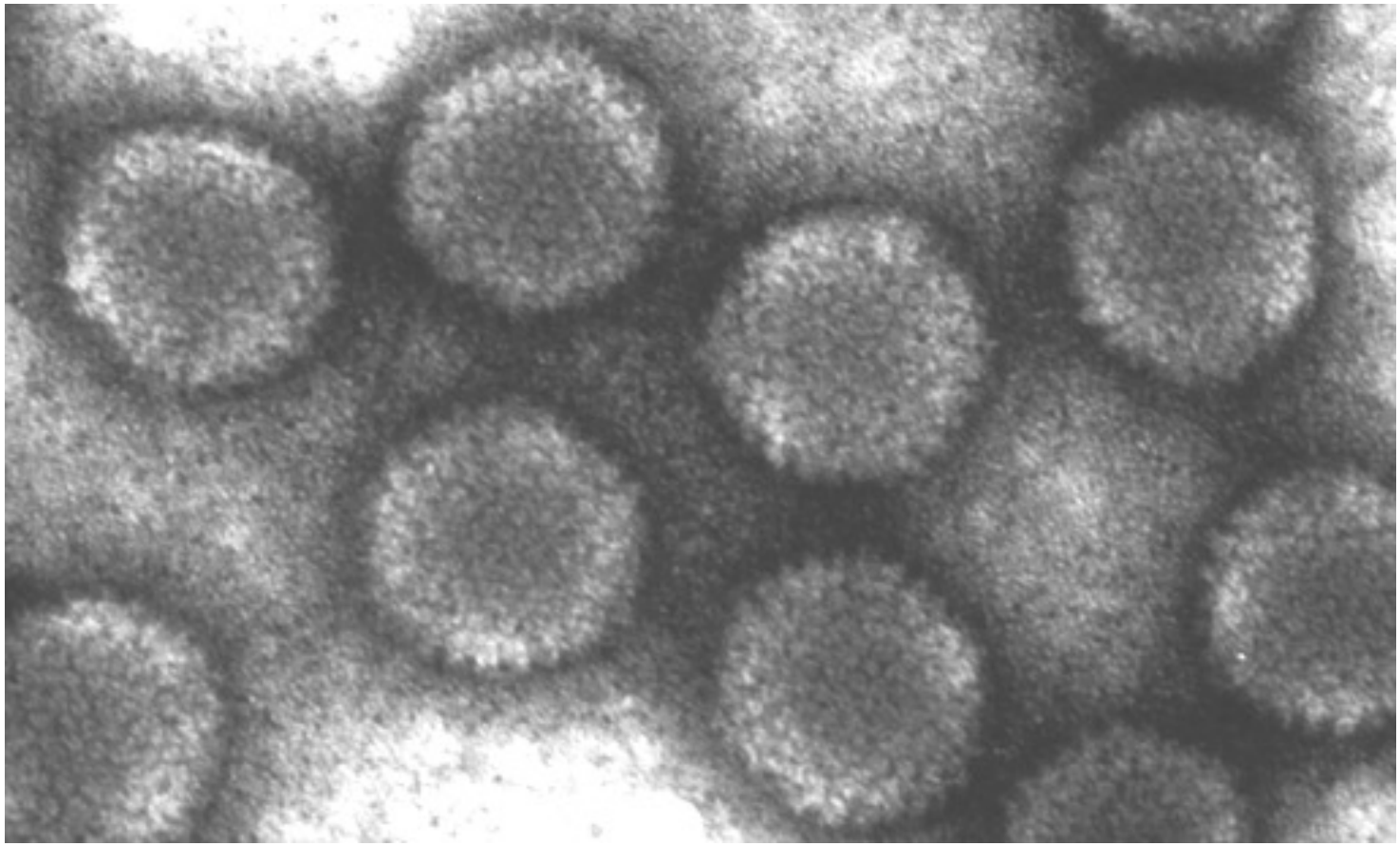
Science is a beautiful gift to
humanity; we should not distort it.

A. P. J. Abdul Kalam

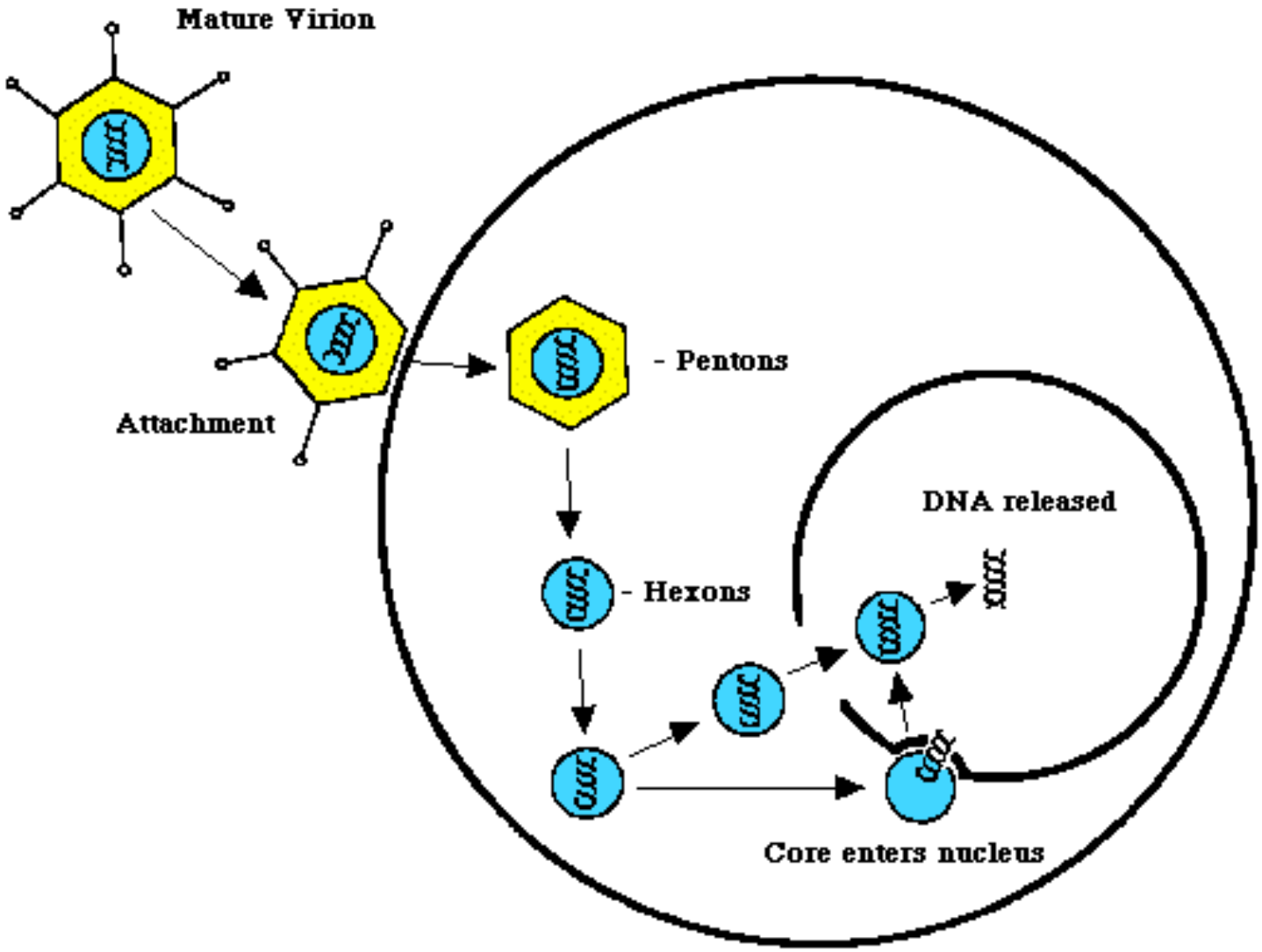
Ad2



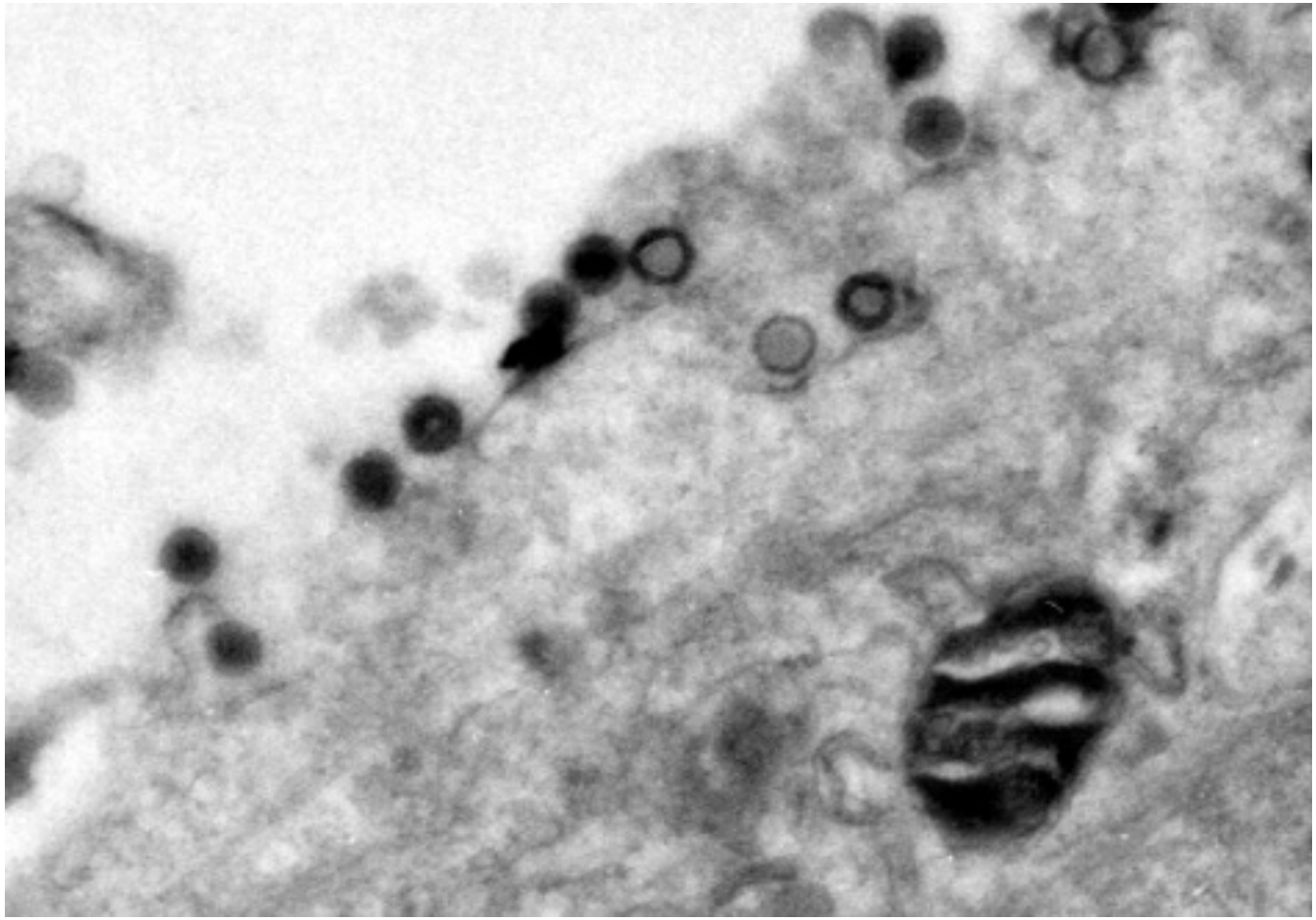
Electron micrograph of negatively stained Ad2 (source, K. Boucke). For further information see (1; 2).

(1). Valentine, R. C., and Pereira, H. G. (1965) J. Mol. Biol. 13, 13-20.
(2). Greber, U. F., et al. (1998) in Adenovirus entry into cells: A quantitative fluorescence microscopy approach, ed. W. S. M. Wold (Humana Press, Inc, Totowa, NJ USA), pp. 217-230.

Ad entry into the cell

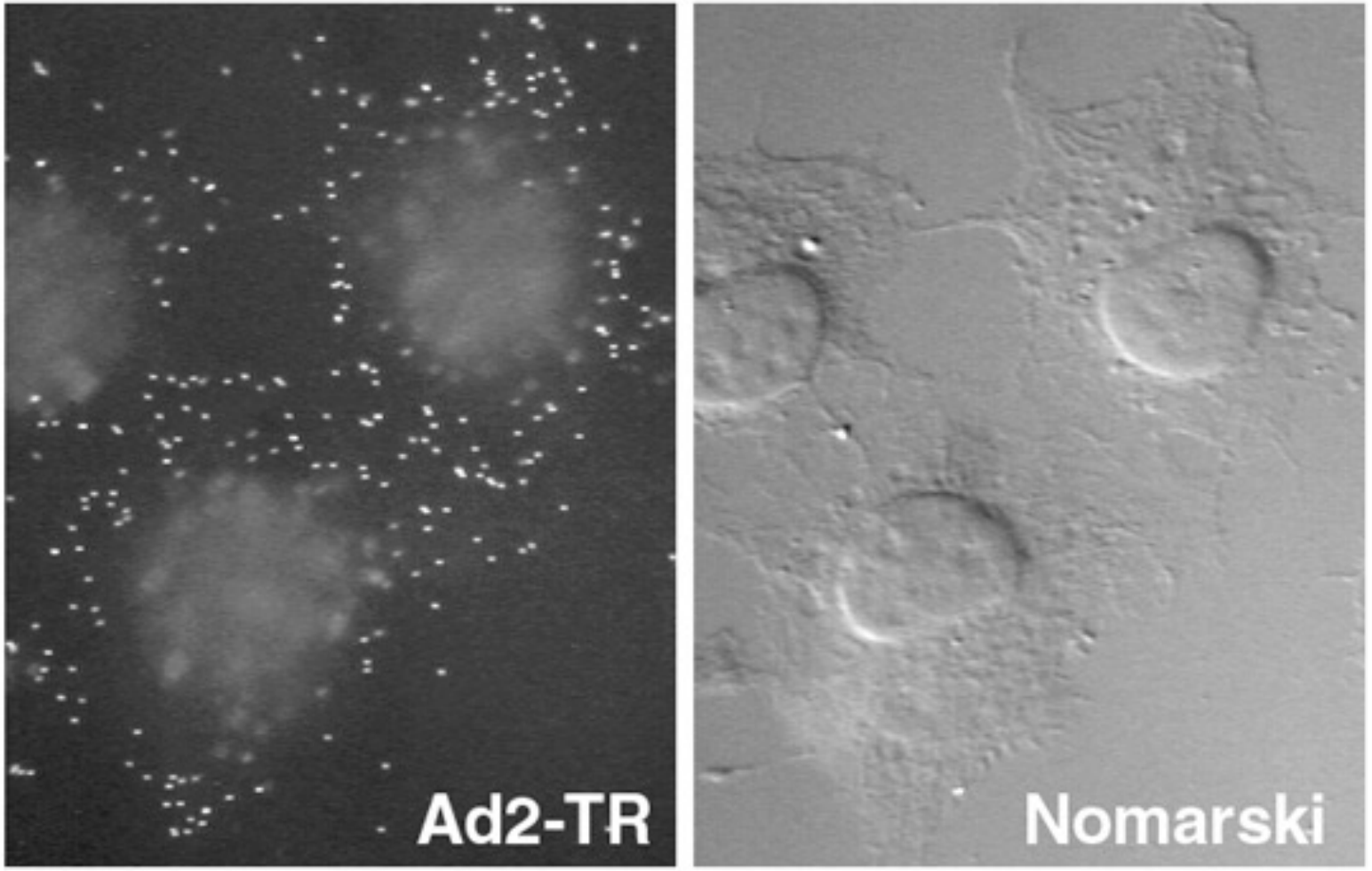


Ad-binding

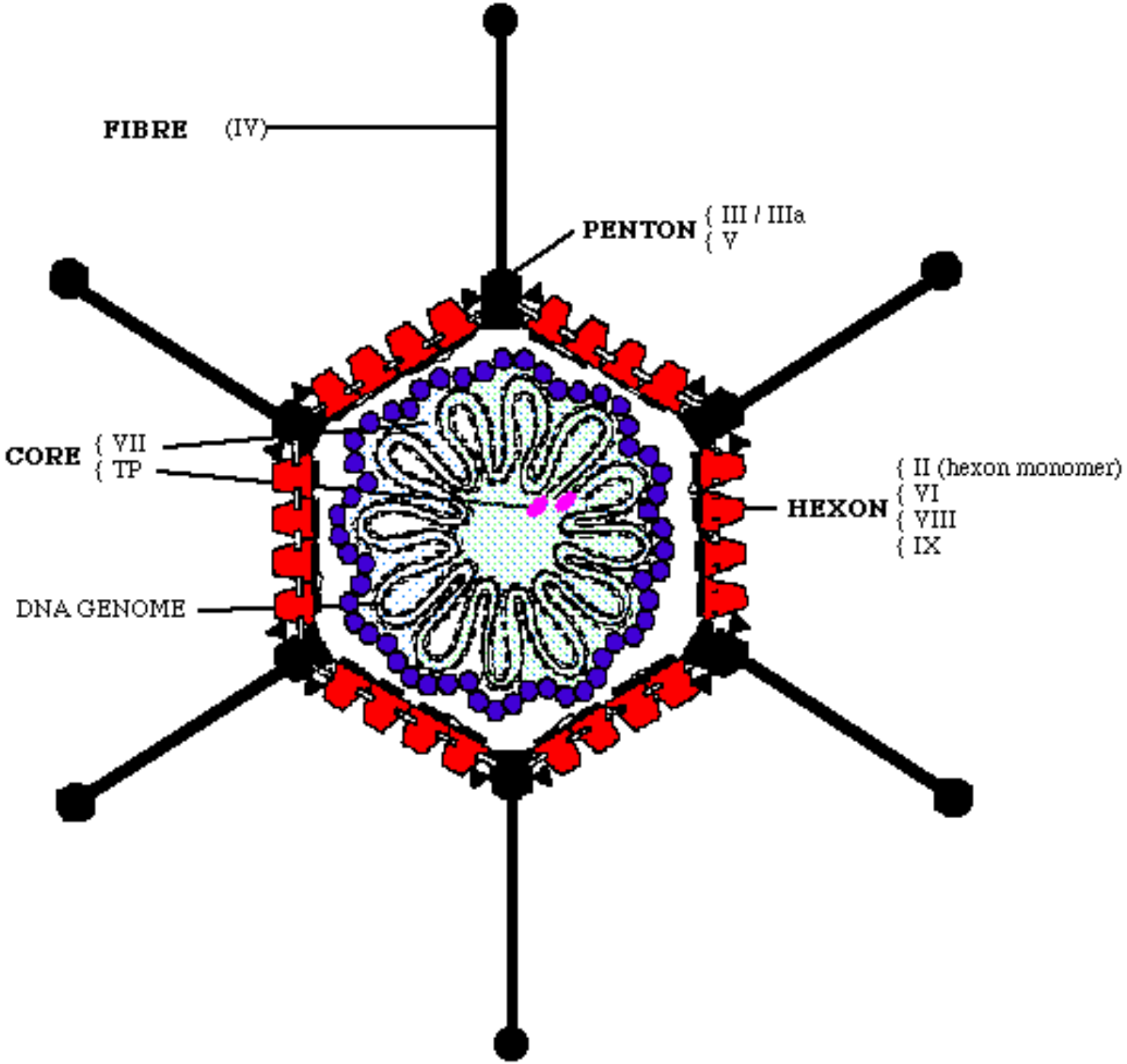


Electron micrograph of Ad2 attached to the HeLa cell surface (source, K. Boucke)

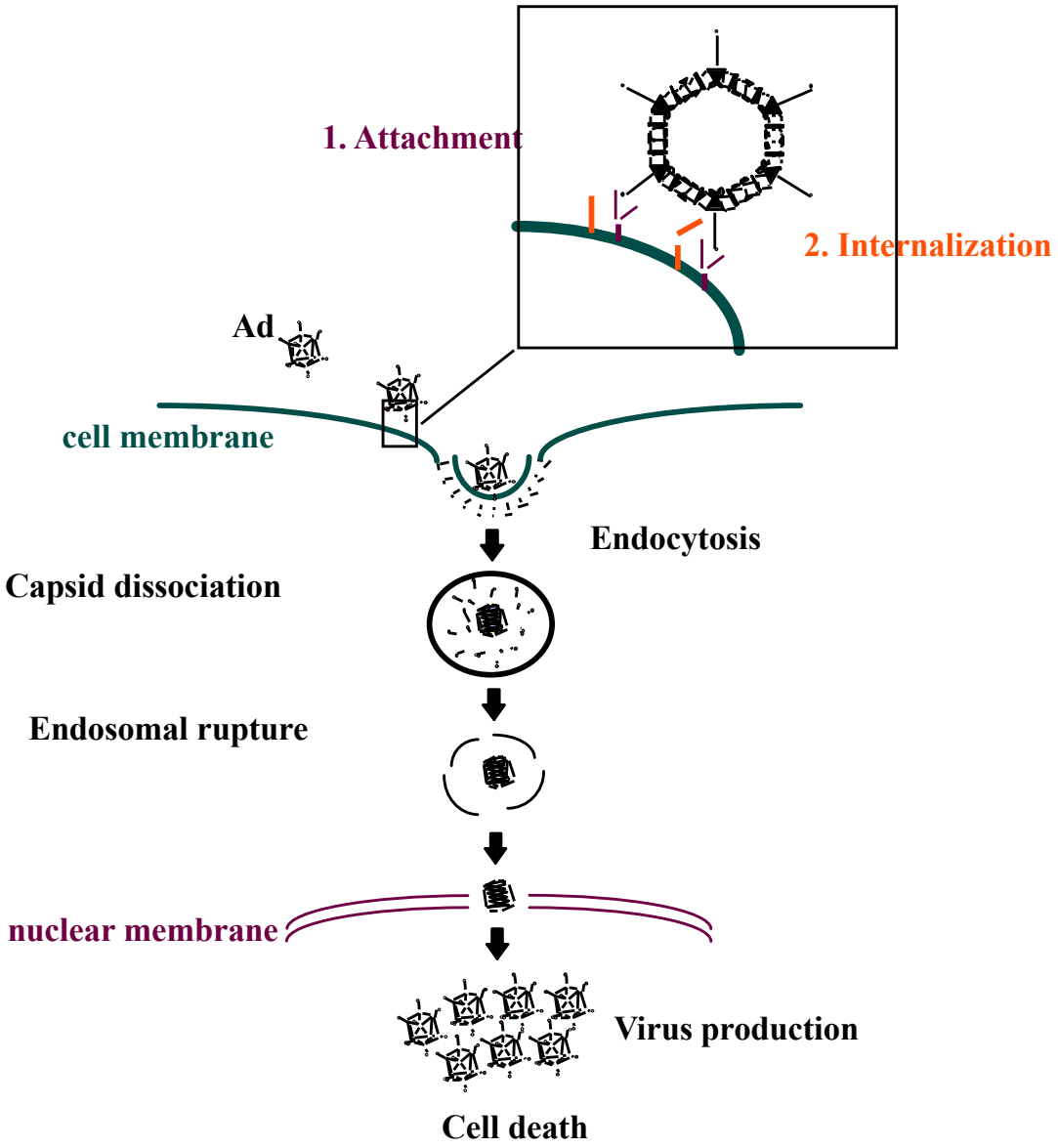
Ad-binding



Fluorescence micrograph of texas red-labeled Ad2 bound to the surface of HeLa cells. Corresponding Nomarski image is shown on the right side (source, U. Greber).

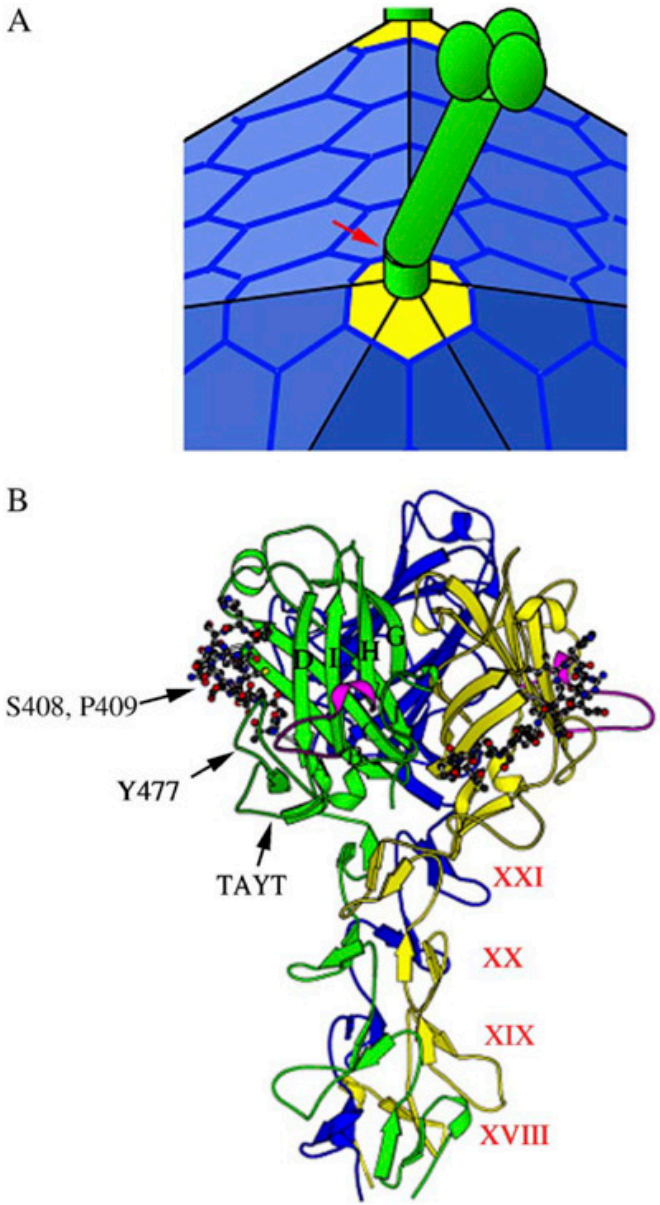

FIBRE (IV)
PENTON { III / IIIa
{ V
CORE { VII
{ TP
DNA GENOME
HEXON { II (hexon monomer)
{ VI
{ VIII
{ IX

Ad entry into cells



Fibre

(A) Fiber **trimers** (green) protrude from each penton complex (yellow) of the icosahedral capsid of adenovirus. The fiber trimer comprises N-terminal tails (thin tubes), a central shaft, and a globular knob (ovals). The third -repeat of the shaft is indicated by a red arrow. (B) Critical features of the fiber are shown in the crystal structure of Ad 2 fiber. beta-strands of the fiber knob are lettered from A to J, according to the nomenclature of Xia et al. 97. The CAR binding site, which is made up mostly by the AB loop (ball and stick), lies along the side of the fiber knob trimer. **Locations of some mutations that abolish CAR binding are indicated by arrows**. The HI loop is shown in magenta. The final four -repeats of the fiber shaft (18–21) are shown with Roman numerals. This image was made using Molscript98. (Features were omitted in blue model for clarity).



Ad-Hexon

Coagulation factor FX binds to the Ad5 hexon, not fiber, via an interaction between the FX Gla domain and hypervariable regions of the hexon surface. Liver infection by the FX-Ad5 complex is mediated through a heparin-binding exosite in the FX serine protease domain. This study reveals an unanticipated function for hexon in mediating liver gene transfer invivo.

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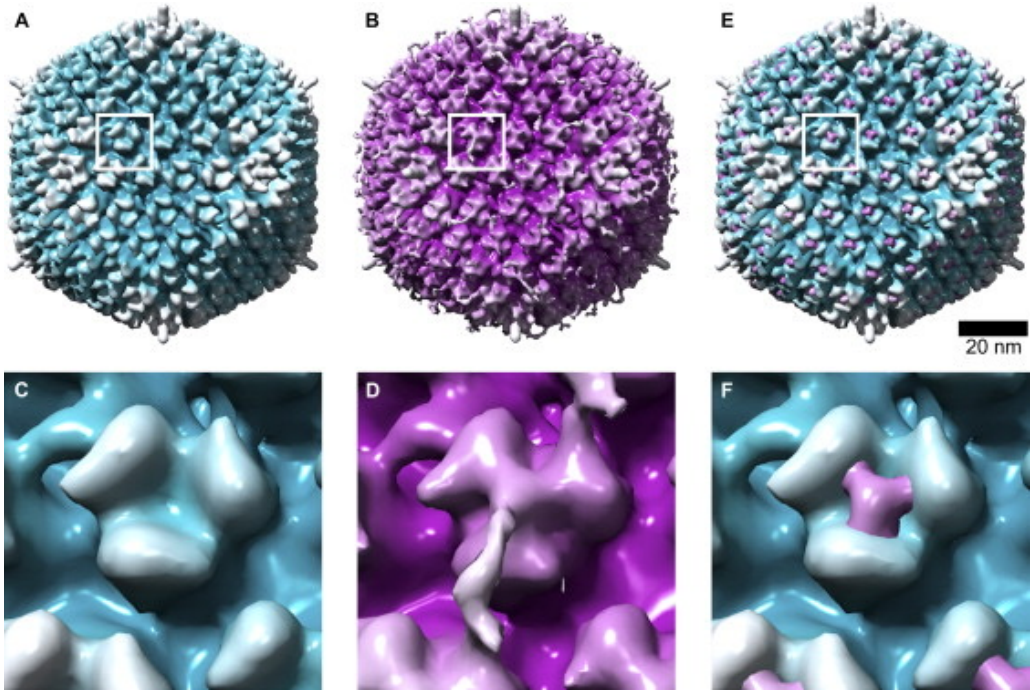
Ad-Hexon



Azzurro esone
Violetto FX

(A and B) Three-dimensional reconstructions of uncomplexed Ad5 (A) and FX-Ad5 complex (B) surface contoured to include density above the mean plus one standard deviation of total map density.

(C and D) Closeup view of an uncomplexed (C) and FX-complexed (D) hexon.

(E) Overlaid reconstructions of uncomplexed Ad5 (blue) and FX-Ad5 complex (purple). The scale bar represents 20 nm. The surface threshold level of the FX-Ad5 structure is raised to highlight the point of contact between FX and Ad5 hexon.

(F) Closeup view of FX-labeled hexon.

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Ad-Hexon

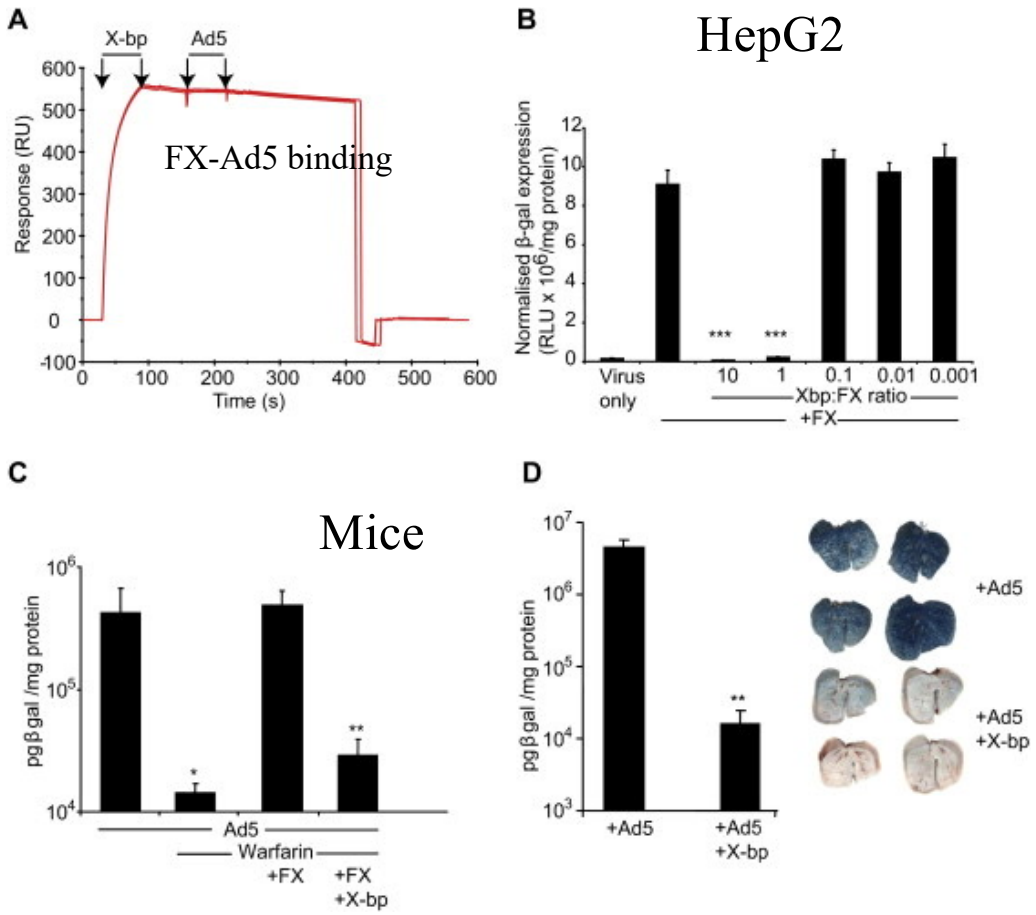

Figure 6. Pharmacological Blockade of Ad5 Binding to FX by **Snake Venom Protein X-bp** Blocks Liver Transduction In Vivo

(A) Subtracted SPR sensorgram (FX-FXI) shows X-bp binding with high affinity (increase in RU following X-bp injection) and ablates subsequent FX-Ad5 binding (no change in RU following Ad5 injection). Arrows indicate the start and end of reagent injection.

(B) HepG2 cells were exposed to AdKO1 in the presence of FX alone or FX preincubated with X-bp at different FX:X-bp molar ratios (as shown). ***p < 0.0001 versus no X-bp. Error bars represent SEM.

(C) MF-1 mice were pretreated with control peanut oil or warfarin and injected with 4 × 1011 VP/mouse Ad5 with or without preinjection of FX alone or preincubated with three-fold molar excess of X-bp. *p = 0.006; **p = 0.0002. Error bars represent SEM.

(D) MF-1 mice (nonwarfarinized) were injected with X-bp 30 min prior to Ad5 injection. Forty-eight hours later liver gene transfer was quantified by ELISA (bar graph) and visualized by staining for β-galactosidase (pictures). (**p = 0.0002). Error bars represent SEM.

Warfarin: blocks reductase -> blocks vitK-> blocks FX
Xbp: binds and blocks directly FX

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Gene therapy project

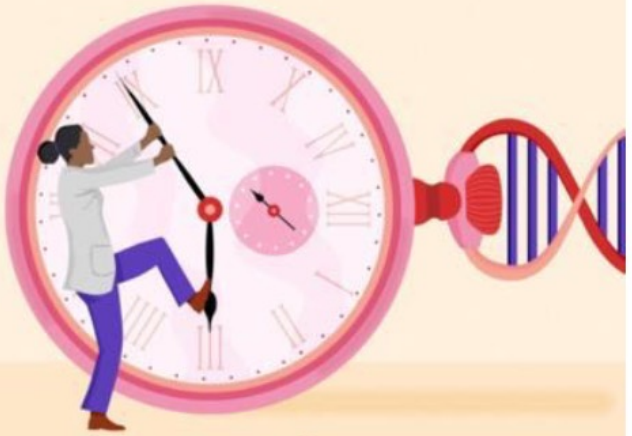
Theme I: Aging
Group A: Bernardi, Ilie, Colonnelli, Bastianelli
Charcot marie tooth – pmp22
Group B: Hazrati, Bartolini, Glaudo, Montrone, Pourali
Spastic paraplegias 3a – atl1

Theme II: Cancer
Group C: Belvedere, Jeong, Majaliwa, Virgilio
Retinoblastoma – rb1
Group D: Santacroce, Pace, Serra, Fanelli, Duarte
Hepatic cancer - plk1

Gene therapy project – example 2022

Defeating Huntington: mHTT degradation by AAV5-TERT non-canonical autophagy induction

N. Salvi, A. Tognon, E. Roscioli, G. Spina, K. Jalilian



Prof. Isabella Saggio
Tutors: Dr M. La Torre, Dr R. Burla

SAPIENZA
UNIVERSITÀ DI ROMA

Gene therapy project

30/10/23

1 slide/group

Title
General Idea
Aim