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Assembly of the Fungal SC3 Hydrophobin into Functional Amyloid Fibrils Depends on Its Concentration and Is Promoted by Cell Wall Polysaccharides

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LEGENDS SUPPLEMENTARY DATA

Fig. 1. Circular dichroism (CD) shows that SC3 in the α -helical state can be removed from a hydrophobic solid by addition of 0.1 % Tween 20 pH 7, while the β -sheet state remains adsorbed. (A) Addition of PTFE to an aqueous SC3 solution results in a change in the CD spectrum from that of the water-soluble form (black line) to that of the α -helical state (dotted line). Subsequent addition of Tween-20 at pH 7 reverses the spectrum back to that of the soluble state (grey line). (B) Treatment of SC3 adsorbed to PTFE with 0.1% Tween-20 at pH 2 results in the β -sheet spectrum (black line), which is not affected by increasing the pH to pH 7 (grey line).

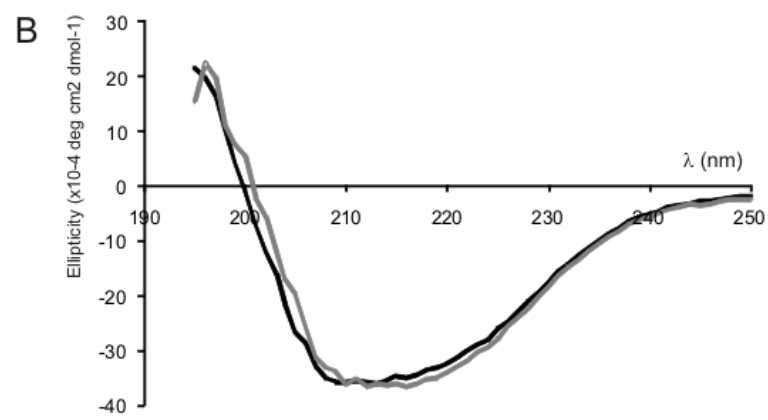
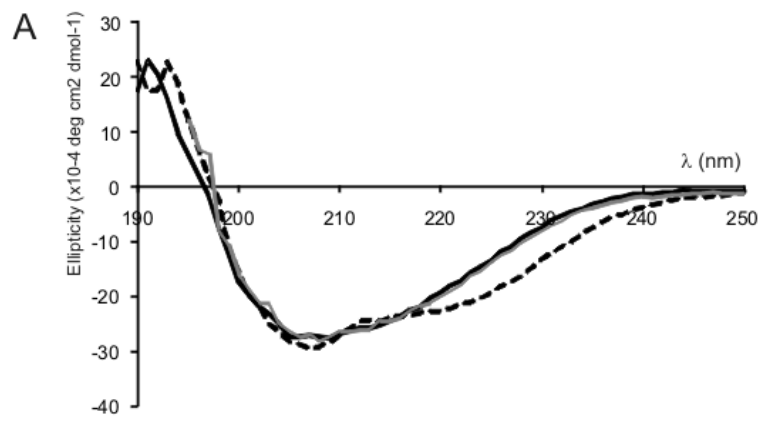
Fig. 2. Schizophyllan promotes the transition of SC3 to the β -sheet state at the water-PTFE interface. WCA measurements of PTFE-sheets coated with SC3 at concentrations of 1, 2.5 and 5 $\mu\text{g ml}^{-1}$ (pH 2) after washing with water (5W-0W) or 0.1 % Tween-20 pH 7 (5T-0T). Coating was performed for 16 h in the absence (white bars) or presence of 0.3 mg ml^{-1} schizophyllan (black bars). Incubation of Teflon in the absence of SC3 served as a control (samples 0 W and 0 T).

Fig. 3. Schizophyllan promotes the transition of SC3 to the β -sheet state at the water-PTFE interface at concentrations $\geq 0.3 \text{ mg ml}^{-1}$. WCA measurements of PTFE-sheets coated with 5 $\mu\text{g ml}^{-1}$ SC3 (pH 2) washed with water (5WW-5WT) or treated with 1 % SDS at 95°C (5 SDSW-5 SDST) followed by washing with either water (5WW-5 SDSW) or 0.1 % Tween-20 pH 7 (5WT-5 SDST). Coating was performed for 16 h in the absence (white bars) or presence of 0.04 mg ml^{-1} (light grey bars), 0.08 mg ml^{-1} (hatched bars), 0.17 mg ml^{-1} (dark grey bars) and 0.30 mg ml^{-1} (black bars) schizophyllan.

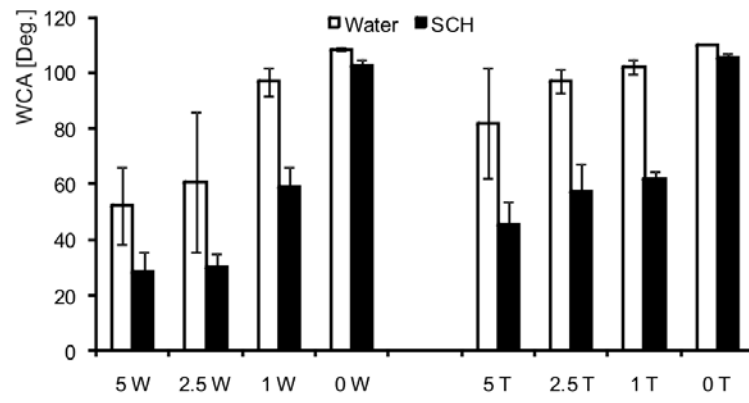
Fig. 4. Schizophyllan accelerates the formation of SC3 into the β -sheet II amyloid state. ThT fluorescence of colloidal PTFE coated with an aqueous solution of SC3 in the absence (W) or presence of 0.3 mg ml^{-1} schizophyllan (SCH). Fluorescence was measured immediately after addition of ThT (white bars), or after 2 h (light grey bars) or 16 h (dark grey bars) of incubation. The black bars represent the total fluorescence obtained after addition of Tween 20 to a final concentration of 0.1% (pH2), which induces formation of amyloid fibrils at the PTFE surface.

Fig. 5. Scleroglucan promotes the transition of SC3 to the β -sheet state at the water-PTFE interface. WCA measurements of PTFE-sheets coated with 5 $\mu\text{g ml}^{-1}$ SC3 washed with water (W) or treated with 0.1 % Tween-20 pH 7 (T). Coating was performed for 16 h in the absence (5W-5T) or presence of 1 mg ml^{-1} scleroglucan (5W Sc-5T Sc). Coating was performed at pH 2 (white bars), pH 5 (grey bars) or pH 7 (black bars).

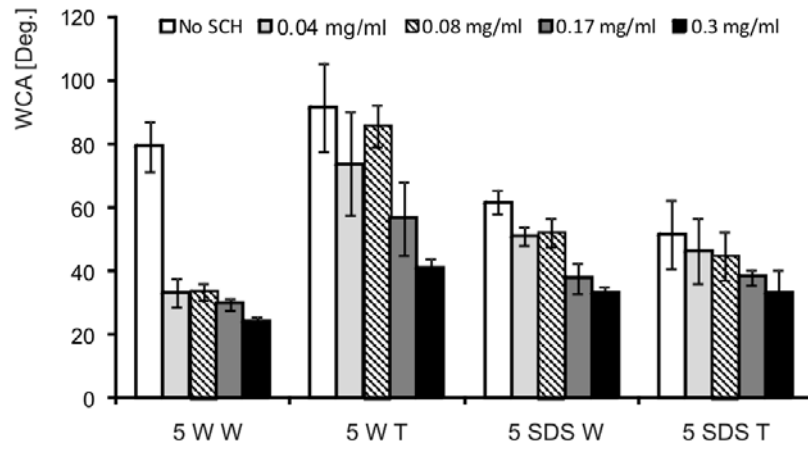
Suppl. Figure 1



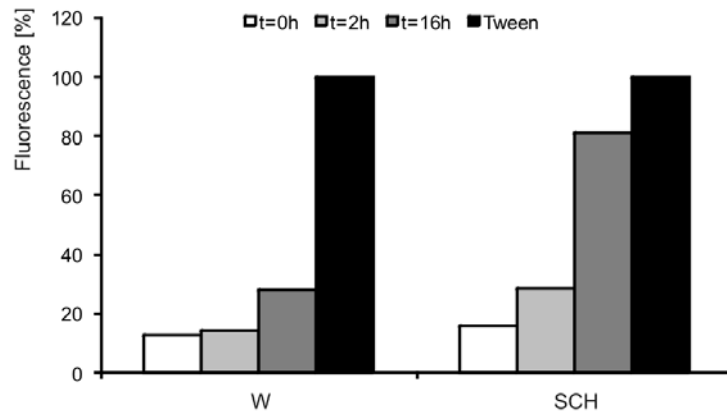
Suppl. Figure 2



Suppl. Figure 3



Suppl. Figure 4



Suppl. Figure 5

