A simple and reliable method is described to produce inorganic nanoparticles functionalized asymmetrically with domains of hydrophobic and hydrophilic ligands on their respective hemispheres. These amphiphilic, Janus-type particles form spontaneously by a thermodynamically controlled process, in which the particle cores and two competing ligands assemble at the interface between two immiscible liquids to reduce the interfacial energy. The asymmetric surface chemistry resulting from this process was confirmed using contact angle measurements of water droplets on nanoparticle monolayers deposited onto hydrophobic and hydrophilic substrates—particles presenting their hydrophobic face give contact angles of 96°, those presenting their hydrophilic face 19°. The spontaneous assembly process is rationalized by a thermodynamic model, which accounts both for the energetic contributions driving the assembly and for the entropic penalties that must be overcome. Consistent with the model, amphiphilic NPs form only when there is sufficient interfacial area to accommodate them; however, this potential limitation is easily overcome by mechanical agitation of the two-phase mixture. While it is straightforward to vary the ratio of hydrophobic and hydrophilic ligands, the accumulation of amphiphilic particles at the interface is maximal for ligand ratios near 1:1. In addition to gold nanoparticles and thiolate ligands, we demonstrate the generality of this approach by extending it to the preparation of amphiphilic iron oxide nanoparticles using two types of diol-terminated ligands. Depending on the material properties of the inorganic cores, the resulting amphiphilic particles should find applications as responsive particle surfactants that respond dynamically to optical (plasmonic particles) and/or magnetic (magnetic particles) fields.