



JBS Methylation Kit

Methylating the primary amines on a protein's surface (in lysine side chains and at the N-terminus) is a rescue strategy often applied to proteins that are reluctant to crystallize. The kit is used on the purified protein prior to crystallization and yields an engineered protein surface within one day. The increased radius of methylated amines allows stronger intra- and intermolecular interactions resulting in more ordered crystal packing and better diffraction. In most cases methylated proteins fully maintain their biochemical function, and the structures of native and methylated proteins are very similar.^[1,2] The successful application of JBS Methylation Kit is shown in several recent publications.^[3-7]

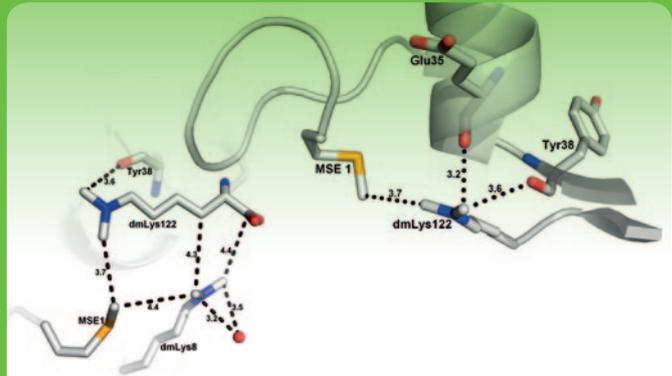


Figure 1: The JBS Methylation Kit changes a protein's surface by replacing the two hydrogens of primary amino groups with methyl groups. The resulting dimethylated lysines (dmLys) and the N-terminus are well ordered and involved in several types of intramolecular interactions with protein side chains, main chain carbonyls and solvent.^[1] (PDB code 2QH0)

JBS Floppy-Choppy

Limited proteolysis is another rescue strategy used for challenging crystallization projects. It can be applied in situ, in which trace amounts of protease are added to the protein immediately prior crystallization set up. Floppy parts of the protein, primarily at the N- or C-termini, are chopped off producing more rigid truncations with enhanced likelihood of crystal formation. Various proteases have been used so far however, chymotrypsin and trypsin are the most successful ones.^[8-12]

JBS Floppy-Choppy can also be applied as pre-proteolysis screen to identify the protease and reaction conditions yielding the most stable/promising truncation. Proteolysis can easily be monitored by SDS-PAGE or MS, and the result can be directly used for producing a larger batch of truncated protein with subsequent purification.

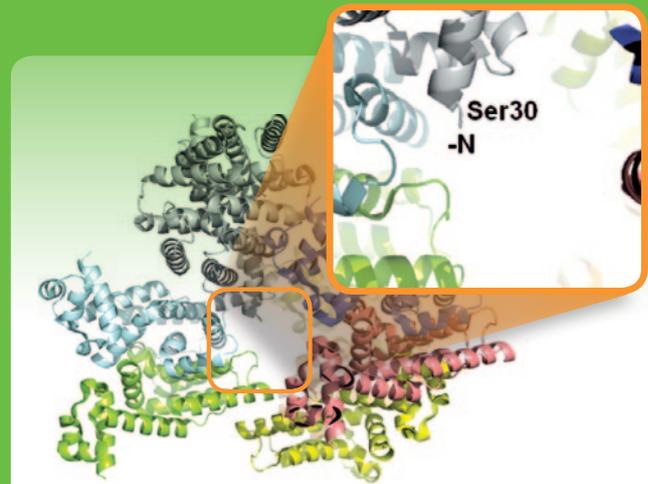


Figure 2: Crystal lattice of a putative transcriptional regulator SCO4942 from *Streptomyces coelicolor* (PDB 2PZ9). In situ proteolysis allowed crystal formation by removal of an N-terminal fragment (amino acids 1-29).^[9]

Product	Cat. No.	Amount	Price (EUR)
JBS Methylation Kit	CS-510	6 reactions	197,00
JBS Floppy-Choppy	CO-110	1 Kit	178,00

References:

- [1] Kim *et al.* (2008) Large-scale evaluation of protein reductive methylation for improving protein crystallization. *Nature Methods* **5**:853.
- [2] Walter *et al.* (2006) Lysine methylation as a routine rescue strategy for protein crystallization. *Structure* **14**:1617.
- [3] Barden *et al.* (2013) A Helical RGD Motif Promoting Cell Adhesion: Crystal Structures of the Helicobacter pylori Type IV Secretion System Pilus Protein CagL. *Structure* **21**:1931.
- [4] Peat *et al.* (2013) Cyanuric acid hydrolase: evolutionary innovation by structural concatenation. *Molecular Microbiology* **88**:1149.
- [5] Koval *et al.* (2013) Plant multifunctional nuclease TBN1 with unexpected phospholipase activity: structural study and reaction-mechanism analysis. *Acta Cryst. D* **69**:213.
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- [7] Cima *et al.* (2012) Insight on an Arginine Synthesis Metabolite from the Tetrameric Structure of Yeast Acetylglutamate Kinase. *PLoS one* **7**:e34734.
- [8] Dong *et al.* (2007) In situ proteolysis for protein crystallization and structure determination. *Nature Methods* **4**:1019.
- [9] Wernimont *et al.* (2009) In Situ Proteolysis to Generate Crystals for Structure Determination: An Update. *PLoS ONE* **4**:e5094.
- [10] Faim *et al.* (2013) Crystallization and preliminary X-ray diffraction analysis of selenophosphate synthetases from *Trypanosoma brucei* and *Leishmania major*. *Acta Cryst. F* **69**:864.
- [11] Ochi *et al.* (2012) Structural Insights into the Role of Domain Flexibility in Human DNA Ligase IV. *Structure* **20**(7):1212.
- [12] Abskharon *et al.* (2011) Combining in-situ proteolysis and microseed matrix screening to promote crystallization of PrP^{Sc}-nanobody complexes. *PEDS* **24**(9):737.