

Continued from page:

- SA- Apt grafting

5

(A) (No Cys) $\rightarrow \oplus$: AS415 (linker modified) $\rightarrow 0.5 \text{ mg}$
 $\rightarrow \ominus$: AS413 (linker modified) $\rightarrow 0.5 \text{ mg}$

10

(B) (Cys) $\rightarrow \oplus$: AS415 (linker modified) $\rightarrow 0.5 \text{ mg}$
 $\rightarrow \ominus$: AS413 (linker modified) $\rightarrow 0.5 \text{ mg}$

• Integrate the aliquots: $10 \mu\text{l}$ each (1 nmol) + $40 \mu\text{l}$ PBS + 5 mM MgCl_2 $\rightarrow 5 \text{ min}$, 90°C

• Cool down 15 min , at,

• Prep TCEP sol: 0.48 mg/mL in Tris-HCl $\rightarrow$ put $50 \mu\text{l}$ per $10 \mu\text{l}$ of apt aliqu $\rightarrow 100 \mu\text{l}$ incub, 2 h , rt (1500 rpm)

25

NPs $\rightarrow$ resusp in 0.5 mL PBS + 5 mM MgCl_2 $\rightarrow$ wash (very long pellet) $\rightarrow$ put reduced-apt on top ($50 \mu\text{l}$ each + washes:

30

- Tween 20 0.01% (1x)

- Hybrid buffer (1x)

- PBS + 5 mM MgCl_2 (1x)

Final resusp: 0.5 mL
 $\rightarrow (1 \text{ mg/mL})$

Take $20 \mu\text{l}$ for OLS

OLS results:

35

(A) $\oplus$ PBS: $142.3 \pm 1.5 \text{ nm}$ (PDI: 0.12)

DMA: $233.4 \pm 33.1 \text{ nm}$ (PDI: 0.16)

pH 7: $-36.6 \pm 0.8 \text{ mV}$

pH 3: $-20.3 \pm 4.1 \text{ mV}$

40

(A) $\ominus$

PBS: $117.4 \pm 3.9 \text{ nm}$ (PDI: 0.15)

DMA: $139.7 \pm 1.5 \text{ nm}$ (PDI: 0.21)

pH 7: $-30.1 \pm 1.4 \text{ mV}$

pH 3: $-13.3 \pm 2.5 \text{ mV}$

45

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