Remarks for osmometer article

1. What is the chemical identity of the IBP being used?

"..We tested a 58 kDa hyperactive IBP from the Antarctic bacterium *Marinomonas primoryensis* (*Mp*IBP) (9) tagged with eGFP, constructed by Peter Davies' group (Queens University),.."

2. What concentration of IBP is used?

".. (*Mp*IBP-GFP 2.4 µM in 20 mM $CaCl_2$ and 25 mM Tris-HCl at pH 8).."

3. How is the IBP added to the protein drop?

"1.5) Slowly insert the glass capillary into the prepared IBP protein sample tube (*Mp*IBP-GFP 2.4 µM in 20 mM $CaCl_2$ and 25 mM Tris-HCl at pH 8) and pull the glass syringe until the glass capillary contains 0.1 µL of the protein solution."

4. How is the duration of exposure to the IBP controlled?

"Exposure time of an ice crystal to IBPs in the solution is defined as the time period from the formation of the crystal (the end of the melting process) until the sudden growth of ice around the crystal (crystal burst). "

5. Please also provide information about what protein solution is being used in step 1.5 of your protocol.

See above answer to 3.

6. JoVE requires at least ten references. Your manuscript only has eight. Please add two more references.

We added several references to the total of 12

7. Finally, we have noticed that Figure 1 is taken from a published paper. Do you have permission to reprint the image? If not, we require that you modify the image or obtain permission to use it as is before we can publish your article.

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