Extreme amyloid polymorphism in *Staphylococcus aureus* virulent PSMα peptides

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Members of the *Staphylococcus aureus* phenol-soluble modulin (PSM) peptide family are secreted as functional amyloids that serve diverse roles in pathogenicity and may be present as full-length peptides or as naturally occurring truncations. We recently showed that the activity of PSMα3, the most toxic member, stems from the formation of cross-α fibrils, which are at variance with the cross-β fibrils linked with eukaryotic amyloid pathologies. Here, we show that PSMα1 and PSMα4, involved in biofilm structuring, form canonical cross-β amyloid fibrils wherein β-sheets tightly mate through steric zipper interfaces, conferring high stability. Contrastingly, a truncated PSMα3 has antibacterial activity, forms reversible fibrils, and reveals two polymorphic and atypical β-rich fibril architectures. These architectures are radically different from both the cross-α fibrils formed by full-length PSMα3, and from the canonical cross-β fibrils. Our results point to structural plasticity being at the basis of the functional diversity exhibited by *S. aureus* PSMαs.
myelins designate peptides and proteins capable of self-assembling into structured oligomers and fibrils, and they are mostly known for their involvement in fatal neurodegenerative diseases, such as Alzheimer’s and Parkinson’s diseases. Some amyloids are functional, in that they participate in specific physiological activities. In humans, functional amyloids partake in immunity, reproduction and hormone secretion. In microorganisms, they act as key virulence factors, and may thus represent novel targets for antibacterial drugs. Amyloid fibrils are found in the self-produced polymeric matrix that embeds biofilm-forming bacteria, where they act as a physical barrier that increases their resilience and resistance to antimicrobial drugs and to the immune system. Functional bacterial amyloids may also act as toxins, killing non-self-cells and thereby increasing virulence. The structural hallmarks of functional amyloids—if any—and how they can be distinguished from disease-associated amyloids remain unclear. To date, only a single atomic resolution structure of a functional bacterial amyloid has been made available, namely, that of phenol-soluble modulin α3 (PSMa3), the most cytotoxic member of the *Staphylococcus aureus* PSMS peptide family. The structure of the full-length PSMα3 revealed cross-α fibrils, a newly discovered mode of self-assembly characterized by the piling of α-helices perpendicular to the fibril axis, in place of β-strands in canonical cross-β amyloid fibrils. Cross-α fibrils form through the tight mating of α-helical sheets, just as cross-β fibrils form through the tight mating of β-sheets; it was thus proposed that they are amyloid-like.

*Staphylococcus aureus* is a prominent cause of threatening infections and its PSMS family members serve as key virulence factors that stimulate inflammatory responses, alter the host cell cycle, lyse human cells and contribute to biofilm structuring. High expression of PSMS, which are four peptides of about 20 residues in length, increases virulence potential of methicillin-resistant *S. aureus* (MRSA). Amyloid aggregation plays roles in PSMS activities in *S. aureus*. For example, fibrillation of PSMα1 promotes biofilm stability by preventing disassembly by matrix-degrading enzymes and mechanical stress. In addition, fibrillation of PSMα3, enhances its toxicity against human cells. Still, the extent of amyloid fibrillation in all PSMS activities remains unclear, especially since PSMS are known to undergo truncation in vivo in response to various external stimuli, yielding truncated PSMS with new functions such as antibacterial activities. The structural hallmarks of functional amyloids—if any—and how they can be distinguished from disease-associated amyloids remain unclear. To date, only a single atomic resolution structure of a functional bacterial amyloid has been made available, namely, that of phenol-soluble modulin α3 (PSMa3), the most cytotoxic member of the *Staphylococcus aureus* PSMS peptide family. The structure of the full-length PSMα3 revealed cross-α fibrils, a newly discovered mode of self-assembly characterized by the piling of α-helices perpendicular to the fibril axis, in place of β-strands in canonical cross-β amyloid fibrils. Cross-α fibrils form through the tight mating of α-helical sheets, just as cross-β fibrils form through the tight mating of β-sheets; it was thus proposed that they are amyloid-like.

**Results**

The biofilm-associated PSMα1 and PSMα4 form cross-β fibrils. We found that in contrast to PSMα3, which forms cross-α fibrils, the homologous and biofilm-associated PSMα1 and PSMα4 form prototypical amyloid fibrils (Fig. 1a, b and Supplementary Fig. 1) which exhibit the cross-β signature in X-ray diffraction patterns (Fig. 1c, d), and bind the amyloid-indicator dye thiolfavin T22 (Supplementary Fig. 2). It was indeed previously shown that, in solution, PSMα1 and PSMα4 transition from α-helices to parallel β-sheets within several days, whereas PSMα3 remains helical. To obtain atomic-level insights into the architecture of PSMα1 and PSMα4 fibrils, we computationally identified their amyloidogenic spine segments (Supplementary Fig. 1), and determined their high-resolution structures by means of X-ray microcrystallography (Table 1). Segments PSMα1-IIKVIK and PSMα4-IIKIIK, both of which are conserved within naturally occurring PSM truncations, adopt the canonical cross-β fibril architecture, wherein pairs of β-sheets tightly mate through a dry interface, forming a steric zipper (Fig. 2, Supplementary Fig. 3a). Both structures belong to the class 1 of steric zippers, indicating that parallel β-sheets mate face-to-face in the fibrils formed by the PSMα1-IIKVIK and PSMα4-IIKIIK segments. X-ray diffraction from these fibrils accordingly exhibit the cross-β pattern (Fig. 2c). Proline substitutions in these spine regions abolished fibril formation of the full-length PSMα1 and PSMα4 (Supplementary Fig. 2). Structural indicators of fibril stability of the IKVIK and IIKIIK segments, i.e., buried surface area and shape complementarity between sheets, calculated from the crystal structures, resemble those of eukaryotic steric-zipper structures (Supplementary Table 1). The similarity between PSMα1 and PSMα4 segments and human pathological amyloids demonstrate that cross-β amyloids are a structural trait shared across species, from bacteria to human.

**PSMα3-LFKFFK displays antibacterial activity.** PSMα3 is toxic to human cells but certain single-point mutations are known to confer antibacterial activity. In addition, natural truncations of PSMS, which occur via proteolysis, could lead to gains in antibacterial activities. We found that the LFKFFK segment from PSMα3, identified as fibril-forming by computational methods and which indeed forms fibrils (Fig. 3b), shows dose-dependent antibacterial activity against Gram-positive *Micrococcus luteus* and *Staphylococcus hominis*, but is nontoxic to *S. aureus*, the secreting bacterium (Fig. 3a, Supplementary Fig. 4). The observation that the steric-zipper-forming segments PSMα1-IIKVIK and PSMα4-IIKIIK do not elicit antibacterial activity (Fig. 3a, Supplementary Fig. 4) indicates that fibrillation is, in itself, insufficient to confer toxicity. Likewise, toxicity of PSM segments cannot be ascribed to charges of their amino acids, since...
all three segments feature two basic and four hydrophobic side chains. Thus, the antibacterial activity of the PSMα3-LFKFFK segment could stem from the specific fibril architecture it adopts.

LFKFFK forms reversible fibrils and atypical structures. We obtained two atomic resolution structures of LFKFFK; both revealed a departure from cross-β fibrils and atypical amyloid architectures (Fig. 4). One polymorph was fundamentally different from steric-zippers, displaying no dry interface between pairs of β-sheets. Instead, hexamers of β-sheets formed cylindrical channels running along the fibril-like structure, effectively yielding nanotubes (Fig. 4a). The second polymorph was composed of out-of-register β-sheets (Fig. 4b), meaning that unlike in canonical cross-β fibrils, β-strands are not perpendicular to the fibril axis. Such extreme polymorphism is exceptional within the hundreds of structures of amyloid-like spine segments solved to date. Quantitative measures of amyloid stability based on the crystal structures (Supplementary Table 1) suggest that both polymorphs, and especially the hexameric configuration, form less stable fibrils compared to canonical steric zippers. The hexameric configuration lacks the tight interdigitation of β-sheets characteristic of canonical cross-β fibrils, such as PSMα4-IIKVIK and eukaryotic steric-zipper amyloid segments, resulting in a smaller solvent-accessible surface area buried and lower shape complementarity at the interface between the β-sheets (Supplementary Table 1). The second polymorph, featuring out-of-register β-sheets, shows intermediate quantitative measures of

| Table 1 Data collection and refinement statistics (molecular replacement) |
|-----------------------------------------------|
| **IIKVIK (PSMα1)** | **IIKIK (PSMα4)** | **LFKFFK polymorph I (PSMα3)** | **LFKFFK polymorph II (PSMα3)** |
| PDB accession code | 6FG4 | 6FGR | 6FHC | 6FHD |
| Beamline | ESRF ID23-2 | ESRF ID23-2 | ESRF ID23-2 | EMBL P14 PETRA III |
| Date | October 8th, 2014 | October 8th, 2014 | July 24th, 2014 | May 2nd, 2016 |
| **Data collection** | | | |
| Space group | C 1 2 1 | P 1 | P 6 5 | C 1 2 1 |
| Cell dimensions | | | |
| a, b, c (Å) | 45.27 4.80 22.90 | 48.22 23.06 | 35.79 9.63 | 41.03 11.73 |
| α, β, γ (°) | 90.00 107.65 90.00 | 107.00 90.01 96.20 | 90.00 121.89 90.00 | 0.9763 |
| Resolution (Å) | 21.8-1.1 (1.13-1.10) | 22.1-1.5 (1.56-1.50) | 100-1.5 (1.55-1.5) | 20.9-1.8 (1.96-1.85) |
| R-factor observed (%) | 13.3 (75.2) | 20.6 (62.2) | 8.2 (67) | 28.4 (54.7) |
| R-meas (%) | 14.0 (82.4) | 21.7 (69.4) | 13.1 (83.1) | 29.2 (56.3) |
| I / σ | 10.1 (2.1) | 7.2 (2.4) | 20.6 (2.3) | 9.1 (5.6) |
| Total reflections | 23,104 (876) | 13,816 (770) | 16,288 | 16,102 (2590) |
| Unique reflections | 2070 (136) | 1397 (158) | 1179 (118) | 913 (148) |
| Completeness (%) | 93.9 (80.0) | 95.0 (89.3) | 97.4 (98.3) | 96.3 (91.9) |
| Redundancy (%) | 11.2 (6.4) | 9.9 (4.9) | 13.8 (9.6) | 17.6 (17.5) |
| CC1/2 (%) | 99.8 (96.7) | 99.4 (97.6) | 96.5 (97.6) | 99.3 (94.6) |
| **Refinement** | | | |
| Resolution (Å) | 18.4-1.1 (1.23-1.10) | 18.2-1.5 (1.54-1.50) | 31.0-1.5 (1.55-1.51) | 19.3-1.8 (1.90-1.85) |
| Completeness (%) | 94.0 (83.5) | 95.1 (93.9) | 97.8 (98.9) | 97.3 (98.8) |
| d No. reflections | 1863 | 1257 | 1057 | 821 |
| e Rwork (%) | 15.9 (21.2) | 18.3 (26.4) | 11.8 (27.7) | 17.1 (29.7) |
| Rfree (%) | 19.4 (26.4) | 22.1 (26.1) | 16.2 (21.2) | 18.7 (43.6) |
| No. atoms | 77 | 127 | 86 | 136 |
| Protein | 65 | 102 | 60 | 120 |
| Ligand/ion | 10 | 22 | 21 | 11 |
| Water | 2 | 3 | 5 | 5 |
| B-factors | | | |
| Protein | 8.8 | 7.7 (Chain A) | 10.2 | 9.6 (Chain A) |
| Ligand/ion | 14.5 (SO4) | 37.3 (SO4) | 25.1 (CO3) | 29.1 (SO4) |
| Water | 33.6 | 26.9 | 46.5 (Cl) | 23.5 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.010 | 0.010 | 0.016 | 0.017 |
| Bond angles (°) | 1.973 | 1.903 | 1.995 | 1.893 |
| Clash score | 0.00 | 0.00 | 6.1 | 0 |
| Molprobity score | 1.33 | 0.5 | 1.33 | 1.20 |
| Molprobity percentile | 85th percentile | 100th percentile | 94th percentile | 99th percentile |
| Number of xtal used for scaling | Four spots from one crystal were used. | Four spots from one crystal were used. | One crystal | Three spots from one crystal were used. |

Values in parentheses are for highest-resolution shell
*Factor square
bR-meas is a redundancy-independent R-factor defined in
*CC1/2 is percentage of correlation between intensities from random half-datasets
*Number of reflections corresponds to the working set
*Work corresponds to working set

canonical cross-β fibrils, β-strands are not perpendicular to the fibril axis. Such extreme polymorphism is exceptional within the hundreds of structures of amyloid-like spine segments solved to date. Quantitative measures of amyloid stability based on the crystal structures (Supplementary Table 1) suggest that both polymorphs, and especially the hexameric configuration, form less stable fibrils compared to canonical steric zippers. The hexameric configuration lacks the tight interdigitation of β-sheets characteristic of canonical cross-β fibrils, such as PSMα4-IIKVIK and eukaryotic steric-zipper amyloid segments, resulting in a smaller solvent-accessible surface area buried and lower shape complementarity at the interface between the β-sheets (Supplementary Table 1). The second polymorph, featuring out-of-register β-sheets, shows intermediate quantitative measures of
behavior is reminiscent of the reversible fibril formation displayed by low-complexity protein segments associated with membraneless assemblies, which form fibrils with kinked β-sheets28,29, and by the TAR DNA-binding protein 43 (TDP-43) that can aggregate both in reversible stress granules and in irreversible pathogenic amyloid35. Similar to these human functional amyloids, the labile fibril formation by PSMa3-LFKFFK could underlie a functional role.

**LFKFFK and KLFKFFK share fibril properties and function.** The antibacterial activity of LFKFFK is preserved in the one-residue longer PSMa3 segment KLFKFFK (Fig. 3a, Supplementary Fig. 4). LFKFFK and KLFKFFK both form polymorphic fibrous structures (Fig. 3b), and the secondary structure of their fibrils analyzed using attenuated total-internal reflection Fourier transform infrared (ATR-FTIR) spectroscopy showed similar spectra with the presences of β-rich species (Supplementary Fig. 5). Specifically, the steric-zipper segment PSMa1-IIKVIK showed a peak at 1621 cm$^{-1}$ corresponding to rigid cross-β amyloid fibrils33–35, in accordance with the crystal structure (Fig. 2). Contrastingly, PSMa3-LFKFFK displayed two main peaks at 1622 cm$^{-1}$ and 1633 cm$^{-1}$, and PSMa3-KLFKFFK displayed a peak at 1633 cm$^{-1}$. The latter is typical of bent β-sheets in protein structures33–35 and was associated to disorder within amyloid fibrils33–35, in accordance with the atypical and polymorphic β-rich crystal structures of LFKFFK (Fig. 4). We propose that the antibacterial activities of LFKFFK and KLFKFFK fibrils are encoded in their unique structural properties, including their disordered and polymorphic nature.

**Discussion**

Work on human disease-associated amyloids has shown that the vast majority of amyloids are β-rich and polymorphic by nature28, which was suggested to encode different levels of neurotoxicity and prion strains36. We hypothesize that functional amyloids are even richer in polymorphisms, as they encode highly diverse functions in a reduced number of related sequences. In the present study, we found considerable structural diversity among close homologs (Supplementary Fig. 6). While PSMa3 forms cross-α fibrils and enhances toxicity against human cells10, PSMa1 and PSMa4 form cross-β fibrils (Fig. 1) which likely play a role in stabilizing the biofilm matrix14. Biofilms formed by pathogenic S. aureus strains displaying robust amyloid fibril formation indeed contained a high level of PSMa1 and PSMa4 fibrils14,37. The role of amyloid in biofilm development is evidenced in other β-rich microbial amyloids, e.g., curli CsgA from Escherichia coli7 and FabC in pseudomonas38. The highly stable steric zipper structures (Fig. 2, Supplementary Fig. 3), forming the spines of the cross-β fibrils, putatively serve as the building block cementing the biofilm and creating the rigidity that can explain the resistance of amyloid-containing biofilms8,14.

A noticeable difference between the steric zipper structures of the PSMa1 and PSMa4 segments, and the two polymorphs of the LFKFFK segment, is the orientation of the β-strands, namely parallel vs. antiparallel, respectively (Figs. 2 and 4). Fibrils with β-strands arranged in an antiparallel orientation are generally more toxic than those with a parallel arrangement39–42. Moreover, the cylindrical architecture of LFKFFK polymorph I (Fig. 4a) is reminiscent of that displayed by a polymorph of amyloid-β fibrils43, and a HET-s prion fragment44. Similarly, stable toxic oligomeric species of α-synuclein45 and a segment of the human αB-crystalline, named cylindrin29, display antiparallel β-strand orientation and cylindrical architectures. The second polymorph of LFKFFK is composed of out-of-register β-sheets (Fig. 4b), a morphology that was also suggested to serve as a pathway to toxic
amyloid aggregates\textsuperscript{26}, and the interface between the sheets is reminiscent of the KLVFFA segment of amyloid-\(\beta\).\textsuperscript{22} Thus, the two different polymorphs of the antibacterial peptide LFKFFK display features which, in human disease-associated amyloids, correlate with toxicity. Classical steric-zippers and cross-\(\beta\) mature fibrils of amyloids are considered to lack the neurotoxicity that has been attributed to smaller, transient, oligomers\textsuperscript{46}. This points to transient, less stable and often reversible species with self-assembly properties as the toxic entity in pathological amyloids. We correspondingly suggest that atypical fibrils of LFKFFK encode toxicity to bacteria, while the reversible fibril formation provides means to regulate activity.

![Fig. 3](image-url) Antibacterial activity of the fibril-forming LFKFFK from S. aureus PSM\(\alpha\)3. Disc diffusion assay testing antibacterial activity against different bacteria. In this assay, the antibacterial agent diffuses into the agar and inhibits germination and growth of the test microorganism. a LFKFFK and KLFKFFK segments from PSM\(\alpha\)3, but not the steric-zipper forming segments PSM\(\alpha\)1-IIKVIK and PSM\(\alpha\)4-IIKIIK, showed dose-dependent antibacterial activity against M. luteus and S. hominis. LFKFFK and KLFKFFK were not toxic to S. aureus, the bacterium which secretes PSM\(\alpha\)3. Discs soaked with only DDW or Dimethyl-sulfoxide (DMSO) served as controls. The results of the disc diffusion assay coincide with the antibacterial activity seen in solution (Supplementary Fig. 4). b Electron micrographs visualizing fibrils and nano-crystals formed by LFKFFK, scale bar represents 400 nm, and straight and twisted crystalline fibrils of KLFKFFK, scale bar represents 200 nm.

![Fig. 4](image-url) Structural polymorphism of the LFKFFK segment from S. aureus PSM\(\alpha\)3. a Crystal structure of polymorph I of the PSM\(\alpha\)3 spine segment LFKFFK determined at 1.5 Å resolution. The structure reveals a unique amyloid-like hexameric architecture, which forms elongated cylindrical cavities along the fibril axis. The view is down the fibril axis. The segments are shown in ribbon representation, with side chains shown as sticks with gray carbons and blue nitrogen atoms. Water molecules (oxygen in red) and chloride ions (green) that counteract the charge of the lysine side chains, are shown as small spheres. b Crystal structure of LFKFFK polymorph II determined at 1.85 Å resolution, revealed a rare amyloid-like architecture of out-of-register \(\beta\)-sheets, in which each \(\beta\)-strand is at an angle of ~50° from the fibril axis, instead of the close to 90° angle found for in-register sheets. In both polymorphs, the \(\beta\)-sheets are composed of anti-parallel strands. In the left panel, the view is perpendicular to the fibril axis, and in the right panel, the view is down the fibril axis. The segments are shown in ribbon representation, with side chain shown as sticks. The carbons within each \(\beta\)-sheet are colored either gray or light blue, and nitrogen atoms in side chains are colored blue.
The PSMα3 sequence offers a fascinating peek at the complexity of the amyloid fold, populating β-rich amyloid states in the truncated antibacterial LFKFFK form, but forming cross-α fibril in the context of cytotoxic full-length PSMα3. By not representing a spine that recapitulates the properties of the full-length PSMα3, the LFKFFK segment exemplifies how an array of activities can be derived from a single polypeptide, through generation of shorter derivatives with different structural properties. This proposal is in agreement with previous observations that PSMs undergo truncations in vivo via proteolysis, yielding derivatives endowed with new functions such as antibacterial activities.

The biological relevance of the LFKFFK segment is supported by the observation that LFKFFK is nontoxic to the PSMα3-secreting S. aureus, but toxic to the closely related S. hominis and to M. luteus (Fig. 3). This suggests a mechanism by which S. aureus is able to adapt to the toxic fold of LFKFFK fibrils.

A link between amyloids and antibacterial activity has been previously proposed, following the observations that several human host defense peptides form amyloid-like fibrils and that several human pathological amyloids display antimicrobial action. LFKFFK presents an additional example. Moreover, LFKFFK associates antibacterial activity with atypical and reversible fibril architectures, which differ from those yielding the highly stable fibrils playing a structural role in biofilms. Remarkably, the shortest recognized peptide that can facilitate antimicrobial activity by molecular self-assembly is diphenylalanine (FF), which was first identified as the core recognition module of amyloid-β involved in Alzheimer’s disease. FF forms stiff nanotubes, and its crystal structure revealed channels formed via hexagonal symmetry forming elongated tubes. The FF motif is present in the LFKFFK segment, and the hexameric structure of LFKFFK resembles the FF nanotubes. This further support the role of self-assembly in antibacterial activity and specifically the hexameric arrangement that forms channels along the fibril axis.

Together we found that PSMs demonstrate a vast structural diversity of amyloid-like structures, including cross-α, cross-β, out-of-register β-sheets, and hexameric configurations (Supplementary Fig. 6). By simultaneously secreting PSMs located at the same operon, and by truncations of these peptides, the S. aureus bacterium generates various virulent activities encoded by diverse amyloid morphologies. PSMs have critical and diverse roles during infection and represent a promising target for anti-staphylococcal therapy. The atomic structures of the PSMα1 and PSMα4 derivatives offer templates for the design of anti-biofilm compounds. In addition, the antibacterial activity of LFKFFK and KLFKFFK may be advantageous in the development of antimicrobial peptides based on amyloid segments.

**Methods**

**Peptides and reagents.** PSM native peptides and shorter derivatives (custom synthesis) at >98% purity (see Table 2) were purchased from GL Biochem (Shanghai) Ltd., as well as from GenScript. The short (6–7 residues) PSMα segments were synthesized with capped termini (acylated in the N-terminus and amidated in the C-terminus) or with unmodified termini for crystallography. PSMα full-length peptides were synthesized with unmodified termini. Hexa-fluorosorpanol (HFIP) and Thioflavin T (ThT) were purchased from Sigma-Aldrich. Ultra-pure water was purchased from Biological Industries.

**Peptides pre-treatment.** Lyophilized synthetic PSMs were dissolved in HFIP to a concentration of 0.5 mg ml\(^{-1}\) followed by a 10 min sonication in a bath-sonicator at room temperature. The organic solvent was evaporated using a mini rotational vacuum concentrator (Christ, Germany) at 1000 rpm for 2 h at room temperature. Treated peptides were aliquoted and stored at −20 °C prior to use.

**Computational prediction of amyloid spine segments.** Amyloidogenic propensity of segments from PSMα were predicted using combined information from several computational methods, including ZipperDB, Tango, Waltz, and Zyggregator.

**Fibril X-ray diffraction.** Pre-treated PSMα1 and PSMα4 peptide aliquots were redissolved in DMSO to 20 mM and immediately diluted 2-fold in ultra-pure water. Short spin peptide with capped termini (IIKVIK from PSMα1, IKVIK from PSMα4) were re-dissolved to 20 mg ml\(^{-1}\) in ultra-pure water. To prepare the sample, 2 μl droplets of the peptide solution were placed between two sealed-end...
Table 2 PSMα wild type (WT) and mutant peptide sequences (Uniprot accession codes are in parenthesis)

| Peptide name | Sequence |
|--------------|----------|
| PSM α1 (H98RQ5) | MIAGAIVIKIKSLIEQFTGK |
| PSM α1 mutant I7/P/K9 | MIAGAIPVIKIKSLIEQFTGK |
| PSM α2 (H98RQT) | MEVARKFLKFKDLGKFLGGN |
| PSM α4 (H98RQ8) | MAIVGTIKIKAI1DIFAK |
| PSM α4 mutant I6/P/110P | MAIVGTIPKIKAI1DIFAK |

Segments of short-derivatives used here are marked in bold and introdused preline substitutions are underlined.

glass capillaries. PSMα1 and PSMα4 were incubated at 37 °C for 3 days to allow fibril formation. IVKIK and IIKIK were incubated at room temperature until the drop dried completely. X-ray diffraction of PSMα1 fibrils was collected at the micro-focus beamline P14 operated by EMBL, Hamburg at the PETRA III storage ring (DESY, Hamburg, Germany). X-ray diffraction of PSMα4 fibrils was collected at the micro-focus beamline ID32-2 of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. X-ray diffraction data from fibrils of IIVIKIK and IIKIK was collected at the micro-focus beamline MASSIF-3 (ID30A-3) of ESRF, Grenoble, France.

Thioflavin T fluorescence fibrillation kinetics assay. Thioflavin T (ThT) is a widely used “gold standard” stain for identifying and exploring formation kinetics of amyloid fibrils, both in vivo and in vitro. Fibrillation curves in presence of ThT commonly show a lag time for the nucleation step, followed by rapid aggregation. Pre-treated PSMα1, PSMα1 I7/P/K9, PSMα4, and PSMα4 I6/P/110P peptide aliquots were dissolved in DMSO to 10 mM and immediately diluted to 50 μM in Tris buffer pH 7.5 for PSMα1 and PSMα1 I7/P/K9, or in ultra-pure water for PSMα4 and PSMα4 I6/P/110P, containing filtered ThT diluted from stock made in ultra-pure water. Final concentrations for each reaction were 50 μM peptide (PSMα1, PSMα4, or their mutants) and 200 μM ThT. Blank solutions were also prepared for each reaction containing everything but the peptides. The reaction mixture was carried out in a black 96-well flat-bottom plate (Greiner bio-one) covered with a thermal seal film (EXCEL scientific) and incubated in a plate reader (CLARION BMG LABTECH) at a temperature of 37 °C with 500 rpm shaking for 85 s before each reading cycle, and up to 1000 cycles of 6 min each. Measurements were made in triplicates. Fluorescence was measured by excitation at 438 ± 20 nm and emission at 490 ± 20 nm over a period of about 100 h. All triplicate values were averaged, appropriate blanks were subtracted, and the resulting values were plotted against time. Calculated standard errors of the mean are presented as error bars. The entire experiment was repeated at least three times on different days.

Transmission electron microscopy. Fibrillated PSMα1 and PSMα4 samples were collected following the ThT fibrillation kinetics assay (as described above) by combining the contents of 2–3 wells from the plate. Solutions were centrifuged at 21,000 × g and the supernatant was discarded. The pellet containing the fibrils was re-suspended in 20 μl of ultra-pure water.

Fibril formation of the short segments, IIVIKIK, IIVIKIK, LFKFFK and KLFKFFK was examined for peptides dissolved directly from the powder form.

Secondary structure analysis using ATR-FTIR spectroscopy. The short spine segments (IIVIKIK from PSMα1, LFKFFK and KLFKFFK from PSMα4) were dissolved to 1 mg ml−1 in 5 mM cholic acid (sodium salt) and 0.1 M sodium borate (pH 9.0) or BHI-agar according to the bacterial strain. The antibacterial activity of PSMα4 was determined by an inoculation in BHI-agar (0.2 M Ammonium sulfate; 20% polyethylene glycol 3350. LFKFFK polymorph I; 0.2 M Sodium acetate pH 5.1; 45% polyethylene glycol 400; 0.09 M Lithium thiocyanate, 20% polyethylene glycol 3350. LFKFFK polymorph II; 0.1 M Sodium acetate pH 5.1; 45% polyethylene glycol 400; 0.09 M Lithium thiocyanate. Micro-crystals grew after a few days and were mounted on glass needles glued to brass pins. Crystals were kept at room temperature prior to data collection.

Structure determination and refinement. X-ray diffraction data was collected at 100 K. The X-ray diffraction data for IIVIKIK, IIVIKIK, and LFKFFK polymorph I was collected at the micro-focus beamline ID33 EH2 of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France; wavelength of data collection was 0.8729 Å. The X-ray diffraction data for LFKFFK polymorph II was collected at the micro-focus beamline P14 operated by EMBL at the PETRAIII storage ring (DESY, Hamburg, Germany); wavelength of data collection was 0.8729 Å. Data indexing, integration and scaling were performed using XDS/XSCALE67 or with DENZO/SCALEPACK68. Molecular replacement solutions for all segments were obtained using the program Phaser67 within the CCP4 suite.67,68 The search models consisted of available structures of geometrically idealized β-strands. Crystallographic refinements were performed with the program REFMAC.51 Structure determination and refinement were completed in the space group P2₁ 2₁ 2₁ at 1.5 Å. Refinement was done at 1.5 Å. All structure solutions were obtained using methods of data collection.

Calculations of structural properties. The Lawrence and Colman’s shape complementarity index was used to calculate the shape complementarity between pairs of sheets forming the dry interface. Area buried was calculated using AREADMOL55,55 with a probe radius of 1.4 Å. Calculations were performed using the CCP4 program.51 The summation of the differences between the accessible surface areas of one molecule alone and in contact with the other strands on the same sheet or opposite sheets, as indicated in Supplementary Table 1, constitutes the reported area buried.

Bacterial strains and culture media. Micrococcus luteus (an environmental isolate) was a kind gift from Prof. Charles Greenblatt from the Hebrew university in Jerusalem (Israel). An inoculum was grown in Luria-Bertani (LB) medium at 37 °C with 220 rpm shaking overnight. Staphylococcus hominis (ATCC 25923) was purchased from ATCC, USA. An inoculum was grown in brain-heart infusion (BHI) medium at 37 °C with 220 rpm shaking overnight.

Disc diffusion assay. Bacterial cultures were grown overnight as described above and diluted 1000-fold to a fresh media until growth reached to optical density of ~0.4 measured at 600 nm (OD600). The reference cultures were plated on LB-agar or BHI-agar according to the bacterial strain. The antibacterial activity of PSMα4 segments was examined: Lyophilized peptides were dissolved in ultra-pure water (LFKFFK and KLFKFFK) or DMSO (IIVIKIK and IIVIKIK) to a concentration of 50 mg ml⁻¹. The concentrated peptide solutions were loaded on blank anti-bacterial susceptibility discs (Oxoid, UK). Discs loaded with ultra-pure water or DMSO were used as controls. The discs were gently placed on bacteria plated agar and incubated over-night at the appropriate temperatures.

NATURE COMMUNICATIONS | DOI: 10.1038/s41467-018-05490-0
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Antibacterial activity in solution. Bacterial cultures were grown overnight as described above and diluted 1000-fold to a fresh media until growth reached to OD 600 of 0.4. Peptides were dissolved to a concentration of 10 μM (LFKFK and KLFKFK in ultra-pure water, IKVIK and IGIKI in DMSO) and diluted to 1 mM in growth media (LB for *M. luteus* and BHI for *S. hominis*). Two-fold serial dilutions of the tested peptides in LB or BHI media ranging from 250 μM to 1 μM were performed in a sterile 96-well plate. Final DMSO concentration was fixed to 2.5% in all samples. Wells containing nothing but the peptide served as controls. Bacterial growth was determined by measuring the OD 600 during a 24 h incubation at 30°/37 °C according to the bacterial strain with 250 rpm shaking in a plate reader (FLUOstar omega or CLARIOstar, BMG LABTECH). The experiment was performed in triplicates. All triplicate values were averaged; appropriate blanks were subtracted at each time point and the resulting values were plotted against peptide concentration. Calculated standard errors of the mean are presented as error bars. The entire experiment was repeated at least three times on different days.

Data availability

The data that support the findings of this study are available on request from the corresponding author. Coordinates and structure factors for the X-ray crystal structures have been deposited in the protein data bank (PDB) with accession codes 6FG4 (IKVIK), 6FGR (IGIK), 6HIC (LFKFK polymorph I) and 6FHD (LFKFK polymorph II).

Received: 12 April 2018 Accepted: 10 July 2018

Published online: 29 August 2018

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Acknowledgements
We are grateful to M. Sawaya, N. Ben-Tal, M. Chapman, N. Jain, M. Evans, A. Syed, Z. Hayouka, H. Bochunik-Tamir, O. Tabachnikov, T. Gera, E. Tamar, I. Yelin, O. Smitser, and R. Edrei for help with experiments and fruitful discussions. We acknowledge Y. Pazy-Benhar and D. Hya at the Technion Center for Structural Biology (TCSB), and the Electron Microscopy Center located at the Technion’s Department of Materials Science & Engineering, and the Russell Berrie Electron Microscopy Center of Soft Matter. This research was supported by the I-CORE Program of the Planning and Budgeting Committee and The Israel Science Foundation, Center of Excellence in Integrated Structural Cell Biology (grant no. 1775/12; DFG: Deutsch-IsraelischeProjektkooperation (DIP) (Grant No. LA 3655/1-1), Israel Science Foundation (grant no. 560/16), University of Michigan—Israel Collaborative Research Grant, and BioStruct-X, funded by FP7, J.-P. C. acknowledges financial support by CEA, CNRS, Université Grenoble Alpes, and the Agence Nationale de la Recherche (Grant No. ANR-15-CE18-0005-02). The synchrotron MX data collection experiments were performed at beamlines ID23-EX2 and MASSIF-3 (ID30A-3) at the European Synchrotron Radiation Facility (ESRF), Grenoble, France, and at beamline P14, operated by EMBL Hamburg at the PETRA III storage ring (DESY, Hamburg, Germany). We are grateful to the teams at ESRF and EMBL Hamburg.

Author contributions
N.S. and M.L. conceived the study and designed the experiments. All authors collected X-ray data. A.M. initiated experiments. N.S. performed the experiments. N.S. and M.L. solved the crystal structures with contributions from J.-P.C. N.S., J.-P.C. and M.L. wrote the paper.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-05490-0.

Competing interests: The authors declare no competing interests.

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