Supplementary Material (ESI) for Molecular BioSystems

Supplementary Data

Role of liposomes in the receptor-independent modulation of reconstituted $G\alpha_i$ protein

Paola Luciani,[1] Debora Berti,[1,] Martina Fortini [1] Piero Baglioni,[1] Carla Ghelardini,[2] Alessandra Pacini,[3] Dina Manetti,[4] Fulvio Gualtieri,[4] Alessandro Bartolini,[2] Lorenzo Di Cesare Mannelli[2]*

[1] Department of Chemistry and CSGI, University of Florence, Via della Lastruccia 3, 50019 – Sesto Fiorentino, Florence ITALY

[2] Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Pieraccini 6, 50134 – Florence ITALY

[3] Department of Anatomy, Histology and Forensic Medicine, Anatomy Section, University of Florence, Viale Morgagni 85, 50134 – Florence ITALY

[4] Department of Pharmaceutical Sciences, University of Florence, Via U. Schiff 6, 50019 – Sesto Fiorentino, Florence ITALY

Figure 1. CD spectra of Gα-mir; first upscan from 20ºC to 60ºC.

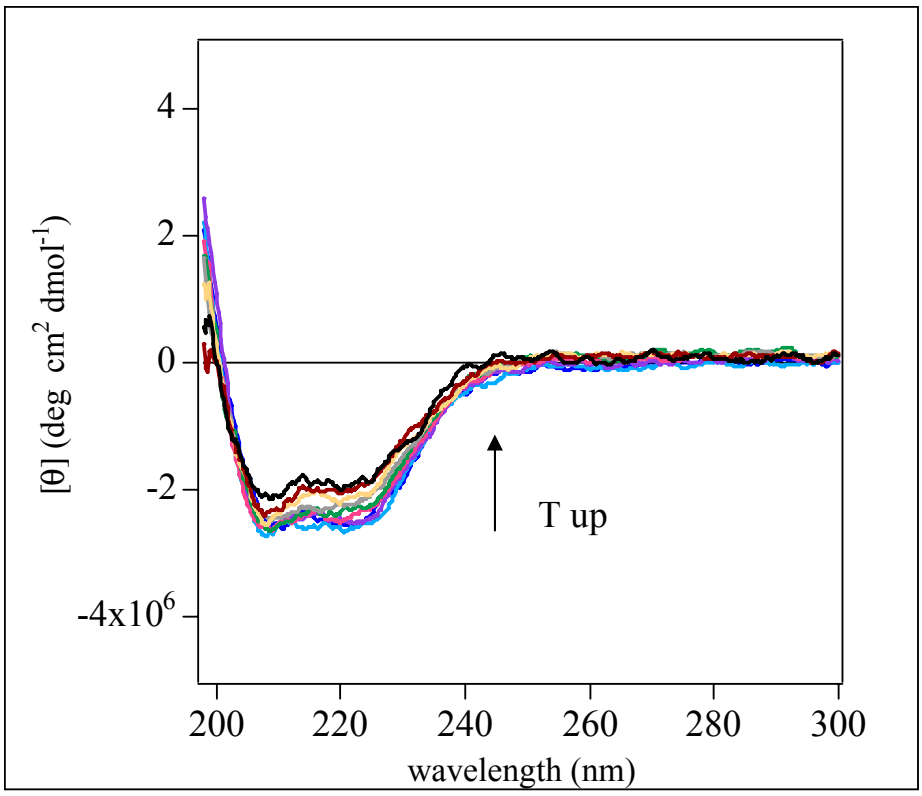


Figure 2. CD spectra of Gα-mir; second upscan on the same solution from 20ºC to 60ºC.

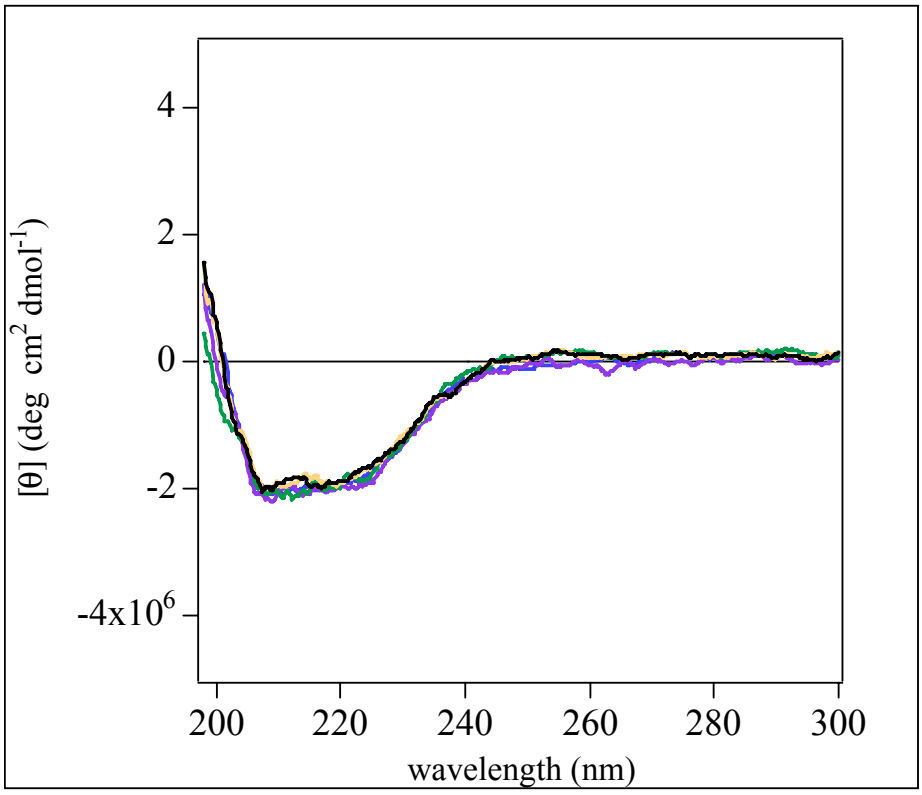
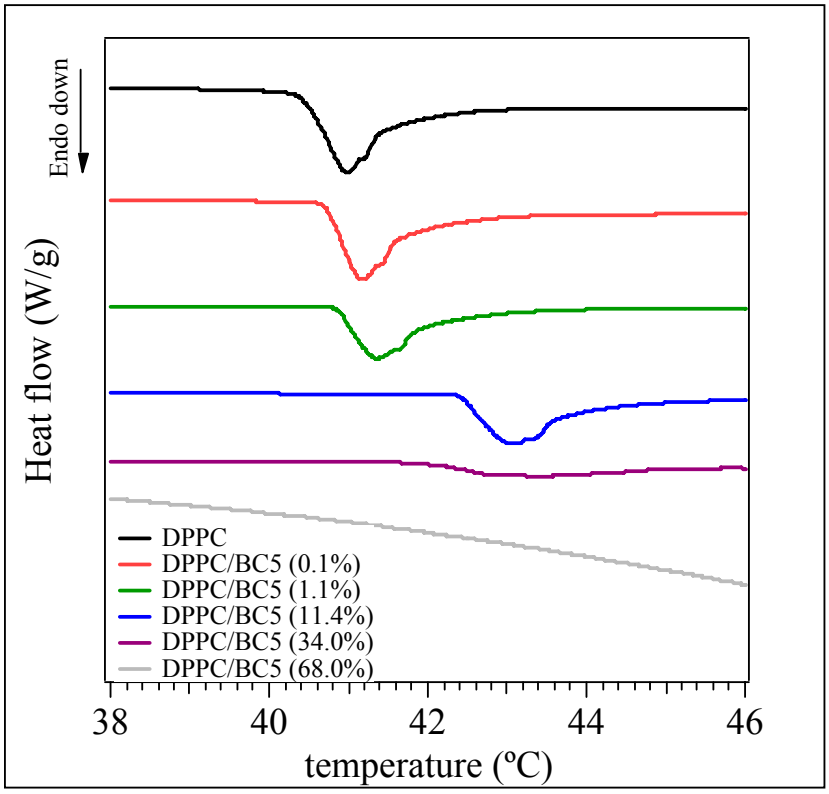


Figure 3. DSC thermograms relative to the formulations described in Table A



Expression and purification of $G\alpha_{i1}$ protein

The entire ORF (Open Reading Frame) of both human G inhibitory a subunit isoform 1 and yeast myristoyl transferase were obtained by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The cDNA coding for the human ai1 isoform was generated by two sequential amplification reactions as described in 23. For MT, RT-PCR was carried out in one-step with upstream primers annealing to the 5'-end starting from ATG and downstream primers complementary to the 3'UTR (UnTranslated Region). The primers used for PCRs were as follows: Gαi1 (ORF 1) forward 5'-GACGACGACAAGATGGGCTGCACGCTGAG-3', and Gαi1 reverse 5'-CGCGGGCGGCCGTTAAAAGAGACCACAATCTTTTAGATT-3' (GenBank accession number NM_002069); MT (ORF2) forward 5'-GCGGGCCCGGCCTTGATGTCAGAAGAGGATAAAGC-3', and MT reverse 5'-GAGGAGAAGCCCGGTCTACAACATAACAACACCGA-3' (GenBank accession number M23726). PCR products were purified by SpinPrep PCR Clean-Up Kit (Novagen) and, in order to generate compatible overhangs, ORF1 and ORF2 were treated with T4 DNA Polymerase (Novagen). Then, the ORFs were annealed to the vector LIC Duet Mini Adaptor (Novagen), a vector designed to anneal to the 3'-end of the ORF-1 and the 5'-end of the ORF-2. The obtained ORF1-Adaptor-ORF2 complex was subsequently cloned in the expression vector pRSF2 Ek LIC (Novagen). Plasmid pRSF2 containing both Gai1 and MT sequences (pRSF2/Gαi1/MT) was used to transform competent E.Coli BL21(DE3) cells (Novagen). For large-scale production of recombinant proteins, 100 mL of Luria-Bertani broth were inoculated with 3 mL of a 0.5 O.D. culture. Cultures were grown up to 0.5-1.0 O.D. and then expression was induced by adding isopropyl-β-D-thiogalactoside (IPTG) to a final concentration of 1mM. After 16h of incubation, bacteria were collected by centrifugation and conserved at -80°C. Myris4oylated Gαi1 protein was purified by immobilized metal affinity chromatography (IMAC) exploiting the 6xHistiding-tag fused to the recombinant protein. Finally, the fusion proteins were cleaved by Enterokinase digestion according to the manufacturer's protocol (Invitrogen).

Liposome preparation

Multilamellar vesicles (A) (0.44 mM total lipid concentration) have been prepared by dispersing a dry lipid film in a protein-containing buffer (50 mM TRIS–HCl buffer, pH 8.0; 150 nM Gα protein). These lipids were vortexed for 1 minute then thermostated in a water bath at 60º C for six times. MLV-FT (B) were prepared as MLV, then subjected to 5 minutes of forceful shaking, frozen (liquid N2), and thawed (60º C) six times. SV-NFT (C) were prepared as MLV and then sized down to homogeneous vesicles of approximately 50 nm diameter by 10 repeated extrusion through two stacked polycarbonate filters with 50 nm-sized pores membranes. SUV (D) were prepared as MLV-FT and then extruded down to about 50 nm. Extrusion was performed at 37ºC with an apparatus described elsewhere [see 34, 35 in the manuscript] and Nucleopore polycarbonate membranes. The size distribution of unilamellar liposomes was verified by dynamic light scattering. Besides hydrating DMPC dry films with a Gα-containing buffer, dry DMPC films were hydrated with 50 mM TRIS buffer pH 8 and only after the preparation of MLV and SUV the proper amount of protein was added to liposomes; protein uptake was performed maintaining the protein-liposome dispersion at 30º C overnight.