Probing the allosteric states of the lactose repressor protein

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Lac repressor (LacR) is a textbook example for understanding genetic control and allostery, tracking back to the pioneering work of Jacob, Monod and Changeux and presents one of the first described heterotropic allosteric regulators[1,2]. In spite of the fact, that the LacR allosteric regulation has been applied to a variety of cellular signalling pathways in all organisms and used as a model system for experimental and theoretical understanding of the allosteric mechanisms[3], the knowledge of structures at a detailed atomic level of all its dynamic allosteric states are still poorly described. Crystal structures exist only for a few allosteric states of LacR, insufficient for understanding all regulatory events.

In order to compliment our understanding of heterotropic allostery, not only of LacR but also in general, we studied this repressor as a dimer alone and in presence of ligands using high-resolution NMR in combination with SAXS and ligand binding studies of NMR active LacR, labelled traditionally with $^{2}H^{13}C^{15}N$ and also with $^{1}H^{13}C$–methyl-labeled Ile, Leu and Val. Additionally, in order to study the changes in structure and the dynamics of LacR upon ligand binding with NMR, we tagged LacR with lanthanide chelating probes, which will allow following pseudo contact shifts and relaxation broadening. For this, fully functional repressors were designed and prepared, to specifically react with Cys–reacting lanthanide probes. Several different positions on the LacR were chosen to introduce surface exposed single- or double Cys- mutations to test two types of tags, single- and double-Cys reacting tags[4,5].

References:
1. Monod, J., Changeux, J. P. and Jacob, F., J Mol Biol 6, 306 – 329 (1963)
2. Lewis, M., Cr Biol 328, 521 – 548 (2005)
3. Wilson, C. J., Zhan, H., Swint-Kruse, L. and Matthews, K. S., CMLS 64, 3 – 16, (2007)
4. Peters, F., Maestre-Martinez, M., Leonov, A., Kovačič, L., Becker, S., Boelens, R., Griesinger, C. J., Biomol Nmr 51, 329 – 337 (2011)
5. Keizers, P.H., Saragliadis, A., Hiruma, Y., Overhand, M., Ubbink, M., J Am Chem Soc. 130, 14802 – 14812 (2008)

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