

Research article

Open Access

Amyloidogenic determinants are usually not buried

Kimon K Frousios[†], Vassiliki A Iconomidou[†], Carolina-Maria Karletidi and Stavros J Hamodrakas*

Address: Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis, Athens 157 01, Greece

Email: Kimon K Frousios - kfrousios@biol.uoa.gr; Vassiliki A Iconomidou - veconom@biol.uoa.gr; Carolina-Maria Karletidi - carolinamaria05@gmail.com; Stavros J Hamodrakas* - shamodr@biol.uoa.gr

* Corresponding author †Equal contributors

Published: 9 July 2009

Received: 7 April 2009

BMC Structural Biology 2009, 9:44 doi:10.1186/1472-6807-9-44

Accepted: 9 July 2009

This article is available from: <http://www.biomedcentral.com/1472-6807/9/44>

© 2009 Frousios et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Amyloidoses are a group of usually fatal diseases, probably caused by protein misfolding and subsequent aggregation into amyloid fibrillar deposits. The mechanisms involved in amyloid fibril formation are largely unknown and are the subject of current, intensive research. In an attempt to identify possible amyloidogenic regions in proteins for further experimental investigation, we have developed and present here a publicly available online tool that utilizes five different and independently published methods, to form a consensus prediction of amyloidogenic regions in proteins, using only protein primary structure data.

Results: It appears that the consensus prediction tool is slightly more objective than individual prediction methods alone and suggests several previously not identified amino acid stretches as potential amyloidogenic determinants, which (although several of them may be overpredictions) require further experimental studies. The tool is available at: <http://biophysics.biol.uoa.gr/AMYLPRD>. Utilizing molecular graphics programs, like O and PyMOL, as well as the algorithm DSSP, it was found that nearly all experimentally verified amyloidogenic determinants (short peptide stretches favouring aggregation and subsequent amyloid formation), and several predicted, with the aid of the tool AMYLPRD, but not experimentally verified amyloidogenic determinants, are located on the surface of the relevant amyloidogenic proteins. This finding may be important in efforts directed towards inhibiting amyloid fibril formation.

Conclusion: The most significant result of this work is the observation that virtually all, to date, experimentally determined amyloidogenic determinants and the majority of predicted, but not yet experimentally verified short amyloidogenic stretches, lie 'exposed' on the surface of the relevant amyloidogenic proteins, and also several of them have the ability to act as conformational 'switches'. Experiments, focused on these fragments, should be performed to test this idea.

Background

Amyloidoses are diseases that occur when soluble proteins undergo conformational re-arrangements and form fibrillar aggregates known as amyloid deposits. Such diseases include Alzheimer's, Parkinson's, Creutzfeldt-

Jacob's and Huntington's neurodegenerative diseases, as well as type II diabetes, prion diseases and many more. Amyloidogenic proteins are quite diverse, with little similarity in sequence and native 3D-structure [1,2]. Additionally, several proteins and peptides not related to

amyloidoses have also been shown to have the potential to form amyloid fibrils *in vitro*, suggesting that this ability for structural rearrangement and aggregation may be inherent to proteins [3].

Despite the diversity of origin, all amyloid fibrils share the same cross-beta architecture and several functional proteins found in bacteria, fungi, insects and humans have also been found to adopt the same architecture under physiological conditions, as part of their functional role [[4-8] and references therein], following our proposal for the existence of natural protective amyloids [9,10].

Evidence indicates that short sequence stretches may be responsible for amyloid formation [11,12] and several methods have been published recently, that attempt to predict amyloidogenic regions, based on various properties of proteins [[13] (TANGO), [14-20] (PASTA), [21] (AGGRESCAN), [22,23] (SALSA), [24] (Zygggregator)].

Each method makes its own assumptions and implements its own predictors, which range from quite simplistic to quite complex. The ability to form β -strands is a predominant feature in most works, either in the form of statistical propensities or in the form of structural stability. Yoon and Welsh (2004) searched for hidden beta-propensity in sequences, in other words regions that appear to be natively α -helical but have nonetheless the ability to form β -strands. Hamodrakas et al. [25] have similarly looked for "conformational switches" in sequences - regions with a high predicted tendency to form both α -helices and β -strands- using the consensus secondary structure prediction program SecStr [26] and Zibae et al. [23] looked for β -contiguity, essentially a derivative of β -strand propensity based on the Chou and Fasman [27,28] set of secondary structure preference values. In a more structural approach, Thompson et al. [19] and Zhang et al. [22] identified regions computationally that can be stable as β -strands in a stacked β -sheet crystal, similar to the one obtained from the peptides GNNQQNY and NNQQNY [29], known amyloidogenic regions from the yeast prion Sup35, while Trovato et al. [20], looked for regions with the ability to pair with each other and form β -sheets, with their program termed "PASTA".

The formation of β -strands is not the only predictor though. Conchillo-Solé et al. [21] defined a set of aggregation propensities, upon which they calculate the presence of aggregation "hot-spots" in sequences. Galzitskaya et al. [17,18] also defined a novel intrinsic property for amino acid residues, the average expected packing density, which they found to be correlated to amyloidogenesis, while Lopez de la Paz and Serrano [11] identified a sequence pattern that is involved in the formation of amyloid-like fibrils.

A variety of multi-parametric methods exist as well. Pawar et al. [16] and Tartaglia et al. [24] combine intrinsic properties of amino acid sequences to calculate aggregation propensities, while Tartaglia et al. [24] and Fernandez-Escamilla et al. [13] additionally include the effect of environmental variables in their equations for calculating aggregation rates.

We demonstrated that a consensus approach might be better suited for the task of predicting amyloidogenic stretches [25] and we developed a consensus algorithm, AMYLPRED, described below (freely available for academic users at <http://biophysics.biol.uoa.gr/AMYLPRED>), which combines some of these methods, representing most of the above mentioned categories.

As mentioned above, amyloidogenic proteins are quite diverse, with little similarity in sequence and native 3D-structure [1,2,30]. Therefore, we tried to determine a common molecular denominator to all amyloid fibril favouring regions, the so-called amyloidogenic determinants, which may dictate their ability at molecular level to form amyloid fibrils. Our efforts were guided by the superb work of Sawaya et al. [31], who reported that as many as 30 segments from fibril-forming proteins that form amyloid-fibrils, microcrystals, or usually both, all form dry 'steric zippers', which are pairs of β -sheets, with the facing side chains of the two sheets interdigitated, from 13 crystal structures of such segments.

In this work, we examined 23 proteins related to amyloidoses, taken from the detailed compilations of Harrison et al. [4] and Uversky and Fink [2]. 18 of them have experimentally determined amyloidogenic regions (hereinafter called amyloidogenic determinants) [[4] and refs. therein, [17,18] and refs. therein], and of these, 7 have experimentally solved structures. The remaining 5 proteins have experimentally solved structures, but no experimentally determined amyloidogenic regions. We thoroughly examined the experimentally solved structures and the experimentally determined amyloidogenic determinants and we have found that almost all experimentally determined, and a large percentage of predicted amyloidogenic regions by our consensus prediction algorithm AMYLPRED (found at <http://biophysics.biol.uoa.gr/AMYLPRED>), which predicts amyloidogenic determinants from sequence, are only partially buried into the hydrophobic cores of the solved protein structures, thus requiring only a slight (perhaps local) unfolding to occur, for the formation of aggregates and subsequent formation of amyloid fibrils.

Results

Since proteins related to amyloidoses vary in sequence and 3D-structure and there are no profound similarities

either in sequence or structure of these proteins [1,2,30], 23 such proteins were extracted from the detailed works of Harrison et al. [4] and Uversky and Fink [2] and our attempts were focused on identifying common structural features for these proteins.

18 of them have experimentally determined amyloidogenic short stretches ('amyloidogenic determinants') [[4] and refs. therein, [17,18] and refs. therein] and they are shown in Additional file 1. 7 of these 18 proteins, clearly indicated in Additional file 1, have experimentally determined 3D-structures (the relevant PDB ID's of these structures are given in this file).

The remaining 5 amyloidogenic proteins, shown in Additional file 2, have experimentally solved 3D-structures, as shown in this file, but, unfortunately, no experimentally determined amyloidogenic regions.

We developed a consensus prediction algorithm of amyloidogenic determinants, from sequence alone, called AMYLPRED (see 'Materials and Methods') and we wanted to compare its results against those of the five individual methods it combines. Therefore, we used as a test set these 23 proteins and the results are presented in detail, in Additional files 1 and 2. Table 1 presents for each method separately and for the consensus method AMYLPRED, sensitivity, specificity, the index Q_{α} and correlation coefficient values, as these measures of accuracy were defined by Baldi et al. [32], for the 18 proteins of Additional file 1, with experimentally verified amyloidogenic regions on a per aminoacid residue basis. It also contains actual true/false positive and true/false negative values for each method to better demonstrate the bias of each individual

method. On the basis of these measures, it can be seen that, AMYLPRED, performs slightly better than each individual method, as perhaps expected. In Additional file 3, the results of AMYLPRED against those of a recently developed prediction algorithm of 'hot spots' of aggregation in polypeptides, AGGRESCAN, [21] are also compared. The results of AMYLPRED on this test set, against those of two other recently presented prediction algorithms, PASTA [20] and Zyggregator [24] were also compared (data not shown).

The crystal structures of 12 proteins from the set of the 23 chosen proteins related to amyloidoses are known (Additional files 1 and 2). Of these 12 proteins, 7 have experimentally determined amyloidogenic regions, shown in yellow in Figure 1, which contains cartoon representations of the determined structures. These include prolactin, apolipoprotein A-I, transthyretin, lactoferrin, lysozyme C, gelsolin and β_2 -microglobulin. Theoretically predicted amyloidogenic regions by AMYLPRED, which coincide with experimentally determined amyloidogenic determinants, are shown in red in Figure 1, whereas theoretically predicted amyloidogenic determinants by AMYLPRED, but not experimentally verified as such, are shown in blue in Figure 1.

Figure 2 contains cartoon representations of the remaining 5 protein structures, for which no experimental information for amyloidogenic determinants is currently available. These are: immunoglobulin κ -4 light chain, superoxide dismutase, immunoglobulin G1 heavy chain, insulin and cystatin C. Theoretically predicted amyloidogenic determinants by AMYLPRED on these protein structures are coloured in blue, in Figure 2.

Table 1: Accuracy indices of the consensus method and of its subordinate methods applied on the set of the 18 amyloidogenic proteins (see text)

| Method | Sensitivity | Specificity | Q_{α} | Correlation coefficient | TP | TN | FP | FN |
|-----------------------|-------------|-------------|--------------|-------------------------|-----|------|-----|-----|
| Av. Packing Density | 0.29 | 0.87 | 0.58 | 0.15 | 191 | 3769 | 575 | 471 |
| SecStr | 0.10 | 0.95 | 0.52 | 0.07 | 67 | 4107 | 237 | 595 |
| Pattern | 0.08 | 0.95 | 0.52 | 0.05 | 53 | 4127 | 217 | 609 |
| TANGO | 0.13 | 0.97 | 0.55 | 0.17 | 88 | 4207 | 137 | 574 |
| Conf. Energy | 0.39 | 0.79 | 0.59 | 0.14 | 256 | 3429 | 915 | 406 |
| Consensus (3 methods) | 0.13 | 0.95 | 0.54 | 0.11 | 84 | 4118 | 226 | 578 |
| Consensus (2 methods) | 0.31 | 0.88 | 0.59 | 0.18 | 206 | 3807 | 537 | 456 |

True/false positives (TP, FP) and true/false negatives (TN, FN) for each method are also shown to demonstrate better the bias of each individual method (see also text). The results for a consensus based on 3 methods are also shown.

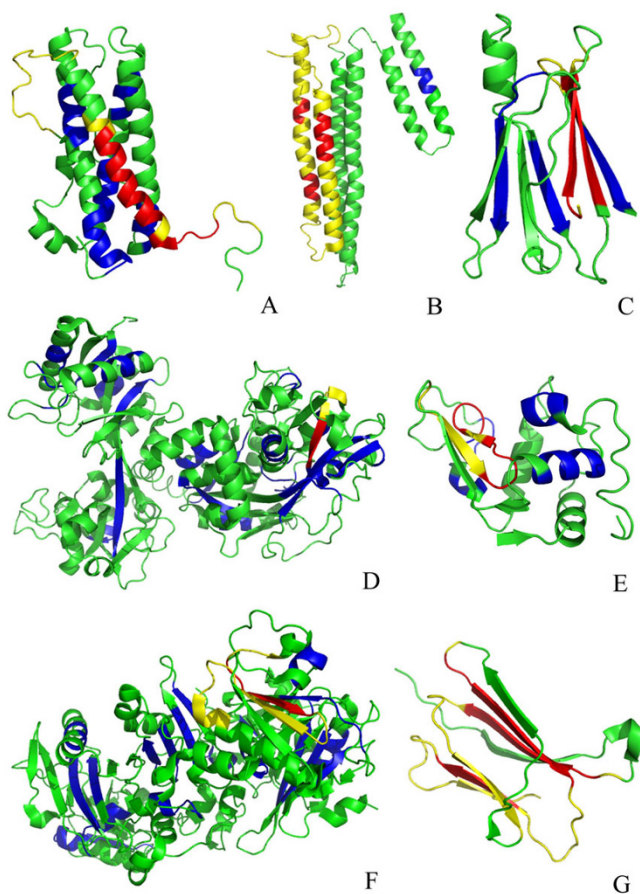


Figure 1
Cartoon representations of 7 proteins related to amyloidoses, with experimentally determined structures, which contain experimentally determined amyloidogenic regions. These 7 protein models, (see also Additional file 1), which were produced utilizing PyMOL [34] are: (A) Prolactin (PDB ID: [1RWVS](#)); (B) Apolipoprotein A-I ([2A01](#)); (C) Transthyretin ([1BMZ](#)); (D) Lactoferrin ([1LCB6](#)); (E) Lysozyme C ([1LZI](#)); (F) Gelsolin ([2FGH](#)); (G) β_2 -Microglobulin ([1LDS](#)). Experimentally determined amyloidogenic regions are shown in yellow. Theoretically predicted amyloidogenic regions, utilizing AMYLPRED (see Results), which coincide with experimentally determined regions are coloured red, whereas predicted amyloidogenic regions by AMYLPRED are shown in blue. The remainder of each protein is shown in green.

A close examination of the 12 solved protein structures (Figures 1 and 2) by molecular graphics programs such as O [33] and PyMOL [34] and also utilizing the algorithm DSSP [35], reveals that 100% of the experimentally determined amyloidogenic determinants and ca. 70% of theoretically predicted by AMYLPRED amyloidogenic determinants, have at least one 'face' of these amyloidog-

enic determinants on the surface of the relevant structures, clearly not buried in the hydrophobic interiors of the protein structures. This finding was somewhat unexpected and intriguing. Furthermore, surprisingly, several of these 'not-buried' amyloidogenic determinants were predicted by the algorithm SecStr (see 'Materials and Methods') to have ambivalent propensities both for α -helix and β -sheet, in other words to have the properties of 'chameleon' sequences [36], and, also, were observed experimentally to adopt either type of secondary structure.

Quantitative estimates of total and per-residue accessible surface areas, in \AA^2 , calculated using DSSP [35], for the experimentally determined amyloidogenic determinants, for the theoretically predicted by AMYLPRED amyloidogenic determinants and for other peptides, either 'exposed' on the surface or 'buried' into a protein's interior are provided in Additional file 4, for the 12 amyloidogenic proteins with experimentally determined 3D-structures of Additional files 1 & 2. Additional file 5 contains quantitative estimates of total and per-residue accessible surface areas, in \AA^2 , calculated using DSSP [35], for 'exposed' on the surface peptides (generally of comparable length to experimentally determined amyloidogenic determinants), taken from the structures of 9, non-amyloidogenic globular proteins, selected at random, belonging to the structural classes α , β , $\alpha + \beta$, α/β . It can clearly be seen from these files that all experimentally found amyloidogenic determinants and a large percentage (ca. 70%) of theoretically predicted determinants, are, indeed, 'exposed' on the surface. However, it is also seen that this feature is not found only in proteins related to disease, but it is a property of globular proteins in general.

Discussion

Amyloids are formed under protein-denaturing conditions or as a result of mutations, but they have also been observed to be the native fold of certain proteins under physiological conditions. As research continues for the understanding of the mechanisms involved in amyloid formation, the development of prediction methods is an important complement to experimental approaches.

Although, clearly, prediction tools cannot substitute experimental work, they might contribute in locating potential regions of interest for further experimental studies. Therefore, we have developed a publicly available online tool for the prediction of amyloidogenic determinants in amino acid sequences, based on the consensus of five independent prediction methods that rely on different properties of these amyloidogenic determinant-regions. In addition, we have tested the consensus method against each of its subordinate methods on the same set of 18 proteins for which experimental data is available and we have found that its results tend to be

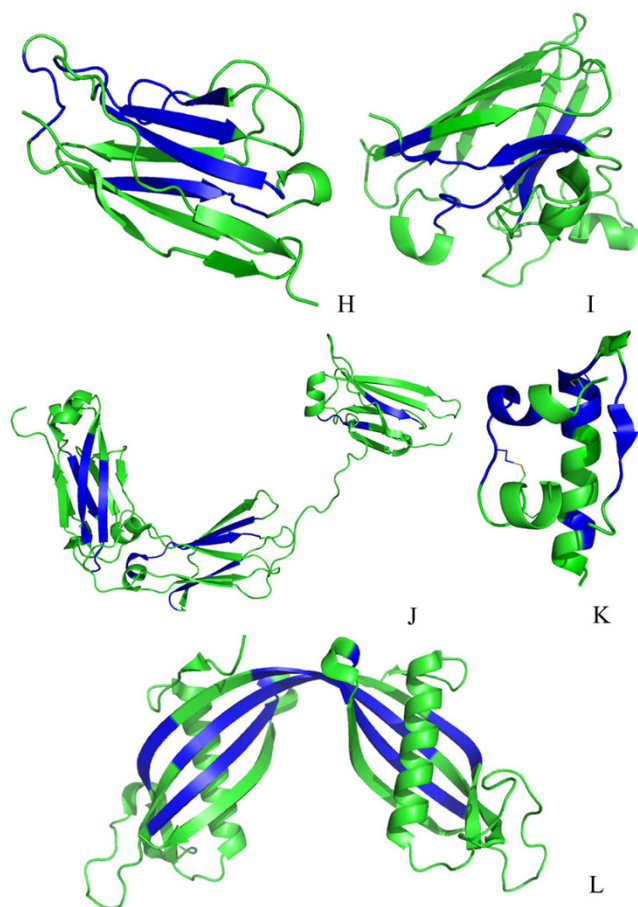


Figure 2
Cartoon representations of 5 proteins related to amyloidoses, with experimentally determined structures, which do not contain experimentally determined amyloidogenic regions. These 5 protein models, (see also Additional file 2), which were produced utilizing PyMOL [34] are: (H) Immunoglobulin κ -4 light chain (PDB ID: 1LVE); (I) Superoxide dismutase (2C9V); (J) Immunoglobulin G1 heavy chain (1HZH); (K) Insulin (1ZNI); (L) Cystatin C (1R4C). Predicted amyloidogenic regions by AMYLPRED are shown in blue (see also Additional file 2). The remainder of each protein is shown in green.

slightly more accurate than those of the individual predictors.

An intriguing finding is that signal peptides, when present (data not included in the results provided here), tend to be detected as amyloidogenic regions, usually by 4 out of the 5 methods and consequently are also shown as strong consensus hits. We currently have no explanation as to what the relation between the two features may be, besides the highly biased hydrophobic composition of the signal peptides' central region.

It is important, however, to note that the numbers shown in Table 1 are subject to change as more experimental data is acquired, because regions currently marked as non-amyloidogenic are not necessarily so and may prove to be in fact amyloidogenic in the future. Indeed, several regions were found in these 18 proteins, for which there was a strong agreement among all methods but are currently marked as non-amyloidogenic. Such prediction results may suggest amyloidogenic determinants currently unknown and methods like the one presented here might therefore provide valuable hints to experimental researchers. It should perhaps be mentioned at this point that, recently, we synthesized and structurally studied six (6) peptides from different proteins of Additional file 1, that the AMYLPRED tool predicts as amyloidogenic determinants (not previously experimentally verified as such) and we have found that five (5) of them produce amyloid fibrils, in water, at physiological pH, temperature and ionic strength, apparently having the ability to act as amyloidogenic determinants (Iconomidou & Hamodrakas, In preparation). This, most probably indicates that AMYLPRED might be a useful tool to experimental researchers. However, it should perhaps be emphasized that the use of AMYLPRED does not provide insights on the molecular rules underlying the aggregation event, as other tools, like TANGO [13], actually do.

Of course, it may be argued that most false positives, which result in the low correlation values presented in Table 1, are mainly due to consensual overpredictions, since all currently available amyloid prediction methods are notorious for their high degree of overprediction, and this perhaps is further seen in Additional file 3, where the results of AMYLPRED are compared with those derived by a recently developed method AGGRESCAN [21] and also when compared against those of two other recently presented prediction algorithms PASTA [20] and Zyggregator [24] (data not shown). However, it is obvious that further experiments are needed, which may reveal important clues for the amyloidogenic properties of the relevant proteins.

Also, it should be said that questions may be raised about the statistics provided in Table 1, as well as the data set used to generate these statistics: ideally the data set should be composed of more or less equal amounts of experimentally verified positives and negatives, allowing to score both false negatives and false positives. As the experimentally verified set is only composed of positives, only false negatives can be scored.

Nevertheless, in this study, we demonstrated rather conclusively that, practically all experimentally determined amyloidogenic determinants, to date, and more than 70% of predicted, but not yet experimentally verified short

potential amyloidogenic stretches, are placed on the surface of the amyloidogenic proteins (see 'Results'). Furthermore, several of them have the ability to act as conformational 'switches' (see 'Results'). This may signify that aggregation and amyloid formation is mediated via such short stretches, which may be achieved by partial local unfolding. It is perhaps difficult to reconcile this observation with the hypothesis that protein unfolding should occur prior to aggregation, however, these short stretches may act as 'switches', for partial unfolding of the whole protein. Experiments, focused on these fragments, should be performed to test this idea. In this respect, it is interesting to note that, the peptide VEALYL, which appears in the crystal structure of insulin (PDB ID [1ZNI](#), chain B, residues 12–17) adopts an α -helical conformation, with Leu(15) buried into the hydrophobic interior of the insulin monomer (chains A and B), whereas, when crystallized alone, forms a steric zipper, class 7 (see [31]), adopting an extended β -strand conformation. At the same time, AMYLPRED predicts it as an amyloidogenic determinant (Additional file 2), with its subordinate program SecStr classifying it as a 'chameleon' sequence, that is, a sequence with ambivalent propensity both for α -helix and β -sheet. This observation may have important implications for the amyloidosis related to insulin, namely iatrogenic amyloidosis [4], presumably by finding factors that may stabilize the conformation of this peptide as α -helical, *in vivo*. Thinking along similar lines may lead to a number of interesting practical consequences for other amyloidoses related to proteins of known 3D-structure, with experimentally verified amyloidogenic determinants, accessible on the surface of the proteins (Figure 1 and Additional file 1).

Conclusion

The results of this study clearly suggest that nearly all experimentally determined amyloidogenic determinants and a large percentage of predicted, but not yet experimentally verified short potential amyloidogenic stretches, are found on the surface of the relevant proteins, 'exposed' to the surrounding solvent and to interactions with neighbouring molecules. Furthermore, several of them have the ability to act as conformational 'switches', for partial unfolding of the whole protein. Experiments, focused on these fragments, should be performed to test this idea.

Methods

Tools and databases used

Amino acid sequences of the proteins used in this study were retrieved from UniprotKB [37], <http://www.uniprot.org>. Protein structures were retrieved from PDB [38], <http://www.pdb.org>. For each protein structure used, residues accessible to the solvent or buried into a protein's hydrophobic interior were determined utilizing the algorithm DSSP [35] and checked by visual inspection

of the relevant structures utilizing the molecular graphics programs PyMOL [34], <http://pymol.sourceforge.net> and O [33]. Cartoon drawings of the structures were obtained using PyMOL [34], <http://pymol.sourceforge.net>.

The consensus prediction tool AMYLPRED

For the purpose of this work, to produce a web-tool that would perform a consensus prediction of amyloidogenic determinants from protein sequences, utilizing available algorithms, we have used five different methods whose algorithms are publicly available or readily implementable and whose input is protein primary structure data.

The first method relies on average packing density profiles [17,18]. No algorithm has been published for this method, therefore we implemented our own.

The second method used is the online consensus secondary structure prediction algorithm SecStr [26] that has been shown to be able to predict amyloidogenic regions as conformational switches [25], which are identified as regions predicted both as α -helices and β -strands. SecStr [26], predicts separately α -helices and beta-strands. Regions predicted both as α -helices and beta-strands, by three individual methods of SecStr at least, are considered as conformational switches (chameleon sequences) [25]. These are easily identified, inspecting the text output file of SecStr <http://athina.biol.uoa.gr/SecStr/>.

Locating the amyloidogenic pattern {P}-{PKRHW}-{VLS-CWFNQE}-{ILTYWFNE}-{FIY}-{PKRH} [11] is another method used for our consensus prediction and is carried out by a short custom-written script.

The TANGO algorithm [13] is the next method used (version 2.1). It calculates the tendency of peptides to form beta aggregates and aside from the primary sequence, it also requires a set of environmental variables to be set. As a universal approach applicable to all proteins, the default values for these variables from the TANGO web-server submission page have been chosen.

Finally, an algorithm that maps all hexapeptides of a sequence onto the microcrystalline structure of NNQQNY and calculates the resulting conformational energy is also used [22]. Minor modifications to the source code of this algorithm have been made in order to allow for its automated execution.

The consensus prediction was found to produce the best results when the threshold is set to require overlapping hits by at least two of the five methods used. The consensus prediction is presented in the web browser window, while the complete predictions by all methods are made

available as a downloadable text file. The consensus prediction tool is freely available to academic users at: <http://biophysics.biol.uoa.gr/AMYPRED>. However, non-academic users of this algorithm should obtain permission of its use from the authors of the original algorithms and the corresponding author of this article.

Amyloidogenic protein data sets

23 proteins related to amyloidoses, were taken from the detailed compilations of Harrison et al. [4] and Uversky and Fink [2]. 18 of them have experimentally determined amyloidogenic regions (hereinafter called amyloidogenic determinants) [[4] and refs. therein, [17] and refs. therein] and of these 18 proteins, 7 have experimentally solved structures. The remaining 5 proteins have experimentally solved structures, but no experimentally determined amyloidogenic regions yet. All proteins were cleared of signal peptides, pro-peptides and other chains that are present in their database entries in UniprotKB [37] but are not part of the mature protein, and the exact locations of the experimental regions were identified by referring to the respective original publications [[17,18] and references therein, [4] and references therein].

Here, it should perhaps be mentioned that, the list of the 23 proteins contains also proteins that their structure is known either for fragments or in conditions that is not at all certain that they are similar to the conditions *in vivo*. Such proteins are calcitonin (for example PDB ID [2GLH](#), in sodium dodecyl sulfate micelles), major prion protein (the structure of several fragments of the prion protein is known), IAPP (for example [1KUIW](#) in detergent micelles or [2F48](#)-human insulin-degrading enzyme in complex with IAPP), α -synuclein (for example [1XQ8](#), micelle bound α -synuclein). Although the results of this study apply to these proteins too, they were not taken into account.

Specific points concerning the quality of the Amyloidogenic protein data sets and application of AMYPRED and of its subordinate methods

There is an average of less than 1.5 known amyloidogenic regions per protein in the dataset, while the consensus method offers an average of 5 predicted stretches per protein and similar if not even more over-predictive results are given by the methods of Galzitskaya et al. [17,18] and Zang et al. [22]. The total dataset collectively amounts to 5006 aminoacid residues and the total amount of experimentally positive residues is only 662, which represents 13% of the dataset. The set is thus highly biased by negatives. This imbalance is responsible for the low correlation values (see 'Results'). However we chose this data set, not as a test set of the performance of the consensus algorithm, AMYPRED, but as a representative set of well known proteins related to amyloidoses, with well defined amyloidogenic properties, structure and experimentally

known determinants (if possible), in our attempts to find common features of predicted, or experimentally known amyloidogenic determinants.

Results are judged based on the correlation coefficient values in order to offer a better overall prediction. Consensus is based on 2 methods instead of 3, because a consensus based on 3 methods produces a considerably lower correlation coefficient value (0.11 instead of 0.18) as a result of positive results being greatly reduced and lowering the sensitivity from 0.31 to 0.13 (see Table 1).

As it is apparent from the 'Results' section, the performance of the consensus method, AMYPRED, is not spectacularly better than that of the individual methods. This is likely because combining the strengths of the algorithms without mixing their weak points is not a task that can be attained with a linear combination of the methods.

This is better seen when all algorithms and AMYPRED were applied to a relatively well-balanced data set of 179 peptides used by Serrano and co-workers [[13], Additional file 1] to test the TANGO algorithm performance [13]. This data set contains 66 peptides, experimentally found to aggregate and 113 peptides also known not to aggregate by experiment. The results are shown in Additional file 6. The consensus algorithm, AMYPRED (correlation coefficient, $C = 0.58$) performs slightly worse than the Conformational energy [22] algorithm ($C = 0.65$) and equally well to TANGO [13] ($C = 0.58$) with default parameters that was used by us in this work. However, they all perform worse than TANGO ($C = 0.75$), with the environmental variables set by its authors [13]. It should perhaps be mentioned at this point that, the correlation coefficient values were calculated on a per segment basis rather than a per residue basis in this case.

There has been consideration for an implementation of a weighted contribution of the methods, possibly performed by a neural network. The gain from such an endeavor however would probably not be very great and would be overshadowed by the inherent flaws of all methods.

However, the main result of AMYPRED is not the list of consensus hits. It is the agreement profile of the methods (graph in the text output file) that provides a better insight on the results. The consensus hits shown on the webpage are presented as a convenient output for quick scans, but they lack the crucial information of how strong a prediction actually is, which is perhaps the most important factor in choosing regions for potential experimental research. When applying AMYPRED, care should be taken that it requires a minimal overlap of 2 residues at least, in order to indicate a positive result.

Authors' contributions

KKF developed the web-tool, analyzed the data, and drafted the manuscript. VAI participated in the coordination of the study, re-analyzed and checked the data and helped editing the manuscript. CMK retrieved the protein data set, examined the solved structures, calculated the accessibility of the peptides and prepared the protein cartoons. SJH designed and co-ordinated the study and was responsible for overall supervision. All authors read and approved the final manuscript.

Additional material

Additional file 1

Prediction of amyloidogenic determinants, for 18 proteins, with experimentally verified amyloidogenic regions, in the evaluation set, for each method separately and for the consensus method (AMYPRED). Details are given in the file.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S1.xls>]

Additional file 2

Prediction of amyloidogenic determinants, for 5 proteins, without experimentally verified amyloidogenic regions, for each method separately and for the consensus method (AMYPRED). Details are given in the file.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S2.xls>]

Additional file 3

Prediction of amyloidogenic determinants, for the 23 proteins of Additional files 1 & 2, both by AMYPRED and AGGRESCAN (Conchillo-Solé et al., 2007), for comparison. Details are given in the file.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S3.xls>]

Additional file 4

Quantitative estimates of total and per-residue accessible surface areas, in Å², calculated using DSSP [35], for the experimentally determined amyloidogenic determinants, for the theoretically predicted by AMYPRED amyloidogenic determinants and for other peptides, either 'exposed' to the solvent or 'buried', for the 12 amyloidogenic proteins with experimentally determined 3D-structures of Additional files 1 & 2. Details are given in the file.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S4.xls>]

Additional file 5

Quantitative estimates of total and per-residue accessible surface areas, in Å², calculated using DSSP [35], for 'exposed' to the surface peptides (generally of comparable length with those of experimentally determined amyloidogenic determinants), taken from the structures of 9 non-amyloidogenic globular proteins, belonging to the structural classes: alpha, beta, alpha+beta, alpha/beta. Details are given in the file.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S5.xls>]

Additional file 6

Accuracy indices of the consensus method, AmylPred, and of its subordinate methods, applied on a balanced set of 179 peptides, used by Serrano and co-workers to test the TANGO algorithm (see text). True/false positives (TP, FP) and true/false negatives (TN, FN) for each method are also shown to demonstrate better the bias of each individual method (see also text). The results for a consensus, AmylPred, based on 2 methods are also shown. TANGO* is the algorithm we used, with default parameters, whereas, TANGO** is the algorithm used by Serrano and co-workers, with the environmental variables set by its authors [13]. Correlation coefficient values were calculated on a per segment, rather than a per residue basis.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1472-6807-9-44-S6.doc>]

Acknowledgements

We thank the University of Athens for financial support. Also, we should like to thank all scientists, whose algorithms were used into this study, for their valuable contributions to the field and the reviewers of this manuscript for their useful and constructive criticism.

References

- Chiti F, Dobson CM: **Protein misfolding, functional amyloid, and human disease.** *Annu Rev Biochem* 2006, **75**:333-366.
- Uversky VN, Fink AL: **Conformational constraints for amyloid fibrillation: the importance of being unfolded.** *Biochim Biophys Acta* 2004, **1698**:131-153.
- Dobson CM: **Protein misfolding, evolution and disease.** *Trends in Biochem Sci* 1999, **24**:329-332.
- Harrison RS, Sharpe DC, Singh Y, Fairlie DP: **Amyloid peptides and proteins in review.** *Rev Physiol Biochem Pharmacol* 2007, **159**:1-77.
- Fowler DM, Koulov AV, Balch WE, Kelly JW: **Functional amyloid-from bacteria to humans.** *Trends Biochem Sci* 2007, **32**:217-224.
- Otzen D, Nielsen PH: **We find them here, we find them there: Functional bacterial amyloid.** *Cell Mol Life Sci* 2008, **65**:910-927.
- Fändrich M: **On the structural definitions of amyloid fibrils and other polypeptide aggregates.** *Cell. Cell Mol Life Sci* 2007, **64**:2066-2078.
- Maji SK, Schubert D, Rivier C, Lee S, Rivier JE, Riek R: **Amyloid as a depot for the formulation of long-acting drugs.** *PLoS Biology* 2008, **6**:240-252.
- Iconomidou VA, Vriend G, Hamodrakas SJ: **Amyloids protect the silkworm oocyte and embryo.** *FEBS Letters* 2000, **479**:141-145.
- Iconomidou VA, Hamodrakas SJ: **Natural protective amyloids.** *Curr Prot Pept Sci* 2008, **9**:291-309.
- López de la Paz M, Serrano L: **Sequence determinants of amyloid fibril formation.** *Proc Natl Acad Sci* 2004, **101**:87-92.
- Esteras-Chopo A, Serrano L, López de la Paz M: **The amyloid stretch hypothesis: recruiting proteins toward the dark side.** *Proc Natl Acad Sci* 2005, **102**:1639-1648.
- Fernandez-Escamilla AM, Rousseaux F, Schymkowitz J, Serrano L: **Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins.** *Nature Biotechnology* 2004, **22**:1302-1306.
- Yoon S, Welsh WJ: **Detecting hidden sequence propensity for amyloid fibril formation.** *Protein Science* 2004, **13**:2149-2160.
- Tartaglia GG, Cavalli A, Pellarin A, Caffiesch A: **Prediction of aggregation rate and aggregation-prone segments in polypeptide sequences.** *Protein Science* 2005, **14**:2723-2734.
- Pawar AP, DuBay KF, Zurdo J, Chiti F, Vendruscolo M, Dobson CM: **Prediction of "aggregation-prone" and "aggregation-susceptible" regions in protein associated with neurodegenerative diseases.** *Mol Biol* 2005, **350**:379-392.
- Galzitskaya OV, Garbuzynskiy SG, Lobanov MV: **Prediction of amyloidogenic and disordered regions in protein chains.** *PLoS Comput Biol* 2006, **2**:1639-1648.

18. Galzitskaya OV, Garbuzynskiy SO, Lobanov MY: **A search for amyloidogenic regions in protein chains.** *Molecular Biology* 2006, **40**:821-828.
19. Thompson MJ, Sievers SA, Karanicolas J, Ivanova MI, Baker D, Eisenberg D: **The 3D profile method for identifying fibril-forming segments of proteins.** *Proc Natl Acad Sci* 2006, **103**:4074-4078.
20. Trovato A, Chiti F, Maritan A, Seno F: **Insight into the structure of amyloid fibrils from the analysis of globular proteins.** *PLoS Comp Biol* 2006, **2**:1608-1618.
21. Conchillo-Solé O, de Groot NS, Aviles FX, Vendrell J, Daura X, Ventura S: **AGGRESCAN: a server for the prediction and evaluation of "hot spots" of aggregation in polypeptides.** *BMC Bioinformatics* 2007, **8**:65-81.
22. Zhang Z, Chen H, Lai L: **Identification of amyloid fibril-forming segments based on structure and residue-based statistical potential.** *Bioinformatics* 2007, **23**:2218-2225.
23. Zibae S, Makin OS, Goedert M, Serpell LC: **A simple algorithm locates β -strands in the amyloid fibril core of α -synuclein, $A\beta$, and tau using the amino acid sequence alone.** *Protein Science* 2007, **16**:906-918.
24. Tartaglia GG, Pawar AP, Campioni S, Dobson CM, Chiti F, Vendruscolo M: **Prediction of aggregation-prone regions in structured proteins.** *J Mol Biol* 2008, **380**:425-436.
25. Hamodrakas SJ, Liappa C, Iconomidou VA: **Consensus prediction of amyloidogenic determinants in amyloid-forming proteins.** *Int J Biol Macromol* 2007, **41**:295-300.
26. Hamodrakas SJ: **A protein secondary structure prediction scheme for the IBM PC and compatibles.** *Comput Appl Biosci* 1988, **4**:473-477.
27. Chou PY, Fasman GD: **Conformational parameters for amino acids in α -helical, β -sheet, and random coil regions calculated from proteins.** *Biochemistry* 1974, **13**:211-222.
28. Chou PY, Fasman GD: **Prediction of protein conformation.** *Biochemistry* 1974, **13**:222-245.
29. Nelson R, Sawaya MR, Balbirnie M, Madsen AØ, Riekel C, Grothe R, Eisenberg D: **Structure of the cross- β spine of amyloid-like fibrils.** *Nature* 2005, **435**:773-778.
30. Pawlicki S, Le Béche A, Delamarche C: **AMYpdb: A database dedicated to amyloid precursor proteins.** *BMC Bioinformatics* 2008, **9**:273-284.
31. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJW, McFarlane HT, Madsen AØ, Riekel C, Eisenberg D: **Atomic structures of amyloid cross- β spines reveal varied steric zippers.** *Nature* 2007, **447**:453-457.
32. Baldi P, Brunak S, Chauvin Y, Andersen CAF, and Nielsen H: **Assessing the accuracy of prediction algorithms for classification: an overview.** *Bioinformatics* 2000, **16**:412-424.
33. Jones TA, Zou JY, Cowan SW, Kjeldgaard M: **Improved methods for building protein models in electron density maps and the location of errors in these models.** *Acta Crystallogr* 1991, **A47**:110-119.
34. Delano WL: **The PyMOL molecular graphics system.** In *DeLano Scientific LLC* 400, Oyster Point Blvd., Suite 213, South San Francisco, CA 94080-1918 USA; 2005.
35. Kabsch W, Sander C: **Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features.** *Biopolymers* 1983, **22**:2577-2637.
36. Guo JT, Jaromczyk JW, Xu Y: **Analysis of chameleon sequences and their implications in biological processes.** *Proteins: Structure, Function and Bioinformatics* 2007, **67**:548-558.
37. The UniProt Consortium: **The Universal Protein Resource (UniProt).** *Nucleic Acids Res* 2007, **35**:D193-D197.
38. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE: **The Protein Data Bank.** *Nucleic Acids Research* 2000, **28**:235-242.