MS1 – e-P3: Can we obtain new information from old protein crystals? The plasma retinol-binding protein case.

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RBP4 (plasma retinol-binding protein) is the 21 kDa transporter of all-trans retinol that circulates in plasma as a moderately tight 1:1 molar complex of the vitamin with the protein. RBP4 is primarily synthesised in the liver but is also produced by adipose tissue and circulates bound to a larger protein, transthyretin, TTR, that serves to increase its molecular mass and thus avoid its elimination by glomerular filtration [1].
Many years ago we published the structure of RBP4 holo and believed to be apo at 2.5 Å resolution [2] and wrote at the end of the abstract "In the case of the unliganded form, the central cavity that is occupied by the vitamin in the two human crystalline holo RBPs, is filled by electron density that, at the present resolution we interpret as solvent." The crystals used for that study had been prepared by microdialysis using protein purified from human plasma and, since they were still kept in our laboratory, we decided to test them at the ESRF a couple of years ago. We collected a full data set of both the liganded and unliganded crystals at a resolution of 1.5 Å and 2.0 Å respectively and were able to refine the unliganded form using about 20,000 reflections instead of the 10,000 that we had used in the original paper. The result was that we identified a fatty acid in the ligand-binding site of the protein believed to be apo.
It is remarkable that the data for the holo and "apo" protein purified from plasma could be collected using crystals from the same batch as those of the structures published more than 20 years ago. The significantly increased resolution can be attributed to the amazing developments in the X-ray data collection methods that occurred during the intervening years and was only possible thanks to the stability of protein crystals prepared by equilibrium dialysis. We also prepared crystals of RBP4 purified from human urine and amniotic fluid, two sources of protein that contain non fluorescent RBP4, i.e. not bound to retinol, and for that reason believed to be the apo form of the protein. In every case we found a fatty acid in the central cavity of the RBP4 molecule, a result that we confirmed by GC-MS analysis of the samples used in the crystallization experiments. This result changes substantially our perception of this protein that has so far been considered to be specific for retinol and is a good example of how simply increasing the quality of the diffraction data can change the perception of the function of a protein [3].

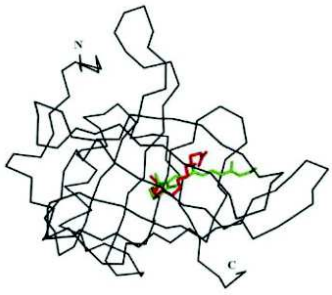

Figure 1. Alternative binding of retinol and palmitic acid to human RBP4.

[1] D.S. Goodman (1984*). Plasma retinol-binding protein. In "The Retinoids" (M.B. Sporn, A.B. Roberts & D.S. Goodman, Eds.) vol 2,* pp41-88. Academic Press, New York.
[2] G. Zanotti, S. Ottonello, R. Berni, H.L. Monaco *J. Mol. Biol.* 1993 **230**, 613-624.
[3] M. Perduca, S. Nicolis, B. Mannucci. M. Galliano, H.L. Monaco *BBA - Molecular and Cell Biology of Lipids* 2018 **1863** 458-466.