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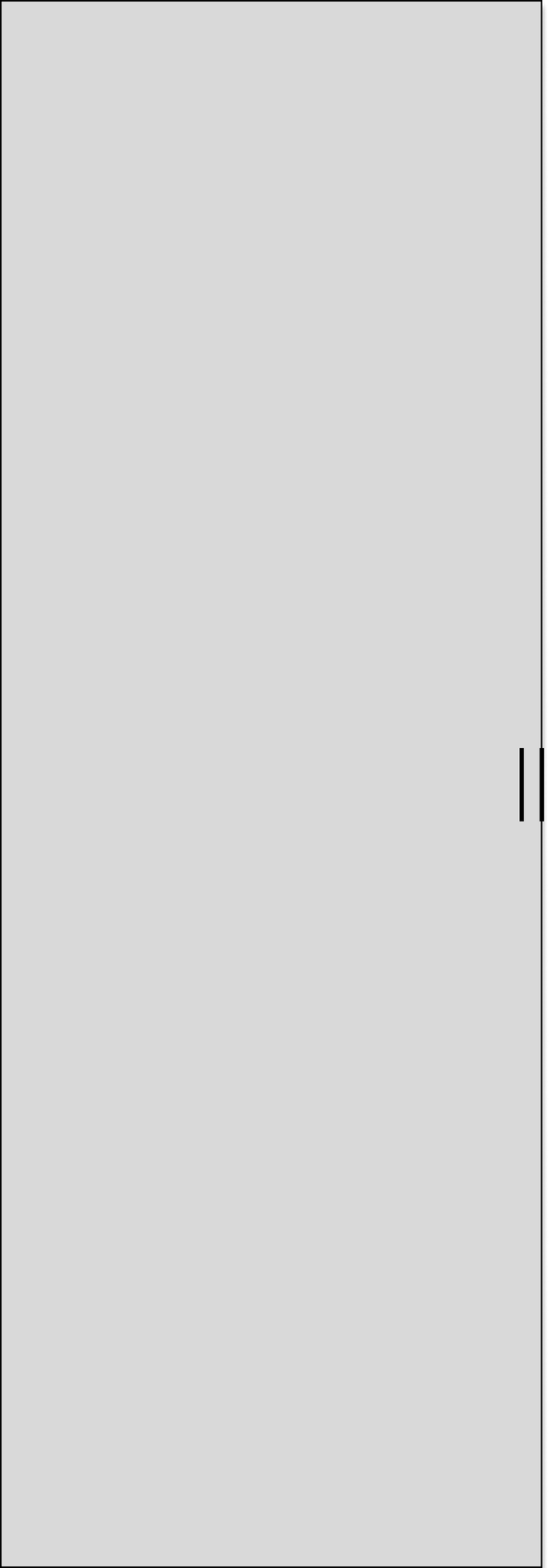

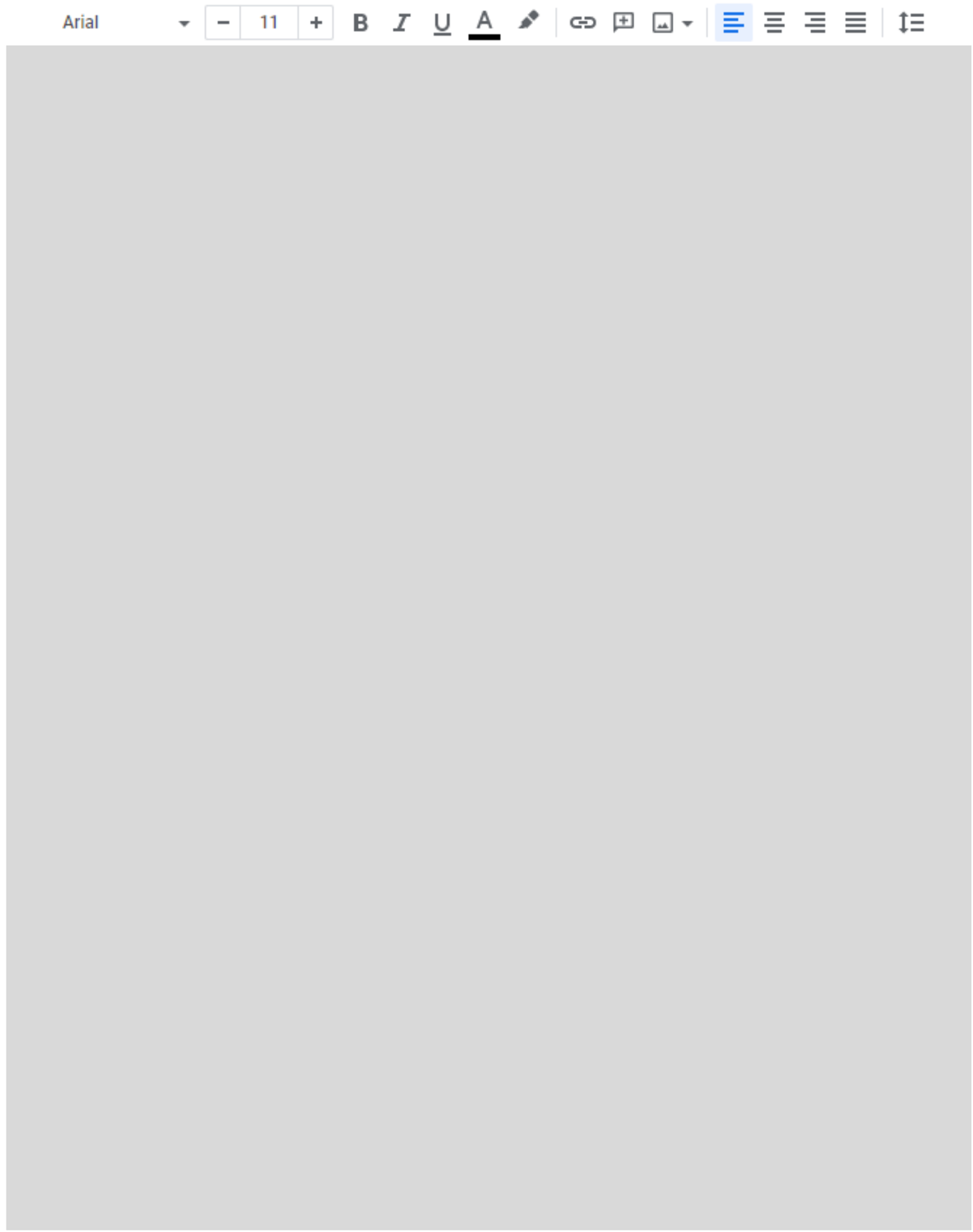

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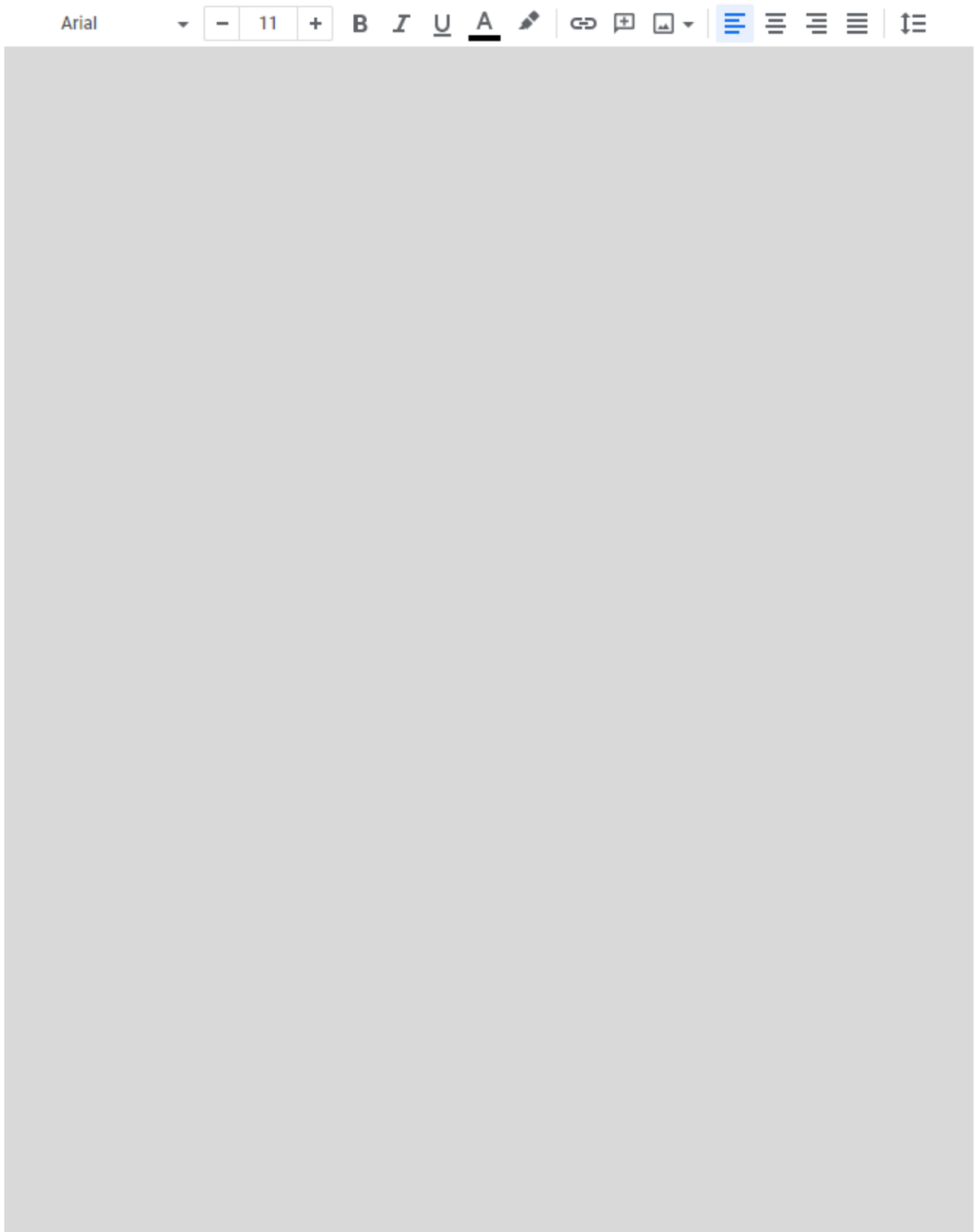

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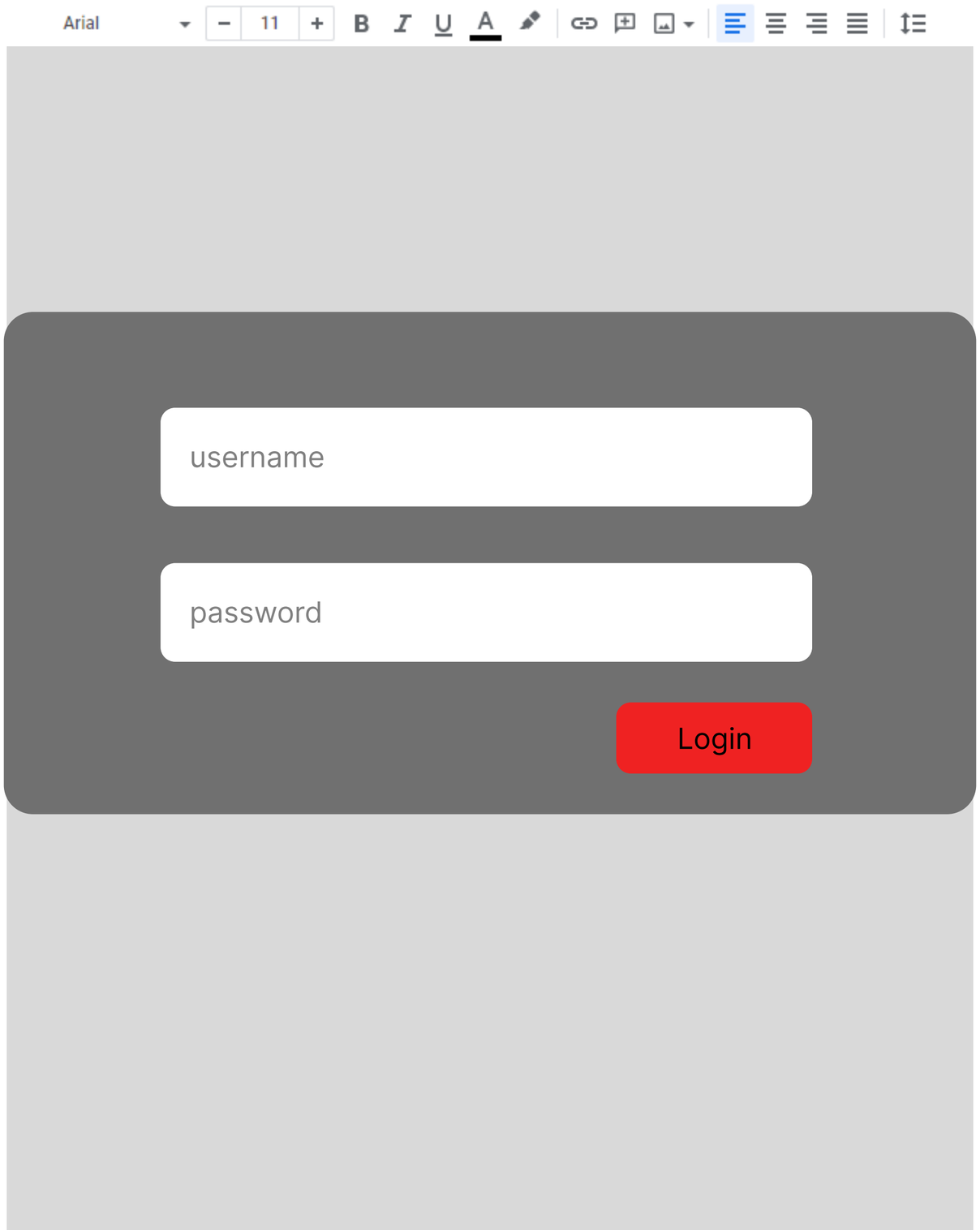

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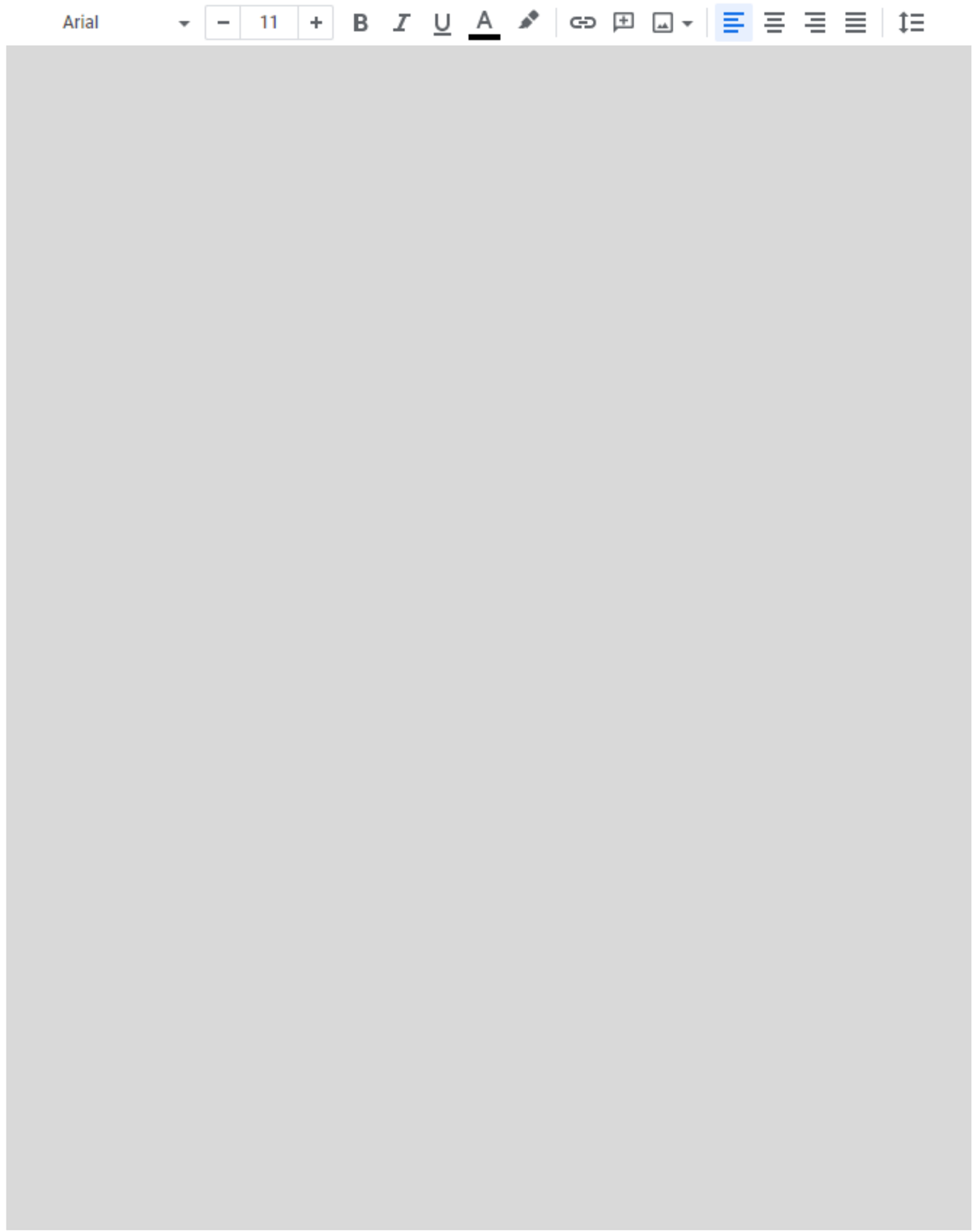

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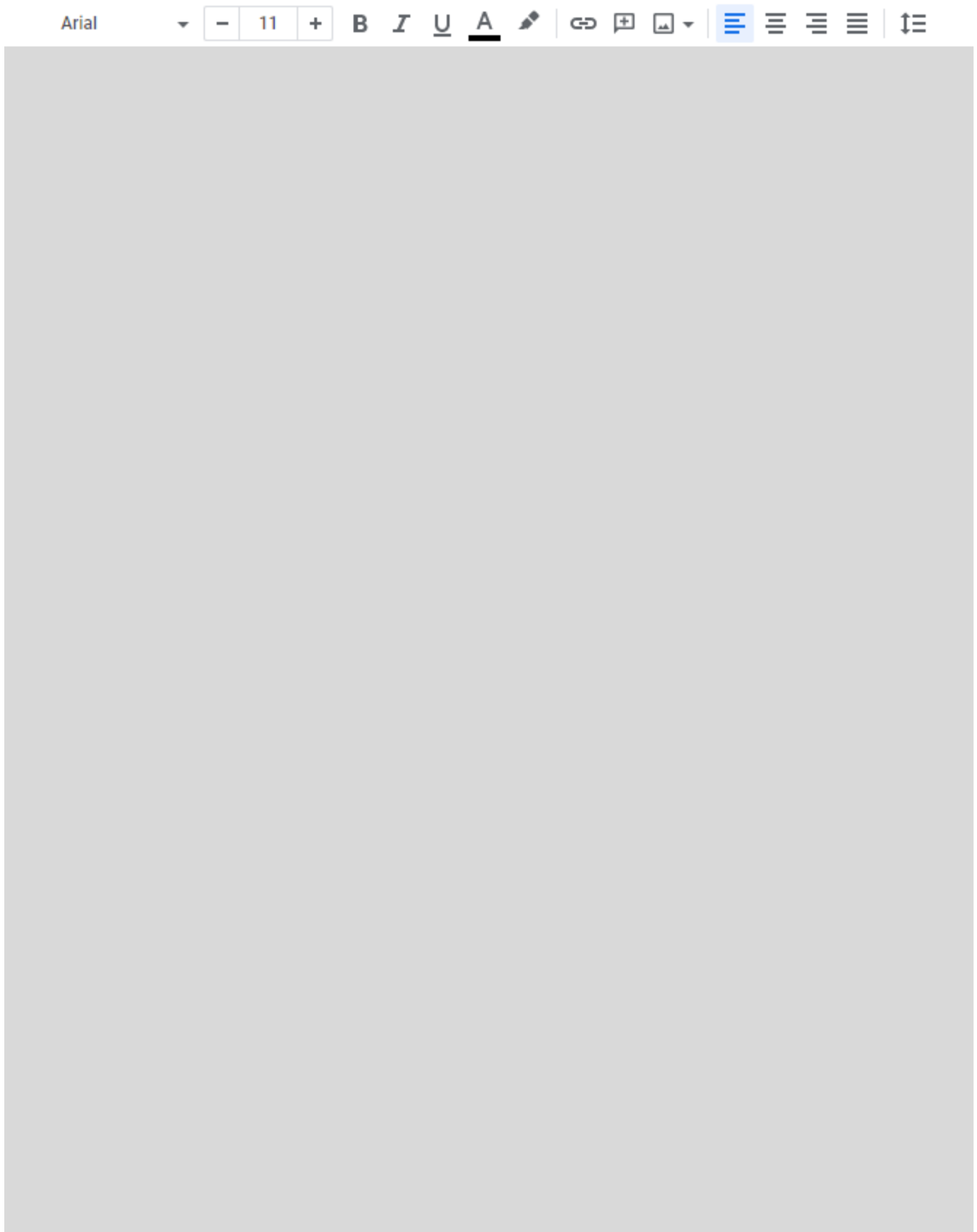

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Guanine Nucleotide Exchange Factor (GEF) Assay SOP

NOTE: This assay is very sensitive to salt, pH, and temperature. Make sure all proteins are in the same buffer before you run the assay.

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- Read for ~150seconds and then add Mix II to Mix I, pipetting very carefully to avoid bubbles
  - If you are using a cuvette, you will have to use a gel loading tip to mix
- Almost immediately, you should see a steep increase in the fluorescence.
- Keep reading until the reaction has plateaued

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Arial 11

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NOTE: The buffer that I found worked well is 10mM HEPES pH 7.0, 50mM NaCl, 2mM MgCl2, and 1mM DTT. I turned on the temperature control on the fluorimeter, but the plate reader should be relatively insensitive to temperature change.

NOTE: This reaction is based on Mant-GTP fluorescence. When Mant-GTP is loaded into the GTPase, you will see an increase in fluorescence.

NOTE: You will need to optimize protein concentrations for your assay. I found that 0.4uM Tiam1 GEF worked well for me, but I had to use 10uM Vav2 GEF to see similar activity. You will need to do a titration to see. You may also need to vary the GTPase concentration and the Mant-GTP concentration.

NOTE: GEF activity is dependent on the DH domain, but almost all GEFs contain a tandem DH-PH domain. The PH domain helps increase the activity of the GEF protein, removing it will decrease the activity of the protein.

- Start up the fluorimeter or the plate reader. Set the excitation to 350nm and the emission to 440nm, read every 2s for ~10 minutes (the reaction is incredibly quick and will be over before that)
- Make Mix I and add it to the cuvette and start reading. The components are:
    - GEF Buffer to fill the volume to 150uL
    - GTPase at the appropriate concentration (I used 10uM protein)
    - Mant-GTP to the final concentration (I used 2uM)
- While Mix I is reading, make Mix II. The components are:
    - GEF Buffer to fill the volume to 150uL
    - GEF protein to the appropriate concentration (I used 0.4uM for Tiam1 and 10uM for Vav2).
    - Mant-GTP to the same concentration as in Mix I
        - NOTE: c is critically important. If you do not add Mant-GTP to both mixes the fluorescence will drop from a reduction in concentration, not anything to do with your assay.
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