CeTPD Journal Club

August – September 2024

Targeted protein degradation, medicinal chemistry, chemical structural biology & cell biology

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
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Nature, **2024**, 633, 232–239

MEET THIS MONTH'S EDITORS

Click here for info on the editor

SUZANNE O'CONNOR

Suzanne received her undergraduate degree in Medicinal Chemistry from Trinity College Dublin and her MRes in Drug Discovery & Development from Imperial College London, where her research focused on the development of antibody drug conjugates. She then joined AstraZeneca working on a variety of oncology drug discovery projects. She moved to the Institute of Cancer Research for her PhD in Fragment Based Drug Discovery with Professor Ian Collins and then joined Evotec as a senior scientist developing molecules for the treatment of chronic pain. Suzanne joined the Ciulli group in 2021 where she led multidisciplinary teams for PROTAC drug discovery projects in oncology, in collaboration with Boehringer Ingelheim and since July 2024 is senior lead scientist for our KOODAC^3 team.

LinkedIn: www.linkedin.com/in/suzanne-o-connor/

KENTARO ISO

Kentaro began studying organic chemistry at Tohoku University, where he completed both his MSc and PhD in the field of natural product synthesis. He joined Eisai to pursue a career in medicinal chemistry. During his career, he also worked at Harvard University as a visiting scholar. His research interests include peptide mimetics, natural products, peptide synthesis, antibody-drug conjugates, and protein degraders.

LinkedIn: https://www.linkedin.com/in/kentaro-iso-0a17721a9/

YUTING CAO

Yuting comes from Hebei, China. She completed her master's degree in medicinal chemistry at Nankai University. Then, she moved to the Artemisinin Research Centre where she worked as a research assistant. Since October 2021, she joined the Ciulli lab as a PhD student through China Scholarship Council (CSC) Programme working on new scaffold PROTACs.

LinkedIn: https://www.linkedin.com/in/yuting-cao-b95b74195/

KEVIN HAUBRICH

Kevin studied Chemistry at the University of Heidelberg and obtained his PhD from the European Molecular Biology Laboratory where he used protein NMR and SAXS to investigate RNA binding of the E3 Ligase TRIM25. Since 2020, he is a Postdoc in the group of Alessio Ciulli where he works on the fragment-based discovery of new E3 ligase binders.

FEATURE OF THE MONTH

| *Dylan & Alex*

Dundee hosts the 4th Annual Meeting of the EUbOPEN Project

Image courtesy of Mark Nakasone

The first week of September saw the **EUbOPEN annual meeting** come to Dundee, hosted by Alex, Kevin, Dylan, and Alessio. This event saw the EUbOPEN consortium members from across Europe (and also the USA!) join CeTPD members in a two-day meeting of chemical probe discussion and exciting new E3 ligase handle development. Kevin presented his **NMR screening work** against the SOCS2 E3 ligase, and Alessio gave an update on **new twists in PROTAC and molecular glue design**. As the project enters its fifth and final year, discussions naturally turned to the legacy of EUbOPEN and how its impact can be measured in the future.

The attendees enjoyed the conference dinner at Discovery Point, with tours of the HMS Discovery and other attractions. Feedback was overwhelmingly positive, and the organising committee would like to thank the admin team for all the excellent support throughout the planning process and during the event.

For more information on the EUbOPEN Project, please visit the website: https://www.eubopen.org/

TARGETED PROTEIN DEGRADATION

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

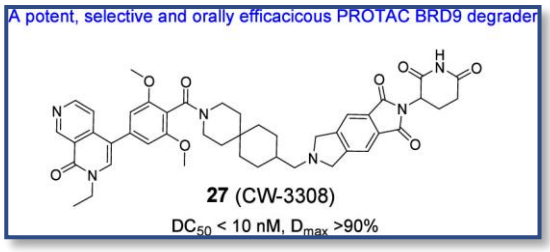
"Every two months, we spotlight the latest and most significant literature in the field of targeted protein degradation, spanning chemistry, biophysics, cell biology, and computational modeling"

Literature review from 21st July to 20th September 2024

| *Suzanne*

Discovery of CW-3308 as a Potent, Selective, and Orally Efficacious PROTAC Degrader of BRD9

Changwei Wang, Mi Wang ….Shaomeng Wang*
J. Med. Chem., ***2024***, *67, 14125−14154*



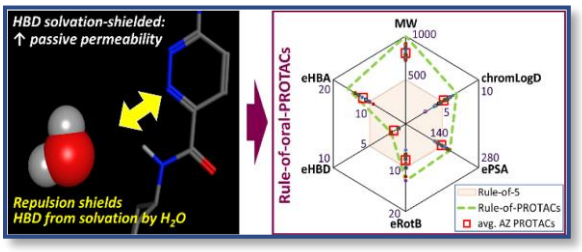
Targeting BRD9 is a promising therapeutic strategy for the treatment of synovial sarcoma and malignant rhabdoid tumours as these cancers are dependent on BRD9 for survival. The authors describe their medicinal chemistry campaign to improve potency and degradation efficiency of BRD9 PROTACs using their previously described CRBN binders, achieving highly effective BRD9 degradation. Highlights included using a CRBN binder with good permeability and no P-gp efflux issue, rigidification of the linker and removal of a basic centre combined with small changes in the BRD9 ligand. Despite poor stability in cell culture media, CW-3308 is more effective than the inhibitor from which it is derived from (BI-7273) in cell growth inhibition and a single oral administration of 50 mg/kg reduced BRD9 protein levels by >90% in the tumour tissue after 3 hours. CC-3308 was well tolerated in mice and successfully achieved tumour growth inhibition in a synovial sarcoma xenograft tumour model.

Shaomeng Wang's group have rationally designed potent BRD9 PROTACs with selectivity over the closely related BRD4 and BRD7 proteins and remarkable oral bioavailability (91%) in mice. Proteomics data showing the selectivity profile and further experiments proving the PROTAC mechanism of action would be necessary for a more complete assessment of the compound. Both clinical BRD9 degraders, FHD609 and CFT8634, have been discontinued in phase 1 clinical trials due to either safety or efficacy concerns, highlighting the need to learn more about this target to reach its clinical potential.

| Suzanne

Structural and Physicochemical Feature of Oral PROTACs

Markus Shade* *et al*
J. Med. Chem, ***2024****, 67 (15), 13106-13116*



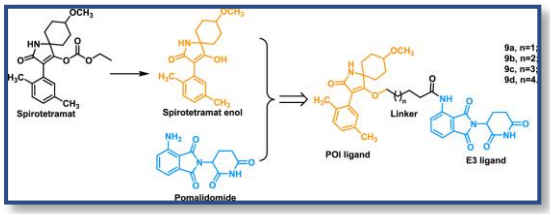
Shade *et al* have suggested a "Rule-of-oral-PROTACs" based on experimental analysis of four orally bioavailable PRO
TACs with known structures in the clinic and thirty in-house PROTACs at AstraZeneca. They highlight the importance of using experimentally measured properties such as ChromLogD and ePSA to measure lipophilicity and polarity, as calculated properties such as cLogP and TPSA can vary significantly from measured values for PROTACs. Importantly, they use proton and Variable Temperature NMR for determining hydrogen bond donor shielding that can occur in apolar environments.
For the best chance of achieving orally bioavailable PROTACs they suggest a strict upper limit of solvent exposed H-bond donors (eHBD) ≤2, ePSA ≤170, eRotB ≤13, MW ≤ 1000, chromLogD ≤7 and eHBA ≤16, with an emphasis on limiting experimentally determined exposed hydrogen bond donors to an absolute maximum of two.

Anyone who has attempted to synthesize orally bioavailable PROTACs can appreciate the challenges associated with their rational design. Hopefully these new guidelines will help the community rationally develop orally bioavailable PROTACs!

| Kevin

Acetyl-CoA Carboxylase Proteolysis-Targeting Chimeras: Conceptual Design and Application as Insecticides

Qi Xu, Hao Feng, Zhong Li and Xusheng Shao*
J. Agric. Food Chem. ***2024****, 72, 34, 18809–18815*



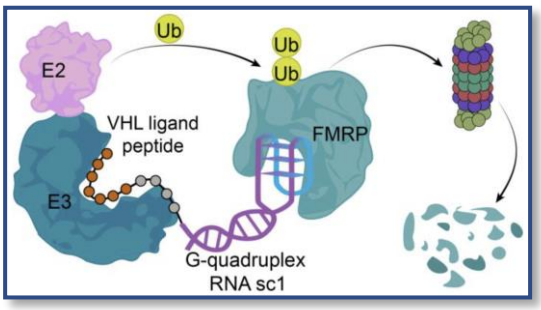
Acetyl-CoA carboxylase (ACC) inhibitors are a staple for both herbicides and insecticides. This paper explores the possibility of using PROTACs against ACC rather than inhibitors as insecticides. PROTACs based on the ACC ligand spirotetramat enol and pomalidomide readily degrade ACC in Sf9 cells with sub-micromolar potency, but show reduced insecticidal performance compared to the parent inhibitor against aphid nymphs. It remains unclear if this is due to reduced bioavailability or other reasons.

PROTAC based insecticides have potential advantages such as higher specific for the target species and lower resistance due to induced PPIs, efficacy at lower concentration and easier biodegradation due to their modular nature. While this paper provides an interesting proof of principle, it suggests no advantage of PROTAC based insecticides over the inhibitors they derive from. Nevertheless, it gives an interesting glimpse into applications of TPD beyond medicine.

| Kevin

G-quadruplex RNA Based PROTAC Enables Targeted Degradation of RNA Binding Protein FMRP for Tumor Immunotherapy

Ruixi Peng, …, Xia Chu
Angew. Chem. Int. Ed. ***2024****, e202402715*



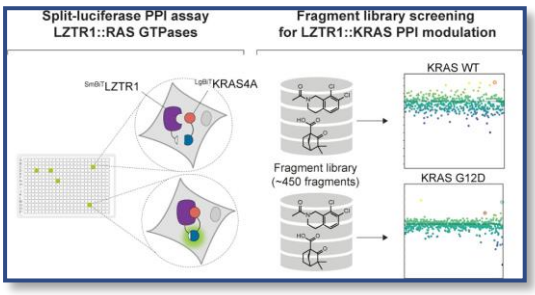
Fragile X mental retardation protein (FMRP) is an RNA-binding protein known to stimulate the expression of immunosuppressive factors such as PROS1, IL-33 and exosomes. There are no small molecule inhibitors and since it relies on four independent RNA binding domains to recognise its target complete inhibition is challenging. In this study the authors combine a G-quadruplex RNA known to bind FMRP with nanomolar affinity and a VHL-recruiting peptide to construct FMRP-degrading PROTACs. Even though the resulting PROTAC showed only moderate degradation in cell culture, after packaging in liposome nanoparticle and intravenous administration it significantly reduced tumour growth in a CT26 mouse model, especially in combination with PD-L1 antibody treatment. This is remarkable as PD-L1 antibody treatment was shown to be ineffective against cold tumours such as CT26. However, degradation of FMRP increases the infiltration of the tumour by lymphocytes and CD8 T cells, thereby rendering them sensitive to immunotherapy.

It is exciting to see how after the success of mRNA vaccines more and more RNA-based therapeutics are explored. Both RNA binding proteins and RNA targets are very difficult to target by small molecules, but the design of targeting RNA sequences is much more straightforward, making such approaches promising if the difficulties in delivery can be overcome.

| Kevin

Identification and Characterization of Novel Small-Molecule Enhancers of the CUL3^{LZTR1} E3 Ligase KRAS Complex

Sophie Piech, …, Johannes W. Bigenzahn and Giulio Superti-Furga**
ACS Chem. Biol. ***2024****, 19, 1942−195*



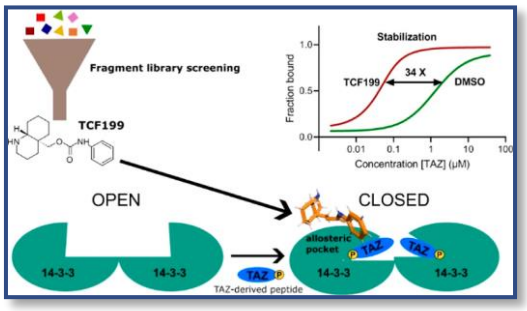
CUL3^{LZTR1} is an E3 Ligase interacting with KRAS and controlling its expression levels through proteasomal degradation. This paper describes the fragment-based discovery of glues that enhance this interaction using Split-Luciferase-Based Assays. BioID shows increased co-purification of KRAS and LZTR1 after treatment with this glue. Interestingly, thermal stabilisation assays indicate that the compound stabilises KRAS, but not LZTR1 in cell. Protein NMR allowed to map the binding site and chemical shift perturbations were used as restrains for docking of the compound into a KRAS structure.

The idea of identifying weak intrinsic POI/E3 ligase interactions to use as a starting point for the rational design of molecular glues has caused considerable excitement lately. This is one of the first example where this approach was successful. Unfortunately, while a nice proof of principle, the paper doesn't address if stabilisation of the KRAS/LZTR1 complex causes degradation of KRAS and therefore has therapeutic potential.

| *Kentaro*

Fragment-Based Interrogation of the 14–3–3/TAZ Protein–Protein Interaction

Blaž AndlovicDario, …, Mattia Mori* Isabelle Landrieu*, Dimitrios Tzalis*, Jan Eickhoff*, and Christian Ottmann*
Biochemistry, **2024**, 63, 2196−2206



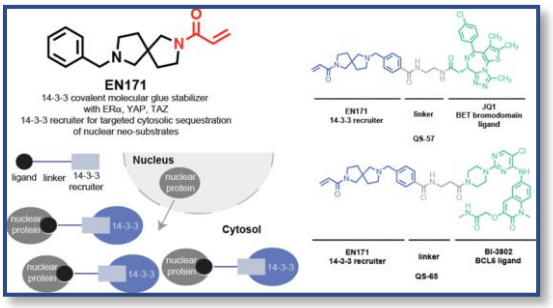
14-3-3 is known as an adaptor protein that interacts with many proteins. In this paper, the authors focused on TAZ, which regulates cellular proliferation, and identified a compound (TCF-199) that stabilizes the complex with 14-3-3ζ through fragment screening. The interaction of the obtained compound was analysed using MD simulations, NMR, and X-ray crystallography, revealing that it binds to an allosteric site distinct from the TAZ binding region. Based on these analyses, the authors proposed a mechanism that the compound stabilises the 14-3-3ζ/TAZ complex by reducing the flexibility of alpha helix 9.

It is interesting that the compounds bind to an allosteric site rather than the ligand-binding pocket. At the same time, when it comes to improving the compounds, further strategies will likely be necessary due to the shallow nature of the binding site. Additionally, as the authors mentioned, the compounds also stabilise complexes with proteins other than TAZ, meaning further innovations will be required if a specific protein is to be targeted. But it would be interesting if the activity could be enhanced through combination with other molecular glues such as Fusicoccin, as the binding region is different.

| *Kentaro*

Targeted Protein Localization by Covalent 14–3–3 Recruitment

Qian Shao, Tuong Nghi Duong, Inji Park, Lauren M. Orr, and Daniel K. Nomura*
J. Am. Chem. Soc. ***2024****, 146, 24788−24799*



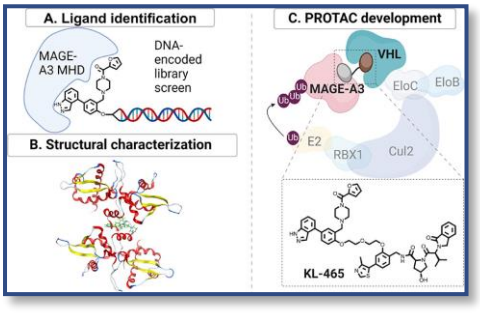
The authors performed covalent ligand screening and identified the molecular glue EN171, which stabilizes the complex between 14-3-3σ and phosphorylated ERα peptide. EN171 was found to stabilize not only ERα but also complexes with YAP, TAZ, c-Myc, and CDC25B peptides, but it did not affect complexes with USP8, p65, c-RAF, TP53, SOS1, or ABL1. MS/MS analysis revealed that EN171 binds to both Cys38 and Cys96 of 14-3-3σ. Interestingly, while EN171 binds to either the C38S or C96S mutant, each of these mutants showed significantly reduced ability to stabilise complexes with ERα and TAZ. The authors confirmed that EN171 inhibits the transcriptional activities of ERα and Hippo. FLAG pull-down assays demonstrated the formation of a ternary complex between 14-3-3σ, ERα, and EN171. Additionally, EN171 was utilized as a 14-3-3σ recruiter to synthesize heterobifunctional compounds targeting BRD4 and BCL6, which resulted in the cytoplasmic accumulation of BRD4 and BCL6.

The authors have discovered a new covalent type of molecular glue EN171 for 14-3-3. EN171 also acts as a warhead of a heterobifunctional binder, enabling it to form complexes with proteins that are not its intrinsic substrates. It would be interesting if this compound could be further developed to achieve target protein selectivity in the form of a molecular glue in the future.

| *Yuting*

Development of Ligands and Degraders Targeting MAGE-A3

Ke Li§, Mackenzie W. Krone§, Arseniy Butrin§, … Craig M. Crews*
J. Am. Chem. Soc. ***2024****, 146, 36, 24884–24891*



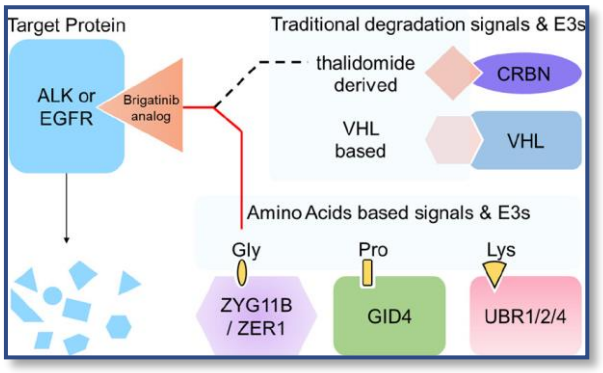
MAGE-A3 stands out as promising target for PROTAC development because it serves as a substrate recruitment module for an E3 ligase such as tripartite motif containing 28 (TRIM28) and is selectively expressed in numerous tumour types. In this paper, the authors developed novel, small-molecule ligands for MAGE-A3 through DNA-encoded library (DEL) screening, ITC experiment revealed the ligands bind to MAGE-A3 at 1:2 stochiometric ratio. Further structural characterization suggested the ligands bind at dimer surface. They then utilized this ligand to develop VHL based PROTAC molecules that induce MAGE-A3 degradation and inhibit the proliferation of MAGE-A3 positive cell lines. These ligands and degraders may serve as valuable probes for investigating MAGE-A3 biology and as foundations for the ongoing development of tumour-specific PROTACs.

Tumour specific degraders are very promising strategies, and it is interesting to see how ligand discovery with DEL screening can be utilized into TPD workflows.

| Yuting

Distinct Amino Acid-Based PROTACs Targeted Oncogenic Kinases for Degradation in Non-Small Cell lung Cancer (NSCLC)

Jianchao Zhang§, Xiao Chen§, Congli Chen§ … Lijing Fang*, Hai Rao*
J. Med. Chem. ***2024***, *67, 13666-13680*



Only a few out of 600 E3 ligases have been adapted in TPD field, of which VHL and cereblon are the most widely used. However, there are some limitations, such as lack of cell- or tissue-specialty and poor bioavailability. The authors previously designed the PROTACs by leveraging N-end rule pathway. In this paper, they devised novel AATacs which utilize either Pro or Gly as E3 ligands connecting to a Brigatinib analogue by a PEG linker, recruiting GID, $CRL^{ZYG11B/ZER1}$ to close to EML4-ALK and EGFR mutant, and demonstrated their degradation potency in H3122 and H1975 cells. They further found that the protein reduction can be fine-tuned by using different degradation signals. Following experiments also showed that these AATacs hindered proliferation and induced cell cycle arrest and apoptosis of NSCLC cells in vitro.

Their work expanded the degradation toolbox and brought unique feature to TPD field. It might have the potential such as improving permeability and bioavailability due to the smaller size of Pro and Gly.

PRE-PRINTS

| *Suzanne*

VIPER-TACs leverage viral E3 ligases for disease-specific targeted protein degradation

*Kyle Mangano Patrick... Ryan Potts**

A nice paper from Amgen scientists introducing a proof of concept study on VIPER-TACs (Viral E3 Pan-Essential Removing Targeting Chimeras) which are an Interesting way of achieving tissue selectivity using viral E3 ubiquitin ligases. Because VIPER-tacs do not induce degradation in healthy cells they have the potential to target pan-essential proteins to treat viral infections or cancers without causing toxicity.

| *Suzanne*

Leveraging dual-ligase recruitment to enhance degradation via a heterotrivalent PROTAC

*Adam G. Bond… Alessio Ciulli**

The authors sought to boost PROTAC degradation by recruiting two E3 ligases with a single degrader molecule and have designed heterotrivalent PROTACs composed of a CRBN, VHL and BET targeting ligand, separately tethered via a branched trifunctional linker. Interesting that degradation was found to occur via both VHL and CRBN in an additive fashion!

| *Yuting*

Genesis and regulation of C-terminal cyclic imides from protein damage

*Wenqing Xu... Christina M. Woo**

The authors utilized proteomic, computational, and chemical biology approaches to characterize the genesis and regulation of C-terminal cyclic imide modifications from protein damage. This paper provides insight to the regions of the proteome that are prone to these unexpectedly frequent modifications, the effects of this form of protein damage on protein stability, and the biological role of CRBN.

| *Aitana*

Unveiling the hidden interactome of CRBN molecular glues with chemoproteomics

Kheewoong Baek§, Rebecca J. Metivier§... Katherine A. Donovan, Eric S. Fisher**

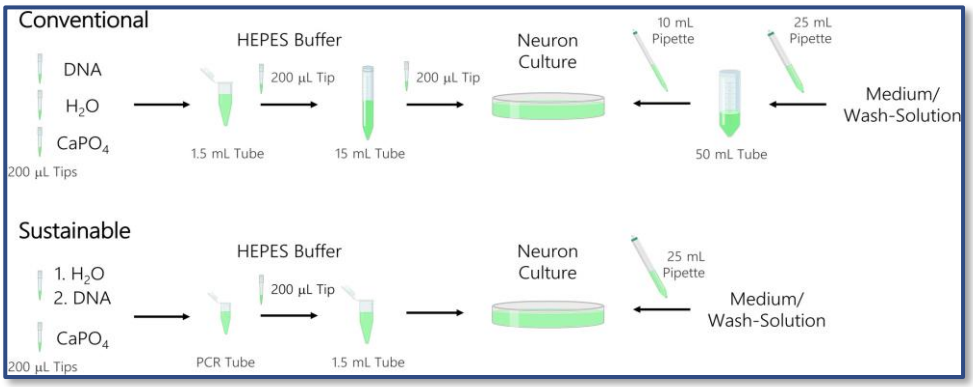
This preprint introduces a new workflow for the identification of molecular glue targets, based on immunopurification and mass spectrometry in cell lysates. The authors showcase this novel methodology, highly sensitive and high throughput, and they identify 298 CRBN-binding molecular glues (MG). It will be very interesting to see this workflow applied for the screening of MGs beyond this system.

OTHER PAPER HIGHLIGHTS

| *Manon*

Reducing plastic waste in scientific protocols by 65% — practical steps for sustainable research

Patrick Penndorf
FEBS Letters, 598, ***2024****, 1331-1334*



This interesting paper highlights how we can change our protocol to reduce day-to-day plastic waste in the lab.
The majority of laboratory waste is not recycled but sent to landfill or incinerated, and is needed to be autoclaved, increasing the laboratory's carbon footprint. Moreover, there is a lack of education when it comes to sustainable lab practice, leading to a high usage of single use plastics. To adopt a more sustainable approach, it is important to implement changes in the experimental procedure. The authors illustrate this by applying three fundamental principles (reduce, reuse, miniaturise) to a protocol under sterile conditions.

Reduction consists of using consumables if only absolutely required by preparing reagents by mixing in one container rather than separate ones or using glassware when possible.

Reusing whenever contamination is avoidable by optimising the use of pipette tips.

Miniaturisation is using the smallest possible size of consumable according to the need.

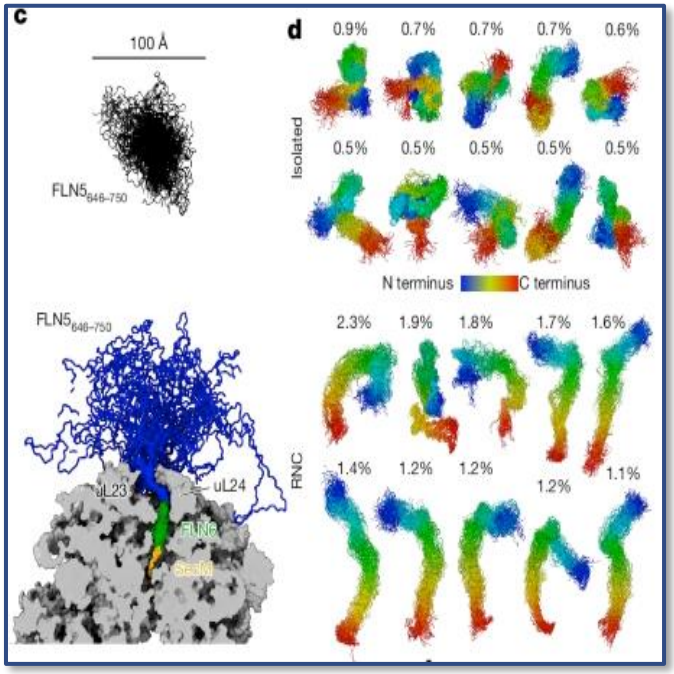
This sustainable approach has been applied to a neuron transfection protocol. Pipette tips have been reused by using first water then DNA (reuse, 33% reduction) rather than two different ones. The dilution was done in PCR microtubes instead of 1.5 mL tubes (miniaturisation, 52% reduction).15 mL tubes were replaced by 1.5 mL for combining solutions with buffer (miniaturisation, 84% reduction). Finally, the suppressant and wash solution were directly mixed avoiding the use of 50 mL tube (reduction, 12.8g less). Overall, simple approach helped reduce 65% of plastic waste.

Sustainability is a hot topic and the number of papers on this subject are increasing. Having a concrete example helps the reader to project and consider it for their own experiment. Scientists have the power to become more sustainable with limited effort.

| *Kevin*

The ribosome lowers the entropic penalty of protein folding

Julian O. Streit, Ivana V. Bukvin, Sammy H. S. Chan, …, Anaïs M. E. Cassaignau & John Christodoulou
Nature, ***2024***, *633, 232–239*



Protein folding natively occurs mostly co-translational on the Ribosome, but the exact role of the ribosome in this process remains unclear. Streit *et al.* use paramagnetic relaxation enhancement (PRE-) NMR and molecular dynamics simulations to model the unfolded state of model-proteins like FLN5 both in isolation and as nascent chain on the ribosome and explore their energy landscape. They found that the structural ensemble is more expanded on the ribosome and show that this is due to a destabilisation of the unfolded state on the ribosome. This destabilisation is due to increased solvation, steric exclusion and tethering rather than direct interaction of the nascent chain with the ribosome. In addition, this work shows that ribosomal tethering acts as a buffer that alleviates the effects of destabilising mutations, with the ribosome thereby acting as a universal foldase.

This seminal paper experimentally determines the basis of co-translational folding, largely confirming earlier theoretical work. It is also an extremely impressive demonstration of the power of NMR to gain structural information into heterogeneous, dynamic systems and extract biophysical parameters nearly impossible to obtain from any other technique.

Centre for Targeted Protein Degradation
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Centre for Targeted Protein Degradation
School of Life Sciences
1 James Lindsay Place,
Dundee,
DD1 5JJ

Journal club – website

@alessiociulli @Farnaby84 @FarnabyLab @UoDCeTPD @DeLaCuadraBaste @Holmqvist89