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Recombinant cyclins and their CDKs in *Escherichia coli*: expression and purification for soluble active products and characterization with putative inhibitors

Nikolopoulos Vaios[1], **Grigoroudis Asterios**[2] and **Kontopidis George**[1]
[1]University of Thessaly, Greece
[2]American College of Thessaloniki, Greece

Targeting cyclins enables us to interfere with cell cycle in order to inhibit the cancerous process which is the result of a non-prober regulation of this cell cycle control. 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 cyclin-dependent kinase inhibitors (CKIs), thus they have been widely held as anti-cancer targets. 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. Although, the path from expression to high levels of yield and purity, as well as from purified protein solution to well-diffracting crystal is rarely a straightforward highway. Moreover an overexpressed or purified protein is either unfolded or misfolded leading to uncertain results. Furthermore protein stability such as salt concentration precipitates the product. So a number of parameters are taking place to find promising lead conditions. Seeking targets for drug development, tumor suppression, anti-cancer targets or other inhibition, more and more screening and optimization is needed and required different strategies while struggling with insoluble aggregates (inclusion bodies formation) and the stability of the protein of each step of the process. So how do we compete with these challenges? To answer these emerging questions, the lecture will give a glimpse on ours experience working on proteins with very low expression in appropriate bacterial vectors, in *E. coli*, as one of the most widely used expression hosts.

Figure 1: The protein quality control network

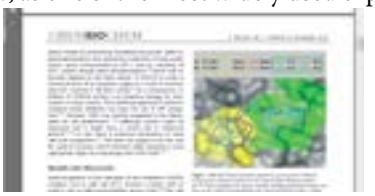

Figure 2: REPLACE strategy. N- terminal HAKRRLIF binding motif from native CGI p21waf1, synthetic peptides were designing while tested as putative inhibitors

Recent Publications:

1. Andrews M J I, Kontopidis G, McInnes et al. (2006) REPLACE: A strategy for iterative design of cyclin-binding groove inhibitors. ChemBioChem 7(12):1909–1915.
2. C Papaneophytou and G Kontopidis (2016) A comparison of statistical approaches used for the optimization of soluble protein expression in *Escherichia coli*. Protein Expression and Purification 120:126-37.
3. Qing-di Cheng et al. (2017) Effect of the weather conditions during solution preparation on lysozyme crystallization. J. Appl. Cryst. 50:1341-1351.
4. Chou C P (2007) Engineering cell physiology to enhance recombinant protein production in *Escherichia coli*. Appl Microbiol Biotechnol 76(3):521–532.
5. Kyratsous C A, Silverstein S J, DeLong C R and Panagiotidis C A (2009) Chaperone-fusion expression plasmid vectors for improved solubility of recombinant proteins in *Escherichia coli*. Gene 440(1–2):9–15.

Biography

Nikolopoulos Vaios is a Biologist, MSc and 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 elaborate 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.

nikvaios@bio.uth.gr