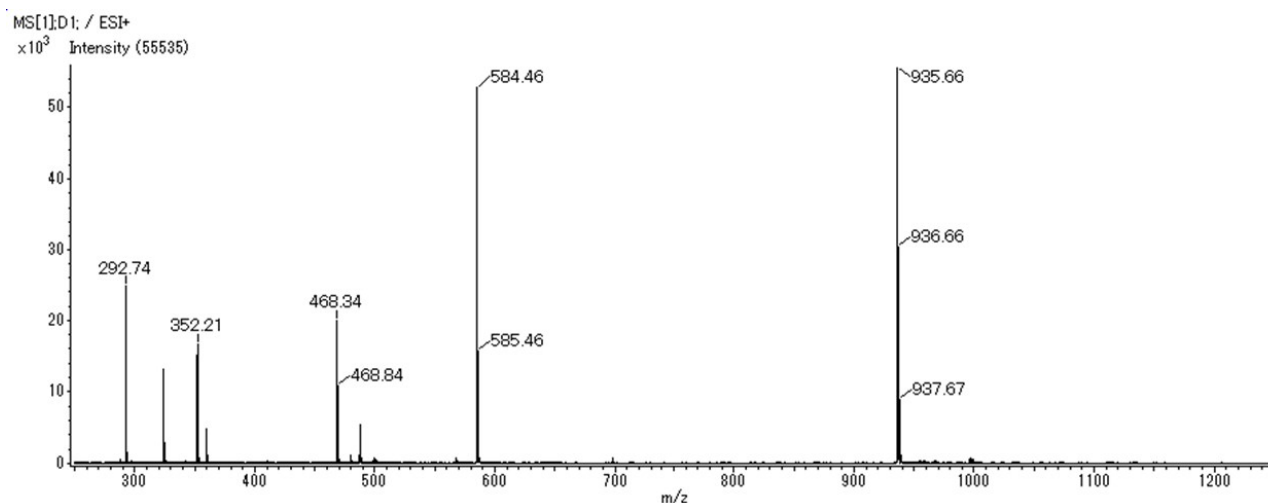


Figure S13. ESI⁺ mass spectrum of azo-peptide **14**: $m/z = 935.66$ [$M+H$]⁺ (calculated mass for the most abundant isotope: 935.54), $m/z = 584.46$ [Pro-Lys-Ala-Ile-Arg+H]⁺ and 352.21 [MeO-Azo-Ile]⁺ (calculated mass for the fragments of the parent azo-peptide **14** cleaved at the amide linkage between the Ile and Pro : 584.38 and 352.16 respectively), $m/z = 468.34$ [$M+2H$]²⁺ (calculated mass for the most abundant isotope: 468.27).

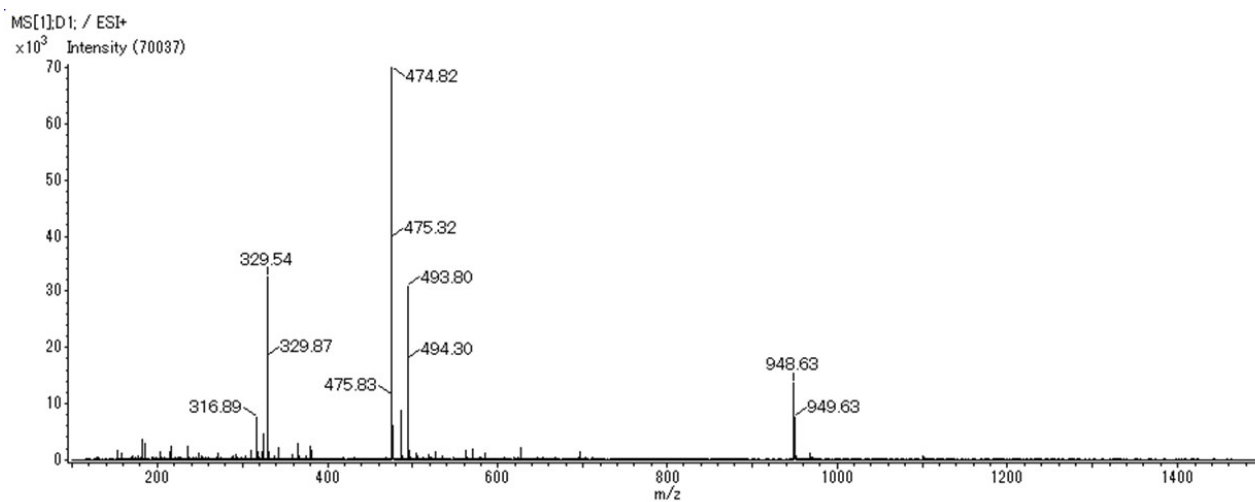


Figure S14. ESI⁺ mass spectrum of azo-peptide **15**: $m/z = 948.63$ [$M+H$]⁺ (calculated mass for the most abundant isotope: 948.57)

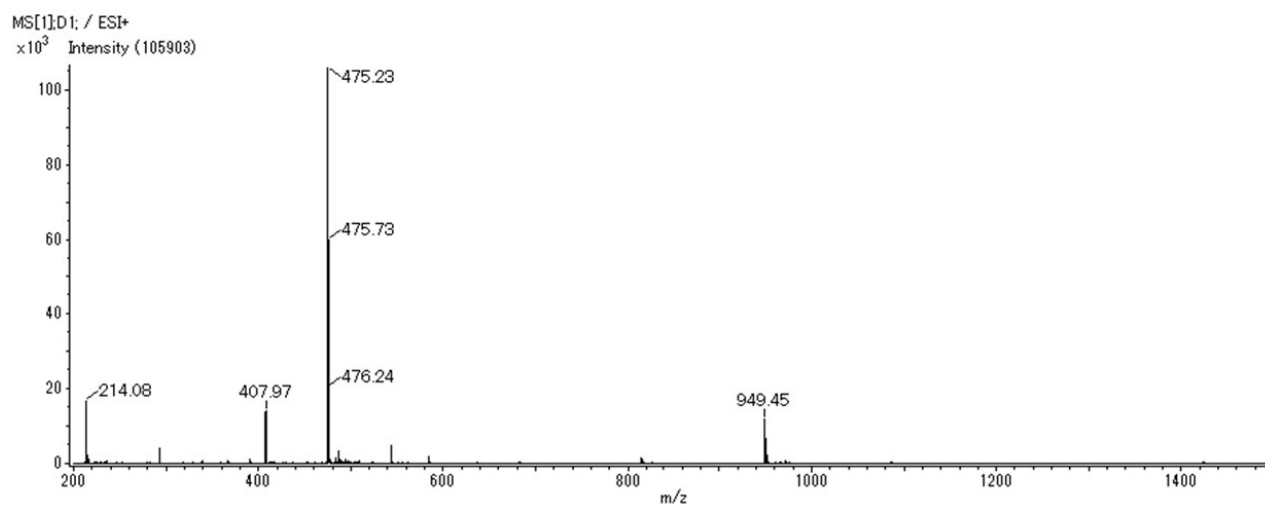


Figure S15. ESI⁺ mass spectrum of azo-peptide **16**: $m/z = 949.45$ [M+H]⁺ (calculated mass for the most abundant isotope: 949.52)

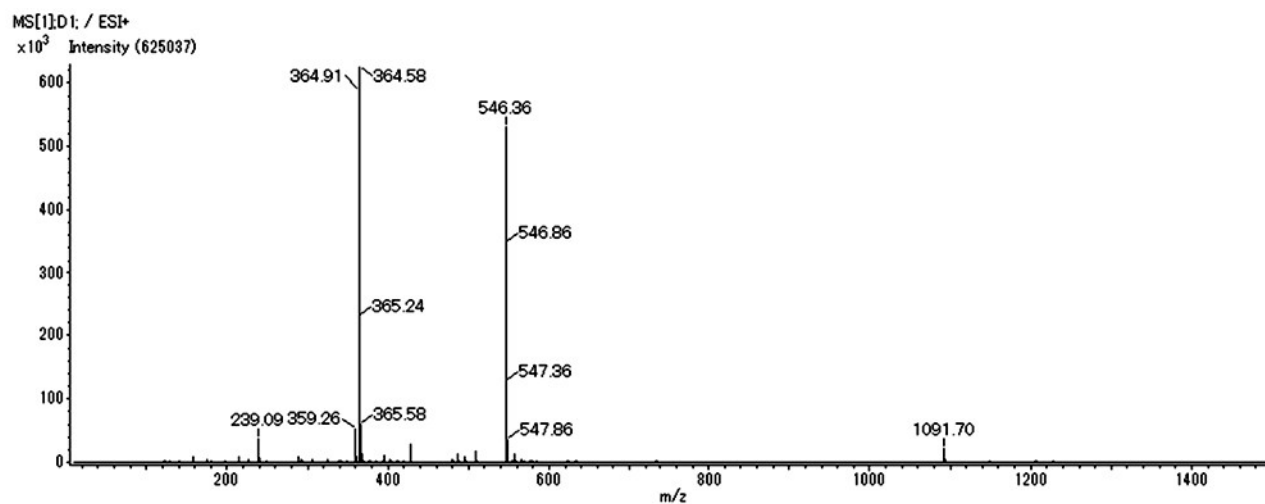
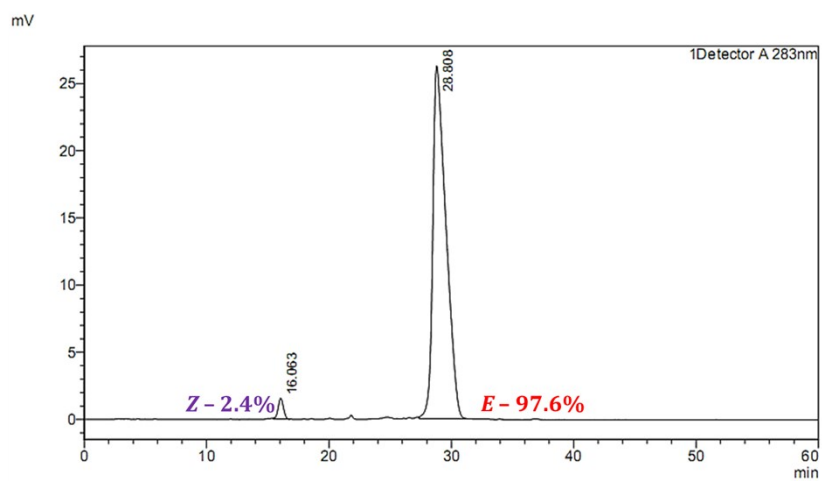


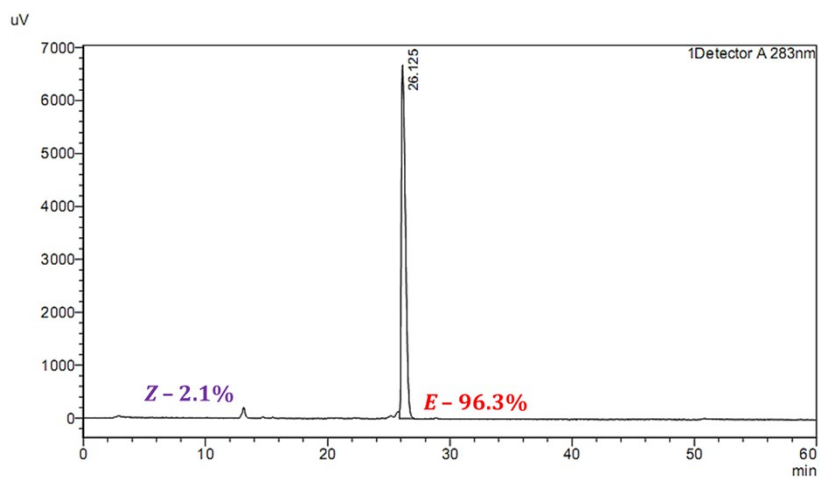
Figure S16. ESI⁺ mass spectrum of azo-peptide **17**: $m/z = 1091.70$ [M+H]⁺ (calculated mass for the most abundant isotope: 1091.64)

2. Purity of the azo-peptides by HPLC analysis

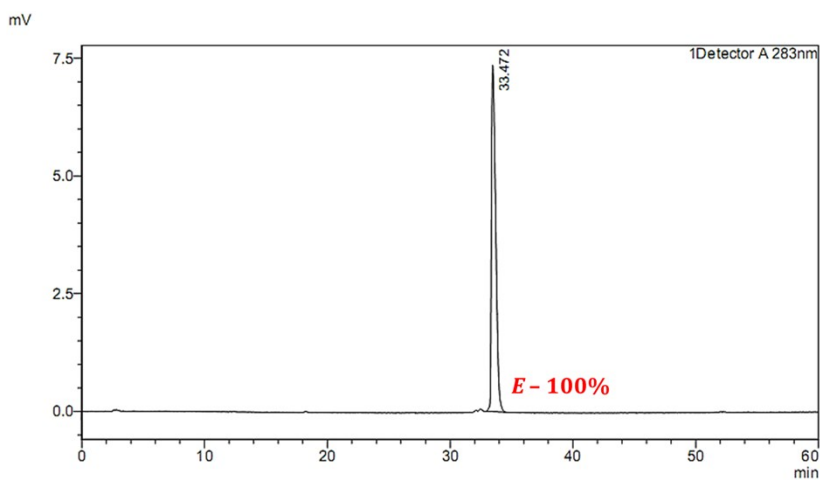
(a) azo-peptide 2



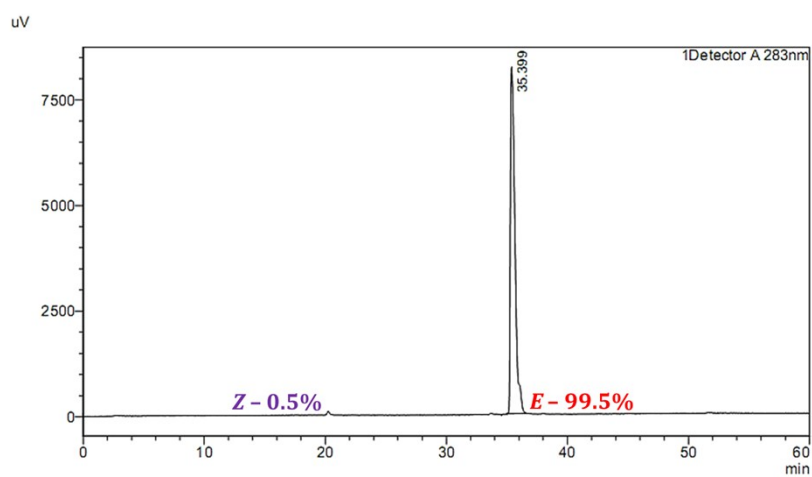
(b) azo-peptide 3



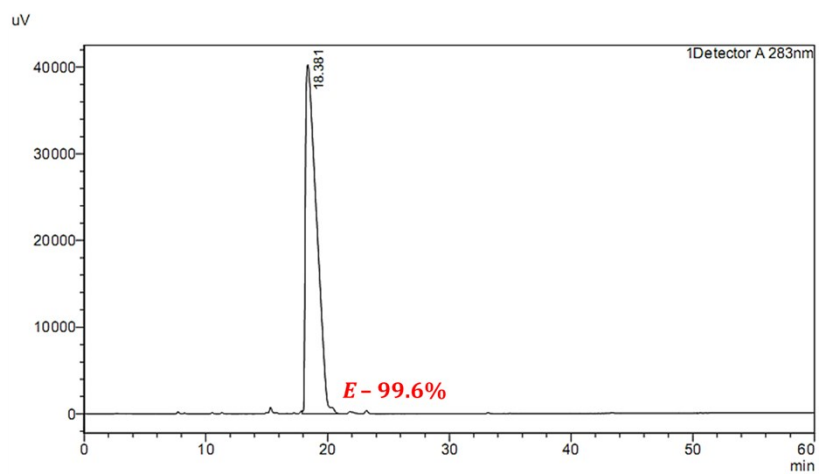
(c) azo-peptide 4



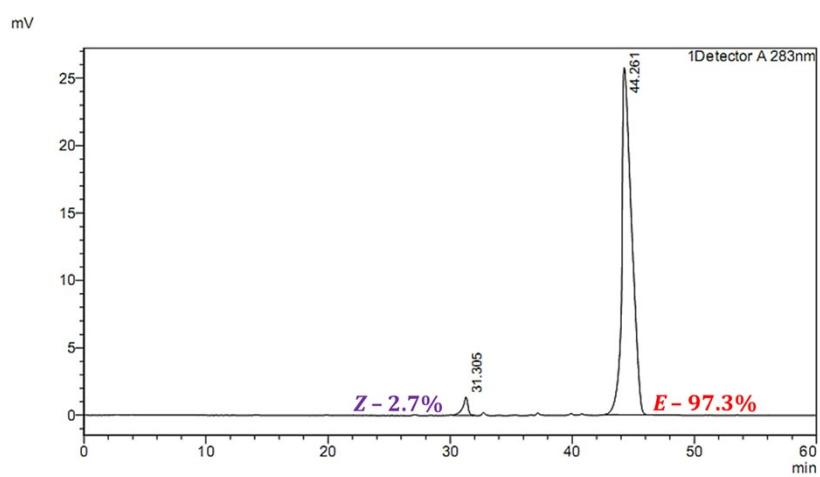
(d) azo-peptide 5



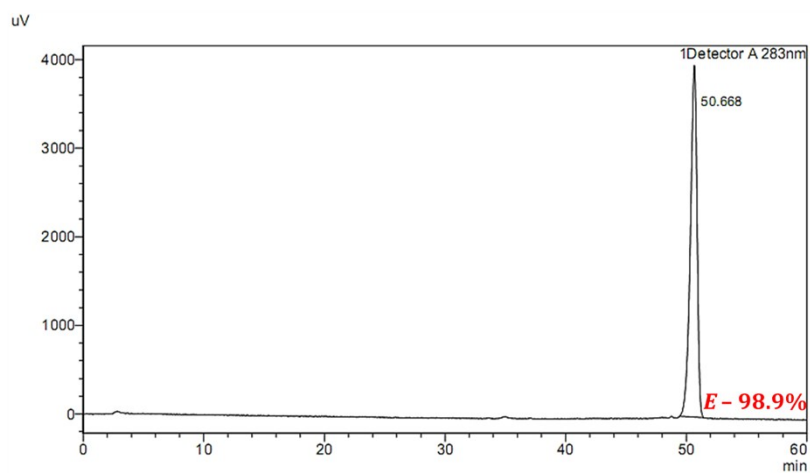
(e) azo-peptide 6



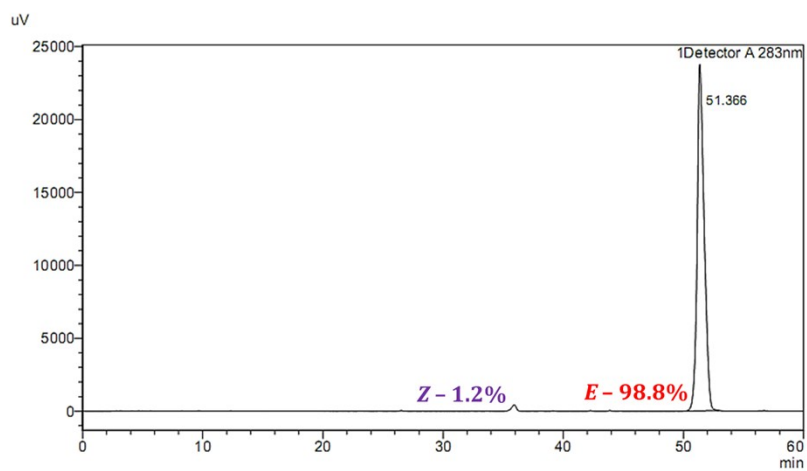
(f) azo-peptide 7



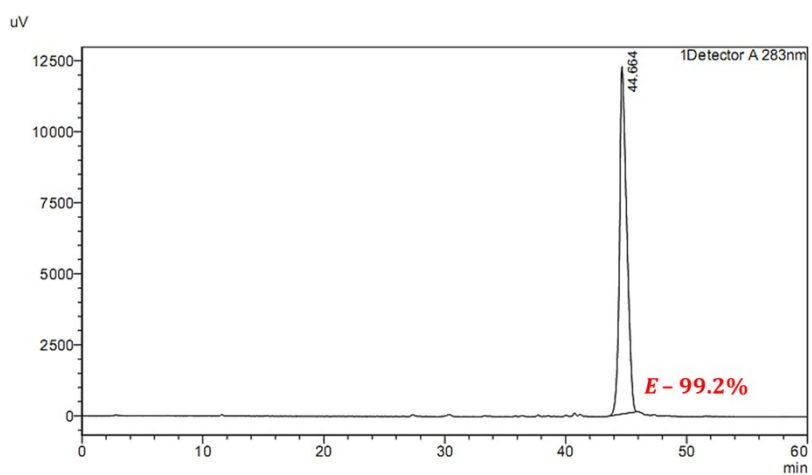
(g) azo-peptide **8**



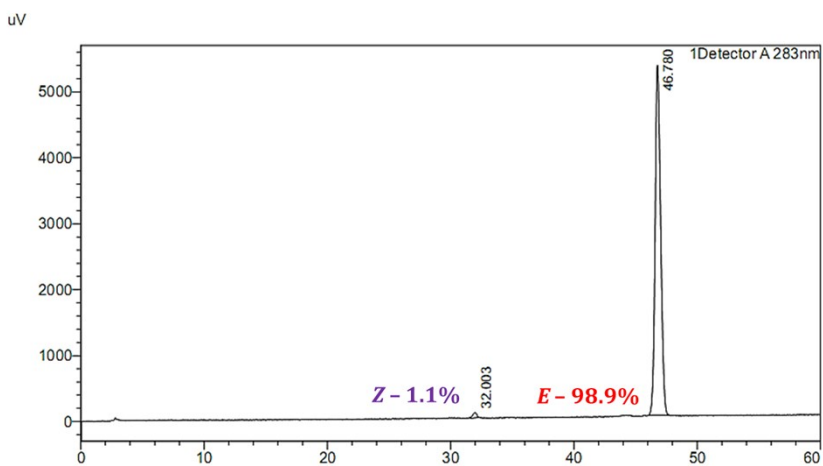
(h) azo-peptide **9**



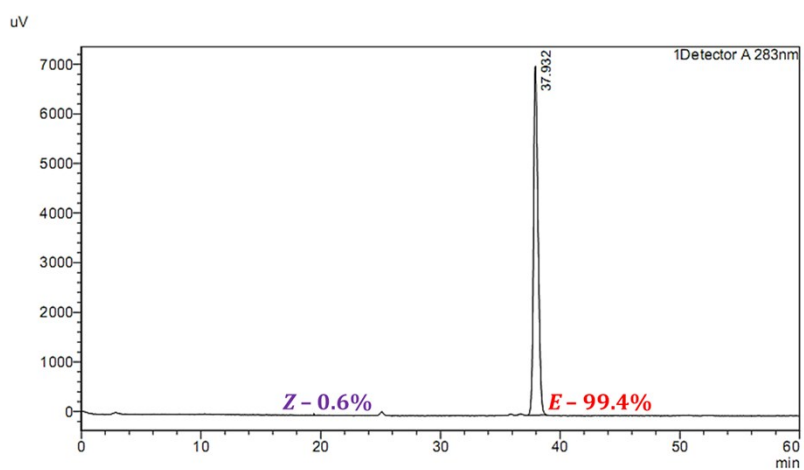
(i) azo-peptide **10**



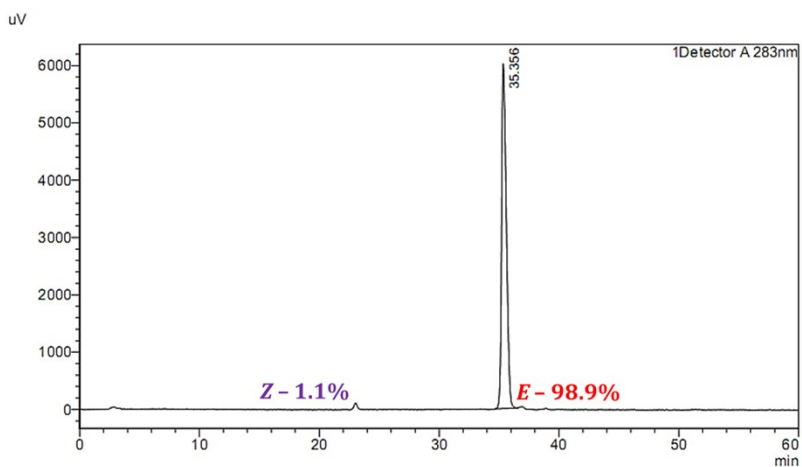
(j) azo-peptide 11



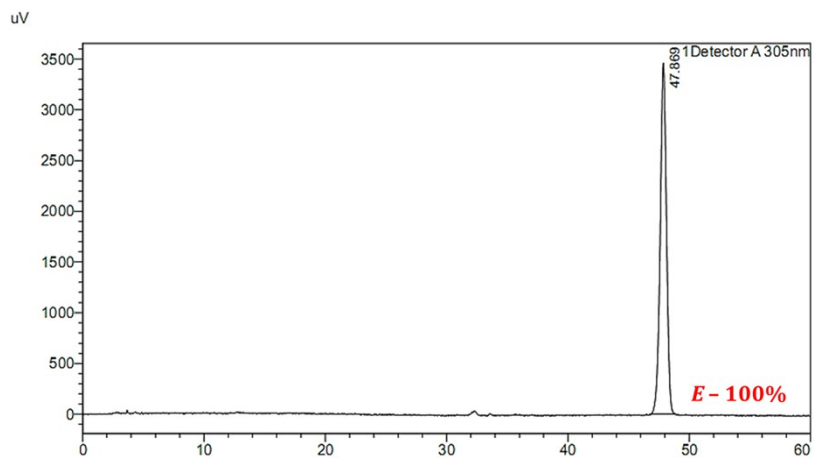
(k) azo-peptide 12



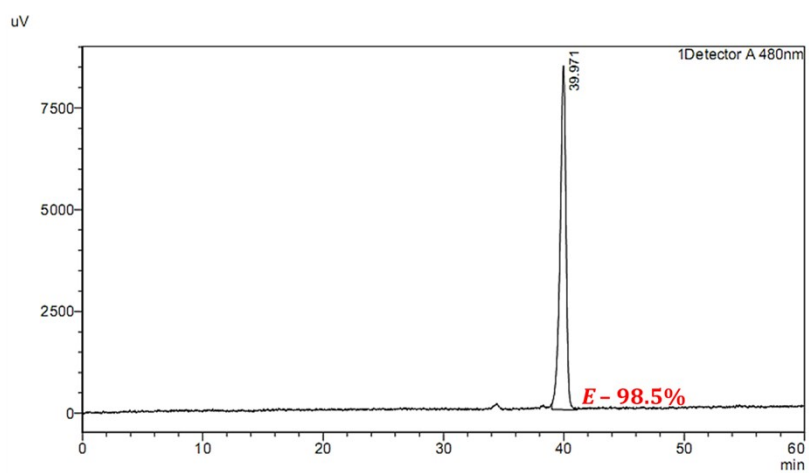
(l) azo-peptide 13



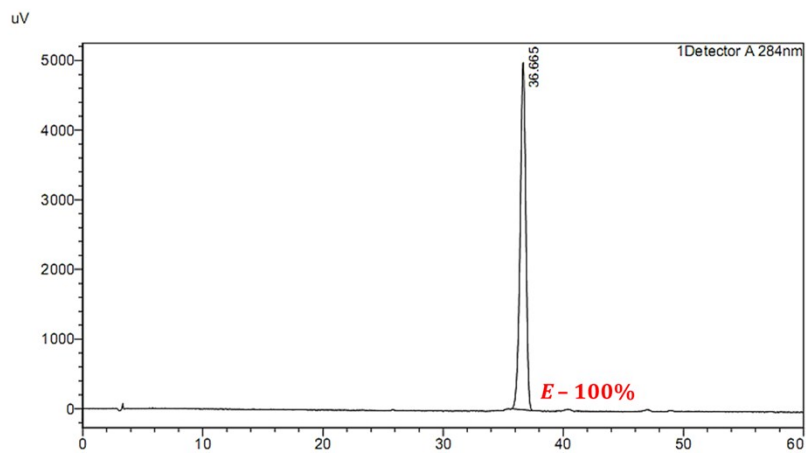
(m) azo-peptide **14**



(n) azo-peptide **15**



(o) azo-peptide **16**



(p) azo-peptide 17

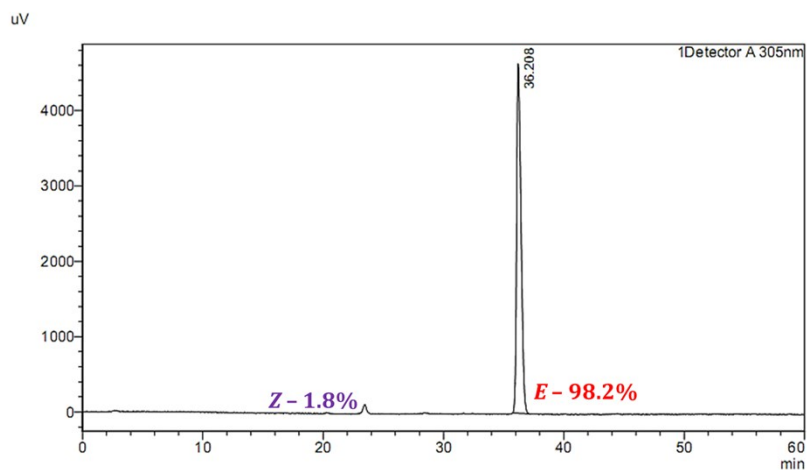


Figure S17. HPLC chromatograms are showing > 95% purity. Conditions of the RP-HPLC analysis; Column - 5C₁₈-MS-II, 4.6×250 mm (Nacalai Tesque, Inc.); Eluent - CH₃CN/H₂O containing 0.1% TFA; Solvent gradient - 20 to 45% of acetonitrile in water for 1 h; Flow rate - 1 mL/min. Injection volume - 20 µL was used to analyze the purity of each azo-peptide.

3. Photoisomerization of the azo-peptides:

Photoisomerization experiments of compounds **11**, **14**, **15**, **16** were performed in BRB-80 buffer at 25 °C.

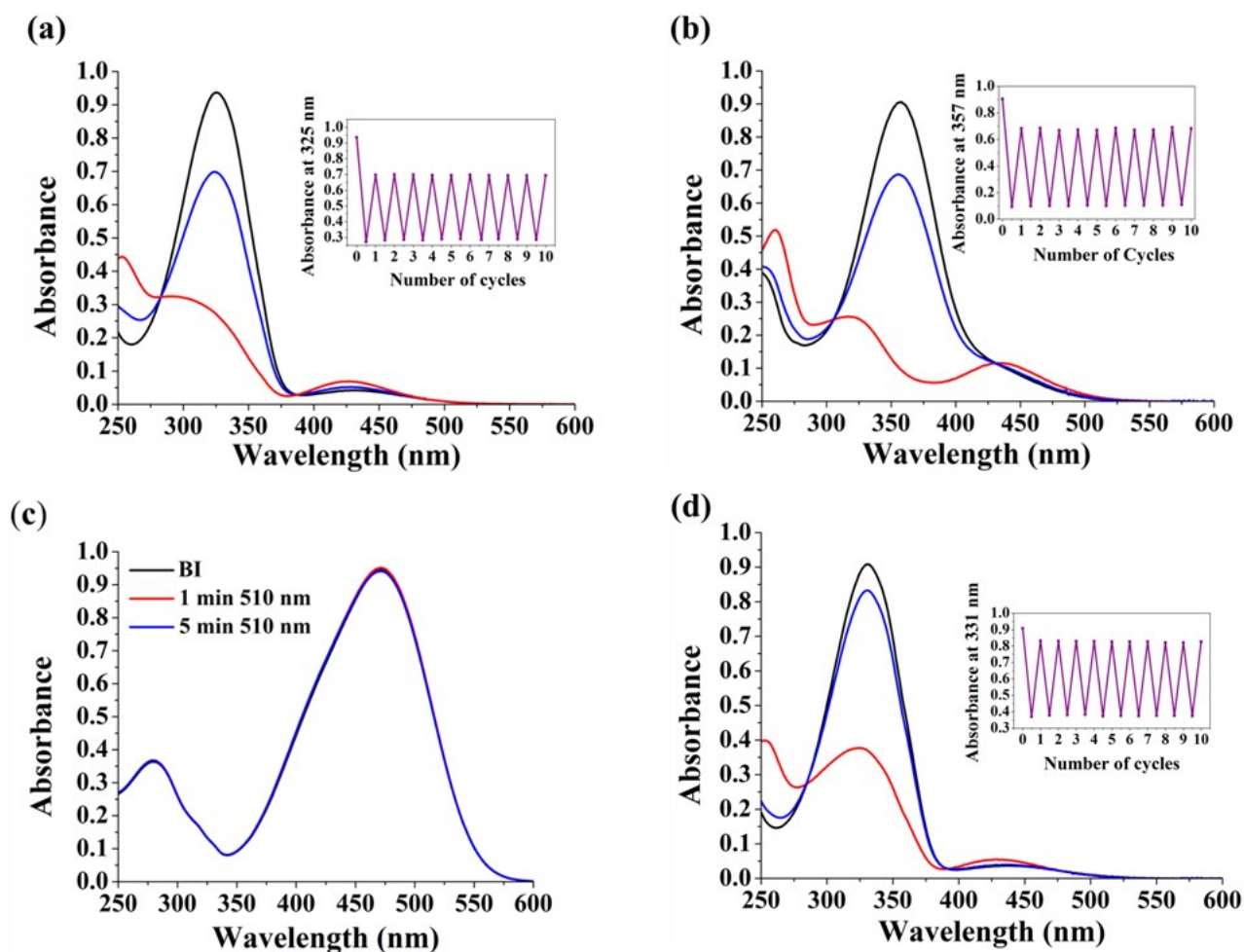


Figure S18. (a), (b), (c) and (d) are the UV-Vis absorption spectra of azo-peptides **11**, **14**, **15** and **16** respectively in BRB-80 buffer solution at 25 °C; before irradiation (black line), UV PSS (red line), Vis PSS (blue line). Inset of (a), (b), and (d): Absorbance changes at 325 nm, 357 nm and 331 nm respectively after the alternate irradiations of 365 and 436 nm for azo-peptides **11** and **16**, 365 and 510 nm for azo-peptide **14** for 10 cycles. Azo-peptide **15** was irradiated with 510 nm light.

4. Thermal stability of the *cis* isomers:

Thermal stability studies of the *cis* isomers of azo-peptides **14** and **16** were performed in BRB-80 buffer at 25 °C.

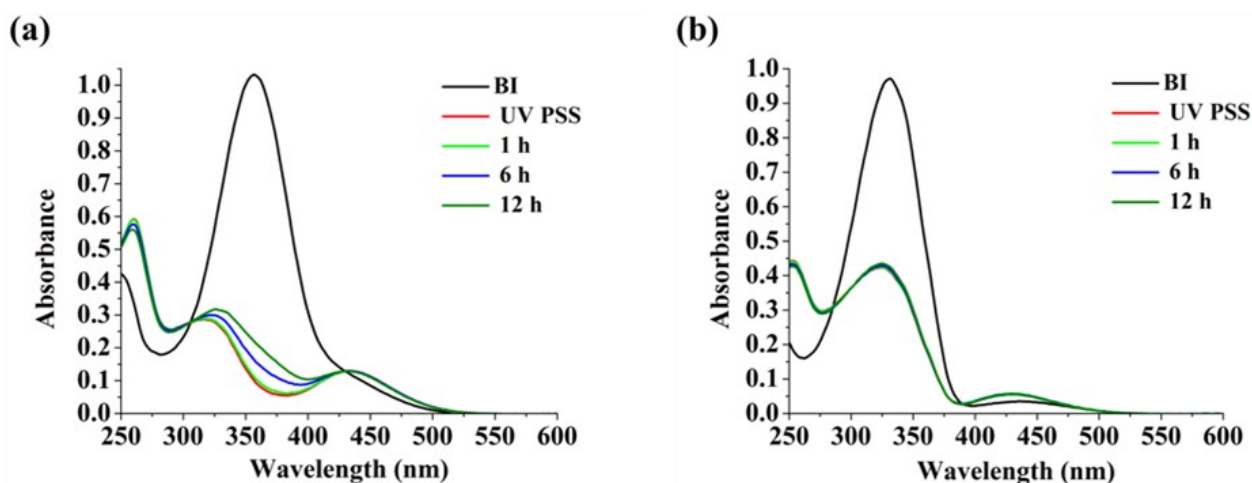
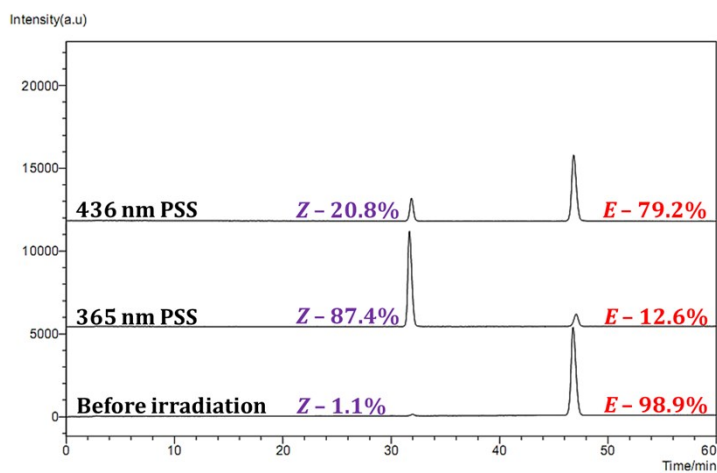


Figure S19. UV-Vis absorption spectra of azo-peptides **14** and **16** showing their thermal stability in BRB-80 buffer solution are represented by (a) and (b) respectively after irradiating with 365 nm light up to their PSSs and then incubation in the dark at 25 °C.

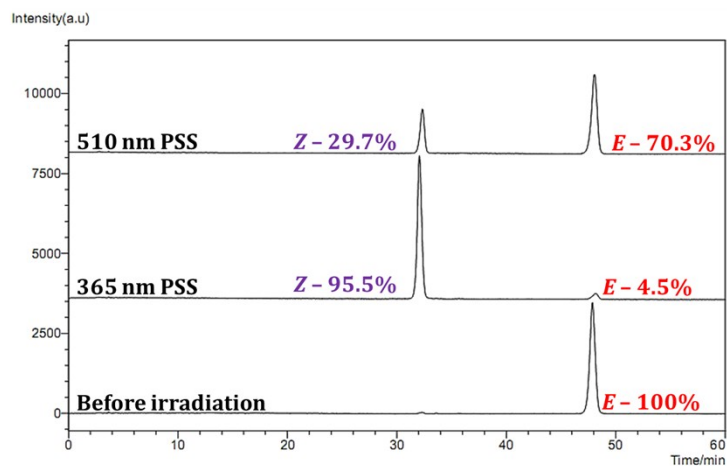
5. HPLC analysis on the conversion ratio from *trans* to *cis* and *cis* to *trans* forms:

The photo conversion ratio from *trans* (*E*) to *cis* (*Z*) and *cis* (*Z*) to *trans* (*E*) of the azo unit in azo-peptides **11**, **14** and **16** upon irradiation with 365 nm light and 436 nm or 510 nm light was measured with HPLC analyses. Conditions of the RP-HPLC analysis; Column - 5C₁₈-MS-II, 4.6×250 mm (Nacalai Tesque, Inc.); Eluent - CH₃CN/H₂O containing 0.1% TFA; Solvent gradient - 20 to 45% of acetonitrile in water for 1 h; Flow rate – 1 mL/min. Injection volume -20 µL was used to analyze the isomer ratio and the isosbestic point in this eluent condition (283 nm for **11**, 305 nm for **14**, 284 nm for **16**) was used as the monitoring wavelength.

(a) azo-peptide 11



(b) azo-peptide 14



(c) azo-peptide 16

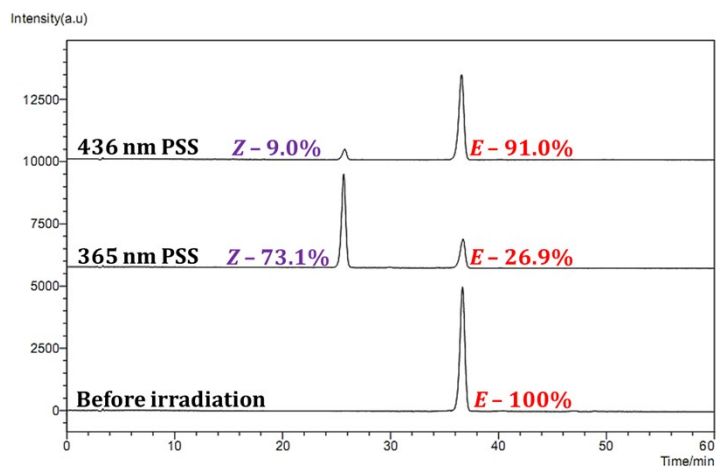
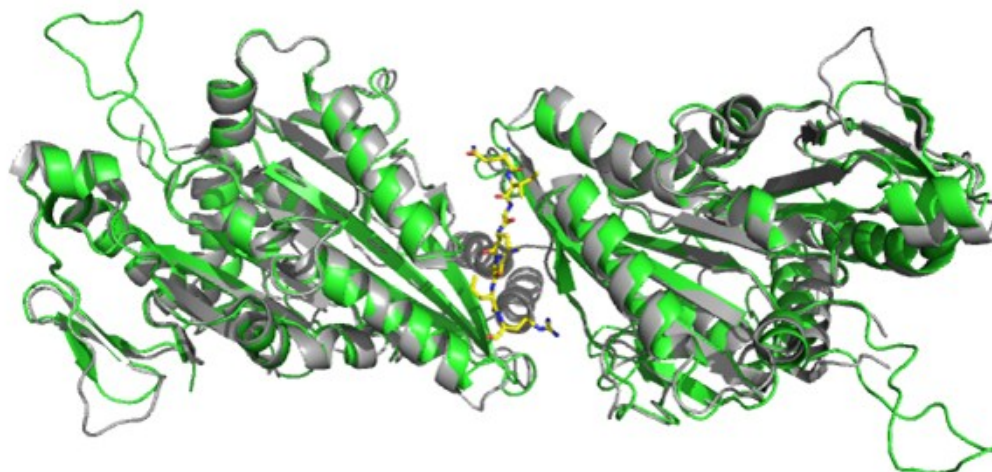


Figure S20. HPLC chromatograms showing the *cis* (Z) and *trans* (E) isomer ratio at before irradiation, after 365 nm light irradiation at PSS and after 436 nm or 510 nm light irradiation at PSS.

6. Structural comparison between the *Drosophila melanogaster* kinesin-1 and human kinesin-1 motor domains

(a)



(b)

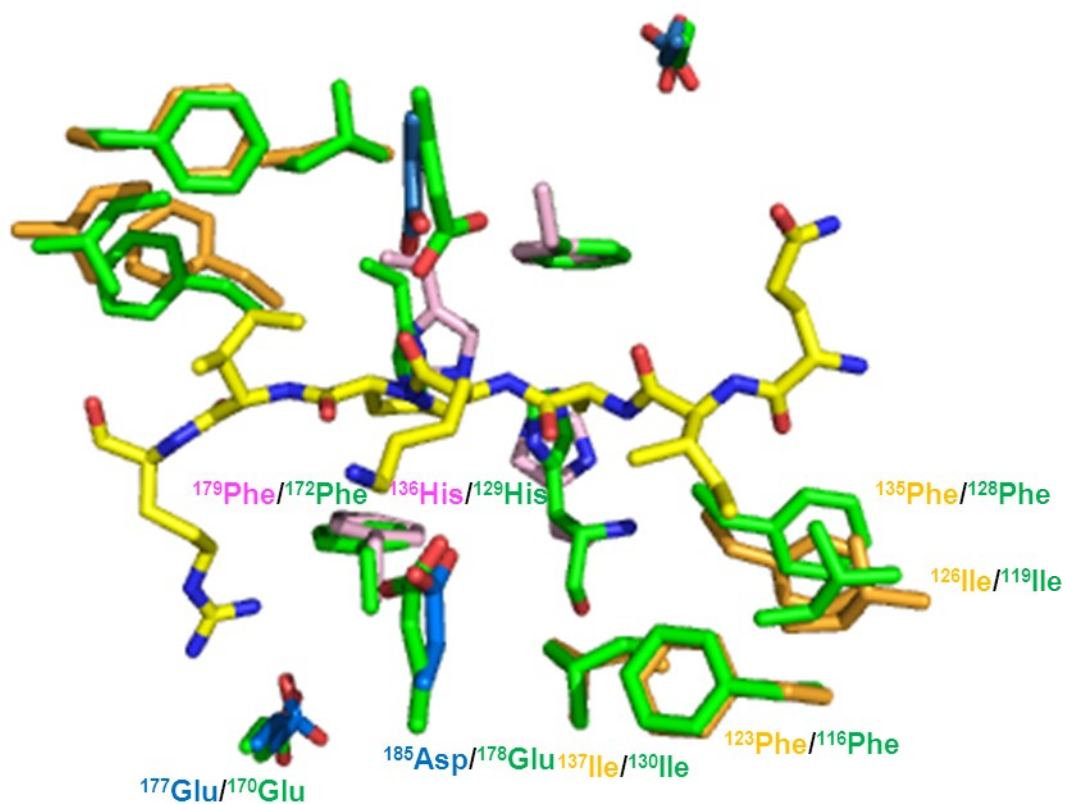


Figure S21. (a) Overlay of *Drosophila melanogaster* kinesin-1 (gray, PDB: 2Y65) and human kinesin motor domains (green, PDB: 1BG2). (b) Detailed similarity in critical amino acids for tail binding.