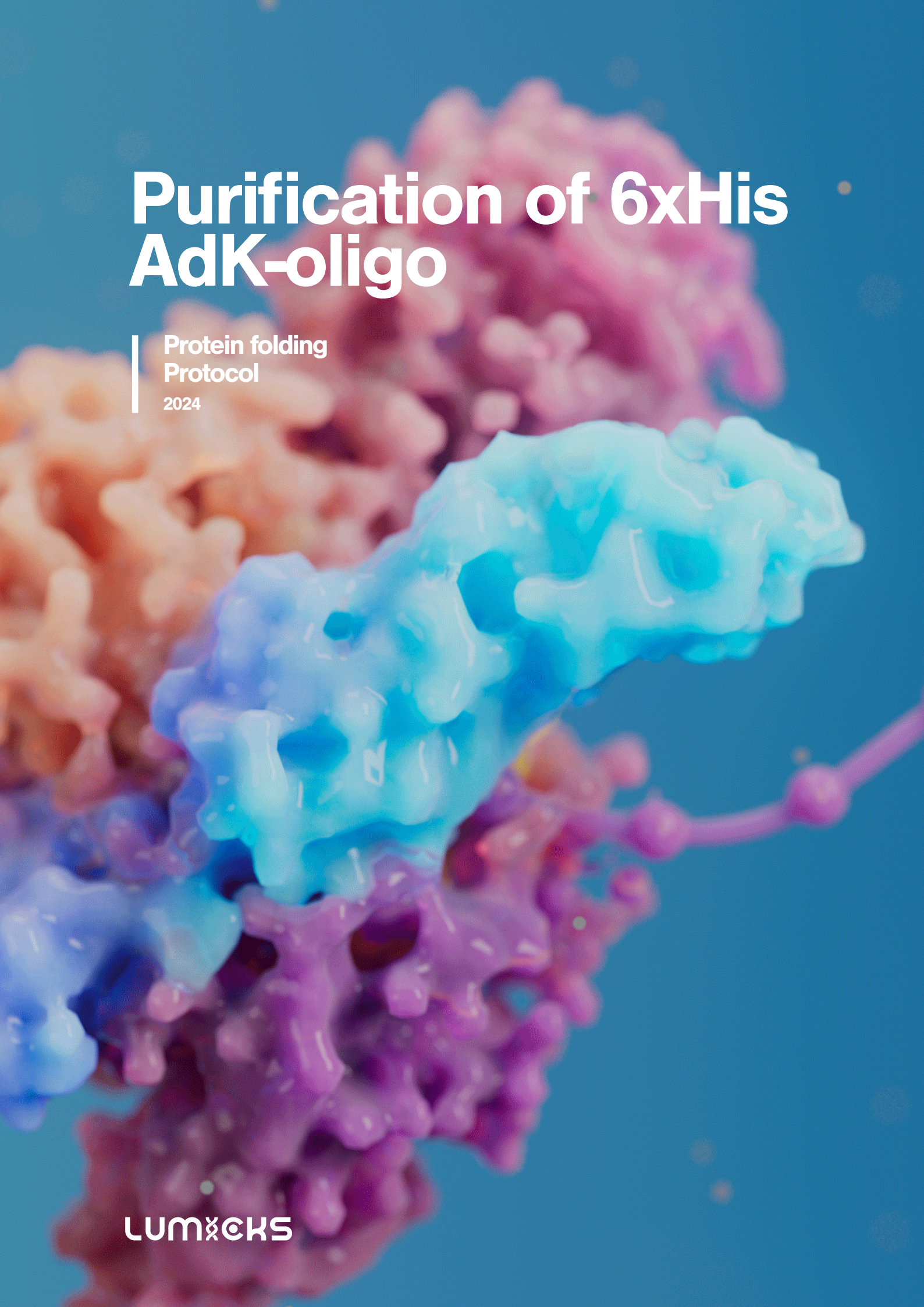
Purification of 6xHis AdK-oligo

Protein folding
Protocol
2024



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Protocol introduction

Purification of 6xHis AdK-oligo using His SpinTrap™ (GE28-4013-53)

Notes before starting the purifcation

- Modifications applied to the instructions of the manufacturer (SpinTrap™) are underlined and are recommended to improve the yield of the protein-oligo purifications.
- It is highly recommended to follow handling instructions of the manufacturer, including safety warnings and precautions.

Buffer preparation

Note: Make sure that the imidazole solution used to prepare the following buffers has a pH ranging between 7 and 8.

- Prepare binding buffer: 40 mM Tris-HCl pH 7.5, 300 mM NaCl, 20 mM imidazole.
- Prepare elution buffer: 40 mM Tris-HCl pH 7.5, 300 mM NaCl, 500 mM imidazole.

Column equilibration

- Following the manufacturer's instructions:
    1. Invert and shake the column repeatedly to resuspend the medium containing the His SpinTrap™ beads (resin)
    2. Loosen the top cap one-quarter of a turn and twist off the bottom closure.
    3. Place the column in a 2 ml microcentrifuge tube and centrifuge for 30 s at 70 to 100 x g.
    4. Discard the flow-through.
    5. Remove and discard the top cap.
- Add 600 ul of binding buffer to the resin in the column and mix by pipetting with a cut tip until the resin is homogenously resuspended.
- Centrifuge for 30 s at 70 to 100 x g, discard flow-through, and re-use the 2 ml tube for the next step.

Binding of 6xHis AdK-oligo to the resin

- Upon completion of the Step 1 “Purification of protein-oligo chimera” of Protein labeling (cysteine or ybbR), add 80 µl of binding buffer to the 80 µl of 6xHis AdK-oligo (Cys) solution or 115 µl of binding buffer to the 45 µl of 6xHis AdK-oligo (ybbR). **Note**: all the following steps are identical for 6xHis AdK-oligo (Cys) and 6xHis AdK-oligo (ybbR).
- Add the 6xHis Adk-oligo solution diluted with binding buffer to the resin and mix by pipetting with a cut tip until the resin is homogenously resuspended.
- Incubate for 3 minutes. Then resuspend the resin again homogenously.
- Repeat the previous step for additional two times to obtain a total incubation time of AdK-oligo with resin of ~9-10 min. **Note:** if a shorter incubation time is preferred for the protein of interest, go to the next step after the first 3 minutes incubation with the resin.
- Centrifuge for 30 s at 70 to 100 x g, discard flow-through, and re-use the 2 ml tube for the next step.

Clean up from the excess of unreacted oligos

- Add 600 µl of binding buffer to the resin and resuspend the resin homogenously.
- Centrifuge for 30 s at 70 to 100 x g, discard flow-through, and re-use the 2 ml tube for the next step.
- Repeat the previous two steps for three additional times to ensure a complete clean up from the excess of unreacted oligos.

Elution

- Place the column in a new 2 ml tube.
- Add 200 ul elution buffer and resuspend the resin homogenously.
- Incubate for 3-5 min.
- Centrifuge for 30 s at 70 to 100 × g.
- Using the same 2 ml tube, add 200 ul elution buffer and resuspend the resin homogenously.
- Incubate for 3-5 min.
- Centrifuge for 30 s at 70 to 100 × g and collect the purified 6xHis AdK-oligo protein.

You are now ready to continue with the buffer exchange from the first bullet point in Step 2 Purification of protein-oligo chimera.

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