Biannual report

March 2018 – December 2018

PI Name: Stephen Cusack

Global summary to explain your project(s):

Cusack: Structural analysis of protein-RNA complexes. (1) polymerases of segmented negative RNA viruses (influenza, La Crosse, salmon anaemia) and their interacting host factors. (2) Pattern recognition receptors of the innate immune system (RIG-I like helicases, NOD proteins), their signalling pathways (downstream adapters like RIP2 and ubiquitin ligases like XIAP, TRIM25) and viral antagonists. (3) Proteins involved in sorting of Pol II transcripts (CBC, ARS2, PHAX, NELF, nCBP3).

Kowalinski: Structural analysis of protein-RNA complexes in the context of RNA modifications. (1) Compontents of the trypanosoma brucei RNA editing complex, (2) human and trypanosoma brucei RNA methyltransferases (3) RNA and DNA deaminases of the APOBEC family.

Marcia: We study the molecular interactions between long non-coding RNAs (lncRNAs) and epigenetic transcription regulator proteins. Our research aims to identify the recognition motifs that guide formation of tight complexes between lncRNAs and chromatin remodelling enzymes, determine the structures of such ribonucleoproteins, and, building on structural insights, understand the molecular mechanism by which lncRNAs exert their cellular functions.

McCarthy: We study a range of different proteins involved in neuronal development, RNA methyl transferase, lysophospholipase activities, MAP Kinase signalling networks and their control by parasites such as *Toxoplasma gondii* (GRA24), and phosphoryl transfer proteins involved in metabolism (βPGM). In addition we develop a number of methods for optimised data collection and analysis of MX and SAXS experiments using standard proteins (Lysozyme, trypsin etc).

Marquez: We develop new approaches in macromolecular crystallography, including the CrystalDirect technology for automated crystal mounting soaking and cryo-cooling. We apply these technologies to study proteins involved in biotechnologically relevant processes, as well as to the development of inhibitors through X-ray based small-molecule screening including i) development of inhibitors against the Human Pirin redox sensor involved in the regulation of cancer cell metabolism i) Characterization of PDE-delta small molecule inhibitors as potential modulator of k-Ras signaling

Jamin: Structural characterization of proteins and protein-protein complexes involved in viral replication and in virus host interactions. (1) Transcription/replication machines of Rhabdoviridae and Paramyxoviridae. (2) Structure and function of non-structural proteins. (3) Interference with essential protein-protein interactions.

Palencia: We study novel drug targets involved in infectious human diseases. A major focus of our research is leucyl-tRNA synthetases (LeuRS) from microbial pathogens; and proteins involved in cleavage and polyadenylation of pre-mRNAs such as CPSF3 and polyadenosine polymerase (PAP).

Burmeister: We work on the DNA polymerase holoenzyme of vaccinia virus composed of E9, A20 and D4. One objective is the analysis of complex-disrupting inhibitors (small molecules and optimized peptides) in complex with D4. A side project was the structure of the complex between adenovirus Ad3 fibre heads and the desmoglein receptor.

Crépin: Structural analysis of protein- and RNA-protein complexes involved in viral replication. (1) nucleoproteins, (2) human splicing RED-SMU1 complex and (3) nuclear cargos involved in influenza replication, (4) proteins that form the replication machinery of Borna disease virus.

Results obtained since the last biannual review:

- several crystal structures of influenza B with capped RNA primer and incoming NTPs with
- several crystal structures of RIP2 kinase with inhibitors
- Crystal structures of Autotaxin in complex with several novel inhibitors.
- Structure of a variant of the vesicular stomatitis virus phosphoprotein.
- Structure of the complex between human dynein light chain 8 and the rabies virus phosphoprotein.
- Structure of full-length Nipah virus phosphoprotein
- Crystal structures of *Cryptosporidium hominii* PAP.
- Crystal structures of Ecoli LeuRS mutants in complex with cognate tRNA-leu.
- Crystal structure of Ecoli LeuRS in complex with tRNA and post-transfer editing analogue of norvaline.
- Phased and solved a structure of a component of the trypanosoma brucei RNA editing complex at ID30B by native SAD.
- SAXS structure of Nuc
- SAXS structures of MEG3
- SAXS structures of C1CAN in Nuc-free forms
- SAXS structure of RNA-bound SCML
- SAXS structures of C1VAR in Nuc-bound forms
- crystal structures of C1VAR and C1CAN
- crystal structures of MEG3
- Structure of the adenovirus Ad3 fibre head/desmoglein complex.
- Crystal structures of the oligomerization domain of Borna disease virus phosphoprotein
- Multiple Crystal structures of Pirin in complex with different inhibitors.
- 5 structures of SerPIN proteins from gut microbiota.
- Multiple structures of PDEdelta in complex with small molecule compounds
- Developed a method based on the CrystalDirect technology for the analysis of membrane protein crystals grown in LCP through Serial synchrotron Crystallography (SSX)
-Crystal structure of DH domain of SpNOX

Structure still in progress since the last biannual review:

- New crystal form of influenza A polymerase with capped RNA primer diffracting to 1.95Å resolution
- Crystal structure of P38 in complex with inhibitors and parasite derived peptide fragments.
- Crystal structure of ERK1 in complex with parasite derived peptide fragments.
- Crystal structure of JNKa1 in complex with parasite derived peptide fragments.
- structure of the nucleoprotein-phosphoprotein complex of rabies virus
- structure of the C protein of tupaia virus
- structures of X domain of Nipah virus
- Crystal structures of PAP in complex with small molecule compounds.
- Structure of a monomeric form of vaccinia virus D4
- structure of the nucleoprotein of Wuhan Asiatic toad-Influenza virus.
- Structure of full-length SpNOX

Papers over the past 2 years(s):

Numbers: **23**

List:

1. Structural snapshots of actively transcribing influenza polymerase. Kouba T, Drncová P, Cusack S. Nat Struct Mol Biol. 2019;26(6):460-470. PMID:31160782
2. RIP2 filament formation is required for NOD2 dependent NF-κB signalling. Pellegrini E, Desfosses A, Wallmann A, Schulze WM, Rehbein K, Mas P, Signor L, Gaudon S, Zenkeviciute G, Hons M, Malet H, Gutsche I, Sachse C, Schoehn G, Oschkinat H, Cusack S. Nat Commun. 2018, 9(1):4043. PMID:30279485
3. Destabilisation of the human RED-SMU1 splicing complex as a basis for host-directed anit-influenza therapy. Ashraf, U., Tengo, L., Le Corre, L. Fournier, G., Busca, P., McCarthy, A. A., Rameix-Welti, M-A., Gravier-Pelletier, C., Ruigrok, R. W. H., Jacob, Y., Vidalain, P-O., Pietrancosta, N., Crépin, T. Naffakh, N. *Proc*. *Natl*. *Acad*. *Sci*. USA 2019 116, 10968-10977. doi: 10.1073/pnas.1901214116.
4. Structural Principles in Robo Activation and Auto-Inhibitions. Barak, R., Yom-Tov, G., Guez-Haddad, J., Gasri-Plotnitsky, L., Maimon, R., Cohen-Berkman, M., McCarthy, A. A., Perlson, E., Henis-Korenblit, S., Isupov, M. N., Opatowsky, Y. *Cell* 2019 177, 272-285. doi: 10.1016/j.cell.2019.02.004.
5. Structural basis for the activation of the deubiquitinase Calypso by the Polycomb protein ASX. De, I., Chittock, E. C., Grötsch, H., Miller, T. C. R., McCarthy, A. A., Müller, C. W. *Structure* 2019 27, 528-536. doi: 10.1016/j.str.2018.11.013.
6. Controlled dehydration, structural flexibility, and Gadolinium MRI contrast compound binding in human plasma glycoprotein afamin. Naschberger, A., Juyoux, P., von Velsen, J., Rupp, B. and Bowler, M. W. *Acta Cryst*. 2019 D75, 1071–1083. doi: 10.1107/S2059798319013500.
7. Fully Autonomous Characterization and Data Collection from Crystals of Biological Macromolecules. Hutin, S., Van Laer, B., Mueller-Dieckmann, C., Leonard, G., Nurizzo, D., Bowler, M. W. *J. Vis. Exp.* 2019 145, e59032, doi:10.3791/59032
8. Calibration of rotation axes for multi-axis goniometers in macromolecular crystallography. White, K. I., Bugris, V., McCarthy, A. A., Ravelli, R. B. G., Csankó, Cassetta, A., Brockhauser, S. *J*. *Appl*. *Cryst*. 2018 51, 1421-1427. doi: 10.1107/S1600576718010956.
9. Methylation of structured RNA by the m^6A writer METTL16 is essential for mouse embryonic development. Mendel, M., Chen, K-M., Homolka, D., Gos, P., Pandey, R. R., McCarthy, A. A., Pillai, R. S. *Mol*. *Cell* 2018 71, 986-1000. doi: 10.1016/j.molcel.2018.08.004.
10. ID30B – A versatile beamline for macromolecular crystallography experiments at the ESRF McCarthy, A. A., Barrett, R., Beteva, A., Caserotto, H., Dobias, F., Felisaz, F., Giraud, T., Guijarro, M., Janocha, R., Khadrouche, A., Lentini, M., Leonard, G. A., Lopez Marrero, M., Malbet-Monaco, S., McSweeney, S., Nurizzo, D., Papp, G., Rossi, C., Sinoir, J., Sorez, C., Surr, J., Svensson, O., Zander, U., Cipriani, F., Theveneau, P. Mueller-Dieckmann, C *J*. *Synchrotron Radiat*. 2018 25, 1249-1260. doi: 10.1107/S1600577518007166.

11. Structural basis for the bi-functionality of human oxaloacetate decarboxylase FAHD1. Weiss AKH, Naschberger A, Loeffler JR, Gstach H, Bowler M. W, Holzknecht M, Cappuccio E, Pittl A, Etemad S, Dunzendorfer-Matt T, Scheffzek K, Liedl KR, Jansen-Dürr P. *Biochem. J.* 2018 475, 3561-3576. doi: 10.1042/BCJ20180750.
12. Structural basis for reactivating the mutant TERT promoter by cooperative binding of p52 and ETS1. Xu, X., Li, Y., Bharath, S.R., Ozturk, M.B., Bowler, M. W., Loo, B.Z.L., Tergaonkar, V. and Song, H. *Nature Commun* 2018 9 doi:10.1038/s41467-018-05644-0
13. The LC8-RavP ensemble Structure Evinces A Role for LC8 in Regulating Lyssavirus Polymerase Functionality. Jespersen NE, Leyrat C, Gérard FC, Bourhis JM, Blondel D, Jamin M, Barbar E. (2019) J Mol Biol. 431, 4959-4977. doi: 10.1016/j.jmb.2019.10.011
14. Vesicular stomatitis virus phosphoprotein dimerization domain is dispensable for virus growth. Gérard FC, Jamin M, Blackledge M, Blondel D, Bourhis JM. (2019) J Virol. In press doi: 10.1128/JVI.01789-19
15. Structural description of Nipah virus phosphoprotein and its interaction with STAT1. Jensen MR, Yabukarski F, Communie , Condamine E, Mas C. Volchkova V, Tarbouriech N, Bourhis JM, Volchkov V, Blackledge M and Jamin M (2020) Biophys. J. In press
16. *Kinetic origin of substrate specificity in post-transfer editing by leucyl-tRNA synthetase. Dulic, M., Cvetesic N., Zivkovic I.,Palencia, A., Cusack, S., Bertosa, B. & Gruic-Sovulj, I. 2018. Journal of Mol. Biol. 17:30517. PMID: 29111343*
17. *Molecular mechanism of influenza A NS1-mediated TRIM25 recognition and inhibition Koliopoulos MG, Lethier M, van der Veen AG, Haubrich K, Hennig J, Kowalinski E, Stevens RV, Martin SR, Reis E Sousa C, Cusack S, Rittinger K. Nat Commun. 2018 May 8;9(1):1820. doi: 10.1038/s41467-018-04214-8. PMID:29739942.*
18. Topology and enzymatic properties of a canonical Polycomb repressive complex 1 isoform Colombo M, Pessey O, Marcia M. *Febs Letters*. 2019. 593:1837-1848. doi: 10.1002/1873-3468.13442. PMID: 31093962.
19. Conserved Pseudoknots in lncRNA MEG3 Are Essential for Stimulation of the p53 Pathway. Uroda T, Anastasakou E, Rossi A, Teulon JM, Pellequer JL, Annibale P, Pessey O, Inga A, Chillon I, Marcia M. *Mol Cell. 2019* 75:982-995 e989. doi: 10.1016/j.molcel.2019.07.025. PMID: 31444106.
20. *Intermediate-resolution crystal structure of the human adenovirus B serotype 3 fibre knob in complex with the EC2-EC3 fragment of desmoglein 2. Vassal-Stermann, E., Hutin S., Fender, P., Burmeister, W. P. Acta Crystallogr F Struct Biol Commun. 75, 750-757. doi: 10.1107/S2053230X19015784 (2019).*
21. Principles and methods used to grow and optimize crystals of protein-metallodrug adducts, to determine metal binding sites and to assign metal ligands. Russo Krauss I, Ferraro G, Pica A, Márquez JA, Helliwell JR, Merlino A. (2017). Metallomics 9(11) doi: 10.1039/c7mt00219j
22. Destabilisation of the human RED-SMU1 splicing complex as a basis for host-directed anti-influenza strategy. Ashraf U, Tengo L, Le Corre L, Fournier G, Busca P, McCarthy AA, Rameix-Welti M-A, Gravier-Pelletier C, Ruigrok RW, Jacob Y, Vidalain P-O, Pietrancosta N, Crépin T, Naffakh N. Proc Natl Acad Sci USA. 2019, 116:10968-10977. doi: 10.1073/pnas.1901214116.PMID: 31076555
23. The structure of the nucleoprotein of Influenza D shows that all Orthomyxoviridae nucleoproteins have a similar NP_{CORE}, with or without a NP_{TAIL} for nuclear transport. Donchet A, Oliva J, Labaronne A, Tengo L, Miloudi M, Gérard CA, Mas C, Schoehn G, Ruigrok RW, Ducatez M, Crépin T. Sci Rep. 2019, 9:600. doi: 10.1038/s41598-018-37306-y. PMID: 30679709

PDB over the past 2 years(s)

Numbers: 27

List:
6GFJ, 6QCX, 6QCW, 6QCV, 6GFK, 6GFN, 6GT5, 6FID, 6FJ2, 6FJ4, 6FJ6, 6FJ8, 6FJ9, 6RQ7, 6FAK, 6FOH, 6FOG, 5ZMC, 4GJW,5ONH, 50MW, 5ON3, 5ON2, 6SIT, 6Q8F, 6Q8I, 6Q8J

Beamline performance (your opinion on beamlines and/or the improvements you will need):

The beamlines performed extremely well. We have obtained excellent results by using the crystal direct technology to prepare crystals for diffraction experiments at ESRF. We recommend CrystalDirect technology to be introduced as a constitutive component of the beamlines. ID30B was used for native phasing (S-SAD) it would be nice to establish a ready-to-run protocol for this. We are extremely grateful for the generous support of ESRF and EMBL colleagues.

RESEARCH Highlights

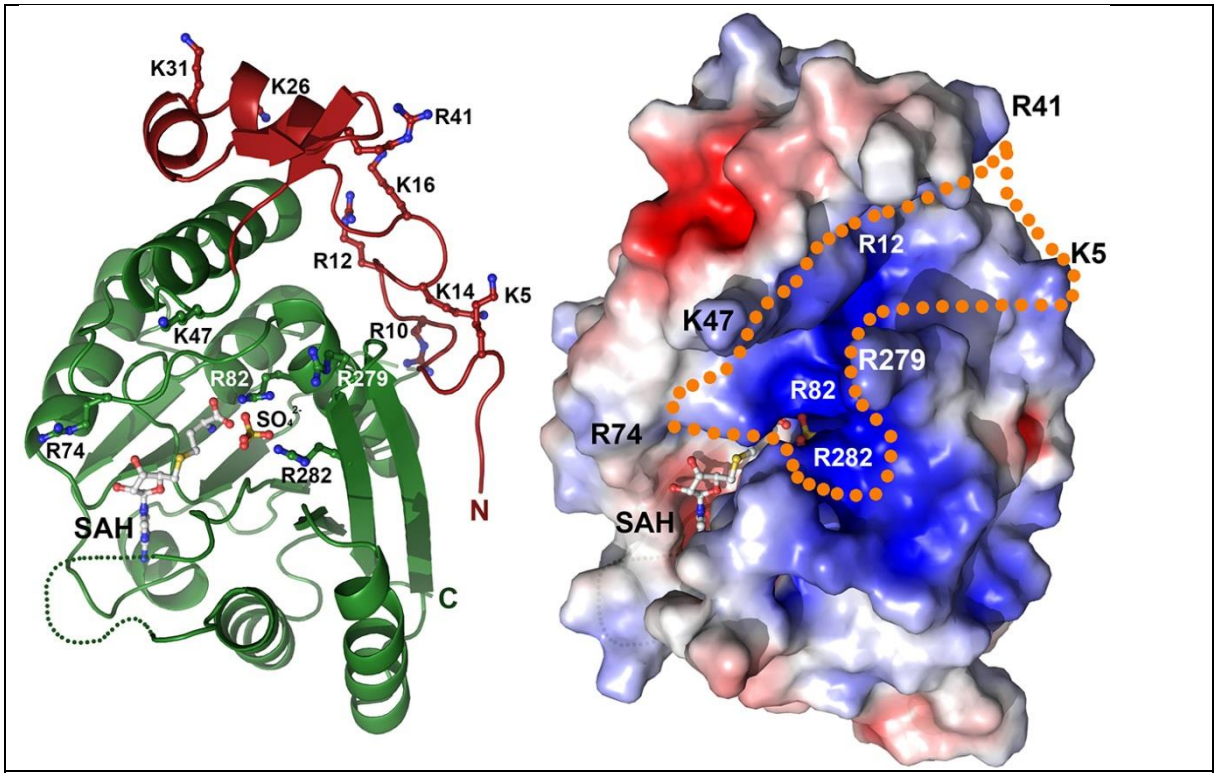
See research highlights below

RESEARCH Highlights

Crystal structure of METTL16, an RNA m^{6}A writer that is essential for mouse embryonic development

Mendel, M., Chen, K-M., Homolka, D., Gos, P., Pandey, R. R., McCarthy, A. A., Pillai, R. S. Methylation of structured RNA by the m^{6}A writer METTL16 is essential for mouse embryonic development. (2018) *Mol*. *Cell* **71**, 986-1000.

RNA *N*^{6}-methyladenosine (m^{6}A) modifications are highly conserved and widely used for gene expression control. While the METTL3/METTL14 heterodimer adds this mark to thousands of single-stranded transcripts, the substrate requirements and exact physiological roles of the second m^{6}A writer METTL16 remains elusive. Here we present the crystal structure of human METTL16 at 2.5 Å resolution. The structure reveals a SAM methyltransferase domain furnished by an additional N-terminal module, which we show is essential for RNA binding (Fig. 1). Together these two domains form a deep, continuous and electropositive groove that is required for RNA binding, as confirmed by mutagenesis and methylation assays. Furthermore, METTL16, when presented with a random pool of RNAs, only selects structured RNAs for methylation that contain a critical adenosine in a bulge region. Lastly, mouse 16-cell embryos (E3.5 blastocyte) lacking *Mettl16* display reduced mRNA levels of its methylation target, the SAM synthetase *Mat2a*. The consequence of this is a massive transcriptome dysregulation in 64-cell blastocysts that are unfit for further development. These results highlight the crucial role of the METTL16 m^{6}A RNA methyltransferase in early development through regulation of SAM availability



METTL16 core MTase domain (left) and positively charged RNA-binding groove (right).

Conserved Pseudoknots in lncRNA MEG3 Are Essential for Stimulation of the p53 Pathway.
Authors: Uroda T, Anastasakou E, Rossi A, Teulon JM, Pellequer JL, Annibale P, Pessey O, Inga A, Chillon I, Marcia M.
Published in: *Mol Cell. 2019* 75:982-995 e989. doi: 10.1016/j.molcel.2019.07.025. PMID: 31444106.

Our group – in collaboration with the IBS platform for atomic force microscopy led by Jean-Luc Pellequer and with the support of ESRF BioSAXS beamline BM29 and other PSB facilities – discovered unexpected molecular properties in a long non-coding RNA (lncRNA) molecule that acts as a tumor suppressor in human brain and endocrine glands, called MEG3. MEG3 potentiates protein p53, a key transcription factor controlling cell proliferation, whose role is to arrest the growth of unhealthy cells before they degenerate into cancerous tissues. Using an original approach – crucially fostered by the PSB environment – we have integrated biochemistry, cell biology and single-particle atomic force microscopy imaging to identify specific MEG3 building blocks essential for p53 stimulation and for the control of cell proliferation. Interestingly, these functional building blocks comprise RNA hairpins connected by so-called "kissing loops", which are three-dimensional motifs characteristic of highly-structured RNAs. The researchers demonstrated that disruption of these kissing loops resulted in concomitant disruption of both the overall 3D structure and the tumor suppression function of MEG3 (**Figure 1**). Their findings prove experimentally what had so far remained a speculative molecular mechanism assigned to lncRNAs: tertiary structure motifs can guide lncRNA function.

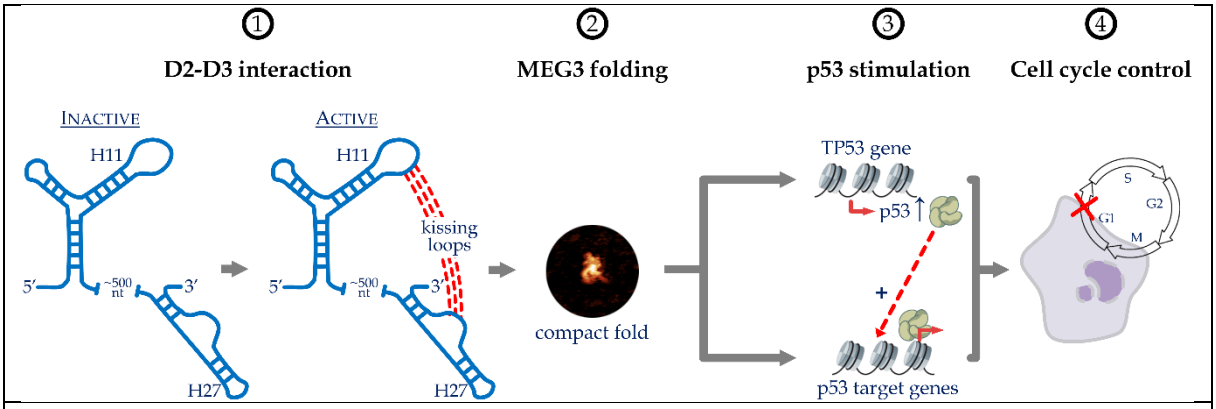


Figure Legend: Structure-functional relationships in human lncRNA MEG3. Formation of an intramolecular "kissing loop" interaction (1) is necessary for MEG3 folding into a compact, globular structure (2), which is responsible for stimulation of p53 and p53 target genes (3) and ultimately for inducing cell cycle arrest or apoptosis (4).

Destabilization of the human RED–SMU1 splicing complex as a basis for host-directed antiinfluenza strategy

Authors: Ashraf U, Tengo L, Le Corre L, Fournier G, Busca P, McCarthy AA, Rameix-Welti M-A, Gravier-Pelletier C, Ruigrok RW, Jacob Y, Vidalain P-O, Pietrancosta N, Crépin T, Naffakh N.

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New therapeutic strategies targeting influenza are actively sought due to limitations in current drugs available. Host-directed therapy is an emerging concept to target host functions involved in pathogen life cycle and/or pathogenesis, rather than pathogen components themselves. Therefore, scientists form the IBS and EMBL, in collaboration with colleagues from Institut Pasteur and the Universities Paris Diderot and Paris Descartes, focused on an essential host partner of influenza viruses, the RED–SMU1 splicing complex. Combining structural biology and molecular dynamics, they identified two synthetic molecules targeting an interface essential for RED–SMU1 complex assembly. They solved the structure of the SMU1 N-terminal domain in complex with RED or bound to one inhibitor identified to disrupt this complex. They show that these compounds also decrease endogenous RED-SMU1 levels and inhibit viral mRNA splicing and viral multiplication, while preserving cell viability. Overall, their data demonstrate the potential of RED-SMU1 destabilizing molecules as an antiviral therapy that could be active against a wide range of influenza viruses and be less prone to drug resistance.

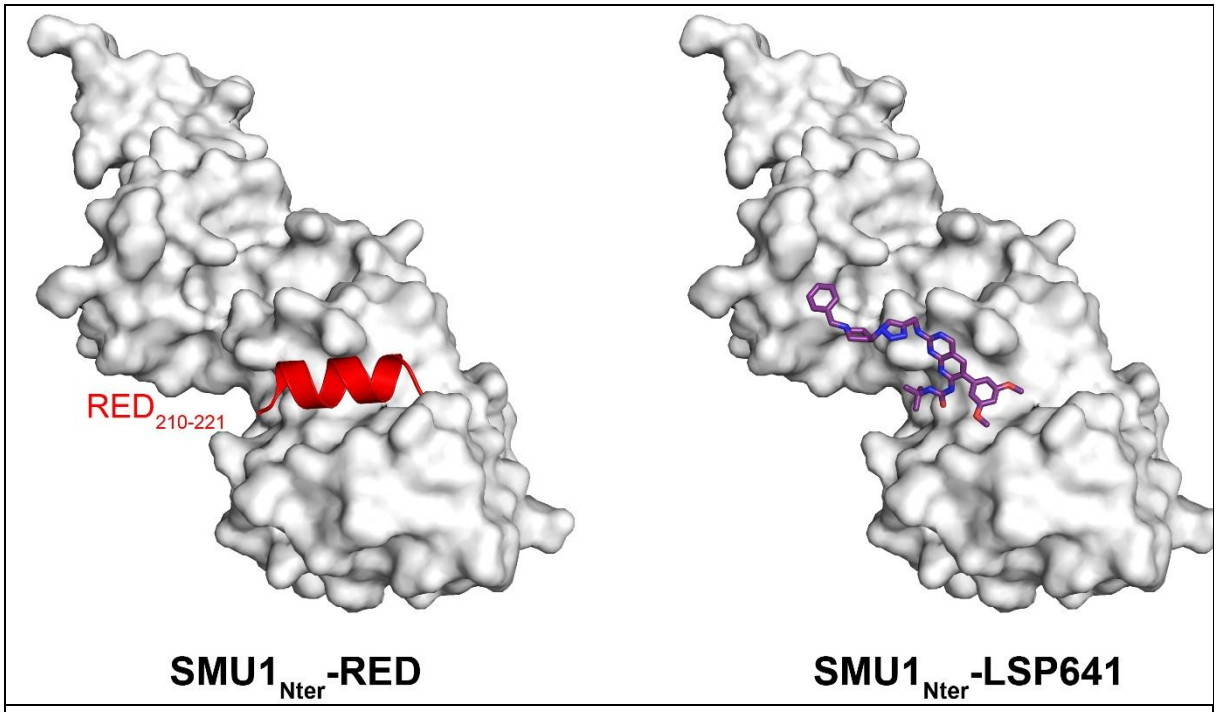

Figure legend: LSP641 occupies the same groove of SMU1 than RED.