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RESEARCH ARTICLE

# In silico study of peptide inhibitors against BACE 1

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**Abstract** Peptides are increasingly used as inhibitors of various disease specific targets. Several naturally occurring and synthetically developed peptides are undergoing clinical trials. Our work explores the possibility of reusing the non-expressing DNA sequences to predict potential drug-target specific peptides. Recently, we experimentally demonstrated the artificial synthesis of novel proteins from non-coding regions of *Escherichia coli* genome. In this study, a library of synthetic peptides (*Synpeps*) was constructed from 2500 intergenic *E. coli* sequences and screened against Beta-secretase 1 protein, a known drug target for Alzheimer's disease (AD). Secondary and tertiary protein structure predictions followed by protein–protein docking studies were performed to identify the most promising enzyme inhibitors. Interacting residues and favorable binding poses of lead peptide inhibitors were studied. Though initial results are encouraging, experimental validation is required in future to develop efficient target specific inhibitors against AD.

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## Background

The traditional drug discovery pipeline has nearly dried up. Emergence of new approaches like synthetic biology, synthetic chemistry and nanotechnology driven by computer-aided design approaches, have brought in fresh perspectives and interesting opportunities. Among several possibilities, small peptides have seen a revival, of late. Peptides fit into the space between the small molecule based drugs (molecular weights <500 Da) and larger biologics (usually >5 kDa) and optimally suit both membrane permeability and target specificity.

Here we present a novel strategy and preliminary evidence in support of mining therapeutic peptides from an unusual source—the non-coding DNA historically considered junk DNA. We asked questions as to: Why did nature use specific sequences to make proteins? Did every possible sequence combination available in the adjoining regions explored optimally? If not, is there a possibility of further experimentation to explore novel genes from sequences that nature did not utilize for encoding RNA and proteins.

To answer these questions, a simple protocol was devised (Dhar et al. 2009). Six intergenic sequences, for which there were no known natural protein equivalents, were artificially synthesized and expressed in *Escherichia coli* using pBAD 202/D-TOPO vector. One of the expressed protein resulted in significant growth inhibitory effects. Furthermore, good structural homology was found in two of the six expressed proteins. Encouraged by the proof-of-the-concept data that we observed, we conducted

this study to explore the possibility of reusing non-coding DNA sequences to design peptide inhibitors against Alzheimer specific target protein.

## Introduction

Reading DNA has been predominant activity since early 1990s (Venter et al. 2001). As a result of mega human genome sequencing project, a large number of data was generated on the protein coding and RNA coding regions. The recent ENCODE project has shown that most of the genome codes for RNA, some sequences code for protein and a large number of sequences encode neither RNA nor proteins (ENCODE Project Consortium 2012).

The protein coding regions of a genome has been in the forefront of genomic research for many decades, the non-coding regions, lightly termed 'junk' are recent interests of the research community at large, while not-coding sequences are still unexplored territory. Though recent reports (Kageyama et al. 2011; Zhao et al. 2014) indicate significant role of non-coding DNA, the story is still unclear.

In *E. coli*, the non-coding DNA accounts for 12 % of the 4.6 MB genome (Blattner et al. 1997). The intergenic DNA, a subset of non-coding DNA covers around 80 % of human and 15 % of bacterial genome. These sequences are located between functional genes and are considered to have a regulatory role on gene expression. Recent studies correlate intergenic regions with functional RNA coding capability (Raghavan et al. 2011). In this paper, we move beyond protein-coding and RNA coding genome and instead focus on 'not-coding DNA′—sequences that neither encode RNA nor proteins. Recently, we found evidence of anti-malarial peptides from the yeast not-coding genome (Joshi et al. 2013). Furthermore, pseudogenic regions have been studied for the presence of putative functional peptides (Shidhi et al. 2014). Here we study not-coding regions of *E. coli* genome in search of novel therapeutic peptides against AD.

Peptides are defined as molecules with <50 amino acids, having high specificity and low toxicity when considered as drugs (Craik et al. 2013). Even though high biodegradability, low bioavailability and low cellular uptake may be some of the limitations of peptide drugs, they can benefit from novel synthetic strategies for enhancing their drug-like properties (Pandey et al. 2009; Vlieghe et al. 2010). Currently, modifications that a prospective peptide drug candidate can undergo to enhance their efficacy include peptide stapling (Verdine and Hilinski 2012), backbone replacement (Fernández-Llamazares et al. 2014), non-natural amino acid incorporation, pseudo peptide bonds, cyclization (Gentilucci et al. 2010), conjugation (Gauthier and Klok 2008; Ahrens et al. 2012) and so on.

In the past, therapeutic peptides have been found in a variety of unexpected animal and plant sources ranging from venom of cone snails (Han et al. 2008), spiders (Saez et al. 2010; Gui et al. 2014) to sea anemones (Rodríguez et al. 2014). Genetic or recombinant libraries and chemical libraries (Meloen et al. 2004) have also been the source of therapeutic peptides and synthetic combinatorial libraries have been used to identify bioactive peptides (Falciani et al. 2005). In this study, a library of peptides predicted from the non-coding DNA sequences was created in search of novel drug candidates with enzyme inhibitory potential.

Alzheimer's disease (AD), a progressive neurodegenerative disease of the brain, is also known as Senile Dementia of the Alzheimer Type (SDAT) (Brookmeyer et al. 1998). This disease is characterized by the loss of intellectual capacity and personality integration due to damage or loss of neurons in the brain resulting in the disruption of neurotransmission. The global burden of AD is increasing at an alarming rate and predicted to impact 1 in 85 people by 2050 (Brookmeyer et al. 2007). As the progress in developing new drugs against AD has been extremely small, novel and effective therapeutic approaches are needed.

Peptide research for neurodegenerative diseases has recently made considerable contribution similar to that of cancer (Thundimadathil 2012), type 2 diabetes mellitus (Pinelli and Hurren 2011; Kaspar and Reichert 2013) and cardiovascular diseases (Lorber 2013). The major focus of drug design against AD is the inhibition of amyloid beta (Aβ) peptide aggregation (Sciarretta et al. 2006; Wang et al. 2014), the causative pathogenic process as supported and established by the Amyloid cascade hypothesis (Hardy and Higgins 1992). According to this hypothesis, the proteolysis of an integral membrane protein—amyloid precursor protein (APP) causes the formation of amyloid beta peptides. Enzymes involved in the cleavage of APP are attractive therapeutic targets for the treatment of AD (Lahiri et al. 2002; Long and Lahiri 2011).

Beta-secretase 1 (BACE1) is an APP cleaving enzyme and is a prime therapeutic target for reducing cerebral amyloid beta concentrations (Yan and Vassar 2014). The crystal structure of catalytic domain of BACE1 co-crystallized with a peptide inhibitor has been described (Hong et al. 2000). BACE1 is an aspartic protease with an open and less hydrophobic active site compared to other aspartic proteases and recent molecular dynamics study has revealed the presence of a flexible flap over the active site region (Xu et al. 2012). Peptides derived from BACE1 have also been studied for their effect on APP processing (Yeon et al. 2007). Computational docking studies of BACE1 using small molecule and peptide inhibitors have been reported (Niu et al. 2012; Hamada et al. 2012). In this study, we attempt to find a novel peptide inhibitor against BACE1 target protein, originating from the non-coding DNA of *E. coli*.

## Methodology

Intergenic sequences of *E. coli* K12 sub strain MG1655 genome downloaded from Ecogene 3.0 database (http://www.ecogene.org/) was the dataset for this study. We screened sequences for the presence of open reading frames (ORFs) and matched them against known protein databases using BLAST X tool (Altschul et al. 1990). Sequences with non-similarity to known proteins, 30–100 amino acids long and without stop codons were considered (Gasteiger et al. 2003). Sequences were sent for secondary structure prediction followed by ab initio tertiary structure prediction using I-TASSER (Zhang 2008). The resulting 3D structures were added to a database along with features like molecular weight, molecular and cellular function, localization and so on.

To explore the target binding potential of 'non-coding DNA derived' peptides, we performed a virtual screening against the known drug target, Beta-secretase 1 (BACE1) involved in AD. The 3D structures of the target enzyme BACE1 were downloaded from the protein databank (http://www.rcsb.org/pdb). Active site residues were identified from published literature and by interaction analysis of BACE1-peptide inhibitor complexes available in PDB. Cast P server (Dundas et al. 2006) was also employed to calculate the surface pockets of the target receptor. Protein–protein docking study was performed employing ligand flexibility focusing the ligand binding site. Docking calculations were carried out using BACE1 (PDB ID: 1FKN) as the target receptor and synthetic peptides in our library as ligands using the online server Patchdock (Schneidman-Duhovny et al. 2005). The docked complexes were visualized using Discovery Studio 4.0 software suite (Accelrys.inc, USA). The native peptide ligand present in the receptor structure was re-docked to the binding site of BACE1 for comparison. The best docking poses of all the interacting peptides (total 424) were sent for refinement using Firedock (Mashiach et al. 2008) and ranked based on the least global energy. The ligand-binding mode and interacting residues of the top listed complexes were analyzed using Discovery studio visualizer 4.0 and leads were shortlisted. The basic molecular properties and physiochemical properties were predicted for the lead peptides (Gasteiger et al. 2005). The possible functional sites of post-translation modification were also predicted (De Castro et al. 2006). The strategy is outlined in Fig. 1.

## Results and discussion

In this study, we used 2500 intergenic sequences of *E. coli* K12 genome. The initial sequence similarity search against known proteins showed 931 sequences with no known identity to existing protein entries in the NCBI database. After computational translation, 424 peptides with potential to generate stable secondary structure were identified. We predicted the tertiary structure of these peptides using a combined ab initio approach and the best structures were added to the database. Figure 2 shows the possible 3D structure of representative peptides.

We call these non-coding DNA derived peptides 'synthetic' as they are not naturally expressed in the organism but generated in the computer. To synthesize and characterize these peptides, chemical synthesis (Merrifield 1963) seems to be a good option, instead of cloning. Currently we do not know the exact molecular and cellular role of these peptides but predictions help us to narrow down the function search space. They may be a part of the overall peptidome of the organism, but remain unidentified. Some of these peptides may also represent evolutionary remnants that were functional earlier. Here we have considered peptides of length between 30 and 100 amino acids, as this range of ORFs is less explored for their protein-coding potential. Moreover, the fundamental criterion to distinguish between a long non-coding RNA (ncRNA) from protein coding sequence is the ORF length. The conventional ORF cutoff used in most of the annotation projects is 300 nucleotides (Dinger et al. 2008).

The purpose of using BACE1 as an example for our approach was to demonstrate the possibility of discovering novel target specific peptide leads of junk origin. The co-crystallized catalytic domain structure of BACE1 selected

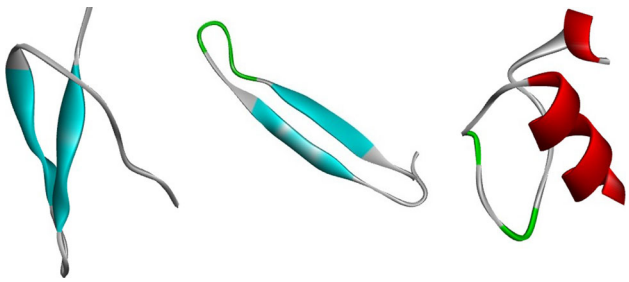

**Fig. 2** Representative synthetic peptides (*synpeps)* of junk origin

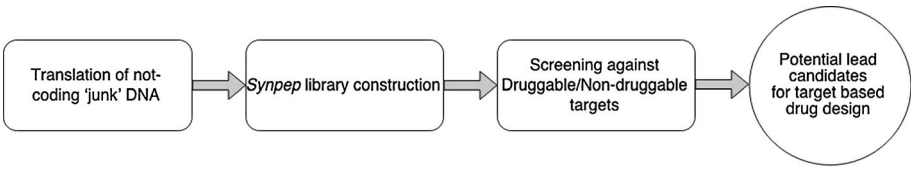


**Fig. 1** Strategy for peptide inhibitor lead identification from junk DNA

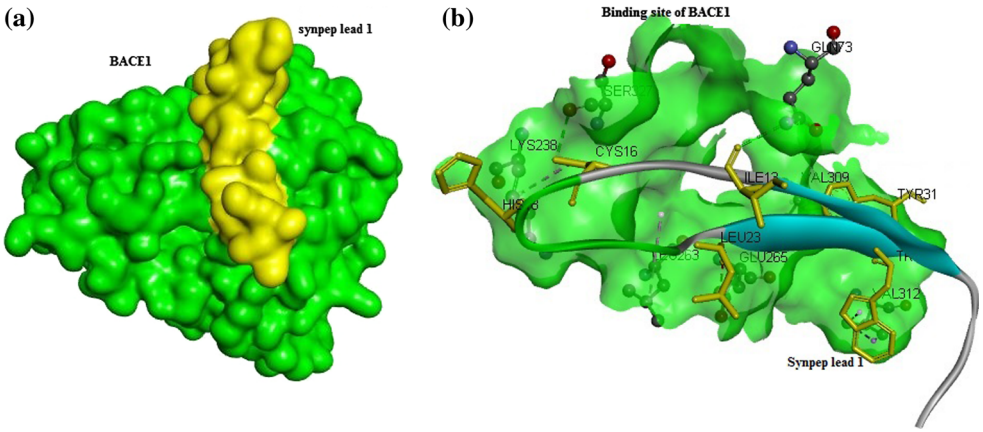


**Fig. 3** Protein-protein docking of BACE1 receptor with synthetic peptides. **a** Docked complex of one of the lead synthetic peptide (*colored yellow*) with BACE1 receptor (*colored green*). **b** Receptor–ligand interactions at the binding site of BACE1 (interacting residues of ligand in *yellow colored stick* representation and residues of receptor in ball and stick representation). (Color figure online)

**Table 1** Physico-chemical properties of the *Synpep* leads

| Top 5 *synpep* leads | No. of residues | Molecular weights (kDa) | Theoretical pI |
|---|---|---|---|
| Syn_Eka 001 | 35 | 4.2 | 4.86 |
| Syn_Eka 002 | 31 | 3.7 | 7.09 |
| Syn_Eka 003 | 35 | 3.7 | 4.37 |
| Syn_Eka 004 | 31 | 3.6 | 6.74 |
| Syn_Eka 005 | 32 | 3.5 | 9.19 |

for the study (1FKN) had a 7 amino-acid long peptide inhibitor OM99-2 attached to it. The key active site residues of BACE1 identified through literature, Cast P server and analysis of experimental structures (PDB Ids: 1fkn, 2zhr, 1xn2, 1xn3 and 1m4h) are Leu30, Asp32, Gly34, Pro70, Tyr71, Thr72, Gln73, Phe108, Trp115, Tyr198, Lys224, Asp228, Gly230, Thr232, Arg235, Arg307 and Lys321. Our protein–protein docking studies provided encouraging results. A total of 424 *synpeps* were docked to the target receptor and the best docked complexes after refinement by Firedock were ranked based on the global energy (of relative units). The global energy function of the docked complex corresponds to the free binding energy and higher negative values are preferred as they have high interaction probability. 233 ligands out of 424 were found to have negative global energy values. Top 25 peptides were shortlisted further and the interacting residues were analyzed. Many of the shortlisted peptides were found to interact with the key residues of BACE1. The most commonly interacting residues of BACE1 were identified to be Gln73, Phe108, Arg235, Arg307, Asp311 and Lys321. Based on the least global energy and the interacting residues, 5 peptides were proposed as the lead molecules. The global energy of native ligand was found to be −42.16 whereas that of our *synpep* leads 1–5 were found to be −33.47, −28.74, −27.29, −27.19 and −26.78 respectively. The probable binding conformation of one of the lead peptide with BACE1 and the possible molecular interactions occurring between the residues of receptor and ligand is shown in Fig. 3.

The physiochemical property prediction (Table 1) shows that our leads are in the chemical middle space when molecular weight is considered i.e. between 500 and 5000 Da. Extracting the shortest region from each peptide having key interactions with the target may help further in reducing the molecular weight. Functional sites prediction results showed the presence of potential N-glycosylation, myristoylation and phosphorylation sites in the lead peptides. This may help in their further design as previous reports shows the correlation between glycosylation sites and anti-bacterial activity in peptides (Strub et al. 1996). The functional sites can also help in conjugating these peptides with carbohydrates by the help of the newly emerging click chemistry protocols (Li et al. 2013) thereby altering the bioavailability of the peptides (Dutot et al. 2009).

The library of *synpeps* constructed in this study can be used for performing extensive screening to assess the multi-target inhibitory potential of each peptide. As the design of multi-targeted drugs based on quantitative structure activity relationship (QSAR) is emerging as a promising area (Speck-Planche et al. 2013; Geldenhuys and Van der Schyf 2013; Viayna et al. 2013), our peptide library analysis can provide new insights in this direction. Very short peptides (<30 amino acids) can also be screened from the intergenic space and considered for this strategy.

Synthetic peptides identified as candidate drug molecules against AD are merely first line of evidence. In future, chemical synthesis followed by in vitro and in vivo studies are required to establish the significance of this discovery. New technological developments in the field of peptidomics (Menschaert et al. 2010; Mason 2010), peptidomimetics (Hruby and Cai 2013; Avan et al. 2014) and drug design holds promise in modifying a primary peptide into a powerful inhibitor and subsequently to a peptide based drug.

## Conclusion

This work shows the potential of making synthetic molecules from the dark matter of DNA towards novel functional endpoints. In this paper, we provide preliminary evidence in favor of non-coding DNA derived molecules to interact with disease specific targets. In future, extensive molecular dynamics studies and experimental validation of our results using relevant bio-molecular assays are required to establish the therapeutic usability of these peptides.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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