Immobilization of E2 protein and its supramolecular complexes, and activity retention on graphene

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Protein immobilization is central to the development of new bio-assays or sensing platforms as it is directly linked to such issues as protein conformation and subsequently to whether they remain active or not after immobilization. Immobilized enzymes are routinely used in the biotechnology industry but there are numerous problems with their immobilization for industrial usage [1]. Here we are exploring graphene as a surface for enzyme immobilization and activity detection. Graphene has shown extreme (down to single-molecule) sensitivity to environmental changes [2] and progress in its synthesis now allows its growth on large areas [3], thus making it a potentially superior candidate for the bio/inorganic interface.

The 2-oxo acid dehydrogenase protein complex (OADHC) (Figure 1c) is one of the largest enzyme complexes, which is central to energy metabolism [4]. It consists of a dihydrolipoyl acyl-transferase (E2) protein core, on which 2-oxo acid decarboxylase (E1) and dihydrolipoamide dehydrogenase (E3) proteins bind non-covalently, but specifically [4]. In this work we first succeeded in immobilizing the E2 monomer *directly* onto the graphene surface and then investigated the supramolecular assembly of the E2 core as well as of the whole 2-OADHC complex. Finally, the retention of activity of the whole complex is tested through its redox electron transfer to a Field-Effect graphene sensor.

Our approach is different to those taken in prior studies of protein binding on graphene, where only small proteins, simple in structure and with single function have been used (see for example [5]). Contrary to them, our thermostable E2 protein offers extensive flexibility for tailoring protein-protein interactions and resulting functions, and with its enhanced thermostability, constitutes a model system for scaffolding of complex, multidomain protein systems.

References

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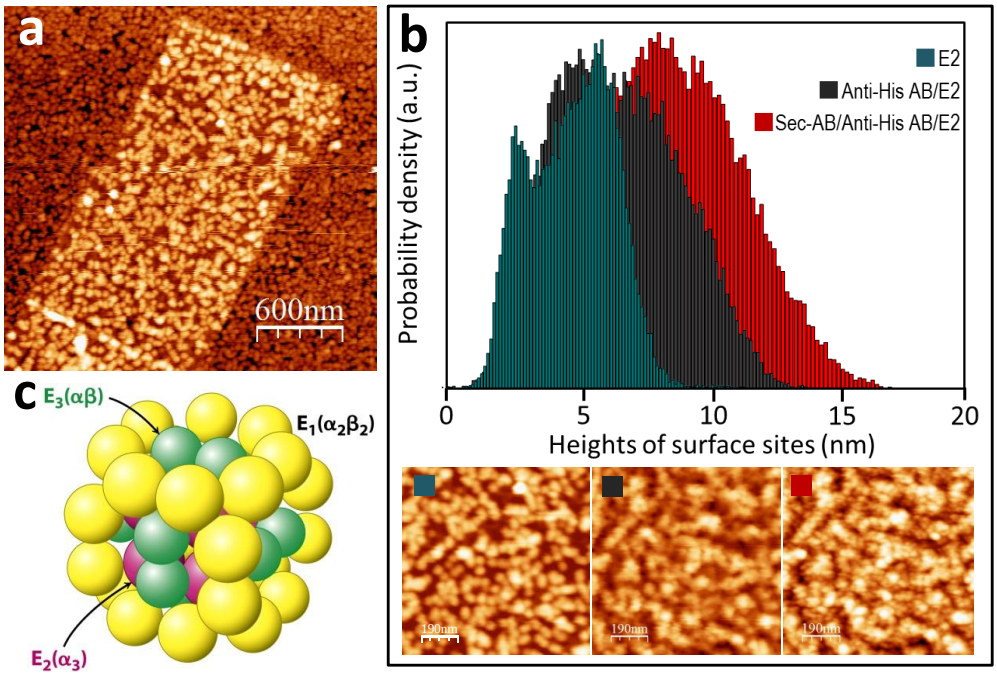

Figure 1: (a) E2 has attached non-covalently onto graphene (thus preserving graphene electronic properties); (b) shows that the His-tag of the E2 protein is accessible for specific binding, tested here with an Anti-His Antibody (Anti-His AB), whose presence is then confirmed by the attachment of a complementary, secondary antibody (Sec-AB).The build-up of this scaffolding system is shown through topographic changes obtained through Atomic Force Microscopy, directly in images as well as in changes in the distribution of surface site heights; (c) the full 2-OADHC complex.