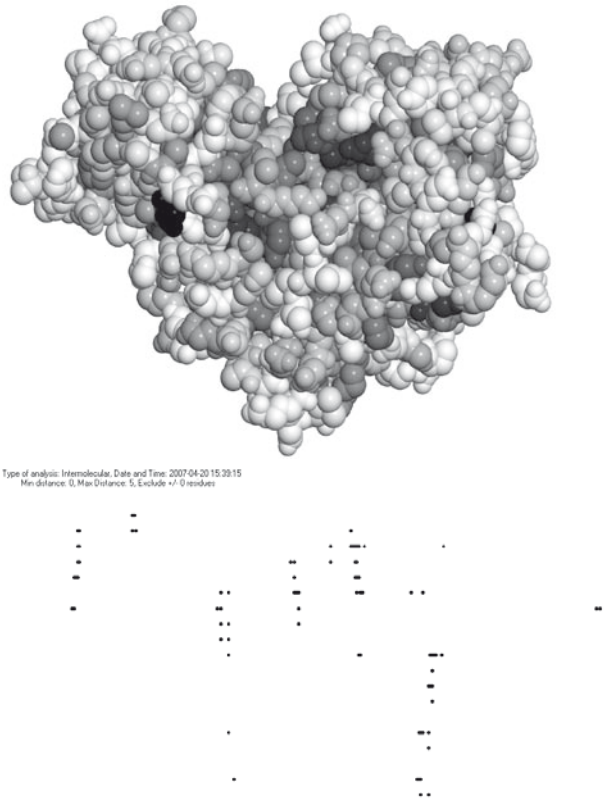

Figure 1. A) Variability profile imposed on a 3D kinase structure by a script file generated by Talana. More conservative positions are shown as the darker ones. B) Intermolecular contact map between a kinase and it's peptide inhibitor — results obtained using Nitano.

The described tools are useful to study locations that are important for catalytic activity, or are located in direct neighborhood of active sites.

The results obtained with our applications are important for drug design, in particular for refinement of designed inhibitors, increasing their specificity. All described applications are freely available for non-commercial purposes at: http://www.bioware.republika.pl and http://www.bioexploratorium.edu.pl. The source code is available from the authors upon direct request.

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WP3

In silico design and implementation of a polyketide synthesis system for production of virtual libraries of macrolides

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The biochemical structure and biological activity of type I polyketide natural products are prescribed by modular multienzymic polyketide synthases(PKSs). The action of multifunctional PKS domains assemble a production-line pathway for biosynthesis of polyketide with a strong combinatorial potential, leading to an extraordinary diversity both in structure and function of these compounds. The astronomical number of these products make their experimental generation impractical. Thus developing knowledge based in silico approaches to produce virtual compounds is essential to discovery of novel polyketide structures and measuring their biological activities. Based on combinatorial chemistry and cheminformatics method, we developed a computational workbench capable of simulating the biosynthesis pathway of polyketide and producing virtual libraries of macrolides as a result of in silico manipulation of modules in the PKS system. The produced libraries may further be filtered by vHTS methods to pass the stages of hits and leads to identification of drug candidates. Moreover, as a result of using this in silico approach, the selected virtual molecules provide guidelines for their genetic engineering regarding the genes encode the catalyzing enzymes in their biosynthesis reactions.

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WP4

Details of the JAK–STAT signaling pathway model

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The JAK–STAT signaling pathways, highly conserved in eukaryotic organisms, provide a direct route to the nucleus, where in effect gene transcription is altered. This mechanism allows for fast cell response to external stimuli and has co–evolved with multitude cellular signaling events. JAK–STAT signaling is responsible for a variety of functions. Pathways activated by type I or type II interferon, that include STAT1 and STAT2 proteins, control antiviral, innate and addaptive immunity, and are important for antitumor immune responses [1]. On the other hand, many cytokine–activated pathways containing mainly STAT3, but also STAT5 proteins, regulate cell growth and apoptosis processes, and are persistently activated in a large number of human cancers, making their components attractive drug targets [2].
Revealed interactions inside the JAK–STAT pathways, as well as crosstalk with other pathways, adding to the already very complex interactions network, cause intuition to fail in understanding what will be the outcome of the pathway interference. To comprehend the role of each of the design parts (phosphatases, dimerization steps, feedback loops) and identify the key elements (parameters), with respect to the dynamics of the pathways, it is crucial to develop a mathematical (computational) model.

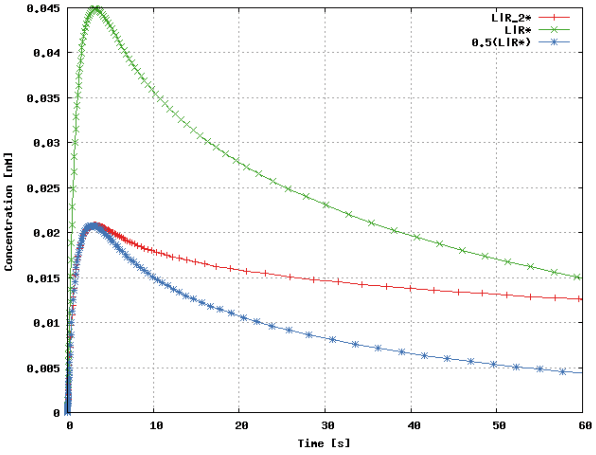

Figure 1. Differential equation simulations of simple cytokine receptor models.
Plotted lines present differences in dynamics during first 60 seconds of the active receptor-ligand complexes (activity is measured in the concentration of the species, though it should be noted that, for receptors residing on the cell surface, it's only an abstraction of the real units). Models differ from the one that is assumed to be original (red line; receptor creates dimer but there is no distinction between receptor chains) by the absence of the dimerization step (green line) and with an additional half of the initial receptor ammount (blue line).

Although some models have already been proposed (e.g. [3]), a good starting point for further analysis, there are still numerous unknowns, especially in subtle details which may play an important role in pathway behaviour.

Our work focuses on cytokine receptors activation process (see Fig. 1), in particular, the importance of the dimerization step [4, 5]. We emphasize stochastic effects, that are important especially in interactions with receptor (e.g. [7]) that acitvate STATs. Furthermore, we intend to apply novel techniques in this field, allowing for exhaustive stochastic analysis and verification of queries expressed in temporal logic [8, 9].

Finally, we are planning to develop the method for automatic inference [10], based on experimental data, of the design details (e.g. receptor dimerization, cytokine unbinding, certain species nuclear imports and exports) of highly constrained pathway networks (i.e. with well established, unchangeable core mechanism: STAT life–cycle, negative and positive regulation by e.g. SOCS, PIAS). This is required to perform further, realiable, in silico experiments.

Additionally, following [11, 12], proteins interactions map is being created, representing current knowledge on the JAK–STAT pathway. It is a manual effort, assembled in iterative process, based on literature and on–line databases. The map is encoded in strict, machine–readable language, allowing for easy exchange between, and manipulation in, different systems biology software tools.

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WP5

Modeling of Possible Binding Modes of Caffeic Acid Derivatives to JAK2 Kinase

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Janus kinases (JAK1, JAK2, JAK3 and TYK2) belong to a family of receptor-associated protein tyrosine kinases. They play a critical role in the JAK/STAT (Signal Transducer and Activator of Transcription) signaling pathway that is responsible for intracellular transduction of growth factors and cytokine-mediated signals. We focused our attention on the JAK2/STAT3 pathway whose abnormal activation was observed in many types of human neoplasia, including highly resistant brain and pancreatic cancers, prostate cancer and hematopoetic malignancies, making it an important and unexplored target for therapeutic intervention.

Among the different classes of Jak2/STAT3 pathway inhibitors, the importance of the natural product caffeic acid and its derivatives is well recognized. Synthetic analog AG490 was extensively studied, however, its low potency (IC_{50}>50mM *in vitro*), and the lack of activity *in vivo* in experimental tumor models, prevented its development as an anticancer drug. We have successfully used

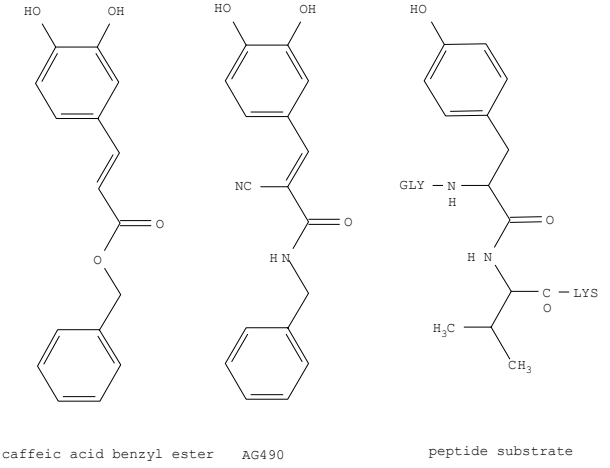

Figure 1. Structural similarity between caffeic acid benzyl ester, AG490 and a part of JAK2 peptide substrate.

The only known crystallographic structure of JAK2 does not contain any peptide inhibitor. In order to obtain the kinase in a conformation suitable for the analysis of potential binding modes, we carried out an explicit solvent molecular dynamics (MD) simulation of JAK2 with a penta-peptide substrate that was artificially placed on the basis of similarity to the already known Insulin Receptor Kinase (IRK) — peptide substrate complex. After an initial conformational change, the simulated complex remained stable for subsequent 2ns MD simulations. At present we exchange the peptide substrate with a series of known caffeic acid based JAK2 inhibitors, and evaluate energy and analyse stability of the obtained complexes.

the scaffold of caffeic acid and its analogs to design and synthesize novel potent JAK2 inhibitors exemplified by WP1066, WP1130 and WP1193.Unfortunately, the limited knowledge of the binding mode of AG490 and other caffeic acid derivatives to JAK2 hampered our ability to efficiently design more potent inhibitors.

In the current study we attempt to propose a binding mode of caffeic acid derivatives including AG490 to JAK2 kinase, using molecular modeling techniques. These derivatives are more likely to be competitive inhibitors for the tyrosine-containing kinase substrate rather than for ATP [1], which indicates that their potential binding site is probably located in the vicinity of the peptide substrate binding site. We further noted a similarity between the caffeic acid scaffold and a part of a structurally conserved region of protein substrates that binds directly to the active site of JAK2 and similar kinases (Fig. 1). It suggests that analysis of the kinase — peptide substrate complex may be of use in finding the JAK2 inhibitors binding modes.

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