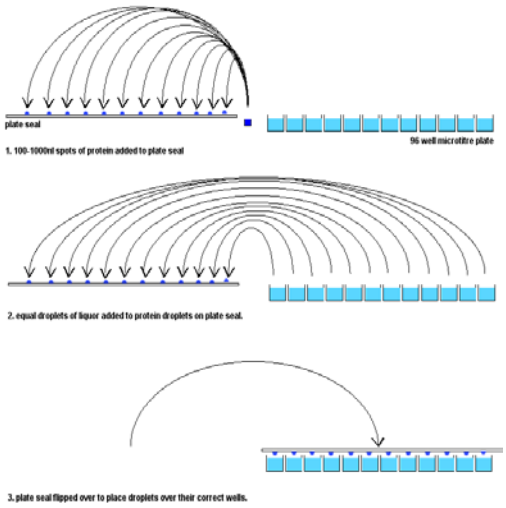
A prerequisite for efficient high throughput protein crystallisation screening is the accurate pipetting and positioning of the low volume drops used in hanging and sitting drop setups. Screening the many different conditions under which a protein crystal may form lends itself to automation, since it 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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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