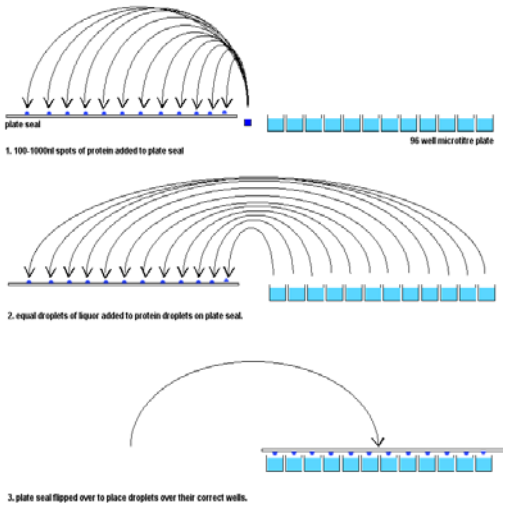
A prerequisite for efficient high throughput protein crystallisation screening is the accurate pipetting and positioning of the low volume drops used in hanging and sitting drop setups. Screening the many different conditions under which a protein crystal may form lends itself to automation, since it requires hundreds of similar experiments to be set up to find the few 'hits'. Automated solutions exist for low volume pipetting, however, the variable viscosities of protein and reservoir/screen solutions present significant challenges for many liquid handling systems. Another challenge is that of drop positioning. The mosquito® (TTP LabTech) offers fast positive displacement pipetting for accurate and reproducible aspiration and dispensing throughout the 50 nL - 1.2 µL range, producing CVs of <8% at 50 nL irrespective of viscosity. This, plus its columnar arrangement of pipettes, allows it to automate hanging drop as well as sitting drop set-ups. Mosquito's micropipettes are also disposable, thus guaranteeing zero cross-contamination where required.

Figure: Automated hanging drop setup



MS06 P13

Development of a new microplate for micro-scale vapour diffusion. Marek Brzowzowski1, Justyna Korczynska1, Ting-Chou Hu1, David K Smith1,Joby Jenkins2, Rob Lewis2

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The automation of the crystallisation process has contributed significantly to the rapid progress of crystallography-based structural biology. For example, 96-well plates have been seamlessly incorporated into automated protein crystallography set-ups enabling much higher process throughputs. This development has delivered a plethora of crystallization plates suitable for both automated and manual set-ups. However, practically all these plates (except microfluidic channel chips) are based on a very similar design and well volume to drop ratios (50-100uL to 25-150nL).
TTP LabTech and the York Structural Biology Laboratory have pooled their expertise in engineering and protein crystallography to develop and test a new type of crystallization plate (μplate) that still employs classical vapour diffusion technique but minimizes the precipitant well volume down to 1.2-10µL. This enables:

- a very significant saving on the total bulk of screens

the use of rare and chemically expensive solutions for automated screening procedures.

MS09 P04

Structure and activity of Kunjin virus NS3 helicase domain Eloise Mastrangelo[a], Mario Milani[a], Michela Bollati[a], Graziella Sorrentino[a], Bruno Canard[b], Dmitri I. Svergun[c] and Martino Bolognesi[a] [a]*Department of Biomolecular Sciences and Biotechnology, University of Milano, Italy.* [b]*Laboratoire Architecture et Fonction des Macromolécules Biologiques, AFMB-CNRS-ESIL, Marseille, France.* [c]*E.M.B.L. Hamburg, Germany*
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Keywords: helicase structure; Kunjin virus; flavivirus NS3 protein

Flaviviral NS3 is a multifunctional protein displaying N-terminal protease activity in addition to C-terminal helicase, nucleoside 5'-triphosphatase (NTPase), and 5'-terminal RNA triphosphatase (RTPase) activities. NS3 is held to support the separation of RNA daughter and template strands during viral replication. We solved the three-dimensional structure of the NS3 helicase domain (residues NS3:186-619) from Kunjin virus, an Australian variant of the West Nile virus. As for homologous helicases, NS3:186-619 is composed of three domains, two of which are structurally related and held to host the NTPase and RTPase active sites. The third domain is involved in RNA binding/recognition. Normal mode analysis of the NS3:186-619 helicase construct indicates the presence of a scissors-like oscillation involving domains II and III, resulting in opening/closure of the ssRNA binding cleft entrance. Such intramolecular scissors-movements may be part of the inchworm mechanism by providing a strain component for dsRNA unwinding [1]. NS3:186-619 displays both ATPase and RTPase activity and can unwind a dsRNA substrate. Analysis of different constructs shows that full length NS3 displays increased helicase activity, suggesting that the protease domain plays an assisting role in the RNA unwinding process. The structural interaction between the helicase and protease domain has been assessed using small angle X-ray scattering on full length NS3, disclosing that the protease and helicase domains build a rather elongated molecular assembly differing from that observed in the NS3 protein from HCV.

[1] Mastrangelo, E. et al. (2007). Crystal structure and activity of Kunjin Virus NS3 Helicase; Protease and Helicase Domain assembly in the Full Length NS3 Protein. *JMB.* [Epub ahead of print]

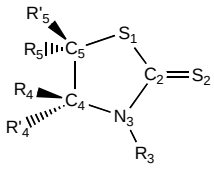
MS13 P29

Energetic and structural studies in

thiazolidines-2-thiones series and its comparison with a conformational study. A. LAKNIFLI, A. HAMINE, A. ELHAMMADI. Department of Chemistry, University of Ibn Zohr, Faculty of Sciences, PO 8106, Agadir, *Morocco.* E-mail: abdelaknifli@yahoo.fr

Keywords: Thiazolidine 2-thione, conformation, Heterocycle

In the prospect to study the heterocyclic thiazolidin-2-thione and its derivatives variously substituted (see figure below), our investigation in the solid state will be completed by *AM1* and *ab-initio* calculations.[5,6].
Both of the two theoretical methods leads us to preferential conformations and the comparison between the most stable molecular conformations of the different molecules investigated, helps us to estimate the "weight" of each effect in the real structure of the compound. Our interest has essentially been carried on the conformational behaviour of the heterocycle th-2-th studied and more exactly on the
Csp3-Csp3 part which is the soft part of cycle.



R3 = H, Methyl, tBu, iPr, Phenyl, Cyclohexyl
R4 = H, Methyl, tBu; **R'4** = H, Methyl
R5 = H, tBu, Phenyl ; **R'5** = H, Methyl

The detailed analysis of the conformations in the solid state [1,2,3,4] show**s** the flexibility of the heterocycle pentagonal form S1C2N3C4C5 and reveals a relationship between the flexibility of the cycle which is defined by the twisting angle S1C5C4N3 and the distance C4-C5. The quasi-planar and planar conformations are associated together with very short distances C4-C5 (up to 1.40 Å).

The study of the heterocycle by the semi-empirical methods [6] shows that the evolution towards the planar state entails a stabilization of the molecule by covering of ***s*** and ***p*** orbitals and particularly in part C4-C5.
At the end, it's possible to envisage that the combination of steric effects and electronic effects could lead to an efficient model in the treatment of the heterocyclic systems.

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MS16 P16

Coumarin 120 - crystallization experiments leading to a new type of polymorphism. Zofia Urbanczyk-Lipkowska, Dorota Niedziałek, *Institute of Organic Chemistry, Polish Academy of Sciences.* 01-224 Warsaw, Poland. E-mail: ocryst@icho.edu.pl

Keywords: coumarins, new polymorph, electronic structure

Coumarins are natural or synthetic compounds used as pharmaceutics and herbicides. They exhibit fluorescent properties (due to presence of benzopyrone moiety) and therefore, are used as high quantum yield laser dyes (e.g. coumarin 4, coumarin 120, etc). Their frequent use as a model compounds in photophysical studies prompted us to look for their polymorphs.
Crystallization experiments involved recrystallization from polar and non-polar solvents and their mixtures, co-crystallization with 5 % or equimolar amount of another coumarin derivative. We also investigated the influence of the crystallization solvent and additives on crystal habit. The transition behavior of the crystalline forms of coumarin 120, its melting point, and enthalpy were investigated by DSC, FTIR and X-ray crystallography.
These experiments allowed to obtain a new crystalline form of 7-amino-4,methyl-coumarin (coumarin 120) with unprecedented crystal structure, containing two types of molecules – nonpolar and polar. These crystals exhibit also a new type of polymorphism – where molecules of two different electronic structures co-exist in the same crystal. Both types of molecules are bound by the same hydrogen bonding pattern, similar tu that found in first polymorph [1]. Although, polar molecules are bound slightly tighter (m.p. of a new form higher by 3 ^{o}C) during melting new polymorph transforms irreversibly into the first form. These studies confirm that fluorescence quenching observed for coumarin 120 [2] is due to the presence of equilibrium of various electronic structures in solution.

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MS21 P08

Crystal structure of the new titanium phosphate $Na_3CaTi(PO_4)_3$

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Key Word : Titanium phosphate, Nasicon – type

Titanium phosphates are currently of interest for their chemical and physical properties as catalysts, non-linear materials and ionic conductors. In this context the new titanium phosphate $Na_3CaTi(PO_4)_3$ has been synthesized and structurally characterized. Single crystals were obtained by melting a mixture of Na_2CO_3, $CaCO_3$, TiO_2 and $(NH_4)_2HPO_4$ in stoichiometric proportion, at 900°C, followed by slow cooling (3°C/hr).

$Na_3CaTi(PO_4)_3$ crystallizes in the space group R32 with a = 8.985(1)Å, c = 21.920(3)Å, V = 1532.6(3)Å^3, and Z = 6. The refinement factors are R_1 = 0.0365 and wR_2 = 0.0944. The structure of $Na_3CaTi(PO_4)_3$ belongs to the Nasicon-type family. It consists of a three dimensional network of PO_4 tetrahedra and AO_6 (A = Ti, Ca) octahedra sharing corners. A 2-2 ordered distribution of titanium and calcium occurs along the c axis giving rise to two different units $[Ti_2(PO_4)_3]$ and $[Ca_2(PO_4)_3]$. Within this covalent framework exist two additional cationic sites usually labeled M1 and M2 where the sodium ions are located.