RSEQ
Real Sociedad Española de Química

ugr Universidad de Granada

XXXVIII REUNIÓN BIENAL

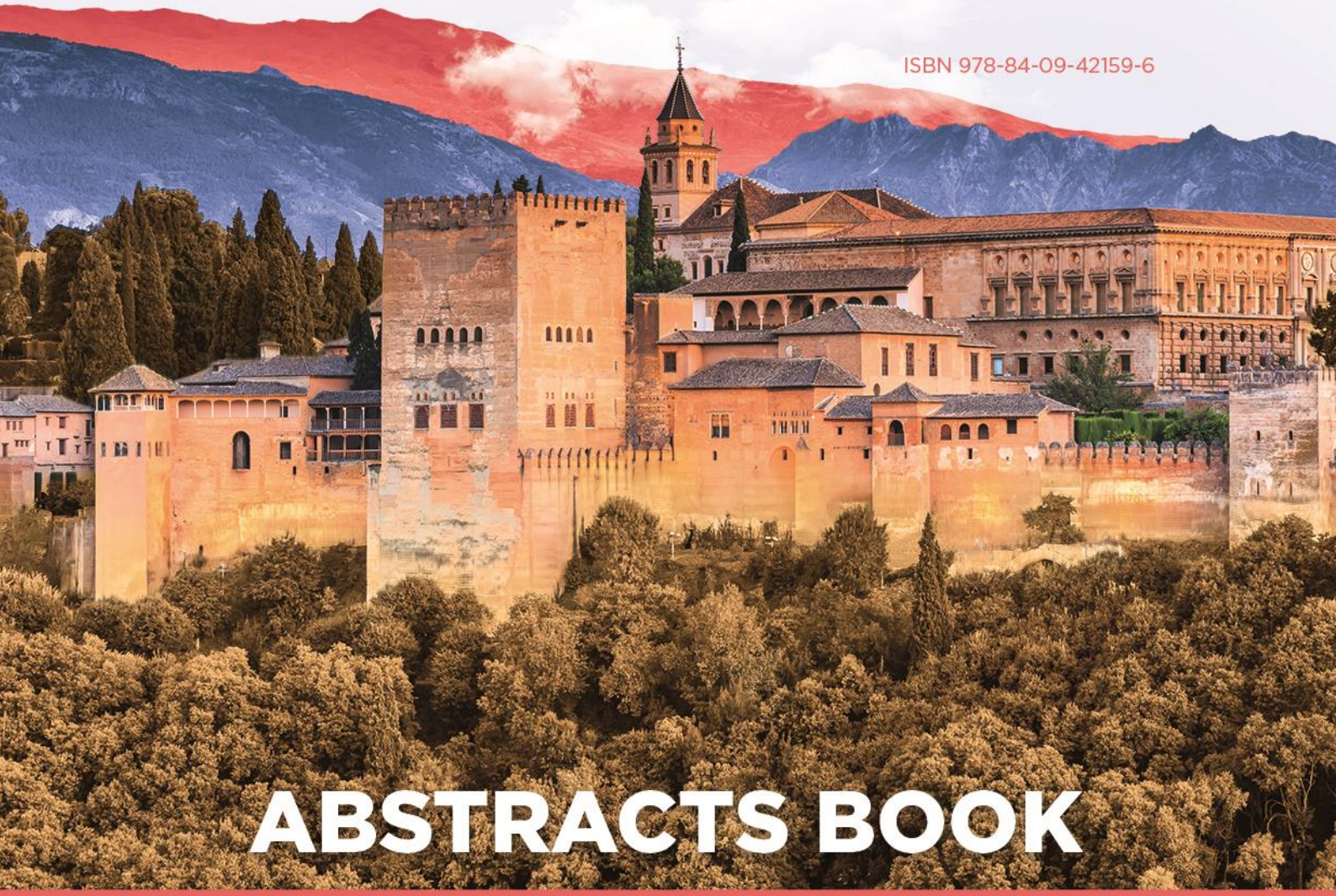
RSEQ

GRANADA 2022

27 - 30 DE JUNIO 2022

REAL SOCIEDAD ESPAÑOLA DE QUÍMICA

ISBN 978-84-09-42159-6



ABSTRACTS BOOK

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S08-OC03

OPTICAL CONTROL OF THE FOLDING AND MEMBRANE TOPOLOGY OF HELICAL TRANSMEMBRANE PROTEIN FRAGMENTS

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Keywords: *infrared spectroscopy, membrane folding, helix tilt, photoswitches, azobenzene*

The spontaneous (translocon-unassisted) folding/insertion of helical transmembrane (TM) protein fragments into lipid bilayers is driven by three sequential equilibria: solution-to-membrane interface (MI) partition, unstructured-to-helical folding, and MI-to-TM helix insertion [1]. Understanding the molecular mechanism underlaying these steps has been challenged by the lack of suitable experimental approaches to perturb protein fragments rapidly and reversibly out of equilibrium once reconstituted in membranes.

We have been actively working in implementing a strategy to control/perturb the folding and membrane topology of protein fragments with light. We have started with a 24-residues-long hydrophobic α-helical polypeptide from the KALP family as a model for a helical transmembrane protein fragment. We introduced two Cys to KALP and coupled it to an azobenzene photoswitch (KCALP-azo), as done before for helical soluble protein fragments [2]. By polarized FTIR spectroscopy we determined that KCALP-azo (*trans* azobenzene) folds as a TM α-helix, as KALP does, with a helix tilt angle of 32 ± 5. After *trans*-to-*cis* photoisomerization of the azobenzene moiety with UV light (reversed with blue light), we confirmed the optical control of the membrane topology of KCALP-azo: its helix tilt changes reversibly from 32 ± 5° (TM topology, blue light) to 79 ± 8° (MI topology, UV light) [3].

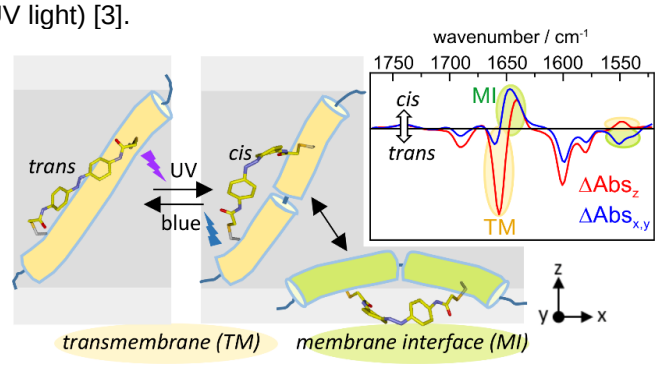

Figure 1 – Model for the light-induced changes in the membrane topology of the peptide KCALP-azo (helical structure represented as a cylinder) as a function of the isomeric state of the covalently attached azobenzene group. Inset: light-induced FTIR difference spectrum in the plane (x,y) and perpendicular (z) to the lipidic membrane surface, reporting on changes in the helix orientation of KCALP-azo from 32º to 79º.

We have started to use short UV and blue light pulses in combination with time-resolved FTIR difference spectroscopy [4]. These experiments exploit the here achieved optical control to provide a detailed picture on how a membrane interface helical fragment inserts in lipidic membrane, a relevant information currently only available from MD simulations [5].

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