Supporting Information

Formation of α -Helical Nanofibers by Mixing β-Structured and α-Helical Coiled Coil Peptides

Enrico Brandenburg,[a‡] Hans v. Berlepsch,[b‡] Jork Leiterer,[c] Franziska Emmerling,[c]

and Beate Koksch*[a]

[a] *Department of Chemistry and Biochemistry, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany*

[b] *Research Center for Electron Microscopy, Freie Universität Berlin, Fabeckstrasse 36a, 14195 Berlin, Germany*

[c] *BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Straße 11, 12489 Berlin, Germany*

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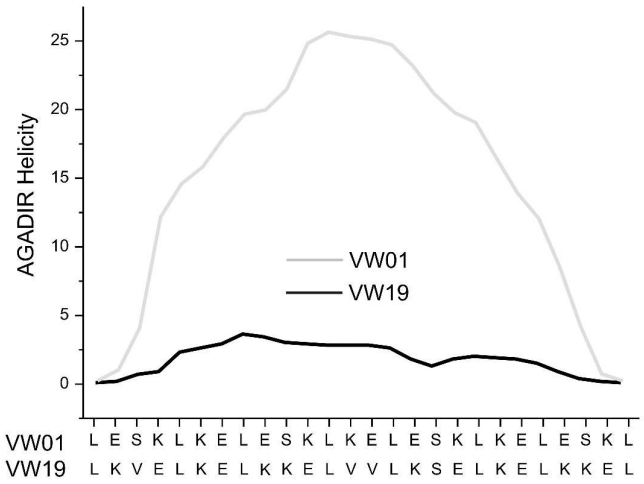

Figure 1. Helicity scores predicted by the AGADIR algorithm for VW19 (black line) and VW01 (gray line) at pH 4.0 related to the primary sequence.

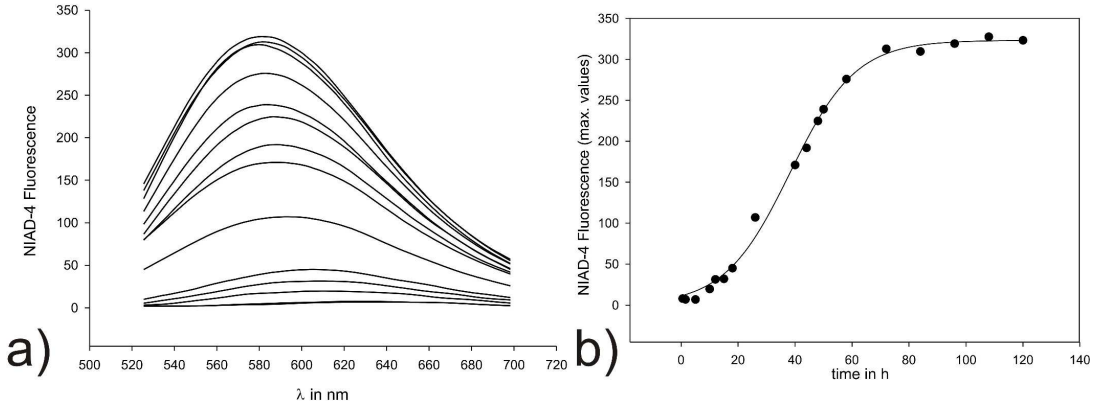

Figure 2. Kinetics of amyloid fibril formation of a 200 µM VW19 solution in 10 mM acetate buffer at pH 4.0 within 120 hours of incubation monitored by NIAD-4 fluorescence. (a) Complete set of fluorescence emission spectra. (b) Maximum fluorescence intensity as a function of incubation time.

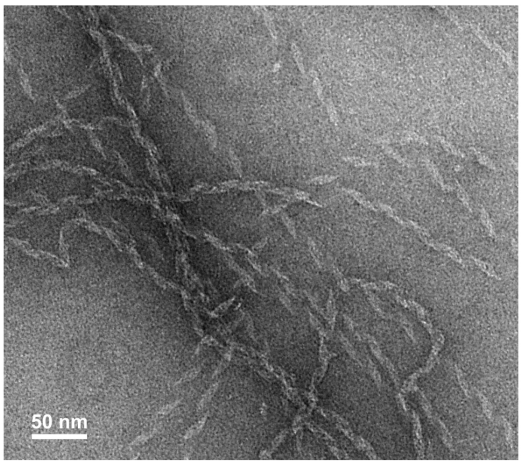

Figure 3. TEM micrograph of PTA-stained helically twisted fibrils of peptide VW19 (100 µM, acetate buffer at pH 4.0).

Experimental conditions: Samples for staining electron microscopy were prepared by adsorbing 7 µL aliquots of peptide solution to a glow discharged carbon coated collodium film on a 400-mesh copper grid. The grids were blotted, stained with 1% phosphotungstic acid (PTA), and air dried. TEM micrographs were taken at a primary magnification of 58300× using a defocus of 0.8 µm.

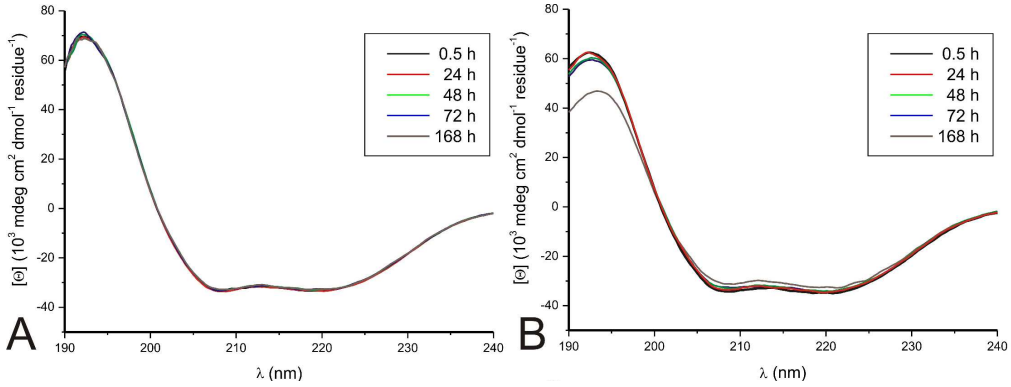

Figure 4a*:* Overlaid CD spectra of VW01 solutions measured after different incubation times up to 168 hours for different pH values and different peptide concentrations. *pH 4,0: (A) 100 µM,* (B) 300 µM (10 mM Acetate-Buffer, pH 4,0; 10 mM Phosphate-Buffer, pH 7,4; 10 mM Carbonate-Buffer, pH 9,0)

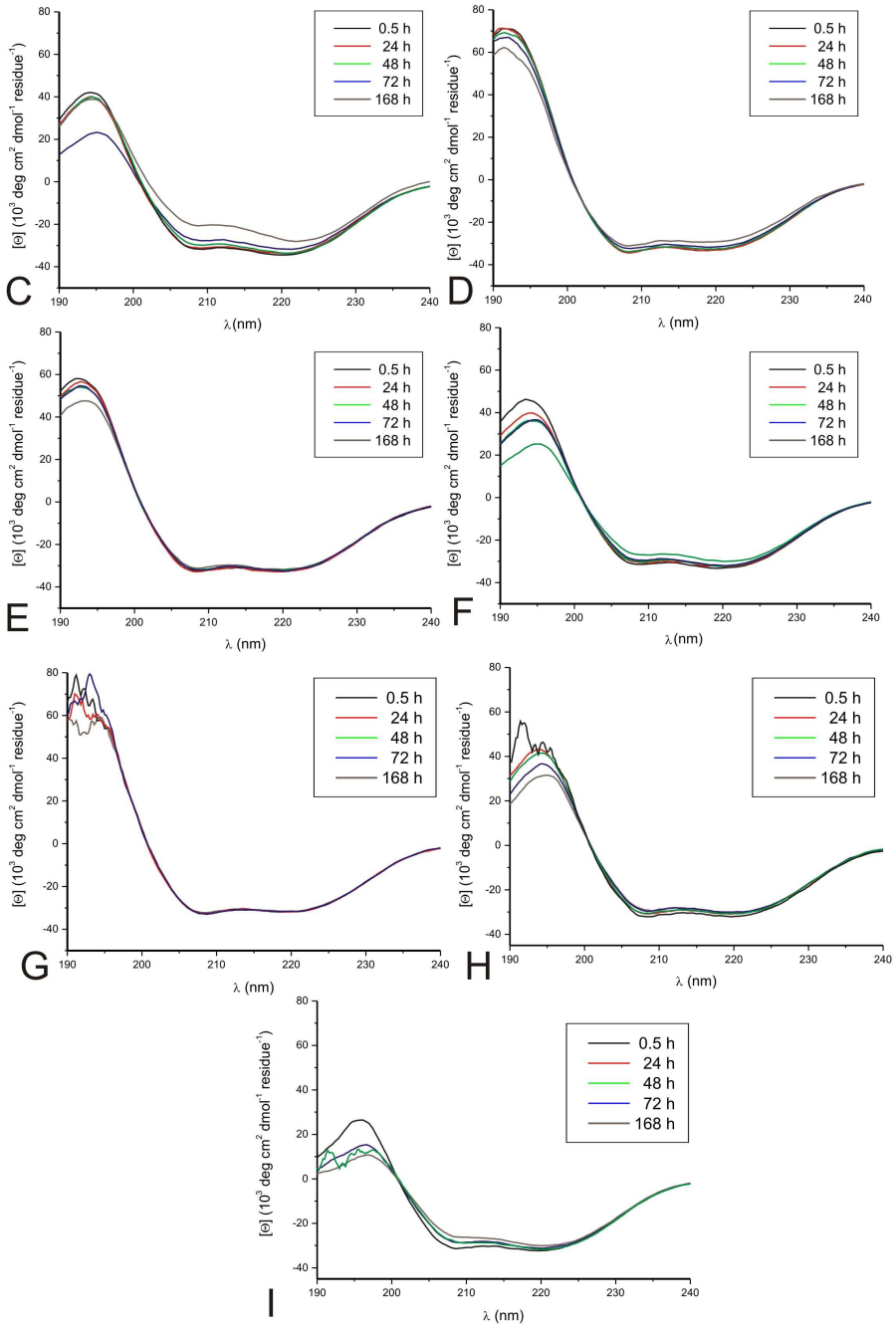

Figure 4b: Overlaid CD spectra of VW01 solutions measured after different incubation times up to 168 hours for different pH values and different peptide concentrations (C) 500 µM; pH 7.4: (D) 100 µM, (E) 300 µM, (F) 500 µM ;pH 9 (G) 100 µM, (H) 300 µM, (I) 500 µM (10 mM Azetat-Puffer, pH 4,0; 10 mM Phosphat-Puffer, pH 7,4; 10 mM Carbonat-Puffer, pH 9,0)

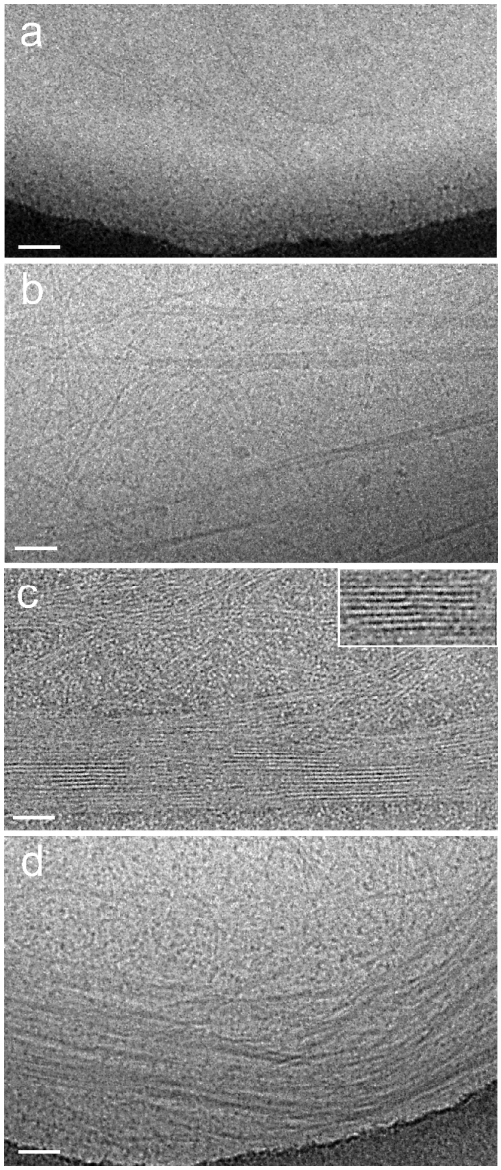

Figure 5. Electron micrographs of 500 μM VW01 solutions at different pH values. (a) Cryo-TEM; acetate buffer at pH 4.0. (b) Cryo-TEM; phosphate buffer, at pH 7.4. (c) TEM after PTA staining; carbonate buffer at pH 9.0. The inset shows the parallel alignment of individual fibers within a fiber bundle at large magnification. (d) Cryo-TEM; carbonate buffer at pH 9.0. Scale bars: 30 nm.

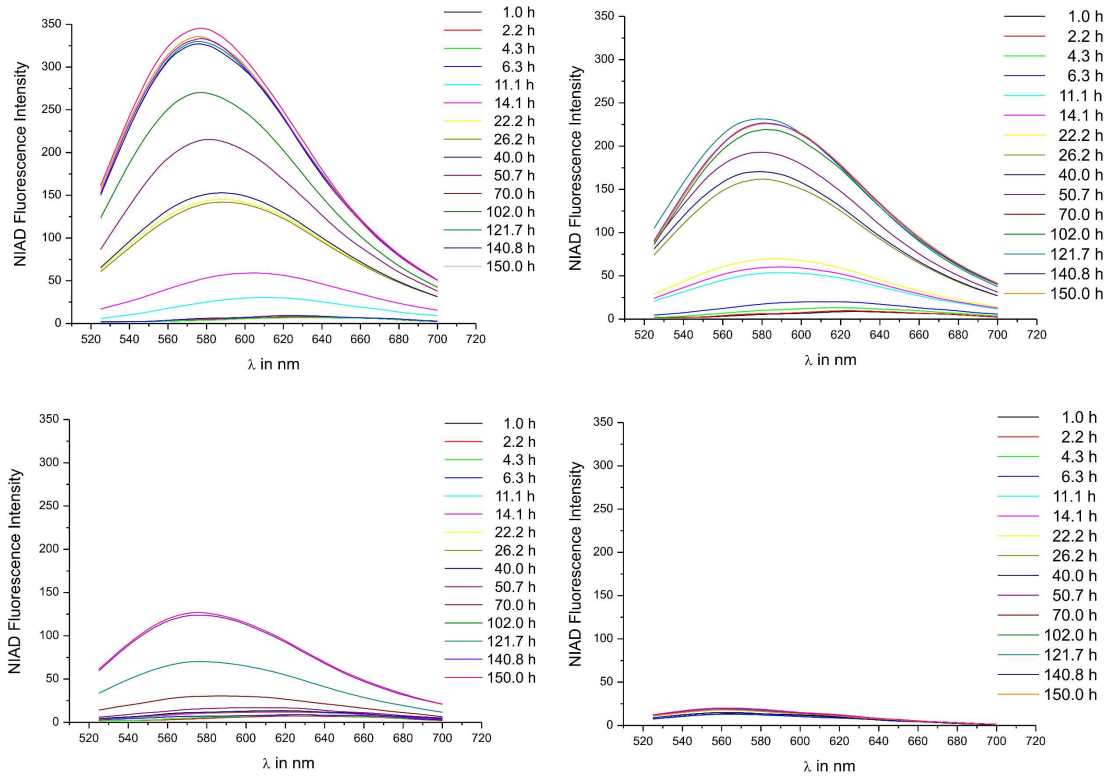

Figure 6. Change of NIAD-4 fluorescence of VW19 alone and of VW19/VW01 mixtures as a function of incubation time (from bottom to top). (a) VW19 alone, (b) VW19/VW01 mixing ratio: r = 2, (c) VW19/VW01 mixing ratio: r = 1, (d) VW19/VW01 mixing ratio: r = 0.5. All samples contained 150 µM of VW19 and were prepared in 10 mM acetate buffer at pH 4.0.

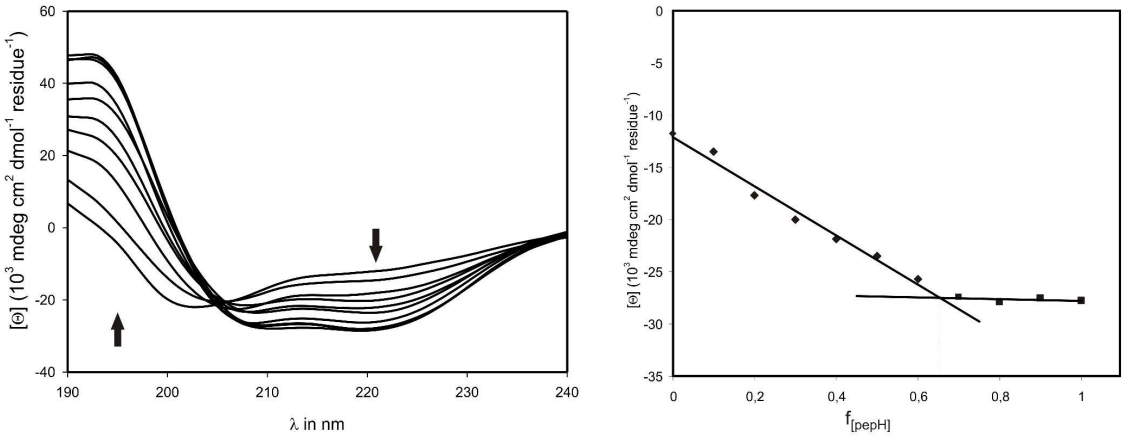

Figure 7. (left) Change of CD spectra of VW19/VW01 mixtures for increasing VW01 mole fractions, $f_{[VW01]}$ = [VW01]/([VW19]+[VW01], between 0.1 and 1.0. The effect of increasing $f_{[VW01]}$ is indicated by the arrows. (right) Change of the molar ellipticity per residue at 222 nm, $[\Theta]_{222}$, as a function of $f_{[VW01]}$. The experiments were carried out in 10 mM phosphate buffer at pH 7.4 at a total peptide concentration of 100 µM.

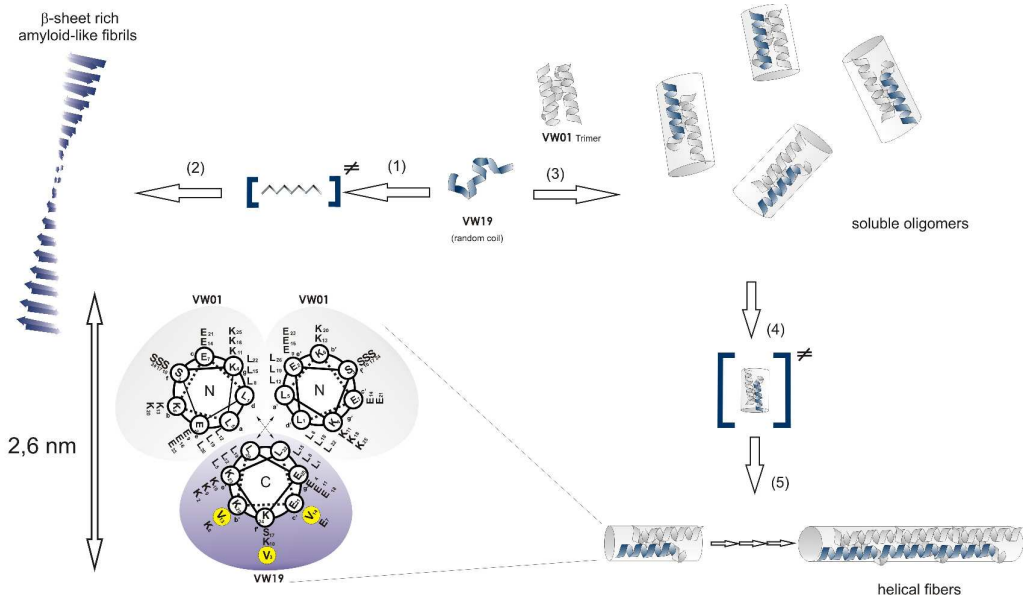

Figure 8. Speculative representation of the aggregation pathways leading to the formation of amyloid fibrils for pure VW19 (steps 1 and 2) and of heteromeric helical fibers for the VW19/VW01 mixture (steps 3, 4 and 5). (1) Peptide concentration-dependent nucleation. (2) Formation of β-sheet rich amyloid fibrils. (3) Assisted secondary structure formation. (4) Peptide concentration-dependent nucleation. (5) Supramolecular polymerization into long helical fibers. The proposed coiled coil arrangement within the helical fibers is visualized by the helical wheel diagram (left).

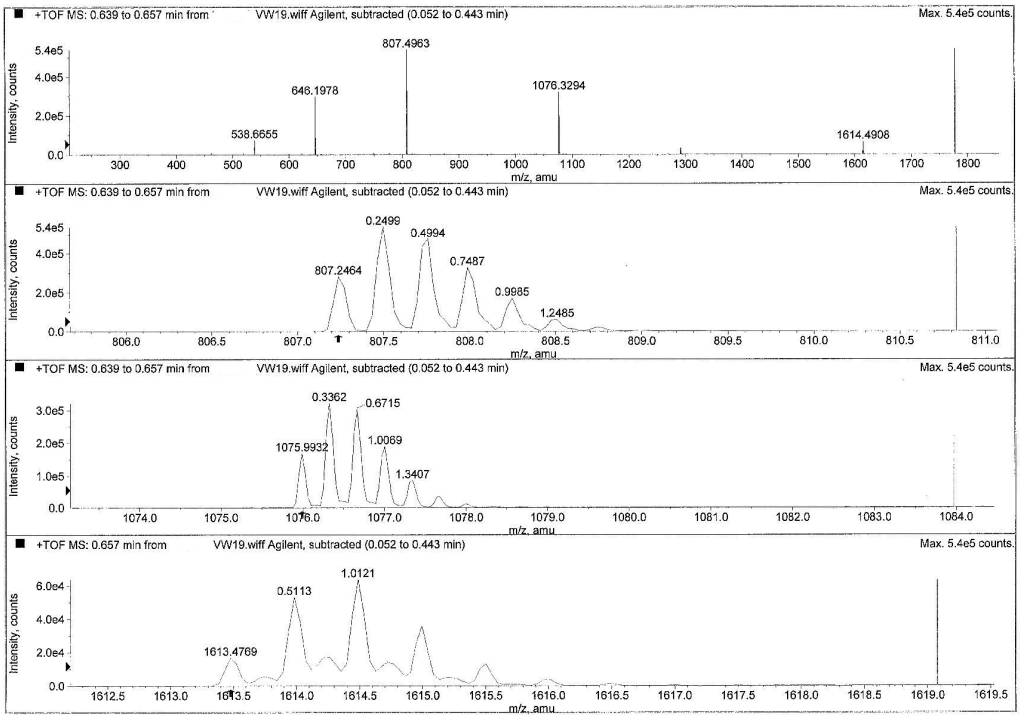

Figure 9. ESI-TOF MS of VW19.

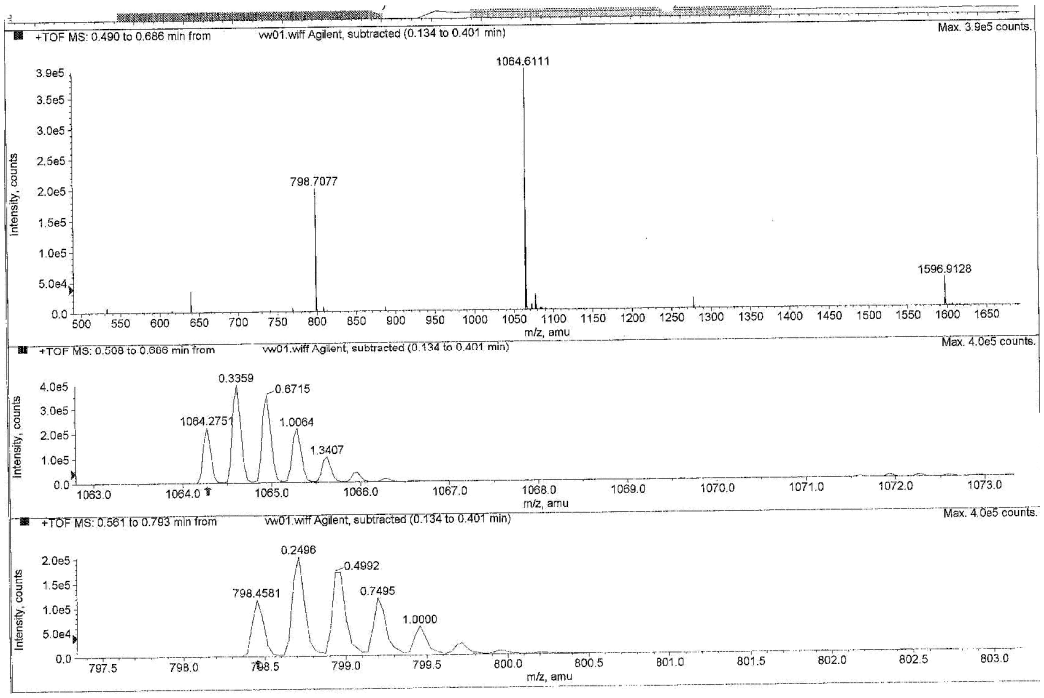

Figure 10. ESI-TOF MS of VW01.

Table S1. Identification of synthesized polypeptides by ESI-TOF mass spectrometry

Model Peptides	Observed		Calculated	
	$[M+3H]^{3+}$	$[M+4H]^{4+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$
VW01	1064.6111	798.7077	1064.2658	798.4513
VW19	1075.9932	807.2464	1075.9863	807.2417
VW01-ran	1064.6071	798.7064	1064.2658	798.4513