Supplemental Information

The cytotoxic Staphylococcus aureus PSMα3 inhibits the aggregation of human insulin *in vitro*

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Table S1 PSMα3 peptide analytical data.

Name	Sequence	Formula	Mass		Analitical HPLC
			theoretical	observed	$t_{ret.}$[min]
PSMα3	MEFVAKLFKFFKDL LGKFLGNN	$C_{127}H_{193}N_{29}O_{28}S$	[(M+2H)/2] 1304.57 [(M+3H)/3] 870.05 [(M+4H)/4] 652.79	[(M+2H)/2] 1304.73 [(M+3H)/3] 870.15 [(M+4H)/4] 652.87	15.213

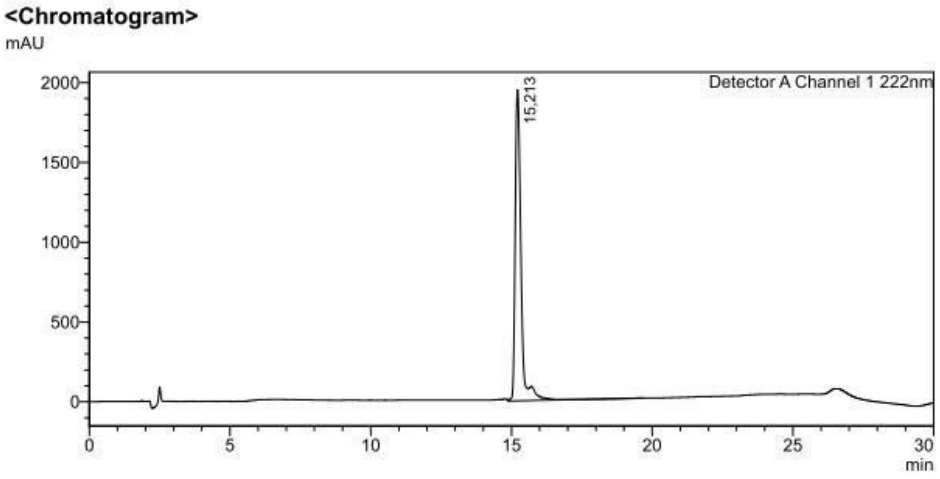


Fig. S1 Analytical RP-HPLC chromatogram of PSMα3.

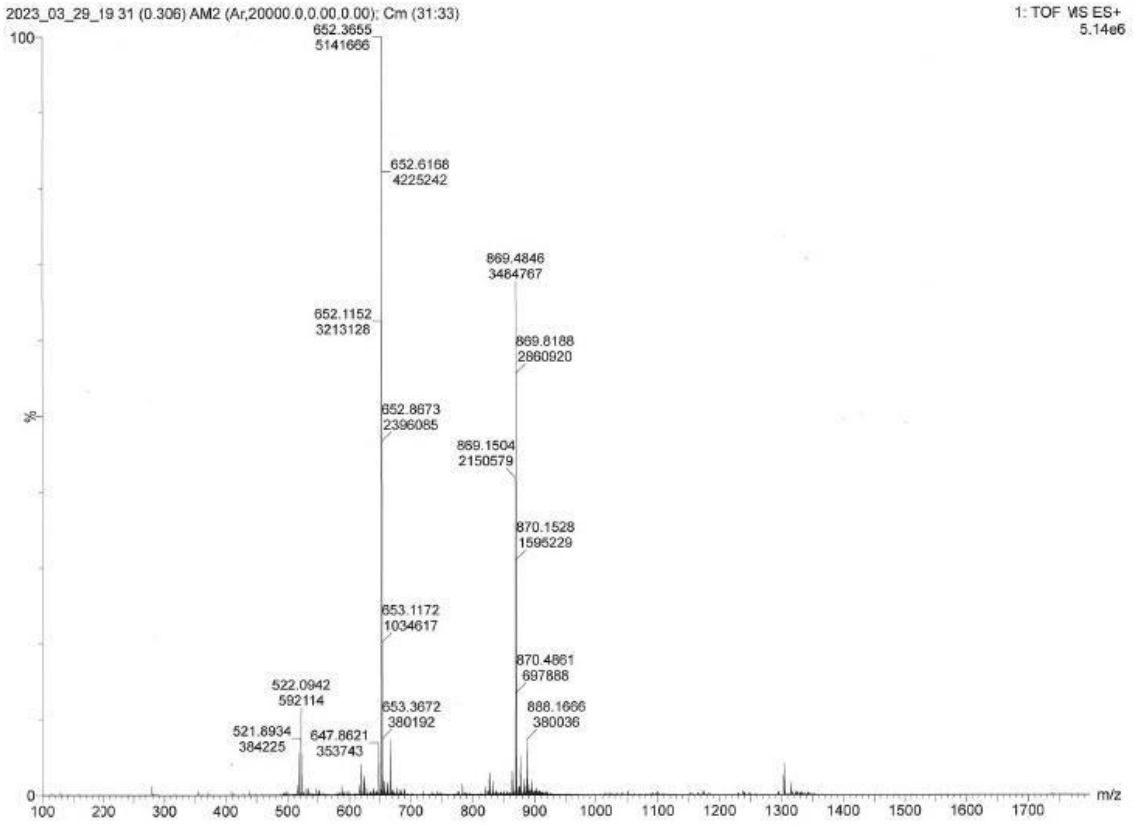
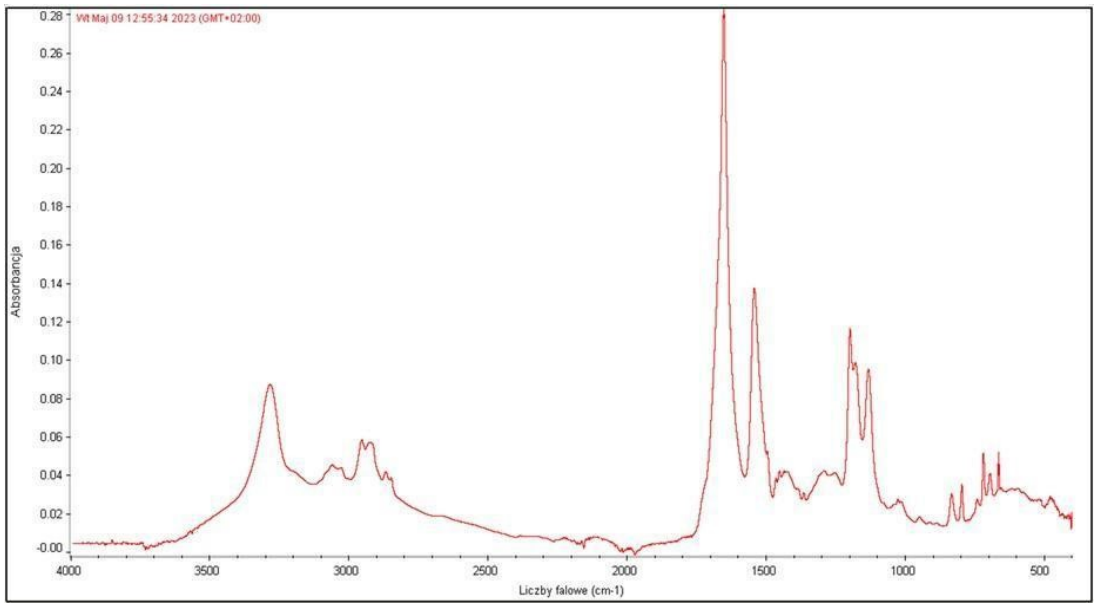


Fig. S2 Mass spectrum of PSMα3.

A



B

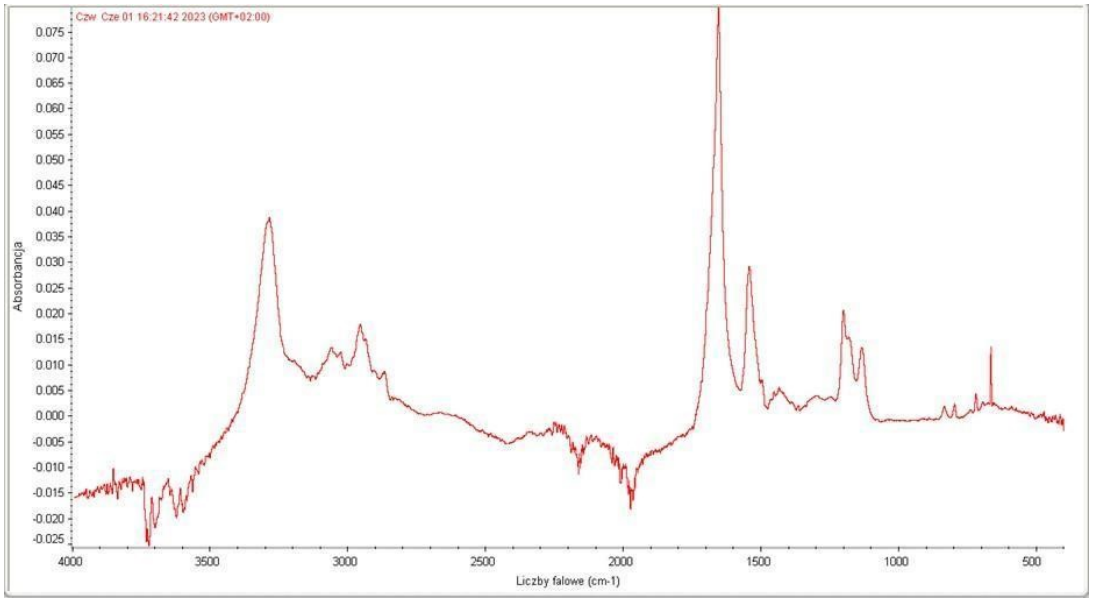


Fig. S3 Raw FTIR spectra of monomerized PSMα3 (**A**) before, and (**B**) after incubation for 72 h at 37°C.

A **B**

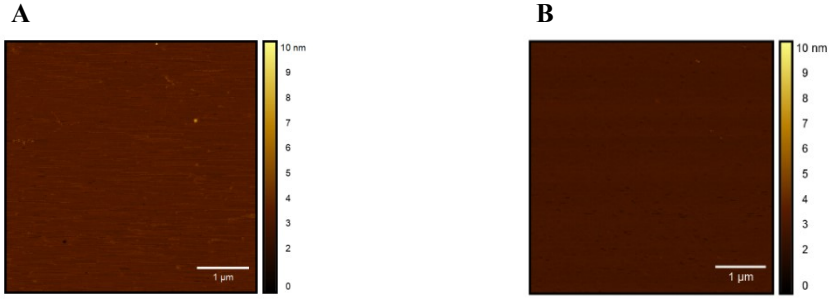


Fig. S4 AFM images of monomerized insulin (**A**) and PSMα3 (**B**) freshly dissolved in water before starting the aggregation assay. Peptide concentrations are equal to 0.5 mg/mL.

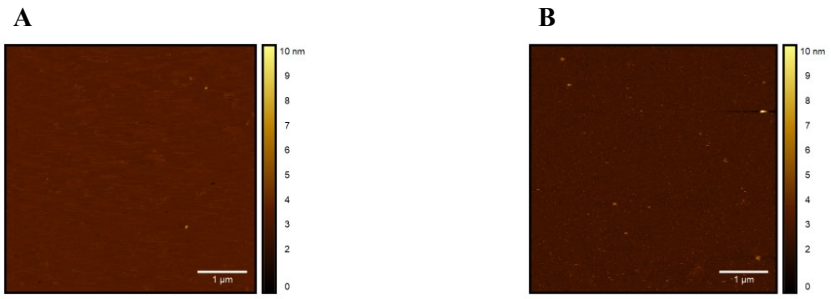


Fig. S5 AFM images of PSMα3 freshly dissolved in water (**A**) and after incubation for 72 h at 37 °C (**B**) without HFIP pre-treatment for monomerization.

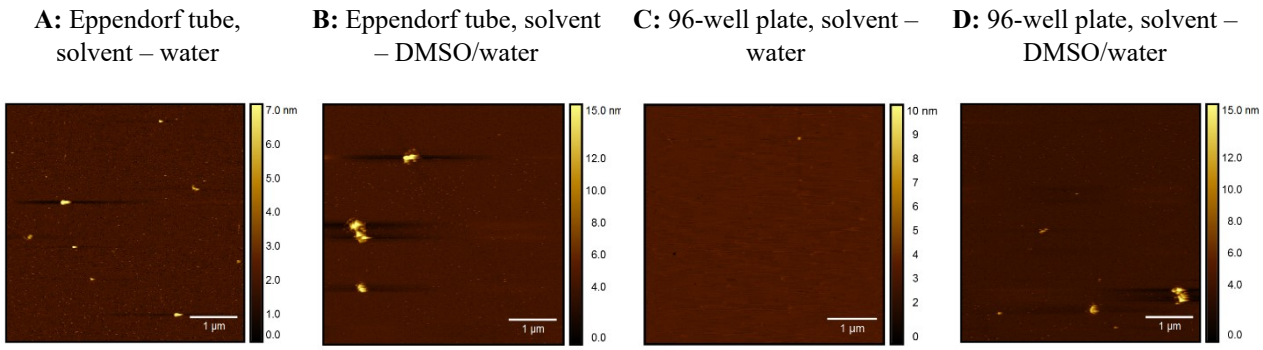


Fig. S6 AFM images of PSMα3 after incubation for 72 h at 37 °C: (**A** and **B**) in Eppendorf tubes and (**C** and **D**) in a 96-well plate; (**A** and **C**) the peptide was dissolved purely in water; (**B** and **D**) peptide was dissolved in DMSO and then diluted with water.

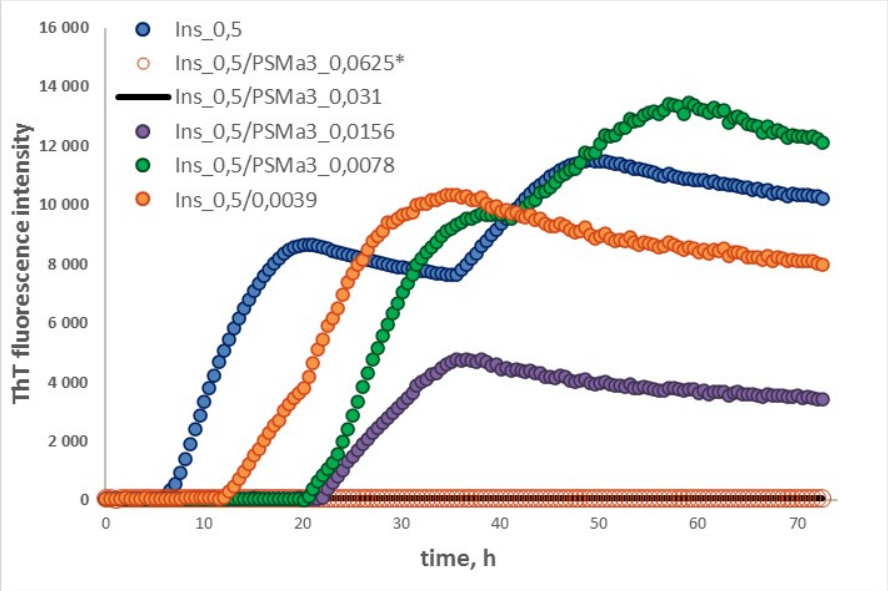
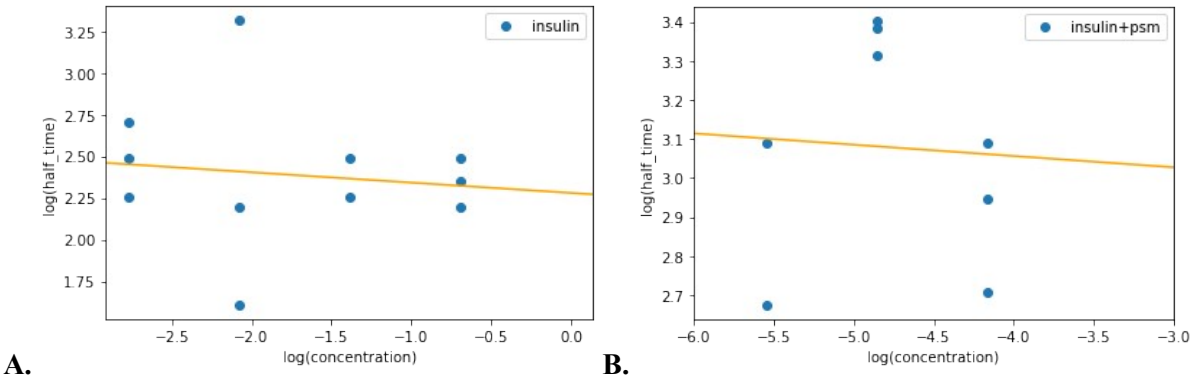


Fig. S7 ThT aggregation kinetics of insulin incubated for 72 h at 37 °C alone and co-incubated with PSMα3 in different concentrations. * There is also a full inhibition (no ThT fluorescence increase) observed in the concentrations of PSMα3 0.125; 0.25, and 0.5 mg/mL per 0.5 mg/mL of Insulin.



A. **B.**

Fig. S8 Log(half time)~log(concentration) plot **A)** for insulin and **B)** for insulin+PSMα3

A: Ins_0.5mg/mL/ PSMα3_0.5mg/mL

B: Ins_0.5mg/mL/ PSMα3_0.25mg/mL

C: Ins_0.5mg/mL/ PSMα3_0.125mg/mL

D: Ins_0.5mg/mL/ PSMα3_0.0625mg/mL

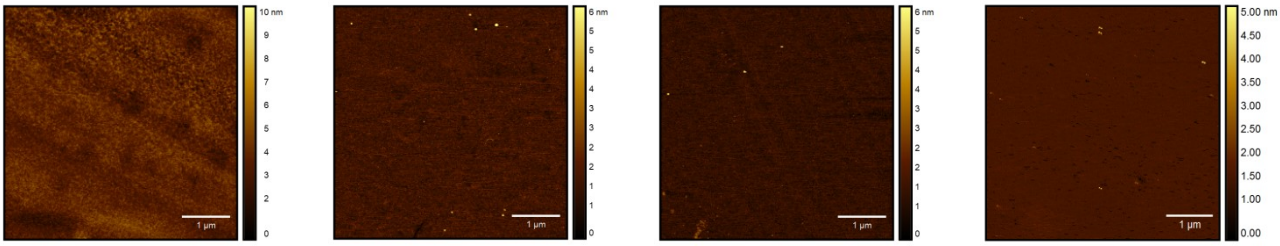
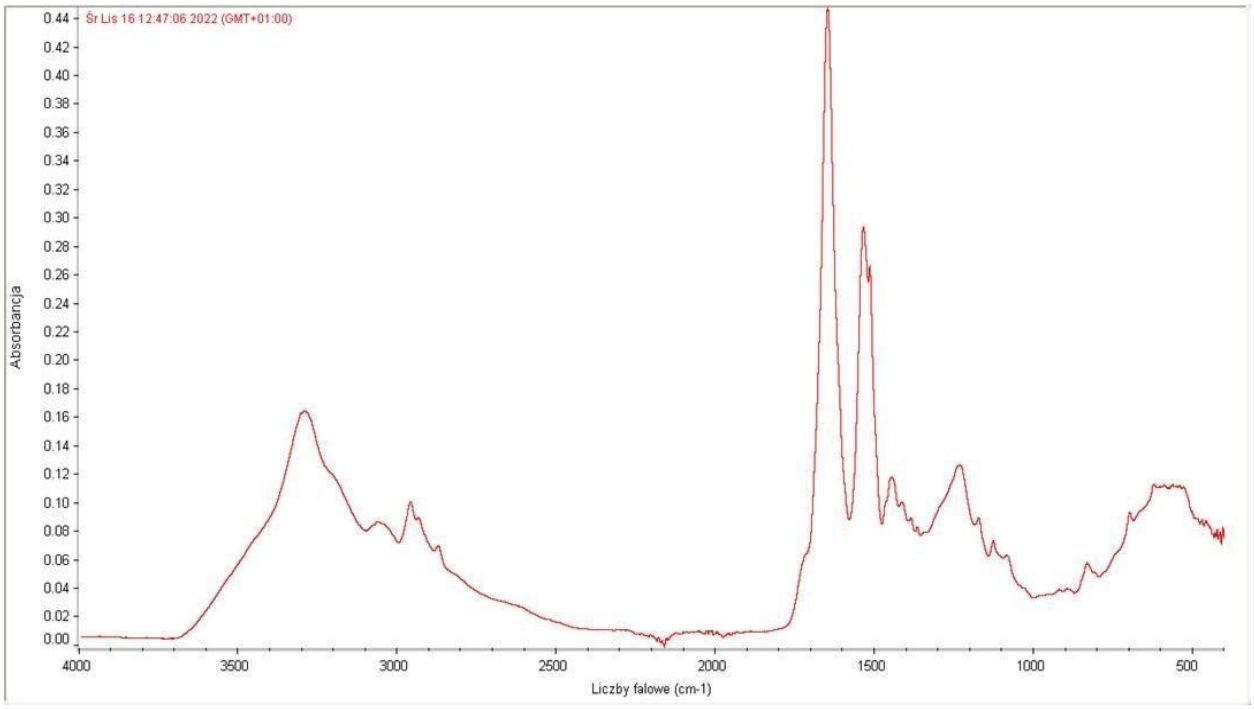


Fig. S9 AFM images of insulin after incubation alone and with PSMα3 in different concentrations.

A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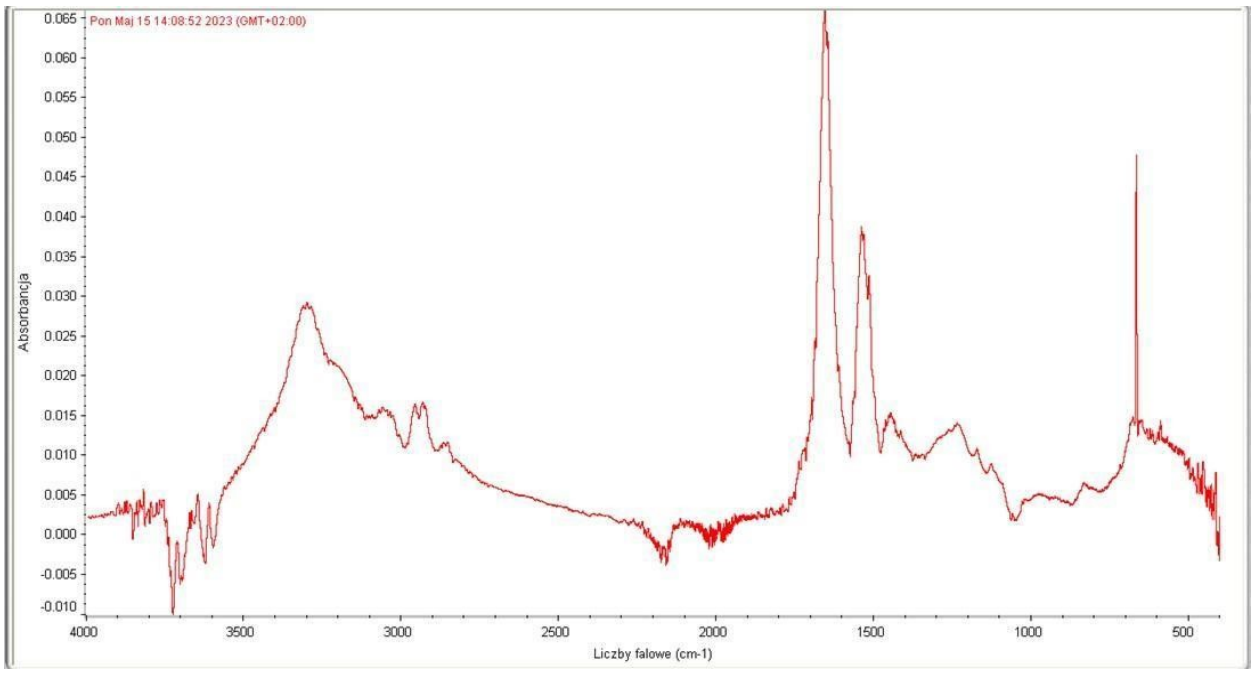
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
4000
3500
3000
2500
2000
1500
1000
500
Liczby falowe (cm-1)

B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Absorbancja
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0.060
0.055
0.050
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0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
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4000
3500
3000
2500
2000
1500
1000
500
Liczby falowe (cm-1)

C

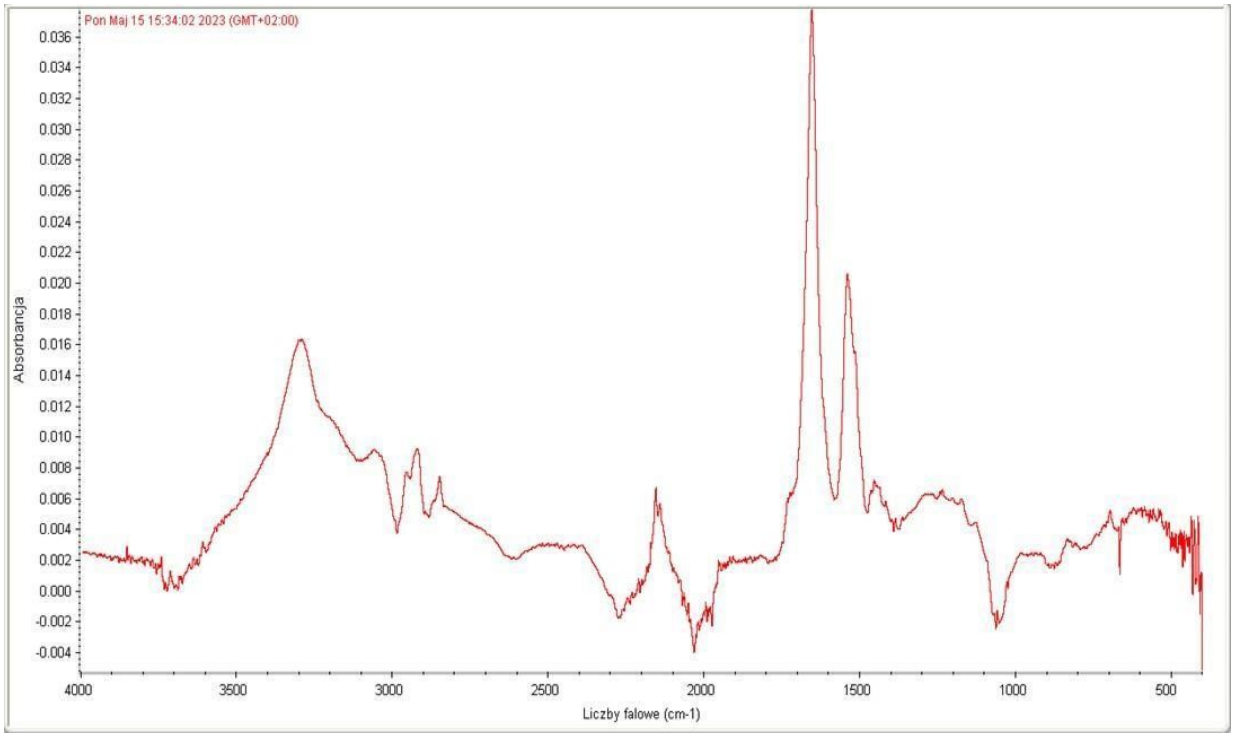

Fig. S10 Raw FTIR spectra of insulin in a concentration of 0.5 mg/mL, freshly dissolved (**A**) and after incubation for 72 h at 37 ºC, alone (**B**) and with PSMα3 at 0.031 mg/mL (**C**)

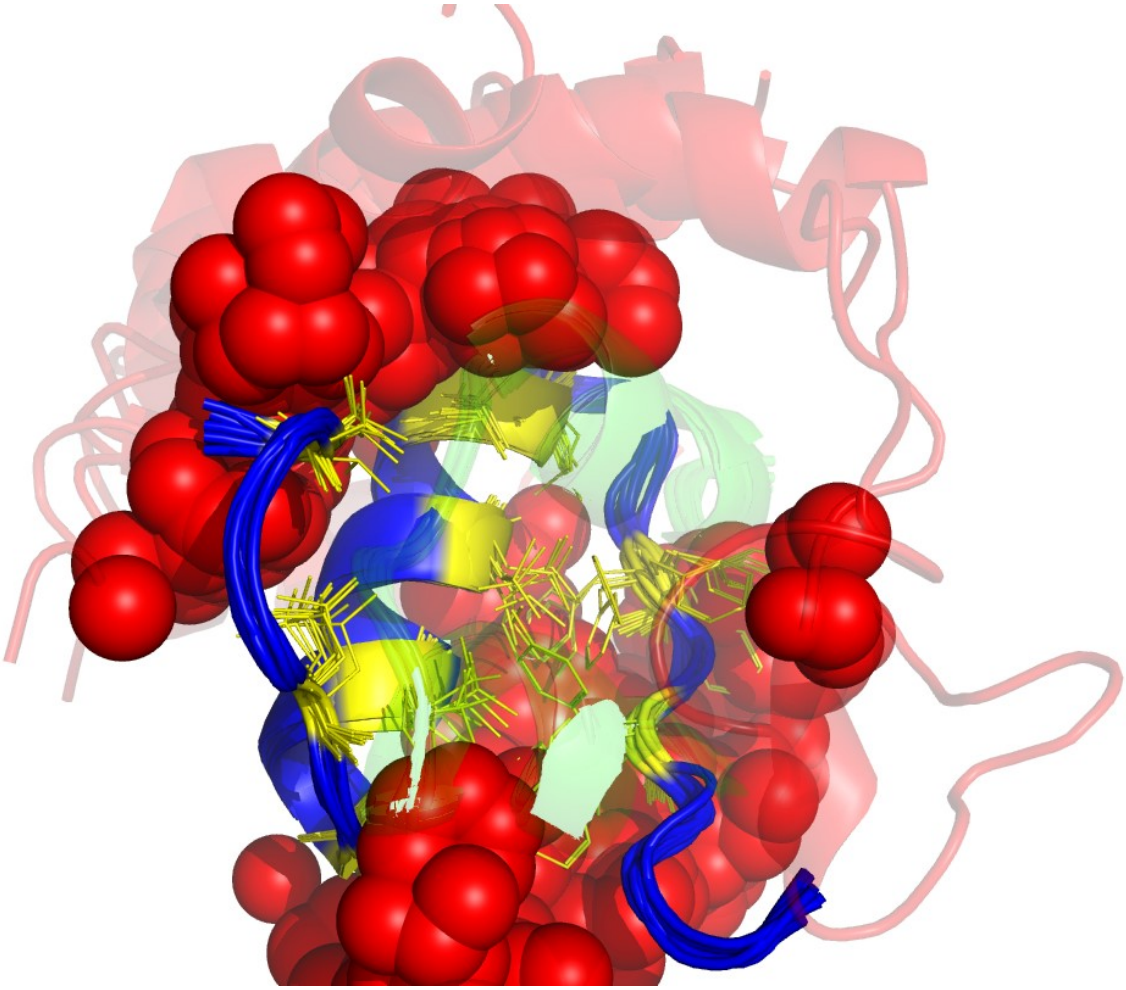

Fig. S11. Visualisation of molecular details of residue-residue contacts in the structure of PSMα3 docked to the experimental insulin structure. Insulin chain A is depicted in green, chain B in blue and PSMα3 in red. Insulin residues involved in contacts are highlighted in yellow, with sticks representing atomistic details of the contacts, and red balls represent atoms in PSMα3 which are involved in the contacts.