

Challenges in peptide solubilization – amyloids case study

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Table S1 Parameters describing the physicochemical properties of peptides that help determine their solubility.

Parameter	Description
Number of residues	Peptides shorter than 5 residues are generally soluble in water or aqueous buffer, except when the entire sequence consists of hydrophobic amino acids (Thr, Val, Met, Trp, Leu, Ile, Phe) [1].
pI	pI is the pH at which a peptide does not carry a net electrical charge [2][3][4]. At this point, a peptide is the least soluble [5] and its aggregation capacity is the highest [6].
Net charge (at pH 7)	The arithmetic average of the charges of all peptide residues depends on the pH of the solvent. It allows the determination whether a peptide is acidic (net charge < 0, peptide is positively charged), basic (net charge > 0, negatively charged) or neutral (net charge = 0). The simplest way to estimate the charge of a peptide is to assign a value of -1 to each acid residue (D, E and C-terminal -COOH) and then assign a value of +1 to each basic residue (R, K, H and N-terminal -NH ₂) [7][8][9]. There are also more complicated algorithms for calculating the value of this parameter, considering the value of pKa. Each algorithm has its limitations and can use different values of pKa, so the results may vary slightly [4][10][11]. Typically, the lower the charge, the greater the propensity for aggregation [12].
Ratio of hydrophilic residues / total number of residues*	This is the percentage of hydrophilic amino acids in the total number of residues present in the peptide sequence [11].
Average hydrophilicity*	The average hydrophilicity is the average value of the hydrophilicity assigned to each amino acid in the peptide sequence [11]. There are many different hydrophobicity scales, i.e., the Kyte-Doolittle scale or the Hopp-Woods scale [13][14]. The Kyte-Doolittle scale is widely used to detect hydrophobic regions in proteins. Regions with a positive value are hydrophobic, while a negative result indicates hydrophilicity [13]. Hopp and Woods developed their scale to identify potentially antigenic sites in proteins. The scale is essentially a hydrophilic index, in which apolar (hydrophobic) residues are assigned negative values. It is a ranking of amino acids according to their water solubility [14]. Many hydrophobic residues in a peptide make it insoluble in aqueous solutions and prone to aggregation [15][16][17].
GRAVY (Grand Average of Hydropathy)*	The hydropathy scale was also developed by Kyte and Doolittle and is related to the hydrophobicity of proteins and peptides. It considers the hydrophilic and hydrophobic properties of each amino acid side chain [5][18]. The GRAVY value for a peptide is calculated as the sum of hydropathy values of all the amino acids divided by the number of residues in the sequence. A low GRAVY range indicates well-soluble, hydrophilic peptides, while positive values are characteristic for poorly soluble, hydrophobic peptides [2][13].

*These are the parameters used most frequently in calculators to determine the hydrophobicity of peptides.

Table S2 Characteristics of selected organic solvents commonly used for the solubilisation of peptides.

Name	Characteristics of solvent
DMSO	<p>Dimethyl sulfoxide (DMSO) is one of the most used solvents to dissolve difficult-to-solubilise proteins and peptides such as amyloids [19]. It is nonvolatile, polar, aprotic, and very hygroscopic. DMSO can effectively dissolve both polar and nonpolar compounds. It can be mixed with water or other organic solvents. From a molecular dynamics perspective, DMSO functions as a hydrogen bond acceptor, removing water from the surface area of the peptide and interacting with the hydrogen bonding network of the peptide [20]. At higher concentrations of DMSO, dissociation of the aggregates occurs, and in pure DMSO the peptides are devoid of secondary structure and unfolded [21][6]. For low concentrations of DMSO, there was no clear effect on the secondary structure, but a decrease in thermal stability occurred [21]. However, the DMSO present in working solutions can cause changes in the properties and kinetics of peptide aggregation processes [19][22][23][24][16]. For example, in the case of Aβ, it was indicated that as the concentration of DMSO increases, aggregation is inhibited [20]. On the other hand, aqueous solutions with DMSO often promote aggregation (e.g., of globular proteins such as myoglobin or concanavalin A) [21][19][22]. The use of DMSO should be avoided for peptides containing Cys, Met, or Trp due to instability and oxidation of the side chains (with the formation of disulphides or sulfoxides) [25][26][16]. This type of peptides should be prepared using 1,2-ethanediol or dithiothreitol (DTT) instead [16]. Even small amounts of DMSO can disrupt UV absorbance measurements, so its use may be incompatible with some analytical methods [24][27]. Furthermore, DMSO is difficult to remove by lyophilization [28]. Taking this into account, it is recommended to use DMSO in peptide studies at a final concentration of 5% (v/v) [29].</p>
HFIP	<p>Hexafluoroisopropanol (HFIP) is a polar organic solvent characterised by low refractive index, transparency to UV light, volatility, low viscosity, and high density. These characteristics contribute to the effective use of HFIP as a solvent for studies of fluorescence and circular dichroism [19]. HFIP is thermally stable, and it is miscible with water and other polar organic solvents. Additionally, its low boiling point enables distillation processes [30][31]. From a chemical point of view, HFIP possesses strong hydrogen-bonding properties. Thus, it is possible to dissolve substances that serve as hydrogen bond acceptors [19][31].</p> <p>HFIP is widely used in the preparation of monomeric forms of synthetic amyloidogenic peptides and the solubilisation of amyloid peptides [19][24]. HFIP causes the distortion of hydrophobic interactions in amyloid aggregates, altering the existing β-sheet structure and stabilising the α-helical structure [23]. Since low concentrations of HFIP may be ineffective in disrupting oligomers [24], concentrated solutions are recommended [20]. Protocols using HFIP mostly recommend preincubation in the presence of this solvent, followed by evaporation to dryness and use of the resulting film with another solvent, such as DMSO (for complete dissociation of amyloid fibrils [20] or NH₃·H₂O [24]). In addition, a combination of HFIP with trichloromethane (TCM) or dichloromethane (DCM) can be very effective [8].</p>
TFE	<p>2,2,2-Trifluoroethanol (TFE) is a highly electrophilic solvent, miscible with water. It has decreased nucleophilicity as an effect of the electron-withdrawing connected with the presence of the three fluorine atoms. Compared to DMSO, TFE is relatively volatile, which allows it to be removed under vacuum conditions at even low temperatures [19][32]. Depending on the concentration, TFE can have an impact on the structure of the peptides and on the aggregation kinetics. It preserves secondary structures, but disrupts tertiary protein interactions by suppressing non-polar interactions [8]. TFE reduces the strength of hydrogen bonds between amides and nearby water molecules, resulting in intramolecular hydrogen bonds that stabilise the secondary structure of peptides and proteins [19][6][32]. TFE can induce and stabilise α-helices (changes from β-sheet to α-helical structure) and induce β-turns, β-hairpins and β-strands [33]. It is recommended</p>

	<p>when other solvents prove to be ineffective, also as a combination with TCM or DCM [8]. In terms of HFIP, a combination of TFE with TCM or DCM can be effective in peptide dissolution. In addition, TFE is proposed as a cosolvent in NMR spectroscopy folding studies [8] and to improve SPPS protocols [32].</p>
TFA	<p>Trifluoroacetic acid (TFA) is a strong and volatile carboxylic acid. Salts, ions, or other TFA compounds can be present in the lyophilized peptide powder because TFA can bind to the free amino terminus and side chains of positively charged amino acids and it is commonly used in peptide synthesis protocols [6][19][16][34][8][22]. As a consequence, TFA counterions can alter the properties of the synthesis product, influence secondary structure, mass, peptide solubility, or aggregation kinetics, and consequently the results of experiments [22][8].</p> <p>Low concentrations of TFA proved to be ineffective in preventing aggregation and self-assembly of Aβ, whereas pre-treatment of Aβ with concentrated TFA followed by lyophilization yielded preparations that, when dissolved in biological buffers, were in the form of monomers exhibiting random secondary structure [6].</p> <p>TFA is often recommended for highly hydrophobic peptides [16]. For IR spectroscopy techniques, it is important to note that the compound absorbs strongly in the amide I band region ($\sim 1674\text{ cm}^{-1}$). In this case, replacement of TFA with suitable ions is suggested [22], by dissolving the peptide in a solution of hydrogen chloride or phosphoric acid [34] and replacing the TFA ions with strong ions such as HCl hydrochloride [8]. These compounds lead to the formation of ion pairs that vary in hydrophobicity ($\text{Cl}^- < \text{TFA}^-$) and size ($\text{Cl}^- < \text{TFA}^-$) [22].</p>
ACN	<p>Acetonitrile (ACN) exhibits the properties of a very weak acid and a relatively weak base, much weaker than water. Its low reactivity and relatively high degree of inertness influence its popular use as a solvent [35]. It is a polar aprotic solvent acting as a hydrogen-bond acceptor. As a result of the reduced polarity of the solvent, in aqueous solutions, ACN disrupts the tertiary structure of peptides by dissolving hydrophobic regions. However, it can enhance the stability of secondary structures by altering the strength of the peptide-peptide <i>versus</i> the peptide-water hydrogen bond. The presence of ACN in water decreases the dielectric constant and thus intensifies electrostatic repulsion at low pH [27]. ACN is recommended as a solvent for hydrophobic and neutral peptides containing many uncharged hydrophobic or polar amino acids [8].</p>

Table S3 Procedures for solubilization of selected peptides.

Name of peptide	Procedure	Resulting structure	Source
Human $A\beta(1-42)^*$	<ol style="list-style-type: none"> 1. Dissolve the peptide in 10% $NH_3 \cdot H_2O$ at 0.5 mg/ml. 2. Incubate it for 10 min at room temperature (RT). 3. Sonicate for 5 min and then dispense (0.5 ml) into microfuge tubes. 4. Remove the remaining ammonium hydroxide by lyophilisation to yield a fluffy white peptide free of salt. 5. All aliquots should be stored at $-80^\circ C$. 6. ***$A\beta$ reconstituted in 60 mM NaOH at a stock concentration $\sim 200 \mu M$ was diluted to a final concentration of $5 \mu M$ in PBS (136.89 mM NaCl, 2.68 mM KCl, 6.39 mM Na_2PO_4, 1.47 mM KH_2PO_4, pH 7.4). 	Monomers	[24]
Human $A\beta(1-42)^*$	<ol style="list-style-type: none"> 1. Dissolve the peptide in TFA and dry in a nitrogen stream. 2. Dissolve the remaining film in 100% HFIP at a concentration of 1 mg/ml. 3. Sonicate for 5 min. and dry under a nitrogen stream. 4. Repeat the HFIP treatment twice more and, on final dissolution, dispense the peptide into microcentrifuge tubes. 5. After drying under a nitrogen stream, dry the peptide under vacuum for 1–2 h to give a clear film. 	Monomers	[24]
$A\beta(1-42)^{**}$ $A\beta(1-40)^{**}$	<ol style="list-style-type: none"> 1. Dissolve 1 mg of the peptide in HFIP at a concentration of $500 \mu M$ to disassemble the preformed aggregates. 2. Evaporate HFIP in vacuum and the tubes with the peptide film keep at $-80^\circ C$ until use. 3. Dissolve $A\beta$-HFIP film in water containing 0.02% NH_3 at a concentration of 10-20 μM. 4. Incubate for 5 minutes. 5. ***Dissolve with buffer (20 mM HEPES and 100 mM NaCl, pH 7.4 to a final concentration of $5 \mu M$). 	Monomers	[36]
$A\beta(1-40)$	<ol style="list-style-type: none"> 1. Dissolve the peptide in 10% $NH_3 \cdot H_2O$ and lyophilize. 2. ***Dissolve the peptide at a concentration of $77 \mu M$ in 20 mM potassium phosphate buffer with 50 mM NaCl at pH 7.3 using a solution of 93% $H_2O/7\% D_2O$. The buffer should initially be chilled to $4^\circ C$ before adding to the peptide and the sample should be kept in ice before further experiments. 	Monomers	[37]
$A\beta(1-42)^*$ $A\beta(1-40)^*$	<ol style="list-style-type: none"> 1. Dissolve the peptide in 2 mM NaOH to produce a peptide concentration of < 1 mg/ml. The peptide should be completely wetted, and the pH of the resulting solution should be ≥ 10.5. Can gently agitate. 2. Sonicate for 1 min. 3. Add 20 mM phosphate buffer, pH 7.4, to obtain a peptide concentration < 1 mg/ml. 4. Lyophilize the solution and store at $-20^\circ C$. 5. Perform filtration or SEC: Filtration: 	Mixture of monomers and low- order oligomers	[6]

	<ul style="list-style-type: none"> - The wanted filter should be placed in a new microcentrifuge tube. - The peptide should be dissolved in water at a concentration of 4 mg/ml. - Add 20 mM phosphate buffer, pH 7.4 in equal volume. - Sonicate for 1 min and transfer to the filter assembly. <p>SEC:</p> <ul style="list-style-type: none"> - Prepare 10 mM sodium phosphate, pH 7.4, using high purity water and filtrate using a 0.22 μm membrane. - Use this buffer to wash and equilibrate a 10/30 Superdex 75 HR column at a flow rate of 0.5 ml/min until a flat ultraviolet trace is observed. - Dissolve 350–400 μg of peptide in DMSO at a concentration of 2 mg/ml. - Sonicate for 1 min. - Centrifuge the peptide solution at 16,000\timesg for 10 min. - Inject 160–180 μl of the supernate into the equilibrated column and monitor the eluate using a UV detector. The protein peaks may be detected at several wavelengths (215 nm - peptide bonds, 254 nm - mercury line, 280 nm - tyrosine absorbance). - The fraction should be used immediately after isolation. 		
Human <i>Aβ</i> (1-42) <i>Aβ</i> (1-40)	<ol style="list-style-type: none"> 1. Dissolve ~100–200 μg of lyophilized peptide in cold HFIP (250ml bottle requires 10-15 min). 2. Sonicate for 5 min at RT. 3. Vortex and incubate at RT for 30 min. 4. Place the tubes on ice for 1 min. 5. Remove HFIP through evaporation under nitrogen stream/ leave the tube open in the fume hood overnight. 6. Exsiccate the remaining HFIP in a lyophilizer or centrifugal concentrator for 30 min, or in an exsiccator attached to a vacuum inlet for 2 h. 7. Store the received peptide film airtight at -20 or -80 $^{\circ}$C. 	Aggregate-free preparation	[38]
<i>Aβ</i> (1-42)	<ol style="list-style-type: none"> 1. Dissolve the peptide in cold HFIP. 2. Incubate the peptide for at least 1 h at RT. 3. Remove HFIP by evaporation. 4. Store at -20 or -80 $^{\circ}$C. 	Monomers	[39]
<i>Aβ</i> (1-42)* <i>Aβ</i> (1-40)*	<ol style="list-style-type: none"> 1. Dissolve the peptides by brief vortexing in 0.02% $\text{NH}_3\cdot\text{H}_2\text{O}$ solution at a concentration of 500 μM (2.2 mg/mL) and 250 μM respectively at 4 $^{\circ}$C. 2. Store at -80 $^{\circ}$C before assay. 3. ***Add 50 mM phosphate buffer, pH 7.5, 100 mM NaCl. 	Dissolving the peptide to then form fibres	[40]
<i>Aβ</i> (1-42)	<ol style="list-style-type: none"> 1. Dissolve the peptide at 1 mg/ml in HFIP. 2. Remove the solvent under vacuum. 3. Store the peptide film at -20 $^{\circ}$C. 4. ***Resuspend prior to use in Tris buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.4). 	Monomeric form dominates for 6 h	[41] [42]
<i>Aβ</i> (1-42)* <i>Aβ</i> (1-40)*	<ol style="list-style-type: none"> 1. Dissolve 1 mg of the peptide in 1 ml of 2 mM NaOH to the final pH 10.5. 	Oligomers 3-12 nm	[43]

	<ol style="list-style-type: none"> Sonicate for 1 min at RT. Lyophilize. ***Dissolve 200 µg of peptide in 250 µl of water and briefly vortex. Filter the solution through 8 mm diameter 0.2 µm Whatman microfuge filters at 5,000×g for 10 min at RT. Add 250 µl of filtered phosphate buffer (10 mM sodium phosphate, pH 7.4, containing 200 mM sodium fluoride) to the peptide filtrate and vortex. 		
<i>Aβ</i> (1-42)* <i>Aβ</i> (1-40)*	<ol style="list-style-type: none"> Dissolve the peptide in 2 mM HCl to receive solution at a concentration of 1 mg/ml. Sonicate for 1 min at RT. Lyophilize. ***Dissolve 200 µg of peptide in 250 µl of water and briefly vortex. Filter the solution through 8 mm diameter 0.2 µm Whatman microfuge filters at 5,000×g for 10 min at RT. Add 250 µl of filtered phosphate buffer (10 mM sodium phosphate, pH 7.4, containing 200 mM sodium fluoride) to the peptide filtrate and vortex. 	Oligomers 3-12 nm	[43]
Human <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Dissolve the peptide in 10 mM NaOH. Vortex gently to mix (less than 1 minute). To completely remove pre-aggregates, perform SEC. 	Monomers (after SEC)	[44]
Human <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Dissolve the peptide in 1% NH₃·H₂O (~80 µl for 1 mg of peptide). Dilute to a concentration of 1 mg/ml or less with your buffer (e.g. PBS). Vortex gently (less than 1 minute). To completely remove pre-aggregates, perform SEC. 	Monomers (after SEC)	[44]
Human <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Dissolve the peptide in 100% HFIP. Vortex gently. Dry the solution under a stream of nitrogen. Redissolve the remaining film in 100% HFIP at a concentration of 1 mg/ml. Sonicate for 5 minutes and dry under a nitrogen stream. Repeat the HFIP treatment twice. Aliquot into smaller volumes and dry under a nitrogen stream. Dry the peptide under vacuum for 1-2 hours to give a clear film. To completely remove pre-aggregates, perform SEC. 	Monomers (after SEC)	[44]
Rat <i>Aβ</i> (1-40)* or ** <i>Aβ</i> (1-42)* or **	<ol style="list-style-type: none"> Dissolve the peptide to a final concentration of 1 mM in 100% HFIP in glass vials and aliquote into microcentrifuge tubes. Evaporate HFIP using a SpeedVac and store it at -20 °C until use. Peptide can be resuspended in anhydrous DMSO to 5 mM concentration and sonicate for 10 min. 	Unaggregated peptides	[45] [46]
Human	<ol style="list-style-type: none"> Dissolve 0.5 mg of the peptide in 500 µl HFIP. 	Aggregate-	[20]

<i>Aβ</i> (1-40)** <i>Aβ</i> (1-42)**	<ol style="list-style-type: none"> Vortex for 1 min and visually inspect for efficient solubilization. Evaporate HFIP using a gentle stream of oxygen-free nitrogen or argon gas. Redissolve the film in 500 μl DMSO. Vortex for 1 min and visually inspect for efficient solubilization. Separated the DMSO by means of a desalting column (HiTrap™ Desalting column (cat. 17-1408-01), GE Healthcare) pre-equilibrated with 25 ml 50 mM Tris-HCl, 1 mM EDTA buffer, pH 7.4. Other buffer systems, including phosphate buffered saline, can also be used. The Aβ-containing samples should be collected in pre-cooled low-adhesion resin-coated polypropylene centrifuge tubes. 	free preparation	
Human <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Dissolve the peptide in 100 μl HFIP (purity ≥ 95%). Incubate it in RT for 3 h. Lyophilize. Store at -20 °C. Before ThT experiments, the peptide should be dissolved in 0.1% NH₃·H₂O. Then NH₃·H₂O should be evaporate by opening the vial for 20 min. 	Monomers	[47]
Human <i>Aβ</i> (1-40)* <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Dissolve the peptide at 0.7 mg/ml in water at pH 10.5. Rock gently at 4 °C for 72 h (pH can be maintained using NaOH). 	Mainly monomers	[48]
Human <i>Aβ</i> (1-42)*	<ol style="list-style-type: none"> Add 1 ml of HFIP to each glass bottle with 1 mg of peptide and seal the bottle cap with parafilm. Allow the peptide to fully dissolve in the HFIP solution for 2 h. Sonicate for 30 min. Transfer the 1 ml mixture to a 1.5 ml centrifuge tube and centrifuge the mixture solution at 13.148×g for 30 min at 4 °C. Extract 80% (0.8 ml) of the top solution, save it in four 500 μl micro tubes, each containing 0.2 ml of the top solution. Freeze the extracted solutions in a refrigerator at -80 °C and freeze-dry at -110 °C and 10 mTorr. 	Monomers	[49]
Human <i>Aβ</i> (1-42)**	<ol style="list-style-type: none"> Dissolve the peptide in HFIP. Sonicate for 30 minutes. Evaporate HFIP in a stream of gaseous N₂. Lyophilization and storage at -20 °C. Dissolving the peptide in 10 mM NaOH at a peptide concentration of 1 mg/ml (pH 12.0). ***Samples for the ThT-assay were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 μM EDTA, 1 mM NaN₃ and 20 μM ThT. 	Preaggregates present (half are monomers)	[50]
Human <i>Aβ</i> (1-42)**	<ol style="list-style-type: none"> Dissolve the peptide in 10 mM NaOH to a final concentration of 1 mg/ml. Sonicate for 30 minutes. Froze the peptide by immersion in liquid N₂ and store at - 	Preaggregates present (half are monomers)	[50]

	<p>80 °C.</p> <p>4. ***Samples for the ThT assay were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 µM EDTA, 1 mM NaN₃, and 20 µM ThT.</p>		
Human Aβ(1-42)**	<ol style="list-style-type: none"> 1. Dissolve the peptide in 50 mM NaOH at a final concentration of 1 mg/ml. 2. Sonicate for 5 minutes. 3. Froze the peptide by immersion in liquid N₂ and store at -80 °C. 4. ***Samples for the ThT test were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 µM EDTA, 1 mM NaN₃, and 20 µM ThT. 	Monomers	[50]
Human insulin (USP standard)	<ol style="list-style-type: none"> 1. Filter insulin on 25x1 cm Sephadex G-25 columns equilibrated with 1% acetic acid (removing large soluble aggregates). The solution applied to the column should be approximately 30 mg/ml in 1% acetic acid. 2. Collect the filtrate by eluting by the same eluent. 3. Store the filtrate in a fridge overnight on Bio-Rad Chelex 100 resin. 4. Lyophilize. 5. Use H₂O/D₂O:CD₃CN (65/35 vol%) as the solvent. Solutions of at least 1.7 mM concentration and pH 3.6 - adjusted by adding HCl or NaOH. 	Monomers	[51]
Human (91077C) and bovine insulin (I6634)	<ol style="list-style-type: none"> 1. Dissolve insulin in a solution at pH 1.6 (25 mM HCl) containing 0.1 M NaCl in deionized water. 2. Chill the solution to 10 °C and filter twice through a 0.2 µm syringe filter. 3. Finally, use pH cycling to precipitate insulin and then redissolve it to maximise purity and homogeneity. 	Probably dimers and monomers	[52]
Human insulin	<ol style="list-style-type: none"> 1. Dissolve peptide in a buffer containing 0.1 M NaCl. 2. Adjust the pH to 1.6 by adding HCl if the solvent is H₂O or DCl if D₂O. The final insulin solution should have a concentration of 10 mg/ml. 3. Filter with a 0.22 µm polyethersulfone membrane filter. 	Mainly dimers	[53]
Human insulin**	<ol style="list-style-type: none"> 1. Dissolve the peptide in insulin buffer at a concentration of 2 mg/ml and adjust the pH to 1.6. 2. Perform defiltration in an Amicon 10 ml filtration cell (Millipore Corporation, Lexington, MA) equipped with a solvent reservoir for diafiltration. Maintain transmembrane pressure at 30 psi using compressed nitrogen. Use a 25 mm diameter regenerated cellulose filter membrane of 3 and 100 kDa MW cut off utilize (Millipore, Bedford, MA). 3. Filtrate the peptide at 10 mg/ml with a 3 kDa membrane for a total of 10 diavolumes, then reduce the volume to the original volume of the insulin sample. 4. Change the membrane to a 100 kDa membrane, filter the solution through the membrane, and collect. 	Microaggregate-free	[54]
Bovine pancreatic	<ol style="list-style-type: none"> 1. Dissolve insulin in 20 mM phosphate buffer at pH 2.0 2. Dialyze solution overnight at 4 °C against the buffer with 	No information	[55] [56]

insulin	at least three changes of it.		
Human insulin with zinc	<ol style="list-style-type: none"> 1. Dissolve the peptide in 25 mM HCl (pH 1.6). 2. Add 1M HEPES buffer to obtain a final concentration of 50 mM HEPES and adjust the pH to 7.4 with NaOH. 	Hexamers	[57]
Human insulin with zinc	<ol style="list-style-type: none"> 1. Dissolve peptide in 25 mM HCl (pH 1.6). 	Dimers	[57]
Human insulin with zinc	<ol style="list-style-type: none"> 1. Dissolve peptide in 20% acetic acid (pH 2.0). 	Monomers	[57] [58] [59]
Bovine insulin	<ol style="list-style-type: none"> 1. Dilute HCl with double-distilled water to a pH of 1.6, 2, 2.5 or 3. 2. Filter the HCl solution through a syringe filter (0.45 μm pore diameter). 3. Dissolve 5.8 mg of peptide in the previously prepared solvent to obtain a final solution of 50 mM. 	Mainly monomers	[60]
Bovine insulin and its mutants*	<ol style="list-style-type: none"> 1. Dissolve lyophilized insulin in 20% acetic acid at the highest concentration of 5.0 mM (29.0 mg/ml). 2. Remove impurities using a 0.20 μm filter membrane. 	No information	[61]
Bovine and human mutants insulin	<ol style="list-style-type: none"> 1. Dissolve the peptide in 20% acetic acid, 0.1 M NaCl, pH 2. 	Monomers	[62]
Bovine insulin	<ol style="list-style-type: none"> 1. Dissolve peptide in 20% (3.4 M) acetic acid at a concentration of 2 mg/ml. 	Monomers	[63]
Bovine insulin	<ol style="list-style-type: none"> 1. Dissolve 2 mg of insulin fibrils in 1 ml of 25% (14 M) $\text{NH}_3\cdot\text{H}_2\text{O}$ at 60 °C for 2 h. 2. Add 3 μl of 30% (10 M) H_2O_2 and incubate at RT for 12 h. 	Completely dissolved - monomers ~2 nm	[63]
<i>S. aureus</i> PSM α (1-4) (phenol-soluble modulins) ** (formylation)	<ol style="list-style-type: none"> 1. Dissolve peptides at a concentration of 0.5 mg/ml in a 1:1 mixture of HFIP and TFA. 2. Sonicate 5\times20 s at 30-s intervals. 3. Incubate in RT for 1 h. 4. Evaporate the HFIP/TFA mixture with a speedway at 1000 rpm for 3 h at RT. 5. Store the dried peptide at -80 °C. 	Monomers (after use DMSO)	[64] [65]
<i>S. aureus</i> PSM α (1,4) *	<ol style="list-style-type: none"> 1. Dissolve the peptides in HFIP at a concentration of 0.5 mg/ml. 2. Sonicate for 10 minutes in an ultrasonic bath at RT. 3. Evaporate the HFIP using a mini rotary vacuum concentrator at 1000 rpm for 2 hours at RT. 4. Divide the peptides into aliquots and store at -20 °C until use. 	Monomers (after use DMSO)	[66]

	<ol style="list-style-type: none"> Dissolve the peptide in DMSO at 10 mM. ***Peptides were diluted to 50 μM in Tris buffer pH 7.5 or in ultrapure water. The final concentrations of the peptides were 50 μM. 		
<i>S. aureus</i> PSM α (1-4)* (formylation)	<ol style="list-style-type: none"> Dissolve the peptide in TFA-HFIP (1:1) at a concentration of 1 mg/ml. Sonicate for 10 minutes. Evaporate the solvents using a centrifugal vacuum concentrator (miVac) for 1 day. Store the peptide at -20 $^{\circ}$C. 	No information	[67]
<i>S. aureus</i> PSM α (1-4)*	<ol style="list-style-type: none"> Dissolve the peptides in HFIP and titrate with NH₃·H₂O (30%) to ensure clarity of the solution - monomeric and soluble forms. Prepare and store 10 mM stock solution at -20 $^{\circ}$C until use. Thaw the solution for the experiment, separate the required amount and evaporate (0–40 mbar) for 2 hours to eliminate HFIP. ***Dissolve the dried sample in DIW (doubly ionized water), vortex, and incubate at RT (peptide concentration 444 μM). ***Dilute the sample to a concentration of 400 μM adding DIW and buffer (HEPES, pH = 7.4, 50 mM in kinetic experiments, 5 mM in CD experiments, prepared with DIW, 18.2 MΩcm). 	Probably monomers	[68]
<i>S. aureus</i> PSM α (1-4)*	<ol style="list-style-type: none"> Dissolve the peptide at a final concentration of 0.5 mg/ml in a 1:1 mixture of TFA and HFIP. Sonicate for 10 minutes. Incubate for 1 hour at RT. Evaporate the TFA/HFIP solvent. Store samples at -80 $^{\circ}$C. *** If necessary, the samples were resuspended in anhydrous DMSO (5%) and sonicated for 10 minutes. ***Samples were dissolved in MilliQ water to obtain a final concentration of peptides of 200 μM. 	Monomers	[69]

*Synthetic peptide, **recombinant peptide, and *** additional steps of the procedure, intended to form fibrils by incubating the peptides in a specific solvent.

Amyloid beta (A β) derived from the amyloid precursor protein (APP), which is cleaved by beta secretase and gamma secretase to yield A β in a cholesterol-dependent process and substrate presentation [70]. Of the numerous types of A β proteins: A β (1-40) and A β (1-42) are the most abundant. The difference between A β (1-42) and A β (1-40) is that A β (1-42) has two extra residues at the C-terminus (I and A). The dimers of these proteins are arranged to form two hydrophobic cores (fragments 17-21 and 32-C-terminus are hydrophobic) terminated by a salt bridge at the end with a hydrophilic outer surface [71]. A β (1-42), due to the presence of two additional hydrophobic amino acids, is generally more hydrophobic than A β (1-40), which is associated with a greater propensity for its aggregation [24][72].

Insulin, like A β , shows the ability to form amyloid fibrils [73]. In organisms, it acts as a hormone, taking part in glucose management, blood glucose regulation, and metabolism. Insulin is identified as a small two-chain protein with a predominantly α -helical structure. Insulin chain A is composed of 21 residues, while insulin chain B has 30 residues [74][75]. These chains are connected by two interchain disulphide bonds, while a third disulphide bridge is found within the A chain [75]. Insulin easily forms fibrils under experimental conditions [74][75]. They can be formed under conditions of elevated temperature and low pH [74][75]. The fibrillar structure takes the form of two to six braided protofilaments, and its model indicates that most of the insulin molecule (A and B chain) is integrated into the core of the cross- β fibril [75].

Phenol-soluble modulins peptides (PSMs) from *S. aureus* is an example of a functional amyloid. PSMs are involved in many biological functions important for staphylococcal pathogenesis [64]. For example, they serve as key virulence factors that stimulate inflammatory responses [66]. Moreover, they are capable of aggregation, with the resulting fibrils likely to stabilise and strengthen the biofilm [64][65]. PSMs are classified by length into α -type (PSM α 1 - PSM α 4) and β -type (PSM β 1 and PSM β 2) [64]. They can exist as full- or shortened-length peptides [66]. All PSM α s have a conserved α -helical structure under physiological conditions. Their lytic activity may be related to the hydrophobic nature of the α -helical motif, since these properties promote aggregation. Aggregated peptides are less able to interact with membranes because they have a hidden site that allows this process, which reduces their destructive action. Therefore, for example, PSM α 3 remains in the α -helical conformation for a long time, while PSM α 4 aggregates quickly by switching to a β -sheet structure [69]. Furthermore, even though PSM α 1 and PSM α 3 possess 7 identical and 10 similar amino acids in their sequence, they show distinct aggregation behaviour as PSM α 3 aggregates approximately 50 times faster than PSM α 1 [65]. Helical propensities alone cannot explain the differences in amyloid potential and toxicity of peptides PSM α 1 and PSM α 2, although the results suggest that the functions of PSMs rely on a fine balance of hydrophobic/hydrophilic forces and α -helical propensity [69].

Table S4 Sequences of the peptides from Table S3.

Name of peptide	Sequence source	Sequence
<i>Aβ</i> (1-40)	P05067 (672-711)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
<i>Aβ</i> (1-42)	P05067 (672-713)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Human insulin	A6XGL2-1 [76]	GIVEQCCTSICSLYQLENYCN (chain A) FVNQHLCGSHLVEALYLVCGERGFFYTPKT (chain B)
Bovine insulin	A5PJB2-1 [76]	GIVEQCCASVCSLYQLENYCN (chain A) FVNQHLCGSHLVEALYLVCGERGFFYTPKA (chain B)
PSM α 1	[65]	MGIAGIIKVIKSLIEQFTGK
PSM α 2	[65]	MGIAGIIKFIKGLIEKFTGK
PSM α 3	[65]	MEFVAKLFKFFKDLLGKFLGNN
PSM α 4	[65]	MAIVGTIIKIKAIDIFAK

Table S5 Physicochemical properties of the peptides from Table S3. Part I.

Name of peptide	Number of residues [77]	Net charge at pH 7 [11]	pI [77]	pI [11]	pI [78]	GRAVY (Kyte-Doolittle) [77]	Ratio of hydrophilic residues / total number of residues [%][Hopp-Woods][11]	Average hydrophilicity [Hopp-Woods] [11]
<i>Aβ</i> (1-40)	40	-2.72*	5.31	5.76	5.42	0.06	33	-0.06
<i>Aβ</i> (1-42)	42	-2.72	5.31	5.76	5.42	0.20	31	-0.11
Human insulin	51	-2.08	5.39	6.04	5.46	0.22	29	-0.50
Bovine insulin	51	-2.08	5.39	6.04	5.46	0.31	29	-0.50
PSM α 1	21	3.00	9.70	10.89	10.50	0.96	29	-0.30
PSM α 2	21	4.00	10.00	11.06	10.80	0.89	24	-0.23
PSM α 3	22	3.00	9.52	10.60	10.32	0.31	36	-0.21
PSM α 4	20	3.00	9.70	10.89	10.50	1.70	20	-0.48

*Individual numerical values were determined using available chemical calculators.

Table S6 Physicochemical properties of the peptides from Table S3. Part II.

Name of peptide	Amino acids [%] [78]*						
	Aliphatic	Aromatic	Non-polar	Polar	Charged	Basic	Acidic
<i>Aβ(1-40)</i>	32.5	17.5	60.0	40.0	30.0	15.0	15.0
<i>Aβ(1-42)</i>	35.7	16.7	61.9	38.1	28.6	14.3	14.3
Human insulin	25.5	17.6	60.8	39.2	15.7	7.8	7.8
Bovine insulin	29.4	17.6	64.7	35.3	15.7	7.8	7.8
PSMα1	42.9	4.8	66.7	33.3	19.0	14.3	4.8
PSMα2	38.1	9.5	71.4	28.6	23.8	19.0	4.8
PSMα3	27.3	22.7	63.6	36.4	27.3	18.2	9.1
PSMα4	60.0	5.0	75.0	25.0	20.0	15.0	5.0

*Individual numerical values were determined using available chemical calculators.

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