SAPdb: A database of nanostructures formed by self-assembly of short peptides

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Abstract

Nanostructures generated by self-assembly of peptides yield nanomaterial that have many therapeutic applications including drug delivery and biomedical engineering due to their low cytotoxicity and higher uptake by targeted cells owing to their high affinity and specificity towards cell surface receptors. Despite promising implications of this rapidly expanding field, there is no dedicated resource to study peptide nanostructures. This study endeavors to create a dedicated repository of short peptides, which may prove to be the best models to study ordered nanostructures formed by peptide self-assembly. SAPdb has a repertoire of 637 entries of experimentally validated nanostructures formed by self-assembling of dipeptides and tripeptides. Each entry encompasses information about the terminal and non-terminal modifications in the peptide sequence, the technique used to study self-assembly, the stability of the nanostructure, type of nanostructure formed and conditions like pH, temperature and solvent required for the self-assembly of the peptide. User-friendly browsing, searching and analysis modules are integrated for easy retrieval and comparison of data and examination of properties. We anticipate SAPdb to be a valuable repository for researchers engaged in the burgeoning arena of nanobiotechnology.

Introduction

Peptides have been reported as key players in diverse fields like immunotherapeutic[1-6], disease biomarkers[7-10], antibacterial[11-15] antiviral[16-20], anticancer[21-25], antiparasitic[26-30], antihypertensive[31-33] drugs owing to their properties such as cell-penetration[34], stability[35-37] and low toxicity[38,39]. Beside these areas, peptides are rapidly gaining the attention of researchers in the field of

nanobiotechnology[40-43] by the virtue of their property to self-assembly into well-defined nanostructures. Advantages of self-assembled short peptides for use as nanomaterial relative to conventional materials include simple structure, fast and low-cost synthesis, better chemical and physical stability, diversity in morphology, ease of synthesis in large quantities. In addition, biocompatibility, biodegradability, low cytotoxicity and higher uptake by targeted cells of SAPs (self-assembling peptides) play significant role in their therapeutic applications[44,45]. Owing to these attractive properties, bioactive peptides with the ability to undergo self-assembly are being explored to serve as building blocks of hydrogels and scaffolds in cell culture[46] and tissue engineering[47,48], controlled drug delivery in response to changes in pH[49,50], for diagnostics and biosensors[51], as well as in the field of bioelectronics[52] and material sciences[53].

Self-assembly of peptides can lead to the formation of well-defined nanostructures like nanofibers, nanorods, nanoparticles, hydrogels, nanotubes, etc [54-62]. The assembly of these nanostructures depends on weak non-covalent interactions like Van der Waals force, hydrophobic interactions, hydrogen bonds and π-π stacking [63-66]. The lack of comprehensive understanding of the properties and mechanisms of the self-assembly of peptide nanostructure formation affect their configuration and utility. Thus, it is a challenge to control the shape, size and stability of these nanostructures during synthesis for successful integration and translation into biosensors and controlled release devices for drug delivery.

Though various studies have been carried out to design peptide based nanoparticles, to the best of the authors' knowledge there is no dedicated platform which maintains comprehensive information about nanoparticles formed by the self-assembly of peptides. A systematic collection and compilation of experimental data is needed to examine the mechanisms and interactions governing self-assembly of peptides into nanostructures for facilitating rational designing of

morphologies and size of peptide assemblies. The present report is the first attempt to develop a repository of short peptides that undergo self-assembly to form nanostructures. Dipeptides and tripeptides are the smallest of known peptide self-assemblers, which show fascinating morphologies, and functionalities besides being cost efficient and fast to synthesize. “SAPdb” database of such short peptides will be very useful to study how experimental conditions and chemical modifications in the amino acid sequence of peptides affect the bottom-up process of self-assembly to form well-defined ordered nanostructures.

Methods

Data Collection

To collate the relevant information for di- and tri-peptides that self-assemble to form discrete and ordered nanostructure, PubMed was queried to obtain the research articles. Keywords like “(tripeptide AND self-assembly)”, “(tripeptide AND nanostructure)”, “(tripeptide AND nanotube)”, “(tripeptide AND nanofiber)”, “(tripeptide AND nanorod)”, “(tripeptide AND hydrogel)”, “(tripeptide AND nanosphere)” and “(tripeptide AND nanoparticle)” for self-assembling tripeptides, while to collect research articles relevant to self-assembling dipeptides keywords “(dipeptide AND self-assembly)”, “(dipeptide AND nanotube)”, “(dipeptide AND nanostructure)”, “(dipeptide AND nanorod)”, “(dipeptide AND nanosphere)”, “(dipeptide AND nanofiber)”, “(dipeptide AND hydrogel)”, “(dipeptide AND nanoparticle)” were used. We screened the articles obtained and included only those for further data curation, where information about peptides forming self-assembled nanostructures were available.

After thorough screening, relevant information extracted from selected articles concerning the sequence of peptides, N-terminal modifications, C-terminal modification, technique to analyze

self-assembled structure, method, type of self assembly, size of self-assembled structure and the conditions under which peptide formed the self-assembled structure such as solvent, concentration, temperature, pH and incubation time etc. To provide information regarding the effect of experimental conditions on self assembly of peptides like concentration, solvent, pH, incubation time, etc. on the self-assembly of peptides, we made multiple entries of the same peptide if it was reported under different experimental conditions. This extensive information systematically cataloged in a tabulated manner. Consequently, 637 entries were collated in SAPdb database from 143 research articles.

Architecture and web - interface of the Database

SAPdb was designed using Apache HTTP Server (version 2.2.17) on Linux system following collection and compilation of significant information. SAPdb is based on MySQL at backend that was implemented to maintain the information and HTML5, PHP and JavaScript were deployed to execute the front end, in order to build a mobile, tablet and desktop compatible web-resource. Different modules were integrated into SAPdb for data compilation, retrieval and exploration. Complete architecture depicting information and tools integrated in SAPdb represented in Figure 1.

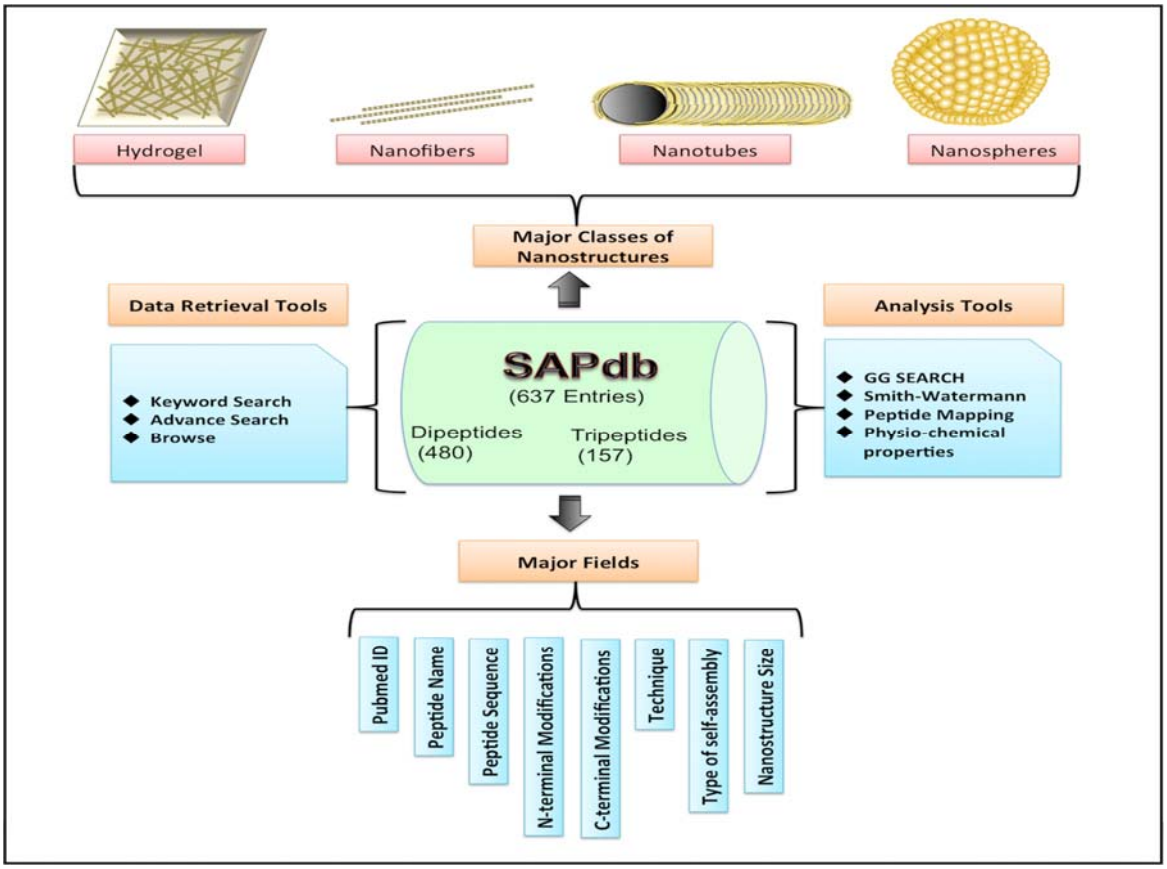

Figure 1: Architecture depicting the information and tools integrated in SAPdb.

Organization of Database

In SAPdb, collated information was classified into primary and secondary information. The primary information include fields like (i) PubMed ID, (ii) peptide sequence, (iii) N-terminal modification, (iv) C-terminal modification, (v) non-terminal modification, (vi) technique, (vii) method, (viii) solvent, (ix) concentration of peptide, (x) pH, (xi) temperature, (xii) incubation time, (xiii) type of self-assembly, (xiv) size of self-assembled structure and (xv) stability of self-assembled structure. While in secondary information (xvi) SMILES and (xvii) tertiary structure of peptides were included.

To determine the structure of SAPs, all the sequences were mapped on PDB[67]. We obtained 10 exact matches from PDB. The structures of the remaining sequences were determined using PEPstrMOD[68] and in-house scripts. The dihedral angles were taken as 180° and the structures after energy minimization and MD simulations using AMBER 11 were regarded as the final structures. Open Babel[69] was employed to convert PDB structures of SAPs to SMILES notation.

Evaluation of prediction

Natural and unique datasets were prepared to analyse the properties which could be used to distinguish between the self-assembling and non-self-assembling classes of peptides. The performances were evaluated using sensitivity (Sn), specificity (Sp), accuracy (Ac) and Matthews correlation coefficient (MCC) parameters which have been widely used in the literature [70], [71], [72]. The equations used to determine these parameters are-

$$Sn = \frac{TP}{TP+FN} \times 100 \qquad (1)$$

$$Sp = \frac{TN}{TN+FP} \times 100 \qquad (2)$$

$$Ac = \frac{TP+TN}{TP+FP+TN+FN} \times 100 \qquad (3)$$

$$MCC = \frac{(TP \times TN)\text{-}(FP \times FN)}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}} \qquad (4)$$

Where TP (True Positive) refers to rightly predicted positive instances and TN (True Negative) represent rightly predicted negative instances. Likewise, FP (False Positive) are wrongly preicted positive and FN (False Negative) are wrongly predicted negative instances.

Results

Statistical analysis of data of SAPdb

SAPdb is a collection of 637 entries of experimentally validated short peptides which undergo self-assembly to form ordered nanostructures manually curated and compiled from 143 research articles published in the recent past with the rise of interest in nanobiotechnology (Figure 2C). Of these 637 entries, 480 entries are of dipeptides while 157 entries belong to tripeptides. 12 of the peptides were cyclic while the rest were linear. These model short peptides form 13 major types of nanostructures as shown in Figure 2A. Hydrogel (242 entries) is the most common type of structure followed by nanofibers (66 entries), nanotubes (54 entries) and nanospheres (46 entries). Different techniques have been described in literature to study the phenomenon of self-assembly of peptides. The most commonly techniques were scanning electron microscopy, transmission electron microscopy, atomic force microscopy, Fourier transform infrared spectroscopy (Figure 2D).

To give different functionality, stability and shape to nanostructure, some peptides have been cyclized while various terminal modifications and non-natural amino acids have been incorporated in various other peptide sequences (Figure 2B). There are total 382 entries in SAPdb encompass modifications at the N-terminal. Most commonly reported N-terminal modifications include naphthalene and its derivatives (94 entries), tert-butyloxycarbonyl (Boc) derivatives (90 entries) and fluorenylmethyloxycarbonyl (Fmoc) (73 entries) protecting groups

attached to their N-terminal. Benzylation (33 entries), methoxylation (28 entries) and amidation (25 entries) are most commonly reported C-terminal modifications. Beside this, 46 reported entries had non-natural amino acids like dehydrophenylalanine (ΔPhe) (26 entries), amino benzoic acid (ABA) (8 entries), amino isobutyric acid (Aib) (8 entries), beta-Alanine (β-Ala) (6 entries), etc. in their sequences.

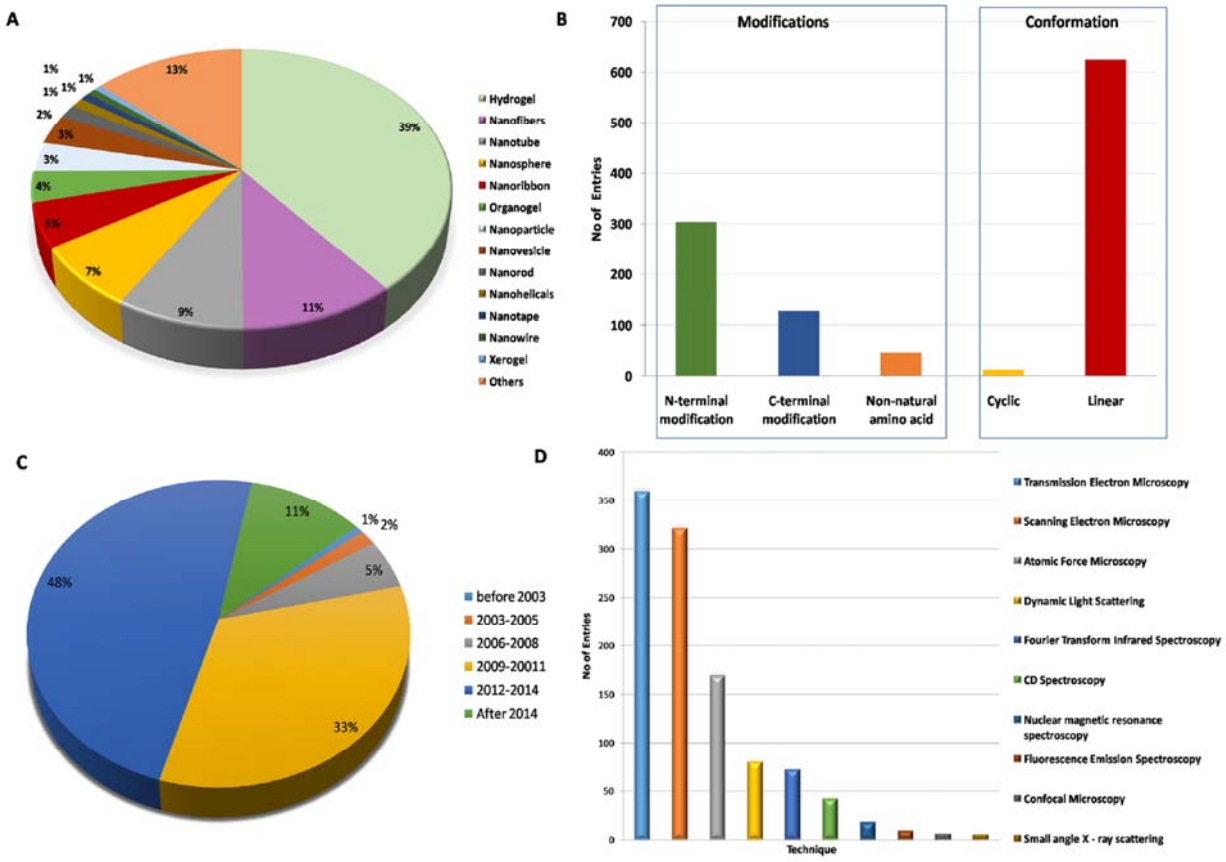

Figure 2: Distribution of peptide entries in SAPdb according to (a) types of nanostructures, (b) modifications and conformation of peptides, (c) year of publication and (d) techniques used to study self-assembly of peptides.

Effect of experimental conditions

Previous studies have shown that the variation in experimental conditions like type of solvent, change in concentration of peptide used, temperature etc. can lead to formation of different nanostructures by the same peptide (ref). SAPdb allows easy access to such information leading to better understanding of control of the 3D shape of nanostructures formed by altering experimental conditions. For instance, diphenylalanine form different nanostructure like nanofibers, nanotubes, nanovesicles *etc* under different experimental conditions as shown in **Table 1**

Table 1: Effect of various experimental conditions on the type of nanostructure formation by diphenylalanine.

SAPdb ID	Nano-structure	Size of nano-structure	Solvent	Concentration	Temperature	PMID
1217	Nanofiber	Diameter ~ 160nm, Length >10μm	Water	2mM	- 20°C	25621167
1502	Nanoribbon and Nanowires	Width: 100 - 1000 nm	Dichloromethane	4mg/ml	Room temperature	19198495
1499	Nanotube	Diameter: 100 nm to 5 μm; length: 100nm to μm	Water	4mg/ml	Room temperature	19198495
1277	Nanovesicle	Diameter : 0.2-2 μm	Acetone	2mg/ml	80°C	20000323

1535	PQD (Peptide quantum dots)	Diameter of 2.12 +/- 0.15 nm	HFIP (1,1,1,3,3,3-hexafluoro-2-Isopropanol) + Methanol	2 - 10mg/ml	Not mentioned	20958029

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Further, our analysis shows that the concentration of peptide is also an important factor for controlling the shape and size of nanostructure. For example, KFG tripeptide forms vesicles at low concentration of 0.5mg/ml while at higher concentration of 5mg/ml it forms nanotubes [73]. Furthermore, the role of solvent in directing the type of nanostructure formation is also revealed through this analysis. Most of the nanostructures are formed using water as a solvent, followed by phosphate buffer, and organic solvents like methanol, ethanol, hexafluoropropanol, chloroform and acetone. FF is reported to form nanovesicles in acetone [74] while in water it forms nanotubes [75].

This analysis also shows some specific experimental conditions favor the formation of nanostructure as evidenced by the observation that majority of the nanostructures are reported to be formed by self-assembly under room temperature (356 entries) and at acidic pH of less than 6 (99 entries) followed by neutral pH of 7 (78 entries).

Analysis and prediction of self-assembling peptides

In order to understand the properties that affect the self-assembly of short peptides, we analyzed the amino acid composition (Figure 3 and 4) and physicochemical properties (Table 2) of experimentally validated, natural and unique 58 self-assembling and 11 non self-assembling peptides present in SAPdb. The average amino acid composition revealed that self-assembling peptides are rich in valine, tryptophan, leucine, and alanine while they are depleted in isoleucine, aspartic acid and histidine compared to non-self-assembling peptides. As discussed by Frederix et al, 2014 [76] aromatic residues phenylalanine and tyrosine are favoured at second and third position in tripeptides undergoing self-assembly while negatively charged residues (aspartic acid and glutamic acid) are favoured at the C-terminal and positively charged residue (like lysine) are preferred at the N-terminal (Figure 4). Beside this, our analysis also showed that non self-assembling peptides were rich in histidine at the second position unlike the self-assembling tripeptides.

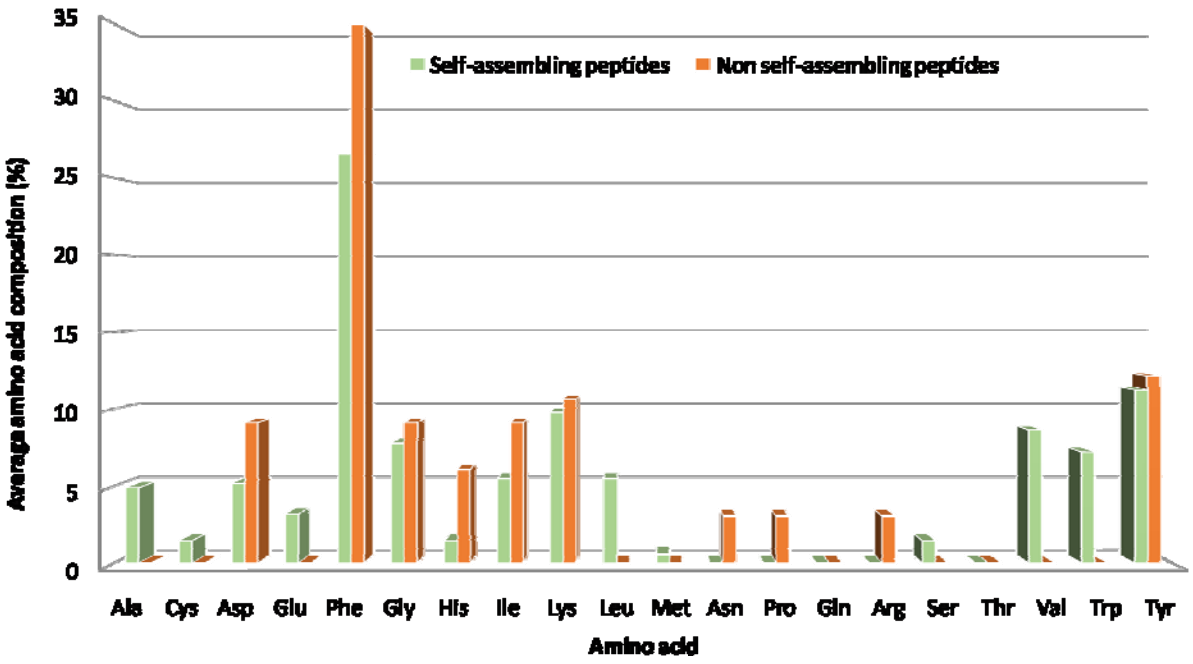

Figure 3: Bargraph displaying the comparison of average amino acid composition between self-assembling and non-self- assembling peptides.

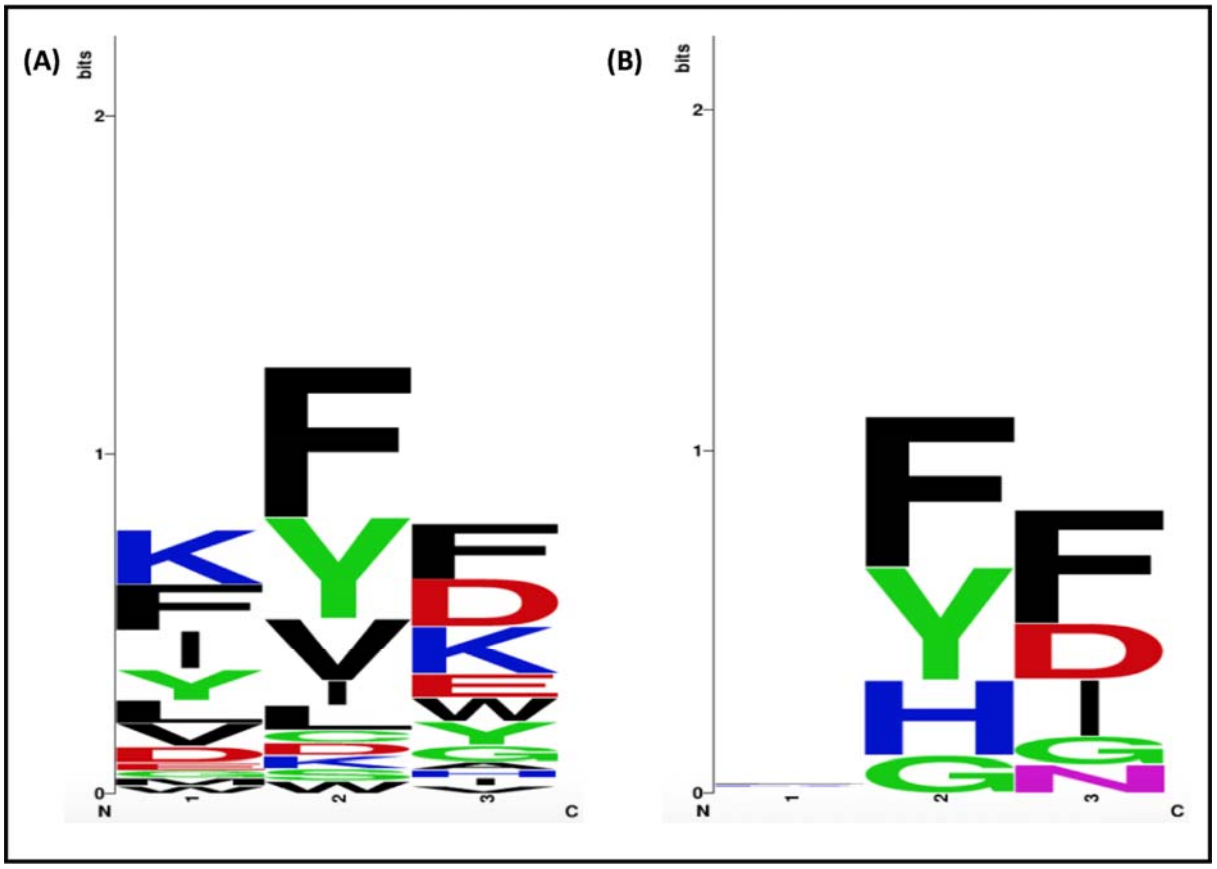

Figure 4: Weblogo of (a) self-assembling and (b) non-self-assembling tripeptides.

Analysis of the difference between the average physicochemical properties between self-assembling (positive dataset) and non-self-assembling (negative dataset) suggested that net hydrogen content (which might be helpful in creation of more number of H-bonds in self-assembled structures) and the isoelectric point (pI determines the net charge at a given pH which

might also affect the bonding between peptides in a self-assembled nanostructure) might be significant while differentiating between these two classes (Table 2). The prediction based on these physicochemical properties revealed that the accuracy of differentiating between self-assembling and non-self-assembling peptides was best on the basis of net charge (72.46%), followed by charge (69.57%), pI (66.67%) and hydrophobicity (63.77%). The aggregation propensity (AP) score which is the ratio between the solvent-accessible surface area before and after simulation reported for dipeptides [77] and tripeptides [77] by Frederix et al. and has been proposed as an indicator for identifying self-assembling peptides. AP score greater than 2 indicates a strong propensity of the peptide to undergo self-assembly. Taking AP score as a feature, the accuracy achieved was 62.32% with a sensitivity of 63.79% and specificity of 54.55%. Though other physicochemical properties performed better than AP score (Table 4. 3), none of them could precisely distinguish between these two classes of peptides.

Table 4.2- Shows mean and standard deviation of composition of different physicochemical properties on experimentally validated self-assembling (positive) and non-self-assembling (negative) peptides.

Physicochemical Property	**Mean (positive dataset)**	**SD (positive dataset)**	**Mean (negative dataset)**	**SD (negative dataset)**	**Mean difference between Positive and Negative dataset**
Hydrophobicity	0.11	0.38	0.01	0.43	0.10
Hydrophilicity	-0.81	1.32	-0.66	1.45	0.15

Net hydrogen	1.40	1.41	1.91	1.64	0.51
Steric hindrance	0.64	0.20	0.66	0.23	0.02
Solvation	1.10	0.75	0.99	0.92	0.11
pI	6.24	1.93	6.53	1.54	0.28
Volume	421.12	153.75	472.53	140.70	51.41
Charge	0.09	0.76	0.09	0.54	0
AP score	1.69	0.58	1.74	0.36	0.05

Table 3: The performance of threshold-based methods developed using different features (e.g., physicochemical properties) developed for predicting self-assembling peptides.

Feature	Sensitivity (%)	Specificity (%)	Accuracy (%)	MCC
Hydrophobicity	67.24	45.45	63.77	0.08
Hydrophilicity	63.79	54.55	62.32	0.10
Net hydrogen	79.31	36.36	72.46	0.13
Steric hindrance	70.69	18.18	62.32	-0.08
Solvation	53.45	54.55	53.62	0.04
pI	72.41	36.36	66.67	0.06

Volume	58.62	45.45	56.52	0.02
Charge	79.31	18.18	69.57	-0.02
AP score	63.79	54.55	62.32	0.10

Implementation of Web Tools

Various modules *i.e.* search tools, browse tools and analysis tools were integrated into the database for users to explore SAPdb.

Search Tools

Two distinctive modules are implemented in SAPdb *i.e.* Simple search, Advance search under search tool to assist the user for amiable data retrieval.

Simple search: This module offers the fundamental facility for data retrieval from the database. Here one can perform a keyword query by selecting any required field of SAPdb such as the type of self-assembly, technique, peptide sequence, etc. Besides, this module also permits the users to select anticipated fields to be displayed in the result.

Advance search: To retrieve relevant information from SAPdb, the advance search module presents the facility for the user to implement multiple query system. By default, it simultaneously executes four queries, but the user can select desired keyword search from any selected field. Moreover, this module allows implementation of standard logical operators (=, >, < and LIKE). Beside this, advance search permits the user to integrate the output of different

queries by employing operators like 'AND and OR'. Further, the user can also add or remove the queries to be executed.

Browsing Tools

Browsing facility has been integrated to facilitate the user for data mining in systematic mode from SAPdb. In this module, the user can fetch information on peptides by browsing six different classes (i) Chemical modification, (ii) Type of Nanostructure, (iii) Size of Nanostructure, (iv) Technique, (v) Publication year, (vi) Dipeptides and (vii) Tripeptides.

Chemical modification field assists the user to retrieve data on peptides that have different chemical modification at the N or C termini such as acetylation (28 entires), amidation (25 entries), Fmoc (fluorenylmethoxycarbonyl; 73 entries), naphthalene and its derivatives (94 entries) and Boc (N-t-Butoxycarbonyl; 90 entries), benzylation (33 entries) etc. Type of nanostructure category offers the user to fetch detailed information on peptides that form particular nanostructure such as nanotube, nanosphere, nanofibers, hydrogel, etc. Size of nanostructure category allows users to browse entries on the basis of the size of nanostructure reported. Further technique category permits the user to extract information regarding peptides whose nanostructure was studied using a particular technique such as TEM (359 entries), SEM (321 entries) and AFM (169 entries), etc. Additionally, users can also retrieve information of the peptides by the year of publication. Different entries of dipeptide and tripeptide sequences can be explored under the dipeptide and tripeptide modules of the browse facility.

Analysis Tools

This module enables the users to execute diverse examinations such as sequence similarity, peptide mapping and physicochemical properties of peptides.

GGSearch: This tool allows the user to perform an efficient similarity-based search against the short peptides in SAPdb. It is based on Needleman-Wunsch alignment[78].

Smith – Waterman Algorithm: This algorithm[79] permits the user to search peptides in SAPdb database similar to their query peptides. Under this option, the user can submit concurrently multiple peptide sequences in FASTA format.

Peptide Mapping: This tool offers the facility to map SAPdb peptides over the query protein sequence. It will be useful for identifying motifs within proteins that have a tendency to undergo self-assembly.

Physicochemical Properties: This module facilitates users to examine properties like charge, polarity, volume, hydrophobicity, etc. of the desired peptide sequences.

DISCUSSION

With the rising interest in nanobiotechnology, it is important to understand the properties governing the self-assembly of peptides into nanostructures. Previously databases like AmyLoad[80], CPAD[81] and AMYPdb[82] have curated protein and peptide aggregation. Since there was no repository of experimentally validated and well-ordered self-assembling peptide nanostructures, we have developed SAPdb, a platform with comprehensive information about nanostructure formed by short peptides. Dipeptides and tripeptides are the shortest peptides that can assemble into higher order structures. They are the model candidates to understand the process of self-assembly. SAPdb holds information of 167 unique dipeptides and 96 unique tripeptides that have been experimentally validated for undergoing ordered nanostructure formation by self-assembly.

Comprehensive analysis of SAPdb data have shown that different environmental conditions like temperature, pH, solvent, concentration *etc* direct the shape or type of nanostructures formation. Further, the specific conditions *i.e.* room temperature, acidic pH favors the formation of nanostructure of self assembling peptides. In addition, amino acid composition analysis of peptides of SAPdb revealed that self-assembling peptides are rich in valine, tryptophan, leucine, and alanine while they are depleted in isoleucine, aspartic acid and histidine compared to non-self-assembling peptides. Further, aromatic residues like phenylalanine and tyrosine are favoured at second and third position in tripeptides undergoing self-assembly. In addition negatively charged residues are preferred at the C-terminal and while positively charged residue are at the N-terminal.

Furthermore, the insilico prediction based on various physicochemical properties *i.e.* hydrophobicity, hydrophilicity, charge, pI, net hydrogen *etc* were employed to distinguish self-assembling from non-self-assembling peptides. This analysis indicates although these properties correctly classified positive peptides (self-assembling petides) with reasonable performance, but it misclassified non-self assembling peptides as self-assembling peptides.

Utilization of SAPdb

SAPdb maintains nanostructure-forming small peptides, which have numerous applications in diverse areas as illustrated by the following examples:

Drug, gene and other biomolecules delivery vehicles

One of major applications of SAPs lies in the delivery of drugs and biomolecules. This attributed to their biocompatible nature, small size of self-assembling dipeptides and tripeptides [83-87]. For instance EΔF dipeptide based nanovesicles employed to deliver vitamin B-12 in the HeLa cells[88],

while MΔF, IΔF, and LΔF were used for the invitro delivery of curcumin in L-929 cells and invivo delivery in mouse [89].

Cell culture scaffold biomaterial for tissue engineering

Another major application of the peptide based self-assembled hydrogels is their efficiency for 3D-cell culture[90]. It has been observed that dipeptide FΔF based hydrogel sustained 3D cell growth of HeLa cells and L929 (mouse fibroblast) cells for more than 14 days, with substantial multiplication rate and cell viability [91].

Models for nanofabrication and biomineralization

In the past the self-assembling peptides were explored as templates for nanofabrication like nanowires, biomineralization, nanocircuits owing to their potential of self-assembly[92,93]. For example, Phe–Phe tagged with ferrocence forms uniform nanowires having diameter of 100 nm with length 5–10 mm. After adsorbing gold nanoparticles and antibody molecules onto these Fc-tagged nanowires surface, these nanowires were employed as a detection probe for sensitive electrochemical immunosensing[92].

We have incorporated peptides with such far-reaching applications in SAPdb so that the users can easily retrieve the SAPs of their interest. Using different modules one can examine how the substitution of D-amino acids or non-natural amino acids in the peptide sequence and the experimental conditions like solvent, concentration, pH, etc. influence the self-assembling property of the peptides. Moreover, to facilitate the users, we have integrated chemical modification browsing tool to highlight the impact of different modifications on the type of nanostructure formed by the same peptide sequence. The users can easily analyze sequence similarity of their query peptide with different SAPs, at a single platform i.e. SAPdb, which are

otherwise scattered in literature and difficult to access. Besides, SAPdb provides a dataset of experimentally validated small peptides that form nanostructures by self-assembly for the development of computational algorithms for rational designing of peptide nanostructures.

Future Developments

As the scientific community is actively publishing articles pertaining to this emerging field, in future, our first concern will be to update data available about short model peptides as well as to expand the database further to include longer peptides which form ordered nanostructures on self-assembly.

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Author contributions

D.M. and H.K. collected and compiled the data, developed the website and wrote the manuscript. D.M. predicted the structural information. G.P.S.R. conceived the idea and coordinated the project.

Competing interests

The authors declare no competing financial interests.

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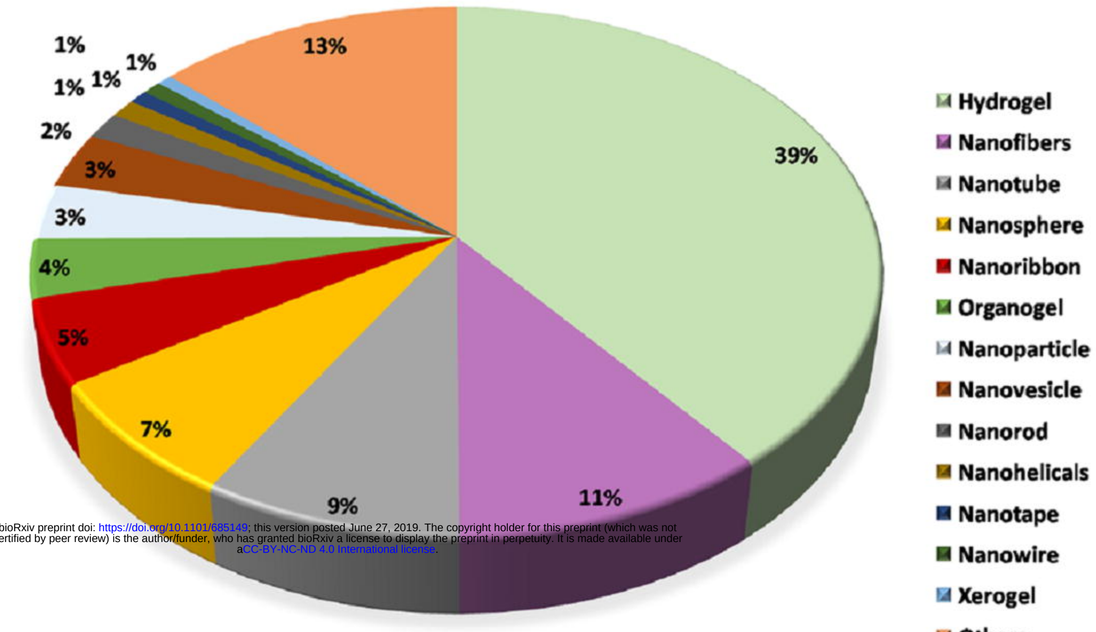

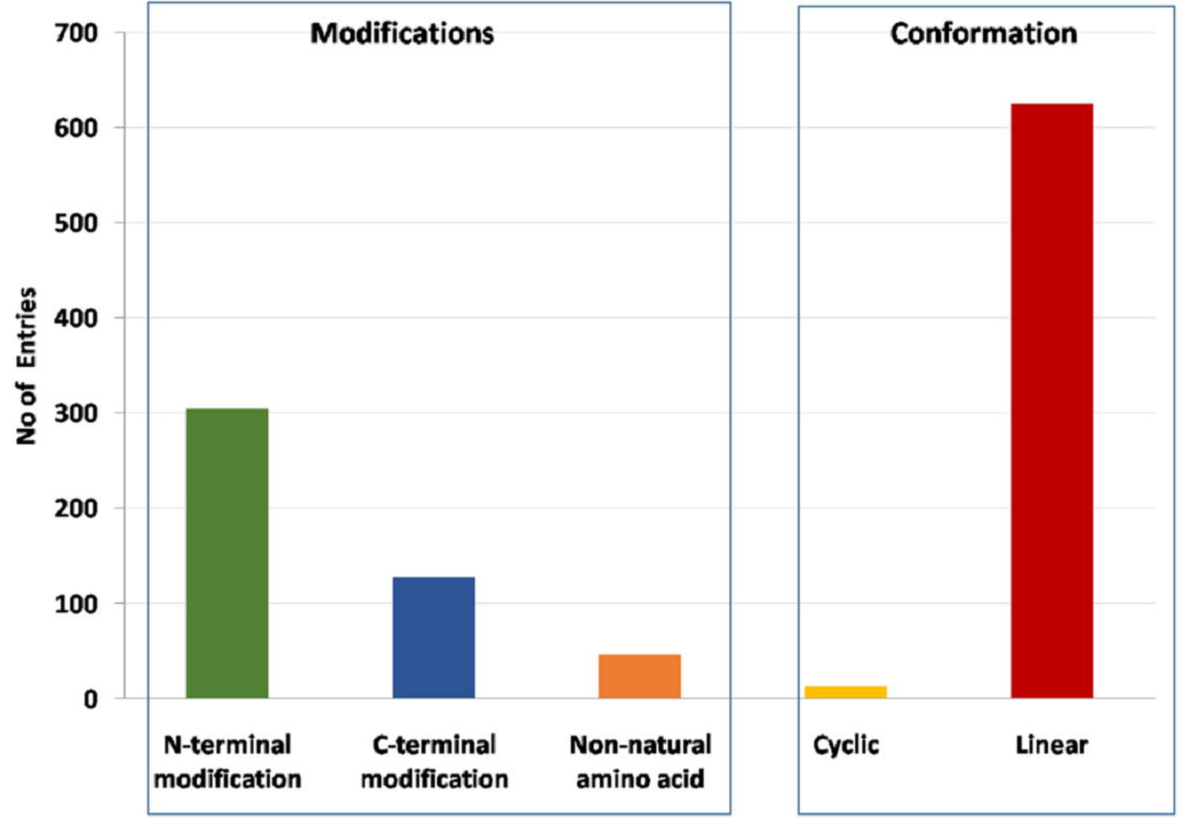

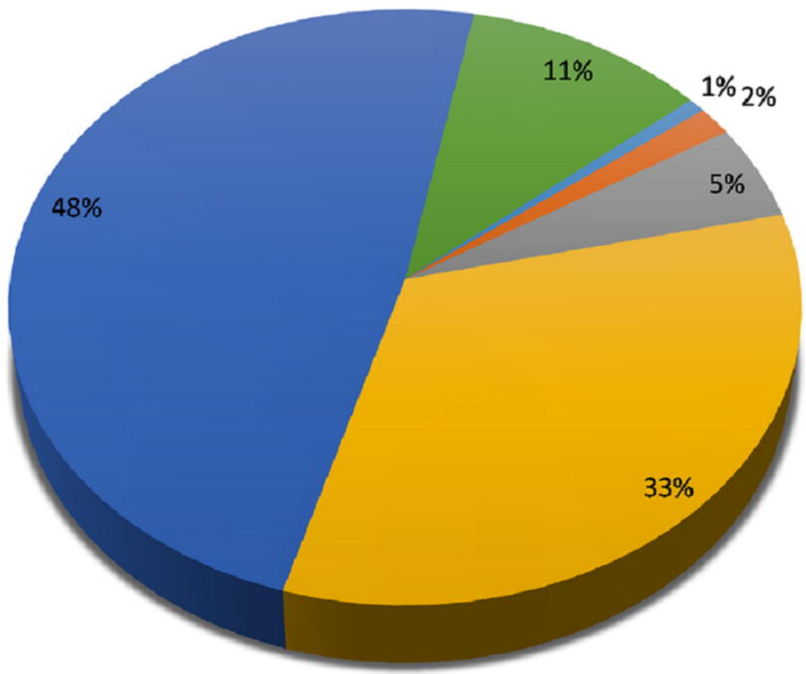

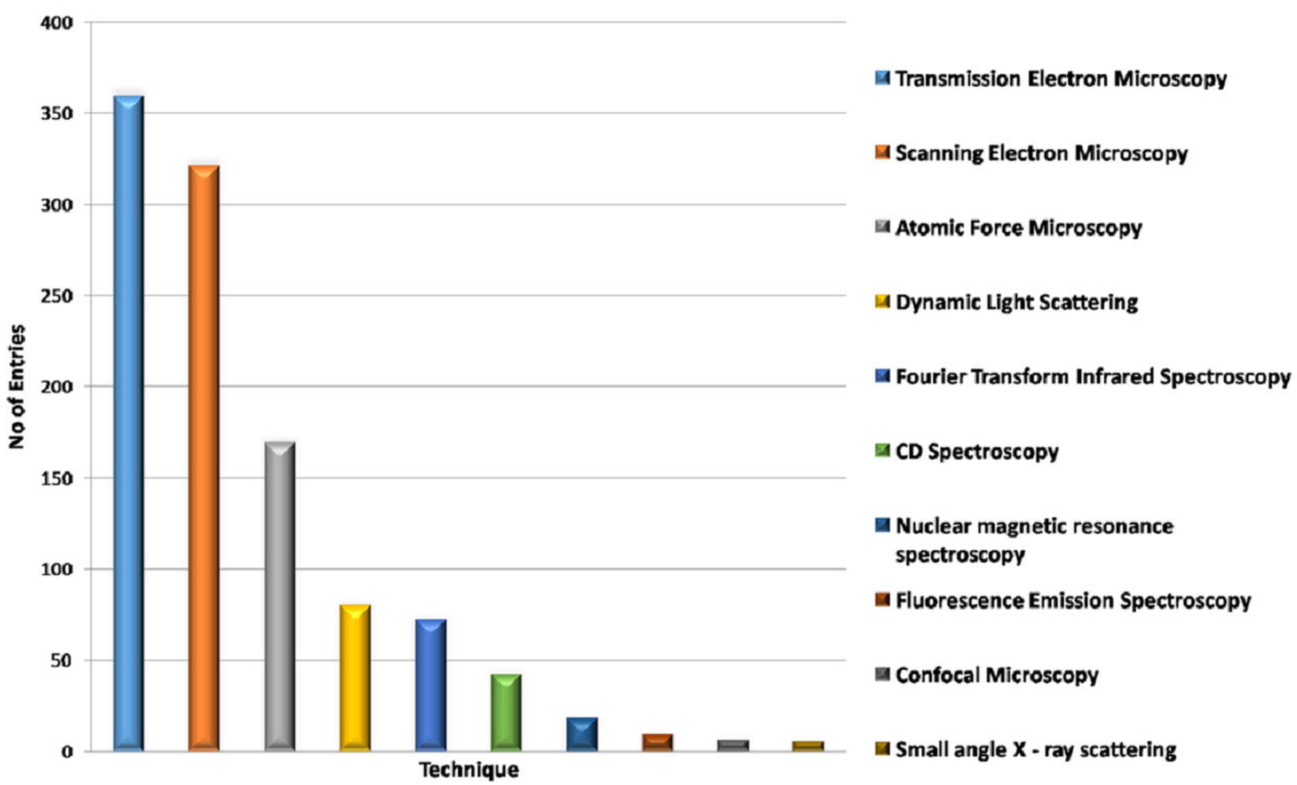

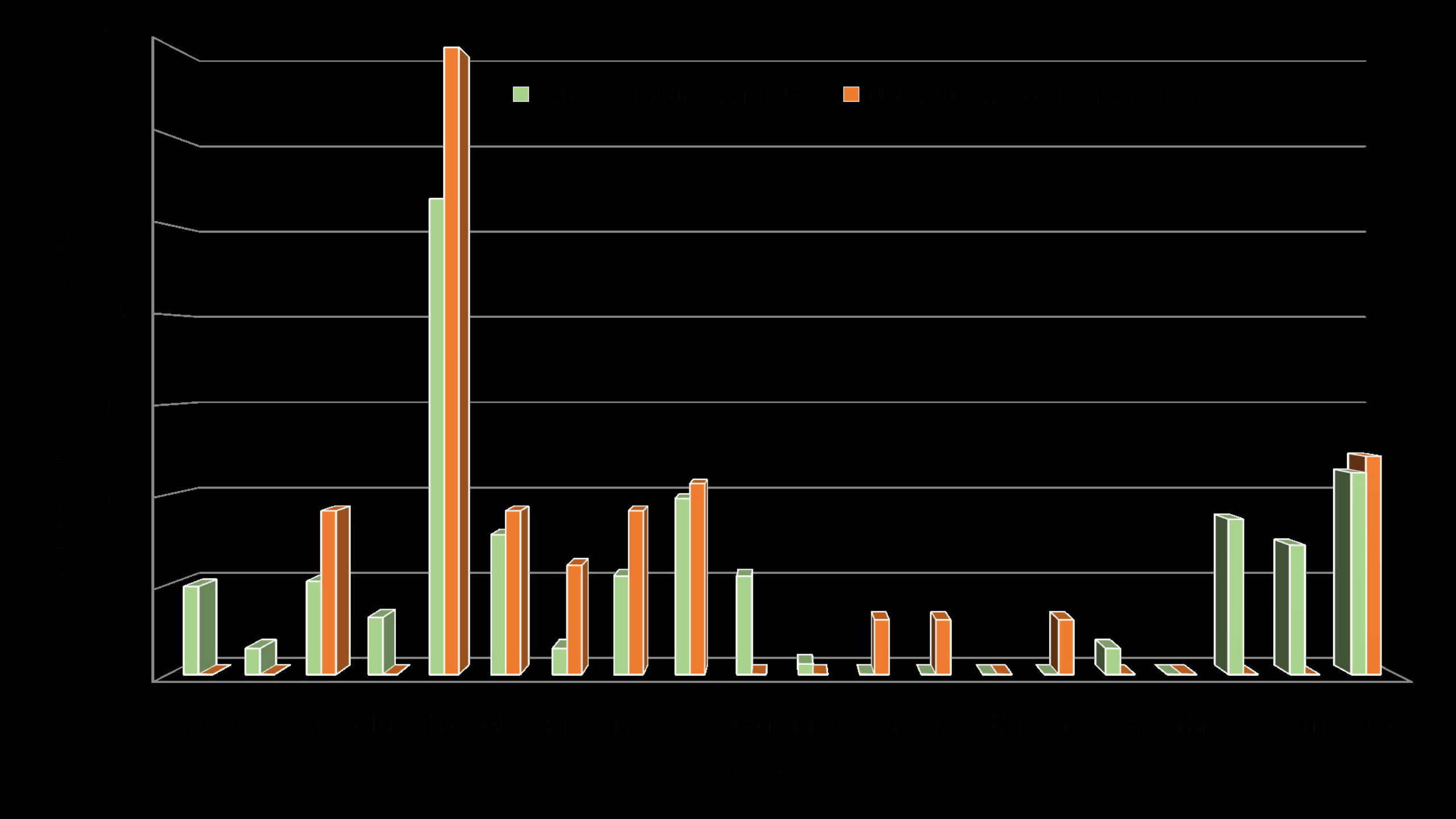

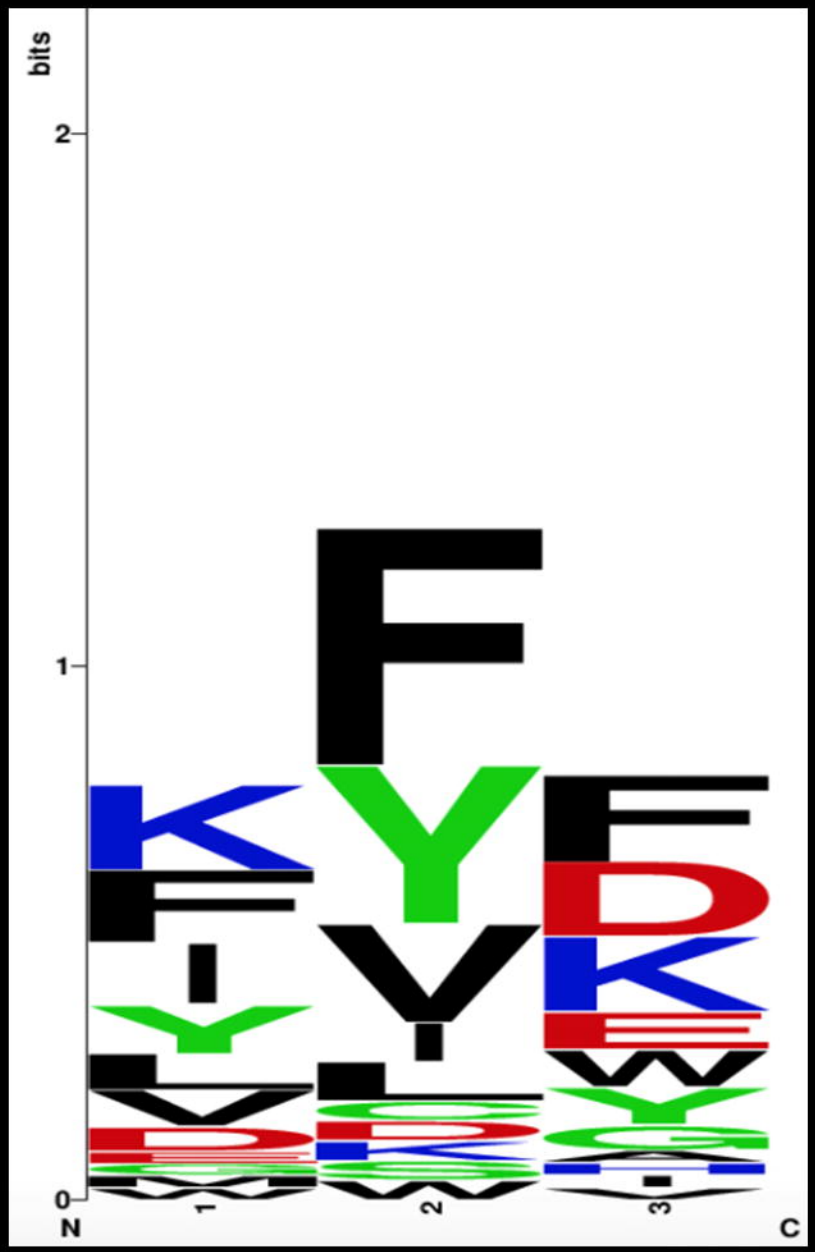
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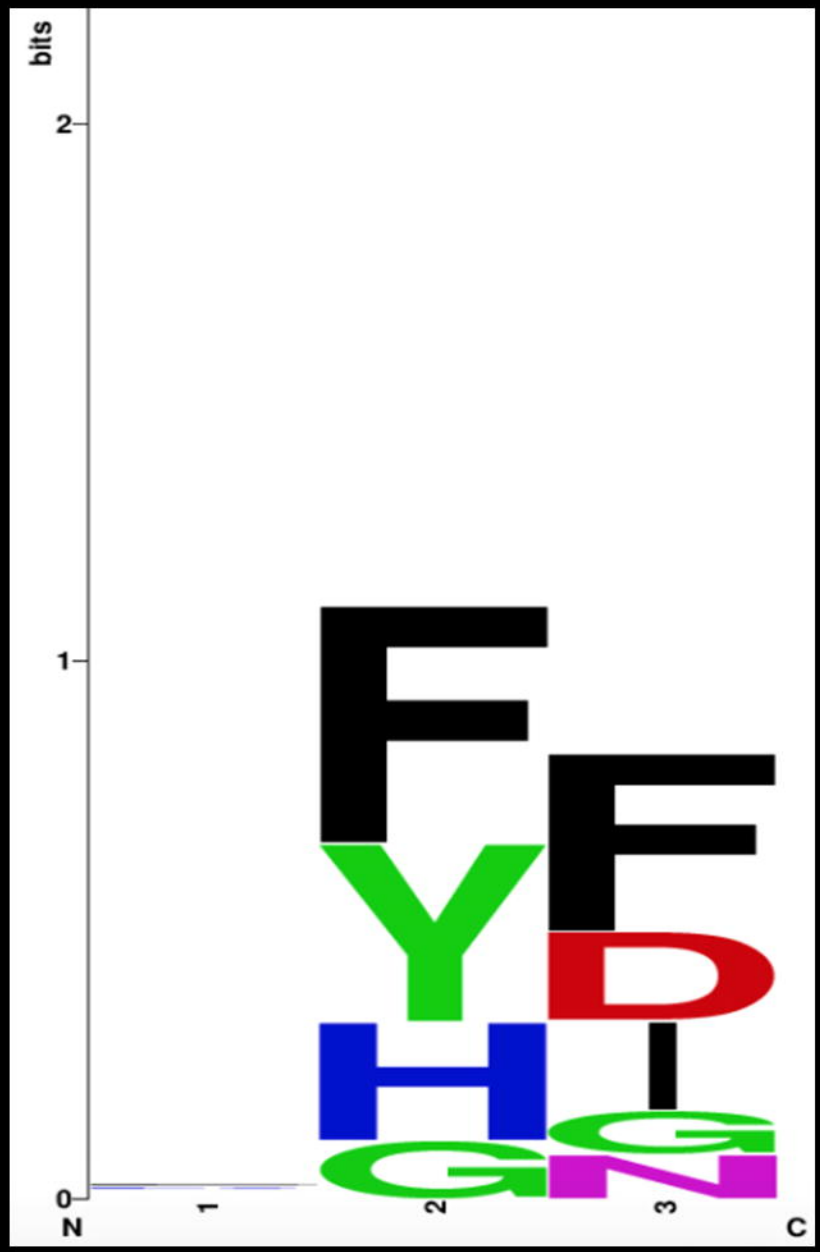
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Hydrogel

Nanofibers

Nanotubes

Nanospheres

Major Classes of Nanostructures

Data Retrieval Tools

- Keyword Search
- Advance Search
- Browse

SAPdb

(637 Entries)

Dipeptides (480)

Tripeptides (157)

Analysis Tools

- GG SEARCH
- Smith-Watermann
- Peptide Mapping
- Physio-chemical properties

Major Fields

- Pubmed ID
- Peptide Name
- Peptide Sequence
- N-terminal Modifications
- C-terminal Modifications
- Technique
- Type of self-assembly
- Nanostructure Size