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

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Abstract

Background: Nanostructures generated by self-assembly of peptides yield nanomaterial that has 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 the promising implications of this rapidly expanding field, there is no dedicated resource to study peptide nanostructures.

Result: This study endeavours 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 1,049 entries of experimentally validated nanostructures formed by the self-assembling of small peptides. It includes 701 entries are of dipeptides, 328 entries belong to tripeptides, and 20 entries of single amino acid with some conjugated partners. Each entry encompasses comprehensive information about the peptide such as chemical modifications in the peptide sequences, the type of nanostructure formed, and experimental conditions like pH, temperature, and solvent required for the self-assembly of the peptide, etc. Further, our analysis has shown that the occurrence of aromatic amino acids favours the formation of self-assembling nanostructures, as indicated by a large number of entries in SAPdb contain aromatics amino acids. Besides, we have observed that these peptides form different nanostructures under different experimental conditions. SAPdb provides this comprehensive information in a hassle-free tabulated manner at a glance. 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.

Availability: The database can be accessed on the web at https://webs.iiitd.edu.in/raghava/sapdb.

Keywords: Nanostructure; Dipeptide; Tripeptide; Self-assembly; Resource

Introduction

Peptides have been reported as key players in diverse fields like immunotherapeutic [1–7], disease biomarkers [8–10], 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]. Besides these areas, peptides are rapidly gaining the attention of researchers in the field of nanobiotechnology [40–43] by 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 a 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 [52].

Self-assembly of peptides can lead to the formation of well-defined nanostructures like nanofibers, nanorods, nanoparticles, hydrogels, nanotubes, etc. [53–61]. The assembly of these nanostructures depends on weak non-covalent interactions like Van der Waals force, hydrophobic interactions, hydrogen bonds, and π-π stacking [62–65]. The lack of a comprehensive understanding of the

properties and mechanisms of the self-assembly of peptide nanostructure formation affects 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 that 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 the self-assembly of peptides into nanostructures for facilitating the rational designing of morphologies and the 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 beneficial 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 collected around ~1500 publications till July 2019. We screened the articles obtained and included only those for further data curation, where information about peptides forming self-assembled nanostructures was available. These self-assembling peptides form various nanostructures, i.e., nanosphere, nanotube, hydrogel, nanovesicle, nanofibers, etc. as represented in Figure 1.

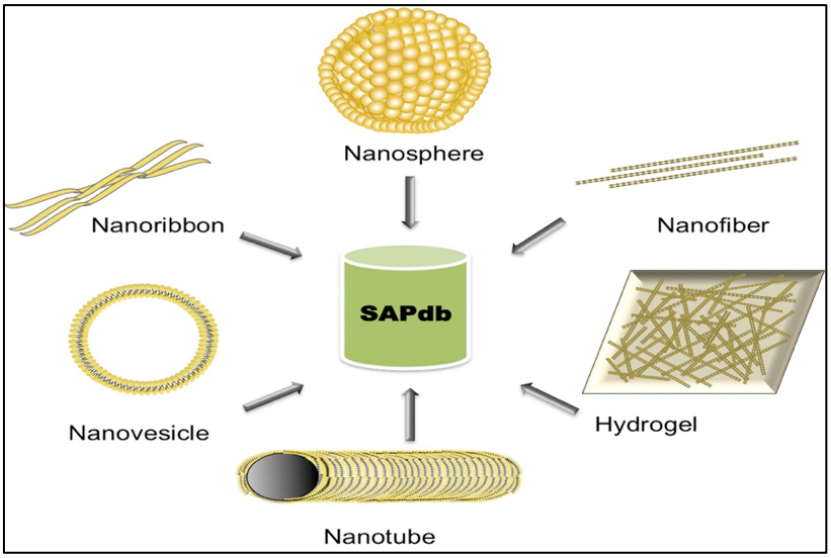


Figure 1: Description of the formation of different nanostructures from the self-assembly of peptides.

After the thorough screening, relevant information extracted from selected articles concerning the sequence of peptides, N-terminal modifications, C-terminal modification, a technique to analyze self-assembled structure, method, type of self-assembly, size of the 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 the 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 is systematically cataloged in a tabulated manner. Consequently, 1,049 entries were collated in the SAPdb database from 301 research articles.

Architecture and web - interface of the Database

SAPdb was designed using Apache HTTP Server (version 2.2.17) on the Linux system following the collection and compilation of significant information. SAPdb is based on MySQL at the back end that was implemented to maintain the information and HTML5, PHP, and JavaScript. They 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. The complete architecture depicting information and tools embedded into SAPdb represented in Figure 2.

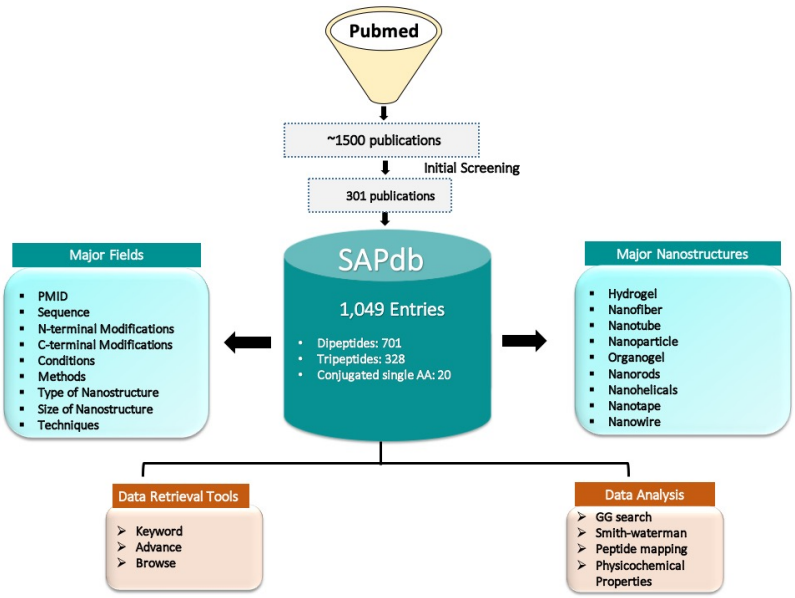


Figure 2: Architecture depicting the information and tools integrated in SAPdb.

Organization of Database

In SAPdb, collated information was classified into primary and secondary information. The primary information includes 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.

Results

Statistical analysis of data of SAPdb

SAPdb is a collection of 1,049 entries of experimentally validated short peptides that undergo self-assembly to form ordered nanostructures manually curated and compiled from 301 research articles published in the recent past with the rise of interest in nanobiotechnology (Figure 3C). Of these 1,049 entries, 701 entries are of dipeptides, 328 entries belong to tripeptides, and 20 entries belong to single amino acid with their conjugate partners. 48 of the peptides were cyclic, while the rest were linear. These model short peptides form 13 major types of nanostructures, as shown in Figure 3A. Hydrogel (297 entries) is the most common type of structure followed by nanofibers (127 entries), nanotubes (67 entries), nanospheres (50 entries), nanoparticles (47), and hundreds of entries of mix nanostructures. Different techniques have been described in the literature to study the phenomenon of self-assembly of peptides. The most common techniques were scanning

electron microscopy, transmission electron microscopy, atomic force microscopy, Fourier transform infrared spectroscopy (Figure 3D).

To give different functionality, stability, and shape to nanostructure, some peptides have been cyclized. In contrast, various terminal modifications and non-natural amino acids have been incorporated in various other peptide sequences (Figure 3B). Thereis a total of 382 entries in SAPdb encompass modifications at the N-terminal. Most commonly reported N-terminal modifications include Fmoc (fluorenylmethoxycarbonyl; 160 entries), Boc (N-t-Butoxycarbonyl; 113 entries) and acetylation (46 entries); while, methoxylation (73), amidation (49 entries), benzylation (33 entries), were reported as primary modifications at C-terminal. Besides this, 54 reported entries had non-natural amino acids like dehydrophenylalanine (ΔPhe) (30 entries), beta-alanine (β-Ala) (16 entries), amino benzoic acid (ABA) (9 entries), amino isobutyric acid (Aib) (9 entries), *etc*. in their sequences.

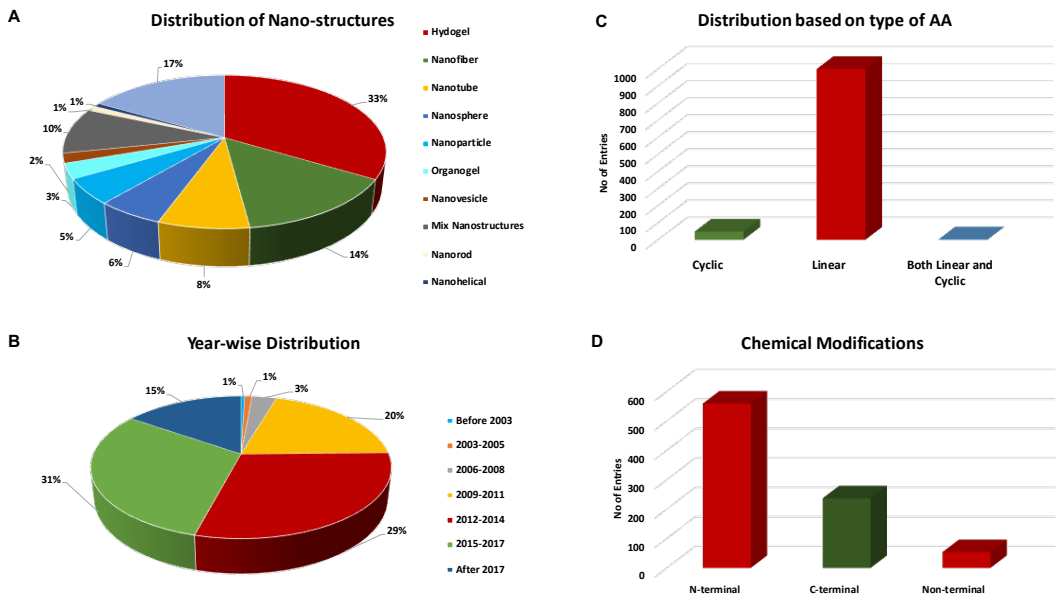


Figure 3: Statistics of distribution of entries in the SAPdb: (A) Types of nanostructures, (B) Year-wise distribution, (C) Based on Peptide, and (D) terminal modification.

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

142 of self-assembly, technique, peptide sequence, etc. Besides, this module also permits the users to
143 select anticipated fields to be displayed in the result.

144 **Advance search:** To retrieve relevant information from SAPdb, the advance search module
145 presents the facility for the user to implement multiple query system. By default, it executes four
146 queries simultaneously, but the user can select the desired keyword search from any selected field.
147 Moreover, this module allows the implementation of standard logical operators (=, >, <, and
148 LIKE). Besides this, advance search permits the user to integrate the output of different queries by
149 employing operators like 'AND and OR'. Further, the user can also add or remove the queries to
150 be executed.

151 **Browsing Tools**

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

156 The chemical modification field assists the user in retrieving data on peptides that have different
157 chemical modifications at the N or C termini. For instance, Fmoc (fluorenylmethoxycarbonyl; 160
158 entries), Boc (N-t-Butoxycarbonyl; 113 entries), and acetylation (47 entries) are major
159 modification at N-terminal; while methoxy (73), amidation (49 entries), benzylation (33 entries),
160 were reported as primary modifications at C-terminal. Type of nanostructure category offers the
161 user to fetch detailed information on peptides that form a particular nanostructure such as
162 nanotube, nanosphere, nanofibers, hydrogel, etc. The size of the nanostructure category allows
163 users to browse entries based on the size of the nanostructure reported. Further technique category

164 permits the user to extract information regarding peptides whose nanostructure was studied using
165 a particular technique such like Transmission Electron Microscopy (TEM), Scanning Electron
166 Microscopy (SEM), and Atomic Force Microscopy (AFM), and Fourier Transform Infrared
167 (FTIR), etc. Additionally, users can also retrieve information of the peptides by the year of
168 publication. Different entries of dipeptide and tripeptide sequences can be explored under the
169 dipeptide and tripeptide modules of the browse facility.

170 **Analysis Tools**

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

173 **GGSearch:** This tool allows the user to perform an efficient similarity-based search against the
174 short peptides in SAPdb. It is based on Needleman-Wunsch alignment [66].

175 **Smith–Waterman Algorithm:** This algorithm [66] permits the user to search peptides in the
176 SAPdb database similar to their query peptides. Under this option, the user can submit concurrently
177 multiple peptide sequences in FASTA format.

178 **Peptide Mapping**: This tool offers the facility to map SAPdb peptides over the query protein
179 sequence. It will be useful for identifying motifs within proteins that tend to undergo self-
180 assembly.

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

183 **Effect of Experimental Conditions**

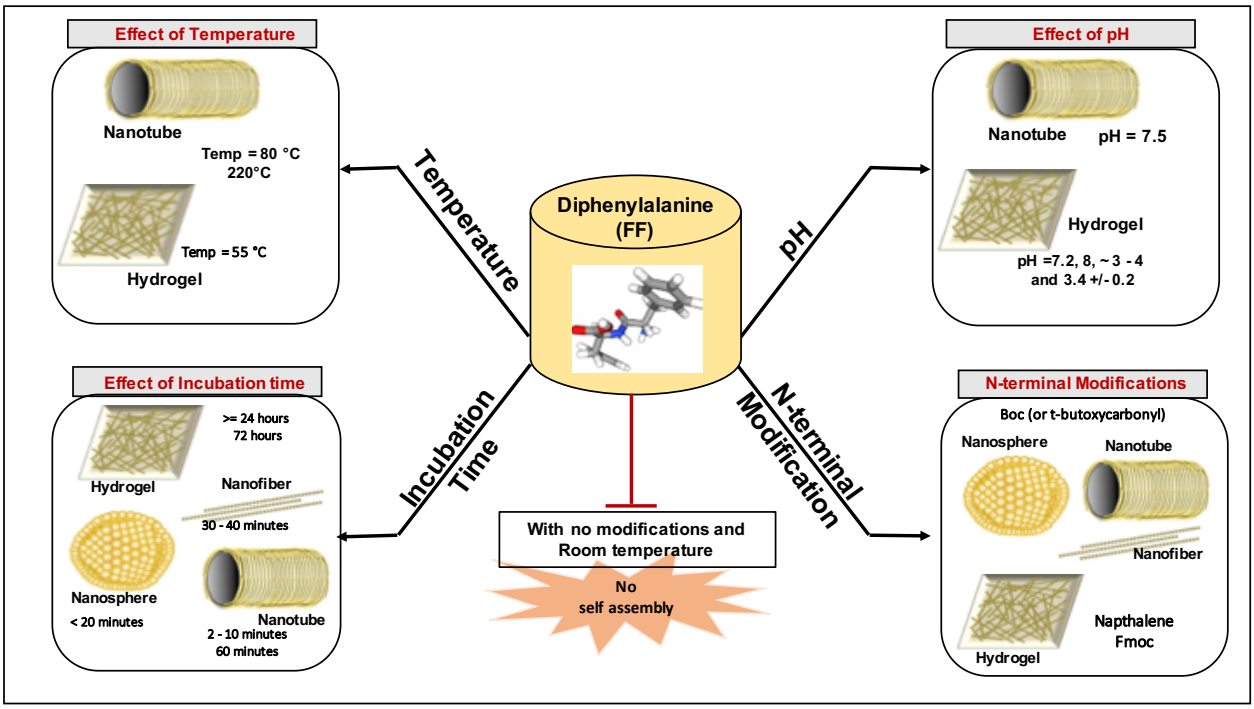
184 Previous studies have shown that the variation in experimental conditions like the type of solvent,
185 change in concentration of peptide, temperature, incubation time, pH, etc. can lead to the formation
186 of different nanostructures by the same peptide [67]. Further, our analysis shows that the
187 concentration of peptide is also an important factor for controlling the shape and size of the
188 nanostructure. For example, KFG tripeptide forms vesicles at a low concentration of 0.5mg/ml
189 while at higher concentration of 5mg/ml forms nanotubes [67]. Furthermore, the role of solvent in
190 directing the type of nanostructure formation is also revealed through this analysis. Most of the
191 nanostructures are formed using water as a solvent, followed by phosphate buffer and organic
192 solvents like methanol, ethanol, hexafluoropropanol, chloroform, and acetone. FF is reported to
193 form nanovesicles in acetone, while in water, it forms nanotubes [68]. This analysis also shows
194 some specific experimental conditions favor the formation of nanostructure as evidenced by the
195 observation that the majority of the nanostructures are reported to be formed by self-assembly
196 under room temperature or 25 °C (499 entries) and in the range of neutral pH of 7 to physiological
197 pH of 7.4 (178 entries) followed by acidic pH of less than 6 (118). Besides, we have observed that
198 there 258 entries (see Table 1), where, peptides form different nanostructures without any chemical
199 modifications or conjugate partners. Out of 258 entries, 180 (70%) entries contain any of aromatics
200 amino acids. This indicates presence of aromatics amino acids favored the self-assembly
201 formation.

202 **Case Study**

203 Our analysis also shown that the different experimental condition changes the nature of self-
204 assemblies. In order to understand the effect of conditions, we have considered an example of
205 diphenylalanine (FF). FF forms hydrogels at different experimental condition such as temperature
206 = 55°C, pH= ~3-4, 7.2 and 8, incubation time >24 hours etc. On the other side, it forms

207 nanostructure like nanosphere, nanotubes, and nanofibers at various conditions like pH, N-terminal

208 modification, temperature, and incubation time, as represented in **Figure 4.**



209

210 **Figure 4: The demonstration of the effect of experimental conditions (pH, temperature, incubation**

211 **time, and N-terminal modification) in the formation of different self-assembled structures like**

212 **nanosphere, hydrogel, nanofiber, and nanotube.**

213 In the absence of any user-friendly web-platform, the user needs to go through widely spread

214 literature in the form of the bulky text publications. SAPdb allows easy access to such information

215 in tabulated form, which leads to a better understanding of the 3D shape of nanostructures formed

216 by altering experimental conditions.

217 **Working of SAPdb database**

218 To understand how a user can be queried and retrieve data from the SAPdb database, we have

219 demonstrated (Figure 5) with an example of a dipeptide, i.e., dipleneyalanine (FF). Figure 5

representing the step by step information on how users can query/search SAPdb using a simple search module by a keyword like PMID, Peptide sequence, Peptide name, year of publication, etc. For instance, here, we queried the search module with peptide sequence, i.e., FF in the given search space (Figure 5A) of the database. User can further select various display fields that are given on a simple search page. On submitting the desired query (e.g., FF) using the "Submit" button, the next page will display all the entries related to the query; for instance, here we retrieved 362 entries of FF, as shown in Figure 5B. Each entry has a unique SAPdb ID, and the user can get the detailed information regarding peptide by further clicking on the SAPdb ID. The next page provides complete information about the selected SAPdb ID, as represented in Figure 5C. It gives the peptide's primary information (SAPdb ID, PMID, Year, Name, Sequence, N-terminal modification, C-terminal modification, non-terminal modification, peptide/conjugate/Mixture, conjugate partner, Technique, solvent, Method, concentration, pH, Temperature, etc. The secondary information provides Physiochemical properties, Structure, and SMILS of the given peptide. Users can further explore these properties, as shown in Figure 5D.

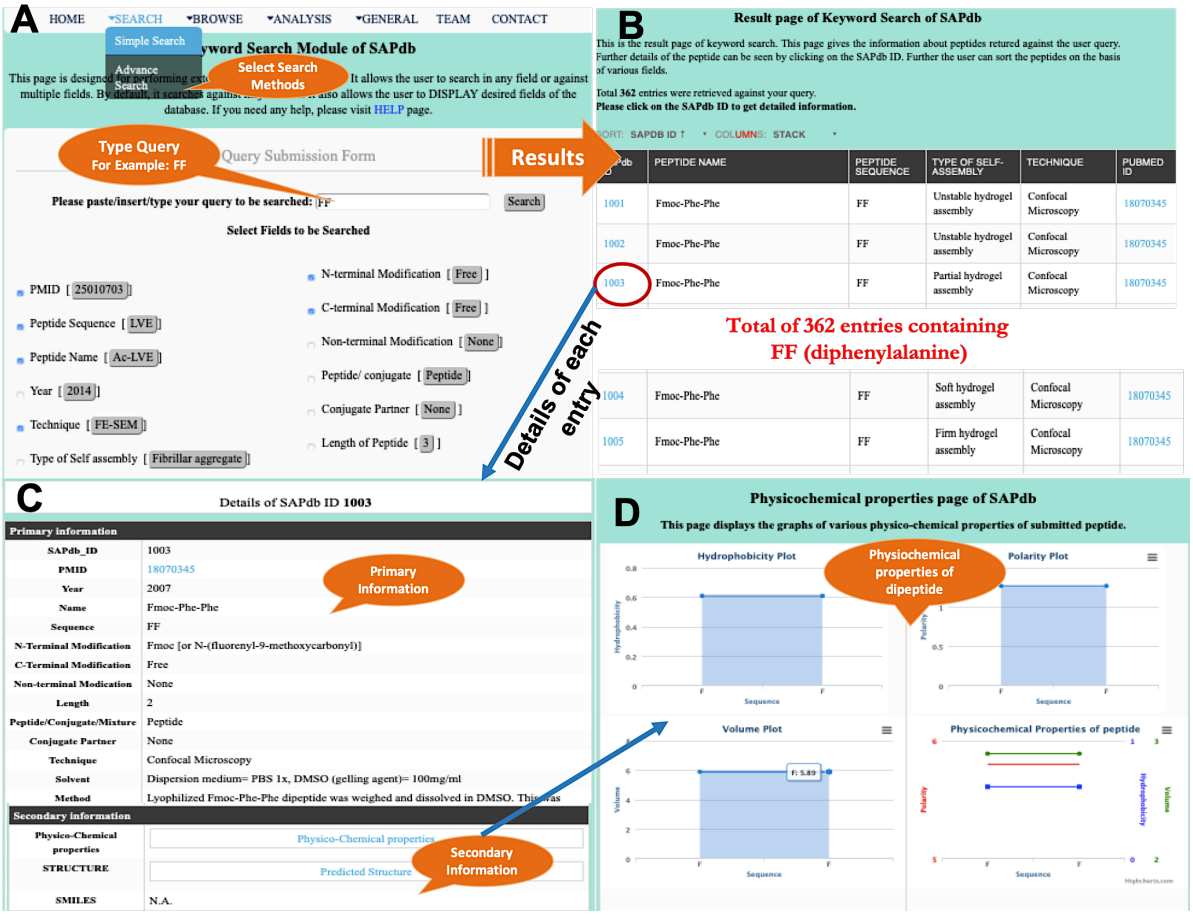


Figure 5: Demonstration of querying and retrieval of information from SAPdb using the Simple Search module.

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 [69], CPAD [70] and AMYPdb [71] 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

244 can assemble into higher-order structures. They are the model candidates to understand the process
245 of self-assembly. SAPdb holds information of 167 unique dipeptides and 96 unique tripeptides that
246 have been experimentally validated for undergoing ordered nanostructure formation by self-
247 assembly.

248 Comprehensive analysis of SAPdb data have shown that different environmental conditions like
249 temperature, pH, solvent, concentration, etc. direct the shape or type of nanostructures formation.
250 Further, the specific conditions, i.e., room temperature, acidic pH, favors the formation of
251 nanostructure of self-assembling peptides. Besides, amino acid composition analysis of peptides
252 of SAPdb revealed that self-assembling peptides are rich in valine, tryptophan, leucine, and
253 alanine. At the same time, they are depleted in isoleucine, aspartic acid, and histidine compared to
254 non-self-assembling peptides. Further, aromatic residues like phenylalanine and tyrosine are
255 favored at the second and third positions in tripeptides undergoing self-assembly. Besides,
256 negatively charged residues are preferred at the C-terminal and while positively charged residue
257 are at the N-terminal.

258 **Utilization of SAPdb**

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

261 **The drug, gene, and other biomolecules delivery vehicles**

262 One of the major applications of SAPs lies in the delivery of drugs and biomolecules. This is
263 attributed to their biocompatible nature, the small size of self-assembling dipeptides and tripeptides
264 [72–76]. For instance, EΔF dipeptide based nanovesicles employed to deliver vitamin B-12 in the

265 HeLa cells [77], while MΔF, IΔF, and LΔF were used for the invitro delivery of curcumin in L-
266 929 cells and in vivo delivery in mouse [78].

267 **Cell culture scaffold biomaterial for tissue engineering**

268 Another major application of the peptide based self-assembled hydrogels is their efficiency for
269 3D-cell culture (Jayawarna). It has been observed that dipeptide FΔF based hydrogel sustained
270 3D cell growth of HeLa cells and L929 (mouse fibroblast) cells for more than 14 days, with
271 substantial multiplication rate and cell viability [79].

272 **Models for nanofabrication and biomineralization**

273 In the past, self-assembling peptides were explored as templates for nanofabrication like
274 nanowires, biomineralization, nanocircuits owing to their potential of self-assembly [80, 81]. For
275 example, Phe–Phe tagged with ferrocence forms uniform nanowires having a diameter of 100 nm
276 with a length 5–10 mm. After adsorbing gold nanoparticles and antibody molecules onto these Fc-
277 tagged nanowires surface, these nanowires were employed as a detection probe for sensitive
278 electrochemical immunosensing [82].

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

286 similarity of their query peptide with different SAPs at a single platform, i.e., SAPdb, which are
287 otherwise scattered in the literature and difficult to access.

288 **Future Developments**

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

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298 **Author contributions**

299 D.M., H.K., A.D., and N.S., collected and compiled the data. D.M., H.K., developed the website
300 and penned the manuscript. G.P.S.R. conceived the idea and coordinated the project.

301 **Competing interests**

302 The authors declare no competing financial and non-financial interests.

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