Modeling peptide-protein complexes: docking, simulations, and machine learning

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Abstract

41 Peptides mediate up to 40% of protein interactions, their high specificity and ability to bind in

42 places where small molecules cannot make them potential drug candidates. However, predicting

43 peptide-protein complexes remains more challenging than protein-protein or protein-small

44 molecule interactions, in part due to the high flexibility peptides have. In this review we look at the

45 advances in docking, molecular simulations, and machine learning to tackle problems related to

46 peptides such as predicting structures, binding affinities or even kinetics. We specifically focus on

47 explaining the number of docking programs and force fields used in molecular simulations, so a

48 prospective user can have an educated guess as to why choose one modeling tool or another to

49 address their scientific questions.

50

Introduction

52

53 Protein-protein interactions (PPI) are a vital component of pathways regulating the behavior of

54 cells. In disease, some of these pathways become aberrant, therefore identifying ways of

55 inhibiting them is of great therapeutic importance. Inhibiting PPI with small molecules is not always

56 possible due to the large interface region, lack of binding cavities and specificity of the interaction.

57 However, between 15 and 40% of PPI(London et al., 2013) are mediated by peptide epitopes,

58 giving rise to opportunities for peptide-based inhibition of PPIs. There is a growing market for

59 peptide-based therapeutic agents(Martins et al., 2021a; L. Wang et al., 2022), and there are

60 already more than 60 peptide drugs approved in the United States(Lau & Dunn, 2018; Usmani et

61 al., 2017). Peptides have well known degradation pathways, lower toxicity than small molecules

62 and are highly specific. Some of the challenges for peptide-based therapeutics such as the rapid

63 degradation by proteases or limited ability to cross membranes(Fosgerau & Hoffmann, 2015) can

64 be overcome by using modified amino acids and cyclization techniques(Bechtler & Lamers, 2021).

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65   Others, like efficient delivery strategies (e.g., oral delivery) limit broader interest(Ganesh et al.,

66   2021).

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68   The rational design of peptide based therapeutic requires structural knowledge of peptide-protein

69   complexes at the atomistic level. Experimental studies are challenging and expensive as peptide

70   degrades fast, and the spectrum of target candidate is too broad to explore experimentally. While

71   the use of computational pipelines is well-stablished in the early stages of drug discovery for small

72   molecules, peptides present some unique challenges that have limited the success of

73   computational pipelines for inhibitory peptide design. Structurally, peptides interact with proteins

74   in different ways(Arkin et al., 2014): 1) as coils through specific amino acid interaction; 2) by

75   adopting well-defined secondary structures (e.g., hairpins or helices); 3) through discontinuous

76   interactions along the peptide chain. In these interactions, peptide flexibility is important, as they

77   are often intrinsically disordered in their free form and adopt well defined structures upon

78   binding—unlike small molecules where flexibility is more limited.

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80   Several excellent and exhaustive reviews on peptides as therapeutics[10,11], and the role of docking

81   to identify peptide-protein interactions(Apostolopoulos et al., 2021; Ciemny et al., 2018a;

82   Fosgerau & Hoffmann, 2015). In this review we describe three major classes of computational

83   methods that are routinely used to elucidate different aspects of peptide-protein interactions: 1)

84   docking; 2) molecular dynamics simulations; and 3) machine learning approach. Docking

85   approaches have traditionally been the most successful at exploring the possible orientations and

86   interaction sites between proteins and peptides. Their use for peptide systems has evolved from

87   protein-small molecule and protein-protein docking tools and face different challenges when

88   accounting for the highly flexible nature of peptides. Hence, these methods are described in most

89   detail. Molecular dynamics methods draw from the wealth of enhance simulation methods

90   available in the literature. Their purpose is to confer detail either about the binding energy

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91   landscape or binding mechanism. They are generally not high-throughput methods and

92   complement studies where the bound structure is known. Finally, machine learning approaches

93   are rapidly evolving thanks to AlphaFold's recent success in protein structure prediction. These

94   methods have rapidly been ported to other applications such as peptide-protein structure

95   prediction with a success rate to match those of the best performing docking programs. Although

96   different in methodology, the three classes of methods need to account for the challenges in

97   sampling bound conformations as well as identifying them through some scoring function. Our

98   goal with this review is to identify the current strategies to approach these challenges and provide

99   a broad understanding of the advances and limitations in the field.

100

101   **Docking section**

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103   Docking remains an efficient approach to sample bound conformations given the receptor

104   structure and the ligand. Their success in small molecule-protein docking and their ease-of-use

105   through webservers and standalone software have popularized this method for virtual screening

106   in the early stages of drug discovery(Taylor et al., 2002). Some of this success has been

107   translated into the protein-protein docking field, as seen from the evolution of predictions in the

108   CAPRI (Critical Assessment of PRediction of Interaction) competition(Lensink et al., 2017, 2020).

109   Despite these successes, peptide-protein docking remains a more challenging problem(Ciemny

110   et al., 2018b). The success of docking relies on the ability to sample bound conformations, and

111   the ability to identify native-like poses using a scoring function. The flexible nature of the peptides

112   significantly increases the sampling problem with respect to small molecule docking and limits

113   sampling native-like poses(Rentzsch & Renard, 2015). Similarly, the flexibility challenges the

114   ability to transfer standard scoring functions to identify peptide-protein complexes. For example,

115   despite the poly-aminoacidic nature of peptides, a straightforward application of protein scoring

116   functions has limited success. Thus, modifications to protein-protein scoring functions are needed

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117 to correctly identify native-like poses(Agrawal et al., 2019b; Weng, Gao, Wang, Wang, Hu, Yao,

118 Cao, Hou, et al., 2020). Innovation in the peptide docking field come from strategies for more

119 efficient handling of flexibility and overcoming limitations in scoring. Along with these

120 improvements, the curation of peptide-protein databases is crucial for systematic testing,

121 benchmarking, and assessing these docking methods(Hauser & Windshügel, 2016). There are

122 already excellent reviews and benchmark studies assessing the performance of different methods

123 (Agrawal et al., 2019a; Ciemny et al., 2018b; A. C.-L. Lee et al., 2019; Z. Wang et al., 2016; Weng,

124 Gao, Wang, Wang, Hu, Yao, Cao, & Hou, 2020a). Hence, we will limit this section to the nature

125 of the databases and different sampling/scoring strategies prevalent in the field.

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127

128 **Databases:**

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130 Many peptide docking methods have evolved from either protein-protein or protein-small molecule

131 docking tools. Their modification includes better handling of flexibility and specific scoring

132 functions. To test their performance on peptide-protein complexes several efforts distil the

133 structural information from the PDB, identifying sets of peptide-protein complexes amongst the

134 ~150,000 structures deposited in the RCSB-PDB(Berman et al., 2002). Thus, the emergence of

135 databases for peptide-protein complexes streamlines the process and accelerates the

136 advancement of the field. Several databases are available, each with a specific purpose in mind

137 – ranging from properties such as length of the peptides to types of binding motifs. Here we

138 provide an overview of the widely used databases. Table 1 provides a quick reference summary.

139

140 *LEADS-PEP*(Hauser & Windshügel, 2016) consists of 53 peptide-protein complexes with

141 peptides ranging from 3 to 12 amino acids with resolution lower than 2 Å. The entries originate

6

142   from a clustering on sequence space, retaining only complexes that are diverse in terms of

143   sequence – excluding those that interact with DNA or RNA. Due to the short length of the peptides

144   in this database, it is a suitable for benchmarking docking tools originating from small molecule

145   docking programs.

146

147   *PeptiDB*(London et al., 2010) is a non-redundant database of 103 high resolution peptide-protein

148   complexes. These peptides are 5 to 15 amino acids long with diverse bound conformations (helix,

149   β-strand, and coil) and functionalities (such as signal-transduction, antibody binding, protein

150   trafficking and transporting). The set includes complexes with a significant conformational change

151   upon binding. These characteristics make PeptiDB appropriate for benchmarking docking tools

152   that account for peptide flexibility.

153

154   *PPDbench*(Agrawal et al., 2019a) database has been used to benchmark 6 common docking

155   programs which contains 133 peptide-protein complexes with less than 40% sequence similarity.

156   The set is diverse with respect to functionality, but the range of peptide lengths is narrower (9 to

157   15 amino acids). The benchmark study with this database by Agarwal et. al. showed that different

158   docking methods perform best on different class of peptides, classified in terms of their

159   functionality such as enzymatic, signaling and many others(Agrawal et al., 2019a). In another

160   study, Weng et. al. created and used the *PepSet*(Weng, Gao, Wang, Wang, Hu, Yao, Cao, & Hou,

161   2020a) database to benchmark 14 docking programs. This database contains 185 high resolution

162   peptide-protein complexes with less than 30% sequence similarity and peptide lengths ranging

163   from 5 to 20 amino acids.

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165   Both LEADS-PEP and PeptiDB have been widely used for benchmarking sampling and scoring

166   ability, but they are limited by peptide length. Peptides longer than 20 amino acids are very

167   common in nature. The *PepPro*(Xu & Zou, 2020) database contains 89 non-redundant peptide-

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168   protein complexes with longer peptide sequences (5 to 30 resides), and diverse peptide

169   secondary structures. As a useful feature of PepPro*,* the database contains 58 structures of the

170   unbound receptor proteins --making it ideal to benchmark docking methods for predicting *apo* to

171   *holo* conformational changes.

172

173   While the above databases have a limited number of complexes, a few databases are more

174   inclusive in their search parameters. For example, *PepX*(Vanhee et al., 2010) contains 1431 non

175   redundant complexes from the PDB with peptide size ranging from 5 to 35 amino acids and

176   resolution less than 2.5 Å. There are redundancies in the database and the number of complexes

177   can be reduced by clustering on their interaction interface reduces, resulting to 505 unique cluster

178   centers. Similarly, the *PepBind*(Das et al., 2013) database is built on similar principles to PepX,

179   without accounting for sequence/structural redundancy. PepBind contains 3100 protein peptide

180   complexes. While larger databases may help in assessing the applicability of docking methods

181   on predicting longer peptides, the databases are not well curated (e.g., complexes in the

182   databases might contain non-interacting chains, small molecule ligands or ions which might lead

183   to erroneous assessment of docking tools(Wen et al., 2018). To overcome this limitation, a

184   curated database *PepBDB*(Wen et al., 2018) was developed, containing - peptides up to 50 amino

185   acids, with nearly 13,000 complexes in the dataset. A more recent database, *Propedia*(Martins et

186   al., 2021b) contains over 20,000 high resolution complexes with peptides ranging from 2 to 50

187   residues. Propedia features a hybrid clustering based on sequence, interface structure or binding

188   site that retains a lower number of clusters (1,845, 1,891, or 1,466 respectively), allowing the user

189   to be flexible for benchmarking purposes. Finally, *PixelDB*(Frappier et al., 2018) contains close to

190   2,000 high resolution and non-redundant complexes. Unlike previous databases, this one relies

191   on a machine learning algorithm along with a chain length cutoff to identify the receptor and

192   peptide in a complex. This overcomes the issue of defining protein and peptide, for cases where

193   the receptor size is smaller than its peptide binder.

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195

196   **Sampling:**

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198   Docking methods can be classified in two major categories depending on their use of templates

199   for modeling the complex (template-based and template-free docking).

200

201   *Template-Based Docking:*

202

203   Template-based docking methods take advantage of known structures of either protein

204   monomers or complexes and extract structural features to predict the unknown peptide-protein

205   complex structure. We summarize these methods in Table 2, and provide further detail below.

206   Template-based methods are grounded on the premise that the peptide-protein interaction

207   interface is conserved and similar to either the protein–protein interaction interface or the different

208   interacting fragments in a protein. Based on conserved interfaces, these methods build a

209   modelling scaffold for the target systems. Indeed, 80% of the peptide-protein interfaces can be

210   derived from fragment interactions in monomeric proteins(Obarska-Kosinska et al., 2016). The

211   first step of these class of methods is finding suitable templates for the target system from different

212   databases. The most popular template-based docking method is *GalaxyPepDock*(H. Lee et al.,

213   2015). It takes a peptide sequence and a receptor structure as inputs to search for structural

214   similarity in the PepBind database. It then uses a score (*S_complex*) for each hit in the database,

215   which is calculated combining the TM score of the receptor in the database with respect to the

216   target receptor, and an Interaction similarity score which is calculated based on the protein

217   structure, peptide sequence, and the interacting residue pairs(H. Lee et al., 2015; "Modeling

218   Peptide-Protein Interactions, Methods and Protocols," 2017). The top 10 scoring templates with

219   scores higher than 90% of the maximum score in the database are selected. These templates are

220 then used to build models with GalaxyTBM predicting an estimated accuracy for each model.

221 Testing on the PeptiDB database where it predicted 37 out of 57 complexes with acceptable or

222 higher quality. In general, the GalaxyPepDock approach is quite reliably when the receptor TM

223 score is greater than 0.7(H. Lee & Seok, 2017). GalaxyPepDock takes 2 to 3 hours to complete

224 a prediction, making it fast compared to methods that rely on computationally expensive molecular

225 simulations. The recently developed InterPep2 (and InterPep2-Refined) also belong to the

226 template-based category, with a similar performance to GalaxyPepDock when tested on unbound

227 receptors set and slightly better when tested on the bound dataset(Johansson-Åkhe et al., 2020).

228

229 *PepComposer*(Obarska-Kosinska et al., 2016) is an example of tool which uses structural

230 knowledge of complexes to design a novel peptide sequence and dock it to the given receptor.

231 This method, in the first step, finds a structurally similar fragment based on a given a binding site

232 and retrieves continuous backbone fragments from a structural database based on contacts to

233 the prior fragment. In the next step, it predicts novel peptide sequences and bound complex

234 structures using Monte Carlo moves embedded in a python-based tool (pyRosetta(Chaudhury et

235 al., 2010)). Testing the method on the LEADS-PEP dataset returned a 50% success rate

236 considering only the top model. However, the caveat here is that the designed peptides are

237 generally shorter than the native which decreases the RMSD value(Obarska-Kosinska et al.,

238 2016).

239

240 *Template-Free Docking:*

241

242 This class of methods sample different peptide-protein orientations and positions as well as

243 generate a diverse set of peptide conformations. Depending on the available knowledge of the

244 binding site, these methods can be further divided into two subclasses: Local and Global docking.

245 In local docking, the binding site is known, reducing search space. In Global docking, no prior

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246   knowledge of the binding site is used, and the peptides explore the whole receptor surface (see

247   Fig. 1). We will discuss these two subgroups in two different sections.

248

249   Local Docking:

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251   As input, local docking methods need a set of user defined information about the binding site,

252   restricting the search of the ligand to the vicinity of this region, so we only need to sample the

253   peptide conformations and orientations. Success of this group of docking methods depends on

254   the accuracy of the initial information(Ciemny et al., 2018b). Knowledge of the binding site can

255   come from diverse sources such as protein-protein complex interfaces, a docked pose from

256   another docking tool, hotspot prediction, or even experimental data. Each method has its own

257   specific input and types of information it can handle, requiring the right fit between prior

258   information and local docking program used. There are also limitations regarding how much

259   sampling of the protein and peptide conformations are needed. As an example, *HADDOCK* uses

260   Ambiguous Interaction Restraints (AIR) based on hotspot residues (e.g., from NMR chemical shift

261   perturbation data) on the protein surface to guide sampling – but requires different peptide

262   conformations as input (e.g., alpha helix, extended and polyproline II) to limit sampling to the

263   relative position of the protein/peptide without sampling the peptide conformations(Geng et al.,

264   2017; Trellet et al., 2013, 2014). Once the preferred bound conformation is found, HADDOCK

265   introduces peptide backbone flexibility to sample diverse conformations. HADDOCK has a 14.5%

266   success rate when tested on the PeptiDB database. *HPEPDOCK*-local, using binding site hotspot

267   information and shape complementarity followed by energy minimization, produces fast and

268   accurate predictions with 33.9% success rate when tested under the same condition as of

269   HADDOCK(Johansson-Åkhe et al., 2019; P. Zhou et al., 2018). HPEPDOCK relies on *MODPEP*

270   to generate an ensemble of unbound peptide conformations. An advantage of HADDOCK over

271   HPEPDOCK is its ability to handle ambiguous data for the binding site(Deplazes et al., 2016;

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272   Williamson, 2013). Finally, Rosetta FlexPepDock *ab initio*, can also produce a diverse set of

273   peptide conformations for binding, but this comes at a higher computationally expense(Raveh et

274   al., 2010, 2011).

275

276   The second group of local docking methods are derived from small molecule docking programs

277   (such as *AutoDock Vina*(Rentzsch & Renard, 2015), *GOLD*(Verdonk et al., 2003), or *Surflex-*

278   *Dock*(Spitzer & Jain, 2012) to name a few). Although no initial conformation of the peptide is

279   required, the accuracy rapidly decays when sampling beyond 10 flexible bonds, limiting peptide

280   size(Ciemny et al., 2018b). In this approach, the peptide is placed in the binding site and peptide

281   conformations are sampled using either Monte Carlo moves (AutoDock Vina and GOLD) or

282   rotamer libraries (Surflex-Dock). AutoDock is most reliable with peptide lengths between 2 to 4

283   amino acids. Several methods have been developed to tackle longer peptides through

284   incremental docking approaches (e.g., *DINC 2 .0*(Antunes et al., 2017), *DLPepDock*(Sun et al.,

285   2021))(Antunes et al., 2017). The incremental pipeline in DINC 2.0 has several stages: 1) dock a

286   small fragment (preferably 6 rotatable bonds, roughly 2 amino acids) with AutoDock 4; 2) increase

287   the peptide size by adding 3 more rotatable bonds and freezing 3 of the 6 previous rotatable

288   bonds; 3) dock the peptide again(Antunes et al., 2017). Using this approach DINC 2.0 has been

289   successful with up to 25 flexible bonds. The selection of the initial fragment is done based on

290   heuristics, while the extension of the fragment follows the potential to maximize H-bonding with

291   the receptor. The benchmark test included a custom dataset of 73 protein peptide complexes with

292   multiple successes, including the docking of a B2 chicken MHC class I receptor and an 8-mer

293   chicken peptide (1.61 Å RMSD from the native structure). The *Glide SP-PEP* method also uses

294   fragment-based docking with Iterative Residue Docking and Linking (IRDL) to dock peptides

295   smaller than 8 amino acids(Diharce et al., 2019). It uses Glide's SP-PEP module to dock each

296   residue iteratively to the binding site and then uses the covalent module to create bonds between

297   them. The success rate for this method was high in a custom-made benchmark set with 10 out of

298   11 successful docking examples.

299

300   The third group of local docking methods can be termed as refinement methods (*DynaDock*(Antes,

301   2010), *PepCrawler*(Donsky & Wolfson, 2011), *Rosetta FlexPepDock*(Raveh et al., 2010)) or

302   peptide inhibitor design methods (*PepCrawler*) rather than strictly docking tools. These methods

303   need input structures of either coarse peptide-protein complexes (for refinement) or protein-

304   protein complexes (for inhibitor design)(Ciemny et al., 2018b). DynaDock, In the first step,

305   generates broad sampling of the peptide conformation at the binding site by performing random

306   rotation of backbone torsion and sidechain. In the next step, it uses an MD (OPMD) based

307   refinement of the bound modes which allow the full flexibility to the receptor(Antes, 2010). Rosetta

308   FlexPepDock uses Monte Carlo moves to sample diverse peptide conformations with full receptor

309   flexibility with on-the-fly energy minimization(Raveh et al., 2010). Unlike these two methods,

310   PepCrawler can be used in two ways – refinement and inhibitor peptide design. For refinement,

311   it uses an initial protein peptide complex structure as input and samples a diverse range of peptide

312   conformations with a Rapidly-exploring Random Trees (RRT) based algorithm. To design inhibitor

313   peptides, it uses provided protein-protein complexes to generate the peptide fragment with lowest

314   binding energy in the first step, followed by the RRT algorithm as refinement to dock the diverse

315   peptide conformation. PepCrawler allows peptide and protein sidechains flexibility and only the

316   peptide backbone flexibility(Donsky & Wolfson, 2011). This group of methods produce best results

317   for short peptides (< 15 amino acids) and when the initial conformation of the peptide is below 5Å

318   RMSD from the native protein peptide complex(Ciemny et al., 2018b).

319

320   Some of the popular local docking tools are summarized in Table 3, together with suggested

321   applications where each method is most successful. The first two groups of docking methods can

322   be used when we only have binding site information and do not have any structural information

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323   of the complex. They are often used to generate initial models which can be later refined with

324   other methods. Tools such as *PeptiMap*(Lavi et al., 2013), *PepSite*(Trabuco et al., 2012), *PEP-*

325   *SiteFinder*(Saladin et al., 2014), *SPRINT-str*(Taherzadeh et al., 2017), *ANCHORSmap*(Ben-

326   Shimon & Eisenstein, 2010), or *InterPep*(Johansson-Åkhe et al., 2019) can predict the binding

327   site, when no other structural information is available. The most recent method, InterPep uses

328   template-based knowledge and a machine learning based model to predict the binding site,

329   outperforming most the other existing tools(Johansson-Åkhe et al., 2019). Experiments such as

330   Chemical Shift Perturbation, alanine scan mutagenesis or ligand foot printing mass spectrometry

331   provide information about the binding site and can be used alternatively to binding site predictors.

332

333

334   Global Docking:

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336   This class of template free docking programs becomes specially useful when there is no

337   information about the binding site (see Fig.1). This class of methods is the most general as it

338   requires the least amount of information provided by the user. However, the additional

339   computational effort required to simultaneously sample the binding site as well as peptide

340   conformations limits the success rate when compared to the previous classes of methods. Many

341   of these approaches use a two-step procedure composed of a fast rigid docking stage to identify

342   the bound state followed by a refinement strategy. Thus, the local docking methods described

343   above can be used as part of the refining strategy.

344

345   There are several strategies to generate initial peptide conformations for the rigid docking stage:

346   1) methods such as *MDockPeP*(Yan et al., 2016), *MDockPeP2*(Xu & Zou, 2022), *Cluspro*

347   *PeptiDock*(Porter et al., 2017), use a *MODELLER*(Webb & Sali, 2014) based algorithm and

348   *PIPER- FlexPepDock* (*PFPD*)(Alam et al., 2017) uses the Rosetta fragment picker to extract a

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349 fragment from an interacting partner of a protein-protein complex with similar sequence.

350 MDockPep2 additionally considers the physiochemical environment similarity of the binding

351 interface along with sequence similarity in the fragment picking stage; 2) *pepATTRACT*(Schindler

352 et al., 2015; Vries et al., 2017) threads through three major secondary conformations (i.e. alpha,

353 beta and coil) using *PeptideBuilder*(Tien et al., 2013); and 3) *HPEPDOCK*-global uses MODPEP

354 to generate an ensemble of peptide conformations(P. Zhou et al., 2018). Once the peptide

355 conformation is identified, each method relies on their own rigid docking strategies. Cluspro

356 PeptiDock and PFPD use a PIPER based protocol(Kozakov et al., 2006); MDockPeP uses a

357 modified version of AutoDock vina whereas MDockPeP2 uses ZDock (protein-protein docking

358 tool) to carry to out the rigid docking step(Xu & Zou, 2022; Yan et al., 2016); pepATTRACT uses

359 ATTRACT to carry out rigid body docking of the peptide with the ATTRAC coarse grained

360 representation of the protein and peptide; and HPEPDOCK uses a modified version of MDock

361 making it suitable for protein –peptide systems to perform rigid body docking. At the end of the

362 rigid docking step, these methods have their own ways to include flexibility in the system and

363 refine the docked structures. These strategies include using local docking methods, MD or MC

364 simulations, or other energy minimizers. For instance, PFPD uses Rosetta FlexPepDock;

365 pepATTRACT uses iATTRACT and AMBER molecular dynamics simulation for refinement; and

366 HPEPDOCK uses a SIMPLEX energy minimizer as the fully flexible refinement step(Alam et al.,

367 2017; Schindler et al., 2015; P. Zhou et al., 2018).

368

369 Methods like *CABS-dock*(Kurcinski et al., 2015, 2019), *AnchorDock*(Ben-Shimon & Niv, 2015),

370 *AutoDock CrankPep* (ADCP)(Zhang & Sanner, 2019) allow flexibility to the peptide during the

371 whole docking process. CABS-dock generates peptide conformations in explicit solvent in the

372 presence of the interacting partner, allowing the peptide to adopt its bound conformation. Thus,

373 this allows full flexibility on both the receptor and peptide side. It uses a coarse-grained

374 representation of each amino acid where backbone and sidechains are represented by two

375   pseudo atoms each for computationally efficiency(Kurcinski et al., 2015, 2019). The limitation in

376   this case is the need for the peptide's secondary structure, which is not available in most cases.

377   PSIPRED(McGuffin et al., 2000) is used when the secondary structure is not known -- even

378   though it is not ideal for predicting the secondary structure of peptides, which are typically

379   intrinsically disordered in their free form(Yan et al., 2016). Unlike other global docking tools,

380   AnchorDock uses the prediction tool *ANCHORSmap* to identify the anchoring spot and then

381   performs an anchor-guided MD simulation(Ben-Shimon & Niv, 2015). This strategy combines the

382   speed-up of the restraints with full flexibility of the peptide/protein system. A recent method, *ADCP*,

383   uses Monte Carlo moves to sample peptide conformations under the influence of the potential

384   landscape generated by the receptor which helps it to find correct fold upon binding making it

385   highly successful allowing fully flexible docking(Zhang & Sanner, 2019).

386

387   Methods which use molecular simulations (extensive MD or MC) either in the docking stage or in

388   refinement stage, generally have higher accuracy than the other methods(Agrawal et al., 2019a;

389   J. Wang et al., 2019; Weng, Gao, Wang, Wang, Hu, Yao, Cao, & Hou, 2020b). For example,

390   despite the global nature of pepATTRACT, it can be as successful as some of the local docking

391   approaches, with a local version of the method (pepATTRACT-local) having a higher success

392   rate(Schindler et al., 2015). PeptiDock+ GaMD which refines Cluspro PeptiDock results with

393   Gaussian accelerated MD (discussed in the MD section) performs significantly better than the

394   traditional Cluspro PeptiDock(J. Wang et al., 2019). AnchorDock correctly predicted 10 of 13

395   complexes (RMSD < 2.2Å) in a custom dataset(Ben-Shimon & Niv, 2015). ADCP has shown

396   better performance than most of the other existing docking tool for peptide ranging 16 to 20

397   residues with an 87% success rate (considering 10 models) when tested on LEADS-PEP

398   dataset(Zhang & Sanner, 2019). Finally, *PFPD*, considered one of the state-of-the-art methods,

399   can produce near native complex structures in 70% for bound test sets and 40% for unbound test

400   sets(Alam et al., 2017). However, due to the nature of the simulation, these methods are

401   significantly slower compared to others. The computational resource requirements increase with

402   longer peptides while maintaining the accuracy (e.g., simulations running for hours on GPUs or

403   longer in CPUs)(Ciemny et al., 2018b). These expensive simulations are needed to achieve the

404   higher accuracy as shown by the pepATTRACT web server which removes the refinement

405   step(Vries et al., 2017). When using the PeptiDB benchmark set, the webserver predicts 14 out

406   of 80 complexes correctly (within 2 Å RMSD of the experimental structure), whereas the full

407   pepATTRACT version correctly predicts 38 complexes(Vries et al., 2017).

408

409   Recently, the Furman lab introduced *patchMAN*, a motif search method (using the MASTER

410   algorithm) combined with Rosetta FlexPepDock refinement, which outperforms other

411   methods(Khramushin et al., 2022). At the first step, it searches for the receptor surface motifs in

412   a non-redundant protein database followed the finding peptide templates that interact with these

413   motifs and the target peptide sequence is threaded through the models. In the end, Rosetta

414   FlexPepDock is used to refine all the models. This method allows fully flexibility of the binding site

415   of the receptor as well as the peptide. When tested on the custom-made PFPD database, it

416   outperforms PFPD and even recent machine learning based revolutionary AlphaFold(Jumper et

417   al., 2021a), considering the success criteria as a 2Å RMSD cutoff from the native. However, on a

418   different dataset (LNR) its performance is comparable to AlphaFold (Khramushin et al., 2022).

419   Table 4 summarizes the for global docking tools listing their features and suitable application

420   cases.

421

422   **Scoring:**

423   At the sampling step, docking methods obtain an ensemble of docked poses -- some of them are

424   native-like, while some are far from native. The state of the art in peptide docking is reliable at

425   sampling the correct binding site. For example, when we only consider sampling efficiency,

426   MDockPeP has success rate of 95% when starting from bound conformations and 93% when

17

427   starting with challenging unbound structures(Yan et al., 2016). The recent method patchMAN can

428   sample within 5Å RMSD from the native complex in 100% cases(Khramushin et al., 2022). This

429   implies that currently, the limitation and overall successes of the docking tools can be attributed

430   to the scoring stage majorly. Thus, the next crucial step is to find the best docked model,

431   representing the native complex, in the ensembles of the docked poses(Ciemny et al., 2018b;

432   Weng et al., 2019a) (see Fig. 1). These methods can be classified in several major groups such

433   as using a knowledge-based scoring function, energy-based method, clustering-based method,

434   and integrative or combinational approach. One important feature of a good scoring method is

435   that it should consider entropic contribution due to conformational change as well as the

436   interaction energy.

437

438   There are a series of scoring function used successfully in small molecule and protein-protein

439   docking field. End point methods like MM/PBSA and MM/GBSA, mentioned in the MD section,

440   are widely used for small molecule binding free affinity calculation and scoring(Hou et al., 2011;

441   Pu et al., 2017; E. Wang et al., 2019b; Weng et al., 2019a). When applied to peptide-protein

442   systems, if these methods are used with appropriate parameter, they outperform pepATTRACT

443   (which uses ATTRACT scoring function) and produce similar quality as HPEPDOCK-local (which

444   uses an iterative knowledge-based scoring function coming from protein–protein docking tool

445   MDock)(Weng et al., 2019b). However, these methods do not consider entropic contributions due

446   to peptide conformational changes, limiting their success to binding processes without significant

447   changes in the peptide conformation. Ideally, these methods should be modified or combined with

448   others for generalized use(Spiliotopoulos et al., 2016; Tao et al., 2020). As an improvement,

449   BiPPred and HADDOCK uses a dampened version of MMPBSA named dMMPBSA algorithm as

450   a scoring function to calculate the free energy and rank docked poses (Spiliotopoulos et al., 2016).

451   In this approach they reduce the Coulombic interaction and polar solvation term by factor of 5 to

452 compensate the overestimation of free energy due to the omission of entropy. Another recent

453 approach by H. Tao et. al. combines MM-GBSA scoring function with knowledge-based scoring

454 function ITScorePP to consider the conformational entropy part(Tao et al., 2020). ITScorePP is

455 derived from atomic distance-based energies parametrized iteratively using statistical mechanics.

456 Their work has shown rescoring the pose clustering with this combined scoring function makes

457 the results significantly better when compared against pepATTRACT, CABS-dock and

458 HPEPDOCK result with LEADS-PEP dataset.

459

460 A group of methods use clustering algorithms on the ensemble of docked poses (or filtered docked

461 poses) based on structural RMSD – but they have their own way to use clustering for selecting

462 structure. Cluspro PeptiDock assigns highest score to the most populated cluster's medoid,

463 whereas CABS-dock selects the consensus medoid obtained from different clustering protocols

464 as the best model(Kurcinski et al., 2015, 2019; Porter et al., 2017). Rosetta FlexPepDock and

465 PFPD perform clustering and score the top clusters with a modified Rosetta ab-initio energy

466 function(Alam et al., 2017; Raveh et al., 2010). The modified version of the Rosetta ab-initio

467 energy function has been shown be successful as it combines standard all atom Rosetta energy

468 with internal peptide energy and interaction energy. AnchorDock also uses clustering algorithm

469 on all snapshots from molecular simulation trajectories and scores the clusters based on the

470 average potential energies of the best 15 models (in term of binding energy) in each cluster(Ben-

471 Shimon & Niv, 2015). pepATTRACT's ATTRACT scoring function is based on modified Lenard-

472 Jones function to select 1000 models and those are further refined by amber followed by

473 performing clustering on simulated trajectory. The clusters are ranked based on the average

474 ATTRACT energy of 4 lowest energy models(Schindler et al., 2015).

475

476 Integrative scoring methods combine external information like agreement with co-evolutionary

477 data or mutagenesis data with energy based or clustering-based scoring which have performed

478   very well in the recent CAPRI competitions(Lensink et al., 2020; Yu et al., 2017). Machine learning

479   is recently becoming one of the methods of choice to derive scoring functions.

480   InterPepRank(Johansson-Åkhe et al., 2021) is an such example which uses deep graph based

481   neural network mapping the protein peptide complex, and DockQ(Johansson-Åkhe et al., 2020)

482   uses a Random Forest model to score and rank the docked poses. Recently, the field of docking

483   has started using combined multiple scoring method together to compensate each other limitation.

484   For example, when InterPepRank is combined with PFPD pipeline as rescoring, success rate

485   increases significantly (40% for the high-quality prediction). Simultaneously, it filters out some the

486   non-native dock poses using a InterPepRank score cutoff, reducing the number of hits for

487   refinement steps which increases the computation efficiency(Johansson-Åkhe et al., 2021).

488

489   **Summary:**

490   The wealth and diversity of available docking programs and servers for peptide-protein complexes

491   poses a barrier of entry to newcomers to the field or would-be users. It is difficult to answer a

492   question like, "which is the best?" as it will depend on the system and available information. Each

493   method has advantages and disadvantages such as the ability to work on peptides of different

494   size, or the ability to explore large conformational changes upon binding. In general, local docking

495   tools outperform the global tools, but the latter does not need any information about the binding

496   site. Most of the global docking tools can be used as local docking tools when the binding site

497   information is provided leading to higher success rates(Schindler et al., 2015; P. Zhou et al., 2018).

498   A benchmark study by Weng el. al. showed that performance of local docking methods that are

499   based on AutoDock, drop significantly depending on erroneous inputs of the size of binding sites,

500   especially for peptides longer than 10 residues(Weng, Gao, Wang, Wang, Hu, Yao, Cao, & Hou,

501   2020b). In summary, for a user when the target receptor can find a template with enough

502   homology (TM score >0.7), template-based methods generally offer the highest success rates.

503   Alternatively, when the TM score is lower than 0.7, template free methods should be used. For

504   peptide smaller than 5 residues, the docking tools coming from small molecule docking work the

505   best(Ciemny et al., 2018b). For the longer peptides, computationally extensive molecular

506   simulation-based tools such as PFPD, or ADCP and patchMAN become more relevant. Running

507   short MD simulation-based refinements have shown higher successes, however computational

508   expenses also rise. In future, coarse graining on the non-interacting residues might be a way to

509   reduce the computer time required for these molecular simulation-based tools. Moreover, the

510   docking field has started to apply combined approaches like combined predictions from

511   InterPep2-refined and PFPD which has shown better performance than the individual

512   one(Johansson-Åkhe et al., 2020).

513

514

515   **MD section**

516   The nature of molecular dynamics (MD) simulations allows, in principle, to obtain thermodynamics,

517   kinetics and mechanistic understanding of the peptide-protein binding and unbinding process. As

518   in the application to other biological problems, MD is limited by the accuracy of the physics model

519   used and the ability to sample the complex energy landscape, which typically requires

520   computational resources beyond our current capacity.

521

522   **Modeling peptide-protein interactions with modern force fields.**

523

524   The interactions of molecules can be theoretically determined using quantum mechanics (QM)

525   but remain unaffordable in practical terms for large biomolecules. In practice an empirical force

526   field is used to model such interactions along with Newton's equations of motion to simulate the

527   dynamics. MD simulations have been shown to accurately predict the binding potency of diverse

528   small molecule binders(L. Wang et al., 2015). However, there are key differences between protein

21

529   binding with small molecule and peptide that makes the latter more computationally challenging.

530   First, while small molecules have a few hot-spot interactions that dominate recognition, peptide

531   recognition can stem from many weak interactions. Second, the structure of peptides can be

532   highly flexible, requiring finely tuned parameters that are sensitive to the change in conformational

533   preferences between the free conformation of peptide and its bound form. Thus, peptides have

534   been specially affected by known biases in secondary structure preferences present in some force

535   fields(Perez et al., 2015; Robinson et al., 2016).

536

537   Early force field development was an art, guided by great scientific insights (e.g., some

538   parameters originated from "guesses" that have remained as part of the force field for decades),

539   and carried out by a few expert groups. One of the challenges is the unexpected consequence of

540   parameter changes as modifying one parameter might affect the accuracy of another parameter

541   that was not adjusted due to the coupling between different terms. Even long MD simulations on

542   a set of systems with times series, distributions, behavior, and stability analysis might not be

543   enough to capture all possible issues. Some issues might arise in timescales beyond those

544   studied during development or in systems not included in the benchmark test. While some groups

545   might have made *ad hoc* modifications to deal with problems in specific systems, these

546   modifications were not often properly benchmarked and rarely made it back into the main force

547   field branch. Such trend has dramatically changed in recent years as measured by the number of

548   force fields as well as involvement of many groups representing these improvements. Despite

549   this, a general problem is the lack of a golden standard benchmark set for parameter development

550   (e.g., for proteins, nucleic acids, lipids). The availability of open sharing resources would make

551   the preparation and dissemination of such a benchmark test an easy endeavor. Community efforts

552   such as the OpenFF are already on their way for continuous optimization of small molecule force

553   fields(Qiu et al., 2021).

554

555   Here, we focus on recent efforts to improve the description of peptides and intrinsically disordered

556   proteins (IDPs) while maintaining the stable properties for folded proteins (Fig. 2), more extensive

557   surveys can be found elsewhere(Mu et al., 2021; Rahman et al., 2020). Force field development

558   follows three main strategies: 1) modifying the dihedral angle parameters against experiments

559   and/or quantum mechanical calculation; 2) adjusting non-bonded protein-water interactions; and

560   3) balancing dihedrals with grid-based energy correction maps (CMAP).

561

562   Most effort is concentrated on directly refining the global backbone dihedral parameters including

563   Amber ff99SB* and ff03*(Best & Hummer, 2009), ff14SB(Maier et al., 2015), ff99SB-

564   ILDN(Lindorff-Larsen et al., 2010), CHARMM22*(Piana et al., 2011), OPLS-AA/M(Robertson et

565   al., 2015), and OPLS3(Harder et al., 2016), while further improvements also involve refined side-

566   chain dihedral terms. The residue specific force field (RSFF) approach involves residue-specific

567   dihedral parameter refitting to achieve better agreement with experimental data. RSFF1 force

568   field(Jiang et al., 2014) is developed based on OPLS/AA(Kaminski et al., 2001), while RSFF2(C.-

569   Y. Zhou et al., 2015) is based on the ff99SB force field(Hornak et al., 2006). Protein-water

570   interactions are actively involved in peptide-protein binding process, where the ensemble of

571   peptide conformations in its free form are highly sensitive to the solvation model used (e.g., overall

572   compactness as defined by the radius of gyration). Not surprisingly, the Amber ff03ws combined

573   with a refined TIP4P/2005 solvation model(Best et al., 2014) and the CHARMM36m with an

574   optimized TIP3P model(Huang et al., 2017) have shown improvement in modeling IDPs

575   ensembles. While some combinations of solvent force fields are designed to work with specific

576   protein force fields, others such as the TIP4P-D(Piana et al., 2015) aim to improve general

577   deficiencies such as underestimation of London interactions by developing larger water dispersion

578   coefficient, resulting in improved agreement with experimental observables over a broad range of

579   force fields. The CMAP strategy was first introduced as a grid-based correction to the CHARMM22

580   force field(MacKerell et al., 1998) to account for coupling of ψ/ϕ torsion angles

581 (CHARMM22/CMAP)(Mackerell et al., 2004). The latest iteration (CHARMM36)(Huang &

582 MacKerell, 2013) has been the starting point for new CMAP potentials that better balance

583 between folded proteins and IDPs (CHARMM36m). The CMAP approximation has been adopted

584 in other families of force fields such as AMBER(Perez et al., 2015; Tian et al., 2019) or

585 OPLS(Yang et al., 2019). Over recent years, the CMAP strategy has led amino acid specific

586 potentials, rather than using a few transferable potentials for all (e.g., non-glycine or proline). Thus,

587 ff99IDPs(W. Wang et al., 2014) and ff14IDPs(Song et al., 2017) develop specific CMAP

588 parameters for eight disordered-promoting amino acids. More recently, ff14IDPSFF(Song et al.,

589 2017) and CHARMM36IDPSFF(Liu et al., 2018) add a different CMAP correction to each of the

590 20 amino acids.

591

592 Although such optimizations can better describe the more extended conformation of disordered

593 peptides, some modified force fields generate unstable structures for folded proteins. Ideally, a

594 force field that allows accurate descriptions of both folded and unfolded ensembles is preferable

595 because it would better simulate transitions of peptides between disordered state to ordered state.

596 The a99SB-disp force field developed by Robustelli et al. modified the ff99SB-ILDN parameters

597 and adjusted TIP4P-D water model against experimental measurements, the resulting force field

598 has shown great improvement for modeling disordered ensembles and still maintains the

599 accuracy for folded proteins(Robustelli et al., 2018). Another environment specific force field

600 (ESFF1) was recently developed based on CMAP corrections of 71 different sequence

601 environments(Song et al., 2020). These force fields have demonstrated an improved balance

602 between modeling IDPs and folded proteins. With the number of choices available, it might be

603 daunting to choose the right force field for your system. Many MD packages such as

604 AMBER(Case et al., 2005), CHARMM(Brooks et al., 2009), NAMD(Phillips et al., 2005),

605 Gromacs(Abraham et al., 2015), Tinker(Lagardère et al., 2017) or OpenMM(Eastman et al., 2017)

606 provide user a wide selection of force fields, even originating from different force field families

607   (e.g., AMBER or CHARMM family of force fields). It is important that for whichever force field is

608   selected the compatible solvent model and ion parameters tested by benchmark studies should

609   also be used correspondingly.

610

611

612   **Characterizing peptide binding poses and affinities by molecular dynamics simulations**.

613

614   MD is not high-throughput enough in its own to routinely determine the structures of peptide-

615   protein complexes when the bound state is unknown. The three major applications for MD are:

616   1) Refinement of docking results; 2) Estimating binding affinities based on known bound

617   complexes; and 3) use of integrative modeling strategies to determine structures of the complexes.

618

619   Docking approaches described in the previous section favor speed at the expense of accuracy,

620   while MD approaches are accurate, but inefficient at identifying where and how a peptide binds

621   from conventional MD simulations. Thus, short MD simulations are often the last step of docking

622   calculations to eliminate steric overlap, account for local conformational changes and identify

623   structures based on physico-chemical principles rather than relying on a scoring function. While

624   this application is standard, it does not leverage the full potential of MD such as calculating binding

625   affinities. Thus, recent integration of docking and MD based techniques such as the combination

626   of ClusPro PeptiDock with Gaussian accelerated molecular dynamics (GaMD) goes beyond

627   refining the structure to provide free energy profiles(J. Wang et al., 2019). In this work, the authors

628   benchmarked their method on three distinct model peptides achieving 0.6-2.7 Å improvement in

629   peptide backbone structures. Moreover, the unbiased free energy profiles help identify key

630   residues involved in significant conformational changes upon binding that can later be used for

631   peptide sequence optimization and design.

632

633   When the experimental structure of the complex is known, end-point methods based on MD are

634   typically used to determine binding affinities. MM/GBSA and MM/PBSA are among the most

635   popular methods in this category, introduced by the Kollman group two decades ago, the method

636   is grounded on robust physico-chemical principles(E. Wang et al., 2019a). The method has been

637   well received by the community and still favored over empirical and semi-empirical scoring

638   functions designed for protein–peptide docking (see discussion in docking section). Despite its

639   robust theoretical framework, its practical implementation results in approximations (such as

640   flexibility, solvation, and entropy) that limit the accuracy of the results. For example, prediction of

641   peptide binding affinities for peptide–MHC complexes is highly desirable for vaccine design, but

642   the flexible nature of the peptides make routine affinity prediction using bioinformatic pipelines

643   insufficient. The inclusion of structural information is crucial to explore relevant molecular

644   conformations of the peptide-protein complex, and therefore, key to understand its dynamic

645   behavior. Wan et al. combined the MM/PBSA and the conformational entropy method to compute

646   peptide–MHC binding affinities from molecular dynamics simulations where both the bound and

647   unbound peptide were simulated(Wan et al., 2015). The method achieves highly correlated

648   binding affinity rankings with experimental estimates after normalizing $\Delta G_{MM/PBSA}$ with the

649   hydrophobicity of peptides. Ochoa et al. generated conformations of the complexes from MD

650   simulation, then using a scoring function to predict binding affinities in better agreement with

651   experiments than either sequence-based predictions or single docking scoring methods(Ochoa

652   et al., 2019). Pathway based free energy calculation methods such as free energy perturbation

653   (FEP) have achieved unprecedented accuracy in modeling protein binding with small molecule

654   for a large set of ligands(L. Wang et al., 2015). However, directly transferring such approaches to

655   estimate protein-peptide binding free energy is challenging due to the flexibility and size of

656   peptides. Kilburg et al. introduced a single-decoupling alchemical method that successfully

657   calculated the free energy for HIV1-IN binding with a series of cyclic peptides(Kilburg & Gallicchio,

658   2018). The calculation convergence is largely affected by the ladder parameters in Hamiltonian

659   and temperature replica exchange, specifically, more dense parameters are required to increase

660   the overlap between phase space of alchemical states in large ligand system. FEP based

661   simulations have also been applied to estimate the mutation effect in binding specificity change

662   of PDZ-peptide system(Panel et al., 2018) and help select a potent blocker for Kv1.3

663   channel(Rashid et al., 2013).

664

665   Integrative approaches combine computational modeling with experimental information to

666   determine structures of peptide-protein complexes. Methods such as Rosetta and MODELLER,

667   are examples involving different types of modeling strategies. Other methods such as maximum

668   entropy(Pitera & Chodera, 2012) or Bayesian inference(MacCallum et al., 2015) aim to identify

669   distributions that agree with experimental data. Analyzing such distributions yields the number of

670   states that best represent the data. Our use of the modeling employing limited data (MELD)

671   approach for peptide binding has been successful for harnessing chemical shift perturbation NMR

672   (Mondal et al., 2022)and ALA scan mutagenesis data in predicting conformations of the bound

673   complex(Morrone, Perez, MacCallum, et al., 2017b). Furthermore, its physico-chemical

674   foundation allows the user to recover relative binding free energies using a competitive binding

675   protocol. These simulations sample peptides conformations in binding while allowing their full

676   flexibility and accurately match experimental results for a series of peptides inhibiting the p53-

677   MDM2 and MDMX interaction(Morrone, Perez, Deng, et al., 2017; Morrone, Perez, MacCallum,

678   et al., 2017a).

679

680

681   **Unveiling peptide binding/unbinding kinetics through enhanced sampling.**

682

683   Accurate prediction of peptide-protein binding/unbinding kinetics from MD simulations requires

684   extensive sampling of bound/unbound states, the transitions between them, and possible

27

685   intermediate states. However, the structural flexibility of many peptides challenges estimation of

686   association and dissociation rates, represented by k_{on} and k_{off} respectively, as complicated

687   binding mechanism arises including folding upon binding of peptides to the receptor, and inherent

688   structural heterogeneity arises from weak interactions in the binding interface. Recent simulation

689   studies on peptide-protein interactions employs various advanced sampling methods with critical

690   thermodynamic and kinetic analysis. Markov state models (MSM) has been widely applied to

691   estimate kinetic quantities of biomolecular conformational dynamics from a set of short atomistic

692   MD simulations(Chodera & Noé, 2014). Paul et al. used multi-ensemble Markov models, which

693   combine conventional MD with Hamiltonian replica exchange enhanced sampling simulations, to

694   characterize peptide-protein binding mechanism and kinetics beyond the seconds timescale of a

695   nano-molar peptide inhibitor PMI to the MDM2 receptor(Paul et al., 2017). Zhou et al. studied the

696   p53 binding with MDM2 by running near 1 ms unbiased simulations on a distributed computing

697   platform(G. Zhou et al., 2017). Two key intermediate states were identified from a four-state

698   kinetic model using MSM analysis and k_{on} was predicted in good agreement with experimental

699   estimation. Zwier et al. generated hundreds of continuous binding pathways from weighted

700   ensemble (WE) simulations and obtained similar on-rate estimates(Zwier et al., 2016). In addition,

701   they identified residue F19 from p53 might be a kinetically important residue for binding as the

702   majority of conformations involve its partial or complete burial.

703

704   Metadynamics employs biasing potential as a function of collective variables (CVs) by which the

705   system is allowed to cross high-energy barriers that are conventionally difficult to sample(Bussi &

706   Laio, 2020). Zou et al. investigated the folding and binding process of p53 to MDM2 using two

707   metadynamics based methods yielding a reasonable estimation for the on/off-rate constants and

708   the binding free energy profile(Zou et al., 2020). The anchor residues F19 and W23 of p53 were

709   identified to follow the stepwise binding pattern. This finding helps explain certain mutants can be

710   regulated by weak non-native interactions near bound state due to the disorder nature of p53.

711   The consequence of secondary interactions on the binding mechanism was also addressed by

712   extensive unbiased simulations combined with umbrella sampling to perform MSM analysis for a

713   coronavirus-derived peptide, bound to a prevalent MHC receptor in humans(Abella et al., 2020).

714   The model reaffirms the major role of anchor positions in the peptide for establishing stable

715   interactions and reveals the underestimated importance of a non-anchor position. The conclusion

716   was confirmed by simulating the impact of specific peptide mutations and validated these

717   predictions through competitive binding assays where stark differences in unbinding pathways

718   were identified by comparing the MSM of the wild-type system with those of the D4A and D4P

719   mutants.

720

721

722

723   **Machine Learning**

724   The role of machine learning in structural biology was greatly accelerated by the success of

725   AlphaFold (AF) in the 13th installment of the Critical Assessment for Structure Prediction (CASP)

726   event(Senior et al., 2020). Two years later, after the field had replicated all the previous successes,

727   a complete re-design of AF produced even higher accuracy structure predictions that surpassed

728   any previous expectation(Baek et al., 2021; Jumper et al., 2021b). The accuracy of such ML

729   predictions is sometimes in better agreement with NMR data than the models generated by

730   standard NMR pipelines(Tejero et al., 2022). Not surprisingly, the field was soon ready to test the

731   limits and possibilities of the AF approach. Early on, adding poly-glycine linker successfully tricked

732   AF into predicting the structures of complexes where the linker remained unstructured. This

733   strategy has produced a level of accuracy for peptide-protein complex structure prediction that

734   surpasses state-of-the-art docking programs in a recent benchmark test, especially for complexes

735   with binding motifs. A retraining of AF for complexes was soon published online (AF-multimer),

736 but the weights have not been as extensively refined as the ones for the original AF(Evans et al.,

737 2021).

738

739 The success of such approaches begs the question of why AF is performing well on complexes

740 such as peptides. One observation points to the structural complementarity between peptides and

741 proteins, where many peptides adopt well-defined secondary structures upon binding. Indeed,

742 peptides that bind as coils are not predicted as well, although generally the binding site is still

743 identified. The field of docking uses scoring functions to rank the different poses and compounds

744 – a strategy that is very successful for small molecules, but which has not reached the same level

745 of maturity for peptides (as described above in the docking section). AF's pLDDT measure also

746 lacks the possibility of ranking different peptides as different sets of peptides might be predicted

747 with similar pLDDT scores despite very different binding affinities. Other ML approaches directly

748 use structural ensembles and a measure of accuracy such as RMSD to assess the quality of the

749 predicted structures(Townshend et al., 2021). This begs the question of whether AF or other ML

750 algorithms can learn something about the biophysical energy function that governs binding (or

751 folding) and how it can be used towards predicting peptide-protein complex structures.

752

753 AlQuraishi's group first addressed this question using a bespoke Hierarchical Statistical Modeling

754 ML approach to learn the biophysical function that scores multiple peptides binding a receptor

755 motif(Cunningham et al., 2020). Unfortunately, lack of data meant that this approach could only

756 be used for eight protein families. Could AF capture such as biophysical function from its training?

757 Recent work from Ovchinnikov's group suggests that indeed AF has learnt such a function(Roney

758 & Ovchinnikov, 2022). According to this study, MSAs serve the purpose of global sampling,

759 focusing the search space in regions of interest, and the biophysical energy learned through the

760 network is able to identify the best local structure. This is especially interesting for peptide-protein

761 systems where the problem can be separated in two parts: (1) a template or MSA for the receptor

762   and (2) a single sequence for the peptide. In this way the learned function is responsible for finding

763   where the peptide interacts, its conformation, and any conformational changes required for the

764   receptor. We put this notion to the test by using competitive binding in AF to determine which

765   peptides had higher affinity to a series of receptors. Surprisingly, the method was very successful

766   in ranking the strongest binders. As the differences in binding affinity become small, the method

767   reflects this uncertainty, and the method is not suitable when both peptides are weak binders. A

768   caveat of the above developments is the requirement to fit within the original hypothesis of

769   peptide-protein complementarity – when the structure of the complex is not correctly predicted

770   then the competitive binding will also not work.

771

772   Turning this argument on its head, the Baker group uses the idealized version of proteins that

773   neural networks learn to design new proteins. The process of deep neural network hallucination

774   has already produced several structures that have been confirmed experimentally(Anishchenko

775   et al., 2021). Adding constraints into the hallucination process can direct the design into areas of

776   complementarity or desired functionality. As such, the complementary nature of peptides to

777   receptor binding motifs can lead to the design of peptides or mini-proteins based on constrained

778   hallucination to the known binding site(J. Wang et al., 2021).

779

780   The advances in ML for structural prediction and accurate scoring, gives rise to the ability to query

781   increasing large libraries of peptides(Chang & Perez, 2022). Along these lines, peptide-protein

782   interaction predictors are also starting to emerge to predict which peptides will interact with a

783   certain protein and give insight into the peptide residues involved in the interaction(Casadio et al.,

784   2022; Lei et al., 2021). Furthermore, ML is also offering ways to identify peptide sequences which

785   are likely to have high biological activity against a particular pathology(Wu et al., 2019) (e.g.,

786   anticancer(Chen et al., 2021) or antimicrobial(Dee, 2022; E. Y. Lee et al., 2017; Plisson et al.,

787   2020) peptides). Thus, we expect combining ML pipelines that act at the sequence level with

31

788 those at the structural level will be able to create peptide libraries specific to a type of disease that

789 can then be screened to predict structures and their relative binding affinities. This is a rapidly

790 changing field, where ML is already having a big impact, and where many questions regarding

791 the interpretability and applicability of the current technology need to be answered.

792

793 **Conclusions**

794 Our focus in this review has been to identify the different approaches that docking, molecular

795 simulations and machine learning use to study peptide-protein systems. We expect new synergies

796 between these three types of technologies will lead to more robust methodologies to capture

797 peptide-protein systems. For example, docking can reliably identify the binding region and   might

798 provide good templates for ML to refine, to predict structures and screen peptides for binding free

799 energies. Meanwhile, the emphasis of new force fields in correctly describing intrinsically

800 disordered peptides together with enhanced sampling can benefit from initial models to determine

801 kinetic constants and binding affinities through an orthogonal approach (on a more limited set of

802 systems to refine). A current limitation in machine learning is the dependence on natural amino

803 acids. MD on the other hand has transferable potentials and can be used as an end-stage in

804 peptide optimization for studying the effect of controlling flexibility (e.g., through chemical staples)

805 or of replacing some residues with non-natural aminoacids such as peptoids.

806
807
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810 **Conflict of interest**

811 The authors declare no conflicts of interest.

812 **Author contributions**

32

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814   AM,LC,AP

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1360 **Table, Figures and captions.**

Dataset	Number of complexes	Length of peptide	Special Features	Specific application	Availability
LEADS-PEP	53	3-12 residues	Diverse sequence of peptides, complexes do not interact with nucleic acids.	Due to smaller peptide size, suitable for testing tools adapted from small molecule docking tools.	www.leads-x.org
PeptiDB	105	5-15 residues	Diverse secondary structure of peptides including conformational change upon binding, complexes with diverse biological functions.	Suitable for testing tools that tackle peptide flexibility.	RCSB code of the complexes: https://ars.els-cdn.com/content/image/1-s2.0-S096921260900478X-mmc1.pdf
PPDbench	133	9-15 residues	Diverse in term peptide sequences (<40% sequence similarity) and biological functionalities.	Suitable for testing docking tools on different complexes categorized with different functionalities.	https://webs.iiitd.edu.in/raghava/ppdbench/
PepPro	89	5-30 residues	Contains 58 unbound receptors structures.	Useful for testing tools whether they can predict apo-holo conformational change.	http://zoulab.dalton.missouri.edu/PepPro_benchmark
Propedia	~20000	2-50 residues	Contains subsets of complexes based on clustering on different features such as sequence, interface structure or binding site.	Broader range of peptide length allows it to test different type of docking tools. Also, different subset gives flexibility to user on testing their tools.	https://bioinfo.dcc.ufmg.br/propedia
PixelDB	1966	NA	Uses machine learning to identify protein and peptide. This helps to overcome the issue of incorrectly identifying them when peptide is larger than the receptor.	Broader range of peptide length allows any docking tools to be tested on.	https://github.com/KeatingLab/PixelDB

1361 Table.1: Summary of the popular protein-peptide complexes datasets that are widely used for testing and benchmarking
1362 different docking tool.
1363 (** This is supposed to be after the "Databases" section)
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Tool	Input	Link to Server/ Standalone	Peptide Flexibility	Receptor Flexibility	Specific applications/ Best cases to apply on
Galaxy PepDock	Protein structure +peptide sequence	Server: https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=PEPDOCK	Full Flexibility at the refinement stage	Full Flexibility at the refinement stage	Tested on the PepBind dataset. Predictions are reliable when templates can be found with TM score>0.7
PepComposer	Binding site information	Server: http://biocomputing.it/pepcomposer/webserver	Sidechain rotamer and small change in backbone	Sidechain rotamer and small change in backbone	Suitable for small peptides, when tested on the LEADS-PEP dataset with 50% successes. Can also be used as inhibitor peptide design tool.
InterPep2-Refined	Protein structure +peptide sequence	Standalone: http://wallnerlab.org/InterPep2	SC flexibility at the refinement stage	Full flexibility at the refinement stage	Predictions are reliable when templates can be found with TM score>0.7. Overall performs slightly better than GalaxyPepDock.

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Table.2: Summary of highlighted templated based docking tools.
(**This is supposed to be right after "Template Based Docking" section.)

Tool	Input	Link to Server/ Standalone	Peptide Flexibility	Receptor Flexibility	Specific applications/ Best cases to apply on
HADDOCK	Pstr+pconf ensemble + ambiguous information of binding site	Server: https://wenmr.science.uu.nl/haddock2.4/submit/1	SC are flexible but can be extended to the BB of the provided binding site residues.	Fully flexible	HADDOCK can use ambiguous information about binding residues on protein and/or peptide. Reliable when there is no significant peptide conformational change upon binding.
HPEPDOCK -local	Pstr+pconf ensemble + Information of binding site	Server: http://huanglab.phys.hust.edu.cn/hpepdock/	Not Flexible	Flexibility is considered generating an ensemble of peptide conformation	34% success rate on PeptiDB database compared to HADDOCK's 14.5% but needs accurate information of binding residues.
AutoDock Vina	Pstr+pseq + Binding site coordinate	Standalone: https://github.com/ccsb-scripps/AutoDock-Vina	SC flexibility is default but can be extended to the BB	Fully flexible	Reliable when binding peptide length is less than 5 residues.
DINC 2.0	Pstr+pconf + Binding site coordinate	Server: http://dinc.kavrakilab.org	No flexibility	Fully flexible	AutoDock based method with fragmentation of peptide. This allows it to tackle peptides up to 8 residues.
PepCrawler	Initial coarse protein – peptide with peptide at the binding site / protein - protein complex	Server: http://bioinfo3d.cs.tau.ac.il/PepCrawler/php.php	SC flexibility	Fully flexible	Can be used as a refinement method. Predictions are reliable when the starting model is with 5Å RMSD from the experimental structure, and peptide is shorter than 15 residues.
Rosetta FlexPep Dock	Initial coarse protein peptide complex with peptide at the binding site	Server: https://www.sciencedirect.com/science/article/pii/S1359644617305937#bib0165	SC flexibility but can be extended to the BB	Full flexible	Can be used as a refinement method. Predictions are reliable when the starting model is with 5Å RMSD from the experimental structure, and peptide is shorted than 15 residues.

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1371 Table.3: Summary of highlighted "local docking" tools. Here, acronyms are used as follow: Pstr- protein structure; pseq-
1372 peptide sequence; pconf- initial peptide conformation; BB- backbone; SC- sidechain.
1373 (**This is supposed to be right after "Local Docking" section.)
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Tool	Input	Link to Server/ Standalone	Peptide Flexibility	Receptor Flexibility	Specific applications/ Best cases to apply on
MDockPeP	Pstr+pseq	Server: https://zougrouptoolkit.missouri.edu/mdockpep/	Small change in conformation at the refinement stage	Full flexibility at the refinement stage	Performs well on smaller peptides with <15 residues
MDockPeP 2	Pstr+pseq	Standalone: https://zougrouptoolkit.missouri.edu/mdockpep2/download.html	Full flexible	Fully flexible at the refinement stage	Can be applied on peptides up to 29 residues but success rate decreases beyond 15 residues.
Anchor Dock	Pstr+pconf	Not available	Fully flexible	Fully flexible	Uses expensive molecular simulations. Suitable for large peptides (>15 residues) which show conformational changes.
Pep ATTRACT	Pstr+pseq	Server: https://bioserv.rpbs.univ-paris-diderot.fr/services/pepATTRACT/	Fully flexible in the full pepATTRACT version but no flexibility in the web server	Fully flexible in the full pepATTRACT version but the server just uses 3 major peptide conformations to dock.	Full version uses expensive molecular simulations. Suitable for large peptides (>15 residues) which shows conformational changes. Web version is useful for smaller peptides.
CABS-dock	Pstr+pseq + Bound peptide secondary structure (optional)	Server: http://biocomp.chem.uw.edu.pl/CABSdock	Fully flexibly at the peptide conformation generation stage.	Fully flexible	Suitable when bound peptide conformation is known.
PIPER-FlexPep Dock	Pstr+pseq	Server: http://piperfpd.furmanlab.cs.huji.ac.il	Fully flexible at the refinement stage	Fully flexible at the refinement stage	Uses expensive molecular simulations. Suitable for large peptides (>15 residues) which shows conformational changes.
AutoDock CrankPep	Pstr+pseq	Standalone: https://github.com/ccsb-scripps/ADCP	Fully flexible	Fully flexible	Uses expensive molecular simulations. Suitable for large peptides (>15 residues) which shows conformational changes.
patchMAN	Pstr+pseq	Server: https://furmanlab.cs.huji.ac.il/patchman/	Fully flexible at the refinement stage	Fully flexible at the refinement stage	Most successful when tested on custom made PFPD dataset outperforming AlphaFold.

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1377 Table.4: Summary of highlighted "global docking" tools. Here, acronyms are used as follow: Pstr- protein structure;
1378 pseq- peptide sequence; BB- backbone; SC- sidechain.
1379 (**This is supposed to be right after "Global Docking" section.)
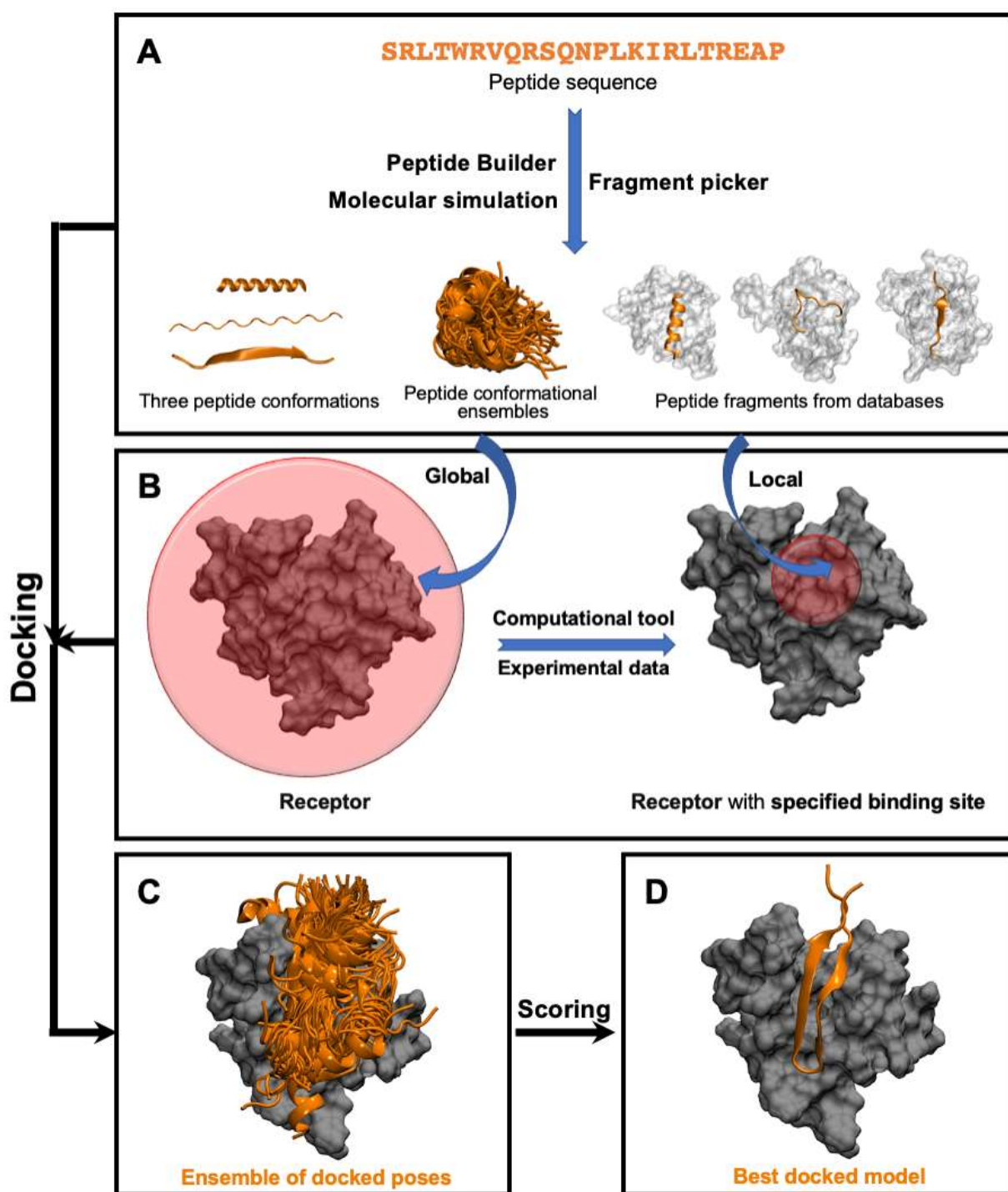1380
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1385 **Figure 1. Pipeline in popular template free docking methods.** (A) Input peptide conformations are generated in 3
1386 major ways: 1) using peptide builder to generate major 3 conformations (alpha, polyproline II, extended); 2) molecular
1387 simulations are used to generate an ensemble of peptide conformations; and 3) fragment pickers are used to select
1388 peptide fragments in the structural databases based on the peptide sequence. (B) If the binding site known, peptides

1389 are guided towards the binding site (local docking), else, peptides explore the whole protein surface (global docking).
1390 (C) Ensemble of docked poses. (D) Top score docked model representing the native structure.
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1392 (**This is supposed to be in the Docking section, right after the table**)
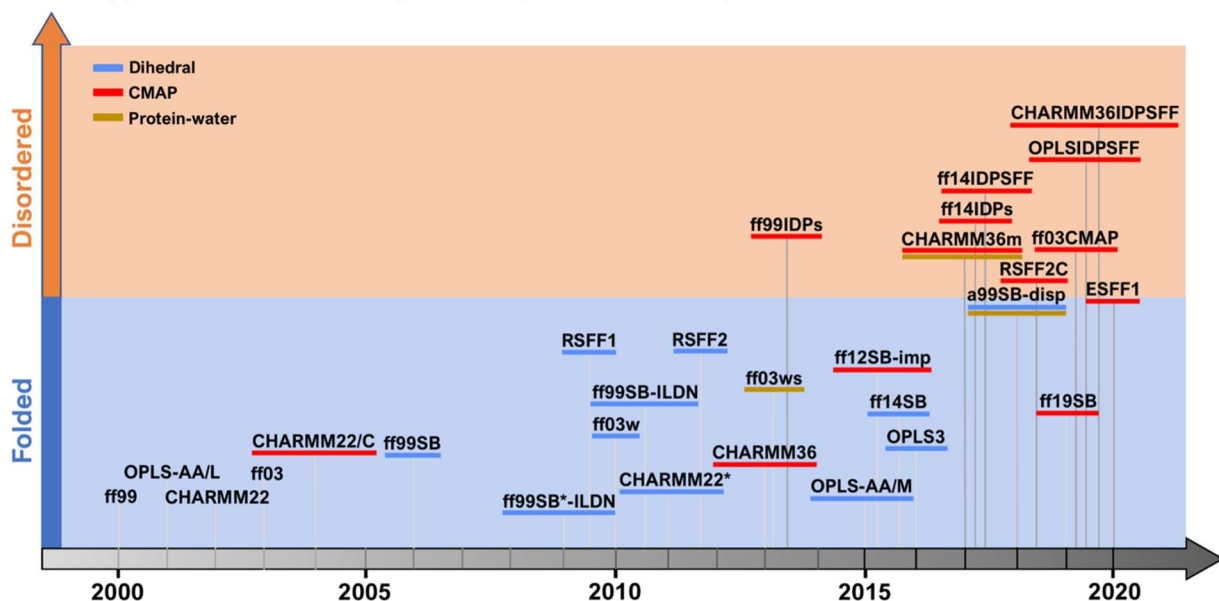
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1394 **Figure 2. Overview of protein force field development after 2000.** Each protein force field is classified by the year
1395 of publication, target systems for optimization (folded, disordered or both), and additional underscores indicating
1396 whether it is a modification version of previous force fields using strategies including dihedral parameter adjustment
1397 (blue), CMAP correction (red), or parameter modification for protein-water interaction (gold).
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1399 (** This is supposed to be right before "**Characterizing peptide binding poses and affinities by molecular dynamics**
1400 **simulations**." in the MD section)
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