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Evaluation of CDK6 and p16/INK4a-Derived Peptides Interaction

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ABSTRACT

The goal of this work is the development of novel peptides with high efficacy of inhibiting activity of CDK6/CyclinD complex. The peptides were derived from primary sequence of P16 protein and its homologues. The interactions between CDK6 and P16/INK4a-derived peptides are studied with molecular dynamics simulation employing umbrella sampling method. The SASA implicit solvent model was used for simulation, which was accelerated using NVIDIA GPUs.

Keywords: Cyclin-Dependent Kinases; Molecular Dynamics; Umbrella Sampling; GPU; SASA

1. Introduction

Cyclin-dependent kinases (CDKs) play a major role in cell cycle regulation and cell division progression. The correct performance of various cyclin-dependent kinases secures the sequential progression of numerous metabolic processes required for cell division. CyclinD-activated kinases CDK4 and CDK6 are quintessential for cell progression from growth phase (G_1) to synthesis phase (S). The CyclinD-CDK4/6 complex activity is regulated by proteins of CKI (Cyclin-dependent kinase inhibitors) family. Many pathological processes including malignant tumors of various locations associated with abnormalities in cell division process are caused by defects in CyclinD-CDK4/6 complex functioning, which are conditioned by hyperexpression of CyclinD or CDK-4/6, mutation of intracellular inhibitors or a number of other processes. CDK4 and CDK6 are attractive molecular targets since it was shown that pharmacological inhibition of CDK4/6 leads to growth suppression in tumors having declension in CDK4/6-involving control pathway.

Currently, numerous low-molecular CDK inhibitors are known [1]. However, their major disadvantage is low selectivity since they are mimicking ATP and are competing with it for binding sites, which are homologous in most kinases, thus the problem of cross-reactivity arises

[2]. More preferable from our point of view is the use of peptides derived from natural CKI proteins. *In vivo* and *in vitro* experiments have shown that short (up to 10 residues) peptides from p16/INK4a, p18/INK4c, p21/WAF1 and p27/Kip1 proteins have inhibitory properties comparable with activity of full-sized protein [3]. The use of peptides as pharmaceutical intracellular drugs became possible after discovery advancement of "cell penetrating peptides" (CPP) technology [4], allowing delivery of almost-arbitrary peptide sequence into intracellular compartments with nearly 100% efficacy. The combination of CPP technology with highly selective functional sequences derived from natural regulatory proteins appears to be a promising approach for development of targeted drugs.

Numerical modeling is routinely used as a way to lower the drug developments costs by significantly reducing the number of potential pharmaceutical compounds. However there is no mastered methodology for evaluation of peptide drugs.

In this work protein docking and molecular dynamics methods are applied to the problem of predicting the efficacy of given peptides. The CDK6 protein is used as drug target, and the tested peptides were derived from p16/INK4a protein sequence.

2. Numerical Model

To calculate the free energy of protein-peptide interaction we use SASA implicit solvent model implemented in GPU-accelerated MDis package [5]. Since the main task is to bolt out the most of *a priory* ineffective peptides the use of such simplified model is justified.

MDis package implements a CHARMM19 forcefield [6] in conjunction with SASA implicit solvent model [7-9].

3. Biological System

The major role in cell division control pathway is played by Retinoblastoma protein (pRb) (**Figure 1**). In activated (non-phosphorylated) state this protein suppresses cell cycle progression by inhibiting E2F transcription factor. In healthy cell ready for division, the phosphorylation (deactivation) of pRb is performed by complex of cyclin-dependent kinases CDK4 and CDK6 with cyclinD proteins (D1, D2, D3). The cancer cells often exhibit the mutations in one or more proteins involved in this pathway [10-14]. The natural inhibitors of CDK4 and CDK6 bound to CyclinD are proteins of INK4 family, including P16/INK4a protein, whose functioning is also known to be disrupted in cancerous cell [11,12]. The introduction of P16 protein into cell has been shown to arrest cell cycle progression from G_1 to S phase.

The clinical use of P16, however, is not practical due to its relatively large size (156 amino acid residues). It might be more practical to develop shorter (up to 10 amino acid residues long) peptides with inhibitory activity on level with full-length P16.

As a foundation for this study we used the works of Fåhraeus *et al.* [15,16] measuring *in vitro* and *in vivo* the efficiency of CDK6 and CDK4 inhibition by various


Figure 1. CDK4/6 regulatory pathway schematics.

parts of P16 protein and its homologues.

The first series of experiments [15] measures the stability of complex formed by CDK6 and 20-residue-long peptides derived from all subsequences of P16 with 15-residue step. Afterwards, the activity of CDK6 was measured *in vitro* in presence of same peptides, as well as their effect on cell cycle progression *in vivo*. In all of these experiments the peptide codenamed "p6" (residues 84 - 103 of full-sized P16) have shown best results.

Reference [16] describes the experiments measuring the efficiency of various mutations of "p6" peptide, including its shortening.

The main goal of this work is to reproduce the results of [15,16] *in silico* to approbate the suggested protocol.

4. Methodology

The proteins of INK4 family are allosteric inhibitors [17, 18]. However, the characteristic times of structural transitions in proteins are of order of milliseconds which is beyond what is currently reachable by all-atom molecular dynamics simulations. So an assumption was made that the stable complex formation is required for peptide inhibitory activity since otherwise it would be unable to cause sufficient structural change. For this reason the energy profiles of association of CDK6 with peptides were studied.

Initial protein structures were taken from Protein Data Bank (CDK6: 1BLX [19], P16: 1BI7 [20]). Each structure was minimized with steepest-descend algorithm in MDis package.

In this work peptides codenamed P2-P9 from [15] are studied. The structure of peptides was obtained by cutting them from 3D structure of whole protein and subsequent equilibration for 5 ns at 300 K temperature.

Initial conformations of peptides bound to CDK6 were obtained using Autodock Vina software [21]. For each peptide ten most energetically favorable conformations were chosen, from which from 2 to 4 significantly different ones were chosen for further investigation.

To measure interaction energy the umbrella sampling technique [22] was used. The distance between peptide and protein centers of mass was taken as reaction coordinate. For each conformation in consideration the generation of initial trajectory was performed by pulling peptide apart from the protein with force applied to peptides center of mass and C_α atoms of CDK6 restrained (Langevin integrator with $T = 300$ K, $\gamma = 0.15$, $\Delta t = 1$ fs, harmonic restraint on C_α atoms of CDK6 16 kcal/mol/Å^2, pulling speed 5 Å/ns, pulling spring constant 2 kcal/mol/Å^2). The trajectories were used to extract initial conformations for further sampling. The conformations were taken with 0.5 Å step of reaction coordinate. For each sampling window the 30 ns of equilibration simulation
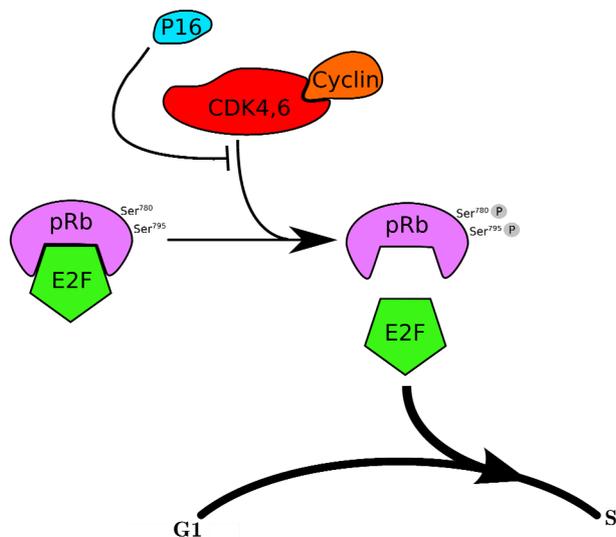
was performed with additional harmonic restraint applied to reaction coordinate (Langevin integrator with $T = 300$ K, $\gamma = 0.15$, $\Delta t = 1$ fs; umbrella spring constant 1 kcal/mol/Å^2; restraint on C$_\alpha$ atoms of CDK6 2.4 kcal/mol/Å^2).

After analyzing resulting trajectories with weighted histogram analysis method (WHAM) [23] the free energy profiles were obtained. We used implementation of WHAM by A. Grossfield [24].

The free energy difference between bound state and the highest point of energy barrier separating bound and unbound states were chosen as resulting value for each peptide studied.

5. Results and Discussion

The conformations obtained from protein docking study are shown in **Figure 2**, and the primary sequences of peptides are listed in the **Table 1**. As one can see, all peptides prefer hydrophobic pockets between two lobes of CDK6 which coheres with proposed structural model of CDK4/6 inhibition by proteins of INK4 family [17].

Figures 3 and **4** show the obtained results for binding energy compared to *in vitro* results from [15,16]. As could be seen in **Figure 4** although numerical modeling yields correct interaction energy it is not always directly related to inhibition efficiency.
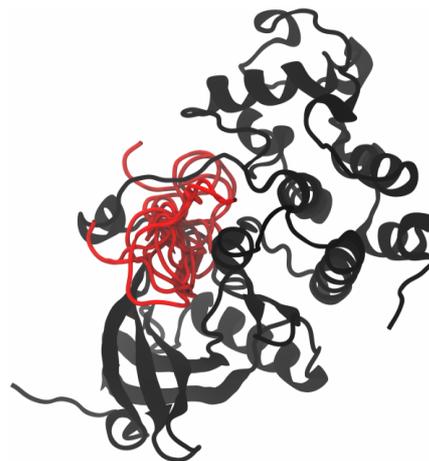
Figure 2. CDK6 (black) and peptides (red) bound conformations as predicted by Autodock Vina [21].
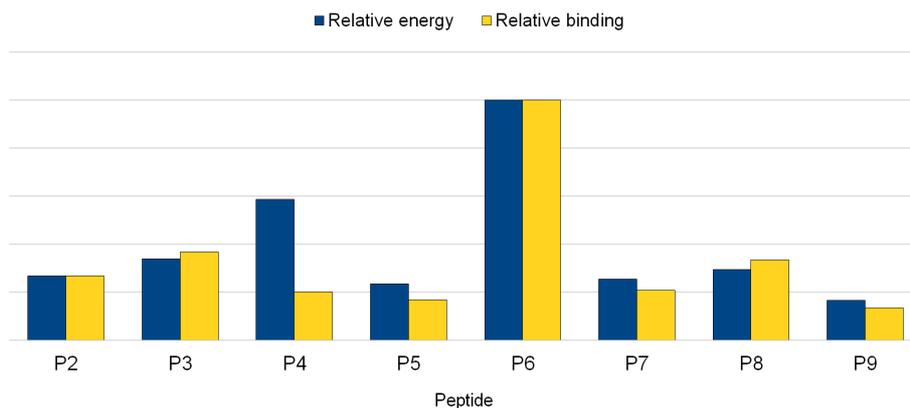

Figure 3. The comparison of *in silico* (blue) and *in vitro* (yellow) results for the first series of experiments. For *in silico*, the energy of binding obtained with Umbrella sampling is shown. For *in vitro*, the data on relative binding on agarose gel is shown.
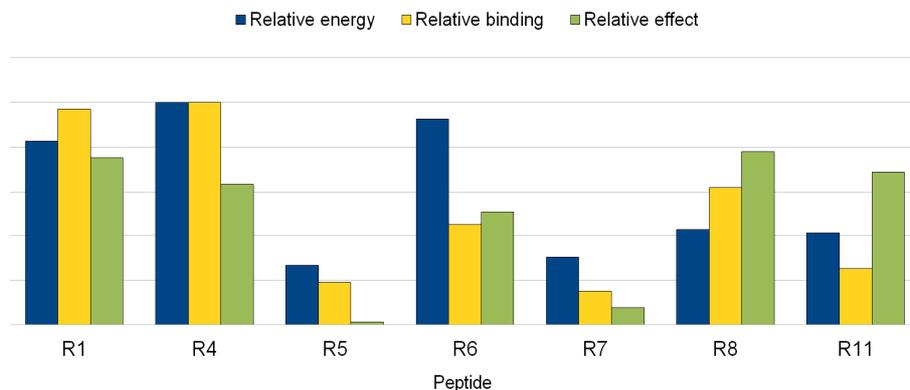

Figure 4. The comparison of *in silico* (blue) and *in vitro* (yellow, green) results for the second series of experiments. For *in silico*, the energy of binding obtained with Umbrella sampling is shown. For *in vitro*, the data on relative binding on agarose gel (yellow) and relative kinase activity in presence of studied peptide (green) is shown.

Table 1. Studied peptides.

Peptide	Sequence	Source
P2	RVEEVRALLEAGANPNAPNS	[15]
P3	NAPNSYGRRPIQVMMMGSAR	[15]
P4	MGSARVAELLLLHGAEPNCA	[15]
P5	EPNCADPATLTRPVHDAARE	[15]
P6	DAAREGFLDTLVVLHRAGAR	[15]
P7	RAGARLDVRDAWGRLPVDLA	[15]
P8	PVDLAEELGHRDVARYLRAA	[15]
P9	YLRAAAGGTRGSNHARIDAA	[15]
P10	DAARAGFLDTLQTLLEFQAD	[15]
P11	DAAREGFLDTLVVLHRAGAR	[15]
R1	DAAREGFLDTLVVLHRAG	[16]
R4	DAAREGFLDTLVVLHR	[16]
R5	AREGFLDTLVVLHRAGAR	[16]
R6	EGFLDTLVVLHRAGAR	[16]
R7	FLDTLVVLHR	[16]
R8	RVEEVRALLEAGANPNAPNS	[16]
R11	NAPNSYGRRPIQVMMMGSAR	[16]

6. Conclusion

The result of this work is the approbation of methodology for numerical evaluation of binding energy of given peptides with chosen target protein. The results could be improved at the expense of increased simulation wall time by using more accurate implicit solvent models (e.g. GB/SA [25] or FACTS [26]) or explicit solvent. Alternative approach to improve the accuracy is to use more initial conformations.

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