not compatible with any stable crystalline state. A helpful term in description of the controlled crystallization process is "***dominating adhesion mode" (DAM)***, i.e. the most probable adhesion realized between two macromolecules under the given conditions, usually but not necessary, the highest affinity adhesion mode. Preferences of adhesion modes can be regulated e.g. by a selective mutation of protein surface but also much more easily by changing pH, buffer composition, anions, additives, precipitants etc. Any chemicals adhering temporarily to the specific places on the protein surface and blocking efficiently unwanted adhesion modes between proteins are called "***protein surface shielding agents"(PSSA)***. Because of complex effects of components used in the crystallization solution, many different low-efficiency PSSA's have already been intuitively used long time in crystallization screens under the concepts of additives, precipitants, anions. In the case that DAM leads to formation of a single crystal we receive a unique ***group of crystal contact areas (GCCA)*** related directly to the specific crystal properties (space group, water contents, diffraction quality, twinning, etc.). It is evident that well diffracting crystals are formed only when a single adhesion mode dominates but a reverse statement need not be true. Because of the possible strain induced by deposition of macromolecules in the growing molecular cluster, the contact areas realized in the crystal are not necessarily identical with the contact areas in solution. However, the contact areas of the dominating adhesion mode should be very similar in both states. Hydrophilic polymers are generally very efficient PSSAs, because they protect a much larger area on protein surface than their low-molecular weight equivalents upon binding to protein surface. Thus the polymers have generally better propensity to eliminate unwanted adhesion modes between protein molecules forming a crystal.

Practical part: We made an experimental scan of an efficiency of a number of water soluble polymers and co-polymers as novel ***protein surface shielding agents*** in the crystallization process. Sixteen novel water soluble polymers were tested for their efficiency in protein crystallization. Six of them were selected as precipitants to two new polymer crystallization screens (POLYA, POLYB) /2/. The screens were independently tested in six laboratories on 30 different globular proteins. Supported by GA AV IAA500500701.

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Keywords: polymers; co-polymers; protein crystallization; protein surface shielding agents; molecular adhesion

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Expression, Purification and Crystallization of Human Bile Acid-coA: Amino Acid N-Acyltransferase (BAAT).

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Bile acid-coenzyme A:Amino Acid N-Acyltransferase (BAAT) is the sole enzyme responsible for conjugation of primary and secondary bile acids to taurine and glycine [1].

The human cDNA BAAT sequence was cloned into the pET15 vector and its expresssion was tested in several *E.coli* strains. Because of the toxicity associated with the overexpression of BAAT in several strains, BL21 (DE3) C41 was selected to express the recombinant protein. Only with this specific strain, a reasonable amount of the protein was obtained in the soluble fraction.

The purification steps involved Nickel-sepharose chromatography followed by reverse IMAC after histidine-tag cleavage. Size-exclusion and hydrophobic interaction chromatography steps were also included to increase the purity of the sample. After these steps the protein shows as a single band in both SDS and native PAGE, The purified enzyme was assessed for activity and was found to be able to catalyze the conjugation of taurine to CoA-fatty acids [2].

The presence of non physiological inter- and/or intramolecular disulfide bounds between the three cysteine residues present in the protein sequence as a source of sample microhetetogenity was also examined. Three cysteine mutants (C235A, C372A and C373A, and the triple mutant without cysteines) were constructed and tested following the same protocols used for the wild type protein and no significant differences in the behaviour of the four species were found.

Crystallization trials with the apo- and holo-wild type enzyme, as well as with the three mutants, are in progress but up to now only microcrystals not adequate for X-ray diffraction studies have been obtained.

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Keywords: BAAT; bile acids; conjugation

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Novel Crystallisation Plate Imaging System Using ANS-Based Fluorescence at the EMBL Hamburg High-Throughput Crystallisation Facility.

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We have previously demonstrated that the addition of low concentrations (μM-nM) of a nonspecific fluorescent dye (1,8-ANS) does not significantly hinder crystal growth in a set of trial proteins [1]. The resulting fluorescence images collected on a high-resolution fluorescence microscope show that these protein crystals are strongly contrasted against other common crystallisation drop phenomena, such as precipitate and phase separation. However, the experiments were performed on a fluorescence microscope and no specialised equipment currently exists for the visualisation of ANS-based fluorescence (excited at 365 nm). Here we present a novel and cost-effective device for visualisation