Protein phosphorylation transduces a large set of intracellular signals. One mechanism by which phosphorylation mediates signal transduction is by prompting conformational changes in the target protein or interacting proteins. Previous work described an allosteric site mediating phosphorylation-dependent activation of AGC protein kinases. The AGC kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) is activated by the docking of a phosphorylated motif from substrates.
PDK1 is a central component of the growth factor and insulin signaling pathways. PDK1 is responsible for the stimulus-dependent phosphorylation and activation of many AGC kinases like Akt/PKB, S6K, RSK and SGK. Thus, our studies on PDK1 are relevant both for the growth factor/cancer field (PDK1 is a validated drug target for cancer treatment) and for insulin/diabetes research. PDK1 is also required for the constitutive phosphorylation of the activation loop of other protein kinases, such as all 12 protein kinase C (PKC) isoforms and the protein kinase C-related protein kinases (PRKs). In all, at least 23 protein kinases are known to be phosphorylated by PDK1.
We present the crystal structure[1] of PDK1 bound to a rationally developed low-molecular-weight activator[2] and describe the conformational changes induced by small compounds in the crystal and in solution using a fluorescence-based assay and deuterium exchange experiments. Our results indicate that the binding of the compound produces local changes at the target site, the PIF binding pocket, and also allosteric changes at the ATP binding site and the activation loop. Altogether, we present molecular details of the allosteric changes induced by small compounds that trigger the activation of PDK1 through mimicry of phosphorylation-dependent conformational changes.

[1] Hindie V, Stroba A, Zhang H, Lopez-Garcia LA, Idrissova L, Zeuzem S, Hirschberg D, Schaeffer F, Jørgensen TJ, Engel M, Alzari PM, Biondi RM, *Nature Chem Biol*, 2009, 5, 758.
[2] Stroba A, Schaeffer F, Hindie V, Lopez-Garcia L, Adrian I, Fröhner W, Hartmann RW, Biondi RM, Engel M, *J Med Chem* 2009, 52, 4683.

Keywords: protein kinases, allostery, phosphorylation

FA1-MS06-P12

Structural investigation of antidepressant drug and dipalmitoyl phosphatidylcholine interactions by Small Angle X-Ray Scattering method. Dilek Yonar[a], Elif Hilal Soylu[b], M. Maral Sunnetcioglu[a], Semra Ide[a]. [a]*Hacettepe University, Faculty of Engineering, Dept. of Physics Engineering, 06800, Beytepe, Ankara, Turkey.* [b]*Karadeniz Technical University, Faculty of Science and Literature, Dept. of Physics, 61080, Trabzon, Turkey.*
E-mail: ddogan@hacettepe.edu.tr

In this work, interaction of antidepressant drugs clomipramine hydrochloride and trazadone hydrochloride with dipalmitoyl phosphatidylcholine (DPPC) liposomes with/without cholesterol have been investigated. On these samples EPR spin labeling studies were performed recently [1, 2]. To receive further structural information on membrane thickness and the change of it by drug incorporation Small Angle X-Ray Scattering (SAXS) profiles have been recorded. The components were mixed in a wide capillary and left for 1 h at room temperature. The resulting complex was centrifuged into a narrow X-ray capillary. SAXS experiment was carried out with a Hecus SWAXS camera (Hecus X-Ray Systems Graz-Austria). X-ray generator operating at 50 kV and 50 mA with Cu anode (λ= 1.54 Å) in q range of 0.008 – 1.22 ($Å^{-1}$). Measurements were made by a step –scanning procedure and in the fixed time mode, with a sampling time 300 s for each step.
SAXS scans were also carried out from 15 to 55 ^{o}C by using an external temperature control unit causing temperature increasing in 2 ^{o}C to investigate thermal effects on the structure of drug incorporated and pure liposomes.
In the result of these studies, the changing in the lamellar repeat distances of DPPC have been determined after the incorporation of three drug compounds. The observed increase in the bilayer thickness which may allow to PBS and drug compound locations was in the range of 12-15 Å. As known as, these interactions occur by four effective types of forces defined as van der Waals, electrostatic, hydration, and undulation forces between DPPC bilayers, and during the incorporation of drug molecule into the DPPC structure [3]. The possible 3-D molecular conformations have been also investigated by using molecular mechanic calculations to explain the interaction of the drug molecules with DPPC. The results have been compared and discussed by using the related literature knowledge [4, 5].

[1] EPR Investigation of Surface active Antidepressant Drug-Membrane Interactions, TBAG-U / 140 (105T064). [2] Spectroscopic investigation of antidepressant drug Trazodone Hydrochloride -model membrane interactions, TBAG-109T549. [3] Y. Kobayashi, K. Fukada, Biochimica et Biophysica Acta, 1371 (1998) 363-370. [4] K. Sakai, H. Tomizawa, K. Tsuchiya, N. Ishida, H. Sakai, M. Abe, Colloids and Surfaces B: Biointerfaces, 67 (2008) 73-78. [5] J. J. McManus, J. O. Radler, K. A. Dawson, Langmuir, 19 (2003) 9630-9637.

Keywords: liposomes, antidepressants, SAXS

FA1-MS06-P13

Drug-stabilized and drug-free complexes of topo IV from *S. pneumoniae* shed light on the mechanisms of reversible DNA scission and selective drug resistance. Ivan Laponogov[a,b], Xiao-Su Pan[b], Dennis A. Veselkov[a], Katherine McAuley[c], L. Mark Fisher[b], Mark R. Sanderson[a]. [a]*Randall Division of Cell and Molecular Biophysics, King's College London, 3rd Floor New Hunt's House, Guy's Campus, University of London, London, UK.* [b]*Molecular Genetics Group, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St. George's, University of London, London, UK.* [c]*Diamond Light Source, Didcot, Oxford, UK.*
E-mail: mark.sanderson@kcl.ac.uk, lfisher@sgul.ac.uk, ivan.laponogov@kcl.ac.uk

Topoisomerase IV belongs to the type II class of DNA topoisomerases, which are responsible for changing and stabilizing DNA supercoiling and are also involved in chromosome segregation in prokaryotes. Topo IIs are essential enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change DNA topology by forming a transient covalent cleavage complex with a gate-DNA (G-segment) duplex and transporting the second duplex (T-segment) though a double-stranded break in the formed protein-DNA gate. Although the biological importance of these enzymes is well known,