MS1-P9 **Fyn, Fab, Xap: Evaluation of different protein binders as crystallization aids.** Armin Ruf [a], David W. Banner[ax], Jörg Benz[a], Julian Bertschinger[b], Dominique Burger[a], Marco Crisci[ax], Simon Cuppuleri [ax], Maja Debulpaep [c], Dragan Grabulovski [b], Bernard Gsell [a], Walter Huber [a], Eric Kusznir[a], Toon Laeremans[c], Hugues Matile[a], Valeria Pecoraro [ax], Arne Rufer[a], Daniel Schlatter[a], Jan Steyeart [c] , Martine Stihle [a], Ralf Thoma [a], Martin Weber [a], Andrea Wiget [ax] *[a]F. Hoffmann-La Roche AG, Pharma Research and Early Development pRED, Small Molecule Research SMR, Discovery Technologies, Grenzacherstrassse 124, 4070 Basel, Switzerland, [b]Covagen AG, Wagis-Strassse 25, 8952 Zurich-Schlieren, Switzerland, [c] Xaperones, Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Elsene, Belgium, [x]These Roche contributors now have other affiliations*
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Iterative high-resolution structures of target proteins in complex with different ligands provide guidance for rational drug design. Easily obtainable and well-ordered crystals are prerequisite to obtain such structures, so robust crystallization systems are required that reproducibly yield well diffracting crystals that withstand soaking with small molecules. There are several techniques to improve protein crystallization. While genetic engineering by the variation of the protein construct length or by the introduction of surface mutations[1] is routinely used, co-crystallization with crystallization aid proteins, a more laborious alternative, is increasingly resorted to when proteins refuse to crystallize. Initially, antibody Fab-fragments which might stabilize the target protein or increase the surface area available for crystal lattice contacts were used for co-crystallization[2]. Recently, novel classes of smaller and very versatile binding proteins such as DARPins[3], Fynomers[4] or Nanobodies/Xaperones[5] have been reported as crystallization aids. Our goal was to evaluate the potential of various differently sized protein scaffolds as crystallization aids. We therefore tested in direct comparison three binding proteins specifically raised against the same challenging crystallization target, BACE-2, and benchmarked the crystallization success against surface mutants. The protein binder scaffolds used were: antibody Fab-fragments (quadruple IG domains), Fynomers (SH3 domain of Fyn), and Nanobodies/Xaperones (single IG domain). The protein target was BACE-2, an aspartic protease very homologous to the Alzheimer's disease beta-secretase, BACE-1. A role for BACE-2 in the early stages of diabetes has been postulated. The structure of BACE-2 has previously been published at only low resolution, although the protein was engineered specifically for crystallization[6]. While all three different "crystallization facilitators" and the surface mutants yielded diffracting BACE-2 crystals, only some of them allowed us to establish reproducible crystallization systems suitable to efficiently support the drug discovery process with many high resolution complex structures of BACE-2 from inhibitor soaks. We will report how the different co-crystallization helpers performed, and how they differed with regards to crystallization success, crystal quality and time and resources needed to obtain suitable crystals.

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Keywords: protein crystallization, antibodies, drug discovery and design

MS1-P10 **Mixing high oligomerization with low resolution, winding way! What's Key in Invertases?** María Angela Sainz-Polo,[a] Beatriz González,[a] Miguel Alvaro,[b] María Fernández-Lobato,[b] Alvaro Lafraya,[c] Julia Marín,[c] Julio Polaina[c], Julia Sanz-Aparicio.[a] *[a]Department of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid. [b]Department of Molecular Biology, Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Madrid (Spain). [c]Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Valencia (Spain).*
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Fructans are polymeric sugars derived from sucrose. They are most interesting because of their physiological characteristics, such as preventing colon cancer and dental caries, selectively stimulating the growth of bifidobacteria and lactobacilli, decreasing total cholesterol and triacylglycerol lipids in blood serum and promoting the absorption of calcium and magnesium ions. Therefore, the enzymes involved in fructans processing attract great biotechnological attention for the production of functional foods and pharmaceuticals. In particular, a detailed knowledge of the molecular mechanisms involved in substrate recognition, transfructosylating efficiency and product specificity of the enzymes used as catalyst for these processes is essential.

We have solved the crystal structure of three invertases from yeast. First, the Schwanniomyces occidentalis Invertase, complexed with long substrates, revealed for the first time that the ancillary domain plays a direct role in oligomerization and substrate binding [1], which is a unique feature that shed light on the molecular mechanism regulating specificity within the GH32 enzymes from eukariota. We report also the Phaffia rhodozyma Invertase structure [2], an atypical highly glycosylated enzyme, with an unique insertion in the sequence of the β-sandwich that folds over the catalytic domain and is involved in a new oligomerization pattern conferring high stability to the enzyme. Finnally, we have studied the Saccharomyces cerevisiae Invertase, an enzyme reported to adopt different aggregation states upon changes in the environment. The crystal structure revealed a sophisticated mechanism of molecular interaction between subunits that form higher aggregates throughout further involvement of the ancillary domains. Our results assign a direct catalytic role to the supplementary ß-sandwich domain of these enzimes, the first time that such a role has been observed within GH32 enzimes.

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Keywords: Invertases, enzymatic specificity, crystal structure