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Strategic recovery of recombinant human truncated Cyclin A and Cyclin D in *Escherichia coli* for characterization with putative inhibitors

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Targeting cyclins enables us to interfere with cell cycle in order to inhibit the cancerous process which is the result of a non-proper regulation of this cell cycle control¹. Through cell cycle, Cyclin dependent kinases (CDKs) as well as their activation partners, Cyclins, are regulators of progression and proliferation through activation of cell cycle checkpoints and inhibited by CKIs, thus they have been widely held as anti-cancer targets². This work attempts the over-expression of recombinant truncated forms of human Cyclin A (His-tagged CCNA2) and Cyclin D (GST-tagged CCND1) seeking the high levels of yield and purity for crystallization and ligand characterization purposes. Seeking to optimize binding affinity of CCNA2/inhibitor and CCND1/inhibitor complexes, in order to develop more active inhibitors against CDks/Cyclins activity for cancer research. CCNA2 and CCND1 fused to the appropriate bacterial vectors³, in *E. coli*, as one of the most widely used expression hosts. Following heterologous over-expression, the recombinant proteins often fail to properly fold, resulting in formation of insoluble aggregates (inclusion bodies - IB). It is known that co-expression with chaperone proteins facilitates their folding process, while increasing solubility in a bacterial over-expression system⁴. Optimizing the high levels of yield and purity required a number of strategies took place for the recombinant proteins. BL21 (DE3) expression host is preferred for both recombinant proteins⁵. Homogenization increases the levels of regained protein from IB. Following denaturation of IB, Urea buffer is suggested for refolding. CCNA2 and CCND1 refolding was more efficient with GSH/GSSG rather than DTT, while stability of cyclins was achieved with elevated concentrations of MgCl₂. In case of CCND1 co-expression with chaperone plasmid pTf16 increases substantially soluble protein. Existing synthesized peptides, designed with REPLACE (REplacement with Partial Ligand Alternatives through Computational Enrichment). This structure-activity co-relation with non-fluorescent peptides as cyclin groove putative inhibitors (CGI) where tested. Image

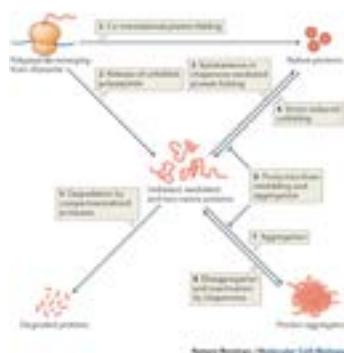


Figure1: The protein quality control network⁶

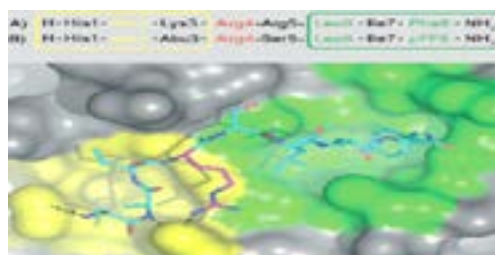


Figure2: REPLACE strategy. N- terminal HAKRRLIF binding motif from native CGI p21waf1, synthetic peptides were designing while tested as putative inhibitors²

Biography

Vaios Nikolopoulos is a Biologist MSc and as a PhD candidate, has his expertise in molecular biology and diagnostics. His passion is to combine more aspects and functions of molecules and their pathways in order to see the big picture in a pathologic state either this is a cancerous process or diabetes etc. Currently he is working in protein purification and ligand binding studies while studying crystallography and also elaborates with other colleagues in various clinical studies. His goal is to be a part of this "thinking tank" community to share his inquiring mind and to absorb new ideas in this highly competitive area of biosciences.

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