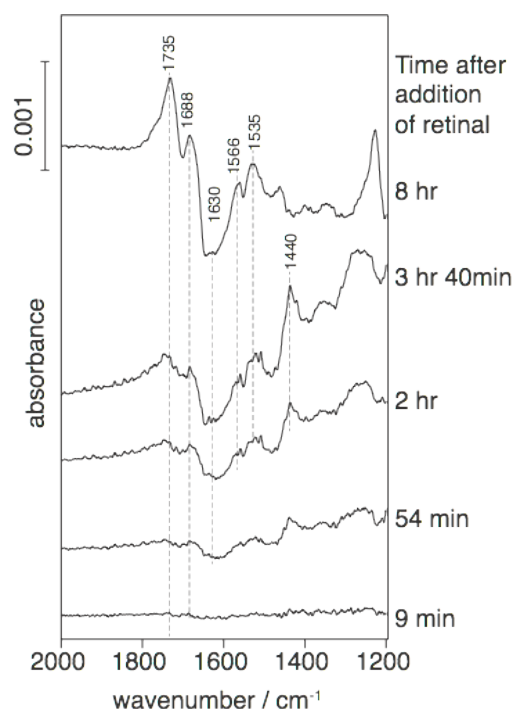


## S8: Post-addition of retinal to bO folding into nanodiscs

Fig L shows a series of IR spectra taken after late addition of the retinal to already expressed bO in nanodiscs. Apo-protein, bacterioopsin, was expressed in the cell-free system without addition of retinal for 4.5 hours (See figure 4b in the main text). A new background spectrum was taken at this time point. In order to see an effect on folding post-translationally by addition of retinal, 17  $\mu\text{M}$  retinal was added to the solution and the sample spectra were taken. In Fig L solely changes affected by the addition of the retinal are observed. IR absorption bands appeared at 1735, 1688, 1566, 1535  $\text{cm}^{-1}$ , which were assigned to retinal molecules adsorbed to the nanodiscs. Despite the appearance of these retinal bands, no changes of the amide mode from the apo-protein could be observed. This result suggests that the post-addition of retinal did not induce correct folding of the bO.



**Fig L:** SEIRA spectra after addition of retinal to misfolded bO in nanodiscs. A background spectrum was taken after bO was cell-free expressed for 4.5 hours. Then, 17  $\mu\text{M}$  retinal was added to the solution. Spectra were taken after times as indicated.