AutoDock CrankPep: Combining folding and docking to predict protein-peptide complexes

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Abstract

Protein-peptide interactions mediate a wide variety of cellular and biological functions. Methods for predicting these interactions have garnered a lot of interest over the past few years, as witnessed by the rapidly growing number of peptide-based therapeutic molecules currently in clinical trials. The size and flexibility of peptides has shown to be challenging for existing automated docking software programs. Here we present *AutoDock CrankPep* or *ADCP* in short, a novel approach to dock flexible peptides into rigid receptors. *ADCP* folds a peptide in the potential field created by the protein to predict the protein-peptide complex. We show that it outperforms leading peptide docking methods on two protein-peptide datasets commonly used for benchmarking docking methods: *LEADS-PEP* and *peptiDB*, comprised of peptides with up to 15 amino acids in length. Beyond these datasets, *ADCP* reliably redocked a set of protein-peptide complexes containing peptides ranging in lengths from 16 to 20 amino acids. The robust performance of *ADCP* on these longer peptides enables accurate modeling of peptide-mediated protein-protein interactions and interactions with disordered proteins.

Availability:

ADCP is distributed under the LGPL 2.0 open source license and is available at http://adcp.scripps.edu. The source code is available at https://github.com/ccsb-scripps/ADCP.

Introduction

Protein-peptide interactions are essential to many biological functions (Petsalaki and Russell, 2008). Thus, peptide-based therapeutic approaches have recently attracted increasing interest (Fosgerau and Hoffmann, 2015; Lau and Dunn, 2018). Moreover, many protein-protein interactions, especially the ones involving intrinsically disordered proteins, are mediated by a peptide-like segment (Wright and Dyson, 2014; Stein and Aloy, 2008). Predicting protein-peptide interactions using automated docking methods remains challenging mainly due to the significantly larger number of rotatable bonds in peptides, making them more flexible than small drug-like molecules. Small molecule docking methods have been shown to perform rather poorly for peptides longer than 5 amino acids (Rentzsch and Renard, 2015; Hauser and Windshügel, 2016). Meanwhile, efforts have been put into developing accurate and efficient peptide docking methods (London *et al.*, 2013; Ciemny *et al.*, 2018). These methods can be segregated into the following three categories: template docking, ensemble docking, and *de novo* methods (see Table 1).

The success of template docking methods for docking peptides (Lee *et al.*, 2015) depends on the availability of homologue structures for both the receptor and the peptide , thus limiting the range of their applicability. Ensemble docking methods sample peptide conformations as a pre-processing step without knowledge of the receptor. Next, these conformations are docked rigidly or semi-rigidly into the receptors (Zhou, Jin, *et al.*, 2018; Zhou, Li, *et al.*, 2018; Schindler *et al.*, 2015; Yan *et al.*, 2016). While, these methods yield good accuracy for small and medium sized peptides (typically $\leqslant$ 9 amino acids), their success rates tend to drop rapidly with longer peptides. Finally, *de novo* methods sample the peptide's conformation on-the-fly during the

docking (Ben-Shimon and Niv, 2015; Raveh *et al.*, 2011; Alam *et al.*, 2017; Trellet *et al.*, 2013; Kurcinski *et al.*, 2015). While *de novo* methods yield high accuracy and are less affected by the length of the peptides, these methods are often computationally expensive and often rely on lengthy molecular dynamics simulations to refine solutions.

Table 1. Summary of three categories of peptide docking methods

Category	Peptide Flexibility	Description	Examples
Template Docking	None or little	Use sequence-based homology model to predict docking poses	*GalaxyPepDock*
Ensemble Docking	Conformation ensemble	Prepare a conformation ensemble to describe peptide flexibility and then dock the conformations back into receptor	*HPepDock* *MDockPep* *pepATTRACT*
***De novo* Method**	Fully flexible	Model peptide flexibility with the respect to the receptor	***ADCP*** *AnchorDock* *CABS-Dock* *FlexPepDock* *HADDOCK*

Here we present *AutoDock CrankPep* or *ADCP* in short, an efficient *de novo* method for protein-peptide docking that folds the peptide in the potential energy landscape created by a given receptor. *ADCP* provides an efficient and accurate way to dock flexible peptides into rigid receptors. We show that it achieves 85.7% success rate on the *LEADS-PEP* dataset within its top 10 predictions. Furthermore, while existing peptide docking methods have typically limited themselves to peptides with less than 16 amino acids, we evaluate *ADCP's* ability to dock a set of peptides ranging in length from 16 to 20 amino acids. For these peptides, *ADCP* achieves re-docking success rates of 64% and 91% when considering the top or top 5 solutions, respectively. These results indicate that *ADCP* is a robust peptide docking tool that can be used to model protein-protein interactions mediated by protein segments such as loops or disordered fragments.

Methods

Small molecule docking methods typically perform best with ligands containing less than 20

rotatable bonds (Hauser and Windshügel, 2016; Rentzsch and Renard, 2015). Peptides with 5 or more amino acids can easily exceed this number. A medium sized peptide of 10 amino acids typically has around 40 rotatable bonds, rendering these methods ineffective.

CRANKITE is an efficient software package originally developed for protein and peptide conformation sampling and folding (Podtelezhnikov and Wild, 2005; Várnai *et al.*, 2013; Burkoff *et al.*, 2012; Podtelezhnikov and Wild, 2008). It samples conformational space of proteins or peptides using a Metropolis *Monte Carlo* (MC) search and a *Gō*-Type representation of amino acid side-chain (Takada, 1999; Taketomi *et al.*, 1975). *CRANKITE* can rapidly explore the conformational space of sequences of amino acids by performing the two MC moves illustrated in Figure 1: *i*) a crankshaft motion along two selected C_α atoms and *ii*) a rotation near the end of the chain.

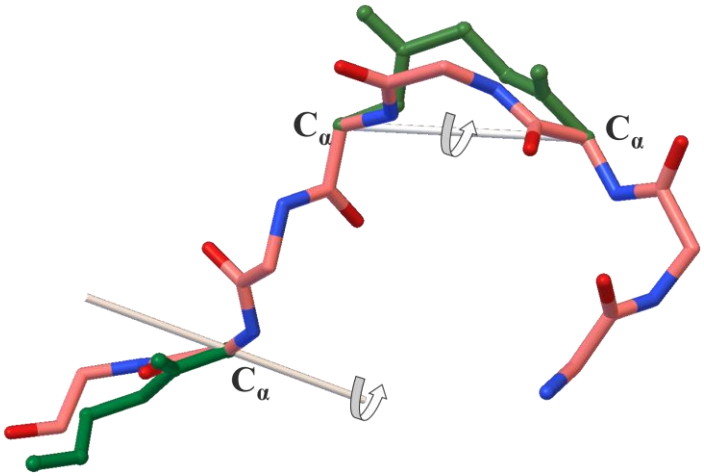

Figure 1. *CRANKITE*'s Monte Carlo moves. A crankshaft motion along two selected C_α atoms or a rotation near the end of the chain.

ADCP combines *CRANKITE*'s conformation sampling ability with the *AutoDock* representation of a rigid receptor (Huey *et al.*, 2007; Morris *et al.*, 2009) to concurrently optimize the peptide conformation and its interactions with the receptor, thus yielding docking poses. *ADCP* was

implemented based on *CRANKITE*. The notable modifications and additions are as follows: *i*) the addition of new MC moves to boost the exploration of peptide position and orientation relative to the receptor; *ii*) the addition of an energy term based on the *AutoDock* affinity grids to describe the peptide-receptor interactions; *iii*) the use of a rotamer library to interactively construct side-chain atoms (Dunbrack Jr and Cohen, 1997); and *iv*) the addition of a pose cache swapping mechanism to enhance the search.

The overall workflow of the MC procedure implemented by *ADCP* is depicted in Figure 1. First, a randomly selected MC move is applied to alter the current pose. The altered pose is then scored, and the move is either accepted or rejected based on a metropolis-like MC criterion. If the move is rejected, the pose before the move is restored and another move is attempted. If it is accepted, the altered pose becomes the current one and is used to update the cache of docking poses. This procedure repeats until one of the termination criteria is met. More details about the various elements of this workflow are provided below.

Input*: ADCP* requires a description of the receptor and the peptide. The receptor is represented by affinity maps calculated using *AutoGridFR* (Ravindranath *et al.*, 2015). *AutoGridFR* produces a single zip file that contains affinity maps for all atom types in the peptide calculated by *AutoGrid4* (Morris *et al.*, 2009), along with metadata about the docking box (*e.g.* the size and position of the box, A list of favorable locations in the affinity called *translational points*, *etc.*). The peptide can be specified as a 3D structure in the PDB file format or by its sequence of amino acids. In the latter case, a starting conformation is constructed automatically. This initial conformation can be generated in an extended or alpha helical conformation using lowercase and uppercase letters respectively. The user can also specify the maximum number of MC steps for the simulation. As conventional docking methods can dock short peptides with reasonable accuracy, *ADCP* was designed to support peptides with 5 or more amino acids.

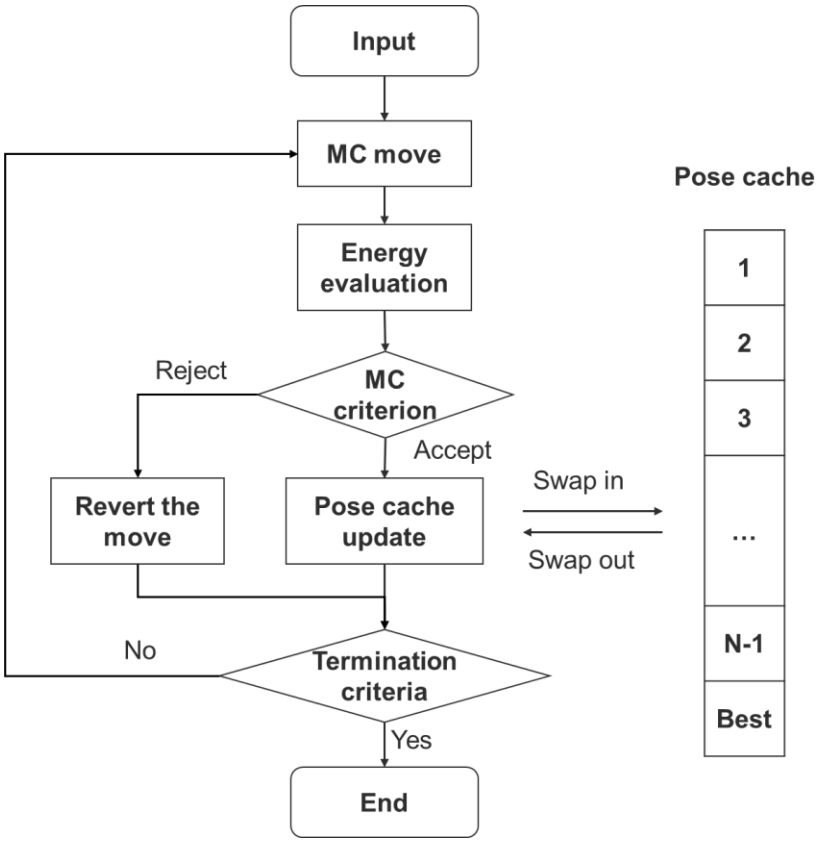

Figure 2. Flow chart of *AutoDock CrankPep Monte Carlo* procedure.

Monte Carlo moves*:* During the MC search the docking pose is modified using MC moves. We extended the original set of MC moves with: *i*) a local translation to perturb the peptide position; and *ii*) a translational jump that translates the peptide to move a central peptide atom called the "*root atom*" to a new "*translational point*" in the docking box (Figure S1). The "*translational points*" are a set of positions with high affinities identified by *AutoGridFR* (Ravindranath and Sanner, 2016). These moves were added to facilitate the peptide's exploration of the potential field created by the receptor. Each MC move will trigger the reconstruction of the all-atom representation of the side-chain using a rotamer library (see below).

Scoring Function*:* The scoring function of *ADCP* consists of two components: a score for the conformation of the peptide; and a score for the interaction between the peptide and the receptor. We will refer to these scores as the internal and the interaction scores, respectively. The internal

score is based on *CRANKITE*'s *Gō*-Type potential (Podtelezhnikov and Wild, 2005, 2008) where each side-chain is represented by a single bead. We extended the internal score function with a new term based on Ramachandran propensities for backbone φ and ψ angles (Lovell *et al.*, 2003). These Ramachandran propensities are transformed into energies according to the Boltzman distribution. The interaction score between the peptide and the protein is calculated using the *AutoDock* affinity grids. Calculating this score requires a full-atom representation of the peptide which is constructed using a rotamer library (Dunbrack Jr and Cohen, 1997). Every time a pose is scored, we iterate over the peptide either forward (N to C-terminus) or backwards (C- to N-terminus) with the same probability. For each amino acid, we construct all rotameric conformations and score them using the affinity grids while avoiding clashes with the peptide backbone and already built side-chains. The energetically most favorable side-chain conformation is selected to represent this amino acid. Once the full atom representation of the peptide is built, the interaction score between the peptide and the receptor is obtained by summing up the scores from the affinity grids for all atoms in the peptide.

Monte Carlo Criterion*: ADCP* uses a metropolis-like MC criterion given in equation 1 to accept a move

$$exp(-k_T \Delta E \times HillClimb) > random \qquad \text{(Equation 1)}$$

where: ΔE is the score difference between the pose before and after the MC move; k_T is a temperature factor that can be used to adjust the probability of the MC criteria; and *random* is a random number ranging from 0.0 to 1.0. We found that the traditional metropolis MC criterion did not yield an efficient exploration of solution space. The *HillClimb* term was introduced to boost the search power. If the score worsens by more than 5.9 kcal/mol (10 k_BT at room temperature), *HillClimb* is set to 0.05, otherwise *HillClimb* remains at 1. With this hill climbing feature, a score increase of 5.9 kcal/mol has a 60.6% probability to be accepted, and a score

increase of 11.8 kcal/mol has 36.8% chance to be accepted, and so on. Using a *HillClimb* factor ranging from 0.05 to 0.20 does not affect the results substantially.

***Pose cache update**:* The presence of the receptor creates a complex energy landscape for the peptide to fold while maximizing its interaction with the receptor. We found that the traditional MC search often got stuck in local minima. Thus, we implemented the pose cache to maintain a pool of docking poses encountered during the search and restart the search under certain circumstances. Every pose accepted by the Metropolis criterion is compared with the ones present in the cache and can: either be added to the cache (e.g. if it has the best energy found so far) or it can replace an entry in the cache (e.g. a similar solution is in the cache but with a worse energy). More details on this process are provided in Chart S1. Every time a pose improves on the best score seen so far, it is appended to the output multi-model pdb file. If the search generates 100,000 consecutive poses each having a score 3 kcal/mol higher than the best score found so far, or the best score has not improved for one million steps, the search restarts from a randomly selected pose from the cache.

Termination criteria: The program stops if the maximum number of steps is reached or the best score has not been improved for 10 million steps.

***Using the software*:** The affinity maps are calculated using *AutoGridFR* for a user-defined docking box. The results presented here were docked with a 4 Å padding on every side of the peptide. The stochastic nature of the MC search is usually addressed by performing multiple searches called replicas. The number of replicas is specified by the user in *ADCP*. Unless otherwise specified, the results presented here were obtained using 80 replicas with 60 simulations started from extended conformations and 20 simulations started from helical conformations constructed from amino acid sequence. Each replica performed 3 million MC steps per amino acid in the peptide (*i.e.*, 15 million steps for a 5-mer peptide and 36 million steps for 12-mer peptide, etc.). While we routinely observed conformational changes between helical

conformation and extended conformation and vice versa during the MC runs, we found that statistically, starting the MC with a mix of initial conformation speeds up the process of identifying the correct solution. Users can customize these parameters according to their specific needs. We suggest using more replicas and longer simulations for larger peptides and/or larger docking boxes.

After all replicas finish their search, the docking poses are clustered to produce the final docking poses. The clustering can be performed using the *AutoDock*FR clustering algorithm (Ravindranath *et al.*, 2015; Morris *et al.*, 2009), or using pairs of peptide-receptor residues in contact. See the supplementary information for a detailed description of this contact-based clustering algorithm.

Datasets:

The *peptiDB* (London *et al.*, 2010) and *LEADS-PEP* (Hauser and Windshügel, 2016) protein-peptide datasets have been widely used for benchmarking peptide docking methods (Zhou, Li, *et al.*, 2018; Raveh *et al.*, 2011; Tubert-Brohman *et al.*, 2013). *PeptiDB* contains 102 protein-peptide complexes varying from 3 to 15 amino acids. We benchmarked *ADCP* with the *Glide SP-PEP* dataset (Tubert-Brohman *et al.*, 2013), a subset of *peptiDB* comprised of 19 high-quality, non-α-helical systems ranging from 5 to 11 amino acids. The *Glide SP-PEP* dataset has been used to benchmark *FlexPepDock*, *Glide SP-PEP*, and *HPepDock*. The *LEADS-PEP* dataset is a more recent, and manually curated dataset of 53 complexes with peptides ranging from 3 to 12 amino acids. In this study we used the subset of 42 complexes from *LEADS-PEP* containing peptides with 5 or more amino acids. We consider the peptides in these datasets as medium-sized peptides for docking purposes.

Current available peptide docking methods are mostly tested on peptides with15 amino acids or less. To further test *ADCP*, we compiled at set of 11 protein peptide complexes from the protein

data bank (PDB) (Berman *et al.*, 2000) with longer peptides ranging in length from 16 to 20 amino acids. These structures were obtained by selecting PDB entries with crystallographic resolution of 2.2 Å or better and containing a peptide comprised of 16 to 20 standard amino acids. The peptides in this set are neither cyclic nor covalently bound to the receptor; they have no significant clashes between peptide and receptor atoms and have no significant contacts between the peptide and crystal mates of the receptor. These complexes are listed in Table S1.

Success metrics:

All atom Root-Mean-Square Deviation (RMSD) is typically used to assess success while docking small molecules. As ligands grow larger this metric becomes less appropriate and RMSD of backbone atoms (N, CA, C) has been used for assessing docking success rate for small peptides (Méndez *et al.*, 2003; Irving *et al.*, 2000). For instance, (Hauser and Windshügel, 2016; Zhou, Li, *et al.*, 2018) used a 2.5 Å backbone RMSD cutoff to define successful peptide redocking on the *Lead-Pep* dataset. Other studies (Raveh *et al.*, 2011; Tubert-Brohman *et al.*, 2013) used the iRMSD (interface RMSD) defined as the RMSD of the backbone atoms of the "*interface residues*". Interface residues are amino acids of the peptide having their C_β atom within 8 Å of any receptor C_β atom. Poses with iRMSD values under 3.0 Å are typically considered to be successful dockings. To facilitate the comparison with other methods, we used the same metric as used in previously published studies, *i.e.* backbone RMSD for comparison on the *LEADS-PEP* dataset and iRMSD for the *Glide SP-PEP* dataset.

When docking longer peptides, RMSD-based metrics do not provide a precise measure for success. For these cases, we assess success using *native contacts*: a metric borrowed from the protein-protein docking field (Méndez *et al.*, 2003; Irving *et al.*, 2000). Native contacts are defined as the list of all pairs of peptide-receptor amino acids located within 5 Å of each other. Similar to (Méndez *et al.*, 2003; Yan *et al.*, 2016; Peterson *et al.*, 2017), we identify successful

redockings of peptides ranging from 16 to 20 amino acids in length when the docking poses reproduce more than 50% of the native contacts (*i.e.* $f_{nc} > 0.5$).

Results and Discussion

We compared the success rate of *ADCP* with previously published results from leading peptide docking methods on the datasets containing medium size peptides (*i.e.* up to 12 amino acids). We also demonstrate that *ADCP* achieves remarkable success rates in rdocking longer peptide (*i.e.* up to 20 amino acids).

Accurate docking of medium size peptides

HPepDock (Zhou, Jin, *et al.*, 2018; Zhou, Li, *et al.*, 2018) is a recent peptide docking method that falls into the ensemble docking category. It uses *MODPEP* (Yan *et al.*, 2017) to generate 1,000 peptide conformations and then docks these peptide conformations semi-rigidly using *MDock* (Huang and Zou, 2006). *HPepDock* has been shown to achieve better accuracy than traditional small-molecule docking methods as well as other leading peptide docking methods including *FlexPepDock*, *Glide-SP-PEP*, *HADDOCK* and *pepATTRACT* (Zhou, Li, *et al.*, 2018; Zhou, Jin, *et al.*, 2018). As such, it can be considered the state of the art at the time of writing this paper. Here we compare the success rates of *ADCP* with the *HPepDock* results on these datasets using the same metrics. For the complexes from the *LEADS-PEP* dataset, *ADCP* consistently outperforms *HPepDock* as shown in Figure 3. Considering the top 10 solutions, *ADCP* achieves 85.7% success rate for this dataset compared to 66.7% for *HPepDock*. *ADCP* significantly improves success rate at 1.0 Å RMSD cutoff, predicting 76.2% of the systems with a sub-angstrom backbone RMSD precision within top 1000 predictions, and 100% systems have a successful prediction model (backbone RMSD $\leqslant$ 2.5 Å). The system-specific comparison is provided in Table S2.

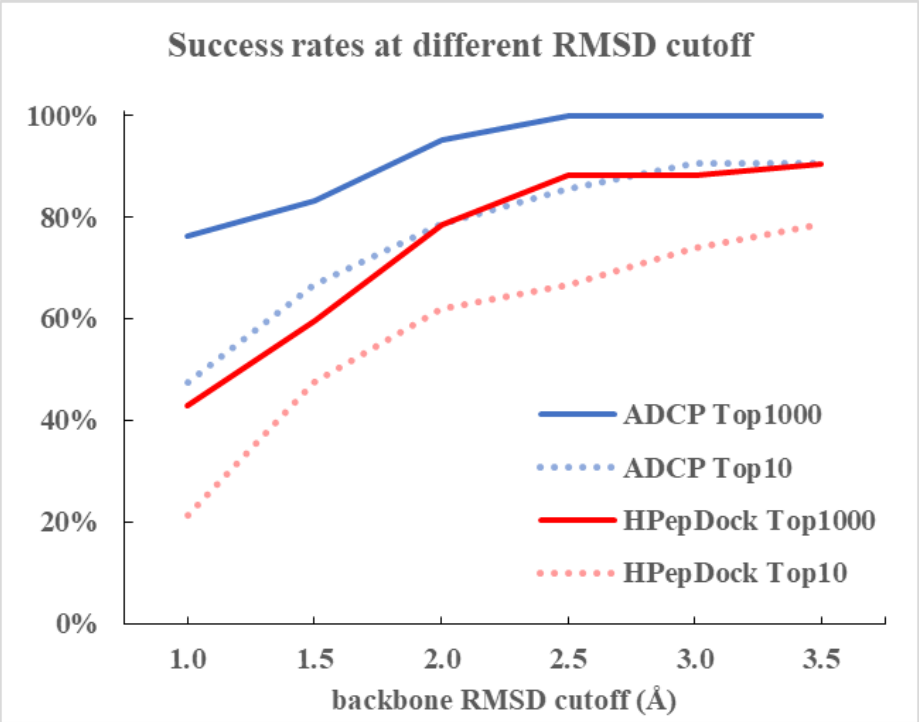

Figure 3. The comparison of success rates for *ADCP* and *HPepDock* with different success criteria. Blue solid line represents the success rate for *ADCP* if top 1000 solutions are considered and blue dashed line represents the success rate if top 10 solutions are considered. Red solid and dashed lines represent the success rate for *HPepDock* if top 1000 solutions and top 10 solutions are considered, respectively.

HPepDock's performance deteriorates significantly for the longer peptides in this data set (Table 2). While it's overall success rate at 2.5 Å backbone RMSD for the top 10 solution is 66.6% (28/42), when considering only peptides with 9 or more amino acids the success rate drops top 35% (7/20). *ADCP* on the other hand maintains its 85% overall success rate on the subset of peptides with 9 or more amino acids.

Table 2. Success rates at 2.5 Å backbone RMSD considering top 10 solutions. Out of the 42 peptides, 20 have more than 8 amino acids (48%) and are classified as "*longer peptides*".

bbRMSD ≤ 2.5 Å	***ADCP***	***HPepDock***

All	85.7%	66.7%
Longer peptides	85.0%	35.0%

A possible explanation for this could be that for longer peptides, the conformational space, which increases exponentially with the length of the peptide, eventually requires a prohibitively large numbers of conformers to be used in ensemble docking methods. *ADCP* models the peptide flexibility during the docking process thus it is less affected by increasing peptide lengths. *ADCP* maintains a consistent success rate across the peptides lengths in this dataset.

On the smaller *Glide SP* data set, *ADCP* performs similarly to other methods for both *holo* (19 systems) and *apo* (10 systems) receptor conformations, as shown in Table 3.

Table 3. Success rates on the *Glide SP-PEP* dataset. Among the 19 complexes in the dataset, 10 receptors have *apo* conformation available. Here a docking is deemed successful if one of the top 10 solutions has an interface RMSD (iRMSD) lower than 2.0 Å or 3.0 Å respectively.

iRMSD		**ADCP**	**FlexPepDock**	**Glide SP-PEP**	**HPepDock**
holo	**≤ 2 Å**	13	13	11	12
	≤ 3 Å	15	13	13	15
apo	**≤ 2 Å**	4	6	4	5
	≤ 3 Å	8	6	4	8

Reliable docking long peptides and protein segments

Currently available peptide docking methods have mostly been tested and benchmarked on small and medium-sized peptides up to 15 amino acids in length. However, a considerable portion of protein protein interactions are mediated by flexible protein loops, disordered chains segments, or intrinsically disordered proteins, involving sequences that can easily exceed 15 amino acids. Therefore, we tested *ADCP* on a set of 11 complexes containing peptides with 16 to 20 amino acids in length. For these dockings we performed 80 MC simulations, allotting 7x*N* million MC moves (where *N* is the number of amino acids in the peptide) to each run. The docking poses

from the 80 MC runs were clustered using contacts with a cutoff value of 80%. Results are shown in Table 4.

Table 4. Docking results for long peptides and protein segments.

PDB ID	Length	Torsions	Fraction of native contacts				
			Top 1	Top 3	Top 5	Top 20	All
2IVZ	16	51	0.73	0.73	0.73	0.73	0.80
2OBH	16	74	0.89	0.89	0.89	0.89	0.89
2XAP	16	65	0.03	0.52	0.52	0.53	0.58
4AK4	16	53	0.59	0.63	0.80	0.83	0.89
5UWI	16	58	0.76	0.80	0.80	0.80	0.80
5N4B	17	62	0.24	0.24	0.46	0.46	0.51
6CIT	17	72	0.32	0.71	0.71	0.71	0.71
1CM1	18	74	0.94	0.94	0.94	0.94	0.94
4YZ6	18	79	0.81	0.81	0.85	0.90	0.90
4RS9	19	83	0.05	0.49	0.54	0.78	0.78
2F31	20	75	0.66	0.66	0.66	0.66	0.66
Avg. f_{nc}			0.55	0.67	0.72	0.75	0.77
f_{nc} > 0.5 percentage			63.6%	90.9%	90.9%	90.9%	100.0%

Considering only the top-ranking solution, *ADCP* identifies solution with at least 50% native contacts for 7 out of 11 complexes (63.6%). Within the top 5 solutions, the success rates increase to 90.9% (10 out of 11 systems). Figure 4 shows some examples of docked pose with respect to the crystal structure along with the fraction of reproduced native contacts.

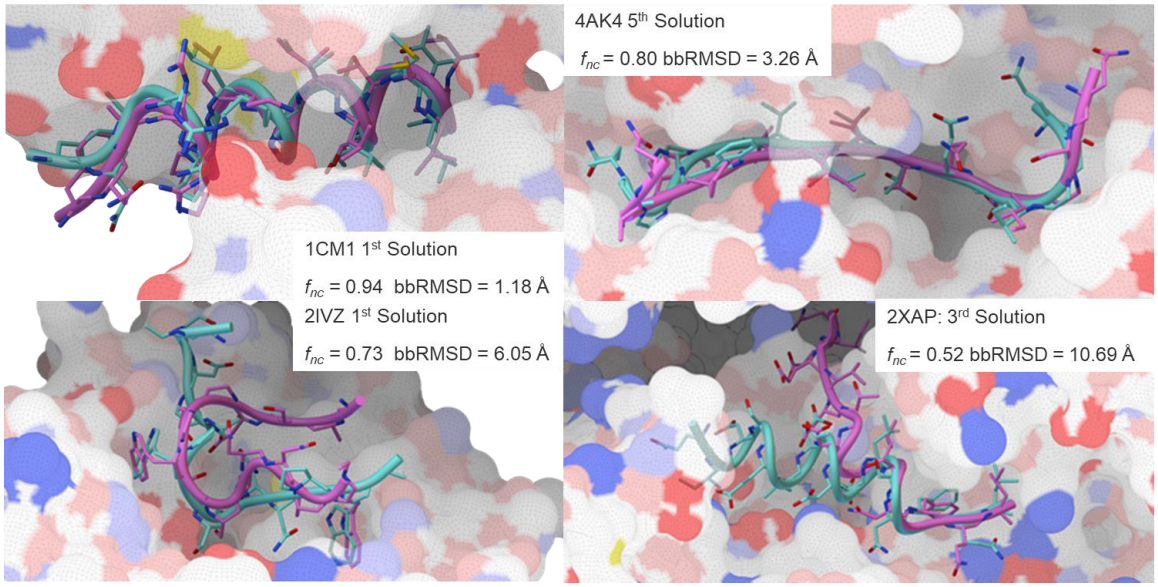

Figure 4. Comparison of selected docked poses (cyan) and experimentally determined structures (magenta). The receptor surface is provided for context and colored using a polarity scheme developed by Dr. D. Goodsell. These Figures are generated using PMV 1.5.7 (Sanner, 1999) and MSMS (Sanner *et al.*, 1996).

ADCP demonstrates a robust ability to redock long peptides and protein segments. With more MC replicas and more steps for each replica, *ADCP* could potentially be applied to even longer peptide-like segments.

It is noteworthy that the current scoring function in *ADCP* has not been optimized or calibrated for protein-peptide interactions. The interaction energy between peptide and receptor relies on *AutoDock*4 affinity maps that were initially developed and calibrated for docking small, drug-like molecules. While we have started incorporating peptide-specific elements, such as a potential for Ramachandran backbone angles into our scoring function, further refinements of the current scoring function could improve docking success rates. Alternatively, re-scoring top-ranked poses with scoring functions designed for protein-peptide interactions (Huang and Zou, 2008; Spiliotopoulos *et al.*, 2016) could also improve the ranking of the docking predictions.

Timing

ADCP is computationally efficient compared with other *de novo* methods. Based on 3 million MC moves per amino acid, a MC search typically takes from ~10 minutes for a 5-mer to ~1 hour for a 12-mer, using a single thread on an *Intel Xeon E5-1620* processor (2014). These times are rough averages and can vary depending on the peptide sequence. See Table S2 for a detailed timing results on *LEADS-PEP* dataset. Each MC simulation can be run independently and thus the MC can be trivially parallelized locally or on high performance computing clusters. The time for the final clustering is a function of the clustering cutoff but typically only takes a few minutes. While *HPepDock* and other ensemble docking methods requires less computational resources, *ADCP* achieves better success rates especially for longer peptides. *HPepDock* remains a powerful peptide docking tool for medium size peptides.

Conclusions

In this paper, we presented *ADCP*, a novel approach for predicting protein-peptide interactions for peptides of substantial length. The approach leverages an algorithm developed initially for protein folding and combines it with a representation of a rigid receptor developed for docking. With a success rate of 85.7% on the *LEADS-PEP* dataset when considering the top 10 predictions, *ADCP* outperforms leading peptide docking approaches. Moreover, we show that *ADCP* is able to dock peptides with up to 20 amino acids to their receptors. *ADCP* expand the fully-flexible peptide docking to predict certain types of protein-protein interactions, *e.g.* disordered tails or flexible protein loops interacting with itself or another protein.

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