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DOI

[10.1002/2211-5463.12449](https://doi.org/10.1002/2211-5463.12449)

Publication date

2018

Document Version

Final published version

Published in

FEBS OPEN BIO

[Link to publication](#)

Citation for published version (APA):

Corthals, G., de Jong, L., de Koning, E. A., Roseboom, W., Buncherd, H., Wanner, M., Dapic, I., Jansen, P., van Maarseveen, J., Lewis, P., Hamoen, L., & de Koster, C. (2018). Identification of dynamic protein interactions from live cells. *FEBS OPEN BIO*, 8(S1), 17-18. [S.32-5]. <https://doi.org/10.1002/2211-5463.12449>

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S.32-5**Identification of dynamic protein interactions from live cells**

G. Corthals, L. de Jong, E. A. de Koning, W. Roseboom, H. Buncherd, M. Wanner, I. Dapic, P. Jansen, J. van Maarseveen, P. Lewis, L. Hamoen, C. de Koster
University of Amsterdam, Amsterdam, Netherlands

Understanding how biological assemblies function at the molecular level requires knowledge of the spatial arrangement of their composite proteins. Such information can be obtained from the mapping of protein-protein interactions. However, both stable and dynamic interactions are omnipresent in living cells, which makes these approaches overall quite challenging. In this lecture I will describe for the first time a system that fulfils all requirements for efficient trapping and identification of both stable and dynamic interactions in living cells. The method rapidly cross-links growing cells with the specifically designed lysine cross-linker bis(succinimidyl)-3-azidomethyl-glutarate (BAMG). Characteristics of the BAMG reagent are that it (i) rapidly crosses membranes by diffusion, (ii) reacts specifically with primary amine groups and (iii) greatly facilitates mass spectrometric identification of cross-linked peptides. We will review examples of the

application of our approach to the Gram positive model *Bacillus subtilis*, widely studied for processes guided by dynamic protein-protein interactions involved in gene expression, cell division, sporulation and germination. New to this method, is that identification and validation of inter-protein cross-linked peptides occurs with less than a 1% false discovery rate by mass spectrometry. It was found that approx. 60% of all cross-links occurred in assemblies involved in transcription and translation. Numerous interactions were new, and we identified an interaction between the δ and β' subunits of RNA polymerase close to the downstream DNA channel, providing insight into how δ regulates promoter selectivity and promotes RNA polymerase recycling. Fellow colleagues will enjoy the simplicity, high efficiency and accuracy of this new methodology, which we believe opens new avenues to investigate the functional dynamic organisation of complex protein assemblies at the cellular level.