Carbon-Binding Designer Proteins that Discriminate Between Graphitic and Diamond Surfaces

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Raman Spectroscopy Analysis of Carbon Allotropes. The 5 different carbon substrates used in this study were analyzed by Raman spectroscopy (Fig. S1). MWNT, glassy carbon and detonation diamond powders were washed with ddH_2O, deposited on a glass slide and allowed to dry. Spectra were acquired for 10 seconds on a Renishaw inVia Raman microspectrometer through a Leica DM IRBE optical microscope. A 514 nm argon source was used to irradiate samples through a 50x objective lens. Raman-scattered light was captured on a cooled charge-coupled device. The location of the D peaks and G peaks is consistent with literature values. [1-4]

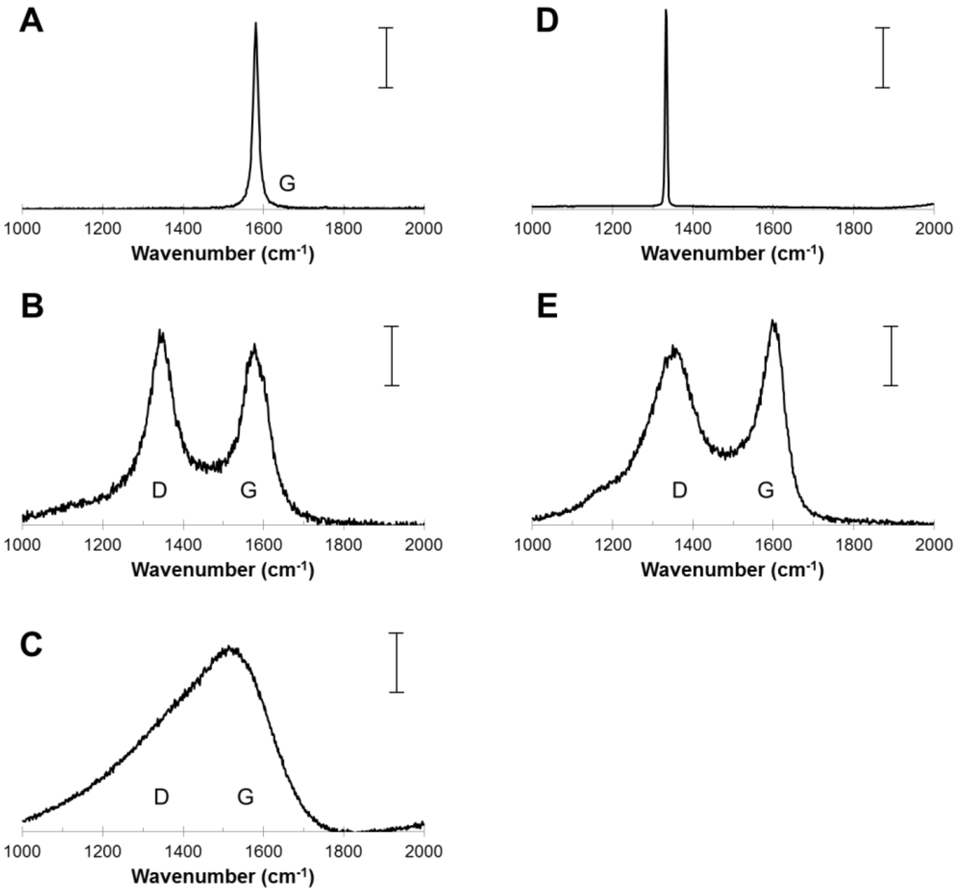

Figure S1. Raman spectra of carbon substrates used in this study. (A) HOPG (bar :110 counts/sec); (B) MWNT (bar: 30 counts/sec); (C) evaporated carbon (bar: 160 counts/sec); deconvoluted peaks occur at 1364 and 1536 $cm^{-1;}$ (D) diamond powder (bar 3000 counts/sec); (E) glassy carbon powder (bar 55 counts/sec).

Determination of K_d by Surface Plasmon Resonance (SPR). Surface Plasmon Resonance measurements were conducted on homemade SPR glass chips coated with a ≈ 2 nm titanium adhesion layer, a ≈ 48 nm evaporated gold film, and a ≈ 4 nm evaporated carbon film. Chips were cleaned with ethanol and UV-ozone as above and mounted on a 4-channel flow cell SPR sensor from the Institute of Photonics and Electronics (Prague, Czech Republic) which includes temperature control (25°C) and a peristaltic pump for controlled flow at 50 µL/min. The SPR design is based on an attenuated total reflection and measures wavelength modulation.

To account for variations in the thickness of deposited carbon films, K_d values were extracted from sensograms recorded at 4 different concentrations on the same 4-channel SPR chip. We assumed 1st order Langmuir association kinetics and determined K_d from the knowledge of the observable rate constants (k_{obs}) at each protein concentration. Briefly, Equation 1 was used to fit each SPR curve using a least squares fit method while varying both σ_∞ and k_{obs}. The k_{obs} values were plotted as a function of protein concentration (Fig. S2) and a linear fit was used to determine k_a and k_d values according to Equation 2. Finally, K_d was obtained as the ratio of these constants (Equation 3).

$$\boldsymbol{\sigma_{obs}(t) = \sigma_\infty(1 - e^{(-k_{obs}t)})} \qquad \textbf{Equation 1}$$

$$\boldsymbol{k_{obs} = k_a C + k_d} \qquad \textbf{Equation 2}$$

$$\boldsymbol{K_d = \frac{k_d}{k_a}} \qquad \textbf{Equation 3}$$

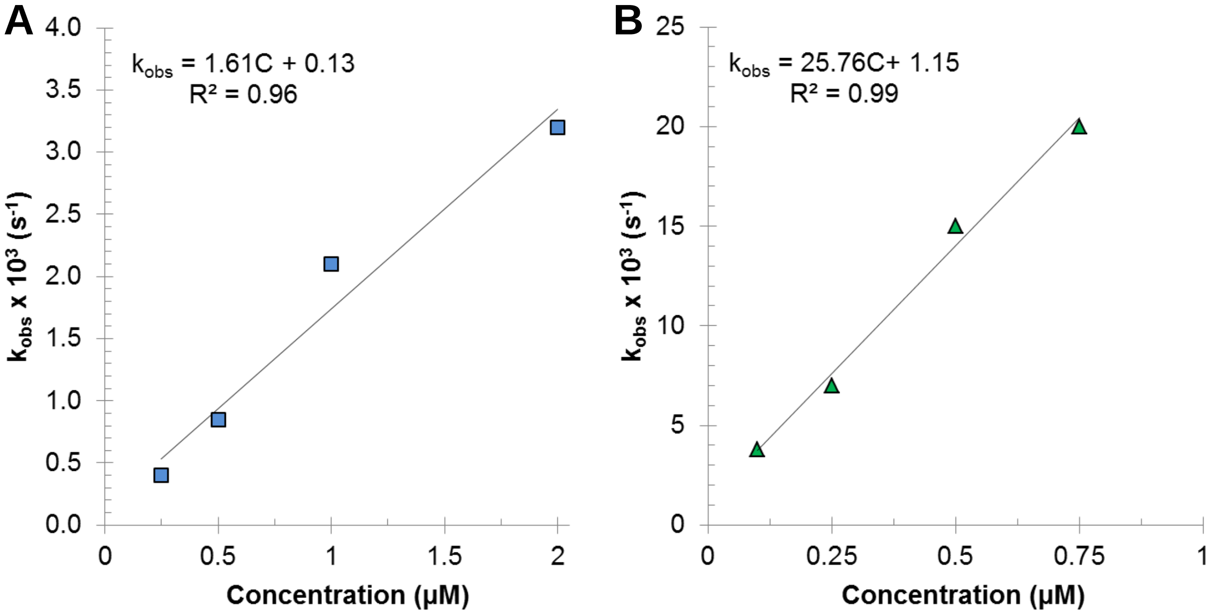

Figure S2. Linear fit models used to determine the equilibrium dissociation constant (K_d) of TrxA::Car15 (A) or TrxA::Car9 (B) on carbon surfaces through SPR.

AFM Analysis of the Adsorption of Carbon-Binding TrxA Derivatives to HOPG

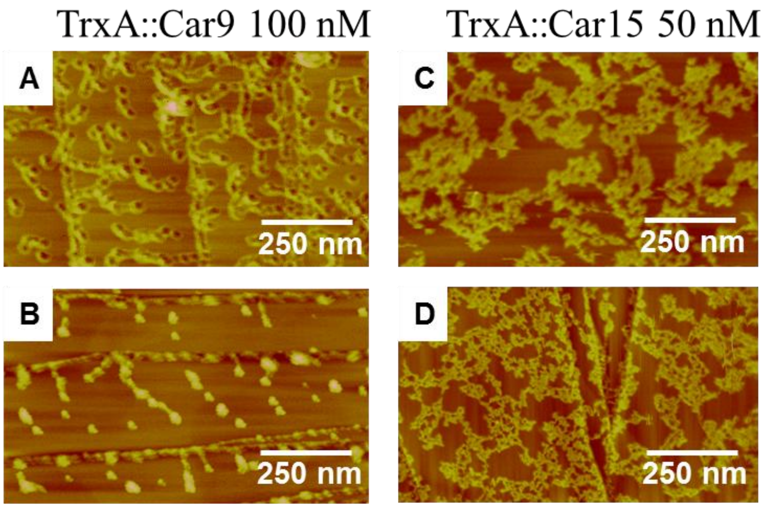

Figure S3. AFM images of the adsorption of 100 nM TrxA::Car9 (A and B) and 50 nM TrxA::Car15 (C and D) on freshly cleaved HOPG. All solutions were contacted for 10 min with freshly cleaved HOPG. Surfaces were imaged by AFM after washing with ddH_2O and drying.

AFM Analysis of the Adsorption of Authentic TrxA on HOPG

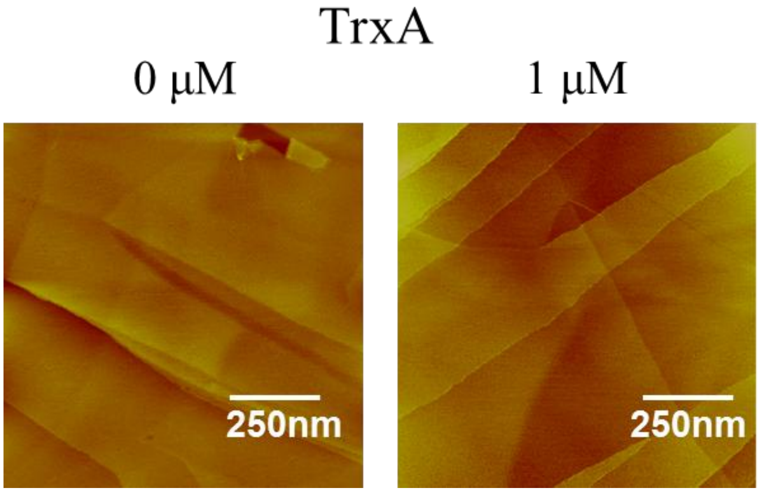

Figure S4. Pure 50 mM sodium phosphate buffer pH 7.5 (left) or authentic TrxA at 1 µM concentration were contacted for 10 min with freshly cleaved HOPG. Surfaces were imaged by AFM after washing and drying.

REFERENCES

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