

## Concurrent Session 5: Report for EuMelaNet members

Dear Colleagues,

The 21<sup>st</sup> iPPC in Bordeaux is just over and we would like to provide a brief overview of the main activities of the group during the meeting. After a preliminary informal meeting held on Tuesday, September 20, and chaired by Shosuke Ito and Kazumasa Wakamatsu, the first official meeting of the EuMelaNet Special Interest group took place on Wednesday September 21, on the occasion of Concurrent Session 5 (CS5).

CS5 was chaired by Marco d'Ischia, Josè-Carlos García-Borrón, Shosuke Ito and John Simon (who sent apologies for not being able to come) and was attended by nearly 30 persons. This session was entitled “**Methods in Melanin Research**” and was formatted as a round table centered around the launching project of the EuMelanet group, that is, the definition of a set of recommended protocols and procedures to be followed for studies of melanins and melanogenesis.

**Marco d'Ischia** opened the session with a brief summary of the main aims and scope of the EuMelaNet group, and gave an overview of the round table programme.

**Jose-Carlos Garcia-Borrón** raised important and relevant issues concerning enzyme sources and sample preparation for studies of melanogenesis: Are crude melanoma extracts with defined tyrosinase activity suitable enzyme preparations for studies of melanogenesis ? Is the fungal enzyme suitable for model studies of mammalian melanogenesis ?

**Shosuke Ito** illustrated the method developed by his group for analysis of tissue melanins based on alkaline hydrogen peroxide degradation. He recommended TTCA (thiazole tricarboxylic acid) as a useful marker of pheomelanins allowing together with PTCA (pyrroletricarboxylic acid) quantitation, the simultaneous determination of eumelanins and pheomelanins.

In the following presentation he went on to review convenient methods for preparation of 5,6-dihydroxyindoles and of cysteinyl dopas by the classical dopa/tyrosinase based protocols with modifications. He also addressed the issue of the preparation of analytical markers, namely pyrrolecarboxylic acids (PTCA and PDCA), thiazolecarboxylic acids (TTCA and TDCA), AHP and synthetic eumelanins.

In the subsequent presentation **Alessandra Napolitano** advocated the value of the benzothiazole BTCA as a more selective pheomelanin marker, easily available by a one pot biomimetic procedure and highly pigment-specific. She also showed the most convenient approach to BTCA synthesis and the alternate methods for gram-scale synthesis of cysteinyl dopas. A lively debate followed in which the various methods were critically examined by the Japanese and Neapolitan groups.

**Alessandro Pezzella** talked about synthetic eumelanins to show that substrates, reaction conditions and isolation/storage protocols may affect significantly the overall structure and properties of synthetic eumelanins. To this aim, the potential of mass spectrometric analysis to distinguish synthetic eumelanins was discussed. It was concluded that preparation protocols may vary depending on the purpose and research goal (structural/biomimetic study or material synthesis).

**Paul Meredith** contributed with a presentation recapitulating the basics of the chemical disorder model and arguing against major differences between various synthetic eumelanins as judged by morphological analysis, in which stacked architectures were invariably detected. He emphasized that, from the materials science perspective, cost effectiveness and substrate availability are more critical parameters than close control of structural features.

Finally a presentation by **Hidekazu Okuda** underlined the potential of the theoretical approach to investigate and predict melanin structure besides the classical biosynthetic and degradative methodologies.

Overall, the meeting featured a series of well tuned presentations from different viewpoints which stimulated lively and constructive discussions. Eventually it was agreed that the presentations debated at CS5 were worthy of further assessment and elaboration by the group to generate a consensus paper for submission to a peer-reviewed journal, possibly Pigment Cell and Melanoma Research. The proposed paper should be directed to guide pigment cell researchers into the experimental details of melanin-relating protocols with an easy-to-do approach also for non-chemists. A draft of the paper will be circulated soon through the EuMelaNet mailing list, and will be available to all interested members for comments, modifications and suggestions.

Marco d'Ischia, Shosuke Ito, Josè-Carlos Garcia-Borron