Supporting Information

Challenges in Peptide Solubilization – Amyloids Case Study

Oliwia Polańska, Natalia Szulc, Rafał Stottko, Mateusz Olek, Julita Nadwodna, Marlena Gąsior-Głogowska, and Monika Szefczyk*

Challenges in peptide solubilization – amyloids case study

Oliwia Polańska, Natalia Szulc, Rafał Stottko, Mateusz Olek, Julita Nadwodna,
Marlena Gąsior-Głogowska, Monika Szefczyk

Content	Page Number
Table S1 Parameters describing the physicochemical properties of peptides that help determine their solubility.	S2
Table S2 Characteristics of selected organic solvents commonly used for peptide solubilization.	S3
Table S3 Procedures for solubilization of selected peptides.	S5
Table S4 Sequences of the peptides from Table S3.	S12
Table S5 Physicochemical properties of the peptides from Table S3. Part I.	S12
Table S6 Physicochemical properties of the peptides from Table S3. Part II.	S12

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

Parameters	Description
Number of residues	Peptides shorter than 5 residues are usually 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 carries no net electrical charge [2][3][4]. It is at this point that a peptide is least soluble [5] and its ability to aggregate is the highest [6].
Net charge (at pH 7)	The arithmetic average of charges of all peptide's residues depends on solvent pH. 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 acidic 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 to 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 for determining peptides hydrophobicity that are most often used in calculators.

Table S2 Characteristics of selected organic solvents commonly used for peptide solubilization.

Name	Characteristics of solvent
DMSO	Dimethyl sulfoxide (DMSO) is one of the most used solvents to dissolve hard-to-solubilize proteins and peptides such as amyloids [19]. It is non-volatile, 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 out of the peptide surface area and interacting with the peptide hydrogen bonding network [20]. At higher DMSO concentrations, dissociation of 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, DMSO present in working solutions can cause changes in properties and kinetics of peptide aggregation processes [19][22][23][24][16]. For instance, 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 the instability and the oxidation of 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]. Also, DMSO is difficult to remove by lyophilization [28]. Taking the above into account, it is recommended to use DMSO in peptide studies at a final concentration of 5% (v/v) [29].
HFIP	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 fluorescence and circular dichroism studies of peptides [19]. HFIP is thermally stable, 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]. HFIP is widely used in the preparation of monomeric forms of synthetic amyloidogenic peptides and solubilization of amyloid peptides [19][24]. HFIP causes distortion of hydrophobic interactions in amyloid aggregates, disrupting 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 pre-incubation 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 $NH_3{\cdot}H_2O$ [24]). Also, a combination of HFIP with trichloromethane (TCM) or dichloromethane (DCM) can be very effective [8].
TFE	2,2,2-Trifluoroethanol (TFE) is a highly electrophilic solvent, miscible with water. It has a 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 peptides structure and aggregation kinetics. It preserves secondary structures but disrupts protein tertiary 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 when other solvents prove to be ineffective, also as a combination with TCM or DCM [8]. As for HFIP, a combination of TFE with TCM or DCM can be effective to dissolve peptides. In addition, TFE is proposed as a co-solvent in NMR spectroscopy folding studies [8] and to improve SPPS protocols [32].
TFA	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]. In 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]. 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]. 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 (~1674 cm^{-1}). It this case, replacement of the TFA with suitable ions is suggested [22], by performing a dissolution of 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 ($Cl^- < TFA^-$) and size ($Cl^- < TFA^-$) [22].
ACN	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 *vs.* 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 hydrophobic or polar uncharged amino acids [8].

Table S3 Detailed procedures for solubilization of selected peptides.

Name of peptide	Procedure	Resulting structure	Source
Human Aβ(1-42)*	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 salt free fluffy white peptide. 5. All aliquots should be stored at −80 °C. 6. ***Aβ reconstituted in 60 mM NaOH to a stock concentration ~200 μM was diluted to a final concentration of 5 μM 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 Aβ(1-42)*	1. Dissolve the peptide in TFA and dry under a nitrogen stream. 2. Dissolve the remaining film in 100% HFIP to 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 dissolving 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]
Aβ(1-42)** Aβ(1-40)**	1. 1 mg of the peptide dissolve in HFIP at a concentration 500 μM to disassemble preformed aggregates. 2. Evaporate HFIP in vacuum and the tubes with the peptide film keep at −80 °C until use. 3. Dissolve Aβ-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 μM).	Monomers	[36]
Aβ(1-40)	1. Dissolve the peptide in 10% $NH_3{\cdot}H_2O$ and lyophilize. 2. ***Dissolve peptide to 77 μM concentration in 20 mM potassium phosphate buffer with 50 mM NaCl at pH 7.3 using a 93% H_2O/7% D_2O solution. The buffer should be initially chilled to 4 °C before adding to the peptide and the sample should be kept in ice before further experiments.	Monomers	[37]
Aβ(1-42)* Aβ(1-40)*	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 < 1mg/mL. 4. Lyophilize the solution and store at -20 °C. 5. Perform filtration or SEC: Filtration: – Washed filter place in a new microcentrifuge tube. – Peptide dissolve in water at a concentration of 4 mg/mL. – Add 20 mM phosphate buffer, pH 7.4 an equal volume. – Sonicate for 1 min and transferred to the filter assembly. SEC: – Prepare 10 mM sodium phosphate, pH 7.4, using	Mixture of monomers and low-order oligomers	[6]

	high-purity water and filtrate it 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×g for 10 min. – Inject 160–180 ul of the supernate onto the equili-brated column and monitor the eluate using a UV detector. Protein peaks eluting 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 Aβ(1-42) Aβ(1-40)	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 a centrifugal concentrator for 30 min, or in an exsiccator attached to a vacuum inlet for 2 h. 7. Received peptide film store airtight at -20 or -80 °C.	Aggregate-free preparation	[38]
Aβ(1-42)	1. Dissolve the peptide in cold HFIP. 2. Incubate peptide for at least 1 h at RT. 3. Remove HFIP by evaporation. 4. Store at -20 or -80 °C.	Monomers	[39]
Aβ(1-42)* Aβ(1-40)*	1. Dissolve the peptides by brief vortexing in 0.02% $NH_3 \cdot H_2O$ solution at a concentration of 500 µM (2.2 mg/mL) and 250 µM respectively in a 4 °C. 2. Store at -80 °C before assaying. 3. ***Add 50 mM phosphate buffer, pH 7.5, 100 mM NaCl.	Dissolving the peptide to then form fibres	[40]
Aβ(1-42)	1. Dissolve the peptide at 1 mg/mL in HFIP. 2. Remove solvent under vacuum. 3. Store peptide film at -20 °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]
Aβ(1-42)* Aβ(1-40)*	1. Dissolve 1 mg peptide in 1 mL of 2 mM NaOH to the final pH 10.5. 2. Sonicated for 1 min at RT. 3. Lyophilizate. 4. ***Dissolve 200 µg of peptide in 250 µl of water and briefly vortex. 5. Filter the solution through 8 mm diameter 0.2 µm Whatman microfuge filters at 5,000×g for 10 min at RT. 6. 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]
Aβ(1-42)* Aβ(1-40)*	1. Dissolve the peptide in 2 mM HCl to receive solution at concentration 1 mg/mL. 2. Sonicated for 1 min at RT.	Oligomers 3-12 nm	[43]

	3. Lyophilizate. 4. ***Dissolve 200 μg of peptide in 250 μl of water and briefly vortex. 5. Filter the solution through 8 mm diameter 0.2 μm Whatman microfuge filters at 5,000×g for 10 min at RT. 6. 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.		
Human Aβ(1-42)*	1. Dissolve the peptide in 10 mM NaOH. 2. Vortex gently to mix (less than 1 minute). 3. To completely remove pre-aggregates, perform SEC.	Monomers (after SEC)	[44]
Human Aβ(1-42)*	1. Dissolve the peptide in 1% $NH_3 \cdot H_2O$ (~80 μl for 1 mg of peptide). 2. Dilute to a concentration of 1 mg/mL or less with your buffer (e.g. PBS). 3. Vortex gently to mix (less than 1 minute). 4. To completely remove pre-aggregates, perform SEC.	Monomers (after SEC)	[44]
Human Aβ(1-42)*	1. Dissolve the peptide in 100% HFIP. Vortex gently to mix. 2. Dry the solution under a stream of nitrogen. 3. Redissolve the remaining film in 100% HFIP to a concentration of 1 mg/mL. 4. Sonicate for 5 minutes and dry under a nitrogen stream. 5. Repeat the HFIP treatment twice. 6. Aliquot into smaller volumes and dry under a nitrogen stream. 7. Afterwards, dry the peptide further under vacuum for 1-2 hours to give a clear film. 8. To completely remove pre-aggregates, perform SEC.	Monomers (after SEC)	[44]
Aβ(1-40)	1. Dissolve 1 mg of lyophilized peptide in a glass vial in TFA. 2. Sonicate for 10 min. 3. Remove the TFA under a stream of gaseous argon. 4. Dissolve the peptides in 1 mL of HFIP. 5. Incubate the solution for 1 h at 37 °C. 6. Determine the amount of peptide in the solution (by HPLC sedimentation assay). 7. Remove the TFA under a stream of argon gas. 8. Dissolve the peptide in 2 mL of HFIP. 9. Based on the HPLC assay, split the sample 0.25 mg per tube. 10. Remove the HFIP under a stream of gaseous argon. 11. Dry the peptide for 30-60 minutes under vacuum to remove all TFA and HFIP. 12. Add 0.5 mL of 2 mM NaOH and incubate for 5 min. 13. Add 0.5 mL of 2×PBS with 0.1% sodium azide per tube. 14. Centrifuge the peptide at 386,000g overnight at 4 °C. 15. Remove supernatant and determine peptide concentration (HPLC sedimentation assay). 16. Quickly freeze the sample in dry ice and ethanol or liquid nitrogen and store at -80 °C.	Monomers	[45]
Rat Aβ(1-40)* or ** Aβ(1-42)* or **	1. Dissolve the peptide to a final concentration of 1 mM in 100% HFIP in glass vials and aliquote into microcentrifuge tubes. 2. Evaporate HFIP using a SpeedVac and store it at -20 °C until use. 3. Peptide can be resuspended in anhydrous DMSO to 5 mM	Unaggregat ed peptides	[46] [47]

	concentration and sonicate for 10 min.		
Human Aβ(1-40)** Aβ(1-42)**	1. Dissolve 0.5 mg peptide in 500 µl HFIP. Vortex for 1 min and visually inspect for efficient solubilization. 2. Evaporate HFIP using a gentle stream of oxygen-free nitrogen or argon gas. 3. Redissolve the film in 500 µl DMSO. Samples should be mixed using a vortex for 1 min and visually inspected for efficient solubilization. 4. Separated the DMSO by means of a desalting column (HiTrapTM 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.	Agregate-free	[20]
Human Aβ(1-42)*	1. Dissolve the peptide in 100 µl HFIP (purity ≥ 95%). 2. Incubate it at RT for 3 h. 3. Lyophilizate. 4. Store at -20 °C. 5. Before ThT experiments, peptide dissolve in 0.1% $NH_3{\cdot}H_2O$. Then $NH_3{\cdot}H_2O$ evaporate by opening the vial for 20 min.	Monomers	[48]
Human Aβ(1-40)* Aβ(1-42)*	1. Dissolve the peptide at 0.7 mg/mL in water at pH 10.5 and gently rocked at 4 °C for 72 h (pH maintaine using NaOH)	Mainly monomers	[49]
Human Aβ(1-42)*	1. Add 1 mL HFIP into each glass bottle with 1 mg peptide and seal the bottle cap with parafilm. 2. Allow the peptide to fully dissolve in HFIP solution for 2 h 3. Sonicate for 30 min. 4. 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. 5. 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. 6. Freeze the extracted solutions in a -80 °C refrigerator and freeze-dry at -110 °C and 10 mTorr.	Monomers	[50]
Human Aβ(1-42)**	1. Dissolve the peptide in HFIP. 2. Sonicated for 30 minutes. 3. HFIP was evaporated in a stream of gaseous N_2. 4. Lyophilization and storage at -20 °C. 5. Dissolving the peptide in 10 mM NaOH to a peptide concentration of 1 mg/mL (pH 12.0). 6. ***Samples for ThT-assay were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 µM EDTA, 1 mM NaN_3 and 20 µM ThT.	Preaggregates present (half are monomers)	[51]
Human Aβ(1-42)**	1. Dissolve the peptide in 10 mM NaOH to a final concentration of 1 mg/mL. 2. Sonication for 30 minutes. 3. Peptide was frozen by immersion in liquid N_2 and stored at -80 °C. 4. ***Samples for ThT-assay were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 µM EDTA, 1 mM NaN_3 and 20 µM ThT.	Preaggregates present (half are monomers)	[51]

Human Aβ(1-42)**	1. Dissolve the peptide in 50 mM NaOH to a final concentration of 1 mg/mL. 2. Sonification for 5 minutes. 3. Peptide was frozen by immersion in liquid N_2 and stored at -80 °C. 4. ***Samples for ThT-assay were dissolved in 20 mM sodium phosphate buffer (pH 8) containing 200 μM EDTA, 1 mM NaN_3 and 20 μM ThT.	Monomers	[51]
Human (91077C) and bovine insulin (I6634)	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 the insulin and then redissolve it to maximize purity and homogeneity.	Probably dimers and monomers	[52]
Human insulin	1. Dissolve peptide in a buffer containing 0.1 M NaCl. Adjust the pH to 1.6 by adding HCl if the solvent is H_2O, or DCl if D_2O. The final insulin solution has a concentration of 10 mg/mL. 2. Filter with a 0.22 μm polyethersulfone membrane filter.	Mainly dimers	[53]
Human insulin**	1. Dissolve peptide in insulin buffer to a concentration of 2 mg/mL and pH adjusted to 1.6. 2. Perform defiltration in an Amicon 10 mL deadend 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. Defiltrate 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 collecte.	Microaggre gate-free	[54]
Bovine pancreatic insulin	1. Dissolve insulin in 20 mM phosphate buffer at pH 2.0 2. Dialyze solution overnight at 4 °C against buffer with at least three changes of it.	No information	[55] [56]
Human insulin with zinc	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.	Dimersk hexamers	[57]
Human insulin with zinc	1. Dissolve peptide in 20% acetic acid (pH 2.0).	Monomers	[57] [58] [59]
Bovine insulin	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 um pore diameter). 3. Dissolve 5.8 mg of peptide in the previously prepared solvent to obtain a 50 mM final solution.	Mainly monomers	[60]
Bovine insulin and its mutants*	1. Dissolve the 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	1. Dissolve the peptide in 20% acetic acid, 0.1 M NaCl, pH 2.	Monomers	[62]
Bovine insulin	1. Dissolve peptide in 20% (3.4 M) acetic acid at a concentration of 2 mg/mL.	Monomers	[63]
Bovine insulin	1. Dissolve 2 mg of insulin fibrils in 1 mL of 25% (14 M) $NH_3{\cdot}H_2O$ 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]
S. aureus PSMα(1-4) (phenol-soluble modulins) **(formylation)	1. Dissolve peptides to a concentration of 0.5 mg/mL in a 1:1 mixture of HFIP and TFA. 2. Sonicate 5×20 s at 30 s intervals. 3. Incubate at 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]
S. aureus PSMα(1,4) *	1. Dissolve the peptides in HFIP to 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. 5. Peptides were dissolved in DMSO to 10 mM. 6. ***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.	Monomers (after use DMSO)	[66]
S. aureus PSMα(1-4)* (formylation)	1. Dissolve the peptides in TFA-HFIP (1:1) to a concentration of 1 mg/mL. 2. Sonicate for 10 minutes. 3. Evaporate the solvents using a centrifugal vacuum concentrator (miVac) for 1 day. 4. Store peptides at -20 °C.	No information	[67]
S. aureus PSMα(1-4)*	1. Dissolve the peptides in HFIP and titrate with $NH_3{\cdot}H_2O$ (30%) to ensure clarity of the solution - monomeric and soluble forms. 2. Prepare and store 10 mM stock solution at -20 °C until use. 3. Thaw the solution for experiment, separate the required amount and evaporate (0–40 mbar) for 2 hours to get rid of the HFIP. 4. ***Dissolve the dried sample in DIW (doubly ionized water), vortex and incubate at RT (peptide concentration 444 µM). 7. ***Dilute the sample to 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]
S. aureus PSMα(1-4)*	1. The peptides were dissolved to a final concentration of 0.5 mg/mL in a 1:1 mixture of TFA and HFIP. 2. Sonicate for 10 minutes. 3. Incubate for 1 hour at RT. 4. Evaporate the TFA/HFIP solvent. 5. Store samples at -80 °C.	Monomers	[69]

	6. *** If necessary, samples were resuspended in anhydrous DMSO (5%) and sonicated for 10 minutes. 7. ***Samples were dissolved in MilliQ water to obtain a final peptide concentration of 200 μM.		

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

A**myloid beta** (**Aβ**) derived from amyloid precursor protein (APP), which is cleaved by beta secretase and gamma secretase to yield Aβ in a cholesterol-dependent process and substrate presentation [70]. Of the numerous types of Aβ proteins: Aβ(1-40) and Aβ(1-42) are the most abundant. Difference between Aβ(1-42) and Aβ(1-40) is that Aβ(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]. Aβ(1-42), due to the presence of two additional hydrophobic amino acids, is generally more hydrophobic than Aβ(1-40), which is associated with a greater propensity for its aggregation [24][72].

Insulin, like Aβ, 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. The A chain of insulin is composed of 21 residues, while the B chain 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 modulin 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 (PSMα1 - PSMα4) and β-type (PSMβ1 and PSMβ2) [64]. They can exist as full or shortened length peptides [66]. All PSMαs have a conserved α-helical structure in physiological conditions. Their lytic activity may be related to the hydrophobic nature of the α-helical motif, as such properties promote aggregation. Aggregated peptides are less able to interact with membranes, as they have a hidden site that allows this process, which reduces their destructive action. And thus, for example, PSMα3 remains in the α-helical conformation for a long time, while PSMα4 aggregates quickly by switching to a β-sheet structure [69]. Moreover, even though PSMα1 and PSMα3 possess 7 identical and additional 10 similar amino acids in their sequence, they show distinct aggregation behaviour as PSMα3 aggregates approximately 50 times faster than PSMα1 [65]. Helical propensities alone cannot explain the differences in amyloid potential and toxicity of peptides PSMα1 and PSMα2, although results suggest that PSM functions 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
Aβ(1-40)	P05067 (672-711)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
Aβ(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)
PSMα1	[65]	MGIIAGIIKVIKSLIEQFTGK
PSMα2	[65]	MGIIAGIIKFIKGLIEKFTGK
PSMα3	[65]	MEFVAKLFKFFKDLLGKFLGNN
PSMα4	[65]	MAIVGTIIKIIKAIIDIFAK

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 hydro-philicity** [Hopp-Woods] [11]
Aβ(1-40)	40	-2.72	5.31	5.76	5.42	0.06	33	-0.06
Aβ(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
PSMα1	21	3.00	9.70	10.89	10.50	0.96	29	-0.30
PSMα2	21	4.00	10.00	11.06	10.80	0.89	24	-0.23
PSMα3	22	3.00	9.52	10.60	10.32	0.31	36	-0.21
PSMα4	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**
Aβ(1-40)	32.5	17.5	60.0	40.0	30.0	15.0	15.0
Aβ(1-42)	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.

References

[1] Thermo, Peptide solubility guidelines, *http://wolfson.huji.ac.il/purification/PDF/Others/PIERCE_ peptideSolubGuide.pdf*.

[2] S. Enany, *Journal of Infection and Public Health*. **2014**, *4*, 296–307.

[3] E. Gasteiger, C. Hoogland, A. Gattiker, S. Duvaud, M. R. Wilkins, R. D. Appel, A. Bairoch, *The Proteomics Protocols Handbook*. **2005**, 571–608.

[4] INNOVAGEN, Documentation of the peptide calculator, *https://pepcalc.com/notes.php?all*.

[5] Univeristy of Hyderabad, A database for Physico-chemical properties of cyanobacterial proteins, *http://bif.uohyd.ac.in/cpc/help_pcp.php*.

[6] D. B. Teplow, *Methods in Enzymology*. **2006**, *06*, 20–33.

[7] Smartox Biotechnology, Peptide solubility guidelines, *https://www.sb-peptide.com/support/ solubility/*.

[8] LifeTein, Peptide Synthesis: Handling and Storage of Synthetic Peptides, *https://www.lifetein.com/handling_and_storage_of_synthetic_peptides.htmL*.

[9] GenScript, Guidelines for Dissolving Peptides, *https://www.genscript.com/site2/document/ 12464_20100407015038.PDF*.

[10] D. R. Lide, *Journal of the American Chemical Society*. **2007**, *3*, 724.

[11] Bachem, Peptide calculator, *https://www.bachem.com/knowledge-center/peptide-calculator/*.

[12] K. L. Zapadka, F. J. Becher, A. L. Gomes dos Santos, S. E. Jackson, *Interface Focus*. **2017**, *6*.

[13] J. Kyte, R. F. Doolittle, *Journal of Molecular Biology*. **1982**, *1*, 105–132.

[14] T. P. Hopp, K. R. Woods, *Molecular Immunology*. **1983**, *4*, 483–489.

[15] A. J. Luo J, WärmLänder SK, Gräslund A, *Journal of Biological Chemistry*. **2017**, *5*, 2046.

[16] ProImmune, Think peptides: the source for all peptides for your research, *https://www.proimmune.com/wp-content/uploads/2021/08/ST55.pdf?fbclid=IwAR0swtpFwM6oa9WBuq1Z9w8MjNzU-xBlZ91FO1kbnLSXQbBmyDNoOciWKno*, **2012**.

[17] P. Smialowski, A. J. Martin-Galiano, A. Mikolajka, T. Girschick, T. A. Holak, D. Frishman, *Bioinformatics*. **2007**, *19*, 2536–2542.

[18] M. Akbarian, M. Kianpour, R. Yousefi, A. A. Moosavi-Movahedi, *RSC Advances*. **2020**, *50*, 29885–29899.

[19] K. Gade Malmos, L. M. Blancas-Mejia, B. Weber, J. Buchner, M. Ramirez-Alvarado, H. Naiki, D. Otzen, *Amyloid*. **2017**, *1*, 1–16.

[20] K. Broersen, W. Jonckheere, J. Rozenski, A. Vandersteen, K. Pauwels, A. Pastore, F. Rousseau, J. Schymkowitz, *Protein Engineering, Design and Selection*. **2011**, *9*, 743–750.

[21] M. Jackson, H. H. Mantsch, *Biochimica et Biophysica Acta (BBA)/Protein Structure and Molecular*. **1991**, *2*, 231–235.

[22] M. R. Nilsson, *Methods*. **2004**, *1*, 151–160.

[23] Z. Szabó, É. Klement, K. Jost, M. Zarándi, K. Soós, B. Penke, *Biochemical and Biophysical Research Communications*. **1999**, *2*, 297–300.

[24] T. M. Ryan, J. Caine, H. D. T. Mertens, N. Kirby, J. Nigro, K. Breheney, L. J. Waddington, V. A. Streltsov, C. Curtain, C. L. Masters, et al., *PeerJ*. **2013**, *1*, 1–20.

[25] S. H. Lipton, C. E. Bodwell, *Journal of Agricultural and Food Chemistry*. **1973**, *2*, 235–237.

[26] W. E. Savige, A. Fontana, *Chemical Modification*. **1976**, *1961*, 442–453.

[27] C. L. Shen, R. M. Murphy, *Biophysical Journal*. **1995**, *2*, 640–651.

[28] Aapptec, Handling and Storage of Peptides, *https://www.peptide.com/faqs/handling-and-storage-of-peptides/*.

[29] R. 2020-11-27 Biomatic, Version 8.1, Peptide Handling (Solubility & Storage) Guideline, *https://www.biomatik.com/content/service_docs/peptide_handling_guideline.pdf*, **2020**.

[30] I. Colomer, A. E. R. Chamberlain, M. B. Haughey, T. J. Donohoe, *Catalysis from A to Z*. **2020**, 1–16.

[31] I. Colomer, A. E. R. Chamberlain, M. B. Haughey, T. J. Donohoe, *Nature Reviews Chemistry*. **2017**, 0088.

[32] D. Gimenez, A. Dose, N. L. Robson, G. Sandford, S. L. Cobb, C. R. Coxon, *Organic and Biomolecular Chemistry*. **2017**, *19*, 4081–4085.

[33] H. Reiersen, A. R. Rees, *Protein Engineering*. **2000**, *11*, 739–743.

[34] S. A. Tatulian, *Methods in Molecular Biology*. **2013**, 177–218.

[35] J. F. Coetzee, G. P. Cunningham, D. K. McGuire, G. R. Padmanabhan, *Analytical Chemistry*. **1962**, *9*, 1139–1143.

[36] A. Tiiman, J. Krishtal, P. Palumaa, V. Tõugu, *AIP Advances*. **2015**, *9*.

[37] S. Vivekanandan, B.-P. W. O. R. S.-2009 T. aggregation kinetics of A. s -amyloid peptide is controlled By.pdf, file:///C:/Users/User/OneDrive-P. W. O. R. P. O. T. AMYLOID.pdf, J. R. render, S. Y. Lee, A. Ramamoorthy, *Biochemical and Biophysical Research Communications*. **2011**, *2*, 312–316.

[38] F. Rahimi, P. Maiti, G. Bitan, *Journal of Visualized Experiments*. **2009**, *23*, 10–12.

[39] B. A. Chromy, R. J. Nowak, M. P. Lambert, K. L. Viola, L. Chang, P. T. Velasco, B. W. Jones, S. J. Fernandez, P. N. Lacor, P. Horowitz, et al., *Biochemistry*. **2003**, *44*, 12749–12760.

[40] K. Ono, K. Hasegawa, H. Naiki, M. Yamada, *Journal of Neuroscience Research*. **2004**, *6*, 742–750.

[41] H. Ahyayauch, M. Masserini, F. M. Goñi, A. Alonso, *International Journal of Biological Macromolecules*. **2021**, 611–619.

[42] H. Ahyayauch, M. Raab, J. V. Busto, N. Andraka, J. L. R. Arrondo, M. Masserini, I. Tvaroska, F. M. Goñi, *Biophysical Journal*. **2012**, *3*, 453–463.

[43] Y. Fezoui, D. M. Hartley, J. D. Harper, R. Khurana, D. M. Walsh, M. M. Condron, D. J. Selkoe, J. Lansbury, A. L. Fink, D. B. Teplow, *Amyloid*. **2000**, *3*, 166–178.

[44]

[45] B. O'Nuallain, A. K. Thakur, A. D. Williams, A. M. Bhattacharyya, S. Chen, G. Thiagarajan, R. Wetzel, *Methods in Enzymology*. **2006**, *06*, 34–74.

[46] K. L. Youmans, L. M. Tai, T. Kanekiyo, W. B. Stine, S. C. Michon, E. Nwabuisi-Heath, A. M. Manelli, Y. Fu, S. Riordan, W. A. Eimer, et al., *Molecular Neurodegeneration*. **2012**, *1*, 1–14.

[47] W. B. Stine, K. N. Dahlgren, G. A. Krafft, M. J. LaDu, *Journal of Biological Chemistry*. **2003**, *13*, 11612–11622.

[48] I. Javed, Z. Zhang, J. Adamcik, N. Andrikopoulos, Y. Li, D. E. Otzen, S. Lin, R. Mezzenga, T. P. Davis, F. Ding, et al., *Advanced Science*. **2020**, *18*, 1–15.

[49] N. D. Younan, C. J. Sarell, P. Davies, D. R. Brown, J. H. Viles, *FASEB Journal*. **2013**, *5*, 1847–1858.

[50] B. Ren, R. Hu, M. Zhang, Y. Liu, L. Xu, B. Jiang, J. Ma, B. Ma, R. Nussinov, J. Zheng, *Methods in Molecular Biology*. **2018**, 429–447.

[51] P. A. I. Taylor, P. J. Davis, L. D. Aubrey, J. B. R. White, Z. N. Parton, R. A. Staniforth, *ACS Chemical Neuroscience*. **2023**, *1*, 53–71.

[52] S. A. McBride, C. F. Tilger, S. P. Sanford, P. M. Tessier, A. H. Hirsa, *Journal of Physical Chemistry B*. **2015**, *33*, 10426–10433.

[53] A. Nayak, M. Sorci, S. Krueger, G. Belfort, *Proteins: Structure, Function and Bioinformatics*. **2009**, *3*, 556–565.

[54] C. L. Heldt, M. Sorci, D. Posada, A. Hirsa, G. Belfort, *Biotechnology and Bioengineering*. **2011**, *1*, 237–241.

[55] S. Choudhary, N. Kishore, R. V. Hosur, *Scientific Reports*. **2015**, 1–10.

[56] S. Choudhary, S. N. Save, N. Kishore, R. V. Hosur, *PLoS ONE*. **2016**, *11*, 1–18.

[57] A. Ahmad, I. S. Millett, S. Doniach, V. N. Uversky, A. L. Fink, *Journal of Biological Chemistry*. **2004**, *15*, 14999–15013.

[58] A. Ahmad, V. N. Uversky, D. Hong, A. L. Fink, *Journal of Biological Chemistry*. **2005**, *52*, 42669–42675.

[59] A. Ahmad, I. S. Millett, S. Doniach, V. N. Uversky, A. L. Fink, *Biochemistry*. **2003**, *39*, 11404–11416.

[60] J. Haas, E. Vöhrunger-Martinez, A. Bögenhold, D. Matthes, U. Hensen, A. Pelah, B. Abel, H. Grubmüller, *ChemBioChem*. **2009**, *11*, 1816–1822.

[61] P. Wang, X. Wang, L. Liu, H. Zhao, W. Qi, M. He, *Biophysical Journal*. **2019**, *3*, 533–541.

[62] L. Nielsen, S. Frokjaer, J. Brange, V. N. Uversky, A. L. Fink, *Biochemistry*. **2001**, *28*, 8397–8409.

[63] R. Liu, R. Su, Y. Yu, W. Qi, L. Wang, Z. He, *Biotechnology letters*. **2012**, *10*, 1959–1964.

[64] M. Zaman, M. Andreasen, *Microorganisms*. **2021**, *1*, 1–17.

[65] M. Zaman, M. Andreasen, *eLife*. **2020**, e59776.

[66] N. Salinas, J. P. Colletier, A. Moshe, M. Landau, *Nature Communications*. **2018**, *1*, 3512.

[67] X. Zhou, Y. Zheng, Q. Lv, D. Kong, B. Ji, X. Han, D. Zhou, Z. Sun, L. Zhu, P. Liu, et al., *Virulence*. **2021**, *1*, 1418–1437.

[68] E. Arad, K. B. Pedersen, O. Malka, S. Mambram Kunnath, N. Golan, P. Aibinder, B. Schiøtt, H. Rapaport, M. Landau, R. Jelinek, *Nature Communications*. **2023**.

[69] P. Marinelli, I. Pallares, S. Navarro, S. Ventura, *Scientific Reports*. **2016**, 1–13.

[70] I. W. HamLey, *Chemical r*. **2012**, *10*, 5147–92.

[71] P. C. Ke, M. A. Sani, F. Ding, A. Kakinen, I. Javed, F. Separovic, T. P. Davis, R. Mezzenga,

Chemical Society Reviews. **2017**, *21*, 6492–6531.

[72] J. Bridstrup, J. M. Yuan, J. S. Schreck, *Journal of the Chinese Chemical Society*. **2022**.

[73] B. Rosetti, S. Marchesan, *International Journal of Molecular Sciences*. **2023**, *2*, 1306.

[74] K. Yuzu, N. Yamamoto, M. Noji, M. So, Y. Goto, T. Iwasaki, M. Tsubaki, E. Chatani, *Biophysical Journal*. **2021**, *2*, 284–295.

[75] G. L. Devlin, T. P. J. Knowles, A. Squires, M. G. McCammon, S. L. Gras, M. R. Nilsson, C. V. Robinson, C. M. Dobson, C. E. MacPhee, *Journal of Molecular Biology*. **2006**, *2*, 497–509.

[76] A. Iyire, C. Russell, T. Dennison, R. Rajoli, I. Saleem, A. Rahman, A. Mohammed, *Journal of Advances in Biotechnology*. **2018**, *1*, 984–998.

[77] Expasy, ProtParam, *https://web.expasy.org/protparam/*.

[78] EMBL-EBI, EMBOSS Pepstats, *https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/*.